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(54) Title: MODIFIED GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) AND CHIMERIC CYTOKINE RECEPTORS BINDING SAME

(57) Abstract: Described herein are methods and compositions for selective activation of cells using variant cytokine receptor and cytokine pairs, wherein the cytokine receptors comprise an extracellular domain (ECD) of granulocyte-colony stimulating factor receptor (G-CSFR). In certain embodiments, the methods and compositions described herein are useful for exclusive activation of cells for adoptive cell transfer therapy. Thus, included herein are methods of producing cells expressing variant receptors that are selectively activated by a cytokine that does not bind its native receptor. Also disclosed herein are methods of treating a subject in need thereof, comprising administering to the subject cells expressing an variant receptor comprising an extracellular domain of G-CSFR and co-administering a variant cytokine that activates the variant receptor.

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MODIFIED GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) AND CHIMERIC CYTOKINE RECEPTORS BINDING SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos: 63/171,933 filed April 7, 2021; 63/171,950 filed April 7, 2021; 63/171,980 filed April 7, 2021 and 63/172,025 filed April 7, 2021, each of which is hereby incorporated in its entirety by reference for all purposes.

SEQUENCE LISTING

[0002] Not Applicable.

BACKGROUND

Field

[0003] In certain aspects, described herein are methods and compositions for selective activation of cells using variant cytokine receptor and cytokine pairs, wherein the cytokine receptors comprise a variant extracellular domain (ECD) of granulocyte-colony stimulating factor receptor (G-CSFR). Also disclosed herein are methods of treating a subject by adoptive cell transfer, comprising administering to the subject cells expressing variant receptors and administering variant cytokines to send signals to the cells expressing variant receptors. Included with this disclosure are nucleic acids, expression vectors and kits for producing cells expressing variant cytokines and receptors, and kits which also provide cytokines for binding variant receptors. In certain aspects, described herein are chimeric cytokine receptors comprising G-CSFR (Granulocyte-Colony Stimulating Factor Receptor) extracellular domains and the intracellular domains of various cytokine receptors for selective activation of cytokine signaling in cells of interest. The present disclosure also comprises methods, cells and kits for use in adoptive cell transfer (ACT), comprising cells expressing the chimeric cytokine receptors and/or expression vectors encoding chimeric cytokine receptors and/or cytokines that bind the chimeric cytokine receptors. In certain aspects, described herein are systems and methods for controlled paracrine signaling comprising chimeric cytokine receptors comprising G-CSFR (Granulocyte-Colony Stimulating Factor Receptor) extracellular domains and the intracellular domains of various cytokine receptors for selective activation of cytokine signaling in cells of interest.

[0004] Also described herein are methods and compositions for selective activation of cells using variant cytokine receptor and cytokine pairs, wherein the cytokine receptors comprise an extracellular domain (ECD) of Interleukin-7 Receptor Alpha (IL-7Rα).

[0005] The past two decades have brought much progress in the treatment of cancer by adoptive cell transfer (ACT). ACT with naturally occurring tumor-infiltrating T cells (TIL) is now reproducibly yielding >50% objective clinical response rates in advanced melanoma. ACT with T cells engineered to recognize B-lineage leukemias (using CD19-directed chimeric antigen receptors, or CD19 CARs) is yielding up to 90% complete response rates, with the majority of patients achieving durable responses. ACT with T cells expressing engineered T Cell Receptors (TCRs) is showing promise against various solid tumors. Successful ACT has also been reported using other effector cell types in place of T cells, including Natural Killer (NK) cells, Natural Killer T cells (NKT cells) and macrophages. Motivated by these remarkable results, several companies are commercializing the TIL, CAR and engineered TCR ACT approaches.

[0006] The engraftment, expansion and persistence of T cells or other effector cells for ACT are important determinants of clinical safety and efficacy. In the case of T cells, this is often addressed by administration of systemic IL-2 after ACT transfer, as well as expanding the T cells outside the body with IL-2 prior to transfer. In addition to the intended immune stimulatory effects, systemic IL-2 treatment can also lead to severe toxicities such as vascular leakage syndrome that need to be tightly controlled for patient safety. To manage these risks, patients typically need to be hospitalized for 2-3 weeks and have access to an ICU as a precautionary measure. Furthermore, IL-2 induces the proliferation of both effector and regulatory (inhibitory) T cells (5); therefore, giving a patient IL-2 is analogous to pressing the gas and brake pedals at the same time. CAR T cells bring the converse problem in that T-cell expansion and persistence exceed safe levels in some patients, and falter prematurely in others. Moreover, they universally eradicate normal B cells (which also express CD19), leaving patients partly immune-deficient. Ideally, one would like to have precise control over the number of tumor-reactive T cells after ACT, including the abilities to safely enhance the proliferation, persistence and potency of T cells and to eliminate transferred cells once the cancer has been eradicated. Other cell-based therapies, such as stem cell therapies, would also benefit from improved control over the expansion, differentiation, and persistence of infused cells.

[0007] Human G-CSF (Neupogen ®, Filgrastim) and a pegylated version of human G-CSF (Neulasta ®, Pegfilgrastim) are approved therapeutics used to treat Neutropenia in cancer patients. G-CSF is a four-helix bundle (Hill, CP *et al.* Proc Natl Acad Sci U S A. 1993 Jun 1;90(11):5167-71), and the structure of G-CSF in complex with its receptor G-CSFR is well characterized (Tamada, T *et al.* Proc Natl Acad Sci U S A. 2006 Feb 28;103(9):3135-40). The G-CSF:G-CSFR complex is a 2:2 heterodimer. G-CSF has two binding interfaces with G-CSFR. One interface is referred to as *site II*; it is the larger interface between G-CSF and the Cytokine Receptor Homologous (CRH) domain of G-CSFR. The second interface is referred to as *site III*; it is the smaller interface between G-CSF and the N-terminal Ig-like domain of G-CSFR.

[0008] Interleukin 7 (IL-7) is an example of safe, well-tolerated cytokine but with limited potency in the setting of cancer immunotherapy. IL-7 is a growth factor for T cells and B cells and is important for thymic development and supporting the survival and homeostasis of naïve and memory T cells. Unlike IL-2, IL-7 induces minimal Treg proliferation, as IL-7Rα is expressed at low levels on this suppressive lymphocyte population. IL-7 is not produced by hematopoietic cells but rather is secreted by stromal cells. IL-7 has been used in cancer immunotherapy with the goal of increasing T cell numbers, persistence and activity (Barata JT et al. Nat Immunol. 2019 Dec;20(12):1584-1593). It has been shown to be well-tolerated; however, it has shown negligible anti-cancer efficacy as a monotherapy (Rosenberg et al. J Immunother. 2006 May-Jun;29(3):313-9), (Sportès C. et al., Clin Cancer Res. 2010 Jan 15;16(2):727-35), and (Sportès C., et al., J Exp Med. 2008 Jul 7;205(7):1701-14). Moreover, recombinant IL-7 made in *E. coli* proved to be highly immunogenic, although this problem was ameliorated by producing IL-7 in mammalian cells (Conlon KC, et al., J Interferon Cytokine Res. 2019 Jan;39(1):6-21). For these reasons, there has been limited clinical development of IL-7 in oncology relative to more therapeutically potent cytokines such as IL-2 and IL-15. However, IL-7 is attractive for use as a ligand for chimeric receptors that induce more potent intracellular signals than achieved by the native IL-7 receptor.

[0009] To mitigate the toxicity issues associated with systemic delivery of cytokines, several groups have engineered T cells or NK cells to produce and secrete cytokines in an autocrine/paracrine manner. The goal is to have the engineered T/NK cells produce enough cytokine for their own consumption, and that of neighboring cells, but not enough to cause systemic toxicities. For example, this strategy has been applied to improve Chimeric Antigen Receptor (CAR) T cell and CAR NK cell therapy using cytokines such as IL-2, IL-15, IL-12

and IL-18. A common approach is to use retroviruses or lentiviruses to stably introduce a CAR gene plus cytokine gene in a T cell or NK cell population. This can be achieved by a variety of approaches, such as co-transduction of two viral vectors, or by using a bicistronic viral vector that carries two transgenes. T cells and NK cells that co-express CARs and cytokine transgenes are commonly referred to as “armored CARs” or “TRUCKS”.

[0010] Armored CARs have been evaluated in murine tumor models and, to a lesser extent, in human clinical trials. In several studies, armored CAR T/NK cells have proven both safe and effective relative to standard CAR T/NK cells (i.e. cells with a CAR but no cytokine transgene). However, toxicities have been observed in some mouse studies (Ataca Atilla P., et al., J Immunother Cancer. 2020 Sep;8(2):e001229) and clinical trials (Zhang L., et al., Clin Cancer Res. 2015 May 15;21(10):2278-88. Moreover, there will always be theoretical concerns about the concept of endowing T/NK cells (or any cell type) with the ability to produce an autocrine growth factor; this has the potential to lead to uncontrolled T/NK cell growth, resulting in a secondary lymphoproliferative disease or malignancy. Some investigators attempt to mitigate this risk by co-expressing a “suicide gene” that can be used to kill the T/NK cells in response to a drug, should toxicities, uncontrolled growth or other concerns arise. However, deployment of a suicide gene by definition terminates the cell therapy, potentially leaving the patient with residual tumor burden. Therefore, novel compositions and methods are needed for reducing the toxicity and safety risks associated with the armored CAR approach to enable therapeutically relevant cytokine signals to be delivered to immune cells in a safe and controlled manner.

SUMMARY

[0011] Described herein are variant Granulocyte Colony-Stimulating Factors (G-CSF), wherein the variant G-CSF comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof; wherein the at least one mutation in the site II interface region is selected from the group of mutations consisting of: L108R, D112R, E122R E122K, E123K and E123R and combinations thereof; wherein the site II interface region mutations are relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; wherein the at least one mutation in the site III interface region comprises mutation E46R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the variant G-CSF binds selectively to a receptor comprising a variant extracellular domain (ECD) of Granulocyte Colony-Stimulating Factor Receptor (G-CSFR).

[0012] In certain aspects, described herein is a system for selective activation of a receptor expressed on a cell surface, the system comprising:(a) a variant G-CSF corresponding to SEQ ID NO: 83 or 84; and (b) a receptor comprising a variant ECD of G-CSFR; wherein the variant G-CSF preferentially binds the receptor comprising the variant ECD of G-CSFR as compared to an otherwise identical wild type G-CSFR ECD, and the receptor comprising the variant ECD of G-CSFR preferentially binds the variant G-CSF as compared to an otherwise identical wild type G-CSF; and wherein the variant G-CSFR comprises G2R-3 or G12/2R-1.

[0013] In some embodiments, the variant G-CSF binds a receptor comprising a variant ECD of G-CSFR that is expressed by a cell. In some embodiments, the cell expressing the receptor comprising the variant ECD of G-CSFR is an immune cell. In some embodiments, the immune cell expressing the receptor comprising the variant ECD of G-CSFR is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T-cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve CD8+T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

[0014] In some embodiments, the selective binding of the variant G-CSF to the receptor comprising the variant ECD of G-CSFR causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor. In some embodiments, the receptor comprising the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof. In some embodiments, the receptor comprising the variant ECD of G-CSFR comprises at least one mutation in the site II interface region of the G-CSFR ECD comprising one or both of a R141E or a R167D mutation; wherein the at least one mutation in the site III interface region of the G-CSFR ECD comprises a R41E mutation; and wherein the variant G-CSFR mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 2. In some embodiments, the receptor comprising the variant ECD of G-CSFR is a chimeric receptor.

[0015] In certain aspects, the present disclosure describes one or more nucleic acid sequence(s) encoding the variant G-CSF described herein. In certain aspects, the present disclosure describes one or more expression vector(s) comprising the nucleic acid sequence. In certain aspects, the present disclosure describes cells engineered to express a variant G-

CSF described herein. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell, is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD8^+$ T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

[0016] In certain aspects, described herein is a system for selective activation of a receptor expressed on a cell surface, the system comprising: (a) the variant G-CSF of any one of claims 1 - [0014]; and (b) a receptor comprising a variant ECD of G-CSFR; wherein the variant G-CSF preferentially binds the receptor comprising the variant ECD of G-CSFR as compared to an otherwise identical wild type G-CSFR ECD, and the receptor comprising the variant ECD of G-CSFR preferentially binds the variant G-CSF as compared to an otherwise identical wild type G-CSF. In some embodiments, the variant G-CSF comprises a combination of mutations of a site II and a site III interface of a G-CSF variant number of Table 4A; wherein the variant G-CSF mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 1.

[0017] In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, E122R, and E123R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2. In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, and E122K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2. In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, and E123K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2. In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, and E122R relative to the corresponding amino acid positions of the

sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2. In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, and E123R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2. In some embodiments, the variant G-CSF comprises mutations E46R, L108K, D112R, E122K, and E123K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

[0018] In some embodiments, the system further comprises one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the system further comprises an antigen binding signaling receptor. In some embodiments, the antigen binding signaling receptor comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, and a pattern recognition receptor. In some embodiments, the antigen binding signaling receptor is a CAR. In some embodiments, the cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, and CCL19, and the receptor NKG2D. In some embodiments, the cytokine is IL-18. In some embodiments, the cytokine is human.

[0019] In certain aspects, described herein is a method of selective activation of a receptor expressed on the surface of a cell, comprising contacting a receptor comprising a variant ECD of G-CSFR with a variant G-CSF described herein. In some embodiments, the receptor comprising a variant ECD of G-CSFR is expressed on an immune cell, and, optionally, the immune cell is:

a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD8^+$ T cells, naïve $CD4^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells. In some embodiments, the selective activation of the immune cell causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor.

[0020] In certain aspects, the present disclosure describes a method of increasing an immune response in a subject in need thereof, comprising: administering cell(s) expressing a receptor comprising a variant ECD of G-CSFR and administering or providing a variant G-CSF described herein. In certain aspects, the present disclosure describes a method of treating a disease in a subject in need thereof, comprising: administering cell(s) expressing a receptor comprising a variant ECD of G-CSFR and administering or providing a variant G-CSF described herein to the subject. In some embodiments, the method is used to treat cancer. In some embodiments, the method is used to treat an inflammatory condition. In some embodiments, the method is used to treat an autoimmune disease. In some embodiments, the method is used to treat a degenerative disease. In some embodiments, the method is used to generate natural or engineered cells, tissues or organs for transplantation. In some embodiments. the method is used to prevent or treat graft rejection. In some embodiments, the method is used to treat an infectious disease. In some embodiments, the methods further comprise administering or providing one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

[0021] In some embodiments, the subject is administered two or more populations of cells each expressing one or both of: (i) a distinct chimeric receptor comprising a G-CSFR ECD and (ii) at least one distinct variant form of G-CSF. wherein at least one population(s) of cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR(s). In some embodiments, one or both of the first and second population(s) of immune cells further expresses one or both of: (a)) at least one additional agonistic or antagonistic

signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor. In some embodiments, the method further comprises one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a distinct variant ECD of G-CSFR, (ii) a distinct variant G-CSF, (iii) a distinct an agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

[0022] In some embodiments of the methods, the cells expressing a receptor comprising a variant ECD of G-CSFR further express at least one antigen binding signaling receptor(s). In some embodiments, the antigen binding signaling receptor(s) comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the antigen binding signaling receptor(s) comprises one or more CAR(s). In some embodiments, the cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, and CCL19, and the receptor NKG2D, and combinations thereof. In some embodiments, the cytokine is IL-18. In some embodiments, the cytokine is human.

[0023] In certain aspects, described herein are methods of treating a subject in need thereof, wherein the method comprises: i) isolating an immune cell-containing sample; (ii) transducing or transfecting the immune cell(s) with a nucleic acid sequence(s) encoding at least one receptor(s) comprising a variant ECD of G-CSFR; (iii) administering the immune cell(s) from (ii) to the subject; and (iv) contacting the immune cell(s) with one or more variant G-CSF described herein that selectively binds the receptor(s). In some embodiments, the subject has undergone an immuno-depletion treatment prior to administering the cells to the subject. In some embodiments, the immune cell-containing sample is isolated from the subject to whom the cells will be administered. In some embodiments, the immune cell-containing sample is generated from cells derived from the subject to whom the cells are administered or from a subject distinct from a subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells. In

some embodiments, the immune cell(s) are contacted with at least one variant G-CSF *in vitro* prior to administering the cells to the subject. In some embodiments, the immune cell(s) are contacted with the at least one variant G-CSF that binds the receptor(s) for a sufficient time to activate signaling from the receptor(s).

[0024] In certain aspects, described herein are kits comprising: cells encoding at least one receptor(s) comprising a variant ECD of G-CSFR and instructions for use; and wherein the kit comprises at least one variant G-CSF of claims 1 - [0014]; and, optionally, wherein the cells are immune cells. In certain aspects, described herein are kits comprising: (a) one or more nucleic acid sequence(s) encoding one or more receptor(s) comprising the variant ECD of G-CSFR; (b) a at least one variant G-CSF described herein, a nucleic acid sequence(s) described herein or one or more expression vector(s) described herein; and (c) instructions for use. In some embodiments, the kit further comprises one or more expression vector(s) that encode one or more cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, and CCL19, and the receptor NKG2D, and combinations thereof. In some embodiments, the kit further comprises one or more expression vector(s) that encodes at least one antigen binding receptor(s). In some embodiments, the at least one antigen binding receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the kit further comprises one or more expression vector(s) that encodes a chimeric antigen receptor. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, and CCL19, and the receptor NKG2D, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s). In some embodiments, the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations

thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one CAR(s), and, optionally the CAR is a mesothelin CAR.

[0025] In certain aspects, described herein are chimeric receptors, comprising: (a) an extracellular domain (ECD) operatively linked to at least one second domain; the second domain comprising: (b) an intracellular domain (ICD) comprising at least one signaling molecule binding site from an intracellular domain of a cytokine receptor; wherein the at least one signaling molecule binding site is selected from the group consisting of: a SHC binding site of Interleukin (IL)-2Rβ; a STAT5 binding site of IL-2Rβ, an IRS-1 or IRS-2 binding site of IL-4Rα, a STAT6 binding site of IL-4Rα, a SHP-2 binding site of gp130, a STAT3 binding site of gp130, a SHP-1 or SHP-2 binding site of EPOR, a STAT5 binding site of Erythropoietin Receptor (EPOR), a STAT1 or STAT2 binding site of Interferon Alpha And Beta Receptor Subunit 2 (IFNAR2), and a STAT1 binding site of Interferon Gamma Receptor 1 (IFNγR1), or combinations thereof; wherein the ICD further comprises at least one Box 1 region and at least one Box 2 region of at least one protein selected from the group consisting of G-CSFR, gp130, EPOR, and Interferon Gamma Receptor 2 (IFNγR2), or combinations thereof; and (c) at least one third domain comprising a transmembrane domain (TMD); wherein the ECD is N-terminal to the TMD, and the TMD is N-terminal to the ICD.

[0026] In certain aspects, described herein are chimeric receptors, comprising: an ECD operatively linked to a second domain; the second domain comprising an ICD, wherein the ICD comprises:

(i)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of IL-4Rα; or

(ii)

(a) a Box 1 and a Box 2 region of gp130;

(b) at least one signaling molecule binding site of gp130; or

(iii)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) at least one signaling molecule binding site of EPOR; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(v)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) at least one signaling molecule binding site of Interferon Gamma Receptor 1 (IFN γR1); wherein the ECD is N-terminal to a TMD, and the TMD is N-terminal to the ICD.

[0027] In certain embodiments, the ECD of the chimeric receptor is an ECD of G-CSFR (Granulocyte-Colony Stimulating Factor Receptor). In certain embodiments, the TMD of the chiomeric receptor is a TMD of G-CSFR and, optionally, the TMD is a wild-type TMD. In certain embodiments, an activated form of the chimeric receptor forms a homodimer, and, optionally, activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with a G-CSF, and, optionally, the G-CSF is a wild-type G-CSF, and, optionally, the extracellular domain of the G-CSFR is a wild-type extracellular domain.

[0028] In certain embodiments, the chimeric receptor is expressed in a cell, and, optionally, an immune cell, and, optionally the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In certain embodiments, the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

[0029] In certain embodiments, the ICD comprises:

(a) an amino acid sequence of one or both of SEQ ID NO. 90 or 91; or

(b) an amino acid sequence of one or both of SEQ ID NO. 90 or 92; or

(c) an amino acid sequence of SEQ ID NO. 93; or

(d) an amino acid sequence of SEQ ID NO. 94; or

(e) an amino acid sequence of one or both of SEQ ID NO. 95 or 96; or

(f) an amino acid sequence of SEQ ID NO. 97 or 98; or

(g) an amino acid sequence of SEQ ID NO. 99 or 100.

[0030] In certain embodiments, the transmembrane domain comprises a sequence set forth in SEQ ID NO. 88.

[0031] In certain aspects, this disclosure describes one or more nucleic acid sequence(s) encoding a chimeric receptor described herein. In certain embodiments, the nucleic acid sequence(s) of the ECD of the G-CSFR is encoded by nucleic acid sequence(s) set forth in any one of SEQ ID NO. 85, 86, or 87. In certain embodiments, this disclosure describes one or more expression vector(s) comprising the nucleic acid sequence(s). In certain embodiments, the expression vector(s) are selected from the group consisting of: a retroviral vector, a lentiviral vector, an adenoviral vector and a plasmid.

[0032] In certain aspects, this disclosure describes one or more nucleic acid sequence(s) encoding a chimeric receptor; wherein the chimeric receptor comprises: an ECD operatively linked to a second domain; the second domain comprising:

(i)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of IL-4Rα; or

(ii)

(a) a Box 1 and a Box 2 region of gp130;

(b) at least one signaling molecule binding site of gp130; or

(iii)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) at least one signaling molecule binding site of EPOR; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(v)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) at least one signaling molecule binding site of Interferon Gamma Receptor 1 (IFN γR1).

[0033] In certain embodiments of the nucleic acid(s) described herein, the ECD is an ECD of G-CSFR (Granulocyte-Colony Stimulating Factor Receptor). In certain embodiments of the nucleic acid(s) described herein, the TMD is a TMD of G-CSFR and, optionally, the TMD is a wild-type TMD. In certain embodiments of the nucleic acid(s) described herein, the ECD of the G-CSFR is encoded by a nucleic acid sequence set forth in any one of SEQ ID NO. 85, 86 or 87. In certain embodiments, the nucleic acid sequence(s) comprises: (a) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 90 or 91; or (b) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 90 or 92; or (c) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 93; or (d) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 94; or (e) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 95 or 96; or (f) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 97 or 98; or (g) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 99 or 100.

[0034] In certain embodiments, one or more expression vector(s) comprise the nucleic acid sequence(s) described herein. In certain embodiments, the expression vector(s) are selected from the group consisting of: a retroviral vector, a lentiviral vector, an adenoviral vector and a plasmid.

[0035] In certain aspects, described herein is a cell comprising a nucleic acid sequence(s) encoding a chimeric receptor described herein. In certain embodiments, the cell is an immune cell, and, optionally the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In certain embodiments, the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell. In certain embodiments, the cell comprises a nucleic acid sequence(s) described herein. In certain embodiments, the cell comprises an expression vector(s) described herein.

[0036] In certain aspects, described herein are methods of selective activation of a chimeric receptor expressed on the surface of a cell, comprising contacting a chimeric receptor with a cytokine that selectively binds the chimeric receptor. In certain embodiments, an activated form of the chimeric receptor forms a homodimer, and, optionally, activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with the cytokine.

[0037] In certain embodiments, the cytokine that selectively binds the chimeric receptor is a G-CSF, and, optionally, the chimeric receptor is activated upon contact with a G-CSF, and, optionally, the G-CSF is a wild-type G-CSF, and, optionally, the extracellular domain of the G-CSFR is a wild-type extracellular domain. In certain embodiments, the chimeric receptor is expressed in a cell, and, optionally, an immune cell, and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell.

[0038] In some embodiments, a first population of immune cells expresses the chimeric receptor and a second population of immune cells express a cytokine that binds the chimeric receptor; optionally, wherein one or both of the first and second population(s) of immune cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR. In some embodiments, one or both of the first and second population(s) of immune cells further expresses one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor. In some embodiments, each of the first and second populations of immune cells express a distinct chimeric receptor comprising a distinct variant ECD of G-CSFR and a distinct variant G-CSF. In some embodiments, the methods further comprise one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a

distinct variant ECD of G-CSFR, (ii) a distinct variant G-CSF, (iii) a distinct an agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

[0039] In certain aspects, described herein are methods of producing a chimeric receptor in a cell, comprising: introducing into the cell one or more nucleic acid sequence(s) described herein or one or more expression vector(s) described herein; and, optionally, the method comprises gene editing; and, optionally, the cell is an immune cell, and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell.

[0040] In certain aspects, described herein are methods of treating a subject in need thereof, comprising: administering to the subject a cell expressing a chimeric receptor described herein, and providing to the subject a cytokine that specifically binds the chimeric receptor. In certain embodiments, an activated form of the chimeric receptor forms a homodimer; and, optionally, activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with the cytokine. In certain embodiments, the cytokine is G-CSF; and, optionally, the G-CSF is a wild-type G-CSF, and, optionally, the extracellular domain of the G-CSFR is a wild-type extracellular domain.

[0041] In certain embodiments of the methods described herein, the chimeric receptor is expressed in a cell, and, optionally, an immune cell, and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell.

[0042] In certain embodiments, the methods further comprise administering or providing at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s). In certain embodiments, the subject is administered two or more populations of cells each expressing a distinct chimeric receptor comprising a G-CSFR ECD and each expressing a distinct variant form of G-CSF. In certain embodiments, the cells

expressing the chimeric receptor further express at least one antigen binding signaling receptor. In certain embodiments, the antigen binding signaling receptor comprises at least one receptor(s) selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In certain embodiments, the antigen binding signaling receptor is a CAR. In certain embodiments, the at least one cytokine(s) or chemokine(s) is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In certain embodiments, the cytokine is IL-18. In certain embodiments, the cytokine is human.

[0043] In certain embodiments, the method is used to treat cancer such as, but not limited to, bile duct cancer, bladder cancer, breast cancer, cervical cancer, ovarian cancer, colon cancer, endometrial cancer, hematologic malignancies, kidney cancer (renal cell), leukemia, lymphoma, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, sarcoma and thyroid cancer. In certain embodiments, method is used to treat an autoimmune disease. In certain embodiments, the method is used to treat an inflammatory condition. In certain embodiments, the method is used to treat a degenerative disease. In certain embodiments, the method is used to generate natural or engineered cells, tissues or organs for transplantation. In certain embodiments, the method is used to treat or prevent allograft rejection.

[0044] In certain embodiments, the method further comprises administering or providing at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s). In some embodiments, the subject is administered two or more populations of cells each expressing a distinct chimeric receptor and each expressing a distinct variant form of a cytokine.

[0045] In certain embodiments, the method comprises: i) isolating an immune cell-containing sample; (ii) introducing to the immune cells a nucleic acid sequence encoding the chimeric cytokine receptor; (iii) administering the immune cells from (ii) to the subject; and (iv) contacting the immune cells with the cytokine that binds the chimeric receptor. In certain

embodiments, the subject has undergone an immuno-depletion treatment prior to administering or infusing the cells to the subject. In certain embodiments, the immune cell-containing sample is isolated from a subject to whom the cells will be administered.
In certain embodiments, the immune cell-containing sample is isolated from a subject distinct from a subject to whom the cells will be administered. In some embodiments, the immune cell-containing sample is generated from cells derived from a subject to whom the cells will be administered, or a subject distinct from a subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells. In certain embodiments, the immune cells are contacted with the cytokine *in vitro* prior to administering or infusing the cells to the subject. In certain embodiments, the immune cells are contacted with the cytokine for a sufficient time to activate signaling from the chimeric receptor. In certain embodiments, the cytokine is G-CSF; and, optionally, the G-CSF is a wild-type G-CSF, and, optionally, the extracellular domain of the G-CSFR is a wild-type extracellular domain.

[0046] In certain aspects, described herein are kits comprising: at least one expression vector(s) encoding one or more chimeric receptor(s) described herein and instructions for use; and, optionally, the kit comprises at least one cytokine(s) that binds the chimeric receptor(s). In some embodiments, the kit further comprises one or more expression vector(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s). In some embodiments, the kits further comprise one or more expression vector(s) that encode one or more cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In some embodiments, the kit further comprises one or more expression vector(s) that encodes at least one antigen binding receptor(s). In some embodiments, the at least one antigen binding receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some

embodiments, the kit further comprises one or more expression vector(s) that encodes one or more CAR(s), and, optionally, wherein the CAR(s) is a mesothelin CAR. In some embodiments, the kit further comprises one or more expression vector(s) encoding one or more distinct chimeric receptor(s) described herein.

[0047] In certain aspects, described herein are kit comprising cells encoding one or more chimeric receptor(s) described herein and, optionally, the cells are immune cells; and instructions for use; and, optionally, the kit comprises at least one cytokine(s) that binds the chimeric receptor(s). In some embodiments, the cells further comprise one or more expression vector(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s). In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s). In some embodiments, the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one CAR(s), and, optionally, wherein CAR(s) is a mesothelin CAR. In some embodiments, the cells further comprise one or more expression vector(s) encoding at least one distinct chimeric receptor described herein.

[0048] In certain aspects, described herein are systems for selective activation of a cell, the system comprising: (i) a receptor comprising a variant extracellular domain (ECD) of Granulocyte Colony-Stimulating Factor Receptor (G-CSFR); and (ii) a variant G-CSF that selectively binds the receptor of (i); and one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) and (b) at least one antigen binding signaling receptor(s). In some embodiments, the at least one additional cytokine(s) or chemokine(s) comprises at least one of interleukin (IL)-18, IL-21, interferon-a, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof. In some embodiments, the at least one additional cytokine(s) comprises IL-18. In some embodiments, the antigen binding signaling receptor(s) comprises at least one of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the antigen binding signaling receptor comprises a CAR; and, optionally, the CAR is a mesothelin CAR. In some embodiments, the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof. In some embodiments, the at least one mutation in the site II interface region is located at an amino acid position of the G-CSFR ECD selected from the group consisting of amino acid position 141,167, 168, 171, 172, 173, 174, 197, 199, 200, 202 and 288 of the sequence shown in SEQ ID NO. 2. In some embodiments, the at least one mutation in the site II interface region of the G-CSFR ECD is selected from the group consisting of R141E, R167D, K168D, K168E, L171E, L172E, Y173K, Q174E, D197K, D197R, M199D, D200K, D200R, V202D, R288D, and R288E of the sequence shown in SEQ ID NO. 2.

[0049] In some embodiments, the at least one mutation in the site III interface region of the G-CSFR ECD is selected from the group consisting of amino acid position 30, 41, 73, 75, 79, 86, 87, 88, 89, 91, and 93 of amino acids 2-308 of the sequence shown in SEQ ID NO. 2. In some embodiments, the at least one mutation in the site III interface region of the G-CSFR ECD is selected from the group consisting of S30D, R41E, Q73W, F75KF, S79D, L86D, Q87D, I88E, L89A, Q91D, Q91K, and E93K of the sequence shown in SEQ ID NO. 2. In

some embodiments, the G-CSFR ECD comprises a combination of a plurality of mutations of a design number shown in Table 4, 22 and 23; wherein the mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 2.

[0050] In some embodiments, the G-CSFR ECD comprises the mutations: R41E, R141E, and R167D of the sequence shown in SEQ ID NO. 2. In some embodiments, the receptor comprising a variant ECD of G-CSFR is a chimeric receptor.

[0051] In some embodiments, the chimeric receptor is operatively linked to at least one second domain; the second domain comprising at least one signaling molecule binding site from an intracellular domain (ICD) of one or more cytokine receptor(s); wherein the at least one signaling molecule binding site is selected from the group consisting of: a STAT3 binding site of G-CSFR, a STAT3 binding site of glycoprotein 130 (gp130), a SHP-2 binding site of gp130, a SHC binding site of IL-2Rβ, a STAT5 binding site of IL-2Rβ, a STAT3 binding site of IL-2Rβ, a STAT1 binding site of IL-2Rβ, a STAT5 binding site of IL-7Rα, a phosphatidylinositol 3-kinase (PI3K) binding site of IL-7Rα, a STAT4 binding site of IL-12Rβ_2, a STAT5 binding site of IL-12Rβ_2, a STAT3 binding site of IL-12Rβ_2, a STAT5 binding site of IL-21R, a STAT3 binding site of IL-21R a STAT1 binding site of IL-21R, an IRS-1 or IRS-2 binding site of IL-4Rα, a STAT6 binding site of IL-4Rα, a SHP-1 or SHP-2 binding site of Erythropoietin Receptor (EPOR), a STAT5 binding site of EPOR, a STAT1 or STAT2 binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2), and a STAT1 binding site of Interferon Gamma Receptor 1 (IFNγR1), or combinations thereof; optionally, the ICD comprises a Box 1 region and a Box 2 region of a protein selected from the group consisting of G-CSFR, gp130 EPOR, and Interferon Gamma Receptor 2 (IFNγR2), or combinations thereof; and, optionally, the chimeric receptor comprises a third domain comprising a transmembrane domain (TMD) of a protein selected from the group consisting of: G-CSFR, gp130 (Glycoprotein 130), and IL-2Rβ, and, optionally, the TMD is a wild-type TMD.

[0052] In some embodiments, the chimeric receptor is operatively linked to at least one second domain; the second domain comprising:

(i)

(a) a Box 1 and a Box 2 region of gp130; and

(b) a C-terminal region of IL-2Rβ; or

(ii)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-2Rβ; or

(iii)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-12Rβ_2; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-21R; or

(v)

(a) a Box 1 and a Box 2 region of IL-2Rβ; and

(b) a C-terminal region of IL-2Rβ; or

(vi)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-7Rα; or

(vii)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) a C-terminal region of IL-4Rα; or

(viii)

(a) a Box 1 and a Box 2 region of gp130;

(b) a C-terminal region of gp130; or

(ix)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) a C-terminal region of EPOR; or

(x)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) a C-terminal region of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(xi)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) a C-terminal region of Interferon Gamma Receptor 1 (IFN γR1).

[0053] In some embodiments, the ECD is N-terminal to the TMD, and TMD N-terminal to the ICD. In some embodiments, the receptor comprising a variant ECD of G-CSFR is expressed on the cell. In some embodiments, the cell is an immune cell and, optionally the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell. In some embodiments, activation of the receptor comprising a variant ECD of G-CSFR by the variant G-CSF causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, enhanced activity of a cell expressing the receptor, and combinations thereof. In some embodiments, the variant G-CSF comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof. In some embodiments, theat least one mutation in the site II interface region of the variant G-CSF is located at an amino acid position selected from the group consisting of amino acid position 12, 16, 19, 20, 104, 108, 109, 112, 115, 116, 118, 119, 122 and 123 of the sequence shown in SEQ ID NO. 1. In some embodiments, the at least one mutation in the site II interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of: S12E, S12K, S12R, K16D, L18F, E19K, Q20E, D104K, D104R, L108K, L108R, D109R, D112R, D112K, T115E, T115K, T116D, Q119E, Q119R, E122K, E122R, and E123R. In some embodiments, the at least one mutation in the site III interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of: 38, 39, 40, 41, 46, 47, 48, 49, and 147 of the sequence shown in SEQ ID NO. 1.

[0054] In some embodiments, the at least one mutation in the site III interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of:

T38R, Y39E, K40D, K40F, L41D, L41E, L41K, E46R, L47D, V48K, V48R, L49K, and R147E. In some embodiments, the cell expresses both the receptor comprising the variant ECD of G-CSFR and the at least one additional cytokine(s) or chemokine(s). In some embodiments, two or more populations of cells each express a one or more distinct chimeric receptor(s) comprising a G-CSFR ECD and each express one or more distinct variant forms of G-CSF. In some embodiments, the first population of immune cells further expresses one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor(s). In some embodiments, the cell is an immune cell; and wherein the immune cell further expresses the antigen binding signaling receptor; and wherein the antigen binding signaling receptor selectively binds to an antigen expressed on a second cell. In some embodiments, the antigen binding signaling receptor(s) comprises a chimeric antigen receptor (CAR), and, optionally, a mesothelin CAR. In some embodiments, the additional cytokine comprises IL-18. In some embodiments, the second cell is a cancer cell.

[0055] In certain aspects, the present disclosure describes one or more nucleic acid sequence(s) encoding a system described herein. In certain asptect, the present disclosure describes one or more expression vector(s) comprising a nucleic acid sequence(s) described herein. In certain aspects, the present disclosure describes one or more cell(s) engineered to express a system described herein. In some embodiments, the cell(s) is an immune cell and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T-cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve CD8+T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

[0056] In certain aspects, described herein are methods of selective activation of a receptor comprising a variant ECD of G-CSFR expressed on the surface of a cell, comprising: introducing into the cell one or more nucleic acid sequence(s) encoding one or more receptor(s) comprising a variant ECD of G-CSFR of a system described herein; and one or both of (i)) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises

one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein; and (ii) at least one antigen binding signaling receptor(s) of a system described herein; and contacting the receptor(s) comprising the variant ECD of G-CSFR with one or more variant G-CSF or the variant G-CSF of a system described herein. In some embodiments, the receptor(s) is expressed on an immune cell, and, optionally the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell. In some embodiments, the selective activation of the receptor(s) expressed on the immune cell causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, enhanced activity of the immune cell, and combinations thereof. In some embodiments, a first population of immune cells expresses the receptor comprising the variant ECD of G-CSFR and a second population of immune cells express the variant G-CSF; optionally, wherein one or both of the first and second population(s) of immune cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR. In some embodiments, one or both of the first and second population(s) of immune cells further expresses one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor(s).

[0057] In some embodiments, each of the first and second populations of immune cells express at least one distinct receptor(s) comprising a distinct variant ECD of G-CSFR and at least one distinct variant G-CSF. In some embodiments, the method further comprises one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a distinct variant ECD of G-CSFR, (ii) a distinct variant G-CSF, (iii) a distinct an agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

[0058] In certain aspects, the present disclosure describes methods of producing a cell expressing one or more receptor(s) comprising a variant ECD of G-CSFR of a system described herein; and one or both of: (i)) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein; and (ii) at least one antigen binding signaling receptor of the system of a system described herein; the method comprising introducing to the cells one or more nucleic acid(s) or expression vector(s) encoding the receptor, and one or both of (i), and (ii). In some embodiments, a first population of immune cells expresses the receptor comprising the variant ECD of G-CSFR and a second population of immune cells express the variant G-CSF. In some embodiments, or both of the first and second population(s) of immune cells further expresses one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor(s).

[0059] In certain aspects, described herein are methods of increasing an immune response in a subject in need thereof, comprising administering to the subject the immune cell(s) described herein. In certain aspects, described herein are methods of treating a disease in a subject in need thereof, comprising: administering to the subject the immune cell(s) herein. In some embodiments, the methods further comprise administering or providing a variant G-CSF to the subject. In some embodiments, the method is used to treat cancer. In some embodiments, the method is used to treat an inflammatory condition. In some embodiments, the method is used to treat an autoimmune disease or condition. In some embodiments, the method is used to treat a degenerative disease. In some embodiments, the method is used to generate natural or engineered cells, tissues or organs for transplantation. In some embodiments, the method is used to prevent or treat graft rejection. In some embodiments, the method is used to treat an infectious disease. In some embodiments, the method further comprises administering or providing at least one additional active agent; optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or

antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s).

[0060] In certain aspects, described herein are methods of treating a subject in need thereof, wherein the method comprises: (i) isolating an immune cell-containing sample; (ii) introducing the immune cells with one or more nucleic acid sequence(s) encoding one or more receptor(s) comprising the variant ECD of G-CSFR of the system of any one of claims [0048]-[0054]; and one or both of: (a) at least one additional active agent; optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein ; and optionally, (b) the antigen binding signaling receptor of a system described herein; (iii) administering the immune cell(s) from (ii) to the subject; and (iv) contacting the immune cell(s) with a variant G-CSF that specifically binds the receptor(s) comprising the variant ECD of G-CSFR. In some embodiments, the subject has undergone an immuno-depletion treatment prior to administering or infusing the immune cell(s) to the subject. In some embodiments, the immune cell-containing sample is isolated from the subject to whom the cell(s) are administered. In some embodiments, the immune cell-containing sample is isolated from a subject distinct from the subject to whom the cell(s) are administered. In some embodiments, the immune cell-containing sample is generated from source cells derived from a subject to whom the source cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells. In some embodiments, the immune cell-containing sample is generated from source cells derived from a subject distinct from a subject to whom the source cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells. In some embodiments, the immune cell(s) are contacted with the variant G-CSF or additional cytokine(s) or chemokine(s) *in vitro* prior to administering the immune cell(s) to the subject. In some embodiments, the immune cell(s) are contacted with the variant G-CSF for a sufficient time to activate signaling from the receptor(s) comprising the variant ECD of G-CSFR of a system described herein.

[0061] In certain aspects, described herein are kits comprising cells encoding: one or more receptor(s) comprising the variant ECD of G-CSFR of a system described herein; and one or both of: (a) one or more additional cytokine(s) and chemokine(s) of a system described

herein; and, (b) one or more antigen binding signaling receptor(s) of a system described herein; and instructions for use; and optionally, wherein the cells are immune cells. In certain aspects, described herein are kits comprising: (i) one or more nucleic acid sequence(s) or expression vector(s) encoding the receptor(s) comprising the variant ECD of G-CSFR of a system described herein; and one or both of: (a) at least one additional active agent(s); optionally wherein the at least one additional active agent(s) comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein; and, (b) one or more antigen binding signaling receptors of a system described herein; and (ii) one or more variant G-CSF; and (iii) instructions for use; wherein the receptor and one or both of (a) and (b) are located on the same or separate nucleic acid sequence or expression vector.

[0062] In certain aspects, described herein are kits comprising: (i) cells comprising one or more nucleic acid sequence(s) or expression vector(s) encoding the receptor(s) comprising variant ECD of G-CSFR of a system described herein; and one or both of: (a) at least one additional active agent(s); optionally wherein the at least one additional active agent(s) comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein; and (b) at least one antigen binding signaling receptor(s) of a system described herein; and (ii) instructions for use; and, optionally, wherein the kit comprises one or more variant G-CSF that specifically binds the receptor(s) comprising the variant ECD of G-CSFR.

[0063] In certain aspects, disclosed herein are chimeric receptors, comprising: (i) an extracellular domain (ECD) of Interleukin Receptor alpha (IL-7Rα); (ii) a transmembrane domain (TMD); and (iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from a wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD. In some embodiments, the carboxy terminus (C-terminus) of the ECD is linked to the amino terminus (N-terminus) of the TMD, and the C-terminus of TMD is linked to the N-terminus of the ICD. In some embodiments, the ECD is the ECD of native human IL-7Rα. In some embodiments, the TMD is the TMD of IL-7Rα. In some embodiments, the TMD is the TMD of native human IL-7Rα.

In some embodiments, the ICD comprises at least one signaling molecule binding site from an intracellular domain of a cytokine receptor, and, optionally, the at least one signaling molecule binding site comprises: (a) a JAK1 binding site (Box 1 and 2 region) of IL-2Rβ, IL-4Rα, IL-7Rα, IL-21R, or gp130; (b) a SHC binding site of IL-2Rβ; (c) a STAT5 binding site of IL-2Rβ or IL-7Rα; (d) a STAT3 binding site of IL-21R or gp130; (e) a STAT4 binding site of IL-12Rβ2; (f) a STAT6 binding site of IL-4Rα; (g) an IRS-1 or IRS-2 binding site of IL-4Rα; and (h) a SHP-2 binding site of gp130; (i) a PI3K binding site of IL-7Rα; or combinations thereof. In some embodiments, the ICD comprises at least an intracellular signaling domain of a receptor that is activated homodimerization or by heterodimerization with the common gamma chain (gc). In some embodiments, the ICD comprises at least an intracellular signaling domain of a cytokine receptor selected from the group consisting of: IL-2Rβ (Interleukin-2 receptor beta), IL-4Rα (Interleukin-4 Receptor alpha), IL-9Rα (Interleukin-9 Receptor alpha), IL-12R β2 (Interleukin-12 Receptor), IL-21R (Interleukin-21 Receptor) and glycoprotein 130 (gp130), and combinations thereof.

[0064] In certain aspects, described herein are chimeric receptors, comprising an ECD of IL-7Rα and a TMD operatively linked to an ICD, the ICD comprising:

(i)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(ii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(iii)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(iv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(v)

(a) a Box 1 and a Box 2 region of IL-21R; and

(b) a STAT3 binding site of IL-21R; or

(vi)

(a) a Box 1 and a Box 2 region of IL-7Rα; and

(b) a STAT3 binding site of IL-21R; or

(vii)

(a) a Box 1 and a Box 2 region of IL-21R;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(viii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(ix)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(x)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(xi)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xiii)

(a) a Box 1 and a Box 2 region of IL-4Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4Rα; and

(c) a STAT6 binding site of IL-4Rα; or

(xiv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4α; and

(c) a STAT6 binding site of IL-4α; or

(xv)

(a) a Box 1 and a Box 2 region of gp130;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130; or

(xvi)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130.

In some embodiments, (b) is N-terminal to (c); or (c) is N-terminal to (b); or (c) is N-terminal to (d); or (d) is N-terminal to (c); or (d) is N-terminal to (e); or (e) is N-terminal to (d).

[0065] In some embodiments, the ICD comprises a sequence at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a sequence shown in at least one of SEQ ID NO: 192-214.

[0066] In some embodiments, an activated form of the chimeric receptor forms a heterodimer and, optionally, the activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the chimeric receptor, and, optionally, the chimeric receptor is activated upon contact with interleukin (IL)-7. In some embodiments, the IL-7 is a wild-type, human IL-7. In some embodiments, the IL-7 harbors 1, 2, 3, 4 or 5 mutations compared to wild-type IL-7. In some embodiments, the IL-7 comprises one or more chemical modifications.

[0067] In some embodiments, the chimeric receptor is expressed on a cell. the cell is an immune cell and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell, is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell. In some embodiments, activation of the receptor by IL-7 causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor.

[0068] In certain aspects, the present disclosure describes one or more nucleic acid sequence(s) encoding a receptor described herein. In certain aspects, the present disclosure

describes one or more expression vector(s) comprising a nucleic acid sequence(s) described herein. In certain aspects, the present disclosure describes a cell comprising the nucleic acid sequence(s), or the expression vector(s) described herein. In some embodiments, the cell is an immune cell and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally, an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD4^+$ T cells, naïve $CD8^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells.

[0069] In certain aspects, the present disclosure describes a system for activation of a receptor expressed on a cell surface, the system comprising: (a) a chimeric receptor described herein; and (b) IL-7.

[0070] In certain aspects, the present disclosure describes a system for activation of an immune cell, the system comprising: (a) a chimeric receptor described herein; (b) IL-7; and (c) an antigen binding signaling receptor.

[0071] In certain aspects, the present disclosure describes a system for activation of an immune cell, the system comprising: (a) a chimeric receptor described herein; (b) IL-7; and (c) at least one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

[0072] In some embodiments, the system further comprises at least one antigen binding signaling receptor. In some embodiments, the at least one antigen binding signaling receptor comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the at least one antigen binding signaling receptor is a CAR. In some embodiments, the cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the

receptor NKG2D, and combinations thereof. In some embodiments, the cytokine is IL-18. In some embodiments, the cytokine is human.

[0073] In certain aspects, described herein are methods of activation of a chimeric receptor expressed on the surface of a cell, comprising: contacting the chimeric receptor with IL-7 to activate the chimeric receptor; wherein the chimeric receptor comprises: (i) an extracellular domain (ECD) of IL-7Rα; (ii) a transmembrane domain (TMD); and (iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from the wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD. In some embodiments, the chimeric receptor is a chimeric receptor described herein.

[0074] In certain aspects, described herein is a method of producing a chimeric receptor in a cell, the method comprising: introducing into the cell a nucleic acid sequence(s) described herein or the expression vector(s) described herein. In some embodiments, the method further comprises editing the sequence(s) or sequence(s) of the vector(s) into the genome of the cell.

[0075] In some embodiments of the methods described herein, the cell is an immune cell; and, optionally, the immune cell is: a T cell, and, optionally, an NK cell, and, optionally,an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally, the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD4^+$ T cells, naïve $CD8^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells.

[0076] In certain aspects, described herein is a method of increasing an immune response in a subject in need thereof, comprising: administering to the subject cell(s) expressing a chimeric receptor described herein, and administering or providing IL-7 to the subject.

[0077] In certain aspects, described herein is a method of treating a subject in need thereof, comprising: administering to the subject cell(s) expressing a chimeric receptor described herein, and administering or providing IL-7 to the subject. In some embodiments, the method is used to treat cancer. In some embodiments, the method is used to treat an autoimmune disease. In some embodiments, the method is used to treat an inflammatory condition. In some embodiments, the method is used to treat a degenerative disease. In some embodiments, the method is used to generate natural or engineered cells, tissues or organs for

transplantation. In some embodiments, the method is used to prevent or treat graft rejection. In some embodiments, the method is used to treat an infectious disease.

[0078] In certain embodiments, the method is used to treat a degenerative disease or condition. Examples of degenerative diseases or conditions include, but are not limited to, neurodegenerative diseases and conditions related to aging.

[0079] In certain embodiments, the method is used to generate natural or engineered cells, tissues or organs for transplantation.

[0080] In some embodiments, the methods further comprise administering or providing at least one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the subject is administered cells expressing at least one additional distinct chimeric receptor. In some embodiments, the at least one additional distinct chimeric receptor is a chimeric receptor comprising a variant ECD of Granulocyte Cell Stimulating Factor Receptor (G-CSFR). In some embodiments, the cells expressing the at least one additional distinct chimeric receptor comprising a variant ECD of G-CSFR are contacted with one or more variant G-CSF, and optionally, the subject is administered one or more variant G-CSF. In some embodiments, the method comprises: i) isolating an immune cell-containing sample; (ii) transducing or transfecting the immune cell(s) with nucleic acid sequence(s) encoding the chimeric cytokine receptor(s); (iii) administering the immune cell(s) from (ii) to the subject; and (iv) contacting the immune cells with IL-7.

[0081] In some embodiments, the methods further comprise introducing to the immune cell(s) with nucleic acid sequence(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the at least one cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1,CCL19, the receptor NKG2D, and combinations thereof. In some embodiments, the methods further comprise introducing to the immune cell(s) with nucleic acid sequence(s) encoding at least one antigen binding signaling receptor. In some embodiments, the at least one antigen binding signaling receptor is selected from the group consisting of: a native T

Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the subject has undergone an immuno-depletion treatment prior to administering the cells to the subject. In some embodiments, the immune cell-containing sample is isolated from the subject to whom the cells are administered. In some embodiments, the immune cell-containing sample is isolated from a subject distinct from a subject to whom the cells will be administered. In some embodiments, the immune cell-containing sample is generated from cells derived from a subject distinct from a subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells. In some embodiments, the immune cells are contacted with one or both of IL-7 or a variant G-CSF *in vitro* prior to administering the cells to the subject. In some embodiments, the immune cells are contacted with one or both of IL-7 or a variant G-CSF for a sufficient time to activate signaling from a chimeric receptor described herein.

[0082] In some embodiments, the cells administered to the subject further express at least one antigen binding signaling receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the cells administered to the subject further express a receptor comprising a variant ECD of G-CSFR. In some embodiments, the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof. In some embodiments, the cells administered to the subject further express IL-18.

[0083] In certain aspects, described herein are kits, comprising: cells encoding a chimeric receptor described herein, and, optionally, the cells are immune cells; and instructions for use; and, optionally, the kit comprises IL-7 and, optionally, the kit comprises a variant G-CSF described herein. In certain aspects, described herein are kits, comprising: one or more expression vector(s) comprising the nucleic acid sequence(s) encoding a chimeric receptor described herein and instructions for use; and, optionally, the kit comprises IL-7 and, optionally, the kit comprises a variant G-CSF described herein. In some embodiments, the kit further comprises one or more expression vector(s) that encode at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s),

chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the kit further comprises one or more expression vector(s) that encode a cytokine or chemokine selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof. In some embodiments, the kit further comprises one or more expression vector(s) that encodes at least one receptor selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the kit further comprises an expression vector that encodes a chimeric antigen receptor. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one cytokine or chemokine selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s). In some embodiments, the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one CAR(s).

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0084] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0085] Figure 1 presents a diagram showing the structures of the Site II and III interfaces of the 2:2 G-CSF:G-CSFR heterodimeric complex.

[0086] Figure 2 presents a diagram outlining the strategy used for the G-CSF:G-CSFR interface design.

[0087] Figure 3 presents a graph showing the energetic components of site II interface interactions.

[0088] Figure 4 presents a diagram showing the structure of the site II interface and the interactions of Arg167 (left) and Arg141 (right) of G-CSFR(CRH) with G-CSF residues at site II interface.

[0089] Figure 5 presents a diagram showing wild type G-CSF at site II (left) and overlay of the same region of a triplicate ZymeCAD™ mean-field pack of site II design #35 (right).

[0090] Figure 6 presents images of SDS-PAGE showing results of G-CSF pulldown assays of G-CSF designs #: 6, 7, 8, 9, 15, 17, 30, 34, 35, and 36 (top panel) and co-expressed and purified site II design complexes #: 6, 7, 8, 9, 15, 17, 30, 34, 35, and 36 (bottom panel), first lane WT G-CSF control, second lane WT G-CSF:G-CSFR(CRH) control.

[0091] Figure 7 presents images of SDS-PAGE showing results of G-CSF pulldown assays of G-CSF designs #: 6, 7, 8, 9, 15, 17, 30, 34, 35, and 36 co-expressed with WT-G-CSFR (top panel) and G-CSF pulldown assays of WT G-CSF co-expressed with G-CSFR designs #: 6, 7, 8, 9, 15, 17, 30, 34, 35, and 36 (bottom panel, first lane WT:WT G-CSF:G-CSFR pulldown control.

[0092] Figure 8 presents a graph showing the energetic components of site III interface interactions.

[0093] Figure 9 presents a diagram showing the structure of the site III interface and interactions of R41 (left) and E93 (right) of G-CSFR(Ig) with G-CSF residues at site III interface.

[0094] Figure 10 presents images of SDS-PAGE showing results of G-CSF pulldown assays of designs #401 and 402 (top panel) and co-expressed and purified site II/III design complexes #401 and 402, and with WT G-CSFR and pulldown of WT G-CSF co-expressed

with design #401 and 402 G-CSFR (bottom panel), first lane WT G-CSF control, second lane WT G-CSF:G-CSFR(Ig-CRH) control.

[0095] Figure 11 presents graphs showing size-exclusion chromatography profile of WT (SX75) G-CSF and design 130 (SX75), 303 (SX200) and 401 (SX200) G-CSF_E mutants post TEV cleavage.

[0096] Figure 12 presents graphs showing size-exclusion chromatography profile of purified WT (SX75) and purified design 401(SX200) and 402 (SX200) G-CSFR(Ig-$CRH)_E$ mutants post TEV cleavage.

[0097] Figure 13 presents graphs showing binding SPR sensorgrams for WT, designs #130, #401, #402 G-CSF, binding to their cognate or mispaired G-CSFR(Ig-CRH). Each G-CSF vs G-CSFR pair is labelled in the bottom of each panel. Representative steady state fit used to derive KDs for the cognate pair of design #401 and #402 are shown under their respective sesorgrams.

[0098] Figure 14 presents graphs showing: Top left panel: DSC thermograms of WT G-CSF, design #130 and #134 G-CSF_E; Top right panel: DSC thermograms of WT G-CSFR(Ig-CRH), design #130 and #134 G-CSFR(Ig-$CRH)_E$; Bottom left panel: DSC thermograms of design #401 and #402 G-CSF_E; Bottom right: DSC thermograms of design #300, #303, #304 and #307 G-CSF_E.

[0099] Figure 15 presents graphs showing results of bromo-deoxyuridine (BrdU) assays showing proliferation 32D-IL-2RβIL2Rb cells expressing A) G-$CSFR_{WT}$-$ICD_{IL\text{-}2Rb}$ (homodimer) or B) G-$CSFR_{WT}$-$ICD_{IL\text{-}2Rb}$ + G-$CSFR_{WT}$-ICD_{gc} (heterodimer). Cells were stimulated with no cytokine, IL-2 (300 IU/ml) or G-CSF_{WT} (100 ng/ml in A or 30 ng/ml in B).

[00100] Figure 16 presents graphs showing results of BrdU assays showing proliferation of 32D-IL-2Rβ cells expressing A) G-$CSFR_{137}$-$ICD_{gp130\text{-}IL\text{-}2Rb}$ (homodimer); or B) G-$CSFR_{137}$-$ICD_{IL\text{-}2Rb}$ + G-$CSFR_{137}$-ICD_{gc} (heterodimer). Cells were stimulated with no cytokine, IL-2 (300 IU/ml), G-CSF_{WT} (30 ng/ml), or G-$CSFR_{137}$ (30 ng/ml).

[00101] Figure 17 presents graphs showing results of BrdU assays showing proliferation of 32D- IL-Rβ cells expressing: A) G-$CSFR_{WT}$-$ICD_{gp130\text{-}IL\text{-}2Rb}$ (homodimer); or B) G-$CSFR_{WT}$-$ICD_{IL\text{-}2Rb}$ + G-$CSFR_{WT}$-ICD_{gc} (heterodimer). Cells were stimulated with no cytokine, IL-2 (300 IU/ml), G-CSF_{WT} (30 ng/ml), or G-$CSFR_{137}$ (30 ng/ml).

[00102] Figure 18 presents western blots showing signaling of 32D-IL-2Rβ cells expressing: G-$CSFR_{WT}$-$ICD_{gp130\text{-}IL\text{-}2Rb}$ (homodimer), G-$CSFR_{137}$-$ICD_{gp130\text{-}IL\text{-}2Rb}$ (homodimer),

G-CSFR$_{WT}$-ICD$_{IL-2Rb}$ + G-CSFR$_{WT}$-ICD$_{gc}$ (heterodimer) or G-CSFR$_{137}$-ICD$_{IL-2Rb}$ + G-CSFR$_{137}$-ICD$_{gc}$ (heterodimer). Cells were stimulated with no cytokine, IL-2 (300 IU/ml), G-CSF$_{WT}$ (30 ng/ml), or G-CSFR$_{137}$ (30 ng/ml).

[00103] **Figure 19** presents graphs showing the results of BrdU incorporation assays to assess cell cycle progression of primary murine T cells expressing the indicated chimeric receptors (or non-transduced cells) in response to stimulation with no cytokine, IL-2 or WT, 130, 304 or 307 cytokine. A and B represent experimental replicates.

[00104] **Figure 20** presents a schematic of native IL-2Rβ, IL-2Rγc, and G-CSFR subunits, as well as the G2R-1 receptor subunit designs.

[00105] **Figure 21** presents graphs showing the expansion (fold change in cell number) of 32D-IL-2Rβ cells (which is the 32D cell line stably expressing the human IL-2Rβ subunit) expressing the indicated G-CSFR chimeric receptor subunits and stimulated with WT G-CSF, IL-2 or no cytokine. G/γc was tagged at its N-terminus with a Myc epitope (Myc/G/γc), and G/IL-2Rβ was tagged at its N-terminus with a Flag epitope (Flag/G/IL-2Rβ); these epitope tags aid detection by flow cytometry and do not impact the function of the receptors. In addition, the lower panels in B-D show the percentage of cells expressing the G-CSFR ECD (% G-CSFR+) under each of the culture conditions. Squares represent cells stimulated with IL-2. Triangles represent cells stimulated with G-CSF. Circles represent cells not stimulated with a cytokine.

[00106] **Figure 22** presents graphs showing the expansion (fold change in cell number) of human T cells expressing the Flag-tagged G/IL-2Rβ subunit alone, the Myc-tagged G/γc subunit alone, or the full-length G-CSFR**.** A-D) PBMC-derived T cells; E-H) tumor-associated lymphocytes (TAL). Squares represent cells stimulated with IL-2. Triangles represent cells stimulated with G-CSF. Circles represent cells not stimulated with a cytokine.

[00107] **Figure 23** presents a schematic of native and chimeric receptors, showing JAK, STAT, Shc, SHP-2 and PI3K binding sites. The shading scheme includes receptors from Figure 20.

[00108] **Figure 24** presents a schematic of chimeric receptors, showing JAK, STAT, Shc, SHP-2 and PI3K binding sites. The shading scheme includes receptors from Figures 20 and 23.

[00109] **Figure 25** presents a diagram of the lentiviral plasmid containing the G2R-2 cDNA insert.

[00110] **Figure 26** presents graphs showing G-CSFR ECD expression assessed by flow cytometry in cells transduced with G2R-2. A) 32D-IL-2Rβ cell line; B) PBMC-derived human T cells and human tumor-associated lymphocytes (TAL).

[00111] **Figure 27** presents graphs showing the expansion (fold change in cell number) of cells expressing G2R-2 compared to non-transduced cells**.** A) Human PBMC-derived T cells; B, C) Human tumor-associated lymphocytes (TAL) from two independent experiments. Squares represent cells stimulated with IL-2. Triangles represent cells stimulated with G-CSF. Circles represent cells not stimulated with cytokine.

[00112] **Figure 28** presents graphs showing expansion (fold change in cell number) of CD4- or CD8-selected human tumor-associated lymphocytes expressing G2R-2 compared to non-transduced cells. A) Non-transduced CD4-selected cells; B) Non-transduced CD8-selected cells; C) CD4-selected cells transduced with G2R-2; D) CD8-selected cells transduced with G2R-2. Dotted gray line represents cells stimulated with IL-2. Solid black line represents cells stimulated with G-CSF. Dashed gray line represents cells not stimulated with a cytokine.

[00113] **Figure 29** presents graphs showing expansion (fold change in cell number) of CD4+ or CD8+ tumor-associated lymphocytes expressing G2R-2. The cells were initially expanded in G-CSF or IL-2, as indicated. Cells were then plated in either IL-2, G-CSF or medium only. Solid gray line represents cells stimulated with IL-2. Solid gray line represents cells stimulated with IL-2. Solid black line represents cells stimulated with G-CSF. Dashed light gray line represents cells expanded in IL-2 and then stimulated with medium only. Dashed dark gray line represents cells expanded in G-CSF and then stimulated with medium only.

[00114] **Figure 30** presents a graph showing immunophenotype (by flow cytometry) of CD4- or CD8-selected tumor-associated lymphocytes (TAL) expressing G2R-2 chimeric receptor construct versus non-transduced cells, after expansion in G-CSF or IL-2. A) Percentage of live cells showing a CD4+, CD8+ or CD3-CD56+ cell surface phenotype. B)

Percentage of live cells showing the indicated cell surface phenotypes based on CD45RA and CCR7 expression.

[00115] **Figure 31** presents graphs showing the results of BrdU incorporation assays to assess proliferation of primary human T cells expressing G2R-2 versus non-transduced cells. T cells were selected by culture in IL-2 or G-CSF, as indicated, prior to the assay. A) Tumor-associated lymphocytes; B) PBMC-derived T cells.

[00116] **Figure 32** presents graphs showing the results of BrdU incorporation assays to assess proliferation of primary murine T cells expressing G2R-2 or the single-chain G/IL-2Rβ (a component of G2R-1) versus mock-transduced cells. A) Transduction efficiency as reflected by the percentage of cells expressing the G-CSFR ECD (by flow cytometry) after culture in the indicated cytokines; B) Percent BrdU incorporation in all live cells in response to the indicated cytokines; C) Percent BrdU incorporation by cells expressing the G-CSFR ECD (G-CSFR+ cells). All cells were expanded in IL-2 for 3 days prior to assay. Squares represent cells stimulated with IL-2. Triangles represent cells stimulated with G-CSF. Circles represent cells not stimulated with a cytokine.

[00117] **Figure 33** presents western blots to detect the indicated cytokine signaling events in human primary T cells expressing G2R-2 versus non-transduced cells. β-actin, total Akt and histone H3 serve as a protein loading controls. A, B) Tumor-associated lymphocytes (TALs); C) PBMC-derived T cells.

[00118] **Figure 34** presents western blots to detect the indicated cytokine signaling events in primary murine T cells expressing G2R-2 or the single-chain G/IL-2Rβ (from G2R-1) versus mock-transduced cells. Arrow indicates the specific phospho-JAK2 band; other larger bands are presumed to be the result of cross-reactivity of the primary anti-phospho-JAK2 antibody with phospho-JAK1. β-actin and histone H3 serve as protein loading controls.

[00119] **Figure 35** presents a graph showing the results of a BrdU incorporation assay to assess cell cycle progression of 32D-IL-2Rβ cells expressing the indicated chimeric receptors (or non-transduced cells) in response to stimulation with no cytokine, IL-2 (300 IU/mL), WT G-CSF (30 ng/mL) or 130 G-CSF (30 ng/mL).

[00120] **Figure 36** presents graphs showing the results of BrdU incorporation assays to assess cell cycle progression of primary murine T cells expressing the indicated chimeric

receptors (or non-transduced cells) in response to stimulation with no cytokine, IL-2, WT G-CSF or G-CSF variants 130, 304 or 307. A and B represent experimental replicates.

[00121] **Figure 37** presents western blots to detect the indicated cytokine signaling events in 32D-IL-2Rβ cells expressing the indicated chimeric receptor subunits (or non-transduced cells) in response to stimulation with no cytokine, IL-2, WT G-CSF or 130 G-CSF. β-actin and histone H3 serve as protein loading controls.

[00122] **Figure 38** presents A) western blots to detect the indicated cytokine signaling events in primary murine T cells expressing the indicated chimeric receptor subunits in response to stimulation with no cytokine, IL-2, WT G-CSF, 130 G-CSF or 304 G-CSF. β-actin and histone H3 serve as protein loading controls. B) Transduction efficiency of cells used in panel A, as assessed by flow cytometry with an antibody specific for the extracellular domain of the human G-CSF receptor.

[00123] **Figure 39** presents plots showing G-CSFR ECD expression by flow cytometry in primary human tumor-associated lymphocytes (TAL) transduced with the indicated chimeric receptor constructs. Live CD3+, CD56- cells were gated on CD8 or CD4, and G-CSFR ECD expression is shown for each population.

[00124] **Figure 40** presents graphs and images showing the expansion, proliferation and signaling of primary human tumor-associated lymphocytes (TAL) expressing G2R-3 versus non-transduced cells. A) Graph showing the results of a T-cell expansion assay, where cells were transduced with G2R-3-encoding lentivirus, washed, and re-plated in IL-2 (300 IU/ml), wild type G-CSF (100 ng/ml) or no cytokine. Live cells were counted every 3-4 days. Squares represent cells stimulated with IL-2. Triangles represent cells stimulated with G-CSF. Circles represent cells not stimulated with a cytokine. B) Western blot to assess intracellular signaling events. Cells were harvested from the expansion assay and stimulated with IL-2 (300 IU/ml) or wildtype G-CSF (100 ng/ml). Arrow indicates the specific phospho-JAK2 band at 125kDa; larger bands are presumed to be the result of cross-reactivity of the primary anti-phospho-JAK2 antibody with phospho-JAK1. β-actin and histone H3 serve as protein loading controls. C) Graph showing the results of a BrdU incorporation assay to assess T-cell proliferation. Cells were harvested from the expansion assay, washed, and re-plated in IL-2 (300 IU/ml), wild type G-CSF (100 ng/ml) or no cytokine.

[00125] **Figure 41** presents graphs showing the fold expansion and G-CSFR ECD expression of primary human PBMC-derived T cells expressing G2R-3 with WT ECD versus non-transduced cells. A) Graph showing the results of a T-cell expansion assay, where cells were transduced with G2R-3-encoding lentivirus. On Day 1, WT G-CSF (100 ng/ml) or no cytokine (medium alone) were added to the culture. Thereafter, to Day 21, cells were replenished with medium containing WT G-CSF or no cytokine. On Day 21 of expansion, cells were washed and re-plated in WT G-CSF (100 ng/mL), IL-7 (20 ng/mL) and IL-15 (20 ng/mL), or no cytokine. Live cells were counted every 2-4 days. Squares represent cells stimulated with G-CSF. Triangles represent cells stimulated with G-CSF and re-plated in IL-7 and IL-15 on Day 21. Circles represent cells not stimulated with a cytokine. Diamonds represent cells stimulated with G-CSF, and re-plated in medium only on Day 21. B) Graph showing the expression of the G-CSFR ECD, as determined by flow cytometry, on Day 21 or 42 of expansion.

[00126] **Figure 42** presents graphs showing the intracellular signaling and immunophenotype of primary human PBMC-derived T cells expressing G2R-3 versus non-transduced cells. **A)** Western blot to assess intracellular signaling events. Cells were harvested from an expansion assay and stimulated with IL-2 (300 IU/ml) or wildtype G-CSF (100 ng/ml). β-actin serves as protein loading control. B, C) Representative flow cytometry plots and graphs showing the immunophenotype, assessed by flow cytometry, of cells expressing G2R-3 versus non-transduced cells on Day 42 of expansion.

[00127] **Figure 43** presents graphs showing the fold expansion of primary human PBMC-derived T cells expressing G2R-3 with 304 or 307 ECD versus non-transduced cells. A) Graph showing the results of a T-cell expansion assay, where cells were transduced with G2R-3 304 ECD-encoding lentivirus. B) Graph showing the results of a T-cell expansion assay, where cells were transduced with G2R-3 307 ECD-encoding lentivirus. C) Graph showing the results of a T-cell expansion assay with non-transduced cells. On Day 2 IL-2 (300 IU/mL), 304 G-CSF (100 ng/ml), 307 G-CSF (100 ng/mL) or no cytokine (medium alone) were added to the culture, as indicated, and replenished every two days thereafter. Live cells were counted every 3-4 days. Diamonds represent cells stimulated with 304 G-CSF. Squares represent cells stimulated with 307 G-CSF. Triangles represent cells stimulated with IL-2. Inverted triangles represent cells not stimulated with a cytokine.

[00128] **Figure 44** presents a graph showing the results of a BrdU incorporation assay to assess proliferation of primary human PBMC-derived T cells expressing G2R-3 with 304 or 307 ECD versus non-transduced cells. Cells were transduced with G2R-3 304 ECD- or 307 ECD-encoding lentivirus and expanded in the 304 or 307 G-CSF (100 ng/mL). Non-transduced cells were expanded in IL-2 (300 IU/mL). On Day 12 of expansion cells were washed, and re-plated in IL-2 (300 IU/ml), 130 G-CSF (100 ng/ml), 304 G-CSF (100 ng/ml), 307 G-CSF (100 ng/ml) or no cytokine.

