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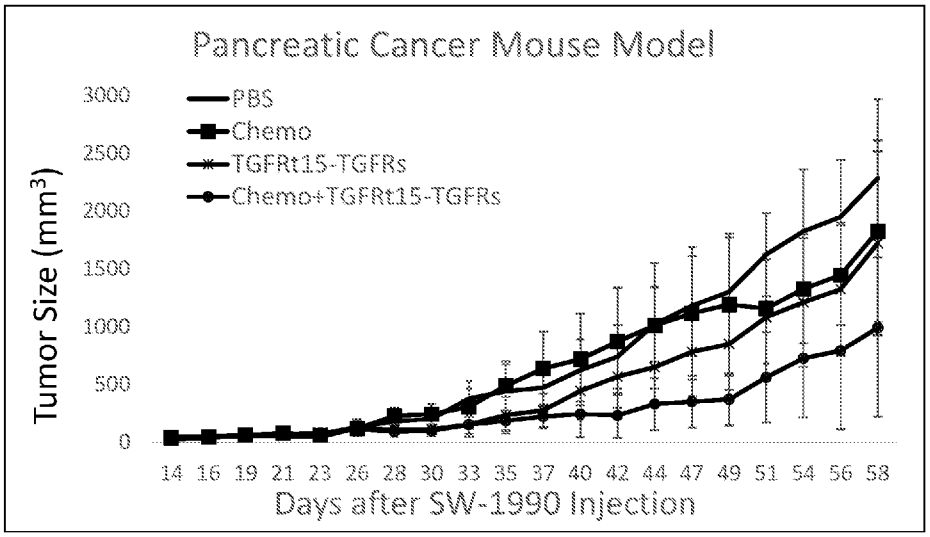


Figure 107

(57) **Abstract:** Provided herein are methods of treating an aging-related disease or condition in a subject in need thereof, killing or reducing the number of senescent cells in a subject in need thereof, improving the texture and/or appearance of skin and/or hair in a subject in need thereof, and assisting in the treatment of obesity in a subject in need thereof, that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells.

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METHODS OF TREATING AGING-RELATED DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to: U.S. Patent Application Serial No. 62/816,683, filed March 11, 2019; U.S. Patent Application Serial No. 62/725,038, filed August 30, 2018; U.S. Patent Application Serial No. 62/817,244, filed March 12, 2019; U.S. Patent Application Serial No. 62/881,039, filed July 31, 2019; and U.S. Patent Application Serial No. 62/724,969, filed August 30, 2018; U.S. Patent Application Serial No. 62/817,230, filed March 12, 2019; U.S. Patent Application Serial No. 62/725,043, filed August 30, 2018; U.S. Patent Application Serial No. 62/725,010, filed August 30, 2018; U.S. Patent Application Serial No. 62/749,007, filed October 22, 2018; U.S. Patent Application Serial No. 62/746,832, filed October 17, 2018; U.S. Patent Application Serial No. 62/749,506, filed October 23, 2018; U.S. Patent Application Serial No. 62/817,241, filed March 12, 2019; and U.S. Patent Application Serial No. 62/881,088, filed July 31, 2019, each of which is incorporated hereby reference in its entirety.

TECHNICAL FIELD

The present disclosure relates to the field of immunology and cell biology.

BACKGROUND

Senescence is a form of irreversible growth arrest accompanied by phenotypic changes, resistance to apoptosis, and activation of damage-sensing signaling pathways. Cellular senescence was first described in cultured human fibroblast cells that lost their ability to proliferate, reaching permanent arrest after about 50 population doublings (referred to as the Hayflick limit). Senescence is considered a stress response that can be induced by a wide range of intrinsic and extrinsic insults, including oxidative and genotoxic stress, DNA damage, telomere attrition, oncogenic activation, mitochondrial dysfunction, or chemotherapeutic agents.

Senescent cells remain metabolically active and can influence tissue hemostasis, disease, and aging through their secretory phenotype. Senescence is considered as a

physiologic process and is important in promoting wound healing, tissue homeostasis, regeneration, and regulation of fibrosis. For instance, transient induction of senescent cells is observed during would healing and contributes to wound resolution. Senescence also plays a role in tumor suppression. The accumulation of senescent cells also drives aging and aging-related diseases and conditions. The senescent phenotype also can trigger chronic inflammatory responses and consequently augment chronic inflammatory conditions to promote tumor growth. The connection between senescence and aging was initially based on the observation that senescent cells accumulate in aged tissue. The use of transgenic models has enabled the detection of senescent cells systematically in many aging-related disorders. Strategies to selectively eliminate senescent cells have demonstrated that senescent cells play a causal role in aging-related disorders.

SUMMARY

The present invention is based on the discovery that administration of NK cell activating agents to a mammal having a cancer resulted in a tumor inhibition and administration of NK cell activating agents to a diabetic animal model demonstrated improved skin and hair appearance and texture, and decreased blood glucose levels. In view of this discovery provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent (s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s) and/or or a therapeutically effective number of activated NK cells. Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof

over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells.

Provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s). In some embodiments of any of the methods described herein, the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells. In some embodiments of any of the methods described herein, the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells. In some embodiments of any of the methods described herein, the subject has been identified or diagnosed as having an aging-related disease or condition.

In some embodiments of any of the methods described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease. In some embodiments of any of the methods described herein, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate

cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some embodiments of any of the methods described herein, the autoimmune disease is type-1 diabetes.

In some embodiments of any of the methods described herein, the metabolic disease is selected from the group of: obesity, a lipodystrophy, and type-2 diabetes mellitus.

In some embodiments of any of the methods described herein, the neurodegenerative disease is selected from the group of: Alzheimer's disease, Parkinson's disease, and dementia.

In some embodiments of any of the methods described herein, the cardiovascular disease is selected from the group of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

In some embodiments of any of the methods described herein, the skin disease is selected from the group of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

In some embodiments of any of the methods described herein, the progeria disease is selected from the group of: progeria and Hutchinson-Gilford Progeria Syndrome.

In some embodiments of any of the methods described herein, the fragility disease is selected from the group of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some embodiments of any of the methods described herein, the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

In some embodiments of any of the methods described herein, the aging-related disease or condition is type-2 diabetes or atherosclerosis.

In some embodiments of any of the methods described herein, the administering results in a decrease in the number of senescent cells in a target tissue in the subject. In some embodiments of any of the methods described herein, the target tissue is selected from the group of: adipose tissue, pancreatic tissue, liver tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

In some embodiments of any of the methods described herein, the administering results in an increase in the expression levels of CD25, CD69, mTORC1, SREBP1, IFN-γ, and granzyme B in activated NK cells.

Also provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective number of activated NK cells.

Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective number of activated NK cells. In some embodiments of any of the methods described herein, the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells. In some embodiments of any of the methods described herein, the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells. In some embodiments of any of the methods described herein, the subject has been identified or diagnosed as having an aging-related disease or condition.

In some embodiments of any of the methods described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease. In some embodiments of any of the methods described herein, the cancer is selected from the group of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma,

rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

In some embodiments of any of the methods described herein, the autoimmune disease is type-1 diabetes.

In some embodiments of any of the methods described herein, the metabolic disease is selected from the group of: obesity, a lipodystrophy, and type-2 diabetes mellitus.

In some embodiments of any of the methods described herein, the neurodegenerative disease is selected from the group of: Alzheimer's disease, Parkinson's disease, and dementia.

In some embodiments of any of the methods described herein, the cardiovascular disease is selected from the group of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

In some embodiments of any of the methods described herein, the skin disease is selected from the group of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

In some embodiments of any of the methods described herein, the progeria disease is selected from the group of: progeria and Hutchinson-Gilford Progeria Syndrome.

In some embodiments of any of the methods described herein, the fragility disease is selected from the group of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some embodiments of any of the methods described herein, the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

Some embodiments of any of the methods described herein further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some embodiments of any of the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some embodiments of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is an artificial NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some embodiments of any of the methods described herein further include isolating the activated NK cells before the activated NK cells are administered to the subject. Some embodiments of any of the methods described herein further include introducing a nucleic acid that encodes a chimeric antigen receptor or a recombinant T cell receptor into the resting NK cell or the activated NK cell prior to administration to the subject.

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective number of activated NK cells. Some embodiments of any of the methods described herein further include: obtaining a

resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some embodiments of any of the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some embodiments of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is an artificial NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some embodiments of any of the methods described herein further include isolating the activated NK cells before the activated NK cells are administered to the subject.

In some embodiments of any of the methods described herein, the method provides for an improvement in the texture and/or appearance of skin of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the rate of formation of wrinkles in the skin of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in an improvement in the coloration of skin of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in an improvement in the texture of skin of the subject over the period of time. In some embodiments of any of the methods described herein, the method provides for an improvement in the texture and/or appearance of hair of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the rate of formation of gray hair in the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the number of gray hairs of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the rate of hair loss in the subject over time. In some embodiments of any of the methods

described herein, the method results in an improvement in the texture of hair of the subject over the period of time.

In some embodiments of any of the methods described herein, the period of time is between about one month and about 10 years. In some embodiments of any of the methods described herein, the method results in a decrease in the number of senescent dermal fibroblasts in the skin of the subject over the period of time.

Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective number of activated NK cells. Some embodiments of any of the methods described herein further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some embodiments of any of the methods described herein, the resting NK cell is an autologous NK cell obtained from the subject. In some embodiments of any of the methods described herein, the resting NK cell is an allogeneic resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is an artificial NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a haploidentical resting NK cell. In some embodiments of any of the methods described herein, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor. Some embodiments of any of the methods described herein further include isolating the activated NK cells before the activated NK cells are administered to the subject.

In some embodiments of any of the methods described herein, the method results in a decrease in the mass of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the body mass

index (BMI) of the subject over the period of time. In some embodiments of any of the methods described herein, the method results in a decrease in the rate of progression from pre-diabetes to type-2 diabetes in the subject. In some embodiments of any of the methods described herein, the method results in a decrease in fasting serum glucose level in the subject. In some embodiments of any of the methods described herein, the method results in an increase in insulin sensitivity in the subject. In some embodiments of any of the methods described herein, the method results in a decrease in the severity of atherosclerosis in the subject. In some embodiments of any of the methods described herein, the period of time is between about two weeks and about 10 years.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) results in activation of one or more of: a receptor for IL-2, a receptor for IL-7, a receptor for IL-12, a receptor for IL-15, a receptor for IL-18, a receptor for IL-21, a receptor for IL-33, CD16, CD69, CD25, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, and KIR3DS1.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-2 is a soluble IL-2 or an agonistic antibody that binds specifically to an IL-2 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-7 is a soluble IL-7 or an agonistic antibody that binds specifically to an IL-7 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-12 is a soluble IL-12 or an agonistic antibody that binds specifically to an IL-12 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-15 is a soluble IL-15 or an agonistic antibody that binds specifically to an IL-15 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-21 is a soluble IL-21 or an agonistic antibody that binds specifically to an IL-21 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-33 is a soluble IL-33 or an agonistic antibody that binds specifically to an IL-33 receptor.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD16 is an agonistic antibody that binds specifically to a CD16.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD69 is an agonistic antibody that binds specifically to a CD69.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD25 or CD59 is an agonistic antibody that binds specifically to CD25 or CD59.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD352 is an agonistic antibody that binds specifically to a CD352.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp80 is an agonistic antibody that binds specifically to an NKp80.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for DNAM-1 is an agonistic antibody that binds specifically to a DNAM-1.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for 2B4 is an agonistic antibody that binds specifically to a 2B4.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp30 is an agonistic antibody that binds specifically to an NKp30.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp44 is an agonistic antibody that binds specifically to an NKp44.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp46 is an agonistic antibody that binds specifically to an NKp46.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKG2D is an agonistic antibody that binds specifically to an NKG2D.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS1 is an agonistic antibody that binds specifically to a KIR2DS1.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS2/3 is an agonistic antibody that binds specifically to a KIR2DS2/3.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DL4 is an agonistic antibody that binds specifically to a KIR2DL4.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS4 is an agonistic antibody that binds specifically to a KIR2DS4.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS5 is an agonistic antibody that binds specifically to a KIR2DS5.

In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR3DS1 is an agonistic antibody that binds specifically to a KIR3DS1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) results in a decrease in the activation of one or more of: PD-1, a TGF-β receptor, TIGIT, CD1, TIM-3, Siglec-7, IRP60, Tactile, IL1R8, NKG2A/KLRD1, KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1, KIR3DL2, ILT2/LIR-1, and LAG-2. In some embodiments of any of the methods described herein, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the

activation of PD-1 is an antagonistic antibody that binds specifically to PD-1, a soluble PD-1, a soluble PD-L1, or an antibody that binds specifically to PD-L1. In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of a TGF-β receptor is a soluble TGF-β receptor, an antibody that binds specifically to TGF-β, or an antagonistic antibody that binds specifically to a TGF-β receptor.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIGIT is an antagonistic antibody that binds specifically to TIGIT, a soluble TIGIT, or an antibody that binds specifically to a ligand of TIGIT.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of CD1 is an antagonistic antibody that binds specifically to CD1, a soluble CD1, or an antibody that binds specifically to a ligand of CD1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIM-3 is an antagonistic antibody that binds specifically to TIM-3, a soluble TIM-3, or an antibody that binds specifically to a ligand of TIM-3.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Siglec-7 is an antagonistic antibody that binds specifically to Siglec-7 or an antibody that binds specifically to a ligand of Siglec-7.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IRP60 is an antagonistic antibody that binds specifically to IRP60 or an antibody that binds specifically to a ligand of IRP60.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Tactile is an antagonistic antibody that binds specifically to Tactile or an antibody that binds specifically to a ligand of Tactile.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IL1R8 is an antagonistic antibody that binds specifically to IL1R8 or an antibody that binds specifically to a ligand of IL1R8.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of NKG2A/KLRD1 is an antagonistic antibody that binds specifically to NKG2A/KLRD1 or an antibody that binds specifically to a ligand of NKG2A/KLRD1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL1 is an antagonistic antibody that binds specifically to KIR2DL1 or an antibody that binds specifically to a ligand of KIR2DL1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL2/3 is an antagonistic antibody that binds specifically to KIR2DL2/3 or an antibody that binds specifically to a ligand of KIR2DL2/3.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL5 is an antagonistic antibody that binds specifically to KIR2DL5 or an antibody that binds specifically to a ligand of KIR2DL5.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL1 is an antagonistic antibody that binds specifically to KIR3DL1 or an antibody that binds specifically to a ligand of KIR3DL1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL2 is an antagonistic antibody that binds specifically to KIR3DL2 or an antibody that binds specifically to a ligand of KIR3DL2.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of

ILT2/LIR-1 is an antagonistic antibody that binds specifically to ILT2/LIR-1 or an antibody that binds specifically to a ligand of ILT2/LIR-1.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of LAG-2is an antagonistic antibody that binds specifically to LAG-2 or an antibody that binds specifically to a ligand of LAG-2.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a single-chain chimeric polypeptide that includes: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a second target-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain. In some embodiments of any of the methods described herein, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the second target-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain directly abut each other. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the first target-binding domain and the second target-binding domain. In some embodiments of any of the methods described herein, the second target-binding domain and the soluble tissue factor domain directly abut each other. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between the second target-binding domain and the soluble tissue factor domain.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of any of the methods described herein, the first target-

binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain are each an antigen-binding domain. In some embodiments of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some embodiments of any of the methods described herein, the

soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some embodiments of any of the methods described herein, the soluble interleukin or cytokine receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some embodiments of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 93. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 93. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 93. In some embodiments of any of the methods described herein, the soluble human tissue factor domain does not include one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some embodiments of any of the methods described herein, the soluble human tissue factor domain does not include any of: a lysine at an amino acid position that

corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some embodiments of any of the methods described herein, the soluble tissue factor domain is not capable of binding Factor VIIa. In some embodiments of any of the methods described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide does not blood stimulate coagulation in a mammal. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes one or more additional target-binding domains at its N- and/or C-terminus.

In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target-binding domains at its N-terminus. In some embodiments of any of the methods described herein, one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target-binding domains at its C-terminus. In some embodiments of any of the methods described herein, one of the one

or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide includes one or more additional target binding domains at its N-terminus and the C-terminus. In some embodiments of any of the methods described herein, one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the one or more additional antigen-binding domains at the N-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some embodiments of any of the methods described herein, one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain. In some embodiments of any of the methods described herein, the single-chain chimeric polypeptide further includes a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

In some embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the

same amino acid sequence. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain. In some embodiments of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a

receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein. In some embodiments of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor. In some embodiments of any of the methods described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide that includes: (a) a first chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

In some embodiments of any of the methods described herein, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the

first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some embodiments of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2,

HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some embodiments of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some embodiments of any of the methods described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some embodiments

of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains. In some embodiments of any of the methods described herein, the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

In some embodiments of any of the methods described herein, at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of

affinity domains in the first chimeric polypeptide. In some embodiments of any of the methods described herein, the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

In some embodiments of any of the methods described herein, the second chimeric polypeptide further includes one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide. In some embodiments of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide. In some embodiments of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide. In some embodiments of any of the methods described herein, at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide. In some embodiments of any of the methods described herein, the second chimeric polypeptide further includes a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some

embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments of any of the methods described herein, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain. In some embodiments of any of the methods described herein, the antigen-binding domain includes a scFv.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3,

AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein. In some embodiments of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the methods described herein, one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor. In some embodiments of any of the methods described herein, the soluble receptor a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some embodiments of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 80% identical to SEQ ID NO: 93. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 90% identical to SEQ ID NO: 93. In some embodiments of any of the methods described herein, the soluble human tissue factor domain includes a sequence that is at least 95% identical to SEQ ID NO: 93.

In some embodiments of any of the methods described herein, the soluble human tissue factor domain does not include one or more of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor

protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some embodiments of any of the methods described herein, the soluble human tissue factor domain does not include any of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

In some embodiments of any of the methods described herein, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some embodiments of any of the methods described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some embodiments of any of the methods described herein, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal. In some embodiments of any of the methods described herein, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15. In some embodiments of any of the methods described herein, the soluble

IL-15 has a D8N or D8A amino acid substitution. In some embodiments of any of the methods described herein, the human IL-15Rα is a mature full-length IL-15Rα.

In some embodiments of any of the methods described herein, the pair of affinity domains is selected from the group of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

In some embodiments of any of the methods described herein, at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide that includes: (a) a first and second chimeric polypeptides, where each includes: (i) a first target-binding domain; (ii) a Fc domain; and (iii) a first domain of a pair of affinity domains; and (b) a third and fourth chimeric polypeptide, where each includes: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first and second chimeric polypeptides and the third and fourth chimeric polypeptides associate through the binding of the first domain and the second domain of the pair of affinity domains, and the first and second chimeric polypeptides associate through their Fc domains.

In some embodiments of any of the methods described herein, the first target-binding domain and the Fc domain directly abut each other in the first and second chimeric polypeptides. In some embodiments of any of the methods described herein, the first and second chimeric polypeptides further include a linker sequence between the first target-binding domain and the Fc domain in the first and second chimeric polypeptides. In some embodiments of any of the methods described herein, the Fc domain and the first domain of the pair of affinity domains directly abut each other in the first and second chimeric polypeptides. In some embodiments of any of the methods described herein, the first chimeric polypeptide further includes a linker sequence between the Fc domain and the first domain of the pair of affinity domains in the first and second chimeric polypeptides.

In some embodiments of any of the methods described herein, the second domain of the pair of affinity domains and the second target-binding domain directly abut each

other in the third and fourth chimeric polypeptides. In some embodiments of any of the methods described herein, the third and fourth chimeric polypeptides further include a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the third and fourth chimeric polypeptides.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the methods described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some embodiments of any of the methods described herein, the antigen-binding domain includes a scFv or a single domain antibody.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand

(FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some embodiments of any of the methods described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the methods described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some embodiments of any of the methods described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

In some embodiments of any of the methods described herein, the soluble tissue factor domain is a soluble human tissue factor domain that does not stimulate blood coagulation. In some embodiments of any of the methods described herein, the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

As used herein, the term "chimeric" refers to a polypeptide that includes amino acid sequences (e.g., domains) originally derived from two different sources (e.g., two different naturally-occurring proteins, e.g., from the same or different species). For example, a chimeric polypeptide can include domains from at least two different naturally occurring human proteins. In some examples, a chimeric polypeptide can include a domain that is a synthetic sequence (e.g., a scFv) and a domain that is derived from a naturally-occurring protein (e.g., a naturally-occurring human protein). In some embodiments, a chimeric polypeptide can include at least two different domains that are synthetic sequences (e.g., two different scFvs).

An "activated NK cell" is a NK cell demonstrating increased expression levels of two or more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to a resting NK cell. Exemplary

methods for identifying the expression levels of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B) are described herein.

A "resting NK cell" is a NK cell that has a reduced expression of two or more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to an activated NK cell.

An "NK cell activating agent" is an agent that induces or promotes (alone or in combination with additional NK cell activating agents) a resting NK cell to develop into an activated NK cell. Non-limiting examples and aspects of NK cell activating agents are described herein.

An "antigen-binding domain" is one or more protein domain(s) (e.g., formed from amino acids from a single polypeptide or formed from amino acids from two or more polypeptides (e.g., the same or different polypeptides) that is capable of specifically binding to one or more different antigen(s). In some examples, an antigen-binding domain can bind to an antigen or epitope with specificity and affinity similar to that of naturally-occurring antibodies. In some embodiments, the antigen-binding domain can be an antibody or a fragment thereof. In some embodiments, an antigen-binding domain can include an alternative scaffold. Non-limiting examples of antigen-binding domains are described herein. Additional examples of antigen-binding domains are known in the art.

A "soluble tissue factor domain" refers to a polypeptide having at least 70% identity (e.g., at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 99% identity, or 100% identical) to a segment of a wildtype mammalian tissue factor protein (e.g., a wildtype human tissue factor protein) that lacks the transmembrane domain and the intracellular domain. Non-limiting examples of soluble tissue factor domains are described herein.

The term "soluble interleukin protein" is used herein to refer to a mature and secreted interleukin protein or a biologically active fragment thereof. In some examples, a soluble interleukin protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and

secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble interleukin proteins are described herein.

The term "soluble cytokine protein" is used herein to refer to a mature and secreted cytokine protein or a biologically active fragment thereof. In some examples, a soluble cytokine protein can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to a wildtype mature and secreted mammalian interleukin protein (e.g., a wildtype human interleukin protein) and retains its biological activity. Non-limiting examples of soluble cytokine proteins are described herein.

The term "soluble interleukin receptor" is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble interleukin receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype interleukin receptor and retains its ability to specifically bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble interleukin receptors are described herein.

The term "soluble cytokine receptor" is used herein in the broadest sense to refer to a polypeptide that lacks a transmembrane domain (and optionally an intracellular domain) that is capable of binding one or more of its natural ligands (e.g., under physiological conditions, e.g., in phosphate buffered saline at room temperature). For example, a soluble cytokine receptor can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to an extracellular domain of wildtype cytokine receptor and retains its ability to specifically

bind to one or more of its natural ligands, but lacks its transmembrane domain (and optionally, further lacks its intracellular domain). Non-limiting examples of soluble cytokine receptors are described herein.

The term "antibody" is used herein in its broadest sense and includes certain types of immunoglobulin molecules that include one or more antigen-binding domains that specifically bind to an antigen or epitope. An antibody specifically includes, e.g., intact antibodies (e.g., intact immunoglobulins), antibody fragments, and multi-specific antibodies. One example of an antigen-binding domain is an antigen-binding domain formed by a VH -VL dimer. Additional examples of an antibody are described herein. Additional examples of an antibody are known in the art.

"Affinity" refers to the strength of the sum total of non-covalent interactions between an antigen-binding site and its binding partner (e.g., an antigen or epitope). Unless indicated otherwise, as used herein, "affinity" refers to intrinsic binding affinity, which reflects a 1:1 interaction between members of an antigen-binding domain and an antigen or epitope. The affinity of a molecule X for its partner Y can be represented by the dissociation equilibrium constant (K_D). The kinetic components that contribute to the dissociation equilibrium constant are described in more detail below. Affinity can be measured by common methods known in the art, including those described herein. Affinity can be determined, for example, using surface plasmon resonance (SPR) technology (e.g., BIACORE®) or biolayer interferometry (e.g., FORTEBIO®). Additional methods for determining the affinity for an antigen-binding domain and its corresponding antigen or epitope are known in the art.

A "single-chain polypeptide" as used herein to refers to a single protein chain.

A "multi-chain polypeptide" as used herein to refers to a polypeptide comprising two or more (e.g., three, four, five, six, seven, eight, nine, or ten) protein chains (e.g., at least a first chimeric polypeptide and a second polypeptide), where the two or more proteins chains associate through non-covalent bonds to form a quaternary structure.

The term "pair of affinity domains" is two different protein domain(s) that bind specifically to each other with a K_D of less than of less than 1×10^{-7} M (e.g., less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, or less than 1×10^{-11} M). In some

examples, a pair of affinity domains can be a pair of naturally-occurring proteins. In some embodiments, a pair of affinity domains can be a pair of synthetic proteins. Non-limiting examples of pairs of affinity domains are described herein.

The term "epitope" means a portion of an antigen that specifically binds to an antigen-binding domain. Epitopes can, e.g., consist of surface-accessible amino acid residues and/or sugar side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter may be lost in the presence of denaturing solvents. An epitope may comprise amino acid residues that are directly involved in the binding, and other amino acid residues, which are not directly involved in the binding. Methods for identifying an epitope to which an antigen-binding domain binds are known in the art.

The term "treatment" means to ameliorate at least one symptom of a disorder. In some examples, the disorder being treated is cancer and to ameliorate at least one symptom of cancer includes reducing aberrant proliferation, gene expression, signaling, translation, and/or secretion of factors. Generally, the methods of treatment include administering a therapeutically effective amount of a composition that reduces at least one symptom of a disorder to a subject who is in need of, or who has been determined to be in need of such treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A-1B show the results of immunostimulation of an exemplary multi-chain polypeptide in C57BL/6 mice. Figure 1A shows the spleen weight of mice treated with increasing dosage of the exemplary multi-chain polypeptide as compared to mice treated with the control solution. Figure 1B shows the percentages of immune cell types present in the spleen of mice treated with increasing dosage of the exemplary multi-chain polypeptide as compared to mice treated with the control solution.

Figures 2A-2B show the duration of immunostimulation of an exemplary multi-chain polypeptide in C57BL/6 mice. Figure 2A shows the spleen weight over a period of 92 hours in mice treated with 3mg/kg of the exemplary multi-chain polypeptide. Figure 2B shows the percentages of immune cell types present in the spleen over a period of 92 hours in mice treated with 3mg/kg of the exemplary multi-chain polypeptide.

Figures 3A-3B show the expression of Ki67 and Granzyme B in immune cells induced by the exemplary multi-chain polypeptide. Figure 3A shows the expression of Ki67 in $CD4^+$ T cells, $CD8^+$ T cells, natural killer (NK) cells, and $CD19^+$ B cells at various time points post-treatment with the multi-chain polypeptide. Figure 3B shows the expression of Granzyme B in $CD4^+$ T cells, $CD8^+$ T cells, natural killer (NK) cells, and $CD19^+$ B cells at various time points post-treatment with the multi-chain polypeptide.

Figure 4 shows the effect of tumor inhibition by splenocytes prepared from mice treated with an exemplary multi-chain polypeptide at various time points after treatment.

Figures 5A-5B show the percentages and the proliferation rate of $CD4^+$ T cells, $CD8^+$ T cells, Natural Killer (NK) cells, and $CD19^+$ B cells in the blood of B6.129P2-$ApoE^{tm1Unc}$/J mice (purchased from The Jackson Laboratory) fed a control diet, a high fat diet and untreated, and mice fed a high fat diet and treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs. Figure 5A shows the percentages of the different cell types in each control and experimental group. Figure 5B shows the proliferation rate of the of the different cell types in each control and experimental group.

Figures 6A-6E show exemplary physical appearance of mice fed either a control or high fat diet and were either untreated or treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs.

Figure 7 shows the fasting body weight of mice fed either a control or a high fat diet and were either untreated or treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs.

Figure 8 shows the fasting blood glucose levels of mice fed either a control or a high fat diet and were either untreated or treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs.

Figures 9A-9F show chemotherapy-induced senescent B16F10 cells and expression of senescent genes. Figure 9A shows chemotherapy induction of senescent B16F10 cells visualized using SA β-gal staining. Figures 9B-9F show expression of p21, IL6, DPP4, RATE1E, and ULBP1 over time in the chemotherapy-induced senescent B16F10 cells.

Figures 10A-10F show colony formation and expression of stem cell markers by chemotherapy-induced senescent B16F10 cells. Figure 10A shows colony formation by chemotherapy-induced senescent B16F10 cells. Figures 10B and 10C show expression of Oct4 mRNA and Notch4 mRNA by chemotherapy-induced senescent B16F10 cells as compared to control B16F10 cells. Figures 10D-10F show percentage of chemotherapy-induced senescent B16F10 cells double-positive for two out of the three stem cell markers including CD44, CD24, and CD133.

Figures 11A-11C show migratory and invasive properties of chemotherapy-induced senescent B16F10 cells. Figure 11A shows the results of a migration assay comparing chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC) with control B16F10 cells. Figures 11B and 11C show the results of an invasion assay comparing chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC) with control B16F10 cells.

Figures 12A and 12B show in vitro expanded NK cells and their cytotoxicity against chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC) or control B16F10 cells. Figure 12A shows an exemplary schematic of a process of obtaining in vitro expanded NK cells. Figure 12 B shows cytotoxicity of the expanded NK cells against chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC) or control B16F10 cells.

Figures 13A-13C show results of combination treatment using a mouse melanoma model. Figure 13A shows an exemplary schematic for treating melanoma in a mouse model. Figures 13B and 13C show the change in tumor volume over time with combination treatments including TGFRt15-TGFRs as compared to chemotherapy or TA99 treatment alone.

Figure 14 shows induction of senescence in the human pancreatic tumor cell line SW1990 and expression of CD44 and CD24 in senescent SW1990 cells as compared to control SW1990 cells.

Figure 15 shows expression of senescent markers by chemotherapy-induced senescent SW1990 cells.

Figure 16 shows the cytotoxicity of in vitro activated human NK cells against chemotherapy-induced senescent SW1990 cells or control SW1990 cells.

Figure 17 shows a schematic diagram of an exemplary IL-12/IL-15RαSu DNA construct.

Figure 18 shows a schematic diagram of an exemplary IL-18/TF/IL-15 DNA construct.

Figure 19 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu and IL-18/TF/IL-15 DNA constructs.

Figure 20 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins resulting in IL-18/TF/IL-15:IL-12/IL-15RαSu complex (18t15-12s).

Figure 21 shows a chromatograph of 18t15-12s purification elution from an anti-TF antibody affinity column.

Figure 22 shows an exemplary chromatographic profile of anti-TF Ab /SEC-purified 18t15-12s protein following elution on an analytical size exclusion column, demonstrating separation of monomeric multiprotein 18t15-12s complexes from protein aggregates.

Figure 23 shows an example of a 4-12% SDS-PAGE of the 18t15-12s complex following disulfide bond reduction. Lane 1: SeeBlue Plus2 marker; Lane 2: anti-TF Ab-purified 18t15-12s (0.5 μg); Lane 3: anti-TF Ab-purified 18t15-12s (1 μg).

Figure 24 shows SDS PAGE analysis of deglycosylated and non-deglycosylated 18t15-12s. Lane 1: anti-TF Ab-purified 18t15-12s (0.5 μg), non-deglycosylated; Lane 2: anti-TF Ab -purified 18t15-12s (1 μg), non-deglycosylated; Lane 3: 18t15-12s (1 μg), deglycosylated, Lane 4: Mark12 unstained maker.

Figure 25 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor antibody capture and a biotinylated anti-human IL-12 detection antibody (BAF 219).

Figure 26 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor antibody capture and a biotinylated anti-human IL-15 detection antibody (BAM 247).

Figure 27 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor antibody capture and a biotinylated anti-human IL-18 detection antibody (D045-6).

Figure 28 shows a sandwich ELISA for the 18t15-12s complex, comprising an anti-human tissue factor (I43) capture antibody and an anti-human tissue factor detection antibody.

Figure 29 shows proliferation of IL-15-dependent 32Dβ cells mediated by the 18t15-12s complex (open squares) and recombinant IL-15 (black squares).

Figure 30 shows biological activity of IL-18 within the 18t15-12s complex (open squares), where recombinant IL-18 (black squares) and recombinant IL-12 (black circles) serve as positive and negative controls, respectively.

Figure 31 shows biological activity of IL-12 within the 18t15-12s complex (open squares), where recombinant IL-12 (black circles) and recombinant IL-18 (open squares) serve as positive and negative controls, respectively.

Figures 32A and 32B show cell-surface expression of CD25 on NK cells induced by the 18t15-12s complex and cell-surface CD69 expression of NK cells induced by the 18t15-12s complex.

Figure 33 shows a flow cytometry graph of intracellular IFN-γ expression of NK cells induced by the 18t15-12s complex.

Figure 34 shows cytotoxicity of 18t15-12s induced human NK cells against K562 cells.

Figure 35 shows a schematic diagram of an exemplary IL-12/IL-15RαSu/αCD16 DNA construct.

Figure 36 shows a schematic diagram of an exemplary IL-18/TF/IL-15 DNA construct.

Figure 37 shows a schematic diagram of the interaction between the exemplary IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs.

Figure 38 shows a schematic diagram of an exemplary 18t15-12s/αCD16 protein complex.

Figure 39 shows a sandwich ELISA for the 18t15-12s16 complex, comprising an anti-human tissue factor antibody capture antibody and a biotinylated anti-human IL-12 (BAF 219) (dark line) or an anti-human tissue factor detection antibody (light line).

Figure 40 shows a schematic diagram of an exemplary TGFβRII/IL-15RαSu DNA construct.

Figure 41 shows a schematic diagram of an exemplary IL-21/TF/IL-15 construct.

Figure 42 shows a schematic diagram of the interaction between the exemplary IL- IL-21/TF/IL-15 and TGFβRII/IL-15RαSu constructs.

Figure 43 shows a schematic diagram of the interaction between the exemplary TGFβRII/IL-15RαSu and IL-21/TF/IL-15 fusion proteins, resulting in an IL-21/TF/IL-15/TGFβRII/IL-15RαSu complex (21t15-TGFRs).

Figure 44 shows a chromatograph of 21t15-TGFRs purification elution from an anti-TF antibody affinity column.

Figure 45 shows an exemplary 21t15-TGFRs size exclusion chromatograph showing a main protein peak and a high molecular weight peak

Figure 46 shows an example of a 4-12% SDS-PAGE of the 21t15-TGFRs complex following disulfide bond reduction. Lane 1: Mark12 unstained marker (numbers on the left side indicate molecular weights in kDa); Lane 2: 21t15-TGFRs (0.5 μg); Lane 3: 21t15-TGFRs (1 μg); Lane 4: 21t15-TGFRs, deglycosylated (1 μg), wherein the MW was the expected size of 53kDa and 39.08 kDa.

Figure 47 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor capture and a biotinylated anti-human IL-21 detection antibody (13-7218-81, BioLegend).

Figure 48 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor antibody capture and a biotinylated anti-human IL-15 detection antibody (BAM 247, R&D Systems).

Figure 49 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor antibody capture and a biotinylated anti-human TGFβRII detection antibody (BAF241, R&D Systems).

Figure 50 shows a sandwich ELISA for the 21t15-TGFRs complex, comprising an anti-human tissue factor (I43) capture antibody and an anti-human tissue factor detection antibody.

Figure 51 shows IL-15-dependent proliferation of 32Dβ cells mediated by the 21t15-TGFRs complex (open squares) compared to IL-15 (black squares).

Figure 52 shows biological activity of the TGFβRII domain within the 21t15-TGFRs complex (open squares). TGFβRII/Fc (black squares) served as a positive control.

Figure 53 shows a flow cytometry graph of cell-surface CD25 expression of NK cells induced by the 21t15-TGFRs complex.

Figure 54 shows a flow cytometry graph of cell-surface CD69 expression of NK cells induced by the 21t15-TGFRs complex.

Figure 55 shows a flow cytometry graph of intracellular IFN-γ expression of NK cells induced by the 21t15-TGFRs complex.

Figure 56 shows cytotoxicity of 21t15-TGFRs-induced human NK cells against K562 cells.

Figure 57 are schematic diagrams of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide.

Figure 58 is a chromatograph showing the elution of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide from an anti-tissue factor affinity column.

Figure 59 is a chromatograph showing the elution of a Superdex 200 Increase 10/300 GL gel filtration column loaded with an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide.

Figure 60 is a sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide purified using an anti-tissue factor affinity column.

Figure 61 is a graph showing the ELISA quantitation of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide performed using the methods described in Example 1. Purified tissue factor was used as the control.

Figure 62 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD25 expression in $CD4^+$ T-cells isolated from blood from two donors. The experiments were performed as described in Example 2.

Figure 63 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD25 expression in $CD8^+$ T-cells isolated from blood from two donors. The experiments were performed as described in Example 2.

Figure 64 is a graph showing the ability of an exemplary αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide to stimulate CD69 expression in $CD4^+$ T-cells isolated from blood from two donors. The experiments were performed as described in Example 2.

Figure 65 shows a schematic diagram of an exemplary IL-7/IL-15RαSu DNA construct.

Figure 66 shows a schematic diagram of an exemplary IL-21/TF/IL-15 DNA construct.

Figure 67 shows a schematic diagram of the interaction between the exemplary IL-7/IL-15RαSu and IL-21/TF/IL-15 DNA constructs.

Figure 68 shows a schematic diagram of the interaction between the exemplary IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins resulting in an IL-21/TF/IL-15:IL-7/IL-15RαSu complex (21t15-7s).

Figure 69 shows a schematic diagram of an exemplary IL-21/IL-15RαSu DNA construct.

Figure 70 shows a schematic diagram of an exemplary IL-7/TF/IL-15 DNA construct.

Figure 71 shows a schematic diagram of the interaction between the exemplary IL-21/IL-15RαSu and IL-7/TF/IL-15 DNA constructs.

Figure 72 shows a schematic diagram of the interaction between the exemplary IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins resulting in an IL-7/TF/IL-15:IL-21/IL-15RαSU complex (7t15-21s).

Figure 73 shows the oxygen consumption rate (OCR) in pmoles/min for human NK cells isolated from blood (2 x 10^6 cells/mL) of two different donors.

Figure 74 shows the extracellular acidification rate (ECAR) in mpH/minute for human NK cells isolated from blood (2 x 10^6 cells/mL) of two different donors.

Figure 75 shows a schematic of the 7t15-16s21 construct.

Figure 76 shows an additional schematic of the 7t15-16s21 construct.

Figures 77A and 77B show binding of 7t15-16s21 to CHO cells expressing human CD16b as compared to a control protein.

Figures 78A-78C are results from ELISA experiments using antibodies against IL-15, IL-21, and IL-7 in detecting 7t15-16s21.

Figure 79 shows results of the 32Dβ cell proliferation assay with 7t15-16s21 or recombinant IL-15.

Figure 80 shows the chromatographic profile of 7t15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 81 shows the analytical SEC Profile of 7t15-16s21.

Figure 82 shows a schematic of the TGFRt15-16s21 construct.

Figure 83 shows an additional schematic of the TGFRt15-16s21 construct.

Figures 84A and 84B show binding affinity of TGFRT15-16S21 and 7t15-21s with CHO cells expressing human CD16b. Figure 84A shows binding affinity of

TGFRT15-16S21 with CHO cells expressing human CD16b. Figure 84B shows binding affinity of 7t15-21s with CHO cells expressing human CD16b.

Figure 85 shows results of TGFβ1 inhibition by TGFRt15-16s21 and TGFR-Fc.

Figure 86 shows results of 32Dβ cell proliferation assay with TGFRt15-16s21 or recombinant IL-15.

Figures 87A-87C show results of detecting IL-15, IL-21, and TGFβRII in TGFRt15-16s21 with corresponding antibodies using ELISA.

Figure 88 shows the chromatographic profile of TGFRt15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 89 shows results of a reduced SDS-PAGE analysis of TGFRt15-16s21.

Figure 90 shows a schematic of the 7t15-7s construct.

Figure 91 shows an additional schematic of the 7t15-7s construct.

Figure 92 shows the chromatographic profile of 7t15-7s protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 93 shows detection of TF, IL-15 and IL-7 in 7t15-7s using ELISA.

Figures 94A and 94B show spleen weight and the percentages of immune cell types in 7t15-7s -treated and control-treated mice. Figure 94A shows spleen weight in mice treated with 7t15-7s as compared to PBS control. Figure 94B shows the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in mice treated with 7t15-7s as compared to PBS control.

Figure 95 shows a schematic of the TGFRt15-TGFRs construct.

Figure 96 shows an additional schematic of the TGFRt15-TGFRs construct.

Figure 97 shows results of TGFβ1 inhibition by TGFRt15-TGFRs and TGFR-Fc.

Figure 98 shows results of 32Dβ cell proliferation assay with TGFRt15-TGFRs or recombinant IL-15

Figures 99A and 99B show results of detecting IL-15 and TGFβRII in TGFRt15-TGFRs with corresponding antibodies using ELISA.

Figure 100 is a line graph showing the chromatographic profile of TGFRt15-TGFRs protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 101 shows the analytical SEC profile of TGFRt15-TGFRs.

Figure 102 shows TGFRt15-TGFRs before and after deglycosylation as analyzed by reduced SDS-PAGE.

Figures 103A and 103B show spleen weight and the percentages of immune cell types in TGFRt15-TGFRs-treated and control-treated mice. Figure 103A shows spleen weight in mice treated with TGFRt15-TGFRs as compared to PBS control. Figure 103B shows the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in mice treated with TGFRt15-TGFRs as compared to PBS control.

Figure 104A and 104B show the spleen weight and immunostimulation over 92 hours in mice treated with TGFRt15-TGFRs. Figure 104A shows spleen weight of mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment. Figure 104B shows the percentages of immune cells in mice treated with TGFRt15-TGFRs at 16, 24, 48, 72, and 92 hours after treatment.

Figure 105A and 105B show Ki67 and Granzyme B expression in mice treated with TGFRt15-TGFRs over time.

Figure 106 shows enhancement of cytotoxicity of splenocytes by TGFRt15-TGFRs in C57BL/6 Mice.

Figure 107 shows changes in tumor size in response to PBS treatment, chemotherapy alone, TGFRt15-TGFRs alone, or chemotherapy and TGFRt15-TGFRs combination, in a pancreatic cancer mouse model.

Figure 108 shows the cytotoxicity of NK cells isolated from mice treated with TGFRt15-TGFRs.

Figure 109 shows a schematic of the 7t15-21s137L (long version) construct.

Figure 110 shows an additional schematic of the 7t15-21s137L (long version) construct.

Figure 111 is a line graph showing the chromatographic profile of 7t15-21s137L (long version) protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 112 shows the analytical SEC profile of 7t15-21s137L (long version).

Figure 113 shows binding of 7t15-21s137L (short version) to CD137L (4.1BBL)

Figures 114A-114C show detection of IL-15, IL21, and IL7 in 7t15-21s137L (short version) with ELISA. Figure 114A shows detection of IL-15 in 7t15-21s137L (short version) with ELISA. Figure 114B shows detection of IL21 in 7t15-21s137L (short version) with ELISA. Figure 114C shows detection of IL7 in 7t15-21s137L (short version) with ELISA.

Figure 115 shows results from a CTLL-2 cell proliferation assay.

Figure 116 shows the activity of 7t15-1s137L (short version) in promoting IL21R containing B9 cell proliferation.

Figure 117 shows a schematic of the 7t15-TGFRs construct.

Figure 118 shows an additional schematic of the 7t15-TGFRs construct.

Figure 119 shows results of TGFβ1 inhibition by 7t15-TGFRs and TGFR-Fc.

Figures 120A-120C show detection of IL-15, TGFβRII, and IL-7 in 7t15-TGFRs with ELISA.

Figure 121 shows results of a 32Dβ cell proliferation assay with 7t15-TGFRs or recombinant IL-15.

Figure 122 is a line graph showing the chromatographic profile of 7t15-TGFRs protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 123 shows 7t15-TGFRs before and after deglycosylation as analyzed using reduced SDS-PAGE.

Figure 124 shows ELISA detection of IL-7, IL-15 and TGFβRII in the 7t15-TGFRs protein.

Figures 125A and 125B show spleen weight and the percentages of immune cell types in 7t15-TGFRs-treated and control-treated mice. Figure 125A shows spleen weight in mice treated with 7t15-TGFRs at various dosages, as compared to PBS control. Figure

125B shows the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells in mice treated with 7t15-TGFRs at various dosages, as compared to PBS control.

Figures 126A and 126B show upregulation of CD44 expression of $CD4^+$ and $CD8^+$ T cells by 7t15-TGFRs in C57BL/6 mice.

Figures 127A and 127B show upregulation of Ki67 expression and Granzyme B expression of $CD8^+$ T cells and NK Cells by 7t15-TGFRs in C57BL/6 mice.

Figure 128 shows enhancement of cytotoxicity of splenocytes by 7t15-TGFRs in C57BL/6 mice.

Figure 129 shows a schematic of the TGFRt15-21s137L construct.

Figure 130 shows an additional schematic of the TGFRt15-21s137L construct.

Figure 131 is a line graph showing the chromatographic profile of TGFRt15-21s137L protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 132 shows a schematic of the TGFRt15-TGFRs21 construct.

Figure 133 shows an additional schematic of the TGFRt15-TGFRs21 construct.

Figure 134 is a line graph showing the chromatographic profile of TGFRt15-TGFRs21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 135 shows TGFRt15-TGFRs21 before and after deglycosylation as analyzed by reduced SDS-PAGE.

Figures 136A and 136B show detection of components of TGFRt15-TGFRs21 using ELISA.

Figures 137A and 137B show the percentages and proliferation of $CD4^+$ T cells, $CD8^+$ T cells, and natural killer (NK) cells present in the spleen of control-treated and TGFRt15-TGFRs21-treated mice.

Figure 138 shows upregulation of Granzyme B expression of splenocytes in mice treated with TGFRt15-TGFRs21.

Figure 139 shows enhancement of cytotoxicity of splenocytes by TGFRt15-TGFRs21 in C57BL/6 Mice.

Figure 140 shows a schematic of the TGFRt15-TGFRs16 construct.

Figure 141 shows an additional schematic of the TGFRt15-TGFRs16 construct.

Figure 142 shows a schematic of the TGFRt15-TGFRs137L construct.

Figure 143 shows an additional schematic of the TGFRt15-TGFRs137L construct.

Figure 144 are schematic diagrams of an exemplary 2t2 single-chain chimeric polypeptide.

Figure 145 shows IL-2 activity in 2t2 as compared to recombinant IL-2 using a 32Dβ cell proliferation assay.

Figure 146 shows IL-2 activity in 2t2 as compared to recombinant IL-2 using a CTLL-2 cell proliferation assay.

Figure 147 shows the fasting blood glucose levels in $ApoE^{-/-}$ mice fed with standard chow or a high fat diet and treated with a PBS control (untreated) or with 2t2.

Figure 148 shows the ratio of $CD4^{+}CD25^{+}FoxP3^{+}$ T regulatory cells in blood lymphocytes from $ApoE^{-/-}$ mice fed with standard chow or a high fat diet and treated with a PBS control (untreated) or with 2t2.

Figure 149 is a line graph showing the chromatographic profile of 2t2 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figure 150 shows an analytical SEC profile of 2t2.

Figures 151A and 151B show reduced SDS-PAGE analysis of 2t2 before and after deglycosylation. Figure 151A shows reduced SDS-PAGE analysis of 2t2 before deglycosylation. Figure 151B shows reduced SDS-PAGE analysis of 2t2 after deglycosylation.

Figures 152A and152B show results of immunostimulation in C57BL/6 mice using 2t2. Figure 152A shows spleen weight following treatment with 2t2. Figure 152B shows the percentages of immune cell types following 2t2 treatment.

Figure 153 shows upregulation of CD25 expression of $CD4^{+}$ T cells in mice treated with 2t2.

Figure 154 shows the pharmacokinetics of 2t2 in C57BL/6 mice.

Figures 155A and 155B show effects of 2t2 in attenuating the formation of high fat-induced atherosclerotic plaques in $ApoE^{-/-}$ mice. Figure 155A shows a representative view of atherosclerotic plaques from $ApoE^{-/-}$ mice fed with standard chow or a high fat diet and treated with either PBS control or 2t2. Figure 155B shows the results of quantitative analysis of atherosclerotic plaques of each group.

Figure 156 shows fasting glucose levels in 2t2 treated-mice as compared to control-treated mice.

Figure 157 shows the percentage of $CD4^+CD25^+FoxP3^+$ Tregs in blood lymphocytes from mice treated with 2t2 and control-treated mice.

Figure 158 are schematic diagrams of an exemplary 15t15 single-chain chimeric polypeptide.

Figure 159 shows the IL-15 activity of 15t15 as compared to recombinant IL-15 in a 32Dβ cell proliferation assay.

Figure 160 is a line graph showing the chromatographic profile of 15t15 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin.

Figures 161A and 161B show reduced SDS-PAGE analysis of 15t15 before and after deglycosylation. Figure 161A shows reduced SDS-PAGE analysis of 15t15 before deglycosylation. Figure 161B shows reduced SDS-PAGE analysis of 15t15 after deglycosylation.

Figures 162A and 162B is a set of histograms (Figure 162A) and a set of graphs (Figure 162B) showing the change in the surface phenotype of NK cells after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s + anti-TF antibody.

Figure 163 is a set of graphs showing changes in the surface phenotype of lymphocyte populations after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s.

Figure 164 is a set of graphs showing an increase in glycolysis in NK cells following treatment with 18t15-12s.

Figure 165 is a set of graphs showing an increase in phospho-STAT4 and phospho-STAT5 levels in NK cells after stimulation with 18t15-12s.

Figure 166 is a set of graphs showing that overnight stimulation of NK cells with 18t15-12s enhances cell metabolism.

Figure 167A-C is a set of graphs showing immunostimulation in C57BL/6 mice following treatment with 2t2.

Figure 168A-B is a set of graphs showing immunostimulation in C57BL/6 mice following treatment with TGFRt15-TGFRs.

Figure 169A-C is a set of graphs showing *in vivo* stimulation of Tregs, NK cells, and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with a Western diet and treated with TGFRt15-TGFRs or 2t2.

Figure 170A-B is a set of graphs showing induction of splenocyte proliferation by 2t2 in C57BL/6 mice.

Figure 171A-C is a set of graphs showing immunostimulation in C57BL/6 mice following treatment with TGFRt15-TGFRs.

Figure 172A-B is a set of graphs showing *in vivo* induction of proliferation of NK cells and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with a Western diet and treated with TGFRt15-TGFRs or 2t2.

Figure 173 is a schematic and a set of graphs showing the persistence of 7t15-21s and anti-TF antibody-expanded NK cells in NSG mice following treatment with 7t15-21, TGFRt15-TGFRs or 2t2.

Figure 174A-B is a set of graphs showing enhancement of cytotoxicity of NK cells following treatment of NK cells with TGFRt15-TGFRs.

Figure 175A-B is a set of graphs showing enhancement of ADCC activity of NK cells following treatment of NK cells with TGFRt15-TGFRs.

Figure 176 is a graph of in vitro killing of senescent B16F10 melanoma cells by TGFRt15-TGFRs/2t2-activated mouse NK cells.

Figure 177A-H is a set of graphs showing antitumor activity of TGFRt15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model.

Figure 178A-C is a set of graphs showing amelioration of the Western diet-induced hyperglycemia in $ApoE^{-/-}$ mice by 2t2.

Figure 179 is a set of graphs showing cell surface staining summarizing the differentiation of NK cells into cytokine-induced memory like NK cells (CIML-NK Cells) after stimulation with 18t15-12s and cultured in rhIL-15.

Figure 180 shows upregulation of CD44 memory T cells. The upper panel shows upregulation of CD44 memory T cells upon treatment with TGFRt15-TGFRs. The lower panel shows upregulation of CD44 memory T cells upon treatment with 2t2.

Figures 181A and 181B show improvement in hair regrowth following depilation in mice treated with 2t2 or IL-2. Figure 181A shows skin pigmentation 10 days after depilation in PBS-, 2t2-, or IL-2-treated mice. Figure 181B shows percent pigmentation in PBS-, 2t2-, or IL-2-treated mice as analyzed using the ImageJ software.

Figure 182 shows skin pigmentation 14 days after depilation in PBS-, 2t2-, or IL-2-treated mice.

Figure 183 shows a graph of Factor X (FX) activation following treatment with single-chain or multi-chain chimeric polypeptides.

Figure 184 shows clotting time for a buffer with varying concentrations of Innovin in a prothrombin time (PT) test.

Figure 185 shows clotting time for multi-chain chimeric polypeptides in a PT Assay.

Figure 186 shows clotting time of the multi-chain chimeric polypeptides in a PT assay when mixed with 32DB cells.

Figure 187 shows clotting time of multi-chain chimeric polypeptides in a PT assay when mixed with human PBMC.

Figure 188 shows binding of 7t15-21s137L (long version) and 7t15-21s137L (short version) to CD137 (4.1BB).

Figure 189A-189D show detection of IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) by the respective antibodies using ELISA.

Figure 190 shows IL-15 activity of 7t15-21s137L (long version) and 7t15-21s137L (short version) as evaluated by a IL2R$\alpha\beta\gamma$-containing CTLL2 cell proliferation assay.

Figures 191A-191C show human blood lymphocyte pStat5a responses in $CD4^+CD25^{hi}T_{reg}$ cells, $CD4^+CD25^-T_{con}$ cells, or in $CD8^+$ T_{con} cells in response to 2t2 or IL2 treatment. Figure 191A shows pSTAT5 responses in $CD4^+CD25^{hi}T_{reg}$ cells. Figure C191B shows pSTAT5 responses in $CD4^+CD25^-T_{con}$ cells. Figure 191C shows pSTAT5 responses in $CD8^+$ T_{con} cells.

Figures 192A-192E is a set of imaging showing that treatment with an IL-2 based molecule (2t2) can induce formation of hair follicles following depilation in mouse model. Figure 192A is an image from a control mouse - only depilation done after hair was shaved, Figure 192B is an image from a mouse where depilation was followed by low dose IL-2 (1 mg/kg) administration, and Figures 192C-192E show images from mice where depilation was followed by 2t2 at 0.3 mg/kg, (Figure 192C), 1 mg/kg (Figure 192D), and (Figure 192E) 3 mg/kg. Black arrows indicate anagen-phase hair follicles that will later extend into dermis and facilitate hair growth.

Figure 193 shows the total number of anagen phase hair follicles counted per 10 fields for each treatment group.

Figure 194 is a graph showing the percentage different in DNA demethylation in NK cells (relative to unexposed NK cells) from two different donors following expansion with 7t15-21s+ anti-tissue factor (TF)-antibody (IgG1) (50 nM).

DETAILED DESCRIPTION

Provided herein are methods of treating an aging-related disease or condition in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of killing or reducing the number of senescent cells in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time that include administering to the subject a

therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells. Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) and/or a therapeutically effective number of activated NK cells.

Activated NK Cells

Some embodiments of any of the methods described herein can include administering to a subject (e.g., any of the exemplary subjects described herein) a therapeutically effective number of activated NK cells (e.g., human activated NK cells). An activated NK cell is an NK cell (e.g., a human NK cell) that has increased expression levels of two or more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP1, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to a resting NK cell (e.g., a human resting NK cell). For example, an activated NK cell can have at least a 10% increase (e.g., at least a 15% increase, at least a 20% increase, at least a 25% increase, at least a 30% increase, at least a 35% increase, at least a 40% increase, at least a 45% increase, at least a 50% increase, at least a 55% increase, at least a 60% increase, at least a 65% increase, at least a 70% increase, at least a 75% increase, at least a 80% increase, at least a 85% increase, at least a 90% increase, at least a 95% increase, at least a 100% increase, at least a 120% increase, at least a 140% increase, at least a 160% increase, at least a 180% increase, at least a 200% increase, at least a 220% increase, at least a 240% increase, at least a 260% increase, at least a 280% increase, or at least a 300% increase) in the expression levels of two of more (e.g., three, four, five, or six) of CD25, CD69, MTOR-C1, SREBP1, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to a resting NK cell (e.g., a human activated NK cell).

In some embodiments, an activated NK cell can optionally further can have at least a 10% increase (e.g., at least a 15% increase, at least a 20% increase, at least a 25% increase, at least a 30% increase, at least a 35% increase, at least a 40% increase, at least a 45% increase, at least a 50% increase, at least a 55% increase, at least a 60% increase,

at least a 65% increase, at least a 70% increase, at least a 75% increase, at least a 80% increase, at least a 85% increase, at least a 90% increase, at least a 95% increase, at least a 100% increase, at least a 120% increase, at least a 140% increase, at least a 160% increase, at least a 180% increase, at least a 200% increase, at least a 220% increase, at least a 240% increase, at least a 260% increase, at least a 280% increase, or at least a 300% increase) in the expression levels of two of more (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of CD25, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, CD16, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, KIR3DS1, NKG2C, CCR7, CXCR3, L-Selectin, CXCR1, CXCR2, CX3CR1, ChemR23, CXCR4, CCR5, S1P5, c-Kit, mTORC1, e.g., as compared to a resting NK cell (e.g., a human activated NK cell).

For example, an activated NK cell (e.g., a human activated NK cell) can have about a 10% increase to about a 500% increase, about a 10% increase to about a 450% increase, about a 10% increase to about a 400% increase, about a 10% increase to about a 350% increase, about a 10% increase to about a 300% increase, about a 10% increase to about a 280% increase, about a 10% increase to about a 260% increase, about a 10% increase to about a 240% increase, about a 10% increase to about a 220% increase, about a 10% increase to about a 200% increase, about a 10% increase to about a 180% increase, about a 10% increase to about a 160% increase, about a 10% increase to about a 140% increase, about a 10% increase to about a 120% increase, about a 10% increase to about a 100% increase, about a 10% increase to about a 80% increase, about a 10% increase to about a 60% increase, about a 10% increase to about a 40% increase, about a 10% increase to about a 20% increase, a 20% increase to about a 500% increase, about a 20% increase to about a 450% increase, about a 20% increase to about a 400% increase, about a 20% increase to about a 350% increase, about a 20% increase to about a 300% increase, about a 20% increase to about a 280% increase, about a 20% increase to about a 260% increase, about a 20% increase to about a 240% increase, about a 20% increase to about a 220% increase, about a 20% increase to about a 200% increase, about a 20% increase to about a 180% increase, about a 20% increase to about a 160% increase, about a 20% increase to about a 140% increase, about a 20% increase to about a 120% increase, about

a 20% increase to about a 100% increase, about a 20% increase to about a 80% increase, about a 20% increase to about a 60% increase, about a 20% increase to about a 40% increase, a 40% increase to about a 500% increase, about a 40% increase to about a 450% increase, about a 40% increase to about a 400% increase, about a 40% increase to about a 350% increase, about a 40% increase to about a 300% increase, about a 40% increase to about a 280% increase, about a 40% increase to about a 260% increase, about a 40% increase to about a 240% increase, about a 40% increase to about a 220% increase, about a 40% increase to about a 200% increase, about a 40% increase to about a 180% increase, about a 40% increase to about a 160% increase, about a 40% increase to about a 140% increase, about a 40% increase to about a 120% increase, about a 40% increase to about a 100% increase, about a 40% increase to about a 80% increase, about a 40% increase to about a 60% increase, a 60% increase to about a 500% increase, about a 60% increase to about a 450% increase, about a 60% increase to about a 400% increase, about a 60% increase to about a 350% increase, about a 60% increase to about a 300% increase, about a 60% increase to about a 280% increase, about a 60% increase to about a 260% increase, about a 60% increase to about a 240% increase, about a 60% increase to about a 220% increase, about a 60% increase to about a 200% increase, about a 60% increase to about a 180% increase, about a 60% increase to about a 160% increase, about a 60% increase to about a 140% increase, about a 60% increase to about a 120% increase, about a 60% increase to about a 100% increase, about a 60% increase to about a 80% increase, a 80% increase to about a 500% increase, about a 80% increase to about a 450% increase, about a 80% increase to about a 400% increase, about a 80% increase to about a 350% increase, about a 80% increase to about a 300% increase, about a 80% increase to about a 280% increase, about a 80% increase to about a 260% increase, about a 80% increase to about a 240% increase, about a 80% increase to about a 220% increase, about a 80% increase to about a 200% increase, about a 80% increase to about a 180% increase, about a 80% increase to about a 160% increase, about a 80% increase to about a 140% increase, about a 80% increase to about a 120% increase, about a 80% increase to about a 100% increase, a 100% increase to about a 500% increase, about a 100% increase to about a 450% increase, about a 100% increase to about a 400% increase, about a 100% increase to

about a 350% increase, about a 100% increase to about a 300% increase, about a 100% increase to about a 280% increase, about a 100% increase to about a 260% increase, about a 100% increase to about a 240% increase, about a 100% increase to about a 220% increase, about a 100% increase to about a 200% increase, about a 100% increase to about a 180% increase, about a 100% increase to about a 160% increase, about a 100% increase to about a 140% increase, about a 100% increase to about a 120% increase, a 120% increase to about a 500% increase, about a 120% increase to about a 450% increase, about a 120% increase to about a 400% increase, about a 120% increase to about a 350% increase, about a 120% increase to about a 300% increase, about a 120% increase to about a 280% increase, about a 120% increase to about a 260% increase, about a 120% increase to about a 240% increase, about a 120% increase to about a 220% increase, about a 120% increase to about a 200% increase, about a 120% increase to about a 180% increase, about a 120% increase to about a 160% increase, about a 120% increase to about a 140% increase, a 140% increase to about a 500% increase, about a 140% increase to about a 450% increase, about a 140% increase to about a 400% increase, about a 140% increase to about a 350% increase, about a 140% increase to about a 300% increase, about a 140% increase to about a 280% increase, about a 140% increase to about a 260% increase, about a 140% increase to about a 240% increase, about a 140% increase to about a 220% increase, about a 140% increase to about a 200% increase, about a 140% increase to about a 180% increase, about a 140% increase to about a 160% increase, a 160% increase to about a 500% increase, about a 160% increase to about a 450% increase, about a 160% increase to about a 400% increase, about a 160% increase to about a 350% increase, about a 160% increase to about a 300% increase, about a 160% increase to about a 280% increase, about a 160% increase to about a 260% increase, about a 160% increase to about a 240% increase, about a 160% increase to about a 220% increase, about a 160% increase to about a 200% increase, about a 160% increase to about a 180% increase, a 180% increase to about a 500% increase, about a 180% increase to about a 450% increase, about a 180% increase to about a 400% increase, about a 180% increase to about a 350% increase, about a 180% increase to about a 300% increase, about a 180% increase to about a 280% increase, about a 180%

increase to about a 260% increase, about a 180% increase to about a 240% increase, about a 180% increase to about a 220% increase, about a 180% increase to about a 200% increase, a 200% increase to about a 500% increase, about a 200% increase to about a 450% increase, about a 200% increase to about a 400% increase, about a 200% increase to about a 350% increase, about a 200% increase to about a 300% increase, about a 200% increase to about a 280% increase, about a 200% increase to about a 260% increase, about a 200% increase to about a 240% increase, about a 200% increase to about a 220% increase, a 220% increase to about a 500% increase, about a 220% increase to about a 450% increase, about a 220% increase to about a 400% increase, about a 220% increase to about a 350% increase, about a 220% increase to about a 300% increase, about a 220% increase to about a 280% increase, about a 220% increase to about a 260% increase, about a 220% increase to about a 240% increase, a 240% increase to about a 500% increase, about a 240% increase to about a 450% increase, about a 240% increase to about a 400% increase, about a 240% increase to about a 350% increase, about a 240% increase to about a 300% increase, about a 240% increase to about a 280% increase, about a 240% increase to about a 260% increase, a 260% increase to about a 500% increase, about a 260% increase to about a 450% increase, about a 260% increase to about a 400% increase, about a 260% increase to about a 350% increase, about a 260% increase to about a 300% increase, about a 260% increase to about a 280% increase, a 280% increase to about a 500% increase, about a 280% increase to about a 450% increase, about a 280% increase to about a 400% increase, about a 280% increase to about a 350% increase, about a 280% increase to about a 300% increase, a 300% increase to about a 500% increase, about a 300% increase to about a 450% increase, about a 300% increase to about a 400% increase, about a 300% increase to about a 350% increase, a 350% increase to about a 500% increase, about a 350% increase to about a 450% increase, about a 350% increase to about a 400% increase, a 400% increase to about a 500% increase, about a 400% increase to about a 450% increase, or a 400% increase to about a 500% increase, in the expression levels of two of more (e.g., three, four, five, or six) of CD25, CD69, mTORC1, SREBP1, IFN-γ, and a granzyme (e.g., granzyme B), e.g., as compared to a resting NK cell (e.g., a human resting NK cell).

In some embodiments, an activated NK cell can further have about a 10% increase to about a 500% increase (e.g., or any of the subranges of this range described herein) in the expression levels of two of more (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of CD25, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, CD16, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, KIR3DS1, NKG2C, CCR7, CXCR3, L-Selectin, CXCR1, CXCR2, CX3CR1, ChemR23, CXCR4, CCR5, S1P5, c-Kit, mTORC1, e.g., as compared to a resting NK cell (e.g., a human activated NK cell).

Non-limiting examples of assays that can be used to determining the expression level of CD25, CD69, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, CD16, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, KIR3DS1, NKG2C, CCR7, CXCR3, L-Selectin, CXCR1, CXCR2, CX3CR1, ChemR23, CXCR4, CCR5, S1P5, c-Kit, mTORC1, MYC, SREBP1, IFN-γ, and a granzyme (e.g., granzyme B) include, e.g., immunoblotting, fluorescence-assisted cell sorting, enzyme-linked immunosorbent assays, and RT-PCR.

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of CD25 are available from Diaclone, Covalab Biotechnology, and Caltag Medsystems. The protein and cDNA sequences for mature human CD25 are shown below.

Mature Human CD25 Protein (SEQ ID NO: 1)

```
elcdddppe iphatfkama ykegtmlnce ckrgfrriks gslymlctgn sshsswdnqc
qctssatrnt tkqvtpqpee qkerkttemq spmqpvdqas lpghcreppp weneateriy
hfvvgqmvyy qcvqgyralh rgpaesvckm thgktrwtqp qlictgemet sqfpgeekpq
aspegrpese tsclvtttdf qiqtemaatm etsiftteyq vavagcvfll isvlllsglt
wqrrqrksrr ti
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Human CD25 cDNA (SEQ ID NO: 2)

```
gagctctg tgacgatgac ccgccagaga tcccacacgc cacattcaaa gccatggcct
acaaggaagg aaccatgttg aactgtgaat gcaagagagg tttccgcaga ataaaaagcg
ggtcactcta tatgctctgt acaggaaact ctagccactc gtcctgggac aaccaatgtc
aatgcacaag ctctgccact cggaacacaa cgaaacaagt gacacctcaa cctgaagaac
agaaagaaag gaaaaccaca gaaatgcaaa gtccaatgca gccagtggac caagcgagcc
ttccaggtca ctgcagggaa cctccaccat gggaaaatga agccacagag agaatttatc
```

atttcgtggt ggggcagatg gtttattatc agtgcgtcca gggatacagg gctctacaca
gaggtcctgc tgagagcgtc tgcaaaatga cccacgggaa gacaaggtgg acccagcccc
agctcatatg cacaggtgaa atggagacca gtcagtttcc aggtgaagag aagcctcagg
caagccccga aggccgtcct gagagtgaga cttcctgcct cgtcacaaca acagattttc
aaatacagac agaaatggct gcaaccatgg agacgtccat atttacaaca gagtaccagg
tagcagtggc cggctgtgtt ttcctgctga tcagcgtcct cctcctgagt gggctcacct
ggcagcggag acagaggaag agtagaagaa caatc

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of CD69 are available from RayBiotech, Novus Biologicals, and Aviscera Bioscience. The protein and cDNA sequences for mature human CD69 are shown below.

Mature Human CD69 Protein (SEQ ID NO: 3)

mssencfvae nsslhpesgq endatsphfs trhegsfqvp vlcavmnvvf itiliialia
lsvgqyncpg qytfsmpsds hvsscsedwv gyqrkcyfis tvkrswtsaq nacsehgatl
avidsekdmn flkryagree hwvglkkepg hpwkwsngke fnnwfnvtgs dkcvflknte
vssmeceknl ywicnkpyk

Human CD69 cDNA (SEQ ID NO: 4)

atgagctctg aaaattgttt cgtagcagag aacagctctt tgcatccgga gagtggacaa
gaaaatgatg ccaccagtcc ccatttctca acacgtcatg aagggtcctt ccaagttcct
gtcctgtgtg ctgtaatgaa tgtggtcttc atcaccattt taatcatagc tctcattgcc
ttatcagtgg gccaatacaa ttgtccaggc caatacacat tctcaatgcc atcagacagc
catgtttctt catgctctga ggactgggtt ggctaccaga ggaaatgcta ctttatttct
actgtgaaga ggagctggac ttcagcccaa aatgcttgtt ctgaacatgg tgctactctt
gctgtcattg attctgaaaa ggacatgaac tttctaaaac gatacgcagg tagagaggaa
cactgggttg gactgaaaaa ggaacctggt cacccatgga agtggtcaaa tggcaaagaa
tttaacaact ggttcaacgt tacagggtct gacaagtgtg tttttctgaa aaacacagag
gtcagcagca tggaatgtga gaagaattta tactggatat gtaacaaacc ttacaaataa

The protein and cDNA sequences for mature human CD59 are shown below.

Mature Human CD59 Protein (SEQ ID NO: 5)

lqcyncpnptadckt avncssdfda clitkaglqv ynkcwkfehc nfndvttrlr
eneltyycck kdlcnfneql en

Human CD59 cDNA (SEQ ID NO: 6)

atgggaatcc aaggagggtc tgtcctgttc gggctgctgc tcgtcctggc tgtcttctgc
cattcaggtc atagcctgca gtgctacaac tgtcctaacc caactgctga ctgcaaaaca

gccgtcaatt gttcatctga ttttgatgcg tgtctcatta ccaaagctgg gttacaagtg
tataacaagt gttggaagtt tgagcattgc aatttcaacg acgtcacaac ccgcttgagg
gaaaatgagc taacgtacta ctgctgcaag aaggacctgt gtaactttaa cgaacagctt
gaaaatggtg ggacatcctt atcagagaaa acagttcttc tgctggtgac tccatttctg
gcagcagcct ggagccttca tccctaa

The protein and cDNA sequences for mature human CD352 are shown below.

Mature Human CD352 Protein (SEQ ID NO: 7)

qssltplmv ngilgesvtl plefpagekv nfitwlfnet slafivphet kspeihvtnp
kqgkrlnftq syslqlsnlk medtgsyraq istktsakls sytlrilrql rniqvtnhsq
lfqnmtcelh ltcsvedadd nvsfrwealg ntlssqpnlt vswdprisse qdytciaena
vsnlsfsvsa qklcedvkiq ytdtkmilfm vsgicivfgf iillllvlrk rrdslslstq
rtqgpaesar nleyvsvspt nntvyasvth snreteiwtp rendtitiys tinhskeskp
tfsrataldn vv

Human CD352 cDNA (SEQ ID NO: 8)

atgttgtggc tgttccaatc gctcctgttt gtcttctgct ttggcccagg gaatgtagtt
tcacaaagca gcttaacccc attgatggtg aacgggattc tgggggagtc agtaactctt
cccctggagt ttcctgcagg agagaaggtc aacttcatca cttggctttt caatgaaaca
tctcttgcct tcatagtacc ccatgaaacc aaaagtccag aaatccacgt gactaatccg
aaacagggaa agcgactgaa cttcacccag tcctactccc tgcaactcag caacctgaag
atggaagaca caggctctta cagagcccag atatccacaa agacctctgc aaagctgtcc
agttacactc tgaggatatt aagacaactg aggaacatac aagttaccaa tcacagtcag
ctatttcaga atatgacctg tgagctccat ctgacttgct ctgtggagga tgcagatgac
aatgtctcat tcagatggga ggccttggga aacacacttt caagtcagcc aaacctcact
gtctcctggg accccaggat ttccagtgaa caggactaca cctgcatagc agagaatgct
gtcagtaatt tatccttctc tgtctctgcc cagaagcttt gcgaagatgt taaaattcaa
tatacagata ccaaaatgat tctgtttatg gtttctggga tatgcatagt cttcggtttc
atcatactgc tgttacttgt tttgaggaaa agaagagatt ccctatcttt gtctactcag
cgaacacagg gccccgagtc cgcaaggaac ctagagtatg tttcagtgtc tccaacgaac
aacactgtgt atgcttcagt cactcattca aacagggaaa cagaaatctg gacacctaga
gaaaatgata ctatcacaat ttactccaca attaatcatt ccaaagagag taaacccact
ttttccaggg caactgccct tgacaatgtc gtgtaa

The protein and cDNA sequences for mature human NKp80 are shown below.

Mature Human NKp80 Protein (SEQ ID NO: 9)

mqdeerymtl nvqskkrssa qtsqltfkdy svtlhwykil lgisgtvngi ltltlislil
lvsqgvllkc qkgscsnatq yedtgdlkvn ngtrrnisnk dlcasrsadq tvlcqsewlk
yqgkcywfsn emkswsdsyv yclerkshll iihdqlemaf iqknlrqlny vwiglnftsl
kmtwtwvdgs pidskiffik gpakenscaa ikeskifset cssvfkwicq y

Human NKp80 cDNA (SEQ ID NO: 10)

atgcaagatg aagaaagata catgacattg aatgtacagt caaagaaaag gagttctgcc
caaacatctc aacttacatt taaagattat tcagtgacgt tgcactggta taaaatctta
ctgggaatat ctggaaccgt gaatggtatt ctcactttga ctttgatctc cttgatcctg
ttggtactat gccaatcaga atggctcaaa taccaaggga agtgttattg gttctctaat
gagatgaaaa gctggagtga cagttatgtg tattgtttgg aaagaaaatc tcatctacta
atcatacatg accaacttga aatggctttt atacagaaaa acctaagaca attaaactac
gtatggattg ggcttaactt tacctccttg aaaatgacat ggacttgggt ggatggttct
ccaatagatt caaagatatt cttcataaag ggaccagcta aagaaaacag ctgtgctgcc
attaaggaaa gcaaaatttt ctctgaaacc tgcagcagtg ttttcaaatg gatttgtcag
tattag

The protein and cDNA sequences for mature human DNAM-1 are shown below.

Mature Human DNAM-1 Protein (SEQ ID NO: 11)

ee vlwhtsvpfa enmslecvyp smgiltqvew fkigtqqdsi aifspthgmv
irkpyaervy flnstmasnn mtlffrnase ddvgyyscsl ytypqgtwqk viqvvqsdsf
eaavpsnshi vsepgknvtl tcqpqmtwpv qavrwekiqp rqidlltycn lvhgrnftsk
fprqivsncs hgrwsvivip dvtvsdsgly rcylqasage netfvmrltv aegktdnqyt
lfvaggtvll llfvisitti iviflnrrrr rerrdlftes wdtqkapnny rspistsqpt
nqsmddtred iyvnyptfsr rpktrv

Human DNAM-1 cDNA (SEQ ID NO: 12)

atggattatc ctactttact tttggctctt cttcatgtat acagagctct atgtgaagag
gtgctttggc atacatcagt tccctttgcc gagaacatgt ctctagaatg tgtgtatcca
tcaatgggca tcttaacaca ggtggagtgg ttcaagatcg ggacccagca ggattccata
gccattttca gccctactca tggcatggtc ataaggaagc cctatgctga gagggtttac
tttttgaatt caacgatggc ttccaataac atgactcttt tctttcggaa tgcctctgaa
gatgatgttg gctactattc ctgctctctt tacacttacc cacagggaac ttggcagaag
gtgatacagg tggttcagtc agatagtttt gaggcagctg tgccatcaaa tagccacatt
gtttcggaac ctggaaagaa tgtcacactc acttgtcagc ctcagatgac gtggcctgtg
caggcagtga ggtgggaaaa gatccagccc cgtcagatcg acctcttaac ttactgcaac
ttggtccatg gcagaaattt cacctccaag ttcccaagac aaatagtgag caactgcagc
cacggaaggt ggagcgtcat cgtcatcccc gatgtcacag tctcagactc ggggctttac
cgctgctact tgcaggccag cgcaggagaa aacgaaacct tcgtgatgag attgactgta
gccgagggta aaaccgataa ccaatatacc ctctttgtgg ctggagggac agttttattg
ttgttgtttg ttatctcaat taccaccatc attgtcattt tccttaacag aaggagaagg
agagagagaa gagatctatt tacagagtcc tgggatacac agaaggcacc caataactat
agaagtccca tctctaccag tcaacctacc aatcaatcca tggatgatac aagagaggat
atttatgtca actatccaac cttctctcgc agaccaaaga ctagagttta a

The protein and cDNA sequences for mature human 2B4 are shown below.

Mature Human 2B4 Protein (SEQ ID NO: 13)

gk gcqgsadhvv sisgvplqlq pnsiqtkvds iawkkllpsq ngfhhilkwe
ngslpsntsn drfsfivknl sllikaaqqq dsglyclevt sisgkvqtat fqvfvfdkve
kprlqgqgki ldrgrcqval sclvsrdgnv syawyrgskl iqtagnltyl deevdingth
tytcnvsnpv sweshtlnlt qdcqnahqef rfwpflviiv ilsalflgtl acfcvwrrkr
kekqsetspk efltiyedvk dlktrrnheq eqtfpgggst iysmiqsqss aptsqepayt
lysliqpsrk sgsrkrnhsp sfnstiyevi gksqpkaqnp arlsrkelen fdvys

Human 2B4 cDNA (SEQ ID NO: 14)

atgctggggc aagtggtcac cctcatactc ctcctgctcc tcaaggtgta tcagggcaaa
ggatgccagg gatcagctga ccatgtggtt agcatctcgg gagtgcctct tcagttacaa
ccaaacagca tacagacgaa ggttgacagc attgcatgga agaagttgct gccctcacaa
aatggatttc atcacatatt gaagtgggag aatggctctt tgccttccaa tacttccaat
gatagattca gttttatagt caagaacttg agtcttctca tcaaggcagc tcagcagcag
gacagtggcc tctactgcct ggaggtcacc agtatatctg gaaaagttca gacagccacg
ttccaggttt ttgtatttga taaagttgag aaaccccgcc tacaggggca ggggaagatc
ctggacagag ggagatgcca agtggctctg tcttgcttgg tctccaggga tggcaatgtg
tcctatgctt ggtacagagg gagcaagctg atccagacag cagggaacct cacctacctg
gacgaggagg ttgacattaa tggcactcac acatatacct gcaatgtcag caatcctgtt
agctgggaaa gccacaccct gaatctcact caggactgtc agaatgccca tcaggaattc
agattttggc cgtttttggt gatcatcgtg attctaagcg cactgttcct tggcaccctt
gcctgcttct gtgtgtggag gagaaagagg aaggagaagc agtcagagac cagtcccaag
gaatttttga caatttacga agatgtcaag gatctgaaaa ccaggagaaa tcacgagcag
gagcagactt ttcctggagg ggggagcacc atctactcta tgatccagtc ccagtcttct
gctcccacgt cacaagaacc tgcatataca ttatattcat taattcagcc ttccaggaag
tctggatcca ggaagaggaa ccacagccct tccttcaata gcactatcta tgaagtgatt
ggaaagagtc aacctaaagc ccagaaccct gctcgattga gccgcaaaga gctggagaac
tttgatgttt attcctag

The protein and cDNA sequences for mature human NKp30 are shown below.

Mature Human NKp30 Protein (SEQ ID NO: 15)

lw vsqppeirtl egssaflpcs fnasqgrlai gsvtwfrdev vpgkevrngt
pefrgrlapl assrflhdhq aelhirdvrg hdasiyvcrv evlglgvgtg ngtrlvveke
hpqlgagtvl llragfyavs flsvavgstv yyqgkcltwk gprrqlpavv paplpppcgs
sahllppvpg g

Human NKp30 cDNA (SEQ ID NO: 16)

atggcctgga tgctgttgct catcttgatc atggtccatc caggatcctg tgctctctgg
gtgtcccagc cccctgagat tcgtaccctg gaaggatcct ctgccttcct gccctgctcc
ttcaatgcca gccaagggag actggccatt ggctccgtca cgtggttccg agatgaggtg

gttccaggga aggaggtgag gaatggaacc ccagagttca ggggccgcct ggccccactt
gcttcttccc gtttcctcca tgaccaccag gctgagctgc acatccggga cgtgcgaggc
catgacgcca gcatctacgt gtgcagagtg gaggtgctgg gccttggtgt cgggacaggg
aatgggactc ggctggtggt ggagaaagaa catcctcagc taggggctgg tacagtcctc
ctccttcggg ctggattcta tgctgtcagc tttctctctg tggccgtggg cagcaccgtc
tattaccagg gcaaatgcca ctgtcacatg ggaacacact gccactcctc agatgggccc
cgaggagtga ttccagagcc cagatgtccc tag

The protein and cDNA sequences for mature human NKp44 are shown below.

Mature Human NKp44 Protein (SEQ ID NO: 17)

qskaqvlqs vagqtltvrc qypptgslye kkgwckeasa lvcirlvtss kprtmawtsr
ftiwddpdag fftvtmtdlr eedsghywcr iyrpsdnsvs ksvrfylvvs pasastqtsw
tprdlvssqt qtqscvppta garqapesps tipvpsqpqn stlrpgpaap ialvpvfcgl
lvakslvlsa llvwwgdiww ktmmelrsld tqkatchlqq vtdlpwtsvs spvereilyh
tvartkisdd ddehtl

Human NKp44 cDNA (SEQ ID NO: 18)

atggcctggc gagccctaca cccactgcta ctgctgctgc tgctgttccc aggctctcag
gcacaatcca aggctcaggt acttcaaagt gtggcagggc agacgctaac cgtgagatgc
cagtacccgc ccacgggcag tctctacgag aagaaaggct ggtgtaagga ggcttcagca
cttgtgtgca tcaggttagt caccagctcc aagcccagga cgatggcttg gacctctcga
ttcacaatct gggacgaccc tgatgctggc ttcttcactg tcaccatgac tgatctgaga
gaggaagact caggacatta ctggtgtaga atctaccgcc cttctgacaa ctctgtctct
aagtccgtca gattctatct ggtggtatct ccagcctctg cctccacaca gacctcctgg
actccccgcg acctggtctc ttcacagacc cagacccaga gctgtgtgcc tcccactgca
ggagccagac aagcccctga gtctccatct accatccctg tcccttcaca gccacagaac
tccacgctcc gccctggccc tgcagccccc attgccctgg tgcctgtgtt ctgtggactc
ctcgtagcca agagcctggt gctgtcagcc ctgctcgtct ggtgggtttt aaggaatcgg
cacatgcagc atcaagggag gtctctgctg cacccagctc agcccaggcc ccaggcccat
agacacttcc cactgagcca cagggcacca ggggggacat atggtggaaa accatga

The protein and cDNA sequences for mature human NKp46 are shown below.

Mature Human NKp46 Protein (SEQ ID NO: 19)

qqqtlpkpf iwaephfmvp kekqvticcq gnygaveyql hfegslfavd rpkpperink
vqfyipdmns rmagqysciy rvgelwseps nlldlvvtem ydtptlsvhp gpevisgekv
tfycrldtat smflllkegr sshvqrgygk vqaefplgpv ttahrgtyrc fgsynnhaws
fpsepvkllv tgdientsla pedptfpadt wgtyllttet glqkdhalwd htaqnllrmg
laflvlvalv wflvedwlsr krtrerasra stwegrrrln tqtl

Human NKp46 cDNA (SEQ ID NO: 20)

atggcctggc gagccctaca cccactgcta ctgctgctgc tgctgttccc aggctctcag
gcacaatcca aggctcaggt acttcaaagt gtggcagggc agacgctaac cgtgagatgc
cagtacccgc ccacgggcag tctctacgag aagaaaggct ggtgtaagga ggcttcagca
cttgtgtgca tcaggttagt caccagctcc aagcccagga cgatggcttg gacctctcga
ttcacaatct gggacgaccc tgatgctggc ttcttcactg tcaccatgac tgatctgaga
gaggaagact caggacatta ctggtgtaga atctaccgcc cttctgacaa ctctgtctct
aagtccgtca gattctatct ggtggtatct ccagcctctg cctccacaca gacctcctgg
actccccgcg acctggtctc ttcacagacc cagacccaga gctgtgtgcc tcccactgca
ggagccagac aagcccctga gtctccatct accatccctg tcccttcaca gccacagaac
tccacgctcc gccctggccc tgcagccccc attgccctgg tgcctgtgtt ctgtggactc
ctcgtagcca agagcctggt gctgtcagcc ctgctcgtct ggtgggtttt aaggaatcgg
cacatgcagc atcaagggag gtctctgctg cacccagctc agcccaggcc ccaggcccat
agacacttcc cactgagcca cagggcacca ggggggacat atggtggaaa accatga

The protein and cDNA sequences for mature human NKG2D are shown below.

Mature Human NKG2D Protein (SEQ ID NO: 21)

mgwirgrrsr hswemsefhn ynldlkksdf strwqkqrcp vvkskcrena spfffccfia
vamgirfiim vaiwsavfln slfnqevqip ltesycgpcp knwicyknnc yqffdesknw
yesqascmsq nasllkvysk edqdllklvk syhwmglvhi ptngswqwed gsilspnllt
iiemqkgdca lyassfkgyi encstpntyi cmqrtv

Human NKG2D cDNA (SEQ ID NO: 22)

atggggtgga ttcgtggtcg gaggtctcga cacagctggg agatgagtga atttcataat
tataacttgg atctgaagaa gagtgatttt tcaacacgat ggcaaaagca aagatgtcca
gtagtcaaaa gcaaatgtag agaaaatgca tctccatttt ttttctgctg cttcatcgct
gtagccatgg gaatccgttt cattattatg gtaacaatat ggagtgctgt attcctaaac
tcattattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct
aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg
tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa
gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt
ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca
ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata
gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgta a

The protein and cDNA sequences for mature human CD16a are shown below.

Mature Human CD16a Protein (SEQ ID NO: 23)

maegtlwqil cvssdaqpqt fegvkgadpp tlppgsflpg pvlwwgslar lqteksdevs
rkgnwwvtem gggagerlft ssclvglvpl glrislvtcp lqcgimwqll lptallllvs
agmrtedlpk avvflepqwy rvlekdsvtl kcqgaysped nstqwfhnes lissqassyf
idaatvddsg eyrcqtnlst lsdpvqlevh igwlllqapr wvfkeedpih lrchswknta

lhkvtylqng kgrkyfhhns dfyipkatlk dsgsyfcrgl fgsknvsset vnititqgla
vstissffpp gyqvsfclvm vllfavdtgl yfsvktnirs strdwkdhkf kwrkdpqdk

Human CD16a cDNA (SEQ ID NO: 24)

atggctgagggcacactctggcagattctgtgtgtgtcctcagatgctca
gccacagacctttgagggagtaaaggggcagacccacccaccttgcctc
caggctctttccttcctggtcctgttctatggtggggctcccttgccaga
cttcagactgagaagtcagatgaagtttcaagaaaaggaaattggtgggt
gacagagatgggtggaggggctggggaaaggctgtttacttcctcctgtc
tagtcggtttggtccctttagggctccggatatctttggtgacttgtcca
ctccagtgtggcatcatgtggcagctgctcctcccaactgctctgctact
tctagtttcagctggcatgcggactgaagatctcccaaaggctgtggtgt
tcctggagcctcaatggtacagggtgctcgagaaggacagtgtgactctg
aagtgccagggagcctactcccctgaggacaattccacacagtggtttca
caatgagagcctcatctcaagccaggcctcgagctacttcattgacgctg
ccacagtcgacgacagtggagagtacaggtgccagacaaacctctccacc
ctcagtgacccggtgcagctagaagtccatatcggctggctgttgctcca
ggcccctcggtgggtgttcaaggaggaagaccctattcacctgaggtgtc
acagctggaagaacactgctctgcataaggtcacatatttacagaatggc
aaaggcaggaagtattttcatcataattctgacttctacattccaaaagc
cacactcaaagacagcggctcctacttctgcaggggggcttttgggagta
aaaatgtgtcttcagagactgtgaacatcaccatcactcaaggtttggca
gtgtcaaccatctcatcattctttccacctgggtaccaagtctctttctg
cttggtgatggtactccttttgcagtggacacaggactatatttctctg
tgaagacaaacattcgaagctcaacaagagactggaaggaccataaattt
aaatggagaaaggaccctcaagacaaatga

The protein and cDNA sequences for mature human CD16b are shown below.

Mature Human CD16b Protein (SEQ ID NO: 25)

mwqlllptal lllvsagmrt edlpkavvfl epqwysvlek dsvtlkcqga yspednstqw
fhneslissq assyfidaat vndsgeyrcq tnlstlsdpv qlevhigwll lqaprwvfke
edpihlrchs wkntalhkvt ylqngkdrky fhhnsdfhip katlkdsgsy fcrglvgskn
vssetvniti tqglavstis sfsppgyqvs fclvmvllfa vdtglyfsvk tni

Human CD16b cDNA (SEQ ID NO: 26)

atgtggcagctgctcctcccaactgctctgctacttctagtttcagctgg
catgcggactgaagatctcccaaaggctgtggtgttcctggagcctcaat
ggtacagcgtgcttgagaaggacagtgtgactctgaagtgccagggagcc
tactcccctgaggacaattccacacagtggtttcacaatgagaacctcat
ctcaagccaggcctcgagctacttcattgacgctgccacagtcaacgaca
gtggagagtacaggtgccagacaaacctctccaccctcagtgacccggtg
cagctagaagtccatatcggctggctgttgctccaggcccctcggtgggt
gttcaaggaggaagaccctattcacctgaggtgtcacagctggaagaaca
ctgctctgcataaggtcacatatttacagaatggcaaagacaggaagtat
tttcatcataattctgacttccacattccaaaagccacactcaaagatag

cggctcctacttctgcaggggcttgttgggagtaaaaatgtgtcttcag
agactgtgaacatcaccatcactcaaggtttggcagtgtcaaccatctca
tcattctctccacctgggtaccaagtctctttctgcttggtgatggtact
ccttttgcagtggacacaggactatatttctctgtgaagacaaacattt
ga

The protein and cDNA sequences for mature human KIR2DS1 are shown below.

Human KIR2DS1 Protein (SEQ ID NO: 27)

msltvvsmac vgffllqgaw phegvhrkps llahpgrlvk seetvilqcw sdvmfehfll
hregmfndtl rligehhdgv skanfsisrm kqdlagtyrc ygsvthspyq vsapsdpldi
viiglyekps lsaqpgptvl agesvtlscs srssydmyhl sregeaherr lpagtkvngt
fqanfplgpa thggtyrcfg sfrdspyews kssdpllvsv tgnpsnswps ptepssetgn
prhlhvligt svvkipftil lffllhrwcs dkknaavmdq epagnrtvns edsdeqdhqe
vsya

Human KIR2DS1 cDNA (SEQ ID NO: 28)

atgtcgctcacggtcgtcagcatggcgtgtgttgggttcttcttgctgca
gggggcctggccacatgagggagtccacagaaaaccttccctcctggccc
acccaggtcgcctggtgaaatcagaagagacagtcatcctgcaatgttgg
tcagatgtcatgtttgaacacttccttctgcacagagaggggatgtttaa
cgacactttgcgcctcattggagaacaccatgatggggtctccaaggcca
acttctccatcagtcgcatgaagcaagacctggcagggacctacagatgc
tacggttctgttactcactccccctatcagttgtcagctcccagtgaccc
tctggacatcgtgatcataggtctatatgagaaaccttctctctcagccc
agccgggccccacggttctggcaggagagaatgtgaccttgtcctgcagc
tcccggagctcctatgacatgtaccatctatccagggaaggggaggccca
tgaacgtaggctccctgcagggaccaaggtcaacggaacattccaggcca
actttcctctgggccctgccacccatggagggacctacagatgcttcggc
tctttccgtgactctccatacgagtggtcaaagtcaagtgacccactgct
tgtttctgtcacaggaaacccttcaaatagttggccttcacccactgaac
caagctccgaaaccggtaaccccagacacctacatgttctgattgggacc
tcagtggtcaaaatccctttcaccatcctcctcttctttctccttcatcg
ctggtgctccgacaaaaaaaatgctgctgtaatggaccaagagcctgcag
ggaacagaacagtgaacagcgaggattctgatgaacaagaccatcaggag
gtgtcatacgcataa

The protein and cDNA sequences for mature human KIR2DS2 are shown below.

Human KIR2DS2 Protein (SEQ ID NO: 29)

mslmvvsmvc vgffllqgaw phegvhrkps llahpgplvk seetvilqcw sdvrfehfll
hregkykdtl hligehhdgv skanfsigpm mqdlagtyrc ygsvthspyq lsapsdpldi
vitglyekps lsaqpgptvl agesvtlscs srssydmyhl sregeaherr fsagpkvngt
fqadfplgpa thggtyrcfg sfrdspyews nssdpllvsv tgnpsnswps ptepssktgn

prhlhvligt svvkipftil lffllhrwcs nkknaavmdq epagnrtvns edsdeqdhqe vsya

Human KIR2DS2 cDNA (SEQ ID NO: 30)

atgtcgctcatggtcgtcagcatggcgtgtgttgggttcttcttgctgca
gggggcctggccacatgagggagtccacagaaaaccttccctcctggccc
acccaggtcccctggtgaaatcagaagagacagtcatcctgcaatgttgg
tcagatgtcaggtttgagcacttccttctgcacagagaggggaagtataa
ggacactttgcacctcattggagagcaccatgatggggtctccaaggcca
acttctccatcggtcccatgatgcaagaccttgcagggacctacagatgc
tacggttctgttactcactccccctatcagttgtcagctcccagtgaccc
tctggacatcgtcatcacaggtctatatgagaaaccttctctctcagccc
agccgggccccacggttttggcaggagagagcgtgaccttgtcctgcagc
tcccggagctcctatgacatgtaccatctatccagggaggggaggccca
tgaacgtaggttctctgcagggcccaaggtcaacggaacattccaggccg
actttcctctgggccctgccacccacggaggaacctacagatgcttcggc
tctttccgtgactctccctatgagtggtcaaactcgagtgacccactgct
tgtttctgtcacaggaaacccttcaaatagttggccttcacccactgaac
caagctccaaaaccggtaaccccagacacctgcatgttctgattgggacc
tcagtggtcaaaatccctttcaccatcctcctcttctttctccttcatcg
ctggtgctccaacaaaaaaaatgctgctgtaatggaccaagagcctgcag
ggaacagaacagtgaacagcgaggactctgatgaacaagaccctcaggag
gtgacatacacacagttgaatcactgcgttttcacacagagaaaaatcac
tcgcccttctcagaggcccaagacacccccaacagatatcatcgtgtaca
cggaacttccaaatgctgagtccaga

The protein and cDNA sequences for mature human KIR2DS3 are shown below.

Mature Human KIR2DS3 Protein (SEQ ID NO: 31)

mslmvismac vgffwlqgaw phegfrrkps llahpgrlvk seetvilqcw sdvmfehfll
hregtfndtl rligehidgv skanfsigrm rqdlagtyrc ygsvphspyq fsapsdpldi
vitglyekps lsaqpgptvl agesvtlscs swssydmyhl stegeaherr fsagpkvngt
fqadfplgpa tqggtyrcfg sfhdspyews kssdpllvsv tgnpsnswps ptepssktgn
prhlhvligt svvklpftil lffllhrwcs dkknasvmdq gpagnrtvnr edsdeqdhqe
vsya

Human KIR2DS3 cDNA (SEQ ID NO: 32)

atgtcgctcatggtcatcagcatggcatgtgttgggttcttctggctgca
gggggcctggccacatgagggattccgcagaaaaccttccctcctggccc
acccaggtcgcctggtgaaatcagaagagacagtcatcctgcaatgttgg
tcagatgtcatgtttgagcacttccttctgcacagagaggggacgtttaa
cgacactttgcgcctcattggagagcacattgatggggtctccaaggcca
acttctccatcggtcgcatgaggcaagacctggcagggacctacagatgc
tacggttctgttcctcactccccctatcagttttcagctcccagtgaccc
tctggacatcgtgatcacaggtctatatgagaaaccttctctctcagccc

agccgggccccacggttctggcaggagagagcgtgaccttgtcctgcagc
tcctggagctcctatgacatgtaccatctatccacggagggggaggccca
tgaacgtaggttctctgcagggcccaaggtcaacggaacattccaggccg
actttcctctgggccctgccacccaaggaggaacctacagatgcttcggc
tctttccatgactctccctacgagtggtcaaagtcaagtgacccactgct
tgtttctgtcacaggaaacccttcaaatagttggccttcacccactgaac
caagctccaaaaccggtaaccccagacacctacacgttctgattgggacc
tcagtggtcaaactccctttcaccatcctcctcttctttctccttcatcg
ctggtgctccgacaaaaaaaatgcatctgtaatggaccaagggcctgcgg
ggaacagaacagtgaacagggaggattctgatgaacaggaccatcaggag
gtgtcatacgcataa

The protein and cDNA sequences for mature human KIR2DL4 are shown below.

Mature Human KIR2DL4 Protein (SEQ ID NO: 33)

hvggqdk pfcsawpsav vpqgghatlr chcrrgfnif tlykkdgvpv pelynrifwn
sflispvtpa hagtyrcrgf hphsptewsa psnplvimvt glyekpslta rpgptvrage
nvtlscssqs sfdiyhlsre geahelrlpa vpsingtfqa dfplgpathg etyrcfgsfh
gspyewsdps dplpvsvtgn pssswpspte psfktgiarh lhavirysva iilftilpff
llhrwcskkk naavmnqepa ghrtvnreds deqdpqevty aqldhciftq rkitgpsqrs
krpstdtsvc ielpnaepra lspahehhsq almgssrett alsqtqlass nvpaagi

Human KIR2DL4 cDNA (SEQ ID NO: 34)

atgtccccttcacatgttgtggtcaatgtgtcaactgcacgatccgggcc
cctcaccacatcctctgcaccggtcagtcgagccgagtcactgcgtcctg
gcagcagaagctgcaccatgtccatgtcacccacggtcatcatcctggca
tgtcttgggttcttcttggaccagagtgtgtgggcacacgtgggtggtca
ggacaagcccttctgctctgcctggcccagcgctgtggtgcctcaaggag
gacacgtgactcttcggtgtcactatcgtcgtgggtttaacatcttcacg
ctgtacaagaaagatggggtccctgtccctgagctctacaacagaatatt
ctggaacagtttcctcattagccctgtgaccccagcacacgcagggacct
acagatgtcgaggttttcacccgcactcccccactgagtggtcggcaccc
agcaaccccctggtgatcatggtcacaggtctatatgagaaaccttcgct
tacagcccggccgggccccacggttcgcgcaggagagaacgtgaccttgt
cctgcagctcccagagctcctttgacatctaccatctatccagggagggg
gaagcccatgaacttaggctccctgcagtgcccagcatcaatggaacatt
ccaggccgacttccctctgggtcctgccacccacggagagacctacagat
gcttcggctctttccatggatctccctacgagtggtcagacccgagtgac
ccactgcctgtttctgtcacaggaaacccttctagtagttggccttcacc
cactgaaccaagcttcaaaactggtatcgccagacacctgcatgctgtga
ttaggtactcagtggccatcatcctctttaccatccttcccttctttctc
cttcatcgctggtgctccaaaaaaaaagatgctgctgtaatgaaccaaga
gcctgcgggacacagaacagtgaacagggaggactctgatgaacaagacc
ctcaggaggtgacatacgcacagttggatcactgcattttcacacagaga
aaaatcactggcccttctcagaggagcaagagaccctcaacagataccag
cgtgtgtatagaacttccaaatgctgagcccagagcgttgtctcctgccc

atgagcaccacagtcaggccttgatgggatcttctagggagacaacagcc
ctgtctcaaacccagcttgccagctctaatgtaccagcagctggaatctg
a

The protein and cDNA sequences for mature human KIR2DS4 are shown below.

Mature Human KIR2DS4 Protein (SEQ ID NO: 35)

qegvhrkps flalpghlvk seetvilqcw sdvmfehfll hregkfnntl hligehhdgv
skanfsigpm mpvlagtyrc yssvphspyq lsapsdpldm viiglyekps lsaqpgptvq
agenvslscs siypgrgrpm nvgslqcaas tehsrptflw alpptegptd asalsvtlpt
sgqtrvihcl fpsqetlqiv glhplnqapk pvtpdtymf

Human KIR2DS4 cDNA (SEQ ID NO: 36)

atgtcgctcatggtcatcatcatggcgtgtgttgggttcttcttgctgca
gggggcctggccacaggagggagtccacagaaaaccttccttcctggccc
tcccaggtcacctggtgaaatcagaagagacagtcatcctgcaatgttgg
tcggatgtcatgtttgagcacttccttctgcacagagaggggaagtttaa
caacactttgcacctcattggagagcaccatgatggggtttccaaggcca
acttctccattggtcccatgatgcctgtccttgcaggaacctacagatgc
tacggttctgttcctcactccccctatcagttgtcagctcccagtgaccc
tctggacatggtgatcataggtctatatgagaaaccttctctctcagccc
agccgggccccacggttcaggcaggagagaatgtgaccttgtcctgcagc
tccatctatccagggaaggggaggcccatgaacgtaggctccctgcagtg
cgcagcatcaacggaacattccaggccgactttcctctgggccctgccac
ccacggagggacctacagatgcttcggctctttccgtgacgctccctacg
agtggtcaaactcgagtgatccactgcttgtttccgtcacaggaaaccct
tcaaatagttggccttcacccactgaaccaagctccaaaaccggtaaccc
cagacacctacatgttctgattgggacctcagtggtcaaaatccctttca
ccatcctcctcttctttctccttcatcgctggtgctccgacaaaaaaaat
gctgctgtaatggaccaagagcctgcagggaacagaacagtgaacagcga
ggattctgatgaacaagaccatcaggaggtgtcatacgcataa

The protein and cDNA sequences for mature human KIR2DS5 are shown below.

Mature Human KIR2DS5 (SEQ ID NO: 37)

hegfrrkps llahpgplvk seetvilqcw sdvmfehfll hregtfnhtl rligehidgv
skgnfsigrm tqdlagtyrc ygsvthspyq lsapsdpldi vitglyekps lsaqpgptvl
agesvtlscs srssydmyhl sregeaherr lpagtkvngt fqadfpldpa thggtyrcfg
sfrdspyews kssdpllvsv tgntsnswps ptepssktgn prhlhvligt svvklpftil
lfflhrwcs nkknasvmdq gpagnrtvnr edsdeqdhqe vsya

Human KIR2DS5 cDNA (SEQ ID NO: 38)

```
atgtcgctcatggtcatcagcatggcgtgtgttgcgttcttcttgctgca
gggggcctggccacatgagggattccgcagaaaaccttccctcctggccc
acccaggtcccctggtgaaatcagaagagacagtcatcctgcaatgttgg
tcagatgtcatgtttgagcacttccttctgcacagagaggggacgtttaa
ccacactttgcgcctcattggagagcacattgatggggtctccaagggca
acttctccatcggtcgcatgacacaagacctggcagggacctacagatgc
tacggttctgttactcactccccctatcagttgtcagcgcccagtgaccc
tctggacatcgtgatcacaggtctatatgagaaaccttctctctcagccc
agccgggccccacggttctggcaggagagagcgtgaccttgtcctgcagc
tcccggagctcctatgacatgtaccatctatccagggaaggggaggccca
tgaacgtaggctccctgcagggcccaaggtcaacagaacattccaggccg
actttcctctggaccctgccacccacggagggacctacagatgcttcggc
tctttccgtgactctccatacgagtggtcaaagtcaagtgacccactgct
tgtttctgtcacaggaaactcttcaaatagttggccttcacccactgaac
caagctccgaaaccggtaaccccagacacctacacgttctgattgggacc
tcagtggtcaaactccctttcaccatcctcctcttctttctccttcatcg
ctggtgctccaacaaaaaaaatgcatctgtaatggaccaagggcctgcgg
ggaacagaacagtgaacagggaggattctgatgaacaggaccatcaggag
gtgtcatacgcataa
```

The protein and cDNA sequences for mature human KIR3DS1 are shown below.

Mature Human KIR3DS1 cDNA (SEQ ID NO: 39)

```
hmggqdkpf lsawpsavvp rgghvtlrch yrhrfnnfml ykedrihvpi fhgrifqegf
nmspvttaha gnytcrgshp hsptgwsaps npmvimvtgn hrkpsllahp gplvksgerv
ilqcwsdimf ehfflhkegi skdpsrlvgq ihdgvskanf sigsmmrala gtyrcygsvt
htpyqlsaps dpldivvtgl yekpslsaqp gpkvqagesv tlscssrssy dmyhlsregg
aherrlpavr kvnrtfqadf plgpathggt yrcfgsfrhs pyewsdpsdp llvsvtgnps
sswpspteps sksgnlrhlh iligtsvvki pftillffll hrwcsnkkkc ccngpracre
qk
```

Human KIR3DS1 cDNA (SEQ ID NO: 40)

```
atgttgctcatggtcgtcagcatggcgtgtgttgggttgttcttggtcca
gagggccggtccacacatgggtggtcaggacaagcccttcctgtctgcct
ggcccagcgctgtggtgcctcgcggaggacacgtgactcttcggtgtcac
tatcgtcataggtttaacaatttcatgctatacaaagaagacagaatcca
cgttcccatcttccatggcagaatattccaggagggcttcaacatgagcc
ctgtgaccacagcacatgcagggaactacacatgtcggggttcacaccca
cactcccccactgggtggtcggcacccagcaaccccatggtgatcatggt
cacaggaaaccacagaaaaccttccctcctggcccacccaggtcccctgg
tgaaatcaggagagagagtcatcctgcaatgttggtcagatatcatgttt
gagcacttctttctgcacaaagagtggatctctaaggacccctcacgcct
cgttggacagatccatgatggggtctccaaggccaatttctccatcggtt
```

ccatgatgcgtgcccttgcagggacctacagatgctacggttctgttact
cacacccccctatcagttgtcagctcccagtgatcccctggacatcgtggt
cacaggtctatatgagaaaccttctctctcagcccagccgggccccaagg
ttcaggcaggagagagcgtgaccttgtcctgtagctcccggagctcctat
gacatgtaccatctatccagggaggggggagcccatgaacgtaggctccc
tgcagtgcgcaaggtcaacagaacattccaggcagatttccctctgggcc
ctgccacccacggagggacctacagatgcttcggctctttccgtcactct
ccctacgagtggtcagacccgagtgacccactgcttgtttctgtcacagg
aaacccttcaagtagttggccttcacccacagaaccaagctccaaatctg
gtaacctcagacacctgcacattctgattgggacctcagtggtcaaaatc
cctttcaccatcctcctcttctttctccttcatcgctggtgctccaacaa
aaaaaaatgctgctgtaatggaccaagagcctgcagggaacagaagtga

The protein and cDNA sequences for mature human NKG2C are shown below.

Mature Human NKG2C Protein (SEQ ID NO: 41)

mskqrgtfse vslaqdpkrq qrkpkgnkss isgteqeifq velnlqnpsl nhqgidkiyd
cqgllpppek ltaevlgiic ivlmatvlkt ivlipfleqn nsspntrtqk arhcghcpee
witysnscyy igkerrtwee sllactskns sllsidneee mkflasilps swigvfrnss
hhpwvtingl afkhkikdsd naelncavlq vnrlksaqcg ssmiyhckhk l

Human NKG2C cDNA (SEQ ID NO: 42)

atgaataaacaaagaggaaccttctcagaagtgagtctggcccaggaccc
aaagcggcagcaaaggaaacctaaaggcaataaaagctccatttcaggaa
ccgaacaggaaatattccaagtagaattaaatcttcaaaatccttccctg
aatcatcaagggattgataaaatatatgactgccaaggtttactgccacc
tccagagaagctcactgccgaggtcctaggaatcatttgcattgtcctga
tggccactgtgttaaaaacaatagttcttattcctttcctggagcagaac
aatttttccccgaatacaagaacgcagaaagcacgtcattgtggccattg
tcctgaggagtggattacatattccaacagttgttattacattggtaagg
aaagaagaacttgggaagagagtttgctggcctgtacttcgaagaactcc
agtctgctttctatagataatgaagaagaaatgaaatttctggccagcat
tttaccttcctcatggattggtgtgtttcgtaacagcagtcatcatccat
gggtgacaataaatggtttggctttcaaacataagataaaagactcagat
aatgctgaacttaactgtgcagtgctacaagtaaatcgacttaaatcagc
ccagtgtggatcttcaatgatatatcattgtaagcataagctttag

The protein and cDNA sequences for mature human CCR7 are shown below.

Mature Human CCR7 Protein (SEQ ID NO: 43)

qdevtd dyigdnttvd ytlfeslcsk kdvrnfkawf lpimysiicf vgllgnglvv
ltyiyfkrlk tmtdtyllnl avadilfllt lpfwaysaak swvfgvhfck lifaiykmsf
fsgmllllci sidryvaivq avsahrhrar vllisklscv giwilatvls ipellysdlq

rssseqamrc slitehveaf itiqvaqmvi gflvpllams fcylviirtl lqarnfernk
aikviiavvv vfivfqlpyn gvvlaqtvan fnitsstcel skqlniaydv tyslacvrcc
vnpflyafig vkfrndlfkl fkdlgclsqe qlrqwsscrh irrssmsvea ettttfsp

Human CCR7 cDNA (SEQ ID NO: 44)

atggacctggggaaaccaatgaaaagcgtgctggtggtggctctccttgt
catttccaggtatgcctgtgtcaagatgaggtcacggacgattacatcg
gagacaacaccacagtggactacactttgttcgagtctttgtgctccaag
aaggacgtgcggaactttaaagcctggttcctccctatcatgtactccat
catttgtttcgtgggcctactgggcaatgggctggtcgtgttgacctata
tctatttcaagaggctcaagaccatgaccgatacctacctgctcaacctg
gcggtggcagacatcctcttcctcctgacccttcccttctgggcctacag
cgcggccaagtcctgggtcttcggtgtccacttttgcaagctcatctttg
ccatctacaagatgagcttcttcagtggcatgctcctacttctttgcatc
agcattgaccgctacgtggccatcgtccaggctgtctcagctcaccgcca
ccgtgcccgcgtccttctcatcagcaagctgtcctgtgtgggcatctgga
tactagccacagtgctctccatcccagagctcctgtacagtgacctccag
aggagcagcagtgagcaagcgatgcgatgctctctcatcacagagcatgt
ggaggcctttatcaccatccaggtggcccagatggtgatcggctttctgg
tccccctgctggccatgagcttctgttaccttgtcatcatccgcaccctg
ctccaggcacgcaactttgagcgcaacaaggccatcaaggtgatcatcgc
tgtggtcgtggtcttcatagtcttccagctgccctacaatggggtggtcc
tggcccagacggtggccaacttcaacatcaccagtagcacctgtgagctc
agtaagcaactcaacatcgcctacgacgtcacctacagcctggcctgcgt
ccgctgctgcgtcaacccttttcttgtacgccttcatcggcgtcaagttcc
gcaacgatctcttcaagctcttcaaggacctgggctgcctcagccaggag
cagctccggcagtggtcttcctgtcggcacatccggcgctcctccatgag
tgtggaggccgagaccaccaccaccttctccccatag

The protein and cDNA sequences for mature human CXCR3 are shown below.

Mature Human CXCR3 Protein (SEQ ID NO: 45)

mvlevsdhqv lndaevaall enfsssydyg enesdsccts ppcpqdfsln fdraflpaly
sllfllgllg ngavaavlls rrtalsstdt fllhlavadt llvltlplwa vdaavqwvfg
sglckvagal fninfyagal llacisfdry lnivhatqly rrgpparvtl tclavwglcl
lfalpdfifl sahhderlna thcqynfpqv grtalrvlql vagfllpllv maycyahila
vllvsrgqrr lramrlvvvv vvafalcwtp yhlvvlvdil mdlgalarnc gresrvdvak
svtsglgymh cclnpllyaf vgvkfrermw mlllrlgcpn qrglqrqpss srrdsswset
seasysgl

Human CXCR3 cDNA (SEQ ID NO: 46)

atggagttgaggaagtacggccctggaagactggcggggacagttatagg
aggagctgctcagagtaaatcacagactaaatcagactcaatcacaaaag
agttcctgccaggcctttacacagcccttcctccccgttcccgccctca

caggtgagtgaccaccaagtgctaaatgacgccgaggttgccgccctcct
ggagaacttcagctcttcctatgactatggagaaaacgagagtgactcgt
gctgtacctccccgccctgcccacaggacttcagcctgaacttcgaccgg
gccttcctgccagccctctacagcctcctctttctgctggggctgctggg
caacggcgcggtggcagccgtgctgctgagccggcggacagccctgagca
gcaccgacaccttcctgctccacctagctgtagcagacacgctgctggtg
ctgacactgccgctctgggcagtggacgctgccgtccagtgggtctttgg
ctctggcctctgcaaagtggcaggtgccctcttcaacatcaacttctacg
caggagccctcctgctggcctgcatcagctttgaccgctacctgaacata
gttcatgccacccagctctaccgccgggggccccccggcccgcgtgaccct
cacctgcctggctgtctgggggctctgcctgcttttcgccctcccagact
tcatcttcctgtcggcccaccacgacgagcgcctcaacgccacccactgc
caatacaacttcccacaggtgggccgcacggctctgcgggtgctgcagct
ggtggctggctttctgctgcccctgctggtcatggcctactgctatgccc
acatcctggccgtgctgctggtttccaggggccagcggcgcctgcgggcc
atgcggctggtggtggtggtcgtggtggcctttgccctctgctggacccc
ctatcacctggtggtgctggtggacatcctcatggacctgggcgctttgg
cccgcaactgtggccgagaaagcagggtagacgtggccaagtcggtcacc
tcaggcctgggctacatgcactgctgcctcaacccgctgctctatgcctt
tgtaggggtcaagttccgggagcggatgtggatgctgctcttgcgcctgg
gctgccccaaccagagagggctccagaggcagccatcgtcttcccgccgg
gattcatcctggtctgagacctcagaggcctcctactcgggcttgtga

The protein and cDNA sequences for mature human L-selectin are shown below.

Mature Human L-Selectin Protein (SEQ ID NO: 47)

df lahhgtdcwt yhysekpmnw qrarrfcrdn ytdlvaiqnk aeieylektl
pfsrsyywig irkiggiwtw vgtnksltee aenwgdgepn nkknkedcve iyikrnkdag
kwnddachkl kaalcytasc qpwscsghge cveiinnytc ncdvgyygpq cqfviqcepl
eapelgtmdc thplgnfsfs sqcafscseg tnltgieett cgpfgnwssp eptcqviqce
plsapdlgim ncshplasfs ftsactfics egteligkkk ticessgiws npspicqkld
ksfsmikegd ynplfipvav mvtafsglaf iiwlarrlkk gkkskrsmnd py

Human L-Selectin cDNA (SEQ ID NO: 48)

atgggctgcagaagaactagagaaggaccaagcaaagccatgatatttcc
atggaaatgtcagagcacccagagggacttatggaacatcttcaagttgt
gggggtggacaatgctctgttgtgatttcctggcacatcatggaaccgac
tgctggacttaccattattctgaaaaacccatgaactggcaaagggctag
aagattctgccgagacaattacacagatttagttgccatacaaaacaagg
cggaaattgagtatctggagaagactctgcctttcagtcgttcttactac
tggataggaatccggaagataggaggaatatggacgtgggtgggaaccaa
caaatctcttactgaagaagcagagaactggggagatggtgagcccaaca
acaagaagaacaaggaggactgcgtggagatctatatcaagagaaacaaa
gatgcaggcaaatggaacgatgacgcctgccacaaactaaaggcagccct
ctgttacacagcttcttgccagccctggtcatgcagtggccatggagaat
gtgtagaaatcatcaataattacacctgcaactgtgatgtggggtactat

gggccccagtgtcagtttgtgattcagtgtgagcctttggaggccccaga
gctgggtaccatggactgtactcacccttgggaaacttcagcttcagct
cacagtgtgccttcagctgctctgaaggaacaaacttaactgggattgaa
gaaaccacctgtggaccatttggaaactggtcatctccagaaccaacctg
tcaagtgattcagtgtgagcctctatcagcaccagatttggggatcatga
actgtagccatcccctggccagcttcagctttacctctgcatgtaccttc
atctgctcagaaggaactgagttaattgggaagaagaaaaccatttgtga
atcatctggaatctggtcaaatcctagtccaatatgtcaaaaattggaca
aaagtttctcaatgattaaggagggtgattataacccccctcttcattcca
gtggcagtcatggttactgcattctctgggttggcatttatcatttggct
ggcaaggagattaaaaaaaggcaagaaatccaagagaagtatgaatgacc
catattaa

The protein and cDNA sequences for mature human CXCR1 are shown below.

Mature Human CXCR1 Protein (SEQ ID NO: 49)

msnitdpqmw dfddlnftgm ppadedyspc xletetlnky vviiayalvf llslllgnslv
mlvilysrvg rsvtdvylln laladllfal tlpiwaaskv ngwifgtflc kvvsllkevn
fysgilllac isvdrylaiv hatrtltqkr hlvkfvclgc wglsmnlslp fflfrqayhp
nnsspvcyev lgndtakwrm vlrilphtfg fivplfvmlf cygftlrtlf kahmgqkhra
mrvifavvli fllcwlpynl vlladtlmrt qviqescerr nnigraldat eilgflhscl
npiiyafigq nfrhgflkil amhglvskef larhrvtsyt sssvnvssnl

Human CXCR1 cDNA (SEQ ID NO: 50)

atgtcaaatattacagatccacagatgtgggattttgatgatctaaattt
cactggcatgccacctgcagatgaagattacagcccctgtatgctagaaa
ctgagacactcaacaagtatgttgtgatcatcgcctatgccctagtgttc
ctgctgagcctgctgggaaactccctggtgatgctggtcatcttatacag
cagggtcggccgctccgtcactgatgtctacctgctgaacctggccttgg
ccgacctactctttgccctgaccttgcccatctgggccgcctccaaggtg
aatggctggatttttggcacattcctgtgcaaggtggtctcactcctgaa
ggaagtcaacttctacagtggcatcctgctgttggcctgcatcagtgtgg
accgttacctggccattgtccatgccacacgcacactgacccagaagcgt
cacttggtcaagtttgtttgtcttggctgctggggactgtctatgaatct
gtccctgcccttcttccttttccgccaggcttaccatccaaacaattcca
gtccagtttgctatgaggtcctgggaaatgacacagcaaaatggcggatg
gtgttgcggatcctgcctcacacctttggcttcatcgtgccgctgtttgt
catgctgttctgctatggattcaccctgcgtacactgtttaaggcccaca
tggggcagaagcaccgagccatgagggtcatctttgctgtcgtcctcatc
ttcctgctttgctggctgccctacaacctggtcctgctggcagacaccct
catgaggacccaggtgatccaggagagctgtgagcgccgcaacaacatcg
gccgggccctggatgccactgagattctgggatttctccatagctgcctc
aaccccatcatctacgccttcatcggccaaaattttcgccatggattcct
caagatcctggctatgcatggcctggtcagcaaggagttcttggcacgtc
atcgtgttacctcctacacttcttcgtctgtcaatgtctcttccaacctc
tga

The protein and cDNA sequences for mature human CXCR2 are shown below.

Mature Human CXCR2 Protein (SEQ ID NO: 51)

```
medfnmesds fedfwkgedl snysysstlp pflldaapce pesleinkyf vviiyalvfl
lsllgnslvm lvilysrvgr svtdvyllnl aladllfalt lpiwaaskvn gwifgtflck
vvsllkevnf ysgilllaci svdrylaivh atrtltqkry lvkficlsiw glslllalpv
llfrrtvyss nvspacyedm gnntanwrml lrilpqsfgf ivpllimlfc ygftlrtlfk
ahmgqkhram rvifavvlif llcwlpynlv lladtlmrtq viqetcerrn hidraldate
ilgilhscln pliyafigqk frhgllkila ihgliskdsl pkdsrpsfvg sssghtsttl
```

Human CXCR2 cDNA (SEQ ID NO: 52)

```
atggaagattttaacatggagagtgacagctttgaagatttctggaaagg
tgaagatcttagtaattacagttacagctctaccctgccccctttctac
tagatgccgccccatgtgaaccagaatccctggaaatcaacaagtatttt
gtggtcattatctatgccctggtattcctgctgagcctgctgggaaactc
cctcgtgatgctggtcatcttatacagcagggtcggccgctccgtcactg
atgtctacctgctgaacctagccttggccgacctactctttgccctgacc
ttgcccatctgggccgcctccaaggtgaatggctggatttttggcacatt
cctgtgcaaggtggtctcactcctgaaggaagtcaacttctatagtggca
tcctgctactggcctgcatcagtgtggaccgttacctggccattgtccat
gccacacgcacactgacccagaagcgctacttggtcaaattcatatgtct
cagcatctggggtctgtccttgctcctggccctgcctgtcttacttttcc
gaaggaccgtctactcatccaatgttagcccagcctgctatgaggacatg
ggcaacaatacagcaaactggcggatgctgttacggatcctgccccagtc
ctttggcttcatcgtgccactgctgatcatgctgttctgctacggattca
ccctgcgtacgctgtttaaggcccacatggggcagaagcaccgggccatg
cgggtcatctttgctgtcgtcctcatcttcctgctctgctggctgcccta
caacctggtcctgctggcagacaccctcatgaggacccaggtgatccagg
agacctgtgagcgccgcaatcacatcgaccgggctctggatgccaccgag
attctgggcatccttcacagctgcctcaacccccctcatctacgccttcat
tggccagaagtttcgccatggactcctcaagattctagctatacatggct
tgatcagcaaggactccctgcccaaagacagcaggccttcctttgttggc
tcttcttcagggcacacttccactactctctaa
```

The protein and cDNA sequences for mature human CX3CR1 are shown below.

Mature Human CX3CR1 Protein (SEQ ID NO: 53)

```
mdqfpesvte nfeyddlaea cyigdivvfg tvflsifysv ifaiglvgnl lvvfaltnsk
kpksvtdiyl lnlalsdllf vatlpfwthy linekglhna mckfttafff igffgsiffi
tvisidryla ivlaansmnn rtvqhgvtis lgvwaaailv aapqfmftkq keneclgdyp
evlqeiwpvl rnvetnflgf llpllimsyc yfriiqtlfs cknhkkakai klillvvivf
flfwtpynvm ifletlklyd ffpscdmrkd lrlalsvtet vafshcclnp liyafagekf
rrylyhlygk clavlcgrsv hvdfsssesq rsrhgsvlss nftyhtsdgd allll
```

Human CX3CR1 cDNA (SEQ ID NO: 54)

```
atggatcagttccctgaatcagtgacagaaaactttgagtacgatgattt
ggctgaggcctgttatattggggacatcgtggtctttgggactgtgttcc
tgtccatattctactccgtcatctttgccattggcctggtgggaaatttg
ttggtagtgtttgccctcaccaacagcaagaagcccaagagtgtcaccga
catttacctcctgaacctggccttgtctgatctgctgtttgtagccactt
tgcccttctggactcactatttgataaatgaaaagggcctccacaatgcc
atgtgcaaattcactaccgccttcttcttcatcggctttttggaagcat
attcttcatcaccgtcatcagcattgataggtacctggccatcgtcctgg
ccgccaactccatgaacaaccggaccgtgcagcatggcgtcaccatcagc
ctaggcgtctgggcagcagccattttggtggcagcaccccagttcatgtt
cacaaagcagaaagaaaatgaatgccttggtgactaccccgaggtcctcc
aggaaatctggcccgtgctccgcaatgtggaaacaaattttcttggcttc
ctactccccctgctcattatgagttattgctacttcagaatcatccagac
gctgttttcctgcaagaaccacaagaaagccaaagccattaaactgatcc
ttctggtggtcatcgtgtttttcctcttctggacaccctacaacgttatg
attttcctggagacgcttaagctctatgacttctttcccagttgtgacat
gaggaaggatctgaggctggccctcagtgtgactgagacggttgcattta
gccattgttgcctgaatcctctcatctatgcatttgctggggagaagttc
agaagataccttaccacctgtatgggaaatgcctggctgtcctgtgtgg
gcgctcagtccacgttgatttctcctcatctgaatcacaaaggagcaggc
atggaagtgttctgagcagcaattttacttaccacacgagtgatggagat
gcattgctccttctctga
```

The protein and cDNA sequences for mature human ChemR23 are shown below.

Mature Human ChemR23 Protein (SEQ ID NO: 55)

```
mrmededynt sisygdeypd yldsivvled lsplearvtr iflvvvysiv cflgilgngl
viiiatfkmk ktvnmvwfln lavadflfnv flpihityaa mdyhwvfgta mckisnflli
hnmftsvfll tiissdrcis vllpvwsqnh rsvrlaymac mviwvlaffl sspslvfrdt
anlhgkiscf nnfslstpgs sswpthsqmd pvgysrhmvv tvtrflcgfl vpvliitacy
ltivcklqrn rlaktkkpfk iivtiiitff lcwcpyhtln llelhhtamp gsvfslglpl
atalaiansc mnpilyvfmg qdfkkfkval fsrlvnalse dtghssypsh rsftkmssmn
ertsmneret gml
```

Human ChemR23 cDNA (SEQ ID NO: 56)

