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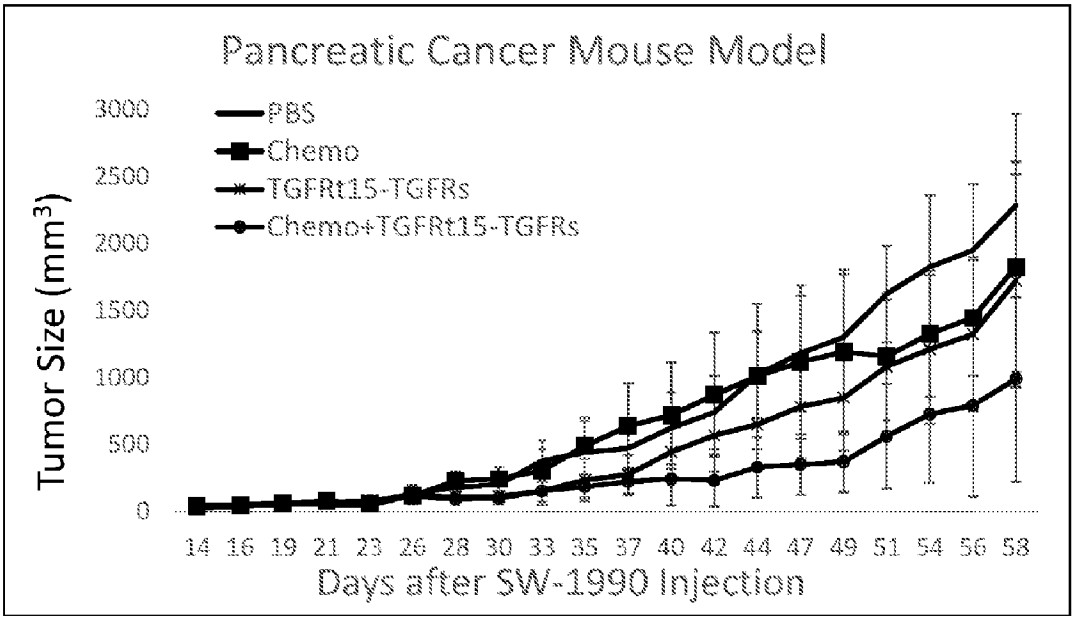


Figure 107

(57) Abrégé/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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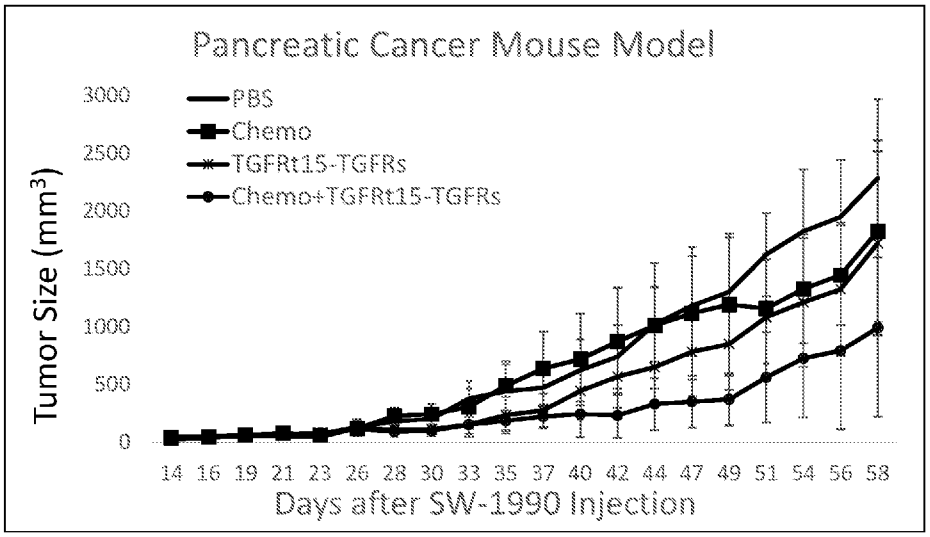


Figure 107

(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
cattttccaggtatgcctgtgtcaagatgaggtcacggacgattacatcg
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
agttcctgccaggcctttacacagccccttcctccccgttcccgccctca

caggtgagtgaccaccaagtgctaaatgacgccgaggttgccgccctcct
ggagaacttcagctcttcctatgactatggagaaaacgagagtgactcgt
gctgtacctccccgccctgcccacaggacttcagcctgaacttcgaccgg
gccttcctgccagccctctacagcctcctctttctgctggggctgctggg
caacggcgcggtggcagccgtgctgctgagccggcggacagccctgagca
gcaccgacaccttcctgctccacctagctgtagcagacacgctgctggtg
ctgacactgccgctctgggcagtggacgctgccgtccagtgggtctttgg
ctctggcctctgcaaagtggcaggtgccctcttcaacatcaacttctacg
caggagccctcctgctggcctgcatcagctttgaccgctacctgaacata
gttcatgccacccagctctaccgccgggggcccccggcccgcgtgaccct
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 llsllgnslv
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
attctgggcatccttcacagctgcctcaaccccctcatctacgccttcat
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
gcagagatggccctggctgggcccagtggctgctgccccagtggtctgg
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
CATCCCCCCCCATGTGCAAAAGAGCGTGAACAACGATATGATCGTGACCGAC

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

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 3
CONTENANT LES PAGES 1 À 273

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 3
CONTAINING PAGES 1 TO 273

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. 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).

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).

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 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.

6. 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.

7. The method of any one of claims 1-6, wherein the subject has been identified or diagnosed as having an aging-related disease or condition.

8. The method of claim 7, 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.

9. The method of claim 8, 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.

10. The method of claim 8, wherein the autoimmune disease is type-1 diabetes.

11. The method of claim 8, wherein the metabolic disease is selected from the group consisting of: obesity, a lipodystrophy, and type 2 diabetes mellitus.

12. The method of claim 8, wherein the neurodegenerative disease is selected from the group consisting of: Alzheimer's disease, Parkinson's disease, and dementia.

13. The method of claim 8, wherein the cardiovascular disease is selected from the group consisting of: coronary artery disease, atherosclerosis, and pulmonary arterial hypertension.

14. The method of claim 8, wherein the skin disease is selected from the group consisting of: wound healing, alopecia, wrinkles, senile lentigo, skin thinning, xeroderma pigmentosum, and dyskeratosis congenita.

15. The method of claim 8, wherein the progeria disease is selected from the group consisting of: progeria and Hutchinson-Gilford Progeria Syndrome.

16. The method of claim 8, wherein the fragility disease is selected from the group consisting of: frailty, responsiveness to vaccination, osteoporosis, and sarcopenia.

17. The method of any one of claims 1-6, 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.

18. The method of any one of claims 1-6, wherein the aging-related disease or condition is type 2 diabetes or atherosclerosis.

19. 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).

20. 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.

21. The method of claim 19 or 20, wherein the method provides for an improvement in the texture and/or appearance of skin of the subject over the period of time.

22. The method of claim 21, 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.

23. The method of claim 21 or 22, wherein the method results in an improvement in the coloration of skin of the subject over the period of time.

24. The method of any one of claims 21-23, wherein the method results in an improvement in the texture of skin of the subject over the period of time.

25. The method of any one of claims 20-24, wherein the method provides for an improvement in the texture and/or appearance of hair of the subject over the period of time.

26. The method of claim 25, wherein the method results in a decrease in the rate of formation of gray hair in the subject over the period of time.

27. The method of claim 25 or 26, wherein the method results in a decrease in the number of gray hairs of the subject over the period of time.

28. The method of any one of claims 25-27, wherein the method results in a decrease in the rate of hair loss in the subject over time.

29. The method of any one of claims 25-28, wherein the method results in an improvement in the texture of hair of the subject over the period of time.

30. The method of any one of claims 19-29, 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.

31. 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).

32. 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.

33. The method of any one of claims 1-32, 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.

34. The method of claim 33, wherein the resting NK cell is a genetically-engineered NK cell carrying a chimeric antigen receptor or recombinant T cell receptor.

35. The method of claim 33, 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.

36. The method of any one of claims 31-35, wherein the method results in a decrease in the mass of the subject over the period of time.

37. The method of any one of claims 31-36, wherein the method results in a decrease in the body mass index (BMI) of the subject over the period of time.

38. The method of any one of claims 31-35, wherein the method results in a decrease in the rate of progression from pre-diabetes to type 2 diabetes in the subject.

39. The method of any one of claims 31-35, wherein the method results in a decrease in fasting serum glucose level in the subject.

40. The method of any one of claims 31-35, wherein the method results in an increase in insulin sensitivity in the subject.

41. The method of any one of claims 31-35, wherein the method results in a decrease in the severity of atherosclerosis in the subject.

42. The method of any one of claims 1-41, 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.

43. The method of any one of claims 1-42, 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.

44. The method of any one of claims 1-41, 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.

45. The method of claim 44, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other.

46. The method of claim 44, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the first target-binding domain and the soluble tissue factor domain.

47. The method of any one of claims 44-46, wherein the soluble tissue factor domain and the second target-binding domain directly abut each other.

48. The method of any one of claims 44-46, wherein the single-chain chimeric polypeptide further comprises a linker sequence between the soluble tissue factor domain and the second target-binding domain.

49. The method of any one of claims 1-41, wherein at least one of the one or more NK cell activating agent(s) is 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.

50. The method of claim 49, wherein the first target-binding domain and the soluble tissue factor domain directly abut each other in the first chimeric polypeptide.

51. The method of claim 49, 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.

52. The method of any one of claims 49-51, 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.

53. The method of any one of claims 49-51, 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.

54. The method of any one of claims 49-53, 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.

55. The method of any one of claims 49-53, 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.

56. The method of any one of claims 1-41, 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.

57. The method of any one of claims 44-56, 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.

58. The method of any one of claims 44-56, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine protein.

59. The method of claim 58, 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.

60. The method of any one of claims 44-56, wherein one or both of the first target-binding domain and the second target-binding domain is a soluble interleukin or cytokine receptor.

61. The method of claim 60, 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.

62. The method of any one of claims 44-55, wherein the soluble tissue factor domain is a soluble human tissue factor domain that does not stimulate blood coagulation.

63. The method of any one of claims 43-55, wherein the soluble tissue factor domain comprises or consists of a sequence from a wildtype soluble human tissue factor.

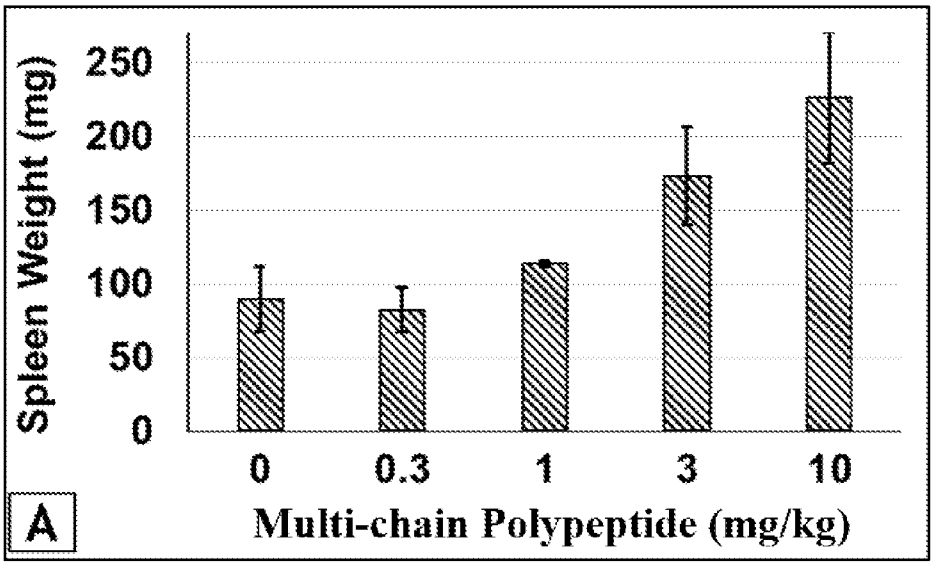


Figure 1A

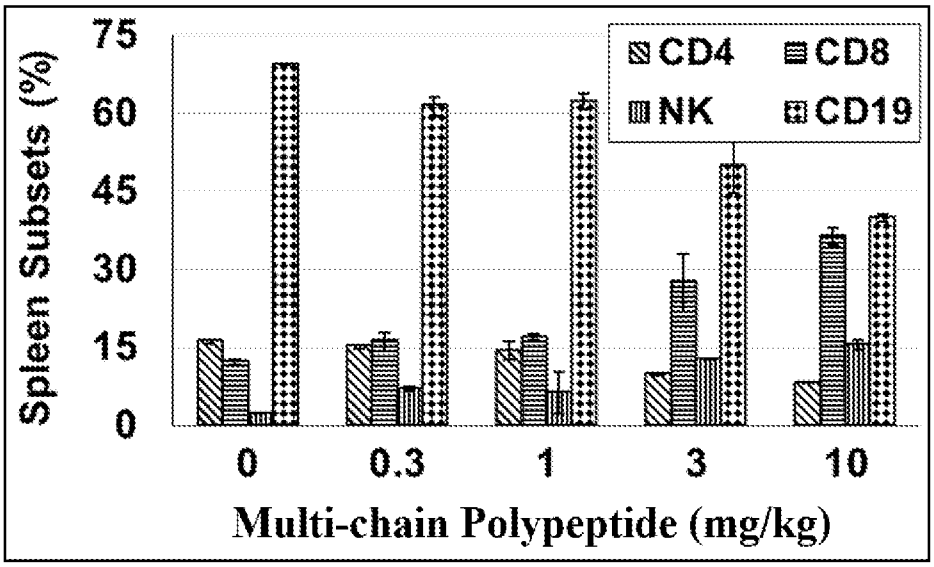


Figure 1B

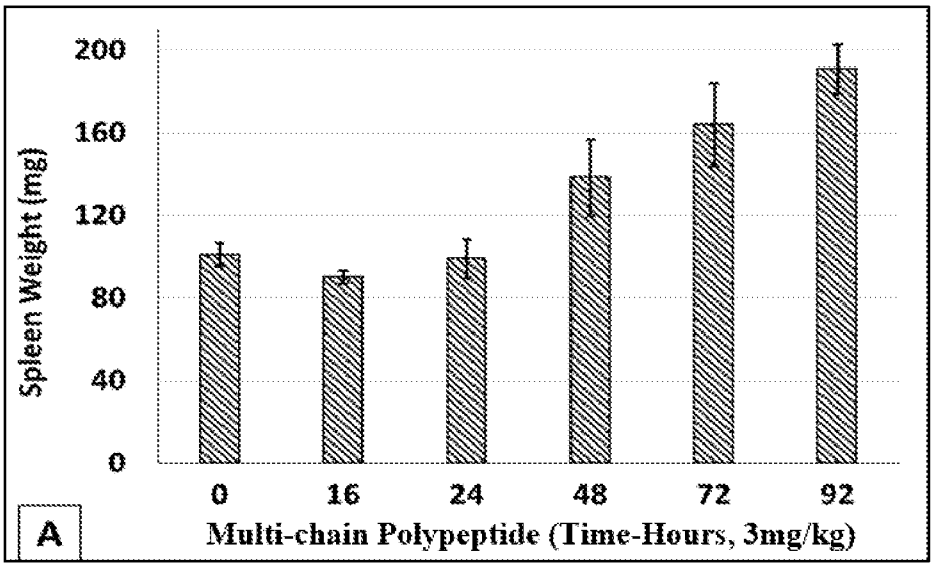


Figure 2A

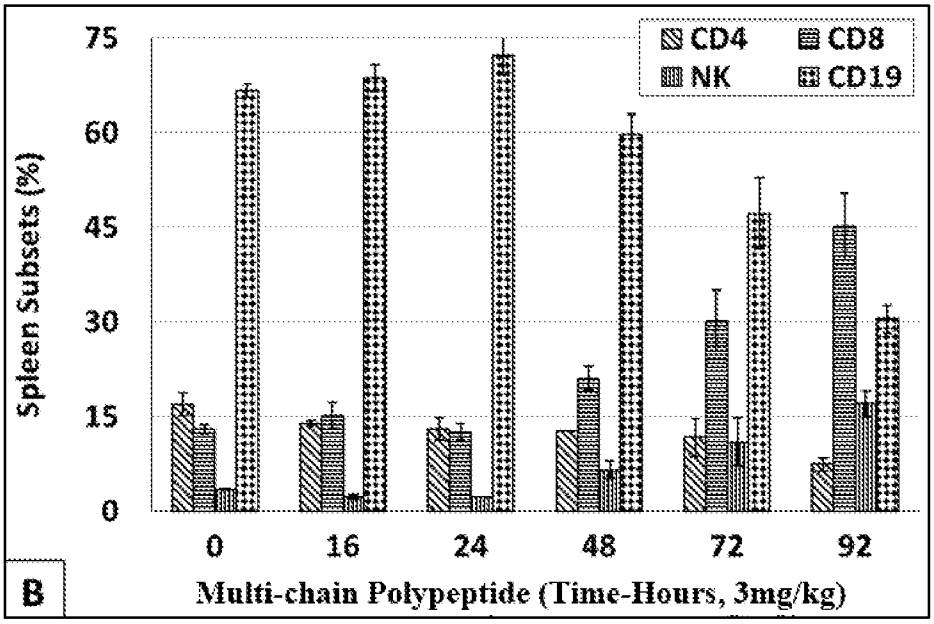


Figure 2B

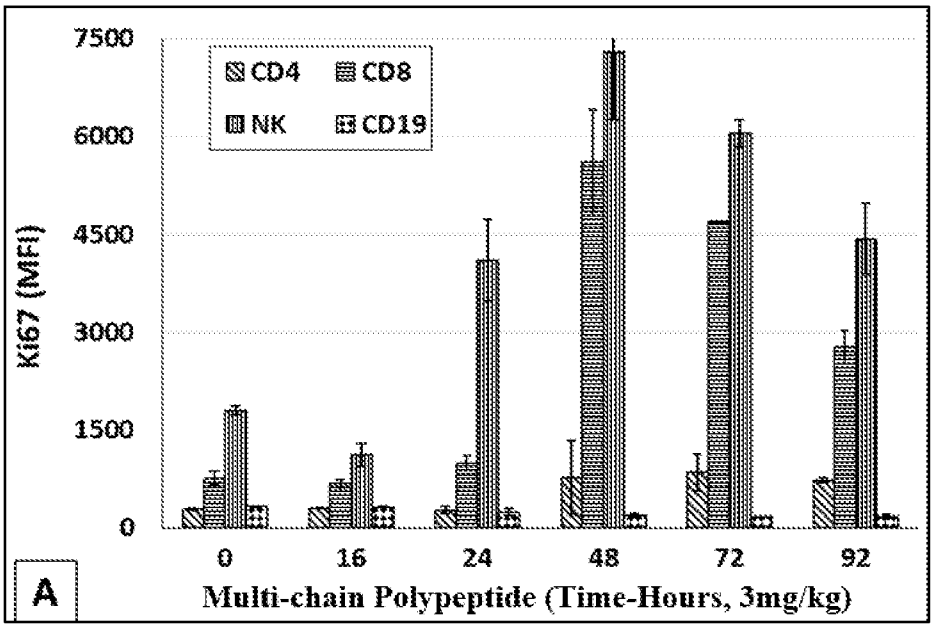


Figure 3A

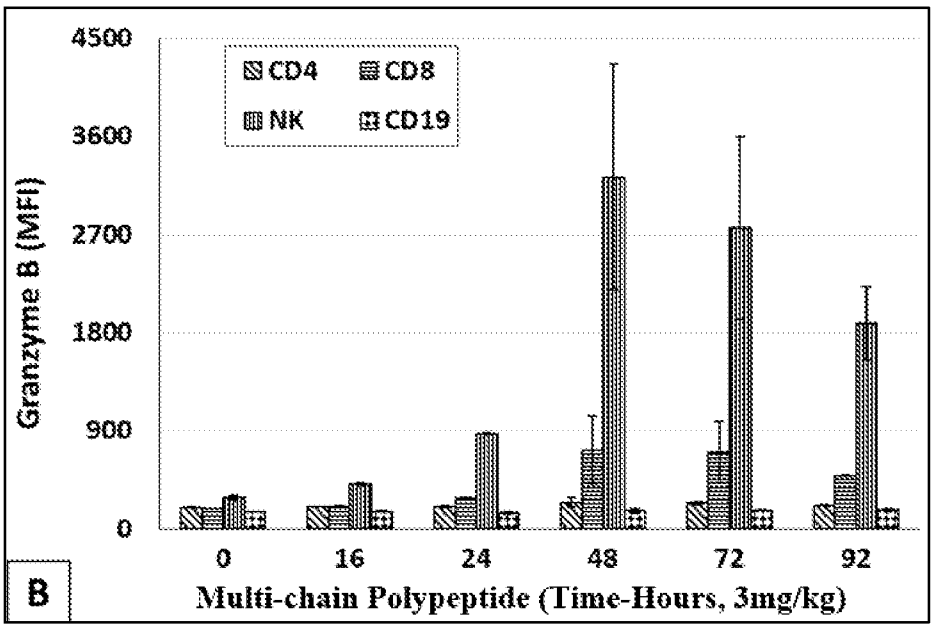


Figure 3B

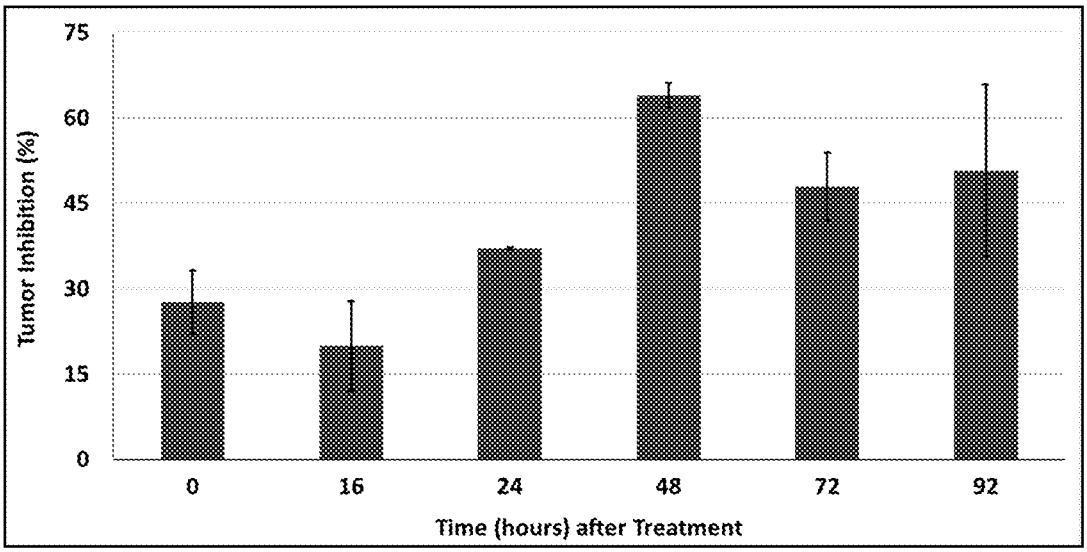
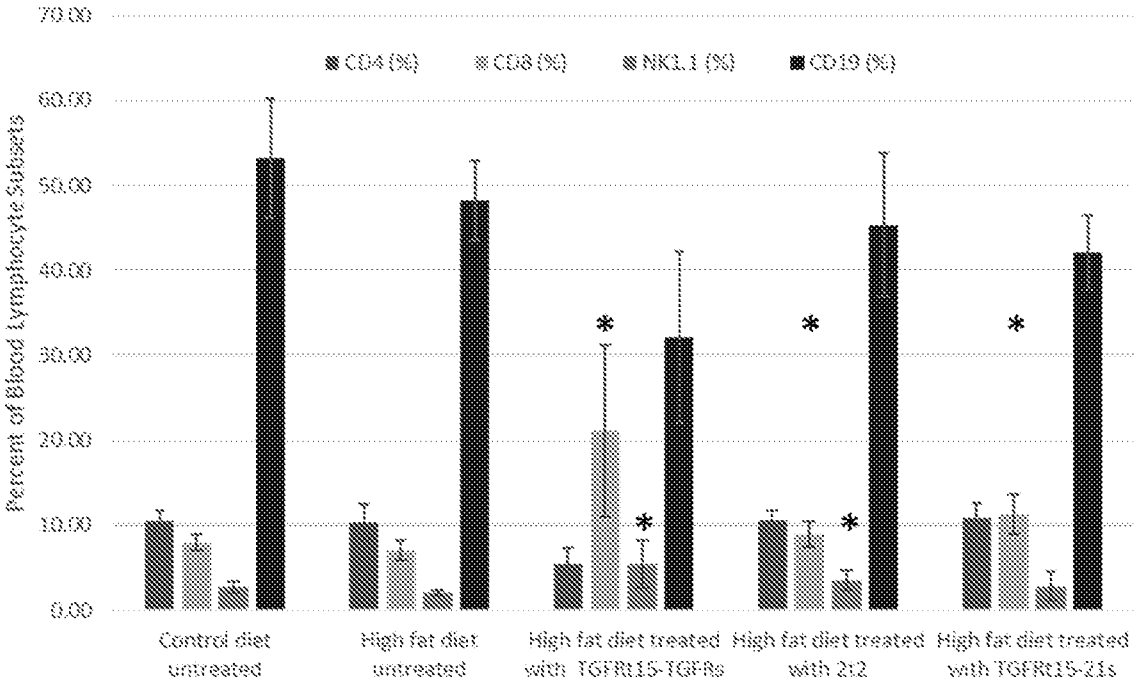
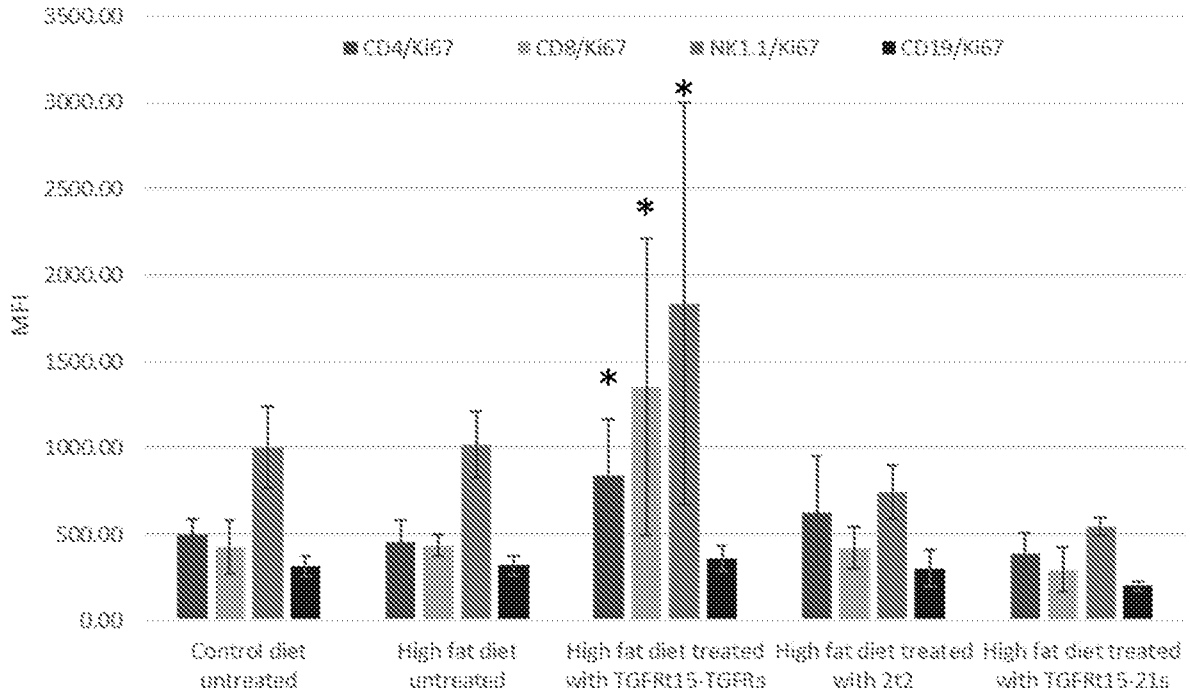

75
60
45
30
15
0
Tumor Inhibition (%)
0
16
24
48
72
92
Time (hours) after Treatment

Figure 4



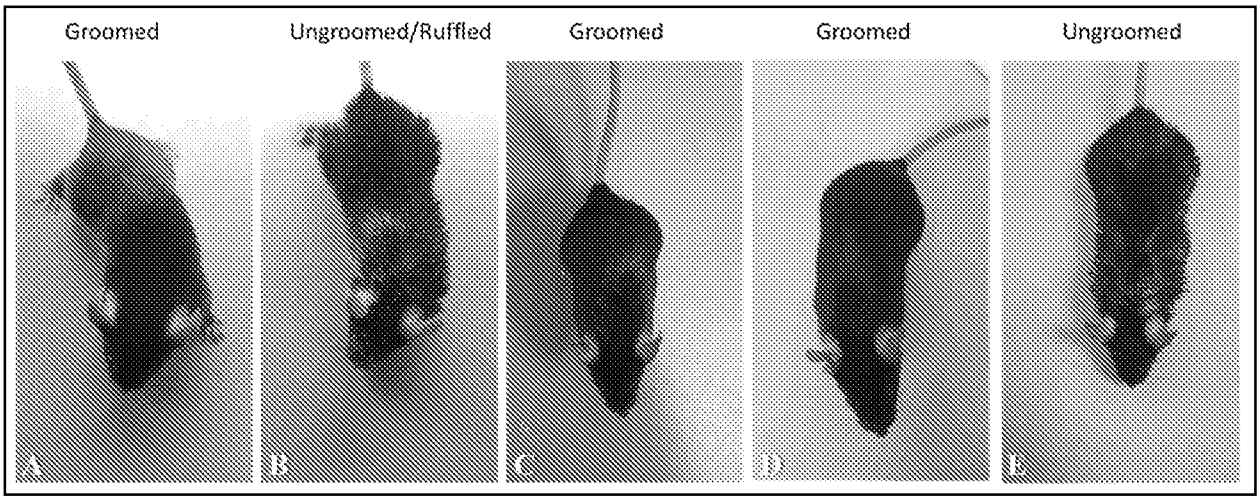
* Statistically significant from High Fat Diet untreated Group

Figure 5A

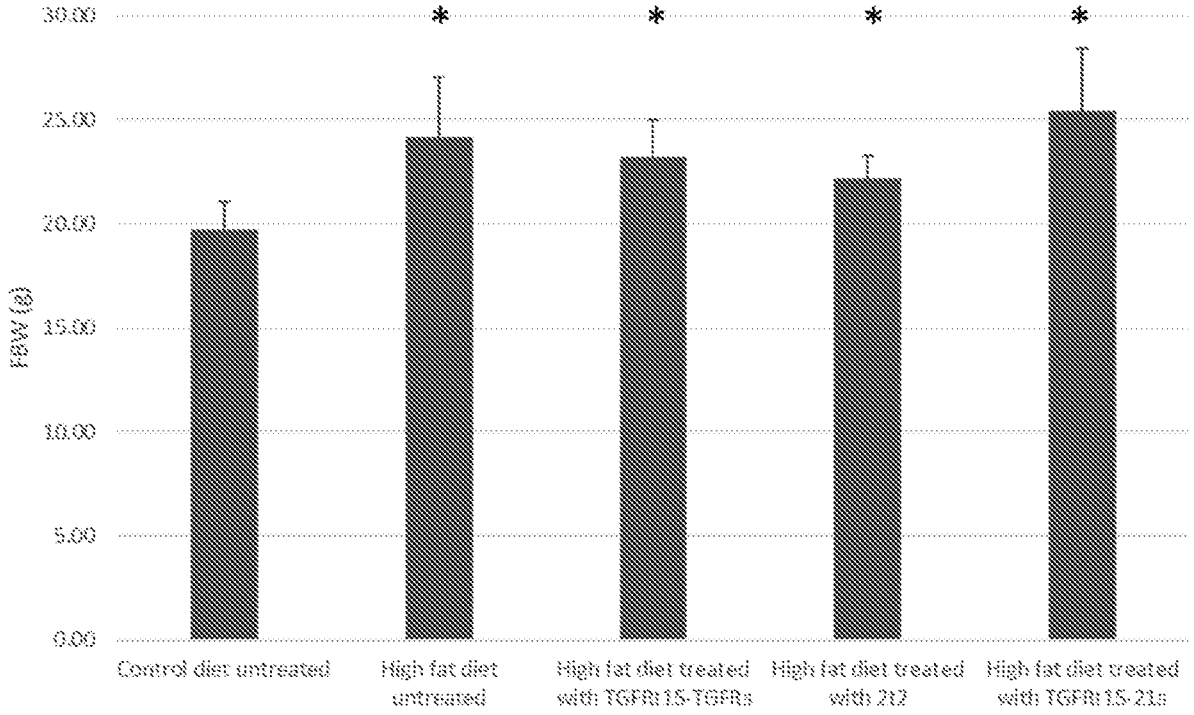


* Statistically significant from High fat diet untreated Group

Figure 5B



Figures 6A-6E



* Statistically significant from Control Normal Diet Group

Figure 7

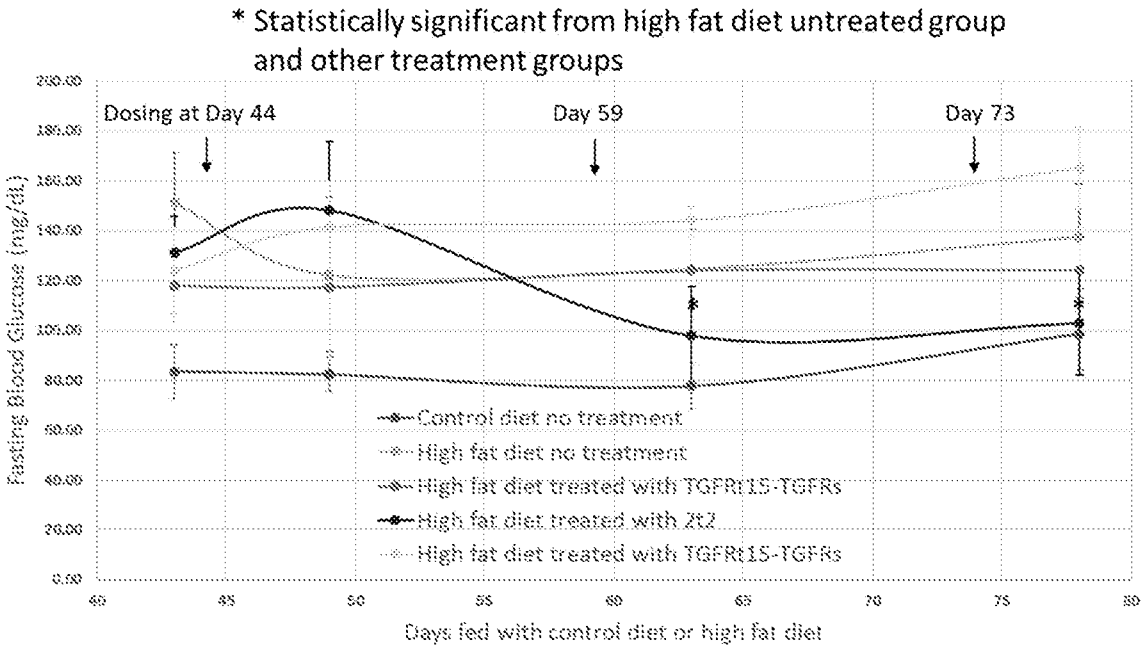
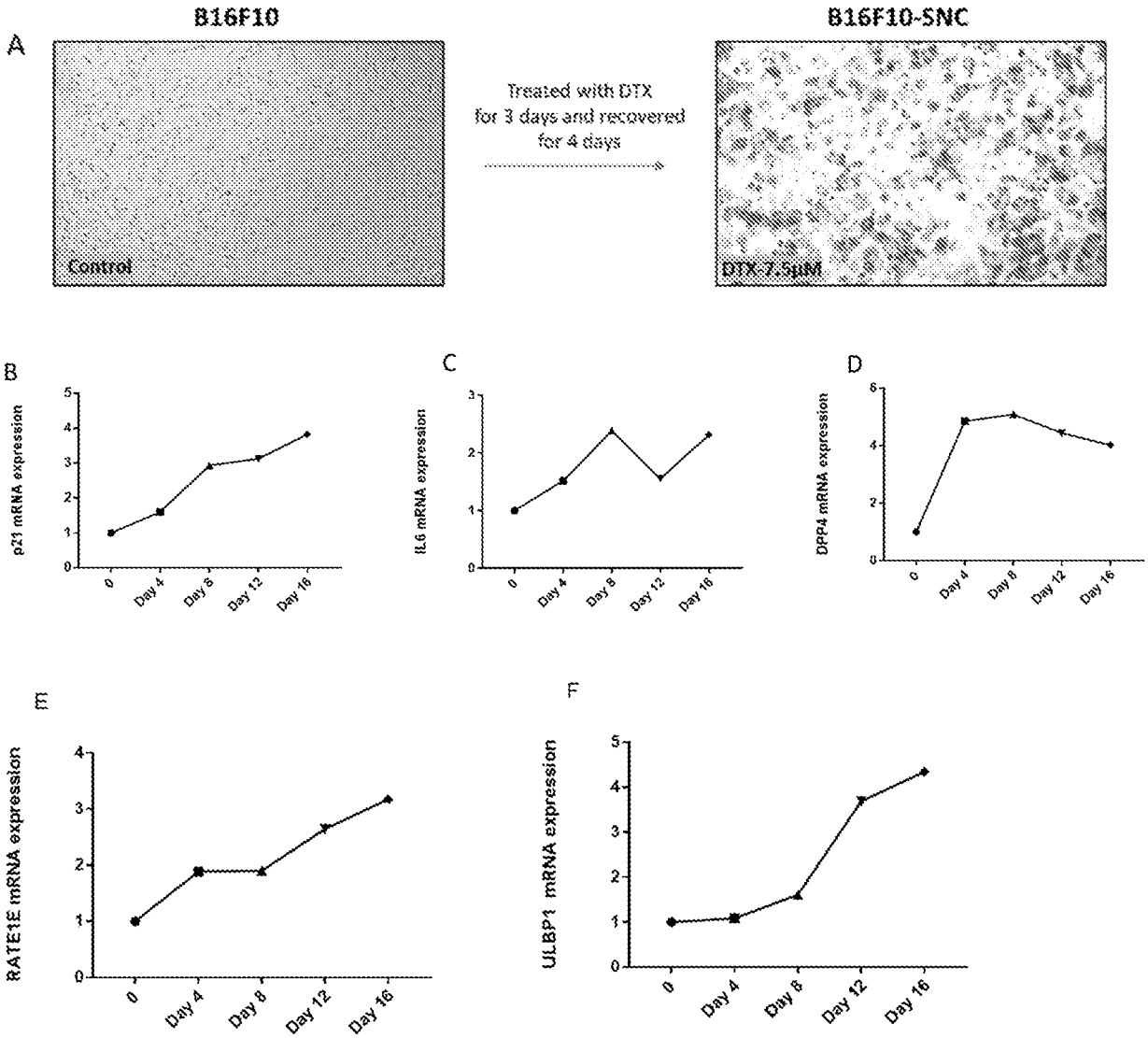
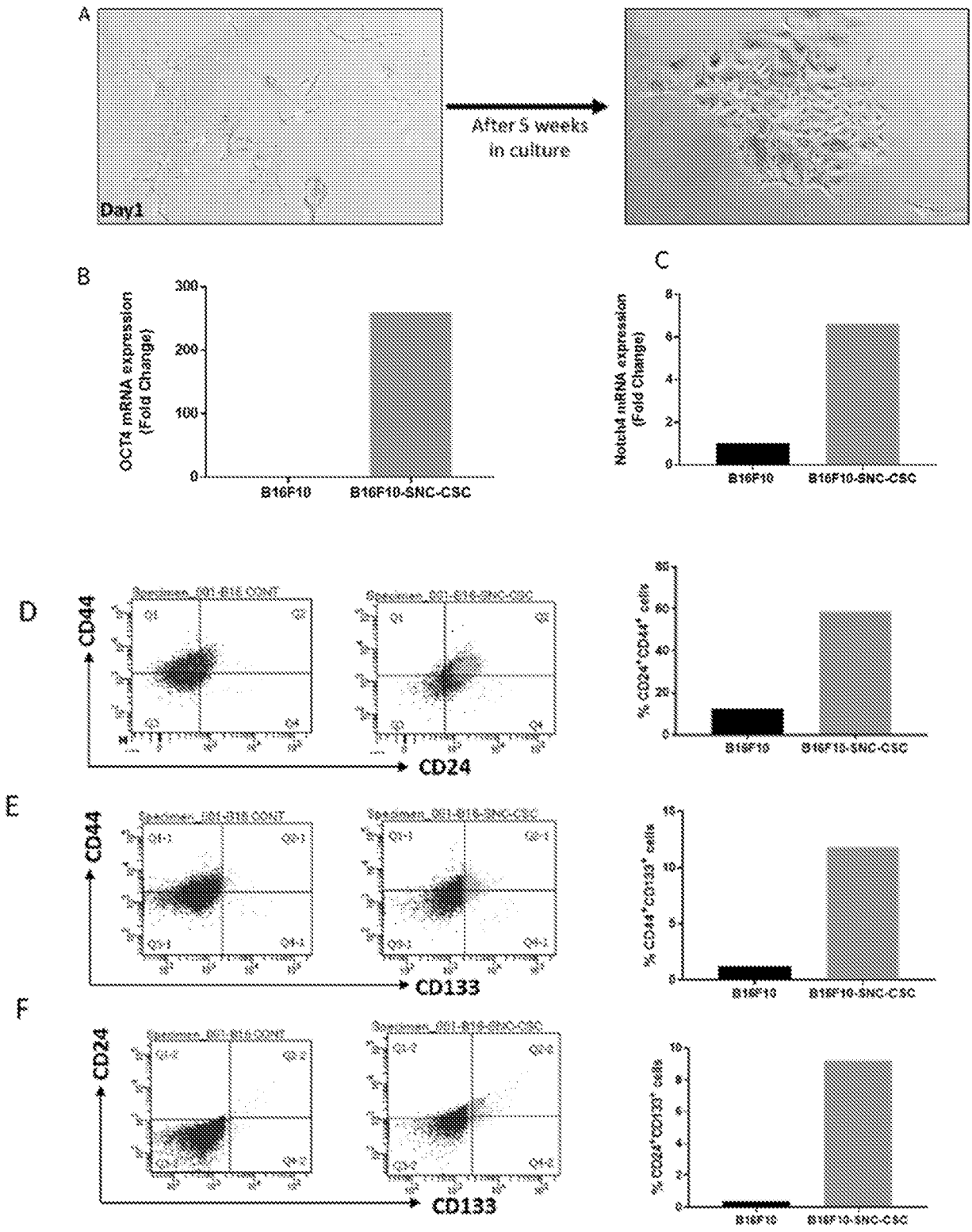

* Statistically significant from high fat diet untreated group and other treatment groups
Dosing at Day 44
Day 59
Day 73
Fasting Blood Glucose (mg/dL)
Control diet no treatment
High fat diet no treatment
High fat diet treated with TGFRt1S-TGFRs
High fat diet treated with Zt2
High fat diet treated with TGFRt1S-TGFRs
Days fed with control diet or high fat diet

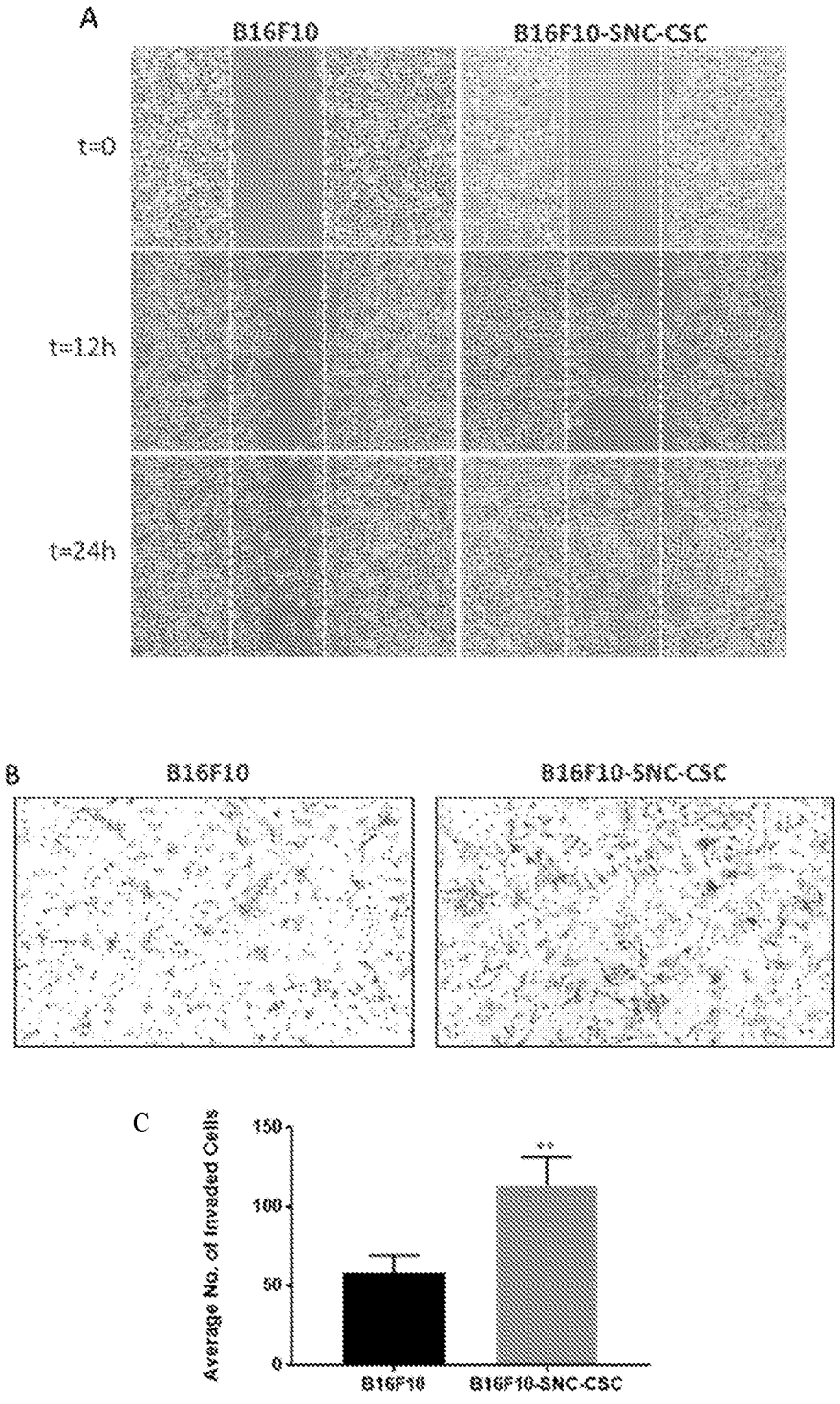
Figure 8



Figures 9A-9F

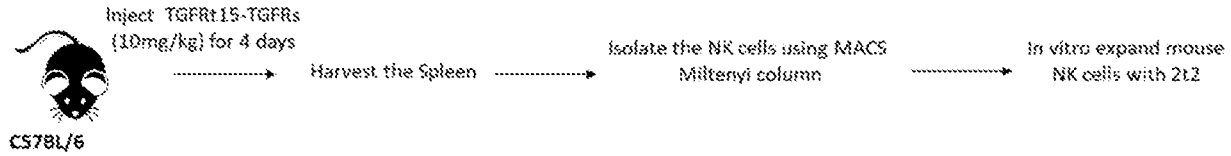


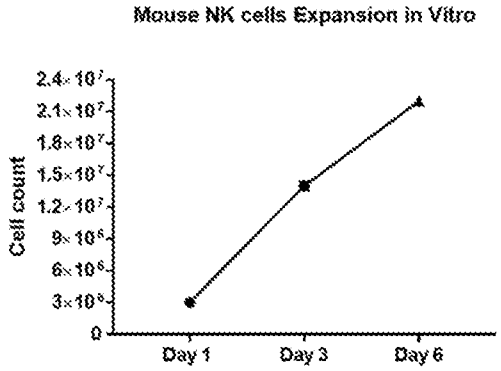
Figures 10A-10F



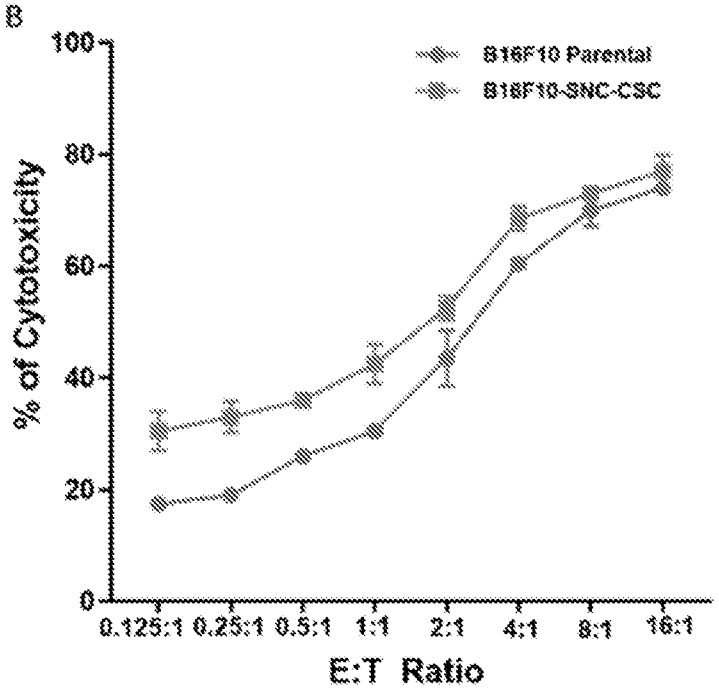
Figures 11A-11C

A



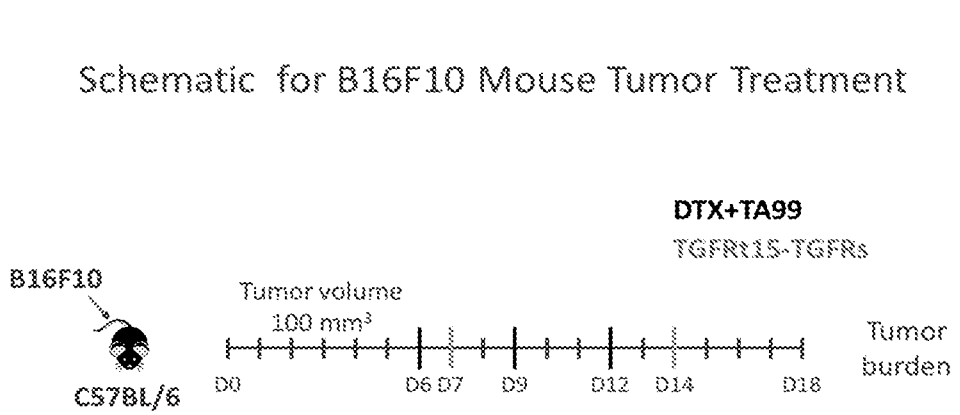


B

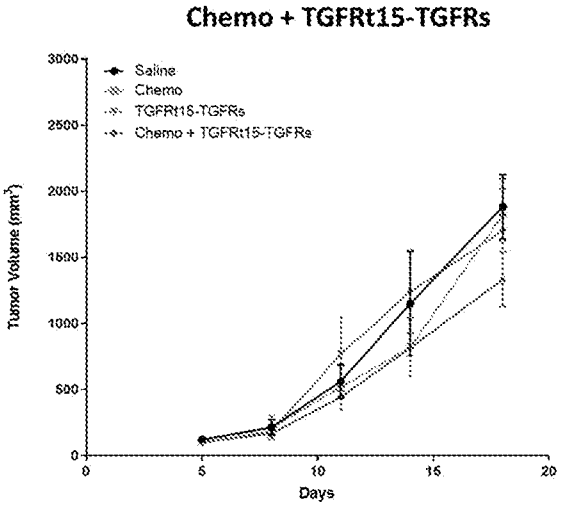


Figures 12A-12B

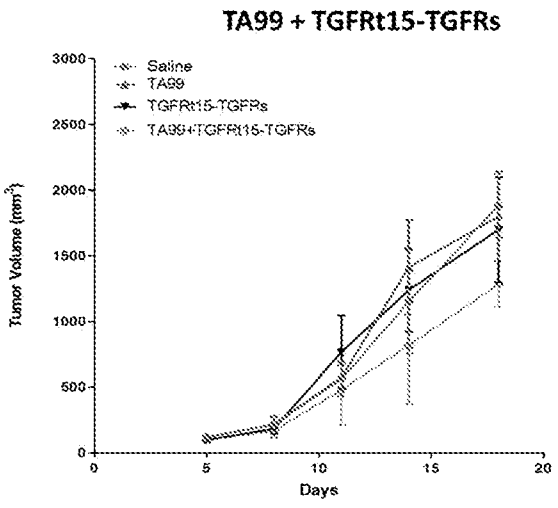
A



B



C



Figures 13A-13C

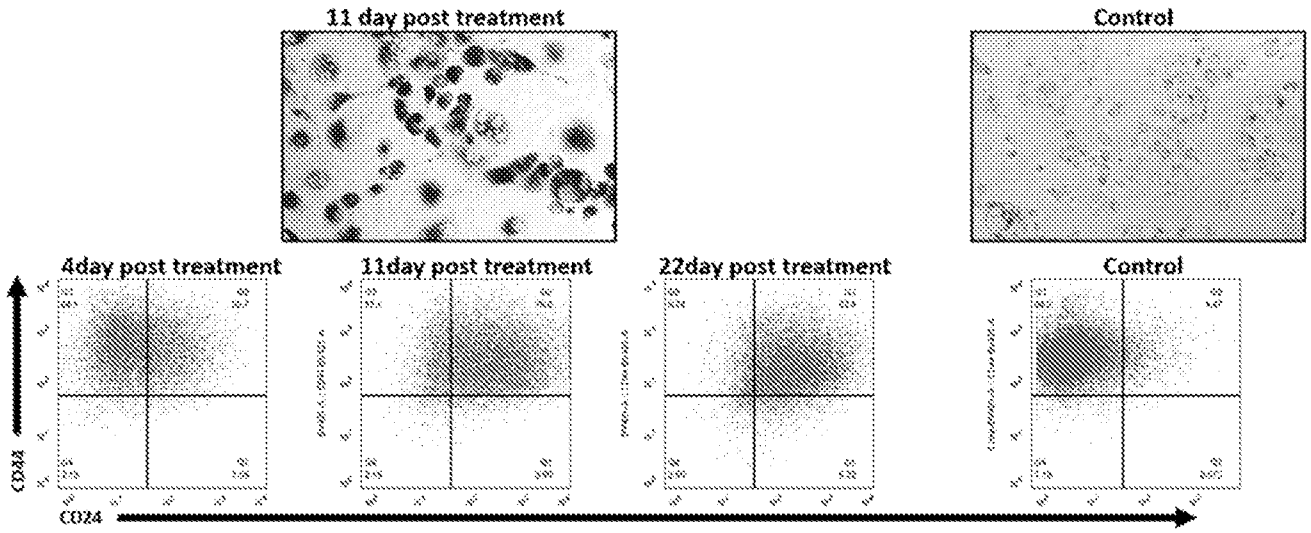

11 day post treatment
Control
4day post treatment
11day post treatment
22day post treatment
Control
CD28
CD24

Figure 14

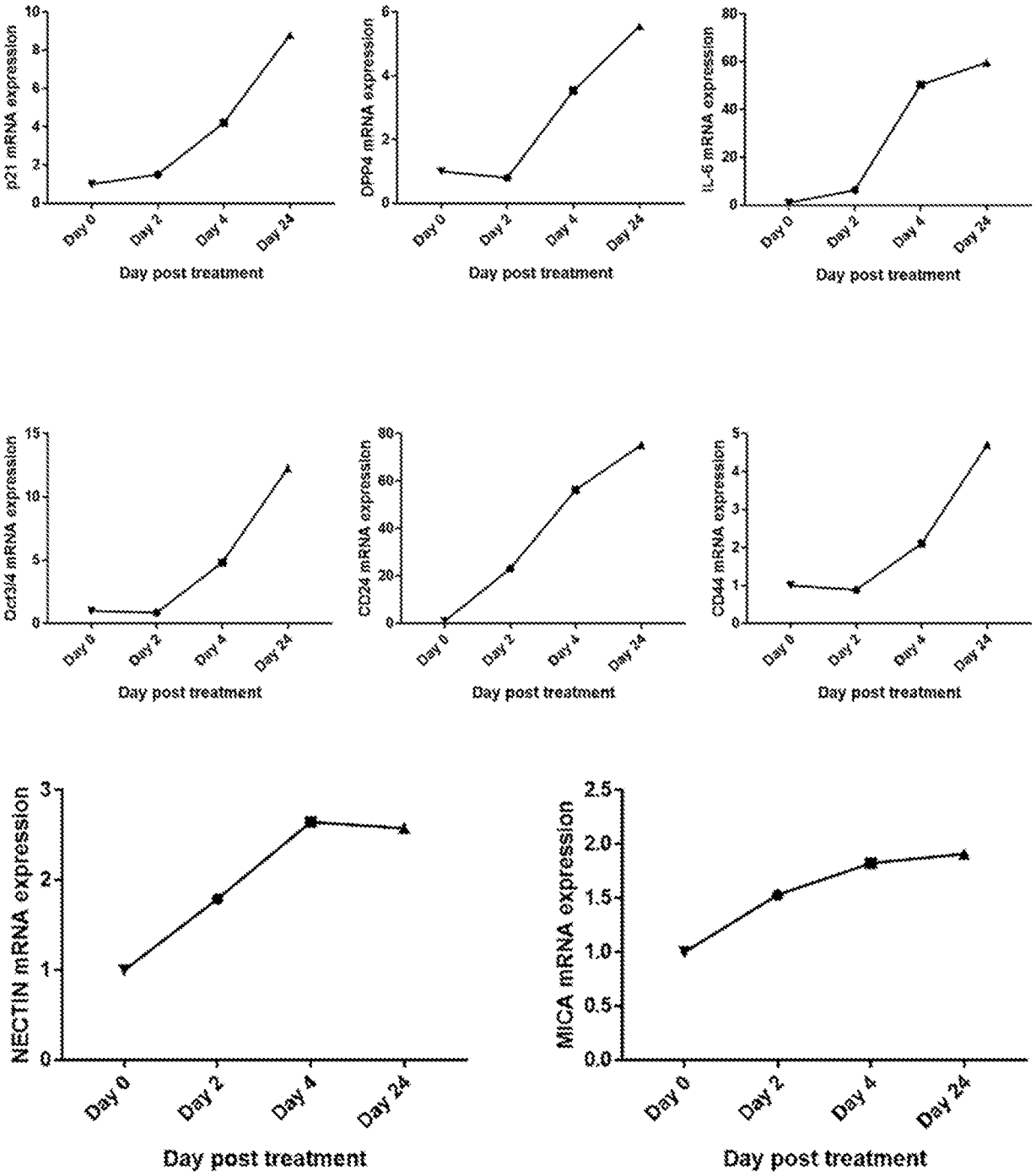

p21 mRNA expression
DPP4 mRNA expression
IL-6 mRNA expression
Oct3/4 mRNA expression
CD24 mRNA expression
CD44 mRNA expression
NECTIN mRNA expression
MICA mRNA expression
Day 0
Day 2
Day 4
Day 24
Day post treatment

Figure 15

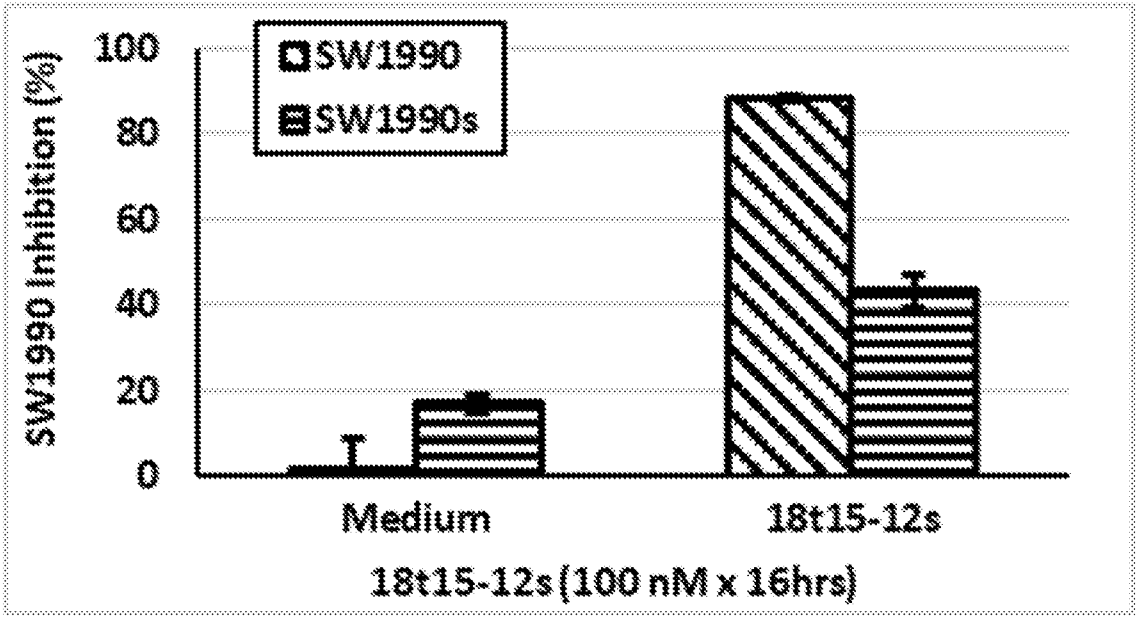

SW1990 Inhibition (%)
100
80
60
40
20
0
SW1990
SW1990s
Medium
18t15-12s
18t15-12s (100 nM x 16hrs)