[00129] **Figure 45** presents a graph showing G-CSFR ECD expression by flow cytometry in primary murine T cells transduced with the indicated chimeric receptor constructs.

[00130] **Figure 46** shows G-CSF-induced phosphorylation of STAT3 (detected by flow cytometry) in primary PBMC-derived human T cells expressing G21R-1 or G21R-2. Cells were subdivided (i.e., gated) into G-CSFR-positive (upper panels) or G-CSFR-negative (lower panels) populations.

[00131] **Figure 47** presents graphs and images showing G-CSF-induced biochemical signaling events in primary murine T cells expressing G21R-1 or G12R-1. A) Graph showing phosphorylation of STAT3 (detected by flow cytometry) in CD4+ or CD8+ cells transduced with G21R-1 and stimulated with no cytokine, IL-21 or G-CSF. B) Graph showing the percentage of cells staining positive for phospho-STAT3 after stimulation with no cytokine (black circles), IL-21 (squares) or WT G-CSF (gray circles). Live cells were gated on CD8 or CD4, and the percentage of phospho-STAT3-positive cells is shown for each population. C) Western blots to assess the indicated cytokine signaling events in cells expressing G21R-1 or G12R-1 and stimulated with IL-21, IL-12 or WT G-CSF. β–actin and histone H3 serve as protein loading controls.

[00132] **Figure 48** presents graphs and images showing proliferation, G-CSFR ECD expression and WT G-CSF-induced intracellular signaling events in primary murine T cells expressing G2R-2, G2R-3, G7R-1, G21/7R-1 and G27/2R-1, or mock-transduced T cells. A, B) Graphs showing the results of BrdU incorporation assays to assess T-cell proliferation. Cells were harvested, washed, and re-plated in IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. Panels A and B are experimental replicates. C) Graph showing G-CSFR ECD expression by flow cytometry in primary murine T cells transduced with the indicated chimeric receptor constructs. D) Western blots to assess the indicated cytokine

signaling events in cells expressing G2R-2, G2R-3, G7R-1, G21/7R-1 and G27/2R-1, or mock-transduced T cells. Cells were stimulated with IL-2 (300 IU/mL), IL-7 (10 ng/mL), IL-21 (10 ng/mL), IL-27 (50 ng/mL) or G-CSF (100 ng/mL). β–actin and histone H3 serve as protein loading controls.

[00133] **Figure 49** presents graphs and images showing proliferation, G-CSFR ECD expression and G-CSF-induced biochemical signaling events in primary murine T cells expressing G21/2R-1, G12/2R-1 and 21/12/2R-1, or mock-transduced T cells. A, B) Graphs showing the results of BrdU incorporation assays to assess T-cell proliferation. Cells were harvested, washed, and re-plated in IL-2 (300 IU/ml), wild type G-CSF (100 ng/ml) or no cytokine. Panels A and B are experimental replicates. C) Graph showing G-CSFR ECD expression by flow cytometry in primary murine T cells transduced with the indicated chimeric receptor constructs. D) Western blots to assess the indicated cytokine signaling events in cells expressing G21/2R-1, G12/2R-1 and G21/12/2R-1 or mock-transduced T cells. Cells were stimulated with IL-2 (300 IU/mL), IL-21 (10 ng/mL), IL-12 (10 ng/mL) or G-CSF (100 ng/mL). β–actin and histone H3 serve as protein loading controls.

[00134] **Figure 50** presents graphs showing the fold expansion and G-CSFR ECD expression of primary human PBMC-derived T cells expressing G12/2R-1 with 134 ECD, versus non-transduced cells. A) Graph showing the results of a T-cell expansion assay, where cells were transduced with lentivirus encoding G12/2R-1 134 ECD and expanded in IL-2 (300 IU/mL), 130 G-CSF (100 ng/ml) or medium. Live cells were counted every 4-5 days. Squares represent cells not stimulated with a cytokine. Triangles represent cells stimulated with 130 G-CSF. Diamonds represent cells stimulated with IL-2. B) Graph showing the results of a T-cell expansion assay, where cells were transduced as in panel A. On Day 19 of expansion, cells were washed and re-plated in IL-2, 130 G-CSF or medium only. Live cells were counted every 4-5 days. Squares represent cells not stimulated with a cytokine. Light grey diamonds represent cells stimulated with 130 G-CSF. Dark grey diamonds represent cells stimulated with IL-2. Light grey inverted triangles represent cells initially stimulated with IL-2, and then re-plated in medium only on Day 19. Dark grey triangles represent cells initially stimulated with 130 G-CSF, and then re-plated in medium alone on Day 19. C) Graph showing the expression of the G-CSFR ECD, as determined by flow cytometry, on Day 4 or 16 of expansion.

[00135] **Figure 51** presents graphs showing the proliferation and immunophenotype of primary human PBMC-derived T cells expressing G12/2R-1 134 ECD, versus non-transduced cells. A) Graph showing the results of a BrdU incorporation assay to assess T-cell proliferation. Cells were harvested, washed, and re-plated in IL-2 (300 IU/ml), IL-2 + IL-12 (300 IU/ml and 10 ng/mL, respectively), 130 G-CSF (300 ng/ml) or medium alone. B, C) Representative flow cytometry plots and graph showing the immunophenotype, assessed by flow cytometry, of cells expressing G12/2R-1 with 134 ECD, versus non-transduced cells, on Day 16 of expansion.

[00136] **Figure 52** presents graphs showing the fold expansion and proliferation of primary human PBMC-derived T cells expressing G12/2R-1 with 304 ECD, versus non-transduced cells. A) Graph showing the results of a T-cell expansion assay, where cells were transduced with lentivirus encoding G12/2R-1 134 ECD, and expanded in IL-2 (300 IU/mL), 130 G-CSF (100 ng/ml), 304 G-CSF (100 ng/ml) or medium alone. Non-transduced cells were cultured in IL-2, 130 G-CSF, 304 G-CSF or medium alone. Live cells were counted every 3-4 days. Inverted triangles represent cells not stimulated with cytokine. Triangles represent cells stimulated with IL-2. Circles represent cells stimulated with 130 G-CSF. Diamonds represent cells stimulated with 304 G-CSF. B) Cells were harvested from the expansion assay on Day 12, and were washed and re-plated in IL-2 (300 IU/ml), 130 G-CSF (300 ng/ml), 304 G-CSF (100 ng/ml), 307 G-CSF (100 ng/ml) or medium alone.

[00137] **Figure 53** presents western blots to detect the indicated cytokine signaling events in primary PBMC-derived T cells expressing G2R-3 with 304 ECD, G12/2R-1 with 304 ECD, or non-transduced T cells. Cells were harvested from the expansion assay and stimulated with 304 G-CSF (100 ng/mL), IL-2 (300 IU/mL), IL-2 and IL-12 (10 ng/mL), or medium alone, as indicated. Black arrows and small outcropped panel on the right indicate the molecular weight markers at 115 kDa and 140 kDa from a protein ladder. β-actin and histone H3 serve as protein loading controls.

[00138] **Figure 54** presents a size exclusion UV trace of refolded 130a1 G-CSF (labelled as GCSF_130a1) and the corresponding SDS PAGE purity gel stained with Coomassie blue. Refolded 130a1 G-CSF eluted at the expected volume relative to standards of known molecular weight.

[00139] **Figure 55** presents the results of BrdU incorporation assays to assess proliferation of (A, B, C) OCI-AML1 cells, which naturally express wild-type (WT) human G-CSFR, or (D, E) 32D clone 3 cells, which naturally express WT murine G-CSFR. Cells were stimulated with the following cytokines: (A) WT G-CSF or the G-CSF variants 130, 130a1, 130b1, 130a2, or 130b2; (B) WT G-CSF or the G-CSF variants 130, 130a1, or 130b1, or medium alone (no added cytokine); (C) WT G-CSF or the G-CSF variants 130, 130a1, or 130a1b1, or medium alone; (D) WT G-CSF or the G-CSF variants 130, 130a1, or 130b1, or medium alone; and (E) WT G-CSF or the G-CSF variants 130, 130a1, or 130a1b1, or medium alone. Solid squares represent WT G-CSF; solid triangles represent 130 G-CSF; solid inverted triangles represent 130a1 G-CSF; open circles represent 130a2 G-CSF; solid diamonds represent 130b1 G-CSF; open squares represent 130bd G-CSF; and open diamonds represent 130a1b1 G-CSF.

[00140] **Figure 56** presents the results of BrdU incorporation assays to assess proliferation of PBMC-derived human T cells expressing G12/2R-1 134 ECD. Cells were stimulated with (A) medium alone or medium plus IL-2, IL-2 + IL-12, or the G-CSF variants 130, 130a1, 130b1, 130a2 or 130b2; or (B) medium alone or medium plus IL-2, IL-2 + IL-12 or the G-CSF variants 130 or 130a1b1. Results are shown for T cells expressing G12/2R-1 134 ECD (i.e., T cells that were G-CSFR+ by flow cytometry).

[00141] **Figure 57** presents the results of an *in vivo* experiment to determine the safety and efficacy of adoptive cell therapy with tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) that were retrovirally transduced to express G2R-3 134 ECD and then infused into syngeneic, immune competent mice bearing established mammary tumors (NOP23 tumor line). Beginning on the day of T cell infusion (day 0), mice were randomized and treated with vehicle (n = 4 animals) or 130a1 G-CSF (10 μg/dose; n = 4 animals) daily for 14 days, followed by every other day for 14 days, for a total of 21 doses. (A) Tumor area (length x width) from day -2 to 80. Each line represents results from an individual animal. (B) Higher resolution view of tumor area from day -2 to 20. (C) Kaplan-Meier plot showing the percent of animals alive from day 0 to 80. (D) Expansion of OT-I T cells in peripheral blood shown as the average percentage of Thy1.1+ (OT-I) cells relative to all CD8+ T cells (mean +/- SEM). (E) Percentage of neutrophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (F) Percentage of eosinophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (G) Percentage of monocytes relative to all CD45+ cells in peripheral blood (mean +/-

SEM). Gray circles represent vehicle-treated animals and black triangles represent 130a1 G-CSF-treated animals.

[00142] **Figure 58** presents the results of an *in vivo* experiment to determine the safety and efficacy of adoptive cell therapy with tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) that were retrovirally transduced to express G12/2R-1 134 ECD and then infused into syngeneic, immune competent mice bearing established mammary tumors (NOP23 tumor line). Beginning on the day of T cell infusion (day 0), mice were randomized and treated with vehicle (n = 4 animals) or 130a1 G-CSF (10 μg/dose; n = 3 animals) daily for 14 days, followed by every other day for 14 days, for a total of 21 doses. (A) Tumor area (length x width) from day -2 to 80. Each line represents results from an individual animal. (B) Higher resolution view of tumor area from day -2 to 20. (C) Kaplan-Meier plot showing the percent of animals alive from day 0 to 80. (D) Expansion of OT-I T cells in peripheral blood shown as the average percentage of Thy1.1+ (OT-I) cells relative to all CD8+ T cells (mean +/- SEM). (E) Percentage of neutrophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (F) Percentage of eosinophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (G) Percentage of monocytes relative to all CD45+ cells in peripheral blood (mean +/- SEM). Gray circles represent vehicle-treated animals and black triangles represent 130a1 G-CSF-treated animals.

[00143] **Figure 59** presents the results for three control groups from the experiments shown in Figures 4 and 5. Tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) underwent a mock retroviral transduction procedure and were then infused into syngeneic, immune competent mice bearing established mammary tumors (NOP23 tumor line). Beginning on the day of T cell infusion (day 0), mice were randomized and treated with vehicle (n = 4 animals), IL-2 (30,000 IU/dose; n = 5 animals) or 130a1 (10 μg/dose; n = 5 animals) daily for 14 days, followed by every other day for 14 days, for a total of 21 doses. (A) Tumor area (length x width) from day -2 to 80. Each line represents results from an individual animal. (B) Higher resolution view of tumor area from day -2 to 20. (C) Kaplan-Meier plot showing the percent of animals alive from day 0 to 80. (D) Expansion of OT-I T cells in peripheral blood shown as the average percentage of Thy1.1+ (OT-I) cells relative to all CD8+ T cells (mean +/- SEM). (E) Percentage of neutrophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (F) Percentage of eosinophils relative to all CD45+ cells in peripheral blood (mean +/- SEM). (G) Percentage of monocytes relative to all CD45+ cells in peripheral blood (mean

+/- SEM). Gray circles represent vehicle-treated animals; gray squares represent IL-2-treated animals; and black triangles represent 130a1 G-CSF-treated animals.

[00144] **Figure 60** presents schematics of wild type cytokine receptor subunits and additional chimeric receptor designs.

[00145] **Figure 61** presents flow cytometry data showing cell surface expression of the G-CSFR 134 ECD on human PBMC-derived CD3+ T cells transduced with G4R 134 ECD. Non-transduced T cells served as a negative control.

[00146] **Figure 62** presents graphs showing the expansion (fold change in cell number) of human PBMC-derived T cells expressing (A) G4R 134 ECD cultured with medium alone or medium plus IL-2, 130a1 G-CSF or 130a1 G-CSF + IL-2. (B) Results for non-transduced T cells cultured with medium alone or medium plus IL-2 or 130a1 G-CSF + 307 G-CSF. The 307 G-CSF variant was included in this latter condition to serve as a control for another arm of this experiment (not shown); we previously established, and confirm here, that neither 130a1 G-CSF nor 307 G-CSF induces proliferation of non-transduced T cells. Diamonds represent cells cultured with 130a1 G-CSF (panel A) or 130a1 G-CSF + 307 G-CSF (panel B). Triangles represent cells cultured with IL-2. Light-grey circles represent cells cultured with 130a1 G-CSF and IL-2. Black circles represent cells cultured with medium alone (no added cytokine).

[00147] **Figure 63** presents graphs showing the results of a BrdU incorporation assay to assess proliferation of primary human T cells expressing (A) G4R 134 ECD versus (B) non-transduced T cells. Cells were stimulated with medium alone, IL-2, IL-4, IL2 + IL-4, 130a1 G-CSF, or 130a1 G-CSF + IL-2. Data is presented separately for the CD4+ and CD8+ T cell subsets. Data in panel A is gated on T cells expressing G4R 134 ECD (detected using an antibody against human G-CSFR).

[00148] **Figure 64** presents western blots to detect the indicated biochemical signaling events in human PBMC-derived T cells expressing G4R 134 ECD versus non-transduced cells. Cells were stimulated with IL-2, IL-4 or 130a1 G-CSF. β-actin and histone H3 served as protein loading controls.

[00149] **Figure 65** presents flow cytometry data showing cell surface expression of the G-CSFR 134 ECD on human PBMC-derived CD3+ T cells transduced with a lentivirus encoding G6R 134 ECD. Non-transduced T cells served as a negative control.

[00150] **Figure 66** presents graphs showing the expansion (fold change in cell number) of human PBMC-derived T cells expressing (A) G6R 134 ECD or (B) non-transduced T cells cultured in medium alone or medium with IL-2, 130a1 G-CSF, or 130a1 G-CSF + IL-2. Diamonds represent cells stimulated with 130a1 G-CSF. Triangles represent cells stimulated with IL-2. Light-grey circles represent cells stimulated with 130a1 G-CSF + IL-2. Black circles represent cells stimulated with medium alone (no added cytokine).

[00151] **Figure 67** presents graphs showing the results of a BrdU incorporation assay to assess proliferation of PBMC-derived human T cells expressing (A) G6R 134 ECD compared to (B) non-transduced T cells. Cells were stimulated with medium alone or medium plus IL-2, IL-6, IL-2 + IL-6, 130a1 G-CSF, or 130a1 G-CSF + IL-2. Data is shown separately for the CD4+ and CD8+ T cell subsets. Data in panel A was gated on T cells expressing G4R 134 ECD (detected using an antibody against human G-CSFR).

[00152] **Figure 68** presents western blots to detect the indicated biochemical signaling events in human PBMC-derived T cells expressing G6R 134 ECD versus non-transduced cells. Cells were stimulated with IL-2, IL-6 or 130a1 G-CSF. Histone H3 served as a protein loading control. Note that on the P-STAT3 image a dark, higher molecular band appears in the IL-2-stimulated conditions (especially in the non-transduced T cells). This is remnant signal from a previous probing of this membrane with phospho-STAT5 antibody. The lower molecular weight band (arrow) represents phospho-STAT3.

[00153] **Figure 69** presents flow cytometry data showing cell surface expression of the G-CSFR 134 ECD on human PBMC-derived CD3+ T cells transduced with GEPOR 134 ECD. Non-transduced T cells served as a negative control.

[00154] **Figure 70** presents graphs showing the expansion (fold change in cell number) of primary human PBMC-derived T cells expressing (A) GEPOR 134 ECD or (B) non-transduced T cells cultured in medium alone or medium with IL-2, 130a1 G-CSF, or 130a1 G-CSF + IL-2. For non-transduced T cells, 307 G-CSF was added to the 130a1 G-CSF condition to serve as a control for another arm of this experiment (not shown); we previously

established, and confirm here, that neither 130a1 G-CSF nor 307 G-CSF induces proliferation of non-transduced T cells. Diamonds represent cells cultured in 130a1 G-CSF +/- 307 G-CSF. Triangles represent cells cultured in IL-2. Light-grey circles represent cells cultured in 130a1 G-CSF + IL-2. Black circles represent cells cultured in medium alone.

[00155] **Figure 71** presents western blots to detect the indicated biochemical signaling events in human PBMC-derived T cells expressing GEPOR 134 ECD versus non-transduced cells. Cells were stimulated with medium alone or medium plus IL-2 or 130a1 G-CSF. β-actin and histone H3 served as protein loading controls.

[00156] **Figure 72** presents flow cytometry data showing cell surface expression of the G-CSFR 134 ECD on human PBMC-derived CD3+ T cells transduced with GIFNAR 134 ECD. Non-transduced T cells served as a negative control.

[00157] **Figure 73** presents graphs showing the expansion (fold change in cell number) of primary human PBMC-derived T cells that were (A) non-transduced or (B) transduced to express GIFNAR 134 ECD and cultured in medium alone or medium with IL-2, 130a1 G-CSF, or 130a1 G-CSF + IL-2. Diamonds represent cells cultured in 130a1 G-CSF. Triangles represent cells cultured in IL-2. Light-grey circles represent cells cultured in 130a1 G-CSF + IL-2. Black circles represent cells cultured in medium alone.

[00158] **Figure 74** presents western blots to detect the indicated biochemical signaling events in human PBMC-derived T cells expressing GIFNAR 134 ECD versus non-transduced cells. Cells were stimulated with medium alone or medium plus IL-2, IFNα or 130a1 G-CSF. β-actin and histone H3 served as protein loading controls.

[00159] **Figure 75** presents flow cytometric data showing Myc-tag and Flag-tag expression on the surface of human PBMC-derived T cells transduced with (A) GIFNGR-1 307 ECD, (B) G2R3 134 ECD, (C) GIFNGR-1 307 ECD and G2R3 134 ECD or (D) non-transduced T cells. G2R3 134 ECD was tagged at its N-terminus with a Myc epitope (EQKLISEEDL) and GIFNGR-1 307 ECD was tagged at its N-terminus with a Flag epitope (DYKDDDDK); the epitope tags aid detection by flow cytometry and do not impact the function of the receptors. Plots were gated on CD3+ cells.

[00160] **Figure 76** presents flow cytometric data showing Myc-tag and Flag-tag expression on the surface of human PBMC-derived T cells transduced with (A) GIFNGR-2

307 ECD, (B) G2R3 134 ECD, (C) GIFNGR-2 307 ECD and G2R3 134 ECD, or (D) non-transduced T cells. G2R3 134 ECD was tagged at its N-terminus with a Myc epitope (EQKLISEEDL), and GIFNGR-2 307 ECD was tagged at its N-terminus with a Flag epitope (DYKDDDDK); the epitope tags aid detection by flow cytometry and do not impact the function of the receptors. Plots were gated on CD3+ cells.

[00161] **Figure 77** presents graphs showing the expansion (fold change in cell number) of primary human PBMC-derived T cells that were (A) non-transduced, (B) transduced to express GIFNGR-1 307 ECD, or (C) co-transduced to express GIFNGR-1 307 ECD and G2R-3 134 ECD. T cells were cultured in medium alone or medium with the indicated combinations of IL-2, 130a1 G-CSF and 307 G-CSF. Diamonds and light-grey circles represent cells cultured in the indicated combinations of 130a1 G-CSF, 307 G-CSF and IL-2. Triangles represent cells cultured in IL-2. Black circles represent cells cultured in medium alone.

[00162] **Figure 78** presents graphs showing the expansion (fold change in cell number) of primary human PBMC-derived T cells that were (A) non-transduced, (B) transduced to express GIFNGR-2 307 ECD, or (C) co-transduced to express GIFNGR-2 307 ECD and G2R-3 134 ECD. T cells were cultured in medium alone or medium with the indicated combinations of IL-2, 130a1 G-CSF and 307 G-CSF. Diamonds and light-grey circles represent cells cultured in the indicated combinations of 130a1 G-CSF, 307 G-CSF and IL-2. Triangles represent cells cultured in IL-2. Black circles represent cells cultured in medium alone.

[00163] **Figure 79** presents graphs showing the results of a BrdU incorporation assay to assess proliferation of human PBMC-derived T cells expressing G2R-3 134 ECD and/or GIFNGR-1 307 ECD. Data are shown for (A) non-transduced T cells or (B) CD4+ and (C) CD8+ T cells expressing the indicated chimeric receptors (see X-axis). Cells were stimulated with the indicated combinations of IL-2, IFNγ, 130a1 G-CSF, 307 G-CSF or medium alone.

[00164] **Figure 80** presents graphs showing the results of a BrdU incorporation assay to assess proliferation of human PBMC-derived T cells expressing G2R-3 134 ECD and/or GIFNGR-2 307 ECD. Data are shown for (A) non-transduced T cells or (B) CD4+ and (C) CD8+ T cells expressing the indicated chimeric receptors (see X-axis). Cells were stimulated with the indicated combinations of IL-2, IFNγ, 130a1 G-CSF, 307 G-CSF or medium alone.

[00165] **Figure 81** presents western blots to detect the indicated biochemical signaling events in human PBMC-derived T cells expressing GIFNGR-1 307 ECD or GIFNGR-2 307 ECD versus non-transduced cells. Cells were stimulated with medium alone or medium plus IL-2, IFNγ or 307 G-CSF. β-actin and histone H3 served as protein loading controls.

[00166] **Figure 82** presents presents flow cytometry data showing cell surface expression of G2R-3 134 ECD and a mesothelin-specific CAR on human PBMC-derived CD3+ T cells transduced with the following mono- or bi-cistronic lentiviral constructs: (A) Non-transduced T cells (negative control); (B) mesothelin CAR alone; (C) G2R-3 134 ECD alone; (D) CAR_T2A_G2R-3-134 ECD, which has gene segments in the following order: mesothelin CAR, T2A site and G2R-3 134 ECD; and (E) G2R-3-134 ECD_T2A_CAR, which has gene segments in the following order: mesothelin CAR, T2A site and G2R-3 134 ECD.

[00167] **Figure 83** presents flow cytometry data showing cell surface expression of G12/2R-1 134 ECD and a mesothelin-specific CAR on human PBMC-derived CD3+ T cells transduced with the following mono- or bi-cistronic lentiviral constructs: (A) Non-transduced T cells (negative control); (B) mesothelin CAR alone; (C) G2R-3 134 ECD alone; (D) CAR_T2A_G12/2R-1-134 ECD, which has gene segments in the following order: mesothelin CAR, T2A site and G12/2R-1 134 ECD; and (E) G12/2R-1-134 ECD_T2A_CAR, which has gene segments in the following order: mesothelin CAR, T2A site and G12/2R-1 134 ECD.

[00168] **Figure 84** presents the results of BrdU incorporation assays to assess proliferation of PBMC-derived human T cells modified as follows: (A) non-transduced or expressing mesothelin CAR only; or (B) expressing G2R3 134 ECD, or bicistronic CAR_T2A_G2R3-134 ECD, or bicistronic CAR_T2A_G12/2R-1-134 ECD, or bicistronic G12/2R-1-134ECD_T2A_CAR constructs. Cells were stimulated with medium alone or medium plus IL-2 or 130a1 G-CSF. Results in panel B are shown for T cells that were positive for G-CSFR ECD expression by flow cytometry.

[00169] **Figure 85** presents the results of *in vitro* co-culture assays to assess the functional properties of a mesothelin CAR in PBMC-derived human CD4+ T cells expressing: (A) mesothelin CAR only; (B) G12/2R-1 134 ECD only, or the following bicistronic constructs containing a mesothelin CAR: (C) CAR_T2A_G2R3-134 ECD, (D) G2R3-134 ECD_T2A_CAR, (E) CAR_T2A_G12/2R-1-134 ECD or (F) G12/2R-1-134ECD_T2A_CAR. Cells were left non-stimulated or stimulated with OVCAR3 cells for 13

hours, followed by intracellular flow cytometry to detect expression of the cytokines IFNγ, TNFα and IL-2, as well as the surface molecules CD69 and CD137. Results in panels A, C, D, E and F are gated on cells expressing the mesothelin CAR.

[00170] **Figure 86** is a schematic of native cytokine receptors, including IL-7Rα, IL-2Rβ, gp130, IL-21R, IL-12Rβ2 and IL-4Rα.

[00171] **Figure 87** is a schematic of 7/2R-1, 7/2R-2, 7/2/12R-1, 7/2/12R-2, 7/21R-1, 7/21R-2, 7/7/21R-1, 7/7/21R-2, 7/2/21R-1, 7/2/21R-27/2/12/21R-1, 7/2/12/21R-2, 7/2/12/21R-3, 7/2/12/21R-4, 7/4R-1, 7/4R-2, 7/6R-1, and 7/6R-2 receptor subunit designs.

[00172] **Figure 88** presents graphs showing human CD127 (IL-7Rα) and Flag-tag expression assessed by flow cytometry in (A) primary human PBMC-derived T cells transduced with 7/2R-1, (B) non-transduced T cells, or (C) T cells transduced with 7/2R-1 but stained as a fluorescence-minus-one (FMO) control (i.e., the antibody to CD127 was left out of the antibody staining panel). 7/2R-1 was tagged at its N-terminus with a Flag epitope; the epitope tag aids detection by flow cytometry and does not impact the function of the receptors. Receptor expression was analyzed 12 days after lentiviral transduction.

[00173] **Figure 89** presents graphs showing the expansion (fold change in cell number) of primary human PBMC-derived T cells (A) expressing 7/2R-1 versus (B) non-transduced T cells and cultured with IL-7, IL-7 + IL-15, or no cytokine. Diamonds represent cells stimulated with IL-7. Triangles represent cells stimulated with IL-7 and IL-15. Circles represent cells cultured in medium alone (no added cytokine).

[00174] **Figure 90** presents graphs showing the results of a BrdU incorporation assay to assess proliferation of primary human T cells expressing (A) 7/2R-1 versus (B) non-transduced T cells. Cells were stimulated with IL-7, IL-2, IL-2 + IL-7, or medium alone (no added cytokine). Results are shown separately for the CD4+ and CD8+ T cell subsets.

[00175] **Figure 91** presents western blots to detect the indicated cytokine signaling events in human primary T cells expressing 7/2R-1 versus non-transduced T cells. β-actin and histone H3 serve as protein loading controls. Cells were stimulated with IL-7, IL-2, IL-2 + IL-7, or medium alone (no added cytokine).

[00176] **Figure 92** presents an SDS-PAGE gel to detect unmodified 130a1 G-CSF, and pegylated forms of 130a1 G-CSF (PEG20k_G-CSF_130a1) and 307 G-CSF (PEG20k_G-CSF_307). Gel was stained with Coomassie brilliant blue.

[00177] **Figure 93** presents the results of an *in vivo* experiment to determine the safety and efficacy of adoptive cell therapy with tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) that were retrovirally transduced to express G4R 134 ECD and then infused into syngeneic, immune competent mice bearing established mammary tumors (NOP23 tumor line). Beginning on the day of T cell infusion (day 0), mice were randomized and treated with vehicle (n = 3 animals) or 130a1 G-CSF (10 μg/dose; n = 3 animals) daily for 14 days, followed by every other day for 14 days, for a total of 21 doses. (A) Tumor area (length x width) from day 0 to 46. Each line represents results from an individual animal. (B) Expansion of OT-I T cells in peripheral blood shown as the mean percentage of Thy1.1+ (OT-I) cells relative to all CD8+ T cells (mean +/- standard deviation [SD]). (C) Percentage of OT-I T cells (Thy1.1+) exhibiting a T effector memory (Tem; CD44+ CD62L-) phenotype (mean +/- SD). (D) Percentage of host (Thy1.1-) CD8+ T cells exhibiting a Tem phenotype (mean +/- SD). (E) Percentage of OT-I T cells (Thy1.1+) expressing Programmed Death-1 (PD-1) (mean +/- SD). (F) Percentage of host (Thy1.1-) CD8+ T cells expressing PD-1 (mean +/- SD). (G) Percentage of host (Thy1.1-) CD3+ T cells relative to all CD45+ cells in peripheral blood (mean +/- SD). (H) Percentage of host (Thy1.1-) CD19+ B cells relative to all CD45+ cells in peripheral blood (mean +/- SD). Gray circles represent vehicle-treated animals and black triangles represent 130a1 G-CSF-treated animals.

[00178] **Figure 94** presents the results of an *in vitro* experiment to compare pegylated versus non-pegylated versions of G-CSF (wildtype, 130a1 and 307) for the ability to stimulate proliferation of cells expressing G2R-3 134 ECD or the native G-CSF receptor. Data is presented as the mean and standard deviation for duplicate wells. (A) Human PBMC-derived CD4 and CD8 T cells expressing G2R-3 134 ECD were stimulated with the indicated cytokines: human IL-2 (Proleukin, 300 IU/ml, positive control), medium alone (negative control), or the indicated concentrations of wildtype G-CSF, pegylated wildtype G-CSF, 130a1 G-CSF, or pegylated 130a1 G-CSF. Cells were cultured for 48 hours and assessed by BrdU incorporation assay. Results are shown for T cells that were positive for human G-CSFR. (B) A similar experiment was performed using the human myeloid cell line OCI-AML-1, which naturally expresses the wildtype human G-CSF receptor. Cells were

stimulated with the indicated cytokines: human GM-CSF (20 ng/ml, positive control), medium alone (negative control), or the indicated concentrations of wildtype G-CSF, pegylated wildtype G-CSF, 130a1 G-CSF, pegylated 130a1 G-CSF, 307 G-CSF, or pegylated 307 G-CSF. Cells were cultured for 48 hours and assessed by BrdU incorporation assay.

[00179] **Figure 95** presents the results of an *in vitro* Western blot experiment comparing the ability of pegylated versus non-pegylated versions of 130a1 G-CSF to induce the indicated biochemical signaling events in human PBMC-derived T cells expressing G2R-3 134 ECD. T cells were stimulated for 20 min with human IL-2 (Proleukin, 300 IU/ml) or the indicated concentrations (in ng/ml) of non-pegylated or pegylated (PEG) versions of 130a1 G-CSF. Histone H3 and Total S6 serve as gel loading controls.

[00180] **Figure 96** presents the results of an *in vivo* experiment comparing the potency of pegylated (PEG) versus non-pegylated versions of 130a1 G-CSF given at daily, every three days, or weekly intervals. Tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) were retrovirally transduced to express G2R-3 134 ECD and then infused into syngeneic, immune competent mice bearing established mammary tumors (NOP23 tumor line). Tumor size (length x width) is plotted over a 46-day period. Each line represents results from an individual animal. The right panels are an expanded version of the left panels. Beginning on the day of T cell infusion (day 0), mice were randomized and treated with vehicle, 130a1 G-CSF, or PEG-130a1 G-CSF as indicated. (A, B) The "daily" dosing cohort received the indicated cytokines (or vehicle) daily for 14 days, followed by every other day for 14 days, for a total of 21 doses. (C, D) The "every three days" dosing cohort received the indicated cytokines every three days for a total of 9 doses. (E,F) The "weekly" dosing cohort received the indicated cytokines every seven days for a total of 4 doses.

[00181] **Figure 97** shows the expansion and effector memory (Tem) phenotype of OT-I T cells in peripheral blood at the indicated time points from the experiment shown in Figure 96. Left panels show the percentage of Thy1.1+ (OT-I) T cells relative to all CD8+ T cells (mean +/-SD). Right panels show the percentage of Thy1.1+ (OT-I) T cells that have a Tem (CD44+ CD62L-) phenotype (mean +/- SD). (A, D) "Daily" dosing cohort. (B, E) "Every three days" dosing cohort. (C,F) "Weekly" dosing cohort.

[00182] **Figure 98** shows the percentage of the indicated immune cell subsets (relative to all CD45+ cells) in peripheral blood at the indicated time points from the "every three days" cohort from the experiment shown in Figures 102 and 103. Mean and standard deviation are

plotted. (A) Neutrophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G+). (B) Monocytes (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC-low (Ly6C+ or Ly6C-). (C) Eosinophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC-high). (D) CD3+ T cells. (E) CD19+ B cells. (F) NK1.1+ Natural Killer cells.

[00183] **Figure 99** shows *in vitro* data from an IL-18 Controlled Paracrine Signaling (CPS) experiment. Human PBMC-derived T cells were transduced with either a bi-cistronic vector encoding a mesothelin-specific CAR, T2A site, and G12/2R-1 134 ECD (Meso CAR_G12/2R-1), or a tri-cistronic vector encoding a mesothelin-specific CAR, T2A site, G12/2R-1 134 ECD and human IL-18 (Meso CAR_G12/2R-1 + h18). (A) T cells were assessed by flow cytometry for expression of the mesothelin CAR (X-axis) and G12/2R-1 134 ECD (Y-axis). (B) An *in vitro* BrdU assay was used to assess T cell proliferation in response to medium alone; human IL-2 (Proleukin, 300 IU/ml); human IL-18 (100 ng/ml); human IL-2 + human IL-12 (20 ng/ml); IL-2 + IL-12 + IL-18; or 130a1 G-CSF (100 ng/ml). (C) ELISA was used to assess human IL-18 levels in culture supernatants from T cells stimulated for 48 hours with media alone; human IL-2 (Proleukin, 300 IU/ml) + human IL-12 (20 ng/ml); or 130a1 G-CSF (100 ng/ml).

[00184] **Figure 100** shows the results of an *in vitro* IL-18 CPS experiment with murine T cells. Mouse CD4 and CD8 T cells were transduced with either a mono-cistronic vector encoding G12/2R-1 134 ECD, or a bi-cistronic vector encoding G12/2R 134 ECD, T2A site and murine IL-18 (G12/2R-1 134 ECD + m18). (A) T cells were assessed by flow cytometry for expression of the G12/2R-1 134 ECD. (B) An *in vitro* BrdU assay was used to assess T cell proliferation in response to medium alone; human IL-2 (Proleukin, 300 IU/ml); murine IL-18 (100 ng/ml); human IL-2 + murine IL-12 (10 ng/ml); IL-2 + IL-12 + IL-18; or 130a1 G-CSF (100 ng/ml). (C) ELISA was used to assess murine IL-18 levels in culture supernatants from T cells stimulated for 48 hours with medium alone; human IL-2 (Proleukin, 300 IU/ml) + murine IL-12 (10 ng/ml); or 130a1 G-CSF (100 ng/ml).

[00185] **Figure 101** shows the results of an *in vitro* IL-18 CPS experiment with murine OT-I T cells. OT-I T cells were transduced with retroviral vectors encoding G2R-2 134 ECD, G2R-3 134 ECD, G12/2R-1 134 ECD or G12/2R-1 134 ECD + m18 (abbreviated as G12/2R/18C in the figure). To detect secreted cytokines, T cells were washed five days later and cultured for 48 hours in medium alone (negative control) or medium with human IL-2

(Proleukin 300 IU/ml), human IL-2 + murine IL-12 (10 ng/ml), or pegylated (Peg) 130a1 (100 ng/ml), followed by a multiplex assay to detect 32 cytokines. Results are shown for (A) murine IL-18, and (B) murine IFN-gamma. Results for other cytokines are shown in Table 35.

[00186] **Figure 102** presents the results of an *in vivo* experiment comparing the properties of G7R-1, G2R-2, G12/2R-1, and G12/2R-1 + m18 (abbreviated as G12/2R/18C) (all with the 134 ECD) in OT-I T cells in the NOP23 mammary tumor model. Tumor-specific CD8 T cells (Thy1.1+ OT-I T cells) were retrovirally transduced to express G7R-1, G2R-2, G12/2R-1 or G12/2R-1 + m18 (all with the 134 ECD) and then infused into syngeneic, immune competent mice bearing established NOP23 mammary tumors. Beginning on the day of T cell infusion (day 0), mice were randomized to receive vehicle or pegylated (PEG) 130a1 G-CSF (10 μg/dose) on a weekly basis for a total of four treatments. (A-D) Flow cytometry results showing receptor (G-CSFR) expression on transduced OT-I T cells on the day of T cell infusion (Day 0). (E-H) Tumor size (length x width) over a 50-day period. Each line represents an individual animal. (I-L) Expansion and persistence of Thy1.1+ OT-I cells in serial blood samples. (M-P) Percentage of Thy1.1+ OT-I T cells exhibiting a T effector memory (Tem) phenotype (CD44+CD62L-) in blood.

[00187] **Figure 103** presents the results of an *in vivo* experiment in the NOP23 mammary tumor model comparing different doses of pegylated (PEG) 130a1 G-CSF, or wild type IL-2. Thy1.1+ OT-I T cells expressing G2R-3 134 ECD were infused into syngeneic, immune competent mice bearing established tumors. Beginning on the day of T cell infusion (day 0), mice were randomized to six cytokine treatment groups: vehicle alone (shown in all panels); PEG-130a1 G-CSF 2 μg/dose, four weekly doses (panels A, F, K, P); (3) PEG-130a1 G-CSF 0.4 μg/dose, four weekly doses (panels B, G, L, Q); (4) PEG-130a1 G-CSF 0.08 μg/dose, four weekly doses (panels C, H, M, R); (5) PEG-130a1 G-CSF four daily doses of 0.1 μg followed by three weekly doses of 0.4 μg (panels D, I, N, S); or (6) human IL-2 (Proleukin) at 30,000 IU/day for 14 days followed by 30,000 IU every two days for 14 days (panels E, J, O, T). Serial blood samples were analyzed by flow cytometry for the following parameters: (A-E) Percentage of Thy1.1+ OT-I cells relative to all CD8+ T cells (mean +/- SD). (F-J) Percentage of Thy1.1+ OT-I cells with a T effector memory (Tem) phenotype (CD44+CD62L-) (mean +/- SD). (K-O) Percentage of neutrophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G+) relative to all CD45+ cells (mean +/- SD). (P-T) Percentage of

eosinophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC-high) relative to all CD45+ cells (mean +/- SD).

DETAILED DESCRIPTION

Briefly, and as described in more detail below, described herein are methods and compositions for selective activation of cells using variant cytokine receptor and cytokine pairs, wherein the cytokine receptors comprise a variant extracellular domain (ECD) of granulocyte-colony stimulating factor receptor (G-CSFR). In certain embodiments, the methods and compositions described herein are useful for exclusive activation of cells for adoptive cell transfer (ACT) therapy. Thus, included herein are methods for producing cells expressing variant receptors that are selectively activated by a cytokine that does not bind its native receptor. Also disclosed herein, are methods of treating a subject in need thereof, comprising administering to the subject a cell expressing receptors comprising a variant ECD of G-CSFR and co-administering a variant of G-CSF that binds to the variant ECD of G-CSFR. In certain aspects, the compositions and methods described herein address an unmet need for the selective activation of cells for adoptive cell transfer methods and may reduce or eliminate the need for immuno-depletion of a subject prior to adoptive cell transfer or for administering broad-acting stimulatory cytokines such as IL-2.

Definitions

[00188] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[00189] The term “treatment” refers to any therapeutically beneficial result in the treatment of a disease state, e.g., a cancer disease state, lessening in the severity or progression, remission, or cure thereof.

[00190] The term “in vivo” refers to processes that occur in a living organism.

[00191] The term “mammal” as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[00192] The term “sufficient amount” means an amount sufficient to produce a desired effect, e.g., an amount sufficient to selectively activate a receptor expressed on a cell.

[00193] The term “therapeutically effective amount” is an amount that is effective to ameliorate a symptom of a disease.

[00194] The term "operatively linked" refers to nucleic acid or amino acid sequences that are placed into a functional relationship with another nucleic acid or amino acid sequence, respectively. Generally, "operatively linked" means that nucleic acid sequences or amino acid sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase.

[00195] As used herein, the term "extracellular domain" (ECD) refers to the domain of a receptor (e.g., G-CSFR) that, when expressed on the surface of a cell, is external to the plasma membrane. In certain embodiments the ECD of G-CSFR comprises at least a portion of SEQ ID NO. 2 or SEQ ID NO. 7.

[00196] As used herein, the term "intracellular domain" (ICD) refers to the domain of a receptor that is located within the cell when the receptor is expressed on a cell surface.

[00197] As used herein, the term "transmembrane domain" (TMD or TM) refers to the domain or region of a cell surface receptor that is located within the plasma membrane when the receptor is expressed on a cell surface.

[00198] The term "cytokine" refers to small proteins (about 5–20 kDa) that bind to cytokine receptors and can induce cell signaling upon binding to and activation of a cytokine receptor expressed on a cell. Examples of cytokines include, but are not limited to: interleukins, lymphokines, colony stimulating factors and chemokines.

[00199] The term "cytokine receptor" refers to receptors that bind to cytokines, including type 1 and type 2 cytokine receptors. Cytokine receptors include, but are not limited to, G-CSFR, IL-2R (Interleukin-2 receptor), IL-7R (Interleukin-7 receptor), IL-12R (Interleukin-12 Receptor), and IL-21R (Interleukin-21 Receptor).

[00200] The term "chimeric receptors," as used herein, refers to a transmembrane receptor that is engineered to have at least a portion of at least one domain (e.g., ECD, ICD, TMD, or C-terminal region) that is derived from sequences of one or more different transmembrane proteins or receptors.

[00201] The term "Site II interface," "Site II region," "Site II interface region," or "Site II," as used herein, refers to the larger of the G-CSF:G-CSFR 2:2 heterodimer binding interfaces of G-CSF with G-CSFR, located at the interface between G-CSF and the Cytokine Receptor Homologous (CRH) domain of G-CSFR.

[00202] The term "Site III interface," "Site III region," "Site III interface region," or "Site III," as used herein, refers to the smaller of the G-CSF:G-CSFR 2:2 heterodimer binding

interfaces of G-CSF with G-CSFR, and is located at the interface between G-CSF and the N-terminal Ig-like domain of G-CSFR.

[00203] The term "at least a portion of" or "a portion of," as used herein, in certain aspects refers to greater than 75%, greater than 80%, greater than 90%, greater than 95%, greater than 99% of the length of contiguous nucleic acid bases or amino acids of a SEQ ID NO described herein. In certain aspects, at least a portion of a domain or binding site (e.g., ECD, ICD, transmembrane, C-terminal region or signaling molecule binding site) described herein can be greater than 75%, greater than 80%, greater than 90%, greater than 95%, greater than 99% identical to a SEQ ID NO. described herein.

[00204] The term "wild-type" refers to the native amino acid sequence of a polypeptide or native nucleic acid sequence of a gene coding for a polypeptide described herein. The wild-type sequence of a protein or gene is the most common sequence of the polypeptide or gene for a species for that protein or gene.

[00205] The terms "variant cytokine-receptor pair," "variant cytokine and receptor pairs," "variant cytokine and receptor design(s)," "variant cytokine-receptor switch," or "orthogonal cytokine-receptor pair" refers to genetically engineered pairs of proteins that are modified by amino acid changes to (a) lack binding to the native cytokine or cognate receptor; and (b) to specifically bind to the counterpart engineered (variant) ligand or receptor.

[00206] The term "variant receptor," or "orthogonal receptor" as used herein, refers to the genetically engineered receptor of a variant cytokine-receptor pair and includes chimeric receptors.

[00207] The term "variant ECD," as used herein, refers to the genetically engineered extracellular domain of a receptor (e.g., G-CSFR) of a variant cytokine-receptor pair.

[00208] The term "variant cytokine," "variant G-CSF," or "orthogonal cytokine" as used herein, refers to the genetically engineered cytokine of a variant cytokine-receptor pair.

[00209] As used herein, "do not bind, " "does not bind" or "incapable of binding" refers to no detectable binding, or an insignificant binding, i.e., having a binding affinity much lower than that of the natural ligand.

[00210] The term "selectively activates," or "selective activation," as used herein when referring to a cytokine and avariant receptor, refers to a cytokine that binds preferentially to a variant receptor and the receptor is activated upon binding of a cytokine to the variant receptor. In certain aspects, the cytokine selectively activates a chimeric receptor that has

been co-evolved to specifically bind the cytokine. In certain aspects, the cytokine is a wild-type cytokine and it selectively activates a chimeric receptor that is expressed on cells, whereas the native, wild-type receptor to the cytokine is not expressed in the cells.

[00211] The term, "enhanced activity," as used herein, refers to increased activity of a variant receptor expressed on a cell upon stimulation with a variant cytokine, wherein the activity is an activity observed for a native receptor upon stimulation with a native cytokine.

[00212] The term, "antigen binding signaling receptor" refers to any cell surface protein or protein complex that can bind an antigen and generate an intracellular signal upon binding to the antigen.

[00213] The term "agonistic signaling protein", as used herein, refers to a protein that binds a target binding molecule (e.g., protein receptor or antigen), and the binding induces one or more signaling events in the target cell harboring the target binding molecule. Conversely, the term, "antagonistic signaling protein" refers to a protein that binds a target binding molecule (e.g., protein receptor or antigen), and the binding inhibits one or more signaling events in the target cell harboring the target binding molecule (by e.g., interfering with other agonistic proteins to bind the same target molecule).

[00214] The term "affinity reagent", as used herein, refers to any molecule (e.g, protein, nucleic acid, etc.) that has an ability to bind any target molecule.

[00215] The term "immune cell" refers to any cell that is known to function to support the immune system of an organism (including innate and adaptive immune responses), and includes, but is not limited to, Lymphocytes (e.g., B cells, plasma cells and T cells), Natural Killer Cells (NK cells), Macrophages, Monocytes, Dendritic cells, Neutrophils, and Granulocytes. Immune cells include stem cells, immature immune cells and differentiated cells. Immune cells also include any sub-population of cells, however rare or abundant in an organism. In certain embodiments, an immune cell is identified as such by harboring known markers (e.g., cell surface markers) of immune cell types and sub-populations.

[00216] The term "T cells" refers to mammalian immune effector cells that may be characterized by expression of CD3 and/or T cell antigen receptor, which cells may be engineered to express an orthologous cytokine receptor . In some embodiments, the T cells are selected from naïve $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD4^+$ T cells, helper T cells, e. g., T_H2 , T_H9 , T_H11 , T_H22, T_{FH}; regulatory T cells, e.g., T_R1 , natural T_{Reg}, inducible T_{Reg};

memory T cells, e.g., central memory T cells, effector memory T cells, NKT cells, and γδT cells.

[00217] The term "G-CSFR" refers to Granulocyte Colony-Stimulating Factor Receptor. G-CSFR can also be referred to as: GCSFR, G-CSF Receptor, Colony Stimulating Factor 3 Receptor, CSF3R, CD114 Antigen, or SCN7. Human G-CSFR is encoded by the gene having an Ensembl identification number of: ENSG00000119535. Human G-CSFR is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_156039.3.

[00218] The term "G-CSF" refers to Granulocyte Colony Stimulating Factor. G-CSF can also be called Colony Stimulating Factor 3 and CSF3. Human G-CSF is encoded by the gene having an Ensembl identification number of: ENSG00000108342. Human G-CSF is encoded by the cDNA sequence corresponding to GeneBank Accession number KP271008.1.

[00219] "JAK" can also be referred to as Janus Kinase. JAK is a family of intracellular, nonreceptor tyrosine kinases that transduce cytokine-mediated signals via the JAK-STAT pathway and includes JAK1, JAK2, JAK3 and TYK2. Human JAK1 is encoded by the gene having an Ensembl identification number of: ENSG00000162434. Human JAK1 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_002227. Human JAK2 is encoded by the gene having an Ensembl identification number of: ENSG00000096968. Human JAK2 is encoded by the cDNA sequence corresponding to GeneBank Accession number_NM_001322194. Human JAK3 is encoded by the gene having an Ensembl identification number of: ENSG00000105639. Human JAK3 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_000215. Human_TYK2 is encoded by the gene having an Ensembl identification number of: ENSG00000105397. Human TYK2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_001385197.

[00220] STAT can also be referred to as Signal Transducer and Activator of Transcription. STAT is a family of 7 STAT proteins: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. Human STAT1 is encoded by the gene having an Ensembl identification number of: ENSG00000115415. Human STAT1 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_007315. Human_STAT2 is encoded by the gene having an Ensembl identification number of: ENSG00000170581. Human STAT2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_005419. Human_STAT3

is encoded by the gene having an Ensembl identification number of: ENSG00000168610. Human STAT3 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_139276. Human STAT4 is encoded by the gene having an Ensembl identification number of: ENSG00000138378. Human STAT4 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_003151. Human STAT5A is encoded by the gene having an Ensembl identification number of: ENSG00000126561. Human STAT5A is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_003152. Human STAT5B is encoded by the gene having an Ensembl identification number of: ENSG00000173757. Human STAT5B is encoded by the cDNA sequence corresponding to GeneBank Accession number_NM_012448. Human STAT6 is encoded by the gene having an Ensembl identification number of: ENSG00000166888. Human STAT6 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_003153.

[00221] SHC can also be referred to as Src Homology 2 Domain Containing Transforming Protein. Shc is a family of three isoforms and includes p66Shc, p52Shc and p46Shc, SHC1, SHC2 and SHC3. Human SHC1 is encoded by the gene having an Ensembl identification number of: ENSG00000160691. Human SHC1 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_183001. Human SHC2 is encoded by the gene having an Ensembl identification number of: ENSG00000129946. Human SHC2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_012435. Human SHC3 is encoded by the gene having an Ensembl identification number of: ENSG00000148082. Human SHC3 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_016848.

[00222] SHP-2 can also be referred to as Protein Tyrosine Phosphatase Non-Receptor Type 11 (PTPN11) and Protein-Tyrosine Phosphatase 1D (PTP-1D). Human SHP-2 is encoded by the gene having an Ensembl identification number of: ENSG00000179295. Human SHP-2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_001330437.

[00223] PI3K can also be referred to as Phosphatidylinositol-4,5-Bisphosphate 3-Kinase. The catalytic subunit of PI3K can be referred to as PIK3CA. Human PIK3CA is encoded by the gene having an Ensembl identification number of: ENSG00000121879. Human PIK3CA

is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_006218.

[00224] EPOR can also be referred to as Erythropoietin Receptor. Human EPOR is encoded by the gene having an Ensembl identification number of: ENSG00000187266. Human EPOR is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_000121.

[00225] IFNγR1 can also be referred to as Interferon Gamma Receptor 1. Human IFNγR1 is encoded by the gene having an Ensembl identification number of: ENSG00000027697. Human IFNγR1 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_000416.

[00226] IFNγR2 can also be referred to as Interferon Gamma Receptor 2. Human IFNγR2 is encoded by the gene having an Ensembl identification number of: ENSG00000159128. Human IFNγR2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_001329128.

[00227] IFNAR2 can also be referred to as Interferon Alpha and Beta Receptor Subunit 2 Human IFNAR2 is encoded by the gene having an Ensembl identification number of: ENSG00000159110. Human IFNAR2 is encoded by the cDNA sequence corresponding to GeneBank Accession number NM_000874.

[00228] Abbreviations used in this application include the following: ECD (extracellular domain), ICD (intracellular domain), TMD (transmembrane domain), a G-CSFR (Granulocyte-Colony Stimulating Factor Receptor), a G-CSF (Granulocyte-Colony Stimulating Factor), IL (Interleukin), IL-2R (Interleukin-2 receptor), IL-12R (Interleukin 12 Receptor), IL-21R (Interleukin-21 Receptor) and IL-7R or IL-7Rα (Interleukin-7 receptor), IL-18 (Interleukin-18), IL-21 (Interleukin-21), IL-17 (Interleukin-17), TNF- α (Tumor Necrosis Factor Alpha), CXCL13 (C-X-C Motif Chemokine Ligand 13), CCL3 (C-C Motif Chemokine Ligand 3 or MIP-1α), CCL4 (C-C Motif Chemokine Ligand 4 or MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21 (C-C Motif Chemokine Ligand 21), CCL5 (C-C Motif Chemokine Ligand 5), XCL1 (X-C Motif Chemokine Ligand 1), CCL19 (C-C Motif Chemokine Ligand 19), and NKG2D (Killer Cell Lectin Like Receptor K1). IL-2Rγ can also be referred to herein as: IL-2RG, IL-2Rgc, γc, or IL-2Rγ. For

select chimeric cytokine receptor designs: "G-CSFRwt-ICDIL-2Rb" is herein also referred to as "G/IL-2Rb"; "G-CSFRwt-ICDgc" is herein also referred to as "G/gc"; "G-CSFR137-ICDgp130-IL-2Rb" is herein also referred to as "G2R-2 with 137 ECD"; and "G-CSFR137-ICDIL-2Rb + GCSFR137-ICDgc" is herein also referred to as "G2R-1 with 137 ECD".

[00229] It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[00230] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Variant cytokine and receptor designs

[00231] Described herein are variant cytokine and receptor pairs for selective activation of the variant receptors. The variant receptors of the instant disclosure comprise an extracellular domain of G-CSFR; and the variant cytokine comprises a G-CSF (Granulocyte Colony-Stimulating Factor), which binds to and activates the variant receptor. In certain embodiments, the variant receptors are chimeric receptors comprising an ECD of G-CSFR and at least a portion of an ICD of a receptor different from G-CSFR.

Varaint G-CSF and Variant G-CSFR ECD pairs

[00232] In certain aspects, the variant G-CSF and receptor designs described herein comprise at least one Site II interface region mutation, at least one Site III interface region mutation, and combinations thereof. In certain aspects, the variant G-CSF and receptor designs described herein comprise at least one Site II or Site III interface region mutation listed in Tables 2, 2A, 4 or 6.

[00233] In certain aspects, at least one mutation on the variant receptor in the site II interface region is located at an amino acid position of the G-CSFR extracellular domain

selected from the group consisting of amino acid position: 141,167, 168, 171, 172, 173, 174, 197, 199, 200, 202 and 288 of the G-CSFR extracellular domain (SEQ ID NO. 2).

[00234] In certain aspects, at least one mutation on the variant G-CSF site II interface region is located at an amino acid position of the G-CSF selected from the group consisting of amino acid position: 12, 16, 19, 20, 104, 108, 109, 112, 115, 116, 118, 119, 122 and 123 of G-CSF (SEQ ID NO. 1).

[00235] In certain aspects, at least one mutation on the variant receptor site II interface region is selected from the group of mutations of the G-CSFR extracellular domains consisting of: R141E, R167D, K168D, K168E, L171E, L172E, Y173K, Q174E, D197K, D197R, M199D, D200K, D200R, V202D, R288D, and R288E.

[00236] In certain aspects, at least one mutation on the variant G-CSF site II interface region is selected from the group of mutations of G-CSF consisting of: K16D, R, S12E, S12K, S12R, K16D, L18F, E19K, E19R,Q20E, D104K, D104R, L108K, L108R, D109R, D112R, D112K, T115E, T115K, T116D, Q119E, Q119R, E122K, E122R, and E123R.

[00237] In certain aspects, at least one mutation on the variant site III interface region is selected from the group of mutations of the G-CSFR extracellular domain selected for the group consisting of amino acid position: 30, 41, 73, 75, 79, 86, 87, 88, 89, 91, and 93 of SEQ ID NO. 2.

[00238] In certain aspects, at least one mutation on the variant site III interface region is selected from the group of mutations of the G-CSF selected for the group consisting of amino acid position: 38, 39, 40, 41, 46, 47, 48, 49, and 147 of SEQ ID NO. 1.

[00239] In certain aspects, at least one mutation on the variant receptor site III interface region is selected from the group of mutations of the G-CSFR extracellular domains consisting of S30D, R41E, Q73W, F75K, S79D, L86D, Q87D, I88E, L89A, Q91D, Q91K, and E93K.

[00240] In certain aspects, at least one mutation on the variant G-CSF site III interface region is selected from the group of mutations of G-CSF consisting of: T38R, Y39E, K40D, K40F, L41D, L41E, L41K, E46R, L47D, V48K, V48R, L49K, and R147E.

[00241] The variant cytokine and receptor pairs described herein can comprise mutations in either the site II region alone, the site III region alone or both the site II and site III regions.

[00242] The variant cytokine and receptor pairs described herein can have any number of site II and/or site III mutations described herein. In certain aspects, the variant G-CSF and receptors have the mutations listed in Table 4. In certain aspects, the variant receptor and/or

variant G-CSF may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mutations described herein. In certain aspects, the variant cytockines described herein share at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to a variant cytokine described herein. In certain aspects, the variant cytockines described herein share at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to a variant cytokine of Table 21.

[00243] In certain aspects, the variant receptors described herein comprise G-CSFR ECD domains that share at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to a G-CSFR ECD SEQ ID NO. described herein. In certain aspects, the chimeric receptor comprises the ECD of G-CSFR having an amino acid sequence of SEQ ID NO. 2, 3, 6 or 8.

[00244] In certain aspects, the variant G-CSF described herein comprise an amino acid sequence that shares at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to a G-CSFR ECD SEQ ID NO. 1.

[00245] In certain aspects, the ECD of G-CSFR comprises at least one amino acid substitution selected from the group consisting of R41E, R141E, and R167D.

[00246] A variant cytokine and/or receptor may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g. a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression the native signal sequence may be used, or other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders. In certain embodiments, the signal sequence is the signal sequence of G-CSFR or GM-CSFR. In certain embodiments, the signal sequence is SEQ ID NO: 11 or SEQ ID NO: 12.

[00247] In certain aspects, the variant receptor and/or variant G-CSF are modified, e.g., sugar groups, and polyethylene glycol (PEG), either naturally or synthetically to enhance stability. For example, in certain embodiments, variant cytokines are fused to the Fc domain

of IgG, albumin, or other molecules to extend its half-life, e.g., by pegylation, glycosylation, and the like as known in the art. In certain embodiments, the variant cytokines described herein are modified by chemical pegylation. In certain embodiments, the variant G-CSF cytokines of Table 21 are pegylated. In certain embodiments, the variant G-CSF cytokines corresponding to SEQ ID NO: 83, 83-1, 83-2, 83-3, 83-4, 83-5 and 84 are modified by pegylation. In certain embodiments, the variant G-CSF cytokines and/or chimeric cytokine receptors described herein are modified by addition of PEG to the N-terminus and/or C-terminus of the protein. In certain embodiments, the variant G-CSF cytokines and/or chimeric cytokine receptors described herein are modified by addition of a compound comprising PEG. In certain embodimetns, the PEG or PEG-containing compound is about 20kDa or less. In certain embodiments, the PEG or PEG-containing compound is 20kDa or less, 15kDa or less, 10kDa or less, 5kDa or less, or 1kDa or less.

[00248] Fc-fusion can also promote alternative Fc receptor mediated properties *in vivo*. The "Fc region" can be a naturally occurring or synthetic polypeptide that is homologous to an IgG C-terminal domain produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50kDa. The variant cytokines can include the entire Fc region, or a smaller portion that retains the ability to extend the circulating half-life of a chimeric polypeptide of which it is a part. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule.

[00249] Upon binding of the variant cytokine to the variant receptor, the variant receptor activates signaling that is transduced through native cellular elements to provide for a biological activity that mimics that native response, but which is specific to a cell engineered to express the variant receptor. In certain aspects, the variant receptor and G-CSF pair do not bind their native, wild-type G-CSF or native, wild-type G-CSFR. Thus, in certain embodiments, the variant receptor does not bind to the endogenous counterpart cytokine, including the native counterpart of the variant cytokine, while the variant cytokine does not bind to any endogenous receptors, including the native counterpart of the variant receptor. In certain embodiments, the variant cytokine binds the native receptor with significantly reduced affinity compared to binding of the native cytokine to the native cytokine receptor. In certain embodiments, the affinity of the variant cytokine for the native receptor is less than 10X, less than 100X, less than 1,000X or less than 10,000X of the affinity of the native cytokine to the native cytokine receptor. In certain embodiments, the variant cytokine binds the native receptor with a K_D of greater than $1X10^{-4}$ M, $1X10^{-5}$ M, greater than $1X10^{-6}$ M; greater

than $1X10^{-7}$ M, greater than $1X10^{-8}$ M, or greater than $1X10^{-9}$ M. In certain embodiments, the variant cytokine receptor binds the native cytokine with significantly reduced affinity compared to the binding of the native cytokine receptor to the native cytokine. In certain embodiments, the variant cytokine receptor binds the native cytokine less than 10X, less than 100X, less than 1,000X or less than 10,000X the native cytokine to the native cytokine receptor. In certain embodiments, the variant cytokine receptor binds the native cytokine with a K_D of greater than $1X10^{-4}$ M, $1X10^{-5}$ M, greater than $1X10^{-6}$ M; or greater than $1X10^{-7}$ M, greater than $1X10^{-8}$ M, or greater than $1X10^{-9}$ M. In some embodiments, the affinity of the variant cytokine for the variant receptor is comparable to the affinity of the native cytokine for the native receptor, e.g. having an affinity that is least about 1% of the native cytokine receptor pair affinity, at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, and may be higher, e.g. 2X, 3X, 4X, 5X, 10X or more of the affinity of the native cytokine for the native receptor. The affinity can be determined by any number of assays well known to one of skill in the art. For example, affinity can be determined with competitive binding experiments that measure the binding of a receptor using a single concentration of labeled ligand in the presence of various concentrations of unlabeled ligand. Typically, the concentration of unlabeled ligand varies over at least six orders of magnitude. Through competitive binding experiments, IC_{50} can be determined. As used herein, " IC_{50} " refers to the concentration of the unlabeled ligand that is required for 50% inhibition of the association between receptor and the labeled ligand. IC_{50} is an indicator of the ligand - receptor binding affinity. Low IC_{50} represents high affinity, while high IC_{50} represents low affinity.

[00250] Binding of a variant cytokine to the variant cytokine receptor expressed on the surface of a cell, may or may not affect the function of the variant cytokine receptor (as compared to native cytokine receptor activity); native activity is not necessary or desired in all cases. In certain embodiments, the binding of a variant cytokine to the variant cytokine receptor will induce one or more aspects of native cytokine signaling. In certain embodiments, the binding of a variant cytokine to the variant cytokine receptor expressed on the surface of a cell causes a cellular response selected from the group consisting of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity.

Table 1: Sequence of human WT G-CSF and human WT G-CSFR Ig-CRH domain

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
G-CSF	A0A0B4U5E3	1-174	TPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP	1
Human G-CSF recombinantly produced in E. coli			MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKLCATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLAGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP	90
G-CSFR(Ig-CRH)	Q99062	Ig: 1-97 CRH: 98-308	EECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILWRLGAELQPGGRQQRLSDGTQESIITLPHLNHTQAFLSCSLNWGNSLQILDQVELRAGYPPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKSRGNCQTQGDSILDCVPKDGQSHCSIPRKHLLLYQNMGIWVQAENALGTSMSPQLCLDPMDVVKLEPPMLRTMDPSPEAAPPQAGCLQLSWEPWQPGLHINQKCELRHKPQRGEASWALVGPLPLEALQYELCGLLPATAYTLQIRCIRWPLPGHWSDWSPSLELRTTE	2

Table 1A: Sequence of human WT G-CSF and human WT G-CSFR Ig-CRH domain

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
G-CSFR(Ig-CRH)	Q99062	Ig: 2-97 CRH: 98-308	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDP EPQILWRLGAELQPGGRQQRLSDGTQESIITL PHLNHTQAFLSCSLNWGNSLQILDQVELRAG YPPAIPHNLSCLMNLTTSSLICQWEPGPETHL PTSFTLKSFKSRGNCQTQGDSILDCVPKDGQS HCSIPRKHLLLYQNMGIWVQAENALGTSMSP QLCLDPMDVVKLEPPMLRTMDPSPEAAPPQ AGCLQLSWEPWQPGLHINQKCELRHKPQRG EASWALVGPLPLEALQYELCGLLPATAYTLQ IRCIRWPLPGHWSDWSPSLELRTTE	101

[00251] The G-CSFR amino acid sequence set forth in SEQ ID NO. 2 of Table 1 corresponds to the G-CSFR amino acid positions for the mutation numbering used herein. The G-CSFR sequence set forth in SEQ ID NO. 101 listed in Table 1A is identical to SEQ ID NO. 2, but for one amino acid (glutamic acid) at the N-terminus that is removed. Thus, the G-CSFR amino acid positions for the mutation numbering used herein corresponds to amino acids 2-308 of SEQ ID NO. 101.

Table 2: *Site II* designs with mutations for G-CSF$_E$ and G-CSFR$_E$.

Design	Mutations G-CSF$_E$	Mutations G-CSFR$_E$
1	E19K	R288E
2	S12K_E19K	R288E
6	D104K	K168D
7	D104R_L108R_T115K	168D_L171E_Q174E
8	S12R_L18F_D104R_L108K_D112R	R167D_K168D_D197E
9	D104R_L108K_D112R_Q119R_E122K	R141E_R167D_K168D_Q174D
15	K16D_T115E_T116D_Q119E	L171R_Y173V_D197R
17	E122R_E123R	R141E
18	E19K_Q20E	Y173K_R288E
19	K16E_E19K	D200R_R288E
20	E19K_D104K	K168D_R288E

21	E19K_D112R	L172E_R288E
22	E19K_D104K	K168E_M199D_R288D
23	S12E_K16D_E19K	D197K_D200K_R288E
24	E19R_D112K	R167D_V202D_R288E
25	E19K_D109R_D112R	R167D_M199D_R288D
27	E19R_D104R_L108K_D112R	R167D_K168D_V202E_I239V_R288E
28	E19K_D109K_D112R_E122R_E123R	R141E_R167D_M199D_R288E
30	L108K D112R	R167D
34	L108K_D112R_E122R_E123R	R141E_R167D
35	T115K_E122R_E123R	R141E_L171E_Q174E
36	S12E_K16E_T115E_Q119E	L171R_D197K_D200R
42	E19K_D109K_D112R	R167D_L172E_M199D_R288D
43	E19R_D104R_D112K	R167D_K168D_V202D_R288E
44	E19K_D104R_L108R_D112R	R167D_K168D_R288D
45	E19K_D112K_T115K	R167E_L171E_Q174E_R288E
46	E19K_D104K_E122R_E123R	R141E_K168D_R288E
47	E19R_E122R_E123R	R141E_V202E_I239V_R288E
48	E19K_D109K_D112R	R167F_L172E_M199D_R288D
49	K16E_E19R_T115E_Q119E	L171R_D197K_D200K_R288E
50	E19K_D104R_L108K_D112R	R167D_K168D_V202E_R288E
80	D112K T115K	R167E Q174E
81	E19K	R288D
82	L108K_D112R	R141E_R167D
83	E19R_D112K	R167D_R288E

Table 3: Site III designs.

Design #	Mutations G-CSF$_E$	Mutations G-CSFR$_E$
51	E46R_L49K	S30D_R41E
52	K40D_L41D	F75K_Q91K
53	L41K_E46R	R41E_Q91D
54	L41E_L47D	I88K
55	T38R_E46R	R41E_Q73E
56	K40D_R147E	F75K_E93K
57	E46R_V48R	R41E_Q87E

58	E46R	R41E_L86D
59	K40D_E46R	R41E_F75K
60	E46R	R41E_I88D
61	E46R_L49F	R41E_L89A
62	L41D	Q91K
63	T38R	Q73E
64	Y39E_K40D	F75K
65	L41K	I88E_Q91D
66	E46R	R41E
67	E46R	R41E_S79D
68	K40D	F75K
69	V48K	Q87D
70	R147E	E93K
71	L41K	Q91D
72	K40F	F75K

Table 4: Examples of designs resulting from combinations of site II and III designs.

Design Number	Mutations G-CSF$_E$	Mutations G-CSFR$_E$	Parental site II design	Parental site III design
106	E46R_D104K	R41E_K168D	**6**	**66**
117	E46R_E122R_E123R	R41E_R141E	**17**	**66**
130	E46R_L108K_D112R	R41E_R167D	**30**	**66**
134	E46R_L108K_D112R_E122R_E123R	R41E_R141E_R167D	**34**	**66**
135	E46R_T115K_E122R_E123R	R41E_R141E_L171E_Q174E	**35**	**66**
137	E46R_L108K_D112R	R41E_R141E_R167D	**82**	**66**
300	K40D_L41D_L108K_D112R	F75K_Q91K_R167D	**30**	**52**
301	T38R_E46R_L108K_D112R	R41E_Q73E_R167D	**30**	**55**
302	E46R_L108K_D112R	R41E_L86D_R167D	**30**	**58**
303	L108K D112R_R147E	E93K_R167D	**30**	**70**
304	E46R_L108K_D112R_R147E	R41E_E93K_R167D	**30**	**66,70**
305	E19K_E46R_L108K_D112R	R41E_R167D_R288E	**1,30**	**66**
307	S12E_K16D_E19K_E46R	R41E_D197K_D200K_R288E	**23**	**66**
308	E19R_E46R_D112K	R41E_R167D_V202D_R288E	**24**	**66**

400	E19K_E46R_D109R_D112R	R41E_R167D_M199D_R288D	**25**	**66**
401	E19K_E46R_L108K_D112R	R41E_R167D_R288D	**30,81**	**66**
402	E19K_E46R_D112K_T115K	R41E_R167E_Q174E_R288E	**1,80**	**66**
403	E19R_E46R_D112K	R41E_R167D_R288E	**83**	**66**

Table 4A: Exemplary G-CSFR and G-CSF pairs

G-CSF Variant Number	**G-CSF Mutations**	**Corresponding G-CSFR Mutations**
130	E46R_L108K_D112R	R41E_R167D
130a1	E46R_L108K_D112R_E122K	R41E_R141E_R167D
130b1	E46R_L108K_D112R_ E123K	
130a2	E46R_L108K_D112R_E122R	
130b2	E46R_L108K_D112R_ E123R	
130a1b1	E46R_L108K_D112R_E122K_E123K	
134	E46R_L108K_D112R_E122R_E123R	

Chimeric Receptors

[00252] In certain aspects, the variant receptors described herein are chimeric receptors. A chimeric receptor can comprise any of the variant G-CSFR ECD domains described herein. In certain aspects, the chimeric receptor further comprises at least a portion of the intracellular domain (ICD) of a different cytokine receptor. The intracellular domain of the different cytokine receptor can be selected from the group consisting of: gp130 (glycoprotein 130, a subunit of the interleukin-6 receptor or IL-6R), IL-2Rβ or IL-2Rb (interleukin-2 receptor beta), IL-2Rγ or γc or IL-2RG (interleukin-2 receptor gamma), IL-7Rα (interleukin-7 receptor alpha), IL-12Rβ_2 (interleukin-12 receptor beta 2), IL-21R (interleukin-21

receptor), IL-4R (interleukin-4 receptor), EPOR (erythropoietin receptor), IFNAR (interferon alpha/beta receptor) or IFNγR (interferon gamma receptor). In certain aspects, at least a portion of the intracellular domain comprises an amino acid sequence that shares at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to the amino acid sequence of a cytokine receptor ICD described herein. In certain aspects, at least a portion of a cytokine receptor ICD shares at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to SEQ ID NO. 4, 7 or 9. In certain aspects, at least a portion of the intracellular domain comprises an amino acid sequence that shares at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to the amino acid sequence of a cytokine receptor ICD of a cytokine receptor listed in Table 26.