```
atgagaatggaggatgaagattacaacacttccatcagttacggtgatga
ataccctgattatttagactccattgtggttttggaggacttatccccct
tggaagccagggtgaccaggatcttcctggtggtggtctacagcatcgtc
tgcttcctcgggattctgggcaatggtctggtgatcatcattgccacctt
caagatgaagaagacagtgaacatggtctggttcctcaacctggcagtgg
cagatttcctgttcaacgtcttcctcccaatccatatcacctatgccgcc
atggactaccactgggttttcgggacagccatgtgcaagatcagcaactt
ccttctcatccacaacatgttcaccagcgtcttcctgctgaccatcatca
gctctgaccgctgcatctctgtgctcctccctgtctggtcccagaaccac
cgcagcgttcgcctggcttacatggcctgcatggtcatctgggtcctggc
```

tttcttcttgagttccccatctctcgtcttccgggacacagccaacctgc
atgggaaaatatcctgcttcaacaacttcagcctgtccacacctgggtct
tcctcgtggcccactcactcccaaatggaccctgtggggtatagccggca
catggtggtgactgtcacccgcttcctctgtggcttcctggtcccagtcc
tcatcatcacagcttgctacctcaccatcgtgtgcaaactgcagcgcaac
cgcctggccaagaccaagaagcccttcaagattattgtgaccatcatcat
taccttcttcctctgctggtgcccctaccacacactcaacctcctagagc
tccaccacactgccatgcctggctctgtcttcagcctgggtttgcccctg
gccactgcccttgccattgccaacagctgcatgaaccccattctgtatgt
tttcatgggtcaggacttcaagaagttcaaggtggccctcttctctcgcc
tggtcaatgctctaagtgaagatacaggccactcttcctaccccagccat
agaagctttaccaagatgtcatcaatgaatgagaggacttctatgaatga
gagggagaccggcatgctttga

The protein and cDNA sequences for mature human CXCR4 are shown below.

Mature Human CXCR4 Protein (SEQ ID NO: 57)

megisiytsd nyteemgsgd ydsmkepcfr eenanfnkif lptiysiifl tgivgnglvi
lvmgyqkklr smtdkyrlhl svadllfvit lpfwavdava nwyfgnflck avhviytvnl
yssvlilafi sldrylaivh atnsqrprkl laekvvyvgv wipallltip dfifanvsea
ddryicdrfy pndlwvvvfq fqhimvglil pgivilscyc iiisklshsk ghqkrkalkt
tvililaffa cwlpyyigis idsfilleii kqgcefentv hkwisiteal affhcclnpi
lyaflgakfk tsaqhaltsv srgsslkils kgkrgghssv stesesssfh ss

Human CXCR4 cDNA (SEQ ID NO: 58)

atgtccattcctttgcctcttttgcagatatacacttcagataactacac
cgaggaaatgggctcaggggactatgactccatgaaggaaccctgtttcc
gtgaagaaaatgctaatttcaataaaatcttcctgcccaccatctactcc
atcatcttcttaactggcattgtgggcaatggattggtcatcctggtcat
gggttaccagaagaaactgagaagcatgacggacaagtacaggctgcacc
tgtcagtggccgacctcctctttgtcatcacgcttcccttctgggcagtt
gatgccgtggcaaactggtactttgggaacttcctatgcaaggcagtcca
tgtcatctacacagtcaacctctacagcagtgtcctcatcctggccttca
tcagtctggaccgctacctggccatcgtccacgccaccaacagtcagagg
ccaaggaagctgttggctgaaaaggtggtctatgttggcgtctggatccc
tgccctcctgctgactattcccgacttcatctttgccaacgtcagtgagg
cagatgacagatatatctgtgaccgcttctaccccaatgacttgtgggtg
gttgtgttccagtttcagcacatcatggttggccttatcctgcctggtat
tgtcatcctgtcctgctattgcattatcatctccaagctgtcacactcca
agggccaccagaagcgcaaggccctcaagaccacagtcatcctcatcctg
gctttcttcgcctgttggctgccttactacattgggatcagcatcgactc
cttcatcctcctggaaatcatcaagcaagggtgtgagtttgagaacactg
tgcacaagtggatttccatcaccgaggccctagctttcttccactgttgt
ctgaaccccatcctctatgctttccttggagccaaatttaaaacctctgc
ccagcacgcactcacctctgtgagcagagggtccagcctcaagatcctct
ccaaaggaaagcgaggtggacattcatctgtttccactgagtctgagtct

tcaagttttcactccagctaa

The protein and cDNA sequences for mature human CCR5 are shown below.

Mature Human CCR5 Protein (SEQ ID NO: 59)

mdyqvsspiy dinyytsepc qkinvkqiaa rllpplyslv fifgfvgnml vililinckr
lksmtdiyll nlaisdlffl ltvpfwahya aaqwdfgntm cqlltglyfi gffsgiffii
lltidrylav vhavfalkar tvtfgvvtsv itwvvavfas lpgiiftrsq keglhytcss
hfpysqyqfw knfqtlkivi lglvlpllvm vicysgilkt llrcrnekkr hravrlifti
mivyflfwap ynivlllntf qeffglnncs ssnrldqamq vtetlgmthc cinpiiyafv
gekfrnyllv ffqkhiakrf ckccsifqqe aperassvyt rstgeqeisv gl

Human CCR5 cDNA (SEQ ID NO: 60)

atggattatcaagtgtcaagtccaatctatgacatcaattattatacatc
ggagccctgccaaaaaatcaatgtgaagcaaatcgcagcccgcctcctgc
ctccgctctactcactggtgttcatctttggttttgtgggcaacatgctg
gtcatcctcatcctgataaactgcaaaaggctgaagagcatgactgacat
ctacctgctcaacctggccatctctgacctgtttttccttcttactgtcc
ccttctgggctcactatgctgccgcccagtgggactttggaaatacaatg
tgtcaactcttgacagggctctattttataggcttcttctctggaatctt
cttcatcatcctcctgacaatcgataggtacctggctgtcgtccatgctg
tgtttgctttaaaagccaggacggtcacctttggggtggtgacaagtgtg
atcacttgggtggtggctgtgtttgcgtctctcccaggaatcatctttac
cagatctcaaaaagaaggtcttcattacacctgcagctctcattttccat
acagtcagtatcaattctggaagaatttccagacattaaagatagtcatc
ttggggctggtcctgccgctgcttgtcatggtcatctgctactcgggaat
cctaaaaactctgcttcggtgtcgaaatgagaagaagaggcacagggctg
tgaggcttatcttcaccatcatgattgtttattttctcttctgggctccc
tacaacattgtccttctcctgaacaccttccaggaattctttggcctgaa
taattgcagtagctctaacaggttggaccaagctatgcaggtgacagaga
ctcttgggatgacgcactgctgcatcaaccccatcatctatgcctttgtc
ggggagaagttcagaaactacctcttagtcttcttccaaaagcacattgc
caaacgcttctgcaaatgctgttctattttccagcaagaggctcccgagc
gagcaagctcagtttacacccgatccactggggagcaggaaatatctgtg
ggcttgtga

The protein and cDNA sequences for mature human S1P5 are shown below.

Mature Human S1P5 Protein (SEQ ID NO: 61)

mesgllrpap vsevivlhyn ytgklrgary qpgaglrada vvclavcafi vlenlavllv
lgrhprfhap mflllgsltl sdllagaaya anillsgplt lklspalwfa reggvfvalt
asvlsllaia lersltmarr gpapvssrgr tlamaaaawg vslllgllpa lgwnclgrld
acstvlplya kayvlfcvla fvgilaaica lyariycqvr anarrlparp gtagttstra
rrkprslall rtlsvvllaf vacwgplfll llldvacpar tcpvllqadp flglamansl

lnpiiytltn rdlrhallrl vccgrhscgr dpsgsqqsas aaeasgglrr clppgldgsf
sgsersspqr dgldtsgstg spgaptaart lvsepaad

Human S1P5 cDNA (SEQ ID NO: 62)

atggagtcggggctgctgcggccggcgccggtgagcgaggtcatcgtcct
gcattacaactacaccggcaagctccgcggtgcgcgctaccagccgggtg
ccggcctgcgcgccgacgccgtggtgtgcctggcggtgtgcgccttcatc
gtgctagagaatctagccgtgttgttggtgctcggacgccacccgcgctt
ccacgctcccatgttcctgctcctgggcagcctcacgttgtcggatctgc
tggcaggcgccgcctacgccgccaacatcctactgtcggggccgctcacg
ctgaaactgtcccccgcgctctggttcgcacgggagggaggcgtcttcgt
ggcactcactgcgtccgtgctgagcctcctggccatcgcgctggagcgca
gcctcaccatggcgcgcaggggcccgcgcccgtctccagtcgggggcgc
acgctggcgatggcagccgcggcctggggcgtgtcgctgctcctcgggct
cctgccagcgctgggctggaattgcctgggtcgcctggacgcttgctcca
ctgtcttgccgctctacgccaaggcctacgtgctcttctgcgtgctcgcc
ttcgtgggcatcctggccgctatctgtgcactctacgcgcgcatctactg
ccaggtacgcgccaacgcgcggcgcctgccggcacggcccgggactgcgg
ggaccacctcgacccgggcgcgtcgcaagccgcgctcgctggccttgctg
cgcacgctcagcgtggtgctcctggcctttgtggcatgttggggccccct
cttcctgctgctgttgctcgacgtggcgtgcccggcgcgcacctgtcctg
tactcctgcaggccgatcccttcctgggactggccatggccaactcactt
ctgaaccccatcatctacacgctcaccaaccgcgacctgcgccacgcgct
cctgcgcctggtctgctgcggacgccactcctgcggcagagacccgagtg
gctcccagcagtcggcgagcgcggctgaggcttccgggggcctgcgccgc
tgcctgcccccgggccttgatgggagcttcagcggctcggagcgctcatc
gccccagcgcgacgggctggacaccagcggctccacaggcagccccggtg
cacccacagccgcccggactctggtatcagaaccggctgcagactga

The protein and cDNA sequences for mature human C-kit are shown below.

Mature Human C-kit Protein (SEQ ID NO: 63)

qpsvs pgepsppsih pgksdlivrv gdeirllctd pgfvkwtfei ldetnenkqn
ewitekaeat ntgkytctnk hglsnsiyvf vrdpaklflv drslygkedn dtlvrcpltd
pevtnyslkg cqgkplpkdl rfipdpkagi miksvkrayh rlclhcsvdq egksvlsekf
ilkvrpafka vpvvsvskas yllregeeft vtctikdvss svystwkren sqtklqekyn
swhhgdfnye rqatltissa rvndsgvfmc yanntfgsan vtttlevvdk gfinifpmin
ttvfvndgen vdliveyeaf pkpehqqwiy mnrtftdkwe dypksenesn iryvselhlt
rlkgteggty tflvsnsdvn aaiafnvyvn tkpeiltydr lvngmlqcva agfpeptidw
yfcpgteqrc sasvlpvdvq tlnssgppfg klvvqssids safkhngtve ckayndvgkt
sayfnfafkg nnkeqihpht lftplligfv ivagmmciiv miltykylqk pmyevqwkvv
eeingnnyvy idptqlpydh kwefprnrls fgktlgagaf gkvveatayg liksdaamtv
avkmlkpsah lterealmse lkvlsylgnh mnivnllgac tiggptlvit eyccygdlln
flrrkrdsfi cskqedhaea alyknllhsk esscsdstne ymdmkpgvsy vvptkadkrr
svrigsyier dvtpaimedd elaldledll sfsyqvakgm aflaskncih rdlaarnill

thgritkicd fglardiknd snyvvkgnar lpvkwmapes ifncvytfes dvwsygiflw
elfslgsspy pgmpvdskfy kmikegfrml spehapaemy dimktcwdad plkrptfkqi
vqliekqise stnhiysnla ncspnrqkpv vdhsvrinsv gstasssqpl lvhddv

Human C-kit cDNA (SEQ ID NO: 64)

atgagaggcgctcgcggcgcctgggattttctctgcgttctgctcctact
gcttcgcgtccagacaggctcttctcaaccatctgtgagtccaggggaac
cgtctccaccatccatccatccaggaaaatcagacttaatagtccgcgtg
ggcgacgagattaggctgttatgcactgatccgggctttgtcaaatggac
ttttgagatcctggatgaaacgaatgagaataagcagaatgaatggatca
cggaaaaggcagaagccaccaacaccggcaaatacacgtgcaccaacaaa
cacggcttaagcaattccatttatgtgtttgttagagatcctgccaagct
tttccttgttgaccgctccttgtatgggaaagaagacaacgacacgctgg
tccgctgtcctctcacagacccagaagtgaccaattattccctcaagggg
tgccaggggaagcctcttcccaaggacttgaggtttattcctgaccccaa
ggcgggcatcatgatcaaaagtgtgaaacgcgcctaccatcggctctgtc
tgcattgttctgtggaccaggagggcaagtcagtgctgtcggaaaaattc
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caaagcaagctatcttcttagggaaggggaagaattcacagtgacgtgca
caataaaagatgtgtctagttctgtgtactcaacgtggaaaagagaaaac
agtcagactaaactacaggagaaatataatagctggcatcacggtgactt
caattatgaacgtcaggcaacgttgactatcagttcagcgagagttaatg
attctggagtgttcatgtgttatgccaataatacttttggatcagcaaat
gtcacaacaaccttggaagtagtagataaaggattcattaatatcttccc
catgataaacactacagtatttgtaaacgatggagaaaatgtagatttga
ttgttgaatatgaagcattccccaaacctgaacaccagcagtggatctat
atgaacagaaccttcactgataaatgggaagattatcccaagtctgagaa
tgaaagtaatatcagatacgtaagtgaacttcatctaacgagattaaaag
gcaccgaaggaggcacttacacattcctagtgtccaattctgacgtcaat
gctgccatagcatttaatgtttatgtgaatacaaaaccagaaatcctgac
ttacgacaggctcgtgaatggcatgctccaatgtgtggcagcaggattcc
cagagcccacaatagattggtatttttgtccaggaactgagcagagatgc
tctgcttctgtactgccagtggatgtgcagacactaaactcatctgggcc
accgtttggaaagctagtggttcagagttctatagattctagtgcattca
agcacaatggcacggttgaatgtaaggcttacaacgatgtgggcaagact
tctgcctattttaactttgcatttaaaggtaacaacaaagagcaaatcca
tccccacaccctgttcactcctttgctgattggtttcgtaatcgtagctg
gcatgatgtgcattattgtgatgattctgacctacaaatatttacagaaa
cccatgtatgaagtacagtggaaggttgttgaggagataaatggaaacaa
ttatgtttacatagacccaacacaacttccttatgatcacaaatgggagt
ttcccagaaacaggctgagttttgggaaaaccctgggtgctggagctttc
gggaaggttgttgaggcaactgcttatggcttaattaagtcagatgcggc
catgactgtcgctgtaaagatgctcaagccgagtgcccatttgacagaac
gggaagccctcatgtctgaactcaaagtcctgagttaccttggtaatcac
atgaatattgtgaatctacttggagcctgcaccattggagggcccaccct
ggtcattacagaatattgttgctatggtgatcttttgaatttttgagaa
gaaaacgtgattcatttatttgttcaaagcaggaagatcatgcagaagct

gcactttataagaatcttctgcattcaaaggagtcttcctgcagcgatag
tactaatgagtacatggacatgaaacctggagtttcttatgttgtcccaa
ccaaggccgacaaaaggagatctgtgagaataggctcatacatagaaaga
gatgtgactcccgccatcatggaggatgacgagttggccctagacttaga
agacttgctgagcttttcttaccaggtggcaaagggcatggctttcctcg
cctccaagaattgtattcacagagacttggcagccagaaatatcctcctt
actcatggtcggatcacaaagatttgtgattttggtctagccagagacat
caagaatgattctaattatgtggttaaaggaaacgctcgactacctgtga
agtggatggcacctgaaagcattttcaactgtgtatacacgtttgaaagt
gacgtctggtcctatgggatttttctttgggagctgttctctttaggaag
cagcccctatcctggaatgccggtcgattctaagttctacaagatgatca
aggaaggcttccggatgctcagccctgaacacgcacctgctgaaatgtat
gacataatgaagacttgctgggatgcagatcccctaaaaagaccaacatt
caagcaaattgttcagctaattgagaagcagatttcagagagcaccaatc
atatttactccaacttagcaaactgcagccccaaccgacagaagcccgtg
gtagaccattctgtgcggatcaattctgtcggcagcaccgcttcctcctc
ccagcctctgcttgtgcacgacgatgtctga

The protein and cDNA sequences for mature human mTOR are shown below.

Mature Human mTOR Protein (SEQ ID NO: 65)

mlgtgpaaat taattssnvs vlqqfasglk srneetraka akelqhyvtm elremsqees
trfydqlnhh ifelvsssda nerkggilai asligveggn atrigrfany lrnllpsndp
vvmemaskai grlamagdtf taeyvefevk ralewlgadr negrrhaavl vlrelaisvp
tfffqqvqpf fdnifvavwd pkqairegav aalraclilt tqrepkemqk pqwyrhtfee
aekgfdetla kekgmnrddr ihgallilne lvrissmege rlreemeeit qqqlvhdkyc
kdlmgfgtkp rhitpftsfq avqpqqsnal vgllgysshq glmgfgtsps pakstlvesr
ccrdlmeekf dqvcqwvlkc rnsknsliqm tilnllprla afrpsaftdt qylqdtmnhv
lscvkkeker taafqalgll svavrsefkv ylprvldiir aalppkdfah krqkamqvda
tvftcismla ramgpgiqqd ikellepmla vglspaltav lydlsrqipq lkkdiqdgll
kmlslvlmhk plrhpgmpkg lahqlaspgl ttlpeasdvg sitlalrtlg sfefeghslt
qfvrhcadhf lnsehkeirm eaartcsrll tpsihlisgh ahvvsqtavq vvadvlskll
vvgitdpdpd irycvlasld erfdahlaqa enlqalfval ndqvfeirel aictvgrlss
mnpafvmpfl rkmliqilte lehsgigrik eqsarmlghl vsnaprlirp ymepilkali
lklkdpdpdp npgvinnvla tigelaqvsg lemrkwvdel fiiimdmlqd ssllakrqva
lwtlgqlvas tgyvvepyrk yptllevlln flkteqnqgt rreairvlgl lgaldpykhk
vnigmidqsr dasavslses kssqdssdys tsemlvnmgn lpldefypav smvalmrifr
dqslshhhtm vvqaitfifk slglkcvqfl pqvmptflnv irvcdgaire flfqqlgmlv
sfvkshirpy mdeivtlmre fwvmntsiqs tiillieqiv valggefkly lpqliphmlr
vfmhdnspgr ivsikllaai qlfganlddy lhlllppivk lfdapeaplp srkaaletvd
rltesldftd yasriihpiv rtldqspelr stamdtlssl vfqlgkkyqi fipmvnkvlv
rhrinhqryd vlicrivkgy tladeeedpl iyqhrmlrsg qgdalasgpv etgpmkklhv
stinlqkawg aarrvskddw lewlrrlsle llkdssspsl rscwalaqay npmardlfna
afvscwseln edqqdelirs ielaltsqdi aevtqtllnl aefmehsdkg plplrddngi
vllgeraakc rayakalhyk elefqkgptp aileslisin nklqqpeaaa gvleyamkhf
geleiqatwy eklhewedal vaydkkmdtn kddpelmlgr mrclealgew gqlhqqccek
wtlvndetqa kmarmaaaaa wglgqwdsme eytcmiprdt hdgafyravl alhqdlfsla

qqcidkardl ldaeltamag esysraygam vschmlsele eviqyklvpe rreiirqiww
erlqgcqriv edwqkilmvr slvvsphedm rtwlkyaslc gksgrlalah ktlvlllgvd
psrqldhplp tvhpqvtyay mknmwksark idafqhmqhf vqtmqqqaqh aiatedqqhk
qelhklmarc flklgewqln lqginestip kvlqyysaat ehdrswykaw hawavmnfea
vlhykhqnqa rdekkklrha sganitnatt aattaatatt tastegsnse seaestensp
tpsplqkkvt edlsktllmy tvpavqgffr sislsrgnnl qdtlrvltlw fdyghwpdvn
ealvegvkai qidtwlqvip qliaridtpr plvgrlihql ltdigryhpq aliypltvas
kstttarhna ankilknmce hsntlvqqam mvseelirva ilwhemwheg leeasrlyfg
ernvkgmfev leplhammer gpqtlketsf nqaygrdlme aqewcrkymk sgnvkdltqa
wdlyyhvfrr iskqlpqlts lelqyvspkl lmcrdlelav pgtydpnqpi iriqsiapsl
qvitskqrpr kltlmgsngh efvfllkghe dlrqdervmq lfglvntlla ndptslrknl
siqryavipl stnsgligwv phcdtlhali rdyrekkkil lniehrimlr mapdydhltl
mqkvevfeha vnntagddla kllwlkspss evwfdrrtny trslavmsmv gyilglgdrh
psnlmldrls gkilhidfgd cfevamtrek fpekipfrlt rmltnamevt gldgnyritc
htvmevlreh kdsvmavlea fvydpllnwr lmdtntkgnk rsrtrtdsys agqsveildg
velgepahkk tgttvpesih sfigdglvkp ealnkkaiqi inrvrdkltg rdfshddtld
vptqvellik qatshenlcq cyigwcpfw

Human mTOR cDNA (SEQ ID NO: 66)

atgcttgga accggacctg ccgccgccac caccgctgcc accacatcta gcaatgtgag
cgtcctgcag cagtttgcca gtggcctaaa gagccggaat gaggaaacca gggccaaagc
cgccaaggag ctccagcact atgtcaccat ggaactccga gagatgagtc aagaggagtc
tactcgcttc tatgaccaac tgaaccatca ctttttgaa ttggtttcca gctcagatgc
caatgagagg aaaggtggca tcttggccat agctagcctc ataggagtgg aaggtgggaa
tgccacccga attggcagat ttgccaacta tcttcggaac ctcctcccct ccaatgaccc
agttgtcatg gaaatggcat ccaaggccat tggccgtctt gccatggcag gggacacttt
taccgctgag tacgtggaat ttgaggtgaa gcgagccctg gaatggctgg gtgctgaccg
caatgagggc cggagacatg cagctgtcct ggttctccgt gagctggcca tcagcgtccc
taccttcttc ttccagcaag tgcaaccctt ctttgacaac atttttgtgg ccgtgtggga
ccccaaacag gccatccgtg agggagctgt agccgccctt cgtgcctgtc tgattctcac
aacccagcgt gagccgaagg agatgcagaa gcctcagtgg tacaggcaca catttgaaga
agcagagaag ggatttgatg agaccttggc caaagagaag ggcatgaatc gggatgatcg
gatccatgga gccttgttga tccttaacga gctggtccga atcagcagca tggagggaga
gcgtctgaga gaagaaatgg aagaaatcac acagcagcag ctggtacacg acaagtactg
caaagatctc atgggcttcg gaacaaaacc tcgtcacatt acccccttca ccagtttcca
ggctgtacag ccccagcagt caaatgcctt ggtggggctg ctggggtaca gctctcacca
aggcctcatg ggatttggga cctcccccag tccagctaag tccaccctgg tggagagccg
gtgttgcaga gacttgatgg aggagaaatt tgatcaggtg tgccagtggg tgctgaaatg
caggaatagc aagaactcgc tgatccaaat gacaatcctt aatttgttgc cccgcttggc
tgcattccga ccttctgcct tcacagatac ccagtatctc caagatacca tgaaccatgt
cctaagctgt gtcaagaagg agaaggaacg tacagcggcc ttccaagccc tggggctact
ttctgtggct gtgaggtctg agtttaaggt ctatttgcct cgcgtgctgg acatcatccg

agcggccctg cccccaaagg acttcgccca taagaggcag aaggcaatgc aggtggatgc
cacagtcttc acttgcatca gcatgctggc tcgagcaatg gggccaggca tccagcagga
tatcaaggag ctgctggagc ccatgctggc agtgggacta agccctgccc tcactgcagt
gctctacgac ctgagccgtc agattccaca gctaaagaag gacattcaag atgggctact
gaaaatgctg tccctggtcc ttatgcacaa acccccttcgc cacccaggca tgcccaaggg
cctggcccat cagctggcct ctcctggcct cacgaccctc cctgaggcca gcgatgtggg
cagcatcact cttgccctcc gaacgcttgg cagctttgaa tttgaaggcc actctctgac
ccaatttgtt cgccactgtg cggatcattt cctgaacagt gagcacaagg agatccgcat
ggaggctgcc cgcacctgct cccgcctgct cacaccctcc atccacctca tcagtggcca
tgctcatgtg gttagccaga ccgcagtgca agtggtggca gatgtgctta gcaaactgct
cgtagttggg ataacagatc ctgaccctga cattcgctac tgtgtcttgg cgtccctgga
cgagcgcttt gatgcacacc tggcccaggc ggagaacttg caggccttgt ttgtggctct
gaatgaccag gtgtttgaga tccgggagct ggccatctgc actgtgggcc gactcagtag
catgaaccct gcctttgtca tgcctttcct gcgcaagatg ctcatccaga ttttgacaga
gttggagcac agtgggattg gaagaatcaa agagcagagt gcccgcatgc tggggcacct
ggtctccaat gccccccgac tcatccgccc ctacatggag cctattctga aggcattaat
tttgaaactg aaagatccag accctgatcc aaacccaggt gtgatcaata atgtcctggc
aacaatagga gaattggcac aggttagtgg cctggaaatg aggaaatggg ttgatgaact
ttttattatc atcatggaca tgctccagga ttcctctttg ttggccaaaa ggcaggtggc
tctgtggacc ctgggacagt tggtggccag cactggctat gtagtagagc cctacaggaa
gtaccctact ttgcttgagg tgctactgaa ttttctgaag actgagcaga accagggtac
acgcagagag gccatccgtg tgttagggct tttaggggct ttggatcctt acaagcacaa
agtgaacatt ggcatgatag accagtcccg ggatgcctct gctgtcagcc tgtcagaatc
caagtcaagt caggattcct ctgactatag cactagtgaa atgctggtca acatgggaaa
cttgcctctg gatgagttct acccagctgt gtccatggtg gccctgatgc ggatcttccg
agaccagtca ctctctcatc atcacaccat ggttgtccag gccatcacct tcatcttcaa
gtccctggga ctcaaatgtg tgcagttcct gccccaggtc atgcccacgt tccttaacgt
cattcgagtc tgtgatgggg ccatccggga atttttgttc cagcagctgg gaatgttggt
gtcctttgtg aagagccaca tcagacctta tatggatgaa atagtcaccc tcatgagaga
attctgggtc atgaacacct caattcagag cacgatcatt cttctcattg agcaaattgt
ggtagctctt gggggtgaat ttaagctcta cctgccccag ctgatcccac acatgctgcg
tgtcttcatg catgacaaca gcccaggccg cattgtctct atcaagttac tggctgcaat
ccagctgttt ggcgccaacc tggatgacta cctgcattta ctgctgcctc ctattgttaa
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ccgcctgacg gagtccctgg atttcactga ctatgcctcc cggatcattc accctattgt
tcgaacactg gaccagagcc cagaactgcg ctccacagcc atggacacgc tgtcttcact
tgtttttcag ctggggaaga agtaccaaat tttcattcca atggtgaata aagttctggt
gcgacaccga atcaatcatc agcgctatga tgtgctcatc tgcagaattg tcaagggata
cacacttgct gatgaagagg aggatccttt gatttaccag catcggatgc ttaggagtgg
ccaaggggat gcattggcta gtggaccagt ggaaacagga cccatgaaga aactgcacgt

cagcaccatc aacctccaaa aggcctgggg cgctgccagg agggtctcca aagatgactg
gctggaatgg ctgagacggc tgagcctgga gctgctgaag gactcatcat cgccctccct
gcgctcctgc tgggccctgg cacaggccta caacccgatg gccagggatc tcttcaatgc
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catcgagttg gccctcacct cacaagacat cgctgaagtc acacagaccc tcttaaactt
ggctgaattc atggaacaca gtgacaaggg ccccctgcca ctgagagatg acaatggcat
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agaactggag ttccagaaag gccccacccc tgccattcta gaatctctca tcagcattaa
taataagcta cagcagccgg aggcagcggc cggagtgtta gaatatgcca tgaaacactt
tggagagctg gagatccagg ctacctggta tgagaaactg cacgagtggg aggatgccct
tgtggcctat gacaagaaaa tggacaccaa caaggacgac ccagagctga tgctgggccg
catgcgctgc ctcgaggcct tgggggaatg gggtcaactc caccagcagt gctgtgaaaa
gtggaccctg gttaatgatg agacccaagc caagatggcc cggatggctg ctgcagctgc
atggggttta ggtcagtggg acagcatgga agaatacacc tgtatgatcc ctcgggacac
ccatgatggg gcattttata gagctgtgct ggcactgcat caggacctct tctccttggc
acaacagtgc attgacaagg ccagggacct gctggatgct gaattaactg cgatggcagg
agagagttac agtcgggcat atggggccat ggtttcttgc cacatgctgt ccgagctgga
ggaggttatc cagtacaaac ttgtccccga gcgacgagag atcatccgcc agatctggtg
ggagagactg cagggctgcc agcgtatcgt agaggactgg cagaaaatcc ttatggtgcg
gtcccttgtg gtcagccctc atgaagacat gagaacctgg ctcaagtatg caagcctgtg
cggcaagagt ggcaggctgg ctcttgctca taaaacttta gtgttgctcc tgggagttga
tccgtctcgg caacttgacc atcctctgcc aacagttcac cctcaggtga cctatgccta
catgaaaaac atgtggaaga gtgcccgcaa gatcgatgcc ttccagcaca tgcagcattt
tgtccagacc atgcagcaac aggcccagca tgccatcgct actgaggacc agcagcataa
gcaggaactg cacaagctca tggcccgatg cttcctgaaa cttggagagt ggcagctgaa
tctacagggc atcaatgaga gcacaatccc caaagtgctg cagtactaca gcgccgccac
agagcacgac cgcagctggt acaaggcctg gcatgcgtgg gcagtgatga acttcgaagc
tgtgctacac tacaaacatc agaaccaagc ccgcgatgag aagaagaaac tgcgtcatgc
cagcggggcc aacatcacca acgccaccac tgccgccacc acggccgcca ctgccaccac
cactgccagc accgagggca gcaacagtga gagcgaggcc gagagcaccg agaacagccc
caccccatcg ccgctgcaga agaaggtcac tgaggatctg tccaaaaccc tcctgatgta
cacggtgcct gccgtccagg gcttcttccg ttccatctcc ttgtcacgag gcaacaacct
ccaggataca ctcagagttc tcaccttatg gtttgattat ggtcactggc cagatgtcaa
tgaggcctta gtggaggggg tgaaagccat ccagattgat acctggctac aggttatacc
tcagctcatt gcaagaattg atacgcccag acccttggtg ggacgtctca ttcaccagct
tctcacagac attggtcggt accaccccca ggccctcatc tacccactga cagtggcttc
taagtctacc acgacagccc ggcacaatgc agccaacaag attctgaaga acatgtgtga
gcacagcaac accctggtcc agcaggccat gatggtgagc gaggagctga tccgagtggc
catcctctgg catgagatgt ggcatgaagg cctggaagag gcatctcgtt tgtactttgg
ggaaaggaac gtgaaaggca tgtttgaggt gctggagccc ttgcatgcta tgatggaacg

gggccccccag actctgaagg aaacatcctt taatcaggcc tatggtcgag atttaatgga
ggcccaagag tggtgcagga agtacatgaa atcagggaat gtcaaggacc tcacccaagc
ctgggacctc tattatcatg tgttccgacg aatctcaaag cagctgcctc agctcacatc
cttagagctg caatatgttt ccccaaaact tctgatgtgc cgggaccttg aattggctgt
gccaggaaca tatgacccca accagccaat cattcgcatt cagtccatag caccgtcttt
gcaagtcatc acatccaagc agaggccccg gaaattgaca cttatgggca gcaacggaca
tgagtttgtt ttccttctaa aaggccatga agatctgcgc caggatgagc gtgtgatgca
gctcttcggc ctggttaaca cccttctggc caatgaccca acatctcttc ggaaaaacct
cagcatccag agatacgctg tcatcccttt atcgaccaac tcgggcctca ttggctgggt
tccccactgt gacacactgc acgccctcat ccgggactac agggagaaga agaagatcct
tctcaacatc gagcatcgca tcatgttgcg gatggctccg gactatgacc acttgactct
gatgcagaag gtggaggtgt ttgagcatgc cgtcaataat acagctgggg acgacctggc
caagctgctg tggctgaaaa gccccagctc cgaggtgtgg tttgaccgaa gaaccaatta
tacccgttct ttagcggtca tgtcaatggt tgggtatatt ttaggcctgg gagatagaca
cccatccaac ctgatgctgg accgtctgag tgggaagatc ctgcacattg actttgggga
ctgctttgag gttgctatga cccgagagaa gtttccagag aagattccat ttagactaac
aagaatgttg accaatgcta tggaggttac aggcctggat ggcaactaca gaatcacatg
ccacacagtg atggaggtgc tgcgagagca caaggacagt gtcatggccg tgctggaagc
ctttgtctat gacccttgc tgaactggag gctgatggac acaaatacca aaggcaacaa
gcgatcccga acgaggacgg attcctactc tgctggccag tcagtcgaaa ttttggacgg
tgtggaactt ggagagccag cccataagaa aacggggacc acagtgccag aatctattca
ttctttcatt ggagacggtt tggtgaaacc agaggcccta aataagaaag ctatccagat
tattaacagg gttcgagata agctcactgg tcgggacttc tctcatgatg acactttgga
tgttccaacg caagttgagc tgctcatcaa acaagcgaca tcccatgaaa acctctgcca
gtgctatatt ggctggtgcc ctttctggta a

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of SREBP1 are available from Novus Biologicals and Abcam. The protein and cDNA sequences for mature human SREBP1 are shown below.

Mature Human SREBP1 Protein (SEQ ID NO: 67)

MDEPPFSEAALEQALGEPCDLDAALLTDIEDMLQLINNQDSDFPGLFDPPYAGSG
AGGTDPASPDTSSPGSLSPPPATLSSSLEAFLSGPQAAPSPLSPPQPAPTPLKMYPS
MPAFSPGPGIKEESVPLSILQTPTPQPLPGALLPQSFPAPAPPQFSSTPVLGYPSPPG
GFSTGSPPGNTQQPLPGLPLASPPGVPPVSLHTQVQSVVPQQLLTVTAAPTAAPV
TTTVTSQIQQVPVLLQPHFIKADSLLLTAMKTDGATVKAAGLSPLVSGTTVQTGP

LPTLVSGGTILATVPLVVDAEKLPINRLAAGSKAPASAQSRGEKRTAHNAIEKRY
RSSINDKIIELKDLVVGTEAKLNKSAVLRKAIDYIRFLQHSNQKLKQENLSLRTAV
HKSKSLKDLVSACGSGGNTDVLMEGVKTEVEDTLTPPPSDAGSPFQSSPLSLGSR
GSGSGGSGSDSEPDSPVFEDSKAKPEQRPSLHSRGMLDRSRLALCTLVFLCLSCN
PLASLLGARGLPSPSDTTSVYHSPGRNVLGTESRDGPGWAQWLLPPVVWLLNGL
LVLVSLVLLFVYGEPVTRPHSGPAVYFWRHRKQADLDLARGDFAQAAQQLWLA
LRALGRPLPTSHLDLACSLLWNLIRHLLQRLWVGRWLAGRAGGLQQDCALRVD
ASASARDAALVYHKLHQLHTMGKHTGGHLTATNLALSALNLAECAGDAVSVA
TLAEIYVAAALRVKTSLPRALHFLTRFFLSSARQACLAQSGSVPPAMQWLCHPV
GHRFFVDGDWSVLSTPWESLYSLAGNPVDPLAQVTQLFREHLLERALNCVTQPN
PSPGSADGDKEFSDALGYLQLLNSCSDAAGAPAYSFSISSSMATTTGVDPVAKW
WASLTAVVIHWLRRDEEAAERLCPLVEHLPRVLQESERPLPRAALHSFKAARAL
LGCAKAESGPASLTICEKASGYLQDSLATTPASSSIDKAVQLFLCDLLLVVRTSL
WRQQQPPAPAPAAQGTSSRPQASALELRGFQRDLSSLRRLAQSFRPAMRRVFLH
EATARLMAGASPTRTHQLLDRSLRRRAGPGGKGGAVAELEPRPTRREHAEALLL
ASCYLPPGFLSAPGQRVGMLAEAARTLEKLGDRRLLHDCQQMLMRLGGGTTVT
SS

Human SREBP1 cDNA (SEQ ID NO: 68)

atggacgagccacccttcagcgaggcggctttggagcaggcgctgggcga
gccgtgcgatctggacgcggcgctgctgaccgacatcgaagacatgcttc
agcttatcaacaaccaagacagtgacttccctggcctatttgacccaccc
tatgctgggagtggggcaggggcacagaccctgccagccccgataccag
ctccccaggcagcttgtctccacctcctgccacattgagctcctctcttg
aagccttcctgagcgggccgcaggcagcgccctcacccctgtcccctccc
cagcctgcacccactccattgaagatgtacccgtccatgcccgctttctc
ccctgggcctggtatcaaggaagagtcagtgccactgagcatcctgcaga
cccccacccacagcccctgccagggccctcctgccacagagcttccca
gccccagccccaccgcagttcagctccacccctgtgttaggctaccccag
ccctccgggaggcttctctacaggaagccctcccgggaacacccagcagc
cgctgcctggcctgccactggcttccccgccaggggtcccgcccgtctcc
ttgcacacccaggtccagagtgtggtcccccagcagctactgacagtcac
agctgcccccacggcagcccctgtaacgaccactgtgacctcgcagatcc
agcaggtcccggtcctgctgcagccccacttcatcaaggcagactcgctg
cttctgacagccatgaagacagacggagccactgtgaaggcggcaggtct
cagtcccctggtctctggcaccactgtgcagacagggcctttgccgaccc
tggtgagtggcggaaccatcttggcaacagtcccactggtcgtagatgcg

gagaagctgcctatcaaccggctcgcagctggcagcaaggccccggcctc
tgcccagagccgtggagagaagcgcacagcccacaacgccattgagaagc
gctaccgctcctccatcaatgacaaaatcattgagctcaaggatctggtg
gtgggcactgaggcaaagctgaataaatctgctgtcttgcgcaaggccat
cgactacattcgctttctgcaacacagcaaccagaaactcaagcaggaga
acctaagtctgcgcactgctgtccacaaaagcaaatctctgaaggatctg
gtgtcggcctgtggcagtggagggaacacagacgtgctcatggagggcgt
gaagactgaggtggaggacacactgaccccacccccctcggatgctggct
caccttttccagagcagccccttgtcccttggcagcaggggcagtggcagc
ggtggcagtggcagtgactcggagcctgacagcccagtctttgaggacag
caaggcaaagccagagcagcggccgtctctgcacagccggggcatgctgg
accgctcccgcctggccctgtgcacgctcgtcttcctctgcctgtcctgc
aacccccttggcctccttgctgggggcccgggggcttcccagcccctcaga
taccaccagcgtctaccatagccctgggcgcaacgtgctgggcaccgaga
gcagagatggccctggctgggcccagtggctgctgcccccagtggtctgg
ctgctcaatgggctgttggtgctcgtctccttggtgcttctctttgtcta
cggtgagccagtcacacggccccactcaggccccgccgtgtacttctgga
ggcatcgcaagcaggctgacctggacctggcccggggagactttgcccag
gctgcccagcagctgtggctggccctgcgggcactgggccggcccctgcc
cacctcccacctggacctggcttgtagcctcctctggaacctcatccgtc
acctgctgcagcgtctctgggtgggccgctggctggcaggccgggcaggg
ggcctgcagcaggactgtgctctgcgagtggatgctagcgccagcgcccg
agacgcagccctggtctaccataagctgcaccagctgcacaccatgggga
agcacacaggcgggcacctcactgccaccaacctggcgctgagtgccctg
aacctggcagagtgtgcaggggatgccgtgtctgtggcgacgctggccga
gatctatgtggcggctgcattgagagtgaagaccagtctcccacgggcct
tgcattttctgacacgcttcttcctgagcagtgcccgccaggcctgcctg
gcacagagtggctcagtgcctcctgccatgcagtggctctgccaccccgt
gggccaccgtttcttcgtggatggggactggtccgtgctcagtaccccat
gggagagcctgtacagcttggccgggaacccagtggacccctggcccag
gtgactcagctattccgggaacatctcttagagcgagcactgaactgtgt
gacccagcccaaccccagccctgggtcagctgatggggacaaggaattct
cggatgccctcgggtacctgcagctgctgaacagctgttctgatgctgcg
ggggctcctgcctacagcttctccatcagttccagcatggccaccaccac
cggcgtagacccggtggccaagtggtgggcctctctgacagctgtggtga
tccactggctgcggcgggatgaggaggcggctgagcggctgtgcccgctg
gtggagcacctgccccgggtgctgcaggagtctgagagacccctgcccag
ggcagctctgcactccttcaaggctgcccgggccctgctgggctgtgcca
aggcagagtctggtccagccagcctgaccatctgtgagaaggccagtggg
tacctgcaggacagcctggctaccacaccagccagcagctccattgacaa
ggccgtgcagctgttcctgtgtgacctgcttcttgtggtgcgcaccagcc
tgtggcggcagcagcagcccccggcccccggccccagcagcccagggcacc
agcagcaggccccaggcttccgcccttgagctgcgtggcttccaacggga
cctgagcagcctgaggcggctggcacagagcttccggcccgccatgcgga
gggtgttcctacatgaggccacggcccggctgatggcgggggccagcccc
acacggacacaccagctcctcgaccgcagtctgaggcggcgggcaggccc
cggtggcaaaggaggcgcggtggcggagctggagccgcggcccacgcggc
gggagcacgcggaggccttgctgctggcctcctgctacctgccccccggc
ttcctgtcggcgcccgggcagcgcgtgggcatgctggctgaggcggcgcg

cacactcgagaagcttggcgatcgccggctgctgcacgactgtcagcaga
tgctcatgcgcctgggcggtgggaccactgtcacttccagctag

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of IFN-γ are available from R&D Systems, Thermo Fisher Scientific, Abcam, Enzo Life Sciences, and RayBiotech. The protein and cDNA sequences for mature human IFN-γ are shown below.

Mature Human IFN-γ (SEQ ID NO: 69)

qdpyvke aenlkkyfna ghsdvadngt lflgilknwk eesdrkimqs qivsfyfklf
knfkddqsiq ksvetikedm nvkffnsnkk krddfekltn ysvtdlnvqr kaiheliqvm
aelspaaktg krkrsqmlfr g

Human IFN-γ cDNA (SEQ ID NO: 70)

caggac ccatatgtaa aagaagcaga aaaccttaag aaatatttta atgcaggtca
ttcagatgta gcggataatg gaactctttt cttaggcatt ttgaagaatt ggaaagagga
gagtgacaga aaaataatgc agagccaaat tgtctccttt tacttcaaac tttttaaaaa
ctttaaagat gaccagagca tccaaaagag tgtggagacc atcaaggaag acatgaatgt
caagtttttc aatagcaaca aaaagaaacg agatgacttc gaaaagctga ctaattattc
ggtaactgac ttgaatgtcc aacgcaaagc aatacatgaa ctcatccaag tgatggctga
actgtcgcca gcagctaaaa cagggaagcg aaaaaggagt cagatgctgt ttcgaggt

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of granzyme B are available from RayBiotech, Thermo Fisher Scientific, and R&D Systems. The protein and cDNA sequences for mature human granzyme B are shown below.

Mature Human Granzyme B (SEQ ID NO: 71)

iiggheakph srpymaylmi wdqkslkrcg gflirddfvl taahcwgssi nvtlgahnik
eqeptqqfip vkrpiphpay npknfsndim llqlerkakr travqplrlp snkaqvkpgq
tcsvagwgqt aplgkhshtl qevkmtvqed rkcesdlrhy ydstielcvg dpeikktsfk
gdsggplvcn kvaqgivsyg rnngmpprac tkvssfvhwi kktmkry

Human Granzyme B cDNA (SEQ ID NO: 72)

atcatcgggg gacatgaggc caagccccac tcccgcccct acatggctta tcttatgatc
tgggatcaga agtctctgaa gaggtgcggt ggcttcctga tacgagacga cttcgtgctg
acagctgctc actgttgggg aagctccata aatgtcacct tgggggccca caatatcaaa
gaacaggagc cgacccagca gtttatccct gtgaaaagac ccatccccca tccagcctat
aatcctaaga acttctccaa cgacatcatg ctactgcagc tggagagaaa ggccaagcgg
accagagctg tgcagcccct caggctacct agcaacaagg cccaggtgaa gccagggcag
acatgcagtg tggccggctg ggggcagacg gccccctgg gaaaacactc acacacacta
caagaggtga agatgacagt gcaggaagat cgaaagtgcg aatctgactt acgccattat
tacgacagta ccattgagtt gtgcgtgggg gacccagaga ttaaaaagac ttcctttaag
ggggactctg gaggccctct tgtgtgtaac aaggtggccc agggcattgt ctcctatgga
cgaaacaatg gcatgcctcc acgagcctgc accaaagtct caagctttgt acactggata
aagaaaacca tgaaacgcta c

Non-limiting examples of commercial ELISA assays that can be used to determine the expression level of MYC are available from Invitrogen, LSBio, Biocodon Technologies, and Elisa Genie. The protein and cDNA sequences for mature human MYC are shown below.

Human Myc Protien (SEQ ID NO: 329)

mdffrvvenq qppatmplnv sftnrnydld ydsvqpyfyc deeenfyqqq qqselqppap
sediwkkfel lptpplspsr rsglcspsyv avtpfslrgd ndggggsfst adqlemvtel
lggdmvnqsf icdpddetfi kniiiqdcmw sgfsaaaklv seklasyqaa rkdsgspnpa
rghsvcstss lylqdlsaaa secidpsvvf pyplndsssp kscasqdssa fspssdslls
stesspqgsp eplvlheetp pttssdseee qedeeeidvv svekrqapgk rsesgspsag
ghskpphspl vlkrchvsth qhnyaappst rkdypaakrv kldsvrvlrq isnnrkctsp
rssdteenvk rrthnvlerq rrnelkrsff alrdqipele nnekapkvvi lkkatayils
vqaeeqklis eedllrkrre qlkhkleqlr nsca

Human Myc cDNA (SEQ ID NO: 330)

ctggatt tttttcgggt agtggaaaac cagcagcctc ccgcgacgat gcccctcaac
gttagcttca ccaacaggaa ctatgacctc gactacgact cggtgcagcc gtatttctac
tgcgacgagg aggagaactt ctaccagcag cagcagcaga gcgagctgca gcccccggcg
cccagcgagg atatctggaa gaaattcgag ctgctgccca ccccgccccct gtcccctagc
cgccgctccg ggctctgctc gccctcctac gttgcggtca cacccttctc ccttcgggga

gacaacgacg gcggtggcgg gagcttctcc acggccgacc agctggagat ggtgaccgag
ctgctgggag gagacatggt gaaccagagt ttcatctgcg acccggacga cgagaccttc
atcaaaaaca tcatcatcca ggactgtatg tggagcggct tctcggccgc cgccaagctc
gtctcagaga agctggcctc ctaccaggct gcgcgcaaag acagcggcag cccgaacccc
gcccgcggcc acagcgtctg ctccacctcc agcttgtacc tgcaggatct gagcgccgcc
gcctcagagt gcatcgaccc ctcggtggtc ttcccctacc ctctcaacga cagcagctcg
cccaagtcct gcgcctcgca agactccagc gccttctctc cgtcctcgga ttctctgctc
tcctcgacgg agtcctcccc gcagggcagc cccgagcccc tggtgctcca tgaggagaca
ccgcccacca ccagcagcga ctctgaggag gaacaagaag atgaggaaga aatcgatgtt
gtttctgtgg aaaagaggca ggctcctggc aaaaggtcag agtctggatc accttctgct
ggaggccaca gcaaacctcc tcacagccca ctggtcctca agaggtgcca cgtctccaca
catcagcaca actacgcagc gcctccctcc actcggaagg actatcctgc tgccaagagg
gtcaagttgg acagtgtcag agtcctgaga cagatcagca acaaccgaaa atgcaccagc
cccaggtcct cggacaccga ggagaatgtc aagaggcgaa cacacaacgt cttggagcgc
cagaggagga acgagctaaa acggagcttt tttgccctgc gtgaccagat cccggagttg
gaaaacaatg aaaaggcccc caaggtagtt atccttaaaa aagccacagc atacatcctg
tccgtccaag cagaggagca aaagctcatt tctgaagagg acttgttgcg gaaacgacga
gaacagttga aacacaaact tgaacagcta cggaactctt gtgcgtaa

In some embodiments, activated NK cells (e.g., human activated NK cells) can show increased (e.g., at least a 10% increase, at least a 20% increase, at least a 30% increase, at least a 40% increase, at least a 50% increase, at least a 60% increase, at least a 70% increase, at least 80% increase, at least a 90% increase, at least a 100% increase, at least a 120% increase, at least a 140% increase, at least a 160% increase, at least a 180% increase, at least a 200% increase, at least a 220% increase, at least a 240% increase, at least a 260% increase, at least a 280% increase, or at least a 300% increase) ability to kill senescent cells (e.g., any of the senescent cells described herein) in a subject (e.g., any of the subjects described herein) or *in vitro* as compared to resting NK cells (e.g., human resting NK cells).

In some embodiments, activated NK cells (e.g., human activated NK cells) can show about a 10% increase to about a 500% increase (or any of the subranges of this range described herein) ability to kill senescent cells (e.g., any of the senescent cells

described herein) in a subject (e.g., any of the subjects described herein) or *in vivo* as compared to resting NK cells (e.g., human resting NK cells).

In some embodiments, activated NK cells (e.g., human activated NK cells) can show increased (e.g., at least a 10% increase, at least a 20% increase, at least a 30% increase, at least a 40% increase, at least a 50% increase, at least a 60% increase, at least a 70% increase, at least 80% increase, at least a 90% increase, at least a 100% increase, at least a 120% increase, at least a 140% increase, at least a 160% increase, at least a 180% increase, at least a 200% increase, at least a 220% increase, at least a 240% increase, at least a 260% increase, at least a 280% increase, or at least a 300% increase) cytotoxic activity in a contact-cytotoxicity assay in the presence of an antibody that binds specifically to an antigen present on a senescent or target cell, e.g., as compared to a resting NK cell (e.g., human resting NK cells).

In some embodiments, activated NK cells (e.g., human activated NK cells) can show increased (e.g., about a 10% increase to about a 500% increase, or any of the subranges of this range described herein) cytotoxic activity in a contact-cytotoxicity assay in the presence of an antibody that binds specifically to an antigen present on a senescent or target cell, e.g., as compared to a resting NK cell (e.g., human resting NK cells).

In some embodiments, an activated NK cell can be produced by a method that includes obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an haploidentical resting NK cells. In some examples of these methods, the resting NK cell is an allogeneic resting NK cell. In some examples of these methods, the resting NK cell is an artificial NK cell. In some examples of any of these methods, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

In some examples of these methods, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium. Some examples of these methods further include isolating the activated NK cells (and optionally further administering a therapeutically effective amount of the activated NK cells to a subject, e.g., any of the subjects described herein).

In some embodiments of these methods, the contacting step is performed for a period of about 2 hours to about 20 days (e.g., about 2 hours to about 18 days, about 2 hours to about 16 days, about 2 hours to about 14 days, about 2 hours to about 12 days, about 2 hours to about 10 days, about 2 hours to about 8 days, about 2 hours to about 7 days, about 2 hours to about 6 days, about 2 hours to about 5 days, about 2 hours to about 4 days, about 2 hours to about 3 days, about 2 hours to about 2 days, about 2 hours to about 1 day, about 6 hours to about 18 days, about 6 hours to about 16 days, about 6 hours to about 14 days, about 6 hours to about 12 days, about 6 hours to about 10 days, about 6 hours to about 8 days, about 6 hours to about 7 days, about 6 hours to about 6 days, about 6 hours to about 5 days, about 6 hours to about 4 days, about 6 hours to about 3 days, about 6 hours to about 2 days, about 6 hours to about 1 day, about 12 hours to about 18 days, about 12 hours to about 16 days, about 12 hours to about 14 days, about 12 hours to about 12 days, about 12 hours to about 10 days, about 12 hours to about 8 days, about 12 hours to about 7 days, about 12 hours to about 6 days, about 12 hours to about 5 days, about 12 hours to about 4 days, about 12 hours to about 3 days, about 12 hours to about 2 days, about 12 hours to about 1 day, about 1 day to about 18 days, about 1 day to about 16 days, about 1 day to about 15 days, about 1 day to about 14 days, about 1 day to about 12 days, about 1 day to about 10 days, about 1 day to about 8 days, about 1 day to about 7 days, about 1 day to about 6 days, about 1 day to about 5 days, about 1 day to about 4 days, about 1 day to about 3 days, about 1 day to about 2 days, about 2 days to about 18 days, about 2 days to about 16 days, about 2 days to about 14 days, about 2 days to about 12 days, about 2 days to about 10 days, about 2 days to about 8 days, about 2 days to about 7 days, about 2 days to about 6 days, about 2 days to about 5 days, about 2 days to about 4 days, about 2 days to about 3 days, about 3 days to about 18 days, about 3

days to about 16 days, about 3 days to about 14 days, about 3 days to about 12 days, about 3 days to about 10 days, about 3 days to about 8 days, about 3 days to about 7 days, about 3 days to about 6 days, about 3 days to about 5 days, about 3 days to about 4 days, about 4 days to about 18 days, about 4 days to about 16 days, about 4 days to about 14 days, about 4 days to about 12 days, about 4 days to about 10 days, about 4 days to about 8 days, about 4 days to about 7 days, about 4 days to about 6 days, about 4 days to about 5 days, about 5 days to about 18 days, about 5 days to about 16 days, about 5 days to about 14 days, about 5 days to about 12 days, about 5 days to about 10 days, about 5 days to about 8 days, about 5 days to about 7 days, about 5 days to about 6 days, about 6 days to about 18 days, about 6 days to about 16 days, about 6 days to about 14 days, about 6 days to about 12 days, about 6 days to about 10 days, about 6 days to about 8 days, about 6 days to about 7 days, about 7 days to about 18 days, about 7 days to about 16 days, about 7 days to about 14 days, about 7 days to about 12 days, about 7 days to about 10 days, about 7 days to about 8 days, about 8 days to about 18 days, about 8 days to about 16 days, about 8 days to about 14 days, about 8 days to about 12 days, about 8 days to about 10 days, about 9 days to about 18 days, about 9 days to about 16 days, about 9 days to about 14 days, about 9 days to about 12 days, about 12 days to about 18 days, about 12 days to about 16 days, about 12 days to about 14 days, about 14 days to about 18 days, about 14 days to about 16 days, or about 16 days to about 18 days.

NK Cell Activating Agents

Provided herein are methods that include the use or administration of one or more NK cell activating agents. In some embodiments, an NK cell activating agent can be a protein. In some embodiments, an NK cell activating agent can be a single-chain chimeric polypeptide (e.g. any of the single-chain chimeric polypeptides described herein), a multi-chain chimeric polypeptide (e.g. any of the multi-chain chimeric polypeptides described herein, e.g., the exemplary type A and type B multi-chain chimeric polypeptides described herein), an antibody, a recombinant cytokine or an interleukin (e.g. any of the recombinant cytokines or interleukins described herein), and a soluble interleukin or cytokine receptor (e.g. any of the soluble interleukin or cytokine

receptors described herein). In some embodiments, the NK cell activating agent can be a small molecule (e.g., a glycogen synthase kinase-3 (GSK3) inhibitor, e.g., CHIR99021 as described in Cichocki et al., *Cancer Res.* 77:5664-5675, 2017) or an aptamer.

In some embodiments of any of the one or more NK cell activating agents provided herein, at least one of the one or more NK cell activating agent(s) results in activation of one or more (e.g., two, three, four, five, six, seven, or eight) of: a receptor for IL-2, a receptor for IL-7, a receptor for IL-12, a receptor for IL-15, a receptor for IL-18, a receptor for IL-21, a receptor for IL-33, CD16, CD69, CD25, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, and KIR3DS1 (e.g., in an immune cell, e.g., a human immune cell, e.g., a human NK cell) as compared to the level of activation in the absence of the one or more NK cell activating agent(s).

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-2 is a soluble IL-2 or an agonistic antibody that binds specifically to an IL-2 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-7 is a soluble IL-7 or an agonistic antibody that binds specifically to an IL-7 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-12 is a soluble IL-12 or an agonistic antibody that binds specifically to an IL-12 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-15 is a soluble IL-15 or an agonistic antibody that binds specifically to an IL-15 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-21 is a soluble IL-21 or an agonistic antibody that binds specifically to an IL-21 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-33 is a soluble IL-33 or an agonistic antibody that binds specifically to an IL-33 receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of CD16 is an agonistic antibody that binds specifically to CD16.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of CD69 is an agonistic antibody that binds specifically to CD69.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of CD25, CD59 is an agonistic antibody that binds specifically to CD25, CD59.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of CD352 is an agonistic antibody that binds specifically to CD352.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of NKp80 is an agonistic antibody that binds specifically to NKp80.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of DNAM-1 is an agonistic antibody that binds specifically to DNAM-1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of 2B4 is an agonistic antibody that binds specifically to 2B4.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of NKp30 is an agonistic antibody that binds specifically to NKp30.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of NKp44 is an agonistic antibody that binds specifically to NKp44.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of NKp46 is an agonistic antibody that binds specifically to NKp46.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of NKG2D is an agonistic antibody that binds specifically to NKG2D.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR2DS1 is an agonistic antibody that binds specifically to KIT2DS1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR2DS2/3 is an agonistic antibody that binds specifically to KIT2DS2/3.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR2DL4 is an agonistic antibody that binds specifically to KIT2DL4.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR2DS4 is an agonistic antibody that binds specifically to KIT2DS4.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR2DS5 is an agonistic antibody that binds specifically to KIT2DS5.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in activation of KIR3DS1 is an agonistic antibody that binds specifically to KIT3DS1.

In some embodiments of any of the one or more NK cell activating agents provided herein, at least one (e.g., two, three, four, or five) of the one or more NK cell activating agent(s) results in a decrease in the activation of one or more of: PD-1, a TGF-β receptor, TIGIT, CD1, TIM-3, Siglec-7, IRP60, Tactile, IL1R8, NKG2A/KLRD1, KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1, KIR3DL2, ILT2/LIR-1, and LAG-2 (e.g., in an immune cell, e.g., a human immune cell, e.g., a human NK cell) as compared to the level of activation in the absence of the one or more NK cell activating agent(s).

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of a TGF-β receptor is a soluble TGF-β

receptor, an antibody that binds specifically to TGF-β, or an antagonistic antibody that binds specifically to a TGF-β receptor.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIGIT is an antagonistic antibody that binds specifically to TIGIT, a soluble TIGIT, or an antibody that binds specifically to a ligand of TIGIT.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of CD1 is an antagonistic antibody that binds specifically to CD1, a soluble CD1, or an antibody that binds specifically to a ligand of CD1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIM-3 is an antagonistic antibody that binds specifically to TIM-3, a soluble TIM-3, or an antibody that binds specifically to a ligand of TIM-3.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Siglec-7 is an antagonistic antibody that binds specifically to Siglec-7, a soluble Siglec-7, or an antibody that binds specifically to a ligand of Siglec-7.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IRP-60 is an antagonistic antibody that binds specifically to IRP-60, a soluble IRP-60, or an antibody that binds specifically to a ligand of IRP-60.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Tactile is an antagonistic antibody that binds specifically to Tactile, a soluble Tactile, or an antibody that binds specifically to a ligand of Tactile.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IL1R8 is an antagonistic antibody that binds specifically to IL1R8, a soluble IL1R8, or an antibody that binds specifically to a ligand of IL1R8.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of NKG2A/KLRD1 is an antagonistic antibody that binds specifically to NKG2A/KLRD1, a soluble NKG2A/KLRD1, or an antibody that binds specifically to a ligand of NKG2A/KLRD1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL1 is an antagonistic antibody that binds specifically to KIR2DL1, a soluble KIR2DL1, or an antibody that binds specifically to a ligand of KIR2DL1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL2/3 is an antagonistic antibody that binds specifically to KIR2DL2/3, a soluble KIR2DL2/3, or an antibody that binds specifically to a ligand of KIR2DL2/3.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL5 is an antagonistic antibody that binds specifically to KIR2DL5, a soluble KIR2DL5, or an antibody that binds specifically to a ligand of KIR2DL5.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL1 is an antagonistic antibody that binds specifically to KIR3DL1, a soluble KIR3DL1, or an antibody that binds specifically to a ligand of KIR3DL1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL2 is an antagonistic antibody that binds specifically to KIR3DL2, a soluble KIR3DL2, or an antibody that binds specifically to a ligand of KIR3DL2.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of ILT2/LIR-1 is an antagonistic antibody that binds specifically to ILT2/LIR-1, a soluble ILT2/LIR-1, or an antibody that binds specifically to a ligand of ILT2/LIR-1.

In some embodiments, the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of LAG2 is an antagonistic antibody

that binds specifically to LAG2, a soluble LAG2, or an antibody that binds specifically to a ligand of LAG2.

Non-limiting examples of NK cell activating agents are described below and can be used in any combination.

In some examples, an NK cell activating agents can be a soluble PD-1, a soluble PD-L1, a soluble TIGIT, a soluble CD1, or a soluble TIM-3. Non-limiting examples of soluble PD-1, PD-L1, TIGIT, CD1, and TIM-3 are provided below.

Human Soluble PD-1 (SEQ ID NO: 73)

pgwfldspdr pwnpptfspa llvvtegdna tftcsfsnts esfvlnwyrm
spsnqtdkla afpedrsqpg qdcrfrvtql pngrdfhmsv vrarrndsgt
ylcgaislap kaqikeslra elrvterrae vptahpspsp rpagqfqtlv
vgvvggllgs lvllvwvlav icsraargti garrtgqplk edpsavpvfs
vdygeldfqw rektpeppvp cvpeqteyat ivfpsgmgts sparrgsadg
prsaqplrpe dghcswpl

Human Soluble PD-L1 (SEQ ID NO: 74)

ftvtvpkdlyvv eygsnmtiec kfpvekqldl aalivyweme dkniiqfvhg
eedlkvqhss yrqrarllkd qlslgnaalq itdvklqdag vyrcmisygg
adykritvkv napynkinqr ilvvdpvtse heltcqaegy pkaeviwtss
dhqvlsgktt ttnskreekl fnvtstlrin tttneifyct frrldpeenh
taelvipelp lahppnerth lvilgaillc lgvaltfifr lrkgrmmdvk
kcgiqdtnsk kqsdthleet

Human Soluble TIGIT (SEQ ID NO: 75)

mmtgtiett gnisaekggs iilqchlsst taqvtqvnwe qqdqllaicn
adlgwhisps fkdrvapgpg lgltlqsltv ndtgeyfciy htypdgtytg
riflevless vaehgarfqi pllgamaatl vvictavivv valtrkkkal
rihsvegdlr rksagqeews psapsppgsc vqaeaapagl cgeqrgedca
elhdyfnvls yrslgncsff tetg

Human Soluble CD1A (SEQ ID NO: 76)

nadglkeplsfhvt wiasfynhsw kqnlvsgwls dlqthtwdsn sstivflcpw
srgnfsneew keletlfrir tirsfegirr yahelqfeyp feiqvtggce
lhsgkvsgsf lqlayqgsdf vsfqnnswlp ypvagnmakh fckvlnqnqh
endithnlls dtcprfilgl ldagkahlqr qvkpeawlsh gpspgpghlq
lvchvsgfyp kpvwvmwmrg eqeqqgtqrg dilpsadgtw ylratlevaa
geaadlscrv khsslegqdi vlywehhssv gfiilavivp lllliglalw
frkrcfc

Human Soluble TIM3 (SEQ ID NO: 77)

seveyraev gqnaylpcfy tpaapgnlvp vcwgkgacpv fecgnvvlrt
derdvnywts rywlngdfrk gdvsltienv tladsgiycc riqipgimnd
ekfnlklvik pakvtpaptr qrdftaafpr mlttrghgpa etqtlgslpd
inltqistla nelrdsrlan dlrdsgatir igiyigagic aglalalifg
alifkwyshs kekiqnlsli slanlppsgl anavaegirs eeniytieen
vyeveepney ycyvssrqqp sqplgcrfam

In some embodiments, a soluble PD-1 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 73.

In some embodiments, a soluble PD-L1 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 74.

In some embodiments, a soluble TIGIT protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94%

identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 75.

In some embodiments, a soluble CD1A protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 76.

In some embodiments, a soluble TIM3 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 77.

Recombinant Antibodies

In some examples, NK activating agent can be: an agonistic antibody that binds specifically to an IL-2 receptor (see, e.g., those described in Gaulton et al., *Clinical Immunology and Immunopathology* 36(1):18-29, 1985), an agonistic antibody that binds specifically to an IL-7 receptor, an agonistic antibody that binds specifically to IL-12 receptor (see, e.g., those described in Rogge et al., *J. Immunol.* 162(7): 3926-3932, 1999), an agonistic antibody that binds specifically to IL-15 receptor, an agonistic antibody that binds specifically to IL-21 receptor (see, e.g., those described in U.S. Patent Application Publication No. 2006/159655), an agonistic antibody that binds specifically to IL-33 receptor (see, e.g., those described in U.S. Patent Application Publication No. 2007/160579), an antagonistic antibody that binds specifically to PD-1 (see, e.g., those described in U.S. Patent No. 7,521,051), an antibody that binds specifically to PD-L1 (see, e.g., those described in U.S. Patent No. 8,217,149), an antibody that binds specifically to TGF-β, an antagonistic antibody that binds specifically to TGF-β receptor (see, e.g., those described in European Patent Application Publication No. 1245676 A1), an antagonistic antibody that binds specifically to TIGIT (see, e.g., those described in WO 2017/053748), an antibody that binds specifically to a ligand of TIGIT (see, e.g.,

those described in WO 2011/127324), an antagonistic antibody that binds specifically to CD1 (see, e.g., those described in Szalay et al., *J. Immunol.* 162(12):6955-6958, 1999), an antibody that binds specifically to a ligand of CD1 (see, e.g., those described in Kain et al., *Immunity* 41(4):543-554, 2014), an antagonistic antibody that binds specifically to TIM-3 (see, e.g., those described in U.S. Patent Application Publication No. 2015/218274), an antibody that binds specifically to a ligand of TIM-3 (see, e.g., those described in U.S. Patent Application Publication No. 2017/283499), an agonistic antibody that binds specifically to CD69 (see, e.g., those described in Moretta et al., Journal of Experimental Medicine 174:1393, 1991), an agonistic antibody that binds specifically to CD25, CD59, an agonistic antibody that binds specifically to CD352 (see, e.g., those described in Yigit et al., *Oncotarget* 7:26346-26360, 2016), an agonistic antibody that binds specifically to NKp80 (see, e.g., those described in Peipp et al., *Oncotarget* 6:32075-32088, 2015), an agonistic antibody that binds specifically to DNAM-1, an agonistic antibody that binds specifically to 2B4 (see, e.g., those described in Sandusky et al., *European J. Immunol.* 36:3268-3276, 2006), an agonistic antibody that binds specifically to NKp30 (see, e.g., those described in Kellner et al., *OncoImmunology* 5:1-12, 2016), an agonistic antibody that binds specifically to NKp44, an agonistic antibody that binds specifically to NKp46 (see, e.g., those described in Xiong et al., *J. Clin. Invest.* 123:4264-4272, 2013), an agonistic antibody that binds specifically to NKG2D (see, e.g., those described in Kellner et al., *OncoImmunology* 5:1-12, 2016), an agonistic antibody that binds specifically to KIR2DS1 (see, e.g., those described in Xiong et al., *J. Clin. Invest.* 123:4264-4272, 2013), an agonistic antibody that binds specifically to KIR2Ds2/3 (see, e.g., those described in Borgerding et al., *Exp. Hematology* 38:213-221, 2010), an agonistic antibody that binds specifically to KIR2DL4 (see, e.g., those described in Miah et al., *J. Immunol.* 180:2922-32, 2008), an agonistic antibody that binds specifically to KIR2DS4 (see, e.g., those described in Czaja et al., *Genes and Immunity* 15:33-37, 2014), an agonistic antibody that binds specifically to KIR2DS5 (see, e.g., those described in Czaja et al., *Genes and Immunity* 15:33-37, 2014), an agonistic antibody that binds specifically to KIR3DS1 (see, e.g., those described in Czaja et al., *Genes and Immunity* 15:33-37, 2014), an antagonistic antibody that binds

specifically to Siglec-7 (see, e.g., those described in Hudak et al., *Nature Chemical Biology* 10:69-75, 2014), an antagonistic antibody that binds specifically to IRP60 (see, e.g., those described in Bachelet et al., *J. Biol. Chem.* 281:27190-27196, 2006), an antagonistic antibody that binds specifically to Tactile (see, e.g., those described in Brooks et al., *Eur. J. Cancer* 61(Suppl. 1):S189, 2016), an antagonistic antibody that binds specifically to IL1R8 (see, e.g., those described in Molgora et al., *Frontiers Immunol.* 7:1, 2016), an antagonistic antibody that binds specifically to NKG2A/KLRD1 (see, e.g., those described in Kim et al., *Infection Immunity* 76:5873-5882, 2008), an antagonistic antibody that binds specifically to KIR2DL1 (see, e.g., those described in Weiner et al., *Cell* 148:1081-1084, 2012), an antagonistic antibody that binds specifically to KIR2DL2/3 (see, e.g., those described in Weiner et al., *Cell* 148:1081-1084, 2012), an antagonistic antibody that binds specifically to KIR2DL5 (see, e.g., those described in US 9,067,997), and an antagonistic antibody that binds specifically KIR3DL1 (see, e.g., those described in US 9,067,997), an antagonistic antibody that binds specifically to KIR3DL2 (see, e.g., those described in US 9,067,997), an antagonistic antibody that binds specifically to ILT2/LIR-1 (see, e.g., those described in US 8,133,485), and an antagonistic antibody that binds specifically to LAG-2.

A recombinant antibody that is an NK cell activating agent can be any of exemplary types of antibodies (e.g., a human or humanized antibody) or any of the exemplary antibody fragments described herein. A recombinant antibody that is an NK cell activating agent can include, e.g., any of the antigen-binding domains described herein.

Recombinant Interleukins or Cytokines

In some examples, NK activating agents can be, e.g., a soluble IL-2, a soluble IL-7, a soluble IL-12, a soluble IL-15, a soluble IL-21, and a soluble IL-33. Non-limiting examples of soluble IL-12, IL-15, IL-21, and IL-33. are provided below.

Human Soluble IL-2 (SEQ ID NO: 78)

aptssstkkt qlqlehllld lqmilnginn yknpkltrml tfkfympkka
telkhlqcle eelkpleevl nlaqsknfhl rprdlisnin vivlelkgse
ttfmceyade tativeflnr witfcqsiis tlt

Human Soluble IL-7 (SEQ ID NO: 79)

dcdiegkdgkqyesv lmvsidqlld smkeigsncl nnefnffkrh icdankegmf
lfraarklrq flkmnstgdf dlhllkvseg ttillnctgq vkgrkpaalg
eaqptkslee nkslkeqkkl ndlcflkrll qeiktcwnki lmgtkeh

Human Soluble IL-12 subunit alpha (SEQ ID NO: 80)

rnlpvatp dpgmfpclhh sqnllravsn mlqkarqtle fypctseeid
heditkdkts tveaclplel tknesclnsr etsfitngsc lasrktsfmm
alclssiyed lkmyqvefkt mnakllmdpk rqifldqnml avidelmqal
nfnsetvpqk ssleepdfyk tkiklcillh afriravtid rvmsylnas

Human Soluble IL-12 subunit beta (SEQ ID NO: 81)

iwelkkdv yvveldwypd apgemvvltc dtpeedgitw tldqssevlg
sgktltiqvk efgdagqytc hkggevlshs llllhkkedg iwstdilkdq
kepknktflr ceaknysgrf tcwwlttist dltfsvkssr gssdpqgvtc
gaatlsaerv rgdnkeyeys vecqedsacp aaeeslpiev mvdavhklky
enytssffir diikpdppkn lqlkplknsr qvevsweypd twstphsyfs
ltfcvqvqgk skrekkdrvf tdktsatvic rknasisvra qdryysssws
ewasvpcs

Human Soluble IL-15 (SEQ ID NO: 82)

Nwvnvisdlkki edliqsmhid atlytesdvh psckvtamkc fllelqvisl
esgdasihdt venliilann slssngnvte sgckeceele eknikeflqs
fvhivqmfin ts

Human Soluble IL-21 (SEQ ID NO: 83)

qgqdrhmi rmrqlidivd qlknyvndlv peflpapedv etncewsafs
cfqkaqlksa ntgnneriin vsikklkrkp pstnagrrqk hrltcpscds
yekkppkefl erfksllqkm ihqhlssrth gseds

Human Soluble IL-33 (SEQ ID NO: 84)

mkpkmkystn kistakwknt askalcfklg ksqqkakevc pmyfmklrsg
lmikkeacyf rrettkrpsl ktgrkhkrhl vlaacqqqst vecfafgisg
vqkytralhd ssitgispit eylaslstyn dqsitfaled esyeiyvedl
kkdekkdkvl lsyyesqhps nesgdgvdgk mlmvtlsptk dfwlhannke
hsvelhkcek plpdqaffvl hnmhsncvsf ecktdpgvfi gvkdnhlali
kvdssenlct enilfklset

In some embodiments, a soluble IL-2 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 78.

In some embodiments, a soluble IL-7 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 79.

In some embodiments, a soluble IL-2 protein includes a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 80 and a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical,

at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 81.

In some embodiments, a soluble IL-15 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 82.

In some embodiments, a soluble IL-21 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 83.

In some embodiments, a soluble IL-33 protein can include a sequence that is at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 84.

Soluble Cytokine or Interleukin Receptors

In some examples of any of the soluble cytokine or interleukin receptors described herein, the soluble cytokine or interleukin receptors can be a soluble TGF-β receptor. In some examples, the soluble TGF-β receptor is a soluble TGF-β receptor I (TGF-βRI) (see, e.g., those described in Docagne et al., *Journal of Biological Chemistry* 276(49):46243-46250, 2001), a soluble TGF-β receptor II (TGF-βRII) (see, e.g., those described in Yung et al., *Am. J. Resp. Crit. Care Med.* 194(9):1140-1151, 2016), a soluble TGF-βRIII (see, e.g., those described in Heng et al., *Placenta* 57:320, 2017). In some examples, the soluble TGF-β receptor is a receptor "trap" for TGF-β (see, e.g., those described in Zwaagstra et al., *Mol. Cancer Ther.* 11(7):1477-1487, 2012, and those described in De Crescenzo et al. *Transforming Growth Factor-β in Cancer Therapy, Volume II*, pp 671-684).

Additional examples of soluble cytokine or soluble interleukin receptors are known in the art.

Single Chain Chimeric Polypeptides

Non-limiting examples of NK cell activating agents are single-chain chimeric polypeptides that include: (i) a first target-binding domain (e.g., any of the target-binding domains described herein or known in the art), (ii) a soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art), and (iii) as second target-binding domain (e.g., any of the target-binding domains described herein or known in the art).

In some examples of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide can have a total length of about 50 amino acids to about 3000 amino acids, about 50 amino acids to about 2500 amino acids, about 50 amino acids to about 2000 amino acids, about 50 amino acids to about 1500 amino acids, about 50 amino acids to about 1000 amino acids, about 50 amino acids to about 950 amino acids, about 50 amino acids to about 900 amino acids, about 50 amino acids to about 850 amino acids, about 50 amino acids to about 800 amino acids, about 50 amino acids to about 750 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 480 amino acids, about 50 amino acids to about 460 amino acids, about 50 amino acids to about 440 amino acids, about 50 amino acids to about 420 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 380 amino acids, about 50 amino acids to about 360 amino acids, about 50 amino acids to about 340 amino acids, about 50 amino acids to about 320 amino acids, about 50 amino acids to about 300 amino acids, about 50 amino acids to about 280 amino acids, about 50 amino acids to about 260 amino acids, about 50 amino acids to about 240 amino acids, about 50 amino acids to about 220 amino acids, about 50 amino acids to about 200 amino acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about 100 amino acids, about 100 amino acids to about 3000 amino acids, about

100 amino acids to about 2500 amino acids, about 100 amino acids to about 2000 amino acids, about 100 amino acids to about 1500 amino acids, about 100 amino acids to about 1000 amino acids, about 100 amino acids to about 950 amino acids, about 100 amino acids to about 900 amino acids, about 100 amino acids to about 850 amino acids, about 100 amino acids to about 800 amino acids, about 100 amino acids to about 750 amino acids, about 100 amino acids to about 700 amino acids, about 100 amino acids to about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 480 amino acids, about 100 amino acids to about 460 amino acids, about 100 amino acids to about 440 amino acids, about 100 amino acids to about 420 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 380 amino acids, about 100 amino acids to about 360 amino acids, about 100 amino acids to about 340 amino acids, about 100 amino acids to about 320 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 280 amino acids, about 100 amino acids to about 260 amino acids, about 100 amino acids to about 240 amino acids, about 100 amino acids to about 220 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 150 amino acids, about 150 amino acids to about 3000 amino acids, about 150 amino acids to about 2500 amino acids, about 150 amino acids to about 2000 amino acids, about 150 amino acids to about 1500 amino acids, about 150 amino acids to about 1000 amino acids, about 150 amino acids to about 950 amino acids, about 150 amino acids to about 900 amino acids, about 150 amino acids to about 850 amino acids, about 150 amino acids to about 800 amino acids, about 150 amino acids to about 750 amino acids, about 150 amino acids to about 700 amino acids, about 150 amino acids to about 650 amino acids, about 150 amino acids to about 600 amino acids, about 150 amino acids to about 550 amino acids, about 150 amino acids to about 500 amino acids, about 150 amino acids to about 480 amino acids, about 150 amino acids to about 460 amino acids, about 150 amino acids to about 440 amino acids, about 150 amino acids to about 420 amino acids, about 150 amino acids to about 400 amino acids, about 150 amino acids to about 380 amino acids, about 150 amino acids to about 360 amino acids, about 150 amino acids to about 340

amino acids, about 150 amino acids to about 320 amino acids, about 150 amino acids to about 300 amino acids, about 150 amino acids to about 280 amino acids, about 150 amino acids to about 260 amino acids, about 150 amino acids to about 240 amino acids, about 150 amino acids to about 220 amino acids, about 150 amino acids to about 200 amino acids, about 200 amino acids to about 3000 amino acids, about 200 amino acids to about 2500 amino acids, about 200 amino acids to about 2000 amino acids, about 200 amino acids to about 1500 amino acids, about 200 amino acids to about 1000 amino acids, about 200 amino acids to about 950 amino acids, about 200 amino acids to about 900 amino acids, about 200 amino acids to about 850 amino acids, about 200 amino acids to about 800 amino acids, about 200 amino acids to about 750 amino acids, about 200 amino acids to about 700 amino acids, about 200 amino acids to about 650 amino acids, about 200 amino acids to about 600 amino acids, about 200 amino acids to about 550 amino acids, about 200 amino acids to about 500 amino acids, about 200 amino acids to about 480 amino acids, about 200 amino acids to about 460 amino acids, about 200 amino acids to about 440 amino acids, about 200 amino acids to about 420 amino acids, about 200 amino acids to about 400 amino acids, about 200 amino acids to about 380 amino acids, about 200 amino acids to about 360 amino acids, about 200 amino acids to about 340 amino acids, about 200 amino acids to about 320 amino acids, about 200 amino acids to about 300 amino acids, about 200 amino acids to about 280 amino acids, about 200 amino acids to about 260 amino acids, about 200 amino acids to about 240 amino acids, about 200 amino acids to about 220 amino acids, about 220 amino acids to about 3000 amino acids, about 220 amino acids to about 2500 amino acids, about 220 amino acids to about 2000 amino acids, about 220 amino acids to about 1500 amino acids, about 220 amino acids to about 1000 amino acids, about 220 amino acids to about 950 amino acids, about 220 amino acids to about 900 amino acids, about 220 amino acids to about 850 amino acids, about 220 amino acids to about 800 amino acids, about 220 amino acids to about 750 amino acids, about 220 amino acids to about 700 amino acids, about 220 amino acids to about 650 amino acids, about 220 amino acids to about 600 amino acids, about 220 amino acids to about 550 amino acids, about 220 amino acids to about 500 amino acids, about 220 amino acids to about 480 amino acids, about 220

amino acids to about 460 amino acids, about 220 amino acids to about 440 amino acids, about 220 amino acids to about 420 amino acids, about 220 amino acids to about 400 amino acids, about 220 amino acids to about 380 amino acids, about 220 amino acids to about 360 amino acids, about 220 amino acids to about 340 amino acids, about 220 amino acids to about 320 amino acids, about 220 amino acids to about 300 amino acids, about 220 amino acids to about 280 amino acids, about 220 amino acids to about 260 amino acids, about 220 amino acids to about 240 amino acids, about 240 amino acids to about 3000 amino acids, about 240 amino acids to about 2500 amino acids, about 240 amino acids to about 2000 amino acids, about 240 amino acids to about 1500 amino acids, about 240 amino acids to about 1000 amino acids, about 240 amino acids to about 950 amino acids, about 240 amino acids to about 900 amino acids, about 240 amino acids to about 850 amino acids, about 240 amino acids to about 800 amino acids, about 240 amino acids to about 750 amino acids, about 240 amino acids to about 700 amino acids, about 240 amino acids to about 650 amino acids, about 240 amino acids to about 600 amino acids, about 240 amino acids to about 550 amino acids, about 240 amino acids to about 500 amino acids, about 240 amino acids to about 480 amino acids, about 240 amino acids to about 460 amino acids, about 240 amino acids to about 440 amino acids, about 240 amino acids to about 420 amino acids, about 240 amino acids to about 400 amino acids, about 240 amino acids to about 380 amino acids, about 240 amino acids to about 360 amino acids, about 240 amino acids to about 340 amino acids, about 240 amino acids to about 320 amino acids, about 240 amino acids to about 300 amino acids, about 240 amino acids to about 280 amino acids, about 240 amino acids to about 260 amino acids, about 260 amino acids to about 3000 amino acids, about 260 amino acids to about 2500 amino acids, about 260 amino acids to about 2000 amino acids, about 260 amino acids to about 1500 amino acids, about 260 amino acids to about 1000 amino acids, about 260 amino acids to about 950 amino acids, about 260 amino acids to about 900 amino acids, about 260 amino acids to about 850 amino acids, about 260 amino acids to about 800 amino acids, about 260 amino acids to about 750 amino acids, about 260 amino acids to about 700 amino acids, about 260 amino acids to about 650 amino acids, about 260 amino acids to about 600 amino acids, about 260 amino acids to about 550

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In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) directly abut each other. In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein). In some embodiments of any of the single-chain chimeric polypeptides described herein, the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abut each other. In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein

or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art).

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abut each other. In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art). In some embodiments of any of the single-chain chimeric polypeptides described herein, the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) directly abut each other. In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGG
GGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQ

RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
YYCARYYDDHYCLDYWGQGTTLTVSSSGTTNTVAAYNLTWKSTNFKTILEWEP
KPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAG
NVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQ
AVIPSRTVNRKSTDSPVECMGQEKGEFREVQLQQSGPELVKPGASVKMSCKASG
YTFTSYVIQWVKQKPGQGLEWIGSINPYNDYTKYNEKFKGKATLTSDKSSITAY
MEFSSLTSEDSALYYCARWGDGNYWGRGTTLTVSSGGGGSGGGGSGGGGSDIE
MTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPGSSPKLCIYSTSNLASG
VPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGGGTKLETKR (SEQ ID
NO: 85).

In some embodiments, a single-chain chimeric polypeptide is encoded by a nucleic acid that includes a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGTGAGA
AGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACTGGTAT
CAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCAGCAAG
CTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCACCAGCTA
CTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACTATTGCC
AGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCTCGAAAT
CAATCGTGGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAG
CCAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCGGCGCCTCC
GTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAATGCA
TTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATATCAAC
CCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCCACTT
TAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTTAACC
AGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCACTACTG
TTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGCTCCGGCACC
ACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGA

CAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGAT
CTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACC
GAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGG
CTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGC
GAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTT
AGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTC
ACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCC
TCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCC
AGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTG
ACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTC
TCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAA
GAAAAGGGCGAGTTCCGGGAGGTCCAGCTGCAGCAGAGCGGACCCGAACTC
GTGAAACCCGGTGCTTCCGTGAAAATGTCTTGTAAGGCCAGCGGATACACCT
TCACCTCCTATGTGATCCAGTGGGTCAAACAGAAGCCCGGACAAGGTCTCGA
GTGGATCGGCAGCATCAACCCTTACAACGACTATACCAAATACAACGAGAAG
TTTAAGGGAAAGGCTACTTTAACCTCCGACAAAAGCTCCATCACAGCCTACA
TGGAGTTCAGCTCTTTAACATCCGAGGACAGCGCTCTGTACTATTGCGCCCGG
TGGGGCGACGGCAATTACTGGGGACGGGGCACAACACTGACCGTGAGCAGC
GGAGGCGGAGGCTCCGGCGGAGGCGGATCTGGCGGTGGCGGCTCCGACATC
GAGATGACCCAGTCCCCCGCTATCATGTCCGCCTCTTTAGGCGAGCGGGTCA
CAATGACTTGTACAGCCTCCTCCAGCGTCTCCTCCTCCTACTTCCATTGGTAC
CAACAGAAACCCGGAAGCTCCCCTAAACTGTGCATCTACAGCACCAGCAATC
TCGCCAGCGGCGTGCCCCCTAGGTTTTCCGGAAGCGGAAGCACCAGCTACTC
TTTAACCATCTCCTCCATGGAGGCTGAGGATGCCGCCACCTACTTTTGTCACC
AGTACCACCGGTCCCCCACCTTCGGAGGCGGCACCAAACTGGAGACAAAGA
GG (SEQ ID NO: 86).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

MKWVTFISLLFLFSSAYSQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQ
KSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWS
SNPFTFGSGTKLEINRGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSCK
ASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSS
STAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFREVQLQQSGPEL
VKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGSINPYNDYTKYNEKF
KGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNYWGRGTTLTVSSGGG
GSGGGGSGGGGSDIEMTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPG
SSPKLCIYSTSNLASGVPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGG
GTKLETKR (SEQ ID NO: 87).

In some embodiments, a single-chain chimeric polypeptide is encoded by a nucleic acid that includes a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

ATGAAGTGGGTGACCTTCATCAGCTTATTATTTTTATTCAGCTCCGCCTATTCC
CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGTGAGA
AGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACTGGTAT
CAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCAGCAAG
CTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCACCAGCTA
CTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACTATTGCC
AGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCTCGAAAT
CAATCGTGGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAG
CCAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCGGCGCCTCC
GTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAATGCA
TTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATATCAAC
CCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCCACTT

TAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTTAACC
AGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCACTACTG
TTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGCTCCGGCACC
ACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGA
CAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGAT
CTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACC
GAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGG
CTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGC
GAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTT
AGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTC
ACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCC
TCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCC
AGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTG
ACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTC
TCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAA
GAAAAGGGCGAGTTCCGGGAGGTCCAGCTGCAGCAGAGCGGACCCGAACTC
GTGAAACCCGGTGCTTCCGTGAAAATGTCTTGTAAGGCCAGCGGATACACCT
TCACCTCCTATGTGATCCAGTGGGTCAAACAGAAGCCCGGACAAGGTCTCGA
GTGGATCGGCAGCATCAACCCTTACAACGACTATACCAAATACAACGAGAAG
TTTAAGGGAAAGGCTACTTTAACCTCCGACAAAAGCTCCATCACAGCCTACA
TGGAGTTCAGCTCTTTAACATCCGAGGACAGCGCTCTGTACTATTGCGCCCGG
TGGGGCGACGGCAATTACTGGGGACGGGGCACAACACTGACCGTGAGCAGC
GGAGGCGGAGGCTCCGGCGGAGGCGGATCTGGCGGTGGCGGCTCCGACATC
GAGATGACCCAGTCCCCCGCTATCATGTCCGCCTCTTTAGGCGAGCGGGTCA
CAATGACTTGTACAGCCTCCTCCAGCGTCTCCTCCTCCTACTTCCATTGGTAC
CAACAGAAACCCGGAAGCTCCCCTAAACTGTGCATCTACAGCACCAGCAATC
TCGCCAGCGGCGTGCCCCCTAGGTTTTCCGGAAGCGGAAGCACCAGCTACTC
TTTAACCATCTCCTCCATGGAGGCTGAGGATGCCGCCACCTACTTTTGTCACC
AGTACCACCGGTCCCCCACCTTCGGAGGCGGCACCAAACTGGAGACAAAGA
GG (SEQ ID NO: 88).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

VQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGSINPYN
DYTKYNEKFKGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNYWGRG
TTLTVSSGGGGSGGGGSGGGGSDIEMTQSPAIMSASLGERVTMTCTASSSVSSSY
FHWYQQKPGSSPKLCIYSTSNLASGVPPRFSGSGSTSYSLTISSMEAEDAATYFCH
QYHRSPTFGGGTKLETKRSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTV
QISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAG
EPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDV
FGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRK
STDSPVECMGQEKGEFREQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQ
QKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQW
SSNPFTFGSGTKLEINRGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSC
KASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKS
SSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO: 89).

In some embodiments, a single-chain chimeric polypeptide is encoded by a nucleic acid that includes a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to

GTGCAGCTGCAGCAGTCCGGACCCGAACTGGTCAAGCCCGGTGCCTCCGTGA
AAATGTCTTGTAAGGCTTCTGGCTACACCTTTACCTCCTACGTCATCCAATGG
GTGAAGCAGAAGCCCGGTCAAGGTCTCGAGTGGATCGGCAGCATCAATCCCT
ACAACGATTACACCAAGTATAACGAAAAGTTTAAGGGCAAGGCCACTCTGAC
AAGCGACAAGAGCTCCATTACCGCCTACATGGAGTTTTCCTCTTTAACTTCTG
AGGACTCCGCTTTATACTATTGCGCTCGTTGGGGCGATGGCAATTATTGGGGC
CGGGGAACTACTTTAACAGTGAGCTCCGGCGGCGGCGGAAGCGGAGGTGGA
GGATCTGGCGGTGGAGGCAGCGACATCGAGATGACACAGTCCCCCGCTATCA

TGAGCGCCTCTTTAGGAGAACGTGTGACCATGACTTGTACAGCTTCCTCCAGC
GTGAGCAGCTCCTATTTCCACTGGTACCAGCAGAAACCCGGCTCCTCCCCTAA
ACTGTGTATCTACTCCACAAGCAATTTAGCTAGCGGCGTGCCTCCTCGTTTTA
GCGGCTCCGGCAGCACCTCTTACTCTTTAACCATTAGCTCTATGGAGGCCGAA
GATGCCGCCACATACTTTTGCCATCAGTACCACCGGTCCCCTACCTTTGGCGG
AGGCACAAAGCTGGAGACCAAGCGGAGCGGCACCACCAACACAGTGGCCGC
CTACAATCTGACTTGGAAATCCACCAACTTCAAGACCATCCTCGAGTGGGAG
CCCAAGCCCGTTAATCAAGTTTATACCGTGCAGATTTCCACCAAGAGCGGCG
ACTGGAAATCCAAGTGCTTCTATACCACAGACACCGAGTGCGATCTCACCGA
CGAGATCGTCAAAGACGTGAAGCAGACATATTTAGCTAGGGTGTTCTCCTAC
CCCGCTGGAAACGTGGAGAGCACCGGATCCGCTGGAGAGCCTTTATACGAGA
ACTCCCCCGAATTCACCCCCTATCTGGAAACCAATTTAGGCCAGCCCACCATC
CAGAGCTTCGAACAAGTTGGCACAAAGGTGAACGTCACCGTCGAAGATGAG
AGGACTTTAGTGCGGAGGAACAATACATTTTTATCCTTACGTGACGTCTTCGG
CAAGGATTTAATCTACACACTGTATTACTGGAAGTCTAGCTCCTCCGGCAAGA
AGACCGCCAAGACCAATACCAACGAATTTTTAATTGACGTGGACAAGGGCGA
GAACTACTGCTTCTCCGTGCAAGCTGTGATCCCCTCCCGGACAGTGAACCGG
AAGTCCACCGACTCCCCCGTGGAGTGCATGGGCCAAGAGAAGGGAGAGTTTC
GTGAGCAGATCGTGCTGACCCAGTCCCCCGCTATTATGAGCGCTAGCCCCGG
TGAAAAGGTGACTATGACATGCAGCGCCAGCTCTTCCGTGAGCTACATGAAC
TGGTATCAGCAGAAGTCCGGCACCAGCCCTAAAAGGTGGATCTACGACACCA
GCAAGCTGGCCAGCGGCGTCCCCGCTCACTTTCGGGGCTCCGGCTCCGGAAC
AAGCTACTCTCTGACCATCAGCGGCATGGAAGCCGAGGATGCCGCTACCTAT
TACTGTCAGCAGTGGAGCTCCAACCCCTTCACCTTTGGATCCGGCACCAAGCT
CGAGATTAATCGTGGAGGCGGAGGTAGCGGAGGAGGCGGATCCGGCGGTGG
AGGTAGCCAAGTTCAGCTCCAGCAAAGCGGCGCCGAACTCGCTCGGCCCGGC
GCTTCCGTGAAGATGTCTTGTAAGGCCTCCGGCTATACCTTCACCCGGTACAC
AATGCACTGGGTCAAGCAACGGCCCGGTCAAGGTTTAGAGTGGATTGGCTAT
ATCAACCCCTCCCGGGGCTATACCAACTACAACCAGAAGTTCAAGGACAAAG
CCACCCTCACCACCGACAAGTCCAGCAGCACCGCTTACATGCAGCTGAGCTC

TTTAACATCCGAGGATTCCGCCGTGTACTACTGCGCTCGGTACTACGACGATC
ATTACTGCCTCGATTACTGGGGCCAAGGTACCACCTTAACAGTCTCCTCC
(SEQ ID NO: 90).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to
MKWVTFISLLFLFSSAYSVQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWV
KQKPGQGLEWIGSINPYNDYTKYNEKFKGKATLTSDKSSITAYMEFSSLTSEDSA
LYYCARWGDGNYWGRGTTLTVSSGGGGSGGGGSGGGGSDIEMTQSPAIMSASL
GERVTMTCTASSSVSSSYFHWYQQKPGSSPKLCIYSTSNLASGVPPRFSGSGSTSY
SLTISSMEAEDAATYFCHQYHRSPTFGGGTKLETKRSGTTNTVAAYNLTWKSTN
FKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYL
ARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTV
EDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKG
ENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFREQIVLTQSPAIMSASPGEK
VTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSL
TISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGGGGSGGGGSGGGGSQVQL
QQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGY
TNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWG
QGTTLTVSS (SEQ ID NO: 91).

In some embodiments, a single-chain chimeric polypeptide is encoded by a nucleic acid that includes a sequence that is at least 70% identical (e.g., at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical) to
ATGAAATGGGTCACCTTCATCTCTTTACTGTTTTTATTTAGCAGCGCCTACAG
CGTGCAGCTGCAGCAGTCCGGACCCGAACTGGTCAAGCCCGGTGCCTCCGTG
AAAATGTCTTGTAAGGCTTCTGGCTACACCTTTACCTCCTACGTCATCCAATG
GGTGAAGCAGAAGCCCGGTCAAGGTCTCGAGTGGATCGGCAGCATCAATCCC
TACAACGATTACACCAAGTATAACGAAAAGTTTAAGGGCAAGGCCACTCTGA

CAAGCGACAAGAGCTCCATTACCGCCTACATGGAGTTTTCCTCTTTAACTTCT
GAGGACTCCGCTTTATACTATTGCGCTCGTTGGGGCGATGGCAATTATTGGGG
CCGGGGAACTACTTTAACAGTGAGCTCCGGCGGCGGCGGAAGCGGAGGTGG
AGGATCTGGCGGTGGAGGCAGCGACATCGAGATGACACAGTCCCCCGCTATC
ATGAGCGCCTCTTTAGGAGAACGTGTGACCATGACTTGTACAGCTTCCTCCAG
CGTGAGCAGCTCCTATTTCCACTGGTACCAGCAGAAACCCGGCTCCTCCCCTA
AACTGTGTATCTACTCCACAAGCAATTTAGCTAGCGGCGTGCCTCCTCGTTTT
AGCGGCTCCGGCAGCACCTCTTACTCTTTAACCATTAGCTCTATGGAGGCCGA
AGATGCCGCCACATACTTTTGCCATCAGTACCACCGGTCCCCTACCTTTGGCG
GAGGCACAAAGCTGGAGACCAAGCGGAGCGGCACCACCAACACAGTGGCCG
CCTACAATCTGACTTGGAAATCCACCAACTTCAAGACCATCCTCGAGTGGGA
GCCCAAGCCCGTTAATCAAGTTTATACCGTGCAGATTTCCACCAAGAGCGGC
GACTGGAAATCCAAGTGCTTCTATACCACAGACACCGAGTGCGATCTCACCG
ACGAGATCGTCAAAGACGTGAAGCAGACATATTTAGCTAGGGTGTTCTCCTA
CCCCGCTGGAAACGTGGAGAGCACCGGATCCGCTGGAGAGCCTTTATACGAG
AACTCCCCCGAATTCACCCCCTATCTGGAAACCAATTTAGGCCAGCCCACCAT
CCAGAGCTTCGAACAAGTTGGCACAAAGGTGAACGTCACCGTCGAAGATGAG
AGGACTTTAGTGCGGAGGAACAATACATTTTTATCCTTACGTGACGTCTTCGG
CAAGGATTTAATCTACACACTGTATTACTGGAAGTCTAGCTCCTCCGGCAAGA
AGACCGCCAAGACCAATACCAACGAATTTTTAATTGACGTGGACAAGGGCGA
GAACTACTGCTTCTCCGTGCAAGCTGTGATCCCCTCCCGGACAGTGAACCGG
AAGTCCACCGACTCCCCCGTGGAGTGCATGGGCCAAGAGAAGGGAGAGTTTC
GTGAGCAGATCGTGCTGACCCAGTCCCCCGCTATTATGAGCGCTAGCCCCGG
TGAAAAGGTGACTATGACATGCAGCGCCAGCTCTTCCGTGAGCTACATGAAC
TGGTATCAGCAGAAGTCCGGCACCAGCCCTAAAAGGTGGATCTACGACACCA
GCAAGCTGGCCAGCGGCGTCCCCGCTCACTTTCGGGGCTCCGGCTCCGGAAC
AAGCTACTCTCTGACCATCAGCGGCATGGAAGCCGAGGATGCCGCTACCTAT
TACTGTCAGCAGTGGAGCTCCAACCCCTTCACCTTTGGATCCGGCACCAAGCT
CGAGATTAATCGTGGAGGCGGAGGTAGCGGAGGAGGCGGATCCGGCGGTGG
AGGTAGCCAAGTTCAGCTCCAGCAAAGCGGCGCCGAACTCGCTCGGCCCGGC

GCTTCCGTGAAGATGTCTTGTAAGGCCTCCGGCTATACCTTCACCCGGTACAC
AATGCACTGGGTCAAGCAACGGCCCGGTCAAGGTTTAGAGTGGATTGGCTAT
ATCAACCCCTCCCGGGGCTATACCAACTACAACCAGAAGTTCAAGGACAAAG
CCACCCTCACCACCGACAAGTCCAGCAGCACCGCTTACATGCAGCTGAGCTC
TTTAACATCCGAGGATTCCGCCGTGTACTACTGCGCTCGGTACTACGACGATC
ATTACTGCCTCGATTACTGGGGCCAAGGTACCACCTTAACAGTCTCCTCC
(SEQ ID NO: 92).

Some embodiments of any of the single-chain chimeric polypeptides described herein can further include one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at its N- and/or C-terminus.

In some embodiments, the single-chain chimeric polypeptides can include one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at its N-terminus. In some embodiments, one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminus of the single-chain chimeric polypeptide can directly abut the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein). In some embodiments, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between one of the at least one additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminus of the single-chain chimeric polypeptide and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein).

In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at its C-terminus. In some embodiments, one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the C-terminus of the single-chain chimeric polypeptide directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art). In some embodiments, the single-chain chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between one of the at least one additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the C-terminus of the single-chain chimeric polypeptide and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein).

In some embodiments of any of the single-chain chimeric polypeptides described herein, the single-chain chimeric polypeptide comprises one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at its N-terminus and its C-terminus. In some embodiments, one of the one or more additional antigen binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminus of the single-chain chimeric polypeptide directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the

soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein). In some embodiments, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between one of the one or more additional antigen-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminus and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains). In some embodiments, one of the one or more additional antigen binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the C-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains). In some embodiments, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between one of the one or more additional antigen-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the C-terminus and the first target-binding domain(e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), or the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein).

In some embodiments of any of the single-chain chimeric polypeptides described herein, two or more (e.g., three, four, five, six, seven, eight, nine, or ten) of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains

described herein or known in the art) bind specifically to the same antigen. In some embodiments, two or more (e.g., three, four, five, six, seven, eight, nine, or ten) of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) bind specifically to the same epitope. In some embodiments, two or more (e.g., three, four, five, six, seven, eight, nine, or ten) of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain(e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) include the same amino acid sequence.

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) each bind specifically to the same antigen. In some embodiments, the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain(e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) each bind specifically to the same epitope. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains each comprise the same amino acid sequence.

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain(e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) bind specifically to different antigens.

In some embodiments of any of the single-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain (e.g., any of the exemplary antigen-binding domains described herein or known in the art). In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain (e.g., any of the exemplary antigen-binding domains described herein or known in the art). In some embodiments, the antigen-binding domain can include a scFv or a single domain antibody.

In some embodiments of any of the single-chain chimeric polypeptides described herein, one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain(e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D,

a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

In some embodiments of any of the single-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine protein. Non-limiting examples of soluble interleukin proteins and soluble cytokine proteins include: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the single-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine receptor. Non-limiting examples of soluble interleukin receptors and soluble cytokine receptors include: a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKP30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the target-binding domains described herein), the second target-binding domain (e.g., any of the target-binding domains

described herein), and the one or more additional target-binding domains (e.g., any of the target-binding domains described herein) can each, independently, bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the single-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine protein. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the single-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble

TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Multi-Chain Chimeric Polypeptides- Type A

Non-limiting examples of NK cell activating agents are multi-chain chimeric polypeptides that include: (a) a first chimeric polypeptide including: (i) a first target-binding domain; (ii) a soluble tissue factor domain; and (iii) a first domain of a pair of affinity domains; and (b) a second chimeric polypeptide including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

In some examples of any of the multi-chain chimeric polypeptides described herein the total length of first chimeric polypeptide and/or the second chimeric polypeptide can each independently be about 50 amino acids to about 3000 amino acids, about 50 amino acids to about 2500 amino acids, about 50 amino acids to about 2000 amino acids, about 50 amino acids to about 1500 amino acids, about 50 amino acids to about 1000 amino acids, about 50 amino acids to about 950 amino acids, about 50 amino acids to about 900 amino acids, about 50 amino acids to about 850 amino acids, about 50 amino acids to about 800 amino acids, about 50 amino acids to about 750 amino acids, about 50 amino acids to about 700 amino acids, about 50 amino acids to about 650 amino acids, about 50 amino acids to about 600 amino acids, about 50 amino acids to about 550 amino acids, about 50 amino acids to about 500 amino acids, about 50 amino acids to about 480 amino acids, about 50 amino acids to about 460 amino acids, about 50 amino acids to about 440 amino acids, about 50 amino acids to about 420 amino acids, about 50 amino acids to about 400 amino acids, about 50 amino acids to about 380 amino acids, about 50 amino acids to about 360 amino acids, about 50 amino acids to about 340 amino acids, about 50 amino acids to about 320 amino acids, about 50 amino acids to about 300 amino acids, about 50 amino acids to about 280 amino acids, about 50 amino acids to about 260 amino acids, about 50 amino acids to about 240 amino acids, about 50 amino acids to about 220 amino acids, about 50 amino acids to about 200 amino acids, about 50

amino acids to about 150 amino acids, about 50 amino acids to about 100 amino acids, about 100 amino acids to about 3000 amino acids, about 100 amino acids to about 2500 amino acids, about 100 amino acids to about 2000 amino acids, about 100 amino acids to about 1500 amino acids, about 100 amino acids to about 1000 amino acids, about 100 amino acids to about 950 amino acids, about 100 amino acids to about 900 amino acids, about 100 amino acids to about 850 amino acids, about 100 amino acids to about 800 amino acids, about 100 amino acids to about 750 amino acids, about 100 amino acids to about 700 amino acids, about 100 amino acids to about 650 amino acids, about 100 amino acids to about 600 amino acids, about 100 amino acids to about 550 amino acids, about 100 amino acids to about 500 amino acids, about 100 amino acids to about 480 amino acids, about 100 amino acids to about 460 amino acids, about 100 amino acids to about 440 amino acids, about 100 amino acids to about 420 amino acids, about 100 amino acids to about 400 amino acids, about 100 amino acids to about 380 amino acids, about 100 amino acids to about 360 amino acids, about 100 amino acids to about 340 amino acids, about 100 amino acids to about 320 amino acids, about 100 amino acids to about 300 amino acids, about 100 amino acids to about 280 amino acids, about 100 amino acids to about 260 amino acids, about 100 amino acids to about 240 amino acids, about 100 amino acids to about 220 amino acids, about 100 amino acids to about 200 amino acids, about 100 amino acids to about 150 amino acids, about 150 amino acids to about 3000 amino acids, about 150 amino acids to about 2500 amino acids, about 150 amino acids to about 2000 amino acids, about 150 amino acids to about 1500 amino acids, about 150 amino acids to about 1000 amino acids, about 150 amino acids to about 950 amino acids, about 150 amino acids to about 900 amino acids, about 150 amino acids to about 850 amino acids, about 150 amino acids to about 800 amino acids, about 150 amino acids to about 750 amino acids, about 150 amino acids to about 700 amino acids, about 150 amino acids to about 650 amino acids, about 150 amino acids to about 600 amino acids, about 150 amino acids to about 550 amino acids, about 150 amino acids to about 500 amino acids, about 150 amino acids to about 480 amino acids, about 150 amino acids to about 460 amino acids, about 150 amino acids to about 440 amino acids, about 150 amino acids to about 420 amino acids, about 150 amino acids to about 400

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In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) directly abut each other in the first chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the first target-binding domain (e.g., any of the exemplary first target-binding domains described herein) and the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) in the first chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) directly abut each other in the first chimeric polypeptide. In some

embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) directly abut each other in the second chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) in the second chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein). In some embodiments, the first chimeric polypeptide can further include a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue

factor domains described herein) and the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art), and/or a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domain(s) (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein). In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in

the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein or known in the art) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain (e.g., any of the exemplary target-binding domains described

herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) disposed between the at least one additional target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) or the first domain of the pair of affinity domains (e.g., any of the exemplary first domains described herein of any of the exemplary pairs of affinity domains described herein) in the first chimeric polypeptide. In some embodiments, the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the first domains described herein or any of the exemplary pairs of affinity domains described herein), directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains. In some embodiments, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) disposed (i) between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) positioned between the soluble tissue factor domain (e.g., any of the exemplary soluble tissue factor domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein), and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) at the N-terminal end and/or the C-terminal end of the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) and the second domain of the pair of affinity domains (e.g., any of the second domains described herein of any of the exemplary pairs of affinity domains described herein) in the second chimeric polypeptide. In some embodiments, at least one of the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) directly abuts the second target-binding domain (e.g., any of the target-binding domains described herein or known in the art) in the second chimeric polypeptide. In some embodiments, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between at least one of the one or more additional target-binding domains (e.g., any of the exemplary target binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target binding domains described herein or known in the art) in the second chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains

bind specifically to the same antigen. In some embodiments, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more (e.g., three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains include the same amino acid sequence. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each include the same amino acid sequence.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens. In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more (e.g., two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more) of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is an antigen-binding domain. In some embodiments, the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain (e.g., a scFv or a single-domain antibody).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more (e.g., two, three, four, five, six, seven, eight, nine, or ten) of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more

additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine protein. Non-limiting examples of soluble interleukin proteins and soluble cytokine proteins include: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or more of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art), and the one or more additional target-binding domains (e.g., any of the exemplary target-

binding domains described herein or known in the art) is a soluble interleukin or cytokine receptor. Non-limiting examples of soluble interleukin receptors and soluble cytokine receptors include: a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKP30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the target-binding domains described herein, the second target-binding domain (e.g., any of the target-binding domains described herein), and the one or more additional target-binding domains (e.g., any of the target-binding domains described herein) can each, independently, bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain (e.g., any of the target-binding domains described herein), the second target-binding domain (e.g., any of the target-binding domains described herein), and the one or more additional binding domains (e.g., any of the target-binding described herein) is a soluble interleukin or cytokine protein. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble interleukin or cytokine protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Multi-Chain Chimeric Polypeptides- Type B

Non-limiting examples of NK cell activating agents are multi-chain chimeric polypeptides that include: (a) a first and second chimeric polypeptide each including: (i) a first target-binding domain; (ii) a Fc domain; and (iii) a first domain of a pair of affinity domains; and (b) a third and fourth chimeric polypeptide each including: (i) a second domain of a pair of affinity domains; and (ii) a second target-binding domain, where the first and second chimeric polypeptides and the third and fourth chimeric polypeptides associate through the binding of the first domain and the second domain of the pair of affinity domains, and the first and second chimeric polypeptides associate through their Fc domains.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain (e.g., any of the first target-binding domains described herein) and the Fc domain (e.g., any of the exemplary Fc domains described herein) directly abut each other in the first and second chimeric polypeptides. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first and second chimeric polypeptides further comprise a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the first target-bnding domain (e.g., any of the exemplary first target-binding domains described herein) and the Fc domain (e.g., any of the exemplary Fc domains described herein) in the first and second chimeric polypeptides.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the Fc domain (e.g., any of the exemplary Fc domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of

any of the exemplary pairs of affinity domains described herein) directly abut each other in the first and second chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first and second chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the Fc domain (e.g., any of the exemplary Fc domains described herein) and the first domain of the pair of affinity domains (e.g., any of the exemplary first domains of any of the exemplary pairs of affinity domains described herein) in the first and second chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) directly abut each other in the third and fourth chimeric polypeptide. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the third and fourth chimeric polypeptide further comprise a linker sequence (e.g., any of the exemplary linker sequences described herein or known in the art) between the second domain of the pair of affinity domains (e.g., any of the exemplary second domains of any of the exemplary pairs of affinity domains described herein) and the second target-binding domain (e.g., any of the exemplary second target-binding domains described herein) in the third and fourth chimeric polypeptide.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain include the same amino acid sequence. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens. In some embodiments of any of the multi-chain chimeric polypeptides

described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain (e.g., any of the exemplary second target-binding domains described herein). In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains (e.g., any of the exemplary second target-binding domains described herein). In some embodiments of any of the multi-chain chimeric polypeptides described herein, the antigen-binding domain (e.g., any of the exemplary second target-binding domains described herein) includes a scFv or a single domain antibody.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain (e.g., any of the exemplary target-

binding domains described herein or known in the art) and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine protein. Non-limiting examples of soluble interleukin proteins and soluble cytokine proteins include: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain (e.g., any of the exemplary target-binding domains described herein or known in the art) is a soluble interleukin or cytokine receptor. Non-limiting examples of soluble interleukin receptors and soluble cytokine receptors include: a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain can each, independently, bind specifically to a target selected from the group of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble interleukin or cytokine

protein is selected from the group of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Tissue Factor

Human tissue factor is a 263 amino-acid transmembrane protein containing three domains: (1) a 219-amino acid N-terminal extracellular domain (residues 1-219); (2) a 22-amino acid transmembrane domain (residues 220-242); and (3) a 21-amino acid cytoplasmic C-terminal tail (residues 242-263) ((UniProtKB Identifier Number: P13726). The cytoplasmic tail contains two phosphorylation sites at Ser253 and Ser258, and one S-palmitoylation site at Cys245. Deletion or mutation of the cytoplasmic domain was not found to affect tissue factor coagulation activity. Tissue factor has one S-palmitoylation site in the intracellular domain of the protein at Cys245. The Cys245 is located at the amino acid terminus of the intracellular domain and close to the membrane surface. The tissue factor transmembrane domain is composed of a single-spanning α-helix.

The extracellular domain of tissue factor, composed of two fibronectin type III domains, is connected to the transmembrane domain through a six-amino acid linker. This linker provides conformational flexibility to decouple the tissue factor extracellular domain from its transmembrane and cytoplasmic domains. Each tissue factor fibronectin type III module is composed of two overlapping β sheets with the top sheet domain containing three antiparallel β-strands and the bottom sheet containing four β-strands. The β-strands are connected by β-loops between strand βA and βB, βC and βD, and βE and βF, all of which are conserved in conformation in the two modules. There are three short α-helix segments connecting the β-strands. A unique feature of tissue factor is a 17-amino acid β-hairpin between strand β10 and strand β11, which is not a common element

of the fibronectin superfamily. The N-terminal domain also contains a 12 amino acid loop between β6F and β7G that is not present in the C-terminal domain and is unique to tissue factor. Such a fibronectin type III domain structure is a feature of the immunoglobulin-like family of protein folds and is conserved among a wide variety of extracellular proteins.

The zymogen FVII is rapidly converted to FVIIa by limited proteolysis once it binds to tissue to form the active tissue factor-FVIIa complex. The FVIIa, which circulates as an enzyme at a concentration of approximately 0.1 nM (1% of plasma FVII), can also bind directly to tissue factor. The allosteric interaction between tissue factor and FVIIa on the tissue factor-FVIIa complex greatly increases the enzymatic activity of FVIIa: an approximate 20- to 100-fold increase in the rate of hydrolysis of small, chromogenic peptidyl substrates, and nearly a million-fold increase in the rate of activation of the natural macromolecular substrates FIX and FX. In concert with allosteric activation of the active site of FVIIa upon binding to tissue factor, the formation of tissue factor-FVIIa complex on phospholipid bilayer (i.e., upon exposure of phosphatidyl-L-serine on membrane surfaces) increases the rate of FIX or FX activation, in a Ca^{2+}-dependent manner, an additional 1,000-fold. The roughly million-fold overall increase in FX activation by tissue factor-FVIIa-phospholipid complex relative to free FVIIa is a critical regulatory point for the coagulation cascade.

FVII is a ~50 kDa, single-chain polypeptide consisting of 406 amino acid residues, with an N-terminal γ-carboxyglutamate-rich (GLA) domain, two epidermal growth factor-like domains (EGF1 and EFG2), and a C-terminal serine protease domain. FVII is activated to FVIIa by a specific proteolytic cleavage of the Ile-^{154}-Arg^{152} bond in the short linker region between the EGF2 and the protease domain. This cleavage results in the light and heavy chains being held together by a single disulfide bond of Cys^{135} and Cys^{262}. FVIIa binds phospholipid membrane in a Ca^{2+}-dependent manner through its N-terminal GLA-domain. Immediately C-terminal to the GLA domain is an aromatic stack and two EGF domains. The aromatic stack connects the GLA to EGF1 domain which binds a single Ca^{2+} ion. Occupancy of this Ca^{2+}-binding site increases FVIIa amidolytic activity and tissue factor association. The catalytic triad consist of His^{193}, Asp^{242}, and

Ser^{344}, and binding of a single Ca^{2+} ion within the FVIIa protease domain is critical for its catalytic activity. Proteolytic activation of FVII to FVIIa frees the newly formed amino terminus at Ile^{153} to fold back and be inserted into the activation pocket forming a salt bridge with the carboxylate of Asp^{343} to generate the oxyanion hole. Formation of this salt bridge is critical for FVIIa activity. However, oxyanion hole formation does not occur in free FVIIa upon proteolytic activation. As a result, FVIIa circulates in a zymogen-like state that is poorly recognized by plasma protease inhibitors, allowing it to circulate with a half-life of approximately 90 minutes.

Tissue factor-mediated positioning of the FVIIa active site above the membrane surface is important for FVIIa towards cognate substrates. Free FVIIa adopts a stable, extended structure when bound to the membrane with its active site positioned ~80Å above the membrane surface. Upon FVIIa binding to tissue factor, the FVa active site is repositioned ~6Å closer to the membrane. This modulation may aid in a proper alignment of the FVIIa catalytic triad with the target substrate cleavage site. Using GLA-domainless FVIIa, it has been shown that the active site was still positioned a similar distance above the membrane, demonstrating that tissue factor is able to fully support FVIIa active site positioning even in the absence of FVIIa-membrane interaction. Additional data showed that tissue factor supported full FVIIa proteolytic activity as long as the tissue factor extracellular domain was tethered in some way to the membrane surface. However, raising the active site of FVIIa greater than 80Å above the membrane surface greatly reduced the ability of the tissue factor-FVIIa complex to activate FX but did not diminish tissue factor-FVIIa amidolytic activity.

Alanine scanning mutagenesis has been used to assess the role of specific amino acid side chains in the tissue factor extracellular domain for interaction with FVIIa (Gibbs et al., *Biochemistry* 33(47): 14003-14010, 1994; Schullek et al., *J Biol Chem* 269(30): 19399-19403, 1994). Alanine substitution identified a limited number of residue positions at which alanine replacements cause 5- to 10-fold lower affinity for FVIIa binding. Most of these residue side chains were found to be well-exposed to solvent in the crystal structure, concordant with macromolecular ligand interaction. The FVIIa ligand-binding site is located over an extensive region at the boundary between the

two modules. In the C-module, residues Arg^{135} and Phe^{140} located on the protruding B-C loop provide an independent contact with FVIIa. Leu^{133} is located at the base of the fingerlike structure and packed into the cleft between the two modules. This provides continuity to a major cluster of important binding residues consisting of Lys^{20}, Thr^{60}, Asp^{58}, and Ile^{22}. Thr^{60} is only partially solvent-exposed and may play a local structural role rather than making a significant contact with ligand. The binding site extends onto the concave side of the intermodule angle involving Glu^{24} and Gln^{110}, and potentially the more distant residue Val^{207}. The binding region extends from Asp58 onto a convex surface area formed by Lys^{48}, Lys^{46}, Gln^{37}, Asp^{44}, and Trp^{45}. Trp^{45} and Asp^{44} do not interact independently with FVIIa, indicating that the mutational effect at the Trp^{45} position may reflect a structural importance of this side chain for the local packing of the adjacent Asp^{44} and Gln^{37} side chain. The interactive area further includes two surface-exposed aromatic residues, Phe^{76} and Tyr^{78}, which form part of the hydrophobic cluster in the N-module.

The known physiologic substrates of tissue factor-FVIIa are FVII, FIX, and FX and certain proteinase-activated receptors. Mutational analysis has identified a number of residues that, when mutated, support full FVIIa amidolytic activity towards small peptidyl substrates but are deficient in their ability to support macromolecular substrate (i.e., FVII, FIX, and FX) activation (Ruf et al., *J Biol Chem* 267(31): 22206-22210, 1992; Ruf et al., *J Biol Chem* 267(9): 6375-6381, 1992; Huang et al., *J Biol Chem* 271(36): 21752-21757, 1996; Kirchhofer et al., *Biochemistry* 39(25): 7380-7387, 2000). The tissue factor loop region at residues 159-165, and residues in or adjacent to this flexible loop have been shown to be critical for the proteolytic activity of the tissue factor-FVIIa complex. This defines the proposed substrate-binding exosite region of tissue factor that is quite distant from the FVIIa active site. A substitution of the glycine residue by a marginally bulkier residue alanine, significantly impairs tissue factor-FVIIa proteolytic activity. This suggests that the flexibility afforded by glycine is critical for the loop of residues 159-165 for tissue factor macromolecular substrate recognition.

The residues Lys^{165} and Lys^{166} have also been demonstrated to be important for substrate recognition and binding. Mutation of either of these residues to alanine results

in a significant decrease in the tissue factor co-factor function. Lys^{165} and Lys^{166} face away from each other, with Lys^{165} pointing towards FVIIa in most tissue factor-FVIIa structures, and Lys^{166} pointing into the substrate binding exosite region in the crystal structure. Putative salt bridge formation between Lys^{165} of and Gla^{35} of FVIIa would support the notion that tissue factor interaction with the GLA domain of FVIIa modulates substrate recognition. These results suggest that the C-terminal portion of the tissue factor ectodomain directly interacts with the GLA-domain, the possible adjacent EGF1 domains, of FIX and FX, and that the presence of the FVIIa GLA-domain may modulate these interactions either directly or indirectly.

Soluble Tissue Factor Domain

In some embodiments of any of the polypeptides, compositions, or methods described herein, the soluble tissue factor domain can be a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain. In some examples, the soluble tissue factor domain can be a tissue factor mutant, wherein a wildtype tissue factor polypeptide lacking the signal sequence, the transmembrane domain, and the intracellular domain, and has been further modified at selected amino acids. In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some examples, the soluble tissue factor domain can be a soluble rat tissue factor domain. Non-limiting examples of soluble human tissue factor domains, a mouse soluble tissue factor domain, a rat soluble tissue factor domain, and mutant soluble tissue factor domains are shown below.

Exemplary Soluble Human Tissue Factor Domain (SEQ ID NO: 93)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQ
PTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGK
KTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRE

Exemplary Nucleic Acid Encoding Soluble Human Tissue Factor Domain (SEQ ID NO: 94)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACC
GTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCAC
CGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACC
TACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTC
CGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGA
CCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGT
GAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTT
CTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGG
AAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTT
TAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATC
CCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGG
GCCAAGAAAAGGGCGAGTTCCGGGAG

Exemplary Soluble Mouse Tissue Factor Domain (SEQ ID NO: 95)

agipekafnltwistdfktilewqpkptnytytvqisdrsrnwknkcfstt
dtecdltdeivkdvtwayeakvlsvprrnsvhgdgdqlvihgeeppftnap
kflpyrdtnlgqpviqqfeqdgrklnvvvkdsltlvrkngtfltlrqvfgk
dlgyiityrkgsstgkktnitntnefsidveegvsycffvqamifsrktnq
nspgsstvcteqwksflge

Exemplary Soluble Rat Tissue Factor Domain (SEQ ID NO: 96)

Agtppgkafnltwistdfktilewqpkptnytytvqisdrsrnwkykctgt
tdtecdltdeivkdvnwtyearvlsvpwrnsthgketlfgthgeeppftna
rkflpyrdtkigqpviqkyeqggtklkvtvkdsftlvrkngtfltlrqvfg
ndlgyiltyrkdsstgrktntthtneflidvekgvsycffaqavifsrktn
hkspesitkcteqwksvlge

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 97)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDWKSKCFYTT
DTECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNL

GQPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSS
GKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEF
RE

Exemplary Mutant Soluble Human Tissue Factor Domain (SEQ ID NO: 98)

SGTTNTVAAYNLTWKSTNFATALEWEPKPVNQVYTVQISTKSGDAKSKCFYTTD
TECALTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLAENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVARNNTALSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

In some embodiments, a soluble tissue factor domain can include a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 93, 95, 96, 97 or 98. In some embodiments, a soluble tissue factor domain can include a sequence of SEQ ID NO: 93, 95, 96, 97, or 98, with one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its N-terminus and/or one to twenty amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids removed from its C-terminus.

As can be appreciated in the art, one skilled in the art would understand that mutation of amino acids that are conserved between different mammalian species is more likely to decrease the activity and/or structural stability of the protein, while mutation of amino acids that are not conserved between different mammalian species is less likely to decrease the activity and/or structural stability of the protein.

In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain is not capable of binding to Factor VIIa. In some examples of any of the multi-chain chimeric polypeptides described herein, the soluble tissue factor domain does not convert inactive Factor X into Factor Xa. In some

embodiments of any of the multi-chain chimeric polypeptides described herein, the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

In some examples, the soluble tissue factor domain can be a soluble human tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble mouse tissue factor domain. In some embodiments, the soluble tissue factor domain can be a soluble rat tissue factor domain.

In some examples, the soluble tissue factor domain does not include one or more (e.g., two, three, four, five, six, or seven) of: a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein; an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein; a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein; an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein; a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein; an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein. In some embodiments, the mutant soluble tissue factor possesses the amino acid sequence of SEQ ID NO: 97 or SEQ ID NO: 98.

In some examples, the soluble tissue factor domain can be encoded by a nucleic acid including a sequence that is at least 70% identical, at least 72% identical, at least 74% identical, at least 76% identical, at least 78% identical, at least 80% identical, at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical to SEQ ID NO: 94.

In some embodiments, the soluble tissue factor domain can have a total length of about 20 amino acids to about 220 amino acids, about 20 amino acids to about 215 amino acids, about 20 amino acids to about 210 amino acids, about 20 amino acids to about 205

amino acids, about 20 amino acids to about 200 amino acids, about 20 amino acids to about 195 amino acids, about 20 amino acids to about 190 amino acids, about 20 amino acids to about 185 amino acids, about 20 amino acids to about 180 amino acids, about 20 amino acids to about 175 amino acids, about 20 amino acids to about 170 amino acids, about 20 amino acids to about 165 amino acids, about 20 amino acids to about 160 amino acids, about 20 amino acids to about 155 amino acids, about 20 amino acids to about 150 amino acids, about 20 amino acids to about 145 amino acids, about 20 amino acids to about 140 amino acids, about 20 amino acids to about 135 amino acids, about 20 amino acids to about 130 amino acids, about 20 amino acids to about 125 amino acids, about 20 amino acids to about 120 amino acids, about 20 amino acids to about 115 amino acids, about 20 amino acids to about 110 amino acids, about 20 amino acids to about 105 amino acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about 95 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids to about 85 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 75 amino acids, about 20 amino acids to about 70 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 30 amino acids, about 30 amino acids to about 220 amino acids, about 30 amino acids to about 215 amino acids, about 30 amino acids to about 210 amino acids, about 30 amino acids to about 205 amino acids, about 30 amino acids to about 200 amino acids, about 30 amino acids to about 195 amino acids, about 30 amino acids to about 190 amino acids, about 30 amino acids to about 185 amino acids, about 30 amino acids to about 180 amino acids, about 30 amino acids to about 175 amino acids, about 30 amino acids to about 170 amino acids, about 30 amino acids to about 165 amino acids, about 30 amino acids to about 160 amino acids, about 30 amino acids to about 155 amino acids, about 30 amino acids to about 150 amino acids, about 30 amino acids to about 145 amino acids, about 30 amino acids to about 140 amino acids, about 30 amino acids to about 135 amino acids, about 30 amino acids to about 130 amino acids, about 30 amino acids to about 125 amino acids, about 30 amino acids to about 120 amino acids, about 30 amino acids to about 115 amino acids, about 30 amino acids to about 110 amino acids, about 30 amino acids to about 105

amino acids, about 30 amino acids to about 100 amino acids, about 30 amino acids to about 95 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids to about 85 amino acids, about 30 amino acids to about 80 amino acids, about 30 amino acids to about 75 amino acids, about 30 amino acids to about 70 amino acids, about 30 amino acids to about 60 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 40 amino acids, about 40 amino acids to about 220 amino acids, about 40 amino acids to about 215 amino acids, about 40 amino acids to about 210 amino acids, about 40 amino acids to about 205 amino acids, about 40 amino acids to about 200 amino acids, about 40 amino acids to about 195 amino acids, about 40 amino acids to about 190 amino acids, about 40 amino acids to about 185 amino acids, about 40 amino acids to about 180 amino acids, about 40 amino acids to about 175 amino acids, about 40 amino acids to about 170 amino acids, about 40 amino acids to about 165 amino acids, about 40 amino acids to about 160 amino acids, about 40 amino acids to about 155 amino acids, about 40 amino acids to about 150 amino acids, about 40 amino acids to about 145 amino acids, about 40 amino acids to about 140 amino acids, about 40 amino acids to about 135 amino acids, about 40 amino acids to about 130 amino acids, about 40 amino acids to about 125 amino acids, about 40 amino acids to about 120 amino acids, about 40 amino acids to about 115 amino acids, about 40 amino acids to about 110 amino acids, about 40 amino acids to about 105 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 95 amino acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about 85 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 75 amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino acids to about 50 amino acids, about 50 amino acids to about 220 amino acids, about 50 amino acids to about 215 amino acids, about 50 amino acids to about 210 amino acids, about 50 amino acids to about 205 amino acids, about 50 amino acids to about 200 amino acids, about 50 amino acids to about 195 amino acids, about 50 amino acids to about 190 amino acids, about 50 amino acids to about 185 amino acids, about 50 amino acids to about 180 amino acids, about 50 amino acids to about 175 amino acids, about 50 amino acids to about 170 amino acids, about 50 amino acids to about 165

amino acids, about 50 amino acids to about 160 amino acids, about 50 amino acids to about 155 amino acids, about 50 amino acids to about 150 amino acids, about 50 amino acids to about 145 amino acids, about 50 amino acids to about 140 amino acids, about 50 amino acids to about 135 amino acids, about 50 amino acids to about 130 amino acids, about 50 amino acids to about 125 amino acids, about 50 amino acids to about 120 amino acids, about 50 amino acids to about 115 amino acids, about 50 amino acids to about 110 amino acids, about 50 amino acids to about 105 amino acids, about 50 amino acids to about 100 amino acids, about 50 amino acids to about 95 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 85 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 75 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 60 amino acids, about 60 amino acids to about 220 amino acids, about 60 amino acids to about 215 amino acids, about 60 amino acids to about 210 amino acids, about 60 amino acids to about 205 amino acids, about 60 amino acids to about 200 amino acids, about 60 amino acids to about 195 amino acids, about 60 amino acids to about 190 amino acids, about 60 amino acids to about 185 amino acids, about 60 amino acids to about 180 amino acids, about 60 amino acids to about 175 amino acids, about 60 amino acids to about 170 amino acids, about 60 amino acids to about 165 amino acids, about 60 amino acids to about 160 amino acids, about 60 amino acids to about 155 amino acids, about 60 amino acids to about 150 amino acids, about 60 amino acids to about 145 amino acids, about 60 amino acids to about 140 amino acids, about 60 amino acids to about 135 amino acids, about 60 amino acids to about 130 amino acids, about 60 amino acids to about 125 amino acids, about 60 amino acids to about 120 amino acids, about 60 amino acids to about 115 amino acids, about 60 amino acids to about 110 amino acids, about 60 amino acids to about 105 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 95 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 85 amino acids, about 60 amino acids to about 80 amino acids, about 60 amino acids to about 75 amino acids, about 60 amino acids to about 70 amino acids, about 70 amino acids to about 220 amino acids, about 70 amino acids to about 215 amino acids, about 70 amino acids to about 210 amino acids, about 70 amino acids to about 205

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about 120 amino acids to about 130 amino acids, about 120 amino acids to about 125 amino acids, about 125 amino acids to about 220 amino acids, about 125 amino acids to about 215 amino acids, about 125 amino acids to about 210 amino acids, about 125 amino acids to about 205 amino acids, about 125 amino acids to about 200 amino acids, about 125 amino acids to about 195 amino acids, about 125 amino acids to about 190 amino acids, about 125 amino acids to about 185 amino acids, about 125 amino acids to about 180 amino acids, about 125 amino acids to about 175 amino acids, about 125 amino acids to about 170 amino acids, about 125 amino acids to about 165 amino acids, about 125 amino acids to about 160 amino acids, about 125 amino acids to about 155 amino acids, about 125 amino acids to about 150 amino acids, about 125 amino acids to about 145 amino acids, about 125 amino acids to about 140 amino acids, about 125 amino acids to about 135 amino acids, about 125 amino acids to about 130 amino acids, about 130 amino acids to about 220 amino acids, about 130 amino acids to about 215 amino acids, about 130 amino acids to about 210 amino acids, about 130 amino acids to about 205 amino acids, about 130 amino acids to about 200 amino acids, about 130 amino acids to about 195 amino acids, about 130 amino acids to about 190 amino acids, about 130 amino acids to about 185 amino acids, about 130 amino acids to about 180 amino acids, about 130 amino acids to about 175 amino acids, about 130 amino acids to about 170 amino acids, about 130 amino acids to about 165 amino acids, about 130 amino acids to about 160 amino acids, about 130 amino acids to about 155 amino acids, about 130 amino acids to about 150 amino acids, about 130 amino acids to about 145 amino acids, about 130 amino acids to about 140 amino acids, about 130 amino acids to about 135 amino acids, about 135 amino acids to about 220 amino acids, about 135 amino acids to about 215 amino acids, about 135 amino acids to about 210 amino acids, about 135 amino acids to about 205 amino acids, about 135 amino acids to about 200 amino acids, about 135 amino acids to about 195 amino acids, about 135 amino acids to about 190 amino acids, about 135 amino acids to about 185 amino acids, about 135 amino acids to about 180 amino acids, about 135 amino acids to about 175 amino acids, about 135 amino acids to about 170 amino acids, about 135 amino acids to about 165 amino acids, about 135 amino acids to about 160 amino acids, about 135 amino acids to

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about 215 amino acids, about 180 amino acids to about 210 amino acids, about 180 amino acids to about 205 amino acids, about 180 amino acids to about 200 amino acids, about 180 amino acids to about 195 amino acids, about 180 amino acids to about 190 amino acids, about 180 amino acids to about 185 amino acids, about 185 amino acids to about 220 amino acids, about 185 amino acids to about 215 amino acids, about 185 amino acids to about 210 amino acids, about 185 amino acids to about 205 amino acids, about 185 amino acids to about 200 amino acids, about 185 amino acids to about 195 amino acids, about 185 amino acids to about 190 amino acids, about 190 amino acids to about 220 amino acids, about 190 amino acids to about 215 amino acids, about 190 amino acids to about 210 amino acids, about 190 amino acids to about 205 amino acids, about 190 amino acids to about 200 amino acids, about 190 amino acids to about 195 amino acids, about 195 amino acids to about 220 amino acids, about 195 amino acids to about 215 amino acids, about 195 amino acids to about 210 amino acids, about 195 amino acids to about 205 amino acids, about 195 amino acids to about 200 amino acids, about 200 amino acids to about 220 amino acids, about 200 amino acids to about 215 amino acids, about 200 amino acids to about 210 amino acids, about 200 amino acids to about 205 amino acids, about 205 amino acids to about 220 amino acids, about 205 amino acids to about 215 amino acids, about 205 amino acids to about 210 amino acids, about 210 amino acids to about 220 amino acids, about 210 amino acids to about 215 amino acids, or about 215 amino acids to about 220 amino acids.

Linker Sequences

In some embodiments, the linker sequence can be a flexible linker sequence. Non-limiting examples of linker sequences that can be used are described in Klein et al., *Protein Engineering, Design & Selection* 27(10):325–330, 2014; Priyanka et al., *Protein Sci.* 22(2):153–167, 2013. In some examples, the linker sequence is a synthetic linker sequence.

In some embodiments of any of the single-chain chimeric polypeptides described herein can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker

sequences described herein or known in the art). In some embodiments of any of the single-chain chimeric polypeptides described herein can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art). In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide can include one, two, three, four, five, six, seven, eight, nine, or ten linker sequence(s) (e.g., the same or different linker sequences, e.g., any of the exemplary linker sequences described herein or known in the art).

In some embodiments, a linker sequence can have a total length of 1 amino acid to about 100 amino acids, 1 amino acid to about 90 amino acids, 1 amino acid to about 80 amino acids, 1 amino acid to about 70 amino acids, 1 amino acid to about 60 amino acids, 1 amino acid to about 50 amino acids, 1 amino acid to about 45 amino acids, 1 amino acid to about 40 amino acids, 1 amino acid to about 35 amino acids, 1 amino acid to about 30 amino acids, 1 amino acid to about 25 amino acids, 1 amino acid to about 24 amino acids, 1 amino acid to about 22 amino acids, 1 amino acid to about 20 amino acids, 1 amino acid to about 18 amino acids, 1 amino acid to about 16 amino acids, 1 amino acid to about 14 amino acids, 1 amino acid to about 12 amino acids, 1 amino acid to about 10 amino acids, 1 amino acid to about 8 amino acids, 1 amino acid to about 6 amino acids, 1 amino acid to about 4 amino acids, about 2 amino acids to about 100 amino acids, about 2 amino acids to about 90 amino acids, about 2 amino acids to about 80 amino acids, about 2 amino acids to about 70 amino acids, about 2 amino acids to about 60 amino acids, about 2 amino acids to about 50 amino acids, about 2 amino acids to about 45 amino acids, about 2 amino acids to about 40 amino acids, about 2 amino acids to about 35 amino acids, about 2 amino acids to about 30 amino acids, about 2 amino acids to about 25 amino acids, about 2 amino acids to about 24 amino acids, about

2 amino acids to about 22 amino acids, about 2 amino acids to about 20 amino acids, about 2 amino acids to about 18 amino acids, about 2 amino acids to about 16 amino acids, about 2 amino acids to about 14 amino acids, about 2 amino acids to about 12 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 6 amino acids, about 2 amino acids to about 4 amino acids, about 4 amino acids to about 100 amino acids, about 4 amino acids to about 90 amino acids, about 4 amino acids to about 80 amino acids, about 4 amino acids to about 70 amino acids, about 4 amino acids to about 60 amino acids, about 4 amino acids to about 50 amino acids, about 4 amino acids to about 45 amino acids, about 4 amino acids to about 40 amino acids, about 4 amino acids to about 35 amino acids, about 4 amino acids to about 30 amino acids, about 4 amino acids to about 25 amino acids, about 4 amino acids to about 24 amino acids, about 4 amino acids to about 22 amino acids, about 4 amino acids to about 20 amino acids, about 4 amino acids to about 18 amino acids, about 4 amino acids to about 16 amino acids, about 4 amino acids to about 14 amino acids, about 4 amino acids to about 12 amino acids, about 4 amino acids to about 10 amino acids, about 4 amino acids to about 8 amino acids, about 4 amino acids to about 6 amino acids, about 6 amino acids to about 100 amino acids, about 6 amino acids to about 90 amino acids, about 6 amino acids to about 80 amino acids, about 6 amino acids to about 70 amino acids, about 6 amino acids to about 60 amino acids, about 6 amino acids to about 50 amino acids, about 6 amino acids to about 45 amino acids, about 6 amino acids to about 40 amino acids, about 6 amino acids to about 35 amino acids, about 6 amino acids to about 30 amino acids, about 6 amino acids to about 25 amino acids, about 6 amino acids to about 24 amino acids, about 6 amino acids to about 22 amino acids, about 6 amino acids to about 20 amino acids, about 6 amino acids to about 18 amino acids, about 6 amino acids to about 16 amino acids, about 6 amino acids to about 14 amino acids, about 6 amino acids to about 12 amino acids, about 6 amino acids to about 10 amino acids, about 6 amino acids to about 8 amino acids, about 8 amino acids to about 100 amino acids, about 8 amino acids to about 90 amino acids, about 8 amino acids to about 80 amino acids, about 8 amino acids to about 70 amino acids, about 8 amino acids to about 60 amino acids, about 8 amino acids to about 50 amino acids, about 8

amino acids to about 45 amino acids, about 8 amino acids to about 40 amino acids, about 8 amino acids to about 35 amino acids, about 8 amino acids to about 30 amino acids, about 8 amino acids to about 25 amino acids, about 8 amino acids to about 24 amino acids, about 8 amino acids to about 22 amino acids, about 8 amino acids to about 20 amino acids, about 8 amino acids to about 18 amino acids, about 8 amino acids to about 16 amino acids, about 8 amino acids to about 14 amino acids, about 8 amino acids to about 12 amino acids, about 8 amino acids to about 10 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 90 amino acids, about 10 amino acids to about 80 amino acids, about 10 amino acids to about 70 amino acids, about 10 amino acids to about 60 amino acids, about 10 amino acids to about 50 amino acids, about 10 amino acids to about 45 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 35 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 25 amino acids, about 10 amino acids to about 24 amino acids, about 10 amino acids to about 22 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 18 amino acids, about 10 amino acids to about 16 amino acids, about 10 amino acids to about 14 amino acids, about 10 amino acids to about 12 amino acids, about 12 amino acids to about 100 amino acids, about 12 amino acids to about 90 amino acids, about 12 amino acids to about 80 amino acids, about 12 amino acids to about 70 amino acids, about 12 amino acids to about 60 amino acids, about 12 amino acids to about 50 amino acids, about 12 amino acids to about 45 amino acids, about 12 amino acids to about 40 amino acids, about 12 amino acids to about 35 amino acids, about 12 amino acids to about 30 amino acids, about 12 amino acids to about 25 amino acids, about 12 amino acids to about 24 amino acids, about 12 amino acids to about 22 amino acids, about 12 amino acids to about 20 amino acids, about 12 amino acids to about 18 amino acids, about 12 amino acids to about 16 amino acids, about 12 amino acids to about 14 amino acids, about 14 amino acids to about 100 amino acids, about 14 amino acids to about 90 amino acids, about 14 amino acids to about 80 amino acids, about 14 amino acids to about 70 amino acids, about 14 amino acids to about 60 amino acids, about 14 amino acids to about 50 amino acids, about 14 amino acids to about 45 amino acids, about 14 amino acids to about 40

amino acids, about 14 amino acids to about 35 amino acids, about 14 amino acids to about 30 amino acids, about 14 amino acids to about 25 amino acids, about 14 amino acids to about 24 amino acids, about 14 amino acids to about 22 amino acids, about 14 amino acids to about 20 amino acids, about 14 amino acids to about 18 amino acids, about 14 amino acids to about 16 amino acids, about 16 amino acids to about 100 amino acids, about 16 amino acids to about 90 amino acids, about 16 amino acids to about 80 amino acids, about 16 amino acids to about 70 amino acids, about 16 amino acids to about 60 amino acids, about 16 amino acids to about 50 amino acids, about 16 amino acids to about 45 amino acids, about 16 amino acids to about 40 amino acids, about 16 amino acids to about 35 amino acids, about 16 amino acids to about 30 amino acids, about 16 amino acids to about 25 amino acids, about 16 amino acids to about 24 amino acids, about 16 amino acids to about 22 amino acids, about 16 amino acids to about 20 amino acids, about 16 amino acids to about 18 amino acids, about 18 amino acids to about 100 amino acids, about 18 amino acids to about 90 amino acids, about 18 amino acids to about 80 amino acids, about 18 amino acids to about 70 amino acids, about 18 amino acids to about 60 amino acids, about 18 amino acids to about 50 amino acids, about 18 amino acids to about 45 amino acids, about 18 amino acids to about 40 amino acids, about 18 amino acids to about 35 amino acids, about 18 amino acids to about 30 amino acids, about 18 amino acids to about 25 amino acids, about 18 amino acids to about 24 amino acids, about 18 amino acids to about 22 amino acids, about 18 amino acids to about 20 amino acids, about 20 amino acids to about 100 amino acids, about 20 amino acids to about 90 amino acids, about 20 amino acids to about 80 amino acids, about 20 amino acids to about 70 amino acids, about 20 amino acids to about 60 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 45 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 35 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 25 amino acids, about 20 amino acids to about 24 amino acids, about 20 amino acids to about 22 amino acids, about 22 amino acids to about 100 amino acids, about 22 amino acids to about 90 amino acids, about 22 amino acids to about 80 amino acids, about 22 amino acids to about 70 amino acids, about 22 amino acids to about 60

amino acids, about 22 amino acids to about 50 amino acids, about 22 amino acids to about 45 amino acids, about 22 amino acids to about 40 amino acids, about 22 amino acids to about 35 amino acids, about 22 amino acids to about 30 amino acids, about 22 amino acids to about 25 amino acids, about 22 amino acids to about 24 amino acids, about 25 amino acids to about 100 amino acids, about 25 amino acids to about 90 amino acids, about 25 amino acids to about 80 amino acids, about 25 amino acids to about 70 amino acids, about 25 amino acids to about 60 amino acids, about 25 amino acids to about 50 amino acids, about 25 amino acids to about 45 amino acids, about 25 amino acids to about 40 amino acids, about 25 amino acids to about 35 amino acids, about 25 amino acids to about 30 amino acids, about 30 amino acids to about 100 amino acids, about 30 amino acids to about 90 amino acids, about 30 amino acids to about 80 amino acids, about 30 amino acids to about 70 amino acids, about 30 amino acids to about 60 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 45 amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to about 35 amino acids, about 35 amino acids to about 100 amino acids, about 35 amino acids to about 90 amino acids, about 35 amino acids to about 80 amino acids, about 35 amino acids to about 70 amino acids, about 35 amino acids to about 60 amino acids, about 35 amino acids to about 50 amino acids, about 35 amino acids to about 45 amino acids, about 35 amino acids to about 40 amino acids, about 40 amino acids to about 100 amino acids, about 40 amino acids to about 90 amino acids, about 40 amino acids to about 80 amino acids, about 40 amino acids to about 70 amino acids, about 40 amino acids to about 60 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 45 amino acids, about 45 amino acids to about 100 amino acids, about 45 amino acids to about 90 amino acids, about 45 amino acids to about 80 amino acids, about 45 amino acids to about 70 amino acids, about 45 amino acids to about 60 amino acids, about 45 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, about 50 amino acids to about 90 amino acids, about 50 amino acids to about 80 amino acids, about 50 amino acids to about 70 amino acids, about 50 amino acids to about 60 amino acids, about 60 amino acids to about 100 amino acids, about 60 amino acids to about 90 amino acids, about 60 amino acids to about 80

amino acids, about 60 amino acids to about 70 amino acids, about 70 amino acids to about 100 amino acids, about 70 amino acids to about 90 amino acids, about 70 amino acids to about 80 amino acids, about 80 amino acids to about 100 amino acids, about 80 amino acids to about 90 amino acids, or about 90 amino acids to about 100 amino acids.

In some embodiments, the linker is rich in glycine (Gly or G) residues. In some embodiments, the linker is rich in serine (Ser or S) residues. In some embodiments, the linker is rich in glycine and serine residues. In some embodiments, the linker has one or more glycine-serine residue pairs (GS), e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GS pairs. In some embodiments, the linker has one or more Gly-Gly-Gly-Ser (GGGS) (SEQ ID NO: 99) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGS (SEQ ID NO: 99) sequences. In some embodiments, the linker has one or more Gly-Gly-Gly-Gly-Ser (GGGGS) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGGGS (SEQ ID NO: 100) sequences. In some embodiments, the linker has one or more Gly-Gly-Ser-Gly (GGSG) (SEQ ID NO: 101) sequences, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more GGSG (SEQ ID NO: 101) sequences.

In some embodiments, the linker sequence can comprise or consist of GGGGSGGGGSGGGGS (SEQ ID NO: 102). In some embodiments, the linker sequence can be encoded by a nucleic acid comprising or consisting of: GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT (SEQ ID NO: 103). In some embodiments, the linker sequence can comprise or consist of: GGGSGGGS (SEQ ID NO: 104),

Target-Binding Domains

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the additional one or more target-binding domains can be an antigen-binding domain (e.g., any of the exemplary antigen-binding domains described herein or known in the art), a soluble interleukin or cytokine protein (e.g., any of the exemplary soluble interleukin proteins or soluble cytokine proteins described herein), and a soluble interleukin or

cytokine receptor (e.g., any of the exemplary soluble interleukin receptors or soluble cytokine receptors described herein).

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain, the second target-binding domain, and/or the one or more additional target-binding domains can each independent have a total number of amino acids of about 5 amino acids to about 1000 amino acids, about 5 amino acids to about 950 amino acids, about 5 amino acids to about 900 amino acids, about 5 amino acids to about 850 amino acids, about 5 amino acids to about 800 amino acids, about 5 amino acids to about 750 amino acids, about 5 amino acids to about 700 amino acids, about 5 amino acids to about 650 amino acids, about 5 amino acids to about 600 amino acids, about 5 amino acids to about 550 amino acids, about 5 amino acids to about 500 amino acids, about 5 amino acids to about 450 amino acids, about 5 amino acids to about 400 amino acids, about 5 amino acids to about 350 amino acids, about 5 amino acids to about 300 amino acids, about 5 amino acids to about 280 amino acids, about 5 amino acids to about 260 amino acids, about 5 amino acids to about 240 amino acids, about 5 amino acids to about 220 amino acids, about 5 amino acids to about 200 amino acids, about 5 amino acids to about 195 amino acids, about 5 amino acids to about 190 amino acids, about 5 amino acids to about 185 amino acids, about 5 amino acids to about 180 amino acids, about 5 amino acids to about 175 amino acids, about 5 amino acids to about 170 amino acids, about 5 amino acids to about 165 amino acids, about 5 amino acids to about 160 amino acids, about 5 amino acids to about 155 amino acids, about 5 amino acids to about 150 amino acids, about 5 amino acids to about 145 amino acids, about 5 amino acids to about 140 amino acids, about 5 amino acids to about 135 amino acids, about 5 amino acids to about 130 amino acids, about 5 amino acids to about 125 amino acids, about 5 amino acids to about 120 amino acids, about 5 amino acids to about 115 amino acids, about 5 amino acids to about 110 amino acids, about 5 amino acids to about 105 amino acids, about 5 amino acids to about 100 amino acids, about 5 amino acids to about 95 amino acids, about 5 amino acids to about 90 amino acids, about 5 amino acids to about 85 amino acids, about 5 amino acids to about 80 amino acids, about 5 amino acids to about 75 amino acids, about 5 amino acids to about 70 amino acids, about 5

amino acids to about 65 amino acids, about 5 amino acids to about 60 amino acids, about 5 amino acids to about 55 amino acids, about 5 amino acids to about 50 amino acids, about 5 amino acids to about 45 amino acids, about 5 amino acids to about 40 amino acids, about 5 amino acids to about 35 amino acids, about 5 amino acids to about 30 amino acids, about 5 amino acids to about 25 amino acids, about 5 amino acids to about 20 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 10 amino acids, about 10 amino acids to about 1000 amino acids, about 10 amino acids to about 950 amino acids, about 10 amino acids to about 900 amino acids, about 10 amino acids to about 850 amino acids, about 10 amino acids to about 800 amino acids, about 10 amino acids to about 750 amino acids, about 10 amino acids to about 700 amino acids, about 10 amino acids to about 650 amino acids, about 10 amino acids to about 600 amino acids, about 10 amino acids to about 550 amino acids, about 10 amino acids to about 500 amino acids, about 10 amino acids to about 450 amino acids, about 10 amino acids to about 400 amino acids, about 10 amino acids to about 350 amino acids, about 10 amino acids to about 300 amino acids, about 10 amino acids to about 280 amino acids, about 10 amino acids to about 260 amino acids, about 10 amino acids to about 240 amino acids, about 10 amino acids to about 220 amino acids, about 10 amino acids to about 200 amino acids, about 10 amino acids to about 195 amino acids, about 10 amino acids to about 190 amino acids, about 10 amino acids to about 185 amino acids, about 10 amino acids to about 180 amino acids, about 10 amino acids to about 175 amino acids, about 10 amino acids to about 170 amino acids, about 10 amino acids to about 165 amino acids, about 10 amino acids to about 160 amino acids, about 10 amino acids to about 155 amino acids, about 10 amino acids to about 150 amino acids, about 10 amino acids to about 145 amino acids, about 10 amino acids to about 140 amino acids, about 10 amino acids to about 135 amino acids, about 10 amino acids to about 130 amino acids, about 10 amino acids to about 125 amino acids, about 10 amino acids to about 120 amino acids, about 10 amino acids to about 115 amino acids, about 10 amino acids to about 110 amino acids, about 10 amino acids to about 105 amino acids, about 10 amino acids to about 100 amino acids, about 10 amino acids to about 95 amino acids, about 10 amino acids to about 90 amino acids, about 10 amino acids to about 85 amino acids, about 10 amino acids to

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Any of the target-binding domains described herein can bind to its target with a dissociation equilibrium constant (K_D) of less than 1 x 10^{-7} M, less than 1 x 10^{-8} M, less than 1 x 10^{-9} M, less than 1 x 10^{-10} M, less than 1 x 10^{-11} M, less than 1 x 10^{-12} M, or less than 1 x 10^{-13} M. In some embodiments, the antigen-binding protein construct provided herein can bind to an identifying antigen with a K_D of about 1 x 10^{-3} M to about 1 x 10^{-5} M, about 1 x 10^{-4} M to about 1 x 10^{-6} M, about 1 x 10^{-5} M to about 1 x 10^{-7} M, about 1 x 10^{-6} M to about 1 x 10^{-8} M, about 1 x 10^{-7} M to about 1 x 10^{-9} M, about 1 x 10^{-8} M to about 1 x 10^{-10} M, or about 1 x 10^{-9} M to about 1 x 10^{-11} M (inclusive).

Any of the target-binding domains described herein can bind to its target with a K_D of between about 1 pM to about 30 nM (e.g., about 1 pM to about 25 nM, about 1 pM to about 20 nM, about 1 pM to about 15 nM, about 1 pM to about 10 nM, about 1 pM to about 5 nM, about 1 pM to about 2 nM, about 1 pM to about 1 nM, about 1 pM to about 950 pM, about 1 pM to about 900 pM, about 1 pM to about 850 pM, about 1 pM to about 800 pM, about 1 pM to about 750 pM, about 1 pM to about 700 pM, about 1 pM to about 650 pM, about 1 pM to about 600 pM, about 1 pM to about 550 pM,about 1 pM to about 500 pM, about 1 pM to about 450 pM, about 1 pM to about 400 pM, about 1 pM to about 350 pM, about 1 pM to about 300 pM, about 1 pM to about 250 pM, about 1 pM to about 200 pM, about 1 pM to about 150 pM, about 1 pM to about 100 pM, about 1 pM to about 90 pM, about 1 pM to about 80 pM, about 1 pM to about 70 pM, about 1 pM to about 60 pM, about 1 pM to about 50 pM, about 1 pM to about 40 pM, about 1 pM to about 30 pM, about 1 pM to about 20 pM, about 1 pM to about 10 pM, about 1 pM to about 5 pM, about 1 pM to about 4 pM, about 1 pM to about 3 pM, about 1 pM to about 2 pM, about 2 pM to about 30 nM, about 2 pM to about 25 nM, about 2 pM to about 20 nM, about 2 pM to about 15 nM, about 2 pM to about 10 nM, about 2 pM to about 5 nM, about 2 pM to about 2 nM, about 2 pM to about 1 nM, about 2 pM to about 950 pM, about 2 pM to about 900 pM, about 2 pM to about 850 pM, about 2 pM to about 800 pM, about 2 pM to about 750 pM, about 2 pM to about 700 pM, about 2 pM to about 650 pM, about 2 pM to about 600 pM, about 2 pM to about 550 pM, about 2 pM to about 500 pM, about 2 pM to about 450 pM, about 2 pM to about 400 pM, about 2 pM to about 350 pM, about 2 pM to about 300 pM, about 2 pM to about 250 pM, about 2 pM to about 200 pM, about 2 pM to

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about 60 pM to about 300 pM, about 60 pM to about 250 pM, about 60 pM to about 200 pM, about 60 pM to about 150 pM, about 60 pM to about 100 pM, about 60 pM to about 90 pM, about 60 pM to about 80 pM, about 60 pM to about 70 pM, about 70 pM to about 30 nM, about 70 pM to about 25 nM, about 70 pM to about 30 nM, about 70 pM to about 15 nM, about 70 pM to about 10 nM, about 70 pM to about 5 nM, about 70 pM to about 2 nM, about 70 pM to about 1 nM, about 70 pM to about 950 pM, about 70 pM to about 900 pM, about 70 pM to about 850 pM, about 70 pM to about 800 pM, about 70 pM to about 750 pM, about 70 pM to about 700 pM, about 70 pM to about 650 pM, about 70 pM to about 600 pM, about 70 pM to about 550 pM, about 70 pM to about 500 pM, about 70 pM to about 450 pM, about 70 pM to about 400 pM, about 70 pM to about 350 pM, about 70 pM to about 300 pM, about 70 pM to about 250 pM, about 70 pM to about 200 pM, about 70 pM to about 150 pM, about 70 pM to about 100 pM, about 70 pM to about 90 pM, about 70 pM to about 80 pM, about 80 pM to about 30 nM, about 80 pM to about 25 nM, about 80 pM to about 30 nM, about 80 pM to about 15 nM, about 80 pM to about 10 nM, about 80 pM to about 5 nM, about 80 pM to about 2 nM, about 80 pM to about 1 nM, about 80 pM to about 950 pM, about 80 pM to about 900 pM, about 80 pM to about 850 pM, about 80 pM to about 800 pM, about 80 pM to about 750 pM, about 80 pM to about 700 pM, about 80 pM to about 650 pM, about 80 pM to about 600 pM, about 80 pM to about 550 pM, about 80 pM to about 500 pM, about 80 pM to about 450 pM, about 80 pM to about 400 pM, about 80 pM to about 350 pM, about 80 pM to about 300 pM, about 80 pM to about 250 pM, about 80 pM to about 200 pM, about 80 pM to about 150 pM, about 80 pM to about 100 pM, about 80 pM to about 90 pM, about 90 pM to about 30 nM, about 90 pM to about 25 nM, about 90 pM to about 30 nM, about 90 pM to about 15 nM, about 90 pM to about 10 nM, about 90 pM to about 5 nM, about 90 pM to about 2 nM, about 90 pM to about 1 nM, about 90 pM to about 950 pM, about 90 pM to about 900 pM, about 90 pM to about 850 pM, about 90 pM to about 800 pM, about 90 pM to about 750 pM, about 90 pM to about 700 pM, about 90 pM to about 650 pM, about 90 pM to about 600 pM, about 90 pM to about 550 pM, about 90 pM to about 500 pM, about 90 pM to about 450 pM, about 90 pM to about 400 pM, about 90 pM to about 350 pM, about 90 pM to about 300 pM, about 90 pM to about 250 pM, about 90 pM to about 200

pM, about 90 pM to about 150 pM, about 90 pM to about 100 pM, about 100 pM to about 30 nM, about 100 pM to about 25 nM, about 100 pM to about 30 nM, about 100 pM to about 15 nM, about 100 pM to about 10 nM, about 100 pM to about 5 nM, about 100 pM to about 2 nM, about 100 pM to about 1 nM, about 100 pM to about 950 pM, about 100 pM to about 900 pM, about 100 pM to about 850 pM, about 100 pM to about 800 pM, about 100 pM to about 750 pM, about 100 pM to about 700 pM, about 100 pM to about 650 pM, about 100 pM to about 600 pM, about 100 pM to about 550 pM, about 100 pM to about 500 pM, about 100 pM to about 450 pM, about 100 pM to about 400 pM, about 100 pM to about 350 pM, about 100 pM to about 300 pM, about 100 pM to about 250 pM, about 100 pM to about 200 pM, about 100 pM to about 150 pM, about 150 pM to about 30 nM, about 150 pM to about 25 nM, about 150 pM to about 30 nM, about 150 pM to about 15 nM, about 150 pM to about 10 nM, about 150 pM to about 5 nM, about 150 pM to about 2 nM, about 150 pM to about 1 nM, about 150 pM to about 950 pM, about 150 pM to about 900 pM, about 150 pM to about 850 pM, about 150 pM to about 800 pM, about 150 pM to about 750 pM, about 150 pM to about 700 pM, about 150 pM to about 650 pM, about 150 pM to about 600 pM, about 150 pM to about 550 pM, about 150 pM to about 500 pM, about 150 pM to about 450 pM, about 150 pM to about 400 pM, about 150 pM to about 350 pM, about 150 pM to about 300 pM, about 150 pM to about 250 pM, about 150 pM to about 200 pM, about 200 pM to about 30 nM, about 200 pM to about 25 nM, about 200 pM to about 30 nM, about 200 pM to about 15 nM, about 200 pM to about 10 nM, about 200 pM to about 5 nM, about 200 pM to about 2 nM, about 200 pM to about 1 nM, about 200 pM to about 950 pM, about 200 pM to about 900 pM, about 200 pM to about 850 pM, about 200 pM to about 800 pM, about 200 pM to about 750 pM, about 200 pM to about 700 pM, about 200 pM to about 650 pM, about 200 pM to about 600 pM, about 200 pM to about 550 pM, about 200 pM to about 500 pM, about 200 pM to about 450 pM, about 200 pM to about 400 pM, about 200 pM to about 350 pM, about 200 pM to about 300 pM, about 200 pM to about 250 pM, about 300 pM to about 30 nM, about 300 pM to about 25 nM, about 300 pM to about 30 nM, about 300 pM to about 15 nM, about 300 pM to about 10 nM, about 300 pM to about 5 nM, about 300 pM to about 2 nM, about 300 pM to about 1 nM, about 300

pM to about 950 pM, about 300 pM to about 900 pM, about 300 pM to about 850 pM, about 300 pM to about 800 pM, about 300 pM to about 750 pM, about 300 pM to about 700 pM, about 300 pM to about 650 pM, about 300 pM to about 600 pM, about 300 pM to about 550 pM, about 300 pM to about 500 pM, about 300 pM to about 450 pM, about 300 pM to about 400 pM, about 300 pM to about 350 pM, about 400 pM to about 30 nM, about 400 pM to about 25 nM, about 400 pM to about 30 nM, about 400 pM to about 15 nM, about 400 pM to about 10 nM, about 400 pM to about 5 nM, about 400 pM to about 2 nM, about 400 pM to about 1 nM, about 400 pM to about 950 pM, about 400 pM to about 900 pM, about 400 pM to about 850 pM, about 400 pM to about 800 pM, about 400 pM to about 750 pM, about 400 pM to about 700 pM, about 400 pM to about 650 pM, about 400 pM to about 600 pM, about 400 pM to about 550 pM, about 400 pM to about 500 pM, about 500 pM to about 30 nM, about 500 pM to about 25 nM, about 500 pM to about 30 nM, about 500 pM to about 15 nM, about 500 pM to about 10 nM, about 500 pM to about 5 nM, about 500 pM to about 2 nM, about 500 pM to about 1 nM, about 500 pM to about 950 pM, about 500 pM to about 900 pM, about 500 pM to about 850 pM, about 500 pM to about 800 pM, about 500 pM to about 750 pM, about 500 pM to about 700 pM, about 500 pM to about 650 pM, about 500 pM to about 600 pM, about 500 pM to about 550 pM, about 600 pM to about 30 nM, about 600 pM to about 25 nM, about 600 pM to about 30 nM, about 600 pM to about 15 nM, about 600 pM to about 10 nM, about 600 pM to about 5 nM, about 600 pM to about 2 nM, about 600 pM to about 1 nM, about 600 pM to about 950 pM, about 600 pM to about 900 pM, about 600 pM to about 850 pM, about 600 pM to about 800 pM, about 600 pM to about 750 pM, about 600 pM to about 700 pM, about 600 pM to about 650 pM, about 700 pM to about 30 nM, about 700 pM to about 25 nM, about 700 pM to about 30 nM, about 700 pM to about 15 nM, about 700 pM to about 10 nM, about 700 pM to about 5 nM, about 700 pM to about 2 nM, about 700 pM to about 1 nM, about 700 pM to about 950 pM, about 700 pM to about 900 pM, about 700 pM to about 850 pM, about 700 pM to about 800 pM, about 700 pM to about 750 pM, about 800 pM to about 30 nM, about 800 pM to about 25 nM, about 800 pM to about 30 nM, about 800 pM to about 15 nM, about 800 pM to about 10 nM, about 800 pM to about 5 nM, about 800 pM to about 2 nM, about 800 pM to about 1

nM, about 800 pM to about 950 pM, about 800 pM to about 900 pM, about 800 pM to about 850 pM, about 900 pM to about 30 nM, about 900 pM to about 25 nM, about 900 pM to about 30 nM, about 900 pM to about 15 nM, about 900 pM to about 10 nM, about 900 pM to about 5 nM, about 900 pM to about 2 nM, about 900 pM to about 1 nM, about 900 pM to about 950 pM, about 1 nM to about 30 nM, about 1 nM to about 25 nM, about 1 nM to about 20 nM, about 1 nM to about 15 nM, about 1 nM to about 10 nM, about 1 nM to about 5 nM, about 2 nM to about 30 nM, about 2 nM to about 25 nM, about 2 nM to about 20 nM, about 2 nM to about 15 nM, about 2 nM to about 10 nM, about 2 nM to about 5 nM, about 4 nM to about 30 nM, about 4 nM to about 25 nM, about 4 nM to about 20 nM, about 4 nM to about 15 nM, about 4 nM to about 10 nM, about 4 nM to about 5 nM, about 5 nM to about 30 nM, about 5 nM to about 25 nM, about 5 nM to about 20 nM, about 5 nM to about 15 nM, about 5 nM to about 10 nM, about 10 nM to about 30 nM, about 10 nM to about 25 nM, about 10 nM to about 20 nM, about 10 nM to about 15 nM, about 15 nM to about 30 nM, about 15 nM to about 25 nM, about 15 nM to about 20 nM, about 20 nM to about 30 nM, and about 20 nM to about 25 nM).

Any of the target-binding domains described herein can bind to its target with a K_D of between about 1 nM to about 10 nM (e.g., about 1 nM to about 9 nM, about 1 nM to about 8 nM, about 1 nM to about 7 nM, about 1 nM to about 6 nM, about 1 nM to about 5 nM, about 1 nM to about 4 nM, about 1 nM to about 3 nM, about 1 nM to about 2 nM, about 2 nM to about 10 nM, about 2 nM to about 9 nM, about 2 nM to about 8 nM, about 2 nM to about 7 nM, about 2 nM to about 6 nM, about 2 nM to about 5 nM, about 2 nM to about 4 nM, about 2 nM to about 3 nM, about 3 nM to about 10 nM, about 3 nM to about 9 nM, about 3 nM to about 8 nM, about 3 nM to about 7 nM, about 3 nM to about 6 nM, about 3 nM to about 5 nM, about 3 nM to about 4 nM, about 4 nM to about 10 nM, about 4 nM to about 9 nM, about 4 nM to about 8 nM, about 4 nM to about 7 nM, about 4 nM to about 6 nM, about 4 nM to about 5 nM, about 5 nM to about 10 nM, about 5 nM to about 9 nM, about 5 nM to about 8 nM, about 5 nM to about 7 nM, about 5 nM to about 6 nM, about 6 nM to about 10 nM, about 6 nM to about 9 nM, about 6 nM to about 8 nM, about 6 nM to about 7 nM, about 7 nM to about 10 nM, about 7 nM to about 9 nM, about

7 nM to about 8 nM, about 8 nM to about 10 nM, about 8 nM to about 9 nM, and about 9 nM to about 10 nM).

A variety of different methods known in the art can be used to determine the K_D values of any of the antigen-binding protein constructs described herein (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

Antigen-Binding Domains

In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to the same antigen. In some embodiments of these single-chain or multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these single-chain or multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain bind specifically to different antigens.

In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, the first target-binding domain and the second target-binding domain are each antigen-binding domains. In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, the antigen-binding domain includes or is a scFv or a single domain antibody (e.g., a V_{HH} or a V_{NAR} domain).

In some examples, an antigen-binding domain (e.g., any of the antigen-binding domains described herein) can bind specifically to any one of CD16a (see, e.g., those described in U.S. Patent No. 9,035,026), CD28 (see, e.g., those described in U.S. Patent

No. 7,723,482), CD3 (see, e.g., those described in U.S. Patent No. 9,226,962), CD33 (see, e.g., those described in U.S. Patent No. 8,759,494), CD20 (see, e.g., those described in WO 2014/026054), CD19 (see, e.g., those described in U.S. Patent No. 9,701,758), CD22 (see, e.g., those described in WO 2003/104425), CD123 (see, e.g., those described in WO 2014/130635), IL-1R (see, e.g., those described in U.S. Patent No. 8,741,604), IL-1 (see, e.g., those described in WO 2014/095808), VEGF (see, e.g., those described in U.S. Patent No. 9,090,684), IL-6R (see, e.g., those described in U.S. Patent No. 7,482,436), IL-4 (see, e.g., those described in U.S. Patent Application Publication No. 2012/0171197), IL-10 (see, e.g., those described in U.S. Patent Application Publication No. 2016/0340413), PDL-1 (see, e.g., those described in Drees et al., *Protein Express. Purif.* 94:60-66, 2014), TIGIT (see, e.g., those described in U.S. Patent Application Publication No. 2017/0198042), PD-1 (see, e.g., those described in U.S. Patent No. 7,488,802), TIM3 (see, e.g., those described in U.S. Patent No. 8,552,156), CTLA4 (see, e.g., those described in WO 2012/120125), MICA (see, e.g., those described in WO 2016/154585), MICB (see, e.g., those described in U.S. Patent No. 8,753,640), IL-6 (see, e.g., those described in Gejima et al., *Human Antibodies* 11(4):121-129, 2002), IL-8 (see, e.g., those described in U.S. Patent No. 6,117,980), TNFα (see, e.g., those described in Geng et al., *Immunol. Res.* 62(3):377-385, 2015), CD26 (see, e.g., those described in WO 2017/189526), CD36 (see, e.g., those described in U.S. Patent Application Publication No. 2015/0259429), ULBP2 (see, e.g., those described in U.S. Patent No. 9,273,136), CD30 (see, e.g., those described in Homach et al., *Scand. J. Immunol.* 48(5):497-501, 1998), CD200 (see, e.g., those described in U.S. Patent No. 9,085,623), IGF-1R (see, e.g., those described in U.S. Patent Application Publication No. 2017/0051063), MUC4AC (see, e.g., those described in WO 2012/170470), MUC5AC (see, e.g., those described in U.S. Patent No. 9,238,084), Trop-2 (see, e.g., those described in WO 2013/068946), CMET (see, e.g., those described in Edwardraja et al., *Biotechnol. Bioeng.* 106(3):367-375, 2010), EGFR (see, e.g., those described in Akbari et al., *Protein Expr. Purif.* 127:8-15, 2016), HER1 (see, e.g., those described in U.S. Patent Application Publication No. 2013/0274446), HER2 (see, e.g., those described in Cao et al., *Biotechnol. Lett.* 37(7):1347-1354, 2015), HER3 (see, e.g., those described in U.S. Patent No. 9,505,843),

PSMA (see, e.g., those described in Parker et al., *Protein Expr. Purif.* 89(2):136-145, 2013), CEA (see, e.g., those described in WO 1995/015341), B7H3 (see, e.g., those described in U.S. Patent No. 9,371,395), EPCAM (see, e.g., those described in WO 2014/159531), BCMA (see, e.g., those described in Smith et al., *Mol. Ther.* 26(6):1447-1456, 2018), P-cadherin (see, e.g., those described in U.S. Patent No. 7,452,537), CEACAM5 (see, e.g., those described in U.S. Patent No. 9,617,345), a UL16-binding protein (see, e.g., those described in WO 2017/083612), HLA-DR (see, e.g., Pistillo et al., *Exp. Clin. Immunogenet.* 14(2):123-130, 1997), DLL4 (see, e.g., those described in WO 2014/007513), TYRO3 (see, e.g., those described in WO 2016/166348), AXL (see, e.g., those described in WO 2012/175692), MER (see, e.g., those described in WO 2016/106221), CD122 (see, e.g., those described in U.S. Patent Application Publication No. 2016/0367664), CD155 (see, e.g., those described in WO 2017/149538), or PDGF-DD (see, e.g., those described in U.S. Patent No. 9,441,034).

The antigen-binding domains present in any of the single-chain or multi-chain chimeric polypeptides described herein are each independently selected from the group consisting of: a VHH domain, a VNAR domain, and a scFv. In some embodiments, any of the antigen-binding domains described herein is a BiTe, a $(scFv)_2$, a nanobody, a nanobody-HSA, a DART, a TandAb, a scDiabody, a scDiabody-CH3, scFv-CH-CL-scFv, a HSAbody, scDiabody-HAS, or a tandem-scFv. Additional examples of antigen-binding domains that can be used in any of the single-chain or multi-chain chimeric polypeptide are known in the art.

A VHH domain is a single monomeric variable antibody domain that can be found in camelids. A VNAR domain is a single monomeric variable antibody domain that can be found in cartilaginous fish. Non-limiting aspects of V_HH domains and V_{NAR} domains are described in, e.g., Cromie et al., *Curr. Top. Med. Chem.* 15:2543-2557, 2016; De Genst et al., *Dev. Comp. Immunol.* 30:187-198, 2006; De Meyer et al., *Trends Biotechnol.* 32:263-270, 2014; Kijanka et al., *Nanomedicine* 10:161-174, 2015; Kovaleva et al., *Expert. Opin. Biol. Ther.* 14:1527-1539, 2014; Krah et al., *Immunopharmacol. Immunotoxicol.* 38:21-28, 2016; Mujic-Delic et al., *Trends Pharmacol. Sci.* 35:247-255, 2014; Muyldermans, *J. Biotechnol.* 74:277-302, 2001; Muyldermans et al., *Trends*

Biochem. Sci. 26:230-235, 2001; Muyldermans, *Ann. Rev. Biochem.* 82:775-797, 2013; Rahbarizadeh et al., *Immunol. Invest.* 40:299-338, 2011; Van Audenhove et al., *EBioMedicine* 8:40-48, 2016; Van Bockstaele et al., *Curr. Opin. Investig. Drugs* 10:1212-1224, 2009; Vincke et al., *Methods Mol. Biol.* 911:15-26, 2012; and Wesolowski et al., *Med. Microbiol. Immunol.* 198:157-174, 2009.

In some embodiments, each of the antigen-binding domains in the single-chain or multi-chain chimeric polypeptides described herein are both VHH domains, or at least one antigen-binding domain is a VHH domain. In some embodiments, each of the antigen-binding domains in the single-chain or multi-chain chimeric polypeptides described herein are both VNAR domains, or at least one antigen-binding domain is a VNAR domain. In some embodiments, each of the antigen-binding domains in the single-chain or multi-chain chimeric polypeptides described herein are both scFv domains, or at least one antigen-binding domain is a scFv domain.

In some embodiments, two or more of polypeptides present in the single-chain or multi-chain chimeric polypeptide can assemble (e.g., non-covalently assemble) to form any of the antigen-binding domains described herein, e.g., an antigen-binding fragment of an antibody (e.g., any of the antigen-binding fragments of an antibody described herein), a VHH-scAb, a VHH-Fab, a Dual scFab, a F(ab')2, a diabody, a crossMab, a DAF (two-in-one), a DAF (four-in-one), a DutaMab, a DT-IgG, a knobs-in-holes common light chain, a knobs-in-holes assembly, a charge pair, a Fab-arm exchange, a SEEDbody, a LUZ-Y, a Fcab, a κλ-body, an orthogonal Fab, a DVD-IgG, a IgG(H)-scFv, a scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG, Diabody-CH3, a triple body, a miniantibody, a minibody, a TriBi minibody, scFv-CH3 KIH, Fab-scFv, a F(ab')2-scFv2, a scFv-KIH, a Fab-scFv-Fc, a tetravalent HCAb, a scDiabody-Fc, a Diabody-Fc, a tandem scFv-Fc, an Intrabody, a dock and lock, a lmmTAC, an IgG-IgG conjugate, a Cov-X-Body, and a scFv1-PEG-scFv2. See, e.g., Spiess et al., *Mol. Immunol.* 67:95-106, 2015, incorporated in its entirety herewith, for a description of these elements. Non-limiting examples of an antigen-binding fragment of an antibody include an Fv fragment, a Fab fragment, a $F(ab')_2$ fragment, and a Fab' fragment. Additional

examples of an antigen-binding fragment of an antibody is an antigen-binding fragment of an IgG (e.g., an antigen-binding fragment of IgG1, IgG2, IgG3, or IgG4) (e.g., an antigen-binding fragment of a human or humanized IgG, e.g., human or humanized IgG1, IgG2, IgG3, or IgG4); an antigen-binding fragment of an IgA (e.g., an antigen-binding fragment of IgA1 or IgA2) (e.g., an antigen-binding fragment of a human or humanized IgA, e.g., a human or humanized IgA1 or IgA2); an antigen-binding fragment of an IgD (e.g., an antigen-binding fragment of a human or humanized IgD); an antigen-binding fragment of an IgE (e.g., an antigen-binding fragment of a human or humanized IgE); or an antigen-binding fragment of an IgM (e.g., an antigen-binding fragment of a human or humanized IgM).

An "Fv" fragment includes a non-covalently-linked dimer of one heavy chain variable domain and one light chain variable domain.

A "Fab" fragment includes, the constant domain of the light chain and the first constant domain (C_{H1}) of the heavy chain, in addition to the heavy and light chain variable domains of the Fv fragment.

A "F(ab')$_2$" fragment includes two Fab fragments joined, near the hinge region, by disulfide bonds.

A "dual variable domain immunoglobulin" or "DVD-Ig" refers to multivalent and multispecific binding proteins as described, e.g., in DiGiammarino et al., *Methods Mol. Biol.* 899:145-156, 2012; Jakob et al., *MABs* 5:358-363, 2013; and U.S. Patent Nos. 7,612,181; 8,258,268; 8,586,714; 8,716,450; 8,722,855; 8,735,546; and 8,822,645, each of which is incorporated by reference in its entirety.

DARTs are described in, e.g., Garber, *Nature Reviews Drug Discovery* 13:799-801, 2014.

In some embodiments of any of the antigen-binding domains described herein can bind to an antigen selected from the group consisting of: a protein, a carbohydrate, a lipid, and a combination thereof.

Additional examples and aspects of antigen-binding domains are known in the art.

Soluble Interleukin or Cytokine Protein

In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain can be a soluble interleukin protein or soluble cytokine protein. In some embodiments, the soluble interleukin or soluble cytokine protein is selected from the group of: IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF. Non-limiting examples of soluble IL-2, IL-3, IL-7, IL-8, IL-10, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF are provided below.

Human Soluble IL-3 (SEQ ID NO: 105)

apmtqttplkt swvncsnmid eiithlkqpp lplldfnnln gedqdilmen nlrrpnleaf nravkslqna saiesilknl lpclplataa ptrhpihikd gdwnefrrkl tfylktlena qaqqttlsla if

Human Soluble IL-8 (SEQ ID NO: 106)

egavlprsak elrcqcikty skpfhpkfik elrviesgph canteiivkl sdgrelcldp kenwvqrvve kflkraens

Human Soluble IL-10 (SEQ ID NO: 107)

spgqgtqsensc thfpgnlpnm lrdlrdafsr vktffqmkdq ldnlllkesl ledfkgylgc qalsemiqfy leevmpqaen qdpdikahvn slgenlktlr lrlrrchrfl pcenkskave qvknafnklq ekgiykamse fdifinyiea ymtmkirn

Human Soluble IL-17 (SEQ ID NO: 108)

gitiprn pgcpnsedkn fprtvmvnln ihnrntntnp krssdyynrs tspwnlhrne dperypsviw eakcrhlgci nadgnvdyhm nsvpiqqeil vlrrepphcp nsfrlekilv svgctcvtpi vhhva

Human Soluble IL-18 (SEQ ID NO: 109)

yfgklesklsvirn lndqvlfidq gnrplfedmt dsdcrdnapr tifiismykd
sqprgmavti svkcekistl scenkiisfk emnppdnikd tksdiiffqr
svpghdnkmq fesssyegyf lacekerdlf klilkkedel gdrsimftvq ned

Human Soluble PDGF-DD (SEQ ID NO: 110)

rdtsatpqsasi kalrnanlrr desnhltdly rrdetiqvkg ngyvqsprfp
nsyprnlllt wrlhsqentr iqlvfdnqfg leeaendicr ydfvevedis
etstiirgrw cghkevppri ksrtnqikit fksddyfvak pgfkiyysll
edfqpaaase tnwesvtssi sgvsynspsv tdptliadal dkkiaefdtv
edllkyfnpe swqedlenmy ldtpryrgrs yhdrkskvdl drlnddakry
sctprnysvn ireelklanv vffprcllvq rcggncgcgt vnwrsctcns
gktvkkyhev lqfepghikr rgraktmalv diqldhherc dcicssrppr

Human Soluble SCF (SEQ ID NO: 111)

egicrnrvtnnvkdv tklvanlpkd ymitlkyvpg mdvlpshcwi semvvqlsds
ltdlldkfsn iseglsnysi idklvnivdd lvecvkenss kdlkksfksp
eprlftpeef frifnrsida fkdfvvaset sdcvvsstls pekdsrvsvt
kpfmlppvaa sslrndssss nrkaknppgd sslhwaamal palfsliigf
afgalywkkr qpsltraven iqineednei smlqekeref qev

Human Soluble FLT3L (SEQ ID NO: 112)

tqdcsfqhspissd favkirelsd yllqdypvtv asnlqdeelc gglwrlvlaq
rwmerlktva gskmqgller vnteihfvtk cafqpppscl rfvqtnisrl
lqetseqlva lkpwitrqnf srclelqcqp dsstlpppws prpleatapt
apqppllllll llpvglllla aawclhwqrt rrrtprpgeq vppvpspqdl
llveh

Additional examples of soluble interleukin proteins and soluble cytokine proteins are known in the art.

Soluble Receptor

In some embodiments of any of the single-chain or multi-chain chimeric polypeptides described herein, one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin receptor or a soluble cytokine receptor. In some embodiments, the soluble receptor is a soluble TGF-β receptor II (TGF-β RII) (see, e.g., those described in Yung et al., *Am. J. Resp. Crit. Care Med.* 194(9):1140-1151, 2016), a soluble TGF-βRIII (see, e.g., those described in Heng et al., *Placenta* 57:320, 2017), a soluble NKG2D (see, e.g., Cosman et al., *Immunity* 14(2):123-133, 2001; Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp30 (see, e.g., Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp44 (see, e.g., those described in Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble NKp46 (see, e.g., Mandelboim et al., *Nature* 409:1055-1060, 2001; Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a soluble DNAM1 (see, e.g., those described in Costa et al., *Front. Immunol.*, Vol. 9, Article 1150, May 29, 2018; doi: 10.3389/fimmu.2018.01150), a scMHCI (see, e.g., those described in Washburn et al., *PLoS One* 6(3):e18439, 2011), a scMHCII (see, e.g., those described in Bishwajit et al., *Cellular Immunol.* 170(1):25-33, 1996), a scTCR (see, e.g., those described in Weber et al., *Nature* 356(6372):793-796, 1992), a soluble CD155 (see, e.g., those described in Tahara-Hanaoka et al., *Int. Immunol.* 16(4):533-538, 2004), or a soluble CD28 (see, e.g., Hebbar et al., *Clin. Exp. Immunol.* 136:388-392, 2004).

Additional examples of soluble interleukin receptors and soluble cytokine receptors are known in the art.

Pairs of Affinity Domains

In some embodiments, a multi-chain chimeric polypeptide includes: 1) a first chimeric polypeptide that includes a first domain of a pair of affinity domains, and 2) a second chimeric polypeptide that includes a second domain of a pair of affinity domains such that the first chimeric polypeptide and the second chimeric polypeptide associate

through the binding of the first domain and the second domain of the pair of affinity domains. In some embodiments, the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15. A sushi domain, also known as a short consensus repeat or type 1 glycoprotein motif, is a common motif in protein-protein interaction. Sushi domains have been identified on a number of protein-binding molecules, including complement components C1r, C1s, factor H, and C2m, as well as the nonimmunologic molecules factor XIII and β2-glycoprotein. A typical Sushi domain has approximately 60 amino acid residues and contains four cysteines (Ranganathan, Pac. Symp Biocomput. 2000:155-67). The first cysteine can form a disulfide bond with the third cysteine, and the second cysteine can form a disulfide bridge with the fourth cysteine. In some embodiments in which one member of the pair of affinity domains is a soluble IL-15, the soluble IL-15 has a D8N or D8A amino acid substitution. In some embodiments in which one member of the pair of affinity domains is an alpha chain of human IL-15 receptor (IL-15Rα), the human IL-15Rα is a mature full-length IL-15Rα. In some embodiments, the pair of affinity domains is barnase and barnstar. In some embodiments, the pair of affinity domains is a PKA and an AKAP. In some embodiments, the pair of affinity domains is an adapter/docking tag module based on mutated RNase I fragments (Rossi, *Proc Natl Acad Sci USA*. 103:6841-6846, 2006; Sharkey et al., *Cancer Res*. 68:5282-5290, 2008; Rossi et al., *Trends Pharmacol Sci*. 33:474-481, 2012) or SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25 (Deyev et al., *Nat Biotechnol*. 1486-1492, 2003).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains and the second domain of the pair of affinity domains bind to each other with a dissociation equilibrium constant (K_D) of less than 1×10^{-7} M, less than 1×10^{-8} M, less than 1×10^{-9} M, less than 1×10^{-10} M, less than 1×10^{-11} M, less than 1×10^{-12} M, or less than 1×10^{-13} M. In some embodiments, the first domain of the pair of affinity domains and the second

domain of the pair of affinity domains bind to each other with a K_D of about 1 x 10^{-4} M to about 1 x 10^{-6} M, about 1 x 10^{-5} M to about 1 x 10^{-7} M, about 1 x 10^{-6} M to about 1 x 10^{-8} M, about 1 x 10^{-7} M to about 1 x 10^{-9} M, about 1 x 10^{-8} M to about 1 x 10^{-10} M, about 1 x 10^{-9} M to about 1 x 10^{-11} M, about 1 x 10^{-10} M to about 1 x 10^{-12} M, about 1 x 10^{-11} M to about 1 x 10^{-13} M, about 1 x 10^{-4} M to about 1 x 10^{-5} M, about 1 x 10^{-5} M to about 1 x 10^{-6} M, about 1 x 10^{-6} M to about 1 x 10^{-7} M, about 1 x 10^{-7} M to about 1 x 10^{-8} M, about 1 x 10^{-8} M to about 1 x 10^{-9} M, about 1 x 10^{-9} M to about 1 x 10^{-10} M, about 1 x 10^{-10} M to about 1 x 10^{-11} M, about 1 x 10^{-11} M to about 1 x 10^{-12} M, or about 1 x 10^{-12} M to about 1 x 10^{-13} M (inclusive). Any of a variety of different methods known in the art can be used to determine the K_D value of the binding of the first domain of the pair of affinity domains and the second domain of the pair of affinity domains (e.g., an electrophoretic mobility shift assay, a filter binding assay, surface plasmon resonance, and a biomolecular binding kinetics assay, etc.).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide includes a first domain of a pair of affinity domains and a second chimeric polypeptide of the multi-chain chimeric polypeptide includes a second domain of a pair of affinity domains, wherein the first domain of the pair of affinity domains, the second domain of the pair of affinity domains, or both is about 10 to 100 amino acids in length. For example, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about

10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a first domain of a pair of affinity domains, a second domain of a pair of affinity domains, or both is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the first and/or second domains of a pair of affinity domains disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the first and/or second domains of a pair of affinity domains remains intact. For example, a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a soluble IL-15. Additionally or alternatively, a soluble IL-15 can include one or more additional amino acids at the N-terminus and/or the C-terminus, while still retaining the ability to bind to a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα).

A non-limiting example of a sushi domain from an alpha chain of IL-15 receptor alpha (IL-15Rα) can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH WTTPSLKCIR (SEQ ID NO: 113). In some embodiments, a sushi domain from an alpha chain of IL-15Rα can be encoded by a nucleic acid including

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAG
CTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGA
AGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGT
GGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 114).

In some embodiments, a soluble IL-15 can include a sequence that is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 99% identical, or 100% identical to NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGD ASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINT S (SEQ ID NO: 115). In some embodiments, a soluble IL-15 can be encoded by a nucleic acid including the sequence of AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTC CATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAA GGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAG CGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATA ACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGA AGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTG TCCAGATGTTCATCAATACCTCC (SEQ ID NO: 116).

Signal Sequence

In some embodiments, a single-chain chimeric polypeptide comprises a signal sequence at its N-terminal end. In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a signal sequence at its N-terminal end. In some embodiments, both the first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a signal sequence. As will be understood by those of ordinary skill in the art, a signal sequence is an amino acid sequence that is present at the N-terminus of a number of endogenously produced proteins that directs the protein to the secretory pathway (e.g.,

the protein is directed to reside in certain intracellular organelles, to reside in the cell membrane, or to be secreted from the cell). Signal sequences are heterogeneous and differ greatly in their primary amino acid sequences. However, signal sequences are typically 16 to 30 amino acids in length and include a hydrophilic, usually positively charged N-terminal region, a central hydrophobic domain, and a C-terminal region that contains the cleavage site for signal peptidase.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence having an amino acid sequence MKWVTFISLLFLFSSAYS (SEQ ID NO: 117). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence encoded by the nucleic acid sequence ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC (SEQ ID NO: 118), ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC (SEQ ID NO: 119), or ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC (SEQ ID NO: 120).

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence having an amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 121). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence having an amino acid sequence MGQIVTMFEALPHIIDEVINIVIIVLIIITSIKAVYNFATCGILALVSFLFLAGRSCG (SEQ ID NO: 122). In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence

having an amino acid sequence:
MPNHQSGSPTGSSDLLLSGKKQRPHLALRRKRRREMRKINRKVRRMNLAPIKEK
TAWQHLQALISEAEEVLKTSQTPQNSLTLFLALLSVLGPPVTG (SEQ ID NO: 123).
In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence having an amino acid sequence MDSKGSSQKGSRLLLLLVVSNLLLCQGVVS (SEQ ID NO: 124). Those of ordinary skill in the art will be aware of other appropriate signal sequences for use in a first chimeric polypeptide and/or a second chimeric polypeptide of multi-chain chimeric polypeptides, or single-chain chimeric polypeptides described herein.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence that is about 10 to 100 amino acids in length. For example, a signal sequence can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about

40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a signal sequence is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

In some embodiments, any of the signal sequences disclosed herein can include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at its N-terminus and/or C-terminus, so long as the function of the signal sequence remains intact. For example, a signal sequence having the amino acid sequence MKCLLYLAFLFLGVNC (SEQ ID NO: 125) can include one or more additional amino acids at the N-terminus or C-terminus, while still retaining the ability to direct the a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, to the secretory pathway.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a signal sequence that directs the multi-chain chimeric polypeptide into the extracellular space. Such embodiments are useful in producing single-chain or multi-chain chimeric polypeptides that are relatively easy to be isolated and/or purified.

Peptide Tags

In some embodiments, a single-chain chimeric polypeptide includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the chimeric polypeptide). In some embodiments, a multi-chain chimeric polypeptide includes a first chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal end or the C-terminal end of the first chimeric polypeptide). In some embodiments, a multi-chain chimeric polypeptide includes a second chimeric polypeptide that includes a peptide tag (e.g., at the N-terminal

end or the C-terminal end of the second chimeric polypeptide). In some embodiments, both the first chimeric polypeptide of a multi-chain chimeric polypeptide and a second chimeric polypeptide of the multi-chain chimeric polypeptide include a peptide tag. In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes two or more peptide tags.

Exemplary peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide include, without limitation, AviTag (GLNDIFEAQKIEWHE; SEQ ID NO: 126), a calmodulin-tag (KRRWKKNFIAVSAANRFKKISSSGAL; SEQ ID NO: 127), a polyglutamate tag (EEEEEE; SEQ ID NO: 128), an E-tag (GAPVPYPDPLEPR; SEQ ID NO: 129), a FLAG-tag (DYKDDDDK; SEQ ID NO: 130), an HA-tag, a peptide from hemagglutinin (YPYDVPDYA; SEQ ID NO: 131), a his-tag (HHHHH (SEQ ID NO: 132); HHHHHH (SEQ ID NO: 133); HHHHHHH (SEQ ID NO: 134); HHHHHHHH (SEQ ID NO: 135); HHHHHHHHH (SEQ ID NO: 136); or HHHHHHHHHH (SEQ ID NO: 137)), a myc-tag (EQKLISEEDL; SEQ ID NO: 138), NE-tag (TKENPRSNQEESYDDNES; SEQ ID NO: 139), S-tag, (KETAAAKFERQHMDS; SEQ ID NO: 140), SBP-tag (MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP; SEQ ID NO: 141), Softag 1 (SLAELLNAGLGGS; SEQ ID NO: 142), Softag 3 (TQDPSRVG; SEQ ID NO: 143), Spot-tag (PDRVRAVSHWSS; SEQ ID NO: 144), Strep-tag (WSHPQFEK; SEQ ID NO: 145), TC tag (CCPGCC; SEQ ID NO: 146), Ty tag (EVHTNQDPLD; SEQ ID NO: 147), V5 tag (GKPIPNPLLGLDST; SEQ ID NO: 148), VSV-tag (YTDIEMNRLGK; SEQ ID NO: 149), and Xpress tag (DLYDDDDK; SEQ ID NO: 150). In some embodiments, tissue factor protein is a peptide tag.

Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide can be used in any of a variety of applications related to the multi-chain or single-chain chimeric polypeptide, respectively. For example, a peptide tag can be used in the purification of a multi-chain

or single-chain chimeric polypeptide. As one non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both, or a single-chain chimeric polypeptide, can include a myc tag; the multi-chain chimeric polypeptide that includes the myc-tagged first chimeric polypeptide, the myc-tagged second chimeric polypeptide, or both, or the myc-tagged single-chain chimeric polypeptide can be purified using an antibody that recognizes the myc tag(s). One non-limiting example of an antibody that recognizes a myc tag is 9E10, available from the non-commercial Developmental Studies Hybridoma Bank. As another non-limiting example, a first chimeric polypeptide of a multi-chain chimeric polypeptide (e.g., a recombinantly expressed first chimeric polypeptide), a second chimeric polypeptide of the multi-chain chimeric polypeptide (e.g., a recombinantly expressed second chimeric polypeptide), or both, or a single-chain chimeric polypeptide, can include a histidine tag; the multi-chain chimeric polypeptide that includes the histidine-tagged first chimeric polypeptide, the histidine-tagged second chimeric polypeptide, or both, or the histidine-tagged single-chain chimeric polypeptide can be purified using a nickel or cobalt chelate. Those of ordinary skill in the art will be aware of other suitable tags and agents that bind those tags for use in purifying a single-chain or multi-chain chimeric polypeptide. In some embodiments, a peptide tag is removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide, or the single-chain chimeric polypeptide after purification. In some embodiments, a peptide tag is not removed from the first chimeric polypeptide and/or the second chimeric polypeptide of the multi-chain chimeric polypeptide, or the single-chain chimeric polypeptide, after purification.

Peptide tags that can be included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, can be used, for example, in immunoprecipitation of the multi-chain chimeric polypeptide or single-chain chimeric polypeptide, respectively, imaging of the multi-chain chimeric polypeptide or single-

chain chimeric polypeptide, respectively (e.g., via Western blotting, ELISA, flow cytometry, and/or immunocytochemistry), and/or solubilization of the multi-chain chimeric polypeptide or single-chain chimeric polypeptide, respectively.

In some embodiments, a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, includes a peptide tag that is about 10 to 100 amino acids in length. For example, a peptide tag can be about 10 to 100 amino acids in length, about 15 to 100 amino acids in length, about 20 to 100 amino acids in length, about 25 to 100 amino acids in length, about 30 to 100 amino acids in length, about 35 to 100 amino acids in length, about 40 to 100 amino acids in length, about 45 to 100 amino acids in length, about 50 to 100 amino acids in length, about 55 to 100 amino acids in length, about 60 to 100 amino acids in length, about 65 to 100 amino acids in length, about 70 to 100 amino acids in length, about 75 to 100 amino acids in length, about 80 to 100 amino acids in length, about 85 to 100 amino acids in length, about 90 to 100 amino acids in length, about 95 to 100 amino acids in length, about 10 to 95 amino acids in length, about 10 to 90 amino acids in length, about 10 to 85 amino acids in length, about 10 to 80 amino acids in length, about 10 to 75 amino acids in length, about 10 to 70 amino acids in length, about 10 to 65 amino acids in length, about 10 to 60 amino acids in length, about 10 to 55 amino acids in length, about 10 to 50 amino acids in length, about 10 to 45 amino acids in length, about 10 to 40 amino acids in length, about 10 to 35 amino acids in length, about 10 to 30 amino acids in length, about 10 to 25 amino acids in length, about 10 to 20 amino acids in length, about 10 to 15 amino acids in length, about 20 to 30 amino acids in length, about 30 to 40 amino acids in length, about 40 to 50 amino acids in length, about 50 to 60 amino acids in length, about 60 to 70 amino acids in length, about 70 to 80 amino acids in length, about 80 to 90 amino acids in length, about 90 to 100 amino acids in length, about 20 to 90 amino acids in length, about 30 to 80 amino acids in length, about 40 to 70 amino acids in length, about 50 to 60 amino acids in length, or any range in between. In some embodiments, a peptide tag is about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