Figure 16



Figure 17

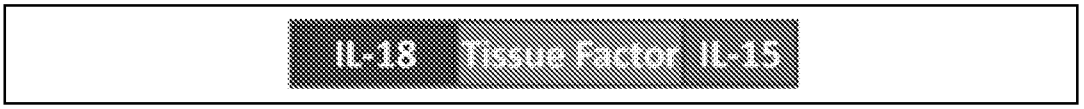


Figure 18

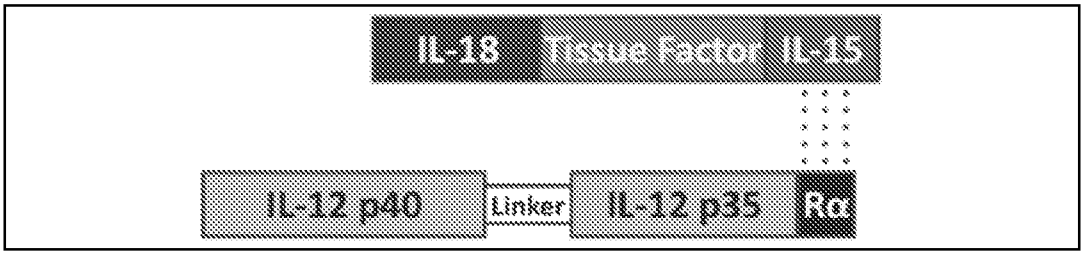


Figure 19

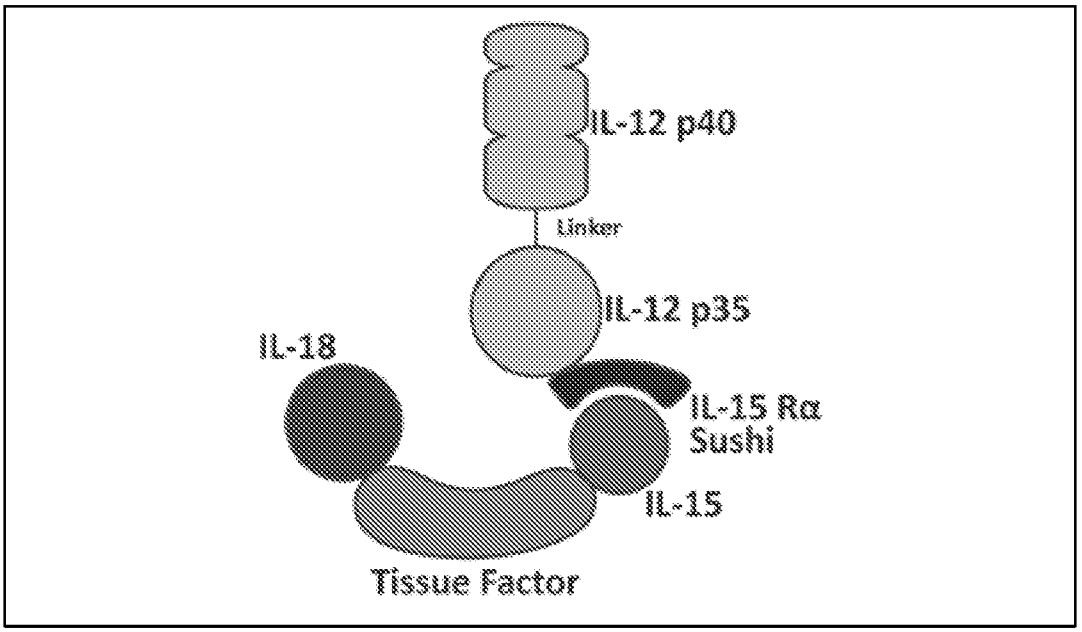


Figure 20

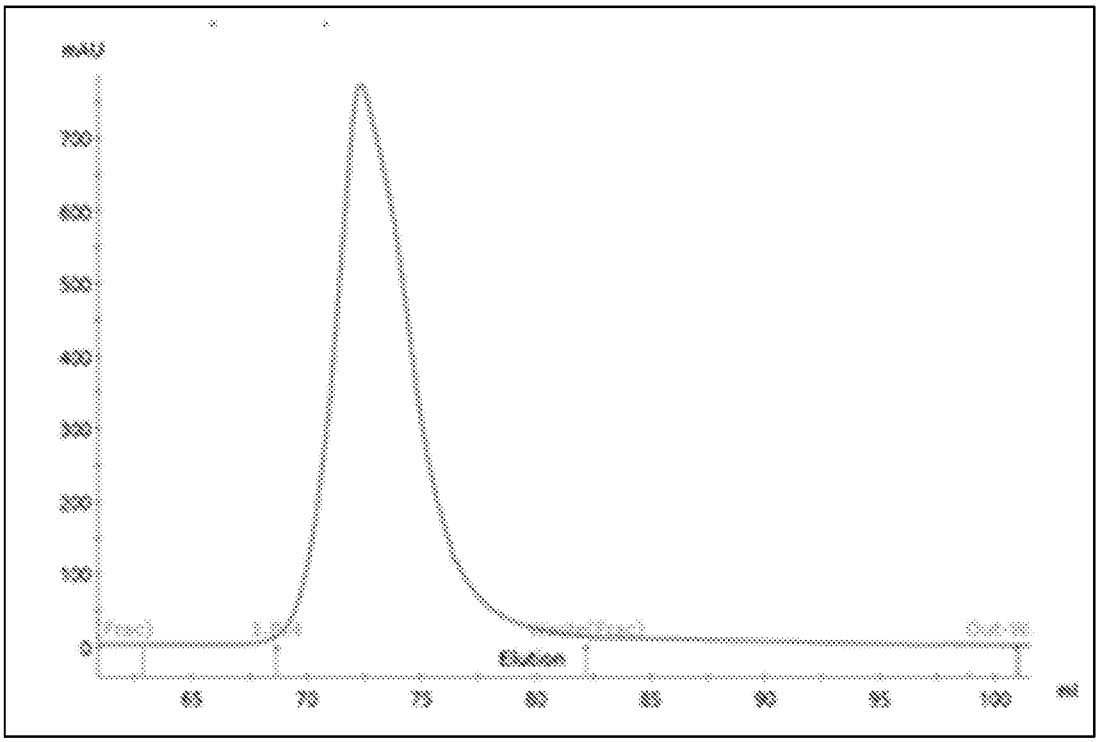


Figure 21

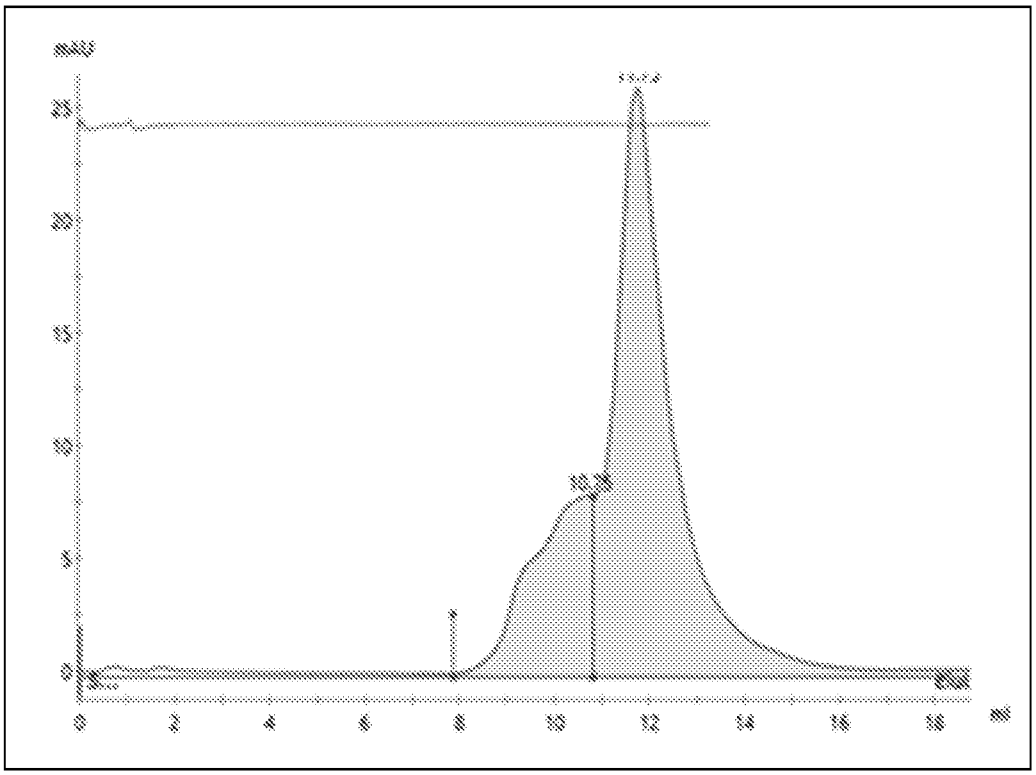


Figure 22

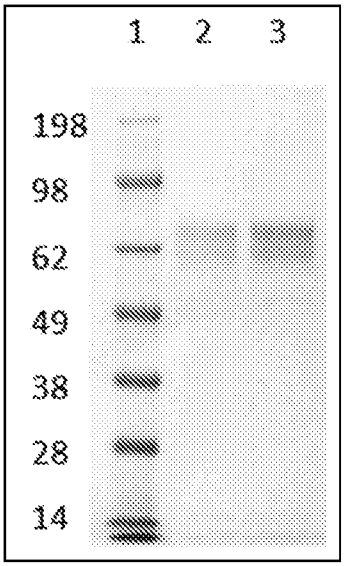


Figure 23

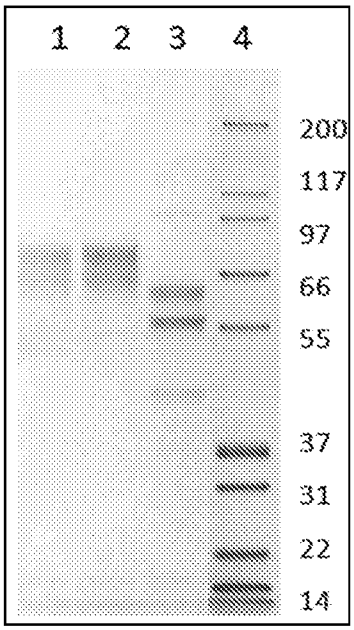


Figure 24

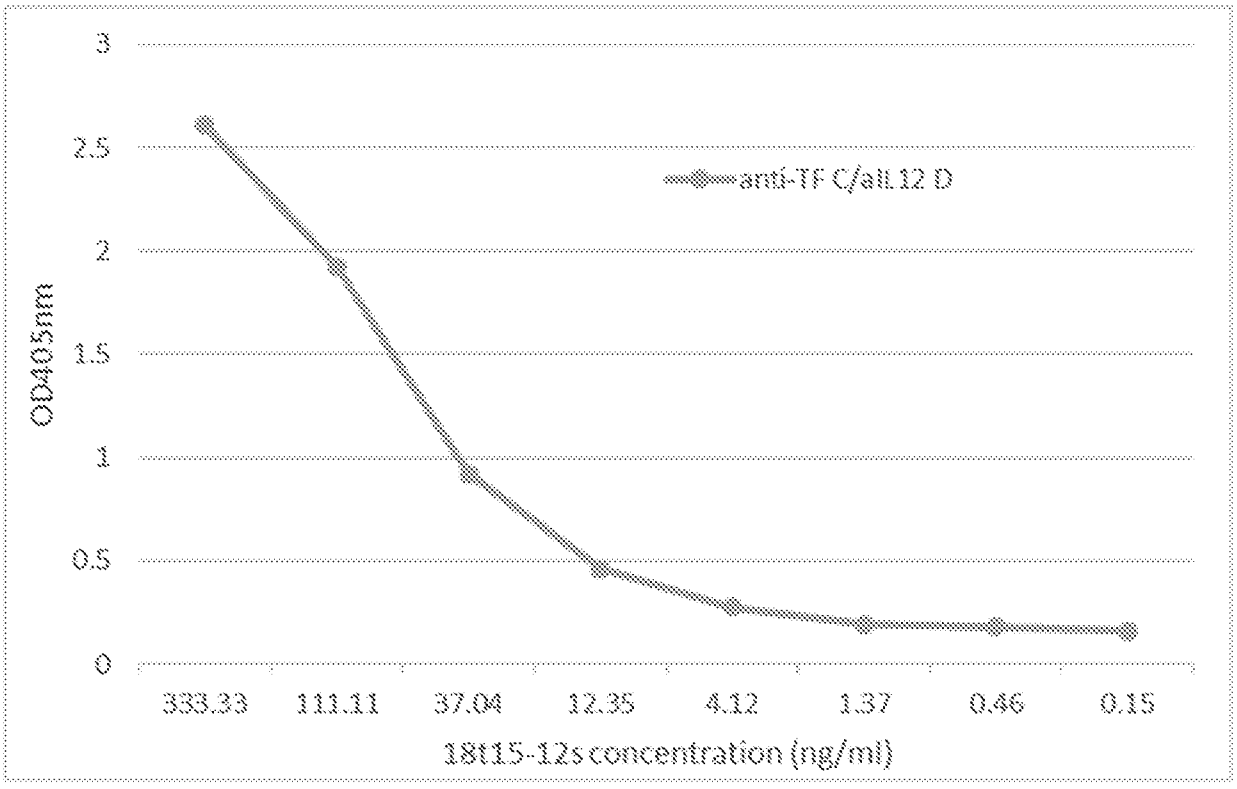


Figure 25

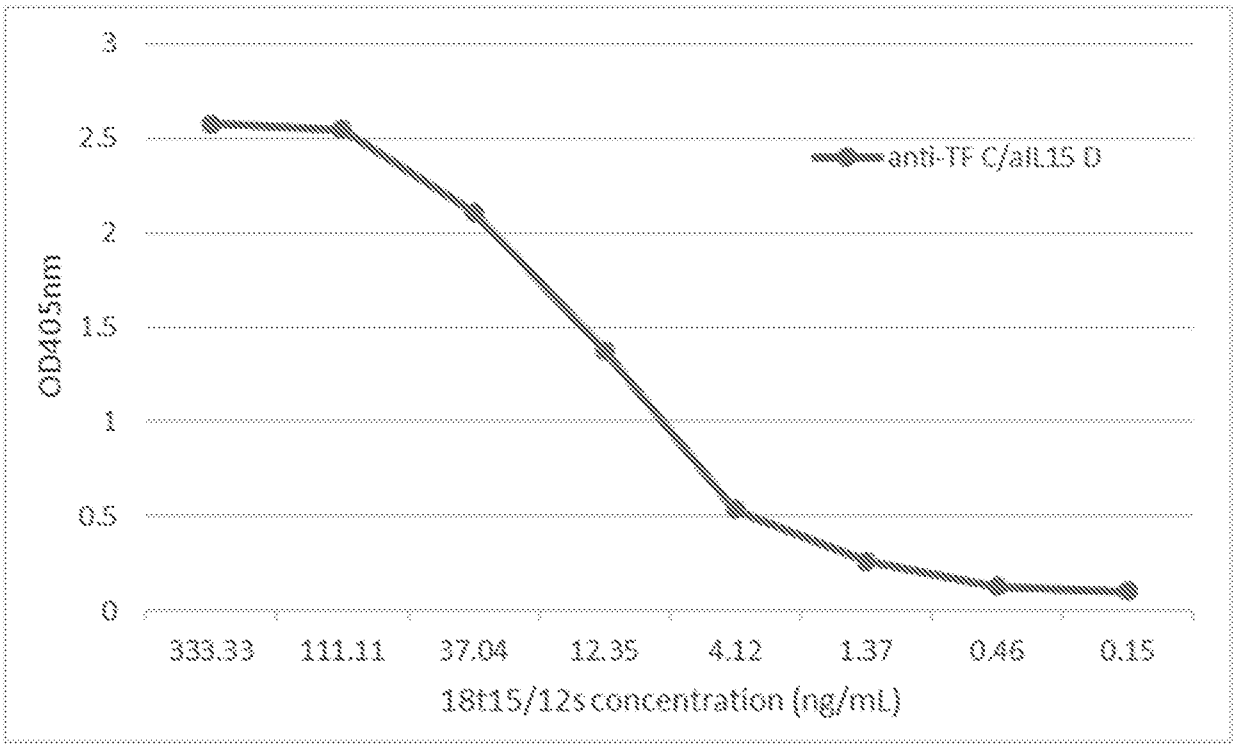


Figure 26

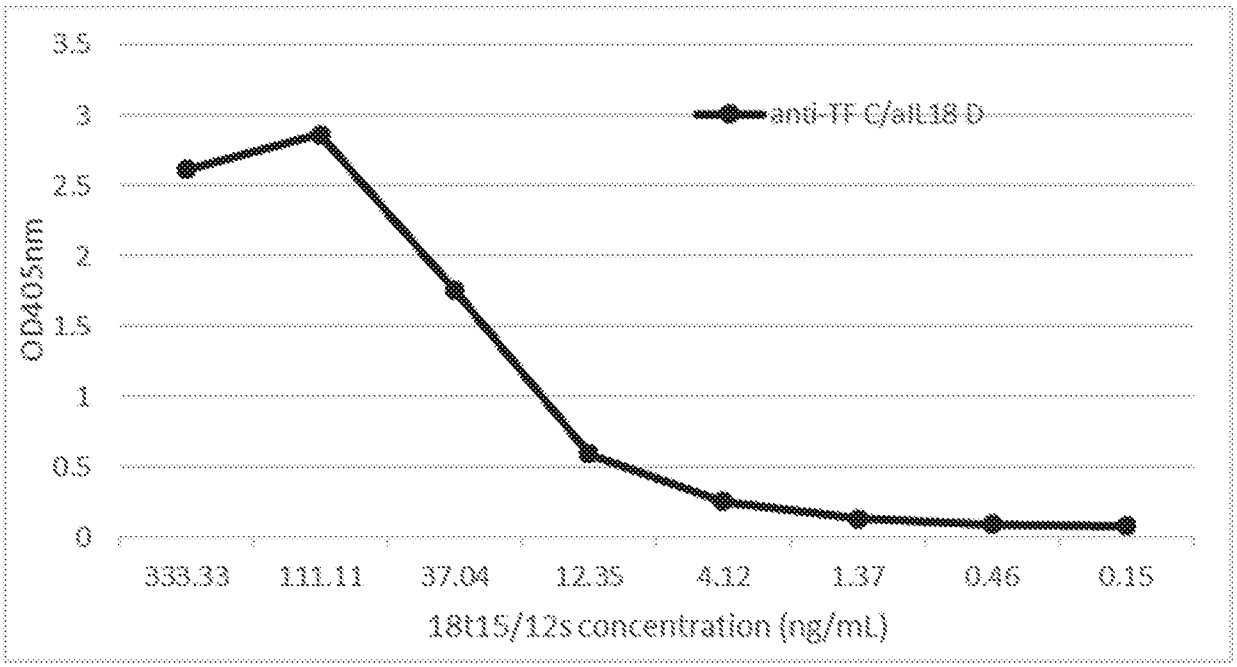


Figure 27

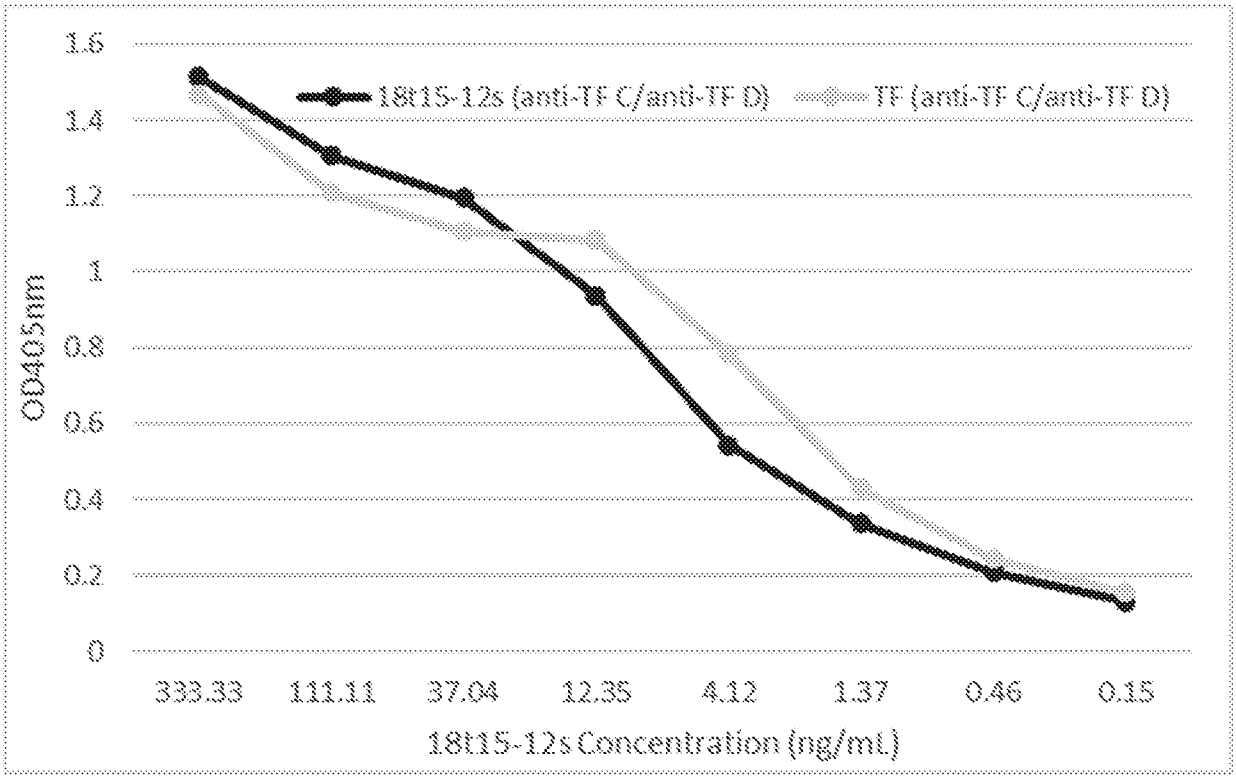

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD405nm
18t15-12s (anti-TF C/anti-TF D)
TF (anti-TF C/anti-TF D)
333.33
111.11
37.04
12.35
4.12
1.37
0.46
0.15
18t15-12s Concentration (ng/mL)

Figure 28

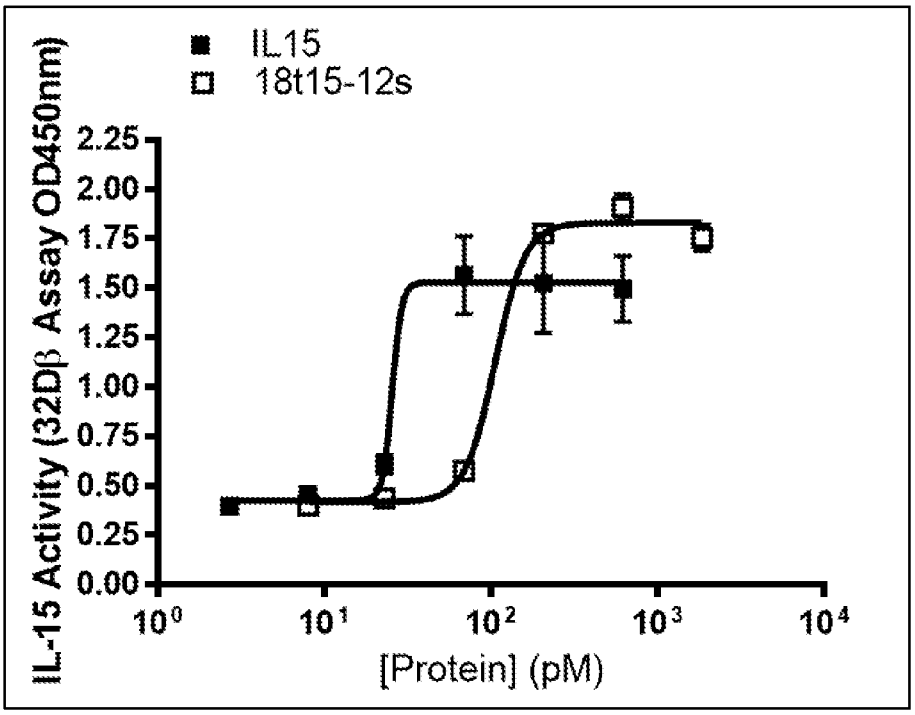


Figure 29

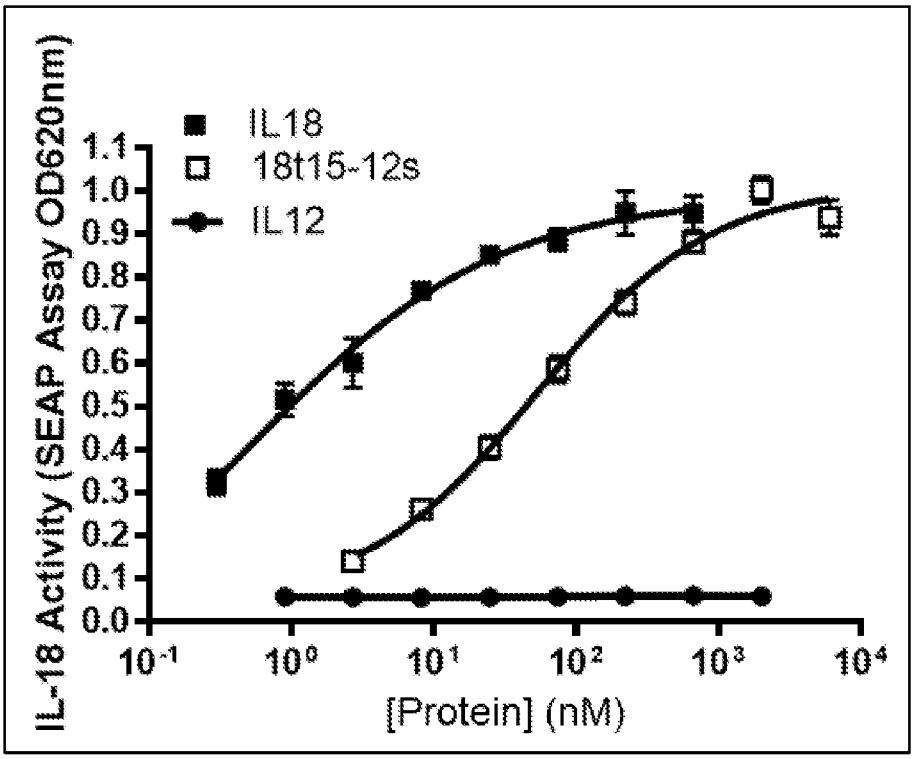


Figure 30

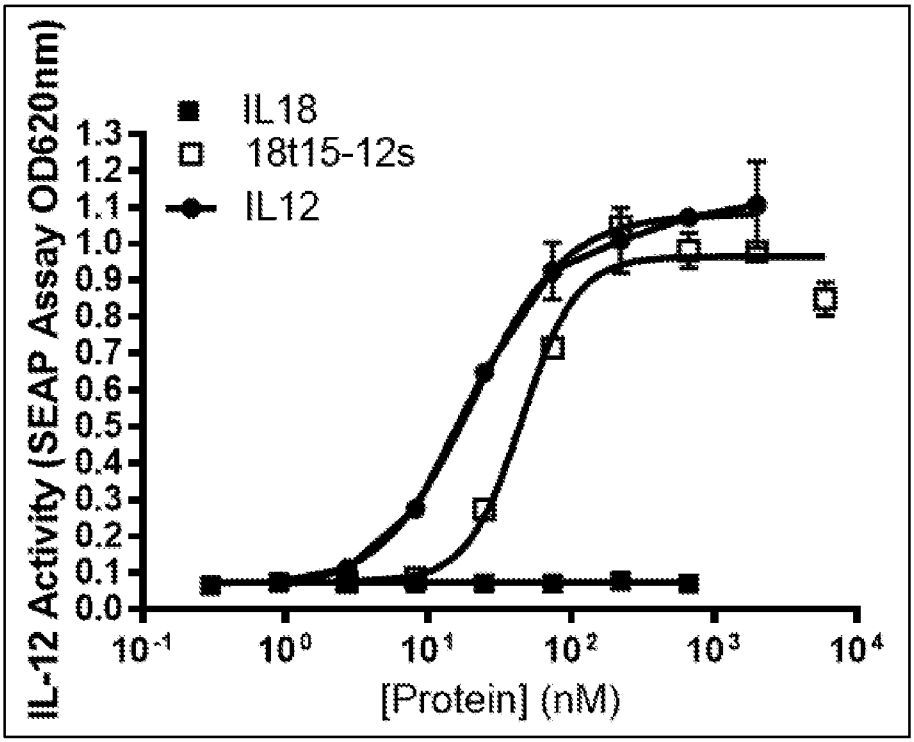

IL18
18t15-12s
IL12
IL-12 Activity (SEAP Assay OD620nm)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10-1
100
101
102
103
104
[Protein] (nM)

Figure 31

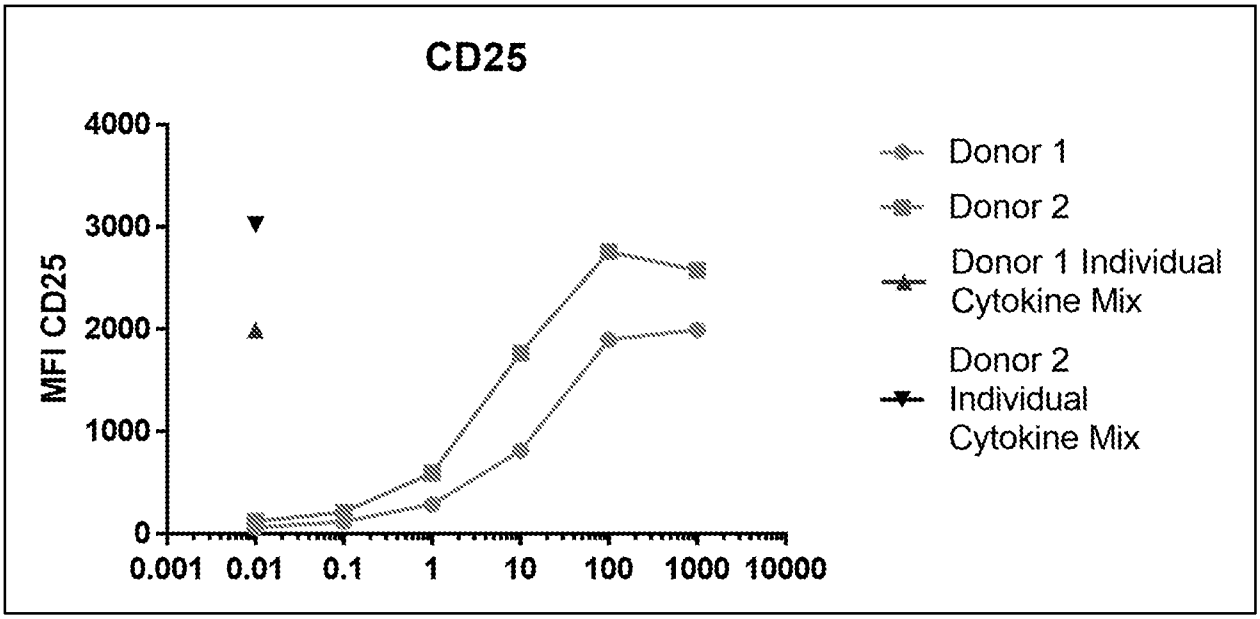


Figure 32A

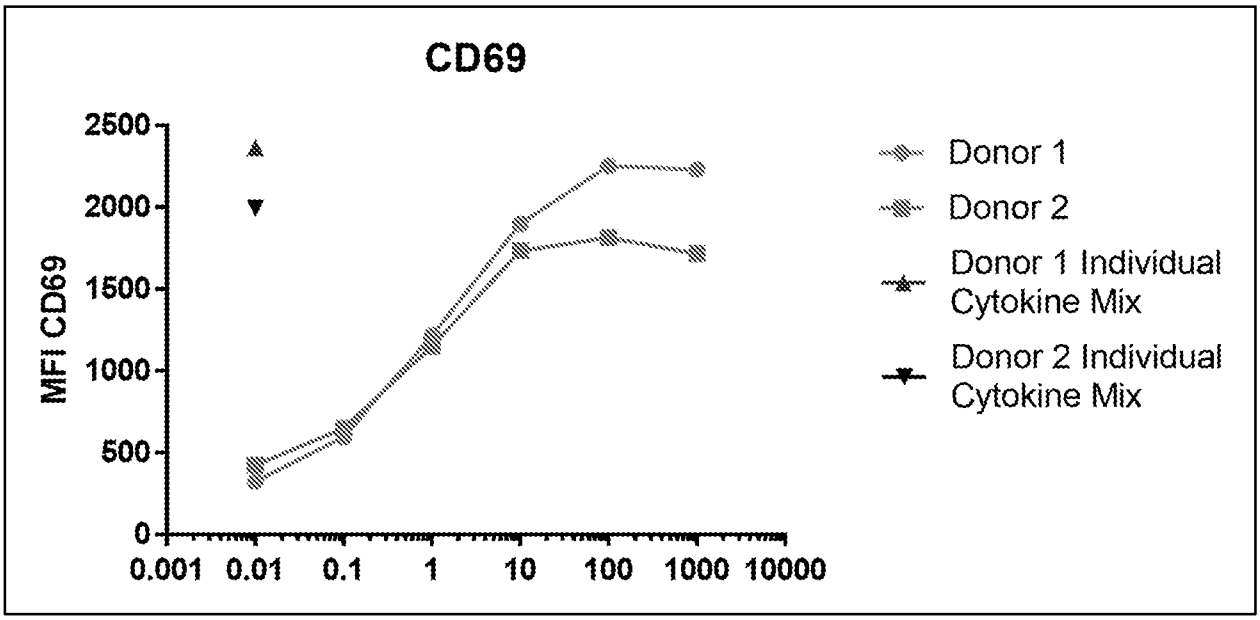


Figure 32B

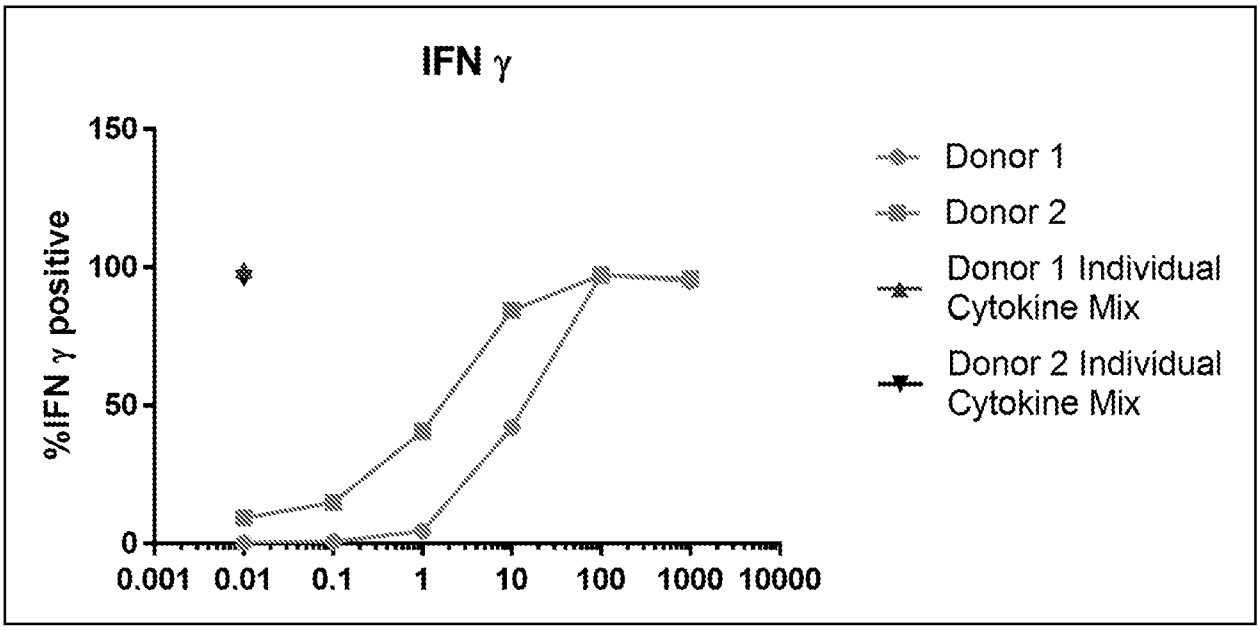


Figure 33

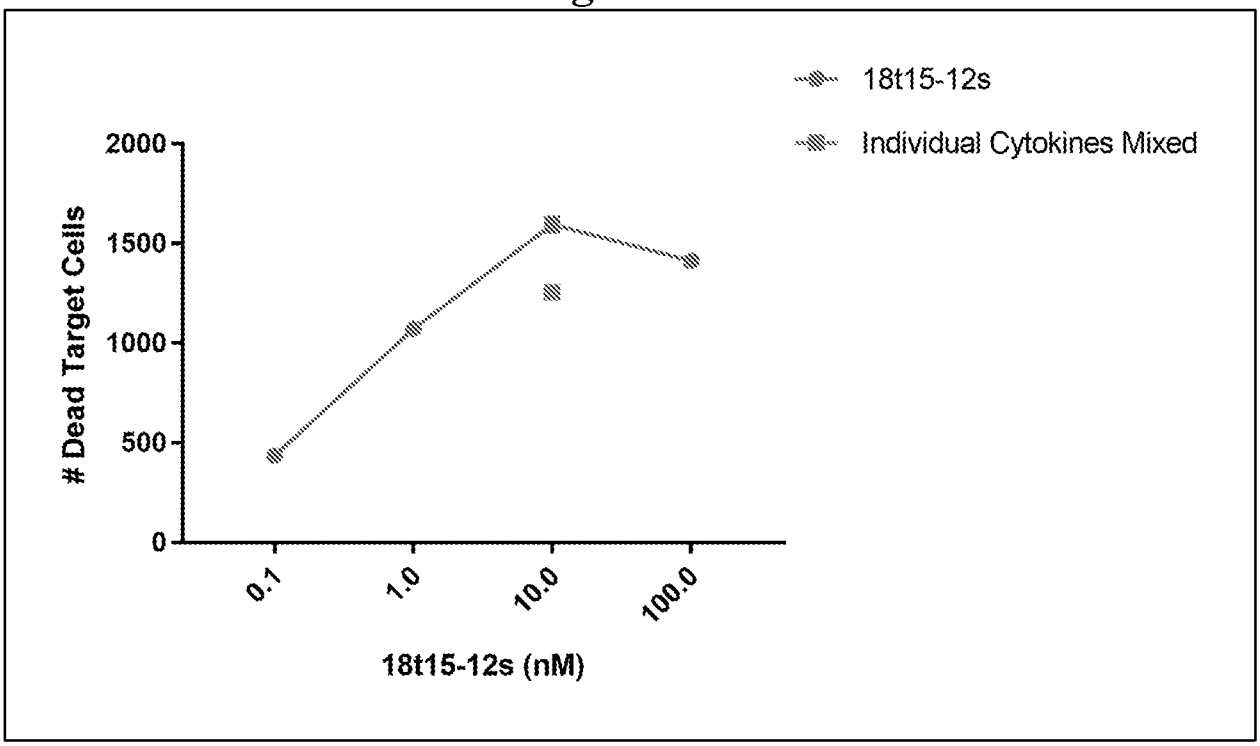


Figure 34

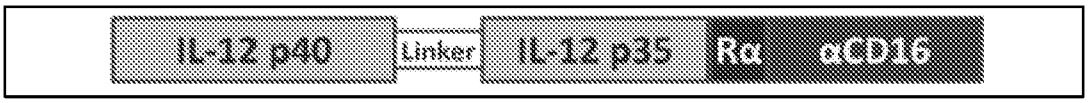


Figure 35

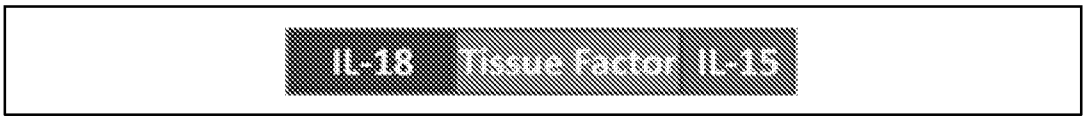


Figure 36

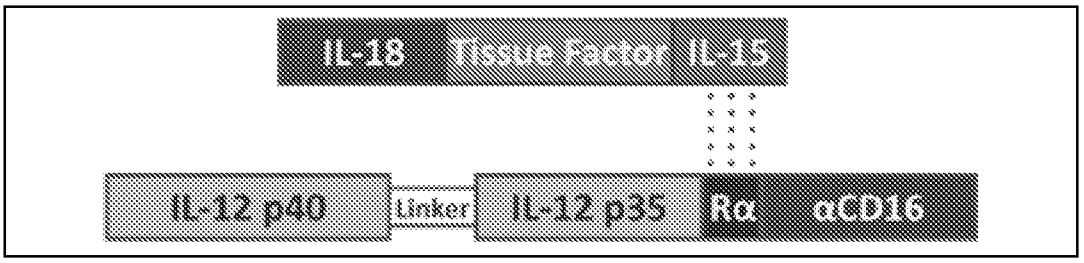


Figure 37

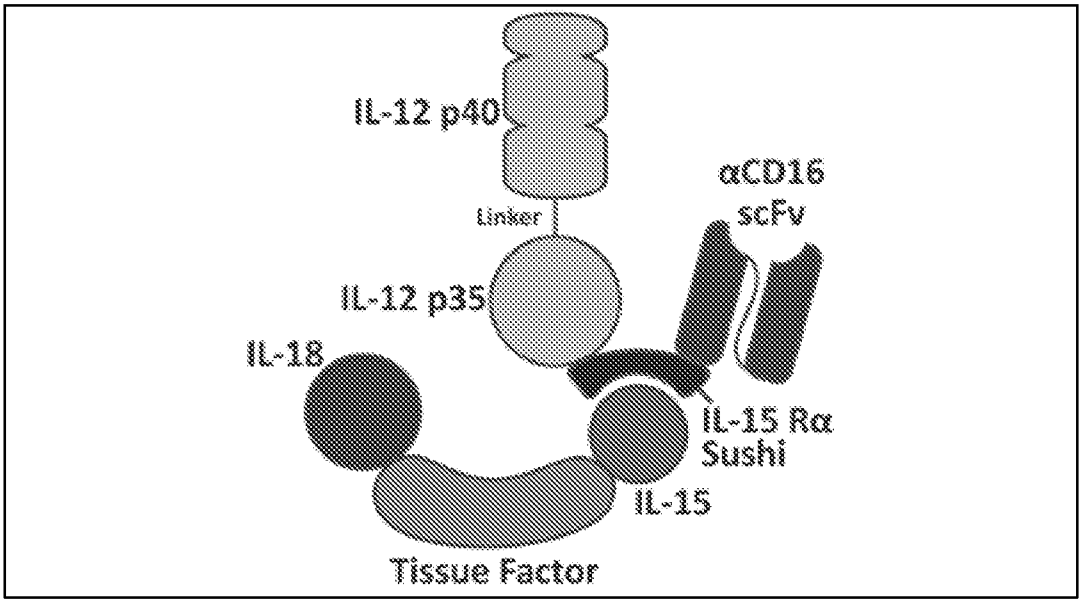


Figure 38

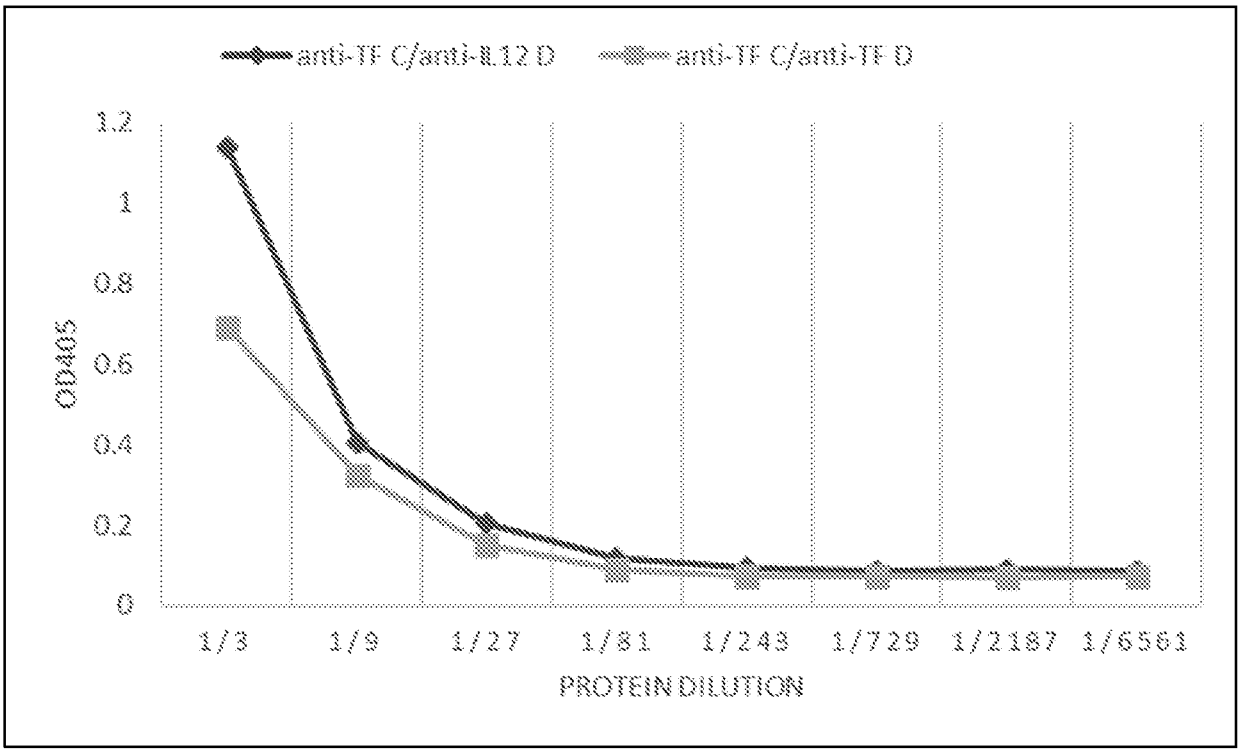

anti-TF C/anti-IL12 D
anti-TF C/anti-TF D
OD405
1.2
1
0.8
0.6
0.4
0.2
0
1/3
1/9
1/27
1/81
1/243
1/729
1/2187
1/6561
PROTEIN DILUTION

Figure 39



Figure 40



Figure 41

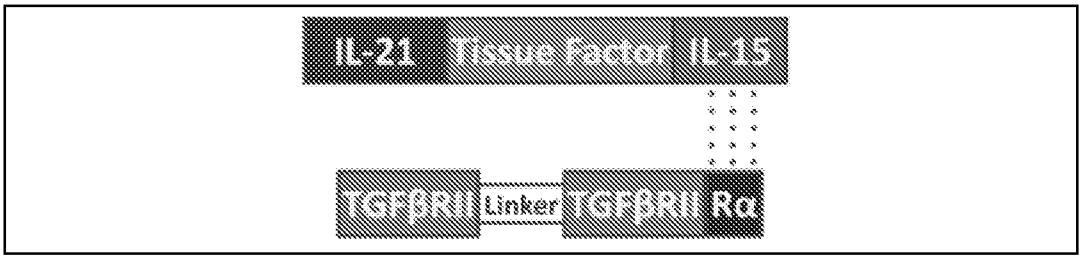


Figure 42

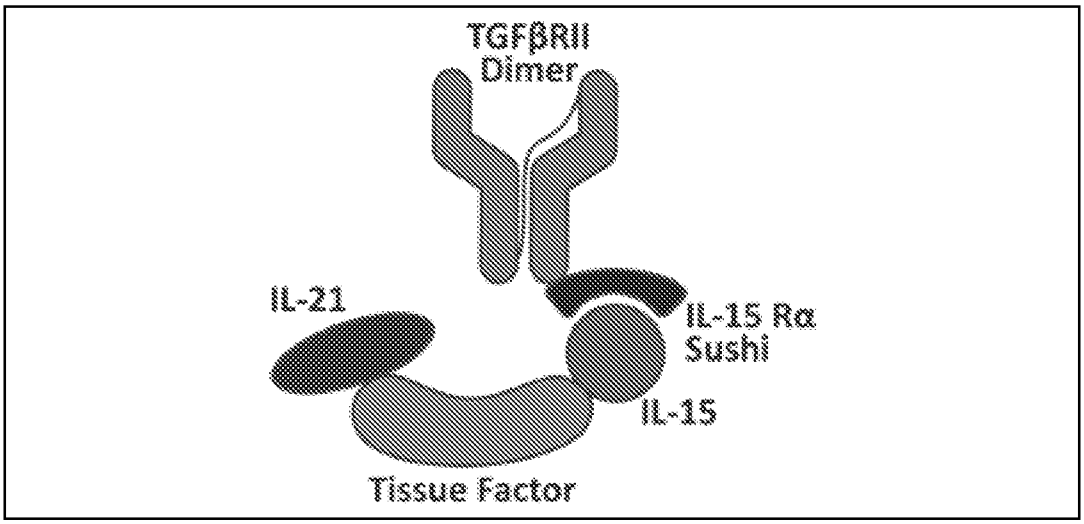


Figure 43

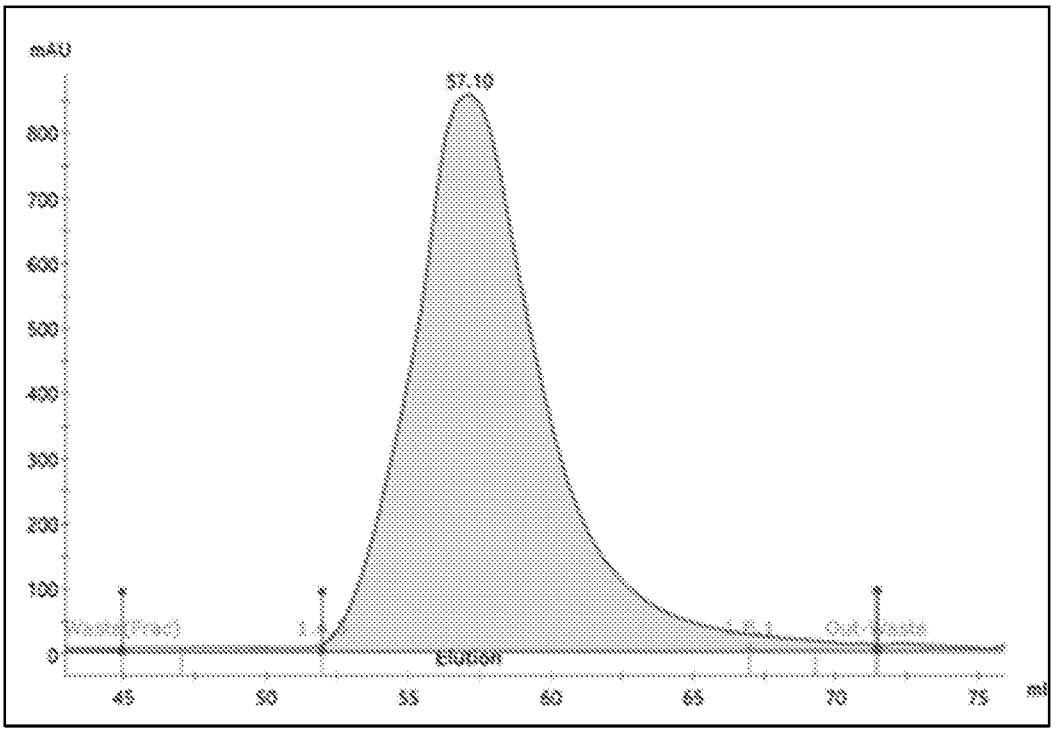


Figure 44

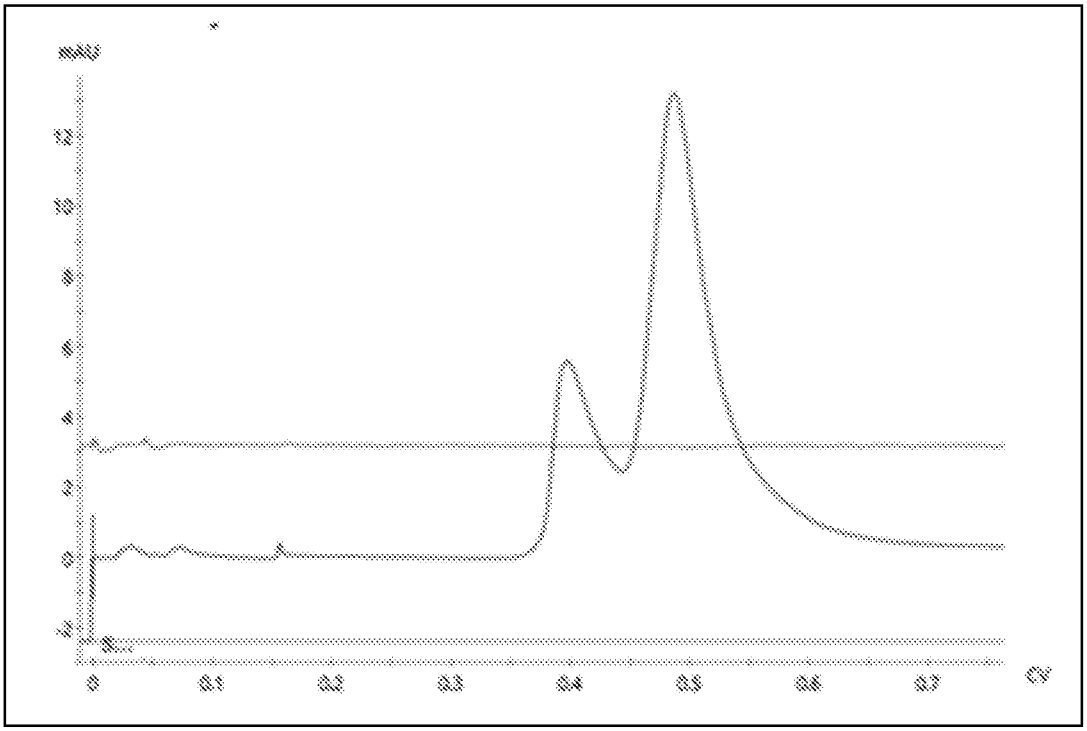


Figure 45

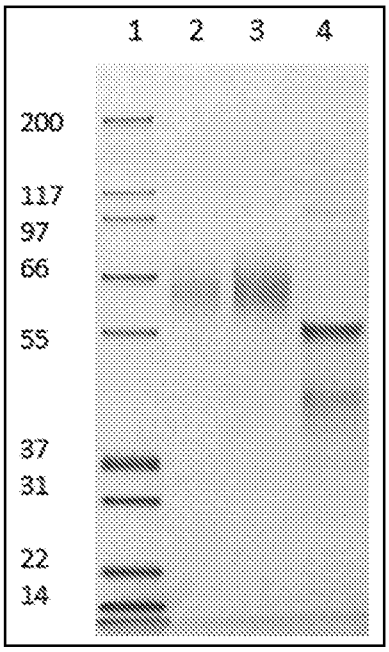


Figure 46

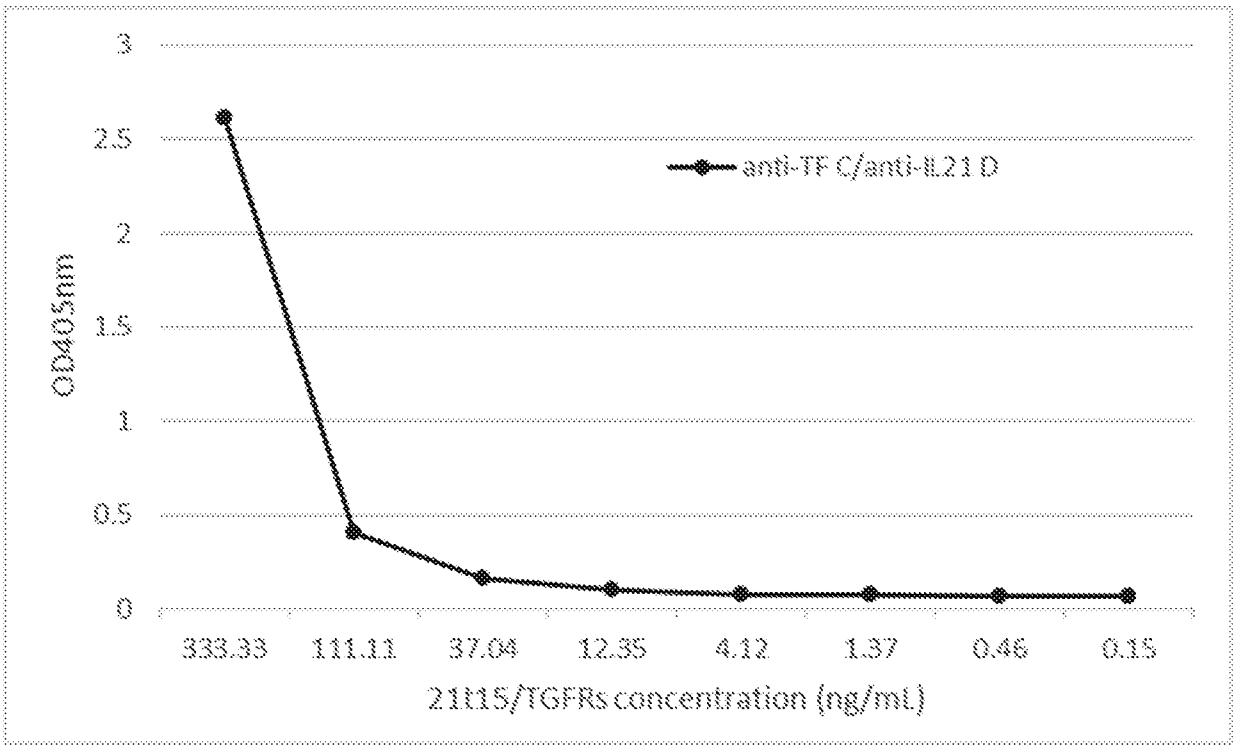


Figure 47

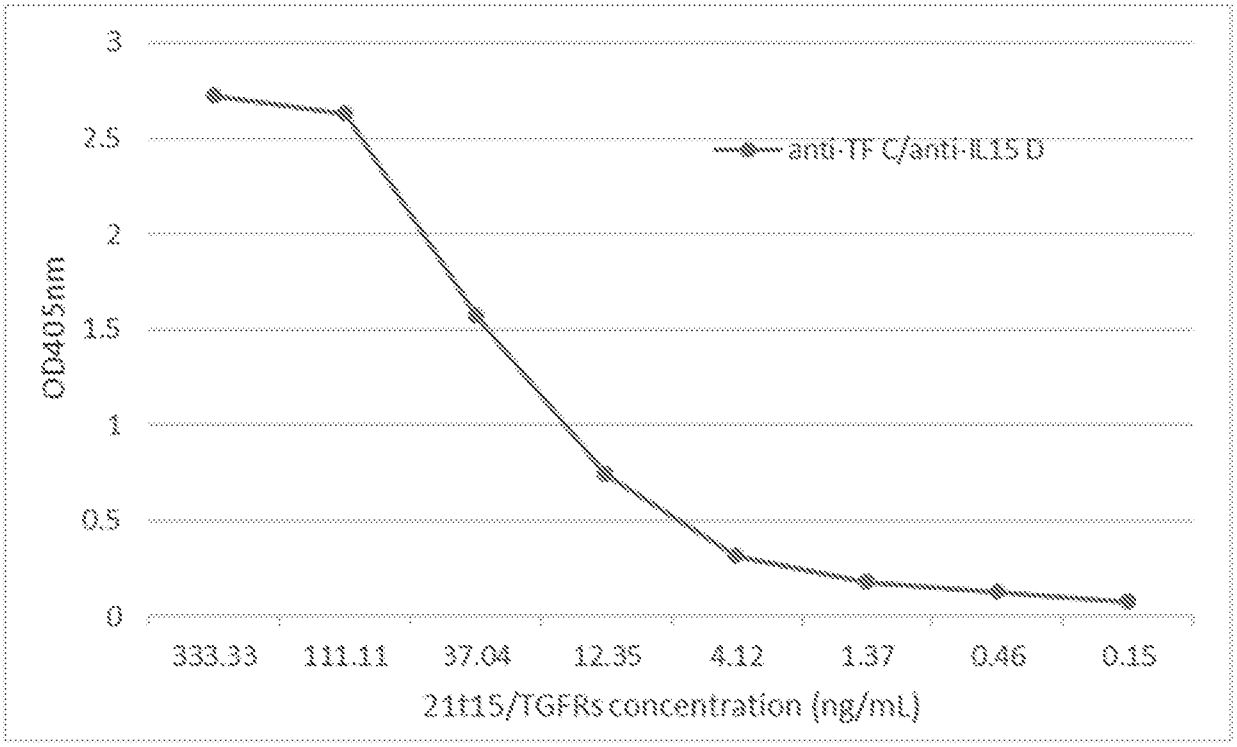


Figure 48

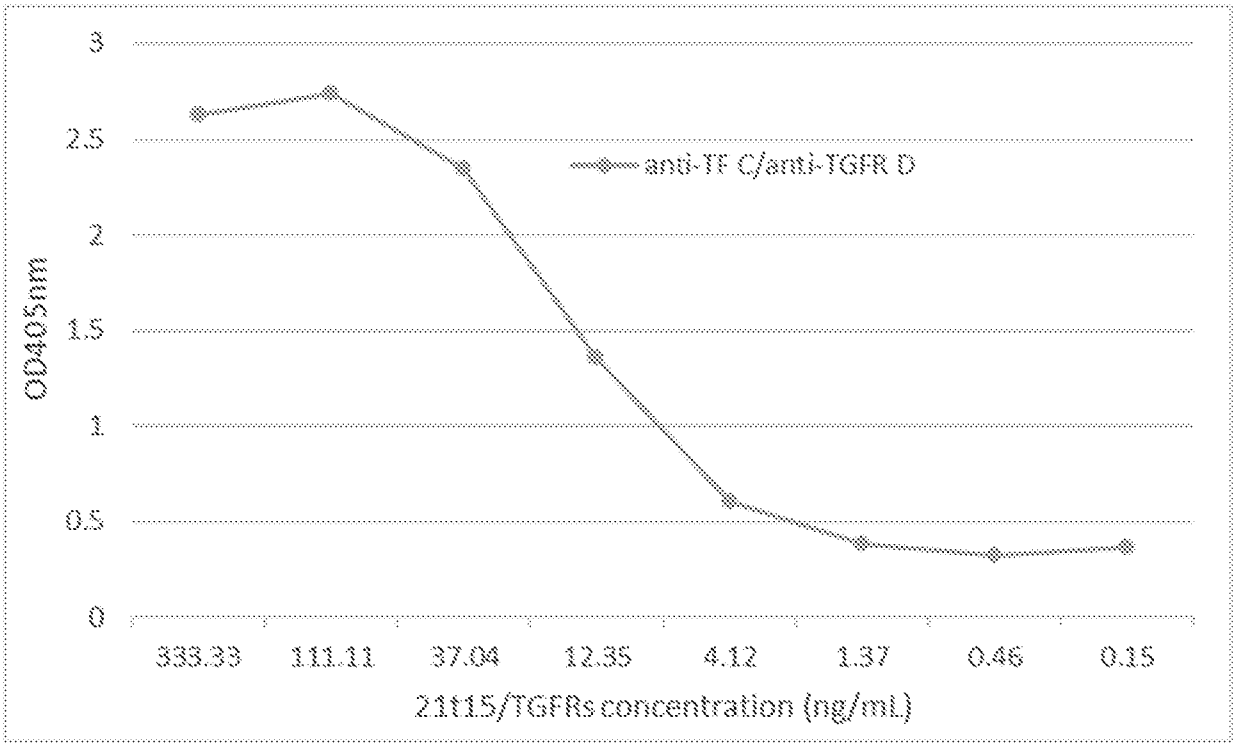


Figure 49

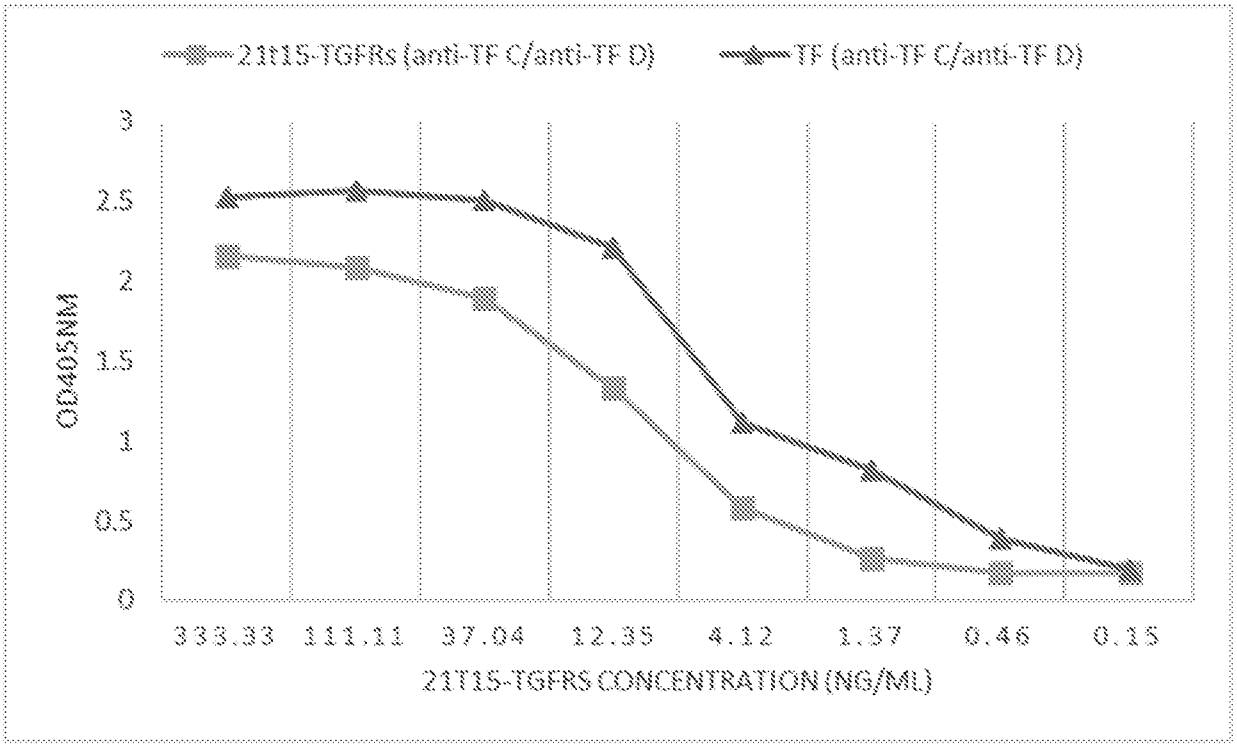

21T15-TGFRs (anti-TF C/anti-TF D)
TF (anti-TF C/anti-TF D)
OD405NM
3
2.5
2
1.5
1
0.5
0
333.33
111.11
37.04
12.35
4.12
1.37
0.46
0.15
21T15-TGFRS CONCENTRATION (NG/ML)

