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(54) **Title:** MULTI-FUNCTIONAL AND MULTI-VALENT INTERLEUKIN-TGF-BETA RECEPTOR FUSION POLYPEPTIDES

(57) **Abstract:** Provided are multi-functional and multi-valent fusion polypeptides comprising an interleukin polypeptide and two or more TGFβ ligand-binding polypeptides. The compositions and methods provided herein are useful in the application of preventing tumorigenesis and treating cancer.

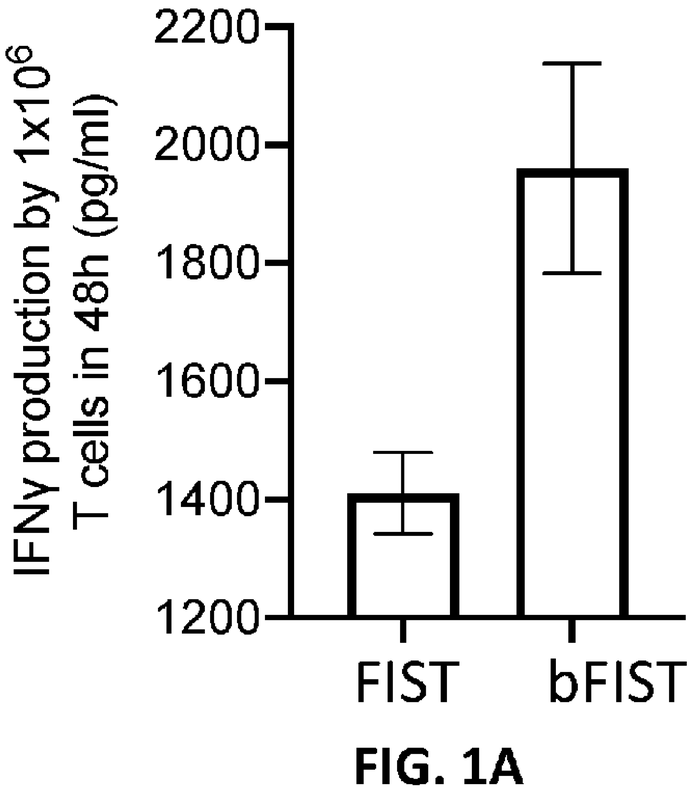



FIG. 1A

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# MULTI-FUNCTIONAL AND MULTI-VALENT INTERLEUKIN-TGF-BETA RECEPTOR FUSION POLYPEPTIDES

## CROSS-REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 63/094,277 filed on October 20, 2020, which application is incorporated herein by reference.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 15, 2021, is named 57949-701_601_SL.txt and is 82,739 bytes in size.

## BACKGROUND

**[0003]** Tumorigenesis that drives and leads to the development of cancer can be generally hallmarked by aberrant cellular processes that lead to, for example, sustained proliferative signaling, a reduced response to growth suppressors, increased resistance to cell death, replicative immortality, and increased angiogenesis. These processes are thought to promote tumor invasion and metastasis. Accordingly, the ability to target molecules associated with these processes may be important for the development of cancer therapeutics.

**[0004]** Transforming growth factor-β (TGF-β) family or proteins are pleiotropic cytokines that promotes angiogenesis and/or immunosuppression in tumorigenic environments. TGF-β proteins were originally named for their ability to transform normal fibroblasts to cells capable of anchorage-independent growth. Produced primarily by hematopoietic and tumor cells, TGF-β proteins can regulate (e.g., stimulate or inhibit) the growth and differentiation of cells from a diversity of normal or neoplastic tissue origins. Notably, TGF-β proteins are known to be involved in many proliferative and non-proliferative cellular processes such as, e.g., cell proliferation and differentiation, embryonic development, extracellular matrix formation, bone development, wound healing, hematopoiesis, and immune and inflammatory responses. Increased levels of TGF-β expression and activity are involved in a large number of pathologic conditions, including, but not limited to, tumorigenesis, wherein members of the TGF-β protein family are known to have a number of biological activities related to tumorigenic processes (e.g., angiogenesis and immunosuppression) and metastasis.

**[0005]** Interleukin-2 (IL-2) is a potent cytokine that acts on the immune system to generate primarily a cell-mediated immune response. Generally, IL-2 is locally produced by immune cells at high concentrations near the site of an antigen to supply the necessary co-stimulatory signals for generating an immune system response to the antigen. IL-2 is therefore an immune-activating molecule and may hold the potential to promote immunotherapeutic responses in treating tumors. For example, in addition to stimulating T cells, IL-2 has also been shown to stimulate lymphocytes (T cells, B cells, NK cells, NKT cells). However, High dose of IL-2 based therapies have previously been associated with toxicity observed with IL-2 systemic administration. Such deleterious effects present hurdles for the successful utilization of IL-2 as an effective therapeutic.

## SUMMARY

**[0006]** Provided herein are fusion proteins that function to activate an immune response and inhibit pro-tumorigenic immunosuppressive signaling. As described herein, immune activation and the inhibition of immune suppression is generally achieved by fusion proteins that comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGF-β activity (e.g., two or more polypeptides that bind to a TGF-β protein). The compositions provided herein are also useful for or in the inhibition of tumorigenesis (e.g., angiogenesis or metastasis). Accordingly, also disclosed herein are methods of inhibiting and/or treating cancer using the fusion proteins disclosed herein.

**[0007]** Provided herein are fusion polypeptides, comprising: an interleukin (IL) polypeptide; a first TGFβ superfamily receptor polypeptide; and a second TGFβ superfamily receptor polypeptide. Also provided are fusion polypeptides comprising: an IL polypeptide that binds to and agonizes an IL receptor; a first TGFβ superfamily receptor polypeptide that binds to and sequesters soluble TGFβ; and a second TGFβ superfamily receptor polypeptide that binds to and sequesters soluble TGFβ

**[0008]** In some embodiments, the IL polypeptide comprises an IL-2 polypeptide or IL-15. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide. In some embodiments, the IL-2 polypeptide comprises SEQ ID NO: 2. In some embodiments, the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL-2 polypeptide consists of SEQ ID NO: 2. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide or IL-15. In some embodiments, the IL polypeptide comprises an IL-2 polypeptide. In some embodiments, the

IL-2 polypeptide comprises SEQ ID NO: 3. In some embodiments, the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In some embodiments, the IL-2 polypeptide consists of SEQ ID NO: 3. In some embodiments, the IL polypeptide comprises an IL-15 polypeptide. In some embodiments, the IL-15 polypeptide comprises SEQ ID NO: 5. In some embodiments, the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5. In some embodiments, the IL-15 polypeptide consists of SEQ ID NO: 5.

**[0009]** In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises an activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, or a TGFβ receptor II polypeptide or fragment thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a TGFβ receptor II (TBRII) polypeptide or fragment thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide or fragment thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGFβ superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In some embodiments, the soluble TGFβ receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9 In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGF-β1 polypeptide, a TGF-β2 polypeptide, a TGF-β3 polypeptide, a activin βA polypeptide, a activin βB polypeptide, a activin βC polypeptide, a activin βE polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal

polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an arteman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide.

**[0010]** In some embodiments, the truncated TGFβ superfamily receptor polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof. In some embodiments, the truncated TGFβ superfamily receptor polypeptide is a truncated soluble TGFβ receptor II (sTβRII) polypeptide. In some embodiments, the truncation comprises greater than 2, 5, 7, 10, 15, 20, 25, or 30 amino acids. In some embodiments, the IL polypeptide comprises a truncated IL polypeptide. In some embodiments, the truncated IL polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof. In some embodiments, the truncated IL polypeptide comprises a truncated IL-2 polypeptide or IL-15 polypeptide. In some embodiments, the fusion polypeptides comprise a linker polypeptide or a linker molecule attaching the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide. In some embodiments, the fusion polypeptides comprise a linker polypeptide fusing the IL polypeptide and the first TGFβ superfamily receptor polypeptide. In some embodiments, the fusion polypeptides comprise a pharmacokinetic (PK) modulator.

**[0011]** In some embodiments, the pharmacokinetic modulator comprises an immunoglobulin constant (Fc) region polypeptide. In some embodiments, the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide. In some embodiments, the immunoglobulin Fc region is an IgG Fc region. In some embodiments, the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments, the PK modulator comprises an albumin polypeptide. In some embodiments, the albumin polypeptide is a human albumin polypeptide.

**[0012]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10-25. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an

amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25. In some embodiments, the fusion polypeptide consists of any one of SEQ ID NOs: 10-25. In some embodiments, the fusion polypeptide activates target cells expressing an IL-2 or IL-15 receptor. In some embodiments, the target cell is an immune cell. In some embodiments, the immune cell is a T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells. Further provided are pharmaceuticals composition comprising a fusion polypeptide, comprising a pharmaceutically acceptable carrier or excipient.

**[0013]** Also provided herein are methods of inhibiting or reducing tumor growth and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGFβ superfamily receptor polypeptide; and a second TGFβ superfamily receptor polypeptide, thereby inhibiting or reducing tumor growth and/or progression in the individual. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2.

**[0014]** In some embodiments, the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cells within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer a cancer comprising a solid tumor. Provided are methods of neutralizing or killing tumor cells in an individual having cancer,

comprising administering to the individual a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGFβ superfamily receptor polypeptide; and a second TGFβ superfamily receptor polypeptide; thereby neutralizing or killing tumor cells in the individual. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer comprises a solid tumor.

**[0015]** Additionally provided are methods of treating or ameliorating cancer in a subject having a cancer, comprising administering to the subject a fusion polypeptide comprising a an interleukin (IL) polypeptide; a first TGFβ superfamily receptor polypeptide; and a second TGFβ superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the subject. In some embodiments, the tumor is a primary tumor. In some embodiments, the tumor is a metastatic tumor. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the fusion polypeptide activates immune cells within a tumor microenvironment. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer comprises a solid tumor. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof.

**[0016]** Further provided are methods of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising: an interleukin (IL) polypeptide; a first TGFβ superfamily receptor polypeptide; and a second TGFβ superfamily receptor polypeptide. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell comprises T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells. In some embodiments, the immunes cells are contacted in vivo. In some embodiments, the immunes cells are contacted ex

vivo. In some embodiments, a leukopack or leukapheresis product comprise the immunes cells. In some embodiments, the immunes cells are derived from an apheresis sample. In some embodiments, the immune cells are subsequently used for a cell-based therapy. In some embodiments, IL polypeptide is IL-2 or IL-15. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide or fragment. A host cell, comprising a nucleic acid molecule comprising a nucleic acid sequence encoding the recombinant fusion polypeptide as described herein.

## INCORPORATION BY REFERENCE

**[0017]** All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0018]** The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

**[0019]** **FIGs. 1A and 1B** show data depicting the quantification of IFN-gamma production by human T cells previously stimulated by human monovalent FIST (a fusion protein comprising an interleukin-2 (IL-2) polypeptide, a single sTBRII receptor polypeptide) or bivalent FIST (also referred to as bFIST - a fusion polypeptide comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide), and CXCL10 production by human NK cells stimulated with human FIST (monovalent FIST) or, human bivalent FIST.

**[0020]** **FIG. 2** shows data depicting the quantification of active TGFβ1 detected in solution after 30 minutes incubation period with 5-20 nanomoles of human monovalent FIST (monovalent FIST), bivalent FIST (bFIST), or sTBRII.

**[0021]** **FIG. 3** shows a schematic representation of exemplary bivalent FIST (bFIST) fusion polypeptides.

**[0022]** **FIGs. 4A, 4B, and 4C** show comparison between bivalent FIST protein and monovalent FIST in the blockage of TGFβ isoforms.

**[0023]** **FIGs. 5A, 5B, 5C, 5D, 5E, and 5F** show inhibition of TGFb1-mediated suppression of cytotoxic T cell proliferation.

**[0024]** **FIGs. 6A, 6B, 6C, 6D, 6E, and 6F** show proliferation of effector memory T cells and terminal differentiated effector memory cells, and the quantification of IFNγ production by stimulated T cells with the bivalent FIST, monovalent FIST (monovalent FIST), and controls.

**[0025]** **FIGs. 7A, 7B, 7C, 7D, and 7E** show bivalent FIST inhibition and/or reduction of TGFb1-mediated suppression of primary NK cell proliferation and IFNγ and CXC10 production.

**[0026]** **FIGs. 8A and 8B** show that treatment of NK cells with bivalent FIST increases NK cell cytotoxicity and activation and cytotoxicity against cancer cells.

**[0027]** **FIGS. 9A, 9B, 9C, and 9D** show that treatment of B cells with bivalent FIST increases maturation, proliferation and IFNγ production.

**[0028]** **FIGs. 10A, 10B, and 10C** show that treatment of lung cancer cells with bivalent FIST inhibits the upregulation of TGFb1-induced Epithelial to Mesenchymal transition (EMT) markers on lung cancer cells.

## DETAILED DESCRIPTION

**[0029]** Provided herein are multi-functional fusion proteins that are capable of, e.g., activating an immune response, inhibiting and/or reducing immunosuppressive signaling, and/or suppressing or inhibiting angiogenic or invasive signaling. In the context of cancer, immune activation is thought to be the physiological function that promotes the recognition and neutralization of transformed cells before they grow into tumors, or to kill (e.g., neutralize) tumors after they are formed. The specific recognition of antigens based on foreignness, molecular weight, complexity, level of expression, and degradability is thought to give rise to the capacities of the immune system to limit tumor expansion. In opposition, tumor cells are thought to possess the capacity to limit responsiveness through various mechanisms, for example, via production of immunosuppressive factors or molecules (e.g., immune suppressive cytokines).

**[0030]** In order to activate an immune response and inhibit immunosuppressive signaling, the fusion proteins disclosed herein generally comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity (e.g., two or more polypeptides that bind a TGFβ protein). The balance between activating and inhibitory signals is an important factor in

maintaining immune homeostasis. During tumorigenesis, immunostimulatory (e.g., immune activating) signals (e.g., proinflammatory cytokines) are antagonized by tumor-derived immunosuppressive signaling molecules (e.g., immunosuppressive cytokines). Among the group of immunosuppressive cytokines thought to be important in driving tumorigenesis are members of the TGF superfamily of proteins (e.g., TGFβ).

**[0031]** The Transforming Growth Factor Beta (TGFβ) superfamily includes the TGFβ proteins, Bone Morphogenetic Proteins (BMPs), Growth Differentiation Factors (GDFs), Glial-derived Neurotrophic Factors (GDNFs), Activins, Inhibins, Nodal, Lefty, and Müllerian Inhibiting Substance (MIS). Generally, ligands of the TGFβ superfamily form dimers that bind to heterodimeric receptor complexes consisting of type I and type II receptor subunits with serine/threonine kinase domains.

**[0032]** Transforming Growth Factor β (TGFβ) superfamily of proteins are cytokines involved in essential cellular functions such as proliferation, differentiation, apoptosis, tissue remodeling, angiogenesis, immune response, and cell adhesion (see, for example, Massagué, J. TGFβ signaling in context. Nat Rev Mol Cell Biol 13, 616–630 (2012)). TGFβ superfamily proteins are also important factors in the pathophysiology of disease states such as chronic inflammatory conditions and cancer. The members of this family include the three isoforms of TGFβs, β1, β2, β3; bone morphogenetic proteins (BMPs) and activins. In addition to immunosuppression, TGFβ proteins can act as a prometastatic and proangiogenic factor in late-stage cancer by constitutively inducing epithelial to mesenchymal transition (EMT) and tumor associated angiogenesis.

**[0033]** Blocking tumor-derived active TGFβ for therapeutic proposes has been extensively explored. Many therapeutic approaches target TGFβ pathway for the treatment of invasive cancers such as breast cancer and melanoma. For example, antagonists of TGFβ binding to its heteromeric receptor have shown reduction of tumor cell motility, intravasation, and metastases in three experimental models of breast cancer. However, these treatment strategies in general do not affect cellular proliferation, indicating that TGFβ blocking agents are not sufficient to promote the neutralization or killing of tumor cells.

**[0034]** The utilization of an interleukin polypeptide (e.g., interleukin-2 or interleukin-15) in the fusion proteins disclosed herein is useful for activating an immune response in addition to the inhibition of TGFβ. The interleukin family of proteins generally promote the activation, development, and differentiation of immune cells (e.g., T cells, NK cells, and other lymphocytes), and play essential roles in both innate and adaptive immunity. Accordingly, immune cell

activation, development, and differentiation can be achieved by the fusion proteins disclosed herein.

**[0035]** Proinflammatory or immune-stimulating interleukins (e.g., interleukin-2 or interleukin-15) constitute useful adjuvants for activating an immune response. For example, interleukin-2 (IL-2) is a factor for lymphocyte activation and clonal expansion, promoting the activation, development, and differentiation of cytotoxic T cells. IL-2 and IL-15 also stimulate NK cell proliferation and cytotoxicity. However, IL-2 can operate as both an immunostimulatory (e.g., immune-activating) and immunosuppressive agent. As immunosuppressor, IL-2 maintains peripheral tolerance by inducing the generation of regulatory cells. For these reasons, IL-2 is considered a double-edge sword. The versatility of interleukin function is influenced by the environment and the interaction with signaling agents.

**[0036]** Accordingly, the fusion proteins disclosed herein are multi-functional in that the fusion proteins are useful for immune activation, the inhibition of immunosuppression, and/or the suppression or inhibition of angiogenic or invasive signaling. In some embodiments, immune activation, the inhibition of immunosuppression, and/or the suppression or inhibition of angiogenic or invasive signaling can be used to treat a tumor. In some embodiments, activating an immune (e.g., activating cells expressing an IL-2 receptor), inhibiting of immunosuppression of immune cells (e.g., blocking or inhibiting an interaction between a cell and TGFβa), and/or the suppression or inhibition of angiogenic or invasive signaling in a subject can be used to treat a tumor or cancer. As disclosed, the activation of an immune response and inhibition of an immunosuppressive response is, at least in part, achieved by the interleukin polypeptide. In turn, the inhibition or reduction of an immunosuppressive signaling or signaling molecules and/or inhibition of angiogenic or invasive signaling or signaling molecules is achieved, at least in part, through the use of one or more polypeptides that inhibit activity of TGFβ proteins.

**[0037]** As used herein, the term "fusion protein" generally refers to a protein that includes polypeptide components derived from more than one parental protein or polypeptide. Generally, a fusion protein is expressed from a fusion gene in which a nucleotide sequence encoding a polypeptide sequence from one protein is appended in frame with, and optionally separated by a linker from, a nucleotide sequence encoding a polypeptide sequence from a different protein. The fusion gene can then be expressed by a recombinant host cell as a single protein.

**[0038]** A "domain" of a protein, as used herein, generally is any portion of the entire protein, up to and including the complete protein, but typically comprising less than the complete protein. A domain can, but need not, fold independently of the rest of the protein chain and/or be correlated

with a particular biological, biochemical, or structural function or location (e.g., a ligand binding domain, or a cytosolic, transmembrane or extracellular domain).

**[0039]** The term "recombinant" indicates that the material (e.g., a nucleic acid or a polypeptide) has been artificially or synthetically (e.g., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a "recombinant nucleic acid" is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling, or other well-known molecular biological procedures. A "recombinant DNA molecule," is comprised of segments of DNA joined together by means of such molecular biological techniques. The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule. A "recombinant host cell" is a cell that contains and/or expresses a recombinant nucleic acid.

**[0040]** A "polynucleotide sequence" or "nucleotide sequence" or "nucleic acid sequence," as used interchangeably herein, is a polymer of nucleotides, including an oligonucleotide, a DNA, and RNA, a nucleic acid, or a character string representing a nucleotide polymer, depending on context. From any specified polynucleotide sequence, either the given nucleic acid or the complementary polynucleotide sequence can be determined. Included are DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

**[0041]** Polynucleotide and polypeptide sequences of the current disclosure can be defined in terms of particular identity and/or similarity with certain polynucleotides and polypeptides described herein. In some embodiments, sequence identity will typically be greater than 60%, greater than 75%, greater than 80%, greater than 90%, and/or greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence disclosed herein. Optimal alignment can be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAST, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, BLAST searches are be performed with the BLAST program, score=100, wordlength=12, to obtain sequences with the desired percent sequence identity. In some

embodiments, to obtain gapped alignments for comparison purposes, Gapped BLAST. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. In some embodiments, Clustal Omega is used.

**[0042]** As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," "nucleic acid sequence encoding" or "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of ribonucleotides along the mRNA chain, and also determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the RNA sequence and for the amino acid sequence.

**[0043]** "Expression of a gene" or "expression of a nucleic acid" means transcription of DNA into RNA (optionally including modification of the RNA, e.g., splicing), translation of RNA into a polypeptide (possibly including subsequent post-translational modification of the polypeptide), or both transcription and translation, as indicated by the context.

**[0044]** The term "gene" is used broadly to refer to any nucleic acid associated with a biological function. Genes typically include coding sequences and/or the regulatory sequences required for expression of such coding sequences. The term "gene" applies to a specific genomic or recombinant sequence, as well as to a cDNA or mRNA encoded by that sequence. A "fusion gene" contains a coding region that encodes a transgene. Genes also include non-expressed nucleic acid segments that, for example, form recognition sequences for other proteins. Non-expressed regulatory sequences including transcriptional control elements to which regulatory proteins, such as transcription factors, bind, resulting in transcription of adjacent or nearby sequences.

**[0045]** As used herein the term "coding region" or "coding sequence" when used in reference to a structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of an mRNA molecule. The coding region is bounded, in eukaryotes, on the 5′ side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3′ side by one of the three triplets which specify stop codons (e.g., TAA, TAG, TGA). Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect, and mammalian cells and viruses (analogous control elements, e.g.,, promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein

of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types.

**[0046]** The term "expression vector" refers to a recombinant DNA molecule containing a desired coding sequence and appropriate nucleic acid sequences necessary for the expression of the operably linked coding sequence in a particular host cell. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, optionally an operator sequence, a ribosome binding site and possibly other sequences. Eukaryotic cells are known to utilize promoters, enhancers, and termination and polyadenylation signals. A secretory signal peptide sequence can also, optionally, be encoded by the expression vector, operably linked to the coding sequence for the inventive toxin peptide analog, so that the expressed toxin peptide analog can be secreted by the recombinant host cell, for more facile isolation of the toxin peptide analog from the cell, if desired. Such techniques are well known in the art.

**[0047]** As used herein, individual is synonymous with patient and/or subject and includes and/or refers to a human and may be a human that has been diagnosed as needing to treat a disease or condition as disclosed herein. However, examples are not limited to humans and include, chimpanzees, marmosets, cows, horses, sheep, goats, pigs, rabbits, dogs, cats, rats, mice, guinea pigs, and the like. The individual is typically a human and may be a human that has been diagnosed as needing to treat a disease or condition as disclosed herein.

**[0048]** As used herein, the term "inhibition" or "inhibiting" includes and/or refers to the reduction or suppression of a given condition, symptom, disorder, or disease, and/or a decrease in the baseline activity of a biological activity or process.

**[0049]** As used herein, the term "treating" or "treatment" of includes and/or refers to ameliorating the disease or disorder or symptoms thereof (e.g., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In certain embodiments, "treating" or "treatment" also includes and/or refers to alleviating or ameliorating at least one physical and/or biological parameters including those which may not be discernible by the patient. In certain embodiments, "treating" or "treatment" includes and/or refers to modulating a disease, disorder, or biological process either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical and/or biological parameter), or both. In certain embodiments, "treating" or "treatment" includes and/or refers to preventing or delaying the onset or development or progression of the disease or disorder. In certain embodiments, "treating" or "treatment" includes and/or refers to preventing or delaying or inhibiting the

deterioration of (i) a healthy physiological state or (ii) a baseline physiological state (e.g., the progression of a disease or disorder).

**[0050]** As used herein, "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification includes and/or refers to "one" and also consistent with the meaning of "one or more", "at least one", and "one or more than one". Similarly, the word "another" may mean at least a second or more.

**[0051]** As used herein, the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "include" and "includes") or "containing" (and any form of containing, such as "contain" and "contains"), are inclusive or open-ended and do not exclude additional, unrecited elements or process steps.

**[0052]** As used herein, the term "about" in the context of a given value or range includes and/or refers to a value or range that is within 20%, within 10%, and/or within 5% of the given value or range.

**[0053]** As used herein, the term "and/or" is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each were set out individually herein.

**[0054]** As used herein, a "sample" includes and/or refers to any fluid or liquid sample which is being analyzed in order to detect and/or quantify an analyte. In some embodiments, a sample is a biological sample. Examples of samples include without limitation a bodily fluid, an extract, a solution containing proteins and/or DNA, a cell extract, a cell lysate, or a tissue lysate. Non-limiting examples of bodily fluids include urine, saliva, blood, serum, plasma, cerebrospinal fluid, tears, semen, sweat, pleural effusion, liquified fecal matter, and lacrimal gland secretion.

**[0055]** As used herein, in any instance or embodiment described herein, "comprising" may be replaced with "consisting essentially of" and/or "consisting of". used herein, in any instance or embodiment described herein, "comprises" may be replaced with "consists essentially of" and/or "consists of".

***Interleukins***

**[0056]** The fusion proteins provided herein generally comprise an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity (e.g., two or more polypeptides that bind a TGFβ protein). Interleukins (IL) are a class of cytokines first thought to

be expressed by leukocytes alone but have later been found to be produced by many other body cells. Interleukins play important roles in, e.g., the activation and differentiation of immune cells, as well as cell proliferation, maturation, migration, and adhesion. The function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses. Accordingly, the interleukin polypeptide of the fusion protein is useful for the regulation (e.g., activation) of an immune response.

**[0057]** Interleukin-2 (IL-2) is an interleukin useful for activating the immune response in the context of a fusion protein comprising an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity. Interleukin-2 (IL-2) is a pleiotropic cytokine that is induced by antigen stimulation and thus plays a significant role in regulating the immune response (see, for example, Spolski, R., Li, P. & Leonard, W.J. Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat Rev Immunol 18, 648–659 (2018)). For example, can promote an innate antitumor response due to its effectiveness at inducing locoregional tumor rejection, acting as autocrine factor for T cells and supporting the development of cytotoxic T cells, stimulating NK cells proliferation and cytolytic activity

**[0058]** IL-2 is a small, 15.5-kDa four α-helical bundle cytokine. It is produced predominately by antigen-simulated CD4+ T cells, while it can also be produced by CD8+ cells, natural killer (NK) cells, and activated dendritic cells (DC). IL-2 is an important factor for the maintenance of CD4+ regulatory T cells and plays a critical role in the differentiation of CD4+ T cells into a variety of T cell subsets. It can promote CD8+ T-cell and NK cell cytotoxicity activity, and modulate T-cell differentiation programs in response to antigen, promoting naive CD4+ T-cell differentiation into T helper-1 (Th1) and T helper-2 (Th2) cells while inhibiting T helper-17 (Th17) differentiation. Notably, IL-2 was one of the first FDA-approved immunotherapy drugs for metastatic melanoma and renal cell cancer. However, IL-2 immunotherapy has not been widely applied due to its short half-life in vivo and severe toxicity at the therapeutic dosage.

**[0059]** The term "interleukin-2" or "IL-2" refers to any native IL-2 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless otherwise indicated. IL-2 includes unprocessed IL-2 as well as any form of IL-2 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-2, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-2 is shown in SEQ ID NO: 1. Unprocessed human IL-2 additionally comprises an N-terminal 20 amino acid signal peptide, which is absent in the mature IL-2 molecule. IL-2 also includes "wild-type IL-2" or a naturally occurring IL-2. The sequence of a native human IL-2 molecule is shown in SEQ ID NO:

1. For the purpose of the present disclosure, the term wild-type also encompasses forms of IL-2 comprising one or more amino acid mutations. For example, the fusion proteins described herein can comprise an IL-2 polypeptide having greater than 80 %, 85 %, 90 % 95 %, 96 %, 97 %, 98 %, 99 % sequence identity to the amino acid sequence of SEQ ID NO: 1.

**[0060]** In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 1 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

**[0061]** The IL-2 polypeptide can comprise a full-length native sequence or truncations thereof. In some embodiments, the IL-2 polypeptide is a truncated IL-2 polypeptide. The truncated IL-2 polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about

10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-2 polypeptide). In some embodiments, the fusion protein comprises an IL-2 polypeptide having an amino acid sequence of SEQ ID NO: 2. In some embodiments, the fusion protein comprises an IL-2 polypeptide having an amino acid sequence of SEQ ID NO: 3. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

**[0062]** In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 2 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino

acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

**[0063]** In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-2 sequence having sequence identity to SEQ ID NO: 3 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-2 sequences or variant sequences (e.g., having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

**[0064]** Interleukin-15 (IL-15) is also an interleukin useful for the activating the immune response in the context of a fusion protein comprising an interleukin polypeptide fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity. Interleukin 15 (IL-15) is a cytokine of about 12–14 kilodaltons comprising a four-α-helix structure. IL-15 belongs to the family of cytokines consisting of interleukins IL-2, IL-4, IL-7, IL-9, and IL-21. IL-15 signals through a receptor complex composed of the IL-2/IL-15 receptor β (IL-15Rβ) (CD122) subunit, which is shared with IL-2 and the common gamma chain (γC) (CD132) receptor subunit, which is also utilized by all of the additional family members. Notably, IL-15 is a growth factor for T cells and NK cells, and plays an important role in the development, proliferation, and activation of these immune cells. Although IL-15 has a potential for therapeutic use, natural IL-15 has therapeutic development issues, namely a low biological potency and a short half-life.

**[0065]** The term "interleukin-15" or "IL-15" refers to any native IL-15 from any vertebrate source, including mammals such as primates (e.g., humans) and rodents (e.g., mice and rats), unless

otherwise indicated. IL-15 includes unprocessed IL-15 as well as any form of IL-15 that results from processing in the cell. The term also encompasses naturally occurring variants of IL-15, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human IL-15 is shown in SEQ ID NO: 4. IL-15 also includes "wild-type IL-15" or a naturally occurring IL-15. The sequence of a native human IL-15 molecule is shown in SEQ ID NO: 4. For the purpose of the present disclosure, the term wild-type IL-15 also encompasses forms of IL-15 comprising one or more amino acid mutations. For example, the fusion proteins described herein can comprise an IL-15 polypeptide having greater than 80%, 85%, 90% 95%, 96%, 97%, 98%, 99% sequence identity to the amino acid sequence of SEQ ID NO: 4.

**[0066]** In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 4 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %. Suitable IL-15 sequences or variant sequences (e.g., a IL-15 polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods described herein (e.g., as described in any one of Examples 1-3).

**[0067]** The IL-15 polypeptide can comprise a full-length native sequence or truncations thereof. In some embodiments, the IL-15 polypeptide is a truncated IL-15 polypeptide. The truncated IL-15 polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino

acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about 10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length IL-15 polypeptide). In some embodiments, the fusion protein comprises an IL-15 polypeptide having an amino acid sequence of SEQ ID NO: 5.

**[0068]** In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %.

In some embodiments, the fusion protein comprises an IL-15 sequence having sequence identity to SEQ ID NO: 5 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

***TGFβ inhibiting polypeptides***

**[0069]** As described herein, the fusion proteins provided herein generally comprise an interleukin polypeptide (e.g., IL-2 or IL-15) fused (e.g., linked) to two or more polypeptides that inhibit TGFβ activity (e.g., two or more polypeptides that bind a TGFβ protein). The transforming growth factor β (TGFβ) superfamily is a large group of soluble factors (e.g., proteins) that initiate and control activation, proliferation, and differentiation of many cell types, thus playing important roles in embryonal development and homeostasis. The TGFβ superfamily includes several subfamilies: the activin/inhibin family, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), the TGFβ subfamily, and glial cell line-derived neurotrophic factor (GDNF) family. TGFβ superfamily has three types: TGFβ 1, TGFβ 2, and TGFβ 3. TGFβ superfamily proteins have been discovered in a variety of species, including invertebrates as well as vertebrates. TGFβ malfunction can lead to developmental disorders, severe defects in organ function, and is associated with several diseases, including various types of cancer.

**[0070]** The inhibition and suppression of TGFβ superfamily activity can be achieved through the use of fusion proteins comprising, in addition to an interleukin polypeptide, two or more TGFβ superfamily receptor polypeptides. In the context of the fusion proteins described herein, the two or more TGFβ superfamily receptor polypeptides yield a multi-valent single chain polypeptide capable of binding two or more TGFβ proteins. Generally, ligands of the TGFβ superfamily bind to receptor complexes consisting of type I and type II receptor subunits. As disclosed herein, polypeptides derived from TGFβ superfamily receptors are useful for inhibiting or suppressing TGFβ superfamily ligands. For example, in some embodiments, the TGFβ superfamily receptor is selected from the group consisting of: an activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, and a TGFβ receptor polypeptide or fragment thereof.