[00253] In certain embodiments, described herein are chimeric cytokine receptors comprising an extracellular domain (ECD) of a G-CSFR (Granulocyte-Colony Stimulating Factor Receptor) operatively linked to a second domain; the second domain comprising at least a portion of an intracellular domain (ICD) of a multi-subunit cytokine receptor, e.g., IL-2R. In certain aspects, the chimeric cytokine receptor comprises a portion of an ICD from Table 15A, Table 15B, Table 23 and Table 24. In certain aspects, the chimeric cytokine receptor comprises a transmembrane domain selected from Table 15A and Table 15B. In certain aspects, the chimeric cytokine receptor ICD comprises Box1 and Box 2 regions from Table 15A, Table 15B, Table 16 Table 23 and Table 24.

[00254] In certain aspects, the chimeric cytokine receptor comprises at least one signaling molecule binding site from Table 15A, Table 15B, Table 16, Table 23, Table 24 and Table 27. In some embodiments, the signaling molecule binding site(s) of the ICD comprise a sequence set forth in SEQ ID NO. 118-146. In some embodiments, the signaling molecule binding site(s) of the ICD comprise a sequence at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a sequence set forth in at least one of SEQ ID NO. 118-146.

[00255] In certain aspects, the chimeric receptors described herein comprise amino acid sequences in N-terminal to C-terminal order of the sequences disclosed in each of Tables 17-20, 23, 24, 26 and 32. In certain aspects, the sequences of the chimeric receptors described herein comprise nucleic acid sequences in 5' to 3' order of the sequences disclosed in each of

Tables 17-20. In certain aspects, the chimeric cytokine receptor shares at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to the amino acid sequences in N-terminal to C-terminal order of the amino acid sequences disclosed in each of Tables 17-20 and 23, 24, 26 and 32. In certain aspects, the chimeric cytokine receptor shares at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleic acid identity to the nucleic acid sequences in 5' to 3' order of the nucleic acid sequences disclosed in each of Tables 17-20.

[00256] In certain aspects, the chimeric receptors described herein comprise at least a portion of an ICD of a cytokine receptor that shares at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid or nucleic acid sequence identity to an ICD SEQ ID NO. described herein. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-2Rβ having an amino acid sequence of SEQ ID NO. 26, 29, 31, 39, 41, 43, 45, 47, or 49. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-2Rβ, i.e., IL-2Rb, having a nucleic acid sequence of SEQ ID NO. 54, 57, 59, 67, 69, 71, 73, 75, or 77. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-7Rα having an amino acid sequence of SEQ ID NO. 51 or a nucleic acid sequence of SEQ ID NO. 79. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-7R having an amino acid sequence of SEQ ID NO. 53 or a nucleic acid sequence of SEQ ID NO. 81. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-21R having an amino acid sequence of SEQ ID NO. 35 or 37. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-21R having a nucleic acid sequence of SEQ ID NO. 63 or 65 In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-12Rβ_2 having an amino acid sequence of SEQ ID NO. 33, 42, or 46. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-12Rβ_2 having a nucleic acid sequence of SEQ ID NO. 61, 70 or 74. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of G-CSFR having an amino acid sequence of SEQ ID NO. 30, 32, 34, 36, 38, 40, 44, 50 or 52. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of G-CSFR having a nucleic acid sequence of SEQ ID NO. 58, 60, 62, 64, 66, 68, 72, 78 or 80. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of gp130 having an amino acid sequence of SEQ ID NO. 28 or 48. In certain aspects, the chimeric receptor comprises at least

a portion of an ICD of gp130 having a nucleic acid sequence of SEQ ID NO. 56 or 76. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-2Rγ (i.e., IL-2RG, IL-2Rgc, γc, or IL-2Rγ) having an amino acid sequence of SEQ ID NO. 27. In certain aspects, the chimeric receptor comprises at least a portion of an ICD of IL-2Rγ (i.e., IL-2RG, IL-2Rgc, γc, or IL-2Rγ) having a nucleic acid sequence of SEQ ID NO. 55.

[00257] In certain aspects, at least a portion of the ICDs described herein comprise at least one signaling molecule binding site. In certain aspects, at least one signaling molecule binding site is a STAT3 binding site of G-CSFR; a STAT3 binding site of gp130; a SHP-2 binding site of gp130; a Shc binding site of IL-2Rβ; a STAT5 binding site of IL-2Rβ; a STAT3 binding site of IL-2Rβ; a STAT1 binding site of IL-2Rβ; a STAT5 binding site of IL-7Rα; a phosphatidylinositol 3-kinase (PI3K) binding site of IL-7Rα; a STAT5 binding site of IL-12Rβ_2; a STAT4 binding site of IL-12Rβ_2; a STAT3 binding site of IL-12Rβ_2; a STAT5 binding site of IL-21R; a STAT3 binding site of IL-21R; and a STAT1 binding site of IL-21R. In certain aspects, at least one signaling molecule binding site comprises a sequence that further comprises an amino acid listed in Table 16.

[00258] In certain embodiments, the chimeric receptor comprises at least one signaling molecule binding site from an intracellular domain of a cytokine receptor. In certain embodiments, the chimeric receptor comprises at least one signaling molecule binding site from an intracellular domain in Table 24. In certain embodiments, the at least one signaling molecule binding site is selected from the group consisting of: a SHC binding site of Interleukin (IL)-2Rβ; a STAT5 binding site of IL-2Rβ, an IRS-1 or IRS-2 binding site of IL-4Rα, a STAT6 binding site of IL-4Rα, a SHP-2 binding site of gp130, a STAT3 binding site of gp130, a SHP-1 or SHP-2 binding site of Erythropoietin Receptor (EPOR), a STAT5 binding site of EPOR, a STAT1 or STAT2 binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2), and a STAT1 binding site of Interferon Gamma Receptor 1 (IFNγR1), or combinations thereof.

[00259] In certain embodiments, the chimeric receptor ICD further comprises at least one Box 1 region and at least one Box 2 region of at least one protein selected from the group consisting of G-CSFR, gp130, EPOR, and Interferon Gamma Receptor 2 (IFNγR2), or combinations thereof.

[00260] In certain aspects, at least a portion of the ICDs described herein comprise the Box 1 and Box regions of gp130 or G-CSFR. In certain aspects the Box 1 region comprises a sequence of amino acids listed in Table 2. In certain aspects the Box 1 region comprises an amino acid sequence that is greater than 50% identical to a Box 1 sequence listed in Table 16.

[00261] In certain aspects, the ICD comprises: (a) an amino acid sequence of one or both of SEQ ID NO. 90 or 91; or (b) an amino acid sequence of one or both of SEQ ID NO. 90 or 92; or (c) an amino acid sequence of SEQ ID NO. 93; or (d) an amino acid sequence of SEQ ID NO. 94; or (e) an amino acid sequence of one or both of SEQ ID NO. 95 or 96; or (f) an amino acid sequence of SEQ ID NO. 97 or 98; or (g) an amino acid sequence of SEQ ID NO. 99 or 100.

[00262] In certain aspects, the intracellular domain of the different cytokine receptor is a wild-type intracellular domain.

[00263] In certain aspects, the chimeric variant receptors described herein further comprise at least a portion of the transmembrane domain (TMD) of G-CSFR. In certain aspects, the chimeric variant receptors described herein further comprise at least a portion of the transmembrane domain (TMD) of a different cytokine receptor. The TMD of the different cytokine receptor can be selected from the group consisting of: G-CSFR, gp130 (glycoprotein 130), IL-2Rβ (interleukin-2 receptor beta), IL-2Rγ or γc (IL-2 receptor gamma), IL-7Rα (interleukin-7 receptor alpha), IL-12Rβ_2 (interleukin-12 receptor beta 2) and IL-21R (interleukin-21 receptor). In certain aspects, at least a portion of the TMD comprises an amino acid sequence that shares at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to the amino acid sequence of a cytokine receptor TMD described herein. In certain aspects, at least a portion of a cytokine receptor TMD shares at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to SEQ ID NO. 4, 5, 7 or 9.

[00264] In certain aspects, the chimeric receptors described herein comprise a G-CSFR ECD domain, a transmembrane domain (TMD), and at least one portion of one ICD arranged in N-terminal to C-terminal order, as shown in the chimeric receptor designs of Figures 20, 23 and 24.

[00265] In certain aspects, the chimeric receptor comprises the G-CSFR ECD of SEQ ID NO. 3, a portion of the gp130 TMD and ICD of SEQ ID NO 4, and a portion of the IL-2Rβ

ICD of SEQ ID NO. 5. In certain aspects, the chimeric receptor comprises the G-CSFR ECD of SEQ ID NO. 6, and a portion of the IL-2Rβ ICD of SEQ ID NO. 7. In certain aspects, the chimeric receptor comprises the G-CSFR ECD of SEQ ID NO. 8, and a portion of the IL-2Rγ ICD of SEQ ID NO. 9.

[00266] Binding of a variant or wild type cytokine to the chimeric cytokine receptor expressed on the surface of a cell, may or may not affect the function of the variant cytokine receptor (as compared to native cytokine receptor activity); native activity is not necessary or desired in all cases. In certain embodiments, the binding of a variant cytokine to the chimeric cytokine receptor will induce one or more aspects of native cytokine signaling. In certain embodiments, the binding of a variant cytokine to the chimeric cytokine receptor expressed on the surface of a cell causes a cellular response selected from the group consisting of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity.

Receptors comprising an ECD of IL-7Rα

[00267] In certain aspects, disclosed herein are chimeric receptors, comprising: (i) an extracellular domain (ECD) of Interleukin-7 Receptor alpha (IL-7Rα); (ii) a transmembrane domain (TMD); and (iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from a wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD. In some embodiments, the carboxy terminus (C-terminus) of the ECD is linked to the amino terminus (N-terminus) of the TMD, and the C-terminus of TMD is linked to the N-terminus of the ICD. In some embodiments, the ECD is the ECD of native human IL-7Rα. In some embodiments, the TMD is the TMD of IL-7Rα. In some embodiments, the TMD is the TMD of native human IL-7Rα. In some embodiments, the ICD comprises at least one signaling molecule binding site from an intracellular domain of a cytokine receptor, and, optionally, the at least one signaling molecule binding site comprises: (a) a JAK1 binding site (Box 1 and 2 region) of IL-2Rβ, IL-4Rα, IL-7Rα, IL-21R, or gp130; (b) a SHC binding site of IL-2Rβ; (c) a STAT5 binding site of IL-2Rβ or IL-7Rα; (d) a STAT3 binding site of IL-21R or gp130; (e) a STAT4 binding site of IL-12Rβ2; (f) a STAT6 binding site of IL-4Rα; (g) an IRS-1 or IRS-2 binding site of IL-4Rα; (h) a SHP-2 binding site of gp130; (i) a PI3K binding site of IL-7Rα; or combinations thereof. In some embodiments, the ICD comprises at least an intracellular signaling domain of a receptor that is activated by heterodimerization with the common

gamma chain (γc) when the ICD is part of its native receptor. In some embodiments, the ICD of the chimeric receptor does not heterodimerize with the common gamma chain. In some embodiments, the ICD of the chimeric receptor homodimerizes upon activation. In some embodiments, the ICD comprises at least an intracellular signaling domain of a cytokine receptor selected from the group consisting of: IL-2Rβ (Interleukin-2 receptor beta), IL-4Rα (Interleukin-4 Receptor alpha), IL-9Rα (Interleukin-9 Receptor alpha), IL-12Rβ2 (Interleukin-12 Receptor), IL-21R (Interleukin-21 Receptor) and glycoprotein 130 (gp130), and combinations thereof.

[00268] In certain aspects, described herein are chimeric receptors, comprising an ECD of IL-7Rα and a TMD operatively linked to an ICD, the ICD comprising:

(i)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(ii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(iii)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(iv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(v)

(a) a Box 1 and a Box 2 region of IL-21R; and

(b) a STAT3 binding site of IL-21R; or

(vi)

(a) a Box 1 and a Box 2 region of IL-7Rα; and

(b) a STAT3 binding site of IL-21R; or

(vii)

(a) a Box 1 and a Box 2 region of IL-21R;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(viii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(ix)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(x)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(xi)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xiii)

(a) a Box 1 and a Box 2 region of IL-4Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4Rα; and

(c) a STAT6 binding site of IL-4Rα; or

(xiv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4α; and

(c) a STAT6 binding site of IL-4α; or

(xv)

(a) a Box 1 and a Box 2 region of gp130;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130; or

(xvi)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130.

In some embodiments, (b) is N-terminal to (c); or (c) is N-terminal to (b); or (c) is N-terminal to (d); or (d) is N-terminal to (c); or (d) is N-terminal to (e); or (e) is N-terminal to (d).

[00269] In some embodiments, the ICDs described herein comprise the Box 1 and Box 2 regions of gp130 or G-CSFR. In certain aspects the Box 1 region comprises a sequence of amino acids listed in Table 16. In certain aspects the Box 1 region comprises an amino acid sequence that is greater than 50% identical to a Box 1 sequence listed in Table 16.

[00270] In some embodiments, the ICD comprises a sequence at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a sequence set forth in at least one of SEQ ID NO: 192-214.

[00271] In some embodiments, the signaling molecule binding site(s) of the ICD comprise a sequence set forth in SEQ ID NO. 118-146. In some embodiments, the signaling molecule binding site(s) of the ICD comprise a sequence at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a sequence set forth in at least one of SEQ ID NO. 118-146. In certain aspects, the chimeric cytokine receptor shares at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid identity to the amino acid sequences in N-terminal to C-terminal order of the amino acid sequences disclosed in Table 32.

Systems Comprising Chimeric Receptors and IL-7

[00272] In certain aspects, the present disclosure describes a system for selective activation of a receptor expressed on a cell surface, the system comprising: (a) a chimeric receptor described herein; and (b) IL-7.

[00273] In certain aspects, the present disclosure describes a system for selective activation of an immune cell, the system comprising: (a) a chimeric receptor described herein; (b) IL-7; and (c) an antigen binding signaling receptor.

[00274] In certain aspects, the present disclosure describes a system for selective activation of an immune cell, the system comprising: (a) a chimeric receptor described herein; (b) IL-7; and (c) at least one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the cytokine or

chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, NKG2D, and combinations thereof. In some embodiments, the cytokine is IL-18. In some embodiments, the cytokine is human.

[00275] In some embodiments, the system further comprises at least one antigen binding signaling receptor. In some embodiments, the at least one antigen binding signaling receptor comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In some embodiments, the at least one antigen binding signaling receptor is a CAR.

Agonistic or antagonistic signaling proteins

[00276] In certain aspects, the systems and method described herein comprise at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor(s). In some embodiments, the at least one additional cytokine(s) or chemokine(s) comprises at least one of interleukin (IL)-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, and the receptor NKG2D, and combinations thereof.

Antigen binding signaling receptors

[00277] In certain aspects, the systems and method described herein comprise an antigen binding signaling receptor(s). In some embodiments, the antigen binding signaling receptor(s) comprises at least one of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. Any CAR known in the art can be used, including but not limited to, a mesothelin CAR, or any CAR described herein. Any TCR known in the art can be used, including but not limited to, a any TCR described herein.

Nucleic Acids encoding variant cytokines and receptors

[00278] Included in this disclosure are nucleic acids encoding any one of the receptors and variant G-CSF described herein.

[00279] A variant receptor or variant G-CSF may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g., a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, the native signal sequence may be used, or other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders. In certain aspects, the signal sequence can be an amino acid sequence comprising the signal sequence at the N-terminal region of SEQ ID NO. 2, 3, 6 or 8. In certain aspects, the signal sequence can be the amino acid sequence of MARLGNCSLTWAALIILLLPGSLE (SEQ ID NO. 11).

[00280] Included in this disclosure are nucleic acids encoding any one of the receptors, IL-7, and variant G-CSF described herein.

[00281] A variant receptor, or wildtype or variant IL-7, or variant G-CSF may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g., a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression, the native signal sequence may be used, or other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders. In certain aspects, the signal sequence can be an amino acid sequence comprising the signal sequence at the N-terminal region of SEQ ID NO. 2, 3, 6 or 8. In certain aspects, the signal sequence can be the amino acid sequence of MTILGTTFGMVFSLLQVVSG (SEQ ID NO. 84). In certain aspects, the signal sequence can be the amino acid sequence of MARLGNCSLTWAALIILLLPGSLE (SEQ ID NO. 11).

Expression vectors encoding variant cytokines or receptors

[00282] Described herein are also expression vectors, and kits of expression vectors, which comprise one or more nucleic acid sequence(s) encoding one or more of the variant receptors, variant G-CSF, or wild type or variant IL-7 described herein.

[00283] In certain embodiments, the nucleic acid encoding a variant receptor or variant G-CSF is inserted into a replicable vector for expression. Such a vector may be used to introduce the nucleic acid sequence(s) into a host cell so that it expresses a variant receptor or cytokine described herein. Many such vectors are available. The vector components generally include, but are not limited to, one or more of the following: an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Vectors include viral vectors, plasmid vectors, integrating vectors, and the like. The vector may, for example, be a plasmid or a viral vector, such as a retroviral vector, adenoviral vector, lentiviral vector, or a transposon-based vector or synthetic mRNA. The vector may be capable of transfecting or transducing a cell (e.g., a T cell, an NK cell or other cells).

[00284] Expression vectors usually contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media.

[00285] In certain aspects, expression vectors contain a promoter that is recognized by the host organism and is operably linked to a variant protein coding sequence. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. A large number of promoters recognized by a variety of potential host cells are well known.

[00286] Transcription from vectors in mammalian host cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox

virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus (such as murine stem cell virus), hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter, PGK (phosphoglycerate kinase), or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication.

[00287] Transcription by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, which act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' and 3' to the transcription unit, within an intron, as well as within the coding sequence itself. Many enhancer sequences are known from mammalian genes (globin, elastase, albumin, fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the expression vector at a position 5' or 3' to the coding sequence, but is preferably located at a site 5' from the promoter.

[00288] Expression vectors used in eukaryotic host cells will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. Construction of suitable vectors containing one or more of the above-listed components employs standard techniques.

[00289] In certain aspects, disclosed herein are lentiviral vectors encoding the chimeric receptors disclosed herein. In certain aspects, the lentiviral vector comprises the HIV-1 5' LT and a 3' LTR. In certain aspects, the lentiviral vector comprises an EF1a promoter. In certain aspects, the lentiviral vector comprises an SV40 poly a terminator sequence. In certain aspects, the vector is psPAX2, Addgene® 12260, pCMV-VSV-G, or Addgene® 8454.

[00290] In some embodiments, also described are nucleic acid and polypeptide sequence with high sequence identity, e.g., 95, 96, 97, 98, 99% or more sequence identity to sequences described herein. The term percent sequence "identity," in the context of two or more nucleic acid or polypeptide sequences, refers to two or more sequences or subsequences that have a

specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[00291] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00292] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

[00293] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

Cells expressing variant receptors and variant cytokines

[00294] Also described herein are cells expressing the variant receptors. Host cells, including engineered immune cells, can be transfected or transduced with the above-described expression vectors for variant cytokine or receptor expression.

[00295] In certain embodiments, the present disclosure provides a cell which comprises one or more of a variant receptor or variant cytokine described herein. The cell may comprise a nucleic acid or a vector encoding a variant receptor or variant cytokine described herein. This disclosure also provides methods of producing cells expressing a variant receptor. In certain

aspects, the cells are produced by introducing into a cell the nucleic acid or expression vector described herein. The nucleic acid or expression vector can be introduced into the cell by any process including, but not limited to, transfection, transduction of a viral vector, transposition or gene editing. Any gene editing technique known in the art may be used including, but not limited to, techniques comprising clustered regularly interspaced short palindromic repeats (CRISPR-Cas) systems, zinc finger nucleases, transcription activator-like effector-based nucleases and meganucleases.

[00296] The host cell can be any cell in the body. In certain embodiments, the cell is an immune cell. In some embodiments, the cell is a T cell, including, but not limited to, naïve $CD8^+$ T cells, cytotoxic $CD8^+$ T cells , naïve $CD4^+$ T cells, helper T cells, e.g., T_H1 , T_H2 , T_H9 , T_H11 , T_H22, T_{FH}; regulatory T cells, e.g., T_R1 , natural T_{Reg}, inducible T_{Reg}; memory T cells, e.g., central memory T cells, effector memory T cells, NKT cells, γδT cells; etc. In certain embodiments, the cell is a B cell, including, but not limited to, naïve B cells, germinal center B cells, memory B cells, cytotoxic B cells, cytokine-producing B cells, regulatory B cells (Bregs), centroblasts, centrocytes, antibody-secreting cells, plasma cells, etc. In certain embodiments, the cell is an innate lymphoid cell, including, but not limited to, NK cells, etc. In certain embodiments, the cell is a myeloid cell, including, but not limited to, macrophages, dendritic cells, myeloid-derived suppressor cells, etc.

[00297] In certain embodiments, the cell is a stem cell, including, but not limited to, hematopoietic stem cells, mesenchymal stem cells, neural stem cells, etc.

[00298] In some embodiments, the cell is genetically modified in an *ex vivo* procedure, prior to transfer into a subject. The cell can be provided in a unit dose for therapy, and can be allogeneic, autologous, etc. with respect to an intended recipient.

[00299] T cells or T lymphocytes are a type of lymphocyte that play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. There are various types of T cell, as summarized below.

[00300] Helper T helper cells (Th cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. Th cells express CD4 on their surface. Th cells become activated when they are presented with peptide antigens by MHC class II

molecules on the surface of antigen presenting cells (APCs). These cells can differentiate into one of several subtypes, including Th1, Th2, Th3, Th17, Th9, or Tfh, which secrete different cytokines to facilitate different types of immune responses.

[00301] Cytolytic T cells (TC cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. Most CTLs express the CD8 at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of all nucleated cells.

[00302] Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T cells comprise three subtypes: central memory T cells (TCM cells) and two types of effector memory T cells (TEM cells and TEMRA cells). Memory cells may be either CD4+ or CD8+. Memory T cells typically express the cell surface protein CD45RO.

[00303] Regulatory T cells (Treg cells), formerly known as suppressor T cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus. Two major classes of CD4+ Treg cells have been described— naturally occurring Treg cells and adaptive Treg cells.

[00304] Naturally occurring Treg cells (also known as CD4+CD25+FoxP3+ Treg cells) arise in the thymus and have been linked to interactions between developing T cells with both myeloid (CD1 1c+) and plasmacytoid (CD123+) dendritic cells that have been activated with TSLP. Treg cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP3.

[00305] Adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response. The cell may be a Natural Killer cell (or NK cell). NK cells form part of the innate immune system. NK cells provide rapid responses to innate signals from virally infected cells in an MHC independent manner.

[00306] In certain aspects, the cells expressing the variant receptors or variant cytokines described herein are tumor-infiltrating lymphocytes (TILs) or tumor-associated lymphocytes (TALs). In certain aspects, the TILs or TALs comprise CD4+ T cells, CD8+ T cells, Natural Killer (NK) cells, and combinations thereof.

[00307] In certain embodiments, the T cells described herein are chimeric antigen receptor T cells (CAR-T cells) that have been genetically engineered to produce an artificial T-cell receptor for use in immunotherapy. In certain aspects, the CAR-T cells derived from T cells in a patient's own blood (i.e., autologous). In certain aspects, the CAR-T are derived from the T cells of another healthy donor (i.e., allogeneic). In certain aspects, the CAR-T cells are derived or synthesized from non-immune cell types such as pluripotent stem cells.

[00308] In certain embodiments, the T cells described herein are engineered T Cell Receptor (eTCR-T cells) that have been genetically engineered to produce a particular T Cell Receptor for use in immunotherapy. In certain aspects, the eTCR-T cells are derived from T cells in a patient's own blood (i.e., autologous). In certain aspects, the eTCR-T cells are derived from the T cells of a donor (i.e., allogeneic). In certain aspects, the eTCR-T cells are derived or synthesized from non-immune cell types such as pluripotent stem cells.

[00309] In certain aspects, the cells expressing chimeric cytokine receptors described herein are NK cells. NK cells (belonging to the group of innate lymphoid cells) are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils and thymus where they then enter into the circulation. In certain aspects, the NK cells are derived from NK cells in a patient's own blood (i.e., autologous). In certain aspects, the NK cells are derived from the NK cells of a donor (i.e., allogeneic). In certain aspects, the NK cells are derived or synthesized from non-immune cell types such as pluripotent stem cells.

[00310] In certain aspects, the cells expressing chimeric cytokine receptors described herein are B cells. B cells include, but are not limited to, naïve B cells, germinal center B cells, memory B cells, cytotoxic B cells, cytokine-producing B cells, regulatory B cells (Bregs), centroblasts, centrocytes, antibody-secreting cells, plasma cells, etc. In certain aspects, the B cells are derived from B cells in a patient's own blood (i.e., autologous). In certain aspects, the B cells are derived from the B cells of a donor (i.e., allogeneic). In certain aspects, the B cells are derived or synthesized from non-immune cell types such as pluripotent stem cells.

[00311] In certain aspects, the cells expressing chimeric cytokine receptors described herein are myeloid cells, including, but not limited to, macrophages, dendritic cells, myeloid-derved suppressor cells, etc. In certain aspects, the myeloid cells are derived from myeloid

cells in a patient's own blood (i.e., autologous). In certain aspects, the myeloid cells are derived from the myeloid cells of a donor (i.e., allogeneic). In certain aspects, the myeloid cells are derived or synthesized from non-immune cell types such as pluripotent stem cells.

[00312] The cells expressing a variant receptor or variant cytokine described herein may be of any cell type. In certain aspects, the cells expressing a variant receptor or variant cytokine described herein is a cell of the hematopoietic system. Immune cells (e.g., T cells or NK cells) according to the invention may either be created ex vivo either from a patient's own peripheral blood (1st party), or in the setting of a hematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party). Alternatively, immune cells described herein may be derived from ex vivo differentiation of inducible progenitor cells or embryonic progenitor cells to immune cells. Alternatively, an immortalized immune cell line which retains its effector function (e.g., a T-cell or NK-cell line that retains its lytic function; a plasma cell line that retains its antibody producing function, or a dendritic cell line or macrophage that retains its phagocytic and antigen presentation function) and could act as a therapeutic may be used. In all these embodiments, variant receptor-expressing cells are generated by introducing DNA or RNA coding for each variant receptor(s) by one of many means including transduction with a viral vector or transfection with DNA or RNA.

[00313] The cells described herein can be immune cells derived from a subject engineered ex vivo to express a variant receptor and/or variant cytokine. The immune cell may be from a peripheral blood mononuclear cell (PBMC) sample or a tumor sample. Immune cells may be activated and/or expanded prior to being transduced with nucleic acid encoding the molecules providing the variant receptor or variant cytokine according to the first aspect of the invention, for example by treatment with an anti-CD3 monoclonal antibody and/or IL-2. The immune cell of the invention may be made by: (i) isolation of an immune cell-containing sample from a subject or other sources listed above; and (ii) transduction or transfection of the immune cells with one or more nucleic acid sequence(s) encoding a variant receptor or variant cytokine.

[00314] Cells can be cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Mammalian host cells may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI 1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are

suitable for culturing the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics, trace elements, and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[00315] The immune cells may then by purified, for example, selected on the basis of expression of the antigen-binding domain of the antigen-binding polypeptide. In certain embodiments, the cells are selected by expression of a selectable marker (e.g., a protein, a fluorescent marker, or an epitope tag) or by any method known in the art for selection, isolation and/or purification of the cells.

Kits

[00316] This disclosure also describes kits for producing a cell expressing at least one of any of the variant receptors or variant G-CSF described herein. In certain aspects, described herein are kits comprising: cells encoding a chimeric receptor described herein, and, optionally, the cells are immune cells; and instructions for use; and, optionally, the kit comprises IL-7 and/or a variant G-CSF. In certain aspects, described herein are kits, comprising: one or more expression vector(s) comprising the nucleic acid sequence(s) encoding a chimeric receptor described herein and instructions for use; and, optionally, the kit comprises IL-7 and/or a variant G-CSF. In certain embodiments, the kits comprise at least one expression vector encoding at least one variant receptor and instructions for use. In certain aspects, the kit further comprises at least one variant cytokine in a pharmaceutical formulation or an expression vector encoding a variant G-CSF that binds to at least one of the variant receptors described herein. In certain embodiments, the kits comprise a cell comprising an expression vector encoding a variant receptor described herein.

[00317] In certain embodiments, the kits comprise a cell comprising an expression vector encoding a (CAR)/engineered T cell receptor (eTCR) or the like (e.g., engineered non-native TCR receptors). In certain embodiments the kits comprise an expression vector encoding a Chimeric Antigen Receptor (CAR)/engineered T cell receptor (eTCR) or the like. In certain

embodiments the kits comprise an expression vector encoding a variant receptor described herein and a Chimeric Antigen Receptor (CAR)/ engineered T cell receptor (eTCR) or the like.

[00318] In certain aspects, the kits described herein further comprise a variant cytokine. In certain embodiments, the kit further comprises at least one additional variant cytokine. In certain aspects, the kits further comprise at least one variant cytokine in a pharmaceutical formulation. In certain aspects, the kits described herein further comprise at least one a cytokine (e.g., IL-7 and/or a variant G-CSF). In some embodiments, the kits comprise at least one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent. In some embodiments, the kits comprise one or more expression vector(s) eonding at least one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

[00319] In certain embodiments, the components are provided in a dosage form, in liquid or solid form in any convenient packaging.

[00320] In some embodiments, the kit comprises one or more expression vectors that encode at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); In certain embodiments, the kits further comprise one or more expression vector(s) that encode a cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In certain embodiments, the kits further comprise one or more expression vector(s) that encodes at least one antigen binding receptor(s). In certain embodiments, the at least one antigen binding receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In certain

embodiments, the kits further comprise an expression vector that encodes a chimeric antigen receptor.

[00321] In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s).

[00322] In certain aspects, the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor and combinations thereof. In some embodiments, the cells further comprise one or more expression vector(s) that encode at least one CAR(s), and optionally, the CAR is a mesothelin CAR. In certain embodiments, the components are provided in a dosage form, in liquid or solid form in any convenient packaging.

[00323] Additional reagents may be provided for the growth, selection and preparation of the cells provided or cells produced as described herein. For example, the kit can include components for cell culture, growth factors, differentiation agents, reagents for transfection or transduction, etc.

[00324] In certain embodiments, in additional to the above components, the kits may also include instructions for use. Instructions can be provided in any convenient form. For example, the instructions may be provided as printed information, in the packaging of the kit, in a package insert, etc. The instructions can also be provided as a computer readable medium on which the information has been recorded. In addition, the instructions may be provided on a website address which can be used to access the information.

Methods of selective activation of a variant receptor

[00325] This disclosure provides methods for selective activation of a variant receptor expressed on the surface of a cell, comprising contacting the variant receptor described herein with a cytokine that selectively activates the chimeric receptor. In certain aspects, the cytokine that selectively activates the chimeric receptor is a variant G-CSF. The G-CSF can

be a wild-type G-CSF or a G-CSF comprising one or more mutations that confer preferential binding and activation of the G-CSF to a variant receptor compared to the native (wild-type) cytokine receptor.

[00326] In certain aspects, the selective activation of the variant receptor by binding of the cytokine to the variant receptor leads to homodimerization, heterodimerization, or combinations thereof.

[00327] In certain aspects, activation of the variant receptor leads to activation of downstream signaling molecules. In certain aspects, the variant receptor activates signaling molecules or pathways that are transduced through native cellular signaling molecules to provide for a biological activity that mimics that native response, but which is specific to a cell engineered to express the variant receptor. In certain embodiments, an activated form of the chimeric receptor forms a homodimer; and, optionally, activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and combinations thereof, and optionally, the chimeric receptor is activated upon contact with the cytokine.

[00328] In certain aspects, the activation of the downstream signaling molecules includes activation of cellular signaling pathways that stimulate cell cycle progression, proliferation, viability, and/or enhanced activity. In certain aspects, the signaling pathways or molecules that are activated are, but not limited to, JAK1, JAK2, JAK3, TYK2, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6, Shc, ERK1/2, IRS-1, IRS-2, and Akt. In certain aspects, activation of the variant receptor leads to increased proliferation of the cell after administration of a cytokine that binds the receptor. In certain aspects, the extent of proliferation is between 0.1-10-fold the proliferation observed when the cells are stimulated with IL-2.

[00329] In certain aspects, described herein are methods of activation of one or more chimeric receptor(s) expressed on the surface of a cell, comprising: contacting the one or more chimeric receptor(s) with IL-7 to activate the chimeric receptor; wherein the chimeric receptor comprises: (i) an extracellular domain (ECD) of IL-7Rα; (ii) a transmembrane domain (TMD); and (iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from the wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO: 109; wherein the ECD and TMD are each operatively linked to the ICD. In some

embodiments, the chimeric receptor(s) is a chimeric receptor comprising an ICD described herein.

Methods and systems for selective activation of a cell

In certain aspects, described herein are methods and systems for selective activation of a cell comprising (i) a receptor comprising a variant extracellular domain (ECD) of Granulocyte Colony-Stimulating Factor Receptor (G-CSFR); and (ii) a variant G-CSF that selectively binds the receptor of (i); and one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor. In some embodiments, the receptor is expressed on an immune cell, and, optionally the immune cell is: a T cell, and, optionally, an NK cell, and, optionally,an NKT cell, and, optionally, a B cell, and, optionally, a plasma cell, and, optionally, a macrophage, and, optionally, a dendritic cell, and, optionally, the cell is a stem cell, and, optionally,the cell is a primary cell, and, optionally, the cell is a human cell. In some embodiments, the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell. In some embodiments, the at least one additional cytokine(s) or chemokine(s) comprises at least one of interleukin (IL)-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, and CCL19, and the receptor NKG2D, and combinations thereof. In certain embodiments, the receptor comprising a variant ECD of G-CSFR is a chimeric receptor described herein.

[00330] In certain embodiments, the system further comprises an antigen binding signaling receptor. In certain embodiments, the antigen binding signaling receptor comprises at least one of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. Any CAR known in the art could be used, including but not limited to a mesothelin CAR.

[00331] In certain aspects, described heren are methods of producing a cell expressing the receptor comprising the variant ECD of G-CSFR of a system described herein; and one or both of: (i)) at least one additional agonistic or antagonistic signaling protein(s); and,

optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of a system described herein; and (ii) at least one antigen binding signaling receptor of a system described herein. In some embodiments. the method comprises introducing to the cells one or more nucleic acid(s) or expression vector(s) encoding the receptor, and one or both of (i), and (ii). In some embodiments, a first population of immune cells expresses the receptor comprising the variant ECD of G-CSFR and a second population of immune cells express the variant G-CSF In some embodiments, one or both of the first and second population(s) of immune cells further expresses one or both of: (a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and (b) at least one antigen binding signaling receptor.

[00332] In certain aspects, described herein are methods and systems for activation of a cell comprising a chimeric receptor, comprising: (a) an extracellular domain (ECD) of Interleukin Receptor alpha (IL-7Rα); (b) a transmembrane domain (TMD); and (c) an intracellular domain (ICD) of a cytokine receptor that is distinct from a wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD.

Methods of adoptive cell transfer

[00333] The present invention provides a method for treating and/or preventing a disease which comprises the step of administering the cells expressing a variant receptor and/or a variant cytokine described herein (for example in a pharmaceutical composition as described below) to a subject.

[00334] A method for treating a disease relates to the therapeutic use of the cells described herein, e.g., T cells, NK cells, or any other immune or non-immune cells expressing a variant receptor. The cells can be administered to a subject having an existing disease or condition in order to lessen, reduce or improve at least one symptom associated with the disease and/or to slow down, reduce or block the progression of the disease. The method for preventing a disease relates to the prophylactic use of the cells of the present disclosure. Such cells may be administered to a subject who has not yet contracted the disease and/or who is not showing any symptoms of the disease to prevent or impair the cause of the disease or to reduce or

prevent development of at least one symptom associated with the disease. The subject may have a predisposition for, or be thought to be at risk of developing, the disease.

[00335] In some embodiments, the subject compositions, methods and kits are used to enhance an immune response. In some embodiments the immune response is directed towards a condition where it is desirable to deplete or regulate target cells, e.g., cancer cells, infected cells, immune cells involved in autoimmune disease, etc. by systemic administration of cytokine, e.g. intramuscular, intraperitoneal, intravenous, and the like.

[00336] The method can involve the steps of: (i) isolating an immune cell-containing sample; (ii) transducing or transfecting such cells with a nucleic acid sequence or vector e.g., expressing a variant receptor; (iii) administering (i.e., infusing) the cells from (ii) to the subject, and (iv) administering a variant cytokine that stimulates the infused cells. In certain aspects, the subject has undergone an immuno-depletion treatment prior to administering the cells to the subject. In certain aspects, the subject has not undergone an immuno-depletion treatment prior to administering the cells to the subject. In certain aspects, the subject has undergone an immuno-depletion treatment reduced in severity, dose and/or duration that would otherwise be necessary without the use of the variant receptors described herein prior to administering the cells to the subject.

[00337] In certain embodiments, the method further comprises administering or providing at least one additional active agent; and, optionally, at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s). In certain embodiments, the subject is administered two or more populations of cells each expressing a distinct chimeric receptor and each expressing a distinct variant form of a cytokine. In certain embodiments, the cells expressing the chimeric receptor further express at least one antigen binding signaling receptor. In certain embodiments, the antigen binding signaling receptor comprises at least one receptor(s) selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof. In certain embodiments, the antigen binding signaling receptor is a CAR. In certain embodiments, the at least one cytokine(s) or chemokine(s) is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-

β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof.

[00338] The immune cell-containing sample can be isolated from a subject or from other sources, for example as described above. The immune cells can be isolated from a subject's own peripheral blood (1st party), or in the setting of a hematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party). The immune cells can also be derived by *in vitro* methods, such as induced differentiation from stem cells or other forms of precursor cell.

[00339] In some embodiments, the immune cells are contacted with the variant cytokine *in vivo*, i.e., where the immune cells are transferred to a recipient, and an effective dose of the variant cytokine is administered to the recipient and allowed to contact the immune cells in their native location, e.g. in lymph nodes, etc. In some embodiments, the contacting is performed *in vitro*. Where the cells are contacted with the variant cytokine *in vitro*, the cytokine is added to the cells in a dose and for a period of time sufficient to activate signaling from the receptor, which can utilize aspects of the native cellular machinery, e.g. accessory proteins, co-receptors, etc. The activated cells can be used for any purpose, including, but not limited to, experimental purposes relating to determination of antigen specificity, cytokine profiling, and for delivery *in vivo*.

[00340] In certain aspects, a therapeutically effective number of cells are administered to the subject. In certain aspects, the subject is administered or infused with cells expressing variant receptors on a plurality of separate occasions. In certain embodiments, at least $1x10^6$ cells/kg, at least $1x10^7$ cells/kg, at least $1x10^8$ cells/kg, at least $1x10^9$ cells/kg, at least $1x10^{10}$ cells/kg, or more are administered, sometimes being limited by the number of cells, e.g., transfected T cells, obtained during collection. The transfected cells may be infused to the subject in any physiologically acceptable medium, normally intravascularly, although they may also be introduced into any other convenient site, where the cells may find an appropriate site for growth.

[00341] In certain aspects, a therapeutically effective amount of variant cytokine is administered to the subject. In certain aspects, the subject is administered the variant cytokine on a plurality of separate occasions. In certain aspects, the amount of variant cytokine that is administered is an amount sufficient to achieve a therapeutically desired

result (e.g., reduce symptoms of a disease in a subject). In certain aspects, the amount of variant cytokine that is administered is an amount sufficient to stimulate cell cycle progression, proliferation, viability and/or functional activity of a cell expressing a variant cytokine receptor described herein. In certain aspects, the variant cytokine is administered at a dose and/or duration that would is necessary to achieve a therapeutically desired result. In certain aspects, the variant cytokine is administered at a dose and/or duration sufficient to stimulate cell cycle progression, proliferation, viability and/or functional activity of a cell expressing a variant cytokine receptor described herein. Dosage and frequency may vary depending on the agent; mode of administration; nature of the cytokine; and the like. It will be understood by one of skill in the art that such guidelines will be adjusted for the individual circumstances. The dosage may also be varied for localized administration, e.g. intranasal, inhalation, etc., for systemic administration, e.g., intramuscular, intraperitoneal, intravascular, and the like.

Indications for adoptive cell transfer

[00342] The present disclosure provides a cell expressing a variant receptor described herein for use in treating and/or preventing a disease. The invention also relates to the use of a cell expressing a variant receptor described herein in the manufacture of a medicament for the treatment and/or prevention of a disease.

[00343] The disease to be treated and/or prevented by the methods of the present invention can be a cancerous disease, such as, but not limited to, bile duct cancer, bladder cancer, breast cancer, cervical cancer, ovarian cancer, colon cancer, endometrial cancer, hematologic malignancies, kidney cancer (renal cell), leukemia, lymphoma, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer, sarcoma and thyroid cancer.

[00344] The disease to be treated and/or prevented can be an autoimmune disease. Autoimmune diseases are characterized by T and B lymphocytes that aberrantly target self-proteins, polypeptides, peptides, and/or other self-molecules causing injury and or malfunction of an organ, tissue, or cell-type within the body (for example, pancreas, brain, thyroid or gastrointestinal tract) to cause the clinical manifestations of the disease. Autoimmune diseases include diseases that affect specific tissues as well as diseases that can affect multiple tissues, which can depend, in part on whether the responses are directed to an antigen confined to a particular tissue or to an antigen that is widely distributed in the body.

Autoimmune diseases include, but are not limited to, Type 1 diabetes, systemic lupus erythematosus, Rheumatoid arthritis, autoimmune thyroid diseases and Graves' disease.

[00345] The disease to be treated and/or prevented can be an inflammatory condition, such as cardiac fibrosis. In general, inflammatory conditions or disorders typically result in the immune system attacking the body's own cells or tissues and may cause abnormal inflammation, which can result in chronic pain, redness, swelling, stiffness, and damage to normal tissues. Inflammatory conditions are characterized by or caused by inflammation and include, but are not limited to, celiac disease, vasculitis, lupus, chronic obstructive pulmonary disease (COPD), irritable bowel disease, atherosclerosis, arthritis, myositis, scleroderma, gout, Sjorgren's syndrome, ankylosing spondylitis, antiphospholipid antibody syndrome, and psoriasis.

[00346] In certain embodiments, the method is used to treat an infectious disease.

[00347] In certain embodiments, the method is used to treat a degenerative disease or condition. Examples of degenerative diseases or conditions include, but are not limited to, neurodegenerative diseases and conditions related to aging.

[00348] In certain embodiments, the method is used to generate natural or engineered cells, tissues or organs for transplantation.

[00349] In certain embodiments, the condition to be treated is to prevent and treat graft rejection. In certain embodiments, the condition to be treated and/or prevented is allograft rejection. In certain aspects, the allograft rejection is acute allograft rejection.

[00350] The disease to be treated and/or prevented can involve the transplantation of cells, tissues, organs or other anatomical structures to an affected individual. The cells, tissues, organs or other anatomical structures can be from the same individual (autologous or "auto" transplantation) or from a different individual (allogeneic or "allo" transplantation). The cells, tissues, organs or other anatomical structures can also be produced using *in vitro* methods, including cell cloning, induced cell differentiation, or fabrication with synthetic biomaterials.

[00351] The present invention provides a method for treating and/or preventing a disease which comprises one or more steps of administering the variant cytokine and/or cells described herein (for example in a pharmaceutical composition as described above) to a subject.

[00352] A method for treating and/or preventing a disease relates to the therapeutic use of the cells of the present disclosure. Herein the cells may be administered to a subject having an existing disease or condition in order to lessen, reduce or improve at least one symptom associated with the disease and/or to slow down, reduce or block the progression of the disease. The method for preventing a disease relates to the prophylactic use of the cells of the present disclosure. Such cells may be administered to a subject who has not yet contracted the disease and/or who is not showing any symptoms of the disease to prevent or impair the cause of the disease or to reduce or prevent development of at least one symptom associated with the disease. The subject may have a predisposition for, or be thought to be at risk of developing, the disease. The method may involve the steps of: (i) isolating an immune cell-containing sample; (ii) transducing or transfecting such cells with a nucleic acid sequence or vector provided by the present invention; (iii) administering the cells from (ii) to a subject, and (iv) administering a variant cytokine that stimulates the infused cells. The immune cell-containing sample may be isolated from a subject or from other sources, for example as described above. The immune cells may be isolated from a subject's own peripheral blood (1st party), or in the setting of a hematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party).

[00353] Treatment can be combined with other active agents, such as, but not limited to, antibiotics, anti-cancer agents, anti-viral agents, and other immune modulating agents (e.g., antibodies against the Programmed Cell Death Protein-1 [PD-1] pathway or antibodies against CTLA-4). Additional cytokines may also be included (e.g., interferon γ, tumor necrosis factor α, interleukin 12, etc.).

Methods using stem cells expressing variant cytokine receptors

[00354] The present invention provides a method for treating and/or preventing a condition or disease which comprises the step of administering stem cells expressing a variant receptor and/or a variant cytokine described herein. In certain embodiments, stem cells expressing the variant cytokine receptors and/or variant cytokines described herein are used for regenerative medicine, cell/tissue/organ transplantation, tissue reconstruction, or tissue repair.

Pharmaceutical compositions

[00355] The present disclosure also relates to a pharmaceutical composition containing a plurality of cells expressing a variant receptor described herein and/or the cytokines described

herein. The present disclosure also relates to a pharmaceutical composition containing a variant cytokine described herein. The cells of the invention can be formulated in pharmaceutical compositions. These compositions can comprise, in addition to one or more of the cells expressing the variant receptor described herein, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The pharmaceutical composition may optionally comprise one or more further pharmaceutically active polypeptides and/or compounds. Such a formulation may, for example, be in a form suitable for intravenous infusion.

[00356] For cells expressing a variant receptor, and variant cytokines described herein, according to the present disclosure that are to be given to an individual, administration is preferably in a "therapeutically effective amount" that is sufficient to show benefit to the individual. A "prophylactically effective amount" can also be administered, when sufficient to show benefit to the individual. The actual amount of cytokine or number of cells administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

[00357] A pharmaceutical composition can be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

EXAMPLES

[00358] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[00359] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques, cell culture, adoptive cell transfer, and pharmacology, within the skill of the art. Such techniques

are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Rational design of an exclusive site II interface of G-CSF:G-CSFR(CRH)

[00360] The wild type (WT) G-CSF$_{WT}$:G-CSFR$_{WT}$ complex is a 2:2 heterodimer. G-CSF has two binding interfaces with the extracellular domain (ECD) of G-CSFR. The larger interface between G-CSF and the extracellular Cytokine Receptor Homologous (CRH) domain of G-CSFR is referred to as site II. The smaller interface between G-CSF and the N-terminal Ig-like (Ig) extracellular domain of G-CSFR is referred to as site III (see, Figure 1).

[00361] To design co-evolved, engineered (E) G-CSF$_E$:G-CSFR$_E$ cytokine: receptor pairs, we separated the 2:2 complex between WT G-CSF and the Ig-CRH extracellular domain of G-CSFR (Protein data Bank ID 2D9Q, Tamada et al. PNAS 2006) into the two distinct sub-complexes encompassing the site II and site III interface, consisting of G-CSF:G-CSFR(CRH) and G-CSF:G-CSFR(Ig) (see, Figure 1), respectively, see Table 1 and Table 1A for sequences. The G-CSFR amino acid sequence set forth in SEQ ID NO. 2 of Table 1 corresponds to the amino acid positions used for the mutation numbering used herein for G-CSFR. The G-CSFR sequence set forth in SEQ ID NO. 101 listed in Table 1A is identical to SEQ ID NO. 2, but for one amino acid (glutamic acid) removed at the N-terminus. Thus, the amino acid positions for the mutation numbering used herien corresponds to amino acids 2-308 of SEQ ID NO. 101.

Methods

[00362] To create co-evolved, exclusive G-CSF$_E$:G-CSFR$_E$ mutant pairs (designs), we employed a computational design workflow (see Figure 2). First, we created exclusive designs at site II.

[00363] *In silico* structural analysis of site II interface interactions of G-CSF$_{WT}$:G-CSFR(CRH)$_{WT}$ showed that most molecular interactions, that contribute to the total attractive AMBER energy at site II of 109.64 kcal/mol, are made by charged residues (see, Figure 3). In depth inspection reveals mostly electrostatic and hydrogen-bonding interactions, for example

between R167 of G-CSFR(CRH) and D112/D109 of G-CSF, which contributes 28% of the total attractive AMBER energy at site II. Another important electrostatic interaction is present between R141 of G-CSFR(CRH) and E122/E123 of G-CSF (see, Figure 4), which contributes 21.4% of the total attractive AMBER energy at site II (See Figure 3). Likewise, the salt bridge between E19 of the cytokine and R288 of the receptor CRH domain contributes 17.3%, the arginine is further stabilized through electrostatic and hydrogen-bonding interaction to D200 of the receptor CRH domain.

[00364] Each design at site II consists of a mutant pair of G-CSF$_E$ and G-CSFR(CRH)$_E$. First, we created positive designs at site II, for example through inverting charges, by mutating basic residues to acidic residues and vice versa on the binding partner, while maintaining packing and hydrophobic interactions through additional mutations if necessary. Mutant pairs G-CSF$_E$:G-CSFR(CRH)$_E$ were packed with the mean-field packing workflow of ZymeCAD^{TM}. The packed *in silico* models of designs at site II were visually inspected for structural integrity and they were assessed by ZymeCAD^{TM} metrics. Specifically, we aimed to design G-CSF$_E$ mutants to have ZymeCAD^{TM} *in silico* dAMBER binding affinity of < 10 kcal/mol for their corresponding G-CSFR(CRH)$_E$ mutant (paired interaction). This metric compares AMBER affinity, the sum of Lennard Jones affinity and Electrostatics affinity, to AMBER affinity of the WT:WT cytokine-receptor pair. Designs with dAMBER_folding > 80 kcal/mol were excluded. The dAMBER folding metric scores the change in the sum of Lennard Jones-bonded folding and Electrostatics folding upon mutation. We also excluded designs that scored higher than 400 kcal/mol in dDDRW apostability. This metric describes the change in knowledge-based potential stability upon mutation of a protein from its apo form.

[00365] Next we packed G-CSF$_E$ mutants of each design in a complex with WT G-CSFR (and vice versa G-CSFR$_E$ with G-CSF$_{WT}$) with ZymeCAD^{TM}, to assess the metrics of each positive design under conditions of mispairing when the following two complexes would form: G-CSF$_{WT}$:G-CSFR(CRH)$_E$ and G-CSF$_E$:G-CSFR(CRH)$_{WT}$. We calculated *in silico* ddAMBER metrics for the mispaired orientations with ZymeCAD^{TM} (DdAMBER_affinity_Awt_Bmut is the AMBER affinity of the paired, engineered complex subtracted by the AMBER affinity of the mispaired complex G-CSF$_{WT}$:G-CSFR(CRH)$_E$, ddAMBER_affinity_Amut_Bwt is the AMBER affinity of the paired, engineered complex subtracted by the AMBER affinity of the mispaired complex G-CSF$_E$:G-CSFR(CRH)$_{WT}$. Designs that minimized mispaired ddAMBER affinity metrics were considered to be more

selective for their paired binding partner over binding to wild type cytokine or receptor binding.

[00366] All *site II* designs were clustered and the packing metrics of G-CSF$_E$:G-CSFR(CRH)$_E$, G-CSF$_{WT}$:G-CSFR(CRH)$_E$, G-CSF$_E$:G-CSFR(CRH)$_{WT}$ were considered to assess the strength of pairing (positive design) and selectivity against mispairing with WT G-CSF and G-CSFR(CRH) (negative design) and rank designs.

Results

[00367] Designs listed in Table 2 have packing metrics in ZymeCAD™ that favour pairing of G-CSF$_E$:G-CSFR(CRH)$_E$ *in silico* (see, Table 5) and showed satisfying interactions *in silico* in the visual inspection such as the presence of salt bridges, hydrogen bonds and absence of severe clashes (see, Figure 5). These designs also showed high selectivity against mispairing with WT G-CSF or G-CSFR(CRH) (see, Table 5).

[00368] Therefore, the site II mutations of the same variant G-CSF and receptor designs shown in Table 2 are predicted to exhibit preferential binding, over wild type G-CSFR and G-CSF, respectively.

Table 5: *Site II* designs with AMBER metrics in kcal/mol from triplicate *in silico* mean-field packs with ZymeCAD^{Tm}.

	G-CSF$_E$:G-CSFR(CRH)$_E$		G-CSF$_E$:G-CSFR(CRH)$_{WT}$	G-CSF$_{WT}$:G-CSFR(CRH)$_E$
Design	**dAMBER_aff**	**dAMBER_folding**	**ddAMBER_aff_E_WT**	**ddAMBER_aff_WT_E**
1	-13.2	36.0	-35.2	-47.3
2	-24.2	35.6	-41.0	-55.8
6	8.9	-7.5	-8.8	-13.3
7	-24.1	-7.8	-45.8	-51.7
8	-30.5	12.2	-73.5	-86.5
9	-25.1	17.4	-89.6	-119.5
15	-53.3	59.5	-72.4	-71.0
17	-0.2	19.3	-23.2	-28.5
18	-17.6	75.2	-44.2	-27.8
19	20.6	38.1	-10.7	-17.3
20	-9.9	28.5	-52.7	-64.4
21	-18.0	51.5	-55.4	-47.8
22	-32.3	52.2	-75.0	-53.3
24	-13.0	57.7	-50.8	-86.4
25	-49.8	83.5	-100.2	-91.3

27	-60.5	43.0	-131.4	-156.4
28	-61.6	82.6	-148.8	-135.9
30	9.6	-5.0	-31.4	-29.1
34	2.9	16.7	-60.4	-63.1
35	-27.4	0.3	-50.1	-63.0
36	-92.2	35.1	-110.7	-93.5
42	-82.1	67.7	-137.0	-119.7
43	-14.9	51.0	-87.4	-111.2
44	-20.2	53.6	-86.4	-108.4
45	-35.5	38.1	-70.7	-107.0
46	-11.6	44.2	-75.0	-95.8
47	-27.8	75.8	-72.4	-91.5
48	-69.7	76.8	-127.8	-87.7
49	-55.9	79.3	-94.7	-84.7
50	-52.0	38.6	-123.5	-134.9
80	15.3	13.0	-16.2	10.8
81	-2.4	52.7	-24.5	-28.9
82	29.8	10.9	1.6	0.7
83	36.3	54.6	-21.6	5.5

Example 2: In vitro screening of site II designs

[00369] Selected site II designs were screened in a pulldown experiment in the format G-CSF$_E$:G-CSFR(CRH)$_E$ for their ability to form a site II complex when co-expressed in a Baculovirus based insect cell system. We also assessed whether designs could form a mispaired complex with WT receptor or cytokine by co-expressing each designs G-CSF$_E$ mutant with G-CSFR(CRH)$_{WT}$, and vice versa each design G-CSFR(CRH)$_E$ mutant was co-expressed with GCSF$_{WT}$. The expression of G-CSF$_E$ alone was verified by single infections of the cytokine mutant.

Methods

[00370] In brief, site II G-CSF designs and corresponding, paired G-CSFR(CRH) mutants were cloned separately into insect cell transfection vectors. G-CSF WT (residues 1-173, Table 1) and mutants were cloned into a modified pAcGP67b transfer vector (Pharmingen), in frame with an N-terminal secretion signal and a C-terminal TEV-cleavable Twin Strep tag

with the sequence AAA**ENLYFQ/G**SA**WSHPQFEK**GGGSGGGSGGSA**WSHPQFEK.** Of the receptor extracellular domain, only the CRH domain (residues 98-308, Table 1) with site II design mutations was cloned into a modified pAcGP67b transfer vector, in frame, with an N-terminal secretion signal and TEV-cleavable Hexahistidine tag of the sequence **HHHHHH**SSGR**ENLYFQ/G**SMG . All constructs were synthesized and codon optimized for insect cell expression (Genscript). Transfer vector DNA was prepared by Midi-prep (ThermoScientific, cat. K0481), was endotoxin-free and had a $A_{260/280}$ absorbance ratio of 1.8-2.0. Recombinant virus generation was achieved by co-transfection of recombinant, linearized Baculovirus DNA with the vector DNA in *Spodoptera frugiperda 9* (Sf9) cells, using the adherent method, as described by the manufacturer (Expression Systems, California). Approximately 1 h prior to transfection, 2 mL of healthy, log-phase Sf9 cells at 0.46 x 10^6 cells ml^{-1} were seeded per well of a 6-well tissue culture plate (Greiner, cat. 657-160). Transfection mixtures were prepared as follows: 100 µl Transfection Medium (Expression Systems, California, cat. 95-020-100) was placed into each of two sterile 1.5 ml microfuge tubes, A and B. To Tube A, 0.4 µg recombinant BestBac 2.0 Δ v-cath/chiA linearized DNA (Expression Systems, California, cat. 91-002) and 2 µg vector DNA was added. To Tube B, 1.2 µl 5X Express^{2}TR transfection reagent (Expres2ION, cat. S2-55A-001) was added. Solutions A and B were incubated at approximately 24°C for 5 minutes and then combined and incubated for 30 minutes. After incubation 800 µl Transfection Medium was added to each transfection reaction to increase the volume to 1 ml. The old ESF 921 medium was removed from the wells and replaced with 1 mL transfection mixture applied drop-by-drop so as not to disturb the cell monolayer. Plate(s) were gently rocked back-and-forth and from side-to-side to evenly distribute the transfection mixtures, and incubated at 27°C for 4 h. After 4 h, the transfection mixture was removed from the co-transfection plate(s) and 2 mL fresh ESF 921 insect cell culture medium (Expression Systems, California, cat. 96-001-01) containing gentamicin at 10 µg/ml (cat. 15750-060) was added drop-by-drop. To prevent evaporation, plates were wrapped in saran wrap, placed in a sterile plastic box and incubated for 4-5 days at 27°C. At day 4 or 5 post-transfection P1 supernatants were collected and clarified by centrifugation at 5000 rpm for 5 minutes and transferred to a new sterile tube and stored at 4°C, protected from light.

[00371] The recombinant P1 stocks produced as described above were further amplified to high-titre, low passage P2 stocks for protein expression studies. The following workflow produced 50-100 mL virus using the P1 seed stock of virus harvested from the co-transfection

as inoculum. 50 mL of log-phase Sf9 cells at 1.5 x 10^6 cells ml^{-1} were seeded into a 250 mL shake flask (FisherScientific, cat. PBV 250), and 0.5 mL P1 virus stock was added. Cells were incubated at 27°C, with shaking at 135 rpm and monitored for infection. P2 virus supernatant was harvested 5-7 days post infection and clarified by centrifugation at 4000 rpm for 10 minutes. To minimize titre loss, 10% heat-inactivated FBS (VWR, cat. 97068-085) was added and P2 virus stored at 4°C in the dark.

[00372] The P2 viral stocks were tested for protein expression in small-scale. P2 virus was used to co-infect G-CSF_E mutants with their corresponding G-$CSFR_E$ mutant in *Trichoplusia ni* (Tni) cells in 12-well plates. Separate co-infections for each design mutant were performed using the P2 stocks of G-CSF_{WT} and GCSFR$(CRH)_{WT}$, as well as for G-CSF_E mutants alone. For each reaction, 2 mL of healthy log-phase Tni at 2 x 10^6 cells ml^{-1} was inoculated with 20 µl P2 virus. Plates were incubated at 27°C for approximately 70 h with shaking at 135 rpm. Supernatants were clarified by centrifugation at 5000 rpm for 3 minutes and secreted protein was pulled down, via the Twin-Strep Tag (TST) on G-CSF_E mutants and G-CSF_{WT}, in batch mode with streptactin-XT superflow (IBA Lifesciences, cat. 2-4010-010). Briefly, to each 1.8 mL reaction supernatant 0.2 mL 10X HEPES Buffered Saline (HBS: 20mM HEPES pH8, 150mM NaCl) was added to 1X, 20 µl bed volume (b.v) of purification beads added and reactions incubated for 30 minutes with end-over-end mixing at 24°C. An additional 20 µl b.v of purification beads was added followed by a second 30 minute incubation. Beads were pelleted by centrifugation at 2200 rpm for 3 minutes, supernatant was removed and the beads washed with 1X HBS buffer. Protein was eluted with 30 µl BXT elution buffer (100mM Tris-CL pH8, 150mM NaCl, 1mM EDTA, 50mM Biotin, (IBA Lifesciences, cat. 2-1042-025)), boiled with SDS-PAGE sample buffer and analyzed on a 12% Bolt Bis-Tris plus, 12 well gel (Thermo Fisher Scientific, cat. NW00122BOX) run at 200V for 30 minutes under reducing conditions.

Results

[00373] Site II designs #6,7,8,9,15,17,30,34,35,36 showed expression for G-CSF_E alone, and formed sufficiently stable, paired G-CSF_E:G-$CSFR_E$ complexes that were pulled down through the Twin Strep tag on G-CSF_E (see, Figure 6). For example, site II design #6 post pulldown of the engineered complex showed a band on SDS-PAGE for its G-CSF_E mutant at ~22 kDa as well as for its corresponding, co-expressed G-CSFR$(CRH)_E$ mutant at ~33 kDa (see, Figure 6).

[00374] Site II designs #8,9,15 and 34 were also selective against mispairing with WT G-CSF and WT G-CSFR (see, Figure 7) in the co-expression assay because they were not pulled down by WT G-CSF (see, Figure 7 bottom panel), and WT receptor was not pulled down by the G-CSF$_E$ mutant (see, Figure 7 top panel). Site II G-CSFR$_E$ design 30 and 35 were selective against mispairing with the WT G-CSF in the co-expression assay because they were not pulled down by WT G-CSF (see, Figure 7 bottom panel), however, the reciprocal G-CSF$_E$ design was not selective against mispairing with the WT G-CSFR in co-expression assay because WT G-CSFR was pulled down (see, Figure 7 top panel). Site II designs 6, 7, 17 and 36 were not selective against mispairing with the WT G-CSF or WT G-CSFR (see, Figure 7) in the co-expression assay because they pulled down WT G-CSFR and were pulled down by WT G-CSF.

[00375] Site II receptor mutants with mutations at residue R288 did not express in the G-CSFR(CRH) receptor chain format without Ig domain. R288 containing designs were evaluated for paired complex formation by SPR in combination with site III designs on the Ig domain (see Example 6). Therefore, several site II designs were identified that formed sufficiently stable, paired G-CSF$_E$:G-CSFR$_E$ complexes that were also selective against mispairing with WT G-CSF and WT G-CSFR.

[00376] Therefore, various site II mutations of the same variant G-CSF and receptor designs shown in Table 2 exhibit preferential binding over wild type G-CSFR and G-CSF, respectively.

Example 3: Rational design of an exclusive site III interface of G-CSF:G-CSFR(Ig)

[00377] The avidity effect of the site III receptor Ig domain binding to G-CSF contributes to the formation of the 2:2 heterodimer stoichiometry of the G-CSF:G-CSFR(Ig-CRH) complex (see, Figure 1). A selective design present only at site II with a WT site III interface appears to be insufficient to create a fully exclusive 2:2 G-CSF$_E$:G-CSFR(Ig-CRH)$_E$ complex. To favor binding of a paired, co-evolved design to be selective over mispaired binding to WT cytokine or receptor, we applied the *in silico* design workflow described in Example 1 to the site III interface to create selective site III designs (see Figure 2).

Methods

[00378] First, we conducted a structural analysis of site III interface interactions. The site III interface contributes 55.64 kcal/mol AMBER energy to the G-CSF:G-CSFR complex and it has an overall smaller interface area with 571.5 Å^2 compared to site II with an interface

area of 692.6 $Å^2$. In depth inspection of site III interface reveals less electrostatic and hydrogen-bonding interactions compared to the site II interface (see, Figure 8). Key interactions at site III are for example a salt bridge between E46 of G-CSF and R41 of the receptor Ig domain, furthermore between R147 of G-CSF and E93 of the receptor Ig domain (see, Figure 9). Both interactions contribute 15.9% and 15.4%, respectively, to the total attractive AMBER energy of site III. Further interactions are for example made through Q87 of the receptor Ig domain, which forms a hydrogen-bonding interaction with its side chain amide to the backbone of G-CSF site III, and has Lennard Jones interactions with surrounding side chains of the cytokine such as E46 and L49. This interaction makes up 12.3% of the total attractive AMBER energy at site III.

[00379] Next, we created positive designs at site III where G-CSF_E mutant has a favorable AMBER binding affinity *in silico* for its co-evolved G-CSFR$(Ig)_E$ mutant (paired interaction). This was done for example through inverting charges or changing shape complementarity while maintaining favorable Lennard Jones and hydrogen bonding interactions. Mutant pairs G-CSF_E:G-CSFR$(Ig)_E$ were packed with the mean-field packing workflow of ZymeCAD™. The packed *in silico* models of designs at site III were visually inspected for structural integrity and they were assessed by ZymeCAD™ metrics as described in Example 1. Next, we packed G-CSF_E mutants of each design with WT G-CSFR(Ig) (and vice versa G-CSFR$(Ig)_E$ with G-CSF_{WT}) with ZymeCAD™ to assess the metrics under conditions of mispairing with WT cytokine and receptor. We calculated ddAMBER affinity metrics (G-CSF_{WT}:G-CSFR$(Ig)_E$, G-CSF_E:G-CSFR$(Ig)_{WT}$) for site III designs with ZymeCAD™ (see Table 6) as described before for site II in Example 1.

[00380] *Site III* designs were clustered and the packing metrics of all three *in silico* complexes G-CSF_E:G-CSFR$(Ig)_E$, G-CSF_{WT}:G-CSFR$(Ig)_E$, G-CSF_E:G-CSFR$(Ig)_{WT}$ were considered together with visual inspection to assess the strength of pairing and selectivity against mispairing with WT in order to rank designs.

Results

[00381] Designs listed in Table 3 have *in silico* packing metrics in ZymeCAD™, that favour pairing of G-CSF_E:G-CSFR$(Ig)_E$, with high selectivity over mispairing with WT G-CSF or G-CSFR(Ig).

Therefore, various site III mutations of the same variant G-CSF and receptor designs shown in Table 3 are predicted to exhibit preferential binding, over wild type G-CSFR and G-CSF, respectively.

Table 6: Site III designs with AMBER metrics from triplicate *in silico* mean-field packs in kcal/mol in ZymeCAD^{Tm}.

	G-CSF$_E$:G-CSFR(Ig)$_E$		G-CSF$_E$:G-CSFR(Ig)$_{WT}$	G-CSF$_{WT}$:G-CSFR(Ig)$_E$
Design #	**dAmber_affinity**	**dAmber_folding**	**ddAmber_affinity_E_WT**	**ddAmber_affinity_WT_E**
51	-31.84	21.42	-28.60	-49.04
52	-31.81	21.57	-36.14	-42.08
53	-28.72	30.24	-36.29	-48.49
54	-27.30	36.15	-34.29	-31.51
55	-23.32	21.36	-33.71	-37.62
56	-21.25	28.70	-40.40	-36.60
57	-18.31	15.77	-24.68	-51.81
58	-17.69	17.14	-24.62	-36.77
59	-17.64	28.39	-27.76	-40.42
60	-16.01	30.54	-22.94	-45.27
61	-15.84	29.64	-9.07	-33.76
62	-14.29	2.89	-21.18	-12.78
63	-14.20	3.57	-14.42	-14.65
64	-13.68	34.79	-22.85	-22.93
65	-13.51	28.01	-15.43	-34.93
66	-12.58	10.88	-19.51	-26.53
67	-11.43	35.72	-21.79	-27.46
68	-10.95	15.99	-12.52	-20.20
69	-9.53	18.46	-7.37	-32.23
70	-9.34	13.13	-27.74	-10.28
71	-7.63	15.05	-7.95	-15.03
72	-5.68	10.95	-1.97	-16.54

Example 4: Combination of site II and III to create a variant, co-evolved cytokine-receptor switch

[00382] To develop a fully selective design pair G-CSF$_E$:G-CSF(Ig-CRH)$_E$, that enables binding and signaling through the 2:2 heterodimeric engineered complex and has low or completely abolished cross-reactivity with wild type cytokine or receptor, selected site II and III designs from Examples 1 and 3 were combined (see, Table 4) and tested in vitro.

[00383] In the same way the designs in Table 4 were combined, any other combination of a site II design of Example 1 (Table 2) with a site III design of Example 3 (Table 3) could result in a combined fully selective G-CSF$_E$:G-CSFR(Ig-CRH)$_E$ design that enables variant

signaling. Combination designs 401 and 402 were tested in the co-expression assay described in Example 2 for their ability to form an engineered G:CSF$_E$:G-CSFR(Ig-CRH)$_E$ complex, as well as for their ability to bind WT cytokine or receptor.