Figure 50

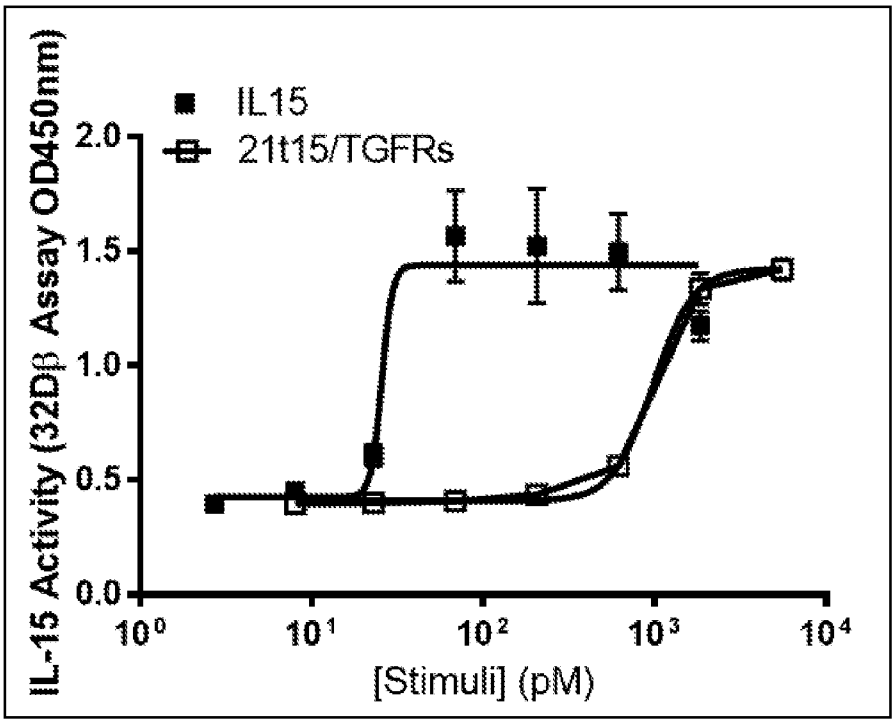


Figure 51

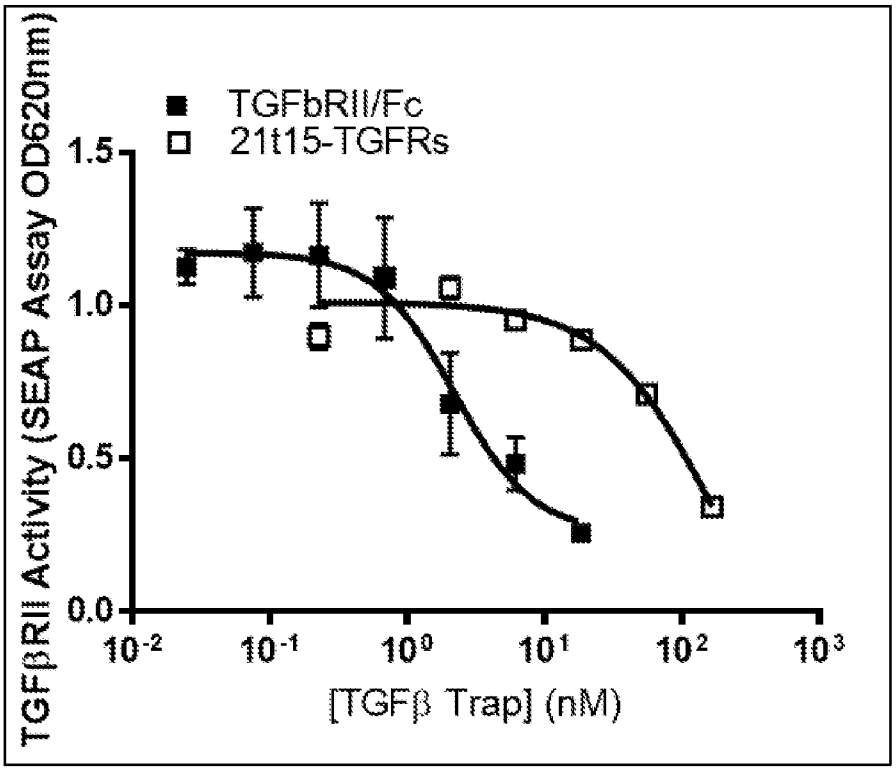


Figure 52

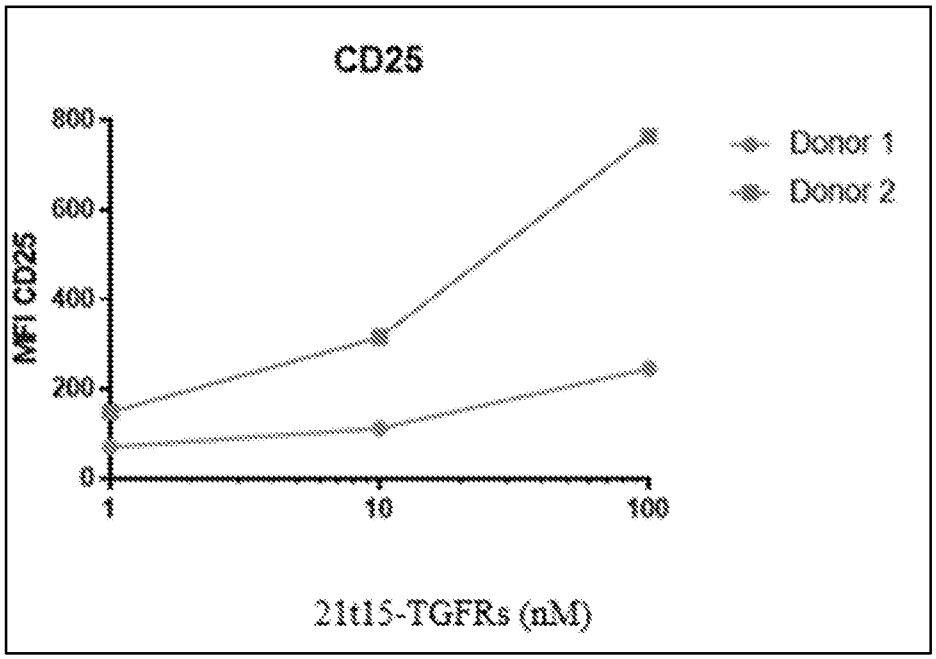


Figure 53

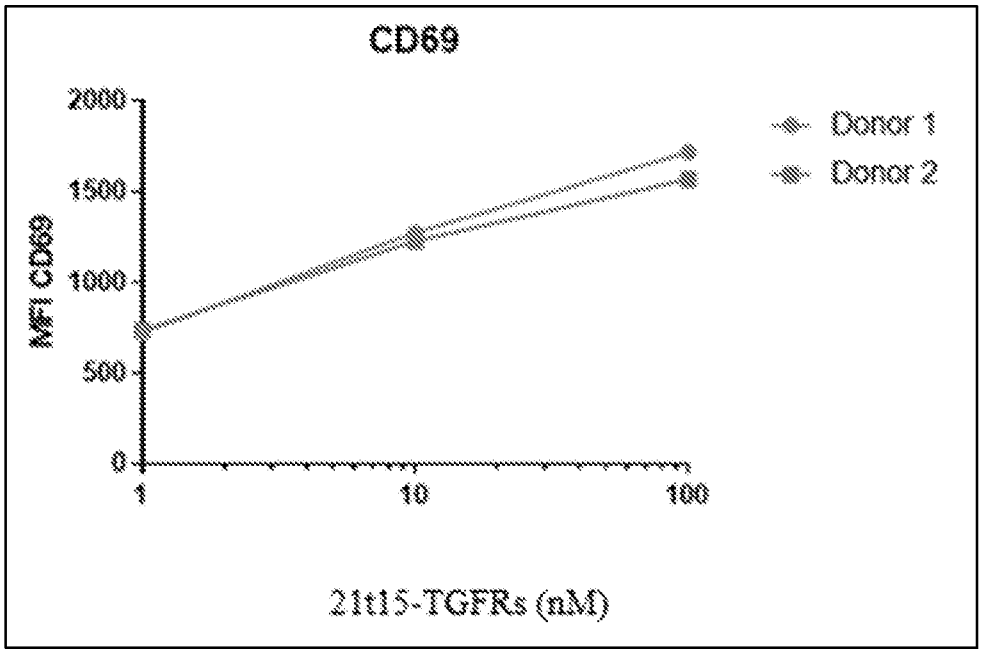


Figure 54

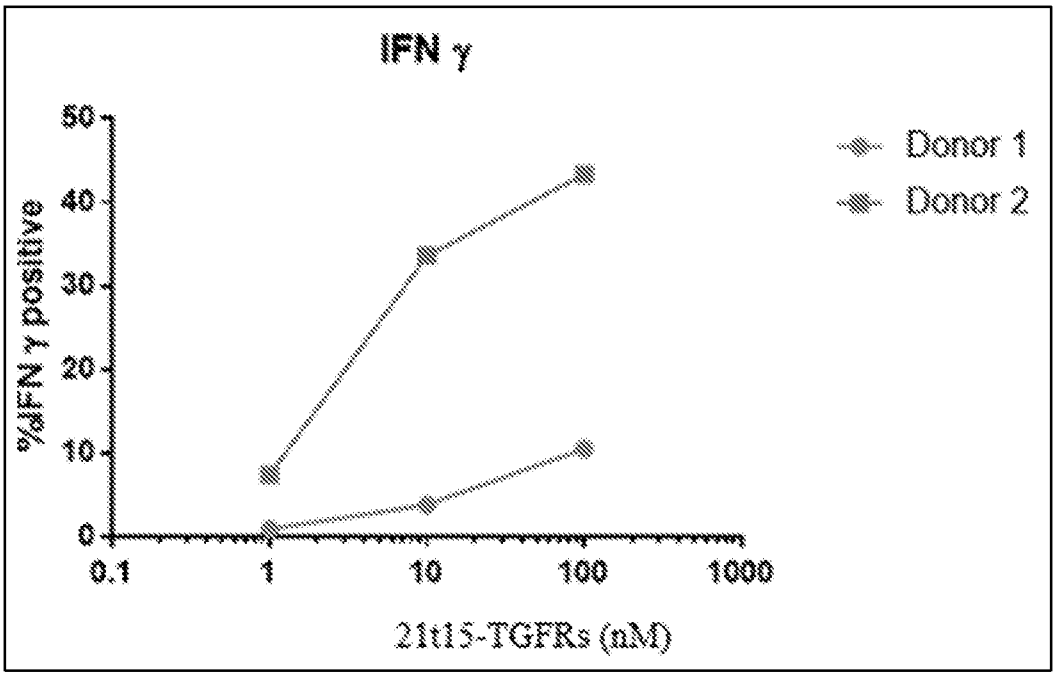


Figure 55

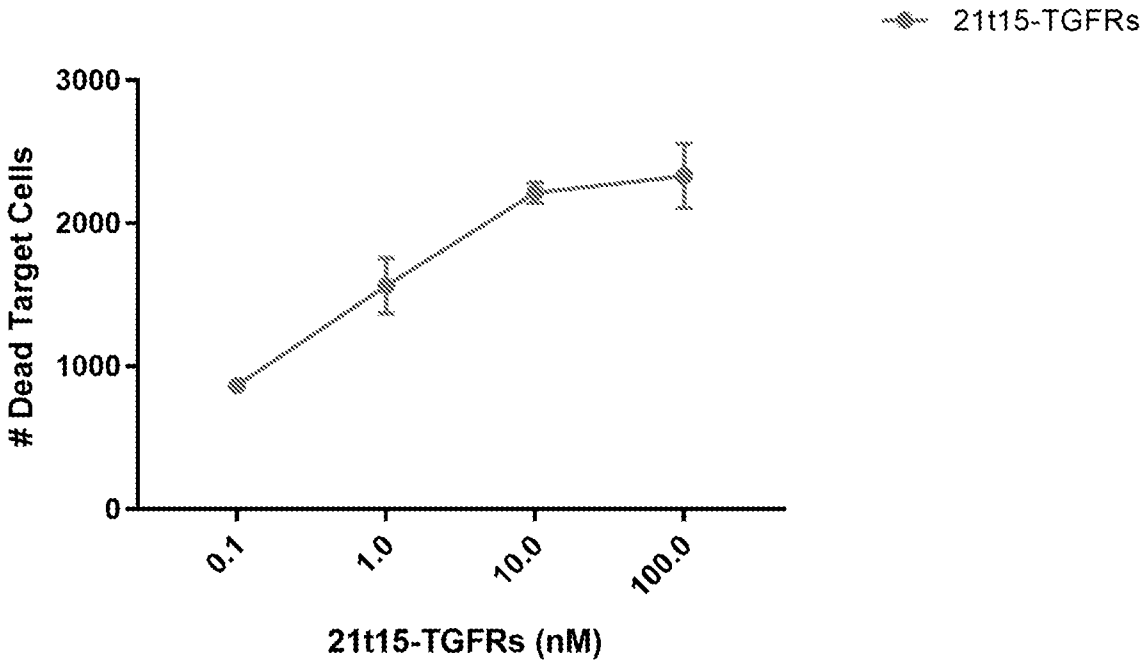


Figure 56

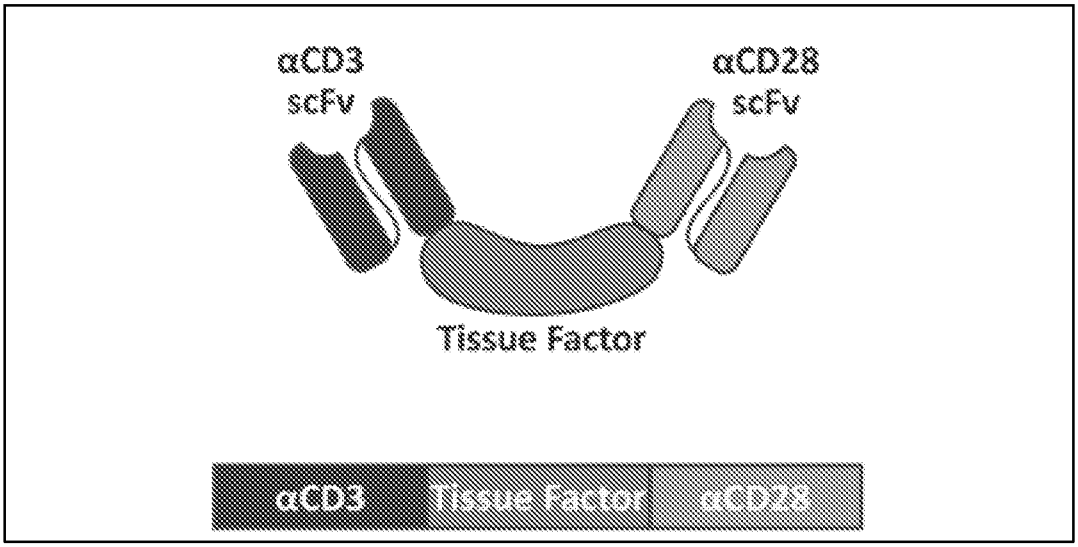

αCD3
scFv
αCD28
scFv
Tissue Factor
αCD3
Tissue Factor
αCD28

Figure 57

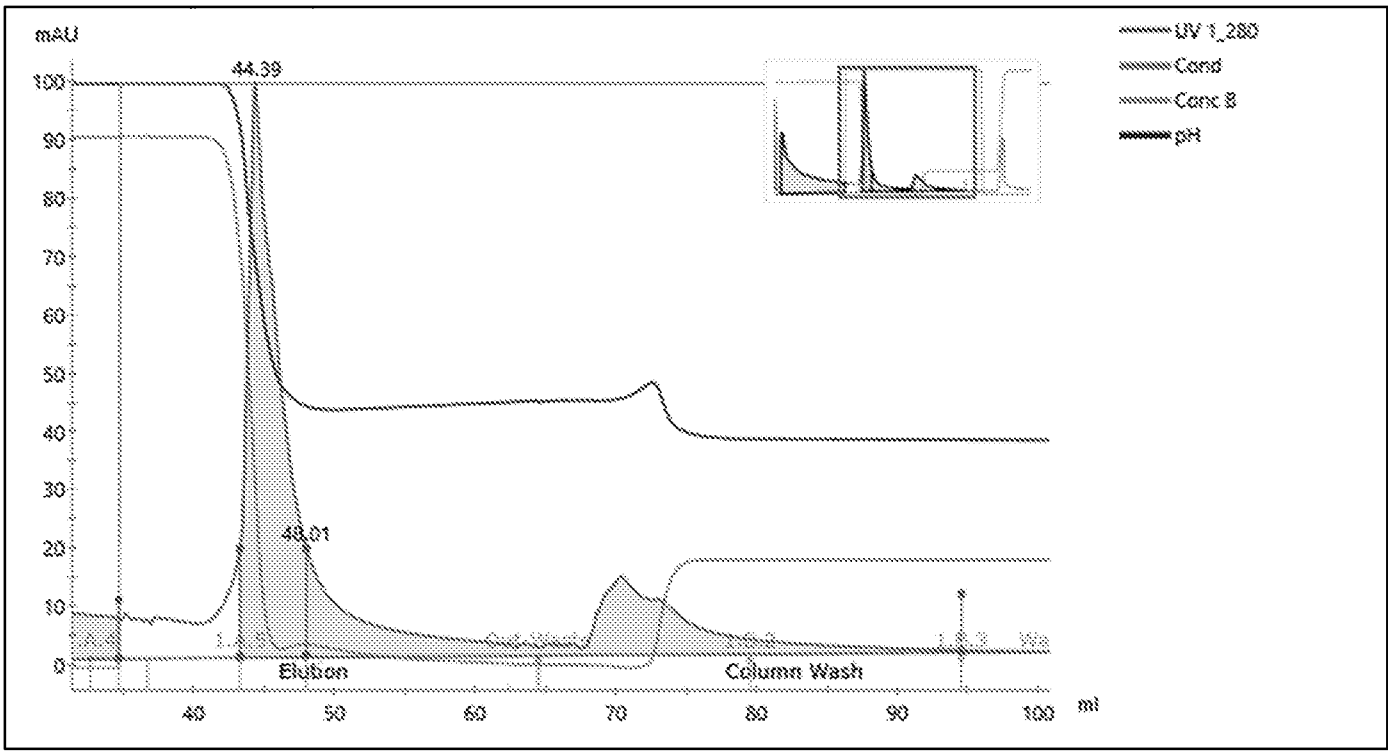

mAU
100
90
80
70
60
50
40
30
20
10
0
44.39
49.01
Elution
Column Wash
40
50
60
70
80
90
100
ml
UV 1_280
Cond
Conc B
pH

Figure 58

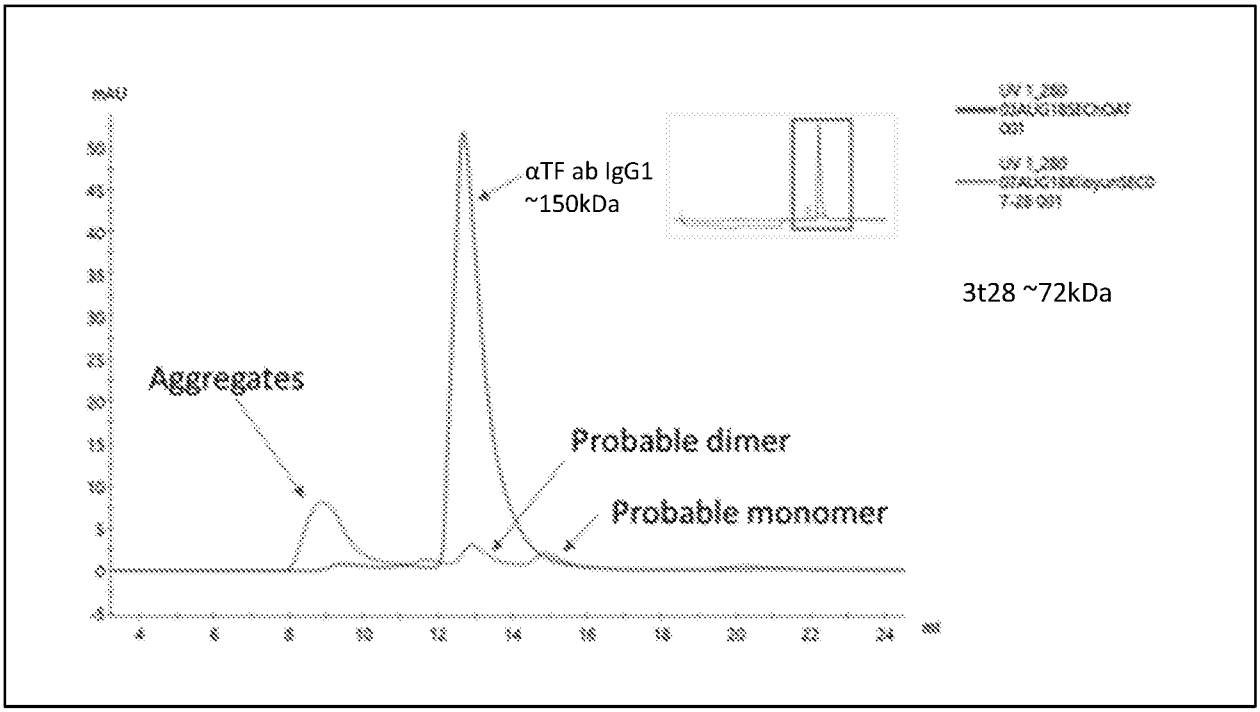

αTF ab IgG1
~150kDa
3t28 ~72kDa
Aggregates
Probable dimer
Probable monomer

Figure 59

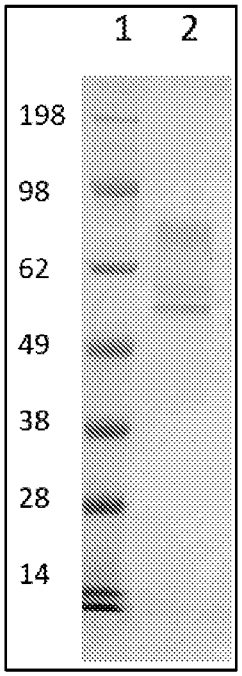


Figure 60

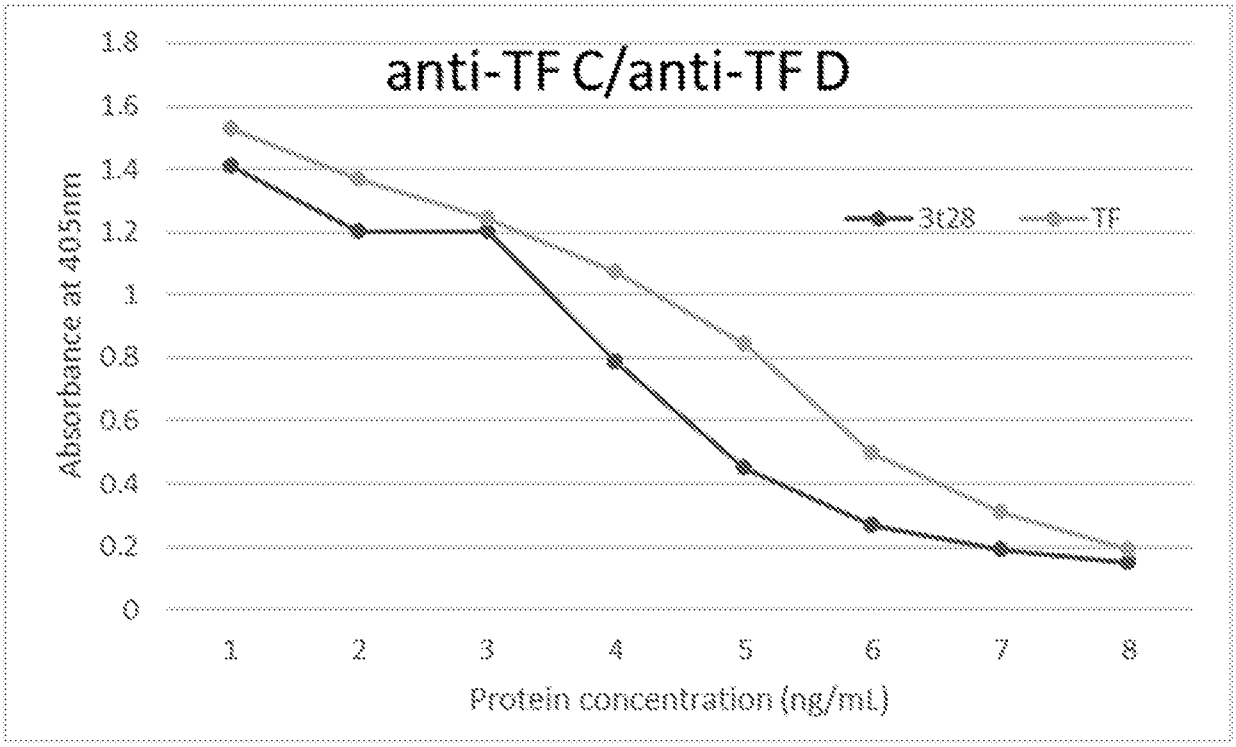


Figure 61

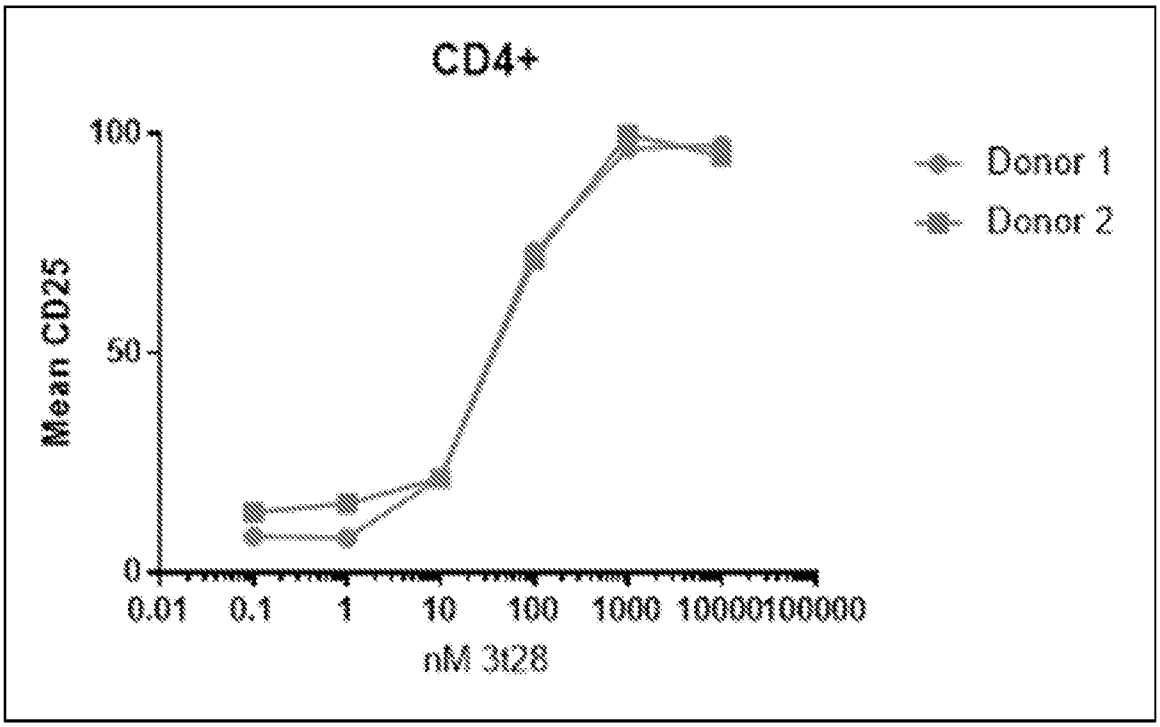


Figure 62

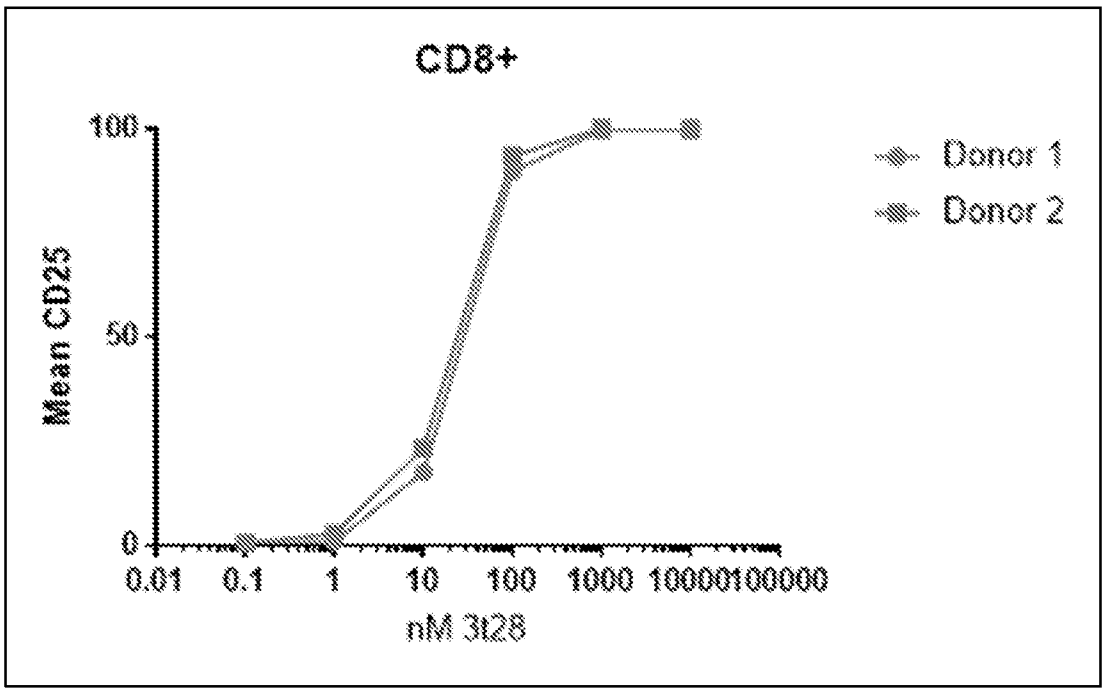


Figure 63

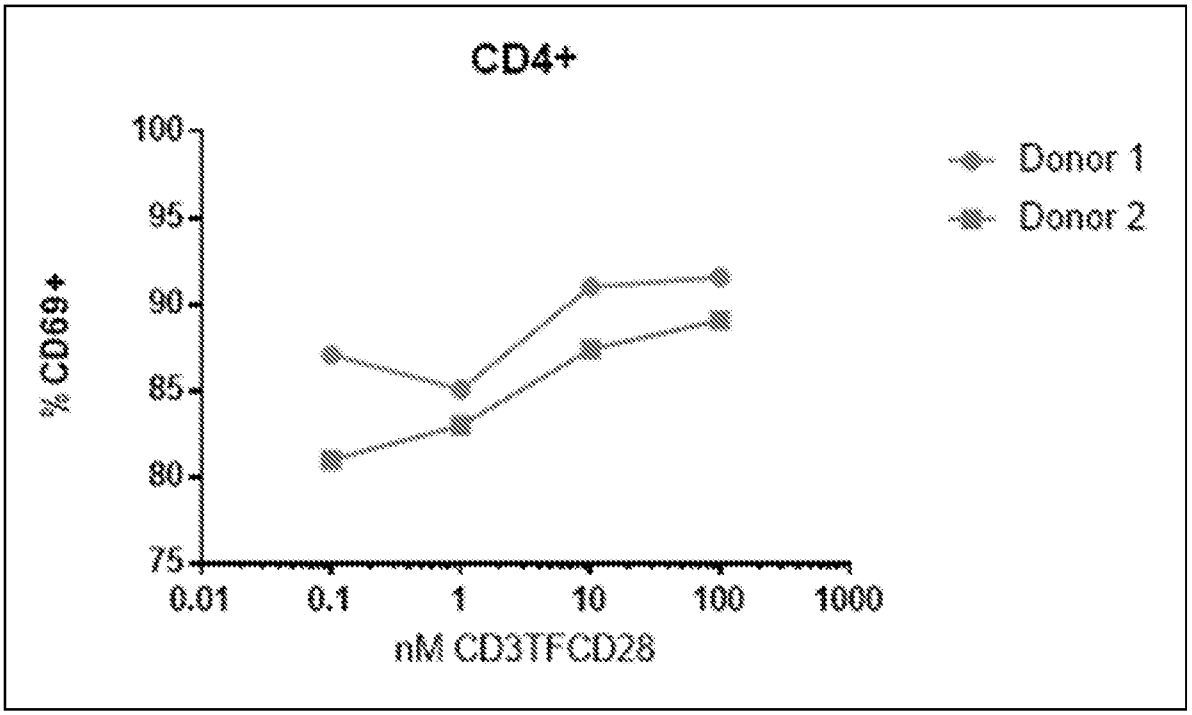

CD4+
100
95
90
85
80
75
% CD69+
0.01
0.1
1
10
100
1000
nM CD3TFCD28
Donor 1
Donor 2

Figure 64



Figure 65

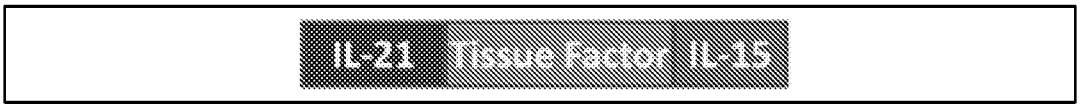


Figure 66

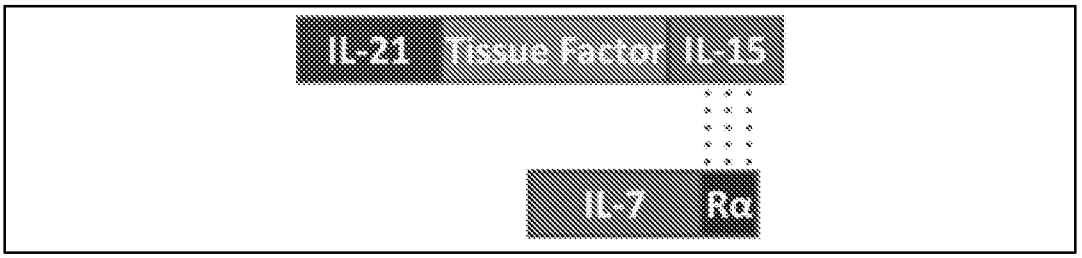


Figure 67

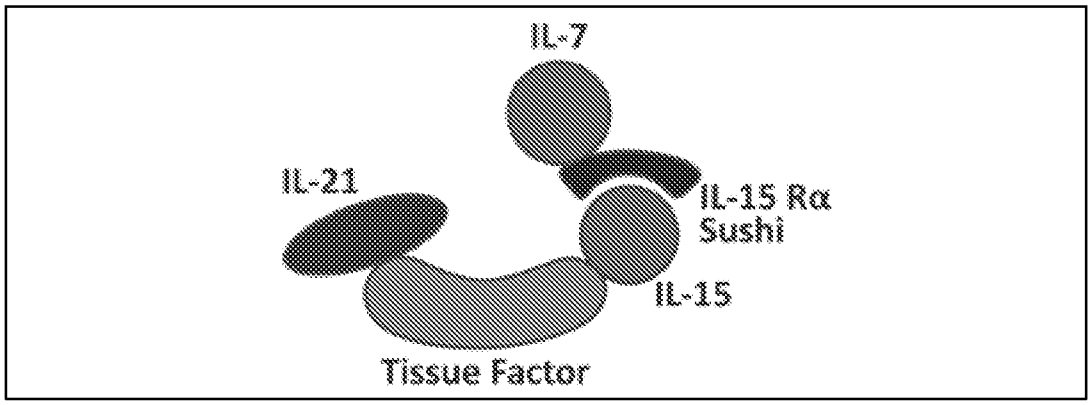


Figure 68



Figure 69

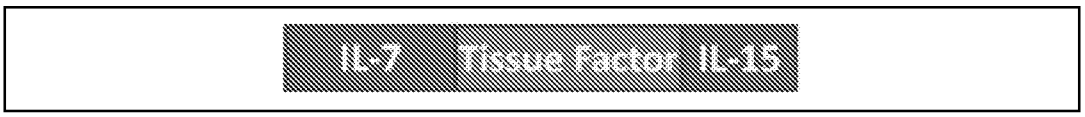


Figure 70

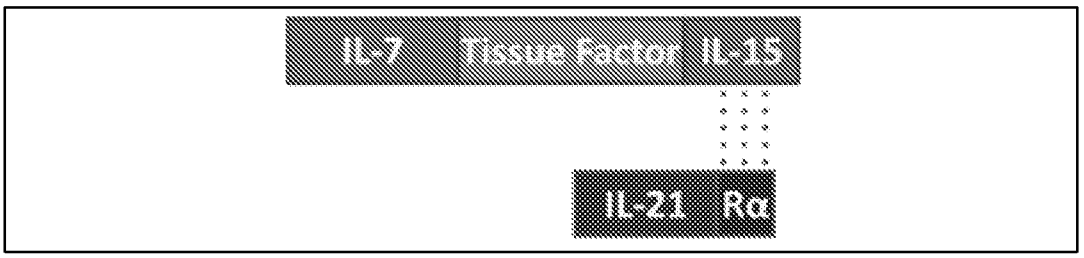


Figure 71

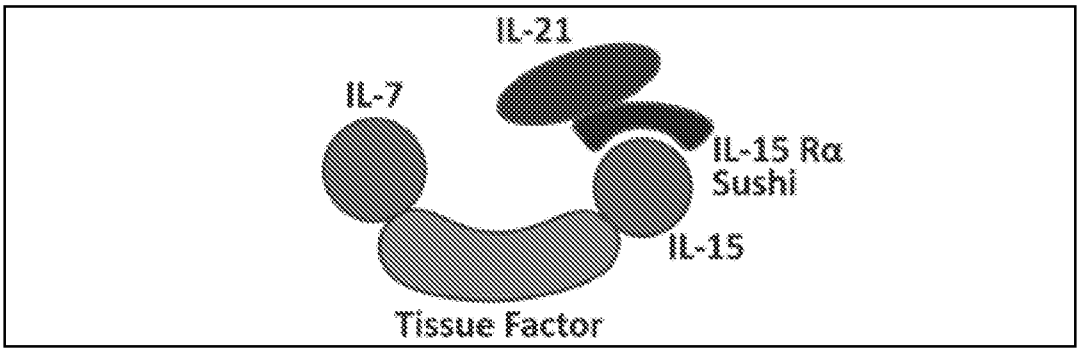


Figure 72

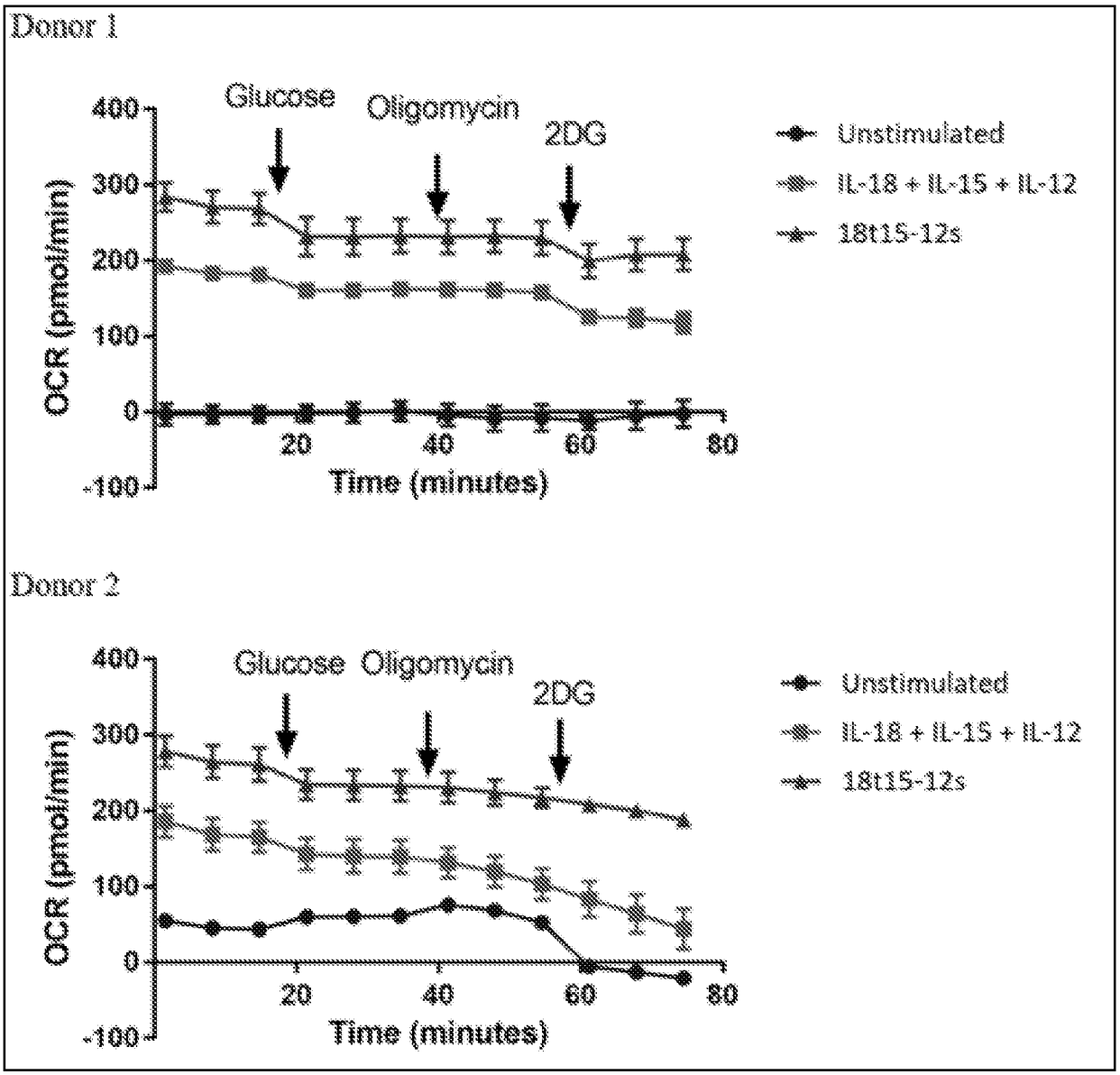

Donor 1
Glucose
Oligomycin
2DG
OCR (pmol/min)
400
300
200
100
0
-100
20
40
60
80
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s
Donor 2
Glucose
Oligomycin
2DG
OCR (pmol/min)
400
300
200
100
0
-100
20
40
60
80
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s

Figure 73

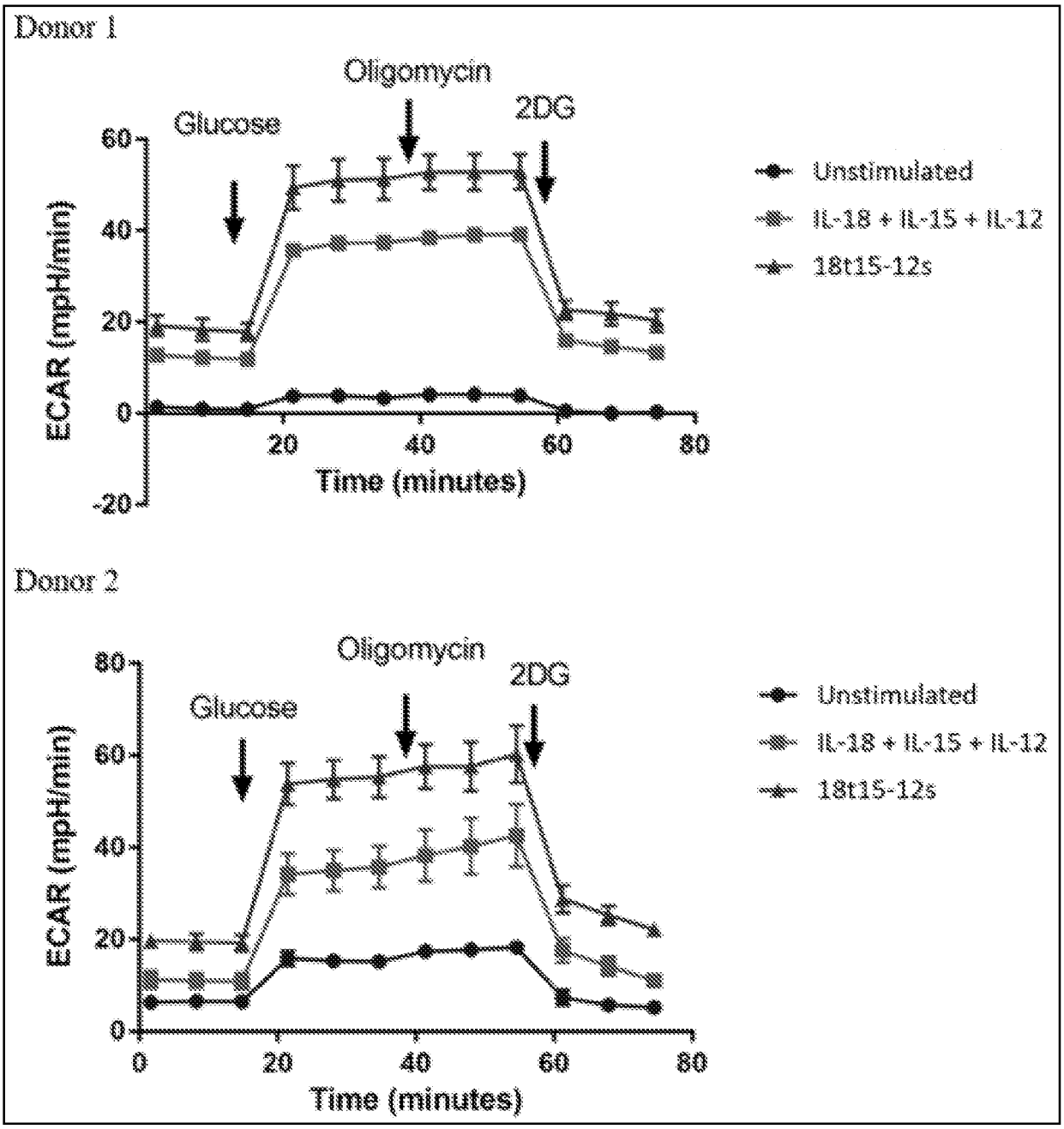

Donor 1
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s
Donor 2
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Unstimulated
IL-18 + IL-15 + IL-12
18t15-12s

Figure 74

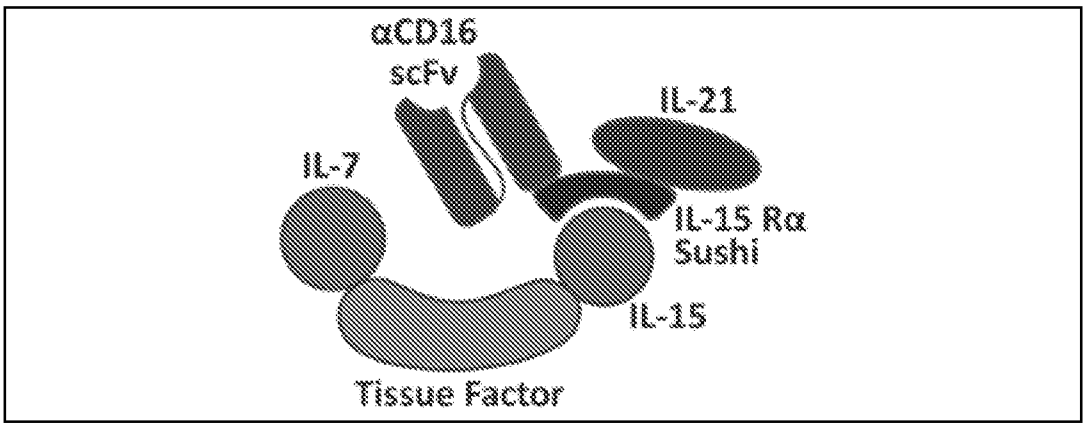


Figure 75

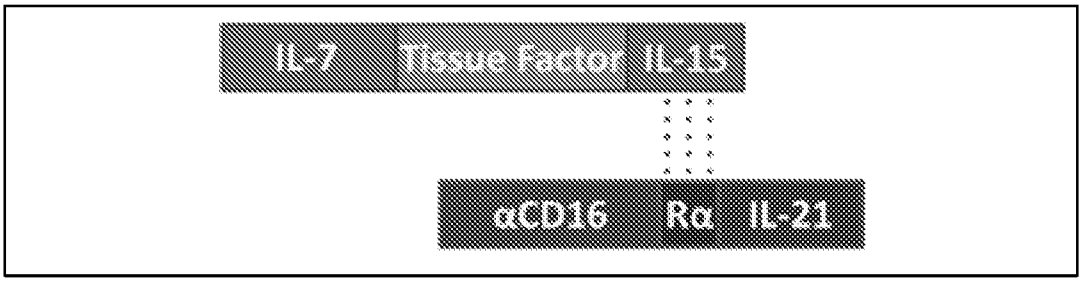


Figure 76

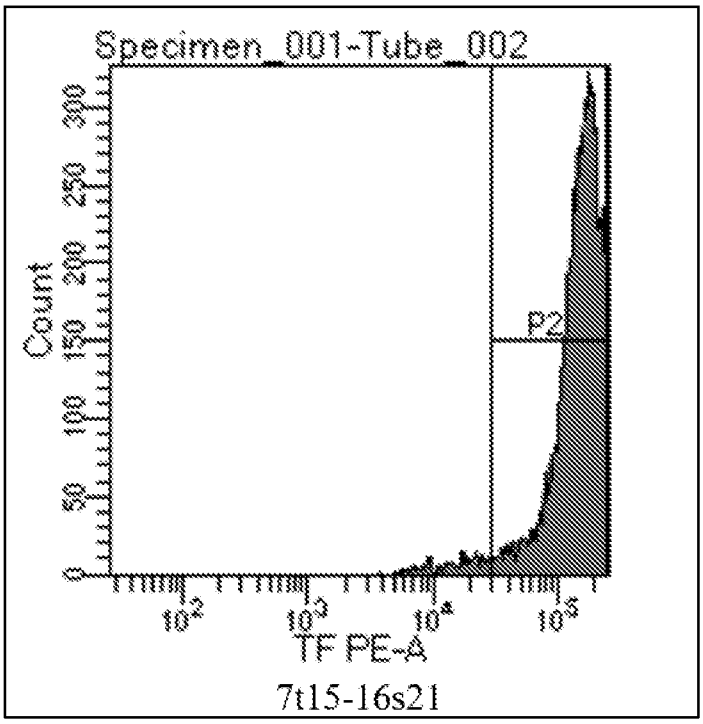


Figure 77A

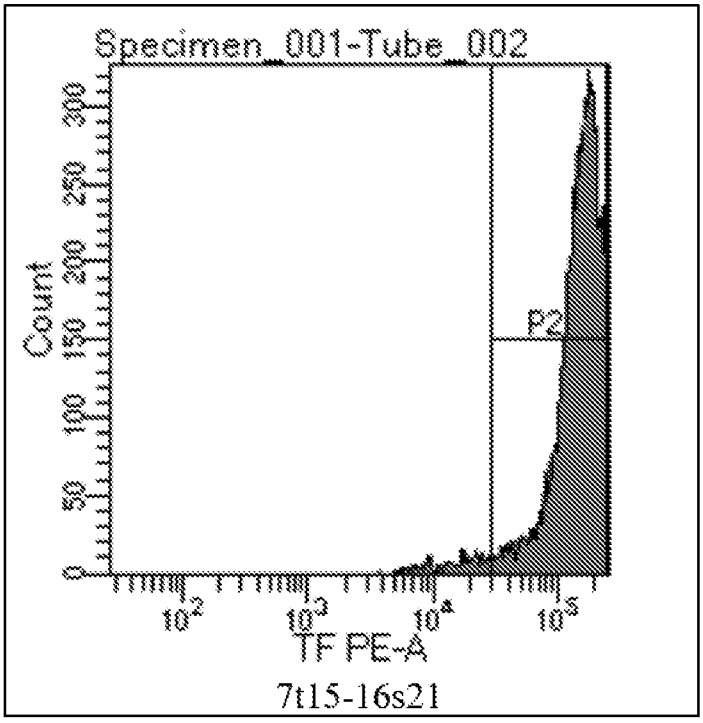


Figure 77B

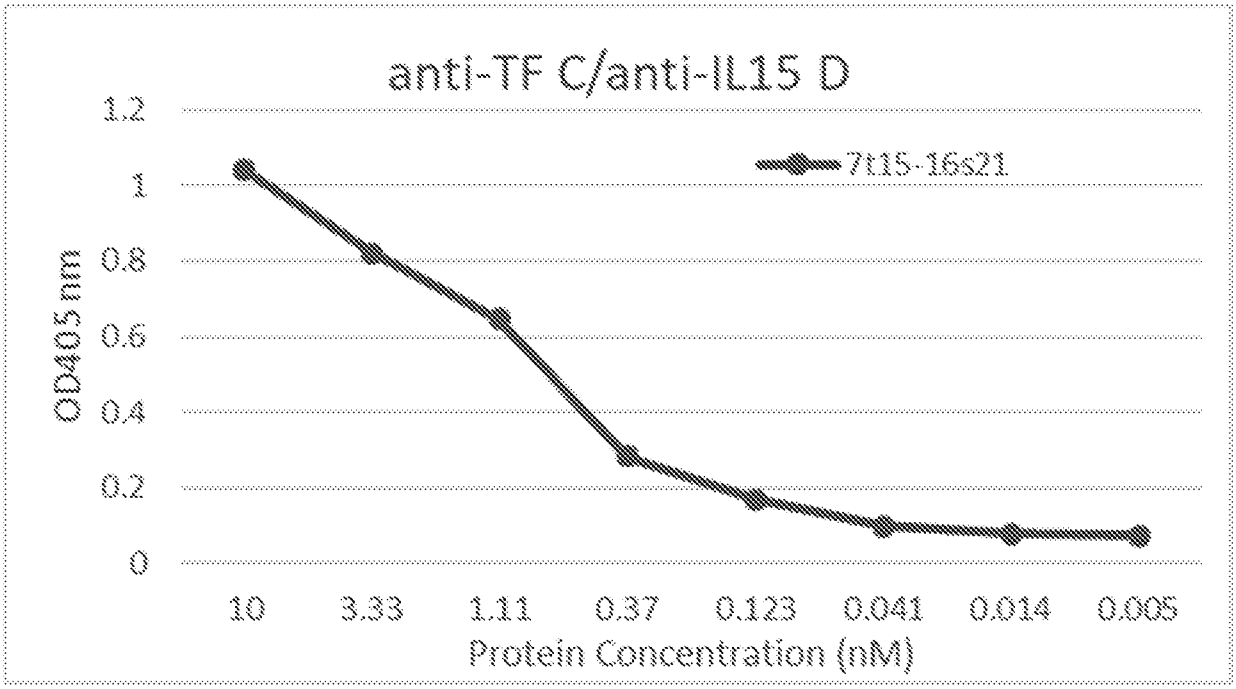


Figure 78A

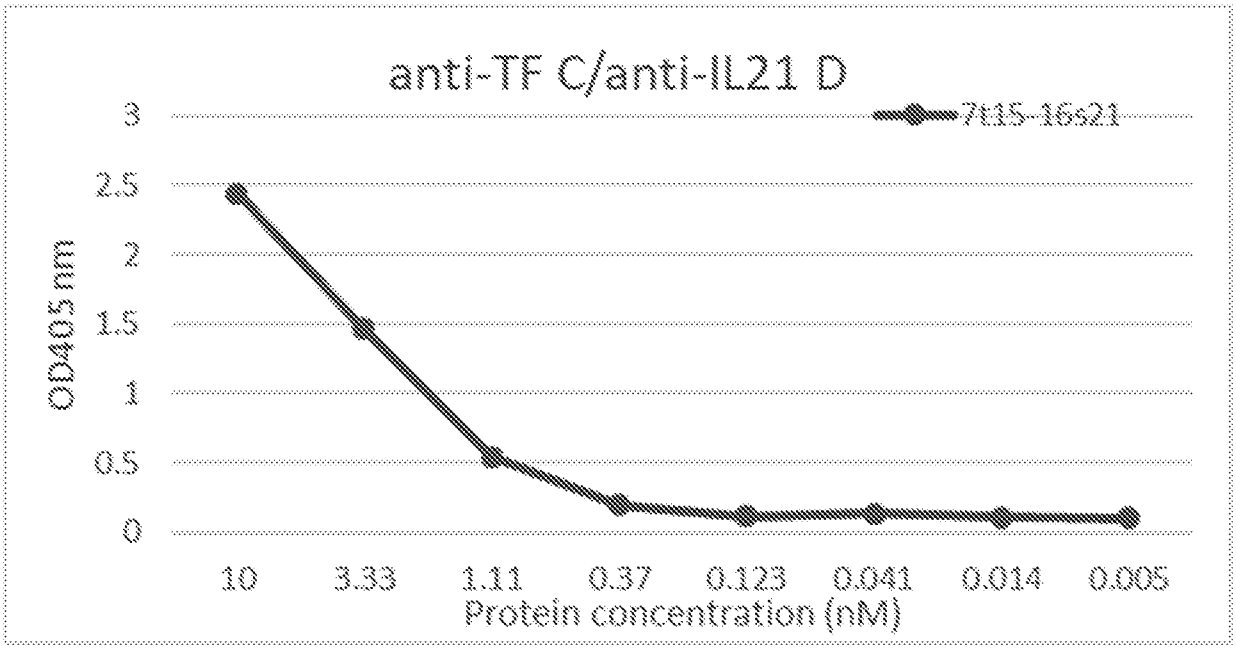


Figure 78B

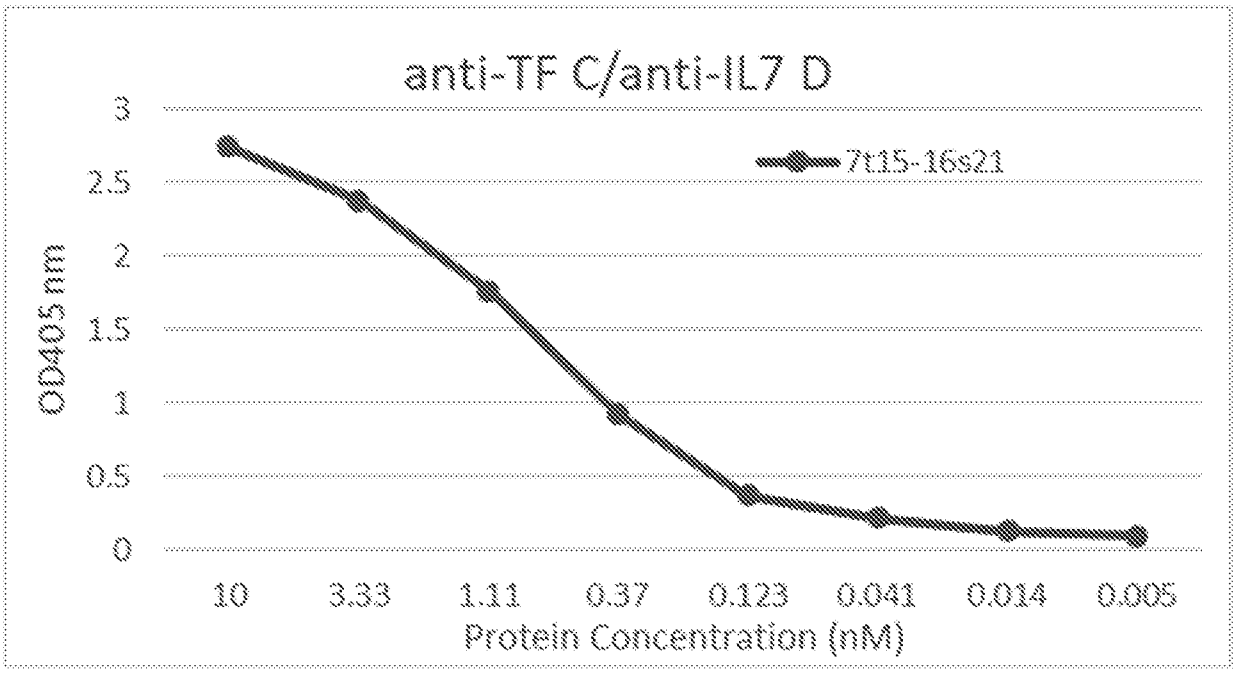

anti-TF C/anti-IL7 D
7t15-16s21
OD405 nm
3
2.5
2
1.5
1
0.5
0
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein Concentration (nM)

Figure 78C

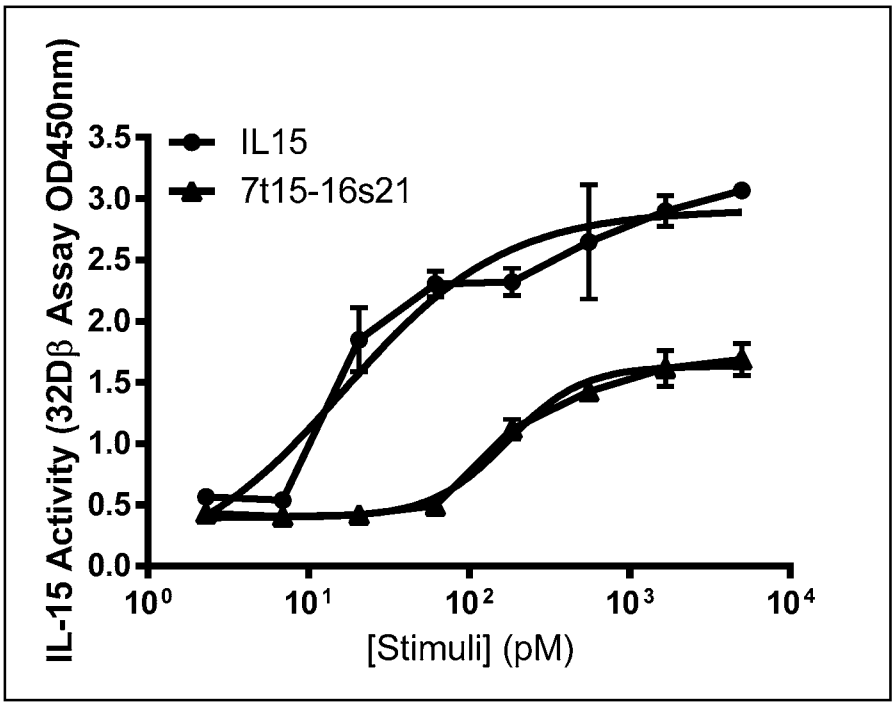

IL-15 Activity (32Dβ Assay OD450nm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
IL15
7t15-16s21
10^0
10^1
10^2
10^3
10^4
[Stimuli] (pM)

Figure 79

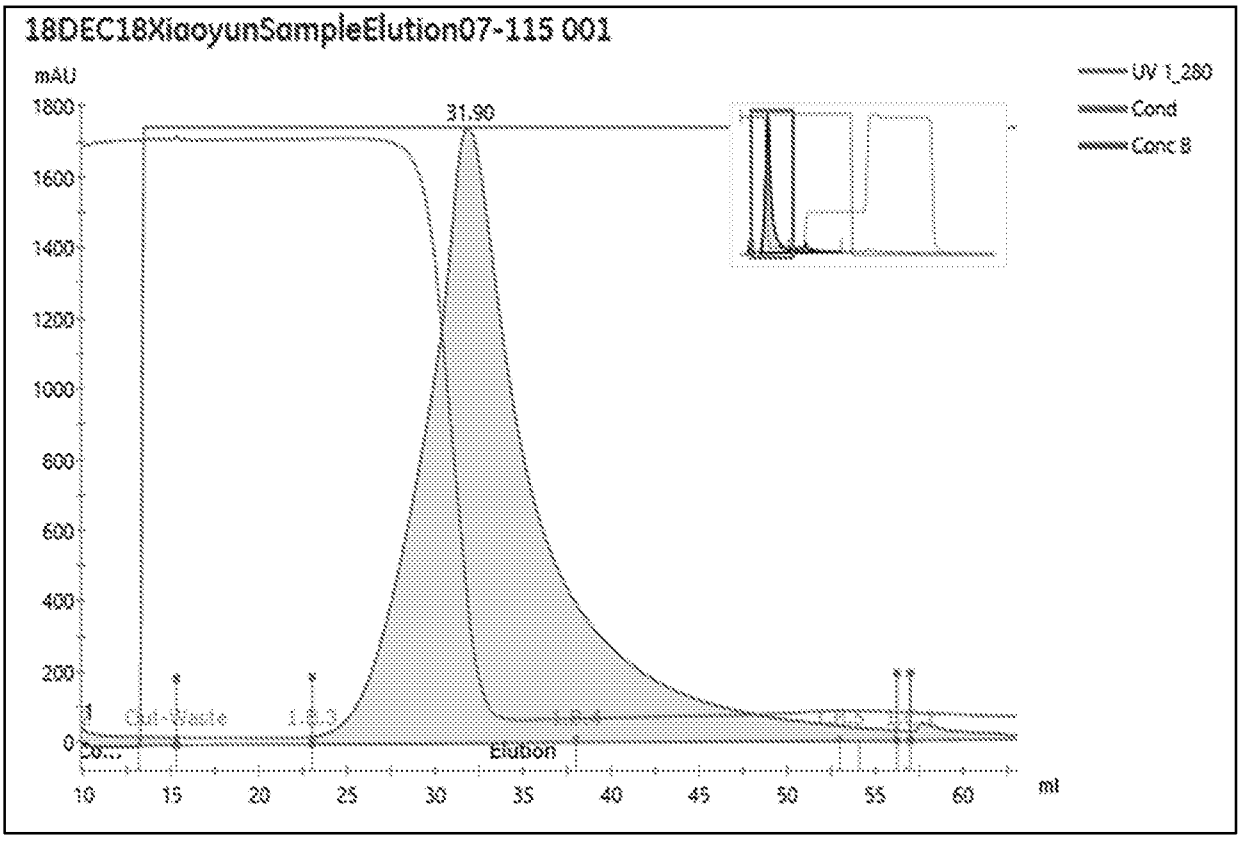

18DEC18XiaoyunSampleElution07-115 001
mAU
1800
1600
1400
1200
1000
800
600
400
200
0
31.90
UV 1_280
Cond
Conc B
Elution
ml
10
15
20
25
30
35
40
45
50
55
60