Peptide tags included in a first chimeric polypeptide of a multi-chain chimeric polypeptide, a second chimeric polypeptide of the multi-chain chimeric polypeptide, or both, or a single-chain chimeric polypeptide, can be of any suitable length. For example, peptide tags can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids in length. In embodiments in which a single-chain or multi-chain chimeric polypeptide includes two or more peptide tags, the two or more peptide tags can be of the same or different lengths. In some embodiments, any of the peptide tags disclosed herein may include one or more additional amino acids (e.g., 1, 2, 3, 5, 6, 7, 8, 9, 10, or more amino acids) at the N-terminus and/or C-terminus, so long as the function of the peptide tag remains intact. For example, a myc tag having the amino acid sequence EQKLISEEDL (SEQ ID NO: 138) can include one or more additional amino acids (e.g., at the N-terminus and/or the C- terminus of the peptide tag), while still retaining the ability to be bound by an antibody (e.g., 9E10).

Exemplary Embodiments of Single-Chain Chimeric Polypeptides- Type A

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain and/or the second target-binding domain can independently bind specifically to CD3 (e.g., human CD3) or CD28 (e.g., human CD28). In some embodiments, the first target-binding domain binds specifically to CD3 (e.g., human CD3) and the second target-binding domain binds specifically to CD28 (e.g., human CD28). In some embodiments, the first target-binding domain binds specifically to CD28 (e.g., human CD28) and the second target-binding domain binds specifically to CD3 (e.g., human CD3).

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain.

In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the second target-binding domain.

In some embodiments of these single-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain. In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each an antigen-binding domain (e.g., any of the exemplary antigen-binding domains described herein). In some embodiments of these single-chain chimeric polypeptides, the antigen-binding domain includes a scFv or a single domain antibody.

A non-limiting example of an scFv that binds specifically to CD3 can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGG
GGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQ
RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
YYCARYYDDHYCLDYWGQGTTLTVSS (SEQ ID NO: 151).

In some embodiments, an scFv that binds specifically to CD3 can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGTGAGA
AGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACTGGTAT
CAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCAGCAAG
CTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCACCAGCTA

CTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACTATTGCC
AGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCTCGAAAT
CAATCGTGGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAG
CCAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCGGCGCCTCC
GTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAATGCA
TTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATATCAAC
CCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCCACTT
TAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTTAACC
AGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCACTACTG
TTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGC (SEQ ID NO: 152).

A non-limiting example of an scFv that binds specifically to CD28 can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

VQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGSINPYN
DYTKYNEKFKGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNYWGRG
TTLTVSSGGGGSGGGGSGGGGSDIEMTQSPAIMSASLGERVTMTCTASSSVSSSY
FHWYQQKPGSSPKLCIYSTSNLASGVPPRFSGSGSTSYSLTISSMEAEDAATYFCH
QYHRSPTFGGGTKLETKR (SEQ ID NO: 153).

In some embodiments, an scFv that binds specifically to CD28 can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GTCCAGCTGCAGCAGAGCGGACCCGAACTCGTGAAACCCGGTGCTTCCGTGA
AAATGTCTTGTAAGGCCAGCGGATACACCTTCACCTCCTATGTGATCCAGTGG
GTCAAACAGAAGCCCGGACAAGGTCTCGAGTGGATCGGCAGCATCAACCCTT
ACAACGACTATACCAAATACAACGAGAAGTTTAAGGGAAAGGCTACTTTAAC
CTCCGACAAAAGCTCCATCACAGCCTACATGGAGTTCAGCTCTTTAACATCCG
AGGACAGCGCTCTGTACTATTGCGCCCGGTGGGGCGACGGCAATTACTGGGG
ACGGGGCACAACACTGACCGTGAGCAGCGGAGGCGGAGGCTCCGGCGGAGG
CGGATCTGGCGGTGGCGGCTCCGACATCGAGATGACCCAGTCCCCCGCTATC
ATGTCCGCCTCTTTAGGCGAGCGGGTCACAATGACTTGTACAGCCTCCTCCAG
CGTCTCCTCCTCCTACTTCCATTGGTACCAACAGAAACCCGGAAGCTCCCCTA
AACTGTGCATCTACAGCACCAGCAATCTCGCCAGCGGCGTGCCCCCTAGGTT

TTCCGGAAGCGGAAGCACCAGCTACTCTTTAACCATCTCCTCCATGGAGGCT
GAGGATGCCGCCACCTACTTTTGTCACCAGTACCACCGGTCCCCCACCTTCGG
AGGCGGCACCAAACTGGAGACAAAGAGG (SEQ ID NO: 154).

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and/or the second target-binding domain is a soluble receptor (e.g., a soluble CD28 receptor or a soluble CD3 receptor). In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein.

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDTSKLA
SGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEINRGG
GGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQ
RPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAV
YYCARYYDDHYCLDYWGQGTTLTVSSSGTTNTVAAYNLTWKSTNFKTILEWEP
KPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAG
NVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQ
AVIPSRTVNRKSTDSPVECMGQEKGEFREVQLQQSGPELVKPGASVKMSCKASG
YTFTSYVIQWVKQKPGQGLEWIGSINPYNDYTKYNEKFKGKATLTSDKSSITAY
MEFSSLTSEDSALYYCARWGDGNYWGRGTTLTVSSGGGGSGGGGSGGGGSDIE
MTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPGSSPKLCIYSTSNLASG
VPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGGGTKLETKR (SEQ ID
NO: 155).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGTGAGA
AGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACTGGTAT
CAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCAGCAAG

CTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCACCAGCTA
CTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACTATTGCC
AGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCTCGAAAT
CAATCGTGGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAG
CCAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCGGCGCCTCC
GTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAATGCA
TTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATATCAAC
CCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCCACTT
TAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTTAACC
AGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCACTACTG
TTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGCTCCGGCACC
ACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGA
CAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGAT
CTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACC
GAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGG
CTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGC
GAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTT
AGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTC
ACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCC
TCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCC
AGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTG
ACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTC
TCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAA
GAAAAGGGCGAGTTCCGGGAGGTCCAGCTGCAGCAGAGCGGACCCGAACTC
GTGAAACCCGGTGCTTCCGTGAAAATGTCTTGTAAGGCCAGCGGATACACCT
TCACCTCCTATGTGATCCAGTGGGTCAAACAGAAGCCCGGACAAGGTCTCGA
GTGGATCGGCAGCATCAACCCTTACAACGACTATACCAAATACAACGAGAAG
TTTAAGGGAAAGGCTACTTTAACCTCCGACAAAAGCTCCATCACAGCCTACA
TGGAGTTCAGCTCTTTAACATCCGAGGACAGCGCTCTGTACTATTGCGCCCGG
TGGGGCGACGGCAATTACTGGGGACGGGGCACAACACTGACCGTGAGCAGC
GGAGGCGGAGGCTCCGGCGGAGGCGGATCTGGCGGTGGCGGCTCCGACATC
GAGATGACCCAGTCCCCCGCTATCATGTCCGCCTCTTTAGGCGAGCGGGTCA
CAATGACTTGTACAGCCTCCTCCAGCGTCTCCTCCTCCTACTTCCATTGGTAC
CAACAGAAACCCGGAAGCTCCCCTAAACTGTGCATCTACAGCACCAGCAATC
TCGCCAGCGGCGTGCCCCCTAGGTTTTCCGGAAGCGGAAGCACCAGCTACTC
TTTAACCATCTCCTCCATGGAGGCTGAGGATGCCGCCACCTACTTTTGTCACC
AGTACCACCGGTCCCCCACCTTCGGAGGCGGCACCAAACTGGAGACAAAGA
GG (SEQ ID NO: 156).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical,

at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQ
KSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWS
SNPFTFGSGTKLEINRGGGGSGGGGSGGGGSQVQLQQSGAELARPGASVKMSCK
ASGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSS
STAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFREVQLQQSGPEL
VKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGSINPYNDYTKYNEKF
KGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNYWGRGTTLTVSSGGG
GSGGGGSGGGGSDIEMTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPG
SSPKLCIYSTSNLASGVPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGG
GTKLETKR (SEQ ID NO: 157).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCTTATTATTTTTATTCAGCTCCGCCTATTCC
CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGTGAGA
AGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACTGGTAT
CAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCAGCAAG
CTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCACCAGCTA
CTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACTATTGCC
AGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCTCGAAAT
CAATCGTGGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAG
CCAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCCGGCGCCTCC
GTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAATGCA
TTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATATCAAC
CCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCCACTT
TAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTTAACC
AGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCACTACTG
TTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGCTCCGGCACC
ACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGA

CAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGAT
CTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACC
GAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGG
CTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGC
GAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTT
AGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTC
ACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCC
TCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCC
AGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTG
ACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTC
TCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAA
GAAAAGGGCGAGTTCCGGGAGGTCCAGCTGCAGCAGAGCGGACCCGAACTC
GTGAAACCCGGTGCTTCCGTGAAAATGTCTTGTAAGGCCAGCGGATACACCT
TCACCTCCTATGTGATCCAGTGGGTCAAACAGAAGCCCGGACAAGGTCTCGA
GTGGATCGGCAGCATCAACCCTTACAACGACTATACCAAATACAACGAGAAG
TTTAAGGGAAAGGCTACTTTAACCTCCGACAAAAGCTCCATCACAGCCTACA
TGGAGTTCAGCTCTTTAACATCCGAGGACAGCGCTCTGTACTATTGCGCCCGG
TGGGGCGACGGCAATTACTGGGGACGGGGCACAACACTGACCGTGAGCAGC
GGAGGCGGAGGCTCCGGCGGAGGCGGATCTGGCGGTGGCGGCTCCGACATC
GAGATGACCCAGTCCCCCGCTATCATGTCCGCCTCTTTAGGCGAGCGGGTCA
CAATGACTTGTACAGCCTCCTCCAGCGTCTCCTCCTCCTACTTCCATTGGTAC
CAACAGAAACCCGGAAGCTCCCCTAAACTGTGCATCTACAGCACCAGCAATC
TCGCCAGCGGCGTGCCCCCTAGGTTTTCCGGAAGCGGAAGCACCAGCTACTC
TTTAACCATCTCCTCCATGGAGGCTGAGGATGCCGCCACCTACTTTTGTCACC
AGTACCACCGGTCCCCCACCTTCGGAGGCGGCACCAAACTGGAGACAAAGA
GG (SEQ ID NO: 158).

Exemplary Embodiments of Single-Chain Chimeric Polypeptides- Type B

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain and/or the second target-binding domain can independently bind specifically to an IL-2 receptor (e.g., human IL-2 receptor).

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain.

In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the second target-binding domain.

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain is a soluble human IL-2 protein. A non-limiting example of an IL-2 protein that binds specifically to an IL-2 receptor can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADE TATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 78).

In some embodiments, an IL-2 protein that binds specifically to an IL-2 receptor can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAACTGGAGCATTTAC TGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCCCAA ACTCACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGGCCACAGAA CTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGC TAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAATCAG CAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTCATG TGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGATGGA TTACCTTTTGTCAAAGCATCATCTCAACACTAACT (SEQ ID NO: 159).

In some embodiments, an IL-2 protein that binds specifically to an IL-2 receptor can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical,

at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GCCCCCACCTCCTCCTCCACCAAGAAGACCCAGCTGCAGCTGGAGCATTTAC
TGCTGGATTTACAGATGATTTTAAACGGCATCAACAACTACAAGAACCCCAA
GCTGACTCGTATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAG
CTGAAGCATTTACAGTGTTTAGAGGAGGAGCTGAAGCCCCTCGAGGAGGTGC
TGAATTTAGCCCAGTCCAAGAATTTCCATTTAAGGCCCCGGGATTTAATCAGC
AACATCAACGTGATCGTTTTAGAGCTGAAGGGCTCCGAGACCACCTTCATGT
GCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGAT
CACCTTCTGCCAGTCCATCATCTCCACTTTAACC (SEQ ID NO: 160).

In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein.

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELK
HLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADE
TATIVEFLNRWITFCQSIISTLTSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQV
YTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTG
SAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSL
RDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTV
NRKSTDSPVECMGQEKGEFREAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNP
KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNI
NVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT (SEQ ID NO: 161).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GCCCCCACCTCCTCCTCCACCAAGAAGACCCAGCTGCAGCTGGAGCATTTAC
TGCTGGATTTACAGATGATTTTAAACGGCATCAACAACTACAAGAACCCCAA
GCTGACTCGTATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGAG

CTGAAGCATTTACAGTGTTTAGAGGAGGAGCTGAAGCCCCTCGAGGAGGTGC
TGAATTTAGCCCAGTCCAAGAATTTCCATTTAAGGCCCCGGGATTTAATCAGC
AACATCAACGTGATCGTTTTAGAGCTGAAGGGCTCCGAGACCACCTTCATGT
GCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGAT
CACCTTCTGCCAGTCCATCATCTCCACTTTAACCAGCGGCACAACCAACACAG
TCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGA
ATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAG
TCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATC
TCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTT
TAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTA
TACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGC
CCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGA
GGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGAT
GTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTC
CGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGAT
AAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCG
TGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGG
GCGAGTTCCGGGAGGCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACA
ACTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATT
ACAAGAATCCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGCCCAA
GAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCT
CTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGACCCA
GGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGA
AACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTT
CTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTAACT (SEQ ID NO: 162).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTR
MLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVL
ELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTSGTTNTVAAYNLTWKST
NFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTY
LARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVT
VEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDK
GENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFREAPTSSSTKKTQLQLEHL
LLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLN

LAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSII STLT (SEQ ID NO: 163).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGCCCCCACCTCCTCCTCCACCAAGAAGACCCAGCTGCAGCTGGAGCATTTA
CTGCTGGATTTACAGATGATTTTAAACGGCATCAACAACTACAAGAACCCCA
AGCTGACTCGTATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCACCGA
GCTGAAGCATTTACAGTGTTTAGAGGAGGAGCTGAAGCCCCTCGAGGAGGTG
CTGAATTTAGCCCAGTCCAAGAATTTCCATTTAAGGCCCCGGGATTTAATCAG
CAACATCAACGTGATCGTTTTAGAGCTGAAGGGCTCCGAGACCACCTTCATG
TGCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTTGGA
TCACCTTCTGCCAGTCCATCATCTCCACTTTAACCAGCGGCACAACCAACACA
GTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCG
AATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAA
GTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGAT
CTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGT
TTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTT
ATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAG
CCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGG
AGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGA
TGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCT
CCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGA
TAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACC
GTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAG
GGCGAGTTCCGGGAGGCACCTACTTCAAGTTCTACAAAGAAAACACAGCTAC
AACTGGAGCATTTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAAT
TACAAGAATCCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGCCCAA
GAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCT
CTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGACCCA
GGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGA
AACAACATTCATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTT
CTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTAACT (SEQ ID NO: 164).

Exemplary Embodiments of Single-Chain Chimeric Polypeptides- Type C

In some embodiments of any of the single-chain chimeric polypeptides described herein, the first target-binding domain and/or the second target-binding domain can independently bind specifically to an IL-15 receptor (e.g., a human IL-15 receptor).

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain.

In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain and the second target-binding domain directly abut each other. In some embodiments of these single-chain chimeric polypeptides, the single-chain chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the second target-binding domain.

In some embodiments of these single-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain is a soluble human IL-15 protein. A non-limiting example of an IL-15 protein that binds specifically to an IL-15 receptor can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS (SEQ ID NO: 82).

In some embodiments, an IL-15 protein that binds specifically to an IL-15 receptor can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

AACTGGGTGAACGTGATCAGCGATTTAAAGAAGATCGAGGATTTAATCCAGA
GCATGCACATCGACGCCACTCTGTACACTGAGAGCGACGTGCACCCTAGCTG
CAAGGTGACTGCCATGAAGTGCTTTTTACTGGAGCTGCAAGTTATCTCTTTAG
AGAGCGGCGATGCCAGCATCCACGACACTGTGGAGAATTTAATCATTTTAGC
CAACAACTCTTTAAGCAGCAACGGCAACGTGACAGAGAGCGGCTGCAAGGA
GTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAGTTTTTACAGAGCTTCGTG
CACATCGTGCAGATGTTCATCAACACTAGC (SEQ ID NO: 165).

In some embodiments, an IL-15 protein that binds specifically to an IL-15 receptor can be encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGT
CCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGT
AAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGA
GAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCC
AATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGT
GCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCA
CATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 166).

In some embodiments of these single-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein.

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYT
TDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETN
LGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSS
SGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGE
FRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLE

SGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMF
INTS (SEQ ID NO: 167).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

AACTGGGTGAACGTGATCAGCGATTTAAAGAAGATCGAGGATTTAATCCAGA
GCATGCACATCGACGCCACTCTGTACACTGAGAGCGACGTGCACCCTAGCTG
CAAGGTGACTGCCATGAAGTGCTTTTTACTGGAGCTGCAAGTTATCTCTTTAG
AGAGCGGCGATGCCAGCATCCACGACACTGTGGAGAATTTAATCATTTTAGC
CAACAACTCTTTAAGCAGCAACGGCAACGTGACAGAGAGCGGCTGCAAGGA
GTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAGTTTTTACAGAGCTTCGTG
CACATCGTGCAGATGTTCATCAACACTAGCAGCGGCACAACCAACACAGTCG
CTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATG
GGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCC
GGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCA
CCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAG
CTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATAC
GAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCA
CCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGA
CGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTG
TTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGG
CAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 168).

In some embodiments, a single-chain chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92%

identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT
AMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEK
NIKEFLQSFVHIVQMFINTSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYT
VQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSA
GEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRD
VFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNR
KSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCK
VTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELE
EKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 169).

In some embodiments, a single-chain chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CAACTGGGTGAACGTGATCAGCGATTTAAAGAAGATCGAGGATTTAATCCAG
AGCATGCACATCGACGCCACTCTGTACACTGAGAGCGACGTGCACCCTAGCT
GCAAGGTGACTGCCATGAAGTGCTTTTTACTGGAGCTGCAAGTTATCTCTTTA
GAGAGCGGCGATGCCAGCATCCACGACACTGTGGAGAATTTAATCATTTTAG
CCAACAACTCTTTAAGCAGCAACGGCAACGTGACAGAGAGCGGCTGCAAGG
AGTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAGTTTTTACAGAGCTTCGT
GCACATCGTGCAGATGTTCATCAACACTAGCAGCGGCACAACCAACACAGTC
GCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAAT
GGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTC
CGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTC
ACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTA
GCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATA
CGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCC
ACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGG
ACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGT
GTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCG
GCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAA
AGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTG
AATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGC
GAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAG
ATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTG

CACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGT
TATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTA
ATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCG
GCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGC
AATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 170).

Exemplary Multi-Chain Chimeric Polypeptides- Type A

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-18 or a receptor of IL-12. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain

chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-18 (e.g., a soluble human IL-18).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 109).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
(SEQ ID NO: 171).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-12 (e.g., a soluble human IL-12). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-15 includes a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-15 human IL-15 further includes a linker sequence (e.g., any of the exemplary linker sequences described herein) between the sequence of soluble IL-12β (p40) and the sequence of soluble human IL-12α (p35). In some examples of these multi-chain chimeric polypeptides, the linker sequence comprises GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN

KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12β (p40) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCC (SEQ ID NO: 172).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKD
KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM
YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD
FYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTACACCA
CAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAGGCAG
ACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGATATCA
CCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGACAAA
GAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGGCTCTT
GTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTCCATCT
ACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCCAAGCT
GCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGGCTGTG
ATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCTCAGA
AGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGTGCAT
TTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATGAGCT
ATTTAAACGCCAGC (SEQ ID NO: 173).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM
QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD
VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGT
KVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEF
LIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLK
KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLI
ILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:
174).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 175).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSD
CRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKD

TKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM
FTVQNEDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 176).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC
TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC

CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 177).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPV
ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTV
EACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEF
KTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTK
IKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHADIWVKSYSLYSRERYICN
SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 178).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA

CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 179).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGI
TWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIW
STDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQV
QGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGG
GSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYP
CTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNS
ETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHA
DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 180).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC
ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 181).

Exemplary Multi-Chain Chimeric Polypeptides- Type B

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or to TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21

(e.g., a soluble human IL-21 polypeptide) or a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain bind specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-21 (e.g., a soluble human IL-21). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble TGF-β receptor (e.g., a soluble TGFRβRII receptor (e.g., a soluble human TGFRβRII receptor)). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at

least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCA TCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGA GAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGAT TTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGA AGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGA CAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the human TGFβRII receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQ
VYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVEST
GSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLS
LRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECE
ELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 189).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCTCCGGCACCACCAAT
ACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGACAATTC
TGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGATCTCCAC
CAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACCGAGTGT
GATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGGCTCGGG
TCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGCGAGCCT
CTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTTAGGCCA
GCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTCACCGTC
GAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCCTCCGGG
ATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCCAGCTCC
TCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTGACGTGG
ACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTCTCGTAC
CGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAAGAAAA
GGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATC
GAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGA

CGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGC
AAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAA
TTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGT
CCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTC
TGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 190).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTW
KSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQ
TYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVN
VTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDV
DKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIED
LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAN
NSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 191).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCTCCGGCACCACCAA
TACCGTGGCCGCTTATAACCTCACATGGAAGAGCACCAACTTCAAGACAATT
CTGGAATGGGAACCCAAGCCCGTCAATCAAGTTTACACCGTGCAGATCTCCA
CCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTACACAACAGACACCGAGT

GTGATTTAACCGACGAAATCGTCAAGGACGTCAAGCAAACCTATCTGGCTCG
GGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCACCGGCTCCGCTGGCGAGC
CTCTCTACGAGAATTCCCCCGAATTCACCCCTTATTTAGAGACCAATTTAGGC
CAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCACCAAGGTGAACGTCACCG
TCGAGGATGAAAGGACTTTAGTGCGGCGGAATAACACATTTTTATCCCTCCG
GGATGTGTTCGGCAAAGACCTCATCTACACACTGTACTATTGGAAGTCCAGCT
CCTCCGGCAAAAAGACCGCTAAGACCAACACCAACGAGTTTTTAATTGACGT
GGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAGCCGTGATCCCTTCTCGT
ACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGAGTGCATGGGCCAAGAAA
AGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGAT
CGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCG
ACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTG
CAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGA
ATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAG
TCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTT
CTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 192).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 193).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCCA

TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATCACGTGTCCTCCTCCTATGTCC
GTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAGC
GGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGAC
GGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGT
CTCAAATGTATTAGA (SEQ ID NO: 194).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 195).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC

AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCACAATCACCTCCATCTGTGAGAAGCCT
CAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGG
AAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGA
CGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGAC
CTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTA
GCGAGGAATACAATACCAGCAACCCCGACATCACGTGTCCTCCTCCTATGTC
CGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTCCAGGGAG
CGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCCAGCCTGAC
GGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAACCCCCAGT
CTCAAATGTATTAGA (SEQ ID NO: 196).

Exemplary Multi-Chain Chimeric Polypeptides- Type C

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or a receptor of IL-21. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor

domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21 polypeptide) or a soluble IL-7 (e.g., a soluble human IL-7 polypeptide). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a

receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-21 (e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 197).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG

GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-7 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 198).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQ
VYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVEST
GSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLS
LRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRT
VNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECE
ELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 199).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCCTCAGGCACTACAAATAC
TGTGGCAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTGG
AGTGGGAACCCAAACCCGTCAATCAAGTCTACACTGTTCAAATAAGCACTAA
GTCAGGAGATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGAC
CTCACCGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCT
TCTCCTACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCT
GTATGAGAACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAG
CCAACAATTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAG
AAGATGAACGGACTTTAGTCAGAAGGAACAACACTTTCCTAAGCCTCCGGGA
TGTTTTTGGCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTC
AGGAAAGAAAACAGCCAAAACAAACACTAATGAGTTTTTGATTGATGTGGAT
AAAGGAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAG
TTAACCGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAG
GGGAATTCAGAGAAAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCG
AAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGAC
GTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCA
AGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAAT
TTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTC

CGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCT
GCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 200).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MGVKVLFALICIAVAEAQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSSGTTNTVAAYNLTW
KSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQ
TYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVN
VTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDV
DKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIED
LIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILAN
NSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 201).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCCCA
AGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTGATC
AGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCAGAA
GATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGGCCCA
ACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATCAATT
AAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACAGAAA
CACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCCAAAG
AATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGCATCTG
TCCTCTAGAACACACGGAAGTGAAGATTCCTCAGGCACTACAAATACTGTGG
CAGCATATAATTTAACTTGGAAATCAACTAATTTCAAGACAATTTTGGAGTGG
GAACCCAAACCCGTCAATCAAGTCTACACTGTTCAAATAAGCACTAAGTCAG
GAGATTGGAAAAGCAAATGCTTTTACACAACAGACACAGAGTGTGACCTCAC
CGACGAGATTGTGAAGGATGTGAAGCAGACGTACTTGGCACGGGTCTTCTCC
TACCCGGCAGGGAATGTGGAGAGCACCGGTTCTGCTGGGGAGCCTCTGTATG

AGAACTCCCCAGAGTTCACACCTTACCTGGAGACAAACCTCGGACAGCCAAC
AATTCAGAGTTTTGAACAGGTGGGAACAAAAGTGAATGTGACCGTAGAAGAT
GAACGGACTTTAGTCAGAAGGAACAACACTTTCCTAAGCCTCCGGGATGTTT
TTGGCAAGGACTTAATTTATACACTTTATTATTGGAAATCTTCAAGTTCAGGA
AAGAAAACAGCCAAAACAAACACTAATGAGTTTTTGATTGATGTGGATAAAG
GAGAAAACTACTGTTTCAGTGTTCAAGCAGTGATTCCCTCCCGAACAGTTAAC
CGGAAGAGTACAGACAGCCCGGTAGAGTGTATGGGCCAGGAGAAAGGGGAA
TTCAGAGAAAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATT
TAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCAC
CCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTAT
CTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCT
GCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAAT
CCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 202)

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 203)

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA

ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTC
CCCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTA
CTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACG
TCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGA
CAACCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 204).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MGVKVLFALICIAVAEADCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK
ATNVAHWTTPSLKCIR (SEQ ID NO: 205).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGGGAGTGAAAGTTCTTTTGCCCTTATTTGTATTGCTGTGGCCGAGGCCGA
TTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGGTC
AGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCTGA
ATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAAGGT
ATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAATAG
CACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAATAC
TGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGGTGA
AGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACAGAA
AAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAAACT
TGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTCCCC
CCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTACTC
CAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACGTCC
AGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGACAA
CCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 206).

Exemplary Multi-Chain Chimeric Polypeptides- Type D

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or a receptor of IL-21. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21

(e.g., a soluble human IL-21 polypeptide) or a soluble IL-7 (e.g., a soluble human IL-7 polypeptide). In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21.

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA

GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 197).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-7 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 198).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 207).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC

AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 208).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%

identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 210).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 211).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 212).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ
ID NO: 213).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 214).

Exemplary Multi-Chain Chimeric Polypeptides- Type E

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-18 (e.g., a soluble human IL-18), a receptor for IL-12 (e.g., a soluble human IL-12), or CD16 (e.g., an anti-CD16 scFv). In some embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-12.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to CD16, and the second target-binding domain binds specifically to a receptor for IL-18. In some embodiments of these multi-chain

chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-18 (e.g., a soluble human IL-18).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED (SEQ ID NO: 109).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-18 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC

GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
(SEQ ID NO: 171).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-12 (e.g., a soluble human IL-12). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-15 includes a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-15 (e.g., soluble human IL-15) further includes a linker sequence (e.g., any of the exemplary linker sequences described herein) between the sequence of soluble IL-12β (p40) and the sequence of soluble human IL-12α (p35). In some examples of these multi-chain chimeric polypeptides, the linker sequence comprises GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCS (SEQ ID NO: 81).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12β (p40) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC

CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCC (SEQ ID NO: 172).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKD
KTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKM
YQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPD
FYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS (SEQ ID NO: 80).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-12α (p35) is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTACACCA
CAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAGGCAG
ACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGATATCA
CCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGACAAA
GAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGGCTCTT
GTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTCCATCT
ACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCCAAGCT
GCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGGCTGTG

ATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCTCAGA
AGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGTGCAT
TTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATGAGCT
ATTTAAACGCCAGC (SEQ ID NO: 173).

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain includes an scFv that specifically binds to CD16 (e.g., an anti-CD16 scFv).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH
(SEQ ID NO: 215).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCAT (SEQ ID NO: 216).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92%

identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 217).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 218).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQ
PRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKM
QFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNEDSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD
VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGT
KVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEF
LIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLK
KIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLI
ILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 174).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 175).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSD
CRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKD
TKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIM
FTVQNEDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 176).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCTACAGC
TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAACGACC
AAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGACCGAC
TCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGTACAA
GGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGAGAA
AATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATGAACC
CCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGCGGTCC
GTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGAGGGCT
ACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCAAGAA
GGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGAGGAT
AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT

GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCA
GCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCAC
TTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAAT
GTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATC
CACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAA
CGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAA
GAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCA
ATACCTCC (SEQ ID NO: 177).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLT
IQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTFLR
CEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDN
KEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKN
LQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKT
SATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPV
ATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHEDITKDKTSTV
EACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYEDLKMYQVEF
KTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTK
IKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHADIWVKSYSLYSRERYICN
SGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVRIT
CQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITG
AQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQL
VESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGG
STGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQ
GTLVTVSR (SEQ ID NO: 223).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG

CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG
ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGTCCGAGCTGACCCAGGACCCTGCTGTGT
CCGTGGCTCTGGGCCAGACCGTGAGGATCACCTGCCAGGGCGACTCCCTGAG
GTCCTACTACGCCTCCTGGTACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTG
GTGATCTACGGCAAGAACAACAGGCCCTCCGGCATCCCTGACAGGTTCTCCG
GATCCTCCTCCGGCAACACCGCCTCCCTGACCATCACAGGCGCTCAGGCCGA
GGACGAGGCTGACTACTACTGCAACTCCAGGGACTCCTCCGGCAACCATGTG
GTGTTCGGCGGCGGCACCAAGCTGACCGTGGGCCATGGCGGCGGCGGCTCCG
GAGGCGGCGGCAGCGGCGGAGGAGGATCCGAGGTGCAGCTGGTGGAGTCCG
GAGGAGGAGTGGTGAGGCCTGGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTC
CGGCTTCACCTTCGACGACTACGGCATGTCCTGGGTGAGGCAGGCTCCTGGA
AAGGGCCTGGAGTGGGTGTCCGGCATCAACTGGAACGGCGGATCCACCGGCT

ACGCCGATTCCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAGA
ACTCCCTGTACCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTA
CTACTGCGCCAGGGGCAGGTCCCTGCTGTTCGACTACTGGGGACAGGGCACC
CTGGTGACCGTGTCCAGG (SEQ ID NO: 224).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGI
TWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIW
STDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQG
VTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYE
NYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQV
QGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCSGGG
GSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYP
CTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMM
ALCLSSIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNS
ETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASITCPPPMSVEHA
DIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSE
LTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGI
PDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGG
GGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQA
PGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV
YYCARGRSLLFDYWGQGTLVTVSR (SEQ ID NO: 225).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC
ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTATCCCG
ATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAGACGG
CATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAAGACC
CTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGCCACA
AGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGGAAGA
CGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGAATAAG

ACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGTTGGTG
GCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCGGGGA
AGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCTGAGA
GGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAAGAAG
ATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGGTGGAC
GCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATCCGGGA
CATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCAAAAATA
GCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCACACCCCA
CAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCAAGCGGG
AGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCATCTGTCG
GAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCCAGCAGC
TGGTCCGAGTGGGCCAGCGTGCCTTGTTCCGGCGGTGGAGGATCCGGAGGAG
GTGGCTCCGGCGGCGGAGGATCTCGTAACCTCCCCGTGGCTACCCCCGATCC
CGGAATGTTCCCTTGTTTACACCACAGCCAGAATTTACTGAGGGCCGTGAGC
AACATGCTGCAGAAAGCTAGGCAGACTTTAGAATTTTACCCTTGCACCAGCG
AGGAGATCGACCATGAAGATATCACCAAGGACAAGACATCCACCGTGGAGG
CTTGTTTACCTCTGGAGCTGACAAAGAACGAGTCTTGTCTCAACTCTCGTGAA
ACCAGCTTCATCACAAATGGCTCTTGTTTAGCTTCCCGGAAGACCTCCTTTAT
GATGGCTTTATGCCTCAGCTCCATCTACGAGGATTTAAAGATGTACCAAGTGG
AGTTCAAGACCATGAACGCCAAGCTGCTCATGGACCCTAAACGGCAGATCTT
TTTAGACCAGAACATGCTGGCTGTGATTGATGAGCTGATGCAAGCTTTAAACT
TCAACTCCGAGACCGTCCCTCAGAAGTCCTCCCTCGAGGAGCCCGATTTTTAC
AAGACAAAGATCAAACTGTGCATTTTACTCCACGCCTTTAGGATCCGGGCCG
TGACCATTGACCGGGTCATGAGCTATTTAAACGCCAGCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGTCCGAGCTGACCCAGGACCCTGCTGTGT
CCGTGGCTCTGGGCCAGACCGTGAGGATCACCTGCCAGGGCGACTCCCTGAG
GTCCTACTACGCCTCCTGGTACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTG
GTGATCTACGGCAAGAACAACAGGCCCTCCGGCATCCCTGACAGGTTCTCCG
GATCCTCCTCCGGCAACACCGCCTCCCTGACCATCACAGGCGCTCAGGCCGA
GGACGAGGCTGACTACTACTGCAACTCCAGGGACTCCTCCGGCAACCATGTG
GTGTTCGGCGGCGGCACCAAGCTGACCGTGGGCCATGGCGGCGGCGGCTCCG
GAGGCGGCGGCAGCGGCGGAGGAGGATCCGAGGTGCAGCTGGTGGAGTCCG
GAGGAGGAGTGGTGAGGCCTGGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTC
CGGCTTCACCTTCGACGACTACGGCATGTCCTGGGTGAGGCAGGCTCCTGGA
AAGGGCCTGGAGTGGGTGTCCGGCATCAACTGGAACGGCGGATCCACCGGCT
ACGCCGATTCCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAGA
ACTCCCTGTACCTGCAGATGAACTCCCTGAGGGCCGAGGACACCGCCGTGTA
CTACTGCGCCAGGGGCAGGTCCCTGCTGTTCGACTACTGGGGACAGGGCACC
CTGGTGACCGTGTCCAGG (SEQ ID NO: 226).

Exemplary Multi-Chain Chimeric Polypeptides- Type F

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7 (e.g., a soluble human IL-7), CD16 (e.g., an anti-CD16 scFv), or a receptor for IL-21 (e.g., a soluble human IL-21). In some embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-21.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second

domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to CD16 or a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain includes a soluble IL-7 protein. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-7 protein is a soluble human IL-7. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes a target-binding domain that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes an scFv that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the additional target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the

second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-7 (e.g., a soluble human IL-7).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC (SEQ ID NO: 198).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 227).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-21 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 197).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182)

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain includes an scFv that specifically binds to CD16 (e.g., an anti-CD16 scFv).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH
(SEQ ID NO: 215).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC

TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCAT (SEQ ID NO: 216).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 217).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 218).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK

DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 207).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA

GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 208).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG

GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 210).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHG
GGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQ
APGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEHADIWVKSYSLYSRERYIC
NSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVD
QLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKK
LKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTH
GSEDS (SEQ ID NO: 232).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG

CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACATG
CCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGC
CTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCG
GCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCA
CTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACATG
ATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTGA
ACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACTG
CGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAAC
ACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCGG
AAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTGC
CCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGGT
TCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCCA
CGGCTCCGAGGACTCC (SEQ ID NO: 233).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQ
KPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSS
GNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSC
AASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNA
KNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQ
GQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQL
KSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERF
KSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 234).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACC
AGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAG
GCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCT
CCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAA
CTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTG
ACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGA
GGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGA
GGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGG
CATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGC
ATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGT
TCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTC
CCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTG
CTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACAT
GCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAG
CCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCC
GGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTC
ACTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACAT
GATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTG
AACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACT
GCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAA
CACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCG
GAAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTG
CCCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGG
TTCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCC
ACGGCTCCGAGGACTCC (SEQ ID NO: 235).

Exemplary Multi-Chain Chimeric Polypeptides- Type G

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGFβ (e.g., a human TGFβRII receptor), CD16 (e.g., an anti-CD16 scFv), or a receptor for IL-21 (e.g., a soluble human IL-21). In some

embodiments of these multi-chain chimeric polypeptides described herein, the first chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 or a receptor for IL-21.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional antigen-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional antigen-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, CD16, or a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to CD16 or a receptor of IL-21. In some embodiments of any

of the multi-chain chimeric polypeptides described herein, the first target-binding domain is a soluble TGF-β receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, soluble TGF-β receptor is a soluble TGFβRII receptor. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes an antigen-binding domain that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second antigen-binding domain includes an scFv that binds specifically to CD16. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second target-binding domain includes a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to a receptor for IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the additional target-binding domain includes a soluble IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the soluble IL-21 is a soluble human IL-21. In some embodiments of any of the multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes an additional target-binding domain that binds specifically to CD16.

In some embodiments of these multi-chain chimeric polypeptides, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen. In some embodiments, two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope. In some embodiments, two or more of the first target-binding domain, the

second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a TGFβRII receptor (e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94%

identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185)

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK

NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the sequence of soluble human IL-21 comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATTGTTG
ATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGCTCCA
GAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGAAGG
CCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATGTATC
AATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAAGACA
GAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACCACCC
AAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCATCAGC
ATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC (SEQ ID NO: 197).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH
(SEQ ID NO: 215).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at

least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCAT (SEQ ID NO: 216).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 217).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 218).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKS
GDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYEN
SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
YTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPV
ECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKC
FLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL
QSFVHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC

TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP
AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV
RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
ESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC

TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHG
GGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQ
APGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTA
VYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEHADIWVKSYSLYSRERYIC
NSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVD
QLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKK
LKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTH
GSEDS (SEQ ID NO: 232).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA
GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACATG
CCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGC
CTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCG

GCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCA
CTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACATG
ATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTGA
ACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACTG
CGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAAC
ACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCGG
AAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTGC
CCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGGT
TCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCCA
CGGCTCCGAGGACTCC (SEQ ID NO: 233).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQ
KPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSS
GNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSC
AASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNA
KNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSRITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQ
GQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQL
KSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERF
KSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 234).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACC
AGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAG
GCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCT
CCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAA
CTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTG
ACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGA

GGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGA
GGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGG
CATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGC
ATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGT
TCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTC
CCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTG
CTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGGATTACAT
GCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAG
CCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCC
GGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTC
ACTGGACAACACCCTCTTTAAAGTGCATCCGGCAGGGCCAGGACAGGCACAT
GATCCGGATGAGGCAGCTCATCGACATCGTCGACCAGCTGAAGAACTACGTG
AACGACCTGGTGCCCGAGTTTCTGCCTGCCCCCGAGGACGTGGAGACCAACT
GCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAGGCCCAGCTGAAGTCCGCCAA
CACCGGCAACAACGAGCGGATCATCAACGTGAGCATCAAGAAGCTGAAGCG
GAAGCCTCCCTCCACAAACGCCGGCAGGAGGCAGAAGCACAGGCTGACCTG
CCCCAGCTGTGACTCCTACGAGAAGAAGCCCCCCAAGGAGTTCCTGGAGAGG
TTCAAGTCCCTGCTGCAGAAGATGATCCATCAGCACCTGTCCTCCAGGACCC
ACGGCTCCGAGGACTCC (SEQ ID NO: 235).

Exemplary Multi-Chain Chimeric Polypeptides- Type H

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor

domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor for IL-7. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include a soluble IL-7 (e.g., a soluble human IL-7). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 227).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 207).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT
TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 208).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP

YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC

AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 210).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHITCPPPMSVEH
ADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR
(SEQ ID NO: 203).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTAATGG
TCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATTGCCT
GAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAGGAA
GGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAATGAA
TAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAACAA
TACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCTGGG
TGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGAACA
GAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATAAAA
ACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACACATCACGTGCCCTC
CCCCCATGTCCGTGGAACACGCAGACATCTGGGTCAAGAGCTACAGCTTGTA
CTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAAGCGTAAAGCCGGCACG
TCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACGAATGTCGCCCACTGGA
CAACCCCCAGTCTCAAATGCATTAGA (SEQ ID NO: 204).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNK
ATNVAHWTTPSLKCIR (SEQ ID NO: 250).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC
CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATATTACATGC
CCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCC
TCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGG
CACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCAC
TGGACAACACCCTCTTTAAAGTGCATCCGG (SEQ ID NO: 251).

Exemplary Multi-Chain Chimeric Polypeptides- Type I

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first

target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain each independently bind specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain bind specifically to the same epitope. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain include the same amino acid sequence.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain and the second target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII). In some

embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT

GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKS
GDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYEN
SPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLI
YTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPV
ECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKC
FLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFL
QSFVHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%

identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEW
EPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYP
AGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLV
RRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSV
QAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATL
YTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVT
ESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC

GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGC
TGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGG
GAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCG
GCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGCGATCTCAC
CGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGC
TACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACG
AGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAGCCCAC
CATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGGAGGAC
GAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGATGTGT
TCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTCCGGC
AAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGATAAA
GGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGTGA
ATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGCG
AGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGA
TTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTT
ATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAA
TCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGC
TGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAA
TCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLT
ECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 193).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
CGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
TTAAAGTGCATCCGG (SEQ ID NO: 257).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRER
YICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 195).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least

86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC
AACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTT
CAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCC
ATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGAC
GAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACG
ACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAA
GAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAAC
GACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTG
GCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGT
GCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTG
AAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAA
CCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGC
GTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGA
GGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCAC
CGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCT
TTAAAGTGCATCCGG (SEQ ID NO: 259).

Exemplary Multi-Chain Chimeric Polypeptides- Type J

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7, a receptor of IL-21, or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a receptor for CD137L (e.g., a soluble CD137L, e.g., a soluble human CD137L).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments, the second chimeric polypeptide can include an additional target-binding domain. In some embodiments, the additional target-binding domain and the

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L. In some embodiments, the additional target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble IL-7 (e.g., a soluble human IL-7). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 227).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain is a soluble IL-21 (e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain and/or the additional target-binding domain is a soluble CD137L (e.g., a soluble human CD137L).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 260).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least

90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 261).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP
PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEI (SEQ ID NO: 262).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA

GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 263).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAY
NLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVK
DVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVG
TKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNE
FLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDL
KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVEN
LIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID
NO: 207).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACA
ACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAA
CCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGAT
CAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACC
GAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCG
CCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGG
CGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATT

TAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGT
GACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGC
CTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTC
CTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATC
GACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCT
CCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCA
AGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAA
GAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACA
GAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACT
GGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACC
GTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGT
GACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAA
GGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTCC
(SEQ ID NO: 208).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKS
KCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTP
YLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYY
WKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMG
QEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLEL
QVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CGATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGAT
GGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGC

CTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGG
AGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGAT
GAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACC
ACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTC
TGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGG
AGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGAT
CAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCAC
AACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAA
ACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGA
TCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACAC
CGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTC
GCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTG
GCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAA
TTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAAT
GTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCA
GCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAG
TCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAA
TCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCC
CTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGC
CAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTA
AAGAAGATCGAAGATTTAATTCAGTCCATGCATATCGACGCCACTTTATACA
CAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTA
CTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACA
CCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCCAGCAACGGCAAC
GTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATC
AAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTTCATCAATACCTC
C (SEQ ID NO: 210).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELS
PDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTK
ELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVD

LPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLF
RVTPEIPAGLPSPRSE (SEQ ID NO: 268).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCT
CCGGCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGC
CGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAAT
GTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAG
GCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGG
TGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGC
GTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGC
CACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCA
CCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCT
GCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGG
GCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCC
GGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 269).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPG
LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHL
QPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEAR
ARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 270).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGC
TCCGGCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCG
CCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAA
TGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCA
GGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTG
GTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCG
CGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAG
CCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCC
ACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGC
TGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAG
GGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTC
CGGGTGACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ
ID NO: 271).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSDPAGLL
DLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKA
GVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEA
RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEI
(SEQ ID NO: 272).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTC
CCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAG
CCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAG
CAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACA
ACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCT
CCGGCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCAT
GTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCT
GGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTA
CAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTC
TTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCG
TTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCC
CTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGC

CTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGC
GTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGG
GCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 273).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTG
GLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAG
AAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQL
TQGATVLGLFRVTPEI (SEQ ID NO: 274).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
CCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGT
GAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAG
GCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCC
CCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATC
AGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCT
CCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACA
GCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCA
GCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGAC
AACACCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGC
TCCGGCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCA
TGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGC
TGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCT

ACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATG
TCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTC
CGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCG
CCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCG
GCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGG
GCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 275).

Exemplary Multi-Chain Chimeric Polypeptides- Type K

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-7 or TGF-β. In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to a receptor for IL-7.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain includes a soluble IL-7 protein (e.g., a soluble human IL-7 protein). In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 protein includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH (SEQ ID NO: 79).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-7 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC

AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT (SEQ ID NO: 227).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain comprises a target-binding domain that binds specifically to TGF-β. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEHSGTTNTVAAYN
LTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKD

VKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTK
VNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLI
DVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIE
DLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILA
NNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 207).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATGG
TGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCCTC
AACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGAGG
GCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATGAA
CTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCACC
ATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCTGG
GAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGAGC
AGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATCAA
GACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACAACC
AACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCAT
CCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCA
CCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGC
GATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGT
GTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTT
TATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAG
CCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGG
AGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGA
TGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTC
CGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGAT
AAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGT
GAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGC
GAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAG
ATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTA
TCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTG
CAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCC
TTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 208).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSDCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNN
EFNFFKRHICDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC
TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKIL
MGTKEHSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSK
CFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWK
SSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEK
GEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS (SEQ ID NO: 209).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATGG
TGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCCTC
AACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGAGG
GCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATGAA
CTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCACC
ATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCTGG
GAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGAGC
AGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATCAA
GACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCATAGCGGCACAACC
AACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCAACTTCAAAACCAT
CCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACACCGTGCAGATCAGCA
CCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACCACCGACACCGAGTGC
GATCTCACCGATGAGATCGTGAAAGATGTGAAACAGACCTACCTCGCCCGGGT
GTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGGTTCCGCTGGCGAGCCTT
TATACGAGAACAGCCCCGAATTTACCCCTTACCTCGAGACCAATTTAGGACAG
CCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAAGGTGAATGTGACAGTGG

AGGACGAGCGGACTTTAGTGCGGCGGAACAACACCTTTCTCAGCCTCCGGGA
TGTGTTCGGCAAAGATTTAATCTACACACTGTATTACTGGAAGTCCTCTTCCTC
CGGCAAGAAGACAGCTAAAACCAACACAAACGAGTTTTTAATCGACGTGGAT
AAAGGCGAAAACTACTGTTTCAGCGTGCAAGCTGTGATCCCCTCCCGGACCGT
GAATAGGAAAAGCACCGATAGCCCCGTTGAGTGCATGGGCCAAGAAAAGGGC
GAGTTCCGGGAGAACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAG
ATTTAATTCAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGC
ACCCCTCTTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTA
TCTCTTTAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATC
ATTTTAGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTG
CAAGGAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCC
TTTGTGCACATTGTCCAGATGTTCATCAATACCTCC (SEQ ID NO: 210).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIR (SEQ ID NO: 193).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC

CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
G (SEQ ID NO: 257).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIR (SEQ ID NO: 195).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC

CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
G (SEQ ID NO: 259).

Exemplary Multi-Chain Chimeric Polypeptides- Type L

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, a receptor of IL-21, or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a receptor for CD137L (e.g., a soluble CD137L, e.g., a soluble human CD137L).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut

each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described

herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, one or more of the first target-binding domain, the second target-binding domain and the additional target-binding domain is an agonistic antigen-binding domain. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain, the second target-binding domain, and the additional target-binding domain are each agonistic antigen-binding domains. In some embodiments of these multi-chain chimeric polypeptides, the antigen-binding domain includes a scFv or single-domain antibody.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA

GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO:
186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain includes a soluble IL-21(e.g., a soluble human IL-21).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain or the additional target-binding domain binds specifically to a receptor for CD137L. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain and/or the additional target-binding domain includes a soluble CD137L (e.g., a soluble human CD137L).

In some embodiments of these multi-chain chimeric polypeptides, a soluble CD137L includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 260).

In some embodiments of these multi-chain chimeric polypeptides, a soluble CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 261).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR VTPEI (SEQ ID NO: 262).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA
GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 263).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least

94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%

identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC

ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELSP
DDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKE
LVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPP
ASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVT
PEIPAGLPSPRSE (SEQ ID NO: 268).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGG
CCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTC
TGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGT
GTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGT
GGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGG
TGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACT
GCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCG
CCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCAC
CTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCAC
GCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTG
ACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 269)

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPG
LAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQ
PLRSAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARA
RHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 270).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTCGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGG
CCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTC
TGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGT
GTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGT
GGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGG
TGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACT
GCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCG
CCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCAC
CTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCAC
GCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTG
ACCCCCGAAATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 271)

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER

FKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIWVKSYSLYSRERYICNSGF
KRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSDPAGLLD
LRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGV
YYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNS
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEI (SEQ ID NO: 272).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTT
GCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTA
CAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAA
GAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCT
TTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTC
ACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGG
CTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTC
GGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCC
ATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCC
ACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 273).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at

least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPED
VETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLT
CPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDSITCPPPMSVEHADIW
VKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGS
GGGGSGGGGSDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGG
LSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQG
ATVLGLFRVTPEI (SEQ ID NO: 274).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTGA
GCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGGC
AGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCCC
CAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCAG
CACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCCATTACATGCCCCCCTCC
CATGAGCGTGGAGCACGCCGACATCTGGGTGAAGAGCTATAGCCTCTACAGCC
GGGAGAGGTATATCTGTAACAGCGGCTTCAAGAGGAAGGCCGGCACCAGCAG
CCTCACCGAGTGCGTGCTGAATAAGGCTACCAACGTGGCTCACTGGACAACA
CCCTCTTTAAAGTGCATCCGGGGCGGTGGAGGATCCGGAGGAGGTGGCTCCG
GCGGCGGAGGATCTGATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTT
GCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTA
CAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAA
GAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCT
TTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTC
ACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGG
CTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTC
GGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCC
ATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCC
ACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 275).

Exemplary Multi-Chain Chimeric Polypeptides- Type M

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor of IL-21. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor for IL-21 (e.g., a soluble IL-21, e.g., a soluble human IL-21) or a TGF-β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to TGF-β or a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In

some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA
ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT
TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT
CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG
AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT
CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG

AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to a receptor for IL-21. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble IL-21 (e.g., a human soluble IL-21). In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-21 includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 83).

In some embodiments of these multi-chain chimeric polypeptides, the soluble IL-21 is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC

CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 182).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG

CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE

SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC

ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAP
EDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHR
LTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDS (SEQ ID NO: 300).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG

CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 301).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRQGQDRHMIRMRQLIDIV
DQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIK
KLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRT
HGSEDS (SEQ ID NO: 302).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA

CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GCAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTC
GACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCC
CCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAA
GGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC (SEQ ID NO: 303).

Exemplary Multi-Chain Chimeric Polypeptides- Type N

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or CD16. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to CD16 (e.g., an anti-CD16 scFv) or a TGF-β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor).

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to TGF-β or CD16. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor). In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT

CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA
GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC
AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO:
186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain binds specifically to CD16. In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes an anti-CD16 scFv. In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a light chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPS GIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGH (SEQ ID NO: 215).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a light chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTGA GGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA CCGTGGGCCAT (SEQ ID NO: 216).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 includes a heavy chain variable domain that includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINW
NGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDY
WGQGTLVTVSR (SEQ ID NO: 217).

In some embodiments of these multi-chain chimeric polypeptides, the scFv that binds specifically to CD16 is encoded by a heavy chain variable domain sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGGCTCC
CTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCATGTC
CTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCATCAAC
TGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTCACCA
TCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCCTGAG
GGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCTGTTC
GACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 218).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86%

identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC

CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQ
QKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDS
SGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLS
CAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDN
AKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQGTLVTVSR (SEQ ID NO:
308).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 309).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY
ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRSELTQDPAVSVALGQTVR
ITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTIT
GAQAEDEADYYCNSRDSSGNHVVFGGGTKLTVGHGGGGSGGGGSGGGGSEVQ
LVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGG
STGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRSLLFDYWGQG
TLVTVSR (SEQ ID NO: 310).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC

CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GTCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACCGTG
AGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGTACCA
GCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAACAGG
CCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCGCCTC
CCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTGCAAC
TCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAGCTGA
CCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAG
GATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAG
GCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGC
ATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCA
TCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTT
CACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCC
CTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGC
TGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG (SEQ ID NO: 311).

Exemplary Multi-Chain Chimeric Polypeptides- Type O

In some embodiments of any of the multi-chain chimeric polypeptides described herein, the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor of CD137L. In some embodiments of these multi-chain chimeric polypeptides described herein, the second chimeric polypeptide further includes the additional target-binding domain. In some embodiments of these multi-chain chimeric polypeptides described herein, the additional target-binding domain binds specifically to a receptor to TGF- β (e.g., a soluble TGF-β receptor, e.g., a soluble TGFβRII receptor) or CD137L.

In some examples of these multi-chain chimeric polypeptides, the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide. In some examples of these multi-chain chimeric polypeptides, the first chimeric polypeptide further comprises a linker sequence (e.g., any of the exemplary

linkers described herein) between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the first chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

In some embodiments, the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second domain of the pair of affinity domains directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g., any of the exemplary linkers described herein) between the second domain of the pair of affinity domains and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the additional target-binding domain and the second target-binding domain directly abut each other in the second chimeric polypeptide. In some embodiments of these multi-chain chimeric polypeptides, the second chimeric polypeptide further includes a linker sequence (e.g.,

any of the exemplary linkers described herein) between the second target-binding domain and the additional target-binding domain in the second chimeric polypeptide.

In some embodiments of these multi-chain chimeric polypeptides, the soluble tissue factor domain can be any of the exemplary soluble tissue factor domains described herein. In some embodiments of these multi-chain chimeric polypeptides, the pair of affinity domains can be any of the exemplary pairs of affinity domains described herein.

In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain binds specifically to TGF-β, and the second target-binding domain binds specifically to CD137L. In some embodiments of these multi-chain chimeric polypeptides, the first target-binding domain or the additional target-binding domain is a soluble TGF-β receptor (e.g., a soluble TGFβRII receptor, e.g., a soluble human TGFβRII receptor).

In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a first sequence of soluble human TGFRβRII and a second sequence of soluble human TGFRβRII. In some embodiments of these multi-chain chimeric polypeptides, the soluble human TGFRβRII includes a linker disposed between the first sequence of soluble human TGFRβRII and the second sequence of soluble human TGFRβRII. In some examples of these multi-chain chimeric polypeptides, the linker includes the sequence GGGGSGGGGSGGGGS (SEQ ID NO: 102).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 183).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor comprises a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at

least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 184).

In some embodiments of these multi-chain chimeric polypeptides, the first sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT (SEQ ID NO: 185).

In some embodiments of these multi-chain chimeric polypeptides, the second sequence of soluble human TGFRβRII receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATA ACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTT TCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCAT CTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAG AATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTT CATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAA GCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGAC AATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 186).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor includes a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRK
NDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD (SEQ ID NO: 188).

In some embodiments of these multi-chain chimeric polypeptides, the soluble TGF-β receptor is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACA
ACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTC
AGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCA
TCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACG
AGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGA
CTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAG
AAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACG
ACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGG
CGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTG
CAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGA
AATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAAC
CAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTC
AGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGA
AACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGAC
GCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACC
TTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAG
CGAGGAATACAATACCAGCAACCCCGAC (SEQ ID NO: 187).

In some embodiments of these multi-chain chimeric polypeptides, the second target-binding domain includes a soluble CD137L protein (e.g., a soluble human CD137L protein). In some embodiments of these multi-chain chimeric polypeptides, a soluble

human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE (SEQ ID NO: 260).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 261).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L includes a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKEL
VVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLP
PASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEI (SEQ ID NO: 262).

In some embodiments of these multi-chain chimeric polypeptides, a soluble human CD137L is encoded by a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to:

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTGGTGG
CCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACCCAGG
CCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACACGAA
GGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTAGAGC
TGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGCTGCA
CCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACCGTGG
ACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCAGGGC
CGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCACACTGA
GGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCTTGGGA
CTCTTCCGGGTGACCCCCGAAATC (SEQ ID NO: 263).

In some embodiments, the first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGD
WKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPE
FTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTL
YYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECM
GQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLE
LQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSF
VHIVQMFINTS (SEQ ID NO: 236).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC

ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG
GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 237).

In some embodiments, a first chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG

GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDSGTTNTVAAYNLTWKSTNFKTILEWE
PKPVNQVYTVQISTKSGDWKSKCFYTTDTECDLTDEIVKDVKQTYLARVFSYPA
GNVESTGSAGEPLYENSPEFTPYLETNLGQPTIQSFEQVGTKVNVTVEDERTLVRR
NNTFLSLRDVFGKDLIYTLYYWKSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQA
VIPSRTVNRKSTDSPVECMGQEKGEFRENWVNVISDLKKIEDLIQSMHIDATLYTE
SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESG
CKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO: 238).

In some embodiments, a first chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACAGCGGCACAACCAACACAGTCGCTGCCTATAACCTC
ACTTGGAAGAGCACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCG
TTAACCAAGTTTACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTC
CAAATGTTTCTATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGA
AAGATGTGAAACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAAT
GTGGAGAGCACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATT
TACCCCTTACCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGC
AAGTTGGCACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCG

GCGGAACAACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCT
ACACACTGTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACC
AACACAAACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAG
CGTGCAAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCC
CCGTTGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAGAACTGGGTGA
ACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGTCCATGCATATCG
ACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGTAAGGTGACCGCC
ATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGAGAGCGGAGACGCT
AGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCCAATAACTCTTTATCC
AGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGTGCGAAGAGCTGGAG
GAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCACATTGTCCAGATGTT
CATCAATACCTCC (SEQ ID NO: 239).

In some embodiments, the second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTEC
VLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSREGPELSPDDPAGLLDLRQ
GMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYV
FFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFG
FQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRS
E (SEQ ID NO: 316).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA

ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GGGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCTCGCGA
GGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAG
GGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCT
GAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTG
AGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACT
ATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGC
TCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGC
CGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACT
CGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTG
GGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCGGA
CTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 317).

In some embodiments, a second chimeric polypeptide can include a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

MKWVTFISLLFLFSSAYSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILED
AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSG
GGGSIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKP
GETFFMCSCSSDECNDNIIFSEEYNTSNPDITCPPPMSVEHADIWVKSYSLYSRERY

ICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRGGGGSGGGGSGGGGSRE
GPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYK
EDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALAL
TVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVL
GLFRVTPEIPAGLPSPRSE (SEQ ID NO: 318).

In some embodiments, a second chimeric polypeptide is encoded by a sequence that is at least 80% identical (e.g., at least 82% identical, at least 84% identical, at least 86% identical, at least 88% identical, at least 90% identical, at least 92% identical, at least 94% identical, at least 96% identical, at least 98% identical, at least 99% identical, or 100% identical) to:

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC
ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGACAA
CAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCAGGTTCA
GCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCACCTCCATCT
GCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAATGACGAGA
ACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATCACGACTTC
ATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAAGAAGAAGC
CCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGTAACGACAAC
ATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAGGTGGCGGATC
CGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCACGTGCAGAAG
AGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCCGTGAAATTTCC
CCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGACAACCAGAAGT
CCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAGCCTCAGGAGGTG
TGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCCTGGAAACCGTCTG
CCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGAAGACGCCGCCAGCC
CTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGAGACCTTTTTCATGTGC
TCCTGCAGCAGCGACGAATGCAACGACAATATCATCTTTAGCGAGGAATACAA
TACCAGCAACCCCGACATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCG
ACATCTGGGTGAAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAAC
AGCGGCTTCAAGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTG
AATAAGGCTACCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCG
GGGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCTCGCGA
GGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGCGGCAG
GGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCT
GAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTG
AGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACT
ATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGC
TCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGC
CGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACT

CGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTG
GGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCA
GGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATCCCAGCCGGA
CTCCCTTCACCGAGGTCGGAA (SEQ ID NO: 319).

Methods of Treating an Aging-Related Disease or Condition

Provided herein are methods of treating an aging-related disease or condition (e.g. any of the exemplary types of aging-related disease or condition described herein or known in the art) in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition (e.g. any of the exemplary types of aging-related disease or condition described herein or known in the art) a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) (e.g. any of the natural killer (NK) cell activating agent(s) described herein or known in the art).

Provided herein are methods of treating an aging-related disease or condition (e.g. any of the exemplary types of aging-related disease or condition described herein or known in the art) in a subject in need thereof that include administering to a subject identified as having an aging-related disease or condition (e.g. any of the exemplary types of aging-related disease or condition described herein or known in the art) a therapeutically effective amount of activated NK cells (e.g. any of the activated NK cells described herein or known in the art).

Some embodiments of these methods further include: obtaining a resting NK cell; and contacting the resting NK cell *in vitro* in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is a haploidentical NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an allogeneic resting NK cell. In some examples of these methods, the resting NK cell is an artificial NK cell. In some examples of any of these methods, the resting NK cell is a

genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

In some examples of these methods, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium. Some examples of these methods further include isolating the activated NK cells (and optionally further administering a therapeutically effective amount of the activated NK cells to a subject, e.g., any of the subjects described herein). In some embodiments of these methods, the contacting step is performed for a period of about 2 hours to about 20 days (or any of the subranges of this range described herein).

In some embodiments of any of the methods described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease.

Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

A non-limiting example of an autoimmune disease is type-1 diabetes.

Non-limiting examples of metabolic disease include: obesity, a lipodystrophy, and type-2 diabetes mellitus.

Non-limiting examples of neurodegenerative disease include: Alzheimer's disease, Parkinson's disease, and dementia.

Non-limiting examples of cardiovascular disease include: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

Non-limiting examples of skin disease include: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

Non-limiting examples of progeria disease include: progeria and Hutchinson-Gilford Progeria Syndrome.

Non-limiting examples of fragility disease include: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some embodiments of any of the aging-related disease or condition described herein, the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

In some embodiments of any of the aging-related disease or condition described herein, the aging-related disease or condition is type-2 diabetes or atherosclerosis.

In some embodiments of any of the methods described herein, the subject has been diagnosed or identified as having an aging-related disease or condition (e.g., any of the exemplary aging-related diseases or conditions described herein). Some embodiments of any of the methods described herein can include a step of selecting a subject identified or diagnosed as having an aging-related disease or condition (e.g., any of the exemplary aging-related diseases or conditions described herein).

In some embodiments of these methods, the administering results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 10% decrease to about a 99% decrease,

about a 10% decrease to about a 95% decrease, about a 10% decrease to about a 90% decrease, about a 10% decrease to about a 85% decrease, about a 10% decrease to about a 80% decrease, about a 10% decrease to about a 75% decrease, about a 10% decrease to about a 70% decrease, about a 10% decrease to about a 65% decrease, about a 10% decrease to about a 60% decrease, about a 10% decrease to about a 55% decrease, about a 10% decrease to about a 50% decrease, about a 10% decrease to about a 45% decrease, about a 10% decrease to about a 40% decrease, about a 10% decrease to about a 35% decrease, about a 10% decrease to about a 30% decrease, about a 10% decrease to about a 25% decrease, about a 10% decrease to about a 20% decrease, about a 10% decrease to about a 15% decrease, about a 15% decrease to about a 99% decrease, about a 15% decrease to about a 95% decrease, about a 15% decrease to about a 90% decrease, about a 15% decrease to about a 85% decrease, about a 15% decrease to about a 80% decrease, about a 15% decrease to about a 75% decrease, about a 15% decrease to about a 70% decrease, about a 15% decrease to about a 65% decrease, about a 15% decrease to about a 60% decrease, about a 15% decrease to about a 55% decrease, about a 15% decrease to about a 50% decrease, about a 15% decrease to about a 45% decrease, about a 15% decrease to about a 40% decrease, about a 15% decrease to about a 35% decrease, about a 15% decrease to about a 30% decrease, about a 15% decrease to about a 25% decrease, about a 15% decrease to about a 20% decrease, about a 20% decrease to about a 99% decrease, about a 20% decrease to about a 95% decrease, about a 20% decrease to about a 90% decrease, about a 20% decrease to about a 85% decrease, about a 20% decrease to about a 80% decrease, about a 20% decrease to about a 75% decrease, about a 20% decrease to about a 70% decrease, about a 20% decrease to about a 65% decrease, about a 20% decrease to about a 60% decrease, about a 20% decrease to about a 55% decrease, about a 20% decrease to about a 50% decrease, about a 20% decrease to about a 45% decrease, about a 20% decrease to about a 40% decrease, about a 20% decrease to about a 35% decrease, about a 20% decrease to about a 30% decrease, about a 20% decrease to about a 25% decrease, about a 25% decrease to about a 99% decrease, about a 25% decrease to about a 95% decrease, about a 25% decrease to about a 90% decrease, about a 25% decrease to about a 85% decrease, about a 25% decrease to about a 80% decrease,

about a 25% decrease to about a 75% decrease, about a 25% decrease to about a 70% decrease, about a 25% decrease to about a 65% decrease, about a 25% decrease to about a 60% decrease, about a 25% decrease to about a 55% decrease, about a 25% decrease to about a 50% decrease, about a 25% decrease to about a 45% decrease, about a 25% decrease to about a 40% decrease, about a 25% decrease to about a 35% decrease, about a 25% decrease to about a 30% decrease, about a 30% decrease to about a 99% decrease, about a 30% decrease to about a 95% decrease, about a 30% decrease to about a 90% decrease, about a 30% decrease to about a 85% decrease, about a 30% decrease to about a 80% decrease, about a 30% decrease to about a 75% decrease, about a 30% decrease to about a 70% decrease, about a 30% decrease to about a 65% decrease, about a 30% decrease to about a 60% decrease, about a 30% decrease to about a 55% decrease, about a 30% decrease to about a 50% decrease, about a 30% decrease to about a 45% decrease, about a 30% decrease to about a 40% decrease, about a 30% decrease to about a 35% decrease, about a 35% decrease to about a 99% decrease, about a 35% decrease to about a 95% decrease, about a 35% decrease to about a 90% decrease, about a 35% decrease to about a 85% decrease, about a 35% decrease to about a 80% decrease, about a 35% decrease to about a 75% decrease, about a 35% decrease to about a 70% decrease, about a 35% decrease to about a 65% decrease, about a 35% decrease to about a 60% decrease, about a 35% decrease to about a 55% decrease, about a 35% decrease to about a 50% decrease, about a 35% decrease to about a 45% decrease, about a 35% decrease to about a 40% decrease, about a 40% decrease to about a 99% decrease, about a 40% decrease to about a 95% decrease, about a 40% decrease to about a 90% decrease, about a 40% decrease to about a 85% decrease, about a 40% decrease to about a 80% decrease, about a 40% decrease to about a 75% decrease, about a 40% decrease to about a 70% decrease, about a 40% decrease to about a 65% decrease, about a 40% decrease to about a 60% decrease, about a 40% decrease to about a 55% decrease, about a 40% decrease to about a 50% decrease, about a 40% decrease to about a 45% decrease, about a 45% decrease to about a 99% decrease, about a 45% decrease to about a 95% decrease, about a 45% decrease to about a 90% decrease, about a 45% decrease to about a 85% decrease, about a 45% decrease to about a 80% decrease, about a 45% decrease to about a 75% decrease,

about a 45% decrease to about a 70% decrease, about a 45% decrease to about a 65% decrease, about a 45% decrease to about a 60% decrease, about a 45% decrease to about a 55% decrease, about a 45% decrease to about a 50% decrease, about a 50% decrease to about a 99% decrease, about a 50% decrease to about a 95% decrease, about a 50% decrease to about a 90% decrease, about a 50% decrease to about a 85% decrease, about a 50% decrease to about a 80% decrease, about a 50% decrease to about a 75% decrease, about a 50% decrease to about a 70% decrease, about a 50% decrease to about a 65% decrease, about a 50% decrease to about a 60% decrease, about a 50% decrease to about a 55% decrease, about a 55% decrease to about a 99% decrease, about a 55% decrease to about a 95% decrease, about a 55% decrease to about a 90% decrease, about a 55% decrease to about a 85% decrease, about a 55% decrease to about a 80% decrease, about a 55% decrease to about a 75% decrease, about a 55% decrease to about a 70% decrease, about a 55% decrease to about a 65% decrease, about a 55% decrease to about a 60% decrease, about a 60% decrease to about a 99% decrease, about a 60% decrease to about a 95% decrease, about a 60% decrease to about a 90% decrease, about a 60% decrease to about a 85% decrease, about a 60% decrease to about a 80% decrease, about a 60% decrease to about a 75% decrease, about a 60% decrease to about a 70% decrease, about a 60% decrease to about a 65% decrease, about a 65% decrease to about a 99% decrease, about a 65% decrease to about a 95% decrease, about a 65% decrease to about a 90% decrease, about a 65% decrease to about a 85% decrease, about a 65% decrease to about a 80% decrease, about a 65% decrease to about a 75% decrease, about a 65% decrease to about a 70% decrease, about a 70% decrease to about a 99% decrease, about a 70% decrease to about a 95% decrease, about a 70% decrease to about a 90% decrease, about a 70% decrease to about a 85% decrease, about a 70% decrease to about a 80% decrease, about a 70% decrease to about a 75% decrease, about a 75% decrease to about a 99% decrease, about a 75% decrease to about a 95% decrease, about a 75% decrease to about a 90% decrease, about a 75% decrease to about a 85% decrease, about a 75% decrease to about a 80% decrease, about a 80% decrease to about a 99% decrease, about a 80% decrease to about a 95% decrease, about a 80% decrease to about a 90% decrease, about a 80% decrease to about a 85% decrease, about a 85% decrease to about a 99% decrease,

about a 85% decrease to about a 95% decrease, about a 85% decrease to about a 90% decrease, about a 90% decrease to about a 99% decrease, about a 90% decrease to about a 95% decrease, or about a 95% decrease to about a 99% decrease) in the number of senescent cells in a target tissue in the subject, e.g., as compared to the number of senescent cells in the target tissue in the subject prior to treatment.

In some embodiments of these methods, the administering results in an increase (e.g., at least a 5% increase, at least a 10% increase, at least a 15% increase, at least a 20% increase, at least a 25% increase, at least a 30% increase, at least a 35% increase, at least a 40% increase, at least a 45% increase, at least a 50% increase, at least a 55% increase, at least a 60% increase, at least a 65% increase, at least a 70% increase, at least a 75% increase, at least a 80% increase, at least a 85% increase, at least a 90% increase, at least a 95% increase, or at least a 99% increase, or about a 10% increase to about a 500% increase (or any of the subranges of this range described herein) in the levels of IFN-γ, a cytotoxic granule granzyme, and/or perforin in the subject, as compared to the levels in a subject prior to treatment or a similar control subject who has not received a treatment.

In some embodiments, these methods can result in a reduction in the number, severity, or frequency of one or more symptoms of the cancer in the subject (e.g., as compared to the number, severity, or frequency of the one or more symptoms of the cancer in the subject prior to treatment). In some embodiments, these methods can result in a reduction (e.g., about 1% reduction to about 99% reduction, about 1% reduction to about 95% reduction, about 1% reduction to about 90% reduction, about 1% reduction to about 85% reduction, about 1% reduction to about 80% reduction, about 1% reduction to about 75% reduction, about 1% reduction to about 70% reduction, about 1% reduction to about 65% reduction, about 1% reduction to about 60% reduction, about 1% reduction to about 55% reduction, about 1% reduction to about 50% reduction, about 1% reduction to about 45% reduction, about 1% reduction to about 40% reduction, about 1% reduction to about 35% reduction, about 1% reduction to about 30% reduction, about 1% reduction to about 25% reduction, about 1% reduction to about 20% reduction, about 1% reduction to about 15% reduction, about 1% reduction to about 10% reduction, about 1% reduction to

about 5% reduction, about 5% reduction to about 99% reduction, about 5% reduction to about 95% reduction, about 5% reduction to about 90% reduction, about 5% reduction to about 85% reduction, about 5% reduction to about 80% reduction, about 5% reduction to about 75% reduction, about 5% reduction to about 70% reduction, about 5% reduction to about 65% reduction, about 5% reduction to about 60% reduction, about 5% reduction to about 55% reduction, about 5% reduction to about 50% reduction, about 5% reduction to about 45% reduction, about 5% reduction to about 40% reduction, about 5% reduction to about 35% reduction, about 5% reduction to about 30% reduction, about 5% reduction to about 25% reduction, about 5% reduction to about 20% reduction, about 5% reduction to about 15% reduction, about 5% reduction to about 10% reduction, about 10% reduction to about 99% reduction, about 10% reduction to about 95% reduction, about 10% reduction to about 90% reduction, about 10% reduction to about 85% reduction, about 10% reduction to about 80% reduction, about 10% reduction to about 75% reduction, about 10% reduction to about 70% reduction, about 10% reduction to about 65% reduction, about 10% reduction to about 60% reduction, about 10% reduction to about 55% reduction, about 10% reduction to about 50% reduction, about 10% reduction to about 45% reduction, about 10% reduction to about 40% reduction, about 10% reduction to about 35% reduction, about 10% reduction to about 30% reduction, about 10% reduction to about 25% reduction, about 10% reduction to about 20% reduction, about 10% reduction to about 15% reduction, about 15% reduction to about 99% reduction, about 15% reduction to about 95% reduction, about 15% reduction to about 90% reduction, about 15% reduction to about 85% reduction, about 15% reduction to about 80% reduction, about 15% reduction to about 75% reduction, about 15% reduction to about 70% reduction, about 15% reduction to about 65% reduction, about 15% reduction to about 60% reduction, about 15% reduction to about 55% reduction, about 15% reduction to about 50% reduction, about 15% reduction to about 45% reduction, about 15% reduction to about 40% reduction, about 15% reduction to about 35% reduction, about 15% reduction to about 30% reduction, about 15% reduction to about 25% reduction, about 15% reduction to about 20% reduction, about 20% reduction to about 99% reduction, about 20% reduction to about 95% reduction, about 20% reduction to

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In some embodiments, these methods can result in treatment of metabolic disease in the subject. In some embodiments, the treatment of metabolic disease can result in, e.g., one or more (e.g., two, three, four, five, or six) improved glucose tolerance, improved glucose utilization, decreased severity or progression of diabetic osteoarthropathy, decreased severity or progression of skin lesions, decreased severity or progression of ketosis, decreased generation of autoantibodies against islet cells, increased insulin sensitivity, decreased mass, and decreased body mass index. The response of a subject to treatment can be monitored by determining fasting glucose or glucose tolerance according to standard techniques. Typically, in accordance with the method, blood glucose is lowered so as to achieve a blood glucose level characterized by

a fasting blood glucose of less than 100 mg/dL or a two-hour 75-g oral glucose tolerance test values of less than 140 mg/dL. In some embodiments, response to treatment may include determining other factors relevant to pre-diabetes, new-onset diabetes, or active diabetes including blood pressure, body mass index, PPARy function, lipid metabolism, glycated hemoglobin (H1c), and renal function.

In some embodiments, these methods can eliminate or reduce the risk, lessen the severity, or delay the outset of the neurodegenerative disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease.

In some embodiments, effective treatment of a skin disease can be assessed by any method described herein or known in the art, including inspecting skin conditions that include skin color, moisture, temperature, texture, mobility and turgor, and skin lesions, as compared to the skin conditions prior to treatment.

In some embodiments, effective treatment of an autoimmune disease can be assessed by any method described herein or known in the art, including monitoring full blood count analysis on freshly isolated PBMCs, total Ig levels, and analysis of serum autoantibody titers.

In some embodiments, effective treatment of a fragility disease can be assessed by any method described herein or known in the art, including monitoring bone mineral density, bone architecture and geometry, biomedical markers of bone turnover, vitamin D measurement, Karnofsky performance status and ECOG scores, and responsiveness to vaccination.

Methods of Killing or Reducing the Number of Senescent Cells in a Subject

Provided herein are methods of killing or reducing the number of senescent cells (e.g. any of the exemplary types of senescent cells described herein or known in the art) in a subject in need thereof that include administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s) (e.g. any of the NK cell activating agent(s) described herein or known in the art).

Also provided herein are methods of killing or reducing the number of senescent cells (e.g. any of the exemplary types of senescent cells described herein or known in the art) in a subject in need thereof that include administering to the subject a therapeutically effective amount of activated NK cells (e.g. any of the activated NK cells described herein or known in the art).

Some embodiments of these methods further include: obtaining a resting NK cell; and contacting the resting NK cell in vitro in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is a haploidentical NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an allogeneic resting NK cell. In some examples of these methods, the resting NK cell is an artificial NK cell. In some examples of any of these methods, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

In some examples of these methods, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium. Some examples of these methods further include isolating the activated NK cells (and further administering a therapeutically effective amount of the activated NK cells to a subject, e.g., any of the subjects described herein). In some embodiments of these methods, the contacting step is performed for a period of about 2 hours to about 20 days (or any of the subranges of this range described herein).

In some embodiments of these methods, the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells. In some embodiments of these methods, senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells.

In some embodiments of these methods, the subject has been identified or diagnosed as having an aging-related disease or condition (e.g., any of the aging-related diseases or conditions described herein or known in the art). In some embodiments of any of the aging-related disease or condition described herein, the aging-related disease or condition is selected from the group of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease.

Non-limiting examples of cancer include: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

A non-limiting example of an autoimmune disease is type-1 diabetes.

Non-limiting examples of metabolic disease include: obesity, a lipodystrophy, and type-2 diabetes mellitus.

Non-limiting examples of neurodegenerative disease include: Alzheimer's disease, Parkinson's disease, and dementia.

Non-limiting examples of cardiovascular disease include: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

Non-limiting examples of skin disease include: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

Non-limiting examples of progeria disease include: progeria and Hutchinson-Gilford Progeria Syndrome.

Non-limiting examples of fragility disease include: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

In some embodiments of any of the aging-related disease or condition described herein, the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

In some embodiments of any of the aging-related disease or condition described herein, the aging-related disease or condition is type-2 diabetes or atherosclerosis.

In some embodiments of these methods, the administering results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 10% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the number of senescent cells in a target tissue in the subject, e.g., as compared to the number of senescent cells in the target tissue in the subject prior to treatment. In some embodiments of these methods, the target tissue in the subject can be one or more of an adipose tissue, pancreatic tissue, liver tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

In some embodiments of these methods, the administering results in an increase (e.g., at least a 5% increase, at least a 10% increase, at least a 15% increase, at least a 20% increase, at least a 25% increase, at least a 30% increase, at least a 35% increase, at least a 40% increase, at least a 45% increase, at least a 50% increase, at least a 55% increase, at least a 60% increase, at least a 65% increase, at least a 70% increase, at least a 75% increase, at least a 80% increase, at least a 85% increase, at least a 90% increase, at least a 95% increase, or at least a 99% increase, or about a 10% increase to about a

500% increase (or any of the subranges of this range described herein)) in the levels of IFN-γ, a cytotoxic granule granzyme, and/or perforin in the subject, as compared to the levels in a subject prior to treatment or a similar control subject who has not received a treatment.

In some embodiments of these methods, the number of senescent cells in a target tissue (e.g. any of the target tissues described herein) can be determined by performing immunostaining on a biopsy sample. In some embodiments of these methods, the number of senescent cells in a target tissue (e.g. any of the target tissues described herein) can be observed indirectly through an improvement in one or more symptoms of an aging-related disease or condition (e.g. any of the symptoms of an aging-related disease or condition described herein) in a subject.

Senescent Cells

Senescent cells display important and unique properties which include changes in morphology, chromatin organization, gene expression, and metabolism. There are several biochemical and functional properties associated with cellular senescence, such as (i) increased expression of p16 and p21, inhibitors of cyclin-dependent kinases, (ii) presence of senescence-associated β-galactosidase, a marker of lysosomal activity, (iii) appearance of senescence-associated heterochromatin foci and downregulation of lamin B1 levels, (iv) resistance to apoptosis caused by an increased expression of anti-apoptotic BCL-family protein, and (v) upregulation of CD26 (DPP4), CD36 (Scavenger receptor), forkhead box 4 (FOXO4), and secretory carrier membrane protein 4 (SCAMP4). Senescent cells also express an inflammatory signature, the so-called senescence-associated secretory phenotype (SASP). Through SASP, the senescent cells produce a wide range of inflammatory cytokines (IL-6, IL-8), growth factors (TGF-β), chemokines (CCL-2), and matrix metalloproteinases (MMP-3, MMP-9) that operate in a cell-autonomous manner to reinforce senescence (autocrine effects) and communicate with and modify the microenvironment (paracrine effects). SASP factors can contribute to tumor suppression by triggering senescence surveillance, an immune-mediated clearance of senescent cells. However, chronic inflammation is also a known driver of

tumorigenesis, and accumulating evidence indicates that chronic SASP can also boost cancer metastasis and aging-related diseases.

The secretion profile of senescent cells is context dependent. For instance, the mitochondrial dysfunction-associated senescence (MiDAS), induced by different mitochondrial dysfunction in human fibroblasts, led to the appearance of a SASP that was deficient in IL-1-dependent inflammatory factors. A decrease in the NAD+/NADH ratio activated AMPK signaling which induced MiDAS through the activation of p53. As a result, p53 inhibited NF-κB signaling which is a crucial inducer of pro-inflammatory SASP. In contrast, the cellular senescence caused by persistent DNA damage in human cells induced an inflammatory SASP, which was dependent on the activation of ataxia-telangiectasia mutated (ATM) kinase but not on that of p53. In particular, the expression and secretion levels of IL-6 and IL-8 were increased. It was also demonstrated that cellular senescence caused by the ectopic expression p16INK4a and p21CIP1 induced the senescent phenotype in human fibroblasts without an inflammatory SASP indicating that the growth arrest itself did not stimulate SASP.

One of the most defining characteristics of senescence is stable growth arrest. This is achieved by two important pathways, the p16/Rb and the p53/p21, both of which are central in tumor suppression. DNA damage results in: (1) high deposition of γH2Ax (histone coding gene) and 53BP1 (involved in DNA damage response) in chromatin: this leads to activation of a kinase cascade eventually resulting in p53 activation, and (2) activation of p16INK4a and ARF (both encoded by CDKN2A) and P15INK4b (encoded by CDKN2B): p53 induces transcription of cyclin-dependent kinase inhibitor (p21) and along with both p16INK4a and p15INK4b block genes for cell cycle progression (CDK4 and CDK6). This eventually leads to hypophosphorylation of Retinoblastoma protein (Rb) and cell cycle arrest at the G1 phase.

Selectively killing senescent cells has been shown to significantly improve the health span of mice in the context of normal aging and ameliorates the consequences of age-related disease or cancer therapy (Ovadya, *J Clin Invest.* 128(4):1247-1254, 2018). In nature, the senescent cells are normally removed by the innate immune cells. Induction of senescence not only prevents the potential proliferation and transformation of

damaged/altered cells, but also favors tissue repair through the production of SASP factors that function as chemoattractants mainly for Natural Killer (NK) cells (such as IL-15 and CCL2) and macrophages (such as CFS-1 and CCL2). These innate immune cells mediate the immunosurveillance mechanism for eliminating stressed cells. Senescent cells usually up-regulate the NK-cell activating receptor NKG2D and DNAM1 ligands, which belong to a family of stress-inducible ligands: an important component of the frontline immune defense against infectious diseases and malignancies. Upon receptor activation, NK cells can then specifically induce the death of senescent cells through their cytolytic machinery. A role for NK cells in the immune surveillance of senescent cells has been pointed out in liver fibrosis (Sagiv, Oncogene 32(15): 1971-1977, 2013), hepatocellular carcinoma (Iannello, *J Exp Med* 210(10): 2057-2069, 2013), multiple myeloma (Soriani, *Blood* 113(15): 3503-3511, 2009), and glioma cells stressed by dysfunction of the mevalonate pathway (Ciaglia, *Int J Cancer* 142(1): 176-190, 2018). Endometrial cells undergo acute cellular senescence and do not differentiate into decidual cells. The differentiated decidual cells secrete IL-15 and thereby recruit uterine NK cells to target and eliminate the undifferentiated senescent cells thus helping to re-model and rejuvenate the endometrium (Brighton, *Elife* 6: e31274, 2017). With a similar mechanism, during liver fibrosis, p53-expressing senescent liver satellite cells skewed the polarization of resident Kupfer macrophages and freshly infiltrated macrophages toward the pro-inflammatory M1 phenotype, which display senolytic activity. F4/80+ macrophages have been shown to play a key role in the clearance of mouse uterine senescent cells to maintain postpartum uterine function.

Senescent cells recruit NK cells by mainly upregulating ligands to NKG2D (expressed on NK cells), chemokines, and other SASP factors. In vivo models of liver fibrosis have shown effective clearance of senescent cells by activated NK cells (Krizhanovsky, *Cell* 134(4): 657-667, 2008). Studies have described various models to study senescence including liver fibrosis (Krizhanovsky, *Cell* 134(4): 657-667, 2008), osteoarthritis (Xu, *J Gerontol A Biol Sci Med Sci* 72(6): 780-785, 2017), and Parkinson's disease (Chinta, *Cell Rep* 22(4): 930-940, 2018). Animal models for studying senescent cells are described in: Krizhanovsky, *Cell* 134(4): 657-667, 2008; Baker, *Nature*

479(7372): 232-236, 2011; Farr, *Nat Med* 23(9): 1072-1079, 2017; Bourgeois, *FEBS Lett* 592(12): 2083-2097, 2018; Xu, *Nat Med* 24(8): 1246-1256, 2018).

Methods of Improving the Texture and/or Appearance of Skin and/or Hair

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time (e.g. any of the periods of time described herein) that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) (e.g. any of the NK cell activating agent(s) described herein or known in the art).

Also provided herein are methods of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time (e.g. any of the periods of time described herein) that include administering to the subject a therapeutically effective number of activated NK cells (e.g. any of the activated NK cells described herein or known in the art).

Some embodiments of these methods further include: obtaining a resting NK cell; and contacting the resting NK cell in vitro in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is a haploidentical NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an allogeneic resting NK cell. In some examples of these methods, the resting NK cell is an artificial NK cell. In some examples of any of these methods, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

In some examples of these methods, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium. Some examples of these methods further include isolating the activated NK cells (and further administering a therapeutically effective amount of the activated NK cells to a subject, e.g., any of the

subjects described herein). In some embodiments of these methods, the contacting step is performed for a period of about 2 hours to about 20 days (or any of the subranges of this range described herein).

In some embodiments of these methods, the method provides for an improvement in the texture and/or appearance of skin of the subject over the period of time (e.g. any of periods of time described herein).

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the rate of formation of wrinkles in the skin of the subject over the period of time (e.g., any of the periods of time described herein), e.g., as compared to the rate of formulation of wrinkles in the subject prior to treatment or the rate of formulation of wrinkles in a similar subject not receiving a treatment.

In some embodiments of these methods, the method results in an improvement in the coloration of skin of the subject over the period of time (e.g. any of the periods of time described herein).

In some embodiments of these methods, the method results in an improvement in the texture of skin of the subject over the period of time (e.g. any of the periods of time described herein).

In some embodiments of these methods, the method provides for an improvement in the texture and/or appearance of hair of the subject over the period of time (e.g. any of the periods of time described herein).

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at

least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the rate of formation of gray hair in the subject over the period of time (e.g. any of the range of time period described herein), e.g., as compared to the rate of formulation of gray hair in the subject prior to treatment or the rate of formulation of gray hair in a similar subject not receiving a treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the number of gray hairs of the subject over the period of time (e.g. any of the periods of time described herein), e.g., as compared to the number of gray hairs in the subject prior to treatment or the rate of formation of gray hairs in a similar subject not receiving a treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the rate of hair loss in the subject over the period of time (e.g., any of the periods of time described herein), e.g., as

compared to the rate of hair loss in the subject prior to treatment or the rate of hair loss in a similar subject not receiving a treatment.

In some embodiments of these methods, the method results in an improvement in the texture of hair of the subject over the period of time (e.g. any of the periods of time described herein).

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the number of senescent dermal fibroblasts in the skin of the subject over the period of time (e.g., any of the periods of time described herein), e.g., as compared to the number of senescent dermal cells in the subject prior to treatment or the number of senescent dermal cells in a similar subject not receiving a treatment.

In some embodiments of these methods, improvement in the texture and/or appearance of skin of the subject over the period of time (e.g. any of the periods of time described herein) can be assessed by any method described herein or known in the art, including inspecting the presence, size and shape of skin lesions, skin color and pigmentation, skin moisture, temperature, elasticity, and vascularity.

In some embodiments of these methods, improvement in the texture and/or appearance of hair of the subject over the period of time (e.g., any of periods of time described herein) can be assessed by any method described herein or known in the art,

In some embodiments of these methods, the period of time is, e.g., one month to ten years, one month to nine years, one month to eight years, one month to seven years, one month to six years, one month to five years, one month to four years, one month to three years, one month to two years, one month to eighteen months, one month to twelve months, one month to ten months, one month to eight months, one month to six months,

one month to four months, one month to two months, one month to six weeks, six weeks to ten years, six weeks to nine years, six weeks to eight years, six weeks to seven years, six weeks to six years, six weeks to five years, six weeks to four years, six weeks to three years, six weeks to two years, six weeks to eighteen months, six weeks to twelve months, six weeks to ten months, six weeks to eight months, six weeks to six months, six weeks to four months, six weeks to two months, two months to ten years, two months to nine years, two months to eight years, two months to seven years, two months to six years, two months to five years, two months to four years, two months to three years, two months to two years, two months to eighteen months, two months to twelve months, two months to ten months, two months to eight months, two months to six months, two months to four months, four months to ten years, four months to nine years, four months to eight years, four months to seven years, four months to six years, four months to five years, four months to four years, four months to three years, four months to two years, four months to eighteen months, four months to twelve months, four months to ten months, four months to eight months, four months to six months, six months to ten years, six months to nine years, six months to eight years, six months to seven years, six months to six years, six months to five years, six months to four years, six months to three years, six months to two years, six months to eighteen months, six months to twelve months, six months to ten months, six months to eight months, eight months to ten years, eight months to nine years, eight months to eight years, eight months to seven years, eight months to six years, eight months to five years, eight months to four years, eight months to three years, eight months to two years, months to eighteen months, eight months to twelve months, eight months to ten months, ten months to ten years, ten months to nine years, ten months to eight years, ten months to seven years, ten months to six years, ten months to five years, ten months to four years, ten months to three years, ten months to two years, ten months to eighteen months, ten months to twelve months, twelve months to ten years, twelve months to nine years, twelve months to eight years, twelve months to seven years, twelve months to six years, twelve months to five years, twelve months to four years, twelve months to three years, twelve months to two years, twelve months to eighteen months, eighteen months to ten years, eighteen months to nine years, eighteen

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In some embodiments of these methods, the age of the subject is between about 30 to about 35, about 35 to about 40, about 40 to about 45, about 45 to about 50, about 50 to about 55, about 55 to about 60, about 60 to about 65, about 65 to about 70, about 70 to about 75, about 75 to about 80, about 80 to about 85, about 85 to about 90, about 90 to about 95, about 95 to about 100, about 100 to about 105, about 105 to about 110, about 110 to about 115, or about 115 to about 120.

Methods of Assisting in the Treatment of Obesity in a Subject

Provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time (e.g. any of the range of time period described herein), that include administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s) (e.g. any of the NK cell activating agent(s) described herein or known in the art).

Also provided herein are methods of assisting in the treatment of obesity in a subject in need thereof over a period of time (e.g. any of the range of time period described herein) that include administering to the subject a therapeutically effective

number of activated NK cells (e.g. any of the activated NK cells described herein or known in the art).

Some embodiments of these methods further include: obtaining a resting NK cell; and contacting the resting NK cell in vitro in a liquid culture medium including one or more NK cell activating agent(s), where the contacting results in the generation of the activated NK cells that are subsequently administered to the subject. In some examples of these methods, the resting NK cell is an autologous NK cell obtained from the subject. In some examples of these methods, the resting NK cell is a haploidentical NK cell obtained from the subject. In some examples of these methods, the resting NK cell is an allogeneic resting NK cell. In some examples of these methods, the resting NK cell is an artificial NK cell. In some examples of any of these methods, the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

In some examples of these methods, the liquid culture medium is a serum-free liquid culture medium. In some embodiments of any of the methods described herein, the liquid culture medium is a chemically-defined liquid culture medium. Some examples of these methods further include isolating the activated NK cells (and further administering a therapeutically effective amount of the activated NK cells to a subject, e.g., any of the subjects described herein). In some embodiments of these methods, the contacting step is performed for a period of about 2 hours to about 20 days (or any of the subranges of this range described herein).

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the mass of the subject over the

period of time (e.g. any of the periods of time described herein), e.g., as compared to the mass of the subject prior to treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the body mass index (BMI) of the subject over the period of time (e.g. any of periods of time described herein), e.g., as compared to the BMI of the subject prior to treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the rate of progression from pre-diabetes to type 2 diabetes in the subject, e.g., as compared to the rate of progression from pre-diabetes to type 2 diabetes in the subject prior to treatment or the rate of progression from pre-diabetes to type 2 diabetes in a similar subject not receiving a treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90%

decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in fasting serum glucose level in the subject, e.g., as compared to the fasting serum glucose level in the subject prior to treatment.

In some embodiments of these methods, the method results in an increase (e.g., at least a 5% increase, at least a 10% increase, at least a 15% increase, at least a 20% increase, at least a 25% increase, at least a 30% increase, at least a 35% increase, at least a 40% increase, at least a 45% increase, at least a 50% increase, at least a 55% increase, at least a 60% increase, at least a 65% increase, at least a 70% increase, at least a 75% increase, at least a 80% increase, at least a 85% increase, at least a 90% increase, at least a 95% increase, or at least a 99% increase, or about a 10% increase to about a 500% increase (or any of the subranges of this range described herein) in insulin sensitivity in the subject, e.g., as compared to the insulin sensitivity in the subject prior to treatment.

In some embodiments of these methods, the method results in a decrease (e.g., at least a 5% decrease, at least a 10% decrease, at least a 15% decrease, at least a 20% decrease, at least a 25% decrease, at least a 30% decrease, at least a 35% decrease, at least a 40% decrease, at least a 45% decrease, at least a 50% decrease, at least a 55% decrease, at least a 60% decrease, at least a 65% decrease, at least a 70% decrease, at least a 75% decrease, at least a 80% decrease, at least a 85% decrease, at least a 90% decrease, or at least a 95% decrease, or about a 5% decrease to about a 99% decrease (or any of the subranges of this range described herein)) in the severity of atherosclerosis in the subject, e.g., as compared to the severity of atherosclerosis in the subject prior to treatment.

In some embodiments of these methods, treatment of obesity in the subject over the period of time (e.g. any of the periods of time described herein) can be assessed by any method described herein or known in the art, including, e.g., measurement of body weight and/or body dimensions, total body fat, total or regional adiposity, and body mass index (BMI).

In some embodiments of these methods, the response of a subject to the treatment can be monitored by determining fasting serum glucose level or glucose tolerance

according to standard techniques. In some embodiments of these methods, insulin sensitivity can be measured using any method described herein or known in the art, including hyperinsulinemic euglycemic clamp and intravenous glucose tolerance test, homeostasis model assessment (HOMA), and quantitative insulin sensitivity check index (QUICKI).

In some embodiments of these methods, the severity of atherosclerosis in the subject can be measured using any method described herein or known in the art, including cardiac catheterization, Doppler sonography, blood pressure comparison, MUGA/radionuclide angiography, Thallium/myocardial perfusion scan, and computerized tomography.

In some embodiments of these methods, the period of time is one month to ten years (or any of the subranges of this range described herein).

In some embodiments of these methods, the age range for the subject is between about 1 to about 5, about 5 to about 10, about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, about 45 to about 50, about 50 to about 55, about 55 to about 60, about 60 to about 65, about 65 to about 70, about 70 to about 75, about 75 to about 80, about 80 to about 85, about 85 to about 90, about 90 to about 95, about 95 to about 100, about 100 to about 105, about 105 to about 110, about 110 to about 115, or about 115 to about 120.

Additional Therapeutic Agents

Some embodiments of any of the methods described herein can further include administering to a subject (e.g., any of the subjects described herein) a therapeutically effective amount of one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered to the subject at substantially the same time as the NK cell activating agent(s) or activated NK cells (e.g., administered as a single formulation or two or more formulations to the subject). In some embodiments, one or more additional therapeutic agents can be administered to the subject prior to administration of the NK cell activating agent(s) or activated NK cells. In some embodiments, one or more additional therapeutic agents can be administered to the

subject after administration of the NK cell activating agent(s) or activated NK cells to the subject.

Non-limiting examples of additional therapeutic agents include: anti-cancer drugs, activating receptor agonists, immune checkpoint inhibitors, agents for blocking HLA-specific inhibitory receptors, Glucogen Synthase Kinase (GSK) 3 inhibitors, and antibodies.

Non-limiting examples of anticancer drugs include antimetabolic drugs (e.g., 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cytarabine, floxuridine, fludarabine, gemcitabine, hydroxycarbamide, methotrexate, 6-thioguanine, cladribine, nelarabine, pentostatin, or pemetrexed), plant alkaloids (e.g., vinblastine, vincristine, vindesine, camptothecin, 9-methoxycamptothecin, coronaridine, taxol, naucleaorals, diprenylated indole alkaloid, montamine, schischkiniin, protoberberine, berberine, sanguinarine, chelerythrine, chelidonine, liriodenine, clivorine, β-carboline, antofine, tylophorine, cryptolepine, neocryptolepine, corynoline, sampangine, carbazole, crinamine, montanine, ellipticine, paclitaxel, docetaxel, etoposide, tenisopide, irinotecan, topotecan, or acridone alkaloids), proteasome inhibitors (e.g., lactacystin, disulfiram, epigallocatechin-3-gallate, marizomib (salinosporamide A), oprozomib (ONX-0912), delanzomib (CEP-18770), epoxomicin, MG132, beta-hydroxy beta-methylbutyrate, bortezomib, carfilzomib, or ixazomib), antitumor antibiotics (e.g., doxorubicin, daunorubicin, epirubicin, mitoxantrone, idarubicin, actinomycin, plicamycin, mitomycin, or bleomycin), histone deacetylase inhibitors (e.g., vorinostat, panobinostat, belinostat, givinostat, abexinostat, depsipeptide, entinostat, phenyl butyrate, valproic acid, trichostatin A, dacinostat, mocetinostat, pracinostat, nicotinamide, cambinol, tenovin 1, tenovin 6, sirtinol, ricolinostat, tefinostat, kevetrin, quisinostat, resminostat, tacedinaline, chidamide, or selisistat), tyrosine kinase inhibitors (e.g., axitinib, dasatinib, encorafinib, erlotinib, imatinib, nilotinib, pazopanib, and sunitinib), and chemotherapeutic agents (e.g., all-trans retinoic acid, azacitidine, azathioprine, doxifluridine, epothilone, hydroxyurea, imatinib, teniposide, tioguanine, valrubicin, vemurafenib, and lenalidomide). Additional examples of chemotherapeutic agents include alkylating agents, e.g., mechlorethamine, cyclophosphamide, chlorambucil, melphalan, ifosfamide,

thiotepa, hexamethylmelamine, busulfan, altretamine, procarbazine, dacarbazine, temozolomide, carmustine, lumustine, streptozocin, carboplatin, cisplatin, and oxaliplatin.

Non-limiting examples of activating receptor agonists include any agonists for activating receptors which activate and enhance the cytotoxicity of NK cells, including anti-CD16 antibodies (e.g., anti-CD16/CD30 bispecific monoclonal antibody (BiMAb)) and Fc-based fusion proteins. Non-limiting examples of checkpoint inhibitors include anti-PD-1 antibodies (e.g., MEDI0680), anti-PD-L1 antibodies (e.g., BCD-135, BGB-A333, CBT-502, CK-301, CS1001, FAZ053, KN035, MDX-1105, MSB2311, SHR-1316, anti-PD-L1/CTLA-4 bispecific antibody KN046, anti-PD-L1/TGFβRII fusion protein M7824, anti-PD-L1/TIM-3 bispecific antibody LY3415244, atezolizumab, or avelumab), anti-TIM3 antibodies (e.g., TSR-022, Sym023, or MBG453) and anti-CTLA-4 antibodies (e.g., AGEN1884, MK-1308, or an anti-CTLA-4/OX40 bispecific antibody ATOR-1015). Non-limiting examples of agents for blocking HLA-specific inhibitory receptors include monalizumab (e.g., an anti-HLA-E NKG2A inhibitory receptor monoclonal antibody). Non-limiting examples of GSK3 inhibitor include tideglusib or CHIR99021. Non-limiting examples of antibodies that can be used as additional therapeutic agents include anti-CD26 antibodies (e.g., YS110), anti-CD36 antibodies, and any other antibody or antibody construct that can bind to and activate an Fc receptor (e.g., CD16) on a NK cell. In some embodiments, an additional therapeutic agent can be insulin or metformin.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1: Immunostimulation in C57BL/6 mice using a multi-chain polypeptide

Materials and Methods

An exemplary multi-chain polypeptide (a type A multi-chain polypeptide described herein) was generated that includes a first polypeptide and a second polypeptide, where the first polypeptide is a soluble fusion of two TGFβRII domains, a human tissue factor 219 fragment, and a human IL-15, and the second polypeptide is a soluble fusion of two TGFβRII domains and the sushi domain of human IL-15Rα chain.

Results

Immunostimulation in C57BL/6 mice

Wild type C57BL/6 mice were treated subcutaneously with either a control PBS solution or with the multi-chain polypeptide at a dosage of 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg, respectively. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. Specifically, single splenocyte suspensions were generated and stained with fluorochrome-conjugated antibodies including anti-CD4, anti-CD8, anti-NK1.1, and anti-CD19. The percentages of $CD4^+$ T cells, $CD8^+$ T cells, Natural Killer (NK) cells, and $CD19^+$ B cells present in the spleen of mice treated with either the control solution or the multi-chain polypeptide were evaluated using flow cytometry. As shown in Figure 1A, the spleen weight in mice treated with the multi-chain polypeptide increased with increasing dosage of the multi-chain polypeptide. Moreover, the spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of the multi-chain polypeptide were significantly higher as compared to mice treated with the control solution, respectively. As shown in Figure 1B, in the spleens of mice treated with the multi-chain polypeptide, the percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of the multi-chain polypeptide. Specifically, the percentages of $CD8^+$ T cells were higher in mice treated with 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of the multi-chain polypeptide compared to control-treated mice, and the percentages of NK cells were higher in mice treated with 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of the multi-chain polypeptide compared to control-treated mice.

These results demonstrate that the exemplary multi-chain polypeptide is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells.

Pharmacokinetics

The pharmacokinetics of the exemplary multi-chain polypeptide were evaluated in wild type C57BL/6 mice. Mice were treated subcutaneously with the multi-chain polypeptide at a dosage of 3 mg/kg. Blood was collected at various time points via tail vein, and serum was prepared. The concentration of the multi-chain polypeptide in the serum was determined with ELISA. Briefly, the multi-chain polypeptide was captured using an anti-human tissue factor antibody, and detected using a biotinylated anti-human TGFβ receptor, a peroxidase conjugated streptavidin, and ABTS substrate. The results showed that the half-life of the exemplary multi-chain polypeptide was 12.66 hours.

Immunostimulation over time in C57BL/6 mice

To evaluate the effect of immunostimulation by the multi-chain polypeptide over time, mice were treated with a single dose of the multi-chain polypeptide at 3mg/kg and the spleen weight and percentages of immune cell types present in the spleen were evaluated immediately upon treatment and at 16, 24, 48, 72, and 92 hours after treatment, using techniques described above. As shown in Figure 2A, the spleen weight of mice treated with the multi-chain polypeptide increased at 48 hours after treatment, and continued to increase over the next 44 hours. Moreover, as shown in Figure 2B, in the spleens of mice treated with the multi-chain polypeptide, the percentages of $CD8^+$ T cells and NK cells both increased at 48 hours after treatment and continued to increase over the next 44 hours. These results further demonstrate that the exemplary multi-chain polypeptide is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells, over time.

Increased proliferation and Granzyme B expression by $CD8^+$ T cells and NK cells

To evaluate the proliferation and cytotoxic potential of the immune cells induced by the multi-chain polypeptide, mice were treated with a single dose of the multi-chain

polypeptide at 3mg/kg, and the spleens of these mice were evaluated immediately after, and at 16, 24, 48, 72, and 92 hours after treatment. Briefly, single splenocyte suspensions were generated and stained with fluorochrome-conjugated antibodies for the various cell types including anti-CD4, anti-CD8, anti-NK1.1, and anti-CD19, and with an anti-Ki67 antibody (i.e. a cell proliferation marker) and an anti-Granzyme B antibody (i.e. a cytotoxic marker). The mean fluorescent intensity (MFI) of Ki67 and Granzyme B for each immune cell type was analyzed by flow cytometry. As shown in Figures 3A and 3B, the expression of Ki67 and Granzyme B by NK cells showed an increase at 24 hours as well as each time point evaluated thereafter as compared to immediately after treatment (0 hours). Moreover, the expression of Ki67 and Granzyme B by $CD8^+$ T cells showed an increase at 48 hours as well as each time point evaluated thereafter as compared to immediately after treatment (0 hours). As such, a single dose of the multi-chain polypeptide resulted in proliferation of $CD8^+$ T cells and NK cells for up to at least 4 days post-treatment.

These results demonstrate that the multi-chain polypeptide not only increased the number of $CD8^+$ T cells and NK cells in the spleen, but also enhanced the proliferation and cytotoxicity of these cells.

Cytotoxicity against tumor cells

Next, the cytotoxicity of the splenocytes activated by the multi-chain polypeptide against tumor cells were evaluated in C57BL/6 mice. Mouse Moloney leukemia cells (Yac-1) were labeled with CellTrace Violet and used as tumor target cells. C57BL/6 mice were treated with a single dose of the multi-chain polypeptide at 3mg/kg, and splenocytes were prepared at various time points thereafter and used as effector cells. The target tumor cells were mixed with the effector cells at an effector:target (E:T) ratio of 10:1, and incubated at 37°C for 20 hours. Target cell viability was assessed by analyzing Propidium Iodide (PI)-positive, violet-labeled Yac-1 cells using flow cytometry. The percentage of Yac-1 tumor inhibition was calculated using the formula:

Percentage of Yac-1 tumor inhibition = (1-viable Yac-1 cell number in experimental sample/viable Yac-1 cell number in the sample without splenocytes) x 100

As shown in Figure 4, splenocytes from mice after 24-hour or more treatment with the multi-chain polypeptide showed increased cytotoxicity against Yac-1 cells as compared to the splenocytes from untreated mice.

Example 2: Immunostimulation in C57BL/6 mice using a high fat diet-based Type-2 diabetes mouse model

Materials and Methods

TGFRt15-TGFRs is a multi-chain chimeric polypeptide (a type A multi-chain chimeric polypeptide described herein) that includes two TGFβ-binding domains which a soluble human TGFβRII dimer (aa24-159). 21t15-TGFRs is a multi-chain chimeric polypeptide (a type A multi-chain chimeric polypeptide described herein) that includes IL-21 and a TGFβ-binding domain. 3t28 is a chimeric polypeptide (a type B chimeric polypeptide described herein) that include two IL-2 polypeptides.

Results

To evaluate the effect of TGFRt15-TGFRs, 2t2, and 21t15-TGFRs in treating Type-2 diabetes, a high fat diet-based Type-2 diabetes mouse model (B6.129P2-ApoE^{tm1Unc}/J from The Jackson Laboratory) was used. Mice were fed either a control diet or a high fat diet for 11 weeks. A subset of mice fed with the high fat diet were also treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs. Mice fed the control diet, high fat diet, and mice fed with the high fat diet and treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs were evaluated 4 days post-treatment. Briefly, single splenocyte suspensions were generated and stained with fluorochrome-conjugated antibodies including anti-CD4, anti-CD8, anti-NK1.1, and anti-CD19. The percentages of $CD4^+$ T cells, $CD8^+$ T cells, Natural Killer (NK) cells, and $CD19^+$ B cells present in the spleen of mice in each group were evaluated using flow cytometry.

As shown in Figure 5A, in mice fed a high fat diet, the percentage of NK cells in PBMCs was significantly increased after treatment with TGFRt15-TGFRs or 2t2 compared to untreated mice, but not after treatment with 21t15-TGFRs. Furthermore, the

percentage of $CD8^+$ T cells in PBMCs was significantly increased after treatment with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs compared to untreated mice. Moreover, the proliferation of $CD4^+$ T cells, $CD8^+$ T cells, Natural Killer (NK) cells, and $CD19^+$ B cells in PBMCs were also evaluated using an anti-Ki67 antibody. As shown in Figure 5B, the number of proliferating NK cells, $CD4^+$ T cells, and $CD8^+$ T cells were significantly increased after treatment with TGFRt15-TGFRs, but not after treatment with 2t2 or 21t15-TGFRs.

To examine the effect of TGFRt15-TGFRs, 2t2 and 21t15-TGFRs on the appearance and texture of skin and hair in animals, mice were fed either a control or a high fat diet for 7 weeks, and a subset of the mice fed a high fat diet were also treated with TGFRt15-TGFRs, 2t2 or 21t15-TGFRs. One week post-treatment, the appearance of the mice was evaluated. Mice fed a high fat diet and untreated, or a high diet and treated with 21t15-TGFRs appeared ungroomed and ruffled, and had increased gray hair/hair loss as compared to mice fed a control diet (Figure 6A, 6B and 6E). Surprisingly, mice fed a high fat diet that received TGFRt15-TGFRs or 2t2 treatment appeared groomed and healthier (less gray hair/hair loss) (Figure 6C and 6D) as compared to mice fed a high fat diet that did not receive TGFRt15-TGFRs or 2t2 treatment (Figure 6B). Specifically, TGFRt15-TGFRs or 2t2-treated mice showed superior skin and hair appearance and texture as compared to control mice. These results demonstrate that treatment with TGFRt15-TGFRs or 2t2 improves the appearance and texture of skin and hair in mammals.

Next, mice were fed either a control or high fat diet for 9 weeks, and a subset of the mice fed a high fat diet were treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs. Four days post-treatment, the fasting body weight of mice in each group were measured. The fasting body weight of mice fed with the high fat diet and untreated, as well as mice fed with the high fat diet and treated with 21t15-TGFRs were significantly increased compared to mice fed a control diet. However, the fasting body weight of mice fed a high fat diet and treated with TGFRt15-TGFRs or 2t2 were decreased compared to the other two high fat diet groups mentioned above. The fasting body weight of the mice at the end of the study (9 weeks) is shown in Figure 7.

To evaluate the fasting glucose levels in the mice of each group, mice were fed either a control or a high fat diet and were either untreated or treated with TGFRt15-TGFRs, 2t2, or 21t15-TGFRs on days 44, 59 and 73. The fasting blood glucose in the mice of each group were measured 4 days post-treatment. As shown in Figure 8, after the second and third doses (on Days 59 and 73, respectively), the fasting blood glucose level was significantly reduced for mice fed a high fat diet and treated with 2t2 (red line) as compared to mice fed a high fat diet but untreated (yellow line). The fasting blood glucose level remained constant for mice fed a high fat diet and treated with TGFRt15-TGFRs (green line), whereas the fasting blood glucose level increased for mice fed a high fat diet and treated with 21t15-TGFRs (blue line).

Example 3: Chemotherapy-induced Senescent B16F10 Melanoma Cells express NK ligands

Material and Methods

Cellular senescence in B16F10 melanoma cells was induced by treating the cells with docetaxel (7.5μM, Sigma) for 3 days followed by recovery in complete media for 4 days. Cellular senescence was accessed by staining the cells with senescence-associated β-galactosidase (SA β-gal). Briefly, B16F10 control and senescence cells (B16F10-SNC) were washed once with PBS, fixed with 0.5% glutaraldehyde (PBS (pH 7.2)), for 30 minutes. Cells were stained in X-gal solution (1 mg/mL X-gal, 0.12 mM $K_3Fe[CN]_6$, 0.12 mM $K_4Fe[CN]_6$, and 1 mM $MgCl_2$ in PBS at pH 6.0) overnight at 37 °C, and were imaged using a Nikon optical light microscope.

Results

Cellular senescence in B16F10 melanoma cells was induced using chemotherapy as described above. As shown in Figure 9A, chemotherapy-induced senescent B16F10 cells (B16F10-SNC) were positive for SA β-gal staining, while the control B16F10 cells were not stained. Next, expression of senescence genes was analyzed using RT-qPCR with RNA isolated on day 0 or following senescence induction on days 4, 8, 12 and 16,

respectively. The expression levels were normalized to control B16F10 cells. As shown in Figures 9B-9D, the expression of p21, IL6 and DPP4 were upregulated in RNA isolated from the senescent cells over the duration of the experiment. Moreover, as shown in Figures 9E and 9F, the expression of RATE1E and ULBP1 (NK activating receptor NKG2D ligands) were also induced in senescent cells, with the highest expression level being on day 16. These results demonstrate that the chemotherapy-induced senescent B16F10 cells are subjected to stronger cytotoxicity of activated NK cells than control B16F10 cells.

Acquisition of Stem-cell Properties in Chemotherapy-induced Senescent B16F10 Melanoma Cells

To examine whether chemotherapy-induced senescent B16F10 melanoma cells acquired stem cell properties, a colony formation assay was performed. Briefly, 1000 cells/well were seeded on a six well plate, and the media was changed every third day. As shown in Figure 10A (images taken at 100x magnification), after 5 weeks in culture the senescent cells were able to form colonies. To evaluate stem cell marker expression by the colonies, RNA was isolated from the colonies and the expression of Oct4 and Notch4 mRNA were determined by RT-qPCR. As compared to control B16F10 cells, chemotherapy-induced senescent B16F10 melanoma cells showed upregulation of Oct4 and Notch 4, which are cancer stem cell markers (Figures 10B and 10C). Moreover, cell surface expression of stem cell markers CD44, CD24 and CD133 were evaluated by staining with antibodies against CD44, CD24, and CD133 followed by flow cytometry. As shown in Figures 10D-10F, double positive populations ($CD44^+CD24^+$, $CD44^+CD133^+$, and $CD24^+CD133^+$) were increased in the chemotherapy induced senescence stem cells (B16F10-SNC-CSC) compared to control B16F10.

Chemotherapy-induced senescent (CIS) melanoma cells with stem cell properties are more "Migratory" and "Invasive" than control B16F10 cells

The migratory properties of chemotherapy-induced senescent (CIS) melanoma cells with stem cell properties (B16F10-SNC-CSC) were analyzed using a migration

assay. Briefly, control B16F10 cells and B16F10-SNC-CSC cells were plated on six well plates and wounded with a p20 pipette tip. Movement of cells were imaged at 0, 12, and 24 hours after. As shown in Figure 11A, chemotherapy-induced senescent (CIS) melanoma cells with stem cell properties (B16F10-SNC-CSC) were more migratory in the *in vitro* migration assay, as compared to control B16F10 cells.

Next, the invasive properties of chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC) were analyzed using an invasion assay. The invasion assay was carried out on 24-well transwell inserts coated with Matrigel. Briefly, $0.5x10^6$ control B16F10 cells and B16F10-SNC-CSC cells were seeded in serum-free media onto the upper chamber, and the lower chamber was filled with media supplemented with 10% FBS. After 16 hours of incubation, the cells on the upper surface of the filter were removed, and cells underneath the filter were fixed and stained with a 0.02% crystal violet solution. The number of cells were counted in three fields at 100× magnification. As shown in Figures 11B and 11C, chemotherapy-induced senescent cells with stem cell properties were more aggressive in invading the Matrigel coated membrane as compared to control B16F10 cells. These results demonstrate that chemotherapy-induced senescent B16F10 tumor cells are able to regain their proliferation capability, obtain stem-cell features, and have increased migratory abilities and invasiveness for metastasis.

Cytotoxic Activity of Mouse NK Cells on Chemotherapy-induced Senescent Cells with Stem Cell Properties

To expand NK cells *in vivo*, C57BL/6 mice were injected subcutaneously with TGFRt15-TGFRs (10 mg/kg) for 4 days. The spleens from these mice were obtained and NK cells were purified using MACS Miltenyi column. The purified NK cells were then expanded *in vitro* with 2t2 (Figure 12A).

To evaluate the cytotoxicity of the expanded NK cells, chemotherapy-induced senescent stem cells (B16F10-SNC-CSC) or control B16F10 cells were labelled with CellTrace violet and incubated with *in vitro* activated 2t2 mouse NK cells (isolated from spleen of C57BL/6 mice injected with 10 mg/kg TGFRt15-TGFRs for 4 days) at various E:T ratios for 16 hrs. The B16F10-SNC-CSC and control B16F10 cells were trypsinized,

washed and re-suspended in complete media containing a Propidium Iodide (PI) solution, and cytotoxicity was accessed by flow cytometry. As shown in Figure 12B, NK cells were more effective at killing chemotherapy-induced senescent cells with stem cell properties (B16F10-SNC-CSC), as compared to control B16F10 cells.

Combination Treatment in Melanoma Mouse Model

The effect of TGFRt15-TGFRs in treating melanoma was evaluated in a mouse melanoma model. Briefly, $5x10^5$ B16F10 cells were injected subcutaneously into C57BL/6 mice. When the tumor volume reached ~100 mm^3, mice were treated with docetaxel (chemotherapy) (5 mg/kg) or TA99 (200 μg) either as a single agent or in combination every third day, and TGFRt15-TGFRs (3 mg/kg) was given once a week (Figure 13A). Mice that received saline, docetaxel (chemotherapy)/TA99 alone, or TGFRt15-TGFRs alone were used as controls. Five mice were tested in each experimental and control group. Tumor volume was measured every third day. As shown in Figures 13B and 13C, combinations of TGFRt15-TGFRs with either chemotherapy or TA99 slowed down tumor progression as compared to mice treated with saline or mice treated with chemotherapy or TA99 alone in the syngeneic melanoma mouse model.

Example 4: Chemotherapeutic Induction of Senescence in Human Pancreatic Cell Line SW1990

Materials and Methods

β-galactosidase staining: Confirmation of chemotherapy induced senescence was carried out by standard β-galactosidase staining at pH 6.0 using commercially available kit (Cell Signaling Technology) according to manufacturer's instructions. The following day, the staining solution was removed, and cells were washed with phosphate buffered saline, and 70% glycerol was added to the wells. The β-galactosidase positive cells will be stained blue, while control untreated cells will not stain.

Flow cytometry: One million control and senescent cells were obtained and stained using commercially available antibodies to surface markers of stem cells such as anti-CD44 and anti-CD24 antibodies (Biolegend) according to manufacturers' instructions. The cells were then washed and analyzed using the BD Celesta flow cytometer. Cells showing stem cell-like properties will be doubly positive for both CD44 and CD24.

Gene expression assay: One million control and senescent cells were obtained and lysed using Trizol (Thermofisher), followed by RNA purification using an RNA isolation kit (Qiagen). The RNA was quantified and converted to cDNA using a Qiagen cDNA Quantitect kit. The cDNA was then used as a template for standard Taqman gene expression assays (Thermofisher) to quantify the relative abundance of senescent, stem cell markers as well as NK ligands.

NK cell cytotoxicity assay: NK cells were isolated from healthy human donors (n=2) using a commercially available NK isolation kit (Stem Cell), and were activated overnight using the cytokine fusion molecule 18t15-12s (100nM). On the following day, NK cells were washed to remove cytokine molecules and mixed with either CellTrace Violet labelled control untreated tumor cells or chemotherapy-induced senescent tumor cells at an E:T ratio of 4:1 for 20 hours. On the following day, cells were trypsinized, and complete contents of each well were analyzed using flow cytometry and percent inhibition of cells was analyzed.

Results

Senescence in the human pancreatic tumor cell line SW1990 was induced through treatment with chemotherapeutic drugs Abraxane (Celgene) and Gemcitabine (Sigma Aldrich) for 3 days at 2.5μM and 6.25μM, respectively. SW1990 cells that were untreated were used as controls. Media was changed after 3 days and cells were allowed to rest in the culture media for 4 days. As shown in Figure 14, senescent cells treated with the chemotherapeutic drugs were positive for β-galactosidase staining (blue), while

control cells were not stained. Senescent cells and control cells were evaluated for their expression of senescence and stem cell markers at 4 days, 11 days, and 22 days post-treatment. As shown in Figure 14, senescent cells showed increased double positive staining for CD44 and CD24 over time as compared to the control cells. Moreover, the chemotherapy-induced senescent SW1990 cells were also analyzed for their expression of senescent markers including DPP4, IL6, and p21, stem cell markers including Oct3/4, CD24, and CD44, and NK ligands including Nectin and MICA, on day 0, and days 2, 4, and 24 post-treatment using the gene expression assay described above. As shown in Figure 15, the expression of all of the markers mentioned showed an increase over time.

Cytotoxicity of in vitro activated Human NK Cells

To evaluate the cytotoxicity of *in vitro* activated human NK Cells (treated with 18t15-12s), senescence in the human pancreatic tumor cell line SW1990 was induced through treatment with chemotherapeutic drugs Abraxane (Celgene) and Gemcitabine (Sigma Aldrich) for 3 days at 2.5μM and 6.25μM, respectively. SW1990 cells that were untreated were used as controls. Media was changed after 3 days and cells were allowed to rest in the culture media for 30 days. The culture media was changed every 4 days. Activated NK cells were obtained and their cytotoxicity for chemotherapy-induced senescent tumor cells and untreated control tumor cells were evaluated using the NK cell cytotoxicity assay described above. As shown in Figure 16, activated NK cells showed increased cytotoxicity against both control SW1990 cells (SW1990) and senescent SW1990 cells (SW1990s).

Example 5: Creation of an IL-12/IL-15RαSu DNA construct

In a non-limiting example, an IL-12/IL-15RαSu DNA construct was created (Figure 17). The human IL-12 subunit sequences, human IL-15RαSu sequence, human IL-15 sequence, human tissue factor 219 sequence, and human IL-18 sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-12 subunit beta (p40) to IL-12 subunit alpha (p35) with a GS (3) linker to generate a single chain version of IL-12 and

then directly linking the IL-12 sequence to the IL-15RαSu sequence. The final IL-12/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence of the IL12/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 181):

(*Signal peptide*)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCT
ACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA
AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(Linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human IL-12 subunit alpha (p35))

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT
CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT
GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

Example 6: Creation of an IL-18/TF/IL-15 DNA construct

In a non-limiting example, an IL-18/TF/IL-15 construct was made (Figure 18) linking the IL-18 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-18/TF construct with the N-terminus coding region of IL-15. The nucleic acid sequence of the IL-18/TF/IL-15 construct (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 177):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG

GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 7: Secretion of IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins

The IL-12/IL-15RαSu and IL-18/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-18/TF/IL-15:IL-12/IL-15RαSu protein complex (referred to as 18t15-12s; Figure 19 and Figure 20). The 18t15-12s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-12/IL-15RαSu and IL-18/TF/IL-15 fusion proteins.

The amino acid sequence of the IL12/IL-15RαSu fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 180):

(Signal peptide)
MKWVTFISLLFLFSSAYS
(Human IL-12 subunit beta (p40))
IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS
GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV
RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD
PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF
TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS
(*Linker*)
GGGGSGGGGSGGGGS
(*Human IL-12 subunit alpha (p35))*

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE
DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS
LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

The amino acid sequence of the IL-18/TF/IL-15 fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 176):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM
YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG
HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

In some cases, the leader (signal sequence) peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 8: Purification of 18t15-12s by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare™ AKTA Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 18t15-12s was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280 nm was collected and then the sample was neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. Figure 21 shows that the 18t15-12s complex binds the anti-TF antibody affinity column, wherein TF is an 18t15-12s binding partner. The buffer-exchanged protein sample is stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide and stored at 2-8°C.

Example 9: Size exclusion chromatography of 18t15-12s

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.8 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 18t15-12s complex onto the column. The injection was chased with 1.25 column volumes of PBS. The SEC chromatograph is shown in Figure 22. There is a main 18t15-12s protein peak with a minor high molecular weight peak, likely due to differing degrees of glycosylation of 18t15-12s dimers or aggregates.

Example 10: SDS-PAGE of 18t15-12s

To determine the purity and protein molecular weight, the purified 18t15-12s protein sample was analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE. The gel was stained with InstantBlue™ for about 30 min, followed by destaining overnight in purified water. Figure 23 shows an example SDS gel of anti-TF antibody affinity purified 18t15-12s, with bands at the expected molecular weights (66 kDa and 56 kDa).

Example 11: Glycosylation of 18t15-12s in CHO-K1 cells

Glycosylation of 18t15-12s in CHO-K1 cells was confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs), according to the manufacturer's instructions. Figure 24 shows an example SDS PAGE of deglycosylated and non-deglycosylated 18t15-12s. Deglycosylation reduces the molecular weight of 18t15-12s as seen in Figure 24, lane 4.

Example 12: Recombinant protein quantitation of 18t15-12s complexes

The 18t15-12s complex was detected and quantified using standard sandwich ELISA methods (Figures 25-28). Anti-human tissue factor antibody served as the capture antibody and biotinylated anti-human IL-12, IL-15, or IL-18 antibody (BAF 219, BAM 247, D045-6, all R&D Systems) served as the detection antibody. Tissue factor in purified 18t15-12s protein complexes was also detected using an anti-human tissue factor capture antibody (I43), and anti-human tissue factor antibody detection. The I43/anti-TF antibody ELISA was compared to purified tissue factor at similar concentrations.

Example 13: Immunostimulatory capacity of the 18t15-12s complex

To assess the IL-15 immunostimulatory activity of the 18t15-12s complex, increasing concentrations of 18t15-12s was added to 32Dβ cells (104 cell/well) in 200 µL IMDM:10% FBS media. The 32Dβ cells were incubated for 3 days at 37°C. On the fourth day, WST-1 proliferation reagent (10 µL/well) was added and after 4 hours, absorbance was measured at 450 nm to determine cell proliferation based on cleavage of WST-1 to a soluble formazan dye. Bioactivity of human recombinant IL-15 was assessed

as a positive control. As shown in Figure 29, 18t15-12s demonstrated IL-15-dependent cell proliferation of 32Dβ cells. The 18t15-12s complex demonstrated reduced activity compared to human recombinant IL-15, possibly due to the linkage of IL-18 and tissue factor to the IL-15 domain.

In order to assess the individual activities of IL-12 and IL-18 in the 18t15-12s complex, 18t15-12s was added to HEK-Blue IL-12 and HEK-Blue IL-18 reporter cells ($5x10^4$ cell/well; hkb-il12 and hkb-hmil18, InvivoGen) in 200 µL IMDM:10% heat-inactivated FBS media. Cells were incubated for overnight at 37°C. 20 µl of induced HEK-Blue IL-12 and HEK-Blue IL-18 reporter cell supernatant was added to 180 µl of QUANTI-Blue (InvivoGen), and incubated for 1-3 hours at 37°C. IL-12 or IL-18 activity was assessed by measuring absorbance at 620 nm. Human recombinant IL-12 or IL-18 was assessed as a positive or negative control. As shown in Figure 30 and Figure 31, each of the cytokine domains of the 18t15-12s complex retain specific biological activity. The activity of 18t15-12s was reduced compared to that of human recombinant IL-18 or IL-12, possibly due to linkage of IL-15 and tissue factor to the IL-18 domain and linkage of IL-12 to the IL-15Rα sushi domain.

Example 14: Induction of cytokine-induced memory-like NK cells by the 18t15-12s complex

Cytokine-induced memory-like NK cells can be induced *ex vivo* following overnight stimulation of purified NK cells with saturating amounts of IL-12 (10 ng/mL), IL-15 (50 ng/mL), and IL-18 (50 ng/mL). These memory-like properties have been measured through expression of IL-2 receptor α (IL-2Rα, CD25), CD69 (and other activation markers), and increased IFN-γ production. To evaluate the ability of 18t15-12s complexes to promote generation of cytokine-induced memory-like NK cells, purified human NK cells (>95% CD56+) were stimulated for 14-18 hours with 0.01nM to 10000nM of the 18t15-12s complex or a combination of individual cytokines (recombinant IL-12 (10 ng/ml), IL-18 (50 ng/ml), and IL-15 (50 ng/ml)). Cell-surface CD25 and CD 69 expression and intracellular IFN-γ levels were assessed by antibody-staining and flow cytometry.

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either a mixture of cytokines hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL) (R&D Systems) and hIL-15 (50 ng/mL) (NCI) or with 0.01 nM to 10000nM of the 18t15-12s at 37°C, 5% CO_2 for 14-18 hrs. The cells were then harvested and surface stained for CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend) for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone), with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, cells were analyzed using a BD FACSCelesta™ flow cytometer (Plotted Data-Mean Fluorescence Intensity; Figure. 32A and Figure 32B).

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either a cytokine mix of hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL) (R&D), and hIL-15 (50 ng/mL) (NCI), or 0.01 nM to 10000 nM of the 18t15-12s complex at 37°C, 5% CO2 for 14-18 hrs. The cells were then treated with 10 µg/mL of Brefeldin A (Sigma) and 1X of Monensin (eBioscience) for 4 hrs before harvesting and staining for CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes in room temperature) in FACS

buffer (1X PBS (Hyclone), with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and fixed for 10 minutes at room temperature. After fixation, cells were washed (1500 RPM for 5 minutes in room temperature) in 1x permeabilized buffer (eBioscience) and stained with IFN-γ- PE (Biolegend) for 30 minutes at room temperature. Cells were washed once again with 1x permeabilized buffer and then washed with FACS buffer. Cell pellets were resuspended in 300 µls of FACS buffer and analyzed using a BD FACSCelesta™ flow cytometer (Plotted % of IFN-γ Positive Cells; Figure 33).