Methods

[00384] The co-expression assay with pulldown through the cytokine TST tag was performed as described above in Example 2, with the difference that the receptor constructs contained the Ig and CRH domains (residues 2-308, Table 1) referred to as G-CSFR(Ig-CRH).

Results

[00385] Combination designs 401 and 402 were fully selective in the co-expression assay, in that the design cytokines pulled down their co-evolved, engineered receptor but not WT receptor, and vice versa WT G-CSF did not pull down the engineered receptor (see, Figure 10).

[00386] These results show that variant G-CSF and receptors comprising variant G-CSFR ECD designs combining select site II and site III mutations are capable of specific binding to engineered cytokine receptor pair and do not bind the wild type receptor or cytokine, respectively.

Example 5: Production of G-CSF and G-CSFR wildtype and mutants

[00387] To produce wild type and engineered cytokine and receptor variants and compare their biophysical properties, recombinant proteins were expressed and purified from insect cells.

Methods

[00388] G-CSF$_E$ and G-CSF$_{WT}$ were cloned as described above. Preparative scale production of recombinant proteins was performed in 2-4 L healthy, log-phase Tni cells as follows: 800 mL Tni at 2 x 10^6 cells ml^{-1} were inoculated with 20 µl cytokine variant P2 virus per 2ml cells, and incubated for 70 h at 27°C with shaking at 135 rpm. After incubation, the cells were pelleted by centrifugation at 5500 rpm for 15 minutes and the supernatant filtered twice, first through a 1 µm Type A/E glass fiber filter (PALL, cat. 61631) followed by 0.45 µm PVDF membrane filter (Sigma Aldrich, cat. HVLP04700). Protease inhibitor cocktail III (Sigma Aldrich, cat. 539134) was added and the supernatant buffer exchanged into HBS (20mM HEPES pH8, 150mM NaCl) and concentrated to 300 mL on the tangential flow. Protein was purified in batch mode with 3 x 3 mL b.v Streptactin-XT superflow and incubated for 2 x 1 h, and 1 x overnight at 4°C with stirring. Prior to elution the resin was

washed with 10 CV HBS buffer. Protein was eluted in 4 x 5 mL of BXT elution buffer. Eluates were analyzed by nanodrop A280 and reducing SDS-PAGE, concentrated to ~2 mL and the TST purification tag cleaved by incubation with TEV at 1:80 ratio of TEV: protein at 18°C overnight with end-over-end mixing. Cut protein was confirmed by SDS-PAGE prior to being loaded onto either a SX75 16/600 or SX200 16/600 size exclusion column (GE Healthcare, cat. 28-9893-33 or 28-9893-35) equilibrated in 20 mM BisTris pH 6.5, 150 mM NaCl (see, Figure 11). Protein containing fractions were analyzed by reducing SDS-PAGE, pooled and the concentration was measured by nanodrop A280 measurement.

[00389] For protein purification of receptor variants, wild type and G-CSFR(Ig-CRH)$_E$ mutants were cloned as described above, receptor constructs for purification included the Ig domain in addition to the CRH domain (residues 3-308 of Uniprot ID Q99062, Table 1). Viral stocks were prepared and used for infection at 2-4 L scale as described above. Clarified supernatants were buffer exchanged into Ni-NTA binding buffer (20 mM HEPES pH8, 1 M NaCl, 30 mM Imidazole) and concentrated to 300 mM as described above. Protein was purified in batch bind mode with 3 x 3 mL b.v Ni-NTA superflow and incubated for 2 x 1 h, and 1 x overnight at 4°C with stirring. Prior to elution the resin was washed with 10 CV binding buffer. Protein was eluted in 4 x 5 mL Ni-NTA elution buffer (20 mM HEPES pH8, 1 M NaCl, 250 mM Imidazole). Eluates were analyzed, buffer exchanged into 20 mM Bis-Tris pH 6.5, 150 mM NaCl, concentrated and cleaved overnight as described above. Cut protein was subsequently loaded onto a SX 75 16/600 or SX200 16/600 size exclusion column (GE Healthcare) equilibrated in 20 mM BisTris pH 6.5, 150 mM NaCl (see, Figure 12). Protein containing fractions were analyzed by reducing SDS-PAGE, pooled and the concentration was measured by nanodrop A280 measurement.

Results

[00390] Wild type, G-CSF$_E$ and G-CSFR$_E$ mutants were > 90% pure post SEC as judged by reducing SDS-PAGE (see, Figures 11 and 12). The yield post SEC per 1 L culture of design 401 and 402 G-CSF$_E$ was 2.7 mgs and 1.6 mgs, respectively. The yield post SEC per 1 L production of design 401 and 402 G-CSFR$_E$ was 1.7 mgs and 1.5 mgs, respectively. WT G-CSF was purified with a yield of 2.1 mgs post SEC per 1 L culture, WT G-CSFR was purified with a yield of 3.1 mg post SEC per 1 L of culture.

[00391] These results confirm that the methods used to purify variant G-CSF and receptors were efficient to yield purified protein to be used for analysis of biophysical properties in vitro.

Example 6: Determination of the affinity of designs for their paired and mispaired binding partner by SPR

[00392] In order to determine the affinity of design cytokines for their co-evolved receptor mutant, we measured the affinity of G-CSF$_E$ for G-CSFR$_E$. We also determined the affinity of G-CSF$_E$ for G-CSFR$_{WT}$ and the affinity of G-CSF$_{WT}$ for G-CSFR$_E$ for a subset of designs by SPR (mispaired).

Methods

[00393] The SPR binding assays were carried out on a Biacore T200 instrument (GE Healthcare, Mississauga, ON, Canada) with PBS-T (PBS + 0.05% (v/v) Tween 20) running buffer at a temperature of 25°C. CM5 Series S sensor chip, Biacore amine coupling kit (NHS, EDC and 1 M ethanolamine), and 10 mM sodium acetate buffers were all purchased from GE Healthcare. PBS running buffer with 0.05% Tween20 (PBS-T) was purchased from Teknova Inc. (Hollister, CA). Designs were assessed in three different immobilization orientations.

[00394] To determine binding affinity of G-CSF$_E$ to G-CSFR$_E$ the G-CSFR$_E$ mutant was captured by standard amine coupling as described by the manufacturer (GE LifeSciences). Briefly, immediately after EDC/NHS activation, a 5 µg/mL solution of G-CSFR$_E$ in 10 mM NaOAc, pH 5.0, was injected at a flow rate of 5 µL/min until a receptor density of ~700-900 RU was reached. The remaining active groups were quenched by a 420 s injection of 1 M ethanolamine hydrochloride-NaOH pH 8.5 at 10 µL/min. Using single-cycle kinetics, six concentrations of a two-fold dilution series of the corresponding G-CSF$_E$ mutant starting at 200 nM with a blank buffer control were sequentially injected at 25 µL/min for 300s with a 1800s dissociation phase, resulting in a set of sensorgrams with a buffer blank reference. The same sample titration was also performed on a reference cell with no variants captured. The chip was regenerated to prepare for the next injection cycle by one pulse of 10 mM glycine/HCl, pH 2.0, for 30 s at 30 µL/min.

[00395] To assess binding affinity of G-CSF$_{WT}$ to G-CSFR$_E$, G-CSF$_E$ was captured on the chip at a density of ~700-900 RU as described above. Using single-cycle kinetics, six concentrations of a two-fold dilution series of each G-CSFR$_{WT}$ starting at 200 nM with a blank buffer control were sequentially injected at 25 µL/min for 300s with a 1800s total dissociation time, resulting in a set of sensorgrams with a buffer blank reference. The same sample titration was also performed on a reference cell with no variants captured and the chip was regenerated as described above.

[00396] To assess binding affinity of G-CSF$_E$ to G-CSFR$_{WT}$, recombinant G-CSFR$_{WT}$ purified as described in Example 5 was captured as described above in this Example. Using single-cycle kinetics, six concentrations of a two-fold dilution series of each G-CSF$_E$ mutant starting at 200 mM with a blank buffer control were sequentially injected as described above. The same sample titration was also performed on a reference cell with no variants captured. The G-CSFR$_{WT}$ surface was regenerated as described above.

[00397] As a control, the binding of WT G-CSF to WT G-CSFR(Ig-CRH) was assessed in each experiment and used to calculate the K_D fold change within each independent measurement.

[00398] Double-referenced sensorgrams from duplicate or triplicate repeat injections were analyzed using Biacore^{TM} T200 Evaluation Software v3.0 and fit to the 1:1 Langmuir binding model.

Results

[00399] Kinetic-derived affinity constants (K_D) where obtained by fitting the association and dissociation phases of the curves. The K_D of WT G-CSF for WT G-CSFR(Ig-CRH) ranged between 1.8-2.5E-9. For the cases in which the kinetic parameters could not be fit an attempt was made to derive a steady state affinity constant. In those cases, the KD fold change was calculated from the steady state derived K_D for the WT G-CSF:WT G-CSFR(Ig-CRH) pair and is indicated in Table 7.

[00400] Designs 9, 130, 134, 137, 307, 401 and 402 showed affinities for their co-evolved binding partner not more than 2x different from the WT:WT KD (see Table 7 and Figure 13).

[00401] Design 9, 30 and 34 G-CSFR(Ig-CRH)$_E$ mutants showed more than >700x weaker affinity for WT G-CSF compared to WT G-CSFR(Ig-CRH). Design #35 G-CSFR(Ig-CRH)$_E$ was less selective against mispairing with WT cytokine, its affinity for WT G-CSF was reduced ~19x compared to WT:WT affinity. Designs 130, 134, 401, 402, 300, 3003, 304 and 307 G-CSFR(Ig-CRH)$_E$ mutant were the most selective against mispairing with WT G-CSF and did not show appreciable binding to WT G-CSF at the concentrations titrated (see, Table 8, Figure 13).

[00402] Design 124, 130, 401, 402, 300, 303, 304 and 307 G-CSF$_E$ mutants did not show appreciable binding to WT G-CSFR(Ig-CRH) at the concentrations titrated. Design 9, 30 and 34 G-CSF$_E$ mutants showed at least a ~20x weaker K_D for WT G-CSFR(Ig-CRH) compared to the WT:WT KD. Design #134 G-CSF$_E$ showed ~500x weaker affinity for WT G-CSFR(Ig-

CRH) (see, Table 9, Figure 13). Design 35 and 117 G-CSF$_E$ mutants show binding to the WT G-CSFR(Ig-CRH) similar to the WT:WT KD.

[00403] These results show that selected variant G-CSF designs do not bind wild type G-CSFR ECD or bind with a significantly reduced affinity to wild type G-CSFR ECD (at least ~20x weaker KD).

Table 7: Change in binding affinity (KD) of G-CSF$_E$ mutants for their corresponding G-CSFR(Ig-CRH)$_E$ mutant compared to WT:WT binding affinity determined by SPR

	K_D fold change
WT:WT control	-
Design G-CSF$_E$:G-CSFR(Ig-CRH)$_E$	
9	1.2
30	11
34	5.0
35	3.3
130	0.9
134	1.0
137	0.3
401	0.6[ss]
402	0.8[ss]
300	194
301	Not measured
303	2.2[ss]
304	6.5
307	0.6[ss]

[ss] denotes a steady state derived affinity constant.

Table 8: Change in binding affinity of WT G-CSF to design G-CSFR(Ig-CRH)$_E$ compared to WT:WT binding affinity determined by SPR

	K_D fold change
WT:WT control	-
Design G-CSFR(Ig-CRH)$_E$	

9	8807[ss]
30	774
34	4258[ss]
35	19
130	No binding
134	No binding
401	No binding
402	No binding
300	No binding
301	Not determined
303	No binding
304	No binding
307	No binding

[ss] denotes a steady state derived affinity constant

Table 9: Change in binding affinity of G-CSF$_E$ to wild type G-CSFR(Ig-CRH) compared to WT: WT binding affinity determined by SPR

	K_D fold change
WT:WT control	-
Design G-CSF$_E$	
9	79
30	20
34	33
35	5.0
117	3.4
124	No binding
130	No binding
134	465
401	No binding
402	No binding
300	No binding
301	Not determined

303	No binding
304	No binding
307	No binding

Example 7: Determination of the thermal stability of design G-CSF$_E$ mutants

[00404] To determine the thermal stability of G-CSF$_E$ and G-CSFR$_E$ mutants in comparison to WT cytokine and receptor we performed differential scanning calorimetry (DSC).

Methods

[00405] The thermal stability of variants was assessed by Differential Scanning Calorimetry (DSC) as follows: 950 mL of purified samples at concentrations of 1-2 mg/mL were used for DSC analysis with a Nano DSC (TA instruments, New Castle, DE). At the start of each run, buffer blank injections were performed for baseline stabilization. Each sample was scanned from 25 to 95˚C at a 60˚C/hr rate, with 60 psi nitrogen pressure. The resulting thermograms were referenced and analyzed using NanoAnalyze software to determine melting temperature (Tm) as an indicator of thermal stability.

Results

[00406] Thermal stability of the engineered variants is reported as the difference between the most prominent transition (highest enthalpy) of the engineered and the equivalent wild type molecule, measured under the same conditions and experimental set up. Measured WT GCSF Tm vary between 52.2 and 55.4°C among independent experiments, while WT G-CSFR displays a Tm of 50.5°C. G-CSF$_E$ mutants tested with the exception of designs #15 and 34 showed a Tm less than 5 °C different from the Tm for WT G-CSF (see, Table 10 and Figure 14). All receptor mutants tested showed the same thermal stability as WT receptor (see, Table 10 and Figure 14).

[00407] These results show that the variant G-CSF and receptors with the variant G-CSFR ECDs designs have similar thermal stability as wild type G-CSF and G-CSFR; and the site II and/or site III mutations do not disrupt thermal stability of either G-CSF or the G-CSFR ECD.

Table 10: Change in melting temperature (Tm) for design cytokine and receptor mutants compared to wild type determined by DSC.

GCSF$_E$ design mutant	ΔTm (°C)
WT	-
#15	-9.9
#34	-6.1
#35	-3.5
#106	+7.5
#130	-1.8
#134	+2.0
#135	-0.8
#300	+1.0
#301	-3.2
#303	+4.2
#304	+2.7
#307	-3.8
#401	-2.5
#402	-3.8
GCSFR (Ig-CRH)$_E$ design mutant	
WT	-
#9	+1.4
#30	+1.4
#34	+1.8
#35	+1.8
#130*	-1.9
#134	-0.9
#300	Not measured
#301	Not measured
#303	Not measured
#304	Not measured
#307	Not measured

#401	Not measured
#402	Not measured

*determined in 150 mM NaCl, 20 mM BisTris pH 6.5

Example 8: Determination of the monodispersity of G-CSF$_E$ mutants by UPLC-SEC

[00408] To determine the monodispersity of G-CSF$_E$ mutants in comparison to WT G-CSF we analyzed mutants by UPLC-SEC.

Methods

[00409] UPLC-SEC was performed on SEC purified protein samples using an Acquity BEH125 SEC column (4.6 x 150 mm, stainless steel, 1.7 µm particles) (Waters LTD, Mississauga, ON) set to 30°C and mounted on an Agilent Technologies 1260 infinity II system with a PDA detector. Run times consisted of 7 minutes with a running buffer of 150 mM NaCl, 20 mM HEPES pH 8.0 or 150 mM NaCl, 20 mM BisTris pH 6.5 at a flow rate of 0.4 mL/min. Elution was monitored by UV absorbance in the range 210-500 nm, and chromatograms were extracted at 280 nm. Peak integration was performed using OpenLAB™ CDS ChemStation™ software.

Results

[00410] WT G-CSF and design 34, 35 and 130 G-CSF$_E$ mutants were 100% monodisperse (see, Table 11). Mutants 8, 9, 15, 117, 135 showed lower monodispersity between 65.3-79.5%. Design #134 cytokine showed 57.3% monodispersity at pH 8.0 which improved to 86.6% monodispersity at pH 6.5. The improvement in monodispersity at lower pH of the mobile phase could be due to a shift of pI, for example from a calculated pI of 5.41 for WT G-CSF to a calculated pI of 8.35 for design #134 G-CSF$_E$.

[00411] These results show that some of the variant G-CSF designs have 100% monodispersity as wild type G-CSF; indicating that a sub-set of the site II and/or site III mutations do not disrupt monodispersity of G-CSF; whereas other variant G-CSF designs led to lowered monodispersity that was increased at lower pH.

Table 11: Monodispersity of G-CSF$_E$ design mutants determined by UPLC-SEC.

G-CSF$_E$ Design Mutant	Monodispersity %
WT	100.0 (96.7*)

8	73.7
9	65.3
15	57.3
34	100.0
35	100.0
117	74.7*
130	100.0*
134	57.3 (86.6*)
135	79.5*
401	89.6*

*in 150 mM NaCl, BisTris pH 6.5

Example 9: Construction of chimeric G-CSF receptors with intracellular IL-2 receptor signalling domains

[00412] To investigate whether design G-CSF$_E$ cytokine mutants are able to signal and cause immune cell proliferation through the design G-CSFR(Ig-CRH)$_E$ receptor mutants, we constructed a single-chain chimeric G-CSF receptor using the G-CSFR ECD fused to the gp130 transmembrane (TM) domain and intracellular signaling domain (ICD), and an IL-2Rβ intracellular signaling domain (G-CSFR$_{WT}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$). We also utilized a chimeric G-CSFR that consists of two subunits designed to be co-expressed as a heterodimeric receptor: 1) The G-CSFR$_{WT}$-ICD$_{IL\text{-}2R\beta}$ subunit consists of the G-CSFR ECD fused to the IL-2Rβ TM and ICD; and 2) The G-CSFR$_{WT}$-ICD$_{\gamma c}$ subunit consists of the G-CSFR ECD fused to the common gamma chain (γc, IL-2Rγ) TM and ICD.

Methods

[00413] The single-chain chimeric receptor construct was designed to include the G-CSFR signal peptide and ECD, followed by the gp130 TM and partial ICD, and IL-2Rβ partial ICD (Table 12). The heterodimeric chimeric receptor construct was designed to include: 1) the G-CSFR signal peptide and ECD, followed by the IL-2Rβ TM and ICD (Table 13); and 2) the G-CSFR signal peptide and ECD, followed by the γc TM and ICD (Table 14). The chimeric receptor constructs were cloned into a lentiviral transfer plasmid and the construct sequences were verified by Sanger sequencing. The transfer plasmid and lentiviral packaging plasmids (psPAX2, pVSVG) were co-transfected into lentiviral packaging cell line HEK293T/17 cells (ATCC) as follows: Cells were plated overnight in DMEM containing 10% fetal bovine

serum and penicillin/streptomycin, and medium changed 2-4 hours prior to transfection. Plasmid DNA and water were mixed in a polypropylene tube, and $CaCl_2$ (0.25M) was added dropwise. After a 2 to 5 minute incubation, the DNA was precipitated by mixing 1:1 with 2x HEPES-buffered saline (0.28M NaCl, 1.5mM Na_2HPO_4, 0.1M HEPES). The precipitated DNA mixture was added onto the cells, which were incubated overnight at 37°C, 5% CO_2. The following day the HEK293T/17 medium was changed, and the cells were incubated for another 24 hours. The following morning, the cellular supernatant was collected from the plates, centrifuged briefly to remove debris, and filtered through a 0.45μm filter. The supernatant was spun for 90 minutes at 25,000 rpm using a SW-32Ti rotor in a Beckman Optima L-XP Ultracentrifuge. The supernatant was removed, and the pellet was resuspended in a suitable volume of Opti-MEM medium. The viral titer was determined by adding serial dilutions of virus onto BAF3 cells (grown RPMI containing 10% fetal bovine serum, penicillin, streptomycin and 100 IU/ml hIL-2). 48-72 hours after transduction, the cells were incubated with an anti-human G-CSFR APC-conjugated antibody (1:50 dilution) and eBioscience™ Fixable Viability Dye eFluor™ 450 (1:1000 dilution) for 15 minutes at 4°C, washed, and analyzed on a Cytek Aurora or BD FACS Calibur flow cytometer. Using the estimated titer determined by this method, the 32D-IL-2Rβ cell line (grown in RPMI containing 10% fetal bovine serum, penicillin, streptomycin and 300 IU/ml hIL-2) was transduced with the lentiviral supernatant encoding the chimeric receptor construct at an MOI of 0.5. Transduction was performed by adding the relevant amount of viral supernatant to the cells, incubating for 24 hours, and replacing the cell medium. 3-4 days after transduction, we verified expression of the human G-CSFR by flow cytometry, as described above. Cells were expanded in $G\text{-}CSF_{WT}$ for approximately 14-28 days before performing the BrdU assay.

[00414] 32D-IL-2Rβ cells expanded in $G\text{-}CSF_{WT}$ as described above were washed three times in PBS, and re-plated in fresh medium containing the relevant assay cytokine (no cytokine, hIL-2 (300 IU/ml), $G\text{-}CSF_{WT}$ (30 ng/ml) or $G\text{-}CSF_{E}$ (30 ng/ml) for 48 hours. The BrdU assay procedure followed the instruction manual for the BD Pharmingen™ APC BrdU Flow Kit (557892), with the following addition: cells were co-incubated with BrdU and eBioscience™ Fixable Viability Dye eFluor™ 450 (1:5000) for 30 minutes. Analytical flow cytometry was performed using a Cytek Aurora instrument.

Results

[00415] In a BrdU assay, cells transfected with the single-chain chimeric receptor construct $G\text{-}CSFR_{WT}\text{-}ICD_{gp130\text{-}IL\text{-}2R\beta}$ or the heterodimeric receptor construct $G\text{-}CSFR_{WT}$-

$ICD_{IL\text{-}2R\beta}$ plus G-$CSFR_{WT}$-$ICD_{\gamma c}$ exhibited similar or superior proliferation in response to G-CSF_{WT} (30 ng/ml) compared to hIL-2 (300 IU/ml). Cells did not proliferate in the absence of cytokine (see Figure 15).

[00416] These results show that, upon stimulation by G-CSF, single chain and heterodimeric chimeric receptor constructs can be activated to induce cell proliferation .

Example 10: Proliferation of 32D-IL-2Rβ cells transduced with design 137 G-$CSFR_E$-$ICD_{IL\text{-}2}$ and treated with wild type or design G-CSF_{137} examined by BrdU

[00417] Site II/III combination designs that were sufficiently selective as judged by SPR or co-expression assays were tested *in vitro* for the ability to induce proliferation of 32D-IL-2Rβ cells.

Methods

[00418] Point mutations for design 137 were introduced into the constructs described in Tables 12-14. Cloning and expression of the G-$CSFR_{137}$-$ICD_{gp130\text{-}IL\text{-}2R\beta}$ (homodimer) or G-$CSFR_{137}$-$ICD_{IL\text{-}2R\beta}$ plus G-$CSFR_{137}$-$ICD_{\gamma c}$ (heterodimer) constructs followed the same procedures as described above. Before performing the BrdU assay, cells were expanded in G-CSF_{137} for approximately 14-28 days.

[00419] 32D-IL-2Rβ cells expanded in G-CSF_{137} were assayed for proliferation in G-CSF_{137} (30 ng/ml), G-CSF_{WT} (30 ng/ml), hIL-2 (300 IU/ml) or no cytokine using the BrdU assay procedure described above.

Results

[00420] In a BrdU assay, cells transduced with the single-chain chimeric receptor construct G-$CSFR_{137}$-$ICD_{gp130\text{-}IL\text{-}2R\beta}$ or the heterodimeric receptor construct G-$CSFR_{137}$-$ICD_{IL\text{-}2R\beta}$ plus G-$CSFR_{137}$-$ICD_{\gamma c}$ exhibited similar or superior proliferation in G-CSF_{137} (30 ng/ml) compared to hIL-2 (300 IU/ml). Cells did not proliferate in absence of cytokine and exhibited inferior proliferation in G-CSF_{WT} (30 ng/ml) (Figure 16).

[00421] These results indicate that the variant G-CSF specifically activates the engineered receptor; and conversely, the engineered receptor is activated by the variant G-CSF but markedly less so than wild type G-CSF. Therefore, variant G-CSF can specifically activate chimeric receptors with variant G-CSFR ECD to specifically induce proliferation of cells expressing the chimeric receptors.

Example 11: Proliferation of 32D-IL-2Rβ cells transduced with wild-type G-CSFR-ICD$_{IL-2}$ and treated with wild type or design G-CSF$_E$ examined by BrdU

[00422] Site II/III combination designs that were able to restore paired signaling in 32D-IL-2R2Rβ cells were subsequently tested for their ability to induce proliferation of 32D-IL-2R2Rβ cells transduced with the single-chain chimeric receptor construct G-CSFR$_{WT}$-ICD$_{gp130-IL-2R\beta}$ or the heterodimeric receptor construct G-CSFR$_{WT}$-ICD$_{IL-2R\beta}$ plus G-CSFR$_{WT}$-ICD$_{\gamma c}$.

Methods

[00423] Cloning and expression of the G-CSFR$_{WT}$-ICD$_{gp130-IL-2R\beta}$ (homodimer) or G-CSFR$_{WT}$-ICD$_{IL-2R\beta}$ plus G-CSFR$_{WT}$-ICD$_{\gamma c}$ (heterodimer) constructs followed the same procedures as described above. Before performing the BrdU assay, cells were expanded in G-CSF$_{WT}$ for approximately 14-28 days.

[00424] 32D-IL-2Rβ cells expanded in G-CSF$_{WT}$ were assayed for proliferation in G-CSF$_{137}$ (30 ng/ml), G-CSF$_{WT}$ (30 ng/ml), hIL-2 (300 IU/ml) or no cytokine using the BrdU assay procedure described above.

Results

[00425] In a BrdU assay, cells transduced with the single-chain chimeric receptor construct G-CSFR$_{WT}$-ICD$_{gp130-IL-2R\beta}$ or the heterodimeric receptor construct G-CSFR$_{WT}$-ICD$_{IL-2R\beta}$ and G-CSFR$_{WT}$-ICD$_{\gamma c}$ exhibited inferior proliferation in G-CSF$_{137}$ (30 ng/ml) compared to G-CSF$_{WT}$ (30 ng/ml). Cells did not proliferate in the absence of cytokine (see, Figure 17).

[00426] These results indicate that the variant G-CSF does not efficiently bind wild type G-CSFR, and the variant G-CSF specifically activates the engineered receptor, but not wild type G-CSFR, to induce cell proliferation.

Example 12: Signaling in 32D-IL-2Rβ cells transduced with WT or design 137 G-CSFR$_E$-ICD$_{IL-2}$ and treated with wild type or design G-CSF$_{137}$ analyzed by Western Blot

[00427] Site II/III combination designs, that were able to restore proliferative signaling through the engineered cytokine-receptor complex in 32D-IL-2Rβ cells and did not significantly signal through binding G-CSF$_{WT}$ or WT-GCSFR-ICD-IL2, were assessed by western blot for the ability to activate downstream signaling molecules in response to G-CSF$_{WT}$ or G-CSF$_{137}$.

Methods

[00428] Cloning and expression of the G-CSFR$_{WT}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$ (homodimer) or G-CSFR$_{WT}$-ICD$_{IL\text{-}2R\beta}$ plus G-CSFR$_{WT}$-ICD$_{\gamma c}$ (heterodimer) constructs followed the same procedures as described above. Cells were expanded in G-CSF$_{137}$ or G-CSF$_{WT}$ for approximately 14-28 days before performing the western blot assay. Non-transduced cells were maintained in IL-2.

[00429] To perform western blots, cells were washed three times in PBS and rested in medium containing no cytokine for 16-20 hours. Cells were stimulated with no cytokine, IL-2 (300 IU/ml), G-CSF$_{137}$ (30 ng/ml) or G-CSF$_{WT}$ (30 ng/ml) for 20 minutes at 37°C. Cells were washed once in a wash buffer containing 10 mM HEPES, pH 777.9, 1 mM $MgCl_2$, 0.05 mM EGTA, 0.5 mM EDTA, pH 8.0, 1 mM DTT, and 1x Pierce Protease and Phosphatase Inhibitor Mini Tablets (A32961). Cells were lysed in the wash buffer above, with the addition of 0.2% Igepal CA630 (Sigma), for 10 minutes on ice and centrifuged for 10 minutes at 13,000 rpm at 4°C, after which supernatant (cytoplasmic fraction) was collected. The pellet was resuspended and lysed in the above wash buffer with the addition of 0.42M NaCl and 20% glycerol. Cells were lysed for 30 minutes on ice, with frequent vortexing, and centrifuged for 20 minutes at 13,000 rpm at 4°C, after which the nuclear fraction (supernatant) was collected. The cytoplasmic and nuclear fractions were reduced (70°C) for 10 minutes and run on a NuPAGE™ 4-12% Bis-Tris Protein Gel. The gels were transferred to nitrocellulose membrane (60 min at 20V in a Trans-Blot® SD Semi-Dry Transfer Cell), dried, and blocked for 1hr in Odyssey® Blocking Buffer in TBS (927-50000). The blots were incubated with primary antibodies (1:1,000) overnight at 4°C in Odyssey® Blocking Buffer in TBS containing 0.1% Tween20. The primary antibodies utilized were obtained from Cell Signaling Technologies: Phospho-Shc (Tyr239/240) Antibody #2434, Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060, Phospho-S6 Ribosomal Protein (Ser235/236) Antibody #2211, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody #9101, β-Actin (13E5) Rabbit mAb #4970, Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145, Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359, and Histone H3 (96C10) Mouse mAb #3638. Blots were washed three times in TBS containing 0.1% Tween20 and incubated with secondary antibodies (1:10,000) in TBS buffer containing 0.1% Tween20 for 30-60 minutes at room temperature. The secondary antibodies obtained from Cell Signaling Technologies: Anti-mouse IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5257 and Anti-rabbit IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5151. Blots were washed and exposed on a LI-COR Odyssey imager.

Results

[00430] In non-transduced 32D-IL-2Rβ cells, we detected activated IL-2R-associated signaling molecules in response to stimulation with IL-2 only. We observed similar patterns of activated signaling molecules in response to IL-2 or G-CSF$_{WT}$ in 32D-IL-2Rβ cells expressing either G-CSFR$_{WT}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$ or G-CSFR$_{WT}$-ICD$_{IL\text{-}2R\beta}$ plus G-CSFR$_{WT}$-ICD$_{\gamma c}$. We did not observe activation of IL-2R-associated signaling molecules in response to G-CSF$_{137}$ stimulation of 32D-IL-2Rβ cells expressing G-CSFR$_{WT}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$ or cells expressing G-CSFR$_{WT}$-ICD$_{IL\text{-}2R\beta}$ plus G-CSFR$_{WT}$-ICD$_{\gamma c}$. We observed similar patterns of activated signaling molecules in response to IL-2 or G-CSF$_{137}$ in 32D-IL-2Rβ cells expressing G-CSFR$_{137}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$ or G-CSFR$_{137}$-ICD$_{IL\text{-}2R\beta}$ plus G-CSFR$_{137}$-ICD$_{\gamma c}$. We did not observe activation of IL-2R2R2R-associated signaling molecules in response to G-CSF$_{WT}$ stimulation in 32D-IL-2Rβ cells expressing G-CSFR$_{137}$-ICD$_{gp130\text{-}IL\text{-}2R\beta}$ or cells expressing G-CSFR$_{137}$-ICD$_{IL\text{-}2R\beta}$ plus G-CSFR$_{137}$-ICD$_{\gamma c}$ (see Figure 18).

[00431] These results demonstrate that variant G-CSF is capable of activating chimeric receptors expressing variant G-CSFR ECD to induce aspects of native cytokine signaling in cells expressing the chimeric receptors.

Methods for Examples 13-30

[00432] **Primary cells and cell lines:** The lentiviral packaging cell line HEK293T/17 (ATCC) was cultured in DMEM containing 10% fetal bovine serum and penicillin/streptomycin. BAF3-IL-2Rβ cells were previously generated by stable transfection of the human IL-2Rβ subunit into the BAF3 cell line, and were grown in RPMI-1640 containing 10% fetal bovine serum, penicillin, streptomycin and 100 IU/ml human IL-2 (hIL-2) (PROLEUKIN®, Novartis Pharmaceuticals Canada). The 32D-IL-2Rβ cell line was previously generated by stable transfection of the human IL-2Rβ subunit into the 32D cell line, and was grown in RPMI-1640 containing 10% fetal bovine serum, penicillin, streptomycin and 300 IU/ml hIL-2, or other cytokines, as indicated. Human PBMC-derived T cells (Hemacare) were grown in TexMACS™ Medium (Milenyi Biotec, 130-097-196), containing 3% Human AB Serum (Sigma-Aldrich, H4522), and 300 IU/ml hIL-2, or other cytokines, as indicated. Human tumor-associated lymphocytes (TAL) were generated by culture of primary ascites samples for 14 days in T cell medium (a 50:50 mixture of the following: 1) RPMI-1640 containing 10% fetal bovine serum, 50 uM β-mercaptoethanol, 10 mM HEPES, 2 mM L-glutamine, penicillin, streptomycin; and 2) AIM V™ Medium (ThermoFisher, 12055083)

containing a final concentration of 3000 IU/ml hIL-2. Following this high-dose IL-2 expansion, TAL were cultured in T cell medium containing 300 IU/ml hIL-2 or other cytokines, as indicated. The retroviral packaging cell line Platinum-E (Cell Biolabs, RV-101) was cultured in DMEM containing 10% FBS, penicillin/streptomycin, puromycin (1 mcg/ml), and blasticidin (10 mcg/ml).

[00433] Lentiviral production and transduction of 32D-IL-2Rβ cells: Chimeric receptor constructs were cloned into a lentiviral transfer plasmid and the resulting sequences were verified by Sanger sequencing. The transfer plasmid and lentiviral packaging plasmids were co-transfected into HEK293T/17 cells using the calcium phosphate transfection method as follows: Cells were plated overnight, and medium changed 2-4 hours prior to transfection. Plasmid DNA and water were mixed in a polypropylene tube, and $CaCl_2$ (0.25 M) was added dropwise. After a 2 to 5 minute incubation, the DNA was precipitated by mixing 1:1 with 2x HEPES-buffered saline (0.28M NaCl, 1.5mM Na_2HPO_4, 0.1M HEPES). The precipitated DNA mixture was added onto the cells, which were incubated overnight at 37°C, 5% CO_2. The following day the HEK293T/17 medium was changed, and the cells were incubated for another 24 hours. The following morning, the cellular supernatant was collected from the plates, centrifuged briefly to remove debris, and the supernatant was filtered through a 0.45 micron filter. The supernatant was spun for 90 minutes at 25000 rpm with a SW-32Ti rotor in a Beckman Optima L-XP Ultracentrifuge. The supernatant was removed and the pellet was resuspended in a suitable volume of Opti-MEM medium. The viral titer was determined by adding serial dilutions of virus onto BAF3-IL-2Rβ cells. At 48-72 hours after transduction, the cells were incubated with an anti-human G-CSFR APC-conjugated antibody (1:50; Miltenyi Biotec, 130-097-308) and Fixable Viability Dye eFluor™ 450 (1:1000, eBioscience™, 65-0863-14) for 15 minutes at 4°C, washed, and analyzed on a Cytek Aurora or BD FACS Calibur flow cytometer. Using the estimated titer determined by this method, the 32D-IL-2Rβ cell line was transduced with the lentiviral supernatant encoding the chimeric receptor construct at a Multiplicity of Infection (MOI) of 0.5. Transduction was performed by adding the relevant amount of viral supernatant to the cells, incubating for 24 hours, and then replacing the medium. At 3-4 days after transduction, the expression of human G-CSFR was determined by flow cytometry, as described above.

[00434] Lentiviral transduction of human primary T cells: For transduction of PBMC-derived T cells and TAL, cells were thawed and plated in the presence of Human T Cell

TransAct™ (Miltenyi Biotec, 130-111-160), according to the manufacturer's guidelines. 24 hours after activation, lentiviral supernatant was added, at MOI of 0.125-0.5. 48 hours after activation, the cells split into fresh medium, to remove residual virus and activation reagent. Two to four days after transduction, the transduction efficiency was determined by flow cytometry, as described above. For experiments in which the transduction efficiency of CD4+ and CD8+ fractions was determined separately, antibodies against human G-CSFR, CD4 (1:50, Alexa Fluor® 700 conjugate, BioLegend, 300526), CD8 (1:50, PerCP conjugate, BioLegend, 301030), CD3 (1:50, Brilliant Violet 510™ conjugate, BioLegend, 300448) and CD56 (1:50, Brilliant Violet 711™ conjugate, BioLegend, 318336) were utilized, along with Fixable Viability Dye eFluor™ 450 (1:1000).

[00435] Human T cell and 32D-IL-2Rβ expansion assays: Human primary T cells or 32-IL-2Rβ cells expressing the indicated chimeric receptor constructs, generated above, were washed three times in PBS and re-plated in fresh medium, or had their medium gradually changed, as indicated. Complete medium was changed to contain either wild-type human G-CSF (generated in-house or NEUPOGEN®, Amgen Canada), mutant G-CSF (generated in-house), hIL-2, or no cytokine. Every 3-5 days, cell viability and density were determined by Trypan Blue exclusion, and fold expansion was calculated relative to the starting cell number. G-CSFR expression was assessed by flow cytometry as described above.

[00436] CD4+ and CD8+ human TAL expansion assay: To examine the expansion of the CD4+ and CD8+ fractions of TAL, *ex vivo* ascites samples were thawed, and the CD4+ and CD8+ fractions were enriched using the Human CD4+ T Cell Isolation Kit (Miltenyi Biotec, 130-096-533) and Human CD8+ T Cell Isolation Kit (Miltenyi Biotec, 130-096-495), respectively. After expansion in cytokine-containing medium, the immunophenotype of the cells was assessed by flow cytometry, utilizing antibodies against human G-CSFR, CD4 (1:50, Alexa Fluor® 700 conjugate, BioLegend, 300526), CD8 (1:50, PerCP conjugate, BioLegend, 301030), CD3 (1:50, Brilliant Violet 510™ conjugate, BioLegend, 300448) and CD56 (1:50, Brilliant Violet 711™ conjugate, BioLegend, 318336), along with Fixable Viability Dye eFluor™ 450 (1:1000).

[00437] Primary human T cell immunophenotyping assay: After expansion in cytokine-containing medium, the immunophenotype of T cells was assessed by flow cytometry, utilizing antibodies against human G-CSFR, CD4 (1:100, Alexa Fluor® 700 conjugate, BioLegend,

300526 or PE conjugate, eBioscience™, 12-0048-42, or Brilliant Violet 570™ conjugate, Biolegend, 317445), CD8 (1:100, PerCP conjugate, BioLegend, 301030), CD3 (1:100, Brilliant Violet 510™ or Brilliant Violet 750™ conjugate, BioLegend, 300448 or 344845), CD56 (1:100, Brilliant Violet 711™ conjugate, BioLegend, 318336), CCR7 (1:50, APC/Fire™ 750 conjugate, Biolegend, 353246), CD62L (1:33, PE/Dazzle™ 594 conjugate, Biolegend, 304842), CD45RA (1:33, FITC conjugate, Biolegend, 304148), CD45RO (1:25, PerCP-eFluor® 710 conjugate, eBioscience™, 46-0457-42), CD95 (1:33, PE-Cyanine7 conjugate, eBioscience™, 25-0959-42), along with Fixable Viability Dye eFluor™ 450 or 5106 (1:1000).

[00438] **Retroviral transduction:** The pMIG transfer plasmid (plasmid #9044, Addgene) was altered by restriction endonuclease cloning to remove the IRES-GFP (BglII to PacI sites), and introduce annealed primers encoding a custom multiple cloning site. The chimeric receptor constructs were cloned into the customized transfer plasmid and the resulting sequences were verified by Sanger sequencing. The transfer plasmid was transfected into Platinum-E cells using the calcium phosphate transfection method, as described above. 24 hours after transfection the medium was changed to 5ml fresh complete medium. 48 hours after transfection the cellular supernatant was collected from the plates and filtered through a 0.45 micron filter. Hexadimethrine bromide (1.6 mcg/ml, Sigma-Aldrich) and murine IL-2 (2 ng/ml, Peprotech) were added to the supernatant. This purified retroviral supernatant was used to transduce murine lymphocytes as described below.

[00439] 48 hours prior to collection of retroviral supernatant, 24-well adherent plates were coated with unconjugated anti-murine CD3 (5 mcg/ml, BD Biosciences, 553058) and anti-murine CD28 (1 mcg/ml, BD Biosciences, 553294) antibodies, diluted in PBS, and stored at 4 degrees Celsius. 24 hours prior to collection of retroviral supernatant, C57Bl/6J mice (generated in-house) were euthanized under an approved Animal Use Protocol administered by the University of Victoria Animal Care Committee. Spleens were harvested and murine T cells were isolated as follows: Spleens were manually dissociated and filtered through a 100 micron filter. Red blood cells were lysed by incubation in ACK lysis buffer (Gibco, A1049201) for five minutes at room temperature, followed by one wash in serum-containing medium. CD8a-positive or Pan-T cells were isolated using specific bead-based isolation kits (Miltenyi Biotec, 130-104-075 or 130-095-130, respectively). Cells were added to plates coated with anti-CD3- and anti-CD28-antibodies in murine T cell expansion medium (RPMI-1640 containing 10% FBS, penicillin/streptomycin, 0.05 mM β-mercaptoethanol, and 2 ng/ml

murine IL-2 (Peprotech, 212-12)) or 300 IU/mL human IL-2 (Proleukin), and incubated at 37 degrees Celsius, 5% CO_2 for 24 hours. On the day of transduction, approximately half of the medium was replaced with retroviral supernatant, generated above. Cells were spinfected with the retroviral supernatant at 1000g, for 90 minutes, at 30 degrees Celsius. The plates were returned to the incubator for 0-4 hours, and then approximately half of the medium was replaced with fresh T cell expansion medium. The retroviral transduction was repeated 24 hours later, as described above, for a total of two transductions. 24 hours after the final transduction, the T cells were split into 6-well plates and removed from antibody stimulation.

[00440] 48-72 hours after transduction, the transduction efficiency was assessed by flow cytometry to detect human G-CSFR, CD4 (Alexa Fluor 532 conjugate, eBioscience™, 58-0042-82), CD8a (PerCP-eFluor 710 conjugate, eBioscience™, 46-0081-82) and Fixable Viability Dye eFluor™ 450 (1:1000 dilution), as described above.

[00441] **BrdU incorporation assay:** Human primary T cells, 32D-IL-2Rβ cells, or murine primary T cells, generated as described above, were washed three times in PBS, and re-plated in fresh medium containing the relevant assay cytokine: no cytokine, hIL-2 (300IU/ml), wildtype or engineered G-CSF (at concentrations indicated in individual experiments) for 48 hours. The BrdU assay procedure followed the instruction manual for the BD Pharmingen™ APC BrdU Flow Kit (BD Biosciences, 557892), with the following additions: Cells were co-incubated with BrdU and Fixable Viability Dye eFluor™ 450 (1:5000) for 30 minutes to 4 hours at 37 degrees Celsius. Flow cytometry was performed using a Cytek Aurora instrument. To specifically assess the proliferation of murine T cells expressing chimeric receptors, additional staining for human G-CSFR (1:20 dilution), CD4 (1:50 dilution) and CD8 (1:50 dilution) was performed for 15 minutes on ice, prior to fixation.

[00442] **Western blots:** Human primary T cells, 32D-IL-2Rβ cells, or murine primary T cells, generated as described above, were washed three times in PBS and rested in medium containing no cytokine for 16-20 hours. Cells were stimulated with no cytokine, IL-2 (300 IU/ml), wildtype G-CSF (at concentrations indicated in individual experiments), or $G\text{-}CSF_{137}$ (30 ng/ml) for 20 minutes at 37 degrees Celsius. Cells were washed once in a buffer containing 10 mM HEPES, pH7.9, 1 mM $MgCl_2$, 0.05 mM EGTA, 0.5 mM EDTA, pH 8.0, 1 mM DTT, and 1x Pierce Protease and Phosphatase Inhibitor Mini Tablets (A32961). Cells were lysed in the wash buffer above, with the addition of 0.2% NP-40 (Sigma) for 10 minutes on ice. The

lysate was centrifuged for 10 minutes at 13000 rpm at 4 degrees Celsius, and the supernatant (cytoplasmic fraction) was collected. The pellet (containing nuclear proteins) was resuspended in the wash buffer above, with the addition of 0.42M NaCl and 20% glycerol. Nuclei were incubated for 30 minutes on ice, with frequent vortexing, and the supernatant (nuclear fraction) was collected after centrifuging for 20 minutes at 13,000 rpm at 4 degrees Celsius. The cytoplasmic and nuclear fractions were reduced (70 degrees Celsius) for 10 minutes and run on a NuPAGE™ 4-12% Bis-Tris Protein Gel. The gels were transferred to nitrocellulose membrane (60 min at 20V in a Trans-Blot® SD Semi-Dry Transfer Cell), dried, and blocked for 1hr in Odyssey® Blocking Buffer in TBS (927-50000). The blots were incubated with primary antibodies (1:1000) overnight at 4 degrees Celsius in Odyssey® Blocking Buffer in TBS containing 0.1% Tween20. The primary antibodies utilized were obtained from Cell Signaling Technologies: Phospho-JAK1 (Tyr1034/1035) (D7N4Z) Rabbit mAb #74129, Phospho-JAK2 (Tyr1007/1008) #3771, Phospho-JAK3 (Tyr980/981) (D44E3) Rabbit mAb #5031, Phospho-p70 S6 Kinase (Thr421/Ser424) Antibody #9204, Phospho-Shc (Tyr239/240) Antibody #2434, Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060, Phospho-S6 Ribosomal Protein (Ser235/236) Antibody #2211, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody #9101, β-Actin (13E5) Rabbit mAb #4970, Phospho-STAT1 (Tyr701) (58D6) Rabbit mAb #9167, Phospho-STAT3 (Tyr705) (D3A7) XP® Rabbit mAb #9145, Phospho-STAT4 (Tyr693) Antibody #5267, Phospho-STAT5 (Tyr694) (C11C5) Rabbit mAb #9359, and Histone H3 (96C10) Mouse mAb #3638. Blots were washed three times in TBS containing 0.1% Tween20 and incubated with secondary antibodies (1:10,000) in TBS buffer containing 0.1% Tween20 for 30-60 minutes at room temperature. The secondary antibodies obtained from Cell Signaling Technologies were Anti-mouse IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5257 and Anti-rabbit IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5151. Blots were washed and exposed on a LI-COR Odyssey imager.

[00443] Flow cytometry to detect phosphorylated proteins: Human primary T cells, 32D-IL-2Rβ cells, or murine primary T cells, generated as described above, were washed three times in PBS and rested in medium containing no cytokine for 16-20 hours. Cells were stimulated with no cytokine, IL-2 (300 IU/ml) or wild-type G-CSF (100 ng/ml) for 20 minutes at 37 degrees Celsius, in the presence of Fixable Viability Dye eFluor™ 450 (1:1000), and anti-G-CSFR (1:20), anti-CD4 (1:50) and anti-CD8a (1:50), as indicated. Cells were pelleted and fixed with BD Phosflow™ Fix Buffer I (BD Biosciences, 557870) for 15 minutes at room

temperature. Cells were washed, then permeabilized using BD Phosflow™ Perm Buffer III (BD Biosciences, 558050) on ice for 15 minutes. Cells were washed twice and resuspended in buffer containing 20 ul of BD Phosflow™ PE Mouse Anti-Stat3 (pY705) (BD Biosciences, 612569) or PE Mouse IgG2a, κ Isotype Control (BD Biosciences, 558595). Cells were washed and flow cytometry was performed using a Cytek Aurora instrument.

EXAMPLE 13: EXPANSION OF HUMAN T CELLS EXPRESSING G2R-1, G-CSFR/IL-2RB SUBUNIT ALONE, THE MYC-TAGGED G-CSFR/GAMMA-C SUBUNIT ALONE, OR THE FULL-LENGTH G-CSFR.

[00444] PBMC-derived T cells or tumor-associated lymphocytes (TAL) were transduced with lentiviruses encoding the chimeric receptor constructs shown in Figures 20, 24 and 24, and cells were washed and re-plated in the indicated cytokine. Cells were counted every 3-4 days. G/γc was tagged at its N-terminus with a Myc epitope (Myc/G/γc), and G/IL-2Rβ was tagged at its N-terminus with a Flag epitope (Flag/G/IL-2Rβ); these epitope tags aid detection by flow cytometry and do not impact the function of the receptors. As expected, all T cell cultures showed proliferation in response to the positive control cytokine, IL-2 (300 IU/ml). After stimulation with G-CSF (100ng/ml), proliferation was observed only for PMBC-derived T cells and TAL expressing the G2R-1 chimeric cytokine receptor (Figure 22). Note that the lentiviral transduction efficiency was less than 100% such that less than 100% of T cells expressed the indicated chimeric cytokine receptors, which likely accounts for the lower rate of proliferation mediated by G2R-1 relative to IL-2. Similarly, increased proliferation was observed in 32D-IL-2Rβ cells (stably expressing the human IL-2Rβ subunit) expressing G-CSFR chimeric receptor subunits G2R-1 and G2R-2 and stimulated with G-CSF (Figure 21). In contrast to T cells, 32D-IL-2Rβ cells expressing the G/IL-2Rβ chimeric receptor subunit alone proliferated in response G-CSF (Figure 21); G-CSF-induced proliferation was not seen in 32D-IL-2Rβ cells expressing the G/γc chimeric receptor subunit alone (Figure 21).

[00445] These results show that G-CSF is capable of stimulating proliferation and viability of PMBC-derived T cells and TALs expressing the G2R-1 chimeric receptors and of 32D-IL-2Rβ cells expressing the G/IL-2Rβ, G2R-1 and G2R-2 chimeric receptors.

EXAMPLE 14: G-CSFR ECD IS EXPRESSED ON THE SURFACE OF CELLS TRANSDUCED WITH G/IL-2Rβ, G2R-1 AND G2R-2

[00446] Flow cytometry was performed on the 32D-IL-2Rβ cell line, PBMC-derived human T cells and human tumor-associated lymphocytes after transduction with a lentiviral vector

encoding the G2R-2 chimeric cytokine receptor (shown schematically in Figures 23 and 25) to determine if the cells express the G-CSFR ECD on the cell surface. G-CSFR positive cells were detected in all transduced cell types (Figure 26). In a separate experiment, 32D-IL-2Rβ cells expressing the G/IL-2Rβ, G2R-1 and G2R-2 chimeric receptors were positive for the G-CSFR ECD by flow cytometry (lower panels in Figure 21B-D).

[00447] These results indicate that the G/IL-2Rβ, G2R-1 and G2R-2 chimeric receptors are expressed on the cell surface.

EXAMPLE 15: EXPANSION OF CELLS EXPRESSING G2R-2 COMPARED TO NON-TRANSDUCED CELLS

[00448] Human PBMC-derived T cells and human tumor-associated lymphocytes were lentivirally transduced with the G2R-2 receptor construct (Figures 23 and 25), washed, and re-plated with the indicated cytokine. In some experiments, T cells were also re-activated periodically by stimulation with TransAct. Live cells were counted every 3-4 days. Proliferation of the PMBC-derived T cells (Figure 27A) and tumor-associated lymphocytes (two independent experiments in Figure 27B,C) was observed after stimulation with G-CSF (100 ng/ml) in cells expressing the G2R-2 chimeric receptor but not in non-transduced cells.

[00449] These results show that G-CSF-induced activation of the G2R-2 chimeric receptor is sufficient to induce proliferation and viability of immune cells.

EXAMPLE 16: EXPANSION AND IMMUNOPHENOTYPE OF CD4- OR CD8-SELECTED HUMAN TUMOR-ASSOCIATED LYMPHOCYTES EXPRESSING G2R-2 COMPARED TO NON-TRANSDUCED CELLS

[00450] CD4-selected and CD8-selected human T cells were transduced with lentiviral vector encoding G2R-2 (Figure 25), or left non-transduced where indicated. Cells were washed and re-plated with the indicated cytokine and counted every 3-4 days. Proliferation of CD4- or CD8-selected TALs expressing G2R-2 was observed after stimulation with G-CSF (100 ng/ml) or IL-2 (300 IU/ml), but not in the absence of added cytokine (medium alone) (Figures 28 and 29). In Figure 28, each line represents results from one of 5 patient samples.

[00451] Immunophenotyping by flow cytometry demonstrated that T cells cultured in G-CSF or IL-2 retained their CD4+ or CD8+ identity (Figure 30A), lacked an NK cell phenotype (CD3-CD56+) (Figure 30A), and exhibited a CD45RA-CCR7- T effector memory (T_{EM}) phenotype under these culture conditions (Figure 30B).

[00452] BrdU assays were performed to confirm increased cell cycle progression of T cells expressing G2R-2 upon stimulation with G-CSF (Figure 31). T cells were selected by culture in IL-2 or G-CSF, as indicated, prior to the assay. Both tumor-associated lymphocytes (Figure 31A) and PBMC-derived T cells (Figure 31B) were assessed.

[00453] These results show that G-CSF can selectively activate cell cycle progression and long-term expansion of primary human TALs by activation of the chimeric cytokine receptor G2R-2. These results also indicate that activation of the G2R-2 chimeric receptor by homodimer formation is sufficient to activate cytokine-like signaling and proliferation in TALs. Furthermore, TALs expressing G2R-2 remain cytokine dependent in that they undergo cell death upon withdrawal of G-CSF, similar to the response to IL-2 withdrawal. TALs cultured in G-CSF maintain a similar immunophenotype as TALs cultured in IL-2.

EXAMPLE 17: PRIMARY MURINE T CELLS EXPRESSING G2R-2 PROLIFERATE IN RESPONSE TO G-CSF.

[00454] BrdU incorporation assays were performed to assess proliferation of primary murine T cells expressing G2R-2 or the single-chain G/IL-2Rβ (a component of G2R-1) versus mock-transduced cells upon stimulation with G-CSF. All cells were expanded in IL-2 for 3 days prior to assay. Cell surface expression of G2R-2 or G/IL-2Rβ was confirmed by flow cytometry (Figure 32A). As indicated, cells were then plated in IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. Increased cell cycle progression after stimulation with G-CSF was observed in the cells expressing G2R-2 above that of non-transduced cells or cells expressing the single-chain G/IL-2Rβ (Figure 32B,C). Panels B and C show results for all live cells or G-CSFR+ cells, respectively.

[00455] These results indicate that, in response to G-CSF-induced homodimerization, the G2R-2 chimeric receptor is more efficient than the single chain G/IL-2Rβ receptor to activate cytokine-like signaling and proliferation in murine T cells.

EXAMPLE 18: ACTIVATION OF CYTOKINE-ASSOCIATED INTRACELLULAR SIGNALING EVENTS IN OF HUMAN PRIMARY T CELLS EXPRESSING G2R-2 IN RESPONSE TO G-CSF OR IL-2

[00456] To confirm that the chimeric cytokine receptors were indeed capable of activating cytokine signaling similar to that of IL-2, the ability of the cytokine receptors to activate various signaling molecules was assessed. Tumor-associated lymphocytes and PBMC-derived T cells expressing G2R-2 were previously expanded in G-CSF, while non-transduced cells were

previously expanded in IL-2. The cells were washed and then stimulated with IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml), or no cytokine, and western blots were performed on the cell lysates using antibodies directed against the indicated signaling molecules (Figure 33). Panels A and B show results for TAL, and panel C for PBMC-derived T cells. T cells expressing G2R-2 activated IL-2-related signaling molecules upon stimulation with G-CSF to a similar extent as seen after IL-2 stimulation of non-transduced cells or transduced cells, with the expected exception that G-CSF induced JAK2 phosphorylation whereas IL-2 induced JAK3 phosphorylation.

[00457] These results confirm that the G2R-2 chimeric receptor is capable of activating IL-2 receptor-like cytokine receptor signaling upon stimulation with G-CSF.

EXAMPLE 19: CYTOKINE SIGNALING IS ACTIVATED IN RESPONSE TO G-CSF IN MURINE PRIMARY T CELLS EXPRESSING G2R-2.

[00458] To assess whether the chimeric cytokine receptor G2R-2 or the single-chain G/IL-2Rβ (from G2R-1) were capable of activating cytokine signaling, the ability of these cytokine receptors to activate various signaling molecules was assessed by western blot of cell lysates of murine primary T cells expressing G2R-2 or G/IL-2Rβ versus mock-transduced cells. All cells were expanded in IL-2 for 3 days prior to assay. Cells were then washed and stimulated with IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. The cells expressing G2R-2 activated IL-2-related signaling molecules upon stimulation with G-CSF to a similar extent as seen after IL-2 stimulation of non-transduced cells or transduced cells, with the expected exception that G-CSF induced JAK2 phosphorylation whereas IL-2 induced JAK3 phosphorylation (Figure 34). In contrast, G/IL-2Rβ did not activate cytokine signaling upon exposure to G-CSF.

[00459] These results confirm in primary murine T cells that the G2R-2 chimeric receptor is capable of activating IL-2 receptor-like cytokine receptor signaling by homodimerization upon G-CSF stimulation, whereas the single-chain G/IL-2Rβ alone is not capable of activating cytokine signaling by homodimerization in response to G-CSF.

EXAMPLE 20: EXPRESSION OF CHIMERIC RECEPTORS LEADS TO PROLIFERATION OF 32D-IL-2Rβ CELLS AND PRIMARY MURINE T CELLS AFTER STIMULATION WITH AN ORTHOGONAL G-CSF.

[00460] To determine if cells expressing chimeric cytokine receptors could be selectively activated in response to an orthogonal version of G-CSF, 32D-IL-2Rβ cells or primary murine T cells were transduced with the chimeric receptors G2R-1 and G2R-2 that comprises the wild-type G-CSFR ECD (G2R-1 WT ECD, G2R-2 WT ECD) and chimeric receptors G2R-1 and G2R-2 that comprises the G-CSFR ECD which harbors the amino acid substitutions R41E, R141E and R167D (G2R-1 134 ECD, G2R-2 134 ECD). Cells were stimulated with either IL-2, wild type G-CSF or the orthogonal G-CSF (130 G-CSF) capable of binding to G2R-1 134 ECD and G2R-2 134 ECD, but with significantly reduced binding to wild-type G-CSFR. BrdU incorporation assays were performed to assess the ability of the cells to promote cell cycle progression upon cytokine stimulation (Figure 35). 32D-IL-2Rβ cells expressing G2R-2 134 ECD demonstrated cell cycle progression upon stimulation with 130 G-CSF (harboring amino acid substitutions E46R, L108K, and D112R; 30 ng/ml), but did not undergo cell cycle progression upon stimulation with wild-type G-CSF (30 ng/ml). The orthogonal nature of engineered cytokine:receptor ECD pairs was further demonstrated by stimulating primary murine T cells in a "criss-cross" proliferation assay, where cells expressing G2R-3 (Figure 23) with the WT, 130, 134, 304 or 307 ECD were stimulated with WT, 130, 304 or 307 cytokine (100 ng/ml) (Figure 36). The 130 ECD harbors the amino acid substitutions: R41E, and R167D. The 304 ECD harbors the amino acid substitutions: R41E, E93K and R167D; whereas the 304 cytokine harbors the amino acid substitutions: E46R, L108K, D112R and R147E. The 307 ECD harbors the amino acid substitutions: R41E, D197K, D200K and R288E; whereas the 307 cytokine harbors the amino acid substitutions: S12E, K16D, E19K and E46R. Panels A and B in Figure 36 represent experimental replicates.

[00461] These results demonstrate that cells expressing an orthogonal chimeric cytokine receptor are capable of selective activation and cell cycle progression upon stimulation with an orthogonal G-CSF CSF.

EXAMPLE 21: INTRACELLULAR SIGNALING IS ACTIVATED IN 32D-IL2Rβ CELLS AND PRIMARY HUMAN T CELLS EXPRESSING ORTHOGONAL CHIMERIC CYTOKINE RECEPTORS AND STIMULATED WITH AN ORTHOGONAL G-CSF.

[00462] To determine if cells expressing chimeric cytokine receptors could selectively activate intracellular cytokine signaling events in response to an orthogonal version of G-CSF,

32D-IL-2Rβ cells were transduced with the chimeric receptors G2R-1 and G2R-2 that comprises the wild-type G-CSFR ECD (G2R-1 WT ECD and G2R-2 WT ECD) and chimeric receptors G2R-1 and G2R-2 that comprises the G-CSFR ECD which harbors the amino acid substitutions R41E, R141E and R167D (G2R-1 134 ECD, G2R-2 134 ECD). Cells were stimulated with either IL-2 (300 IU/ml), wild type G-CSF (30 ng/ml) or the orthogonal G-CSF (130 G-CSF - E46R_L108K_D112R; 30 ng/ml) capable of binding to G2R-1 134 ECD, G2R-2 134 ECD, but with significantly reduced binding to wild-type G-CSFR. Western blots were performed on cell lysates to assess the ability of the cells to activate cytokine signaling upon exposure to cytokines (Figure 37). Cells expressing G2R-2 134 ECD showed evidence of cytokine signaling upon stimulation with 130 G-CSF but not wild-type G-CSF. Furthermore, cells expressing G2R-2 WT ECD were not able to activate cytokine signaling upon stimulation with 130 G-CSF.

[00463] The orthogonal nature of engineered cytokine:receptor pairs was further demonstrated by subjecting primary murine T cells to western blot analysis, where cells expressing G2R-3 with the WT, 134, or 304 ECD (R41E_E93K_R167D) stimulated with WT, 130, or 304 G-CSF (E46R_L108K_D112R_R147E; 100 ng/ml) and the indicated signaling events were measured (Figure 38A). IL-2 (300 IU/ml) and IL-12 (10 ng/ml) served as control cytokines. Cells expressing G2R-3 WT ECD showed evidence of cytokine signaling upon stimulation with IL-2, IL-12 or WT G-CSF. Cells expressing G2R-3 134 ECD showed evidence of cytokine signaling upon stimulation with IL-2, IL-12 or 130 G-CSF. Cells expressing G2R-3 304 ECD showed evidence of cytokine signaling upon stimulation with IL-2, IL-12 or 304 G-CSF.

[00464] Cell surface expression of the three ECD variants of G2R-3 was confirmed by flow cytometry (Figure 38B).

[00465] These results demonstrate that cells expressing an orthogonal chimeric cytokine receptor are capable of selective activation of intracellular cytokine signaling events upon stimulation with an orthogonal G-CSF.

EXAMPLE 22: EXPRESSION OF G2R-3 LEADS TO EXPANSION, CELL CYCLE PROGRESSION AND CYTOKINE-RELATED INTRACELLULAR SIGNALING AND IMMUNOPHENOTYPE IN PRIMARY HUMAN T CELLS

[00466] To determine whether the G2R-3 chimeric receptor can promote cytokine signaling-related events upon stimulation with G-CSF in primary human T cells, TALs were transduced

with a lentiviral vector encoding G2R-3. T-cell expansion assays were performed to test the proliferation of cells upon stimulation with IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. Live cells were counted every 3-4 days. In contrast to their non-transduced counterparts, primary TALs expressing G2R-3 expanded in culture in response to G-CSF (Figure 40A). To determine whether cytokine signaling events were activated upon stimulation with G-CSF, western blots were performed on cell lysates to assess intracellular signaling. Cells were harvested from the expansion assay, washed, and stimulated with IL-2 (300 IU/ml) or wildtype G-CSF (100 ng/ml). Primary TALs expressing G2R-3 demonstrated IL-2-related signaling events in response to G-CSF, with the expected exception that G-CSF induced JAK2 phosphorylation whereas IL-2 induced JAK3 phosphorylation (Figure 40B).

[00467] BrdU incorporation assays were performed to assess cell cycle progression upon stimulation with G-CSF. Cells were harvested from the expansion assay, washed, and re-plated in IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. Primary TALs expressing G2R-3 demonstrated cell cycle progression in response to G-CSF (Figure 40C).

[00468] G-CSF-induced expansion of cells expressing G2R-3 was also demonstrated using primary PBMC-derived human T cells (Figure 41). Cells expressing G2R-3 WT ECD expanded in response to WT G-CSF but not medium alone (Figure 41A). To demonstrate the continued dependence of cells on exogenous cytokine, on Day 21 of culture, cells from the G-CSF-expanded condition were washed and re-plated in WT G-CSF (100 ng/mL), IL-7 (20 ng/mL) + IL-15 (20 ng/mL), or medium only. Only cells re-plated in the presence of G-CSF or IL-7 + IL-15 remained viable over time.

[00469] Expression of G-CSFR ECD, as assessed by flow cytometry, remained stable on both CD4+ and CD8+ T cells between days 21-42 of expansion (Figure 41B).

[00470] By western blot, primary PBMC-derived T cells expressing G2R-3 demonstrated IL-2-related signaling events in response to G-CSF (Figure 42A). Flow cytometry-based immunophenotyping was performed on primary PBMC-derived T cells expanded for 42 days in WT G-CSF versus IL-7 + IL-15. Cells expressing G2R-3 WT ECD and cultured in G-CSF retained a similar phenotype to non-transduced cells cultured in IL-7 + IL-15, with primarily a CD62L+, CD45RO+ phenotype, which is indicative of a stem cell-like memory T cell phenotype (T_{SCM}) (Figure 42B, C). Likewise, the fractions of central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated (T_{TE}) T cells were similar.

[00471] These results confirm that the G2R-3 chimeric cytokine receptor is capable of activating cytokine signaling events and promoting cell cycle progression and expansion in primary cells. The immunophenotype of T cells expressing G2R-3 and expanded long-term in G-CSF is similar to non-transduced cells expanded in IL-7 + IL-15.

EXAMPLE 23: ORTHOGONAL G-CSF INDUCES EXPANSION AND PROLIFERATION IN PRIMARY HUMAN T CELLS EXPRESSING G2R-3 WITH ORTHOGONAL ECD

[00472] We assessed whether the chimeric cytokine receptor G2R-3 with 304 (R41E_E93K_R167D) or 307 (R41E_D197K_D200K_R288E) ECD was capable of inducing proliferation and expansion in response to stimulation with the orthogonal ligand 130, 304 or 307 G-CSF, primary PBMC-derived human T cells were transduced with lentiviral vectors encoding GR-3 304 ECD or G2R-3 307 (R41E_D197K_D200K_R288E) ECD. T-cell growth assays were performed to assess the fold expansion of cells when cultured with IL-2 (300 IU/ml), 304 G-CSF (100 ng/ml), 307 G-CSF (100 ng/ml) or no cytokine. Live cells were counted every 3-4 days. T cells expressing G2R-3 304 ECD expanded in culture in response to IL-2 or 304 G-CSF (Figure 43A). T cells expressing G2R-3 307 ECD expanded in culture in response to IL-2 or 307 G-CSF (Figure 43B). Non-transduced T cells only expanded in response to IL-2 (Figure 43C).

[00473] BrdU incorporation assays were performed to assess cell cycle progression upon stimulation with 130, 304 and 307 G-CSF in a criss-cross design. Cells were harvested from the expansion assay, washed, and re-plated in IL-2 (300 IU/ml), 130 G-CSF (100 ng/ml), 304 G-CSF (100 ng/ml), 307 G-CSF (100 ng/ml) or no cytokine. Primary human T cells expressing G2R-3 304 ECD demonstrated cell cycle progression in response to 130 or 304 G-CSF, but not in response to 307 G-CSF (Figure 44). T cells expressing G2R-3 307 ECD demonstrated cell cycle progression in response to 307 G-CSF, but not in response to 130 or 304 G-CSF. All T cells demonstrated cell cycle progression in response to IL-2.

[00474] The results show that the chimeric receptors G2R-3 304 ECD and G2R-3 307 ECD are capable of inducing selective cell cycle progression and expansion of primary human CD4+ and CD8+ T cells upon stimulation with orthogonal 304 or 307 G-CSF, respectively. Furthermore, 130 G-CSF can stimulate proliferation of cells expressing G2R-3 304 ECD, but not G2R-3 307 ECD.

EXAMPLE 24: G-CSFR ECD IS EXPRESSED ON THE SURFACE OF PRIMARY HUMAN TUMOR-ASSOCIATED LYMPHOCYTES (TAL) TRANSDUCED WITH G21R-1, G21R-2, G12R-1 AND G2R-3 CHIMERIC RECEPTOR CONSTRUCTS

[00475] To assess whether chimeric cytokine receptor constructs can be expressed on the surface of primary human tumor-associated lymphocytes (TAL), TAL were transduced with lentiviral vectors encoding the G21R-1, G21R-2, G12R-1 and G2R-3 chimeric receptors, and the cells were tested by flow cytometry for G-CSFR ECD expression on the cell surface (Figure 39). For all four chimeric cytokine receptor designs, G-CSFR ECD positive cells were detected.

[00476] These results demonstrate that the G21R-1, G21R-2, G12R-1 and G2R-3 chimeric receptors are capable of being expressed on the surface of primary cells. These results also indicate that G-CSFR ECD chimeric receptor designs are expressed on the surface of primary cells.

EXAMPLE 25: G-CSFR ECD IS EXPRESSED ON THE SURFACE OF PRIMARY MURINE T CELLS TRANSDUCED WITH G12R-1 AND G21R-1 CHIMERIC RECEPTOR CONSTRUCTS

[00477] To determine if the G12R-1 and G21R-1 chimeric receptors are capable of being expressed on the surface of primary T cells, primary murine T cells were transduced with retroviral vectors encoding the G12R-1 and G21R-1 chimeric receptors and analyzed by flow cytometry (Figure 45).

[00478] The results show that G-CSFR ECD is expressed on the surface of primary murine CD4+ and CD8+ T cells transduced with retroviral vectors encoding G12R-1 and G21R-1.

EXAMPLE 26: G-CSF INDUCES CYTOKINE SIGNALING EVENTS IN PRIMARY PBMC-DERIVED HUMAN T CELLS EXPRESSING G21R-1 OR G21R-2

[00479] To determine whether the G21R-1 and G21R-2 constructs are capable of inducing cytokine signaling events in primary cells, primary PBMC-derived human T cells were transduced with lentiviral vectors encoding G21R-1 or G21R-2 chimeric cytokine receptors. Cells were subjected to intracellular staining with phospho-STAT3 (p-STAT3) specific antibody and assessed by flow cytometry to determine the extent of STAT3 phosphorylation, a measure of STAT3 activation (Figure 46). Upon stimulation with G-CSF (100 ng/ml), the number of cells expressing phosphorylated STAT3 increased among the subset of G-CSFR positive cells transduced with either G21R-1 or G21R-2. In contrast, the G-CSFR negative (i.e.

non-expressing) cells did not exhibit an increase in phosphorylated STAT3 upon stimulation with G-CSF but did upon stimulation with IL-21.