**[0071]** Within the TGFβ superfamily of proteins, the TGFβ sub-family of proteins comprises TGFβ1, TGFβ2, and TGFβ3. One of the biological effects of TGFβ proteins is the inhibition of proliferation of most normal epithelial cells using an autocrine mechanism of action, and this suggests a tumor suppressor role for TGFβ. Loss of autocrine TGFβ activity and/or responsiveness

to exogenous TGFβ appears to provide some epithelial cells with a growth advantage leading to malignant progression. This suggests a pro-oncogenic role for TGFβ in addition to its tumor suppressor role. Among TGFβs, β1, β2, β3, TGFβ1 is the most potent immunosuppressive cytokine described to date, exerts deleterious effects (e.g., immunosuppression) on several components of the immune system response against cancer cells, and is most frequently overexpressed by carcinomas. Notably, TGFβ proteins (e.g., TGFβ1) diminishes or reduces the effector functions of macrophages, B cells, cytotoxic T cells, dendritic cells, and NK cells, where TGFβ acts as negative regulator of IFNγ production via its mediators SMAD2, SMAD3, and SMAD4. In addition to immunosuppression, TGFβ proteins can act as a prometastatic and proangiogenic factor in late-stage cancer by constitutively inducing epithelial to mesenchymal transition (EMT) and tumor associated angiogenesis.

**[0072]** Most cell types express three sizes of receptors for TGFβ, designated Type I (53 kDa), Type II (70 - 85 kDa), and Type III (250 - 350 kDa). The Type I receptor is a membrane-bound serine/threonine kinase that apparently requires the presence of the Type II receptor to bind TGFβ. The Type II receptor is also a membrane-bound serine/threonine kinase that binds TGFβ1 and TGFβ3 with high affinity and TGFβ2 with much lower affinity. The Type I and Type II receptors together form a heterodimeric signaling complex that is essential for the transduction of the anti-proliferative signals of TGFβ. The Type III receptor is a transmembrane proteoglycan with a large extracellular domain and a 43 amino acid residue cytoplasmic domain. The cytoplasmic domain of the Type III receptor lacks an obvious signaling motif and the receptor may not be involved directly in signal transduction. Notably, the soluble extracellular domain of the TβRII, consisting of the extracellular domain of the receptor binds TGF-β1 and TGF-β3 with high affinity.

**[0073]** Accordingly, the TGFβ receptor TβRII polypeptides are useful in TGFβ binding polypeptides of the multi-functional fusion proteins described herein. Particularly, TGFβ receptor TβRII polypeptides are useful for inhibiting or reducing TGFβ1 activity or signaling associated therewith. The term "soluble transforming growth factor (TGF) β receptor type II B or sTβRII" as used herein refers to a soluble, or non-membrane form of the alternatively spliced transforming growth factor β type II receptor, preferably the ectodomain of the TGFβ type II receptor from any species or source and includes the full-length ectodomain as well as fragments or portions of the ectodomain. In some embodiments, the sTβRIIB is human or mouse. The human TGFβ receptor II has the amino acid sequence of SEQ ID NOs: 5 or 6 (short and long isoforms, respectively). The term "sTβRII fragment" as used herein means at least 10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %,

80 %, 90 %, 95 %, or more of the entire length of the reference polypeptide. In one embodiment, the IL-2 is truncated at the N-terminal or C-terminal end to permit cloning.

**[0074]** In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 6 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

**[0075]** In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 7 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having

sequence identity to SEQ ID NO: 7 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

**[0076]** As described herein, the two or more sTBRII receptor polypeptides utilize the ectodomain of a native sTBRII receptor. Accordingly, the sTBRII receptor polypeptide used herein comprises SEQ ID NO: 8 (e.g., the short isoform of sTBRII), SEQ ID NO: 9 (e.g., the long isoform of sTBRII), or a combination thereof.

**[0077]** In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 8 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an TBRII sequence having sequence identity to SEQ ID NO: 8 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

**[0078]** In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 % to about 100 %. In some embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 % to about 90 %, about 85 % to about 95 %, about 85 % to about 96 %, about 85 % to about 97 %, about 85 % to about 98 %, about 85 % to about 99 %, about 85 % to about 100 %, about 90 % to about 95 %, about 90 % to about 96 %, about 90 % to about 97 %, about 90 % to about 98 %, about 90 % to about 99 %, about 90 % to about 100 %, about 95 % to about 96 %, about 95 % to about 97 %, about 95 % to about 98 %, about 95 % to about 99 %, about 95 % to about 100 %, about 96 % to about 97 %, about 96 % to about 98 %, about 96 % to about 99 %, about 96 % to about 100 %, about 97 % to about 98 %, about 97 % to about 99 %, about 97 % to about 100 %, about 98 % to about 99 %, about 98 % to about 100 %, or about 99 % to about 100 %. In some

embodiments, the fusion protein comprises an sTBRII sequence having sequence identity to SEQ ID NO: 9 of about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, about 99 %, or about 100 %. In some embodiments, the fusion protein comprises an TBRII sequence having sequence identity to SEQ ID NO: 9 of at least about 85 %, about 90 %, about 95 %, about 96 %, about 97 %, about 98 %, or about 99 %.

**[0079]** The sTBRII polypeptide of SEQ ID NO: 8 or SEQ ID NO: 9 can comprise additional truncations thereof. In some embodiments, the sTBRII polypeptide is a truncated sTBRII polypeptide. The truncated sTBRII polypeptide can comprise N-terminus truncations, C-terminus truncations, or a combination thereof. In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids to about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide). In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids to about 5 amino acids, about 2 amino acids to about 7 amino acids, about 2 amino acids to about 8 amino acids, about 2 amino acids to about 10 amino acids, about 2 amino acids to about 15 amino acids, about 2 amino acids to about 20 amino acids, about 5 amino acids to about 7 amino acids, about 5 amino acids to about 8 amino acids, about 5 amino acids to about 10 amino acids, about 5 amino acids to about 15 amino acids, about 5 amino acids to about 20 amino acids, about 7 amino acids to about 8 amino acids, about 7 amino acids to about 10 amino acids, about 7 amino acids to about 15 amino acids, about 7 amino acids to about 20 amino acids, about 8 amino acids to about 10 amino acids, about 8 amino acids to about 15 amino acids, about 8 amino acids to about 20 amino acids, about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, or about 15 amino acids to about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide). In some embodiments, the fusion protein comprises an sTBRII that is truncated by about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids. In some embodiments, the fusion protein comprises an sTBRII that is truncated by at least about 2 amino acids, about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, or about 15 amino acids. In some embodiments, the fusion protein comprises an sTBRII that is truncated by at most about 5 amino acids, about 7 amino acids, about 8 amino acids, about 10 amino acids, about 15 amino acids, or about 20 amino acids (e.g., relative to a full-length sTBRII polypeptide).

**[0080]** Suitable sTBRII sequences or variant sequences (e.g., sTBRII polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any

of the methods for sequestering or binding TGFβ described herein (e.g., as described in any one of Examples 1-3).

**Multi-Functional and Multi-Valent Fusion Protein Compositions**

**[0081]** Provided herein are fusion polypeptides comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide. Also provided herein are fusion polypeptides comprising: a first IL polypeptide that binds to and agonizes the IL receptor; a second polypeptide that binds to and sequesters soluble TGFβ; and a third polypeptide that binds to and sequesters soluble TGFβ.

**[0082]** In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 2. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 3. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In certain embodiments the IL-15 polypeptide comprises SEQ ID NO: 5. In certain embodiments the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5. In certain embodiments, the IL polypeptide is a truncated polypeptide. In some embodiments, the IL-2 sequence comprises an alanine at position 62 (e.g., as in SEQ ID NO: 3).

**[0083]** In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II polypeptide. In some embodiments, the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGFβ superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In certain embodiments the soluble TGFβ receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

**[0084]** In another embodiment, the second polypeptide that binds to and sequesters soluble TGFβ and/or the third polypeptide that binds to and sequesters soluble TGFβ comprises a soluble TGFβ receptor II polypeptide. In some embodiments, the second polypeptide that binds to and sequesters soluble TGFβ and/or the third polypeptide that binds to and sequesters soluble TGFβ comprises a soluble TGFβ receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In certain embodiments the soluble TGFβ receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

**[0085]** In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide target a TGF-β1 polypeptide, a TGF-β2 polypeptide, a TGF-β3 polypeptide, a activin βA polypeptide, a activin βB polypeptide, a activin βC polypeptide, a activin βE polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an arteman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide.

**[0086]** In another embodiment, the second polypeptide and/or the third polypeptide binds to and sequesters a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof. In certain embodiments, the second polypeptide and/or the third polypeptide binds to and sequesters a TGFβ 1 polypeptide.

**[0087]** A polypeptide of the fusion polypeptide can be "fused" or "linked" via a linker amino acid sequence. The linker may be a polypeptide linker or other linker of suitable flexibility so as not to inhibit binding of either targeting polypeptide (e.g., the IL2 polypeptide or TBRII receptor polypeptide). The linker polypeptide can be unstructured (e.g., lacking secondary structure), structured, or a combination thereof. In some embodiments, the linker sequence is a natural amino

acid sequence of the IL polypeptide or soluble TBRII polypeptide. In some embodiments, the linker is not native to the IL polypeptide or TGF receptor polypeptide. For example, a non-native linker can comprise poly-glycine, poly-alanine, poly-serine amino acid or a combination thereof (e.g., GSSG (SEQ ID NO: 26), GGSS (SEQ ID NO: 27), GSAGG (SEQ ID NO: 28), etc.).

**[0088]** In some embodiments, the linker is a non-nature or synthetic linker. The term "synthetic linker" as used herein includes a chemical moiety comprising or derived from a group of atoms that is covalently attached to a targeting agent, and that is also covalently attached to a cytotoxic moiety. Linkers include compounds comprising or derived from divalent radicals such as an alkylene, an arylene, a heteroarylene, moieties such as: — (CR2)nO(CR2)n— wherein R2 is independently repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g. polyethyleneamino, polyetheramines such as Jeffamine™) and n is independently >1, in particular n may be 1 to 15; compounds including the linkers described in Example 1, N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC) and N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide as well as peptides, such as but not limited to repeating units of G, A and C (for example up to 10) with one or more lys residues or other suitable chemical groups for linking to a targeting agent and a cytotoxic moiety. The linker is optionally Cl-30 alkylene, unsubstituted or substituted with one or more substituents, and/or optionally interrupted with one or more heteromoieties independently selected from O, S, NR1, and/or optionally interrupted with one or more of C(O) and C(S), wherein Rl is independently selected from H, and Cl-6 alkyl. The linker can comprise a non-cleavable (stable linker) or cleavable unit (labile linker) such as a peptide bond or a disulfide bond. The linker can be conjugated to the targeting agent and/or the cytotoxic moiety via reactive functional groups.

**[0089]** Both cleavable and non-cleavable linkers can be used in the synthesis of fusion proteins (ADCs). Cleavable linkers include motifs that are either sensitive to lysosomal proteases or sensitive to an acidic pH (such as hydrazone, which is hydrolysed to cleave the linker in gemtuzumab ozogamicin and inotuzumab ozogamicin), or they can contain disulfide bridges that can be reduced by glutathione. The steric hindrance of disulfide bridges can be optimized to limit premature cleavage inside the cell. Generally, the disulfide linker is initially cleaved to release the thiol compound. Acid-cleavable linkers, such as hydrazone, are designed to remain stable at the neutral pH in the blood circulation, but in acidic cellular compartments they undergo hydrolysis and release the cytotoxic drug.

**[0090]** In some embodiments, the linker (e.g., peptide linker) comprises about 10 amino acids to about 100 amino acids. In some embodiments, the linker comprises about 10 amino acids to about 15 amino acids, about 10 amino acids to about 20 amino acids, about 10 amino acids to about 25 amino acids, about 10 amino acids to about 30 amino acids, about 10 amino acids to about 40 amino acids, about 10 amino acids to about 50 amino acids, about 10 amino acids to about 75 amino acids, about 10 amino acids to about 100 amino acids, about 15 amino acids to about 20 amino acids, about 15 amino acids to about 25 amino acids, about 15 amino acids to about 30 amino acids, about 15 amino acids to about 40 amino acids, about 15 amino acids to about 50 amino acids, about 15 amino acids to about 75 amino acids, about 15 amino acids to about 100 amino acids, about 20 amino acids to about 25 amino acids, about 20 amino acids to about 30 amino acids, about 20 amino acids to about 40 amino acids, about 20 amino acids to about 50 amino acids, about 20 amino acids to about 75 amino acids, about 20 amino acids to about 100 amino acids, about 25 amino acids to about 30 amino acids, about 25 amino acids to about 40 amino acids, about 25 amino acids to about 50 amino acids, about 25 amino acids to about 75 amino acids, about 25 amino acids to about 100 amino acids, about 30 amino acids to about 40 amino acids, about 30 amino acids to about 50 amino acids, about 30 amino acids to about 75 amino acids, about 30 amino acids to about 100 amino acids, about 40 amino acids to about 50 amino acids, about 40 amino acids to about 75 amino acids, about 40 amino acids to about 100 amino acids, about 50 amino acids to about 75 amino acids, about 50 amino acids to about 100 amino acids, or about 75 amino acids to about 100 amino acids. In some embodiments, the linker comprises about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 75 amino acids, or about 100 amino acids. In some embodiments, the linker comprises at least about 10 amino acids, about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, or about 75 amino acids. In some embodiments, the linker comprises at most about 15 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 75 amino acids, or about 100 amino acids.

**[0091]** Generally, different polypeptide elements of the fusion polypeptides can be arranged or ordered, from N-terminus to C-terminus, in any number of different combinations. For example, described herein are fusion polypeptides that comprise, from N-terminus to C-terminus, an IL polypeptide, a first TGFβ superfamily receptor polypeptide, and a second TGFβ superfamily receptor polypeptide. By way of further example, the fusion polypeptides as described herein, can

also comprise, from N-terminus to C-terminus, a first TGFβ superfamily receptor polypeptide, an IL polypeptide, and a second TGFβ superfamily receptor polypeptide. In some embodiments, the fusion polypeptide further comprises a pharmacokinetic extender polypeptide (e.g., an Fc polypeptide or HSA polypeptide), wherein the fusion polypeptides further comprising the pharmacokinetic extender can be arranged or ordered, from N-terminus to C-terminus, in any number of different combinations. **FIG. 3** shows a non-limiting schematic representation of exemplary embodiments of fusion polypeptides that comprise an IL polypeptide, a first TGFβ superfamily receptor polypeptide, and a second TGFβ superfamily receptor polypeptide (e.g., SEQ ID NOs: 10-25)

**[0092]** In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10 to 20.

**[0093]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises

an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 10.

**[0094]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 11. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 11.

**[0095]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 12. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 10. In some embodiments, the fusion comprises SEQ ID NO: 12.

**[0096]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 13. In some embodiments, the fusion comprises SEQ ID NO: 13.

**[0097]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 14. In some embodiments, the fusion comprises SEQ ID NO: 14.

**[0098]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98%

sequence identity to SEQ ID NO: 15. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 15. In some embodiments, the fusion comprises SEQ ID NO: 15.

**[0099]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 16. In some embodiments, the fusion comprises SEQ ID NO: 16.

**[0100]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 17. In some embodiments, the fusion comprises SEQ ID NO: 17.

**[0101]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90%

sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 18. In some embodiments, the fusion comprises SEQ ID NO: 18.

**[0102]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 19. In some embodiments, the fusion comprises SEQ ID NO: 19.

**[0103]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 20. In some embodiments, the fusion comprises SEQ ID NO: 20.

**[0104]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at

least 80% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 21. In some embodiments, the fusion comprises SEQ ID NO: 21.

**[0105]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 22. In some embodiments, the fusion comprises SEQ ID NO: 22.

**[0106]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 23. In some embodiments, the fusion comprises SEQ ID NO: 23.

**[0107]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 24. In some embodiments, the fusion comprises SEQ ID NO: 24.

**[0108]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to of SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 98% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO: 25. In some embodiments, the fusion comprises SEQ ID NO: 25.

**[0109]** In some embodiments, the bivalent FIST fusion polypeptide comprises one or more amino acid modifications (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 15 amino acid modifications mutations (e.g., substitutions,

deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), or about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.) to about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, a fusion polypeptide sequence comprises greater than about 5 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 10 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 15 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), about 20 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.), or about 25 amino acid modifications mutations (e.g., substitutions, deletions, truncations, etc.). In some embodiments, the modification increases an affinity for an IL-2 receptor. In certain embodiments, the IL-2 receptor comprises IL-2Rα (termed CD25). In certain embodiments, the IL-2 receptor comprises IL-2Rβ. In certain embodiments, the IL-2 receptor comprises IL-2Rγ. In certain embodiments, the modification increases an affinity for IL-2Rβ and decreases an affinity for IL-2Rα. Such mutations for affecting IL-2 affinity for an IL-2 receptor include those known in the field. Suitable bivalent FIST sequences or variant sequences (e.g., fusion polypeptide sequences having one or more amino acid deletions, substitutions, and/or insertions) can be identified by any of the methods for activating an immune cell (e.g., increased IFN-gamma secretion, increased proliferation, etc.) and sequestering or binding TGFβ (e.g., TGFβ binding, etc.) described herein (e.g., as described in any one of Examples 1-5).

**[0110]** In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 10. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 11. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 12. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 13. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 14.In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 15. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 16. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 17. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 18. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide consists of an amino acid sequence of SEQ ID NO: 25.

**[0111]** The fusion polypeptides disclosed herein can further comprise a pharmacokinetic modulating polypeptide (e.g., a polypeptide that improves the pharmacokinetic profile of a therapeutic agent). For example, the fusion protein can further comprise an immunoglobulin Fc polypeptide or human serum albumin (HAS) polypeptide fused to the N-terminus of the IL polypeptide. In some embodiments, the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide. In some embodiments, the immunoglobulin Fc region is an IgG Fc region. In some embodiments, the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region. In some embodiments, the PK modulator comprises an albumin polypeptide. In some embodiments, the albumin polypeptide is a human albumin polypeptide.

**[0112]** In certain embodiments the fusion polypeptides of the current disclosure are included in a pharmaceutical composition comprising one or more pharmaceutically acceptable excipients, carriers, and diluents. In certain embodiments, the fusion proteins of the current disclosure are administered suspended in a sterile solution. In certain embodiments, the solution comprises about 0.9% NaCl. In certain embodiments, the solution comprises about 5.0% dextrose. In certain embodiments, the solution further comprises one or more of: buffers, for example, acetate, citrate,

histidine, succinate, phosphate, bicarbonate and hydroxymethylaminomethane (Tris); surfactants, for example, polysorbate 80, polysorbate 20 , and poloxamer 188; polyol/disaccharide/polysaccharides, for example, glucose, dextrose, mannose, mannitol, sorbitol, sucrose, trehalose, and dextran 40; amino acids, for example, glycine or arginine; antioxidants, for example, ascorbic acid, methionine; or chelating agents, for example, Ethylenediaminetetraacetic acid or ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid.

**[0113]** In certain embodiments, the fusion proteins of the current disclosure are shipped/stored lyophilized and reconstituted before administration. In certain embodiments, lyophilized fusion polypeptide formulations comprise a bulking agent such as, mannitol, sorbitol, sucrose, trehalose, dextran 40, or combinations thereof. The lyophilized formulation can be contained in a vial comprised of glass or other suitable non-reactive material. The fusion proteins when formulated, whether reconstituted or not, can be buffered at a certain pH, generally less than 7.0. In certain embodiments, the pH can be between 4.5 and 6.5, 4.5 and 6.0, 4.5 and 5.5, 4.5 and 5.0, or 5.0 and 6.0.

**[0114]** In certain embodiments, described herein is a method of preparing a cancer treatment comprising admixing one or more pharmaceutically acceptable excipients, carriers, or diluents and a fusion polypeptide of the current disclosure. In certain embodiments, described herein is a method of preparing a cancer treatment for storage or shipping comprising lyophilizing one or more fusion polypeptides of the current disclosure.

**[0115]** The fusion polypeptides described herein (e.g., SEQ ID NOs: 10 to 25) can be encoded by a nucleic acid. A nucleic acid is a type of polynucleotide comprising two or more nucleotide bases. In certain embodiments, the nucleic acid is a component of a vector that can be used to transfer the polypeptide encoding polynucleotide into a cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a genomic integrated vector, or "integrated vector," which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an "episomal" vector, e.g., a nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors." Suitable vectors comprise plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, viral vectors and the like. In the expression vectors regulatory elements such as promoters, enhancers, polyadenylation signals for use in controlling transcription can be derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants

may additionally be incorporated. Vectors derived from viruses, such as lentiviruses, retroviruses, adenoviruses, adeno-associated viruses, and the like, may be employed. Plasmid vectors can be linearized for integration into a chromosomal location. Vectors can comprise sequences that direct site-specific integration into a defined location or restricted set of sites in the genome (e.g., AttP-AttB recombination). Additionally, vectors can comprise sequences derived from transposable elements.

**Methods of Use**

**[0116]** The fusion polypeptides disclosed herein are useful as an effective angiostatic and anticancer compound for cancer therapy. For example, the disclosed fusion polypeptides are an effective immunotherapy that induces a unique gene expression profile downstream of IL-2 receptor not achieved by its single components or the combination, which leads to new pharmacological properties. These novel multi-functional proteins can simultaneously activate several immune system mechanisms and signaling pathways that act in synergy to effectively eliminate cancer cells. At the cellular level, the immunotherapy platform activates a panoply of lymphoid cells (e.g., T, B, NK and NK-T cells) and indirectly prime antigen-presenting cells (e.g., dendritic cells and macrophages) due to, in part, GM-CSF induction triggering the cascade of specific immunological reactions against malignant cells. At the molecular level, it activates key transcription factors and signaling molecules crucial to potentiate innate and adaptive immune responses. The fusion polypeptides described herein target and synchronize various arms of the immune system against cancer.

**[0117]** Accordingly, disclosed herein are methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; thereby inhibiting or reducing tumor growth and/or progression in the individual. Furthermore, provided are the fusion polypeptides for use in methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; thereby inhibiting or reducing tumor growth and/or progression in the individual.

**[0118]** Also disclosed herein are methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the

individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; wherein the fusion polypeptide activates an immune cell. Furthermore, provided are the fusion polypeptides for use in methods of inhibiting or reducing tumor growth, angiogenesis, and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; wherein the fusion polypeptide activates an immune cell. In some embodiments the immune cell is selected from the group consisting of: a T cell, an NK cell, a B cell, or a combination thereof. In certain embodiments, activation comprises increasing cell proliferation, increasing cytokine signaling (e.g., IFNγ), and/or decreasing TGFβ1-mediated suppression.

**[0119]** Additionally, disclosed are methods of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide, thereby neutralizing or killing tumor cells in the individual. Furthermore, provided are the fusion polypeptides for use in methods of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide, thereby neutralizing or killing tumor cells in the individual.

**[0120]** Disclosed are also methods of treating or ameliorating cancer in an individual having a cancer, comprising administering to the subject a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the individual. Furthermore, provided are the fusion polypeptides for use methods of treating or ameliorating cancer in an individual having a cancer, comprising administering to the subject a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; thereby treating or ameliorating the cancer in the individual.

**[0121]** Also provided are methods of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide. In some embodiments, the cell is an immune cell. The immune cell can be contacted,

and subsequently activated, in vivo or ex vivo. In vivo, the immune cell can reside within a tumor microenvironment. Furthermore, provided are the fusion polypeptides for use in methods of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide.

**[0122]** Methods utilizing the ex-vivo activation of immune cells using the fusion polypeptides disclosed herein are also embodied. For example, disclosed are methods of activating immune cells ex vivo, comprising contacting an immune cell with a composition comprising: (a) an interleukin (IL) polypeptide; (b) a first TGFβ superfamily receptor polypeptide; and (c) a second TGFβ superfamily receptor polypeptide; thereby producing an activated immune cell. Such methods can be applied to the treatment of cancer or a tumor by administering the activated immune cells to an individual having cancer or a tumor. For example, the fusion polypeptides described herein are useful in Methods for the ex-vivo activation of immune cells. The ex vivo treatment of immune cells is also useful in the generation and expansion of immune cells for the generation of chimeric antigen receptor cell therapies (e.g., CAR-T or CAR-NK cells and B cells). For example, a leukopack (e.g., a leukapheresis product) comprising immune cells can be contacted with the fusion polypeptides disclosed herein to expand T cells or NK cells to be used in a chimeric antigen receptor cell therapy. In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. In some embodiments, the cancer is a hematological cancer.

**[0123]** In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 2. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2. In some embodiments, the IL polypeptide is IL-2. In some embodiments, the IL polypeptide is IL-15. In certain embodiments the IL-2 polypeptide comprises SEQ ID NO: 3. In certain embodiments the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3. In certain embodiments the IL-15 polypeptide comprises SEQ ID NO: 5. In certain embodiments the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99%

sequence identity to SEQ ID NO: 5. In certain embodiments, the IL polypeptide is a truncated polypeptide.

**[0124]** In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprise a soluble TGFβ receptor II polypeptide. In some embodiments, the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide comprise a soluble TGFβ receptor II polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGFβ superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9. In certain embodiments the soluble TGFβ receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9.

**[0125]** In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide target a TGF-β1 polypeptide, a TGF-β2 polypeptide, a TGF-β3 polypeptide, a activin βA polypeptide, a activin βB polypeptide, a activin βC polypeptide, a activin βE polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an arteman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof. In some embodiments, the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide.

**[0126]** In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13.In some embodiments, the fusion polypeptide comprises an

amino acid sequence of SEQ ID NO: 14.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18.In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24. In some embodiments, the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25.

**[0127]** In some embodiments, the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10 to 20.

**[0128]** The term “cancer” includes primary malignant tumors (e.g., those whose cells have not migrated to sites in the subject's body other than the site of the original tumor) and secondary malignant tumors (e.g., those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor).

**[0129]** “Tumor” refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

**[0130]** The methods disclosed herein are useful for treating cancers comprising a solid tumor or treating solid tumors. The term “solid tumor” refers to, for example, breast cancer, ovarian cancer, cancer of the colon and generally the GI (gastro-intestinal) tract, cervix cancer, lung cancer, in particular small-cell lung cancer, and non-small-cell lung cancer, head and neck cancer, bladder cancer, cancer of the prostate or Kaposi's sarcoma. The fusion polypeptides neutralize and/or inhibit the growth of solid tumors. Furthermore, depending on the tumor type and the particular combination used a decrease of the tumor volume can be obtained. The combinations disclosed herein are also suited to prevent the metastatic spread of tumors and the growth or development of micrometastases. The combinations disclosed herein are in particular suitable for the treatment of poor prognosis patients, especially such poor prognosis patients having metastatic melanoma or pancreatic cancer.

**[0131]** Furthermore, the methods disclosed herein are useful in the treatment of hematological cancer. The term "hematological cancer" refers to a cancer of the blood, and includes leukemia and malignant lymphoproliferative disorders, among others. "Leukemia" refers to a cancer of the blood, in which too many white or red blood cells are made, thus crowding out the other parts that make up the blood, such as platelets and normal red blood cells. It is understood that cases of leukemia are classified as acute or chronic. Cancer cells in acute leukemias are blocked at an immature stage, yet they continue to multiply. Chronic leukemias progress more slowly, with cancer cells developing to full maturity. Furthermore, the white blood cells may be myelogenous or lymphoid. Thus, certain forms of leukemia may be, by way of example, acute lymphocytic (or lymphoblastic) leukemia (ALL); acute myelogenic leukemia (AML); chronic lymphocytic leukemia (CLL); or chronic myelogenic leukemia (CML); and myelodysplastic syndrome. "Malignant hematological disorders" may refer to a lymphoma, such as Hodgkin's lymphoma, and non-Hodgkin's lymphoma, or multiple myeloma among others.

**[0132]** In certain embodiments, disclosed herein, are fusion proteins useful for the treatment of a cancer or tumor. Treatment refers to a method that seeks to improve or ameliorate the condition being treated. With respect to cancer, treatment includes, but is not limited to, reduction of tumor volume, reduction in growth of tumor volume, increase in progression-free survival, or overall life expectancy. In certain embodiments, treatment may affect remission of a cancer being treated. In certain embodiments, treatment encompasses use as a prophylactic or maintenance dose intended to prevent reoccurrence or progression of a previously treated cancer or tumor. It is understood by those of skill in the art that not all individuals will respond equally or at all to a treatment that is administered, nevertheless these individuals are considered to be treated.

**[0133]** The fusion polypeptides provided herein are useful for the activation of an immune response (e.g., anti-tumor immune response). In some embodiments, the immune cells express IL-2 or IL-15 receptors. In some embodiments, immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells. In some embodiments, the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment. Accordingly, treating or a treatment, in some embodiments, comprises activating immune effector cells that target a tumor cell and/or reducing the activation of immunosuppressive cells (e.g., Treg cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), tumor-associated neutrophils (TANs), and cancer-associated fibroblasts (CAFs))

**[0134]** The term "T cell" refers to a subset of lymphocytic cells that are present in PBMC and express a surface marker of "CD3" (T-cell receptor). Unless otherwise indicated T cells are intended to include CD4+ (e.g., T-helper cells) and CD8+ (e.g., cytotoxic killer cells).

**[0135]** The methods provided herein are also useful for the suppression or reduction of immunosuppressive immune cells such as T regulatory cells. The terms "Treg" or "regulatory T cell" refer to CD4+CD25+FOxP3+ T cells that suppresses CD4+CD25− and CD8+ T cell proliferation and/or effector function, or that otherwise down-modulate an immune response. Notably, Treg may down-regulate immune responses mediated by CD8 T cells, Natural Killer cells, Natural Killer T cells as well as other immune cells. Further encompassed, in some embodiments, is the blockade or prevention of immunosuppressive cell differentiation or activation.

**[0136]** In certain embodiments, the cancer or tumor is a solid cancer or tumor. In certain embodiments, the cancer or tumor is a blood cancer or tumor. In certain embodiments, the cancer or tumor comprises breast, heart, lung, small intestine, colon, spleen, kidney, bladder, head, neck, ovarian, prostate, brain, pancreatic, skin, bone, bone marrow, blood, thymus, uterine, testicular, and liver tumors. In certain embodiments, tumors which can be treated with the fusion proteins described herein comprise adenoma, adenocarcinoma, angiosarcoma, astrocytoma, epithelial carcinoma, germinoma, glioblastoma, glioma, hemangioendothelioma, hemangiosarcoma, hematoma, hepatoblastoma, leukemia, lymphoma, medulloblastoma, melanoma, neuroblastoma, osteosarcoma, retinoblastoma, rhabdomyosarcoma, sarcoma and/or teratoma. In certain embodiments, the tumor/cancer is selected from the group of acral lentiginous melanoma, actinic keratosis, adenocarcinoma, adenoid cystic carcinoma, adenomas, adenosarcoma, adenosquamous carcinoma, astrocytic tumors, Bartholin gland carcinoma, basal cell carcinoma, bronchial gland carcinoma, capillary carcinoid, carcinoma, carcinosarcoma, cholangiocarcinoma, chondrosarcoma, cystadenoma, endodermal sinus tumor, endometrial hyperplasia, endometrial stromal sarcoma, endometrioid adenocarcinoma, ependymal sarcoma, Swing's sarcoma, focal nodular hyperplasia, gastronoma, germ line tumors, glioblastoma, glucagonoma, hemangioblastoma, hemangioendothelioma, hemangioma, hepatic adenoma, hepatic adenomatosis, hepatocellular carcinoma, insulinite, intraepithelial neoplasia, intraepithelial squamous cell neoplasia, invasive squamous cell carcinoma, large cell carcinoma, liposarcoma, lung carcinoma, lymphoblastic leukemia, lymphocytic leukemia, leiomyosarcoma, melanoma, malignant melanoma, malignant mesothelial tumor, nerve sheath tumor, medulloblastoma, medulloepithelioma, mesothelioma, mucoepidermoid carcinoma, myeloid leukemia,

neuroblastoma, neuroepithelial adenocarcinoma, nodular melanoma, osteosarcoma, ovarian carcinoma, papillary serous adenocarcinoma, pituitary tumors, plasmacytoma, pseudosarcoma, prostate carcinoma, pulmonary blastoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, sarcoma, serous carcinoma, squamous cell carcinoma, small cell carcinoma, soft tissue carcinoma, somatostatin secreting tumor, squamous carcinoma, squamous cell carcinoma, undifferentiated carcinoma, uveal melanoma, verrucous carcinoma, vagina/vulva carcinoma, VIPpoma, and Wilm's tumor. In certain embodiments, the tumor/cancer to be treated with one or more fusion proteins described herein comprise brain cancer, head and neck cancer, colorectal carcinoma, acute myeloid leukemia, pre-B-cell acute lymphoblastic leukemia, bladder cancer, astrocytoma, preferably grade II, III or IV astrocytoma, glioblastoma, glioblastoma multiforme, small cell cancer, and non-small cell cancer, preferably non-small cell lung cancer, lung adenocarcinoma, metastatic melanoma, androgen-independent metastatic prostate cancer, androgen-dependent metastatic prostate cancer, prostate adenocarcinoma, and breast cancer, preferably breast ductal cancer, and/or breast carcinoma. In certain embodiments, the cancer treated with the fusion proteins of this disclosure comprises glioblastoma. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises pancreatic cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises ovarian cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises lung cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises prostate cancer. In certain embodiments, the cancer treated with one or more fusion proteins of this disclosure comprises colon cancer. In certain embodiments, the cancer treated comprises glioblastoma, pancreatic cancer, ovarian cancer, colon cancer, prostate cancer, or lung cancer. In a certain embodiment, the cancer is refractory to other treatment. In a certain embodiment, the cancer treated is relapsed. In a certain embodiment, the cancer is a relapsed/refractory glioblastoma, pancreatic cancer, ovarian cancer, colon cancer, prostate cancer, or lung cancer.