Example 15: In vitro cytotoxicity of NK cells against human tumor cells

Human myelogenous leukemia cells, K562 (CellTrace violet labelled), were incubated with purified human NK cells in the presence of increasing concentrations of the 18t15-12s complex or a mixture of cytokines as a control. After 20 hours, the cultures were harvested, stained with propidium iodide (PI), and assessed by flow cytometry. As shown in Figure 34, the 18t15-12s complex induced human NK cytotoxicity against K562, at levels similar or greater than the cytokine mixture, wherein both the 18t15-12s complex and the cytokine mixture induced greater cytotoxicity than the medium control.

Example 16: Creation of IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs

In a non-limiting example, IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 DNA constructs were created (Figure 35 and Figure 36). The human IL-12 subunit sequences, human IL-15RαSu sequence, human IL-15 sequence, human tissue factor 219 sequence, and human IL-18 sequence were synthesized by Genewiz. A DNA construct was made linking the IL-12 subunit beta (p40) to IL-12 subunit alpha (p35) with a GS (3) linker to generate a single chain version of IL-12, directly linking the IL-12 sequence to the IL-15RαSu sequence, and directly linking the IL-12/ IL-15RαSu construct to the N-terminus coding region of αCD16scFv.

The nucleic acid sequence of the IL-12/IL-15RαSu/αCD16scFv construct is as follows (SEQ ID NO: 226):

(Signal peptide)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCTACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA
AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(Linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human IL-12 subunit alpha (p35))

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT

ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT
CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT
GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(anti-Human CD16 light chain variable domain)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCAT

(*Linker)*

GGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGAGGAGGATCC

(anti-Human CD16 heavy chain variable domain)

GAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCTGGAGG
CTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTACGGCA
TGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCCGGCAT
CAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCAGGTTC
ACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAACTCCC

TGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCCCTGCT
GTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

Constructs were also made linking the IL-18 sequence to the N-terminus coding region of tissue factor 219, and linking the IL-18/TF construct with the N-terminus coding region of IL-15 (Figure 36). The nucleic acid sequence of the IL-18/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 177):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC

AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 17: Secretion of IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 fusion proteins

The IL-12/IL-15RαSu/αCD16scFv and IL-18/TF/IL-15 constructs were cloned into a pMSGV-1 modified retrovirus expression vector (Hughes, *Hum Gene Ther* 16:457–72, 2005, herein incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of a soluble IL-18/TF/IL-15:IL-12/IL-15RαSu/αCD16scFv protein complex (referred to as 18t15-12s/αCD16; Figure 37 and Figure 38). Co-expression of the two constructs in CHO-K1 cells resulted in secretion of the soluble IL-18/TF/IL-15:IL-12/IL-15RαSu/αCD16scFv protein complex (referred to as 18t15-12s/αCD16; Figure 37 and Figure 38), which can be purified by anti-TF Ab affinity and other chromatography methods. In some cases, the signal peptide is cleaved from the intact polypeptide to generate the mature form.

The amino acid sequence of the IL-12/IL-15RαSu/αCD16scFv fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 225):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS
GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN
KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV
RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD
PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF
TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

(*Linker*)

GGGGSGGGGSGGGGS

(*Human IL-12 subunit alpha (p35))*

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE
DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS
LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(anti-Human CD16 light chain variable domain)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGH

(*Linker*)

GGGGSGGGGSGGGGS

(anti-Human CD16 heavy chain variable domain)

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWV
SGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGRS
LLFDYWGQGTLVTVSR

The amino acid sequence of the IL-18/TF/IL-15 fusion protein (including leader sequence) is as follows (SEQ ID NO: 221):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

Example 18: Creation of IL-18/IL-15RαSu and IL-12/TF/IL-15 DNA constructs

In a non-limiting example, IL-18/IL-15RαSu and IL-12/TF/IL-15 DNA constructs were created. The human IL-18 subunit sequences, human IL-15RαSu sequence, human IL-12 sequence, human tissue factor 219 sequence, and human IL-15 sequence were synthesized by Genewiz. A DNA construct was made linking IL-18 directly to IL-15RαSu. An additional construct was also made linking IL-12 sequence to the N-terminus coding region of human tissue factor 219 form, and further linking the IL-12/TF construct to the N-terminus coding region of IL-15. As described above, a single-chain version of IL-12 (p40-linker-p35) was used.

The nucleic acid sequence of the IL-18/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 320):

(Signal peptide)

ATGAAGTGGGTCACATTTATCTCTTTACTGTTCCTCTTCTCCAGCGCCT
ACAGC

(Human IL-18)

TACTTCGGCAAACTGGAATCCAAGCTGAGCGTGATCCGGAATTTAAAC
GACCAAGTTCTGTTTATCGATCAAGGTAACCGGCCTCTGTTCGAGGACATGAC
CGACTCCGATTGCCGGGACAATGCCCCCCGGACCATCTTCATTATCTCCATGT
ACAAGGACAGCCAGCCCCGGGGCATGGCTGTGACAATTAGCGTGAAGTGTGA
GAAAATCAGCACTTTATCTTGTGAGAACAAGATCATCTCCTTTAAGGAAATG
AACCCCCCCCGATAACATCAAGGACACCAAGTCCGATATCATCTTCTTCCAGC
GGTCCGTGCCCGGTCACGATAACAAGATGCAGTTCGAATCCTCCTCCTACGA
GGGCTACTTTTTAGCTTGTGAAAAGGAGAGGGATTTATTCAAGCTGATCCTCA
AGAAGGAGGACGAGCTGGGCGATCGTTCCATCATGTTCACCGTCCAAAACGA
GGAT

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The nucleic acid sequence of the IL-12/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 321):

(Signal peptide)

ATGAAATGGGTGACCTTTATTTCTTTACTGTTCCTCTTTAGCAGCGCCT
ACTCC

(Human IL-12 subunit beta (p40))

ATTTGGGAACTGAAGAAGGACGTCTACGTGGTCGAACTGGACTGGTAT
CCCGATGCTCCCGGCGAAATGGTGGTGCTCACTTGTGACACCCCCGAAGAAG
ACGGCATCACTTGGACCCTCGATCAGAGCAGCGAGGTGCTGGGCTCCGGAAA
GACCCTCACAATCCAAGTTAAGGAGTTCGGAGACGCTGGCCAATACACATGC
CACAAGGGAGGCGAGGTGCTCAGCCATTCCTTATTATTATTACACAAGAAGG
AAGACGGAATCTGGTCCACCGACATTTTAAAAGATCAGAAGGAGCCCAAGA
ATAAGACCTTTTTAAGGTGTGAGGCCAAAAACTACAGCGGTCGTTTCACTTGT
TGGTGGCTGACCACCATTTCCACCGATTTAACCTTCTCCGTGAAAAGCAGCCG
GGGAAGCTCCGACCCTCAAGGTGTGACATGTGGAGCCGCTACCCTCAGCGCT
GAGAGGGTTCGTGGCGATAACAAGGAATACGAGTACAGCGTGGAGTGCCAA
GAAGATAGCGCTTGTCCCGCTGCCGAAGAATCTTTACCCATTGAGGTGATGG
TGGACGCCGTGCACAAACTCAAGTACGAGAACTACACCTCCTCCTTCTTTATC
CGGGACATCATTAAGCCCGATCCTCCTAAGAATTTACAGCTGAAGCCTCTCA
AAAATAGCCGGCAAGTTGAGGTCTCTTGGGAATATCCCGACACTTGGAGCAC
ACCCCACAGCTACTTCTCTTTAACCTTTTGTGTGCAAGTTCAAGGTAAAAGCA
AGCGGGAGAAGAAAGACCGGGTGTTTACCGACAAAACCAGCGCCACCGTCA
TCTGTCGGAAGAACGCCTCCATCAGCGTGAGGGCTCAAGATCGTTATTACTCC
AGCAGCTGGTCCGAGTGGGCCAGCGTGCCTTGTTCC

(*Linker*)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(*Human IL-12 subunit alpha (p35)*)

CGTAACCTCCCCGTGGCTACCCCCGATCCCGGAATGTTCCCTTGTTTAC
ACCACAGCCAGAATTTACTGAGGGCCGTGAGCAACATGCTGCAGAAAGCTAG
GCAGACTTTAGAATTTTACCCTTGCACCAGCGAGGAGATCGACCATGAAGAT
ATCACCAAGGACAAGACATCCACCGTGGAGGCTTGTTTACCTCTGGAGCTGA
CAAAGAACGAGTCTTGTCTCAACTCTCGTGAAACCAGCTTCATCACAAATGG
CTCTTGTTTAGCTTCCCGGAAGACCTCCTTTATGATGGCTTTATGCCTCAGCTC
CATCTACGAGGATTTAAAGATGTACCAAGTGGAGTTCAAGACCATGAACGCC
AAGCTGCTCATGGACCCTAAACGGCAGATCTTTTTAGACCAGAACATGCTGG
CTGTGATTGATGAGCTGATGCAAGCTTTAAACTTCAACTCCGAGACCGTCCCT

CAGAAGTCCTCCCTCGAGGAGCCCGATTTTTACAAGACAAAGATCAAACTGT
GCATTTTACTCCACGCCTTTAGGATCCGGGCCGTGACCATTGACCGGGTCATG
AGCTATTTAAACGCCAGC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 19: Secretion of IL-18/IL-15RαSu and IL-12/TF/IL-15 fusion proteins

The IL-18/IL-15RαSu and IL-12/TF/IL-15 constructs were cloned into a pMSGV-1 modified retrovirus expression vector (Hughes, *Hum Gene Ther* 16:457–72, 2005 herein incorporated by reference), and the expression vector was transfected into CHO-

K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of a soluble IL-12/TF/IL-15:IL-18/IL-15RαSu protein complex (referred to as 12t15/s18), which can be purified by anti-TF Ab affinity and other chromatography methods.

The amino acid sequence of the IL-18/IL-15RαSu fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 322):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-18)

YFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISM YKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPG HDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

The amino acid sequence of the IL-12/TF/IL-15 fusion protein (including leader sequence) is as follows (SEQ ID NO: 323):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-12 subunit beta (p40))

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGS GKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKN KTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERV RGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPD PPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVF TDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS

(*Linker*)

GGGGSGGGGSGGGGS

(*Human IL-12 subunit alpha (p35))*

RNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSEEIDHE
DITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIYE
DLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS
LEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 20: Recombinant protein quantitation of the 18t15-12s16 complex

The 18t15-12s16 complex (comprising IL-12/IL-15RαSu/αCD16scFv;IL-18/TF/IL-15) was detected and quantified using standard sandwich ELISA methods (Figure 39). Anti-human tissue factorantibody/IL-2 or anti-TF Ab /IL-18 served as the capture antibody and biotinylated anti-human IL-12 or IL-18 antibody (BAF 219, D045-6, both R&D Systems) served as the detection antibody. Tissue factor was also detected using an anti-human tissue factor antibody (I43), and anti-human tissue factor antibody detection.

Example 21: Creation of TGFβRII/IL-15RαSu and IL-21/TF/IL-15 DNA constructs

In a non-limiting example, a TGFβRII/IL-15RαSu DNA construct was created (Figure 40). The human TGFβRII dimer and human IL-21 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA

construct was made linking the TGFβRII to another TGFβRII with a linker to generate a single chain version of TGFβRII and then directly linking the TGFβRII single chain dimer sequence to the N-terminal coding region of IL-15RαSu.

The nucleic acid sequences of the TGFβRII/IL-15RαSu construct (including signal sequence) is as follows (SEQ ID NO: 196):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβRII-1st fragment)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT

(*Linker)*

GGAGGTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGT

(Human TGFβRII-2nd fragment)

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACC
GATAACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGA
GGTTTTCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCAC
CTCCATCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAAT
GACGAGAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACC
ACGATTTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAA
AAAGAAGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGC
AACGACAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATCACGTGTCCTCCTCCTATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAA
GCGTAAAGCCGGCACGTCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACG
AATGTCGCCCACTGGACAACCCCCAGTCTCAAATGTATTAGA

Additionally, an IL-21/TF/IL-15 construct was made linking the IL-21 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-21/TF construct to the N-terminus coding region of IL-15 (Figure 41). The nucleic acid sequence of the IL-21/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 192):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human Tissue Factor 219)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCAAGACAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC

CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAAC
ACATTTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTA
CTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAAC
GAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAG
CCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGA
GTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Example 22: Secretion of TGFβRII/IL-15RαSu and IL-21/TF/IL-15 fusion proteins

The TGFβRII/IL-15RαSu and IL-21/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described in Hughes et al., *Hum Gene Ther* 16:457–72, 2005, herein incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells resulted in secretion of the soluble IL-21/TF/IL-15:TGFβRII/IL-15RαSu protein complex (referred to as 21t15-TGFRs; Figure 42 and Figure 43). The 21t15-TGFRs complex was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity chromatography and other chromatography methods.

The amino acid sequence of the TGFβRII/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 195):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβRII-1st fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Linker)

GGGGSGGGGSGGGGS

(Human TGFβRII-2nd fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

The amino acid sequence of the mature IL-21/TF/IL-15 fusion protein (including signal peptide sequence) is as follows (SEQ ID NO: 191):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

Example 23: Purification of 21t15-TGFRs by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare AKTA™ Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 21t15-TGFRs was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280 nm was collected and then the sample was then neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. Figure 44 shows that the 21t15-TGFRs complex binds anti-TF antibody affinity column, wherein TF is a 21t15-TGFRs binding partner. The buffer-exchanged protein sample is stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide, and stored at 2-8°C.

Example 24: Size exclusion chromatography of 21t15-TGFRs

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.8 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 21t15-TGFRs complex onto the

column. The injection was then chased with 1.25 column volumes of PBS. The SEC chromatograph was shown in Figure 45. There were two protein peaks, likely representing a monomer and dimer forms of 21t15-TGFRs.

Example 25: SDS-PAGE of 21t15-TGFRs

To determine the purity and protein molecular weight, the purified 21t15-TGFRs complex protein sample was analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE under reduced conditions. The gel was stained with InstantBlue™ for about 30 min, followed by destaining overnight in purified water. Figure 46 shows an example SDS gel of anti-TF antibody affinity purified 21t15-TGFRs, with bands at 39.08 kDa and 53 kDa

Glycosylation of 21t15-TGFRs in CHO cells was confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs) and the manufacturer's instructions. Deglycosylation reduces the molecular weight of 21t15-TGFRs, as seen in lane 4 of Figure 46.

Example 26: Recombinant protein quantitation of 21t15-TGFRs complexes

The 21t15-TGFRs complex was detected and quantified using standard sandwich ELISA methods (Figures 47-50). Anti-human tissue factor antibody served as the capture antibody and biotinylated anti-human IL-21, IL-15, or TGFβRII served as the detection antibody. Tissue factor was also detected using an anti-human tissue factor capture antibody (I43), and anti-human tissue factor antibody detection. The I43/ anti-TF antibody ELISA was compared to purified tissue factor at similar concentrations.

Example 27: Immunostimulatory capacity of the 21t15-TGFRs complex

To assess the IL-15 immunostimulatory activity of the 21t15-TGFRs complexes, increasing concentrations of 21t15-TGFRs was added to 32Dβ cells (10^4 cell/well) in 200 μL IMDM:10% FBS media and cells were incubated for 3 days at 37°C. On the fourth day, WST-1 proliferation reagent (10 μL/well) then was added and after 4 hours, absorbance was measured at 450 nm to determine cell proliferation based on cleavage of

WST-1 to a soluble formazan dye. Bioactivity of the human recombinant IL-15 was assessed as a positive control. As shown in Figure 51, 21t15-TGFRs demonstrated IL-15-dependent 32Dβ cell proliferation. The 21t15-TGFRs complex was reduced compared to that of human recombinant IL-15, possibly due to the linkage of IL-21 and tissue factor to the IL-15 domain.

Additionally, HEK-Blue TGFβ reporter cells (hkb-tgfb, InvivoGen) were used to measure the ability of 21t15-TGFRs to block TGFβ1 activity (Figure 52). Increasing concentrations of 21t15-TGFRs were mixed with 0.1 nM of TGFβ1 and added to HEK-Blue TGFβ reporter cells ($2.5x10^4$ cell/well) in 200 µL IMDM:10% heat-inactivated FBS media. Cells were incubated overnight at 37°C. The next day, 20 µl of induced HEK-Blue TGFβ reporter cell supernatant was added to 180 µl of QUANTI-Blue (InvivoGen) and incubated for 1-3 hours at 37°C. 21t15-TGFRs activity was assessed by measuring absorbance at 620 nm. Human recombinant TGFβRII/Fc activity was assessed as a positive control.

These results demonstrate that TGFβRII domain of the 21t15-TGFRs complex retains its ability to trap TGFβ1. The ability of 21t15-TGFRs to block TGFβ1 activity was reduced compared to that of human recombinant TGFβRII/Fc, possibly due to the linkage of TGFβRII to the IL-15Rα sushi domain.

Example 28: Induction of cytokine-induced memory-like NK cells by the 21t15-TGFRs complex

Cytokine-induced memory-like NK cells can be induced *ex vivo* following overnight stimulation of purified NK cells with saturating amounts of cytokines. These memory-like properties can be measured through expression of IL-2 receptor α (IL-2Rα, CD25), CD69 (and other activation markers), and increased IFN-γ production. To evaluate the ability of 21t15-TGFRs complexes to promote generation of cytokine-induced memory-like NK cells, purified human NK cells (>95% CD56+) were stimulated for 14-18 hours with 1 nM to 100 nM of the 21t15-TGFRs complex. Cell-surface CD25

and CD 69 expression and intracellular IFN-γ levels were assessed by antibody-staining and flow cytometry.

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 10^6/mL in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-21 (50 ng/ml) (Biolegend) and hIL-15 (50 ng/ml) (NCI) or with 1 nM, 10 nM, or 100 nM 21t15-TGFRs complex overnight at 37°C, 5% CO_2 for 14-18 hrs. The cells were then harvested and surface stained for CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, cells were analyzed using a BD FACSCelesta™ flow cytometer. (Plotted Data-Mean Fluorescence Intensity; Figure 53 and Figure 54).

Fresh human leukocytes were obtained from a blood bank and CD56+ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (BioLegend). Cells were counted and resuspended in 0.2 x 106/ml in a 96 well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco), supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells were stimulated with either mix-cytokines of hIL-21 (50 ng/ml) (Biolegend) and hIL-15 (50 ng/ml) (NCI) or with 1 nM, 10 nM, or 100 nM 21t15-TGFRs complex overnight at 37°C, 5% CO2 for 14-18 hrs. The cells were then treated with 10 μg/ml of Brefeldin A (Sigma) and 1X of Monensin (eBioscience) for 4 hrs. Cells were harvested and surface stained for CD56-BV421,

CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies for 30 minutes. After staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and fixed for 10 minutes at room temperature. After fixation, cells were washed (1500 RPM for 5 minutes at room temperature) with 1x permeabilized buffer (eBioscience) and stained for intracellular IFN-γ- PE (Biolegend) for 30 minutes at room temperature. Cells were washed once again with 1x permeabilized buffer and then washed with FACS buffer. Cell pellets were resuspended in 300 µls of FACS Buffer and analyzed using a BD FACSCelesta™ flow cytometer. (Plotted % of IFN-γ Positive Cells; Figure 55).

Example 29: In vitro cytotoxicity of NK cells against human tumor cells

K562 (CellTrace violet labelled), human myelogenous leukemia cells, were incubated with purified human NK cells (using StemCell human NK cell purification kit (E:T ratio; 2:1)) in the presence of increasing concentrations of the 21t15-TGFRs complex. After 20 hours, the cultures were harvested, stained with propidium iodide (PI), and assessed by flow cytometry. As shown in Figure 56, the 21t15-TGFRs complex induced human NK cytotoxicity against K562, as compared to control.

Example 30: Creation of an IL-21/TF mutant/IL-15 DNA construct and resulting fusion protein complex with TGFβRII /IL-15RαSu

In a non-limiting example, an IL-21/TF mutant/IL-15 DNA construct was made by linking IL-21 directly to the N-terminus coding region of a tissue factor 219 mutant, and further linking the IL-21/TF mutant to the N-terminus coding region of IL-15.

The nucleic acid sequence of the IL-21/TF mutant/IL-15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 324, shaded nucleotides are mutant and the mutant codons are underlined):

(Signal sequence)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human Tissue Factor 219 mutants)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCGCGACAGCTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGCTTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCCGCGGAATAA
CACAGCCTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGT
ACTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAA
CGAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAA
GCCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTG
AGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG

GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of the IL-21/TF mutant/IL-15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 325, substituted residues are shaded):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTKLEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

In some embodiments, the IL-21/TF mutant/IL-15 DNA construct may be combined with an TGFβRII /IL-15RαSu DNA construct, transfected into cells using a retroviral vector as described above, and expressed as IL-21/TF mutant/IL-15 and TGFβRII/IL-15RαSu fusion proteins. The IL-15RαSu domain of the TGFβRII/IL-

15RαSu fusion protein binds to the IL-15 domain of the IL-21/TF mutant/IL-15 fusion protein to create an IL-21/TF mutant/IL-15:TGFβRII /IL-15RαSu complex.

Example 31: Creation of IL-21/IL-15RαSu and TGFβRII/TF/IL-15 DNA constructs and the resulting fusion protein complex

In a non-limiting example, an IL-21/IL-15RαSu DNA construct was made by linking IL-21 directly to the IL-15RαSu subunit sequence. The nucleic acid sequence of the IL-21/IL-15RαSu construct (including signal sequence) is as follows (SEQ ID NO: 214):

(Signal sequence)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of the IL-21/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 213):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate a mature form that may be soluble or secreted.

In some embodiments, the IL-21/IL-15RαSu DNA construct may be combined with a TGFβRII/TF/IL-15 DNA construct, transfected into a retroviral vector as described above, and expressed as IL-21/IL-15RαSu and TGFβRII/TF/IL-15 fusion proteins. The IL-15RαSu domain of the IL-21/IL-15RαSu fusion protein binds to the IL-15 domain of the TGFβRII/TF/IL-15 fusion protein to create a TGFβRII/TF/IL-15:IL-21/IL-15RαSu complex.

The TGFβRII/TF/IL-15RαSu DNA construct was created by linking the TGFβRII sequence to the N-terminus coding region of human tissue factor 219 form, and then linking the TGFβRII/TF construct to the N-terminus coding region of IL-15. As described above, a single-chain version of TGFβRII (TGFβRII-linker-TGFβRII) was used. The nucleic acid sequence of the TGFβRII/TF/IL-15 construct (including leader sequence) is as follows (SEQ ID NO: 239):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβRII-1st fragment)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCACGATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGAT

(*Linker)*

GGAGGTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGT

(Human TGFβRII-2nd fragment)

ATTCCTCCCCACGTGCAGAAGAGCGTGAATAATGACATGATCGTGACC
GATAACAATGGCGCCGTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGA
GGTTTTCCACCTGCGACAACCAGAAGTCCTGTATGAGCAACTGCACAATCAC
CTCCATCTGTGAGAAGCCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAAT
GACGAGAATATCACCCTGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACC
ACGATTTCATCCTGGAAGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAA
AAAGAAGCCTGGCGAGACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGC
AACGACAATATCATCTTTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCAAGACAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAAC
ACATTTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTA

CTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAAC
GAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAG
CCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGA
GTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of the TGFβRII/TF/IL-15 fusion protein (including signal peptide) is as follows (SEQ ID NO: 238):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβRII-1st fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Linker)

GGGGSGGGGSGGGGS

(Human TGFβRII-2nd fragment)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL

ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Example 32. Production of an Exemplary Single-Chain Chimeric Polypeptides

An exemplary single-chain chimeric polypeptide including a first target-binding domain that is an anti-CD3 scFv, a soluble human tissue factor domain, and a second target-binding domain that is an anti-CD28 scFv was generated (αCD3scFv/TF/αCD28scFv) (Figure 57). The nucleic acid and amino acid sequences of this single-chain chimeric polypeptide are shown below.

Nucleic Acid Encoding Exemplary Single-Chain Chimeric Polypeptide (αCD3scFv/TF/αCD28scFv) (SEQ ID NO: 158)

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCTTATTATTTTTATTCAGCTCCGCCT
ATTCC

(αCD3 light chain variable region)

CAGATCGTGCTGACCCAAAGCCCCGCCATCATGAGCGCTAGCCCCGGT
GAGAAGGTGACCATGACATGCTCCGCTTCCAGCTCCGTGTCCTACATGAACT
GGTATCAGCAGAAAAGCGGAACCAGCCCCAAAAGGTGGATCTACGACACCA
GCAAGCTGGCCTCCGGAGTGCCCGCTCATTTCCGGGGCTCTGGATCCGGCAC
CAGCTACTCTTTAACCATTTCCGGCATGGAAGCTGAAGACGCTGCCACCTACT
ATTGCCAGCAATGGAGCAGCAACCCCTTCACATTCGGATCTGGCACCAAGCT
CGAAATCAATCGT

(Linker)

GGAGGAGGTGGCAGCGGCGGCGGTGGATCCGGCGGAGGAGGAAGC

(αCD3 heavy chain variable region)

CAAGTTCAACTCCAGCAGAGCGGCGCTGAACTGGCCCGGCCCGGCGC
CTCCGTCAAGATGAGCTGCAAGGCTTCCGGCTATACATTTACTCGTTACACAA
TGCATTGGGTCAAGCAGAGGCCCGGTCAAGGTTTAGAGTGGATCGGATATAT
CAACCCTTCCCGGGGCTACACCAACTATAACCAAAAGTTCAAGGATAAAGCC
ACTTTAACCACTGACAAGAGCTCCTCCACCGCCTACATGCAGCTGTCCTCTTT
AACCAGCGAGGACTCCGCTGTTTACTACTGCGCTAGGTATTACGACGACCAC
TACTGTTTAGACTATTGGGGACAAGGTACCACTTTAACCGTCAGCAGC

(Human tissue factor 219 form)

TCCGGCACCACCAATACCGTGGCCGCTTATAACCTCACATGGAAGAGC
ACCAACTTCAAGACAATTCTGGAATGGGAACCCAAGCCCGTCAATCAAGTTT
ACACCGTGCAGATCTCCACCAAATCCGGAGACTGGAAGAGCAAGTGCTTCTA
CACAACAGACACCGAGTGTGATTTAACCGACGAAATCGTCAAGGACGTCAAG
CAAACCTATCTGGCTCGGGTCTTTTCCTACCCCGCTGGCAATGTCGAGTCCAC
CGGCTCCGCTGGCGAGCCTCTCTACGAGAATTCCCCCGAATTCACCCCTTATT
TAGAGACCAATTTAGGCCAGCCTACCATCCAGAGCTTCGAGCAAGTTGGCAC
CAAGGTGAACGTCACCGTCGAGGATGAAAGGACTTTAGTGCGGCGGAATAAC
ACATTTTTATCCCTCCGGGATGTGTTCGGCAAAGACCTCATCTACACACTGTA
CTATTGGAAGTCCAGCTCCTCCGGCAAAAAGACCGCTAAGACCAACACCAAC
GAGTTTTTAATTGACGTGGACAAAGGCGAGAACTACTGCTTCAGCGTGCAAG
CCGTGATCCCTTCTCGTACCGTCAACCGGAAGAGCACAGATTCCCCCGTTGA
GTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(αCD28 light chain variable region)

GTCCAGCTGCAGCAGAGCGGACCCGAACTCGTGAAACCCGGTGCTTCC
GTGAAAATGTCTTGTAAGGCCAGCGGATACACCTTCACCTCCTATGTGATCCA
GTGGGTCAAACAGAAGCCCGGACAAGGTCTCGAGTGGATCGGCAGCATCAA
CCCTTACAACGACTATACCAAATACAACGAGAAGTTTAAGGGAAAGGCTACT
TTAACCTCCGACAAAAGCTCCATCACAGCCTACATGGAGTTCAGCTCTTTAAC

ATCCGAGGACAGCGCTCTGTACTATTGCGCCCGGTGGGGCGACGGCAATTAC
TGGGGACGGGGCACAACACTGACCGTGAGCAGC

(Linker)

GGAGGCGGAGGCTCCGGCGGAGGCGGATCTGGCGGTGGCGGCTCC

(αCD28 light chain variable region)

GACATCGAGATGACCCAGTCCCCCGCTATCATGTCCGCCTCTTTAGGCGAGC
GGGTCACAATGACTTGTACAGCCTCCTCCAGCGTCTCCTCCTCCTACTTCCAT
TGGTACCAACAGAAACCCGGAAGCTCCCCTAAACTGTGCATCTACAGCACCA
GCAATCTCGCCAGCGGCGTGCCCCCTAGGTTTTCCGGAAGCGGAAGCACCAG
CTACTCTTTAACCATCTCCTCCATGGAGGCTGAGGATGCCGCCACCTACTTTT
GTCACCAGTACCACCGGTCCCCCACCTTCGGAGGCGGCACCAAACTGGAGAC
AAAGAGG

Exemplary Single-Chain Chimeric Polypeptide (αCD3scFv/TF/αCD28scFv) (SEQ ID NO: 157)

(Signal peptide)

MKWVTFISLLFLFSSAYS

(αCD3 light chain variable region)

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT
SKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEI
NR

(Linker)

GGGGSGGGGSGGGGS

(αCD3 heavy chain variable region)

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWI
GYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDD
HYCLDYWGQGTTLTVSS

(Human tissue factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(αCD28 light chain variable region)

VQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGS
INPYNDYTKYNEKFKGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNY
WGRGTTLTVSS

(Linker)

GGGGSGGGGSGGGGS

(αCD28 heavy chain variable region)

DIEMTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPGSSPKLCIYSTSNLA
SGVPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGGGTKLETKR

A second exemplary single-chain chimeric polypeptide including a first target-binding domain that is an anti-CD28 scFv, a soluble human tissue factor domain, and a second target-binding domain that is an anti-CD3 scFv was generated (αCD28scFv/TF/αCD3scFv) (Figure 57). The nucleic acid and amino acid sequences of this single-chain chimeric polypeptide are shown below.

Nucleic Acid Encoding Exemplary Single-Chain Chimeric Polypeptide (αCD28scFv/TF/αCD3scFv) (SEQ ID NO: 326)

(Signal peptide)

ATGAAATGGGTCACCTTCATCTCTTTACTGTTTTTATTTAGCAGCGCCT
ACAGC

(αCD28 light chain variable region)

GTGCAGCTGCAGCAGTCCGGACCCGAACTGGTCAAGCCCGGTGCCTCC
GTGAAAATGTCTTGTAAGGCTTCTGGCTACACCTTTACCTCCTACGTCATCCA
ATGGGTGAAGCAGAAGCCCGGTCAAGGTCTCGAGTGGATCGGCAGCATCAAT
CCCTACAACGATTACACCAAGTATAACGAAAAGTTTAAGGGCAAGGCCACTC

TGACAAGCGACAAGAGCTCCATTACCGCCTACATGGAGTTTTCCTCTTTAACT
TCTGAGGACTCCGCTTTATACTATTGCGCTCGTTGGGGCGATGGCAATTATTG
GGGCCGGGGAACTACTTTAACAGTGAGCTCC

(*Linker)*

GGCGGCGGCGGAAGCGGAGGTGGAGGATCTGGCGGTGGAGGCAGC

(αCD28 heavy chain variable region)

GACATCGAGATGACACAGTCCCCCGCTATCATGAGCGCCTCTTTAGGA
GAACGTGTGACCATGACTTGTACAGCTTCCTCCAGCGTGAGCAGCTCCTATTT
CCACTGGTACCAGCAGAAACCCGGCTCCTCCCCTAAACTGTGTATCTACTCCA
CAAGCAATTTAGCTAGCGGCGTGCCTCCTCGTTTTAGCGGCTCCGGCAGCACC
TCTTACTCTTTAACCATTAGCTCTATGGAGGCCGAAGATGCCGCCACATACTT
TTGCCATCAGTACCACCGGTCCCCTACCTTTGGCGGAGGCACAAAGCTGGAG
ACCAAGCGG

(Human tissue factor 219 form)

AGCGGCACCACCAACACAGTGGCCGCCTACAATCTGACTTGGAAATCC
ACCAACTTCAAGACCATCCTCGAGTGGGAGCCCAAGCCCGTTAATCAAGTTT
ATACCGTGCAGATTTCCACCAAGAGCGGCGACTGGAAATCCAAGTGCTTCTA
TACCACAGACACCGAGTGCGATCTCACCGACGAGATCGTCAAAGACGTGAAG
CAGACATATTTAGCTAGGGTGTTCTCCTACCCCGCTGGAAACGTGGAGAGCA
CCGGATCCGCTGGAGAGCCTTTATACGAGAACTCCCCCGAATTCACCCCCTAT
CTGGAAACCAATTTAGGCCAGCCCACCATCCAGAGCTTCGAACAAGTTGGCA
CAAAGGTGAACGTCACCGTCGAAGATGAGAGGACTTTAGTGCGGAGGAACA
ATACATTTTTATCCTTACGTGACGTCTTCGGCAAGGATTTAATCTACACACTG
TATTACTGGAAGTCTAGCTCCTCCGGCAAGAAGACCGCCAAGACCAATACCA
ACGAATTTTTAATTGACGTGGACAAGGGCGAGAACTACTGCTTCTCCGTGCA
AGCTGTGATCCCCTCCCGGACAGTGAACCGGAAGTCCACCGACTCCCCCGTG
GAGTGCATGGGCCAAGAGAAGGGAGAGTTTCGTGAG

(αCD3 light chain variable region)

CAGATCGTGCTGACCCAGTCCCCCGCTATTATGAGCGCTAGCCCCGGT
GAAAAGGTGACTATGACATGCAGCGCCAGCTCTTCCGTGAGCTACATGAACT

GGTATCAGCAGAAGTCCGGCACCAGCCCTAAAAGGTGGATCTACGACACCAG
CAAGCTGGCCAGCGGCGTCCCCGCTCACTTTCGGGGCTCCGGCTCCGGAACA
AGCTACTCTCTGACCATCAGCGGCATGGAAGCCGAGGATGCCGCTACCTATT
ACTGTCAGCAGTGGAGCTCCAACCCCTTCACCTTTGGATCCGGCACCAAGCTC
GAGATTAATCGT

(Linker)

GGAGGCGGAGGTAGCGGAGGAGGCGGATCCGGCGGTGGAGGTAGC

(αCD3 heavy chain variable region)

CAAGTTCAGCTCCAGCAAAGCGGCGCCGAACTCGCTCGGCCCGGCGCT
TCCGTGAAGATGTCTTGTAAGGCCTCCGGCTATACCTTCACCCGGTACACAAT
GCACTGGGTCAAGCAACGGCCCGGTCAAGGTTTAGAGTGGATTGGCTATATC
AACCCCTCCCGGGGCTATACCAACTACAACCAGAAGTTCAAGGACAAAGCCA
CCCTCACCACCGACAAGTCCAGCAGCACCGCTTACATGCAGCTGAGCTCTTT
AACATCCGAGGATTCCGCCGTGTACTACTGCGCTCGGTACTACGACGATCATT
ACTGCCTCGATTACTGGGGCCAAGGTACCACCTTAACAGTCTCCTCC

Exemplary Single-Chain Chimeric Polypeptide (αCD28scFv/TF/αCD3scFv) (SEQ ID NO: 327)

(Signal peptide)

MKWVTFISLLFLFSSAYS

(αCD28 light chain variable region)

VQLQQSGPELVKPGASVKMSCKASGYTFTSYVIQWVKQKPGQGLEWIGS
INPYNDYTKYNEKFKGKATLTSDKSSITAYMEFSSLTSEDSALYYCARWGDGNY
WGRGTTLTVSS

(Linker)

GGGGSGGGGSGGGGS

(αCD28 heavy chain variable region)

DIEMTQSPAIMSASLGERVTMTCTASSSVSSSYFHWYQQKPGSSPKLCIYS
TSNLASGVPPRFSGSGSTSYSLTISSMEAEDAATYFCHQYHRSPTFGGGTKLETKR

(Human tissue factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(αCD3 light chain variable region)

QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDT
SKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYCQQWSSNPFTFGSGTKLEI
NR

(Linker)

GGGGSGGGGSGGGGS

(αCD3 heavy chain variable region)

QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWI
GYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDD
HYCLDYWGQGTTLTVSS

The nucleic acid encoding αCD3scFv/TF/αCD28scFv was cloned into a modified retrovirus expression vectors as described previously (Hughes et al., *Hum Gene Ther* 16:457–72, 2005). The expression vector encoding αCD3scFv/TF/αCD28scFv was transfected into CHO-K1 cells. Expression of the expression vector in CHO-K1 cells allowed for secretion of the soluble αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide (referred to as 3t28), which can be purified by anti-TF Ab affinity and other chromatography methods.

An anti-tissue factor affinity column was used to purify the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide. The anti-tissue factor affinity column was connected to a GE Healthcare AKTA Avant system. A flow rate of 4 mL/min was used for all steps except the elution step, which was 2 mL/min.

Cell culture harvest including αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column (described above) which was equilibrated with 5 column

volumes of PBS. After sample loading, the column was washed with 5 column volumes PBS, followed by elution with 6 column volumes 0.1 M acetic acid, pH 2.9. An A280 elution peak was collected and then neutralized to pH 7.5-8.0 by adding 1 M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 kDa molecular weight cutoff. The data in Figure 58 show that the anti-tissue factor affinity column can bind the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide, which contains a human soluble tissue factor domain. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analysis and biological activity testing.

After each elution, the anti-tissue factor affinity column was stripped using 6 column volumes of 0.1 M glycine, pH 2.5. The column was then neutralized using 10 column volumes of PBS, 0.05% NaN_3, and stored at 2-8 °C.

Analytical size exclusion chromatography (SEC) was performed on the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide using a Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare) connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. A flow rate of 0.8 mL/min was used. Two hundred µL of αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide (1 mg/mL) was injected onto the column using a capillary loop. After injection of the single-chain chimeric polypeptide, 1.25 column volumes of PBS were flowed into the column. The SEC chromatograph is shown in Figure 59. The data show that there are 3 protein peaks, likely representing a monomer and dimer or other different forms of the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide.

To determine the purity and protein molecular weight of the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide, the purified αCD3scFv/TF/αCD28scFv protein sample from anti-tissue factor affinity column was analyzed by standard sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced conditions. The gel was stained with InstantBlue for about 30 minutes and destained overnight with purified water. Figure 60 shows the SDS gel of the αCD3scFv/TF/αCD28scFv single-chain

chimeric polypeptide purified using an anti-tissue factor affinity column. The results show that the purified αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide has the expected molecular weight (72 kDa) in reduced SDS gel.

Example 33. Functional Characterization of αCD3scFv/TF/αCD28scFv Single-Chain Chimeric Polypeptide

ELISA-based methods confirmed the formation of the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide. The αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide was detected using an anti-TF antibody (I43)/anti-TF antibody-specific ELISA with a capture antibody, anti-human tissue factor antibody (I43), and a detection antibody, anti-TF antibody(Figure 61). A purified tissue factor protein with a similar concentration was used as a control.

A further in vitro experiment was performed to determine whether the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide is capable of activating human peripheral blood mononuclear cells (PBMCs). Fresh human leukocytes were obtained from the blood bank and peripheral blood mononuclear cells (PBMC) were isolated using density gradient Histopaque (Sigma). The cells were counted and resuspended in 0.2 x 10^6/mL in a 96-well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide from 0.01 nM to 1000 nM for 3 days at 37 °C, 5% CO_2. After 72 hours, the cells were harvested and surface stained for CD4-488, CD8-PerCP Cy5.5, CD25-BV421,CD69-APCFire750, CD62L-PE Cy7, and CD44-PE specific antibodies (Biolegend) for 30 minutes. After surface staining, the cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were resuspended in 300 µL of FACS buffer and analyzed by Flow Cytometry (Celesta-BD Bioscience). The data in Figures 62 and 63 show that the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide is able to stimulate both $CD8^+$ and $CD4^+$ T-cells.

A further experiment was performed, in which PBMCs isolated from blood using Histopaque (Sigma) were counted and resuspended in 0.2 x 10^6/mL in a 96-well flat bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were then stimulated with the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide from 0.01 nM to 1000 nM for 3 days at 37 °C, 5% CO_2. After 72 hours, the cells were harvested and surface stained for CD4-488, CD8-PerCP Cy5.5, CD25-BV421, CD69-APCFire750, CD62L-PE Cy7, and CD44-PE (Biolegend) for 30 minutes. After surface staining, the cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were resuspended in 300 µL of FACS buffer and analyzed by Flow Cytometry (Celesta-BD Bioscience). The data again show that the αCD3scFv/TF/αCD28scFv single-chain chimeric polypeptide was able to stimulate activation of $CD4^+$ T cells (Figure 64).

Example 34: Creation of an IL-7/IL-15RαSu DNA construct

In a non-limiting example, an IL-7/IL-15RαSu DNA construct was created (see Figure 65). The human IL-7 sequence, human IL-15RαSu sequence, human IL-15 sequence, and human tissue factor 219 sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-7 sequence to the IL-15RαSu sequence. The final IL-7/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence encoding the second chimeric polypeptide of IL-7/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 206):

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGG CC

(Human IL-7)

GATTGTGATATTGAAGGTAAAGATGGCAAACAATATGAGAGTGTTCTA
ATGGTCAGCATCGATCAATTATTGGACAGCATGAAAGAAATTGGTAGCAATT
GCCTGAATAATGAATTTAACTTTTTTAAAAGACATATCTGTGATGCTAATAAG
GAAGGTATGTTTTTATTCCGTGCTGCTCGCAAGTTGAGGCAATTTCTTAAAAT
GAATAGCACTGGTGATTTTGATCTCCACTTATTAAAAGTTTCAGAAGGCACAA
CAATACTGTTGAACTGCACTGGCCAGGTTAAAGGAAGAAAACCAGCTGCCCT
GGGTGAAGCCCAACCAACAAAGAGTTTGGAAGAAAATAAATCTTTAAAGGA
ACAGAAAAAACTGAATGACTTGTGTTTCCTAAAGAGACTATTACAAGAGATA
AAAACTTGTTGGAATAAAATTTTGATGGGCACTAAAGAACAC

(Human IL-15R α sushi domain)

ATCACGTGCCCTCCCCCCATGTCCGTGGAACACGCAGACATCTGGGTC
AAGAGCTACAGCTTGTACTCCAGGGAGCGGTACATTTGTAACTCTGGTTTCAA
GCGTAAAGCCGGCACGTCCAGCCTGACGGAGTGCGTGTTGAACAAGGCCACG
AATGTCGCCCACTGGACAACCCCCAGTCTCAAATGCATTAGA

The second chimeric polypeptide of IL-7/IL-15RαSu construct (including signal peptide sequence) is as follows (SEQ ID NO: 205):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Example 35: Creation of an IL-21/TF/IL-15 DNA construct

In a non-limiting example, an IL-21/TF/IL-15 construct was made (Figure 66) by linking the IL-21 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-21/TF construct with the N-terminus coding region of IL-15.

The nucleic acid sequence encoding the first chimeric polypeptide of IL-21/TF/IL-15 construct (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 202):

(Signal peptide)

ATGGGAGTGAAAGTTCTTTTTGCCCTTATTTGTATTGCTGTGGCCGAGG
CC

(Human IL-21 fragment)

CAAGGTCAAGATCGCCACATGATTAGAATGCGTCAACTTATAGATATT
GTTGATCAGCTGAAAAATTATGTGAATGACTTGGTCCCTGAATTTCTGCCAGC
TCCAGAAGATGTAGAGACAAACTGTGAGTGGTCAGCTTTTTCCTGTTTTCAGA
AGGCCCAACTAAAGTCAGCAAATACAGGAAACAATGAAAGGATAATCAATG
TATCAATTAAAAAGCTGAAGAGGAAACCACCTTCCACAAATGCAGGGAGAA
GACAGAAACACAGACTAACATGCCCTTCATGTGATTCTTATGAGAAAAAACC
ACCCAAAGAATTCCTAGAAAGATTCAAATCACTTCTCCAAAAGATGATTCAT
CAGCATCTGTCCTCTAGAACACACGGAAGTGAAGATTCC

(Human Tissue Factor 219)

TCAGGCACTACAAATACTGTGGCAGCATATAATTTAACTTGGAAATCA
ACTAATTTCAAGACAATTTTGGAGTGGGAACCCAAACCCGTCAATCAAGTCT
ACACTGTTCAAATAAGCACTAAGTCAGGAGATTGGAAAAGCAAATGCTTTTA
CACAACAGACACAGAGTGTGACCTCACCGACGAGATTGTGAAGGATGTGAA
GCAGACGTACTTGGCACGGGTCTTCTCCTACCCGGCAGGGAATGTGGAGAGC
ACCGGTTCTGCTGGGGAGCCTCTGTATGAGAACTCCCCAGAGTTCACACCTTA

CCTGGAGACAAACCTCGGACAGCCAACAATTCAGAGTTTTGAACAGGTGGGA
ACAAAAGTGAATGTGACCGTAGAAGATGAACGGACTTTAGTCAGAAGGAAC
AACACTTTCCTAAGCCTCCGGGATGTTTTTGGCAAGGACTTAATTTATACACT
TTATTATTGGAAATCTTCAAGTTCAGGAAAGAAAACAGCCAAAACAAACACT
AATGAGTTTTTGATTGATGTGGATAAAGGAGAAAACTACTGTTTCAGTGTTCA
AGCAGTGATTCCCTCCCGAACAGTTAACCGGAAGAGTACAGACAGCCCGGTA
GAGTGTATGGGCCAGGAGAAAGGGGAATTCAGAGAA

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The first chimeric polypeptide of IL-21/TF/IL-15 construct including leader sequence is SEQ ID NO: 201:

(Signal peptide)

MGVKVLFALICIAVAEA (SEQ ID NO: 328)

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS

Example 36: Secretion of IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins

The IL-7/IL-15RαSu and IL-21/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-21/TF/IL-15:IL-7/IL-15RαSu protein complex (referred to as 21t15-7s; Figures 67 and Figure 68). The 21t15-7s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-7/IL-15RαSu and IL-21/TF/IL-15 fusion proteins.

In some cases, the leader (signal sequence) peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

Example 37: Purification of 21t15-7s by immunoaffinity chromatography

An anti-TF antibody affinity column was connected to a GE Healthcare™ AKTA Avant protein purification system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Cell culture harvest of 21t15-7s was adjusted to pH 7.4 with 1M Tris base and loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After loading the sample, the column was washed with 5 column volumes PBS,

followed by elution with 6 column volumes 0.1M acetic acid, pH 2.9. Absorbance at 280 nm was collected and then the sample was neutralized to pH 7.5-8.0 by adding 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon® centrifugal filters with a 30 KDa molecular weight cutoff. The buffer-exchanged protein sample was stored at 2-8°C for further biochemical analysis and biological activity testing.

After each elution, the anti-TF antibody affinity column was then stripped using 6 column volumes 0.1M glycine, pH 2.5. The column was then neutralized using 10 column volumes PBS, 0.05% sodium azide and stored at 2-8 °C.

Example 38: Size exclusion chromatography

A GE Healthcare Superdex® 200 Increase 10/300 GL gel filtration column was connected to a GE Healthcare AKTA™ Avant protein purification system. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A capillary loop was used to inject 200µL of 1 mg/mL of 7t15-21scomplex onto the column. The injection was chased with 1.25 column volumes of PBS.

Example 39: SDS-PAGE of 21t15-7s and 21t15-TGFRs

To determine the purity and protein molecular weight, the purified 21t15-7s or 21t15-TGFRs protein sample were analyzed using 4-12% NuPage Bis-Tris protein gel SDS-PAGE. The gel will be stained with InstantBlue™ for about 30 min, followed by destaining overnight in purified water.

Example 40: Glycosylation of 21t15-7s and 21t15-TGFRs in CHO-K1 cells

Glycosylation of 21t15-7s in CHO-K1 cells or 21t15-TGFRs in CHO-K1 cells were confirmed using the Protein Deglycosylation Mix II kit (New England Biolabs), according to the manufacturer's instructions.

Example 41: Recombinant protein quantitation of 21t15-7s and 21t15-TGFRs complexes

The 21t15-7s complex or the 21t15-TGFRs complex were detected and quantified using standard sandwich ELISA methods. Anti-human tissue factor antibody (IgG1) served as the capture antibody and biotinylated anti-human IL-21, IL-15, or IL-7 antibody (21t15-7s) or biotinylated anti-human IL-21, IL-15, or TGF-βRII antibody (21t15-TGFRs) served as the detection antibody. Tissue factor in purified 21t15-7s or 21t15-TGFRs protein complexes was detected using an anti-human tissue factor capture antibody, and anti-human tissue factor antibody (IgG1) detection antibody. The anti-TF antibody ELISA will be compared to purified tissue factor at similar concentrations.

Example 42: Creation of an IL-21/IL-15RαSu DNA construct

In a non-limiting example, an IL-21/IL-15RαSu DNA construct was created. The human IL-21 sequence and human IL-15RαSu sequence were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-21 sequence to the IL-15RαSu sequence. The final IL-21/IL-15RαSu DNA construct sequence was synthesized by Genewiz. See Figure 69.

Example 43: Creation of an IL-7/TF/IL-15 DNA construct

In a non-limiting example, an IL-7/TF/IL-15 construct was made by linking the IL-7 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-7/TF construct with the N-terminus coding region of IL-15. See Figure 70.

Example 44: Creation of an IL-21/IL-15Rα Sushi DNA construct

In a non-limiting example, a second chimeric polypeptide of IL-21/IL-15RαSu was generated. The human IL-21 and human IL-15Rα sushi sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. A DNA construct was made linking the IL-21 sequence to the IL-15Rα sushi sequence. The final IL-21/IL-15RαSu DNA construct sequence was synthesized by Genewiz.

The nucleic acid sequence encoding the second chimeric polypeptide of IL-21/IL-15RαSu domain (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 214):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC
CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGA
GCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAG
GAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAAC
GTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The second chimeric polypeptide of IL-21/IL-15Rα sushi domain (including leader sequence) is as follows (SEQ ID NO: 213):

(Signal Sequence)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15Rα sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH
WTTPSLKCIR

Example 45: Creation of an IL-7/TF/IL-15 DNA construct

In a non-limiting example, an exemplary first chimeric polypeptide of IL-7/TF/IL-15 was made by linking the IL-7 sequence to the N-terminus coding region of tissue factor 219, and further linking the IL-7/TF construct with the N-terminus coding region of IL-15. The nucleic acid sequence encoding the first chimeric polypeptide of IL-7/TF/IL-15 (including leader sequence), synthesized by Genewiz, is as follows (SEQ ID NO: 210):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL-7 fragment)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG

AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGT
CCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGT
AAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGA
GAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCC
AATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGT
GCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCA
CATTGTCCAGATGTTCATCAATACCTCC

The first chimeric polypeptide of IL-7/TF/IL-15 (including leader sequence), is as follows (SEQ ID NO: 209):

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN TS

Example 46: Secretion of IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins

The IL-21/IL-15RαSu and IL-7/TF/IL-15 DNA constructs were cloned into a pMSGV-1 modified retrovirus expression vector (as described by Hughes, *Hum Gene Ther* 16:457–72, 2005, hereby incorporated by reference), and the expression vector was transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of a soluble IL-7/TF/IL-15:IL-21/IL-15RαSu protein complex (referred to as 7t15-21s). The 7t15-21s protein was purified from CHO-K1 cell culture supernatant using anti-TF antibody (IgG1) affinity chromatography and size exclusion chromatography resulting in soluble (non-aggregated) protein complexes consisting of IL-21/IL-15RαSu and IL-7/TF/IL-15 fusion proteins. See Figure 71 and Figure 72.

Example 47: Expansion capacity of primary natural killer (NK) cells by 7t15-21s complex + anti-TF IgG1 antibody

To assess the 7t15-21s complex's ability to expand primary natural killer (NK) cells, 7t15-21s complex and 7t15-21s complex + anti-TF IgG1 antibody are added to NK cells obtained from samples of fresh human leukocytes. Cells are stimulated with 50nM of 7t15-21s complex with or without 25 nM of anti-TF IgG1 or anti-TF IgG4 antibody at 37° and 5% CO_2. Cells are maintained at concentration at 0.5 x 10^6/mL not exceeding 2.0 x 10^6/mL by counting every 48-72 hours and media is replenished with fresh stimulator. Cells stimulated with 7t15-21s complex or anti-TF IgG1 antibody or anti-TFIgG4 antibody or anti-TF IgG4 + 7t15-21s complex are maintained up to day 5. Expansion of primary NK cells upon incubation with 21t15-7s complex + anti-TF IgG1 antibody is observed.

Example 48: Activation of expanded NK cells by the 7t15-21s complex + anti-TF IgG1 antibody

Primary NK cells are induced *ex vivo* following overnight stimulation of purified NK cells with 7t15-21s complex + anti-TF IgG1 antibody. Fresh human leukocytes are obtained from a blood bank and CD56+ NK cells are isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells is >80% and is confirmed by staining with CD56-BV421 and CD16-BV510 specific antibodies (BioLegend). Cells are counted and resuspended in 1 x 10^6/mL in a 24 well flat bottom plate in 1 mL of complete media (RPMI 1640 (Gibco), supplemented with 4 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), non-essential amino acid (Thermo Life Technologies), sodium pyruvate (Thermo Life Technologies), and 10% FBS (Hyclone)). Cells are stimulated with 50 nM of 7t15-21s with or without 25 nM of anti-TF IgG1 antibody at 37° and 5% CO_2. Cells are counted every 48-72 hours and maintained at a concentration of 0.5 x 10^6/mL to 2.0 x 10^6/mL until day 14. Media is periodically replenished with fresh stimulator. Cells are harvested and surface stained at day 3 for CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 specific antibodies (Biolegend and analyzed by Flow Cytometry-Celeste-BD Bioscience). The activation marker CD25 MFI are observed to increase with 7t15-21s complex + anti-TF IgG1 antibody stimulation, but not 7t15-21s complex stimulation. The activation marker CD69 MFI is observed to increase with both 7t15-21s complex + anti-TF IgG1 antibody and with 7t15-21s complex, alone.

Example 49: Increase in Glucose Metabolism in NK Cells Using 18t15-12s

A set of experiments was performed to determine the effect of the construct of 18t15-12s on oxygen consumption rate and extracellular acidification rate (ECAR) on NK cells purified from human blood.

In these experiments, fresh human leukocytes were obtained from the blood bank from two different human donors and NK cells were isolated via negative selection using the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells

was >80% and confirmed by staining with CD56-BV421 and CD16-BV510 specific antibodies (BioLegend). The cells were counted and resuspended in 2 x 10^6/mL in 24-well, flat-bottom plates in 1 mL of complete media (RPMI 1640 (Gibco) supplemented with 4 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), non-essential amino acid (Thermo Life Technologies), sodium pyruvate (Thermo Life Technologies) and 10% FBS (Hyclone)). The cells were stimulated with either (1) media alone, (2) 100 nM 18t15-12s, or (3) mixture of single cytokines recombinant human IL-12 (0.25 μg), recombinant human IL-15 (1.25 μg), and recombinant human IL-18 (1.25 μg) overnight at 37 °C, 5% CO_2. On the next day, the cells were harvested and extracellular flux assays on expanded NK cells were performed using a XFp Analyzer (Seahorse Bioscience). The harvested cells washed and plated 2.0 x 10^5 cells/well in at least duplicate for extracellular flux analysis of OCR (Oxygen Consumption Rate) and ECAR (Extracellular Acidification Rate). The glycolysis stress tests were performed in Seahorse Media contain 2 mM of glutamine. The following were used during the assay: 10 mM glucose; 100 nM oligomycin; and 100 mM 2-deoxy-D-glycose (2DG).

The data show that the 18t15-12s results in significantly increased oxygen consumption rate (Figure 73) and extracellular acidification rate (ECAR) as compared to the same cells activated with a combination of recombinant human IL-12, recombinant human IL-15, and recombinant human IL-18 (Figure 74).

Example 50: 7t15-16s21 fusion protein generation and characterization

A fusion protein complex was generated comprising of anti-CD16scFv/IL-15RαSu/IL-21 and IL-7/TF/IL-15 fusion proteins. The human IL-7 and IL-21 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the IL-7/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC

AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of IL-7/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by linking the anti-CD16scFv sequence to the N-terminus coding region of IL-15RαSu chain followed by the N-terminus coding region of IL-21 which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the anti-CD16scFv linked to the N-terminus of IL-15RαSu chain followed by the N-terminus coding region of IL-21 are shown below.

The nucleic acid sequence of the anti-CD16SscFv/IL-15 RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

((Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

The amino acid sequence of the anti-CD16scFv/IL-15RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The anti-CD16scFv/IL-15RαSu/IL-21 and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15:anti-CD16scFv/IL-15RαSu/IL-21 protein complex (referred to as 7t15-16s21; Figure 75 and Figure 76), which can be purified by anti-TF IgG1 antibody-based affinity and other chromatography methods.

Binding of 7t15-16s21 to CHO cells expressing human CD16b

CHO cells were transfected with human CD16b in a pMC plasmid and selected with 10 μg/mL of blasticidin for 10 days. The CHO cells stably expressing CD16b were stained with 1.2 μg/mL of 7t15-16s21, containing anti-human CD16 scFv or 18t15-12s, which does not contain anti-human CD16 scFv, as a negative control, and then stained with biotinylated anti-human tissue factor and PE conjugated streptavidin. Only anti-human CD16scFv containing 7t15-16s21 stained the cells as shown in Figure 77A. 18t15-12s did not stain the CHO cells expressing human CD16b as showed in Figure 77B.

Detection of IL-15, IL-21, and IL-7 in 7t15-16s21 using ELISA

A 96-well plate was coated with 100 μL (8 μg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 μL of 1% BSA in PBS. Serial dilution of 7t15-16s21 (at a 1:3 ratio) were added to the wells, and incubated at RT for 60 min. Following 3 washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL-21 antibody (13-7218-81, R&D Systems), or 500 ng/mL of biotinylated-anti-IL-7 antibody (506602, R&D Systems) was added to the wells and incubated at RT for 60 min. The plate was washed 3 times, and incubated with 0.25 μg/mL of HRP-SA (Jackson ImmunoResearch) at 100 μL per well for 30 min at RT,

followed by 4 washes and incubation with 100 µl of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 78A-78C, the IL-15, IL-21, and IL-7 domains in 7t15-16s21 were detected by the individual antibodies.

The IL-15 in 7t15-16s21 promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To analyze the activity of IL-15 in 7t15-16s21, the IL-15 activity of 7t15-16s21 was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted 7t15-16s21 or IL-15 were added to the cells (Figure 79). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µl of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 79, 7t15-16s21 and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of 7t15-16s21 and IL-15 being 172.2 pM and 16.63 pM, respectively.

Purification elution chromatograph of 7t15-16s21 from anti-TF antibody affinity column

7t15-16s21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. The column was then washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. Figure 80 is a line graph showing the chromatographic profile of 7t15-16s21 protein containing cell culture supernatant following binding and elution on anti-TF antibody resin. As shown in Figure 80, the anti-TF antibody affinity column bound 7t15-16s21 which contains TF. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was

stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of 7t15-16s21

To perform size exclusion chromatography (SEC) analysis for 7t15-16s21, a Superdex 200 Increase 10/300 GL gel filtration column (GE Healthcare) connected to an AKTA Avant system (GE Healthcare) was used. The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing 7t15-16s21 in PBS was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. As shown in Figure 81, the SEC results showed two protein peaks for 7t15-16s21.

Example 51: TGFRt15-16s21 fusion protein generation and characterization

A fusion protein complex was generated comprising anti-human CD16scFv/IL-15RαSu/IL21 and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 82 and 83). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the two TGFβ Receptor II/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT

GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFβ Receptor II/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching anti-human CD16scFv directly linking to the N-terminus coding region of IL-15RαSu chain followed by the N-terminus coding region of IL-21 which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the anti-human CD16scFv linked to the N-terminus of IL-15RαSu followed by the N-terminus coding region of IL-21 are shown below.

The nucleic acid sequence of the anti-CD16scFv/IL-15 RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG
CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

The amino acid sequence of the anti-CD16scFv/IL-15RαSu/IL-21 construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The anti-CD16scFv/IL-15RαSu/IL-21 and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:CD16scFv/IL-15RαSu/IL-21 protein complex (referred to as TGFRt15-16s21), which can be purified by anti-TF IgG1-based affinity and other chromatography methods.

Interaction between TGFRt15-16s21 and CHO cells expressing human CD16b

CHO cells were transfected with human CD16b in a pMC plasmid and selected with 10 μg/mL of blasticidin for10 days. Cells stably expressing CD16b were stained with 1.2 μg/mL of TGFRt15-16s21, containing anti-human CD16 scFv, or 7t15-21s, not containing anti-human CD16 scFv, as a negative control, and with biotinylated anti-human tissue factor antibody and PE conjugated streptavidin. As shown in Figures 84A and 84B, TGFRt15-16s21, which contains anti-human CD16scFv, showed positive binding, while 7t15-21s did not show binding.

Effect of TGFRt15-16s21 on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβRII in TGFRt15-16s21, the effect of TGFRt15-16s21 on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x

glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 µl cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 µL 0.1nM TGFβ1 (R&D systems). TGFRt15-16s21 or TGFR-Fc (R&D Systems) prepared at a 1:3 serial dilution was then added to the plate to reach a total volume of 200 µL. After 24 hrs of incubation at 37°C, 40 µL of induced HEK-Blue TGFβ cell supernatant was added to 160 µL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The IC_{50} of TGFRt15-16s21 and TGFR-Fc were 9127 pM and 460.6 pM respectively. These results showed that the TGFβRII domain in TGFRt15-16s21 was able to block the activity of TGFβ-1 in HEK-Blue TGFβ cells.

The IL-15 in TGFRt15-16s21 promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To analyze the activity of IL-15 in TGFRt15-16s21, the IL-15 activity of TGFRt15-16s21 was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted TGFRt15-16s21 or IL-15 were added to the cells (Figure 86). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. The data are shown in Figure 85. As shown in Figure 86, TGFRt15-16s21 and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of TGFRt15-16s21 and IL-15 being 51298 pM and 10.63 pM, respectively.

Detection of IL-15, IL-21, and TGFβRII in TGFRt15-16s21 using ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were

washed 3 times and blocked with 100 µL of 1% BSA in PBS. TGFRt15-16s21 serially diluted at a 1:3 ratio was added and incubated at RT for 60 min. Following three washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL-21 antibody (13-7218-81, R&D Systems), or 200 ng/mL of biotinylated-anti-TGFβRII antibody (BAF241, R&D Systems) was applied per well, and incubated at RT for 60 min. Following three washes, incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch at 100 µL per well for 30 min at RT was carried out, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 87A-87C, the IL-15, IL-21, and TGFβRII domains in TGFRt15-16s21 were detected by the respective antibodies.

Purification elution chromatograph of TGFRt15-16s21 using anti-TF antibody affinity column

TGFRt15-16s21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 88, the anti-TF antibody affinity column bound to TGFRt15-16s21, which contains tissue factor as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE of TGFRt15-16s21

To determine the purity and molecular weight of the TGFRt15-16s21 protein, protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-16s21 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs according to the manufacturer's instructions. Figure 89 shows results from the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the TGFRt15-16s21 protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (69 kDa and 48 kDa) in the reduced SDS gel. Lane M was loaded with 10μL of SeeBlue Plus2 Prestained Standard.

Example 52: 7t15-7s fusion protein generation and characterization

A fusion protein complex was generated comprising IL-7/TF/IL-15 and IL-7/IL-15RαSu fusion proteins (Figure 90 and Figure 91). The human IL-7, tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC

TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by linking the IL-7 sequence to the N-terminus coding region of IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the IL-7 linked to the N-terminus of IL-15RαSu chain are shown below.

The nucleic acid sequence of 7s construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of 7s fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

The IL-7/TF/IL-15 and IL-7/IL-15RαSu constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15:IL-7/IL-15RαSu protein complex referred to as 7t15-7s, which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Purification elution chromatograph of 7t15-7s using anti-TF antibody affinity column

7t15-7s harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 92, the anti-TF antibody affinity column bound to 7t15-7s which contains tissue factor (TF) as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except the elution step, which was 2 mL/min.

Immunostimulation of 7t15-7s in C57BL/6 mice

7t15-7s is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of human IL-7, human

tissue factor 219 fragment and human IL-15 (7t15), and the second polypeptide that is a soluble fusion of human IL-7 and sushi domain of human IL-15 receptor alpha chain (7s).

CHO cells were co-transfected with the IL7-TF-IL-15 (7t15) and IL7-IL-15Ra sushi domain (7s) vectors. The 7t15-7s complex was purified from the transfected CHO cell culture supernatant. The IL-7, IL-15 and tissue factor (TF) components were demonstrated in the complex by ELISA as shown in Figure 93. A humanized anti-TF antibody monoclonal antibody (anti-TF IgG1) was used as the capture antibody to determine TF in 7t15-7s, and biotinylated anti-human IL-15 antibody (R&D systems) and biotinylated anti-human IL-7 antibody (R&D Systems) were used as the detection antibodies to respectively detect IL-15 and IL-7 in 7t15-7s, followed by peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS substrate (Surmodics IVD, Inc.).

7t15-7s was subcutaneously injected into C57BL/6 mice at 10 mg/kg to determine the immunostimulatory activity of 7t15-7s in vivo. C57BL/6 mice subcutaneously treated with PBS were used as control. The mouse spleens were collected and weighed day 4 post treatment. Single splenocytes suspensions were prepared, and with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 antibodies and the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells was analyzed by flow cytometry. The results showed that 7t15-7s was effective at expanding splenocytes based on spleen weight (Figure 94A) and specifically, the percentages of $CD8^+$ T cells and NK cells were higher compared to control-treated mice (Figure 94B).

Example 53: TGFRt15-TGFRs fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 95 and Figure 96). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then

directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the two TGFβ Receptor II/TF/IL-15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFβ Receptor II/TF/IL-15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN

NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu are shown below.

The nucleic acid sequence of the TGFβ Receptor II/IL-15 RαSu construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Two human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA

GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of the two TGFβ Receptor II/IL-15RαSu construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Two human TGFβ Receptor II extra-cellular domains)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFβR/IL-15RαSu and TGFβR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFβR/TF/IL-15:TGFβR/IL-15RαSu protein complex (referred to as TGFRt15-TGFRs), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Effect of TGFRt15-TGFRs on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβRII in TGFRt15-TGFRs, the effect of TGFRt15-16s21 on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 µL cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 µL 0.1nM TGFβ1 (R&D systems). TGFRt15-16s21 or TGFR-Fc (R&D Systems) prepared at a 1:3 serial dilution was then added to the plate to reach a total volume of 200 µL. After 24hrs of incubation at 37°C, 40 µL of induced HEK-Blue TGFβ cell supernatant was added to 160 µL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM (Figure 97). The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The IC_{50} of TGFRt15-TGFRs and TGFR-Fc were 216.9 pM and 460.6 pM

respectively. These results showed that the TGFβRII domain in TGFRt15-TGFRs was able to block the activity of TGFβ1 in HEK-Blue TGFβ cells.

The IL-15 in TGFRt15-TGFRs promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To evaluate the activity of IL-15 in TGFRt15-TGFRs, the IL-15 activity of TGFRt15-TGFRs was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted TGFRt15-TGFRs or IL-15 were added to the cells (Figure 98). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The absorbance at 450 nm was measured by analyzing the amount of formazan dye produced. As shown in Figure 98, TGFRt15-TGFRs and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of TGFRt15-16s21 and IL-15 being 1901 pM and 10.63 pM, respectively.

Detection of IL-15 and TGFβRII domains in TGFRt15-TGFRs with corresponding antibodies using ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed 3 times and blocked with 100 µL of 1% BSA in PBS. TGFRt15-TGFRs was added at a 1:3 serial dilution, and incubated at RT for 60 min. After 3 washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), or 200 ng/mL of biotinylated-anti-TGFbRII antibody (BAF241, R&D Systems) was added to the wells and incubated at RT for 60 min. Next the plates were washed 3 times, and 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was added and incubated for 30 min at RT, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance at 405 nm was read. As shown in Figure 99A and 99B, the IL-15 and TGFβRII domains in TGFRt15-TGFRs were detected by the individual antibodies.

Purification elution chromatograph of TGFRt15-TGFRs from anti-TF antibody affinity column

TGFRt15-TGFRs harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 100, the anti-TF antibody affinity column bound to TGFRt15-TGFRs which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of TGFRt15-TGFRs

A Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare) was connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing TGFRt15-TGFRs in PBS was injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. The SEC chromatograph of the sample is shown in Figure 101. The SEC results showed four protein peaks for TGFRt15-TGFRs.

Reduced SDS-PAGE analysis of TGFRt15-TGFRs

To determine the purity and molecular weight of the TGFRt15-TGFRs protein, protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-

PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-TGFRs protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 102 shows the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the TGFRt15-TGFRs protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (69 kDa and 39 kDa) in the reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Immunostimulatory activity of TGFRt15-TGFRs in C57BL/6 mice

TGFRt15-TGFRs is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes a first polypeptide that is a soluble fusion of two TGFβRII domains, human tissue factor 219 fragment and human IL-15, and the second polypeptide that is a soluble fusion of two TGFβRII domains and sushi domain of human IL-15 receptor alpha chain.

Wild type C57BL/6 mice were treated subcutaneously with either control solution or with TGFRt15-TGFRs at a dosage of 0.3 mg/kg, 1 mg/kg, 3 mg/kg, or 10 mg/kg. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. As shown in Figure 103A, the spleen weight in mice treated with TGFRt15-TGFRs increased with increasing dosage of TGFRt15-TGFRs. Moreover, the spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs were higher as compared to mice treated with the control solution, respectively. In addition, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and $CD19^+$ B cells present in the spleen of control-treated and TGFRt15-TGFRs-treated mice were evaluated. As shown in Figure 103B, in the spleens of mice treated with TGFRt15-TGFRs, the percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of TGFRt15-TGFRs. Specifically, the percentages of $CD8^+$ T cells

were higher in mice treated with 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice, and the percentages of NK cells were higher in mice treated with 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice. These results demonstrate that TGFRt15-TGFRs is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells.

The pharmacokinetics of TGFRt15-TGFRs molecules were evaluated in wild type C57BL/6 mice. The mice were treated subcutaneously with TGFRt15-TGFRs at a dosage of 3 mg/kg. The mouse blood was drained from tail vein at various time points and the serum was prepared. The TGFRt15-TGFRs concentrations in mouse serum was determined with ELISA (capture: anti-human tissue factor antibody; detection: biotinylated anti-human TGFβ receptor antibody and followed by peroxidase conjugated streptavidin and ABTS substrate). The results showed that the half-life of TGFRt15-TGFRs was 12.66 hours in C57BL/6 mice.

The mouse splenocytes were prepared in order to evaluate the immunostimulatory activity of TGFRt15-TGFRs over time in mice. As shown in Figure 104A, the spleen weight in mice treated with TGFRt15-TGFRs increased 48 hours posttreatment and continued to increase over time. In addition, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and $CD19^+$ B cells present in the spleen of control-treated and TGFRt15-TGFRs-treated mice were evaluated. As shown in Figure 104B, in the spleens of mice treated with TGFRt15-TGFRs, the percentages of $CD8^+$ T cells and NK cells both increased at 48 hours after treatment and were higher and higher overtime after the single dose treatment. These results further demonstrate that TGFRt15-TGFRs is able to stimulate immune cells in the spleen, in particular $CD8^+$ T cells and NK cells.

Furthermore, the dynamic proliferation of immune cells based on Ki67 expression of splenocytes and cytotoxicity potential based on granzyme B expression were evaluated in splenocytes isolated from mice following a single dose (3 mg/kg) of TGFRt15-TGFRs. As shown in Figure 105A and 105B, in the spleens of mice treated with TGFRt15-TGFRs, the expression of Ki67 and granzyme B by NK cells increased at 24 hours after treatment and its expression of $CD8^+$ T cells and NK cells both increased at 48 hours and later time points after the single dose treatment. These results demonstrate that

TGFRt15-TGFRs not only increases the numbers of $CD8^+$ T cells and NK cells but also enhance the cytotoxicity of these cells. The single dose treatment of TGFRt15-TGFRs led $CD8^+$ T cells and NK cells to proliferate for at least 4 days.

The cytotoxicity of the splenocytes from TGFRt15-TGFRs-treated mice against tumor cells was also evaluated. Mouse Moloney leukemia cells (Yac-1) were labeled with CellTrace Violet and were used as tumor target cells. Splenocytes were prepared from TGFRt15-TGFRs (3 mg/kg)-treated mouse spleens at various time points post treatment and were used as effector cells. The target cells were mixed with effector cells at an E:T ratio = 10:1 and incubated at 37°C for 20 hours. Target cell viability was assessed by analysis of propidium iodide positive, violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 tumor inhibition was calculated using the formula, (1-[viable Yac-1 cell number in experimental sample]/[viable Yac-1 cell number in the sample without splenocytes]) x 100. As shown in Figure 106, splenocytes from TGFRt15-TGFRs-treated mice had stronger cytotoxicity against Yac-1 cells than the control mouse splenocytes.

Tumor size analysis in response to chemotherapy and/or TGFRt15-TGFRs

Pancreatic cancer cells (SW1990, ATCC® CRL-2172) were subcutaneously (s.c.) injected into C57BL/6 scid mice (The Jackson Laboratory, 001913, $2x10^6$ cells/mouse, in 100μL HBSS) to establish the pancreatic cancer mouse model. Two weeks after tumor cell injection, chemotherapy was initiated in these mice intraperitoneally with a combination of Abraxane (Celgene, 68817-134, 5 mg/kg, i.p.) and Gemcitabine (Sigma Aldrich, G6423, 40 mg/kg, i.p.), followed by immunotherapy with TGFRt15-TGFRs (3 mg/kg, s.c.) in 2 days. The procedure above was considered one treatment cycle and was repeated for another 3 cycles (1 cycle/week). Control groups were set up as the SW1990-injected mice that received PBS, chemotherapy (Gemcitabine and Abraxane), or TGFRt15-TGFRs alone. Along with the treatment cycles, tumor size of each animal was measured and recorded every other day, until the termination of the experiment 2 months after the SW1990 cells were injected. Measurement of the tumor volumes were analyzed by group and the results indicated that the animals receiving a combination of

chemotherapy and TGFRt15-TGFRs had significantly smaller tumors comparing to the PBS group, whereas neither chemotherapy nor TGFRt15-TGFRs therapy alone work as sufficiently as the combination (Figure 107).

In vitro senescent B16F10 melanoma model

Next, in vitro killing of senescent B16F10 melanoma cells by activated mouse NK cells was evaluated. B16F10 senescence cells (B16F10-SNC) cells were labelled with CellTrace violet and incubated for 16 hrs with different E:T ratio of in vitro 2t2-activated mouse NK cells (isolated from spleen of C57BL/6 mice injected with TGFRt15-TGFRs10 mg/kg for 4 days). The cells were trypsinized, washed and resuspended in complete media containing propidium iodide (PI) solution. The cytotoxicity was assessed by flow cytometry (Figure 108).

Example 54: 7t15-21s137L (long version) fusion protein creation and characterization

A fusion protein complex was generated comprising of IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 fusion proteins (Figure 109 and Figure 110). Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA

AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

The nucleic acid and protein sequences of the 21s137L are shown below. The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA

ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC
TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG
GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA
ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows:

(Signal peptide)

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS
LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA
AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW
QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as 7t15-21s137L), which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Purification elution chromatograph of 7t15-21s137L using anti-TF antibody affinity column

7t15-21s137L harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the

column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 111, the anti-TF antibody affinity column bound to 7t15-21s137L which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min. Figure 112 shows the analytical SEC profile of 7t15-21s137L.

Example 55: 7t15-21s137L (short version) fusion protein generation and characterization

A fusion protein complex was generated comprising of IL-21/IL-15RαSu/CD137L and IL-7/TF/IL-15 fusion proteins. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC

TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

The nucleic acid and protein sequences of the 21s137L (short version) are shown below. The nucleic acid sequence of 21s137L (short version) construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA

ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137 Ligand short version)

GATCCCGCCGGCCTCTTGGACCTGCGGCAGGGCATGTTTGCGCAGCTG
GTGGCCCAAAATGTTCTGCTGATCGATGGGCCCCTGAGCTGGTACAGTGACC
CAGGCCTGGCAGGCGTGTCCCTGACGGGGGGCCTGAGCTACAAAGAGGACA
CGAAGGAGCTGGTGGTGGCCAAGGCTGGAGTCTACTATGTCTTCTTTCAACTA
GAGCTGCGGCGCGTGGTGGCCGGCGAGGGCTCAGGCTCCGTTTCACTTGCGC
TGCACCTGCAGCCACTGCGCTCTGCTGCTGGGGCCGCCGCCCTGGCTTTGACC
GTGGACCTGCCACCCGCCTCCTCCGAGGCTCGGAACTCGGCCTTCGGTTTCCA
GGGCCGCTTGCTGCACCTGAGTGCCGGCCAGCGCCTGGGCGTCCATCTTCAC
ACTGAGGCCAGGGCACGCCATGCCTGGCAGCTTACCCAGGGCGCCACAGTCT
TGGGACTCTTCCGGGTGACCCCCGAAATC

The amino acid sequence of the 21s137L (short version) construct (including signal peptide sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137 Ligand short version)

DPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKE
DTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALAL
TVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATV
LGLFRVTPEI

The IL-21/IL-15RαSu/CD137L (short version) and IL-7/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble IL-7/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as 7t15-21s137L (short version)), which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Binding of 7t15-21s137L (short version) to CD137 (4.1BB)

On day 1, a 96-well plate was coated with 100 µL (2.5 µg/mL) of GAH IgG Fc (G-102-C, R&D Systems) in R5 (coating buffer) or R5 only and incubated at 4°C, overnight. On day 2, the plates were washed three times and blocked with 300 µL of 1% BSA in PBS at 37°C for 2 hrs. 10 ng/mL of 4.1BB/Fc (838-4B, R&D Systems) was added at 100 µL/well and incubated for 2 hrs at RT. After three washes, the 7t15-

21s137L or 7t15-21s serially diluted at a 1/3 ratio (starting at 10 nM), and incubated at 4°C overnight. On day 3, following 3 washes, 300 ng/mL of biotinylated-anti-hTF antibody (BAF2339, R&D Systems) was added at 100 µL per well and incubated at RT for 2 hrs. The plate was then washed three times and incubated with 0.25 µg/mL of HRP-SA (Jackson ImmuneResearch) at 100 µL per well for 30 min, followed by 3 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figure 113, 7t15-21s137L (short version) showed significant interaction with 4.1BB/Fc (blue line) as compared to 7t15-21s.

Detection of IL-15, IL-21, and IL-7 in 7t15-21s137L (short version) with ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF antibody IgG1 in R5 (coating buffer) and incubated at RT for 2 hrs. The plates were washed 3 times and blocked with 100 µL of 1% BSA in PBS. 7t15-21s137L (short version), serially diluted at a 1:3 ratio was added, and incubated at RT for 60 min. After three washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 500 ng/mL of biotinylated-anti-IL21 antibody (13-7218-81, R&D Systems), or 500 ng/mL of biotinylated-anti-IL7 antibody (506602, R&D Systems) was added to the wells and incubated at RT for 60 min. After three washes and incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was carried out for 30 min at RT, followed by four washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 114A-114C, the IL-15, IL-21, and IL-7 domains in 7t15-21s137L (short version) were detected by the respective antibodies.

The IL-15 in 7t15-1s137L (short version) promotes IL2Rαβγ containing CTLL2 cell proliferation

To evaluate the IL-15 activity of 7t15-21s137L (short version), 7t15-21s137L (short version) was compared with recombinant IL-15 in promoting proliferation of IL2R$\alpha\beta\gamma$ expressing CTLL2 cells. IL-15-dependent CTLL2 cells were washed 5 times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serially diluted 7t15-21s137L (short version) or IL-15 were added to the cells (Figure 115). Cells were

incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 μL of WST1 to each well on day 3 and incubated for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 115, 7t15-21s137L (short version) and IL-15 promoted CTLL2 cell proliferation. The EC_{50} of 7t15-21s137L (short version) and IL-15 was 55.91 pM and 6.22 pM, respectively.

The IL-21 in 7t15-1s137L (short version) promotes IL21R containing B9 cell proliferation

To evaluate the IL-21 activity of 7t15-21s137L (short version), 7t15-21s137L (short version) was compared with recombinant IL-21 in promoting proliferation of IL-21R expressing B9 cells. IL-21R containing B9 cells were washed 5 times with RPMI-10% FBS and seeded to the wells at 1 x 10^4 cells/well. Serially diluted 7t15-21s137L (short version) or IL-21 were added to the cells (Figure 116). Cells were incubated in a CO_2 incubator at 37°C for 5 days. Cell proliferation was detected by adding 10 μL of WST1 to each well on day 5 and incubated for an additional 4 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 116, 7t15-21s137L (short version) and IL-21 promoted B9 cell proliferation. The EC_{50} of 7t15-21s137L (short version) and IL-21 was 104.1 nM and 72.55 nM, respectively.

Example 56: 7t15-TGFRs fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu and IL-7/TF/IL-15 fusion proteins (Figure 117 and Figure 118). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, IL-15, and IL-7 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking the IL-7 sequence to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15. The nucleic acid and protein sequences of a construct comprising IL-7 linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCT
GATGGTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAAC
TGCCTCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACA
AGGAGGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAA
GATGAACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGC
ACCACCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTG
CTCTGGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGA
AGGAGCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGG
AGATCAAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDAN
KEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAAL
GEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain which was synthesized by Genewiz. The nucleic acid and protein

sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu are shown below.

The nucleic acid sequence of the TGFRs construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

The amino acid sequence of TGFRs fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

Effect of 7t15-TGFRs on TGFβ1 activity in HEK-Blue TGFβ cells

To evaluate the activity of TGFβR in 7t15-TGFRs, the effect of 7t15-TGFRs on the activity of TGFβ1 in HEK-Blue TGFβ cells was analyzed. HEK-Blue TGFβ cells (Invivogen) were washed twice with pre-warmed PBS and resuspended in the testing medium (DMEM, 10% heat-inactivated FCS, 1x glutamine, 1x anti-anti, and 2x glutamine) at 5 x 10^5 cells/mL. In a flat-bottom 96-well plate, 50 µL cells were added to each well (2.5 x 10^4 cells/well) and followed with 50 µL 0.1nM TGFβ1 (R&D systems). 7t15-TGFRs or TGFR-Fc (R&D Systems) prepared at a1:3 serial dilution was then added to the plate to reach a total volume of 200 µL. After 24hrs of incubation at 37°C, 40 µL of induced HEK-Blue TGFβ cell supernatant was added to 160 µL pre-warmed QUANTI-Blue (Invivogen) in a flat-bottom 96-well plate, and incubated at 37°C for 1-3 hrs. The OD values were then determined using a plate reader (Multiscan Sky) at 620-655 nM. The data are shown in Figure 119. The IC_{50} of each protein sample was calculated with GraphPad Prism 7.04. The IC_{50} of 7t15-TGFRs and TGFR-Fc were 1142 pM and

558.6 pM respectively. These results showed that the TGFβR in 7t15-TGFRs was able to block the activity of TGFβ1 in HEK-Blue TGFβ cells.

Detection of IL-15, TGFβRII, and IL-7 in 7t15-TGFRs with ELISA

A 96-well plate was coated with 100 µL (8 µg/mL) of anti-TF antibody IgG1 in R5 (coating buffer) and incubated at room temperature (RT) for 2 hrs. The plates were washed three times and blocked with 100 µL of 1% BSA in PBS. Serial dilution of 7t15-TGFRs (1:3 ratio) was added, and incubated at RT for 60 mins. After 3 washes, 50 ng/mL of biotinylated-anti-IL-15 antibody (BAM247, R&D Systems), 200 ng/mL of biotinylated-anti-TGFbRII antibody (BAF241, R&D Systems), or 500 ng/mL of biotinylated-anti-IL-7 antibody (506602, R&D Systems) was added and incubated at RT for 60 min. Following three washes, incubation with 0.25 µg/mL of HRP-SA (Jackson ImmunoResearch) at 100 µL per well was carried out for 30 min at RT, followed by 4 washes and incubation with 100 µL of ABTS for 2 mins at RT. Absorbance was read at 405 nm. As shown in Figures 120A-120C, the IL-15, TGFR, and IL-7 in 7t15-TGFRs were detected by the respective antibodies.

The IL-15 in 7t15-TGFRs promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

To evaluate the activity of IL-15 in 7t15-TGFRs, 7t15-TGFRs was compared to recombinant IL-15 using 32Dβ cells that express IL2Rβ and common γ chain, and evaluating their effects on promoting cell proliferation. IL-15 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded in the wells at 2 x 10^4 cells/well. Serially-diluted 7t15-TGFRs or IL-15 were added to the cells (Figure 121). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µL of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 121, 7t15-TGFRs and IL-15 promoted 32Dβ cell proliferation, with the EC_{50} of 7t15-TGFRs and IL-15 being 126 nM and 16.63 pM, respectively.

Purification elution chromatograph of 7t15-TGFRs using anti-TF antibody affinity column

7t15-TGFRs harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 122, the anti-TF antibody affinity column can bind 7t15-TGFRs which contains TF as a fusion partner of 7t15-TGFRs. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE analysis of 7t15-TGFRs

To determine the purity and molecular weight of the protein, 7t15-TGFRs protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the 7t15-TGFRs protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 123 shows reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. These results showed that the protein is glycosylated when it is expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (55 kDa

and 39 kDa) in reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Characterization of 7t15-TGFRs

7t15-TGFRs is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of human IL-7, human tissue factor 219 fragment and human IL-15 (7t15), and the second polypeptide that is a soluble fusion of single chain two TGFβRII domains and sushi domain of human IL-15 receptor alpha chain (TGFRs).

CHO cells were co-transfected with 7t15 and TGFRs vectors. The 7t15-TGFRs complex was purified from the transfected CHO cell culture supernatant. The IL-7, IL-15, TGFβ receptor and tissue factor (TF) components were demonstrated in the complex by ELISA as shown in Figure 124. A humanized anti-TF antibody monoclonal antibody (anti-TF antibody IgG1) was used as the capture antibody to determine TF in 7t15-TGFRs, and biotinylated antibodies against human IL-15 (R&D systems), human IL-7 (Biolegend), anti-TGFβ receptor (R&D Systems) were used as the detection antibodies to respectively determine IL-7, IL-15 and TGFβ receptor in 7t15-TGFRs. Peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS substrate (Surmodics IVD, Inc.) were then used to detect the bound biotinylated antibodies. The results were analyzed by ELISA (Figure 124).

In vivo characterization of 7t15-TGFRs in C57BL/6 mice

To determine the immunostimulatory activity of 7t15-TGFRs in vivo, C57BL/6 mice were subcutaneously treated with control solution (PBS) or 7t15-TGFRs at 0.3, 1, 3 and 10 mg/kg. The treated mice were euthanized. The mouse spleens were collected and weighed day 4 post treatment. Single splenocyte suspensions were prepared and stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 antibodies and the percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells was analyzed by flow cytometry. The results showed that 7t15-TGFRs was effective at expanding splenocytes based on spleen weight (Figure 125A), especially at 1-10 mg/kg. The percentages of $CD8^+$ T cells

and NK cells were higher compared to control-treated mice (Figure 125B) at all doses tested.

CD44 Expression of $CD4^+$ and $CD8^+$ T cells

It has been known that IL-15 induces CD44 expression on T cells and development of memory T cells. CD44 expression of $CD4^+$ and $CD8^+$ T cells in the 7t15-TGFRs treated mice were assessed. C57BL/6 mice were subcutaneously treated with 7t15-TGFRs. The splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8 and anti-CD44 monoclonal antibodies for immunocyte subsets. The percentages of $CD4^+CD44^{high}$ T cells of total CD4+ T cells and $CD8^+CD44^{high}$ T cells of total $CD8^+$ T cells were analyzed by flow cytometry. As shown in Figures 126A and 126B, 7t15-TGFRs significantly activated $CD4^+$ and $CD8^+$ T cells to differentiate into memory T cells.

Furthermore, the dynamic proliferation of immune cells based on Ki67 expression of splenocytes and cytotoxicity potential based on granzyme B expression of the splenocytes induced by 7t15-TGFRs after the single dose treatment of mouse were also evaluated. C57BL/6 mice were subcutaneously treated with 7t15-TGFRs at 3 mg/kg. The treated mice were euthanized and the splenocytes were prepared. The prepared splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies for immunocyte subsets and then intracellularly stained with anti-Ki67 antibody for cell proliferation and anti-granzyme B antibody for cytotoxic marker. The mean fluorescent intensity (MFI) of Ki67 and granzyme B of corresponding immunocyte subsets was analyzed by flow cytometry. As shown in Figures 127A and 127B, in the spleens of mice treated with 7t15-TGFRs, the expression of Ki67 and granzyme B by $CD8^+$ T cells and NK cells increased compared with PBS control treatment. These results demonstrate that 7t15-TGFRs is not only to increase numbers of $CD8^+$ T cells and NK cells but also enhance potential cytotoxicity of these cells.

Additionally, cytotoxicity of the mouse splenocytes against tumor cells was also evaluated. Mouse Yac-1 cells were labeled with CellTrace Violet and used as tumor target cells. The splenocytes were prepared from 7t15-TGFRs-treated mice and used as

effector cells. The target cells were mixed with effector cells at E:T ratio = 10:1 in RPMI-10 medium with or without 7t15-TGFRs at 100 nM and incubated at 37°C for 20 hours. Target Yac-1 cell inhibition was assessed by analysis of viable violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 inhibition was calculated using a formula, (1-viable Yac-1 cell number in experimental sample/viable Yac-1 cell number in the sample without splenocytes) x 100. As shown in Figure 128, 7t15-TGFRs-treated mouse splenocytes had stronger cytotoxicity against Yac-1 cells than the control mouse splenocytes and addition of 7t15-TGFRs during cytotoxic assay further enhanced cytotoxicity of splenocytes against Yac-1 target cells.

Example 57: TGFRt15-21s137L fusion protein generation and characterization

A fusion protein complex was generated comprising IL-21/IL-15RαSu/CD137L and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 129 and Figure 130). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC

ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT

TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

The nucleic acid and protein sequences of the 21s137L are shown below. The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC
TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG

GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK EFLERFKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The IL-21/IL-15RαSu/CD137L and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation

and secretion of the soluble TGFR/TF/IL-15: IL-21/IL-15RαSu/CD137L protein complex (referred to as TGFRt15-21s137L), which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Purification elution chromatograph of TGFRt15-21s137L using anti-TF antibody affinity column

TGFRt15-21s137L harvest from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 131, the anti-TF antibody affinity column bound to TGFRt15-21s137L which contains TF as a fusion partner of TGFRt15-21s137L. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Example 58: TGFRt15-TGFRs21 fusion protein generation and characterization

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/IL-21 and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 132 and Figure 133). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, IL-21, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II

and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN

NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by the N-terminus coding region of IL-21, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with the N-terminus of IL-21 are shown below.

The nucleic acid sequence of the TGFRs21 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC

ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACAT
CGTCGACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCT
GCCCCCGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTC
AGAAGGCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCA
ACGTGAGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCA
GGAGGCAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGA
AGCCCCCCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGAT
CCATCAGCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

The amino acid sequence of TGFRs21 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCF
QKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPK
EFLERFKSLLQKMIHQHLSSRTHGSEDS

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/IL-21 and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/IL-21 protein complex (referred to as TGFRt15-TGFRs21), which can be purified by anti-TF antibody IgG1 affinity and other chromatography methods.

Purification elution chromatograph of TGFRt15-TGFRs21 using anti-TF antibody affinity column

TGFRt15-TGFRs21 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid (pH 2.9). A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 KDa molecular weight cutoff. As shown in Figure 134, the anti-TF antibody affinity column bound to TGFRt15-TGFRs21 which contains TF as a fusion partner. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine (pH 2.5). The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE analysis of TGFRt15-TGFRs21

To determine the purity and molecular weight of the protein, TGFRt15-TGFRs21 protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the TGFRt15-TGFRs21 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figure 135 shows the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. It is clear that the protein is glycosylated when it is expressed in CHO cells. After deglycosylation, the purified sample showed expected molecular weights (69 kDa and 55

kDa) in reduced SDS gel. Lane M was loaded with 10 ul of SeeBlue Plus2 Prestained Standard.

Immunostimulation of TGFRt15-TGFRs21 in C57BL/6 mice

TGFRt15-TGFRs21 is a multi-chain polypeptide (a type A multi-chain polypeptide described herein) that includes the first polypeptide that is a soluble fusion of single chain two TGFβRII domains, human tissue factor 219 fragment and human IL-15 (TGFRt15), and the second polypeptide that is a soluble fusion of single chain two TGFβRII domains, sushi domain of human IL-15 receptor alpha chain and human IL-21 (TGFRs21).

CHO cells were co-transfected with TGFRt15 and TGFRs21 vectors. The TGFRt15-TGFRs21 complex was purified from the transfected CHO cell culture supernatant. The TGFβ receptor, IL-15, IL-21 and tissue factor (TF) components were demonstrated in the complex by ELISA as shown in Figure 136. A humanized anti-TF monoclonal antibody (anti-TF IgG1) was used as the capture antibody to determine TF in TGFRt15-TGFRs21, biotinylated anti-human IL-15 antibody (R&D systems), biotinylated anti-human TGFβ receptor antibody (R&D systems, and biotinylated anti-human IL-21 antibody (R&D Systems) were used as the detection antibodies to respectively determine IL-15, TGFβ receptor, and IL-21 in TGFRt15-TGFRs21. For detection, peroxidase conjugated streptavidin (Jackson ImmunoResearch Lab) and ABTS were used.

Wild type C57BL/6 mice were treated subcutaneously with either control solution (PBS) or with TGFRt15-TGFRs21 at 3 mg/kg. Four days after treatment, spleen weight and the percentages of various immune cell types present in the spleen were evaluated. As shown in Figure 137A, the percentages of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells present in the spleen of control-treated and TGFRt15-TGFRs21-treated mice were evaluated. The dynamic proliferation of immune cells based on Ki67 expression after TGFRt15-TGFRs21 treatment was also evaluated. The splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies and then intracellularly stained with anti-Ki67 antibody. The percentage of $CD4^+$ T cells, $CD8^+$ T

cells, and NK cells and the mean fluorescent intensity (MFI) of Ki67 of corresponding immunocyte subsets were analyzed by flow cytometry (Figures 137A and 137B). Furthermore, cytotoxicity potential based on granzyme B expression of the splenocytes induced by TGFRt15-TGFRs21 after the single dose treatment of mouse was also evaluated. As shown in Figure 138, in the spleens of mice treated with TGFRt15-TGFRs21, the expression of granzyme B by NK cells increased after treatment. The splenocytes from TGFRt15-TGFRs21-treated mice were stained with fluorochrome-conjugated anti-CD4, anti-CD8, and anti–NK1.1 (NK) antibodies and then intracellularly stained with anti-granzyme B antibody. The mean fluorescent intensity (MFI) of granzyme B of corresponding immunocyte subsets was analyzed by flow cytometry (Figure 138).

As shown in Figure 137A, in the spleens of mice treated with TGFRt15-TGFRs21, the percentages of $CD8^+$ T cells and NK cells both increased on day 4 after a single TGFRt15-TGFRs21 treatment. These results demonstrate that TGFRt15-TGFRs21 is able to induce immune cells to proliferate in mouse spleen, in particular $CD8^+$ T cells and NK cells.

Additionally, cytotoxicity of the mouse splenocytes against tumor cells was also evaluated. Mouse Yac-1 cells were labeled with CellTrace Violet and used as tumor target cells. The splenocytes were prepared from TGFRt15-TGFRs21-treated mice and used as effector cells. The target cells were mixed with effector cells at E:T ratio = 10:1 in RPMI-10 medium with or without TGFRt15-TGFRs21 at 100 nM and incubated at 37°C for 24 hours. Target Yac-1 cell inhibition was assessed by analysis of viable violet-labeled Yac-1 cells using flow cytometry. Percentage of Yac-1 inhibition was calculated using a formula, (1-[viable Yac-1 cell number in experimental sample]/[viable Yac-1 cell number in the sample without splenocytes]) x 100. As shown in Figure 139, TGFRt15-TGFRs21-treated mouse splenocytes had stronger cytotoxicity against Yac-1 cells than the control mouse cells in the presence of TGFRt15-TGFRs21 during cytotoxic assay (Figure 139).

Example 59: TGFRt15-TGFRs16 fusion protein generation

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/ anti-CD16scFv and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 140 and Figure 141). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG

CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by the anti-CD16scFv sequence, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with the anti-CD16scFv sequence are shown below.

The nucleic acid sequence of the TGFRs16 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

(Anti-human CD16scFv)

TCCGAGCTGACCCAGGACCCTGCTGTGTCCGTGGCTCTGGGCCAGACC
GTGAGGATCACCTGCCAGGGCGACTCCCTGAGGTCCTACTACGCCTCCTGGT
ACCAGCAGAAGCCCGGCCAGGCTCCTGTGCTGGTGATCTACGGCAAGAACAA
CAGGCCCTCCGGCATCCCTGACAGGTTCTCCGGATCCTCCTCCGGCAACACCG
CCTCCCTGACCATCACAGGCGCTCAGGCCGAGGACGAGGCTGACTACTACTG
CAACTCCAGGGACTCCTCCGGCAACCATGTGGTGTTCGGCGGCGGCACCAAG

CTGACCGTGGGCCATGGCGGCGGCGGCTCCGGAGGCGGCGGCAGCGGCGGA
GGAGGATCCGAGGTGCAGCTGGTGGAGTCCGGAGGAGGAGTGGTGAGGCCT
GGAGGCTCCCTGAGGCTGAGCTGTGCTGCCTCCGGCTTCACCTTCGACGACTA
CGGCATGTCCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAGTGGGTGTCC
GGCATCAACTGGAACGGCGGATCCACCGGCTACGCCGATTCCGTGAAGGGCA
GGTTCACCATCAGCAGGGACAACGCCAAGAACTCCCTGTACCTGCAGATGAA
CTCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCAGGTCC
CTGCTGTTCGACTACTGGGGACAGGGCACCCTGGTGACCGTGTCCAGG

The amino acid sequence of TGFRs16 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

(Anti-human CD16scFv)

SELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGK
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTKL
TVGHGGGGSGGGGSGGGGSEVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGM
SWVRQAPGKGLEWVSGINWNGGSTGYADSVKGRFTISRDNAKNSLYLQMNSLR
AEDTAVYYCARGRSLLFDYWGQGTLVTVSR

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/anti-CD16scFv and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. *Hum Gene Ther* 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/anti-CD16scFv protein complex (referred to as TGFRt15-TGFRs16), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Example 60: The TGFRt15-TGFRs137L fusion protein generation

A fusion protein complex was generated comprising of TGFβ Receptor II/IL-15RαSu/ CD137L and TGFβ Receptor II/TF/IL-15 fusion proteins (Figure 142 and Figure 143). The human TGFβ Receptor II (Ile24-Asp159), tissue factor 219, CD137L, and IL-15 sequences were obtained from the UniProt website and DNA for these sequences was synthesized by Genewiz. Specifically, a construct was made linking two TGFβ Receptor II sequences with a G4S(3) linker to generate a single chain version of TGFβ Receptor II and then directly linking to the N-terminus coding region of tissue factor 219 followed by the N-terminus coding region of IL-15.

The nucleic acid and protein sequences of a construct comprising two TGFβ Receptor II linked to the N-terminus of tissue factor 219 following with the N-terminus of IL-15 are shown below.

The nucleic acid sequence of the TGFRt15 construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA
GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC

AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of TGFRt15 fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

Constructs were also made by attaching two TGFβ Receptor II directly to the IL-15RαSu chain, followed by a (G4S)3 linker and the CD137L sequence, which was synthesized by Genewiz. The nucleic acid and protein sequences of a construct comprising the TGFβ Receptor II linked to the N-terminus of IL-15RαSu following with a (G4S)3 linker and the CD137L sequence are shown below.

The nucleic acid sequence of the TGFRs137L construct (including signal peptide sequence) is as follows:

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(Human TGFβ Receptor II fragments)

ATCCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACC
GACAACAACGGCGCCGTGAAGTTTCCCCAGCTCTGCAAGTTCTGCGATGTCA
GGTTCAGCACCTGCGATAATCAGAAGTCCTGCATGTCCAACTGCAGCATCAC
CTCCATCTGCGAGAAGCCCCAAGAAGTGTGCGTGGCCGTGTGGCGGAAAAAT
GACGAGAACATCACCCTGGAGACCGTGTGTCACGACCCCAAGCTCCCTTATC
ACGACTTCATTCTGGAGGACGCTGCCTCCCCCAAATGCATCATGAAGGAGAA
GAAGAAGCCCGGAGAGACCTTCTTTATGTGTTCCTGTAGCAGCGACGAGTGT
AACGACAACATCATCTTCAGCGAAGAGTACAACACCAGCAACCCTGATGGAG
GTGGCGGATCCGGAGGTGGAGGTTCTGGTGGAGGTGGGAGTATTCCTCCCCA
CGTGCAGAAGAGCGTGAATAATGACATGATCGTGACCGATAACAATGGCGCC
GTGAAATTTCCCCAGCTGTGCAAATTCTGCGATGTGAGGTTTTCCACCTGCGA
CAACCAGAAGTCCTGTATGAGCAACTGCTCCATCACCTCCATCTGTGAGAAG
CCTCAGGAGGTGTGCGTGGCTGTCTGGCGGAAGAATGACGAGAATATCACCC
TGGAAACCGTCTGCCACGATCCCAAGCTGCCCTACCACGATTTCATCCTGGA
AGACGCCGCCAGCCCTAAGTGCATCATGAAAGAGAAAAAGAAGCCTGGCGA

GACCTTTTTCATGTGCTCCTGCAGCAGCGACGAATGCAACGACAATATCATCT
TTAGCGAGGAATACAATACCAGCAACCCCGAC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTG
AAGAGCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCA
AGAGGAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTA
CCAACGTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGAC
CTGCGGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCG
ATGGGCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGAC
GGGGGGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGC
TGGAGTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCG
AGGGCTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCT
GCTGGGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGA
GGCTCGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCG
GCCAGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTG
GCAGCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAA
ATCCCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of TGFRs137L fusion protein (including the leader sequence) is as follows:

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human TGFβ Receptor II)

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI
TSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEYNTSNPDGGGGSGGGGSGGGGSIPPHVQKSVN

NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKAT
NVAHWTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVS
LTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSA
AGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAW
QLTQGATVLGLFRVTPEIPAGLPSPRSE

In some cases, the leader peptide is cleaved from the intact polypeptide to generate the mature form that may be soluble or secreted.

The TGFR/IL-15RαSu/CD137L and TGFR/TF/IL-15 constructs were cloned into a modified retrovirus expression vectors as described previously (Hughes MS, Yu YY, Dudley ME, Zheng Z, Robbins PF, Li Y, et al. Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions. Hum Gene Ther 2005;16:457–72), and the expression vectors were transfected into CHO-K1 cells. Co-expression of the two constructs in CHO-K1 cells allowed for formation and secretion of the soluble TGFR/TF/IL-15:TGFR/IL-15RαSu/CD137L protein complex (referred to as TGFRt15-TGFRs137L), which can be purified by anti-TF IgG1 affinity and other chromatography methods.

Example 61. Production and characterization of the Exemplary Single-Chain Chimeric Polypeptide 2t2

An exemplary single-chain chimeric polypeptide including a first target-binding domain that binds to an IL-2 receptor, a soluble human tissue factor domain, and a second target-binding domain that binds to an IL-2 receptor was generated (IL-2/TF/IL-2;

referred to as 2t2) (Figure 144). The nucleic acid and amino acid sequences of this single-chain chimeric polypeptide are shown below.

Nucleic Acid Encoding Exemplary Single-Chain Chimeric Polypeptide (IL-2/TF/IL-2) (SEQ ID NO: 164)

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(First IL-2 fragment)

GCCCCCACCTCCTCCTCCACCAAGAAGACCCAGCTGCAGCTGGAGCAT
TTACTGCTGGATTTACAGATGATTTTAAACGGCATCAACAACTACAAGAACC
CCAAGCTGACTCGTATGCTGACCTTCAAGTTCTACATGCCCAAGAAGGCCAC
CGAGCTGAAGCATTTACAGTGTTTAGAGGAGGAGCTGAAGCCCCTCGAGGAG
GTGCTGAATTTAGCCCAGTCCAAGAATTTCCATTTAAGGCCCCGGGATTTAAT
CAGCAACATCAACGTGATCGTTTTAGAGCTGAAGGGCTCCGAGACCACCTTC
ATGTGCGAGTACGCCGACGAGACCGCCACCATCGTGGAGTTTTTAAATCGTT
GGATCACCTTCTGCCAGTCCATCATCTCCACTTTAACC

(Human tissue factor 219 form)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC
ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC

AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Second IL-2 fragment)

GCACCTACTTCAAGTTCTACAAAGAAAACACAGCTACAACTGGAGCAT
TTACTGCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCC
CAAACTCACCAGGATGCTCACATTTAAGTTTTACATGCCCAAGAAGGCCACA
GAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAG
TGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAAT
CAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTC
ATGTGTGAATATGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAGAT
GGATTACCTTTTGTCAAAGCATCATCTCAACACTAACT

Exemplary Single-Chain Chimeric Polypeptide (IL-2/TF/IL-2) (SEQ ID NO: 163)

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-2)

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA
TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE
YADETATIVEFLNRWITFCQSIISTLT

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-2)

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKA
TELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCE
YADETATIVEFLNRWITFCQSIISTLT

The nucleic acid encoding IL-2/TF/IL-2 was cloned into a modified retrovirus expression vector as described previously (Hughes et al., *Hum Gene Ther* 16:457–72, 2005). The expression vector encoding IL-2/TF/IL-2 was transfected into CHO-K1 cells. Expression of the expression vector in CHO-K1 cells allowed for secretion of the soluble IL-2/TF/IL-2 single-chain chimeric polypeptide (referred to as 2t2), which can be purified by anti-TF antibody affinity and other chromatography methods.

IL-2 and 2t2 promoted IL-2Rβ and common γ chain containing 32Dβ cell proliferation in a similar manner

To evaluate the IL-2 activity of 2t2, 2t2 was compared with recombinant IL-2 for promoting proliferation of 32Dβ cells that express IL-2Rβ and common γ chain. IL-2 dependent 32Dβ cells were washed 5 times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serial dilutions of 2t2 or IL-2 were added to the cells (Figure 145). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µl of WST1 to each well on day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 145, 2t2 and IL-2 activated 32Dβ cells in a similar manner. The EC_{50} of 2t2 and IL-2 was 158.1 pM and 140 pM. respectively.

2t2 showed improved ability to promote IL-2Rαβγ containing CTLL-2 cell proliferation as compared to IL-2

To evaluate the IL-2 activity of 2t2, 2t2 was compared with recombinant IL-2 for promoting proliferation of CTLL-2 cells that express IL-2Rα, IL-2Rβ and common γ chain. IL-2 dependent CTLL-2 cells were washed 5 times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serial dilutions of 2t2 or IL-2 were added to the cells (Figure 146). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µl of WST1 to each well in the day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan

dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 146, 2t2 promoted CTLL-2 cell proliferation 4-5-fold stronger than IL-2. The EC_{50} of 2t2 was 123.2 pM and IL-2 was 548.2 pM.

2t2 suppressed the increase of the high fat-induced hyperglycemia in ApoE$^{-/-}$ mice

Six-week-old female ApoE$^{-/-}$ mice (Jackson Lab) were fed with standard chow diet or high diet fat containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Harlan Laboratories) and maintained in the standard conditions. At week 7, mice fed with high fat diet were randomly assigned into the control group and treatment group. Mice then received either 2t2 (treatment group) or PBS (chow diet group and control group) per subcutaneous injection at a dosage of 3 mg/kg. Three days post dosing, the mice were fasted overnight, and blood samples were collected through retro-orbital venous plexus puncture. Overnight fasting glucose levels were measured using a OneTouch Glucometer. As shown in Figure 147, the results showed that 2t2 injection effectively suppresses the increase of glucose levels in ApoE$^{-/-}$ mice.

2t2 significantly upregulate the ratio of CD4^{+}CD25^{+}FoxP3^{+} T regulatory (Treg) cells in blood lymphocytes

Six-week-old female ApoE$^{-/-}$ mice (Jackson Lab) were fed with standard chow diet or high diet fat containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Harlan Laboratories) and maintained in the standard conditions. At week 7, mice fed with the high fat diet were randomly assigned into control group and treatment group. Mice then received either 2t2 (treatment group) or PBS (chow diet group and control group) per subcutaneous injection at a dosage of 3mg/kg. Three days after the dosing, overnight fasting blood samples were collected through retro-orbital venous plexus puncture and incubated with ACK lysing buffer (Thermo Fisher Scientific) at 37°C for 5 minutes. Samples were then resuspended in FACS buffer (1 X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and surface stained with FITC-anti-CD4 and APC-anti-CD25 antibodies

(BioLegend) for 30 minutes. Surface-stained samples were further fixed and premetallized with Fix/Perm buffer (BioLegend) and intracellular stained with PE-anti-Foxp3 antibody (BioLegend). After staining, cells were washed twice with FACs buffer followed by centrifugation at 1500 RPM for 5 minutes at room temperature. The cells were analyzed by flow cytometry (Celesta-BD Bioscience). As shown in Figure 148, 2t2 treatment significantly increased Treg populations in blood lymphocytes (3.5%±0.32) compared to the untreated groups (0.4%±0.16 for chow diet group and 0.46%±0.09 for high fat diet group).

Purification elution chromatograph of 2t2 from anti-TF antibody affinity column

2t2 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid, pH 2.9. A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS using Amicon centrifugal filters with a 30 kDa molecular weight cutoff. As shown in Figure 149, the anti-TF antibody affinity column bound to 2t2 which contains TF as a fusion domain. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine, pH 2.5. The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Analytical size exclusion chromatography (SEC) analysis of 2t2

To analyze 2t2 using analytical size exclusion chromatography (SEC), a Superdex 200 Increase 10/300 GL gel filtration column (from GE Healthcare) was connected to an AKTA Avant system (from GE Healthcare). The column was equilibrated with 2 column volumes of PBS. The flow rate was 0.7 mL/min. A sample containing 2t2 in PBS was

injected into the Superdex 200 column using a capillary loop, and analyzed by SEC. The SEC chromatograph of the sample is shown in Figure 150. The SEC results indicated two protein peaks for 2t2.

Reduced SDS-PAGE of 2t2

To determine the purity and molecular weight of the protein, 2t2 protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the 2t2 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs according to the manufacturer's instructions. Figures 151A and 151B show the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results show that the 2t2 protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample ran with expected molecular weights (56 kDa) in reduced SDS gel. Lane M was loaded with 10 μL of SeeBlue Plus2 Prestained Standard.

In vivo characterization of 2t2

2t2 was subcutaneously injected into C57BL/6 mice at various doses to determine the immunostimulatory activity of 2t2 in vivo. Mice were subcutaneously treated with control solution (PBS) or 2t2 at 0.1, 0.4, 2 and 10 mg/kg. The treated mice were euthanized day 3 post treatment. The mouse spleens were collected and weighed day 3 post treatment. Single splenocyte suspensions were prepared, and the prepared splenocytes were stained for $CD4^+$ T cells, $CD8^+$ T cells and NK cells (with fluorochrome-conjugated anti-CD4, -CD8, and –NK1.1 antibodies), and analyzed by flow cytometry. The results showed that 2t2 was effective at expanding splenocytes based on spleen weight (Figure 152A) especially at 0.1-10 mg/kg. The percentage of $CD8^+$ T cells were higher compared to control-treated mice (Figure 152B) at 2 and 10

mg/kg. The percentage of NK cells were higher compared to control-treated mice (Figure 152B) at all doses tested.

It has been known that IL-2 upregulates CD25 expression by immunocytes. We therefore accessed CD25 expression of $CD4^+$ T cells, $CD8^+$ T cells and NK cells in the 2t2 treated mice. C57BL/6 mice were subcutaneously treated with 2t2 as described in the paragraph above. The splenocytes were stained with fluorochrome-conjugated anti-CD4, -CD8, CD25 and NK1.1 monoclonal antibodies. The CD25 expression (MFI) of splenocyte subsets was analyzed by flow cytometry. As shown in Figure 153, at the doses and time points tested, 2t2 significantly upregulated CD25 expression by $CD4^+$ T cells but not CD8+ T cells or NK cells.

The pharmacokinetics of 2t2 in C57BL/6 mice were also investigated. 2t2 was subcutaneously injected into C57BL/6 mice at 1 mg/kg. The mouse blood was drawn from tail vein at various time points as shown in Figure 154 and the serum was prepared. 2t2 concentrations were determined with ELISA (Capture: anti-tissue factor antibody; Detection: biotinylated anti-human IL-2 antibody followed by SA-HRP and ABTS substrate). The half-life of 2t2 was 1.83 hours calculated with PK Solutions 2.0 (Summit Research Services).

2t2 attenuated the formation of high fat-induced atherosclerotic plaques in $ApoE^{-/-}$ mice

Six-week-old female $ApoE^{-/-}$ mice (The Jackson Laboratory) were fed with standard chow diet or high diet fat (21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch) (TD88137, Harlan Laboratories) and maintained in the standard conditions. At week 7, mice fed with high fat diet (HFD) were randomly assigned into control group and treatment group. Mice were then administrated either 2t2 (treatment group) or PBS (chow diet group and control group) subcutaneously at a dosage of 3mg/kg weekly for 4 weeks. At week 12, all mice were euthanized by isoflurane. Aortas were collected, opened longitudinally and stained with Sudan IV solution (0.5%) using *en face* method. The percentage of plaque area (red color as shown in Figure 155A) relative to total aorta area was then quantified with Image J software. Figure 155A shows a representative view of atherosclerotic plaques from each group. Figure 155B shows the

results of quantitative analysis of atherosclerotic plaques of each group. The percentage of plaque areas in control group (HF Diet) was much higher than the treatment group (HFD+2t2), being 10.28% vs 4.68 %.

2t2 suppresses the progression of type 2 diabetes.

Male BKS.Cg-Dock7^{m} +/+ Lepr^{db}/J (db/db (Jackson Lab)) mice were fed with standard chow diet and received drinking water ad libitum. At the age of six weeks, mice were randomly assigned into control group and treatment group. The treatment group received 2t2 by subcutaneous injection at 3 mg/kg bi-weekly, while control group received vehicle (PBS) only. Overnight fasting glucose levels were measure weekly using a OneTouch Glucometer. The results showed that 2t2 effectively suppressed the increase of glucose levels in BKS.Cg-Dock7^{m} +/+ Lepr^{db}/J mice (Figure 156).

2t2 significantly upregulates the ratio of CD4^{+}CD25^{+}FoxP3^{+} T regulatory cells in blood lymphocytes after the first injection

Male BKS.Cg-Dock7^{m} +/+ Lepr^{db}/J (db/db) (The Jackson Laboratory) mice were fed with standard chow diet and received drinking water ad libitum. At the age of six weeks, mice were randomly assigned into control group and treatment group. The treatment group received 2t2 by subcutaneous injection at 3 mg/kg bi-weekly, while the control group received vehicle (PBS) only. Four days after the first drug injection, overnight fasting blood samples were collected and incubated with ACK lysing buffer (Thermo Fisher Scientific) at 37°C for 5 minutes. Samples were then resuspended in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) and surface stained with FITC-anti-CD4 and APC-anti-CD25 antibodies (BioLegend) for 30 minutes. Surface-stained samples were further fixed and premetallized with Fix/Perm buffer (BioLegend) and intracellular stained with PE-anti-Foxp3 antibody (BioLegend). After staining, cells were washed twice with FACs buffer and were analyzed by flow cytometry (Celesta-BD Bioscience). The percentage of CD4^{+}CD25^{+}FoxP3^{+} Tregs in blood lymphocytes were measured. As shown in Figure

157, the results showed that 2t2 significantly upregulated the ratio of Tregs in blood lymphocytes (* $p<0.05$).

Example 62. Production and characterization of the Exemplary Single-Chain Chimeric Polypeptide 15t15

A second exemplary single-chain chimeric polypeptide including a first target-binding domain that binds to an IL-15 receptor, a soluble human tissue factor domain, and a second target-binding domain that binds to an IL-15 receptor was generated (IL-15/TF/IL-15; referred to at 15t15) (Figure 158). The nucleic acid and amino acid sequences of this single-chain chimeric polypeptide are shown below.

Nucleic Acid Encoding Exemplary Single-Chain Chimeric Polypeptide (IL-15/TF/IL-15) (SEQ ID NO: 170)

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCT
ACTCC

(First IL-15 fragment)

AACTGGGTGAACGTGATCAGCGATTTAAAGAAGATCGAGGATTTAATC
CAGAGCATGCACATCGACGCCACTCTGTACACTGAGAGCGACGTGCACCCTA
GCTGCAAGGTGACTGCCATGAAGTGCTTTTTACTGGAGCTGCAAGTTATCTCT
TTAGAGAGCGGCGATGCCAGCATCCACGACACTGTGGAGAATTTAATCATTT
TAGCCAACAACTCTTTAAGCAGCAACGGCAACGTGACAGAGAGCGGCTGCA
AGGAGTGCGAGGAGCTGGAGGAGAAGAACATCAAGGAGTTTTTACAGAGCT
TCGTGCACATCGTGCAGATGTTCATCAACACTAGC

(Human tissue factor 219 form)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAG
CACCAACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTT
TACACCGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCT
ATACCACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAA
ACAGACCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGC

ACTGGTTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTA
CCTCGAGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGC
ACAAAGGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAAC
AACACCTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACT
GTATTACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACA
AACGAGTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGC
AAGCTGTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGT
TGAGTGCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Second IL-15 fragment)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATT
CAGTCCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTC
TTGTAAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTT
TAGAGAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTT
AGCCAATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAG
GAGTGCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTG
TGCACATTGTCCAGATGTTCATCAATACCTCC

Exemplary Single-Chain Chimeric Polypeptide (IL-15/TF/IL-15) (SEQ ID NO: 169)

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS
LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ
MFINTS

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKC
FYTTDTECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYL
ETNLGQPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYW
KSSSSGKKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQE
KGEFRE

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVIS LESGDASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQ MFINTS

The nucleic acid encoding IL-15/TF/IL-15 was cloned into a modified retrovirus expression vector as described previously (Hughes et al., *Hum Gene Ther* 16:457–72, 2005). The expression vector encoding IL-15/TF/IL-15 was transfected into CHO-K1 cells. Expression of the expression vector in CHO-K1 cells allowed for secretion of the soluble IL-15/TF/IL-15 single-chain chimeric polypeptide (referred to as 15t15), which can be purified by anti-TF antibody affinity and other chromatography methods.

15t15 promotes IL-2Rβ and common γ chain containing 32Dβ cell proliferation

IL-15 activity of 15t15 was compared with recombinant IL-15 in IL2Rβ and common γ chain expressed 32Dβ cells. IL-15 dependent 32Dβ cells were washed five times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serial dilutions of 15t15 or IL-15 were added to the cells (Figure 159). Cells were incubated in a CO_2 incubator at 37°C for 3 days. Cell proliferation was detected by adding 10 µl of WST1 to each well in the day 3 and incubating for an additional 3 hours in a CO_2 incubator at 37°C. The amount of formazan dye produced was analyzed by measuring the absorbance at 450 nm. As shown in Figure 159, 15t15 promoted 32Dβ cell proliferation less efficiently as compared to IL-15. The EC_{50} of 15t15 and IL-15 was 161.4 pM and 1.6 pM, respectively.

Purification elution chromatograph of 15t15 from anti-TF antibody affinity column

15t15 harvested from cell culture was loaded onto the anti-TF antibody affinity column equilibrated with 5 column volumes of PBS. After sample loading, the column was washed with 5 column volumes of PBS, followed by elution with 6 column volumes of 0.1M acetic acid, pH 2.9. A280 elution peak was collected and then neutralized to pH 7.5-8.0 with 1M Tris base. The neutralized sample was then buffer exchanged into PBS

using Amicon centrifugal filters with a 30 kDa molecular weight cutoff. As shown in Figure 160, the anti-TF antibody affinity column bound to 15t15 which contains TF as a fusion domain. The buffer-exchanged protein sample was stored at 2-8 °C for further biochemical analyses and biological activity tests. After each elution, the anti-TF antibody affinity column was stripped using 6 column volumes of 0.1M glycine, pH 2.5. The column was then neutralized using 5 column volumes of PBS, and 7 column volumes of 20% ethanol for storage. The anti-TF antibody affinity column was connected to a GE Healthcare AKTA Avant system. The flow rate was 4 mL/min for all steps except for the elution step, which was 2 mL/min.

Reduced SDS-PAGE of 15t15

To determine the purity and molecular weight of the protein, 15t15 protein sample purified with anti-TF antibody affinity column was analyzed by sodium dodecyl sulfate polyacrylamide gel (4-12% NuPage Bis-Tris gel) electrophoresis (SDS-PAGE) method under reduced condition. After electrophoresis, the gel was stained with InstantBlue for about 30 min, followed by destaining overnight in purified water.

To verify that the 15t15 protein undergoes glycosylation after translation in CHO cells, a deglycosylation experiment was conducted using the Protein Deglycosylation Mix II kit from New England Biolabs and the manufacturer's instructions. Figures 161A and 161B show the reduced SDS-PAGE analysis of the sample in non-deglycosylated (lane 1 in red outline) and deglycosylated (lane 2 in yellow outline) state. The results showed that the 15t15 protein is glycosylated when expressed in CHO cells. After deglycosylation, the purified sample ran with expected molecular weights (50 kDa) in reduced SDS gel. Lane M was loaded with 10 µL of SeeBlue Plus2 Prestained Standard.

Example 63: Stimulation of NK cells *in vitro*

A set of experiments was performed to assess the changes in surface phenotype of NK cells after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s + anti-TF antibody. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell

Technologies). The purity of NK cells was >90% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 antibodies (BioLegend). The cells were counted and resuspended at 0.2 x 10^6/mL in a 96-well flat-bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with: 18t15-12s (100 nM); 18t15-12s16 (100 nM); a mixture of single cytokines rhIL-15 (50 ng/mL) (Miltenyi), rhIL18 (50 ng/mL) (Invivogen), and rhIL-12 (10 ng/mL) (Peprotech); 7t15-21s + anti-TF antibody (100 nM-50 nM); 7t15-21s (100 nM); or anti-TF antibody (50nM) at 37 °C and 5% CO_2 for 16 hours. The next day, the cells were harvested and surface stained for 30 minutes with CD56, CD16, CD25, CD69, CD27, CD62L, NKp30, and NKp44 specific antibodies. After surface staining, the cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 162A and 162B shows that overnight incubation of purified NK cells with 18t15-12s, 18t15-12s16, and 7t15-21s + anti-TF antibody resulted in an increase in the percentage of cells expressing CD25, CD69, NKp44, and NKp30 activation markers and a decrease in the percentage of cells expressing CD62L. All activation marker data is from $CD56^+$ gated lymphocytes.

A set of experiments was performed to assess changes in the surface phenotype of lymphocyte populations after stimulation with 18t15-12s, 18t15-12s16, and 7t15-21s. In these experiments, fresh human leukocytes were obtained from the blood bank. Peripheral blood lymphocytes were isolated with the Ficoll-PAQUE Plus (GE Healthcare) density gradient media. The cells were counted and resuspended at 0.2 x 10^6/mL in a 96-well flat-bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with: 18t15-12s (100 nM); 18t15-12s16 (100 nM), a mixture of single cytokines rhIL-15 (50 ng/mL) (Miltenyi), rhIL18 (50 ng/mL) (Invivogen), and rhIL-12 (10 ng/mL) (Peprotech); 7t15-21s (100 nM) + anti-TF antibody (50 nM); 7t15-

21s (100 nM); or anti-TF antibody (50 nM) at 37 °C and 5% CO_2 for 16 hours. The next day, the cells were harvested and surface stained for 30 minutes for CD4 or CD8, CD62L, and CD69 specific antibodies. After surface staining, cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 163 shows that overnight incubation of purified lymphocyte populations (CD4 and CD8 T cells) with 18t15-12s, 18t15-12s16, or 7t15-21s + anti-TF antibody resulted in an increase in the percentage of CD8 and CD4 T cells expressing CD69. Additionally, incubation with 7t15-21s + anti-TF antibody resulted in an increase in the percentage of CD8 and CD4 T cells expressing CD62L (Figure 163).

A set of experiments was performed to determine the effect of 18t15-12s on the extracellular acidification rate (ECAR) of NK cells purified from human blood. ECAR can be used to measure glycolysis. Glycolysis is the intracellular biochemical conversion of one molecule of glucose into two molecules of pyruvate with the concurrent generation of two molecules of ATP. An increase in glycolysis was indicated by an increase in ECAR measured by a Seahorse XF96 Analyzer. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining for CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 antibodies (BioLegend). The cells were counted and resuspended in 0.2 x 10^6/mL in a 96-well flat-bottom plate in 0.2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with either a mixture of single cytokines hIL-12 (10 ng/mL) (Biolegend), hIL-18 (50 ng/mL) (R&D), and hIL-15 (50 ng/mL) (NCI) or 18t15-12s (100 nM) at 37 °C and 5% CO_2 for 14-18 hours. The next day, the cells were harvested and washed two times in Seahorse media. The cells (2×10^5 cells/well) were seeded in 96-well flux plates that were coated with 10 µL of poly-L-lysine (Sigma). NK cells were adhered to plates for 30 minutes prior to the assay.

Glucose, oligomycin, and 2DG solutions were prepared at 10x concentration in buffered Seahorse medium and injected in port A, B, and C of the calibration plate. ECAR readings were taken every 6.5–7 minutes and ECAR results represent the average readings over 80 minutes or average readings at each timepoint. Figure 164 shows overnight stimulation of NK cells with 18t15-12s resulted in increased basal ECAR levels. The addition of glucose and oligomycin further showed enhanced glycolysis and glycolytic capacity, respectively, of NK cells stimulated with 18t15-12s overnight (Figure 164). NK cells treated overnight with media alone or a mixture of IL12, IL18, and IL-15 were used for comparison (Figure 164).

A set of experiments was performed to determine the increase in phospho-STAT4 and phospho-STAT5 levels in NK cells after stimulation with 18t15-12s. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 specific antibodies (BioLegend). The cells were counted and resuspended in 0.05 x 10^6/mL in a 96-well flat-bottom plate in 0.1 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were stimulated with hIL-12 (10 ng/mL) (Biolegend) or hIL-15 (50 ng/mL) (NCI) (Single cytokines), or 18t15-12s (100 nM) at 37 °C and 5% CO_2 for 90 minutes. Unstimulated NK cells (US) were used as a control. The cells were harvested and fixed in paraformaldehyde (Sigma) to a final concentration of 1.6%. Plates were incubated in the dark at room temperature for 10 minutes. FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)) (100 µL) was added and cells were transferred to 96-well "V" bottom plate. The cells were washed for 1500 RPM for 5 minutes at room temperature. The cell pellet was mixed with 100 µL chilled methanol by gently pipetting up and down, and cells were incubated for 30 minutes at 4 °C. The cells were mixed with 100 mL of FACS buffer and washed for 1500 RPM for 5 minutes at room temperature. The cell pellets were mixed with 50 mL of FACS buffer containing 4 mL of pSTAT4 (BD

Bioscience) and pSTAT5 antibodies (BD Bioscience) followed by incubation for 30 minutes at room temperature in the dark. The cells were mixed with 100 mL of FACS buffer and washed for 1500 RPM for 5 minutes at room temperature. The cell pellets were mixed with 50 mL of FACS buffer and cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 165 shows that incubation of NK cells with 18t15-12s induced an increase in pSTAT4 and pSTAT5 (plotted data, normalized fold-change).

A set of experiments was performed to determine the effect of 18t15-12s or a mixture of cytokines (e.g., IL12, IL18, and IL-15) on oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) on NK cells purified from human blood. OCR and ECAR were measured by a Seahorse XF96 Analyzer. In these experiments, fresh human NK cells were isolated from human leukocytes via negative selection using the RosetteSep/human NK cell reagent (StemCell Technologies). Freshly purified NK cells were stimulated overnight (16 h) with either 18t15-12s (100nM) or a mixture of rhIL12 (10 ng/mL), rhIL18 (50 ng/mL), and rhIL-15 (50 ng/mL) cytokines as a control. The next day, the cells were washed, counted, and equal numbers of cells were plated in buffered Seahorse media. Glucose, oligomycin, and 2DG solutions were prepared at 10x concentration in buffered Seahorse medium and injected in port A, B, and C of the calibration plate. Figure 166 shows OCR (left) and ECAR (right) data from two individual donors. Overnight stimulation of NK cells with 18t15-12s resulted in an increase in basal ECAR and OCR levels. Addition of glucose and oligomycin further showed enhanced glycolysis and glycolytic capacity, respectively, of NK cells stimulated with 18t15-12s overnight. NK cells treated overnight with media alone or a mixture of IL12, IL18, and IL-15 were used for comparison.

Example 64: Stimulation of NK cells *in vivo* by 2t2 and/or TGFRt15-TGFRs

A set of experiments was performed to determine the effect of the 2t2 construct on immune stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or 2t2 at 0.1, 0.4, 2, and 10 mg/kg. Treated mice were euthanized 3 days post-treatment. Spleen weight was measured and single splenocyte suspensions were prepared. Splenocytes suspensions were stained with

conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells, and CD25 expression on lymphocyte subsets were analyzed by flow cytometry. Figure 167A shows that 2t2 was effective at expanding splenocytes based on spleen weight especially at a dose level of 0.1-10 mg/kg. Following treatment, the percentage of $CD8^+$ T cells were higher in 2t2-treated mice compared to control-treated mice at 2 and 10 mg/kg (Figure 167B). The percentage of NK cells were also higher in 2t2-treated mice compared to control-treated mice at all doses of 2t2 tested (Figure 167B). Additionally, 2t2 significantly upregulated CD25 expression by $CD4^+$ T cells, but not $CD8^+$ T cells and NK cells following treatment at 0.4 to10 mg/kg (Figure 167C).

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or TGFRt15-TGFRs at 0.3, 1, 3, and 10 mg/kg. The treated mice were euthanized 4 days post-treatment. Spleen weight was measured and single splenocyte suspensions were prepared. The splenocytes suspensions were stained with conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells were analyzed by flow cytometry. Figure 168A shows that spleen weight in mice treated with TGFRt15-TGFRs increased with increasing dosage of TGFRt15-TGFRs. Additionally, spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs were higher as compared to mice treated with the control solution. Figure 168B shows that the percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of TGFRt15-TGFRs. Specifically, the percentages of $CD8^+$ T cells were higher in mice treated with 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice, and the percentages of NK cells were higher in mice treated with 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs compared to control-treated mice.

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct or 2t2 construct on immune stimulation in $ApoE^{-/-}$ mice fed with a Western diet. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice

(Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs or 2t2 at 3 mg/kg. Three days post treatment, mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 μL 0.5 M EDTA, and 20 μL blood was taken for lymphocyte subsets analysis. The red blood cells were lysed with ACK (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and analyzed with a BD FACS Celesta. For Treg staining, ACK treated blood lymphocytes were stained with anti-mouse CD4 and anti-mouse CD25 antibodies for 30 minutes at 4 °C in FACS staining buffer. The cells were washed once and resuspended in fixation/permeabilization working solution and incubated at room temperature for 60 minutes. The cells were washed once and resuspended in permeabilization buffer. The samples were centrifuged at 300-400 x g for 5 minutes at room temperature and the supernatant was then discarded. The cell pellet was resuspended in residual volume and the volume adjusted to about 100 μL with 1 x permeabilization buffer. Anti-Foxp3 antibody was added to the cells, and the cells were incubated for 30 minutes at room temperature. Permeabilization buffer (200 μL) was added to the cells, and the cells were centrifuged at 300-400 x g for 5 minutes at room temperature. The cells were resuspended in flow cytometry staining buffer and analyzed on a flow cytometer. Figures 169B-169C show that treatment with TGFRt15-TGFRs and 2t2 increased the percentage of NK cells and $CD8^+$ T cells in $ApoE^{-/-}$ mice fed with Western diet. Figure 169A shows that treatment with 2t2 also increased the percentage of Treg cells.

Example 65: Induction of proliferation of immune cells *in vivo*

A set of experiments was performed to determine the effect of the 2t2 construct on immune cell stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or 2t2 at 0.1, 0.4, 2, and 10 mg/kg.

Treated mice were euthanized 3 days post-treatment. Spleen weight was measured and single splenocyte suspensions were prepared. The splenocyte suspensions were stained with conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The percentage of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells were analyzed by flow cytometry. Figure 170A shows that 2t2 treatment was effective at expanding splenocytes based on spleen weight especially at 0.1-10 mg/kg. The percentage of $CD8^+$ T cells was higher compared to control-treated mice at 2 and 10 mg/kg (Figure 170B). Additionally, the percentage of NK cells was higher compared to control-treated mice at all doses of 2t2 tested (Figure 170B). These results demonstrate that 2t2 treatment was able to induce proliferation of $CD8^+$ T cells and NK cells in C57BL/6 mice.

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct on immune stimulation in C57BL/6 mice. In these experiments, C57BL/6 mice were subcutaneously treated with control solution (PBS) or TGFRt15-TGFRs at 0.1, 0.3, 1, 3, and 10 mg/kg. The treated mice were euthanized 4 days post-treatment. Spleen weight was measured and splenocyte suspensions were prepared. The splenocyte suspensions were stained with conjugated anti-CD4, anti-CD8, and anti-NK1.1 (NK) antibodies. The cells were additionally stained for proliferation marker Ki67. Figure 171A shows that spleen weight in mice treated with TGFRt15-TGFRs increased with increasing dosage of TGFRt15-TGFRs. Additionally, spleen weight in mice treated with 1 mg/kg, 3 mg/kg, and 10 mg/kg of TGFRt15-TGFRs was higher as compared to mice treated with just the control solution. The percentages of $CD8^+$ T cells and NK cells both increased with increasing dosage of TGFRt15-TGFRs (Figure 171B). Finally, TGFRt15-TGFRs significantly upregulated expression of cell proliferation marker Ki67 in both $CD8^+$ T cells and NK cells at all doses of TGFRt15-TGFRs tested. These results demonstrate that TGFRt15-TGFRs treatment induced proliferation of both $CD8^+$ T cells and NK cells in C57BL/6 mice.

A set of experiments was performed to determine the effect of the TGFRt15-TGFRs construct or the 2t2 construct on immune stimulation in $ApoE^{-/-}$ mice fed with a Western diet. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15%

cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-week of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs or 2t2 at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. The blood was mixed with 10 μL 0.5 M EDTA and 20 μL blood was taken for lymphocyte subsets analysis. The red blood cells were lysed with ACK (0.15 M NH_4Cl, 1.0 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4) and the lymphocytes were stained with anti-mouse CD8a and anti-mouse NK1.1 antibodies for 30 minutes at 4 °C in FACS staining buffer (1% BSA in PBS). The cells were washed once and resuspended in Fixation Buffer (BioLegend Cat# 420801) for 20 minutes at room temperature. The cells were centrifuged at 350 x g for 5 minutes, the fixed cells were resuspended in Intracellular Staining Permeabilization Wash Buffer (BioLegend Cat# 421002) and then centrifuged at 350 x g for 5 minutes. The cells were then stained with anti-Ki67 antibody for 20 minutes at RT. The cells were washed twice with Intracellular Staining Permeabilization Wash Buffer and centrifuged at 350 x g for 5 minutes. The cells were then resuspended in FACS staining buffer. Lymphocyte subsets were analyzed with a BD FACS Celesta. As described in Figure 172A, treatment of $ApoE^{-/-}$ mice with TGFRt15-TGFRs induced proliferation (Ki67-positive staining) in NK and $CD8^+$ T cells. Additionally, Figure 172B shows treatment of $ApoE^{-/-}$ mice with 2t2 also induced proliferation (Ki67-positive staining) in NK and $CD8^+$ T cells.

A set of experiments was performed to determine the effect 7t15-21s + anti-TF antibody-expanded NK cells in NSG mice following treatment with 7t15-21s, TGFRt15-TGFRs, and 2t2. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >90% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 antibodies (BioLegend). The cells were counted and resuspended in 2 x 10^6/mL in a 24-well flat-bottom plate in 2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were

stimulated with: 7t15-21s (100 nM) and anti-TF antibody (50 nM) for 15 days. After every 2 days, the cells were resuspended at 2 x 10^6/mL with fresh media containing 100 nM 7t15-21s and 50 nM of anti-TF antibody. As the volume of the cultures increased, the cells were transferred to higher volume flasks. The cells were counted using trypan blue to access the fold-expansion. 7t15-21s + anti-TF antibody-expanded NK cells were washed three times in warm HBSS Buffer (Hyclone) at 1000 RPM for 10 minutes at room temperature. The 7t15-21s + anti-TF antibody-expanded-NK cells were resuspended in 10 x 10^6/0.2 mL HBSS buffer and injected intravenously into the tail vein of *NSG mice* (NOD scid gamma mouse) (Jackson Laboratories). The transferred NK cells were supported every 48 hours with either 7t15-21s (10 ng/dose, i.p.), TGFRt15-TGFRs (10 ng/dose, i.p.) or 2t2 (10 ng/dose, i.p.) for up to 21 days. Engraftment and persistence of the human 7t15-21s + anti-TF antibody-expanded NK cells were measured every week in blood staining for hCD45, mCD45, hCD56, hCD3, and hCD16 antibodies by flow cytometry (Celesta-BD Bioscience) (Data represent 3 mice per group). Figure 173 indicates that treatment of mice bearing adoptively-transferred 7t15-21s + anti-TF antibody-expanded NK cells with 7t15-21s-, TGFRt15-TGFRs-, or 2t2-induced expansion and persistence of the adoptively transferred NK cells compared to control treated mice.

Example 66: NK-mediated cytotoxicity following treatment with single-chain constructs or multi-chain constructs

A set of experiments was performed to determine if treatment of NK cells with TGFRt15-TGFRs enhanced cytotoxicity of NK cells. In these experiments, Human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post TGFRt15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Human Daudi B lymphoma cells) were mixed with effector cells (either mouse NK

effector cells or human NK effector cells) in the presence of 50 nM TGFRt15-TGFRs or in the absence of TGFRt15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. Target cell (Daudi) viability was assessed by analysis of propidium iodide-positive, CTV-labeled cells using flow cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 174 shows that mouse (Figure 174A) and human (Figure 174B) NK cells had significantly stronger cytotoxicity against Daudi B cells following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

A set of experiments was performed to determine antibody-dependent cellular cytotoxicity (ADCC) of mouse and human NK cells following treatment with TGFRt15-TGFRs. In these experiments, human Daudi B lymphoma cells were labeled with CellTrace Violet (CTV) and used as tumor target cells. Mouse NK effector cells were isolated with NK1.1-positive selection using a magnetic cell sorting method (Miltenyi Biotec) of C57BL/6 female mouse spleens 4 days post-TGFRt15-TGFRs subcutaneous treatment at 3 mg/kg. Human NK effector cells were isolated from peripheral blood mononuclear cells derived from human blood buffy coats with the RosetteSep/human NK cell reagent (Stemcell Technologies). The target cells (Daudi B cells) were mixed with effector cells (either mouse NK effector cells or human NK effector cells) in the presence of anti-CD20 antibody (10 nM Rituximab, Genentech) and in the presence of 50 nM TGFRt15-TGFRs, or in the absence of TGFRt15-TGFRs (control) and incubated at 37 °C for 44 hours for mouse NK cells and for 20 hours for human NK cells. The Daudi B cells express the CD20 targets for the anti-CD20 antibody. Target cell viability was assessed after incubation by analysis of propidium iodide-positive, CTV-labeled target cells using flow cytometry. The percentage of Daudi inhibition was calculated using the formula (1-viable tumor cell number in experimental sample/viable tumor cell number in the sample without NK cells) x 100. Figure 175 shows that mouse NK cells (Figure 175A) and human NK cells (Figure 175B) had stronger ADCC activity against Daudi B cells

following NK cell activation with TGFRt15-TGFRs than in the absence of TGFRt15-TGFRs activation.

A set of experiments was performed to determine cytotoxicity of TGFRt15-TGFRs-activated mouse NK cells towards senescent B16F10 melanoma cells. In these experiments, mouse NK cells were activated *in vivo* by injecting C57BL/6 mice with 10 mg/kg of TGFRt15-TGFRs for 4 days followed by isolation of splenic NK cells. The NK cells were then expanded *in vitro* for 7 days in the presence of 100 nM 2t2. The B16F10 senescent target cells (B16F10-SNC) were labelled with CellTrace Violet (CTV) and incubated at different Effector:Target (E:T) ratios with the activated mouse NK effector cells for 16 hours. The cells were trypsinized, washed, and resuspended in complete media containing propidium iodide (PI) solution. The cytotoxicity of the TGFRt15-TGFRs/2t2-activated NK cells against the senescent cell targets was accessed by flow cytometry based on PI staining of the CTV-labeled cells. The findings demonstrate that *in vivo* activation of NK cells with TGFRt15-TGFRs followed by *in vitro* expansion and activation with 2t2 resulted in increased killing of senescent melanoma tumor cells by the NK cells (Figure 176).

Example 67: Treatment of Cancer, Diabetes, and Atherosclerosis

A set of experiments was performed to assess antitumor activity of TGFRt15-TGFRs plus anti-TRP1 antibody (TA99) in combination with chemotherapy in a melanoma mouse model. In these experiments, C57BL/6 mice were subcutaneously injected with 0.5×10^6 B16F10 melanoma cells. The mice were treated with three doses of chemotherapy docetaxel (10 mg/kg) (DTX) on day 1, day 4, and day 7, followed by treatment with single dose of combination immunotherapy TGFRt15-TGFRs (3 mg/kg) + anti-TRP1 antibody TA99 (200 μg) on day 9. Figure 177A shows a schematic of the treatement regimen. Tumor growth was monitored by caliper measurement, and tumor volume was calculated using the formula $V = (L \times W^2)/2$, where L is the largest tumor diameter and W is the perpendicular tumor diameter. Figure 177B shows that treatment with DTX + TGFRt15-TGFRs + TA99 significantly reduced tumor growth compared to saline control and DTX treatment groups (N=10, ****p <0.001, Multiple t test analyses).

To assess immune cell subsets in the B16F10 tumor model, peripheral blood analysis was performed. In these experiments, C57BL/6 mice were injected with B16F10 cells and treated with DTX, DTX + TGFRt15-TGFRs + TA99, or saline. Blood was drawn from the submandibular vein of B16F10 tumor-bearing mice on days 2, 5, and 8 post-immunotherapy for the DTX + TGFRt15-TGFRs + TA99 group and day 11 post-tumor injection for the DTX and saline groups. RBCs were lysed in ACK lysis buffer and the lymphocytes were washed and stained with anti-NK1.1, anti-CD8, and anti-CD4 antibodies. The cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figures 177C-177E show that DTX + TGFRt15-TGFRs + TA99 treatment induced an increase in the percentage of NK cells and $CD8^+$ T cells in the tumors compared to the saline and DTX treatment groups.

On day 17, total RNA was extracted from tumors of mice treated with saline, DTX or DTX + TGFRt15-TGFRs + TA99 using Trizol. Total RNA (1 μg) was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out with CFX96 Detection System (Bio-Rad) using FAM-labeled predesigned primers for senescence cell markers, (F) p21 (G) DPP4 and (H) IL6. The housekeeping gene 18S ribosomal RNA was used as an internal control to normalize the variability in expression levels. The expression of each target mRNA relative to *18S* rRNA was calculated based on Ct as $2^{-\Delta(\Delta Ct)}$, in which $\Delta Ct = Ct_{target} - Ct_{18S}$. The data is presented as fold-change as compared to saline control. Figure 177F-177H show that DTX treatment induced an increase in senescent tumor cells that were subsequently reduced following treatment with TGFRt15-TGFRs + TA99 immunotherapy.

A set of experiments was performed to investigate amelioration of Western diet-induced hyperglycemia in $ApoE^{-/-}$ mice by 2t2. In these experiments, 6-week old female B6.129P2-$ApoE^{tm1Unc}$/J mice (Jackson Laboratory) were fed with a Western diet containing 21% fat, 0.15% cholesterol, 34.1% sucrose, 19.5% casein, and 15% starch (TD88137, Envigo Laboratories). After 8-weeks of the Western diet, the mice were injected subcutaneously with TGFRt15-TGFRs or 2t2 at 3 mg/kg. Three days post-treatment, the mice were fasted for 16 hours and then blood samples were collected through retro-orbital venous plexus puncture. Blood glucose was detected with a glucose

meter (OneTouch UltraMini) and GenUltimated test strips using a drop of fresh blood. As shown in Figure 178A, 2t2 treatment significantly reduced hyperglycemia induced by the Western diet ($p<0.04$). The plasma insulin and resistin levels were analyzed with Mouse Rat Metabolic Array by Eve Technologies. HOMA-IR was calculated using the following formula: homeostatic model assessment-insulin resistance = Glucose (mg/dL) * Insulin (mU/mL)/405. As shown in Figure 178B, both 2t2 and TGFRt15-TGFRs treatment reduced insulin resistance compared to the untreated group. Both 2t2 ($p<0.02$) and TGFRt15-TGFRs ($p<0.05$) reduced resistin levels significantly compared to the untreated group as shown in Figure 178C, which may relate to the reduced insulin resistance induced by 2t2 and TGFRt15-TGFRs (Figure F3B).

Example 68: Induction of differentiation of NK cells into cytokine-induced memory like NK cells

A set of experiments was performed to assess the differentiation of NK cells into cytokine-induced memory like NK Cells (CIMK-NK Cells) after stimulation with 18t15-12s. In these experiments, fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >90% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, and CD69-APCFire750 antibodies (BioLegend). The cells were counted and resuspended in 2 x 10^6/mL in a 24-well flat-bottom plate in 2 mL of complete media (RPMI 1640 (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), penicillin (Thermo Life Technologies), streptomycin (Thermo Life Technologies), and 10% FBS (Hyclone)). The cells were unstimulated ("No Spike") or stimulated with 18t15-12s (100 nM) or a mixture of single cytokines including rhIL-15 (50 ng/mL) (Miltenyi), rhIL18 (50 ng/mL) (Invivogen), and rhIL-12 (10 ng/mL) (Peprotech) ("single cytokines") at 37 °C and 5% CO_2 for 16 hrs. The next day, the cells were harvested, and washed two times with warm complete media at 1000 RPM for 10 minutes at room temperature. The cells were resuspended at 2 x 10^6/mL in a 24-well flat-bottom plate in 2 mL of complete media with rhIL-15 (1 ng/mL). After every 2 days, half of the medium was replaced with fresh complete media containing rhIL-15.

To assess the change in memory phenotype of NK cells at day 7, the cells were stained with antibodies to cell-surface CD56, CD16, CD27, CD62L, NKp30, and NKp44 (BioLegend). After surface staining, the cells were washed (1500 RPM for 5 minutes at room temperature) in FACS buffer (1X PBS (Hyclone) with 0.5% BSA (EMD Millipore) and 0.001% sodium azide (Sigma)). After two washes, the cells were analyzed by flow cytometry (Celesta-BD Bioscience). Figure 179 shows that incubation of NK cells with 18t15-12s resulted in an increase in the percentage of $CD16^{+}CD56^{+}$ NK cells expressing CD27, CD62L, and NKp44, and an increase in the levels (MFI) of NKp30 in $CD16^{+}CD56^{+}$ NK cells.

Example 69. Upregulation of CD44 memory T cells

C57BL/6 mice were subcutaneously treated with TGFRt15-TGFRs or 2t2. The treated mice were euthanized and the single splenocyte suspensions were prepared 4 days (TGFRt15-TGFRs) or 3 days (2t2) following the treatment. The prepared splenocytes were stained with fluorochrome-conjugated anti-CD4, anti-CD8 and anti-CD44 antibodies and the percentages of $CD44^{high}$ T cells in $CD4^{+}$ T cells or $CD8^{+}$ T cells were analyzed by flow cytometry. The results show that TGFRt15-TGFRs and 2t2 upregulated expression of the memory marker CD44 on CD4+ and CD8+ T cells (Figures 180). These findings indicate that TGFRt15-TGFRs and 2t2 molecules were able to induce mouse T cells to differentiate into memory T cells.

Example 70: Improvement of the texture and/or appearance and/or hair

To examine the effect of 2t2 on hair regrowth, dorsal hair of C57BL6/J mice (Jackson Laboratory) was first shortened with clippers followed by application of depilatory cream (Nair) to the shaved region for a period of 30 seconds before wiping clean. After 4 hours, 2t2 (3 mg/kg, single dose), low dose recombinant IL-2 (25000 IU, 5 consecutive days, 1 dose/day), or PBS were administered subcutaneously. The mice were monitored for skin pigmentation related to hair regrowth and pictures were taken and analyzed using the Image J software. Figure 181A shows skin pigmentation 10 days after depilation in PBS-, 2t2-, or IL-2-treated mice. Figure 181B shows the percent

pigmentation in each group of mice 10 days post-treatment as analyzed using the Image J software. The results showed that treatment of mice with 2t2 or IL-2 promoted hair regrowth following depilation compared to PBS-treated mice.

Dorsal hair of C57BL6/J mice (Jackson Laboratory) was first shortened with clippers before applying depilatory cream (Nair) to the shaved region for a period of exactly 30 seconds before wiping clean. After 4 hours, 2t2 (3 mg/kg, single dose), low dose recombinant IL-2 (25000 IU, 5 consecutive days, 1 dose/day) or PBS were administered subcutaneously. The mice were monitored for skin pigmentation related to hair regrowth and pictures were taken and analyzed using Image J software. Figure 182 shows skin pigmentation 14 days after depilation in PBS-, 2t2-, or IL-2-treated mice. The results showed that treatment of mice with 2t2 or IL-2 promoted hair regrowth following depilation compared to the PBS-treated mice.

Example 71: Tissue factor coagulation assays following treatment with single-chain or multi-chain chimeric polypeptides

A set of experiments was performed to assess blood coagulation following treatment with single-chain or multi-chain chimeric polypeptides. To initiate the blood coagulation cascade pathway, tissue factor (TF) binds to Factor VIIa (FVIIa) to form a TF/FVIIa complex. The TF/FVIIa complex then binds Factor X (FX) and converts FX to FXa.

Factor VIIa (FVIIa) activity Assay

One assay to measure blood coagulation involves measuring Factor VIIa (FVIIa) activity. This type of assay requires the presence of tissue factor and calcium. The TF/FVIIa complex activity can be measured by a small substrate or by a natural protein substrate, for example, Factor X (FX). When FX is used as a substrate, phospholipids are also required for TF/FVIIa activity. In this assay, FVIIa activity is determined with FVIIa-specific chromogenic substrate S-2288 (Diapharma, West Chester, OH). The color change of the S-2288 substrate can be measured spectrophotometrically and is proportional to the proteolytic activity of FVIIa (e.g., the TF/FVIIa complex).

In these experiments, the FVIIa activity of the following groups were compared: the 219-amino acid extracellular domain of tissue factor domain (TF_{219}), a multi-chain chimeric polypeptide with a wild-type tissue factor domain, and a multi-chain chimeric polypeptide with a mutant tissue factor domain. The chimeric polypeptides containing mutant tissue factor molecules were constructed with mutations to the TF domain at amino acid sites: Lys20, Ile22, Asp58, Arg135, and Phe140.

In order to assess activity of FVIIa, FVIIa, and TF_{219} or a TF_{219} -containing multi-chain chimeric polypeptide were mixed at an equal molar concentration (10 nM) in all wells of a 96-well ELISA plate in a total volume of 70 µL. After incubation for 10 minutes at 37 °C, 10 µL of 8 mM S-2288 substrate was added to start the reaction. The incubation was then kept at 37 °C for 20 minutes. Finally, color change was monitored by reading absorbance at 405 nm. The OD values of different TF/VIIa complexes are shown in Table 1 and Table 2. Table 1 shows a comparison of TF_{219}, 21t15-21s wild-type (WT) and 21t15-21s mutant (Mut). Table 2 shows a comparison of TF_{219}, 21t15-TGFRs wild-type (WT), and 21t15-TGFRs mutant (Mut). These data show that TF_{219}-containing multi-chain chimeric polypeptides (e.g., 21t15-21s-WT, 21t15-21s-Mut, 21t15-TGFRS-WT, and 21t15-TGFRS-Mut) have lower FVIIa activity than TF_{219} when the chromogenic S-2288 was used as a substrate. Notably, the multi-chain chimeric polypeptides containing TF_{219} mutations showed much lower FVIIa activity when compared to multi-chain chimeric polypeptides containing wild type TF_{219}.

Table 1. FVIIa activity

Molecule	**OD value at 405 nm**
TF_{219}	0.307
21t15/21S-WT	0.136
21t15/21S-Mut	0.095

WT: wild type of TF_{219}, Mut: TF_{219} containing mutations.

Table 2. FVIIa activity

Molecule	OD value at 405 nm
TF_{219}	0.345
21t15/TGFRS-WT	0.227
21t15/TGFRS-Mut	0.100

WT: wild type of TF_{219}, Mut: TF_{219} containing mutations.

Factor X (FX) Activation Assay

An additional assay to measure blood coagulation involves measuring activation of Factor X (FX). Briefly, TF/VIIa activates blood coagulation Factor X (FX) to Factor Xa (FXa) in the presence of calcium and phospholipids. TF_{243}, which contains the transmembrane domain of TF, has much higher activity in activating FX to FXa than TF_{219}, which does not contain the transmembrane domain. TF/VIIa dependent activation of FX is determined by measuring FXa activity using an FXa-specific chromogenic substrate S-2765 (Diapharma, West Chester, OH). The color change of S-2765 can be monitored spectrophotometrically and is proportional to the proteolytic activity of FXa.

In these experiments, FX activation with a multi-chain chimeric polypeptide (18t15-12s, mouse (m)21t15, 21t15-TGFRs, and 21t15-7s) was compared with a positive control (Innovin) or TF_{219}. TF_{219} (or TF_{219}-containing multi-chain chimeric polypeptides)/FVIIa complexes were mixed at an equal molar concentration (0.1 nM each) in a volume of 50 µL in round bottom wells of a 96-well ELISA plate, after which 10 µL of 180 nM FX was added. After 15 minutes of incubation at 37 °C, during which time FX was converted to FXa, 8 µL of 0.5 M EDTA (which chelates calcium and thus terminates FX activation by TF/VIIa) was added to each well to stop FX activation. Next, 10 µL of 3.2 mM S-2765 substrate was added to the reaction mixture. Immediately, the plate absorbance was measured at 405 nm and was recorded as the absorbance at time 0. The plate was then incubated for 10-20 minutes at 37 °C. The color change was monitored by reading absorbance at 405 nm following the incubation. Results of FX activation as measured by FXa activity using chromogenic substrate S-2765 are shown in Figure 183. In this experiment, Innovin, which is a commercial

prothrombin reagent containing lipidated recombinant human TF_{243}, was used as a positive control for FX activation. Innovin was reconstituted with purified water to about 10 nM of TF_{243}. Next, 0.1 nM TF/VIIa complex was made by mixing an equal volume of 0.2nM of FVIIa with 0.2 nM of Innovin. Innovin demonstrated very potent FX activation activity, while TF_{219} and TF_{219}-containing multi-chain chimeric polypeptides had very low FX activation activity, confirming that TF_{219} is not active in a TF/FVIIa complex for activating natural substrate FX *in vivo*.

Prothrombin Time Test

A third assay to measure blood coagulation is the prothrombin time (PT) test, which measures blood clotting activity. Here, the PT test was performed using commercially available normal human plasma (Ci-Trol Coagulation Control, Level I). For a standard PT test, clot reactions were initiated by addition of Innovin, a lipidated recombinant human TF_{243}, in the presence of calcium. Clotting time was monitored and reported by STart PT analyzer (Diagnostica Stago, Parsippany, N.J.). PT assays were started by injecting 0.2 mL of various dilutions of Innovin diluted in PT assay buffer (50 mM Tris-HCl, pH 7.5, 14.6 mM $CaCl_2$, 0.1% BSA) into cuvettes containing 0.1 mL of normal human plasma prewarmed at 37 °C. In the PT assay, shorter PT time (clotting time) indicates a higher TF-dependent clotting activity while longer PT (clotting time) means lower TF-dependent clotting activity.

As seen in Figure 184, addition of different amounts of Innovin (e.g., Innovin reconstituted with purified water equivalent to 10 nM of lipidated recombinant human TF_{243} was considered to be 100% Innovin) to the PT assay demonstrated a dose-response relationship, where lower concentrations of TF_{243} resulted in a longer PT time (lower clotting activity). For example, 0.001% Innovin had a PT time greater than 110 seconds, which was almost the same as buffer alone.

In another experiment, the PT test was conducted on TF_{219} and multi-chain chimeric polypeptides including: 18t15-12s, 7t15-21s, 21t15-TGFRs-WT, and 21t15-TGFRs-Mut. Figure 185 show that TF_{219} and TF_{219}-containing multi-chain chimeric

polypeptides (at a concentration of 100 nM) had prolonged PT times indicating extremely low or no clotting activity.

Studies were also conducted to evaluate whether incubating the multi-chain chimeric polypeptides in the presence of other cells carrying receptors for the cytokine components of the multi-chain chimeric polypeptide (32Dβ or human PBMCs) would affect the clotting time in the PT assay. To examine whether cells that express IL-15 receptor (32Dβ cells) or IL-15 and IL-21 receptors (PBMCs) would bind IL-15 -containing multi-chain chimeric polypeptides to mimic natural TF as a cellular FVIIa receptor, TF_{219}-containing multi-chain chimeric polypeptides (at a concentration of 100 nM for each molecule) were diluted in the PT assay buffer and preincubated with 32Dβ cells (at 2 x 10^5 cells/mL) or PBMC (at 1 x 10^5 cells/mL) for 20-30 minutes at room temperature. The PT assay was then conducted as described above. Figures 186 and 187 shows that TF_{219} and TF_{219}-containing multi-chain chimeric polypeptides mixed with 32Dβ cells (Figure 186) or PBMC (Figure 187) at a final concentration of 100 nM had prolonged PT times similar to 0.001-0.01% Innovin (equivalent to 0.1 pM to 1.0 pM of TF_{243}). Expressed in percentage of relative TF_{243} activity, TF_{219}-containing multi-chain chimeric polypeptides had 100,000 to 1,000,000 times lower TF dependent clotting activity when compared to Innovin. This demonstrated that TF_{219}-containing multi-chain chimeric polypeptides had extremely low or no TF-dependent clotting activity, even while the molecules were bound to an intact cell membrane surface, such as 32Dβ or PBMCs.

Example 72: Characterization of 7t15-21s137L (long version)

The nucleic acid sequence of the 7t15 construct (including signal peptide sequence) is as follows (SEQ ID NO: 210):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL7)

GATTGCGACATCGAGGGCAAGGACGGCAAGCAGTACGAGAGCGTGCTGATG
GTGTCCATCGACCAGCTGCTGGACAGCATGAAGGAGATCGGCTCCAACTGCC
TCAACAACGAGTTCAACTTCTTCAAGCGGCACATCTGCGACGCCAACAAGGA
GGGCATGTTCCTGTTCAGGGCCGCCAGGAAACTGCGGCAGTTCCTGAAGATG
AACTCCACCGGCGACTTCGACCTGCACCTGCTGAAGGTGTCCGAGGGCACCA
CCATCCTGCTGAACTGCACCGGACAGGTGAAGGGCCGGAAACCTGCTGCTCT
GGGAGAGGCCCAACCCACCAAGAGCCTGGAGGAGAACAAGTCCCTGAAGGA
GCAGAAGAAGCTGAACGACCTGTGCTTCCTGAAGAGGCTGCTGCAGGAGATC
AAGACCTGCTGGAACAAGATCCTGATGGGCACCAAGGAGCAT

(Human Tissue Factor 219)

AGCGGCACAACCAACACAGTCGCTGCCTATAACCTCACTTGGAAGAGCACCA
ACTTCAAAACCATCCTCGAATGGGAACCCAAACCCGTTAACCAAGTTTACAC
CGTGCAGATCAGCACCAAGTCCGGCGACTGGAAGTCCAAATGTTTCTATACC
ACCGACACCGAGTGCGATCTCACCGATGAGATCGTGAAAGATGTGAAACAGA
CCTACCTCGCCCGGGTGTTTAGCTACCCCGCCGGCAATGTGGAGAGCACTGG
TTCCGCTGGCGAGCCTTTATACGAGAACAGCCCCGAATTTACCCCTTACCTCG
AGACCAATTTAGGACAGCCCACCATCCAAAGCTTTGAGCAAGTTGGCACAAA
GGTGAATGTGACAGTGGAGGACGAGCGGACTTTAGTGCGGCGGAACAACAC
CTTTCTCAGCCTCCGGGATGTGTTCGGCAAAGATTTAATCTACACACTGTATT
ACTGGAAGTCCTCTTCCTCCGGCAAGAAGACAGCTAAAACCAACACAAACGA
GTTTTTAATCGACGTGGATAAAGGCGAAAACTACTGTTTCAGCGTGCAAGCT
GTGATCCCCTCCCGGACCGTGAATAGGAAAAGCACCGATAGCCCCGTTGAGT
GCATGGGCCAAGAAAAGGGCGAGTTCCGGGAG

(Human IL-15)

AACTGGGTGAACGTCATCAGCGATTTAAAGAAGATCGAAGATTTAATTCAGT
CCATGCATATCGACGCCACTTTATACACAGAATCCGACGTGCACCCCTCTTGT
AAGGTGACCGCCATGAAATGTTTTTTACTGGAGCTGCAAGTTATCTCTTTAGA
GAGCGGAGACGCTAGCATCCACGACACCGTGGAGAATTTAATCATTTTAGCC
AATAACTCTTTATCCAGCAACGGCAACGTGACAGAGTCCGGCTGCAAGGAGT

GCGAAGAGCTGGAGGAGAAGAACATCAAGGAGTTTCTGCAATCCTTTGTGCA
CATTGTCCAGATGTTCATCAATACCTCC

The amino acid sequence of 7t15 fusion protein (including the leader sequence) is as follows (SEQ ID NO: 209):

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL7)

DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHICDANKEGM
FLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQ
PTKSLEENKSLKEQKKLNDLCFLKRLLQEIKTCWNKILMGTKEH

(Human Tissue Factor 219)

SGTTNTVAAYNLTWKSTNFKTILEWEPKPVNQVYTVQISTKSGDWKSKCFYTTD
TECDLTDEIVKDVKQTYLARVFSYPAGNVESTGSAGEPLYENSPEFTPYLETNLG
QPTIQSFEQVGTKVNVTVEDERTLVRRNNTFLSLRDVFGKDLIYTLYYWKSSSSG
KKTAKTNTNEFLIDVDKGENYCFSVQAVIPSRTVNRKSTDSPVECMGQEKGEFR
E

(Human IL-15)

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESG
DASIHDTVENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFIN
TS

The nucleic acid sequence of the 21s137L construct (including signal peptide sequence) is as follows (SEQ ID NO: 331):

(Signal peptide)

ATGAAGTGGGTGACCTTCATCAGCCTGCTGTTCCTGTTCTCCAGCGCCTACTC
C

(Human IL-21)

CAGGGCCAGGACAGGCACATGATCCGGATGAGGCAGCTCATCGACATCGTCG
ACCAGCTGAAGAACTACGTGAACGACCTGGTGCCCGAGTTTCTGCCTGCCCC

CGAGGACGTGGAGACCAACTGCGAGTGGTCCGCCTTCTCCTGCTTTCAGAAG
GCCCAGCTGAAGTCCGCCAACACCGGCAACAACGAGCGGATCATCAACGTG
AGCATCAAGAAGCTGAAGCGGAAGCCTCCCTCCACAAACGCCGGCAGGAGG
CAGAAGCACAGGCTGACCTGCCCCAGCTGTGACTCCTACGAGAAGAAGCCCC
CCAAGGAGTTCCTGGAGAGGTTCAAGTCCCTGCTGCAGAAGATGATCCATCA
GCACCTGTCCTCCAGGACCCACGGCTCCGAGGACTCC

(Human IL-15R α sushi domain)

ATTACATGCCCCCCTCCCATGAGCGTGGAGCACGCCGACATCTGGGTGAAGA
GCTATAGCCTCTACAGCCGGGAGAGGTATATCTGTAACAGCGGCTTCAAGAG
GAAGGCCGGCACCAGCAGCCTCACCGAGTGCGTGCTGAATAAGGCTACCAAC
GTGGCTCACTGGACAACACCCTCTTTAAAGTGCATCCGG

((G4S)3 linker)

GGCGGTGGAGGATCCGGAGGAGGTGGCTCCGGCGGCGGAGGATCT

(Human CD137L)

CGCGAGGGTCCCGAGCTTTCGCCCGACGATCCCGCCGGCCTCTTGGACCTGC
GGCAGGGCATGTTTGCGCAGCTGGTGGCCCAAAATGTTCTGCTGATCGATGG
GCCCCTGAGCTGGTACAGTGACCCAGGCCTGGCAGGCGTGTCCCTGACGGGG
GGCCTGAGCTACAAAGAGGACACGAAGGAGCTGGTGGTGGCCAAGGCTGGA
GTCTACTATGTCTTCTTTCAACTAGAGCTGCGGCGCGTGGTGGCCGGCGAGGG
CTCAGGCTCCGTTTCACTTGCGCTGCACCTGCAGCCACTGCGCTCTGCTGCTG
GGGCCGCCGCCCTGGCTTTGACCGTGGACCTGCCACCCGCCTCCTCCGAGGCT
CGGAACTCGGCCTTCGGTTTCCAGGGCCGCTTGCTGCACCTGAGTGCCGGCC
AGCGCCTGGGCGTCCATCTTCACACTGAGGCCAGGGCACGCCATGCCTGGCA
GCTTACCCAGGGCGCCACAGTCTTGGGACTCTTCCGGGTGACCCCCGAAATC
CCAGCCGGACTCCCTTCACCGAGGTCGGAA

The amino acid sequence of 21s137L fusion protein (including the leader sequence) is as follows (SEQ ID NO: 332):

(*Signal peptide)*

MKWVTFISLLFLFSSAYS

(Human IL-21)

QGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQ
LKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLER
FKSLLQKMIHQHLSSRTHGSEDS

(Human IL-15R α sushi domain)

ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH
WTTPSLKCIR

((G4S)3 linker)

GGGGSGGGGSGGGGS

(Human CD137L)

REGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGL
SYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRSAAGAA
ALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQ
GATVLGLFRVTPEIPAGLPSPRSE

The following experiment was conducted to evaluate whether the CD137L portion in 7t15-21s137L was intact to bind to CD137 (4.1BB). On day 1, a 96-well plate was coated with 100 μL (2.5 μg/mL) of GAH IgG Fc (G-102-C, R&D Systems) in R5 (coating buffer), overnight. On day 2, the plates were washed three times and blocked with 300 μL of 1% BSA in PBS at 37°C for 2 hrs. 10 ng/ml of 4.1BB/Fc (838-4B, R&D Systems) was added at 100 μl/well for 2 hrs at room temperature. Following three washes, 7t15-21s137L (long version) or 7t15-21s137Ls (short version) was added starting at 10 nM, or recombinant human 4.1BBL starting at 180ng/mL, with 1/3 dilution, followed by incubation at 4°C overnight. On day 3, the plates were washed three times, and 500 ng/mL of biotinylate-goat anti-human 4.1BBL (BAF2295, R&D Systems) was applied at 100 μL per well, followed by incubation at RT for 2 hrs. The plates were washed three times, and incubated with 0.25 μg/mL of HRP-SA (Jackson ImmuneResearch) at 100 μL per well for 30 min. The plates were then washed three times, and incubated with 100 μL of ABTS for 2 mins at RT. The results were read at 405 nm. As shown in Figure 188, both 7t15-21s137L (long version) and 7t15-21s137L

(short version) could interact with 4.1BB/Fc (dark diamond and gray square) compared to the recombinant human 4.1BB ligand (rhCD137L, light gray star). 7t15-21s137L (long version) (dark diamond) interacted better with 4.1BB/Fc as compared to 7t15-21s137L (short version) (gray square).

The following experiments were conducted to evaluate whether the components IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) were intact to be detected by the individual antibody using ELISA. A 96-well plate was coated with 100 μL (4 μg/mL) of anti-TF (human IgG1) in R5 (coating buffer) and incubated at RT for 2 hrs. The plates were washed three times, and blocked with 100 μL of 1% BSA in PBS. Purified 7t15-21s137L (long version) was added starting at 10 nM, and at 1/3 dilution, followed by incubation at RT for 60 min. The plates were washed three times, and 500 ng/mL of biotinylate-anti-IL7 (506602, R&D Systems), 500 ng/mL of biotinylate-anti-IL21 (13-7218-81, R&D Systems), 50 ng/mL of biotinylate-anti-IL15 (BAM247, R&D Systems), or 500 ng/ml of biotinylate-goat anti-human 4.1BBL (BAF2295, R&D Systems) was added per well and incubated at room temperature for 60 min. The plates were washed three times and incubated with 0.25 μg/mL of HRP-SA (Jackson ImmunoResearch) at 100 μL per well for 30 min at RT. The plates were washed four times, and incubated with 100 μL of ABTS for 2 mins at room temperature. The absorbance results were read at 405 nm. As shown in Figure 189A-189D, the components including IL7, IL21, IL15, and 4.1BBL in 7t15-21s137L (long version) were detected by the individual antibodies.

The following experiment was conducted to evaluate the activity of IL15 in 7t15-21s137L (long version) and 7t15-21s137L (short version). The ability of 7t15-21s137L (long version) and 7t15-21s137L (short version) to promote proliferation of IL2Rαβγ-expressing CTLL2 cells was compared with that of recombinant IL15. IL15 dependent CTLL2 cells were washed five times with IMDM-10% FBS and seeded to the wells at 2 x 10^4 cells/well. Serially diluted 7t15-21s137L (long version), 7t15-21s137L (short version), or IL15 were added to the cells. Cells were incubated in a CO_2 incubator at 37 °C for 3 days. Cell proliferation was detected by adding 20 μL of PrestoBlue (A13261,

ThermoFisher) to each well on day 3 and incubated for an additional 4 hours in a CO_2 incubator at 37 °C. Raw absorbance at 570-610 nm was read in a micro-titer plate reader. As shown in Figure 190, 7t15-21s137L (long version), 7t15-21s137L (short version), and IL15 all promoted CTLL2 cell proliferation. The EC_{50} of 7t15-21s137L (long version), 7t15-21s137L (short version), and IL15 is 51.19 pM, 55.75 pM, and 4.947 pM, respectively.

Example 73: Induction of Treg cells by 2t2

The peripheral blood mononuclear cells (PBMC) of a heathy donor (Donor 163) were isolated from 5 mL of whole blood buffy coats by Ficoll Paque Plus (GE17144003). The PBMC were then lysed with ACK to remove red blood cells. Cells were washed with IMDM-10% FBS and counted. 1.8 x10^6 cells (100 µL/tube) were seeded to the flow tubes and incubated with 50 µL of descending 2t2 or IL2 (15000, 1500, 150, 15, 1.5, 0.15, or 0 pM) and 50 µLof pre-staining antibodies (anti-CD8-BV605 and anti-CD127-AF647). Cells were incubated for 30 min at 37 °C in water bath. 200 µL of pre-warmed BD Phosflow Fix Buffer I (Cat# 557870, Becton Dickinson Biosciences) was added for 10 min at 37° C in water bath to stop the stimulation. Cells (4.5 x10^5 cells/100 µL) were transferred to a V-shape 96-well plate and were spun down followed by permeabilization with 100 µL of -20 °C pre-cooled BD Phosflow Perm Buffer III (Cat# BD Biosciences) for 30 min on ice. The cells were then extensively washed x2 with 200 µL of FACS buffer and stained with a panel of fluorescent antibodies (anti-CD25-PE, CD4-PerCP-Cy5.5, CD56-BV421, CD45RA-PE-Cy7 and pSTAT5a-AF488) to distinguish between different lymphocyte subpopulations and evaluate the pSTAT5a status. Cells were spun down and resuspended in 200 µL of FACS buffer for FACSCelesta analysis. As sown in Figure 191A, 6 pM of 2t2 was sufficient to induce the phosphorylation of Stat5a in $CD4^+CD25^{hi}$ T_{reg} cells while 43.11 pM of IL-2 was required to induce phosphorylation of Stat5a in the same population of lymphocytes. In contrast, 2t2 was less active (Figure 191B) or equally active (Figure 191C) as compared to IL2 in inducing phosphorylation of Stat5a in $CD4^+CD25^-T_{con}$ and $CD8^+T_{con}$ cells. These results suggest that 2t2 is superior

as compared to IL2 in activating T_{reg} in human PBMC, and that 2t2 demonstrates increased T_{reg} selectivity compared to IL-2 in human blood lymphocyte pStat5a responses.

Example 74. Improvement in Hair Growth using a Single-Chain Chimeric Polypeptide

The dorsal hair of 7-week-old C57BL6/J mice was shaved and depilated using commercial depilatory cream. The mice were injected on the same day subcutaneously with a single dose of 2t2 or low dose commercially available recombinant IL-2, followed by daily dosing for four additional days. Untreated mice served as controls. On day 10, the mice were sacrificed and skin sections of the shaved areas were prepared. Representative H&E staining of skin sections from C57BL6J mice on day 10 following depilation are shown in Figures 192A – 192E. Figure 192A shows control mice - only depilation done after hair was shaved, Figure 192B shows mice where depilation was followed by low dose IL-2 (1 mg/kg) administration, and Figures 192C-192E shows mice where depilation was followed by 2t2 administered at 0.3 mg/kg (Figure 192C), 1 mg/kg (Figure 192D), and 3 mg/kg (Figure 192E). Black arrows indicate anagen-phase hair follicles that will later extend into dermis and facilitate hair growth. Figure 194 shows the total number of anagen phase hair follicles counted per 10 fields for each treatment group. In summary, the data show that the 2t2 molecule resulted in increased numbers of anagen-phase hair follicles compared to depilation alone. This effect was also dose-dependent.

Example 75: Differentiation of the Immune Cell into a Memory-Like Immune Cell

Fresh human leukocytes were obtained from the blood bank and $CD56^+$ NK cells were isolated with the RosetteSep/human NK cell reagent (StemCell Technologies). The purity of NK cells was >70% and confirmed by staining with CD56-BV421, CD16-BV510, CD25-PE, CD69-APCFire750 (BioLegend). The cells were counted and resuspended at a density of 2×10^6 cells/mL in RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine (Thermo Life Technologies), antibiotics

(penicillin, 10,000 units/mL; streptomycin, 10,000 µg/mL; Thermo Life Technologies), and 10% FBS (Hyclone). The cells (1 mL) were transferred into a 24-well flat bottom plate, and subjected to either: no treatment, or expanded with 7t15-21s + anti-tissue factor (TF)-antibody (IgG1) (50 nM) for 14 days with medium. The cells were replenished with fresh 7t15-21s + anti-TF-antibody (IgG1) (50 nM) to keep the cell density at approximately 1 x 10^6 cells/mL.

Unexpanded NK cells to treatment groups were used as positive controls for full DNA methylation levels (Data not shown). NK cells were pelleted (1 x 10^6), and genomic DNA (nDNA) isolated using the QIAamp UCP DNA Micro Kit (Qiagen). 500 ng of purified nDNA was subjected to sodium bisulfite treatment using the EZ DNA Methylation-Direct kit (Zymo Research) according to the manufacturer's protocol. Bisulfite treatment introduces methylation-dependent changes in the DNA with demethylated cytosines being converted into uracil, whereas methylated cytosines remain unchanged. The bisulfite-treated nDNA (10-50 ng) was used as template to PCR amplify a 228 bp region of the IFNγ promoter containing two CpG sites (CpG -186 and CpG -54, position relative to the transcription start site, TSS), known to be heavily regulated by DNA methylation in T cells, using the Pyromark PCR kit (Qiagen) with the forward primer IFNG127F (5′-ATGGTATAGGTGGGTATAATGG-3′) and the biotinylated reverse primer IFNG355R-bio (biotin-5′-CAATATACTACACCTCCTCTAACTAC-3′) (GENEWIZ). T he PCR conditions were 15 minutes at 95°C, 48 cycles of 30 seconds at 95 °C, 30 seconds at 56°C, 60 seconds at 72°C followed by 10 minutes at 72°C. The integrity and quality of the PCR amplified products were visualized on a 1.2% TAE agarose gel. The DNA methylation status of these two CpG sites was determined by pyrosequencing, which is the gold standard technique to quantitatively measure DNA methylation at single CpG-site. Pyrosequencing reactions were performed at Johns Hopkins University Genetic Resources Core Facility using the DNA sequencing primers C186-IFNG135F (5′-GGTGGGTATAATGGG-3′) (SEQ ID NO: 333) and C54-IFNG261F (5′-ATTATTTTATTTTAAAAAATTTGTG-3′) (SEQ ID NO: 334), specific to the CpG sites -186 and -54, respectively. Commercially available non-methylated and methylated DNA (Zymo Research) were used as controls for DNA methylation. The

methylation percentages of the two CpG sites (-186 and -54) were pooled for each treatment. The percent difference in DNA methylation was calculated relative to the levels of DNA methylation at the two CpG sites observed in unexposed NK cells.

Analysis of the DNA methylation status of these two IFNγ CpG sites revealed higher levels of DNA demethylation in NK cells supported by 7t15-21s + anti-TF-antibody compared to unexposed NK cells (Figure 194). These 7t15-21s + anti-TF-antibody supported NK cells exhibited 47.70% ± 11.76 difference in DNA methylation (i.e., demethylation) compared to unexposed NK cells. The DNA methylation levels of these two IFNγ CpG sites correlated with increased expression of IFNγ following treatment with 7t15-21s + anti-TF-antibody. These data suggest that long-term exposure of NK cells (14 days expansion in culture) with a combination regimen of 7t15-21s + anti-TF-antibody is able to induce DNA demethylation of the two hypomethylated IFNγ CpG sites (-186 and -54) and that 7t15-21s + anti-TF-antibody (IgG1) can epigenetically reprogram gene expression of IFNγ via DNA demethylation of CpG sites leading to interconversion of NK cells into innate immune memory NK cells.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Exemplary Embodiments

Embodiment A1. A single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a second target-binding domain.

Embodiment A2. The single-chain chimeric polypeptide of embodiment A1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment A3. The single-chain chimeric polypeptide of embodiment A1, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

Embodiment A4. The single-chain chimeric polypeptide of any one of embodiments A1-A3, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

Embodiment A5. The single-chain chimeric polypeptide of any one of embodiments A1-A3, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

Embodiment A6. The single-chain chimeric polypeptide of embodiment A1, wherein the first target-binding domain and the second target-binding domain directly abut each other.

Embodiment A7. The single-chain chimeric polypeptide of embodiment A1, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the second target-binding domain.

Embodiment A8. The single-chain chimeric polypeptide of embodiment A6 or A7, wherein the second target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment A9. The single-chain chimeric polypeptide of embodiment A6 or A7, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the second target-binding domain and the soluble tissue factor domain.

Embodiment A10. The single-chain chimeric polypeptide of any one of embodiments A1-A9, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment A11. The single-chain chimeric polypeptide of embodiment A10, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment A12. The single-chain chimeric polypeptide of embodiment A11, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment A13. The single-chain chimeric polypeptide of any one of embodiments A1-A9, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment A14. The single-chain chimeric polypeptide of any one of embodiments A1-A13, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment A15. The single-chain chimeric polypeptide of embodiment A14, wherein the first target-binding domain and the second target-binding domain are each an antigen-binding domain.

Embodiment A16. The single-chain chimeric polypeptide of embodiment A13, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment A17. The single-chain chimeric polypeptide of any one of embodiments A1-A16, wherein one or both of the first target-binding domain and the second target-binding domain bind to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-D, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment A18. The single-chain chimeric polypeptide of any one of embodiments A1-A16, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment A19. The single-chain chimeric polypeptide of embodiment A18, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-D, and SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment A20. The single-chain chimeric polypeptide of any one of embodiments A1-A16, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment A21. The single-chain chimeric polypeptide of embodiment A20, wherein the soluble interleukin or cytokine receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28.

Embodiment A22. The single-chain chimeric polypeptide of any one of embodiments A1-A21, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment A23. The single-chain chimeric polypeptide of embodiment A22, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment A24. The single-chain chimeric polypeptide of embodiment A23, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment A25. The single-chain chimeric polypeptide of embodiment A24, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment A26. The single-chain chimeric polypeptide of any one of embodiments A22-A25, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment A27. The single-chain chimeric polypeptide of embodiment A26, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment A28. The single-chain chimeric polypeptide of any one of embodiments A1-A27, wherein the soluble tissue factor domain is not capable of binding Factor VIIa.

Embodiment A29. The single-chain chimeric polypeptide of any one of embodiments A1-A28, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment A30. The single-chain chimeric polypeptide of any one of embodiments A1-A29, wherein the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment A31. The single-chain chimeric polypeptide of any one of embodiments A1-A30, wherein the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

Embodiment A32. The single-chain chimeric polypeptide of embodiment A31, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its N-terminus.

Embodiment A33. The single-chain chimeric polypeptide of embodiment A32, wherein one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A34. The single-chain chimeric polypeptide of embodiment A33, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A35. The single-chain chimeric polypeptide of embodiment A31, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its C-terminus.

Embodiment A36. The single-chain chimeric polypeptide of embodiment A35, wherein one of the one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A37. The single-chain chimeric polypeptide of embodiment A35, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A38. The single-chain chimeric polypeptide of embodiment A31, wherein the single-chain chimeric polypeptide comprises one or more additional target binding domains at its N-terminus and the C-terminus.

Embodiment A39. The single-chain chimeric polypeptide of embodiment A38, wherein one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A40. The single-chain chimeric polypeptide of embodiment A38, wherein the single-chain chimeric polypeptide further comprises a linker sequence

between one of the one or more additional antigen-binding domains at the N-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A41. The single-chain chimeric polypeptide of embodiment A38, wherein one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A42. The single-chain chimeric polypeptide of embodiment A38, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment A43. The single-chain chimeric polypeptide of any one of embodiments A31-A42, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment A44. The single-chain chimeric polypeptide of embodiment A43, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment A45. The single-chain chimeric polypeptide of embodiment A44, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment A46. The single-chain chimeric polypeptide of embodiment A43, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment A47. The single-chain chimeric polypeptide of embodiment A46, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment A48. The single-chain chimeric polypeptide of embodiment A47, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment A49. The single-chain chimeric polypeptide of any one of embodiments A31-A42, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment A50. The single-chain chimeric polypeptide of any one of embodiments A31-A49, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment A51. The single-chain chimeric polypeptide of embodiment A50, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment A52. The single-chain chimeric polypeptide of embodiment A51, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment A53. The single-chain chimeric polypeptide of any one of embodiments A31-A52, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-D, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment A54. The single-chain chimeric polypeptide of any one of embodiments A31-A52, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment A55. The single-chain chimeric polypeptide of embodiment A54, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-D, and SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment A56. The single-chain chimeric polypeptide of any one of embodiments A31-A52, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment A57. The single-chain chimeric polypeptide of embodiment A56, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