Figure 80

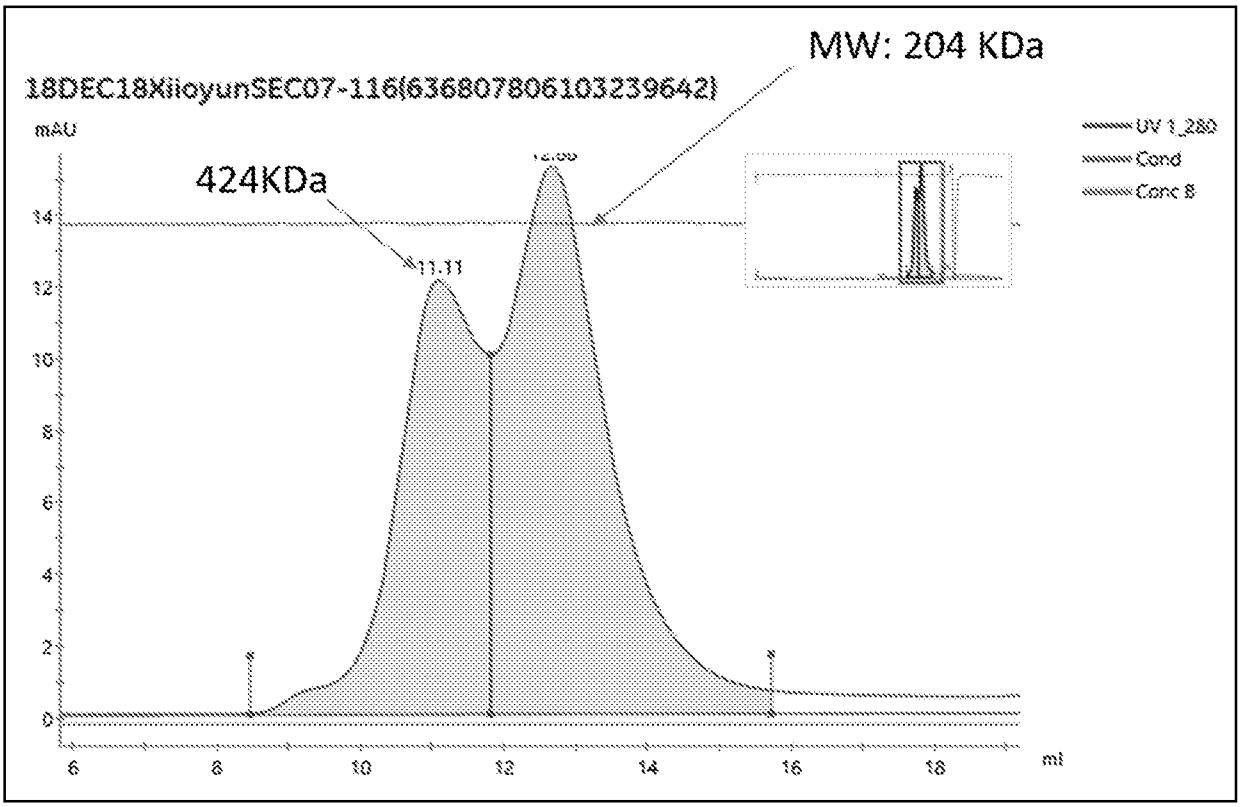

MW: 204 KDa
18DEC18XiloyunSEC07-116(636807806103239642)
mAU
424KDa
11.11
UV 1_280
Cond
Conc B
14
12
10
8
6
4
2
0
6
8
10
12
14
16
18
ml

Figure 81

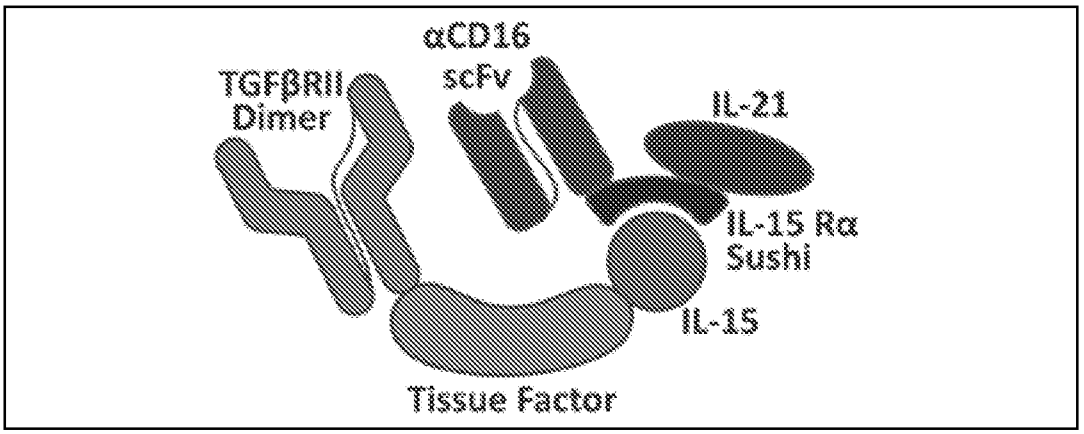


Figure 82

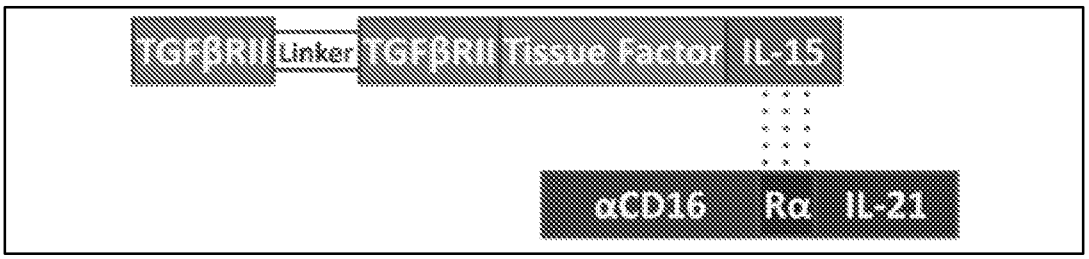


Figure 83

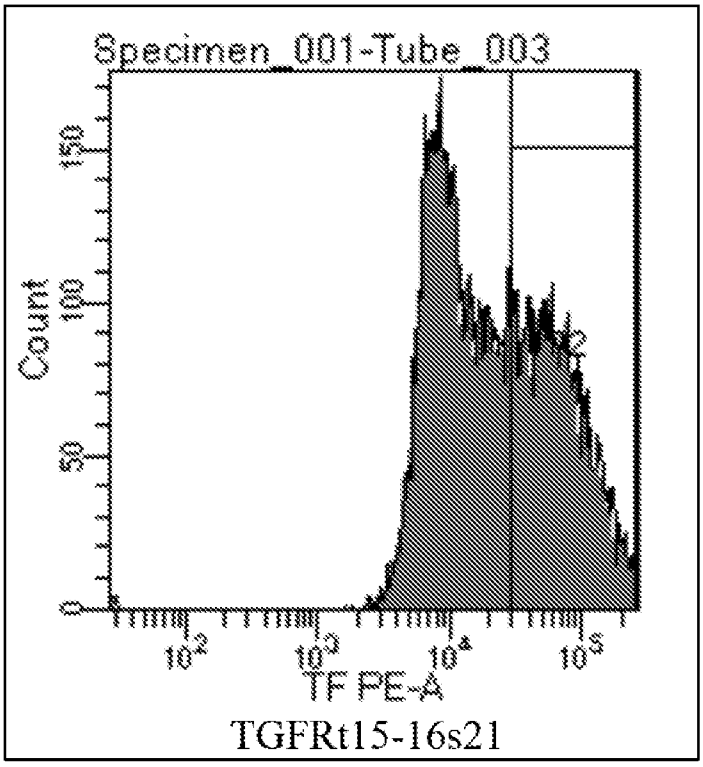

Specimen_001-Tube_003
Count
150
100
50
0
10^2
10^3
10^4
10^5
TF PE-A
TGFRt15-16s21

Figure 84A

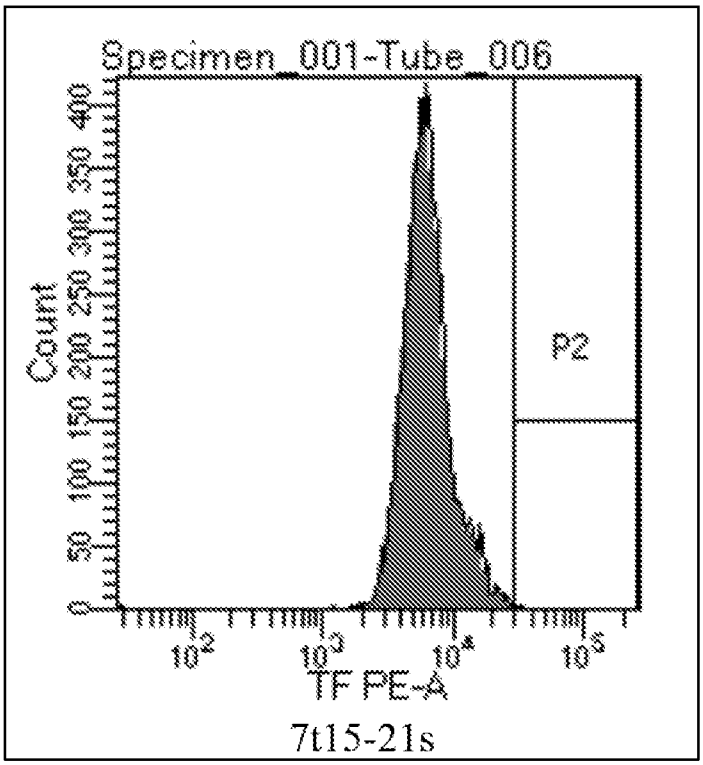


Figure 84B

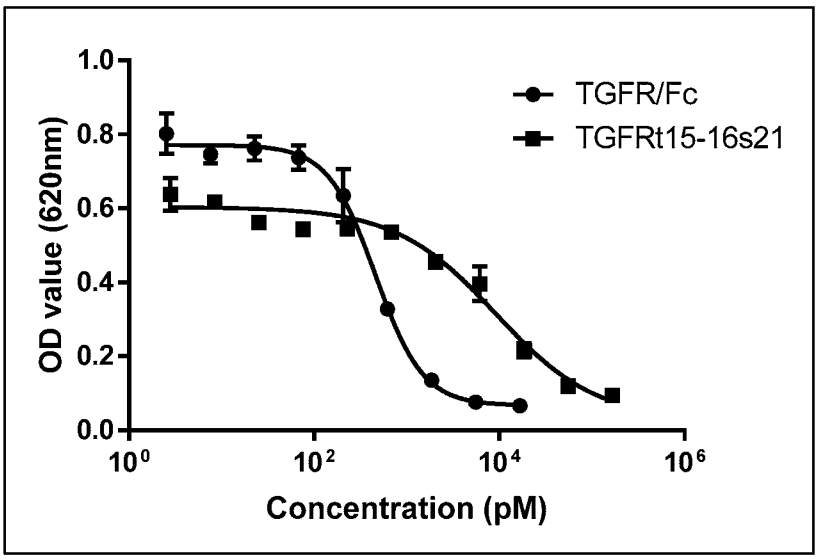


Figure 85

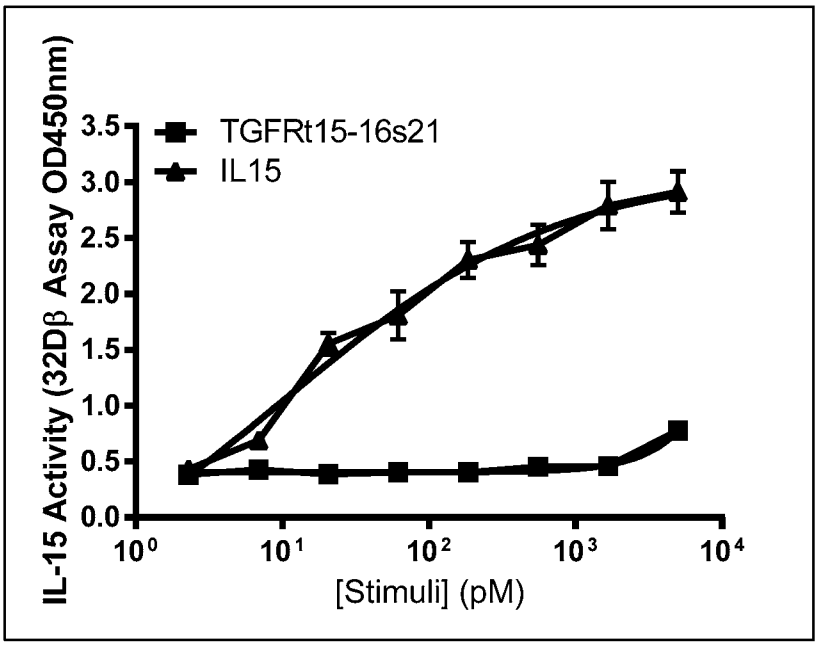


Figure 86

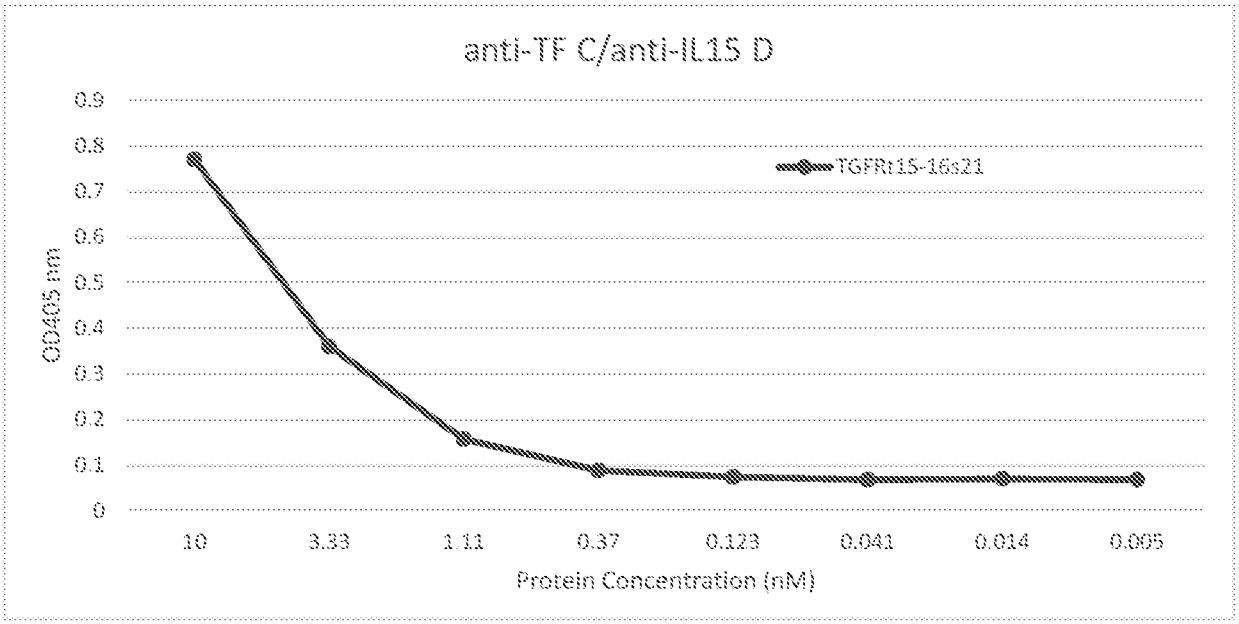


Figure 87A

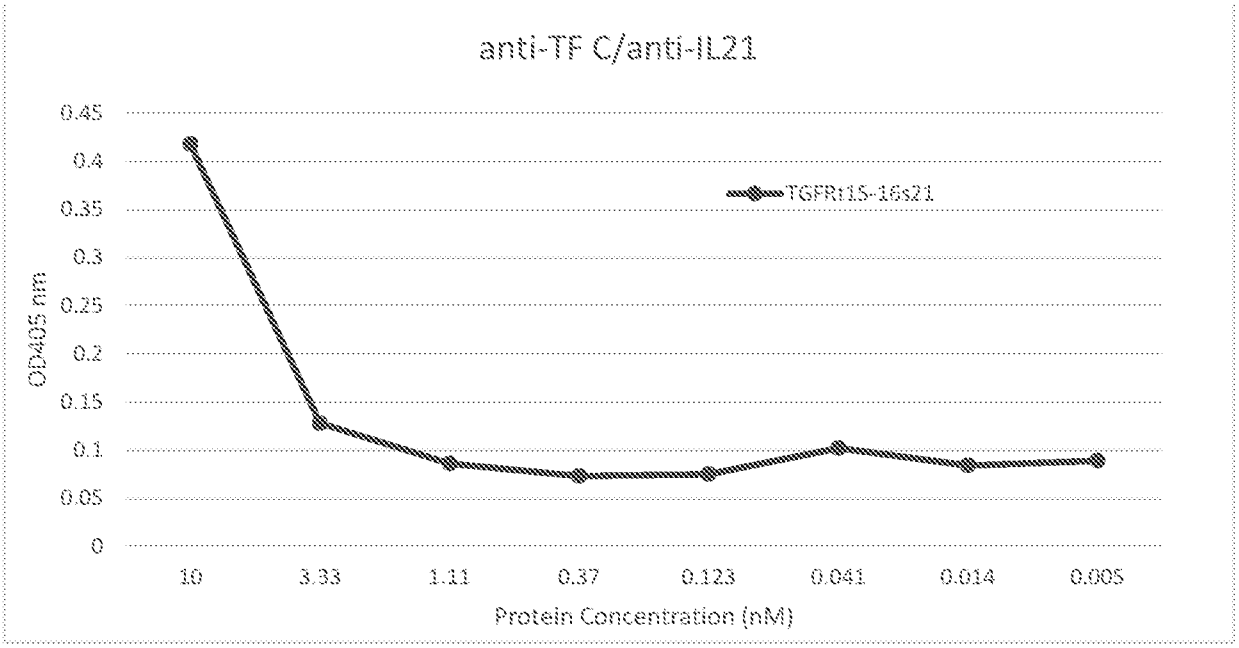


Figure 87B

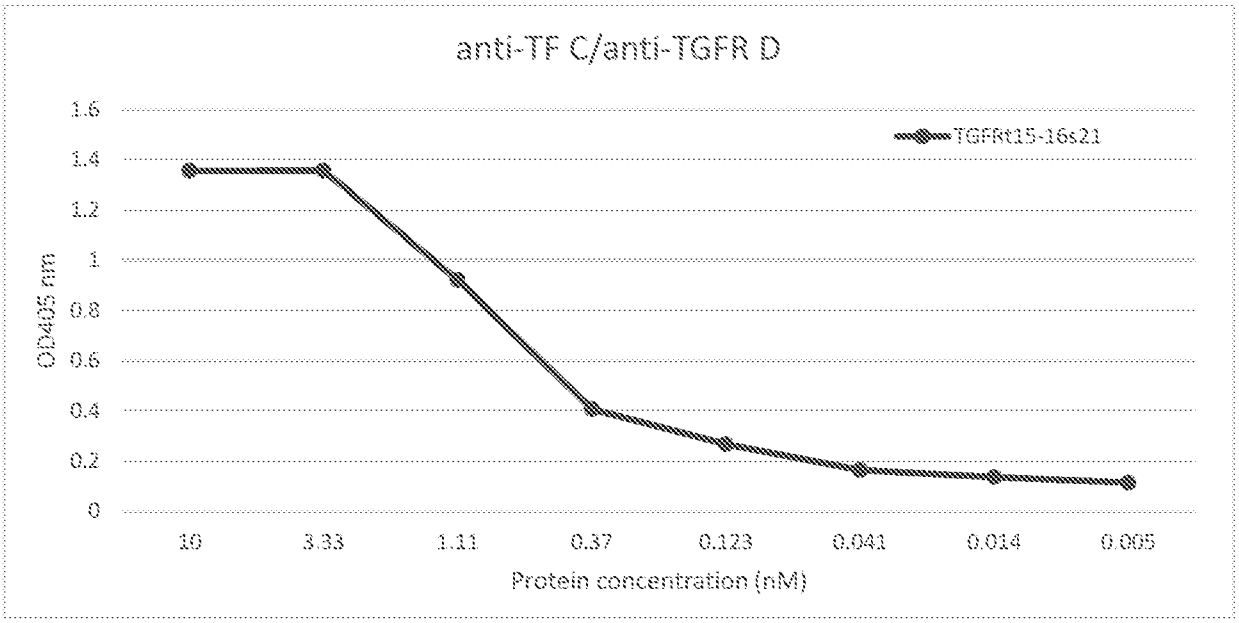


Figure 87C

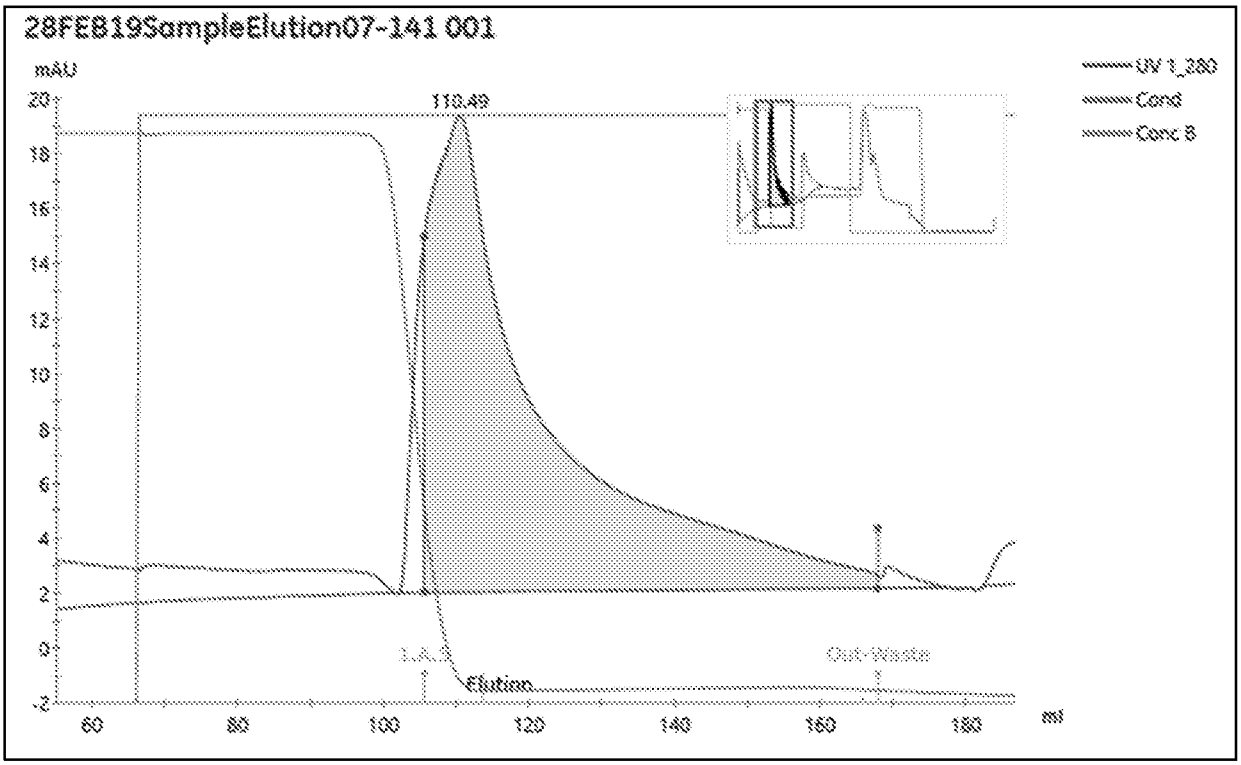


Figure 88

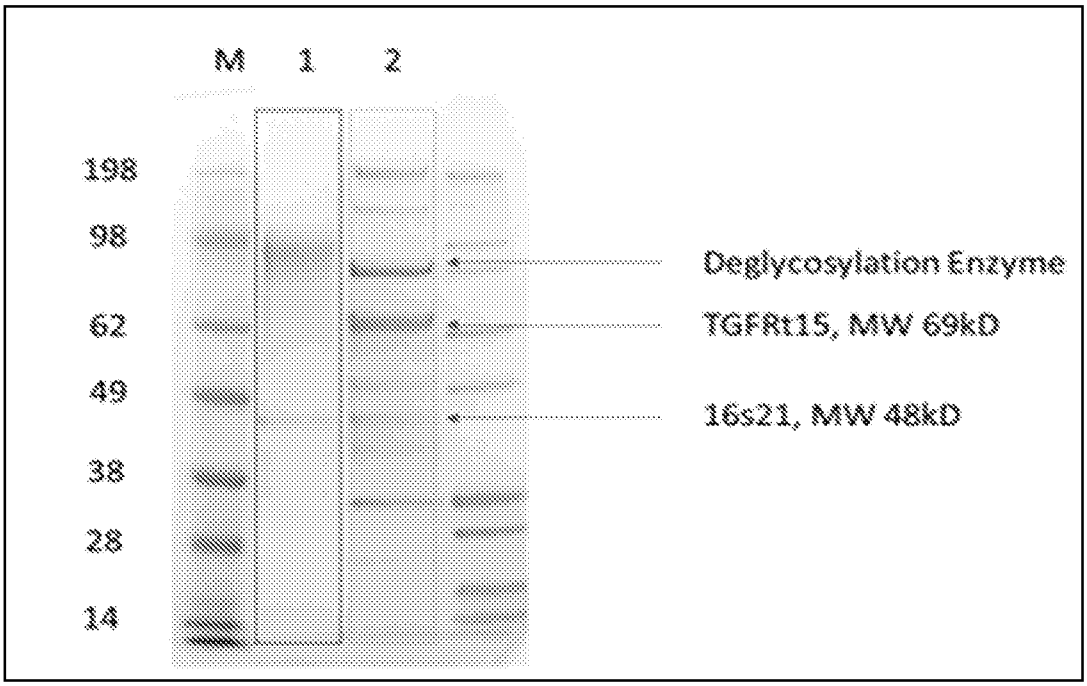


Figure 89

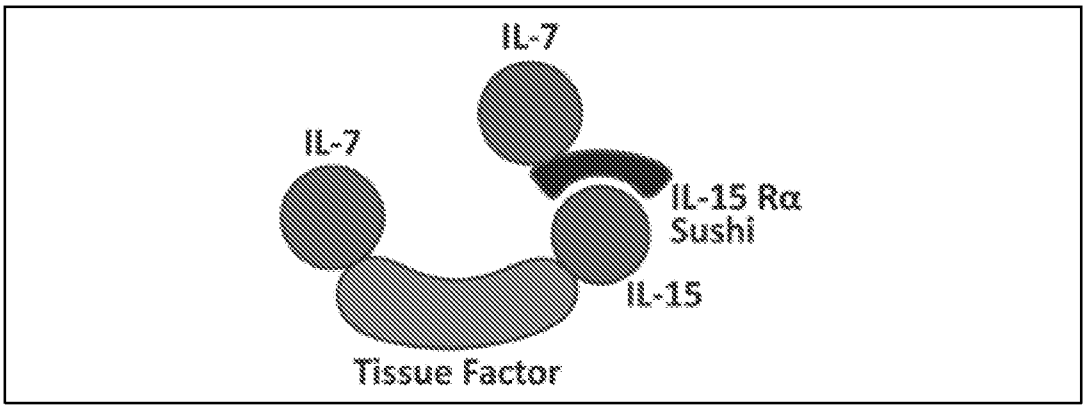


Figure 90

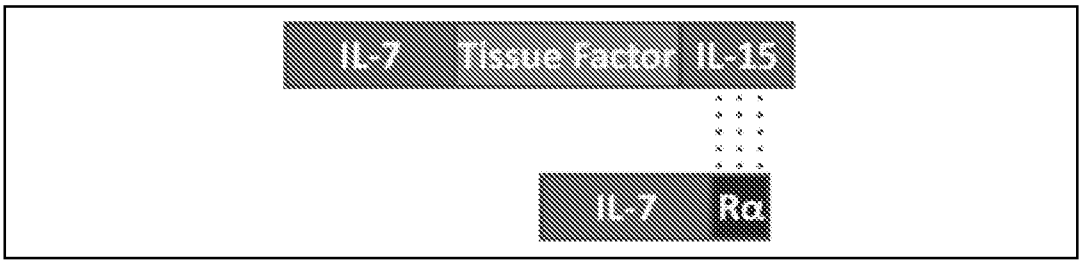


Figure 91

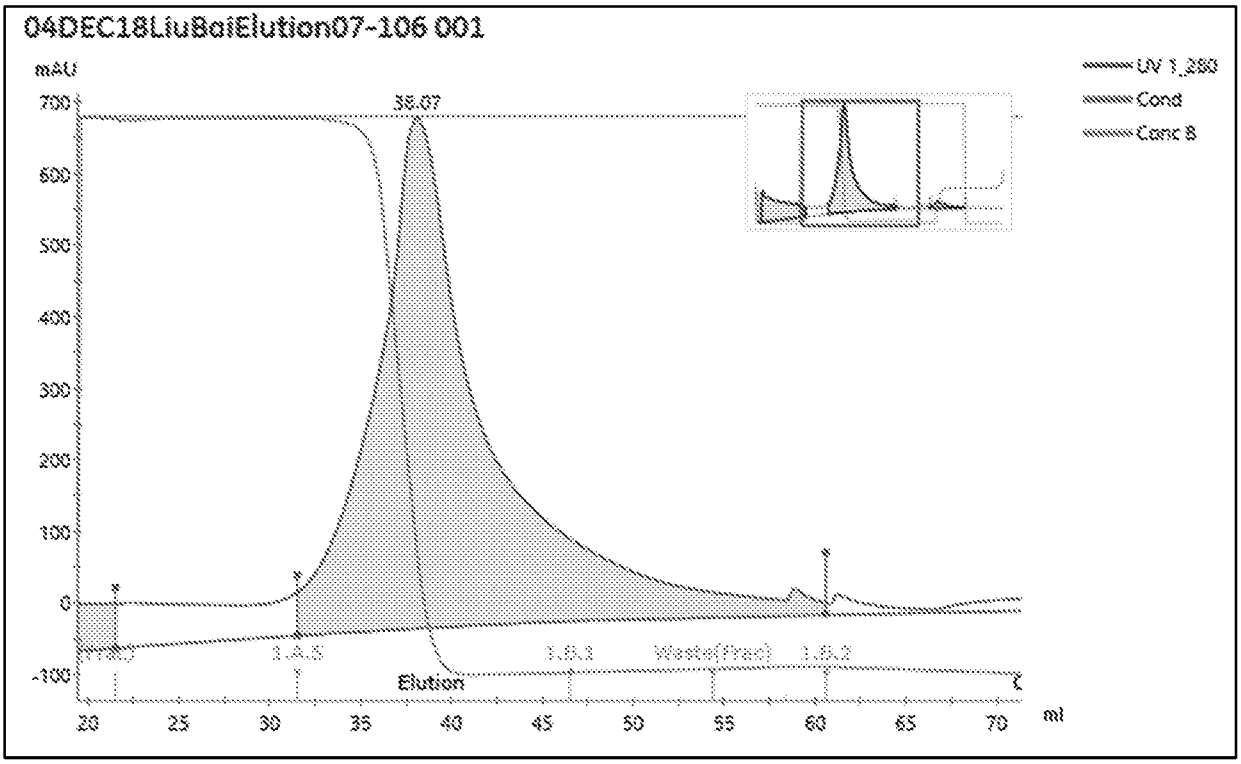


Figure 92

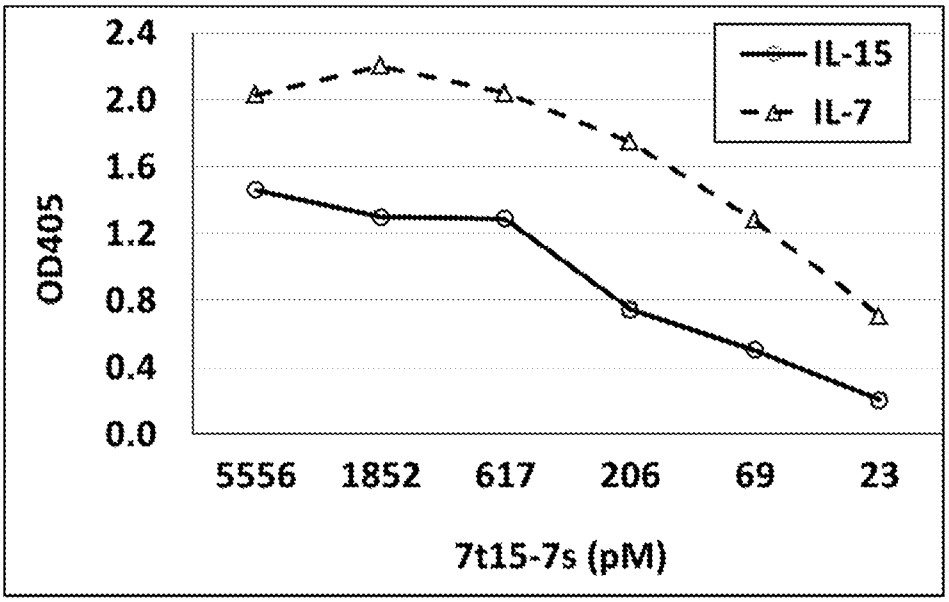

2.4
2.0
1.6
1.2
0.8
0.4
0.0
OD405
IL-15
IL-7
5556
1852
617
206
69
23
7t15-7s (pM)

Figure 93

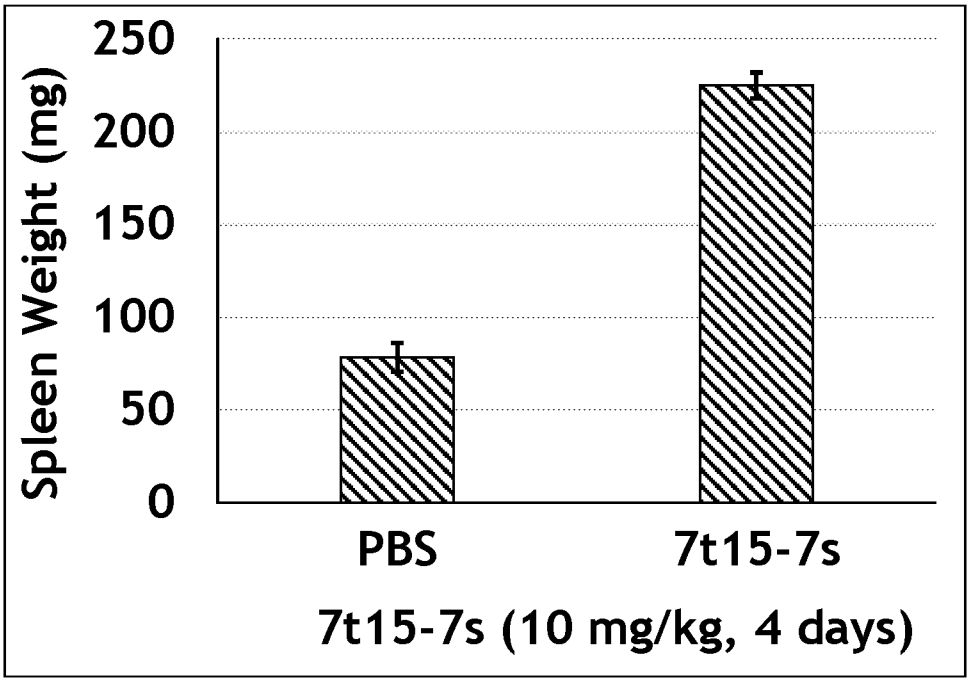


Figure 94A

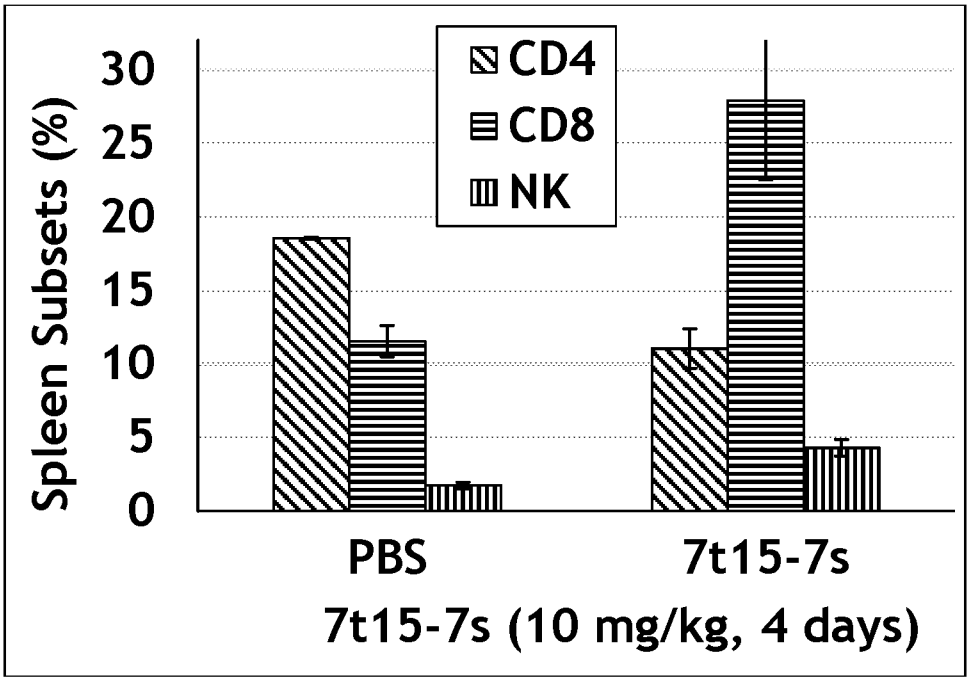


Figure 94B

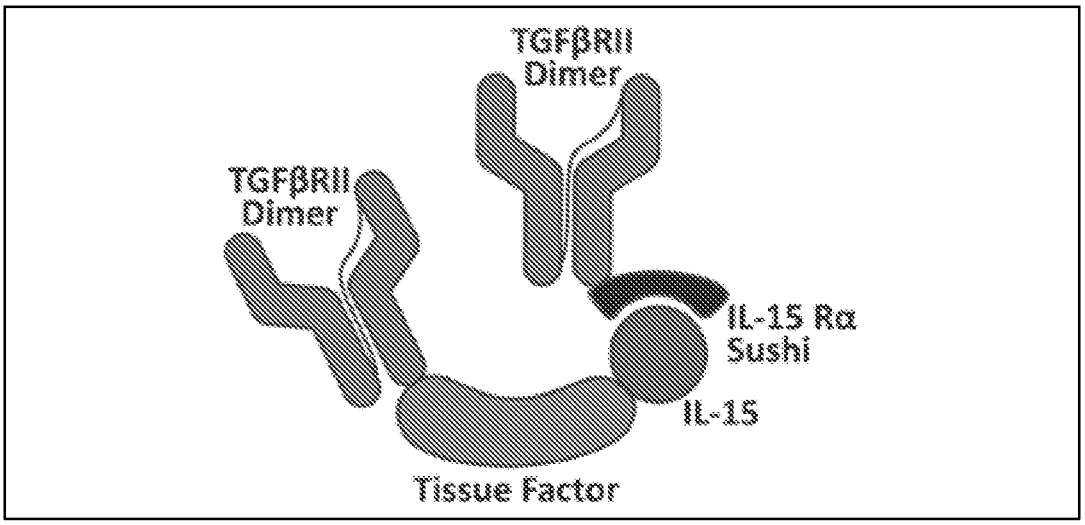


Figure 95

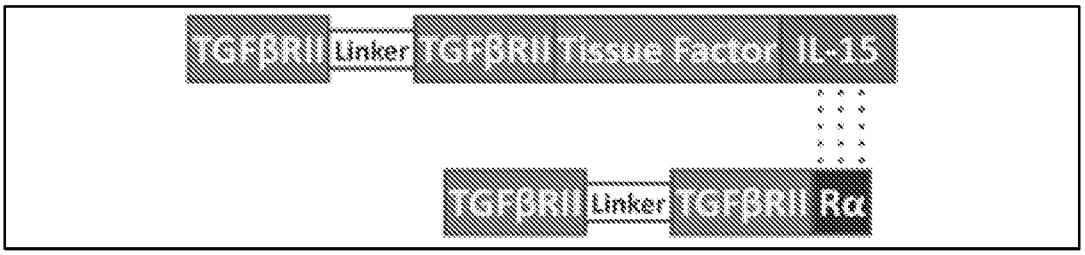


Figure 96

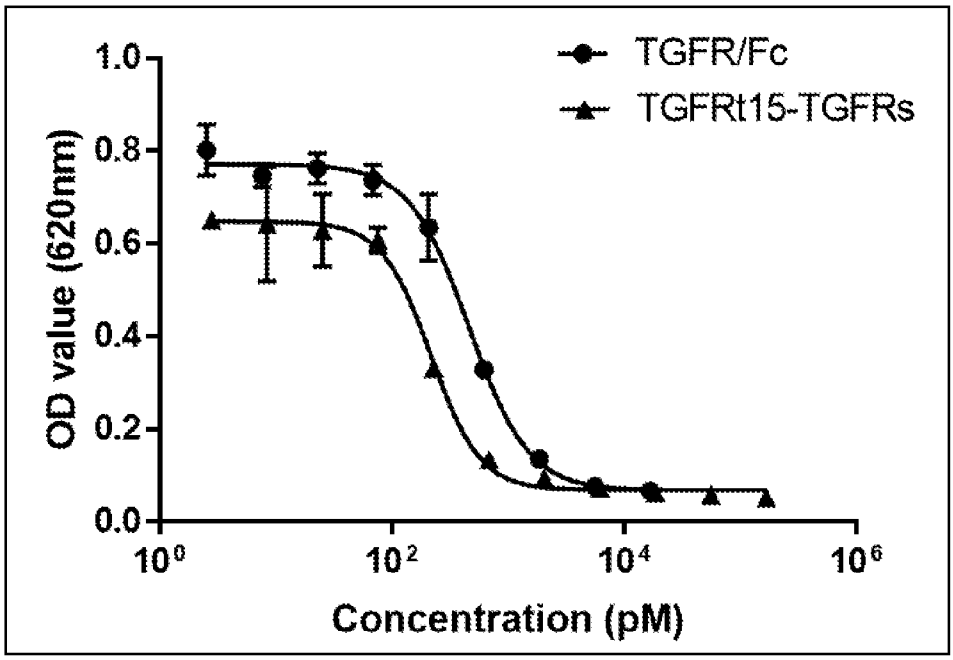


Figure 97

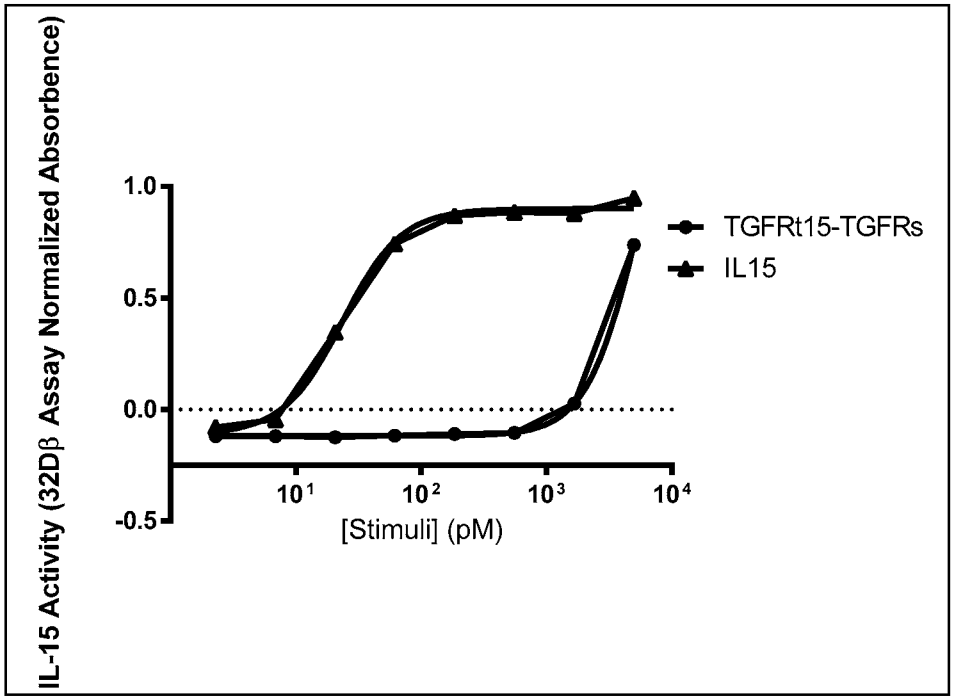


Figure 98

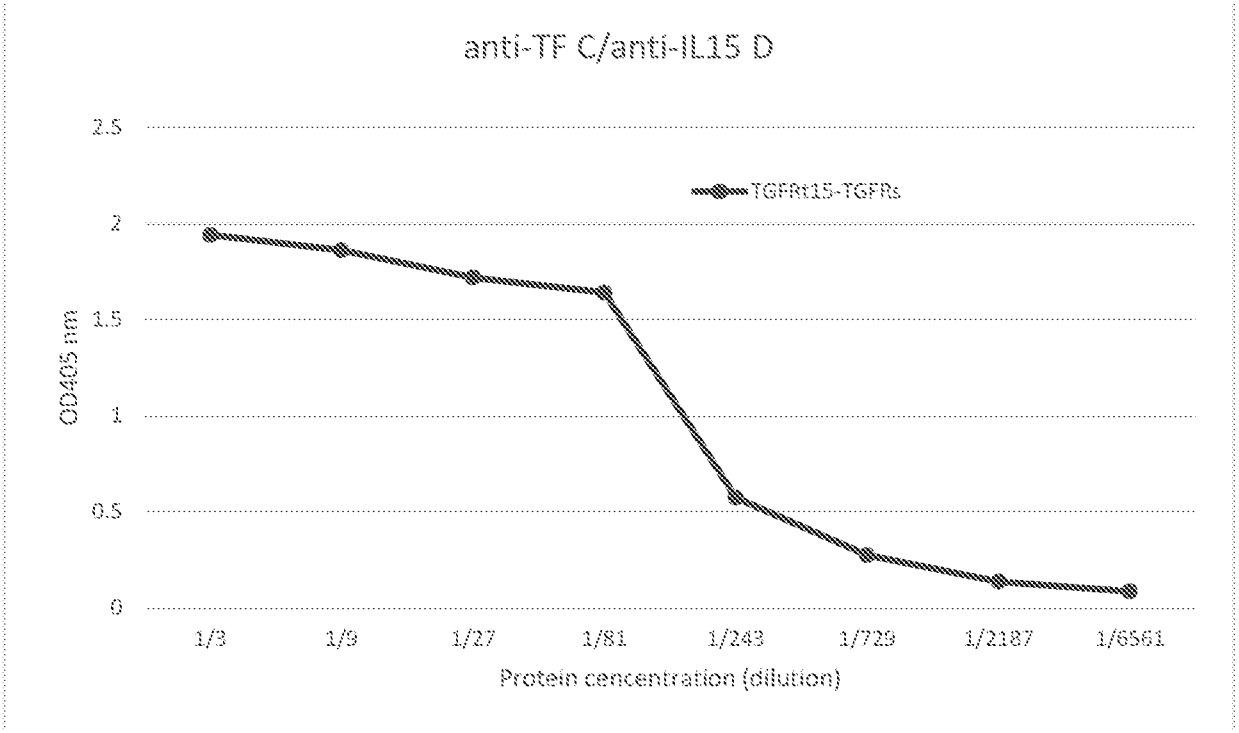


Figure 99A

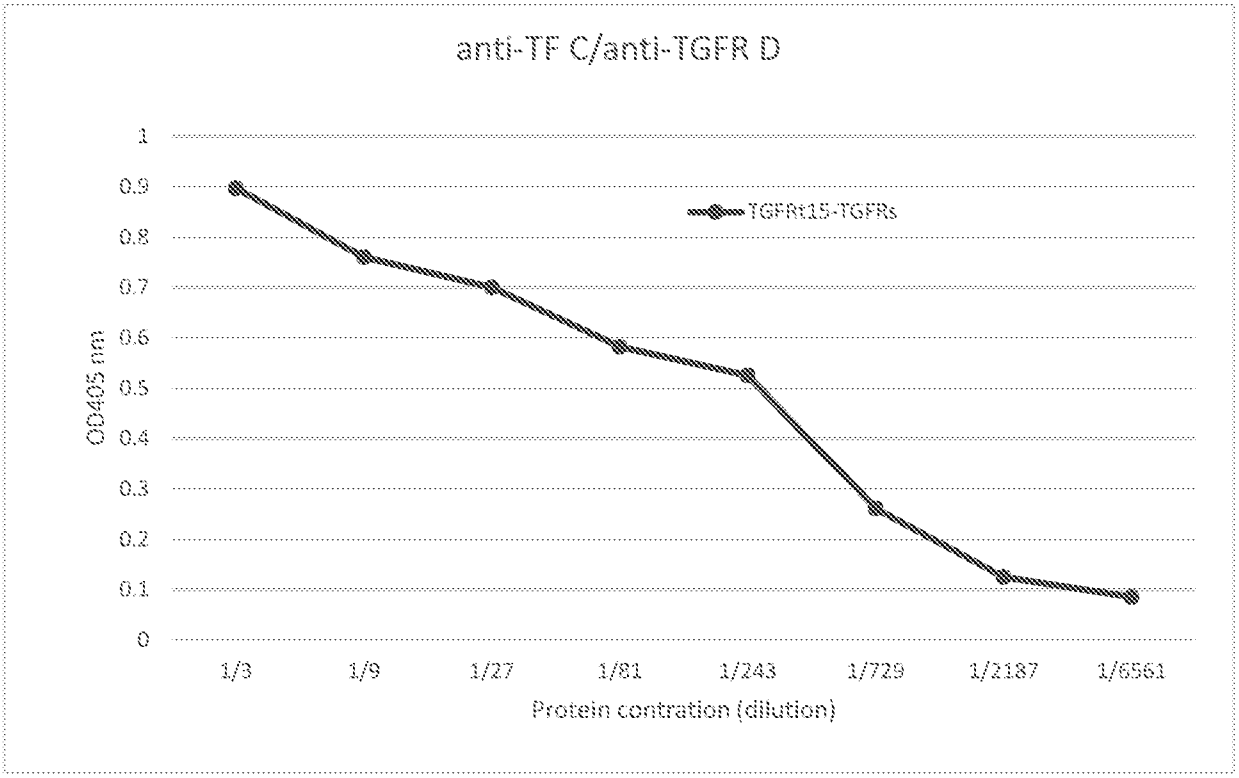


Figure 99B

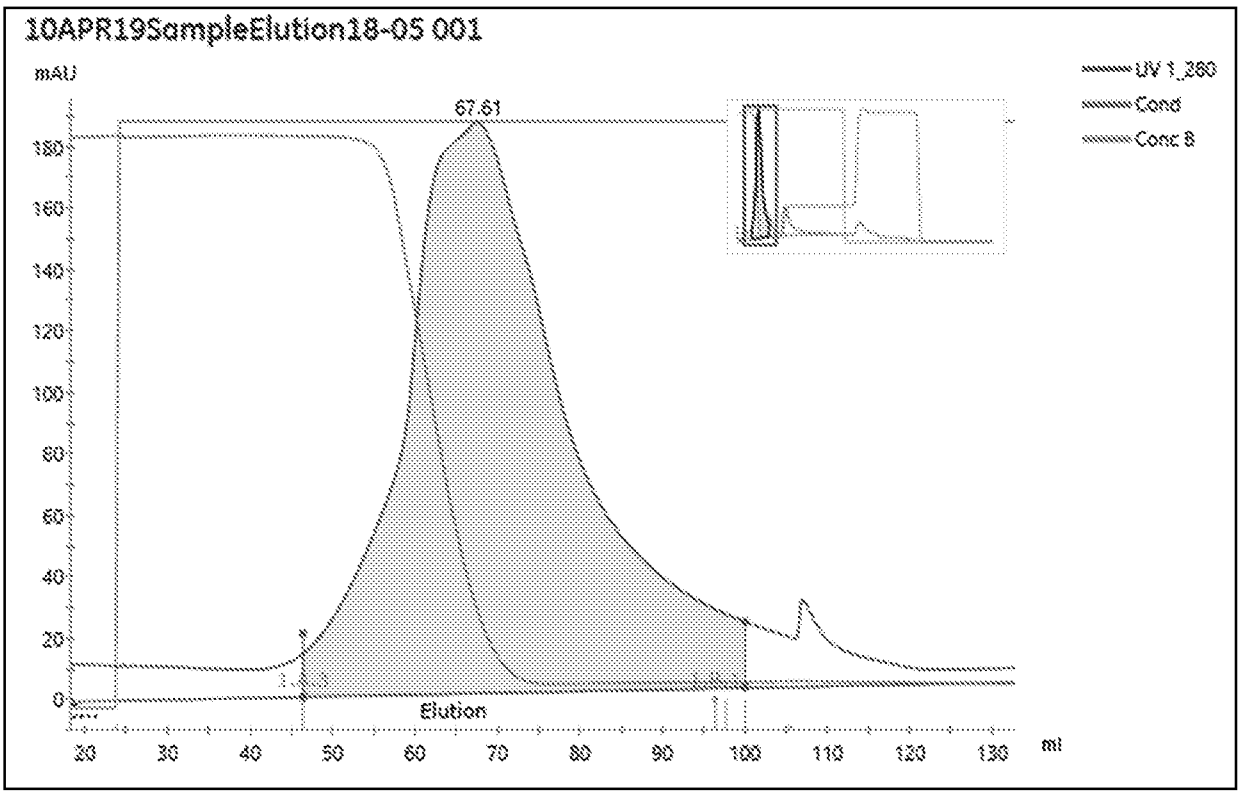

10APR19SampleElution18-05 001
mAU
UV 1_280
Cond
Conc B
67.61
Elution
ml

Figure 100

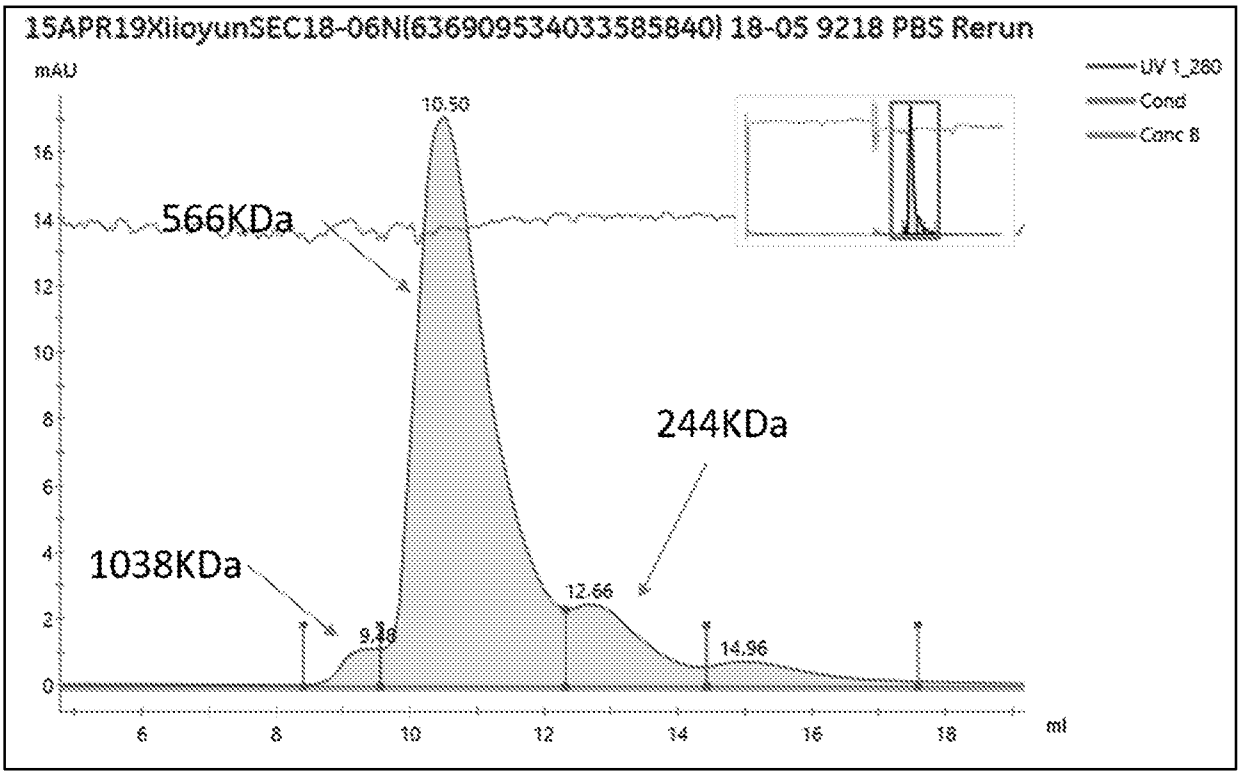

15APR19XiaoyunSEC18-06N(6369095340333585840) 18-05 9218 PBS Rerun
mAU
UV 1_280
Cond
Conc B
16.50
566KDa
244KDa
1038KDa
12.66
9.49
14.96
ml

Figure 101

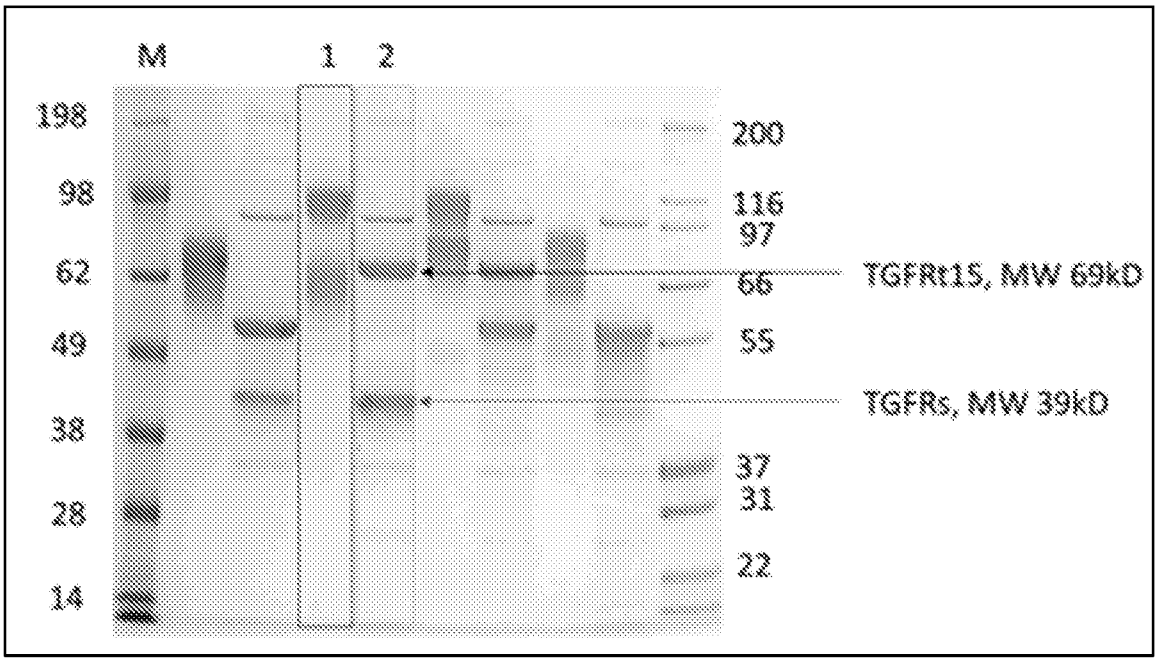

M
1
2
198
98
62
49
38
28
14
200
116
97
66
55
37
31
22
TGFRt15, MW 69kD
TGFRs, MW 39kD