**[0137]** The term "tumor microenvironment" refers to a microenvironment (e.g., a region/area wherein malignant cells or clusters of malignant cells are located) in and surrounding a solid tumor to support the growth and metastasis of the tumor cells. The tumor microenvironment includes surrounding blood vessels, immune cells, fibroblasts, other cells, soluble factors, signaling molecules, an extracellular matrix, and mechanical cues that can promote neoplastic transformation, support tumor growth and invasion, protect the tumor from host immunity, foster therapeutic resistance, and provide niches for dormant metastases to thrive. The tumor and its

surrounding microenvironment are closely related and interact constantly. Tumors can influence their microenvironment by releasing extracellular signals, promoting tumor angiogenesis and inducing peripheral immune tolerance, while the immune cells in the microenvironment can affect the growth and evolution of cancerous cells.

**[0138]** In certain embodiments, the fusion proteins can be administered to a subject in need thereof by any route suitable for the administration of fusion protein-containing pharmaceutical compositions, such as, for example, subcutaneous, intraperitoneal, intravenous, intramuscular, intratumoral, or intracerebral, etc. In certain embodiments, the fusion proteins are administered intravenously. In certain embodiments, the fusion proteins are administered subcutaneously. In certain embodiments, the fusion proteins are administered intratumoral. In certain embodiments, the fusion proteins are administered on a suitable dosage schedule, for example, weekly, twice weekly, monthly, twice monthly, once every two weeks, once every three weeks, or once a month etc. In certain embodiments, the fusion proteins are administered once every three weeks. The fusion proteins can be administered in any therapeutically effective amount. In certain embodiments, the therapeutically acceptable amount is between about 0.1 mg/kg and about 50 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 1 mg/kg and about 40 mg/kg. In certain embodiments, the therapeutically acceptable amount is between about 5 mg/kg and about 30 mg/kg. Therapeutically effective amounts include amounts are those sufficient to ameliorate one or more symptoms associated with the disease or affliction to be treated.

**[0139]** Unless defined otherwise, all terms of art, notations and other technical and scientific terms or terminology used herein are intended to have the same meaning as is commonly understood by one of ordinary skill in the art to which the claimed subject matter pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

**[0140]** As used herein, the terms "treatment" or "treating" are used in reference to a pharmaceutical or other intervention regimen for obtaining beneficial or desired results in the recipient. Beneficial or desired results include but are not limited to a therapeutic benefit and/or a prophylactic benefit. A therapeutic benefit may refer to eradication or amelioration of symptoms or of an underlying disorder being treated. Also, a therapeutic benefit can be achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the

subject may still be afflicted with the underlying disorder. A prophylactic effect includes delaying, preventing, or eliminating the appearance of a disease or condition, delaying or eliminating the onset of symptoms of a disease or condition, slowing, halting, or reversing the progression of a disease or condition, or any combination thereof. For prophylactic benefit, a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease may undergo treatment, even though a diagnosis of this disease may not have been made.

**[0141]** The term "a therapeutically effective amount" of a compound of the present application refers to an amount of the compound of the present application that will elicit the biological or medical response of a subject, for example, reduction or inhibition of tumor cell proliferation, or ameliorate symptoms, alleviate conditions, slow or delay disease progression, or prevent a disease, etc. In one non-limiting embodiment, the term "a therapeutically effective amount" refers to the amount of a compound of the present application that, when administered to a subject, is effective to at least partially alleviate, inhibit, prevent and/or ameliorate a condition, or a disorder or a disease, or at least partially inhibit activity of a targeted enzyme or receptor.

**[0142]** The term "target cells" refers to a type of cell, cell population, or composition of cells which are the desired cells to be collected, isolated, or separated by the present disclosure. Target cells represent cells that various procedures described herein require or are designed to purify, collect, engineer etc. What the specific cells are will depend on the context in which the term is used. For example, if the objective of a procedure is to isolate a particular kind of stem cell, that cell would be the target cell of the procedure. The terms "target cells" and "desired cells" are interchangeable and have the same meaning regarding the present disclosure. Target cells can exist in a genus-species relationship. For example, if target cells comprised leukocytes, the target cells would include T cells.

**[0143]** The methods disclosed herein are particularly useful or comprise the activation of immune cells. The term "immune cell" refers generally to cells of the immune system. Immune cells are derived from myeloid or lymphoid cell linages. Generally, the methods disclosed herein are directed towards, but not limited to, the activation of immune effector cells.

**[0144]** The term "immune effector cell" refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes. In certain embodiments, T cells,

NK cells, B cells, NKT cells and gamma/delta T cells or a combination thereof are activated by the fusion polypeptide.

**[0145]** The term "immune effector function" or "immune effector response," as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack to a target cell. E.g., an immune effector function or response refers to a property of a T cells or NK cells that promotes killing or the inhibition of growth or proliferation, of a target cell. Immune effector function includes direct cytotoxicity, cytokine release, chemokine release, phagocytosis, or other immune function that primes or perpetuates an immune response.

**[0146]** The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

**[0147]** The term "myeloid cell" refers to terminally differentiated cells of the myeloid lineage. These cells include neutrophils, eosinophils and monocytes/macrophages, myeloid dendritic cells. In one embodiment of any aspect of the present disclosure, the myeloid cell is a neutrophil, eosinophil or monocyte/macrophage/dendritic cells.

**[0148]** The term "macrophage" and/or "macrophage-like cells" generally refers to macrophages, monocytes, and cells of macrophage/monocyte lineage, and any other similar cells which perform the functions generally associated with macrophages, such as phagocytosis or antigen presentation to other classes of immune cells such as T-cells and B-cells in order to sensitize these cells to a particular target, including but not limited to viruses, bacterial cells, other foreign cells, cancer cells, and other undesired proliferating cells.

**[0149]** The term "lymphocyte" as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells kill tumors and cells infected by viruses.

**[0150]** The term "natural killer (NK) cells" refers to cells of the immune system that kill target cells in the absence of a specific antigenic stimulus, and without restriction according to MHC class. Target cells may be tumor cells or cells harboring viruses. NK cells are characterized by the presence of CD56 and the absence of CD3 surface markers.

**[0151]** The term "T cell" refers to a subset of lymphocytic cells that express a surface marker of "CD3" (T-cell receptor). Unless otherwise indicated T cells are intended to include $CD4^+$ (e.g., T-helper cells) and $CD8^+$ (e.g., cytotoxic killer cells).

**[0152]** The term "endogenous cells" is used to refer to cells derived from a donor (or the patient), as distinguished from cells from a cell line. Endogenous cells are generally heterogeneous

populations of cells from which a specific cell type can be isolated or enriched. Endogenous cells may be intended for autologous or allogeneic treatment of a patient.

**[0153]** Throughout this application, various embodiments may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

**[0154]** As used in the specification and claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a sample" includes a plurality of samples, including mixtures thereof.

**[0155]** The terms "determining," "measuring," "evaluating," "assessing," "assaying," and "analyzing" are often used interchangeably herein to refer to forms of measurement. The terms include determining if an element is present or not (for example, detection). These terms can include quantitative, qualitative or quantitative and qualitative determinations. Assessing can be relative or absolute. "Detecting the presence of" can include determining the amount of something present in addition to determining whether it is present or absent depending on the context.

**[0156]** The terms "subject," "individual," or "patient" are often used interchangeably herein. A "subject" can be a biological entity containing expressed genetic materials. The biological entity can be a plant, animal, or microorganism, including, for example, bacteria, viruses, fungi, and protozoa. The subject can be tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro. The subject can be a mammal. The mammal can be a human. In some embodiments, the subject may be diagnosed or suspected of being at high risk for cancer. In some embodiments, the subject may be diagnosed or suspected of being at high risk for having a tumor. In some embodiments, the subject is not necessarily diagnosed or suspected of being at high risk for cancer.

**[0157]** The term "*in vivo*" is used to describe an event that takes place in a subject's body.

**[0158]** The term "*ex vivo*" is used to describe an event that takes place outside of a subject's body. An *ex vivo* assay is not performed on a subject. Rather, it is performed upon a sample separate from a subject. An example of an *ex vivo* assay performed on a sample is an "*in vitro*" assay.

**[0159]** The term "*in vitro*" is used to describe an event that takes places contained in a container for holding laboratory reagent such that it is separated from the biological source from which the material is obtained. *In vitro* assays can encompass cell-based assays in which living or dead cells are employed. *In vitro* assays can also encompass a cell-free assay in which no intact cells are employed.

**[0160]** As used herein, the term "about" a number refers to that number plus or minus 10% of that number. The term "about" a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value.

**[0161]** The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

## EXAMPLES

**[0162]** The following examples are included for illustrative purposes only and are not intended to limit the scope of the disclosure.

### Example 1 – Improved IL-2 activation of immune cells

**[0163]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide (bFIST) activate higher levels of IFN-gamma and CXCL10 in human NK cells as compared to fusion proteins comprising an IL-2 polypeptide and single sTBRII receptor polypeptide (monovalent FIST).

**[0164]** Human T cells and NK cells were isolated from PBMC by negative selection and were stimulated with FIST (IL2-sTBRII, monovalent) or a FIST variant with bivalent sTBRII traps (IL2-sTBRII-sTBRII) for 48 hours. After 48h of incubation, the conditioned media were collected to quantify the levels of IFN-gamma from T cells and CXCL10 from NK cells by ELISA. As shown in the data of **FIG. 1**, bFIST demonstrates significantly higher IL-2 receptor activation than FIST (monovalent FIST).

### Example 2 – Improved TGFβ1 binding

**[0165]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide

(bFIST) more effectively bind active TGFβ1 as compared to fusion proteins comprising an IL-2 polypeptide and single sTBRII receptor polypeptide (monovalent FIST).

**[0166]** Human active TGFβ1 (2 ng/mL) previously activated with 4 mM HCl containing 0.1% human or bovine serum albumin (BSA) was incubated with sTBRII isoform 2 (long), FIST (monovalent FIST) or bFIST (5-20 nmoL) for 30 mins at RT and free active TGFβ in solution was quantified by ELISA (ELISA for human TGFβ1 immunoassay).

**[0167]** bFIST effectively blocks active TGFβ1 in solution at significant lower concentrations than sTBRII as illustrated in that **FIG. 2**. These results indicate that IL-2 as part of the fusion protein also modifies the sTBRII portion and its binding properties to active TGFβ. Furthermore, the bivalent sTBRII trap variant has more effective TGFβ blocking activity.

**Example 3 – Blockage of TGFβ Isoforms in Solution**

**[0168]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a first sTBRII receptor polypeptide, and a second sTBRII receptor polypeptide more effectively binds TGFβ isoforms.

**[0169]** bFISTv3 and FIST (monovalent FIST) at equimolar concentrations were compared in their ability to inhibit TGFβ1-mediated suppression of CTLL-2 proliferation (IL-2 dependent T Lymphocyte cell line). The blocking activity of TGFβ isoforms in solution by bFISTv3, FIST (monovalent FIST), or control (TbRII isoform 2) was determined by incubating bFISTv3, FIST (monovalent FIST), or control with 1 ng/mL of active TGF β isoforms. Equimolar concentrations (range of 5-5x$10^{-6}$ nmoL) of bFISTv3, FIST (monovalent FIST) or control (TβRII isoform 2) were compared in their ability to block active TGFβ isoforms in solution. bFISTv3, FIST (monovalent FIST) or control were incubated with 1 ng/mL of active TGFβ isoforms diluted in phosphate buffered saline (PBS) for 30 minutes and the free TGFβ (unblocked) was quantified by ELISAs specific for each TGFβ isoforms.

**[0170]** bFISTv3 demonstrated a significantly increased affinity with picomolar potency for TGFb1 (9 picomoles) and TGFβ3 (70 picomoles) compared to FIST and the control TβRII isoform 2 (**FIG. 4A-C**). **Figure 4A** shows blocking of the TGFb1 binding. **Figure 4B** shows blocking of the TGFb2 binding. **Figure 4C** shows blocking of the TGFb3 binding. **Table 1** shows binding values for TGFβ blocking activity in solution. The TGFβ1 and TGFβ3 isoforms are the most oncogenic isoforms and potent inducers of tumor-immune resistance, angiogenesis, metastases,

and immunosuppression. TGFβ1 and TGFβ3 isoforms are also known inducers of fibrotic diseases. In contrast, TGFβ2 was not blocked by the fusion protein or control. TGFβ2 is an important regulator of hematopoiesis, cardiovascular function and glucose and fatty acid metabolism.

**[0171] Table 1: IC50 values for active TGFβ blocking activity in solution**

| Molecule | TGFβ1 | TGFβ2 | TGFβ3 |
|---|---|---|---|
| **TbRII (isoform II)** | 0.02 nM | NA | 0.26 nM |
| **FIST** | 1.29 nM | NA | 3.6 nM |
| **bFISTv3** | 0.009 nM | NA | 0.07 nM |

**Example 4 – Inhibition of TGFb1-mediated Suppression of CTLL-2 Proliferation**

**[0172]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively induces cytotoxic T cell proliferation and prevents the TGFβ1-mediated suppression.

**[0173]** Equimolar concentrations (range of 50-$5x10^{-5}$ nmoL) of bFISTv3, FIST (monovalent FIST) or controls (IL-2 and TβRII isoform 2) were compared in their ability to inhibit active TGFβ1–mediated suppression of CTLL-2 proliferation. CTLL-2 cells (5 x $10^3$ cells/well) were stimulated with equimolar concentration of bFISTv3, FIST (monovalent FIST) or controls for 72 hours at 37°C with/without active TGFβ1 (1 ng/mL). After the incubation period, cell proliferation was determined by a luminescent cell viability assay. The absolute IC50 was determined with a dose-response curve using nonlinear regression model with a sigmoidal dose response. The surviving rate (%) = ($Lum_{Test\ article}$-$Lum_{Medium\ control}$)/ ($Lum_{Vehicle\ control}$-$Lum_{Medium\ control}$) ×100%. The cell number was quantified by a luminescence signal versus cell dose curve. bFISTv3 induced CTLL-2 cell proliferation but prevents the TGFβ1-mediated suppression of CTLL-2 proliferation more effectively than FIST (monovalent FIST). **Figure 5A**: Proliferation of CTLL-2 stimulated with bFISTv3. **Figure 5B**: Proliferation of CTLL-2 stimulated with bFISTv3 in the presence of active TGFβ1 (1 ng/mL). **Figure 5C**: Proliferation of CTLL-2 stimulated with FIST (monovalent FIST). **Figure 5D**: Proliferation of CTLL-2 stimulated with FIST (monovalent FIST) in the presence of active TGFβ1 (1 ng/mL). **Figure 5E**: Proliferation of CTLL-2 stimulated with IL-2. **Figure 5F**: Proliferation of CTLL-2 stimulated IL-2 in the presence of active TGFβ1 (1 ng/mL). Data are shown as mean ± SD, **p<0.005. TGFβ1 is known to inhibit IL-2 signaling and the proliferation of T cells through multiple pathways. TGFβ1 inhibits the phosphorylation and

activation of components of the JAK/STAT cascade downstream of IL-2R, as well as exerts inhibitory activity at the nuclear level on a subset of IL-2 target genes including c-Myc, cyclin D2, and cyclin.

**[0174] Table 2: IC50 values for CTLL-2 cell proliferation in response to the stimuli of fusion proteins or controls**

| Molecule | IC50 | top | bottom | slope |
|---|---|---|---|---|
| IL-2 | 0.057 | 106 | 1.04 | ~12 |
| IL2+TβRII | 0.131 | 116 | 1.26 | 1.6 |
| FIST | 0.063 | 121 | 3.8 | 1.56 |
| bFISTv3 | 0.459 | 137 | 0.07 | 0.69 |

**[0175] Table 3: IC50 values for the inhibition of TGFb1–dependent suppression of CTLL-2 cell proliferation**

| Molecule | IC50 | top | bottom | slope |
|---|---|---|---|---|
| IL-2 | ~0.051 | 37 | 1.22 | ~6.85 |
| IL2+TβRII | 0.322 | 94 | 2.21 | 1.01 |
| FIST | 0.120 | 54 | -3.4 | 0.39 |
| bFISTv3 | 0.650 | 106 | 0 | 0.90 |

**Example 5 – bFIST Proliferation of Effector Memory T cells and Terminal Differentiated Effector Memory Cells**

**[0176]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively induces cytokine production and proliferation of both effector memory ($T_{EM}$) and terminal differentiated effector memory ($T_{EMRA}$) CD8 T cells.

**[0177]** Purified human T cells derived from PBMC-stimulated with CEF peptide pool were labeled with CellTrace™ Violet to track T cell proliferation (CellTrace™ mean fluorescence (MFI) decreases as the cell proliferation increases). Proliferation of $CD8^+$ T cells, effector memory T cells ($CCR7^-CD45RA^-CD62L^-CD8^+$ T cells), and terminal differentiated effector memory CD8 T cells ($CCR7^-$ $CD45RA^+$ $CD62L^-$ $CD8^+$ T cells) stimulated with equimolar concentration of bFISTv3, FIST (monovalent FIST), or TbRII isoform 2.

Human PBMC from healthy donors were stimulated with CEF peptide pool overnight to induce the generation of antigen-specific T cells. T cells ($CD3^+$) were purified from PBMC derived from

healthy donors by immunomagnetic negative selection using a Human T cell isolation kit. Purified T cells were labeled with CellTrace™ Violet before being stimulated with 5 nmoL of bFISTv3, FIST (monovalent FIST) and IL-2 for 48 hours. Stimulated T cells were labeled with fluorescence-conjugated antibodies specific for the memory T cell populations. After the incubation period, cell supernatants were collected to quantify the concentration of IFNγ by ELISA and the cells were collected to determine the cell proliferation of each T cell subpopulation by flow cytometry. bFISTv3 induces IFNγ production from T cells and significantly higher proliferation of both effector memory ($T_{EM}$) and terminal differentiated effector memory ($T_{EMRA}$) CD8 T cells (e.g., when compared to FIST). **FIG. 6A** shows bFISTv3 induced proliferation of effector memory T cells ($T_{EM}$) and terminal differentiated effector memory cells ($T_{EMRA}$). **FIG. 6B** shows FIST induced proliferation of effector memory T cells ($T_{EM}$) and terminal differentiated effector memory cells ($T_{EMRA}$). **FIG. 6C** shows IL-2 induced proliferation of effector memory T cells ($T_{EM}$) and terminal differentiated effector memory cells ($T_{EMRA}$). **FIG. 6D** shows the average of CellTrace™ MFI values of stimulated $T_{EM}$ derived from two donors (a decrease in MFI denotes increased proliferation). **FIG. 6E** shows average CellTrace™ MFI values of stimulated $T_{EMRA}$ derived from two donors (a decrease in MFI denotes increased proliferation). **FIG. 6F** shows quantification of IFNγ production by T cells stimulated with bFISTv3, FIST (monovalent FIST), or IL-2 for 48 hours. Data are shown as mean ± SD, * $p<0.05$. The effector memory T lymphocyte are antigen-primed lymphocytes that can confer rapid immune protection upon a second challenge with tumor-associated antigens. The memory T cell subpopulations $CCR7^-$ can migrate to inflame tissues (e.g., tumor site) and exert immediate effector functions like cytotoxicity against cancer cells. $CCR7^-$ memory $CD8^+$ T cells are characterized by the production of IFNγ and perforin-containing granules essential for their anti-tumor cytolytic activity. In particular, the perforin expression is prominent in the terminal differentiated effector memory T cells ($CD45RA^+CCR7^-CD62L^-$).

**Example 6 – bFIST inhibits TGFb1-mediated suppression of primary NK cell proliferation and increases IFNγ production**

**[0178]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively inhibits and/or reduces TGFb1-mediated of primary NK cell proliferation and IFNγ production.

**[0179]** Human NK cells were purified from PBMC derived from healthy donors by immunomagnetic negative selection using a human NK cell isolation kit. Purified NK cells were labeled stimulated with equimolar concentrations (25 nM, 12.5 nM and 6.25 nM) of bFISTv3, FIST (monovalent FIST) and IL-2 for 5 days. After the incubation period, the cell culture supernatant was collected to quantify the concentration of IFNγ by ELISA. The number of viable cells was quantified with the fluorescence-based assay using a cell number/luminescence curve and the percentage of cell proliferation was calculated versus the positive control (IL-2+TbRII) as the maximum stimulus of NK cell proliferation for these experimental conditions.

**[0180]** Purified human NK cells derived from peripheral mononuclear cells (PBMC) were stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or control (IL-2 + TbRII) for 5 days with or without active TGFb1 (1 ng/mL). The culture supernatant was collected to quantify the amount of IFNγ by ELISA and the number of viable cells in culture was determined by quantitating the amount of ATP present, which indicates the metabolically active cells. **FIG. 7A** shows the percentage of NK cell proliferation in the absence of active TGFb1. **FIG. 7B** shows the percentage of NK cell proliferation in the presence of active TGFb1. **FIG. 7C** shows the quantification of IFNγ produced by stimulated NK cells cultured in the absence of active TGFb1. **FIG. 7D,** quantification of IFNγ produced by stimulated NK cells cultured with active TGFb1(1 ng/mL). **FIG. 7E,** quantification of CXCL10 produced by stimulated NK cells cultured with of active TGFb1(1 ng/mL). The data are representative of two independent experiments performed in duplicates. Statistic comparisons between bFISTv3 and FIST are indicated (data are shown as mean ± SD, *p<0.05, **p<0.005, ***p<0.0005).

**Example 7 – bFIST Increases NK Cell-mediated Cytotoxicity and Activation**

**[0181]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively increases and/or stimulates natural killer (NK) cell-mediated cytotoxicity and activation.

**[0182]** The NK92 cells were serum-starved for 9 hours before being stimulated with equimolar concentrations of the bFISTv3, FIST (monovalent FIST) and TβRII isoform 2 (control) in the presence or absence of active TGFβ1 (1 ng/mL) for 48 hours. After the incubation period, the amount of IFNγ was quantified by ELISA. The NK92 cells were collected and co-cultured with

target cells (K-562 cells, Chronic Myelogenous Leukemia) at different effector: target ratios (1:1, 3:1, 6:1). The NK92 cytolytic activity against cancer cells was determined by measuring % of specific tumor target death through fixable viability dye staining after NK: K-562 co-culture for three hours at 37℃, 5% $CO_2$ incubator. The % of dead cells was calculated based on the equation:

$$\% \text{ specific lysis} = \frac{100 \text{ x } (\% \text{ lysis in sample} - \% \text{ basal death})}{100 - \% \text{ basal death}}$$

**[0183]** TGF-β is a potent immunosuppressive cytokine that severely affects immune cell functions, including NK cell activation, IFN-γ production, and the expression of activating receptors NKG2D and NKp30, thereby reducing the cytotoxic ability of NK cells and impairing their antitumor function. bFIST induces both robust immune cell activation and effective blockage (with picomolar potency) of active TGFb1. These two effects in tandem promote higher NK cell proliferation, activation, and cytotoxicity against cancer cells. **FIG. 8A** shows quantification of IFNγ production by NK92 cells as indicator of NK92 cell activation (as described above, human NK92 cells were serum-starved for 9 hours before being stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, control (IL-2 + TβRII) for 48 hours with or without active TGFβ1 (1 ng/mL) and the cell supernatant was collected). **FIG. 8B,** shows induced cytotoxicity of bFISTv3- or FIST-stimulated NK92 cells against K-562 cells at 6:1 ratio (effector:target). Statistic comparisons between bFISTv3 and FIST are indicated. Data are shown as mean ± SD, *$p<0.05$.

**Example 8 – bFIST stimulates and increases B cell maturation, proliferation and IFNγ production**

**[0184]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively (1) stimulates and/or increases B cell maturation, proliferation and IFNγ production and (2) reduces and/or inhibits TGFb1 suppression of B cell maturation, proliferation and IFNγ production.

**[0185]** Human B cells were purified from PBMC derived from healthy donors by immunomagnetic negative selection using a Human B cell isolation kit. Purified B cells were pre-labeled with CFSE to trace cell proliferation (the marker intensity decreases as the cell proliferation increases) before being stimulated with equimolar concentrations (20 nM, 10 nM and 5 nM) of bFISTv3, FIST and IL-2 for 5 days. After the incubation period, B cells were labeled with CD86 and HLA-DR and the percentages of double positive ($CD86^+$ $HLA\text{-}DR^+$) B cells and proliferation

were quantified by flow cytometry. The cell culture supernatants were collected to quantify the concentration of IFNγ by ELISA. The statistic comparisons between bFISTv3 and FIST are indicated. Data are shown as mean ± SD, *p<0.05, **p<0.005, ***p<0.0005.

**[0186]** Bivalent FIST effectively increases and/or stimulates B cell activation, proliferation, and IFNγ production (e.g., compared to FIST or IL-2). Bivalent FIST also induces the upregulation of costimulatory molecule (CD86) and HLA-DR (MHC class II) molecule expression that indicates B-cell responsiveness to bivalent FIST stimulatory effects. Further, bivalent FIST also activates B cells into effector cells with high expression of antigen-presenting cell (APC) maturation markers and IFNγ production

**[0187]** **FIG. 9A-D** shows data from purified human B cells ($CD19^-$ $CD3^-$) derived from peripheral mononuclear cells (PBMC) were labeled with Carboxyfluorescein succinimidyl ester (CFSE) to trace cell proliferation before being stimulated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, control (IL-2 + TbRII) for 5 days with and without active TGFb1 (1 ng/mL). Stimulated B cells were labeled with conjugated antibodies specific for HLA-DR and CD86 to quantify the percentage of mature B cells by flow cytometry. **FIG. 9A** shows a comparison between bFISTv3 (20 nmoL) and FIST (20 nmoL) in the induction of double positive $CD86^+HLA\text{-}DR^+$ B cells cultured with/without active TGFb1. **FIG. 9B** shows the percentage of $CD86^+$ $HLA\text{-}DR^+$ B cells stimulated with 20, 10, 5 nmoL of bFISTv3, FIST (monovalent FIST) or, control (IL-2+TbRII) cultured with/without active TGFb1. **FIG. 9C** shows a comparison between bFISTv3 (20 nmoL) and FIST (20 nmoL) in the induction of $CD86^+HLA\text{-}DR^+$ B cell proliferation with/without active TGFb1. The mean fluorescence intensity (MFI) values of CFSE (cell tracer) are indicated. **FIG. 9D** shows quantification of IFNγ production in the supernatant of stimulated B cells cultured with and without active TGFb1.

## Example 9 – bFIST Inhibits the Upregulation of Epithelial to Mesenchymal Transition (EMT) Markers on Lung Cancer Cells

**[0188]** Exemplifying the fusion polypeptide compositions and methods disclosed herein, the flowing example demonstrates that the fusion polypeptides comprising an interleukin-2 (IL-2) polypeptide, a sTβRII receptor polypeptide, and a second sTβRII receptor polypeptide more effectively inhibits and/or reduces the upregulation of Epithelial to Mesenchymal Transition (EMT) markers on lung cancer cells.

**[0189]** A-549 lung cancer cells were treated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, controls (IL-2 and IL-2 + TbRII) with/without active TGFb1 for 72 hours.

After the incubation period, the cells were collected and labeled with conjugated antibodies specific for E-Cadherin, N-Cadherin, and PD-L1. The percentage of cells expressing these EMT markers was quantified by Flow cytometry.

Epithelial-mesenchymal transition (EMT) enables carcinoma cells to suppress their epithelial features changing to mesenchymal ones. As result of the EMT process, carcinoma cells to acquire mobility and the capacity to migrate from the primary tumor site to distant organs and form metastases. The loss of E-Cadherin expression is a crucial step in EMT and a hallmark of mesenchymal phenotype that characterize the transformation from non-metastatic to metastatic cancer cells. The treatment with bFIST reduces the TGFb1-mediated upregulation of EMT markers (decrease of E-cadherin, increase of N-cadherin and increase of PD-L1 expression). **FIG. 10A** shows the percentage of A-549 cells (lung cancer cells) expressing E-Cadherin after being treated with equimolar concentrations of bFISTv3, FIST (monovalent FIST) or, controls (IL-2 and IL-2 + TbRII) with/without active TGFb1 for 72 hours. **FIG. 10B** shows the percentage of A-549 cells expressing N-Cadherin and **FIG. 10C** shows the percentage of A-549 cells expressing PD-L1. The data are representative of two independent experiments performed in duplicates. As shown, The treatment of lung cancer cells with bFIST increases of E-cadherin expression, decreases of N-cadherin, and decreases of PD-L1 expression.