Embodiment A58. The single-chain chimeric polypeptide of any one of embodiments A1-A57, wherein the single-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment A59. The single-chain chimeric polypeptide of any one of embodiments A1-A58, wherein the single-chain chimeric polypeptide further comprises a peptide tag positioned at the N-terminal end or the C-terminal end of the single-chain chimeric polypeptide.

Embodiment A60. A composition comprising any of the single-chain chimeric polypeptides of embodiments A1-A59.

Embodiment A61. The composition of embodiment A60, wherein the composition is a pharmaceutical composition.

Embodiment A62. A kit comprising at least one dose of the composition of embodiment A60 or A61.

Embodiment A63. Nucleic acid encoding any of the single-chain chimeric polypeptides of any one of embodiments A1-A59.

Embodiment A64. A vector comprising the nucleic acid of embodiment A63.

Embodiment A65. The vector of embodiment A64, wherein the vector is an expression vector.

Embodiment A66. A cell comprising the nucleic acid of embodiment A63 or the vector of embodiment A64 or A65.

Embodiment A67. A method of producing a single-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment A66 in a culture medium under conditions sufficient to result in the production of the single-chain chimeric polypeptide; and

recovering the single-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment A68. A single-chain chimeric polypeptide produced by the method of embodiment A67.

Embodiment A69. The single-chain chimeric polypeptide of embodiment A26, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment A70. The single-chain chimeric polypeptide of embodiment A69, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment A71. The single-chain chimeric polypeptide of embodiment A70, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment A72. The single-chain chimeric polypeptide of embodiment A71, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment A73. The single-chain chimeric polypeptide of embodiment A26, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment A74. The single-chain chimeric polypeptide of embodiment A73, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment A75. The single-chain chimeric polypeptide of embodiment A74, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment A76. The single-chain chimeric polypeptide of embodiment A75, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

Embodiment B1. A single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a second target-binding domain,

wherein:

the first target-binding domain and the second target-binding domain each specifically bind to an IL-2 receptor; or

the first target-binding domain and the second target-binding domain each specifically bind to an IL-15 receptor.

Embodiment B2. The single-chain chimeric polypeptide of embodiment B1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment B3. The single-chain chimeric polypeptide of embodiment B1, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

Embodiment B4. The single-chain chimeric polypeptide of any one of embodiments B1-B3, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

Embodiment B5. The single-chain chimeric polypeptide of any one of embodiments B1-B3, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

Embodiment B6. The single-chain chimeric polypeptide of embodiment B1, wherein the first target-binding domain and the second target-binding domain directly abut each other.

Embodiment B7. The single-chain chimeric polypeptide of embodiment B1, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the second target-binding domain.

Embodiment B8. The single-chain chimeric polypeptide of embodiment B6 or B7, wherein the second target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment B9. The single-chain chimeric polypeptide of embodiment B6 or B7, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the second target-binding domain and the soluble tissue factor domain.

Embodiment B10. The single-chain chimeric polypeptide of any one of embodiments B1-B9, wherein both the first target-binding domain and the second target-binding domain is a soluble interleukin protein.

Embodiment B11. The single-chain chimeric polypeptide of embodiment B10, wherein the first target-binding domain and the second target-binding domain is a soluble IL-2 protein.

Embodiment B12. The single-chain chimeric polypeptide of embodiment B11, wherein the soluble IL-2 protein is a soluble human IL-2 protein.

Embodiment B13. The single-chain chimeric polypeptide of embodiment B12, wherein the soluble human IL-2 protein comprises SEQ ID NO: 78.

Embodiment B14. The single-chain chimeric polypeptide of embodiment B10, wherein the first target-binding domain and the second target-binding domain is a soluble IL-15 protein.

Embodiment B15. The single-chain chimeric polypeptide of embodiment B14, wherein the soluble IL-15 protein is a soluble human IL-15 protein.

Embodiment B16. The single-chain chimeric polypeptide of embodiment B15, wherein the soluble human IL-15 protein comprises SEQ ID NO: 82.

Embodiment B17. The single-chain chimeric polypeptide of any one of embodiments B1-B16, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment B18. The single-chain chimeric polypeptide of embodiment B17, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment B19. The single-chain chimeric polypeptide of embodiment B18, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment B20. The single-chain chimeric polypeptide of embodiment B19, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment B21. The single-chain chimeric polypeptide of any one of embodiments B17-B20, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment B22. The single-chain chimeric polypeptide of embodiment B21, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment B23. The single-chain chimeric polypeptide of any one of embodiments B1-B22, wherein the soluble tissue factor domain is not capable of binding Factor VIIa.

Embodiment B24. The single-chain chimeric polypeptide of any one of embodiments B1-B23, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment B25. The single-chain chimeric polypeptide of any one of embodiments B1-B24, wherein the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment B26. The single-chain chimeric polypeptide of any one of embodiments B1-B25, wherein the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

Embodiment B27. The single-chain chimeric polypeptide of embodiment B26, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its N-terminus.

Embodiment B28. The single-chain chimeric polypeptide of embodiment B27, wherein one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B29. The single-chain chimeric polypeptide of embodiment B28, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B30. The single-chain chimeric polypeptide of embodiment B26, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its C-terminus.

Embodiment B31. The single-chain chimeric polypeptide of embodiment B30, wherein one of the one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B32. The single-chain chimeric polypeptide of embodiment B30, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B33. The single-chain chimeric polypeptide of embodiment B26, wherein the single-chain chimeric polypeptide comprises one or more additional target binding domains at its N-terminus and the C-terminus.

Embodiment B34. The single-chain chimeric polypeptide of embodiment B33, wherein one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B35. The single-chain chimeric polypeptide of embodiment B33, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the N-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B36. The single-chain chimeric polypeptide of embodiment B33, wherein one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B37. The single-chain chimeric polypeptide of embodiment B33, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment B38. The single-chain chimeric polypeptide of any one of embodiments B26-B37, wherein each of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to an IL-2 receptor or an IL-15 receptor.

Embodiment B39. The single-chain chimeric polypeptide of embodiment B38, wherein each of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment B40. The single-chain chimeric polypeptide of any one of embodiments B26-B37, wherein the one or more additional target-binding domains is an antigen-binding domain.

Embodiment B41. The single-chain chimeric polypeptide of embodiment B40, wherein the antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment B42. The single-chain chimeric polypeptide of any one of embodiments B26-B37, B40, and B41, wherein the one or more additional target-binding

domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment B43. The single-chain chimeric polypeptide of any one of embodiments B6-B37, B40, and B41, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment B44. The single-chain chimeric polypeptide of embodiment B43, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment B45. The single-chain chimeric polypeptide of any one of embodiments B6-B37, B40, and B41, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment B46. The single-chain chimeric polypeptide of embodiment B45, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) and a soluble TGF-βRIII.

Embodiment B47. The single-chain chimeric polypeptide of any one of embodiments B1-B46, wherein the single-chain chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment B48. The single-chain chimeric polypeptide of any one of embodiments B1-B47, wherein the single-chain chimeric polypeptide further comprises a peptide tag positioned at the N-terminal end or the C-terminal end of the single-chain chimeric polypeptide.

Embodiment B49. A composition comprising any of the single-chain chimeric polypeptides of embodiments B1-B48.

Embodiment B50. The composition of embodiment B49, wherein the composition is a pharmaceutical composition.

Embodiment B51. A kit comprising at least one dose of the composition of embodiment B49 or B50.

Embodiment B52. A nucleic acid encoding any of the single-chain chimeric polypeptides of any one of embodiments B1-B48.

Embodiment B53. A vector comprising the nucleic acid of embodiment B52.

Embodiment B54. The vector of embodiment B53, wherein the vector is an expression vector.

Embodiment B55. A cell comprising the nucleic acid of embodiment B52 or the vector of embodiment B53 or B54.

Embodiment B56. A method of producing a single-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment B55 in a culture medium under conditions sufficient to result in the production of the single-chain chimeric polypeptide; and

recovering the single-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment B57. A single-chain chimeric polypeptide produced by the method of embodiment B56.

Embodiment B58. The single-chain chimeric polypeptide of embodiment B21, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment B59. The single-chain chimeric polypeptide of embodiment B58, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment B60. The single-chain chimeric polypeptide of embodiment B59, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment B61. The single-chain chimeric polypeptide of embodiment B60, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment B62. The single-chain chimeric polypeptide of embodiment B21, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment B63. The single-chain chimeric polypeptide of embodiment B62, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment B64. The single-chain chimeric polypeptide of embodiment B63, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment B65. The single-chain chimeric polypeptide of embodiment B64, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

Embodiment C1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

Embodiment C2. The multi-chain chimeric polypeptide of embodiment C1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment C3. The multi-chain chimeric polypeptide of embodiment C1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment C4. The multi-chain chimeric polypeptide of any one of embodiments C1-C3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment C5. The multi-chain chimeric polypeptide of any one of embodiments C1-C3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C6. The multi-chain chimeric polypeptide of any one of embodiments C1-C5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment C7. The multi-chain chimeric polypeptide of any one of embodiments C1-C5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment C8. The multi-chain chimeric polypeptide of any one of embodiments C1-C7, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment C9. The multi-chain chimeric polypeptide of embodiment C8, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment C10. The multi-chain chimeric polypeptide of embodiment C9, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment C11. The multi-chain chimeric polypeptide of any one of embodiments C1-C7, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment C12. The multi-chain chimeric polypeptide of any one of embodiments C1-C11, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment C13. The multi-chain chimeric polypeptide of embodiment C12, wherein the first target-binding domain and the second target-binding domain are each antigen-binding domains.

Embodiment C14. The multi-chain chimeric polypeptide of embodiment C12 or C13, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment C15. The multi-chain chimeric polypeptide of any one of embodiments C1-C14, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD28.

Embodiment C16. The multi-chain chimeric polypeptide of any one of embodiments C1-C14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment C17. The multi-chain chimeric polypeptide of embodiment C16, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment C18. The multi-chain chimeric polypeptide of any one of embodiments C1-C14, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment C19. The multi-chain chimeric polypeptide of embodiment C18, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, or a soluble CD28.

Embodiment C20. The multi-chain chimeric polypeptide of any one of embodiments C1-C19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment C21. The multi-chain chimeric polypeptide of embodiment C20, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment C22. The multi-chain chimeric polypeptide of any one of embodiments C1-C19, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment C23. The multi-chain chimeric polypeptide of embodiment C22, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C24. The multi-chain chimeric polypeptide of embodiment C22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment C25. The multi-chain chimeric polypeptide of embodiment C22, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment C26. The multi-chain chimeric polypeptide of embodiment C22, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment C27. The multi-chain chimeric polypeptide of embodiment C22, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C28. The multi-chain chimeric polypeptide of embodiment C27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C29. The multi-chain chimeric polypeptide of embodiment C27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C30. The multi-chain chimeric polypeptide of embodiment C27, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C31. The multi-chain chimeric polypeptide of embodiment C27, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment C32. The multi-chain chimeric polypeptide of embodiment C27, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment C33. The multi-chain chimeric polypeptide of embodiment C27, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-

binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment C34. The multi-chain chimeric polypeptide of any one of embodiments C1-C33, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment C35. The multi-chain chimeric polypeptide of embodiment C34, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment C36. The multi-chain chimeric polypeptide of embodiment C34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment C37. The multi-chain chimeric polypeptide of embodiment C34, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment C38. The multi-chain chimeric polypeptide of embodiment C34, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment C39. The multi-chain chimeric polypeptide of any one of embodiments C20-C38, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment C40. The multi-chain chimeric polypeptide of embodiment C39, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment C41. The multi-chain chimeric polypeptide of embodiment C40, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment C42. The multi-chain chimeric polypeptide of embodiment C39, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment C43. The multi-chain chimeric polypeptide of embodiment C42, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment C44. The multi-chain chimeric polypeptide of embodiment C43, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment C45. The multi-chain chimeric polypeptide of any one of embodiments C20-C38, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment C46. The multi-chain chimeric polypeptide of any one of embodiments C20-C45, wherein one or more of the first target-binding domain, the

second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment C47. The multi-chain chimeric polypeptide of embodiment C46, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment C48. The multi-chain chimeric polypeptide of embodiment C47, wherein antigen-binding domain comprises a scFv.

Embodiment C49. The multi-chain chimeric polypeptide of any one of embodiments C20-C48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, a receptor for CD122, and a receptor for CD3, and a receptor for CD28.

Embodiment C50. The multi-chain chimeric polypeptide of any one of embodiments C20-C48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment C51. The multi-chain chimeric polypeptide of embodiment C50, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment C52. The multi-chain chimeric polypeptide of any one of embodiments C20-C48, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment C53. The multi-chain chimeric polypeptide of embodiment C52, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, a soluble CD3, or a soluble CD28.

Embodiment C54. The multi-chain chimeric polypeptide of any one of embodiments C1-C53, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment C55. The multi-chain chimeric polypeptide of any one of embodiments C1-C53, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment C56. The multi-chain chimeric polypeptide of any one of embodiments C1-C55, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment C57. The multi-chain chimeric polypeptide of embodiment C56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment C58. The multi-chain chimeric polypeptide of embodiment C57, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment C59. The multi-chain chimeric polypeptide of embodiment C58, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment C60. The multi-chain chimeric polypeptide of any one of embodiments C56-C59, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment C61. The multi-chain chimeric polypeptide of embodiment C60, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment C62. The multi-chain chimeric polypeptide of any one of embodiments C1-C61, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment C63. The multi-chain chimeric polypeptide of any one of embodiments C1-C62, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment C64. The multi-chain chimeric polypeptide of any one of embodiments C1-C63, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment C65. The multi-chain chimeric polypeptide of any one of embodiments C1-C64, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

Embodiment C66. The multi-chain chimeric polypeptide of embodiment C65, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment C67. The multi-chain chimeric polypeptide of embodiment C65 or C66, wherein the human IL-15Rα is a mature full-length IL-15Rα.

Embodiment C68. The multi-chain chimeric polypeptide of any one of embodiments C1-C64, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment C69. The multi-chain chimeric polypeptide of any one of embodiments C1-C68, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment C70. A composition comprising any of the multi-chain chimeric polypeptides of embodiments C1-C69.

Embodiment C71. The composition of embodiment C70, wherein the composition is a pharmaceutical composition.

Embodiment C72. A kit comprising at least one dose of the composition of embodiment C70 or C71.

Embodiment C73. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments C1-C69.

Embodiment C74. A vector comprising the nucleic acid of embodiment C73.

Embodiment C75. The vector of embodiment C74, wherein the vector is an expression vector.

Embodiment C76. A cell comprising the nucleic acid of embodiment C73 or the vector of embodiment C74 or C75.

Embodiment C77. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment C76 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment C78. A multi-chain chimeric polypeptide produced by the method of embodiment C77.

Embodiment C79. The multi-chain chimeric polypeptide of embodiment A56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment C80. The multi-chain chimeric polypeptide of embodiment C79, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment C81. The multi-chain chimeric polypeptide of embodiment C80, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment C82. The multi-chain chimeric polypeptide of embodiment C81, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment C83. The multi-chain chimeric polypeptide of embodiment C56, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment C84. The multi-chain chimeric polypeptide of embodiment C83, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment C85. The multi-chain chimeric polypeptide of embodiment C84, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment C86. The multi-chain chimeric polypeptide of embodiment C85, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

Embodiment D1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-18 or a receptor of IL-12.

Embodiment D2. The multi-chain chimeric polypeptide of embodiment D1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment D3. The multi-chain chimeric polypeptide of embodiment D1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment D4. The multi-chain chimeric polypeptide of any one of embodiments D1-D3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment D5. The multi-chain chimeric polypeptide of any one of embodiments D1-D3, wherein the first chimeric polypeptide further comprises a linker

sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D6. The multi-chain chimeric polypeptide of any one of embodiments D1-D5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment D7. The multi-chain chimeric polypeptide of any one of embodiments D1-D5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment D8. The multi-chain chimeric polypeptide of any one of embodiments D1-D7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment D9. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment D10. The multi-chain chimeric polypeptide of embodiment D9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment D11. The multi-chain chimeric polypeptide of embodiment D10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment D12. The multi-chain chimeric polypeptide of any one of embodiments D8-D11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment D13. The multi-chain chimeric polypeptide of embodiment D12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment D14. The multi-chain chimeric polypeptide of any one of embodiments D1-D13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment D15. The multi-chain chimeric polypeptide of any one of embodiments D1-D14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment D16. The multi-chain chimeric polypeptide of any one of embodiments D1-D15, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment D17. The multi-chain chimeric polypeptide of any one of embodiments D1-D16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment D18. The multi-chain chimeric polypeptide of any one of embodiments D1-D17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment D19. The multi-chain chimeric polypeptide of any one of embodiments D1-D18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment D20. The multi-chain chimeric polypeptide of embodiment D19, wherein the signal sequence comprises SEQ ID NO: 117.

Embodiment D21. The multi-chain chimeric polypeptide of embodiment D20, wherein the signal sequence is SEQ ID NO: 117.

Embodiment D22. The multi-chain chimeric polypeptide of any one of embodiments D1-D21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

Embodiment D23. The multi-chain chimeric polypeptide of embodiment D22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment D24. The multi-chain chimeric polypeptide of embodiment D22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 82.

Embodiment D25. The multi-chain chimeric polypeptide of embodiment D24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 82.

Embodiment D26. The multi-chain chimeric polypeptide of embodiment D25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 82.

Embodiment D27. The multi-chain chimeric polypeptide of embodiment D26, wherein the soluble IL-15 comprises SEQ ID NO: 82.

Embodiment D28. The multi-chain chimeric polypeptide of any one of embodiments D22-D27, wherein the sushi domain of IL-15Rα comprises a sushi domain from human IL-15Rα.

Embodiment D29. The multi-chain chimeric polypeptide of embodiment D28, wherein the sushi domain from human IL-15Rα comprises a sequence that is 80% identical to SEQ ID NO: 113.

Embodiment D30. The multi-chain chimeric polypeptide of embodiment D29, wherein the sushi domain from human IL-15Rα comprises a sequence that is 90% identical to SEQ ID NO: 113.

Embodiment D31. The multi-chain chimeric polypeptide of embodiment D30, wherein the sushi domain from human IL-15Rα comprises a sequence that is 95% identical to SEQ ID NO: 113.

Embodiment D32. The multi-chain chimeric polypeptide of embodiment D31, wherein the sushi domain from human IL-15Rα comprises SEQ ID NO: 113.

Embodiment D33. The multi-chain chimeric polypeptide of embodiment D28, wherein the sushi domain from human IL-15Rα is a mature full-length IL-15Rα.

Embodiment D34. The multi-chain chimeric polypeptide of any one of embodiments D1-D21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment D35. The multi-chain chimeric polypeptide of any one of embodiments D1-D34, wherein one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain.

Embodiment D36. The multi-chain chimeric polypeptide of embodiment D35, wherein the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains.

Embodiment D37. The multi-chain chimeric polypeptide of embodiment D35 or D36, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment D38. The multi-chain chimeric polypeptide of any one of embodiments D1-D34, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-15 or a soluble IL-18.

Embodiment D39. The multi-chain chimeric polypeptide of embodiment D38, wherein the first target-binding domain and the second target-binding domain are each independently a soluble IL-15 or a soluble IL-18.

Embodiment D40. The multi-chain chimeric polypeptide of any one of embodiments D1-D39, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-18 or a receptor of IL-12.

Embodiment D41. The multi-chain chimeric polypeptide of embodiment B40, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment D42. The multi-chain chimeric polypeptide of embodiment D41, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment D43. The multi-chain chimeric polypeptide of any one of embodiments D1-D39, wherein the first target-binding domain binds specifically to a

receptor for IL-12, and the second target-binding domain binds specifically to a receptor for IL-18.

Embodiment D44. The multi-chain chimeric polypeptide of any one of embodiments D1-D39, wherein the first target-binding domain binds specifically to a receptor for IL-18, and the second target-binding domain bind specifically to a receptor for IL-12.

Embodiment D45. The multi-chain chimeric polypeptide of embodiment D44, wherein the first target-binding domain comprises a soluble IL-18.

Embodiment D46. The multi-chain chimeric polypeptide of embodiment D45, wherein the soluble IL-18 is a soluble human IL-18.

Embodiment D47. The multi-chain chimeric polypeptide of embodiment D46, wherein the soluble human IL-18 comprises a sequence at least 80% identical to SEQ ID NO: 109.

Embodiment D48. The multi-chain chimeric polypeptide of embodiment D47, wherein the soluble human IL-18 comprises a sequence at least 90% identical to SEQ ID NO: 109.

Embodiment D49. The multi-chain chimeric polypeptide of embodiment D48, wherein the soluble human IL-18 comprises a sequence at least 95% identical to SEQ ID NO: 109.

Embodiment D50. The multi-chain chimeric polypeptide of embodiment D49, wherein the soluble human IL-18 comprises a sequence of SEQ ID NO: 109.

Embodiment D51. The multi-chain chimeric polypeptide of any one of embodiments D44-D50, wherein the second target-binding domain comprises a soluble IL-12.

Embodiment D52. The multi-chain chimeric polypeptide of embodiment D51, wherein the soluble IL-18 is a soluble human IL-12.

Embodiment D53. The multi-chain chimeric polypeptide of embodiment D52, wherein the soluble human IL-15 comprises a sequence of soluble human IL-12β (p40) and a sequence of soluble human IL-12α (p35).

Embodiment D54. The multi-chain chimeric polypeptide of embodiment D53, wherein the soluble human IL-15 further comprises a linker sequence between the sequence of soluble IL-12β (p40) and the sequence of soluble human IL-12α (p35).

Embodiment D55. The multi-chain chimeric polypeptide of embodiment D54, wherein the linker sequence comprises SEQ ID NO: 102.

Embodiment D56. The multi-chain chimeric polypeptide of any one of embodiments D53-D55, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 80% identical to SEQ ID NO: 81.

Embodiment D57. The multi-chain chimeric polypeptide of embodiment D56, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 90% identical to SEQ ID NO: 81.

Embodiment D58. The multi-chain chimeric polypeptide of embodiment D57, wherein the sequence of soluble human IL-12β (p40) comprises a sequence that is at least 95% identical to SEQ ID NO: 81.

Embodiment D59. The multi-chain chimeric polypeptide of embodiment D58, wherein the sequence of soluble human IL-12β (p40) comprises SEQ ID NO: 81.

Embodiment D60. The multi-chain chimeric polypeptide of any one of embodiments D53-D59, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 80% identical to SEQ ID NO: 80.

Embodiment D61. The multi-chain chimeric polypeptide of embodiment D60, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 90% identical to SEQ ID NO: 80.

Embodiment D62. The mule-chain chimeric polypeptide of embodiment D61, wherein the sequence of soluble human IL-12α (p35) comprises a sequence that is at least 95% identical to SEQ ID NO: 80.

Embodiment D63. The multi-chain chimeric polypeptide of embodiment D62, wherein the sequence of soluble human IL-12α (p35) comprises SEQ ID NO: 80.

Embodiment D64. The multi-chain chimeric polypeptide of embodiment D1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 174.

Embodiment D65. The multi-chain chimeric polypeptide of embodiment D64, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 174.

Embodiment D66. The multi-chain chimeric polypeptide of embodiment D65, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 174.

Embodiment D67. The multi-chain chimeric polypeptide of embodiment D66, wherein the first chimeric polypeptide comprises SEQ ID NO: 174.

Embodiment D68. The multi-chain chimeric polypeptide of embodiment D67, wherein the first chimeric polypeptide comprises SEQ ID NO: 176.

Embodiment D69. The multi-chain chimeric polypeptide of any one of embodiments D1 and D64-D68, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 178.

Embodiment D70. The multi-chain chimeric polypeptide of embodiment D69, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 178.

Embodiment D71. The multi-chain chimeric polypeptide of embodiment D70, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 178.

Embodiment D72. The multi-chain chimeric polypeptide of embodiment D71, wherein the second chimeric polypeptide comprises SEQ ID NO: 178.

Embodiment D73. The multi-chain chimeric polypeptide of embodiment D72, wherein the second chimeric polypeptide comprises SEQ ID NO: 180.

Embodiment D74. The multi-chain chimeric polypeptide of any one of embodiments D1-D63, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment D75. The multi-chain chimeric polypeptide of embodiment D74, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment D76. The multi-chain chimeric polypeptide of any one of embodiments D1-D63, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment D77. The multi-chain chimeric polypeptide of embodiment D76, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D78. The multi-chain chimeric polypeptide of embodiment D76, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment D79. The multi-chain chimeric polypeptide of embodiment D76, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment D80. The multi-chain chimeric polypeptide of embodiment D76, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment D81. The multi-chain chimeric polypeptide of embodiment D76, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D82. The multi-chain chimeric polypeptide of embodiment D81, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D83. The multi-chain chimeric polypeptide of embodiment D81, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D84. The multi-chain chimeric polypeptide of embodiment D81, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D85. The multi-chain chimeric polypeptide of embodiment D81, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment D86. The multi-chain chimeric polypeptide of embodiment D81, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment D87. The multi-chain chimeric polypeptide of embodiment D81, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment D88. The multi-chain chimeric polypeptide of any one of embodiments D1-D63 and D74-D87, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment D89. The multi-chain chimeric polypeptide of embodiment D88, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment D90. The multi-chain chimeric polypeptide of embodiment D88, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment D91. The multi-chain chimeric polypeptide of embodiment D88, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment D92. The multi-chain chimeric polypeptide of embodiment B88, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment D93. The multi-chain chimeric polypeptide of any one of embodiments D74-D92, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment D94. The multi-chain chimeric polypeptide of embodiment B93, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment D95. The multi-chain chimeric polypeptide of embodiment B94, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment D96. The multi-chain chimeric polypeptide of any one of embodiments D74-D92, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment D97. The multi-chain chimeric polypeptide of any one of embodiments D74-D96, wherein the one or more additional antigen-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment D98. The multi-chain chimeric polypeptide of any one of embodiments D74-D96, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment D99. The multi-chain chimeric polypeptide of embodiment B98, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment D100. The multi-chain chimeric polypeptide of any one of embodiments D74-D96, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment D101. The multi-chain chimeric polypeptide of embodiment B100, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, or a soluble CD28.

Embodiment D102. A composition comprising any of the multi-chain chimeric polypeptides of embodiments D1-D101.

Embodiment D103. The composition of embodiment D102, wherein the composition is a pharmaceutical composition.

Embodiment D104. A kit comprising at least one dose of the composition of embodiment D102 or D103.

Embodiment D105. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments D1-D101.

Embodiment D106. A vector comprising the nucleic acid of embodiment D105.

Embodiment D107. The vector of embodiment D106, wherein the vector is an expression vector.

Embodiment D108. A cell comprising the nucleic acid of embodiment D105 or the vector of embodiment D106 or D107.

Embodiment D109. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment D108 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment D110. A multi-chain chimeric polypeptide produced by the method of embodiment D109.

Embodiment D111. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment D112. The multi-chain chimeric polypeptide of embodiment D111, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment D113. The multi-chain chimeric polypeptide of embodiment D112, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment D114. The multi-chain chimeric polypeptide of embodiment D113, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment D115. The multi-chain chimeric polypeptide of embodiment D8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment D116. The multi-chain chimeric polypeptide of embodiment D115, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment D117. The multi-chain chimeric polypeptide of embodiment D116, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment D118. The multi-chain chimeric polypeptide of embodiment D117, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

Embodiment E1. A multi-chain chimeric polypeptide comprising:

(a) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(b) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or a ligand of tumor growth factor receptor β II (TGFβRII).

Embodiment E2. The multi-chain chimeric polypeptide of embodiment E1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment E3. The multi-chain chimeric polypeptide of embodiments E1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment E4. The multi-chain chimeric polypeptide of any one of embodiments E1-E3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment E5. The multi-chain chimeric polypeptide of any one of embodiments E1-E3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E6. The multi-chain chimeric polypeptide of any one of embodiments E1-E5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment E7. The multi-chain chimeric polypeptide of any one of embodiments E1-E5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment E8. The multi-chain chimeric polypeptide of any one of embodiments E1-E7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment E9. The multi-chain chimeric polypeptide of embodiment E8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment E10. The multi-chain chimeric polypeptide of embodiment E9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment E11. The multi-chain chimeric polypeptide of embodiment E10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment E12. The multi-chain chimeric polypeptide of any one of embodiments E8-E11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment E13. The multi-chain chimeric polypeptide of embodiment E12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment E14. The multi-chain chimeric polypeptide of any one of embodiments E1-E13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment E15. The multi-chain chimeric polypeptide of any one of embodiments E1-E14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment E16. The multi-chain chimeric polypeptide of any one of embodiments E1-E15, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment E17. The multi-chain chimeric polypeptide of any one of embodiments E1-E16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment E18. The multi-chain chimeric polypeptide of any one of embodiments E1-E17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment E19. The multi-chain chimeric polypeptide of any one of embodiments E1-E18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment E20. The multi-chain chimeric polypeptide of embodiment E19, wherein the signal sequence comprises SEQ ID NO: 117.

Embodiment E21. The multi-chain chimeric polypeptide of embodiment E20, wherein the signal sequence is SEQ ID NO: 117.

Embodiment E22. The multi-chain chimeric polypeptide of any one of embodiments E1-E21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα and a soluble IL-15.

Embodiment E23. The multi-chain chimeric polypeptide of embodiment E22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment E24. The multi-chain chimeric polypeptide of embodiment E22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 82.

Embodiment E25. The multi-chain chimeric polypeptide of embodiment E24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 82.

Embodiment E26. The multi-chain chimeric polypeptide of embodiment E25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 82.

Embodiment E27. The multi-chain chimeric polypeptide of embodiment E26, wherein the soluble IL-15 comprises SEQ ID NO: 82.

Embodiment E28. The multi-chain chimeric polypeptide of any one of embodiments E22-E27, wherein the sushi domain of IL-15Rα comprises a sushi domain from human IL-15Rα.

Embodiment E29. The multi-chain chimeric polypeptide of embodiment E28, wherein the sushi domain from human IL-15Rα comprises a sequence that is 80% identical to SEQ ID NO: 113.

Embodiment E30. The multi-chain chimeric polypeptide of embodiment E29, wherein the sushi domain from human IL-15Rα comprises a sequence that is 90% identical to SEQ ID NO: 113.

Embodiment E31. The multi-chain chimeric polypeptide of embodiment E30, wherein the sushi domain from human IL-15Rα comprises a sequence that is 95% identical to SEQ ID NO: 113.

Embodiment E32. The multi-chain chimeric polypeptide of embodiment E31, wherein the sushi domain from human IL-15Rα comprises SEQ ID NO: 113.

Embodiment E33. The multi-chain chimeric polypeptide of embodiment E28, wherein the sushi domain from human IL-15Rα is a mature full-length IL-15Rα.

Embodiment E34. The multi-chain chimeric polypeptide of any one of embodiments E1-E21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment E35. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment E36. The multi-chain chimeric polypeptide of embodiment E35, wherein the first target-binding domain and the second target-binding domain are antigen-binding domains.

Embodiment E37. The multi-chain chimeric polypeptide of embodiment E35 or E36, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment E38. The multi-chain chimeric polypeptide of any one of embodiments E1-E34, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 or a soluble TGFβRII.

Embodiment E39. The multi-chain chimeric polypeptide of any one of embodiments E1-E38, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a ligand of TGFβRII.

Embodiment E40. The multi-chain chimeric polypeptide of embodiment E39, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment E41. The multi-chain chimeric polypeptide of embodiment E40, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment E42. The multi-chain chimeric polypeptide of any one of embodiments E1-E38, wherein the first target-binding domain binds specifically to a ligand of TGFβRII, and the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment E43. The multi-chain chimeric polypeptide of any one of embodiments E1-E38, wherein the first target-binding domain binds specifically to a

receptor for IL-21, and the second target-binding domain bind specifically to a ligand of TGFβRII.

Embodiment E44. The multi-chain chimeric polypeptide of embodiment E43, wherein the first target-binding domain comprises a soluble IL-21.

Embodiment E45. The multi-chain chimeric polypeptide of embodiment E44, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment E46. The multi-chain chimeric polypeptide of embodiment E45, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 83.

Embodiment E47. The multi-chain chimeric polypeptide of embodiment E46, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 83.

Embodiment E48. The multi-chain chimeric polypeptide of embodiment E47, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 83.

Embodiment E49. The multi-chain chimeric polypeptide of embodiment E48, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 83.

Embodiment E50. The multi-chain chimeric polypeptide of any one of embodiments E43-E49, wherein the second target-binding domain comprises a soluble TGFβRII.

Embodiment E51. The multi-chain chimeric polypeptide of embodiment E50, wherein the soluble TGFβRII is a soluble human TGFβRII.

Embodiment E52. The multi-chain chimeric polypeptide of embodiment E51, wherein the soluble human TGFβRII comprises a first sequence of soluble human TGFβRII and a second sequence of soluble human TGFβRII.

Embodiment E53. The multi-chain chimeric polypeptide of embodiment E52, wherein the soluble human TGFβRII further comprises a linker sequence between the first sequence of soluble human TGFβRII and the second sequence of soluble human TGFβRII.

Embodiment E54. The multi-chain chimeric polypeptide of embodiment E53, wherein the linker sequence comprises SEQ ID NO: 102.

Embodiment E55. The multi-chain chimeric polypeptide of any one of embodiments E52-E54, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 80% identical to SEQ ID NO: 183.

Embodiment E56. The multi-chain chimeric polypeptide of embodiment E55, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 90% identical to SEQ ID NO: 183.

Embodiment E57. The multi-chain chimeric polypeptide of embodiment E56, wherein the first sequence of soluble human TGFβRII comprises a sequence that is at least 95% identical to SEQ ID NO: 183.

Embodiment E58. The multi-chain chimeric polypeptide of embodiment E57, wherein the first sequence of soluble human TGFβRII comprises SEQ ID NO: 183.

Embodiment E59. The multi-chain chimeric polypeptide of any one of embodiments E52-E58, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 80% identical to SEQ ID NO: 184.

Embodiment E60. The multi-chain chimeric polypeptide of embodiment E59, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 90% identical to SEQ ID NO: 184.

Embodiment E61. The mule-chain chimeric polypeptide of embodiment E60, wherein the second sequence of soluble human TGFβRII comprises a sequence that is at least 95% identical to SEQ ID NO: 184.

Embodiment E62. The multi-chain chimeric polypeptide of embodiment E61, wherein the second sequence of soluble human TGFβRII comprises SEQ ID NO: 184.

Embodiment E63. The multi-chain chimeric polypeptide of embodiment E1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 189.

Embodiment E64. The multi-chain chimeric polypeptide of embodiment E63, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 189.

Embodiment E65. The multi-chain chimeric polypeptide of embodiment E64, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 189.

Embodiment E66. The multi-chain chimeric polypeptide of embodiment E65, wherein the first chimeric polypeptide comprises SEQ ID NO: 189.

Embodiment E67. The multi-chain chimeric polypeptide of embodiment E66, wherein the first chimeric polypeptide comprises SEQ ID NO: 191.

Embodiment E68. The multi-chain chimeric polypeptide of any one of embodiments E1 and E63-E67, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 193.

Embodiment E69. The multi-chain chimeric polypeptide of embodiment E68, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 193.

Embodiment E70. The multi-chain chimeric polypeptide of embodiment E69, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 193.

Embodiment E71. The multi-chain chimeric polypeptide of embodiment E70, wherein the second chimeric polypeptide comprises SEQ ID NO: 193.

Embodiment E72. The multi-chain chimeric polypeptide of embodiment E71, wherein the second chimeric polypeptide comprises SEQ ID NO: 195.

Embodiment E73. The multi-chain chimeric polypeptide of any one of embodiments E1-E62, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment E74. The multi-chain chimeric polypeptide of embodiment E73, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment E75. The multi-chain chimeric polypeptide of any one of embodiments E1-E62, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment E76. The multi-chain chimeric polypeptide of embodiment E75, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E77. The multi-chain chimeric polypeptide of embodiment E75, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment E78. The multi-chain chimeric polypeptide of embodiment E75, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment E79. The multi-chain chimeric polypeptide of embodiment E75, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment E80. The multi-chain chimeric polypeptide of embodiment E75, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E81. The multi-chain chimeric polypeptide of embodiment E80, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E82. The multi-chain chimeric polypeptide of embodiment E80, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E83. The multi-chain chimeric polypeptide of embodiment E80, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E84. The multi-chain chimeric polypeptide of embodiment E80, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment E85. The multi-chain chimeric polypeptide of embodiment E80, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment E86. The multi-chain chimeric polypeptide of embodiment E80, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment E87. The multi-chain chimeric polypeptide of any one of embodiments E1-E62 and E73-E86, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment E88. The multi-chain chimeric polypeptide of embodiment E87, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment E89. The multi-chain chimeric polypeptide of embodiment E87, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment E90. The multi-chain chimeric polypeptide of embodiment E87, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment E91. The multi-chain chimeric polypeptide of embodiment E87, wherein the second chimeric polypeptide further comprises a linker sequence between at

least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment E92. The multi-chain chimeric polypeptide of any one of embodiments E73-E91, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment E93. The multi-chain chimeric polypeptide of embodiment E92, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment E94. The multi-chain chimeric polypeptide of embodiment E93, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment E95. The multi-chain chimeric polypeptide of any one of embodiments E73-E91, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment E96. The multi-chain chimeric polypeptide of any one of embodiments E73-E95, wherein the one or more additional antigen-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein,

HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-D, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment E97. The multi-chain chimeric polypeptide of any one of embodiments E73-E95, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment E98. The multi-chain chimeric polypeptide of embodiment E97, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment E99. The multi-chain chimeric polypeptide of any one of embodiments E73-E95, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment E100. The multi-chain chimeric polypeptide of embodiment E99, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-β RII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, , or a soluble CD28.

Embodiment E101. A composition comprising any of the multi-chain chimeric polypeptides of embodiments E1-E100.

Embodiment E102. The composition of embodiment E101, wherein the composition is a pharmaceutical composition.

Embodiment E103. A kit comprising at least one dose of the composition of embodiment E101 or E102.

Embodiment E104. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments E1-E100.

Embodiment E105. A vector comprising the nucleic acid of embodiment E104.

Embodiment E106. The vector of embodiment E105, wherein the vector is an expression vector.

Embodiment E107. A cell comprising the nucleic acid of embodiment C104 or the vector of embodiment E105 or E106.

Embodiment E108. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment E107 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment E109. A multi-chain chimeric polypeptide produced by the method of embodiment E108.

Embodiment E110. The multi-chain chimeric polypeptide of embodiment E12, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment E111. The multi-chain chimeric polypeptide of embodiment E110, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment E112. The multi-chain chimeric polypeptide of embodiment E111, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment E113. The multi-chain chimeric polypeptide of embodiment E112, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment E114. The multi-chain chimeric polypeptide of embodiment E12, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment E115. The multi-chain chimeric polypeptide of embodiment E114, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment E116. The multi-chain chimeric polypeptide of embodiment E115, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment E117. The multi-chain chimeric polypeptide of embodiment E116, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

Embodiment F1. A multi-chain chimeric polypeptide comprising:

(c) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(d) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains;

the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor of IL-21 or a receptor of IL-7.

Embodiment F2. The multi-chain chimeric polypeptide of embodiment F1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment F3. The multi-chain chimeric polypeptide of embodiment F1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment F4. The multi-chain chimeric polypeptide of any one of embodiments F1-F3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment F5. The multi-chain chimeric polypeptide of any one of embodiments F1-F3, wherein the first chimeric polypeptide further comprises a linker

sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F6. The multi-chain chimeric polypeptide of any one of embodiments F1-F5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment F7. The multi-chain chimeric polypeptide of any one of embodiments F1-F5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment F8. The multi-chain chimeric polypeptide of any one of embodiments F1-F7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment F9. The multi-chain chimeric polypeptide of embodiment F8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment F10. The multi-chain chimeric polypeptide of embodiment F9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment F11. The multi-chain chimeric polypeptide of embodiment F10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment F12. The multi-chain chimeric polypeptide of embodiment F11, wherein the soluble human tissue factor domain comprises SEQ ID NO: 93.

Embodiment F13. The multi-chain chimeric polypeptide of embodiment F8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment F14. The multi-chain chimeric polypeptide of embodiment F13, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment F15. The multi-chain chimeric polypeptide of embodiment F14, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment F16. The multi-chain chimeric polypeptide of embodiment F15, wherein the soluble human tissue factor domain comprises SEQ ID NO: 97.

Embodiment F17. The multi-chain chimeric polypeptide of embodiment F8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment F18. The multi-chain chimeric polypeptide of embodiment F17, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment F19. The multi-chain chimeric polypeptide of embodiment F18, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment F20. The multi-chain chimeric polypeptide of embodiment F19, wherein the soluble human tissue factor domain comprises SEQ ID NO: 98.

Embodiment F21. The multi-chain chimeric polypeptide of any one of embodiments F8-F11, F13-F15, and F17-F19, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment F22. The multi-chain chimeric polypeptide of embodiment F21, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment F23. The multi-chain chimeric polypeptide of any one of embodiments F1-F22, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment F24. The multi-chain chimeric polypeptide of any one of embodiments F1-F23, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment F25. The multi-chain chimeric polypeptide of any one of embodiments F1-F24, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment F26. The multi-chain chimeric polypeptide of any one of embodiments F1-F25, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment F27. The multi-chain chimeric polypeptide of any one of embodiments F1-F26, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment F28. The multi-chain chimeric polypeptide of any one of embodiments F1-F27, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment F29. The multi-chain chimeric polypeptide of embodiment F28, wherein the signal sequence comprises SEQ ID NO: 117.

Embodiment F30. The multi-chain chimeric polypeptide of embodiment F28, wherein the signal sequence is SEQ ID NO: 328.

Embodiment F31. The multi-chain chimeric polypeptide of any one of embodiments F1-F30, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

Embodiment F32. The multi-chain chimeric polypeptide of embodiment F31, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment F33. The multi-chain chimeric polypeptide of embodiment F31, wherein the soluble IL-15 comprises a sequence that is at least 80% identical to SEQ ID NO: 82.

Embodiment F34. The multi-chain chimeric polypeptide of embodiment F33, wherein the soluble IL-15 comprises a sequence that is at least 90% identical to SEQ ID NO: 82.

Embodiment F35. The multi-chain chimeric polypeptide of embodiment F34, wherein the soluble IL-15 comprises a sequence that is at least 95% identical to SEQ ID NO: 82.

Embodiment F36. The multi-chain chimeric polypeptide of embodiment F35, wherein the soluble IL-15 comprises SEQ ID NO: 82.

Embodiment F37. The multi-chain chimeric polypeptide of any one of embodiments F31-F36, wherein the sushi domain of IL-15Rα comprises a sushi domain from human IL-15Rα.

Embodiment F38. The multi-chain chimeric polypeptide of embodiment F37, wherein the sushi domain from human IL-15Rα comprises a sequence that is at least 80% identical to SEQ ID NO: 113.

Embodiment F39. The multi-chain chimeric polypeptide of embodiment F38, wherein the sushi domain from human IL-15Rα comprises a sequence that is at least 90% identical to SEQ ID NO: 113.

Embodiment F40. The multi-chain chimeric polypeptide of embodiment F39, wherein the sushi domain from human IL-15Rα comprises a sequence that is at least 95% identical to SEQ ID NO: 113.

Embodiment F41. The multi-chain chimeric polypeptide of embodiment F40, wherein the sushi domain from human IL-15Rα comprises SEQ ID NO: 113.

Embodiment F42. The multi-chain chimeric polypeptide of embodiment F37, wherein the sushi domain from human IL-15Rα is a mature full-length IL-15Rα.

Embodiment F43. The multi-chain chimeric polypeptide of any one of embodiments F1-F30, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment F44. The multi-chain chimeric polypeptide of any one of embodiments F1-F43, wherein one or both of the first target-binding domain and the second target-binding domain is an agonistic antigen-binding domain.

Embodiment F45. The multi-chain chimeric polypeptide of embodiment F44, wherein the first target-binding domain and the second target-binding domain are each agonistic antigen-binding domains.

Embodiment F46. The multi-chain chimeric polypeptide of embodiment F44 or F45, wherein antigen-binding domain comprises a scFv or single-domain antibody.

Embodiment F47. The multi-chain chimeric polypeptide of any one of embodiments F1-F43, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble IL-21 or a soluble IL-7.

Embodiment F48. The multi-chain chimeric polypeptide of embodiment F47, wherein the first target-binding domain and the second target-binding domain are each independently a soluble IL-21 or a soluble IL-7.

Embodiment F49. The multi-chain chimeric polypeptide of any one of embodiments F1-F48, wherein the first target-binding domain and the second target-binding domain both bind specifically to a receptor of IL-21 or a receptor of IL-7.

Embodiment F50. The multi-chain chimeric polypeptide of embodiment F49, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment F51. The multi-chain chimeric polypeptide of embodiment F50, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment F52. The multi-chain chimeric polypeptide of any one of embodiments F1-F48, wherein the first target-binding domain binds specifically to a receptor for IL-21, and the second target-binding domain binds specifically to a receptor for IL-7.

Embodiment F53. The multi-chain chimeric polypeptide of any one of embodiments F1-F48, wherein the first target-binding domain binds specifically to a receptor for IL-7, and the second target-binding domain bind specifically to a receptor for IL-21.

Embodiment F54. The multi-chain chimeric polypeptide of embodiment F53, wherein the first target-binding domain comprises a soluble IL-21.

Embodiment F55. The multi-chain chimeric polypeptide of embodiment F54, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment F56. The multi-chain chimeric polypeptide of embodiment F55, wherein the soluble human IL-21 comprises a sequence at least 80% identical to SEQ ID NO: 83.

Embodiment F57. The multi-chain chimeric polypeptide of embodiment F56, wherein the soluble human IL-21 comprises a sequence at least 90% identical to SEQ ID NO: 83.

Embodiment F58. The multi-chain chimeric polypeptide of embodiment F57, wherein the soluble human IL-21 comprises a sequence at least 95% identical to SEQ ID NO: 83.

Embodiment F59. The multi-chain chimeric polypeptide of embodiment F58, wherein the soluble human IL-21 comprises a sequence of SEQ ID NO: 83.

Embodiment F60. The multi-chain chimeric polypeptide of any one of embodiments F53-F59, wherein the second target-binding domain comprises a soluble IL-7.

Embodiment F61. The multi-chain chimeric polypeptide of embodiment D60, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment F62. The multi-chain chimeric polypeptide of embodiment F61, wherein the soluble human IL-7 comprises a sequence at least 80% identical to SEQ ID NO: 79.

Embodiment F63. The multi-chain chimeric polypeptide of embodiment F62, wherein the soluble human IL-7 comprises a sequence at least 90% identical to SEQ ID NO: 79.

Embodiment F64. The multi-chain chimeric polypeptide of embodiment F63, wherein the soluble human IL-7 comprises a sequence at least 95% identical to SEQ ID NO: 79.

Embodiment F65. The multi-chain chimeric polypeptide of embodiment F64, wherein the soluble human IL-7 comprises a sequence of SEQ ID NO: 79.

Embodiment F66. The multi-chain chimeric polypeptide of embodiment F1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment F67. The multi-chain chimeric polypeptide of embodiment F66, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment F68. The multi-chain chimeric polypeptide of embodiment F67, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment F69. The multi-chain chimeric polypeptide of embodiment F68, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment F70. The multi-chain chimeric polypeptide of embodiment F69, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment F71. The multi-chain chimeric polypeptide of any one of embodiments F1 and F66-F70, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 211.

Embodiment F72. The multi-chain chimeric polypeptide of embodiment F71, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 211.

Embodiment F73. The multi-chain chimeric polypeptide of embodiment F72, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 211.

Embodiment F74. The multi-chain chimeric polypeptide of embodiment F73, wherein the second chimeric polypeptide comprises SEQ ID NO: 211.

Embodiment F75. The multi-chain chimeric polypeptide of embodiment F74, wherein the second chimeric polypeptide comprises SEQ ID NO: 213.

Embodiment F76. The multi-chain chimeric polypeptide of embodiment F1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 199.

Embodiment F77. The multi-chain chimeric polypeptide of embodiment F76, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 199.

Embodiment F78. The multi-chain chimeric polypeptide of embodiment F77, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 199.

Embodiment F79. The multi-chain chimeric polypeptide of embodiment F68, wherein the first chimeric polypeptide comprises SEQ ID NO: 199.

Embodiment F80. The multi-chain chimeric polypeptide of embodiment F69, wherein the first chimeric polypeptide comprises SEQ ID NO: 201.

Embodiment F81. The multi-chain chimeric polypeptide of any one of embodiments F1 and F76-F80, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 203.

Embodiment F82. The multi-chain chimeric polypeptide of embodiment F81, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 203.

Embodiment F83. The multi-chain chimeric polypeptide of embodiment F82, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 203.

Embodiment F84. The multi-chain chimeric polypeptide of embodiment F83, wherein the second chimeric polypeptide comprises SEQ ID NO: 203.

Embodiment F85. The multi-chain chimeric polypeptide of embodiment F84, wherein the second chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment F86. The multi-chain chimeric polypeptide of any one of embodiments F1-F65, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment F87. The multi-chain chimeric polypeptide of embodiment F86, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment F88. The multi-chain chimeric polypeptide of any one of embodiments F1-F65, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment F89. The multi-chain chimeric polypeptide of embodiment F88, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F90. The multi-chain chimeric polypeptide of embodiment F88, wherein the first chimeric polypeptide further comprises a linker sequence between the at

least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment F91. The multi-chain chimeric polypeptide of embodiment F88, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment F92. The multi-chain chimeric polypeptide of embodiment F88, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment F93. The multi-chain chimeric polypeptide of embodiment F88, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F94. The multi-chain chimeric polypeptide of embodiment F93, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F95. The multi-chain chimeric polypeptide of embodiment F93, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F96. The multi-chain chimeric polypeptide of embodiment F93, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F97. The multi-chain chimeric polypeptide of embodiment F93, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment F98. The multi-chain chimeric polypeptide of embodiment F93, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment F99. The multi-chain chimeric polypeptide of embodiment F93, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment F100. The multi-chain chimeric polypeptide of any one of embodiments F1-F65 and F86-F99, wherein the second chimeric polypeptide further

comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment F101. The multi-chain chimeric polypeptide of embodiment F100, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment F102. The multi-chain chimeric polypeptide of embodiment F100, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment F103. The multi-chain chimeric polypeptide of embodiment F100, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment F104. The multi-chain chimeric polypeptide of embodiment F100, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment F105. The multi-chain chimeric polypeptide of any one of embodiments F86-F104, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment F106. The multi-chain chimeric polypeptide of embodiment F105, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment F107. The multi-chain chimeric polypeptide of embodiment F106, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment F108. The multi-chain chimeric polypeptide of any one of embodiments F86-F104, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment F109. The multi-chain chimeric polypeptide of any one of embodiments F86-F108, wherein the one or more additional antigen-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD28, CD3, CD33, CD20, CD19, CD22, CD123, IL-1R, IL-1, VEGF, IL-6R, IL-4, IL-10, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for IL-1, a receptor for IL-2, a receptor for IL-3, a receptor for IL-7, a receptor for IL-8, a receptor for IL-10, a receptor for IL-12, a receptor for IL-15, a receptor for IL-17, a receptor for IL-18, a receptor for IL-21, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD28.

Embodiment F110. The multi-chain chimeric polypeptide of any one of embodiments F86-F108, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment F111. The multi-chain chimeric polypeptide of embodiment F110, wherein the soluble interleukin, cytokine, or ligand protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, SCF, FLT3L, MICA, MICB, and a ULP16-binding protein.

Embodiment F112. The multi-chain chimeric polypeptide of any one of embodiments F86-F108, wherein the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment F113. The multi-chain chimeric polypeptide of embodiment F112, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII), a soluble TGF-βRIII, a soluble NKG2D, a soluble NKp30, a soluble NKp44, a soluble NKp46, a soluble DNAM1, a scMHCI, a scMHCII, a scTCR, a soluble CD155, a soluble CD122, or a soluble CD28.

Embodiment F114. A composition comprising any of the multi-chain chimeric polypeptides of embodiments F1-F113.

Embodiment F115. The composition of embodiment F114, wherein the composition is a pharmaceutical composition.

Embodiment F116. A kit comprising at least one dose of the composition of embodiment F114 or F115.

Embodiment F117. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments F1-F113.

Embodiment F118. A vector comprising the nucleic acid of embodiment F117.

Embodiment F119. The vector of embodiment F118, wherein the vector is an expression vector.

Embodiment F120. A cell comprising the nucleic acid of embodiment F117 or the vector of embodiment F118 or F119.

Embodiment F121. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment F120 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment F122. A multi-chain chimeric polypeptide produced by the method of embodiment F121.

Embodiment G1. A multi-chain chimeric polypeptide comprising:

(e) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(f) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein:

the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; and

the first target-binding domain and the second targeting-binding domain each independently bind specifically to: a receptor for IL-7, CD16, a receptor for IL-21, TGF-β, or a receptor for CD137L.

Embodiment G2. The multi-chain chimeric polypeptide of embodiment G1, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment G3. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment G4. The multi-chain chimeric polypeptide of any one of embodiments G1-G3, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment G5. The multi-chain chimeric polypeptide of any one of embodiments G1-G3, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G6. The multi-chain chimeric polypeptide of any one of embodiments G1-G5, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment G7. The multi-chain chimeric polypeptide of any one of embodiments G1-G5, wherein second chimeric polypeptide further comprises a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment G8. The multi-chain chimeric polypeptide of any one of embodiments G1-G7, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment G9. The multi-chain chimeric polypeptide of embodiment G8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment G10. The multi-chain chimeric polypeptide of embodiment G9, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment G11. The multi-chain chimeric polypeptide of embodiment G10, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment G12. The multi-chain chimeric polypeptide of any one of embodiments G8-G11, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment G13. The multi-chain chimeric polypeptide of embodiment G12, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment G14. The multi-chain chimeric polypeptide of any one of embodiments G1-G13, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment G15. The multi-chain chimeric polypeptide of any one of embodiments G1-G14, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment G16. The multi-chain chimeric polypeptide of any one of embodiments G1-G15, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment G17. The multi-chain chimeric polypeptide of any one of embodiments G1-G16, wherein the first chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the first chimeric polypeptide.

Embodiment G18. The multi-chain chimeric polypeptide of any one of embodiments G1-G17, wherein the second chimeric polypeptide further comprises a peptide tag at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment G19. The multi-chain chimeric polypeptide of any one of embodiments G1-G18, wherein the first chimeric polypeptide and/or the second chimeric polypeptide further comprises a signal sequence at its N-terminal end.

Embodiment G20. The multi-chain chimeric polypeptide of embodiment G19, wherein the signal sequence comprises SEQ ID NO: 117.

Embodiment G21. The multi-chain chimeric polypeptide of embodiment G20, wherein the signal sequence is SEQ ID NO: 117.

Embodiment G22. The multi-chain chimeric polypeptide of any one of embodiments G1-G21, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

Embodiment G23. The multi-chain chimeric polypeptide of embodiment G22, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment G24. The multi-chain chimeric polypeptide of embodiment G22, wherein the soluble IL-15 comprises a sequence that is 80% identical to SEQ ID NO: 82.

Embodiment G25. The multi-chain chimeric polypeptide of embodiment G24, wherein the soluble IL-15 comprises a sequence that is 90% identical to SEQ ID NO: 82.

Embodiment G26. The multi-chain chimeric polypeptide of embodiment G25, wherein the soluble IL-15 comprises a sequence that is 95% identical to SEQ ID NO: 82.

Embodiment G27. The multi-chain chimeric polypeptide of embodiment G26, wherein the soluble IL-15 comprises SEQ ID NO: 82.

Embodiment G28. The multi-chain chimeric polypeptide of any one of embodiments G22-G27, wherein the sushi domain of IL-15Rα comprises a sushi domain from human IL-15Rα.

Embodiment G29. The multi-chain chimeric polypeptide of embodiment G28, wherein the sushi domain from human IL-15Rα comprises a sequence that is 80% identical to SEQ ID NO: 113.

Embodiment G30. The multi-chain chimeric polypeptide of embodiment G29, wherein the sushi domain from human IL-15Rα comprises a sequence that is 90% identical to SEQ ID NO: 113.

Embodiment G31. The multi-chain chimeric polypeptide of embodiment G30, wherein the sushi domain from human IL-15Rα comprises a sequence that is 95% identical to SEQ ID NO: 113.

Embodiment G32. The multi-chain chimeric polypeptide of embodiment G31, wherein the sushi domain from human IL-15Rα comprises SEQ ID NO: 113.

Embodiment G33. The multi-chain chimeric polypeptide of embodiment G28, wherein the sushi domain from human IL-15Rα is a mature full-length IL-15Rα.

Embodiment G34. The multi-chain chimeric polypeptide of any one of embodiments G1-G21, wherein the pair of affinity domains is selected from the group consisting of: barnase and barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment G35. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7, CD16, or a receptor for IL-21.

Embodiment G36. The multi-chain chimeric polypeptide of embodiment G35, wherein the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to CD16 or a receptor for IL-21.

Embodiment G37. The multi-chain chimeric polypeptide of embodiment G36, wherein the first target-binding domain comprises a soluble IL-7 protein.

Embodiment G38. The multi-chain chimeric polypeptide of embodiment G37, wherein the soluble IL-7 protein is a soluble human IL-7.

Embodiment G39. The multi-chain chimeric polypeptide of any one of embodiments G36-G38, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment G40. The multi-chain chimeric polypeptide of embodiment G39, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment G41. The multi-chain chimeric polypeptide of any one of embodiments G36-G38, wherein the second antigen-binding domain bind specifically to a receptor for IL-21.

Embodiment G42. The multi-chain chimeric polypeptide of embodiment G41, wherein the second antigen-binding domain comprises a soluble IL-21.

Embodiment G43. The multi-chain chimeric polypeptide of embodiment G42, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment G44. The multi-chain chimeric polypeptide of any one of embodiments G36-G40, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for IL-21.

Embodiment G45. The multi-chain chimeric polypeptide of embodiment G44, wherein the additional target-binding domain comprises a soluble IL-21.

Embodiment G46. The multi-chain chimeric polypeptide of embodiment G45, wherein the soluble IL-21 is a soluble human IL-12.

Embodiment G47. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, CD16, or a receptor for IL-21.

Embodiment G48. The multi-chain chimeric polypeptide of embodiment G47, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to CD16 or a receptor of IL-21.

Embodiment G49. The multi-specific chimeric polypeptide of embodiment G48, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G50. The multi-specific chimeric polypeptide of embodiment G49, wherein soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G51. The multi-specific chimeric polypeptide of any one of embodiments G48-G50, wherein the second target-binding domain binds specifically to CD16.

Embodiment G52. The multi-specific chimeric polypeptide of embodiment G51, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment G53. The multi-chain chimeric polypeptide of embodiment G52, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment G54. The multi-chain chimeric polypeptide of any one of embodiments G48-G50, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment G55. The multi-chain chimeric polypeptide of embodiment G54, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment G56. The multi-chain chimeric polypeptide of embodiment G55, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment G57. The multi-chain chimeric polypeptide of any one of embodiments G48-G53, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for IL-21.

Embodiment G58. The multi-chain chimeric polypeptide of embodiment G57, wherein the additional target-binding domain comprises a soluble IL-21.

Embodiment G59. The multi-chain chimeric polypeptide of embodiment G58, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment G60. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second target-binding domain each independently bind specifically to a receptor for IL-7.

Embodiment G61. The multi-chain chimeric polypeptide of embodiment G60, wherein the first target-binding domain and the second target-binding domain include a soluble IL-7.

Embodiment G62. The multi-chain chimeric polypeptide of embodiment G61, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment G63. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second target-binding domain each independently bind specifically to TGF-β.

Embodiment G64. The multi-specific chimeric polypeptide of embodiment G63, wherein the first target-binding domain and the second target-binding domain is a soluble TGF-β receptor.

Embodiment G65. The multi-specific chimeric polypeptide of embodiment G64, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G66. The multi-specific chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7, a receptor for IL-21, or a receptor for CD137L.

Embodiment G67. The multi-chain chimeric polypeptide of embodiment G66, wherein the first target-binding domain binds specifically to a receptor for IL-7 and the

second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

Embodiment G68. The multi-specific chimeric polypeptide of embodiment G67, wherein the first target-binding domain is a soluble IL-7.

Embodiment G69. The multi-specific chimeric polypeptide of embodiment G68, wherein the soluble IL-7 is a soluble human IL-7.

Embodiment G70. The multi-chain chimeric polypeptide of any one of embodiments G67-G69, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment G71. The multi-chain chimeric polypeptide of embodiment G70, wherein the second target-binding domain is a soluble IL-21.

Embodiment G72. The multi-chain chimeric polypeptide of embodiment G71, wherein the soluble IL-21 is a soluble human IL-21.

Embodiment G73. The multi-chain chimeric polypeptide of any one of embodiments G67-G69, wherein the second antigen-binding domain binds specifically to a receptor for CD137L.

Embodiment G74. The multi-chain chimeric polypeptide of embodiment G73, wherein the second antigen-binding domain is a soluble CD137L.

Embodiment G75. The multi-chain chimeric polypeptide of embodiment G74, wherein the soluble CD137L is a soluble human CD137L.

Embodiment G76. The multi-chain chimeric polypeptide of any one of embodiments G67-G72, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L.

Embodiment G77. The multi-chain chimeric polypeptide of embodiment G76, wherein the additional target-binding domain comprises a soluble CD137L.

Embodiment G78. The multi-chain chimeric polypeptide of embodiment G77, wherein the soluble CD137L is a soluble human CD137L.

Embodiment G79. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a receptor for IL-7 or TGF-β.

Embodiment G80. The multi-chain chimeric polypeptide of embodiment G79, wherein the first target-binding domain binds specifically to a receptor IL-7 and the second target-binding domain binds specifically to TGF-β.

Embodiment G81. The multi-chain chimeric polypeptide of embodiment G80, wherein the first target-binding domain comprises a soluble IL-7 protein.

Embodiment G82. The multi-chain chimeric polypeptide of embodiment G81, wherein the soluble IL-7 protein is a soluble human IL-7.

Embodiment G83. The multi-chain chimeric polypeptide of any one of embodiments G80-G82, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to TGF-β.

Embodiment G84. The multi-specific chimeric polypeptide of embodiment G83, wherein the second target-binding domain is a soluble TGF-β receptor.

Embodiment G85. The multi-specific chimeric polypeptide of embodiment G84, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G86. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β, a receptor for IL-21, or a receptor for CD137L.

Embodiment G87. The multi-chain chimeric polypeptide of embodiment G86, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to a receptor for IL-21 or a receptor for CD137L.

Embodiment G88. The multi-specific chimeric polypeptide of embodiment G87, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G89. The multi-specific chimeric polypeptide of embodiment G88, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G90. The multi-specific chimeric polypeptide of any one of embodiments G87-G89, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment G91. The multi-chain chimeric polypeptide of embodiment G90, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment G92. The multi-chain chimeric polypeptide of embodiment G91, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment G93. The multi-specific chimeric polypeptide of any one of embodiments G87-G89, wherein the second target-binding domain binds specifically to a receptor for CD137L.

Embodiment G94. The multi-chain chimeric polypeptide of embodiment G93, wherein the second target-binding domain comprises a soluble CD137L.

Embodiment G95. The multi-chain chimeric polypeptide of embodiment G94, wherein the second target-binding domain comprises a soluble human CD137L.

Embodiment G96. The multi-chain chimeric polypeptide of any one of embodiments G87-G92, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to a receptor for CD137L.

Embodiment G97. The multi-chain chimeric polypeptide of embodiment G96, wherein the additional target-binding domain comprises a soluble CD137L.

Embodiment G98. The multi-chain chimeric polypeptide of embodiment G97, wherein the soluble CD137L is a soluble human CD137L.

Embodiment G99. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or a receptor for IL-21.

Embodiment G100. The multi-chain chimeric polypeptide of embodiment G99, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to TGF-β or a receptor for IL-21.

Embodiment G101. The multi-specific chimeric polypeptide of embodiment G100, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G102. The multi-specific chimeric polypeptide of embodiment G101, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G103. The multi-specific chimeric polypeptide of any one of embodiments G100-G102, wherein the second target-binding domain binds specifically to a receptor for IL-21.

Embodiment G104. The multi-chain chimeric polypeptide of embodiment G103, wherein the second target-binding domain comprises a soluble IL-21.

Embodiment G105. The multi-chain chimeric polypeptide of embodiment G104, wherein the second target-binding domain comprises a soluble human IL-21.

Embodiment G106. The multi-specific chimeric polypeptide of any one of embodiments G100-G102, wherein the second target-binding domain binds specifically to TGF-β.

Embodiment G107. The multi-specific chimeric polypeptide of embodiment G106, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G108. The multi-specific chimeric polypeptide of embodiment G107, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G109. The multi-specific chimeric polypeptide of any one of embodiments G100-G105, wherein the second polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment G110. The multi-specific chimeric polypeptide of embodiment G109, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G111. The multi-specific chimeric polypeptide of embodiment G110, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G112. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to TGF-β or IL-16.

Embodiment G113. The multi-chain chimeric polypeptide of embodiment G112, wherein the first target-binding domain binds specifically to a TGF-β and the second target-binding domain binds specifically to TGF-β or IL-16.

Embodiment G114. The multi-specific chimeric polypeptide of embodiment G113, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G115. The multi-specific chimeric polypeptide of embodiment G114, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G116. The multi-specific chimeric polypeptide of any one of embodiments G113-G115, wherein the second target-binding domain binds specifically to IL-16.

Embodiment G117. The multi-specific chimeric polypeptide of embodiment G116, wherein the second antigen-binding domain comprises an antigen-binding domain that binds specifically to CD16.

Embodiment G118. The multi-chain chimeric polypeptide of embodiment G117, wherein the second antigen-binding domain comprises an scFv that binds specifically to CD16.

Embodiment G119. The multi-specific chimeric polypeptide of any one of embodiments G113-G115, wherein the second target-binding domain binds specifically to TGF-β.

Embodiment G120. The multi-specific chimeric polypeptide of embodiment G119, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G121. The multi-specific chimeric polypeptide of embodiment G120, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G122. The multi-specific chimeric polypeptide of any one of embodiments G113-G118, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment G123. The multi-specific chimeric polypeptide of embodiment G122, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G124. The multi-specific chimeric polypeptide of embodiment G123, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G125. The multi-chain chimeric polypeptide of any one of embodiments G1-G34, wherein the first target-binding domain and the second targeting-binding domain each independently bind specifically to a TGF-β or a receptor for CD137L.

Embodiment G126. The multi-chain chimeric polypeptide of embodiment G125, wherein the first target-binding domain binds specifically to TGF-β and the second target-binding domain binds specifically to a receptor for CD137L.

Embodiment G127. The multi-specific chimeric polypeptide of embodiment G126, wherein the first target-binding domain is a soluble TGF-β receptor.

Embodiment G128. The multi-specific chimeric polypeptide of embodiment G127, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G129. The multi-chain chimeric polypeptide of embodiment G128, wherein the second target-binding domain comprises a soluble CD137L protein.

Embodiment G130. The multi-chain chimeric polypeptide of embodiment G129, wherein the soluble CD137L protein is a soluble human CD137L.

Embodiment G131. The multi-chain chimeric polypeptide of any one of embodiments G126-G130, wherein the second chimeric polypeptide further comprises an additional target-binding domain that binds specifically to TGF-β.

Embodiment G132. The multi-specific chimeric polypeptide of embodiment G131, wherein the additional target-binding domain is a soluble TGF-β receptor.

Embodiment G133. The multi-specific chimeric polypeptide of embodiment G132, wherein the soluble TGF-β receptor is a soluble TGFβRII receptor.

Embodiment G134. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment G135. The multi-chain chimeric polypeptide of embodiment G134, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment G136. The multi-chain chimeric polypeptide of embodiment G135, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment G137. The multi-chain chimeric polypeptide of embodiment G136, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment G138. The multi-chain chimeric polypeptide of embodiment G137, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment G139. The multi-chain chimeric polypeptide of any one of embodiments G1 and G134-G138, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 232.

Embodiment G140. The multi-chain chimeric polypeptide of embodiment G139, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 232.

Embodiment G141. The multi-chain chimeric polypeptide of embodiment G140, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 232.

Embodiment G142. The multi-chain chimeric polypeptide of embodiment G141, wherein the second chimeric polypeptide comprises SEQ ID NO: 232.

Embodiment G143. The multi-chain chimeric polypeptide of embodiment G142, wherein the second chimeric polypeptide comprises SEQ ID NO: 234.

Embodiment G144. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G145. The multi-chain chimeric polypeptide of embodiment G144, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G146. The multi-chain chimeric polypeptide of embodiment G145, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G147. The multi-chain chimeric polypeptide of embodiment G146, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G148. The multi-chain chimeric polypeptide of embodiment G147, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G149. The multi-chain chimeric polypeptide of any one of embodiments G1 and G144-G148, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 232.

Embodiment G150. The multi-chain chimeric polypeptide of embodiment G149, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 232.

Embodiment G151. The multi-chain chimeric polypeptide of embodiment G150, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 232.

Embodiment G152. The multi-chain chimeric polypeptide of embodiment G151, wherein the second chimeric polypeptide comprises SEQ ID NO: 232.

Embodiment G153. The multi-chain chimeric polypeptide of embodiment G152, wherein the second chimeric polypeptide comprises SEQ ID NO: 234.

Embodiment G154. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment G155. The multi-chain chimeric polypeptide of embodiment G154, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment G156. The multi-chain chimeric polypeptide of embodiment G155, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment G157. The multi-chain chimeric polypeptide of embodiment G156, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment G158. The multi-chain chimeric polypeptide of embodiment G157, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment G159. The multi-chain chimeric polypeptide of any one of embodiments G1 and G154-G158, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 203.

Embodiment G160. The multi-chain chimeric polypeptide of embodiment G159, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 203.

Embodiment G161. The multi-chain chimeric polypeptide of embodiment G160, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 203.

Embodiment G162. The multi-chain chimeric polypeptide of embodiment G161, wherein the second chimeric polypeptide comprises SEQ ID NO: 203.

Embodiment G163. The multi-chain chimeric polypeptide of embodiment G162, wherein the second chimeric polypeptide comprises SEQ ID NO: 250.

Embodiment G164. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G165. The multi-chain chimeric polypeptide of embodiment G164, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G166. The multi-chain chimeric polypeptide of embodiment G165, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G167. The multi-chain chimeric polypeptide of embodiment G166, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G168. The multi-chain chimeric polypeptide of embodiment G167, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G169. The multi-chain chimeric polypeptide of any one of embodiments G1 and G164-G168, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 193.

Embodiment G170. The multi-chain chimeric polypeptide of embodiment G169, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 193.

Embodiment G171. The multi-chain chimeric polypeptide of embodiment G170, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 193.

Embodiment G172. The multi-chain chimeric polypeptide of embodiment G171, wherein the second chimeric polypeptide comprises SEQ ID NO: 193.

Embodiment G173. The multi-chain chimeric polypeptide of embodiment G172, wherein the second chimeric polypeptide comprises SEQ ID NO: 195.

Embodiment G174. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment G175. The multi-chain chimeric polypeptide of embodiment G174, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment G176. The multi-chain chimeric polypeptide of embodiment G175, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment G177. The multi-chain chimeric polypeptide of embodiment G176, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment G178. The multi-chain chimeric polypeptide of embodiment G177, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment G179. The multi-chain chimeric polypeptide of any one of embodiments G1 and G174-G178, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 268.

Embodiment G180. The multi-chain chimeric polypeptide of embodiment G179, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 268.

Embodiment G181. The multi-chain chimeric polypeptide of embodiment G180, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 268.

Embodiment G182. The multi-chain chimeric polypeptide of embodiment G181, wherein the second chimeric polypeptide comprises SEQ ID NO: 268.

Embodiment G183. The multi-chain chimeric polypeptide of embodiment G182, wherein the second chimeric polypeptide comprises SEQ ID NO: 270.

Embodiment G184. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment G185. The multi-chain chimeric polypeptide of embodiment G184, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment G186. The multi-chain chimeric polypeptide of embodiment G185, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment G187. The multi-chain chimeric polypeptide of embodiment G186, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment G188. The multi-chain chimeric polypeptide of embodiment G187, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment G189. The multi-chain chimeric polypeptide of any one of embodiments G1 and G184-G188, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 272.

Embodiment G190. The multi-chain chimeric polypeptide of embodiment G189, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 272.

Embodiment G191. The multi-chain chimeric polypeptide of embodiment G190, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 272.

Embodiment G192. The multi-chain chimeric polypeptide of embodiment G191, wherein the second chimeric polypeptide comprises SEQ ID NO: 272.

Embodiment G193. The multi-chain chimeric polypeptide of embodiment G192, wherein the second chimeric polypeptide comprises SEQ ID NO: 272.

Embodiment G194. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 207.

Embodiment G195. The multi-chain chimeric polypeptide of embodiment G194, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 207.

Embodiment G196. The multi-chain chimeric polypeptide of embodiment G195, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 207.

Embodiment G197. The multi-chain chimeric polypeptide of embodiment G196, wherein the first chimeric polypeptide comprises SEQ ID NO: 207.

Embodiment G198. The multi-chain chimeric polypeptide of embodiment G197, wherein the first chimeric polypeptide comprises SEQ ID NO: 209.

Embodiment G199. The multi-chain chimeric polypeptide of any one of embodiments G1 and G194-G198, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 193.

Embodiment G200. The multi-chain chimeric polypeptide of embodiment G199, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 193.

Embodiment G201. The multi-chain chimeric polypeptide of embodiment G200, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 193.

Embodiment G202. The multi-chain chimeric polypeptide of embodiment G201, wherein the second chimeric polypeptide comprises SEQ ID NO: 193.

Embodiment G203. The multi-chain chimeric polypeptide of embodiment G202, wherein the second chimeric polypeptide comprises SEQ ID NO: 195.

Embodiment G204. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G205. The multi-chain chimeric polypeptide of embodiment G204, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G206. The multi-chain chimeric polypeptide of embodiment G205, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G207. The multi-chain chimeric polypeptide of embodiment G206, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G208. The multi-chain chimeric polypeptide of embodiment G207, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G209. The multi-chain chimeric polypeptide of any one of embodiments G1 and G204-G208, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 268.

Embodiment G210. The multi-chain chimeric polypeptide of embodiment G209, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 268.

Embodiment G211. The multi-chain chimeric polypeptide of embodiment G210, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 268.

Embodiment G212. The multi-chain chimeric polypeptide of embodiment G211, wherein the second chimeric polypeptide comprises SEQ ID NO: 268.

Embodiment G213. The multi-chain chimeric polypeptide of embodiment G212, wherein the second chimeric polypeptide comprises SEQ ID NO: 270.

Embodiment G214. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G215. The multi-chain chimeric polypeptide of embodiment G214, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G216. The multi-chain chimeric polypeptide of embodiment G215, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G217. The multi-chain chimeric polypeptide of embodiment G216, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G218. The multi-chain chimeric polypeptide of embodiment G217, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G219. The multi-chain chimeric polypeptide of any one of embodiments G1 and G214-G218, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 300.

Embodiment G220. The multi-chain chimeric polypeptide of embodiment G219, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 300.

Embodiment G221. The multi-chain chimeric polypeptide of embodiment G220, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 300.

Embodiment G222. The multi-chain chimeric polypeptide of embodiment G221, wherein the second chimeric polypeptide comprises SEQ ID NO: 300.

Embodiment G223. The multi-chain chimeric polypeptide of embodiment G222, wherein the second chimeric polypeptide comprises SEQ ID NO: 302.

Embodiment G224. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G225. The multi-chain chimeric polypeptide of embodiment G224, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G226. The multi-chain chimeric polypeptide of embodiment G225, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G227. The multi-chain chimeric polypeptide of embodiment G226, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G228. The multi-chain chimeric polypeptide of embodiment G227, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G229. The multi-chain chimeric polypeptide of any one of embodiments G1 and G224-G228, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 308.

Embodiment G230. The multi-chain chimeric polypeptide of embodiment G229, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 308.

Embodiment G231. The multi-chain chimeric polypeptide of embodiment G230, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 308.

Embodiment G232. The multi-chain chimeric polypeptide of embodiment G231, wherein the second chimeric polypeptide comprises SEQ ID NO: 308.

Embodiment G233. The multi-chain chimeric polypeptide of embodiment G232, wherein the second chimeric polypeptide comprises SEQ ID NO: 310.

Embodiment G234. The multi-chain chimeric polypeptide of embodiment G1, wherein the first chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 236.

Embodiment G235. The multi-chain chimeric polypeptide of embodiment G234, wherein the first chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 236.

Embodiment G236. The multi-chain chimeric polypeptide of embodiment G235, wherein the first chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 236.

Embodiment G237. The multi-chain chimeric polypeptide of embodiment G236, wherein the first chimeric polypeptide comprises SEQ ID NO: 236.

Embodiment G238. The multi-chain chimeric polypeptide of embodiment G237, wherein the first chimeric polypeptide comprises SEQ ID NO: 238.

Embodiment G239. The multi-chain chimeric polypeptide of any one of embodiments G1 and G234-G238, wherein the second chimeric polypeptide comprises a sequence that is at least 80% identical to SEQ ID NO: 316.

Embodiment G240. The multi-chain chimeric polypeptide of embodiment G239, wherein the second chimeric polypeptide comprises a sequence that is at least 90% identical to SEQ ID NO: 316.

Embodiment G241. The multi-chain chimeric polypeptide of embodiment G240, wherein the second chimeric polypeptide comprises a sequence that is at least 95% identical to SEQ ID NO: 316.

Embodiment G242. The multi-chain chimeric polypeptide of embodiment G241, wherein the second chimeric polypeptide comprises SEQ ID NO: 316.

Embodiment G243. The multi-chain chimeric polypeptide of embodiment G242, wherein the second chimeric polypeptide comprises SEQ ID NO: 318.

Embodiment G244. The multi-chain chimeric polypeptide of any one of embodiments G1-G133, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment G245. The multi-chain chimeric polypeptide of embodiment G244, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s)and the first domain of the pair of affinity domains.

Embodiment G246. The multi-chain chimeric polypeptide of any one of embodiments G1-G133, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment G247. The multi-chain chimeric polypeptide of embodiment G246, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G248. The multi-chain chimeric polypeptide of embodiment G246, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment G249. The multi-chain chimeric polypeptide of embodiment G246, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment G250. The multi-chain chimeric polypeptide of embodiment G246, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment G251. The multi-chain chimeric polypeptide of embodiment G246, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G252. The multi-chain chimeric polypeptide of embodiment G251, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G253. The multi-chain chimeric polypeptide of embodiment G251, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G254. The multi-chain chimeric polypeptide of embodiment G251, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G255. The multi-chain chimeric polypeptide of embodiment G251, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment G256. The multi-chain chimeric polypeptide of embodiment G251, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment G257. The multi-chain chimeric polypeptide of embodiment G251, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-

binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment G258. The multi-chain chimeric polypeptide of any one of embodiments G44-G46, G57-G59, G76-G78, G96-G98, G109-G111, G122-G124, and G131-G133, wherein the second chimeric polypeptide further comprises the additional target-binding domain at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment G259. The multi-chain chimeric polypeptide of embodiment G258, wherein the additional target-binding domain directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment G260. The multi-chain chimeric polypeptide of embodiment G258, wherein the second chimeric polypeptide further comprises a linker sequence between the additional target-binding domain and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment G261. The multi-chain chimeric polypeptide of embodiment G258, wherein the additional target-binding domain directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment G262. The multi-chain chimeric polypeptide of embodiment G258, wherein the second chimeric polypeptide further comprises a linker sequence between the additional target-binding domain and the second target-binding domain in the second chimeric polypeptide.

Embodiment G263. A composition comprising any of the multi-chain chimeric polypeptides of embodiments G1-G262.

Embodiment G264. The composition of embodiment G263, wherein the composition is a pharmaceutical composition.

Embodiment G265. A kit comprising at least one dose of the composition of embodiment G263 or G264.

Embodiment G266. Nucleic acid encoding any of the multi-chain chimeric polypeptides of any one of embodiments G1-G262.

Embodiment G267. A vector comprising the nucleic acid of embodiment G266.

Embodiment G268. The vector of embodiment G267, wherein the vector is an expression vector.

Embodiment G269. A cell comprising the nucleic acid of embodiment G323 or the vector of embodiment G267 or G268.

Embodiment G270. A method of producing a multi-chain chimeric polypeptide, the method comprising:

culturing the cell of embodiment G269 in a culture medium under conditions sufficient to result in the production of the multi-chain chimeric polypeptide; and

recovering the multi-chain chimeric polypeptide from the cell and/or the culture medium.

Embodiment G271. A multi-chain chimeric polypeptide produced by the method of embodiment G270.

Embodiment G272. The multi-chain chimeric polypeptide of embodiment G8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 97.

Embodiment G273. The multi-chain chimeric polypeptide of embodiment G272, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 97.

Embodiment G274. The multi-chain chimeric polypeptide of embodiment G273, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 97.

Embodiment G275. The multi-chain chimeric polypeptide of embodiment G274, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 97.

Embodiment G276. The multi-chain chimeric polypeptide of embodiment G8, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 98.

Embodiment G277. The multi-chain chimeric polypeptide of embodiment G276, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 98.

Embodiment G278. The multi-chain chimeric polypeptide of embodiment G277, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 98.

Embodiment G279. The multi-chain chimeric polypeptide of embodiment G278, wherein the soluble human tissue factor domain comprises a sequence that is 100% identical to SEQ ID NO: 98.

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Embodiment H1. A method of treating an aging-related disease or condition in a subject in need thereof, the method comprising administering to a subject identified as having an aging-related disease or condition a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Embodiment H2. A method of killing or reducing the number of senescent cells in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s).

Embodiment H3. The method of embodiment H2, wherein the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells.

Embodiment H4. The method of embodiment H3, wherein the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells.

Embodiment H5. The method of embodiment H2, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment H6. The method of embodiment H1 or H5, wherein the aging-related disease or condition is selected from the group consisting of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease.

Embodiment H7. The method of embodiment H6, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-

Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment H8. The method of embodiment H6, wherein the autoimmune disease is type-1 diabetes.

Embodiment H9. The method of embodiment H6, wherein the metabolic disease is selected from the group consisting of: obesity, a lipodystrophy, and type 2 diabetes mellitus.

Embodiment H10. The method of embodiment H6, wherein the neurodegenerative disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease, and dementia.

Embodiment H11. The method of embodiment H6, wherein the cardiovascular disease is selected from the group consisting of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

Embodiment H12. The method of embodiment H6, wherein the skin disease is selected from the group consisting of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

Embodiment H13. The method of embodiment H6, wherein the progeria disease is selected from the group consisting of: progeria and Hutchinson-Gilford Progeria Syndrome.

Embodiment H14. The method of embodiment H6, wherein the fragility disease is selected from the group consisting of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

Embodiment H15. The method of embodiment H1 or H5, wherein the aging-related disease or condition is selected from the group of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

Embodiment H16. The method of embodiment H1 or H5, wherein the aging-related disease or condition is type 2 diabetes or atherosclerosis.

Embodiment H17. The method of any one of embodiments H1-H16, wherein the administering results in a decrease in the number of senescent cells in a target tissue in the subject.

Embodiment H18. The method of embodiment H17, wherein the target tissue is selected from the group consisting of: adipose tissue, pancreatic tissue, liver tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

Embodiment H19. The method of any one of embodiments H1-H18, wherein the administering results in an increase in the expression levels of CD25, CD69, MTOR-C1, SREBP1, IFN-γ, and granzyme B in activated NK cells.

Embodiment H20. A method of treating an aging-related disease or condition in a subject in need thereof, the method comprising administering to a subject identified as having an aging-related disease or condition a therapeutically effective number of activated NK cells.

Embodiment H21. A method of killing or reducing the number of senescent cells in a subject in need thereof, the method comprising administering to the subject a therapeutically effective number of activated NK cells.

Embodiment H22. The method of embodiment H21, wherein the senescent cells are senescent cancer cells, senescent monocytes, senescent lymphocytes, senescent astrocytes, senescent microglia, senescent neurons, senescent tissue fibroblasts, senescent dermal fibroblasts, senescent keratinocytes, or other differentiated tissue-specific dividing functional cells.

Embodiment H23. The method of embodiment H22, wherein the senescent cancer cells are chemotherapy-induced senescent cells or radiation-induced senescent cells.

Embodiment H24. The method of embodiment H21, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

Embodiment H25. The method of embodiment H20 or H24, wherein the aging-related disease or condition is selected from the group consisting of: a cancer, an autoimmune disease, a metabolic disease, a neurodegenerative disease, a cardiovascular disease, a skin disease, a progeria disease, and a fragility disease.

Embodiment H26. The method of embodiment H25, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell

non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

Embodiment H27. The method of embodiment H25, wherein the autoimmune disease is type-1 diabetes.

Embodiment H28. The method of embodiment H25, wherein the metabolic disease is selected from the group consisting of: obesity, a lipodystrophy, and type 2 diabetes mellitus.

Embodiment H29. The method of embodiment H25, wherein the neurodegenerative disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease, and dementia.

Embodiment H30. The method of embodiment H25, wherein the cardiovascular disease is selected from the group consisting of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

Embodiment H31. The method of embodiment H25, wherein the skin disease is selected from the group consisting of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

Embodiment H32. The method of embodiment H25, wherein the progeria disease is selected from the group consisting of: progeria and Hutchinson-Gilford Progeria Syndrome.

Embodiment H33. The method of embodiment H25, wherein the fragility disease is selected from the group consisting of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

Embodiment H34. The method of embodiment H20 or H24, wherein the aging-related disease or condition is selected from the group consisting of: age-related macular degeneration, osteoarthritis, adipose atrophy, idiopathic pulmonary fibrosis, kidney transplant failure, liver fibrosis, loss of bone mass, sarcopenia, age-associated loss of lung tissue elasticity, osteoporosis, age-associated renal dysfunction, and chemical-induced renal dysfunction.

Embodiment H35. The method of any one of embodiments H20-H34, wherein the method further comprises:

obtaining a resting NK cell; and

contacting the resting NK cell *in vitro* in a liquid culture medium comprising one or more NK cell activating agent(s), wherein the contacting results in the generation of the activated NK cells that are subsequently administered to the subject.

Embodiment H36. The method of embodiment H35, wherein the resting NK cell is an autologous NK cell obtained from the subject.

Embodiment H37. The method of embodiment H35, wherein the resting NK cell is an allogeneic resting NK cell.

Embodiment H38. The method of embodiment H35, wherein the resting NK cell is an artificial NK cell.

Embodiment H39. The method of embodiment H35, wherein the resting NK cell is a haploidentical resting NK cell.

Embodiment H40. The method of any one of embodiments H35-H39, wherein the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

Embodiment H41. The method of any one of embodiments H35-H40, wherein the method further comprises isolating the activated NK cells before the activated NK cells are administered to the subject.

Embodiment H42. A method of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time, the method comprising administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Embodiment H43. A method of improving the texture and/or appearance of skin and/or hair in a subject in need thereof over a period of time, the method comprising administering to the subject a therapeutically effective number of activated NK cells.

Embodiment H44. The method of embodiment H43, wherein the method further comprises:

obtaining a resting NK cell; and

contacting the resting NK cell *in vitro* in a liquid culture medium comprising one or more NK cell activating agent(s), wherein the contacting results in the generation of the activated NK cells that are subsequently administered to the subject.

Embodiment H45. The method of embodiment H44, wherein the resting NK cell is an autologous NK cell obtained from the subject.

Embodiment H46. The method of embodiment H44, wherein the resting NK cell is an allogeneic resting NK cell.

Embodiment H47. The method of embodiment H44, wherein the resting NK cell is an artificial NK cell.

Embodiment H48. The method of embodiment H44, wherein the resting NK cell is a haploidentical resting NK cell.

Embodiment H49. The method of any one of embodiments H44-H48, wherein the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

Embodiment H50. The method of any one of embodiments H44-H49, wherein the method further comprises isolating the activated NK cells before the activated NK cells are administered to the subject.

Embodiment H51. The method of any one of embodiments H42-H50, wherein the method provides for an improvement in the texture and/or appearance of skin of the subject over the period of time.

Embodiment H52. The method of embodiment H51, wherein the method results in a decrease in the rate of formation of wrinkles in the skin of the subject over the period of time.

Embodiment H53. The method of embodiment H51 or H52, wherein the method results in an improvement in the coloration of skin of the subject over the period of time.

Embodiment H54. The method of any one of embodiments H51-H53, wherein the method results in an improvement in the texture of skin of the subject over the period of time.

Embodiment H55. The method of any one of embodiments H42-H50, wherein the method provides for an improvement in the texture and/or appearance of hair of the subject over the period of time.

Embodiment H56. The method of embodiment H55, wherein the method results in a decrease in the rate of formation of gray hair in the subject over the period of time.

Embodiment H57. The method of embodiment H55 or H56, wherein the method results in a decrease in the number of gray hairs of the subject over the period of time.

Embodiment H58. The method of any one of embodiments H55-H57, wherein the method results in a decrease in the rate of hair loss in the subject over time.

Embodiment H59. The method of any one of embodiments H55-H58, wherein the method results in an improvement in the texture of hair of the subject over the period of time.

Embodiment H60. The method of any one of embodiments H42-H59, wherein the period of time is between about one month and about 10 years.

Embodiment H61. The method of any one of embodiments H42-H60, wherein the method results in a decrease in the number of senescent dermal fibroblasts in the skin of the subject over the period of time.

Embodiment H62. A method of assisting in the treatment of obesity in a subject in need thereof over a period of time, the method comprising administering to the subject a

therapeutically effective amount of one or more natural killer (NK) cell activating agent(s).

Embodiment H63. A method of assisting in the treatment of obesity in a subject in need thereof over a period of time, the method comprising administering to the subject a therapeutically effective number of activated NK cells.

Embodiment H64. The method of embodiment H63, wherein the method further comprises:

obtaining a resting NK cell; and

contacting the resting NK cell *in vitro* in a liquid culture medium comprising one or more NK cell activating agent(s), wherein the contacting results in the generation of the activated NK cells that are subsequently administered to the subject.

Embodiment H65. The method of embodiment H64, wherein the resting NK cell is an autologous NK cell obtained from the subject.

Embodiment H66. The method of embodiment H64, wherein the resting NK cell is an allogeneic resting NK cell.

Embodiment H67. The method of embodiment H64, wherein the resting NK cell is an artificial NK cell.

Embodiment H68. The method of embodiment H64, wherein the resting NK cell is a haploidentical resting NK cell.

Embodiment H69. The method of any one of embodiments H64-H68, wherein the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

Embodiment H70. The method of any one of embodiments H64-H69, wherein the method further comprises isolating the activated NK cells before the activated NK cells are administered to the subject.

Embodiment H71. The method of any one of embodiments H62-H70, wherein the method results in a decrease in the mass of the subject over the period of time.

Embodiment H72. The method of any one of embodiments H62-H71, wherein the method results in a decrease in the body mass index (BMI) of the subject over the period of time.

Embodiment H73. The method of any one of embodiments H62-H70, wherein the method results in a decrease in the rate of progression from pre-diabetes to type 2 diabetes in the subject.

Embodiment H74. The method of any one of embodiments H62-H70, wherein the method results in a decrease in fasting serum glucose level in the subject.

Embodiment H75. The method of any one of embodiments H62-H70, wherein the method results in an increase in insulin sensitivity in the subject.

Embodiment H76. The method of any one of embodiments H62-H70, wherein the method results in a decrease in the severity of atherosclerosis in the subject.

Embodiment H77. The method of any one of embodiments H62-H76, wherein the period of time is between about two weeks and about 10 years.

Embodiment H78. The method of any one of embodiments H1-H19, H35-H42, H44-H62, and H64-H77, wherein at least one of the one or more NK cell activating agent(s) results in activation of one or more of: a receptor for IL-2, a receptor for IL-7, a

receptor for IL-12, a receptor for IL-15, a receptor for IL-18, a receptor for IL-21, a receptor for IL-33, CD16, CD69, CD25, CD36, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, and KIR3DS1.

Embodiment H79. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-2 is a soluble IL-2 or an agonistic antibody that binds specifically to an IL-2 receptor.

Embodiment H80. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-7 is a soluble IL-7 or an agonistic antibody that binds specifically to an IL-7 receptor.

Embodiment H81. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-12 is a soluble IL-12 or an agonistic antibody that binds specifically to an IL-12 receptor.

Embodiment H82. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-15 is a soluble IL-15 or an agonistic antibody that binds specifically to an IL-15 receptor.

Embodiment H83. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-21 is a soluble IL-21 or an agonistic antibody that binds specifically to an IL-21 receptor.

Embodiment H84. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for IL-33 is a soluble IL-33 or an agonistic antibody that binds specifically to an IL-33 receptor.

Embodiment H85. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD16 is an agonistic antibody that binds specifically to a CD16.

Embodiment H86. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD69 is an agonistic antibody that binds specifically to a CD69.

Embodiment H87. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD25, CD36, CD59 is an agonistic antibody that binds specifically to a CD25, CD6, CD59.

Embodiment H88. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for CD352 is an agonistic antibody that binds specifically to a CD352.

Embodiment H89. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp80 is an agonistic antibody that binds specifically to an NKp80.

Embodiment H90. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for DNAM-1 is an agonistic antibody that binds specifically to a DNAM-1.

Embodiment H91. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for 2B4 is an agonistic antibody that binds specifically to a 2B4.

Embodiment H92. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp30 is an agonistic antibody that binds specifically to an NKp30.

Embodiment H93. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp44 is an agonistic antibody that binds specifically to an NKp44.

Embodiment H94. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKp46 is an agonistic antibody that binds specifically to an NKp46.

Embodiment H95. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for NKG2D is an agonistic antibody that binds specifically to an NKG2D.

Embodiment H96. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS1 is an agonistic antibody that binds specifically to a KIR2DS1.

Embodiment H97. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS2/3 is an agonistic antibody that binds specifically to a KIR2DS2/3.

Embodiment H98. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DL4 is an agonistic antibody that binds specifically to a KIR2DL4.

Embodiment H99. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS4 is an agonistic antibody that binds specifically to a KIR2DS4.

Embodiment H100. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR2DS5 is an agonistic antibody that binds specifically to a KIR2DS5.

Embodiment H101. The method of embodiment H78, wherein the at least one of the one or more NK cell activating agent(s) that results in activation of a receptor for KIR3DS1 is an agonistic antibody that binds specifically to a KIR3DS1.

Embodiment H102. The method of any one of embodiments H1-H19, H35-H42, H44-H62, and H64-H101, wherein at least one of the one or more NK cell activating agent(s) results in a decrease in the activation of one or more of: PD-1, a TGF-β receptor, TIGIT, CD1, TIM-3, Siglec-7, IRP60, Tactile, IL1R8, NKG2A/KLRD1, KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1, KIR3DL2, ILT2/LIR-1, and LAG-2.

Embodiment H103. The method of embodiment H102, wherein the at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of PD-1 is an antagonistic antibody that binds specifically to PD-1, a soluble PD-1, a soluble PD-L1, or an antibody that binds specifically to PD-L1.

Embodiment H104. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of a TGF-β receptor is a soluble TGF-β receptor, an antibody that binds specifically to TGF-β, or an antagonistic antibody that binds specifically to a TGF-β receptor.

Embodiment H105. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of

TIGIT is an antagonistic antibody that binds specifically to TIGIT, a soluble TIGIT, or an antibody that binds specifically to a ligand of TIGIT.

Embodiment H106. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of CD1 is an antagonistic antibody that binds specifically to CD1, a soluble CD1, or an antibody that binds specifically to a ligand of CD1.

Embodiment H107. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of TIM-3 is an antagonistic antibody that binds specifically to TIM-3, a soluble TIM-3, or an antibody that binds specifically to a ligand of TIM-3.

Embodiment H108. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Siglec-7 is an antagonistic antibody that binds specifically to Siglec-7 or an antibody that binds specifically to a ligand of Siglec-7.

Embodiment H109. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of IRP60 is an antagonistic antibody that binds specifically to IRP60 or an antibody that binds specifically to a ligand of IRP60.

Embodiment H110. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of Tactile is an antagonistic antibody that binds specifically to Tactile or an antibody that binds specifically to a ligand of Tactile.

Embodiment H111. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of

IL1R8 is an antagonistic antibody that binds specifically to IL1R8 or an antibody that binds specifically to a ligand of IL1R8.

Embodiment H112. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of NKG2A/KLRD1 is an antagonistic antibody that binds specifically to NKG2A/KLRD1 or an antibody that binds specifically to a ligand of NKG2A/KLRD1.

Embodiment H113. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL1 is an antagonistic antibody that binds specifically to KIR2DL1 or an antibody that binds specifically to a ligand of KIR2DL1.

Embodiment H114. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL2/3 is an antagonistic antibody that binds specifically to KIR2DL2/3 or an antibody that binds specifically to a ligand of KIR2DL2/3.

Embodiment H115. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR2DL5 is an antagonistic antibody that binds specifically to KIR2DL5 or an antibody that binds specifically to a ligand of KIR2DL5.

Embodiment H116. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of KIR3DL1 is an antagonistic antibody that binds specifically to KIR3DL1 or an antibody that binds specifically to a ligand of KIR3DL1.

Embodiment H117. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of

KIR3DL2 is an antagonistic antibody that binds specifically to KIR3DL2 or an antibody that binds specifically to a ligand of KIR3DL2.

Embodiment H118. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of ILT2/LIR-1 is an antagonistic antibody that binds specifically to ILT2/LIR-1 or an antibody that binds specifically to a ligand of ILT2/LIR-1.

Embodiment H119. The method of embodiment H102, wherein at least one of the one or more NK cell activating agent(s) that results in a decrease in the activation of LAG-2is an antagonistic antibody that binds specifically to LAG-2 or an antibody that binds specifically to a ligand of LAG-2.

Embodiment H120. The method of any one of embodiments H1-H19, H35-H42, H44-H62, and H64-H77, wherein at least one of the one or more NK cell activating agent(s) is a single-chain chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a second target-binding domain.

Embodiment H121. The method of embodiment H120, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment H122. The method of embodiment H120, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

Embodiment H123. The method of any one of embodiments H120-H122, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

Embodiment H124. The method of any one of embodiments H120-H122, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

Embodiment H125. The method of embodiment H120, wherein the first target-binding domain and the second target-binding domain directly abut each other.

Embodiment H126. The method of embodiment H120, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the second target-binding domain.

Embodiment H127. The method of embodiment H125 or H126, wherein the second target-binding domain and the soluble tissue factor domain directly abut each other.

Embodiment H128. The method of embodiment H125 or H126, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the second target-binding domain and the soluble tissue factor domain.

Embodiment H129. The method of any one of embodiments H120-H128, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment H130. The method of embodiment H129, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment H131. The method of embodiment H130, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment H132. The method of any one of embodiments H120-H128, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment H133. The method of any one of embodiments H120-H132, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment H134. The method of embodiment H133, wherein the first target-binding domain and the second target-binding domain are each an antigen-binding domain.

Embodiment H135. The method of embodiment H134, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment H136. The method of any one of embodiments H120-H135, wherein one or both of the first target-binding domain and the second target-binding domain bind to a target selected from the group consisting of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

Embodiment H137. The method of any one of embodiments H120-H128, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment H138. The method of embodiment H137, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment H139. The method of any one of embodiments H120-H128, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment H140. The method of embodiment H139, wherein the soluble interleukin or cytokine receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Embodiment H141. The method of any one of embodiments H120-H140, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment H142. The method of embodiment H141, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment H143. The method of embodiment H142, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment H144. The method of embodiment H143, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment H145. The method of any one of embodiments H141-H144, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment H146. The method of embodiment H145, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment H147. The method of any one of embodiments H120-H146, wherein the soluble tissue factor domain is not capable of binding Factor VIIa.

Embodiment H148. The method of any one of embodiments H120-H147, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment H149. The method of any one of embodiments H120-H148, wherein the single-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment H150. The method of any one of embodiments H120-H149, wherein the single-chain chimeric polypeptide further comprises one or more additional target-binding domains at its N- and/or C-terminus.

Embodiment H151. The method of embodiment H150, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its N-terminus.

Embodiment H152. The method of embodiment H151, wherein one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H153. The method of embodiment H152, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H154. The method of embodiment H150, wherein the single-chain chimeric polypeptide comprises one or more additional target-binding domains at its C-terminus.

Embodiment H155. The method of embodiment H154, wherein one of the one or more additional target-binding domains directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H156. The method of embodiment H154, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the at least one additional target-binding domains and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H157. The method of embodiment H150, wherein the single-chain chimeric polypeptide comprises one or more additional target binding domains at its N-terminus and the C-terminus.

Embodiment H158. The method of embodiment H157, wherein one of the one or more additional antigen binding domains at the N-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H159. The method of embodiment H157, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the N-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H160. The method of embodiment H157, wherein one of the one or more additional antigen binding domains at the C-terminus directly abuts the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H161. The method of embodiment H157, wherein the single-chain chimeric polypeptide further comprises a linker sequence between one of the one or more additional antigen-binding domains at the C-terminus and the first target-binding domain, the second target-binding domain, or the soluble tissue factor domain.

Embodiment H162. The method of any one of embodiments H150-H161, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment H163. The method of embodiment H162, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment H164. The method of embodiment H163, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment H165. The method of embodiment H162, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment H166. The method of embodiment H165, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment H167. The method of embodiment H166, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment H168. The method of any one of embodiments H150-H161, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment H169. The method of any one of embodiments H150-H168, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment H170. The method of embodiment H169, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment H171. The method of embodiment H170, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment H172. The method of any one of embodiments H150-H171, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor

(SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

Embodiment H173. The method of any one of embodiments H150-H161, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment H174. The method of embodiment H173, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment H175. The method of any one of embodiments H150-H161, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment H176. The method of embodiment H175, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Embodiment H177. The method of any one of embodiments H1-H19, H35-H42, H44-H62, and H64-H77, wherein at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide comprising:

(c) a first chimeric polypeptide comprising:

(i) a first target-binding domain;

(ii) a soluble tissue factor domain; and

(iii) a first domain of a pair of affinity domains;

(d) a second chimeric polypeptide comprising:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

Embodiment H178. The method of embodiment H177, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

Embodiment H179. The method of embodiment H177, wherein the first chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain in the first chimeric polypeptide.

Embodiment H180. The method of any one of embodiments H177-H179, wherein the soluble tissue factor domain and the first domain of the pair of affinity domains directly abut each other in the first chimeric polypeptide.

Embodiment H181. The method of any one of embodiments H177-H179, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H182. The method of any one of embodiments H177-H181, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the second chimeric polypeptide.

Embodiment H183. The method of any one of embodiments H177-H181, wherein second chimeric polypeptide further comprises a linker sequence between the second

domain of the pair of affinity domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment H184. The method of any one of embodiments H177-H183, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment H185. The method of embodiment H184, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment H186. The method of embodiment H185, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment H187. The method of any one of embodiments H177-H183, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment H188. The method of any one of embodiments H177-H187, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment H189. The method of embodiment H188, wherein the first target-binding domain and the second target-binding domain are each antigen-binding domains.

Embodiment H190. The method of embodiment H188 or H189, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment H191. The method of any one of embodiments H177-H190, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKP30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

Embodiment H192. The method of any one of embodiments H177-H183, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment H193. The method of embodiment H192, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment H194. The method of any one of embodiments H177-H183, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment H195. The method of embodiment H194, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Embodiment H196. The method of any one of embodiments H177-H195, wherein the first chimeric polypeptide further comprises one or more additional target-binding domain(s), where at least one of the one or more additional antigen-binding domain(s) is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment H197. The method of embodiment H196, wherein the first chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the at least one of the one or more additional antigen-binding domain(s), and/or a linker sequence between the at least one of the one or more additional antigen-binding domain(s) and the first domain of the pair of affinity domains.

Embodiment H198. The method of any one of embodiments H177-H195, wherein the first chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal and/or C-terminal end of the first chimeric polypeptide.

Embodiment H199. The method of embodiment H198, wherein at least one of the one or more additional target-binding domains directly abuts the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H200. The method of embodiment H198, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first domain of the pair of affinity domains.

Embodiment H201. The method of embodiment H198, wherein the at least one of the one or more additional target-binding domains directly abuts the first target-binding domain in the first chimeric polypeptide.

Embodiment H202. The method of embodiment H198, wherein the first chimeric polypeptide further comprises a linker sequence between the at least one of the one or more additional target-binding domains and the first target-binding domain.

Embodiment H203. The method of embodiment H198, wherein at least one of the one or more additional target-binding domains is disposed at the N- and/or C-terminus of the first chimeric polypeptide, and at least one of the one or more additional target-binding domains is positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H204. The method of embodiment H203, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the N-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H205. The method of embodiment H203, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H206. The method of embodiment H203, wherein the at least one additional target-binding domain of the one or more additional target-binding domains disposed at the C-terminus directly abuts the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H207. The method of embodiment H203, wherein the first chimeric polypeptide further comprises a linker sequence disposed between the at least one additional target-binding domain and the first target-binding domain or the first domain of the pair of affinity domains in the first chimeric polypeptide.

Embodiment H208. The method of embodiment H203, wherein the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, directly abuts the soluble tissue factor domain and/or the first domain of the pair of affinity domains.

Embodiment H209. The method of embodiment H203, wherein the first chimeric polypeptide further comprises a linker sequence disposed (i) between the soluble tissue factor domain and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains, and/or (ii) between the first domain of the pair of affinity domains and the at least one of the one or more additional target-binding domains positioned between the soluble tissue factor domain and the first domain of the pair of affinity domains.

Embodiment H210. The method of any one of embodiments H177-H209, wherein the second chimeric polypeptide further comprises one or more additional target-binding domains at the N-terminal end or the C-terminal end of the second chimeric polypeptide.

Embodiment H211. The method of embodiment H210, wherein at least one of the one or more additional target-binding domains directly abuts the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment H212. The method of embodiment H210, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second domain of the pair of affinity domains in the second chimeric polypeptide.

Embodiment H213. The method of embodiment H210, wherein at least one of the one or more additional target-binding domains directly abuts the second target-binding domain in the second chimeric polypeptide.

Embodiment H214. The method of embodiment H210, wherein the second chimeric polypeptide further comprises a linker sequence between at least one of the one or more additional target-binding domains and the second target-binding domain in the second chimeric polypeptide.

Embodiment H215. The method of any one of embodiments H196-H214, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same antigen.

Embodiment H216. The method of embodiment H215, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to the same epitope.

Embodiment H217. The method of embodiment H216, wherein two or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains comprise the same amino acid sequence.

Embodiment H218. The method of embodiment H215, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same antigen.

Embodiment H219. The method of embodiment H218, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each bind specifically to the same epitope.

Embodiment H220. The method of embodiment H219, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains each comprise the same amino acid sequence.

Embodiment H221. The method of any one of embodiments H196-H214, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains bind specifically to different antigens.

Embodiment H222. The method of any one of embodiments H196-H221, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains is an antigen-binding domain.

Embodiment H223. The method of embodiment H222, wherein the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains are each an antigen-binding domain.

Embodiment H224. The method of embodiment H223, wherein antigen-binding domain comprises a scFv.

Embodiment H225. The method of any one of embodiments H196-H224, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more target-binding domains bind specifically to a target selected from the group consisting of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

Embodiment H226. The method of any one of embodiments H196-H214, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine protein.

Embodiment H227. The method of embodiment H226, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment H228. The method of any one of embodiments H196-H214, wherein one or more of the first target-binding domain, the second target-binding domain, and the one or more additional target-binding domains is a soluble interleukin or cytokine receptor.

Embodiment H229. The method of embodiment H228, wherein the soluble receptor a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

Embodiment H230. The method of any one of embodiments H196-H229, wherein the soluble tissue factor domain is a soluble human tissue factor domain.

Embodiment H231. The method of embodiment H230, wherein the soluble human tissue factor domain comprises a sequence that is at least 80% identical to SEQ ID NO: 93.

Embodiment H232. The method of embodiment H231, wherein the soluble human tissue factor domain comprises a sequence that is at least 90% identical to SEQ ID NO: 93.

Embodiment H233. The method of embodiment H232, wherein the soluble human tissue factor domain comprises a sequence that is at least 95% identical to SEQ ID NO: 93.

Embodiment H234. The method of any one of embodiments H230-H233, wherein the soluble human tissue factor domain does not comprise one or more of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment H235. The method of embodiment H234, wherein the soluble human tissue factor domain does not comprise any of:

a lysine at an amino acid position that corresponds to amino acid position 20 of mature wildtype human tissue factor protein;

an isoleucine at an amino acid position that corresponds to amino acid position 22 of mature wildtype human tissue factor protein;

a tryptophan at an amino acid position that corresponds to amino acid position 45 of mature wildtype human tissue factor protein;

an aspartic acid at an amino acid position that corresponds to amino acid position 58 of mature wildtype human tissue factor protein;

a tyrosine at an amino acid position that corresponds to amino acid position 94 of mature wildtype human tissue factor protein;

an arginine at an amino acid position that corresponds to amino acid position 135 of mature wildtype human tissue factor protein; and

a phenylalanine at an amino acid position that corresponds to amino acid position 140 of mature wildtype human tissue factor protein.

Embodiment H236. The method of any one of embodiments H196-H235, wherein the soluble tissue factor domain is not capable of binding to Factor VIIa.

Embodiment H237. The method of any one of embodiments H196-H236, wherein the soluble tissue factor domain does not convert inactive Factor X into Factor Xa.

Embodiment H238. The method of any one of embodiments H196-H237, wherein the multi-chain chimeric polypeptide does not stimulate blood coagulation in a mammal.

Embodiment H239. The method of any one of embodiments H196-H238, wherein the pair of affinity domains is a sushi domain from an alpha chain of human IL-15 receptor (IL-15Rα) and a soluble IL-15.

Embodiment H240. The method of embodiment H239, wherein the soluble IL-15 has a D8N or D8A amino acid substitution.

Embodiment H241. The method of embodiment H239 or H240, wherein the human IL-15Rα is a mature full-length IL-15Rα.

Embodiment H242. The method of any one of embodiments H196-H238, wherein the pair of affinity domains is selected from the group consisting of: barnase and

barnstar, a PKA and an AKAP, adapter/docking tag modules based on mutated RNase I fragments, and SNARE modules based on interactions of the proteins syntaxin, synaptotagmin, synaptobrevin, and SNAP25.

Embodiment H243. The method of any one of embodiments H1-H19, H35-H42, H44-H62, and H64-H77, wherein at least one of the one or more NK cell activating agent(s) is a multi-chain chimeric polypeptide comprising:

(a) a first and second chimeric polypeptides, wherein each comprises:

(i) a first target-binding domain;

(ii) a Fc domain; and

(iii) a first domain of a pair of affinity domains;

(b) a third and fourth chimeric polypeptide, wherein each comprises:

(i) a second domain of a pair of affinity domains; and

(ii) a second target-binding domain,

wherein the first and second chimeric polypeptides and the third and fourth chimeric polypeptides associate through the binding of the first domain and the second domain of the pair of affinity domains, and the first and second chimeric polypeptides associate through their Fc domains.

Embodiment H244. The method of embodiment H243, wherein the first target-binding domain and the Fc domain directly abut each other in the first and second chimeric polypeptides.

Embodiment H245. The method of embodiment H243, wherein the first and second chimeric polypeptides further comprise a linker sequence between the first target-binding domain and the Fc domain in the first and second chimeric polypeptides.

Embodiment H246. The method of any one of embodiments H243-H245, wherein the Fc domain and the first domain of the pair of affinity domains directly abut each other in the first and second chimeric polypeptides.

Embodiment H247. The method of any one of embodiments H243-H245, wherein the first chimeric polypeptide further comprises a linker sequence between the Fc domain and the first domain of the pair of affinity domains in the first and second chimeric polypeptides.

Embodiment H248. The method of any one of embodiments H243-H247, wherein the second domain of the pair of affinity domains and the second target-binding domain directly abut each other in the third and fourth chimeric polypeptides.

Embodiment H249. The method of any one of embodiments H243-H247, wherein third and fourth chimeric polypeptides further comprise a linker sequence between the second domain of the pair of affinity domains and the second target-binding domain in the third and fourth chimeric polypeptides.

Embodiment H250. The method of any one of embodiments H243-H249, wherein the first target-binding domain and the second target-binding domain bind specifically to the same antigen.

Embodiment H251. The method of embodiment H250, wherein the first target-binding domain and the second target-binding domain bind specifically to the same epitope.

Embodiment H252. The method of embodiment H251, wherein the first target-binding domain and the second target-binding domain comprise the same amino acid sequence.

Embodiment H253. The method of any one of embodiments H243-H249, wherein the first target-binding domain and the second target-binding domain bind specifically to different antigens.

Embodiment H254. The method of any one of embodiments H243-H253, wherein one or both of the first target-binding domain and the second target-binding domain is an antigen-binding domain.

Embodiment H255. The method of embodiment H254, wherein the first target-binding domain and the second target-binding domain are each antigen-binding domains.

Embodiment H256. The method of embodiment H254 or H255, wherein antigen-binding domain comprises a scFv or a single domain antibody.

Embodiment H257. The method of any one of embodiments H243-H256, wherein one or both of the first target-binding domain and the second target-binding domain bind specifically to a target selected from the group consisting of: CD16a, CD33, CD20, CD19, CD22, CD123, PDL-1, TIGIT, PD-1, TIM3, CTLA4, MICA, MICB, IL-6, IL-8, TNFα, CD26, CD36, ULBP2, CD30, CD200, IGF-1R, MUC4AC, MUC5AC, Trop-2, CMET, EGFR, HER1, HER2, HER3, PSMA, CEA, B7H3, EPCAM, BCMA, P-cadherin, CEACAM5, a UL16-binding protein, HLA-DR, DLL4, TYRO3, AXL, MER, CD122, CD155, PDGF-DD, a ligand of TGF-β receptor II (TGF-βRII), a ligand of TGF-βRIII, a ligand of DNAM1, a ligand of NKp46, a ligand of NKp44, a ligand of NKG2D, a ligand of NKp30, a ligand for a scMHCI, a ligand for a scMHCII, a ligand for a scTCR, a receptor for PDGF-DD, a receptor for stem cell factor (SCF), a receptor for stem cell-like tyrosine kinase 3 ligand (FLT3L), a receptor for MICA, a receptor for MICB, a receptor for a ULP16-binding protein, a receptor for CD155, and a receptor for CD122.

Embodiment H258. The method of any one of embodiments H243-H256, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

Embodiment H259. The method of embodiment H258, wherein the soluble interleukin or cytokine protein is selected from the group consisting of: IL-1, IL-2, IL-3, IL-7, IL-8, IL-10, IL-12, IL-15, IL-17, IL-18, IL-21, PDGF-DD, and SCF.

Embodiment H260. The method of any one of embodiments H243-H256, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

Embodiment H261. The method of embodiment H260, wherein the soluble receptor is a soluble TGF-β receptor II (TGF-βRII) a soluble TGF-βRIII, a soluble receptor for TNFα, a soluble receptor for IL-4, or a soluble receptor for IL-10.

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WHAT IS CLAIMED IS:

1. A method of treating cancer in a subject in need thereof, the method comprising administering to a subject identified as having cancer a therapeutically effective amount of a multi-chain chimeric polypeptide comprising: a first chimeric polypeptide comprising: (i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188; (ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and (iii) a first domain of a pair of affinity domains, wherein the first domain of the pair of the affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 115; and a second chimeric polypeptide comprising: (i) a second domain of the pair of affinity domains, wherein the second domain of the pair of affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 113; and (ii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

2. A method of killing or reducing the number of senescent cells in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of one or more NK cell activating agent(s), wherein the one or more NK cell activating agent(s) comprise a multi-chain chimeric polypeptide comprising: a first chimeric polypeptide comprising: (i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188; (ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and (iii) a first domain of a pair of affinity domains, wherein the first domain of the pair of the affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 115; and a second chimeric polypeptide comprising: (i) a second domain of the pair of affinity domains, wherein the second domain of the pair of affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 113; and (ii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

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3. The method of claim 1 or 2, wherein the administering results in a decrease in the number of senescent cells in a target tissue in the subject.

4. The method of claim 3, wherein the target tissue is selected from the group consisting of: adipose tissue, pancreatic tissue, liver tissue, lung tissue, vasculature, bone tissue, central nervous system (CNS) tissue, eye tissue, skin tissue, muscle tissue, and secondary lympho-organ tissue.

5. A method of killing or reducing the number of senescent cells in a subject in need thereof, the method comprising administering to the subject a therapeutically effective number of activated NK cells, wherein the NK cells are activated *ex vivo* by contact with an NK cell activating agent comprising a multi-chain chimeric polypeptide comprising: a first chimeric polypeptide comprising: (i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188; (ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and (iii) a first domain of a pair of affinity domains, wherein the first domain of the pair of the affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 115; and a second chimeric polypeptide comprising: (i) a second domain of the pair of affinity domains, wherein the second domain of the pair of affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 113; and (ii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

6. The method of claim 1, wherein the cancer is selected from the group consisting of: solid tumor, hematological tumor, sarcoma, osteosarcoma, glioblastoma, neuroblastoma, melanoma, rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, B-cell neoplasms, multiple myeloma, B-cell lymphoma, B-cell non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), myelodysplastic syndromes (MDS), cutaneous T-cell lymphoma, retinoblastoma, stomach cancer, urothelial carcinoma, lung

cancer, renal cell carcinoma, gastric and esophageal cancer, pancreatic cancer, prostate cancer, breast cancer, colorectal cancer, ovarian cancer, non-small cell lung carcinoma, squamous cell head and neck carcinoma, endometrial cancer, cervical cancer, liver cancer, and hepatocellular carcinoma.

7. The method of claim 1, wherein the cancer is breast cancer, ovarian cancer, colorectal cancer, hepatocellular carcinoma, melanoma, gastric cancer, or urothelial carcinoma.

8. A method of improving the appearance of hair, wherein the appearance of hair is comprised of graying hair and hair loss, in a subject in need thereof over a period of time, the method comprising administering to the subject a therapeutically effective amount of one or more natural killer (NK) cell activating agent(s), wherein the one or more NK cell activating agent(s) comprise (a) a multi-chain chimeric polypeptide comprising: a first chimeric polypeptide comprising: (i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188; (ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and (iii) a first domain of a pair of affinity domains, wherein the first domain of the pair of the affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 115; and a second chimeric polypeptide comprising: (i) a second domain of the pair of affinity domains, wherein the second domain of the pair of affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 113; and (ii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains; or (b) a single chain chimeric polypeptide comprising: a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 78; a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 78.

9. The method of claim 8, wherein the method results in a decrease in the rate of formation of gray hair in the subject over the period of time.

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10. The method of claim 8 or 9, wherein the method results in a decrease in the number of gray hairs of the subject over the period of time.

11. The method of any one of claims 8-10, wherein the method results in a decrease in the rate of hair loss in the subject over time.

12. The method of claim 1 or claim 2, wherein the method further comprises:

obtaining a resting NK cell; and

contacting the resting NK cell *in vitro* in a liquid culture medium comprising one or more NK cell activating agent(s), wherein the contacting results in the generation of the activated NK cells that are subsequently administered to the subject, wherein the NK cell activating agent comprises a multi-chain chimeric polypeptide comprising: a first chimeric polypeptide comprising: (i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188; (ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and (iii) a first domain of a pair of affinity domains, wherein the first domain of the pair of the affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 115; and a second chimeric polypeptide comprising: (i) a second domain of the pair of affinity domains, wherein the second domain of the pair of affinity domains comprises a sequence that is at least 90% identical to SEQ ID NO: 113; and (ii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 188, wherein the first chimeric polypeptide and the second chimeric polypeptide associate through the binding of the first domain and the second domain of the pair of affinity domains.

13. The method of claim 12, wherein the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

14. The method of claim 12, wherein the method further comprises introducing a nucleic acid that encodes a chimeric antigen receptor or a recombinant T cell receptor into the resting NK cell or the activated NK cell prior to administration to the subject.

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15. The method of any one of claims 12-14, wherein the method results in a decrease in the mass of the subject over the period of time.

16. The method of any one of claims 12-15, wherein the method results in a decrease in the body mass index (BMI) of the subject over the period of time.

17. The method of any one of claims 12-14, wherein the method results in a decrease in the rate of progression from pre-diabetes to type 2 diabetes in the subject.

18. The method of any one of claims 12-14, wherein the method results in a decrease in fasting serum glucose level in the subject.

19. The method of any one of claims 12-14, wherein the method results in an increase in insulin sensitivity in the subject.

20. The method of any one of claims 12-14, wherein the method results in a decrease in the severity of atherosclerosis in the subject.

21. The method of any one of claims 1-20, wherein at least one of the one or more NK cell activating agent(s) results in activation of one or more of: a receptor for IL-2, a receptor for IL-7, a receptor for IL-12, a receptor for IL-15, a receptor for IL-18, a receptor for IL-21, a receptor for IL-33, CD16, CD69, CD25, CD36, CD59, CD352, NKp80, DNAM-1, 2B4, NKp30, NKp44, NKp46, NKG2D, KIR2DS1, KIR2Ds2/3, KIR2DL4, KIR2DS4, KIR2DS5, and KIR3DS1.

22. The method of any one of claims 1-21, wherein at least one of the one or more NK cell activating agent(s) results in a decrease in the activation of one or more of: PD-1, a TGF-β receptor, TIGIT, CD1, TIM-3, Siglec-7, IRP60, Tactile, IL1R8, NKG2A/KLRD1, KIR2DL1, KIR2DL2/3, KIR2DL5, KIR3DL1, KIR3DL2, ILT2/LIR-1, and LAG-2.

23. The method of any one of claims 1-20, wherein at least one of the one or more NK cell activating agent(s) is a single-chain chimeric polypeptide comprising:

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(i) a first target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 78;

(ii) a soluble tissue factor domain comprising a sequence that is at least 90% identical to SEQ ID NO: 93; and

(iii) a second target-binding domain comprising a sequence that is at least 90% identical to SEQ ID NO: 78.

24. The method of claim 23, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

25. The method of claim 23, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

26. The method of any one of claims 22-25, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

27. The method of any one of claims 22-25, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

28. The method of claim 6, wherein the cancer is pancreatic cancer.

29. The method of claim 28, further comprising administering to the subject Abraxane and Gemcitabine.

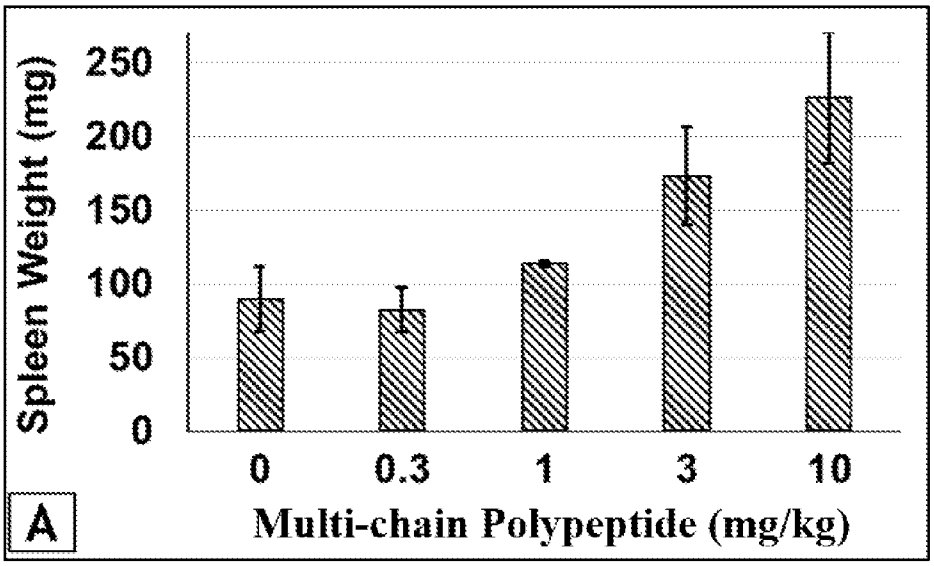


Figure 1A

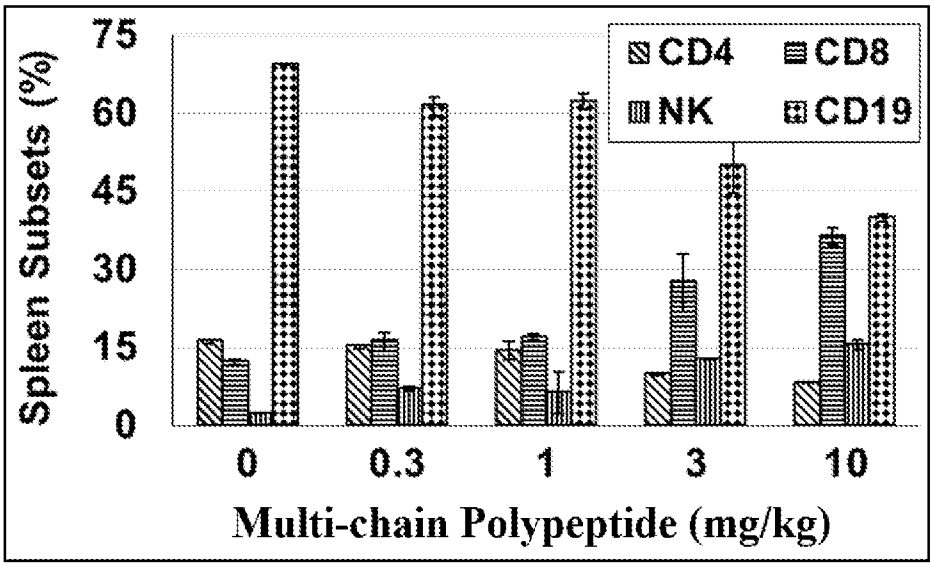


Figure 1B

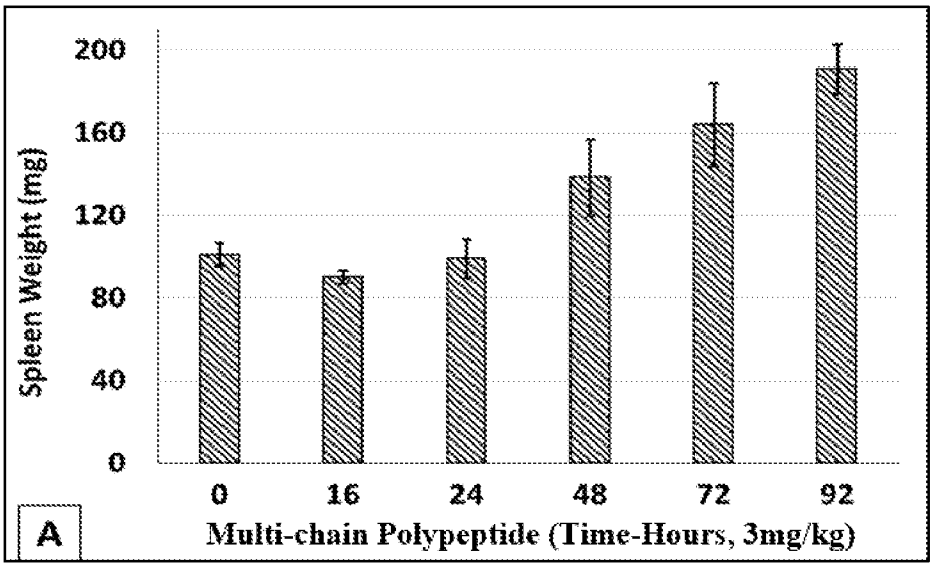


Figure 2A

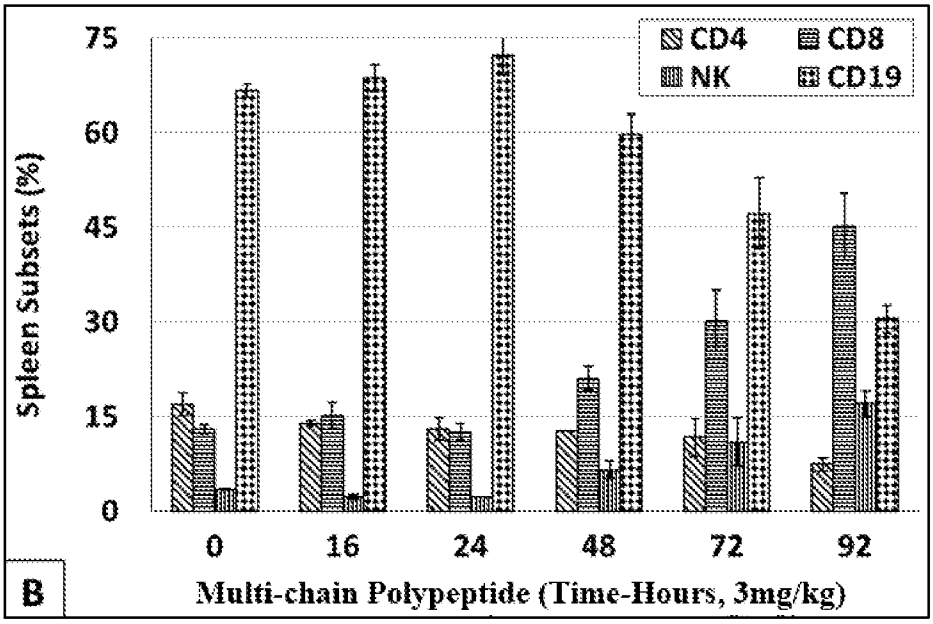


Figure 2B

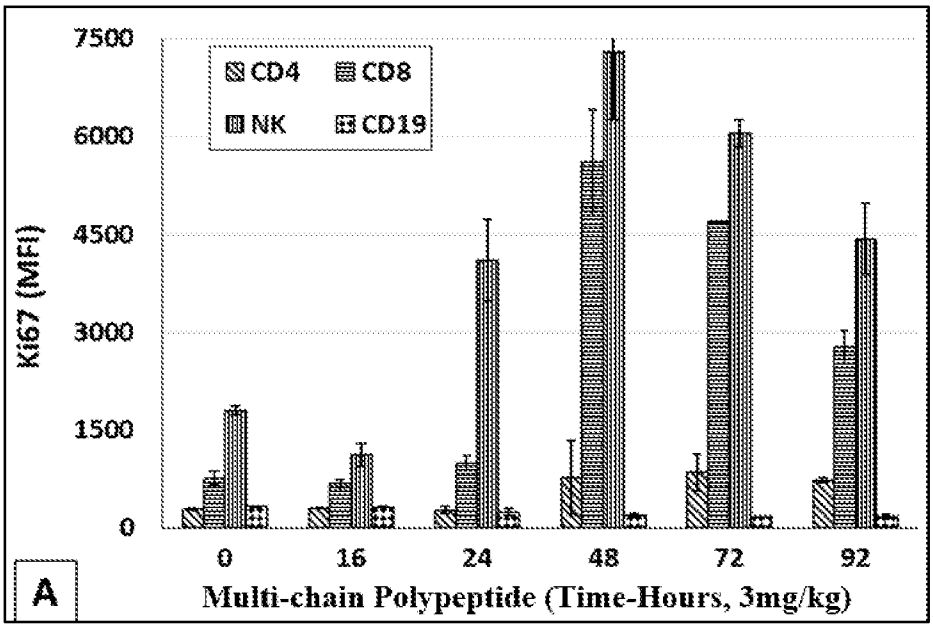


Figure 3A

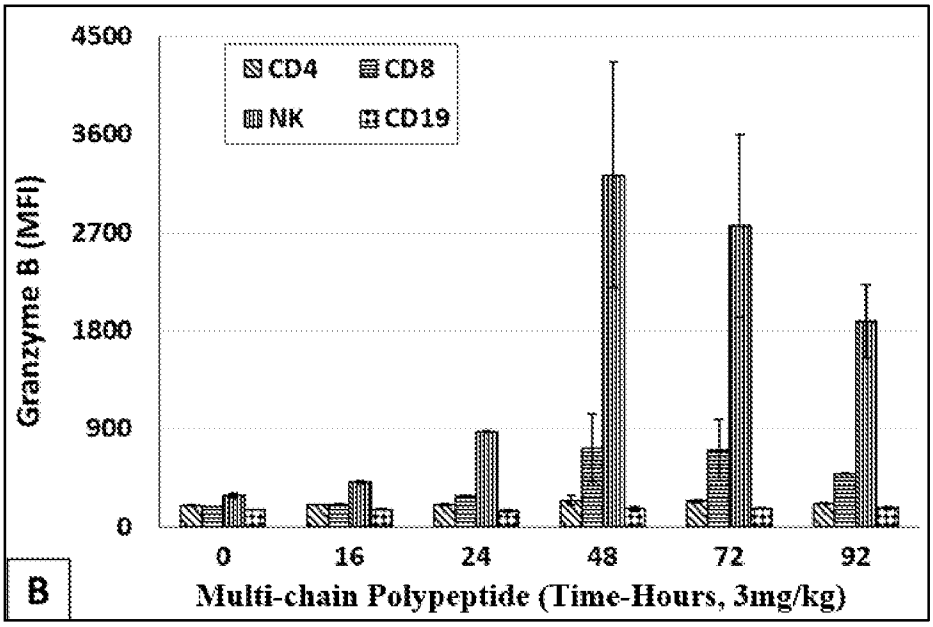


Figure 3B

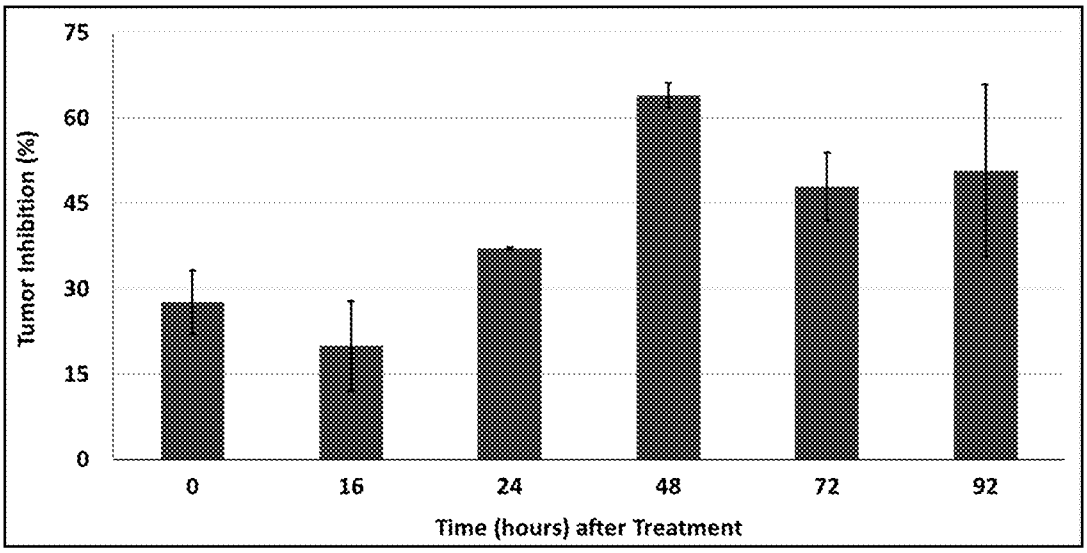
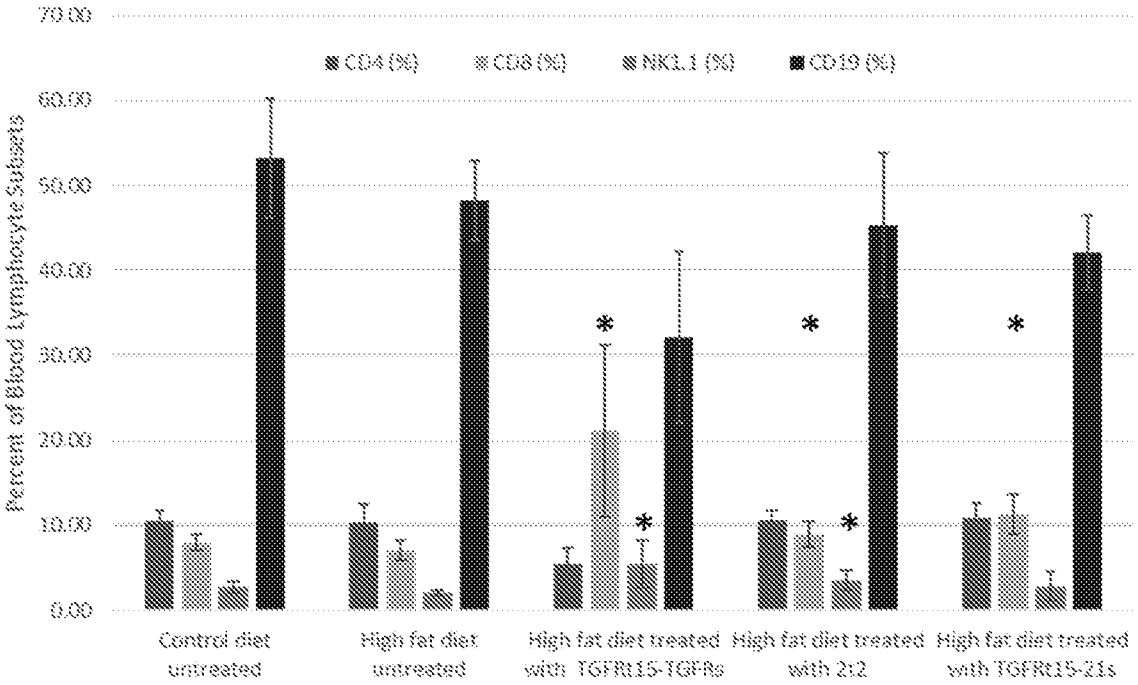
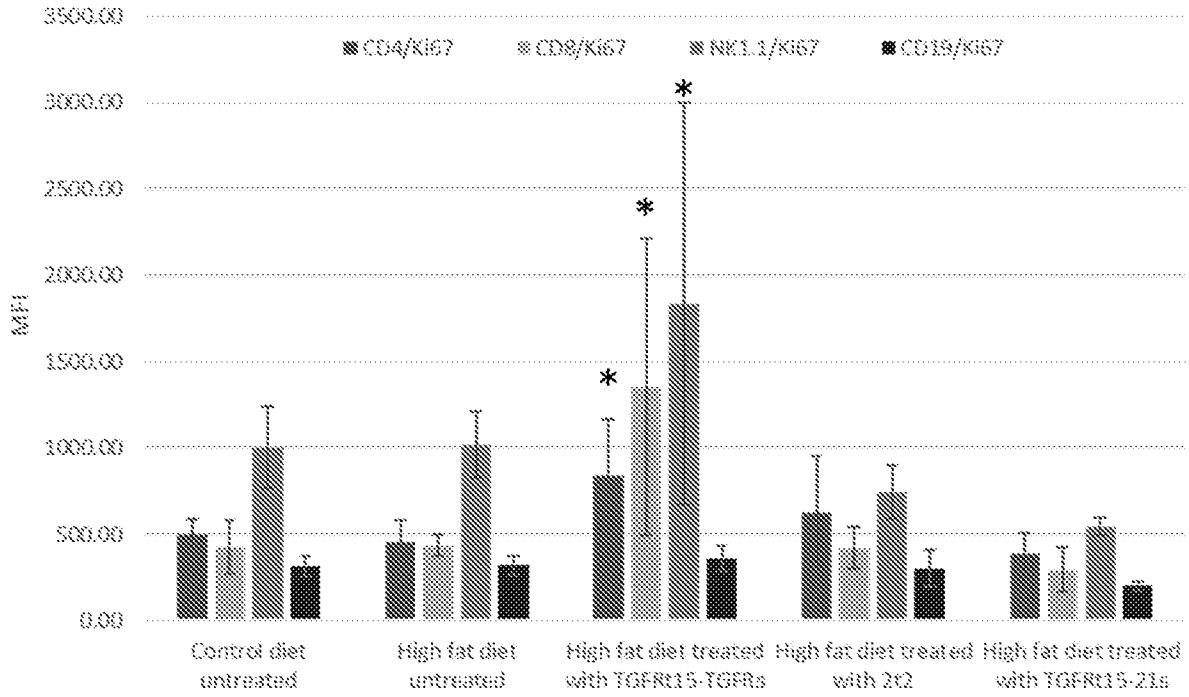

75
60
45
30
15
0
Tumor Inhibition (%)
0
16
24
48
72
92
Time (hours) after Treatment

Figure 4



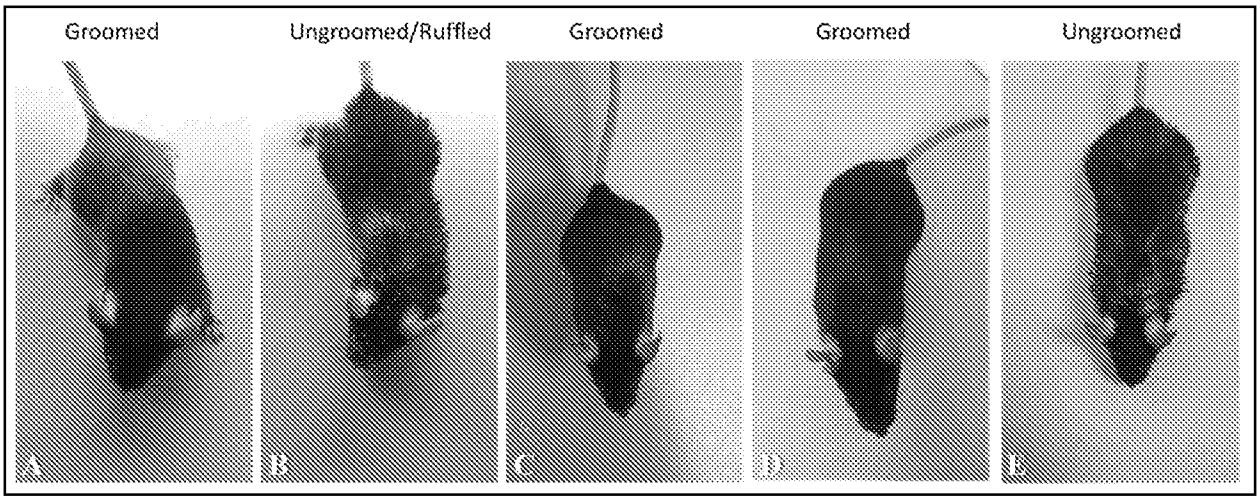
* Statistically significant from High Fat Diet untreated Group

Figure 5A

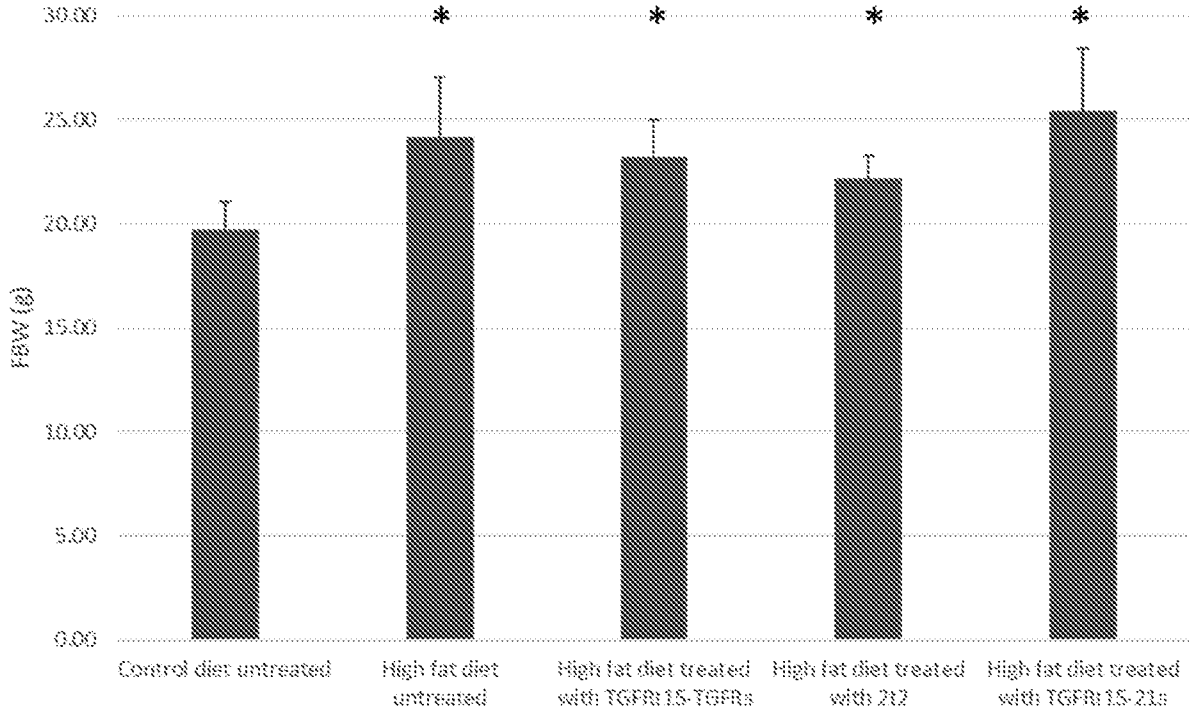


* Statistically significant from High fat diet untreated Group

Figure 5B



Figures 6A-6E



* Statistically significant from Control Normal Diet Group

Figure 7

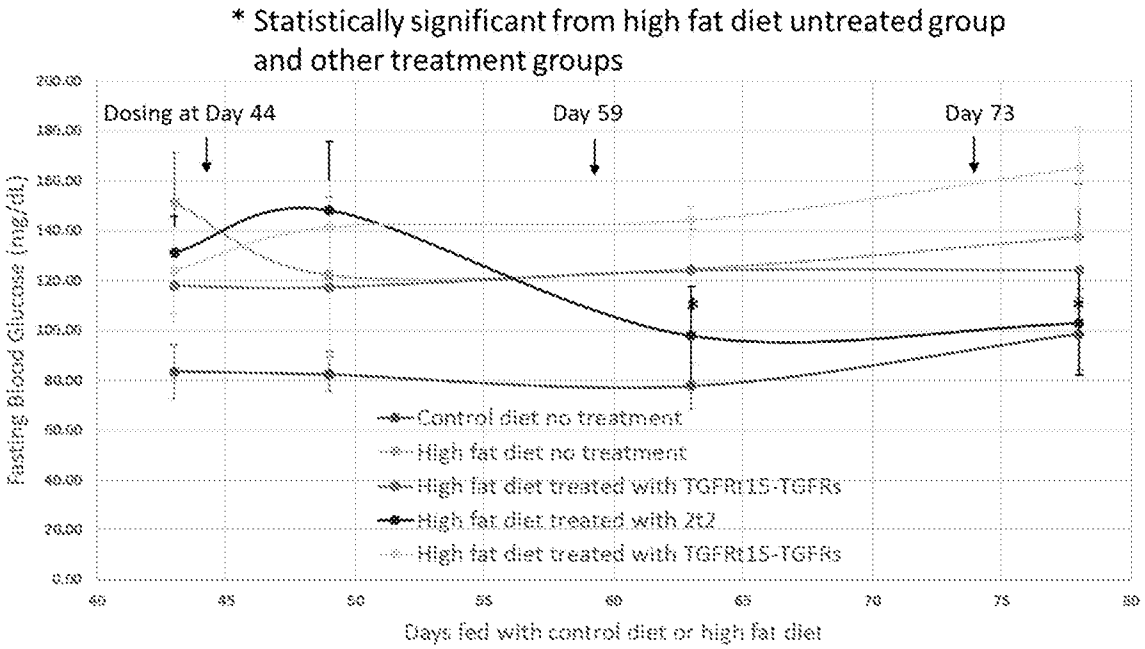
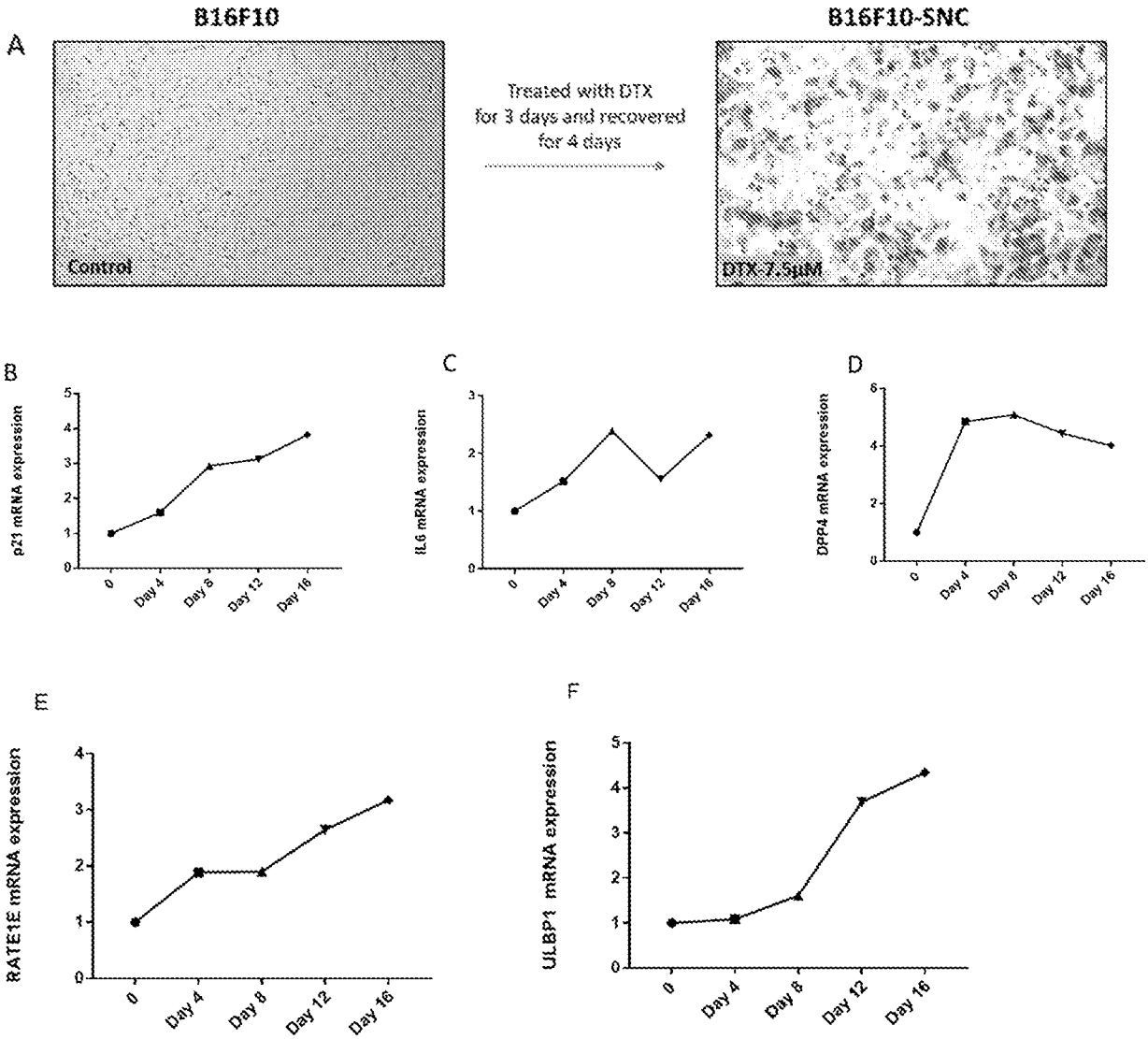
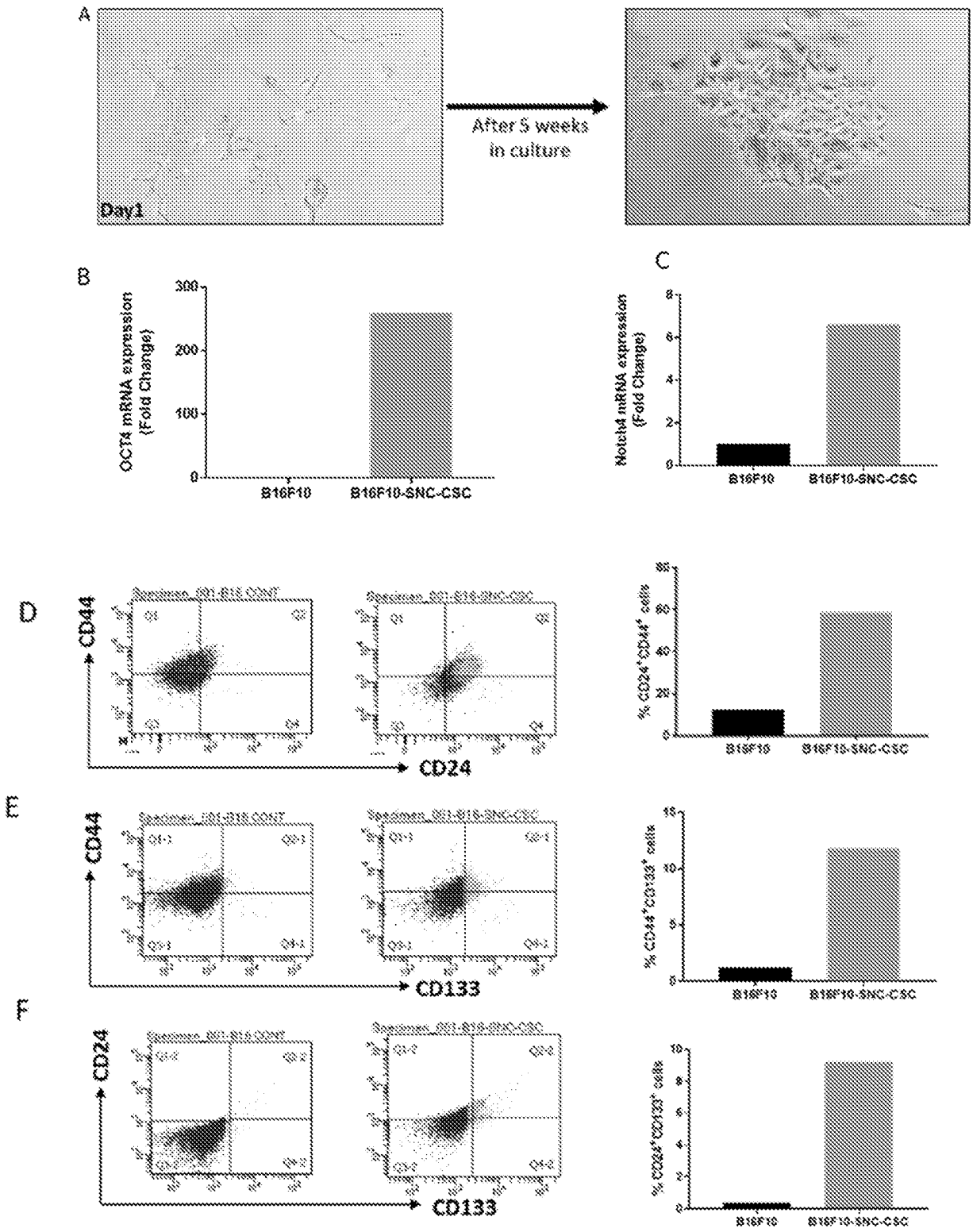

* Statistically significant from high fat diet untreated group and other treatment groups
Dosing at Day 44
Day 59
Day 73
Fasting Blood Glucose (mg/dL)
Control diet no treatment
High fat diet no treatment
High fat diet treated with TGFRt1S-TGFRs
High fat diet treated with Zt2
High fat diet treated with TGFRt1S-TGFRs
Days fed with control diet or high fat diet

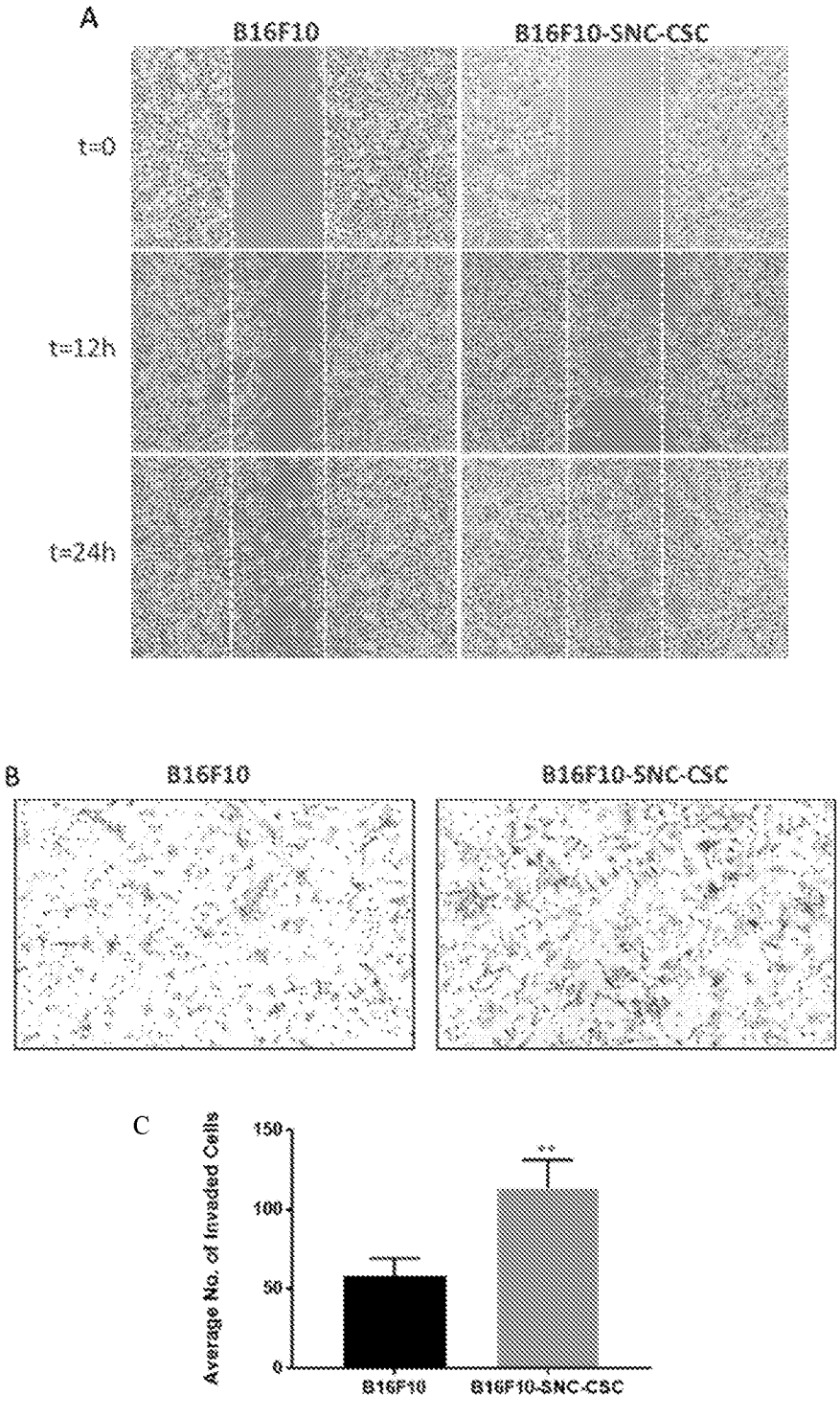
Figure 8



Figures 9A-9F

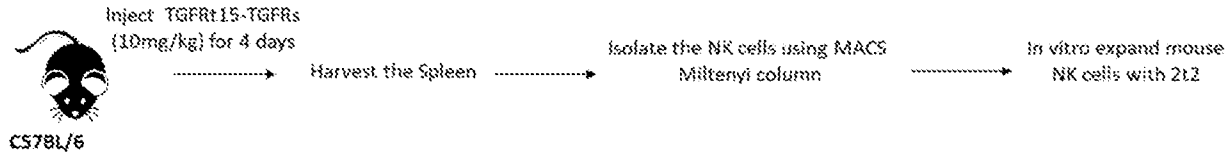


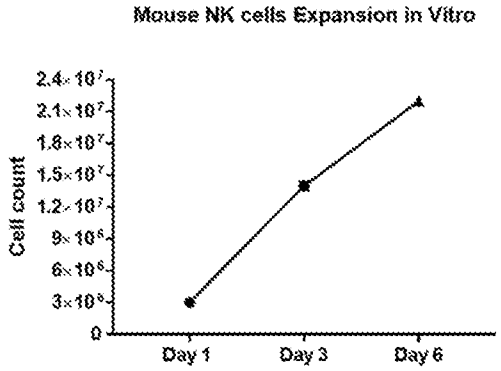
Figures 10A-10F



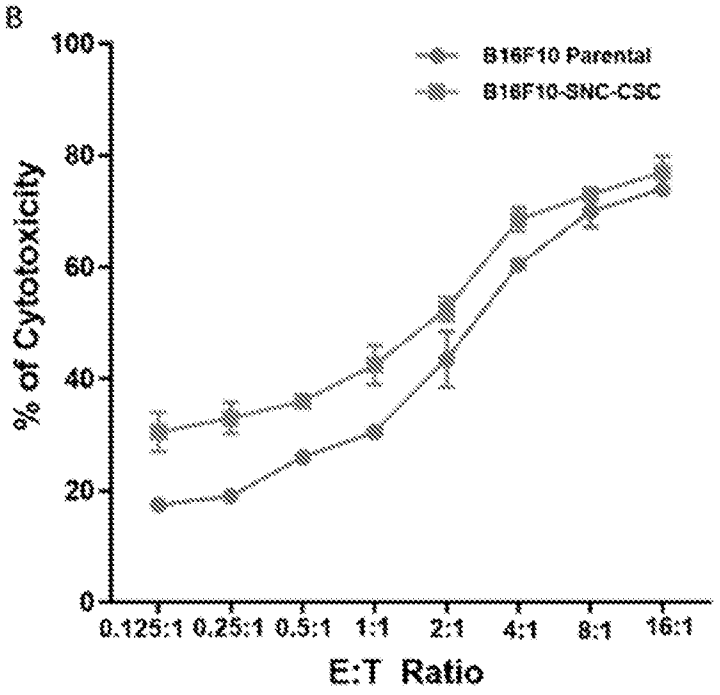
Figures 11A-11C

A



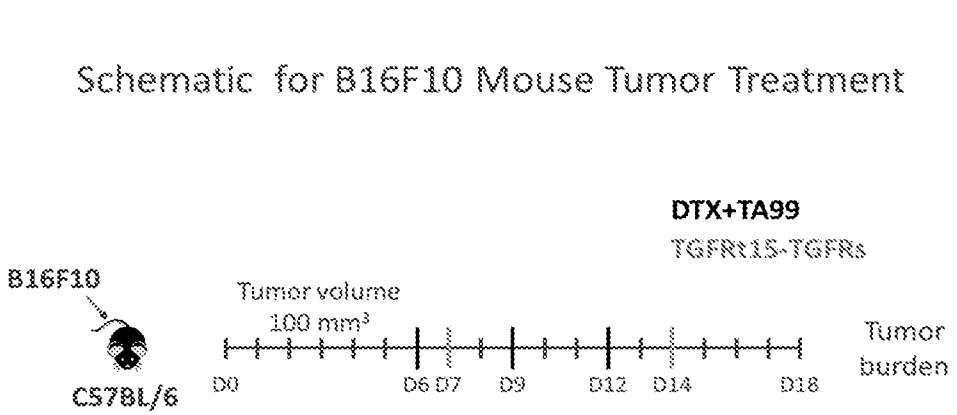


B

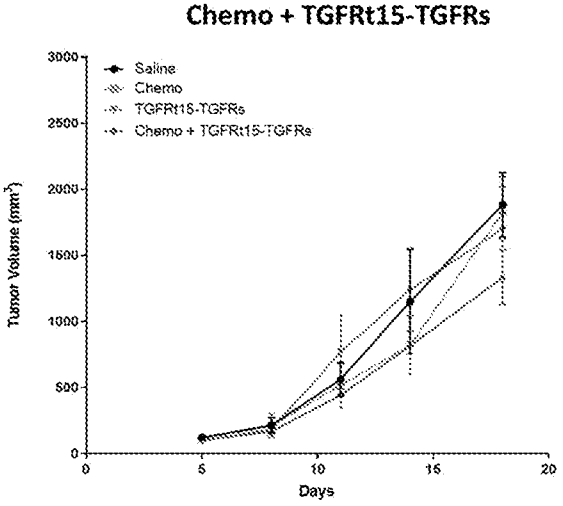


Figures 12A-12B

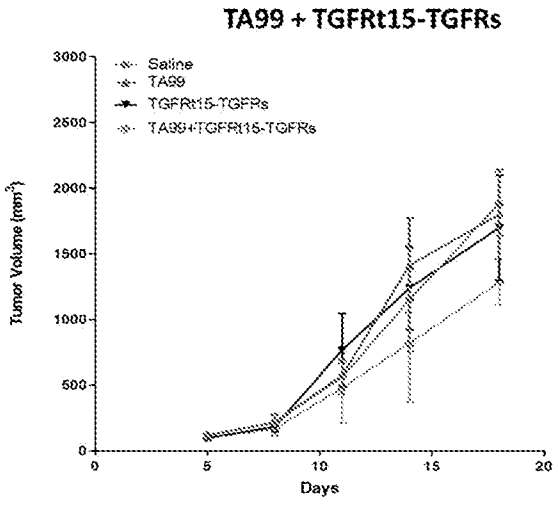
A



B



C



Figures 13A-13C

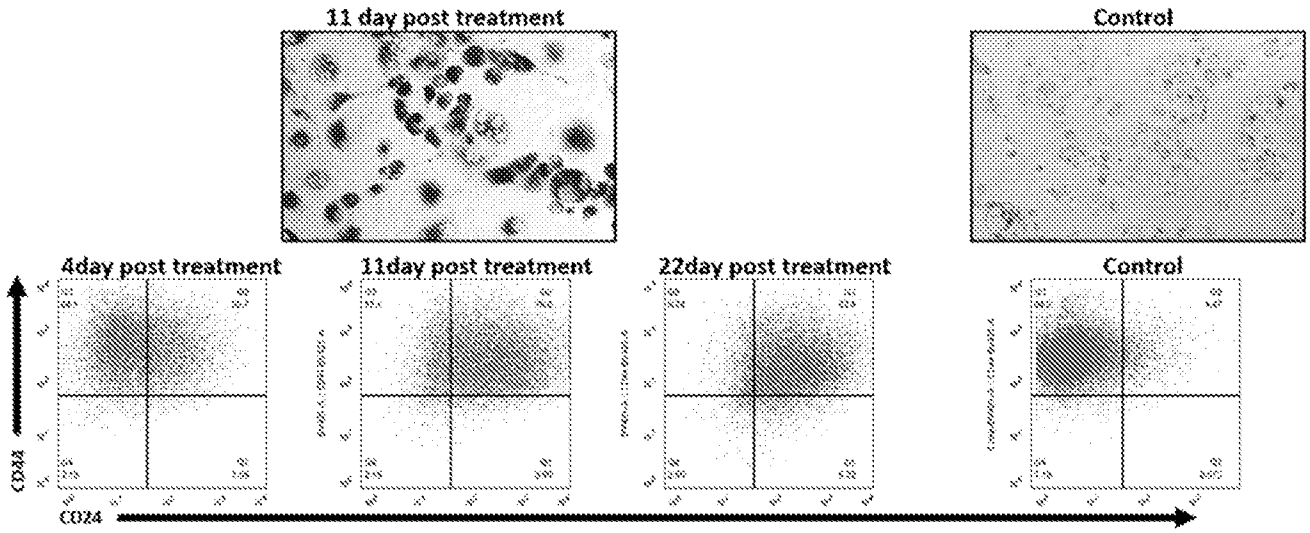

11 day post treatment
Control
4day post treatment
11day post treatment
22day post treatment
Control
CD28
CD24

Figure 14

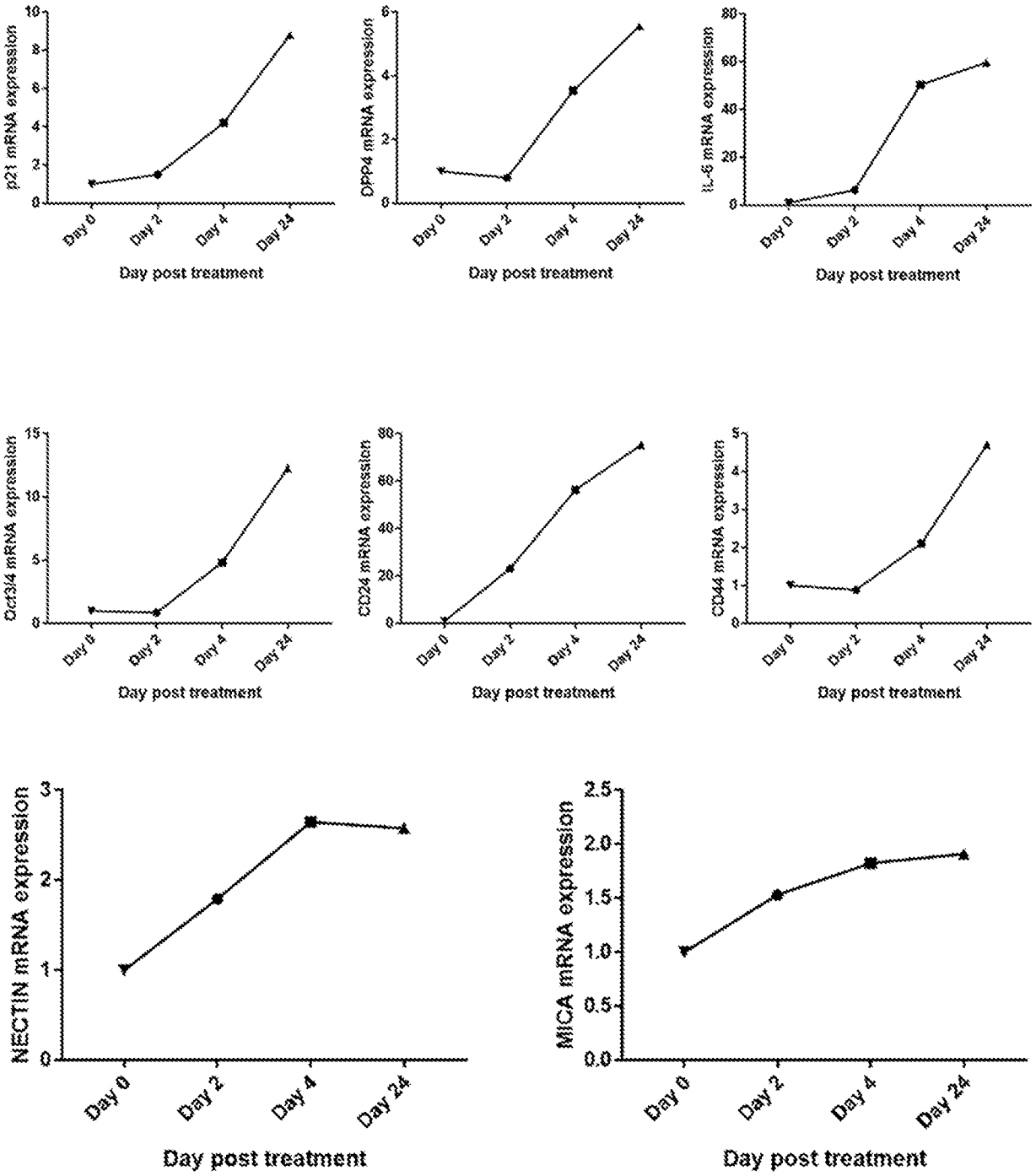

p21 mRNA expression
DPP4 mRNA expression
IL-6 mRNA expression
Oct3/4 mRNA expression
CD24 mRNA expression
CD44 mRNA expression
NECTIN mRNA expression
MICA mRNA expression
Day 0
Day 2
Day 4
Day 24
Day post treatment

Figure 15

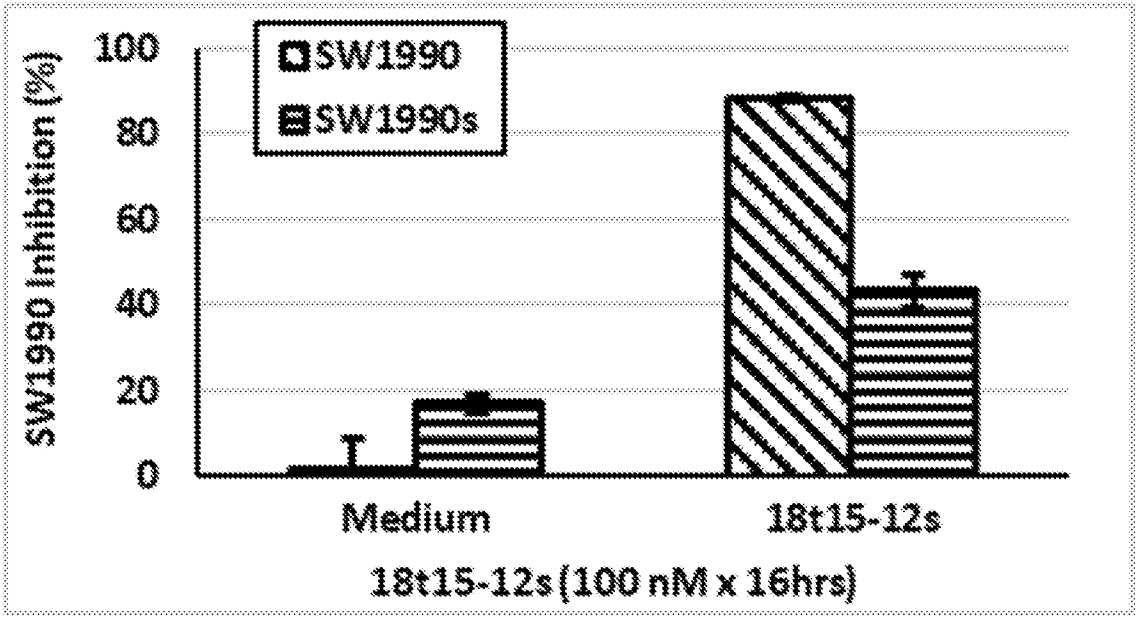

SW1990 Inhibition (%)
100
80
60
40
20
0
SW1990
SW1990s
Medium
18t15-12s
18t15-12s (100 nM x 16hrs)

Figure 16



Figure 17

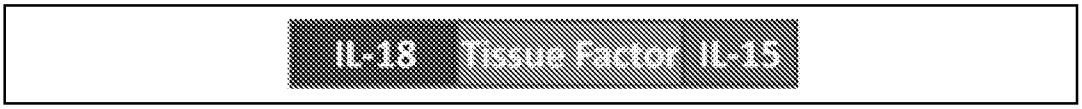


Figure 18

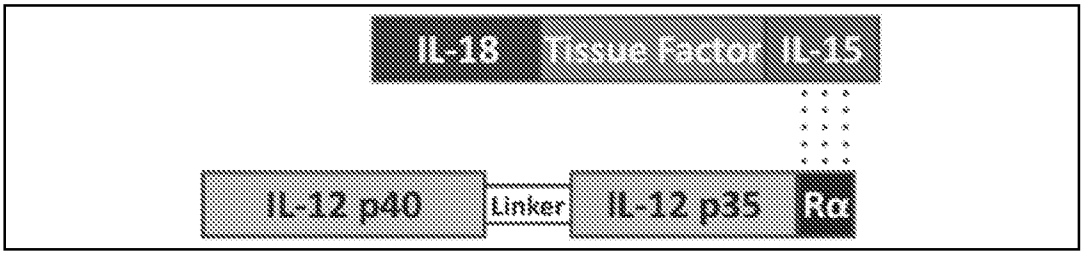


Figure 19

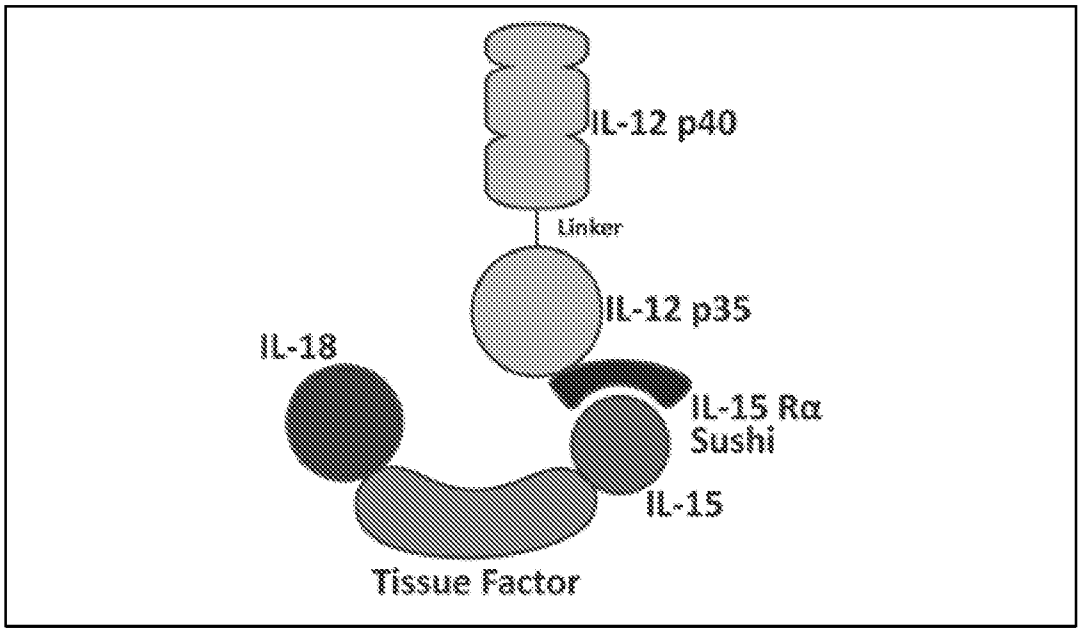


Figure 20

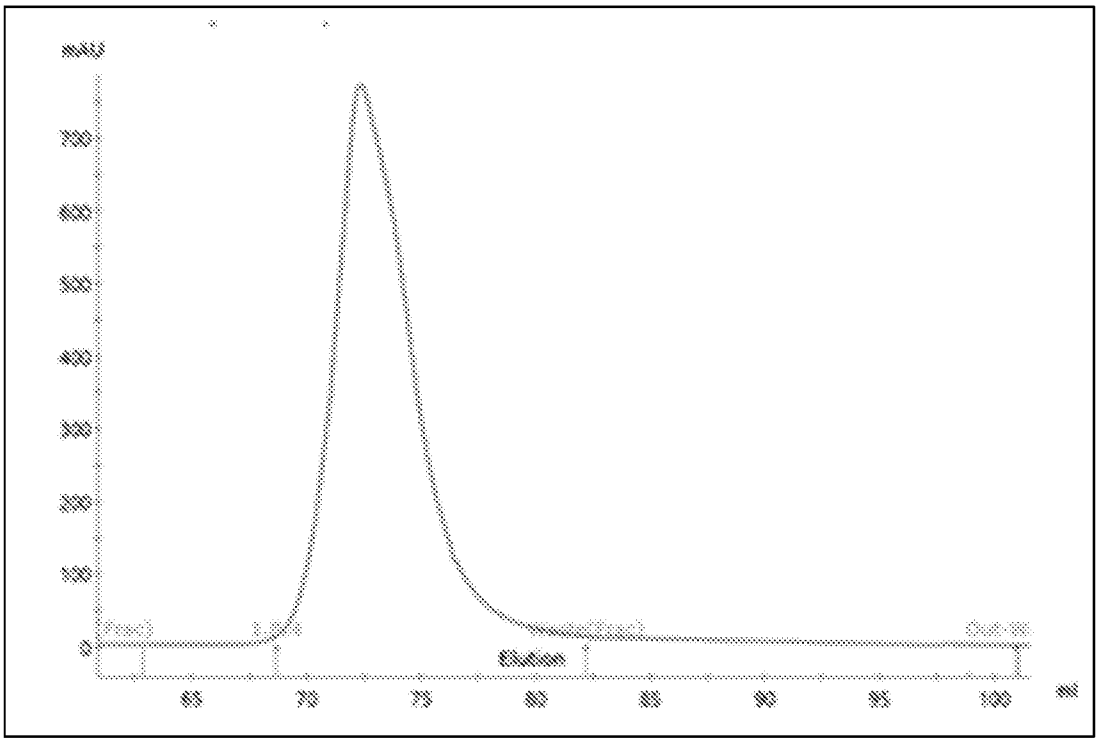


Figure 21

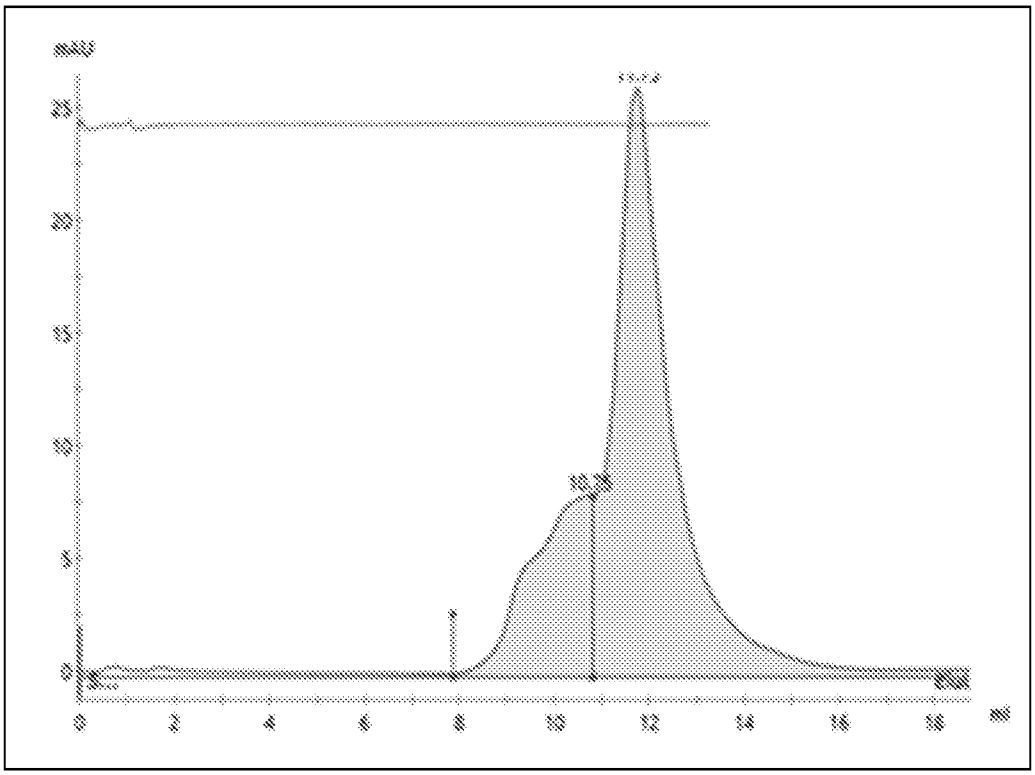


Figure 22

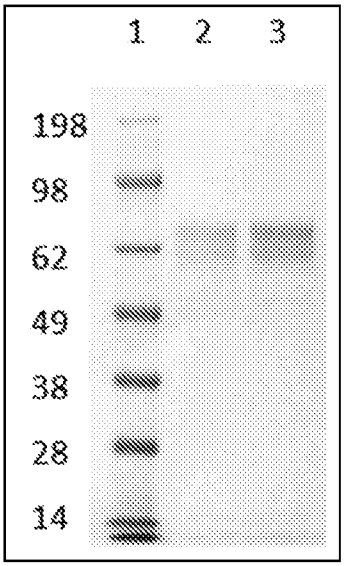


Figure 23

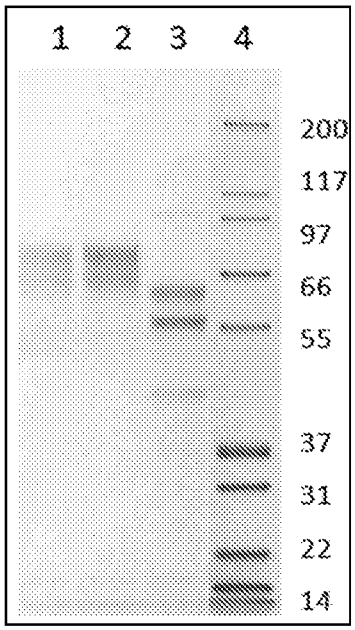


Figure 24

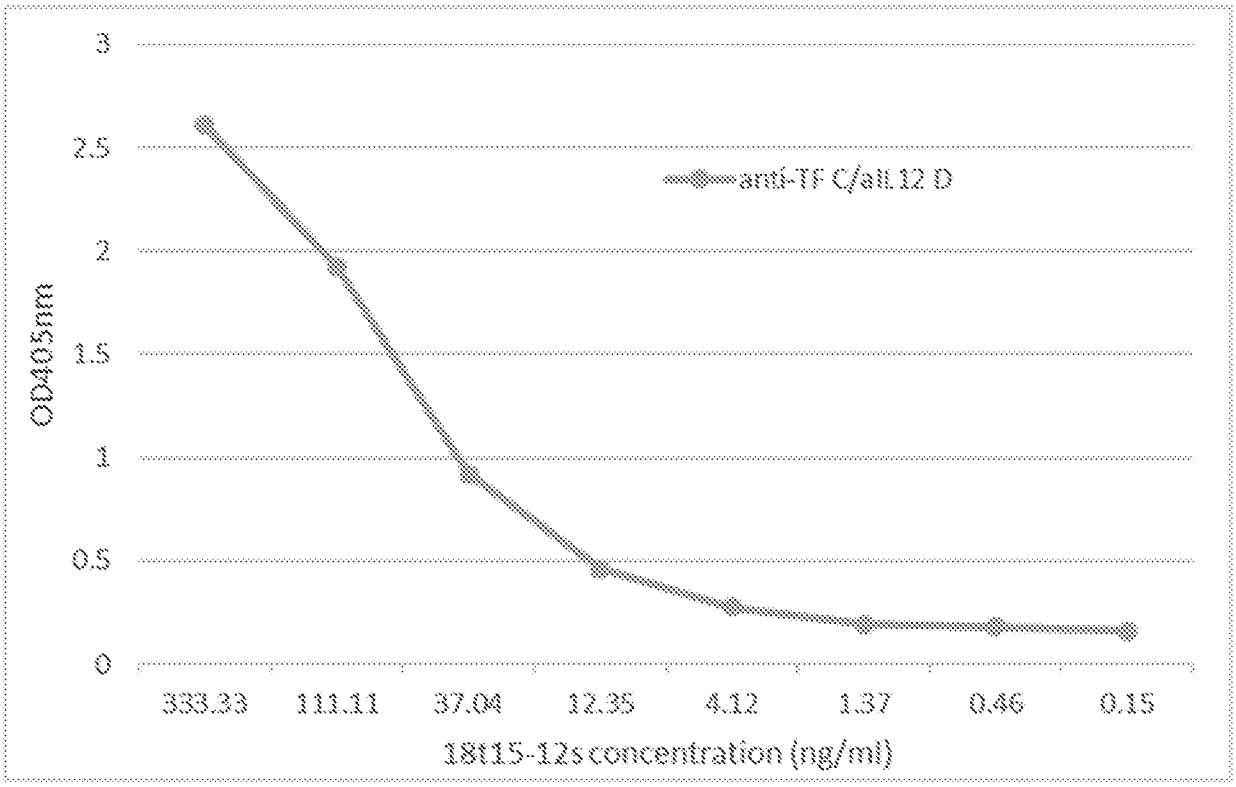


Figure 25

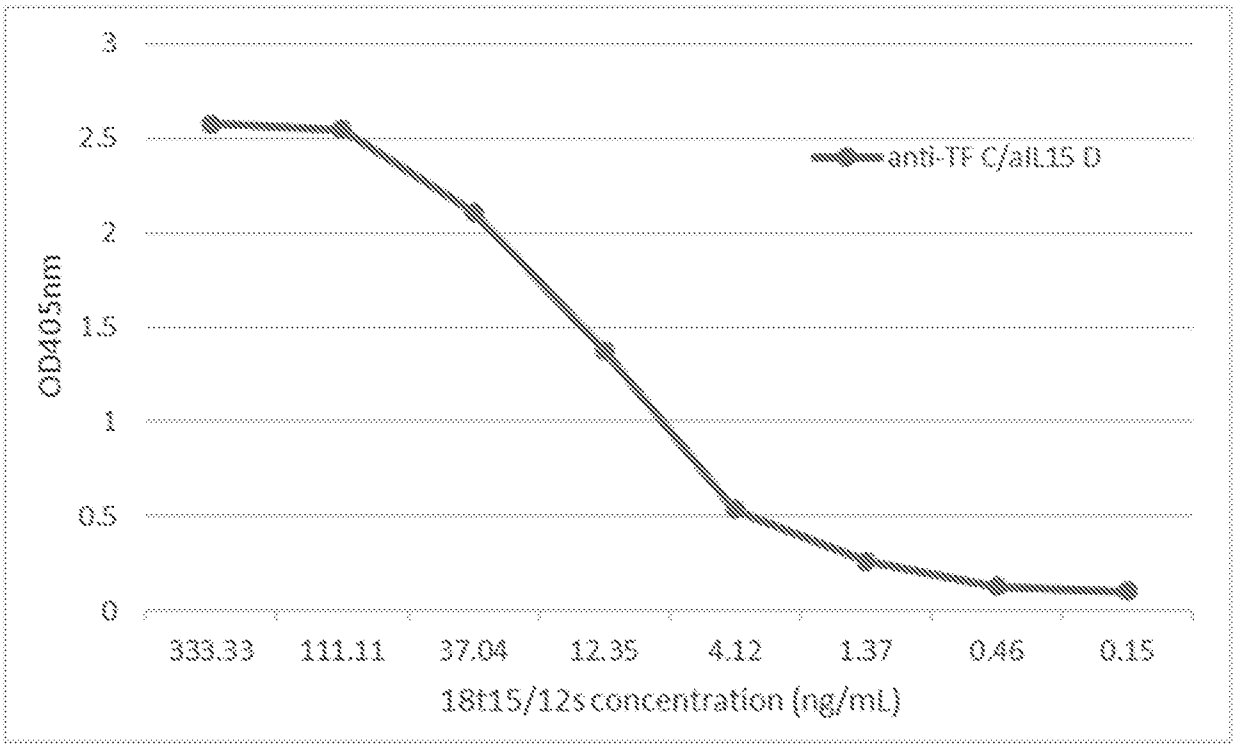


Figure 26

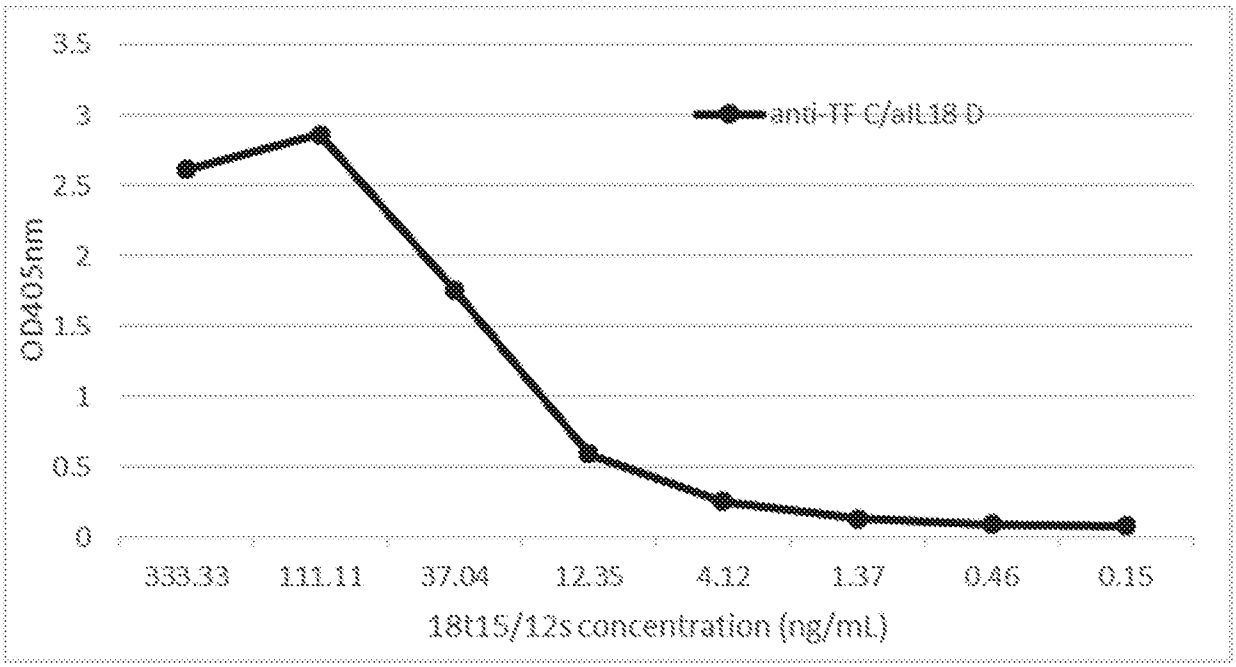


Figure 27

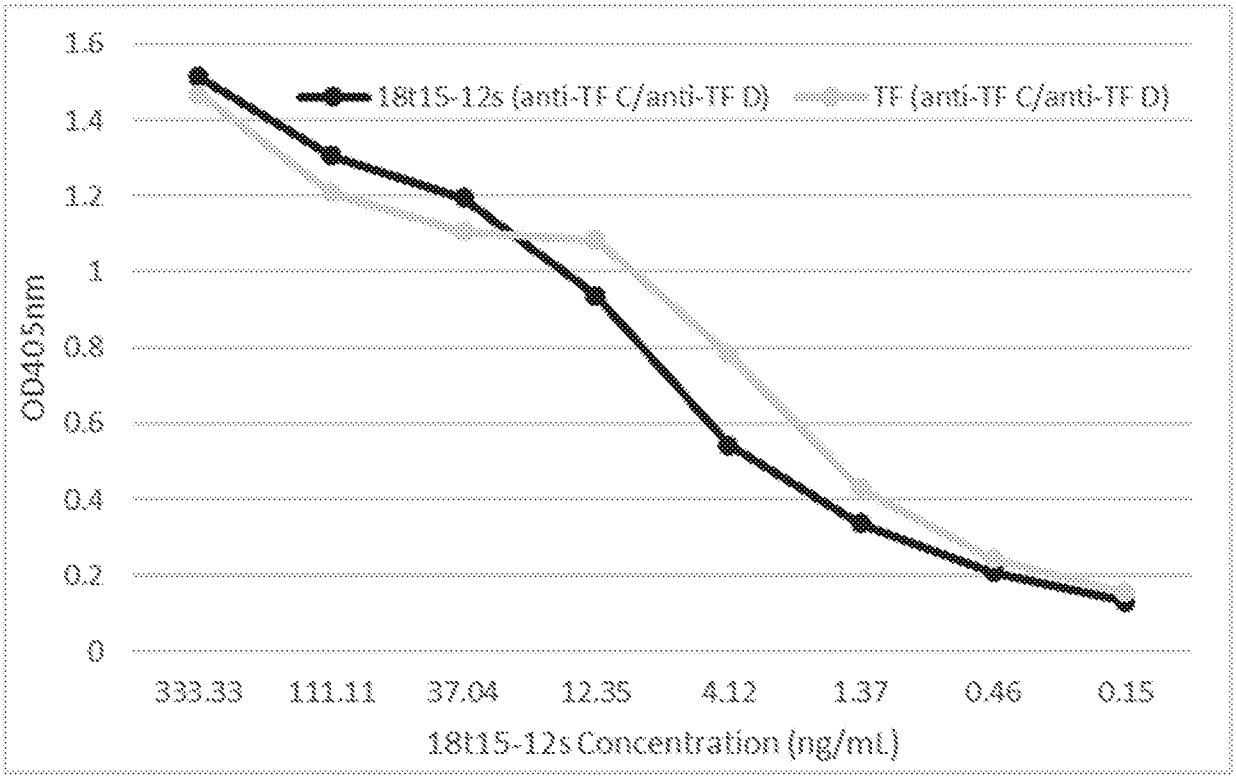

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD405nm
18t15-12s (anti-TF C/anti-TF D)
TF (anti-TF C/anti-TF D)
333.33
111.11
37.04
12.35
4.12
1.37
0.46
0.15
18t15-12s Concentration (ng/mL)

Figure 28

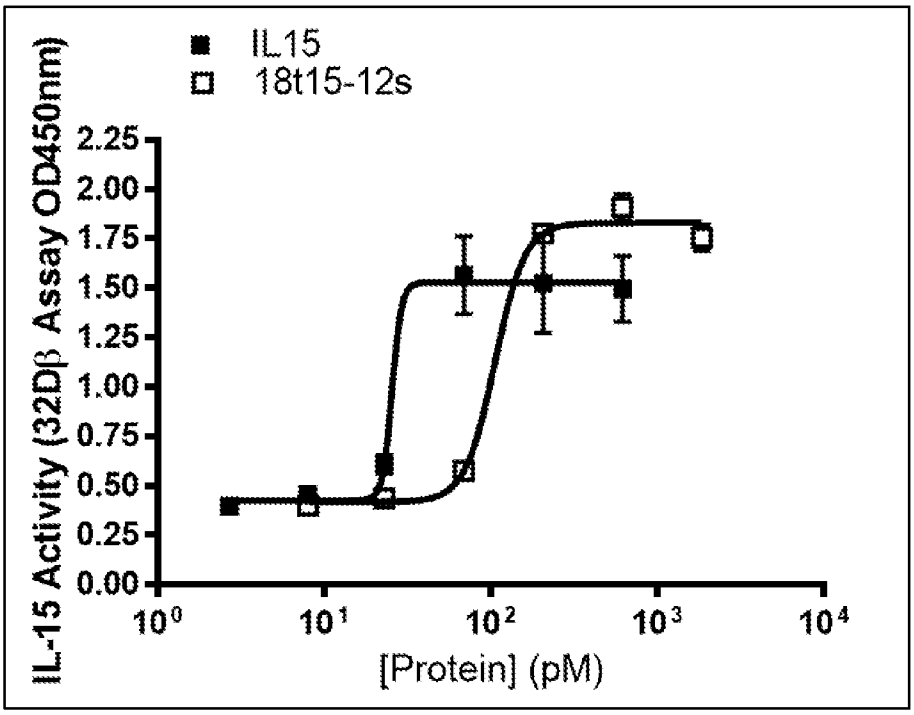


Figure 29

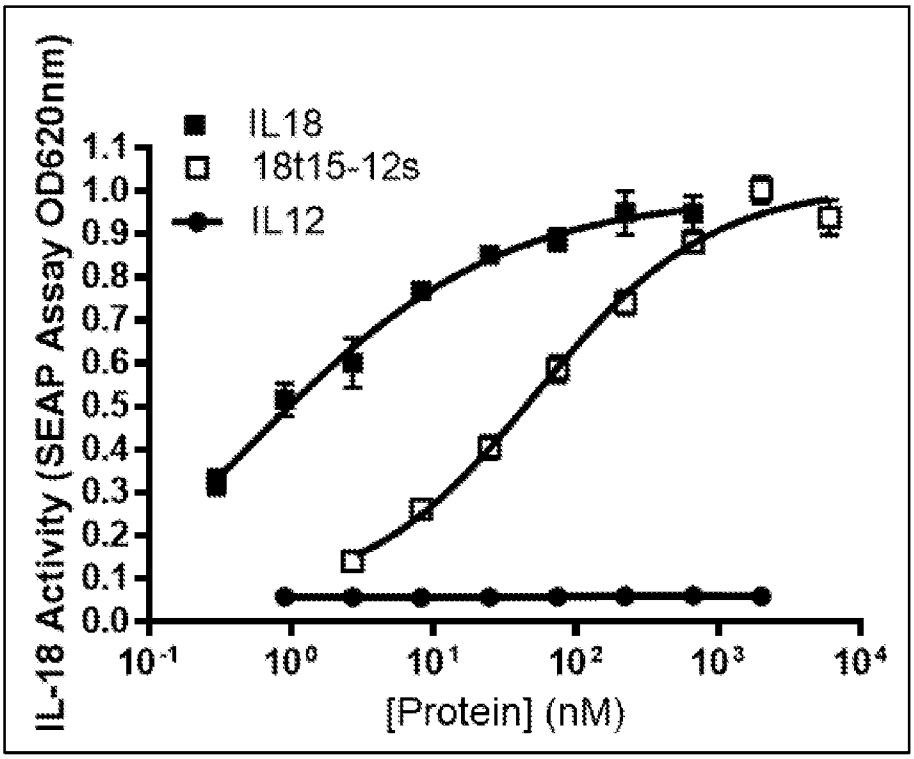


Figure 30

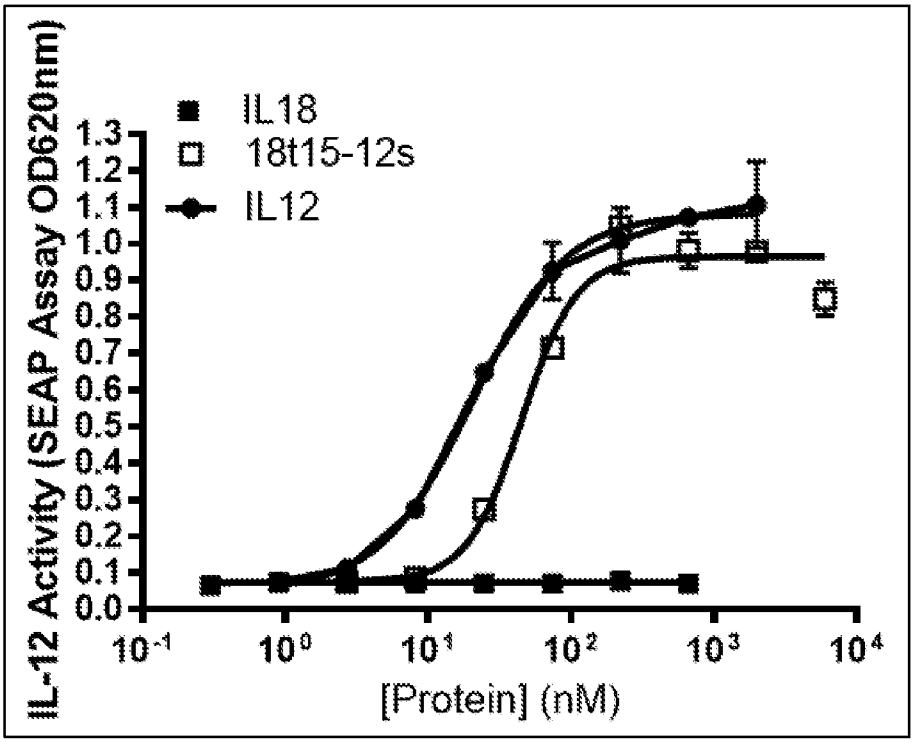

IL18
18t15-12s
IL12
IL-12 Activity (SEAP Assay OD620nm)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10-1
100
101
102
103
104
[Protein] (nM)

Figure 31

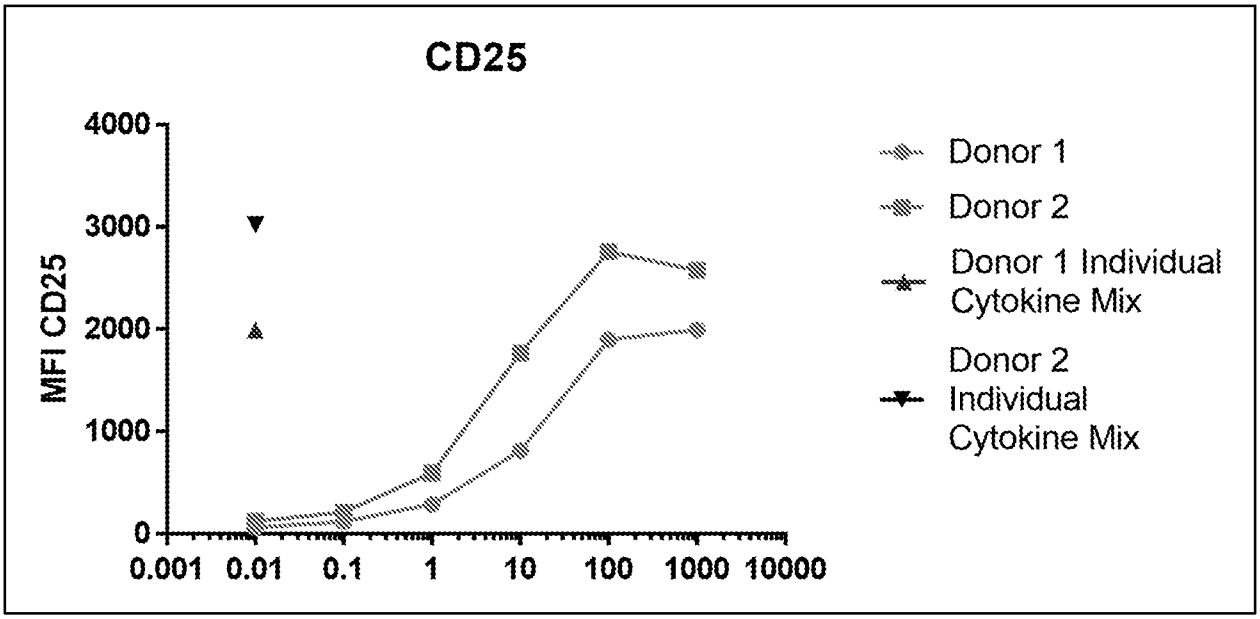


Figure 32A

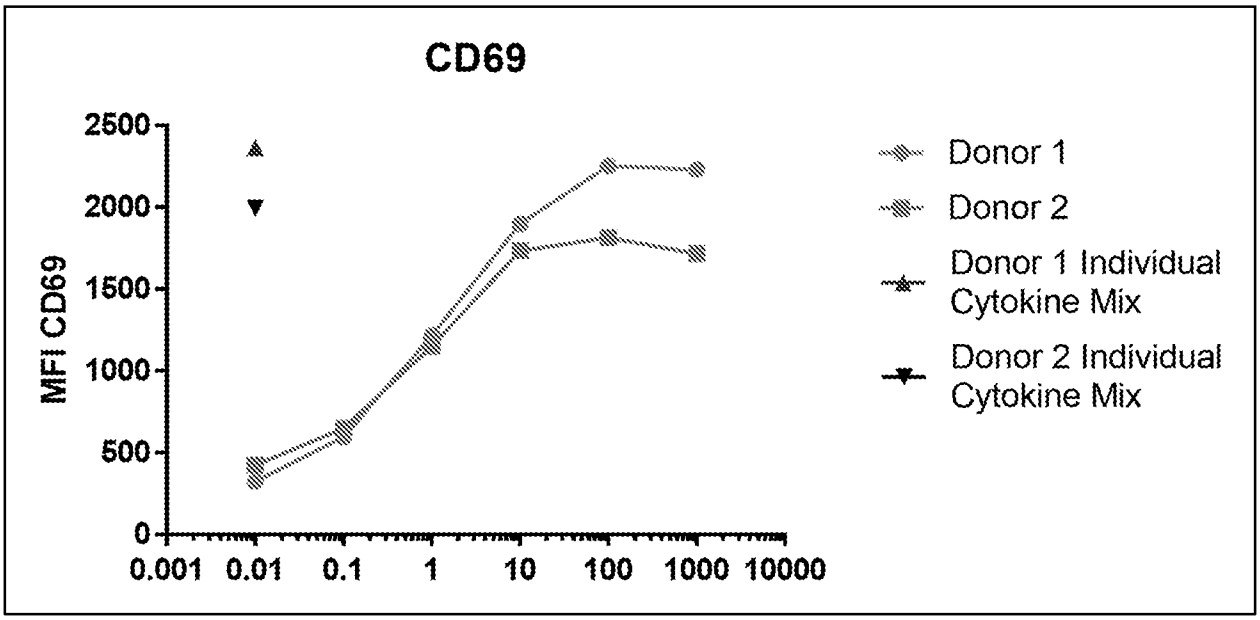


Figure 32B

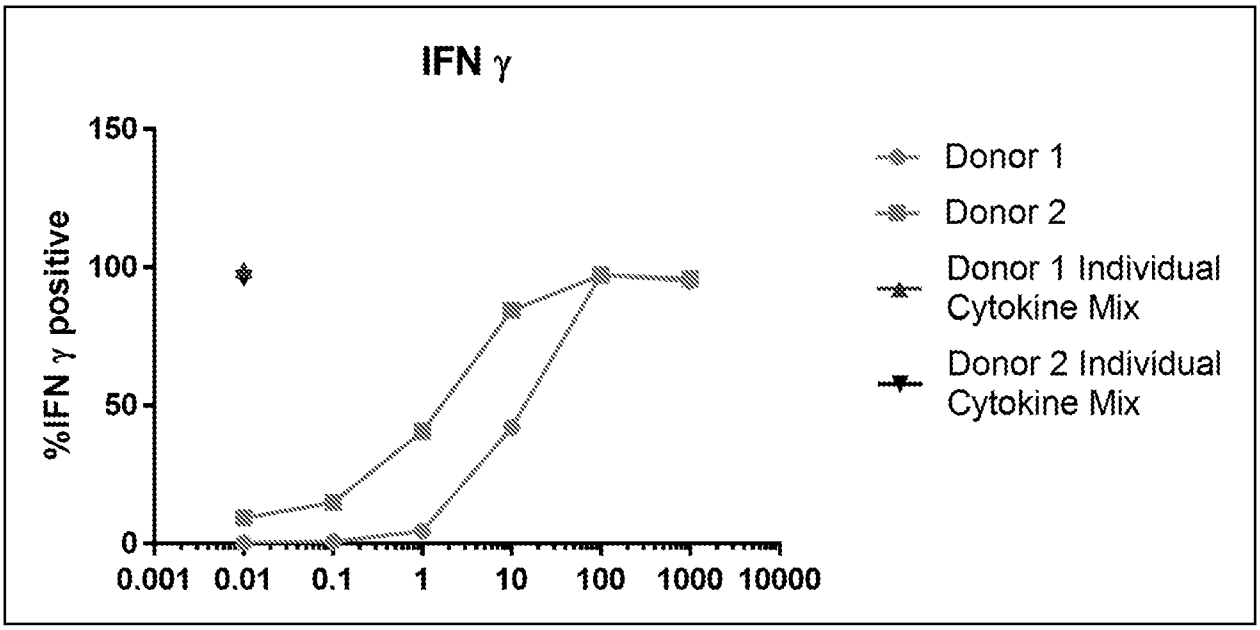


Figure 33

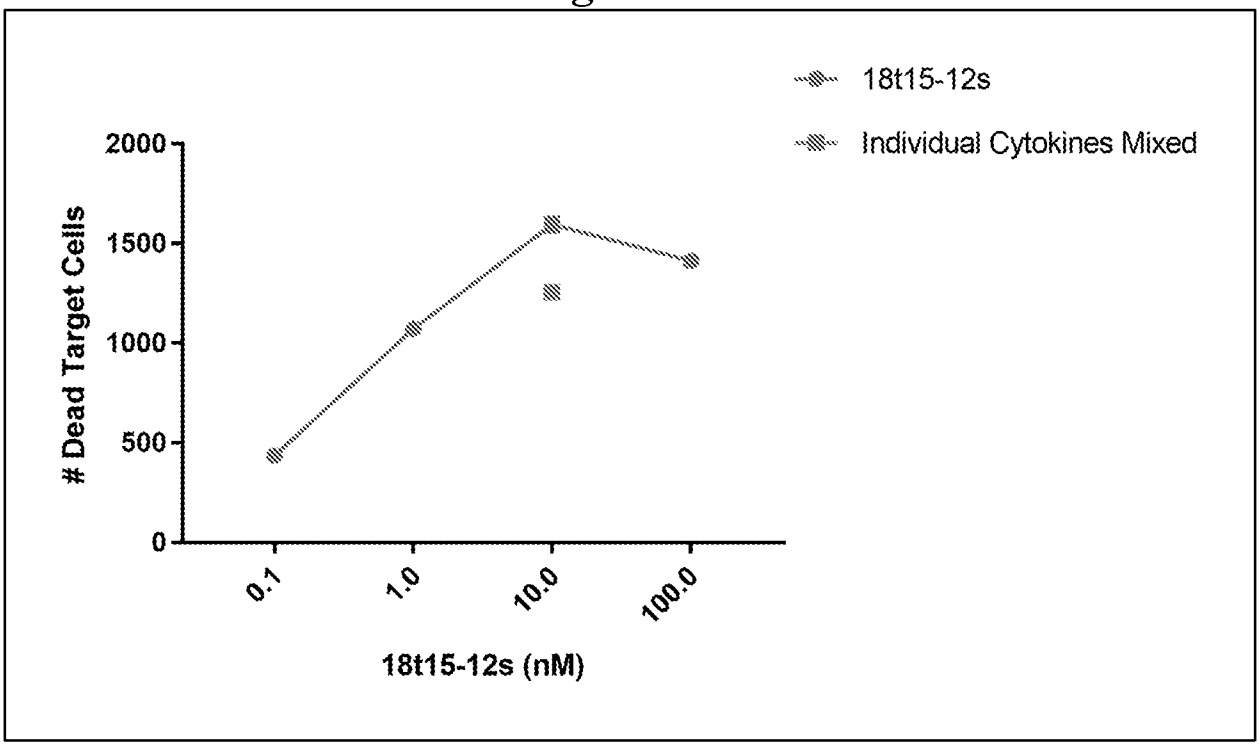


Figure 34

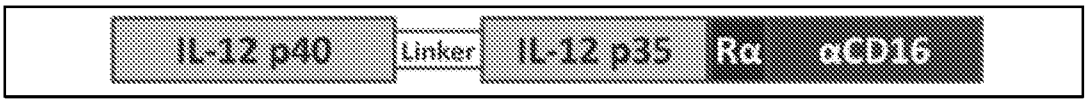


Figure 35

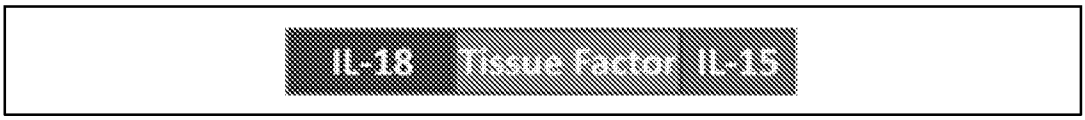


Figure 36

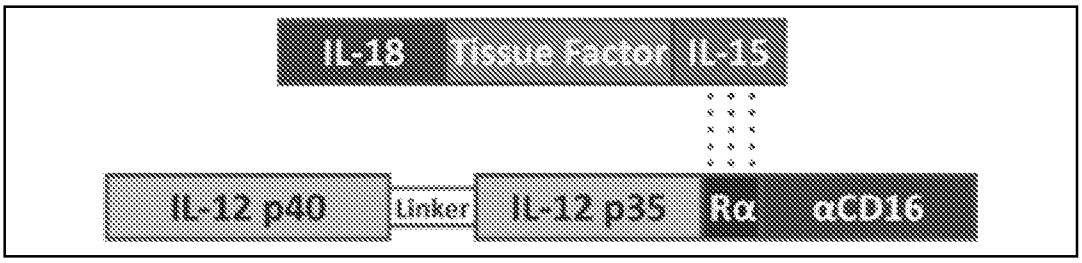


Figure 37

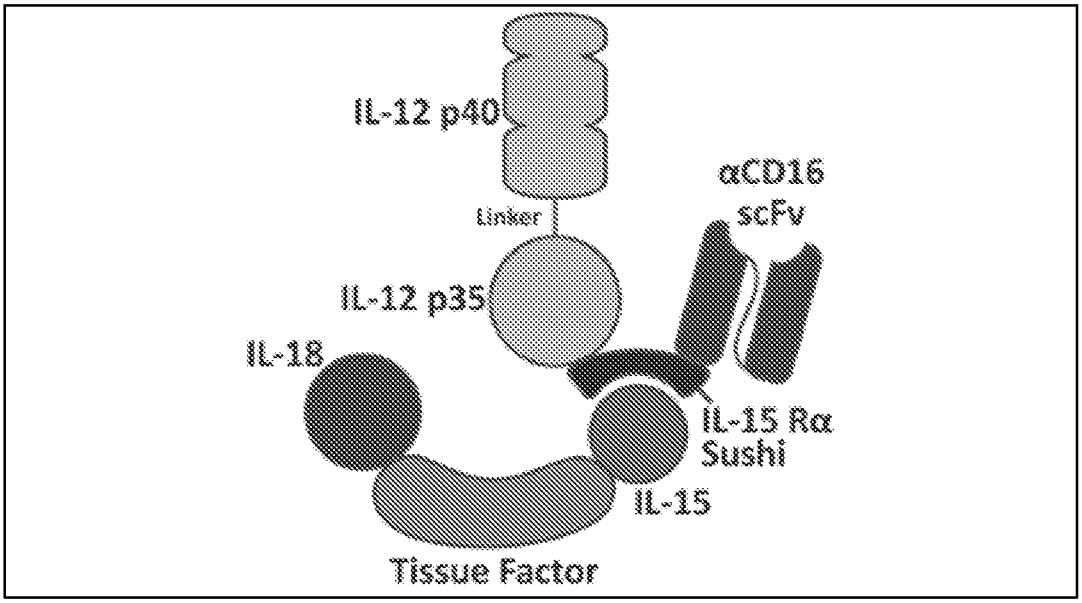


Figure 38

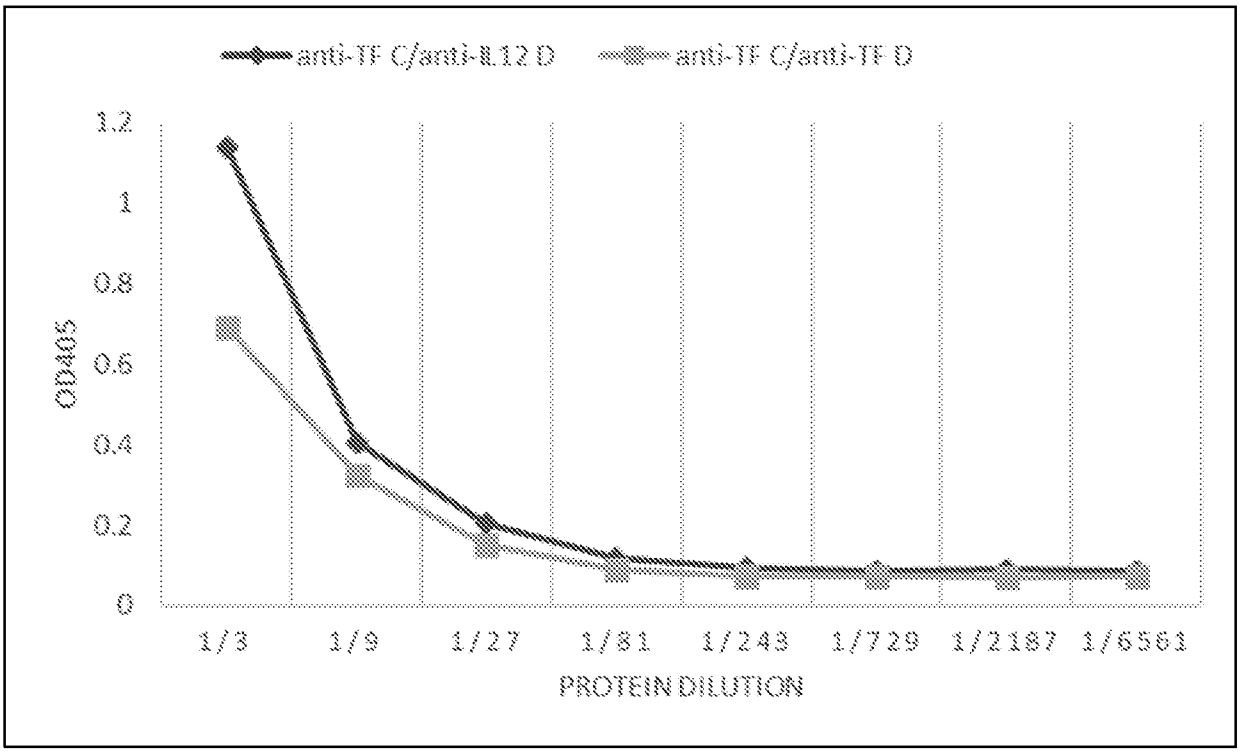

anti-TF C/anti-IL12 D
anti-TF C/anti-TF D
OD405
1.2
1
0.8
0.6
0.4
0.2
0
1/3
1/9
1/27
1/81
1/243
1/729
1/2187
1/6561
PROTEIN DILUTION

Figure 39



Figure 40



Figure 41

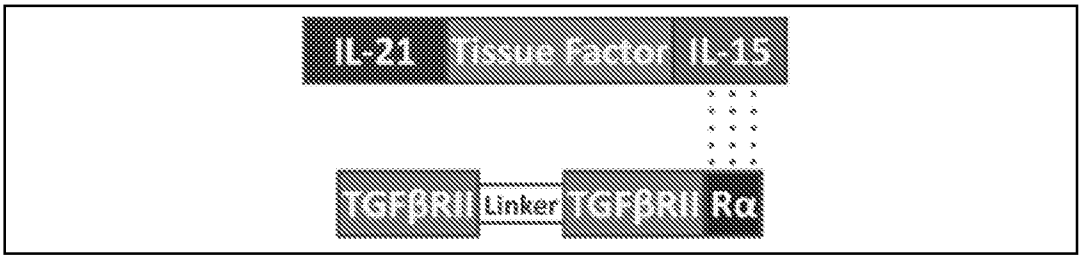


Figure 42

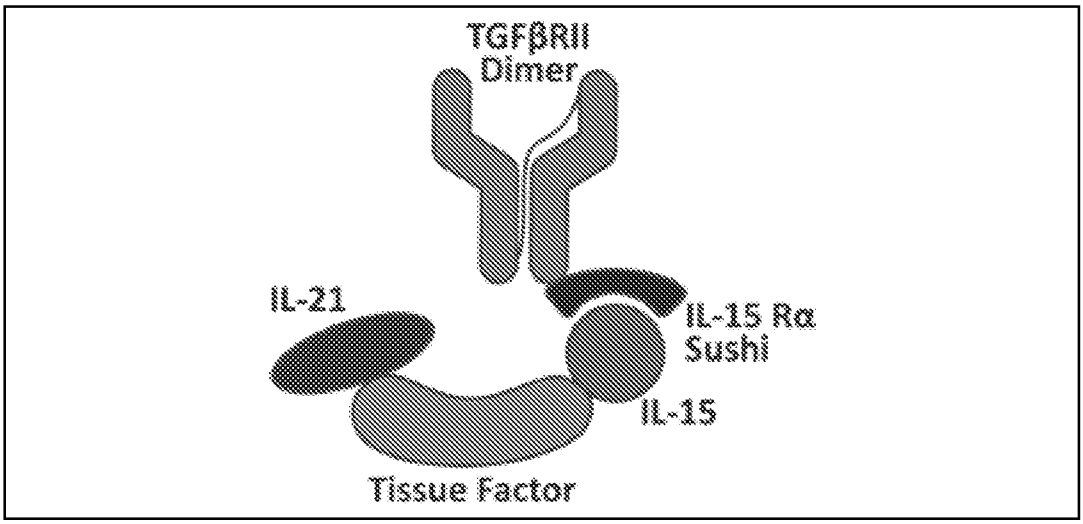


Figure 43

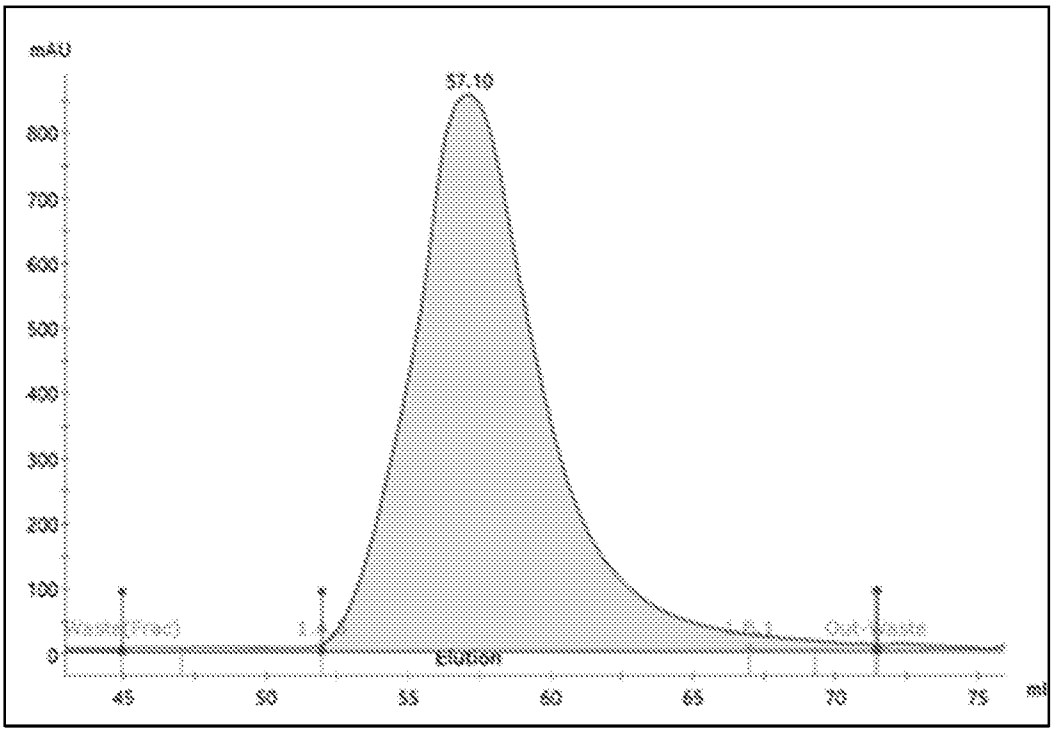


Figure 44

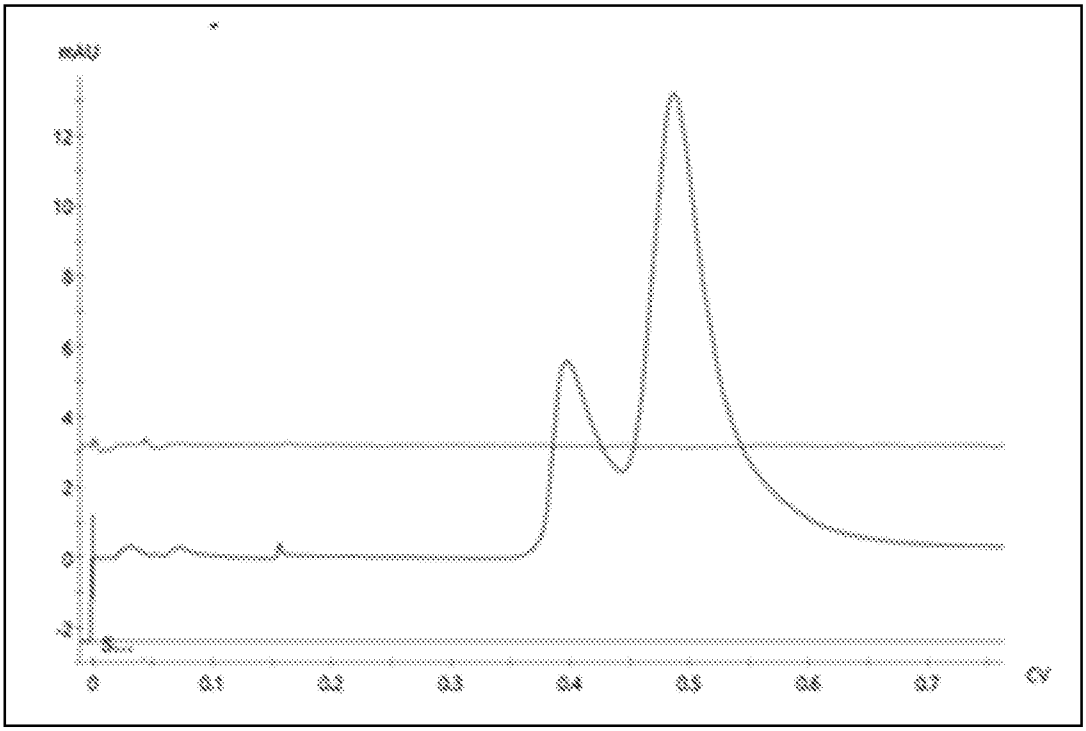


Figure 45

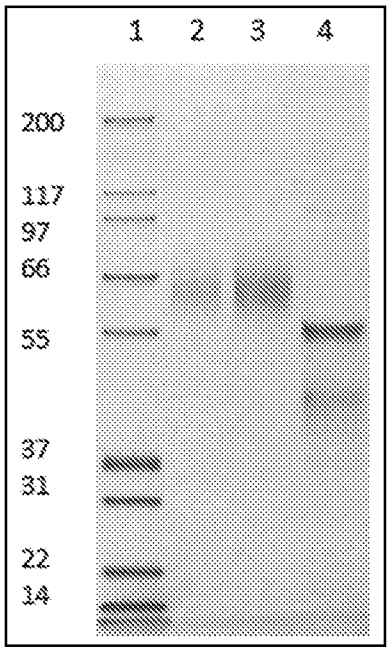


Figure 46

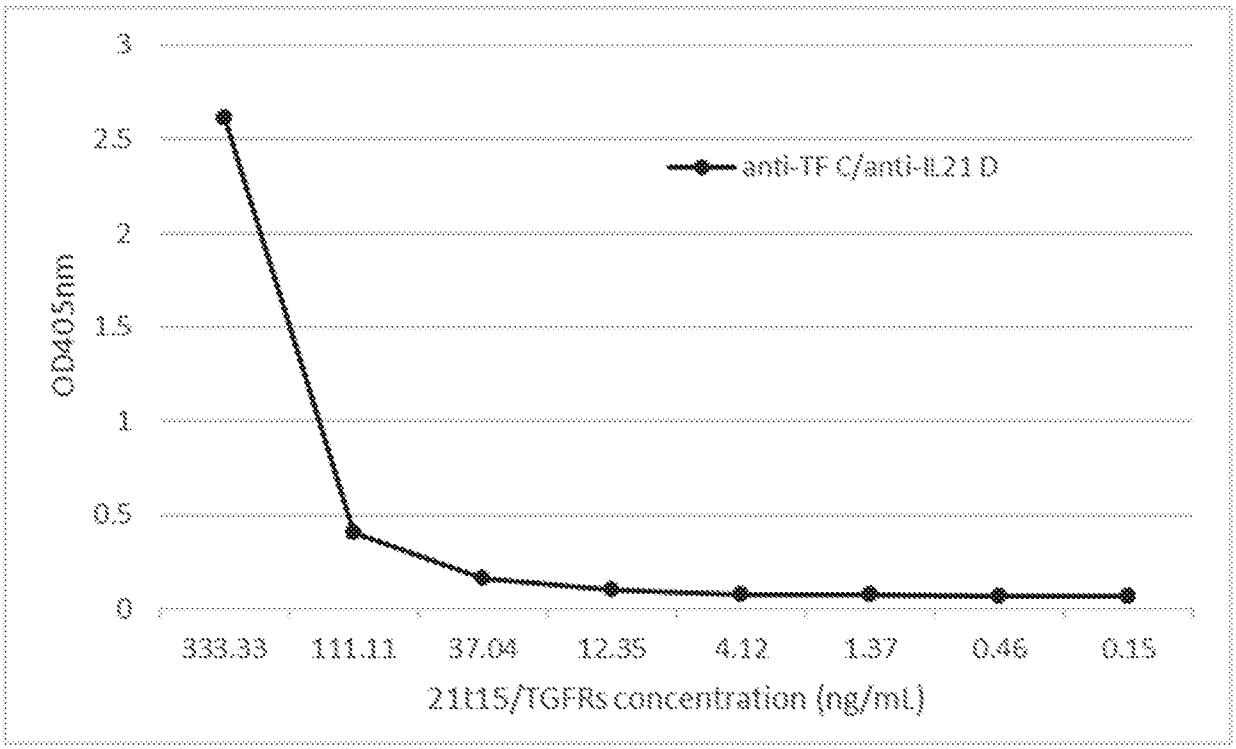


Figure 47

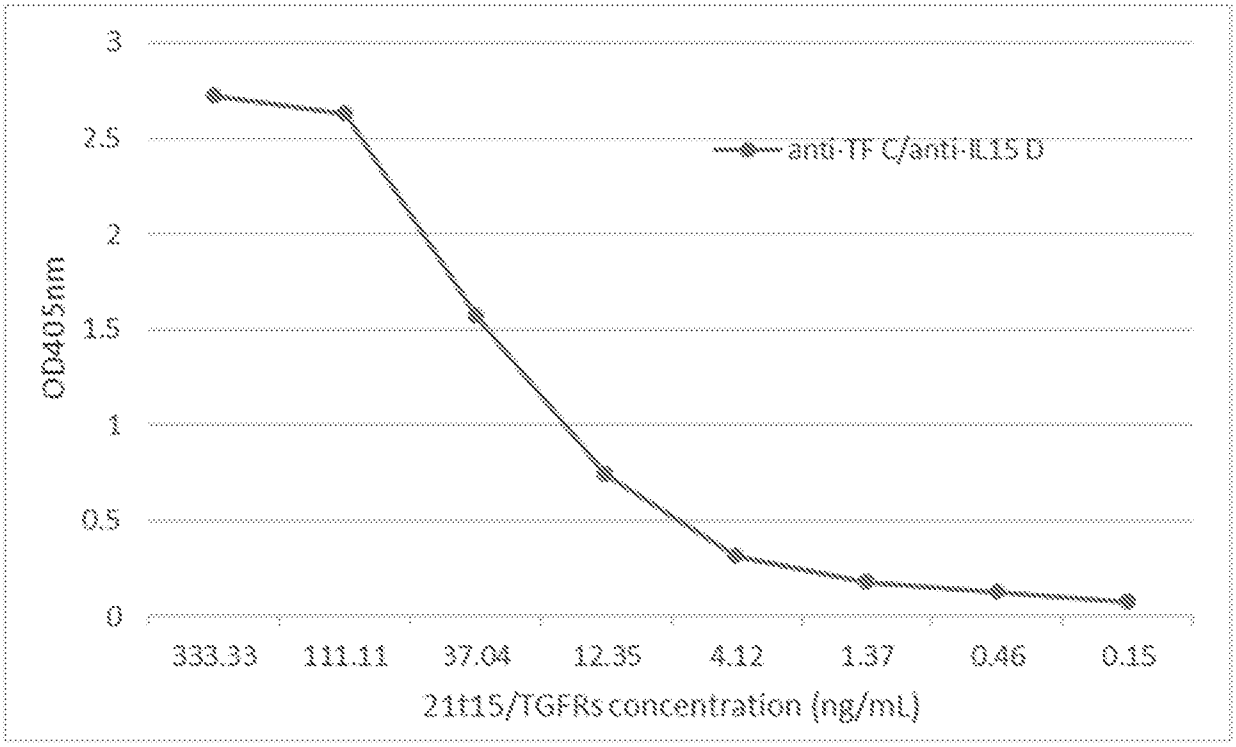


Figure 48

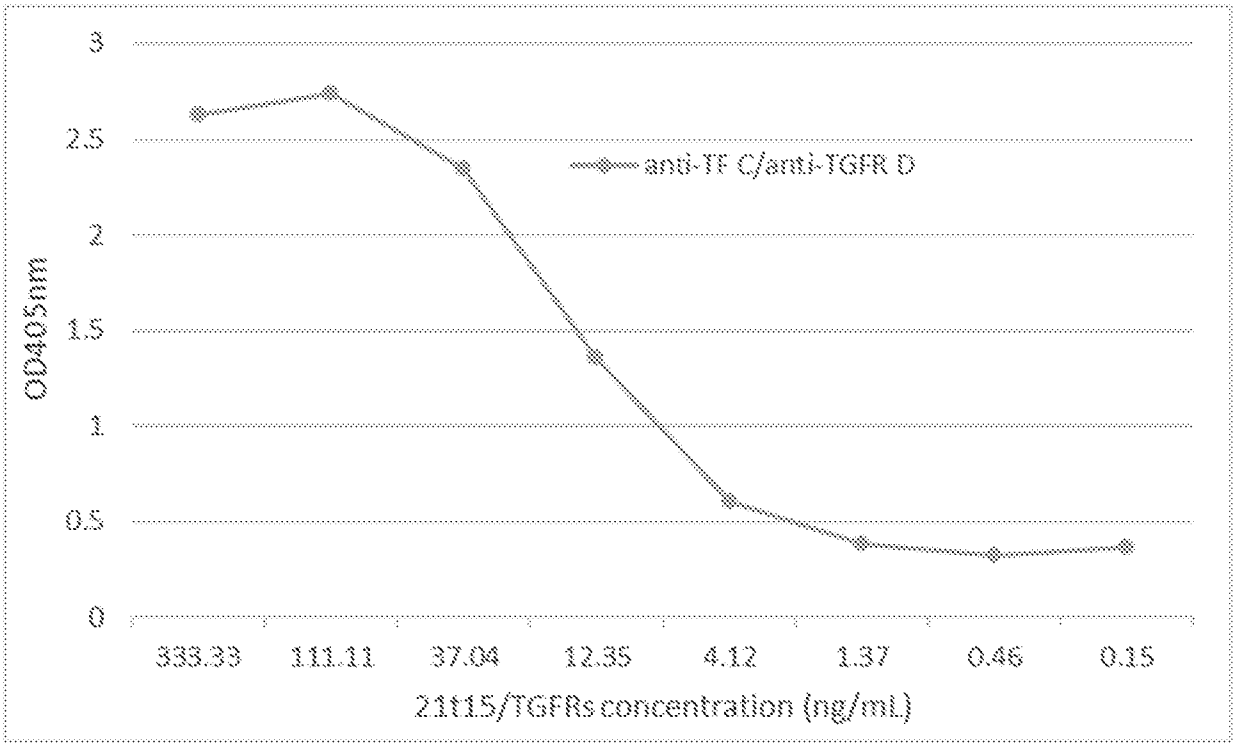


Figure 49

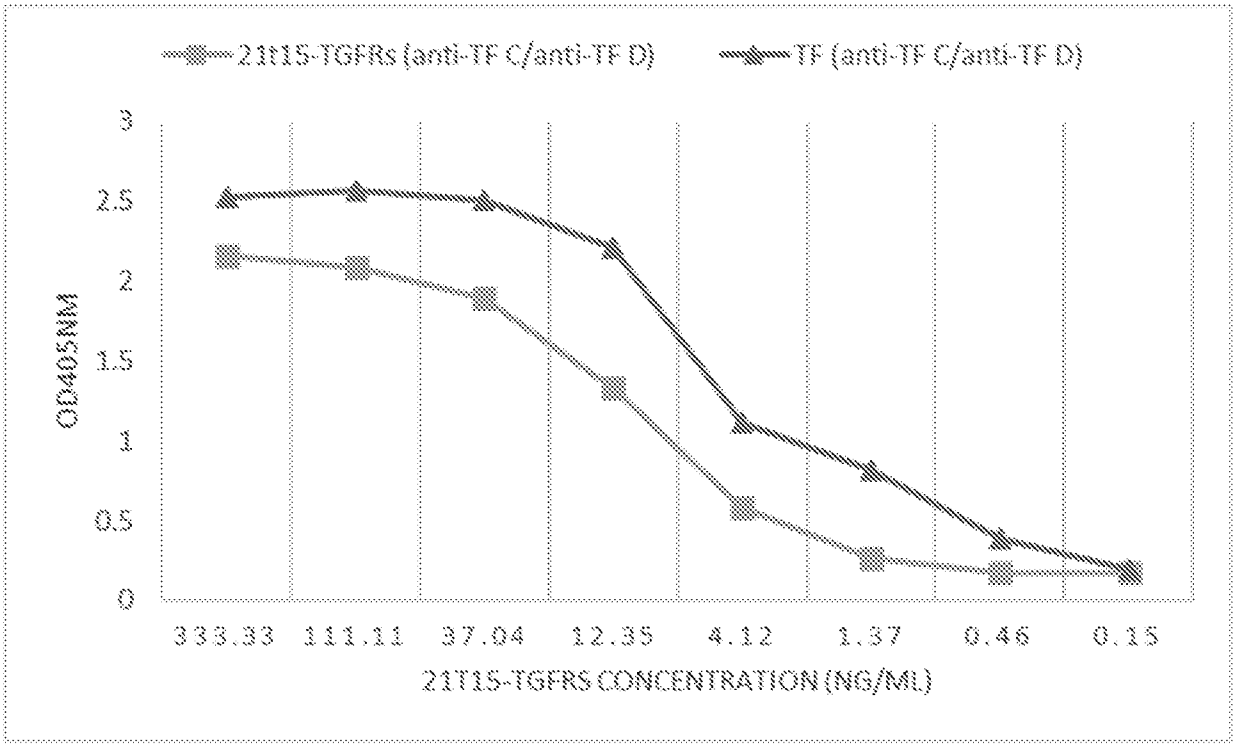

21t15-TGFRs (anti-TF C/anti-TF D)
TF (anti-TF C/anti-TF D)
OD405NM
3
2.5
2
1.5
1
0.5
0
333.33
111.11
37.04
12.35
4.12
1.37
0.46
0.15
21T15-TGFRS CONCENTRATION (NG/ML)

Figure 50

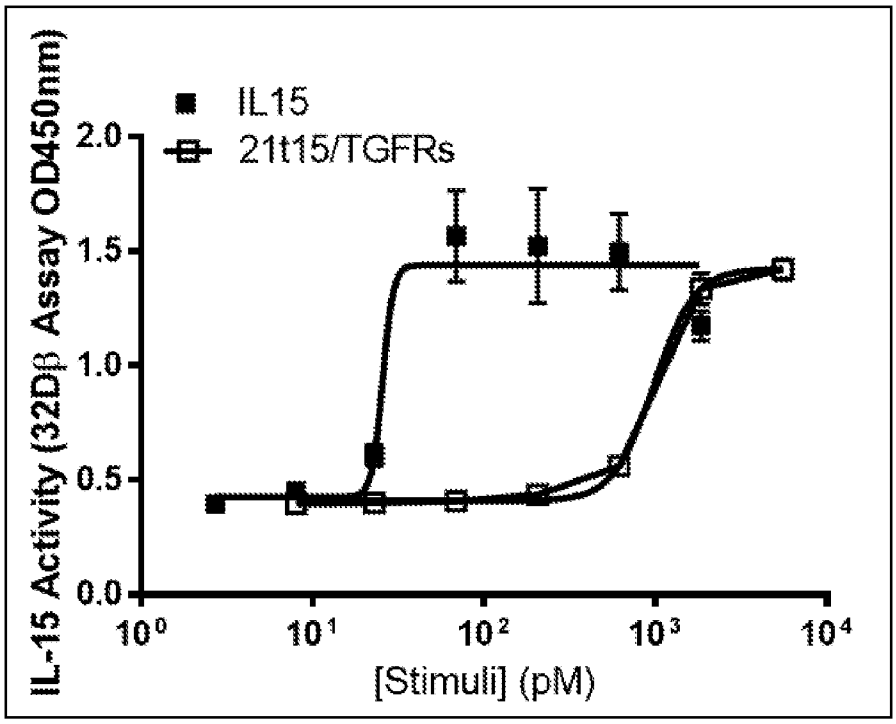


Figure 51

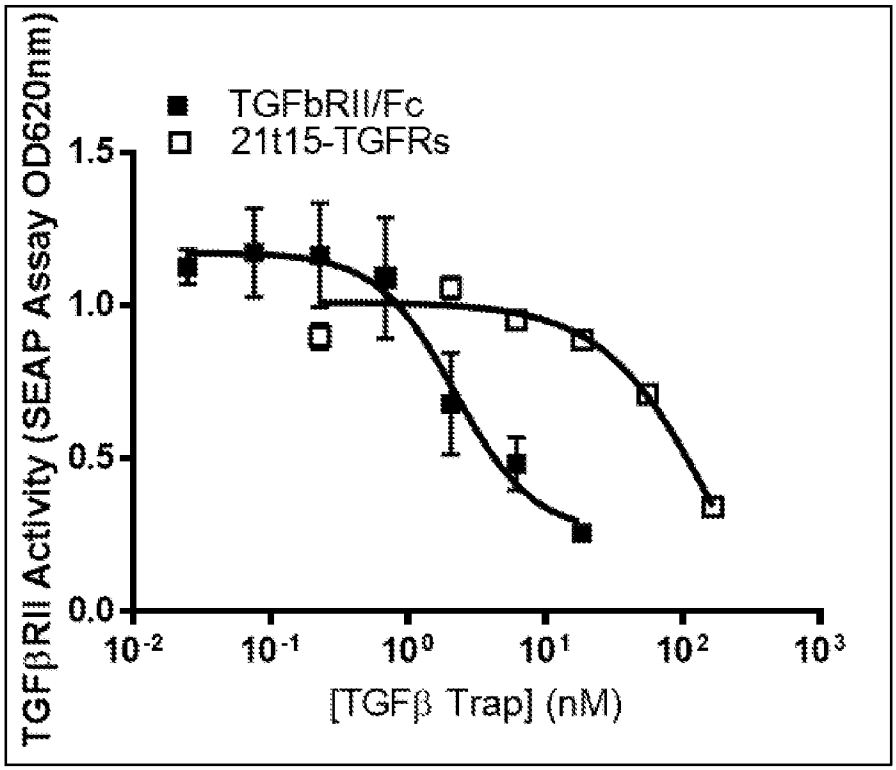


Figure 52

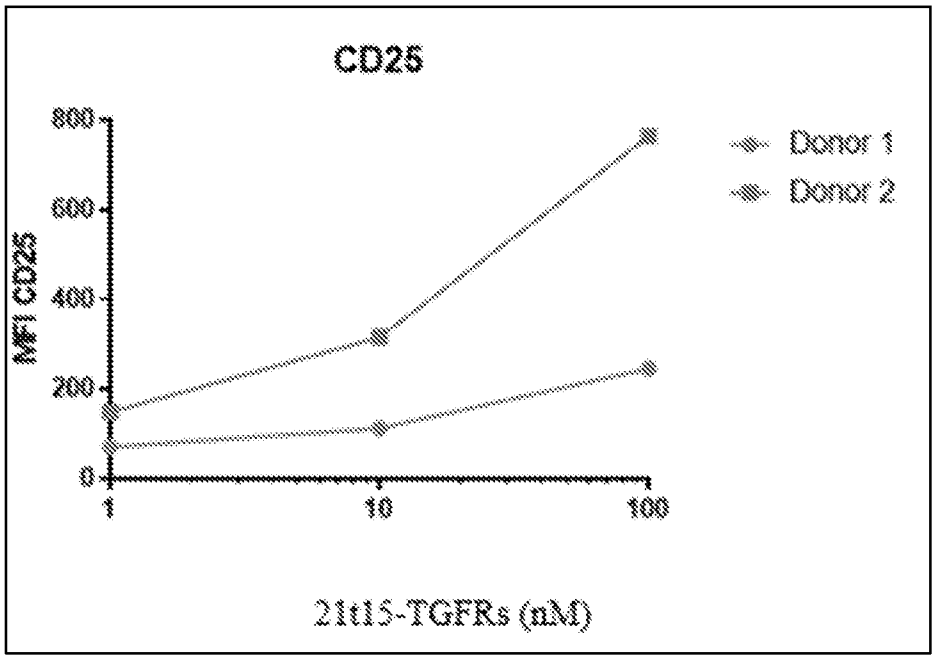


Figure 53

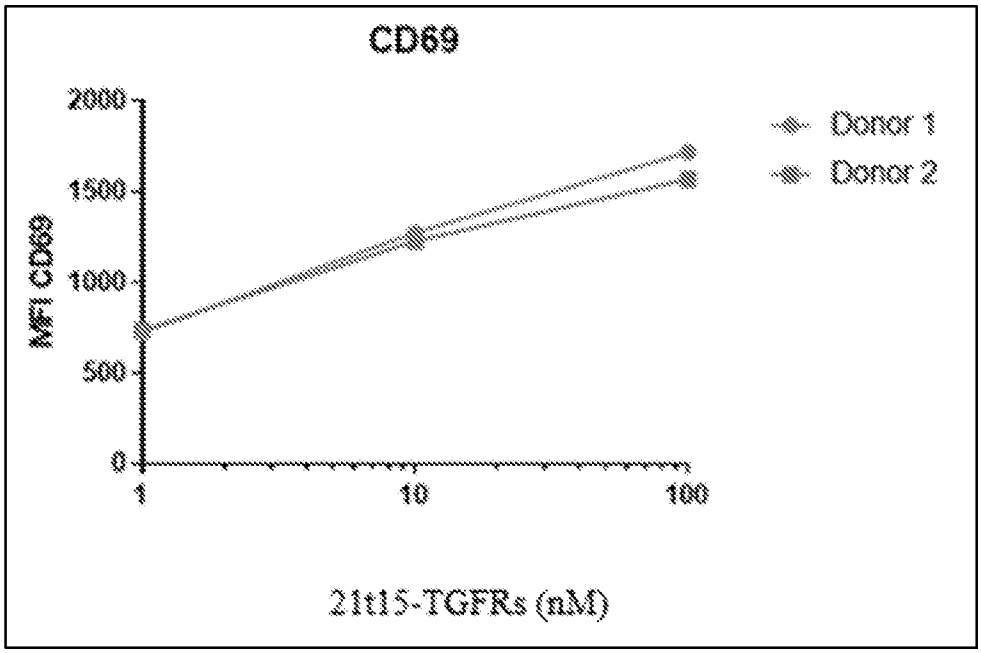


Figure 54

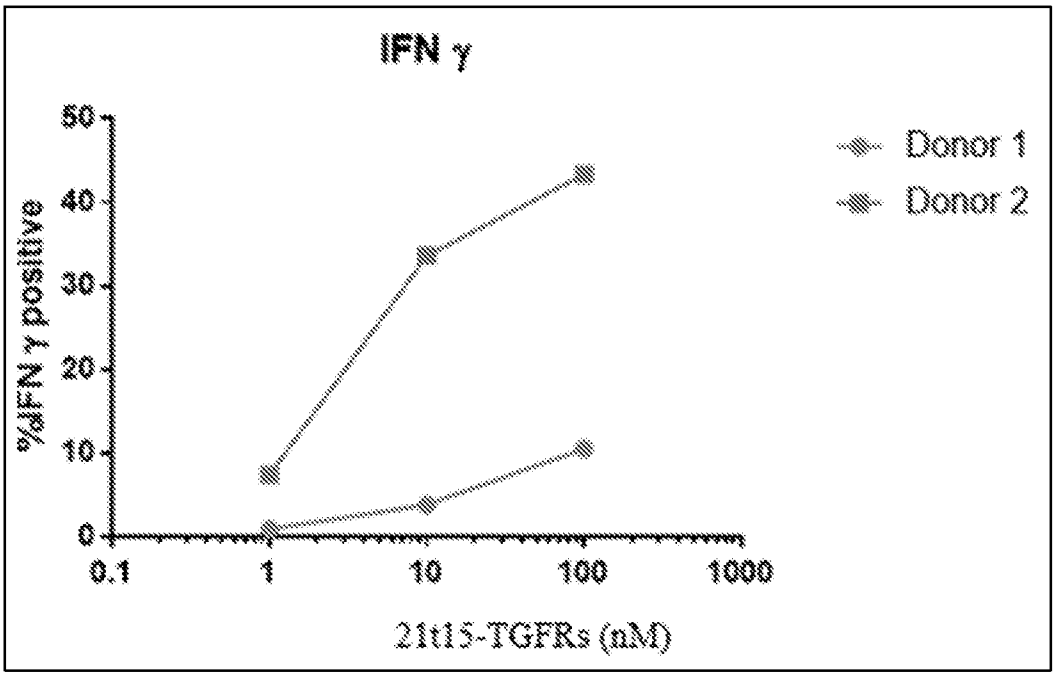


Figure 55

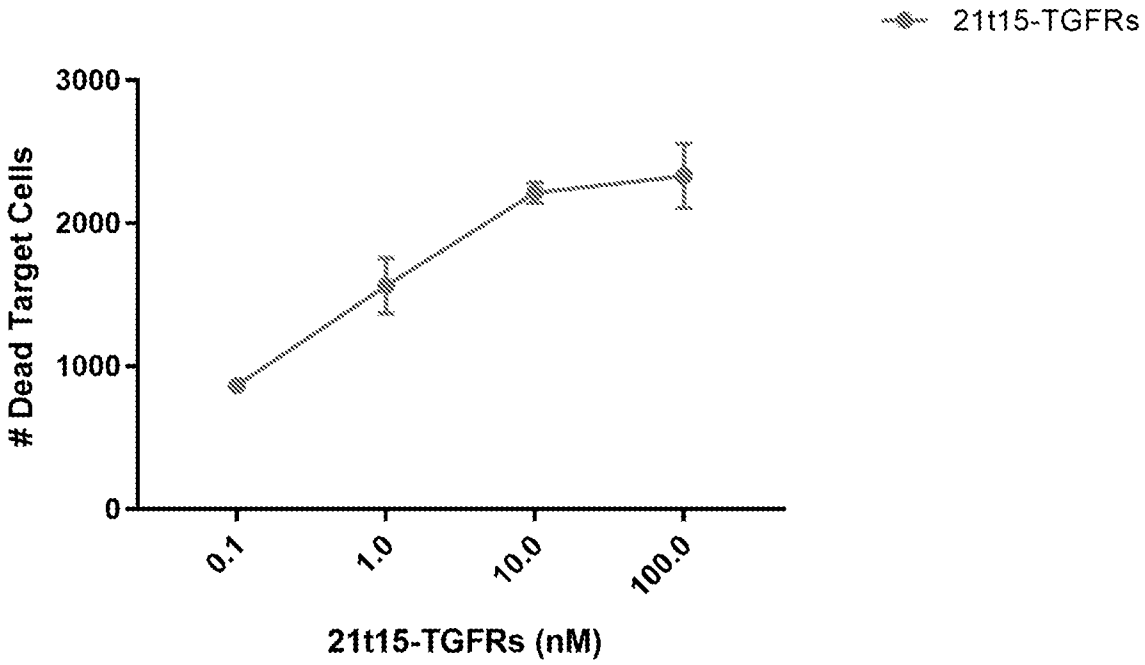


Figure 56

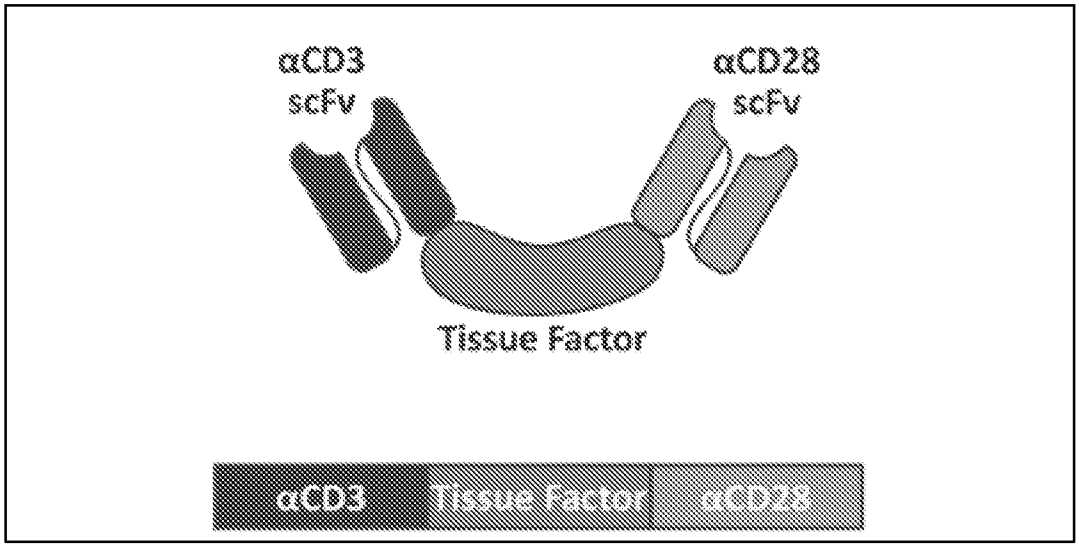

αCD3
scFv
αCD28
scFv
Tissue Factor
αCD3
Tissue Factor
αCD28

Figure 57

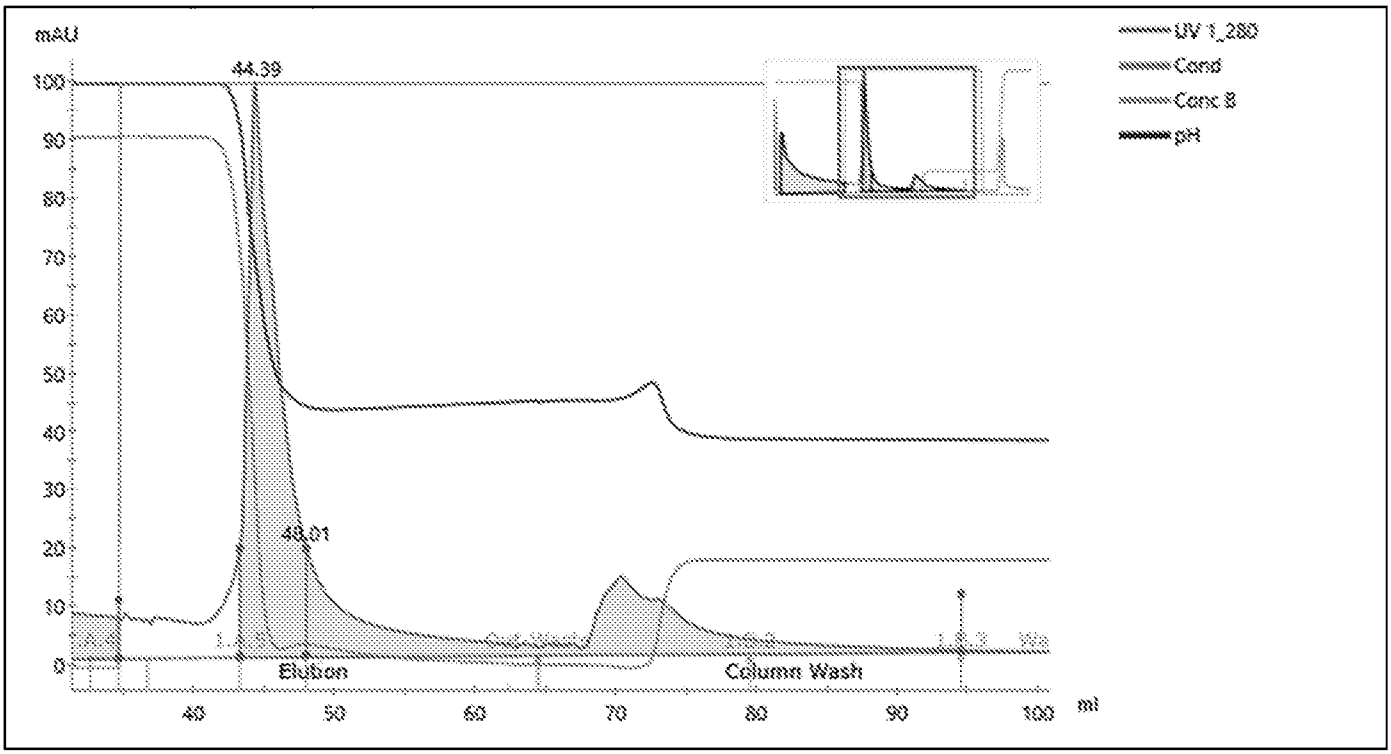

mAU
100
90
80
70
60
50
40
30
20
10
0
44.39
49.01
Elution
Column Wash
40
50
60
70
80
90
100
ml
UV 1_280
Cond
Conc B
pH

Figure 58

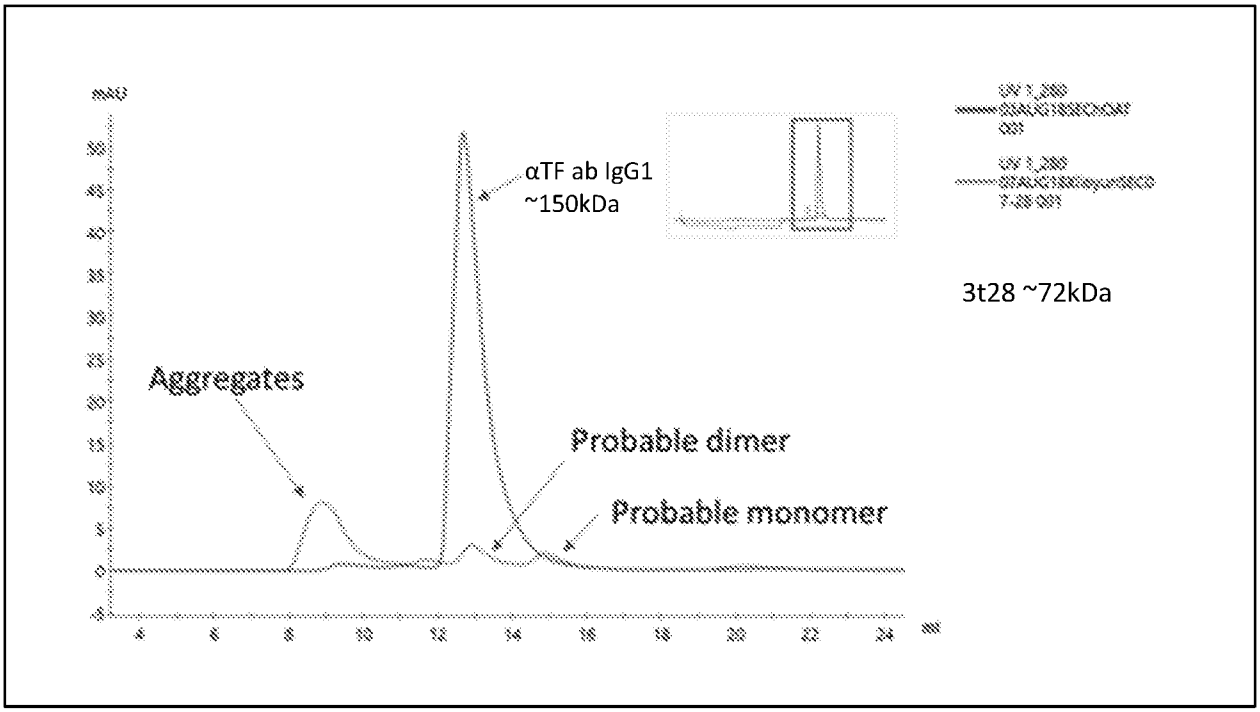

αTF ab IgG1
~150kDa
3t28 ~72kDa
Aggregates
Probable dimer
Probable monomer

Figure 59

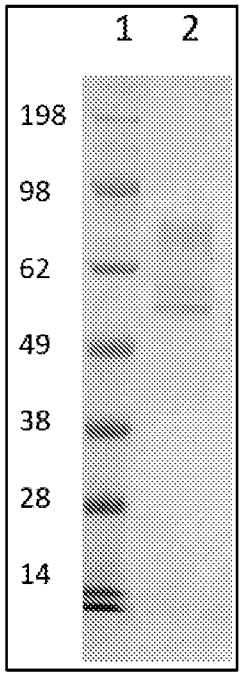


Figure 60

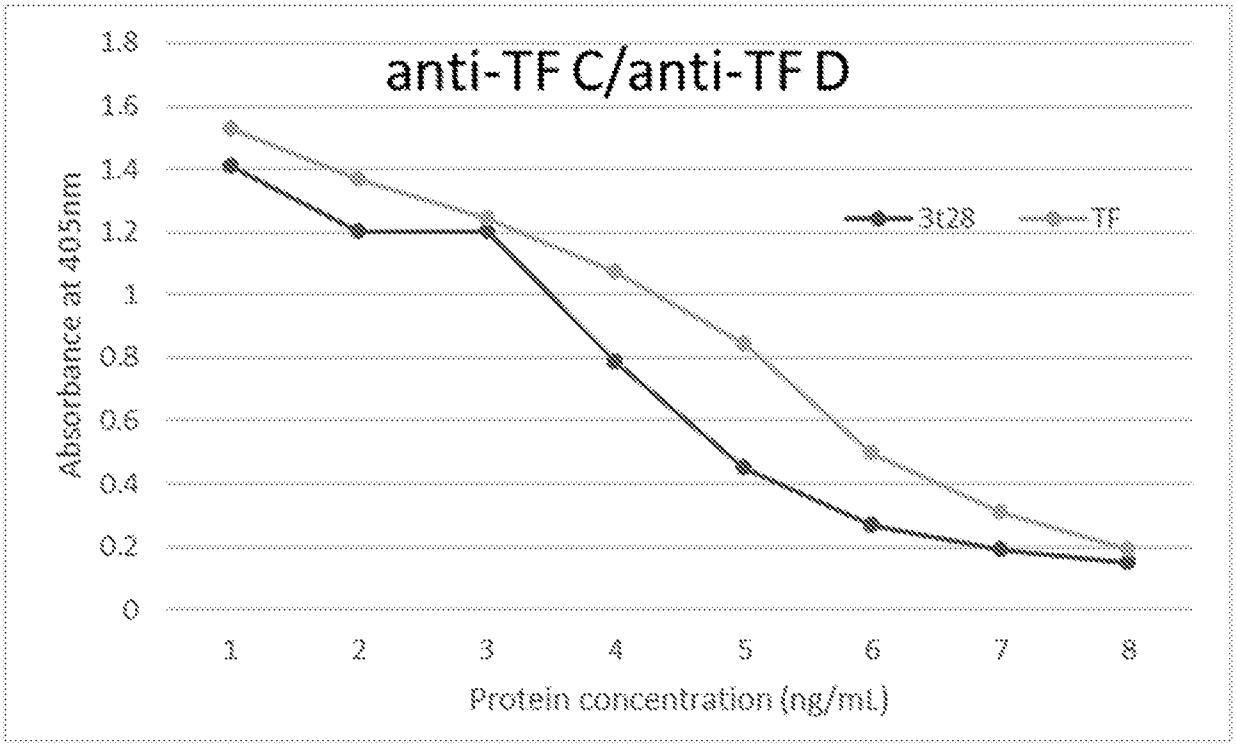


Figure 61

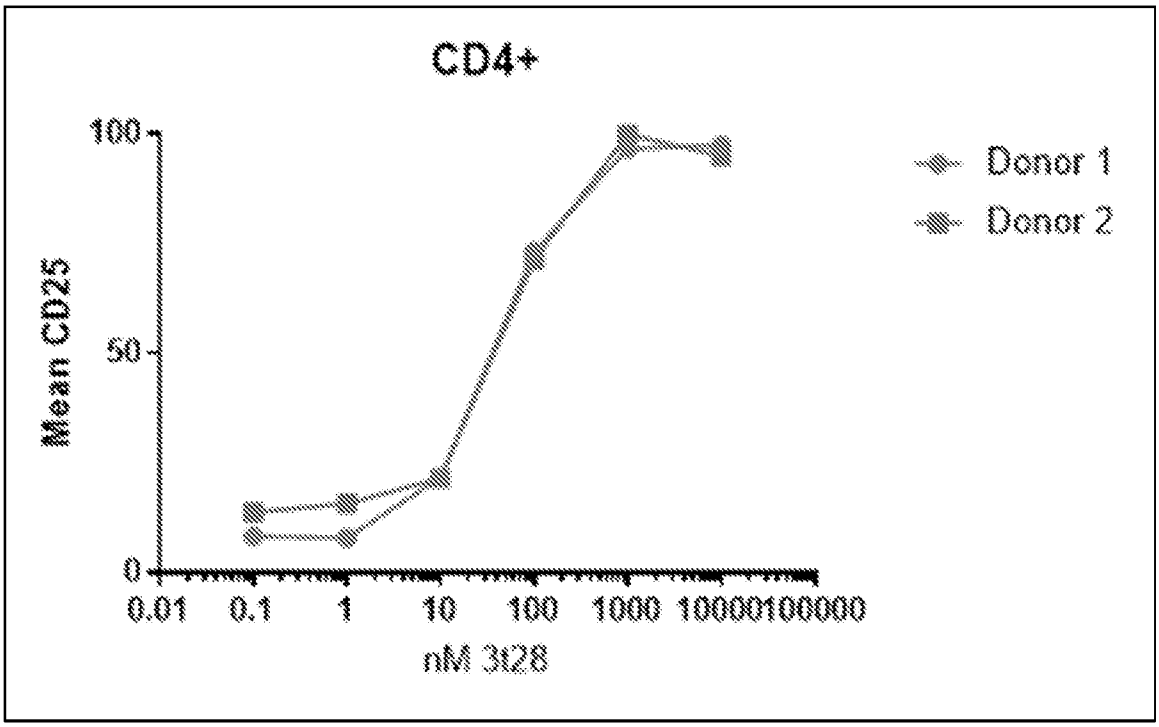


Figure 62

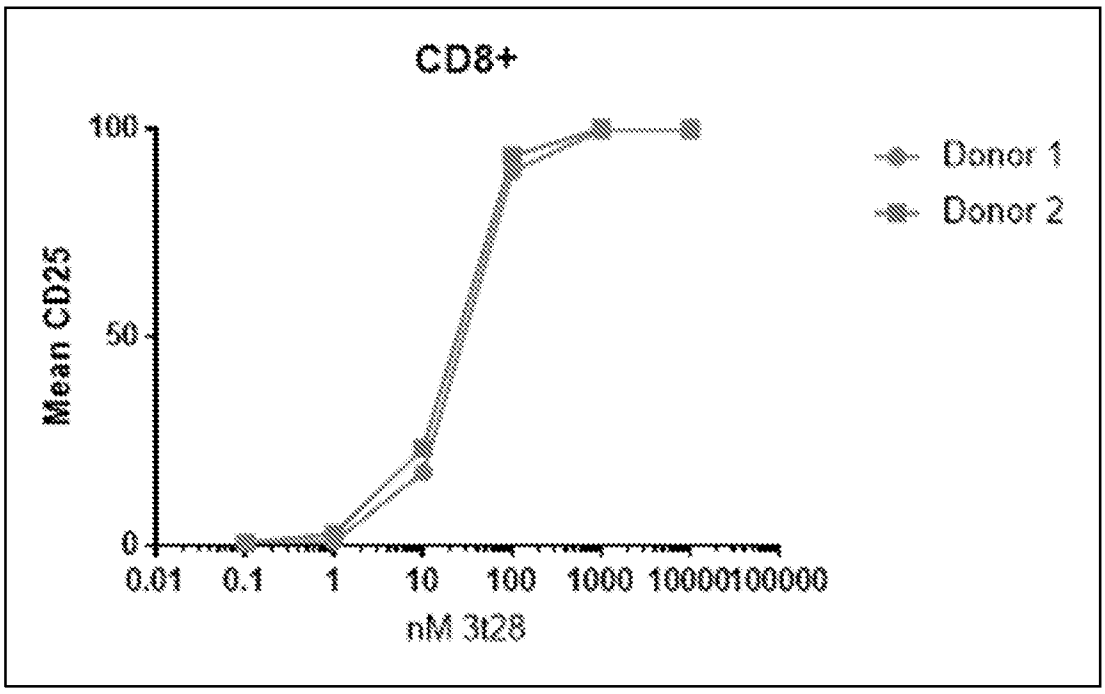


Figure 63

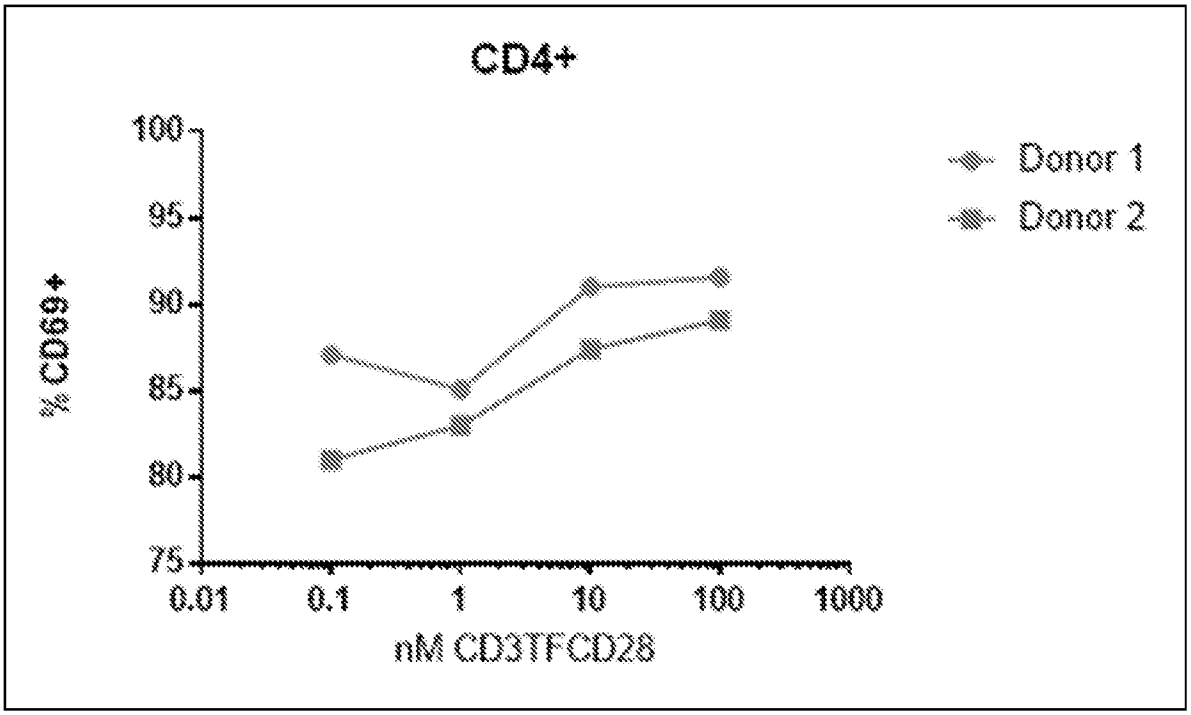

CD4+
100
95
90
85
80
75
% CD69+
0.01
0.1
1
10
100
1000
nM CD3TFCD28
Donor 1
Donor 2

Figure 64



Figure 65

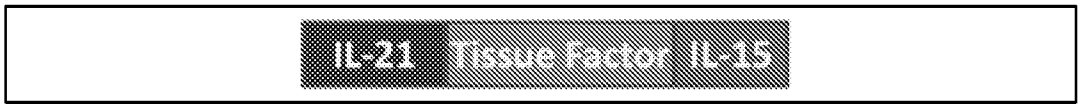


Figure 66

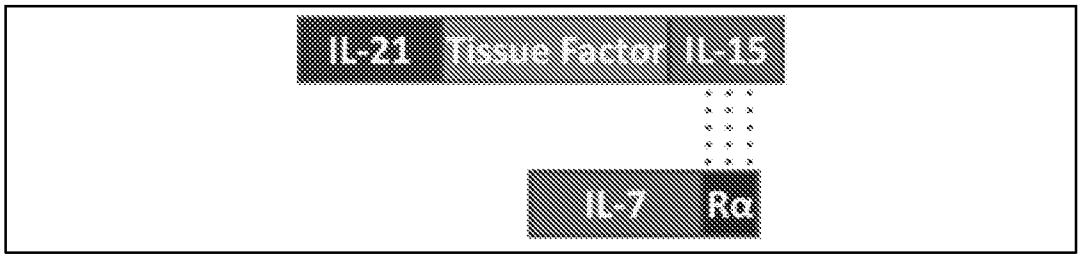


Figure 67

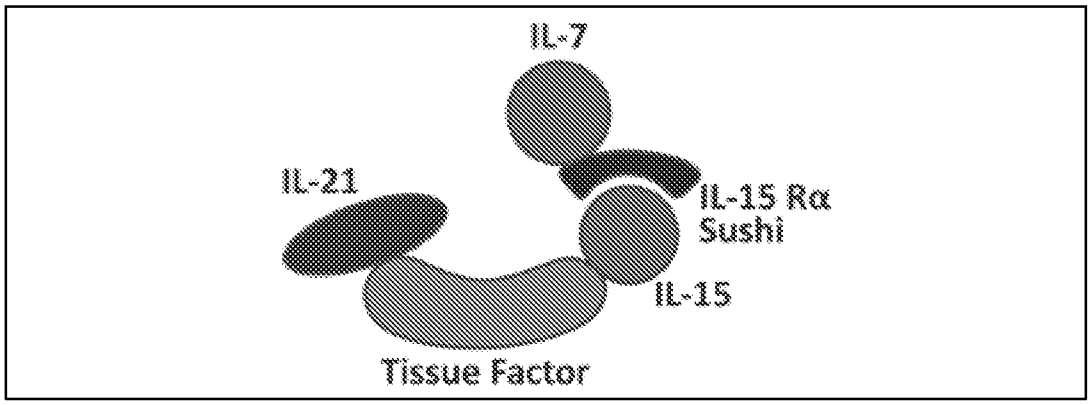


Figure 68

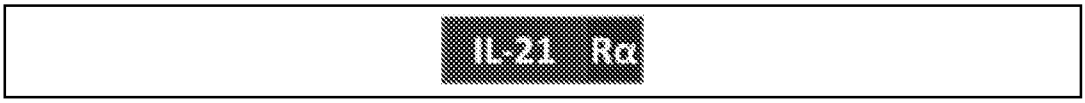


Figure 69

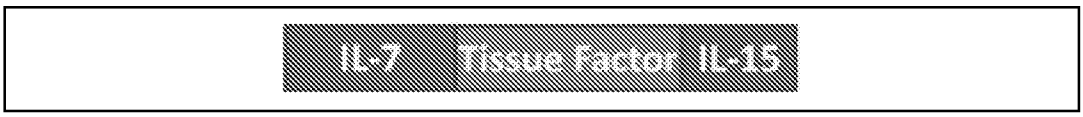


Figure 70

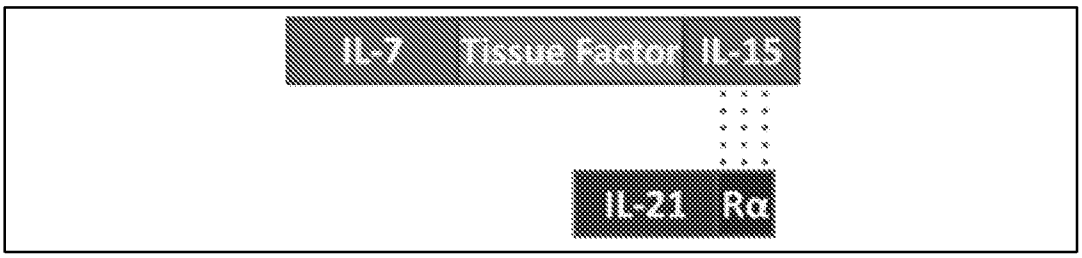


Figure 71

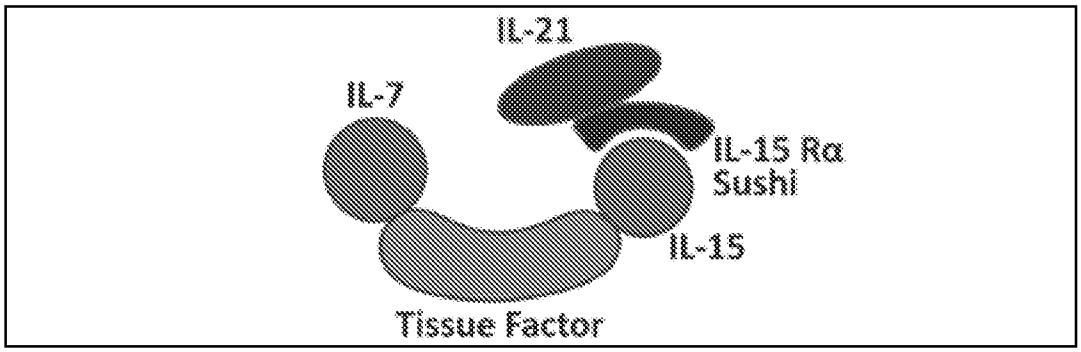


Figure 72

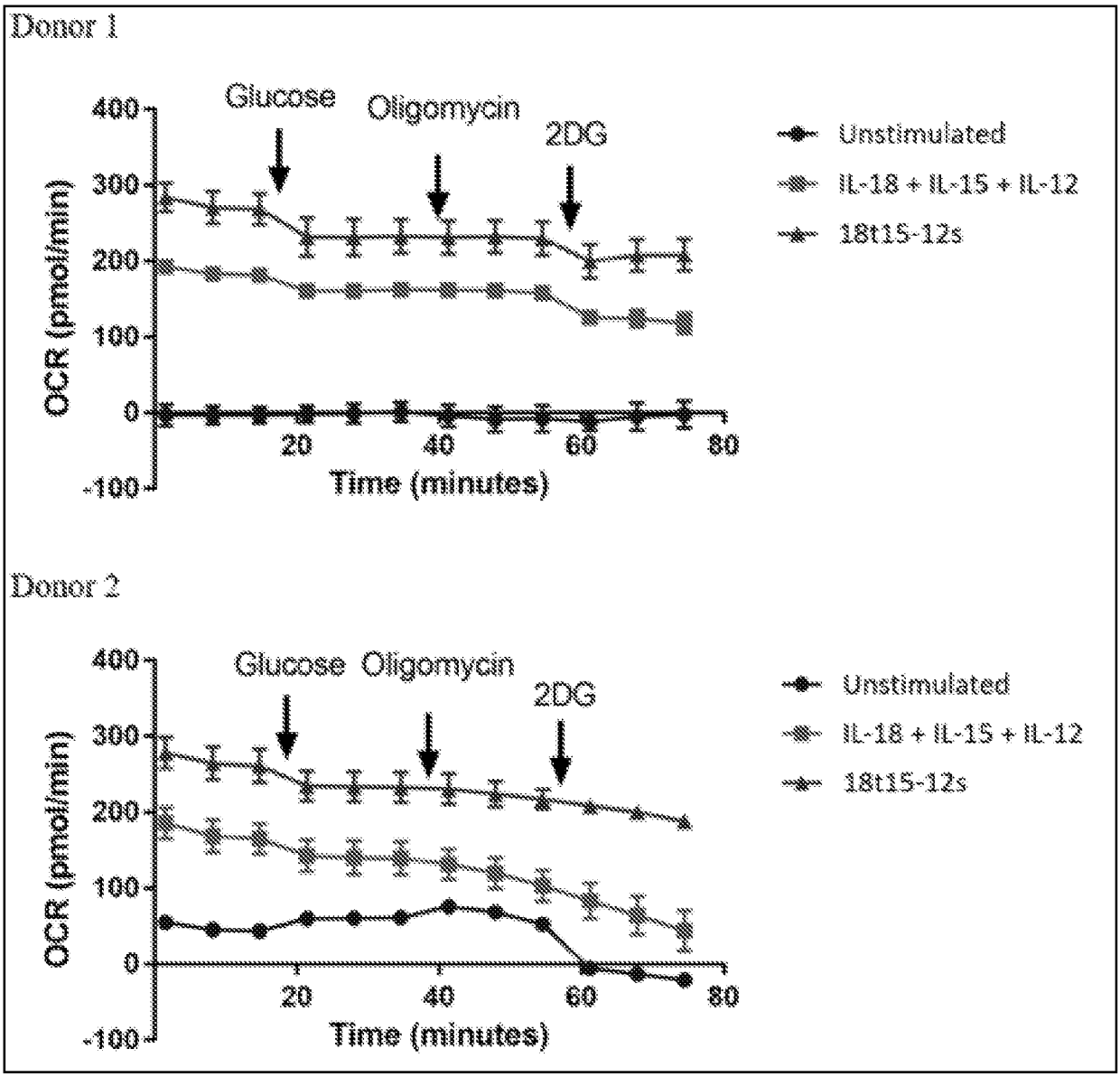

Donor 1
Glucose
Oligomycin
2DG
OCR (pmol/min)
400
300
200
100
0
-100
20
40
60
80
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s
Donor 2
Glucose
Oligomycin
2DG
OCR (pmol/min)
400
300
200
100
0
-100
20
40
60
80
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s

Figure 73

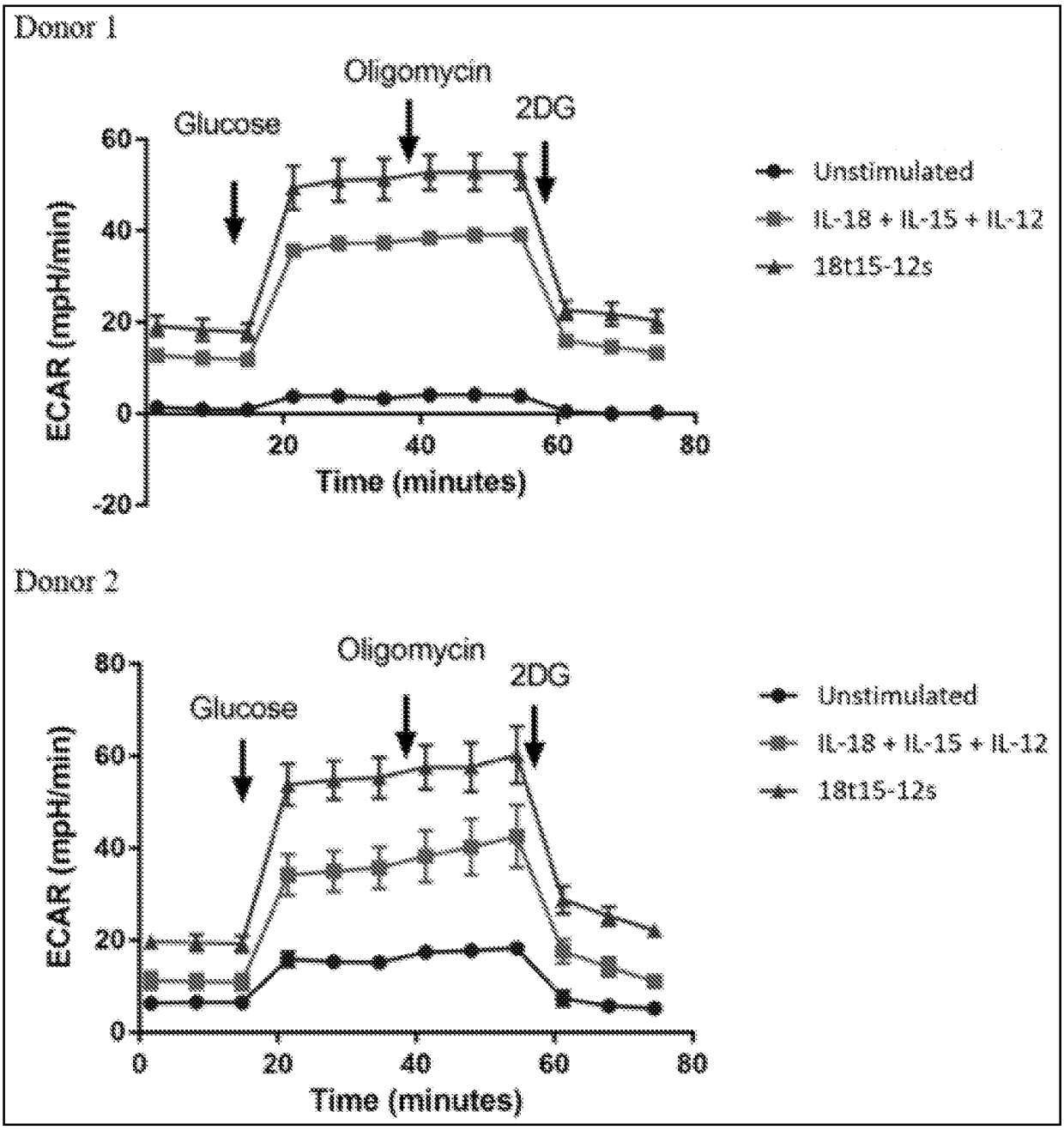

Donor 1
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s
Donor 2
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s

Figure 74

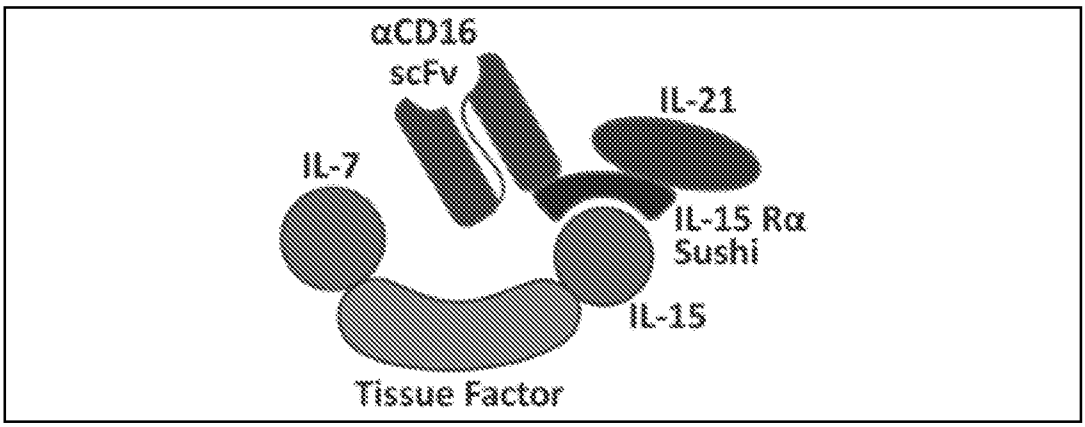


Figure 75

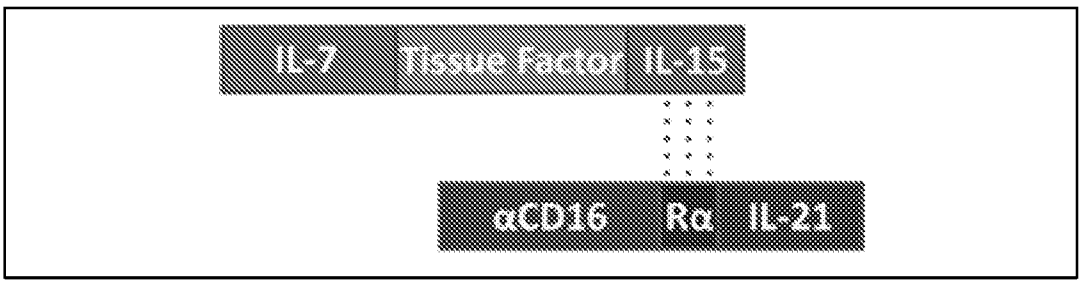


Figure 76

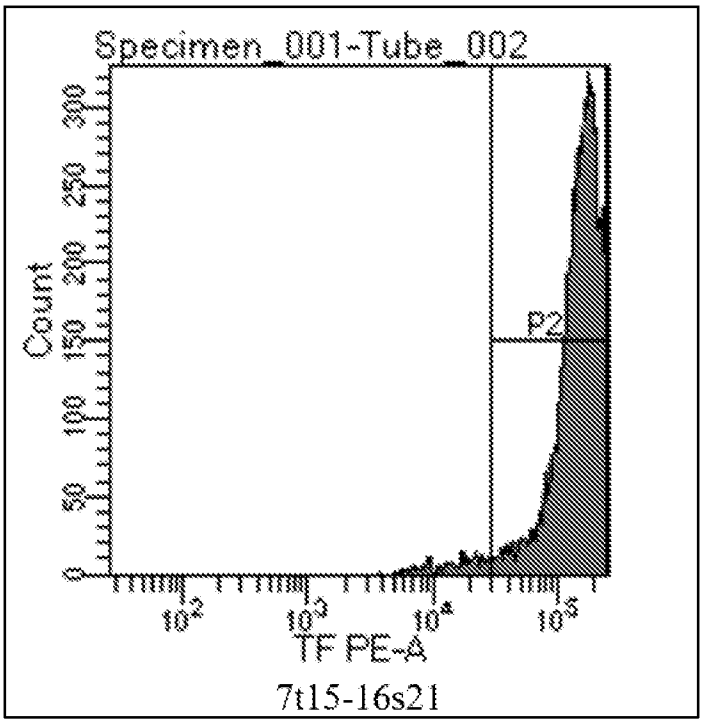


Figure 77A

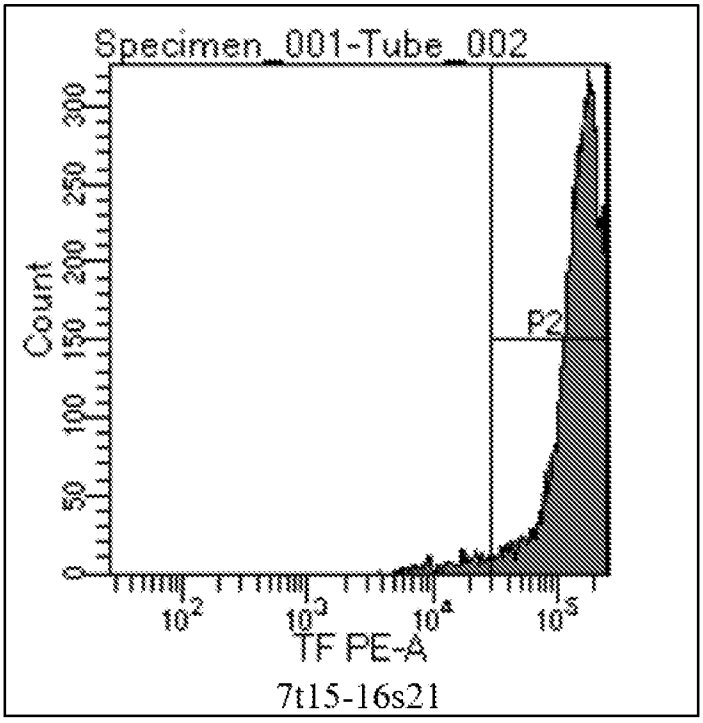


Figure 77B

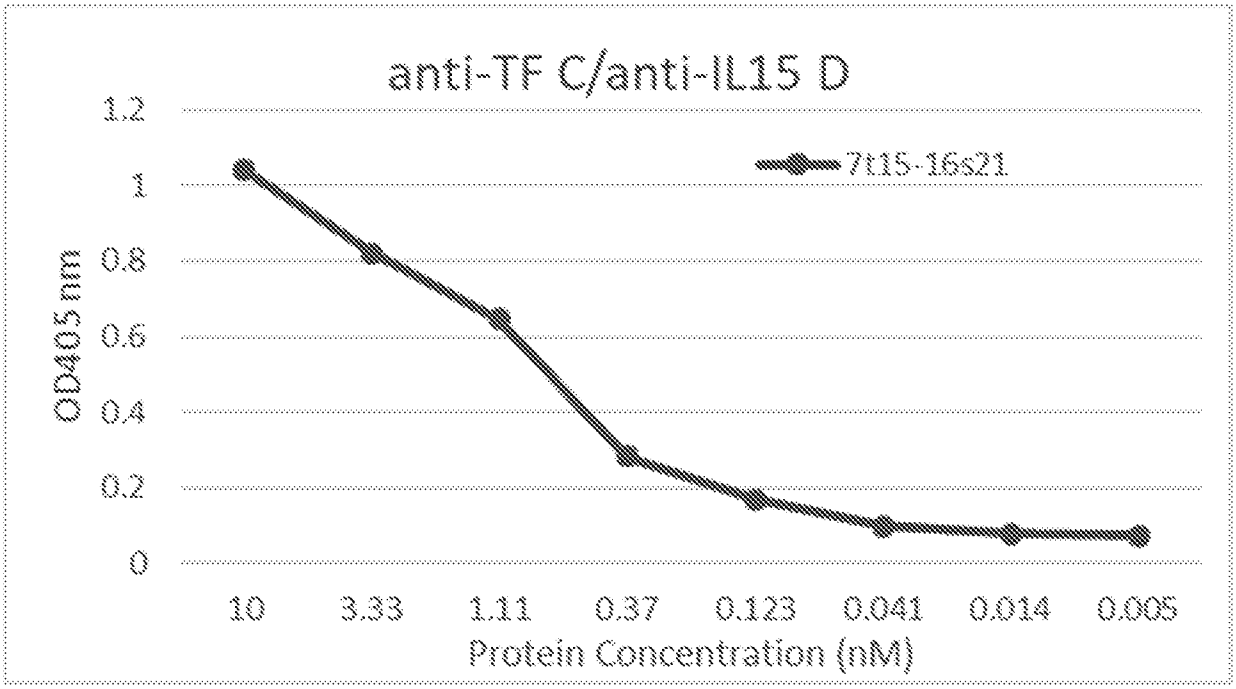


Figure 78A

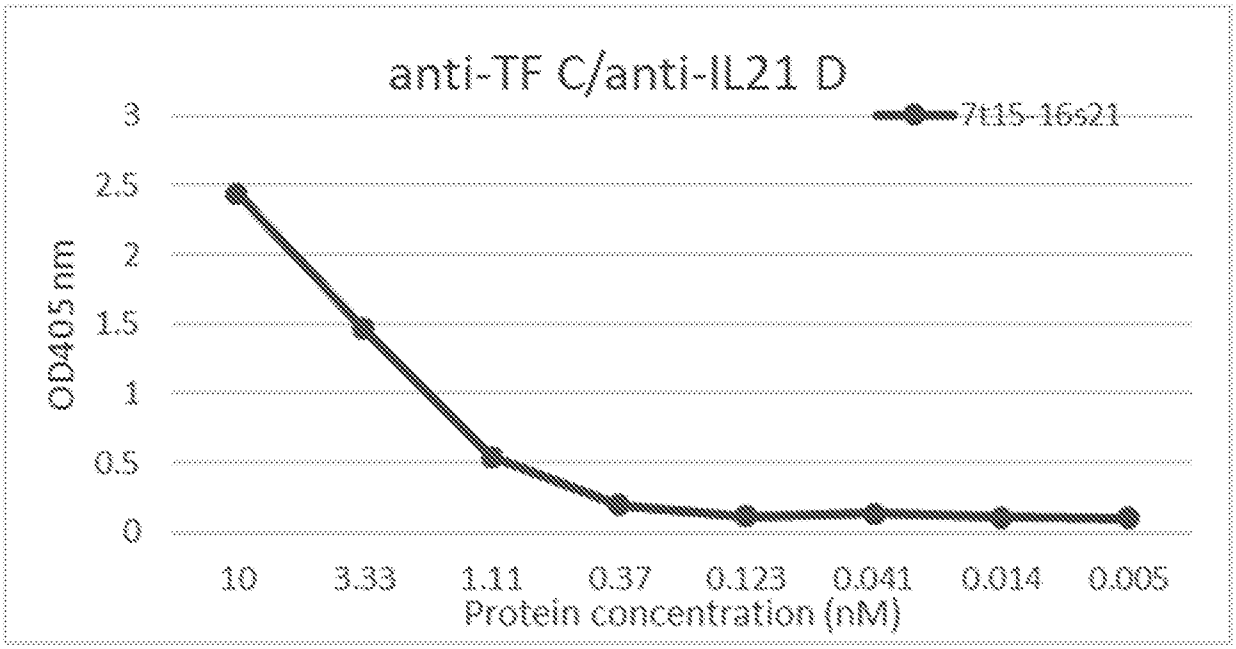


Figure 78B

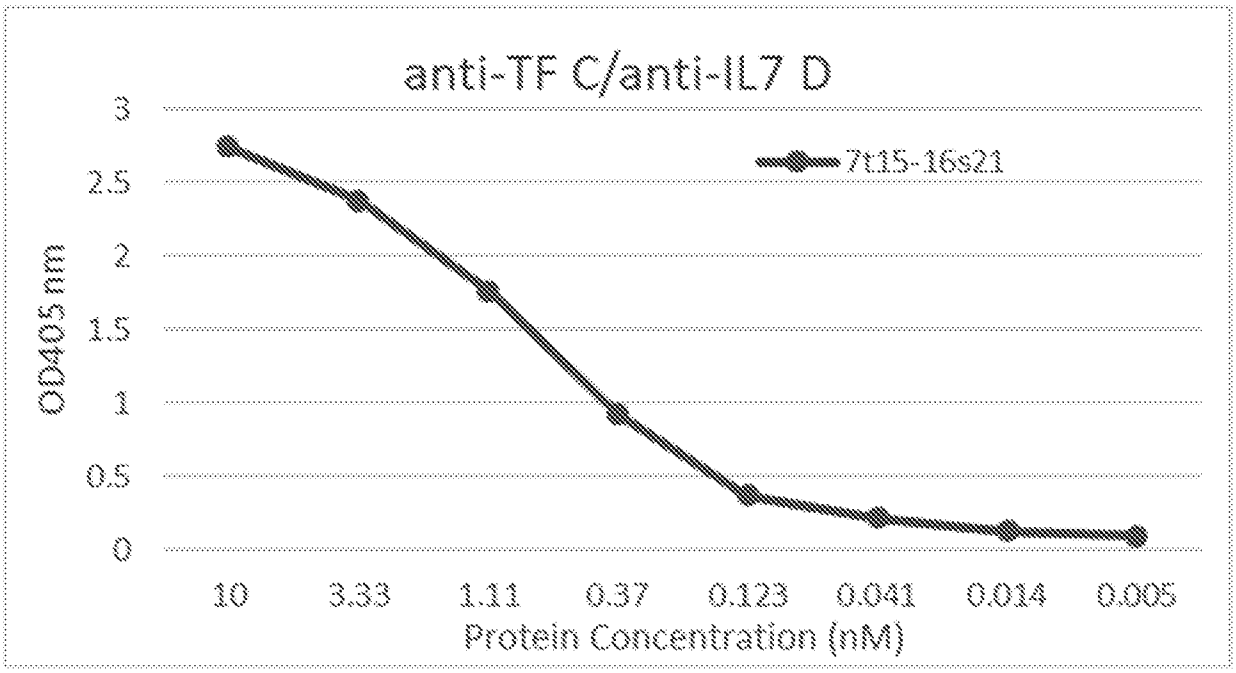

anti-TF C/anti-IL7 D
7t15-16s21
OD405 nm
3
2.5
2
1.5
1
0.5
0
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein Concentration (nM)

Figure 78C

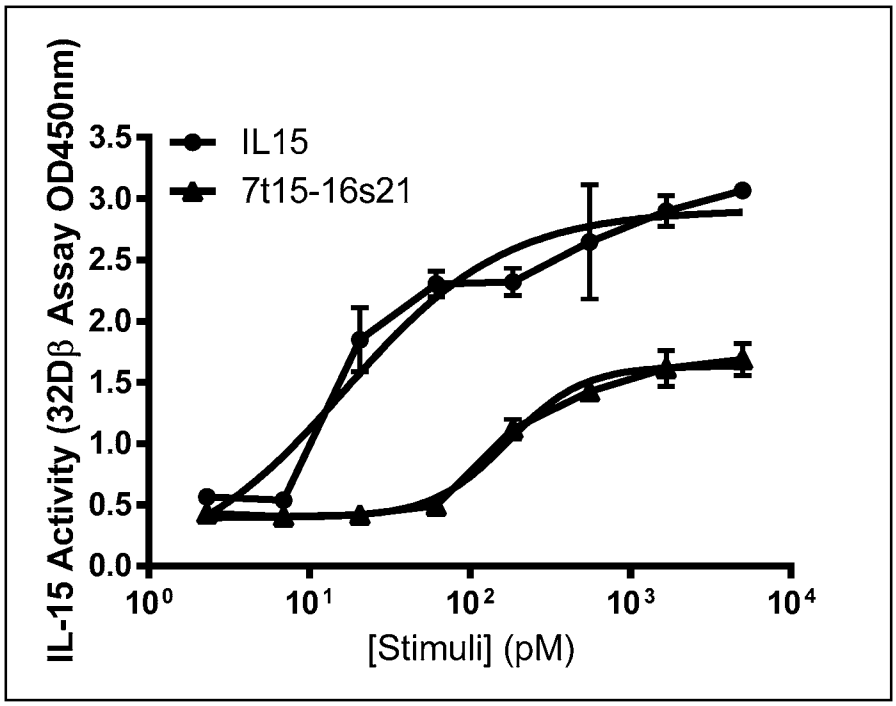

IL-15 Activity (32Dβ Assay OD450nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IL15
7t15-16s21
10⁰
10¹
10²
10³
10⁴
[Stimuli] (pM)

Figure 79

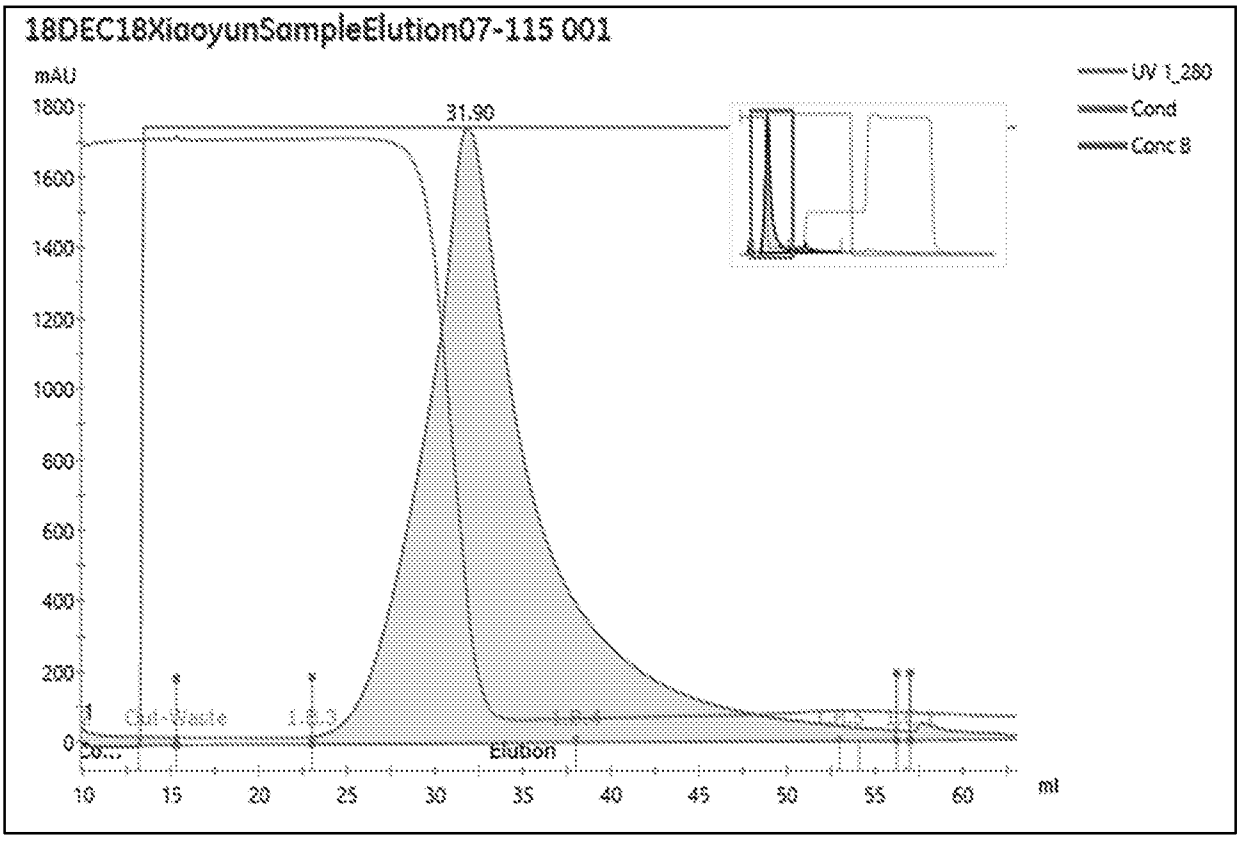

18DEC18XiaoyunSampleElution07-115 001
mAU
1800
1600
1400
1200
1000
800
600
400
200
0
31.90
UV 1_280
Cond
Conc B
Elution
10
15
20
25
30
35
40
45
50
55
60
ml

Figure 80

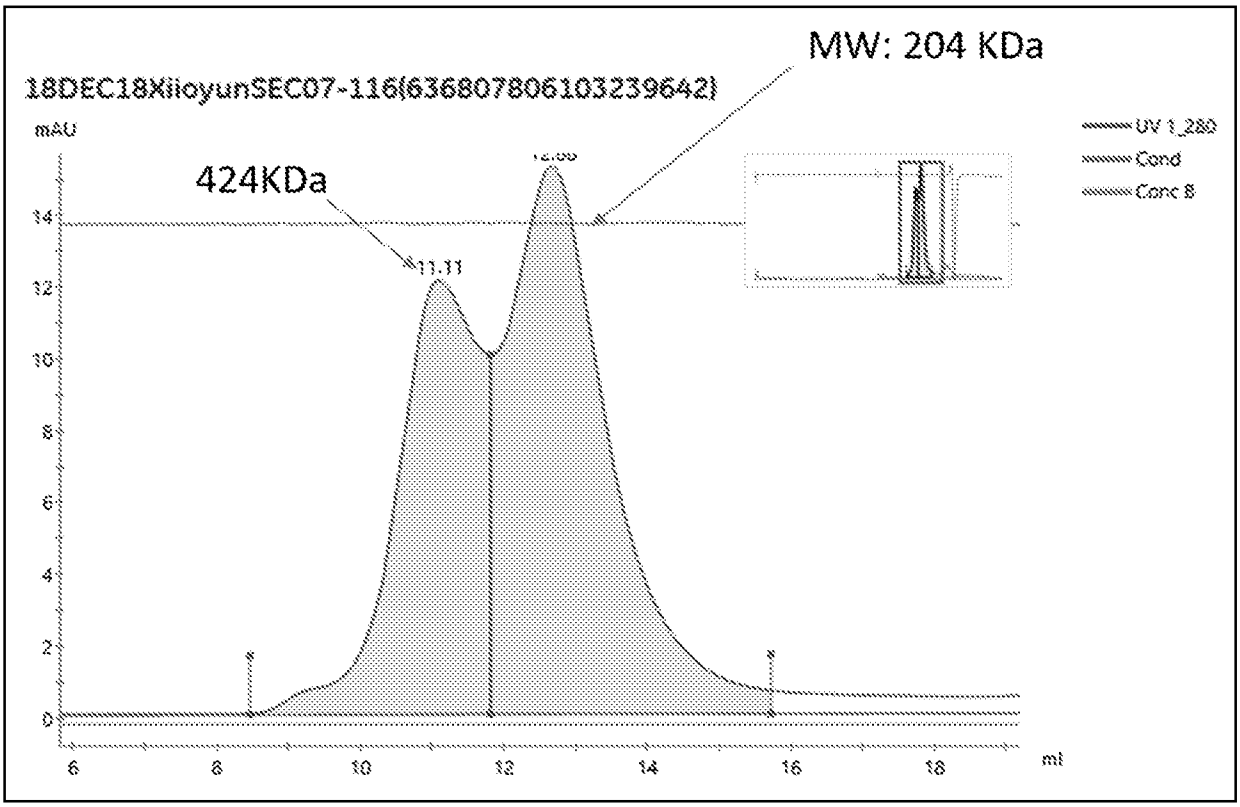

MW: 204 KDa
18DEC18XiloyunSEC07-116(636807806103239642)
mAU
424KDa
11.11
UV 1_280
Cond
Conc B
14
12
10
8
6
4
2
0
6
8
10
12
14
16
18
ml

Figure 81

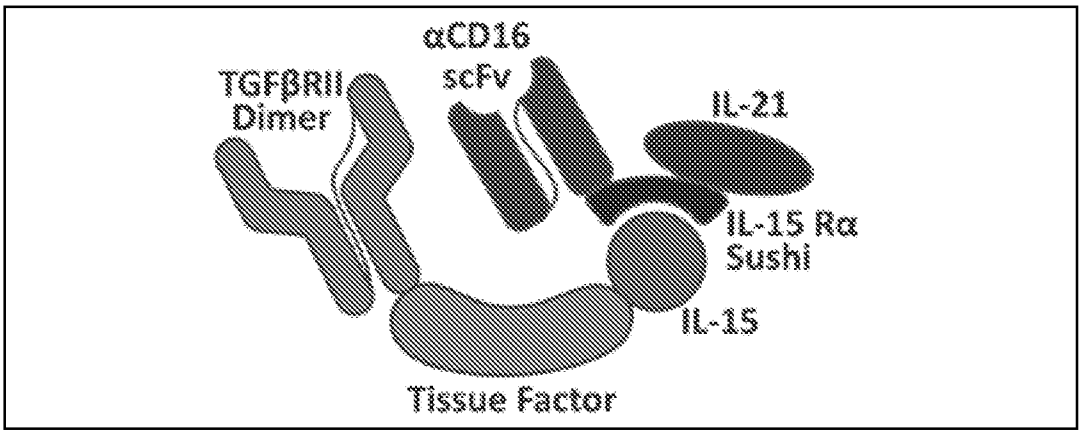


Figure 82

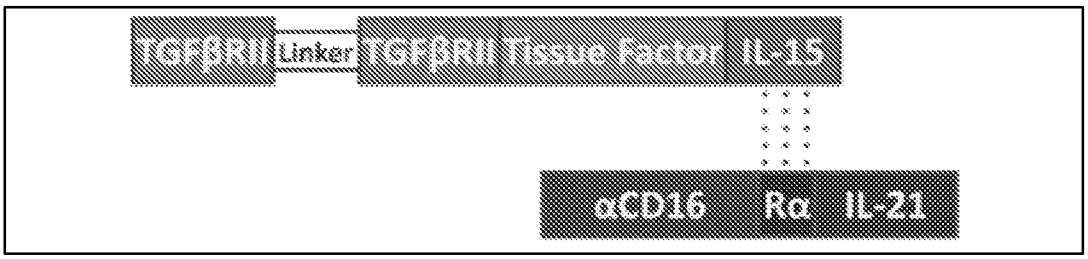


Figure 83

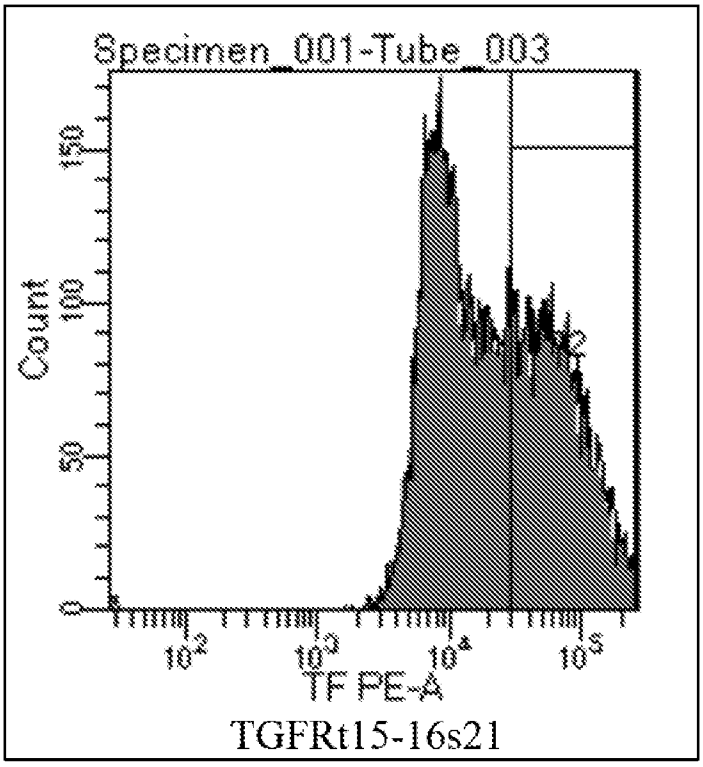

Specimen_001-Tube_003
Count
150
100
50
0
10^2
10^3
10^4
10^5
TF PE-A
TGFRt15-16s21

Figure 84A

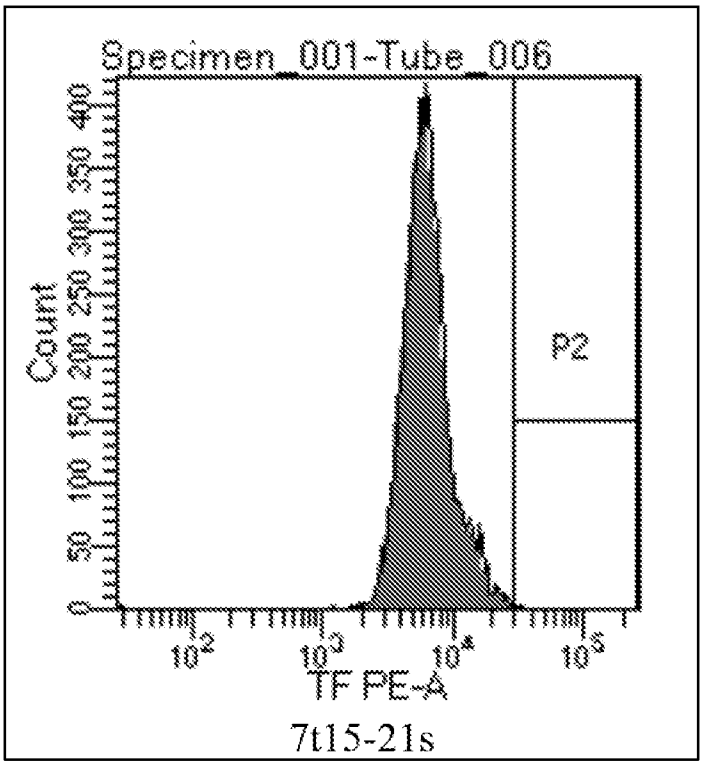


Figure 84B

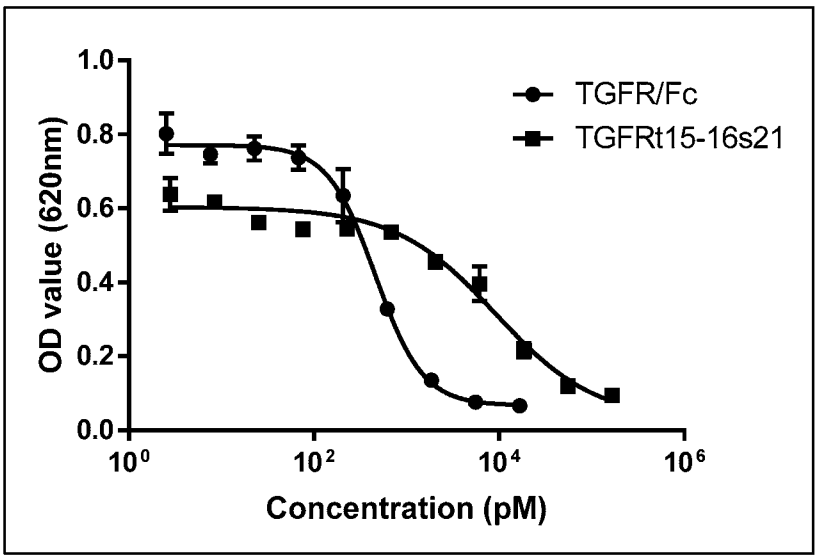


Figure 85

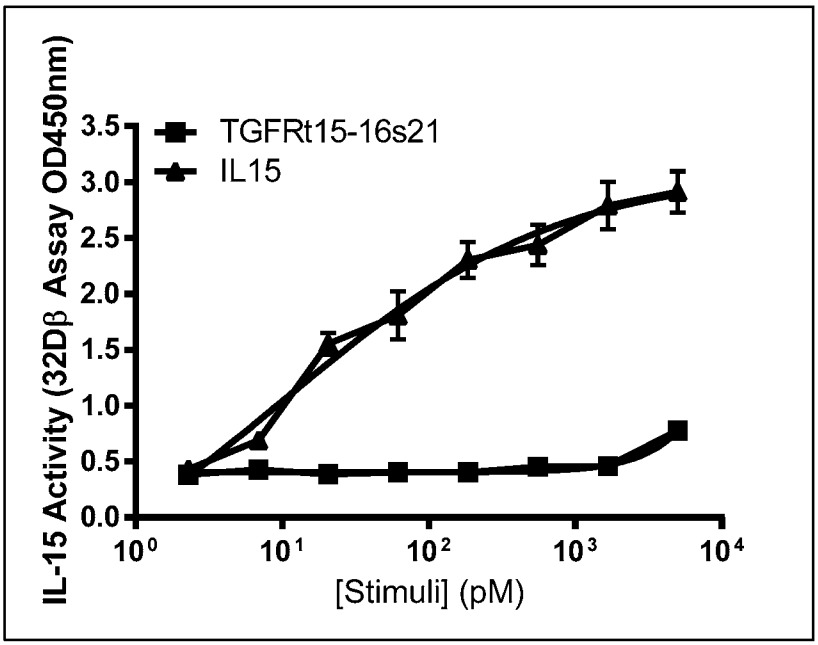


Figure 86

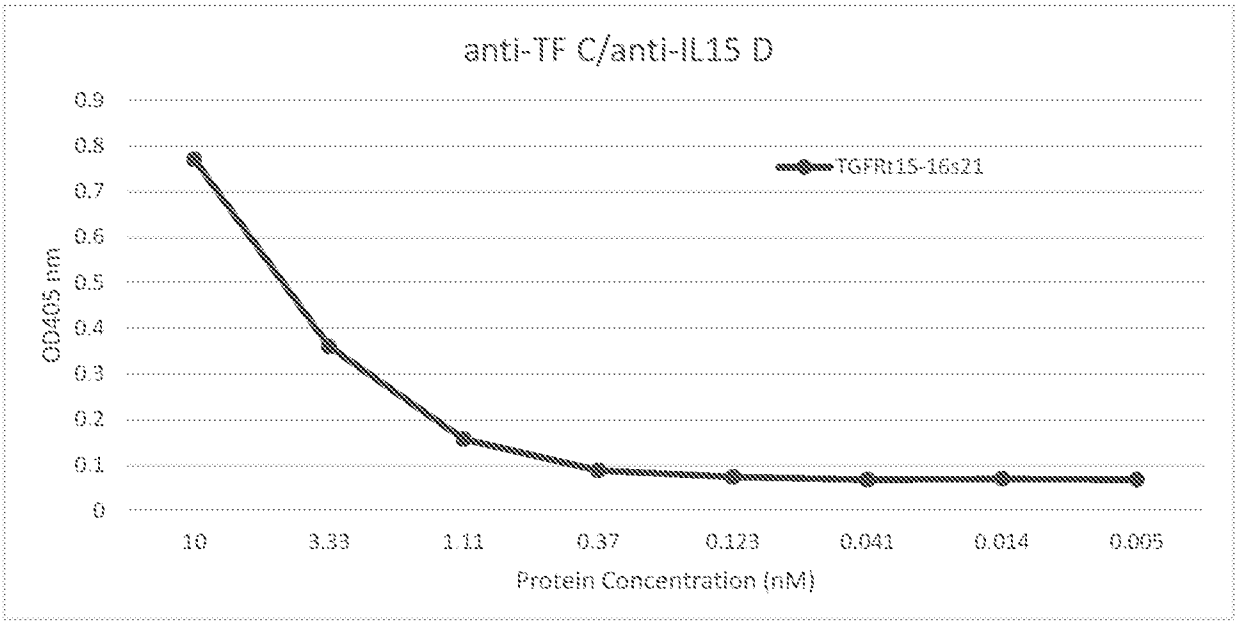


Figure 87A

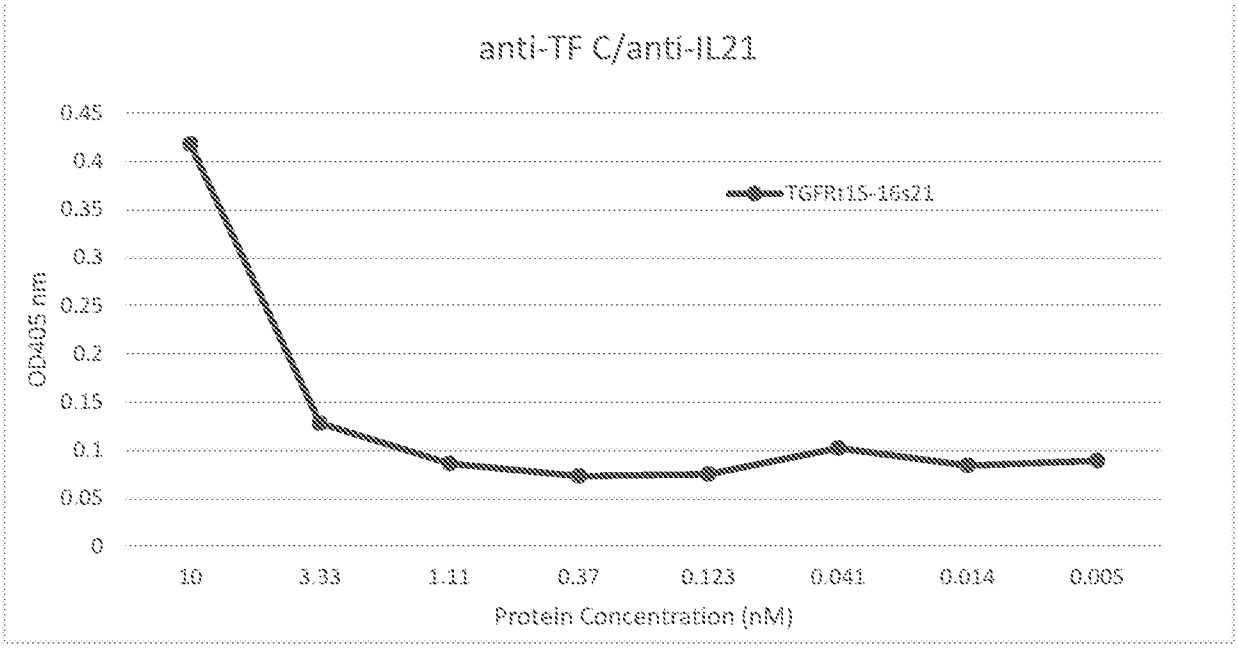


Figure 87B

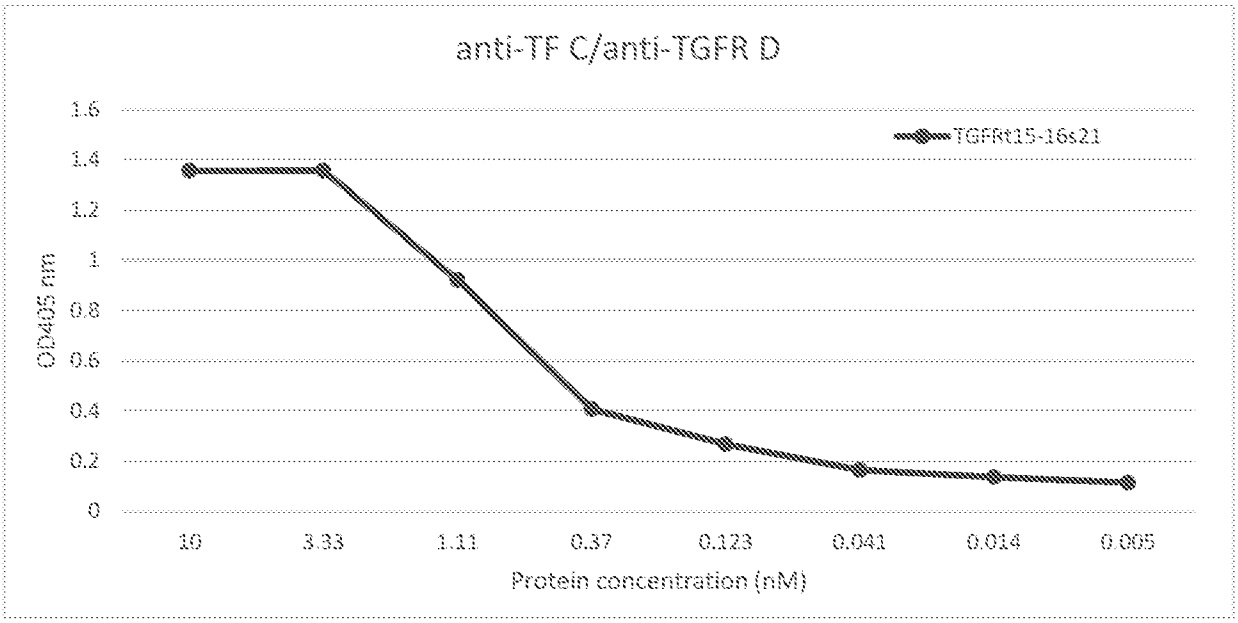


Figure 87C

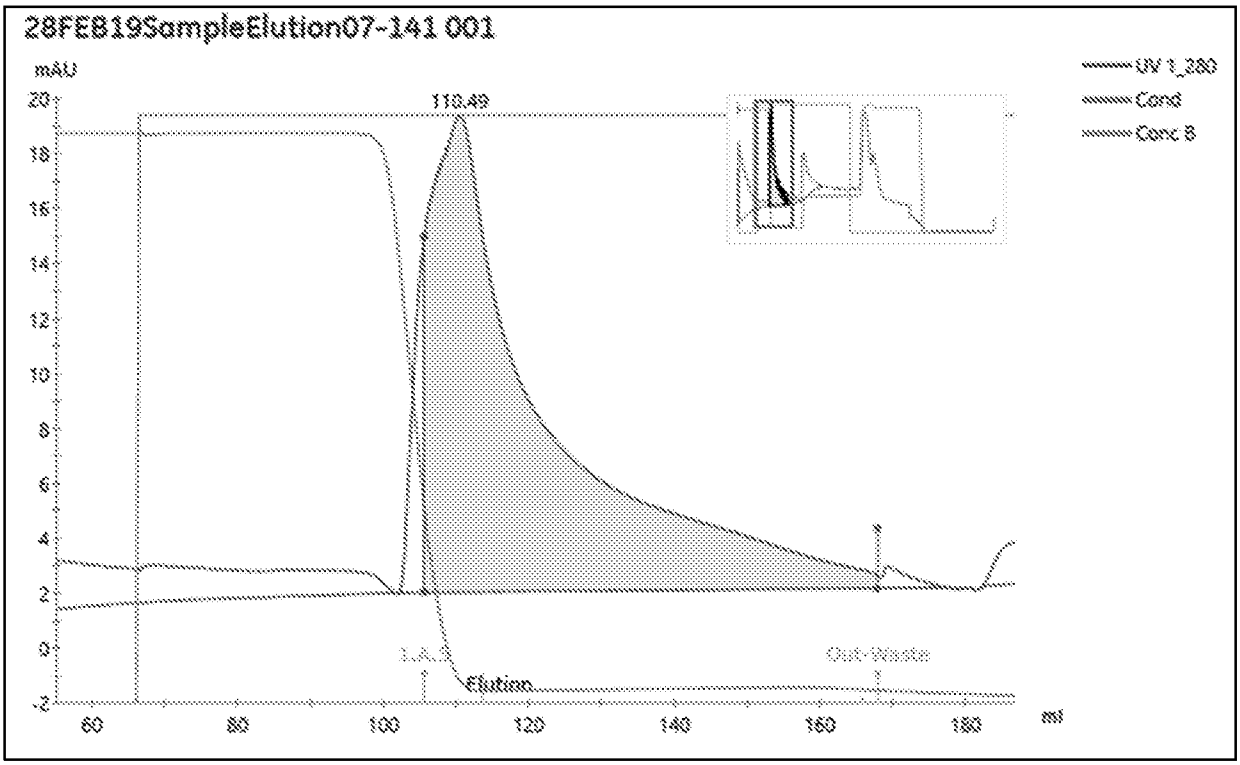


Figure 88

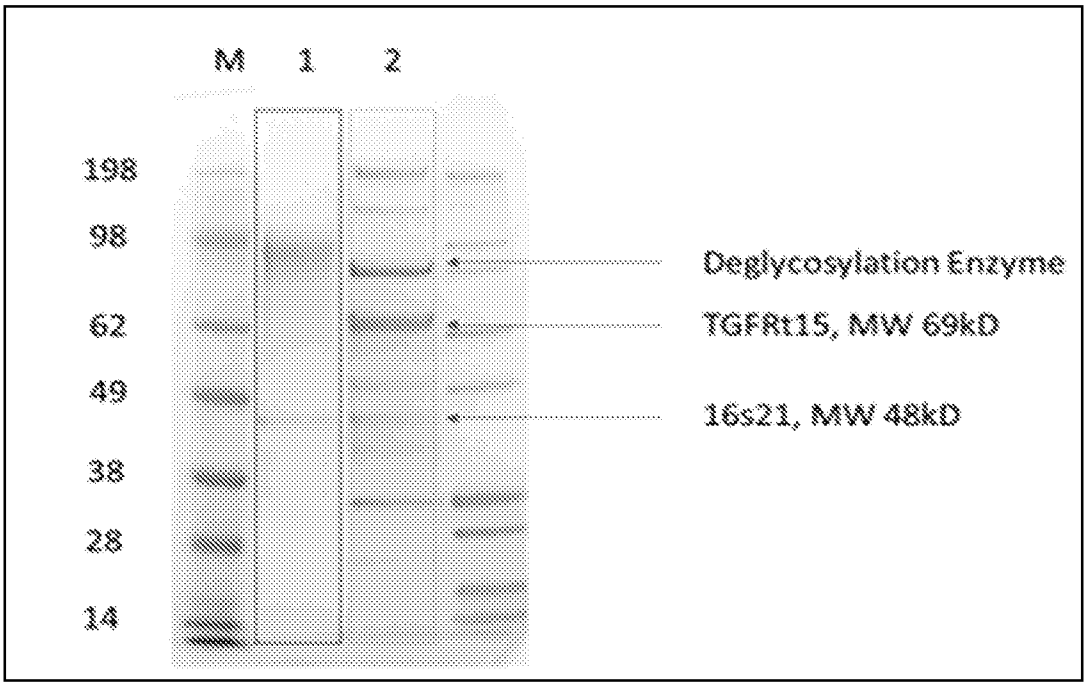


Figure 89

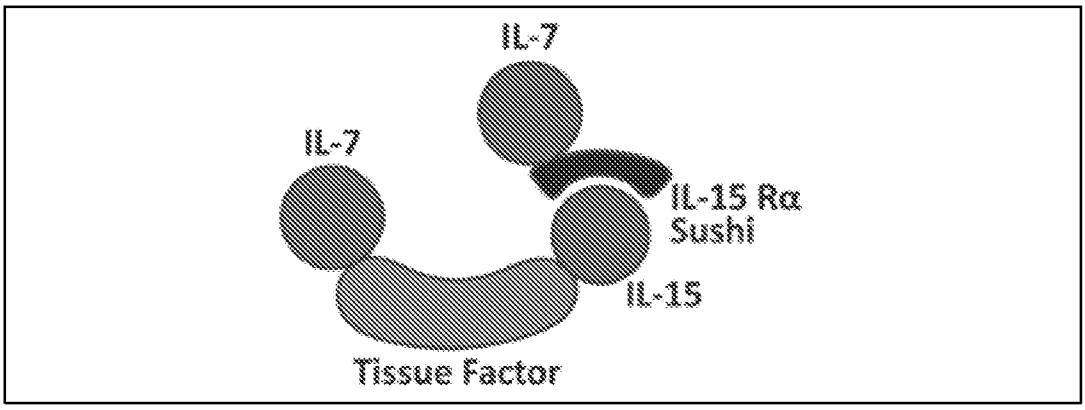


Figure 90

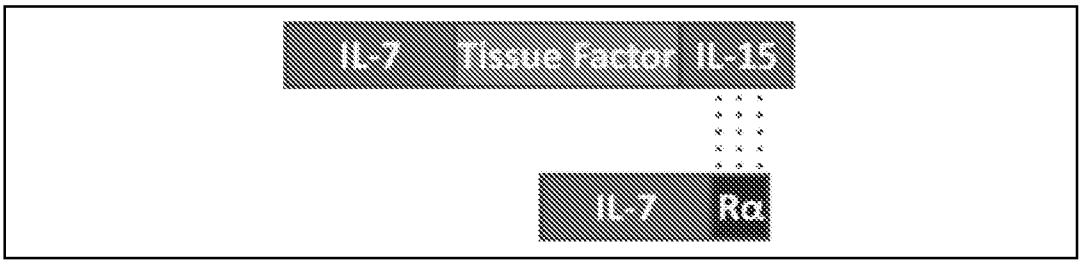


Figure 91

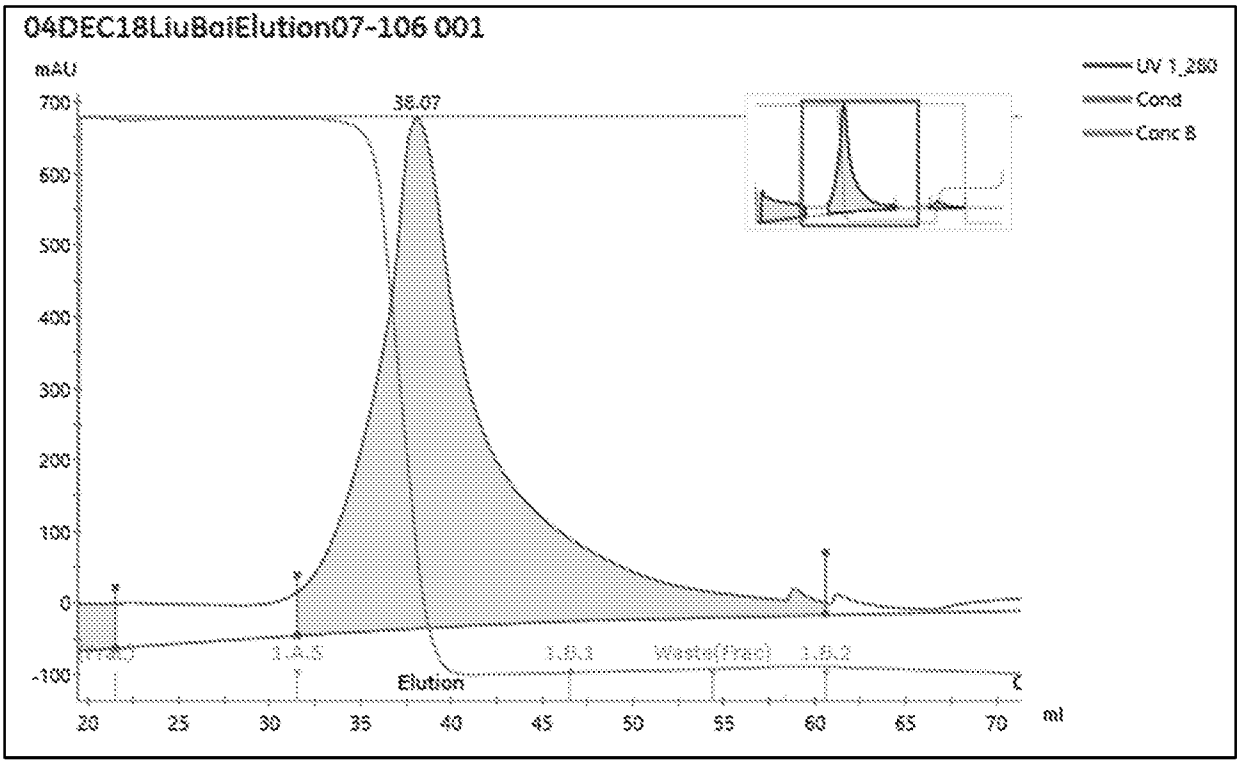


Figure 92

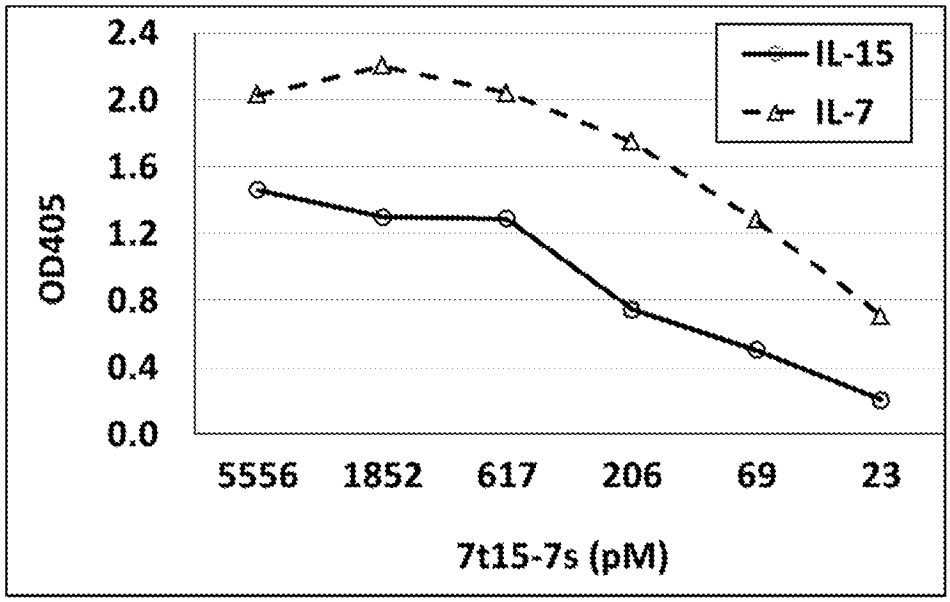

2.4
2.0
1.6
1.2
0.8
0.4
0.0
OD405
IL-15
IL-7
5556
1852
617
206
69
23
7t15-7s (pM)

Figure 93

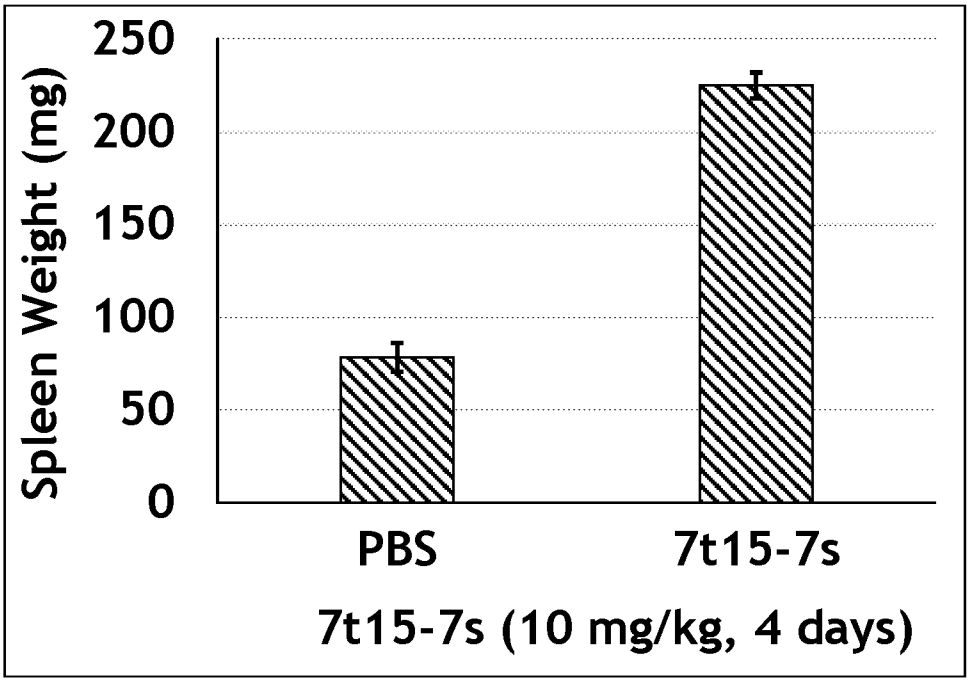


Figure 94A

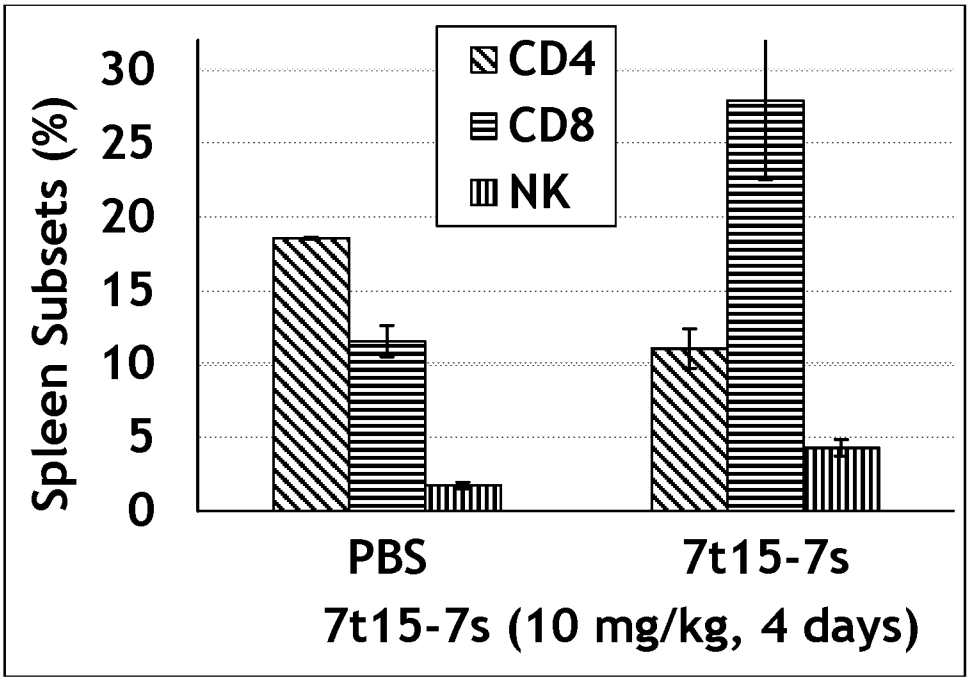


Figure 94B

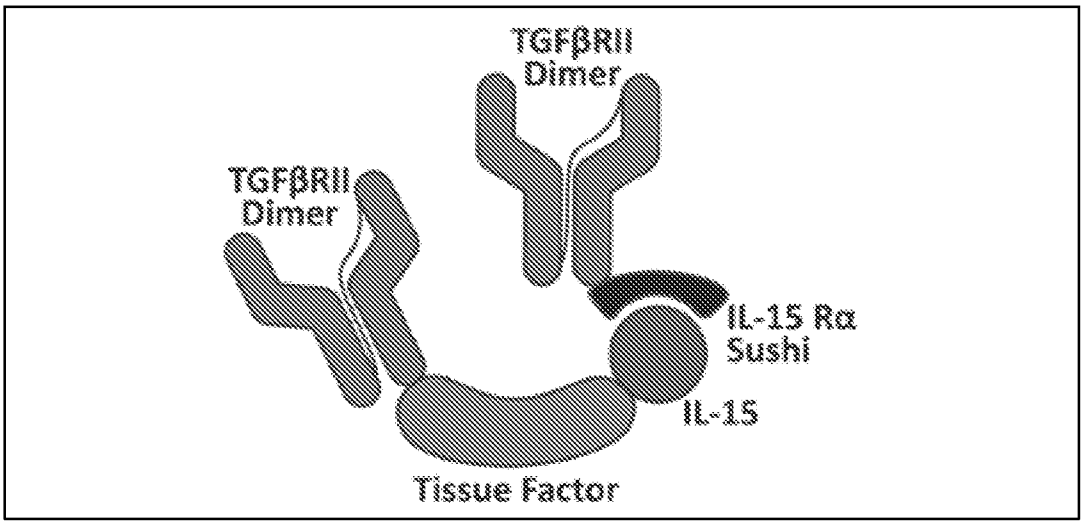


Figure 95

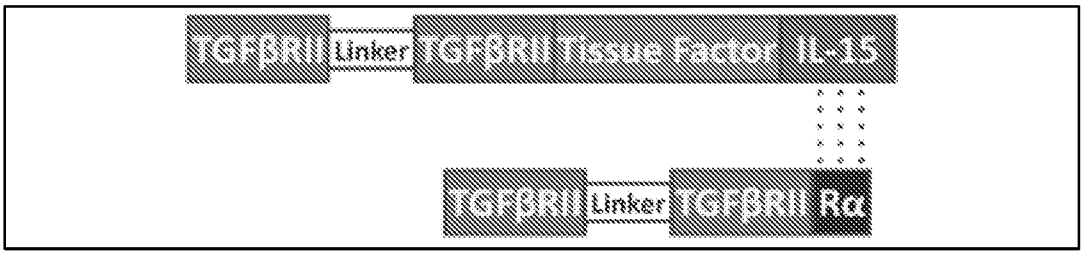


Figure 96

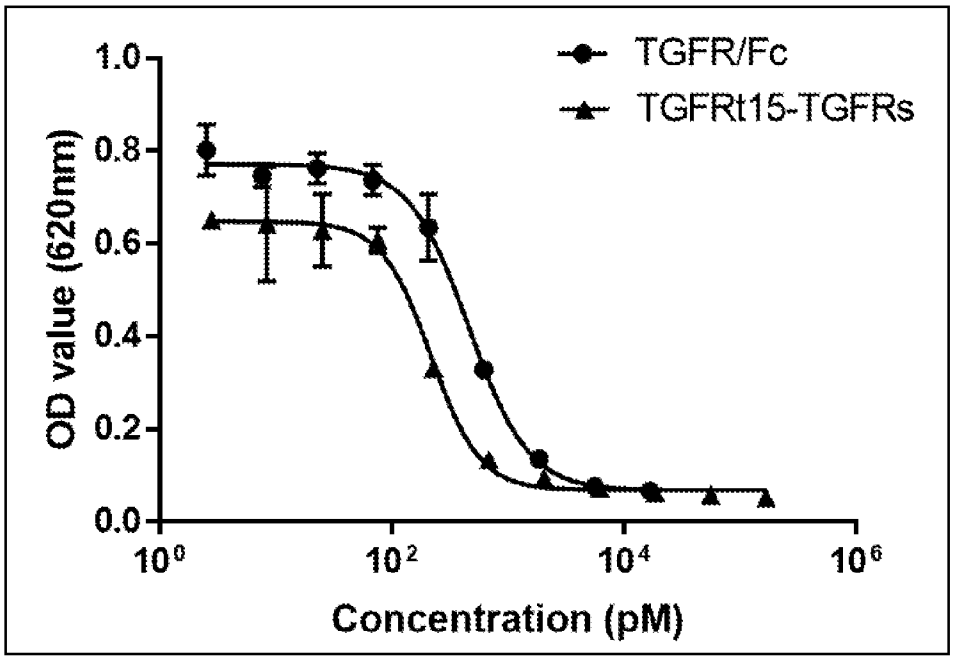


Figure 97

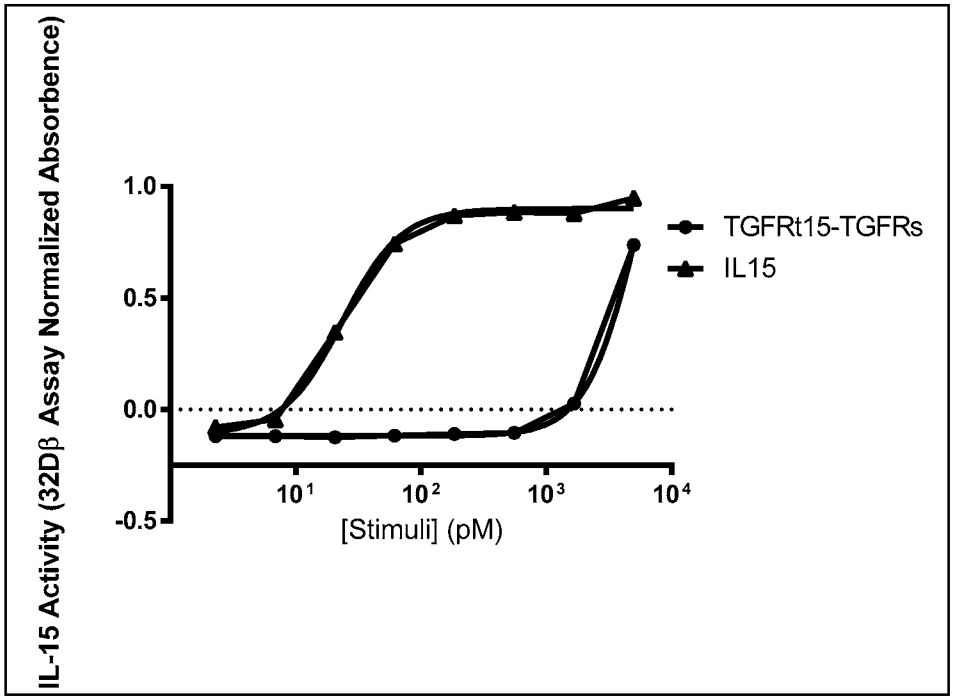


Figure 98

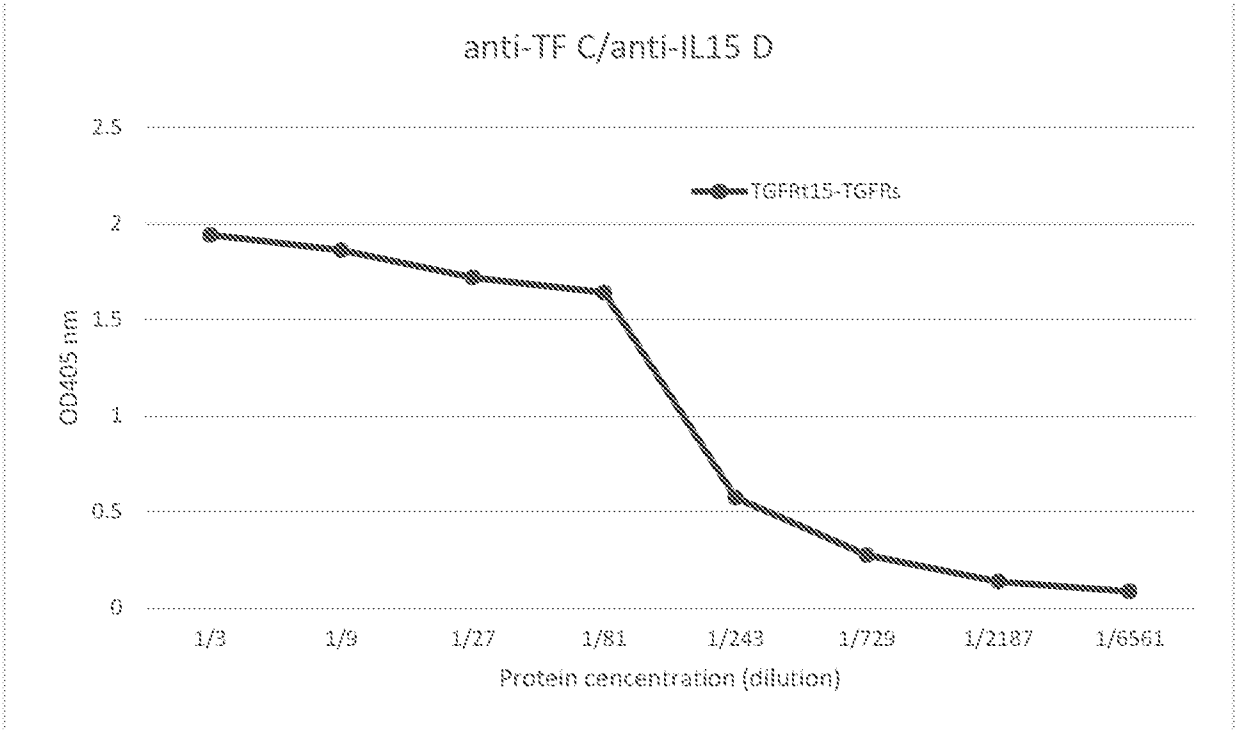


Figure 99A

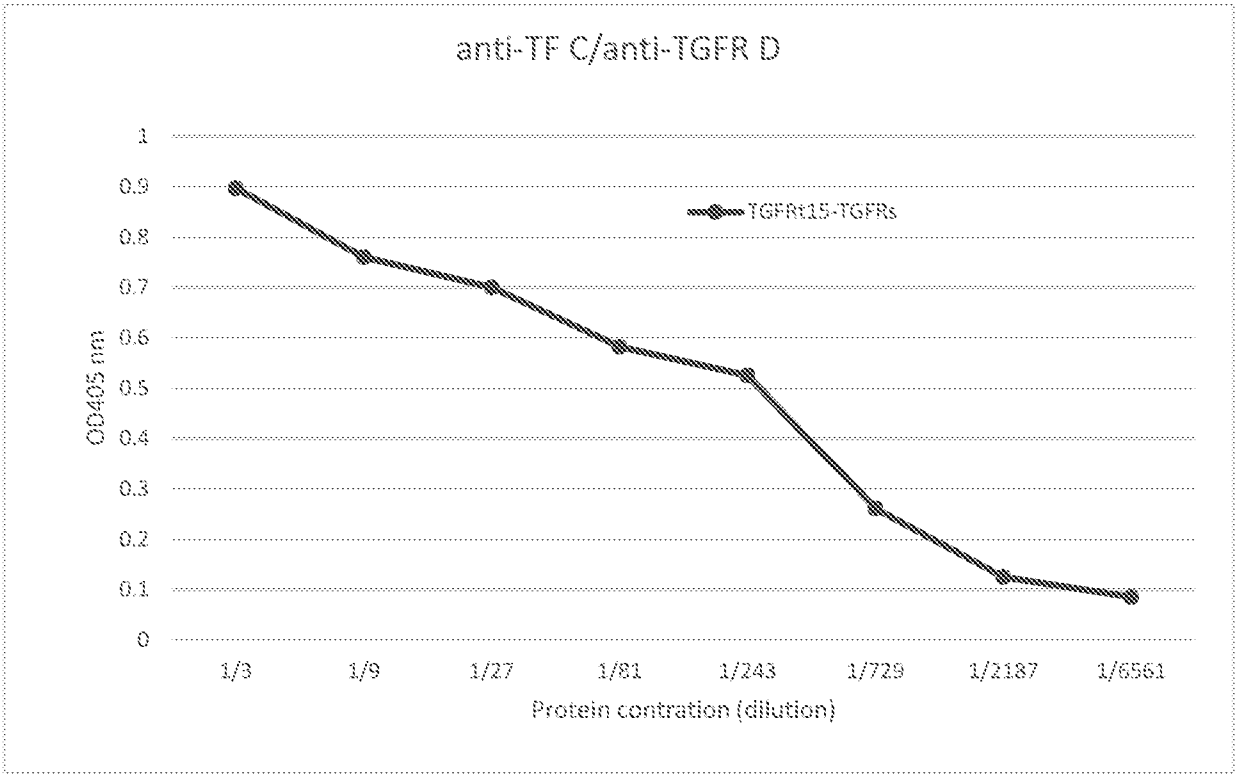


Figure 99B

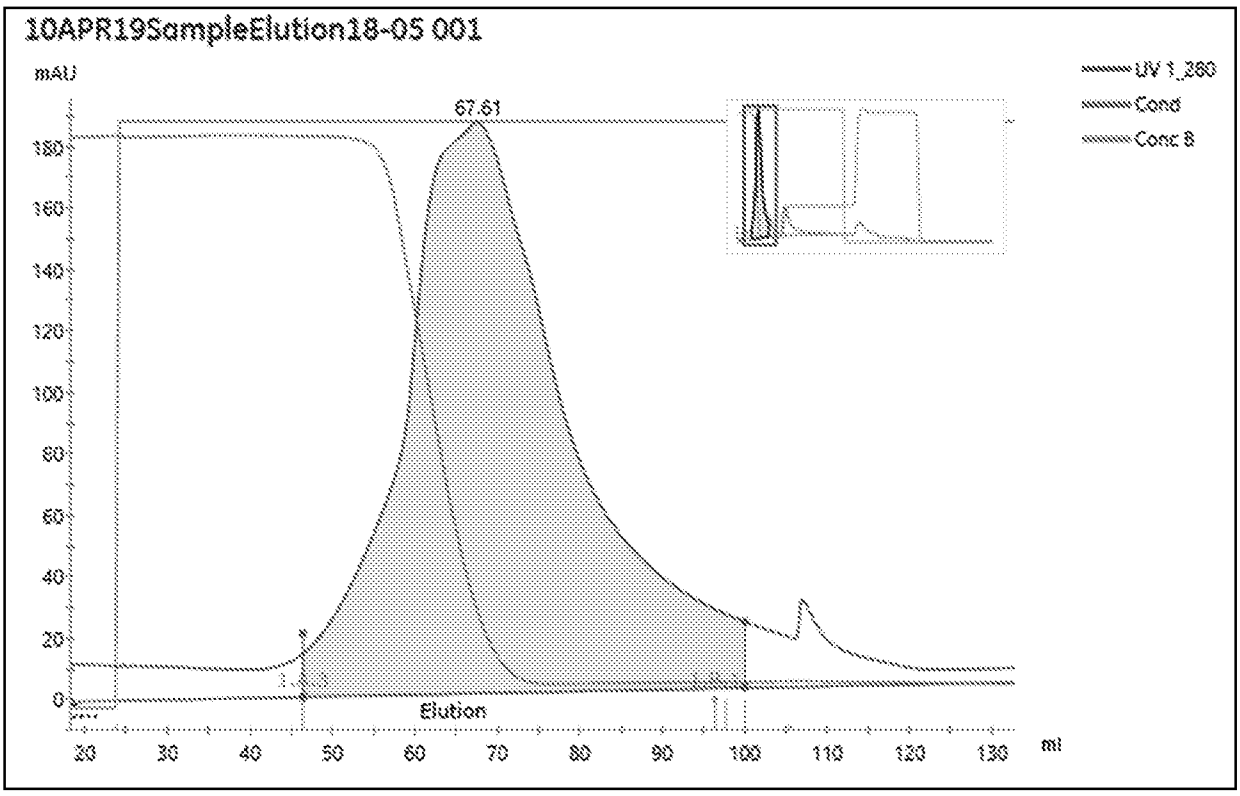

10APR19SampleElution18-05 001
mAU
UV 1_280
Cond
Conc B
67.61
180
160
140
120
100
80
60
40
20
0
Elution
30
40
50
60
70
80
90
100
110
120
130
ml

Figure 100

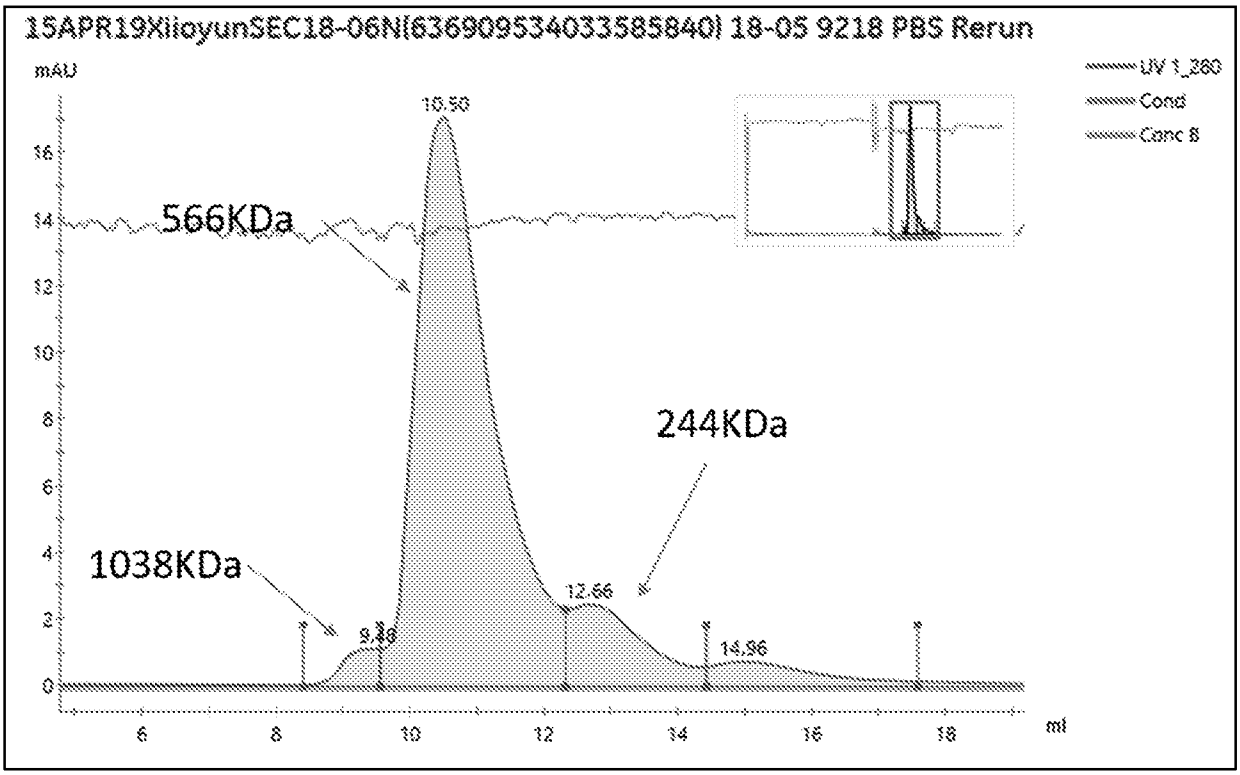

15APR19XiaoyunSEC18-06N(6369095340333585840) 18-05 9218 PBS Rerun
mAU
UV 1_280
Cond
Conc B
16.50
566KDa
244KDa
1038KDa
12.66
14.96
ml

Figure 101

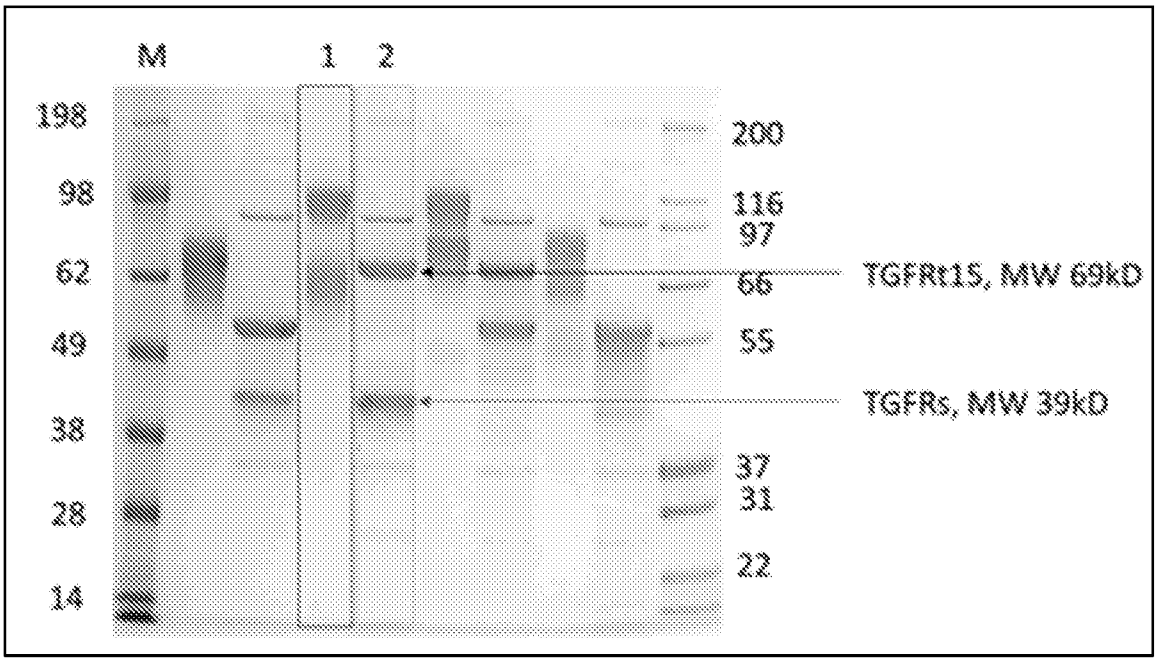

M
1
2
198
98
62
49
38
28
14
200
116
97
66
55
37
31
22
TGFRt15, MW 69kD
TGFRs, MW 39kD

Figure 102

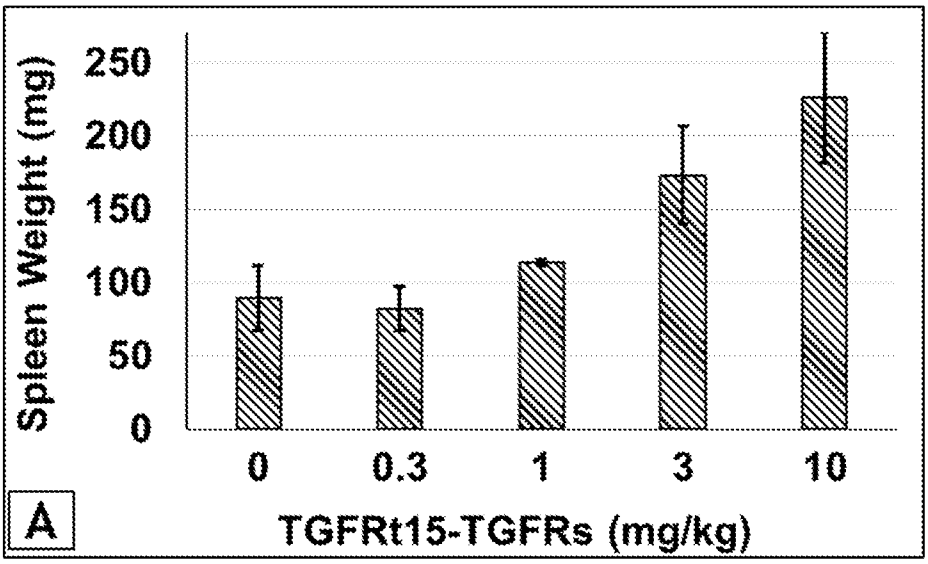


Figure 103A

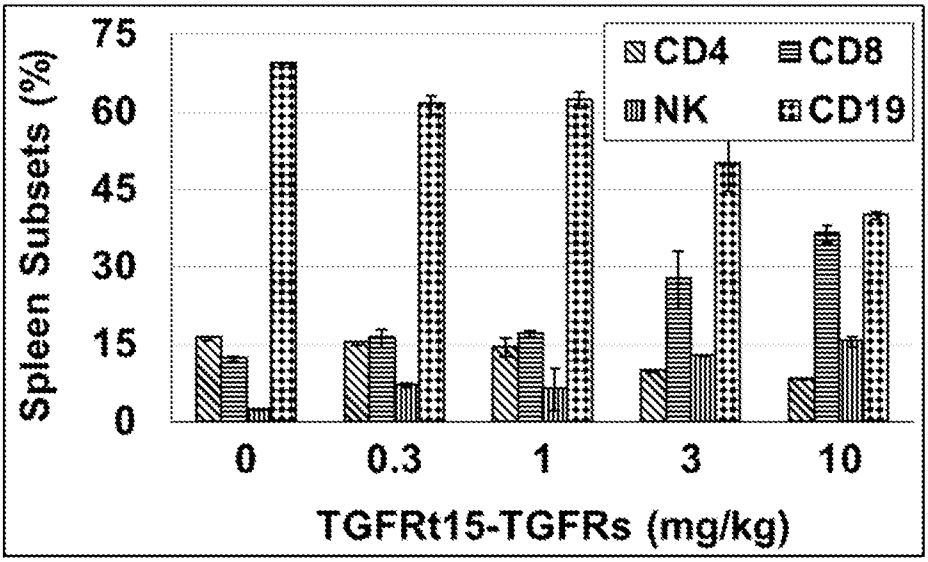


Figure 103B

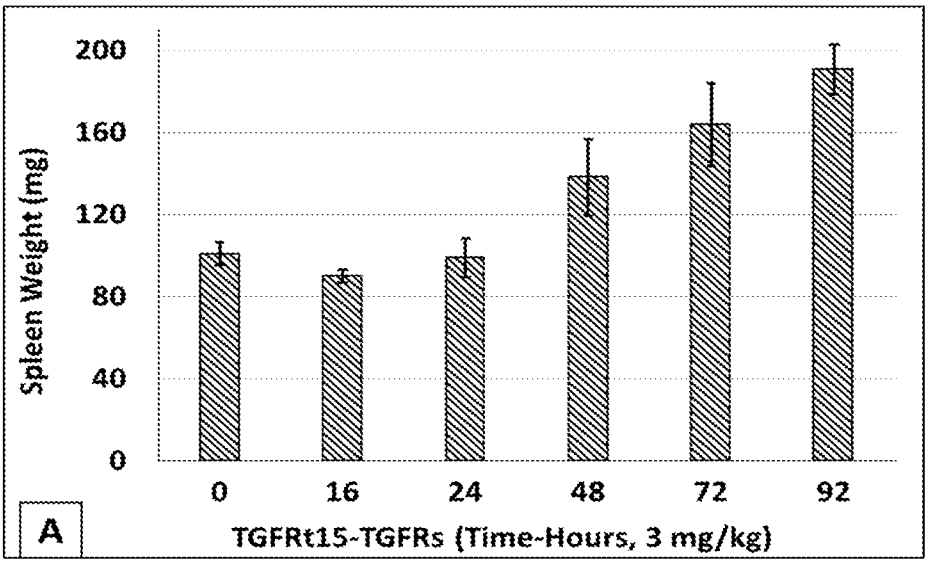


Figure 104A

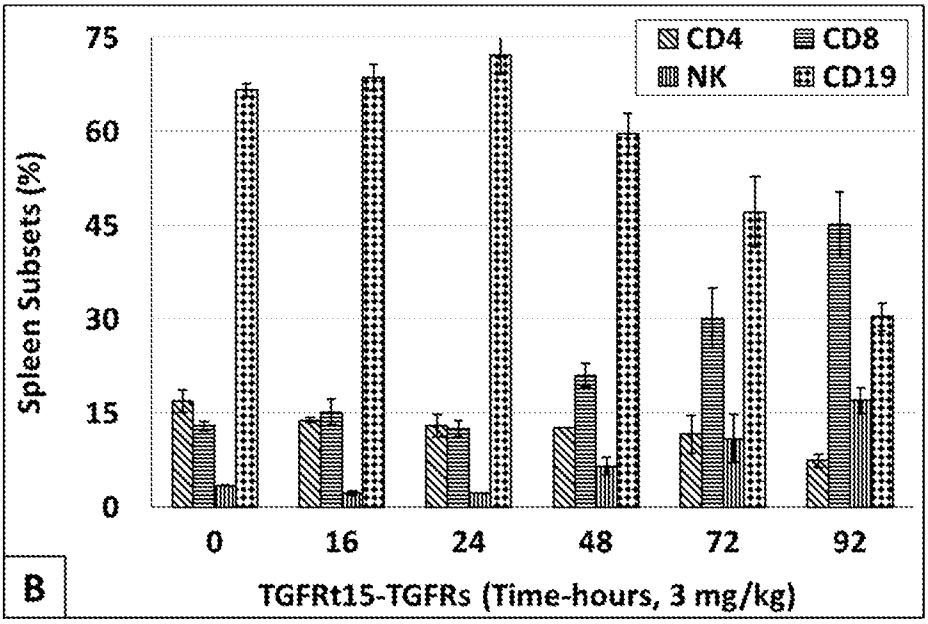


Figure 104B

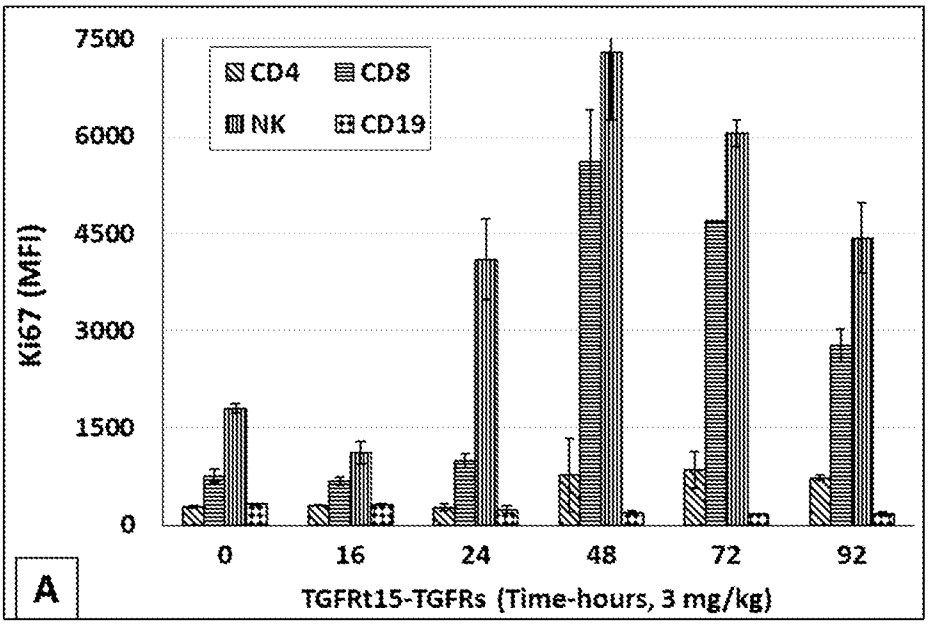


Figure 105A

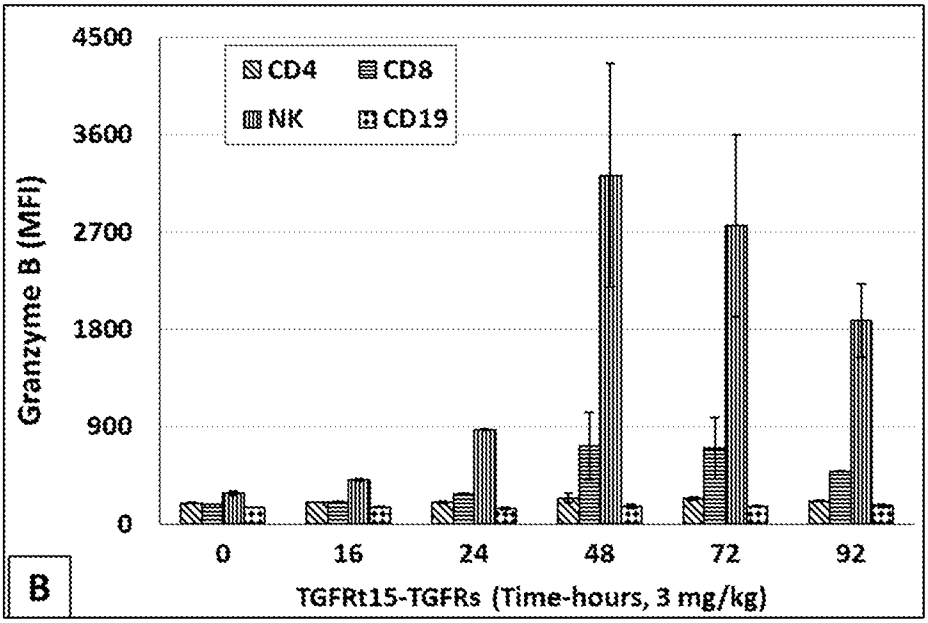


Figure 105B

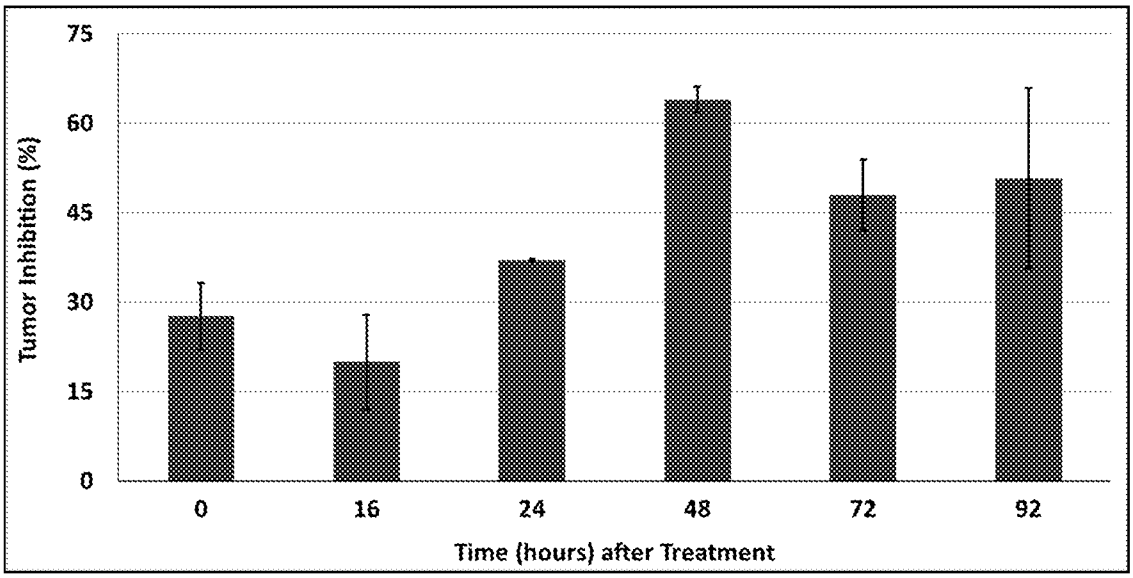

75
60
45
30
15
0
Tumor Inhibition (%)
0
16
24
48
72
92
Time (hours) after Treatment

Figure 106

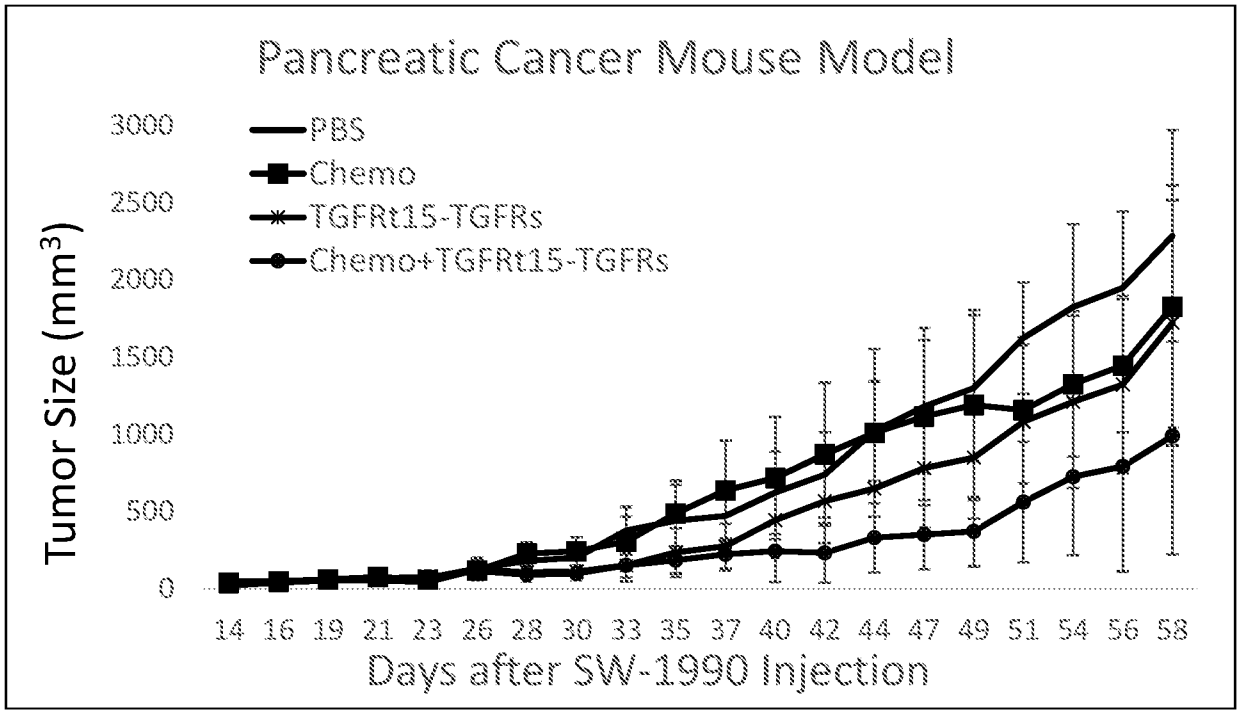

Pancreatic Cancer Mouse Model
PBS
Chemo
TGFRt15-TGFRs
Chemo+TGFRt15-TGFRs
Tumor Size (mm3)
3000
2500
2000
1500
1000
500
0
14 16 19 21 23 26 28 30 33 35 37 40 42 44 47 49 51 54 56 58
Days after SW-1990 Injection

Figure 107

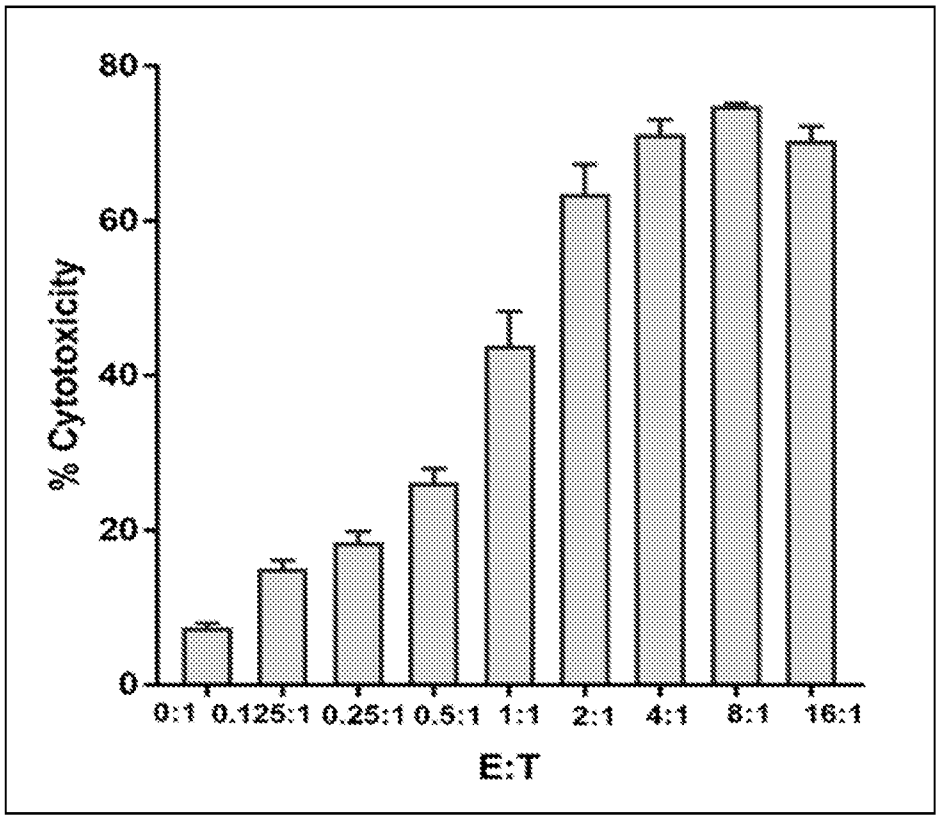


Figure 108

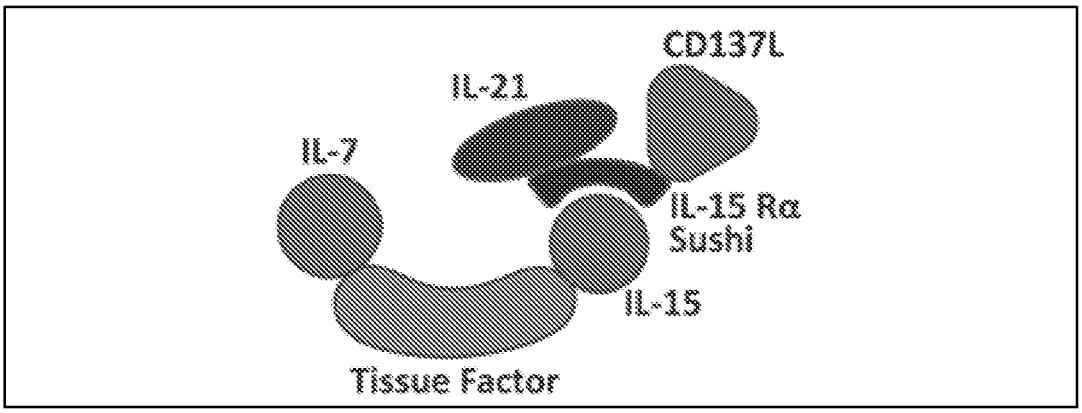


Figure 109

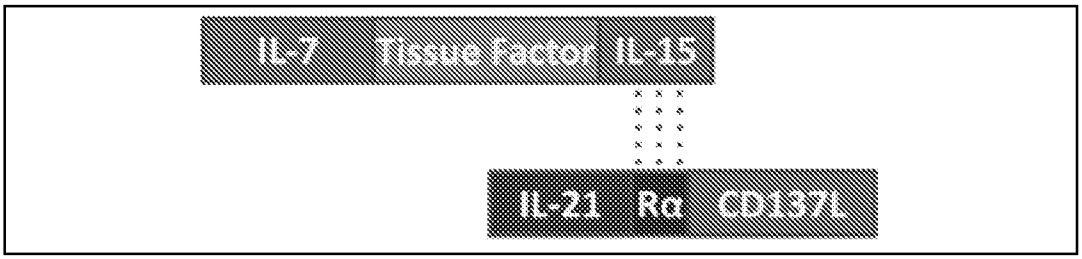


Figure 110

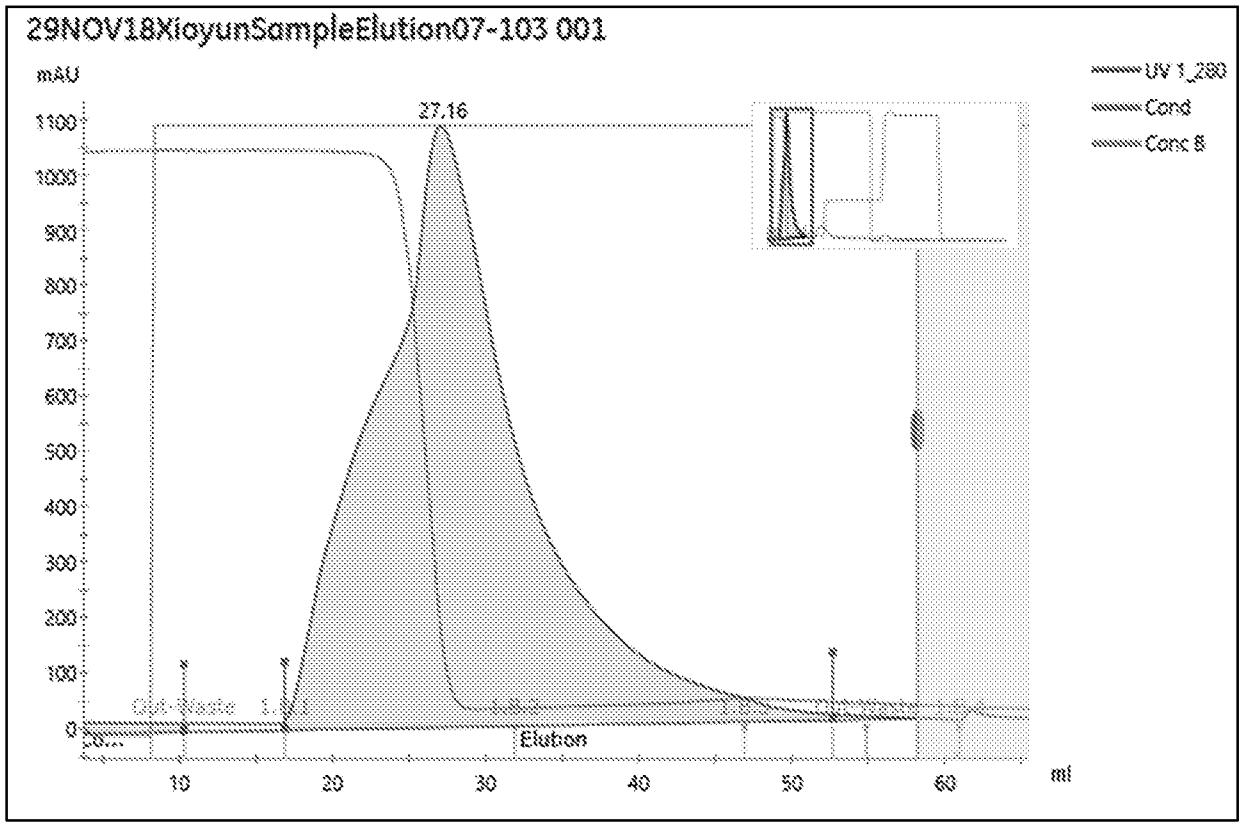

29NOV18XiayunSampleElution07-103 001
mAU
1100
1000
900
800
700
600
500
400
300
200
100
0
27.16
UV 1_280
Cond
Conc B
Elution
10
20
30
40
50
60
ml

Figure 111

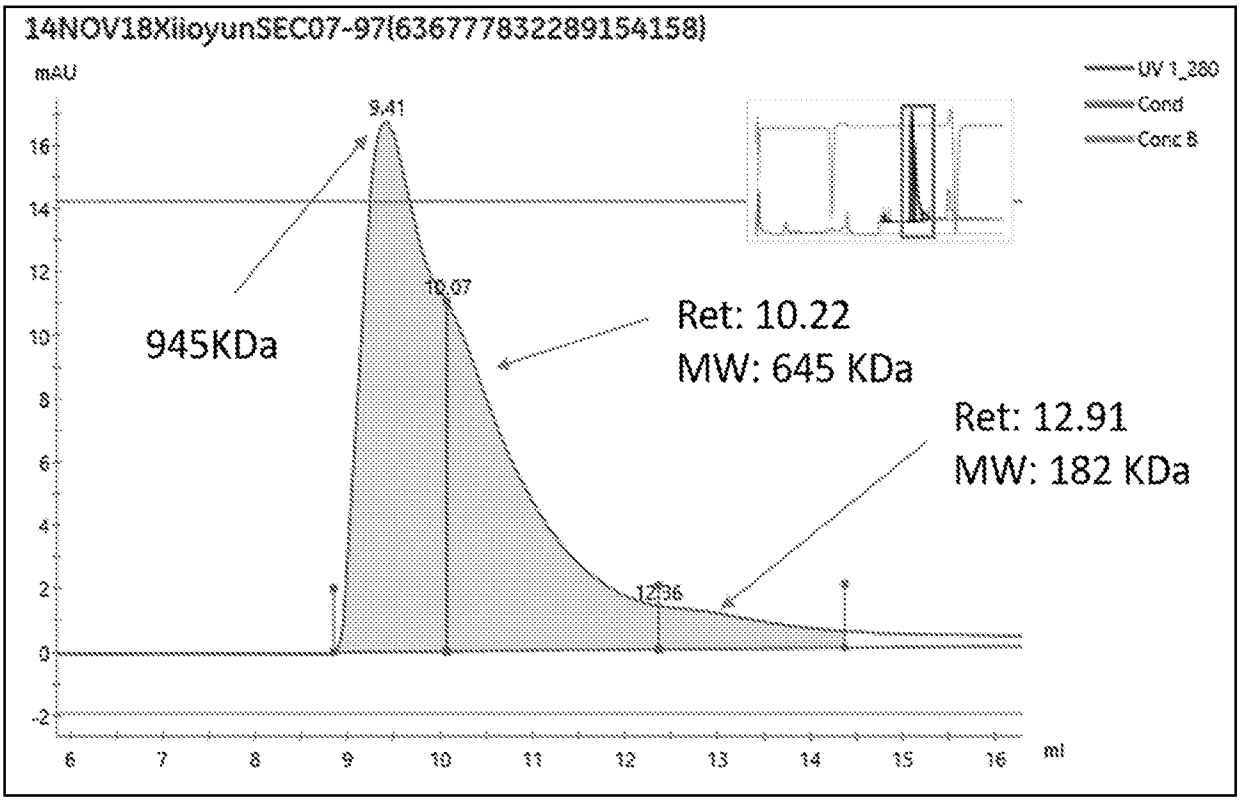

14NOV18XiloyunSEC07-97(636777832289154158)
mAU
UV 1_280
Cond
Conc B
9.41
10.07
12.36
945KDa
Ret: 10.22
MW: 645 KDa
Ret: 12.91
MW: 182 KDa
ml

Figure 112

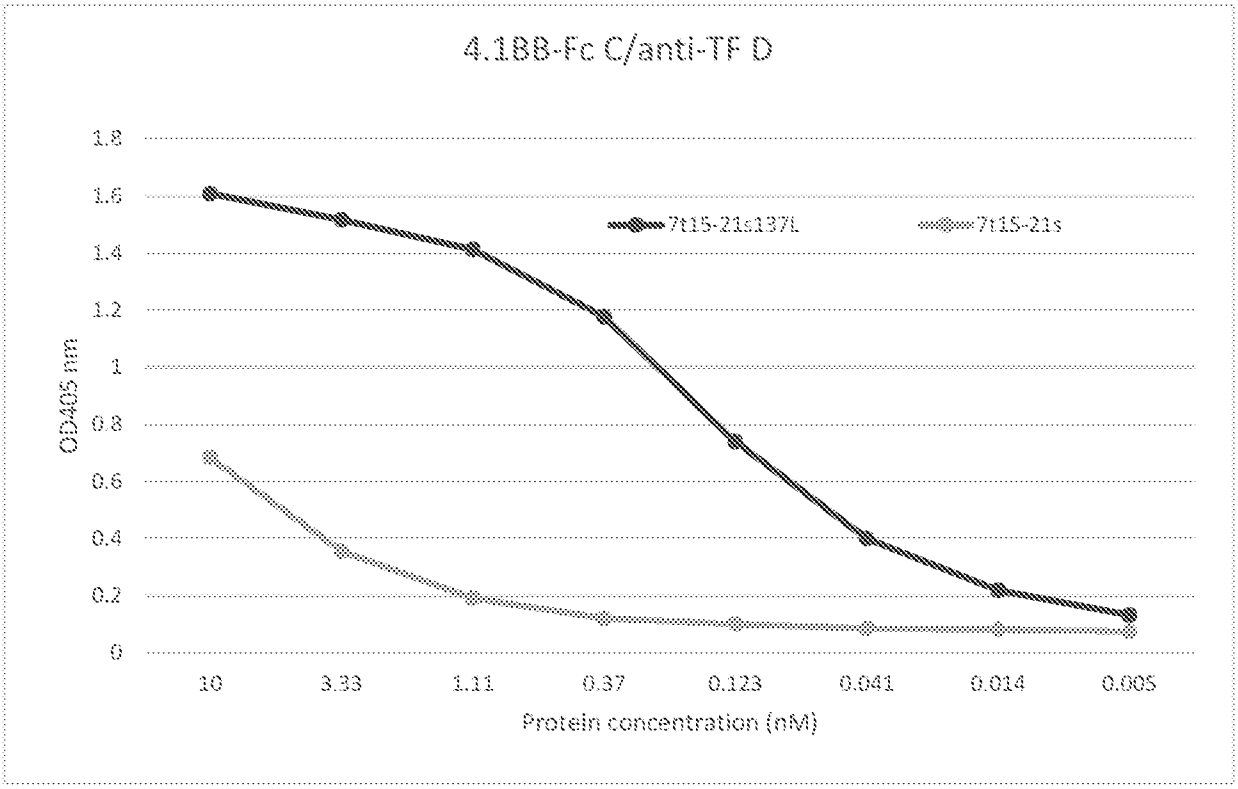

4.1BB-Fc C/anti-TF D
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD405 nm
7t15-21s137L
7t15-21s
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein concentration (nM)

Figure 113

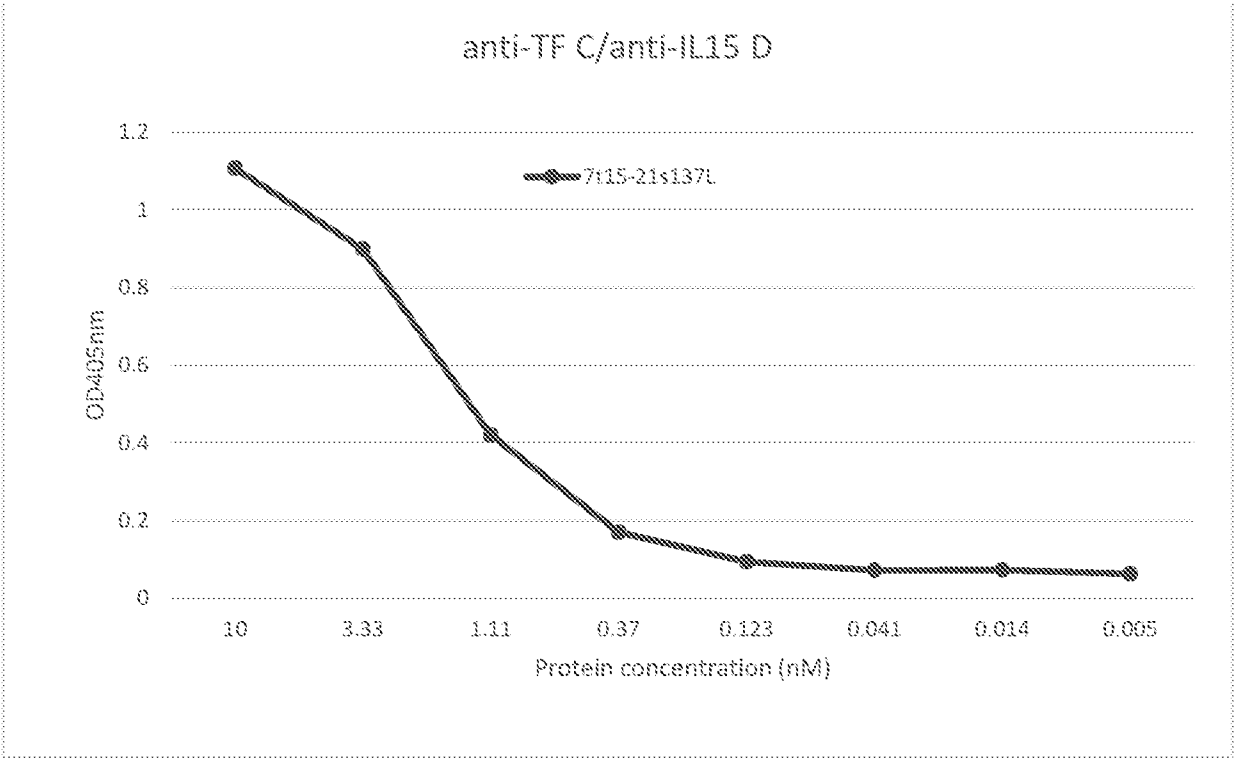


Figure 114A

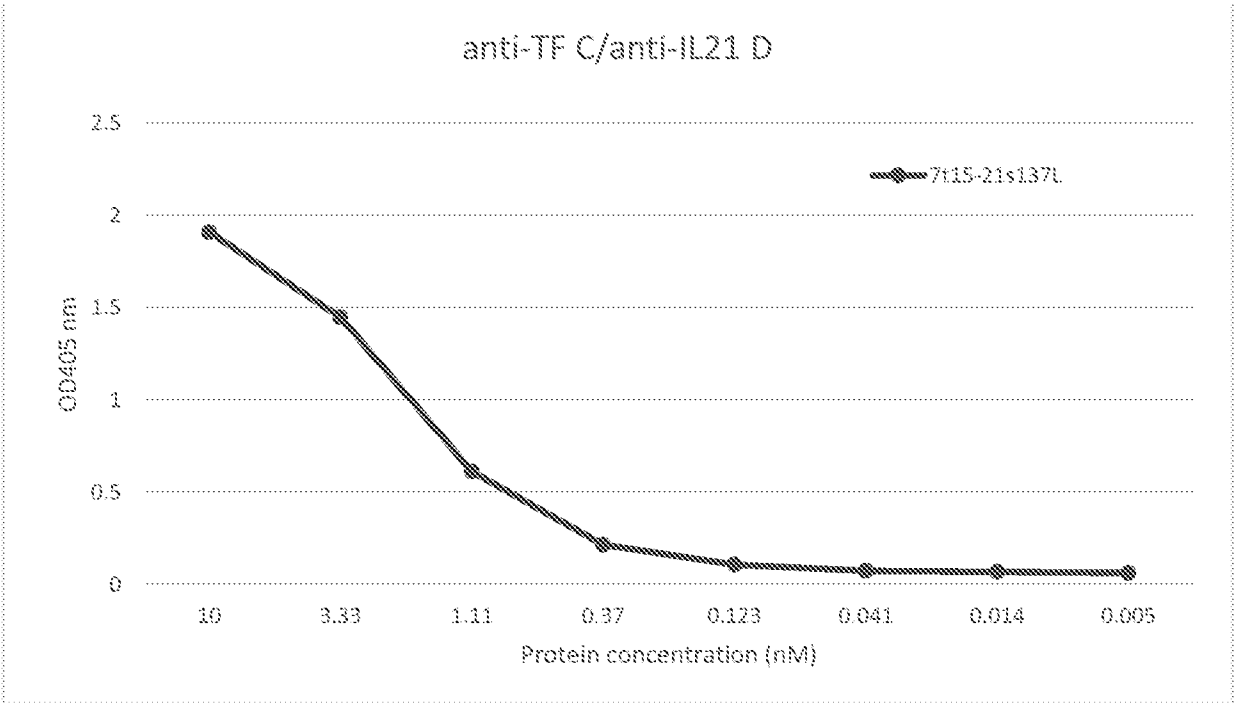


Figure 114B

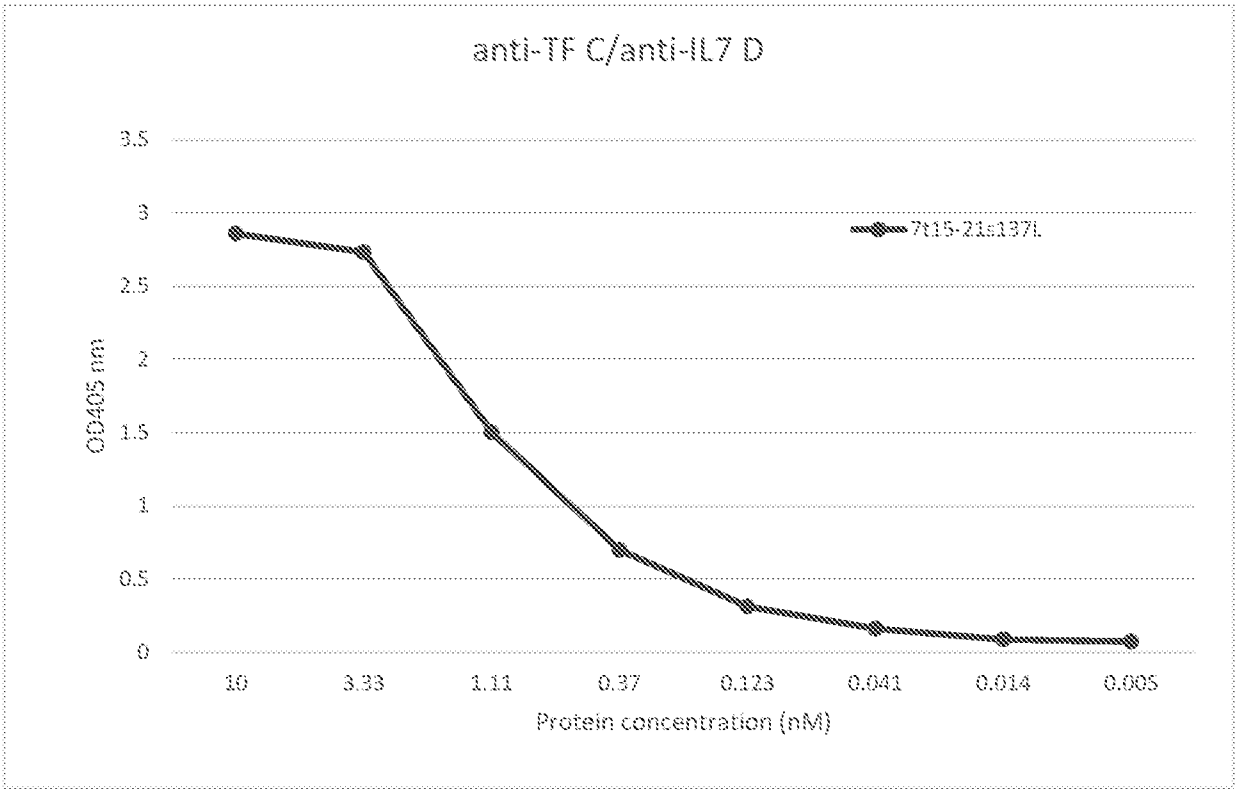

anti-TF C/anti-IL7 D
OD405 nm
3.5
3
2.5
2
1.5
1
0.5
0
7t15-21:137L
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein concentration (nM)

Figure 114C

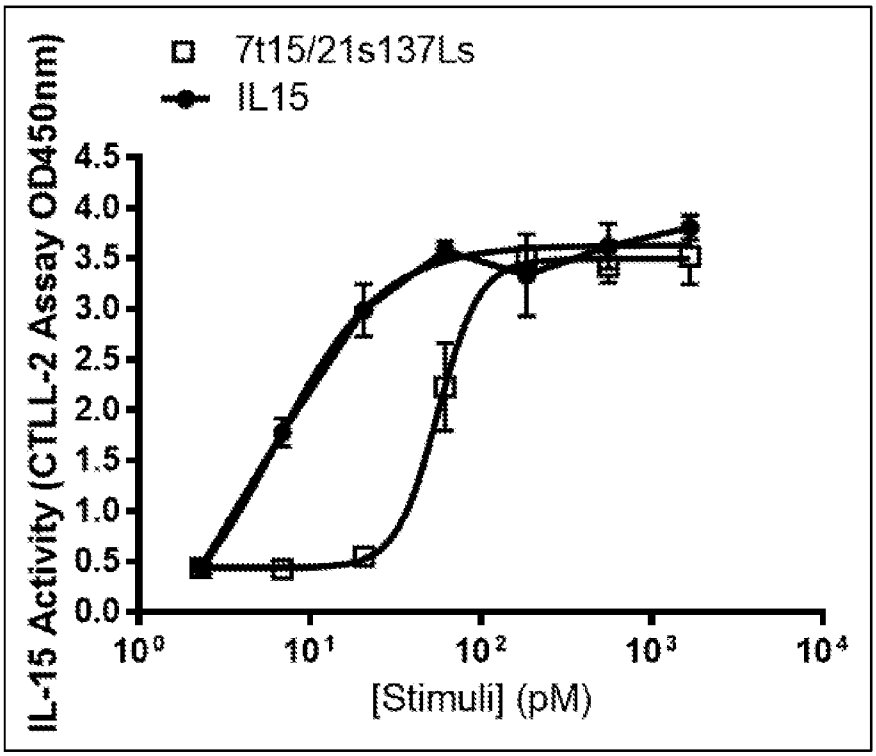


Figure 115

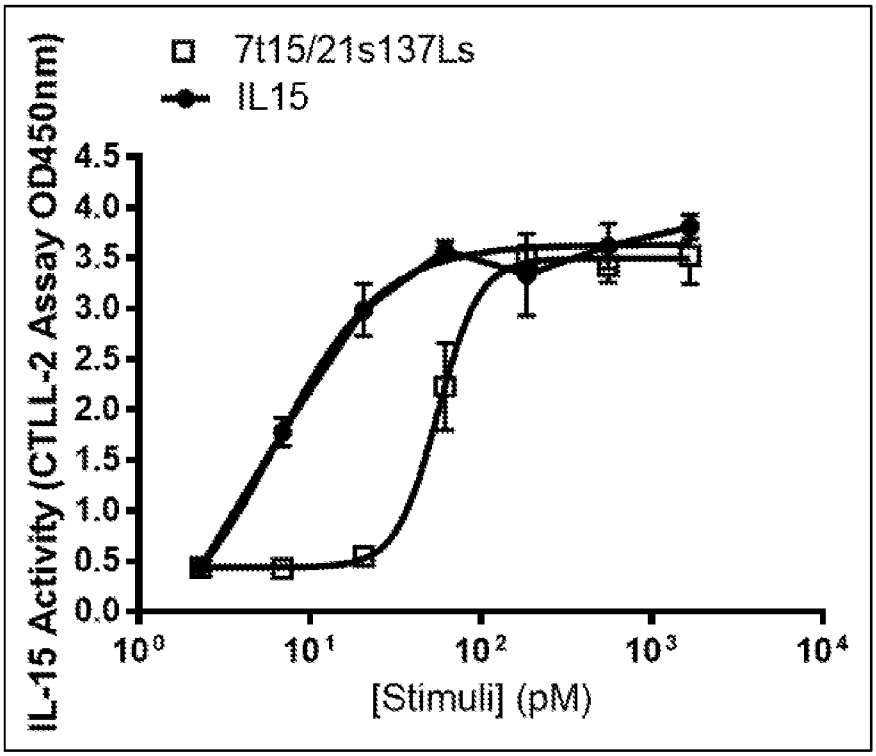


Figure 116

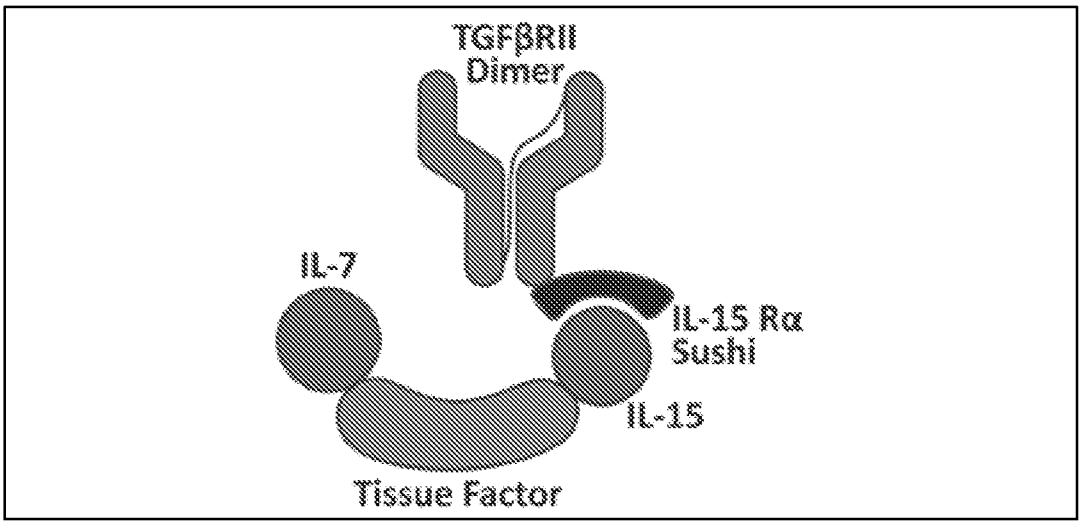


Figure 117

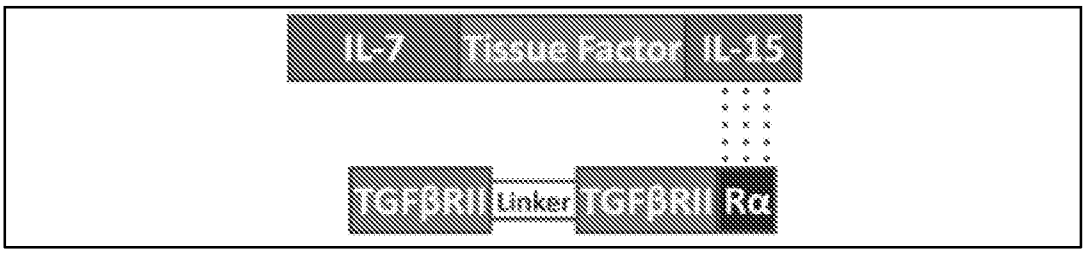


Figure 118

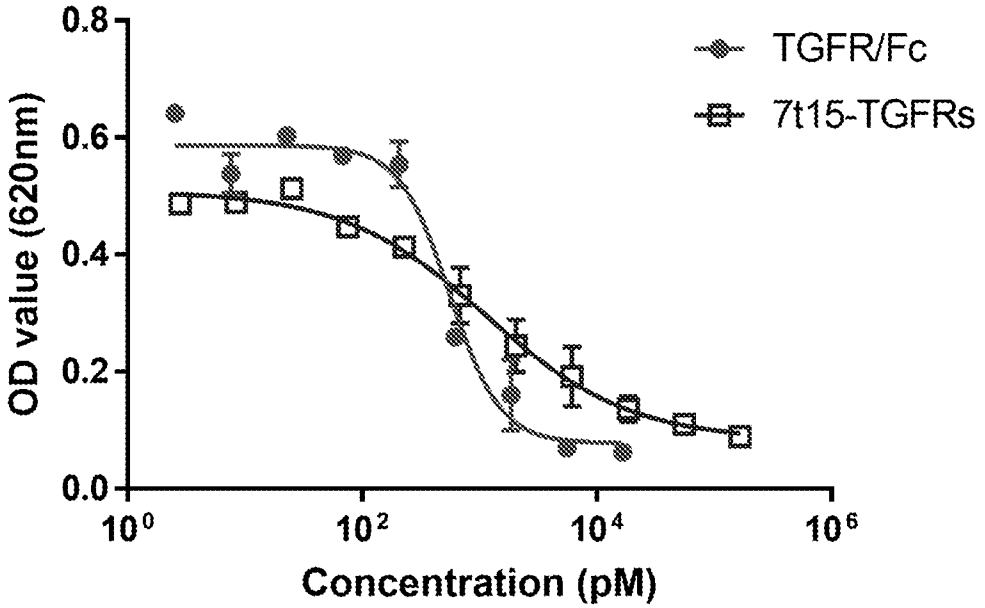

0.8
0.6
0.4
0.2
0.0
OD value (620nm)
10^0
10^2
10^4
10^6
Concentration (pM)
TGFR/Fc
7t15-TGFRs

Figure 119

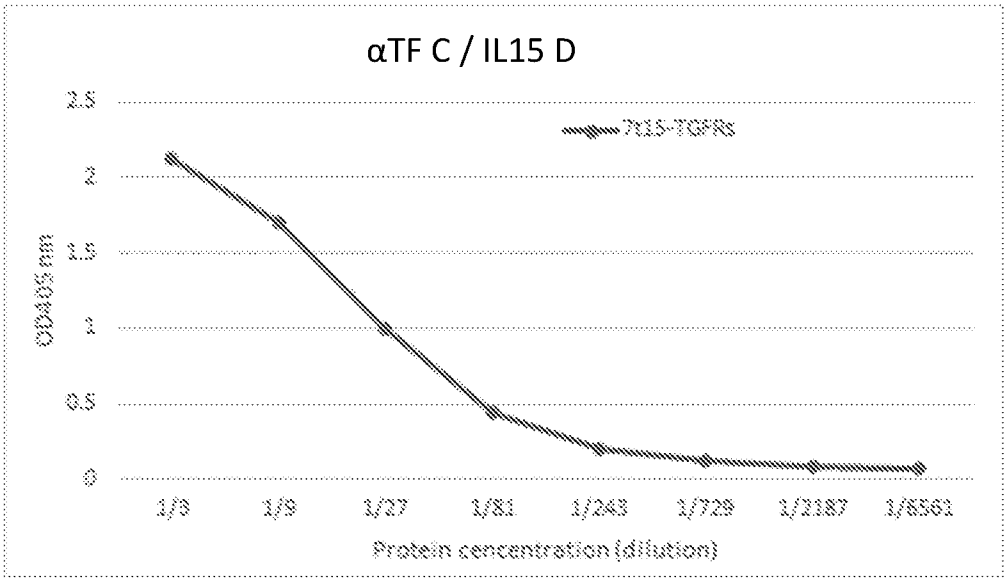


Figure 120A

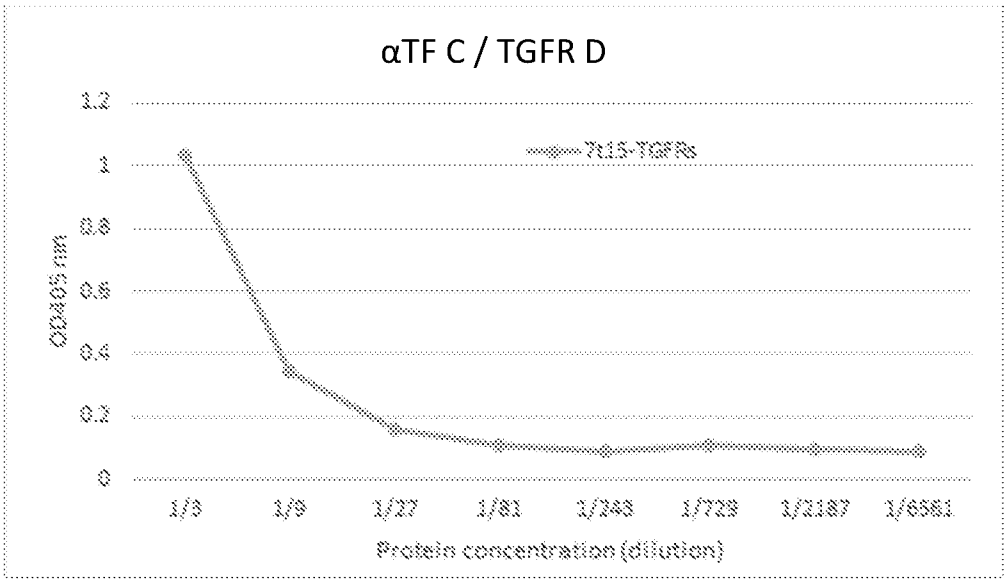


Figure 120B

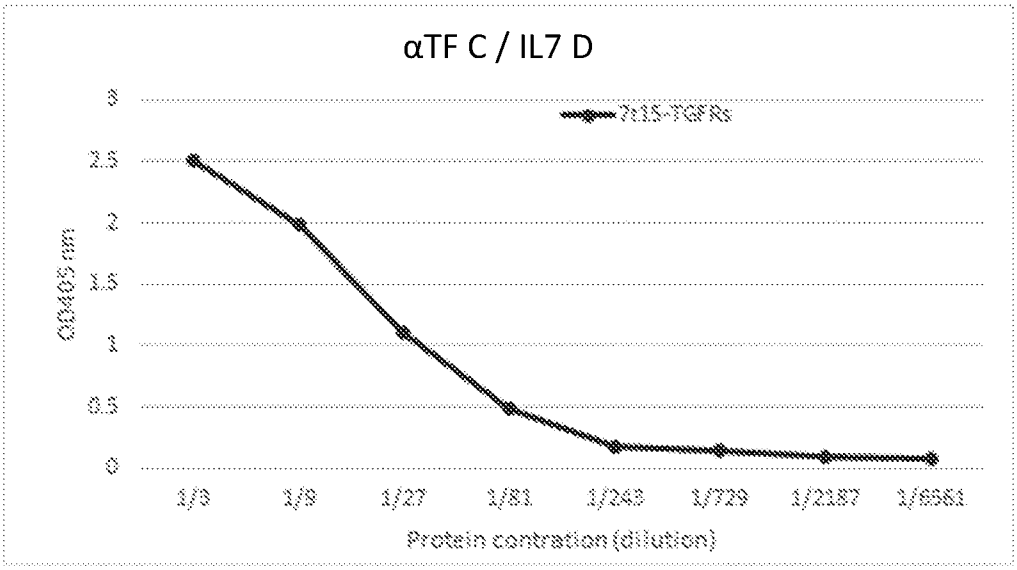

αTF C / IL7 D
7r15-TGFRs
OD405 nm
3
2.5
2
1.5
1
0.5
0
1/3
1/9
1/27
1/81
1/243
1/729
1/2187
1/6561
Protein contration (dilution)

Figure 120C

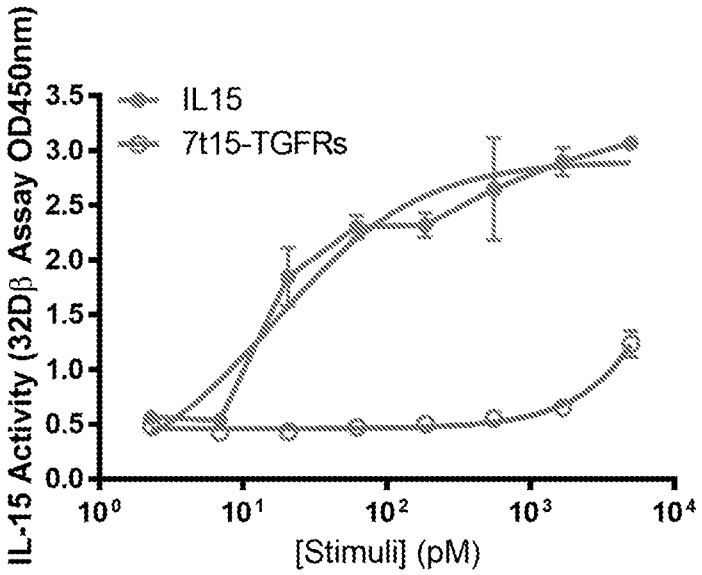


Figure 121

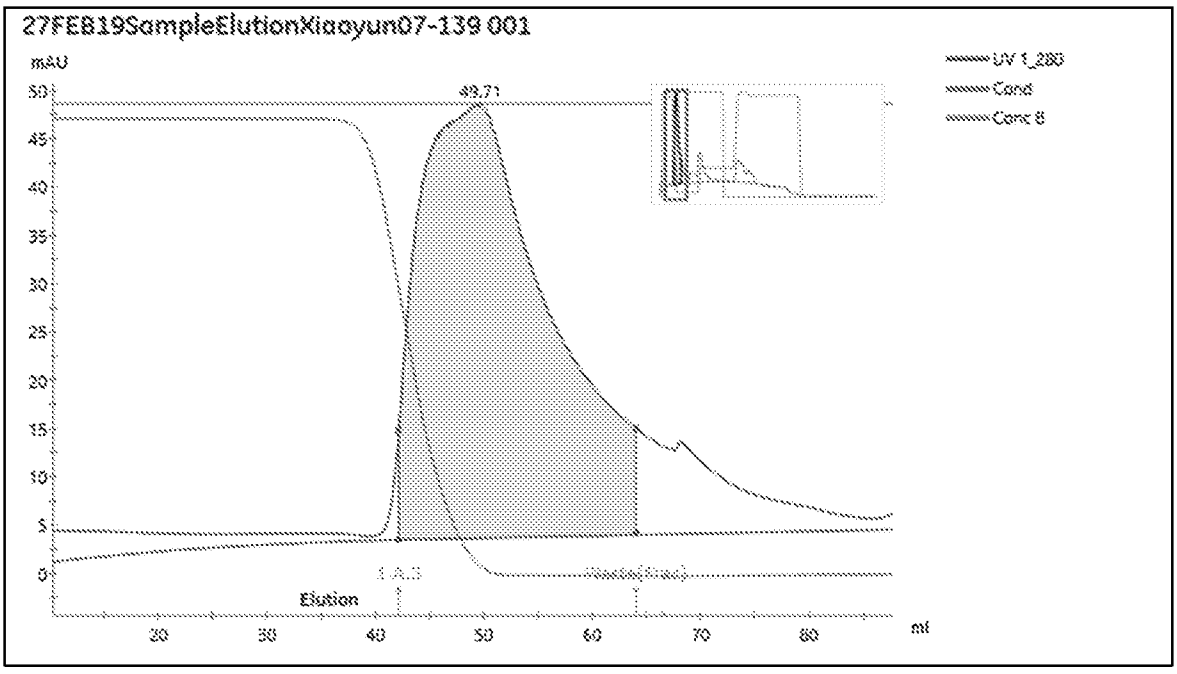


Figure 122

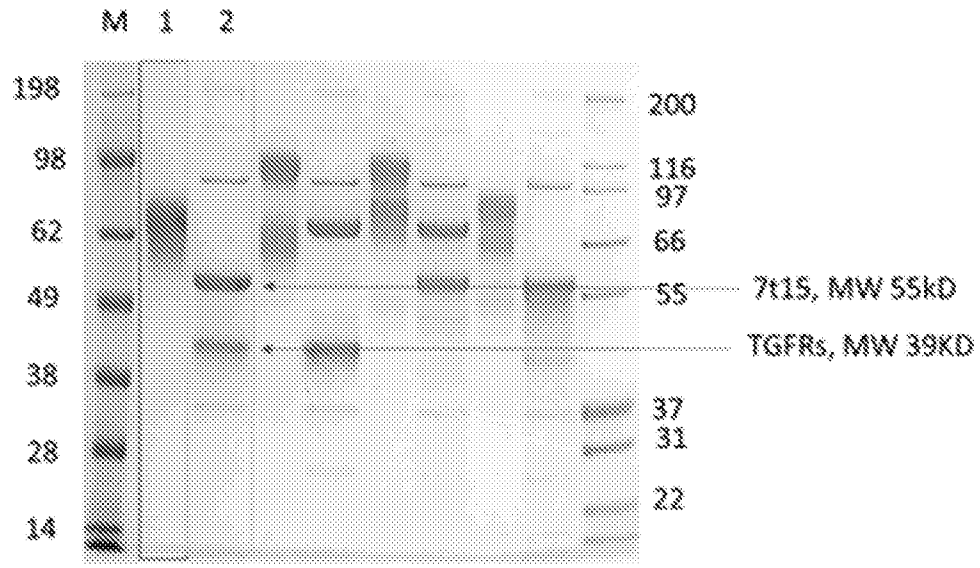


Figure 123

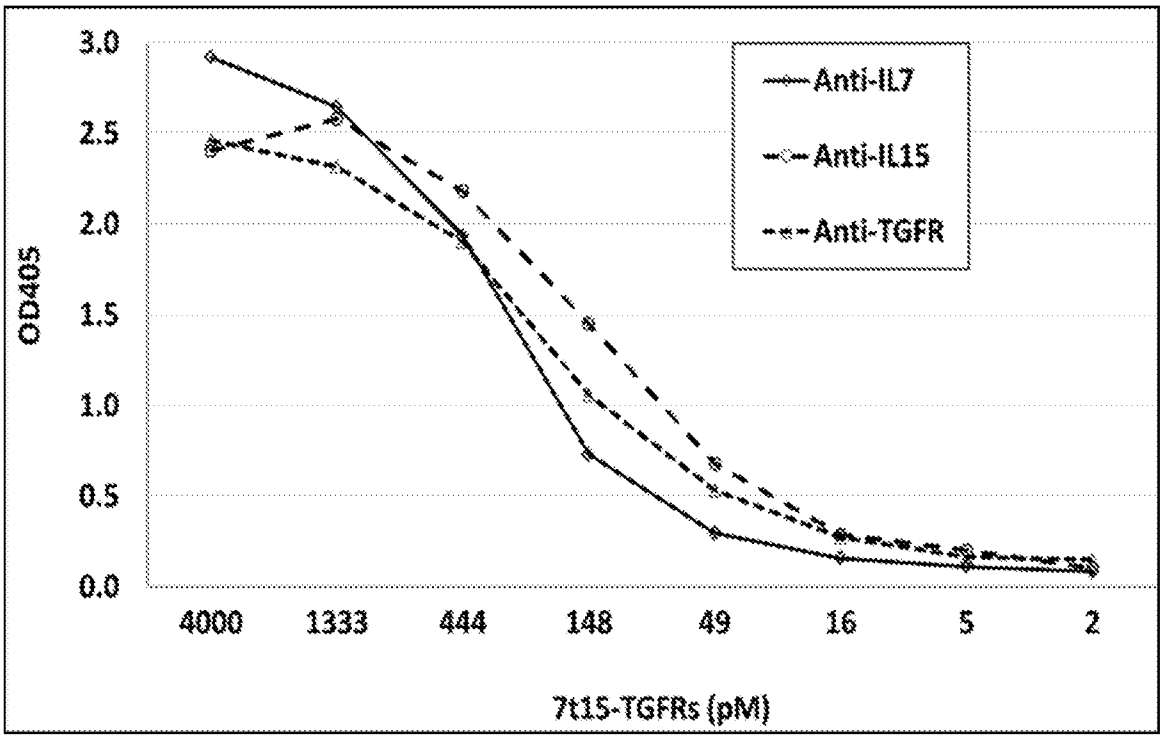


Figure 124

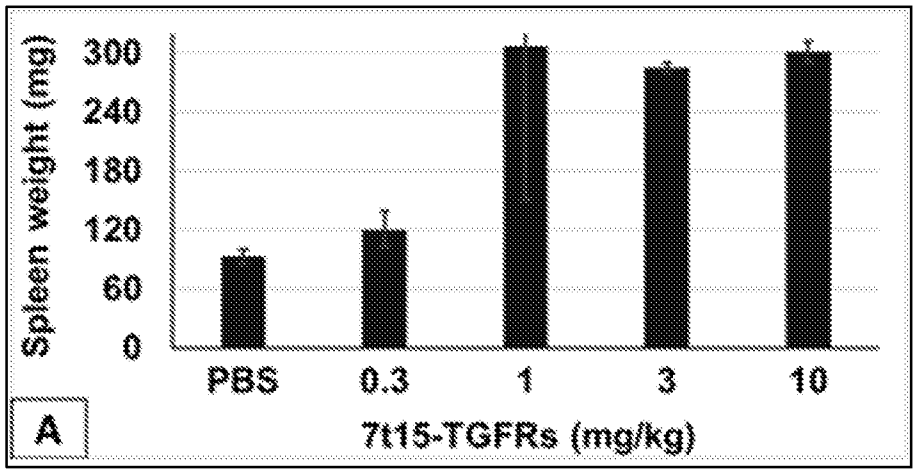


Figure 125A

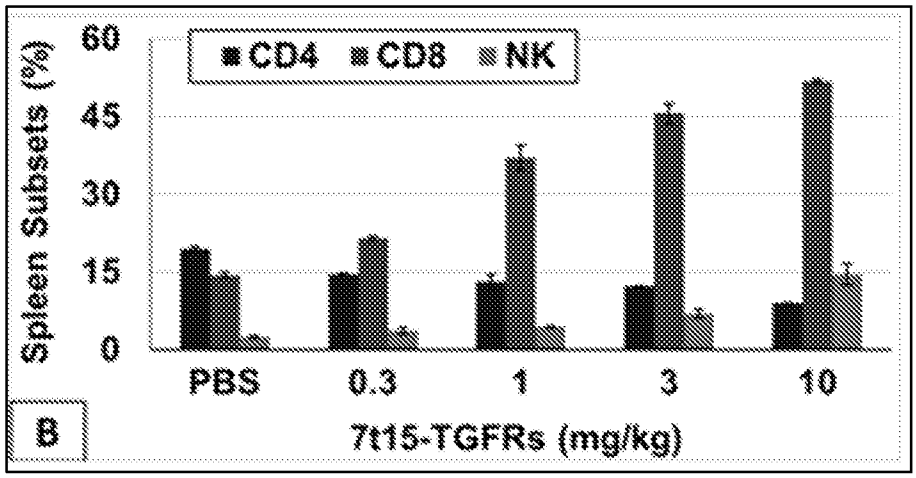


Figure 125B

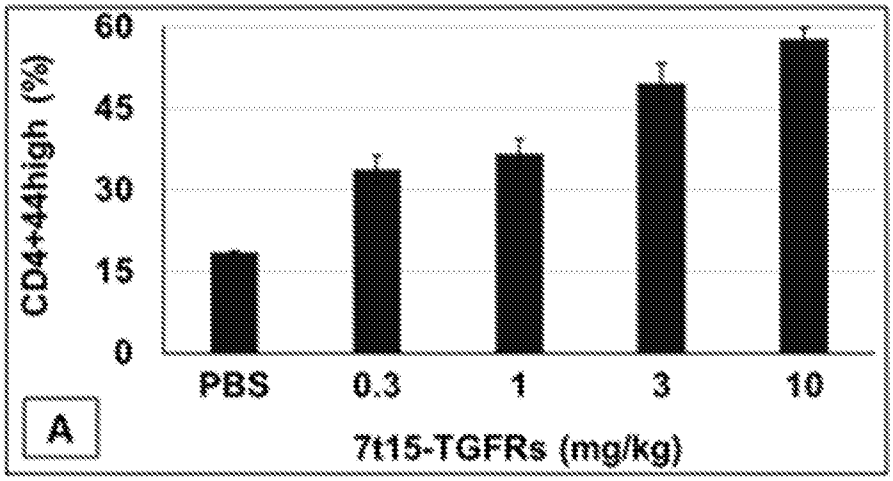


Figure 126A

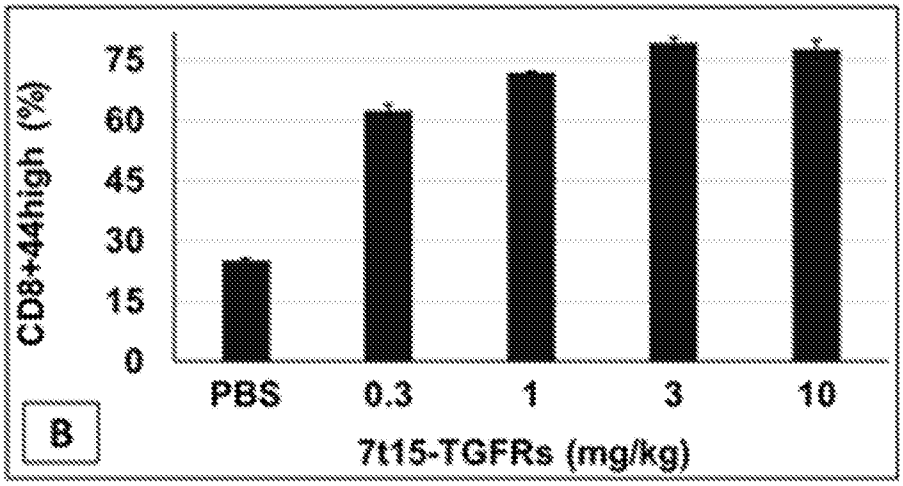


Figure 126B

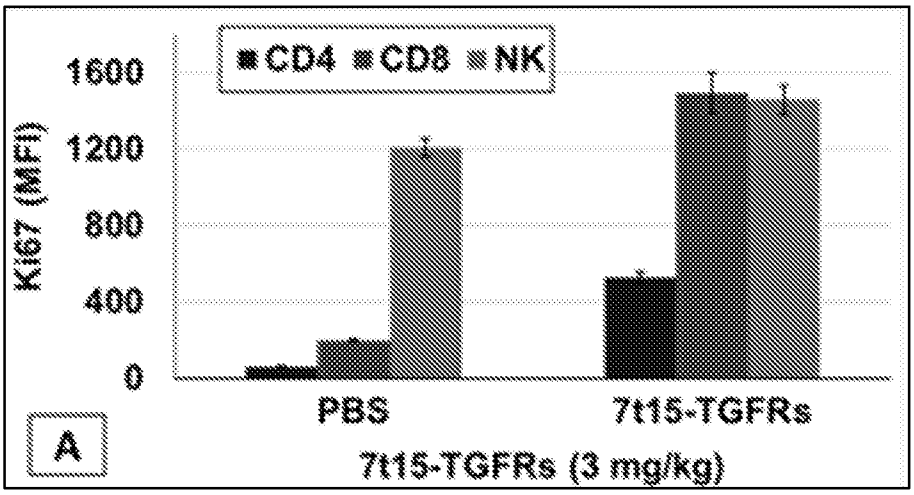


Figure 127A

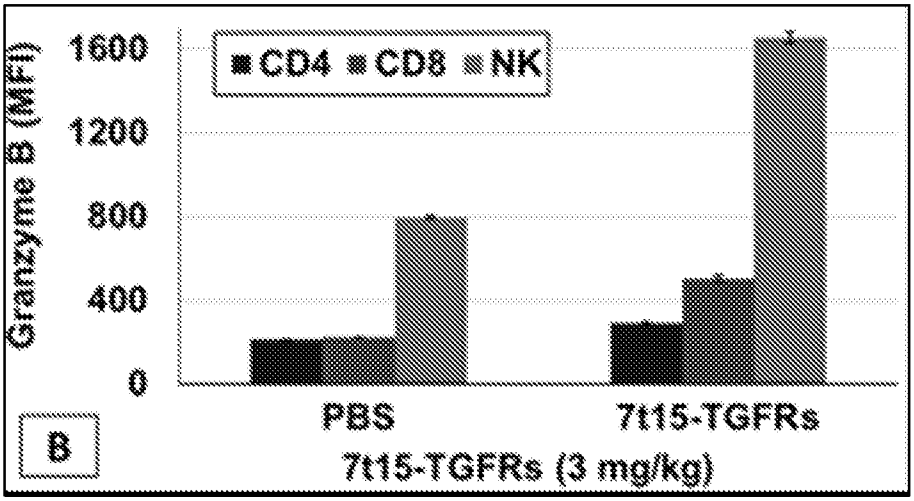


Figure 127B

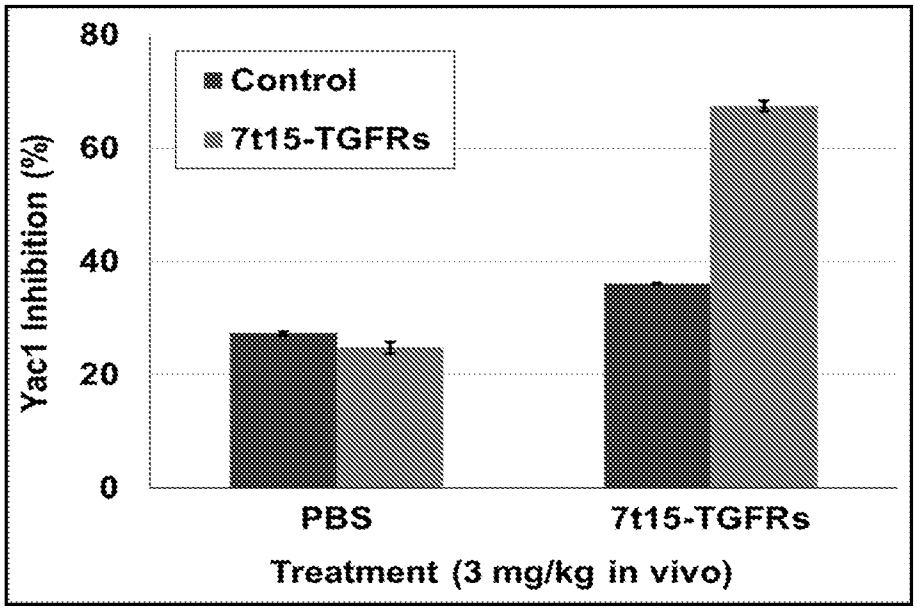

80
60
40
20
0
Yac1 Inhibition (%)
Control
7t15-TGFRs
PBS
7t15-TGFRs
Treatment (3 mg/kg in vivo)

Figure 128

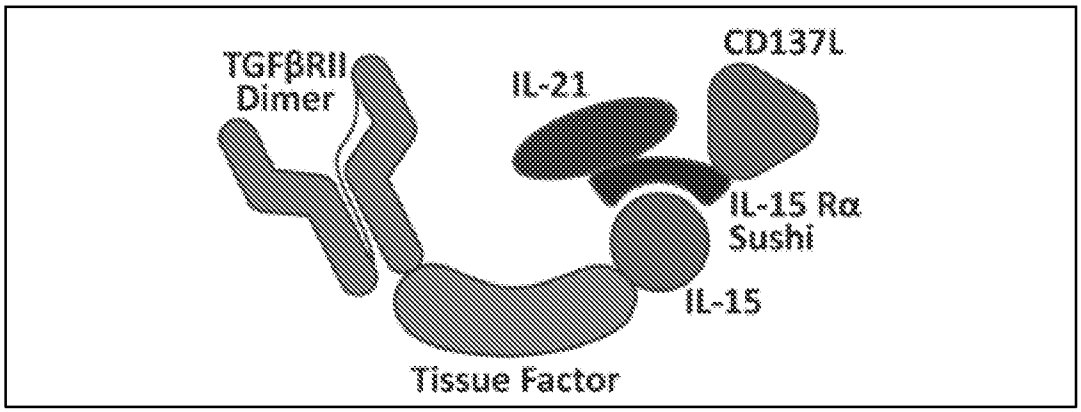


Figure 129

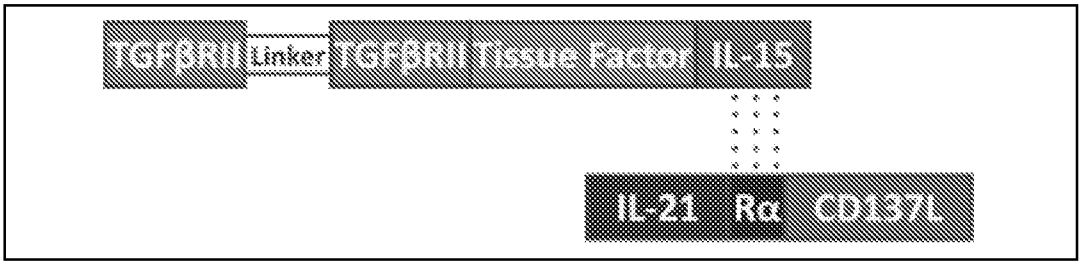


Figure 130

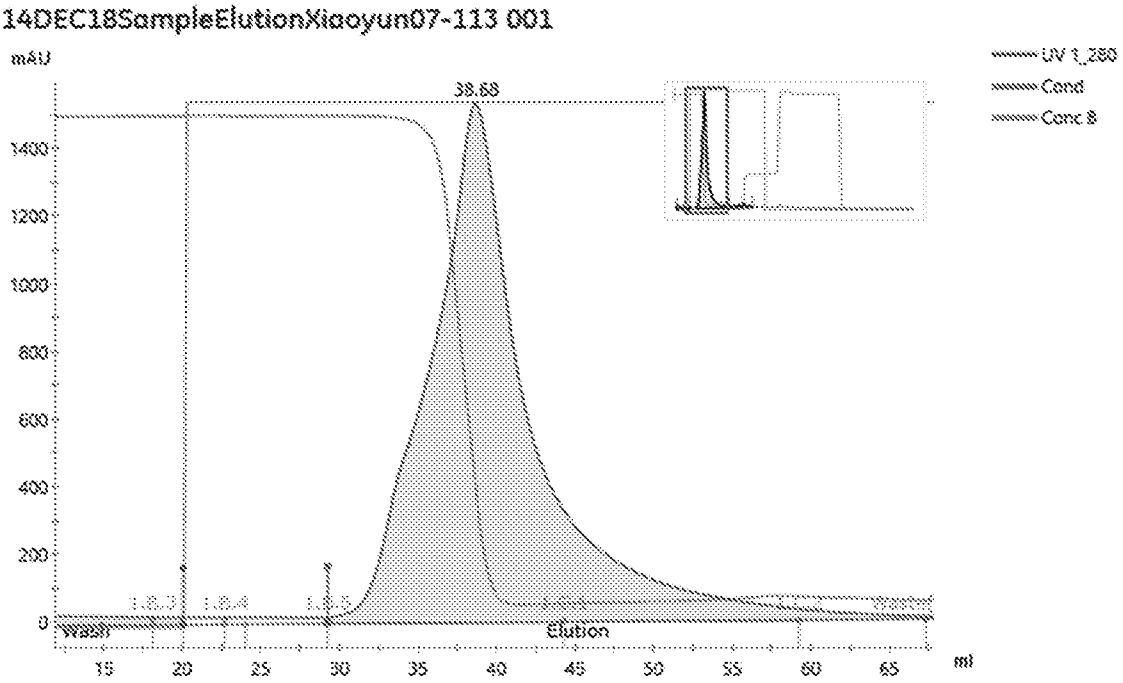

14DEC18SampleElutionXiaoyun07-113 001
mAU
38.68
UV 1_280
Cond
Conc B
1400
1200
1000
800
600
400
200
0
Wash
Elution
15
20
25
30
35
40
45
50
55
60
65
ml

Figure 131

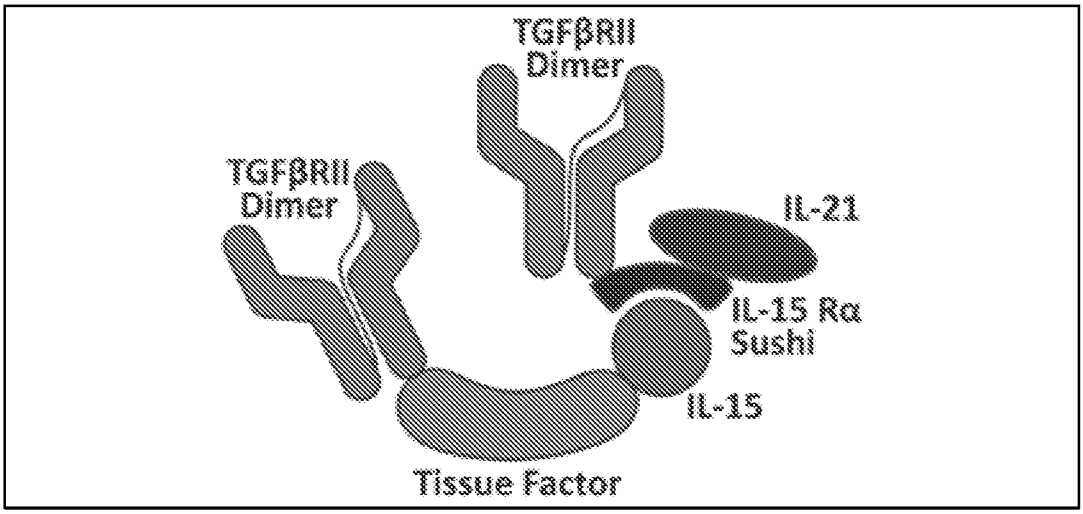


Figure 132

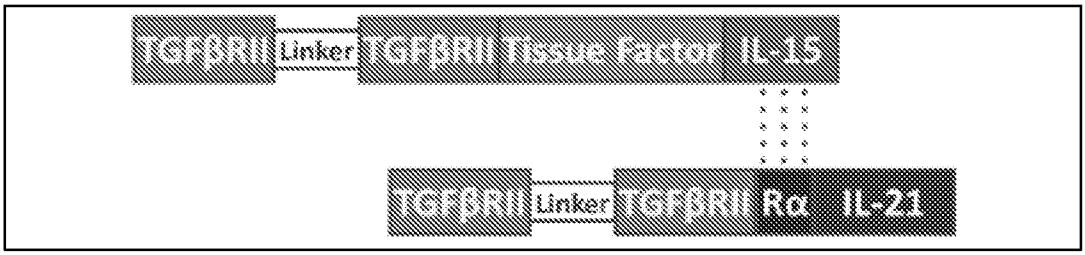


Figure 133

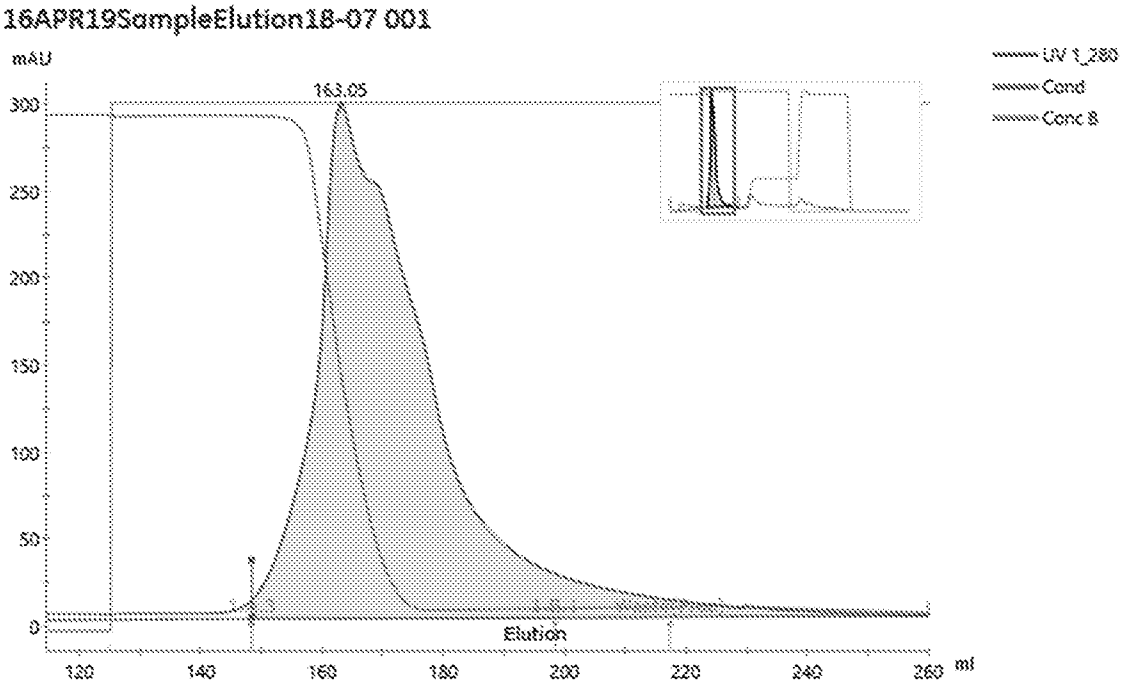


Figure 134

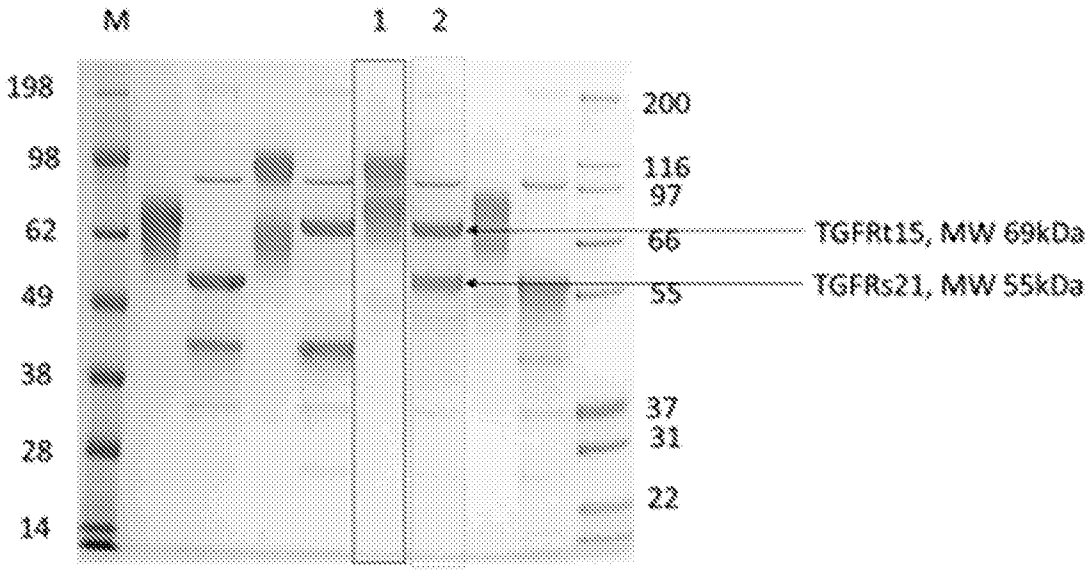


Figure 135

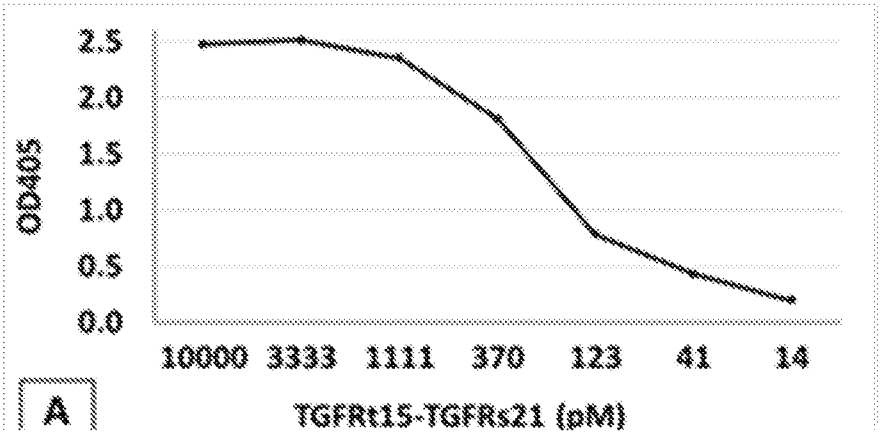


Figure 136A

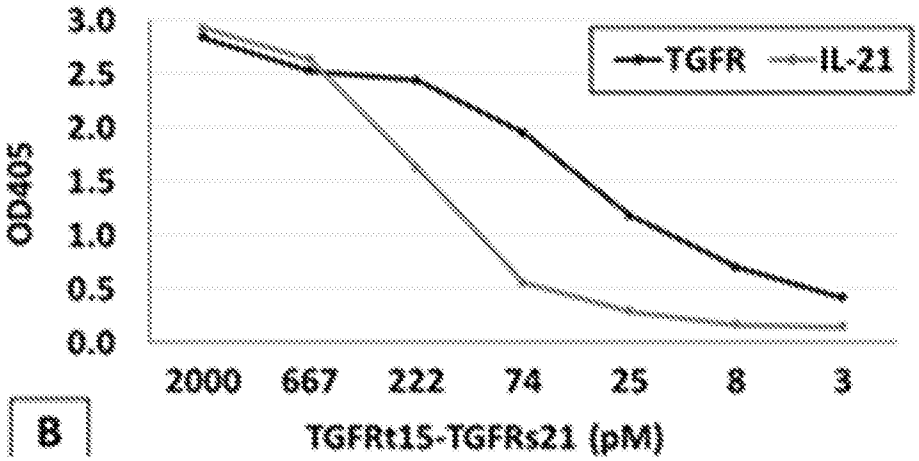


Figure 136B

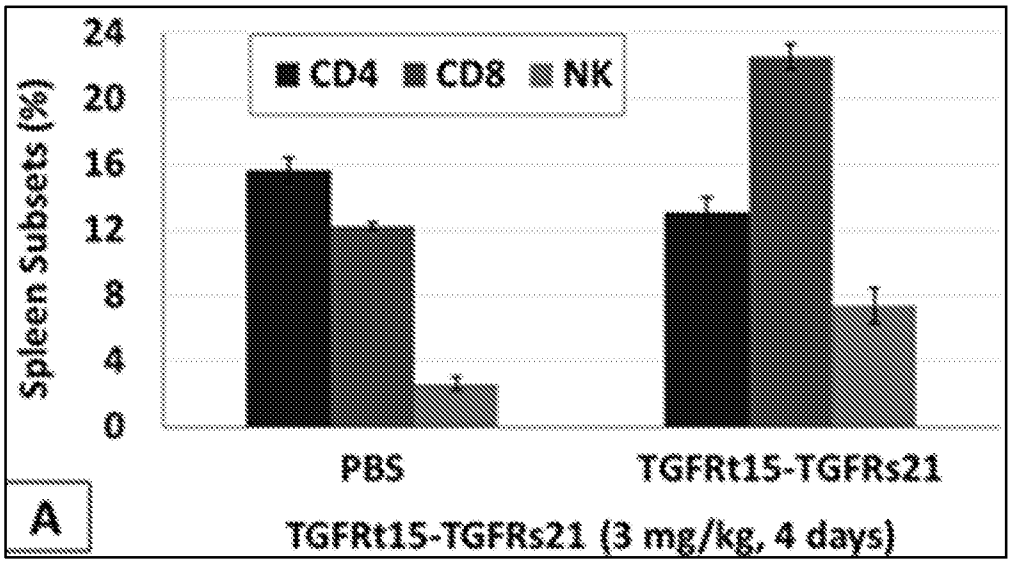


Figure 137A

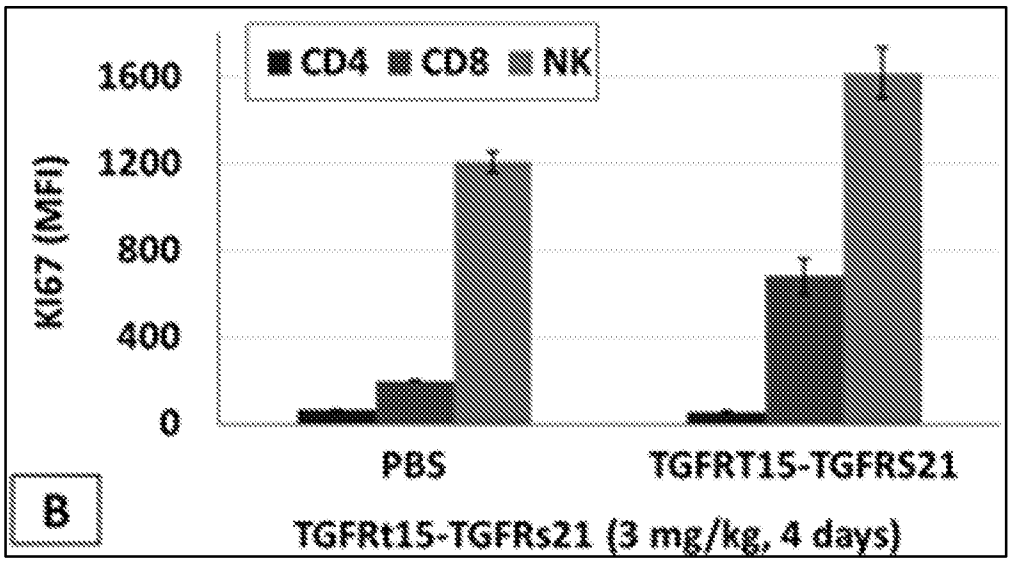


Figure 137B

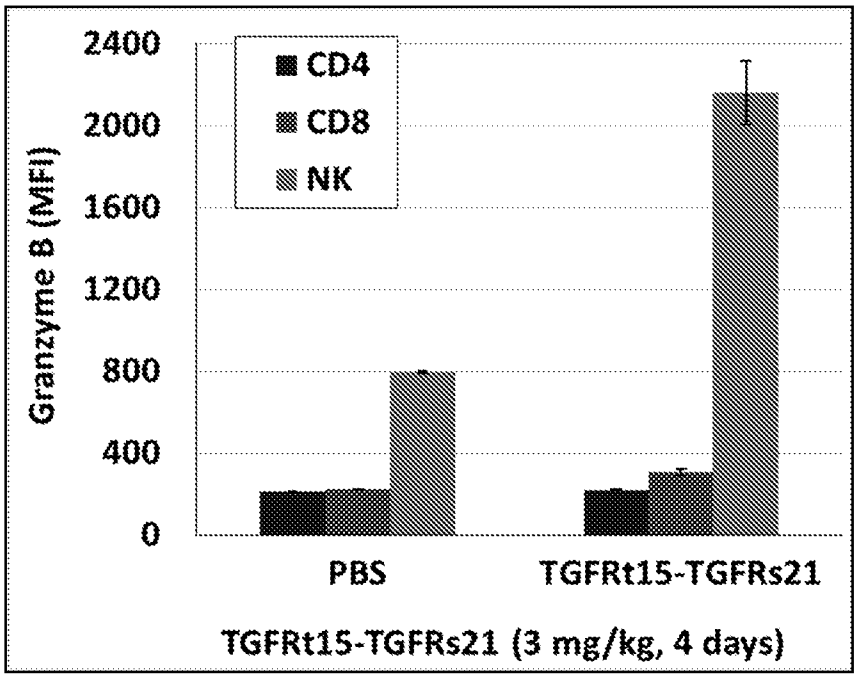


Figure 138

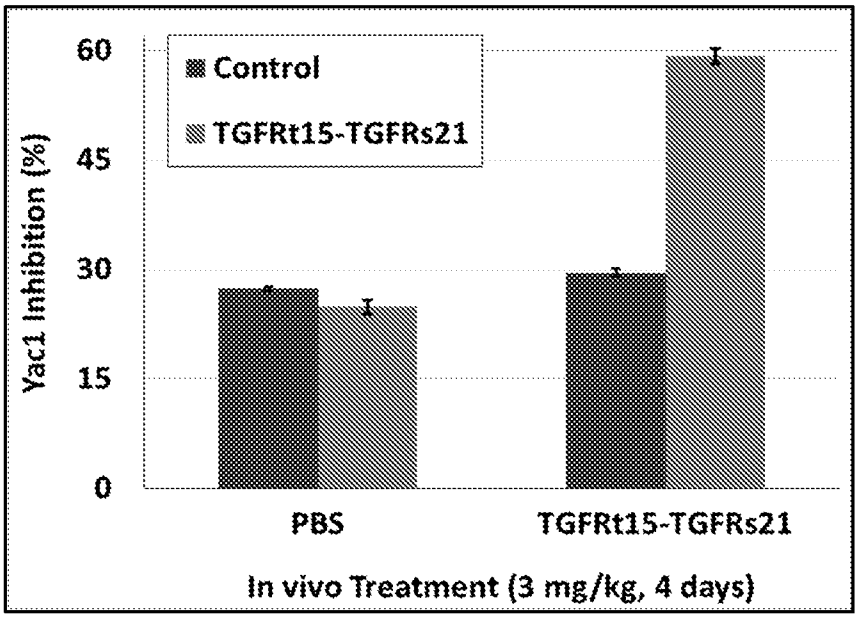


Figure 139

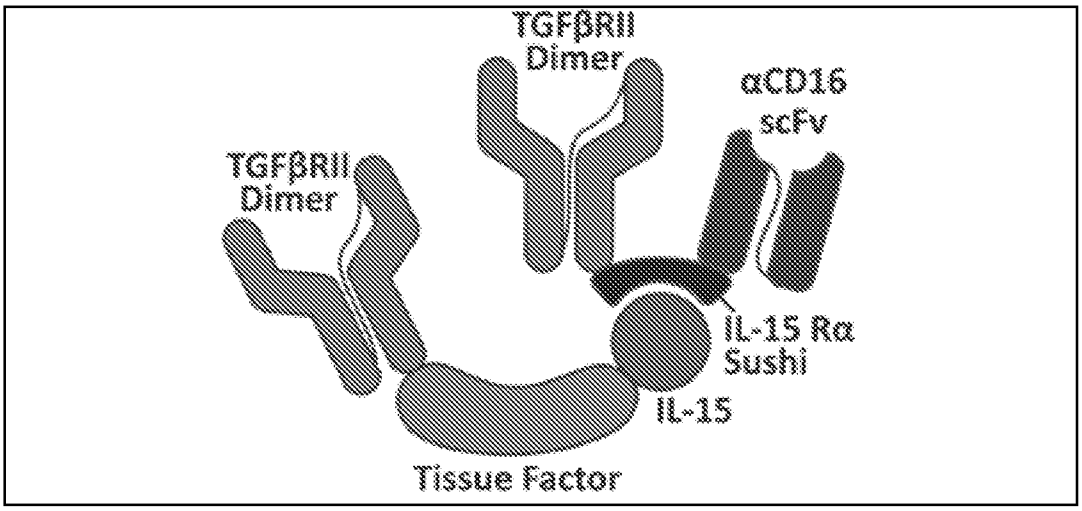


Figure 140

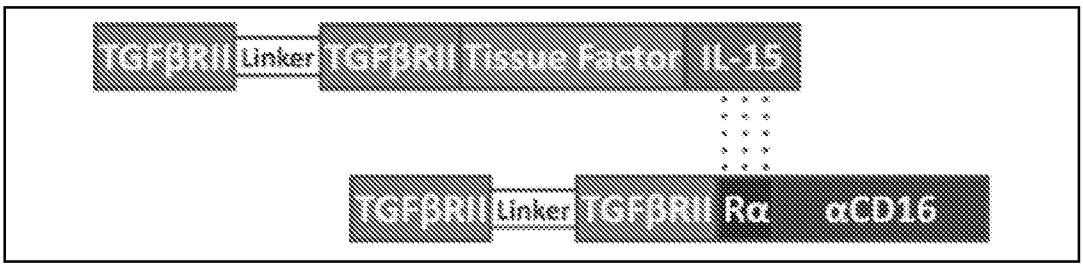


Figure 141

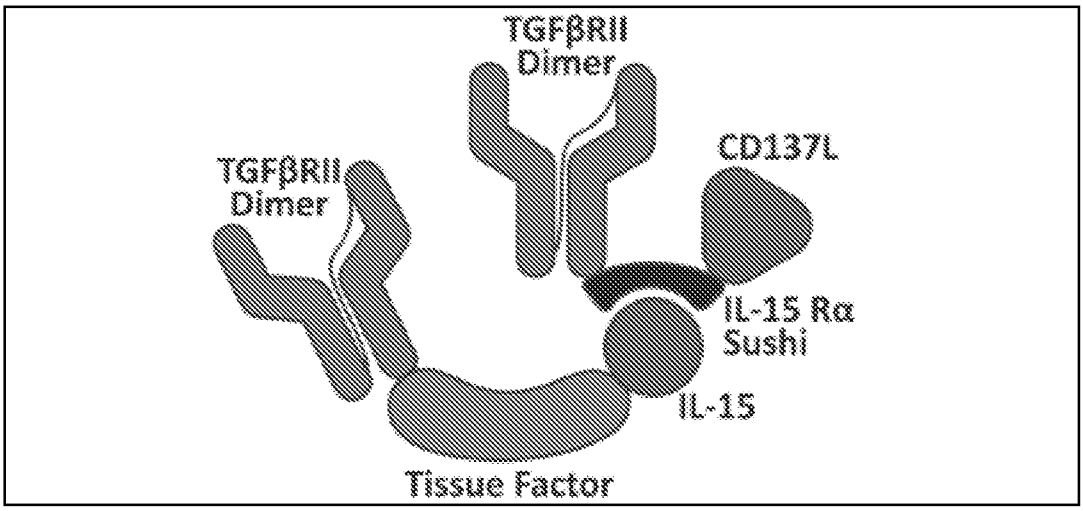


Figure 142

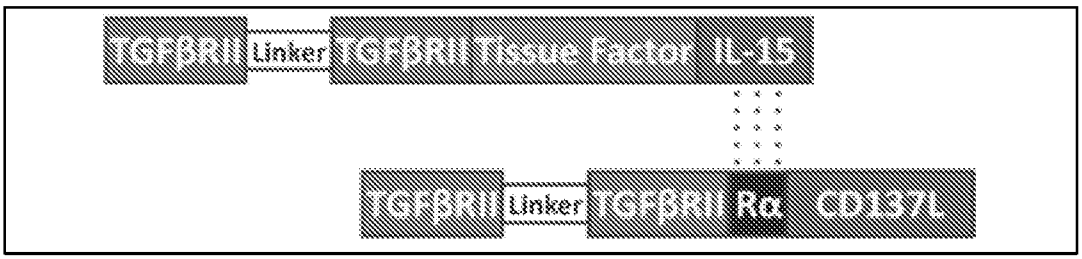


Figure 143

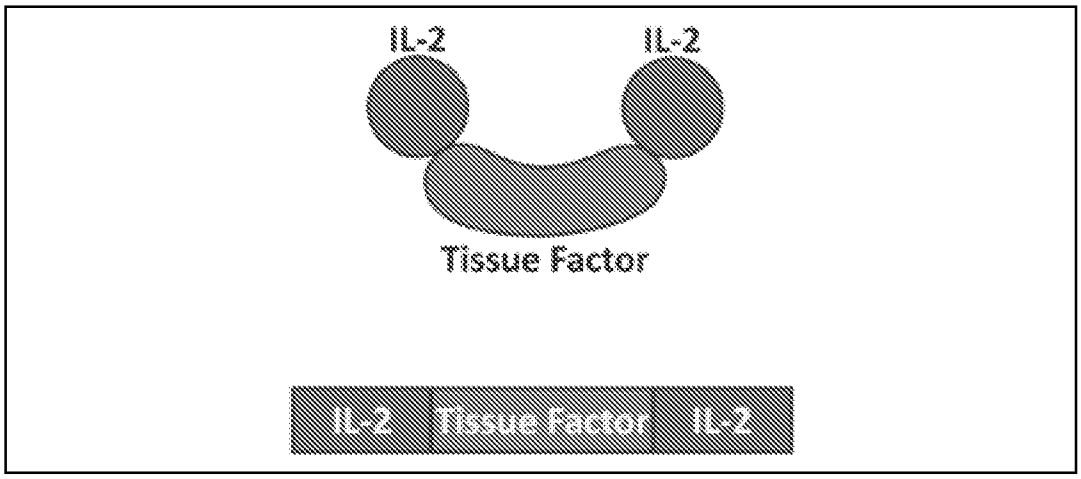


Figure 144

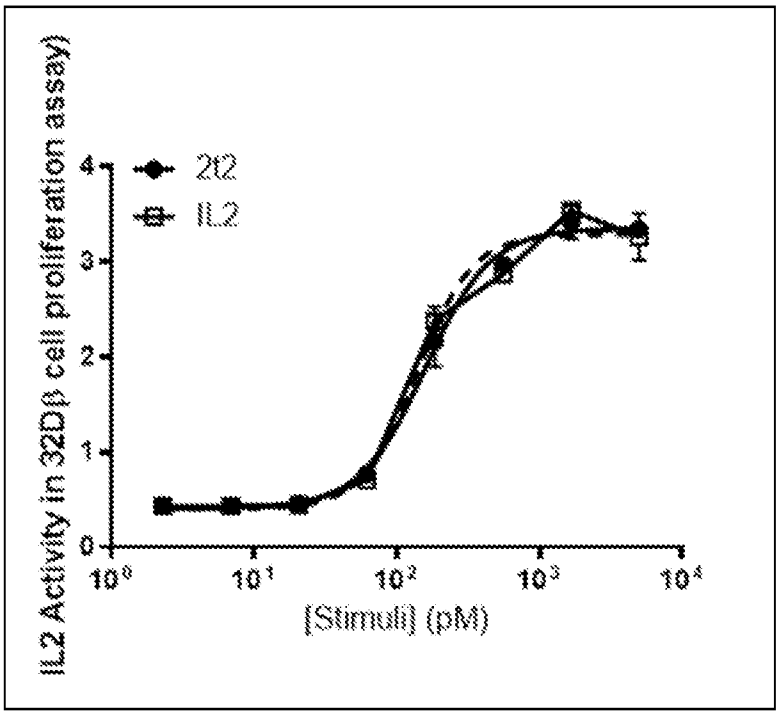


Figure 145

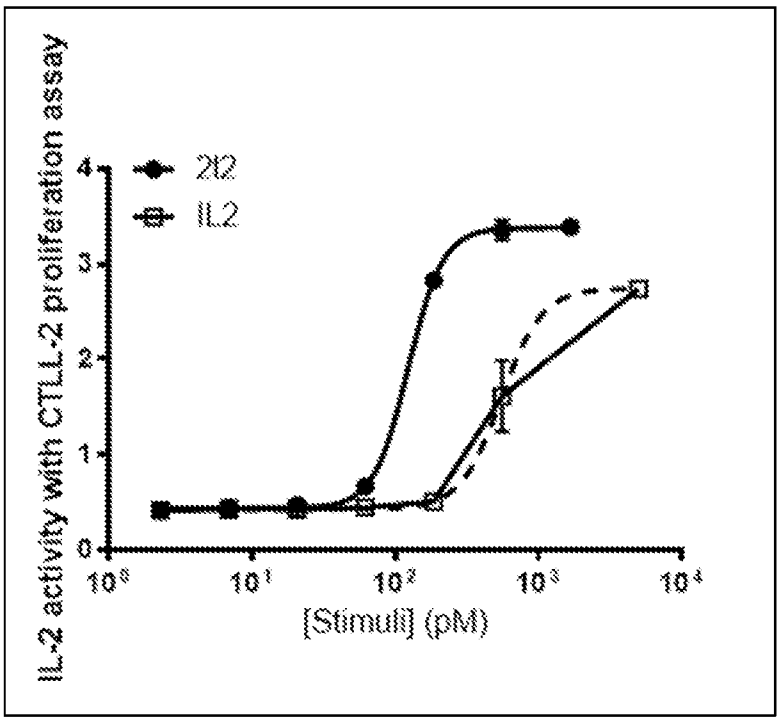


Figure 146

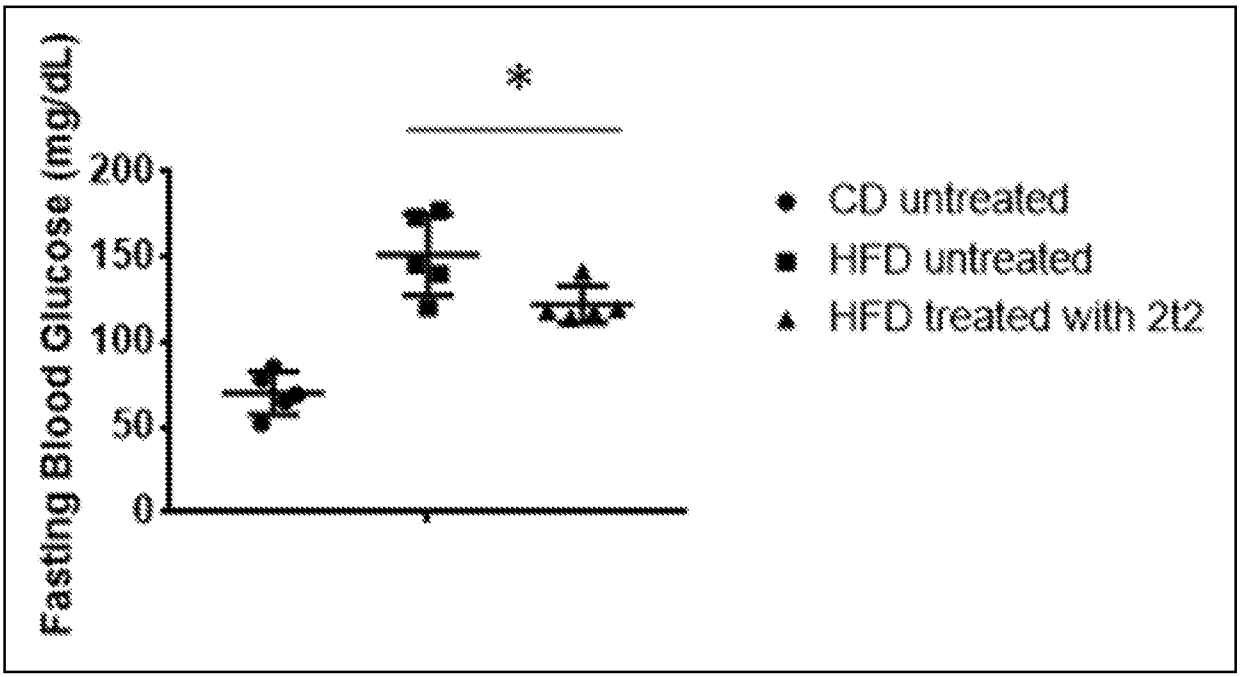


Figure 147

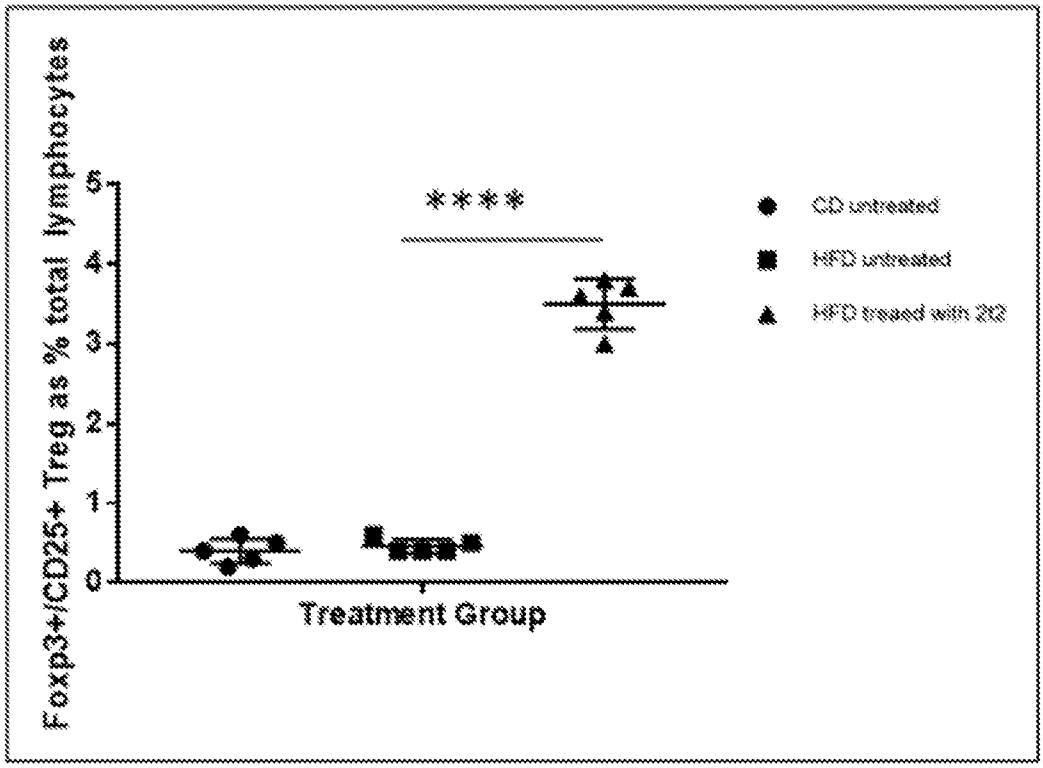


Figure 148

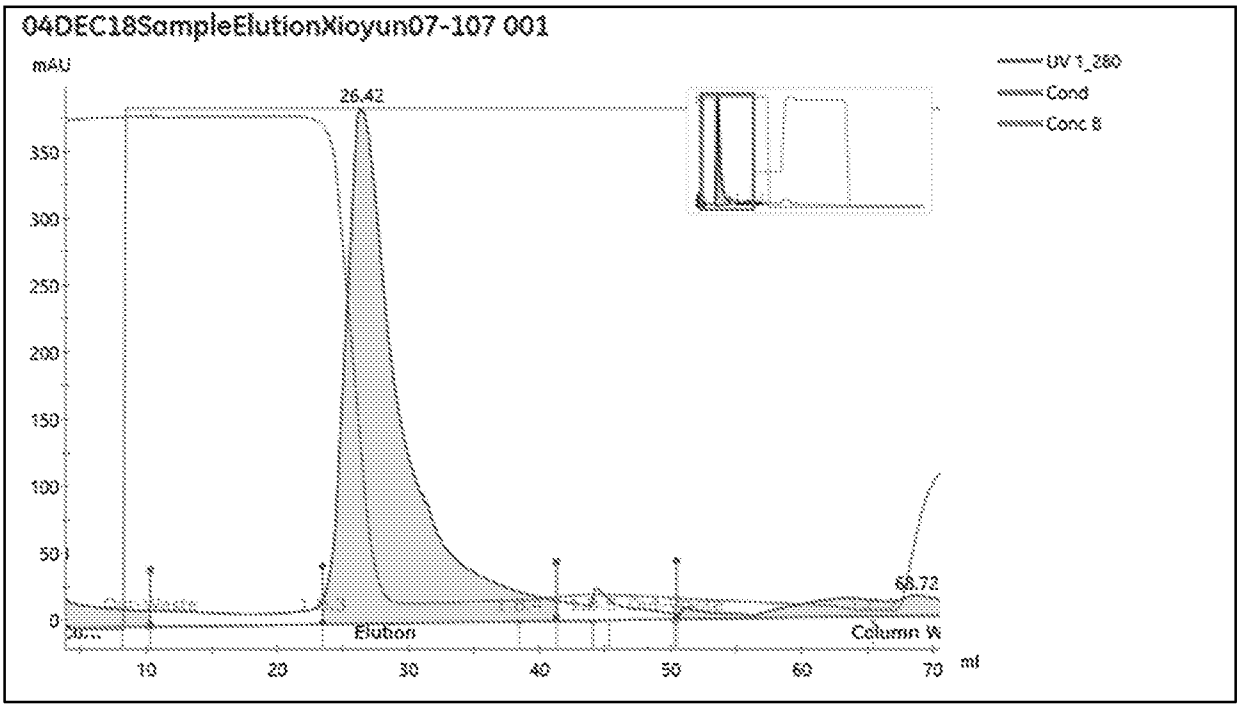


Figure 149

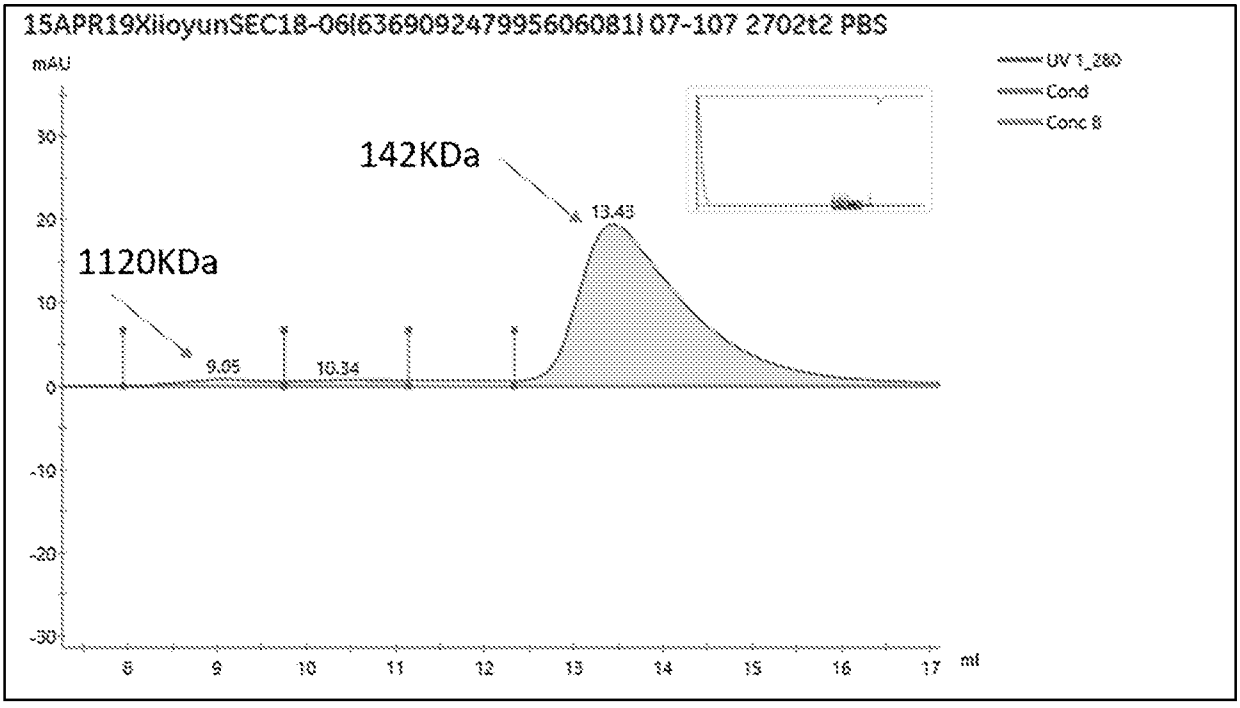


Figure 150

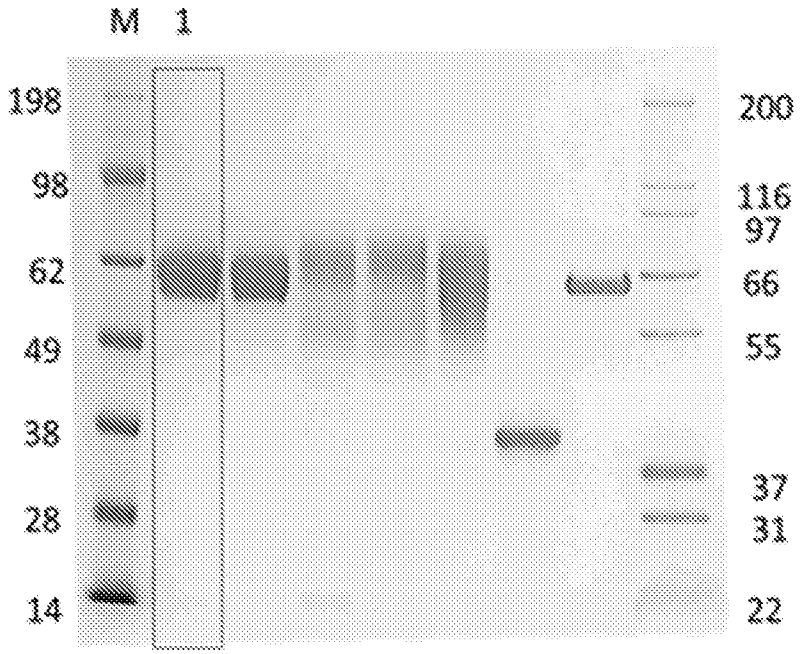


Figure 151A

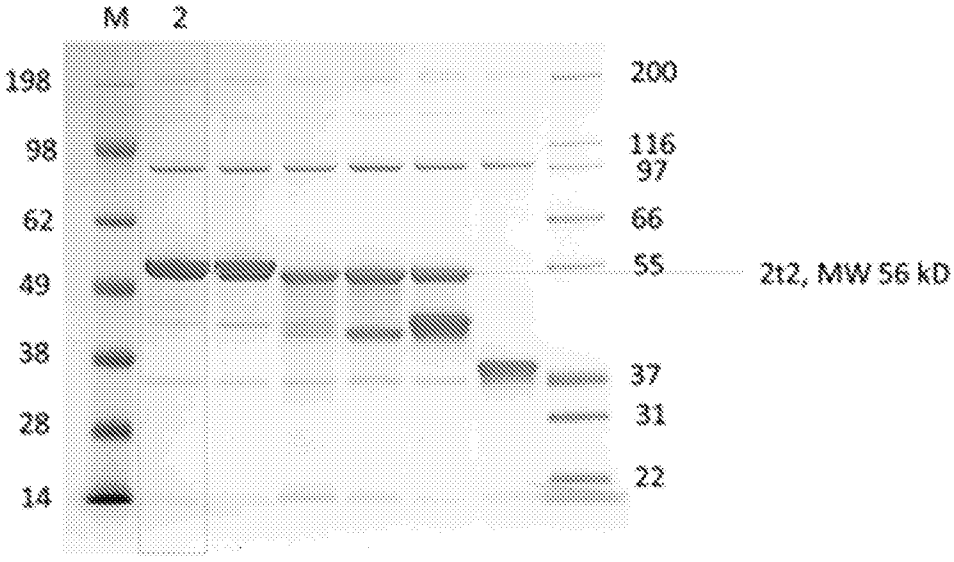


Figure 151B

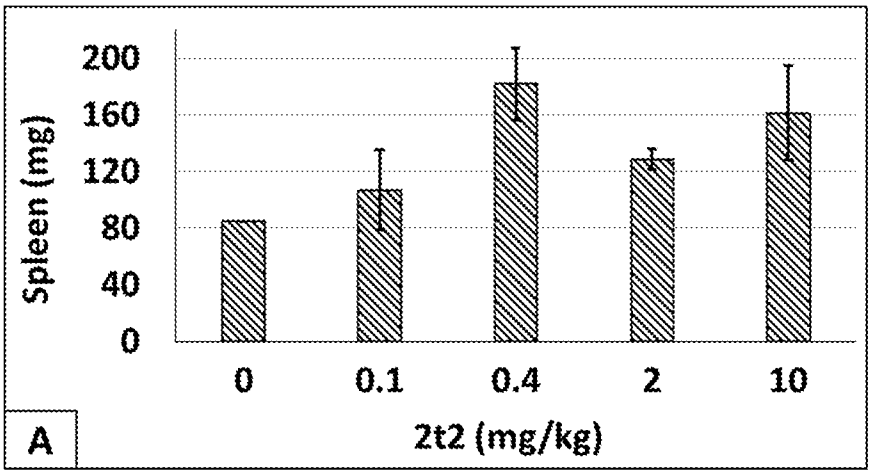


Figure 152A

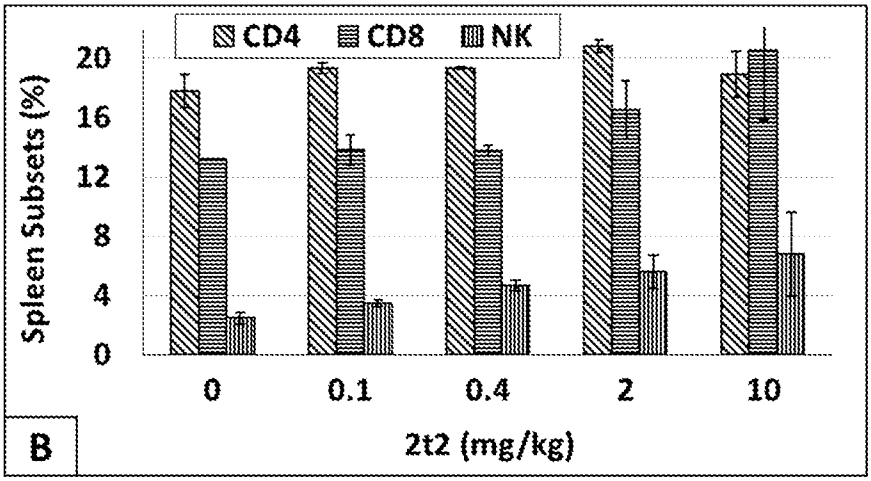


Figure 152B

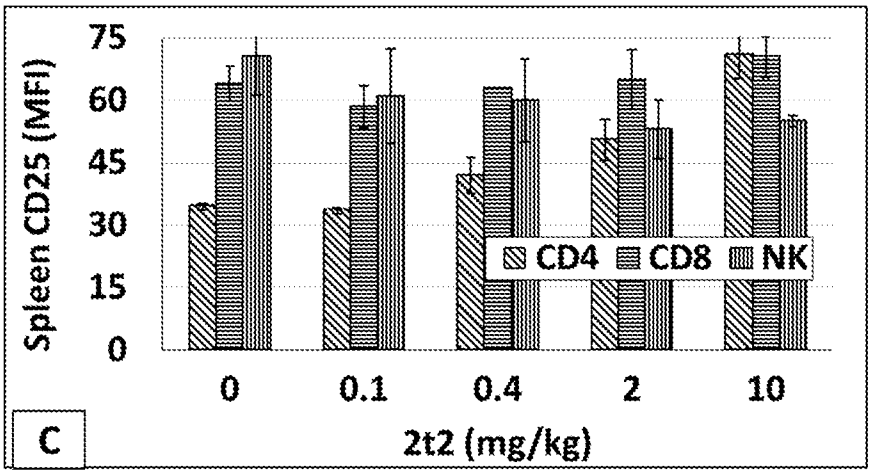


Figure 153

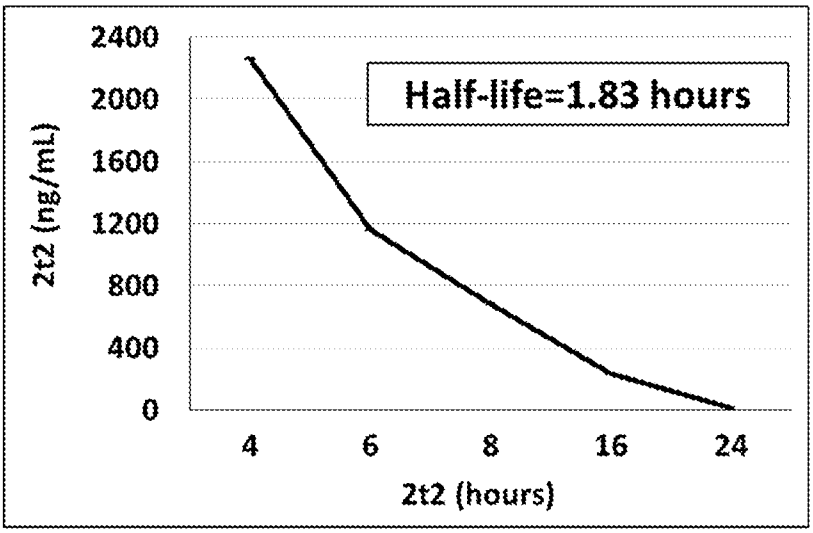


Figure 154

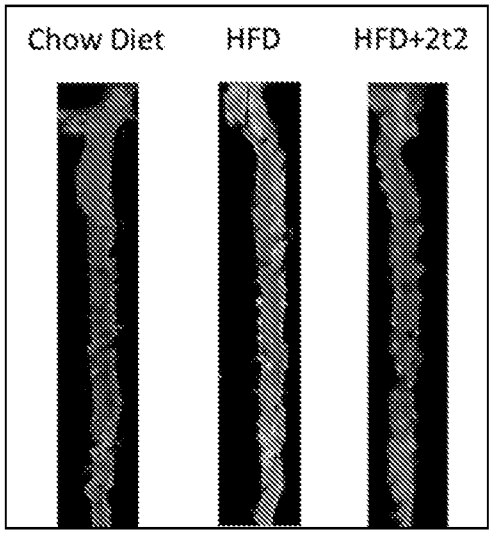


Figure 155A

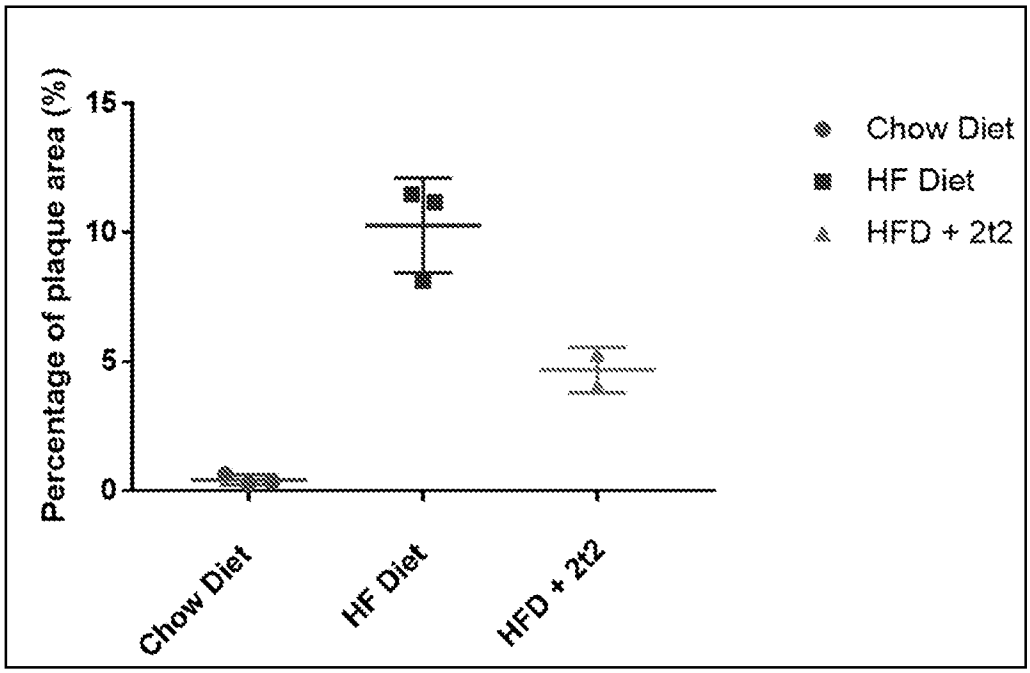


Figure 155B

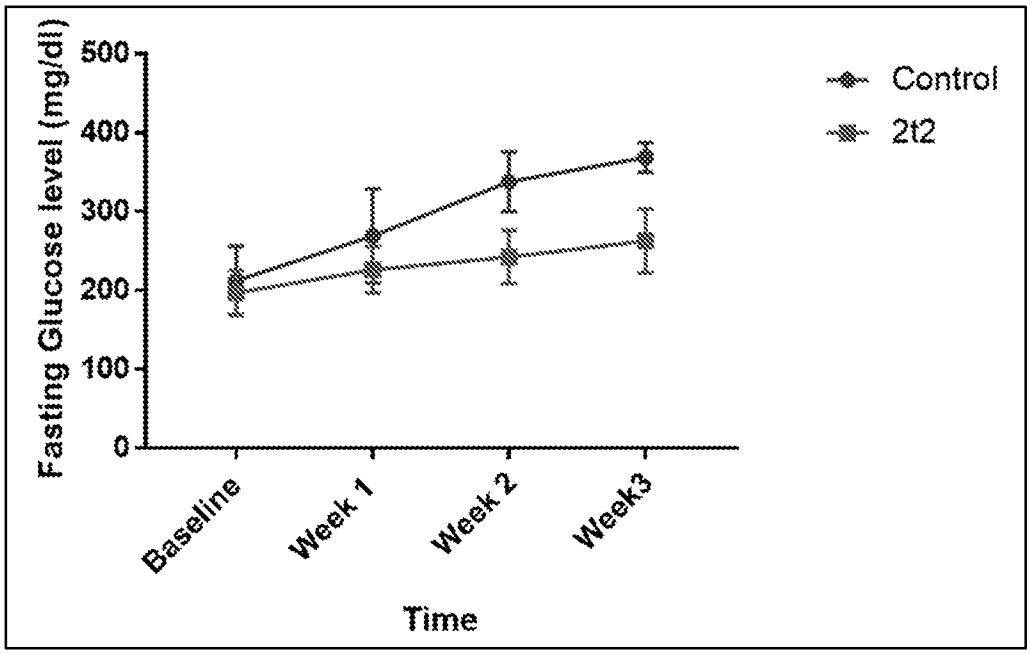


Figure 156

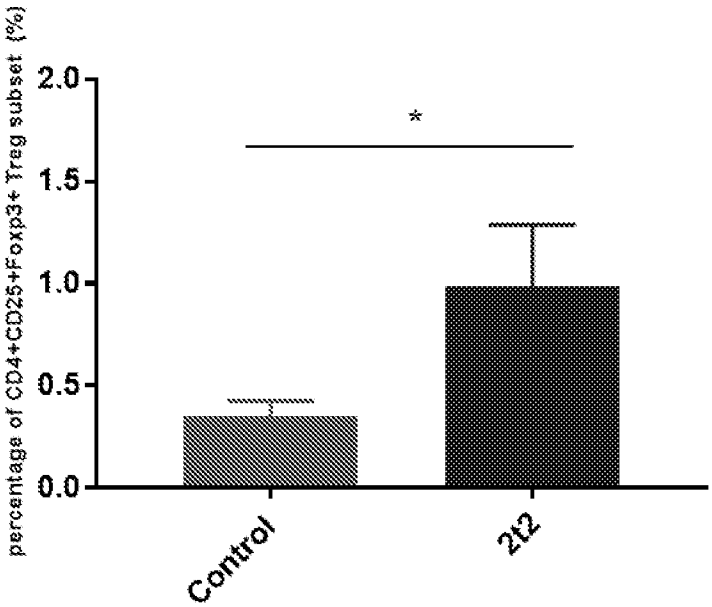


Figure 157

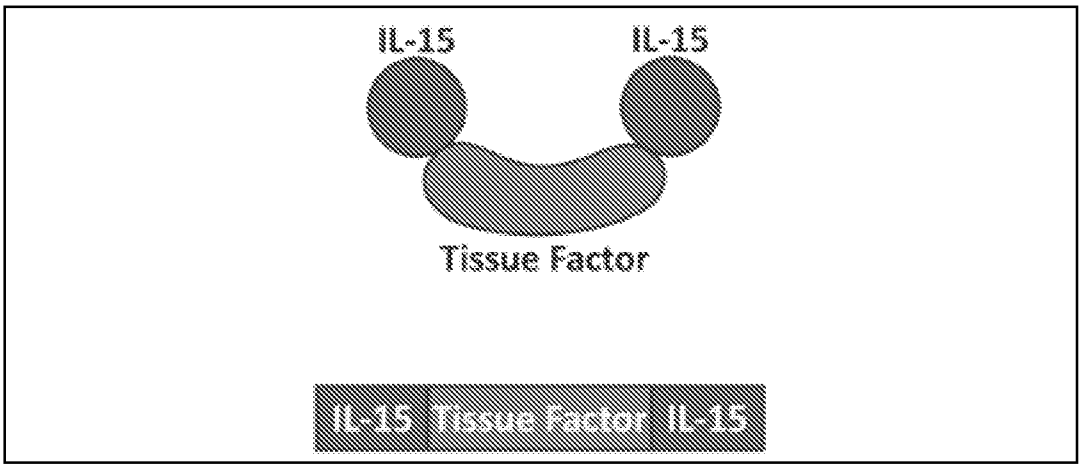


Figure 158

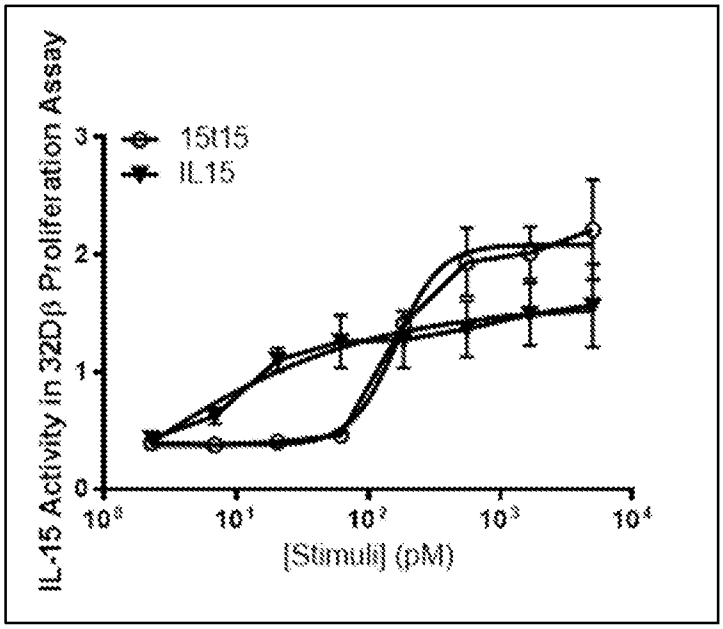

IL-15 Activity in 32Dβ Proliferation Assay
15i15
IL15
3
2
1
0
10⁰
10¹
10²
10³
10⁴
[Stimuli] (pM)

Figure 159

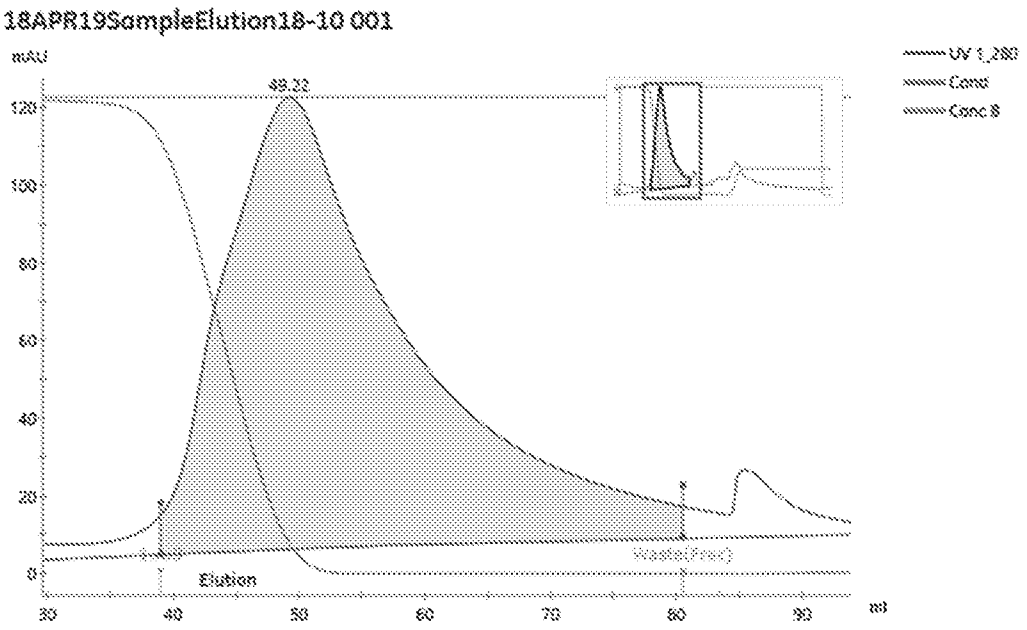

18APR19SampleElution18-10 001
mAU
UV 1_280
Cond
Conc B
Elution
Waste(Frac)
ml

Figure 160

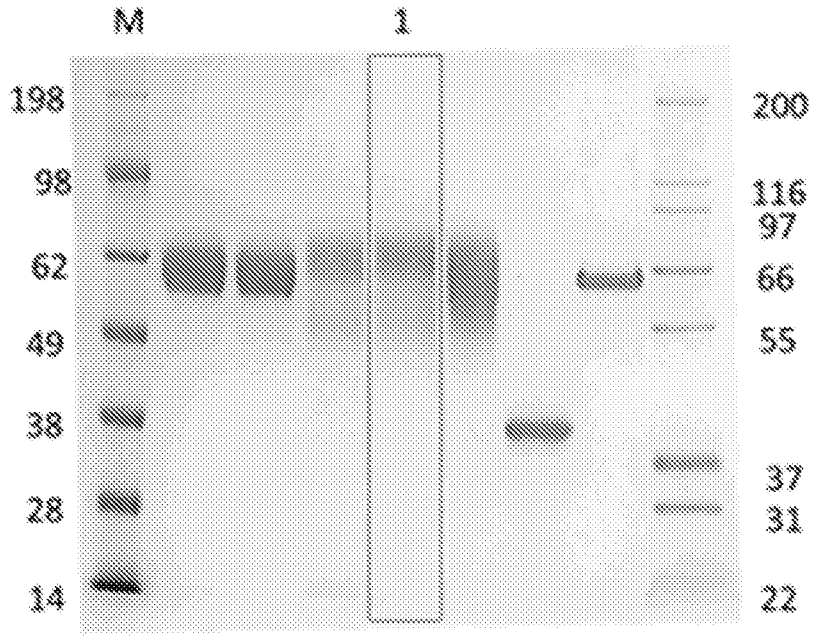


Figure 161A

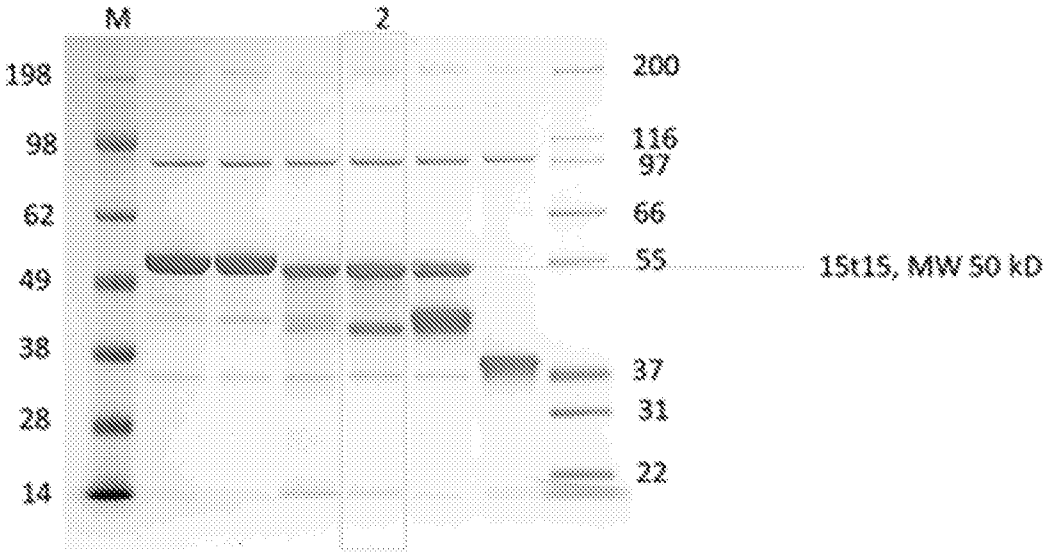
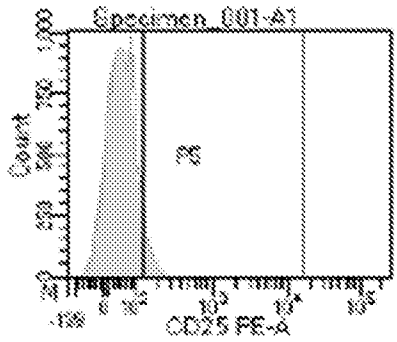
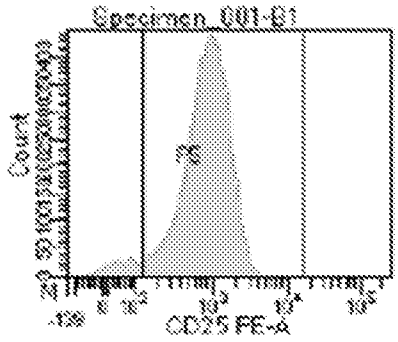


Figure 161B

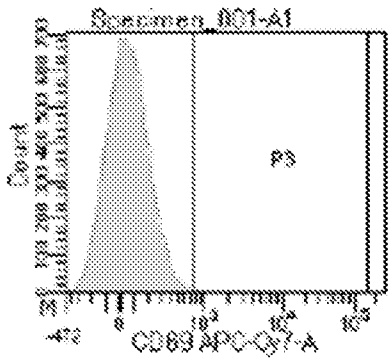
Before Stimulation



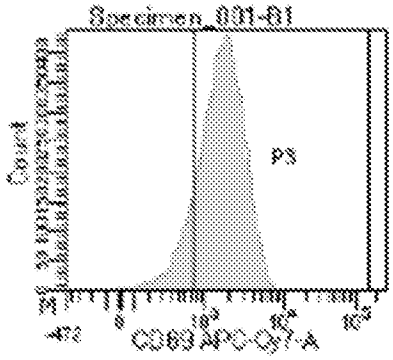
After Overnight Stimulation with 100nM 18t15-12s



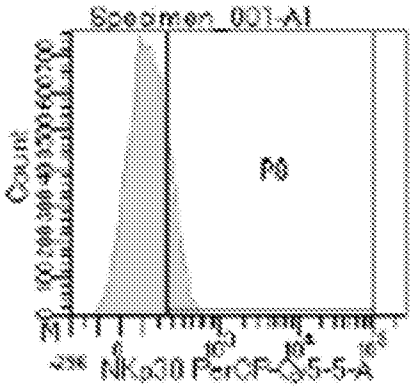
Before Stimulation



After Overnight Stimulation with 100nM 18t15-12s



Before Stimulation



After Overnight Stimulation with 100nM 18t15-12s

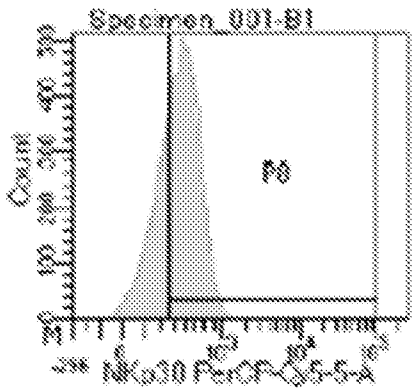


Figure 162A

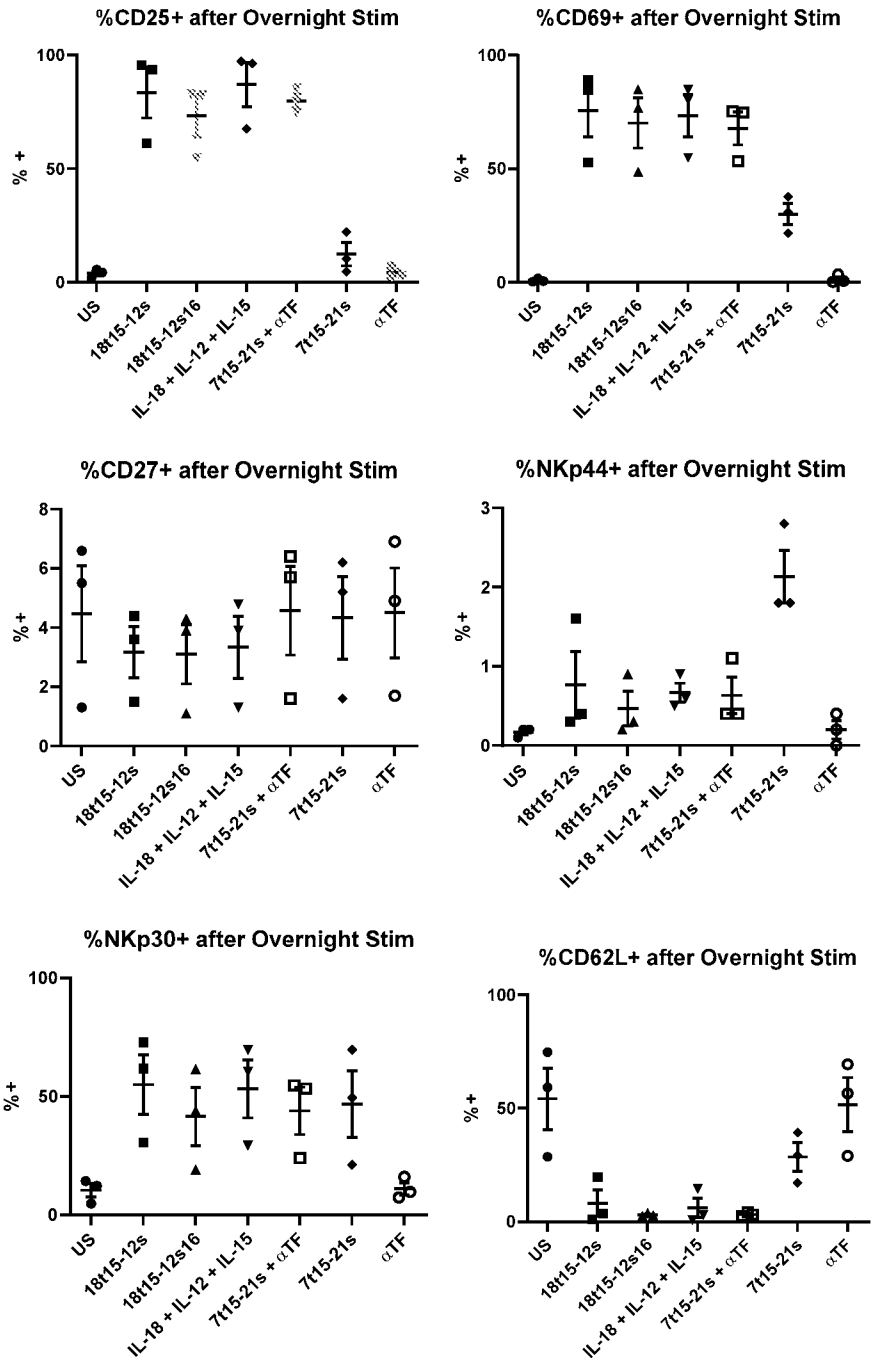

%CD25+ after Overnight Stim
%CD69+ after Overnight Stim
%CD27+ after Overnight Stim
%NKp44+ after Overnight Stim
%NKp30+ after Overnight Stim
%CD62L+ after Overnight Stim
% +
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF

Figure 162B

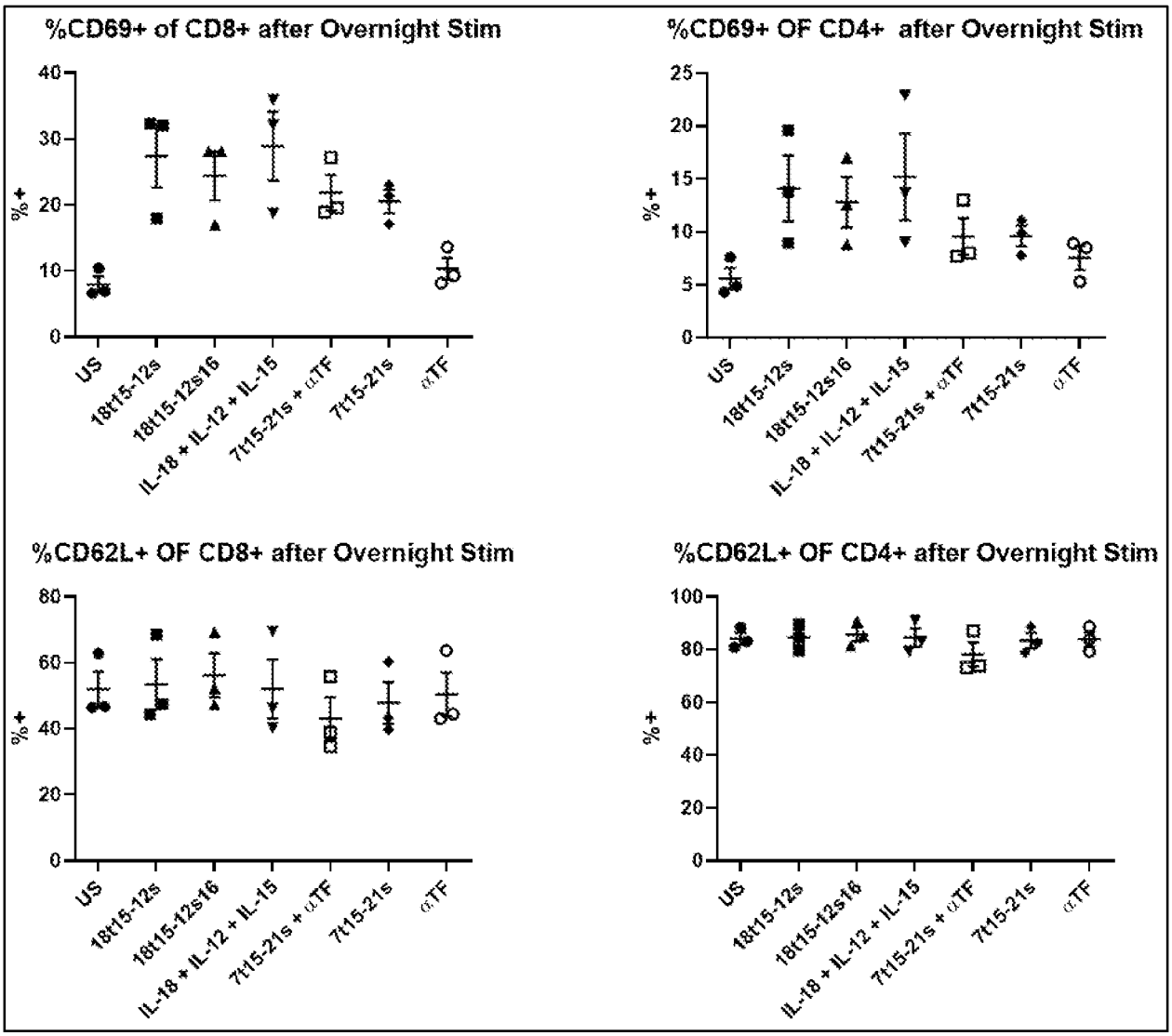

%CD69+ of CD8+ after Overnight Stim
%+
40
30
20
10
0
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF
%CD69+ OF CD4+ after Overnight Stim
%+
25
20
15
10
5
0
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF
%CD62L+ OF CD8+ after Overnight Stim
%+
80
60
40
20
0
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF
%CD62L+ OF CD4+ after Overnight Stim
%+
100
80
60
40
20
0
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF

Figure 163

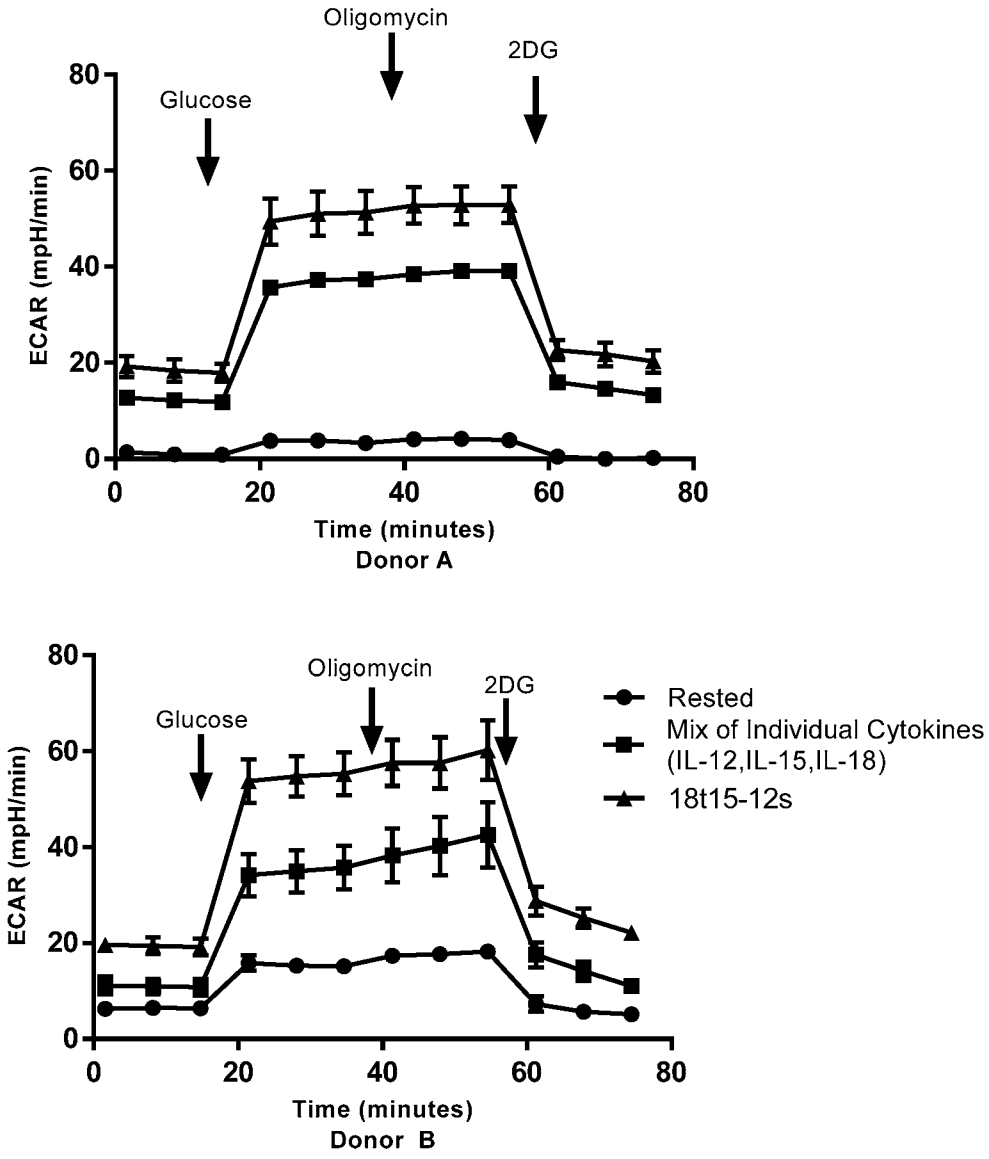

Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Donor A
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Donor B
Rested
Mix of Individual Cytokines
(IL-12,IL-15,IL-18)
18t15-12s

Figure 164

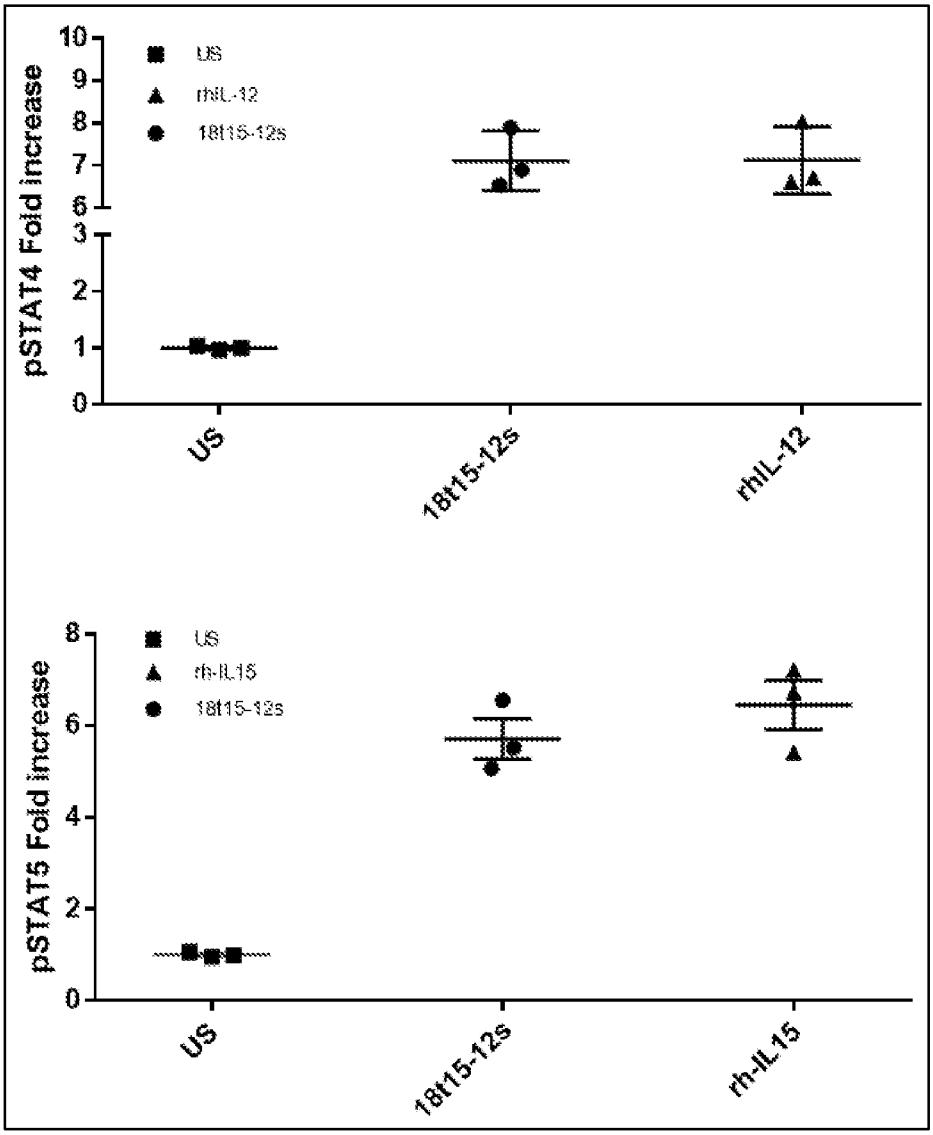

pSTAT4 Foid increase
US
rhIL-12
18t15-12s
pSTAT5 Foid increase
US
rh-IL15
18t15-12s
US
18t15-12s
rhIL-12
US
18t15-12s
rh-IL15

Figure 165

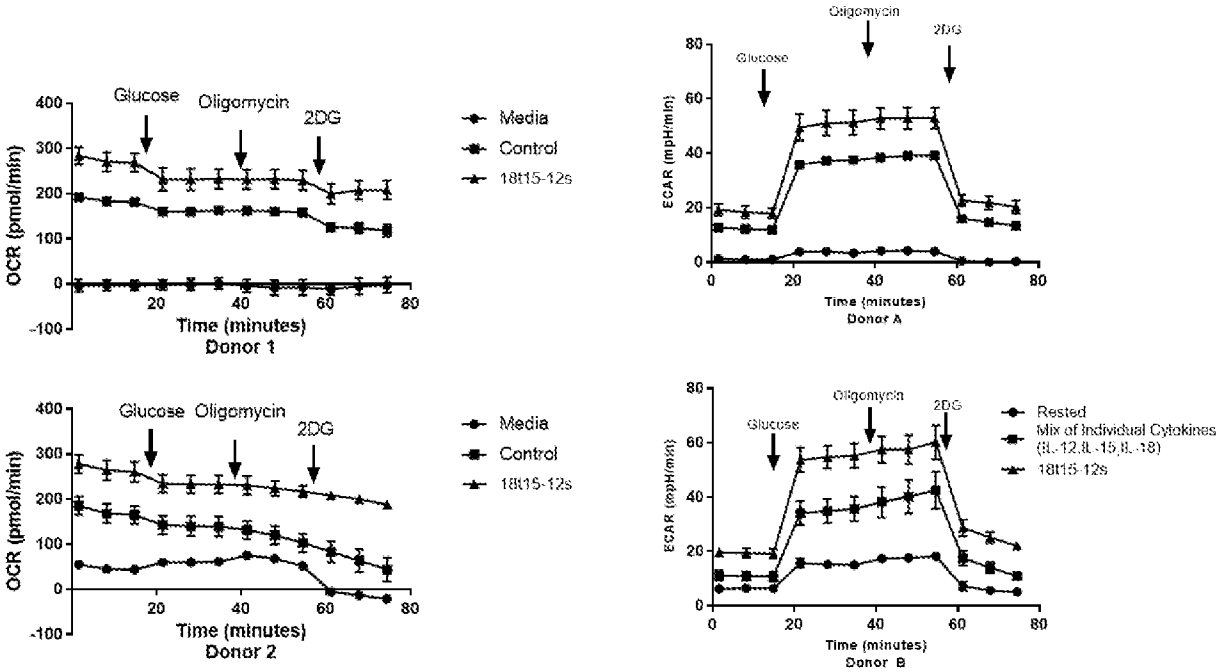

Glucose
Oligomycin
2DG
OCR (pmol/min)
Time (minutes)
Donor 1
Media
Control
18t15-12s
ECAR (mpH/min)
Donor A
Donor 2
Donor B
Rested
Mix of Individual Cytokines
(IL-12,IL-15,IL-18)

Figure 166

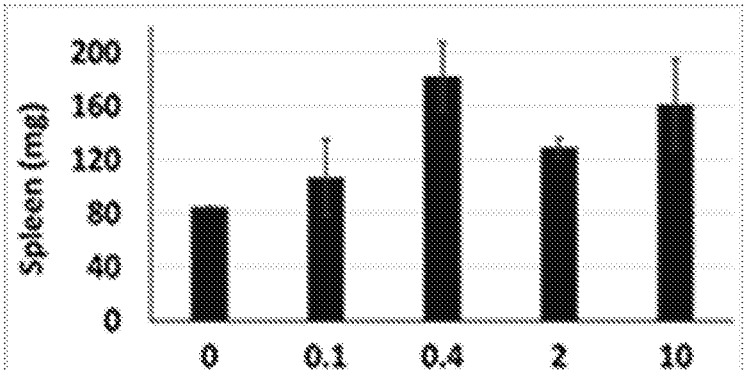


Figure 167A

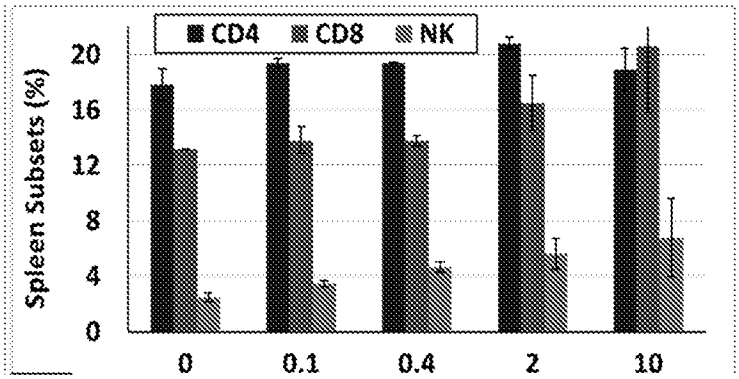


Figure 167B

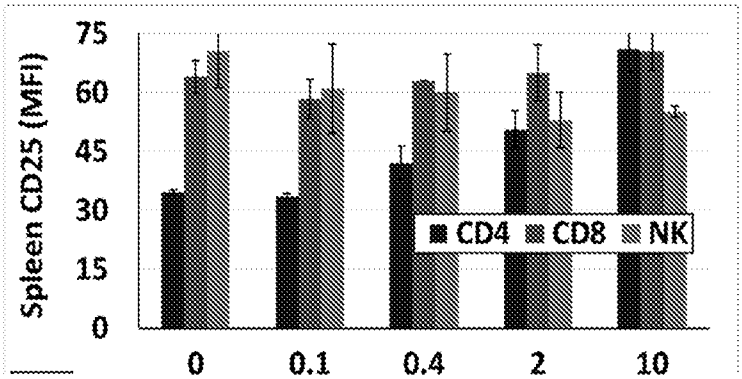


Figure 167C

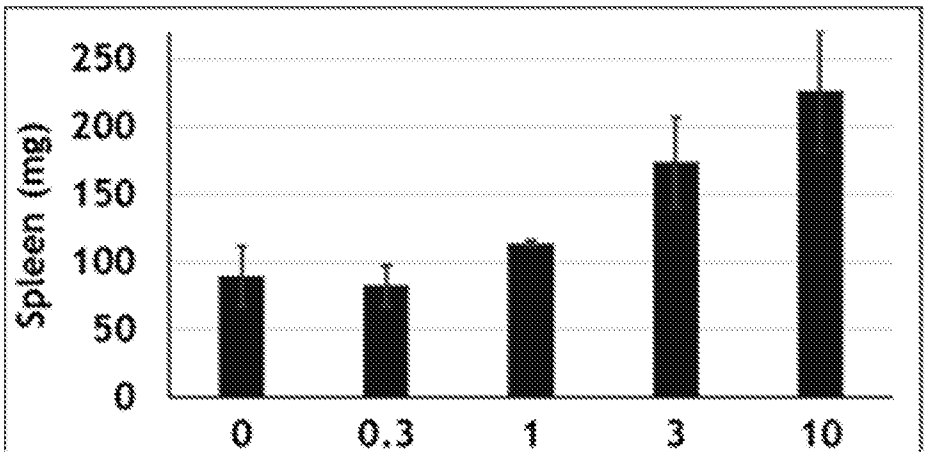


Figure 168A

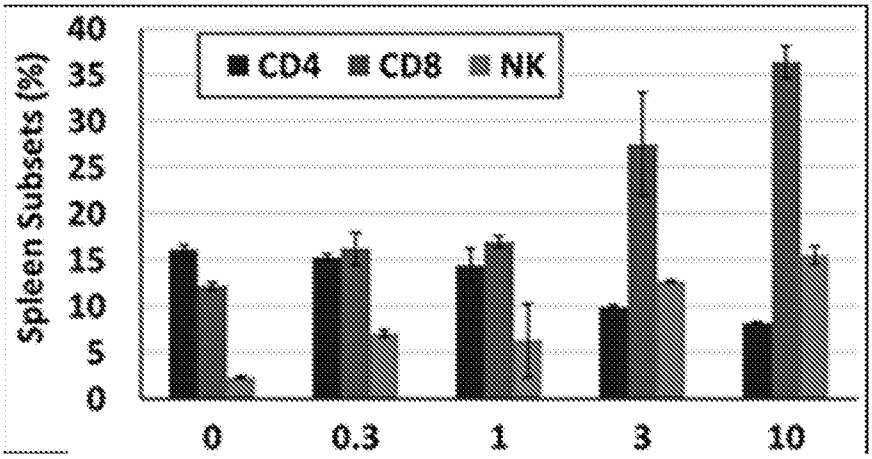


Figure 168B

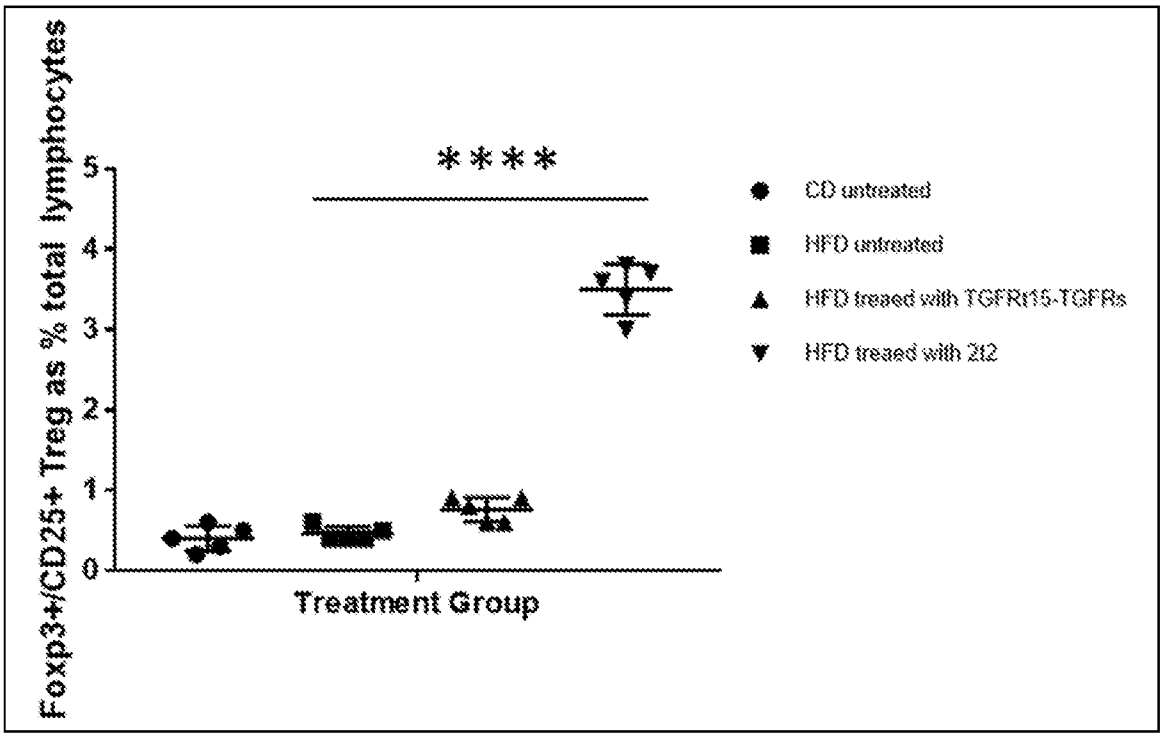


Figure 169A

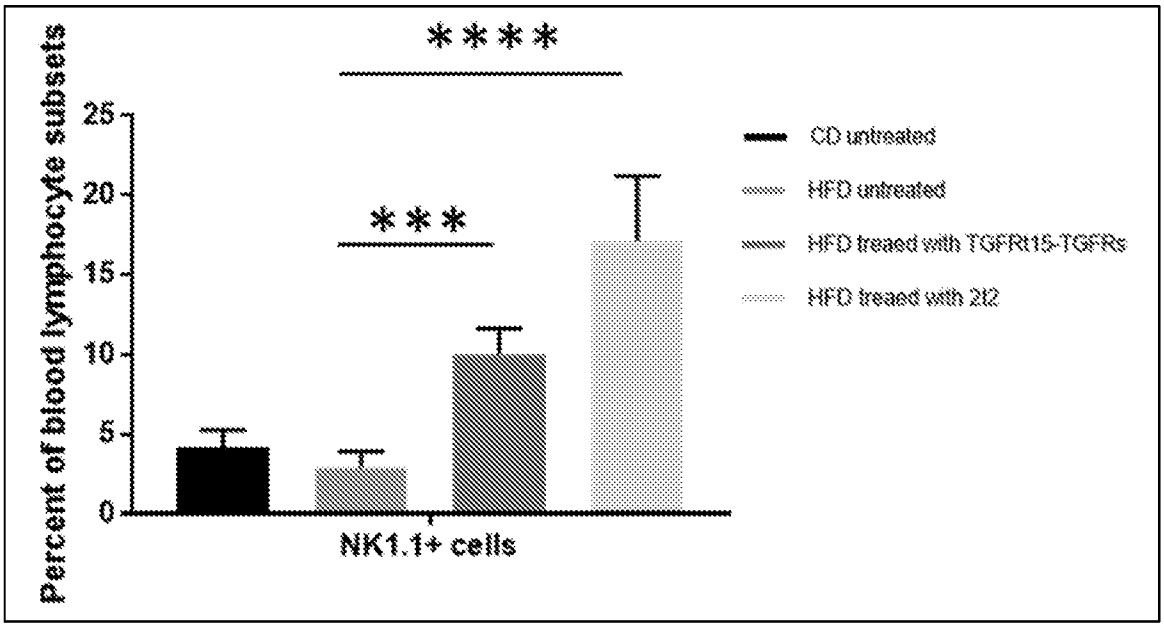


Figure 169B

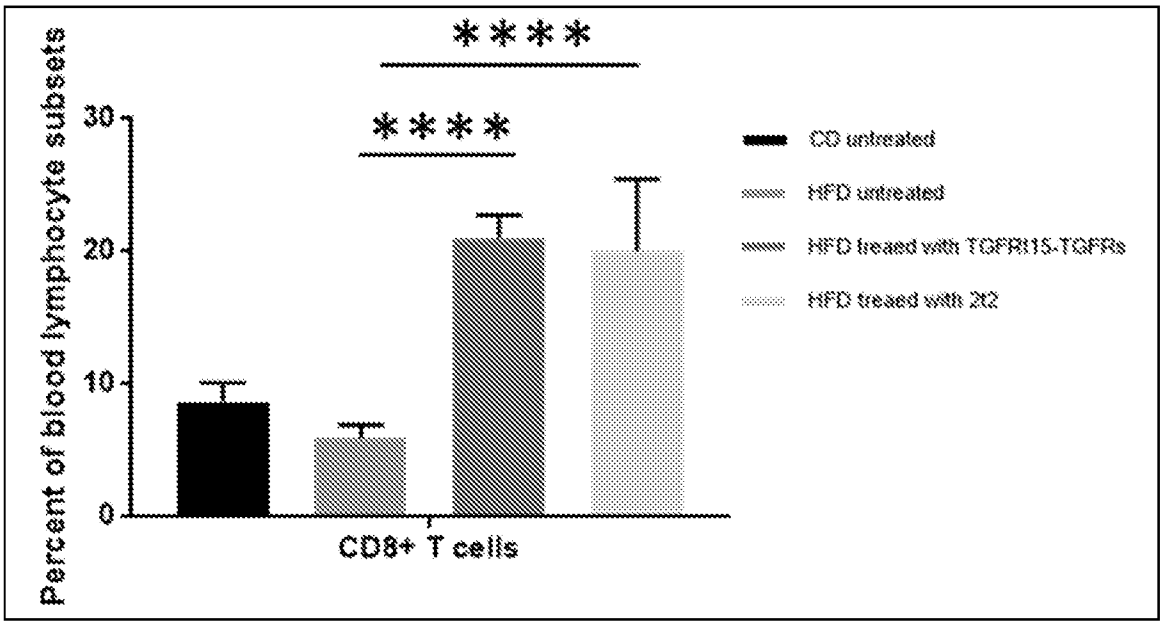

****
****
Percent of blood lymphocyte subsets
30
20
10
0
CD8+ T cells
CD untreated
HFD untreated
HFD treaed with TGFBR1S-TGFBRs
HFD treaed with 2t2

Figure 169C

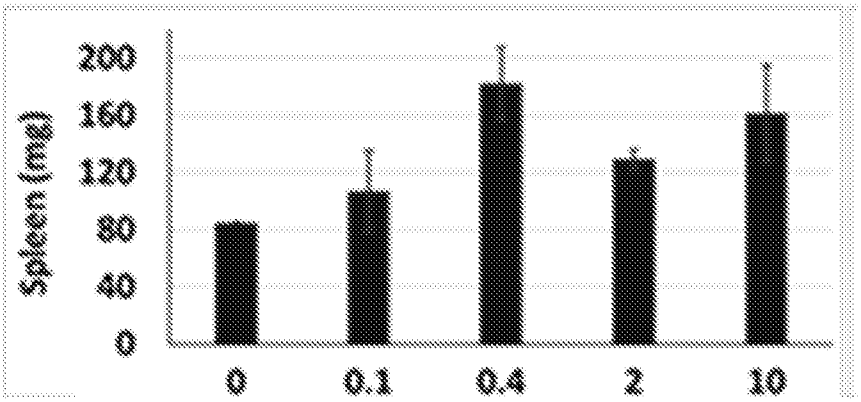


Figure 170A

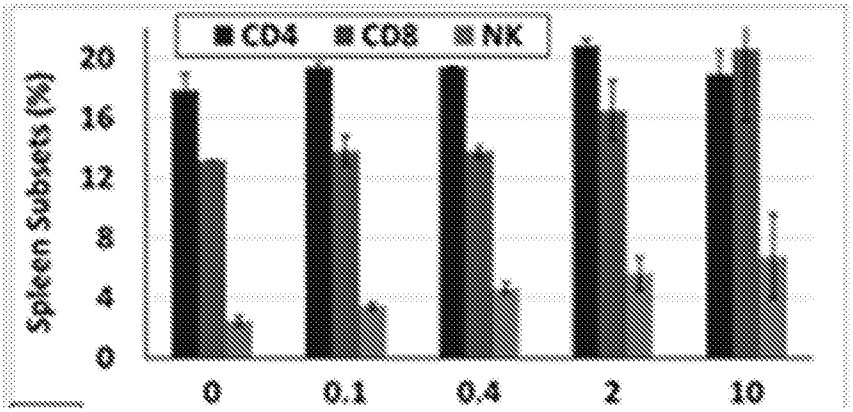


Figure 170B

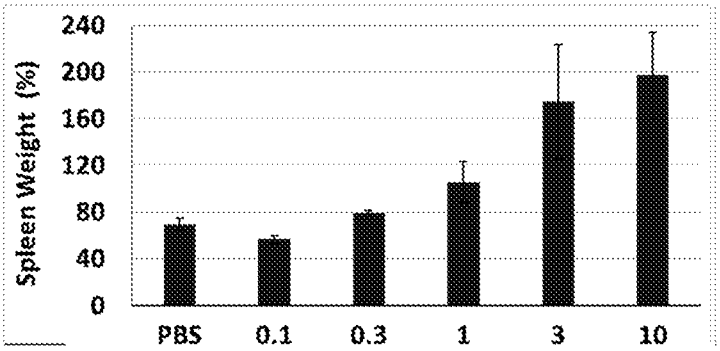


Figure 171A

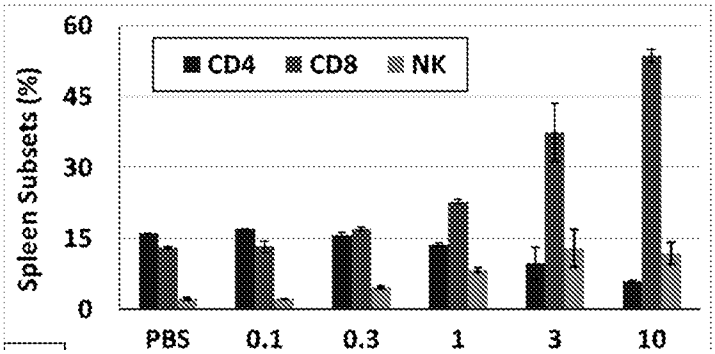


Figure 171B

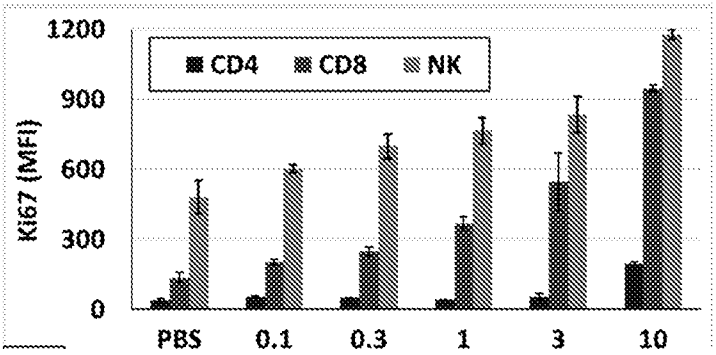


Figure 171C

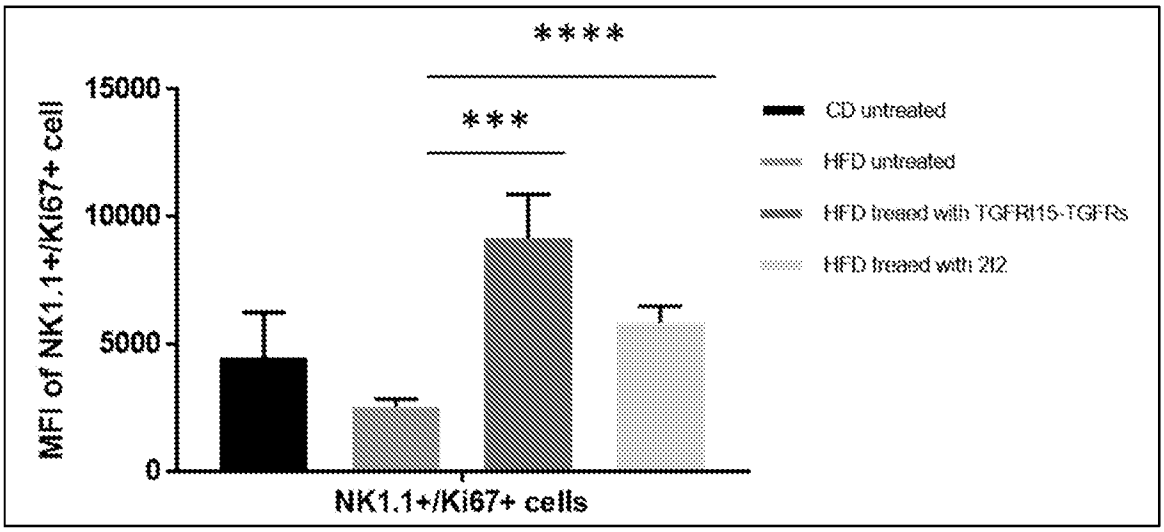


Figure 172A

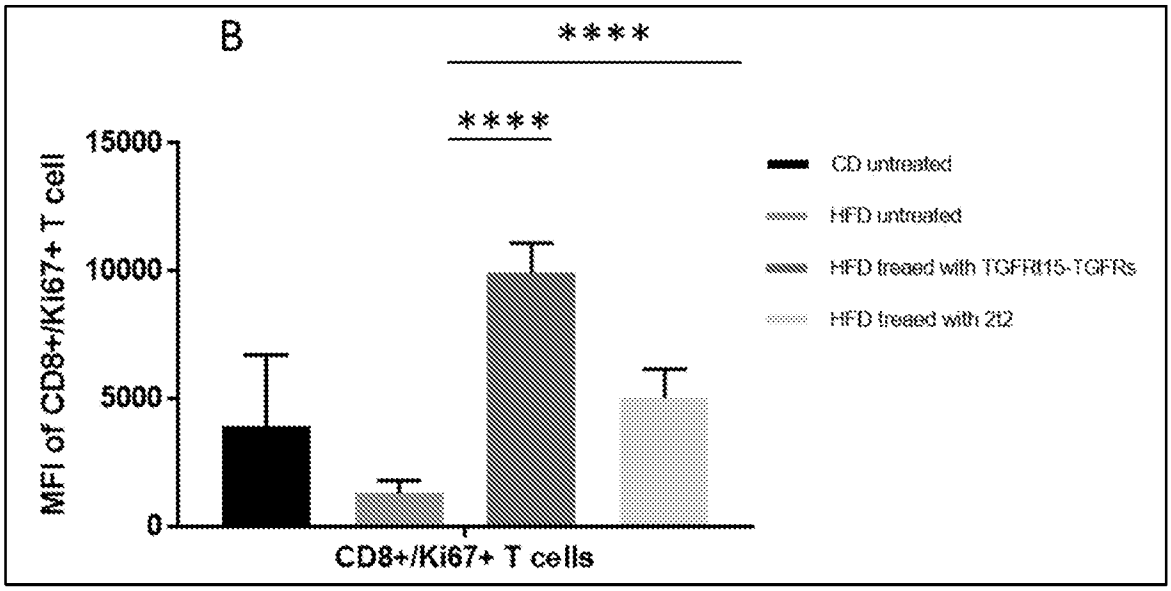
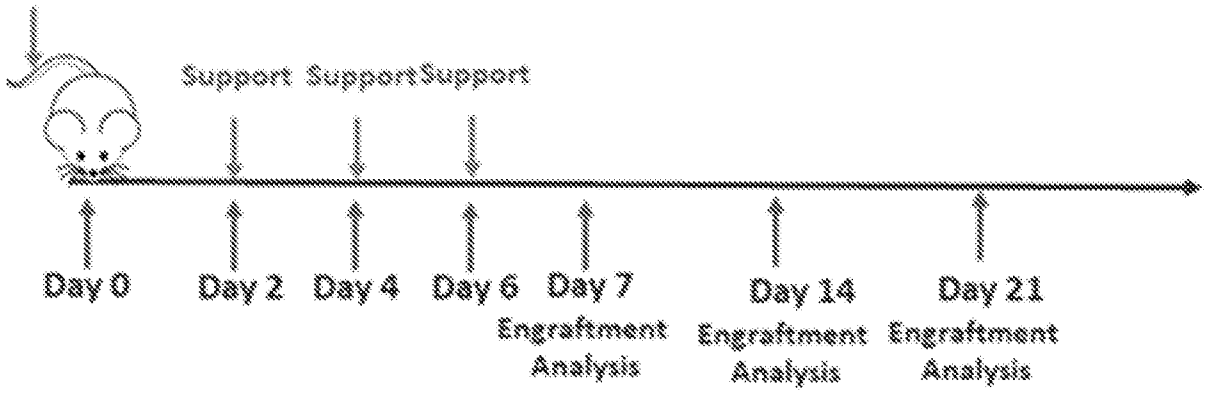


Figure 172B


Support
Support
Support
Day 0
Day 2
Day 4
Day 6
Day 7
Engraftment Analysis
Day 14
Engraftment Analysis
Day 21
Engraftment Analysis

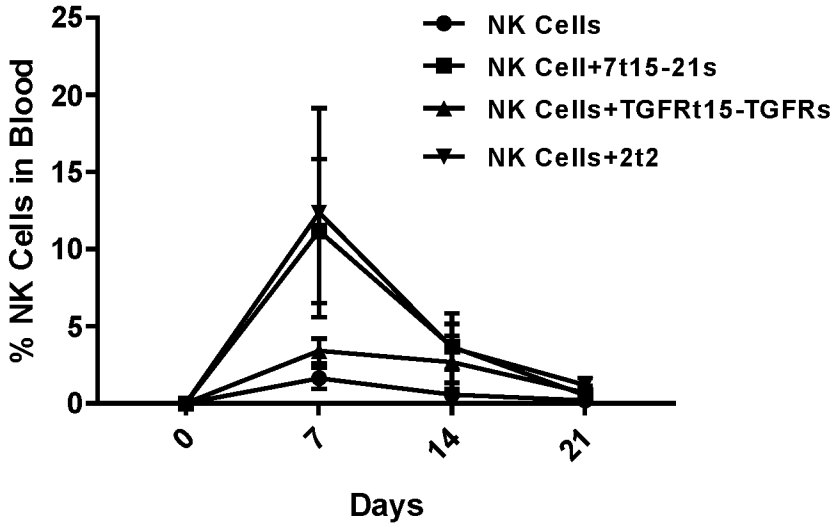

NK Cells
NK Cell+7t15-21s
NK Cells+TGFRt15-TGFRs
NK Cells+2t2
% NK Cells in Blood
25
20
15
10
5
0
0
7
14
21
Days

Figure 173

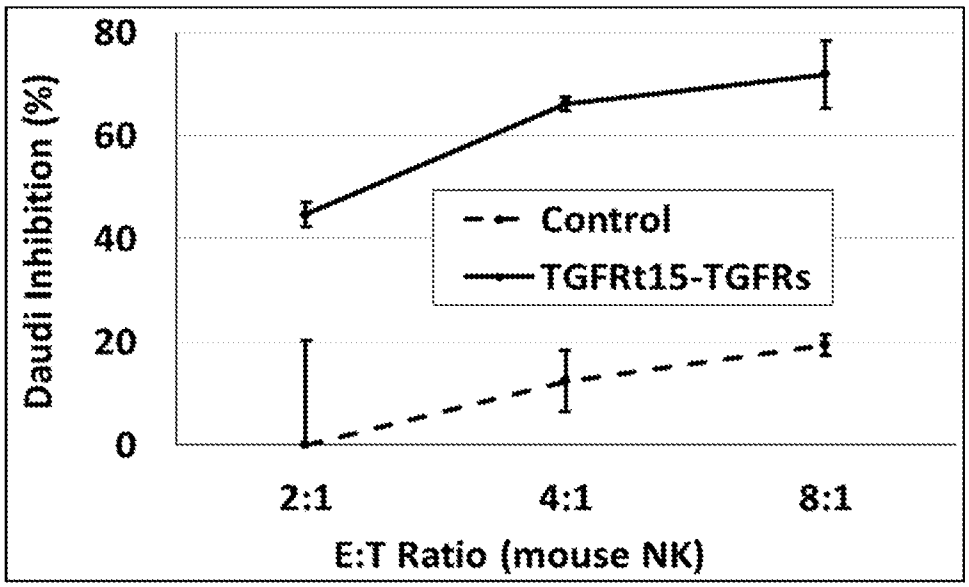


Figure 174A

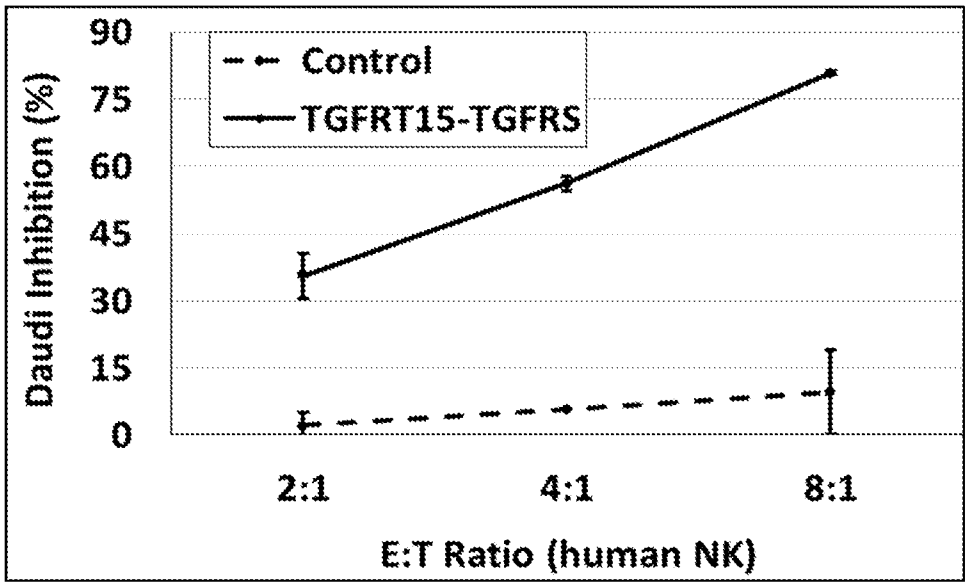


Figure 174B

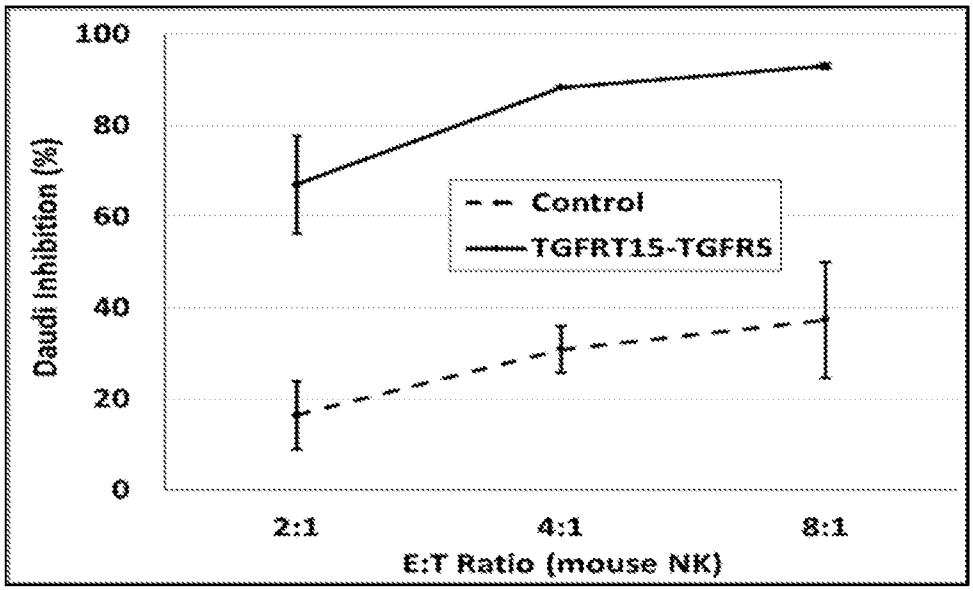


Figure 175A

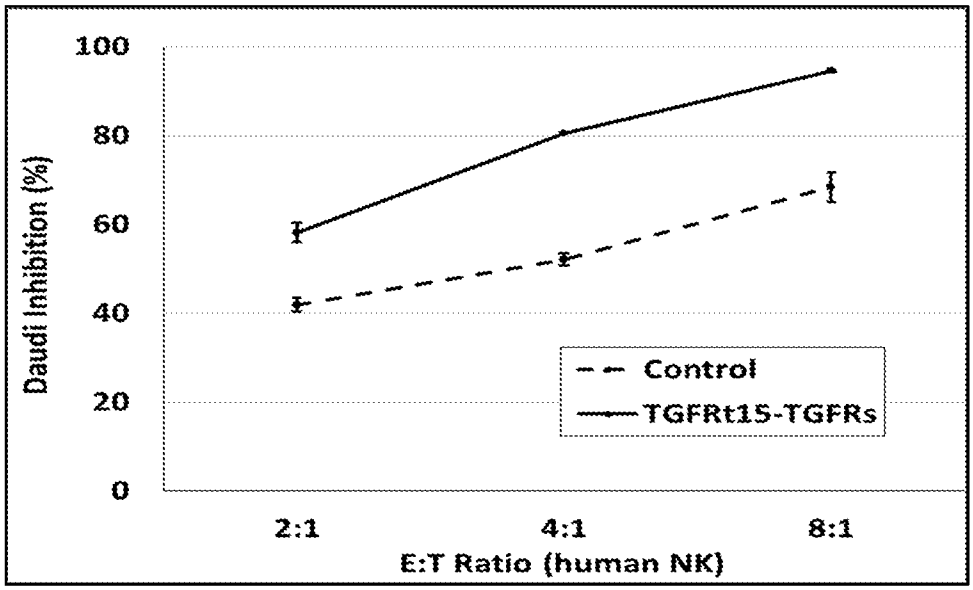


Figure 175B

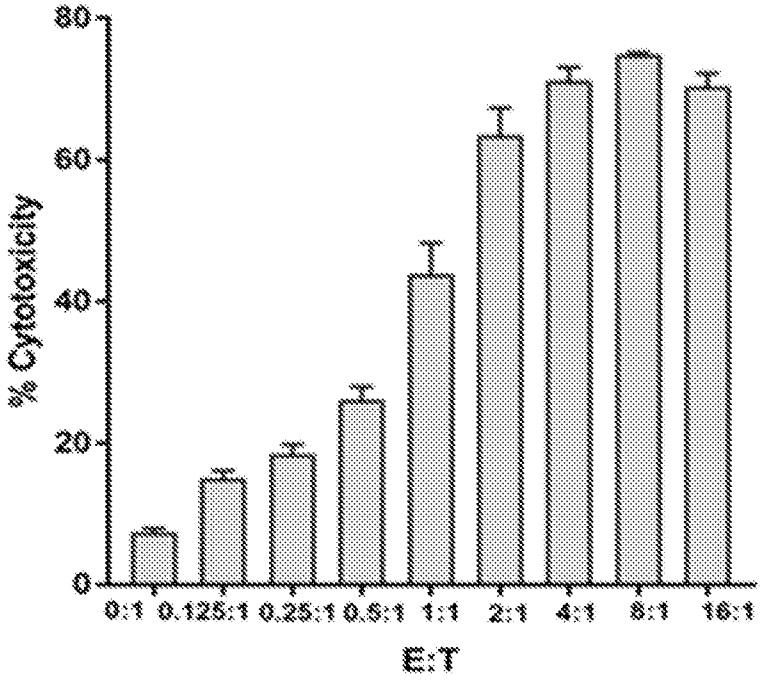


Figure 176

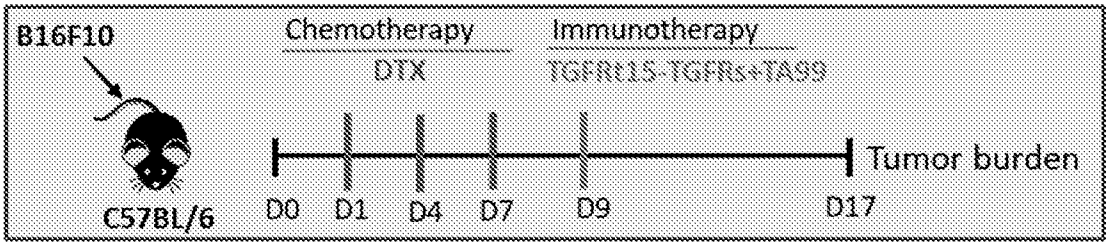


Figure 177A

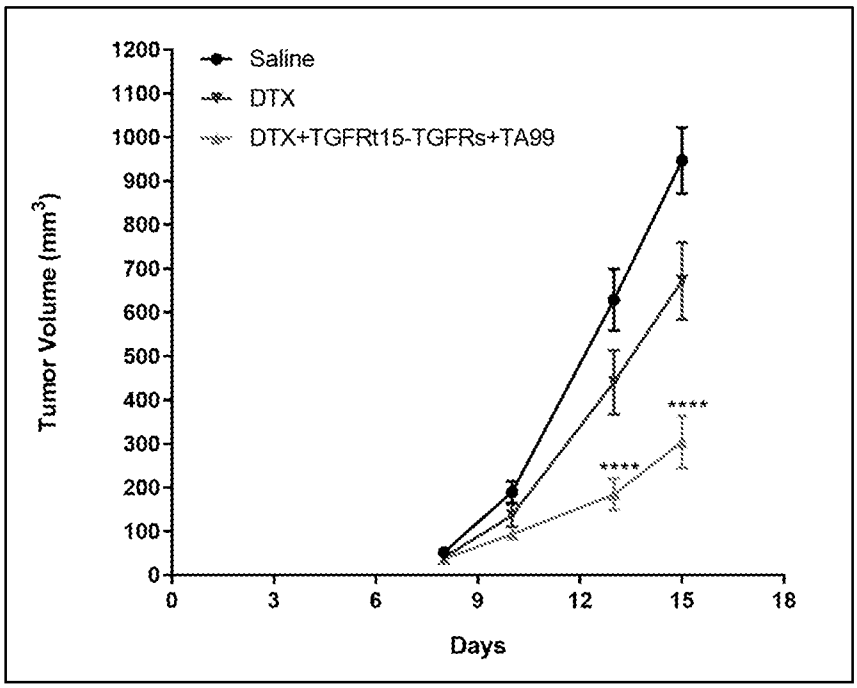


Figure 177B

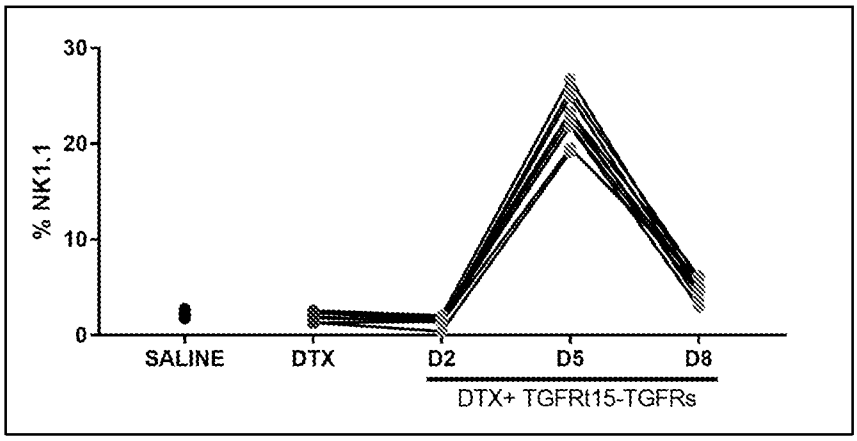


Figure 177C

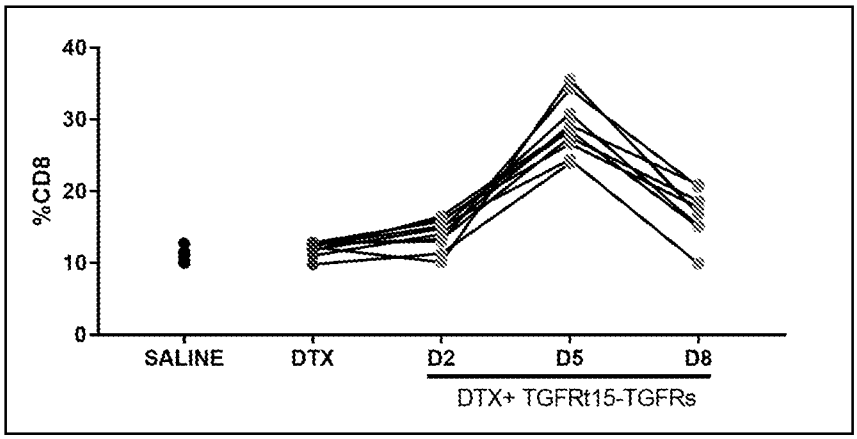


Figure 177D

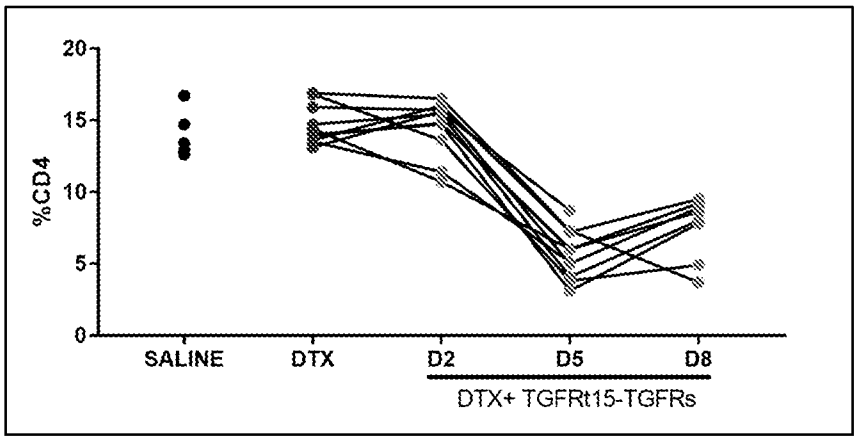


Figure 177E

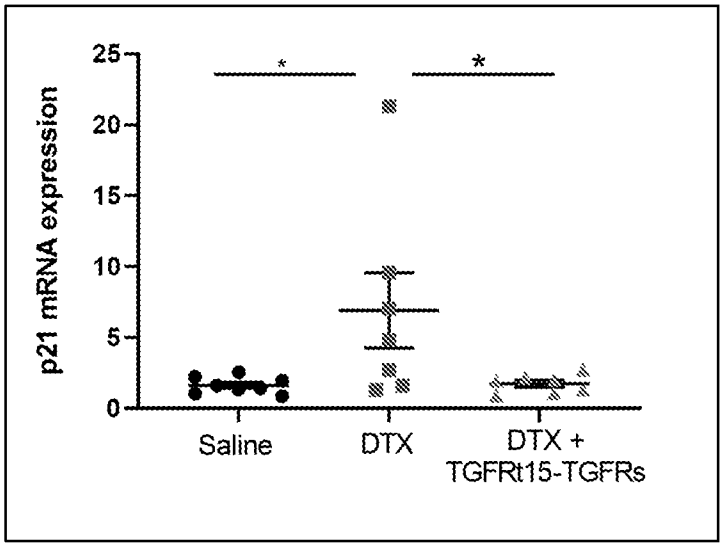


Figure 177F

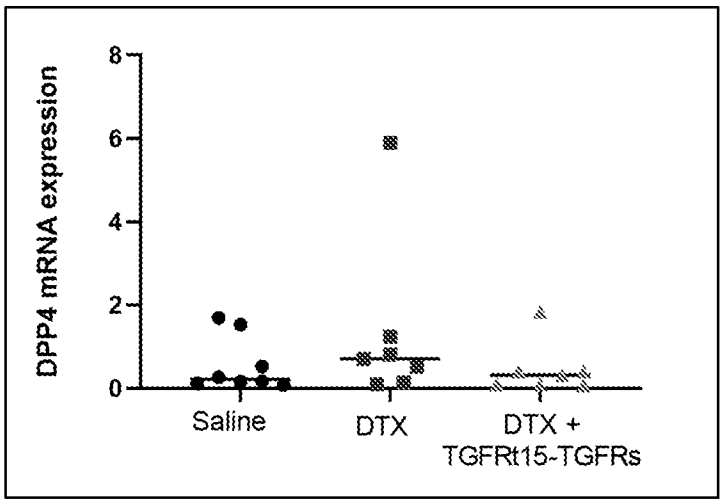


Figure 177G

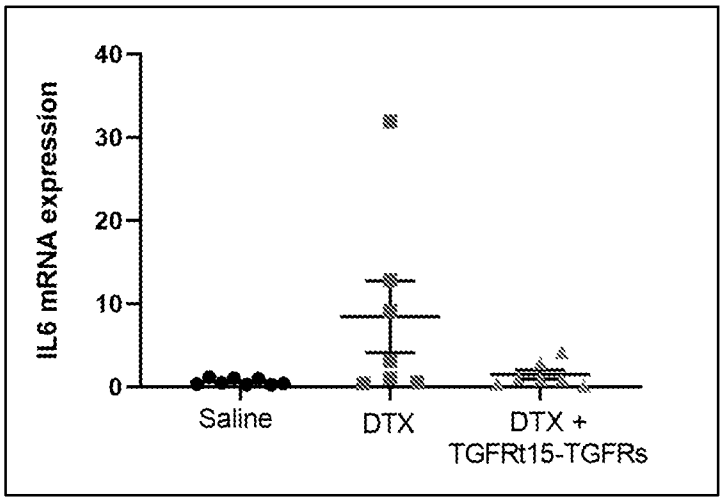

IL6 mRNA expression
40
30
20
10
0
Saline
DTX
DTX +
TGFRt15-TGFRs

Figure 177H

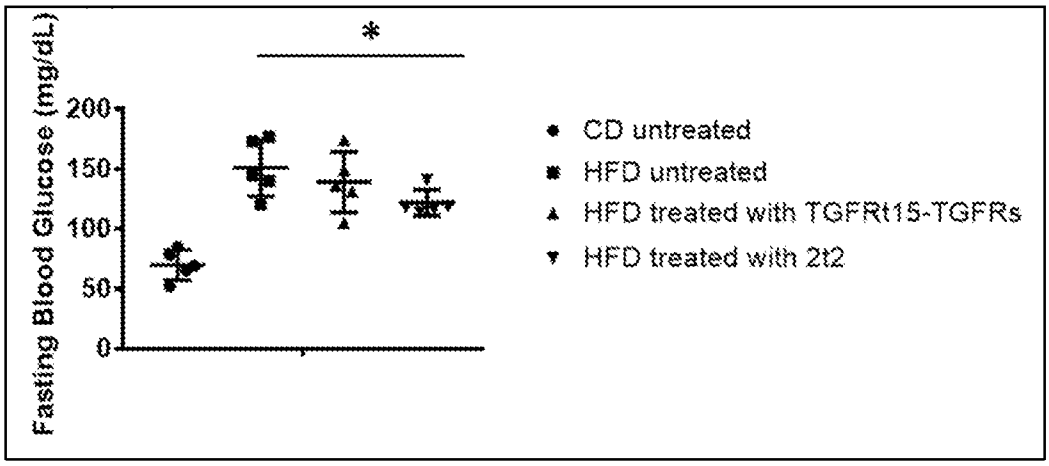


Figure 178A

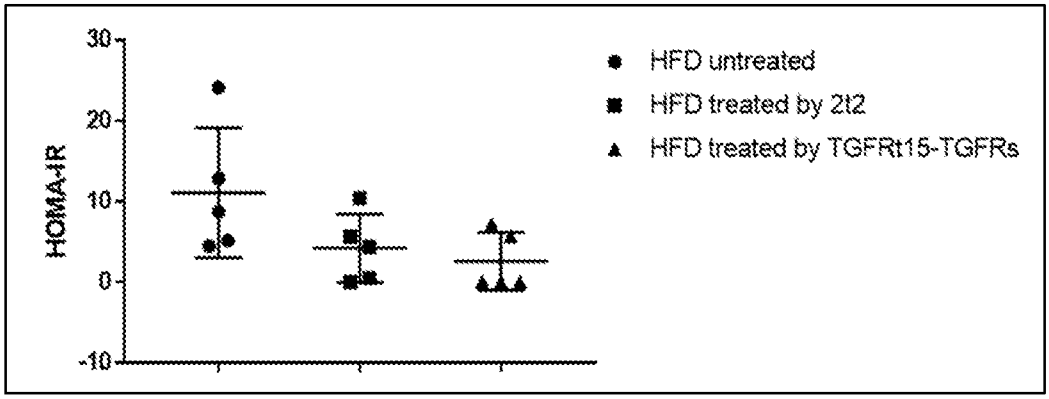


Figure 178B

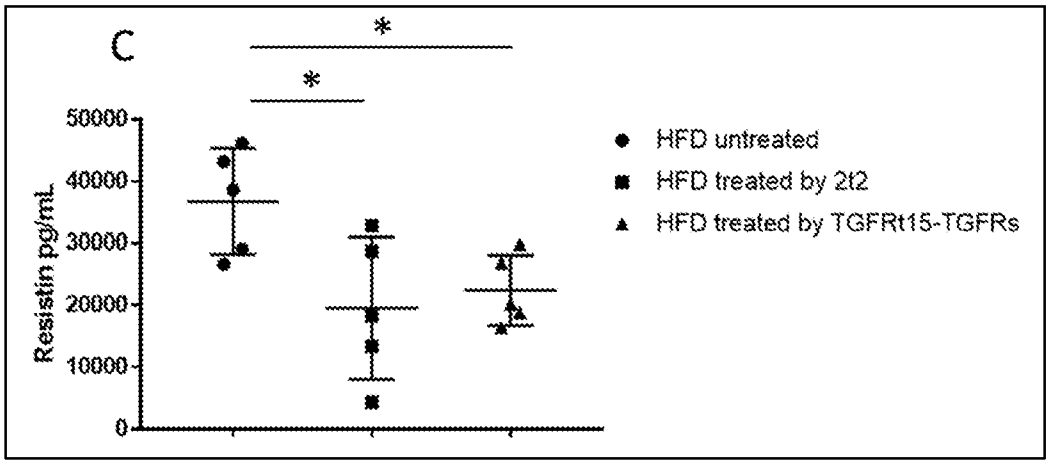


Figure 178C

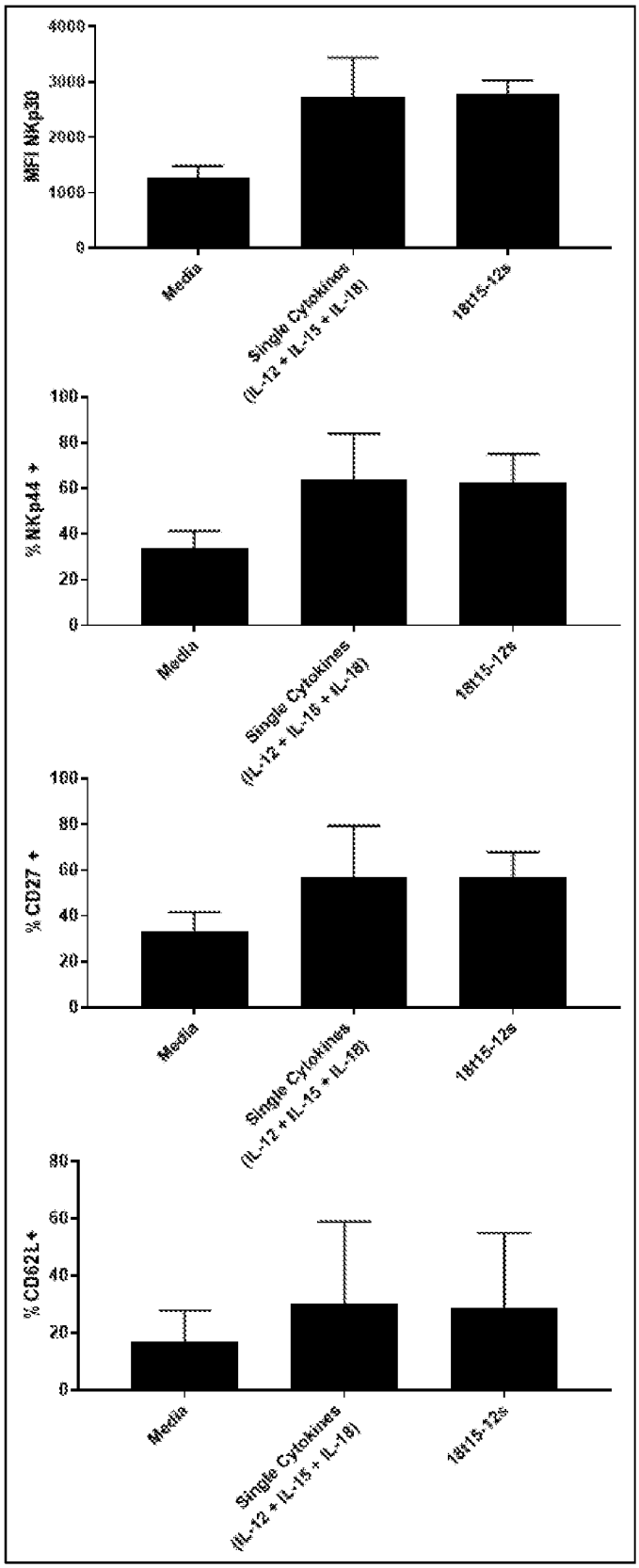

MFI NKp30
4000
3000
2000
1000
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18I15-12s
% NKp44 +
100
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18I15-12s
% CD27 +
100
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18I15-12s
% CD62L +
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18I15-12s

Figure 179

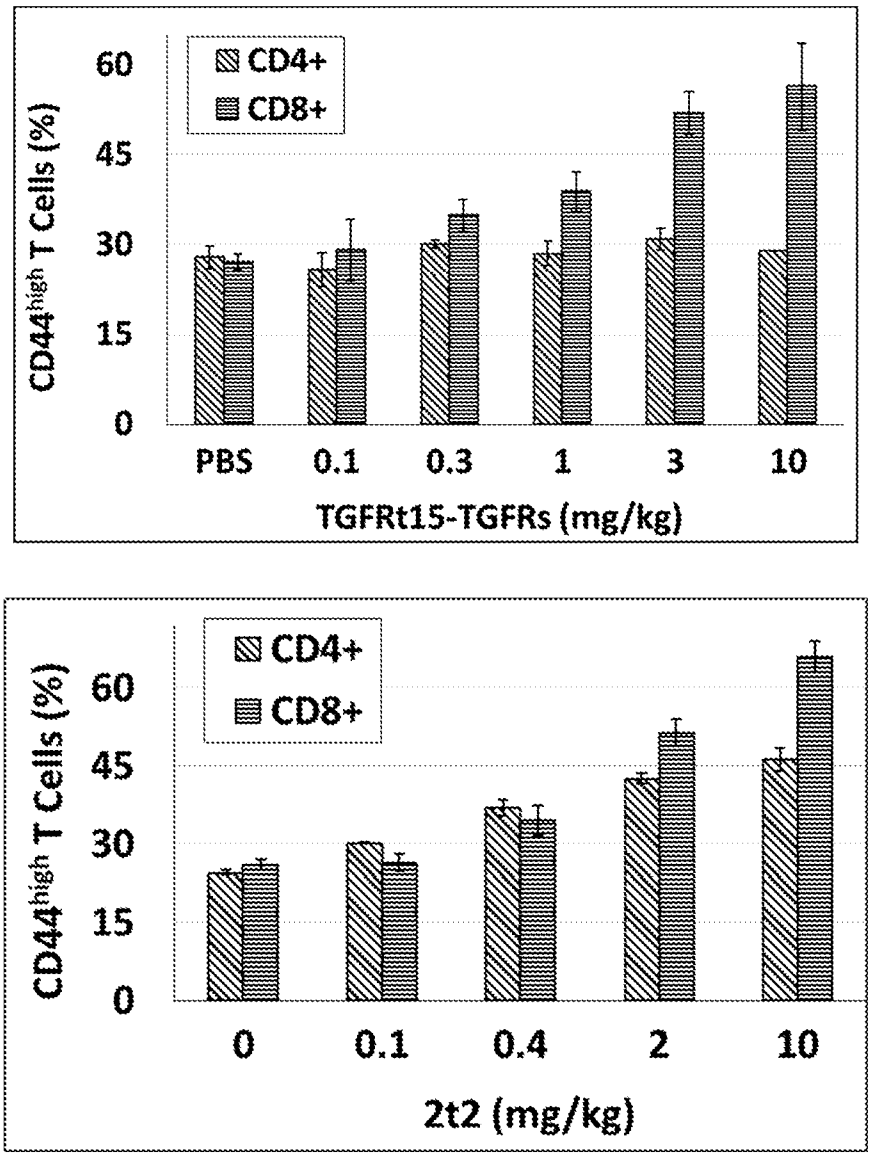

CD4+
CD8+
CD44high T Cells (%)
60
45
30
15
0
PBS
0.1
0.3
1
3
10
TGFRt15-TGFRs (mg/kg)
CD4+
CD8+
CD44high T Cells (%)
60
45
30
15
0
0
0.1
0.4
2
10
2t2 (mg/kg)

Figure 180

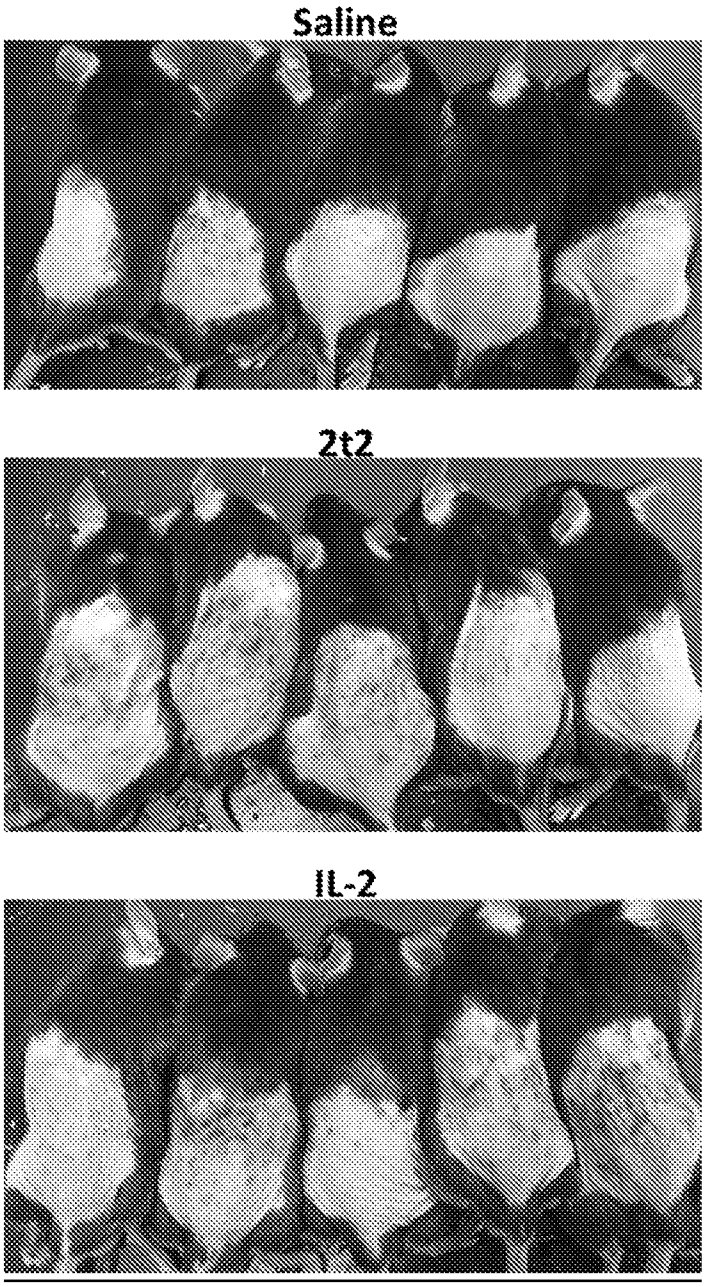


Figure 181A

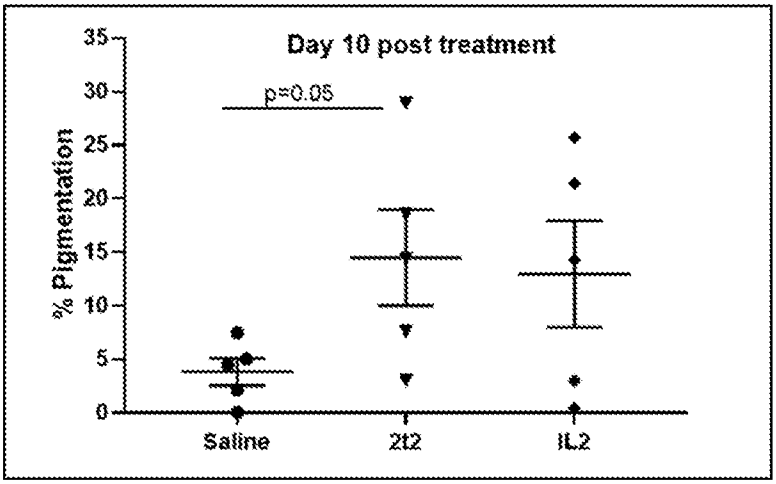

Day 10 post treatment
p=0.05
% Pigmentation
35
30
25
20
15
10
5
0
Saline
2t2
IL2

Figure 181B

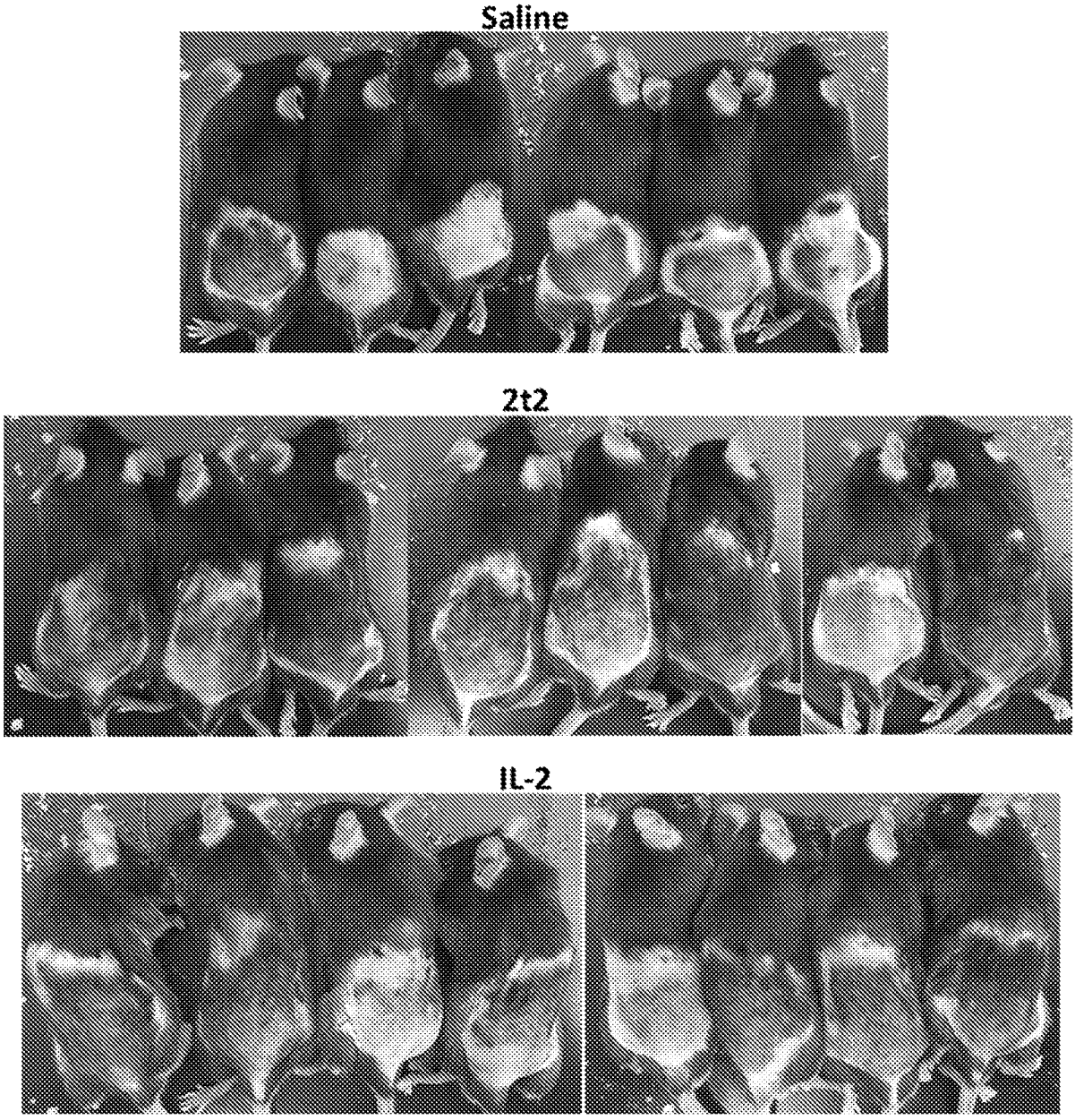


Figure 182

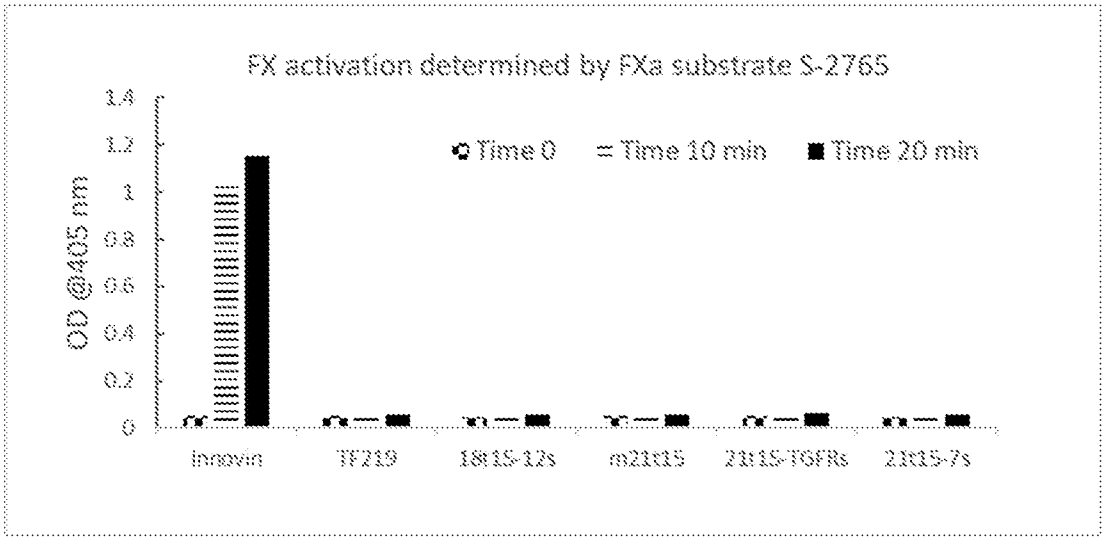


Figure 183

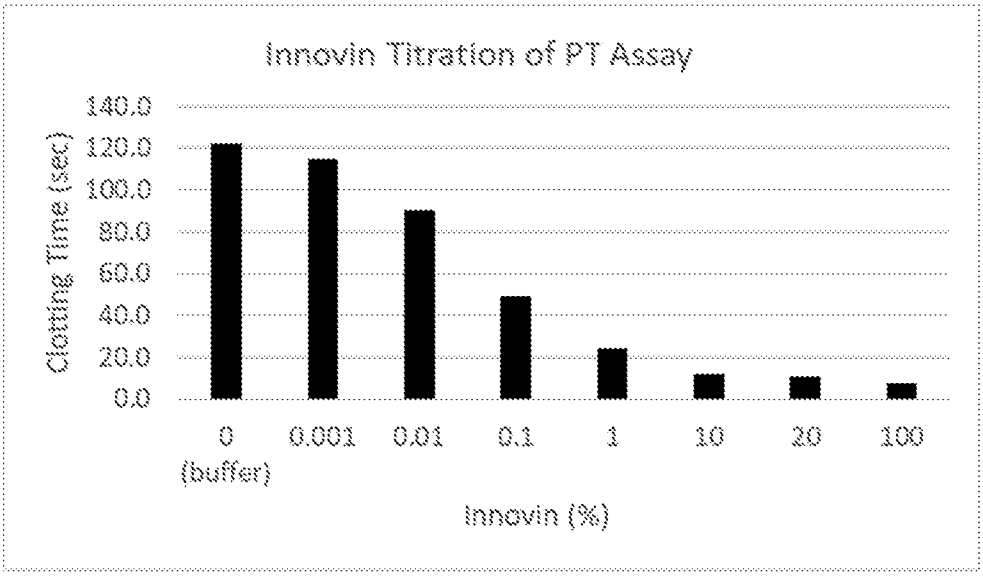


Figure 184

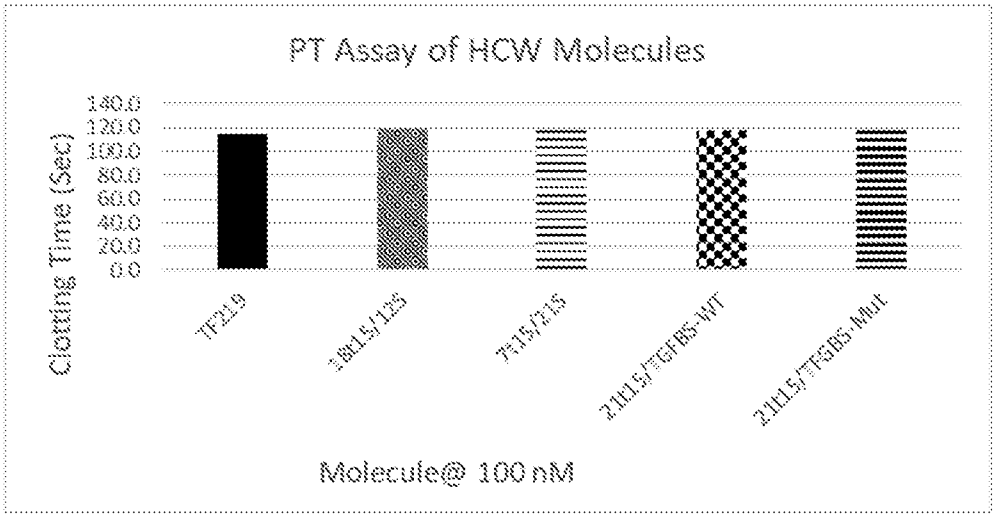


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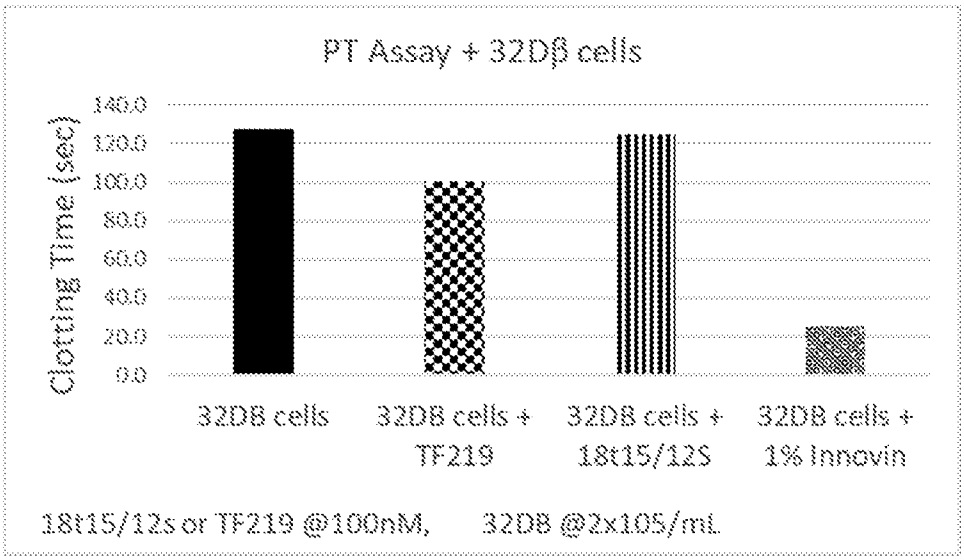


Figure 186

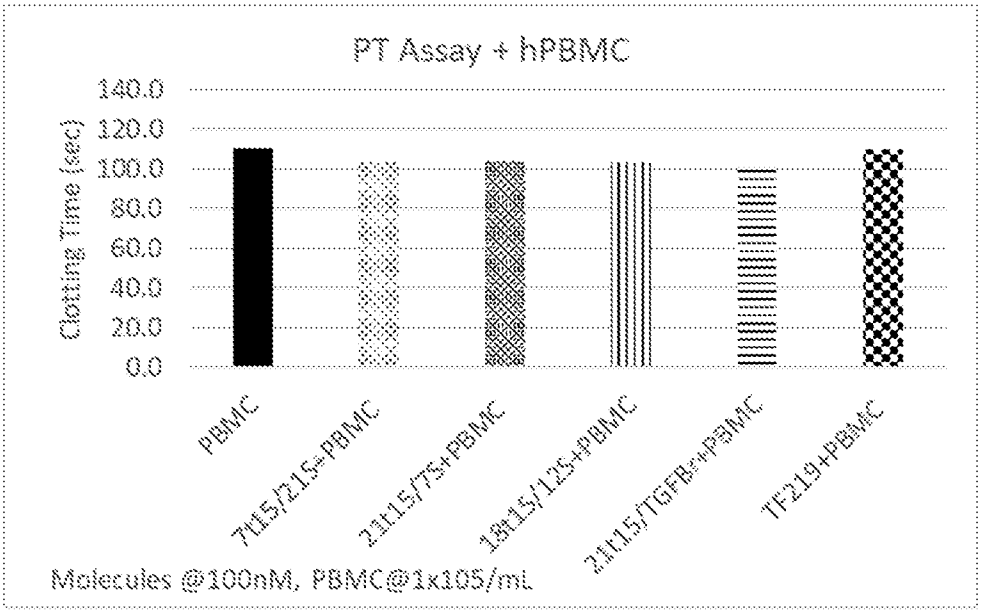


Figure 187

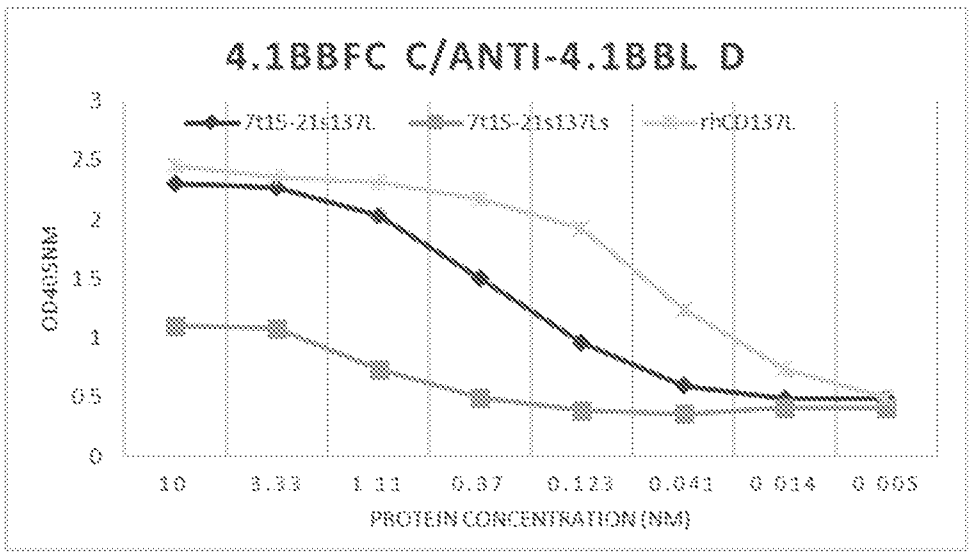


Figure 188

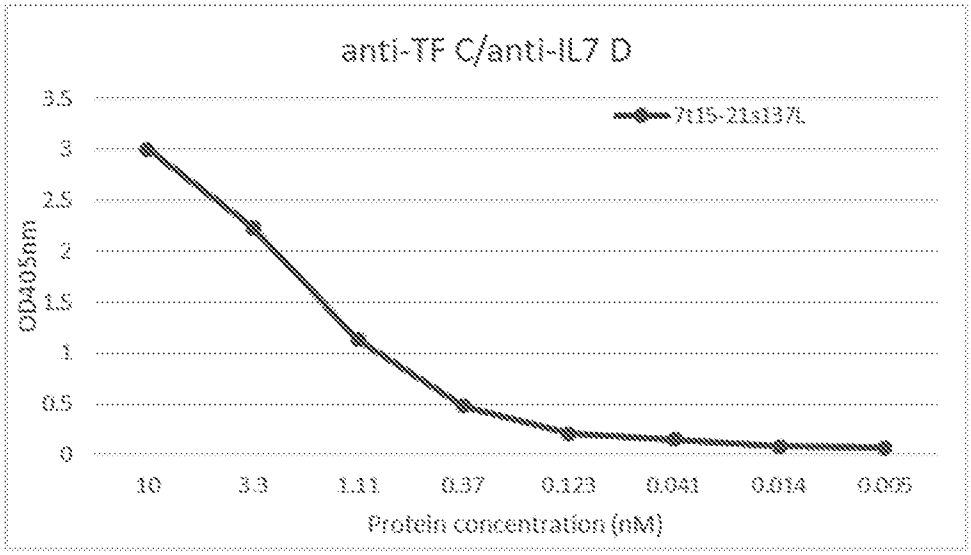


Figure 189A

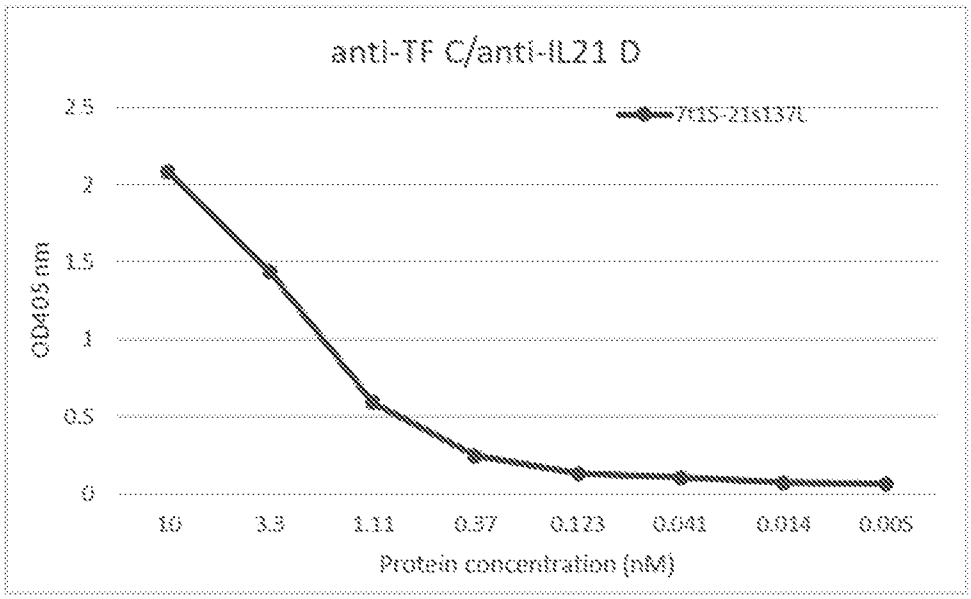


Figure 189B

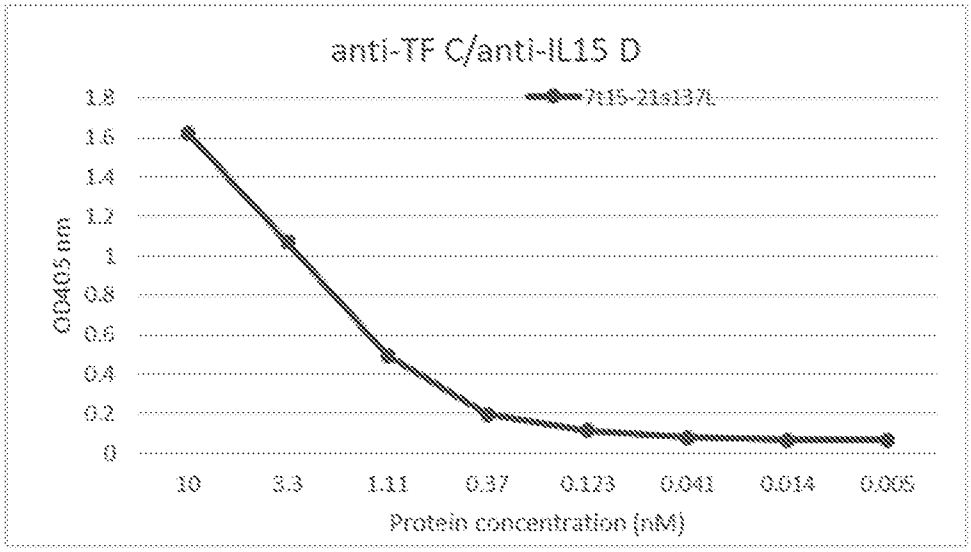


Figure 189C

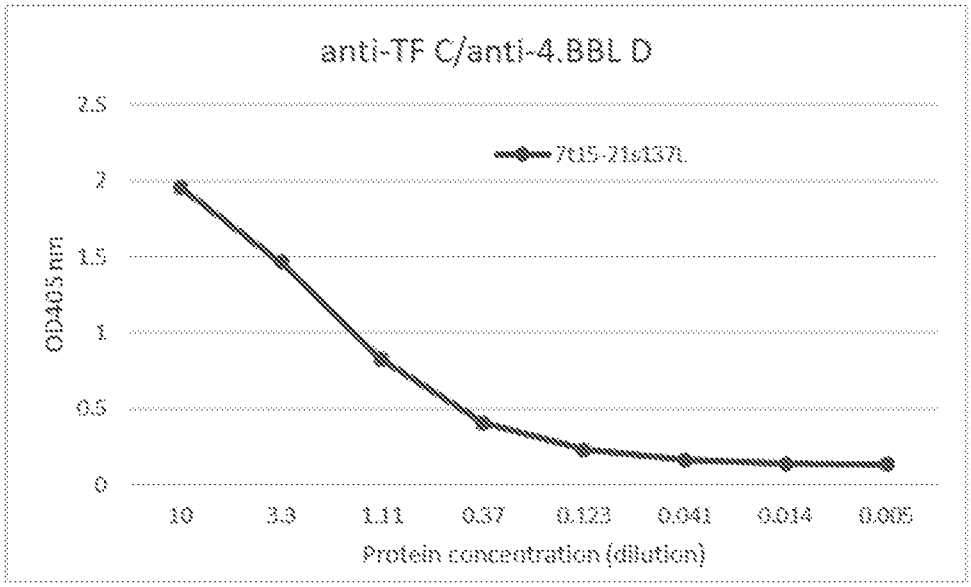


Figure 189D

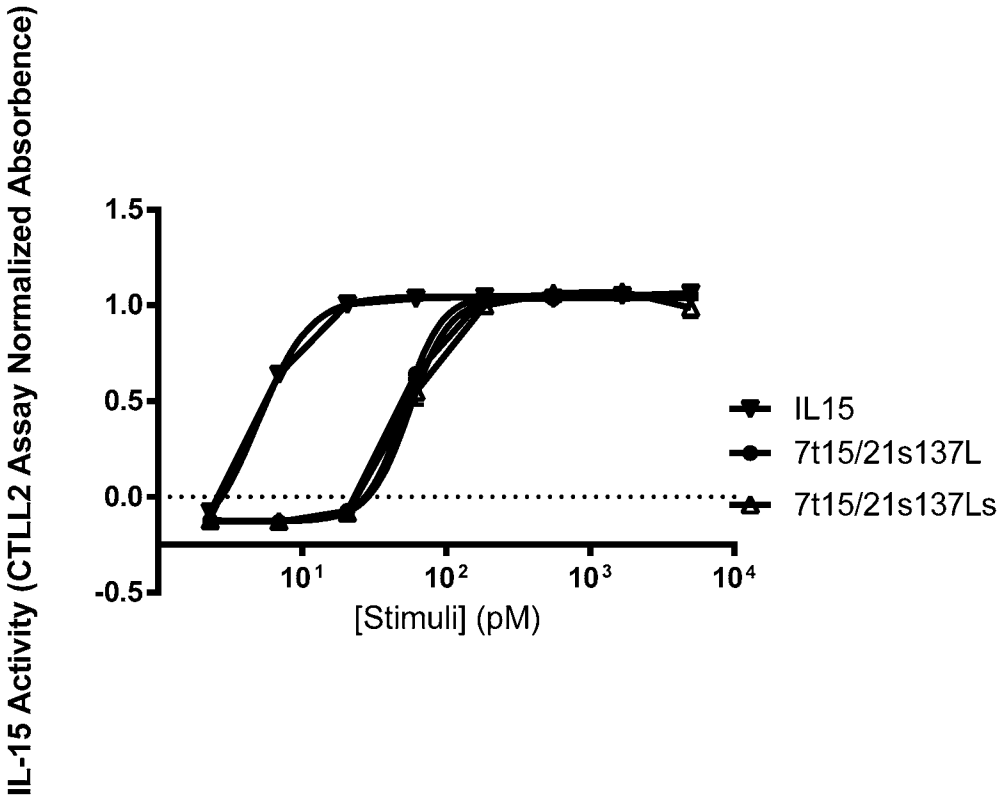

IL-15 Activity (CTLL2 Assay Normalized Absorbence)
1.5
1.0
0.5
0.0
-0.5
10¹
10²
10³
10⁴
[Stimuli] (pM)
IL15
7t15/21s137L
7t15/21s137Ls

Figure 190

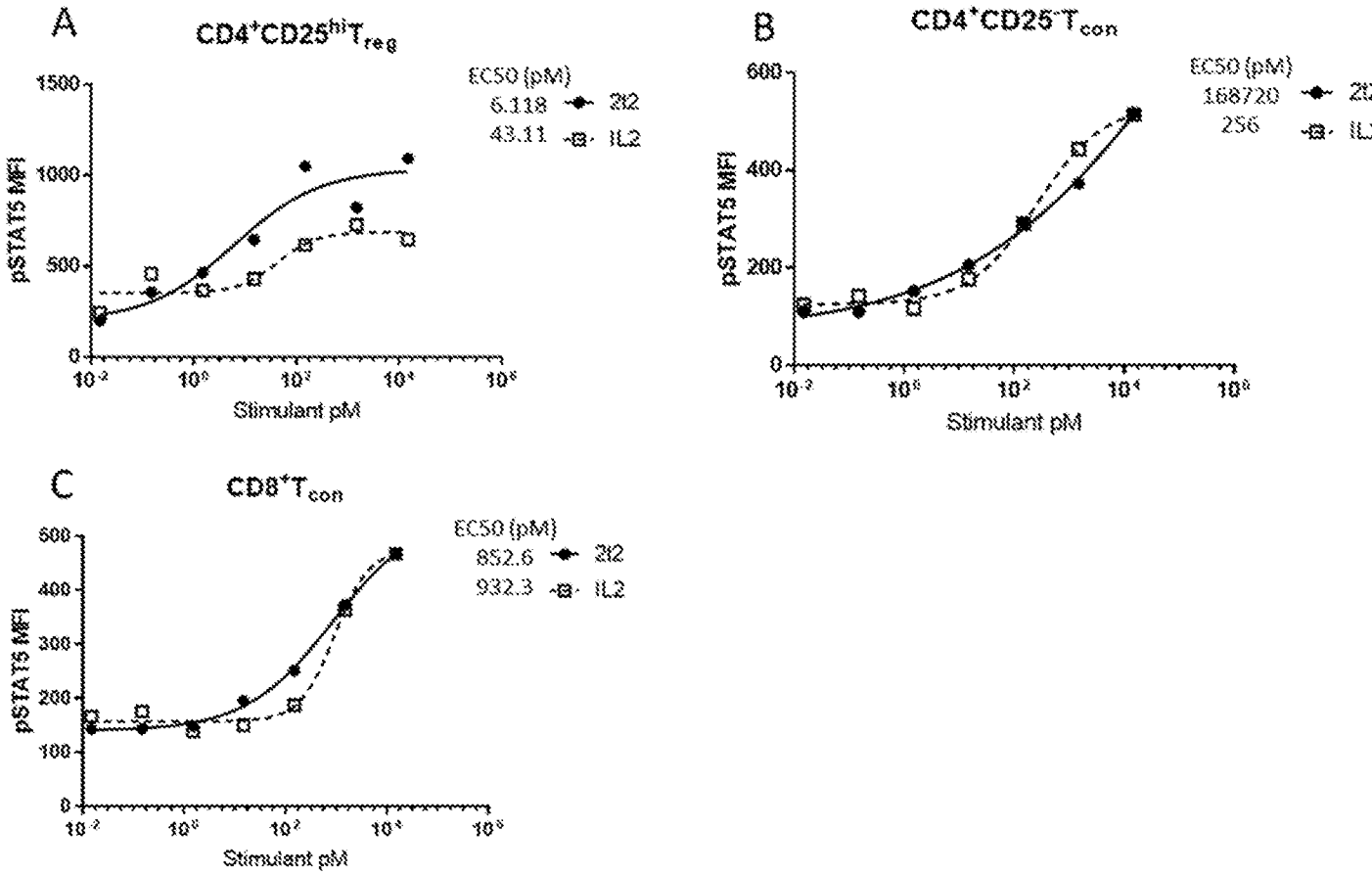

A
CD4+CD25hiTreg
EC50 (pM)
6.118 2I2
43.11 IL2
pSTAT5 MFI
Stimulant pM
B
CD4+CD25-Tcon
EC50 (pM)
168720
256
pSTAT5 MFI
Stimulant pM
C
CD8+Tcon
EC50 (pM)
852.6 2I2
932.3 IL2
pSTAT5 MFI
Stimulant pM

Figure 191

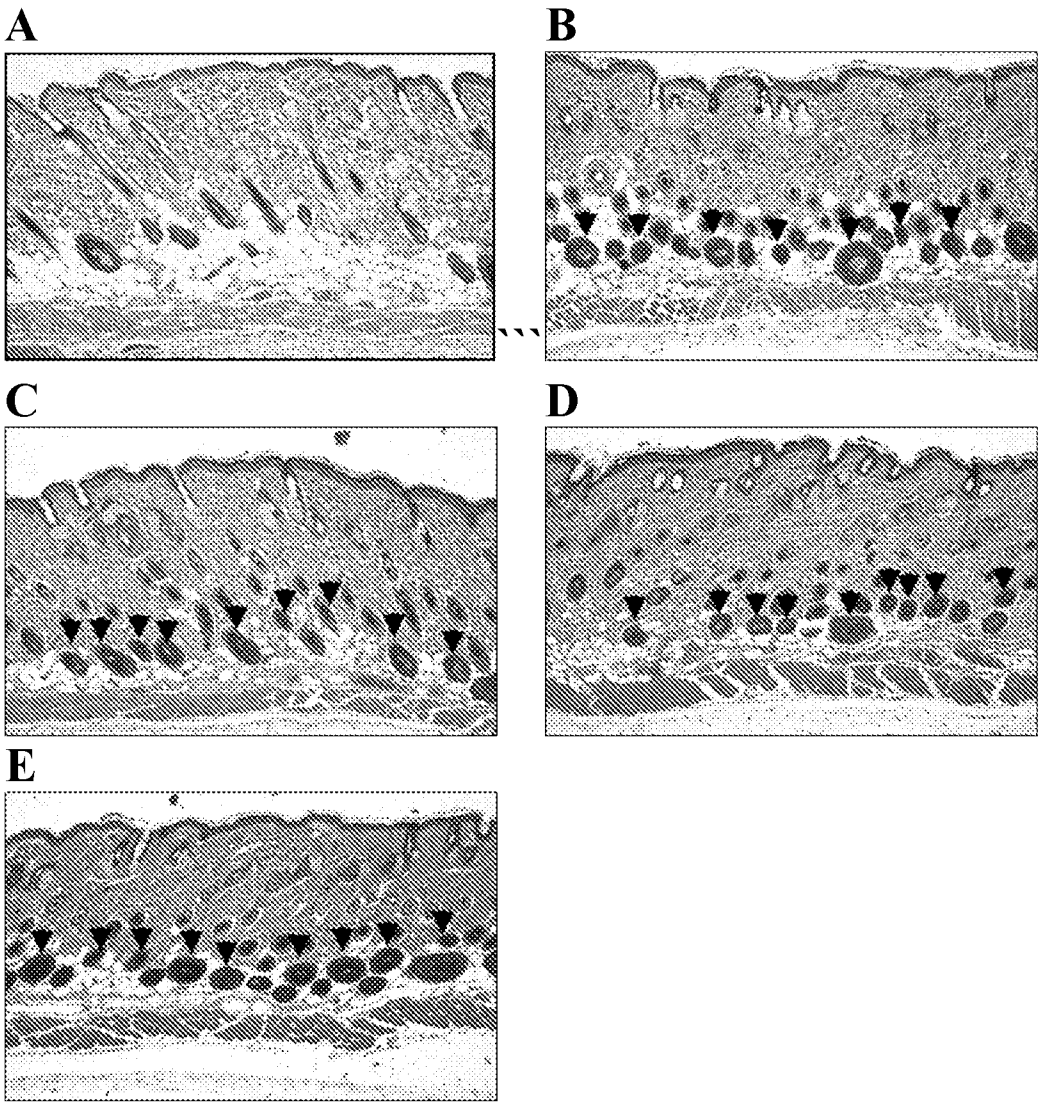


Figure 192

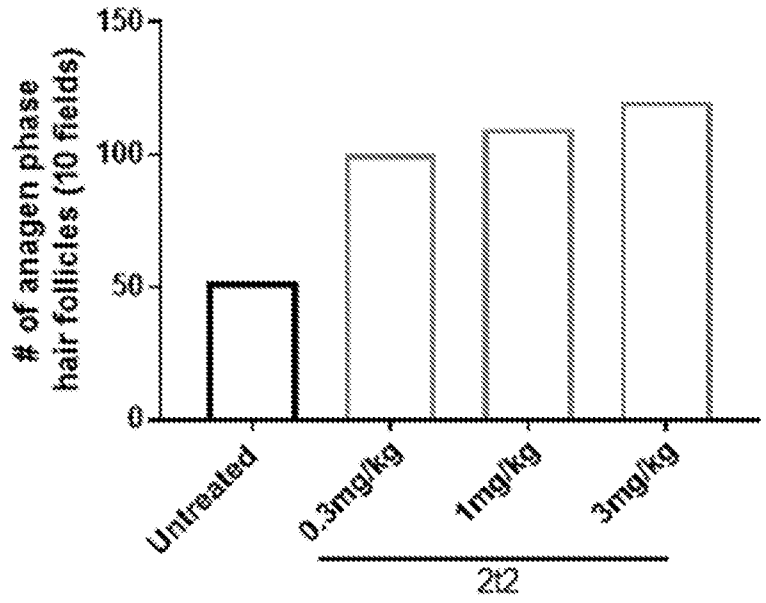


Figure 193

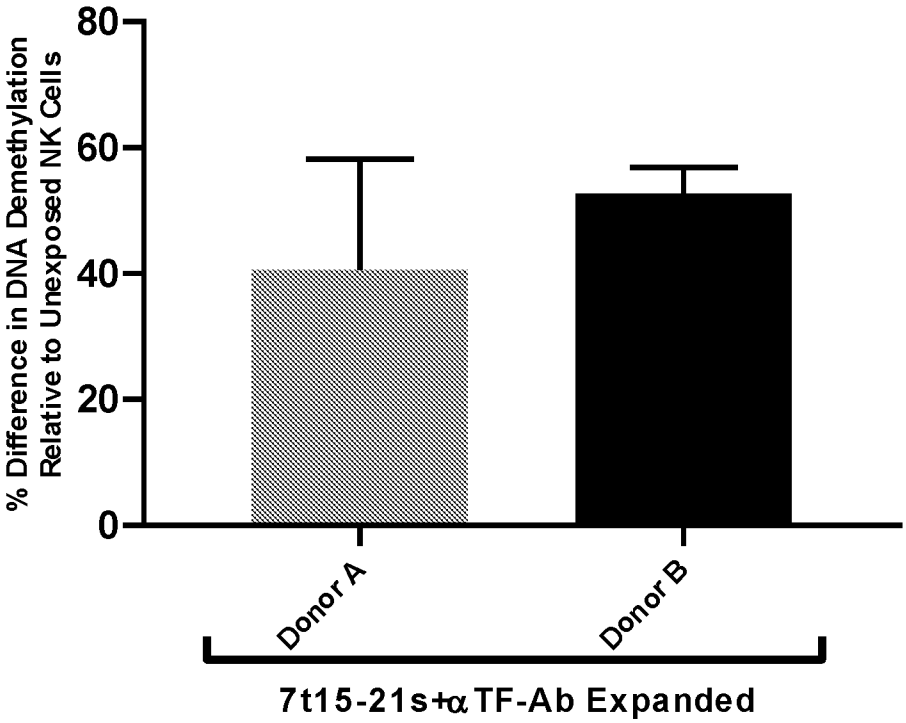


Figure 194

SEQUENCE LISTING

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<120> METHODS OF TREATING AGING-RELATED DISORDER

<130> 47039-0013WO1

<140>
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<151> 2019-07-31

<150> 62/881,039
<151> 2019-07-31

<150> 62/817,244
<151> 2019-03-12

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<151> 2019-03-11

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<151> 2018-10-23

<150> 62/749,007
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<150> 62/746,832
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<151> 2018-08-30

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<150> 62/724,969
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<212> PRT
<213> Homo sapiens

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1   5   10   15

Ala Met Ala Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg
            20   25   30

Gly Phe Arg Arg Ile Lys Ser Gly Ser Leu Tyr Met Leu Cys Thr Gly
        35   40   45

Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys Gln Cys Thr Ser Ser
    50   55   60

Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu Gln
65   70   75   80

Lys Glu Arg Lys Thr Thr Glu Met Gln Ser Pro Met Gln Pro Val Asp
                85   90   95

Gln Ala Ser Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn
            100   105   110

Glu Ala Thr Glu Arg Ile Tyr His Phe Val Val Gly Gln Met Val Tyr
        115   120   125

Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His Arg Gly Pro Ala Glu
    130   135   140