Figure 102

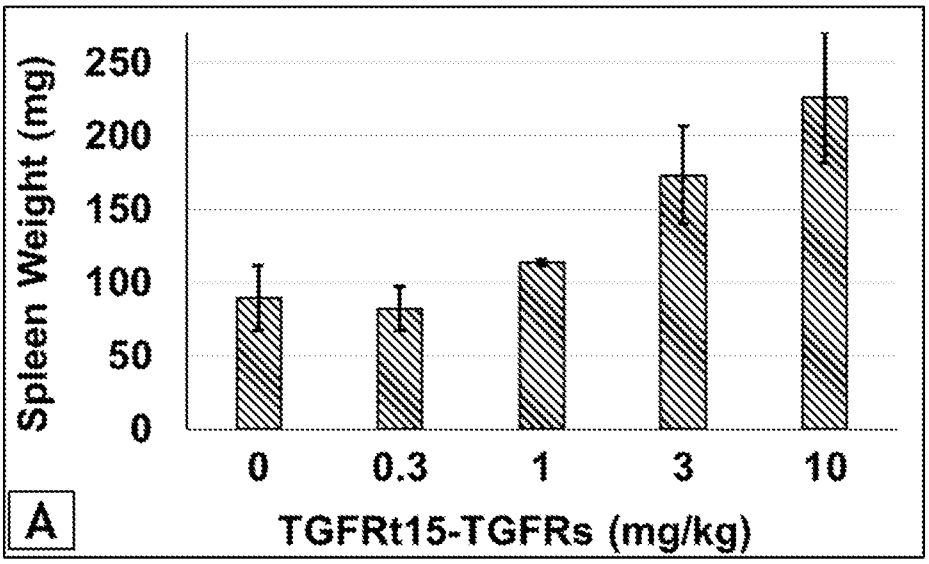


Figure 103A

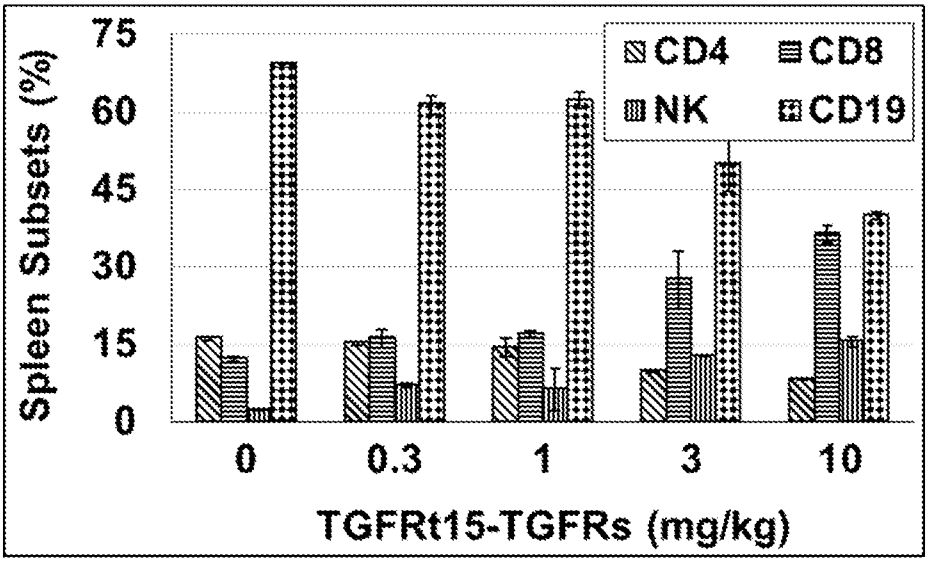


Figure 103B

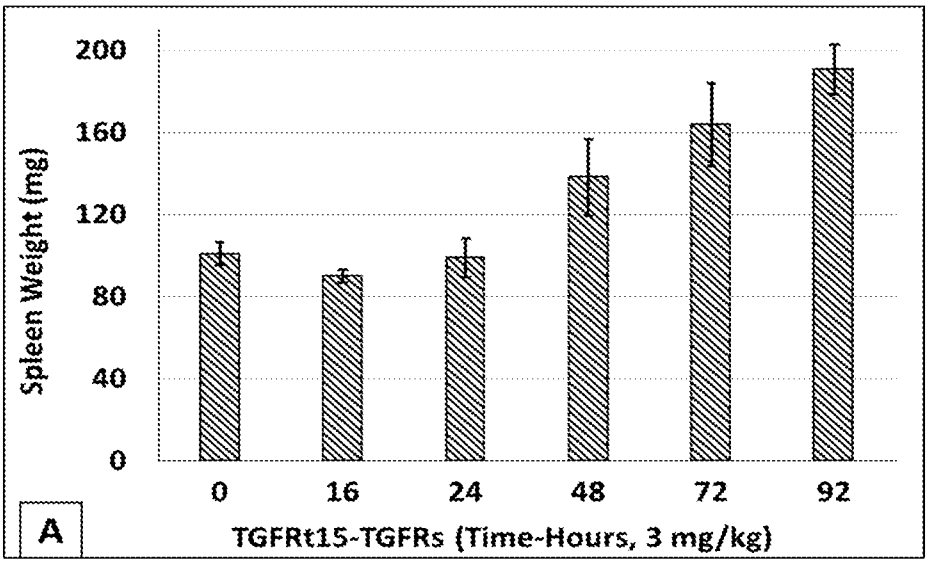


Figure 104A

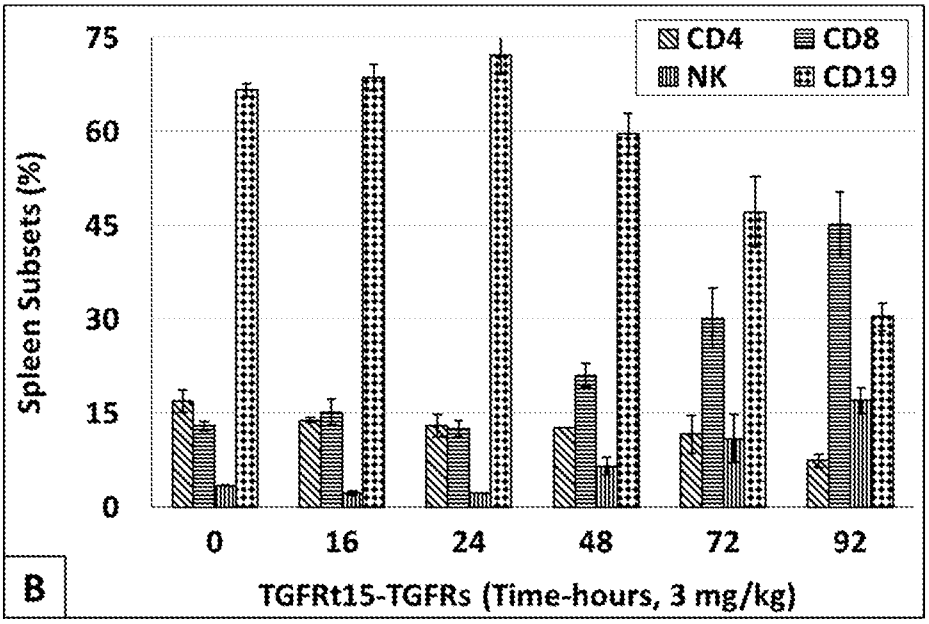


Figure 104B

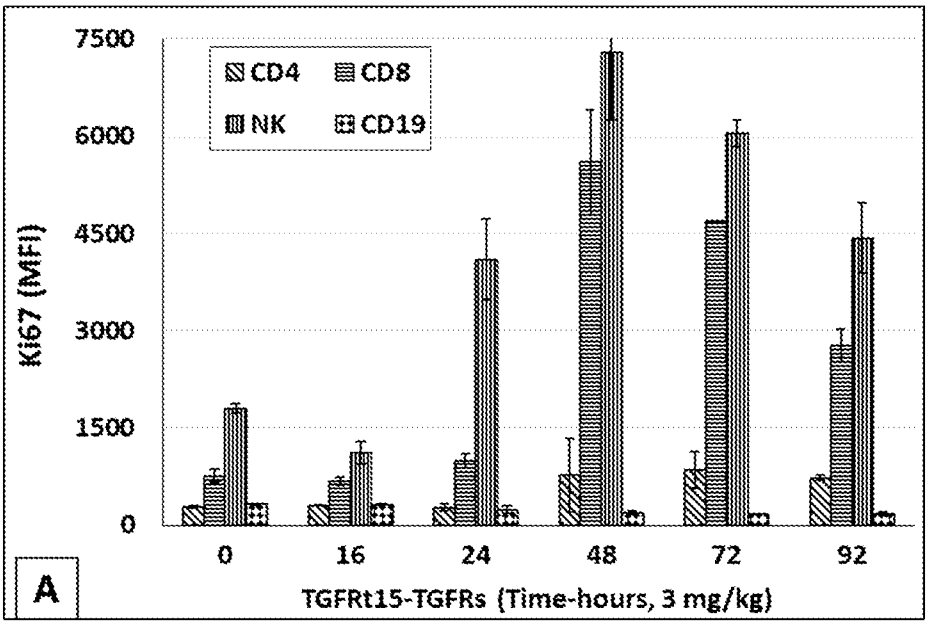


Figure 105A

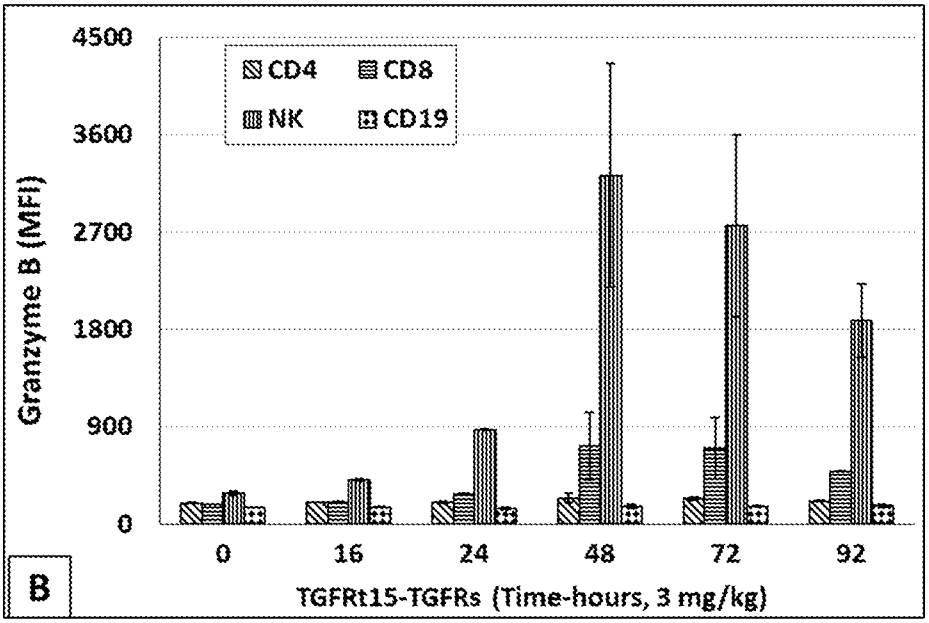


Figure 105B

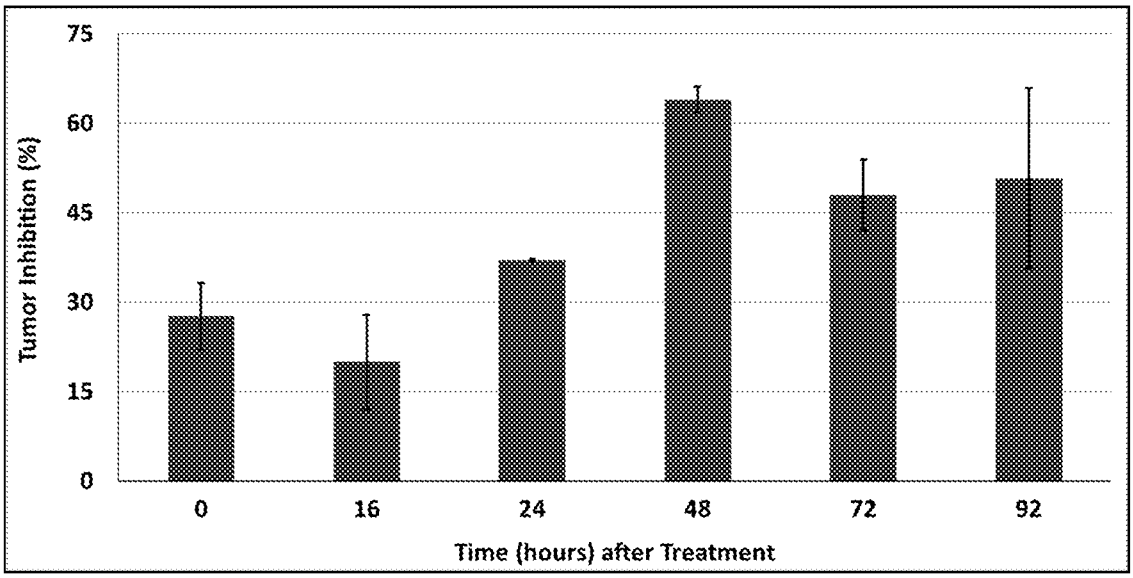

75
60
45
30
15
0
Tumor Inhibition (%)
0
16
24
48
72
92
Time (hours) after Treatment

Figure 106

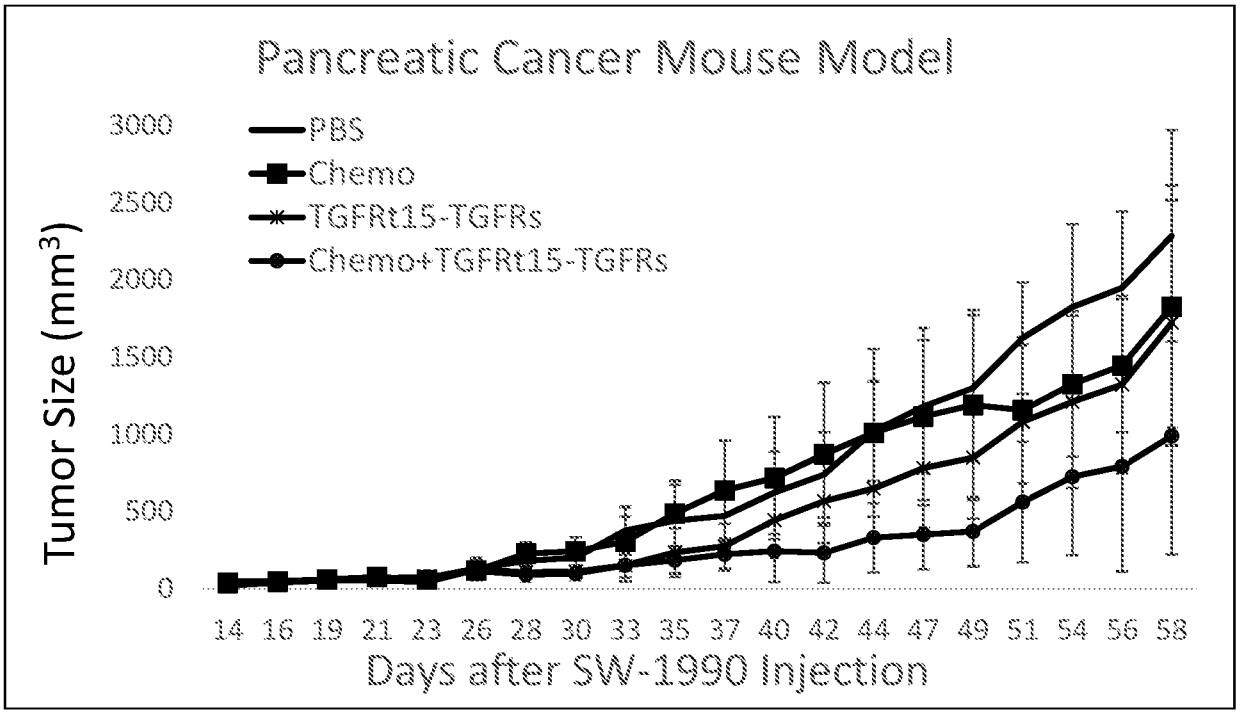

Pancreatic Cancer Mouse Model
PBS
Chemo
TGFRt15-TGFRs
Chemo+TGFRt15-TGFRs
Tumor Size (mm3)
3000
2500
2000
1500
1000
500
0
14 16 19 21 23 26 28 30 33 35 37 40 42 44 47 49 51 54 56 58
Days after SW-1990 Injection

Figure 107

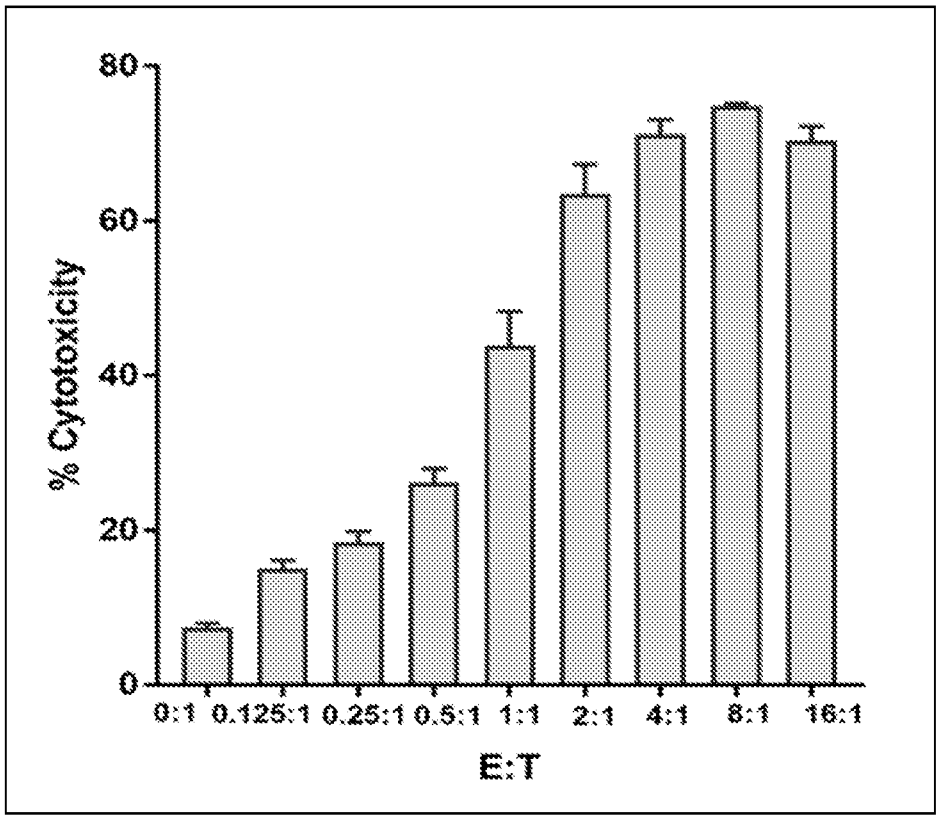


Figure 108

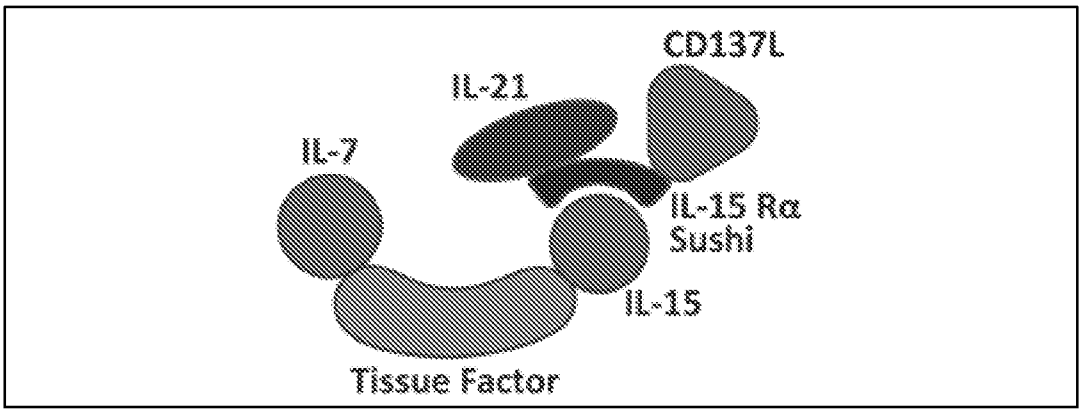


Figure 109

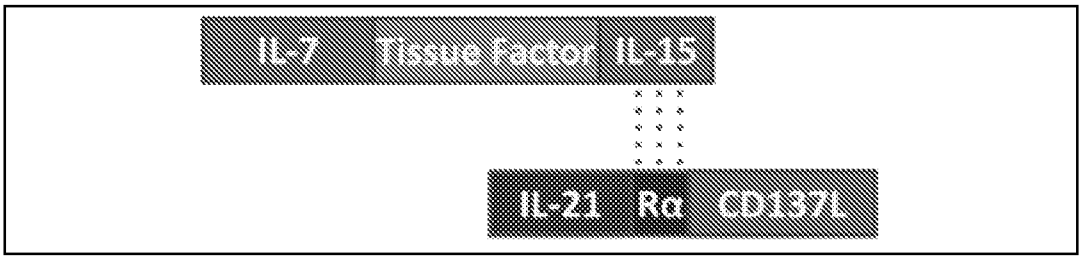


Figure 110

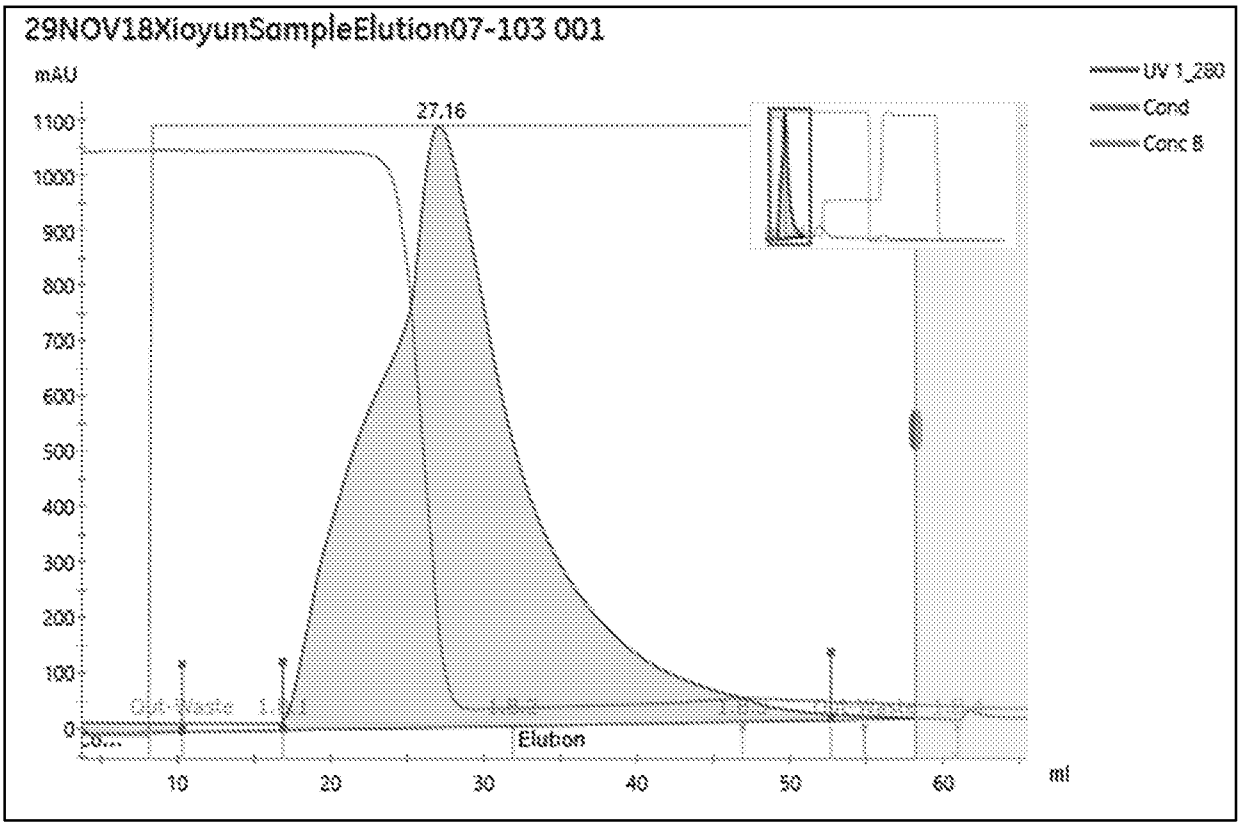

29NOV18XiaoyunSampleElution07-103 001
mAU
1100
1000
900
800
700
600
500
400
300
200
100
0
27.16
UV 1_280
Cond
Conc B
Elution
10
20
30
40
50
60
ml

Figure 111

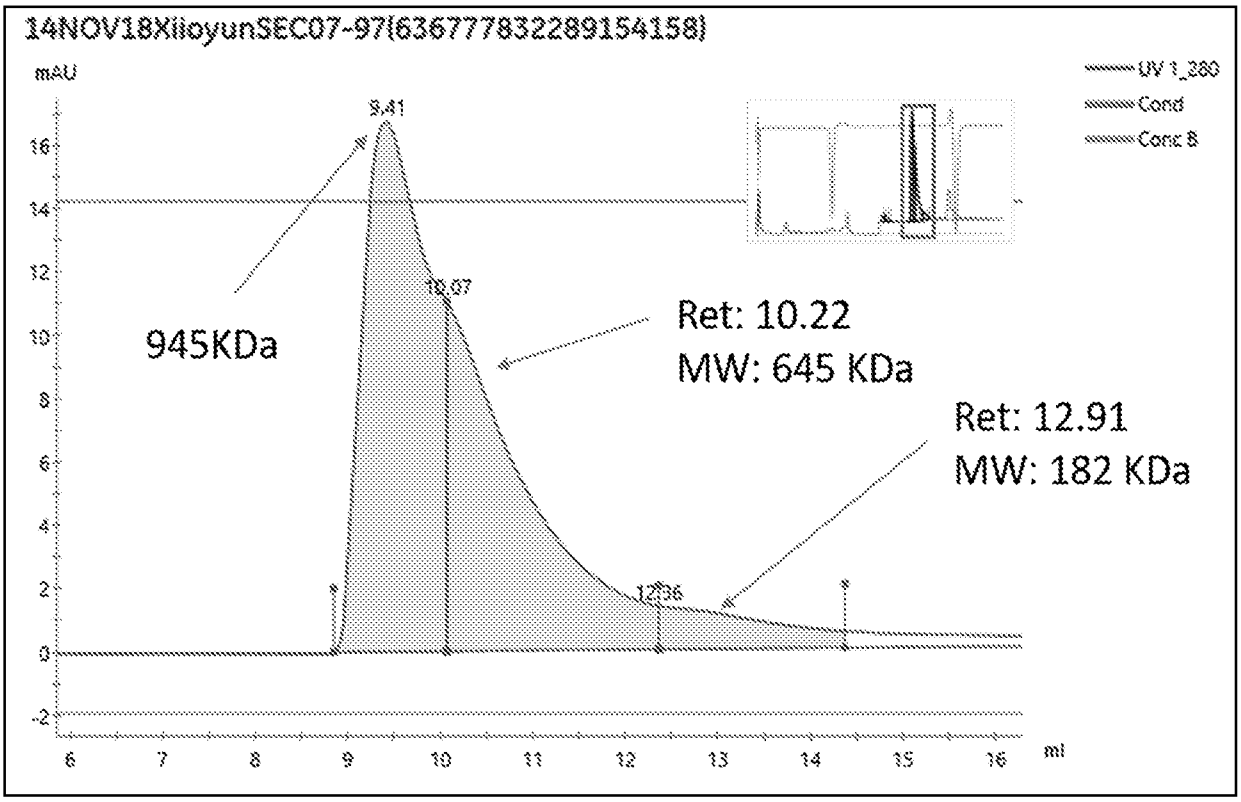

14NOV18XiloyunSEC07-97(636777832289154158)
mAU
UV 1_280
Cond
Conc B
9.41
10.07
12.36
945KDa
Ret: 10.22
MW: 645 KDa
Ret: 12.91
MW: 182 KDa
ml

Figure 112

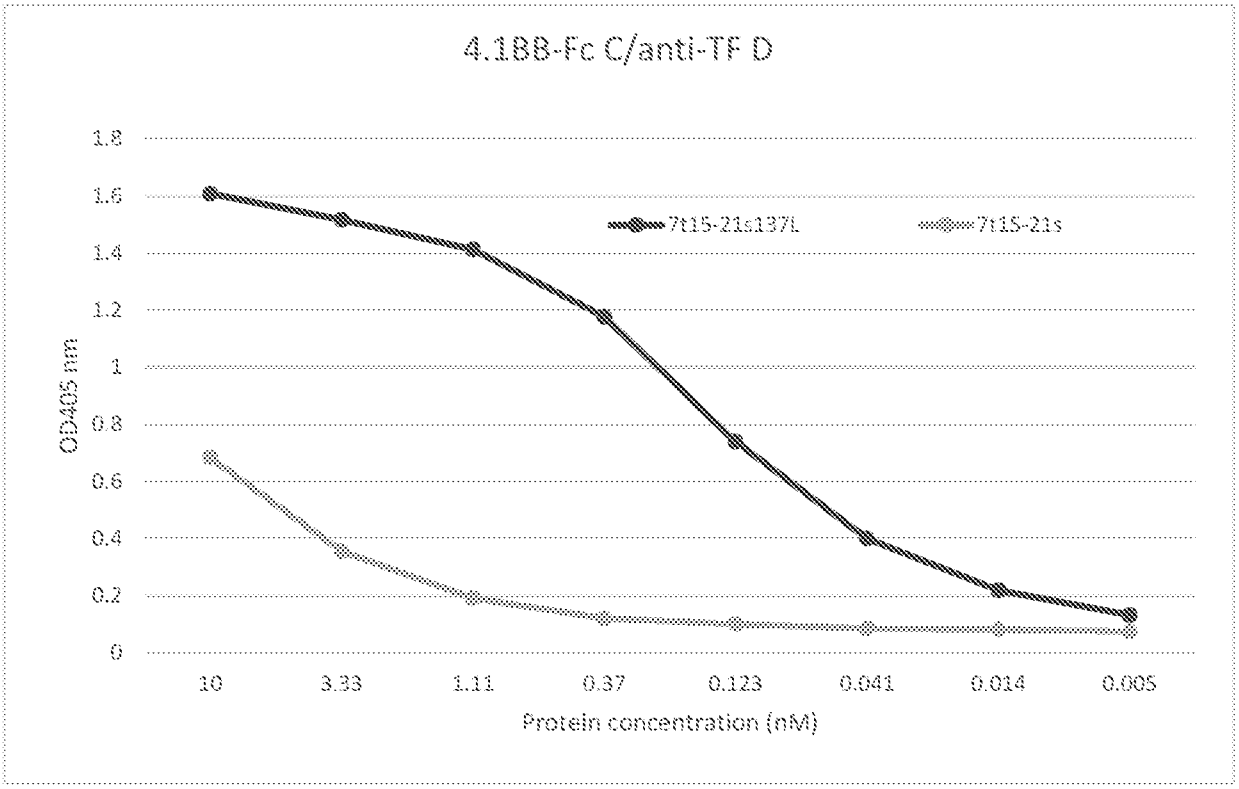

4.1BB-Fc C/anti-TF D
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD405 nm
7t15-21s137L
7t15-21s
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein concentration (nM)

Figure 113

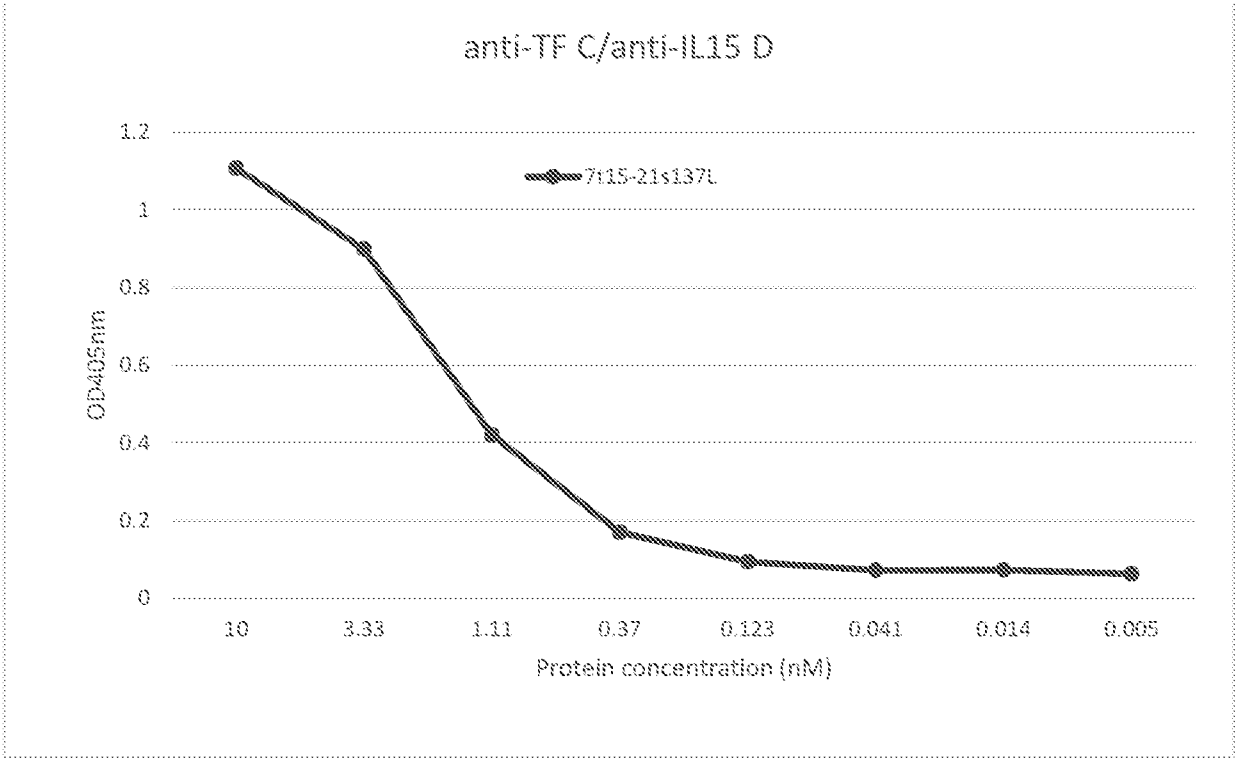


Figure 114A

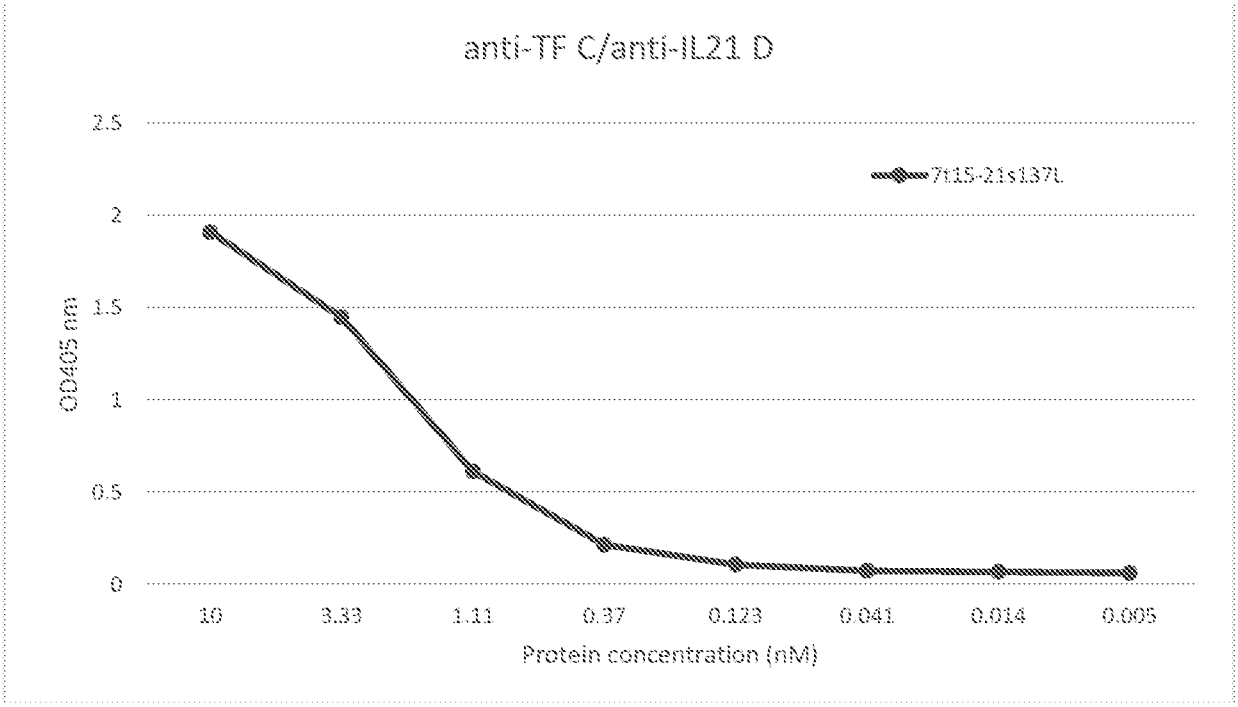


Figure 114B

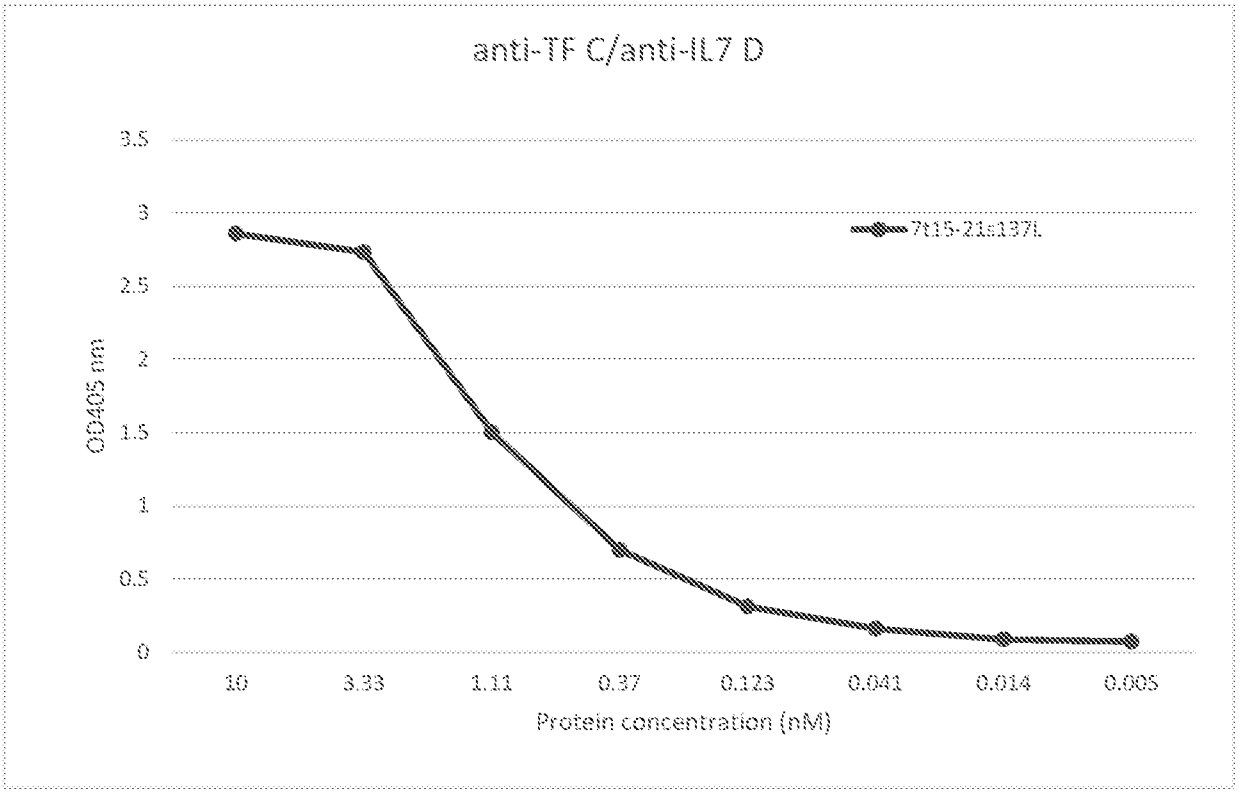

anti-TF C/anti-IL7 D
OD405 nm
3.5
3
2.5
2
1.5
1
0.5
0
7t15-21:137L
10
3.33
1.11
0.37
0.123
0.041
0.014
0.005
Protein concentration (nM)

Figure 114C

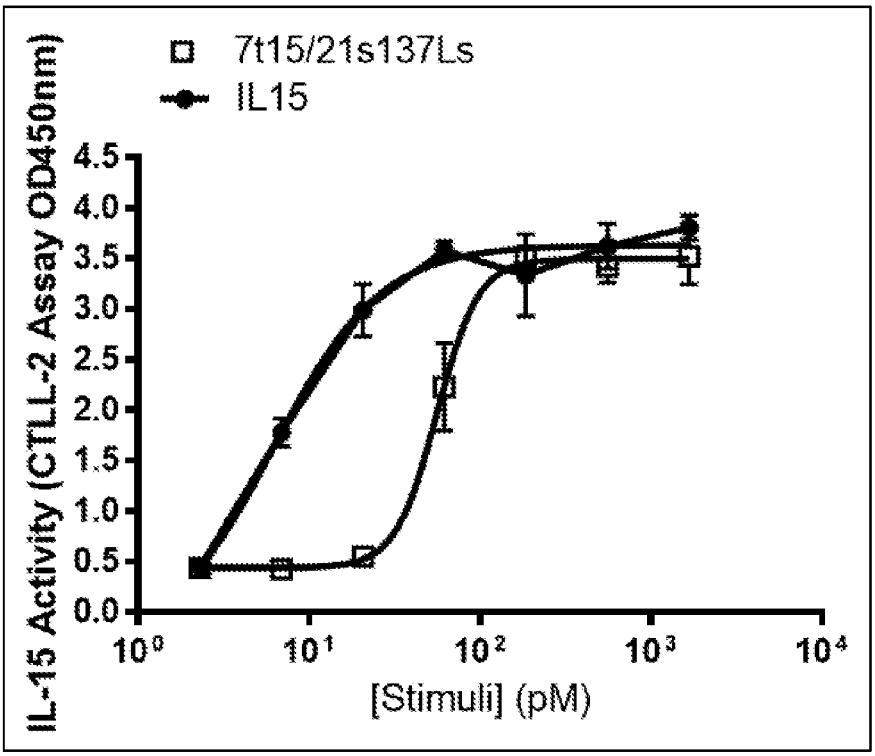


Figure 115

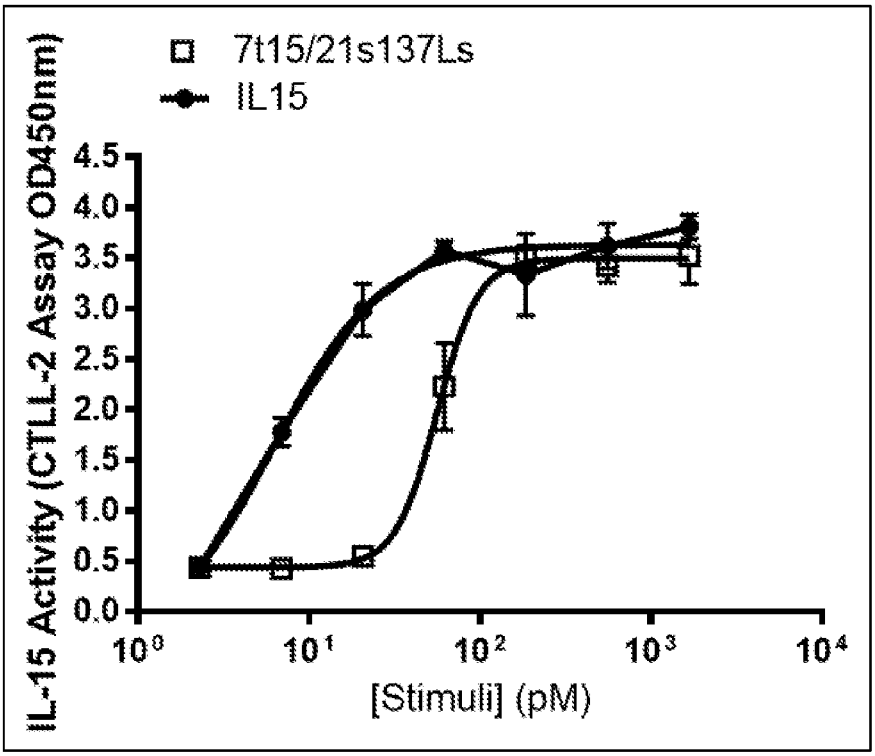


Figure 116

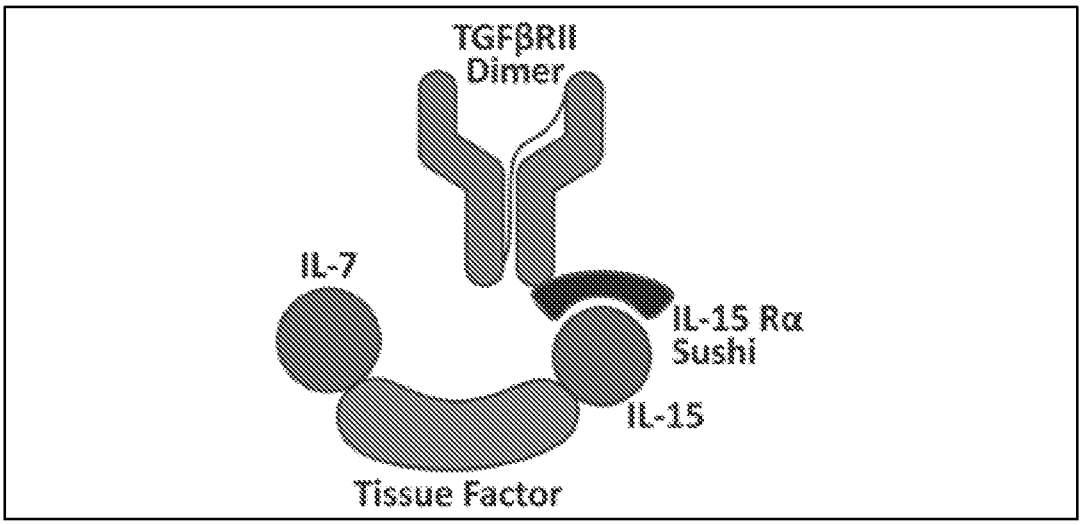


Figure 117

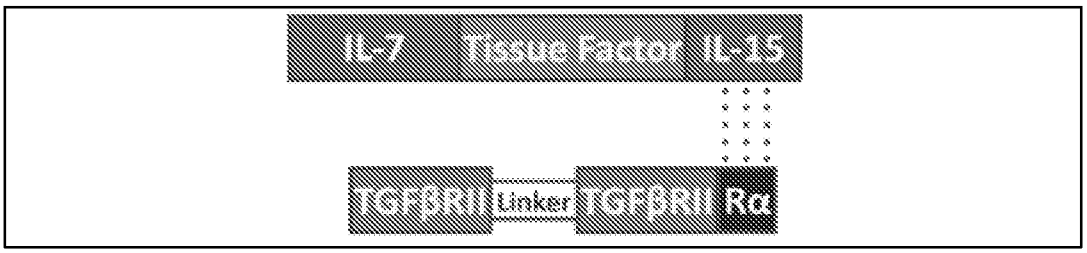


Figure 118

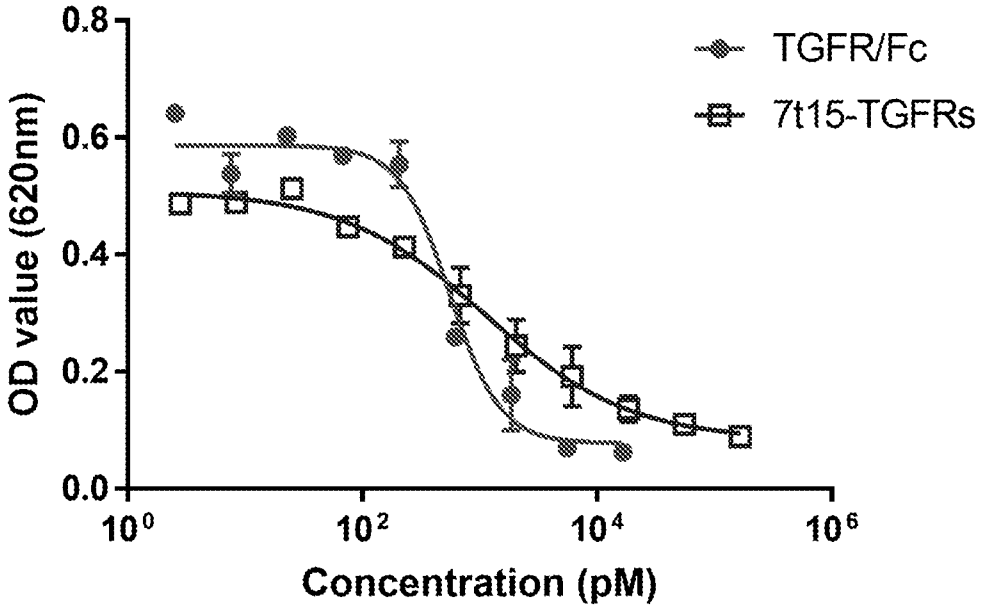

0.8
0.6
0.4
0.2
0.0
OD value (620nm)
10^0
10^2
10^4
10^6
Concentration (pM)
TGFR/Fc
7t15-TGFRs

Figure 119

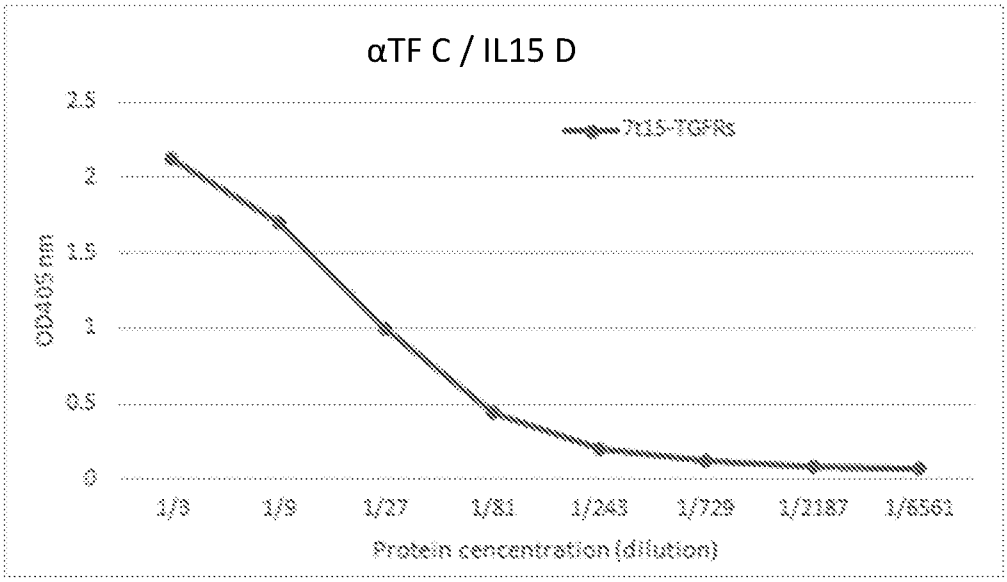


Figure 120A

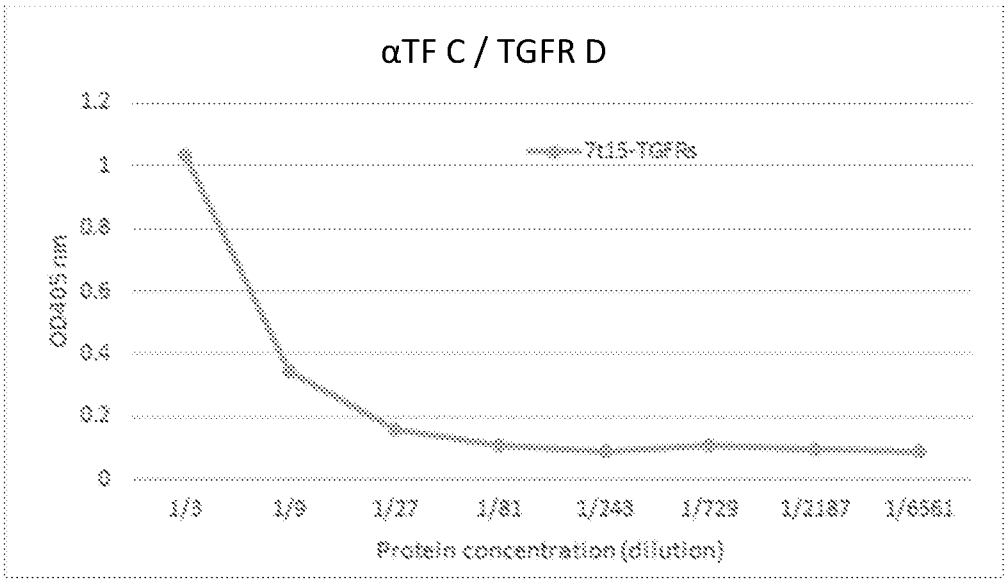


Figure 120B

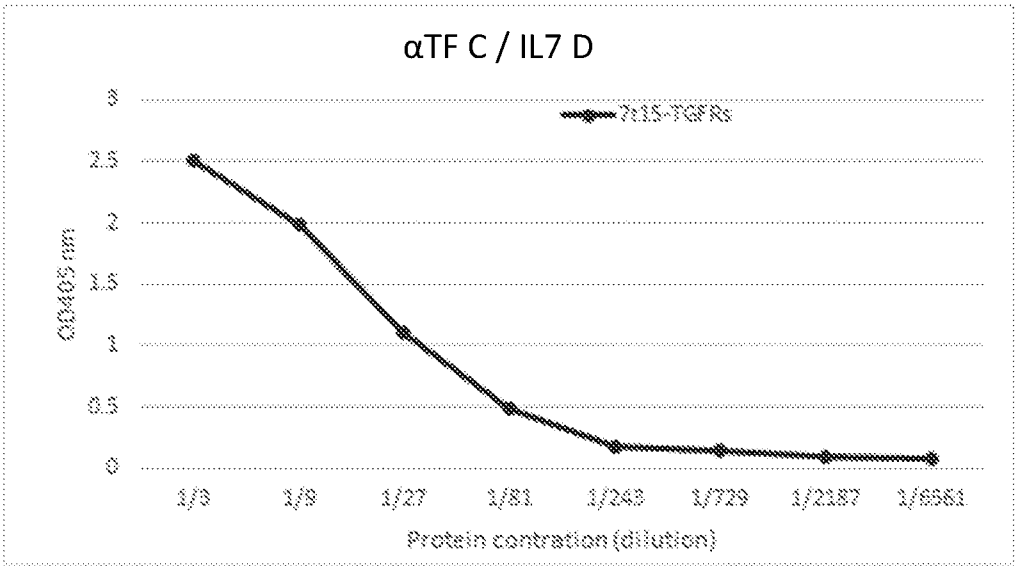

αTF C / IL7 D
7r15-TGFRs
OD405 nm
3
2.5
2
1.5
1
0.5
0
1/3
1/9
1/27
1/81
1/243
1/729
1/2187
1/6561
Protein contration (dilution)

Figure 120C

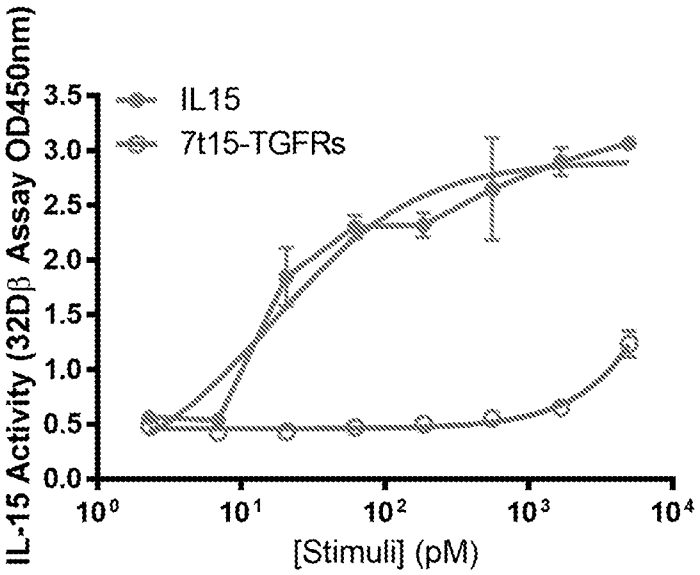


Figure 121

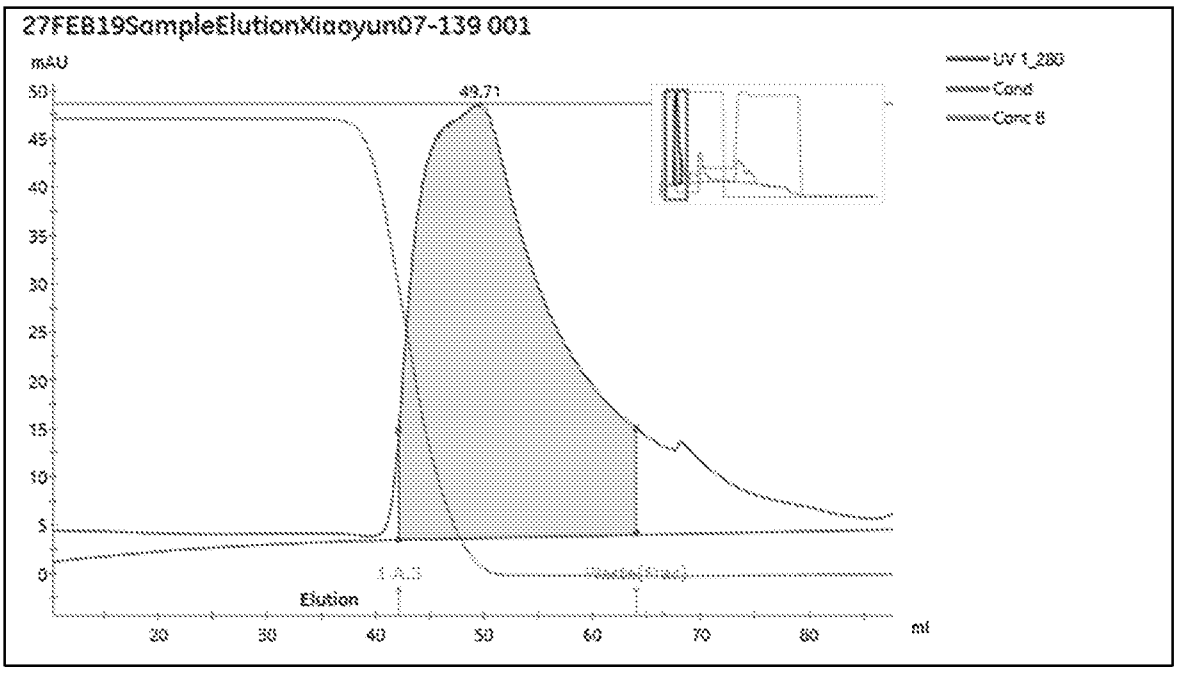


Figure 122

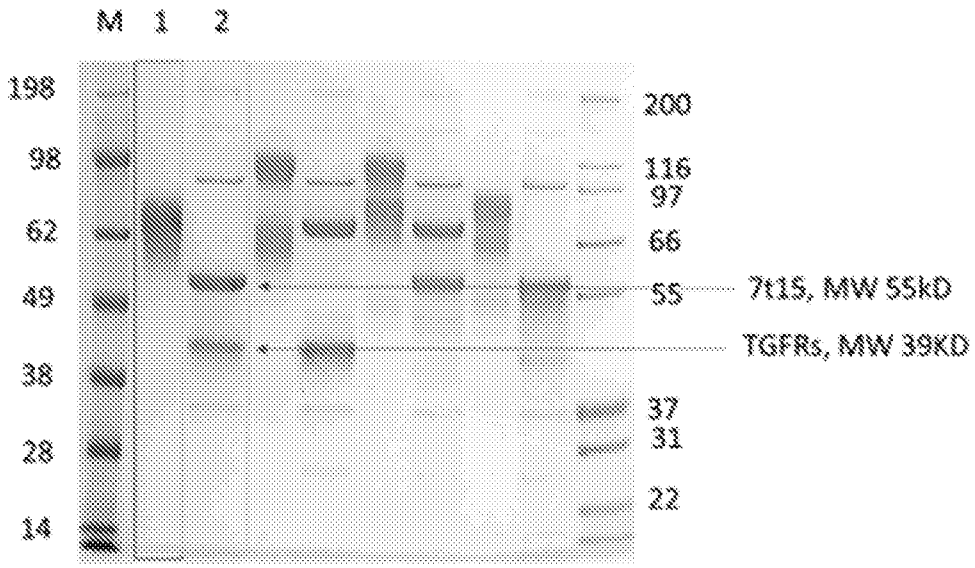


Figure 123

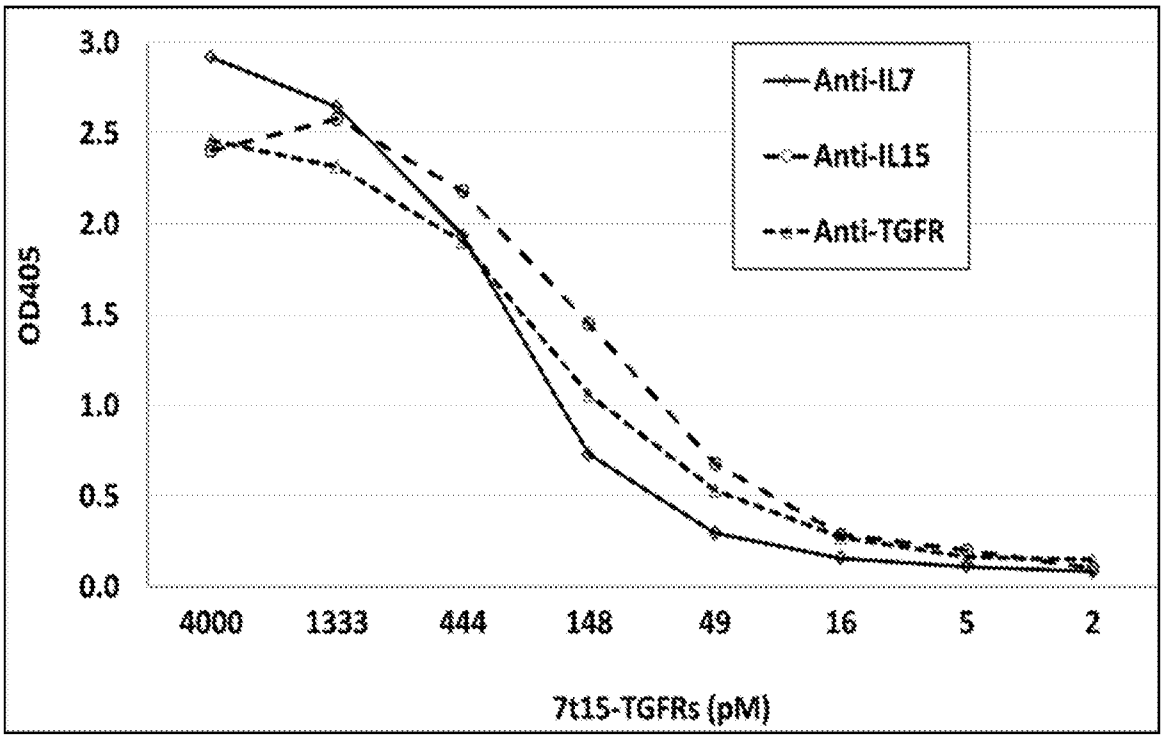


Figure 124

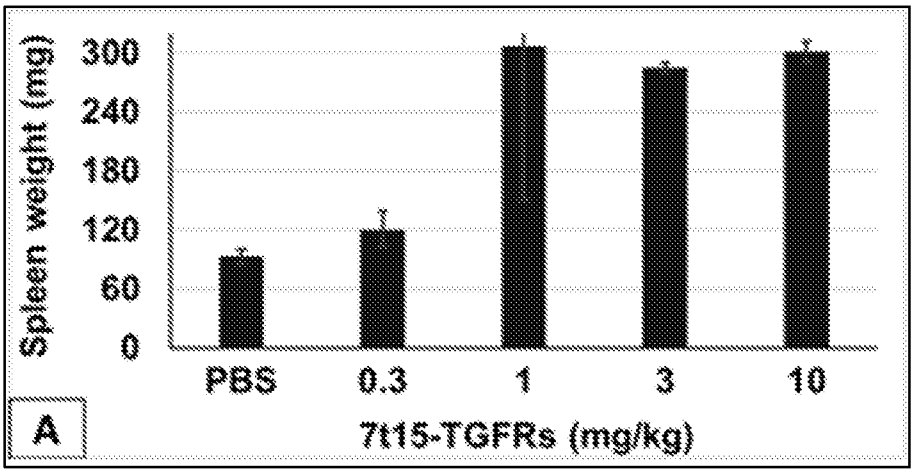


Figure 125A

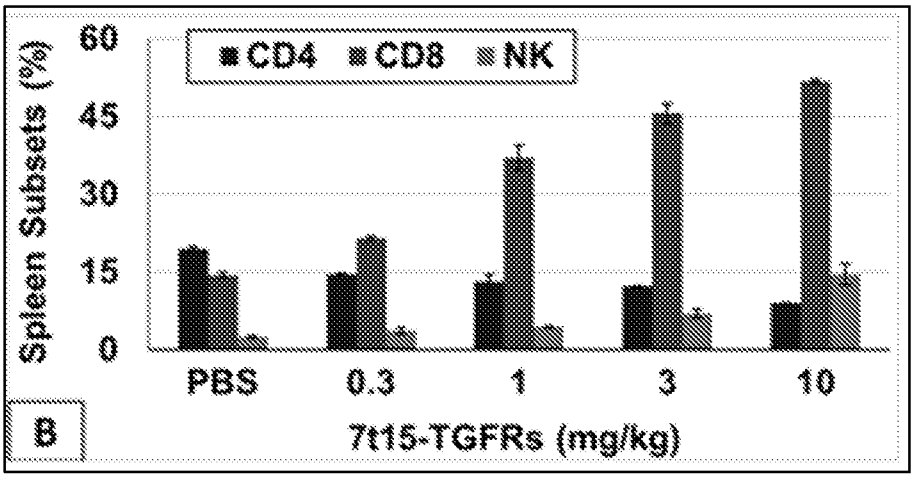


Figure 125B

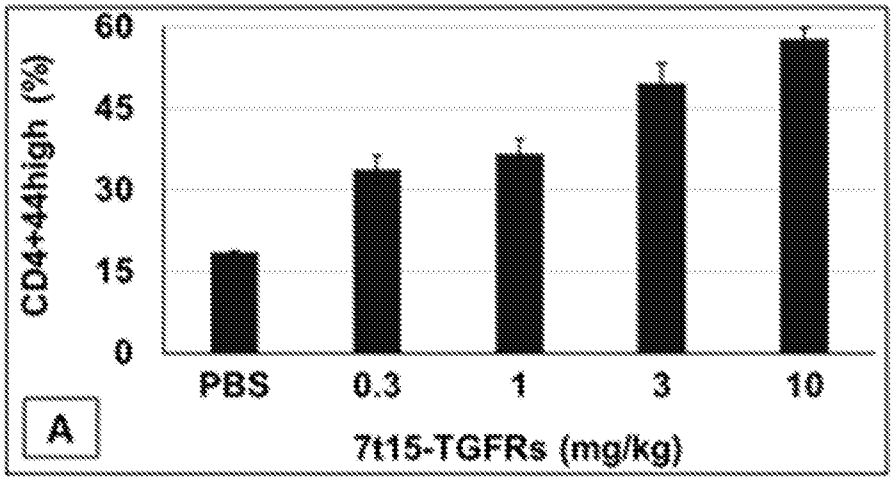


Figure 126A

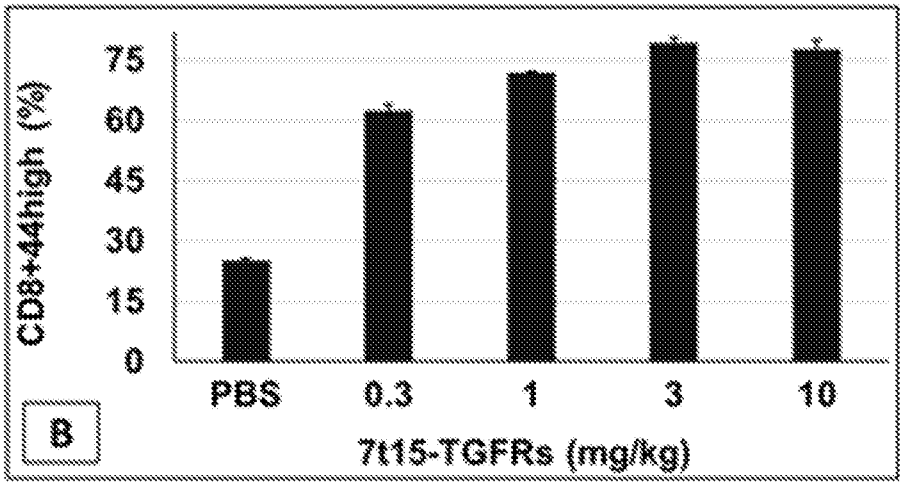


Figure 126B

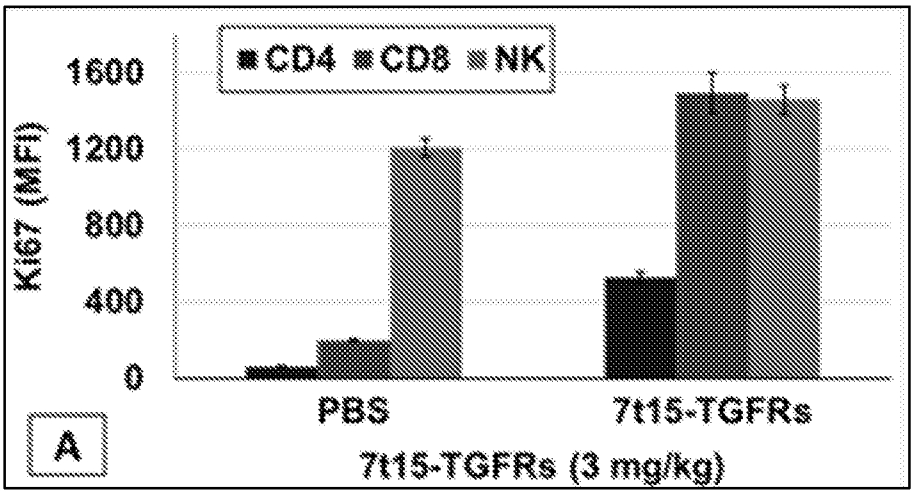


Figure 127A

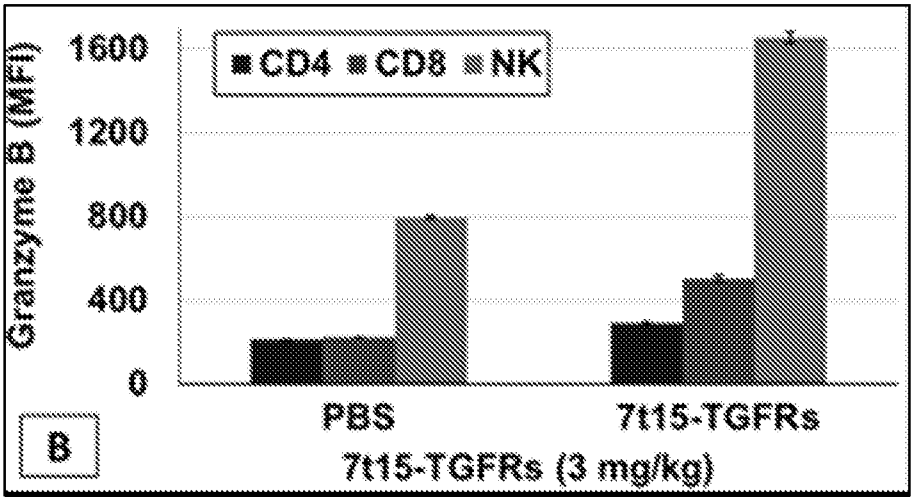


Figure 127B

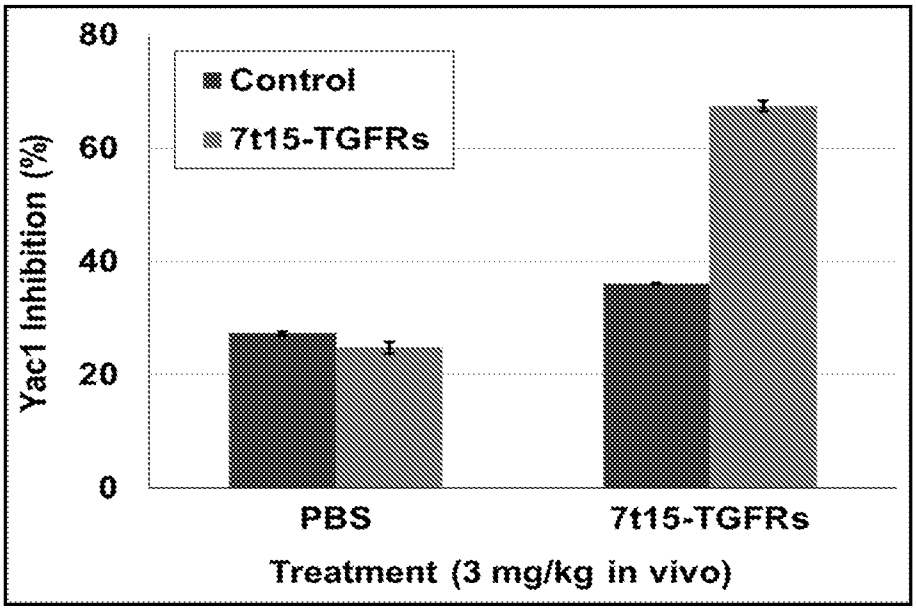

80
60
40
20
0
Yac1 Inhibition (%)
Control
7t15-TGFRs
PBS
7t15-TGFRs
Treatment (3 mg/kg in vivo)