[00480] These results demonstrate that the G21R-1 and G21R-2 chimeric receptors are capable of activating IL-21-related cytokine signaling events upon stimulation with G-CSF in primary human T cells.

EXAMPLE 27: G-CSF INDUCES INTRACELLULAR SIGNALING EVENTS IN PRIMARY MURINE T CELLS EXPRESSING G21R-1 OR G-12R-1

[00481] To determine if the chimeric cytokine receptor G21R-1 was capable of activating cytokine signaling events, primary murine T cells were transduced with a retroviral vector encoding G21R-1 and assessed by flow cytometry to detect phosphorylated STAT3 upon stimulation with G-CSF. Live cells were gated on CD8 or CD4, and the percentage of cells staining positive for phospho-STAT3 after stimulation with no cytokine, IL-21 (1ng/ml) or G-CSF (100 ng/ml) was determined for the CD8 and CD4 cell populations. Upon stimulation with G-CSF, cells expressing G21R-1 (but not non-transduced cells) showed increased amounts of phosphorylated STAT3 (Figures 47A and 47B). Western blots were performed to assess intracellular cytokine signaling in cells expressing G21R-1 or G12R-1 upon stimulation with G-CSF. As expected, cells expressing G21R-1 and stimulated with G-CSF showed increased phosphorylation of STAT3, with minor increases in phospho-STAT4 and phospho-STAT5 (Figure 47C). Also as expected, in cells expressing G12R-1, strong phosphorylation of STAT4 was seen in response to G-CSF. G-CSF did not induce any signaling events in mock transduced cells. (Note that in the G12R-1 group, the positive control (hIL-12 10 ng/ml) did not appear to induce any signaling events; this may be due to poor binding of human IL-12 to the murine IL-12R.)

[00482] The results show that G21R-1 and G12R-1 are capable of inducing cytokine signaling events in primary murine T cells upon stimulation with G-CSF.

EXAMPLE 28: G-CSF INDUCES PROLIFERATION AND INTRACELLULAR SIGNALING EVENTS IN PRIMARY MURINE T CELLS EXPRESSING G2R-2, G2R-3, G7R-1, G21/7R-1, G27/2R-1, G21/2R-1, G12/2R-1 OR G21/12/2R-1

[00483] To assess cytokine signaling events and cell proliferation mediated by chimeric cytokine receptors, primary murine T cells were transduced with retroviral vectors encoding G2R-2, G2R-3, G7R-1, G21/7R-1, G27/2R-1, G21/2R-1, G12/2R-1 or G21/12/2R-1. BrdU incorporation assays were performed to assess cell cycle progression upon stimulation with G-

CSF. Cells were harvested, washed, and re-plated in IL-2 (300 IU/ml), wildtype G-CSF (100 ng/ml) or no cytokine. G-CSF-induced-cell cycle progression was seen in primary murine T cells expressing G2R-2, G2R-3, G7R-1, G21/7R-1 or G27/2R-1 (Figures 48A, 48B) or G21/2R-1, G12/2R-1 or G21/12/2R-1 (Figures 49A, 49B). Expression of the G-CSFR ECD was also detectable by flow cytometry (Figures 48C, 49C).

[00484] By Western blot, multiple cytokine signaling events were observed in response to G-CSF (100 ng/ml) in cells expressing the indicated chimeric cytokine receptors, but not in mock transduced cells (Figures 48D, 49D). In general, the observed cytokine signaling events were as expected based on the signaling domains that were incorporated into the various ICD designs (Figures 23 and 24). As one example, the G7R-1 chimeric receptor induced phosphorylation of STAT5 (Figure 48D), which is expected due to the incorporation of the STAT5 binding site from IL-7Rα (Figure 23). As a second example, the G21/2R-1 chimeric receptor induced phosphorylation of STAT3 (Figure 49D), which is expected due to the incorporation of the STAT3 binding site from G-CSFR (Figure 24). As a third example, the G12/2R-1 chimeric receptor induced phosphorylation of STAT4, which is expected due to the incorporation of the STAT4 binding site from IL-12Rβ2 (Figure 24). Other chimeric cytokine receptors showed other distinct patterns of intracellular signaling events.

[00485] The results show that G2R-2, G2R-3, G7R-1, G21/7R-1, G27/2R-1, G21/2R-1, G12/2R-1 and G21/12/2R-1 are capable of inducing cytokine signaling events and proliferation in primary murine T cells upon stimulation with G-CSF. Furthermore, different patterns of intracellular signaling events can be generated by incorporating different signaling domains into the ICD of the chimeric receptor.

EXAMPLE 29: ORTHOGONAL G-CSF INDUCES EXPANSION, PROLIFERATION, CYTOKINE RELATED INTRACELLULAR SIGNALING AND IMMUNOPHENOTYPE IN PRIMARY HUMAN T CELLS EXPRESSING G12/2R-1 WITH ORTHOGONAL ECD

[00486] To determine if the chimeric cytokine receptor G12/2R-1 with 134 ECD was capable of inducing proliferation and expansion in response to stimulation with the orthogonal ligand 130 G-CSF, primary PBMC-derived human T cells were transduced with a lentiviral vector encoding G12/2R-1 134 ECD. T-cell growth assays were performed to assess the fold expansion of cells when cultured with IL-2 (300 IU/ml), 130 G-CSF (100 ng/ml) or no cytokine. Live cells were counted every 4-5 days. Primary human T cells expressing G12/2R-

1 134 ECD expanded in culture in response to IL-2 or 130 G-CSF (Figure 50A) but showed limited, transient expansion in medium alone.

[00487] On day 19 of this experiment, T cells that had been expanded in 130 G-CSF or IL-2 were washed three times are re-plated in IL-2, 130 G-CSF or medium alone. In medium alone, T cells showed reduced viability and a decline in number (Figure 50B). In contrast, T cells re-plated in IL-2 or G-CSF 130 showed continued viability and stable numbers.

[00488] Expression of the G12/2R-1 134 ECD, detected by flow cytometry with an antibody against the G-CSF receptor, increased between Day 4 and Day 16 on both CD4+ and CD8+ T cells expanded by stimulation with 130 G-CSF (Figure 50C). BrdU incorporation assays were performed to assess cell cycle progression upon stimulation with 130 G-CSF.

[00489] To assess cell cycle progression by BrdU assay, cells were harvested from the expansion assay, washed, and re-plated in IL-2 (300 IU/ml), IL-2 and IL-12 (10 ng/mL), 130 G-CSF (300 ng/ml) or no cytokine. Primary human T cells expressing G12/2R-1 134 ECD demonstrated cell cycle progression in response to 130 G-CSF, IL-2 or IL-2 + IL-12, whereas non-transduced cells responded only to IL-2 or IL-2 + IL-12 (Figure 51A).

[00490] After a 16-day culture period, immunophenotyping was performed by flow cytometry with antibodies to CD62L and CD45RO to compare T cells expressing G12/2R-1 134 ECD expanded in 130 G-CSF to non-transduced cells expanded in IL-2. The two T cell populations showed similar proportions of stem cell-like memory (T_{SCM}), central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated (T_{TE}) phenotypes (Figure 51B, C).

[00491] Similar experiments were performed with the chimeric cytokine receptor G12/2R-1 with 304 ECD (as opposed to 134 ECD). Primary PBMC-derived human T cells were transduced with a lentiviral vector encoding G12/2R-1 304 ECD. T-cell growth assays were performed to assess the fold expansion of cells when cultured with IL-2 (300 IU/ml), 130 G-CSF (100 ng/ml), 304 G-CSF (100 ng/mL) or medium alone. Live cells were counted every 4-5 days. T cells expressing G12/2R-1 with 304 ECD could expand in the presence of IL-2, 130 G-CSF or 304 G-CSF, but not in medium alone, while non-transduced cells could expand only in response to IL-2 (Figure 52A).

[00492] To assess cell cycle progression by BrdU assay, T cells expressing G12/2R-1 304 ECD, previously expanded in either 130 G-CSF or 304 G-CSF, were harvested from the

expansion assay, washed, and re-plated in IL-2 (300 IU/ml), 130 G-CSF (100 ng/ml), 304 G-CSF (100 ng/mL), 307 G-CSF (100 ng/mL), or medium alone. T cells expressing G12/2R-1 304 ECD demonstrated cell cycle progression in response to 130 or 304 G-CSF, but not in response to 307 G-CSF or medium alone (Figure 52B).

[00493] The results show that G12/2R-1 134 ECD is capable of inducing cell cycle progression and expansion of primary human CD4+ and CD8+ T cells upon stimulation with orthogonal 130 G-CSF. The T cell memory phenotype of cells expressing G12/2R-1 134 ECD and expanded with 130 G-CSF is similar to non-transduced cells expanded with IL-2. In addition, G12/2R-1 304 ECD is capable of inducing selective cell cycle progression and expansion of T cells upon stimulation with 130 or 304 G-CSF, but not in response to 307 G-CSF.

EXAMPLE 30: ORTHOGONAL G-CSF INDUCES DISTINCT INTRACELLULAR SIGNALING EVENTS IN PRIMARY HUMAN T CELLS EXPRESSING G2R-3 OR G12/2R-1 WITH ORTHOGONAL ECD

[00494] To assess intracellular signaling events, primary PBMC-derived human T cells were transduced with lentiviral vectors encoding G2R-3 304 ECD or G12/2R-1 304 ECD. Western blots were performed to assess intracellular cytokine signaling in cells expressing G2R-3 304 ECD or G12/2R-1 304 ECD, or non-transduced cells, upon stimulation with 304 G-CSF (100 ng/mL), IL-2 (300 IU/mL), IL-2 and IL-12 (10 ng/mL) or medium alone. In both transduced and non-transduced T cells, strong phosphorylation of STAT5 was detected in response to stimulation with either IL-2 + IL-12, or IL-2 alone (Figure 53). Strong phosphorylation of STAT4 was detected in response to stimulation with both IL-2 + IL-12, but only weak phosphorylation of STAT4 was detected in response to stimulation with IL-2 alone. In cells expressing G2R-3 304 ECD, weak phosphorylation of STAT4 and strong phosphorylation of STAT5 was detected in response to stimulation with 304 G-CSF, similar to the pattern seen in response to IL-2 alone. In cells expressing G12/2R-1 304 ECD, strong phosphorylation of STAT4 and STAT5 was detected in response to stimulation with 304 G-CSF, similar to the pattern seen in response to IL-2 + IL-12. Non-transduced T cells showed no response to 304 G-CSF.

[00495] The results show that G12/2R-1 with 304 ECD is capable of inducing cytokine signaling events, including strong phosphorylation of STAT4 and STAT5, in response to stimulation with 304 G-CSF. A different pattern of signaling events is seen in cells expressing

G2R-3 304 ECD after stimulation with 304 G-CSF, including strong phosphorylation of STAT5 but not STAT4.

Example 31: Design of novel G-CSF variants

[00496] The invention described herein is that of engineered variant G-CSF cytokines that bind selectively to a corresponding engineered extracellular domain (ECD) of G-GCSF receptor (G-CSFR). The threshold for selectivity is established using cell-based assays that measure the degree of binding and induced cell proliferation between variant G-CSF and WT G-CSFR, and between WT G-CSF and variant G-CSFR. As described herein, a panel of first and second generation engineered G-CSF/G-CSFR pairs were developed. In this example, we disclose the third generation of G-CSF designs (130a1, 130b1, 130a2, 130b2, 130a1b1) (Table 4A) which show improved thermal stability and selectivity.

[00497] Second generation G-CSF 130 variant contains three amino acid substitutions (E46R, L108K and D112R) relative to WT G-CSF. To identify additional residues that when mutated had the potential to reduce cytokine binding to WT G-CSFR, we first analyzed the biophysical and cell-based data from all our previously designed G-CSF variants, using the G-CSF/G-CSFR co-crystal structure for further guidance. Through iterative analysis, tandem glutamate residues (E122 and E123) were identified as lead candidates for substitution. Notably, these two glutamates, in addition to E46R, L108K and D112R, were substituted to arginines in the previously designed second generation G-CSF 134 variant, which proved to be unstable. Here, we individually substituted E122 and E123 using a “charge-swapping” strategy with arginine or lysine. We also substituted E122 and E123 simultaneously with lysine. Based on the structural analysis, the new positively charged residues at positions 122 and/or 123 on the cytokine are expected to repel the corresponding positively charged arginine at position 141 on the WT G-CSFR ECD leading to reduced binding and therefore enhanced selectivity. By modifying only one position (i.e., E122 or E123), we expected to reduce the stability problems observed with the previously designed 134 G-CSF in which both positions were simultaneously substituted. Furthermore, based on structural analysis, the incorporation of lysine (instead of arginine) at positions 122 and 123 was expected to yield a more stable variant than G-CSF 134. These new G-CSF variants (designated as 130a1, 130b1, 130a2, 130b2, 130a1b1) (Table 4A) were expected to efficiently pair with G-CSFR 134 ECD due to the complementary R141E substitution.

Example 32: Recombinant production of WT and engineered variant G-CSF cytokines in *E. coli*

[00498] To recombinantly produce WT and engineered G-CSF cytokine variants, an *E. coli*-based expression system was used to generate inclusion bodies from which correctly folded proteins of interest were purified by refolding.

Methods

[00499] Wild-type and engineered G-CSF variants were cloned into pET *E. coli* expression vectors under the control of the T7 viral promoter. Preparative scale *E. coli* production of recombinant cytokine was performed in 2 L cultures of BL21 (DE3) cells, and expression was induced via lactose-based autoinduction, controlled by natural operation of the *lac* operon in ZYP-5052 autoinduction media, after which cell cultures were grown for approximately 16 hrs at 30°C with shaking at 200 rpm. Cells were then pelleted by centrifugation at 8,200 RCF for 10 minutes and the supernatant was discarded. Cell pellets (~10 g) were resuspended in 40 ml lysis buffer (50mM TRIS pH8, 1 mM EDTA), followed by addition of Protease inhibitor cocktail III (Sigma Aldrich, cat. 539134). *E. coli* cells were lysed via French press and lysates were diluted to 250 ml with lysis buffer before being centrifuged at 30,000 RCF for 30 minutes. The supernatant was discarded, and the pellet, containing the inclusion bodies, was thoroughly resuspended in 70 ml wash buffer #1 (50mM TRIS pH8, 5 mM EDTA, 1% triton x-100) and centrifuged again. The cell pellet was then wash twice more; once with 70 ml wash buffer #2 (50mM TRIS pH8, 5 mM EDTA, 1% Sodium deoxycholate), and once with 70 ml wash buffer #3 (50mM TRIS pH8, 5 mM EDTA, 1M NaCl). Finally, inclusion bodies were resuspended in 100 ml solubilization buffer (50mM TRIS pH10, 8M urea, 10 mM 2-mercaptoethanol). This solution was stirred at room temperature for 1-2 h to ensure complete solubilization of inclusion bodies and then centrifuged at 30,000 RCF for 30 minutes to remove any remaining insoluble material. The solubilized inclusion bodies were then diluted to 500 ml with dialysis buffer #1 (50mM TRIS pH8, 1M urea, 100m mM NaCl, 5 mM reduced glutathione). Solubilized G-CSF was dialyzed against 3 L of dialysis buffer #1 for 40 h at 4°C, using dialysis tubing with a molecular weight cutoff (mwco) of 8,000 Da (BioDesign Inc., cat. D104), followed by dialysis against 3 L of dialysis buffer #2 (50mM TRIS pH8, 100m mM NaCl, 1 mM reduced glutathione) for 8 h at 4°C and finally, dialysis against 3 L of dialysis buffer #3 (25mM Sodium acetate pH4, 50 mM NaCl) for 16 h at 4°C. Refolded G-CSF was concentrated using an Amicon® Ultra-15 centrifugal filter unit with a 10 kDa mwco (Millipore Sigma, cat.

UFC901024) and further purified via cation exchange chromatography using an ENrich™ S 5x50 column (Bio-Rad, cat. 7800021) equilibrated in dialysis buffer #3 and eluted with a gradient of 50 mM to 500 mM NaCl. Fractions containing refolded G-CSF were pooled and concentrated prior to being loaded onto an ENrich™ SEC70 10x300 size-exclusion column (Bio-Rad, cat. 7801070) equilibrated in 25 mM Sodium Acetate pH 4.0, 150 mM NaCl (Figure 1). Protein containing fractions were analyzed by reducing SDS-PAGE, pooled and the concentration was measured by nanodrop A280 measurement using an extinction coefficient of 0.836.

Results

[00500] Wild-type and engineered G-CSF variants eluted at the expected volume relative to standard from the SEC 70. consistent with correctly folded protein that displayed sufficient stability to withstand chromatographic purification. Moreover, there was no observed aggregate protein peak, further confirming the effectiveness of the refolding protocol. A representative SEC UV trace and SDS-PAGE gel are shown for G-CSF_130a1 (Figure 54). The yield per Litre of culture ranged from 2.5 – 5 mgs of purified, refolded protein, depending on the G-CSF variant.

Example 33: Determination of the thermal stability of engineeered G-CSF variants

[00501] Differential scanning fluorimetry (DSF) was used to measure the thermal stability of the refolded G-CSF variants compared to WT G-CSF. DSF measures protein unfolding by monitoring changes in fluorescence of a dye with affinity for hydrophobic parts of the protein, which are exposed as the protein unfolds.

Methods

Differential scanning fluorimetry was performed using the Applied Biosystems StepOnePlus™ RT-PCR instrument (ThermoFisher Scientific, cat. 4376600) with the excitation and emission wavelengths set to 587 and 607 nm, respectively. Briefly, 20 µl of purified G-CSF (WT or variant) at a concentration of 1 mg/mL was dispensed into a 96-well plate in triplicate. To approximate the clinically validated formulation used for therapeutic WT G-CSF (10 mM Sodium acetate pH4, 5% sorbitol, 0.004% polysorbate-20) (PMID: 17822802), the assay buffer used for DSF was 10mM Sodium acetate pH4, 25 mM NaCl. SYPRO orange (Invitrogen) was diluted to 2x concentration, from a 5,000x stock. For thermal stability measurements, the temperature scan rate was fixed at 0.5 °C/min and the temperature range spanned 20 °C to 95 °C. Data analysis was performed using Protein

Thermal Shift Software v1.4 (ThermoFisher Scientific, cat. 4466038), which determined melting temperatures (Tms) of individual replicates by fitting fluorescence data to a two-state Boltzman model, after which triplicate Tms for each variant were then averaged.

Results

[00502] All variant G-CSF designs tested were found to have melting temperatures 10-17 °C higher than WT G-CSF when measured in a clinically relevant buffer (Table 6A), demonstrating the significantly improved thermostability conferred by the engineered mutations.

Table 6A: Melting temperature (Tm) for WT and engineered G-CSF variants determined by DSF.

GCSF	Tm (°C)
WT	50.55 ± 0.34
130	61.96 ± 0.12
130a1	67.32 ± 0.02
130b1	66.73 ± 0.24
130a2	65.84 ± 0.02
130b2	66.38 ± 0.07

*determined in 10 mM sodium acetate pH 4.0, 25 mM NaCl

Example 34: Proliferation of human and mouse cell lines expressing wild-type G-CSF receptor in wild-type G-CSF or engineered cytokines 130, 130a1, 130a2, 130b1, 130b2 and 130a1b1

[00503] Cytokines that were predicted to be less cross-reactive than 130 G-CSF for binding to the WT G-CSFR ECD, yet exhibited acceptable biophysical properties and thermal stability by *in vitro* assays in Examples 31 to 33, were tested for their ability to induce proliferation of the cell lines OCI-AML1 (which naturally expresses WT human G-CSFR) and 32D clone 3 (which naturally expresses WT murine G-CSFR.

Methods

[00504] The OCI-AML1 cell line (DSMZ) was maintained in alpha-MEM medium (Gibco) containing 20% fetal bovine serum (Sigma), 20 ng/ml human GM-CSF (Peprotech) and 1% penicillin and streptomycin (Gibco). The 32D clone 3 cell line (ATCC), hereafter referred to

as "32D", was maintained in RPMI 1640 (ATCC modification) medium (Gibco) containing 10% fetal bovine serum, 1 ng/ml murine IL-3 (Peprotech) and 1% penicillin and streptomycin.

[00505] To perform the BrdU incorporation assay, OCI-AML1 or 32D cells were harvested and washed twice in PBS and once in alpha-MEM or RPMI, respectively. Cells were re-plated in fresh complete medium containing the indicated assay cytokines, at the indicated concentrations, and incubated for 48 hours at 37°C, 5% CO_2. The BrdU assay procedure followed the instruction manual for the BD Pharmingen™ FITC BrdU Flow Kit (557891), with the following addition: viability staining was performed using the eFluor™ 450 Fixable Viability Dye (eBioscience™) at a concentration of 1:600. Flow cytometry was performed using a Cytek™ Aurora instrument.

Results

[00506] In a BrdU incorporation assay utilizing the OCI-AML1 cell line, the cells exhibited lower proliferation in response to the G-CSF variants 130, 130a1, 130a2, 130b1 and 130b2 compared to WT G-CSF (Figure 55A). Moreover, the cells exhibited lower proliferation in response to the G-CSF variants 130a1, 130a2, 130b1 and 130b2 compared to 130 (Figure 55A).

[00507] The cytokines WT G-CSF and G-CSF variants 130, 130a1 and 130b1 were further evaluated in a BrdU assay using a wider range of cytokine concentrations (Figure 55B). Compared to WT G-CSF, much higher concentrations of the three variant cytokines were required to induce proliferation of OCI-AML1 cells. Compared to 130 G-CSF, higher concentrations of 130a1 and 130b1 G-CSF were required to induce proliferation of OCI-AML1 cells (Figure 55B).

[00508] An independent BrdU assay was performed to compare WT G-CSF and G-CSF variants 130, 130a1 and 130a1b1 (Figure 55C). Compared to WT G-CSF, much higher concentrations of the three variant cytokines were required to induce proliferation of OCI-AML1 cells. Compared to 130 G-CSF, higher concentrations of 130a1 and 130a1b1 G-CSF were required to induce proliferation of OCI-AML1 cells (Figure 55C).

[00509] Similar results were seen in a BrdU assay involving 32D cells (Figure 55D,E). Compared to WT G-CSF, much higher concentrations of G-CSF variants 130, 130a1, 130b1 and 130a1b1 were required to induce proliferation of 32D cells (Figure 55D,E).

[00510] Thus, compared to human or murine WT G-CSF, G-CSF variants 130, 130a1, 130b1, 130a2, 130b2 and 130a1b1 showed significantly reduced capacity to induce proliferation in cells that express human or murine WT G-CSFR. Furthermore, compared to

130 G-CSF, the variants 130a1, 130b1, 130a2, 130b2 and 130a1b1 showed reduced capacity to induce proliferation of the OCI-AML1 cell line.

Example 35: Proliferation of primary human T cells expressing G12/2R-1 with 134 ECD in engineered cytokines 130, 130a1, 130a2, 130b1, 130b2 and 130a1b1

[00511] The G-CSF variants 130, 130a1, 130a2, 130b1, 130b2 and 130a1b1 were tested for their ability to induce proliferation of PBMC-derived human T cells expressing G12/2R-1 134 ECD.

Methods

[00512] The G12/2R-1 134 ECD cDNA was synthesized and cloned into a lentiviral transfer plasmid. The transfer plasmid and lentiviral packaging plasmids (psPAX2, pMD2.G, Addgene) were co-transfected into the lentiviral packaging cell line HEK293T/17 (ATCC) as follows. Cells were plated overnight in Opti-MEM™ I Reduced Serum Medium, GlutaMAX™ Supplement (Gibco™) containing 5% fetal bovine serum and 0.2mM sodium pyruvate. Plasmid DNA and Lipofectamine™ 3000 Transfection Reagent (Gibco™) were mixed according to the manufacturer's specifications and added dropwise onto cells. The cells were incubated for 6 hours at 37°C, 5% CO_2, followed by replacement of the medium and further incubation overnight. The following day, the cellular supernatant was collected from the plates, stored at 4°C, and medium replaced. The following day, cellular supernatant was again collected from the cells, and pooled with supernatant from the previous day. The supernatant was centrifuged briefly to remove debris and filtered through a 0.45μm filter. The supernatant was spun for 90 minutes at 25,000 rpm using a SW-32Ti rotor in a Beckman Optima L-XP Ultracentrifuge. The supernatant was removed, and the pellet was resuspended with 30 minutes of gentle shaking in Opti-MEM I medium.

[00513] Human T cells were isolated from a healthy donor leukapheresis product as follows. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. CD4+ and CD8+ T cells were isolated using human CD4 and CD8 MicroBeads (Miltenyi Biotec) following the manufacturer's specifications. Isolated T cells were plated at 5 x 10^5 per well in a 48-well plate in TexMACS™ medium (Miltenyi Biotec) containing 3% human serum (Sigma) and 0.5% gentamicin (DIN 0226853) (hereafter referred to as "complete TexMACS"). Human T cell TransAct™ (Miltenyi Biotec) was added (10 μl per well), and cells were incubated at 37°C, 5% CO_2. Twenty to 28 hours following activation, T cells were

transduced with lentivirus encoding the G12/2R-1 134 ECD construct. Twenty-four hours after transduction, fresh medium containing 130a1 G-CSF (100 ng/ml) was added to the T cells. Thereafter, cell cultures were replenished with fresh medium and/or cytokine every two days and kept at a density of approximately 5 x 10^5 to 1 x 10^6 cells per ml.

[00514] For the BrdU incorporation assay, T cells were harvested on day 10-14 of expansion and washed twice in PBS and once in complete TexMACS. Cells were re-plated in complete TexMACS containing medium alone or medium plus the following cytokines: IL-2 (300 IU/ml), IL-2 + IL-12 (10 ng/ml each), or individual G-CSF variants (130, 130a1, 130b1, 130a2, 130b2 or 130a1b1, each at 100 ng/ml). T cells were incubated for 48 hours at 37°C, 5% CO_2. The rest of the BrdU assay was performed as described in Example 34, except that cells were additionally stained with anti-human G-CSFR APC-conjugated and CD3 BV750-conjugated antibodies.

Results

[00515] Compared to IL-2 or IL-2 + IL-12, G-CSF variants 130, 130a1, 130b1, 130a2, 130b2 and 130a1b1 all induced a similar level of proliferation of human T cells expressing G12/2R-1 134 ECD (Figure 56A). Negligible proliferation was seen with medium alone. Similar results were obtained in a repeat BrdU assay with G-CSF variants 130 and 130a1b1 (Figure 56B).

[00516] Thus, G-CSF variants 130, 130a1, 130b1, 130a2, 130b2 and 130a1b1 can efficiently induce proliferation of T cells expressing the chimeric receptor G12/2R-1 134 ECD, resulting in a proliferative response similar to that elicited by IL-2 or IL-2 + IL-12.

Example 36: In a murine mammary carcinoma model, treatment with variant cytokine 130a1 selectively enhances the expansion and anti-tumor activity of tumor-specific T cells expressing G2R-3 134 ECD

[00517] Based on the ability of 130a1 G-CSF to stimulate proliferation of T cells expressing G12/2R-1 134 ECD, and its low cross-reactivity with WT murine and human G-CSFR, it was advanced to in vivo testing in a murine breast cancer model.

Methods

[00518] All procedures followed the Canadian Council for Animal Care guidelines and were approved by the University of Victoria Animal Care Committee. C57Bl/6 mice expressing the $Neu^{OT\text{-}I/OT\text{-}II}$ transgene in mammary epithelium under the control of the MMTV promoter were utilized as tumor hosts (Wall *et al.*, 2007). Tumor-specific CD8+ T cells were obtained from T Cell Receptor (TCR) transgenic OT-I mice (hereafter referred to as "OT-I"

mice; Jackson Laboratories); OT-I T cells recognize residues 257-264 of chicken ovalbumin presented by MHC class I. We also obtained congenic mice harboring the T lymphocyte-specific Thy1a (Thy1.1) allele (hereafter referred to as "Thy1.1+" mice; Jackson Laboratories). The OT-I and Thy1.1+ strains were intercrossed to homozygosity to produce "Thy1.1+ OT-I" mice; this allows the Thy1.1 marker to be used to identify OT-I T cells by flow cytometry. NOP23 is a syngeneic mammary tumor line that was derived from a spontaneous tumor in a transgenic mouse expressing $Neu^{OT-I/OT-II}$ and a dominant negative version of p53 in mammary epithelium (Wall *et al.*, 2007) (Yang *et al.*, 2009). NOP23 cells present residues 257-264 of chicken ovalbumin on MHC class I and hence can be recognized by OT-I T cells. NOP23 cells were maintained at an early passage and were cultured in DMEM-high glucose (Hyclone) containing 10% fetal bovine serum, 1x Insulin-Transferrin-Selenium supplement (Corning), and 1% penicillin/streptomycin (Gibco). The cell line underwent routine testing for mouse pathogens and Mycoplasma species.

[00519] Host MMTV $Neu^{OT-I/OT-II}$ mice were implanted subcutaneously with 1 x 10^6 NOP23 cells on the rear flank. Once tumors had reached a mean area of 20-50 mm^2 (approximately 15 days following implantation), mice received an intravenous infusion of 5 x 10^6 donor Thy1.1+ OT-I lymphocytes that had been retrovirally transduced to express G2R-3 134 ECD as described in the following paragraphs.

[00520] The retroviral packaging cell line Platinum-E (Cell Biolabs, RV-101) was cultured in DMEM containing 10% FBS, 1% penicillin/streptomycin, puromycin (1 μg/ml), and blasticidin (10 μg/ml). Chimeric receptor constructs were synthesized and cloned into a pMIG retroviral transfer plasmid (plasmid #9044, Addgene) that had been altered by restriction endonuclease cloning to remove the IRES-GFP (BglII to PacI sites) and to introduce annealed primers encoding a custom multiple cloning site. The resulting sequences were verified by Sanger sequencing. The transfer plasmid was transfected into Platinum-E cells using the Lipofectamine 3000 transfection method, as described above. The retroviral supernatant was collected from the plates at 24 and 48 hours following transfection and filtered through a 0.45 micron filter. Hexadimethrine bromide (1.6 μg/ml, Sigma-Aldrich) and human IL-2 (300 IU/ml) were added to the supernatant. The purified retroviral supernatant was used to transduce murine lymphocytes as described below.

[00521] Forty-eight hours prior to collection of retroviral supernatant, 24-well adherent plates were coated with unconjugated anti-murine CD3 (10 μg/ml, BD Biosciences, 553058)

and anti-murine CD28 (2 μg/ml, BD Biosciences, 553294) antibodies, diluted in PBS, and stored at 4°C. Twenty-four hours prior to collection of retroviral supernatant, Thy1.1+ OT-I mice were euthanized, and T cells were harvested from spleens as follows. Spleens were manually dissociated and filtered through a 100 micron filter. Red blood cells were lysed by incubation in ACK lysis buffer (Gibco, A1049201) for five minutes at room temperature, followed by one wash in serum-containing medium. CD8a-positive T cells were isolated using a bead-based isolation kit (Miltenyi Biotec, 130-104-075). Cells were added to plates coated with anti-CD3- and anti-CD28 antibodies in murine T cell expansion medium (RPMI-1640 containing 10% FBS, penicillin/streptomycin, 0.05 mM β-mercaptoethanol, and 300 IU/mL human IL-2), and incubated at 37°C, 5% CO_2 for 24 hours.

[00522] Retroviral transduction was performed on two successive days (Day 1 and 2) as follows. Approximately half of the medium was replaced with retroviral supernatant (generated as described above). Cells were spinfected with the retroviral supernatant at 1000g, for 90 minutes, at 30°C. Plates were returned to the incubator for 0-4 hours, and then approximately half of the medium was replaced with fresh T cell expansion medium. The procedure was repeated the next day. Twenty-four hours after the second transduction, T cells were split into 6-well plates, removed from antibody stimulation, and cultured under standard conditions.

[00523] On Day 5, the transduced T cells were harvested, and the transduction efficiency was assessed by flow cytometry with antibodies to human G-CSFR and CD8a (PerCP-eFluor 710 conjugate, eBioscience™, 46-0081-82), as well as Fixable Viability Dye eFluor™ 506 (1:1000 dilution), as described above. Cells were washed three times in PBS, and then infused by tail vein injection into host mice bearing established NOP23 tumors.

[00524] Following T cell infusion, mice were monitored for tumor growth and engraftment of Thy1.1+ OT-I cells in peripheral blood. Mice were randomized to receive intraperitoneal injections of vehicle versus 130a1 G-CSF (10 μg/dose) daily for 14 days, followed by every other day for a further 14 days for a total of 21 doses. Throughout the cytokine treatment period, operators were blinded to the contents of the cytokine syringes (i.e., vehicle versus 130a1 G-CSF). On Days 1, 4, 7, 10, 14 and 19 following ACT, a sample of peripheral blood was collected from each mouse. PBMCs were isolated after ACK lysis (described above), incubated in mouse Fc block and Zombie NIR viability dye (1:4,000), and stained with antibodies specific for murine CD45 (PerCP-conjugated antibody, 1:200), CD3 (AlexaFluor™ 700-conjugated

antibody, 1:100), CD8a (APC-conjugated antibody, 1:80), NK1.1 (PECy7-conjugated antibody, 1:25), CD19 (SuperBright™ 780-conjugated antibody, 1:100), CD11b (FITC-conjugated antibody, 1:25), CD11c (BV510-conjugated antibody, 1:50), Ly6C (BV605-conjugated antibody, 1:10), and Ly6G (PE-conjugated antibody, 1:80). Flow cytometry was performed on a Cytek Aurora instrument.

[00525] Mice were monitored for tumor size, body weight and general health and euthanized when they reached the experimental endpoint (>150 mm^2 tumor area) or, for mice experiencing complete tumor regression, on Day 80 following T cell infusion.

Results

[00526] All mice received an equivalent dose (5×10^6) of Thy1.1+ OT-I cells transduced to express G2R-3 134 ECD. The transduction efficiency was 36%, as assessed by the percentage of Thy1.1+ cells expressing human G-CSFR by flow cytometry. In the 130a1 G-CSF-treated group, 4/4 mice experienced complete regression (CR) of their tumor and remained tumor-free until day 80, when the study was stopped for practical reasons (Figures 57A and 57B). In contrast, in the vehicle-treated group, only 1/4 mice experienced CR, while the remaining 3/4 mice had tumors that progressed to the experimental endpoint. This translated to survival of 100% of mice in the 130a1 G-CSF-treated group, and 25% in the vehicle-treated group (Figure 57C).

[00527] The expansion and persistence of Thy1.1+ OT-I cells was monitored in serial blood samples collected from mice. In the 130a1 G-CSF-treated group, Thy1.1+ OT-I cells reached a mean peak engraftment of 49.8% of all CD8+ T cells on Day 7, whereas in the vehicle-treated group Thy1.1+ OT-I cells reached a mean peak engraftment of only 8.6% of all CD8+ T cells on Day 7 (Figure 57D).

[00528] Peripheral blood samples were also assessed for the percentage of myeloid cell subsets relative to all CD45+ cells. The 130a1 G-CSF- and vehicle-treated groups showed no significant differences in the percentage of neutrophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G+), eosinophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC^{high}) or monocytes (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC^{low}) relative to all CD45+ cells in peripheral blood over time (Figures 57E, 57F and 57G).

[00529] Throughout the study, all mice in both groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00530] Thus, 130a1 G-CSF was able to induce selective and profound expansion of tumor-specific CD8+ T cells expressing G2R-3 134 ECD. This resulted in a 100% durable CR rate compared to 25% in the vehicle-treated group. Treatment with 130a1 G-CSF did not affect neutrophil, eosinophil or monocyte counts in peripheral blood. There were no overt toxicities associated with 130a1 G-CSF treatment.

Example 37: In a murine mammary carcinoma model, treatment with variant G-CSF 130a1 selectively enhances the expansion and anti-tumor activity of tumor-specific T cells expressing G12/2R-1 134 ECD

[00531] The variant cytokine 130a1 G-CSF was further tested for the ability to enhance the expansion and anti-tumor activity of T cells engineered to express G12/2R-1 134 ECD in an *in vivo* murine breast cancer model.

Methods

[00532] We used the same methods as described in Example 36, with the exception that Thy1.1+ OT-I cells were retrovirally transduced to express G12/2R-1 134 ECD instead of G2R-3 134 ECD.

Results

[00533] All mice received an equivalent dose (5×10^6) of Thy1.1+ OT-I cells transduced to express G12/2R-1 134 ECD. The transduction efficiency was 43%, as assessed by the percentage of Thy1.1+ cells expressing human G-CSFR+ by flow cytometry. In the 130a1 G-CSF-treated group, 3/3 mice experienced complete regression (CR) of their tumor and remained tumor-free until day 80, when the study was stopped for practical reasons (Figures 58A and 58B). In contrast, in the vehicle-treated group, only 1/4 mice experienced CR, while the remaining 3/4 mice had tumors that progressed to the experimental endpoint. This translated to survival of 100% of mice in the 130a1 G-CSF-treated group, and 25% in the vehicle-treated group (Figure 58C).

[00534] The expansion and persistence of Thy1.1+ OT-I cells was monitored in serial blood samples collected from mice. In the 130a1 G-CSF-treated group, Thy1.1+ OT-I cells reached a mean peak engraftment of 34.3% of all CD8+ T cells on Day 7, whereas in the vehicle-treated group Thy1.1+ OT-I cells reached a mean peak engraftment of only 7.7% of all CD8+ T cells on Day 7 (Figure 58D).

[00535] Peripheral blood samples were also assessed for the percentage of several myeloid cell subsets relative to all CD45+ cells. The 130a1 G-CSF- and vehicle-treated groups showed no significant differences in the percentage of neutrophils (CD3-, CD19-, NK1.1-, CD11b+,

CD11c-, Ly6G+), eosinophils (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC^{high}) or monocytes (CD3-, CD19-, NK1.1-, CD11b+, CD11c-, Ly6G-, SSC^{low}) relative to all CD45+ cells in peripheral blood over time (Figures 58E, 58F and 58G).

[00536] Throughout the study, all mice in both groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00537] Thus, 130a1 G-CSF was able to induce selective and profound expansion of tumor-specific CD8+ T cells expressing G12/2R-1 134 ECD. This resulted in a 100% durable CR rate compared to 25% in the vehicle-treated group. Treatment with 130a1 G-CSF did not affect neutrophil, eosinophil or monocyte counts in peripheral blood. There were no overt toxicities associated with 130a1 G-CSF treatment.

Example 38: The therapeutic effects of 130a1 G-CSF require the expression of cognate engineered cytokine receptors by tumor-specific OT-I T cells

[00538] To demonstrate that 130a1 G-CSF mediates its immunological and anti-tumor effects through receptors containing the G-CSFR 134 ECD, the above *in vivo* experiment was performed using mock-transduced OT-I T cells (i.e., OT-I T cells that underwent the retroviral transduction procedure but without including a retroviral construct). The activity of 130a1 G-CSF was also compared to that of human IL-2.

Methods

[00539] We used the same methods as described in Example 36, with two exceptions: (a) the Thy1.1+ OT-I cells were mock-transduced using supernatant derived from mock-transfected Platinum-Eco cells; all other aspects of the retroviral transduction process were the same; and (b) we included an additional control group of mice that received human interleukin-2 (30,000 IU/dose; Proleukin) in place of vehicle or 130a1 G-CSF, following the same treatment schedule.

Results

[00540] All mice received an equivalent dose (5×10^6) of Thy1.1+ OT-I cells which had undergone the mock transduction process. Mice were then randomized to receive vehicle, 130a1 G-CSF or IL-2. In the 130a1 G-CSF-treated group, 3/5 mice experienced complete regression (CR) of their tumor and remained tumor-free until day 80, when the study was stopped for practical reasons (Figures 59A and 59B). In the IL-2-treated group, 4/5 mice experienced CR and remained tumor-free until day 80. In the vehicle-treated group, 3/4 mice experienced CR and remained tumor-free until day 80 (Figures 59A and 59B). This translated

to survival of 80% of mice in the 130a1 G-CSF- and IL-2-treated groups, and 75% in the vehicle-treated group (Figure 59C).

[00541] The complete response rates achieved by mock-transduced OT-I T cells were higher than those seen with vehicle-treated OT-I T cells expressing either G2R-3 134 ECD or G12/2R-1 134 ECD (Examples 36 and 37), despite the fact that none of these groups had the benefit of signaling through G2R-3 134 ECD or G12/2R-1 134 ECD. The lower efficacy of transduced vehicle-treated OT-I T cells likely reflects a modest impairment of T cell function caused by retroviral transduction.

[00542] The expansion and persistence of Thy1.1+ OT-I cells was monitored in serial blood samples collected from mice. Thy1.1+ OT-I cell numbers peaked on Day 4 in all three groups, reaching levels of 8.0%, 10.6% and 9.9% of all CD8+ T cells for the 130a1 G-CSF-, IL-2-, and vehicle-treated groups (Figure 59D).

[00543] There were no significant differences in the number of neutrophils or monocytes as a percentage of all peripheral blood CD45+ cells between the 130a1 G-CSF-, IL-2-, or vehicle-treated groups (Figures 59E and 59G).

[00544] On Day 7, eosinophils reached a mean of 6.7% of all CD45+ cells in the IL-2-treated group compared to 2.9% and 2.7% in the 130a1 G-CSF- and vehicle-treated groups, respectively (Figure 59F). Eosinophilial is a known effect of systemic IL-2 administration in mice and humans.

[00545] Throughout the study, all mice in both groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00546] Thus, in mice receiving mock-transduced Thy1.1+ OT-I cells, 130a1 G-CSF failed to mediate anti-tumor activity or induce T cell expansion. As described above, 130a1 G-CSF had no effect on neutrophil, eosinophil or monocyte counts in peripheral blood. There were no overt toxicities associated with 130a1 G-CSF treatment. IL-2 (at the dose and schedule used) had very modest effects on T cell expansion and anti-tumor activity despite inducing transient eosinophilia.

Example 39: Construction and functional evaluation of a chimeric receptor with a variant extracellular domain from the G-CSFR receptor and intracellular signaling domains from IL-4 receptor alpha

[00547] We synthesized a chimeric receptor called G4R 134 ECD (Figure 60) which contained the extracellular domain (134 variant), transmembrane domain, and Box 1 and 2 regions of human G-CSFR fused to a portion of the intracellular domain of human IL-

4 receptor alpha. The chimeric receptor subunit was encoded in a lentiviral vector which was used to transduce human CD4+ and CD8+ T cells. Transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of G4R 134 ECD. The ability of G4R 134 ECD to induce T cell expansion and proliferation was evaluated in vitro. Biochemical signaling events mediated by G4R 134 ECD were evaluated by western blot using phospho-specific antibodies against STAT6 and other signaling intermediaries associated with IL-4 signaling.

[00548] Methods

[00549] The chimeric receptor construct G4R 134 ECD was synthesized and cloned into a lentiviral transfer plasmid (Twist Bioscience). The transfer plasmid and lentiviral packaging plasmids (psPAX2, pMD2.G, Addgene) were co-transfected into the lentiviral packaging cell line HEK293T/17 (ATCC) as follows. Cells were plated overnight in Opti-MEM™ I Reduced Serum Medium, GlutaMAX™ Supplement (Gibco™) containing 5% fetal bovine serum and 0.2mM sodium pyruvate. Plasmid DNA and Lipofectamine™ 3000 Transfection Reagent (Gibco™) were mixed, according to the manufacturer's specifications, and added dropwise onto cells. The cells were incubated for 6 hours at 37°C, 5% CO_2, followed by replacement of the medium and further incubation overnight. The following day, the cellular supernatant was collected from the plates and stored at 4°C, and the medium was replaced. The next day, cellular supernatant was again collected from the cells and pooled with supernatant from the previous day. The supernatant was centrifuged briefly to remove debris and filtered through a 0.45µm filter. The supernatant was spun for 90 minutes at 25,000 rpm using a SW-32Ti rotor in a Beckman Optima L-XP Ultracentrifuge. The supernatant was removed, and the pellet was resuspended with 30 minutes of gentle shaking in Opti-MEM I medium.

[00550] Primary human T cells were isolated from healthy donor leukapheresis product as follows. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. CD4+ and CD8+ T cells were isolated using human CD4 and CD8 MicroBeads (Miltenyi Biotec) following the manufacturer's specifications. For some experiments, T cells were viably frozen and thawed later for analysis. Isolated T cells were plated at 5 x 10^5 per well in a 48-well plate in TexMACS™ medium (Miltenyi Biotec) containing 3% human serum (Sigma) and 0.5% gentamicin (DIN 0226853) (hereafter

referred to as "complete TexMACS"). Human T cell TransAct™ (Miltenyi Biotec) was added (10 ml per well), and cells were incubated at 37°C, 5% CO_2. Twenty to 28 hours following activation, the T cells were transduced with lentivirus encoding the receptor construct. The following day, fresh medium was added to T cells, along with either IL-2 (300 IU/ml), 130a1 G-CSF (100 ng/ml), IL-2 plus 130a1 G-CSF (300 IU/ml or 100 ng/ml, respectively) or no added cytokine. (For the non-transduced T cells, the 307 G-CSF variant was added with 130a1 G-CSF to serve as a control for another arm of the experiment [not shown]; use of this experimental convenience was justified by the fact that neither 130a1 G-CSF nor 307 G-CSF induces proliferation or other signaling events in non-transduced T cells.) T cells were maintained by addition of fresh medium and/or the indicated cytokines every two days, while maintaining a density of approximately 5×10^5 to 1×10^6 cells per ml.

[00551] Eight days after transduction, expression of G4R 134 ECD was assessed by flow cytometry of T cells harvested from the IL-2 culture condition. T cells were incubated for 15 minutes at 4°C with anti-human G-CSFR APC-conjugated antibody (1:50 dilution), anti-human CD3 BV750™-conjugated antibody (1:50 dilution), anti-human CD4 PE-conjugated antibody (1:25 dilution), anti-human CD8 PerCP-conjugated antibody (1:100) and eBioscience™ Fixable Viability Dye eFluor™ 506 (1:1000 dilution). Cells were then washed twice and analyzed on a Cytek™ Aurora flow cytometer.

[00552] To assess T cell expansion, cells from each of the culturing conditions were counted on days 4, 8, 12 and 16 using a Cytek™ Aurora flow cytometer.

[00553] To assess cell cycle progression (BrdU incorporation), cells were harvested on day 12 (from the 130a1 G-CSF + IL-2 culture condition), washed twice in PBS and once in complete TexMACS medium, and re-plated in fresh complete TexMACS medium containing the indicated cytokines: IL-2 (300 IU/ml), IL-4 (50 ng/ml), IL-2 + IL-4 (300 IU/ml and 50 ng/ml, respectively), 130a1 G-CSF (100 ng/ml), 130a1 G-CSF + IL-2, or medium alone (no added cytokine). Cells were cultured for 48 hours at 37°C, 5% CO_2. The BrdU assay procedure followed the instruction manual for the BD Pharmingen™ FITC BrdU Flow Kit (557891), with one exception: cells were also stained to detect cell surface expression of the G-CSFR ECD (as described in Example 35). Flow cytometry was performed using a Cytek™ Aurora instrument.

[00554] For western blot experiments, cells were harvested from the 130a1 G-CSF + IL-2 culture condition, washed three times, and rested overnight in complete TexMACS. The following day, cells were stimulated for 20 minutes at 37°C with medium alone or medium plus IL-2 (300 IU/ml), IL-4 (50ng/ml), or 130a1 G-CSF (100 ng/ml). Cells were washed once in a buffer containing 10 mM HEPES, pH 7.9, 1 mM $MgCl_2$, 0.05 mM EGTA, 0.5 mM EDTA, pH 8.0, 1 mM DTT, and 1x Pierce Protease and Phosphatase Inhibitor Mini Tablets (A32961). Cells were lysed for 10 minutes on ice in wash buffer containing 0.2% NP-40 substitute (Sigma). Lysates were centrifuged for 10 minutes at 13,000 rpm at 4°C, after which supernatant (cytoplasmic fraction) was collected. To extract the nuclear fraction, pellets from the lysis step were resuspended in the above wash buffer with the addition of 0.42M NaCl and 20% glycerol. The nuclear fraction was incubated for 30 minutes on ice, with frequent vortexing, and centrifuged for 20 minutes at 13,000 rpm at 4°C, after which the nuclear fraction (supernatant) was collected. The cytoplasmic and nuclear fractions were heated (70°C) under reducing conditions for 10 minutes and run on NuPAGE™ 4-12% Bis-Tris Protein Gels. The gel contents were transferred to nitrocellulose membranes (60 min at 20V in a Trans-Blot® SD Semi-Dry Transfer Cell), dried, and blocked for 1hr in Intercept® Blocking Buffer in TBS (927-600001, LI-COR). The blots were incubated with primary antibodies (1:1,000) overnight at 4°C in Intercept® Blocking Buffer in TBS containing 0.1% Tween20. Primary antibodies were obtained from Cell Signaling Technologies: Phospho-Shc (Tyr239/240) antibody #2434, Phospho-Akt (Ser473) (D9E) XP® rabbit monoclonal antibody #4060, Phospho-S6 Ribosomal Protein (Ser235/236) antibody #2211, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody #9101, β-Actin (13E5) rabbit monoclonal antibody #4970, Phospho-JAK1 (Tyr1034/1035) (D7N4Z) rabbit monoclonal antibody #74129, Phospho-JAK3 (Tyr980/981) (D44E3) rabbit monoclonal antibody #5031, Phospho-Stat6 (Tyr641) (C11C5) rabbit monoclonal antibody #9361, and Histone H3 (96C10) mouse monoclonal antibody #3638. Blots were washed three times in TBS containing 0.1% Tween20 and incubated with secondary antibodies (1:10,000) in TBS buffer containing 0.1% Tween20 for 30-60 minutes at room temperature. Secondary antibodies were obtained from Cell Signaling Technologies: anti-mouse IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5257 and anti-rabbit IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5151. Blots were washed and exposed on a LI-COR Odyssey imager.

[00555] Results

[00556] By flow cytometry (Figure 61), a large proportion (93.7%) of CD3+ T cells transduced with the lentiviral vector encoding G4R 134 ECD expressed the G-CSFR ECD, whereas non-transduced T cells did not.

[00557] Thus, G4R 134 ECD can be expressed on the surface of engineered T cells.

[00558] As shown in Figure 62, T cells transduced with the lentiviral vector encoding G4R 134 ECD expanded well in medium containing IL-2 or IL-2 + 130a1 G-CSF and showed an intermediate level of expansion in response to 130a1 G-CSF alone (Figure 62A). Non-transduced T cells expanded well in response to IL-2 but showed no response to the combination of 130a1 G-CSF plus the G-CSF variant 307 (the latter was included as a control for a separate arm of this experiment; not shown) (Figure 62B). Neither culture expanded well in medium alone.

[00559] Thus, expression of G4R 134 ECD on T cells leads to increased expansion in response to 130a1 G-CSF, demonstrating that the hybrid intracellular domain of G4R 134 ECD transduces a functional growth signal in T cells.

[00560] As shown in Figure 63, CD4+ and CD8+ T cells expressing G4R 134 ECD demonstrated proliferation (BrdU incorporation) in response to IL-2, IL-4, IL-2 + IL-4, 130a1 G-CSF, or 130a1 G-CSF + IL-2 (Figure 63A). In contrast, non-transduced CD4+ and CD8+ T cells proliferated in response to IL-2, IL-4, IL-2 + IL-4, and 130a1 G-CSF + IL-2 but not in response to 130a1 G-CSF alone (Figure 63B). None of the cultures proliferated in response to medium alone, with the exception of CD8+ T cells expressing G4R 134 ECD, which had elevated background proliferation in this particular experiment.

[00561] Thus, expression of G4R 134 ECD on CD4+ and CD8+ T cells leads to increased proliferation in response to 130a1 G-CSF, achieving levels equivalent to or even exceeding that induced by IL-2 or IL-4. This demonstrates that the hybrid intracellular domain of G4R 134 ECD transduces a functional proliferative signal in T cells.

[00562] As shown in Figure 64, in both non-transduced T cells and T cells expressing G4R 134 ECD, IL-2 induced the phosphorylation of JAK1, JAK3, Shc, Erk1/2, Akt, and S6. IL-4 induced the phosphorylation of STAT6, JAK1 and JAK3, with only modest phosphorylation of Erk1/2, Akt, and S6 and no phosphorylation of Shc. In T cells expressing G4R 134 ECD,

130a1 G-CSF induced the phosphorylation of STAT6 and JAK1, with intermediate phosphorylation of Erk1/2, Akt, and S6 (relative to IL-2), and no phosphorylation of JAK3 or Shc. The lack of phosphorylation of JAK3 is expected given that G4R 134 ECD uses the Box 1 and 2 regions of human G-CSFR, which binds JAK2 instead of JAK3. As expected, 130a did not induce any signaling events in non-transduced T cells.

[00563] Thus, the hybrid intracellular domain of G4R 134 ECD activates many of the same biochemical signaling events as the native IL-4 receptor, in particular tyrosine phosphorylation of STAT6.

Example 40: Construction and functional evaluation of a chimeric receptor with a variant extracellular domain from the G-CSFR receptor and intracellular signaling domains from IL-6 receptor beta (gp130)

[00564] We synthesized a chimeric receptor called G6R 134 ECD (Figure 60) which contained the extracellular domain (134 variant), transmembrane domain, and Box 1 and 2 regions of human G-CSFR fused to a portion of the intracellular domain of human IL-6 receptor beta (gp130). The chimeric receptor subunit was encoded in a lentiviral vector which was used to transduce human CD4+ and CD8+ T cells. Transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of G6R 134 ECD. The ability of G6R 134 ECD to induce T cell expansion and proliferation was evaluated in vitro. Biochemical signaling events mediated by G6R 134 ECD were evaluated by western blot using phospho-specific antibodies against STAT3 and STAT5.

[00565] Methods

[00566] In general, we followed similar methods as described in Example 39; however, the experiments were adapted to focus on IL-6 signaling mechanisms. For example, IL-6 was used as a reference cytokine instead of IL-4.

[00567] Human T cells were engineered to express G6R 134 ECD and transduction efficiency was determined by flow cytometry on Day 8, as per Example 39.

[00568] T cells were expanded in IL-2 (300 IU/ml), 130a1 G-CSF (100 ng/ml), IL-2 + 130a1 G-CSF (300 IU/ml and 100 ng/ml, respectively) or medium alone (no added cytokine) for 16 days, with medium and the indicated cytokines refreshed every two days. On days 4, 8, 12 and 16 of the expansion, T cells were counted using a Cytek™ Aurora flow cytometer.

[00569] For the BrdU incorporation assay, on day 12 of expansion T cells were harvested from the IL-2 culture condition and washed twice in PBS and once in complete TexMACS medium. Cells were re-plated in fresh complete TexMACS medium containing the relevant assay cytokine: IL-2 (300 IU/ml), IL-6 (100 ng/ml), IL-2 + IL-6 (300 IU/ml and 100 ng/ml, respectively), 130a1 G-CSF (100 ng/ml), 130a1 G-CSF + IL-2 (300 IU/ml and 100 ng/ml, respectively), or medium alone (no added cytokine). Cells were cultured for 48 hours at 37°C, 5% CO_2. The BrdU incorporation assay was performed as described in Example 39.

[00570] For the western blot experiment, cells were expanded in IL-2 (300 IU/ml) for 18 days, then washed as above and rested overnight in complete TexMACS medium. The following day, cells were stimulated for 20 minutes at 37°C with medium alone or medium with IL-2 (300 IU/ml), IL-6 (100 ng/ml), or 130a1 G-CSF (100 ng/ml). Cytoplasmic and nuclear fractions were prepared and western blots performed as described in Example 39. Primary antibodies were obtained from Cell Signaling Technologies: Phospho-Stat3 (Tyr705) (D3A7) rabbit mAb #9145, Phospho-Stat5 (Tyr694) (C11C5) rabbit mAb #9359, and Histone H3 (96C10) mouse mAb #3638.

[00571] **Results**

[00572] By flow cytometry (Figure 65), a large proportion (93.7%) of CD3+ T cells transduced with the lentiviral vector encoding G6R 134 ECD expressed the G-CSFR ECD, whereas non-transduced T cells did not.

[00573] Thus, G6R 134 ECD can be expressed on the surface of engineered T cells.

[00574] As shown in Figure 66, T cells transduced with the lentiviral vector encoding G6R 134 ECD expanded well in medium containing IL-2, showed an intermediate level of expansion in response to IL-2 + 130a1 G-CSF, and poor expansion in response to 130a1 G-CSF alone (Figure 66A). Non-transduced T cells expanded well in response to IL-2 but not 130a1 G-CSF (Figure 66B). Neither culture expanded well in medium alone.

[00575] Thus, G6R 134 ECD does not appear to induce a growth signal in T cells, as assessed by this expansion assay.

[00576] As shown in Figure 67, CD4+ and CD8+ T cells expressing G6R 134 ECD demonstrated proliferation (BrdU incorporation) in response to IL-2, IL-2 + IL-6, or 130a1

G-CSF + IL-2; 130a1 G-CSF induced modest proliferation of CD4+ T cells, and weak proliferation of CD8+ T cells (Figure 67A). In contrast, non-transduced CD4+ and CD8+ T cells proliferated in response to IL-2, IL-2 + IL-6, and 130a1 G-CSF + IL-2 but not in response to IL-6 or 130a1 G-CSF alone (Figure 67B). None of the cultures proliferated in response to medium alone, with the exception of CD8+ T cells expressing G6R 134 ECD, which had elevated background proliferation in this particular experiment.

[00577] Thus, G6R 134 ECD appears to induce a weak or moderate proliferative signal in CD8+ and CD4+ T cells, respectively.

[00578] As shown in Figure 68, in both non-transduced T cells and T cells expressing G6R 134 ECD, IL-2 induced the tyrosine phosphorylation of STAT3 (arrow) and STAT5, whereas IL-6 induced the tyrosine phosphorylation of STAT3 but not STAT5. In T cells expressing G6R 134 ECD, 130a1 G-CSF induced the tyrosine phosphorylation of STAT3 but not STAT5. 130a did not induce tyrosine phosphorylation of STAT3 or STAT5 in non-transduced T cells. Note that on the P-STAT3 image a dark, higher molecular band appears in the IL-2-stimulated conditions (especially in the non-transduced T cells). This is remnant signal from a previous probing of this membrane with phospho-STAT5 antibody. The lower molecular weight band (arrow) represents phospho-STAT3.

[00579] Thus, the hybrid intracellular domain of G6R 134 ECD induces a pattern of STAT phosphorylation similar to that induced by IL-6 in T cells.

Example 41: Construction and functional evaluation of a chimeric receptor with a variant extracellular domain from the G-CSFR receptor and the intracellular domain of the EPO receptor

[00580] We synthesized a chimeric receptor called GEPOR 134 ECD (Figure 60) which contained the extracellular domain (134 variant) and transmembrane domain of human G-CSFR fused to the entire intracellular domain of human EPO receptor. The chimeric receptor subunit was encoded in a lentiviral vector which was used to transduce human CD4+ and CD8+ T cells. Transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of GEPOR 134 ECD. The ability of GEPOR 134 ECD to induce T cell expansion was evaluated in vitro. Biochemical signaling events mediated by GEPOR 134 ECD were evaluated by western blot using phospho-specific antibodies against STAT5 and other signaling intermediaries.

[00581] Methods

[00582] In general, we followed similar methods as described in Example 39; however, the experiments were adapted to focus on EPO-R and IL-2 signaling mechanisms.

[00583] Human T cells were engineered to express GEPOR 134 ECD and transduction efficiency was determined by flow cytometry on Day 8, as per Example 39.

[00584] T cells were expanded in IL-2 (300 IU/ml), 130a1 G-CSF (100 ng/ml), IL-2 + 130a1 G-CSF (300 IU/ml and 100 ng/ml, respectively) or medium alone (no added cytokine) for 16 days, with medium and the indicated cytokines refreshed every two days. For the non-transduced T cells, 307 G-CSF was added with 130a1 G-CSF to serve as a control for another arm of the experiment (not shown); use of this experimental convenience was justified by the fact that neither 130a1 G-CSF nor 307 G-CSF induces proliferation or other signaling events in non-transduced T cells. On days 4, 8, 12 and 16 of the expansion, T cells were counted using a Cytek™ Aurora flow cytometer.

[00585] For the western blot experiment, cells were expanded in IL-2 (300 IU/ml) for 18 days, then washed as above and rested overnight in complete TexMACS medium. The following day, cells were stimulated for 20 minutes at 37°C with medium alone or medium with IL-2 (300 IU/ml) or 130a1 G-CSF (100 ng/ml). Cytoplasmic and nuclear fractions were prepared and western blots performed as described in Example 39. Primary antibodies were obtained from Cell Signaling Technologies: Phospho-Stat5 (Tyr694) (C11C5) rabbit mAb #9359, Histone H3 (96C10) mouse mAb #3638, Phospho-JAK2 (Tyr1007/1008) antibody #3771, Phospho-Akt (Ser473) (D9E) XP® rabbit mAb #4060, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody #9101, and β-Actin (13E5) rabbit mAb #4970.

[00586] Results

[00587] By flow cytometry (Figure 69), a large proportion (96.5%) of CD3+ T cells transduced with the lentiviral vector encoding GEPOR 134 ECD expressed the G-CSFR ECD, whereas non-transduced T cells did not.

[00588] Thus, GEPOR 134 ECD can be expressed on the surface of engineered T cells.

[00589] As shown in Figure 70, T cells transduced with the lentiviral vector encoding GEPOR 134 ECD expanded well in medium containing IL-2 or IL-2 + 130a1 G-CSF and

showed an intermediate level of expansion in response to 130a1 G-CSF alone (Figure 64A). Non-transduced T cells expanded well in response to IL-2 but not 130a1 G-CSF + 307 G-CSF (Figure 70B). Neither culture expanded well in medium alone.

[00590] Thus, GEPOR 134 ECD appears to induce an intermediate growth signal in T cells, as assessed by this expansion assay.

[00591] As shown in Figure 71, in both non-transduced T cells and T cells expressing GEPOR 134 ECD, IL-2 induced the phosphorylation of STAT5, Akt and ERK1/2; the apparent phosphorylation of JAK2 in response to IL-2 is due to cross-reactivity of the anti-phospho-JAK2 antibody with phospho-JAK3 (note the difference in protein size compared to actual JAK2, indicated by the arrow). In T cells expressing GEPOR 134 ECD, 130a1 G-CSF induced the phosphorylation of STAT5, JAK2 (arrow), and ERK1/2 and weak phosphorylation of Akt. As expected, 130a1 G-CSF did not induce any biochemical signaling events in non-transduced T cells.

[00592] Thus, GEPOR 134 ECD induces biochemical signaling events that are similar to that induced by IL-2, but with weaker phosphorylation of Akt and with the expected activation of JAK2 in place of JAK3.

Example 42: Construction and functional evaluation of a chimeric receptor with a variant extracellular domain from the G-CSFR receptor and the intracellular domain of interferon alpha receptor 2

[00593] We synthesized a chimeric receptor called GIFNAR 134 ECD (Figure 60) which contains the extracellular domain (134 variant), transmembrane domain, and Box 1 and 2 regions of human G-CSFR fused to a portion of the intracellular domain of human interferon receptor alpha 2. The chimeric receptor subunit was encoded in a lentiviral vector which was used to transduce human CD4+ and CD8+ T cells. Transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of GIFNAR 134 ECD. The ability of GIFNAR 134 ECD to induce T cell expansion was evaluated in vitro. Biochemical signaling events mediated by GIFNAR 134 ECD were evaluated by western blot using phospho-specific antibodies against STAT1, STAT2 and other signaling intermediaries.

[00594] Methods

[00595] In general, we followed similar methods as described in Example 39; however, the experiments were adapted to focus on IFN alpha signaling mechanisms.

[00596] Human T cells were engineered to express GIFNAR 134 ECD, and transduction efficiency was determined by flow cytometry on Day 8, as per Example 39.

[00597] T cells were expanded in IL-2 (300 IU/ml), 130a1 G-CSF (100 ng/ml), IL-2 + 130a1 G-CSF (300 IU/ml and 100 ng/ml, respectively) or medium alone (no added cytokine) for 16 days, with medium and the indicated cytokines refreshed every two days. On days 4, 8, 12 and 16 of the expansion, T cells were counted using a Cytek™ Aurora flow cytometer.

[00598] For the western blot experiment, cells were expanded in IL-2 (300 IU/ml) for 18 days, then washed as above and rested overnight in complete TexMACS medium. The following day, cells were stimulated for 20 minutes at 37°C with medium alone or medium with IL-2 (300 IU/ml), IFNα (300 IU/ml) or 130a1 G-CSF (100 ng/ml). Cytoplasmic and nuclear fractions were prepared and western blots performed as described in Example 39. Primary antibodies were obtained from Cell Signaling Technologies: Phospho-Stat1 (Tyr701) (58D6) rabbit mAb #9167, Phospho-Stat2 (Tyr690) (D3P2P) rabbit mAb #88410, Histone H3 (96C10) mouse mAb #3638, Phospho-JAK1 (Tyr1034/1035) (D7N4Z) rabbit mAb #74129, Phospho-Akt (Ser473) (D9E) XP® rabbit mAb #4060, Phospho-S6 Ribosomal Protein (Ser235/236) antibody #2211, and β-Actin (13E5) rabbit mAb #4970.

[00599] **Results**

[00600] By flow cytometry (Figure 72), a large proportion (97.9%) of CD3+ T cells transduced with the lentiviral vector encoding GIFNAR 134 ECD expressed the G-CSFR ECD, whereas non-transduced T cells did not.

[00601] Thus, GIFNAR 134 ECD can be expressed on the surface of engineered T cells.

[00602] As shown in Figure 73, T cells transduced with the lentiviral vector encoding GIFNAR 134 ECD expanded well in medium containing IL-2 but showed markedly reduced expansion in medium containing 130a1 G-CSF alone or IL-2 + 130a1 G-CSF; indeed, the level of expansion under these latter two conditions appeared to be inferior to that seen with medium alone (Figure 73B). Non-transduced T cells expanded well in response to IL-2 but not 130a1 G-CSF (Figure 73A). Neither culture expanded well in medium alone.

[00603] Thus, GIFNAR 134 ECD appears to induce a growth inhibitory signal in T cells, as assessed by this expansion assay.

[00604] As shown in Figure 74, in both non-transduced T cells and T cells expressing GIFNAR 134 ECD, IL-2 induced weak phosphorylation of STAT1 and typical levels of phosphorylation of JAK1, Akt and S6. In both non-transduced T cells and T cells expressing GIFNAR 134 ECD, IFNα induced strong phosphorylation of STAT1 and STAT2 with no apparent phosphorylation of JAK1, Akt or S6. In T cells expressing GIFNAR 134 ECD, 130a1 G-CSF induced strong phosphorylation of STAT1, STAT2 and JAK1 with no apparent phosphorylation of Akt or S6. 130a did not induce any biochemical signaling events in non-transduced T cells.

[00605] Thus, GIFNAR 134 ECD induces biochemical signaling events that are similar to those induced by IFNα.

Example 43: Construction and functional evaluation of chimeric receptors with a variant extracellular domain from the G-CSFR receptor and intracellular signaling domains from the interferon gamma receptor

[00606] We synthesized two chimeric receptors called GIFNGR-1 307 ECD and GIFNGR-2 307 ECD (Figure 60). GIFNGR-1 307 ECD contains the extracellular domain (307 variant) and transmembrane domain of human G-CSFR fused to the Box 1 and 2 regions of human IFNγR2 and the STAT1 binding site from the intracellular domain of human IFNγR1. GIFNGR-2 307 ECD contains the extracellular domain (307 variant), transmembrane domain, and Box 1 and 2 regions of human G-CSFR fused to the STAT1 binding site from the intracellular domain of human IFNγR1. The chimeric receptor subunits were encoded in separate lentiviral vectors, which were used to transduce human CD4+ and CD8+ T cells. Transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of GIFNGR-1 307 ECD and GIFNGR-2 307 ECD. The ability of GIFNGR-1 307 ECD and GIFNGR-2 307 ECD to induce T cell expansion was evaluated in vitro. Biochemical signaling events mediated by GIFNGR-1 307 ECD and GIFNGR-2 307 ECD were evaluated by western blot using phospho-specific antibodies against STAT1 and other signaling intermediaries.

[00607] Methods

[00608] In general, we followed similar methods as described in Example 39; however, the experiments were adapted to focus on IFN gamma signaling mechanisms.

[00609] Human T cells were transduced with lentiviral vectors encoding GIFNGR-1 307 ECD or GIFNGR-2 307 ECD. In addition, some T cells were co-transduced with lentiviral vectors encoding GIFNGR-1 307 ECD and G2R-3 134 ECD, or GIFNGR-2 307 ECD and G2R-3 134 ECD. G2R-3 134 ECD was tagged at the N-terminus with a Myc epitope (EQKLISEEDL), while GIFNGR-1 307 ECD and GIFNGR-2 307 ECD were tagged at the N-terminus with a Flag epitope (DYKDDDDK), which enabled the different receptor subunits to be distinguished by flow cytometry.

[00610] Transduction efficiency was determined by flow cytometry on Day 8, as per Example 39. The Flag and Myc tags were detected using BV421-conjugated and AlexaFluorTM 488-conjugated antibodies, respectively.

[00611] T cells were expanded in IL-2 (300 IU/ml), 130a1 G-CSF and/or 307 (100 ng/ml each), IL-2 + 130a1 G-CSF (300 IU/ml and 100 ng/ml, respectively), IL-2 + 307 G-CSF (300 IU/ml and 100 ng/ml, respectively) or medium alone (no added cytokine) for 18 days. Medium and the indicated cytokines were refreshed every two days. On days 4, 8, 12 and 16 of the expansion, T cells were counted using a Cytek™ Aurora flow cytometer.

[00612] For BrdU incorporation assays, T cells expanded in IL-2 were harvested on day 12 and washed twice in PBS and once in complete TexMACS medium. Cells were re-plated in fresh complete TexMACS medium containing medium alone or medium with IL-2 (300 IU/ml), IFNγ (100 ng/ml), IL-2 + IFNγ (300 IU/ml and 100 ng/ml, respectively), 130a1 G-CSF (100 ng/ml), 307 G-CSF (100 ng/ml), 130a1 G-CSF + 307 G-CSF (100 ng/ml each), 130a1 G-CSF + IFNγ (100 ng/ml each) or 307 G-CSF + IL-2 (100 ng/ml and 300 IU/ml, respectively). Cells were incubated for 48 hours 37°C, 5% CO2. The BrdU assay was performed as described in Example 39.