```
Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro Gln
145                 150                 155                 160


Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu
                165                 170                 175


Lys Pro Gln Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys
            180                 185                 190


Leu Val Thr Thr Thr Asp Phe Gln Ile Gln Thr Glu Met Ala Ala Thr
        195                 200                 205


Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln Val Ala Val Ala Gly
    210                 215                 220


Cys Val Phe Leu Leu Ile Ser Val Leu Leu Leu Ser Gly Leu Thr Trp
225                 230                 235                 240


Gln Arg Arg Gln Arg Lys Ser Arg Arg Thr Ile
                245                 250


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tcactctata tgctctgtac aggaaactct agccactcgt cctgggacaa ccaatgtcaa      180

tgcacaagct ctgccactcg gaacacaacg aaacaagtga cacctcaacc tgaagaacag      240

aaagaaagga aaaccacaga aatgcaaagt ccaatgcagc cagtggacca agcgagcctt      300

ccaggtcact gcagggaacc tccaccatgg gaaaatgaag ccacagagag aatttatcat      360
```

ttcgtggtgg ggcagatggt ttattatcag tgcgtccagg gatacagggc tctacacaga 420

ggtcctgctg agagcgtctg caaaatgacc cacgggaaga caaggtggac ccagccccag 480

ctcatatgca caggtgaaat ggagaccagt cagtttccag gtgaagagaa gcctcaggca 540

agccccgaag gccgtcctga gagtgagact tcctgcctcg tcacaacaac agattttcaa 600

atacagacag aaatggctgc aaccatggag acgtccatat ttacaacaga gtaccaggta 660

gcagtggccg gctgtgtttt cctgctgatc agcgtcctcc tcctgagtgg gctcacctgg 720

cagcggagac agaggaagag tagaagaaca atc 753

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1 5 10 15

Glu Ser Gly Gln Glu Asn Asp Ala Thr Ser Pro His Phe Ser Thr Arg
20 25 30

His Glu Gly Ser Phe Gln Val Pro Val Leu Cys Ala Val Met Asn Val
35 40 45

Val Phe Ile Thr Ile Leu Ile Ile Ala Leu Ile Ala Leu Ser Val Gly
50 55 60

Gln Tyr Asn Cys Pro Gly Gln Tyr Thr Phe Ser Met Pro Ser Asp Ser
65 70 75 80

His Val Ser Ser Cys Ser Glu Asp Trp Val Gly Tyr Gln Arg Lys Cys
85 90 95

Tyr Phe Ile Ser Thr Val Lys Arg Ser Trp Thr Ser Ala Gln Asn Ala

```
            100                 105                 110

Cys Ser Glu His Gly Ala Thr Leu Ala Val Ile Asp Ser Glu Lys Asp
        115                 120                 125

Met Asn Phe Leu Lys Arg Tyr Ala Gly Arg Glu Glu His Trp Val Gly
    130                 135                 140

Leu Lys Lys Glu Pro Gly His Pro Trp Lys Trp Ser Asn Gly Lys Glu
145                 150                 155                 160

Phe Asn Asn Trp Phe Asn Val Thr Gly Ser Asp Lys Cys Val Phe Leu
                165                 170                 175

Lys Asn Thr Glu Val Ser Ser Met Glu Cys Glu Lys Asn Leu Tyr Trp
            180                 185                 190