Figure 128

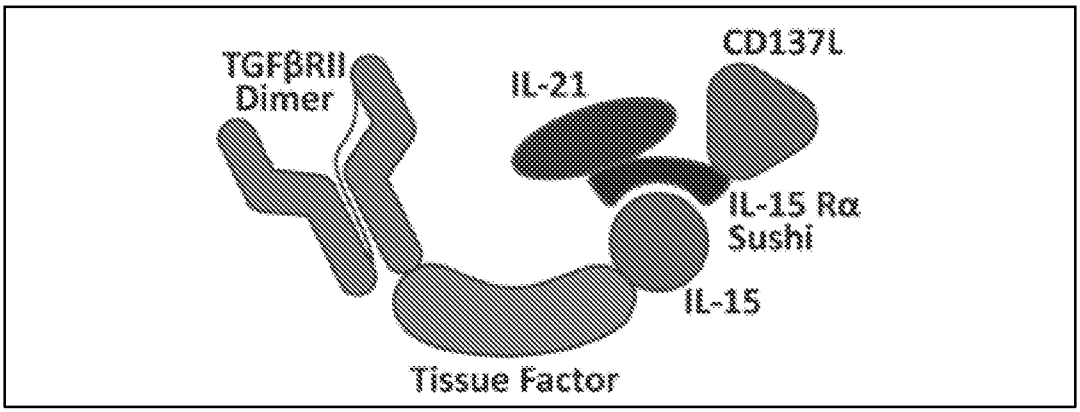


Figure 129

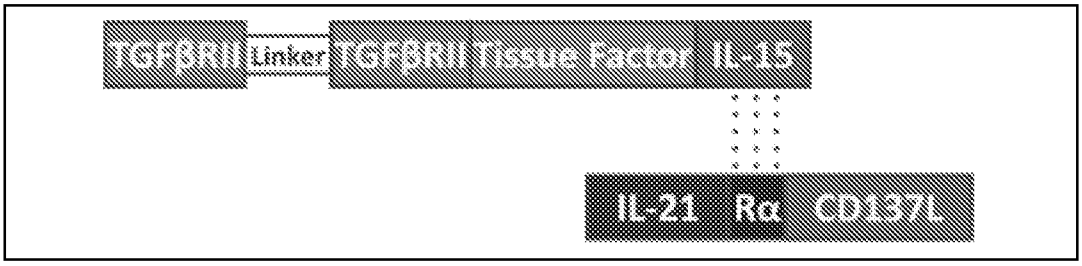


Figure 130

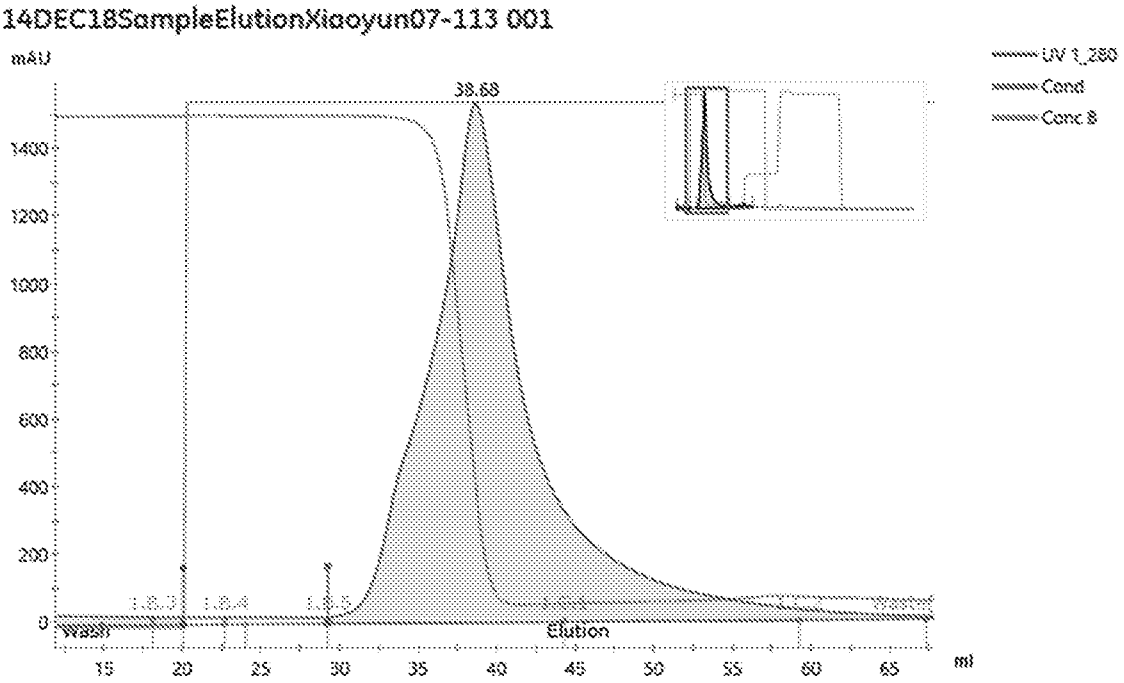

14DEC18SampleElutionXiaoyun07-113 001
mAU
38.68
UV 1_280
Cond
Conc B
1400
1200
1000
800
600
400
200
0
Wash
Elution
15
20
25
30
35
40
45
50
55
60
65
ml

Figure 131

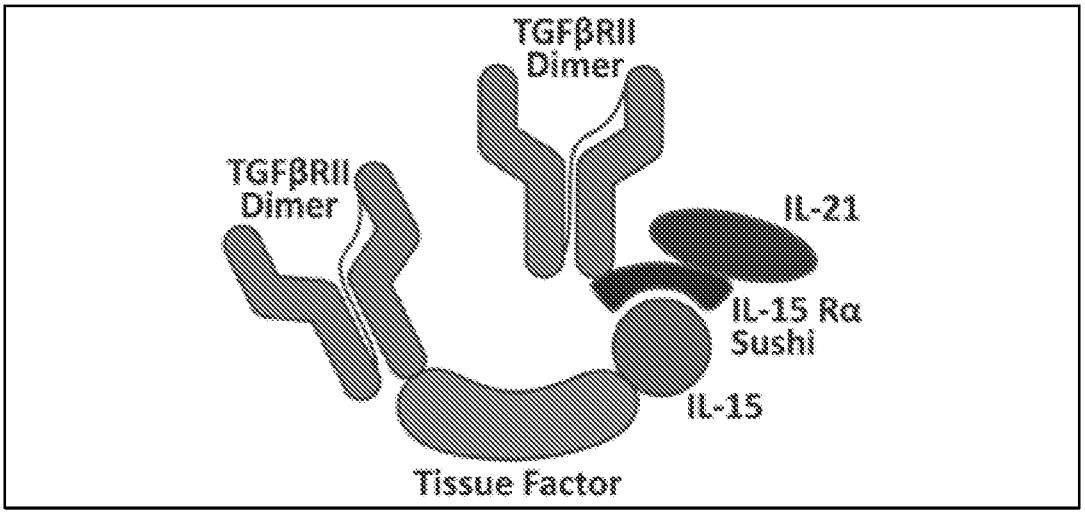


Figure 132

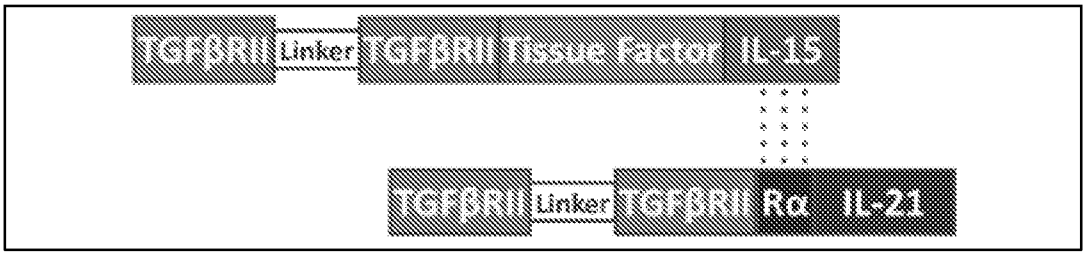


Figure 133

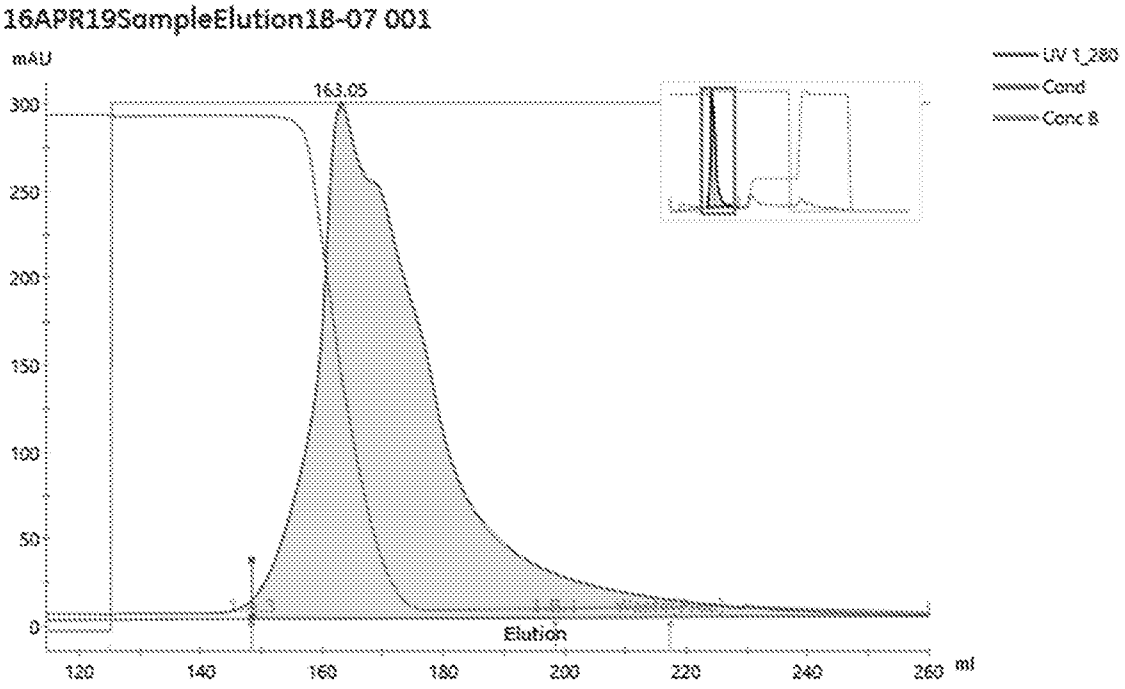


Figure 134

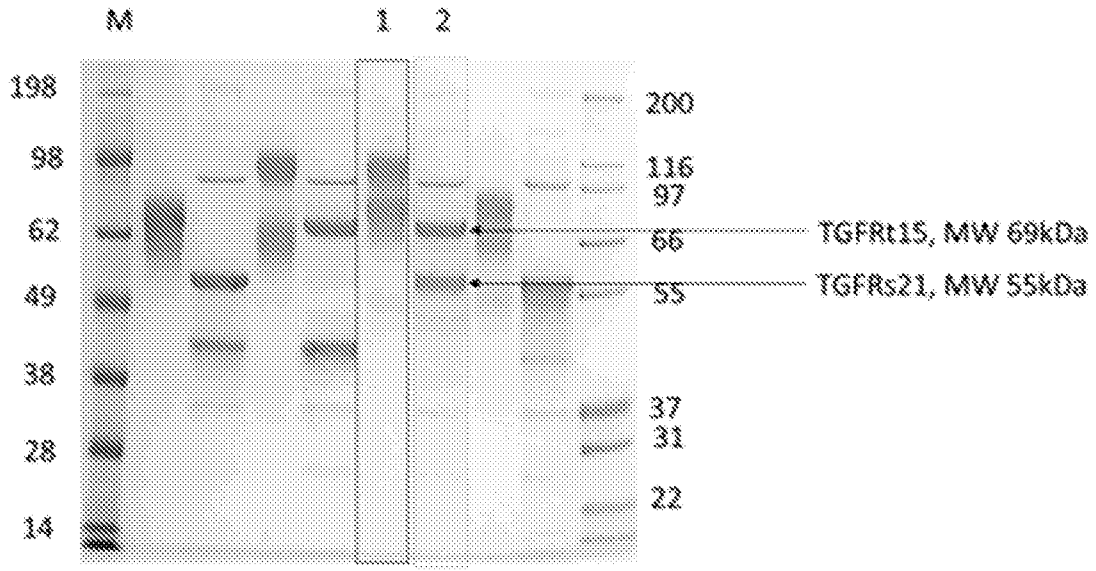


Figure 135

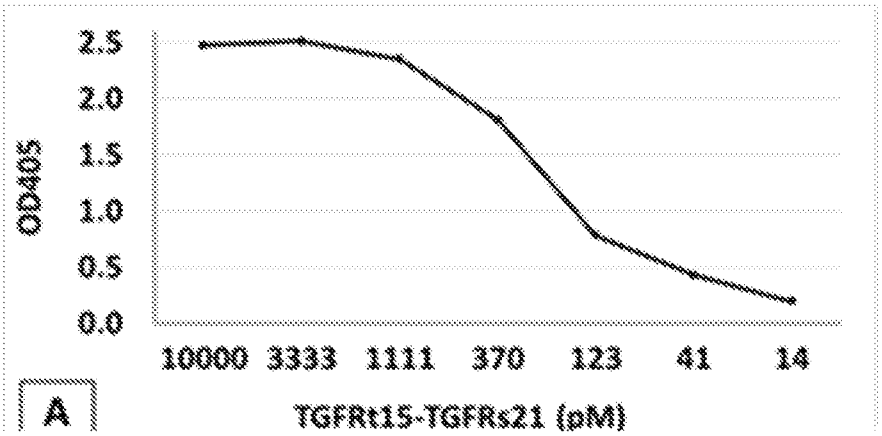


Figure 136A

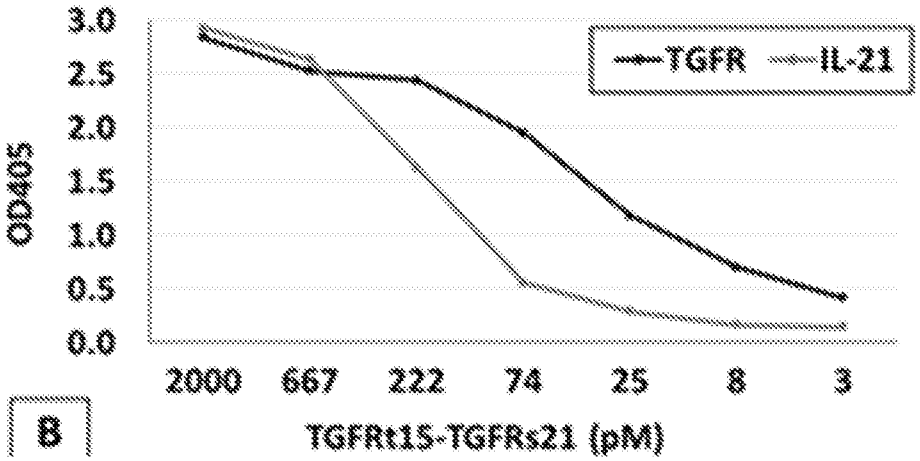


Figure 136B

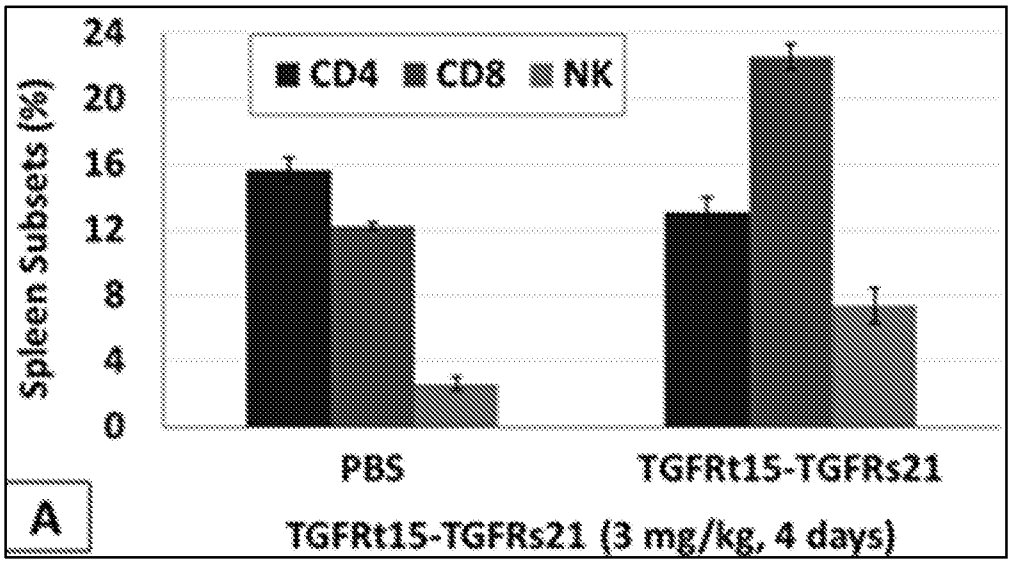


Figure 137A

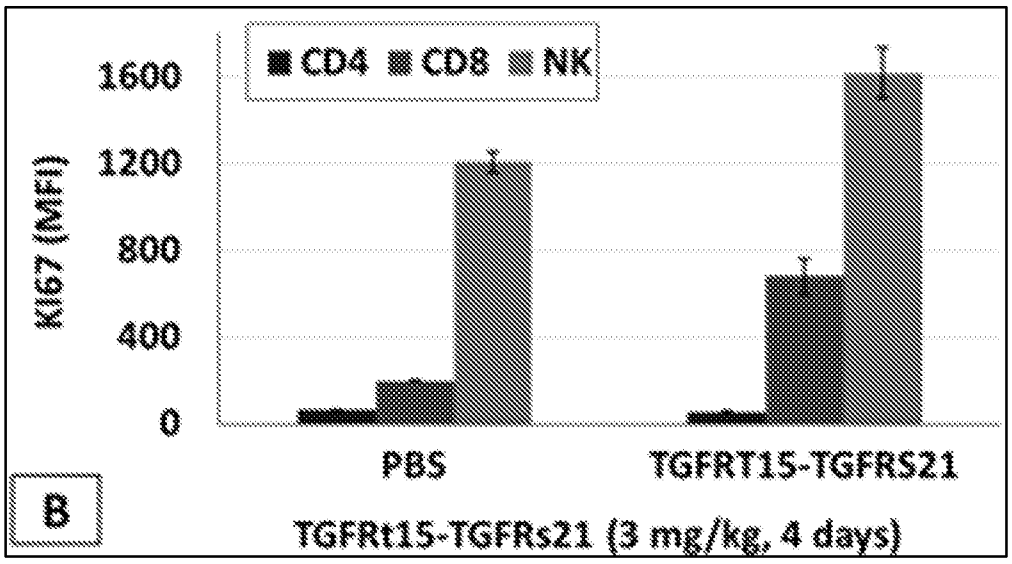


Figure 137B

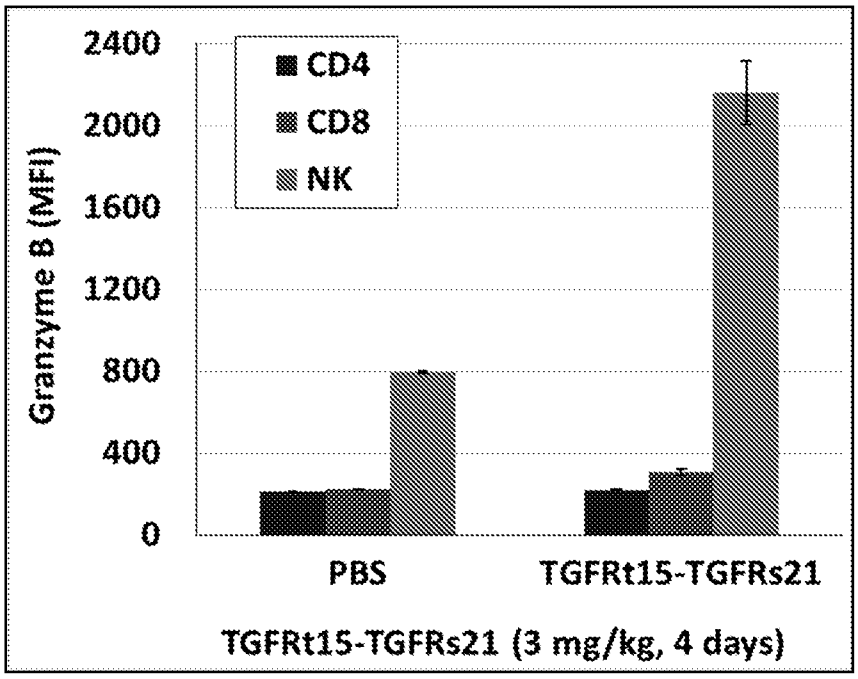


Figure 138

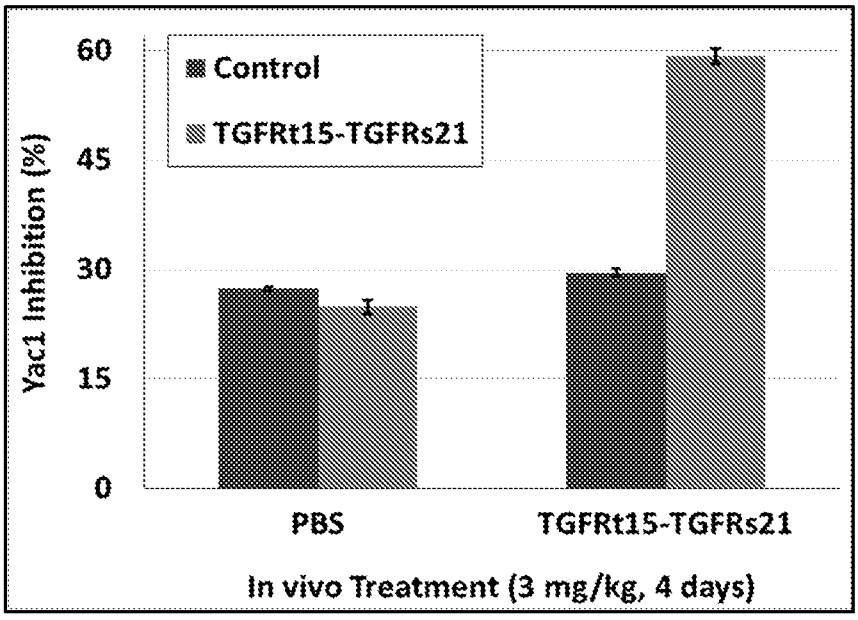


Figure 139

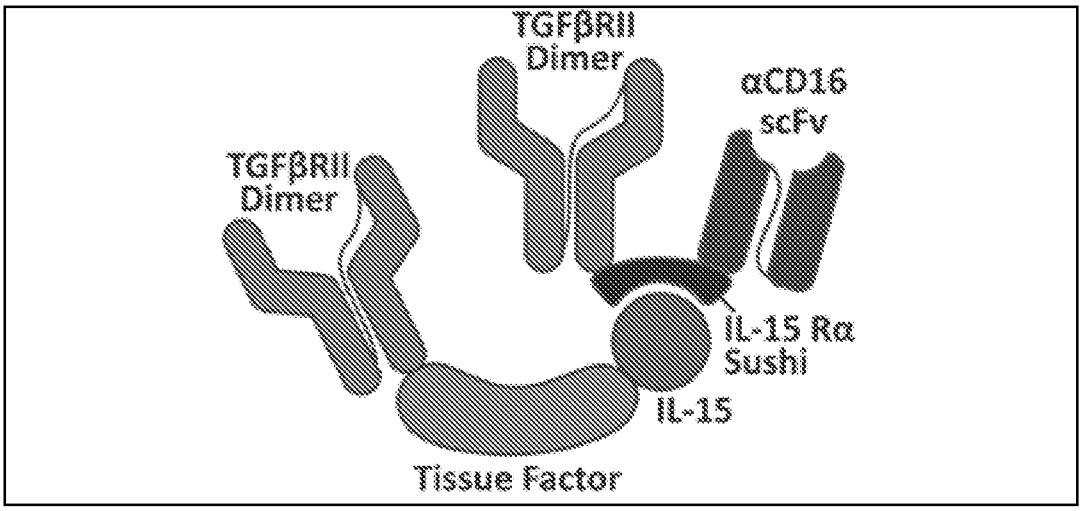


Figure 140

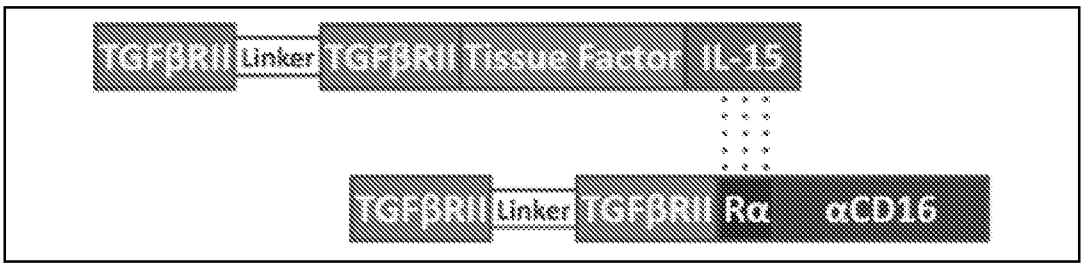


Figure 141

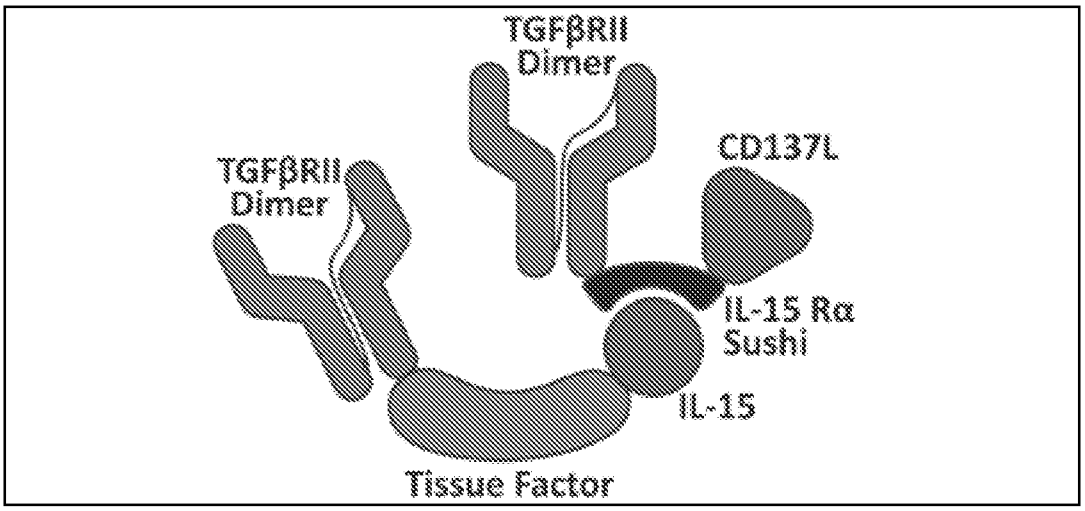


Figure 142

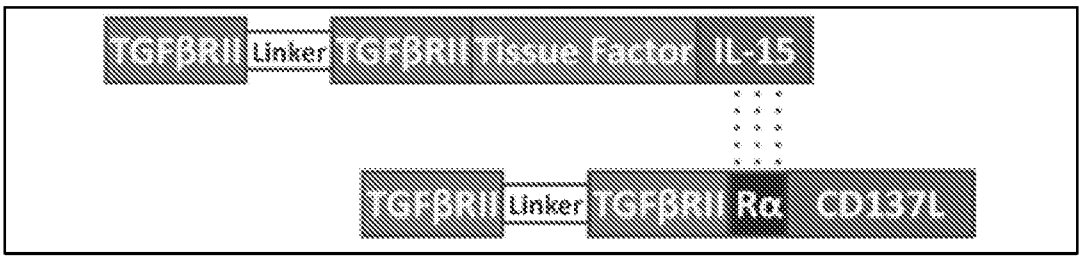


Figure 143

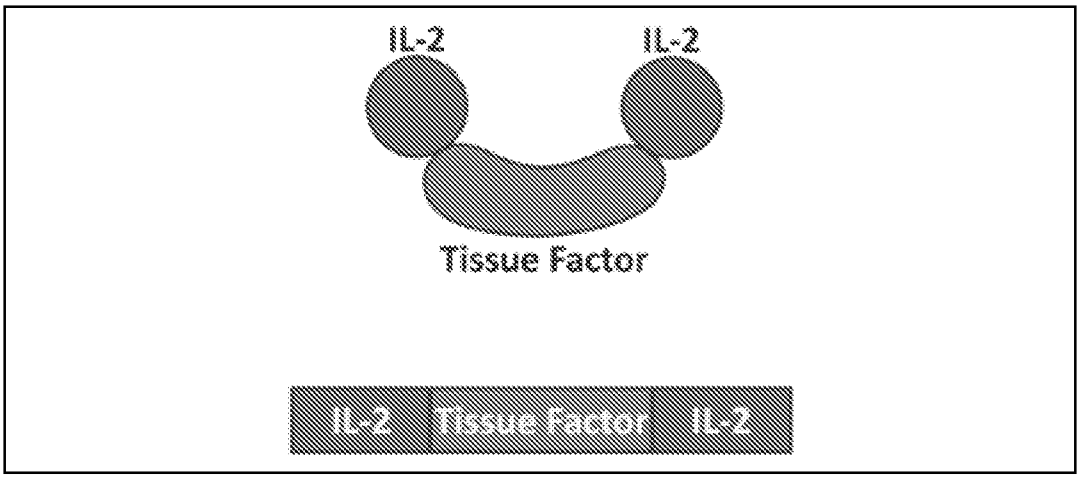


Figure 144

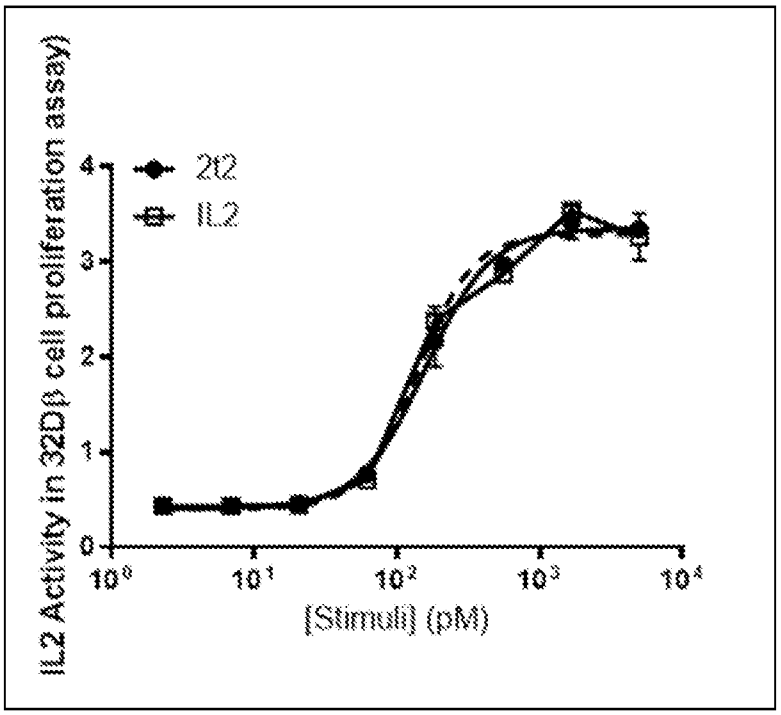


Figure 145

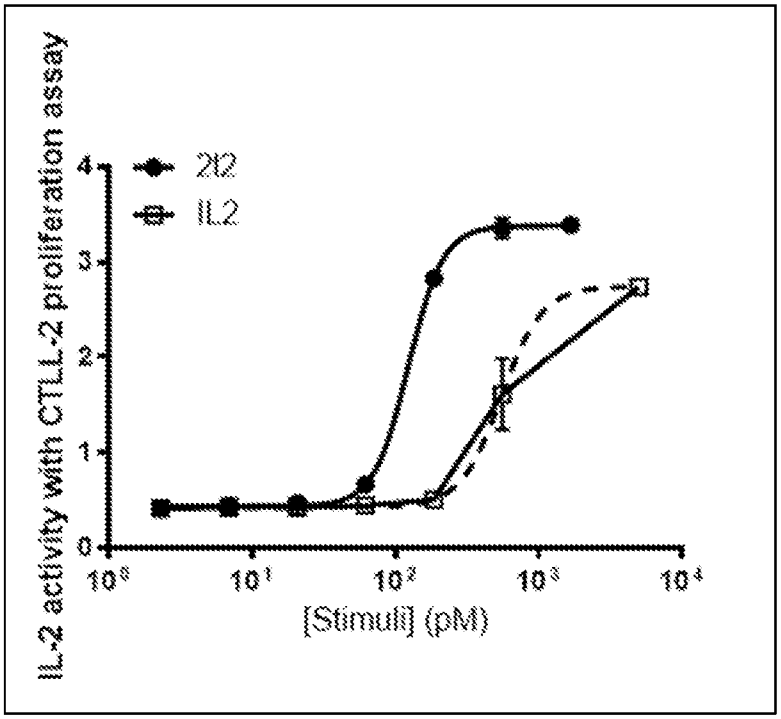


Figure 146

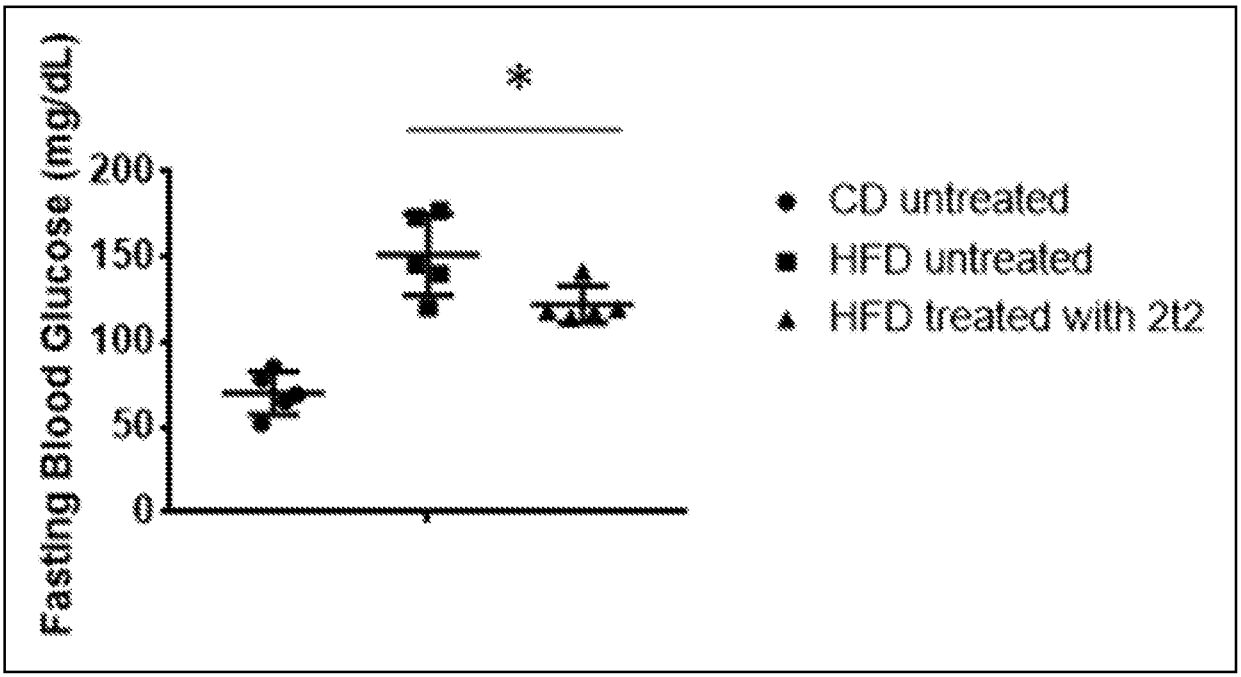


Figure 147

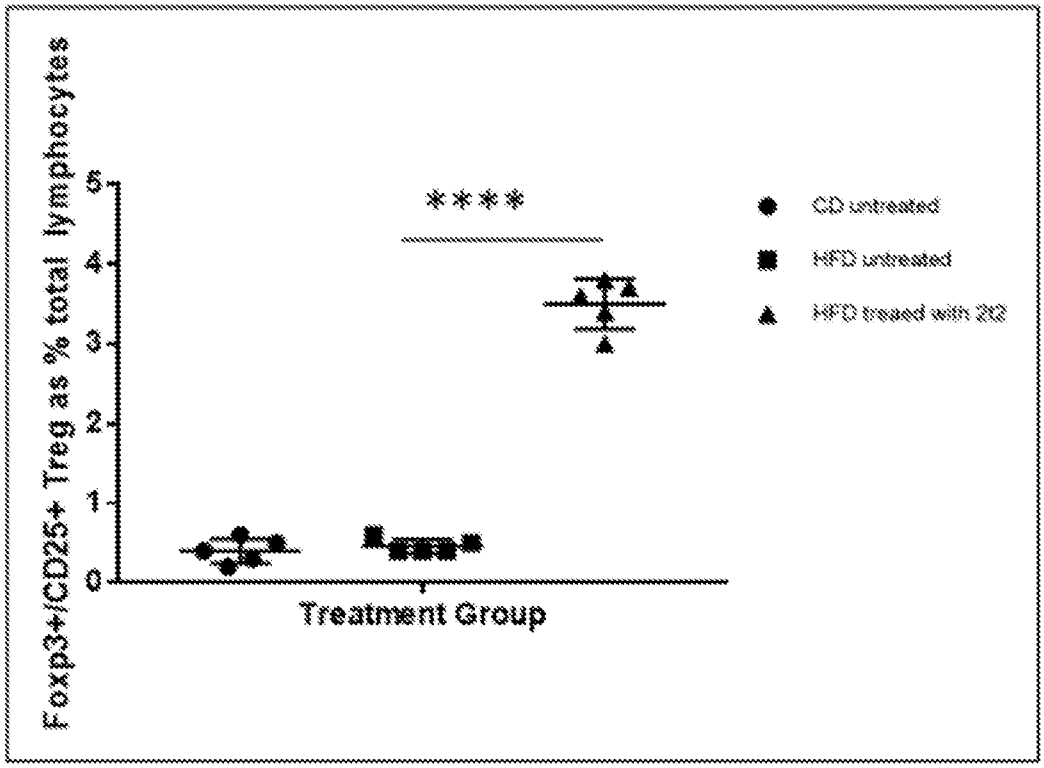


Figure 148

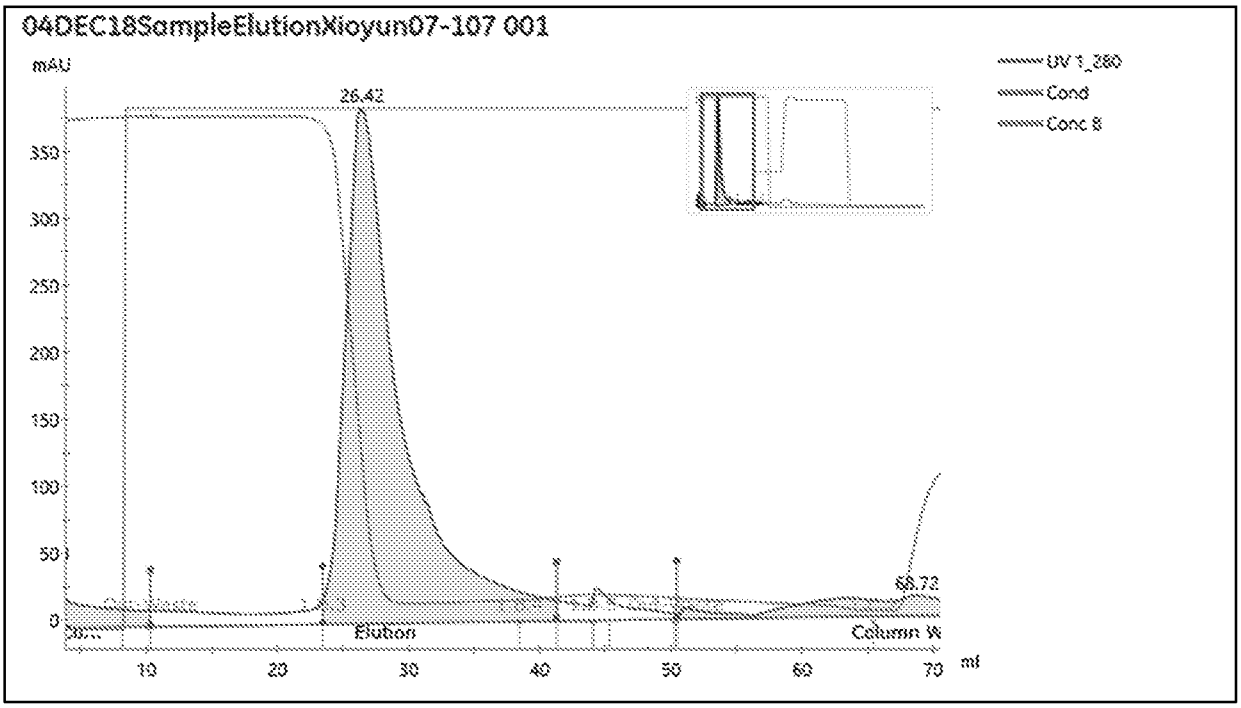


Figure 149

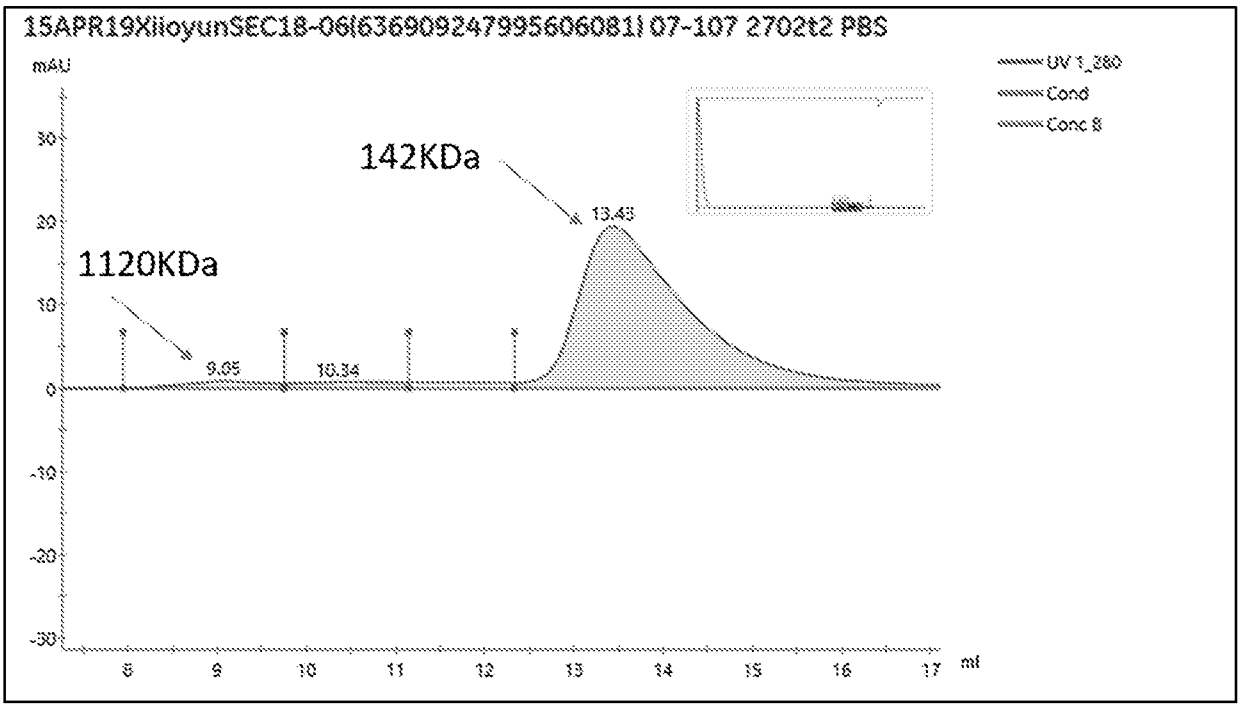


Figure 150

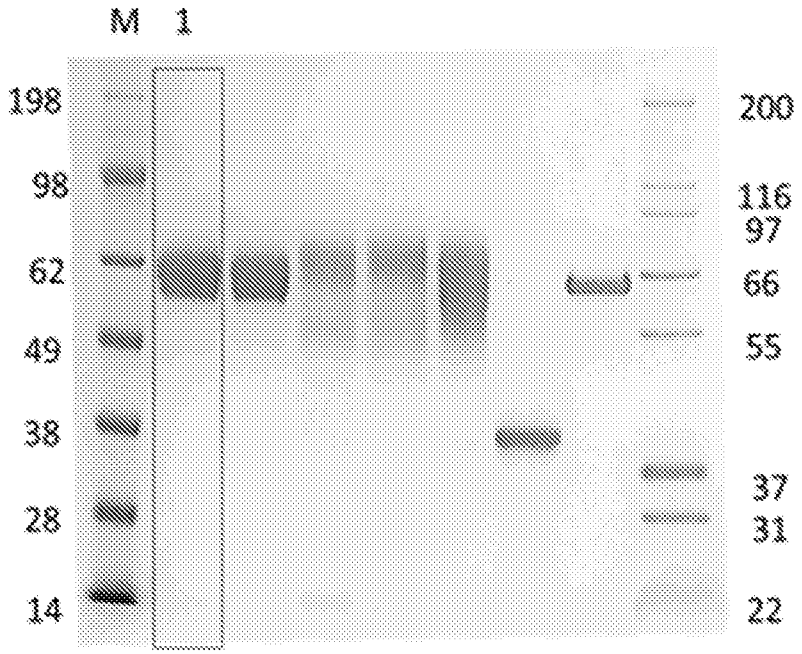


Figure 151A

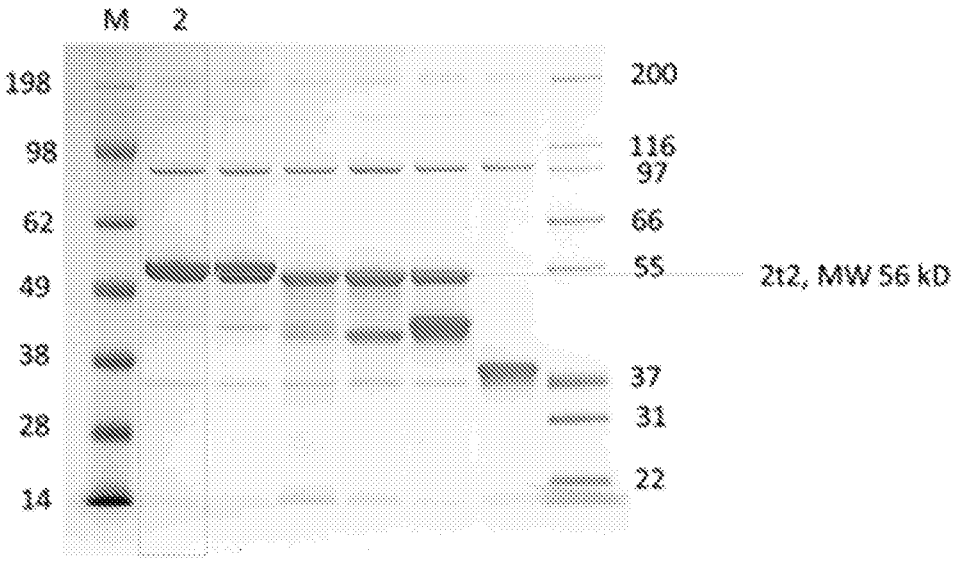


Figure 151B

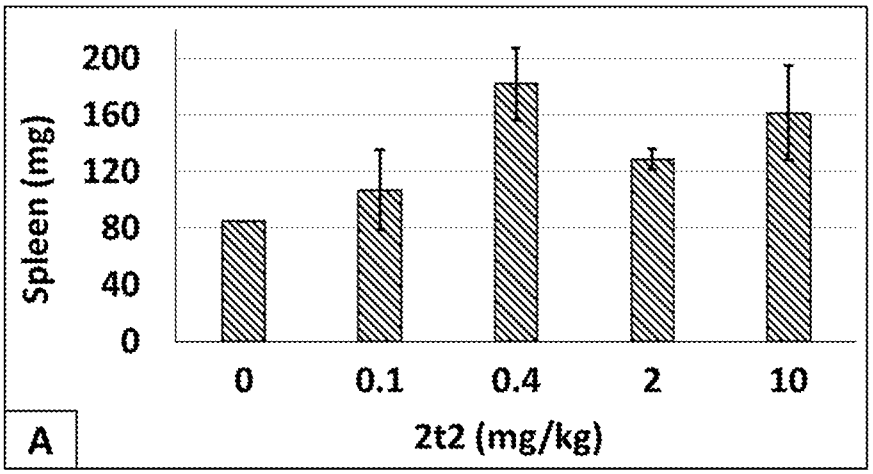


Figure 152A

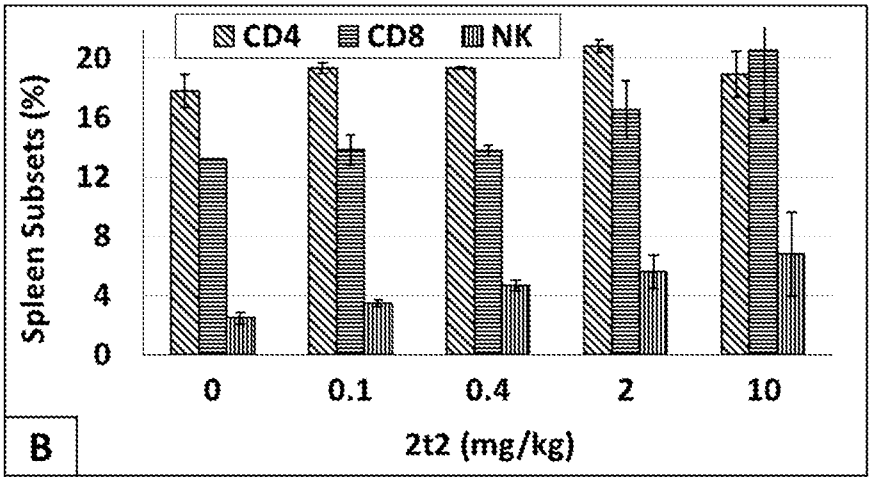


Figure 152B

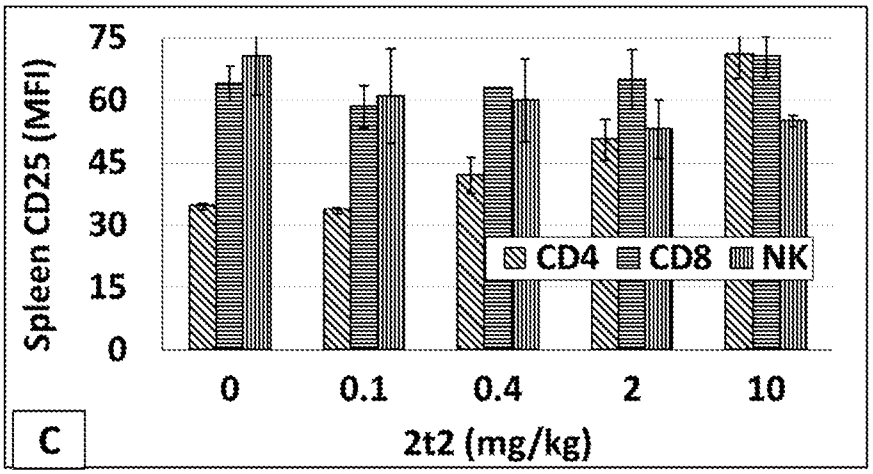


Figure 153

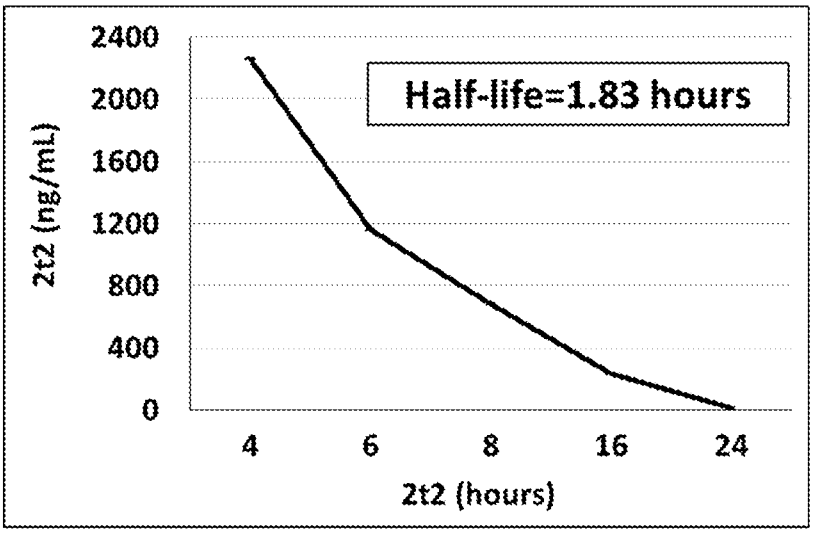


Figure 154

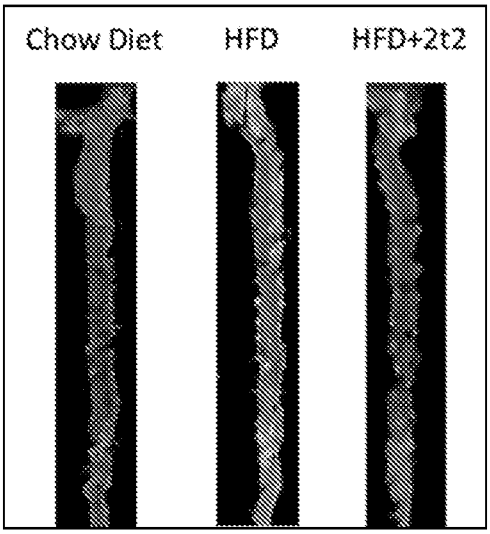


Figure 155A

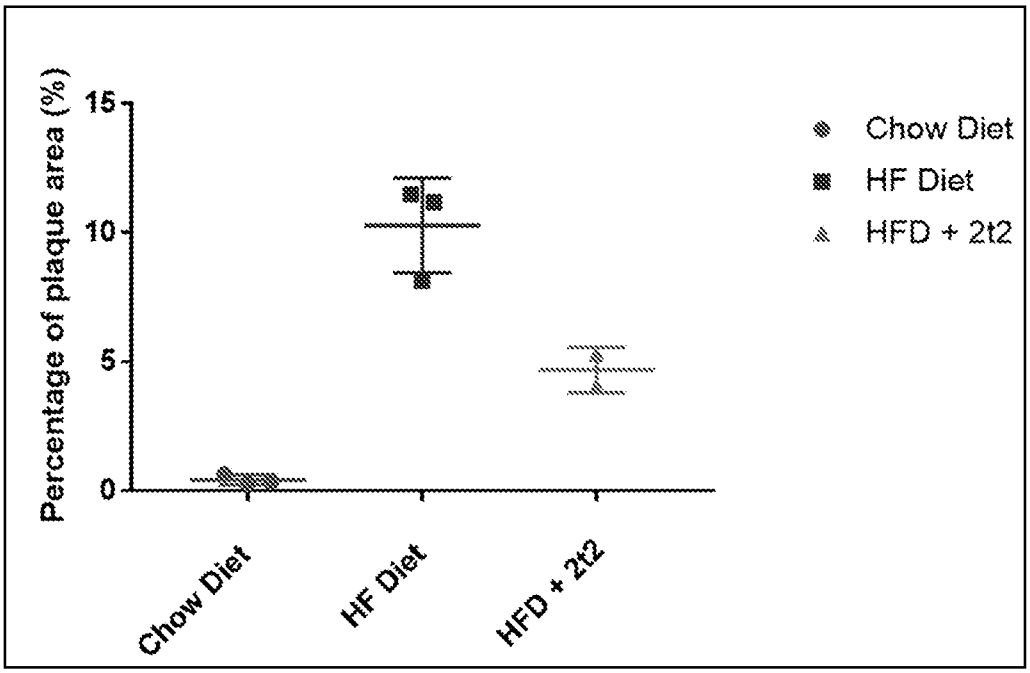


Figure 155B

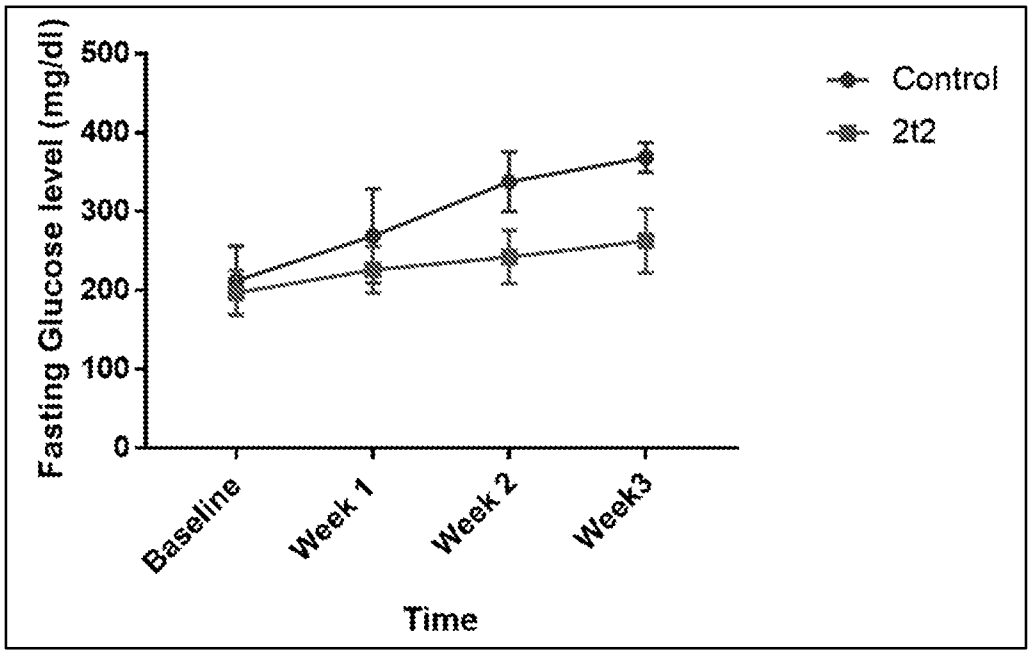


Figure 156

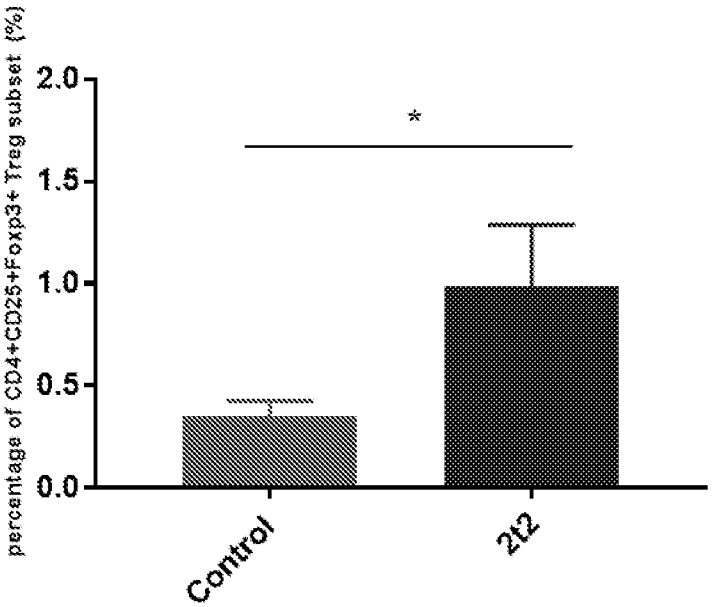


Figure 157

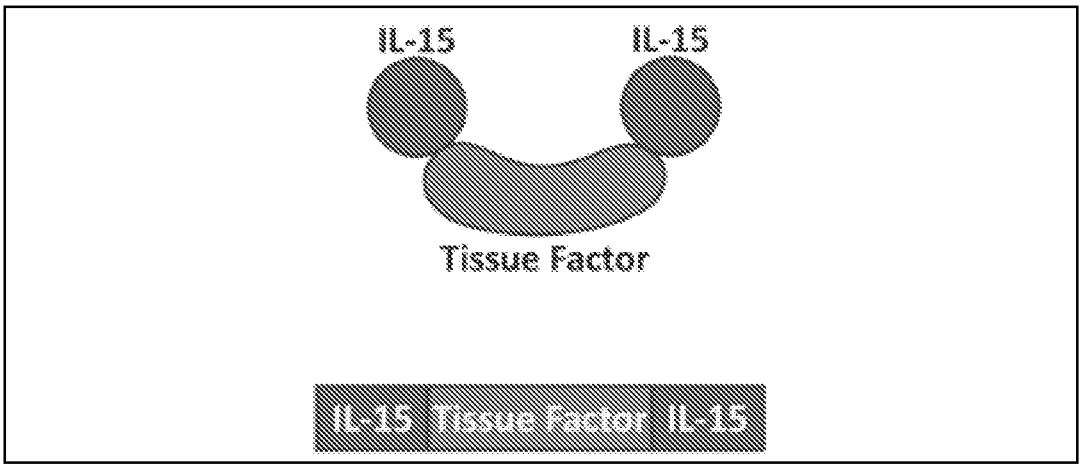


Figure 158

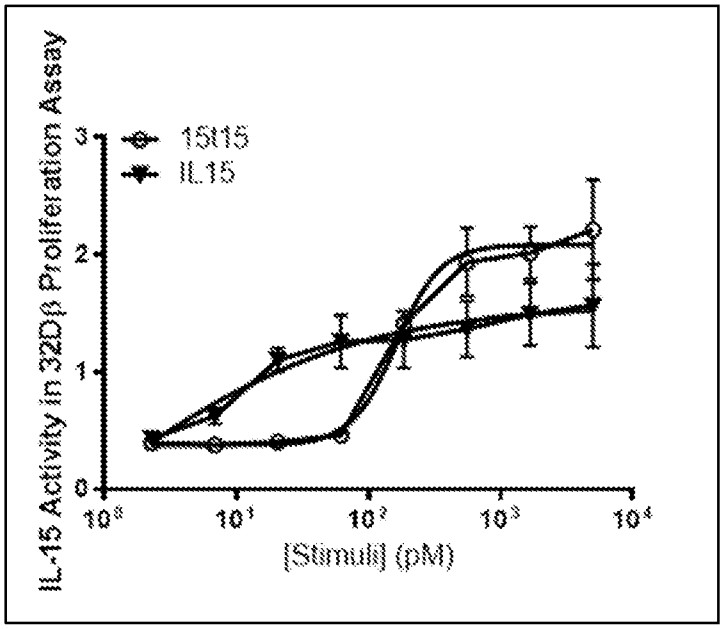

IL-15 Activity in 32Dβ Proliferation Assay
15i15
IL15
3
2
1
0
10⁰
10¹
10²
10³
10⁴
[Stimuli] (pM)