**[0190]** While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

# SEQUENCES

| SEQ ID NO | SEQUENCE | DESCRIPTION |
|---|---|---|
| 1 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLT | Human IL2 |
| 2 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTL | FIST_IL2 |
| 3 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTL | FIST_IL2M IL-2 mutated F62A |
| 4 | MVLGTIDLCSCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTE SDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNG NVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS | Human_IL15 |
| 5 | CFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVT AMKCFLLELQVISLESGDASIHDTVENLIILANNSLSSNGNVTESGCKEC EELEEKNIKEFLQSFVHIVQMFINTS | FIST_IL15 |
| 6 | MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFP QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENIT LETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPDLLLVIFQVTGISLLPPLGVAISVIIIFYCYRVNRQQKL SSTWETGKTRKLMEFSEHCAIILEDDRSDISSTCANNINHNTELLPIELD TLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYEEYASWKTEKDIFS DINLKHENILQFLTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVIS WEDLRKLGSSLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNILVKNDL TCCLCDFGLSLRLDPTLSVDDLANSGQVGTARYMAPEVLESRMNLEN VESFKQTDVYSMALVLWEMTSRCNAVGEVKDYEPPFGSKVREHPCVE SMKDNVLRDRGRPEIPSFWLNHQGIQMVCETLTECWDHDPEARLTAQ CVAERFSELEHLDRLSGRSCSEEKIPEDGSLNTTK | Human TGFBR |

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|---|---|---|
| 7 | MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSDVEMEAQKDEIICPSCNR TAHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNC SITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPK CIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIFQVTGI SLLPPLGVAISVIIIFYCYRVNRQQKLSSTWETGKTRKLMEFSEHCAIILE DDRSDISSTCANNINHNTELLPIELDTLVGKGRFAEVYKAKLKQNTSEQ FETVAVKIFPYEEYASWKTEKDIFSDINLKHENILQFLTAEERKTELGK QYWLITAFHAKGNLQEYLTRHVISWEDLRKLGSSLARGIAHLHSDHTP CGRPKMPIVHRDLKSSNILVKNDLTCCLCDFGLSLRLDPTLSVDDLANS GQVGTARYMAPEVLESRMNLENVESFKQTDVYSMALVLWEMTSRCN AVGEVKDYEPPFGSKVREHPCVESMKDNVLRDRGRPEIPSFWLNHQGI QMVCETLTECWDHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKIP EDGSLNTTK | Human TGFBR long |
| 8 | IPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASP KCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | bFIST_TGFB R_short |
| 9 | IPPHVQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAV KLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDE NITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSSD ECNDNIIFSEDYNTSNPD | bFIST_TGFB R_long |
| 10 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASP KCVMKEKKKPGETFFMCSCSADECNDNIIFIPPHVQKSVNNDMIVTDN NGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWR KNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCS CSADECNDNIIFSEDYNTSNPD | bFISTv.2 |

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| 11 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLE DAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPDIPPH VQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSIT SICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCV MKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | bFISTv.2.1 |
| 12 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSS DECNDNIIFIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHG FTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | bFISTv.3 |
| 13 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQIPPHVKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTD NNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW RKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFM CSCSSDECNDNIIFSEDYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKLP QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENIT LETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADEC NDNIIFSEDYNTSNPD | bFISTv.3.1 |

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| 14 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN<br>NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK<br>NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ<br>SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN<br>CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASP<br>KCVMKEKKKPGETFFMCSCSADECNDNIIFIPPHVQKSDVEMEAQKGE<br>MICVTCNRTAHPLKHVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDN<br>QKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLTYHGF<br>TLEDAASPKCVMKEKKKPGETFFMCSCSSDECNDNIIFSEDYNTSNPD | bFISTv.4 |
| 15 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN<br>NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK<br>NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ<br>SIISTLIPPHVQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKS<br>CMSNCSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLE<br>DAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPDIPPH<br>VQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAVKLP<br>QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENIT<br>LETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSSDECN<br>DNIIFSEDYNTSNPD | bFISTv.4.1 |
| 16 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN<br>NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK<br>NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ<br>SIISTLQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGA<br>VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND<br>ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSS<br>DECNDNIIFIPPHVKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIV<br>TDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVA<br>VWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETF<br>FMCSCSSDECNDNIIFSEDYNTSNPD | bFISTv.5 |

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| 17 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQIPPHVKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTD NNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW RKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFM CSCSSDECNDNIIFSEDYNTSNPDIPPHVKSDVEMEAQKGEMICVTCNR TAHPLKHVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNC SITSICEKPQEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPK CVMKEKKKPGETFFMCSCSSDECNDNIIFSEDYNTSNPD | bFISTv.5.1 |
| 18 | MYRMQLLSCIALSLALVTNSISAMVRSVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGKAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTR MLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKSDV EMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAVKLPQLCKFC DVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCH DPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | bFISTv.6 |

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|---|---|---|
| 19 | MYRMQLLSCIALSLALVTNSISAMVRSVECPPCPAPPVAGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGKAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTR MLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLI SNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKSVN NDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPH EVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKK PGETFFMCSCSADECNDNIIFIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPD | bFISTv.7 |
| 20 | MYRMQLLSCIALSLALVTNSIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPDAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLT RMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKS VNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEK PHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVMKEK KKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | bFISTv.8 |
| 21 | MYRMQLLSCIALSLALVTNSIPPHVQKSVNNDMIVTDNNGAVKLPQLC KFCDVRFSTCDNQKSCMSNCSITSICEKPHEVCVAVWRKNDENITLET VCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSADECNDN IIFSEDYNTSNPDAPTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLT RMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLQKS DVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGAVKLPQLCK FCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLETV CHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSSDECNDNII FSEDYNTSNPD | bFISTv.9 |

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|---|---|---|
| 22 | MYRMQLLSCIALSLALVTNSIPPHVKSDVEMEAQKGEMICVTCNRTAH PLKHVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCV MKEKKKPGETFFMCSCSSDECNDNIIFSEDYNTSNPDAPTSSSTKKTQL QLEHLLLDLQMILNGINNYKNPKLTRMLTAKFYMPKKATELKHLQCL EEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYA DETATIVEFLNRWITFCQSIISTLQKSDVEMEAQKGEMICVTCNRTAHP LKHVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSNCSITSI CEKPQEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASPKCVM KEKKKPGETFFMCSCSSDECNDNIIFSEDYNTSNPD | bFISTv.10 |
| 23 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSS DECNDNIIFSEDYNTSNPD | FISTv.11 |
| 24 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ SIISTLQKSDVEMEAQKGEMICVTCNRTAHPLKHVNNDMIVTDNNGA VKLPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKND ENITLETVCHDPKLTYHGFTLEDAASPKCVMKEKKKPGETFFMCSCSS DECNDNIIFSEDYNTSNPD | FISTv.12 |

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|---|---|---|
| 25 | MYRMQLLSCIALSLALVTNSAPTSSSTKKTQLQLEHLLLDLQMILNGIN<br>NYKNPKLTRMLTAKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSK<br>NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQ<br>SIISTLQKSVNNDMIVTDNNGAVKLPQLCKFCDVRFSTCDNQKSCMSN<br>CSITSICEKPHEVCVAVWRKNDENITLETVCHDPKLTYHGFTLEDAASP<br>KCVMKEKKKPGETFFMCSCSADECNDNIIFSEDYNTSNPD | FISTv.13 |

# CLAIMS

**Listing of Claims:**

1. A fusion polypeptide, comprising:

an interleukin (IL) polypeptide;

a first TGFβ superfamily receptor polypeptide; and

a second TGFβ superfamily receptor polypeptide.

2. A fusion polypeptide comprising:

an IL polypeptide that binds to and agonizes an IL receptor; a first TGFβ superfamily receptor polypeptide that binds to and sequesters soluble TGFβ; and a second TGFβ superfamily receptor polypeptide that binds to and sequesters soluble TGFβ.

3. The fusion polypeptide of any one of claims 1 to 2, wherein the IL polypeptide comprises an IL-2 polypeptide or IL-15.

4. The fusion polypeptide of any one of claims 1 to 3, wherein the IL polypeptide comprises an IL-2 polypeptide.

5. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide comprises SEQ ID NO: 2 or SEQ ID NO: 3.

6. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, or an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 3.

7. The fusion polypeptide of claim 4, wherein the IL-2 polypeptide consists of SEQ ID NO: 2 or SEQ ID NO: 3.

8. The fusion polypeptide of any one of claims 1 to 3, wherein the IL polypeptide comprises an IL-15 polypeptide.

9. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide comprises SEQ ID NO: 5.

10. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 5.

11. The fusion polypeptide of claim 8, wherein the IL-15 polypeptide consists of SEQ ID NO: 5.

12. The fusion polypeptide of any one of claims 1 to 11, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises an

activin receptor polypeptide or fragment thereof, a bone morphogenetic protein (BMP) receptor polypeptide or fragment thereof, a glial cell-derived neurotrophic factor (GDNF) receptor polypeptide or fragment thereof, or a TGFβ receptor II polypeptide or fragment thereof.

13. The fusion polypeptide of any one of claims 1 to 12, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a TGFβ receptor II (TBRII) polypeptide or fragment thereof.

14. The fusion polypeptide of any one of claims 1 to 13, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide or fragment thereof.

15. The fusion polypeptide of any one of claims 1 to 14, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide comprising SEQ ID NO: 8, SEQ ID NO: 9, or a combination thereof.

16. The fusion polypeptide of any one of claims 1 to 15, wherein the first TGFβ superfamily receptor polypeptide comprises an amino acid sequence of SEQ ID NO: 8 and the second TGFβ superfamily receptor polypeptide comprises a truncation of an amino acid sequence of SEQ ID NO: 9.

17. The fusion polypeptide of any one of claims 14 to 16, wherein the soluble TGFβ receptor II polypeptide comprises an amino acid sequence having greater than 80%, 85%, 90%, 95% 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 9

18. The fusion polypeptide of any one of claims 1 to 17, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGF-β1 polypeptide, a TGF-β2 polypeptide, a TGF-β3 polypeptide, a activin βA polypeptide, a activin βB polypeptide, a activin βC polypeptide, a activin βE polypeptide, a bone morphogenic protein (BMP) 2 polypeptide, a BMP 3 polypeptide, a BMP4 polypeptide, a BMP 5 polypeptide, a BMP 6 polypeptide, a BMP 7 polypeptide, a BMP 8 polypeptide, a BMP 9 polypeptide, a BMP 10 polypeptide, a BMP 11 polypeptide, a BMP 12 polypeptide, a BMP 13 polypeptide, a BMP 14 polypeptide, a BMP 15 polypeptide, a growth differentiation factor (GDF) 1 polypeptide, a GDF 3 polypeptide, a GDF 8 polypeptide, a GDF 9 polypeptide, a GDF 15 polypeptide, a Nodal polypeptide, a Inhibin α polypeptide, an anti-Mullerian Hormone polypeptide, a Lefty 1 polypeptide, a Lefty 2 polypeptide, an arteman polypeptide, a Persephin polypeptide, or a Neurturin polypeptide.

19. The fusion polypeptide of any one of claims 1 to 18, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof.

20. The fusion polypeptide of any one of claims 1 to 19, wherein the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide.

21. The fusion polypeptide of any one of claims 1-20, wherein the truncated TGFβ superfamily receptor polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof.

22. The fusion protein of claim 21, wherein the truncated TGFβ superfamily receptor polypeptide is a truncated soluble TGFβ receptor II (sTβRII) polypeptide.

23. The fusion polypeptide of claim 22, wherein the truncation comprises greater than 2, 5, 7, 10, 15, 20, 25, or 30 amino acids.

24. The fusion polypeptide of any one of claims 1 to 23, wherein the IL polypeptide comprises a truncated IL polypeptide.

25. The fusion polypeptide of claim 24, wherein the truncated IL polypeptide comprises an N terminus truncation, a C terminus truncation, or combination thereof.

26. The fusion polypeptide of claim 24, wherein the truncated IL polypeptide comprises a truncated IL-2 polypeptide or IL-15 polypeptide.

27. The fusion polypeptide of any one of claims 1 to 26, further comprising a linker polypeptide or a linker molecule attaching the first TGFβ superfamily receptor polypeptide and the second TGFβ superfamily receptor polypeptide.

28. The fusion polypeptide of any one of claims 1 to 27, further comprising a linker polypeptide fusing the IL polypeptide and the first TGFβ superfamily receptor polypeptide.

29. The fusion polypeptide of any one of claims 1 to 28, further comprising a pharmacokinetic (PK) modulator.

30. The fusion polypeptide of claim 29, wherein the pharmacokinetic modulator comprises an immunoglobulin constant (Fc) region polypeptide.

31. The fusion polypeptide of claim 30, wherein the immunoglobulin Fc region polypeptide is a human immunoglobulin Fc region polypeptide.

32. The fusion polypeptide of claim 30, wherein the immunoglobulin Fc region is an IgG Fc region.

33. The fusion polypeptide of claim 30, wherein the IgG Fc region is an IgG1, IgG2, IgG3, or IgG4 Fc region.

34. The fusion polypeptide of claim 29, wherein the PK modulator comprises an albumin polypeptide.

35. The fusion polypeptide of clam 34, wherein the albumin polypeptide is a human albumin polypeptide.

36. The fusion polypeptide of any one of claims 1 to 35, wherein the fusion polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to any one of SEQ ID NOs: 10-25.

37. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 10.

38. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 11.

39. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 12.

40. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 13.

41. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 14.

42. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 15.

43. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 16.

44. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 17.

45. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 18.

46. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 19.

47. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 20.

48. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 21.

49. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 22.

50. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 23.

51. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 24.

52. The fusion polypeptide of claim 36, wherein the fusion polypeptide comprises an amino acid sequence of SEQ ID NO: 25.

53. The fusion polypeptide of any one of claims 1 to 52, wherein the fusion polypeptide consists of any one of SEQ ID NOs: 10-25.

54. The fusion polypeptide of any one of claims 1 to 53, wherein the fusion polypeptide activates target cells expressing an IL-2 or IL-15 receptor.

55. The fusion polypeptide of claim 54, wherein the target cell is an immune cell.

56. The fusion polypeptide of claim 55, wherein the immune cell is a T cells, natural killer cells NKT cells, B cells, or gamma delta T cells.

57. A pharmaceutical composition comprising the fusion polypeptide of any one of claims 1 to 56, comprising a pharmaceutically acceptable carrier or excipient.

58. A method of inhibiting or reducing tumor growth and/or progression in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising:

(a) an interleukin (IL) polypeptide;

(b) a first TGFβ superfamily receptor polypeptide; and

(c) a second TGFβ superfamily receptor polypeptide;

thereby inhibiting or reducing tumor growth and/or progression in the individual.

59. The method of claim 58, wherein the tumor is a primary tumor.

60. The method of any one of claims 58 to 59, wherein the tumor is a metastatic tumor.

61. The method of any one of claims 58 to 60, wherein the cancer comprises a solid tumor.

62. The method of any one of claims 58 to 61, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.

63. The method of claim 62, wherein the immune cells express IL-2.

64. The method of claim 63, wherein the immune cells comprise T cells, natural killer cells, NKT cells, B cells, or gamma delta T cells.

65. The method of any one of claims 58 to 64, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cells within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.

66. The method of any one of claims 58 to 65, wherein the cancer is a hematological cancer.

67. The method of any one of claims 58 to 66, wherein the cancer a cancer comprising a solid tumor.

68. A method of neutralizing or killing tumor cells in an individual having cancer, comprising administering to the individual a fusion polypeptide comprising:

(a) an interleukin (IL) polypeptide;

(b) a first TGFβ superfamily receptor polypeptide; and

(c) a second TGFβ superfamily receptor polypeptide;

thereby neutralizing or killing tumor cells in the individual.

69. The method of claim 68, wherein the tumor is a primary tumor.

70. The method of any one of claims 68 to 69, wherein the tumor is a metastatic tumor.

71. The method of any one of claims 68 to 70, wherein the cancer comprises a solid tumor.

72. The method of any one of claims 68 to 71, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.

73. The method of claim 72, wherein the immune cells express IL-2 or IL-15 receptors.

74. The method of claim 72, wherein the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells.

75. The method of any one of claims 68 to 74, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.

76. The method of any one of claims 68 to 75, wherein the cancer is a hematological cancer.

77. The method of any one of claims 68 to 76, wherein the cancer comprises a solid tumor.

78. A method of treating or ameliorating cancer in a subject having a cancer, comprising administering to the subject a fusion polypeptide comprising a

(a) an interleukin (IL) polypeptide;

(b) a first TGFβ superfamily receptor polypeptide; and

(c) a second TGFβ superfamily receptor polypeptide;

thereby treating or ameliorating the cancer in the subject.

79. The method of claim 78, wherein the tumor is a primary tumor.

80. The method of any one of claims 78 to 79, wherein the tumor is a metastatic tumor.

81. The method of any one of claims 78 to 80, wherein the cancer comprises a solid tumor.

82. The method of any one of claims 78 to 81, wherein the fusion polypeptide activates immune cells within a tumor microenvironment.

83. The method of claim 82, wherein the immune cells express IL-2 or IL-15 receptors.

84. The method of claim 82, wherein the immune cells comprise T cells, natural killer cells NKT cells, B cells, or gamma delta T cells.

85. The method of any one of claims 78 to 84, wherein the fusion polypeptide inhibits or reduces immunosuppression of immune cell within a tumor microenvironment and/or reduces or inhibits the activation of immunosuppressive cells in the tumor microenvironment.

86. The method of any one of claims 78 to 85, wherein the cancer is a hematological cancer.

87. The method of any one of claims 78 to 86, wherein the cancer comprises a solid tumor.

88. The method of any one of claims 78 to 87, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide binds a TGFβ 1 polypeptide, a TGFβ 2 polypeptide, a TGFβ 3 polypeptide, or any combination thereof.

89. A method of activating a cell expressing an IL receptor comprising: contacting a cell with a fusion polypeptide comprising

(a) an interleukin (IL) polypeptide;

(b) a first TGFβ superfamily receptor polypeptide; and

(c) a second TGFβ superfamily receptor polypeptide.

90. The method of claim 89, wherein the cell is an immune cell.

91. The method of claim 90, wherein the immune cell comprises T cells, natural killer cells NKT cells, B cells, or gamma delta T cells.

92. The method of any one of claims 89 to 91, wherein the immunes cells are contacted in vivo.

93. The method of any one of claims 89 to 91, wherein the immunes cells are contacted ex vivo.

94. The method of claim 93, wherein a leukopack or leukapheresis product comprises the immunes cells.

95. The method of claim 93, wherein the immunes cells are derived from an apheresis sample.

96. The method of claim 93, wherein the immune cells are subsequently used for a cell-based therapy.

97. The method of any one of claims 89 to 96, wherein the IL polypeptide is IL-2 or IL-15.

98. The method of any one of claims 89 to 97, wherein the first TGFβ superfamily receptor polypeptide and/or the second TGFβ superfamily receptor polypeptide comprises a soluble TGFβ receptor II (TBRII) polypeptide or fragment.

99. A host cell, comprising a nucleic acid molecule comprising a nucleic acid sequence encoding the recombinant fusion polypeptide as in any of claims 1 to 56.

100. Use of the fusion polypeptide of any one of claims 1 to 56, for inhibiting or reducing tumor growth and/or progression in an individual having cancer.

101. Use of the fusion polypeptide of any one of claims 1 to 56, for neutralizing or killing tumor cells in an individual having cancer.

102. Use of the fusion polypeptide of any one of claims 1 to 56, for treating or ameliorating cancer in a subject having a cancer.

103. Use of the fusion polypeptide of any one of claims 1 to 56, for activating a cell expressing an IL receptor.

104. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for inhibiting or reducing tumor growth and/or progression in an individual having cancer.

105. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for neutralizing or killing tumor cells in an individual having cancer.

106. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for treating or ameliorating cancer in a subject having a cancer.

107. Use of the fusion polypeptide of any one of claims 1 to 56, in the manufacture of a medicament for activating a cell expressing an IL receptor.

108. The fusion polypeptide of any one of claims 1 to 56, for use in inhibiting or reducing tumor growth and/or progression in an individual having cancer.

109. The fusion polypeptide of any one of claims 1 to 56, for use in neutralizing or killing tumor cells in an individual having cancer.

110. The fusion polypeptide of any one of claims 1 to 56, for use in treating or ameliorating cancer in a subject having a cancer.

111. The fusion polypeptide of any one of claims 1 to 56, for use in activating a cell expressing an IL receptor.

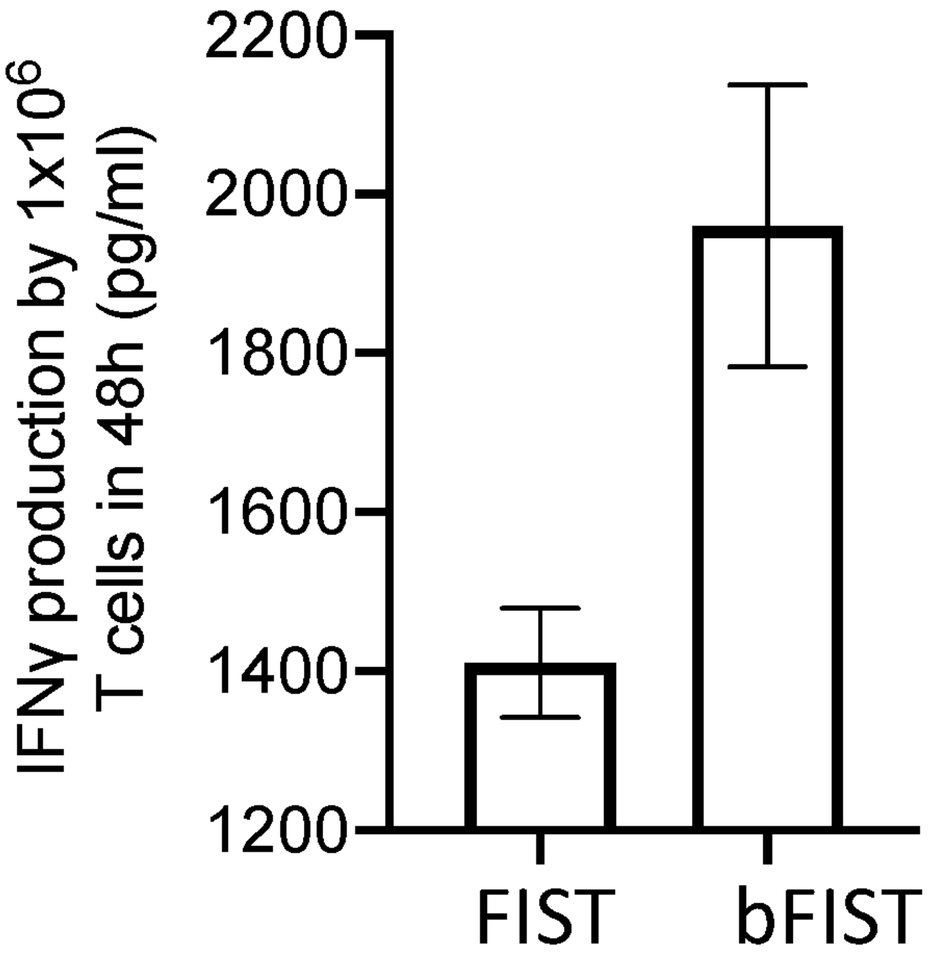



FIG. 1A

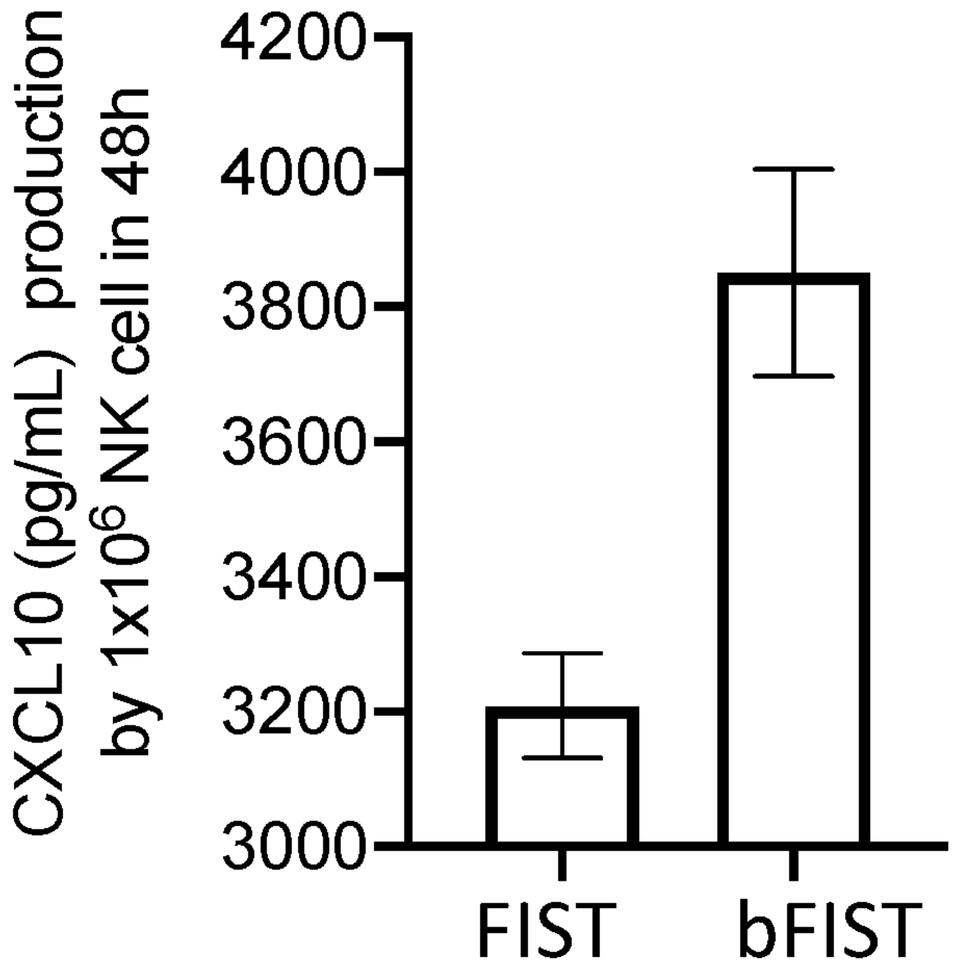



FIG. 1B

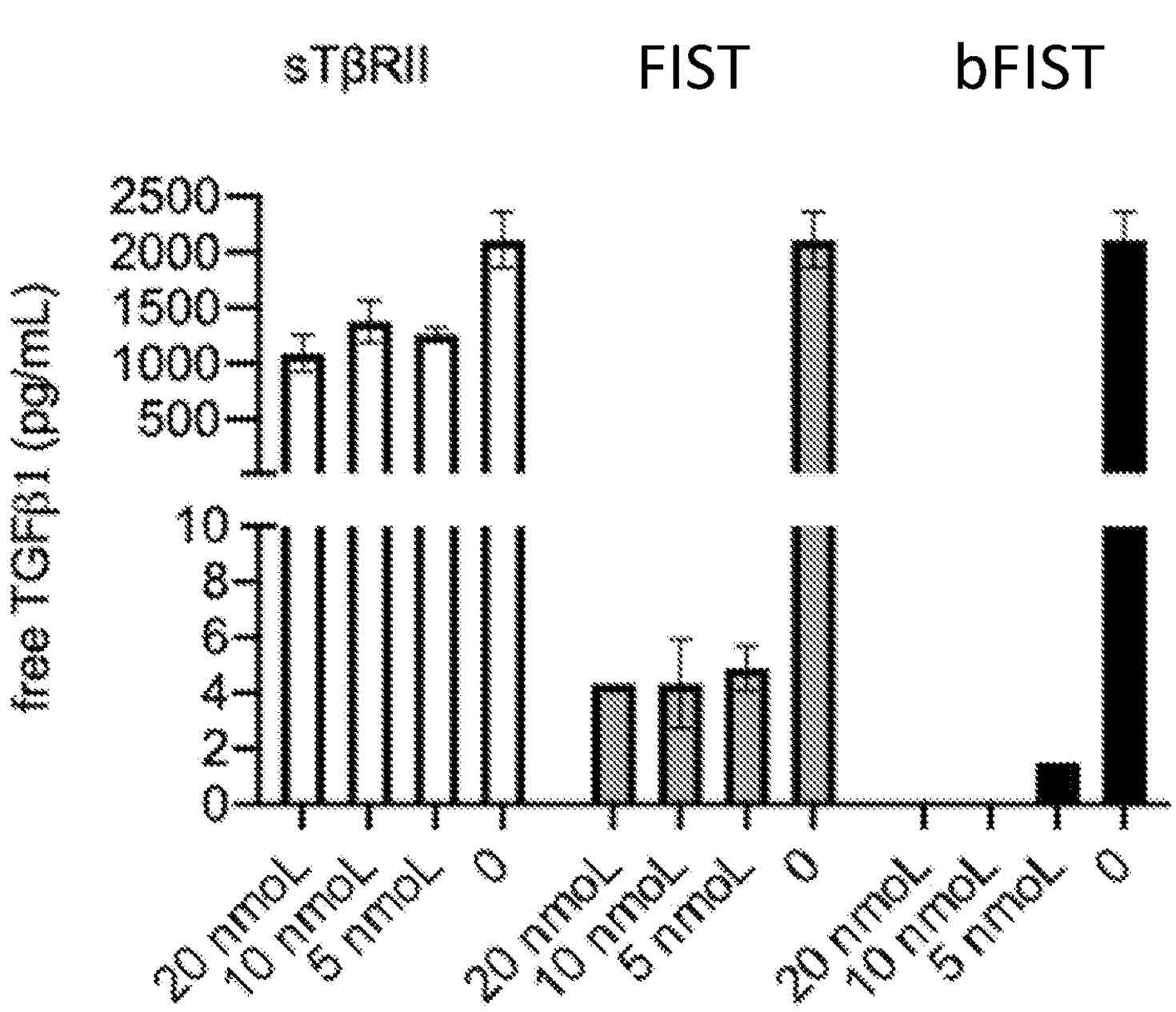



FIG. 2

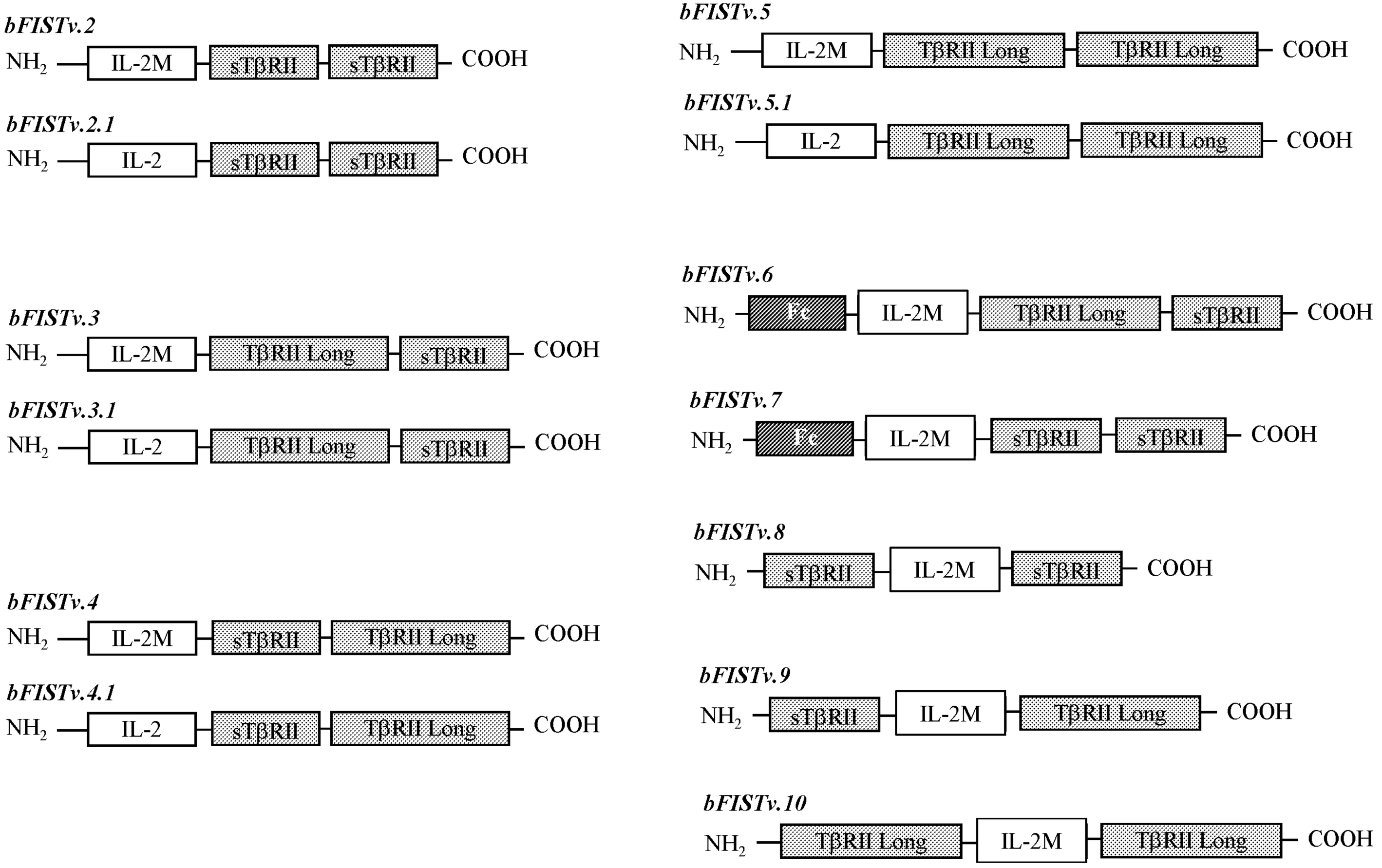

bFISTv.2
NH2
IL-2M
sTβRII
sTβRII
COOH
bFISTv.2.1
NH2
IL-2
sTβRII
sTβRII
COOH
bFISTv.3
NH2
IL-2M
TβRII Long
sTβRII
COOH
bFISTv.3.1
NH2
IL-2
TβRII Long
sTβRII
COOH
bFISTv.4
NH2
IL-2M
sTβRII
TβRII Long
COOH
bFISTv.4.1
NH2
IL-2
sTβRII
TβRII Long
COOH
bFISTv.5
NH2
IL-2M
TβRII Long
TβRII Long
COOH
bFISTv.5.1
NH2
IL-2
TβRII Long
TβRII Long
COOH
bFISTv.6
NH2
Fc
IL-2M
TβRII Long
sTβRII
COOH
bFISTv.7
NH2
Fc
IL-2M
sTβRII
sTβRII
COOH
bFISTv.8
NH2
sTβRII
IL-2M
sTβRII
COOH
bFISTv.9
NH2
sTβRII
IL-2M
TβRII Long
COOH
bFISTv.10
NH2
TβRII Long
IL-2M
TβRII Long
COOH