[00613] For the western blot experiment, T cells were expanded in IL-2 (300 IU/ml) for 18 days, washed as above, and rested overnight in complete TexMACS medium. The following day, cells were stimulated for 20 minutes at 37°C with medium alone or medium with IL-2 (300 IU/ml), IFNγ (100 ng/ml) or 307 G-CSF (100 ng/ml). Cytoplasmic and nuclear fractions were prepared and western blots performed as described in Example 39. Primary

antibodies were obtained from Cell Signaling Technologies: Phospho-Stat1 (Tyr701) (58D6) rabbit mAb #9167, Histone H3 (96C10) mouse mAb #3638, Phospho-JAK2 (Tyr1007/1008) antibody #3771, Phospho-Akt (Ser473) (D9E) XP® rabbit mAb #4060, Phospho-S6 Ribosomal Protein (Ser235/236) antibody #2211, and β-Actin (13E5) rabbit mAb #4970.

[00614] Results

[00615] By flow cytometry (Figure 75), a large proportion (93.8%) of CD3+ T cells transduced with the lentiviral vector encoding GIFNGR-1 307 ECD expressed the Flag epitope on the G-CSFR 307 ECD (Figure 75A). Likewise, a large proportion (51.7%) of CD3+ T cells transduced with the lentiviral vector encoding G2R-3 134 ECD expressed the Myc epitope on the G-CSFR 134 ECD (Figure 75B). For T cells that were co-transduced with lentiviral vectors encoding GIFNGR-1 307 ECD and G2R-3 134 ECD, 32.0% co-expressed both the Flag and Myc epitopes on the G-CSFR 307 and 134 ECDs, respectively (Figure 69C). As expected, non-transduced T cells did not express either the Myc or Flag epitopes (Figure 75D).

[00616] Thus, GIFNGR-1 307 ECD can be expressed on the surface of engineered T cells with or without G2R-3 134 ECD.

[00617] Similar flow cytometry results were obtained with GIFNGR-2 307 ECD (Figure 76). A large proportion (97.6%) of CD3+ T cells transduced with the lentiviral vector encoding GIFNGR-2 307 ECD expressed the Flag epitope on the G-CSFR 307 ECD (Figure 76A). Likewise, a large proportion (51.7%) of CD3+ T cells transduced with the lentiviral vector encoding G2R-3 134 ECD expressed the Myc epitope on the G-CSFR 134 ECD (Figure 76B; this is the same data as in Figure 75B). For T cells that were co-transduced with lentiviral vectors encoding GIFNGR-2 307 ECD and G2R-3 134 ECD, 32.6% co-expressed both the Flag and Myc epitopes on the G-CSFR 307 and 134 ECDs, respectively (Figure 76C). As expected, non-transduced T cells did not express either the Myc or Flag epitopes (Figure 76D; this is the same data as in Figure 75D).

[00618] Thus, GIFNGR-2 307 ECD can be expressed on the surface of engineered T cells with or without G2R-3 134 ECD.

[00619] As shown in Figure 71, T cells transduced with the lentiviral vector encoding GIFNGR-1 307 ECD expanded well in medium containing IL-2 or IL-2 + 307 G-CSF but not

307 G-CSF alone or medium alone (Figure 77B). T cells co-transduced with lentiviral vectors encoding GIFNGR-1 307 ECD and G2R-3 134 ECD expanded well in medium containing IL-2 or IL-2 + 130a1 G-CSF (Figure 77C). When these T cells were cultured in 130a1 G-CSF + 307 G-CSF, they showed reduced expansion on day 12 which increased by day 16 (Figure 77C). Non-transduced T cells expanded well in response to IL-2 but not 130a1 G-CSF + 307 G-CSF (Figure 77A). None of the T cell lines expanded well in medium alone.

[00620] Thus, GIFNGR-1 307 ECD appears to have a negligible effect on T cell growth, as assessed by this expansion assay.

[00621] As shown in Figure 78, T cells transduced with the lentiviral vector encoding GIFNGR-2 307 ECD expanded well in medium containing IL-2 but not in medium containing 307 G-CSF alone, IL-2 + 307 G-CSF, or medium alone (Figure 78B). T cells co-transduced with lentiviral vectors encoding GIFNGR-2 307 ECD and G2R-3 134 ECD expanded well in medium containing IL-2 or IL-2 + 130a1 G-CSF (Figure 78C). These T cells failed to expand when cultured in 130a1 G-CSF + 307 G-CSF (Figure 78C). Non-transduced T cells expanded well in response to IL-2 but not 130a1 G-CSF + 307 G-CSF (Figure 78A, which shows the same data as Figure 77A). None of the T cell lines expanded well in medium alone.

[00622] Thus, GIFNGR-2 307 ECD appears to inhibit T cell growth, as assessed by this expansion assay.

[00623] As shown in Figure 79, non-transduced CD4+ and CD8+ T cells demonstrated proliferation (BrdU incorporation) in response to IL-2, IL-2 + IFNγ, and 307 G-CSF + IL-2 (Figure 79A).

[00624] CD4+ and CD8+ T cells expressing G2R-3 134 ECD alone demonstrated proliferation in response to IL-2, IL-2 + IFNγ, 130a1 G-CSF, 130a1 G-CSF + 307 G-CSF, 130a1 G-CSF + IFNγ, and 307 G-CSF + IL-2 (Figure 79B, C).

[00625] CD4+ and CD8+ T cells expressing GIFNGR-1 307 ECD alone demonstrated proliferation in response to IL-2, IL-2 + IFNγ, and 307 G-CSF + IL-2 (Figure 79B, C).

[00626] CD4+ and CD8+ T cells co-expressing GIFNGR-1 307 ECD and G2R-3 134 ECD demonstrated proliferation in response to IL-2, IL-2 + IFNγ, 130a1 G-CSF, 130a1 G-CSF +

307 G-CSF, 130a1 G-CSF + IFNγ, and 307 G-CSF + IL-2 (Figure 79B, C). They also showed weak proliferation in response to 307 G-CSF alone (Figure 79B, C).

[00627] Thus, GIFNGR-1 307 ECD appears to have a neutral or negligible effect on CD8+ and CD4+ T cell proliferation.

[00628] Figure 80 shows the proliferation results for GIFNGR-2. The data for non-transduced T cells and CD4+ and CD8+ T cells expressing G2R-3 134 ECD alone are the same as shown in Figure 79 and hence are not described again.

[00629] CD4+ and CD8+ T cells expressing GIFNGR-2 307 ECD alone demonstrated proliferation in response to IL-2, IL-2 + IFNγ, 307 G-CSF, 130a1 G-CSF + 307 G-CSF, and 307 G-CSF + IL-2 (Figure 80B, C).

[00630] CD4+ and CD8+ T cells co-expressing GIFNGR-2 307 ECD and G2R-3 134 ECD demonstrated proliferation in response to IL-2, IL-2 + IFNγ, 130a1 G-CSF, 307, 130a1 G-CSF + 307 G-CSF, 130a1 G-CSF + IFNγ, and 307 G-CSF + IL-2 (Figure 80B, C).

[00631] Thus, despite inhibiting T cell expansion in long-term culture (Figure 78), GIFNGR-2 307 ECD appears to enhance CD4+ and CD8+ T cell proliferation, as assessed by BrdU assay.

[00632] As shown in Figure 81, in both non-transduced T cells and T cells expressing GIFNGR-1 307 ECD or GIFNGR-2 307 ECD, IL-2 induced weak/negligible phosphorylation of STAT1, strong phosphorylation of Akt, and (in this particular experiment) weak phosphorylation of S6. IFNγ did not appear to induce any of these signaling events. In T cells expressing GIFNGR-1 307 ECD or GIFNGR-2 307 ECD, 307 G-CSF induced strong phosphorylation of STAT1. In T cells expressing GIFNGR-2 307 ECD, 307 G-CSF also induced strong phosphorylation of JAK2 and Akt.

[00633] Thus, GIFNGR-1 307 ECD and GIFNGR-2 307 ECD induce tyrosine phosphorylation of STAT1 but differ in other ways from the native IFNγ receptor signal.

Example 44: Construction and functional evaluation of bicistronic constructs encoding the chimeric receptor G12/2R-1 134 ECD or G2R-3 134 ECD and a mesothelin-specific chimeric antigen receptor (CAR)

[00634] We synthesized bicistronic constructs called CAR_T2A_G2R-3-134-ECD, G2R-3-134-ECD_T2A_CAR, CAR_T2A_G12/2R-1-134-ECD, and G12/2R-1-134-ECD_T2A_CAR, as well as a monocistronic construct that drives expression of mesothelin CAR alone. The nomenclature of bicistronic contructs indicates how the gene segments are ordered from the 5' to 3' end. For example, CAR_T2A_G2R-3-134-ECD encodes (from 5' to 3') a mesothelin-specific CAR, a T2A ribosomal skip element, and G2R-3 134 ECD. The bicistronic constructs were expressed using a single lentiviral vector encoding the mesothelin CAR and either G2R-3 134 ECD or G12/2R-1 ECD, separated by a T2A ribosomal skip element. These bicistronic constructs were used to transduce human CD4+ and CD8+ T cells, and transduced T cells (and non-transduced control T cells) were analyzed by flow cytometry to detect cell surface expression of the G-CSFR ECD and the CAR. The ability of 130a1 G-CSF to induce T cell proliferation through G2R-3 134 ECD or G12/2R-1 134 ECD was evaluated *in vitro*. T cells were also evaluated by intracellular flow cytometry to measure expression of the cytokines IFNγ, TNFα, and IL-2, as well as the cell surface markers CD69 and CD137 in response to activation of the CAR by co-incubation of T cells with the mesothelin-positive human ovarian cancer cell line OVCAR3.

Methods

[00635] In general, we followed similar methods as described in Example 31; however, the experiments were adapted to focus on co-expression and functionality of the mesothelin CAR and the engineered chimeric cytokine receptors G2R-3 134 ECD and G12/2R-1 134 ECD, as described below.

[00636] For flow cytometry to detect the G-CSFR ECD and mesothelin CAR, cells were expanded in IL-7 and IL-15 (10 ng/ml each) for 8 days. Cells were incubated with antibodies specific for CD3 (BV750-conjugated), CD4 (PE-conjugated), CD8 (PerCP-conjugated) and G-CSFR ECD (APC-conjugated), as well as recombinant Fc-tagged mesothelin protein (FITC-conjugated) and efluor506™ viability dye.

[00637] For intracellular flow cytometry, transduced T cells were expanded in 130a1 G-CSF for 13 days. OVCAR3 cells (ATCC) were grown in ATCC-formulated RPMI-1640 medium containing 0.01 mg/ml bovine insulin and 20% fetal bovine serum. T cells were

washed and replated in complete TexMACS medium in the presence or absence of OVCAR3 cells (at a 2:1 effector:target cell ratio) for 13 hours at 37 degrees Celsius, 5% CO2. The last four hours of incubation were performed in the presence of a protein transport inhibitor (Brefeldin A). At the conclusion of incubation, the cells were stained with Zombie NIR™ Fixable Viability Kit, followed by a cocktail of antibodies against CD3 (BV510-conjugated), CD4 (AlexaFluor™ 700-conjugated), G-CSFR (APC-conjugated), CD69 (BV785-conjugated) and CD137 (BV605-conjugated), as well as recombinant mesoethelin (FITC-conjugated) and Fc block. The cells were then fixed and permeabilized (BD Cytofix/Cytoperm™ set) and stained for the cytokines IFNγ (BV650-conjugated), TNFα (PECy7-conjugated) and IL-2 (PE-CF594-conjugated). Cells were analyzed using a Cytek Aurora flow cytometer.

Results

[00638] By flow cytometry (Figures 82 and 83), non-transduced cells exhibited neglible expression of G-CSFR ECD and mesothelin CAR (Figure 66A and 67A), whereas 55.3% of CD3+ T cells transduced with a lentivirus encoding the mesothelin CAR construct alone expressed the CAR (Figure 82B and 83B), and 13.1% of T cells transduced with a lentivirus encoding G2R-3 134 ECD expressed the G-CSFR ECD (Figure 82C). 26.7% of T cells transduced with a lentivirus encoding CAR_T2A_G2R-3-134ECD expressed both the G-CSFR ECD and mesothelin CAR (Figure 82D), while 25.2% of CD3+ T cells transduced with a lentivirus encoding G2R-3-134ECD_T2A_CAR expressed both the G-CSFR ECD and mesothelin CAR (Figure 82E). Likewise, 13.1% of T cells transduced with a lentivirus encoding G12/2R-1 134 ECD alone expressed the G-CSFR ECD (Figure 83C). 30.8% of T cells transduced with a lentivirus encoding CAR_T2A_G12/2R-1-134ECD expressed both the G-CSFR ECD and mesothelin CAR (Figure 83D), and 36.0% of T cells transduced with a lentivirus encoding G12/2R-1-134ECD_T2A_CAR expressed the both the G-CSFR ECD and mesothelin CAR (Figure 83E).

[00639] Thus, bicistronic lentiviral vectors encoding a mesothelin CAR plus G2R-3 134 ECD or mesothelin CAR plus G12/2R-1 134 ECD can cause cell surface expression of both the CAR and chimeric cytokine receptor.

[00640] By BrdU incorporation assay (Figure 84), non-transduced T cells or T cells engineered to express the mesothelin CAR alone proliferated in response to IL-2, with negligible proliferation in response to 130a1 G-CSF or medium alone. T cells expressing G2R-3 134 ECD alone proliferated in response to IL-2 or 130a1 G-CSF (Figure 84B). T cells transduced with lentiviral vectors encoding CAR_T2A_G2R-3-134ECD, CAR_T2A_G12/2R-

1-134ECD, or G12/2R-1-134ECD_T2A_CAR proliferated in response to IL-2 or 130a1 G-CSF. None of the T cell populations showed significant proliferation in response to medium alone.

[00641] Thus, G2R-3 134 ECD and G12/2R-1 134 ECD retain their functional properties when co-expressed as part of a bicistronic construct with a mesothelin CAR, irrespective of whether they are arranged upstream or downstream of the CAR.

[00642] By intracellular flow cytometry (Figure 85), T cells expressing the monocistronic mesothelin CAR construct upregulated expression of the cytokines IFNγ, TNFα and IL-2, and the activation markers CD69 and CD137, in response to stimulation with OVCAR3 cells (Figure 85A). In contrast, there was no observed change in expression of IFNγ, TNFα, IL-2, CD69 or CD137 in T cells engineered to express G12/2R-1 134 ECD alone and stimulated with OVCAR3 cells (Figure 85B). T cells expressing the bicistronic construct CAR_T2A_G2R-3-134ECD showed increased expression of IFNγ, TNFα, IL-2, CD69 and CD137 in response to OVCAR3 cells (Figure 85C). Likewise, T cells expressing the G2R-3-134ECD_T2A_CAR, CAR_T2A_G12/2R-1-134ECD or G12/2R-1-134ECD_T2A_CAR constructs showed increased expression of these cytokines and markers in response to stimulation with OVCAR3 cells (Figures 85D, 85E and 85F).

[00643] Thus, the mesothelin CAR retains its functional properties when expressed as part of a bicistronic lentiviral construct with G2R-3 134 ECD or G12/2R-1 134 ECD, as evidenced by upregulation of IFNγ, TNFα and IL-2 and the activation markers CD69 and CD137 in response to stimulation with cognate antigen on OVCAR3 cells. The CAR was functional when arranged either upstream or downstream of G2R-3 134 ECD or G12/2R-1 134 ECD.

Example 45: Human T cells co-expressing a mesothelin-specific CAR and an IL-2 emulating chimeric cytokine receptor demonstrate cytotoxicity against mesothelin-expressing target cells

[00644] Experiments are conducted to show that CAR constructs and chimeric cytokine receptors, when co-expressed, exhibit their expected functional properties and that stimulation of chimeric cytokine receptors such as G2R-3 134 ECD enhances CAR-mediated cytotoxity against target cells expressing the appropriate CAR antigen. The following example uses a mesothelin-specific CAR co-expressed with G2R-3 134 ECD.

Methods

[00645] A mammalian expression transfer plasmid encoding firefly luciferase, pCCL Luc Puromycin is introduced into a lentiviral vector using a standard calcium phosphate

transfection protocol and 2nd generation packaging vectors (AddGene). The mesothelin-expressing OVCAR3 cell line is transduced with the luciferase-encoding lentivirus and selected over 2 weeks for resistance to puromycin (1.5 µg/ml, Sigma, Cat# P8833) to generate a puromycin-resistant cell line for use as a target cell population in cytotoxicity assays.

[00646] Luciferase-expressing target OVCAR3 lines are seeded at 2 x 10^4 cells, 100 µl per well in a 96-well plate (Corning, Cat# 3917) and incubated overnight to allow adherence to the plate. Human PBMC-derived T cells are lentivirally transduced to express a mesothelin-specific CAR alone or with G2R-3 134 ECD. T cells are plated (100 µl per well) in a 96-well plate and split over a 2-fold dilution series to achieve a range of effector-to-target (E:T) ratios from 20:1 to 0.625:1. The diluted T cell suspensions are then be added to the adherent tumor cell cultures to achieve a total volume of 200 µl. IL-2 (300 IU/ml), 130a1 G-CSF (100 ng/ml) or PBS are added to some wells to determine whether cytotoxicity is enhanced by IL-2 or 130a1 G-CSF. All conditions are tested in triplicate. Three wells of target cells alone and 3 wells of media alone are plated to determine the maximal and minimal relative luminescence units (RLU) for the assay. Cells are cultured for 24-48 h at 37ºC in 5% CO_2.

[00647] The day of the assay, 22 µl 10X stock of Xenolight™ D-Luciferin (Perkin Elmer, Cat# 122799) are added to each well, and plates are incubated for 10 min at RT in the dark. Plates are scanned on a luminescence plate reader (Perkin Elmer Wallac Envision 2104 Multilabel Reader). RLU from triplicate wells are averaged, and the percent specific cytotoxicity is determined by the following equation: percent specific cytotoxicity = 100 x (Max Luminescence RLU – Test Luminescence RLU) / (Min Luminescence RLU – Max Luminescence RLU). In addition, target cells are imaged for morphological signs of cell death at 24 & 48 hours.

Expected Results

[00648] T cells expressing the mesothelin-specific CAR cause lysis of target cells in a dose-dependent manner. Target cell lysis is increased by the addition of IL-2, as reflected by increased killing at lower E:T ratios. For T cells co-expressing the mesothelin-specific CAR with G2R-3 134 ECD, target cell lysis is increased with the addition of 130a1 G-CSF to a similar extent as seen with IL-2. These results show that, in T cells co-expressing a mesothelin-specific CAR with G2R-3 134 ECD, the CAR mediates target cell recognition and killing as expected and that the cytotoxicity of T cells can be selectively enhanced by stimulation of G2R-3 134 ECD with 130a1 G-CSF.

Example 46: Construction and functional evaluation of a Controlled Paracrine Signaling system paired with a CAR

[00649] Using the methods described in Example 41, a CPS system is paired with a CAR, using the example of G12/2R 134 ECD, IL-18 and a mesothelin-specific CAR (see construct called CAR_P2A_G12-2R1-134_T2A_hIL-18 in Table 31). This combined system is functionally assessed *in vitro* and *in vivo*.

Methods

[00650] The three cDNAs are separated by P2A and T2A sites to allow three proteins to be produced from a single mRNA transcript. With such tricistronic constructs, it is not uncommon for one cDNA to be expressed at higher levels than the other, and the optimal configuration needs to be determined empirically. Therefore, at least three configurations are constructed (listed from 5' to 3'): (a) G12/2R-1 134 ECD, IL-18, Meso CAR; (b) IL-18, G12/2R-1 134 ECD, Meso CAR; and (c) Meso CAR G12/2R-1 134 ECD, IL-18 (Figures 82-85) (Table 31).

[00651] T cell transduction, expansion, proliferation, IL-18 secretion and biochemical signaling will be assessed using similar methods as Example 49. CAR expression and recognition and killing of mesothelin-positive tumor cells will be assessed using similar methods as Example 44.

Expected results

[00652] T cells are transduced with two different lentiviral constructs: one encoding G12/2R-1 134 ECD in tandem with IL-18 and Meso CAR (referred to in this example as $CPS^{2/12+18}$ + Meso CAR; also referred to as CAR_P2A_G12-2R1-134_T2A_hIL-18 in Table 31), and the other encoding G12/2R-1 134 ECD and Meso CAR (Table 31) (to serve as a control).

[00653] By flow cytometry, T cells expressing $CPS^{2/12+18}$ + Meso CAR or G12/2R-1 + Meso CAR demonstrate co-expression of the human G-CSFR ECD and Meso CAR on a significant proportion of T cells (15-90%). Meso CAR-expressing T cells upregulate expression of activation markers such as CD69 or CD137 when exposed to target cells expressing mesothelin, similar to Example 44.

[00654] By BrdU assay, Meso CAR-expressing T cells proliferate in response to target cells expressing mesothelin.

[00655] By IFN-γ ELISA, Meso CAR-expressing T cells secrete IFN-γ in response to target cells expressing mesothelin. IFN-γ secretion is enhanced by addition of IL-2, IL-2 + IL-12, and/or 130a1 G-CSF to cultures. The effect of 130a1 G-CSF is more pronounced in cells expressing $CPS^{2/12+18}$ + Meso CAR, as IL-18 can enhance IFN-γ secretion by T cells, especially when combined with IL-2 and IL-12 signals.

[00656] By *in vitro* cytotoxicity assay (similar to Example 45), Meso CAR-expressing T cells kill target cells expressing mesothelin. This effect is enhanced by addition of IL-2, IL-2 + IL-12, and/or 130a1 G-CSF to cultures, since both IL-2 and IL-12 can enhance the cytotoxicity of T cells. The effect of 130a1 G-CSF is more pronounced in cells expressing $CPS^{2/12+18}$ + Meso CAR, as IL-18 can further enhance the cytotoxicity of T cells when combined with IL-2 and IL-12 signals.

[00657] In *in vivo* tumor regression models, Meso CAR-expressing T cells induce regression of tumor cells expressing mesothelin. This effect is enhanced by administration of IL-2 or 130a1 G-CSF to cultures, since IL-2 can enhance the cytotoxicity of T cells. (IL-12 is too toxic to safely administer systemically.) 130a1 G-CSF has a stronger anti-tumor effect than IL-2, as the combined IL-2 + IL-12 signal from G12/2R 134 ECD should be stronger than IL-2 alone. The effect of 130a1 G-CSF is even more pronounced in cells expressing $CPS^{2/12+18}$ + Meso CAR, as IL-18 further enhance the anti-tumor activity of T cells when combined with IL-2 and IL-12 signals.

[00658] By methods such as flow cytometry and immunohistology, T cells expressing $CPS^{2/12+18}$ + Meso CAR (in comparison to T cells expressing G12/2R-1 + Meso CAR) induce a stronger anti-tumor immune response. This is reflected by increased infiltration of tumors by activated host T cells, NK cells, myeloid cells and other cell types due to the paracrine effects of IL-18 in the tumor microenvironment.

[00659] Thus, the experiments in this example demonstrate superior effector function of T cells expressing $CPS^{2/12+18}$ over T cells expressing G12/2R-1 alone due to the stimulatory effects of IL-18 on such functions as IFN-γ secretion and cytotoxicity by T cells, and paracrine effects on host immune cells.

Example 47: Construction and functional evaluation of a dual-cytokine Controlled Paracrine Signaling system

[00660] Chronic signaling can have undesirable or deleterious effects on cells. For example, prolonged or chronic exposure to an IL-12 signal could promote the terminal differentiation of T cells, thereby limiting their proliferative potential and/or persistence. Therefore, in some circumstances it may be desirable to use a CPS system in which, for example, the IL-2 and IL-12 signals are controlled by distinct, orthogonal cytokines. In this manner, T cells are induced to proliferate in response to one cytokine (for example, via stimulation of G2R-3 with 130a1 G-CSF to emulate an IL-2 signal) and to differentiate at a later time in response to another cytokine (for example, via stimulation of G12R-1 with 307 G-CSF to emulate an IL-12 signal). To enable such control, methods described in the above examples are used to construct and evaluate a CPS system comprised of a tricistronic vector encoding G2R-3 134 ECD, G12R-1 307 ECD and IL-18 (referred to as $CPS^{2+12+18}$). This combined system is functionally assessed *in vitro* and *in vivo*.

Example 48: Construction and functional evaluation of Controlled Paracrine Signaling systems that emulate other cytokine combinations

[00661] Using the methods described in Example 41, other cytokine combinations are emulated by substituting G12/2R-1 with chimeric cytokine receptors encoding other ICDs, such as those shown in Figure 31. These chimeric receptors could use the 134 ECD of human G-CSFR, or other variant ECDs of human G-CSFR (e.g., 307 ECD), or ECDs from other cytokine receptors.

[00662] In place of the IL-18 cDNA, cDNAs encoding other cytokines or chemokines are inserted, such as CXCL13, IL-21, CCL3, CCL4, CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, IFN-γ, IFN-α, IL-17, or TNF-α. Other cell surface proteins are inserted, such as the receptor NKG2D (Table 30). Human, murine or other versions of such factors are used, depending on the intended application.

Example 49: Construction and expression of a chimeric receptor with extracellular and transmembrane domains from the IL-7 alpha receptor subunit and intracellular domain from the IL-2/IL-15 receptor beta subunit

[00663] We synthesized a chimeric receptor called 7/2R-1 (Figures 92 and 93) which contained the extracellular and transmembrane domains of the human IL-7 receptor alpha subunit fused to the intracellular domain of the human IL-2/IL-15 receptor beta subunit. The IL-7 receptor alpha subunit was tagged at its N-terminus with a standard Flag epitope

(DYKDDDDK) to enable discrimination of this chimeric receptor subunit from the native IL-7 receptor alpha subunit, which is also frequently expressed by T cells. The chimeric receptor subunit was encoded in a lentiviral vector which was used to transduce human CD4+ and CD8+ T cells. Twelve days later, transduced cells (and non-transduced control cells) were analyzed by flow cytometry to detect expression of the chimeric receptor subunit relative to the native IL-7 receptor alpha subunit.

Methods

[00664] The Flag-tagged chimeric receptor (7/2R-1) was synthesized and cloned into a lentiviral transfer plasmid (Twist Bioscience). The transfer plasmid and lentiviral packaging plasmids (psPAX2, pMD2.G, Addgene) were co-transfected into the lentiviral packaging cell line HEK293T/17 (ATCC) as follows. Cells were plated overnight in Opti-MEM™ I Reduced Serum Medium, GlutaMAX™ Supplement (Gibco™) containing 5% fetal bovine serum and 0.2 mM sodium pyruvate. Plasmid DNA and Lipofectamine™ 3000 Transfection Reagent (Gibco™) were mixed, according to the manufacturer's specifications, and added dropwise onto cells. The cells were incubated for 6 hours at 37°C, 5% CO_2, followed by replacement of the medium and further incubation overnight. The following day, the cellular supernatant was collected from the plates, stored at 4°C, and medium replaced. The following day, cellular supernatant was collected from the cells, and pooled with supernatant from the previous day. The supernatant was centrifuged briefly, to remove debris, and filtered through a 0.45 μm filter. The supernatant was spun for 90 minutes at 25,000 rpm using a SW-32Ti rotor in a Beckman Optima L-XP Ultracentrifuge. The supernatant was removed, and the pellet was resuspended via 30 minutes of gentle shaking in a suitable volume of Opti-MEM I medium.

[00665] Primary human T cells were isolated from a healthy donor leukapheresis product as follows. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation. CD4+ and CD8+ T cells were isolated using human CD4 and CD8 MicroBeads (Miltenyi Biotec) following manufacturer's specifications. For some experiments, T cells were viably frozen and thawed at a later time point. Isolated T cells were plated at 5 x 10^5 per well in a 48-well plate in TexMACS™ medium (Miltenyi Biotec) containing 3% human serum (Sigma), and 0.5% gentamicin (DIN 0226853) (hereafter referred to as "complete TexMACS"). Human T cell TransAct™ (Miltenyi Biotec) was added (10 μl per well), and cells were incubated at 37°C, 5% CO_2. Twenty to 28 hours following activation, the T cells were transduced with the lentivirus encoding the receptor constructs. Twenty-four hours after

transduction, fresh medium was added to T cells, along with IL-7 (10 ng/ml), IL-7 and IL-15 (10 ng/ml each), or no cytokine, and the cells were incubated for a further 48 hours. On day 12 of expansion, receptor expression was assessed by flow cytometry as follows. Cells were incubated for 15 minutes at 4°C with an anti-human CD127 eFluor™ 450-conjugated antibody (1:50 dilution), anti-Flag antibody conjugated to PE or PECy7 (1:50 dilution), anti-human CD3 antibody conjugated to BV750™ (1:50 dilution), anti-human CD4 antibody conjugated to PE or AlexaFluor™ 700 (1:25 dilution), anti-human CD8 antibody conjugated to PerCP (1:100), and eBioscience™ Fixable Viability Dye eFluor™ 506 (1:1000 dilution). Cells were then washed twice in buffer and analyzed on a Cytek™ Aurora flow cytometer. Figure 88 shows the results for CD3+ T cells, which include both CD4+ and CD8+ subsets.

Results

[00666] A large proportion (48.5%) of CD3+ T cells transduced with the lentiviral vector encoding 7/2R-1 co-expressed CD127 (IL-7Rα) and the Flag epitope tag (Figure 88A), whereas non-transduced T cells showed low/moderate expression of native CD127 but not the Flag epitope tag (Figure 88B). The specificity of CD127 detection was confirmed by the CD127 FMO control (Figure 88C).

[00667] Thus, 7/2R-1 can be expressed on the surface of lentivirally transduced T cells.

Example 50: Expansion of human T cells transduced with 7/2R-1 and stimulated with IL-7

[00668] To determine whether 7/2R-1 promoted T cell expansion (increase in cell number), T cells from the transduced and non-transduced conditions from Example 49 were cultured in medium alone versus IL-7 + IL-15 or versus IL-7 and counted at regular intervals. The combination of IL-7 + IL-15 was chosen as a comparator to IL-7 alone, as IL-7 + IL-15 are commonly used to expand human T cells after lentiviral transduction.

Methods

[00669] Human T cells were isolated and transduced to express 7/2R-1 as described in Example 49 (day 0). The cells were expanded in IL-7 (10 ng/ml), IL-7 and IL-15 (10 ng/ml each) or medium only for a total of 16 days, with medium and cytokine refreshed every two days. On days 4, 8, 12 and 16 of expansion, live T cells were counted using a Cytek™ Aurora flow cytometer to assess fold expansion relative to day 0.

Results

[00670] As shown in Figure 57, CD3+ T cells transduced with the lentiviral vector encoding 7/2R-1 showed equivalent expansion in either IL-7 or IL-7 + IL-15 (Figure 89A), whereas non-transduced CD3+ T cells expanded well in IL-7 + IL-15 but only modestly in IL-7 (Figure 89B). Neither culture expanded well in medium alone.

[00671] Thus, expression of 7/2R-1 on T cells leads to increased expansion in response to IL-7, reaching a cell density equivalent to that induced by IL-7 + IL-15. This provides functional evidence that the intracellular domain of the IL-2/IL-15 receptor beta subunit is functional in the context of the 7/2R-1 chimeric receptor.

Example 51: Proliferation of human T cells transduced with 7/2R-1 and stimulated with IL-7 assessed by BrdU incorporation assay

[00672] To determine whether 7/2R-1 promoted cell proliferation (cell cycling), T cells from the transduced and non-transduced conditions from Example 49 were cultured in medium alone versus medium containing IL-2, IL-7 or the combination of IL-2 + IL-7. Cell proliferation was measured by a 48-hour BrdU assay.

Methods

[00673] Cells were isolated and engineered to express 7/2R-1 as described in Example 49. Cells expressing 7/2R-1 were expanded in IL-7 (10 ng/ml) and non-transduced cells were expanded in IL-7 and IL-15 (10 ng/ml each). On day 12 of expansion, cells were harvested and washed twice in PBS, and once in complete TexMACS medium, and re-plated in fresh complete TexMACS medium containing the relevant assay cytokine (IL-7 at 10 ng/ml, IL-2 at 300 IU/ml, IL-7 and IL-2 at 10 ng/ml and 300 IU/ml, respectively, or no cytokine) for 48 hours at 37°C, 5% CO_2. The BrdU assay procedure followed the instruction manual for the BD Pharmingen™ FITC BrdU Flow Kit (557891), with the following addition: surface staining was performed, as described in Example 49, to assess 7/2R-1 expression in addition to BrdU incorporation. Flow cytometry was performed using a Cytek™ Aurora instrument.

Results

[00674] As shown in Figure 58, CD4+ and CD8+ T cells transduced with the lentiviral vector encoding 7/2R-1 showed equivalent proliferation in response to either IL-2, IL-7 or IL-2 + IL-7 (Figure 90A), whereas non-transduced CD4+ and CD8+ T cells proliferated well in response to IL-2 and IL-2 + IL-7 but only modestly in response to IL-7 (Figure 90B). None of

the cultures proliferated well in medium alone, with the exception of CD8+ T cells expressing 7/2R-1, which had high background proliferation in this particular assay.

[00675] Thus, expression of 7/2R-1 on T cells leads to increased proliferation in response to IL-7, reaching levels equivalent to that induced by IL-2 or IL-2 + IL-7. This provides functional evidence that the intracellular domain of the IL-2/IL-15 receptor beta subunit is functional in the context of the 7/2R-1 chimeric receptor.

Example 52: Western blot detection of biochemical signaling events in human T cells expressing 7/2R-1

[00676] To determine whether 7/2R-1 induced similar biochemical signaling events as the native IL-2 receptor, T cells from the transduced and non-transduced conditions from Example 31 were cultured in medium alone versus medium containing IL-2, IL-7 or the combination of IL-2 + IL-7. Cell lysates were prepared and probed by western blot with antibodies against various proteins involved in IL-7 and IL-2 receptor signaling (Figure 91).

Methods

[00677] Cells were isolated and engineered to express 7/2R-1 as described in Example 49. Cells expressing 7/2R-1 were expanded in IL-7 (10 ng/ml) and non-transduced cells were expanded in IL-7 and IL-15 (10 ng/ml each). On day 12 of expansion, cells were harvested, washed twice PBS, washed once in complete TexMACS medium, and rested overnight in complete TexMACS medium containing no added cytokine at 37°C, 5% CO_2.

[00678] To perform western blots, cells were stimulated with no cytokine, IL-2 (300 IU/ml), IL-7 (10 ng/ml), or IL-2 and IL-7 (at 300 IU/ml and 10 ng/ml, respectively) for 20 minutes at 37°C. Cells were washed once in a buffer containing 10 mM HEPES, pH 7.9, 1 mM $MgCl_2$, 0.05 mM EGTA, 0.5 mM EDTA, pH 8.0, 1 mM DTT, and 1x Pierce Protease and Phosphatase Inhibitor Mini Tablets (A32961). Cells were lysed in the wash buffer above, with the addition of 0.2% NP-40 substitute (Sigma), for 10 minutes on ice and centrifuged for 10 minutes at 13,000 rpm at 4°C, after which supernatant (cytoplasmic fraction) was collected. The nuclear pellet was resuspended and extracted in the above wash buffer with the addition of 0.42M NaCl and 20% glycerol. Nuclear pellets were extracted for 30 minutes on ice, with frequent vortexing, and centrifuged for 20 minutes at 13,000 rpm at 4°C, after which the soluble nuclear fraction (supernatant) was collected. The cytoplasmic and nuclear fractions were heated (70°C) in reducing buffer for 10 minutes and run on NuPAGE™ 4-12% Bis-Tris Protein Gels. The gels were transferred to nitrocellulose membrane (60 min at 20V in a Trans-Blot® SD Semi-Dry Transfer Cell), dried, and blocked for 1hr in Intercept® Blocking Buffer in TBS (927-

50000, LI-COR). The blots were incubated with the indicated primary antibodies (1:1,000) overnight at 4°C in Intercept® Blocking Buffer in TBS containing 0.1% Tween20. The primary antibodies utilized were obtained from Cell Signaling Technologies: Phospho-Shc (Tyr239/240) antibody #2434, Phospho-Akt (Ser473) (D9E) XP® rabbit monoclonal antibody #4060, Phospho-S6 Ribosomal Protein (Ser235/236) antibody #2211, Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody #9101, β-Actin (13E5) rabbit monoclonal antibody #4970, Phospho-JAK1 (Tyr1034/1035) (D7N4Z) rabbit monoclonal antibody #74129, Phospho-JAK3 (Tyr980/981) (D44E3) rabbit monoclonal antibody #5031, Phospho-Stat5 (Tyr694) (C11C5) rabbit monoclonal antibody #9359, and Histone H3 (96C10) mouse monoclonal antibody #3638. Blots were washed three times in TBS containing 0.1% Tween20 and incubated for 30-60 minutes at room temperature with secondary antibodies (1:10,000) in TBS buffer containing 0.1% Tween20. The secondary antibodies were obtained from Cell Signaling Technologies: anti-mouse IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5257 and anti-rabbit IgG (H+L) (DyLight™ 800 4X PEG Conjugate) #5151. Blots were washed and exposed on a LI-COR Odyssey imager.

Results

[00679] As shown in Figure 59, in both non-transduced T cells and T cells expressing 7/2R-1, IL-2 induced the phosphorylation of STAT5, JAK1, JAK3, Shc, Erk1/2, Akt, and S6. A similar pattern was seen in response to IL-2 + IL-7 in both T cell cultures. In non-transduced T cells, IL-7 stimulation induced less phosphorylation of STAT5, Shc, Akt and S6 compared to IL-2 stimulation. In contrast, in T cells expressing 7/2R-1, the phosphorylation patterns induced by IL-7 and IL-2 were indistinguishable. Histone H3 and beta-actin served as loading controls.

[00680] Thus, stimulation of 7/2R-1 by IL-7 results in similar biochemical signaling events in T cells as seen after stimulation of the native IL-2 receptor, providing biochemical evidence that the intracellular domain of the IL-2/IL-15 receptor beta subunit was functional in the context of the 7/2R-1 chimeric receptor.

Example 53: Construction and evaluation of an alternative chimeric receptor containing the extracellular and transmembrane domains of IL-7 receptor alpha domain and the intracellular domain of the IL-2 receptor beta subunit

[00681] We have designed an alternative version of 7/2R-1, which we refer to as 7/2R-2 (Figures 92 and 93). The key difference is that the fusion site between IL-7R alpha and IL-2R beta has been moved such that 7/2R-2 contains the Box 1 and 2 regions of IL-7Rα rather than

IL-2Rβ. This receptor may be advantageous if, for example, the fusion site in 7/2R-1 proves to be immunogenic in humans. Following the methods in Examples 49-52, a Flag epitope tagged version of 7/2-R2 is constructed and encoded in a lentiviral vector, and this lentiviral vector is transduced into T cells. Expression of 7/2R-2 is assessed by flow cytometry; the functional properties of 7/2R-2 are assessed by expansion and proliferation assays, and the biochemical signaling properties of 7/2R-2 are assessed by western blot.

Example 54: Chemical addition of a PEG20k moiety to the N-terminus of 130a1 G-CSF

[00682] We synthesized a modified protein called PEG20K_G-CSF_130a1 (Figure 93) (also referred to as PEG-130a1 G-CSF) that includes the full sequence of 130a1 G-CSF with a 20 kDa polyethylene glycol (PEG) moiety chemically linked to the N-terminus of the protein. In addition, we synthesized an analogous version of 307 G-CSF, which we refer to as PEG20K_G-CSF_307 or PEG-307 G-CSF.

[00683] Methods

[00684] 20 mg of purified 130a1 G-CSF was combined with 44 mg of 20 kDa methoxy-polyethylene glycol propionaldehyde (mPEG-ALD, Creative PEGworks). Reaction buffer (10 mM Sodium acetate pH 5.0, 5 mM NaCl) was added to a final volume of 2 ml. The PEGylation reaction was initiated by adding 40 μl of a 1.0 M solution of sodium cyanoborohydride (Final [$NaBH_3CN$] = 20 mM) and mixing thoroughly. The reaction was then incubated on a rotator at 18 °C for 8 hours. The reaction was terminated by adding 3 ml of termination buffer (10 mM Sodium acetate pH 3.5, 5 mM NaCl). The final 5 ml sample was then run on a cation exchange column to separate mono-PEGylated protein from any remaining unmodified protein or multi-PEGylated species. The same protocol was successfully completed with 307 G-CSF.

[00685] Results

SDS-PAGE showed a homogenous population of mono-PEGylated 130a1 G-CSF and 307 G-CSF, which showed similar signalling activity to unmodified protein *in vitro*.

Example 55: In a murine mammary carcinoma model, treatment with 130a1 G-CSF selectively enhances the expansion and anti-tumor phenotype of tumor-specific T cells expressing G4R-1 134 ECD

[00686] The variant cytokine 130a1 G-CSF was tested for the ability to enhance the expansion, phenotype and anti-tumor activity of T cells engineered to express G4R 134 ECD in an *in vivo* murine breast cancer model.

Methods

[00687] We used similar methods as described in Example 36, with the exception that Thy1.1+ OT-I cells were retrovirally transduced to express G4R 134 ECD instead of G2R-3 134 ECD. The antibody panel used for flow cytometry of blood samples included: murine CD45 (Clone 30-F11, APC-conjugated), CD3 (Clone 17A2, AF700-conjugated), CD8a (Clone 53-6.7, PerCP-conjugated), CD4 (Clone RM4-5, BV510-conjugated), NK1.1 (Clone PK136, PECy7-conjugated), CD19 (Clone 6D5, SparkNIR685-conjugated), CD11b (Clone M1/70, FITC-conjugated), CD11c (Clone N418, BV711-conjugated), Ly6C (Clone HK1.4, BV605-conjugated), and Ly6G (Clone 1A8, PE-conjugated). Flow cytometry was performed on a Cytek Aurora instrument.

Results

[00688] All mice received an equivalent dose (5×10^6) of Thy1.1+ OT-I cells transduced to express G4R 134 ECD. The transduction efficiency was 74.7%, as assessed by the percentage of Thy1.1+ cells expressing human G-CSFR+ by flow cytometry on Day 0.

[00689] In the 130a1 G-CSF-treated group, 1/3 mice experienced complete regression (CR) of their tumor and remained tumor-free until day 46, when the study was stopped for practical reasons (Figure 93A). Similarly, in the vehicle-treated group, 1/3 mice experienced CR. Thus, the 130a1 G-CSF- and vehicle-treated groups did not show statistically significant difference.

[00690] The expansion and persistence of Thy1.1+ OT-I cells were monitored by flow cytometry in serial blood samples collected from mice. In the 130a1 G-CSF-treated group, Thy1.1+ OT-I cells reached a mean peak engraftment of 6.1% of all CD8+ T cells on Day 4 compared to a peak of 3.3% for the vehicle-treated group (Figure 93B).

[00691] The phenotype of Thy1.1+ OT-I cells was monitored by flow cytometry of serial blood samples. In the 130a1 G-CSF-treated group, the majority (mean = 70.4%) of Thy1.1+ OT-I cells adopted a T effector memory (Tem) phenotype (CD44+CD62L-) by Day 8

compared to a mean of 27.9% for the vehicle-treated group (Figure 93C). In contrast, host (Thy1.1-) CD8+ T cells showed no significant difference in the percentage of Tem cells between the 130a1 G-CSF- and vehicle-treated groups at any time point (Figure 93D).

[00692] Expression of the Programmed Death-1 (PD-1) molecule was assessed by flow cytometry. In the 130a1 G-CSF-treated group, only a small minority (mean = 1.3%) of Thy1.1+ OT-I cells expressed PD-1 by Day 12 compared to a mean of 15.5% for the vehicle-treated group (Figure 93E). In contrast, host (Thy1.1-) CD8+ T cells showed no significant difference in the percentage of PD-1+ cells between the 130a1 G-CSF- and vehicle-treated groups at any time point (Figure 93F).

[00693] Flow cytometry demonstrated that the overall percentage of host (Thy1.1-) CD4+ T cells and CD19+ B cells (relative to host CD45+ cells) did not differ significantly between the 130a1 G-CSF- and vehicle-treated groups at any time point (Figure 93G,H). Likewise, treatment with 130a1 G-CSF did not affect neutrophil, eosinophil or monocyte counts in peripheral blood (data not shown).

[00694] Throughout the study, all mice in both groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00695] Thus, 130a1 G-CSF was able to induce modest yet selective expansion of tumor-specific CD8+ T cells expressing G4R 134 ECD. This resulted in a 33% durable CR rate. Treatment with 130a1 G-CSF induced a significant proportion of tumor-specific CD8+ T cells to adopt a Tem phenotype and to decrease expression of PD-1. There were no overt toxicities associated with 130a1 G-CSF treatment.

Example 56: In a cell proliferation assay, pegylated versions of G-CSF 130a1 and 307 show similar potency and selectivity compared to non-pegylated versions

[00696] An *in vitro* cell proliferation experiment was performed to compare pegylated versus non-pegylated versions of G-CSF (wildtype, 130a1 and 307) for the ability to stimulate proliferation of cells expressing G2R-3 134 ECD or the wildtype human G-CSF receptor.

Methods

[00697] In Figure 94A, human CD4 and CD8 T cells derived from PBMC of a healthy donor were lentivirally transduced to express G2R-3 134 ECD using methods similar to Example 35. To assess proliferation (BrdU incorporation), T cells were washed twice in PBS

and once in medium and then re-plated in fresh medium containing the indicated cytokines: human IL-2 (Proleukin, 300 IU/ml, positive control), medium alone (negative control), or the indicated concentrations (in ng/ml) of wildtype G-CSF, pegylated wildtype G-CSF (PEG-WT G-CSF), 130a1 G-CSF, or PEG-130a1 G-CSF. Cells were cultured for 48 hours. The BrdU assay procedure followed the instruction manual for the BD PharmingenTM FITC BrdU Flow Kit (557891), with one exception: cells were also stained to detect cell surface expression of the G-CSFR ECD (as described in Example 35). Flow cytometry was performed using a Cytek Aurora instrument. BrdU incorporation is shown for T cells positive for expression of G-CSFR.

[00698] In Figure 94B, a similar experiment was performed using the human myeloid cell line OCI-AML-1 which naturally expresses the wildtype human G-CSF receptor and proliferates in response to wildtype G-CSF (See Example 34). Cells were washed twice in PBS and once in medium and then re-plated in medium containing the indicated cytokines: human GM-CSF (20 ng/ml, positive control), medium alone (negative control), or the indicated concentrations (in ng/ml) of wildtype G-CSF, PEG-WT G-CSF, 130a1 G-CSF, PEG-130a1 G-CSF, 307 G-CSF, or PEG-307 G-CSF. Cells were cultured for 48 hours and assessed for BrdU incorporation by flow cytometry.

Results

[00699] As shown in Figure 94A, human T cells expressing G2R-3 134 ECD showed a similar proliferative response to pegylated versus non-pegylated 130a1 G-CSF across a range of cytokine concentrations. These cells also showed a similar proliferative response to pegylated versus non-pegylated wildtype G-CSF; however, the response was significantly weaker than that seen with 130a1 versions of G-CSF.

[00700] As shown in Figure 94B, human OCI-AML1 myeloid cells showed a similar proliferative response to pegylated versus non-pegylated 130a1 G-CSF across a range of concentrations; the only noted difference was that slightly higher concentrations of pegylated 130a1 G-CSF were required to induce the same level of cell proliferation. OCI-AML1 cells showed a similar proliferative response to pegylated versus non-pegylated 307 G-CSF. Finally, OCI-AML1 cells showed a similar proliferative response to pegylated versus non-pegylated wildtype G-CSF; at lower concentrations, the response to pegylated G-CSF was greater than that to non-pegylated G-CSF. OCI-AML1 cells showed a much stronger response to wildtype versions of G-CSF than to 130a1 or 307 versions of G-CSF.

[00701] Thus, pegylation of 130a1 G-CSF had negligible effects on the ability to induce cell proliferation through the G2R-3 134 ECD receptor. Moreover, pegylation of 130a1 G-CSF or 307 G-CSF had negligible effects on the specificity of these cytokines for G2R-3 134 ECD versus the wildtype G-CSF receptor.

Example 57: Pegylated and non-pegylated versions of 130a1 G-CSF induce similar biochemical signaling events in T cells

[00702] An *in vitro* western blotting experiment was performed to compare pegylated versus non-pegylated 130a1 G-CSF for the ability to induce biochemical signaling events in human T cells expressing G2R-3 134 ECD.

Methods

[00703] Human PBMC-derived T cells expressing G2R-3 134 ECD were prepared similar to Example 39 and then stimulated for 20 min with medium alone; human IL-2 (Proleukin, 300 IU/ml); or non-pegylated or pegylated versions of 130a1 G-CSF ranging from 1 to 400 ng/ml. Similar to Example 39, cellular and nuclear extracts were prepared and subjected to western blotting using the antibodies indicated in Figure 95 and described in detail in prior examples.

Results

[00704] As shown in Figure 95, both the pegylated and non-pegylated versions of 130a1 G-CSF induced signaling events similar to IL-2, including phosphorylation of STAT5, STAT3, Erk-1/2, Akt and S6. Unlike IL-2, pegylated and non-pegylated 130a1 G-CSF induced phosphorylation of JAK2, which is expected based on their design. Pegylated 130a1 G-CSF appeared slightly less potent than non-pegylated 130a1 G-CSF, as evidenced by slightly diminished phosphorylation events at 1 ng/ml.

[00705] Thus, the pegylated and non-pegylated versions of 130a1 G-CSF induce similar biochemical signaling events in human T cells and with only minor differences in potency.

Example 58: In a murine mammary carcinoma model, treatment with pegylated G-CSF 130a1 selectively enhances the expansion and anti-tumor activity of tumor-specific T cells expressing G2R-3 134 ECD

[00706] The pegylated form of the variant cytokine 130a1 G-CSF was tested across three dosing schedules for the ability to enhance the expansion, phenotype and anti-tumor activity of T cells expressing G2R-3 134 ECD in a murine breast cancer model.

Methods

[00707] We used similar methods as described in Example 36. All mice received an equivalent dose (5 x 10^6) of Thy1.1+ OT-I cells transduced to express G2R-3 134 ECD. The antibody panel used for flow cytometry of blood samples was the same as in Example 59.

Results

[00708] The transduction efficiency was 79.8%, as assessed by the percentage of Thy1.1+ cells expressing human G-CSFR+ by flow cytometry on Day 0. Starting on the day of adoptive cell transfer (Day 0), mice were randomized to different cytokine treatment groups. In the "daily" group, mice were randomized to receive either vehicle, 130a1 G-CSF (10 μg/dose), or pegylated (PEG) 130a1 G-CSF (10 μg/dose) on the following schedule: every day for 14 days, followed by every second day for 14 days. In the "every three days" group, mice were randomized to receive either 130a1 G-CSF or pegylated 130a1 G-CSF every three days for 9 cycles. In the "weekly" group, mice were randomized to receive either 130a1 G-CSF or pegylated 130a1 G-CSF every seven days for four cycles.

[00709] In the vehicle-treated group, 1/4 mice experienced complete regression (CR) of their tumor and remained tumor-free until day 46, when the study was stopped for practical reasons (Figure 96A, B). In both the "daily" 130a1 G-CSF and PEG-130a1 G-CSF-treated groups, 4/4 mice experienced CR. In the "every three days" 130a1 G-CSF and PEG-130a1 G-CSF-treated groups, 3/4 and 4/4 mice experienced CR, respectively (Figure 96C,D). In the "weekly" 130a1 G-CSF and PEG-130a1 G-CSF-treated groups, 3/3 and 4/4 mice experienced CR, respectively (Figure 96E,F).

[00710] The expansion and persistence of Thy1.1+ OT-I cells were monitored by flow cytometry in serial blood samples collected from mice (Figure 97A-C). In the "daily" cohort, on Day 4 the Thy1.1+ OT-I cells reached a mean peak level of 8.85%, 52.75% and 43.63% (relative to all CD8+ T cells) for the vehicle, 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97A). In the "every three days" cohort, on Day 4 the Thy1.1+ OT-I cells reached a mean peak level of 8.41% and 31.13% for the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97B). In the "weekly" cohort, on Day 4 the Thy1.1+ OT-I cells reached a mean peak level of 13.63% and 41.93% for the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97C).

[00711] The T effector memory (Tem, CD44+ CD62L-) phenotype of Thy1.1+ OT-I cells was also monitored by flow cytometry in serial blood samples (Figure 97D-F). In the "daily" cohort, on Day 4 the mean percentage of Thy1.1+ OT-I cells exhibiting a Tem phenotype was 24.50%, 49.35% and 74.40% for the vehicle, 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97D). In the "every three days" cohort, on Day 4 the mean percentage of Thy1.1+ OT-I cells exhibiting a Tem phenotype was 32.03% and 71.53% for the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97E). In the "weekly" cohort, on Day 4 the mean percentage of Thy1.1+ OT-I cells exhibiting a Tem phenotype was 32.33% and 70.58% for the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups, respectively (Figure 97F).

[00712] Expression of the Programmed Death-1 (PD-1) molecule was also assessed by flow cytometry in serial blood samples. No significant differences were observed in the percentage of PD-1+ OT-I T cells between the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups at any time point across any of the three dosing schedules (data not shown).

[00713] Expression of the T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) molecule was also assessed by flow cytometry in serial blood samples. No significant differences were observed in the percentage of TIM-3+ OT-I T cells between the 130a1 G-CSF and PEG-130a1 G-CSF treatment groups at any time point across any of the three dosing schedules (data not shown).

[00714] Figure 98 shows flow cytometry-based assessment of host neutrophils, monocytes, eosinophils, CD3+ T cells, CD19+ B cells and NK1.1+ Natural Killer cells from serial blood samples from the "every three days" dosing cohort. No substantial differences were observed in the percentage of any of these cell types between the vehicle, 130a1 G-CSF or PEG-130a1 G-CSF treatment groups at any time point.

[00715] Throughout the study, all mice in all treatment groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00716] Thus, PEG-130a1 G-CSF appears to be at least equivalent to 130a1 G-CSF in terms of anti-tumor efficacy in this model. Compared to 130a1 G-CSF, PEG-130a1 G-CSF induced substantially greater OT-I T cell expansion with the "every three days" and weekly dosing schedules. With all three dosing schedules, PEG-130a1 G-CSF induced a larger proportion of

OT-I T cells to adopt a Tem phenotype compared to 130a1 G-CSF. There were no overt toxicities associated with treatment with either form of 130a1 G-CSF.

Example 59: *In vitro* demonstration of a Controlled Paracrine Signaling strategy that emulates a combination of IL-2, IL-12 and IL-18 signals in human T cells

[00717] Human CD4 and CD8 T cells from healthy donor PBMCs were transduced with a bi-cistronic vector encoding a mesothelin-specific CAR and G12/2R, or a tri-cistronic vector encoding a mesothelin-specific CAR, G12/2R 134 ECD and human IL-18. T cells were assessed *in vitro* for expression of the CAR and G12/2R-1 134 ECD; for proliferation, and for IL-18 production.

Methods

[00718] Using methods similar to Example 35, human CD4 and CD8 T cells from healthy donor PBMC were transduced with a tri-cistronic lentiviral vector encoding (in order from 5' to 3') a mesothelin-specific CAR, T2A ribosomal skip site, G12/2R-1 134 ECD, T2A ribosomal skip site, and the mature form of human IL-18 (SEQ ID NO: 102). Controls included non-transduced T cells and T cells transduced with a bi-cistronic lentiviral vector encoding a mesothelin-specific CAR, T2A ribosomal skip site, and G12/2R 134 ECD. Non-transduced T cells were expanded for 12 days in IL-2; transduced T cells were expanded in 130a1 G-CSF. After expansion, T cells were subjected to flow cytometry with an antibody specific for G-CSFR ECD (APC-conjugated) and with recombinant Fc-tagged mesothelin protein (FITC-conjugated).

[00719] T cell proliferation was assessed by BrdU incorporation assay as per Example 60 with the following cytokines: human IL-2 (Proleukin, 300 IU/ml); human IL-18 (100 ng/ml); human IL-2 + human IL-12 (100 ng/ml); IL-2 + IL-12 + IL-18; or 130a1 G-CSF (100 ng/ml).

[00720] Human IL-18 production was detected by ELISA. T cells were plated at $1x10^6$/ml and cultured in media containing medium alone; human IL-2 (Proleukin, 300 IU/ml) + human IL-12 (20 ng/ml); or 130a1 G-CSF (100 ng/ml). Supernatants were collected after 48h and subjected to ELISA (R&D Systems, Human Total IL-18 DuoSet ELISA kit, cat. #DY318-05 used with DuoSet ELISA Ancillary Reagent kit, cat. #DY008) according to the manufacturer's instructions. Absolute IL-18 concentrations were determined using a standard curve provided with the kit.

Results

[00721] As shown in Figure 99A, co-expression of the mesothelin CAR and G12/2R 134 ECD was seen in 38.18% of T cells transduced with the bi-cistronic vector and 78.40% of T cells transduced with the tri-cistronic vector. As expected, neither receptor was detected in non-transduced T cells.

[00722] As shown in Figure 99B, T cells transduced with either the bi-cistronic or tri-cistronic vectors proliferated in response to IL-2; IL-2 + IL-12; IL-2 + IL-12 + IL-18; or 130a1 G-CSF; no proliferation was seen in response to medium or IL-18 alone. Similar results were seen for both the CD4+ and CD8+ subsets of T cells (data not shown).

[00723] As shown in Figure 99C, human IL-18 was produced by T cells expressing the tri-cistronic vector and stimulated with 130a1 G-CSF. Negligible levels of IL-18 were detected in the other conditions.

[00724] Thus, in human T cells transduced with the vector encoding G12/2R 134 ECD and human IL-18 (along with a mesothelin-specific CAR in this example), T cell proliferation was induced in response to 130a1 G-CSF, as well as various IL-2-containing cytokine combinations. Expression of IL-18 was induced in response to 130a1 G-CSF.

Example 60: *In vitro* demonstration of an IL-18-based Controlled Paracrine Signaling system in murine T cells

[00725] Murine CD4 and CD8 T cells were transduced with a retroviral vector encoding G12/2R 134 ECD or G12/2R 134 ECD plus the mature form of murine IL-18 (m18). Expression of transduced receptors was assessed by flow cytometry. T cells were assessed for proliferation and production of murine IL-18.

Methods

[00726] Using methods similar to Example 36, bulk CD4 and CD8 T cells were isolated from a healthy donor mouse (C57Bl/6J), activated with soluble anti-CD3 and anti-CD28, and expanded for 4 days in the presence of human IL-2 (300 IU/ml). Cells were transduced with a retroviral vector encoding G12/2R-1 134 ECD or G12/2R-1 134 ECD + m18. Mock-transduced T cells underwent the transduction protocol but without retrovirus. Following expansion, cells were washed and re-plated in the following cytokine conditions: medium alone; human IL-2 (Proleukin, 300 IU/ml); murine IL-18 (100 ng/ml); IL-2 + mIL-12 (10 ng/ml); IL-2 + IL-12

+18; or 130a1 G-CSF (100ng/ml). T cell proliferation was assessed by BrdU incorporation 48 hours later. To assess IL-18 production, transduced T cells were washed and plated at a density of 2 x 10^6 cells/ml for 48 hours in medium alone; human IL-2 (300 IU/ml) + murine IL-12 (10 ng/ml); or 130a1 G-CSF (100ng/ml). Murine IL-18 was detected in cell culture supernatants by ELISA (R&D Systems, Mouse IL-18 ELISA kit, Cat. #7625).

Results

[00727] As shown in Figure 100A, expression of the human G-CSFR ECD was detected on all transduced T cell populations, indicating successful transduction. Expression of G12/2R 134 ECD was lower in the context of the IL-18 CPS compared to on its own; this appears to reflect a technical problem with this retroviral vector, as this issue was not seen in human T cells transduced with the equivalent lentiviral vector (see Example 57).

[00728] As shown in Figure 100B, T cells transduced with G12/2R 134 ECD or G12/2R 134 ECD + m18 proliferated in response to IL-2, IL-2 + IL-12, IL-2 + IL-12 + IL-18, or 130a1 G-CSF.

[00729] As shown in Figure 100C, murine IL-18 was produced by T cells transduced with G12/2R 134 ECD + m18 and stimulated with IL-2 + IL-12 or 130a1 G-CSF.

[00730] Thus, in murine T cells transduced with G12/2R 134 ECD + m18, stimulation with 130a1 G-CSF induces proliferation and murine IL-18 secretion.

Example 61: *In vitro* cytokine secretion profiles of murine T cells stimulated through engineered receptors and IL-18 CPS

[00731] OT-I T cells were transduced with retroviral vectors encoding G2R-2, G2R-3, G12/2R-1, or G12/2R-1 + m18 (all with 134 ECD). Expression of transduced receptors was assessed by flow cytometry. Expression of IL-18 and 32 other cytokines was assessed with a multiplexed bead-based assay.

Methods

[00732] Using methods similar to Example 36, OT-I T cells from a healthy donor mouse were transduced with retroviral vectors encoding G2R-2, G2R-3, G12/2R-1 or G12/2R-1+ m18 (all with 134 ECD). Five days later, T cells were subjected to flow cytometry with an antibody specific for G-CSFR ECD. To detect secreted cytokines, T cells were washed three times with

PBS and cultured in 48-well plates at $2x10^6$ cells/ml in tissue culture medium supplemented with either nothing (media alone, negative control), human IL-2 (Proleukin 300 IU/ml), human IL-2 + murine IL-12 (10 ng/ml), or PEG-130a1 G-CSF (100 ng/ml). After 48 hours, culture supernatants were harvested and frozen at -80 °C. Supernatants were shipped to Eve Technologies (Calgary, Alberta, Canada) and subjected to a bead-based multiplex cytokine assay (Mouse Cytokine/Chemokine 31-Plex Discovery Assay® Array, MD31) and an IL-18 detection assay (Mouse IL-18 Single Plex Discovery Assay® Array, MDIL18).

Results

[00733] As shown in Figure 100A, expression of the human G-CSFR ECD was detected on all transduced T cell populations, indicating successful transduction. Expression of G12/2R 134 ECD was lower in the context of the IL-18 CPS compared to on its own; this appears to reflect a technical problem with this retroviral vector, as this issue was not seen in human T cells transduced with the equivalent lentiviral vector (see Example 57).

[00734] The upper panel of Figure 101 shows results for murine IL-18 production. Only T cells expressing G12/2R 134 ECD + m18 produced detectable levels of murine IL-18. Expression of murine IL-18 by these cells was increased by stimulation with IL-2 + IL-12 or PEG-130a1 G-CSF.

[00735] As shown in Table 35, expression of the following cytokines was induced by IL-2 or IL-2 + IL-12: GM-CSF, TNF-alpha, IL-10 and VEGF. IL-2 + IL-12 additionally induced expression of IFN-gamma (Table 35 and lower panel of Figure 101). In T cells expressing G12/2R 134 ECD + m18, IL-2 + IL-12 additionally induced expression of IP-10 and increased expression of IFN-gamma, TNF-alpha and GM-CSF (Table 35).

[00736] In T cells expressing G2R-2 134 ECD, G2R-3 134 ECD or G12/2R-1 134 ECD (without IL-18 CPS), PEG-130a1 G-CSF induced expression of the following cytokines: GM-CSF, TNF-alpha, IL-10, and VEGF. In T cells expressing G12/2R-1 134 ECD + m18, PEG-130a1 G-CSF additionally induced expression of IFN-gamma and IP-10 and increased expression of GM-CSF, TNF-alpha and IL-10 (Table 35).

[00737] Thus, in response to PEG-130a1 G-CSF, G2R-2 and G2R-3 induced cytokine secretion patterns resembling those induced by IL-2. In cells expressing G2/12R 134 ECD + m18, stimulation with IL-2 + IL-12 or PEG-130a1 G-CSF induced increased production of IL-

18, and stimulation with PEG-130a1 G-CSF induced cytokine secretion patterns resembling those induced by IL-2 + IL-12.

Example 62: A Controlled Paracrine Signaling strategy emulating a combination of IL-2, IL-12 and IL-18 signals leads to enhanced anti-tumor activity in a murine mammary carcinoma model

[00738] Using the NOP 23 mammary tumor model, OT-I T cells engineered with a retroviral vector encoding G12/2R-1 134 ECD + m18 were assessed for *in vivo* expansion, effector phenotype, and anti-tumor activity. For comparison, other groups of mice received OT-I T cells expressing G7R-1, G2R-2 or G12/2R-1 (all with 134 ECD).

[00739] Methods

[00740] We used similar methods as described in Example 36. Thy1.1+ OT-I cells were retrovirally transduced to express G7R-1, G2R-2, G12/2R-1 or G12/2R-1 + m18 (all with 134 ECD). The antibody panel used for flow cytometry of blood samples was similar to that in Example 59.

[00741] Results

[00742] As shown in Figure 102A-D, on the day of adoptive cell transfer (Day 0), transduced OT-I T cells expressed G7R, G2R-2 or G12/2R-1 (all with 134 ECD), as detected using an antibody against human G-CSFR. Expression of G12/2R-1 134 ECD was lower in the context of the IL-18 CPS compared to on its own; this appears to reflect a technical problem with this retroviral vector, as this issue was not seen in human T cells transduced with the equivalent lentiviral vector (see Example 59).

[00743] All mice received an equivalent dose (2.5×10^6) of transduced Thy1.1+ OT-I cells. After adoptive cell transfer (Day 0), mice were randomized to receive either vehicle or PEG-130a1 G-CSF (10 μg/dose) every seven days for four cycles.

[00744] In the G7R-1 cohort, 2/4 mice receiving vehicle alone experienced complete regression (CR) of their tumor compared to 2/4 4 mice in the PEG-130a1 G-CSF treatment group (Figure 102E). In the G2R-2 cohort, 1/5 mice receiving vehicle alone experienced complete regression (CR) of their tumor compared to 3/4 mice in the PEG-130a1 G-CSF treatment group (Figure 102F). In the G12/2R-1 cohort, 1/4 mice receiving vehicle alone experienced CR compared to 3/4 mice in the PEG-130a1 G-CSF treatment group, although one of these latter animals experienced tumor recurrence after about 35 days (Figure 102G). In the G12/2R-1 + m18 cohort, 3/4 mice receiving vehicle alone experienced CR compared to

4/4 mice in the PEG-130a1 G-CSF treatment group (Figure 102H). In all cohorts, mice experiencing durable CR remained tumor-free until day 53, when the study was stopped for practical reasons.

[00745] The expansion and persistence of Thy1.1+ OT-I cells were monitored by flow cytometry in serial blood samples collected from mice. In the G7R cohort, on Day 4 the Thy1.1+ OT-I cells reached a mean peak level of 1.99% and 15.31% (relative to all CD8+ T cells) for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102I). In the G2R-2 cohort, on Day 4 the Thy1.1+ OT-I cells reached a mean peak level of 1.21% and 13.78% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102J). In the G12/2R-1 cohort, the Thy1.1+ OT-I cells reached a mean peak level of 2.18% and 11.63% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102K). In the G12/2R-1 + m18 cohort, the Thy1.1+ OT-I cells reached a mean peak level of 2.35% and 6.21% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102L).

[00746] The T effector memory (Tem, CD44+ CD62L-) phenotype of Thy1.1+ OT-I cells was also monitored by flow cytometry in serial blood samples. In the G7R cohort, on Day 4 the mean percentage of Thy1.1+ OT-I cells exhibiting a Tem phenotype was 17.75% and 34.65% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102M). In the G2R-2 cohort, these values were 15.12% and 57.85% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102N). In the G12/2R-1 cohort, these values were 14.78% and 48.80% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102O). In the G12/2R/18C cohort, these values were 42.03% and 57.55% for the vehicle and PEG-130a1 G-CSF treatment groups, respectively (Figure 102P).

[00747] Throughout the study, all mice in all treatment groups maintained a normal body weight and exhibited good general health, despite tumor progression in some.

[00748] Thus, the extent of OT-I *in vivo* expansion and effector differentiation in response to pegylated 130a1 G-CSF is influenced by the receptor intracellular domain and CPS cytokine. G12/2R-1 + m18 showed the strongest anti-tumor efficacy in this model.

Example 63: *In vivo* dose-response characteristics of PEG-130a1 G-CSF compared to IL-2 in a murine mammary carcinoma model

[00749] Using the NOP23 mammary tumor model, OT-I T cells expressing G2R-3 134 ECD were assessed for *in vivo* expansion and effector phenotype in response to different doses of

PEG-130a1 G-CSF or human IL-2 (Proleukin). The effects of these cytokines on neutrophil and eosinophil counts in peripheral blood were also assessed.

Methods

[00750] We used similar methods as described in Example 36. Thy1.1+ OT-I cells were retrovirally transduced to express G2R-3 134 ECD. All mice received a dose of 2.5 x 10^6 OT-I T cells. On the day of adoptive cell transfer (Day 0), mice were randomized to six cytokine treatment groups: (1) vehicle alone; (2) PEG-130a1 G-CSF 2 μg/dose, four weekly doses; (3) PEG-130a1 G-CSF 0.4 μg/dose, four weekly doses; (4) PEG-130a1 G-CSF 0.08 μg/dose, four weekly doses; (5) PEG-130a1 G-CSF four daily doses of 0.1 μg followed by three weekly doses of 0.4 μg; or (6) human IL-2 (Proleukin) at 30,000 IU/day for 14 days followed by 30,000 IU every two days for 14 days. The antibody panel used for flow cytometry of blood samples was similar to that used in Example 55.

Results

[00751] The expansion and persistence of Thy1.1+ OT-I cells were monitored by flow cytometry in serial blood samples collected from mice. Figure 103A-E shows mean peak percentages of Thy1.1+ OT-I cells (relative to all CD8+ T cells) on Day 4 across the different cytokine treatment groups: vehicle alone group = 0.70% (shown in each panel); (A) 2 μg PEG-130a1 G-CSF group = 9.38%; (B) 0.4 μg PEG-130a1 G-CSF group = 4.09%; (C) 0.08 μg PEG-130a1 G-CSF group = 1.50%; (D) 0.1 then 0.4 μg PEG-130a1 G-CSF group = 18.80%; (E) human IL-2 group = 4.56%.