Figure 159

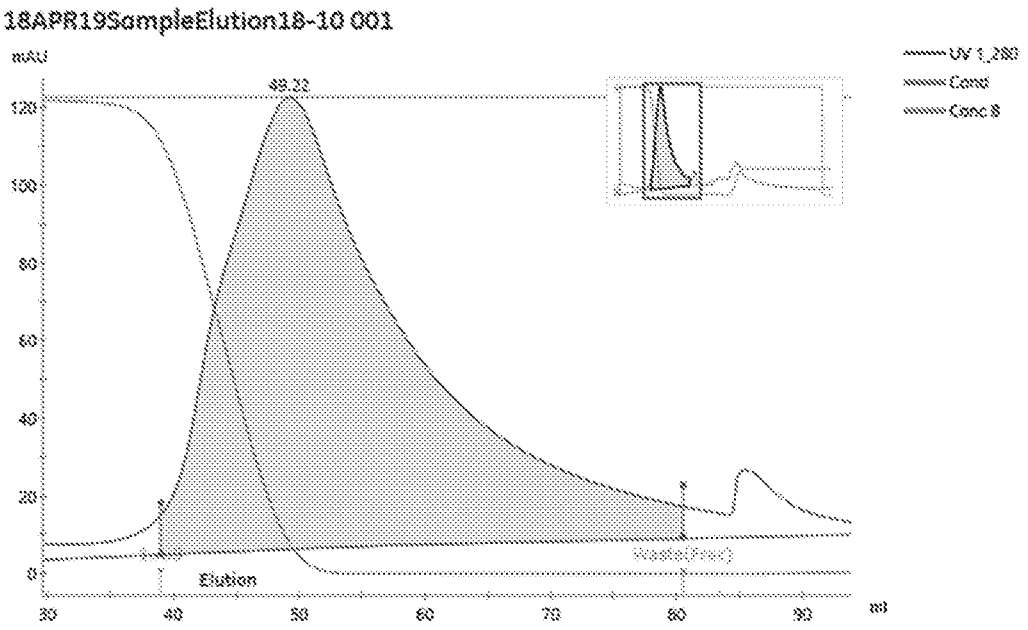

18APR19SampleElution18-10 001
mAU
UV 1_280
Cond
Conc B
Elution
Waste(Pool)
mL

Figure 160

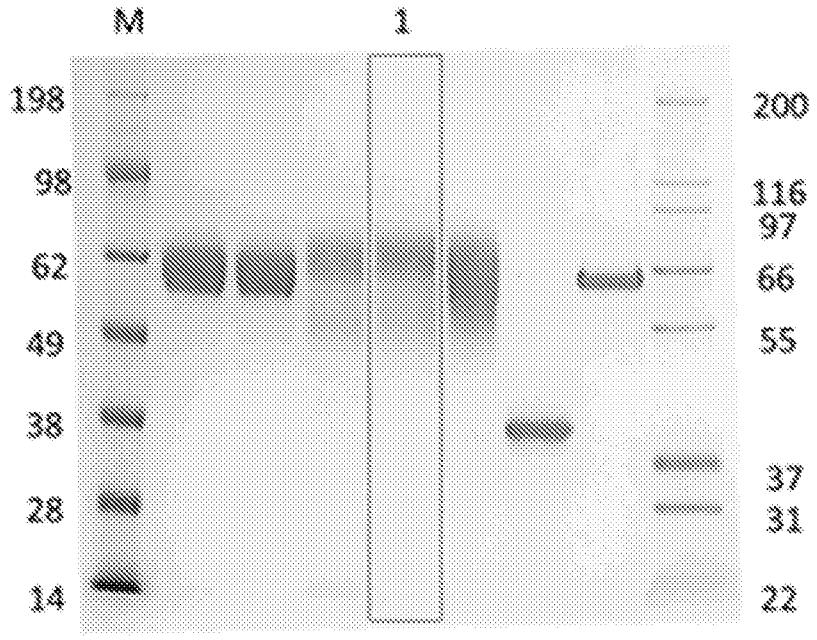


Figure 161A

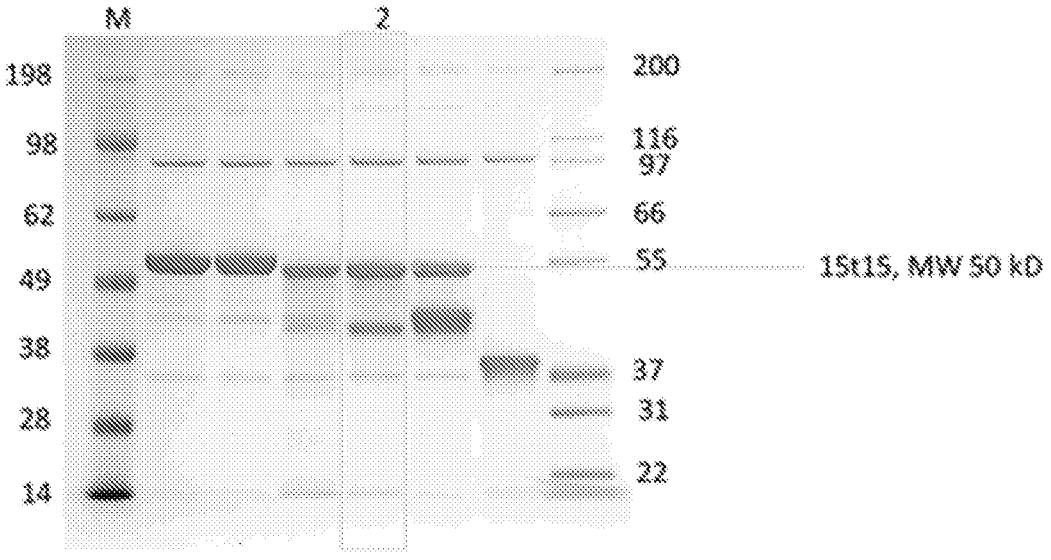


Figure 161B

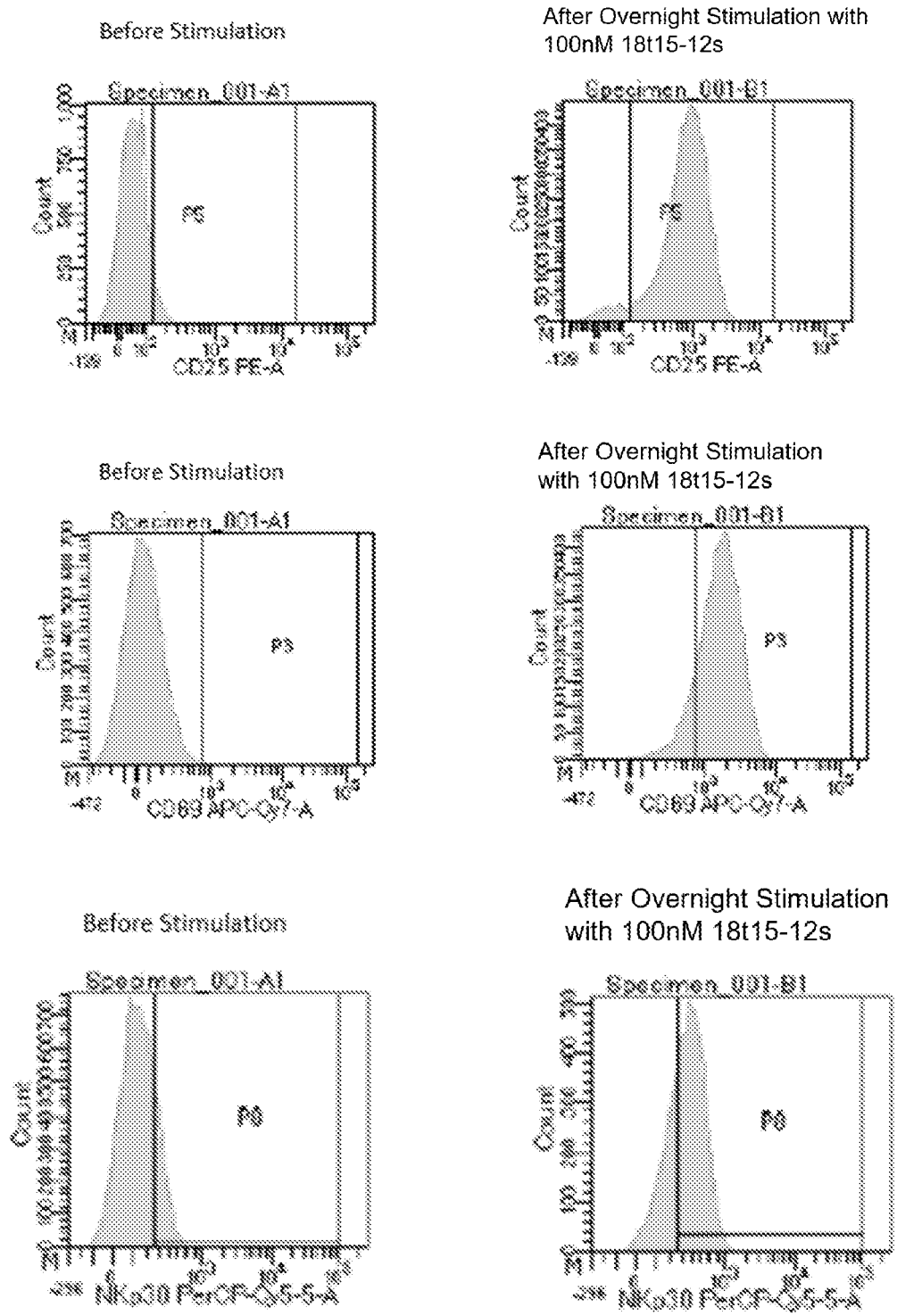

Before Stimulation
After Overnight Stimulation with 100nM 18t15-12s
Specimen_001-A1
Specimen_001-B1
Count
P5
CD25 PE-A
Before Stimulation
After Overnight Stimulation with 100nM 18t15-12s
Specimen_001-A1
Specimen_001-B1
Count
P3
CD69 APC-Cy7-A
Before Stimulation
After Overnight Stimulation with 100nM 18t15-12s
Specimen_001-A1
Specimen_001-B1
Count
P6
NKp30 PerCP-Cy5-5-A

Figure 162A

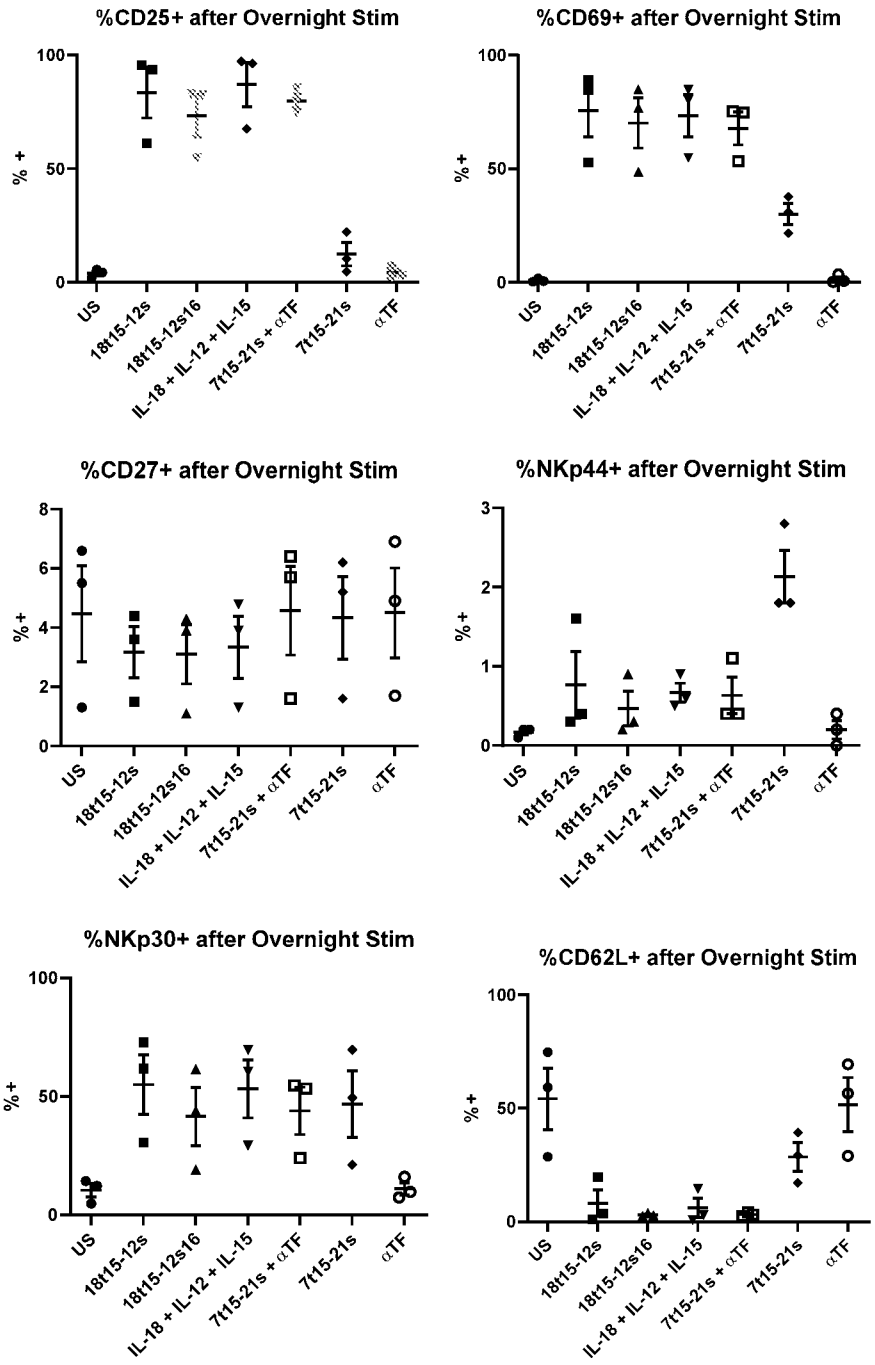

%CD25+ after Overnight Stim
%CD69+ after Overnight Stim
%CD27+ after Overnight Stim
%NKp44+ after Overnight Stim
%NKp30+ after Overnight Stim
%CD62L+ after Overnight Stim
% +
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF

Figure 162B

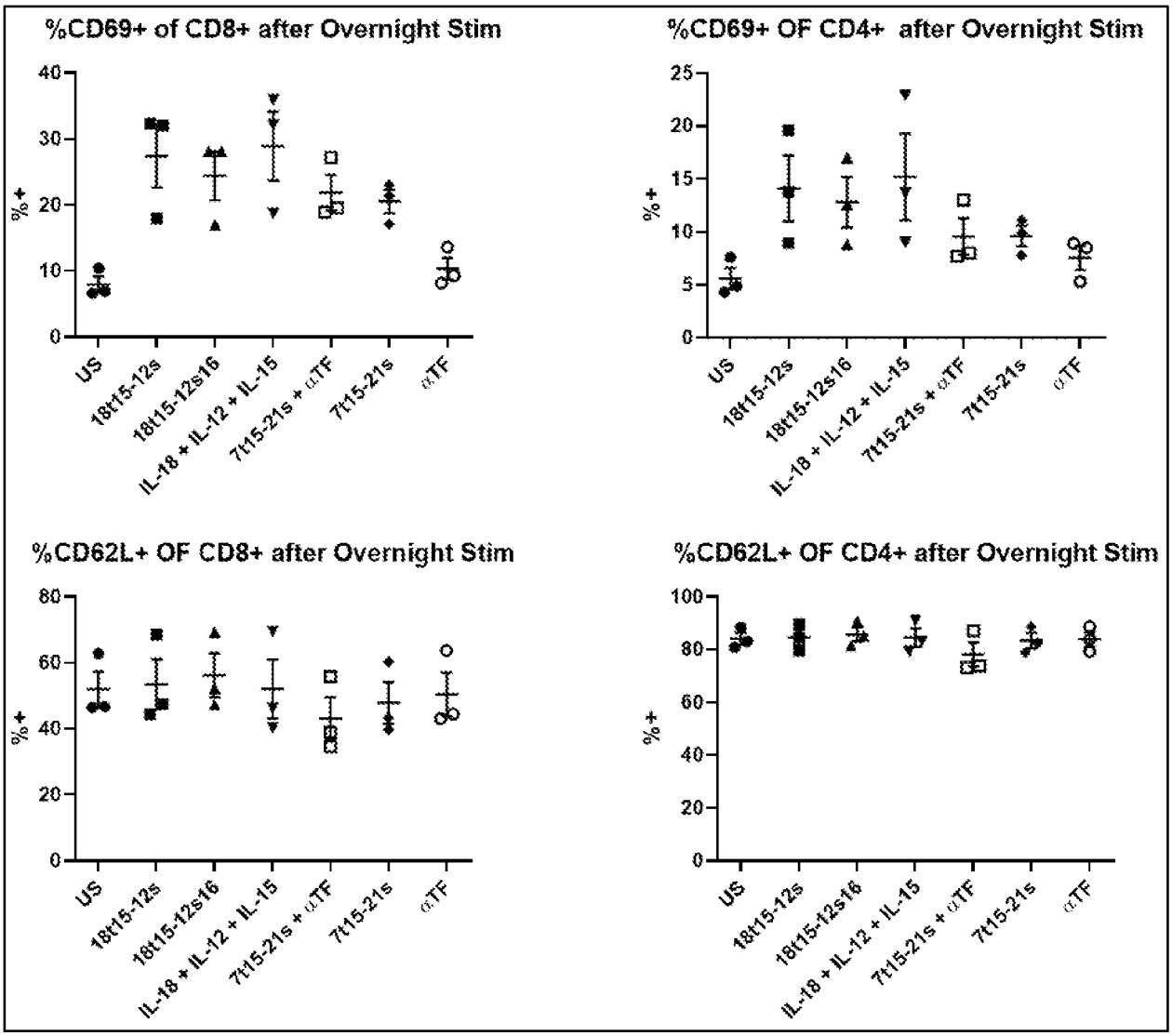

%CD69+ of CD8+ after Overnight Stim
%+
US
18t15-12s
18t15-12s16
IL-18 + IL-12 + IL-15
7t15-21s + αTF
7t15-21s
αTF
%CD69+ OF CD4+ after Overnight Stim
%CD62L+ OF CD8+ after Overnight Stim
%CD62L+ OF CD4+ after Overnight Stim

Figure 163

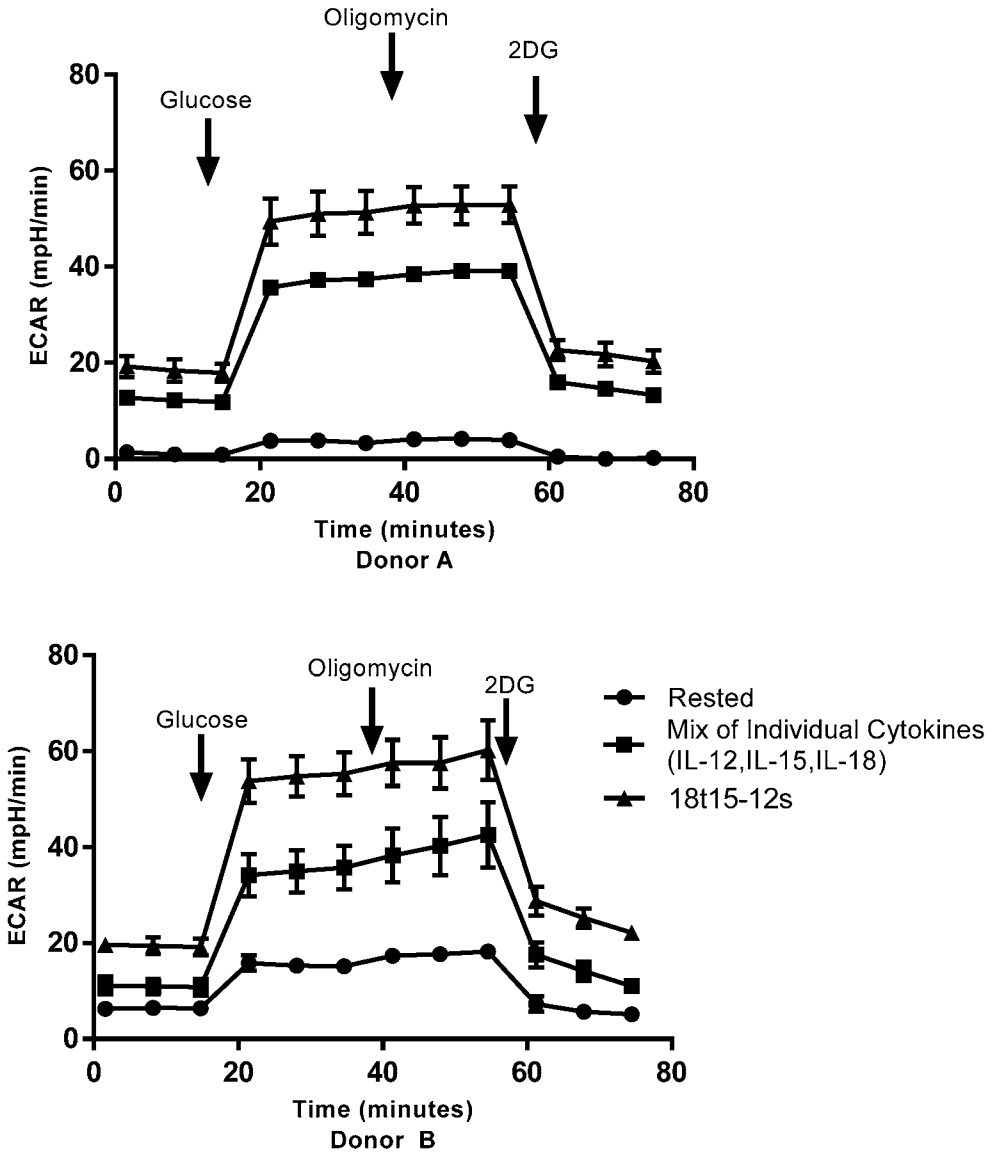

Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Donor A
Oligomycin
2DG
Glucose
ECAR (mpH/min)
Time (minutes)
Donor B
Rested
Mix of Individual Cytokines
(IL-12,IL-15,IL-18)
18t15-12s

Figure 164

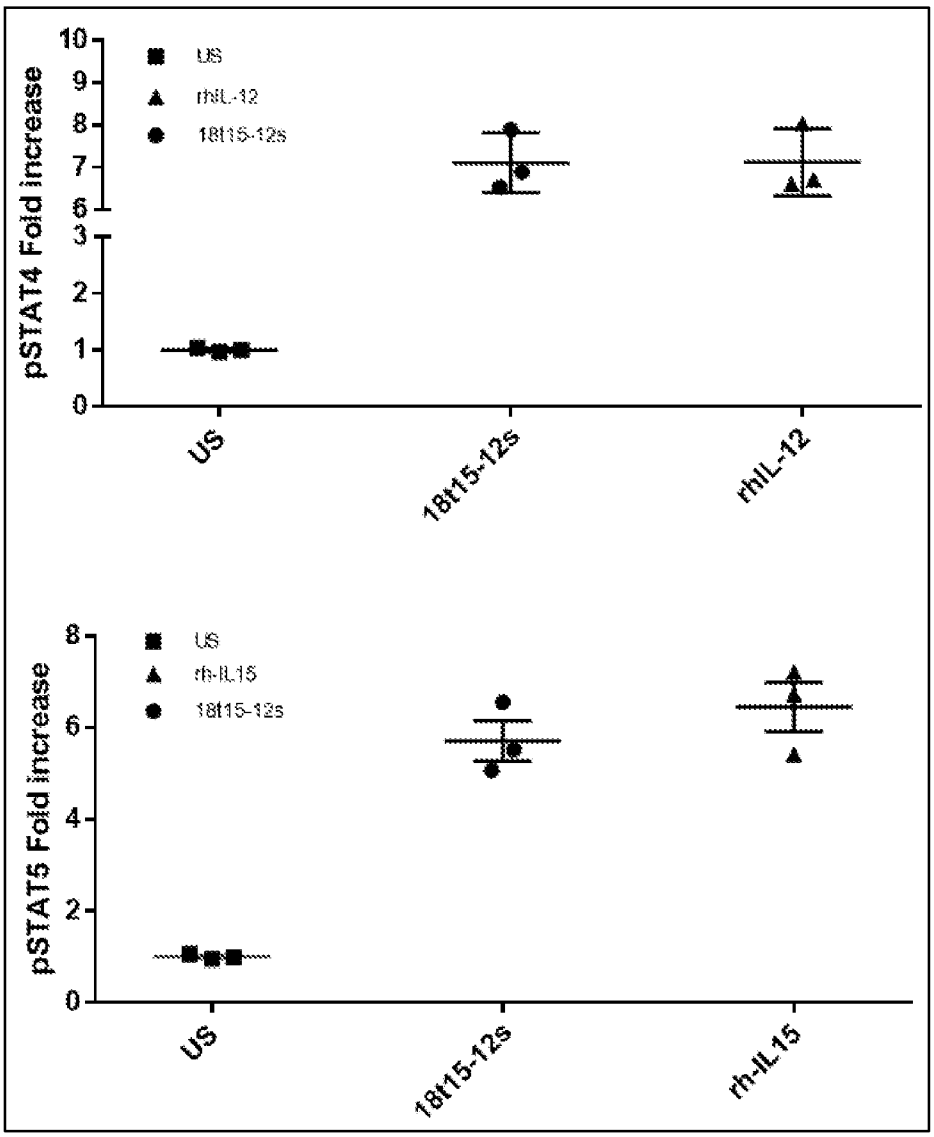

pSTAT4 Foid increase
US
rhIL-12
18t15-12s
10
9
8
7
6
3
2
1
0
US
18t15-12s
rhIL-12
pSTAT5 Foid increase
US
rh-IL15
18t15-12s
8
6
4
2
0
US
18t15-12s
rh-IL15

Figure 165

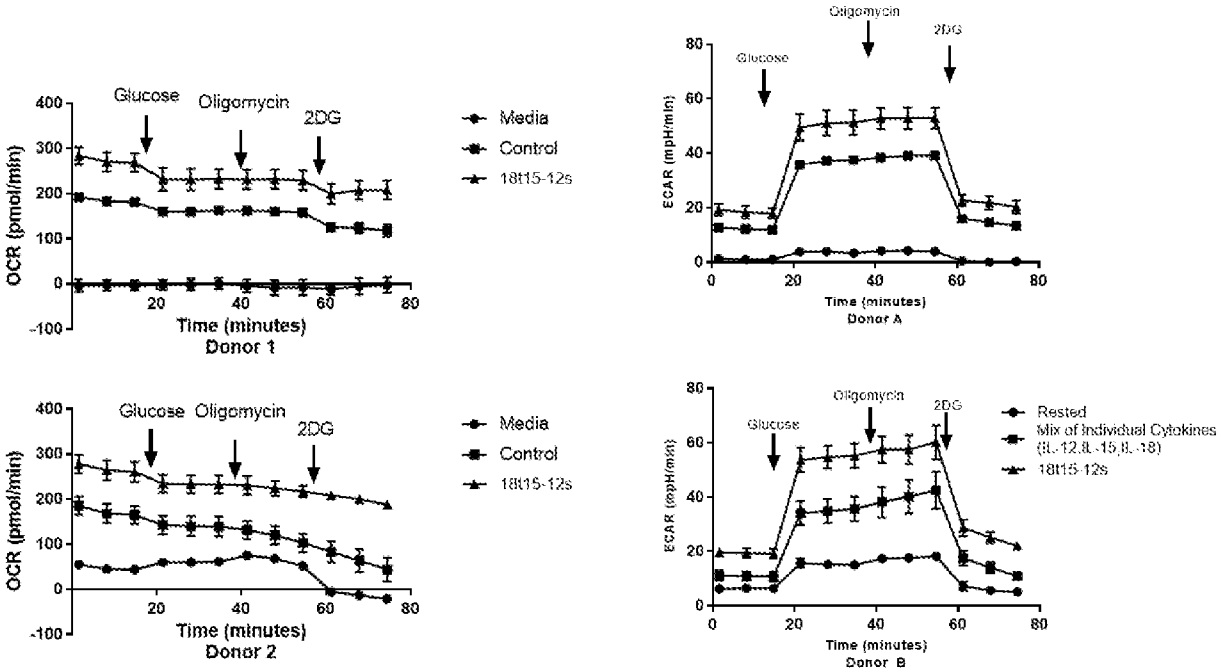

Glucose
Oligomycin
2DG
OCR (pmol/min)
Time (minutes)
Donor 1
Media
Control
18t15-12s
ECAR (mpH/min)
Donor A
Donor 2
Donor B
Rested
Mix of Individual Cytokines
(IL-12,IL-15,IL-18)

Figure 166

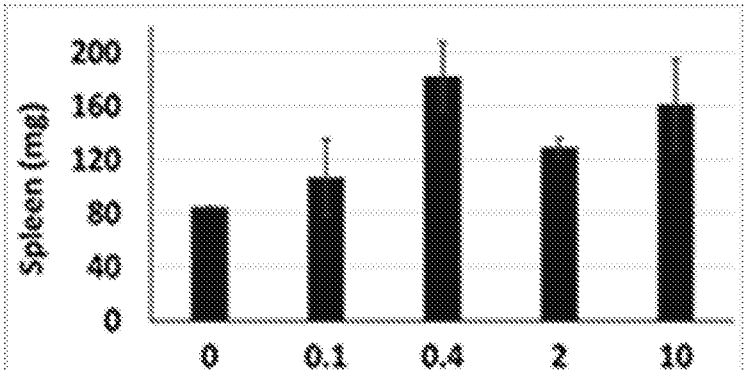


Figure 167A

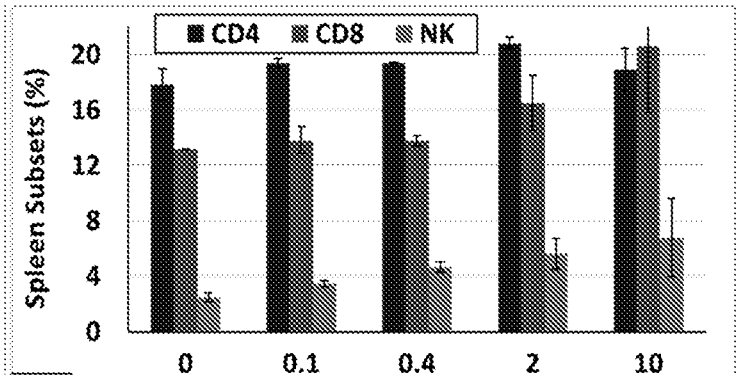


Figure 167B

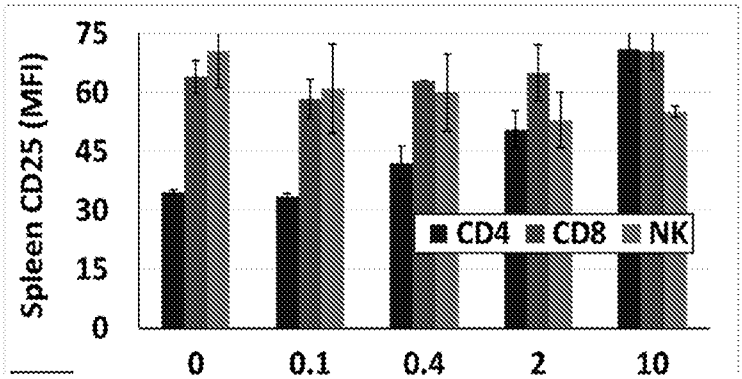


Figure 167C

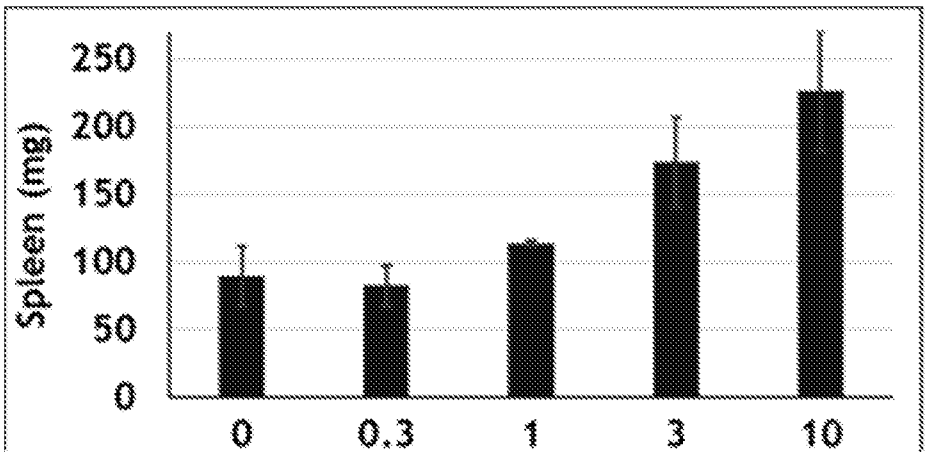


Figure 168A

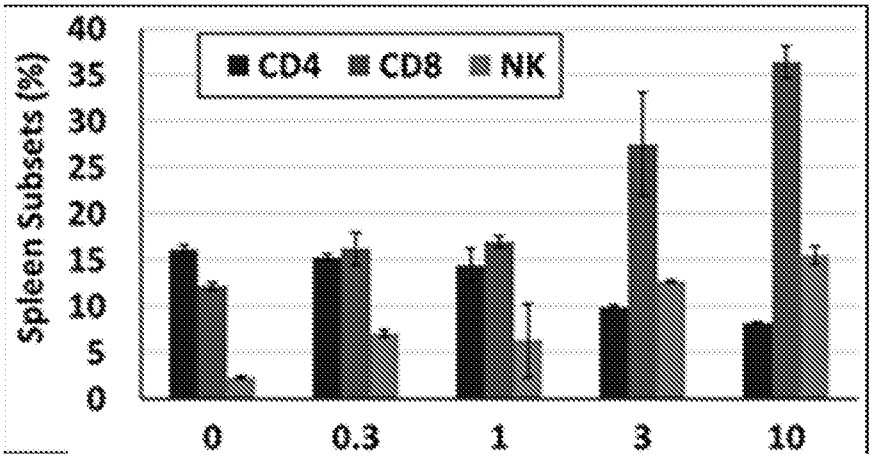


Figure 168B

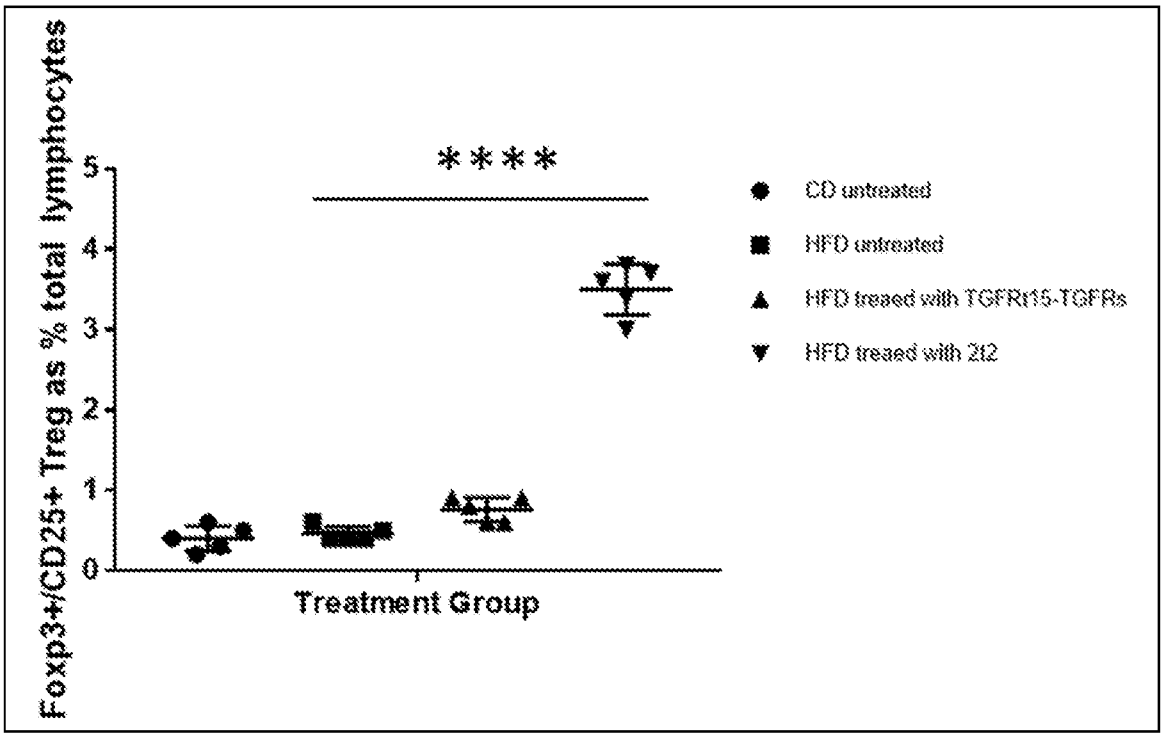


Figure 169A

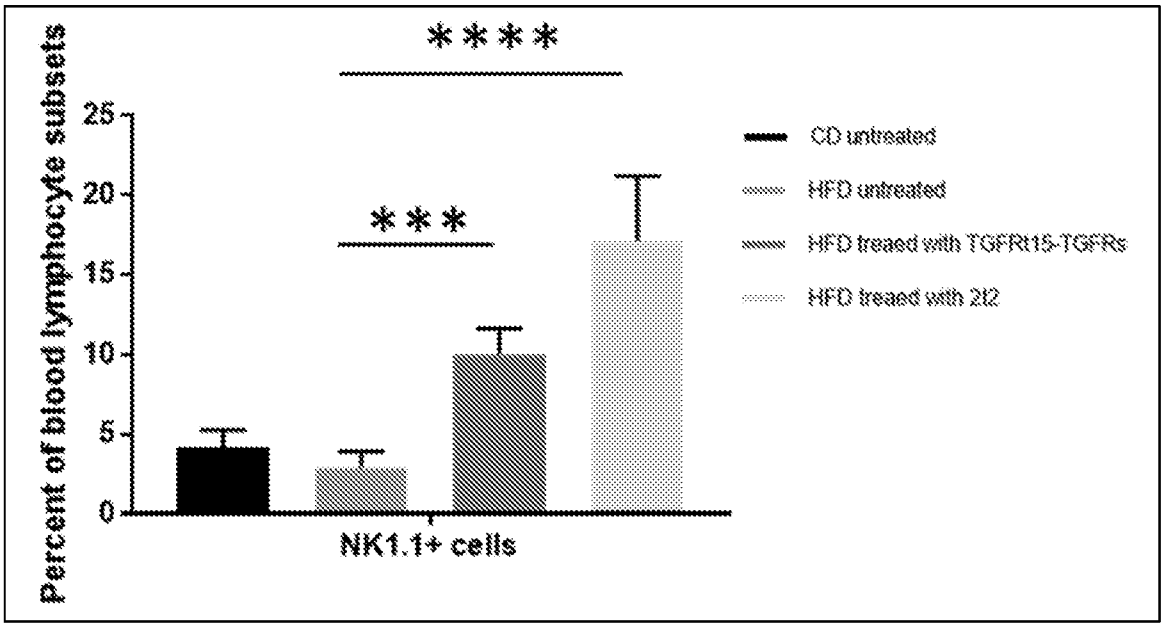


Figure 169B

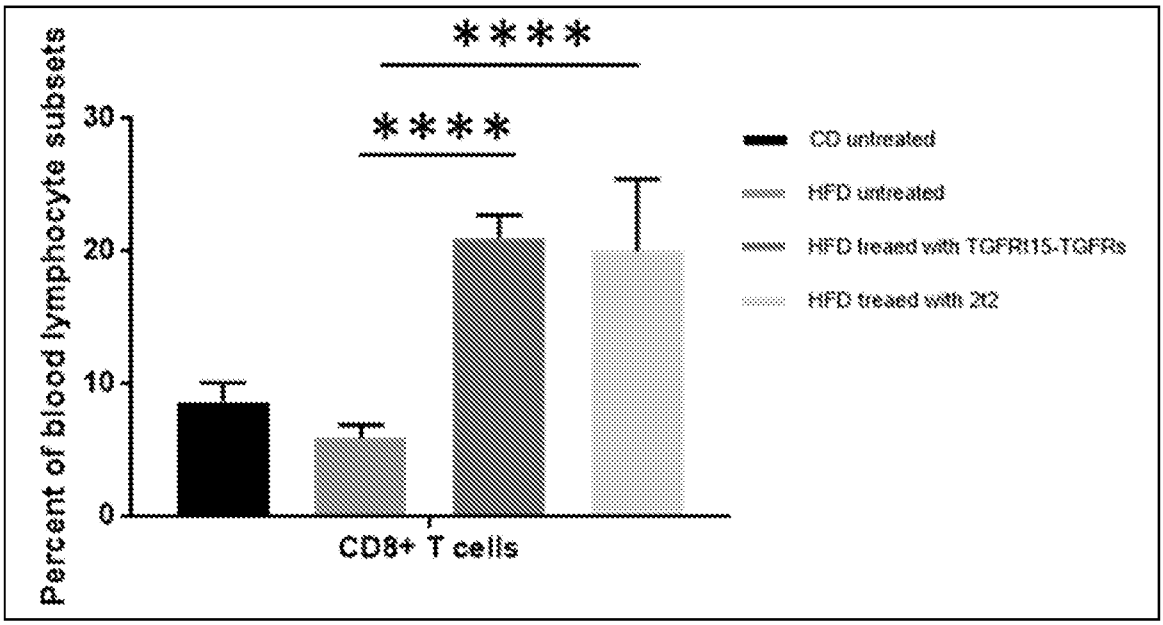

Percent of blood lymphocyte subsets
30
20
10
0
****
****
CD8+ T cells
CD untreated
HFD untreated
HFD treaed with TGFBR1S-TGFBRs
HFD treaed with 2t2

Figure 169C

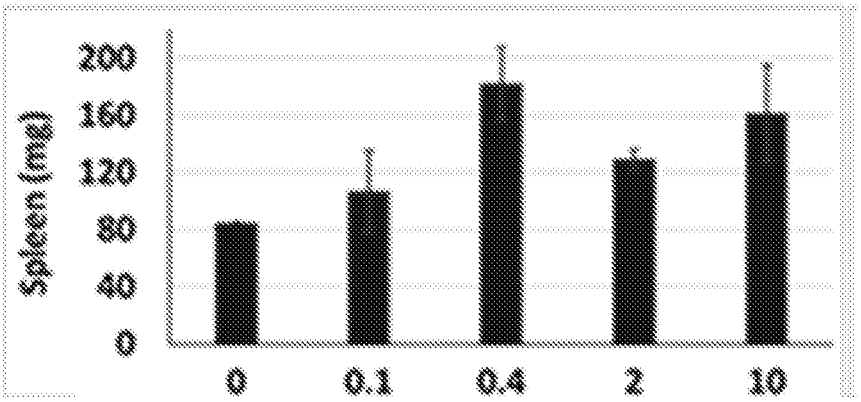


Figure 170A

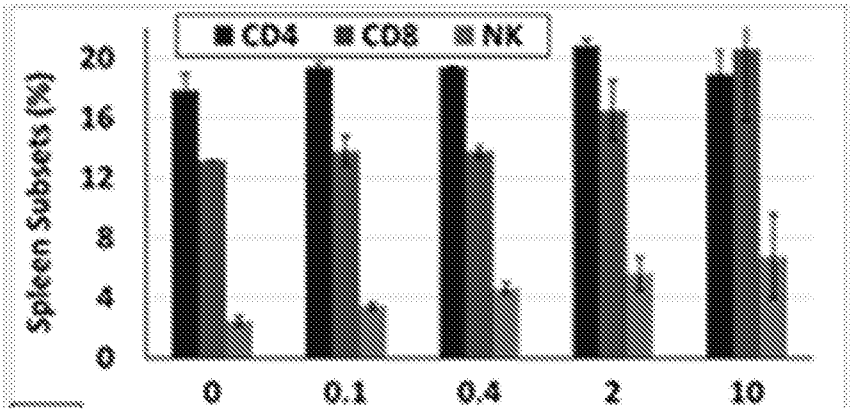


Figure 170B

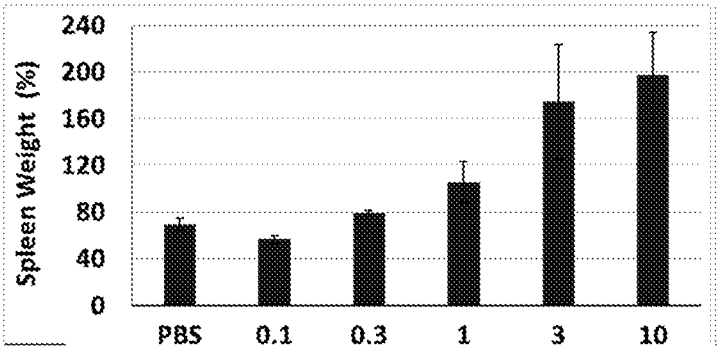


Figure 171A

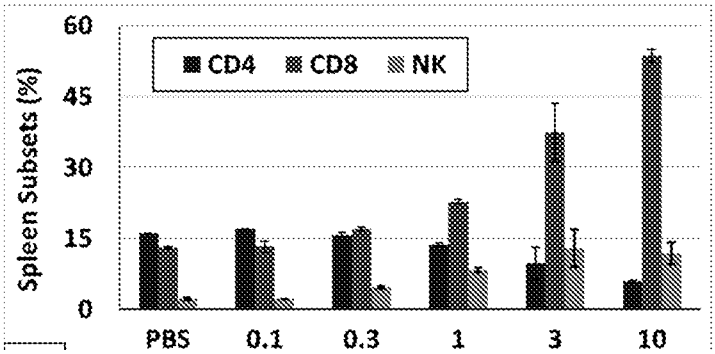


Figure 171B

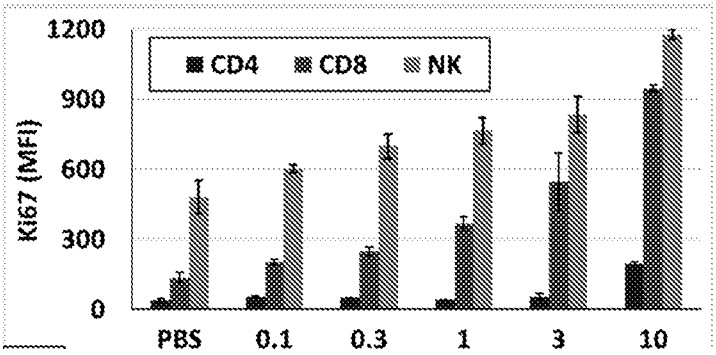


Figure 171C

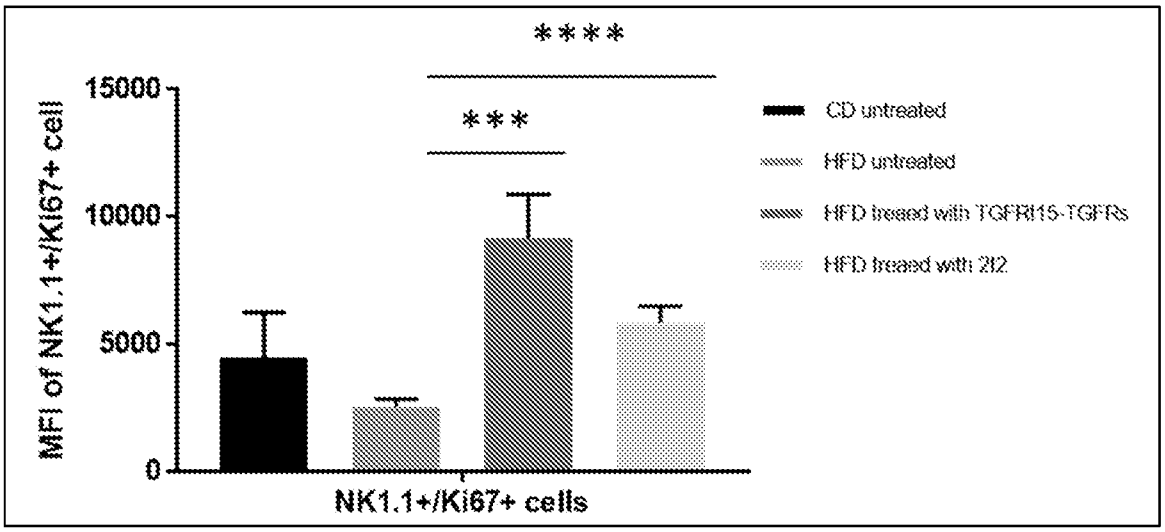


Figure 172A

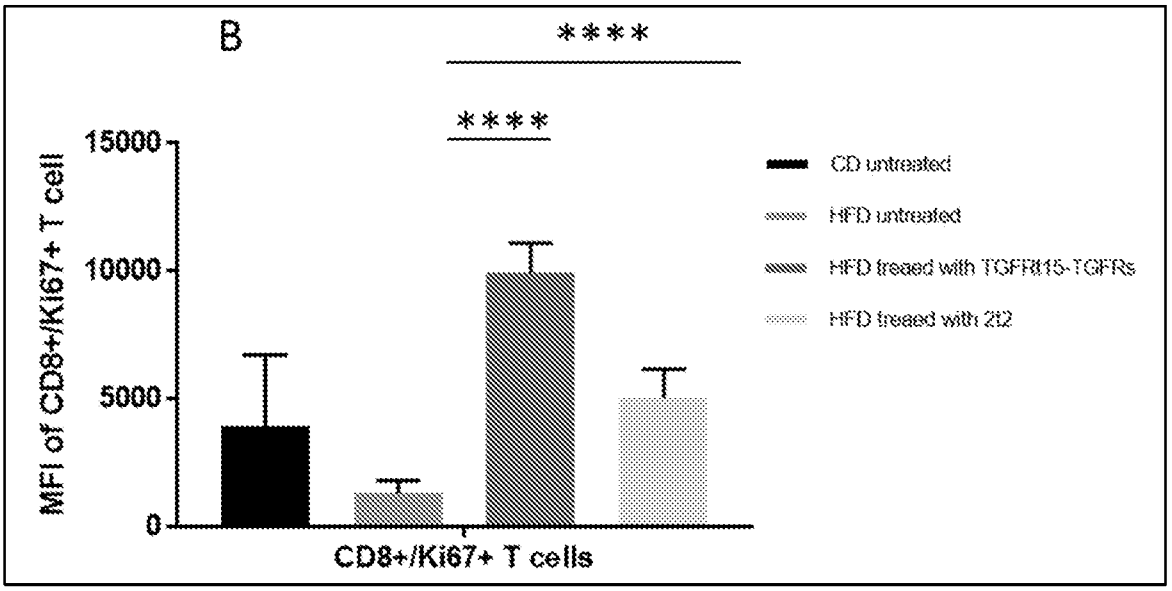
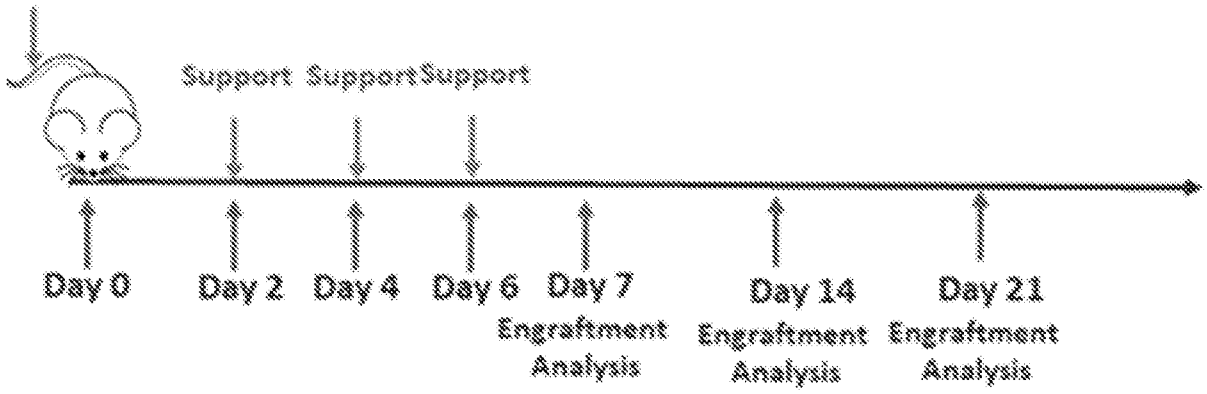


Figure 172B


Support
Support
Support
Day 0
Day 2
Day 4
Day 6
Day 7
Engraftment Analysis
Day 14
Engraftment Analysis
Day 21
Engraftment Analysis

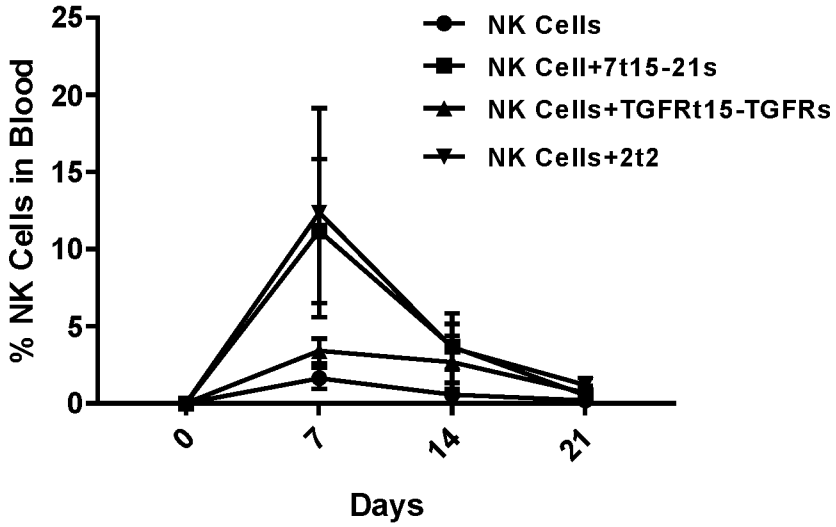

% NK Cells in Blood
25
20
15
10
5
0
0
7
14
21
Days
NK Cells
NK Cell+7t15-21s
NK Cells+TGFRt15-TGFRs
NK Cells+2t2

Figure 173

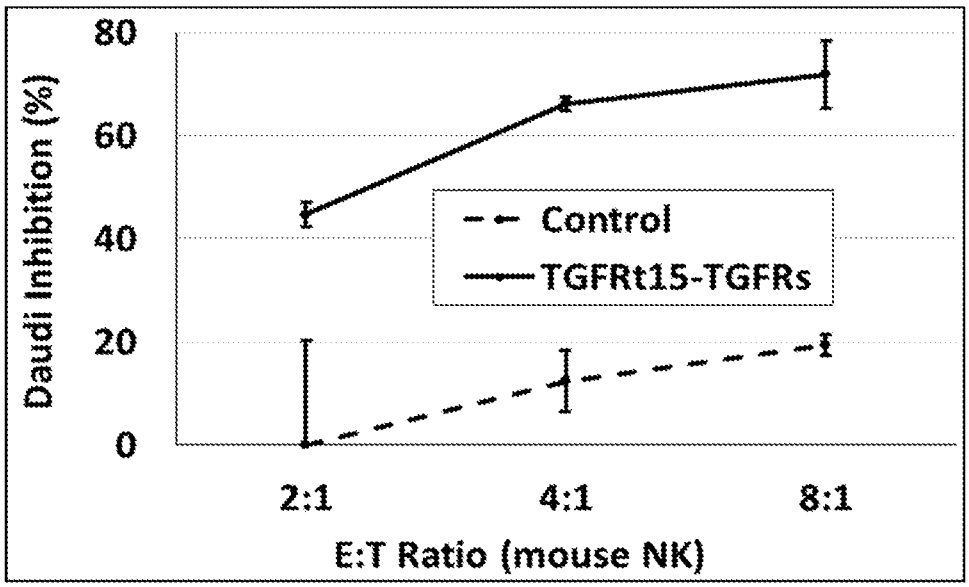


Figure 174A

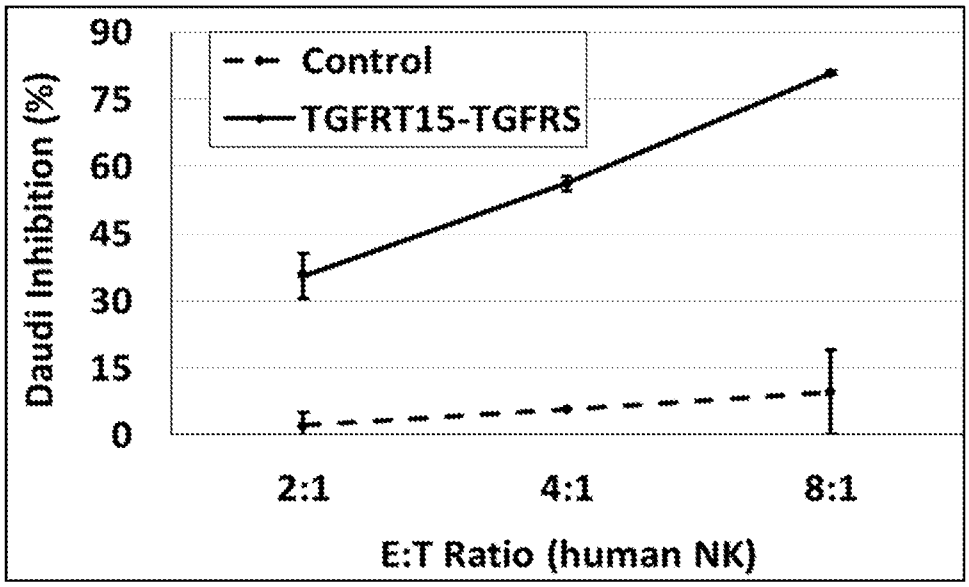


Figure 174B

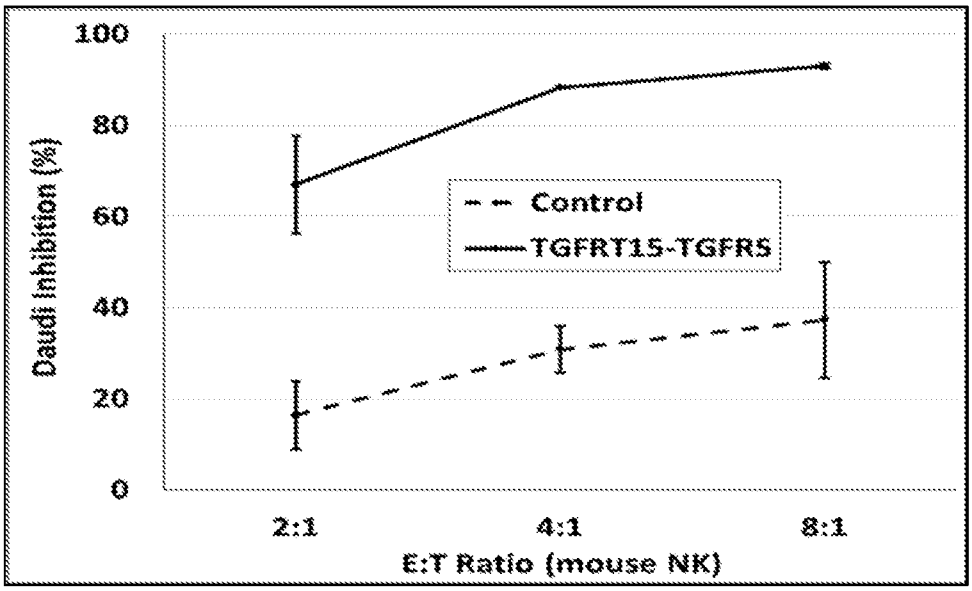


Figure 175A

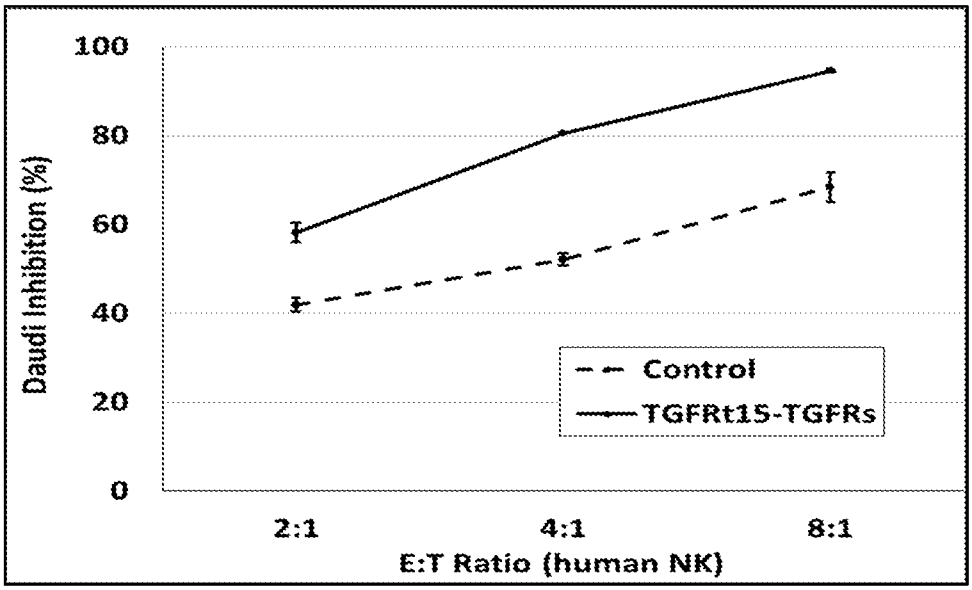


Figure 175B

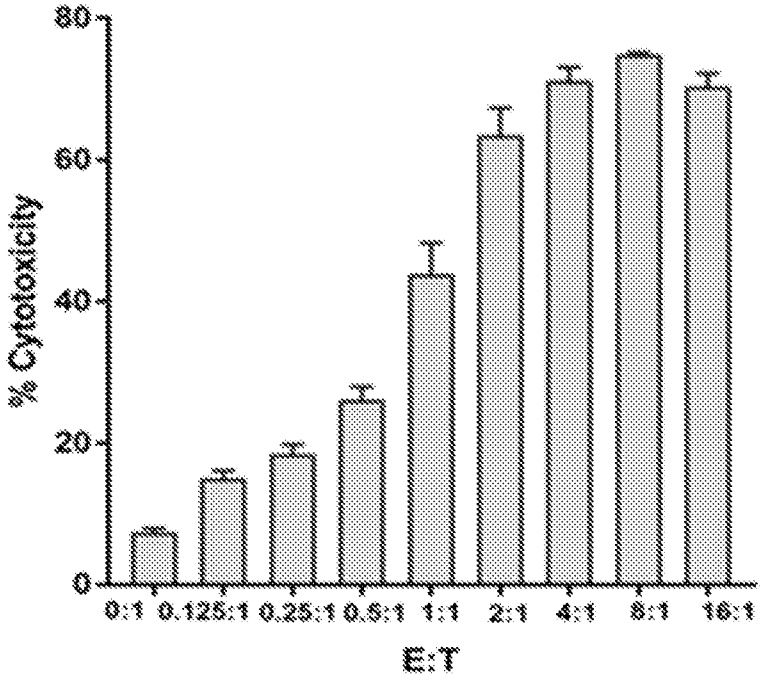
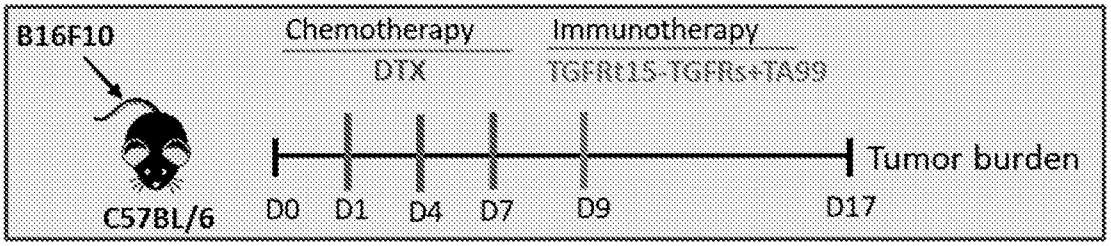