**FIG. 3**

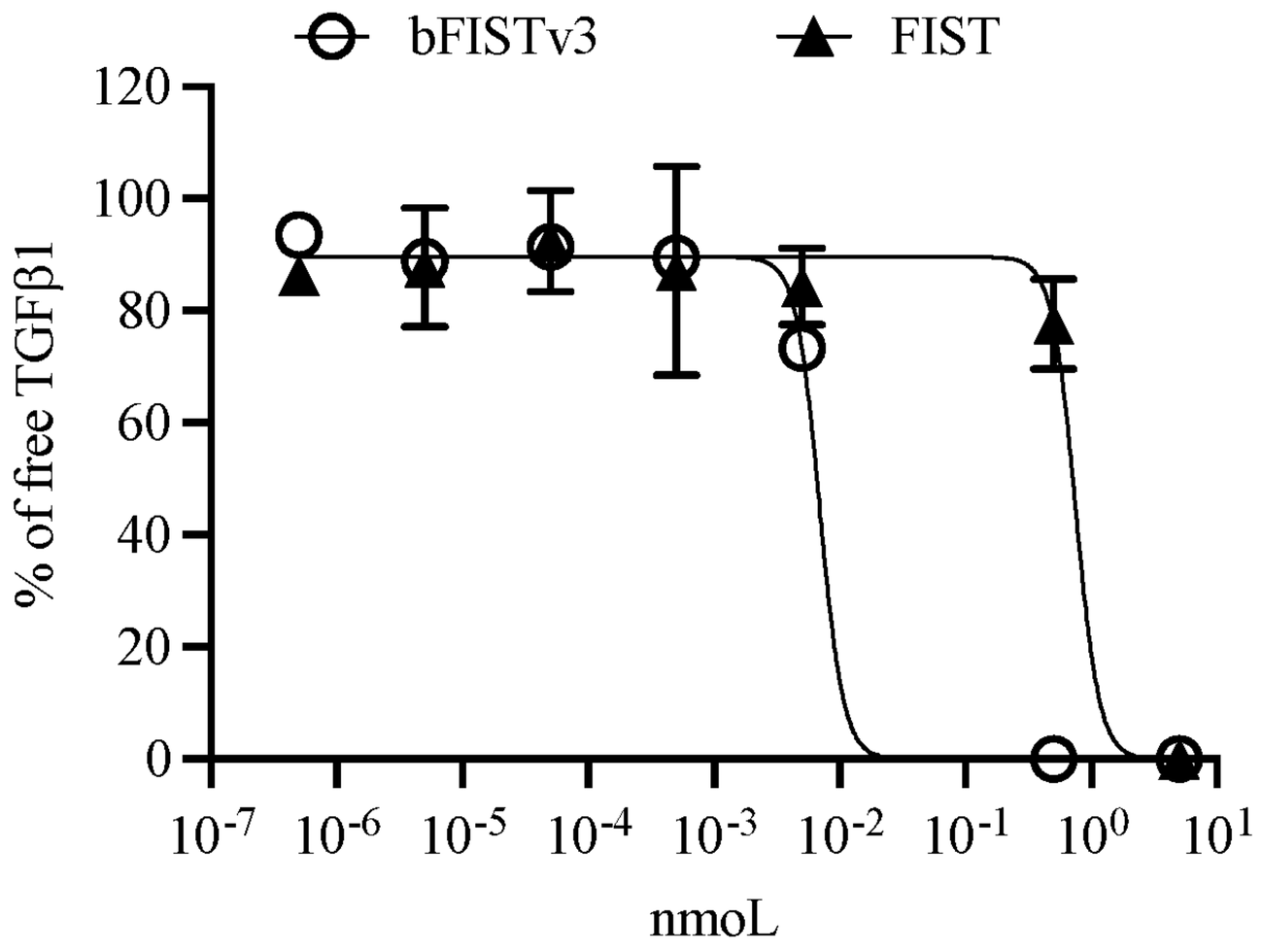

bFISTv3
FIST
120
100
80
60
40
20
0
% of free TGFβ1
$10^{-7}$ $10^{-6}$ $10^{-5}$ $10^{-4}$ $10^{-3}$ $10^{-2}$ $10^{-1}$ $10^{0}$ $10^{1}$
nmoL

FIG. 4A

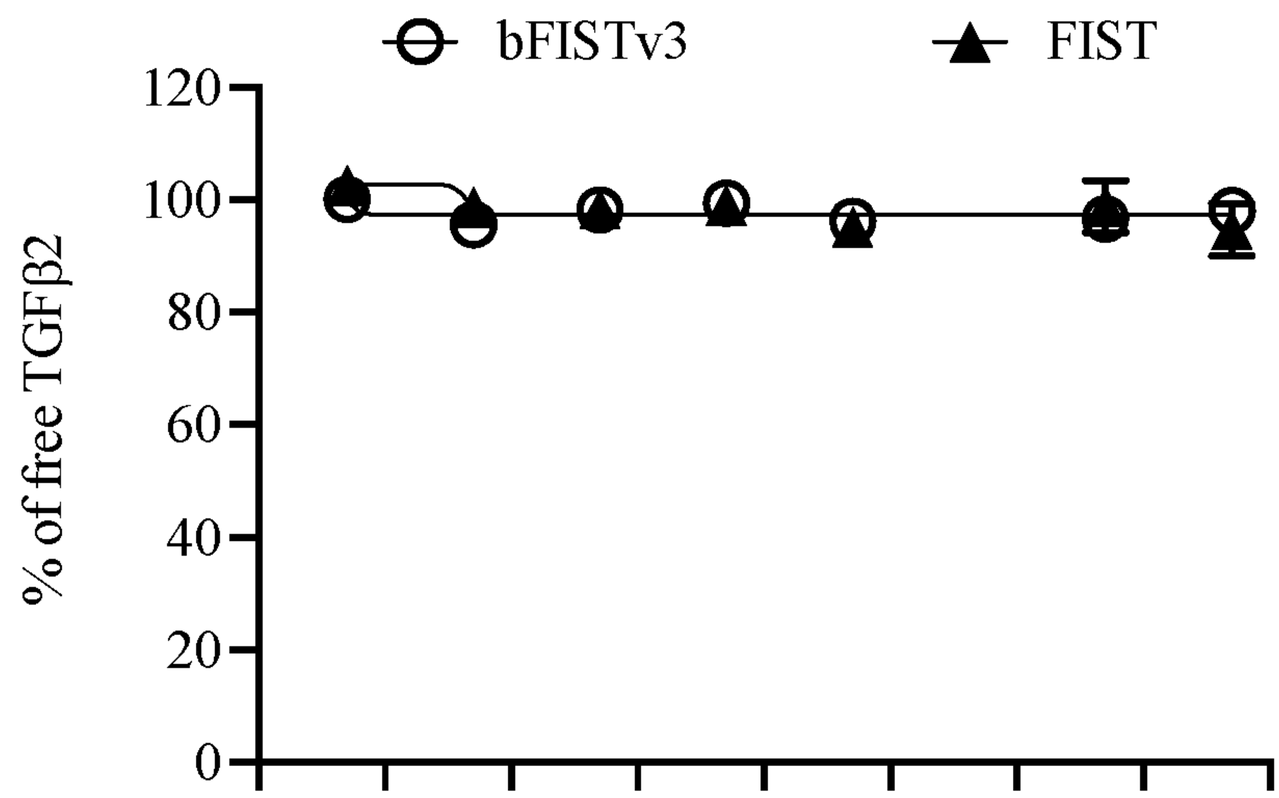

bFISTv3
FIST
120
100
80
60
40
20
0
% of free TGFβ2
$10^{-7}$ $10^{-6}$ $10^{-5}$ $10^{-4}$ $10^{-3}$ $10^{-2}$ $10^{-1}$ $10^{0}$ $10^{1}$
nmoL

FIG. 4B

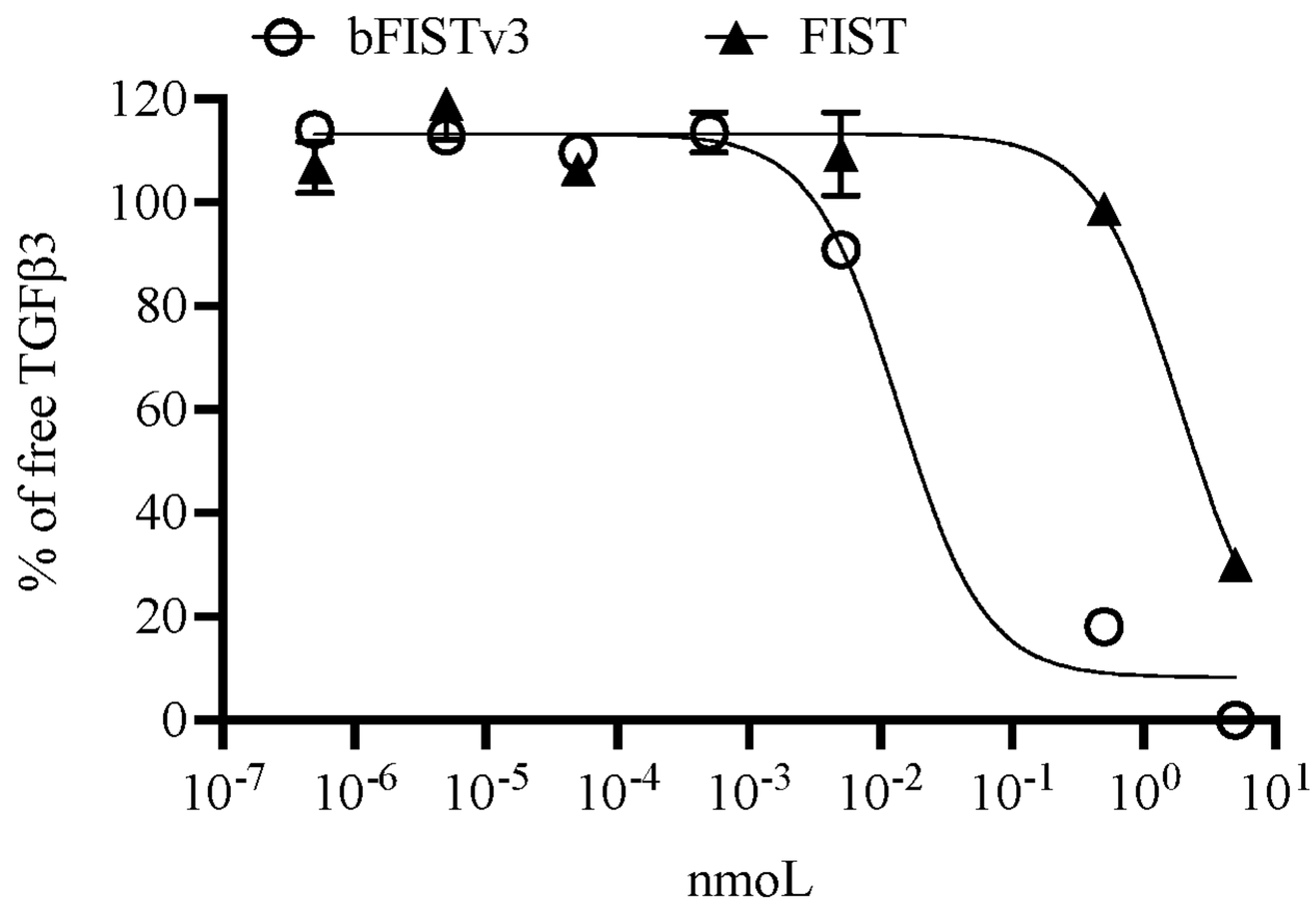

bFISTv3
FIST
120
100
80
60
40
20
0
% of free TGFβ3
$10^{-7}$ $10^{-6}$ $10^{-5}$ $10^{-4}$ $10^{-3}$ $10^{-2}$ $10^{-1}$ $10^{0}$ $10^{1}$
nmoL

**FIG. 4C**

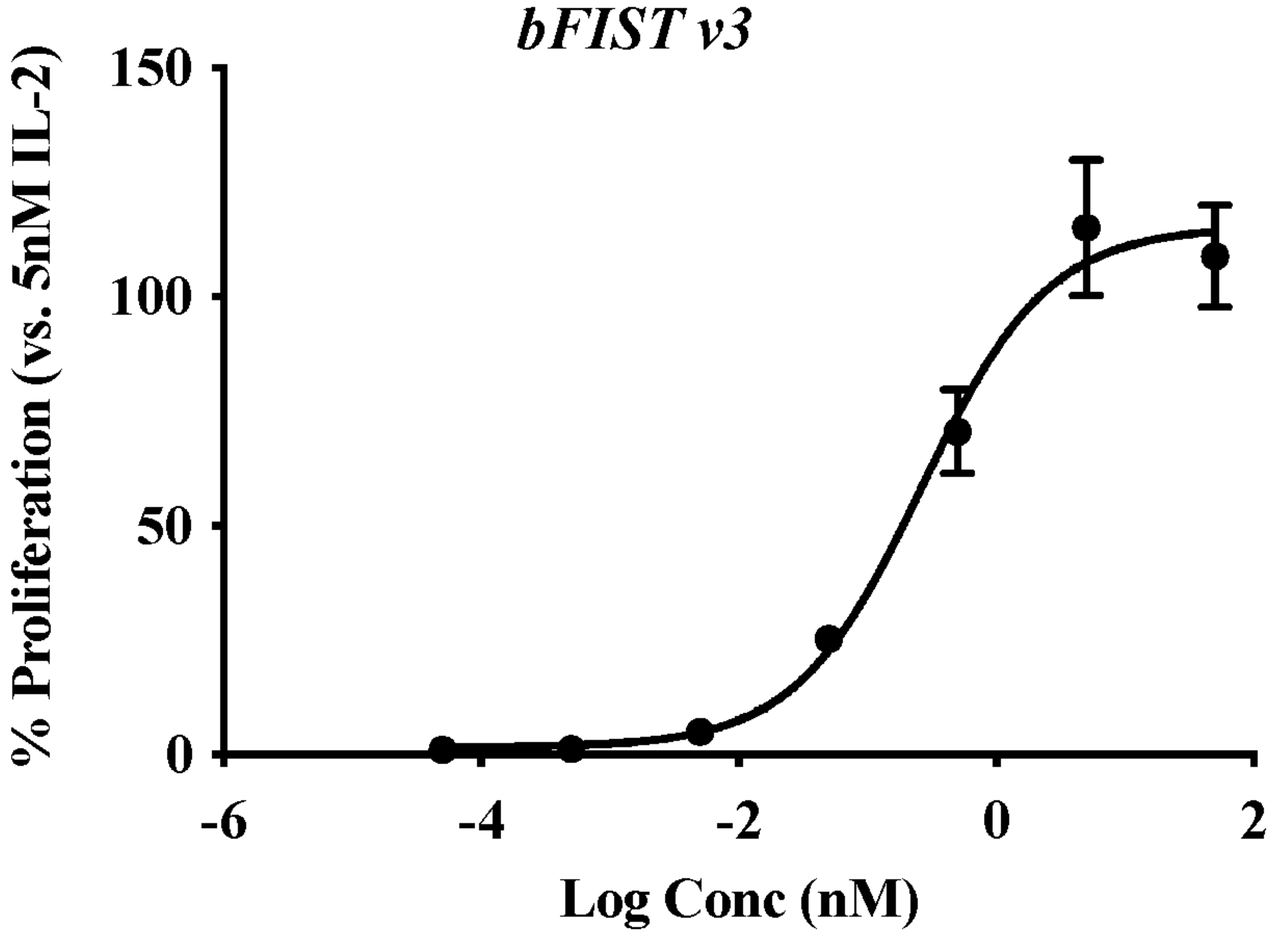

bFIST v3
% Proliferation (vs. 5nM IL-2)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5A

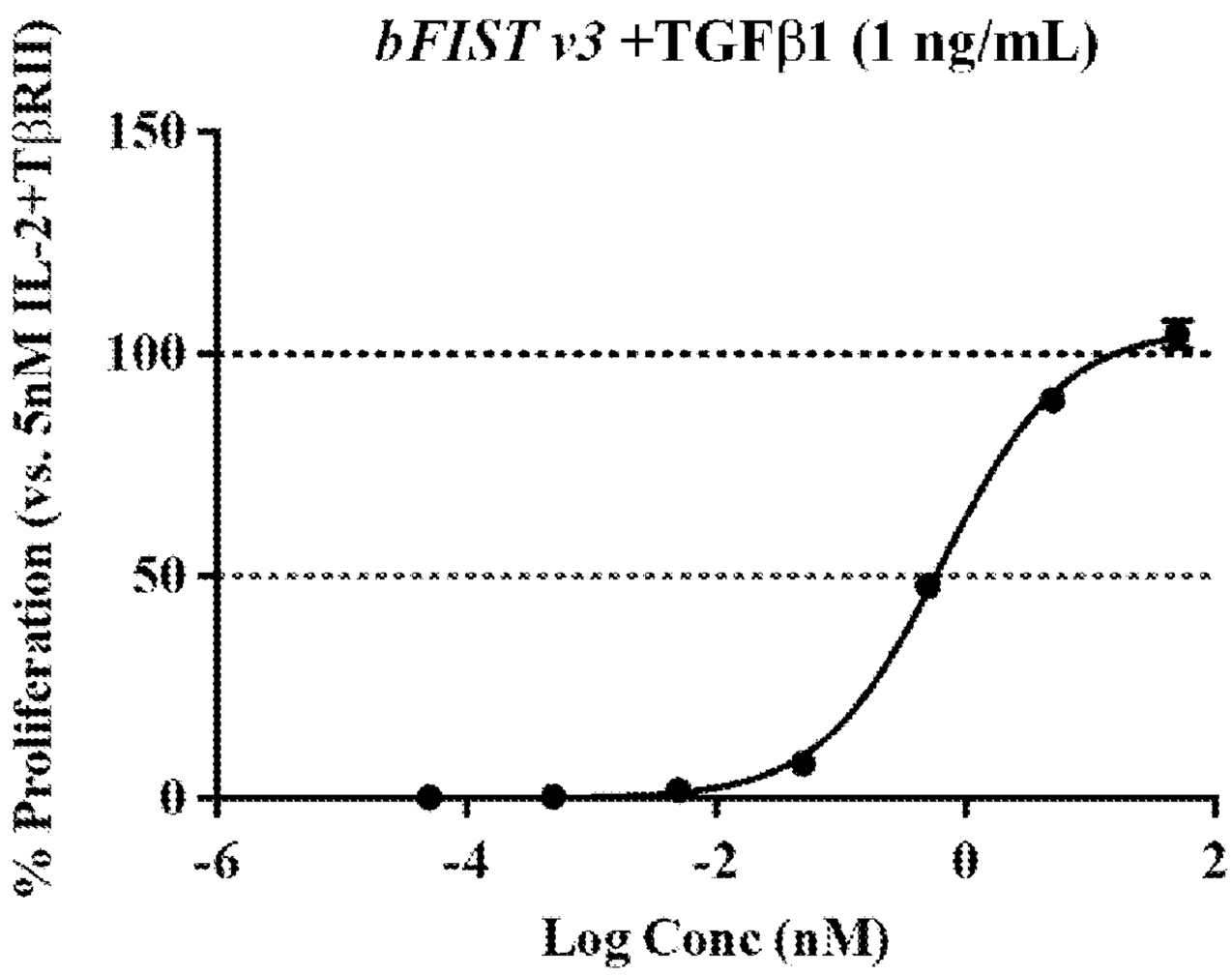

bFIST v3 +TGFβ1 (1 ng/mL)
% Proliferation (vs. 5nM IL-2+TβRII)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5B

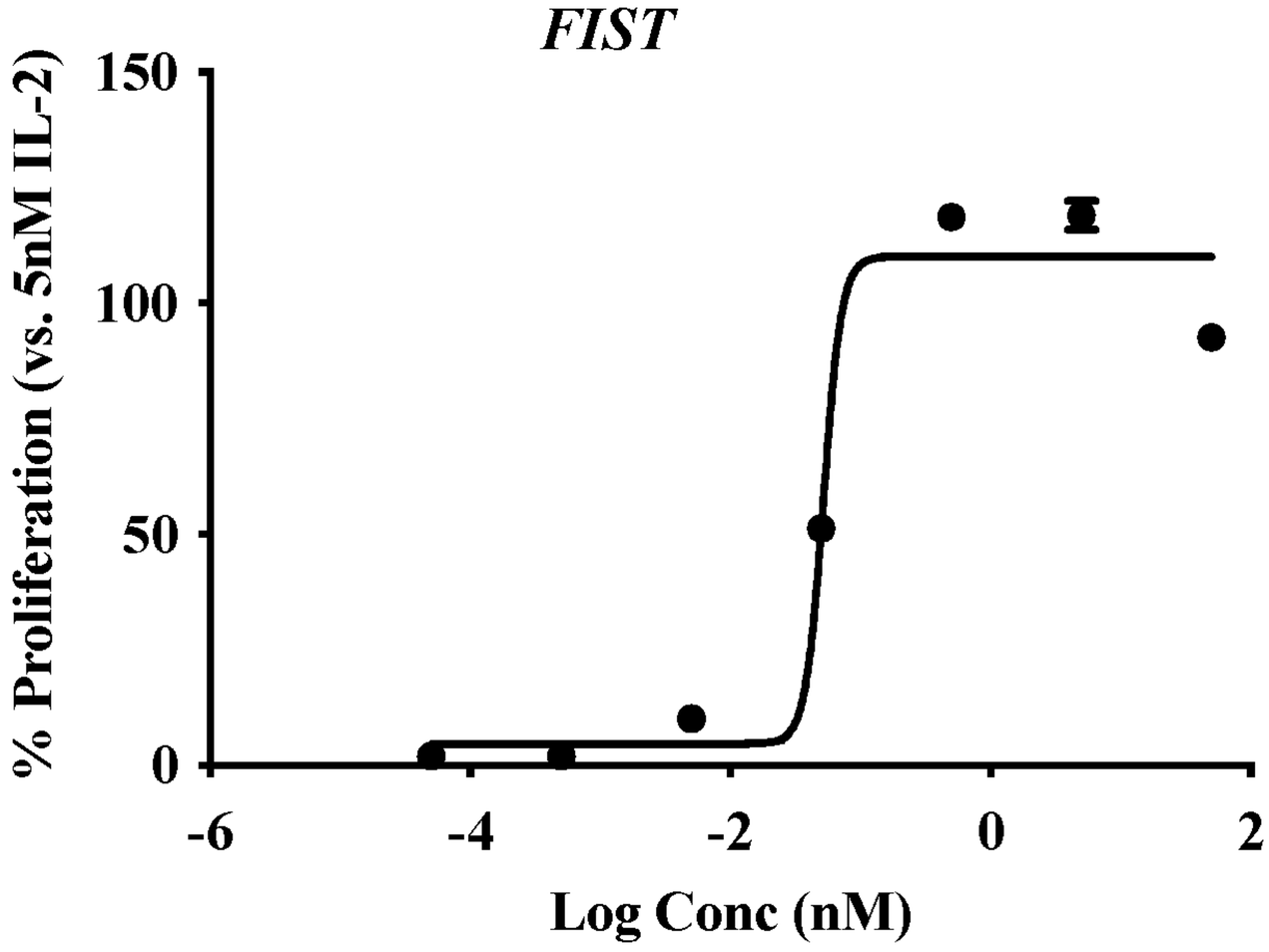

*FIST*
% Proliferation (vs. 5nM IL-2)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5C

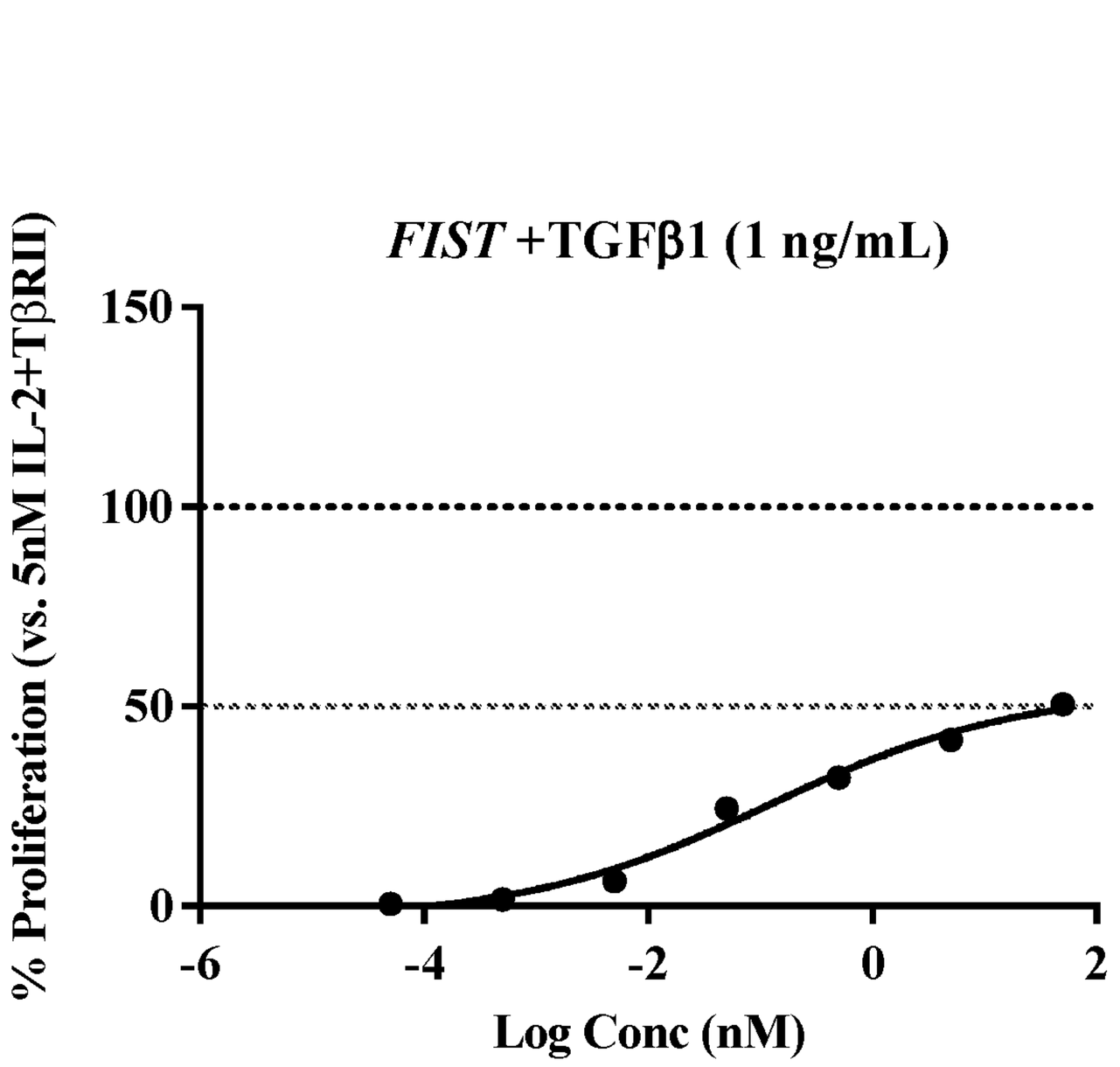

*FIST* +TGFβ1 (1 ng/mL)
% Proliferation (vs. 5nM IL-2+TβRII)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5D

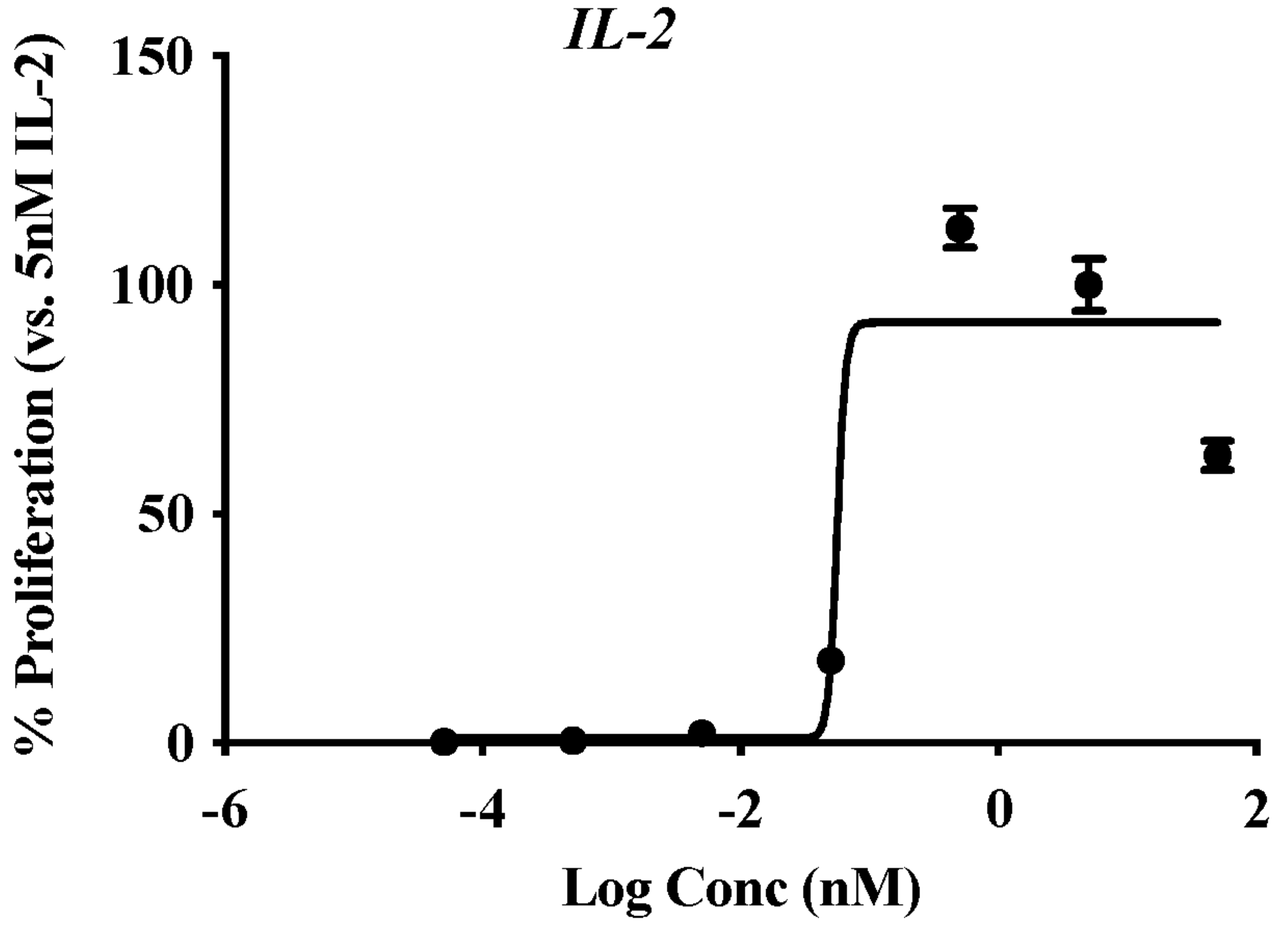

IL-2
% Proliferation (vs. 5nM IL-2)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5E