[00752] The T effector memory (Tem, CD44+ CD62L-) phenotype of Thy1.1+ OT-I cells was also monitored by flow cytometry in serial blood samples. Figure 103F-J shows the mean percentage of Thy1.1+ OT-I cells exhibiting a Tem phenotype on Day 4: vehicle group = 27.05% (shown in each panel); (A) 2 μg PEG-130a1 G-CSF group = 66.38%; (B) 0.4 μg PEG-130a1 G-CSF group = 74.80%; (C) 0.08 μg PEG-130a1 G-CSF group = 33.03%; (D) 0.1 then 0.4 μg PEG-130a1 G-CSF group = 74.90%; (E) human IL-2 group = 64.30%.

[00753] Across time points, all cytokine treatment groups showed similar levels of neutrophils compared to the vehicle group (Figure 103K-O).

[00754] Across time points, all cytokine treatment groups showed similar levels of eosinophils compared to the vehicle group (Figure 103P-S), with the exception of the IL-2-treated group, which showed an increase in eosinophils on Days 8 and 12 (Figure 103T).

[00755] Throughout the study, all mice in all treatment groups maintained a normal body weight and exhibited good general health with one exception: one mouse in the IL-2-treated group died on Day 19, possibly due to IL-2-associated toxicity.

[00756] Thus, the extent of OT-I T cell *in vivo* expansion and effector differentiation in peripheral blood is influenced by the dose of pegylated 130a1 G-CSF. Pegylated 130a1 G-CSF and IL-2 induced similar OT-I T cell expansion and differentiation profiles in blood. At the assessed doses, neither cytokine had a significant impact on neutrophil levels in blood. IL-2 uniquely induced eosinophilia and may have caused one treatment-related death.

[00757]

[00758] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[00759] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

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TABLE 12: SEQUENCE OF G-CSFR$_{WT}$-ICD$_{GP130-IL-2R\beta}$

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
G-CSFR (Signal peptide, ECD)	Q99062	1-627	MARLGNCSLTWAALIILLLPGSLEECGHISVS APIVHLGDPITASCIIKQNCSHLDPEPQILWRL GAELQPGGRQQRLSDGTQESIITLPHLNHTQA FLSCCLNWGNSLQILDQVELRAGYPPAIPHNL SCLMNLTTSSLICQWEPGPETHLPTSFTLKSF KSRGNCQTQGDSILDCVPKDGQSHCCIPRKH LLLYQNMGIWVQAENALGTSMSPQLCLDPM DVVKLEPPMLRTMDPSPEAAPPQAGCLQLC WEPWQPGLHINQKCELRHKPQRGEASWALV GPLPLEALQYELCGLLPATAYTLQIRCIRWPL PGHWSDWSPSLELRTTERAPTVRLDTWWRQ RQLDPRTVQLFWKPVPLEEDSGRIQGYVVSW RPSGQAGAILPLCNTTELSCTFHLPSEAQEVA LVAYNSAGTSRPTPVVFSESRGPALTRLHAM ARDPHSLWVGWEPPNPWPQGYVIEWGLGPP SASNSNKTWRMEQNGRATGFLLKENIRPFQL YEIIVTPLYQDTMGPSQHVYAYSQEMAPSHA PELHLKHIGKTWAQLEWVPEPPELGKSPLTH YTIFWTNAQNQSFSAILNASSRGFVLHGLEPA SLYHIHLMAASQAGATNSTVLTLMTLTPEGS ELH	3
gp130 (TM, ICD)	P40189	628-751	AIVVPVCLAFLLTTLLGVLFCFNKRDLIKKHI WPNVPDPSKSHIAQWSPHTPPRHNFNSKDQM YSDGNFTDVSVVEIEANDKKPFPEDLKSLDLF KKEKINTEGHSSGIGGSSCMSSSRPSIS	4
IL-2RβICD)	P14784	752-857	ASLSSNHSLTSCFTNQGYFFFHLPDALEIEAC QVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQ PLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPS TAPGGSGAGEERMPPSLQERVPRDWDPQPLG PPTPGVPDLVDFQPPPELVLREAGEEVPDAGP	5

			REGVSFPWSRPPGQGEFRALNARLPLNTDAY LSLQELQGQDPTHLV	

TABLE 13: SEQUENCE OF G-CSFR$_{WT}$-ICD$_{IL-2R\beta}$

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
G-CSFR (Signal peptide, ECD)	Q99062	1-627	MARLGNCSLTWAALIILLLPGSLEECGHISVS APIVHLGDPITASCIIKQNCSHLDPEPQILWRL GAELQPGGRQQRLSDGTQESIITLPHLNHTQA FLSCCLNWGNSLQILDQVELRAGYPPAIPHNL SCLMNLTTSSLICQWEPGPETHLPTSFTLKSF KSRGNCQTQGDSILDCVPKDGQSHCCIPRKH LLLYQNMGIWVQAENALGTSMSPQLCLDPM DVVKLEPPMLRTMDPSPEAAPPQAGCLQLC WEPWQPGLHINQKCELRHKPQRGEASWALV GPLPLEALQYELCGLLPATAYTLQIRCIRWPL PGHWSDWSPSLELRTTERAPTVRLDTWWRQ RQLDPRTVQLFWKPVPLEEDSGRIQGYVVSW RPSGQAGAILPLCNTTELSCTFHLPSEAQEVA LVAYNSAGTSRPTPVVFSESRGPALTRLHAM ARDPHSLWVGWEPPNPWPQGYVIEWGLGPP SASNSNKTWRMEQNGRATGFLLKENIRPFQL YEIIVTPLYQDTMGPSQHVYAYSQEMAPSHA PELHLKHIGKTWAQLEWVPEPPELGKSPLTH YTIFWTNAQNQSFSAILNASSRGFVLHGLEPA SLYHIHLMAASQAGATNSTVLTLMTLTPEGS ELH	6
IL-2Rβ (TM and ICD)	P14784	628-956	AIVVPVCLAFLLTTLLGVLFCFNKRDLIKKHI WPNVPDPSKSHIAQWSPHTPPRHNFNSKDQM YSDGNFTDVSVVEIEANDKKPFPEDLKSLDLF KKEKINTEGHSSGIGGSSCMSSSRPSISASLSS NHSLTSCFTNQGYFFFHLPDALEIEACQVYFT YDPYSEEDPDEGVAGAPTGSSPQPLQPLSGED	7

			DAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGS GAGEERMPPSLQERVPRDWDPQPLGPPTPGV PDLVDFQPPPELVLREAGEEVPDAGPREGVSF PWSRPPGQGEFRALNARLPLNTDAYLSLQEL QGQDPTHLV	

TABLE 14: SEQUENCE OF G-CSFR$_{WT}$-ICD$_{\gamma C}$

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
G-CSFR (Signal peptide, ECD)	Q99062	1-627	MARLGNCSLTWAALIILLLPGSLEECGHISVS APIVHLGDPITASCIIKQNCSHLDPEPQILWRL GAELQPGGRQQRLSDGTQESIITLPHLNHTQA FLSCCLNWGNSLQILDQVELRAGYPPAIPHNL SCLMNLTTSSLICQWEPGPETHLPTSFTLKSF KSRGNCQTQGDSILDCVPKDGQSHCCIPRKH LLLYQNMGIWVQAENALGTSMSPQLCLDPM DVVKLEPPMLRTMDPSPEAAPPQAGCLQLC WEPWQPGLHINQKCELRHKPQRGEASWALV GPLPLEALQYELCGLLPATAYTLQIRCIRWPL PGHWSDWSPSLELRTTERAPTVRLDTWWRQ RQLDPRTVQLFWKPVPLEEDSGRIQGYVVSW RPSGQAGAILPLCNTTELSCTFHLPSEAQEVA LVAYNSAGTSRPTPVVFSESRGPALTRLHAM ARDPHSLWVGWEPPNPWPQGYVIEWGLGPP SASNSNKTWRMEQNGRATGFLLKENIRPFQL YEIIVTPLYQDTMGPSQHVYAYSQEMAPSHA PELHLKHIGKTWAQLEWVPEPPELGKSPLTH YTIFWTNAQNQSFSAILNASSRGFVLHGLEPA SLYHIHLMAASQAGATNSTVLTLMTLTPEGS ELH	8
IL-2Rγ (TM and ICD)	P31785	628-735	VVISVGSMGLIISLLCVYFWLERTMPRIPTLK NLEDLVTEYHGNFSAWSGVSKGLAESLQPD YSERLCLVSEIPPKGGALGEGPGASPCNQHSP YWAPPCYTLKPET	9

TABLE 15A: CHIMERIC CYTOKINE RECEPTORS

Name	ECD	TMD	Box1/2	ICD	STAT site(s)	Other site(s)
G2R-1	G-CSFR	IL-2Rβ + γc	IL-2Rβ + γc	IL-2Rβ + γc	5, 5	Shc
G2R-2	G-CSFR	gp130	gp130	IL-2Rβ	5, 5	Shc
G2R-3	G-CSFR	G-CSFR	G-CSFR	IL-2Rβ	5, 5	Shc
G21R-1	G-CSFR	G-CSFR	G-CSFR	IL-21R	3	
G21R-2	G-CSFR	G-CSFR	G-CSFR	IL-21R	3	unknown
G12R-1	G-CSFR	G-CSFR	G-CSFR	IL-12Rβ_2	4	

TABLE 15B: CHIMERIC CYTOKINE RECEPTORS

Name	ECD	TMD	Box1/2	ICD	STAT site(s)	Other site(s)
G21/2R-1	G-CSFR	G-CSFR	G-CSFR	G-CSFR, IL-2Rβ	3, 5, 5	Shc
G12/2R-1	G-CSFR	G-CSFR	G-CSFR	STAT4 site from IL-12Rβ_2 replaces STAT5 site from IL-2Rβ	4, 5	Shc
G21/12/2R-1	G-CSFR	G-CSFR	G-CSFR	Contains STAT3 site from G-CSFR ICD; STAT4 site from IL-12Rβ_2 replaces STAT5 site from IL-2Rβ	3, 4, 5	Shc
G27/2R-1	G-CSFR	gp130	gp130	gp130 + IL-2Rβ	3, 5, 5	SHP-2, Shc
G7R-1	G-CSFR	G-CSFR	G-CSFR	IL-7Rα	5	PI3K

TABLE 16: BOX 1 AND OTHER BINDING SITES ON CYTOKINE RECEPTOR ICDS

Native ICD	Box 1 (source)	SEQ ID NO:	Shc	SHP-2	STAT5	STAT4	STAT3	STAT1	PI3K
G-CSFR	LWPSVPDPA (Q99062)		n/a	n/a	n/a	n/a	Y704	n/a	n/a
gp130	IWPNVPDPS (P40189)		n/a	Y759	n/a	n/a	Y767	n/a	n/a
IL-2Rβ	LKCNTPDPS (P14784)		Y338	n/a	Y392, Y510	n/a	Y392, Y510	Y392, Y510	n/a
IL-21R	KIWAVPSPE (Q9HBE5)		n/a	n/a	n/a	n/a	Y510	Y510	n/a
IL-12Rβ_2	CSREIPDPA (Q99665)		n/a	n/a	Y800	Y800	Y800	n/a	n/a

IL-7Rα	VWPSLPDHK (P16871)		n/a	n/a	Y449	n/a	n/a	n/a	Y449

TABLE 17: SIGNAL PEPTIDES

Domain	**Uniprot ID**	**Polypeptide Sequence**	**SEQ ID NO.**
G-CSFR (Signal peptide)	Q99062	MARLGNCSLTWAALIILLLPGSLE	11
GM-CSFR-alpha (Signal peptide)	P15509	MLLLVTSLLLCELPHPAFLLIP	12
Domain	**Genbank Accession ID**	**Nucleic Acid Sequence**	
G-CSFR (Signal peptide, native sequence)	NP_000751	ATGGCAAGGCTGGGAAACTGCAGCCTGACTTGGGCTGCCCTGATC ATCCTGCTGCTCCCCGGAAGTCTGGAG	13
GM-CSFR-alpha (Signal peptide, native sequence)	NP_034100	ATGCTGCTGCTAGTGACCTCCCTGCTGCTCTGTGAGCTGCCTCACCC GGCGTTCCTGCTGATTCCT	14

TABLE 18: WILD-TYPE G-CSFR EXTRACELLULAR DOMAINS (ECD):

Domain	**Uniprot ID**	**Polypeptide Sequence**	**SEQ ID NO.**
G-CSFR (ECD)	Q99062	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILWRLGAELQPGG RQQRLSDGTQESIITLPHLNHTQAFLSCCLNWGNSLQILDQVELRAGY PPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKSRGNCQTQ GDSILDCVPKDGQSHCCIPRKHLLLYQNMGIWVQAENALGTSMSPQL CLDPMDVVKLEPPMLRTMDPSPEAAPPQAGCLQLCWEPWQPGLHI NQKCELRHKPQRGEASWALVGPLPLEALQYELCGLLPATAYTLQIRCIR WPLPGHWSDWSPSLELRTTERAPTVRLDTWWRQRQLDPRTVQLFW KPVPLEEDSGRIQGYVVSWRPSGQAGAILPLCNTTELSCTFHLPSEAQE VALVAYNSAGTSRPTPVVFSESRGPALTRLHAMARDPHSLWVGWEP PNPWPQGYVIEWGLGPPSASNSNKTWRMEQNGRATGFLLKENIRPF QLYEIIVTPLYQDTMGPSQHVYAYSQEMAPSHAPELHLKHIGKTWAQ LEWVPEPPELGKSPLTHYTIFWTNAQNQSFSAILNASSRGFVLHGLEP ASLYHIHLMAASQAGATNSTVLTLMTLTPEGSELH	15
Domain	**Genbank Accession ID**	**Nucleic Acid Sequence**	

G-CSFR (ECD, native sequence)	NP_000751	GAGTGCGGGCACATCAGTGTCTCAGCCCCCATCGTCCACCTGGGG GATCCCATCACAGCCTCCTGCATCATCAAGCAGAACTGCAGCCATC TGGACCCGGAGCCACAGATTCTGTGGAGACTGGGAGCAGAGCTTC AGCCCGGGGGCAGGCAGCAGCGTCTGTCTGATGGGACCCAGGAA TCTATCATCACCCTGCCCCACCTCAACCACACTCAGGCCTTTCTCTCC TGCTGCCTGAACTGGGGCAACAGCCTGCAGATCCTGGACCAGGTT GAGCTGCGCGCAGGCTACCCTCCAGCCATACCCCACAACCTCTCCT GCCTCATGAACCTCACAACCAGCAGCCTCATCTGCCAGTGGGAGCC AGGACCTGAGACCCACCTACCCACCAGCTTCACTCTGAAGAGTTTC AAGAGCCGGGGCAACTGTCAGACCCAAGGGGACTCCATCCTGGAC TGCGTGCCCAAGGACGGGCAGAGCCACTGCTGCATCCCACGCAAA CACCTGCTGTTGTACCAGAATATGGGCATCTGGGTGCAGGCAGAG AATGCGCTGGGGACCAGCATGTCCCCACAACTGTGTCTTGATCCCA TGGATGTTGTGAAACTGGAGCCCCCCATGCTGCGGACCATGGACC CCAGCCCTGAAGCGGCCCCTCCCCAGGCAGGCTGCCTACAGCTGT GCTGGGAGCCATGGCAGCCAGGCCTGCACATAAATCAGAAGTGTG AGCTGCGCCACAAGCCGCAGCGTGGAGAAGCCAGCTGGGCACTG GTGGGCCCCCTCCCCTTGGAGGCCCTTCAGTATGAGCTCTGCGGGC TCCTCCCAGCCACGGCCTACACCCTGCAGATACGCTGCATCCGCTG GCCCCTGCCTGGCCACTGGAGCGACTGGAGCCCCAGCCTGGAGCT GAGAACTACCGAACGGGCCCCCACTGTCAGACTGGACACATGGTG GCGGCAGAGGCAGCTGGACCCCAGGACAGTGCAGCTGTTCTGGA AGCCAGTGCCCCTGGAGGAAGACAGCGGACGGATCCAAGGTTAT GTGGTTTCTTGGAGACCCTCAGGCCAGGCTGGGGCCATCCTGCCCC TCTGCAACACCACAGAGCTCAGCTGCACCTTCCACCTGCCTTCAGA AGCCCAGGAGGTGGCCCTTGTGGCCTATAACTCAGCCGGGACCTC TCGTCCCACTCCGGTGGTCTTCTCAGAAAGCAGAGGCCCAGCTCTG ACCAGACTCCATGCCATGGCCCGAGACCCTCACAGCCTCTGGGTAG GCTGGGAGCCCCCCAATCCATGGCCTCAGGGCTATGTGATTGAGT GGGGCCTGGGCCCCCCCAGCGCGAGCAATAGCAACAAGACCTGG AGGATGGAACAGAATGGGAGAGCCACGGGGTTTCTGCTGAAGGA GAACATCAGGCCCTTTCAGCTCTATGAGATCATCGTGACTCCCTTGT ACCAGGACACCATGGGACCCTCCCAGCATGTCTATGCCTACTCTCA AGAAATGGCTCCCTCCCATGCCCCAGAGCTGCATCTAAAGCACATT GGCAAGACCTGGGCACAGCTGGAGTGGGTGCCTGAGCCCCCTGA GCTGGGGAAGAGCCCCCTTACCCACTACACCATCTTCTGGACCAAC GCTCAGAACCAGTCCTTCTCCGCCATCCTGAATGCCTCCTCCCGTGG CTTTGTCCTCCATGGCCTGGAGCCCGCCAGTCTGTATCACATCCACC TCATGGCTGCCAGCCAGGCTGGGGCCACCAACAGTACAGTCCTCA CCCTGATGACCTTGACCCCAGAGGGGTCGGAGCTACAC	16
G-CSFR (ECD, 4 codons altered)		GAGTGCGGGCACATCAGTGTCTCAGCCCCCATCGTCCACCTGGGG GATCCCATCACAGCCTCCTGCATCATCAAGCAGAACTGCAGCCATC TGGACCCGGAGCCACAGATTCTGTGGAGACTGGGAGCAGAGCTTC AGCCCGGGGGCAGGCAGCAGCGTCTGTCTGATGGGACCCAGGAA TCTATCATCACCCTGCCCCACCTCAACCACACTCAGGCCTTTCTCTCC TGCTGCCTGAACTGGGGCAACAGCCTGCAGATCCTGGACCAGGTT GAGCTGCGCGCAGGCTACCCTCCAGCCATACCCCACAACCTCTCCT GCCTCATGAACCTCACAACCAGCAGCCTCATCTGCCAGTGGGAGCC	17

		AGGACCTGAGACCCACCTACCCACCAGCTTCACTCTGAAGAGTTTC AAGAGCCGGGGCAACTGTCAGACCCAAGGGGACTCCATCCTGGAC TGCGTGCCCAAGGACGGGCAGAGCCACTGCTGCATCCCACGCAAA CACCTGCTGTTGTACCAGAATATGGGCATCTGGGTGCAGGCAGAG AATGCGCTGGGGACCAGCATGTCCCCACAACTGTGTCTTGATCCCA TGGATGTTGTGAAACTGGAGCCCCCCATGCTGCGGACCATGGACC CCAGCCCTGAAGCGGCCCCTCCCCAGGCAGGCTGCCTACAGCTGT GCTGGGAGCCATGGCAGCCAGGCCTGCACATAAATCAGAAGTGTG AGCTGCGCCACAAGCCGCAGCGTGGAGAAGCCAGCTGGGCACTG GTGGGCCCCCTCCCCTTGGAGGCCCTTCAGTATGAGCTCTGCGGGC TCCTCCCAGCCACGGCCTACACCCTGCAGATACGCTGCATCCGCTG GCCCCTGCCTGGCCACTGGAGCGACTGGAGCCCCAGCCTGGAGCT GAGAACTACCGAACGGGCCCCCACTGTCAGACTGGACACATGGTG GCGGCAGAGGCAGCTGGACCCCAGGACAGTGCAGCTGTTCTGGA AGCCAGTGCCCCTGGAGGAAGACAGCGGACGCATCCAAGGTTATG TGGTTTCTTGGAGACCCTCAGGCCAGGCTGGGGCCATCCTGCCCCT CTGCAACACCACAGAGCTCAGCTGCACCTTCCACCTGCCTTCAGAA GCCCAGGAGGTGGCCCTTGTGGCCTATAACTCAGCCGGGACCTCTC GCCCCACCCCGGTGGTCTTCTCAGAAAGCAGAGGCCCAGCTCTGAC CAGACTCCATGCCATGGCCCGAGACCCTCACAGCCTCTGGGTAGGC TGGGAGCCCCCCAATCCATGGCCTCAGGGCTATGTGATTGAGTGG GGCCTGGGCCCCCCCAGCGCGAGCAATAGCAACAAGACCTGGAG GATGGAACAGAATGGGAGAGCCACGGGGTTTCTGCTGAAGGAGA ACATCAGGCCCTTTCAGCTCTATGAGATCATCGTGACTCCCTTGTAC CAGGACACCATGGGACCCTCCCAGCATGTCTATGCCTACTCTCAAG AAATGGCTCCCTCCCATGCCCCAGAGCTGCATCTAAAGCACATTGG CAAGACCTGGGCACAGCTGGAGTGGGTGCCTGAGCCCCCTGAGCT GGGGAAGAGCCCCCTTACCCACTACACCATCTTCTGGACCAACGCT CAGAACCAGTCCTTCTCCGCCATCCTGAATGCATCCTCCCGTGGCTT TGTCCTCCATGGCCTGGAGCCCGCCAGTCTGTATCACATCCACCTCA TGGCTGCCAGCCAGGCTGGGGCCACCAACAGTACAGTCCTCACCCT GATGACCTTGACCCCAGAGGGGTCGGAGCTACAC	

TABLE 19: TRANSMEMBRANE DOMAINS (TM)

Domain	Uniprot ID	Polypeptide Sequence	SEQ ID NO.
G-CSFR (TM)	Q99062	IILGLFGLLLLLTCLCGTAWLCC	18
gp130 (TM)	P40189	AIVVPVCLAFLLTTLLGVLFCF	19
IL-2Rb (TM)	P14784	IPWLGHLLVGLSGAFGFIILVYLLI	20
IL-2RG (TM)	P31785	VVISVGSMGLIISLLCVYFWL	21
Domain	**Genbank Accession ID**	**Nucleic Acid Sequence**	

G-CSFR (TM)	NP_000751	ATCATCCTGGGCCTGTTCGGCCTCCTGCTGTTGCTCAC CTGCCTCTGTGGAACTGCCTGGCTCTGTTGC	22
gp130 (TM)	NM_002184	GCCATAGTCGTGCCTGTTTGCTTAGCATTCCTATTGAC AACTCTTCTGGGAGTGCTGTTCTGCTTT	23
IL-2Rb (TM)	NM_000878	ATTCCGTGGCTCGGCCACCTCCTCGTGGGTCTCAGCGG GGCTTTTGGCTTCATCATCTTAGTGTACTTGCTGATC	24
IL-2RG (TM)	NP_000197	GTGGTTATCTCTGTTGGCTCCATGGGATTGATTATCAG CCTTCTCTGTGTGTATTTCTGGCTG	25

TABLE 20: INTRACELLULAR DOMAINS (ICD)

Domain	Uniprot ID	Polypeptide Sequence	SEQ ID NO.
IL-2Rb (ICD, G2R-1)	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSF SPGGLAPEISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQ GYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPL SGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSL QERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV*	26
IL-2RG (ICD, G2R-1)	P31785	ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCL VSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET*	27
gp130 (ICD, G2R-2)	P40189	NKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNSKDQMYSDGN FTDVSVVEIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIGGSSCMSSS RPSIS	28
IL-2Rb (ICD, G2R-2)	P14784	ASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEG VAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTA PGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPE LVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLS LQELQGQDPTHLV*	29
G-CSFR (ICD, G2R-3)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESH	30
IL-2Rb (ICD, G2R-3)	P14784	SSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAG APTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVL REAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQ ELQGQDPTHLV*	31
G-CSFR (ICD, G12R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHNSSET	32
IL-12Rb2 (ICD, G12R-1)	Q99665	AGDLPTHDGYLPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPL TFSCGDKLTLDQLKMRCDSLML*	33
G-CSFR (ICD, G21R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHNSSET	34
IL-21R (ICD, G21R-1)	Q9HBE5	SPGDEGPPRSYLRQWVVIPPPLSSPGPQAS*	35

G-CSFR (ICD, G21R-2)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHN	36
IL-21R (ICD, G21R-2)	Q9HBE5	QNSGGSAYSEERDRPYGLVSIDTVTVLDAEGPCTWPCSCEDDGYPAL DLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKP PLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVG SDCSSPVECDFTSPGDEGPPRSYLRQWVVIPPPLSSPGPQAS*	37
G-CSFR (ICD, G21/2R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHNSSETCGLPTLVQT**Y**VLQGDPRAVSTQPQSQ	38
IL-2Rb (ICD, G21/2R-1)	P14784	SSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAG APTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVL REAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQ ELQGQDPTHLV*	39
G-CSFR (ICD, G12/2R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESH	40
IL-2Rb (ICD, G12/2R-1)	P14784	SSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDP	41
IL-12Rb2 (ICD, G12/2R-1)	Q99665	AGDLPTHDGYLPSNIDDLPS	42
IL-2Rb (ICD, G12/2R-1)	P14784	GGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPD LVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNAR LPLNTDAYLSLQELQGQDPTHLV*	43
G-CSFR (ICD, G21/12/2R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHNSSETCGLPTLVQTYVLQGDPRAVSTQPQSQ	44
IL-2Rb (ICD, G21/12/2R-1)	P14784	SSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDP	45
IL-12Rb2 (ICD, G21/12/2R-1)	Q99665	AGDLPTHDGYLPSNIDDLPS	46
IL-2Rb (ICD, G21/12/2R-1)	P14784	GGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPD LVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNAR LPLNTDAYLSLQELQGQDPTHLV*	47
gp130 (ICD, G27/2R-1)	P40189	NKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNSKDQMYSDGN FTDVSVVEIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIGGSSCMSSS RPSISSSDENESSQNTSSTVQYSTVVHSGYRHQVPSVQVFSR	48
IL-2Rb (ICD, G27/2R-1)	P14784	ASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEG VAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTA PGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPE LVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLS LQELQGQDPTHLV*	49
G-CSFR (ICD, G7R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESH	50
IL-7Ra (ICD, G7R-1)	P16871	SGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLG SNQEEAYVTMSSFYQNQ*	51
G-CSFR (ICD, G21/7R-1)	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITKLTV LEEDEKKPVPWESHNSSETCGLPTLVQT**Y**VLQGDPRAVSTQPQSQ	52
IL7R (ICD, G21/7R-1)	P16871	SSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVA QGQPILTSLGSNQEEAYVTMSSFYQNQ*	53

Domain	Genbank Accession ID	Nucleic Acid Sequence	
IL-2RB (ICD, G2R-1)	NM_000878	AACTGCAGGAACACCGGGCCATGGCTGAAGAAGGTCCTGAAGTGT AACACCCCAGACCCCTCGAAGTTCTTTTCCCAGCTGAGCTCAGAGC ATGGAGGAGACGTCCAGAAGTGGCTCTCTTCGCCCTTCCCCTCATC GTCCTTCAGCCCTGGCGGCCTGGCACCTGAGATCTCGCCACTAGAA GTGCTGGAGAGGGACAAGGTGACGCAGCTGCTCCTGCAGCAGGA CAAGGTGCCTGAGCCCGCATCCTTAAGCAGCAACCACTCGCTGACC AGCTGCTTCACCAACCAGGGTTACTTCTTCTTCCACCTCCCGGATGC CTTGGAGATAGAGGCCTGCCAGGTGTACTTTACTTACGACCCCTAC TCAGAGGAAGACCCTGATGAGGGTGTGGCCGGGGCACCCACAGG GTCTTCCCCCCAACCCCTGCAGCCTCTGTCAGGGGAGGACGACGCC TACTGCACCTTCCCCTCCAGGGATGACCTGCTGCTCTTCTCCCCCAG TCTCCTCGGTGGCCCCAGCCCCCCAAGCACTGCCCCTGGGGGCAGT GGGGCCGGTGAAGAGAGGATGCCCCCTTCTTTGCAAGAAAGAGTC CCCAGAGACTGGGACCCCCAGCCCCTGGGGCCTCCCACCCCAGGA GTCCCAGACCTGGTGGATTTTCAGCCACCCCCTGAGCTGGTGCTGC GAGAGGCTGGGGAGGAGGTCCCTGACGCTGGCCCCAGGGAGGGA GTCAGTTTCCCCTGGTCCAGGCCTCCTGGGCAGGGGGAGTTCAGG GCCCTTAATGCTCGCCTGCCCCTGAACACTGATGCCTACTTGTCCCT CCAAGAACTCCAGGGTCAGGACCCAACTCACTTGGTGTAG	54
IL-2RG (ICD, G2R-1)	NP_000197	GAACGGACGATGCCCCGAATTCCCACCCTGAAGAACCTAGAGGAT CTTGTTACTGAATACCACGGGAACTTTTCGGCCTGGAGTGGTGTGT CTAAGGGACTGGCTGAGAGTCTGCAGCCAGACTACAGTGAACGAC TCTGCCTCGTCAGTGAGATTCCCCCAAAAGGAGGGGCCCTTGGGG AGGGGCCTGGGGCCTCCCCATGCAACCAGCATAGCCCCTACTGGG CCCCCCCATGTTACACCCTAAAGCCTGAAACCTGA	55
gp130 (ICD, G2R-2)	NM_002184	AATAAGCGAGACCTAATTAAAAAACACATCTGGCCTAATGTTCCAG ATCCTTCAAAGAGTCATATTGCCCAGTGGTCACCTCACACTCCTCCA AGGCACAATTTTAATTCAAAAGATCAAATGTATTCAGATGGCAATT TCACTGATGTAAGTGTTGTGGAAATAGAAGCAAATGACAAAAAGC CTTTTCCAGAAGATCTGAAATCATTGGACCTGTTCAAAAAGGAAAA AATTAATACTGAAGGACACAGCAGTGGTATTGGGGGGTCTTCATG TATGTCATCTTCTAGGCCAAGCATTTCT	56
IL-2Rb (ICD, G2R-2)	NM_000878	GCATCCTTAAGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACC AGGGTTACTTCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGC CTGCCAGGTGTACTTTACTTACGACCCCTACTCAGAGGAAGACCCT GATGAGGGTGTGGCCGGGGCACCCACAGGGTCTTCCCCCCAACCC CTGCAGCCTCTGTCAGGGGAGGACGACGCCTACTGCACCTTCCCCT CCAGGGATGACCTGCTGCTCTTCTCCCCCAGTCTCCTCGGTGGCCCC AGCCCCCCAAGCACTGCCCCTGGGGGCAGTGGGGCCGGTGAAGA GAGGATGCCCCCTTCTTTGCAAGAAAGAGTCCCCAGAGACTGGGA CCCCCAGCCCCTGGGGCCTCCCACCCCAGGAGTCCCAGACCTGGTG GATTTTCAGCCACCCCCTGAGCTGGTGCTGCGAGAGGCTGGGGAG GAGGTCCCTGACGCTGGCCCCAGGGAGGGAGTCAGTTTCCCCTGG TCCAGGCCTCCTGGGCAGGGGGAGTTCAGGGCCCTTAATGCTCGC	57

		CTGCCCCTGAACACTGATGCCTACTTGTCCCTCCAAGAACTCCAGG GTCAGGACCCAACTCACTTGGTGTAG	
G-CSFR (ICD, G2R-3)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCAT	58
IL-2Rb (ICD, G2R-3)	NM_000878	AGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACCAGGGTTACT TCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGCCTGCCAGGT GTACTTTACTTACGACCCCTACTCAGAGGAAGACCCTGATGAGGGT GTGGCCGGGGCACCCACAGGGTCTTCCCCCCAACCCCTGCAGCCTC TGTCAGGGGAGGACGACGCCTACTGCACCTTCCCCTCCAGGGATG ACCTGCTGCTCTTCTCCCCCAGTCTCCTCGGTGGCCCCAGCCCCCCA AGCACTGCCCCTGGGGGCAGTGGGGCCGGTGAAGAGAGGATGCC CCCTTCTTTGCAAGAAAGAGTCCCCAGAGACTGGGACCCCCAGCCC CTGGGGCCTCCCACCCCAGGAGTCCCAGACCTGGTGGATTTTCAGC CACCCCCTGAGCTGGTGCTGCGAGAGGCTGGGGAGGAGGTCCCTG ACGCTGGCCCCAGGGAGGGAGTCAGTTTCCCCTGGTCCAGGCCTC CTGGGCAGGGGGAGTTCAGGGCCCTTAATGCTCGCCTGCCCCTGA ACACTGATGCCTACTTGTCCCTCCAAGAACTCCAGGGTCAGGACCC AACTCACTTGGTGTAG	59
G-CSFR (ICD, G12R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAACAGCTCAGAGACC	60
IL-12Rb2 (ICD, G12R-1)	NP_001550	GCAGGTGACCTTCCCACCCATGATGGCTACTTACCCTCCAACATAG ATGACCTCCCCTCACATGAGGCACCTCTCGCTGACTCTCTGGAAGA ACTGGAGCCTCAGCACATCTCCCTTTCTGTTTTCCCCTCAAGTTCTCT TCACCCACTCACCTTCTCCTGTGGTGATAAGCTGACTCTGGATCAGT TAAAGATGAGGTGTGACTCCCTCATGCTCTGA	61
G-CSFR (ICD, G21R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAACAGCTCAGAGACC	62
IL-21R (ICD, G21R-1)	NP_068570 (1codon altered)	AGCCCTGGGGACGAAGGACCCCCCCGGAGCTACCTCCGCCAGTGG GTGGTCATTCCTCCGCCACTTTCGAGCCCTGGACCCCAGGCCAGCT AA	63
G-CSFR (ICD, G21R-2)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT	64

		CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAAC	
IL-21R (ICD, G21R-2)	NP_068570 (1codon altered)	CAGAACTCGGGGGGCTCAGCTTACAGTGAGGAGAGGGATCGGCC ATACGGCCTGGTGTCCATTGACACAGTGACTGTGCTAGATGCAGA GGGGCCATGCACCTGGCCCTGCAGCTGTGAGGATGACGGCTACCC AGCCCTGGACCTGGATGCTGGCCTGGAGCCCAGCCCAGGCCTAGA GGACCCACTCTTGGATGCAGGGACCACAGTCCTGTCCTGTGGCTGT GTCTCAGCTGGCAGCCCTGGGCTAGGAGGGCCCCTGGGAAGCCTC CTGGACAGACTAAAGCCACCCCTTGCAGATGGGGAGGACTGGGCT GGGGGACTGCCCTGGGGTGGCCGGTCACCTGGAGGGGTCTCAGA GAGTGAGGCGGGCTCACCCCTGGCCGGCCTGGATATGGACACGTT TGACAGTGGCTTTGTGGGCTCTGACTGCAGCAGCCCTGTGGAGTGT GACTTCACCAGCCCTGGGGACGAAGGACCCCCCCGGAGCTACCTCC GCCAGTGGGTGGTCATTCCTCCGCCACTTTCGAGCCCTGGACCCCA GGCCAGC	65
G-CSFR (ICD, G21/2R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAACAGCTCAGAGACCTGTGGCCTCCCCACTCTGGTCCAGAC CTATGTGCTCCAGGGGGACCCAAGAGCAGTTTCCACCCAGCCCCAA TCCCAG	66
IL-2Rb (ICD, G21/2R-1)	NM_000878	AGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACCAGGGTTACT TCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGCCTGCCAGGT GTACTTTACTTACGACCCCTACTCAGAGGAAGACCCTGATGAGGGT GTGGCCGGGGCACCCACAGGGTCTTCCCCCCAACCCCTGCAGCCTC TGTCAGGGGAGGACGACGCCTACTGCACCTTCCCCTCCAGGGATG ACCTGCTGCTCTTCTCCCCCAGTCTCCTCGGTGGCCCCAGCCCCCCA AGCACTGCCCCTGGGGGCAGTGGGGCCGGTGAAGAGAGGATGCC CCCTTCTTTGCAAGAAAGAGTCCCCAGAGACTGGGACCCCCAGCCC CTGGGGCCTCCCACCCCAGGAGTCCCAGACCTGGTGGATTTTCAGC CACCCCCTGAGCTGGTGCTGCGAGAGGCTGGGGAGGAGGTCCCTG ACGCTGGCCCCAGGGAGGGAGTCAGTTTCCCCTGGTCCAGGCCTC CTGGGCAGGGGGAGTTCAGGGCCCTTAATGCTCGCCTGCCCCTGA ACACTGATGCCTACTTGTCCCTCCAAGAACTCCAGGGTCAGGACCC AACTCACTTGGTGTAG	67
G-CSFR (ICD, G12/2R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCAT	68
IL-2Rb (ICD, G12/2R-1)	NM_000878	AGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACCAGGGTTACT TCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGCCTGCCAGGT GTACTTTACTTACGACCCCTACTCAGAGGAAGACCCT	69

IL-12Rb2 (ICD, G12/2R-1)	NP_001550	GCAGGTGACCTTCCCACCCATGATGGCTACTTACCCTCCAACATAG ATGACCTCCCCTCA	70
IL-2Rb (ICD, G12/2R-1)	NM_000878	GGTGGCCCCAGCCCCCCAAGCACTGCCCCTGGGGGCAGTGGGGCC GGTGAAGAGAGGATGCCCCCTTCTTTGCAAGAAAGAGTCCCCAGA GACTGGGACCCCCAGCCCCTGGGGCCTCCCACCCCAGGAGTCCCA GACCTGGTGGATTTTCAGCCACCCCCTGAGCTGGTGCTGCGAGAG GCTGGGGAGGAGGTCCCTGACGCTGGCCCCAGGGAGGGAGTCAG TTTCCCCTGGTCCAGGCCTCCTGGGCAGGGGGAGTTCAGGGCCCTT AATGCTCGCCTGCCCCTGAACACTGATGCCTACTTGTCCCTCCAAGA ACTCCAGGGTCAGGACCCAACTCACTTGGTGTAG	71
G-CSFR (ICD, G21/12/2R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAACAGCTCAGAGACCTGTGGCCTCCCCACTCTGGTCCAGAC CTATGTGCTCCAGGGGGACCCAAGAGCAGTTTCCACCCAGCCCCAA TCCCAG	72
IL-2Rb (ICD, G21/12/2R-1)	NM_000878	AGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACCAGGGTTACT TCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGCCTGCCAGGT GTACTTTACTTACGACCCCTACTCAGAGGAAGACCCT	73
IL-12Rb2 (ICD, G21/12/2R-1)	NP_001550	GCAGGTGACCTTCCCACCCATGATGGCTACTTACCCTCCAACATAG ATGACCTCCCCTCA	74
IL-2Rb (ICD, G21/12/2R-1)	NM_000878	GGTGGCCCCAGCCCCCCAAGCACTGCCCCTGGGGGCAGTGGGGCC GGTGAAGAGAGGATGCCCCCTTCTTTGCAAGAAAGAGTCCCCAGA GACTGGGACCCCCAGCCCCTGGGGCCTCCCACCCCAGGAGTCCCA GACCTGGTGGATTTTCAGCCACCCCCTGAGCTGGTGCTGCGAGAG GCTGGGGAGGAGGTCCCTGACGCTGGCCCCAGGGAGGGAGTCAG TTTCCCCTGGTCCAGGCCTCCTGGGCAGGGGGAGTTCAGGGCCCTT AATGCTCGCCTGCCCCTGAACACTGATGCCTACTTGTCCCTCCAAGA ACTCCAGGGTCAGGACCCAACTCACTTGGTGTAG	75
gp130 (ICD, G27/2R-1)	NM_002184 (3 codons altered)	AATAAGCGAGACCTAATTAAAAAACACATCTGGCCTAATGTTCCAG ATCCTTCAAAGAGTCATATTGCCCAGTGGTCACCTCACACTCCTCCA AGGCACAATTTCAATTCAAAGGATCAAATGTATTCAGATGGCAATT TCACTGATGTAAGTGTTGTGGAAATAGAAGCAAATGACAAAAAGC CTTTTCCAGAAGATCTGAAATCATTGGACCTGTTCAAAAAGGAAAA AATTAATACTGAAGGACACAGCAGTGGTATTGGGGGGTCTTCATG TATGTCATCTTCTAGGCCAAGCATTTCTAGCAGTGATGAAAATGAA TCTTCACAAAACACTTCGAGCACTGTCCAGTATTCTACCGTGGTACA CAGTGGCTACAGACACCAAGTTCCGTCAGTCCAAGTCTTCTCAAGA	76
IL-2Rb (ICD, G27/2R-1)	NM_000878 (2 codons altered)	GCATCCTTAAGCAGCAACCACTCGCTGACCAGCTGCTTCACCAACC AGGGTTACTTCTTCTTCCACCTCCCGGATGCCTTGGAGATAGAGGC CTGCCAGGTGTACTTTACTTACGACCCCTACTCAGAGGAAGACCCT GATGAGGGTGTGGCCGGGGCACCCACAGGGTCTTCCCCCCAACCC	77

		CTGCAGCCTCTGTCAGGGGAGGACGACGCCTACTGCACCTTCCCCT CCAGGGATGACCTGCTGCTCTTCTCCCCCAGTCTCCTCGGTGGCCCC AGCCCCCCAAGCACTGCCCCTGGGGGCAGTGGGGCCGGTGAAGA GAGGATGCCCCCTTCTTTGCAAGAAAGAGTCCCCAGAGACTGGGA CCCCCAGCCCCTGGGGCCTCCCACCCCAGGAGTCCCAGACCTGGTG GATTTTCAGCCACCCCCTGAGCTGGTGCTGCGAGAGGCTGGGGAG GAGGTCCCTGACGCTGGCCCCAGGGAGGGAGTCAGTTTCCCCTGG TCCAGGCCTCCTGGGCAGGGGGAGTTCAGGGCCCTTAATGCTCGC CTGCCCCTGAACACTGATGCCTACTTGTCCCTCCAAGAACTCCAGG GTCAGGACCCAACTCACTTGGTGTAG	
G-CSFR (ICD, G7R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCAT	78
IL-7Ra (ICD, G7R-1)	NP_002176	AGTGGCAAGAATGGGCCTCATGTGTACCAGGACCTCCTGCTTAGCC TTGGGACTACAAACAGCACGCTGCCCCCTCCATTTTCTCTCCAATCT GGAATCCTGACATTGAACCCAGTTGCTCAGGGTCAGCCCATTCTTA CTTCCCTGGGATCAAATCAAGAAGAAGCATATGTCACCATGTCCAG CTTCTACCAAAACCAGTGA	79
G-CSFR (ICD, G21/7R-1)	NP_000751	AGCCCCAACAGGAAGAATCCCCTCTGGCCAAGTGTCCCAGACCCAG CTCACAGCAGCCTGGGCTCCTGGGTGCCCACAATCATGGAGGAGG ATGCCTTCCAGCTGCCCGGCCTTGGCACGCCACCCATCACCAAGCT CACAGTGCTGGAGGAGGATGAAAAGAAGCCGGTGCCCTGGGAGT CCCATAACAGCTCAGAGACCTGTGGCCTCCCCACTCTGGTCCAGAC CTATGTGCTCCAGGGGGACCCAAGAGCAGTTTCCACCCAGCCCCAA TCCCAG	80
IL7R (ICD, G21/7R-1)	NP_002176	TCCTCTTCCAGGTCCCTAGACTGCAGGGAGAGTGGCAAGAATGGG CCTCATGTGTACCAGGACCTCCTGCTTAGCCTTGGGACTACAAACA GCACGCTGCCCCCTCCATTTTCTCTCCAATCTGGAATCCTGACATTG AACCCAGTTGCTCAGGGTCAGCCCATTCTTACTTCCCTGGGATCAA ATCAAGAAGAAGCATATGTCACCATGTCCAGCTTCTACCAAAACCA GTGA	81

TABLE 21: PROTEIN SEQUENCE OF WT AND VARIANT FORMS OF RECOMBINANT HUMAN G-CSF CYTOKINEAND VARIATIONS OF 130A1 SEQ ID NO 83-1 THROUGH 83-5) DESIGNED TO IMPROVE STABILITY.

Protein	Uniprot ID	Residue numbering	Polypeptide Sequence	SEQ ID NO.
WT G-CSF	P09919	*0-174 (*0 refers to an M residue not present in native G-CSF)	MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKL CATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLA GCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQLD VADFATTIWQQMEELGMAPALQPTQGAMPAFASAF QRRAGGVLVASHLQSFLEVSYRVLRHLAQP	82
130a1 variant with mutations underlined	Derived from P09919	*0-174	MTPLGPASSLPQSFLLKCLEQVRKIQGDGAALQEKL CATYKLCHPE**R**LVLLGHSLGIPWAPLSSCPSQALQL AGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQ**K** DVA**R**FATTIWQQM**K**ELGMAPALQPTQGAMPAFAS AFQRRAGGVLVASHLQSFLEVSYRVLRHLAQP	83
307 variant with mutations underlined	Derived from P09919	*0-174	MTPLGPASSLPQ**E**FLL**D**CL**K**QVRKIQGDGAALQEKL CATYKLCHPE**R**LVLLGHSLGIPWAPLSSCPSQALQL AGCLSQLHSGLFLYQGLLQALEGISPELGPTLDTLQL DVADFATTIWQQMEELGMAPALQPTQGAMPAFASA FQRRAGGVLVASHLQSFLEVSYRVLRHLAQP	84

Table 22: Protein sequence of WT and variant forms of human G-CSF receptor extracellular and transmembrane domains and signal peptide

Protein	Uniprot ID	Domain & residue numbering	Polypeptide Sequence	SEQ ID NO.
WT G-CSFR	Q99062	ECD: 2-308	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILWRLGAELQP GGRQQRLSDGTQESIITLPHLNHTQAFLSCSLNWGNSLQILDQVEL RAGYPPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKSR GNCQTQGDSILDCVPKDGQSHCSIPRKHLLLYQNMGIWVQAENA LGTSMSPQLCLDPMDVVKLEPPMLRTMDPSPEAAPPQAGCLQLS WEPWQPGLHINQKCELRHKPQRGEASWALVGPLPLEALQYELCG LLPATAYTLQIRCIRWPLPGHWSDWSPSLELRTTE	85
134 G-CSFR variant (mutations underlined)	Derived from Q99062	ECD: 2-308	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILW**E**LGAELQP GGRQQRLSDGTQESIITLPHLNHTQAFLSCSLNWGNSLQILDQVEL RAGYPPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKS**E** GNCQTQGDSILDCVPKDGQSHCSIP**D**KHLLLYQNMGIWVQAENA LGTSMSPQLCLDPMDVVKLEPPMLRTMDPSPEAAPPQAGCLQLS WEPWQPGLHINQKCELRHKPQRGEASWALVGPLPLEALQYELCG LLPATAYTLQIRCIRWPLPGHWSDWSPSLELRTTE	86
307 G-CSFR variant (mutations underlined)	Derived from Q99062	ECD: 2-308	ECGHISVSAPIVHLGDPITASCIIKQNCSHLDPEPQILW**E**LGAELQP GGRQQRLSDGTQESIITLPHLNHTQAFLSCSLNWGNSLQILDQVEL RAGYPPAIPHNLSCLMNLTTSSLICQWEPGPETHLPTSFTLKSFKSR GNCQTQGDSILDCVPKDGQSHCSIPRKHLLLYQNMGIWVQAENA LGTSMSPQLCL**K**PM**K**VVKLEPPMLRTMDPSPEAAPPQAGCLQLS WEPWQPGLHINQKCELRHKPQRGEASWALVGPLPLEALQYELCG LLPATAYTLQIRCI**E**WPLPGHWSDWSPSLELRTTE	87
WT G-CSFR	Q99062	TMD: 309-331	IILGLFGLLLLLTCLCGTAWLCC	88
WT G-CSFR	Q99062	Signal peptide	MARLGNCSLTWAALIILLLPGSLE	89

TABLE 23: INTRACELLULAR DOMAINS (ICDS)

Chimeric receptor	Protein (Domain)	Uniprot ID	Polypeptide Sequence	SEQ ID NO.
G2R-3	G-CSFR: Box 1 underlined Box 2 bold underlined	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITK**LTVL**EE DEKKPVPWESH	90
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	SSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAP TGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAG EERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEV PDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPTH LV*	91
G4R	G-CSFR: Box 1 underlined Box 2 bold underlined	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITK**LTVL**EE DEKKPVPWESH	90
	IL4Rα: IRS-1 & 2 and STAT6 binding sites bold underlined *stop codon	P24394	DEDPHKAAKEMPFQGSGKSAWCPVEISKTVLWPESISVVRCVELFEAPVE CEEEEEVEEEKGSFCASPESSRDDFQEGREGIVARLTESLFLDLLGEENGGF CQQDMGESCLLPPSGSTSAHMPWDEFPSAGPKEAPPWGKEQPLHLEPS PPASPTQSPDNLTCTETPLVIAGNPA**Y**RSFSNSLSQSPCPRELGPDPLLARH LEEVEPEMPCVPQLSEPTTVPQPEPETWEQILRRNVLQHGAAAAPVSAPT SGYQEFVHAVEQGGTQASAVVGLGPPGEAG**Y**KAFSSLLASSAVSPEKCGF GASSGEEG**Y**KPFQDLIPGCPGDPAPVPVPLFTFGLDREPPRSPQSSHLPSSS PEHLGLEPGEKVEDMPKPPLPQEQATDPLVDSLGSGIVYSALTCHLCGHLK QCHGQEDGGQTPVMASPCCGCCCGDRSSPPTTPLRAPDPSPGGVPLEAS LCPASLAPSGISEKSKSSSSFHPAPGNAQSSSQTPKIVNFVSVGPTYMRVS*	92
G6R	gp130: Box 1 underlined Box 2 bold underlined SHP-2 and STAT3 binding sites underlined, tyrosines bold *stop codon	P40189	NKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNSKDQMYSDGNFT D**VSVV**EIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIGGSSCMSSSRPSIS SSDENESSQNTSSTVQ**Y**STVVHSG**Y**RHQVPSVQVFSRSESTQPLLDSEERP EDLQLVDHVDGGDGILPRQQ**Y**FKQNCSQHESSPDISHFERSKQVSSVNEE DFVRLKQQISDHISQSCGSGQMKMFQEVSAADAFGPGTEGQVERFETVG MEAATDEGMPKS**Y**LPQTVRQGG**Y**MPQ*	93
GEPOR	EPOR: Box 1 underlined Box 2 bold underlined SHP-1 & 2, STAT5 binding sites bold underlined *stop codon	P19235	HRRALKQKIWPGIPSPESEFEGLFTTHKGNFQ**LWLY**QNDGCLWWSPCTP FTEDPPAS**LEVL**SERCWGTMQAVEPGTDDEGPLLEPVGSEHAQDT**Y**LVL DKWLLPRNPPSEDLPGPGGSVDIVAMDEGSEASSCSSALASKPSPEGASA ASFE**Y**TILDPSSQLLRPWTLCPELPPTPPHLKYL**Y**LVVSDSGISTDYSSGDSQ GAQGGLSDGPYSNPYENSLIPAAEPLPPS**Y**VACS*	94
GIFNAR2	G-CSFR: Box 1 underlined Box 2 bold underlined	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITK**LTVL**EE DEKKPVPWESHNSS	95
	IFNAR2: STAT1 & 2 binding sites bold underlined *stop codon	P48551	KKKVWDYNYDDESDSDTEAAPRTSGGG**Y**TMHGLTVRPLGQASATSTES QLIDPESEEEPDLPEVDVELPTMPKDSPQQLELLSGPCERRKSPLQDPFPEE DYSSTEGSGGRITFNVDLNSVFLRVLDDEDSDDLEAPLMLSSHLEEMVDPE DPDNVQSNHLLASGEGTQPTFPSPSSEGLWSEDAPSDQSDTSESDVDLG DG**Y**IMR*	96

GIFNGR1	IFNγR2: Box 1 underlined Box 2 bold underlined	P38484	KYRGLIKYWFHTPPSIPLQIEEYLKDPTQPILEALDKDSSPKDDVWDS**VSII**S FPEKEQEDVLQTL	97
	IFNγR1: STAT1 binding site bold underlined *stop codon	P15260	EGQELITVIKAPTSFG**Y**DKPHVLVDLLVDDS*	98
GIFNGR2	G-CSFR: Box 1 underlined Box 2 bold underlined	Q99062	SPNRKNPLWPSVPDPAHSSLGSWVPTIMEEDAFQLPGLGTPPITK**LTVL**EE DEKKPVPWESHNS	99
	IFNγR1: STAT1 binding site bold underlined *stop codon	P15260	TEGQELITVIKAPTSFG**Y**DKPHVLVDLLVDDS*	100

TABLE 24: DOMAIN COMPOSITION OF CHIMERIC CYTOKINE RECEPTORS

Name	Signal Peptide (SEQ ID)	ECD (SEQ ID)	TMD (SEQ ID)	Box1/2 (SEQ ID)	ICD (SEQ ID)	STAT site(s)	Other site(s)
G2R-3	G-CSFR (11)	G-CSFR_134 (86)	G-CSFR (18)	G-CSFR (90)	IL-2Rβ (91)	5	Shc
G4R	G-CSFR (11)	G-CSFR_134 (86)	G-CSFR (18)	G-CSFR (90)	IL-4Rα (92)	6	IRS-1& 2
G6R	G-CSFR (11)	G-CSFR_134 (86)	G-CSFR (18)	gp130 (93)	gp130 (93)	3	SHP-2
GEPOR	G-CSFR (11)	G-CSFR_134 (86)	G-CSFR (18)	EPOR (94)	EPOR (94)	5	SHP-1 & 2
GIFNAR	G-CSFR (11)	G-CSFR_134 (86)	G-CSFR (18)	G-CSFR (95)	IFNAR2 (96)	1,2	
GIFNGR-1	G-CSFR (11)	G-CSFR_307 (87)	G-CSFR (18)	IFNγR2 (97)	IFNγR1 (98)	1	
GIFNGR-2	G-CSFR (11)	G-CSFR_307 (87)	G-CSFR (18)	G-CSFR (99)	IFNγR1 (100)	1	

TABLE 25: MUTATIONS IN G-CSF AND G-CSFR VARIANTS

G-CSF Variant Number	G-CSF Mutations	Corresponding G-CSFR Variant Number	Corresponding G-CSFR Mutations
130a1	**E46R_L108K_D112R_E122K**	**134**	**R41E_R141E_R167D**
307	**S12E_K16D_E19K_E46R**	**307**	**R41E_D197K_D200K_R288E**

TABLE 26: PROTEIN SEQUENCE OF HUMAN CYTOKINE RECEPTORS

Protein	Uniprot ID	Domains	Polypeptide Sequence	SEQ ID NO.
IL-2Rβ/ IL-15Rβ	P14784	Signal peptide ECD TM	MAAPALSWRLPLLILLLPLATSWASAAVNGTSQFTCFYNSRANISCV WSQDGALQDTSCQVHAWPDRRRWNQTCELLPVSQASWACNLILG APDSQKLTTVDIVTLRVLCREGVRWRVMAIQDFKPFENLRLMAPISL	111

		ICD	QVVHVETHRCNISWEISQASHYFERHLEFEARTLSPGHTWEEAPLLTL KQKQEWICLETLTPDTQYEFQVRVKPLQGEFTTWSPWSQPLAFRTK PAALGKDTIPWLGHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKC NTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLE RDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEA CQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSR DDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQ PLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPP GQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	
IL-2Rγc	P31785	Signal peptide ECD TM ICD	MLKPSLPFTSLLFLQLPLLGVGLNTTILTPNGNEDTTADFFLTTMPTDS LSVSTLPLPEVQCFVFNVEYMNCTWNSSSEPQPTNLTLHYWYKNSD NDKVQKCSHYLFSEEITSGCQLQKKEIHLYQTFVVQLQDPREPRRQA TQMLKLQNLVIPWAPENLTLHKLSESQLELNWNNRFLNHCLEHLVQ YRTDWDHSWTEQSVDYRHKFSLPSVDGQKRYTFRVRSRFNPLCGSA QHWSEWSHPIHWGSNTSKENPFLFALEAVVISVGSMGLIISLLCVYF WLERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSER LCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET	112
IL-4Rα	P24394	Signal peptide ECD TM ICD	MGWLCSGLLFPVSCLVLLQVASSGNMKVLQEPTCVSDYMSISTCEW KMNGPTNCSTELRLLYQLVFLLSEAHTCIPENNGGAGCVCHLLMDD VVSADNYTLDLWAGQQLLWKGSFKPSEHVKPRAPGNLTVHTNVSD TLLLTWSNPYPPDNYLYNHLTYAVNIWSENDPADFRIYNVTYLEPSLR IAASTLKSGISYRARVRAWAQCYNTTWSEWSPSTKWHNSYREPFEQ HLLLGVSVSCIVILAVCLLCYVSITKIKKEWWDQIPNPARSRLVAIIIQD AQGSQWEKRSRGQEPAKCPHWKNCLTKLLPCFLEHNMKRDEDPHK AAKEMPFQGSGKSAWCPVEISKTVLWPESISVVRCVELFEAPVECEE EEEVEEEKGSFCASPESSRDDFQEGREGIVARLTESLFLDLLGEENGGF CQQDMGESCLLPPSGSTSAHMPWDEFPSAGPKEAPPWGKEQPLHL EPSPPASPTQSPDNLTCTETPLVIAGNPAYRSFSNSLSQSPCPRELGPD PLLARHLEEVEPEMPCVPQLSEPTTVPQPEPETWEQILRRNVLQHGA AAAPVSAPTSGYQEFVHAVEQGGTQASAVVGLGPPGEAGYKAFSSL LASSAVSPEKCGFGASSGEEGYKPFQDLIPGCPGDPAPVPVPLFTFGL DREPPRSPQSSHLPSSSPEHLGLEPGEKVEDMPKPPLPQEQATDPLV DSLGSGIVYSALTCHLCGHLKQCHGQEDGGQTPVMASPCCGCCCG DRSSPPTTPLRAPDPSPGGVPLEASLCPASLAPSGISEKSKSSSSFHPA PGNAQSSSQTPKIVNFVSVGPTYMRVS	113
IL-9R	Q01113	Signal peptide ECD TM ICD	MGLGRCIWEGWTLESEALRRDMGTWLLACICICTCVCLGVSVTGEG QGPRSRTFTCLTNNILRIDCHWSAPELGQGSSPWLLFTSNQAPGGT HKCILRGSECTVVLPPEAVLVPSDNFTITFHHCMSGREQVSLVDPEYL PRRHVKLDPPSDLQSNISSGHCILTWSISPALEPMTTLLSYELAFKKQE EAWEQAQHRDHIVGVTWLILEAFELDPGFIHEARLRVQMATLEDDV VEEERYTGQWSEWSQPVCFQAPQRQGPLIPPWGWPGNTLVAVSIF LLLTGPTYLLFKLSPRVKRIFYQNVPSPAMFFQPLYSVHNGNFQTWM GAHGAGVLLSQDCAGTPQGALEPCVQEATALLTCGPARPWKSVALE EEQEGPGTRLPGNLSSEDVLPAGCTEWRVQTLAYLPQEDWAPTSLT RPAPPDSEGSRSSSSSSSSNNNNYCALGCYGGWHLSALPGNTQSSG	114

			PIPALACGLSCDHQGLETQQGVAWVLAGHCQRPGLHEDLQGMLLP SVLSKARSWTF	
IL-12Rβ2	Q99665	Signal peptide ECD TM ICD	MAHTFRGCSLAFMFIITWLLIKAKIDACKRGDVTVKPSHVILLGSTVNI TCSLKPRQGCFHYSRRNKLILYKFDRRINFHHGHSLNSQVTGLPLGTT LFVCKLACINSDEIQICGAEIFVGVAPEQPQNLSCIQKGEQGTVACTW ERGRDTHLYTEYTLQLSGPKNLTWQKQCKDIYCDYLDFGINLTPESPE SNFTAKVTAVNSLGSSSSLPSTFTFLDIVRPLPPWDIRIKFQKASVSRCT LYWRDEGLVLLNRLRYRPSNSRLWNMVNVTKAKGRHDLLDLKPFTE YEFQISSKLHLYKGSWSDWSESLRAQTPEEEPTGMLDVWYMKRHID YSRQQISLFWKNLSVSEARGKILHYQVTLQELTGGKAMTQNITGHTS WTTVIPRTGNWAVAVSAANSKGSSLPTRINIMNLCEAGLLAPRQVS ANSEGMDNILVTWQPPRKDPSAVQEYVVEWRELHPGGDTQVPLN WLRSRPYNVSALISENIKSYICYEIRVYALSGDQGGCSSILGNSKHKAPL SGPHINAITEEKGSILISWNSIPVQEQMGCLLHYRIYWKERDSNSQPQ LCEIPYRVSQNSHPINSLQPRVTYVLWMTALTAAGESSHGNEREFCL QGKANWMAFVAPSICIAIIMVGIFSTHYFQQKVFVLLAALRPQWCSR EIPDPANSTCAKKYPIAEEKTQLPLDRLLIDWPTPEDPEPLVISEVLHQ VTPVFRHPPCSNWPQREKGIQGHQASEKDMMHSASSPPPPRALQA ESRQLVDLYKVLESRGSDPKPENPACPWTVLPAGDLPTHDGYLPSNI DDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLK MRCDSLML	115
IL-21R	Q9HBE5	Signal peptide ECD TM ICD	MPRGWAAPLLLLLLQGGWGCPDLVCYTDYLQTVICILEMWNLHPST LTLTWQDQYEELKDEATSCSLHRSAHNATHATYTCHMDVFHFMAD DIFSVNITDQSGNYSQECGSFLLAESIKPAPPFNVTVTFSGQYNISWRS DYEDPAFYMLKGKLQYELQYRNRGDPWAVSPRRKLISVDSRSVSLLP LEFRKDSSYELQVRAGPMPGSSYQGTWSEWSDPVIFQTQSEELKEG WNPHLLLLLLLVIVFIPAFWSLKTHPLWRLWKKIWAVPSPERFFMPLY KGCSGDFKKWVGAPFTGSSLELGPWSPEVPSTLEVYSCHPPRSPAKR LQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEERDRPYGLVSI DTVTVLDAEGPCTWPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAGT TVLSCGCVSAGSPGLGGPLGSLLDRLKPPLADGEDWAGGLPWGGRS PGGVSESEAGSPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGP PRSYLRQWVVIPPPLSSPGPQAS	116
gp130	P40189	Signal peptide ECD TM ICD	MLTLQTWLVQALFIFLTTESTGELLDPCGYISPESPVVQLHSNFTAVC VLKEKCMDYFHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTDIASL NIQLTCNILTFGQLEQNVYGITIISGLPPEKPKNLSCIVNEGKKMRCEW DGGRETHLETNFTLKSEWATHKFADCKAKRDTPTSCTVDYSTVYFVN IEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVINSEELSSILKL TWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTASTRSSFTVQDLKP FTEYVFRIRCMKEDGKGYWSDWSEEASGITYEDRPSKAPSFWYKIDP SHTQGYRTVQLVWKTLPPFEANGKILDYEVTLTRWKSHLQNYTVNA TKLTVNLTNDRYLATLTVRNLVGKSDAAVLTIPACDFQATHPVMDLK AFPKDNMLWVEWTTPRESVKKYILEWCVLSDKAPCITDWQQEDGT VHRTYLRGNLAESKCYLITVTPVYADGPGSPESIKAYLKQAPPSKGPTV	117

			RTKKVGKNEAVLEWDQLPVDVQNGFIRNYTIFYRTIIGNETAVNVDS SHTEYTLSSLTSDTLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGEIEA IVVPVCLAFLLTTLLGVLFCFNKRDLIKKHIWPNVPDPSKSHIAQWSP HTPPRHNFNSKDQMYSDGNFTDVSVVEIEANDKKPFPEDLKSLDLFK KEKINTEGHSSGIGGSSCMSSSRPSISSSDENESSQNTSSTVQYSTVVH SGYRHQVPSVQVFSRSESTQPLLDSEERPEDLQLVDHVDGGDGILPR QQYFKQNCSQHESSPDISHFERSKQVSSVNEEDFVRLKQQISDHISQS CGSGQMKMFQEVSAADAFGPGTEGQVERFETVGMEAATDEGMP KSYLPQTVRQGGYMPQ	

TABLE 27: ICD ADAPTOR PROTEIN BINDING SITES.