Figure 176



DTX (10 mg/kg), TGFRt15-TGFRs (3mg/kg), TA99 (200 μg)

Figure 177A

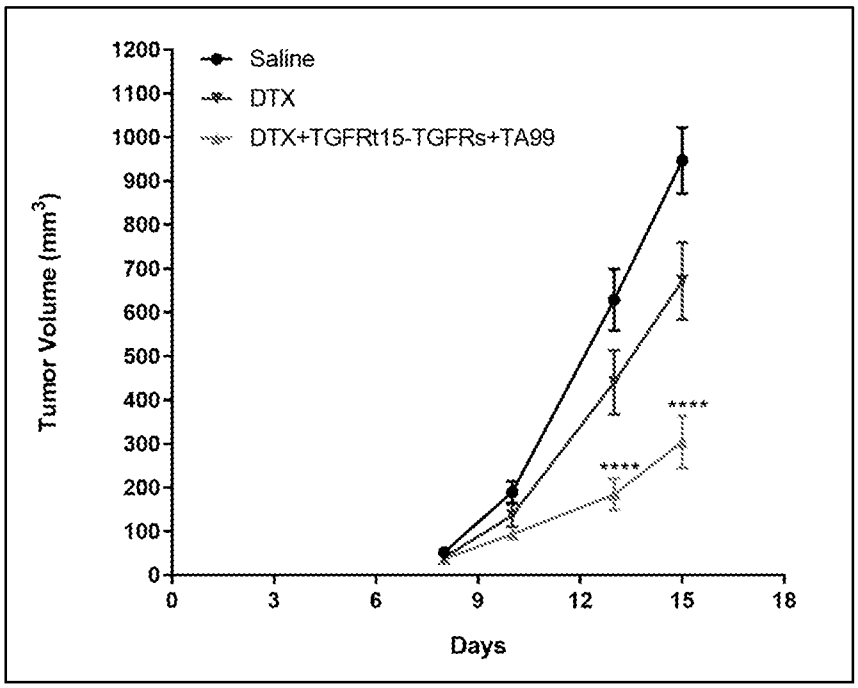


Figure 177B

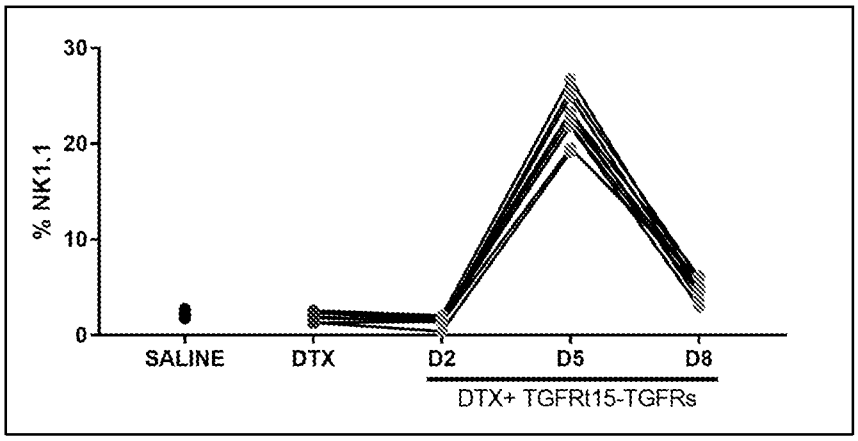


Figure 177C

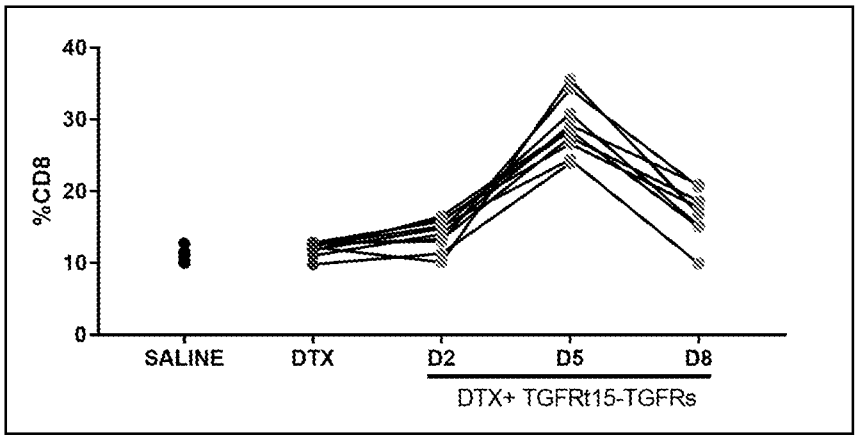


Figure 177D

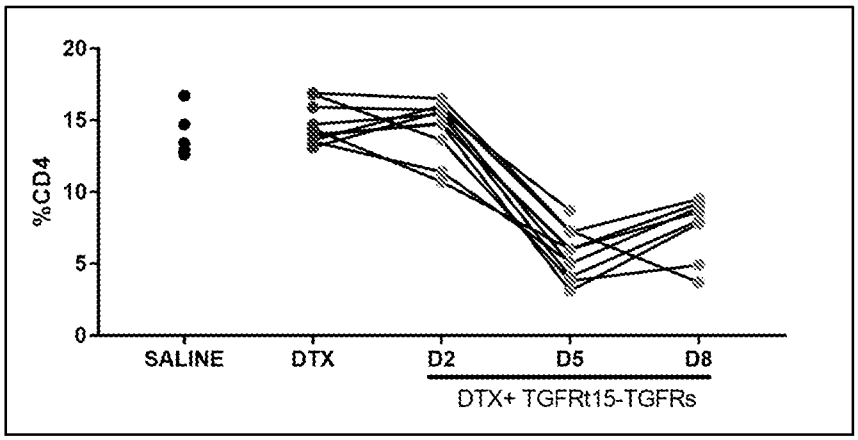


Figure 177E

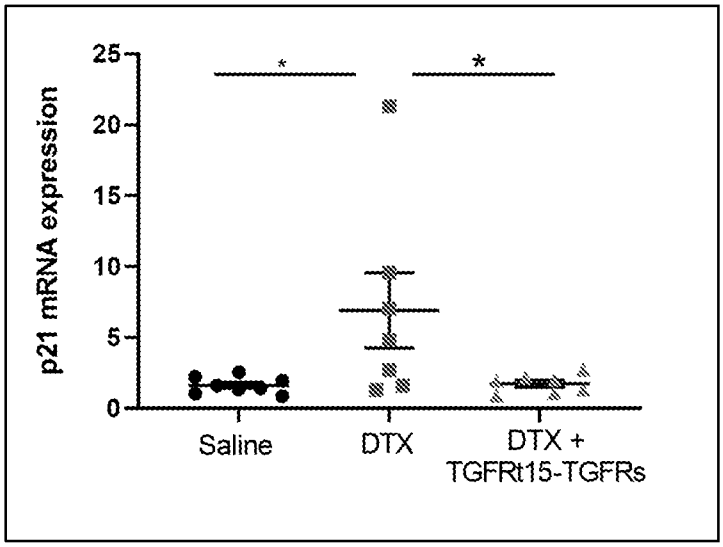


Figure 177F

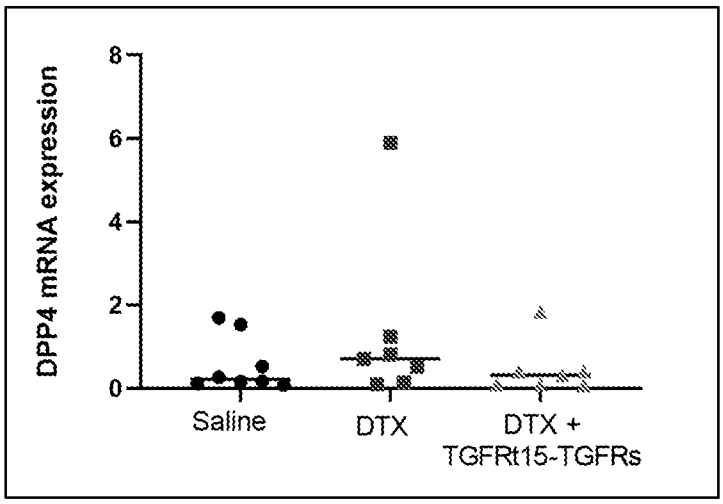


Figure 177G

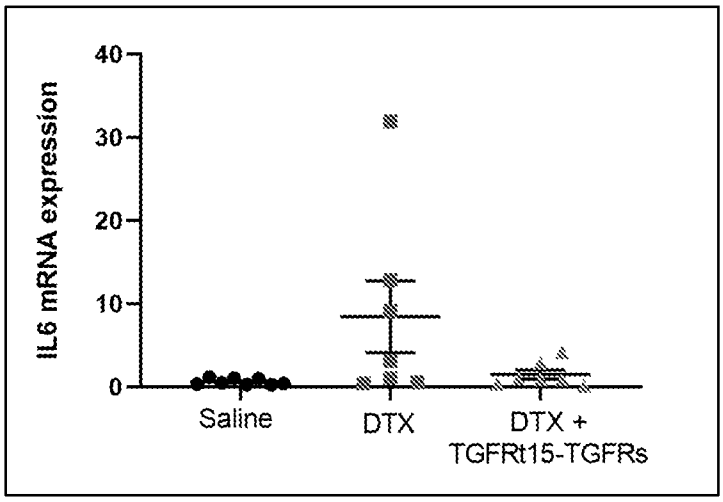

IL6 mRNA expression
40
30
20
10
0
Saline
DTX
DTX +
TGFRt15-TGFRs

Figure 177H

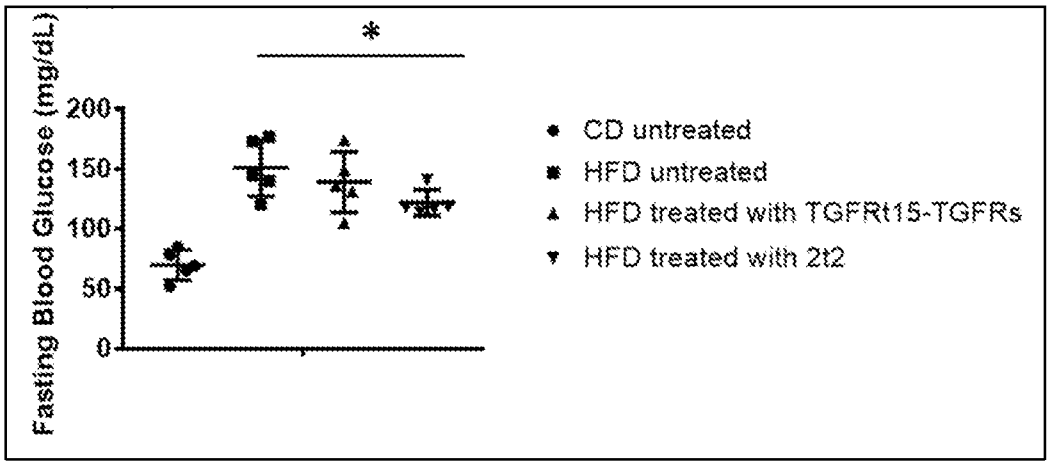


Figure 178A

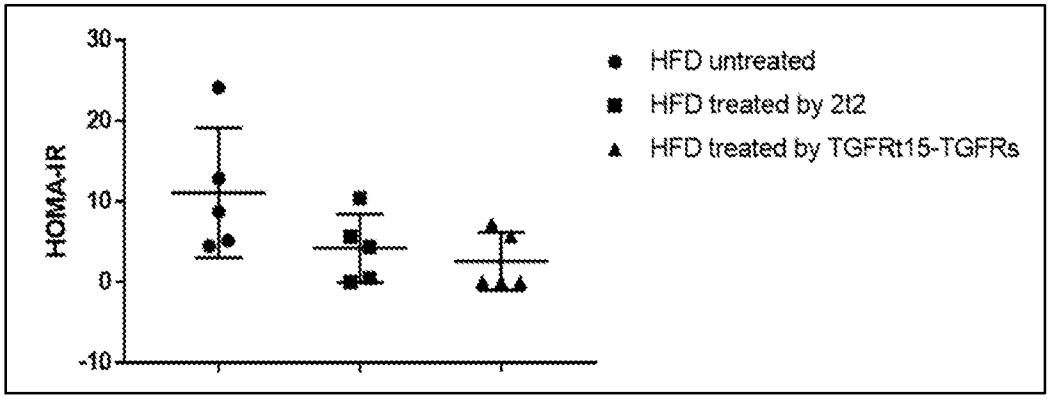


Figure 178B

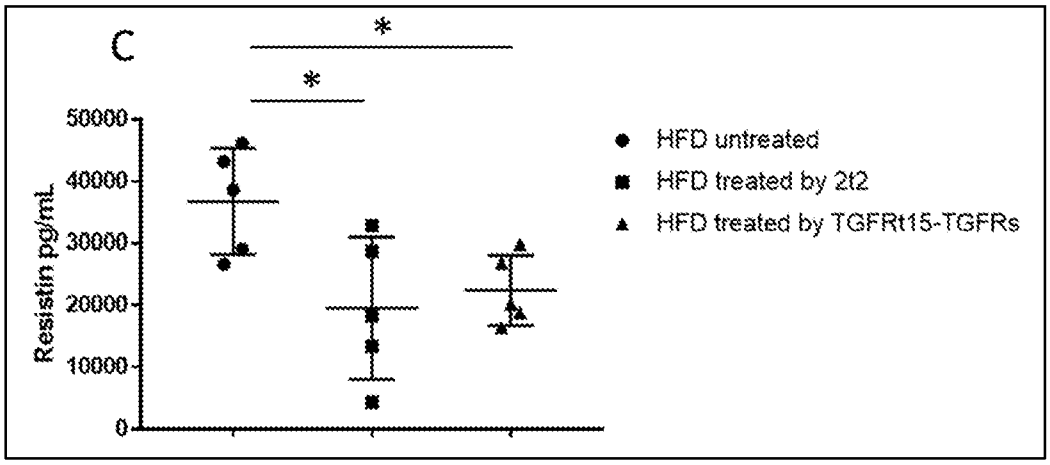


Figure 178C

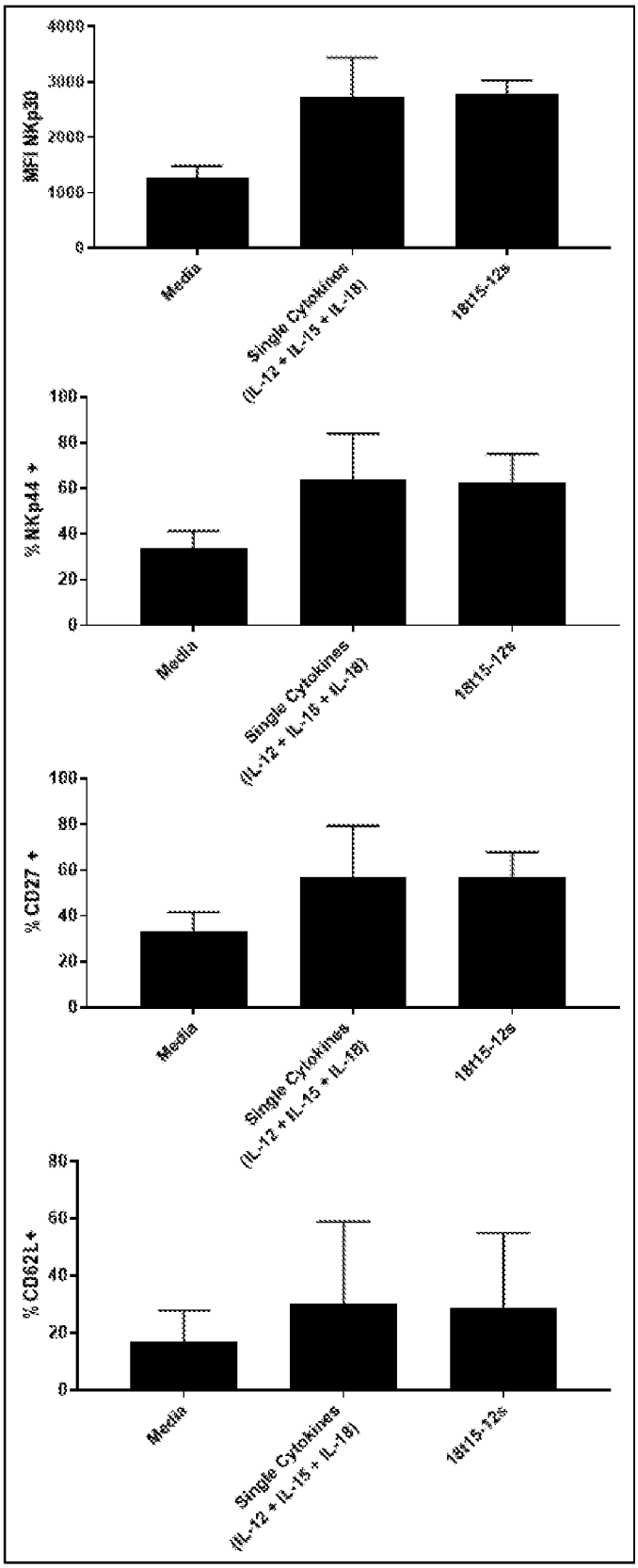

MFI NKp30
4000
3000
2000
1000
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18/15-12s
% NKp44 +
100
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18/15-12s
% CD27 +
100
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18/15-12s
% CD62L +
80
60
40
20
0
Media
Single Cytokines (IL-12 + IL-15 + IL-18)
18/15-12s

Figure 179

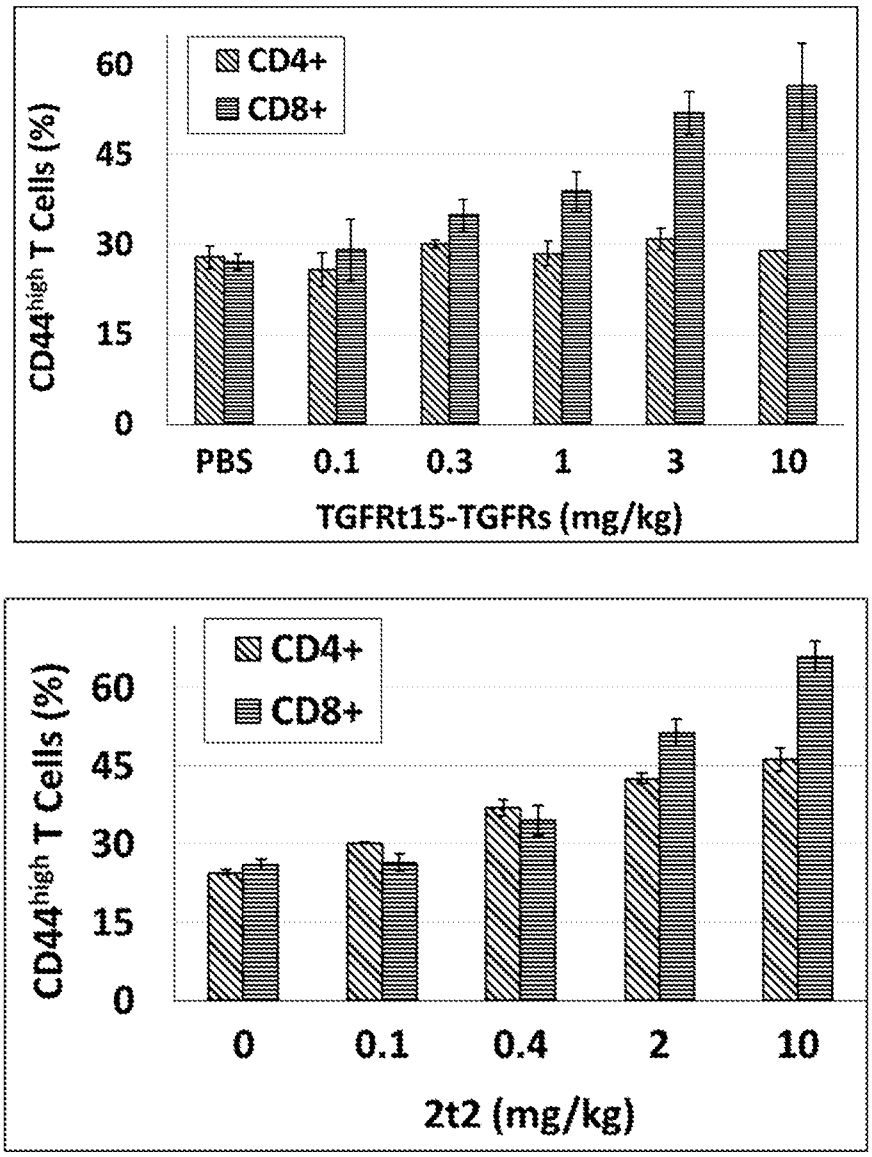

CD4+
CD8+
CD44high T Cells (%)
60
45
30
15
0
PBS
0.1
0.3
1
3
10
TGFRt15-TGFRs (mg/kg)
CD4+
CD8+
CD44high T Cells (%)
60
45
30
15
0
0
0.1
0.4
2
10
2t2 (mg/kg)

Figure 180

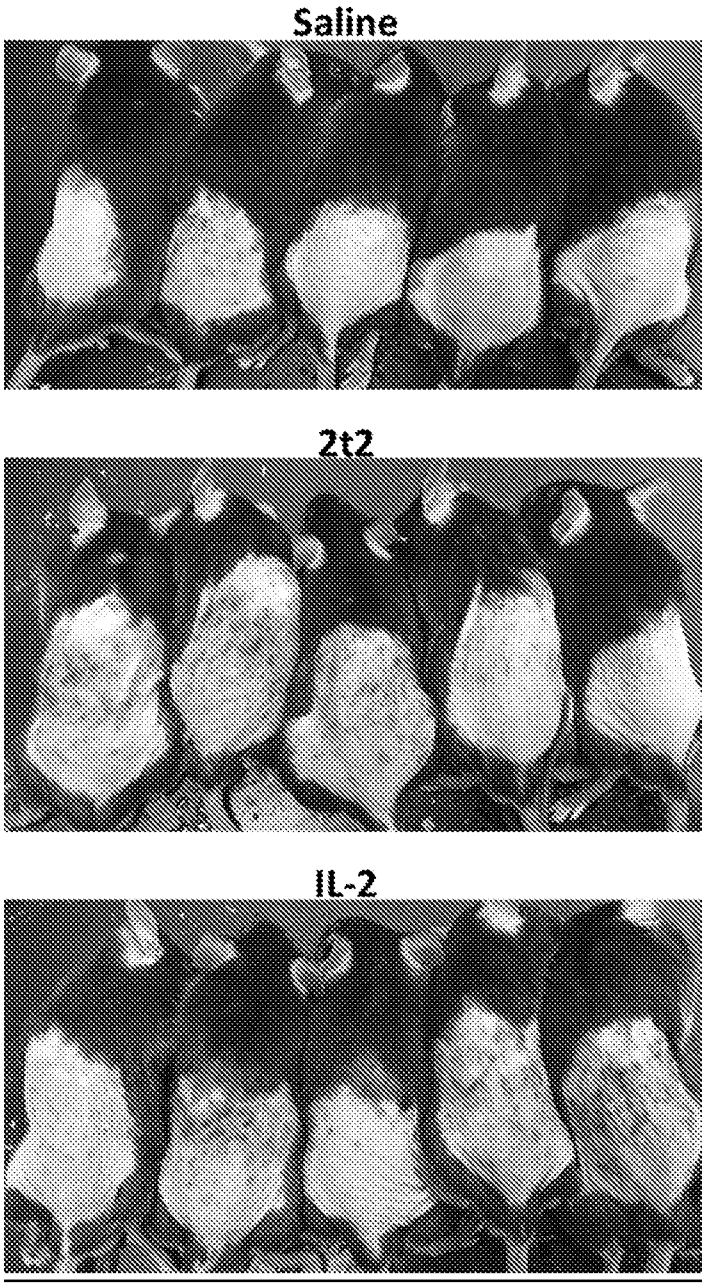


Figure 181A

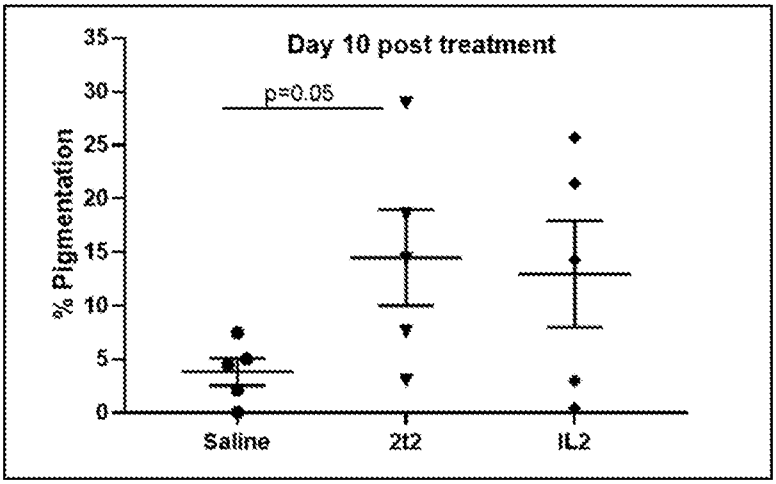

Day 10 post treatment
p=0.05
% Pigmentation
35
30
25
20
15
10
5
0
Saline
2t2
IL2

Figure 181B

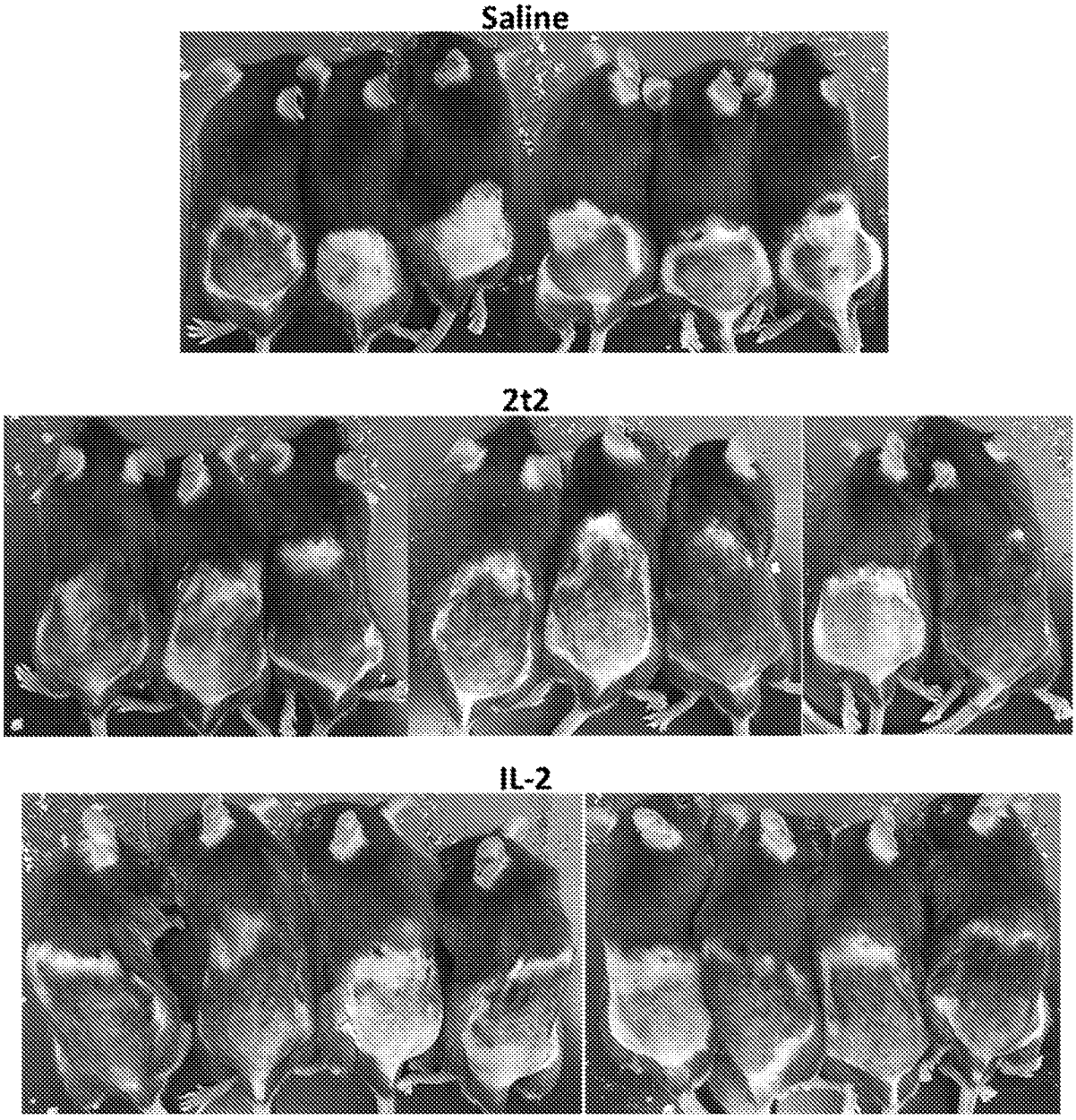


Figure 182

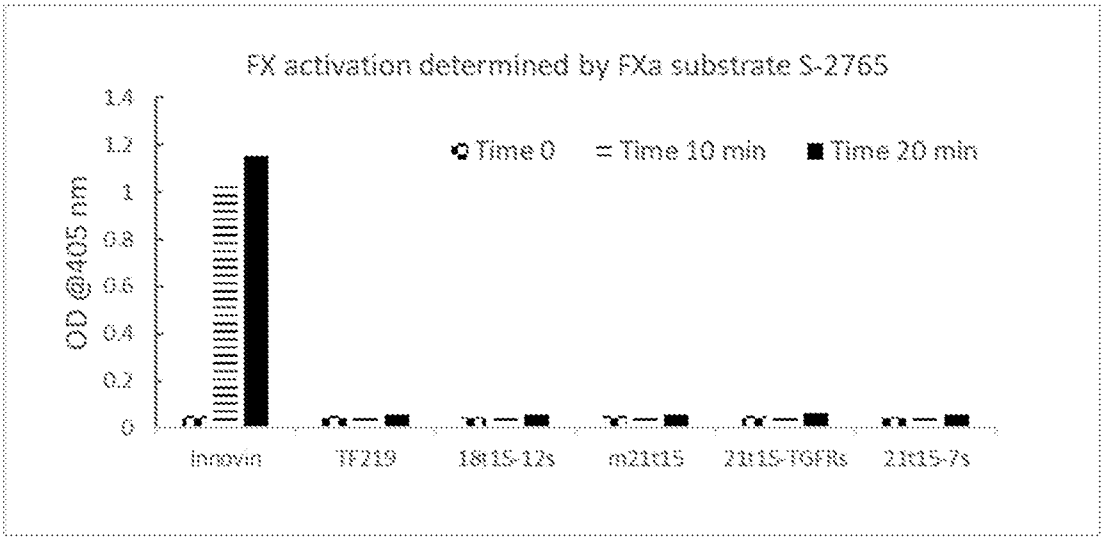


Figure 183

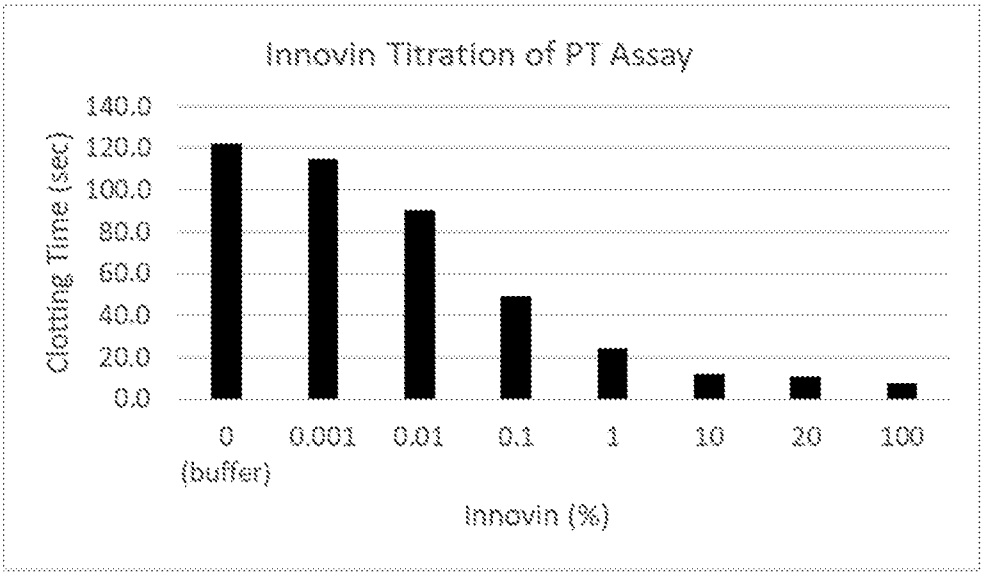


Figure 184

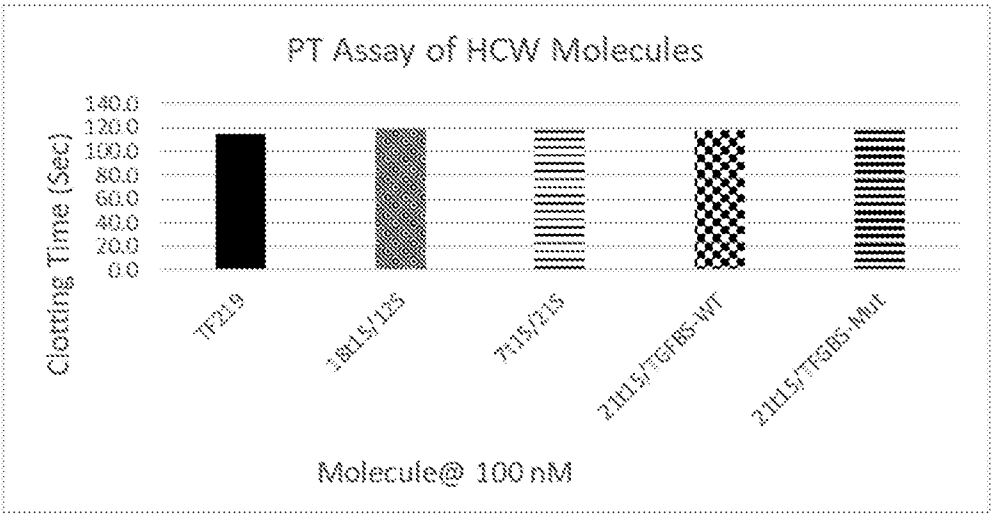


Figure 185

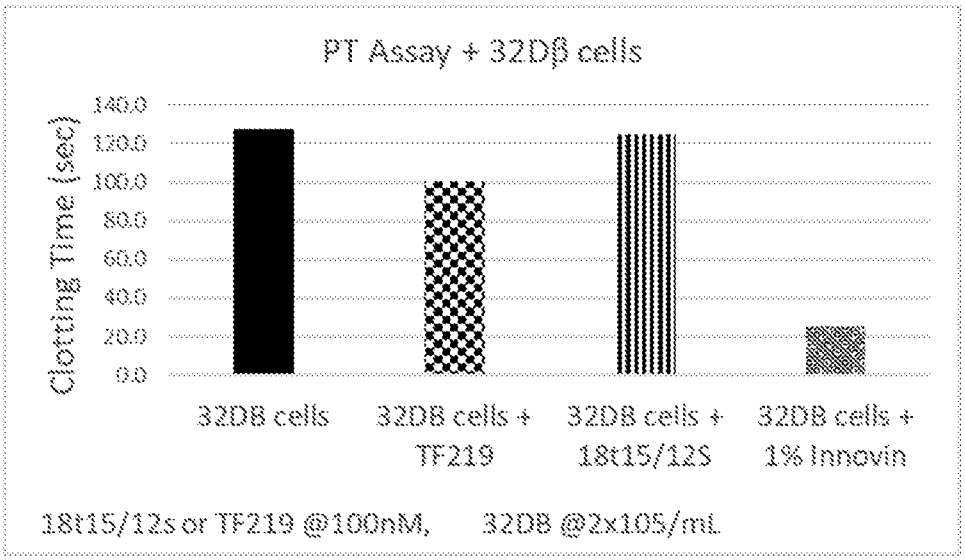


Figure 186

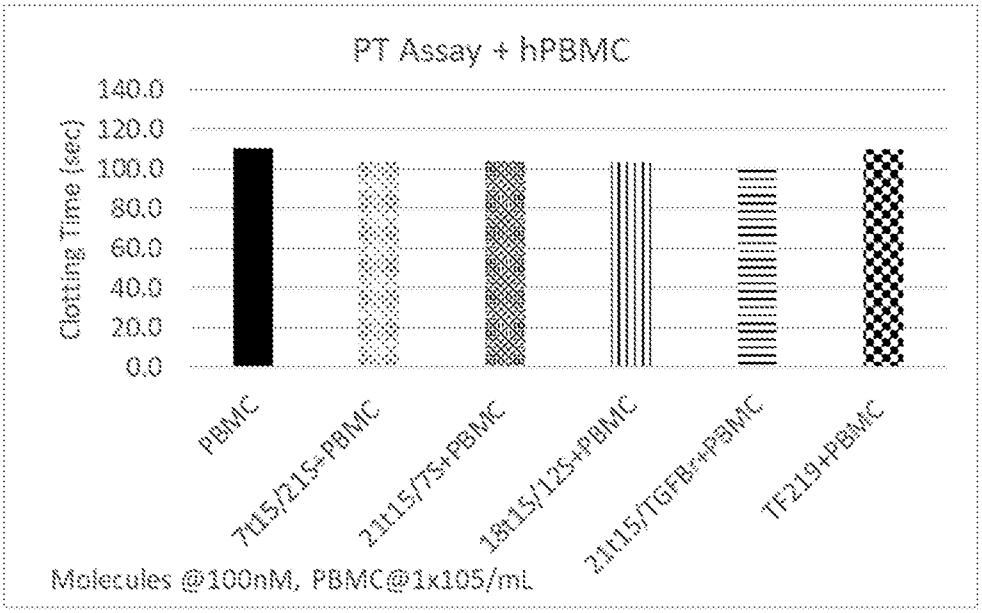


Figure 187

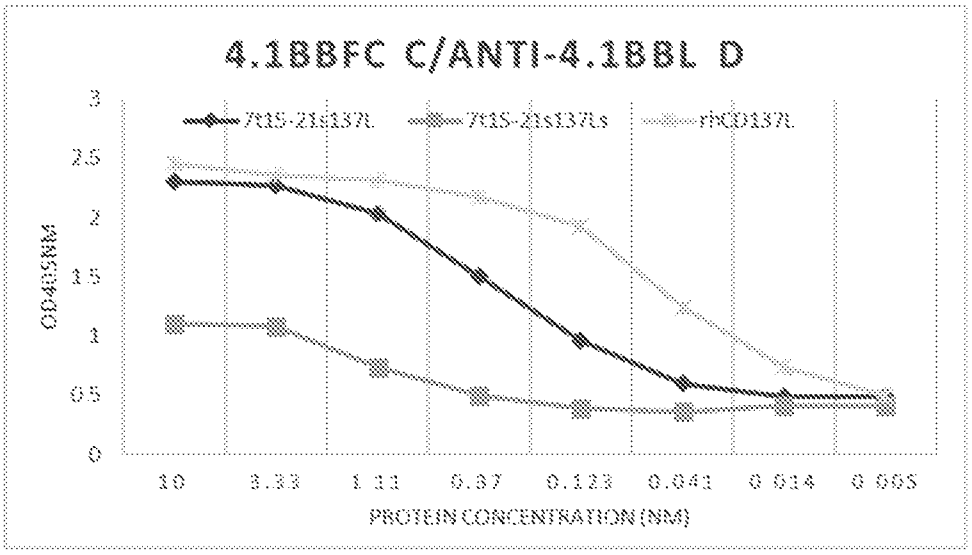


Figure 188

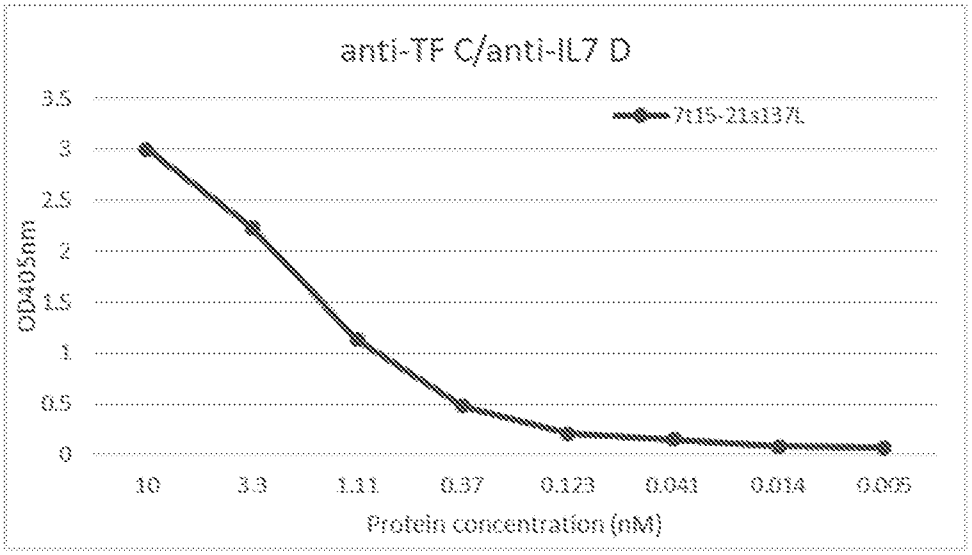


Figure 189A

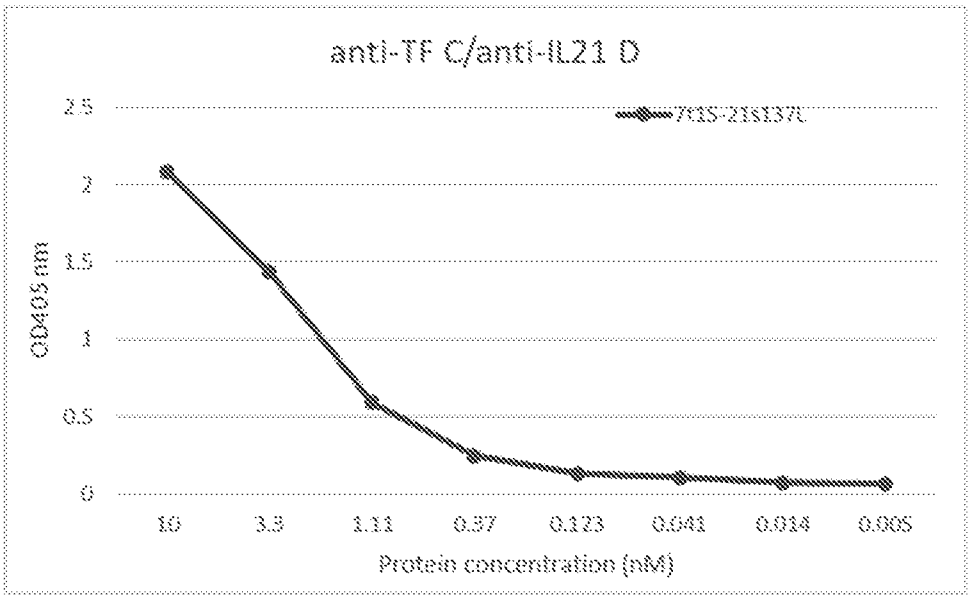


Figure 189B

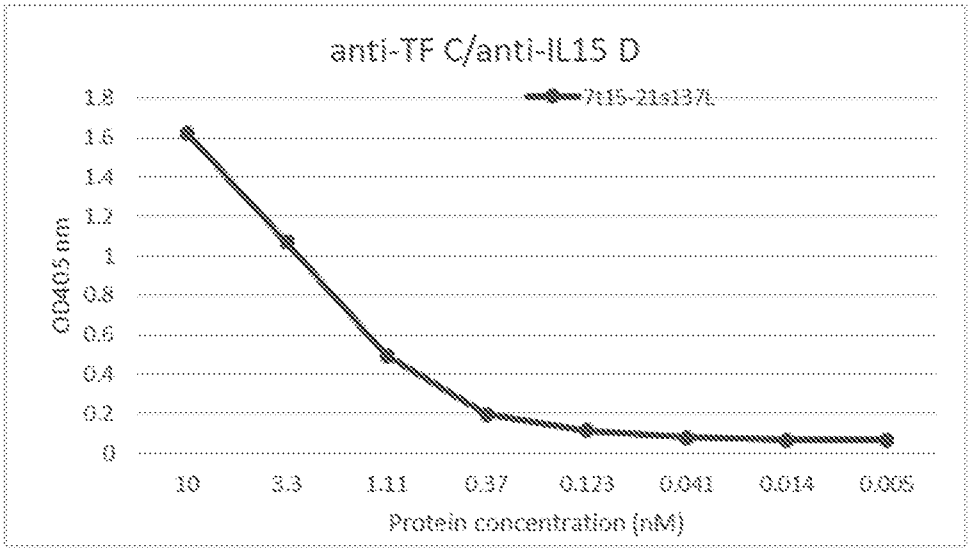


Figure 189C

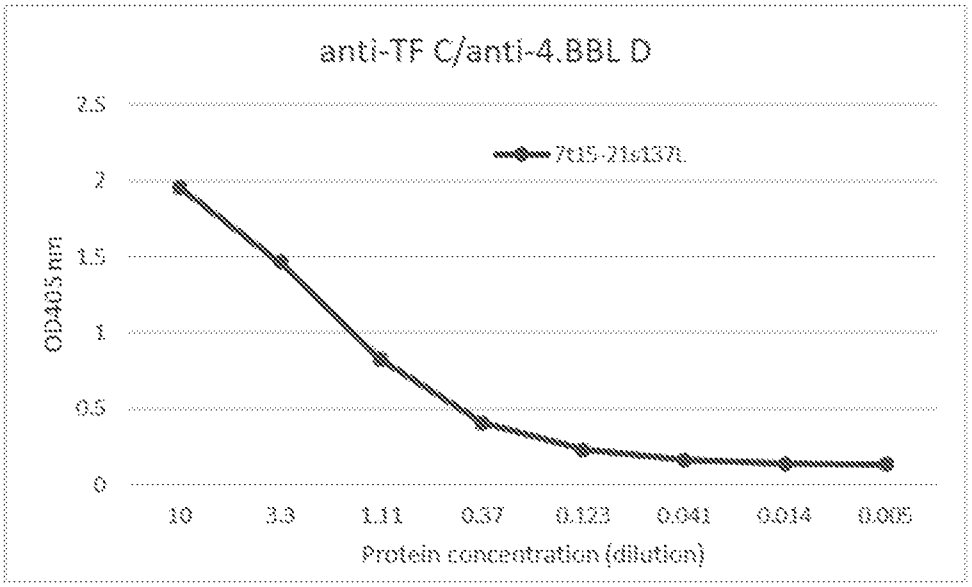


Figure 189D

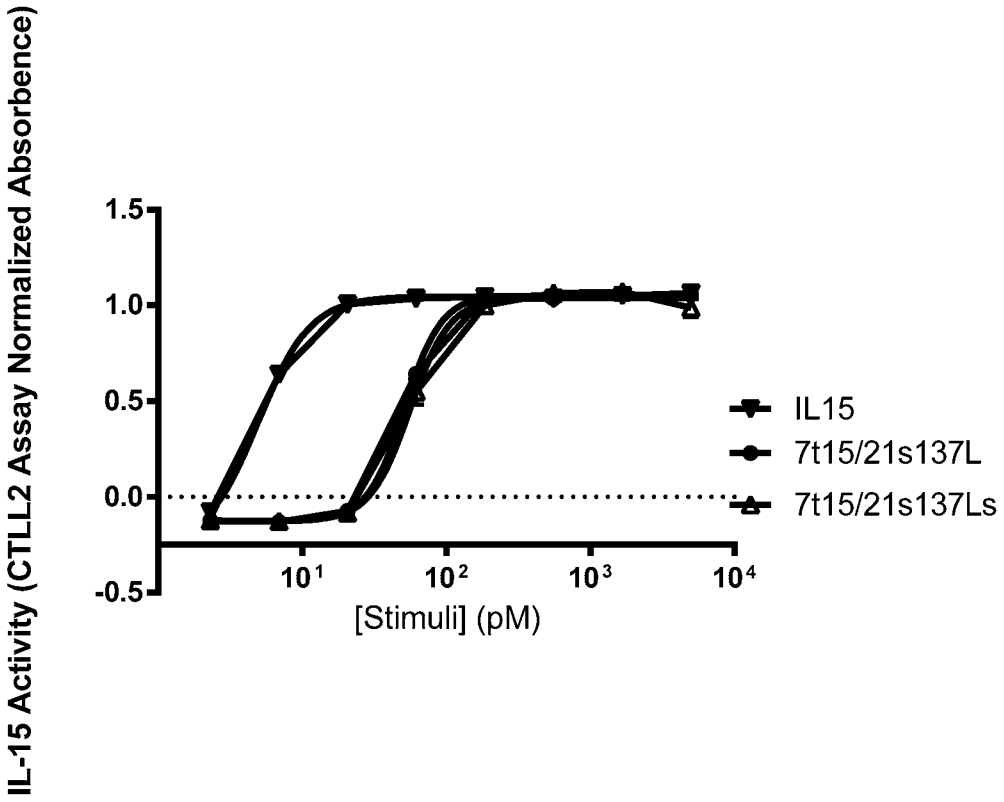

IL-15 Activity (CTLL2 Assay Normalized Absorbence)
1.5
1.0
0.5
0.0
-0.5
10¹
10²
10³
10⁴
[Stimuli] (pM)
IL15
7t15/21s137L
7t15/21s137Ls

Figure 190

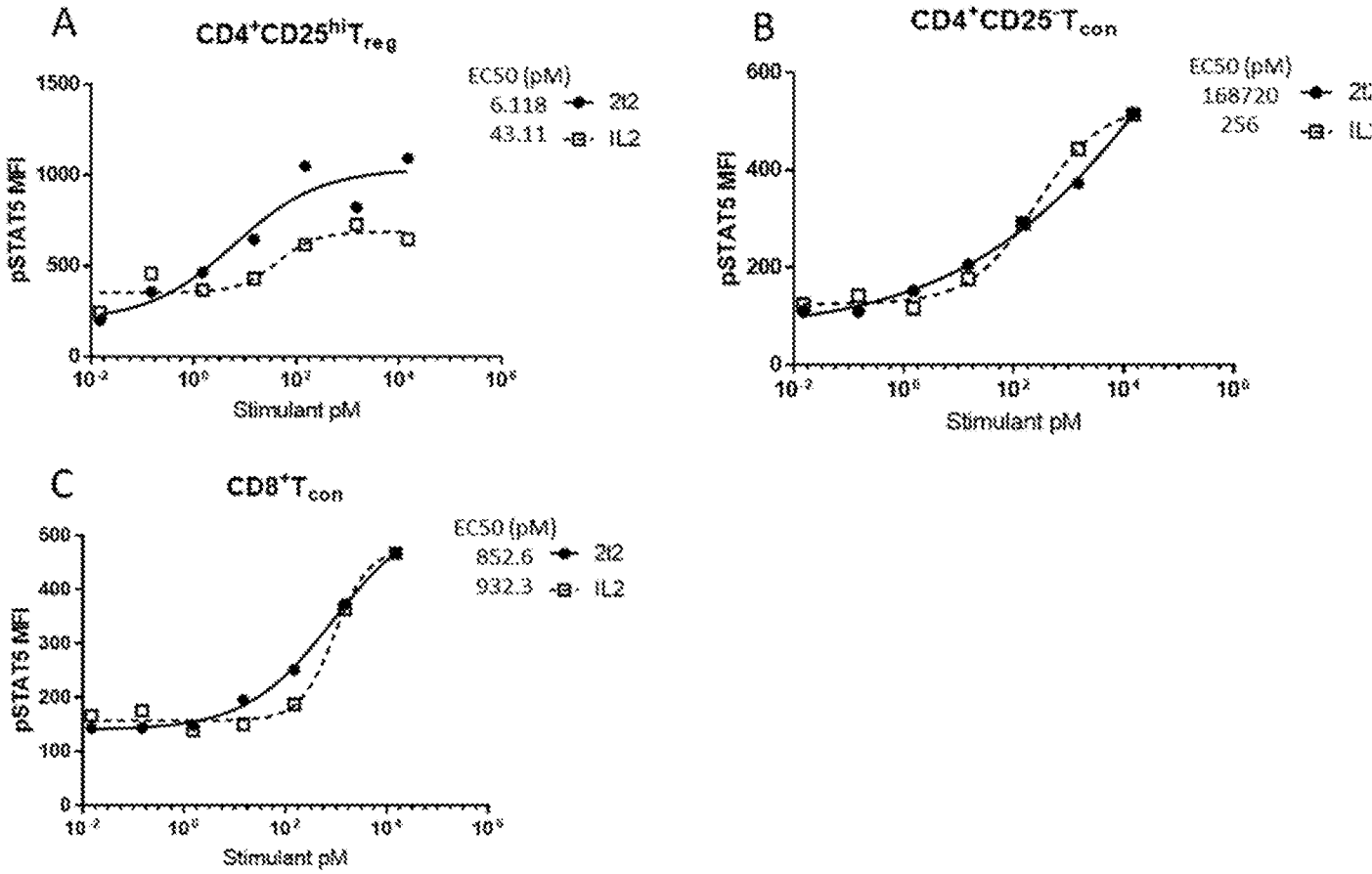

A
CD4+CD25hiTreg
EC50 (pM)
6.118 2t2
43.11 IL2
pSTAT5 MFI
Stimulant pM
B
CD4+CD25-Tcon
EC50 (pM)
168720
256
pSTAT5 MFI
Stimulant pM
C
CD8+Tcon
EC50 (pM)
852.6 2t2
932.3 IL2
pSTAT5 MFI
Stimulant pM

Figure 191

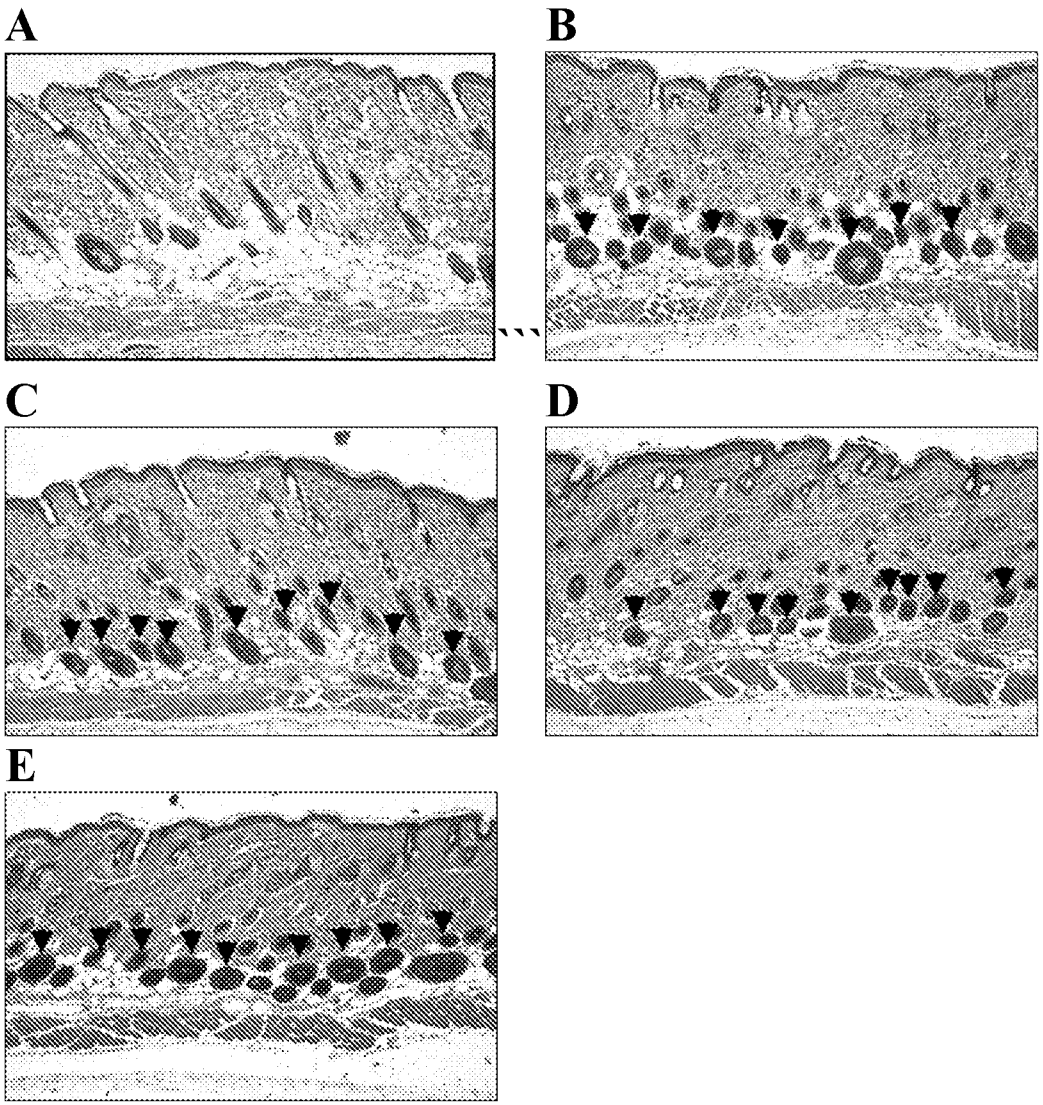


Figure 192

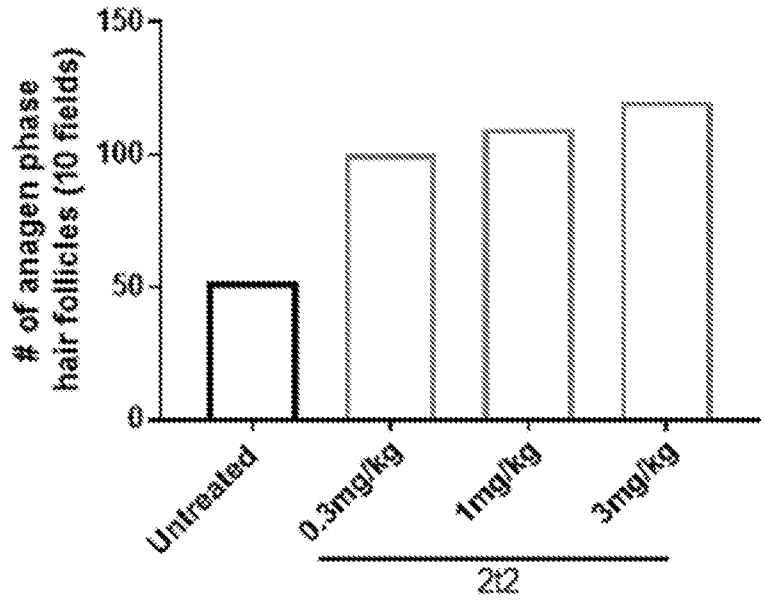


Figure 193

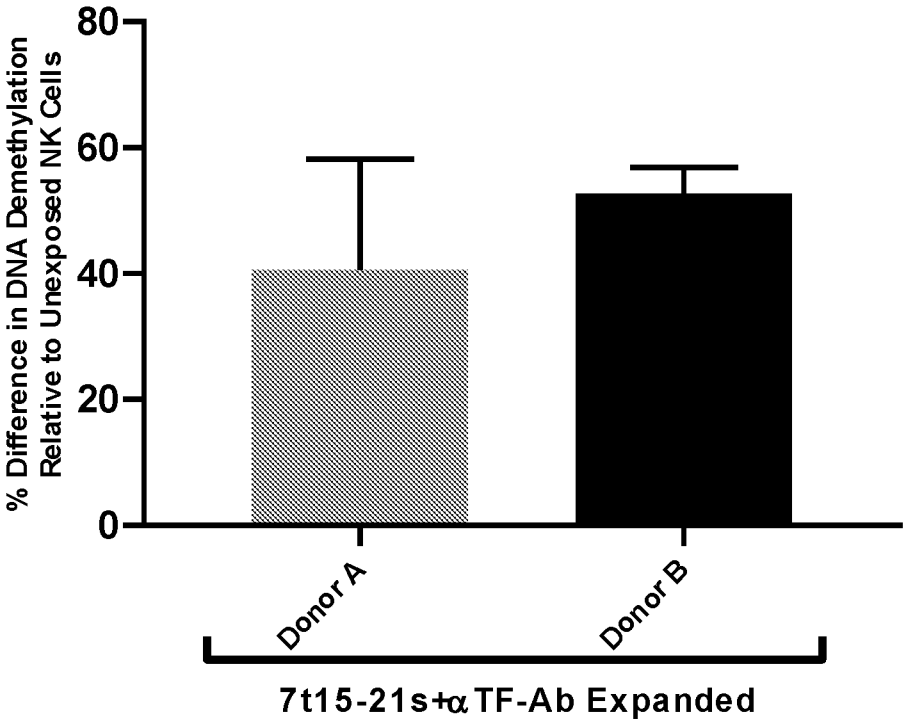


Figure 194

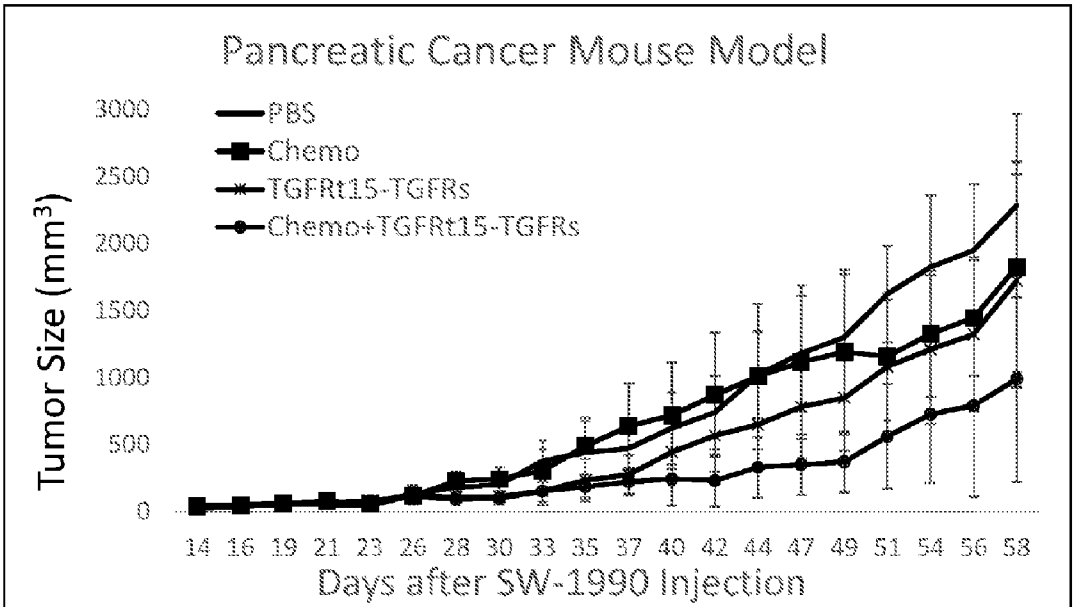

Pancreatic Cancer Mouse Model
PBS
Chemo
TGFRt15-TGFRs
Chemo+TGFRt15-TGFRs
Tumor Size (mm3)
3000
2500
2000
1500
1000
500
0
14 16 19 21 23 26 28 30 33 35 37 40 42 44 47 49 51 54 56 58
Days after SW-1990 Injection

Figure 107