Ile Cys Asn Lys Pro Tyr Lys
        195


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gaaaatgatg ccaccagtcc ccatttctca acacgtcatg aagggtcctt ccaagttcct      120

gtcctgtgtg ctgtaatgaa tgtggtcttc atcaccattt taatcatagc tctcattgcc      180

ttatcagtgg gccaatacaa ttgtccaggc caatacacat tctcaatgcc atcagacagc      240

catgtttctt catgctctga ggactgggtt ggctaccaga ggaaatgcta ctttatttct      300

actgtgaaga ggagctggac ttcagcccaa aatgcttgtt ctgaacatgg tgctactctt      360

gctgtcattg attctgaaaa ggacatgaac tttctaaaac gatacgcagg tagagaggaa      420
```

cactgggttg gactgaaaaa ggaacctggt cacccatgga agtggtcaaa tggcaaagaa 480

tttaacaact ggttcaacgt tacagggtct gacaagtgtg tttttctgaa aaacacagag 540

gtcagcagca tggaatgtga gaagaattta tactggatat gtaacaaacc ttacaaataa 600

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<223> /note="CD59"

<400> 5
Leu Gln Cys Tyr Asn Cys Pro Asn Pro Thr Ala Asp Cys Lys Thr Ala
1 5 10 15

Val Asn Cys Ser Ser Asp Phe Asp Ala Cys Leu Ile Thr Lys Ala Gly
20 25 30

Leu Gln Val Tyr Asn Lys Cys Trp Lys Phe Glu His Cys Asn Phe Asn
35 40 45

Asp Val Thr Thr Arg Leu Arg Glu Asn Glu Leu Thr Tyr Tyr Cys Cys
50 55 60

Lys Lys Asp Leu Cys Asn Phe Asn Glu Gln Leu Glu Asn
65 70 75

<210> 6
<211> 387
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD59"

<400> 6
atgggaatcc aaggagggtc tgtcctgttc gggctgctgc tcgtcctggc tgtcttctgc 60

cattcaggtc atagcctgca gtgctacaac tgtcctaacc caactgctga ctgcaaaaca 120

gccgtcaatt gttcatctga ttttgatgcg tgtctcatta ccaaagctgg gttacaagtg 180

tataacaagt gttggaagtt tgagcattgc aatttcaacg acgtcacaac ccgcttgagg 240

gaaaatgagc taacgtacta ctgctgcaag aaggacctgt gtaactttaa cgaacagctt 300

gaaaatggtg ggacatcctt atcagagaaa acagttcttc tgctggtgac tccatttctg 360

gcagcagcct ggagccttca tccctaa 387

<210> 7
<211> 311
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD352"

<400> 7
Gln Ser Ser Leu Thr Pro Leu Met Val Asn Gly Ile Leu Gly Glu Ser
1 5 10 15

Val Thr Leu Pro Leu Glu Phe Pro Ala Gly Glu Lys Val Asn Phe Ile
20 25 30

Thr Trp Leu Phe Asn Glu Thr Ser Leu Ala Phe Ile Val Pro His Glu
35 40 45

Thr Lys Ser Pro Glu Ile His Val Thr Asn Pro Lys Gln Gly Lys Arg
50 55 60

Leu Asn Phe Thr Gln Ser Tyr Ser Leu Gln Leu Ser Asn Leu Lys Met
65 70 75 80

Glu Asp Thr Gly Ser Tyr Arg Ala Gln Ile Ser Thr Lys Thr Ser Ala
85 90 95

Lys Leu Ser Ser Tyr Thr Leu Arg Ile Leu Arg Gln Leu Arg Asn Ile
100 105 110

```
Gln Val Thr Asn His Ser Gln Leu Phe Gln Asn Met Thr Cys Glu Leu
        115                 120                 125

His Leu Thr Cys Ser Val Glu Asp Ala Asp Asp Asn Val Ser Phe Arg
    130                 135                 140

Trp Glu Ala Leu Gly Asn Thr Leu Ser Ser Gln Pro Asn Leu Thr Val
145                 150                 155                 160

Ser Trp Asp Pro Arg Ile Ser Ser Glu Gln Asp Tyr Thr Cys Ile Ala
                165                 170                 175

Glu Asn Ala Val Ser Asn Leu Ser Phe Ser Val Ser Ala Gln Lys Leu
            180                 185                 190

Cys Glu Asp Val Lys Ile Gln Tyr Thr Asp Thr Lys Met Ile Leu Phe
        195                 200                 205

Met Val Ser Gly Ile Cys Ile Val Phe Gly Phe Ile Ile Leu Leu Leu
    210                 215                 220

Leu Val Leu Arg Lys Arg Arg Asp Ser Leu Ser Leu Ser Thr Gln Arg
225                 230                 235                 240

Thr Gln Gly Pro Ala Glu Ser Ala Arg Asn Leu Glu Tyr Val Ser Val
                245                 250                 255

Ser Pro Thr Asn Asn Thr Val Tyr Ala Ser Val Thr His Ser Asn Arg
            260                 265                 270

Glu Thr Glu Ile Trp Thr Pro Arg Glu Asn Asp Thr Ile Thr Ile Tyr
        275                 280                 285

Ser Thr Ile Asn His Ser Lys Glu Ser Lys Pro Thr Phe Ser Arg Ala
    290                 295                 300

Thr Ala Leu Asp Asn Val Val
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305 310

<210> 8
<211> 996
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD59"

<400> 8
atgttgtggc tgttccaatc gctcctgttt gtcttctgct ttggcccagg gaatgtagtt 60

tcacaaagca gcttaacccc attgatggtg aacgggattc tgggggagtc agtaactctt 120

cccctggagt ttcctgcagg agagaaggtc aacttcatca cttggctttt caatgaaaca 180

tctcttgcct tcatagtacc ccatgaaacc aaaagtccag aaatccacgt gactaatccg 240

aaacagggaa agcgactgaa cttcacccag tcctactccc tgcaactcag caacctgaag 300

atggaagaca caggctctta cagagcccag atatccacaa agacctctgc aaagctgtcc 360

agttacactc tgaggatatt aagacaactg aggaacatac aagttaccaa tcacagtcag 420

ctatttcaga atatgacctg tgagctccat ctgacttgct ctgtggagga tgcagatgac 480

aatgtctcat tcagatggga ggccttggga aacacacttt caagtcagcc aaacctcact 540

gtctcctggg accccaggat ttccagtgaa caggactaca cctgcatagc agagaatgct 600

gtcagtaatt tatccttctc tgtctctgcc cagaagcttt gcgaagatgt taaaattcaa 660

tatacagata ccaaaatgat tctgtttatg gtttctggga tatgcatagt cttcggtttc 720

atcatactgc tgttacttgt tttgaggaaa agaagagatt ccctatcttt gtctactcag 780

cgaacacagg gccccgagtc cgcaaggaac ctagagtatg tttcagtgtc tccaacgaac 840

aacactgtgt atgcttcagt cactcattca aacagggaaa cagaaatctg gacacctaga 900

gaaaatgata ctatcacaat ttactccaca attaatcatt ccaaagagag taaacccact 960

ttttccaggg caactgccct tgacaatgtc gtgtaa 996

<210> 9
<211> 231

<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NKp80"

<400> 9
Met Gln Asp Glu Glu Arg Tyr Met Thr Leu Asn Val Gln Ser Lys Lys
1   5   10   15

Arg Ser Ser Ala Gln Thr Ser Gln Leu Thr Phe Lys Asp Tyr Ser Val
            20   25   30

Thr Leu His Trp Tyr Lys Ile Leu Leu Gly Ile Ser Gly Thr Val Asn
        35   40   45

Gly Ile Leu Thr Leu Thr Leu Ile Ser Leu Ile Leu Leu Val Ser Gln
    50   55   60

Gly Val Leu Leu Lys Cys Gln Lys Gly Ser Cys Ser Asn Ala Thr Gln
65   70   75   80

Tyr Glu Asp Thr Gly Asp Leu Lys Val Asn Asn Gly Thr Arg Arg Asn
                85   90   95

Ile Ser Asn Lys Asp Leu Cys Ala Ser Arg Ser Ala Asp Gln Thr Val
            100   105   110

Leu Cys Gln Ser Glu Trp Leu Lys Tyr Gln Gly Lys Cys Tyr Trp Phe
        115   120   125

Ser Asn Glu Met Lys Ser Trp Ser Asp Ser Tyr Val Tyr Cys Leu Glu
    130   135   140

Arg Lys Ser His Leu Leu Ile Ile His Asp Gln Leu Glu Met Ala Phe
145   150   155   160

Ile Gln Lys Asn Leu Arg Gln Leu Asn Tyr Val Trp Ile Gly Leu Asn
                165   170   175

Phe Thr Ser Leu Lys Met Thr Trp Thr Trp Val Asp Gly Ser Pro Ile
180 185 190

Asp Ser Lys Ile Phe Phe Ile Lys Gly Pro Ala Lys Glu Asn Ser Cys
195 200 205

Ala Ala Ile Lys Glu Ser Lys Ile Phe Ser Glu Thr Cys Ser Ser Val
210 215 220

Phe Lys Trp Ile Cys Gln Tyr
225 230

<210> 10
<211> 546
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="Npk80"

<400> 10
atgcaagatg aagaaagata catgacattg aatgtacagt caaagaaaag gagttctgcc 60

caaacatctc aacttacatt taaagattat tcagtgacgt tgcactggta taaaatctta 120

ctgggaatat ctggaaccgt gaatggtatt ctcactttga ctttgatctc cttgatcctg 180

ttggtactat gccaatcaga atggctcaaa taccaaggga agtgttattg gttctctaat 240

gagatgaaaa gctggagtga cagttatgtg tattgtttgg aaagaaaatc tcatctacta 300

atcatacatg accaacttga aatggctttt atacagaaaa acctaagaca attaaactac 360

gtatggattg ggcttaactt tacctccttg aaaatgacat ggacttgggt ggatggttct 420

ccaatagatt caaagatatt cttcataaag ggaccagcta aagaaaacag ctgtgctgcc 480

attaaggaaa gcaaaatttt ctctgaaacc tgcagcagtg ttttcaaatg gatttgtcag 540

tattag 546

<210> 11

<211> 318
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="DNAM-1"

<400> 11
Glu Glu Val Leu Trp His Thr Ser Val Pro Phe Ala Glu Asn Met Ser
1   5   10   15

Leu Glu Cys Val Tyr Pro Ser Met Gly Ile Leu Thr Gln Val Glu Trp
            20   25   30

Phe Lys Ile Gly Thr Gln Gln Asp Ser Ile Ala Ile Phe Ser Pro Thr
        35   40   45

His Gly Met Val Ile Arg Lys Pro Tyr Ala Glu Arg Val Tyr Phe Leu
    50   55   60

Asn Ser Thr Met Ala Ser Asn Asn Met Thr Leu Phe Phe Arg Asn Ala
65   70   75   80

Ser Glu Asp Asp Val Gly Tyr Tyr Ser Cys Ser Leu Tyr Thr Tyr Pro
                85   90   95

Gln Gly Thr Trp Gln Lys Val Ile Gln Val Val Gln Ser Asp Ser Phe
            100   105   110

Glu Ala Ala Val Pro Ser Asn Ser His Ile Val Ser Glu Pro Gly Lys
        115   120   125

Asn Val Thr Leu Thr Cys Gln Pro Gln Met Thr Trp Pro Val Gln Ala
    130   135   140

Val Arg Trp Glu Lys Ile Gln Pro Arg Gln Ile Asp Leu Leu Thr Tyr
145   150   155   160

Cys Asn Leu Val His Gly Arg Asn Phe Thr Ser Lys Phe Pro Arg Gln

```
                165                 170                 175

Ile Val Ser Asn Cys Ser His Gly Arg Trp Ser Val Ile Val Ile Pro
            180                 185                 190

Asp Val Thr Val Ser Asp Ser Gly Leu Tyr Arg Cys Tyr Leu Gln Ala
        195                 200                 205

Ser Ala Gly Glu Asn Glu Thr Phe Val Met Arg Leu Thr Val Ala Glu
    210                 215                 220

Gly Lys Thr Asp Asn Gln Tyr Thr Leu Phe Val Ala Gly Gly Thr Val
225                 230                 235                 240

Leu Leu Leu Leu Phe Val Ile Ser Ile Thr Thr Ile Ile Val Ile Phe
                245                 250                 255

Leu Asn Arg Arg Arg Arg Arg Glu Arg Arg Asp Leu Phe Thr Glu Ser
            260                 265                 270

Trp Asp Thr Gln Lys Ala Pro Asn Asn Tyr Arg Ser Pro Ile Ser Thr
        275                 280                 285

Ser Gln Pro Thr Asn Gln Ser Met Asp Asp Thr Arg Glu Asp Ile Tyr
    290                 295                 300

Val Asn Tyr Pro Thr Phe Ser Arg Arg Pro Lys Thr Arg Val
305                 310                 315


<210> 12
<211> 1011
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="DNAM-1"

<400> 12
atggattatc ctactttact tttggctctt cttcatgtat acagagctct atgtgaagag       60
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gtgctttggc atacatcagt tccctttgcc gagaacatgt ctctagaatg tgtgtatcca 120

tcaatgggca tcttaacaca ggtggagtgg ttcaagatcg ggacccagca ggattccata 180

gccattttca gccctactca tggcatggtc ataaggaagc cctatgctga gagggtttac 240

tttttgaatt caacgatggc ttccaataac atgactcttt tctttcggaa tgcctctgaa 300

gatgatgttg gctactattc ctgctctctt tacacttacc cacagggaac ttggcagaag 360

gtgatacagg tggttcagtc agatagtttt gaggcagctg tgccatcaaa tagccacatt 420

gtttcggaac ctggaaagaa tgtcacactc acttgtcagc ctcagatgac gtggcctgtg 480

caggcagtga ggtgggaaaa gatccagccc cgtcagatcg acctcttaac ttactgcaac 540

ttggtccatg gcagaaattt cacctccaag ttcccaagac aaatagtgag caactgcagc 600

cacggaaggt ggagcgtcat cgtcatcccc gatgtcacag tctcagactc ggggctttac 660

cgctgctact tgcaggccag cgcaggagaa aacgaaacct tcgtgatgag attgactgta 720

gccgagggta aaaccgataa ccaatatacc ctctttgtgg ctggagggac agttttattg 780

ttgttgtttg ttatctcaat taccaccatc attgtcattt tccttaacag aaggagaagg 840

agagagagaa gagatctatt tacagagtcc tgggatacac agaaggcacc caataactat 900

agaagtccca tctctaccag tcaacctacc aatcaatcca tggatgatac aagagaggat 960

atttatgtca actatccaac cttctctcgc agaccaaaga ctagagttta a 1011

<210> 13
<211> 347
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="HB4"

<400> 13
Gly Lys Gly Cys Gln Gly Ser Ala Asp His Val Val Ser Ile Ser Gly
1 5 10 15

Val Pro Leu Gln Leu Gln Pro Asn Ser Ile Gln Thr Lys Val Asp Ser
20 25 30

```
Ile Ala Trp Lys Lys Leu Leu Pro Ser Gln Asn Gly Phe His His Ile
        35                  40                  45

Leu Lys Trp Glu Asn Gly Ser Leu Pro Ser Asn Thr Ser Asn Asp Arg
    50                  55                  60

Phe Ser Phe Ile Val Lys Asn Leu Ser Leu Leu Ile Lys Ala Ala Gln
65                  70                  75                  80

Gln Gln Asp Ser Gly Leu Tyr Cys Leu Glu Val Thr Ser Ile Ser Gly
                85                  90                  95

Lys Val Gln Thr Ala Thr Phe Gln Val Phe Val Phe Asp Lys Val Glu
            100                 105                 110

Lys Pro Arg Leu Gln Gly Gln Gly Lys Ile Leu Asp Arg Gly Arg Cys
        115                 120                 125

Gln Val Ala Leu Ser Cys Leu Val Ser Arg Asp Gly Asn Val Ser Tyr
    130                 135                 140

Ala Trp Tyr Arg Gly Ser Lys Leu Ile Gln Thr Ala Gly Asn Leu Thr
145                 150                 155                 160

Tyr Leu Asp Glu Glu Val Asp Ile Asn Gly Thr His Thr Tyr Thr Cys
                165                 170                 175

Asn Val Ser Asn Pro Val Ser Trp Glu Ser His Thr Leu Asn Leu Thr
            180                 185                 190

Gln Asp Cys Gln Asn Ala His Gln Glu Phe Arg Phe Trp Pro Phe Leu
        195                 200                 205

Val Ile Ile Val Ile Leu Ser Ala Leu Phe Leu Gly Thr Leu Ala Cys
    210                 215                 220
```

```
Phe Cys Val Trp Arg Arg Lys Arg Lys Glu Lys Gln Ser Glu Thr Ser
225                 230                 235                 240


Pro Lys Glu Phe Leu Thr Ile Tyr Glu Asp Val Lys Asp Leu Lys Thr
                245                 250                 255


Arg Arg Asn His Glu Gln Glu Gln Thr Phe Pro Gly Gly Gly Ser Thr
            260                 265                 270


Ile Tyr Ser Met Ile Gln Ser Gln Ser Ser Ala Pro Thr Ser Gln Glu
        275                 280                 285


Pro Ala Tyr Thr Leu Tyr Ser Leu Ile Gln Pro Ser Arg Lys Ser Gly
    290                 295                 300


Ser Arg Lys Arg Asn His Ser Pro Ser Phe Asn Ser Thr Ile Tyr Glu
305                 310                 315                 320


Val Ile Gly Lys Ser Gln Pro Lys Ala Gln Asn Pro Ala Arg Leu Ser
                325                 330                 335


Arg Lys Glu Leu Glu Asn Phe Asp Val Tyr Ser
            340                 345


<210> 14
<211> 1098
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="HB4"

<400> 14
atgctggggc aagtggtcac cctcatactc ctcctgctcc tcaaggtgta tcagggcaaa       60

ggatgccagg gatcagctga ccatgtggtt agcatctcgg gagtgcctct tcagttacaa      120

ccaaacagca tacagacgaa ggttgacagc attgcatgga agaagttgct gccctcacaa      180

aatggatttc atcacatatt gaagtgggag aatggctctt tgccttccaa tacttccaat      240
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gatagattca gttttatagt caagaacttg agtcttctca tcaaggcagc tcagcagcag 300

gacagtggcc tctactgcct ggaggtcacc agtatatctg gaaaagttca gacagccacg 360

ttccaggttt ttgtatttga taaagttgag aaaccccgcc tacaggggca ggggaagatc 420

ctggacagag ggagatgcca agtggctctg tcttgcttgg tctccaggga tggcaatgtg 480

tcctatgctt ggtacagagg gagcaagctg atccagacag cagggaacct cacctacctg 540

gacgaggagg ttgacattaa tggcactcac acatatacct gcaatgtcag caatcctgtt 600

agctgggaaa gccacaccct gaatctcact caggactgtc agaatgccca tcaggaattc 660

agattttggc cgtttttggt gatcatcgtg attctaagcg cactgttcct tggcaccctt 720

gcctgcttct gtgtgtggag gagaaagagg aaggagaagc agtcagagac cagtcccaag 780

gaatttttga caatttacga agatgtcaag gatctgaaaa ccaggagaaa tcacgagcag 840

gagcagactt ttcctggagg ggggagcacc atctactcta tgatccagtc ccagtcttct 900

gctcccacgt cacaagaacc tgcatataca ttatattcat taattcagcc ttccaggaag 960

tctggatcca ggaagaggaa ccacagccct tccttcaata gcactatcta tgaagtgatt 1020

ggaaagagtc aacctaaagc ccagaaccct gctcgattga gccgcaaaga gctggagaac 1080

tttgatgttt attcctag 1098

<210> 15
<211> 183
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NK-p30"

<400> 15
Leu Trp Val Ser Gln Pro Pro Glu Ile Arg Thr Leu Glu Gly Ser Ser
1 5 10 15

Ala Phe Leu Pro Cys Ser Phe Asn Ala Ser Gln Gly Arg Leu Ala Ile
20 25 30

Gly Ser Val Thr Trp Phe Arg Asp Glu Val Val Pro Gly Lys Glu Val

```
        35                  40                  45

Arg Asn Gly Thr Pro Glu Phe Arg Gly Arg Leu Ala Pro Leu Ala Ser
    50                  55                  60

Ser Arg Phe Leu His Asp His Gln Ala Glu Leu His Ile Arg Asp Val
65                  70                  75                  80

Arg Gly His Asp Ala Ser Ile Tyr Val Cys Arg Val Glu Val Leu Gly
                85                  90                  95

Leu Gly Val Gly Thr Gly Asn Gly Thr Arg Leu Val Val Glu Lys Glu
            100                 105                 110

His Pro Gln Leu Gly Ala Gly Thr Val Leu Leu Leu Arg Ala Gly Phe
        115                 120                 125

Tyr Ala Val Ser Phe Leu Ser Val Ala Val Gly Ser Thr Val Tyr Tyr
    130                 135                 140

Gln Gly Lys Cys Leu Thr Trp Lys Gly Pro Arg Arg Gln Leu Pro Ala
145                 150                 155                 160

Val Val Pro Ala Pro Leu Pro Pro Pro Cys Gly Ser Ser Ala His Leu
                165                 170                 175

Leu Pro Pro Val Pro Gly Gly
            180


<210> 16
<211> 573
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="NKp30"

<400> 16
atggcctgga tgctgttgct catcttgatc atggtccatc caggatcctg tgctctctgg       60
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gtgtcccagc cccctgagat tcgtaccctg gaaggatcct ctgccttcct gccctgctcc 120

ttcaatgcca gccaagggag actggccatt ggctccgtca cgtggttccg agatgaggtg 180

gttccaggga aggaggtgag gaatggaacc ccagagttca ggggccgcct ggccccactt 240

gcttcttccc gtttcctcca tgaccaccag gctgagctgc acatccggga cgtgcgaggc 300

catgacgcca gcatctacgt gtgcagagtg gaggtgctgg gccttggtgt cgggacaggg 360

aatgggactc ggctggtggt ggagaaagaa catcctcagc taggggctgg tacagtcctc 420

ctccttcggg ctggattcta tgctgtcagc tttctctctg tggccgtggg cagcaccgtc 480

tattaccagg gcaaatgcca ctgtcacatg ggaacacact gccactcctc agatgggccc 540

cgaggagtga ttccagagcc cagatgtccc tag 573

<210> 17
<211> 255
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NKp44"

<400> 17
Gln Ser Lys Ala Gln Val Leu Gln Ser Val Ala Gly Gln Thr Leu Thr
1 5 10 15

Val Arg Cys Gln Tyr Pro Pro Thr Gly Ser Leu Tyr Glu Lys Lys Gly
20 25 30

Trp Cys Lys Glu Ala Ser Ala Leu Val Cys Ile Arg Leu Val Thr Ser
35 40 45

Ser Lys Pro Arg Thr Met Ala Trp Thr Ser Arg Phe Thr Ile Trp Asp
50 55 60

Asp Pro Asp Ala Gly Phe Phe Thr Val Thr Met Thr Asp Leu Arg Glu
65 70 75 80

```
Glu Asp Ser Gly His Tyr Trp Cys Arg Ile Tyr Arg Pro Ser Asp Asn
                85                  90                  95

Ser Val Ser Lys Ser Val Arg Phe Tyr Leu Val Val Ser Pro Ala Ser
            100                 105                 110

Ala Ser Thr Gln Thr Ser Trp Thr Pro Arg Asp Leu Val Ser Ser Gln
        115                 120                 125

Thr Gln Thr Gln Ser Cys Val Pro Pro Thr Ala Gly Ala Arg Gln Ala
    130                 135                 140

Pro Glu Ser Pro Ser Thr Ile Pro Val Pro Ser Gln Pro Gln Asn Ser
145                 150                 155                 160

Thr Leu Arg Pro Gly Pro Ala Ala Pro Ile Ala Leu Val Pro Val Phe
                165                 170                 175

Cys Gly Leu Leu Val Ala Lys Ser Leu Val Leu Ser Ala Leu Leu Val
            180                 185                 190

Trp Trp Gly Asp Ile Trp Trp Lys Thr Met Met Glu Leu Arg Ser Leu
        195                 200                 205

Asp Thr Gln Lys Ala Thr Cys His Leu Gln Gln Val Thr Asp Leu Pro
    210                 215                 220

Trp Thr Ser Val Ser Ser Pro Val Glu Arg Glu Ile Leu Tyr His Thr
225                 230                 235                 240

Val Ala Arg Thr Lys Ile Ser Asp Asp Asp Asp Glu His Thr Leu
                245                 250                 255
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<210> 18
<211> 777
<212> DNA
<213> Homo sapiens

<220>

<221> source
<223> /note="NKp44"

<400> 18
atggcctggc gagccctaca cccactgcta ctgctgctgc tgctgttccc aggctctcag 60

gcacaatcca aggctcaggt acttcaaagt gtggcagggc agacgctaac cgtgagatgc 120

cagtacccgc ccacgggcag tctctacgag aagaaaggct ggtgtaagga ggcttcagca 180

cttgtgtgca tcaggttagt caccagctcc aagcccagga cgatggcttg gacctctcga 240

ttcacaatct gggacgaccc tgatgctggc ttcttcactg tcaccatgac tgatctgaga 300

gaggaagact caggacatta ctggtgtaga atctaccgcc cttctgacaa ctctgtctct 360

aagtccgtca gattctatct ggtggtatct ccagcctctg cctccacaca gacctcctgg 420

actccccgcg acctggtctc ttcacagacc cagacccaga gctgtgtgcc tcccactgca 480

ggagccagac aagcccctga gtctccatct accatccctg tcccttcaca gccacagaac 540

tccacgctcc gccctggccc tgcagccccc attgccctgg tgcctgtgtt ctgtggactc 600

ctcgtagcca agagcctggt gctgtcagcc ctgctcgtct ggtgggtttt aaggaatcgg 660

cacatgcagc atcaagggag gtctctgctg cacccagctc agcccaggcc ccaggcccat 720

agacacttcc cactgagcca cagggcacca ggggggacat atggtggaaa accatga 777

<210> 19
<211> 283
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NKp46"

<400> 19
Gln Gln Gln Thr Leu Pro Lys Pro Phe Ile Trp Ala Glu Pro His Phe
1 5 10 15

Met Val Pro Lys Glu Lys Gln Val Thr Ile Cys Cys Gln Gly Asn Tyr
20 25 30

Gly Ala Val Glu Tyr Gln Leu His Phe Glu Gly Ser Leu Phe Ala Val

```
        35                  40                  45

Asp Arg Pro Lys Pro Pro Glu Arg Ile Asn Lys Val Gln Phe Tyr Ile
    50                  55                  60

Pro Asp Met Asn Ser Arg Met Ala Gly Gln Tyr Ser Cys Ile Tyr Arg
65                  70                  75                  80

Val Gly Glu Leu Trp Ser Glu Pro Ser Asn Leu Leu Asp Leu Val Val
                85                  90                  95

Thr Glu Met Tyr Asp Thr Pro Thr Leu Ser Val His Pro Gly Pro Glu
            100                 105                 110

Val Ile Ser Gly Glu Lys Val Thr Phe Tyr Cys Arg Leu Asp Thr Ala
        115                 120                 125

Thr Ser Met Phe Leu Leu Leu Lys Glu Gly Arg Ser Ser His Val Gln
    130                 135                 140

Arg Gly Tyr Gly Lys Val Gln Ala Glu Phe Pro Leu Gly Pro Val Thr
145                 150                 155                 160

Thr Ala His Arg Gly Thr Tyr Arg Cys Phe Gly Ser Tyr Asn Asn His
                165                 170                 175

Ala Trp Ser Phe Pro Ser Glu Pro Val Lys Leu Leu Val Thr Gly Asp
            180                 185                 190

Ile Glu Asn Thr Ser Leu Ala Pro Glu Asp Pro Thr Phe Pro Ala Asp
        195                 200                 205

Thr Trp Gly Thr Tyr Leu Leu Thr Thr Glu Thr Gly Leu Gln Lys Asp
    210                 215                 220

His Ala Leu Trp Asp His Thr Ala Gln Asn Leu Leu Arg Met Gly Leu
225                 230                 235                 240
```

Ala Phe Leu Val Leu Val Ala Leu Val Trp Phe Leu Val Glu Asp Trp
245 250 255

Leu Ser Arg Lys Arg Thr Arg Glu Arg Ala Ser Arg Ala Ser Thr Trp
260 265 270

Glu Gly Arg Arg Arg Leu Asn Thr Gln Thr Leu
275 280

<210> 20
<211> 777
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="NKp46"

<400> 20
atggcctggc gagccctaca cccactgcta ctgctgctgc tgctgttccc aggctctcag 60

gcacaatcca aggctcaggt acttcaaagt gtggcagggc agacgctaac cgtgagatgc 120

cagtacccgc ccacgggcag tctctacgag aagaaaggct ggtgtaagga ggcttcagca 180

cttgtgtgca tcaggttagt caccagctcc aagcccagga cgatggcttg gacctctcga 240

ttcacaatct gggacgaccc tgatgctggc ttcttcactg tcaccatgac tgatctgaga 300

gaggaagact caggacatta ctggtgtaga atctaccgcc cttctgacaa ctctgtctct 360

aagtccgtca gattctatct ggtggtatct ccagcctctg cctccacaca gacctcctgg 420

actccccgcg acctggtctc ttcacagacc cagacccaga gctgtgtgcc tcccactgca 480

ggagccagac aagcccctga gtctccatct accatccctg tcccttcaca gccacagaac 540

tccacgctcc gccctggccc tgcagccccc attgccctgg tgcctgtgtt ctgtggactc 600

ctcgtagcca agagcctggt gctgtcagcc ctgctcgtct ggtgggtttt aaggaatcgg 660

cacatgcagc atcaagggag gtctctgctg cacccagctc agcccaggcc ccaggcccat 720

agacacttcc cactgagcca cagggcacca ggggggacat atggtggaaa accatga 777

<210> 21
<211> 216
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NKG2D"

<400> 21
Met Gly Trp Ile Arg Gly Arg Arg Ser Arg His Ser Trp Glu Met Ser
1   5   10   15

Glu Phe His Asn Tyr Asn Leu Asp Leu Lys Lys Ser Asp Phe Ser Thr
            20   25   30

Arg Trp Gln Lys Gln Arg Cys Pro Val Val Lys Ser Lys Cys Arg Glu
        35   40   45

Asn Ala Ser Pro Phe Phe Phe Cys Cys Phe Ile Ala Val Ala Met Gly
    50   55   60

Ile Arg Phe Ile Ile Met Val Ala Ile Trp Ser Ala Val Phe Leu Asn
65   70   75   80

Ser Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys
                85   90   95

Gly Pro Cys Pro Lys Asn Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln
            100   105   110

Phe Phe Asp Glu Ser Lys Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met
        115   120   125

Ser Gln Asn Ala Ser Leu Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp
    130   135   140

Leu Leu Lys Leu Val Lys Ser Tyr His Trp Met Gly Leu Val His Ile
145   150   155   160

```
Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro
                165                 170                 175

Asn Leu Leu Thr Ile Ile Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr
            180                 185                 190

Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr
        195                 200                 205

Tyr Ile Cys Met Gln Arg Thr Val
    210                 215
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<210> 22
<211> 651
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="NKG2D"

<400> 22

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atggggtgga ttcgtggtcg gaggtctcga cacagctggg agatgagtga atttcataat       60

tataacttgg atctgaagaa gagtgatttt tcaacacgat ggcaaaagca aagatgtcca      120

gtagtcaaaa gcaaatgtag agaaaatgca tctccatttt ttttctgctg cttcatcgct      180

gtagccatgg gaatccgttt cattattatg gtaacaatat ggagtgctgt attcctaaac      240

tcattattca accaagaagt tcaaattccc ttgaccgaaa gttactgtgg cccatgtcct      300

aaaaactgga tatgttacaa aaataactgc taccaatttt ttgatgagag taaaaactgg      360

tatgagagcc aggcttcttg tatgtctcaa aatgccagcc ttctgaaagt atacagcaaa      420

gaggaccagg atttacttaa actggtgaag tcatatcatt ggatgggact agtacacatt      480

ccaacaaatg gatcttggca gtgggaagat ggctccattc tctcacccaa cctactaaca      540

ataattgaaa tgcagaaggg agactgtgca ctctatgcct cgagctttaa aggctatata      600

gaaaactgtt caactccaaa tacgtacatc tgcatgcaaa ggactgtgta a                651
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<210> 23

<211> 359
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 23
Met Ala Glu Gly Thr Leu Trp Gln Ile Leu Cys Val Ser Ser Asp Ala
1   5   10   15

Gln Pro Gln Thr Phe Glu Gly Val Lys Gly Ala Asp Pro Pro Thr Leu
            20   25   30

Pro Pro Gly Ser Phe Leu Pro Gly Pro Val Leu Trp Trp Gly Ser Leu
        35   40   45

Ala Arg Leu Gln Thr Glu Lys Ser Asp Glu Val Ser Arg Lys Gly Asn
    50   55   60

Trp Trp Val Thr Glu Met Gly Gly Gly Ala Gly Glu Arg Leu Phe Thr
65   70   75   80

Ser Ser Cys Leu Val Gly Leu Val Pro Leu Gly Leu Arg Ile Ser Leu
                85   90   95

Val Thr Cys Pro Leu Gln Cys Gly Ile Met Trp Gln Leu Leu Leu Pro
            100   105   110

Thr Ala Leu Leu Leu Leu Val Ser Ala Gly Met Arg Thr Glu Asp Leu
        115   120   125

Pro Lys Ala Val Val Phe Leu Glu Pro Gln Trp Tyr Arg Val Leu Glu
    130   135   140

Lys Asp Ser Val Thr Leu Lys Cys Gln Gly Ala Tyr Ser Pro Glu Asp
145   150   155   160

Asn Ser Thr Gln Trp Phe His Asn Glu Ser Leu Ile Ser Ser Gln Ala

```
                165                 170                 175

Ser Ser Tyr Phe Ile Asp Ala Ala Thr Val Asp Asp Ser Gly Glu Tyr
            180                 185                 190

Arg Cys Gln Thr Asn Leu Ser Thr Leu Ser Asp Pro Val Gln Leu Glu
        195                 200                 205

Val His Ile Gly Trp Leu Leu Leu Gln Ala Pro Arg Trp Val Phe Lys
    210                 215                 220

Glu Glu Asp Pro Ile His Leu Arg Cys His Ser Trp Lys Asn Thr Ala
225                 230                 235                 240

Leu His Lys Val Thr Tyr Leu Gln Asn Gly Lys Gly Arg Lys Tyr Phe
                245                 250                 255

His His Asn Ser Asp Phe Tyr Ile Pro Lys Ala Thr Leu Lys Asp Ser
            260                 265                 270

Gly Ser Tyr Phe Cys Arg Gly Leu Phe Gly Ser Lys Asn Val Ser Ser
        275                 280                 285

Glu Thr Val Asn Ile Thr Ile Thr Gln Gly Leu Ala Val Ser Thr Ile
    290                 295                 300

Ser Ser Phe Phe Pro Pro Gly Tyr Gln Val Ser Phe Cys Leu Val Met
305                 310                 315                 320

Val Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe Ser Val Lys Thr
                325                 330                 335

Asn Ile Arg Ser Ser Thr Arg Asp Trp Lys Asp His Lys Phe Lys Trp
            340                 345                 350

Arg Lys Asp Pro Gln Asp Lys
        355
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<210> 24
<211> 1080
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 24
atggctgagg gcacactctg gcagattctg tgtgtgtcct cagatgctca gccacagacc 60

tttgagggag taaaggggc agacccaccc accttgcctc caggctcttt ccttcctggt 120

cctgttctat ggtggggctc ccttgccaga cttcagactg agaagtcaga tgaagtttca 180

agaaaaggaa attggtgggt gacagagatg ggtggagggg ctggggaaag gctgtttact 240

tcctcctgtc tagtcggttt ggtcccttta gggctccgga tatctttggt gacttgtcca 300

ctccagtgtg gcatcatgtg gcagctgctc ctcccaactg ctctgctact tctagtttca 360

gctggcatgc ggactgaaga tctcccaaag gctgtggtgt tcctggagcc tcaatggtac 420

agggtgctcg agaaggacag tgtgactctg aagtgccagg gagcctactc ccctgaggac 480

aattccacac agtggtttca caatgagagc ctcatctcaa gccaggcctc gagctacttc 540

attgacgctg ccacagtcga cgacagtgga gagtacaggt gccagacaaa cctctccacc 600

ctcagtgacc cggtgcagct agaagtccat atcggctggc tgttgctcca ggcccctcgg 660

tgggtgttca aggaggaaga ccctattcac ctgaggtgtc acagctggaa gaacactgct 720

ctgcataagg tcacatattt acagaatggc aaaggcagga agtattttca tcataattct 780

gacttctaca ttccaaaagc cacactcaaa gacagcggct cctacttctg caggggggctt 840

tttgggagta aaaatgtgtc ttcagagact gtgaacatca ccatcactca aggtttggca 900

gtgtcaacca tctcatcatt ctttccacct gggtaccaag tctctttctg cttggtgatg 960

gtactccttt ttgcagtgga cacaggacta tatttctctg tgaagacaaa cattcgaagc 1020

tcaacaagag actggaagga ccataaattt aaatggagaa aggaccctca agacaaatga 1080

<210> 25
<211> 233

<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 25
Met Trp Gln Leu Leu Leu Pro Thr Ala Leu Leu Leu Leu Val Ser Ala
1   5   10   15

Gly Met Arg Thr Glu Asp Leu Pro Lys Ala Val Val Phe Leu Glu Pro
            20   25   30

Gln Trp Tyr Ser Val Leu Glu Lys Asp Ser Val Thr Leu Lys Cys Gln
        35   40   45

Gly Ala Tyr Ser Pro Glu Asp Asn Ser Thr Gln Trp Phe His Asn Glu
    50   55   60

Ser Leu Ile Ser Ser Gln Ala Ser Ser Tyr Phe Ile Asp Ala Ala Thr
65   70   75   80

Val Asn Asp Ser Gly Glu Tyr Arg Cys Gln Thr Asn Leu Ser Thr Leu
                85   90   95

Ser Asp Pro Val Gln Leu Glu Val His Ile Gly Trp Leu Leu Leu Gln
            100   105   110

Ala Pro Arg Trp Val Phe Lys Glu Glu Asp Pro Ile His Leu Arg Cys
        115   120   125

His Ser Trp Lys Asn Thr Ala Leu His Lys Val Thr Tyr Leu Gln Asn
    130   135   140

Gly Lys Asp Arg Lys Tyr Phe His His Asn Ser Asp Phe His Ile Pro
145   150   155   160

Lys Ala Thr Leu Lys Asp Ser Gly Ser Tyr Phe Cys Arg Gly Leu Val
                165   170   175

Gly Ser Lys Asn Val Ser Ser Glu Thr Val Asn Ile Thr Ile Thr Gln
180 185 190

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Ser Pro Pro Gly Tyr Gln
195 200 205

Val Ser Phe Cys Leu Val Met Val Leu Leu Phe Ala Val Asp Thr Gly
210 215 220

Leu Tyr Phe Ser Val Lys Thr Asn Ile
225 230

<210> 26
<211> 702
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 26
atgtggcagc tgctcctccc aactgctctg ctacttctag tttcagctgg catgcggact 60

gaagatctcc caaaggctgt ggtgttcctg gagcctcaat ggtacagcgt gcttgagaag 120

gacagtgtga ctctgaagtg ccagggagcc tactcccctg aggacaattc cacacagtgg 180

tttcacaatg agaacctcat ctcaagccag gcctcgagct acttcattga cgctgccaca 240

gtcaacgaca gtggagagta caggtgccag acaaacctct ccaccctcag tgacccggtg 300

cagctagaag tccatatcgg ctggctgttg ctccaggccc ctcggtgggt gttcaaggag 360

gaagacccta ttcacctgag gtgtcacagc tggaagaaca ctgctctgca taaggtcaca 420

tatttacaga atggcaaaga caggaagtat tttcatcata attctgactt ccacattcca 480

aaagccacac tcaaagatag cggctcctac ttctgcaggg ggcttgttgg gagtaaaaat 540

gtgtcttcag agactgtgaa catcaccatc actcaaggtt tggcagtgtc aaccatctca 600

tcattctctc cacctgggta ccaagtctct ttctgcttgg tgatggtact ccttttgca 660

gtggacacag gactatattt ctctgtgaag acaaacattt ga 702

<210> 27
<211> 304
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS1"

<400> 27
Met Ser Leu Thr Val Val Ser Met Ala Cys Val Gly Phe Phe Leu Leu
1 5 10 15

Gln Gly Ala Trp Pro His Glu Gly Val His Arg Lys Pro Ser Leu Leu
20 25 30

Ala His Pro Gly Arg Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln
35 40 45

Cys Trp Ser Asp Val Met Phe Glu His Phe Leu Leu His Arg Glu Gly
50 55 60

Met Phe Asn Asp Thr Leu Arg Leu Ile Gly Glu His His Asp Gly Val
65 70 75 80

Ser Lys Ala Asn Phe Ser Ile Ser Arg Met Lys Gln Asp Leu Ala Gly
85 90 95

Thr Tyr Arg Cys Tyr Gly Ser Val Thr His Ser Pro Tyr Gln Val Ser
100 105 110

Ala Pro Ser Asp Pro Leu Asp Ile Val Ile Ile Gly Leu Tyr Glu Lys
115 120 125

Pro Ser Leu Ser Ala Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser
130 135 140

Val Thr Leu Ser Cys Ser Ser Arg Ser Ser Tyr Asp Met Tyr His Leu

145 150 155 160

Ser Arg Glu Gly Glu Ala His Glu Arg Arg Leu Pro Ala Gly Thr Lys
165 170 175

Val Asn Gly Thr Phe Gln Ala Asn Phe Pro Leu Gly Pro Ala Thr His
180 185 190

Gly Gly Thr Tyr Arg Cys Phe Gly Ser Phe Arg Asp Ser Pro Tyr Glu
195 200 205

Trp Ser Lys Ser Ser Asp Pro Leu Leu Val Ser Val Thr Gly Asn Pro
210 215 220

Ser Asn Ser Trp Pro Ser Pro Thr Glu Pro Ser Ser Glu Thr Gly Asn
225 230 235 240

Pro Arg His Leu His Val Leu Ile Gly Thr Ser Val Val Lys Ile Pro
245 250 255

Phe Thr Ile Leu Leu Phe Phe Leu Leu His Arg Trp Cys Ser Asp Lys
260 265 270

Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asn Arg Thr Val
275 280 285

Asn Ser Glu Asp Ser Asp Glu Gln Asp His Gln Glu Val Ser Tyr Ala
290 295 300

<210> 28
<211> 915
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS1"

<400> 28
atgtcgctca cggtcgtcag catggcgtgt gttgggttct tcttgctgca gggggcctgg 60

ccacatgagg gagtccacag aaaaccttcc ctcctggccc acccaggtcg cctggtgaaa 120

tcagaagaga cagtcatcct gcaatgttgg tcagatgtca tgtttgaaca cttccttctg 180

cacagagagg ggatgtttaa cgacactttg cgcctcattg gagaacacca tgatggggtc 240

tccaaggcca acttctccat cagtcgcatg aagcaagacc tggcagggac ctacagatgc 300

tacggttctg ttactcactc cccctatcag ttgtcagctc ccagtgaccc tctggacatc 360

gtgatcatag gtctatatga gaaaccttct ctctcagccc agccgggccc cacggttctg 420

gcaggagaga atgtgacctt gtcctgcagc tcccggagct cctatgacat gtaccatcta 480

tccagggaag gggaggccca tgaacgtagg ctccctgcag ggaccaaggt caacggaaca 540

ttccaggcca actttcctct gggccctgcc acccatggag ggacctacag atgcttcggc 600

tctttccgtg actctccata cgagtggtca aagtcaagtg acccactgct tgtttctgtc 660

acaggaaacc cttcaaatag ttggccttca cccactgaac caagctccga aaccggtaac 720

cccagacacc tacatgttct gattgggacc tcagtggtca aaatcccttt caccatcctc 780

ctcttctttc tccttcatcg ctggtgctcc gacaaaaaaa atgctgctgt aatggaccaa 840

gagcctgcag ggaacagaac agtgaacagc gaggattctg atgaacaaga ccatcaggag 900

gtgtcatacg cataa 915

<210> 29
<211> 304
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS2"

<400> 29
Met Ser Leu Met Val Val Ser Met Val Cys Val Gly Phe Phe Leu Leu
1 5 10 15

Gln Gly Ala Trp Pro His Glu Gly Val His Arg Lys Pro Ser Leu Leu
20 25 30

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Ala His Pro Gly Pro Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln
        35                  40                  45

Cys Trp Ser Asp Val Arg Phe Glu His Phe Leu Leu His Arg Glu Gly
    50                  55                  60

Lys Tyr Lys Asp Thr Leu His Leu Ile Gly Glu His His Asp Gly Val
65                  70                  75                  80

Ser Lys Ala Asn Phe Ser Ile Gly Pro Met Met Gln Asp Leu Ala Gly
                85                  90                  95

Thr Tyr Arg Cys Tyr Gly Ser Val Thr His Ser Pro Tyr Gln Leu Ser
            100                 105                 110

Ala Pro Ser Asp Pro Leu Asp Ile Val Ile Thr Gly Leu Tyr Glu Lys
        115                 120                 125

Pro Ser Leu Ser Ala Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser
    130                 135                 140

Val Thr Leu Ser Cys Ser Ser Arg Ser Ser Tyr Asp Met Tyr His Leu
145                 150                 155                 160

Ser Arg Glu Gly Glu Ala His Glu Arg Arg Phe Ser Ala Gly Pro Lys
                165                 170                 175

Val Asn Gly Thr Phe Gln Ala Asp Phe Pro Leu Gly Pro Ala Thr His
            180                 185                 190

Gly Gly Thr Tyr Arg Cys Phe Gly Ser Phe Arg Asp Ser Pro Tyr Glu
        195                 200                 205

Trp Ser Asn Ser Ser Asp Pro Leu Leu Val Ser Val Thr Gly Asn Pro
    210                 215                 220

Ser Asn Ser Trp Pro Ser Pro Thr Glu Pro Ser Ser Lys Thr Gly Asn
225                 230                 235                 240
```

```
Pro Arg His Leu His Val Leu Ile Gly Thr Ser Val Val Lys Ile Pro
               245                 250                 255

Phe Thr Ile Leu Leu Phe Phe Leu Leu His Arg Trp Cys Ser Asn Lys
            260                 265                 270

Lys Asn Ala Ala Val Met Asp Gln Glu Pro Ala Gly Asn Arg Thr Val
        275                 280                 285

Asn Ser Glu Asp Ser Asp Glu Gln Asp His Gln Glu Val Ser Tyr Ala
    290                 295                 300
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<210> 30
<211> 1026
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS2"

<400> 30
atgtcgctca tggtcgtcag catggcgtgt gttgggttct tcttgctgca gggggcctgg 60

ccacatgagg gagtccacag aaaaccttcc ctcctggccc acccaggtcc cctggtgaaa 120

tcagaagaga cagtcatcct gcaatgttgg tcagatgtca ggtttgagca cttccttctg 180

cacagagagg ggaagtataa ggacactttg cacctcattg gagagcacca tgatggggtc 240

tccaaggcca acttctccat cggtcccatg atgcaagacc ttgcagggac ctacagatgc 300

tacggttctg ttactcactc cccctatcag ttgtcagctc ccagtgaccc tctggacatc 360

gtcatcacag gtctatatga gaaaccttct ctctcagccc agccgggccc cacggttttg 420

gcaggagaga gcgtgacctt gtcctgcagc tcccggagct cctatgacat gtaccatcta 480

tccagggagg gggaggccca tgaacgtagg ttctctgcag ggcccaaggt caacggaaca 540

ttccaggccg actttcctct gggccctgcc acccacggag gaacctacag atgcttcggc 600

tctttccgtg actctcccta tgagtggtca aactcgagtg acccactgct tgtttctgtc 660

acaggaaacc cttcaaatag ttggccttca cccactgaac caagctccaa aaccggtaac 720

cccagacacc tgcatgttct gattgggacc tcagtggtca aaatcccttt caccatcctc 780

ctcttctttc tccttcatcg ctggtgctcc aacaaaaaaa atgctgctgt aatggaccaa 840

gagcctgcag ggaacagaac agtgaacagc gaggactctg atgaacaaga ccctcaggag 900

gtgacataca cacagttgaa tcactgcgtt ttcacacaga gaaaaatcac tcgcccttct 960

cagaggccca agacaccccc aacagatatc atcgtgtaca cggaacttcc aaatgctgag 1020

tccaga 1026

<210> 31
<211> 304
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS3"

<400> 31
Met Ser Leu Met Val Ile Ser Met Ala Cys Val Gly Phe Phe Trp Leu
1 5 10 15

Gln Gly Ala Trp Pro His Glu Gly Phe Arg Arg Lys Pro Ser Leu Leu
20 25 30

Ala His Pro Gly Arg Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln
35 40 45

Cys Trp Ser Asp Val Met Phe Glu His Phe Leu Leu His Arg Glu Gly
50 55 60

Thr Phe Asn Asp Thr Leu Arg Leu Ile Gly Glu His Ile Asp Gly Val
65 70 75 80

Ser Lys Ala Asn Phe Ser Ile Gly Arg Met Arg Gln Asp Leu Ala Gly
85 90 95

Thr Tyr Arg Cys Tyr Gly Ser Val Pro His Ser Pro Tyr Gln Phe Ser

```
            100                 105                 110

Ala Pro Ser Asp Pro Leu Asp Ile Val Ile Thr Gly Leu Tyr Glu Lys
        115                 120                 125

Pro Ser Leu Ser Ala Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser
    130                 135                 140

Val Thr Leu Ser Cys Ser Ser Trp Ser Ser Tyr Asp Met Tyr His Leu
145                 150                 155                 160

Ser Thr Glu Gly Glu Ala His Glu Arg Arg Phe Ser Ala Gly Pro Lys
                165                 170                 175

Val Asn Gly Thr Phe Gln Ala Asp Phe Pro Leu Gly Pro Ala Thr Gln
            180                 185                 190

Gly Gly Thr Tyr Arg Cys Phe Gly Ser Phe His Asp Ser Pro Tyr Glu
        195                 200                 205

Trp Ser Lys Ser Ser Asp Pro Leu Leu Val Ser Val Thr Gly Asn Pro
    210                 215                 220

Ser Asn Ser Trp Pro Ser Pro Thr Glu Pro Ser Ser Lys Thr Gly Asn
225                 230                 235                 240

Pro Arg His Leu His Val Leu Ile Gly Thr Ser Val Val Lys Leu Pro
                245                 250                 255

Phe Thr Ile Leu Leu Phe Phe Leu Leu His Arg Trp Cys Ser Asp Lys
            260                 265                 270

Lys Asn Ala Ser Val Met Asp Gln Gly Pro Ala Gly Asn Arg Thr Val
        275                 280                 285

Asn Arg Glu Asp Ser Asp Glu Gln Asp His Gln Glu Val Ser Tyr Ala
    290                 295                 300
```

<210> 32
<211> 915
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS3"

<400> 32
atgtcgctca tggtcatcag catggcatgt gttgggttct tctggctgca gggggcctgg 60

ccacatgagg gattccgcag aaaaccttcc ctcctggccc acccaggtcg cctggtgaaa 120

tcagaagaga cagtcatcct gcaatgttgg tcagatgtca tgtttgagca cttccttctg 180

cacagagagg ggacgtttaa cgacactttg cgcctcattg gagagcacat tgatggggtc 240

tccaaggcca acttctccat cggtcgcatg aggcaagacc tggcagggac ctacagatgc 300

tacggttctg ttcctcactc cccctatcag ttttcagctc ccagtgaccc tctggacatc 360

gtgatcacag gtctatatga gaaaccttct ctctcagccc agccgggccc cacggttctg 420

gcaggagaga gcgtgacctt gtcctgcagc tcctggagct cctatgacat gtaccatcta 480

tccacggagg gggaggccca tgaacgtagg ttctctgcag ggcccaaggt caacggaaca 540

ttccaggccg actttcctct gggccctgcc acccaaggag gaacctacag atgcttcggc 600

tctttccatg actctcccta cgagtggtca aagtcaagtg acccactgct tgtttctgtc 660

acaggaaacc cttcaaatag ttggccttca cccactgaac caagctccaa aaccggtaac 720

cccagacacc tacacgttct gattgggacc tcagtggtca aactcccttt caccatcctc 780

ctcttctttc tccttcatcg ctggtgctcc gacaaaaaaa atgcatctgt aatggaccaa 840

gggcctgcgg ggaacagaac agtgaacagg gaggattctg atgaacagga ccatcaggag 900

gtgtcatacg cataa 915

<210> 33
<211> 354
<212> PRT
<213> Homo sapiens

<220>

<221> source
<223> /note="KIR2DL4"

<400> 33
His Val Gly Gly Gln Asp Lys Pro Phe Cys Ser Ala Trp Pro Ser Ala
1   5   10   15

Val Val Pro Gln Gly Gly His Ala Thr Leu Arg Cys His Cys Arg Arg
            20   25   30

Gly Phe Asn Ile Phe Thr Leu Tyr Lys Lys Asp Gly Val Pro Val Pro
        35   40   45

Glu Leu Tyr Asn Arg Ile Phe Trp Asn Ser Phe Leu Ile Ser Pro Val
    50   55   60

Thr Pro Ala His Ala Gly Thr Tyr Arg Cys Arg Gly Phe His Pro His
65   70   75   80

Ser Pro Thr Glu Trp Ser Ala Pro Ser Asn Pro Leu Val Ile Met Val
                85   90   95

Thr Gly Leu Tyr Glu Lys Pro Ser Leu Thr Ala Arg Pro Gly Pro Thr
            100   105   110

Val Arg Ala Gly Glu Asn Val Thr Leu Ser Cys Ser Ser Gln Ser Ser
        115   120   125

Phe Asp Ile Tyr His Leu Ser Arg Glu Gly Glu Ala His Glu Leu Arg
    130   135   140

Leu Pro Ala Val Pro Ser Ile Asn Gly Thr Phe Gln Ala Asp Phe Pro
145   150   155   160

Leu Gly Pro Ala Thr His Gly Glu Thr Tyr Arg Cys Phe Gly Ser Phe
                165   170   175

His Gly Ser Pro Tyr Glu Trp Ser Asp Pro Ser Asp Pro Leu Pro Val
            180   185   190

```
Ser Val Thr Gly Asn Pro Ser Ser Ser Trp Pro Ser Pro Thr Glu Pro
        195                 200                 205

Ser Phe Lys Thr Gly Ile Ala Arg His Leu His Ala Val Ile Arg Tyr
    210                 215                 220

Ser Val Ala Ile Ile Leu Phe Thr Ile Leu Pro Phe Phe Leu Leu His
225                 230                 235                 240

Arg Trp Cys Ser Lys Lys Lys Asn Ala Ala Val Met Asn Gln Glu Pro
                245                 250                 255

Ala Gly His Arg Thr Val Asn Arg Glu Asp Ser Asp Glu Gln Asp Pro
            260                 265                 270

Gln Glu Val Thr Tyr Ala Gln Leu Asp His Cys Ile Phe Thr Gln Arg
        275                 280                 285

Lys Ile Thr Gly Pro Ser Gln Arg Ser Lys Arg Pro Ser Thr Asp Thr
    290                 295                 300

Ser Val Cys Ile Glu Leu Pro Asn Ala Glu Pro Arg Ala Leu Ser Pro
305                 310                 315                 320

Ala His Glu His His Ser Gln Ala Leu Met Gly Ser Ser Arg Glu Thr
                325                 330                 335

Thr Ala Leu Ser Gln Thr Gln Leu Ala Ser Ser Asn Val Pro Ala Ala
            340                 345                 350

Gly Ile
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<210> 34
<211> 1251
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DL4"

<400> 34
atgtcccctt cacatgttgt ggtcaatgtg tcaactgcac gatccgggcc cctcaccaca 60

tcctctgcac cggtcagtcg agccgagtca ctgcgtcctg gcagcagaag ctgcaccatg 120

tccatgtcac ccacggtcat catcctggca tgtcttgggt tcttcttgga ccagagtgtg 180

tgggcacacg tgggtggtca ggacaagccc ttctgctctg cctggcccag cgctgtggtg 240

cctcaaggag gacacgtgac tcttcggtgt cactatcgtc gtgggtttaa catcttcacg 300

ctgtacaaga aagatggggt ccctgtccct gagctctaca acagaatatt ctggaacagt 360

ttcctcatta gccctgtgac cccagcacac gcagggacct acagatgtcg aggttttcac 420

ccgcactccc ccactgagtg gtcggcaccc agcaaccccc tggtgatcat ggtcacaggt 480

ctatatgaga aaccttcgct tacagcccgg ccgggcccca cggttcgcgc aggagagaac 540

gtgaccttgt cctgcagctc ccagagctcc tttgacatct accatctatc cagggagggg 600

gaagcccatg aacttaggct ccctgcagtg cccagcatca atggaacatt ccaggccgac 660

ttccctctgg gtcctgccac ccacggagag acctacagat gcttcggctc tttccatgga 720

tctccctacg agtggtcaga cccgagtgac ccactgcctg tttctgtcac aggaaaccct 780

tctagtagtt ggccttcacc cactgaacca agcttcaaaa ctggtatcgc cagacacctg 840

catgctgtga ttaggtactc agtggccatc atcctcttta ccatccttcc cttctttctc 900

cttcatcgct ggtgctccaa aaaaaaagat gctgctgtaa tgaaccaaga gcctgcggga 960

cacagaacag tgaacaggga ggactctgat gaacaagacc ctcaggaggt gacatacgca 1020

cagttggatc actgcatttt cacacagaga aaaatcactg gcccttctca gaggagcaag 1080

agaccctcaa cagataccag cgtgtgtata gaacttccaa atgctgagcc cagagcgttg 1140

tctcctgccc atgagcacca cagtcaggcc ttgatgggat cttctaggga gacaacagcc 1200

ctgtctcaaa cccagcttgc cagctctaat gtaccagcag ctggaatctg a 1251

<210> 35

<211> 218
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS4"

<400> 35
Gln Glu Gly Val His Arg Lys Pro Ser Phe Leu Ala Leu Pro Gly His
1   5   10   15

Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln Cys Trp Ser Asp Val
            20   25   30

Met Phe Glu His Phe Leu Leu His Arg Glu Gly Lys Phe Asn Asn Thr
        35   40   45

Leu His Leu Ile Gly Glu His His Asp Gly Val Ser Lys Ala Asn Phe
    50   55   60

Ser Ile Gly Pro Met Met Pro Val Leu Ala Gly Thr Tyr Arg Cys Tyr
65   70   75   80

Ser Ser Val Pro His Ser Pro Tyr Gln Leu Ser Ala Pro Ser Asp Pro
                85   90   95

Leu Asp Met Val Ile Ile Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala
            100   105   110

Gln Pro Gly Pro Thr Val Gln Ala Gly Glu Asn Val Ser Leu Ser Cys
        115   120   125

Ser Ser Ile Tyr Pro Gly Arg Gly Arg Pro Met Asn Val Gly Ser Leu
    130   135   140

Gln Cys Ala Ala Ser Thr Glu His Ser Arg Pro Thr Phe Leu Trp Ala
145   150   155   160

Leu Pro Pro Thr Glu Gly Pro Thr Asp Ala Ser Ala Leu Ser Val Thr

165 170 175

Leu Pro Thr Ser Gly Gln Thr Arg Val Ile His Cys Leu Phe Pro Ser
180 185 190

Gln Glu Thr Leu Gln Ile Val Gly Leu His Pro Leu Asn Gln Ala Pro
195 200 205

Lys Pro Val Thr Pro Asp Thr Tyr Met Phe
210 215

<210> 36
<211> 893
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS4"

<400> 36
atgtcgctca tggtcatcat catggcgtgt gttgggttct tcttgctgca gggggcctgg 60

ccacaggagg gagtccacag aaaaccttcc ttcctggccc tcccaggtca cctggtgaaa 120

tcagaagaga cagtcatcct gcaatgttgg tcggatgtca tgtttgagca cttccttctg 180

cacagagagg ggaagtttaa caacactttg cacctcattg gagagcacca tgatggggtt 240

tccaaggcca acttctccat tggtcccatg atgcctgtcc ttgcaggaac ctacagatgc 300

tacggttctg ttcctcactc cccctatcag ttgtcagctc ccagtgaccc tctggacatg 360

gtgatcatag gtctatatga gaaaccttct ctctcagccc agccgggccc cacggttcag 420

gcaggagaga atgtgacctt gtcctgcagc tccatctatc cagggaaggg gaggcccatg 480

aacgtaggct ccctgcagtg cgcagcatca acggaacatt ccaggccgac tttcctctgg 540

gccctgccac ccacggaggg acctacagat gcttcggctc tttccgtgac gctccctacg 600

agtggtcaaa ctcgagtgat ccactgcttg tttccgtcac aggaaaccct tcaaatagtt 660

ggccttcacc cactgaacca agctccaaaa ccggtaaccc cagacaccta catgttctga 720

ttgggacctc agtggtcaaa atccctttca ccatcctcct cttctttctc cttcatcgct 780

ggtgctccga caaaaaaaat gctgctgtaa tggaccaaga gcctgcaggg aacagaacag 840

tgaacagcga ggattctgat gaacaagacc atcaggaggt gtcatacgca taa 893

<210> 37
<211> 283
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS5"

<400> 37
His Glu Gly Phe Arg Arg Lys Pro Ser Leu Leu Ala His Pro Gly Pro
1 5 10 15

Leu Val Lys Ser Glu Glu Thr Val Ile Leu Gln Cys Trp Ser Asp Val
20 25 30

Met Phe Glu His Phe Leu Leu His Arg Glu Gly Thr Phe Asn His Thr
35 40 45

Leu Arg Leu Ile Gly Glu His Ile Asp Gly Val Ser Lys Gly Asn Phe
50 55 60

Ser Ile Gly Arg Met Thr Gln Asp Leu Ala Gly Thr Tyr Arg Cys Tyr
65 70 75 80

Gly Ser Val Thr His Ser Pro Tyr Gln Leu Ser Ala Pro Ser Asp Pro
85 90 95

Leu Asp Ile Val Ile Thr Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala
100 105 110

Gln Pro Gly Pro Thr Val Leu Ala Gly Glu Ser Val Thr Leu Ser Cys
115 120 125

Ser Ser Arg Ser Ser Tyr Asp Met Tyr His Leu Ser Arg Glu Gly Glu
130 135 140

```
Ala His Glu Arg Arg Leu Pro Ala Gly Thr Lys Val Asn Gly Thr Phe
145                 150                 155                 160

Gln Ala Asp Phe Pro Leu Asp Pro Ala Thr His Gly Gly Thr Tyr Arg
                165                 170                 175

Cys Phe Gly Ser Phe Arg Asp Ser Pro Tyr Glu Trp Ser Lys Ser Ser
            180                 185                 190

Asp Pro Leu Leu Val Ser Val Thr Gly Asn Thr Ser Asn Ser Trp Pro
        195                 200                 205

Ser Pro Thr Glu Pro Ser Ser Lys Thr Gly Asn Pro Arg His Leu His
    210                 215                 220

Val Leu Ile Gly Thr Ser Val Val Lys Leu Pro Phe Thr Ile Leu Leu
225                 230                 235                 240

Phe Phe Leu Leu His Arg Trp Cys Ser Asn Lys Lys Asn Ala Ser Val
                245                 250                 255

Met Asp Gln Gly Pro Ala Gly Asn Arg Thr Val Asn Arg Glu Asp Ser
            260                 265                 270

Asp Glu Gln Asp His Gln Glu Val Ser Tyr Ala
        275                 280
```

<210> 38
<211> 915
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR2DS"

<400> 38
atgtcgctca tggtcatcag catggcgtgt gttgcgttct tcttgctgca gggggcctgg 60

ccacatgagg gattccgcag aaaaccttcc ctcctggccc acccaggtcc cctggtgaaa 120

tcagaagaga cagtcatcct gcaatgttgg tcagatgtca tgtttgagca cttccttctg 180

cacagagagg ggacgtttaa ccacactttg cgcctcattg gagagcacat tgatggggtc 240

tccaagggca acttctccat cggtcgcatg acacaagacc tggcagggac ctacagatgc 300

tacggttctg ttactcactc cccctatcag ttgtcagcgc ccagtgaccc tctggacatc 360

gtgatcacag gtctatatga gaaaccttct ctctcagccc agccgggccc cacggttctg 420

gcaggagaga gcgtgacctt gtcctgcagc tcccggagct cctatgacat gtaccatcta 480

tccagggaag gggaggccca tgaacgtagg ctccctgcag ggcccaaggt caacagaaca 540

ttccaggccg actttcctct ggaccctgcc acccacggag ggacctacag atgcttcggc 600

tctttccgtg actctccata cgagtggtca aagtcaagtg acccactgct tgtttctgtc 660

acaggaaact cttcaaatag ttggccttca cccactgaac caagctccga aaccggtaac 720

cccagacacc tacacgttct gattgggacc tcagtggtca aactcccttt caccatcctc 780

ctcttctttc tccttcatcg ctggtgctcc aacaaaaaaa atgcatctgt aatggaccaa 840

gggcctgcgg ggaacagaac agtgaacagg gaggattctg atgaacagga ccatcaggag 900

gtgtcatacg cataa 915

<210> 39
<211> 361
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR3DS1"

<400> 39
His Met Gly Gly Gln Asp Lys Pro Phe Leu Ser Ala Trp Pro Ser Ala
1 5 10 15

Val Val Pro Arg Gly Gly His Val Thr Leu Arg Cys His Tyr Arg His
20 25 30

Arg Phe Asn Asn Phe Met Leu Tyr Lys Glu Asp Arg Ile His Val Pro

```
        35                  40                  45

Ile Phe His Gly Arg Ile Phe Gln Glu Gly Phe Asn Met Ser Pro Val
    50                  55                  60

Thr Thr Ala His Ala Gly Asn Tyr Thr Cys Arg Gly Ser His Pro His
65                  70                  75                  80

Ser Pro Thr Gly Trp Ser Ala Pro Ser Asn Pro Met Val Ile Met Val
                85                  90                  95

Thr Gly Asn His Arg Lys Pro Ser Leu Leu Ala His Pro Gly Pro Leu
            100                 105                 110

Val Lys Ser Gly Glu Arg Val Ile Leu Gln Cys Trp Ser Asp Ile Met
        115                 120                 125

Phe Glu His Phe Phe Leu His Lys Glu Gly Ile Ser Lys Asp Pro Ser
    130                 135                 140

Arg Leu Val Gly Gln Ile His Asp Gly Val Ser Lys Ala Asn Phe Ser
145                 150                 155                 160

Ile Gly Ser Met Met Arg Ala Leu Ala Gly Thr Tyr Arg Cys Tyr Gly
                165                 170                 175

Ser Val Thr His Thr Pro Tyr Gln Leu Ser Ala Pro Ser Asp Pro Leu
            180                 185                 190

Asp Ile Val Val Thr Gly Leu Tyr Glu Lys Pro Ser Leu Ser Ala Gln
        195                 200                 205

Pro Gly Pro Lys Val Gln Ala Gly Glu Ser Val Thr Leu Ser Cys Ser
    210                 215                 220

Ser Arg Ser Ser Tyr Asp Met Tyr His Leu Ser Arg Glu Gly Gly Ala
225                 230                 235                 240
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His Glu Arg Arg Leu Pro Ala Val Arg Lys Val Asn Arg Thr Phe Gln
                245                 250                 255

Ala Asp Phe Pro Leu Gly Pro Ala Thr His Gly Gly Thr Tyr Arg Cys
            260                 265                 270

Phe Gly Ser Phe Arg His Ser Pro Tyr Glu Trp Ser Asp Pro Ser Asp
        275                 280                 285

Pro Leu Leu Val Ser Val Thr Gly Asn Pro Ser Ser Ser Trp Pro Ser
    290                 295                 300

Pro Thr Glu Pro Ser Ser Lys Ser Gly Asn Leu Arg His Leu His Ile
305                 310                 315                 320

Leu Ile Gly Thr Ser Val Val Lys Ile Pro Phe Thr Ile Leu Leu Phe
                325                 330                 335

Phe Leu Leu His Arg Trp Cys Ser Asn Lys Lys Lys Cys Cys Cys Asn
            340                 345                 350

Gly Pro Arg Ala Cys Arg Glu Gln Lys
        355                 360


<210> 40
<211> 1149
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="KIR3DS1"

<400> 40
atgttgctca tggtcgtcag catggcgtgt gttgggttgt tcttggtcca gagggccggt       60

ccacacatgg gtggtcagga caagcccttc ctgtctgcct ggcccagcgc tgtggtgcct      120

cgcggaggac acgtgactct tcggtgtcac tatcgtcata ggtttaacaa tttcatgcta      180

tacaaagaag acagaatcca cgttcccatc ttccatggca gaatattcca ggagggcttc      240
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aacatgagcc ctgtgaccac agcacatgca gggaactaca catgtcgggg ttcacaccca 300

cactccccca ctgggtggtc ggcacccagc aaccccatgg tgatcatggt cacaggaaac 360

cacagaaaac cttccctcct ggcccaccca ggtcccctgg tgaaatcagg agagagagtc 420

atcctgcaat gttggtcaga tatcatgttt gagcacttct ttctgcacaa agagtggatc 480

tctaaggacc cctcacgcct cgttggacag atccatgatg gggtctccaa ggccaatttc 540

tccatcggtt ccatgatgcg tgcccttgca gggacctaca gatgctacgg ttctgttact 600

cacaccccct atcagttgtc agctcccagt gatcccctgg acatcgtggt cacaggtcta 660

tatgagaaac cttctctctc agcccagccg ggccccaagg ttcaggcagg agagagcgtg 720

accttgtcct gtagctcccg gagctcctat gacatgtacc atctatccag ggaggggggа 780

gcccatgaac gtaggctccc tgcagtgcgc aaggtcaaca gaacattcca ggcagatttc 840

cctctgggcc ctgccaccca cggagggacc tacagatgct tcggctcttt ccgtcactct 900

ccctacgagt ggtcagaccc gagtgaccca ctgcttgttt ctgtcacagg aaacccttca 960

agtagttggc cttcacccac agaaccaagc tccaaatctg gtaacctcag acacctgcac 1020

attctgattg ggacctcagt ggtcaaaatc cctttcacca tcctcctctt ctttctcctt 1080

catcgctggt gctccaacaa aaaaaaatgc tgctgtaatg gaccaagagc ctgcagggaa 1140

cagaagtga 1149

<210> 41
<211> 231
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="NKG2C"

<400> 41
Met Ser Lys Gln Arg Gly Thr Phe Ser Glu Val Ser Leu Ala Gln Asp
1 5 10 15

Pro Lys Arg Gln Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Ser
20 25 30

```
Gly Thr Glu Gln Glu Ile Phe Gln Val Glu Leu Asn Leu Gln Asn Pro
        35                  40                  45

Ser Leu Asn His Gln Gly Ile Asp Lys Ile Tyr Asp Cys Gln Gly Leu
    50                  55                  60

Leu Pro Pro Pro Glu Lys Leu Thr Ala Glu Val Leu Gly Ile Ile Cys
65                  70                  75                  80

Ile Val Leu Met Ala Thr Val Leu Lys Thr Ile Val Leu Ile Pro Phe
                85                  90                  95

Leu Glu Gln Asn Asn Ser Ser Pro Asn Thr Arg Thr Gln Lys Ala Arg
            100                 105                 110

His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys
        115                 120                 125

Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala
    130                 135                 140

Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu
145                 150                 155                 160

Met Lys Phe Leu Ala Ser Ile Leu Pro Ser Ser Trp Ile Gly Val Phe
                165                 170                 175

Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu Ala Phe
            180                 185                 190

Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
        195                 200                 205

Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Met Ile
    210                 215                 220
```

Tyr His Cys Lys His Lys Leu
225 230

<210> 42
<211> 696
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="NKG2C"

<400> 42
atgaataaac aaagaggaac cttctcagaa gtgagtctgg cccaggaccc aaagcggcag 60

caaaggaaac ctaaaggcaa taaaagctcc atttcaggaa ccgaacagga aatattccaa 120

gtagaattaa atcttcaaaa tccttccctg aatcatcaag ggattgataa aatatatgac 180

tgccaaggtt tactgccacc tccagagaag ctcactgccg aggtcctagg aatcatttgc 240

attgtcctga tggccactgt gttaaaaaca atagttctta ttcctttcct ggagcagaac 300

aatttttccc cgaatacaag aacgcagaaa gcacgtcatt gtggccattg tcctgaggag 360

tggattacat attccaacag ttgttattac attggtaagg aaagaagaac ttgggaagag 420

agtttgctgg cctgtacttc gaagaactcc agtctgcttt ctatagataa tgaagaagaa 480

atgaaatttc tggccagcat tttaccttcc tcatggattg gtgtgtttcg taacagcagt 540

catcatccat gggtgacaat aaatggtttg gctttcaaac ataagataaa agactcagat 600

aatgctgaac ttaactgtgc agtgctacaa gtaaatcgac ttaaatcagc ccagtgtgga 660

tcttcaatga tatatcattg taagcataag ctttag 696

<210> 43
<211> 354
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CCR7"

<400> 43
Gln Asp Glu Val Thr Asp Asp Tyr Ile Gly Asp Asn Thr Thr Val Asp

```
1               5                   10                  15

Tyr Thr Leu Phe Glu Ser Leu Cys Ser Lys Lys Asp Val Arg Asn Phe
            20                  25                  30

Lys Ala Trp Phe Leu Pro Ile Met Tyr Ser Ile Ile Cys Phe Val Gly
        35                  40                  45

Leu Leu Gly Asn Gly Leu Val Val Leu Thr Tyr Ile Tyr Phe Lys Arg
    50                  55                  60

Leu Lys Thr Met Thr Asp Thr Tyr Leu Leu Asn Leu Ala Val Ala Asp
65                  70                  75                  80

Ile Leu Phe Leu Leu Thr Leu Pro Phe Trp Ala Tyr Ser Ala Ala Lys
                85                  90                  95

Ser Trp Val Phe Gly Val His Phe Cys Lys Leu Ile Phe Ala Ile Tyr
            100                 105                 110

Lys Met Ser Phe Phe Ser Gly Met Leu Leu Leu Leu Cys Ile Ser Ile
        115                 120                 125

Asp Arg Tyr Val Ala Ile Val Gln Ala Val Ser Ala His Arg His Arg
    130                 135                 140

Ala Arg Val Leu Leu Ile Ser Lys Leu Ser Cys Val Gly Ile Trp Ile
145                 150                 155                 160

Leu Ala Thr Val Leu Ser Ile Pro Glu Leu Leu Tyr Ser Asp Leu Gln
                165                 170                 175

Arg Ser Ser Ser Glu Gln Ala Met Arg Cys Ser Leu Ile Thr Glu His
            180                 185                 190

Val Glu Ala Phe Ile Thr Ile Gln Val Ala Gln Met Val Ile Gly Phe
        195                 200                 205
```

```
Leu Val Pro Leu Leu Ala Met Ser Phe Cys Tyr Leu Val Ile Ile Arg
    210                 215                 220

Thr Leu Leu Gln Ala Arg Asn Phe Glu Arg Asn Lys Ala Ile Lys Val
225                 230                 235                 240

Ile Ile Ala Val Val Val Val Phe Ile Val Phe Gln Leu Pro Tyr Asn
                245                 250                 255

Gly Val Val Leu Ala Gln Thr Val Ala Asn Phe Asn Ile Thr Ser Ser
            260                 265                 270

Thr Cys Glu Leu Ser Lys Gln Leu Asn Ile Ala Tyr Asp Val Thr Tyr
        275                 280                 285

Ser Leu Ala Cys Val Arg Cys Cys Val Asn Pro Phe Leu Tyr Ala Phe
    290                 295                 300

Ile Gly Val Lys Phe Arg Asn Asp Leu Phe Lys Leu Phe Lys Asp Leu
305                 310                 315                 320

Gly Cys Leu Ser Gln Glu Gln Leu Arg Gln Trp Ser Ser Cys Arg His
                325                 330                 335

Ile Arg Arg Ser Ser Met Ser Val Glu Ala Glu Thr Thr Thr Thr Phe
            340                 345                 350

Ser Pro
```

<210> 44
<211> 1137
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CCR7"

<400> 44
atggacctgg ggaaaccaat gaaaagcgtg ctggtggtgg ctctccttgt cattttccag 60

gtatgcctgt gtcaagatga ggtcacggac gattacatcg gagacaacac cacagtggac 120

tacactttgt tcgagtcttt gtgctccaag aaggacgtgc ggaactttaa agcctggttc 180

ctccctatca tgtactccat catttgtttc gtgggcctac tgggcaatgg gctggtcgtg 240

ttgacctata tctatttcaa gaggctcaag accatgaccg atacctacct gctcaacctg 300

gcggtggcag acatcctctt cctcctgacc cttcccttct gggcctacag cgcggccaag 360

tcctgggtct tcggtgtcca cttttgcaag ctcatctttg ccatctacaa gatgagcttc 420

ttcagtggca tgctcctact tctttgcatc agcattgacc gctacgtggc catcgtccag 480

gctgtctcag ctcaccgcca ccgtgcccgc gtccttctca tcagcaagct gtcctgtgtg 540

ggcatctgga tactagccac agtgctctcc atcccagagc tcctgtacag tgacctccag 600

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atcaccatcc aggtggccca gatggtgatc ggctttctgg tccccctgct ggccatgagc 720

ttctgttacc ttgtcatcat ccgcaccctg ctccaggcac gcaactttga gcgcaacaag 780

gccatcaagg tgatcatcgc tgtggtcgtg gtcttcatag tcttccagct gccctacaat 840

ggggtggtcc tggcccagac ggtggccaac ttcaacatca ccagtagcac ctgtgagctc 900

agtaagcaac tcaacatcgc ctacgacgtc acctacagcc tggcctgcgt ccgctgctgc 960

gtcaaccctt tcttgtacgc cttcatcggc gtcaagttcc gcaacgatct cttcaagctc 1020

ttcaaggacc tgggctgcct cagccaggag cagctccggc agtggtcttc ctgtcggcac 1080

atccggcgct cctccatgag tgtggaggcc gagaccacca ccaccttctc cccatag 1137

<210> 45
<211> 368
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR3"

<400> 45

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Met Val Leu Glu Val Ser Asp His Gln Val Leu Asn Asp Ala Glu Val
1               5                   10                  15

Ala Ala Leu Leu Glu Asn Phe Ser Ser Ser Tyr Asp Tyr Gly Glu Asn
            20                  25                  30

Glu Ser Asp Ser Cys Cys Thr Ser Pro Pro Cys Pro Gln Asp Phe Ser
        35                  40                  45

Leu Asn Phe Asp Arg Ala Phe Leu Pro Ala Leu Tyr Ser Leu Leu Phe
    50                  55                  60

Leu Leu Gly Leu Leu Gly Asn Gly Ala Val Ala Ala Val Leu Leu Ser
65                  70                  75                  80

Arg Arg Thr Ala Leu Ser Ser Thr Asp Thr Phe Leu Leu His Leu Ala
                85                  90                  95

Val Ala Asp Thr Leu Leu Val Leu Thr Leu Pro Leu Trp Ala Val Asp
            100                 105                 110

Ala Ala Val Gln Trp Val Phe Gly Ser Gly Leu Cys Lys Val Ala Gly
        115                 120                 125

Ala Leu Phe Asn Ile Asn Phe Tyr Ala Gly Ala Leu Leu Leu Ala Cys
    130                 135                 140

Ile Ser Phe Asp Arg Tyr Leu Asn Ile Val His Ala Thr Gln Leu Tyr
145                 150                 155                 160

Arg Arg Gly Pro Pro Ala Arg Val Thr Leu Thr Cys Leu Ala Val Trp
                165                 170                 175

Gly Leu Cys Leu Leu Phe Ala Leu Pro Asp Phe Ile Phe Leu Ser Ala
            180                 185                 190

His His Asp Glu Arg Leu Asn Ala Thr His Cys Gln Tyr Asn Phe Pro
        195                 200                 205
```

```
Gln Val Gly Arg Thr Ala Leu Arg Val Leu Gln Leu Val Ala Gly Phe
    210                 215                 220

Leu Leu Pro Leu Leu Val Met Ala Tyr Cys Tyr Ala His Ile Leu Ala
225                 230                 235                 240

Val Leu Leu Val Ser Arg Gly Gln Arg Arg Leu Arg Ala Met Arg Leu
                245                 250                 255

Val Val Val Val Val Val Ala Phe Ala Leu Cys Trp Thr Pro Tyr His
            260                 265                 270

Leu Val Val Leu Val Asp Ile Leu Met Asp Leu Gly Ala Leu Ala Arg
        275                 280                 285

Asn Cys Gly Arg Glu Ser Arg Val Asp Val Ala Lys Ser Val Thr Ser
    290                 295                 300

Gly Leu Gly Tyr Met His Cys Cys Leu Asn Pro Leu Leu Tyr Ala Phe
305                 310                 315                 320

Val Gly Val Lys Phe Arg Glu Arg Met Trp Met Leu Leu Leu Arg Leu
                325                 330                 335

Gly Cys Pro Asn Gln Arg Gly Leu Gln Arg Gln Pro Ser Ser Ser Arg
            340                 345                 350

Arg Asp Ser Ser Trp Ser Glu Thr Ser Glu Ala Ser Tyr Ser Gly Leu
        355                 360                 365
```

<210> 46
<211> 1248
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR3"

<400> 46
atggagttga ggaagtacgg ccctggaaga ctggcgggga cagttatagg aggagctgct 60

cagagtaaat cacagactaa atcagactca atcacaaaag agttcctgcc aggcctttac 120

acagcccctt cctccccgtt cccgccctca caggtgagtg accaccaagt gctaaatgac 180

gccgaggttg ccgccctcct ggagaacttc agctcttcct atgactatgg agaaaacgag 240

agtgactcgt gctgtacctc cccgccctgc ccacaggact tcagcctgaa cttcgaccgg 300

gccttcctgc cagccctcta cagcctcctc tttctgctgg ggctgctggg caacggcgcg 360

gtggcagccg tgctgctgag ccggcggaca gccctgagca gcaccgacac cttcctgctc 420

cacctagctg tagcagacac gctgctggtg ctgacactgc cgctctgggc agtggacgct 480

gccgtccagt gggtctttgg ctctggcctc tgcaaagtgg caggtgccct cttcaacatc 540

aacttctacg caggagccct cctgctggcc tgcatcagct ttgaccgcta cctgaacata 600

gttcatgcca cccagctcta ccgccggggg cccccggccc gcgtgaccct cacctgcctg 660

gctgtctggg ggctctgcct gcttttcgcc ctcccagact tcatcttcct gtcggcccac 720

cacgacgagc gcctcaacgc cacccactgc caatacaact tcccacaggt gggccgcacg 780

gctctgcggg tgctgcagct ggtggctggc tttctgctgc ccctgctggt catggcctac 840

tgctatgccc acatcctggc cgtgctgctg gtttccaggg gccagcggcg cctgcgggcc 900

atgcggctgg tggtggtggt cgtggtggcc tttgccctct gctggacccc ctatcacctg 960

gtggtgctgg tggacatcct catggacctg ggcgctttgg cccgcaactg tggccgagaa 1020

agcagggtag acgtggccaa gtcggtcacc tcaggcctgg gctacatgca ctgctgcctc 1080

aacccgctgc tctatgcctt tgtaggggtc aagttccggg agcggatgtg gatgctgctc 1140

ttgcgcctgg gctgccccaa ccagagaggg ctccagaggc agccatcgtc ttcccgccgg 1200

gattcatcct ggtctgagac ctcagaggcc tcctactcgg gcttgtga 1248

<210> 47
<211> 344
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="L-selectin"

<400> 47
Asp Phe Leu Ala His His Gly Thr Asp Cys Trp Thr Tyr His Tyr Ser
1   5   10   15

Glu Lys Pro Met Asn Trp Gln Arg Ala Arg Arg Phe Cys Arg Asp Asn
            20   25   30

Tyr Thr Asp Leu Val Ala Ile Gln Asn Lys Ala Glu Ile Glu Tyr Leu
        35   40   45

Glu Lys Thr Leu Pro Phe Ser Arg Ser Tyr Tyr Trp Ile Gly Ile Arg
    50   55   60

Lys Ile Gly Gly Ile Trp Thr Trp Val Gly Thr Asn Lys Ser Leu Thr
65   70   75   80

Glu Glu Ala Glu Asn Trp Gly Asp Gly Glu Pro Asn Asn Lys Lys Asn
                85   90   95

Lys Glu Asp Cys Val Glu Ile Tyr Ile Lys Arg Asn Lys Asp Ala Gly
            100   105   110

Lys Trp Asn Asp Asp Ala Cys His Lys Leu Lys Ala Ala Leu Cys Tyr
        115   120   125

Thr Ala Ser Cys Gln Pro Trp Ser Cys Ser Gly His Gly Glu Cys Val
    130   135   140

Glu Ile Ile Asn Asn Tyr Thr Cys Asn Cys Asp Val Gly Tyr Tyr Gly
145   150   155   160

Pro Gln Cys Gln Phe Val Ile Gln Cys Glu Pro Leu Glu Ala Pro Glu
                165   170   175

Leu Gly Thr Met Asp Cys Thr His Pro Leu Gly Asn Phe Ser Phe Ser

```
            180                 185                 190

Ser Gln Cys Ala Phe Ser Cys Ser Glu Gly Thr Asn Leu Thr Gly Ile
        195                 200                 205

Glu Glu Thr Thr Cys Gly Pro Phe Gly Asn Trp Ser Ser Pro Glu Pro
    210                 215                 220

Thr Cys Gln Val Ile Gln Cys Glu Pro Leu Ser Ala Pro Asp Leu Gly
225                 230                 235                 240

Ile Met Asn Cys Ser His Pro Leu Ala Ser Phe Ser Phe Thr Ser Ala
                245                 250                 255

Cys Thr Phe Ile Cys Ser Glu Gly Thr Glu Leu Ile Gly Lys Lys Lys
            260                 265                 270

Thr Ile Cys Glu Ser Ser Gly Ile Trp Ser Asn Pro Ser Pro Ile Cys
        275                 280                 285

Gln Lys Leu Asp Lys Ser Phe Ser Met Ile Lys Glu Gly Asp Tyr Asn
    290                 295                 300

Pro Leu Phe Ile Pro Val Ala Val Met Val Thr Ala Phe Ser Gly Leu
305                 310                 315                 320

Ala Phe Ile Ile Trp Leu Ala Arg Arg Leu Lys Lys Gly Lys Lys Ser
                325                 330                 335

Lys Arg Ser Met Asn Asp Pro Tyr
            340
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<210> 48
<211> 1158
<212> DNA
<213> Homo sapiens

<220>
<221> source

<223> /note="L-selectin"

<400> 48
atgggctgca gaagaactag agaaggacca agcaaagcca tgatatttcc atggaaatgt 60

cagagcaccc agagggactt atggaacatc ttcaagttgt gggggtggac aatgctctgt 120

tgtgatttcc tggcacatca tggaaccgac tgctggactt accattattc tgaaaaaccc 180

atgaactggc aaagggctag aagattctgc cgagacaatt acacagattt agttgccata 240

caaaacaagg cggaaattga gtatctggag aagactctgc ctttcagtcg ttcttactac 300

tggataggaa tccggaagat aggaggaata tggacgtggg tgggaaccaa caaatctctt 360

actgaagaag cagagaactg gggagatggt gagcccaaca acaagaagaa caaggaggac 420

tgcgtggaga tctatatcaa gagaaacaaa gatgcaggca aatggaacga tgacgcctgc 480

cacaaactaa aggcagccct ctgttacaca gcttcttgcc agccctggtc atgcagtggc 540

catggagaat gtgtagaaat catcaataat tacacctgca actgtgatgt ggggtactat 600

gggccccagt gtcagtttgt gattcagtgt gagcctttgg aggccccaga gctgggtacc 660

atggactgta ctcacccttt gggaaacttc agcttcagct cacagtgtgc cttcagctgc 720

tctgaaggaa caaacttaac tgggattgaa gaaaccacct gtggaccatt tggaaactgg 780

tcatctccag aaccaacctg tcaagtgatt cagtgtgagc ctctatcagc accagatttg 840

gggatcatga actgtagcca tcccctggcc agcttcagct ttacctctgc atgtaccttc 900

atctgctcag aaggaactga gttaattggg aagaagaaaa ccatttgtga atcatctgga 960

atctggtcaa atcctagtcc aatatgtcaa aaattggaca aaagtttctc aatgattaag 1020

gagggtgatt ataacccccc cttcattcca gtggcagtca tggttactgc attctctggg 1080

ttggcattta tcatttggct ggcaaggaga ttaaaaaaag gcaagaaatc caagagaagt 1140

atgaatgacc catattaa 1158

<210> 49
<211> 350
<212> PRT
<213> Homo sapiens

<220>

<221> source
<223> /note="CXCR1"

<220>
<221> MOD_RES
<222> (31)..(31)
<223> Any amino acid

<400> 49
Met Ser Asn Ile Thr Asp Pro Gln Met Trp Asp Phe Asp Asp Leu Asn
1   5   10   15

Phe Thr Gly Met Pro Pro Ala Asp Glu Asp Tyr Ser Pro Cys Xaa Leu
            20   25   30

Glu Thr Glu Thr Leu Asn Lys Tyr Val Val Ile Ile Ala Tyr Ala Leu
        35   40   45

Val Phe Leu Leu Ser Leu Leu Gly Asn Ser Leu Val Met Leu Val Ile
    50   55   60

Leu Tyr Ser Arg Val Gly Arg Ser Val Thr Asp Val Tyr Leu Leu Asn
65   70   75   80

Leu Ala Leu Ala Asp Leu Leu Phe Ala Leu Thr Leu Pro Ile Trp Ala
                85   90   95

Ala Ser Lys Val Asn Gly Trp Ile Phe Gly Thr Phe Leu Cys Lys Val
            100   105   110

Val Ser Leu Leu Lys Glu Val Asn Phe Tyr Ser Gly Ile Leu Leu Leu
        115   120   125

Ala Cys Ile Ser Val Asp Arg Tyr Leu Ala Ile Val His Ala Thr Arg
    130   135   140

Thr Leu Thr Gln Lys Arg His Leu Val Lys Phe Val Cys Leu Gly Cys
145   150   155   160

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Trp Gly Leu Ser Met Asn Leu Ser Leu Pro Phe Phe Leu Phe Arg Gln
                165                 170                 175

Ala Tyr His Pro Asn Asn Ser Ser Pro Val Cys Tyr Glu Val Leu Gly
            180                 185                 190

Asn Asp Thr Ala Lys Trp Arg Met Val Leu Arg Ile Leu Pro His Thr
        195                 200                 205

Phe Gly Phe Ile Val Pro Leu Phe Val Met Leu Phe Cys Tyr Gly Phe
    210                 215                 220

Thr Leu Arg Thr Leu Phe Lys Ala His Met Gly Gln Lys His Arg Ala
225                 230                 235                 240

Met Arg Val Ile Phe Ala Val Val Leu Ile Phe Leu Leu Cys Trp Leu
                245                 250                 255

Pro Tyr Asn Leu Val Leu Leu Ala Asp Thr Leu Met Arg Thr Gln Val
            260                 265                 270

Ile Gln Glu Ser Cys Glu Arg Arg Asn Asn Ile Gly Arg Ala Leu Asp
        275                 280                 285

Ala Thr Glu Ile Leu Gly Phe Leu His Ser Cys Leu Asn Pro Ile Ile
    290                 295                 300

Tyr Ala Phe Ile Gly Gln Asn Phe Arg His Gly Phe Leu Lys Ile Leu
305                 310                 315                 320

Ala Met His Gly Leu Val Ser Lys Glu Phe Leu Ala Arg His Arg Val
                325                 330                 335

Thr Ser Tyr Thr Ser Ser Ser Val Asn Val Ser Ser Asn Leu
            340                 345                 350
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<210> 50
<211> 1053

<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR1"

<400> 50
atgtcaaata ttacagatcc acagatgtgg gattttgatg atctaaattt cactggcatg 60

ccacctgcag atgaagatta cagcccctgt atgctagaaa ctgagacact caacaagtat 120

gttgtgatca tcgcctatgc cctagtgttc ctgctgagcc tgctgggaaa ctccctggtg 180

atgctggtca tcttatacag cagggtcggc cgctccgtca ctgatgtcta cctgctgaac 240

ctggccttgg ccgacctact ctttgccctg accttgccca tctgggccgc ctccaaggtg 300

aatggctgga tttttggcac attcctgtgc aaggtggtct cactcctgaa ggaagtcaac 360

ttctacagtg gcatcctgct gttggcctgc atcagtgtgg accgttacct ggccattgtc 420

catgccacac gcacactgac ccagaagcgt cacttggtca agtttgtttg tcttggctgc 480

tggggactgt ctatgaatct gtccctgccc ttcttccttt tccgccaggc ttaccatcca 540

aacaattcca gtccagtttg ctatgaggtc ctgggaaatg acacagcaaa atggcggatg 600

gtgttgcgga tcctgcctca cacctttggc ttcatcgtgc cgctgtttgt catgctgttc 660

tgctatggat tcaccctgcg tacactgttt aaggcccaca tggggcagaa gcaccgagcc 720

atgagggtca tctttgctgt cgtcctcatc ttcctgcttt gctggctgcc ctacaacctg 780

gtcctgctgg cagacaccct catgaggacc caggtgatcc aggagagctg tgagcgccgc 840

aacaacatcg gccgggccct ggatgccact gagattctgg gatttctcca tagctgcctc 900

aaccccatca tctacgcctt catcggccaa aattttcgcc atggattcct caagatcctg 960

gctatgcatg gcctggtcag caaggagttc ttggcacgtc atcgtgttac ctcctacact 1020

tcttcgtctg tcaatgtctc ttccaacctc tga 1053

<210> 51
<211> 360
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR2"

<400> 51
Met Glu Asp Phe Asn Met Glu Ser Asp Ser Phe Glu Asp Phe Trp Lys
1   5   10   15

Gly Glu Asp Leu Ser Asn Tyr Ser Tyr Ser Ser Thr Leu Pro Pro Phe
            20   25   30

Leu Leu Asp Ala Ala Pro Cys Glu Pro Glu Ser Leu Glu Ile Asn Lys
        35   40   45

Tyr Phe Val Val Ile Ile Tyr Ala Leu Val Phe Leu Leu Ser Leu Leu
    50   55   60

Gly Asn Ser Leu Val Met Leu Val Ile Leu Tyr Ser Arg Val Gly Arg
65   70   75   80

Ser Val Thr Asp Val Tyr Leu Leu Asn Leu Ala Leu Ala Asp Leu Leu
                85   90   95

Phe Ala Leu Thr Leu Pro Ile Trp Ala Ala Ser Lys Val Asn Gly Trp
            100   105   110

Ile Phe Gly Thr Phe Leu Cys Lys Val Val Ser Leu Leu Lys Glu Val
        115   120   125

Asn Phe Tyr Ser Gly Ile Leu Leu Leu Ala Cys Ile Ser Val Asp Arg
    130   135   140

Tyr Leu Ala Ile Val His Ala Thr Arg Thr Leu Thr Gln Lys Arg Tyr
145   150   155   160

Leu Val Lys Phe Ile Cys Leu Ser Ile Trp Gly Leu Ser Leu Leu Leu
                165   170   175

Ala Leu Pro Val Leu Leu Phe Arg Arg Thr Val Tyr Ser Ser Asn Val

```
            180                 185                 190

Ser Pro Ala Cys Tyr Glu Asp Met Gly Asn Asn Thr Ala Asn Trp Arg
        195                 200                 205

Met Leu Leu Arg Ile Leu Pro Gln Ser Phe Gly Phe Ile Val Pro Leu
    210                 215                 220

Leu Ile Met Leu Phe Cys Tyr Gly Phe Thr Leu Arg Thr Leu Phe Lys
225                 230                 235                 240

Ala His Met Gly Gln Lys His Arg Ala Met Arg Val Ile Phe Ala Val
                245                 250                 255

Val Leu Ile Phe Leu Leu Cys Trp Leu Pro Tyr Asn Leu Val Leu Leu
            260                 265                 270

Ala Asp Thr Leu Met Arg Thr Gln Val Ile Gln Glu Thr Cys Glu Arg
        275                 280                 285

Arg Asn His Ile Asp Arg Ala Leu Asp Ala Thr Glu Ile Leu Gly Ile
    290                 295                 300

Leu His Ser Cys Leu Asn Pro Leu Ile Tyr Ala Phe Ile Gly Gln Lys
305                 310                 315                 320

Phe Arg His Gly Leu Leu Lys Ile Leu Ala Ile His Gly Leu Ile Ser
                325                 330                 335

Lys Asp Ser Leu Pro Lys Asp Ser Arg Pro Ser Phe Val Gly Ser Ser
            340                 345                 350

Ser Gly His Thr Ser Thr Thr Leu
        355                 360
```

<210> 52
<211> 1083
<212> DNA

<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR2"

<400> 52
atggaagatt ttaacatgga gagtgacagc tttgaagatt tctggaaagg tgaagatctt 60

agtaattaca gttacagctc taccctgccc ccttttctac tagatgccgc cccatgtgaa 120

ccagaatccc tggaaatcaa caagtatttt gtggtcatta tctatgccct ggtattcctg 180

ctgagcctgc tgggaaactc cctcgtgatg ctggtcatct tatacagcag ggtcggccgc 240

tccgtcactg atgtctacct gctgaaccta gccttggccg acctactctt tgccctgacc 300

ttgcccatct gggccgcctc caaggtgaat ggctggattt ttggcacatt cctgtgcaag 360

gtggtctcac tcctgaagga agtcaacttc tatagtggca tcctgctact ggcctgcatc 420

agtgtggacc gttacctggc cattgtccat gccacacgca cactgaccca gaagcgctac 480

ttggtcaaat tcatatgtct cagcatctgg ggtctgtcct tgctcctggc cctgcctgtc 540

ttacttttcc gaaggaccgt ctactcatcc aatgttagcc cagcctgcta tgaggacatg 600

ggcaacaata cagcaaactg gcggatgctg ttacggatcc tgccccagtc ctttggcttc 660

atcgtgccac tgctgatcat gctgttctgc tacggattca ccctgcgtac gctgtttaag 720

gcccacatgg ggcagaagca ccgggccatg cgggtcatct ttgctgtcgt cctcatcttc 780

ctgctctgct ggctgcccta caacctggtc ctgctggcag acaccctcat gaggacccag 840

gtgatccagg agacctgtga gcgccgcaat cacatcgacc gggctctgga tgccaccgag 900

attctgggca tccttcacag ctgcctcaac cccctcatct acgccttcat tggccagaag 960

tttcgccatg gactcctcaa gattctagct atacatggct tgatcagcaa ggactccctg 1020

cccaaagaca gcaggccttc ctttgttggc tcttcttcag ggcacacttc cactactctc 1080

taa 1083

<210> 53
<211> 355
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CXC3CR1"

<400> 53
Met Asp Gln Phe Pro Glu Ser Val Thr Glu Asn Phe Glu Tyr Asp Asp
1   5   10   15

Leu Ala Glu Ala Cys Tyr Ile Gly Asp Ile Val Val Phe Gly Thr Val
            20   25   30

Phe Leu Ser Ile Phe Tyr Ser Val Ile Phe Ala Ile Gly Leu Val Gly
        35   40   45

Asn Leu Leu Val Val Phe Ala Leu Thr Asn Ser Lys Lys Pro Lys Ser
    50   55   60

Val Thr Asp Ile Tyr Leu Leu Asn Leu Ala Leu Ser Asp Leu Leu Phe
65   70   75   80

Val Ala Thr Leu Pro Phe Trp Thr His Tyr Leu Ile Asn Glu Lys Gly
                85   90   95

Leu His Asn Ala Met Cys Lys Phe Thr Thr Ala Phe Phe Phe Ile Gly
            100   105   110

Phe Phe Gly Ser Ile Phe Phe Ile Thr Val Ile Ser Ile Asp Arg Tyr
        115   120   125

Leu Ala Ile Val Leu Ala Ala Asn Ser Met Asn Asn Arg Thr Val Gln
    130   135   140

His Gly Val Thr Ile Ser Leu Gly Val Trp Ala Ala Ala Ile Leu Val
145   150   155   160

Ala Ala Pro Gln Phe Met Phe Thr Lys Gln Lys Glu Asn Glu Cys Leu
                165   170   175

```
Gly Asp Tyr Pro Glu Val Leu Gln Glu Ile Trp Pro Val Leu Arg Asn
            180                 185                 190

Val Glu Thr Asn Phe Leu Gly Phe Leu Leu Pro Leu Leu Ile Met Ser
        195                 200                 205

Tyr Cys Tyr Phe Arg Ile Ile Gln Thr Leu Phe Ser Cys Lys Asn His
    210                 215                 220

Lys Lys Ala Lys Ala Ile Lys Leu Ile Leu Leu Val Val Ile Val Phe
225                 230                 235                 240

Phe Leu Phe Trp Thr Pro Tyr Asn Val Met Ile Phe Leu Glu Thr Leu
                245                 250                 255

Lys Leu Tyr Asp Phe Phe Pro Ser Cys Asp Met Arg Lys Asp Leu Arg
            260                 265                 270

Leu Ala Leu Ser Val Thr Glu Thr Val Ala Phe Ser His Cys Cys Leu
        275                 280                 285

Asn Pro Leu Ile Tyr Ala Phe Ala Gly Glu Lys Phe Arg Arg Tyr Leu
    290                 295                 300

Tyr His Leu Tyr Gly Lys Cys Leu Ala Val Leu Cys Gly Arg Ser Val
305                 310                 315                 320

His Val Asp Phe Ser Ser Ser Glu Ser Gln Arg Ser Arg His Gly Ser
                325                 330                 335

Val Leu Ser Ser Asn Phe Thr Tyr His Thr Ser Asp Gly Asp Ala Leu
            340                 345                 350

Leu Leu Leu
        355
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<210> 54
<211> 1068

<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CXC3CR1"

<400> 54
atggatcagt tccctgaatc agtgacagaa aactttgagt acgatgattt ggctgaggcc 60

tgttatattg gggacatcgt ggtctttggg actgtgttcc tgtccatatt ctactccgtc 120

atctttgcca ttggcctggt gggaaatttg ttggtagtgt ttgccctcac caacagcaag 180

aagcccaaga gtgtcaccga catttacctc ctgaacctgg ccttgtctga tctgctgttt 240

gtagccactt tgcccttctg gactcactat ttgataaatg aaaagggcct ccacaatgcc 300

atgtgcaaat tcactaccgc cttcttcttc atcggctttt ttggaagcat attcttcatc 360

accgtcatca gcattgatag gtacctggcc atcgtcctgg ccgccaactc catgaacaac 420

cggaccgtgc agcatggcgt caccatcagc ctaggcgtct gggcagcagc cattttggtg 480

gcagcacccc agttcatgtt cacaaagcag aaagaaaatg aatgccttgg tgactacccc 540

gaggtcctcc aggaaatctg gcccgtgctc cgcaatgtgg aaacaaattt tcttggcttc 600

ctactccccc tgctcattat gagttattgc tacttcagaa tcatccagac gctgttttcc 660

tgcaagaacc acaagaaagc caaagccatt aaactgatcc ttctggtggt catcgtgttt 720

ttcctcttct ggacacccta caacgttatg attttcctgg agacgcttaa gctctatgac 780

ttctttccca gttgtgacat gaggaaggat ctgaggctgg ccctcagtgt gactgagacg 840

gttgcattta gccattgttg cctgaatcct ctcatctatg catttgctgg ggagaagttc 900

agaagatacc tttaccacct gtatgggaaa tgcctggctg tcctgtgtgg gcgctcagtc 960

cacgttgatt tctcctcatc tgaatcacaa aggagcaggc atggaagtgt tctgagcagc 1020

aattttactt accacacgag tgatggagat gcattgctcc ttctctga 1068

<210> 55
<211> 373
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="ChemR23"

<400> 55
Met Arg Met Glu Asp Glu Asp Tyr Asn Thr Ser Ile Ser Tyr Gly Asp
1   5   10   15

Glu Tyr Pro Asp Tyr Leu Asp Ser Ile Val Val Leu Glu Asp Leu Ser
            20   25   30

Pro Leu Glu Ala Arg Val Thr Arg Ile Phe Leu Val Val Val Tyr Ser
        35   40   45

Ile Val Cys Phe Leu Gly Ile Leu Gly Asn Gly Leu Val Ile Ile Ile
    50   55   60

Ala Thr Phe Lys Met Lys Lys Thr Val Asn Met Val Trp Phe Leu Asn
65   70   75   80

Leu Ala Val Ala Asp Phe Leu Phe Asn Val Phe Leu Pro Ile His Ile
                85   90   95

Thr Tyr Ala Ala Met Asp Tyr His Trp Val Phe Gly Thr Ala Met Cys
            100   105   110

Lys Ile Ser Asn Phe Leu Leu Ile His Asn Met Phe Thr Ser Val Phe
        115   120   125

Leu Leu Thr Ile Ile Ser Ser Asp Arg Cys Ile Ser Val Leu Leu Pro
    130   135   140

Val Trp Ser Gln Asn His Arg Ser Val Arg Leu Ala Tyr Met Ala Cys
145   150   155   160

Met Val Ile Trp Val Leu Ala Phe Phe Leu Ser Ser Pro Ser Leu Val
                165   170   175

Phe Arg Asp Thr Ala Asn Leu His Gly Lys Ile Ser Cys Phe Asn Asn

```
            180                 185                 190

Phe Ser Leu Ser Thr Pro Gly Ser Ser Ser Trp Pro Thr His Ser Gln
        195                 200                 205

Met Asp Pro Val Gly Tyr Ser Arg His Met Val Val Thr Val Thr Arg
    210                 215                 220

Phe Leu Cys Gly Phe Leu Val Pro Val Leu Ile Ile Thr Ala Cys Tyr
225                 230                 235                 240

Leu Thr Ile Val Cys Lys Leu Gln Arg Asn Arg Leu Ala Lys Thr Lys
                245                 250                 255

Lys Pro Phe Lys Ile Ile Val Thr Ile Ile Ile Thr Phe Phe Leu Cys
            260                 265                 270

Trp Cys Pro Tyr His Thr Leu Asn Leu Leu Glu Leu His His Thr Ala
        275                 280                 285

Met Pro Gly Ser Val Phe Ser Leu Gly Leu Pro Leu Ala Thr Ala Leu
    290                 295                 300

Ala Ile Ala Asn Ser Cys Met Asn Pro Ile Leu Tyr Val Phe Met Gly
305                 310                 315                 320

Gln Asp Phe Lys Lys Phe Lys Val Ala Leu Phe Ser Arg Leu Val Asn
                325                 330                 335

Ala Leu Ser Glu Asp Thr Gly His Ser Ser Tyr Pro Ser His Arg Ser
            340                 345                 350

Phe Thr Lys Met Ser Ser Met Asn Glu Arg Thr Ser Met Asn Glu Arg
        355                 360                 365

Glu Thr Gly Met Leu
    370
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<210> 56
<211> 1122
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="ChemR23"

<400> 56
atgagaatgg aggatgaaga ttacaacact tccatcagtt acggtgatga ataccctgat 60

tatttagact ccattgtggt tttggaggac ttatcccccct tggaagccag ggtgaccagg 120

atcttcctgg tggtggtcta cagcatcgtc tgcttcctcg ggattctggg caatggtctg 180

gtgatcatca ttgccacctt caagatgaag aagacagtga acatggtctg gttcctcaac 240

ctggcagtgg cagatttcct gttcaacgtc ttcctcccaa tccatatcac ctatgccgcc 300

atggactacc actgggtttt cgggacagcc atgtgcaaga tcagcaactt ccttctcatc 360

cacaacatgt tcaccagcgt cttcctgctg accatcatca gctctgaccg ctgcatctct 420

gtgctcctcc ctgtctggtc ccagaaccac cgcagcgttc gcctggctta catggcctgc 480

atggtcatct gggtcctggc tttcttcttg agttccccat ctctcgtctt ccgggacaca 540

gccaacctgc atgggaaaat atcctgcttc aacaacttca gcctgtccac acctgggtct 600

tcctcgtggc ccactcactc ccaaatggac cctgtggggt atagccggca catggtggtg 660

actgtcaccc gcttcctctg tggcttcctg gtcccagtcc tcatcatcac agcttgctac 720

ctcaccatcg tgtgcaaact gcagcgcaac cgcctggcca agaccaagaa gcccttcaag 780

attattgtga ccatcatcat taccttcttc ctctgctggt gcccctacca cacactcaac 840

ctcctagagc tccaccacac tgccatgcct ggctctgtct tcagcctggg tttgcccctg 900

gccactgccc ttgccattgc caacagctgc atgaacccca ttctgtatgt tttcatgggt 960

caggacttca agaagttcaa ggtggccctc ttctctcgcc tggtcaatgc tctaagtgaa 1020

gatacaggcc actcttccta ccccagccat agaagcttta ccaagatgtc atcaatgaat 1080

gagaggactt ctatgaatga gagggagacc ggcatgcttt ga 1122

<210> 57
<211> 352
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR4"

<400> 57
Met Glu Gly Ile Ser Ile Tyr Thr Ser Asp Asn Tyr Thr Glu Glu Met
1   5   10   15

Gly Ser Gly Asp Tyr Asp Ser Met Lys Glu Pro Cys Phe Arg Glu Glu
            20   25   30

Asn Ala Asn Phe Asn Lys Ile Phe Leu Pro Thr Ile Tyr Ser Ile Ile
        35   40   45

Phe Leu Thr Gly Ile Val Gly Asn Gly Leu Val Ile Leu Val Met Gly
    50   55   60

Tyr Gln Lys Lys Leu Arg Ser Met Thr Asp Lys Tyr Arg Leu His Leu
65   70   75   80

Ser Val Ala Asp Leu Leu Phe Val Ile Thr Leu Pro Phe Trp Ala Val
                85   90   95

Asp Ala Val Ala Asn Trp Tyr Phe Gly Asn Phe Leu Cys Lys Ala Val
            100   105   110

His Val Ile Tyr Thr Val Asn Leu Tyr Ser Ser Val Leu Ile Leu Ala
        115   120   125

Phe Ile Ser Leu Asp Arg Tyr Leu Ala Ile Val His Ala Thr Asn Ser
    130   135   140

Gln Arg Pro Arg Lys Leu Leu Ala Glu Lys Val Val Tyr Val Gly Val
145   150   155   160

```
Trp Ile Pro Ala Leu Leu Leu Thr Ile Pro Asp Phe Ile Phe Ala Asn
                165                 170                 175

Val Ser Glu Ala Asp Asp Arg Tyr Ile Cys Asp Arg Phe Tyr Pro Asn
            180                 185                 190

Asp Leu Trp Val Val Val Phe Gln Phe Gln His Ile Met Val Gly Leu
        195                 200                 205

Ile Leu Pro Gly Ile Val Ile Leu Ser Cys Tyr Cys Ile Ile Ile Ser
    210                 215                 220

Lys Leu Ser His Ser Lys Gly His Gln Lys Arg Lys Ala Leu Lys Thr
225                 230                 235                 240

Thr Val Ile Leu Ile Leu Ala Phe Phe Ala Cys Trp Leu Pro Tyr Tyr
                245                 250                 255

Ile Gly Ile Ser Ile Asp Ser Phe Ile Leu Leu Glu Ile Ile Lys Gln
            260                 265                 270

Gly Cys Glu Phe Glu Asn Thr Val His Lys Trp Ile Ser Ile Thr Glu
        275                 280                 285

Ala Leu Ala Phe Phe His Cys Cys Leu Asn Pro Ile Leu Tyr Ala Phe
    290                 295                 300

Leu Gly Ala Lys Phe Lys Thr Ser Ala Gln His Ala Leu Thr Ser Val
305                 310                 315                 320

Ser Arg Gly Ser Ser Leu Lys Ile Leu Ser Lys Gly Lys Arg Gly Gly
                325                 330                 335

His Ser Ser Val Ser Thr Glu Ser Glu Ser Ser Ser Phe His Ser Ser
            340                 345                 350
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<210> 58
<211> 1071

<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CXCR4"

<400> 58
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ggctcagggg actatgactc catgaaggaa ccctgtttcc gtgaagaaaa tgctaatttc 120

aataaaatct tcctgcccac catctactcc atcatcttct taactggcat tgtgggcaat 180

ggattggtca tcctggtcat gggttaccag aagaaactga gaagcatgac ggacaagtac 240

aggctgcacc tgtcagtggc cgacctcctc tttgtcatca cgcttccctt ctgggcagtt 300

gatgccgtgg caaactggta ctttgggaac ttcctatgca aggcagtcca tgtcatctac 360

acagtcaacc tctacagcag tgtcctcatc ctggccttca tcagtctgga ccgctacctg 420

gccatcgtcc acgccaccaa cagtcagagg ccaaggaagc tgttggctga aaaggtggtc 480

tatgttggcg tctggatccc tgccctcctg ctgactattc ccgacttcat ctttgccaac 540

gtcagtgagg cagatgacag atatatctgt gaccgcttct accccaatga cttgtgggtg 600

gttgtgttcc agtttcagca catcatggtt ggccttatcc tgcctggtat tgtcatcctg 660

tcctgctatt gcattatcat ctccaagctg tcacactcca agggccacca gaagcgcaag 720

gccctcaaga ccacagtcat cctcatcctg gctttcttcg cctgttggct gccttactac 780

attgggatca gcatcgactc cttcatcctc ctggaaatca tcaagcaagg gtgtgagttt 840

gagaacactg tgcacaagtg gatttccatc accgaggccc tagctttctt ccactgttgt 900

ctgaacccca tcctctatgc tttccttgga gccaaattta aaacctctgc ccagcacgca 960

ctcacctctg tgagcagagg gtccagcctc aagatcctct ccaaaggaaa gcgaggtgga 1020

cattcatctg tttccactga gtctgagtct tcaagttttc actccagcta a 1071

<210> 59
<211> 352
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CCR5"

<400> 59
Met Asp Tyr Gln Val Ser Ser Pro Ile Tyr Asp Ile Asn Tyr Tyr Thr
1   5   10   15

Ser Glu Pro Cys Gln Lys Ile Asn Val Lys Gln Ile Ala Ala Arg Leu
            20   25   30

Leu Pro Pro Leu Tyr Ser Leu Val Phe Ile Phe Gly Phe Val Gly Asn
        35   40   45

Met Leu Val Ile Leu Ile Leu Ile Asn Cys Lys Arg Leu Lys Ser Met
    50   55   60

Thr Asp Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asp Leu Phe Phe Leu
65   70   75   80

Leu Thr Val Pro Phe Trp Ala His Tyr Ala Ala Ala Gln Trp Asp Phe
                85   90   95

Gly Asn Thr Met Cys Gln Leu Leu Thr Gly Leu Tyr Phe Ile Gly Phe
            100   105   110

Phe Ser Gly Ile Phe Phe Ile Ile Leu Leu Thr Ile Asp Arg Tyr Leu
        115   120   125

Ala Val Val His Ala Val Phe Ala Leu Lys Ala Arg Thr Val Thr Phe
    130   135   140

Gly Val Val Thr Ser Val Ile Thr Trp Val Val Ala Val Phe Ala Ser
145   150   155   160

Leu Pro Gly Ile Ile Phe Thr Arg Ser Gln Lys Glu Gly Leu His Tyr
                165   170   175

Thr Cys Ser Ser His Phe Pro Tyr Ser Gln Tyr Gln Phe Trp Lys Asn

```
            180                 185                 190

Phe Gln Thr Leu Lys Ile Val Ile Leu Gly Leu Val Leu Pro Leu Leu
        195                 200                 205

Val Met Val Ile Cys Tyr Ser Gly Ile Leu Lys Thr Leu Leu Arg Cys
    210                 215                 220

Arg Asn Glu Lys Lys Arg His Arg Ala Val Arg Leu Ile Phe Thr Ile
225                 230                 235                 240

Met Ile Val Tyr Phe Leu Phe Trp Ala Pro Tyr Asn Ile Val Leu Leu
                245                 250                 255

Leu Asn Thr Phe Gln Glu Phe Phe Gly Leu Asn Asn Cys Ser Ser Ser
            260                 265                 270

Asn Arg Leu Asp Gln Ala Met Gln Val Thr Glu Thr Leu Gly Met Thr
        275                 280                 285

His Cys Cys Ile Asn Pro Ile Ile Tyr Ala Phe Val Gly Glu Lys Phe
    290                 295                 300

Arg Asn Tyr Leu Leu Val Phe Phe Gln Lys His Ile Ala Lys Arg Phe
305                 310                 315                 320

Cys Lys Cys Cys Ser Ile Phe Gln Gln Glu Ala Pro Glu Arg Ala Ser
                325                 330                 335

Ser Val Tyr Thr Arg Ser Thr Gly Glu Gln Glu Ile Ser Val Gly Leu
            340                 345                 350


<210> 60
<211> 1059
<212> DNA
<213> Homo sapiens

<220>
<221> source
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<223> /note="CCR5"

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caaaaaatca atgtgaagca aatcgcagcc cgcctcctgc ctccgctcta ctcactggtg 120

ttcatctttg gttttgtggg caacatgctg gtcatcctca tcctgataaa ctgcaaaagg 180

ctgaagagca tgactgacat ctacctgctc aacctggcca tctctgacct gtttttcctt 240

cttactgtcc ccttctgggc tcactatgct gccgcccagt gggactttgg aaatacaatg 300

tgtcaactct tgacagggct ctattttata ggcttcttct ctggaatctt cttcatcatc 360

ctcctgacaa tcgataggta cctggctgtc gtccatgctg tgtttgcttt aaaagccagg 420

acggtcacct ttggggtggt gacaagtgtg atcacttggg tggtggctgt gtttgcgtct 480

ctcccaggaa tcatctttac cagatctcaa aaagaaggtc ttcattacac ctgcagctct 540

cattttccat acagtcagta tcaattctgg aagaatttcc agacattaaa gatagtcatc 600

ttggggctgg tcctgccgct gcttgtcatg gtcatctgct actcgggaat cctaaaaact 660

ctgcttcggt gtcgaaatga gaagaagagg cacagggctg tgaggcttat cttcaccatc 720

atgattgttt attttctctt ctgggctccc tacaacattg tccttctcct gaacaccttc 780

caggaattct ttggcctgaa taattgcagt agctctaaca ggttggacca agctatgcag 840

gtgacagaga ctcttgggat gacgcactgc tgcatcaacc ccatcatcta tgcctttgtc 900

ggggagaagt tcagaaacta cctcttagtc ttcttccaaa agcacattgc caaacgcttc 960

tgcaaatgct gttctatttt ccagcaagag gctcccgagc gagcaagctc agtttacacc 1020

cgatccactg gggagcagga aatatctgtg ggcttgtga 1059

<210> 61
<211> 398
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="S1P5"

<400> 61

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Met Glu Ser Gly Leu Leu Arg Pro Ala Pro Val Ser Glu Val Ile Val
1               5                  10                  15

Leu His Tyr Asn Tyr Thr Gly Lys Leu Arg Gly Ala Arg Tyr Gln Pro
            20                  25                  30

Gly Ala Gly Leu Arg Ala Asp Ala Val Val Cys Leu Ala Val Cys Ala
        35                  40                  45

Phe Ile Val Leu Glu Asn Leu Ala Val Leu Leu Val Leu Gly Arg His
    50                  55                  60

Pro Arg Phe His Ala Pro Met Phe Leu Leu Leu Gly Ser Leu Thr Leu
65                  70                  75                  80

Ser Asp Leu Leu Ala Gly Ala Ala Tyr Ala Ala Asn Ile Leu Leu Ser
                85                  90                  95

Gly Pro Leu Thr Leu Lys Leu Ser Pro Ala Leu Trp Phe Ala Arg Glu
            100                 105                 110

Gly Gly Val Phe Val Ala Leu Thr Ala Ser Val Leu Ser Leu Leu Ala
        115                 120                 125

Ile Ala Leu Glu Arg Ser Leu Thr Met Ala Arg Arg Gly Pro Ala Pro
    130                 135                 140

Val Ser Ser Arg Gly Arg Thr Leu Ala Met Ala Ala Ala Ala Trp Gly
145                 150                 155                 160

Val Ser Leu Leu Leu Gly Leu Leu Pro Ala Leu Gly Trp Asn Cys Leu
                165                 170                 175

Gly Arg Leu Asp Ala Cys Ser Thr Val Leu Pro Leu Tyr Ala Lys Ala
            180                 185                 190

Tyr Val Leu Phe Cys Val Leu Ala Phe Val Gly Ile Leu Ala Ala Ile
        195                 200                 205
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Cys Ala Leu Tyr Ala Arg Ile Tyr Cys Gln Val Arg Ala Asn Ala Arg
    210                 215                 220

Arg Leu Pro Ala Arg Pro Gly Thr Ala Gly Thr Thr Ser Thr Arg Ala
225                 230                 235                 240

Arg Arg Lys Pro Arg Ser Leu Ala Leu Leu Arg Thr Leu Ser Val Val
                245                 250                 255

Leu Leu Ala Phe Val Ala Cys Trp Gly Pro Leu Phe Leu Leu Leu Leu
            260                 265                 270

Leu Asp Val Ala Cys Pro Ala Arg Thr Cys Pro Val Leu Leu Gln Ala
        275                 280                 285

Asp Pro Phe Leu Gly Leu Ala Met Ala Asn Ser Leu Leu Asn Pro Ile
    290                 295                 300

Ile Tyr Thr Leu Thr Asn Arg Asp Leu Arg His Ala Leu Leu Arg Leu
305                 310                 315                 320

Val Cys Cys Gly Arg His Ser Cys Gly Arg Asp Pro Ser Gly Ser Gln
                325                 330                 335

Gln Ser Ala Ser Ala Ala Glu Ala Ser Gly Gly Leu Arg Arg Cys Leu
            340                 345                 350

Pro Pro Gly Leu Asp Gly Ser Phe Ser Gly Ser Glu Arg Ser Ser Pro
        355                 360                 365

Gln Arg Asp Gly Leu Asp Thr Ser Gly Ser Thr Gly Ser Pro Gly Ala
    370                 375                 380

Pro Thr Ala Ala Arg Thr Leu Val Ser Glu Pro Ala Ala Asp
385                 390                 395
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<210> 62
<211> 1197
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="S1P5"

<400> 62
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tacaccggca agctccgcgg tgcgcgctac cagccgggtg ccggcctgcg cgccgacgcc 120

gtggtgtgcc tggcggtgtg cgccttcatc gtgctagaga atctagccgt gttgttggtg 180

ctcggacgcc acccgcgctt ccacgctccc atgttcctgc tcctgggcag cctcacgttg 240

tcggatctgc tggcaggcgc cgcctacgcc gccaacatcc tactgtcggg gccgctcacg 300

ctgaaactgt cccccgcgct ctggttcgca cgggagggag gcgtcttcgt ggcactcact 360

gcgtccgtgc tgagcctcct ggccatcgcg ctggagcgca gcctcaccat ggcgcgcagg 420

gggcccgcgc ccgtctccag tcgggggcgc acgctggcga tggcagccgc ggcctggggc 480

gtgtcgctgc tcctcgggct cctgccagcg ctgggctgga attgcctggg tcgcctggac 540

gcttgctcca ctgtcttgcc gctctacgcc aaggcctacg tgctcttctg cgtgctcgcc 600

ttcgtgggca tcctggccgc tatctgtgca ctctacgcgc gcatctactg ccaggtacgc 660

gccaacgcgc ggcgcctgcc ggcacggccc gggactgcgg ggaccacctc gacccgggcg 720

cgtcgcaagc cgcgctcgct ggccttgctg cgcacgctca gcgtggtgct cctggccttt 780

gtggcatgtt ggggccccct cttcctgctg ctgttgctcg acgtggcgtg cccggcgcgc 840

acctgtcctg tactcctgca ggccgatccc ttcctgggac tggccatggc caactcactt 900

ctgaacccca tcatctacac gctcaccaac cgcgacctgc gccacgcgct cctgcgcctg 960

gtctgctgcg gacgccactc ctgcggcaga gacccgagtg gctcccagca gtcggcgagc 1020

gcggctgagg cttccggggg cctgcgccgc tgcctgcccc cgggccttga tgggagcttc 1080

agcggctcgg agcgctcatc gccccagcgc gacgggctgg acaccagcgg ctccacaggc 1140

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<210> 63
<211> 951
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="cKit"

<400> 63
Gln Pro Ser Val Ser Pro Gly Glu Pro Ser Pro Pro Ser Ile His Pro
1   5   10   15

Gly Lys Ser Asp Leu Ile Val Arg Val Gly Asp Glu Ile Arg Leu Leu
            20   25   30

Cys Thr Asp Pro Gly Phe Val Lys Trp Thr Phe Glu Ile Leu Asp Glu
        35   40   45

Thr Asn Glu Asn Lys Gln Asn Glu Trp Ile Thr Glu Lys Ala Glu Ala
    50   55   60

Thr Asn Thr Gly Lys Tyr Thr Cys Thr Asn Lys His Gly Leu Ser Asn
65   70   75   80

Ser Ile Tyr Val Phe Val Arg Asp Pro Ala Lys Leu Phe Leu Val Asp
                85   90   95

Arg Ser Leu Tyr Gly Lys Glu Asp Asn Asp Thr Leu Val Arg Cys Pro
            100   105   110

Leu Thr Asp Pro Glu Val Thr Asn Tyr Ser Leu Lys Gly Cys Gln Gly
        115   120   125

Lys Pro Leu Pro Lys Asp Leu Arg Phe Ile Pro Asp Pro Lys Ala Gly
    130   135   140

Ile Met Ile Lys Ser Val Lys Arg Ala Tyr His Arg Leu Cys Leu His
145   150   155   160

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Cys Ser Val Asp Gln Glu Gly Lys Ser Val Leu Ser Glu Lys Phe Ile
                165                 170                 175

Leu Lys Val Arg Pro Ala Phe Lys Ala Val Pro Val Val Ser Val Ser
            180                 185                 190

Lys Ala Ser Tyr Leu Leu Arg Glu Gly Glu Glu Phe Thr Val Thr Cys
        195                 200                 205

Thr Ile Lys Asp Val Ser Ser Ser Val Tyr Ser Thr Trp Lys Arg Glu
    210                 215                 220

Asn Ser Gln Thr Lys Leu Gln Glu Lys Tyr Asn Ser Trp His His Gly
225                 230                 235                 240

Asp Phe Asn Tyr Glu Arg Gln Ala Thr Leu Thr Ile Ser Ser Ala Arg
                245                 250                 255

Val Asn Asp Ser Gly Val Phe Met Cys Tyr Ala Asn Asn Thr Phe Gly
            260                 265                 270

Ser Ala Asn Val Thr Thr Thr Leu Glu Val Val Asp Lys Gly Phe Ile
        275                 280                 285

Asn Ile Phe Pro Met Ile Asn Thr Thr Val Phe Val Asn Asp Gly Glu
    290                 295                 300

Asn Val Asp Leu Ile Val Glu Tyr Glu Ala Phe Pro Lys Pro Glu His
305                 310                 315                 320

Gln Gln Trp Ile Tyr Met Asn Arg Thr Phe Thr Asp Lys Trp Glu Asp
                325                 330                 335

Tyr Pro Lys Ser Glu Asn Glu Ser Asn Ile Arg Tyr Val Ser Glu Leu
            340                 345                 350

His Leu Thr Arg Leu Lys Gly Thr Glu Gly Gly Thr Tyr Thr Phe Leu
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        355                 360                 365

Val Ser Asn Ser Asp Val Asn Ala Ala Ile Ala Phe Asn Val Tyr Val
    370                 375                 380

Asn Thr Lys Pro Glu Ile Leu Thr Tyr Asp Arg Leu Val Asn Gly Met
385                 390                 395                 400

Leu Gln Cys Val Ala Ala Gly Phe Pro Glu Pro Thr Ile Asp Trp Tyr
                405                 410                 415

Phe Cys Pro Gly Thr Glu Gln Arg Cys Ser Ala Ser Val Leu Pro Val
            420                 425                 430

Asp Val Gln Thr Leu Asn Ser Ser Gly Pro Pro Phe Gly Lys Leu Val
        435                 440                 445

Val Gln Ser Ser Ile Asp Ser Ser Ala Phe Lys His Asn Gly Thr Val
    450                 455                 460

Glu Cys Lys Ala Tyr Asn Asp Val Gly Lys Thr Ser Ala Tyr Phe Asn
465                 470                 475                 480

Phe Ala Phe Lys Gly Asn Asn Lys Glu Gln Ile His Pro His Thr Leu
                485                 490                 495

Phe Thr Pro Leu Leu Ile Gly Phe Val Ile Val Ala Gly Met Met Cys
            500                 505                 510

Ile Ile Val Met Ile Leu Thr Tyr Lys Tyr Leu Gln Lys Pro Met Tyr
        515                 520                 525

Glu Val Gln Trp Lys Val Val Glu Glu Ile Asn Gly Asn Asn Tyr Val
    530                 535                 540

Tyr Ile Asp Pro Thr Gln Leu Pro Tyr Asp His Lys Trp Glu Phe Pro
545                 550                 555                 560
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Arg Asn Arg Leu Ser Phe Gly Lys Thr Leu Gly Ala Gly Ala Phe Gly
                565                 570                 575

Lys Val Val Glu Ala Thr Ala Tyr Gly Leu Ile Lys Ser Asp Ala Ala
            580                 585                 590

Met Thr Val Ala Val Lys Met Leu Lys Pro Ser Ala His Leu Thr Glu
        595                 600                 605

Arg Glu Ala Leu Met Ser Glu Leu Lys Val Leu Ser Tyr Leu Gly Asn
    610                 615                 620

His Met Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Ile Gly Gly Pro
625                 630                 635                 640

Thr Leu Val Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Phe
                645                 650                 655

Leu Arg Arg Lys Arg Asp Ser Phe Ile Cys Ser Lys Gln Glu Asp His
            660                 665                 670

Ala Glu Ala Ala Leu Tyr Lys Asn Leu Leu His Ser Lys Glu Ser Ser
        675                 680                 685

Cys Ser Asp Ser Thr Asn Glu Tyr Met Asp Met Lys Pro Gly Val Ser
    690                 695                 700

Tyr Val Val Pro Thr Lys Ala Asp Lys Arg Arg Ser Val Arg Ile Gly
705                 710                 715                 720

Ser Tyr Ile Glu Arg Asp Val Thr Pro Ala Ile Met Glu Asp Asp Glu
                725                 730                 735

Leu Ala Leu Asp Leu Glu Asp Leu Leu Ser Phe Ser Tyr Gln Val Ala
            740                 745                 750

Lys Gly Met Ala Phe Leu Ala Ser Lys Asn Cys Ile His Arg Asp Leu
```

```
        755                 760                 765

Ala Ala Arg Asn Ile Leu Leu Thr His Gly Arg Ile Thr Lys Ile Cys
    770                 775                 780

Asp Phe Gly Leu Ala Arg Asp Ile Lys Asn Asp Ser Asn Tyr Val Val
785                 790                 795                 800

Lys Gly Asn Ala Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Ile
                805                 810                 815

Phe Asn Cys Val Tyr Thr Phe Glu Ser Asp Val Trp Ser Tyr Gly Ile
            820                 825                 830

Phe Leu Trp Glu Leu Phe Ser Leu Gly Ser Ser Pro Tyr Pro Gly Met
        835                 840                 845

Pro Val Asp Ser Lys Phe Tyr Lys Met Ile Lys Glu Gly Phe Arg Met
    850                 855                 860

Leu Ser Pro Glu His Ala Pro Ala Glu Met Tyr Asp Ile Met Lys Thr
865                 870                 875                 880

Cys Trp Asp Ala Asp Pro Leu Lys Arg Pro Thr Phe Lys Gln Ile Val
                885                 890                 895

Gln Leu Ile Glu Lys Gln Ile Ser Glu Ser Thr Asn His Ile Tyr Ser
            900                 905                 910

Asn Leu Ala Asn Cys Ser Pro Asn Arg Gln Lys Pro Val Val Asp His
        915                 920                 925

Ser Val Arg Ile Asn Ser Val Gly Ser Thr Ala Ser Ser Ser Gln Pro
    930                 935                 940

Leu Leu Val His Asp Asp Val
945                 950
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<210> 64
<211> 2931
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="cKit"

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ccaggaaaat cagacttaat agtccgcgtg ggcgacgaga ttaggctgtt atgcactgat 180

ccgggctttg tcaaatggac ttttgagatc ctggatgaaa cgaatgagaa taagcagaat 240

gaatggatca cggaaaaggc agaagccacc aacaccggca aatacacgtg caccaacaaa 300

cacggcttaa gcaattccat ttatgtgttt gttagagatc ctgccaagct tttccttgtt 360

gaccgctcct tgtatgggaa agaagacaac gacacgctgg tccgctgtcc tctcacagac 420

ccagaagtga ccaattattc cctcaagggg tgccagggga agcctcttcc caaggacttg 480

aggtttattc ctgaccccaa ggcgggcatc atgatcaaaa gtgtgaaacg cgcctaccat 540

cggctctgtc tgcattgttc tgtggaccag gagggcaagt cagtgctgtc ggaaaaattc 600

atcctgaaag tgaggccagc cttcaaagct gtgcctgttg tgtctgtgtc caaagcaagc 660

tatcttctta gggaagggga agaattcaca gtgacgtgca caataaaaga tgtgtctagt 720

tctgtgtact caacgtggaa aagagaaaac agtcagacta aactacagga gaaatataat 780

agctggcatc acggtgactt caattatgaa cgtcaggcaa cgttgactat cagttcagcg 840

agagttaatg attctggagt gttcatgtgt tatgccaata atacttttgg atcagcaaat 900

gtcacaacaa ccttggaagt agtagataaa ggattcatta atatcttccc catgataaac 960

actacagtat ttgtaaacga tggagaaaat gtagatttga ttgttgaata tgaagcattc 1020

cccaaacctg aacaccagca gtggatctat atgaacagaa ccttcactga taaatgggaa 1080

gattatccca agtctgagaa tgaaagtaat atcagatacg taagtgaact tcatctaacg 1140

agattaaaag gcaccgaagg aggcacttac acattcctag tgtccaattc tgacgtcaat 1200

gctgccatag catttaatgt ttatgtgaat acaaaaccag aaatcctgac ttacgacagg 1260

ctcgtgaatg gcatgctcca atgtgtggca gcaggattcc cagagcccac aatagattgg 1320

tatttttgtc caggaactga gcagagatgc tctgcttctg tactgccagt ggatgtgcag 1380

acactaaact catctgggcc accgtttgga aagctagtgg ttcagagttc tatagattct 1440

agtgcattca agcacaatgg cacggttgaa tgtaaggctt acaacgatgt gggcaagact 1500

tctgcctatt ttaactttgc atttaaaggt aacaacaaag agcaaatcca tccccacacc 1560

ctgttcactc ctttgctgat tggtttcgta atcgtagctg gcatgatgtg cattattgtg 1620

atgattctga cctacaaata tttacagaaa cccatgtatg aagtacagtg gaaggttgtt 1680

gaggagataa atggaaacaa ttatgtttac atagacccaa cacaacttcc ttatgatcac 1740

aaatgggagt ttcccagaaa caggctgagt tttgggaaaa ccctgggtgc tggagctttc 1800

gggaaggttg ttgaggcaac tgcttatggc ttaattaagt cagatgcggc catgactgtc 1860

gctgtaaaga tgctcaagcc gagtgcccat ttgacagaac gggaagccct catgtctgaa 1920

ctcaaagtcc tgagttacct tggtaatcac atgaatattg tgaatctact tggagcctgc 1980

accattggag ggcccaccct ggtcattaca gaatattgtt gctatggtga tcttttgaat 2040

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gcactttata agaatcttct gcattcaaag gagtcttcct gcagcgatag tactaatgag 2160

tacatggaca tgaaacctgg agtttcttat gttgtcccaa ccaaggccga caaaaggaga 2220

tctgtgagaa taggctcata catagaaaga gatgtgactc ccgccatcat ggaggatgac 2280

gagttggccc tagacttaga agacttgctg agcttttctt accaggtggc aaagggcatg 2340

gctttcctcg cctccaagaa ttgtattcac agagacttgg cagccagaaa tatcctcctt 2400

actcatggtc ggatcacaaa gatttgtgat tttggtctag ccagagacat caagaatgat 2460

tctaattatg tggttaaagg aaacgctcga ctacctgtga agtggatggc acctgaaagc 2520

attttcaact gtgtatacac gtttgaaagt gacgtctggt cctatgggat ttttctttgg 2580

gagctgttct ctttaggaag cagcccctat cctggaatgc cggtcgattc taagttctac 2640

aagatgatca aggaaggctt ccggatgctc agccctgaac acgcacctgc tgaaatgtat 2700

gacataatga agacttgctg ggatgcagat cccctaaaaa gaccaacatt caagcaaatt 2760

gttcagctaa ttgagaagca gatttcagag agcaccaatc atatttactc caacttagca 2820

aactgcagcc ccaaccgaca gaagcccgtg gtagaccatt ctgtgcggat caattctgtc 2880

ggcagcaccg cttcctcctc ccagcctctg cttgtgcacg acgatgtctg a 2931

<210> 65
<211> 2549
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="mTor"

<400> 65
Met Leu Gly Thr Gly Pro Ala Ala Ala Thr Thr Ala Ala Thr Thr Ser
1 5 10 15

Ser Asn Val Ser Val Leu Gln Gln Phe Ala Ser Gly Leu Lys Ser Arg
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Asn Glu Glu Thr Arg Ala Lys Ala Ala Lys Glu Leu Gln His Tyr Val
35 40 45

Thr Met Glu Leu Arg Glu Met Ser Gln Glu Glu Ser Thr Arg Phe Tyr
50 55 60

Asp Gln Leu Asn His His Ile Phe Glu Leu Val Ser Ser Ser Asp Ala
65 70 75 80

Asn Glu Arg Lys Gly Gly Ile Leu Ala Ile Ala Ser Leu Ile Gly Val
85 90 95

Glu Gly Gly Asn Ala Thr Arg Ile Gly Arg Phe Ala Asn Tyr Leu Arg
100 105 110

Asn Leu Leu Pro Ser Asn Asp Pro Val Val Met Glu Met Ala Ser Lys
115 120 125

```
Ala Ile Gly Arg Leu Ala Met Ala Gly Asp Thr Phe Thr Ala Glu Tyr
    130                 135                 140

Val Glu Phe Glu Val Lys Arg Ala Leu Glu Trp Leu Gly Ala Asp Arg
145                 150                 155                 160

Asn Glu Gly Arg Arg His Ala Ala Val Leu Val Leu Arg Glu Leu Ala
                165                 170                 175

Ile Ser Val Pro Thr Phe Phe Phe Gln Gln Val Gln Pro Phe Phe Asp
            180                 185                 190

Asn Ile Phe Val Ala Val Trp Asp Pro Lys Gln Ala Ile Arg Glu Gly
        195                 200                 205

Ala Val Ala Ala Leu Arg Ala Cys Leu Ile Leu Thr Thr Gln Arg Glu
    210                 215                 220

Pro Lys Glu Met Gln Lys Pro Gln Trp Tyr Arg His Thr Phe Glu Glu
225                 230                 235                 240

Ala Glu Lys Gly Phe Asp Glu Thr Leu Ala Lys Glu Lys Gly Met Asn
                245                 250                 255

Arg Asp Asp Arg Ile His Gly Ala Leu Leu Ile Leu Asn Glu Leu Val
            260                 265                 270

Arg Ile Ser Ser Met Glu Gly Glu Arg Leu Arg Glu Glu Met Glu Glu
        275                 280                 285

Ile Thr Gln Gln Gln Leu Val His Asp Lys Tyr Cys Lys Asp Leu Met
    290                 295                 300

Gly Phe Gly Thr Lys Pro Arg His Ile Thr Pro Phe Thr Ser Phe Gln
305                 310                 315                 320
```

```
Ala Val Gln Pro Gln Gln Ser Asn Ala Leu Val Gly Leu Leu Gly Tyr
                325                 330                 335

Ser Ser His Gln Gly Leu Met Gly Phe Gly Thr Ser Pro Ser Pro Ala
            340                 345                 350

Lys Ser Thr Leu Val Glu Ser Arg Cys Cys Arg Asp Leu Met Glu Glu
        355                 360                 365

Lys Phe Asp Gln Val Cys Gln Trp Val Leu Lys Cys Arg Asn Ser Lys
    370                 375                 380

Asn Ser Leu Ile Gln Met Thr Ile Leu Asn Leu Leu Pro Arg Leu Ala
385                 390                 395                 400

Ala Phe Arg Pro Ser Ala Phe Thr Asp Thr Gln Tyr Leu Gln Asp Thr
                405                 410                 415

Met Asn His Val Leu Ser Cys Val Lys Lys Glu Lys Glu Arg Thr Ala
            420                 425                 430

Ala Phe Gln Ala Leu Gly Leu Leu Ser Val Ala Val Arg Ser Glu Phe
        435                 440                 445

Lys Val Tyr Leu Pro Arg Val Leu Asp Ile Ile Arg Ala Ala Leu Pro
    450                 455                 460

Pro Lys Asp Phe Ala His Lys Arg Gln Lys Ala Met Gln Val Asp Ala
465                 470                 475                 480

Thr Val Phe Thr Cys Ile Ser Met Leu Ala Arg Ala Met Gly Pro Gly
                485                 490                 495

Ile Gln Gln Asp Ile Lys Glu Leu Leu Glu Pro Met Leu Ala Val Gly
            500                 505                 510

Leu Ser Pro Ala Leu Thr Ala Val Leu Tyr Asp Leu Ser Arg Gln Ile
        515                 520                 525
```

```
Pro Gln Leu Lys Lys Asp Ile Gln Asp Gly Leu Leu Lys Met Leu Ser
    530                 535                 540

Leu Val Leu Met His Lys Pro Leu Arg His Pro Gly Met Pro Lys Gly
545                 550                 555                 560

Leu Ala His Gln Leu Ala Ser Pro Gly Leu Thr Thr Leu Pro Glu Ala
                565                 570                 575

Ser Asp Val Gly Ser Ile Thr Leu Ala Leu Arg Thr Leu Gly Ser Phe
            580                 585                 590

Glu Phe Glu Gly His Ser Leu Thr Gln Phe Val Arg His Cys Ala Asp
        595                 600                 605

His Phe Leu Asn Ser Glu His Lys Glu Ile Arg Met Glu Ala Ala Arg
    610                 615                 620

Thr Cys Ser Arg Leu Leu Thr Pro Ser Ile His Leu Ile Ser Gly His
625                 630                 635                 640

Ala His Val Val Ser Gln Thr Ala Val Gln Val Val Ala Asp Val Leu
                645                 650                 655

Ser Lys Leu Leu Val Val Gly Ile Thr Asp Pro Asp Pro Asp Ile Arg
            660                 665                 670

Tyr Cys Val Leu Ala Ser Leu Asp Glu Arg Phe Asp Ala His Leu Ala
        675                 680                 685

Gln Ala Glu Asn Leu Gln Ala Leu Phe Val Ala Leu Asn Asp Gln Val
    690                 695                 700

Phe Glu Ile Arg Glu Leu Ala Ile Cys Thr Val Gly Arg Leu Ser Ser
705                 710                 715                 720
```

```
Met Asn Pro Ala Phe Val Met Pro Phe Leu Arg Lys Met Leu Ile Gln
                725                 730                 735

Ile Leu Thr Glu Leu Glu His Ser Gly Ile Gly Arg Ile Lys Glu Gln
            740                 745                 750

Ser Ala Arg Met Leu Gly His Leu Val Ser Asn Ala Pro Arg Leu Ile
        755                 760                 765

Arg Pro Tyr Met Glu Pro Ile Leu Lys Ala Leu Ile Leu Lys Leu Lys
    770                 775                 780

Asp Pro Asp Pro Asp Pro Asn Pro Gly Val Ile Asn Asn Val Leu Ala
785                 790                 795                 800

Thr Ile Gly Glu Leu Ala Gln Val Ser Gly Leu Glu Met Arg Lys Trp
                805                 810                 815

Val Asp Glu Leu Phe Ile Ile Ile Met Asp Met Leu Gln Asp Ser Ser
            820                 825                 830

Leu Leu Ala Lys Arg Gln Val Ala Leu Trp Thr Leu Gly Gln Leu Val
        835                 840                 845

Ala Ser Thr Gly Tyr Val Val Glu Pro Tyr Arg Lys Tyr Pro Thr Leu
    850                 855                 860

Leu Glu Val Leu Leu Asn Phe Leu Lys Thr Glu Gln Asn Gln Gly Thr
865                 870                 875                 880

Arg Arg Glu Ala Ile Arg Val Leu Gly Leu Leu Gly Ala Leu Asp Pro
                885                 890                 895

Tyr Lys His Lys Val Asn Ile Gly Met Ile Asp Gln Ser Arg Asp Ala
            900                 905                 910

Ser Ala Val Ser Leu Ser Glu Ser Lys Ser Ser Gln Asp Ser Ser Asp
        915                 920                 925
```

```
Tyr Ser Thr Ser Glu Met Leu Val Asn Met Gly Asn Leu Pro Leu Asp
    930                 935                 940

Glu Phe Tyr Pro Ala Val Ser Met Val Ala Leu Met Arg Ile Phe Arg
945                 950                 955                 960

Asp Gln Ser Leu Ser His His His Thr Met Val Val Gln Ala Ile Thr
                965                 970                 975

Phe Ile Phe Lys Ser Leu Gly Leu Lys Cys Val Gln Phe Leu Pro Gln
            980                 985                 990

Val Met Pro Thr Phe Leu Asn Val  Ile Arg Val Cys Asp  Gly Ala Ile
        995                 1000                1005

Arg Glu  Phe Leu Phe Gln Gln  Leu Gly Met Leu Val  Ser Phe Val
    1010                1015                1020

Lys Ser  His Ile Arg Pro Tyr  Met Asp Glu Ile Val  Thr Leu Met
    1025                1030                1035

Arg Glu  Phe Trp Val Met Asn  Thr Ser Ile Gln Ser  Thr Ile Ile
    1040                1045                1050

Leu Leu  Ile Glu Gln Ile Val  Val Ala Leu Gly Gly  Glu Phe Lys
    1055                1060                1065

Leu Tyr  Leu Pro Gln Leu Ile  Pro His Met Leu Arg  Val Phe Met
    1070                1075                1080

His Asp  Asn Ser Pro Gly Arg  Ile Val Ser Ile Lys  Leu Leu Ala
    1085                1090                1095

Ala Ile  Gln Leu Phe Gly Ala  Asn Leu Asp Asp Tyr  Leu His Leu
    1100                1105                1110
```

```
Leu Leu  Pro Pro Ile Val Lys  Leu Phe Asp Ala Pro  Glu Ala Pro
    1115                1120                1125

Leu Pro  Ser Arg Lys Ala Ala  Leu Glu Thr Val Asp  Arg Leu Thr
    1130                1135                1140

Glu Ser  Leu Asp Phe Thr Asp  Tyr Ala Ser Arg Ile  Ile His Pro
    1145                1150                1155

Ile Val  Arg Thr Leu Asp Gln  Ser Pro Glu Leu Arg  Ser Thr Ala
    1160                1165                1170

Met Asp  Thr Leu Ser Ser Leu  Val Phe Gln Leu Gly  Lys Lys Tyr
    1175                1180                1185

Gln Ile  Phe Ile Pro Met Val  Asn Lys Val Leu Val  Arg His Arg
    1190                1195                1200

Ile Asn  His Gln Arg Tyr Asp  Val Leu Ile Cys Arg  Ile Val Lys
    1205                1210                1215

Gly Tyr  Thr Leu Ala Asp Glu  Glu Glu Asp Pro Leu  Ile Tyr Gln
    1220                1225                1230

His Arg  Met Leu Arg Ser Gly  Gln Gly Asp Ala Leu  Ala Ser Gly
    1235                1240                1245

Pro Val  Glu Thr Gly Pro Met  Lys Lys Leu His Val  Ser Thr Ile
    1250                1255                1260

Asn Leu  Gln Lys Ala Trp Gly  Ala Ala Arg Arg Val  Ser Lys Asp
    1265                1270                1275

Asp Trp  Leu Glu Trp Leu Arg  Arg Leu Ser Leu Glu  Leu Leu Lys
    1280                1285                1290

Asp Ser  Ser Ser Pro Ser Leu  Arg Ser Cys Trp Ala  Leu Ala Gln
    1295                1300                1305
```

```
Ala Tyr  Asn Pro Met Ala Arg  Asp Leu Phe Asn Ala  Ala Phe Val
    1310                1315                1320

Ser Cys  Trp Ser Glu Leu Asn  Glu Asp Gln Gln Asp  Glu Leu Ile
    1325                1330                1335

Arg Ser  Ile Glu Leu Ala Leu  Thr Ser Gln Asp Ile  Ala Glu Val
    1340                1345                1350

Thr Gln  Thr Leu Leu Asn Leu  Ala Glu Phe Met Glu  His Ser Asp
    1355                1360                1365

Lys Gly  Pro Leu Pro Leu Arg  Asp Asp Asn Gly Ile  Val Leu Leu
    1370                1375                1380

Gly Glu  Arg Ala Ala Lys Cys  Arg Ala Tyr Ala Lys  Ala Leu His
    1385                1390                1395

Tyr Lys  Glu Leu Glu Phe Gln  Lys Gly Pro Thr Pro  Ala Ile Leu
    1400                1405                1410

Glu Ser  Leu Ile Ser Ile Asn  Asn Lys Leu Gln Gln  Pro Glu Ala
    1415                1420                1425

Ala Ala  Gly Val Leu Glu Tyr  Ala Met Lys His Phe  Gly Glu Leu
    1430                1435                1440

Glu Ile  Gln Ala Thr Trp Tyr  Glu Lys Leu His Glu  Trp Glu Asp
    1445                1450                1455

Ala Leu  Val Ala Tyr Asp Lys  Lys Met Asp Thr Asn  Lys Asp Asp
    1460                1465                1470

Pro Glu  Leu Met Leu Gly Arg  Met Arg Cys Leu Glu  Ala Leu Gly
    1475                1480                1485
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```
Glu Trp  Gly Gln Leu His Gln  Gln Cys Cys Glu Lys  Trp Thr Leu
    1490                 1495                 1500

Val Asn  Asp Glu Thr Gln Ala  Lys Met Ala Arg Met  Ala Ala Ala
    1505                 1510                 1515

Ala Ala  Trp Gly Leu Gly Gln  Trp Asp Ser Met Glu  Glu Tyr Thr
    1520                 1525                 1530

Cys Met  Ile Pro Arg Asp Thr  His Asp Gly Ala Phe  Tyr Arg Ala
    1535                 1540                 1545

Val Leu  Ala Leu His Gln Asp  Leu Phe Ser Leu Ala  Gln Gln Cys
    1550                 1555                 1560

Ile Asp  Lys Ala Arg Asp Leu  Leu Asp Ala Glu Leu  Thr Ala Met
    1565                 1570                 1575

Ala Gly  Glu Ser Tyr Ser Arg  Ala Tyr Gly Ala Met  Val Ser Cys
    1580                 1585                 1590

His Met  Leu Ser Glu Leu Glu  Glu Val Ile Gln Tyr  Lys Leu Val
    1595                 1600                 1605

Pro Glu  Arg Arg Glu Ile Ile  Arg Gln Ile Trp Trp  Glu Arg Leu
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Gln Gly  Cys Gln Arg Ile Val  Glu Asp Trp Gln Lys  Ile Leu Met
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Val Arg  Ser Leu Val Val Ser  Pro His Glu Asp Met  Arg Thr Trp
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Leu Lys  Tyr Ala Ser Leu Cys  Gly Lys Ser Gly Arg  Leu Ala Leu
    1655                 1660                 1665

Ala His  Lys Thr Leu Val Leu  Leu Leu Gly Val Asp  Pro Ser Arg
    1670                 1675                 1680
```

```
Gln Leu  Asp His Pro Leu Pro  Thr Val His Pro Gln  Val Thr Tyr
    1685                1690                1695

Ala Tyr  Met Lys Asn Met Trp  Lys Ser Ala Arg Lys  Ile Asp Ala
    1700                1705                1710

Phe Gln  His Met Gln His Phe  Val Gln Thr Met Gln  Gln Gln Ala
    1715                1720                1725

Gln His  Ala Ile Ala Thr Glu  Asp Gln Gln His Lys  Gln Glu Leu
    1730                1735                1740

His Lys  Leu Met Ala Arg Cys  Phe Leu Lys Leu Gly  Glu Trp Gln
    1745                1750                1755

Leu Asn  Leu Gln Gly Ile Asn  Glu Ser Thr Ile Pro  Lys Val Leu
    1760                1765                1770

Gln Tyr  Tyr Ser Ala Ala Thr  Glu His Asp Arg Ser  Trp Tyr Lys
    1775                1780                1785

Ala Trp  His Ala Trp Ala Val  Met Asn Phe Glu Ala  Val Leu His
    1790                1795                1800

Tyr Lys  His Gln Asn Gln Ala  Arg Asp Glu Lys Lys  Lys Leu Arg
    1805                1810                1815

His Ala  Ser Gly Ala Asn Ile  Thr Asn Ala Thr Thr  Ala Ala Thr
    1820                1825                1830

Thr Ala  Ala Thr Ala Thr Thr  Thr Ala Ser Thr Glu  Gly Ser Asn
    1835                1840                1845

Ser Glu  Ser Glu Ala Glu Ser  Thr Glu Asn Ser Pro  Thr Pro Ser
    1850                1855                1860
```

```
Pro Leu  Gln Lys Lys Val Thr  Glu Asp Leu Ser Lys  Thr Leu Leu
    1865                1870                1875

Met Tyr  Thr Val Pro Ala Val  Gln Gly Phe Phe Arg  Ser Ile Ser
    1880                1885                1890

Leu Ser  Arg Gly Asn Asn Leu  Gln Asp Thr Leu Arg  Val Leu Thr
    1895                1900                1905

Leu Trp  Phe Asp Tyr Gly His  Trp Pro Asp Val Asn  Glu Ala Leu
    1910                1915                1920

Val Glu  Gly Val Lys Ala Ile  Gln Ile Asp Thr Trp  Leu Gln Val
    1925                1930                1935

Ile Pro  Gln Leu Ile Ala Arg  Ile Asp Thr Pro Arg  Pro Leu Val
    1940                1945                1950

Gly Arg  Leu Ile His Gln Leu  Leu Thr Asp Ile Gly  Arg Tyr His
    1955                1960                1965

Pro Gln  Ala Leu Ile Tyr Pro  Leu Thr Val Ala Ser  Lys Ser Thr
    1970                1975                1980

Thr Thr  Ala Arg His Asn Ala  Ala Asn Lys Ile Leu  Lys Asn Met
    1985                1990                1995

Cys Glu  His Ser Asn Thr Leu  Val Gln Gln Ala Met  Met Val Ser
    2000                2005                2010

Glu Glu  Leu Ile Arg Val Ala  Ile Leu Trp His Glu  Met Trp His
    2015                2020                2025

Glu Gly  Leu Glu Glu Ala Ser  Arg Leu Tyr Phe Gly  Glu Arg Asn
    2030                2035                2040

Val Lys  Gly Met Phe Glu Val  Leu Glu Pro Leu His  Ala Met Met
    2045                2050                2055
```

```
Glu Arg  Gly Pro Gln Thr Leu  Lys Glu Thr Ser Phe  Asn Gln Ala
    2060                2065                2070

Tyr Gly  Arg Asp Leu Met Glu  Ala Gln Glu Trp Cys  Arg Lys Tyr
    2075                2080                2085

Met Lys  Ser Gly Asn Val Lys  Asp Leu Thr Gln Ala  Trp Asp Leu
    2090                2095                2100

Tyr Tyr  His Val Phe Arg Arg  Ile Ser Lys Gln Leu  Pro Gln Leu
    2105                2110                2115

Thr Ser  Leu Glu Leu Gln Tyr  Val Ser Pro Lys Leu  Leu Met Cys
    2120                2125                2130

Arg Asp  Leu Glu Leu Ala Val  Pro Gly Thr Tyr Asp  Pro Asn Gln
    2135                2140                2145

Pro Ile  Ile Arg Ile Gln Ser  Ile Ala Pro Ser Leu  Gln Val Ile
    2150                2155                2160

Thr Ser  Lys Gln Arg Pro Arg  Lys Leu Thr Leu Met  Gly Ser Asn
    2165                2170                2175

Gly His  Glu Phe Val Phe Leu  Leu Lys Gly His Glu  Asp Leu Arg
    2180                2185                2190

Gln Asp  Glu Arg Val Met Gln  Leu Phe Gly Leu Val  Asn Thr Leu
    2195                2200                2205

Leu Ala  Asn Asp Pro Thr Ser  Leu Arg Lys Asn Leu  Ser Ile Gln
    2210                2215                2220

Arg Tyr  Ala Val Ile Pro Leu  Ser Thr Asn Ser Gly  Leu Ile Gly
    2225                2230                2235
```

```
Trp Val  Pro His Cys Asp Thr  Leu His Ala Leu Ile  Arg Asp Tyr
    2240                2245                2250

Arg Glu  Lys Lys Lys Ile Leu  Leu Asn Ile Glu His  Arg Ile Met
    2255                2260                2265

Leu Arg  Met Ala Pro Asp Tyr  Asp His Leu Thr Leu  Met Gln Lys
    2270                2275                2280

Val Glu  Val Phe Glu His Ala  Val Asn Asn Thr Ala  Gly Asp Asp
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Leu Ala  Lys Leu Leu Trp Leu  Lys Ser Pro Ser Ser  Glu Val Trp
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Phe Asp  Arg Arg Thr Asn Tyr  Thr Arg Ser Leu Ala  Val Met Ser
    2315                2320                2325

Met Val  Gly Tyr Ile Leu Gly  Leu Gly Asp Arg His  Pro Ser Asn
    2330                2335                2340

Leu Met  Leu Asp Arg Leu Ser  Gly Lys Ile Leu His  Ile Asp Phe
    2345                2350                2355

Gly Asp  Cys Phe Glu Val Ala  Met Thr Arg Glu Lys  Phe Pro Glu
    2360                2365                2370

Lys Ile  Pro Phe Arg Leu Thr  Arg Met Leu Thr Asn  Ala Met Glu
    2375                2380                2385

Val Thr  Gly Leu Asp Gly Asn  Tyr Arg Ile Thr Cys  His Thr Val
    2390                2395                2400

Met Glu  Val Leu Arg Glu His  Lys Asp Ser Val Met  Ala Val Leu
    2405                2410                2415

Glu Ala  Phe Val Tyr Asp Pro  Leu Leu Asn Trp Arg  Leu Met Asp
    2420                2425                2430
```

```
Thr Asn  Thr Lys Gly Asn Lys  Arg Ser Arg Thr Arg  Thr Asp Ser
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Tyr Ser  Ala Gly Gln Ser Val  Glu Ile Leu Asp Gly  Val Glu Leu
    2450                 2455                 2460


Gly Glu  Pro Ala His Lys Lys  Thr Gly Thr Thr Val  Pro Glu Ser
    2465                 2470                 2475


Ile His  Ser Phe Ile Gly Asp  Gly Leu Val Lys Pro  Glu Ala Leu
    2480                 2485                 2490


Asn Lys  Lys Ala Ile Gln Ile  Ile Asn Arg Val Arg  Asp Lys Leu
    2495                 2500                 2505


Thr Gly  Arg Asp Phe Ser His  Asp Asp Thr Leu Asp  Val Pro Thr
    2510                 2515                 2520


Gln Val  Glu Leu Leu Ile Lys  Gln Ala Thr Ser His  Glu Asn Leu
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Cys Gln  Cys Tyr Ile Gly Trp  Cys Pro Phe Trp
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gaactggagt tccagaaagg ccccacccct gccattctag aatctctcat cagcattaat 4260

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tggaccctgg ttaatgatga gacccaagcc aagatggccc ggatggctgc tgcagctgca 4560

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caagtcatca catccaagca gaggccccgg aaattgacac ttatgggcag caacggacat 6540

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ccatccaacc tgatgctgga ccgtctgagt gggaagatcc tgcacattga ctttggggac 7080

tgctttgagg ttgctatgac ccgagagaag tttccagaga agattccatt tagactaaca 7140

agaatgttga ccaatgctat ggaggttaca ggcctggatg gcaactacag aatcacatgc 7200

cacacagtga tggaggtgct gcgagagcac aaggacagtg tcatggccgt gctggaagcc 7260

tttgtctatg accccttgct gaactggagg ctgatggaca caaataccaa aggcaacaag 7320

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gtggaacttg gagagccagc ccataagaaa acggggacca cagtgccaga atctattcat 7440

tctttcattg gagacggttt ggtgaaacca gaggccctaa ataagaaagc tatccagatt 7500

attaacaggg ttcgagataa gctcactggt cgggacttct ctcatgatga cactttggat 7560

gttccaacgc aagttgagct gctcatcaaa caagcgacat cccatgaaaa cctctgccag 7620

tgctatattg gctggtgccc tttctggtaa 7650

<210> 67
<211> 1147
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="SREBP1"

<400> 67
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1   5   10   15

Glu Pro Cys Asp Leu Asp Ala Ala Leu Leu Thr Asp Ile Glu Asp Met
            20   25   30

Leu Gln Leu Ile Asn Asn Gln Asp Ser Asp Phe Pro Gly Leu Phe Asp
        35   40   45

Pro Pro Tyr Ala Gly Ser Gly Ala Gly Gly Thr Asp Pro Ala Ser Pro
    50   55   60

Asp Thr Ser Ser Pro Gly Ser Leu Ser Pro Pro Pro Ala Thr Leu Ser
65   70   75   80

Ser Ser Leu Glu Ala Phe Leu Ser Gly Pro Gln Ala Ala Pro Ser Pro
                85   90   95

Leu Ser Pro Pro Gln Pro Ala Pro Thr Pro Leu Lys Met Tyr Pro Ser
            100   105   110

Met Pro Ala Phe Ser Pro Gly Pro Gly Ile Lys Glu Glu Ser Val Pro
        115   120   125

Leu Ser Ile Leu Gln Thr Pro Thr Pro Gln Pro Leu Pro Gly Ala Leu
    130   135   140

Leu Pro Gln Ser Phe Pro Ala Pro Ala Pro Pro Gln Phe Ser Ser Thr
145   150   155   160

```
Pro Val Leu Gly Tyr Pro Ser Pro Pro Gly Gly Phe Ser Thr Gly Ser
                165                 170                 175

Pro Pro Gly Asn Thr Gln Gln Pro Leu Pro Gly Leu Pro Leu Ala Ser
            180                 185                 190

Pro Pro Gly Val Pro Pro Val Ser Leu His Thr Gln Val Gln Ser Val
        195                 200                 205

Val Pro Gln Gln Leu Leu Thr Val Thr Ala Ala Pro Thr Ala Ala Pro
    210                 215                 220

Val Thr Thr Thr Val Thr Ser Gln Ile Gln Gln Val Pro Val Leu Leu
225                 230                 235                 240

Gln Pro His Phe Ile Lys Ala Asp Ser Leu Leu Leu Thr Ala Met Lys
                245                 250                 255

Thr Asp Gly Ala Thr Val Lys Ala Ala Gly Leu Ser Pro Leu Val Ser
            260                 265                 270

Gly Thr Thr Val Gln Thr Gly Pro Leu Pro Thr Leu Val Ser Gly Gly
        275                 280                 285

Thr Ile Leu Ala Thr Val Pro Leu Val Val Asp Ala Glu Lys Leu Pro
    290                 295                 300

Ile Asn Arg Leu Ala Ala Gly Ser Lys Ala Pro Ala Ser Ala Gln Ser
305                 310                 315                 320

Arg Gly Glu Lys Arg Thr Ala His Asn Ala Ile Glu Lys Arg Tyr Arg
                325                 330                 335

Ser Ser Ile Asn Asp Lys Ile Ile Glu Leu Lys Asp Leu Val Val Gly
            340                 345                 350

Thr Glu Ala Lys Leu Asn Lys Ser Ala Val Leu Arg Lys Ala Ile Asp
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        355                 360                 365

Tyr Ile Arg Phe Leu Gln His Ser Asn Gln Lys Leu Lys Gln Glu Asn
    370                 375                 380

Leu Ser Leu Arg Thr Ala Val His Lys Ser Lys Ser Leu Lys Asp Leu
385                 390                 395                 400

Val Ser Ala Cys Gly Ser Gly Gly Asn Thr Asp Val Leu Met Glu Gly
                405                 410                 415

Val Lys Thr Glu Val Glu Asp Thr Leu Thr Pro Pro Pro Ser Asp Ala
            420                 425                 430

Gly Ser Pro Phe Gln Ser Ser Pro Leu Ser Leu Gly Ser Arg Gly Ser
        435                 440                 445

Gly Ser Gly Gly Ser Gly Ser Asp Ser Glu Pro Asp Ser Pro Val Phe
    450                 455                 460

Glu Asp Ser Lys Ala Lys Pro Glu Gln Arg Pro Ser Leu His Ser Arg
465                 470                 475                 480

Gly Met Leu Asp Arg Ser Arg Leu Ala Leu Cys Thr Leu Val Phe Leu
                485                 490                 495

Cys Leu Ser Cys Asn Pro Leu Ala Ser Leu Leu Gly Ala Arg Gly Leu
            500                 505                 510

Pro Ser Pro Ser Asp Thr Thr Ser Val Tyr His Ser Pro Gly Arg Asn
        515                 520                 525

Val Leu Gly Thr Glu Ser Arg Asp Gly Pro Gly Trp Ala Gln Trp Leu
    530                 535                 540

Leu Pro Pro Val Val Trp Leu Leu Asn Gly Leu Leu Val Leu Val Ser
545                 550                 555                 560
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Leu Val Leu Leu Phe Val Tyr Gly Glu Pro Val Thr Arg Pro His Ser
                565                 570                 575

Gly Pro Ala Val Tyr Phe Trp Arg His Arg Lys Gln Ala Asp Leu Asp
            580                 585                 590

Leu Ala Arg Gly Asp Phe Ala Gln Ala Ala Gln Gln Leu Trp Leu Ala
        595                 600                 605

Leu Arg Ala Leu Gly Arg Pro Leu Pro Thr Ser His Leu Asp Leu Ala
    610                 615                 620

Cys Ser Leu Leu Trp Asn Leu Ile Arg His Leu Leu Gln Arg Leu Trp
625                 630                 635                 640

Val Gly Arg Trp Leu Ala Gly Arg Ala Gly Gly Leu Gln Gln Asp Cys
                645                 650                 655

Ala Leu Arg Val Asp Ala Ser Ala Ser Ala Arg Asp Ala Ala Leu Val
            660                 665                 670

Tyr His Lys Leu His Gln Leu His Thr Met Gly Lys His Thr Gly Gly
        675                 680                 685

His Leu Thr Ala Thr Asn Leu Ala Leu Ser Ala Leu Asn Leu Ala Glu
    690                 695                 700

Cys Ala Gly Asp Ala Val Ser Val Ala Thr Leu Ala Glu Ile Tyr Val
705                 710                 715                 720

Ala Ala Ala Leu Arg Val Lys Thr Ser Leu Pro Arg Ala Leu His Phe
                725                 730                 735

Leu Thr Arg Phe Phe Leu Ser Ser Ala Arg Gln Ala Cys Leu Ala Gln
            740                 745                 750

Ser Gly Ser Val Pro Pro Ala Met Gln Trp Leu Cys His Pro Val Gly
```

```
        755                 760                 765

His Arg Phe Phe Val Asp Gly Asp Trp Ser Val Leu Ser Thr Pro Trp
    770                 775                 780

Glu Ser Leu Tyr Ser Leu Ala Gly Asn Pro Val Asp Pro Leu Ala Gln
785                 790                 795                 800

Val Thr Gln Leu Phe Arg Glu His Leu Leu Glu Arg Ala Leu Asn Cys
                805                 810                 815

Val Thr Gln Pro Asn Pro Ser Pro Gly Ser Ala Asp Gly Asp Lys Glu
            820                 825                 830

Phe Ser Asp Ala Leu Gly Tyr Leu Gln Leu Leu Asn Ser Cys Ser Asp
        835                 840                 845

Ala Ala Gly Ala Pro Ala Tyr Ser Phe Ser Ile Ser Ser Ser Met Ala
    850                 855                 860

Thr Thr Thr Gly Val Asp Pro Val Ala Lys Trp Trp Ala Ser Leu Thr
865                 870                 875                 880

Ala Val Val Ile His Trp Leu Arg Arg Asp Glu Glu Ala Ala Glu Arg
                885                 890                 895

Leu Cys Pro Leu Val Glu His Leu Pro Arg Val Leu Gln Glu Ser Glu
            900                 905                 910

Arg Pro Leu Pro Arg Ala Ala Leu His Ser Phe Lys Ala Ala Arg Ala
        915                 920                 925

Leu Leu Gly Cys Ala Lys Ala Glu Ser Gly Pro Ala Ser Leu Thr Ile
    930                 935                 940

Cys Glu Lys Ala Ser Gly Tyr Leu Gln Asp Ser Leu Ala Thr Thr Pro
945                 950                 955                 960
```

```
Ala Ser Ser Ser Ile Asp Lys Ala Val Gln Leu Phe Leu Cys Asp Leu
               965                 970                 975

Leu Leu Val Val Arg Thr Ser Leu Trp Arg Gln Gln Gln Pro Pro Ala
            980                 985                 990

Pro Ala Pro Ala Ala Gln Gly Thr  Ser Ser Arg Pro Gln  Ala Ser Ala
        995                 1000                1005

Leu Glu  Leu Arg Gly Phe Gln  Arg Asp Leu Ser Ser  Leu Arg Arg
    1010                1015                1020

Leu Ala  Gln Ser Phe Arg Pro  Ala Met Arg Arg Val  Phe Leu His
    1025                1030                1035

Glu Ala  Thr Ala Arg Leu Met  Ala Gly Ala Ser Pro  Thr Arg Thr
    1040                1045                1050

His Gln  Leu Leu Asp Arg Ser  Leu Arg Arg Arg Ala  Gly Pro Gly
    1055                1060                1065

Gly Lys  Gly Gly Ala Val Ala  Glu Leu Glu Pro Arg  Pro Thr Arg
    1070                1075                1080

Arg Glu  His Ala Glu Ala Leu  Leu Leu Ala Ser Cys  Tyr Leu Pro
    1085                1090                1095

Pro Gly  Phe Leu Ser Ala Pro  Gly Gln Arg Val Gly  Met Leu Ala
    1100                1105                1110

Glu Ala  Ala Arg Thr Leu Glu  Lys Leu Gly Asp Arg  Arg Leu Leu
    1115                1120                1125

His Asp  Cys Gln Gln Met Leu  Met Arg Leu Gly Gly  Gly Thr Thr
    1130                1135                1140

Val Thr  Ser Ser
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1145

<210> 68
<211> 3444
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="SREBP1"

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ctggacgcgg cgctgctgac cgacatcgaa gacatgcttc agcttatcaa caaccaagac 120

agtgacttcc ctggcctatt tgacccaccc tatgctggga gtggggcagg gggcacagac 180

cctgccagcc ccgataccag ctccccaggc agcttgtctc cacctcctgc cacattgagc 240

tcctctcttg aagccttcct gagcgggccg caggcagcgc cctcacccct gtcccctccc 300

cagcctgcac ccactccatt gaagatgtac ccgtccatgc ccgctttctc ccctgggcct 360

ggtatcaagg aagagtcagt gccactgagc atcctgcaga cccccacccc acagcccctg 420

ccaggggccc tcctgccaca gagcttccca gccccagccc caccgcagtt cagctccacc 480

cctgtgttag gctaccccag ccctccggga ggcttctcta caggaagccc tcccgggaac 540

acccagcagc cgctgcctgg cctgccactg gcttccccgc caggggtccc gcccgtctcc 600

ttgcacaccc aggtccagag tgtggtcccc cagcagctac tgacagtcac agctgccccc 660

acggcagccc ctgtaacgac cactgtgacc tcgcagatcc agcaggtccc ggtcctgctg 720

cagccccact tcatcaaggc agactcgctg cttctgacag ccatgaagac agacggagcc 780

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gacaaaatca ttgagctcaa ggatctggtg gtgggcactg aggcaaagct gaataaatct 1080

gctgtcttgc gcaaggccat cgactacatt cgctttctgc aacacagcaa ccagaaactc 1140

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cccacgcggc gggagcacgc ggaggccttg ctgctggcct cctgctacct gccccccggc 3300

ttcctgtcgg cgcccgggca gcgcgtgggc atgctggctg aggcggcgcg cacactcgag 3360

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gggaccactg tcacttccag ctag 3444

<210> 69
<211> 138
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="Interferon-gamma"

<400> 69
Gln Asp Pro Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys Tyr Phe Asn
1 5 10 15

Ala Gly His Ser Asp Val Ala Asp Asn Gly Thr Leu Phe Leu Gly Ile
20 25 30

Leu Lys Asn Trp Lys Glu Glu Ser Asp Arg Lys Ile Met Gln Ser Gln
35 40 45

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Ile Val Ser Phe Tyr Phe Lys Leu Phe Lys Asn Phe Lys Asp Asp Gln
    50                  55                  60

Ser Ile Gln Lys Ser Val Glu Thr Ile Lys Glu Asp Met Asn Val Lys
65                  70                  75                  80

Phe Phe Asn Ser Asn Lys Lys Lys Arg Asp Asp Phe Glu Lys Leu Thr
                85                  90                  95

Asn Tyr Ser Val Thr Asp Leu Asn Val Gln Arg Lys Ala Ile His Glu
            100                 105                 110

Leu Ile Gln Val Met Ala Glu Leu Ser Pro Ala Ala Lys Thr Gly Lys
        115                 120                 125

Arg Lys Arg Ser Gln Met Leu Phe Arg Gly
    130                 135
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<210> 70
<211> 414
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="Interferon-gamma"

<400> 70
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gatgtagcgg ataatggaac tcttttctta ggcattttga agaattggaa agaggagagt 120

gacagaaaaa taatgcagag ccaaattgtc tccttttact tcaaactttt taaaaacttt 180

aaagatgacc agagcatcca aaagagtgtg gagaccatca aggaagacat gaatgtcaag 240

tttttcaata gcaacaaaaa gaaacgagat gacttcgaaa agctgactaa ttattcggta 300

actgacttga atgtccaacg caaagcaata catgaactca tccaagtgat ggctgaactg 360

tcgccagcag ctaaaacagg gaagcgaaaa aggagtcaga tgctgtttcg aggt 414

<210> 71
<211> 227
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="Granzyme B"

<400> 71
Ile Ile Gly Gly His Glu Ala Lys Pro His Ser Arg Pro Tyr Met Ala
1   5   10   15

Tyr Leu Met Ile Trp Asp Gln Lys Ser Leu Lys Arg Cys Gly Gly Phe
            20   25   30

Leu Ile Arg Asp Asp Phe Val Leu Thr Ala Ala His Cys Trp Gly Ser
        35   40   45

Ser Ile Asn Val Thr Leu Gly Ala His Asn Ile Lys Glu Gln Glu Pro
    50   55   60

Thr Gln Gln Phe Ile Pro Val Lys Arg Pro Ile Pro His Pro Ala Tyr
65   70   75   80

Asn Pro Lys Asn Phe Ser Asn Asp Ile Met Leu Leu Gln Leu Glu Arg
                85   90   95

Lys Ala Lys Arg Thr Arg Ala Val Gln Pro Leu Arg Leu Pro Ser Asn
            100   105   110

Lys Ala Gln Val Lys Pro Gly Gln Thr Cys Ser Val Ala Gly Trp Gly
        115   120   125

Gln Thr Ala Pro Leu Gly Lys His Ser His Thr Leu Gln Glu Val Lys
    130   135   140

Met Thr Val Gln Glu Asp Arg Lys Cys Glu Ser Asp Leu Arg His Tyr
145   150   155   160

Tyr Asp Ser Thr Ile Glu Leu Cys Val Gly Asp Pro Glu Ile Lys Lys
165 170 175

Thr Ser Phe Lys Gly Asp Ser Gly Gly Pro Leu Val Cys Asn Lys Val
180 185 190

Ala Gln Gly Ile Val Ser Tyr Gly Arg Asn Asn Gly Met Pro Pro Arg
195 200 205

Ala Cys Thr Lys Val Ser Ser Phe Val His Trp Ile Lys Lys Thr Met
210 215 220

Lys Arg Tyr
225

<210> 72
<211> 681
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="Granzyme B"

<400> 72
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tgggatcaga agtctctgaa gaggtgcggt ggcttcctga tacgagacga cttcgtgctg 120

acagctgctc actgttgggg aagctccata aatgtcacct ggggggccca caatatcaaa 180

gaacaggagc cgacccagca gtttatccct gtgaaaagac ccatccccca tccagcctat 240

aatcctaaga acttctccaa cgacatcatg ctactgcagc tggagagaaa ggccaagcgg 300

accagagctg tgcagcccct caggctacct agcaacaagg cccaggtgaa gccagggcag 360

acatgcagtg tggccggctg ggggcagacg gccccctgg gaaaacactc acacacacta 420

caagaggtga agatgacagt gcaggaagat cgaaagtgcg aatctgactt acgccattat 480

tacgacagta ccattgagtt gtgcgtgggg gacccagaga ttaaaaagac ttcctttaag 540

ggggactctg gaggccctct tgtgtgtaac aaggtggccc agggcattgt ctcctatgga 600

cgaaacaatg gcatgcctcc acgagcctgc accaaagtct caagctttgt acactggata 660

aagaaaacca tgaaacgcta c 681

<210> 73
<211> 268
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="PD-1"

<400> 73
Pro Gly Trp Phe Leu Asp Ser Pro Asp Arg Pro Trp Asn Pro Pro Thr
1 5 10 15

Phe Ser Pro Ala Leu Leu Val Val Thr Glu Gly Asp Asn Ala Thr Phe
20 25 30

Thr Cys Ser Phe Ser Asn Thr Ser Glu Ser Phe Val Leu Asn Trp Tyr
35 40 45

Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala Ala Phe Pro Glu
50 55 60

Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg Val Thr Gln Leu
65 70 75 80

Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg Ala Arg Arg Asn
85 90 95

Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala
100 105 110

Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val Thr Glu Arg Arg
115 120 125

Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro Arg Pro Ala Gly
130 135 140

```
Gln Phe Gln Thr Leu Val Val Gly Val Val Gly Gly Leu Leu Gly Ser
145                 150                 155                 160


Leu Val Leu Leu Val Trp Val Leu Ala Val Ile Cys Ser Arg Ala Ala
                165                 170                 175


Arg Gly Thr Ile Gly Ala Arg Arg Thr Gly Gln Pro Leu Lys Glu Asp
            180                 185                 190


Pro Ser Ala Val Pro Val Phe Ser Val Asp Tyr Gly Glu Leu Asp Phe
        195                 200                 205


Gln Trp Arg Glu Lys Thr Pro Glu Pro Pro Val Pro Cys Val Pro Glu
    210                 215                 220


Gln Thr Glu Tyr Ala Thr Ile Val Phe Pro Ser Gly Met Gly Thr Ser
225                 230                 235                 240


Ser Pro Ala Arg Arg Gly Ser Ala Asp Gly Pro Arg Ser Ala Gln Pro
                245                 250                 255


Leu Arg Pro Glu Asp Gly His Cys Ser Trp Pro Leu
            260                 265


<210> 74
<211> 272
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="PD-L1"

<400> 74
Phe Thr Val Thr Val Pro Lys Asp Leu Tyr Val Val Glu Tyr Gly Ser
1               5                   10                  15


Asn Met Thr Ile Glu Cys Lys Phe Pro Val Glu Lys Gln Leu Asp Leu
            20                  25                  30
```

```
Ala Ala Leu Ile Val Tyr Trp Glu Met Glu Asp Lys Asn Ile Ile Gln
        35                  40                  45

Phe Val His Gly Glu Glu Asp Leu Lys Val Gln His Ser Ser Tyr Arg
    50                  55                  60

Gln Arg Ala Arg Leu Leu Lys Asp Gln Leu Ser Leu Gly Asn Ala Ala
65                  70                  75                  80

Leu Gln Ile Thr Asp Val Lys Leu Gln Asp Ala Gly Val Tyr Arg Cys
                85                  90                  95

Met Ile Ser Tyr Gly Gly Ala Asp Tyr Lys Arg Ile Thr Val Lys Val
            100                 105                 110

Asn Ala Pro Tyr Asn Lys Ile Asn Gln Arg Ile Leu Val Val Asp Pro
        115                 120                 125

Val Thr Ser Glu His Glu Leu Thr Cys Gln Ala Glu Gly Tyr Pro Lys
    130                 135                 140

Ala Glu Val Ile Trp Thr Ser Ser Asp His Gln Val Leu Ser Gly Lys
145                 150                 155                 160

Thr Thr Thr Thr Asn Ser Lys Arg Glu Glu Lys Leu Phe Asn Val Thr
                165                 170                 175

Ser Thr Leu Arg Ile Asn Thr Thr Thr Asn Glu Ile Phe Tyr Cys Thr
            180                 185                 190

Phe Arg Arg Leu Asp Pro Glu Glu Asn His Thr Ala Glu Leu Val Ile
        195                 200                 205

Pro Glu Leu Pro Leu Ala His Pro Pro Asn Glu Arg Thr His Leu Val
    210                 215                 220
```

```
Ile Leu Gly Ala Ile Leu Leu Cys Leu Gly Val Ala Leu Thr Phe Ile
225                 230                 235                 240


Phe Arg Leu Arg Lys Gly Arg Met Met Asp Val Lys Lys Cys Gly Ile
                245                 250                 255


Gln Asp Thr Asn Ser Lys Lys Gln Ser Asp Thr His Leu Glu Glu Thr
            260                 265                 270


<210> 75
<211> 223
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="TIGIT"

<400> 75
Met Met Thr Gly Thr Ile Glu Thr Thr Gly Asn Ile Ser Ala Glu Lys
1               5                   10                  15


Gly Gly Ser Ile Ile Leu Gln Cys His Leu Ser Ser Thr Thr Ala Gln
            20                  25                  30


Val Thr Gln Val Asn Trp Glu Gln Gln Asp Gln Leu Leu Ala Ile Cys
        35                  40                  45


Asn Ala Asp Leu Gly Trp His Ile Ser Pro Ser Phe Lys Asp Arg Val
    50                  55                  60


Ala Pro Gly Pro Gly Leu Gly Leu Thr Leu Gln Ser Leu Thr Val Asn
65                  70                  75                  80


Asp Thr Gly Glu Tyr Phe Cys Ile Tyr His Thr Tyr Pro Asp Gly Thr
                85                  90                  95


Tyr Thr Gly Arg Ile Phe Leu Glu Val Leu Glu Ser Ser Val Ala Glu
            100                 105                 110
```

```
His Gly Ala Arg Phe Gln Ile Pro Leu Leu Gly Ala Met Ala Ala Thr
        115                 120                 125


Leu Val Val Ile Cys Thr Ala Val Ile Val Val Val Ala Leu Thr Arg
    130                 135                 140


Lys Lys Lys Ala Leu Arg Ile His Ser Val Glu Gly Asp Leu Arg Arg
145                 150                 155                 160


Lys Ser Ala Gly Gln Glu Glu Trp Ser Pro Ser Ala Pro Ser Pro Pro
                165                 170                 175


Gly Ser Cys Val Gln Ala Glu Ala Ala Pro Ala Gly Leu Cys Gly Glu
            180                 185                 190


Gln Arg Gly Glu Asp Cys Ala Glu Leu His Asp Tyr Phe Asn Val Leu
        195                 200                 205


Ser Tyr Arg Ser Leu Gly Asn Cys Ser Phe Phe Thr Glu Thr Gly
    210                 215                 220


<210> 76
<211> 311
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD1A"

<400> 76
Asn Ala Asp Gly Leu Lys Glu Pro Leu Ser Phe His Val Thr Trp Ile
1               5                   10                  15


Ala Ser Phe Tyr Asn His Ser Trp Lys Gln Asn Leu Val Ser Gly Trp
            20                  25                  30


Leu Ser Asp Leu Gln Thr His Thr Trp Asp Ser Asn Ser Ser Thr Ile
        35                  40                  45
```

```
Val Phe Leu Cys Pro Trp Ser Arg Gly Asn Phe Ser Asn Glu Glu Trp
    50                  55                  60

Lys Glu Leu Glu Thr Leu Phe Arg Ile Arg Thr Ile Arg Ser Phe Glu
65                  70                  75                  80

Gly Ile Arg Arg Tyr Ala His Glu Leu Gln Phe Glu Tyr Pro Phe Glu
                85                  90                  95

Ile Gln Val Thr Gly Gly Cys Glu Leu His Ser Gly Lys Val Ser Gly
            100                 105                 110

Ser Phe Leu Gln Leu Ala Tyr Gln Gly Ser Asp Phe Val Ser Phe Gln
        115                 120                 125

Asn Asn Ser Trp Leu Pro Tyr Pro Val Ala Gly Asn Met Ala Lys His
    130                 135                 140

Phe Cys Lys Val Leu Asn Gln Asn Gln His Glu Asn Asp Ile Thr His
145                 150                 155                 160

Asn Leu Leu Ser Asp Thr Cys Pro Arg Phe Ile Leu Gly Leu Leu Asp
                165                 170                 175

Ala Gly Lys Ala His Leu Gln Arg Gln Val Lys Pro Glu Ala Trp Leu
            180                 185                 190

Ser His Gly Pro Ser Pro Gly Pro Gly His Leu Gln Leu Val Cys His
        195                 200                 205

Val Ser Gly Phe Tyr Pro Lys Pro Val Trp Val Met Trp Met Arg Gly
    210                 215                 220

Glu Gln Glu Gln Gln Gly Thr Gln Arg Gly Asp Ile Leu Pro Ser Ala
225                 230                 235                 240

Asp Gly Thr Trp Tyr Leu Arg Ala Thr Leu Glu Val Ala Ala Gly Glu
                245                 250                 255
```

```
Ala Ala Asp Leu Ser Cys Arg Val Lys His Ser Ser Leu Glu Gly Gln
            260                 265                 270


Asp Ile Val Leu Tyr Trp Glu His His Ser Ser Val Gly Phe Ile Ile
        275                 280                 285


Leu Ala Val Ile Val Pro Leu Leu Leu Leu Ile Gly Leu Ala Leu Trp
    290                 295                 300


Phe Arg Lys Arg Cys Phe Cys
305                 310


<210> 77
<211> 279
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="TIM3"

<400> 77
Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln Asn Ala Tyr Leu Pro
1               5                   10                  15


Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu Val Pro Val Cys Trp
            20                  25                  30


Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly Asn Val Val Leu Arg
        35                  40                  45


Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser Arg Tyr Trp Leu Asn
    50                  55                  60


Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr Ile Glu Asn Val Thr
65                  70                  75                  80


Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile Gln Ile Pro Gly Ile
                85                  90                  95
```

```
Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val Ile Lys Pro Ala Lys
            100                 105                 110

Val Thr Pro Ala Pro Thr Arg Gln Arg Asp Phe Thr Ala Ala Phe Pro
        115                 120                 125

Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala Glu Thr Gln Thr Leu
    130                 135                 140

Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile Ser Thr Leu Ala Asn
145                 150                 155                 160

Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu Arg Asp Ser Gly Ala
                165                 170                 175

Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly Ile Cys Ala Gly Leu
            180                 185                 190

Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe Lys Trp Tyr Ser His
        195                 200                 205

Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile Ser Leu Ala Asn Leu
    210                 215                 220

Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu Gly Ile Arg Ser Glu
225                 230                 235                 240

Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Glu Pro
                245                 250                 255

Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln Gln Pro Ser Gln Pro
            260                 265                 270

Leu Gly Cys Arg Phe Ala Met
        275
```

<210> 78
<211> 133
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-2"

<400> 78
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1   5   10   15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
            20   25   30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
        35   40   45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
    50   55   60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65   70   75   80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
                85   90   95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
            100   105   110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
        115   120   125

Ile Ser Thr Leu Thr
    130

<210> 79
<211> 152
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-7"

<400> 79
Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu
1   5   10   15

Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly Ser
            20   25   30

Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp
        35   40   45

Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg
    50   55   60

Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu Leu
65   70   75   80

Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val
                85   90   95

Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys Ser
            100   105   110

Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu
        115   120   125

Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys
    130   135   140

Ile Leu Met Gly Thr Lys Glu His
145   150

<210> 80
<211> 197
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-12alpha"

<400> 80
Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys Leu
1   5   10   15

His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln Lys
            20   25   30

Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp
        35   40   45

His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys Leu
    50   55   60

Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr
65   70   75   80

Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe
                85   90   95

Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met Tyr
            100   105   110

Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro Lys
        115   120   125

Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu Leu
    130   135   140

Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser Ser
145   150   155   160

Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile Leu
                165   170   175

```
Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met Ser
            180                 185                 190


Tyr Leu Asn Ala Ser
        195


<210> 81
<211> 306
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-12beta"

<400> 81
Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr
1               5                   10                  15


Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr Pro Glu
            20                  25                  30


Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val Leu Gly
        35                  40                  45


Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
    50                  55                  60


Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
65                  70                  75                  80


Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys
                85                  90                  95


Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu Ala Lys
            100                 105                 110


Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile Ser Thr
        115                 120                 125
```

```
Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp Pro Gln
    130                 135                 140

Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val Arg Gly
145                 150                 155                 160

Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp Ser Ala
                165                 170                 175

Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val Asp Ala
            180                 185                 190

Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe Ile Arg
        195                 200                 205

Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys Pro Leu
    210                 215                 220

Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp Thr Trp
225                 230                 235                 240

Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln Val Gln
                245                 250                 255

Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp Lys Thr
            260                 265                 270

Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val Arg Ala
        275                 280                 285

Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser Val Pro
    290                 295                 300

Cys Ser
305
```

<210> 82
<211> 114

<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-15"

<400> 82
Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1   5   10   15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
            20   25   30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
        35   40   45

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
    50   55   60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
65   70   75   80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
                85   90   95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
            100   105   110

Thr Ser

<210> 83
<211> 133
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-21"

<400> 83

```
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1               5                   10                  15


Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
            20                  25                  30


Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
        35                  40                  45


Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
    50                  55                  60


Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
65                  70                  75                  80


Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
                85                  90                  95


Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
            100                 105                 110


Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
        115                 120                 125


Gly Ser Glu Asp Ser
    130


<210> 84
<211> 270
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-33"

<400> 84
Met Lys Pro Lys Met Lys Tyr Ser Thr Asn Lys Ile Ser Thr Ala Lys
1               5                   10                  15
```

```
Trp Lys Asn Thr Ala Ser Lys Ala Leu Cys Phe Lys Leu Gly Lys Ser
            20                  25                  30

Gln Gln Lys Ala Lys Glu Val Cys Pro Met Tyr Phe Met Lys Leu Arg
        35                  40                  45

Ser Gly Leu Met Ile Lys Lys Glu Ala Cys Tyr Phe Arg Arg Glu Thr
    50                  55                  60

Thr Lys Arg Pro Ser Leu Lys Thr Gly Arg Lys His Lys Arg His Leu
65                  70                  75                  80

Val Leu Ala Ala Cys Gln Gln Gln Ser Thr Val Glu Cys Phe Ala Phe
                85                  90                  95

Gly Ile Ser Gly Val Gln Lys Tyr Thr Arg Ala Leu His Asp Ser Ser
            100                 105                 110

Ile Thr Gly Ile Ser Pro Ile Thr Glu Tyr Leu Ala Ser Leu Ser Thr
        115                 120                 125

Tyr Asn Asp Gln Ser Ile Thr Phe Ala Leu Glu Asp Glu Ser Tyr Glu
    130                 135                 140

Ile Tyr Val Glu Asp Leu Lys Lys Asp Glu Lys Lys Asp Lys Val Leu
145                 150                 155                 160

Leu Ser Tyr Tyr Glu Ser Gln His Pro Ser Asn Glu Ser Gly Asp Gly
                165                 170                 175

Val Asp Gly Lys Met Leu Met Val Thr Leu Ser Pro Thr Lys Asp Phe
            180                 185                 190

Trp Leu His Ala Asn Asn Lys Glu His Ser Val Glu Leu His Lys Cys
        195                 200                 205

Glu Lys Pro Leu Pro Asp Gln Ala Phe Phe Val Leu His Asn Met His
    210                 215                 220
```

Ser Asn Cys Val Ser Phe Glu Cys Lys Thr Asp Pro Gly Val Phe Ile
225 230 235 240

Gly Val Lys Asp Asn His Leu Ala Leu Ile Lys Val Asp Ser Ser Glu
245 250 255

Asn Leu Cys Thr Glu Asn Ile Leu Phe Lys Leu Ser Glu Thr
260 265 270

<210> 85
<211> 696
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 85
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Gly Gly Gly Gly Ser

```
            100                 105                 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Gln Gln
        115                 120                 125

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys
    130                 135                 140

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
145                 150                 155                 160

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser
                165                 170                 175

Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu
            180                 185                 190

Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
        195                 200                 205

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp
    210                 215                 220

His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
225                 230                 235                 240

Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys
                245                 250                 255

Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn
            260                 265                 270

Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser
        275                 280                 285

Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile
    290                 295                 300
```

```
Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro
305                 310                 315                 320

Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu
                325                 330                 335

Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro
            340                 345                 350

Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val
        355                 360                 365

Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu
    370                 375                 380

Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys
385                 390                 395                 400

Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe
                405                 410                 415

Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala
            420                 425                 430

Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val
        435                 440                 445

Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Val Gln Leu Gln
    450                 455                 460

Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser
465                 470                 475                 480

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val Ile Gln Trp Val
                485                 490                 495

Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Ser Ile Asn Pro
```

```
            500                 505                 510

Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr
        515                 520                 525

Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met Glu Phe Ser Ser
    530                 535                 540

Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala Arg Trp Gly Asp
545                 550                 555                 560

Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val Ser Ser Gly Gly
                565                 570                 575

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu
            580                 585                 590

Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu Arg Val
        595                 600                 605

Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr Phe His
    610                 615                 620

Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Cys Ile Tyr Ser
625                 630                 635                 640

Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe Ser Gly Ser Gly
                645                 650                 655

Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala
            660                 665                 670

Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro Thr Phe Gly Gly
        675                 680                 685

Gly Thr Lys Leu Glu Thr Lys Arg
    690                 695
```

<210> 86
<211> 2088
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 86
cagatcgtgc tgacccaaag ccccgccatc atgagcgcta gccccggtga gaaggtgacc 60

atgacatgct ccgcttccag ctccgtgtcc tacatgaact ggtatcagca gaaaagcgga 120

accagcccca aaaggtggat ctacgacacc agcaagctgg cctccggagt gcccgctcat 180

ttccggggct ctggatccgg caccagctac tctttaacca tttccggcat ggaagctgaa 240

gacgctgcca cctactattg ccagcaatgg agcagcaacc ccttcacatt cggatctggc 300

accaagctcg aaatcaatcg tggaggaggt ggcagcggcg gcggtggatc cggcggagga 360

ggaagccaag ttcaactcca gcagagcggc gctgaactgg cccggcccgg cgcctccgtc 420

aagatgagct gcaaggcttc cggctataca tttactcgtt acacaatgca ttgggtcaag 480

cagaggcccg gtcaaggttt agagtggatc ggatatatca acccttcccg gggctacacc 540

aactataacc aaaagttcaa ggataaagcc actttaacca ctgacaagag ctcctccacc 600

gcctacatgc agctgtcctc tttaaccagc gaggactccg ctgtttacta ctgcgctagg 660

tattacgacg accactactg tttagactat tggggacaag gtaccacttt aaccgtcagc 720

agctccggca ccaccaatac cgtggccgct tataacctca catggaagag caccaacttc 780

aagacaattc tggaatggga acccaagccc gtcaatcaag tttacaccgt gcagatctcc 840

accaaatccg gagactggaa gagcaagtgc ttctacacaa cagacaccga gtgtgattta 900

accgacgaaa tcgtcaagga cgtcaagcaa acctatctgg ctcgggtctt ttcctacccc 960

gctggcaatg tcgagtccac cggctccgct ggcgagcctc tctacgagaa ttcccccgaa 1020

ttcacccctt atttagagac caatttaggc cagcctacca tccagagctt cgagcaagtt 1080

ggcaccaagg tgaacgtcac cgtcgaggat gaaaggactt tagtgcggcg gaataacaca 1140

```
tttttatccc tccgggatgt gttcggcaaa gacctcatct acacactgta ctattggaag     1200

tccagctcct ccggcaaaaa gaccgctaag accaacacca acgagttttt aattgacgtg     1260

gacaaaggcg agaactactg cttcagcgtg caagccgtga tcccttctcg taccgtcaac     1320

cggaagagca cagattcccc cgttgagtgc atgggccaag aaaagggcga gttccgggag     1380

gtccagctgc agcagagcgg acccgaactc gtgaaacccg gtgcttccgt gaaaatgtct     1440

tgtaaggcca gcggatacac cttcacctcc tatgtgatcc agtgggtcaa acagaagccc     1500

ggacaaggtc tcgagtggat cggcagcatc aacccttaca acgactatac caaatacaac     1560

gagaagttta agggaaaggc tactttaacc tccgacaaaa gctccatcac agcctacatg     1620

gagttcagct ctttaacatc cgaggacagc gctctgtact attgcgcccg gtggggcgac     1680

ggcaattact ggggacgggg cacaacactg accgtgagca gcggaggcgg aggctccggc     1740

ggaggcggat ctggcggtgg cggctccgac atcgagatga cccagtcccc cgctatcatg     1800

tccgcctctt taggcgagcg ggtcacaatg acttgtacag cctcctccag cgtctcctcc     1860

tcctacttcc attggtacca acagaaaccc ggaagctccc ctaaactgtg catctacagc     1920

accagcaatc tcgccagcgg cgtgcccccc aggttttccg gaagcggaag caccagctac     1980

tctttaacca tctcctccat ggaggctgag gatgccgcca cctacttttg tcaccagtac     2040

caccggtccc ccaccttcgg aggcggcacc aaactggaga caaagagg                  2088
```

<210> 87
<211> 714
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 87

```
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                   10                  15


Tyr Ser Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
            20                  25                  30
```

```
Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
        35                  40                  45

Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp
    50                  55                  60

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg
65                  70                  75                  80

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu
                85                  90                  95

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
            100                 105                 110

Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu
    130                 135                 140

Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met
145                 150                 155                 160

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp
                165                 170                 175

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn
            180                 185                 190

Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala
        195                 200                 205

Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
    210                 215                 220
```

```
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr
225                 230                 235                 240

Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
                245                 250                 255

Val Ser Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr
            260                 265                 270

Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro
        275                 280                 285

Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp
    290                 295                 300

Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp
305                 310                 315                 320

Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser
                325                 330                 335

Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu
            340                 345                 350

Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly
        355                 360                 365

Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val
    370                 375                 380

Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu
385                 390                 395                 400

Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr
                405                 410                 415

Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn
            420                 425                 430
```

```
Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val
        435                 440                 445

Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser
    450                 455                 460

Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Val Gln
465                 470                 475                 480

Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys
                485                 490                 495

Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val Ile Gln
            500                 505                 510

Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Ser Ile
        515                 520                 525

Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys Gly Lys
    530                 535                 540

Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met Glu Phe
545                 550                 555                 560

Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala Arg Trp
                565                 570                 575

Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val Ser Ser
            580                 585                 590

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
        595                 600                 605

Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu
    610                 615                 620
```

```
Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr
625                 630                 635                 640


Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Cys Ile
                645                 650                 655


Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe Ser Gly
            660                 665                 670


Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
        675                 680                 685


Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro Thr Phe
    690                 695                 700


Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg
705                 710


<210> 88
<211> 2142
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 88
atgaagtggg tgaccttcat cagcttatta tttttattca gctccgccta ttcccagatc       60

gtgctgaccc aaagccccgc catcatgagc gctagccccg gtgagaaggt gaccatgaca      120

tgctccgctt ccagctccgt gtcctacatg aactggtatc agcagaaaag cggaaccagc      180

cccaaaaggt ggatctacga caccagcaag ctggcctccg gagtgcccgc tcatttccgg      240

ggctctggat ccggcaccag ctactcttta accatttccg gcatggaagc tgaagacgct      300

gccacctact attgccagca atggagcagc aacccccttca cattcggatc tggcaccaag      360

ctcgaaatca atcgtggagg aggtggcagc ggcggcggtg gatccggcgg aggaggaagc      420

caagttcaac tccagcagag cggcgctgaa ctggcccggc ccggcgcctc cgtcaagatg      480
```

agctgcaagg cttccggcta tacatttact cgttacacaa tgcattgggt caagcagagg 540

cccggtcaag gtttagagtg gatcggatat atcaaccctt cccggggcta caccaactat 600

aaccaaaagt tcaaggataa agccacttta accactgaca agagctcctc caccgcctac 660

atgcagctgt cctctttaac cagcgaggac tccgctgttt actactgcgc taggtattac 720

gacgaccact actgtttaga ctattgggga caaggtacca ctttaaccgt cagcagctcc 780

ggcaccacca ataccgtggc cgcttataac ctcacatgga agagcaccaa cttcaagaca 840

attctggaat gggaacccaa gcccgtcaat caagtttaca ccgtgcagat ctccaccaaa 900

tccggagact ggaagagcaa gtgcttctac acaacagaca ccgagtgtga tttaaccgac 960

gaaatcgtca aggacgtcaa gcaaacctat ctggctcggg tcttttccta ccccgctggc 1020

aatgtcgagt ccaccggctc cgctggcgag cctctctacg agaattcccc cgaattcacc 1080

ccttatttag agaccaattt aggccagcct accatccaga gcttcgagca agttggcacc 1140

aaggtgaacg tcaccgtcga ggatgaaagg actttagtgc ggcggaataa cacattttta 1200

tccctccggg atgtgttcgg caaagacctc atctacacac tgtactattg gaagtccagc 1260

tcctccggca aaaagaccgc taagaccaac accaacgagt ttttaattga cgtggacaaa 1320

ggcgagaact actgcttcag cgtgcaagcc gtgatccctt ctcgtaccgt caaccggaag 1380

agcacagatt cccccgttga gtgcatgggc caagaaaagg gcgagttccg ggaggtccag 1440

ctgcagcaga gcggacccga actcgtgaaa cccggtgctt ccgtgaaaat gtcttgtaag 1500

gccagcggat acaccttcac ctcctatgtg atccagtggg tcaaacagaa gcccggacaa 1560

ggtctcgagt ggatcggcag catcaaccct tacaacgact ataccaaata caacgagaag 1620

tttaagggaa aggctacttt aacctccgac aaaagctcca tcacagccta catggagttc 1680

agctctttaa catccgagga cagcgctctg tactattgcg cccggtgggg cgacggcaat 1740

tactggggac ggggcacaac actgaccgtg agcagcggag gcggaggctc cggcggaggc 1800

ggatctggcg gtggcggctc cgacatcgag atgacccagt cccccgctat catgtccgcc 1860

tctttaggcg agcgggtcac aatgacttgt acagcctcct ccagcgtctc ctcctcctac 1920

ttccattggt accaacagaa acccggaagc tcccctaaac tgtgcatcta cagcaccagc 1980

aatctcgcca gcggcgtgcc ccctaggttt tccggaagcg gaagcaccag ctactcttta 2040

accatctcct ccatggaggc tgaggatgcc gccacctact tttgtcacca gtaccaccgg 2100

tcccccacct tcggaggcgg caccaaactg gagacaaaga gg 2142

<210> 89
<211> 696
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 89
Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser
1 5 10 15

Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val
20 25 30

Ile Gln Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly
35 40 45

Ser Ile Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys
50 55 60

Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met
65 70 75 80

Glu Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Trp Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val
100 105 110

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

```
Ser Asp Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu
    130                 135                 140

Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser
145                 150                 155                 160

Ser Tyr Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu
                165                 170                 175

Cys Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe
            180                 185                 190

Ser Gly Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
        195                 200                 205

Ala Glu Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro
    210                 215                 220

Thr Phe Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg Ser Gly Thr Thr
225                 230                 235                 240

Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys
                245                 250                 255

Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val
            260                 265                 270

Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr
        275                 280                 285

Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys
    290                 295                 300

Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu
305                 310                 315                 320

Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe
```

```
                325                 330                 335

Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe
            340                 345                 350

Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr
        355                 360                 365

Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly
    370                 375                 380

Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly
385                 390                 395                 400

Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp
                405                 410                 415

Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg
            420                 425                 430

Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln
        435                 440                 445

Glu Lys Gly Glu Phe Arg Glu Gln Ile Val Leu Thr Gln Ser Pro Ala
    450                 455                 460

Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala
465                 470                 475                 480

Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr
                485                 490                 495

Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val
            500                 505                 510

Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
        515                 520                 525
```

```
Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
    530                 535                 540

Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
545                 550                 555                 560

Asn Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
                565                 570                 575

Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly
            580                 585                 590

Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg
        595                 600                 605

Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp
    610                 615                 620

Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys
625                 630                 635                 640

Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala
                645                 650                 655

Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
            660                 665                 670

Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln
        675                 680                 685

Gly Thr Thr Leu Thr Val Ser Ser
    690                 695
```

<210> 90
<211> 2088
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polynucleotide"

<400> 90
gtgcagctgc agcagtccgg acccgaactg gtcaagcccg gtgcctccgt gaaaatgtct 60

tgtaaggctt ctggctacac ctttacctcc tacgtcatcc aatgggtgaa gcagaagccc 120

ggtcaaggtc tcgagtggat cggcagcatc aatccctaca acgattacac caagtataac 180

gaaaagttta agggcaaggc cactctgaca agcgacaaga gctccattac cgcctacatg 240

gagttttcct ctttaacttc tgaggactcc gctttatact attgcgctcg ttggggcgat 300

ggcaattatt ggggccgggg aactacttta acagtgagct ccggcggcgg cggaagcgga 360

ggtggaggat ctggcggtgg aggcagcgac atcgagatga cacagtcccc cgctatcatg 420

agcgcctctt taggagaacg tgtgaccatg acttgtacag cttcctccag cgtgagcagc 480

tcctatttcc actggtacca gcagaaaccc ggctcctccc ctaaactgtg tatctactcc 540

acaagcaatt tagctagcgg cgtgcctcct cgttttagcg gctccggcag cacctcttac 600

tctttaacca ttagctctat ggaggccgaa gatgccgcca catacttttg ccatcagtac 660

caccggtccc ctacctttgg cggaggcaca aagctggaga ccaagcggag cggcaccacc 720

aacacagtgg ccgcctacaa tctgacttgg aaatccacca acttcaagac catcctcgag 780

tgggagccca agcccgttaa tcaagtttat accgtgcaga tttccaccaa gagcggcgac 840

tggaaatcca agtgcttcta taccacagac accgagtgcg atctcaccga cgagatcgtc 900

aaagacgtga agcagacata tttagctagg gtgttctcct accccgctgg aaacgtggag 960

agcaccggat ccgctggaga gcctttatac gagaactccc ccgaattcac cccctatctg 1020

gaaaccaatt taggccagcc caccatccag agcttcgaac aagttggcac aaaggtgaac 1080

gtcaccgtcg aagatgagag gactttagtg cggaggaaca atacattttt atccttacgt 1140

gacgtcttcg gcaaggattt aatctacaca ctgtattact ggaagtctag ctcctccggc 1200

aagaagaccg ccaagaccaa taccaacgaa tttttaattg acgtggacaa gggcgagaac 1260

tactgcttct ccgtgcaagc tgtgatcccc tcccggacag tgaaccggaa gtccaccgac 1320

tcccccgtgg agtgcatggg ccaagagaag ggagagtttc gtgagcagat cgtgctgacc 1380

cagtcccccg ctattatgag cgctagcccc ggtgaaaagg tgactatgac atgcagcgcc 1440

agctcttccg tgagctacat gaactggtat cagcagaagt ccggcaccag ccctaaaagg 1500

tggatctacg acaccagcaa gctggccagc ggcgtccccg ctcactttcg gggctccggc 1560

tccggaacaa gctactctct gaccatcagc ggcatggaag ccgaggatgc cgctacctat 1620

tactgtcagc agtggagctc caacccccttc acctttggat ccggcaccaa gctcgagatt 1680

aatcgtggag gcggaggtag cggaggaggc ggatccggcg gtggaggtag ccaagttcag 1740

ctccagcaaa gcggcgccga actcgctcgg cccggcgctt ccgtgaagat gtcttgtaag 1800

gcctccggct ataccttcac ccggtacaca atgcactggg tcaagcaacg gcccggtcaa 1860

ggtttagagt ggattggcta tatcaacccc tcccggggct ataccaacta caaccagaag 1920

ttcaaggaca aagccaccct caccaccgac aagtccagca gcaccgctta catgcagctg 1980

agctctttaa catccgagga ttccgccgtg tactactgcg ctcggtacta cgacgatcat 2040

tactgcctcg attactgggg ccaaggtacc accttaacag tctcctcc 2088

<210> 91
<211> 714
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 91
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
20 25 30

Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser
35 40 45

```
Tyr Val Ile Gln Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp
    50                  55                  60

Ile Gly Ser Ile Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys
65                  70                  75                  80

Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala
                85                  90                  95

Tyr Met Glu Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr
            100                 105                 110

Cys Ala Arg Trp Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu
        115                 120                 125

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    130                 135                 140

Gly Gly Ser Asp Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala
145                 150                 155                 160

Ser Leu Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val
                165                 170                 175

Ser Ser Ser Tyr Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro
            180                 185                 190

Lys Leu Cys Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro
        195                 200                 205

Arg Phe Ser Gly Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser
    210                 215                 220

Met Glu Ala Glu Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg
225                 230                 235                 240

Ser Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg Ser Gly
                245                 250                 255
```

```
Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn
            260                 265                 270

Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val Tyr
        275                 280                 285

Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe
    290                 295                 300

Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp
305                 310                 315                 320

Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn
                325                 330                 335

Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro
            340                 345                 350

Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln
        355                 360                 365

Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp Glu
    370                 375                 380

Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val
385                 390                 395                 400

Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser
                405                 410                 415

Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp
            420                 425                 430

Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile Pro
        435                 440                 445
```

```
Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys Met
    450                 455                 460

Gly Gln Glu Lys Gly Glu Phe Arg Glu Gln Ile Val Leu Thr Gln Ser
465                 470                 475                 480

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
                485                 490                 495

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
            500                 505                 510

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
        515                 520                 525

Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser
    530                 535                 540

Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
545                 550                 555                 560

Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
                565                 570                 575

Glu Ile Asn Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            580                 585                 590

Gly Gly Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
        595                 600                 605

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    610                 615                 620

Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
625                 630                 635                 640

Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                645                 650                 655
```