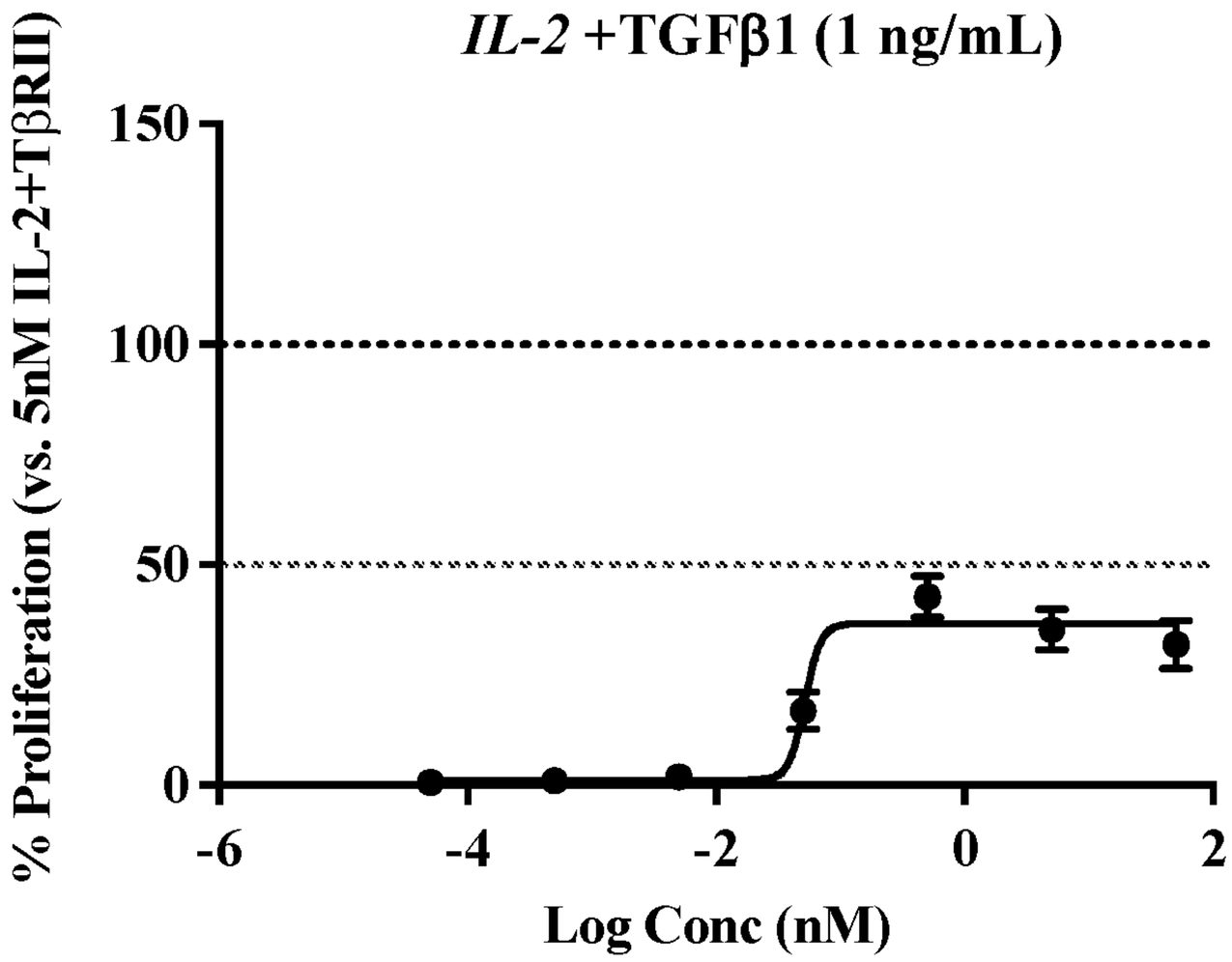

IL-2 +TGFβ1 (1 ng/mL)
% Proliferation (vs. 5nM IL-2+TβRII)
150
100
50
0
-6
-4
-2
0
2
Log Conc (nM)

FIG. 5F

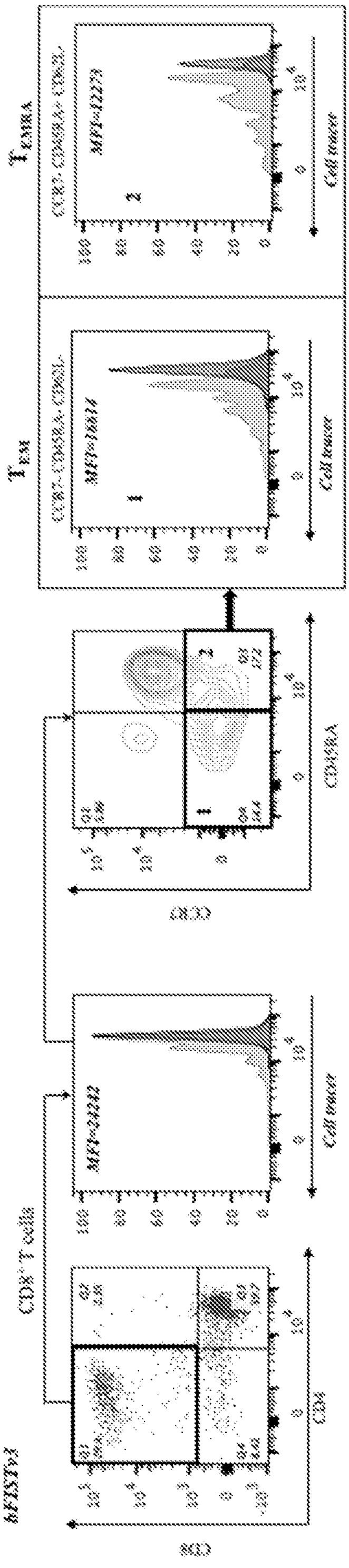

$T_{EMRA}$
CCR7+CD45RA+ CD62L+
MFI=1229
Cell count
$T_{EM}$
CCR7-CD45RA- CD62L-
MFI=1014
Cell count
CD45RA
CCR7
$T_{CM}$
CCR7+CD45RA- CD62L+
MFI=24242
Cell count
CD8+ T cells
APTSTAT1
CD8

FIG. 6A

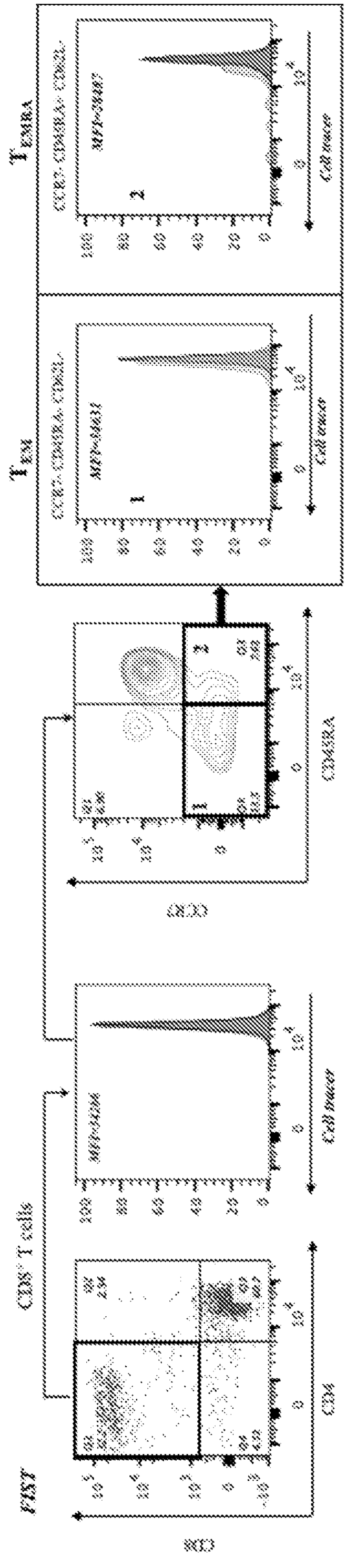

$T_{EMRA}$
CCR7+CD45RA+ CD62L+
MFI=3887
Cell count
$T_{EM}$
CCR7-CD45RA- CD62L-
MFI=8944
Cell count
CD45RA
CCR7
$T_{CM}$
CCR7+CD45RA- CD62L+
MFI=6434
Cell count
CD8+ T cells
APTSTAT1
CD8

FIG. 6B

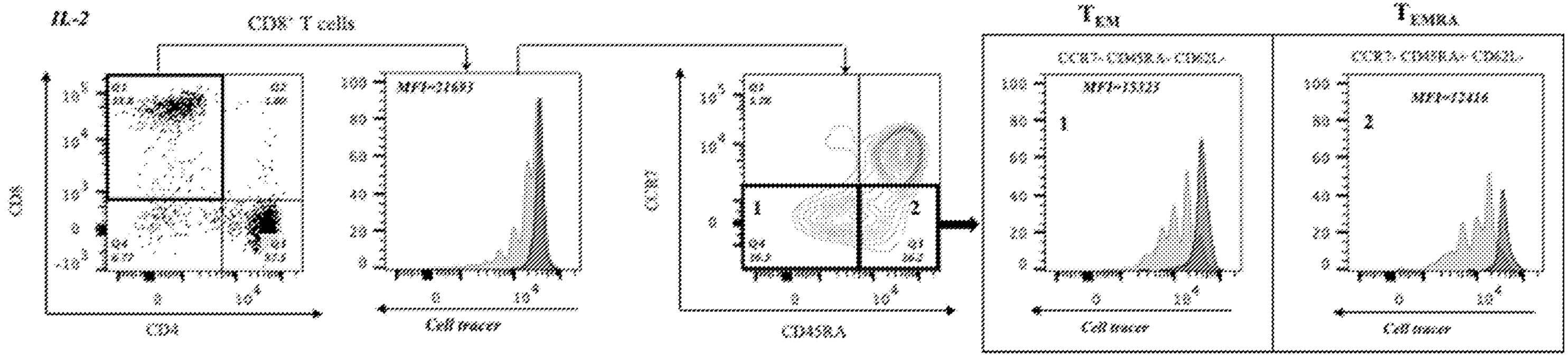



FIG. 6C

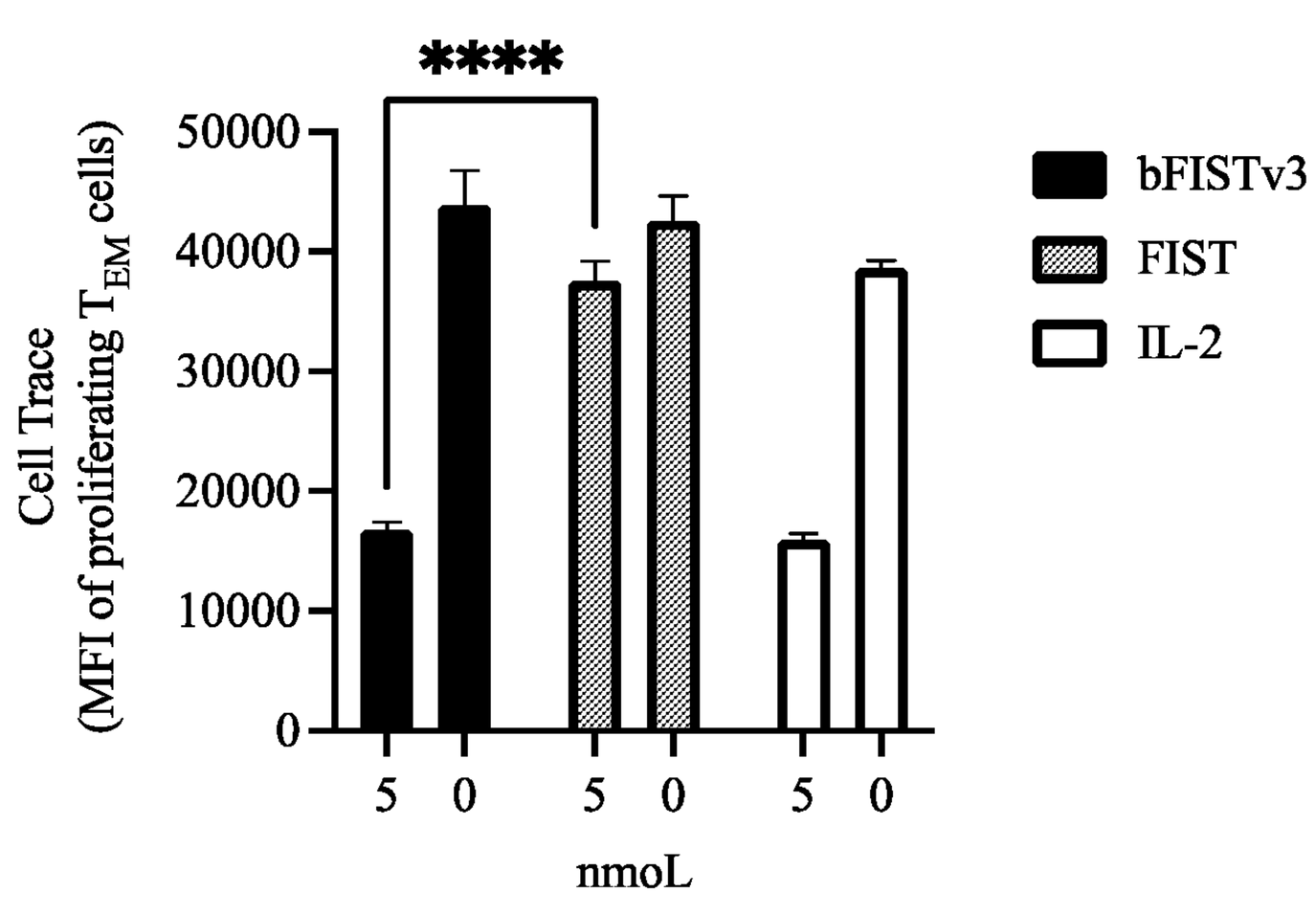



FIG. 6D

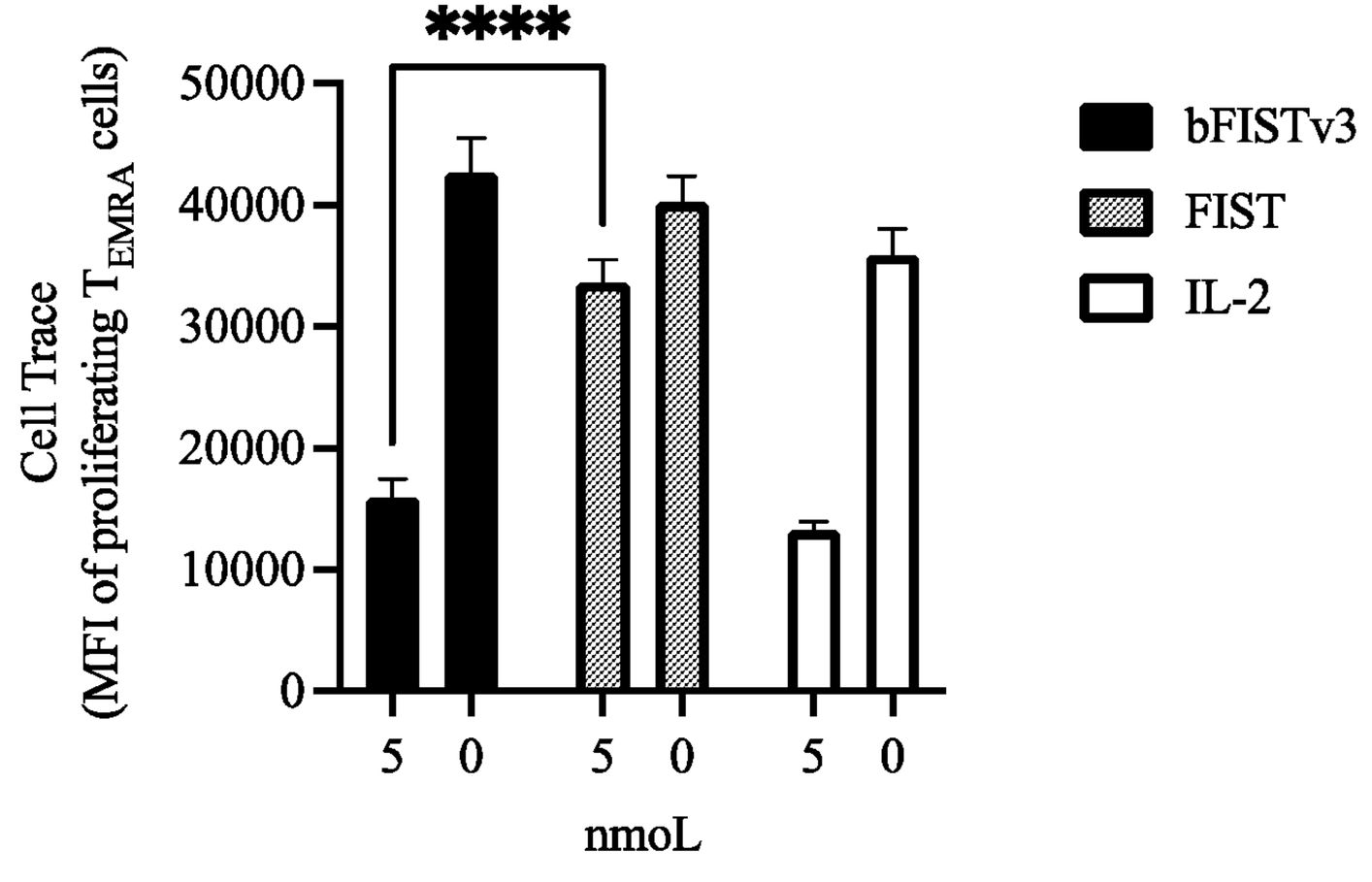



FIG. 6E

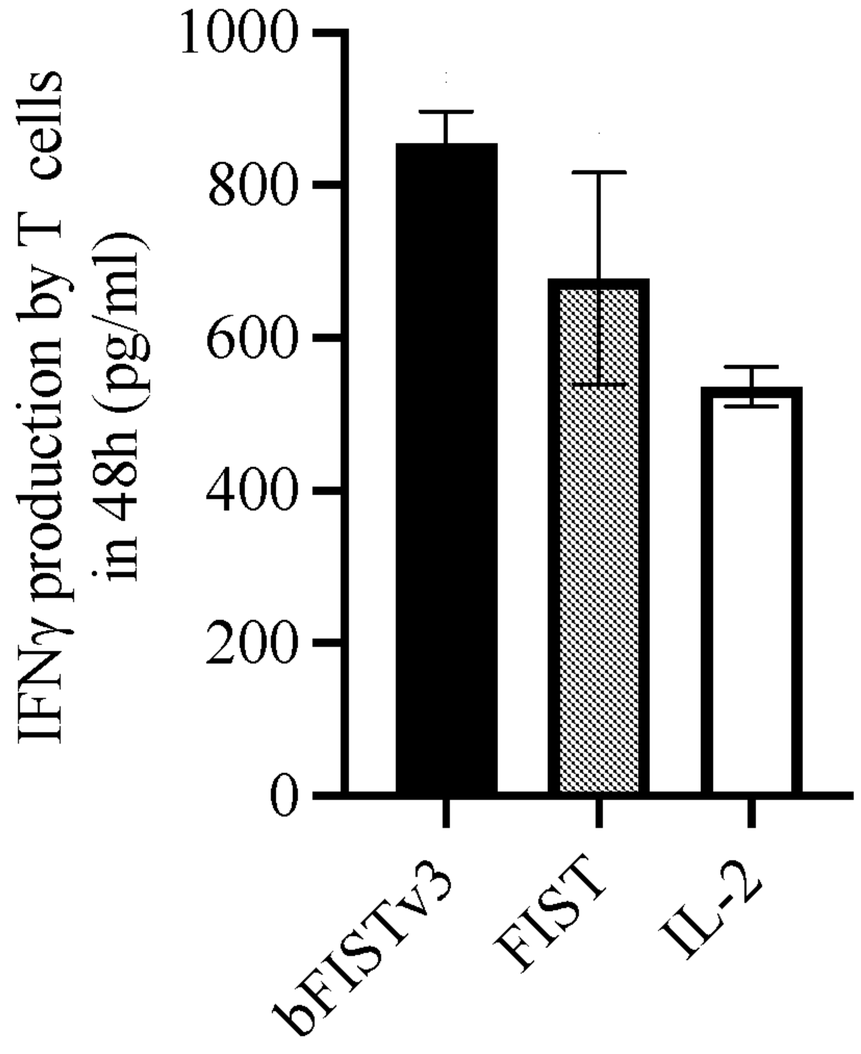

IFNγ production by T cells
in 48h (pg/ml)
1000
800
600
400
200
0
bFISTv3
FIST
IL-2

FIG. 6F

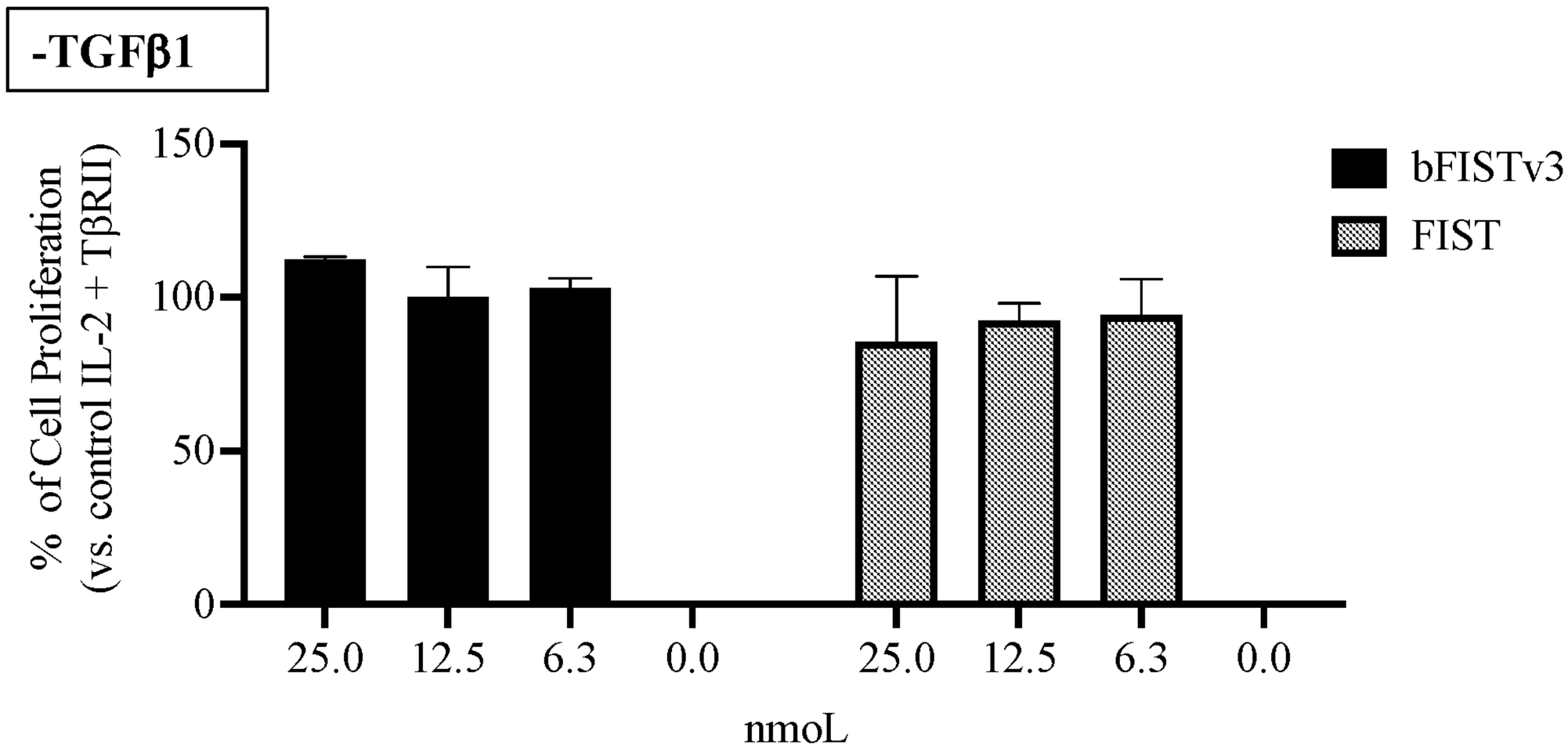

-TGFβ1
% of Cell Proliferation
(vs. control IL-2 + TβRII)
150
100
50
0
25.0
12.5
6.3
0.0
25.0
12.5
6.3
0.0
nmoL
bFISTv3
FIST

FIG. 7A

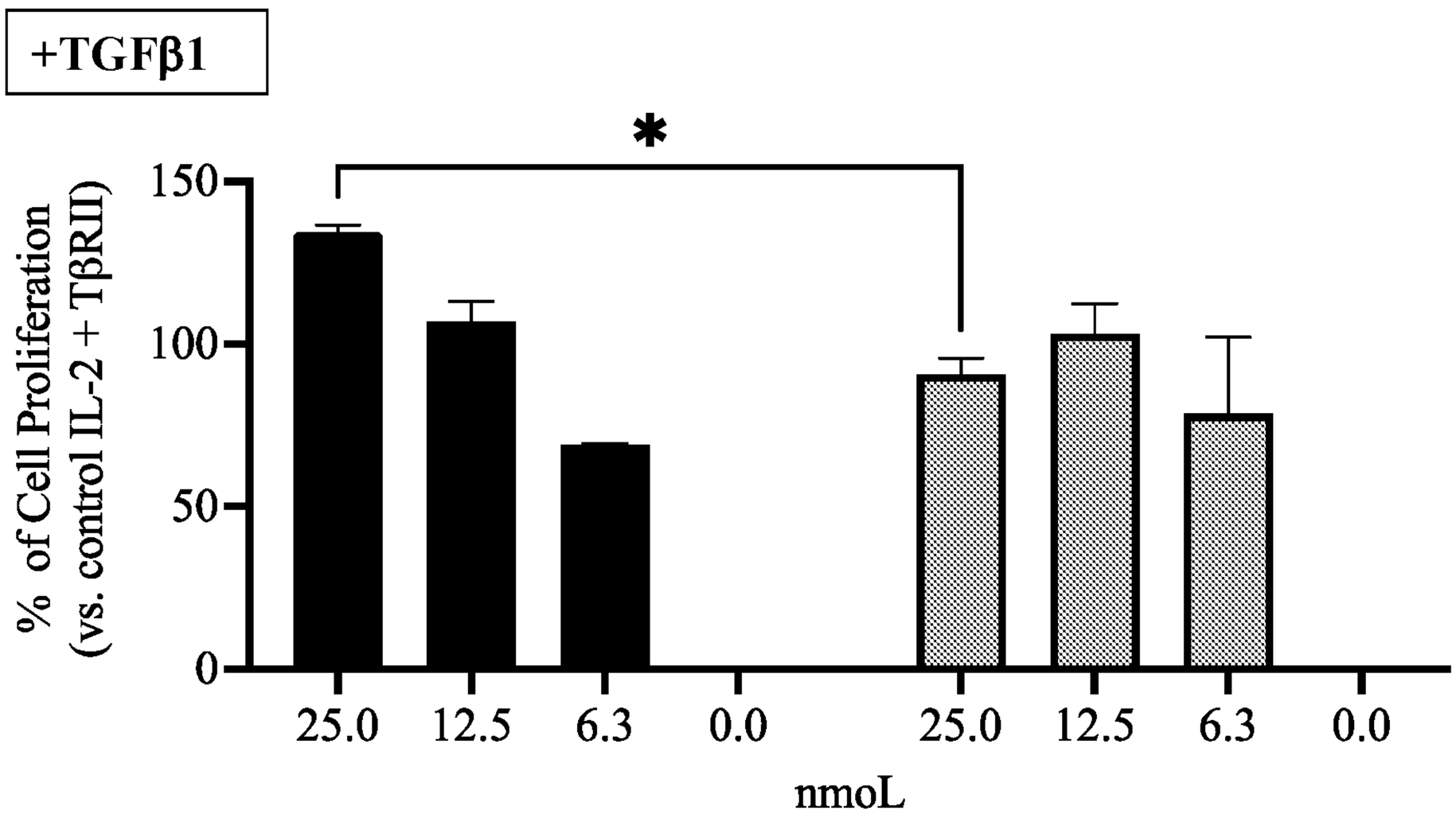

+TGFβ1
*
% of Cell Proliferation
(vs. control IL-2 + TβRII)
150
100
50
0
25.0
12.5
6.3
0.0
25.0
12.5
6.3
0.0
nmoL

FIG. 7B

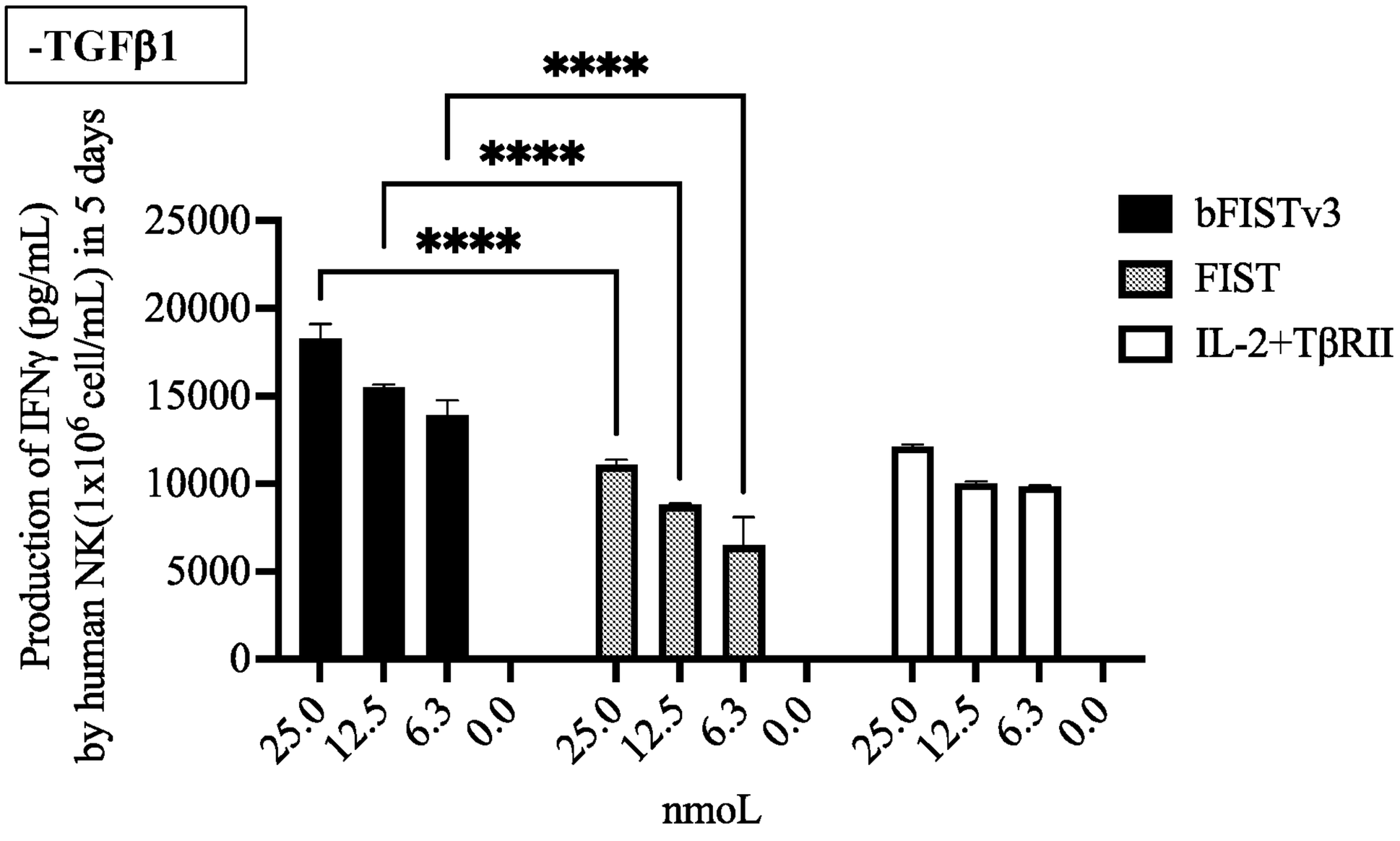

-TGFβ1
Production of IFNγ (pg/mL) by human NK(1x10^6 cell/mL) in 5 days
25000
20000
15000
10000
5000
0
****
****
****
****
25.0
12.5
6.3
0.0
nmoL
bFISTv3
FIST
IL-2+TβRII