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
660 665 670

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
675 680 685

Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
690 695 700

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
705 710

<210> 92
<211> 2142
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 92
atgaaatggg tcaccttcat ctctttactg tttttattta gcagcgccta cagcgtgcag 60

ctgcagcagt ccggacccga actggtcaag cccggtgcct ccgtgaaaat gtcttgtaag 120

gcttctggct acacctttac ctcctacgtc atccaatggg tgaagcagaa gcccggtcaa 180

ggtctcgagt ggatcggcag catcaatccc tacaacgatt acaccaagta taacgaaaag 240

tttaagggca aggccactct gacaagcgac aagagctcca ttaccgccta catggagttt 300

tcctctttaa cttctgagga ctccgcttta tactattgcg ctcgttgggg cgatggcaat 360

tattggggcc ggggaactac tttaacagtg agctccggcg gcggcggaag cggaggtgga 420

ggatctggcg gtggaggcag cgacatcgag atgacacagt cccccgctat catgagcgcc 480

tctttaggag aacgtgtgac catgacttgt acagcttcct ccagcgtgag cagctcctat 540

ttccactggt accagcagaa acccggctcc tcccctaaac tgtgtatcta ctccacaagc 600

aatttagcta gcggcgtgcc tcctcgtttt agcggctccg gcagcacctc ttactcttta 660

accattagct ctatggaggc cgaagatgcc gccacatact tttgccatca gtaccaccgg 720

tcccctacct ttggcggagg cacaaagctg gagaccaagc ggagcggcac caccaacaca 780

gtggccgcct acaatctgac ttggaaatcc accaacttca agaccatcct cgagtgggag 840

cccaagcccg ttaatcaagt ttataccgtg cagatttcca ccaagagcgg cgactggaaa 900

tccaagtgct tctataccac agacaccgag tgcgatctca ccgacgagat cgtcaaagac 960

gtgaagcaga catatttagc tagggtgttc tcctaccccg ctggaaacgt ggagagcacc 1020

ggatccgctg gagagccttt atacgagaac tcccccgaat tcacccccta tctggaaacc 1080

aatttaggcc agcccaccat ccagagcttc gaacaagttg gcacaaaggt gaacgtcacc 1140

gtcgaagatg agaggacttt agtgcggagg aacaatacat ttttatcctt acgtgacgtc 1200

ttcggcaagg atttaatcta cacactgtat tactggaagt ctagctcctc cggcaagaag 1260

accgccaaga ccaataccaa cgaattttta attgacgtgg acaagggcga gaactactgc 1320

ttctccgtgc aagctgtgat cccctcccgg acagtgaacc ggaagtccac cgactccccc 1380

gtggagtgca tgggccaaga gaagggagag tttcgtgagc agatcgtgct gacccagtcc 1440

cccgctatta tgagcgctag ccccggtgaa aaggtgacta tgacatgcag cgccagctct 1500

tccgtgagct acatgaactg gtatcagcag aagtccggca ccagccctaa aaggtggatc 1560

tacgacacca gcaagctggc cagcggcgtc cccgctcact ttcggggctc cggctccgga 1620

acaagctact ctctgaccat cagcggcatg gaagccgagg atgccgctac ctattactgt 1680

cagcagtgga gctccaaccc cttcaccttt ggatccggca ccaagctcga gattaatcgt 1740

ggaggcggag gtagcggagg aggcggatcc ggcggtggag gtagccaagt tcagctccag 1800

caaagcggcg ccgaactcgc tcggcccggc gcttccgtga agatgtcttg taaggcctcc 1860

ggctatacct tcacccggta cacaatgcac tgggtcaagc aacggcccgg tcaaggttta 1920

gagtggattg gctatatcaa cccctcccgg ggctatacca actacaacca gaagttcaag 1980

gacaaagcca ccctcaccac cgacaagtcc agcagcaccg cttacatgca gctgagctct 2040

ttaacatccg aggattccgc cgtgtactac tgcgctcggt actacgacga tcattactgc 2100

ctcgattact ggggccaagg taccacctta acagtctcct cc 2142

<210> 93
<211> 219
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="Tissue factor"

<400> 93
Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser
1   5   10   15

Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln
            20   25   30

Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys
        35   40   45

Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val
    50   55   60

Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala
65   70   75   80

Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn
                85   90   95

Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr
            100   105   110

Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu
        115   120   125

Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg
    130   135   140

Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser
145   150   155   160

```
Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu
                165                 170                 175


Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val
            180                 185                 190


Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu
        195                 200                 205


Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
    210                 215


<210> 94
<211> 657
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="Tissue factor"

<400> 94
agcggcacaa ccaacacagt cgctgcctat aacctcactt ggaagagcac caacttcaaa       60

accatcctcg aatgggaacc caaacccgtt aaccaagttt acaccgtgca gatcagcacc      120

aagtccggcg actggaagtc caaatgtttc tataccaccg acaccgagtg cgatctcacc      180

gatgagatcg tgaaagatgt gaaacagacc tacctcgccc gggtgtttag ctaccccgcc      240

ggcaatgtgg agagcactgg ttccgctggc gagcctttat acgagaacag ccccgaattt      300

acccccttacc tcgagaccaa tttaggacag cccaccatcc aaagctttga gcaagttggc      360

acaaaggtga atgtgacagt ggaggacgag cggactttag tgcggcggaa caacaccttt      420

ctcagcctcc gggatgtgtt cggcaaagat ttaatctaca cactgtatta ctggaagtcc      480

tcttcctccg gcaagaagac agctaaaacc aacacaaacg agtttttaat cgacgtggat      540

aaaggcgaaa actactgttt cagcgtgcaa gctgtgatcc cctcccggac cgtgaatagg      600

aaaagcaccg atagccccgt tgagtgcatg ggccaagaaa agggcgagtt ccgggag         657
```

<210> 95
<211> 223
<212> PRT
<213> Mus musculus

<220>
<221> source
<223> /note="Tissue factor"

<400> 95
Ala Gly Ile Pro Glu Lys Ala Phe Asn Leu Thr Trp Ile Ser Thr Asp
1   5   10   15

Phe Lys Thr Ile Leu Glu Trp Gln Pro Lys Pro Thr Asn Tyr Thr Tyr
            20   25   30

Thr Val Gln Ile Ser Asp Arg Ser Arg Asn Trp Lys Asn Lys Cys Phe
        35   40   45

Ser Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp
    50   55   60

Val Thr Trp Ala Tyr Glu Ala Lys Val Leu Ser Val Pro Arg Arg Asn
65   70   75   80

Ser Val His Gly Asp Gly Asp Gln Leu Val Ile His Gly Glu Glu Pro
                85   90   95

Pro Phe Thr Asn Ala Pro Lys Phe Leu Pro Tyr Arg Asp Thr Asn Leu
            100   105   110

Gly Gln Pro Val Ile Gln Gln Phe Glu Gln Asp Gly Arg Lys Leu Asn
        115   120   125

Val Val Val Lys Asp Ser Leu Thr Leu Val Arg Lys Asn Gly Thr Phe
    130   135   140

Leu Thr Leu Arg Gln Val Phe Gly Lys Asp Leu Gly Tyr Ile Ile Thr
145   150   155   160

```
Tyr Arg Lys Gly Ser Ser Thr Gly Lys Lys Thr Asn Ile Thr Asn Thr
                165                 170                 175


Asn Glu Phe Ser Ile Asp Val Glu Glu Gly Val Ser Tyr Cys Phe Phe
            180                 185                 190


Val Gln Ala Met Ile Phe Ser Arg Lys Thr Asn Gln Asn Ser Pro Gly
        195                 200                 205


Ser Ser Thr Val Cys Thr Glu Gln Trp Lys Ser Phe Leu Gly Glu
    210                 215                 220


<210> 96
<211> 224
<212> PRT
<213> Rattus rattus

<220>
<221> source
<223> /note="Tissue factor"

<400> 96
Ala Gly Thr Pro Pro Gly Lys Ala Phe Asn Leu Thr Trp Ile Ser Thr
1               5                   10                  15


Asp Phe Lys Thr Ile Leu Glu Trp Gln Pro Lys Pro Thr Asn Tyr Thr
            20                  25                  30


Tyr Thr Val Gln Ile Ser Asp Arg Ser Arg Asn Trp Lys Tyr Lys Cys
        35                  40                  45


Thr Gly Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys
    50                  55                  60


Asp Val Asn Trp Thr Tyr Glu Ala Arg Val Leu Ser Val Pro Trp Arg
65                  70                  75                  80


Asn Ser Thr His Gly Lys Glu Thr Leu Phe Gly Thr His Gly Glu Glu
                85                  90                  95
```

```
Pro Pro Phe Thr Asn Ala Arg Lys Phe Leu Pro Tyr Arg Asp Thr Lys
            100                 105                 110

Ile Gly Gln Pro Val Ile Gln Lys Tyr Glu Gln Gly Gly Thr Lys Leu
        115                 120                 125

Lys Val Thr Val Lys Asp Ser Phe Thr Leu Val Arg Lys Asn Gly Thr
    130                 135                 140

Phe Leu Thr Leu Arg Gln Val Phe Gly Asn Asp Leu Gly Tyr Ile Leu
145                 150                 155                 160

Thr Tyr Arg Lys Asp Ser Ser Thr Gly Arg Lys Thr Asn Thr Thr His
                165                 170                 175

Thr Asn Glu Phe Leu Ile Asp Val Glu Lys Gly Val Ser Tyr Cys Phe
            180                 185                 190

Phe Ala Gln Ala Val Ile Phe Ser Arg Lys Thr Asn His Lys Ser Pro
        195                 200                 205

Glu Ser Ile Thr Lys Cys Thr Glu Gln Trp Lys Ser Val Leu Gly Glu
    210                 215                 220


<210> 97
<211> 219
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="Tissue factor"

<400> 97
Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser
1               5                   10                  15

Thr Asn Phe Ala Thr Ala Leu Glu Trp Glu Pro Lys Pro Val Asn Gln
            20                  25                  30
```

```
Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys
        35                  40                  45

Cys Phe Tyr Thr Thr Asp Thr Glu Cys Ala Leu Thr Asp Glu Ile Val
    50                  55                  60

Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala
65                  70                  75                  80

Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn
                85                  90                  95

Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr
            100                 105                 110

Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu
        115                 120                 125

Asp Glu Arg Thr Leu Val Ala Arg Asn Asn Thr Ala Leu Ser Leu Arg
    130                 135                 140

Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser
145                 150                 155                 160

Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu
                165                 170                 175

Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val
            180                 185                 190

Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu
        195                 200                 205

Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
    210                 215
```

<210> 98

<211> 219
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="Tissue factor"

<400> 98
Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser
1   5   10   15

Thr Asn Phe Ala Thr Ala Leu Glu Trp Glu Pro Lys Pro Val Asn Gln
            20   25   30

Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Ala Lys Ser Lys
        35   40   45

Cys Phe Tyr Thr Thr Asp Thr Glu Cys Ala Leu Thr Asp Glu Ile Val
    50   55   60

Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala
65   70   75   80

Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Ala Glu Asn
                85   90   95

Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr
            100   105   110

Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu
        115   120   125

Asp Glu Arg Thr Leu Val Ala Arg Asn Asn Thr Ala Leu Ser Leu Arg
    130   135   140

Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser
145   150   155   160

Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu

```
                165                 170                 175

Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val
            180                 185                 190

Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu
        195                 200                 205

Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
    210                 215
```

<210> 99
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 99
Gly Gly Gly Ser
1

<210> 100
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 100
Gly Gly Gly Gly Ser
1   5

<210> 101
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 101
Gly Gly Ser Gly
1

<210> 102
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 102
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 103
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
oligonucleotide"

<400> 103
ggcggtggag gatccggagg aggtggctcc ggcggcggag gatct 45

<210> 104
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 104

Gly Gly Gly Ser Gly Gly Gly Ser
1   5

<210> 105
<211> 133
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-3"

<400> 105
Ala Pro Met Thr Gln Thr Thr Pro Leu Lys Thr Ser Trp Val Asn Cys
1   5   10   15

Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro Leu
            20   25   30

Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu
        35   40   45

Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala
    50   55   60

Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys Asn
65   70   75   80

Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His Pro
                85   90   95

Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu Thr
            100   105   110

Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu
        115   120   125

Ser Leu Ala Ile Phe
    130

<210> 106
<211> 79
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-8"

<400> 106
Glu Gly Ala Val Leu Pro Arg Ser Ala Lys Glu Leu Arg Cys Gln Cys
1   5   10   15

Ile Lys Thr Tyr Ser Lys Pro Phe His Pro Lys Phe Ile Lys Glu Leu
            20   25   30

Arg Val Ile Glu Ser Gly Pro His Cys Ala Asn Thr Glu Ile Ile Val
        35   40   45

Lys Leu Ser Asp Gly Arg Glu Leu Cys Leu Asp Pro Lys Glu Asn Trp
    50   55   60

Val Gln Arg Val Val Glu Lys Phe Leu Lys Arg Ala Glu Asn Ser
65   70   75

<210> 107
<211> 160
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-10"

<400> 107
Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His Phe Pro
1   5   10   15

Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser Arg
            20   25   30

Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu Leu Leu
        35   40   45

```
Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys Gln Ala
    50                  55                  60

Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro Gln Ala
65                  70                  75                  80

Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu Gly Glu
                85                  90                  95

Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg Phe Leu
            100                 105                 110

Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn Ala Phe
        115                 120                 125

Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu Phe Asp
    130                 135                 140

Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile Arg Asn
145                 150                 155                 160
```

<210> 108
<211> 132
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-17"

<400> 108

```
Gly Ile Thr Ile Pro Arg Asn Pro Gly Cys Pro Asn Ser Glu Asp Lys
1               5                   10                  15

Asn Phe Pro Arg Thr Val Met Val Asn Leu Asn Ile His Asn Arg Asn
            20                  25                  30

Thr Asn Thr Asn Pro Lys Arg Ser Ser Asp Tyr Tyr Asn Arg Ser Thr
        35                  40                  45
```

```
Ser Pro Trp Asn Leu His Arg Asn Glu Asp Pro Glu Arg Tyr Pro Ser
    50                  55                  60

Val Ile Trp Glu Ala Lys Cys Arg His Leu Gly Cys Ile Asn Ala Asp
65                  70                  75                  80

Gly Asn Val Asp Tyr His Met Asn Ser Val Pro Ile Gln Gln Glu Ile
                85                  90                  95

Leu Val Leu Arg Arg Glu Pro Pro His Cys Pro Asn Ser Phe Arg Leu
            100                 105                 110

Glu Lys Ile Leu Val Ser Val Gly Cys Thr Cys Val Thr Pro Ile Val
        115                 120                 125

His His Val Ala
    130


<210> 109
<211> 157
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-18"

<400> 109
Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1               5                   10                  15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
            20                  25                  30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        35                  40                  45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
    50                  55                  60
```

```
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80


Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95


Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110


Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125


Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140


Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145                 150                 155


<210> 110
<211> 352
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="PDGF-DD"

<400> 110
Arg Asp Thr Ser Ala Thr Pro Gln Ser Ala Ser Ile Lys Ala Leu Arg
1               5                   10                  15


Asn Ala Asn Leu Arg Arg Asp Glu Ser Asn His Leu Thr Asp Leu Tyr
            20                  25                  30


Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val Gln Ser
        35                  40                  45


Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr Trp Arg
    50                  55                  60
```

```
Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp Asn Gln
65                  70                  75                  80

Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp Phe Val
                85                  90                  95

Glu Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly Arg Trp
            100                 105                 110

Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr Asn Gln
        115                 120                 125

Ile Lys Ile Thr Phe Lys Ser Asp Asp Tyr Phe Val Ala Lys Pro Gly
    130                 135                 140

Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala Ala Ala
145                 150                 155                 160

Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly Val Ser
                165                 170                 175

Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp Ala Leu
            180                 185                 190

Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Leu Lys Tyr
        195                 200                 205

Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Glu Asn Met Tyr Leu Asp
    210                 215                 220

Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser Lys Val
225                 230                 235                 240

Asp Leu Asp Arg Leu Asn Asp Asp Ala Lys Arg Tyr Ser Cys Thr Pro
                245                 250                 255
```

```
Arg Asn Tyr Ser Val Asn Ile Arg Glu Glu Leu Lys Leu Ala Asn Val
            260                 265                 270

Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly Asn Cys
        275                 280                 285

Gly Cys Gly Thr Val Asn Trp Arg Ser Cys Thr Cys Asn Ser Gly Lys
    290                 295                 300

Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly His Ile
305                 310                 315                 320

Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile Gln Leu
                325                 330                 335

Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro Pro Arg
            340                 345                 350
```

<210> 111
<211> 248
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="SCF"

<400> 111

```
Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val Thr
1               5                   10                  15

Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys Tyr
            20                  25                  30

Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu Met
        35                  40                  45

Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe Ser
    50                  55                  60
```

```
Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Val
65                  70                  75                  80

Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser Lys
                85                  90                  95

Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr Pro
            100                 105                 110

Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        115                 120                 125

Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr Leu
    130                 135                 140

Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145                 150                 155                 160

Pro Pro Val Ala Ala Ser Ser Leu Arg Asn Asp Ser Ser Ser Ser Asn
                165                 170                 175

Arg Lys Ala Lys Asn Pro Pro Gly Asp Ser Ser Leu His Trp Ala Ala
            180                 185                 190

Met Ala Leu Pro Ala Leu Phe Ser Leu Ile Ile Gly Phe Ala Phe Gly
        195                 200                 205

Ala Leu Tyr Trp Lys Lys Arg Gln Pro Ser Leu Thr Arg Ala Val Glu
    210                 215                 220

Asn Ile Gln Ile Asn Glu Glu Asp Asn Glu Ile Ser Met Leu Gln Glu
225                 230                 235                 240

Lys Glu Arg Glu Phe Gln Glu Val
                245
```

<210> 112
<211> 209

<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="FLT3L"

<400> 112
Thr Gln Asp Cys Ser Phe Gln His Ser Pro Ile Ser Ser Asp Phe Ala
1   5   10   15

Val Lys Ile Arg Glu Leu Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val
            20   25   30

Thr Val Ala Ser Asn Leu Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp
        35   40   45

Arg Leu Val Leu Ala Gln Arg Trp Met Glu Arg Leu Lys Thr Val Ala
    50   55   60

Gly Ser Lys Met Gln Gly Leu Leu Glu Arg Val Asn Thr Glu Ile His
65   70   75   80

Phe Val Thr Lys Cys Ala Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe
                85   90   95

Val Gln Thr Asn Ile Ser Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu
            100   105   110

Val Ala Leu Lys Pro Trp Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu
        115   120   125

Glu Leu Gln Cys Gln Pro Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser
    130   135   140

Pro Arg Pro Leu Glu Ala Thr Ala Pro Thr Ala Pro Gln Pro Pro Leu
145   150   155   160

Leu Leu Leu Leu Leu Leu Pro Val Gly Leu Leu Leu Leu Ala Ala Ala
                165   170   175

```
Trp Cys Leu His Trp Gln Arg Thr Arg Arg Arg Thr Pro Arg Pro Gly
            180                 185                 190


Glu Gln Val Pro Pro Val Pro Ser Pro Gln Asp Leu Leu Leu Val Glu
        195                 200                 205


His



<210> 113
<211> 65
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL15Ralpha"

<400> 113
Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val
1               5                   10                  15


Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly
            20                  25                  30


Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn
        35                  40                  45


Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile
    50                  55                  60


Arg
65


<210> 114
<211> 195
<212> DNA
<213> Homo sapiens

<220>
```

<221> source
<223> /note="IL15Ralpha"

<400> 114
attacatgcc cccctcccat gagcgtggag cacgccgaca tctgggtgaa gagctatagc 60

ctctacagcc gggagaggta tatctgtaac agcggcttca agaggaaggc cggcaccagc 120

agcctcaccg agtgcgtgct gaataaggct accaacgtgg ctcactggac aacaccctct 180

ttaaagtgca tccgg 195

<210> 115
<211> 114
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-15"

<400> 115
Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1 5 10 15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
20 25 30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
35 40 45

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
85 90 95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
100 105 110

Thr Ser

<210> 116
<211> 342
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-15"

<400> 116
aactgggtga acgtcatcag cgatttaaag aagatcgaag atttaattca gtccatgcat 60

atcgacgcca ctttatacac agaatccgac gtgcacccct cttgtaaggt gaccgccatg 120

aaatgttttt tactggagct gcaagttatc tctttagaga gcggagacgc tagcatccac 180

gacaccgtgg agaatttaat cattttagcc aataactctt tatccagcaa cggcaacgtg 240

acagagtccg gctgcaagga gtgcgaagag ctggaggaga agaacatcaa ggagtttctg 300

caatcctttg tgcacattgt ccagatgttc atcaatacct cc 342

<210> 117
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
Signal sequence"

<400> 117
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser

<210> 118
<211> 54
<212> DNA

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
Signal sequence"

<400> 118
atgaaatggg tgacctttat ttctttactg ttcctcttta gcagcgccta ctcc 54

<210> 119
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
Signal sequence"

<400> 119
atgaagtggg tcacatttat ctctttactg ttcctcttct ccagcgccta cagc 54

<210> 120
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
Signal sequence"

<400> 120
atgaaatggg tgacctttat ttctttactg ttcctcttta gcagcgccta ctcc 54

<210> 121
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
Signal sequence"

<400> 121

Met Lys Cys Leu Leu Tyr Leu Ala Phe Leu Phe Leu Gly Val Asn Cys
1   5   10   15

<210> 122
<211> 58
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      Signal sequence"

<400> 122
Met Gly Gln Ile Val Thr Met Phe Glu Ala Leu Pro His Ile Ile Asp
1   5   10   15

Glu Val Ile Asn Ile Val Ile Ile Val Leu Ile Ile Ile Thr Ser Ile
            20   25   30

Lys Ala Val Tyr Asn Phe Ala Thr Cys Gly Ile Leu Ala Leu Val Ser
        35   40   45

Phe Leu Phe Leu Ala Gly Arg Ser Cys Gly
    50   55

<210> 123
<211> 97
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      Signal sequence"

<400> 123
Met Pro Asn His Gln Ser Gly Ser Pro Thr Gly Ser Ser Asp Leu Leu
1   5   10   15

Leu Ser Gly Lys Lys Gln Arg Pro His Leu Ala Leu Arg Arg Lys Arg
            20   25   30

Arg Arg Glu Met Arg Lys Ile Asn Arg Lys Val Arg Arg Met Asn Leu
        35   40   45

Ala Pro Ile Lys Glu Lys Thr Ala Trp Gln His Leu Gln Ala Leu Ile
    50   55   60

Ser Glu Ala Glu Glu Val Leu Lys Thr Ser Gln Thr Pro Gln Asn Ser
65   70   75   80

Leu Thr Leu Phe Leu Ala Leu Leu Ser Val Leu Gly Pro Pro Val Thr
                85   90   95

Gly

<210> 124
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      Signal sequence"

<400> 124
Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu
1   5   10   15

Leu Val Val Ser Asn Leu Leu Leu Cys Gln Gly Val Val Ser
            20   25   30

<210> 125
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      Signal sequence"

<400> 125

Met Lys Cys Leu Leu Tyr Leu Ala Phe Leu Phe Leu Gly Val Asn Cys
1   5   10   15

<210> 126
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 126
Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
1   5   10   15

<210> 127
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 127
Lys Arg Arg Trp Lys Lys Asn Phe Ile Ala Val Ser Ala Ala Asn Arg
1   5   10   15

Phe Lys Lys Ile Ser Ser Ser Gly Ala Leu
            20   25

<210> 128
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 128
Glu Glu Glu Glu Glu Glu

1 5

<210> 129
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 129
Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Arg
1 5 10

<210> 130
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 130
Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 131
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 131
Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1 5

<210> 132
<211> 5

<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 132
His His His His His
1   5

<210> 133
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 133
His His His His His His
1   5

<210> 134
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 134
His His His His His His His
1   5

<210> 135
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 135
His His His His His His His His
1 5

<210> 136
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 136
His His His His His His His His His
1 5

<210> 137
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 137
His His His His His His His His His His
1 5 10

<210> 138
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 138
Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu

1 5 10

<210> 139
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 139
Thr Lys Glu Asn Pro Arg Ser Asn Gln Glu Glu Ser Tyr Asp Asp Asn
1 5 10 15

Glu Ser

<210> 140
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 140
Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser
1 5 10 15

<210> 141
<211> 38
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 141
Met Asp Glu Lys Thr Thr Gly Trp Arg Gly Gly His Val Val Glu Gly
1 5 10 15

Leu Ala Gly Glu Leu Glu Gln Leu Arg Ala Arg Leu Glu His His Pro
            20   25   30

Gln Gly Gln Arg Glu Pro
        35

<210> 142
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 142
Ser Leu Ala Glu Leu Leu Asn Ala Gly Leu Gly Gly Ser
1   5   10

<210> 143
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 143
Thr Gln Asp Pro Ser Arg Val Gly
1   5

<210> 144
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 144
Pro Asp Arg Val Arg Ala Val Ser His Trp Ser Ser
1   5   10

<210> 145
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 145
Trp Ser His Pro Gln Phe Glu Lys
1   5

<210> 146
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 146
Cys Cys Pro Gly Cys Cys
1   5

<210> 147
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      7t15-7s sequence"

<400> 147
Glu Val His Thr Asn Gln Asp Pro Leu Asp
1   5   10

<210> 148
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 148
Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr
1 5 10

<210> 149
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 149
Tyr Thr Asp Ile Glu Met Asn Arg Leu Gly Lys
1 5 10

<210> 150
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 150
Asp Leu Tyr Asp Asp Asp Asp Lys
1 5

<210> 151
<211> 241
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD3"

<400> 151
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1   5   10   15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20   25   30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
        35   40   45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
    50   55   60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65   70   75   80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85   90   95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Gly Gly Gly Gly Ser
            100   105   110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Gln Gln
        115   120   125

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys
    130   135   140

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
145   150   155   160

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser
                165   170   175

Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu

180 185 190

Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
195 200 205

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp
210 215 220

His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
225 230 235 240

Ser

<210> 152
<211> 723
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD3"

<400> 152
cagatcgtgc tgacccaaag ccccgccatc atgagcgcta gccccggtga gaaggtgacc 60

atgacatgct ccgcttccag ctccgtgtcc tacatgaact ggtatcagca gaaaagcgga 120

accagcccca aaaggtggat ctacgacacc agcaagctgg cctccggagt gcccgctcat 180

ttccggggct ctggatccgg caccagctac tctttaacca tttccggcat ggaagctgaa 240

gacgctgcca cctactattg ccagcaatgg agcagcaacc ccttcacatt cggatctggc 300

accaagctcg aaatcaatcg tggaggaggt ggcagcggcg gcggtggatc cggcggagga 360

ggaagccaag ttcaactcca gcagagcggc gctgaactgg cccggcccgg cgcctccgtc 420

aagatgagct gcaaggcttc cggctataca tttactcgtt acacaatgca ttgggtcaag 480

cagaggcccg gtcaaggttt agagtggatc ggatatatca acccttcccg gggctacacc 540

aactataacc aaaagttcaa ggataaagcc actttaacca ctgacaagag ctcctccacc 600

gcctacatgc agctgtcctc tttaaccagc gaggactccg ctgtttacta ctgcgctagg 660

tattacgacg accactactg tttagactat tggggacaag gtaccacttt aaccgtcagc 720

agc 723

<210> 153
<211> 236
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD28"

<400> 153
Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser
1 5 10 15

Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val
20 25 30

Ile Gln Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly
35 40 45

Ser Ile Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys
50 55 60

Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met
65 70 75 80

Glu Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala
85 90 95

Arg Trp Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val
100 105 110

Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Asp Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu
130 135 140

```
Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser
145                 150                 155                 160


Ser Tyr Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu
                165                 170                 175


Cys Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe
            180                 185                 190


Ser Gly Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
        195                 200                 205


Ala Glu Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro
    210                 215                 220


Thr Phe Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg
225                 230                 235


<210> 154
<211> 708
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD28"

<400> 154
gtccagctgc agcagagcgg acccgaactc gtgaaacccg gtgcttccgt gaaaatgtct       60

tgtaaggcca gcggatacac cttcacctcc tatgtgatcc agtgggtcaa acagaagccc      120

ggacaaggtc tcgagtggat cggcagcatc aacccttaca acgactatac caaatacaac      180

gagaagttta agggaaaggc tactttaacc tccgacaaaa gctccatcac agcctacatg      240

gagttcagct ctttaacatc cgaggacagc gctctgtact attgcgcccg gtggggcgac      300

ggcaattact ggggacgggg cacaacactg accgtgagca gcggaggcgg aggctccggc      360

ggaggcggat ctggcggtgg cggctccgac atcgagatga cccagtcccc cgctatcatg      420
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tccgcctctt taggcgagcg ggtcacaatg acttgtacag cctcctccag cgtctcctcc 480

tcctacttcc attggtacca acagaaaccc ggaagctccc ctaaactgtg catctacagc 540

accagcaatc tcgccagcgg cgtgcccccc aggttttccg gaagcggaag caccagctac 600

tctttaacca tctcctccat ggaggctgag gatgccgcca cctacttttg tcaccagtac 660

caccggtccc ccaccttcgg aggcggcacc aaactggaga caaagagg 708

<210> 155
<211> 696
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
3t28 sequence"

<400> 155
Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Gly Gly Gly Gly Ser
100 105 110

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Gln Gln
        115                 120                 125

Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys
    130                 135                 140

Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
145                 150                 155                 160

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser
                165                 170                 175

Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu
            180                 185                 190

Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu
        195                 200                 205

Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp
    210                 215                 220

His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
225                 230                 235                 240

Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys
                245                 250                 255

Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn
            260                 265                 270

Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser
        275                 280                 285

Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile
    290                 295                 300

Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro
305                 310                 315                 320
```

```
Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu
                325                 330                 335

Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro
            340                 345                 350

Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val
        355                 360                 365

Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu
    370                 375                 380

Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys
385                 390                 395                 400

Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe
                405                 410                 415

Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala
            420                 425                 430

Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val
        435                 440                 445

Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Val Gln Leu Gln
    450                 455                 460

Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Met Ser
465                 470                 475                 480

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val Ile Gln Trp Val
                485                 490                 495

Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Ser Ile Asn Pro
            500                 505                 510
```

```
Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr
        515                 520                 525

Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met Glu Phe Ser Ser
    530                 535                 540

Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala Arg Trp Gly Asp
545                 550                 555                 560

Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val Ser Ser Gly Gly
                565                 570                 575

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu
            580                 585                 590

Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu Arg Val
        595                 600                 605

Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr Phe His
    610                 615                 620

Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Cys Ile Tyr Ser
625                 630                 635                 640

Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe Ser Gly Ser Gly
                645                 650                 655

Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala
            660                 665                 670

Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro Thr Phe Gly Gly
        675                 680                 685

Gly Thr Lys Leu Glu Thr Lys Arg
    690                 695
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<210> 156
<211> 2088

<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
3t28 sequence"

<400> 156
cagatcgtgc tgacccaaag ccccgccatc atgagcgcta gccccggtga gaaggtgacc 60

atgacatgct ccgcttccag ctccgtgtcc tacatgaact ggtatcagca gaaaagcgga 120

accagcccca aaaggtggat ctacgacacc agcaagctgg cctccggagt gcccgctcat 180

ttccggggct ctggatccgg caccagctac tctttaacca tttccggcat ggaagctgaa 240

gacgctgcca cctactattg ccagcaatgg agcagcaacc ccttcacatt cggatctggc 300

accaagctcg aaatcaatcg tggaggaggt ggcagcggcg gcggtggatc cggcggagga 360

ggaagccaag ttcaactcca gcagagcggc gctgaactgg cccggcccgg cgcctccgtc 420

aagatgagct gcaaggcttc cggctataca tttactcgtt acacaatgca ttgggtcaag 480

cagaggcccg gtcaaggttt agagtggatc ggatatatca acccttcccg gggctacacc 540

aactataacc aaaagttcaa ggataaagcc actttaacca ctgacaagag ctcctccacc 600

gcctacatgc agctgtcctc tttaaccagc gaggactccg ctgtttacta ctgcgctagg 660

tattacgacg accactactg tttagactat tggggacaag gtaccacttt aaccgtcagc 720

agctccggca ccaccaatac cgtggccgct tataacctca catggaagag caccaacttc 780

aagacaattc tggaatggga acccaagccc gtcaatcaag tttacaccgt gcagatctcc 840

accaaatccg gagactggaa gagcaagtgc ttctacacaa cagacaccga gtgtgattta 900

accgacgaaa tcgtcaagga cgtcaagcaa acctatctgg ctcgggtctt ttcctacccc 960

gctggcaatg tcgagtccac cggctccgct ggcgagcctc tctacgagaa ttcccccgaa 1020

ttcacccctt atttagagac caatttaggc cagcctacca tccagagctt cgagcaagtt 1080

ggcaccaagg tgaacgtcac cgtcgaggat gaaaggactt tagtgcggcg gaataacaca 1140

tttttatccc tccgggatgt gttcggcaaa gacctcatct acacactgta ctattggaag 1200

tccagctcct ccggcaaaaa gaccgctaag accaacacca acgagttttt aattgacgtg 1260

gacaaaggcg agaactactg cttcagcgtg caagccgtga tcccttctcg taccgtcaac 1320

cggaagagca cagattcccc cgttgagtgc atgggccaag aaaagggcga gttccgggag 1380

gtccagctgc agcagagcgg acccgaactc gtgaaacccg gtgcttccgt gaaaatgtct 1440

tgtaaggcca gcggatacac cttcacctcc tatgtgatcc agtgggtcaa acagaagccc 1500

ggacaaggtc tcgagtggat cggcagcatc aacccttaca acgactatac caaatacaac 1560

gagaagttta agggaaaggc tactttaacc tccgacaaaa gctccatcac agcctacatg 1620

gagttcagct ctttaacatc cgaggacagc gctctgtact attgcgcccg gtggggcgac 1680

ggcaattact ggggacgggg cacaacactg accgtgagca gcggaggcgg aggctccggc 1740

ggaggcggat ctggcggtgg cggctccgac atcgagatga cccagtcccc cgctatcatg 1800

tccgcctctt taggcgagcg ggtcacaatg acttgtacag cctcctccag cgtctcctcc 1860

tcctacttcc attggtacca acagaaaccc ggaagctccc ctaaactgtg catctacagc 1920

accagcaatc tcgccagcgg cgtgcccccc aggttttccg gaagcggaag caccagctac 1980

tctttaacca tctcctccat ggaggctgag gatgccgcca cctacttttg tcaccagtac 2040

caccggtccc ccaccttcgg aggcggcacc aaactggaga caaagagg 2088

<210> 157
<211> 714
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
3t28 sequence"

<400> 157
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
20 25 30

Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser

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        35                  40                  45

Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp
    50                  55                  60

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala His Phe Arg
65                  70                  75                  80

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Gly Met Glu
                85                  90                  95

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
            100                 105                 110

Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn Arg Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu
    130                 135                 140

Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met
145                 150                 155                 160

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp
                165                 170                 175

Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn
            180                 185                 190

Pro Ser Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala
        195                 200                 205

Thr Leu Thr Thr Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
    210                 215                 220

Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Tyr
225                 230                 235                 240
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Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
                245                 250                 255

Val Ser Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr
            260                 265                 270

Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro
        275                 280                 285

Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp
    290                 295                 300

Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp
305                 310                 315                 320

Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser
                325                 330                 335

Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu
            340                 345                 350

Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly
        355                 360                 365

Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val
    370                 375                 380

Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu
385                 390                 395                 400

Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr
                405                 410                 415

Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn
            420                 425                 430

Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val
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        435                 440                 445

Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser
    450                 455                 460

Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Val Gln
465                 470                 475                 480

Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys
                485                 490                 495

Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Val Ile Gln
            500                 505                 510

Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile Gly Ser Ile
        515                 520                 525

Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys Phe Lys Gly Lys
    530                 535                 540

Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala Tyr Met Glu Phe
545                 550                 555                 560

Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr Cys Ala Arg Trp
                565                 570                 575

Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu Thr Val Ser Ser
            580                 585                 590

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
        595                 600                 605

Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu
    610                 615                 620

Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr
625                 630                 635                 640
```

Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Cys Ile
645 650 655

Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro Arg Phe Ser Gly
660 665 670

Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
675 680 685

Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg Ser Pro Thr Phe
690 695 700

Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg
705 710

<210> 158
<211> 2142
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
3t28 sequence"

<400> 158
atgaagtggg tgaccttcat cagcttatta tttttattca gctccgccta ttcccagatc 60

gtgctgaccc aaagccccgc catcatgagc gctagccccg gtgagaaggt gaccatgaca 120

tgctccgctt ccagctccgt gtcctacatg aactggtatc agcagaaaag cggaaccagc 180

cccaaaaggt ggatctacga caccagcaag ctggcctccg gagtgcccgc tcatttccgg 240

ggctctggat ccggcaccag ctactcttta accatttccg gcatggaagc tgaagacgct 300

gccacctact attgccagca atggagcagc aacccccttca cattcggatc tggcaccaag 360

ctcgaaatca atcgtggagg aggtggcagc ggcggcggtg gatccggcgg aggaggaagc 420

caagttcaac tccagcagag cggcgctgaa ctggcccggc ccggcgcctc cgtcaagatg 480

agctgcaagg cttccggcta tacatttact cgttacacaa tgcattgggt caagcagagg 540

cccggtcaag gtttagagtg gatcggatat atcaaccctt cccggggcta caccaactat 600

aaccaaaagt tcaaggataa agccacttta accactgaca agagctcctc caccgcctac 660

atgcagctgt cctctttaac cagcgaggac tccgctgttt actactgcgc taggtattac 720

gacgaccact actgtttaga ctattgggga caaggtacca ctttaaccgt cagcagctcc 780

ggcaccacca ataccgtggc cgcttataac ctcacatgga agagcaccaa cttcaagaca 840

attctggaat gggaacccaa gcccgtcaat caagtttaca ccgtgcagat ctccaccaaa 900

tccggagact ggaagagcaa gtgcttctac acaacagaca ccgagtgtga tttaaccgac 960

gaaatcgtca aggacgtcaa gcaaacctat ctggctcggg tcttttccta ccccgctggc 1020

aatgtcgagt ccaccggctc cgctggcgag cctctctacg agaattcccc cgaattcacc 1080

ccttatttag agaccaattt aggccagcct accatccaga gcttcgagca agttggcacc 1140

aaggtgaacg tcaccgtcga ggatgaaagg actttagtgc ggcggaataa cacattttta 1200

tccctccggg atgtgttcgg caaagacctc atctacacac tgtactattg gaagtccagc 1260

tcctccggca aaaagaccgc taagaccaac accaacgagt ttttaattga cgtggacaaa 1320

ggcgagaact actgcttcag cgtgcaagcc gtgatccctt ctcgtaccgt caaccggaag 1380

agcacagatt cccccgttga gtgcatgggc caagaaaagg gcgagttccg ggaggtccag 1440

ctgcagcaga gcggacccga actcgtgaaa cccggtgctt ccgtgaaaat gtcttgtaag 1500

gccagcggat acaccttcac ctcctatgtg atccagtggg tcaaacagaa gcccggacaa 1560

ggtctcgagt ggatcggcag catcaaccct tacaacgact ataccaaata caacgagaag 1620

tttaagggaa aggctacttt aacctccgac aaaagctcca tcacagccta catggagttc 1680

agctctttaa catccgagga cagcgctctg tactattgcg cccggtgggg cgacggcaat 1740

tactggggac ggggcacaac actgaccgtg agcagcggag gcggaggctc cggcggaggc 1800

ggatctggcg gtggcggctc cgacatcgag atgacccagt cccccgctat catgtccgcc 1860

tctttaggcg agcgggtcac aatgacttgt acagcctcct ccagcgtctc ctcctcctac 1920

ttccattggt accaacagaa acccggaagc tcccctaaac tgtgcatcta cagcaccagc 1980

aatctcgcca gcggcgtgcc ccctaggttt tccggaagcg gaagcaccag ctactcttta 2040

accatctcct ccatggaggc tgaggatgcc gccacctact tttgtcacca gtaccaccgg 2100

tcccccacct tcggaggcgg caccaaactg gagacaaaga gg 2142

<210> 159
<211> 399
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-2"

<400> 159
gcacctactt caagttctac aaagaaaaca cagctacaac tggagcattt actgctggat 60

ttacagatga ttttgaatgg aattaataat tacaagaatc ccaaactcac caggatgctc 120

acatttaagt tttacatgcc caagaaggcc acagaactga aacatcttca gtgtctagaa 180

gaagaactca aacctctgga ggaagtgcta aatttagctc aaagcaaaaa ctttcactta 240

agacccaggg acttaatcag caatatcaac gtaatagttc tggaactaaa gggatctgaa 300

acaacattca tgtgtgaata tgctgatgag acagcaacca ttgtagaatt tctgaacaga 360

tggattacct tttgtcaaag catcatctca acactaact 399

<210> 160
<211> 399
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-2"

<400> 160
gcccccacct cctcctccac caagaagacc cagctgcagc tggagcattt actgctggat 60

ttacagatga ttttaaacgg catcaacaac tacaagaacc ccaagctgac tcgtatgctg 120

accttcaagt tctacatgcc caagaaggcc accgagctga agcatttaca gtgtttagag 180

gaggagctga agcccctcga ggaggtgctg aatttagccc agtccaagaa tttccattta 240

aggccccggg atttaatcag caacatcaac gtgatcgttt tagagctgaa gggctccgag 300

accaccttca tgtgcgagta cgccgacgag accgccacca tcgtggagtt tttaaatcgt 360

tggatcacct tctgccagtc catcatctcc actttaacc 399

<210> 161
<211> 485
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
2t2 sequence"

<400> 161
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
50 55 60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn
130 135 140

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Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro
145                 150                 155                 160

Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly
                165                 170                 175

Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu
            180                 185                 190

Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val
        195                 200                 205

Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu
    210                 215                 220

Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn
225                 230                 235                 240

Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val
                245                 250                 255

Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr
            260                 265                 270

Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu
        275                 280                 285

Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn
    290                 295                 300

Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe
305                 310                 315                 320

Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr
                325                 330                 335
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Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
            340                 345                 350

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
        355                 360                 365

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
    370                 375                 380

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
385                 390                 395                 400

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
                405                 410                 415

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
            420                 425                 430

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
        435                 440                 445

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
    450                 455                 460

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
465                 470                 475                 480

Ile Ser Thr Leu Thr
                485
```

<210> 162
<211> 1455
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
2t2 sequence"

<400> 162
gcccccacct cctcctccac caagaagacc cagctgcagc tggagcattt actgctggat 60

ttacagatga ttttaaacgg catcaacaac tacaagaacc ccaagctgac tcgtatgctg 120

accttcaagt tctacatgcc caagaaggcc accgagctga agcatttaca gtgtttagag 180

gaggagctga agcccctcga ggaggtgctg aatttagccc agtccaagaa tttccattta 240

aggccccggg atttaatcag caacatcaac gtgatcgttt tagagctgaa gggctccgag 300

accaccttca tgtgcgagta cgccgacgag accgccacca tcgtggagtt tttaaatcgt 360

tggatcacct tctgccagtc catcatctcc actttaacca gcggcacaac caacacagtc 420

gctgcctata acctcacttg gaagagcacc aacttcaaaa ccatcctcga atgggaaccc 480

aaacccgtta accaagttta caccgtgcag atcagcacca agtccggcga ctggaagtcc 540

aaatgtttct ataccaccga caccgagtgc gatctcaccg atgagatcgt gaaagatgtg 600

aaacagacct acctcgcccg ggtgtttagc taccccgccg gcaatgtgga gagcactggt 660

tccgctggcg agcctttata cgagaacagc cccgaattta ccccttacct cgagaccaat 720

ttaggacagc ccaccatcca aagctttgag caagttggca caaaggtgaa tgtgacagtg 780

gaggacgagc ggactttagt gcggcggaac aacacctttc tcagcctccg ggatgtgttc 840

ggcaaagatt taatctacac actgtattac tggaagtcct cttcctccgg caagaagaca 900

gctaaaacca acacaaacga gtttttaatc gacgtggata aaggcgaaaa ctactgtttc 960

agcgtgcaag ctgtgatccc ctcccggacc gtgaatagga aaagcaccga tagccccgtt 1020

gagtgcatgg gccaagaaaa gggcgagttc cgggaggcac ctacttcaag ttctacaaag 1080

aaaacacagc tacaactgga gcatttactg ctggatttac agatgatttt gaatggaatt 1140

aataattaca agaatcccaa actcaccagg atgctcacat ttaagtttta catgcccaag 1200

aaggccacag aactgaaaca tcttcagtgt ctagaagaag aactcaaacc tctggaggaa 1260

gtgctaaatt tagctcaaag caaaaacttt cacttaagac ccagggactt aatcagcaat 1320

atcaacgtaa tagttctgga actaaaggga tctgaaacaa cattcatgtg tgaatatgct 1380

gatgagacag caaccattgt agaatttctg aacagatgga ttacctttg tcaaagcatc 1440

atctcaacac taact 1455

<210> 163
<211> 503
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
2t2 sequence"

<400> 163
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu
20 25 30

Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn
35 40 45

Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met
50 55 60

Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu
65 70 75 80

Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe
85 90 95

His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu
100 105 110

Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu
115 120 125

Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln
130 135 140

Ser Ile Ile Ser Thr Leu Thr Ser Gly Thr Thr Asn Thr Val Ala Ala

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145                 150                 155                 160

Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp
                165                 170                 175

Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys
            180                 185                 190

Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys
        195                 200                 205

Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala
    210                 215                 220

Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala
225                 230                 235                 240

Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu
                245                 250                 255

Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr
            260                 265                 270

Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn
        275                 280                 285

Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr
    290                 295                 300

Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys
305                 310                 315                 320

Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr
                325                 330                 335

Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys
            340                 345                 350
```

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Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe
        355                 360                 365


Arg Glu Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu
    370                 375                 380


Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn
385                 390                 395                 400


Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met
                405                 410                 415


Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu
            420                 425                 430


Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe
        435                 440                 445


His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu
    450                 455                 460


Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu
465                 470                 475                 480


Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln
                485                 490                 495


Ser Ile Ile Ser Thr Leu Thr
            500


<210> 164
<211> 1509
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      2t2 sequence"
```

<400> 164
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccgccccc 60

acctcctcct ccaccaagaa gacccagctg cagctggagc atttactgct ggatttacag 120

atgattttaa acggcatcaa caactacaag aaccccaagc tgactcgtat gctgaccttc 180

aagttctaca tgcccaagaa ggccaccgag ctgaagcatt tacagtgttt agaggaggag 240

ctgaagcccc tcgaggaggt gctgaattta gcccagtcca agaatttcca tttaaggccc 300

cgggatttaa tcagcaacat caacgtgatc gttttagagc tgaagggctc cgagaccacc 360

ttcatgtgcg agtacgccga cgagaccgcc accatcgtgg agtttttaaa tcgttggatc 420

accttctgcc agtccatcat ctccacttta accagcggca caaccaacac agtcgctgcc 480

tataacctca cttggaagag caccaacttc aaaaccatcc tcgaatggga acccaaaccc 540

gttaaccaag tttacaccgt gcagatcagc accaagtccg gcgactggaa gtccaaatgt 600

ttctatacca ccgacaccga gtgcgatctc accgatgaga tcgtgaaaga tgtgaaacag 660

acctacctcg cccgggtgtt tagctacccc gccggcaatg tggagagcac tggttccgct 720

ggcgagcctt tatacgagaa cagccccgaa tttacccctt acctcgagac caatttagga 780

cagcccacca tccaaagctt tgagcaagtt ggcacaaagg tgaatgtgac agtggaggac 840

gagcggactt tagtgcggcg gaacaacacc tttctcagcc tccgggatgt gttcggcaaa 900

gatttaatct acacactgta ttactggaag tcctcttcct ccggcaagaa gacagctaaa 960

accaacacaa acgagttttt aatcgacgtg gataaaggcg aaaactactg tttcagcgtg 1020

caagctgtga tcccctcccg gaccgtgaat aggaaaagca ccgatagccc cgttgagtgc 1080

atgggccaag aaaagggcga gttccgggag gcacctactt caagttctac aaagaaaaca 1140

cagctacaac tggagcattt actgctggat ttacagatga ttttgaatgg aattaataat 1200

tacaagaatc ccaaactcac caggatgctc acatttaagt tttacatgcc caagaaggcc 1260

acagaactga aacatcttca gtgtctagaa gaagaactca aacctctgga ggaagtgcta 1320

aatttagctc aaagcaaaaa ctttcactta agacccaggg acttaatcag caatatcaac 1380

gtaatagttc tggaactaaa gggatctgaa acaacattca tgtgtgaata tgctgatgag 1440

acagcaacca ttgtagaatt tctgaacaga tggattacct tttgtcaaag catcatctca 1500

acactaact 1509

<210> 165
<211> 342
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-15"

<400> 165
aactgggtga acgtgatcag cgatttaaag aagatcgagg atttaatcca gagcatgcac 60

atcgacgcca ctctgtacac tgagagcgac gtgcacccta gctgcaaggt gactgccatg 120

aagtgctttt tactggagct gcaagttatc tctttagaga gcggcgatgc cagcatccac 180

gacactgtgg agaatttaat cattttagcc aacaactctt taagcagcaa cggcaacgtg 240

acagagagcg gctgcaagga gtgcgaggag ctggaggaga agaacatcaa ggagttttta 300

cagagcttcg tgcacatcgt gcagatgttc atcaacacta gc 342

<210> 166
<211> 342
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-15"

<400> 166
aactgggtga acgtcatcag cgatttaaag aagatcgaag atttaattca gtccatgcat 60

atcgacgcca ctttatacac agaatccgac gtgcaccct cttgtaaggt gaccgccatg 120

aaatgttttt tactggagct gcaagttatc tctttagaga gcggagacgc tagcatccac 180

gacaccgtgg agaatttaat cattttagcc aataactctt tatccagcaa cggcaacgtg 240

acagagtccg gctgcaagga gtgcgaagag ctggaggaga agaacatcaa ggagtttctg 300

caatcctttg tgcacattgt ccagatgttc atcaatacct cc 342

<210> 167
<211> 447
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
15t15 sequence"

<400> 167
Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
1   5   10   15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
            20   25   30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
        35   40   45

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
    50   55   60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
65   70   75   80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
                85   90   95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
            100   105   110

Thr Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp
        115   120   125

Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val
    130   135   140

Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys
145   150   155   160

```
Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu
                165                 170                 175

Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr
            180                 185                 190

Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr
        195                 200                 205

Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln
    210                 215                 220

Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr
225                 230                 235                 240

Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser
                245                 250                 255

Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp
            260                 265                 270

Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu
        275                 280                 285

Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln
    290                 295                 300

Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro
305                 310                 315                 320

Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Asn Trp Val
                325                 330                 335

Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met
            340                 345                 350
```

```
His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys
        355                 360                 365

Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser
    370                 375                 380

Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile
385                 390                 395                 400

Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser
                405                 410                 415

Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe
            420                 425                 430

Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
        435                 440                 445
```

<210> 168
<211> 1341
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
15t15 sequence"

<400> 168

```
aactgggtga acgtgatcag cgatttaaag aagatcgagg atttaatcca gagcatgcac       60

atcgacgcca ctctgtacac tgagagcgac gtgcacccta gctgcaaggt gactgccatg      120

aagtgctttt tactggagct gcaagttatc tctttagaga gcggcgatgc cagcatccac      180

gacactgtgg agaatttaat cattttagcc aacaactctt taagcagcaa cggcaacgtg      240

acagagagcg gctgcaagga gtgcgaggag ctggaggaga agaacatcaa ggagttttta      300

cagagcttcg tgcacatcgt gcagatgttc atcaacacta gcagcggcac aaccaacaca      360

gtcgctgcct ataacctcac ttggaagagc accaacttca aaaccatcct cgaatgggaa      420

cccaaacccg ttaaccaagt ttacaccgtg cagatcagca ccaagtccgg cgactggaag      480
```

tccaaatgtt tctataccac cgacaccgag tgcgatctca ccgatgagat cgtgaaagat 540

gtgaaacaga cctacctcgc ccgggtgttt agctaccccg ccggcaatgt ggagagcact 600

ggttccgctg gcgagccttt atacgagaac agccccgaat ttacccctta cctcgagacc 660

aatttaggac agcccaccat ccaaagcttt gagcaagttg gcacaaaggt gaatgtgaca 720

gtggaggacg agcggacttt agtgcggcgg aacaacacct ttctcagcct ccgggatgtg 780

ttcggcaaag atttaatcta cacactgtat tactggaagt cctcttcctc cggcaagaag 840

acagctaaaa ccaacacaaa cgagttttta atcgacgtgg ataaaggcga aaactactgt 900

ttcagcgtgc aagctgtgat cccctcccgg accgtgaata ggaaaagcac cgatagcccc 960

gttgagtgca tgggccaaga aaagggcgag ttccgggaga actgggtgaa cgtcatcagc 1020

gatttaaaga agatcgaaga tttaattcag tccatgcata tcgacgccac tttatacaca 1080

gaatccgacg tgcacccctc ttgtaaggtg accgccatga aatgtttttt actggagctg 1140

caagttatct ctttagagag cggagacgct agcatccacg acaccgtgga gaatttaatc 1200

attttagcca ataactcttt atccagcaac ggcaacgtga cagagtccgg ctgcaaggag 1260

tgcgaagagc tggaggagaa gaacatcaag gagtttctgc aatcctttgt gcacattgtc 1320

cagatgttca tcaatacctc c 1341

<210> 169
<211> 465
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
15t15 sequence"

<400> 169
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp
20 25 30

```
Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp
        35                  40                  45

Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu
    50                  55                  60

Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr
65                  70                  75                  80

Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly
                85                  90                  95

Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys
            100                 105                 110

Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe
        115                 120                 125

Ile Asn Thr Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu
    130                 135                 140

Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys
145                 150                 155                 160

Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp
                165                 170                 175

Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr
            180                 185                 190

Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe
        195                 200                 205

Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro
    210                 215                 220

Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu
```

```
225                 230                 235                 240

Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn
                245                 250                 255

Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe
            260                 265                 270

Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr
        275                 280                 285

Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr
    290                 295                 300

Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser
305                 310                 315                 320

Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp
                325                 330                 335

Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Asn
            340                 345                 350

Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln
        355                 360                 365

Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro
    370                 375                 380

Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val
385                 390                 395                 400

Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn
                405                 410                 415

Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr
            420                 425                 430
```

Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys
435 440 445

Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr
450 455 460

Ser
465

<210> 170
<211> 1395
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
15t15 sequence"

<400> 170
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccaactgg 60

gtgaacgtga tcagcgattt aaagaagatc gaggatttaa tccagagcat gcacatcgac 120

gccactctgt acactgagag cgacgtgcac cctagctgca aggtgactgc catgaagtgc 180

tttttactgg agctgcaagt tatctcttta gagagcggcg atgccagcat ccacgacact 240

gtggagaatt taatcatttt agccaacaac tctttaagca gcaacggcaa cgtgacagag 300

agcggctgca aggagtgcga ggagctggag gagaagaaca tcaaggagtt tttacagagc 360

ttcgtgcaca tcgtgcagat gttcatcaac actagcagcg gcacaaccaa cacagtcgct 420

gcctataacc tcacttggaa gagcaccaac ttcaaaacca tcctcgaatg ggaacccaaa 480

cccgttaacc aagtttacac cgtgcagatc agcaccaagt ccggcgactg gaagtccaaa 540

tgtttctata ccaccgacac cgagtgcgat ctcaccgatg agatcgtgaa agatgtgaaa 600

cagacctacc tcgcccgggt gtttagctac cccgccggca atgtggagag cactggttcc 660

gctggcgagc ctttatacga gaacagcccc gaatttaccc cttacctcga gaccaattta 720

ggacagccca ccatccaaag ctttgagcaa gttggcacaa aggtgaatgt gacagtggag 780

gacgagcgga ctttagtgcg gcggaacaac acctttctca gcctccggga tgtgttcggc 840

aaagatttaa tctacacact gtattactgg aagtcctctt cctccggcaa gaagacagct 900

aaaaccaaca caaacgagtt tttaatcgac gtggataaag gcgaaaacta ctgtttcagc 960

gtgcaagctg tgatcccctc ccggaccgtg aataggaaaa gcaccgatag ccccgttgag 1020

tgcatgggcc aagaaaaggg cgagttccgg gagaactggg tgaacgtcat cagcgattta 1080

aagaagatcg aagatttaat tcagtccatg catatcgacg ccactttata cacagaatcc 1140

gacgtgcacc cctcttgtaa ggtgaccgcc atgaaatgtt ttttactgga gctgcaagtt 1200

atctctttag agagcggaga cgctagcatc cacgacaccg tggagaattt aatcatttta 1260

gccaataact ctttatccag caacggcaac gtgacagagt ccggctgcaa ggagtgcgaa 1320

gagctggagg agaagaacat caaggagttt ctgcaatcct ttgtgcacat tgtccagatg 1380

ttcatcaata cctcc 1395

<210> 171
<211> 471
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-18"

<400> 171
tacttcggca aactggaatc caagctgagc gtgatccgga atttaaacga ccaagttctg 60

tttatcgatc aaggtaaccg gcctctgttc gaggacatga ccgactccga ttgccgggac 120

aatgcccccc ggaccatctt cattatctcc atgtacaagg acagccagcc ccggggcatg 180

gctgtgacaa ttagcgtgaa gtgtgagaaa atcagcactt tatcttgtga gaacaagatc 240

atctccttta aggaaatgaa ccccccccgat aacatcaagg acaccaagtc cgatatcatc 300

ttcttccagc ggtccgtgcc cggtcacgat aacaagatgc agttcgaatc ctcctcctac 360

gagggctact ttttagcttg tgaaaaggag agggatttat tcaagctgat cctcaagaag 420

gaggacgagc tgggcgatcg ttccatcatg ttcaccgtcc aaaacgagga t 471

<210> 172
<211> 918
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL12Beta"

<400> 172
atttgggaac tgaagaagga cgtctacgtg gtcgaactgg actggtatcc cgatgctccc 60

ggcgaaatgg tggtgctcac ttgtgacacc cccgaagaag acggcatcac ttggaccctc 120

gatcagagca gcgaggtgct gggctccgga aagaccctca caatccaagt taaggagttc 180

ggagacgctg gccaatacac atgccacaag ggaggcgagg tgctcagcca ttccttatta 240

ttattacaca agaaggaaga cggaatctgg tccaccgaca ttttaaaaga tcagaaggag 300

cccaagaata agacctttt aaggtgtgag gccaaaaact acagcggtcg tttcacttgt 360

tggtggctga ccaccatttc caccgattta accttctccg tgaaaagcag ccggggaagc 420

tccgaccctc aaggtgtgac atgtggagcc gctaccctca gcgctgagag ggttcgtggc 480

gataacaagg aatacgagta cagcgtggag tgccaagaag atagcgcttg tcccgctgcc 540

gaagaatctt tacccattga ggtgatggtg gacgccgtgc acaaactcaa gtacgagaac 600

tacacctcct ccttctttat ccgggacatc attaagcccg atcctcctaa gaatttacag 660

ctgaagcctc tcaaaaatag ccggcaagtt gaggtctctt gggaatatcc cgacacttgg 720

agcacacccc acagctactt ctctttaacc ttttgtgtgc aagttcaagg taaaagcaag 780

cgggagaaga aagaccgggt gtttaccgac aaaaccagcg ccaccgtcat ctgtcggaag 840

aacgcctcca tcagcgtgag ggctcaagat cgttattact ccagcagctg gtccgagtgg 900

gccagcgtgc cttgttcc 918

<210> 173
<211> 591
<212> DNA
<213> Homo sapiens

<220>
<221> source

<223> /note="IL-12alpha"

<400> 173
cgtaacctcc ccgtggctac ccccgatccc ggaatgttcc cttgtttaca ccacagccag 60

aatttactga gggccgtgag caacatgctg cagaaagcta ggcagacttt agaattttac 120

ccttgcacca gcgaggagat cgaccatgaa gatatcacca aggacaagac atccaccgtg 180

gaggcttgtt tacctctgga gctgacaaag aacgagtctt gtctcaactc tcgtgaaacc 240

agcttcatca caaatggctc ttgtttagct tcccggaaga cctcctttat gatggcttta 300

tgcctcagct ccatctacga ggatttaaag atgtaccaag tggagttcaa gaccatgaac 360

gccaagctgc tcatggaccc taaacggcag atctttttag accagaacat gctggctgtg 420

attgatgagc tgatgcaagc tttaaacttc aactccgaga ccgtccctca gaagtcctcc 480

ctcgaggagc ccgattttta caagacaaag atcaaactgt gcattttact ccacgccttt 540

aggatccggg ccgtgaccat tgaccgggtc atgagctatt taaacgccag c 591

<210> 174
<211> 490
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 174
Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
35 40 45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 55 60

```
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
65                  70                  75                  80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                85                  90                  95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
            100                 105                 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
        115                 120                 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
    130                 135                 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp Ser Gly Thr
145                 150                 155                 160

Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe
                165                 170                 175

Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val Tyr Thr
            180                 185                 190

Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr
        195                 200                 205

Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val
    210                 215                 220

Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val
225                 230                 235                 240

Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu
                245                 250                 255

Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser
```

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            260                 265                 270

Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp Glu Arg
        275                 280                 285

Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe
    290                 295                 300

Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser
305                 310                 315                 320

Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val
                325                 330                 335

Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile Pro Ser
            340                 345                 350

Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys Met Gly
        355                 360                 365

Gln Glu Lys Gly Glu Phe Arg Glu Asn Trp Val Asn Val Ile Ser Asp
    370                 375                 380

Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr
385                 390                 395                 400

Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met
                405                 410                 415

Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp
            420                 425                 430

Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn
        435                 440                 445

Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys
    450                 455                 460
```

Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val
465 470 475 480

His Ile Val Gln Met Phe Ile Asn Thr Ser
485 490

<210> 175
<211> 1470
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 175
tacttcggca aactggaatc caagctgagc gtgatccgga atttaaacga ccaagttctg 60

tttatcgatc aaggtaaccg gcctctgttc gaggacatga ccgactccga ttgccgggac 120

aatgcccccc ggaccatctt cattatctcc atgtacaagg acagccagcc ccggggcatg 180

gctgtgacaa ttagcgtgaa gtgtgagaaa atcagcactt tatcttgtga gaacaagatc 240

atctccttta aggaaatgaa ccccccccgat aacatcaagg acaccaagtc cgatatcatc 300

ttcttccagc ggtccgtgcc cggtcacgat aacaagatgc agttcgaatc ctcctcctac 360

gagggctact ttttagcttg tgaaaaggag agggatttat tcaagctgat cctcaagaag 420

gaggacgagc tgggcgatcg ttccatcatg ttcaccgtcc aaaacgagga tagcggcaca 480

accaacacag tcgctgccta taacctcact tggaagagca ccaacttcaa aaccatcctc 540

gaatgggaac ccaaacccgt taaccaagtt tacaccgtgc agatcagcac caagtccggc 600

gactggaagt ccaaatgttt ctataccacc gacaccgagt gcgatctcac cgatgagatc 660

gtgaaagatg tgaaacagac ctacctcgcc cgggtgttta gctaccccgc cggcaatgtg 720

gagagcactg gttccgctgg cgagccttta tacgagaaca gccccgaatt tacccttac 780

ctcgagacca atttaggaca gcccaccatc caaagctttg agcaagttgg cacaaaggtg 840

aatgtgacag tggaggacga gcggacttta gtgcggcgga acaacacctt tctcagcctc 900

cgggatgtgt tcggcaaaga tttaatctac acactgtatt actggaagtc ctcttcctcc 960

ggcaagaaga cagctaaaac caacacaaac gagtttttaa tcgacgtgga taaaggcgaa 1020

aactactgtt tcagcgtgca agctgtgatc ccctcccgga ccgtgaatag gaaaagcacc 1080

gatagccccg ttgagtgcat gggccaagaa aagggcgagt tccgggagaa ctgggtgaac 1140

gtcatcagcg atttaaagaa gatcgaagat ttaattcagt ccatgcatat cgacgccact 1200

ttatacacag aatccgacgt gcacccctct tgtaaggtga ccgccatgaa atgtttttta 1260

ctggagctgc aagttatctc tttagagagc ggagacgcta gcatccacga caccgtggag 1320

aatttaatca ttttagccaa taactcttta tccagcaacg gcaacgtgac agagtccggc 1380

tgcaaggagt gcgaagagct ggaggagaag aacatcaagg agtttctgca atcctttgtg 1440

cacattgtcc agatgttcat caatacctcc 1470

<210> 176
<211> 508
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 176
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn
20 25 30

Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe
35 40 45

Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile
50 55 60

Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val
65 70 75 80

```
Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn
                85                  90                  95

Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp
            100                 105                 110

Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp
        115                 120                 125

Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala
    130                 135                 140

Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp
145                 150                 155                 160

Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp Ser
                165                 170                 175

Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr
            180                 185                 190

Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val
        195                 200                 205

Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys
    210                 215                 220

Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys
225                 230                 235                 240

Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly
                245                 250                 255

Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser
            260                 265                 270
```

```
Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile
        275                 280                 285

Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp
    290                 295                 300

Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp
305                 310                 315                 320

Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser
                325                 330                 335

Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile
            340                 345                 350

Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile
        355                 360                 365

Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys
    370                 375                 380

Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Asn Trp Val Asn Val Ile
385                 390                 395                 400

Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp
                405                 410                 415

Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr
            420                 425                 430

Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser
        435                 440                 445

Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala
    450                 455                 460

Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys
465                 470                 475                 480
```

Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser
485 490 495

Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
500 505

<210> 177
<211> 1524
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 177
atgaagtggg tcacatttat ctctttactg ttcctcttct ccagcgccta cagctacttc 60

ggcaaactgg aatccaagct gagcgtgatc cggaatttaa acgaccaagt tctgtttatc 120

gatcaaggta accggcctct gttcgaggac atgaccgact ccgattgccg ggacaatgcc 180

ccccggacca tcttcattat ctccatgtac aaggacagcc agccccgggg catggctgtg 240

acaattagcg tgaagtgtga gaaaatcagc actttatctt gtgagaacaa gatcatctcc 300

tttaaggaaa tgaacccccc cgataacatc aaggacacca agtccgatat catcttcttc 360

cagcggtccg tgcccggtca cgataacaag atgcagttcg aatcctcctc ctacgagggc 420

tacttttag cttgtgaaaa ggagagggat ttattcaagc tgatcctcaa gaaggaggac 480

gagctgggcg atcgttccat catgttcacc gtccaaaacg aggatagcgg cacaaccaac 540

acagtcgctg cctataacct cacttggaag agcaccaact tcaaaaccat cctcgaatgg 600

gaacccaaac ccgttaacca agtttacacc gtgcagatca gcaccaagtc cggcgactgg 660

aagtccaaat gtttctatac caccgacacc gagtgcgatc tcaccgatga gatcgtgaaa 720

gatgtgaaac agacctacct cgcccgggtg tttagctacc ccgccggcaa tgtggagagc 780

actggttccg ctggcgagcc tttatacgag aacagccccg aatttacccc ttacctcgag 840

accaatttag gacagcccac catccaaagc tttgagcaag ttggcacaaa ggtgaatgtg 900

acagtggagg acgagcggac tttagtgcgg cggaacaaca cctttctcag cctccgggat 960

gtgttcggca aagatttaat ctacacactg tattactgga agtcctcttc ctccggcaag 1020

aagacagcta aaaccaacac aaacgagttt ttaatcgacg tggataaagg cgaaaactac 1080

tgtttcagcg tgcaagctgt gatcccctcc cggaccgtga ataggaaaag caccgatagc 1140

cccgttgagt gcatgggcca agaaaagggc gagttccggg agaactgggt gaacgtcatc 1200

agcgatttaa agaagatcga agatttaatt cagtccatgc atatcgacgc cactttatac 1260

acagaatccg acgtgcaccc ctcttgtaag gtgaccgcca tgaaatgttt tttactggag 1320

ctgcaagtta tctctttaga gagcggagac gctagcatcc acgacaccgt ggagaattta 1380

atcattttag ccaataactc tttatccagc aacggcaacg tgacagagtc cggctgcaag 1440

gagtgcgaag agctggagga gaagaacatc aaggagtttc tgcaatcctt tgtgcacatt 1500

gtccagatgt tcatcaatac ctcc 1524

<210> 178
<211> 583
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 178
Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr
1 5 10 15

Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr Pro Glu
20 25 30

Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val Leu Gly
35 40 45

Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
50 55 60

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Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
65                  70                  75                  80

Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys
                85                  90                  95

Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu Ala Lys
            100                 105                 110

Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile Ser Thr
        115                 120                 125

Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp Pro Gln
    130                 135                 140

Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val Arg Gly
145                 150                 155                 160

Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp Ser Ala
                165                 170                 175

Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val Asp Ala
            180                 185                 190

Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe Ile Arg
        195                 200                 205

Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys Pro Leu
    210                 215                 220

Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp Thr Trp
225                 230                 235                 240

Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln Val Gln
                245                 250                 255

Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp Lys Thr
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            260                 265                 270

Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val Arg Ala
        275                 280                 285

Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser Val Pro
    290                 295                 300

Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
305                 310                 315                 320

Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys
                325                 330                 335

Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln
            340                 345                 350

Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile
        355                 360                 365

Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys
    370                 375                 380

Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu
385                 390                 395                 400

Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser
                405                 410                 415

Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met
            420                 425                 430

Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro
        435                 440                 445

Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu
    450                 455                 460
```

Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser
465 470 475 480

Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile
485 490 495

Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met
500 505 510

Ser Tyr Leu Asn Ala Ser Ile Thr Cys Pro Pro Pro Met Ser Val Glu
515 520 525

His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg
530 535 540

Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu
545 550 555 560

Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr
565 570 575

Pro Ser Leu Lys Cys Ile Arg
580

<210> 179
<211> 1749
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 179
atttgggaac tgaagaagga cgtctacgtg gtcgaactgg actggtatcc cgatgctccc 60

ggcgaaatgg tggtgctcac ttgtgacacc cccgaagaag acggcatcac ttggaccctc 120

gatcagagca gcgaggtgct gggctccgga aagaccctca caatccaagt taaggagttc 180

ggagacgctg gccaatacac atgccacaag ggaggcgagg tgctcagcca ttccttatta 240

ttattacaca agaaggaaga cggaatctgg tccaccgaca ttttaaaaga tcagaaggag 300

cccaagaata agacctttt aaggtgtgag gccaaaaact acagcggtcg tttcacttgt 360

tggtggctga ccaccatttc caccgattta accttctccg tgaaaagcag ccggggaagc 420

tccgaccctc aaggtgtgac atgtggagcc gctaccctca gcgctgagag ggttcgtggc 480

gataacaagg aatacgagta cagcgtggag tgccaagaag atagcgcttg tcccgctgcc 540

gaagaatctt tacccattga ggtgatggtg gacgccgtgc acaaactcaa gtacgagaac 600

tacacctcct ccttctttat ccgggacatc attaagcccg atcctcctaa gaatttacag 660

ctgaagcctc tcaaaaatag ccggcaagtt gaggtctctt gggaatatcc cgacacttgg 720

agcacacccc acagctactt ctctttaacc ttttgtgtgc aagttcaagg taaaagcaag 780

cgggagaaga aagaccgggt gtttaccgac aaaaccagcg ccaccgtcat ctgtcggaag 840

aacgcctcca tcagcgtgag ggctcaagat cgttattact ccagcagctg gtccgagtgg 900

gccagcgtgc cttgttccgg cggtggagga tccggaggag gtggctccgg cggcggagga 960

tctcgtaacc tccccgtggc taccccgat cccggaatgt tcccttgttt acaccacagc 1020

cagaatttac tgagggccgt gagcaacatg ctgcagaaag ctaggcagac tttagaattt 1080

taccttgca ccagcgagga gatcgaccat gaagatatca ccaaggacaa gacatccacc 1140

gtggaggctt gtttacctct ggagctgaca aagaacgagt cttgtctcaa ctctcgtgaa 1200

accagcttca tcacaaatgg ctcttgttta gcttcccgga agacctcctt tatgatggct 1260

ttatgcctca gctccatcta cgaggattta aagatgtacc aagtggagtt caagaccatg 1320

aacgccaagc tgctcatgga ccctaaacgg cagatctttt tagaccagaa catgctggct 1380

gtgattgatg agctgatgca agctttaaac ttcaactccg agaccgtccc tcagaagtcc 1440

tccctcgagg agcccgattt ttacaagaca aagatcaaac tgtgcatttt actccacgcc 1500

tttaggatcc gggccgtgac cattgaccgg gtcatgagct atttaaacgc cagcattaca 1560

tgcccccctc ccatgagcgt ggagcacgcc gacatctggg tgaagagcta tagcctctac 1620

agccgggaga ggtatatctg taacagcggc ttcaagagga aggccggcac cagcagcctc 1680

accgagtgcg tgctgaataa ggctaccaac gtggctcact ggacaacacc ctctttaaag 1740

tgcatccgg 1749

<210> 180
<211> 601
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 180
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp
20 25 30

Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr
35 40 45

Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val
50 55 60

Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp
65 70 75 80

Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser
85 90 95

Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile
100 105 110

Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu
115 120 125

Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile
130 135 140

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Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp
145                 150                 155                 160

Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val
                165                 170                 175

Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp
            180                 185                 190

Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val
        195                 200                 205

Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe
    210                 215                 220

Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys
225                 230                 235                 240

Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp
                245                 250                 255

Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln
            260                 265                 270

Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp
        275                 280                 285

Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val
    290                 295                 300

Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser
305                 310                 315                 320

Val Pro Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
                325                 330                 335
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Gly Gly Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe
            340                 345                 350

Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met
        355                 360                 365

Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu
    370                 375                 380

Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu
385                 390                 395                 400

Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser
                405                 410                 415

Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys
            420                 425                 430

Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu
        435                 440                 445

Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met
    450                 455                 460

Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile
465                 470                 475                 480

Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln
                485                 490                 495

Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu
            500                 505                 510

Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg
        515                 520                 525

Val Met Ser Tyr Leu Asn Ala Ser Ile Thr Cys Pro Pro Pro Met Ser
    530                 535                 540
```

Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg
545 550 555 560

Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser
565 570 575

Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp
580 585 590

Thr Thr Pro Ser Leu Lys Cys Ile Arg
595 600

<210> 181
<211> 1803
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s sequence"

<400> 181
atgaaatggg tgacctttat ttctttactg ttcctcttta gcagcgccta ctccatttgg 60

gaactgaaga aggacgtcta cgtggtcgaa ctggactggt atcccgatgc tcccggcgaa 120

atggtggtgc tcacttgtga cacccccgaa gaagacggca tcacttggac cctcgatcag 180

agcagcgagg tgctgggctc cggaaagacc ctcacaatcc aagttaagga gttcggagac 240

gctggccaat acacatgcca caagggaggc gaggtgctca gccattcctt attattatta 300

cacaagaagg aagacggaat ctggtccacc gacattttaa aagatcagaa ggagcccaag 360

aataagacct ttttaaggtg tgaggccaaa aactacagcg gtcgtttcac ttgttggtgg 420

ctgaccacca tttccaccga tttaaccttc tccgtgaaaa gcagccgggg aagctccgac 480

cctcaaggtg tgacatgtgg agccgctacc ctcagcgctg agagggttcg tggcgataac 540

aaggaatacg agtacagcgt ggagtgccaa gaagatagcg cttgtcccgc tgccgaagaa 600

tctttaccca ttgaggtgat ggtggacgcc gtgcacaaac tcaagtacga gaactacacc 660

tcctccttct ttatccggga catcattaag cccgatcctc ctaagaattt acagctgaag 720

cctctcaaaa atagccggca agttgaggtc tcttgggaat atcccgacac ttggagcaca 780

ccccacagct acttctcttt aacctttttgt gtgcaagttc aaggtaaaag caagcgggag 840

aagaaagacc gggtgtttac cgacaaaacc agcgccaccg tcatctgtcg gaagaacgcc 900

tccatcagcg tgagggctca agatcgttat tactccagca gctggtccga gtgggccagc 960

gtgccttgtt ccggcggtgg aggatccgga ggaggtggct ccggcggcgg aggatctcgt 1020

aacctccccg tggctacccc cgatcccgga atgttccctt gtttacacca cagccagaat 1080

ttactgaggg ccgtgagcaa catgctgcag aaagctaggc agactttaga attttaccct 1140

tgcaccagcg aggagatcga ccatgaagat atcaccaagg acaagacatc caccgtggag 1200

gcttgtttac ctctggagct gacaaagaac gagtcttgtc tcaactctcg tgaaaccagc 1260

ttcatcacaa atggctcttg tttagcttcc cggaagacct cctttatgat ggctttatgc 1320

ctcagctcca tctacgagga tttaaagatg taccaagtgg agttcaagac catgaacgcc 1380

aagctgctca tggaccctaa acggcagatc tttttagacc agaacatgct ggctgtgatt 1440

gatgagctga tgcaagcttt aaacttcaac tccgagaccg tccctcagaa gtcctccctc 1500

gaggagcccg atttttacaa gacaaagatc aaactgtgca ttttactcca cgcctttagg 1560

atccgggccg tgaccattga ccgggtcatg agctatttaa acgccagcat tacatgcccc 1620

cctcccatga gcgtggagca cgccgacatc tgggtgaaga gctatagcct ctacagccgg 1680

gagaggtata tctgtaacag cggcttcaag aggaaggccg gcaccagcag cctcaccgag 1740

tgcgtgctga ataaggctac caacgtggct cactggacaa caccctcttt aaagtgcatc 1800

cgg 1803

<210> 182
<211> 399
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL-21"

<400> 182
cagggccagg acaggcacat gatccggatg aggcagctca tcgacatcgt cgaccagctg 60

aagaactacg tgaacgacct ggtgcccgag tttctgcctg cccccgagga cgtggagacc 120

aactgcgagt ggtccgcctt ctcctgcttt cagaaggccc agctgaagtc cgccaacacc 180

ggcaacaacg agcggatcat caacgtgagc atcaagaagc tgaagcggaa gcctccctcc 240

acaaacgccg gcaggaggca gaagcacagg ctgacctgcc ccagctgtga ctcctacgag 300

aagaagcccc ccaaggagtt cctggagagg ttcaagtccc tgctgcagaa gatgatccat 360

cagcacctgt cctccaggac ccacggctcc gaggactcc 399

<210> 183
<211> 136
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 183
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
20 25 30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
35 40 45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
50 55 60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65 70 75 80

Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
85 90 95

```
Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110


Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125


Glu Tyr Asn Thr Ser Asn Pro Asp
    130                 135


<210> 184
<211> 136
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 184
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1               5                   10                  15


Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
            20                  25                  30


Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
        35                  40                  45


Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
    50                  55                  60


Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65                  70                  75                  80


Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95


Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110
```

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115   120   125

Glu Tyr Asn Thr Ser Asn Pro Asp
    130   135

<210> 185
<211> 408
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 185
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcacgatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgat 408

<210> 186
<211> 408
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 186
attcctcccc acgtgcagaa gagcgtgaat aatgacatga tcgtgaccga taacaatggc 60

gccgtgaaat ttccccagct gtgcaaattc tgcgatgtga ggttttccac ctgcgacaac 120

cagaagtcct gtatgagcaa ctgcacaatc acctccatct gtgagaagcc tcaggaggtg 180

tgcgtggctg tctggcggaa gaatgacgag aatatcaccc tggaaaccgt ctgccacgat 240

cccaagctgc cctaccacga tttcatcctg gaagacgccg ccagccctaa gtgcatcatg 300

aaagagaaaa agaagcctgg cgagaccttt ttcatgtgct cctgcagcag cgacgaatgc 360

aacgacaata tcatctttag cgaggaatac aataccagca accccgac 408

<210> 187
<211> 861
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 187
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcacgatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgcaca 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga c 861

<210> 188
<211> 287
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="TGFRbetaRII"

<400> 188
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1   5   10   15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
            20   25   30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
        35   40   45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
    50   55   60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65   70   75   80

Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85   90   95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100   105   110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115   120   125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
    130   135   140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145   150   155   160

```
Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175


Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190


Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205


Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220


Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240


Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255


Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
            260                 265                 270


Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp
        275                 280                 285


<210> 189
<211> 466
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      21t15-TGFRs sequence"

<400> 189
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1               5                   10                  15


Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
            20                  25                  30
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Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
        35                  40                  45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
    50                  55                  60

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
65                  70                  75                  80

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
                85                  90                  95

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
            100                 105                 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
        115                 120                 125

Gly Ser Glu Asp Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn
    130                 135                 140

Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro
145                 150                 155                 160

Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly
                165                 170                 175

Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu
            180                 185                 190

Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val
        195                 200                 205

Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu
    210                 215                 220

Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn
```

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225                 230                 235                 240

Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val
                245                 250                 255

Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr
            260                 265                 270

Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu
        275                 280                 285

Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn
    290                 295                 300

Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe
305                 310                 315                 320

Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr
                325                 330                 335

Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
            340                 345                 350

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
        355                 360                 365

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
    370                 375                 380

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
385                 390                 395                 400

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
                405                 410                 415

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
            420                 425                 430
```

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
435 440 445

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
450 455 460

Thr Ser
465

<210> 190
<211> 1398
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 190
cagggccagg acaggcacat gatccggatg aggcagctca tcgacatcgt cgaccagctg 60

aagaactacg tgaacgacct ggtgcccgag tttctgcctg cccccgagga cgtggagacc 120

aactgcgagt ggtccgcctt ctcctgcttt cagaaggccc agctgaagtc cgccaacacc 180

ggcaacaacg agcggatcat caacgtgagc atcaagaagc tgaagcggaa gcctccctcc 240

acaaacgccg gcaggaggca gaagcacagg ctgacctgcc ccagctgtga ctcctacgag 300

aagaagcccc ccaaggagtt cctggagagg ttcaagtccc tgctgcagaa gatgatccat 360

cagcacctgt cctccaggac ccacggctcc gaggactcct ccggcaccac caataccgtg 420

gccgcttata acctcacatg gaagagcacc aacttcaaga caattctgga atgggaaccc 480

aagcccgtca atcaagttta caccgtgcag atctccacca aatccggaga ctggaagagc 540

aagtgcttct acacaacaga caccgagtgt gatttaaccg acgaaatcgt caaggacgtc 600

aagcaaacct atctggctcg ggtcttttcc taccccgctg gcaatgtcga gtccaccggc 660

tccgctggcg agcctctcta cgagaattcc cccgaattca ccccttattt agagaccaat 720

ttaggccagc ctaccatcca gagcttcgag caagttggca ccaaggtgaa cgtcaccgtc 780

gaggatgaaa ggactttagt gcggcggaat aacacatttt tatccctccg ggatgtgttc 840

ggcaaagacc tcatctacac actgtactat tggaagtcca gctcctccgg caaaaagacc 900

gctaagacca acaccaacga gtttttaatt gacgtggaca aaggcgagaa ctactgcttc 960

agcgtgcaag ccgtgatccc ttctcgtacc gtcaaccgga agagcacaga ttcccccgtt 1020

gagtgcatgg gccaagaaaa gggcgagttc cgggagaact gggtgaacgt catcagcgat 1080

ttaaagaaga tcgaagattt aattcagtcc atgcatatcg acgccacttt atacacagaa 1140

tccgacgtgc acccctcttg taaggtgacc gccatgaaat gtttttttact ggagctgcaa 1200

gttatctctt tagagagcgg agacgctagc atccacgaca ccgtggagaa tttaatcatt 1260

ttagccaata actctttatc cagcaacggc aacgtgacag agtccggctg caaggagtgc 1320

gaagagctgg aggagaagaa catcaaggag tttctgcaat cctttgtgca cattgtccag 1380

atgttcatca atacctcc 1398

<210> 191
<211> 484
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 191
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
20 25 30

Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
35 40 45

Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
50 55 60

```
Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65                  70                  75                  80

Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
                85                  90                  95

Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
            100                 105                 110

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
        115                 120                 125

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
    130                 135                 140

Thr His Gly Ser Glu Asp Ser Ser Gly Thr Thr Asn Thr Val Ala Ala
145                 150                 155                 160

Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp
                165                 170                 175

Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys
            180                 185                 190

Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys
        195                 200                 205

Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala
    210                 215                 220

Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala
225                 230                 235                 240

Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu
                245                 250                 255

Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr
            260                 265                 270
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Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn
        275                 280                 285

Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr
    290                 295                 300

Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys
305                 310                 315                 320

Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr
                325                 330                 335

Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys
            340                 345                 350

Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe
        355                 360                 365

Arg Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp
    370                 375                 380

Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp
385                 390                 395                 400

Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu
                405                 410                 415

Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr
            420                 425                 430

Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly
        435                 440                 445

Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys
    450                 455                 460
```

Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe
465 470 475 480

Ile Asn Thr Ser

<210> 192
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 192
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc 60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac 120

tacgtgaacg acctggtgcc cgagtttctg cctgcccccg aggacgtgga gaccaactgc 180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac 240

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tcctccggca ccaccaatac cgtggccgct 480

tataacctca catggaagag caccaacttc aagacaattc tggaatggga acccaagccc 540

gtcaatcaag tttacaccgt gcagatctcc accaaatccg gagactggaa gagcaagtgc 600

ttctacacaa cagacaccga gtgtgattta accgacgaaa tcgtcaagga cgtcaagcaa 660

acctatctgg ctcgggtctt ttcctacccc gctggcaatg tcgagtccac cggctccgct 720

ggcgagcctc tctacgagaa ttcccccgaa ttcacccctt atttagagac caatttaggc 780

cagcctacca tccagagctt cgagcaagtt ggcaccaagg tgaacgtcac cgtcgaggat 840

gaaaggactt tagtgcggcg gaataacaca tttttatccc tccgggatgt gttcggcaaa 900

gacctcatct acacactgta ctattggaag tccagctcct ccggcaaaaa gaccgctaag 960

accaacacca acgagttttt aattgacgtg gacaaaggcg agaactactg cttcagcgtg 1020

caagccgtga tcccttctcg taccgtcaac cggaagagca cagattcccc cgttgagtgc 1080

atgggccaag aaaagggcga gttccgggag aactgggtga acgtcatcag cgatttaaag 1140

aagatcgaag atttaattca gtccatgcat atcgacgcca ctttatacac agaatccgac 1200

gtgcaccccct cttgtaaggt gaccgccatg aaatgttttt tactggagct gcaagttatc 1260

tctttagaga gcggagacgc tagcatccac gacaccgtgg agaatttaat cattttagcc 1320

aataactctt tatccagcaa cggcaacgtg acagagtccg gctgcaagga gtgcgaagag 1380

ctggaggaga agaacatcaa ggagtttctg caatcctttg tgcacattgt ccagatgttc 1440

atcaatacct cc 1452

<210> 193
<211> 352
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 193
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
20 25 30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
35 40 45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
50 55 60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65 70 75 80

```
Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145                 150                 155                 160

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220

Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
            260                 265                 270

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Ile
```

```
        275                 280                 285

Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys
    290                 295                 300

Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe
305                 310                 315                 320

Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys
                325                 330                 335

Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
            340                 345                 350
```

<210> 194
<211> 1056
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 194

```
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc       60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat      120

cagaagtcct gcatgtccaa ctgcacgatc acctccatct gcgagaagcc ccaagaagtg      180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac      240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg      300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt      360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga      420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg      480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa      540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgcaca      600
```

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga catcacgtgt cctcctccta tgtccgtgga acacgcagac 900

atctgggtca agagctacag cttgtactcc agggagcggt acatttgtaa ctctggtttc 960

aagcgtaaag ccggcacgtc cagcctgacg gagtgcgtgt tgaacaaggc cacgaatgtc 1020

gcccactgga caacccccag tctcaaatgt attaga 1056

<210> 195
<211> 370
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-TGFRs sequence"

<400> 195
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile
20 25 30

Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe
35 40 45

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
50 55 60

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
65 70 75 80

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
85 90 95

```
His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala
            100                 105                 110

Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
        115                 120                 125

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
    130                 135                 140

Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys
                165                 170                 175

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
            180                 185                 190

Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
        195                 200                 205

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
    210                 215                 220

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
225                 230                 235                 240

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp
                245                 250                 255

Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys
            260                 265                 270

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
        275                 280                 285
```

```
Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro
    290                 295                 300

Asp Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp
305                 310                 315                 320

Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser
                325                 330                 335

Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu
            340                 345                 350

Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys
        355                 360                 365

Ile Arg
    370
```

<210> 196
<211> 1110
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic TGFRt15-TGFRs21 sequence"

<400> 196

```
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc       60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg      120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag      180

tcctgcatgt ccaactgcac gatcacctcc atctgcgaga agccccaaga agtgtgcgtg      240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag      300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag      360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac      420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga      480
```

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat 540

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg cacaatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacatcac gtgtcctcct cctatgtccg tggaacacgc agacatctgg 960

gtcaagagct acagcttgta ctccagggag cggtacattt gtaactctgg tttcaagcgt 1020

aaagccggca cgtccagcct gacggagtgc gtgttgaaca aggccacgaa tgtcgcccac 1080

tggacaaccc ccagtctcaa atgtattaga 1110

<210> 197
<211> 399
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL21"

<400> 197
caaggtcaag atcgccacat gattagaatg cgtcaactta tagatattgt tgatcagctg 60

aaaaattatg tgaatgactt ggtccctgaa tttctgccag ctccagaaga tgtagagaca 120

aactgtgagt ggtcagcttt ttcctgtttt cagaaggccc aactaaagtc agcaaataca 180

ggaaacaatg aaaggataat caatgtatca attaaaaagc tgaagaggaa accaccttcc 240

acaaatgcag ggagaagaca gaaacacaga ctaacatgcc cttcatgtga ttcttatgag 300

aaaaaaccac ccaaagaatt cctagaaaga ttcaaatcac ttctccaaaa gatgattcat 360

cagcatctgt cctctagaac acacggaagt gaagattcc 399

<210> 198

<211> 456
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL7"

<400> 198
gattgtgata ttgaaggtaa agatggcaaa caatatgaga gtgttctaat ggtcagcatc 60

gatcaattat tggacagcat gaaagaaatt ggtagcaatt gcctgaataa tgaatttaac 120

ttttttaaaa gacatatctg tgatgctaat aaggaaggta tgtttttatt ccgtgctgct 180

cgcaagttga ggcaatttct taaaatgaat agcactggtg attttgatct ccacttatta 240

aaagtttcag aaggcacaac aatactgttg aactgcactg gccaggttaa aggaagaaaa 300

ccagctgccc tgggtgaagc ccaaccaaca aagagtttgg aagaaaataa atctttaaag 360

gaacagaaaa aactgaatga cttgtgtttc ctaaagagac tattacaaga gataaaaact 420

tgttggaata aaattttgat gggcactaaa gaacac 456

<210> 199
<211> 466
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 199
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1 5 10 15

Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
20 25 30

Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
35 40 45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu

```
    50                  55                  60

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
65                  70                  75                  80

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
                85                  90                  95

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
            100                 105                 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
        115                 120                 125

Gly Ser Glu Asp Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn
    130                 135                 140

Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro
145                 150                 155                 160

Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly
                165                 170                 175

Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu
            180                 185                 190

Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val
        195                 200                 205

Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu
    210                 215                 220

Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn
225                 230                 235                 240

Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val
                245                 250                 255
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```
Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr
            260                 265                 270

Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu
        275                 280                 285

Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn
    290                 295                 300

Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe
305                 310                 315                 320

Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr
                325                 330                 335

Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu
            340                 345                 350

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile
        355                 360                 365

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His
    370                 375                 380

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln
385                 390                 395                 400

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu
                405                 410                 415

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val
            420                 425                 430

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile
        435                 440                 445

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn
```

450 455 460

Thr Ser
465

<210> 200
<211> 1398
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 200
caaggtcaag atcgccacat gattagaatg cgtcaactta tagatattgt tgatcagctg 60

aaaaattatg tgaatgactt ggtccctgaa tttctgccag ctccagaaga tgtagagaca 120

aactgtgagt ggtcagcttt ttcctgtttt cagaaggccc aactaaagtc agcaaataca 180

ggaaacaatg aaaggataat caatgtatca attaaaaagc tgaagaggaa accaccttcc 240

acaaatgcag ggagaagaca gaaacacaga ctaacatgcc cttcatgtga ttcttatgag 300

aaaaaaccac ccaaagaatt cctagaaaga ttcaaatcac ttctccaaaa gatgattcat 360

cagcatctgt cctctagaac acacggaagt gaagattcct caggcactac aaatactgtg 420

gcagcatata atttaacttg gaaatcaact aatttcaaga caattttgga gtgggaaccc 480

aaacccgtca atcaagtcta cactgttcaa ataagcacta agtcaggaga ttggaaaagc 540

aaatgctttt acacaacaga cacagagtgt gacctcaccg acgagattgt gaaggatgtg 600

aagcagacgt acttggcacg ggtcttctcc tacccggcag ggaatgtgga gagcaccggt 660

tctgctgggg agcctctgta tgagaactcc ccagagttca caccttacct ggagacaaac 720

ctcggacagc caacaattca gagttttgaa caggtgggaa caaaagtgaa tgtgaccgta 780

gaagatgaac ggactttagt cagaaggaac aacactttcc taagcctccg ggatgttttt 840

ggcaaggact taatttatac actttattat tggaaatctt caagttcagg aaagaaaaca 900

gccaaaacaa acactaatga gtttttgatt gatgtggata aaggagaaaa ctactgtttc 960

agtgttcaag cagtgattcc ctcccgaaca gttaaccgga agagtacaga cagcccggta 1020

gagtgtatgg gccaggagaa aggggaattc agagaaaact gggtgaacgt catcagcgat 1080

ttaaagaaga tcgaagattt aattcagtcc atgcatatcg acgccacttt atacacagaa 1140

tccgacgtgc acccctcttg taaggtgacc gccatgaaat gtttttttact ggagctgcaa 1200

gttatctctt tagagagcgg agacgctagc atccacgaca ccgtggagaa tttaatcatt 1260

ttagccaata actctttatc cagcaacggc aacgtgacag agtccggctg caaggagtgc 1320

gaagagctgg aggagaagaa catcaaggag tttctgcaat cctttgtgca cattgtccag 1380

atgttcatca atacctcc 1398

<210> 201
<211> 483
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 201
Met Gly Val Lys Val Leu Phe Ala Leu Ile Cys Ile Ala Val Ala Glu
1 5 10 15

Ala Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp
20 25 30

Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe
35 40 45

Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe
50 55 60

Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn
65 70 75 80

Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro
85 90 95

```
Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser
            100                 105                 110

Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe
        115                 120                 125

Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr
    130                 135                 140

His Gly Ser Glu Asp Ser Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr
145                 150                 155                 160

Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu
                165                 170                 175

Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser
            180                 185                 190

Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp
        195                 200                 205

Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg
    210                 215                 220

Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly
225                 230                 235                 240

Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr
                245                 250                 255

Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys
            260                 265                 270

Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn
        275                 280                 285
```

```
Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr
    290                 295                 300

Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr
305                 310                 315                 320

Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys
                325                 330                 335

Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser
            340                 345                 350

Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg
        355                 360                 365

Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu
    370                 375                 380

Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val
385                 390                 395                 400

His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu
                405                 410                 415

Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val
            420                 425                 430

Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn
        435                 440                 445

Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn
    450                 455                 460

Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile
465                 470                 475                 480

Asn Thr Ser
```

<210> 202
<211> 1449
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 202
atgggagtga aagttctttt tgcccttatt tgtattgctg tggccgaggc ccaaggtcaa 60

gatcgccaca tgattagaat gcgtcaactt atagatattg ttgatcagct gaaaaattat 120

gtgaatgact tggtccctga atttctgcca gctccagaag atgtagagac aaactgtgag 180

tggtcagctt tttcctgttt tcagaaggcc caactaaagt cagcaaatac aggaaacaat 240

gaaaggataa tcaatgtatc aattaaaaag ctgaagagga aaccaccttc cacaaatgca 300

gggagaagac agaaacacag actaacatgc ccttcatgtg attcttatga gaaaaaacca 360

cccaaagaat tcctagaaag attcaaatca cttctccaaa agatgattca tcagcatctg 420

tcctctagaa cacacggaag tgaagattcc tcaggcacta caaatactgt ggcagcatat 480

aatttaactt ggaaatcaac taatttcaag acaattttgg agtgggaacc caaacccgtc 540

aatcaagtct acactgttca aataagcact aagtcaggag attggaaaag caaatgcttt 600

tacacaacag acacagagtg tgacctcacc gacgagattg tgaaggatgt gaagcagacg 660

tacttggcac gggtcttctc ctacccggca gggaatgtgg agagcaccgg ttctgctggg 720

gagcctctgt atgagaactc cccagagttc acaccttacc tggagacaaa cctcggacag 780

ccaacaattc agagttttga acaggtggga acaaaagtga atgtgaccgt agaagatgaa 840

cggactttag tcagaaggaa caacactttc ctaagcctcc gggatgtttt tggcaaggac 900

ttaatttata cactttatta ttggaaatct tcaagttcag gaaagaaaac agccaaaaca 960

aacactaatg agtttttgat tgatgtggat aaaggagaaa actactgttt cagtgttcaa 1020

gcagtgattc cctcccgaac agttaaccgg aagagtacag acagcccggt agagtgtatg 1080

ggccaggaga aaggggaatt cagagaaaac tgggtgaacg tcatcagcga tttaaagaag 1140

atcgaagatt taattcagtc catgcatatc gacgccactt tatacacaga atccgacgtg 1200

caccctctt gtaaggtgac cgccatgaaa tgtttttttac tggagctgca agttatctct 1260

ttagagagcg gagacgctag catccacgac accgtggaga atttaatcat tttagccaat 1320

aactctttat ccagcaacgg caacgtgaca gagtccggct gcaaggagtg cgaagagctg 1380

gaggagaaga acatcaagga gtttctgcaa tcctttgtgc acattgtcca gatgttcatc 1440

aatacctcc 1449

<210> 203
<211> 217
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 203
Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu
1 5 10 15

Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly Ser
20 25 30

Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp
35 40 45

Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg
50 55 60

Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu Leu
65 70 75 80

Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val
85 90 95

Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys Ser

```
            100                 105                 110

Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu
        115                 120                 125

Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys
    130                 135                 140

Ile Leu Met Gly Thr Lys Glu His Ile Thr Cys Pro Pro Pro Met Ser
145                 150                 155                 160

Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg
                165                 170                 175

Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser
            180                 185                 190

Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp
        195                 200                 205

Thr Thr Pro Ser Leu Lys Cys Ile Arg
    210                 215


<210> 204
<211> 651
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      21t15-7s sequence"

<400> 204
gattgtgata ttgaaggtaa agatggcaaa caatatgaga gtgttctaat ggtcagcatc       60

gatcaattat tggacagcat gaaagaaatt ggtagcaatt gcctgaataa tgaatttaac      120

ttttttaaaa gacatatctg tgatgctaat aaggaaggta tgtttttatt ccgtgctgct      180

cgcaagttga ggcaatttct taaaatgaat agcactggtg attttgatct ccacttatta      240
```

aaagtttcag aaggcacaac aatactgttg aactgcactg gccaggttaa aggaagaaaa 300

ccagctgccc tgggtgaagc ccaaccaaca aagagtttgg aagaaaataa atctttaaag 360

gaacagaaaa aactgaatga cttgtgtttc ctaaagagac tattacaaga gataaaaact 420

tgttggaata aaattttgat gggcactaaa gaacacatca cgtgccctcc ccccatgtcc 480

gtggaacacg cagacatctg ggtcaagagc tacagcttgt actccaggga gcggtacatt 540

tgtaactctg gtttcaagcg taaagccggc acgtccagcc tgacggagtg cgtgttgaac 600

aaggccacga atgtcgccca ctggacaacc cccagtctca aatgcattag a 651

<210> 205
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 205
Met Gly Val Lys Val Leu Phe Ala Leu Ile Cys Ile Ala Val Ala Glu
1 5 10 15

Ala Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val
20 25 30

Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly
35 40 45

Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys
50 55 60

Asp Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu
65 70 75 80

Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu
85 90 95

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Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln
            100                 105                 110

Val Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys
        115                 120                 125

Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp
    130                 135                 140

Leu Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn
145                 150                 155                 160

Lys Ile Leu Met Gly Thr Lys Glu His Ile Thr Cys Pro Pro Pro Met
                165                 170                 175

Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser
            180                 185                 190

Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr
        195                 200                 205

Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His
    210                 215                 220

Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
225                 230
```

<210> 206
<211> 702
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15-7s sequence"

<400> 206
atgggagtga aagttctttt tgcccttatt tgtattgctg tggccgaggc cgattgtgat 60

attgaaggta aagatggcaa acaatatgag agtgttctaa tggtcagcat cgatcaatta 120

ttggacagca tgaaagaaat tggtagcaat tgcctgaata atgaatttaa ctttttaaa 180

agacatatct gtgatgctaa taaggaaggt atgtttttat tccgtgctgc tcgcaagttg 240

aggcaatttc ttaaaatgaa tagcactggt gattttgatc tccacttatt aaaagtttca 300

gaaggcacaa caatactgtt gaactgcact ggccaggtta aaggaagaaa accagctgcc 360

ctgggtgaag cccaaccaac aaagagtttg gaagaaaata aatctttaaa ggaacagaaa 420

aaactgaatg acttgtgttt cctaaagaga ctattacaag agataaaaac ttgttggaat 480

aaaattttga tgggcactaa agaacacatc acgtgccctc cccccatgtc cgtggaacac 540

gcagacatct gggtcaagag ctacagcttg tactccaggg agcggtacat ttgtaactct 600

ggtttcaagc gtaaagccgg cacgtccagc ctgacggagt gcgtgttgaa caaggccacg 660

aatgtcgccc actggacaac ccccagtctc aaatgcatta ga 702

<210> 207
<211> 485
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 207
Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser Val Leu
1 5 10 15

Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile Gly Ser
20 25 30

Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile Cys Asp
35 40 45

Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys Leu Arg
50 55 60

Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His Leu Leu

```
65                  70                  75                  80

Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly Gln Val
                85                  90                  95

Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr Lys Ser
            100                 105                 110

Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn Asp Leu
        115                 120                 125

Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp Asn Lys
    130                 135                 140

Ile Leu Met Gly Thr Lys Glu His Ser Gly Thr Thr Asn Thr Val Ala
145                 150                 155                 160

Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu
                165                 170                 175

Trp Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr
            180                 185                 190

Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu
        195                 200                 205

Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu
    210                 215                 220

Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser
225                 230                 235                 240

Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu
                245                 250                 255

Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly
            260                 265                 270
```

```
Thr Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg
        275                 280                 285

Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile
    290                 295                 300

Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala
305                 310                 315                 320

Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn
                325                 330                 335

Tyr Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg
            340                 345                 350

Lys Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu
        355                 360                 365

Phe Arg Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu
    370                 375                 380

Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser
385                 390                 395                 400

Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu
                405                 410                 415

Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp
            420                 425                 430

Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn
        435                 440                 445

Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu
    450                 455                 460

Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met
```

465 470 475 480

Phe Ile Asn Thr Ser
485

<210> 208
<211> 1455
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 208
gattgcgaca tcgagggcaa ggacggcaag cagtacgaga gcgtgctgat ggtgtccatc 60

gaccagctgc tggacagcat gaaggagatc ggctccaact gcctcaacaa cgagttcaac 120

ttcttcaagc ggcacatctg cgacgccaac aaggagggca tgttcctgtt cagggccgcc 180

aggaaactgc ggcagttcct gaagatgaac tccaccggcg acttcgacct gcacctgctg 240

aaggtgtccg agggcaccac catcctgctg aactgcaccg gacaggtgaa gggccggaaa 300

cctgctgctc tgggagaggc ccaacccacc aagagcctgg aggagaacaa gtccctgaag 360

gagcagaaga agctgaacga cctgtgcttc ctgaagaggc tgctgcagga gatcaagacc 420

tgctggaaca agatcctgat gggcaccaag gagcatagcg gcacaaccaa cacagtcgct 480

gcctataacc tcacttggaa gagcaccaac ttcaaaacca tcctcgaatg ggaacccaaa 540

cccgttaacc aagtttacac cgtgcagatc agcaccaagt ccggcgactg gaagtccaaa 600

tgtttctata ccaccgacac cgagtgcgat ctcaccgatg agatcgtgaa agatgtgaaa 660

cagacctacc tcgcccgggt gtttagctac cccgccggca atgtggagag cactggttcc 720

gctggcgagc ctttatacga gaacagcccc gaatttaccc cttacctcga gaccaattta 780

ggacagccca ccatccaaag ctttgagcaa gttggcacaa aggtgaatgt gacagtggag 840

gacgagcgga ctttagtgcg gcggaacaac acctttctca gcctccggga tgtgttcggc 900

aaagatttaa tctacacact gtattactgg aagtcctctt cctccggcaa gaagacagct 960

aaaaccaaca caaacgagtt tttaatcgac gtggataaag gcgaaaacta ctgtttcagc 1020

gtgcaagctg tgatcccctc ccggaccgtg aataggaaaa gcaccgatag ccccgttgag 1080

tgcatgggcc aagaaaaggg cgagttccgg gagaactggg tgaacgtcat cagcgattta 1140

aagaagatcg aagatttaat tcagtccatg catatcgacg ccactttata cacagaatcc 1200

gacgtgcacc cctcttgtaa ggtgaccgcc atgaaatgtt ttttactgga gctgcaagtt 1260

atctctttag agagcggaga cgctagcatc cacgacaccg tggagaattt aatcatttta 1320

gccaataact ctttatccag caacggcaac gtgacagagt ccggctgcaa ggagtgcgaa 1380

gagctggagg agaagaacat caaggagttt ctgcaatcct ttgtgcacat tgtccagatg 1440

ttcatcaata cctcc 1455

<210> 209
<211> 503
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 209
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser
20 25 30

Val Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile
35 40 45

Gly Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile
50 55 60

Cys Asp Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys
65 70 75 80

```
Leu Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His
                85                  90                  95

Leu Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly
            100                 105                 110

Gln Val Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr
        115                 120                 125

Lys Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn
    130                 135                 140

Asp Leu Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp
145                 150                 155                 160

Asn Lys Ile Leu Met Gly Thr Lys Glu His Ser Gly Thr Thr Asn Thr
                165                 170                 175

Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile
            180                 185                 190

Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile
        195                 200                 205

Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp
    210                 215                 220

Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr
225                 230                 235                 240

Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr
                245                 250                 255

Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro
            260                 265                 270

Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln
        275                 280                 285
```

```
Val Gly Thr Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val
    290                 295                 300

Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp
305                 310                 315                 320

Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys
                325                 330                 335

Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly
            340                 345                 350

Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val
        355                 360                 365

Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys
    370                 375                 380

Gly Glu Phe Arg Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys
385                 390                 395                 400

Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr
                405                 410                 415

Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe
            420                 425                 430

Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile
        435                 440                 445

His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser
    450                 455                 460

Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu
465                 470                 475                 480
```

Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val
485 490 495

Gln Met Phe Ile Asn Thr Ser
500

<210> 210
<211> 1509
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 210
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccgattgc 60

gacatcgagg gcaaggacgg caagcagtac gagagcgtgc tgatggtgtc catcgaccag 120

ctgctggaca gcatgaagga gatcggctcc aactgcctca acaacgagtt caacttcttc 180

aagcggcaca tctgcgacgc caacaaggag ggcatgttcc tgttcagggc cgccaggaaa 240

ctgcggcagt tcctgaagat gaactccacc ggcgacttcg acctgcacct gctgaaggtg 300

tccgagggca ccaccatcct gctgaactgc accggacagg tgaagggccg gaaacctgct 360

gctctgggag aggcccaacc caccaagagc ctggaggaga acaagtccct gaaggagcag 420

aagaagctga acgacctgtg cttcctgaag aggctgctgc aggagatcaa gacctgctgg 480

aacaagatcc tgatgggcac caaggagcat agcggcacaa ccaacacagt cgctgcctat 540

aacctcactt ggaagagcac caacttcaaa accatcctcg aatgggaacc caaacccgtt 600

aaccaagttt acaccgtgca gatcagcacc aagtccggcg actggaagtc caaatgtttc 660

tataccaccg acaccgagtg cgatctcacc gatgagatcg tgaaagatgt gaaacagacc 720

tacctcgccc gggtgtttag ctaccccgcc ggcaatgtgg agagcactgg ttccgctggc 780

gagcctttat acgagaacag ccccgaattt acccccttacc tcgagaccaa tttaggacag 840

cccaccatcc aaagctttga gcaagttggc acaaaggtga atgtgacagt ggaggacgag 900

cggactttag tgcggcggaa caacaccttt ctcagcctcc gggatgtgtt cggcaaagat 960

ttaatctaca cactgtatta ctggaagtcc tcttcctccg gcaagaagac agctaaaacc 1020

aacacaaacg agtttttaat cgacgtggat aaaggcgaaa actactgttt cagcgtgcaa 1080

gctgtgatcc cctcccggac cgtgaatagg aaaagcaccg atagccccgt tgagtgcatg 1140

ggccaagaaa agggcgagtt ccgggagaac tgggtgaacg tcatcagcga tttaaagaag 1200

atcgaagatt taattcagtc catgcatatc gacgccactt tatacacaga atccgacgtg 1260

caccctctt gtaaggtgac cgccatgaaa tgtttttac tggagctgca agttatctct 1320

ttagagagcg gagacgctag catccacgac accgtggaga atttaatcat tttagccaat 1380

aactctttat ccagcaacgg caacgtgaca gagtccggct gcaaggagtg cgaagagctg 1440

gaggagaaga acatcaagga gtttctgcaa tcctttgtgc acattgtcca gatgttcatc 1500

aatacctcc 1509

<210> 211
<211> 198
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 211
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1 5 10 15

Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
20 25 30

Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
35 40 45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
50 55 60

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser

65 70 75 80

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
85 90 95

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
100 105 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
115 120 125

Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val Glu His
130 135 140

Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr
145 150 155 160

Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr
165 170 175

Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro
180 185 190

Ser Leu Lys Cys Ile Arg
195

<210> 212
<211> 594
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 212
cagggccagg acaggcacat gatccggatg aggcagctca tcgacatcgt cgaccagctg 60

aagaactacg tgaacgacct ggtgcccgag tttctgcctg cccccgagga cgtggagacc 120

```
aactgcgagt ggtccgcctt ctcctgcttt cagaaggccc agctgaagtc cgccaacacc      180

ggcaacaacg agcggatcat caacgtgagc atcaagaagc tgaagcggaa gcctccctcc      240

acaaacgccg gcaggaggca gaagcacagg ctgacctgcc ccagctgtga ctcctacgag      300

aagaagcccc ccaaggagtt cctggagagg ttcaagtccc tgctgcagaa gatgatccat      360

cagcacctgt cctccaggac ccacggctcc gaggactcca ttacatgccc ccctcccatg      420

agcgtggagc acgccgacat ctgggtgaag agctatagcc tctacagccg ggagaggtat      480

atctgtaaca gcggcttcaa gaggaaggcc ggcaccagca gcctcaccga gtgcgtgctg      540

aataaggcta ccaacgtggc tcactggaca acaccctctt taaagtgcat ccgg            594


<210> 213
<211> 216
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      7t15-21s sequence"

<400> 213
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                   10                  15


Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
            20                  25                  30


Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
        35                  40                  45


Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
    50                  55                  60


Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65                  70                  75                  80


Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
                85                  90                  95
```

Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
100 105 110

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
115 120 125

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
130 135 140

Thr His Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val
145 150 155 160

Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu
165 170 175

Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser
180 185 190

Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr
195 200 205

Thr Pro Ser Leu Lys Cys Ile Arg
210 215

<210> 214
<211> 648
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21s sequence"

<400> 214
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc 60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac 120

tacgtgaacg acctggtgcc cgagtttctg cctgcccccg aggacgtgga gaccaactgc 180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac 240

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tccattacat gccccccctcc catgagcgtg 480

gagcacgccg acatctgggt gaagagctat agcctctaca gccgggagag gtatatctgt 540

aacagcggct tcaagaggaa ggccggcacc agcagcctca ccgagtgcgt gctgaataag 600

gctaccaacg tggctcactg gacaacaccc tctttaaagt gcatccgg 648

<210> 215
<211> 108
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 215
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln Thr
1 5 10 15

Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
20 25 30

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr Gly
35 40 45

Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser
50 55 60

Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
65 70 75 80

Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val
85 90 95

```
Val Phe Gly Gly Gly Thr Lys Leu Thr Val Gly His
            100                 105


<210> 216
<211> 324
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 216
tccgagctga cccaggaccc tgctgtgtcc gtggctctgg gccagaccgt gaggatcacc       60

tgccagggcg actccctgag gtcctactac gcctcctggt accagcagaa gcccggccag      120

gctcctgtgc tggtgatcta cggcaagaac aacaggccct ccggcatccc tgacaggttc      180

tccggatcct cctccggcaa caccgcctcc ctgaccatca caggcgctca ggccgaggac      240

gaggctgact actactgcaa ctccagggac tcctccggca accatgtggt gttcggcggc      300

ggcaccaagc tgaccgtggg ccat                                             324


<210> 217
<211> 117
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 217
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
            20                  25                  30


Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
```

Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Ser Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Arg
115

<210> 218
<211> 351
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD16"

<400> 218
gaggtgcagc tggtggagtc cggaggagga gtggtgaggc ctggaggctc cctgaggctg 60

agctgtgctg cctccggctt caccttcgac gactacggca tgtcctgggt gaggcaggct 120

cctggaaagg gcctggagtg ggtgtccggc atcaactgga acggcggatc caccggctac 180

gccgattccg tgaagggcag gttcaccatc agcagggaca acgccaagaa ctccctgtac 240

ctgcagatga actccctgag ggccgaggac accgccgtgt actactgcgc caggggcagg 300

tccctgctgt tcgactactg gggacagggc accctggtga ccgtgtccag g 351

<210> 219

<400> 219
000

<210> 220

<400> 220
000

<210> 221

<400> 221
000

<210> 222

<400> 222
000

<210> 223
<211> 823
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s16 sequence"

<400> 223
Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp Trp Tyr
1   5   10   15

Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr Pro Glu
            20   25   30

Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val Leu Gly
        35   40   45

Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp Ala Gly
    50   55   60

Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser Leu Leu
65   70   75   80

Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile Leu Lys

```
                85                  90                  95

Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu Ala Lys
            100                 105                 110

Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile Ser Thr
        115                 120                 125

Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp Pro Gln
    130                 135                 140

Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val Arg Gly
145                 150                 155                 160

Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp Ser Ala
                165                 170                 175

Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val Asp Ala
            180                 185                 190

Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe Ile Arg
        195                 200                 205

Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys Pro Leu
    210                 215                 220

Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp Thr Trp
225                 230                 235                 240

Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln Val Gln
                245                 250                 255

Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp Lys Thr
            260                 265                 270

Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val Arg Ala
        275                 280                 285
```

```
Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser Val Pro
    290                 295                 300

Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
305                 310                 315                 320

Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe Pro Cys
                325                 330                 335

Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met Leu Gln
            340                 345                 350

Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile
        355                 360                 365

Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys
    370                 375                 380

Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu
385                 390                 395                 400

Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser
                405                 410                 415

Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met
            420                 425                 430

Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro
        435                 440                 445

Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile Asp Glu
    450                 455                 460

Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln Lys Ser
465                 470                 475                 480

Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu Cys Ile
```

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                485                 490                 495

Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met
            500                 505                 510

Ser Tyr Leu Asn Ala Ser Ile Thr Cys Pro Pro Pro Met Ser Val Glu
        515                 520                 525

His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg
    530                 535                 540

Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu
545                 550                 555                 560

Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr
                565                 570                 575

Pro Ser Leu Lys Cys Ile Arg Ser Glu Leu Thr Gln Asp Pro Ala Val
            580                 585                 590

Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser
        595                 600                 605

Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala
    610                 615                 620

Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro
625                 630                 635                 640

Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile
                645                 650                 655

Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg
            660                 665                 670

Asp Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr
        675                 680                 685
```

Val Gly His Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
690 695 700

Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro
705 710 715 720

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
725 730 735

Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu
740 745 750

Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp
755 760 765

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser
770 775 780

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
785 790 795 800

Tyr Cys Ala Arg Gly Arg Ser Leu Leu Phe Asp Tyr Trp Gly Gln Gly
805 810 815

Thr Leu Val Thr Val Ser Arg
820

<210> 224
<211> 2469
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s16 sequence"

<400> 224
atttgggaac tgaagaagga cgtctacgtg gtcgaactgg actggtatcc cgatgctccc 60

ggcgaaatgg tggtgctcac ttgtgacacc cccgaagaag acggcatcac ttggaccctc 120

gatcagagca gcgaggtgct gggctccgga aagaccctca caatccaagt taaggagttc 180

ggagacgctg gccaatacac atgccacaag ggaggcgagg tgctcagcca ttccttatta 240

ttattacaca agaaggaaga cggaatctgg tccaccgaca ttttaaaaga tcagaaggag 300

cccaagaata agacctttt aaggtgtgag gccaaaaact acagcggtcg tttcacttgt 360

tggtggctga ccaccatttc caccgattta accttctccg tgaaaagcag ccggggaagc 420

tccgaccctc aaggtgtgac atgtggagcc gctaccctca gcgctgagag ggttcgtggc 480

gataacaagg aatacgagta cagcgtggag tgccaagaag atagcgcttg tcccgctgcc 540

gaagaatctt tacccattga ggtgatggtg gacgccgtgc acaaactcaa gtacgagaac 600

tacacctcct ccttctttat ccgggacatc attaagcccg atcctcctaa gaatttacag 660

ctgaagcctc tcaaaaatag ccggcaagtt gaggtctctt gggaatatcc cgacacttgg 720

agcacacccc acagctactt ctctttaacc ttttgtgtgc aagttcaagg taaaagcaag 780

cgggagaaga aagaccgggt gtttaccgac aaaaccagcg ccaccgtcat ctgtcggaag 840

aacgcctcca tcagcgtgag ggctcaagat cgttattact ccagcagctg gtccgagtgg 900

gccagcgtgc cttgttccgg cggtggagga tccggaggag gtggctccgg cggcggagga 960

tctcgtaacc tccccgtggc taccccgat cccggaatgt tcccttgttt acaccacagc 1020

cagaatttac tgagggccgt gagcaacatg ctgcagaaag ctaggcagac tttagaattt 1080

taccttgca ccagcgagga gatcgaccat gaagatatca ccaaggacaa gacatccacc 1140

gtggaggctt gtttacctct ggagctgaca aagaacgagt cttgtctcaa ctctcgtgaa 1200

accagcttca tcacaaatgg ctcttgttta gcttcccgga agacctcctt tatgatggct 1260

ttatgcctca gctccatcta cgaggattta aagatgtacc aagtggagtt caagaccatg 1320

aacgccaagc tgctcatgga ccctaaacgg cagatctttt tagaccagaa catgctggct 1380

gtgattgatg agctgatgca agctttaaac ttcaactccg agaccgtccc tcagaagtcc 1440

tccctcgagg agcccgattt ttacaagaca aagatcaaac tgtgcatttt actccacgcc 1500

tttaggatcc gggccgtgac cattgaccgg gtcatgagct atttaaacgc cagcattaca 1560

tgccccctc ccatgagcgt ggagcacgcc gacatctggg tgaagagcta tagcctctac 1620

agccgggaga ggtatatctg taacagcggc ttcaagagga aggccggcac cagcagcctc 1680

accgagtgcg tgctgaataa ggctaccaac gtggctcact ggacaacacc ctctttaaag 1740

tgcatccggt ccgagctgac ccaggaccct gctgtgtccg tggctctggg ccagaccgtg 1800

aggatcacct gccagggcga ctccctgagg tcctactacg cctcctggta ccagcagaag 1860

cccggccagg ctcctgtgct ggtgatctac ggcaagaaca acaggccctc cggcatccct 1920

gacaggttct ccggatcctc ctccggcaac accgcctccc tgaccatcac aggcgctcag 1980

gccgaggacg aggctgacta ctactgcaac tccagggact cctccggcaa ccatgtggtg 2040

ttcggcggcg gcaccaagct gaccgtgggc catggcggcg gcggctccgg aggcggcggc 2100

agcggcggag gaggatccga ggtgcagctg gtggagtccg gaggaggagt ggtgaggcct 2160

ggaggctccc tgaggctgag ctgtgctgcc tccggcttca ccttcgacga ctacggcatg 2220

tcctgggtga ggcaggctcc tggaaagggc ctggagtggg tgtccggcat caactggaac 2280

ggcggatcca ccggctacgc cgattccgtg aagggcaggt tcaccatcag cagggacaac 2340

gccaagaact ccctgtacct gcagatgaac tccctgaggg ccgaggacac cgccgtgtac 2400

tactgcgcca ggggcaggtc cctgctgttc gactactggg gacagggcac cctggtgacc 2460

gtgtccagg 2469

<210> 225
<211> 841
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s16 sequence"

<400> 225
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp
20 25 30

```
Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr
        35                  40                  45

Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val
    50                  55                  60

Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp
65                  70                  75                  80

Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser
                85                  90                  95

Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile
            100                 105                 110

Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu
        115                 120                 125

Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile
    130                 135                 140

Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp
145                 150                 155                 160

Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val
                165                 170                 175

Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp
            180                 185                 190

Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val
        195                 200                 205

Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe
    210                 215                 220
```

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Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys
225                 230                 235                 240

Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp
                245                 250                 255

Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln
            260                 265                 270

Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp
        275                 280                 285

Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val
    290                 295                 300

Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser
305                 310                 315                 320

Val Pro Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
                325                 330                 335

Gly Gly Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe
            340                 345                 350

Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met
        355                 360                 365

Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu
    370                 375                 380

Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu
385                 390                 395                 400

Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser
                405                 410                 415

Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys
            420                 425                 430
```

```
Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu
        435                 440                 445

Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met
    450                 455                 460

Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile
465                 470                 475                 480

Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln
                485                 490                 495

Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu
            500                 505                 510

Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg
        515                 520                 525

Val Met Ser Tyr Leu Asn Ala Ser Ile Thr Cys Pro Pro Pro Met Ser
    530                 535                 540

Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg
545                 550                 555                 560

Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser
                565                 570                 575

Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp
            580                 585                 590

Thr Thr Pro Ser Leu Lys Cys Ile Arg Ser Glu Leu Thr Gln Asp Pro
        595                 600                 605

Ala Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly
    610                 615                 620
```

```
Asp Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly
625                 630                 635                 640

Gln Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly
                645                 650                 655

Ile Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu
            660                 665                 670

Thr Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn
        675                 680                 685

Ser Arg Asp Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys
    690                 695                 700

Leu Thr Val Gly His Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
705                 710                 715                 720

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
                725                 730                 735

Arg Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
            740                 745                 750

Phe Asp Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
        755                 760                 765

Leu Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr
    770                 775                 780

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys
785                 790                 795                 800

Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
                805                 810                 815

Val Tyr Tyr Cys Ala Arg Gly Arg Ser Leu Leu Phe Asp Tyr Trp Gly
            820                 825                 830
```

Gln Gly Thr Leu Val Thr Val Ser Arg
835 840

<210> 226
<211> 2523
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18t15-12s16 sequence"

<400> 226
atgaaatggg tgacctttat ttctttactg ttcctcttta gcagcgccta ctccatttgg 60

gaactgaaga aggacgtcta cgtggtcgaa ctggactggt atcccgatgc tcccggcgaa 120

atggtggtgc tcacttgtga caccccgaa gaagacggca tcacttggac cctcgatcag 180

agcagcgagg tgctgggctc cggaaagacc ctcacaatcc aagttaagga gttcggagac 240

gctggccaat acacatgcca caagggaggc gaggtgctca gccattcctt attattatta 300

cacaagaagg aagacggaat ctggtccacc gacattttaa aagatcagaa ggagcccaag 360

aataagacct ttttaaggtg tgaggccaaa aactacagcg gtcgtttcac ttgttggtgg 420

ctgaccacca tttccaccga tttaaccttc tccgtgaaaa gcagccgggg aagctccgac 480

cctcaaggtg tgacatgtgg agccgctacc ctcagcgctg agagggttcg tggcgataac 540

aaggaatacg agtacagcgt ggagtgccaa gaagatagcg cttgtcccgc tgccgaagaa 600

tctttaccca ttgaggtgat ggtggacgcc gtgcacaaac tcaagtacga gaactacacc 660

tcctccttct ttatccggga catcattaag cccgatcctc ctaagaattt acagctgaag 720

cctctcaaaa atagccggca agttgaggtc tcttgggaat atcccgacac ttggagcaca 780

ccccacagct acttctcttt aacctttgt gtgcaagttc aaggtaaaag caagcgggag 840

aagaaagacc gggtgtttac cgacaaaacc agcgccaccg tcatctgtcg gaagaacgcc 900

tccatcagcg tgagggctca agatcgttat tactccagca gctggtccga gtgggccagc 960

gtgccttgtt ccggcggtgg aggatccgga ggaggtggct ccggcggcgg aggatctcgt 1020

aacctccccg tggctacccc cgatcccgga atgttccctt gtttacacca cagccagaat 1080

ttactgaggg ccgtgagcaa catgctgcag aaagctaggc agactttaga attttaccct 1140

tgcaccagcg aggagatcga ccatgaagat atcaccaagg acaagacatc caccgtggag 1200

gcttgtttac ctctggagct gacaaagaac gagtcttgtc tcaactctcg tgaaaccagc 1260

ttcatcacaa atggctcttg tttagcttcc cggaagacct cctttatgat ggctttatgc 1320

ctcagctcca tctacgagga tttaaagatg taccaagtgg agttcaagac catgaacgcc 1380

aagctgctca tggaccctaa acggcagatc tttttagacc agaacatgct ggctgtgatt 1440

gatgagctga tgcaagcttt aaacttcaac tccgagaccg tccctcagaa gtcctccctc 1500

gaggagcccg atttttacaa gacaaagatc aaactgtgca ttttactcca cgcctttagg 1560

atccgggccg tgaccattga ccgggtcatg agctatttaa acgccagcat tacatgcccc 1620

cctcccatga gcgtggagca cgccgacatc tgggtgaaga gctatagcct ctacagccgg 1680

gagaggtata tctgtaacag cggcttcaag aggaaggccg gcaccagcag cctcaccgag 1740

tgcgtgctga ataaggctac caacgtggct cactggacaa caccctcttt aaagtgcatc 1800

cggtccgagc tgacccagga ccctgctgtg tccgtggctc tgggccagac cgtgaggatc 1860

acctgccagg gcgactccct gaggtcctac tacgcctcct ggtaccagca gaagcccggc 1920

caggctcctg tgctggtgat ctacggcaag aacaacaggc cctccggcat ccctgacagg 1980

ttctccggat cctcctccgg caacaccgcc tccctgacca tcacaggcgc tcaggccgag 2040

gacgaggctg actactactg caactccagg gactcctccg gcaaccatgt ggtgttcggc 2100

ggcggcacca agctgaccgt gggccatggc ggcggcggct ccggaggcgg cggcagcggc 2160

ggaggaggat ccgaggtgca gctggtggag tccggaggag gagtggtgag gcctggaggc 2220

tccctgaggc tgagctgtgc tgcctccggc ttcaccttcg acgactacgg catgtcctgg 2280

gtgaggcagg ctcctggaaa gggcctggag tgggtgtccg gcatcaactg gaacggcgga 2340

tccaccggct acgccgattc cgtgaagggc aggttcacca tcagcaggga caacgccaag 2400

aactccctgt acctgcagat gaactccctg agggccgagg acaccgccgt gtactactgc 2460

gccaggggca ggtccctgct gttcgactac tggggacagg gcaccctggt gaccgtgtcc 2520

agg 2523

<210> 227
<211> 456
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="IL7"

<400> 227
gattgcgaca tcgagggcaa ggacggcaag cagtacgaga gcgtgctgat ggtgtccatc 60

gaccagctgc tggacagcat gaaggagatc ggctccaact gcctcaacaa cgagttcaac 120

ttcttcaagc ggcacatctg cgacgccaac aaggagggca tgttcctgtt cagggccgcc 180

aggaaactgc ggcagttcct gaagatgaac tccaccggcg acttcgacct gcacctgctg 240

aaggtgtccg agggcaccac catcctgctg aactgcaccg gacaggtgaa gggccggaaa 300

cctgctgctc tgggagaggc ccaacccacc aagagcctgg aggagaacaa gtccctgaag 360

gagcagaaga agctgaacga cctgtgcttc ctgaagaggc tgctgcagga gatcaagacc 420

tgctggaaca agatcctgat gggcaccaag gagcat 456

<210> 228

<400> 228
000

<210> 229

<400> 229
000

<210> 230

<400> 230
000

<210> 231

<400> 231
000

<210> 232
<211> 438
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-16s21 sequence"

<400> 232
Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln Thr
1 5 10 15

Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
20 25 30

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr Gly
35 40 45

Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser
50 55 60

Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
65 70 75 80

Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val
85 90 95

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Gly His Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val
115 120 125

Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly Ser Leu Arg Leu Ser
130 135 140

```
Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Gly Met Ser Trp Val
145                 150                 155                 160

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp
                165                 170                 175

Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
            180                 185                 190

Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser
        195                 200                 205

Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Ser
    210                 215                 220

Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Arg
225                 230                 235                 240

Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val
                245                 250                 255

Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly
            260                 265                 270

Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn
        275                 280                 285

Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile
    290                 295                 300

Arg Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp
305                 310                 315                 320

Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe
                325                 330                 335
```

Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe
340 345 350

Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn
355 360 365

Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro
370 375 380

Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser
385 390 395 400

Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe
405 410 415

Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr
420 425 430

His Gly Ser Glu Asp Ser
435

<210> 233
<211> 1314
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-16s21 sequence"

<400> 233
tccgagctga cccaggaccc tgctgtgtcc gtggctctgg gccagaccgt gaggatcacc 60

tgccagggcg actccctgag gtcctactac gcctcctggt accagcagaa gcccggccag 120

gctcctgtgc tggtgatcta cggcaagaac aacaggccct ccggcatccc tgacaggttc 180

tccggatcct cctccggcaa caccgcctcc ctgaccatca caggcgctca ggccgaggac 240

gaggctgact actactgcaa ctccagggac tcctccggca accatgtggt gttcggcggc 300

ggcaccaagc tgaccgtggg ccatggcggc ggcggctccg gaggcggcgg cagcggcgga 360

ggaggatccg aggtgcagct ggtggagtcc ggaggaggag tggtgaggcc tggaggctcc 420

ctgaggctga gctgtgctgc ctccggcttc accttcgacg actacggcat gtcctgggtg 480

aggcaggctc ctggaaaggg cctggagtgg gtgtccggca tcaactggaa cggcggatcc 540

accggctacg ccgattccgt gaagggcagg ttcaccatca gcagggacaa cgccaagaac 600

tccctgtacc tgcagatgaa ctccctgagg gccgaggaca ccgccgtgta ctactgcgcc 660

aggggcaggt ccctgctgtt cgactactgg ggacagggca ccctggtgac cgtgtccagg 720

attacatgcc cccctcccat gagcgtggag cacgccgaca tctgggtgaa gagctatagc 780

ctctacagcc gggagaggta tatctgtaac agcggcttca agaggaaggc cggcaccagc 840

agcctcaccg agtgcgtgct gaataaggct accaacgtgg ctcactggac aacaccctct 900

ttaaagtgca tccggcaggg ccaggacagg cacatgatcc ggatgaggca gctcatcgac 960

atcgtcgacc agctgaagaa ctacgtgaac gacctggtgc ccgagtttct gcctgccccc 1020

gaggacgtgg agaccaactg cgagtggtcc gccttctcct gctttcagaa ggcccagctg 1080

aagtccgcca acaccggcaa caacgagcgg atcatcaacg tgagcatcaa gaagctgaag 1140

cggaagcctc cctccacaaa cgccggcagg aggcagaagc acaggctgac ctgccccagc 1200

tgtgactcct acgagaagaa gccccccaag gagttcctgg agaggttcaa gtccctgctg 1260

cagaagatga tccatcagca cctgtcctcc aggacccacg gctccgagga ctcc 1314

<210> 234
<211> 456
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-16s21 sequence"

<400> 234
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly

```
            20                  25                  30

Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr
        35                  40                  45

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile
    50                  55                  60

Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly
65                  70                  75                  80

Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala
                85                  90                  95

Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn
            100                 105                 110

His Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Gly His Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln
    130                 135                 140

Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly Ser Leu Arg
145                 150                 155                 160

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Gly Met Ser
                165                 170                 175

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile
            180                 185                 190

Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys Gly Arg
        195                 200                 205

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met
    210                 215                 220
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Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly
225                 230                 235                 240

Arg Ser Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
                245                 250                 255

Ser Arg Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile
            260                 265                 270

Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn
        275                 280                 285

Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val
    290                 295                 300

Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys
305                 310                 315                 320

Cys Ile Arg Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu
                325                 330                 335

Ile Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro
            340                 345                 350

Glu Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser
        355                 360                 365

Ala Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly
    370                 375                 380

Asn Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys
385                 390                 395                 400

Pro Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys
                405                 410                 415

Pro Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu
```

420 425 430

Arg Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser
435 440 445

Arg Thr His Gly Ser Glu Asp Ser
450 455

<210> 235
<211> 1368
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-16s21 sequence"

<400> 235
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcctccgag 60

ctgacccagg accctgctgt gtccgtggct ctgggccaga ccgtgaggat cacctgccag 120

ggcgactccc tgaggtccta ctacgcctcc tggtaccagc agaagcccgg ccaggctcct 180

gtgctggtga tctacggcaa gaacaacagg ccctccggca tccctgacag gttctccgga 240

tcctcctccg gcaacaccgc ctccctgacc atcacaggcg ctcaggccga ggacgaggct 300

gactactact gcaactccag ggactcctcc ggcaaccatg tggtgttcgg cggcggcacc 360

aagctgaccg tgggccatgg cggcggcggc tccggaggcg gcggcagcgg cggaggagga 420

tccgaggtgc agctggtgga gtccggagga ggagtggtga ggcctggagg ctccctgagg 480

ctgagctgtg ctgcctccgg cttcaccttc gacgactacg gcatgtcctg ggtgaggcag 540

gctcctggaa agggcctgga gtgggtgtcc ggcatcaact ggaacggcgg atccaccggc 600

tacgccgatt ccgtgaaggg caggttcacc atcagcaggg acaacgccaa gaactccctg 660

tacctgcaga tgaactccct gagggccgag gacaccgccg tgtactactg cgccaggggc 720

aggtccctgc tgttcgacta ctggggacag ggcaccctgg tgaccgtgtc caggattaca 780

tgccccctc ccatgagcgt ggagcacgcc gacatctggg tgaagagcta tagcctctac 840

agccgggaga ggtatatctg taacagcggc ttcaagagga aggccggcac cagcagcctc 900

accgagtgcg tgctgaataa ggctaccaac gtggctcact ggacaacacc ctctttaaag 960

tgcatccggc agggccagga caggcacatg atccggatga ggcagctcat cgacatcgtc 1020

gaccagctga agaactacgt gaacgacctg gtgcccgagt ttctgcctgc ccccgaggac 1080

gtggagacca actgcgagtg gtccgccttc tcctgctttc agaaggccca gctgaagtcc 1140

gccaacaccg gcaacaacga gcggatcatc aacgtgagca tcaagaagct gaagcggaag 1200

cctccctcca caaacgccgg caggaggcag aagcacaggc tgacctgccc cagctgtgac 1260

tcctacgaga agaagccccc caaggagttc ctggagaggt tcaagtccct gctgcagaag 1320

atgatccatc agcacctgtc ctccaggacc cacggctccg aggactcc 1368

<210> 236
<211> 620
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-16s21 sequence"

<400> 236
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
20 25 30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
35 40 45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
50 55 60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65 70 75 80

```
Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145                 150                 155                 160

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220

Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
            260                 265                 270

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Ser
        275                 280                 285
```

```
Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr
    290                 295                 300

Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val
305                 310                 315                 320

Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys
                325                 330                 335

Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys
            340                 345                 350

Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly
        355                 360                 365

Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser
    370                 375                 380

Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile
385                 390                 395                 400

Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp
                405                 410                 415

Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp
            420                 425                 430

Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser
        435                 440                 445

Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile
    450                 455                 460

Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile
465                 470                 475                 480
```

```
Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys
                485                 490                 495

Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Asn Trp Val Asn Val Ile
            500                 505                 510

Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp
        515                 520                 525

Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr
    530                 535                 540

Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser
545                 550                 555                 560

Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala
                565                 570                 575

Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys
            580                 585                 590

Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser
        595                 600                 605

Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
    610                 615                 620
```

<210> 237
<211> 1860
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-16s21 sequence"

<400> 237
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcagcatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgctcc 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga cagcggcaca accaacacag tcgctgccta taacctcact 900

tggaagagca ccaacttcaa aaccatcctc gaatgggaac ccaaacccgt taaccaagtt 960

tacaccgtgc agatcagcac caagtccggc gactggaagt ccaaatgttt ctataccacc 1020

gacaccgagt gcgatctcac cgatgagatc gtgaaagatg tgaaacagac ctacctcgcc 1080

cgggtgttta gctaccccgc cggcaatgtg gagagcactg gttccgctgg cgagccttta 1140

tacgagaaca gccccgaatt taccccttac ctcgagacca atttaggaca gcccaccatc 1200

caaagctttg agcaagttgg cacaaaggtg aatgtgacag tggaggacga gcggacttta 1260

gtgcggcgga acaacacctt tctcagcctc cgggatgtgt tcggcaaaga tttaatctac 1320

acactgtatt actggaagtc ctcttcctcc ggcaagaaga cagctaaaac caacacaaac 1380

gagtttttaa tcgacgtgga taaaggcgaa aactactgtt tcagcgtgca agctgtgatc 1440

ccctcccgga ccgtgaatag gaaaagcacc gatagccccg ttgagtgcat gggccaagaa 1500

aagggcgagt tccgggagaa ctgggtgaac gtcatcagcg atttaaagaa gatcgaagat 1560

ttaattcagt ccatgcatat cgacgccact ttatacacag aatccgacgt gcacccctct 1620

tgtaaggtga ccgccatgaa atgtttttta ctggagctgc aagttatctc tttagagagc 1680

ggagacgcta gcatccacga caccgtggag aatttaatca ttttagccaa taactcttta 1740

tccagcaacg gcaacgtgac agagtccggc tgcaaggagt gcgaagagct ggaggagaag 1800

aacatcaagg agtttctgca atcctttgtg cacattgtcc agatgttcat caatacctcc 1860

<210> 238
<211> 638
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-16s21 sequence"

<400> 238
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile
20 25 30

Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe
35 40 45

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
50 55 60

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
65 70 75 80

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
85 90 95

His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala
100 105 110

Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe

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        115                 120                 125

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
    130                 135                 140

Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys
                165                 170                 175

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
            180                 185                 190

Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
        195                 200                 205

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
    210                 215                 220

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
225                 230                 235                 240

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp
                245                 250                 255

Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys
            260                 265                 270

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
        275                 280                 285

Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro
    290                 295                 300

Asp Ser Gly Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys
305                 310                 315                 320
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Ser Thr Asn Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn
                325                 330                 335

Gln Val Tyr Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser
            340                 345                 350

Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile
        355                 360                 365

Val Lys Asp Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro
    370                 375                 380

Ala Gly Asn Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu
385                 390                 395                 400

Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro
                405                 410                 415

Thr Ile Gln Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val
            420                 425                 430

Glu Asp Glu Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu
        435                 440                 445

Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys
    450                 455                 460

Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe
465                 470                 475                 480

Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala
                485                 490                 495

Val Ile Pro Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val
            500                 505                 510

Glu Cys Met Gly Gln Glu Lys Gly Glu Phe Arg Glu Asn Trp Val Asn
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515 520 525

Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His
530 535 540

Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys
545 550 555 560

Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu
565 570 575

Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile
580 585 590

Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly
595 600 605

Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu
610 615 620

Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
625 630 635

<210> 239
<211> 1914
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-16s21 sequence"

<400> 239
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc 60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg 120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag 180

tcctgcatgt ccaactgcag catcacctcc atctgcgaga agccccaaga agtgtgcgtg 240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag 300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag 360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac 420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga 480

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat 540

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg ctccatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacagcgg cacaaccaac acagtcgctg cctataacct cacttggaag 960

agcaccaact tcaaaaccat cctcgaatgg gaacccaaac ccgttaacca agtttacacc 1020

gtgcagatca gcaccaagtc cggcgactgg aagtccaaat gtttctatac caccgacacc 1080

gagtgcgatc tcaccgatga gatcgtgaaa gatgtgaaac agacctacct cgcccgggtg 1140

tttagctacc ccgccggcaa tgtggagagc actggttccg ctggcgagcc tttatacgag 1200

aacagccccg aatttacccc ttacctcgag accaatttag gacagcccac catccaaagc 1260

tttgagcaag ttggcacaaa ggtgaatgtg acagtggagg acgagcggac tttagtgcgg 1320

cggaacaaca cctttctcag cctccgggat gtgttcggca aagatttaat ctacacactg 1380

tattactgga agtcctcttc ctccggcaag aagacagcta aaaccaacac aaacgagttt 1440

ttaatcgacg tggataaagg cgaaaactac tgtttcagcg tgcaagctgt gatcccctcc 1500

cggaccgtga ataggaaaag caccgatagc cccgttgagt gcatgggcca agaaaagggc 1560

gagttccggg agaactgggt gaacgtcatc agcgatttaa agaagatcga agatttaatt 1620

cagtccatgc atatcgacgc cactttatac acagaatccg acgtgcaccc ctcttgtaag 1680

gtgaccgcca tgaaatgttt tttactggag ctgcaagtta tctctttaga gagcggagac 1740

gctagcatcc acgacaccgt ggagaattta atcattttag ccaataactc tttatccagc 1800

aacggcaacg tgacagagtc cggctgcaag gagtgcgaag agctggagga gaagaacatc 1860

aaggagtttc tgcaatcctt tgtgcacatt gtccagatgt tcatcaatac ctcc 1914

<210> 240

<400> 240
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<210> 241

<400> 241
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<210> 242

<400> 242
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<210> 243

<400> 243
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<210> 244

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<210> 245

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<210> 247

<400> 247
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<210> 248

<400> 248
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<210> 249

<400> 249
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<210> 250
<211> 235
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-7s sequence"

<400> 250
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Asp Cys Asp Ile Glu Gly Lys Asp Gly Lys Gln Tyr Glu Ser
20 25 30

Val Leu Met Val Ser Ile Asp Gln Leu Leu Asp Ser Met Lys Glu Ile
35 40 45

Gly Ser Asn Cys Leu Asn Asn Glu Phe Asn Phe Phe Lys Arg His Ile
50 55 60

Cys Asp Ala Asn Lys Glu Gly Met Phe Leu Phe Arg Ala Ala Arg Lys
65 70 75 80

Leu Arg Gln Phe Leu Lys Met Asn Ser Thr Gly Asp Phe Asp Leu His
85 90 95

Leu Leu Lys Val Ser Glu Gly Thr Thr Ile Leu Leu Asn Cys Thr Gly
100 105 110

Gln Val Lys Gly Arg Lys Pro Ala Ala Leu Gly Glu Ala Gln Pro Thr
115 120 125

Lys Ser Leu Glu Glu Asn Lys Ser Leu Lys Glu Gln Lys Lys Leu Asn
130 135 140

Asp Leu Cys Phe Leu Lys Arg Leu Leu Gln Glu Ile Lys Thr Cys Trp
145 150 155 160

Asn Lys Ile Leu Met Gly Thr Lys Glu His Ile Thr Cys Pro Pro Pro
165 170 175

Met Ser Val Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr
180 185 190

Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly
195 200 205

Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala
210 215 220

His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
225 230 235

<210> 251
<211> 705
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-7s sequence"

<400> 251
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccgattgc 60

gacatcgagg gcaaggacgg caagcagtac gagagcgtgc tgatggtgtc catcgaccag 120

ctgctggaca gcatgaagga gatcggctcc aactgcctca acaacgagtt caacttcttc 180

aagcggcaca tctgcgacgc caacaaggag ggcatgttcc tgttcagggc cgccaggaaa 240

ctgcggcagt tcctgaagat gaactccacc ggcgacttcg acctgcacct gctgaaggtg 300

tccgagggca ccaccatcct gctgaactgc accggacagg tgaagggccg gaaacctgct 360

gctctgggag aggcccaacc caccaagagc ctggaggaga acaagtccct gaaggagcag 420

aagaagctga acgacctgtg cttcctgaag aggctgctgc aggagatcaa gacctgctgg 480

aacaagatcc tgatgggcac caaggagcat attacatgcc cccctcccat gagcgtggag 540

cacgccgaca tctgggtgaa gagctatagc ctctacagcc gggagaggta tatctgtaac 600

agcggcttca agaggaaggc cggcaccagc agcctcaccg agtgcgtgct gaataaggct 660

accaacgtgg ctcactggac aacaccctct ttaaagtgca tccgg 705

<210> 252

<400> 252
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<210> 253

<400> 253
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<210> 254

<400> 254
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<210> 255

<400> 255
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<210> 256

<400> 256
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<210> 257
<211> 1056
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs sequence"

<400> 257
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcagcatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgctcc 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga cattacatgc ccccctccca tgagcgtgga gcacgccgac 900

atctgggtga agagctatag cctctacagc cgggagaggt atatctgtaa cagcggcttc 960

aagaggaagg ccggcaccag cagcctcacc gagtgcgtgc tgaataaggc taccaacgtg 1020

gctcactgga caacaccctc tttaaagtgc atccgg 1056

<210> 258

<400> 258
000

<210> 259
<211> 1110
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs sequence"

<400> 259
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc 60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg 120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag 180

tcctgcatgt ccaactgcag catcacctcc atctgcgaga agccccaaga agtgtgcgtg 240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag 300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag 360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac 420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga 480

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat 540

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg ctccatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacattac atgcccccct cccatgagcg tggagcacgc cgacatctgg 960

gtgaagagct atagcctcta cagccgggag aggtatatct gtaacagcgg cttcaagagg 1020

aaggccggca ccagcagcct caccgagtgc gtgctgaata aggctaccaa cgtggctcac 1080

tggacaacac cctctttaaa gtgcatccgg 1110

<210> 260
<211> 184
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="CD137"

<400> 260
Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp
1 5 10 15

Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu
20 25 30

Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val
35 40 45

Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val
50 55 60

Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg
65 70 75 80

Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His
85 90 95

Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr
100 105 110

Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly
115 120 125

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Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val
    130                 135                 140

His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln
145                 150                 155                 160

Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala
                165                 170                 175

Gly Leu Pro Ser Pro Arg Ser Glu
            180
```

<210> 261
<211> 552
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD137"

<400> 261
cgcgagggtc ccgagctttc gcccgacgat cccgccggcc tcttggacct gcggcagggc 60

atgtttgcgc agctggtggc ccaaaatgtt ctgctgatcg atgggcccct gagctggtac 120

agtgacccag gcctggcagg cgtgtccctg acgggggggcc tgagctacaa agaggacacg 180

aaggagctgg tggtggccaa ggctggagtc tactatgtct tctttcaact agagctgcgg 240

cgcgtggtgg ccggcgaggg ctcaggctcc gtttcacttg cgctgcacct gcagccactg 300

cgctctgctg ctggggccgc cgccctggct ttgaccgtgg acctgccacc cgcctcctcc 360

gaggctcgga actcggcctt cggtttccag ggccgcttgc tgcacctgag tgccggccag 420

cgcctgggcg tccatcttca cactgaggcc agggcacgcc atgcctggca gcttacccag 480

ggcgccacag tcttgggact cttccgggtg acccccgaaa tcccagccgg actcccttca 540

ccgaggtcgg aa 552

<210> 262
<211> 165
<212> PRT

<213> Homo sapiens

<220>
<221> source
<223> /note="CD137"

<400> 262
Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu
1   5   10   15

Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser
            20   25   30

Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys
        35   40   45

Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val
    50   55   60

Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly
65   70   75   80

Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly
                85   90   95

Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu
            100   105   110

Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser
        115   120   125

Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg
    130   135   140

His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg
145   150   155   160

Val Thr Pro Glu Ile
                165

<210> 263
<211> 495
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="CD137"

<400> 263
gatcccgccg gcctcttgga cctgcggcag ggcatgtttg cgcagctggt ggcccaaaat 60

gttctgctga tcgatgggcc cctgagctgg tacagtgacc caggcctggc aggcgtgtcc 120

ctgacggggg gcctgagcta caaagaggac acgaaggagc tggtggtggc caaggctgga 180

gtctactatg tcttctttca actagagctg cggcgcgtgg tggccggcga gggctcaggc 240

tccgtttcac ttgcgctgca cctgcagcca ctgcgctctg ctgctggggc cgccgccctg 300

gctttgaccg tggacctgcc acccgcctcc tccgaggctc ggaactcggc cttcggtttc 360

cagggccgct tgctgcacct gagtgccggc cagcgcctgg gcgtccatct tcacactgag 420

gccagggcac gccatgcctg gcagcttacc cagggcgcca cagtcttggg actcttccgg 480

gtgacccccg aaatc 495

<210> 264

<400> 264
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<210> 265

<400> 265
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<210> 266

<400> 266
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<210> 267

<400> 267
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<210> 268
<211> 397
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 268
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1 5 10 15

Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
20 25 30

Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
35 40 45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
50 55 60

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
65 70 75 80

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
85 90 95

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
100 105 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
115 120 125

Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val Glu His
130 135 140

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Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr
145                 150                 155                 160

Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr
                165                 170                 175

Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro
            180                 185                 190

Ser Leu Lys Cys Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        195                 200                 205

Gly Gly Gly Gly Ser Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp Pro
    210                 215                 220

Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala
225                 230                 235                 240

Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro
                245                 250                 255

Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp
            260                 265                 270

Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe
        275                 280                 285

Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val
    290                 295                 300

Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala
305                 310                 315                 320

Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg
                325                 330                 335

Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly
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340 345 350

Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His Ala
355 360 365

Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr
370 375 380

Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
385 390 395

<210> 269
<211> 1191
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 269
cagggccagg acaggcacat gatccggatg aggcagctca tcgacatcgt cgaccagctg 60

aagaactacg tgaacgacct ggtgcccgag tttctgcctg cccccgagga cgtggagacc 120

aactgcgagt ggtccgcctt ctcctgcttt cagaaggccc agctgaagtc cgccaacacc 180

ggcaacaacg agcggatcat caacgtgagc atcaagaagc tgaagcggaa gcctccctcc 240

acaaacgccg gcaggaggca gaagcacagg ctgacctgcc ccagctgtga ctcctacgag 300

aagaagcccc ccaaggagtt cctggagagg ttcaagtccc tgctgcagaa gatgatccat 360

cagcacctgt cctccaggac ccacggctcc gaggactcca ttacatgccc ccctcccatg 420

agcgtggagc acgccgacat ctgggtgaag agctatagcc tctacagccg ggagaggtat 480

atctgtaaca gcggcttcaa gaggaaggcc ggcaccagca gcctcaccga gtgcgtgctg 540

aataaggcta ccaacgtggc tcactggaca acaccctctt taaagtgcat ccggggcggt 600

ggaggatccg gaggaggtgg ctccggcggc ggaggatctc gcgagggtcc cgagctttcg 660

cccgacgatc ccgccggcct cttggacctg cggcagggca tgtttgcgca gctggtggcc 720

caaaatgttc tgctgatcga tgggcccctg agctggtaca gtgacccagg cctggcaggc 780

gtgtccctga cgggggggcct gagctacaaa gaggacacga aggagctggt ggtggccaag 840

gctggagtct actatgtctt ctttcaacta gagctgcggc gcgtggtggc cggcgagggc 900

tcaggctccg tttcacttgc gctgcacctg cagccactgc gctctgctgc tggggccgcc 960

gccctggctt tgaccgtgga cctgccaccc gcctcctccg aggctcggaa ctcggccttc 1020

ggtttccagg gccgcttgct gcacctgagt gccggccagc gcctgggcgt ccatcttcac 1080

actgaggcca gggcacgcca tgcctggcag cttacccagg gcgccacagt cttgggactc 1140

ttccgggtga cccccgaaat cccagccgga ctcccttcac cgaggtcgga a 1191

<210> 270
<211> 415
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 270
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
20 25 30

Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
35 40 45

Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
50 55 60

Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65 70 75 80

Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
85 90 95

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Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
            100                 105                 110

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
        115                 120                 125

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
    130                 135                 140

Thr His Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val
145                 150                 155                 160

Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu
                165                 170                 175

Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser
            180                 185                 190

Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr
        195                 200                 205

Thr Pro Ser Leu Lys Cys Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Arg Glu Gly Pro Glu Leu Ser Pro Asp
225                 230                 235                 240

Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu
                245                 250                 255

Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser
            260                 265                 270

Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys
        275                 280                 285
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Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val
    290                 295                 300

Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly
305                 310                 315                 320

Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly
                325                 330                 335

Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu
            340                 345                 350

Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser
        355                 360                 365

Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg
    370                 375                 380

His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg
385                 390                 395                 400

Val Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
                405                 410                 415


<210> 271
<211> 1245
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      7t15-21CD137 sequence"

<400> 271
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc       60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac      120

tacgtgaacg acctggtgcc cgagtttctg cctgcccccg aggacgtgga gaccaactgc      180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac      240
```

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tccattacat gccccccctcc catgagcgtg 480

gagcacgccg acatctgggt gaagagctat agcctctaca gccgggagag gtatatctgt 540

aacagcggct tcaagaggaa ggccggcacc agcagcctca ccgagtgcgt gctgaataag 600

gctaccaacg tggctcactg gacaacaccc tctttaaagt gcatccgggg cggtggagga 660

tccggaggag gtggctccgg cggcggagga tctcgcgagg gtcccgagct ttcgcccgac 720

gatcccgccg gcctcttgga cctgcggcag ggcatgtttg cgcagctggt ggcccaaaat 780

gttctgctga tcgatgggcc cctgagctgg tacagtgacc caggcctggc aggcgtgtcc 840

ctgacggggg gcctgagcta caaagaggac acgaaggagc tggtggtggc caaggctgga 900

gtctactatg tcttctttca actagagctg cggcgcgtgg tggccggcga gggctcaggc 960

tccgtttcac ttgcgctgca cctgcagcca ctgcgctctg ctgctggggc cgccgccctg 1020

gctttgaccg tggacctgcc acccgcctcc tccgaggctc ggaactcggc cttcggtttc 1080

cagggccgct tgctgcacct gagtgccggc cagcgcctgg gcgtccatct tcacactgag 1140

gccagggcac gccatgcctg gcagcttacc cagggcgcca cagtcttggg actcttccgg 1200

gtgacccccg aaatcccagc cggactccct tcaccgaggt cggaa 1245

<210> 272
<211> 378
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 272
Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
1 5 10 15

```
Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
            20                  25                  30

Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
        35                  40                  45

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
    50                  55                  60

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
65                  70                  75                  80

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
                85                  90                  95

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
            100                 105                 110

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
        115                 120                 125

Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val Glu His
    130                 135                 140

Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr
145                 150                 155                 160

Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr
                165                 170                 175

Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro
            180                 185                 190

Ser Leu Lys Cys Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        195                 200                 205

Gly Gly Gly Gly Ser Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly
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    210                 215                 220

Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro
225                 230                 235                 240

Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly
                245                 250                 255

Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala
            260                 265                 270

Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala
        275                 280                 285

Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu
    290                 295                 300

Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro
305                 310                 315                 320

Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg
                325                 330                 335

Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu His Thr
            340                 345                 350

Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val
        355                 360                 365

Leu Gly Leu Phe Arg Val Thr Pro Glu Ile
    370                 375


<210> 273
<211> 1134
<212> DNA
<213> Artificial Sequence

<220>
<221> source
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<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 273
cagggccagg acaggcacat gatccggatg aggcagctca tcgacatcgt cgaccagctg 60

aagaactacg tgaacgacct ggtgcccgag tttctgcctg cccccgagga cgtggagacc 120

aactgcgagt ggtccgcctt ctcctgcttt cagaaggccc agctgaagtc cgccaacacc 180

ggcaacaacg agcggatcat caacgtgagc atcaagaagc tgaagcggaa gcctccctcc 240

acaaacgccg gcaggaggca gaagcacagg ctgacctgcc ccagctgtga ctcctacgag 300

aagaagcccc ccaaggagtt cctggagagg ttcaagtccc tgctgcagaa gatgatccat 360

cagcacctgt cctccaggac ccacggctcc gaggactcca ttacatgccc ccctcccatg 420

agcgtggagc acgccgacat ctgggtgaag agctatagcc tctacagccg ggagaggtat 480

atctgtaaca gcggcttcaa gaggaaggcc ggcaccagca gcctcaccga gtgcgtgctg 540

aataaggcta ccaacgtggc tcactggaca acaccctctt taaagtgcat ccggggcggt 600

ggaggatccg gaggaggtgg ctccggcggc ggaggatctg atcccgccgg cctcttggac 660

ctgcggcagg gcatgtttgc gcagctggtg gcccaaaatg ttctgctgat cgatgggccc 720

ctgagctggt acagtgaccc aggcctggca ggcgtgtccc tgacgggggg cctgagctac 780

aaagaggaca cgaaggagct ggtggtggcc aaggctggag tctactatgt cttctttcaa 840

ctagagctgc ggcgcgtggt ggccggcgag ggctcaggct ccgtttcact tgcgctgcac 900

ctgcagccac tgcgctctgc tgctggggcc gccgccctgg ctttgaccgt ggacctgcca 960

cccgcctcct ccgaggctcg gaactcggcc ttcggtttcc agggccgctt gctgcacctg 1020

agtgccggcc agcgcctggg cgtccatctt cacactgagg ccagggcacg ccatgcctgg 1080

cagcttaccc agggcgccac agtcttggga ctcttccggg tgacccccga aatc 1134

<210> 274
<211> 396
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 274

```
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                  10                  15


Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
            20                  25                  30


Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
        35                  40                  45


Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
    50                  55                  60


Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65                  70                  75                  80


Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
                85                  90                  95


Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
            100                 105                 110


Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
        115                 120                 125


Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
    130                 135                 140


Thr His Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val
145                 150                 155                 160


Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu
                165                 170                 175


Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser
            180                 185                 190
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Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr
        195                 200                 205

Thr Pro Ser Leu Lys Cys Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Asp Pro Ala Gly Leu Leu Asp Leu Arg
225                 230                 235                 240

Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu Ile Asp
                245                 250                 255

Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser Leu
            260                 265                 270

Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val Ala
        275                 280                 285

Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg Val
    290                 295                 300

Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu Gln
305                 310                 315                 320

Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr Val Asp
                325                 330                 335

Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe Gln
            340                 345                 350

Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His Leu
        355                 360                 365

His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly Ala
    370                 375                 380
```

Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile
385 390 395

<210> 275
<211> 1188
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
7t15-21CD137 sequence"

<400> 275
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc 60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac 120

tacgtgaacg acctggtgcc cgagtttctg cctgccccg aggacgtgga gaccaactgc 180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac 240

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tccattacat gccccctcc catgagcgtg 480

gagcacgccg acatctgggt gaagagctat agcctctaca gccgggagag gtatatctgt 540

aacagcggct tcaagaggaa ggccggcacc agcagcctca ccgagtgcgt gctgaataag 600

gctaccaacg tggctcactg gacaacaccc tctttaaagt gcatccgggg cggtggagga 660

tccggaggag gtggctccgg cggcggagga tctgatcccg ccggcctctt ggacctgcgg 720

cagggcatgt ttgcgcagct ggtggcccaa aatgttctgc tgatcgatgg gcccctgagc 780

tggtacagtg acccaggcct ggcaggcgtg tccctgacgg ggggcctgag ctacaaagag 840

gacacgaagg agctggtggt ggccaaggct ggagtctact atgtcttctt tcaactagag 900

ctgcggcgcg tggtggccgg cgagggctca ggctccgttt cacttgcgct gcacctgcag 960

ccactgcgct ctgctgctgg ggccgccgcc ctggctttga ccgtggacct gccacccgcc 1020

tcctccgagg ctcggaactc ggccttcggt ttccagggcc gcttgctgca cctgagtgcc 1080

ggccagcgcc tgggcgtcca tcttcacact gaggccaggg cacgccatgc ctggcagctt 1140

acccagggcg ccacagtctt gggactcttc cgggtgaccc ccgaaatc 1188

<210> 276

<400> 276
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<210> 277

<400> 277
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<210> 300
<211> 485
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs21 sequence"

<400> 300
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
20 25 30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
35 40 45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
50 55 60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65 70 75 80

Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
85 90 95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
100 105 110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
115 120 125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145 150 155 160

```
Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220

Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
            260                 265                 270

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Ile
        275                 280                 285

Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys
    290                 295                 300

Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe
305                 310                 315                 320

Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys
                325                 330                 335

Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
            340                 345                 350

Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile Asp Ile
```

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        355                 360                 365

Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu Phe Leu
    370                 375                 380

Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala Phe Ser
385                 390                 395                 400

Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn Asn Glu
                405                 410                 415

Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro Pro Ser
            420                 425                 430

Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro Ser Cys
        435                 440                 445

Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg Phe Lys
    450                 455                 460

Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg Thr His
465                 470                 475                 480

Gly Ser Glu Asp Ser
                485
```

<210> 301
<211> 1455
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs21 sequence"

<400> 301
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcagcatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgctcc 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga cattacatgc ccccctccca tgagcgtgga gcacgccgac 900

atctgggtga agagctatag cctctacagc cgggagaggt atatctgtaa cagcggcttc 960

aagaggaagg ccggcaccag cagcctcacc gagtgcgtgc tgaataaggc taccaacgtg 1020

gctcactgga caacaccctc tttaaagtgc atccggcagg gccaggacag gcacatgatc 1080

cggatgaggc agctcatcga catcgtcgac cagctgaaga actacgtgaa cgacctggtg 1140

cccgagtttc tgcctgcccc cgaggacgtg gagaccaact gcgagtggtc cgccttctcc 1200

tgctttcaga aggcccagct gaagtccgcc aacaccggca acaacgagcg gatcatcaac 1260

gtgagcatca agaagctgaa gcggaagcct ccctccacaa acgccggcag gaggcagaag 1320

cacaggctga cctgccccag ctgtgactcc tacgagaaga agccccccaa ggagttcctg 1380

gagaggttca agtccctgct gcagaagatg atccatcagc acctgtcctc caggacccac 1440

ggctccgagg actcc 1455

<210> 302
<211> 503
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs21 sequence"

<400> 302
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile
20 25 30

Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe
35 40 45

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
50 55 60

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
65 70 75 80

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
85 90 95

His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala
100 105 110

Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
115 120 125

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
130 135 140

Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys
165 170 175

```
Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
            180                 185                 190

Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
        195                 200                 205

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
    210                 215                 220

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
225                 230                 235                 240

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp
                245                 250                 255

Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys
            260                 265                 270

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
        275                 280                 285

Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro
    290                 295                 300

Asp Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp
305                 310                 315                 320

Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser
                325                 330                 335

Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu
            340                 345                 350

Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys
        355                 360                 365
```

```
Ile Arg Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
    370                 375                 380

Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
385                 390                 395                 400

Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
                405                 410                 415

Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
            420                 425                 430

Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
        435                 440                 445

Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
    450                 455                 460

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
465                 470                 475                 480

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
                485                 490                 495

Thr His Gly Ser Glu Asp Ser
            500
```

<210> 303
<211> 1509
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs21 sequence"

<400> 303
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc 60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg 120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag 180

tcctgcatgt ccaactgcag catcacctcc atctgcgaga agccccaaga agtgtgcgtg 240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag 300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag 360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac 420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga 480

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat 540

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg ctccatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacattac atgccccct cccatgagcg tggagcacgc cgacatctgg 960

gtgaagagct atagcctcta cagccgggag aggtatatct gtaacagcgg cttcaagagg 1020

aaggccggca ccagcagcct caccgagtgc gtgctgaata aggctaccaa cgtggctcac 1080

tggacaacac cctctttaaa gtgcatccgg cagggccagg acaggcacat gatccggatg 1140

aggcagctca tcgacatcgt cgaccagctg aagaactacg tgaacgacct ggtgcccgag 1200

tttctgcctg cccccgagga cgtggagacc aactgcgagt ggtccgcctt ctcctgcttt 1260

cagaaggccc agctgaagtc cgccaacacc ggcaacaacg agcggatcat caacgtgagc 1320

atcaagaagc tgaagcggaa gcctccctcc acaaacgccg gcaggaggca gaagcacagg 1380

ctgacctgcc ccagctgtga ctcctacgag aagaagcccc ccaaggagtt cctggagagg 1440

ttcaagtccc tgctgcagaa gatgatccat cagcacctgt cctccaggac ccacggctcc 1500

gaggactcc 1509

<210> 304

<400> 304
000

<210> 305

<400> 305
000

<210> 306

<400> 306
000

<210> 307

<400> 307
000

<210> 308
<211> 592
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs16 sequence"

<400> 308
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1   5   10   15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
            20   25   30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
        35   40   45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
    50   55   60

```
Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65                  70                  75                  80

Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145                 150                 155                 160

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220

Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
```

```
            260                 265                 270

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Ile
        275                 280                 285

Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys
    290                 295                 300

Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe
305                 310                 315                 320

Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys
                325                 330                 335

Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
            340                 345                 350

Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly Gln Thr
        355                 360                 365

Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala Ser
    370                 375                 380

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr Gly
385                 390                 395                 400

Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser
                405                 410                 415

Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp
            420                 425                 430

Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val
        435                 440                 445

Val Phe Gly Gly Gly Thr Lys Leu Thr Val Gly His Gly Gly Gly Gly
    450                 455                 460
```

```
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val
465                 470                 475                 480


Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly Ser Leu Arg Leu Ser
                485                 490                 495


Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Gly Met Ser Trp Val
            500                 505                 510


Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp
        515                 520                 525


Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
    530                 535                 540


Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser
545                 550                 555                 560


Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Arg Ser
                565                 570                 575


Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Arg
            580                 585                 590


<210> 309
<211> 1776
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      TGFRt15-TGFRs16 sequence"

<400> 309
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc       60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat      120

cagaagtcct gcatgtccaa ctgcagcatc acctccatct gcgagaagcc ccaagaagtg      180
```

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgctcc 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga cattacatgc ccccctccca tgagcgtgga gcacgccgac 900

atctgggtga agagctatag cctctacagc cgggagaggt atatctgtaa cagcggcttc 960

aagaggaagg ccggcaccag cagcctcacc gagtgcgtgc tgaataaggc taccaacgtg 1020

gctcactgga caacaccctc tttaaagtgc atccggtccg agctgaccca ggaccctgct 1080

gtgtccgtgg ctctgggcca gaccgtgagg atcacctgcc agggcgactc cctgaggtcc 1140

tactacgcct cctggtacca gcagaagccc ggccaggctc ctgtgctggt gatctacggc 1200

aagaacaaca ggccctccgg catccctgac aggttctccg gatcctcctc cggcaacacc 1260

gcctccctga ccatcacagg cgctcaggcc gaggacgagg ctgactacta ctgcaactcc 1320

agggactcct ccggcaacca tgtggtgttc ggcggcggca ccaagctgac cgtgggccat 1380

ggcggcggcg gctccggagg cggcggcagc ggcggaggag gatccgaggt gcagctggtg 1440

gagtccggag gaggagtggt gaggcctgga ggctccctga ggctgagctg tgctgcctcc 1500

ggcttcacct tcgacgacta cggcatgtcc tgggtgaggc aggctcctgg aaagggcctg 1560

gagtgggtgt ccggcatcaa ctggaacggc ggatccaccg gctacgccga ttccgtgaag 1620

ggcaggttca ccatcagcag ggacaacgcc aagaactccc tgtacctgca gatgaactcc 1680

ctgagggccg aggacaccgc cgtgtactac tgcgccaggg gcaggtccct gctgttcgac 1740

tactggggac agggcaccct ggtgaccgtg tccagg 1776

<210> 310
<211> 610
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs16 sequence"

<400> 310
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile
20 25 30

Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe
35 40 45

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
50 55 60

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
65 70 75 80

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
85 90 95

His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala
100 105 110

Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
115 120 125

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
130 135 140

```
Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys
                165                 170                 175

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
            180                 185                 190

Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
        195                 200                 205

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
    210                 215                 220

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
225                 230                 235                 240

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp
                245                 250                 255

Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys
            260                 265                 270

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
        275                 280                 285

Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro
    290                 295                 300

Asp Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp
305                 310                 315                 320

Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser
                325                 330                 335
```

```
Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu
            340                 345                 350

Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys
        355                 360                 365

Ile Arg Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala Leu Gly
    370                 375                 380

Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr
385                 390                 395                 400

Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile
                405                 410                 415

Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly
            420                 425                 430

Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala
        435                 440                 445

Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn
    450                 455                 460

His Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Gly His Gly Gly
465                 470                 475                 480

Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln
                485                 490                 495

Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly Ser Leu Arg
            500                 505                 510

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Gly Met Ser
        515                 520                 525

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gly Ile
    530                 535                 540
```

```
Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys Gly Arg
545                 550                 555                 560


Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met
                565                 570                 575


Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly
            580                 585                 590


Arg Ser Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val
        595                 600                 605


Ser Arg
    610
```

<210> 311
<211> 1830
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs16 sequence"

<400> 311

```
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc       60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg      120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag      180

tcctgcatgt ccaactgcag catcacctcc atctgcgaga agccccaaga agtgtgcgtg      240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag      300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag      360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac      420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga      480

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat      540
```

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg ctccatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacattac atgccccccc cccatgagcg tggagcacgc cgacatctgg 960

gtgaagagct atagcctcta cagccgggag aggtatatct gtaacagcgg cttcaagagg 1020

aaggccggca ccagcagcct caccgagtgc gtgctgaata aggctaccaa cgtggctcac 1080

tggacaacac cctctttaaa gtgcatccgg tccgagctga cccaggaccc tgctgtgtcc 1140

gtggctctgg gccagaccgt gaggatcacc tgccagggcg actccctgag gtcctactac 1200

gcctcctggt accagcagaa gcccggccag gctcctgtgc tggtgatcta cggcaagaac 1260

aacaggccct ccggcatccc tgacaggttc tccggatcct cctccggcaa caccgcctcc 1320

ctgaccatca caggcgctca ggccgaggac gaggctgact actactgcaa ctccagggac 1380

tcctccggca accatgtggt gttcggcggc ggcaccaagc tgaccgtggg ccatggcggc 1440

ggcggctccg gaggcggcgg cagcggcgga ggaggatccg aggtgcagct ggtggagtcc 1500

ggaggaggag tggtgaggcc tggaggctcc ctgaggctga gctgtgctgc ctccggcttc 1560

accttcgacg actacggcat gtcctgggtg aggcaggctc ctggaaaggg cctggagtgg 1620

gtgtccggca tcaactggaa cggcggatcc accggctacg ccgattccgt gaagggcagg 1680

ttcaccatca gcagggacaa cgccaagaac tccctgtacc tgcagatgaa ctccctgagg 1740

gccgaggaca ccgccgtgta ctactgcgcc aggggcaggt ccctgctgtt cgactactgg 1800

ggacagggca ccctggtgac cgtgtccagg 1830

<210> 312

<400> 312
000

<210> 313

<400> 313
000

<210> 314

<400> 314
000

<210> 315

<400> 315
000

<210> 316
<211> 551
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs137 sequence"

<400> 316
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1   5   10   15

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
            20   25   30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
        35   40   45

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
    50   55   60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65   70   75   80

```
Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
                85                  90                  95

Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
            100                 105                 110

Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
        115                 120                 125

Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly Gly Gly
    130                 135                 140

Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys Ser Val
145                 150                 155                 160

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
                165                 170                 175

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
            180                 185                 190

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
        195                 200                 205

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
    210                 215                 220

Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
225                 230                 235                 240

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
                245                 250                 255

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
            260                 265                 270

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Ile
```

```
        275                 280                 285

Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys
    290                 295                 300

Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe
305                 310                 315                 320

Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys
                325                 330                 335

Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
            340                 345                 350

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Arg
        355                 360                 365

Glu Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu Asp Leu
    370                 375                 380

Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu Leu Ile
385                 390                 395                 400

Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly Val Ser
                405                 410                 415

Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu Val Val
            420                 425                 430

Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu Arg Arg
        435                 440                 445

Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu His Leu
    450                 455                 460

Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu Thr Val
465                 470                 475                 480
```

Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe Gly Phe
485 490 495

Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly Val His
500 505 510

Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr Gln Gly
515 520 525

Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro Ala Gly
530 535 540

Leu Pro Ser Pro Arg Ser Glu
545 550

<210> 317
<211> 1653
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic TGFRt15-TGFRs137 sequence"

<400> 317
atccccccc atgtgcaaaa gagcgtgaac aacgatatga tcgtgaccga caacaacggc 60

gccgtgaagt ttccccagct ctgcaagttc tgcgatgtca ggttcagcac ctgcgataat 120

cagaagtcct gcatgtccaa ctgcagcatc acctccatct gcgagaagcc ccaagaagtg 180

tgcgtggccg tgtggcggaa aaatgacgag aacatcaccc tggagaccgt gtgtcacgac 240

cccaagctcc cttatcacga cttcattctg gaggacgctg cctcccccaa atgcatcatg 300

aaggagaaga agaagcccgg agagaccttc tttatgtgtt cctgtagcag cgacgagtgt 360

aacgacaaca tcatcttcag cgaagagtac aacaccagca accctgatgg aggtggcgga 420

tccggaggtg gaggttctgg tggaggtggg agtattcctc cccacgtgca gaagagcgtg 480

aataatgaca tgatcgtgac cgataacaat ggcgccgtga aatttcccca gctgtgcaaa 540

ttctgcgatg tgaggttttc cacctgcgac aaccagaagt cctgtatgag caactgctcc 600

atcacctcca tctgtgagaa gcctcaggag gtgtgcgtgg ctgtctggcg gaagaatgac 660

gagaatatca ccctggaaac cgtctgccac gatcccaagc tgccctacca cgatttcatc 720

ctggaagacg ccgccagccc taagtgcatc atgaaagaga aaaagaagcc tggcgagacc 780

tttttcatgt gctcctgcag cagcgacgaa tgcaacgaca atatcatctt tagcgaggaa 840

tacaatacca gcaaccccga cattacatgc ccccctccca tgagcgtgga gcacgccgac 900

atctgggtga agagctatag cctctacagc cgggagaggt atatctgtaa cagcggcttc 960

aagaggaagg ccggcaccag cagcctcacc gagtgcgtgc tgaataaggc taccaacgtg 1020

gctcactgga caacaccctc tttaaagtgc atccggggcg gtggaggatc cggaggaggt 1080

ggctccggcg gcggaggatc tcgcgagggt cccgagcttt cgcccgacga tcccgccggc 1140

ctcttggacc tgcggcaggg catgtttgcg cagctggtgg cccaaaatgt tctgctgatc 1200

gatgggcccc tgagctggta cagtgaccca ggcctggcag gcgtgtccct gacgggggggc 1260

ctgagctaca aagaggacac gaaggagctg gtggtggcca aggctggagt ctactatgtc 1320

ttctttcaac tagagctgcg gcgcgtggtg gccggcgagg gctcaggctc cgtttcactt 1380

gcgctgcacc tgcagccact gcgctctgct gctggggccg ccgccctggc tttgaccgtg 1440

gacctgccac ccgcctcctc cgaggctcgg aactcggcct tcggtttcca gggccgcttg 1500

ctgcacctga gtgccggcca gcgcctgggc gtccatcttc acactgaggc cagggcacgc 1560

catgcctggc agcttaccca gggcgccaca gtcttgggac tcttccgggt gacccccgaa 1620

atcccagccg gactcccttc accgaggtcg gaa 1653

<210> 318
<211> 569
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs137 sequence"

<400> 318

```
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                  10                  15

Tyr Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile
            20                  25                  30

Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe
        35                  40                  45

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
    50                  55                  60

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
65                  70                  75                  80

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
                85                  90                  95

His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala
            100                 105                 110

Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
        115                 120                 125

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
    130                 135                 140

Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Gly Gly Gly Gly Ser Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ile Pro Pro His Val Gln Lys
                165                 170                 175

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
            180                 185                 190

Phe Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
        195                 200                 205
```

```
Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
    210                 215                 220

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
225                 230                 235                 240

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp
                245                 250                 255

Phe Ile Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys
            260                 265                 270

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
        275                 280                 285

Cys Asn Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro
    290                 295                 300

Asp Ile Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp
305                 310                 315                 320

Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser
                325                 330                 335

Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu
            340                 345                 350

Asn Lys Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys
        355                 360                 365

Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
    370                 375                 380

Ser Arg Glu Gly Pro Glu Leu Ser Pro Asp Asp Pro Ala Gly Leu Leu
385                 390                 395                 400
```

```
Asp Leu Arg Gln Gly Met Phe Ala Gln Leu Val Ala Gln Asn Val Leu
                405                 410                 415

Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser Asp Pro Gly Leu Ala Gly
            420                 425                 430

Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys Glu Asp Thr Lys Glu Leu
        435                 440                 445

Val Val Ala Lys Ala Gly Val Tyr Tyr Val Phe Phe Gln Leu Glu Leu
    450                 455                 460

Arg Arg Val Val Ala Gly Glu Gly Ser Gly Ser Val Ser Leu Ala Leu
465                 470                 475                 480

His Leu Gln Pro Leu Arg Ser Ala Ala Gly Ala Ala Ala Leu Ala Leu
                485                 490                 495

Thr Val Asp Leu Pro Pro Ala Ser Ser Glu Ala Arg Asn Ser Ala Phe
            500                 505                 510

Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly Gln Arg Leu Gly
        515                 520                 525

Val His Leu His Thr Glu Ala Arg Ala Arg His Ala Trp Gln Leu Thr
    530                 535                 540

Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr Pro Glu Ile Pro
545                 550                 555                 560

Ala Gly Leu Pro Ser Pro Arg Ser Glu
                565
```

<210> 319
<211> 1707
<212> DNA
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic
TGFRt15-TGFRs137 sequence"

<400> 319
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctccatcccc 60

ccccatgtgc aaaagagcgt gaacaacgat atgatcgtga ccgacaacaa cggcgccgtg 120

aagtttcccc agctctgcaa gttctgcgat gtcaggttca gcacctgcga taatcagaag 180

tcctgcatgt ccaactgcag catcacctcc atctgcgaga agccccaaga agtgtgcgtg 240

gccgtgtggc ggaaaaatga cgagaacatc accctggaga ccgtgtgtca cgaccccaag 300

ctcccttatc acgacttcat tctggaggac gctgcctccc ccaaatgcat catgaaggag 360

aagaagaagc ccggagagac cttctttatg tgttcctgta gcagcgacga gtgtaacgac 420

aacatcatct tcagcgaaga gtacaacacc agcaaccctg atggaggtgg cggatccgga 480

ggtggaggtt ctggtggagg tgggagtatt cctccccacg tgcagaagag cgtgaataat 540

gacatgatcg tgaccgataa caatggcgcc gtgaaatttc cccagctgtg caaattctgc 600

gatgtgaggt tttccacctg cgacaaccag aagtcctgta tgagcaactg ctccatcacc 660

tccatctgtg agaagcctca ggaggtgtgc gtggctgtct ggcggaagaa tgacgagaat 720

atcaccctgg aaaccgtctg ccacgatccc aagctgccct accacgattt catcctggaa 780

gacgccgcca gccctaagtg catcatgaaa gagaaaaaga agcctggcga gacctttttc 840

atgtgctcct gcagcagcga cgaatgcaac gacaatatca tctttagcga ggaatacaat 900

accagcaacc ccgacattac atgccccct cccatgagcg tggagcacgc cgacatctgg 960

gtgaagagct atagcctcta cagccgggag aggtatatct gtaacagcgg cttcaagagg 1020

aaggccggca ccagcagcct caccgagtgc gtgctgaata aggctaccaa cgtggctcac 1080

tggacaacac cctctttaaa gtgcatccgg ggcggtggag gatccggagg aggtggctcc 1140

ggcggcggag gatctcgcga gggtcccgag ctttcgcccg acgatcccgc cggcctcttg 1200

gacctgcggc agggcatgtt tgcgcagctg gtggcccaaa atgttctgct gatcgatggg 1260

cccctgagct ggtacagtga cccaggcctg gcaggcgtgt ccctgacggg gggcctgagc 1320

tacaaagagg acacgaagga gctggtggtg gccaaggctg gagtctacta tgtcttcttt 1380

caactagagc tgcggcgcgt ggtggccggc gagggctcag gctccgtttc acttgcgctg 1440

cacctgcagc cactgcgctc tgctgctggg gccgccgccc tggctttgac cgtggacctg 1500

ccacccgcct cctccgaggc tcggaactcg gccttcggtt tccagggccg cttgctgcac 1560

ctgagtgccg gccagcgcct gggcgtccat cttcacactg aggccagggc acgccatgcc 1620

tggcagctta cccagggcgc cacagtcttg ggactcttcc gggtgacccc cgaaatccca 1680

gccggactcc cttcaccgag gtcggaa 1707

<210> 320
<211> 720
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18s sequence"

<400> 320
atgaagtggg tcacatttat ctctttactg ttcctcttct ccagcgccta cagctacttc 60

ggcaaactgg aatccaagct gagcgtgatc cggaatttaa acgaccaagt tctgtttatc 120

gatcaaggta accggcctct gttcgaggac atgaccgact ccgattgccg ggacaatgcc 180

ccccggacca tcttcattat ctccatgtac aaggacagcc agccccgggg catggctgtg 240

acaattagcg tgaagtgtga gaaaatcagc actttatctt gtgagaacaa gatcatctcc 300

tttaaggaaa tgaacccccc cgataacatc aaggacacca agtccgatat catcttcttc 360

cagcggtccg tgcccggtca cgataacaag atgcagttcg aatcctcctc ctacgagggc 420

tacttttag cttgtgaaaa ggagagggat ttattcaagc tgatcctcaa gaaggaggac 480

gagctgggcg atcgttccat catgttcacc gtccaaaacg aggatattac atgccccct 540

cccatgagcg tggagcacgc cgacatctgg gtgaagagct atagcctcta cagccgggag 600

aggtatatct gtaacagcgg cttcaagagg aaggccggca ccagcagcct caccgagtgc 660

gtgctgaata aggctaccaa cgtggctcac tggacaacac cctctttaaa gtgcatccgg 720

<210> 321
<211> 2607
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
12t15 sequence"

<400> 321
atgaaatggg tgacctttat ttctttactg ttcctcttta gcagcgccta ctccatttgg 60

gaactgaaga aggacgtcta cgtggtcgaa ctggactggt atcccgatgc tcccggcgaa 120

atggtggtgc tcacttgtga cacccccgaa gaagacggca tcacttggac cctcgatcag 180

agcagcgagg tgctgggctc cggaaagacc ctcacaatcc aagttaagga gttcggagac 240

gctggccaat acacatgcca caagggaggc gaggtgctca gccattcctt attattatta 300

cacaagaagg aagacggaat ctggtccacc gacattttaa aagatcagaa ggagcccaag 360

aataagacct ttttaaggtg tgaggccaaa aactacagcg gtcgtttcac ttgttggtgg 420

ctgaccacca tttccaccga tttaaccttc tccgtgaaaa gcagccgggg aagctccgac 480

cctcaaggtg tgacatgtgg agccgctacc ctcagcgctg agagggttcg tggcgataac 540

aaggaatacg agtacagcgt ggagtgccaa gaagatagcg cttgtcccgc tgccgaagaa 600

tctttaccca ttgaggtgat ggtggacgcc gtgcacaaac tcaagtacga gaactacacc 660

tcctccttct ttatccggga catcattaag cccgatcctc ctaagaattt acagctgaag 720

cctctcaaaa atagccggca agttgaggtc tcttgggaat atcccgacac ttggagcaca 780

ccccacagct acttctcttt aacctttttgt gtgcaagttc aaggtaaaag caagcgggag 840

aagaaagacc gggtgtttac cgacaaaacc agcgccaccg tcatctgtcg gaagaacgcc 900

tccatcagcg tgagggctca agatcgttat tactccagca gctggtccga gtgggccagc 960

gtgccttgtt ccggcggtgg aggatccgga ggaggtggct ccggcggcgg aggatctcgt 1020

aacctccccg tggctacccc cgatcccgga atgttccctt gtttacacca cagccagaat 1080

ttactgaggg ccgtgagcaa catgctgcag aaagctaggc agactttaga attttaccct 1140

tgcaccagcg aggagatcga ccatgaagat atcaccaagg acaagacatc caccgtggag 1200

gcttgtttac ctctggagct gacaaagaac gagtcttgtc tcaactctcg tgaaaccagc 1260

ttcatcacaa atggctcttg tttagcttcc cggaagacct cctttatgat ggctttatgc 1320

ctcagctcca tctacgagga tttaaagatg taccaagtgg agttcaagac catgaacgcc 1380

aagctgctca tggaccctaa acggcagatc tttttagacc agaacatgct ggctgtgatt 1440

gatgagctga tgcaagcttt aaacttcaac tccgagaccg tccctcagaa gtcctccctc 1500

gaggagcccg atttttacaa gacaaagatc aaactgtgca ttttactcca cgcctttagg 1560

atccgggccg tgaccattga ccgggtcatg agctatttaa acgccagcag cggcacaacc 1620

aacacagtcg ctgcctataa cctcacttgg aagagcacca acttcaaaac catcctcgaa 1680

tgggaaccca aacccgttaa ccaagtttac accgtgcaga tcagcaccaa gtccggcgac 1740

tggaagtcca aatgtttcta taccaccgac accgagtgcg atctcaccga tgagatcgtg 1800

aaagatgtga aacagaccta cctcgcccgg gtgtttagct accccgccgg caatgtggag 1860

agcactggtt ccgctggcga gcctttatac gagaacagcc ccgaatttac cccttacctc 1920

gagaccaatt taggacagcc caccatccaa agctttgagc aagttggcac aaaggtgaat 1980

gtgacagtgg aggacgagcg gactttagtg cggcggaaca acacctttct cagcctccgg 2040

gatgtgttcg gcaaagattt aatctacaca ctgtattact ggaagtcctc ttcctccggc 2100

aagaagacag ctaaaaccaa cacaaacgag tttttaatcg acgtggataa aggcgaaaac 2160

tactgtttca gcgtgcaagc tgtgatcccc tcccggaccg tgaataggaa aagcaccgat 2220

agccccgttg agtgcatggg ccaagaaaag ggcgagttcc gggagaactg ggtgaacgtc 2280

atcagcgatt taaagaagat cgaagattta attcagtcca tgcatatcga cgccacttta 2340

tacacagaat ccgacgtgca ccctcttgt aaggtgaccg ccatgaaatg ttttttactg 2400

gagctgcaag ttatctcttt agagagcgga gacgctagca tccacgacac cgtggagaat 2460

ttaatcattt tagccaataa ctctttatcc agcaacggca acgtgacaga gtccggctgc 2520

aaggagtgcg aagagctgga ggagaagaac atcaaggagt ttctgcaatc ctttgtgcac 2580

attgtccaga tgttcatcaa tacctcc 2607

<210> 322
<211> 240
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
18s sequence"

<400> 322
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn
20 25 30

Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe
35 40 45

Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile
50 55 60

Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val
65 70 75 80

Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn
85 90 95

Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp
100 105 110

Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp
115 120 125

Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala
130 135 140

Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp
145 150 155 160

```
Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp Ile
                165                 170                 175


Thr Cys Pro Pro Pro Met Ser Val Glu His Ala Asp Ile Trp Val Lys
            180                 185                 190


Ser Tyr Ser Leu Tyr Ser Arg Glu Arg Tyr Ile Cys Asn Ser Gly Phe
        195                 200                 205


Lys Arg Lys Ala Gly Thr Ser Ser Leu Thr Glu Cys Val Leu Asn Lys
    210                 215                 220


Ala Thr Asn Val Ala His Trp Thr Thr Pro Ser Leu Lys Cys Ile Arg
225                 230                 235                 240


<210> 323
<211> 869
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      12t15 sequence"

<400> 323
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1               5                   10                  15


Tyr Ser Ile Trp Glu Leu Lys Lys Asp Val Tyr Val Val Glu Leu Asp
            20                  25                  30


Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu Thr Cys Asp Thr
        35                  40                  45


Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln Ser Ser Glu Val
    50                  55                  60


Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys Glu Phe Gly Asp
65                  70                  75                  80
```

```
Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val Leu Ser His Ser
                85                  90                  95

Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp Ser Thr Asp Ile
            100                 105                 110

Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe Leu Arg Cys Glu
        115                 120                 125

Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp Leu Thr Thr Ile
    130                 135                 140

Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg Gly Ser Ser Asp
145                 150                 155                 160

Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser Ala Glu Arg Val
                165                 170                 175

Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu Cys Gln Glu Asp
            180                 185                 190

Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile Glu Val Met Val
        195                 200                 205

Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr Ser Ser Phe Phe
    210                 215                 220

Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln Leu Lys
225                 230                 235                 240

Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp Glu Tyr Pro Asp
                245                 250                 255

Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr Phe Cys Val Gln
            260                 265                 270
```

```
Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg Val Phe Thr Asp
        275                 280                 285

Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala Ser Ile Ser Val
    290                 295                 300

Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser Glu Trp Ala Ser
305                 310                 315                 320

Val Pro Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
                325                 330                 335

Gly Gly Ser Arg Asn Leu Pro Val Ala Thr Pro Asp Pro Gly Met Phe
            340                 345                 350

Pro Cys Leu His His Ser Gln Asn Leu Leu Arg Ala Val Ser Asn Met
        355                 360                 365

Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu
    370                 375                 380

Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu
385                 390                 395                 400

Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser
                405                 410                 415

Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys
            420                 425                 430

Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu
        435                 440                 445

Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met
    450                 455                 460

Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn Met Leu Ala Val Ile
465                 470                 475                 480
```

```
Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser Glu Thr Val Pro Gln
                485                 490                 495

Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys Thr Lys Ile Lys Leu
            500                 505                 510

Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg
        515                 520                 525

Val Met Ser Tyr Leu Asn Ala Ser Ser Gly Thr Thr Asn Thr Val Ala
    530                 535                 540

Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Lys Thr Ile Leu Glu
545                 550                 555                 560

Trp Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr
                565                 570                 575

Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu
            580                 585                 590

Cys Asp Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu
        595                 600                 605

Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser
    610                 615                 620

Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu
625                 630                 635                 640

Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly
                645                 650                 655

Thr Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Arg Arg
            660                 665                 670
```

```
Asn Asn Thr Phe Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile
        675                 680                 685

Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala
    690                 695                 700

Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn
705                 710                 715                 720

Tyr Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg
                725                 730                 735

Lys Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu
            740                 745                 750

Phe Arg Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu
        755                 760                 765

Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser
    770                 775                 780

Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu
785                 790                 795                 800

Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp
                805                 810                 815

Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn
            820                 825                 830

Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu
        835                 840                 845

Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met
    850                 855                 860

Phe Ile Asn Thr Ser
865
```

<210> 324
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15 sequence"

<400> 324
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc 60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac 120

tacgtgaacg acctggtgcc cgagtttctg cctgcccccg aggacgtgga gaccaactgc 180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac 240

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tcctccggca ccaccaatac cgtggccgct 480

tataacctca catggaagag caccaacttc gcgacagctc tggaatggga acccaagccc 540

gtcaatcaag tttacaccgt gcagatctcc accaaatccg gagactggaa gagcaagtgc 600

ttctacacaa cagacaccga gtgtgcttta accgacgaaa tcgtcaagga cgtcaagcaa 660

acctatctgg ctcgggtctt ttcctacccc gctggcaatg tcgagtccac cggctccgct 720

ggcgagcctc tctacgagaa ttcccccgaa ttcacccctt atttagagac caatttaggc 780

cagcctacca tccagagctt cgagcaagtt ggcaccaagg tgaacgtcac cgtcgaggat 840

gaaaggactt tagtggcgcg gaataacaca gctttatccc tccgggatgt gttcggcaaa 900

gacctcatct acacactgta ctattggaag tccagctcct ccggcaaaaa gaccgctaag 960

accaacacca acgagttttt aattgacgtg gacaaaggcg agaactactg cttcagcgtg 1020

caagccgtga tcccttctcg taccgtcaac cggaagagca cagattcccc cgttgagtgc 1080

atgggccaag aaaagggcga gttccgggag aactgggtga acgtcatcag cgatttaaag 1140

aagatcgaag atttaattca gtccatgcat atcgacgcca ctttatacac agaatccgac 1200

gtgcacccct cttgtaaggt gaccgccatg aaatgttttt tactggagct gcaagttatc 1260

tctttagaga gcggagacgc tagcatccac gacaccgtgg agaatttaat cattttagcc 1320

aataactctt tatccagcaa cggcaacgtg acagagtccg gctgcaagga gtgcgaagag 1380

ctggaggaga agaacatcaa ggagtttctg caatcctttg tgcacattgt ccagatgttc 1440

atcaatacct cc 1452

<210> 325
<211> 484
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
21t15 sequence"

<400> 325
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
20 25 30

Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
35 40 45

Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
50 55 60

Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65 70 75 80

Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
85 90 95

Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro

```
            100                 105                 110

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
        115                 120                 125

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
    130                 135                 140

Thr His Gly Ser Glu Asp Ser Ser Gly Thr Thr Asn Thr Val Ala Ala
145                 150                 155                 160

Tyr Asn Leu Thr Trp Lys Ser Thr Asn Phe Ala Thr Ala Leu Glu Trp
                165                 170                 175

Glu Pro Lys Pro Val Asn Gln Val Tyr Thr Val Gln Ile Ser Thr Lys
            180                 185                 190

Ser Gly Asp Trp Lys Ser Lys Cys Phe Tyr Thr Thr Asp Thr Glu Cys
        195                 200                 205

Ala Leu Thr Asp Glu Ile Val Lys Asp Val Lys Gln Thr Tyr Leu Ala
    210                 215                 220

Arg Val Phe Ser Tyr Pro Ala Gly Asn Val Glu Ser Thr Gly Ser Ala
225                 230                 235                 240

Gly Glu Pro Leu Tyr Glu Asn Ser Pro Glu Phe Thr Pro Tyr Leu Glu
                245                 250                 255

Thr Asn Leu Gly Gln Pro Thr Ile Gln Ser Phe Glu Gln Val Gly Thr
            260                 265                 270

Lys Val Asn Val Thr Val Glu Asp Glu Arg Thr Leu Val Ala Arg Asn
        275                 280                 285

Asn Thr Ala Leu Ser Leu Arg Asp Val Phe Gly Lys Asp Leu Ile Tyr
    290                 295                 300
```

```
Thr Leu Tyr Tyr Trp Lys Ser Ser Ser Ser Gly Lys Lys Thr Ala Lys
305                 310                 315                 320

Thr Asn Thr Asn Glu Phe Leu Ile Asp Val Asp Lys Gly Glu Asn Tyr
                325                 330                 335

Cys Phe Ser Val Gln Ala Val Ile Pro Ser Arg Thr Val Asn Arg Lys
            340                 345                 350

Ser Thr Asp Ser Pro Val Glu Cys Met Gly Gln Glu Lys Gly Glu Phe
        355                 360                 365

Arg Glu Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp
    370                 375                 380

Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp
385                 390                 395                 400

Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu
                405                 410                 415

Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr
            420                 425                 430

Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly
        435                 440                 445

Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys
    450                 455                 460

Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe
465                 470                 475                 480

Ile Asn Thr Ser
```

<210> 326

<211> 2142
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
CD28tCD3 sequence"

<400> 326
atgaaatggg tcaccttcat ctctttactg tttttattta gcagcgccta cagcgtgcag 60

ctgcagcagt ccggacccga actggtcaag cccggtgcct ccgtgaaaat gtcttgtaag 120

gcttctggct acacctttac ctcctacgtc atccaatggg tgaagcagaa gcccggtcaa 180

ggtctcgagt ggatcggcag catcaatccc tacaacgatt acaccaagta taacgaaaag 240

tttaagggca aggccactct gacaagcgac aagagctcca ttaccgccta catggagttt 300

tcctctttaa cttctgagga ctccgcttta tactattgcg ctcgttgggg cgatggcaat 360

tattggggcc ggggaactac tttaacagtg agctccggcg gcggcggaag cggaggtgga 420

ggatctggcg gtggaggcag cgacatcgag atgacacagt cccccgctat catgagcgcc 480

tctttaggag aacgtgtgac catgacttgt acagcttcct ccagcgtgag cagctcctat 540

ttccactggt accagcagaa acccggctcc tcccctaaac tgtgtatcta ctccacaagc 600

aatttagcta gcggcgtgcc tcctcgtttt agcggctccg gcagcacctc ttactcttta 660

accattagct ctatggaggc cgaagatgcc gccacatact tttgccatca gtaccaccgg 720

tcccctacct ttggcggagg cacaaagctg gagaccaagc ggagcggcac caccaacaca 780

gtggccgcct acaatctgac ttggaaatcc accaacttca agaccatcct cgagtgggag 840

cccaagcccg ttaatcaagt ttataccgtg cagatttcca ccaagagcgg cgactggaaa 900

tccaagtgct tctataccac agacaccgag tgcgatctca ccgacgagat cgtcaaagac 960

gtgaagcaga catatttagc tagggtgttc tcctaccccg ctggaaacgt ggagagcacc 1020

ggatccgctg gagagccttt atacgagaac tcccccgaat tcacccccta tctggaaacc 1080

aatttaggcc agcccaccat ccagagcttc gaacaagttg gcacaaaggt gaacgtcacc 1140

gtcgaagatg agaggacttt agtgcggagg aacaatacat ttttatcctt acgtgacgtc 1200

ttcggcaagg atttaatcta cacactgtat tactggaagt ctagctcctc cggcaagaag 1260

accgccaaga ccaataccaa cgaattttta attgacgtgg acaagggcga gaactactgc 1320

ttctccgtgc aagctgtgat cccctcccgg acagtgaacc ggaagtccac cgactccccc 1380

gtggagtgca tgggccaaga gaagggagag tttcgtgagc agatcgtgct gacccagtcc 1440

cccgctatta tgagcgctag ccccggtgaa aaggtgacta tgacatgcag cgccagctct 1500

tccgtgagct acatgaactg gtatcagcag aagtccggca ccagccctaa aaggtggatc 1560

tacgacacca gcaagctggc cagcggcgtc cccgctcact ttcggggctc cggctccgga 1620

acaagctact ctctgaccat cagcggcatg gaagccgagg atgccgctac ctattactgt 1680

cagcagtgga gctccaaccc cttcaccttt ggatccggca ccaagctcga gattaatcgt 1740

ggaggcggag gtagcggagg aggcggatcc ggcggtggag gtagccaagt tcagctccag 1800

caaagcggcg ccgaactcgc tcggcccggc gcttccgtga agatgtcttg taaggcctcc 1860

ggctatacct tcacccggta cacaatgcac tgggtcaagc aacggcccgg tcaaggttta 1920

gagtggattg gctatatcaa cccctcccgg ggctatacca actacaacca gaagttcaag 1980

gacaaagcca ccctcaccac cgacaagtcc agcagcaccg cttacatgca gctgagctct 2040

ttaacatccg aggattccgc cgtgtactac tgcgctcggt actacgacga tcattactgc 2100

ctcgattact ggggccaagg taccacctta acagtctcct cc 2142

<210> 327
<211> 714
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
CD28tCD3 sequence"

<400> 327
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly
20 25 30

```
Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser
        35                  40                  45

Tyr Val Ile Gln Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp
    50                  55                  60

Ile Gly Ser Ile Asn Pro Tyr Asn Asp Tyr Thr Lys Tyr Asn Glu Lys
65                  70                  75                  80

Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ile Thr Ala
                85                  90                  95

Tyr Met Glu Phe Ser Ser Leu Thr Ser Glu Asp Ser Ala Leu Tyr Tyr
            100                 105                 110

Cys Ala Arg Trp Gly Asp Gly Asn Tyr Trp Gly Arg Gly Thr Thr Leu
        115                 120                 125

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
    130                 135                 140

Gly Gly Ser Asp Ile Glu Met Thr Gln Ser Pro Ala Ile Met Ser Ala
145                 150                 155                 160

Ser Leu Gly Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val
                165                 170                 175

Ser Ser Ser Tyr Phe His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro
            180                 185                 190

Lys Leu Cys Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Pro
        195                 200                 205

Arg Phe Ser Gly Ser Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser
    210                 215                 220
```

```
Met Glu Ala Glu Asp Ala Ala Thr Tyr Phe Cys His Gln Tyr His Arg
225                 230                 235                 240

Ser Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Thr Lys Arg Ser Gly
                245                 250                 255

Thr Thr Asn Thr Val Ala Ala Tyr Asn Leu Thr Trp Lys Ser Thr Asn
            260                 265                 270

Phe Lys Thr Ile Leu Glu Trp Glu Pro Lys Pro Val Asn Gln Val Tyr
        275                 280                 285

Thr Val Gln Ile Ser Thr Lys Ser Gly Asp Trp Lys Ser Lys Cys Phe
    290                 295                 300

Tyr Thr Thr Asp Thr Glu Cys Asp Leu Thr Asp Glu Ile Val Lys Asp
305                 310                 315                 320

Val Lys Gln Thr Tyr Leu Ala Arg Val Phe Ser Tyr Pro Ala Gly Asn
                325                 330                 335

Val Glu Ser Thr Gly Ser Ala Gly Glu Pro Leu Tyr Glu Asn Ser Pro
            340                 345                 350

Glu Phe Thr Pro Tyr Leu Glu Thr Asn Leu Gly Gln Pro Thr Ile Gln
        355                 360                 365

Ser Phe Glu Gln Val Gly Thr Lys Val Asn Val Thr Val Glu Asp Glu
    370                 375                 380

Arg Thr Leu Val Arg Arg Asn Asn Thr Phe Leu Ser Leu Arg Asp Val
385                 390                 395                 400

Phe Gly Lys Asp Leu Ile Tyr Thr Leu Tyr Tyr Trp Lys Ser Ser Ser
                405                 410                 415

Ser Gly Lys Lys Thr Ala Lys Thr Asn Thr Asn Glu Phe Leu Ile Asp
            420                 425                 430
```

```
Val Asp Lys Gly Glu Asn Tyr Cys Phe Ser Val Gln Ala Val Ile Pro
        435                 440                 445

Ser Arg Thr Val Asn Arg Lys Ser Thr Asp Ser Pro Val Glu Cys Met
    450                 455                 460

Gly Gln Glu Lys Gly Glu Phe Arg Glu Gln Ile Val Leu Thr Gln Ser
465                 470                 475                 480

Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys
                485                 490                 495

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser
            500                 505                 510

Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser
        515                 520                 525

Gly Val Pro Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser
    530                 535                 540

Leu Thr Ile Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys
545                 550                 555                 560

Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu
                565                 570                 575

Glu Ile Asn Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
            580                 585                 590

Gly Gly Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
        595                 600                 605

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
    610                 615                 620
```

```
Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
625                 630                 635                 640


Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
                645                 650                 655


Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
            660                 665                 670


Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
        675                 680                 685


Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
    690                 695                 700


Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
705                 710


<210> 328
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      Signal sequence"

<400> 328
Met Gly Val Lys Val Leu Phe Ala Leu Ile Cys Ile Ala Val Ala Glu
1               5                   10                  15


Ala



<210> 329
<211> 454
<212> PRT
<213> Homo sapiens

<220>
<221> source
```

<223> /note="Myc"

<400> 329
Met Asp Phe Phe Arg Val Val Glu Asn Gln Gln Pro Pro Ala Thr Met
1   5   10   15

Pro Leu Asn Val Ser Phe Thr Asn Arg Asn Tyr Asp Leu Asp Tyr Asp
            20   25   30

Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu Glu Glu Asn Phe Tyr Gln
        35   40   45

Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro Ala Pro Ser Glu Asp Ile
    50   55   60

Trp Lys Lys Phe Glu Leu Leu Pro Thr Pro Pro Leu Ser Pro Ser Arg
65   70   75   80

Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val Ala Val Thr Pro Phe Ser
                85   90   95

Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly Ser Phe Ser Thr Ala Asp
            100   105   110

Gln Leu Glu Met Val Thr Glu Leu Leu Gly Gly Asp Met Val Asn Gln
        115   120   125

Ser Phe Ile Cys Asp Pro Asp Asp Glu Thr Phe Ile Lys Asn Ile Ile
    130   135   140

Ile Gln Asp Cys Met Trp Ser Gly Phe Ser Ala Ala Ala Lys Leu Val
145   150   155   160

Ser Glu Lys Leu Ala Ser Tyr Gln Ala Ala Arg Lys Asp Ser Gly Ser
                165   170   175

Pro Asn Pro Ala Arg Gly His Ser Val Cys Ser Thr Ser Ser Leu Tyr
            180   185   190

```
Leu Gln Asp Leu Ser Ala Ala Ala Ser Glu Cys Ile Asp Pro Ser Val
        195                 200                 205

Val Phe Pro Tyr Pro Leu Asn Asp Ser Ser Ser Pro Lys Ser Cys Ala
    210                 215                 220

Ser Gln Asp Ser Ser Ala Phe Ser Pro Ser Ser Asp Ser Leu Leu Ser
225                 230                 235                 240

Ser Thr Glu Ser Ser Pro Gln Gly Ser Pro Glu Pro Leu Val Leu His
                245                 250                 255

Glu Glu Thr Pro Pro Thr Thr Ser Ser Asp Ser Glu Glu Glu Gln Glu
            260                 265                 270

Asp Glu Glu Glu Ile Asp Val Val Ser Val Glu Lys Arg Gln Ala Pro
        275                 280                 285

Gly Lys Arg Ser Glu Ser Gly Ser Pro Ser Ala Gly Gly His Ser Lys
    290                 295                 300

Pro Pro His Ser Pro Leu Val Leu Lys Arg Cys His Val Ser Thr His
305                 310                 315                 320

Gln His Asn Tyr Ala Ala Pro Pro Ser Thr Arg Lys Asp Tyr Pro Ala
                325                 330                 335

Ala Lys Arg Val Lys Leu Asp Ser Val Arg Val Leu Arg Gln Ile Ser
            340                 345                 350

Asn Asn Arg Lys Cys Thr Ser Pro Arg Ser Ser Asp Thr Glu Glu Asn
        355                 360                 365

Val Lys Arg Arg Thr His Asn Val Leu Glu Arg Gln Arg Arg Asn Glu
    370                 375                 380

Leu Lys Arg Ser Phe Phe Ala Leu Arg Asp Gln Ile Pro Glu Leu Glu
```

385 390 395 400

Asn Asn Glu Lys Ala Pro Lys Val Val Ile Leu Lys Lys Ala Thr Ala
405 410 415

Tyr Ile Leu Ser Val Gln Ala Glu Glu Gln Lys Leu Ile Ser Glu Glu
420 425 430

Asp Leu Leu Arg Lys Arg Arg Glu Gln Leu Lys His Lys Leu Glu Gln
435 440 445

Leu Arg Asn Ser Cys Ala
450

<210> 330
<211> 1365
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="Myc"

<400> 330
ctggattttt ttcgggtagt ggaaaaccag cagcctcccg cgacgatgcc cctcaacgtt 60

agcttcacca acaggaacta tgacctcgac tacgactcgg tgcagccgta tttctactgc 120

gacgaggagg agaacttcta ccagcagcag cagcagagcg agctgcagcc cccggcgccc 180

agcgaggata tctggaagaa attcgagctg ctgcccaccc cgcccctgtc ccctagccgc 240

cgctccgggc tctgctcgcc ctcctacgtt gcggtcacac ccttctccct tcggggagac 300

aacgacggcg gtggcgggag cttctccacg gccgaccagc tggagatggt gaccgagctg 360

ctgggaggag acatggtgaa ccagagtttc atctgcgacc cggacgacga gaccttcatc 420

aaaaacatca tcatccagga ctgtatgtgg agcggcttct cggccgccgc caagctcgtc 480

tcagagaagc tggcctccta ccaggctgcg cgcaaagaca gcggcagccc gaaccccgcc 540

cgcggccaca gcgtctgctc cacctccagc ttgtacctgc aggatctgag cgccgccgcc 600

tcagagtgca tcgacccctc ggtggtcttc ccctaccctc tcaacgacag cagctcgccc 660

aagtcctgcg cctcgcaaga ctccagcgcc ttctctccgt cctcggattc tctgctctcc 720

tcgacggagt cctccccgca gggcagcccc gagcccctgg tgctccatga ggagacaccg 780

cccaccacca gcagcgactc tgaggaggaa caagaagatg aggaagaaat cgatgttgtt 840

tctgtggaaa agaggcaggc tcctggcaaa aggtcagagt ctggatcacc ttctgctgga 900

ggccacagca aacctcctca cagcccactg gtcctcaaga ggtgccacgt ctccacacat 960

cagcacaact acgcagcgcc tccctccact cggaaggact atcctgctgc caagagggtc 1020

aagttggaca gtgtcagagt cctgagacag atcagcaaca accgaaaatg caccagcccc 1080

aggtcctcgg acaccgagga gaatgtcaag aggcgaacac acaacgtctt ggagcgccag 1140

aggaggaacg agctaaaacg gagctttttt gccctgcgtg accagatccc ggagttggaa 1200

aacaatgaaa aggcccccaa ggtagttatc cttaaaaaag ccacagcata catcctgtcc 1260

gtccaagcag aggagcaaaa gctcatttct gaagaggact tgttgcggaa acgacgagaa 1320

cagttgaaac acaaacttga acagctacgg aactcttgtg cgtaa 1365

<210> 331
<211> 1245
<212> DNA
<213> Homo sapiens

<220>
<221> source
<223> /note="21s137L"

<400> 331
atgaagtggg tgaccttcat cagcctgctg ttcctgttct ccagcgccta ctcccagggc 60

caggacaggc acatgatccg gatgaggcag ctcatcgaca tcgtcgacca gctgaagaac 120

tacgtgaacg acctggtgcc cgagtttctg cctgcccccg aggacgtgga gaccaactgc 180

gagtggtccg ccttctcctg ctttcagaag gcccagctga agtccgccaa caccggcaac 240

aacgagcgga tcatcaacgt gagcatcaag aagctgaagc ggaagcctcc ctccacaaac 300

gccggcagga ggcagaagca caggctgacc tgccccagct gtgactccta cgagaagaag 360

ccccccaagg agttcctgga gaggttcaag tccctgctgc agaagatgat ccatcagcac 420

ctgtcctcca ggacccacgg ctccgaggac tccattacat gccccctcc catgagcgtg 480

gagcacgccg acatctgggt gaagagctat agcctctaca gccgggagag gtatatctgt 540

aacagcggct tcaagaggaa ggccggcacc agcagcctca ccgagtgcgt gctgaataag 600

gctaccaacg tggctcactg gacaacaccc tctttaaagt gcatccgggg cggtggagga 660

tccggaggag gtggctccgg cggcggagga tctcgcgagg gtcccgagct ttcgcccgac 720

gatcccgccg gcctcttgga cctgcggcag ggcatgtttg cgcagctggt ggcccaaaat 780

gttctgctga tcgatgggcc cctgagctgg tacagtgacc caggcctggc aggcgtgtcc 840

ctgacggggg gcctgagcta caaagaggac acgaaggagc tggtggtggc caaggctgga 900

gtctactatg tcttctttca actagagctg cggcgcgtgg tggccggcga gggctcaggc 960

tccgtttcac ttgcgctgca cctgcagcca ctgcgctctg ctgctggggc cgccgccctg 1020

gctttgaccg tggacctgcc acccgcctcc tccgaggctc ggaactcggc cttcggtttc 1080

cagggccgct tgctgcacct gagtgccggc cagcgcctgg gcgtccatct tcacactgag 1140

gccagggcac gccatgcctg gcagcttacc cagggcgcca cagtcttggg actcttccgg 1200

gtgacccccg aaatcccagc cggactccct tcaccgaggt cggaa 1245

<210> 332
<211> 415
<212> PRT
<213> Homo sapiens

<220>
<221> source
<223> /note="21s137L"

<400> 332
Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Gln Gly Gln Asp Arg His Met Ile Arg Met Arg Gln Leu Ile
20 25 30

Asp Ile Val Asp Gln Leu Lys Asn Tyr Val Asn Asp Leu Val Pro Glu
35 40 45

```
Phe Leu Pro Ala Pro Glu Asp Val Glu Thr Asn Cys Glu Trp Ser Ala
    50                  55                  60

Phe Ser Cys Phe Gln Lys Ala Gln Leu Lys Ser Ala Asn Thr Gly Asn
65                  70                  75                  80

Asn Glu Arg Ile Ile Asn Val Ser Ile Lys Lys Leu Lys Arg Lys Pro
                85                  90                  95

Pro Ser Thr Asn Ala Gly Arg Arg Gln Lys His Arg Leu Thr Cys Pro
            100                 105                 110

Ser Cys Asp Ser Tyr Glu Lys Lys Pro Pro Lys Glu Phe Leu Glu Arg
        115                 120                 125

Phe Lys Ser Leu Leu Gln Lys Met Ile His Gln His Leu Ser Ser Arg
    130                 135                 140

Thr His Gly Ser Glu Asp Ser Ile Thr Cys Pro Pro Pro Met Ser Val
145                 150                 155                 160

Glu His Ala Asp Ile Trp Val Lys Ser Tyr Ser Leu Tyr Ser Arg Glu
                165                 170                 175

Arg Tyr Ile Cys Asn Ser Gly Phe Lys Arg Lys Ala Gly Thr Ser Ser
            180                 185                 190

Leu Thr Glu Cys Val Leu Asn Lys Ala Thr Asn Val Ala His Trp Thr
        195                 200                 205

Thr Pro Ser Leu Lys Cys Ile Arg Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Arg Glu Gly Pro Glu Leu Ser Pro Asp
225                 230                 235                 240

Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu
```

```
                245                 250                 255

Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser
            260                 265                 270

Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys
        275                 280                 285

Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val
    290                 295                 300

Phe Phe Gln Leu Glu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly
305                 310                 315                 320

Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly
                325                 330                 335

Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu
            340                 345                 350

Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser
        355                 360                 365

Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg
    370                 375                 380

His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg
385                 390                 395                 400

Val Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
                405                 410                 415


<210> 333
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<221> source
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<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 333
ggtgggtata atggg 15

<210> 334
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic primer"

<400> 334
attattttat tttaaaaaat ttgtg 25