FIG. 7C

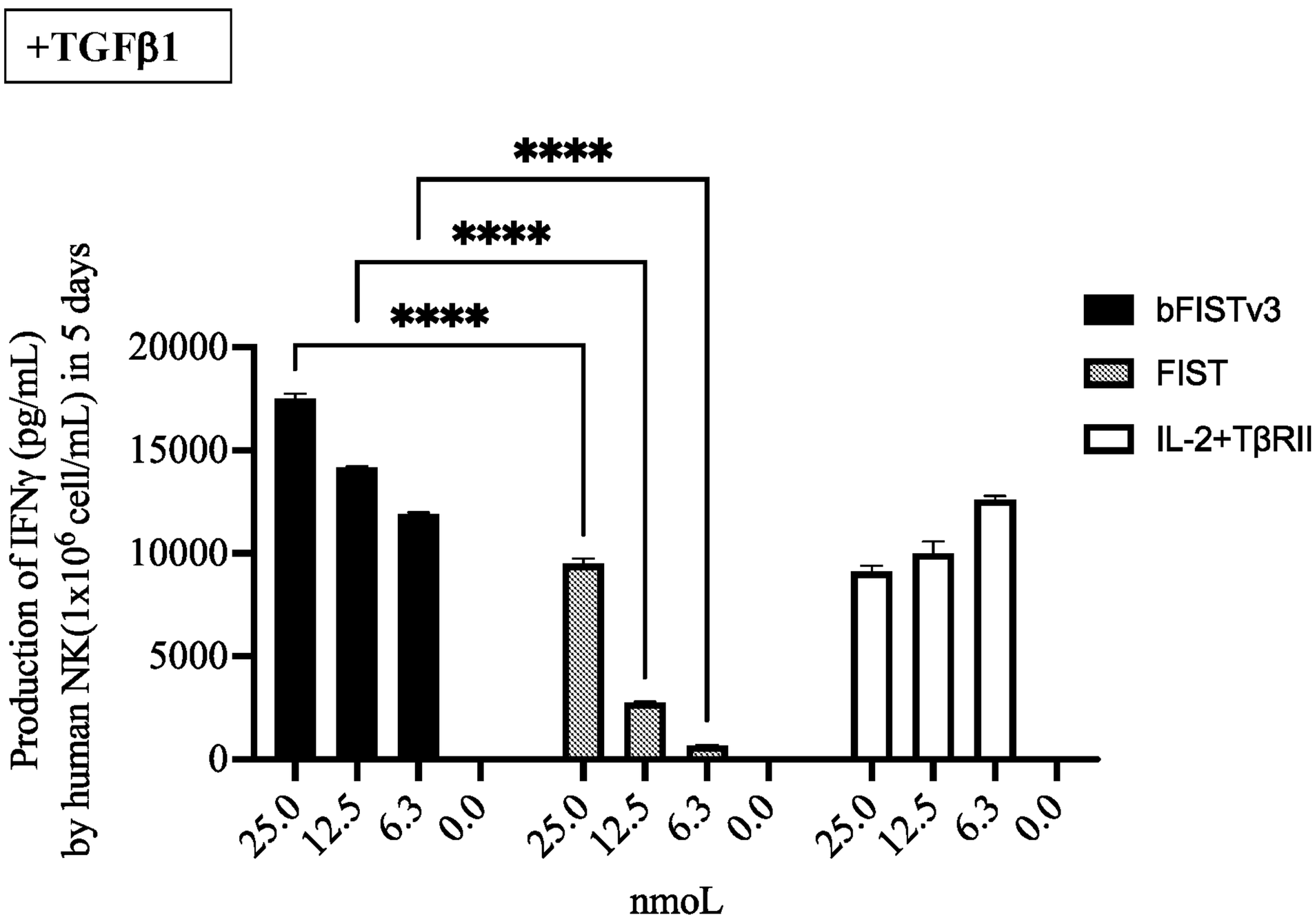

+TGFβ1
Production of IFNγ (pg/mL) by human NK(1x10^6 cell/mL) in 5 days
20000
15000
10000
5000
0
****
****
****
****
25.0
12.5
6.3
0.0
nmoL
bFISTv3
FIST
IL-2+TβRII

FIG. 7D

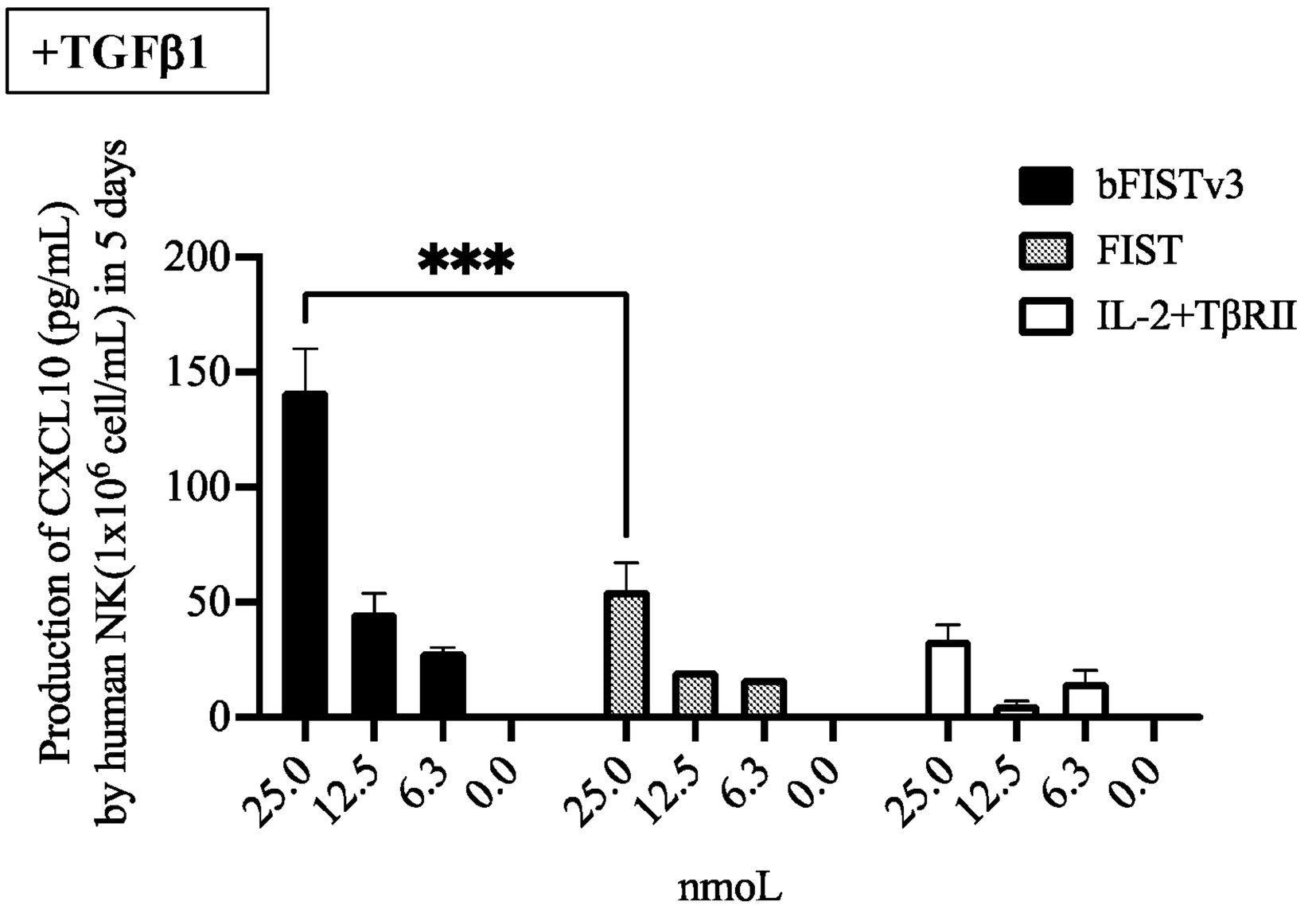

+TGFβ1
Production of CXCL10 (pg/mL) by human NK(1x10^6 cell/mL) in 5 days
200
150
100
50
0
***
25.0
12.5
6.3
0.0
nmoL
bFISTv3
FIST
IL-2+TβRII

FIG. 7E

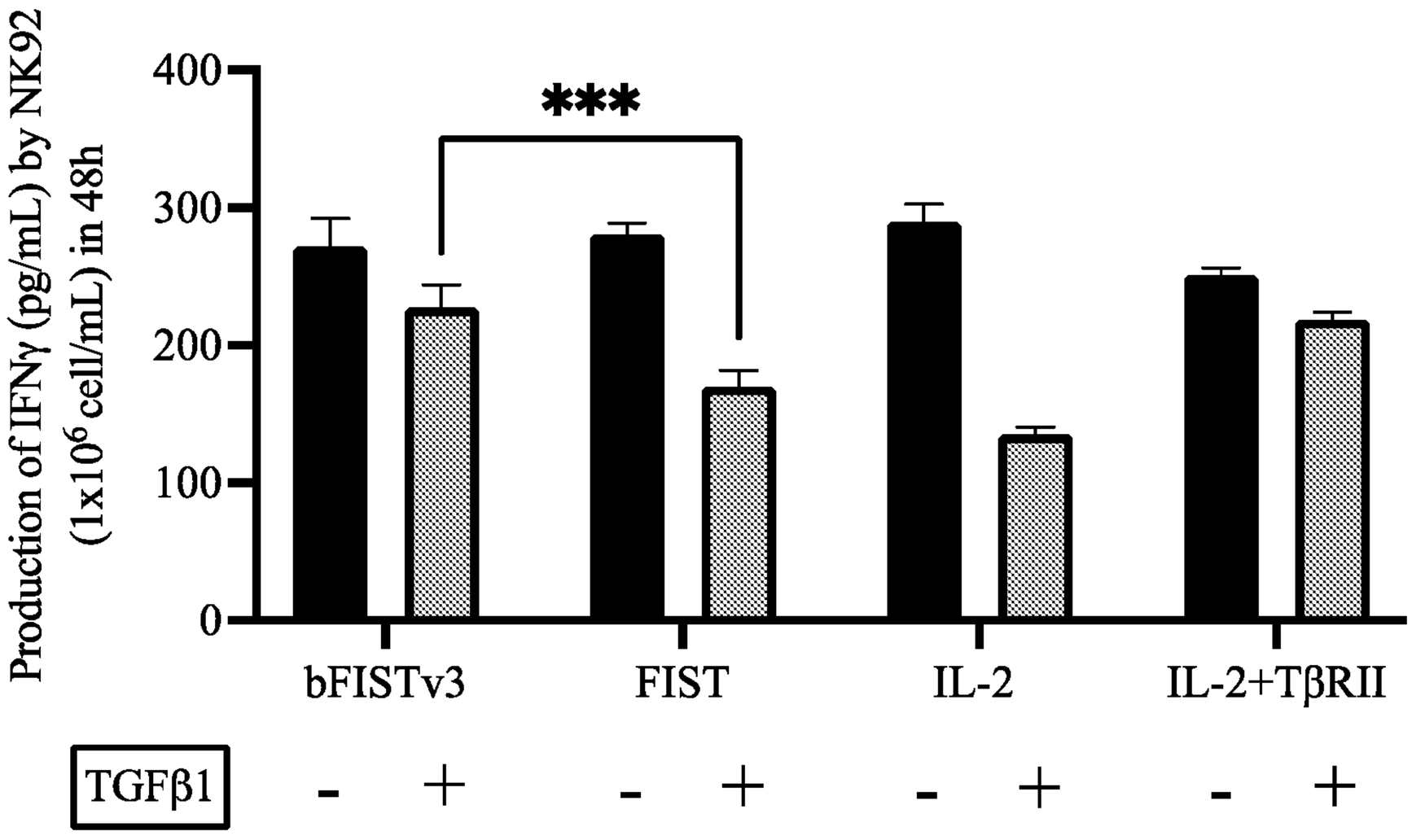

Production of IFNγ (pg/mL) by NK92 (1x10^6 cell/mL) in 48h
400
300
200
100
0
***
bFISTv3
FIST
IL-2
IL-2+TβRII
TGFβ1
-
+

FIG. 8A

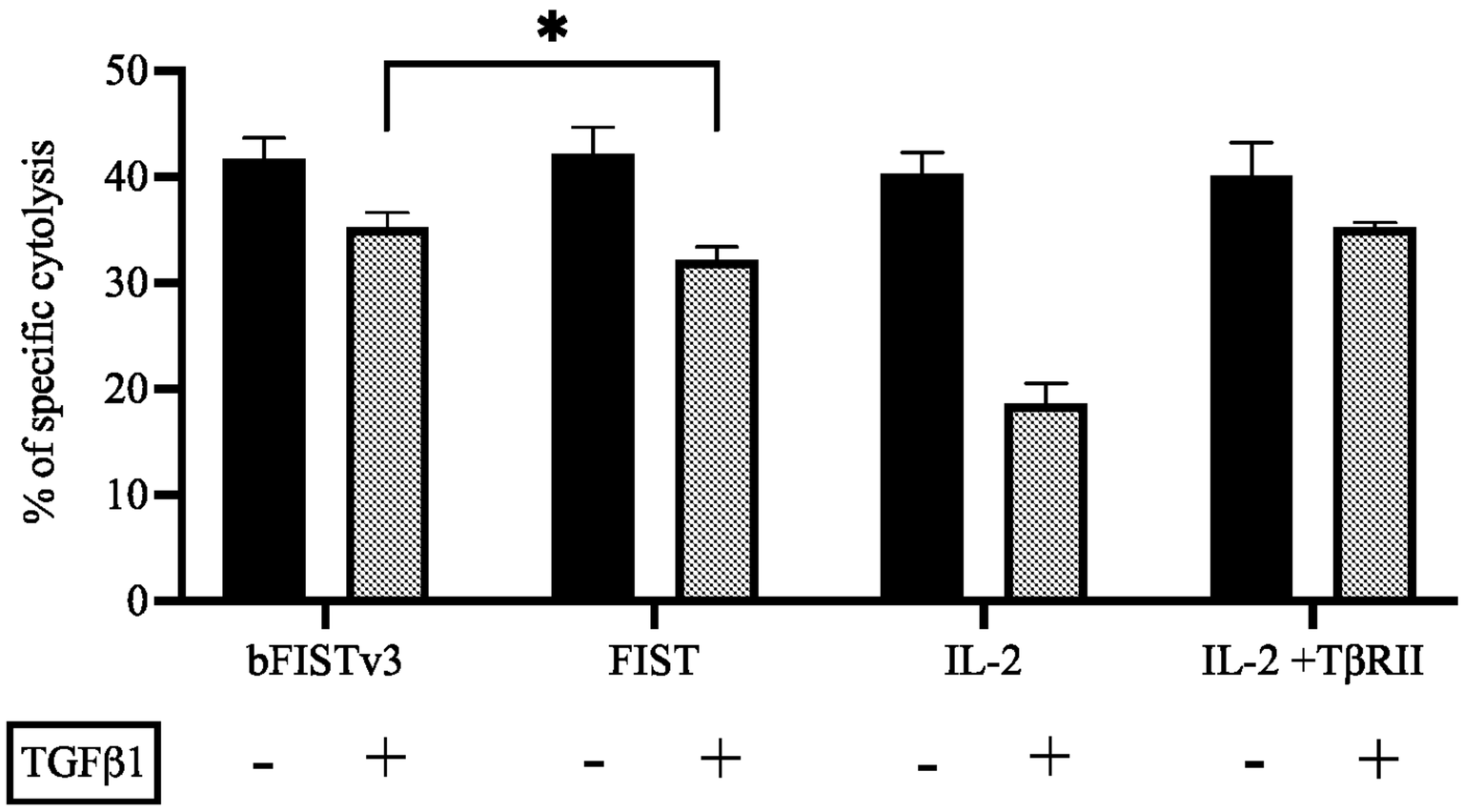

*
50
40
30
20
10
0
% of specific cytolysis
bFISTv3
FIST
IL-2
IL-2 +TβRII
TGFβ1
-
+
-
+
-
+
-
+

FIG. 8B

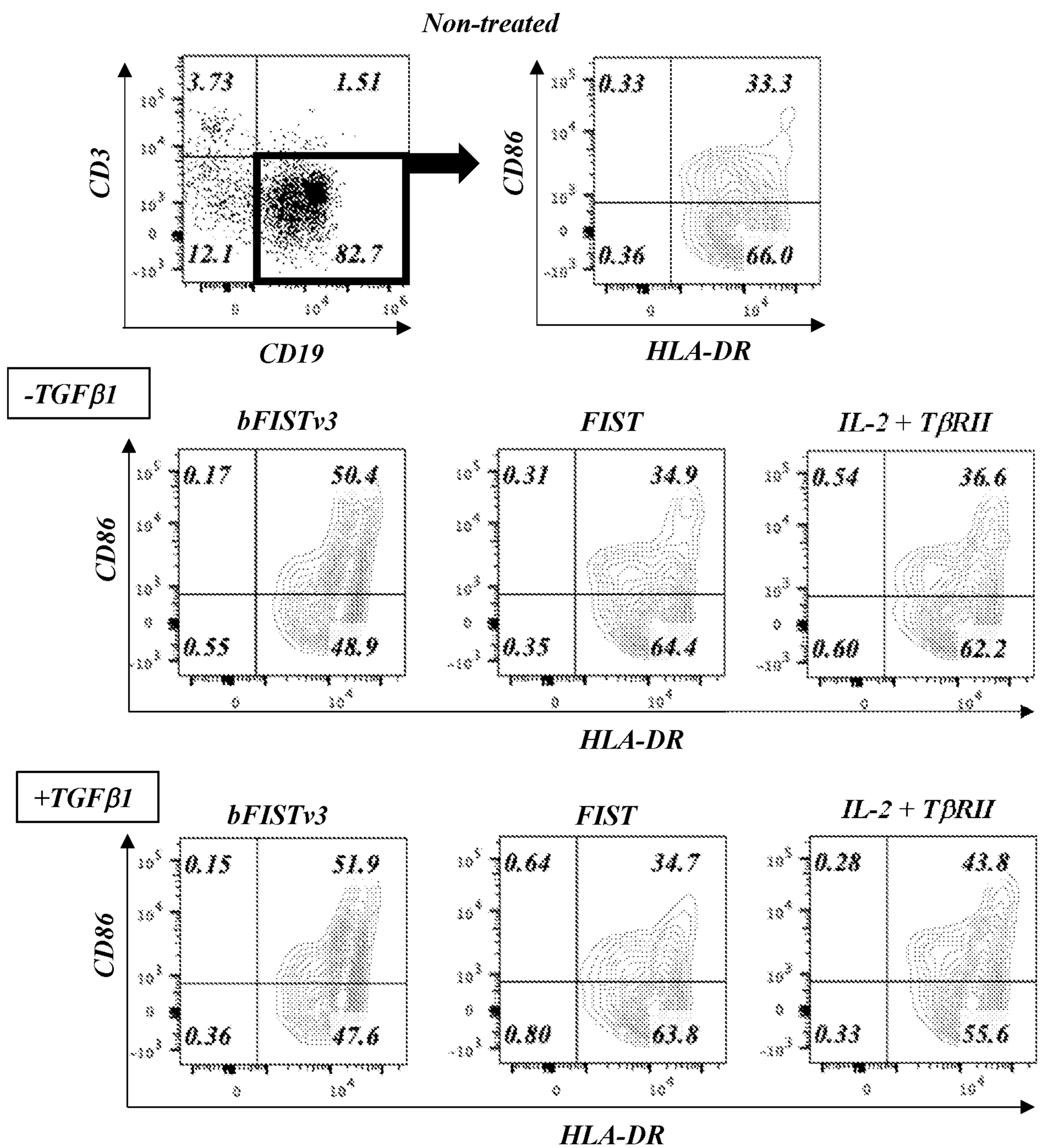

Non-treated
3.73
1.51
12.1
82.7
CD3
CD19
0.33
33.3
0.36
66.0
CD86
HLA-DR
-TGFβ1
bFISTv3
FIST
IL-2 + TβRII
0.17
50.4
0.55
48.9
0.31
34.9
0.35
64.4
0.54
36.6
0.60
62.2
CD86
HLA-DR
+TGFβ1
bFISTv3
FIST
IL-2 + TβRII
0.15
51.9
0.36
47.6
0.64
34.7
0.80
63.8
0.28
43.8
0.33
55.6
CD86
HLA-DR

FIG. 9A

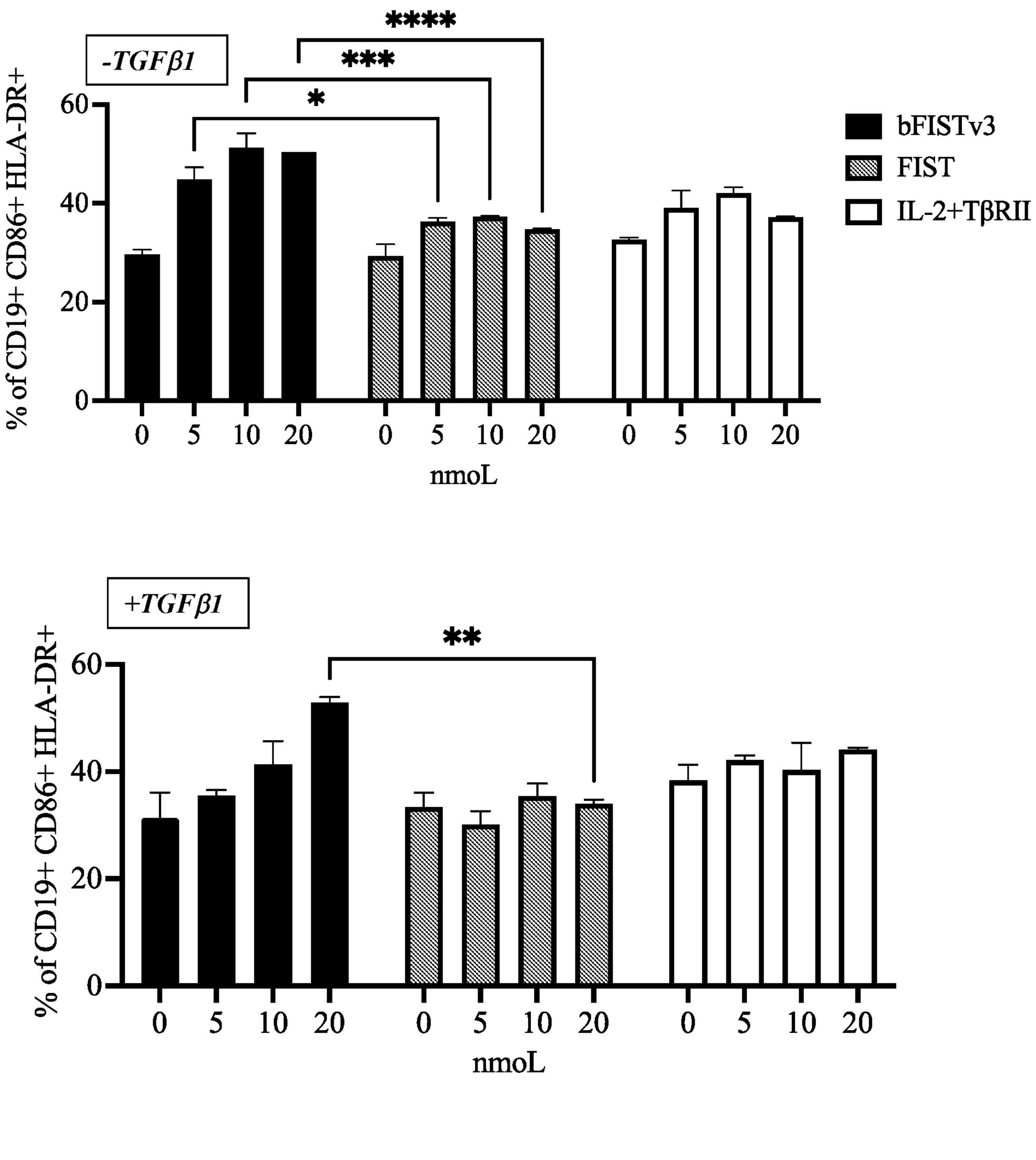

-TGFβ1
+TGFβ1
% of CD19+ CD86+ HLA-DR+
60
40
20
0
****
***
*
**
0 5 10 20 0 5 10 20 0 5 10 20
nmoL
bFISTv3
FIST
IL-2+TβRII

FIG. 9B

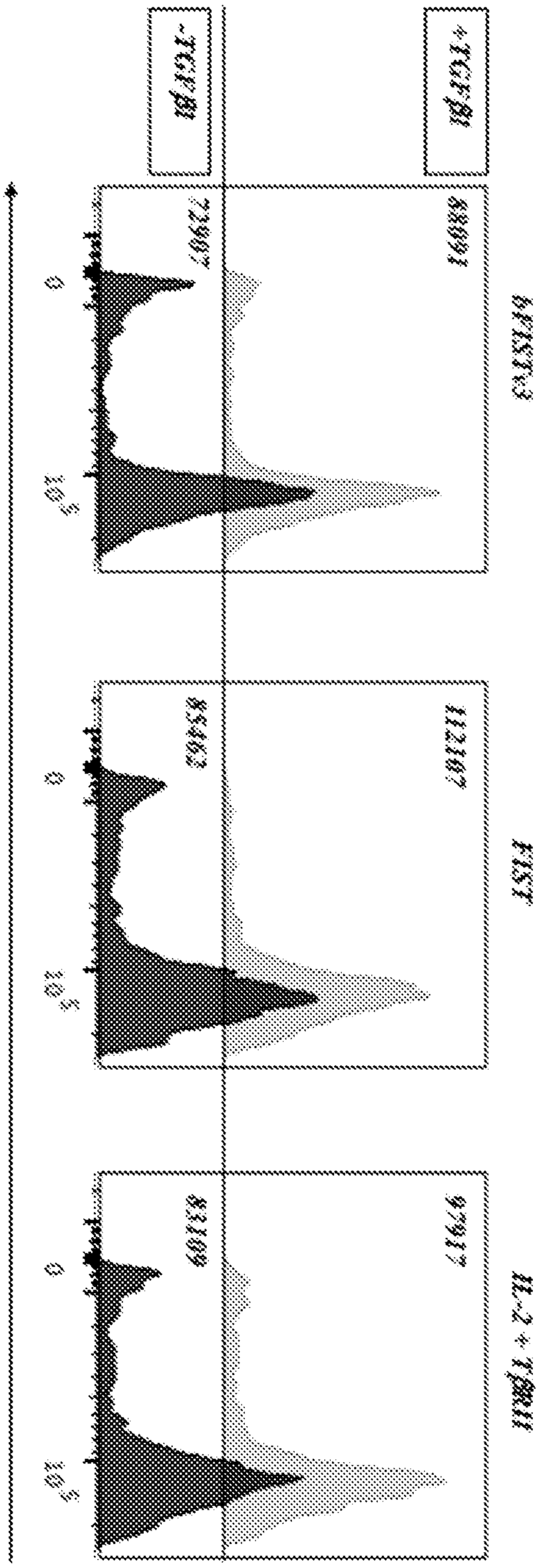

IL-2 + IgM
IL-2 + IgM1
IL-2 + IgM2
CFSE
FAST
FAST
FAST
0
10^5
97917
81189
112107
85463
72987
68891

FIG. 9C

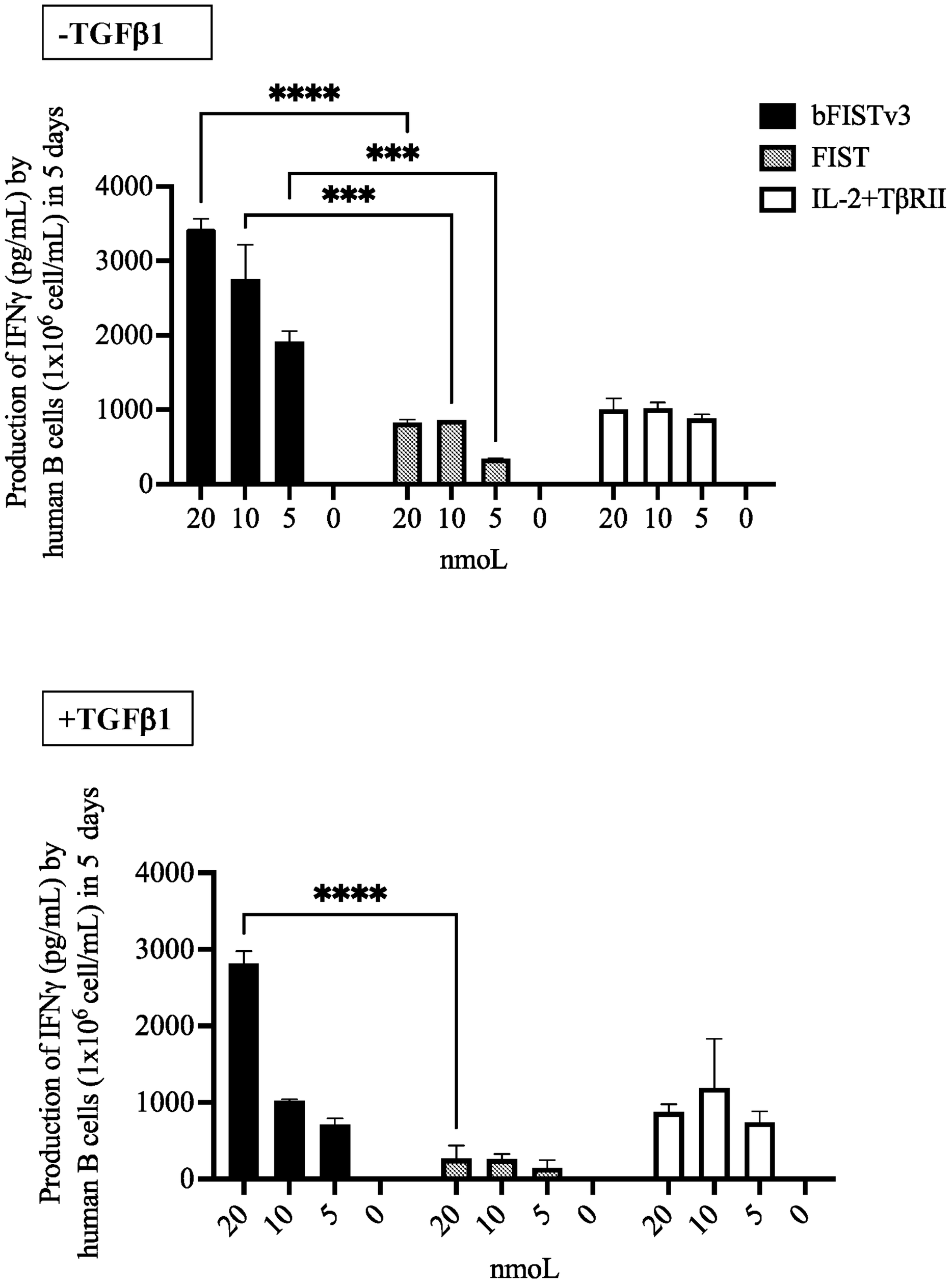

-TGFβ1
Production of IFNγ (pg/mL) by human B cells (1x10^6 cell/mL) in 5 days
4000
3000
2000
1000
0
****
***
***
20 10 5 0 20 10 5 0 20 10 5 0
nmoL
bFISTv3
FIST
IL-2+TβRII
+TGFβ1
Production of IFNγ (pg/mL) by human B cells (1x10^6 cell/mL) in 5 days
4000
3000
2000
1000
0
****
20 10 5 0 20 10 5 0 20 10 5 0
nmoL

FIG. 9D

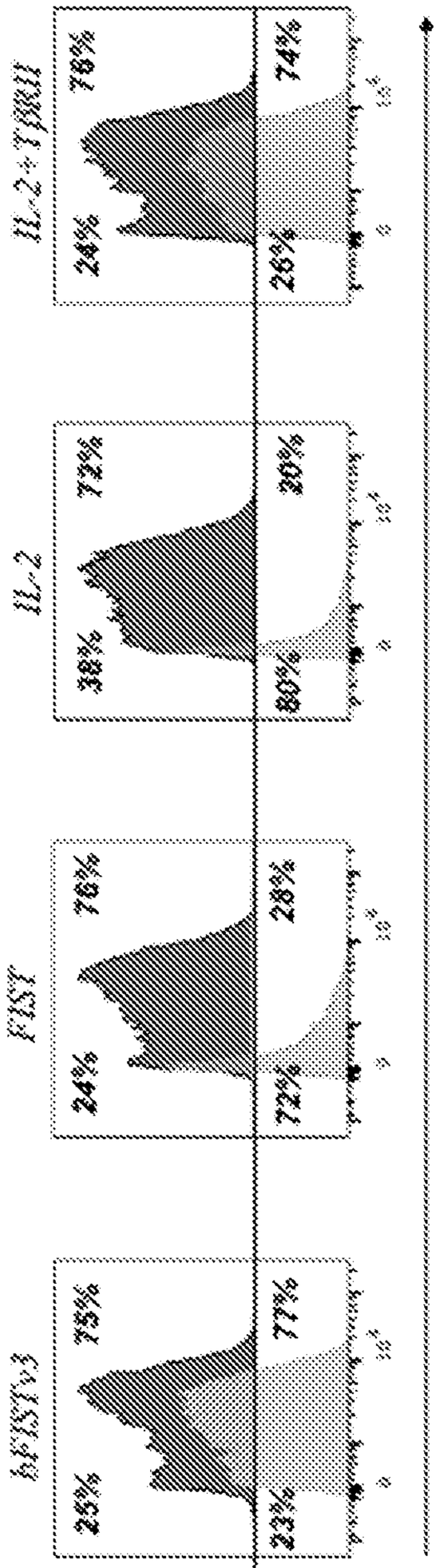



FIG. 10A

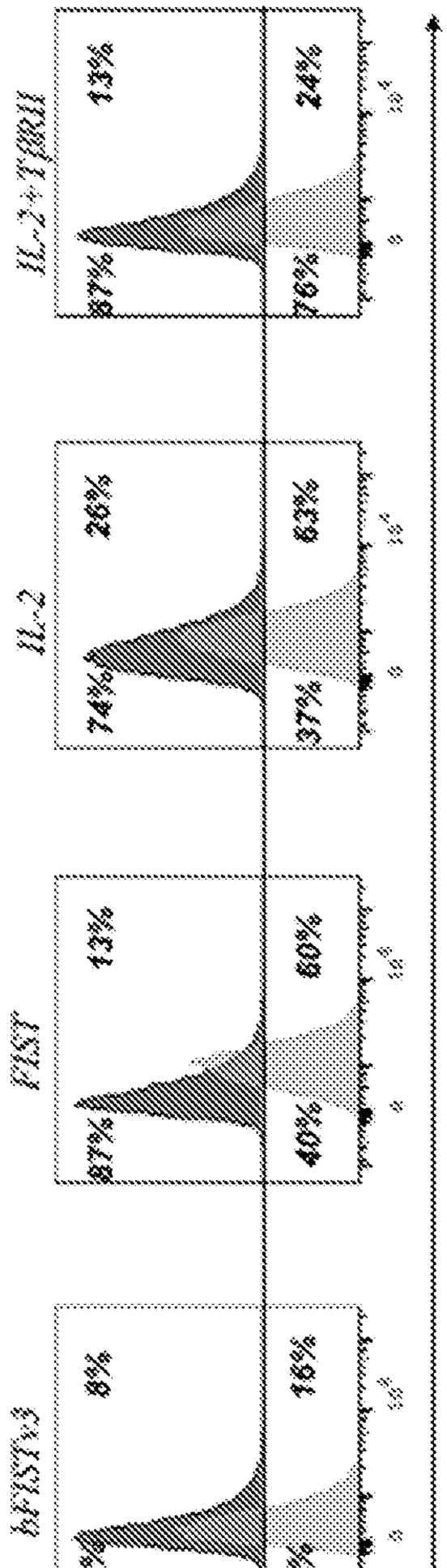
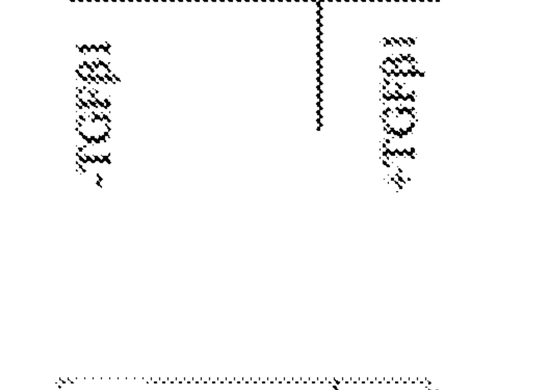
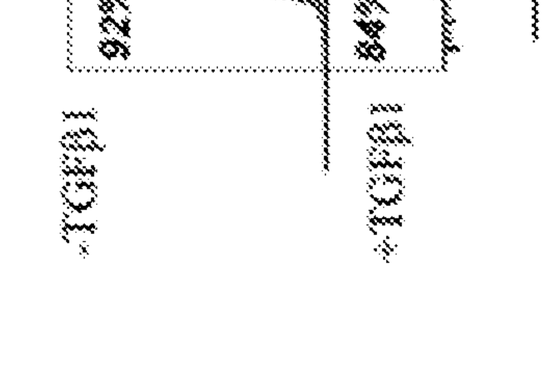



FIG. 10B



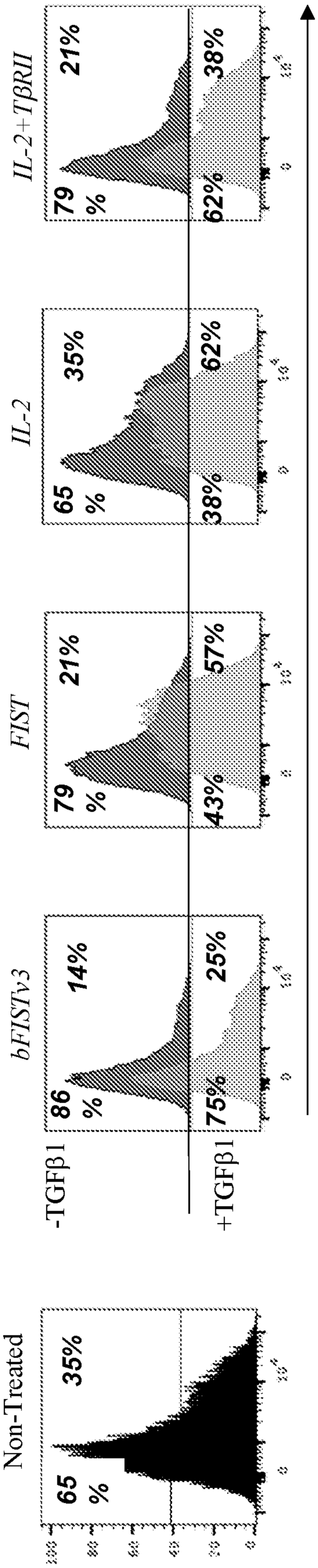

IL-2+TβRII
21%
38%
79 %
62%
IL-2
35%
62%
65 %
38%
FIST
21%
57%
79 %
43%
bFISTv3
14%
25%
86 %
75%
-TGFβ1
+TGFβ1
PD-L1
Non-Treated
35%
65 %

FIG. 10C

SEQUENCE LISTING

<110> CURA THERAPEUTICS

<120> MULTI-FUNCTIONAL AND MULTI-VALENT INTERLEUKIN-TGF-BETA RECEPTOR FUSION POLYPEPTIDES

<130> 57949-701.601

<140>
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<150> 63/094,277
<151> 2020-10-20

<160> 28

<170> PatentIn version 3.5

<210> 1
<211> 153
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                  10                  15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
```

```
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Thr
145                 150
```

<210> 2
<211> 152
<212> PRT
<213> Homo sapiens

<400> 2

```
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu
```

145 150

<210> 3
<211> 152
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 3
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu
145 150

<210> 4

<211> 135
<212> PRT
<213> Homo sapiens

<400> 4
Met Val Leu Gly Thr Ile Asp Leu Cys Ser Cys Phe Ser Ala Gly Leu
1               5                  10                  15

Pro Lys Thr Glu Ala Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys
            20                  25                  30

Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr
        35                  40                  45

Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe
    50                  55                  60

Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile
65                  70                  75                  80

His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser
                85                  90                  95

Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu
            100                 105                 110

Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val
        115                 120                 125

Gln Met Phe Ile Asn Thr Ser
    130                 135

<210> 5
<211> 125
<212> PRT
<213> Homo sapiens

<400> 5
Cys Phe Ser Ala Gly Leu Pro Lys Thr Glu Ala Asn Trp Val Asn Val
1               5                  10                  15

Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile
            20                  25                  30

```
Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His Pro Ser Cys Lys Val
        35                  40                  45

Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu
    50                  55                  60

Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu
65                  70                  75                  80

Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys
                85                  90                  95

Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln
            100                 105                 110

Ser Phe Val His Ile Val Gln Met Phe Ile Asn Thr Ser
        115                 120                 125
```

<210> 6
<211> 567
<212> PRT
<213> Homo sapiens

<400> 6

```
Met Gly Arg Gly Leu Leu Arg Gly Leu Trp Pro Leu His Ile Val Leu
1               5                   10                  15

Trp Thr Arg Ile Ala Ser Thr Ile Pro Pro His Val Gln Lys Ser Val
            20                  25                  30

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Phe Pro
        35                  40                  45

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
    50                  55                  60

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
65                  70                  75                  80

Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
                85                  90                  95
```

```
Leu Glu Thr Val Cys His Asp Pro Lys Leu Pro Tyr His Asp Phe Ile
            100                 105                 110

Leu Glu Asp Ala Ala Ser Pro Lys Cys Ile Met Lys Glu Lys Lys Lys
        115                 120                 125

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn
    130                 135                 140

Asp Asn Ile Ile Phe Ser Glu Glu Tyr Asn Thr Ser Asn Pro Asp Leu
145                 150                 155                 160

Leu Leu Val Ile Phe Gln Val Thr Gly Ile Ser Leu Leu Pro Pro Leu
                165                 170                 175

Gly Val Ala Ile Ser Val Ile Ile Ile Phe Tyr Cys Tyr Arg Val Asn
            180                 185                 190

Arg Gln Gln Lys Leu Ser Ser Thr Trp Glu Thr Gly Lys Thr Arg Lys
        195                 200                 205

Leu Met Glu Phe Ser Glu His Cys Ala Ile Ile Leu Glu Asp Asp Arg
    210                 215                 220

Ser Asp Ile Ser Ser Thr Cys Ala Asn Asn Ile Asn His Asn Thr Glu
225                 230                 235                 240

Leu Leu Pro Ile Glu Leu Asp Thr Leu Val Gly Lys Gly Arg Phe Ala
                245                 250                 255

Glu Val Tyr Lys Ala Lys Leu Lys Gln Asn Thr Ser Glu Gln Phe Glu
            260                 265                 270

Thr Val Ala Val Lys Ile Phe Pro Tyr Glu Glu Tyr Ala Ser Trp Lys
        275                 280                 285

Thr Glu Lys Asp Ile Phe Ser Asp Ile Asn Leu Lys His Glu Asn Ile
    290                 295                 300

Leu Gln Phe Leu Thr Ala Glu Glu Arg Lys Thr Glu Leu Gly Lys Gln
```

```
305                 310                 315                 320

Tyr Trp Leu Ile Thr Ala Phe His Ala Lys Gly Asn Leu Gln Glu Tyr
                325                 330                 335

Leu Thr Arg His Val Ile Ser Trp Glu Asp Leu Arg Lys Leu Gly Ser
            340                 345                 350

Ser Leu Ala Arg Gly Ile Ala His Leu His Ser Asp His Thr Pro Cys
        355                 360                 365

Gly Arg Pro Lys Met Pro Ile Val His Arg Asp Leu Lys Ser Ser Asn
    370                 375                 380

Ile Leu Val Lys Asn Asp Leu Thr Cys Cys Leu Cys Asp Phe Gly Leu
385                 390                 395                 400

Ser Leu Arg Leu Asp Pro Thr Leu Ser Val Asp Asp Leu Ala Asn Ser
                405                 410                 415

Gly Gln Val Gly Thr Ala Arg Tyr Met Ala Pro Glu Val Leu Glu Ser
            420                 425                 430

Arg Met Asn Leu Glu Asn Val Glu Ser Phe Lys Gln Thr Asp Val Tyr
        435                 440                 445

Ser Met Ala Leu Val Leu Trp Glu Met Thr Ser Arg Cys Asn Ala Val
    450                 455                 460

Gly Glu Val Lys Asp Tyr Glu Pro Pro Phe Gly Ser Lys Val Arg Glu
465                 470                 475                 480

His Pro Cys Val Glu Ser Met Lys Asp Asn Val Leu Arg Asp Arg Gly
                485                 490                 495

Arg Pro Glu Ile Pro Ser Phe Trp Leu Asn His Gln Gly Ile Gln Met
            500                 505                 510

Val Cys Glu Thr Leu Thr Glu Cys Trp Asp His Asp Pro Glu Ala Arg
        515                 520                 525
```

Leu Thr Ala Gln Cys Val Ala Glu Arg Phe Ser Glu Leu Glu His Leu
    530                 535                 540

Asp Arg Leu Ser Gly Arg Ser Cys Ser Glu Glu Lys Ile Pro Glu Asp
545                 550                 555                 560

Gly Ser Leu Asn Thr Thr Lys
                565

<210> 7
<211> 592
<212> PRT
<213> Homo sapiens

<400> 7
Met Gly Arg Gly Leu Leu Arg Gly Leu Trp Pro Leu His Ile Val Leu
1               5                   10                  15

Trp Thr Arg Ile Ala Ser Thr Ile Pro Pro His Val Gln Lys Ser Asp
            20                  25                  30

Val Glu Met Glu Ala Gln Lys Asp Glu Ile Ile Cys Pro Ser Cys Asn
        35                  40                  45

Arg Thr Ala His Pro Leu Arg His Ile Asn Asn Asp Met Ile Val Thr
    50                  55                  60

Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys Asp
65                  70                  75                  80

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
                85                  90                  95

Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val
            100                 105                 110

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
        115                 120                 125

Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser Pro
    130                 135                 140

```
Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
145                 150                 155                 160


Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
                165                 170                 175


Glu Tyr Asn Thr Ser Asn Pro Asp Leu Leu Leu Val Ile Phe Gln Val
            180                 185                 190


Thr Gly Ile Ser Leu Leu Pro Pro Leu Gly Val Ala Ile Ser Val Ile
        195                 200                 205


Ile Ile Phe Tyr Cys Tyr Arg Val Asn Arg Gln Gln Lys Leu Ser Ser
    210                 215                 220


Thr Trp Glu Thr Gly Lys Thr Arg Lys Leu Met Glu Phe Ser Glu His
225                 230                 235                 240


Cys Ala Ile Ile Leu Glu Asp Asp Arg Ser Asp Ile Ser Ser Thr Cys
                245                 250                 255


Ala Asn Asn Ile Asn His Asn Thr Glu Leu Leu Pro Ile Glu Leu Asp
            260                 265                 270


Thr Leu Val Gly Lys Gly Arg Phe Ala Glu Val Tyr Lys Ala Lys Leu
        275                 280                 285


Lys Gln Asn Thr Ser Glu Gln Phe Glu Thr Val Ala Val Lys Ile Phe
    290                 295                 300


Pro Tyr Glu Glu Tyr Ala Ser Trp Lys Thr Glu Lys Asp Ile Phe Ser
305                 310                 315                 320


Asp Ile Asn Leu Lys His Glu Asn Ile Leu Gln Phe Leu Thr Ala Glu
                325                 330                 335


Glu Arg Lys Thr Glu Leu Gly Lys Gln Tyr Trp Leu Ile Thr Ala Phe
            340                 345                 350


His Ala Lys Gly Asn Leu Gln Glu Tyr Leu Thr Arg His Val Ile Ser
```

```
        355                 360                 365

Trp Glu Asp Leu Arg Lys Leu Gly Ser Ser Leu Ala Arg Gly Ile Ala
    370                 375                 380

His Leu His Ser Asp His Thr Pro Cys Gly Arg Pro Lys Met Pro Ile
385                 390                 395                 400

Val His Arg Asp Leu Lys Ser Ser Asn Ile Leu Val Lys Asn Asp Leu
                405                 410                 415

Thr Cys Cys Leu Cys Asp Phe Gly Leu Ser Leu Arg Leu Asp Pro Thr
            420                 425                 430

Leu Ser Val Asp Asp Leu Ala Asn Ser Gly Gln Val Gly Thr Ala Arg
        435                 440                 445

Tyr Met Ala Pro Glu Val Leu Glu Ser Arg Met Asn Leu Glu Asn Val
    450                 455                 460

Glu Ser Phe Lys Gln Thr Asp Val Tyr Ser Met Ala Leu Val Leu Trp
465                 470                 475                 480

Glu Met Thr Ser Arg Cys Asn Ala Val Gly Glu Val Lys Asp Tyr Glu
                485                 490                 495

Pro Pro Phe Gly Ser Lys Val Arg Glu His Pro Cys Val Glu Ser Met
            500                 505                 510

Lys Asp Asn Val Leu Arg Asp Arg Gly Arg Pro Glu Ile Pro Ser Phe
        515                 520                 525

Trp Leu Asn His Gln Gly Ile Gln Met Val Cys Glu Thr Leu Thr Glu
    530                 535                 540

Cys Trp Asp His Asp Pro Glu Ala Arg Leu Thr Ala Gln Cys Val Ala
545                 550                 555                 560

Glu Arg Phe Ser Glu Leu Glu His Leu Asp Arg Leu Ser Gly Arg Ser
                565                 570                 575
```

Cys Ser Glu Glu Lys Ile Pro Glu Asp Gly Ser Leu Asn Thr Thr Lys
580 585 590

<210> 8
<211> 136
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 8
Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
1 5 10 15

Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp
20 25 30

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
35 40 45

Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys Val Ala Val
50 55 60

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
65 70 75 80

Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro
85 90 95

Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
100 105 110

Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
115 120 125

Asp Tyr Asn Thr Ser Asn Pro Asp
130 135

<210> 9
<211> 161
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 9
Ile Pro Pro His Val Gln Lys Ser Asp Val Glu Met Glu Ala Gln Lys
1 5 10 15

Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His Pro Leu Lys
20 25 30

His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
35 40 45

Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
50 55 60

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
65 70 75 80

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
85 90 95

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly
100 105 110

Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys
115 120 125

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
130 135 140

Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro
145 150 155 160

Asp

<210> 10
<211> 409
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 10
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Val Asn Asn Asp Met
145 150 155 160

Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys
165 170 175

Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met
180 185 190

```
Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys
        195                 200                 205

Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val
    210                 215                 220

Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala
225                 230                 235                 240

Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr
                245                 250                 255

Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile
            260                 265                 270

Phe Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val
        275                 280                 285

Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys
    290                 295                 300

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
305                 310                 315                 320

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys Val Ala
                325                 330                 335

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
            340                 345                 350

Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser
        355                 360                 365

Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
    370                 375                 380

Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser
385                 390                 395                 400

Glu Asp Tyr Asn Thr Ser Asn Pro Asp
```

405

<210> 11
<211> 424
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 11
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Ile Pro Pro His Val Gln Lys Ser
145 150 155 160

Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu

```
                165                 170                 175

Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn
            180                 185                 190

Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys
        195                 200                 205

Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile
    210                 215                 220

Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe
225                 230                 235                 240

Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys
                245                 250                 255

Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys
            260                 265                 270

Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
        275                 280                 285

Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
    290                 295                 300

Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp
305                 310                 315                 320

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
                325                 330                 335

Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys Val Ala Val
            340                 345                 350

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
        355                 360                 365

Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro
    370                 375                 380
```

Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
385 390 395 400

Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
405 410 415

Asp Tyr Asn Thr Ser Asn Pro Asp
420

<210> 12
<211> 434
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 12
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

```
Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Asp Val Glu Met Glu
145                 150                 155                 160

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
                165                 170                 175

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
            180                 185                 190

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
        195                 200                 205

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
    210                 215                 220

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
225                 230                 235                 240

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
                245                 250                 255

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
            260                 265                 270

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
        275                 280                 285

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ile Pro Pro His Val Gln
    290                 295                 300

Lys Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val
305                 310                 315                 320

Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys
                325                 330                 335

Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys
```

340 345 350

Glu Lys Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu
355 360 365

Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His
370 375 380

Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu
385 390 395 400

Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp
405 410 415

Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn
420 425 430

Pro Asp

<210> 13
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 13
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu

```
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Ile Pro Pro His Val Lys Ser
145                 150                 155                 160

Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys
                165                 170                 175

Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp Met Ile Val
            180                 185                 190

Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys
        195                 200                 205

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
    210                 215                 220

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala
225                 230                 235                 240

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
                245                 250                 255

Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser
            260                 265                 270

Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
        275                 280                 285
```

```
Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser
    290                 295                 300

Glu Asp Tyr Asn Thr Ser Asn Pro Asp Ile Pro Pro His Val Gln Lys
305                 310                 315                 320

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
                325                 330                 335

Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
            340                 345                 350

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
        355                 360                 365

Lys Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
    370                 375                 380

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly
385                 390                 395                 400

Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys
                405                 410                 415

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu
            420                 425                 430

Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro
        435                 440                 445

Asp
```

<210> 14
<211> 434
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 14
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                  10                  15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Val Asn Asn Asp Met
145                 150                 155                 160

Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys
                165                 170                 175

Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met
            180                 185                 190

Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys
        195                 200                 205

Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val

```
    210                 215                 220

Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala
225                 230                 235                 240

Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr
                245                 250                 255

Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile
            260                 265                 270

Phe Ile Pro Pro His Val Gln Lys Ser Asp Val Glu Met Glu Ala Gln
        275                 280                 285

Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His Pro Leu
    290                 295                 300

Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val
305                 310                 315                 320

Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys
                325                 330                 335

Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys
            340                 345                 350

Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu
        355                 360                 365

Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His
    370                 375                 380

Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu
385                 390                 395                 400

Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp
                405                 410                 415

Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn
            420                 425                 430
```

Pro Asp

<210> 15
<211> 449
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 15
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Ile Pro Pro His Val Gln Lys Ser
145 150 155 160

```
Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu
                165                 170                 175

Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn
            180                 185                 190

Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys
        195                 200                 205

Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile
    210                 215                 220

Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe
225                 230                 235                 240

Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys
                245                 250                 255

Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys
            260                 265                 270

Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
        275                 280                 285

Ile Pro Pro His Val Gln Lys Ser Asp Val Glu Met Glu Ala Gln Lys
    290                 295                 300

Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His Pro Leu Lys
305                 310                 315                 320

His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
                325                 330                 335

Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
            340                 345                 350

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
        355                 360                 365

Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
```

370 375 380

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly
385 390 395 400

Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys
405 410 415

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu
420 425 430

Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro
435 440 445

Asp

<210> 16
<211> 458
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 16
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys

```
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Asp Val Glu Met Glu
145                 150                 155                 160

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
                165                 170                 175

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
            180                 185                 190

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
        195                 200                 205

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
    210                 215                 220

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
225                 230                 235                 240

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
                245                 250                 255

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
            260                 265                 270

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
        275                 280                 285

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ile Pro Pro His Val Lys
    290                 295                 300
```

```
Ser Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys Val Thr
305                 310                 315                 320


Cys Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp Met Ile
                325                 330                 335


Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe
            340                 345                 350


Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
        355                 360                 365


Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
    370                 375                 380


Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
385                 390                 395                 400


His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala
                405                 410                 415


Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
            420                 425                 430


Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
        435                 440                 445


Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
    450                 455


<210> 17
<211> 473
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
      polypeptide

<400> 17
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1               5                   10                  15
```

```
Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Ile Pro Pro His Val Lys Ser
145                 150                 155                 160

Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys
                165                 170                 175

Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp Met Ile Val
            180                 185                 190

Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys
        195                 200                 205

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
    210                 215                 220

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala
```

```
225                 230                 235                 240

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
                245                 250                 255

Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser
            260                 265                 270

Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
        275                 280                 285

Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser
    290                 295                 300

Glu Asp Tyr Asn Thr Ser Asn Pro Asp Ile Pro Pro His Val Lys Ser
305                 310                 315                 320

Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys
                325                 330                 335

Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp Met Ile Val
            340                 345                 350

Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys
        355                 360                 365

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn
    370                 375                 380

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala
385                 390                 395                 400

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His
                405                 410                 415

Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser
            420                 425                 430

Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe
        435                 440                 445
```

Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser
450 455 460

Glu Asp Tyr Asn Thr Ser Asn Pro Asp
465 470

<210> 18
<211> 664
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 18
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Val Glu Cys Pro Pro
20 25 30

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
35 40 45

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
50 55 60

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
65 70 75 80

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
85 90 95

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
100 105 110

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
115 120 125

Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
130 135 140

```
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
145                 150                 155                 160

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                165                 170                 175

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            180                 185                 190

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        195                 200                 205

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    210                 215                 220

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
225                 230                 235                 240

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ala Pro Thr Ser Ser Ser
                245                 250                 255

Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
            260                 265                 270

Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
        275                 280                 285

Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys
    290                 295                 300

His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu
305                 310                 315                 320

Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile
                325                 330                 335

Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
            340                 345                 350

Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
```

```
        355                 360                 365

Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys
    370                 375                 380

Ser Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys Val Thr
385                 390                 395                 400

Cys Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp Met Ile
                405                 410                 415

Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe
            420                 425                 430

Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser
        435                 440                 445

Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val
    450                 455                 460

Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys
465                 470                 475                 480

His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala
                485                 490                 495

Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe
            500                 505                 510

Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe
        515                 520                 525

Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr
    530                 535                 540

Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp
545                 550                 555                 560

Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys
                565                 570                 575
```

Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys Val Ala Val
580 585 590

Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp
595 600 605

Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro
610 615 620

Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met
625 630 635 640

Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu
645 650 655

Asp Tyr Asn Thr Ser Asn Pro Asp
660

<210> 19
<211> 639
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 19
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ile Ser Ala Met Val Arg Ser Val Glu Cys Pro Pro
20 25 30

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
35 40 45

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
50 55 60

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
65 70 75 80

```
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                85                  90                  95

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            100                 105                 110

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        115                 120                 125

Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    130                 135                 140

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
145                 150                 155                 160

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                165                 170                 175

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            180                 185                 190

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        195                 200                 205

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    210                 215                 220

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
225                 230                 235                 240

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ala Pro Thr Ser Ser Ser
                245                 250                 255

Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp Leu Gln
            260                 265                 270

Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg
        275                 280                 285

Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys
```

```
    290                 295                 300

His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu Val Leu
305                 310                 315                 320

Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp Leu Ile
                325                 330                 335

Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu Thr Thr
            340                 345                 350

Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu Phe Leu
        355                 360                 365

Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys
    370                 375                 380

Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys
385                 390                 395                 400

Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp
                405                 410                 415

Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu
            420                 425                 430

Lys Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn
        435                 440                 445

Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly
    450                 455                 460

Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys
465                 470                 475                 480

Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu
                485                 490                 495

Cys Asn Asp Asn Ile Ile Phe Ile Pro Pro His Val Gln Lys Ser Val
            500                 505                 510
```

Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro
515 520 525

Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln
530 535 540

Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro
545 550 555 560

His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr
565 570 575

Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr
580 585 590

Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys
595 600 605

Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn
610 615 620

Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
625 630 635

<210> 20
<211> 419
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 20
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp
20 25 30

Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys
35 40 45

```
Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys
    50                  55                  60

Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val
65                  70                  75                  80

Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr
                85                  90                  95

Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp
            100                 105                 110

Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu
        115                 120                 125

Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile
    130                 135                 140

Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp Ala Pro Thr Ser
145                 150                 155                 160

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
                165                 170                 175

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
            180                 185                 190

Thr Arg Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu
        195                 200                 205

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
    210                 215                 220

Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
225                 230                 235                 240

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
                245                 250                 255

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
```

```
            260                 265                 270

Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu
        275                 280                 285

Gln Lys Ser Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly Ala
    290                 295                 300

Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser Thr
305                 310                 315                 320

Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser Ile
                325                 330                 335

Cys Glu Lys Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp
            340                 345                 350

Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr Tyr
        355                 360                 365

His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met Lys
    370                 375                 380

Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser Ala
385                 390                 395                 400

Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr Ser
                405                 410                 415

Asn Pro Asp
```

<210> 21
<211> 444
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 21

```
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
```

```
1               5                   10                  15

Val Thr Asn Ser Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp
            20                  25                  30

Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys
        35                  40                  45

Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys
    50                  55                  60

Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val
65                  70                  75                  80

Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr
                85                  90                  95

Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp
            100                 105                 110

Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu
        115                 120                 125

Thr Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile
    130                 135                 140

Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp Ala Pro Thr Ser
145                 150                 155                 160

Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His Leu Leu Leu Asp
                165                 170                 175

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
            180                 185                 190

Thr Arg Met Leu Thr Ala Lys Phe Tyr Met Pro Lys Lys Ala Thr Glu
        195                 200                 205

Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys Pro Leu Glu Glu
    210                 215                 220
```

```
Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu Arg Pro Arg Asp
225                 230                 235                 240

Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu Lys Gly Ser Glu
                245                 250                 255

Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala Thr Ile Val Glu
            260                 265                 270

Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile Ile Ser Thr Leu
        275                 280                 285

Gln Lys Ser Asp Val Glu Met Glu Ala Gln Lys Gly Glu Met Ile Cys
    290                 295                 300

Val Thr Cys Asn Arg Thr Ala His Pro Leu Lys His Val Asn Asn Asp
305                 310                 315                 320

Met Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys
                325                 330                 335

Lys Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys
            340                 345                 350

Met Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val
        355                 360                 365

Cys Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr
    370                 375                 380

Val Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp
385                 390                 395                 400

Ala Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu
                405                 410                 415

Thr Phe Phe Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile
            420                 425                 430

Ile Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
```

435 440

<210> 22
<211> 468
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 22
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ile Pro Pro His Val Lys Ser Asp Val Glu Met Glu
20 25 30

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
35 40 45

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
50 55 60

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
65 70 75 80

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
85 90 95

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
100 105 110

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
115 120 125

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
130 135 140

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
145 150 155 160

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr

```
                165                 170                 175

Ser Asn Pro Asp Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            180                 185                 190

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        195                 200                 205

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
    210                 215                 220

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
225                 230                 235                 240

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                245                 250                 255

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            260                 265                 270

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        275                 280                 285

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    290                 295                 300

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Asp Val Glu Met Glu
305                 310                 315                 320

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
                325                 330                 335

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
            340                 345                 350

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
        355                 360                 365

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
    370                 375                 380
```

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
385 390 395 400

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
405 410 415

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
420 425 430

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
435 440 445

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr
450 455 460

Ser Asn Pro Asp
465

<210> 23
<211> 308
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 23
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

```
Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Asp Val Glu Met Glu
145                 150                 155                 160

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
                165                 170                 175

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
            180                 185                 190

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
        195                 200                 205

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
    210                 215                 220

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
225                 230                 235                 240

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
                245                 250                 255

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
            260                 265                 270

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
        275                 280                 285

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr
```

290 295 300

Ser Asn Pro Asp
305

<210> 24
<211> 308
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 24
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
1 5 10 15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
20 25 30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
35 40 45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
50 55 60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65 70 75 80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
85 90 95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
100 105 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
115 120 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
130 135 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Asp Val Glu Met Glu

145 150 155 160

Ala Gln Lys Gly Glu Met Ile Cys Val Thr Cys Asn Arg Thr Ala His
165 170 175

Pro Leu Lys His Val Asn Asn Asp Met Ile Val Thr Asp Asn Asn Gly
180 185 190

Ala Val Lys Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Phe Ser
195 200 205

Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ser
210 215 220

Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala Val Trp Arg Lys Asn
225 230 235 240

Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His Asp Pro Lys Leu Thr
245 250 255

Tyr His Gly Phe Thr Leu Glu Asp Ala Ala Ser Pro Lys Cys Val Met
260 265 270

Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe Met Cys Ser Cys Ser
275 280 285

Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser Glu Asp Tyr Asn Thr
290 295 300

Ser Asn Pro Asp
305

<210> 25
<211> 283
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 25
Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu

```
1               5                   10                  15

Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Gln Leu Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile
        35                  40                  45

Asn Asn Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Ala Lys Phe
    50                  55                  60

Tyr Met Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu
65                  70                  75                  80

Glu Glu Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys
                85                  90                  95

Asn Phe His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile
            100                 105                 110

Val Leu Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala
        115                 120                 125

Asp Glu Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe
    130                 135                 140

Cys Gln Ser Ile Ile Ser Thr Leu Gln Lys Ser Val Asn Asn Asp Met
145                 150                 155                 160

Ile Val Thr Asp Asn Asn Gly Ala Val Lys Leu Pro Gln Leu Cys Lys
                165                 170                 175

Phe Cys Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met
            180                 185                 190

Ser Asn Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro His Glu Val Cys
        195                 200                 205

Val Ala Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val
    210                 215                 220
```

Cys His Asp Pro Lys Leu Thr Tyr His Gly Phe Thr Leu Glu Asp Ala
225 230 235 240

Ala Ser Pro Lys Cys Val Met Lys Glu Lys Lys Lys Pro Gly Glu Thr
245 250 255

Phe Phe Met Cys Ser Cys Ser Ala Asp Glu Cys Asn Asp Asn Ile Ile
260 265 270

Phe Ser Glu Asp Tyr Asn Thr Ser Asn Pro Asp
275 280

<210> 26
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 26
Gly Ser Ser Gly
1

<210> 27
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 27
Gly Gly Ser Ser
1

<210> 28
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 28
Gly Ser Ala Gly Gly
1               5