Receptor name	Uniprot ID	Binding site	Polypeptide Sequence	SEQ ID NO.
IL-7Rα	P16871	Box 1	KRIKPIVWPSLPDHKKTLEHL	118
		Box 2 - 1	KKPRKN**LNVS**FNPESF	119
		Box 2 - 2	PESFLD**CQIH**RVDDIQ	120
IL-2Rβ	P14784	Box 1	PWLKKVLKCNTPDPSKFFSQL	121
		Box 2	APEISP**LEVL**ERDKVT	122
		Shc	TSCFTNQG**Y**FFFHLPDA	123
		STAT5	RLPLNTDA**Y**LSLQELQG	124
IL-2Rγ$_c$	P31785	Box 1	ERTMPRIPTLKNLEDL	125
		Box 2	PDYSER**LCLV**SEIPPK	126
IL-4Rα	P24394	Box 1	KIKKEWWDQIPNPARSRLVA	127
		Box 2	LTKLLP**CFLE**HNMKRD	128
		IRS-2	LVIAGNPA**Y**RSFSNSLS	129
		STAT6 - 1	LGPPGEAG**Y**KAFSSLLA	130
		STAT6 - 2	GASSGEEG**Y**KPFQDLIP	131
IL-9R	Q01113	Box 1	SPRVKRIFYQNVPSPAMFFQP	132
		Box 2 - 1	GAHGAG**VLLS**QDCAGT	133
		Box 2 - 2	GTPQGA**LEPC**VQEATA	134
IL-12Rβ$_2$	Q99665	STAT4	AGDLPTHDG**Y**LPSNIDDLPSHEAPLA	135
IL-21R	Q9HBE5	Box 1	LWRLWKKIWAVPSPERFFMPL	136
		Box 2 - 1	PFTGSS**LELG**PWSPEV	137

		Box 2 - 2	PEVPST**LEVY**SCHPPR	138
		STAT3	GDEGPPRS**Y**LRQWVVIP	139
gp130	P40189	Box 1	DLIKKHIWPNVPDPSKSHIAQ	140
		Box 2	DGNFTD**VSVV**EIEAND	141
		SHP-2	QNTSSTVQ**Y**STVVHSG	142
		STAT3 - 1	STVVHSG**Y**RHQVPSVQ	143
		STAT3 - 2	DGILPRQQ**Y**FKQNCSQHESS	144
		STAT3 - 3	EGMPKS**Y**LPQTVRQ	145
		STAT3 - 4	TVRQGG**Y**MPQ	146

TABLE 28: EXAMPLES OF ENGINEERED T CELL RECEPTORS (ETCRS)

Chimeric receptor	**Receptor name**: domains	**Patent**	**Polypeptide Sequence**	**SEQ ID NO.**
HPV 16 E7 eTCR	**LVL-modified chimeric TCR**: β chain Variable region - Murine β chain Constant region - Linker peptide (P2A) - Human α chain Variable region - Murine α chain Constant region - Murine α chain TMD	US 10174098 B2	MAPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQVTLRCSPK**SGHDTV**SWYQQALGQGPQFIFQ**YYEEEE**RQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLC**ASSLGWRGGRYNEQF**FGPGTRLTVLEDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSWWVNGKEVHSGVSTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDKWPEGSPKPVTQNISAEAWGRADCGITSASYQQGVLSA*TILYEILLGKATLYAVLVSTLVV*MAMVKRKNSRAKRSGSGATNFSLLKQAGDVEENPGPMWGVFLLYVSMKMGGTTGQNIDQPTEMTATEGAIVQINCTYQ**TSGFNG**LFWYQQHAGEAPTFLSY**NVLDGL**EEKGRFSSFLSRSKGYSYLLLKELQMKDSASYLC**ASVDGNNRLA**FGKGNQVVVIPNIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKTVLDMKAMDSKSNGAIAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS	147
	Cys-substituted, LVL-modified chimeric TCR: β chain Variable region - Murine β chain Constant region - Linker peptide (P2A) - Human α chain Variable region - Murine α chain Constant region - Murine α chain TMD		MAPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQVTLRCSPK**SGHDTV**SWYQQALGQGPQFIFQ**YYEEEE**RQRGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLC**ASSLGWRGGRYNEQF**FGPGTRLTVLEDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSWWVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDKWPEGSPKPVTQNISAEAWGRADCGITSASYQQGVLSA*TILYEILLGKATLYAVLVSTLVV*MAMVKRKNSRAKRSGSGATNFSLLKQAGDVEENPGPMWGVFLLYVSMKMGGTTGQNIDQPTEMTATEGAIVQINCTYQ**TSGFNG**LFWYQQHAGEAPTFLSY**NVLDGL**EEKGRFSSFLSRSKGYSYLLLKELQ	148

			MKDSASYLC**ASVDGNNRLA**FGKGNQVVVIPNIQNP EPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESG TFITDKCVLDMKAMDSKSNGAIAWSNQTSFTCQDIF KETNATYPSSDVPCDATLTEKSFETDMNLNFQNLLVI VLRILLLKVAGFNLLMTLRLWSS	
NY-ESO-1 eTCR	NY-ESO-1^{c259} TCR a chain	WO 2020/049496 A1	METLLGLLILWLGLGWVSSKGEVTGIPAALSVPEGEN LVLNCSFTDSAIYNLGWFRGDPGKGLTSLLLIGSSGRE GTSGRLNASLDKSSGRSTLYIAASGPGDSATYLCAVR PLYGGSYIPTFGRGTSLIVHPYIONPDPAVYOLRDSKS SDKSVCLFTDFDSOTNVSOSKDSDVYITDKTVLDMR SMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFP SPESSCDVKLVEKSFETDTNLNFCNLSVIGFRILLLKVA GFNLLMTLRLWSS	149
	NY-ESO-1 ^{c259} TCR b chain		RMSIGLLCCAALSLLWAGPVNAGVTGTPKFGVLKTG GSMTLGCAGDMNHEYMSWYRGDPGMGLRLIHYS VGAGITDGGEVPNGYNVSRSTTEDFPLRLLSAAPSGT SVYFCASSYVGNTGELFFGEGSRLTVLEDLKNVFPPE VAVFEPSEAEISHTCKATLVCLATGFYPDHVELSWW VNGKEVHSGVSTDPGPLKEGPALNDSRYCLSSRLRVS ATFWGNPRNHFRCGVGFYGLSENDEWTODRAKPV TOIVSAEAWGRADCGFTSESYOOGVLSATILYEILLG KATLYAVLVSALVLMAMVKRKDSRG	150

TABLE 29: EXAMPLES OF CHIMERIC ANTIGEN RECEPTORS (CARS)

Chimeric receptor	Receptor name Domains	Patent	Polypeptide Sequence	SEQ ID NO.
Second generation 4-1BB containing anti-CD19 CAR	**CTL019**: Leader - VH - Linker - VL - CD8 hinge - CD8 TM - 4-1BB - CD3ζ	WO2014153270A1	MALPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGD RVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRIH SGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT LPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQES GPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKG LEWLGVIWGSETTYYNSALKSRITIIKDNSKSQVFLKM NSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTV SSTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL PPR	151
	Leader		MALPVTALLLPLALLLHAARP	152

	VH		DIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRIHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT	153
	Linker		GGGGSGGGGSGGGGS	154
	VL		EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRITIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSVTVSS	155
	CD8 hinge		TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	156
	CD8 TM		IYIWAPLAGTCGVLLLSLVITLYC	157
	4-1BB		KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	158
	CD3ζ		RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	159
First generation anti-mesothelin CAR	**P4-z**: Leader - VH - Linker - VL - CD8 hinge - CD8 TM - CD3ζ	US 9272002 B2	MALPVTALLLPLALLLHAARPGSQVQLQQSGPGLVTPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRMSINPDTSKNQFSLQLNSVTPEDTAVYYCARGMMTYYYGMDVWGQGTTVTVSSGILGSGGGGSGGGGSGGGGSQPVLTQSSSLSASPGASASLTCTLRSGINVGPYRIYWYQQKPGSPPQYLLNYKSDSDKQQGSGVPSRFSGSKDASANAGVLLISGLRSEDEADYYCMIWHSSAAVFGGGTQLTVLSASTTTPAPRPPTPAPTIASRPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCRVKFSRSADAPAYQNGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELNKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	160
	Leader		MALPVTALLLPLALLLHAARPGSQVQLQQSGPGL	161
	VH		VTPSQTLSLTCAISGDSVSSNSATWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRMSINPDTSKNQFSLQLNSVTPEDTAVYYCARGMMTYYYGMDVWGQGTTVTVSSGIL	162
	Linker		GSGGGGSGGGGSGGGGS	163
	VL		QPVLTQSSSLSASPGASASLTCTLRSGINVGPYRIYWYQQKPGSPPQYLLNYKSDSDKQQGSGVPSRFSGSKDASANAGVLLISGLRSEDEADYYCMIWHSSAAVFGGGTQLTVLSAS	164
	CD8 hinge		TTTPAPRPPTPAPTIASRPLSLRPEACRPAAGGAVHTRGLDFACD	165

	CD8 TM		IYIWAPLAGTCGVLLLSLVITLYC	166
	CD3ζ		RVKFSRSADAPAYQNGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELNKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	167
Second generation CD28 containing anti-mesothelin CAR	**HN1scFv-28Z**: Leader - VH - Linker - VL - CD8 hinge - CD8 TM - CD28 ICD - CD3ζ	US 9359447 B2	MVLLVTSLLLCELPHPAFLLIPDIQAQVQLVQSGAEVKRPGASVQVSCRASGYSINTYYMQWVRQAPGAGLEWMGVINPSGVTSYAQKFQGRVTLTNDTSTNTVYMQLNSLTSADTAVYYCARWALWGDFGMDVWGKGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSTLSASIGDRVTITCRASEGIYHWLAWYQQKPGKAPKLLIYKASSLASGAPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQYSNYPLTFGGGTKLEIKRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	168
	Leader		MVLLVTSLLLCELPHPAFLLIPDIQ	169
	VH		AQVQLVQSGAEVKRPGASVQVSCRASGYSINTYYMQWVRQAPGAGLEWMGVINPSGVTSYAQKFQGRVTLTNDTSTNTVYMQLNSLTSADTAVYYCARWALWGDFGMDVWGKGTLVTVSS	170
	Linker		GGGGSGGGGSGGGGS	171
	VL		DIQMTQSPSTLSASIGDRVTITCRASEGIYHWLAWYQQKPGKAPKLLIYKASSLASGAPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQYSNYPLTFGGGTKLEIKR	172
	CD8 hinge		AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	173
	CD8 TM		FWVLVVVGGVLACYSLLVTVAFIIFWV	174
	CD28 ICD		RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	175
	CD3ζ		RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	176
Third generation CD28 and	**HN1scFv-28BBZ**:		MVLLVTSLLLCELPHPAFLLIPDIQAQVQLVQSGAEVKRPGASVQVSCRASGYSINTYYMQWVRQAPGAGLEWMGVINPSGVTSYAQKFQGRVTLTNDTSTNTVYMQLN	177

4-1BB containing anti-mesothelin CAR	Leader - VH - Linker - VL - CD28 hinge - CD28 TM - CD28 ICD - 4-1BB - CD3ζ		SLTSADTAVYYCARWALWGDFGMDVWGKGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSTLSASIGDRVTITCRASEGIYHWLAWYQQKPGKAPKLLIYKASSLASGAPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQYSNYPLTFGGGTKLEIKRAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	
	Leader -		MVLLVTSLLLCELPHPAFLLIPDIQ	178
	VH -		AQVQLVQSGAEVKRPGASVQVSCRASGYSINTYYMQWVRQAPGAGLEWMGVINPSGVTSYAQKFQGRVTLTNDTSTNTVYMQLNSLTSADTAVYYCARWALWGDFGMDVWGKGTLVTVSS	179
	Linker -		GGGGSGGGGSGGGGS	180
	VL -		DIQMTQSPSTLSASIGDRVTITCRASEGIYHWLAWYQQKPGKAPKLLIYKASSLASGAPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQYSNYPLTFGGGTKLEIKR	181
	CD28 hinge -		AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	182
	CD28 TM -		IYIWAPLAGTCGVLLLSLVITLYCNHRN	183
	CD28 ICD -		RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	184
	4-1BB -		RFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	185
	CD3ζ		RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	186

TABLE 30: CYTOKINES, CHEMOKINES AND OTHER SIGNALING PROTEINS:

Cytokine	Uniprot ID	Polypeptide Sequence	SEQ ID NO.
hIL-18: Signal peptide underlined	Q14116	MAAEPVEDNCINFVAMKFIDNTLYFIAEDDENLESDYFGKLESKLSVIRNLNDQVLFIDQGNRPLFEDMTDSDCRDNAPRTIFIISMYKDSQPRGMAVTISVKCEKISTLSCENKIISFKEMNPPDNIKDTKSDIIFFQRSVPGHDNKMQFESSSYEGYFLACEKERDLFKLILKKEDELGDRSIMFTVQNED	102
hCXCL13: Signal peptide underlined	Q52X90	MKFISTSLLLMLLVSSLSPVQGVLEVYYTSLRCRCVQESSVFIPRRFIDRIQILPRGNGCPRKEIIVWKKNKSIVCVDPQAEWIQRMMEVLRKRSSSTLPVPVFKRKIP	103
hIL-21: Signal peptide underlined	Q9HBE4	MRSSPGNMERIVICLMVIFLGTLVHKSSSQGQDRHMIRMRQLIDIVDQLKNYVNDLVPEFLPAPEDVETNCEWSAFSCFQKAQLKSANTGNNERIINVSIKKLKRKPPSTNAGRRQKHRLTCPSCDSYEKKPPKEFLERFKSLLQKMIHQHLSSRTHGSEDS	104
hCCL3: Signal peptide underlined	P10147	MQVSTAALAVLLCTMALCNQFSASLAADTPTACCFSYTSRQIPQNFIADYFETSSQCSKPGVIFLTKRSRQVCADPSEEWVQKYVSDLELSA	105
hCCL4: Signal peptide underlined	P13236	MKLCVTVLSLLMLVAAFCSPALSAPMGSDPPTACCFSYTARKLPRNFVVDYYETSSLCSQPAVVFQTKRSKQVCADPSESWVQEYVYDLELN	106
hCCL19: Signal peptide underlined	Q99731	MALLLALSLLVLWTSPAPTLSGTNDAEDCCLSVTQKPIPGYIVRNFHYLLIKDGCRVPAVVFTTLRGRQLCAPPDQPWVERIIQRLQRTSAKMKRRSS	107
hIFNγ: Signal peptide underlined	P01579	MKYTSYILAFQLCIVLGSLGCYCQDPYVKEAENLKKYFNAGHSDVADNGTLFLGILKNWKEESDRKIMQSQIVSFYFKLFKNFKDDQSIQKSVETIKEDMNVKFFNSNKKKRDDFEKLTNYSVTDLNVQRKAIHELIQVMAELSPAAKTGKRKRSQMLFRGRRASQ	108
hIFNα-1/13: Signal peptide underlined	P01562	MASPFALLMVLVVLSCKSSCSLGCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDLEACVMQEERVGETPLMNADSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE	109
hIL-17: Signal peptide underlined	Q16552	MTPGKTSLVSLLLLLSLEAIVKAGITIPRNPGCPNSEDKNFPRTVMVNLNIHNRNTNTNPKRSSDYYNRSTSPWNLHRNEDPERYPSVIWEAKCRHLGCINADGNVDYHMNSVPIQQEILVLRREPPHCPNSFRLEKILVSVGCTCVTPIVHHVA	110
hTNF-α: Membrane-bound portion underlined	P01357	MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQREEFPRDLSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL	111
hNKG2-D: ICD-TM-ECD	P26718	MGWIRGRRSRHSWEMSEFHNYNLDLKKSDFSTRWQKQRCPVVKSKCRENASPFFFCCFIAVAMGIRFIIMVAIWSAVFLNSLFNQEVQIPLTESYCGPCPKNWICYKNNCYQFFDESKNWYESQASCMSQNASLLKVYSKEDQDLLKLVKSYHWMGLVHIPTNGSWQWEDGSILSPNLLTIIEMQKGDCALYASSFKGYIENCSTPNTYICMQRTV	112

TABLE 31: DNA SEQUENCES FOR CONTROLLED PARACRINE SIGNALING (CPS) EXEMPLARY CONSTRUCTS

Gene	Ref Seq ID	Residue numbering	DNA Sequence	SEQ ID NO.
CAR_P2A_G12-2R1-134_T2A_hIL-18		1-4758	CCCGCCAGGATCCTGACGCGTATGGTGCTGCTGGTC ACCAGCTTACTCCTGTGCGAGCTCCCCCATCCTGCGT TCTTGCTGATCCCCGACATCCAGCAGGTGCAGCTGC AACAGTCGGGCCCCGAGTTAGAAAAACCCGGCGCCT CCGTGAAGATCTCGTGTAAAGCTTCGGGCTACTCGT TTACCGGCTACACCATGAATTGGGTTAAGCAGAGCC ATGGAAAGTCTCTTGAATGGATAGGCCTGATTACGC CTTATAATGGGGCCTCGAGCTACAACCAGAAATTTC GTGGTAAGGCAACTCTGACTGTAGACAAGTCGTCCT CCACCGCATACATGGATCTTCTGAGCCTAACCTCAG AAGACAGCGCAGTCTACTTCTGCGCCCGTGGTGGTT ACGATGGCCGCGGATTCGACTATTGGGGCCAGGGCA CCACCGTCACCGTGAGCTCCGGCGGTGGCGGGTCCG GTGGCGGCGGCTCAAGCGGAGGAGGTAGTGACATC GAGTTGACCCAGTCGCCTGCCATCATGTCCGCATCC CCCGGCGAGAAGGTGACGATGACGTGTTCTGCTTCC AGCTCTGTATCGTACATGCACTGGTACCAACAGAAG TCCGGCACCTCCCCCAAGCGTTGGATCTACGACACT TCCAAGCTGGCGTCTGGCGTCCCTGGGCGCTTTTCAG GTAGCGGGTCCGGCAATAGCTATTCCTTGACGATCA GTAGCGTGGAGGCTGAGGATGATGCCACCTACTACT GCCAGCAGTGGAGCAAGCACCCGCTCACCTTCGGCG CCGGGACCAAGCTTGAAATCAAGGCGGCGGCGATC GAGGTGATGTATCCTCCTCCCTACCTGGACAACGAG AAATCCAATGGCACTATCATCCACGTGAAGGGGAAG CACCTGTGCCCGAGCCCCCTGTTCCCCGGCCCTTCCA AGCCTTTTTGGGTGCTTGTTGTGGTCGGCGGCGTGCT GGCCTGCTACTCTCTGCTGGTAACCGTAGCGTTCATC ATCTTCTGGGTGCGATCTAAGCGGTCCCGGCTGTTGC ACAGTGACTACATGAACATGACCCCGCGCCGCCCCG GCCCAACCCGCAAGCACTACCAGCCGTACGCTCCAC CTCGCGACTTTGCCGCCTACAGGTCGCGAGTGAAAT TTTCGCGCTCCGCGGACGCGCCGGCCTATCAGCAGG GCCAGAACCAGCTTTACAACGAGTTGAACCTCGGGC GCAGGGAGGAGTACGACGTGCTGGATAAACGTAGA GGTCGGGACCCGGAGATGGGCGGCAAACCACGCCG CAAGAACCCTCAGGAAGGCCTGTACAACGAGCTCCA GAAGGACAAAATGGCTGAGGCGTACTCGGAGATTG GGATGAAAGGCGAGCGGCGCCGCGGCAAGGGCCAC GATGGCCTGTACCAGGGGCTATCTACCGCTACAAAG GACACCTACGACGCCCTACATATGCAGGCCCTGCCA CCGCGTGGCTCTGGCGCAACCAACTTCTCGCTTCTGA AGCAGGCCGGTGATGTGGAGGAAAACCCAGGTCCT	149

			ATGGCCCGCCTGGGCAACTGCTCGCTGACCTGGGCG GCCCTGATCATCCTGCTGCTGCCCGGCTCTCTGGAGG AGTGTGGCCATATTTCCGTGTCAGCTCCAATCGTGCA CCTGGGCGACCCCATCACCGCTTCTTGCATCATCAA GCAGAACTGCTCTCACCTCGACCCTGAGCCCCAAAT ACTTTGGGAGCTGGGCGCGGAGCTCCAGCCTGGAGG ACGCCAGCAGCGTTTGTCAGACGGCACTCAGGAATC CATCATCACCCTCCCACATCTGAATCACACCCAGGC GTTCCTGTCTTGTTGTCTCAACTGGGGCAACTCCTTG CAGATACTGGACCAGGTGGAGCTGAGAGCTGGCTAT CCCCCGGCAATTCCTCACAACCTGTCCTGTCTGATGA ACCTGACCACCTCCAGTCTGATCTGTCAGTGGGAGC CCGGGCCAGAGACTCACCTTCCCACCAGCTTCACCC TTAAGTCGTTCAAATCCGAGGGCAACTGCCAGACCC AGGGTGATTCTATTTTGGATTGCGTGCCCAAGGACG GGCAGTCTCACTGTTGTATTCCCGACAAGCACCTCCT TTTATACCAGAATATGGGCATCTGGGTGCAGGCCGA GAATGCCCTGGGAACGTCTATGTCTCCACAGCTATG CCTGGATCCAATGGACGTAGTGAAGCTGGAGCCCCC CATGCTACGCACTATGGATCCTAGCCCAGAGGCCGC TCCCCCTCAGGCGGGGTGCCTGCAGCTTTGTTGGGA ACCCTGGCAACCCGGGTTGCACATTAACCAGAAGTG CGAGCTGCGCCACAAGCCCCAGAGAGGAGAGGCCT CCTGGGCCCTCGTGGGCCCTCTGCCGTTGGAGGCCTT GCAGTATGAGCTGTGCGGCCTCTTGCCCGCTACAGC TTATACTCTGCAAATCAGGTGTATTCGCTGGCCTTTG CCCGGACACTGGTCCGACTGGTCCCCCAGCCTTGAG CTGAGAACCACGGAGCGCGCCCCCACCGTACGCTTG GACACCTGGTGGCGCCAGAGGCAGCTGGACCCTCGC ACTGTGCAGCTGTTCTGGAAGCCGGTGCCCCTGGAG GAGGACTCCGGACGCATCCAGGGCTACGTGGTCAGC TGGCGTCCGAGTGGGCAGGCCGGGGCCATCCTGCCC CTGTGCAACACGACTGAGCTGAGCTGCACTTTCCAT CTGCCCAGCGAGGCTCAGGAGGTGGCGCTCGTGGCT TACAACTCCGCAGGCACTTCTCGCCCAACCCCCGTG GTGTTCTCTGAGAGTCGGGGCCCCGCCCTGACGCGC CTTCACGCCATGGCGCGCGACCCGCATTCCCTTTGG GTCGGTTGGGAGCCACCTAACCCATGGCCGCAGGGC TACGTGATCGAGTGGGGCCTGGGCCCCCCGAGCGCA TCGAACAGCAACAAGACCTGGAGAATGGAACAGAA CGGCCGCGCAACCGGCTTCCTATTAAAGGAGAACAT CCGCCCGTTCCAGCTGTACGAGATCATCGTGACACC GCTGTACCAGGACACTATGGGGCCTTCTCAGCACGT TTACGCCTACTCCCAGGAGATGGCCCCGAGCCACGC ACCGGAACTGCACCTAAAGCACATCGGTAAGACCTG GGCACAGCTCGAGTGGGTCCCCGAGCCTCCCGAGCT AGGCAAGTCGCCGCTGACCCATTACACCATCTTCTG GACCAACGCGCAGAACCAGTCCTTTAGCGCGATCCT CAATGCCTCCTCCCGCGGTTTCGTCTTGCACGGGCTG GAGCCAGCCTCCTTATACCACATCCACCTGATGGCG GCTTCCCAGGCTGGTGCTACCAACAGCACAGTTTTG ACACTGATGACCCTGACCCCCGAGGGCTCGGAGCTC CACATCATTTTGGGACTCTTCGGACTGCTGCTGCTGC TGACCTGCCTGTGCGGCACTGCTTGGCTCTGCTGTTC	

			TCCCAACCGCAAGAACCCTCTTTGGCCCTCTGTCCCT GACCCGGCCCATTCTAGCCTGGGATCTTGGGTCCCG ACCATCATGGAAGAAGACGCGTTCCAGCTCCCTGGG CTGGGCACCCCTCCGATCACGAAGCTCACGGTCCTG GAGGAGGACGAGAAGAAGCCCGTACCGTGGGAGAG CCACTCCTCGAATCATAGCCTAACCTCCTGCTTTACA AACCAGGGGTATTTTTTTTTCCACTTGCCCGACGCTC TGGAGATCGAGGCCTGCCAGGTGTACTTCACCTACG ACCCCTACAGCGAGGAGGACCCTGCCGGTGATCTGC CCACCCATGACGGCTATCTGCCATCAAACATTGACG ACCTGCCGTCCGGAGGTCCCAGTCCTCCGTCCACCG CCCCCGGCGGTTCTGGTGCCGGCGAAGAACGGATGC CTCCCTCCCTGCAGGAGCGTGTGCCACGGGATTGGG ACCCACAGCCCTTAGGGCCGCCTACCCCCGGCGTGC CCGACCTCGTCGATTTCCAGCCGCCTCCTGAGCTGGT TCTCCGAGAGGCAGGAGAGGAGGTGCCTGATGCTGG ACCCCGTGAGGGCGTGAGCTTTCCTTGGTCTCGCCC GCCAGGACAGGGGGAGTTCCGTGCTCTCAACGCGCG TCTGCCGTTGAACACTGATGCATACCTCAGTCTTCAG GAGCTGCAGGGCCAGGATCCAACCCACCTCGTTGGC TCAGGGGAAGGTCGCGGCTCGCTGTTGACGTGTGGT GACGTGGAGGAGAACCCCGGCCCGTACTTCGGTAAG CTGGAGTCTAAACTGTCGGTGATACGGAACCTGAAC GACCAGGTCCTGTTCATTGACCAGGGTAACCGCCCG CTGTTCGAGGACATGACTGACTCCGATTGCCGCGAC AATGCCCCCCGCACGATCTTCATTATCTCCATGTACA AGGATAGCCAACCCCGTGGTATGGCCGTGACCATTA GCGTCAAATGTGAGAAGATCTCTACTCTGTCCTGTG AGAATAAGATTATCTCCTTCAAGGAGATGAACCCCC CTGATAACATCAAAGACACTAAATCGGACATCATTT TCTTTCAGAGGAGCGTGCCAGGCCACGACAACAAGA TGCAGTTCGAGAGTTCTAGTTACGAGGGATATTTCCT GGCCTGCGAAAAAGAACGTGACCTCTTCAAGCTAAT CCTGAAGAAGGAGGACGAGCTCGGTGACAGGAGCA TCATGTTTACTGTCCAAAACGAGGACTGA	
5' upstream sequence including Kozak		1-21	CCCGCCAGGATCCTGACGCGT	150
Mesothelin CAR	n/a	22-1482	ATGGTGCTGCTGGTCACCAGCTTACTCCTGTGCGAG CTCCCCCATCCTGCGTTCTTGCTGATCCCCGACATCC AGCAGGTGCAGCTGCAACAGTCGGGCCCCGAGTTAG AAAAACCCGGCGCCTCCGTGAAGATCTCGTGTAAAG CTTCGGGCTACTCGTTTACCGGCTACACCATGAATTG GGTTAAGCAGAGCCATGGAAAGTCTCTTGAATGGAT AGGCCTGATTACGCCTTATAATGGGGCCTCGAGCTA CAACCAGAAATTTCGTGGTAAGGCAACTCTGACTGT AGACAAGTCGTCCTCCACCGCATACATGGATCTTCT GAGCCTAACCTCAGAAGACAGCGCAGTCTACTTCTG CGCCCGTGGTGGTTACGATGGCCGCGGATTCGACTA TTGGGGCCAGGGCACCACCGTCACCGTGAGCTCCGG CGGTGGCGGGTCCGGTGGCGGCGGCTCAAGCGGAG	151

			GAGGTAGTGACATCGAGTTGACCCAGTCGCCTGCCA TCATGTCCGCATCCCCCGGCGAGAAGGTGACGATGA CGTGTTCTGCTTCCAGCTCTGTATCGTACATGCACTG GTACCAACAGAAGTCCGGCACCTCCCCCAAGCGTTG GATCTACGACACTTCCAAGCTGGCGTCTGGCGTCCC TGGGCGCTTTTCAGGTAGCGGGTCCGGCAATAGCTA TTCCTTGACGATCAGTAGCGTGGAGGCTGAGGATGA TGCCACCTACTACTGCCAGCAGTGGAGCAAGCACCC GCTCACCTTCGGCGCCGGGACCAAGCTTGAAATCAA GGCGGCGGCGATCGAGGTGATGTATCCTCCTCCCTA CCTGGACAACGAGAAATCCAATGGCACTATCATCCA CGTGAAGGGGAAGCACCTGTGCCCGAGCCCCCTGTT CCCCGGCCCTTCCAAGCCTTTTTGGGTGCTTGTTGTG GTCGGCGGCGTGCTGGCCTGCTACTCTCTGCTGGTA ACCGTAGCGTTCATCATCTTCTGGGTGCGATCTAAGC GGTCCCGGCTGTTGCACAGTGACTACATGAACATGA CCCCGCGCCGCCCCGGCCCAACCCGCAAGCACTACC AGCCGTACGCTCCACCTCGCGACTTTGCCGCCTACA GGTCGCGAGTGAAATTTTCGCGCTCCGCGGACGCGC CGGCCTATCAGCAGGGCCAGAACCAGCTTTACAACG AGTTGAACCTCGGGCGCAGGGAGGAGTACGACGTG CTGGATAAACGTAGAGGTCGGGACCCGGAGATGGG CGGCAAACCACGCCGCAAGAACCCTCAGGAAGGCC TGTACAACGAGCTCCAGAAGGACAAAATGGCTGAG GCGTACTCGGAGATTGGGATGAAAGGCGAGCGGCG CCGCGGCAAGGGCCACGATGGCCTGTACCAGGGGCT ATCTACCGCTACAAAGGACACCTACGACGCCCTACA TATGCAGGCCCTGCCACCGCGT	
P2A		1483-1548	GGCTCTGGCGCAACCAACTTCTCGCTTCTGAAGCAG GCCGGTGATGTGGAGGAAAACCCAGGTCCT	152
G12/2R-1 134 ECD		1549-4221	ATGGCCCGCCTGGGCAACTGCTCGCTGACCTGGGCG GCCCTGATCATCCTGCTGCTGCCCGGCTCTCTGGAGG AGTGTGGCCATATTTCCGTGTCAGCTCCAATCGTGCA CCTGGGCGACCCCATCACCGCTTCTTGCATCATCAA GCAGAACTGCTCTCACCTCGACCCTGAGCCCCAAAT ACTTTGGGAGCTGGGCGCGGAGCTCCAGCCTGGAGG ACGCCAGCAGCGTTTGTCAGACGGCACTCAGGAATC CATCATCACCCTCCCACATCTGAATCACACCCAGGC GTTCCTGTCTTGTTGTCTCAACTGGGGCAACTCCTTG CAGATACTGGACCAGGTGGAGCTGAGAGCTGGCTAT CCCCCGGCAATTCCTCACAACCTGTCCTGTCTGATGA ACCTGACCACCTCCAGTCTGATCTGTCAGTGGGAGC CCGGGCCAGAGACTCACCTTCCCACCAGCTTCACCC TTAAGTCGTTCAAATCCGAGGGCAACTGCCAGACCC AGGGTGATTCTATTTTGGATTGCGTGCCCAAGGACG GGCAGTCTCACTGTTGTATTCCCGACAAGCACCTCCT TTTATACCAGAATATGGGCATCTGGGTGCAGGCCGA GAATGCCCTGGGAACGTCTATGTCTCCACAGCTATG CCTGGATCCAATGGACGTAGTGAAGCTGGAGCCCCC CATGCTACGCACTATGGATCCTAGCCCAGAGGCCGC TCCCCCTCAGGCGGGGTGCCTGCAGCTTTGTTGGGA ACCCTGGCAACCCGGGTTGCACATTAACCAGAAGTG CGAGCTGCGCCACAAGCCCCAGAGAGGAGAGGCCT	153

			CCTGGGCCCTCGTGGGCCCTCTGCCGTTGGAGGCCTT GCAGTATGAGCTGTGCGGCCTCTTGCCCGCTACAGC TTATACTCTGCAAATCAGGTGTATTCGCTGGCCTTTG CCCGGACACTGGTCCGACTGGTCCCCCAGCCTTGAG CTGAGAACCACGGAGCGCGCCCCCACCGTACGCTTG GACACCTGGTGGCGCCAGAGGCAGCTGGACCCTCGC ACTGTGCAGCTGTTCTGGAAGCCGGTGCCCCTGGAG GAGGACTCCGGACGCATCCAGGGCTACGTGGTCAGC TGGCGTCCGAGTGGGCAGGCCGGGGCCATCCTGCCC CTGTGCAACACGACTGAGCTGAGCTGCACTTTCCAT CTGCCCAGCGAGGCTCAGGAGGTGGCGCTCGTGGCT TACAACTCCGCAGGCACTTCTCGCCCAACCCCCGTG GTGTTCTCTGAGAGTCGGGGCCCCGCCCTGACGCGC CTTCACGCCATGGCGCGCGACCCGCATTCCCTTTGG GTCGGTTGGGAGCCACCTAACCCATGGCCGCAGGGC TACGTGATCGAGTGGGGCCTGGGCCCCCCGAGCGCA TCGAACAGCAACAAGACCTGGAGAATGGAACAGAA CGGCCGCGCAACCGGCTTCCTATTAAAGGAGAACAT CCGCCCGTTCCAGCTGTACGAGATCATCGTGACACC GCTGTACCAGGACACTATGGGGCCTTCTCAGCACGT TTACGCCTACTCCCAGGAGATGGCCCCGAGCCACGC ACCGGAACTGCACCTAAAGCACATCGGTAAGACCTG GGCACAGCTCGAGTGGGTCCCCGAGCCTCCCGAGCT AGGCAAGTCGCCGCTGACCCATTACACCATCTTCTG GACCAACGCGCAGAACCAGTCCTTTAGCGCGATCCT CAATGCCTCCTCCCGCGGTTTCGTCTTGCACGGGCTG GAGCCAGCCTCCTTATACCACATCCACCTGATGGCG GCTTCCCAGGCTGGTGCTACCAACAGCACAGTTTTG ACACTGATGACCCTGACCCCCGAGGGCTCGGAGCTC CACATCATTTTGGGACTCTTCGGACTGCTGCTGCTGC TGACCTGCCTGTGCGGCACTGCTTGGCTCTGCTGTTC TCCCAACCGCAAGAACCCTCTTTGGCCCTCTGTCCCT GACCCGGCCCATTCTAGCCTGGGATCTTGGGTCCCG ACCATCATGGAAGAAGACGCGTTCCAGCTCCCTGGG CTGGGCACCCCTCCGATCACGAAGCTCACGGTCCTG GAGGAGGACGAGAAGAAGCCCGTACCGTGGGAGAG CCACTCCTCGAATCATAGCCTAACCTCCTGCTTTACA AACCAGGGGTATTTTTTTTTCCACTTGCCCGACGCTC TGGAGATCGAGGCCTGCCAGGTGTACTTCACCTACG ACCCCTACAGCGAGGAGGACCCTGCCGGTGATCTGC CCACCCATGACGGCTATCTGCCATCAAACATTGACG ACCTGCCGTCCGGAGGTCCCAGTCCTCCGTCCACCG CCCCCGGCGGTTCTGGTGCCGGCGAAGAACGGATGC CTCCCTCCCTGCAGGAGCGTGTGCCACGGGATTGGG ACCCACAGCCCTTAGGGCCGCCTACCCCCGGCGTGC CCGACCTCGTCGATTTCCAGCCGCCTCCTGAGCTGGT TCTCCGAGAGGCAGGAGAGGAGGTGCCTGATGCTGG ACCCCGTGAGGGCGTGAGCTTTCCTTGGTCTCGCCC GCCAGGACAGGGGGAGTTCCGTGCTCTCAACGCGCG TCTGCCGTTGAACACTGATGCATACCTCAGTCTTCAG GAGCTGCAGGGCCAGGATCCAACCCACCTCGTT	
T2A		4222-4284	GGCTCAGGGGAAGGTCGCGGCTCGCTGTTGACGTGT GGTGACGTGGAGGAGAACCCCGGCCCG	154

IL-18	NM_001562.4	4285-4758	TACTTCGGTAAGCTGGAGTCTAAACTGTCGGTGATA CGGAACCTGAACGACCAGGTCCTGTTCATTGACCAG GGTAACCGCCCGCTGTTCGAGGACATGACTGACTCC GATTGCCGCGACAATGCCCCCCGCACGATCTTCATT ATCTCCATGTACAAGGATAGCCAACCCCGTGGTATG GCCGTGACCATTAGCGTCAAATGTGAGAAGATCTCT ACTCTGTCCTGTGAGAATAAGATTATCTCCTTCAAGG AGATGAACCCCCCTGATAACATCAAAGACACTAAAT CGGACATCATTTTCTTTCAGAGGAGCGTGCCAGGCC ACGACAACAAGATGCAGTTCGAGAGTTCTAGTTACG AGGGATATTTCCTGGCCTGCGAAAAAGAACGTGACC TCTTCAAGCTAATCCTGAAGAAGGAGGACGAGCTCG GTGACAGGAGCATCATGTTTACTGTCCAAAACGAGG ACTGA	155
G12-2R1_134_ECD_T2A_hIL-18		1-3210	ATGGCTCGCCTCGGAAATTGTTCCCTGACCTGGGCA GCATTGATCATTCTCCTGCTACCAGGGAGTCTCGAA GAATGTGGCCATATCTCTGTAAGCGCCCCTATTGTGC ACTTGGGAGACCCAATCACAGCATCATGCATCATCA AGCAGAATTGCTCTCATCTCGATCCAGAGCCACAGA TACTCTGGGAGCTGGGCGCAGAACTGCAACCCGGAG GGCGCCAGCAGAGACTGAGTGATGGAACACAGGAG AGCATTATCACTTTACCTCATCTGAACCACACCCAG GCCTTTCTTAGCTGCTGTTTGAACTGGGGCAACAGTC TCCAGATTCTGGACCAGGTGGAGCTGAGGGCGGGTT ACCCGCCCGCCATTCCACACAATTTGTCCTGTCTCAT GAACCTGACAACAAGTAGCCTGATATGCCAGTGGGA GCCAGGGCCGGAGACCCATCTCCCAACTAGTTTCAC GCTCAAGAGCTTCAAGTCAGAGGGGAACTGCCAGAC ACAAGGGGACAGCATTCTCGACTGTGTGCCCAAGGA CGGGCAGAGTCACTGTTGCATTCCAGACAAGCATCT GCTGCTGTATCAGAACATGGGCATTTGGGTTCAGGC CGAAAATGCCCTCGGTACTTCCATGAGTCCACAGTT GTGTCTTGACCCCATGGATGTGGTGAAGCTGGAACC GCCAATGCTGAGGACCATGGATCCCTCACCTGAGGC TGCTCCGCCACAGGCAGGGTGTCTGCAGCTGTGCTG GGAGCCCTGGCAGCCTGGATTGCACATAAACCAAAA ATGTGAGCTGAGGCACAAGCCCCAGAGAGGTGAGG CGAGTTGGGCCCTGGTAGGGCCTCTTCCTCTAGAAG CCCTGCAGTATGAATTGTGTGGATTACTTCCCGCAAC CGCCTACACTCTTCAGATCAGGTGTATCCGGTGGCCT CTGCCCGGCCACTGGAGCGACTGGTCTCCGAGCCTC GAATTGAGAACAACAGAACGGGCTCCTACGGTGCGT CTGGATACATGGTGGAGGCAGCGACAGTTGGACCCG CGTACAGTGCAGCTGTTCTGGAAGCCCGTACCTCTG GAAGAGGATAGTGGTCGGATTCAGGGATATGTGGTC TCTTGGCGGCCTTCCGGTCAGGCTGGGGCTATCCTTC CCTTATGCAACACTACAGAGCTCAGCTGTACCTTTCA TTTACCCAGCGAAGCCCAGGAAGTGGCTCTCGTCGC CTATAATTCCGCTGGAACGTCAAGACCCACCCCTGT CGTGTTCTCTGAGTCTCGGGGACCTGCCCTAACCAG GCTCCATGCAATGGCACGCGATCCTCACAGTCTGTG GGTTGGCTGGGAACCACCAAATCCCTGGCCTCAGGG CTATGTCATCGAATGGGGCCTTGGCCCCCCATCAGC CTCCAATAGCAACAAGACCTGGAGAATGGAGCAAA	156

			ATGGGAGAGCAACCGGGTTCCTGCTGAAGGAGAAC ATCCGACCGTTCCAGCTTTATGAAATTATTGTAACTC CACTGTACCAGGACACAATGGGACCATCTCAGCATG TCTACGCTTATTCCCAGGAGATGGCGCCTAGTCATG CTCCTGAATTACACTTGAAGCACATAGGGAAAACTT GGGCCCAATTAGAGTGGGTCCCGGAGCCTCCCGAGC TGGGGAAAAGCCCATTGACCCATTACACGATATTTT GGACCAATGCTCAGAACCAATCCTTTTCTGCCATCCT GAATGCCTCATCTCGAGGCTTTGTGCTGCATGGCCTA GAGCCTGCATCACTCTACCACATTCACCTGATGGCC GCCAGCCAGGCGGGTGCCACAAACTCCACAGTTCTC ACTTTAATGACTCTAACCCCAGAGGGCAGTGAACTG CACATCATATTAGGGTTATTCGGATTGCTGCTTCTTT TGACTTGCCTCTGTGGCACTGCGTGGCTGTGCTGCTC CCCGAACCGAAAGAATCCTCTCTGGCCTAGCGTTCC CGATCCTGCTCACAGTTCACTAGGATCATGGGTGCC AACCATCATGGAAGAGGATGCCTTCCAGCTACCAGG ACTGGGTACGCCGCCCATAACCAAATTAACGGTCCT TGAAGAGGATGAGAAGAAACCAGTTCCATGGGAAA GCCACTCTTCCAATCATAGCCTCACCTCTTGCTTCAC CAACCAAGGGTATTTTTTTTTCCACCTGCCCGACGCT CTGGAAATTGAGGCCTGCCAAGTGTATTTCACCTAC GACCCTTACTCTGAAGAAGATCCGGCAGGCGACCTA CCCACTCATGATGGCTACCTGCCGTCAAACATCGAC GACTTACCGAGTGGAGGACCCTCCCCACCTTCTACA GCCCCTGGTGGCTCCGGTGCAGGGGAGGAAAGGAT GCCTCCCTCACTGCAGGAGAGAGTCCCCAGAGACTG GGATCCTCAGCCACTTGGACCACCCACACCTGGTGT GCCTGACCTTGTGGATTTTCAGCCCCCTCCTGAGCTT GTTCTTCGAGAAGCAGGTGAGGAGGTTCCTGATGCA GGCCCACGTGAGGGGGTCTCCTTTCCCTGGTCCAGG CCTCCTGGCCAAGGAGAGTTCCGCGCTCTGAATGCA CGCTTGCCTCTGAACACAGATGCTTACCTGAGCCTA CAAGAGTTACAAGGCCAGGATCCGACACACCTGGTG GGATCTGGCGAGGGTAGAGGCAGCCTCCTAACTTGT GGCGACGTGGAGGAGAACCCCGGTCCCTACTTCGGT AAGCTGGAGTCTAAACTGTCGGTGATACGGAACCTG AACGACCAGGTCCTGTTCATTGACCAGGGTAACCGC CCGCTGTTCGAGGACATGACTGACTCCGATTGCCGC GACAATGCCCCCCGCACGATCTTCATTATCTCCATGT ACAAGGATAGCCAACCCCGTGGTATGGCCGTGACCA TTAGCGTCAAATGTGAGAAGATCTCTACTCTGTCCTG TGAGAATAAGATTATCTCCTTCAAGGAGATGAACCC CCCTGATAACATCAAAGACACTAAATCGGACATCAT TTTCTTTCAGAGGAGCGTGCCAGGCCACGACAACAA GATGCAGTTCGAGAGTTCTAGTTACGAGGGATATTT CCTGGCCTGCGAAAAAGAACGTGACCTCTTCAAGCT AATCCTGAAGAAGGAGGACGAGCTCGGTGACAGGA GCATCATGTTTACTGTCCAAAACGAGGACTGATGA	
G12/2R-1 134 ECD		1-2673	ATGGCTCGCCTCGGAAATTGTTCCCTGACCTGGGCA GCATTGATCATTCTCCTGCTACCAGGGAGTCTCGAA GAATGTGGCCATATCTCTGTAAGCGCCCCTATTGTGC ACTTGGGAGACCCAATCACAGCATCATGCATCATCA AGCAGAATTGCTCTCATCTCGATCCAGAGCCACAGA	157

			TACTCTGGGAGCTGGGCGCAGAACTGCAACCCGGAG GGCGCCAGCAGAGACTGAGTGATGGAACACAGGAG AGCATTATCACTTTACCTCATCTGAACCACACCCAG GCCTTTCTTAGCTGCTGTTTGAACTGGGGCAACAGTC TCCAGATTCTGGACCAGGTGGAGCTGAGGGCGGGTT ACCCGCCCGCCATTCCACACAATTTGTCCTGTCTCAT GAACCTGACAACAAGTAGCCTGATATGCCAGTGGGA GCCAGGGCCGGAGACCCATCTCCCAACTAGTTTCAC GCTCAAGAGCTTCAAGTCAGAGGGGAACTGCCAGAC ACAAGGGGACAGCATTCTCGACTGTGTGCCCAAGGA CGGGCAGAGTCACTGTTGCATTCCAGACAAGCATCT GCTGCTGTATCAGAACATGGGCATTTGGGTTCAGGC CGAAAATGCCCTCGGTACTTCCATGAGTCCACAGTT GTGTCTTGACCCCATGGATGTGGTGAAGCTGGAACC GCCAATGCTGAGGACCATGGATCCCTCACCTGAGGC TGCTCCGCCACAGGCAGGGTGTCTGCAGCTGTGCTG GGAGCCCTGGCAGCCTGGATTGCACATAAACCAAAA ATGTGAGCTGAGGCACAAGCCCCAGAGAGGTGAGG CGAGTTGGGCCCTGGTAGGGCCTCTTCCTCTAGAAG CCCTGCAGTATGAATTGTGTGGATTACTTCCCGCAAC CGCCTACACTCTTCAGATCAGGTGTATCCGGTGGCCT CTGCCCGGCCACTGGAGCGACTGGTCTCCGAGCCTC GAATTGAGAACAACAGAACGGGCTCCTACGGTGCGT CTGGATACATGGTGGAGGCAGCGACAGTTGGACCCG CGTACAGTGCAGCTGTTCTGGAAGCCCGTACCTCTG GAAGAGGATAGTGGTCGGATTCAGGGATATGTGGTC TCTTGGCGGCCTTCCGGTCAGGCTGGGGCTATCCTTC CCTTATGCAACACTACAGAGCTCAGCTGTACCTTTCA TTTACCCAGCGAAGCCCAGGAAGTGGCTCTCGTCGC CTATAATTCCGCTGGAACGTCAAGACCCACCCCTGT CGTGTTCTCTGAGTCTCGGGGACCTGCCCTAACCAG GCTCCATGCAATGGCACGCGATCCTCACAGTCTGTG GGTTGGCTGGGAACCACCAAATCCCTGGCCTCAGGG CTATGTCATCGAATGGGGCCTTGGCCCCCCATCAGC CTCCAATAGCAACAAGACCTGGAGAATGGAGCAAA ATGGGAGAGCAACCGGGTTCCTGCTGAAGGAGAAC ATCCGACCGTTCCAGCTTTATGAAATTATTGTAACTC CACTGTACCAGGACACAATGGGACCATCTCAGCATG TCTACGCTTATTCCCAGGAGATGGCGCCTAGTCATG CTCCTGAATTACACTTGAAGCACATAGGGAAAACTT GGGCCCAATTAGAGTGGGTCCCGGAGCCTCCCGAGC TGGGGAAAAGCCCATTGACCCATTACACGATATTTT GGACCAATGCTCAGAACCAATCCTTTTCTGCCATCCT GAATGCCTCATCTCGAGGCTTTGTGCTGCATGGCCTA GAGCCTGCATCACTCTACCACATTCACCTGATGGCC GCCAGCCAGGCGGGTGCCACAAACTCCACAGTTCTC ACTTTAATGACTCTAACCCCAGAGGGCAGTGAACTG CACATCATATTAGGGTTATTCGGATTGCTGCTTCTTT TGACTTGCCTCTGTGGCACTGCGTGGCTGTGCTGCTC CCCGAACCGAAAGAATCCTCTCTGGCCTAGCGTTCC CGATCCTGCTCACAGTTCACTAGGATCATGGGTGCC AACCATCATGGAAGAGGATGCCTTCCAGCTACCAGG ACTGGGTACGCCGCCCATAACCAAATTAACGGTCCT TGAAGAGGATGAGAAGAAACCAGTTCCATGGGAAA	

			GCCACTCTTCCAATCATAGCCTCACCTCTTGCTTCAC CAACCAAGGGTATTTTTTTTTCCACCTGCCCGACGCT CTGGAAATTGAGGCCTGCCAAGTGTATTTCACCTAC GACCCTTACTCTGAAGAAGATCCGGCAGGCGACCTA CCCACTCATGATGGCTACCTGCCGTCAAACATCGAC GACTTACCGAGTGGAGGACCCTCCCCACCTTCTACA GCCCCTGGTGGCTCCGGTGCAGGGGAGGAAAGGAT GCCTCCCTCACTGCAGGAGAGAGTCCCCAGAGACTG GGATCCTCAGCCACTTGGACCACCCACACCTGGTGT GCCTGACCTTGTGGATTTTCAGCCCCCTCCTGAGCTT GTTCTTCGAGAAGCAGGTGAGGAGGTTCCTGATGCA GGCCCACGTGAGGGGGTCTCCTTTCCCTGGTCCAGG CCTCCTGGCCAAGGAGAGTTCCGCGCTCTGAATGCA CGCTTGCCTCTGAACACAGATGCTTACCTGAGCCTA CAAGAGTTACAAGGCCAGGATCCGACACACCTGGTG	
T2A		2674-2736	GGATCTGGCGAGGGTAGAGGCAGCCTCCTAACTTGT GGCGACGTGGAGGAGAACCCCGGTCCC	157

TABLE 32: PROTEIN SEQUENCE OF HUMAN IL-7RA EXTRACELLULAR, TRANSMEMBRANE AND INTRACELLULAR DOMAINS AND SIGNAL PEPTIDE

Protein	Uniprot ID	Domain & residue numbering	Polypeptide Sequence	SEQ ID NO.
IL-7Rα	P16871	ECD: 1-219	ESGYAQNGDLEDAELDDYSFSCYSQLEVNGSQHSLTCAFEDPDV NITNLEFEICGALVEVKCLNFRKLQEIYFIETKKFLLIGKSNICVKVG EKSLTCKKIDLTTIVKPEAPFDLSVVYREGANDFVVTFNTSHLQKK YVKVLMHDVAYRQEKDENKWTHVNLSSTKLTLLQRKLQPAAMY EIKVRSIPDHYFKGFWSEWSPSYYFRTPEINNSSGEMD	187
IL-7Rα	P16871	TMD: 220-244	PILLTISILSFFSVALLVILACVLW	188
IL-7Rα	P16871	ICD: 245-439	KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHR VDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSED VVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPH VYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQ EEAYVTMSSFYQNQ	189
IL-7Rα	P16871	Signal peptide	MTILGTTFGMVFSLLQVVSG	190
IL-7	A8K673	Cytokine: 1-152	DCDIEGKDGKQYESVLMVSIDQLLDSMKEIGSNCLNNEFNFFKRHI CDANKEGMFLFRAARKLRQFLKMNSTGDFDLHLLKVSEGTTILLNC TGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDLCFLKRLLQEIK TCWNKILMGTKEH	191

TABLE 33: INTRACELLULAR DOMAINS (ICDS). THE ICD CONSISTS OF ONE OF THE FOLLOWING:

Engineered Construct	Protein (Domain)	Uniprot ID	Polypeptide Sequence	SEQ ID NO.

7/2R-1	IL-2Rβ: Box 1 underlined Box 2, Shc, & STAT5 binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPTHLV*	192
7/2R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**RVDDIQARDEVEG	193
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPTHLV*	194
7/2/12R-1	IL-2Rβ: Box 1 underlined Box 2, Shc, & STAT5 binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPTHLV	192
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	TVLPAGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDSLML*	195
7/2/12R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**RVDDIQARDEVEG	193
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPTHLV	194
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	TVLPAGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDSLML*	195
7/12/2R-1	IL-2Rβ: Box 1 underlined Box 2 and Shc binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDP	196
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	AGDLPTHDGYLPSNIDDLPS	197

	IL-2Rβ: STAT5 binding site bold underlined *stop codon	P14784	GGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTP GVPDLVDFQPPPELVLREAGEEVPDAGPREGVSFPWSRPPGQG EFRALNARLPLNTDA**Y**LSLQELQGQDPTHLV*	198
7/12/2R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQAR	199
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	AGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSL HPLTFSCGDKLTLDQLKMRC	200
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDA LEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDA YCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQER VPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPT HLV*	194
7/21-R1	IL-21R: Box 1 underlined Box 2 & STAT3 binding site bold underlined	Q9HBE5	LWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSS**LE LG**PWSPEVPST**LEVY**SCHPPRSPAKRLQLTELQEPAELVESDGVP KPSFWPTAQNSGGSAYSEERDRPYGLVSIDTVTVLDAEGPCTWP CSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSP GLGGPLGSLLDRLKPPLADGEDWAGGLPWGGRSPGGVSESEAG SPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGPPRS**Y**LRQ WVVIPPPLSSPGPQAS*	201
7/21-R2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARD	202
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	RSPAKRLQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEE RDRPYGLVSIDTVTVLDAEGPCTWPCSCEDDGYPALDLDAGLEP SPGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLLDRLKPPLAD GEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTFDSGFVGS DCSSPVECDFTSPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	203
7/21/7R-1	IL-21R: Box 1 underlined Box 2 & STAT3 binding site bold underlined	Q9HBE5	LWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSS**LE LG**PWSPEVPST**LEVY**SCHPPRSPAKRLQLTELQEPAELVESDGVP KPSFWPTAQNSGGSAYSEERDRPYGLVSIDTVTVLDAEGPCTWP CSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVSAGSP GLGGPLGSLLDRLKPPLADGEDWAGGLPWGGRSPGGVSESEAG SPLAGLDMDTFDSGFVGSDCSSPVECDFTSPGDEGPPRS**Y**LRQ WVVIPPPLSSPGPQAS	201
	IL-7Rα: STAT5 and PI3K binding sites boldunderlined	P16871	DCRESGKNGPHV**Y**QDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQ GQPILTSLGSNQEEA**Y**VTMSSF**Y**QNQ*	204
7/7/21R-1	IL-7Rα: Box 1 underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSED VVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPH	205

	Box 2, STAT5 & PI3K binding sites bold underlined		V**Y**QDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQ EEA**Y**VTMSSF**Y**QNQ	
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	206
7/2/21R-1	IL-2Rβ: Box 1 underlined Box 2, Shc & STAT5 binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFP SSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLT SCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPT GSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPE LVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD A**Y**LSLQELQGQDPTHLV	192
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	206
7/2/21R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARDEVEG	193
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDA LEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDA YCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQER VPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPT HLV	194
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	206
7/2/12/21R -1	IL-2Rβ: Box 1 underlined Box 2, Shc & STAT5 binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFP SSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLT SCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPT GSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPE LVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD A**Y**LSLQELQGQDPTHLV	192
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	TVLPAGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVF PSSSLHPLTFSCGDKLTLDQLKMRCDSLML	195
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	206
7/2/12/21R -2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARDEVEG	193

	IL-2Rβ: Shc & STAT5 binding sites bold underlined	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDA LEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDA YCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQER VPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPT HLV	194
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	TVLPAGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVF PSSSLHPLTFSCGDKLTLDQLKMRCDSLML	195
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQAS*	206
7/2/21/12R -1	IL-2Rβ: Box 1 underlined Box 2, Shc, & STAT5 binding sites bold underlined	P14784	NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFP SSSFSPGGLAPEISP**LEVL**ERDKVTQLLLQQDKVPEPASLSSNHSLT SCFTNQG**Y**FFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPT GSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGG SGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPE LVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTD A**Y**LSLQELQGQDPTHLV	192
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQA	207
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	AGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSL HPLTFSCGDKLTLDQLKMRCDSLML*	208
7/2/21/12R -2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARDEVEG	193
	IL-2Rβ: Shc & STAT5 binding sites bold underlined *stop codon	P14784	LERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQG**Y**FFFHLPDA LEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDA YCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQER VPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGP REGVSFPWSRPPGQGEFRALNARLPLNTDA**Y**LSLQELQGQDPT HLV	194
	IL-21R: STAT3 binding site bold underlined	Q9HBE5	SPGDEGPPRS**Y**LRQWVVIPPPLSSPGPQA	207
	IL-12Rβ2: STAT4 binding site bold underlined	Q99665	AGDLPTHDG**Y**LPSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSL HPLTFSCGDKLTLDQLKMRCDSLML*	208
7/4R-1	IL-4Rα: Box 1 underlined Box 2, IRS-2 & STAT6 binding sites bold underlined	P24394	KIKKEWWDQIPNPARSRLVAIIIQDAQGSQWEKRSRGQEPAKCP HWKNCLTKLLP**CFLE**HNMKRDEDPHKAAKEMPFQGSGKSAWC PVEISKTVLWPESISVVRCVELFEAPVECEEEEEVEEEKGSFCASPE SSRDDFQEGREGIVARLTESLFLDLLGEENGGFCQQDMGESCLLP PSGSTSAHMPWDEFPSAGPKEAPPWGKEQPLHLEPSPPASPTQ SPDNLTCTETPLVIAGNPA**Y**RSFSNSLSQSPCPRELGPDPLLARHL	209

			EEVEPEMPCVPQLSEPTTVPQPEPETWEQILRRNVLQHGAAAAP VSAPTSGYQEFVHAVEQGGTQASAVVGLGPPGEAG**Y**KAFSSLLA SSAVSPEKCGFGASSGEEG**Y**KPFQDLIPGCPGDPAPVPVPLFTFG LDREPPRSPQSSHLPSSSPEHLGLEPGEKVEDMPKPPLPQEQATD PLVDSLGSGIVYSALTCHLCGHLKQCHGQEDGGQTPVMASPCC GCCCGDRSSPPTTPLRAPDPSPGGVPLEASLCPASLAPSGISEKSK SSSSFHPAPGNAQSSSQTPKIVNFVSVGPTYMRVS*	
7/4R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQA	210
	IL-4Rα: IRS-2 & STAT6 binding sites bold underlined	P24394	DEDPHKAAKEMPFQGSGKSAWCPVEISKTVLWPESISVVRCVEL FEAPVECEEEEEVEEEKGSFCASPESSRDDFQEGREGIVARLTESL FLDLLGEENGGFCQQDMGESCLLPPSGSTSAHMPWDEFPSAGP KEAPPWGKEQPLHLEPSPPASPTQSPDNLTCTETPLVIAGNPA**Y**R SFSNSLSQSPCPRELGPDPLLARHLEEVEPEMPCVPQLSEPTTVP QPEPETWEQILRRNVLQHGAAAAPVSAPTSGYQEFVHAVEQGG TQASAVVGLGPPGEAG**Y**KAFSSLLASSAVSPEKCGFGASSGEEG**Y** KPFQDLIPGCPGDPAPVPVPLFTFGLDREPPRSPQSSHLPSSSPEH LGLEPGEKVEDMPKPPLPQEQATDPLVDSLGSGIVYSALTCHLCG HLKQCHGQEDGGQTPVMASPCCGCCCGDRSSPPTTPLRAPDPS PGGVPLEASLCPASLAPSGISEKSKSSSSFHPAPGNAQSSSQTPKI VNFVSVGPTYMRVS*	211
7/6R-1	Gp130: Box 1 underlined Box 2, SHP-2 & STAT3 binding sites bold underlined	P40189	KNKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNSKDQMY SDGNFTD**VSVV**EIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIG GSSCMSSSRPSISSSDENESSQNTSSTVQ**Y**STVVHSG**Y**RHQVPSV QVFSRSESTQPLLDSEERPEDLQLVDHVDGGDGILPRQQ**Y**FKQN CSQHESSPDISHFERSKQVSSVNEEDFVRLKQQISDHISQSCGSG QMKMFQEVSAADAFGPGTEGQVERFETVGMEAATDEGMPKS **Y**LPQTVRQGG**Y**MPQ*	212
7/6R-2	IL-7Rα: Box 1 underlined Box 2 bold underlined	P16871	KKRIKPIVWPSLPDHKKTLEHLCKKPRKN**LNVS**FNPESFLD**CQIH**R VDDIQARDEVE	213
	Gp130: SHP-2 & STAT3 binding sites bold underlined	P40189	KKPFPEDLKSLDLFKKEKINTEGHSSGIGGSSCMSSSRPSISSSDEN ESSQNTSSTVQ**Y**STVVHSG**Y**RHQVPSVQVFSRSESTQPLLDSEER PEDLQLVDHVDGGDGILPRQQ**Y**FKQNCSQHESSPDISHFERSKQ VSSVNEEDFVRLKQQISDHISQSCGSGQMKMFQEVSAADAFGP GTEGQVERFETVGMEAATDEGMPKS**Y**LPQTVRQGG**Y**MPQ*	214

TABLE 34: DOMAIN COMPOSITION OF CHIMERIC CYTOKINE RECEPTORS

Name	Signal Peptide	ECD	TMD	Box1/2	ICD	STAT site(s)	Other site(s)
7/2R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (192)	IL-2Rβ (192)	5	Shc
7/2R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (193)	IL-2Rβ (194)	5	Shc
7/2/12R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (192)	IL-2Rβ (195)	4,5	Shc

					IL-12Rβ2 (195)		
7/2/12R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (193)	IL-2Rβ (194) IL-12Rβ2 (195)	4,5	Shc
7/12/2R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (196)	IL-2Rβ (196) IL-12Rβ2 (197) IL-2Rβ (198)	4,5	Shc
7/12/2R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (199)	IL-12Rβ2 (200) IL-2Rβ (194)	4,5	Shc
7/21-R1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-21R (201)	IL-21R (201)	3	N/A
7/21-R2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (202)	IL-21R (203)	3	N/A
7/21/7R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-21R (201)	IL-21R (201) IL-7Rα (204)	3,5	PI3K
7/7/21R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (205)	IL-7Rα (205) IL-21R (206)	3,5	PI3K
7/2/21R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (192)	IL-2Rβ (192) IL-21R (206)	3,5	Shc
7/2/21R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (193)	IL-2Rβ (194) IL-21R (206)	3,5	Shc
7/2/12/21 R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (192)	IL-2Rβ (192) IL-12Rβ2 (195) IL-21R (206)	3,4,5	Shc
7/2/12/21 R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (193)	IL-2Rβ (194) IL-12Rβ2 (195) IL-21R (206)	3,4,5	Shc
7/2/21/12 R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-2Rβ (192)	IL-2Rβ (192) IL-21R (207) IL-12Rβ2 (208)	3,4,5	Shc
7/2/21/12 R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (193)	IL-2Rβ (194) IL-21R (207) IL-12Rβ2 (208)	3,4,5	Shc
7/4R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-4Rα (209)	IL-4Rα (209)	6	IRS-2
7/4R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (210)	IL-7Rα (210) IL-4Rα (211)	6	IRS-2
7/6R-1	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	gp130 (212)	gp130 (212)	3	SHP-2
7/6R-2	IL-7Rα (190)	IL-7Rα (187)	IL-7Rα (188)	IL-7Rα (213)	IL-7Rα (213) gp130 (214)	3	SHP-2

Table 35: CONTROLLED PARACRINE SIGNALING AND DETECTION OF SECRETED CYTOKINES IN MURINE OT-I T CELLS TRANSDUCED WITH RETROVIRAL VECTORS ENCODING CHIMERIC CYTOKINE RECEPTORS

Receptor	Cytokine	IL-18	Eotaxin	G-CSF	GM-CSF	IFN-g	IL-1a	IL-1b	IL-2	IL-3	IL-4	IL-5
G2R-2	Medium	OOR <	OOR <	0.79	3.31	0.95	0.58	0.43	OOR <	1.25	0.12	OOR <
G2R-2	IL-2	OOR <	OOR <	1.08	13.70	1.32	OOR <	0.14	OOR <	0.35	0.14	0.57
G2R-2	PEG-130a1	OOR <	0 96	0.91	17.70	2.29	2.51	OOR<	OOR <	0.35	0.12	1.09
G2R-3	Medium	OOR <	OOR <	0.86	3.34	0.83	OOR <	029	OOR <	0.86	0.17	OOR <
G2R-3	IL-2	OOR <	OOR <	1.01	14.09	0.86	OOR <	0.14	OOR <	0.45	0.15	0.30
G2R-3	PEG-130a1	OOR <	OOR <	1.01	14.52	0.95	OOR <	OOR<	OOR <	0.35	0.12	0.06
G12/2R-1	Medium	OOR <	OOR <	0.72	3.34	1.20	OOR <	OOR<	OOR <	0.92	0.16	OOR <
G12/2R-1	IL-2 + IL-12	OOR <	OOR <	0.79	95.76	717.93	7.94	029	OOR <	OOR <	0.18	OOR <
G12/2R-1	PEG-130a1	OOR <	OOR <	1.38	15.79	2.00	0.96	0.43	OOR <	0.19	0.16	OOR <
G12/2R-1 +m18	Medium	155.62	0.18	0.86	12.20	6.92	OOR <	OOR<	OOR <	0.17	021	OOR <
G12/2R-1 + m18	IL-2 + IL-12	288.24	OOR <	1.52	972.75	9871.71	40.19	OOR<	0.59	0.86	023	0.81
G12/2R-1 + m18	PEG-130a1	811.10	OOR <	1.27	271.81	7426.63	19.56	OOR<	OOR <	0.47	020	7.59

Receptor	Cytokine	IL-6	IL-7	IL-9	IL-10	IL-12p40	IL-12p70	IL-13	IL-15	IL-17	IP-10	KC
G2R-2	Medium	0.24	025	OOR <	2.61	OOR <	3.19	OOR <	1.28	OOR<	2.46	0.96
G2R-2	IL-2	0.38	0.54	OOR <	52.28	OOR <	1.77	OOR<	OOR <	0.10	2.97	2.48
G2R-2	PEG-130a1	0.27	0.74	OOR <	300.01	OOR <	526	OOR <	2.56	0.28	3.59	1.20
G2R-3	Medium	0.34	0.30	OOR <	3.29	OOR <	1.73	OOR <	OOR <	0.07	1.98	0.57
G2R-3	IL-2	0.33	0.19	OOR <	44.22	OOR <	2.59	OOR <	OOR <	OOR <	2.74	0.55
G2R-3	PEG-130a1	0.28	0.08	OOR <	68.26	OOR <	2.17	OOR <	OOR <	0.03	2.90	0.21
G12/2R	Medium	0.49	023	OOR <	2.43	OOR <	1.90	OOR<	OOR <	OOR <	2.76	0.55
G12/2R	IL-2 + IL-12	0.33	0.11	020	1402.50	24.79	1166.81	OOR<	OOR <	0.10	4.29	0.96
G12/2R	PEG-130a1	0.29	0.05	020	160.37	OOR <	2.79	OOR<	OOR <	OOR <	3.26	8.23
G12/2R/18C	Medium	0.48	0.30	OOR <	12.99	OOR <	1.77	OOR<	0.65	0.47	8.95	1.05
G12/2R/18C	IL-2 + IL-12	18.08	0.54	0.39	2911.40	19.69	875.39	OOR<	OOR <	1.51	18.77	2.57
G12/2R/18C	PEG-130a1	1.94	0.53	OOR <	1202.95	OOR <	2.99	OOR <	0.97	0.79	11.07	0.44

Receptor	Cytokine	LIF	LIX	MCP-1	M-CSF	MG	MP-1a	MP-1b	MP-2	RANTES	TNFa	VEGF
G2R-2	Medium	11.58	96.40	5.13	0.28	0.02	4.19	OOR <	OOR <	0.33	2.69	3.67
G2R-2	IL-2	11.91	80.86	320	0.73	0.02	OOR <	OOR <	20.29	1.64	6.56	45.30
G2R-2	PEG-130a1	15.25	87.57	0.99	0.23	0.04	4.02	12.86	33.70	3.12	10.33	78.42
G2R-3	Medium	7.70	53.21	6.51	0.28	0.04	OOR <	OOR <	15.72	0.48	3.01	6.90
G2R-3	IL-2	7.55	78.33	4.30	0.46	OOR <	3.38	OOR <	13.24	1.60	5.45	40.10
G2R-3	PEG-130a1	8.86	104.04	4.86	0.32	OOR <	OOR <	OOR <	OOR <	1.95	7.34	76.88
G12/2R	Medium	5.38	69.45	5.96	0.46	OOR <	OOR <	5.61	37.16	1.14	2.44	2.88
G12/2R	IL-2 + IL-12	12.60	80.50	9.57	0.82	0.18	7.98	3.52	7.05	1.92	7.12	38.94
G12/2R	PEG-130a1	7.07	117.52	4.86	0.73	0.07	3.38	OOR <	33.70	2.82	6.57	56.95
G12/2R/18C	Medium	13.79	70.40	4.58	0.82	0.06	3.38	OOR <	39.25	5.58	6.77	8.30
G12/2R/18C	IL-2 + IL-12	39.84	88.99	8.18	0.60	0.39	7.98	7.97	26.47	6.46	26.12	121.10
G12/2R/18C	PEG-130a1	17.54	85.52	7.07	0.87	0.08	OOR <	OOR <	20.86	8.46	16.67	78.03

CLAIMS

1. A variant Granulocyte Colony-Stimulating Factor (G-CSF), wherein

the variant G-CSF comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof; wherein the at least one mutation in the site II interface region comprises at least one of: L108R, D112R, E122R E122K, E123K and E123R and combinations thereof; wherein the site II interface region mutations are relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; wherein

the at least one mutation in the site III interface region comprises mutation E46R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein

the variant G-CSF binds selectively to a receptor comprising a variant extracellular domain (ECD) of Granulocyte Colony-Stimulating Factor Receptor (G-CSFR).

2. A system for selective activation of a receptor expressed on a cell surface, the system comprising:

(a) a variant G-CSF corresponding to SEQ ID NO: 83 or 84; and

(b) a receptor comprising a variant ECD of G-CSFR; wherein

the variant G-CSF preferentially binds the receptor comprising the variant ECD of G-CSFR as compared to an otherwise identical wild type G-CSFR ECD, and the receptor comprising the variant ECD of G-CSFR preferentially binds the variant G-CSF as compared to an otherwise identical wild type G-CSF; and wherein the variant G-CSFR comprises G2R-3 or G12/2R-1.

3. The variant G-CSF of claim 1, wherein the variant G-CSF binds a receptor comprising a variant ECD of G-CSFR that is expressed by a cell.

4. The variant G-CSF of claim 3, wherein the cell expressing the receptor comprising the variant ECD of G-CSFR is an immune cell.

5. The variant G-CSF of claim 4, wherein the immune cell expressing the receptor comprising the variant ECD of G-CSFR is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

6. The variant G-CSF of claim 5, wherein the T-cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve CD8+T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

7. The variant G-CSF of any one of claims 1- 6, wherein

the selective binding of the variant G-CSF to the receptor comprising the variant ECD of G-CSFR causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and combinations thereof.

8. The variant G-CSF of any one of the above claims, wherein the receptor comprising the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof.

9. The variant G-CSF of any one of the above claims, wherein the receptor comprising the variant ECD of G-CSFR comprises at least one mutation in the site II interface region of the G-CSFR ECD comprising one or both of a R141E or a R167D mutation; wherein

the at least one mutation in the site III interface region of the G-CSFR ECD comprises a R41E mutation; and wherein

the variant G-CSFR mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 2.

10. The variant G-CSF of any one of the above claims, wherein the receptor comprising the variant ECD of G-CSFR is a chimeric receptor.

11. The variant G-CSF of any one of the above claims, wherein the G-CSF is chemically modified.

12. The variant G-CSF of claim 11, wherein the chemical modification comprises pegylation.

13. The variant G-CSF of claim 12, wherein the G-CSF is pegylated at the N-terminus or C-terminus of the G-CSF.

14. One or more nucleic acid sequence(s) encoding the variant G-CSF of any one of claims 1 – 6 or 10 - 12.

15. One or more expression vector(s) comprising the nucleic acid sequence of claim 10.

16. A cell engineered to express the variant G-CSF of any one of claims 1 – 6 or 10 - 12.

17. The cell of claim 12, wherein the cell is an immune cell.

18. The cell of claim 13, wherein the immune cell is

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

19. The cell of claim 14, wherein the T cell, is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, cytotoxic $CD4^+$ T cell naïve $CD8^+$ T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

20. A system for selective activation of a receptor expressed on a cell surface, the system comprising:

(a) the variant G-CSF of any one of claims 1 – 6 or 10 - 12; and

(b) a receptor comprising a variant ECD of G-CSFR; wherein

the variant G-CSF preferentially binds the receptor comprising the variant ECD of G-CSFR as compared to an otherwise identical wild type G-CSFR ECD, and the receptor comprising the variant ECD of G-CSFR preferentially binds the variant G-CSF as compared to an otherwise identical wild type G-CSF.

21. The system of claim 16, wherein the variant G-CSF comprises a combination of mutations of a site II and a site III interface of a G-CSF variant number of Table 4A; wherein the variant G-CSF mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 1.

22. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, E122R, and E123R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

23. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, and E122K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

24. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, and E123K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

25. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, and E122R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises

mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

26. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, and E123R relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

27. The system of claim 17, wherein the variant G-CSF comprises mutations E46R, L108K, D112R, E122K, and E123K relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 1; and wherein the receptor comprising a variant ECD of G-CSFR comprises mutations R41E, R141E, and R167D relative to the corresponding amino acid positions of the sequence shown in SEQ ID NO. 2.

28. The system of any one of claims 20- 27, wherein the variant G-CSF is chemically modified.

29. The system of claim 28, wherein the chemical modification of the variant G-CSF comprises pegylation.

30. The system of claim 29, wherein the variant G-CSF is pegylated at the N-terminus or C-terminus of the G-CSF.

31. The system of any one of claims 16 - 30, further comprising one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

32. The system of any one of claims 16 - 31, further comprising one or more antigen binding signaling receptor(s).

33. The system of claim 32, wherein the one or more antigen binding signaling receptor(s) comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

34. The system of claim 33, wherein the antigen binding signaling receptor(s) comprises one or more CAR(s); and, optionally, the CAR is a mesothelin CAR.

35. The system of claim 31, wherein the cytokine(s) or chemokine(s) is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof.

36. The system of claim 35, wherein the cytokine is IL-18.

37. The system of claim 35 or 36 wherein the cytokine is human.

38. A method of selective activation of a receptor expressed on the surface of a cell, comprising contacting a receptor comprising a variant ECD of G-CSFR with a variant G-CSF of claims 1 – 6 or 10 - 12.

39. The method of claim 38, wherein the receptor comprising a variant ECD of G-CSFR is expressed on an immune cell, and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

40. The method of claim 39, wherein the T cell is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, cytotoxic $CD4^+$ T cells, naïve $CD8^+$ T cells, naïve $CD4^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells.

41. The method of claim 39, wherein the selective activation of the immune cell causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor.

42. A method of increasing an immune response in a subject in need thereof, comprising:

administering cell(s) expressing a receptor comprising a variant ECD of G-CSFR and administering or providing the variant G-CSF of any one of claims 1 – 6 or 10 - 12 to the subject.

43. A method of treating a disease in a subject in need thereof, comprising:

administering cell(s) expressing a receptor comprising a variant ECD of G-CSFR and administering or providing the variant G-CSF of any one of claims 1 – 6 or 10 - 12 to the subject.

44. The method of claim 42 or 43, wherein the method is used to treat cancer.

45. The method of claim 42 or 43, wherein the method is used to treat an inflammatory condition.

46. The method of claim 42 or 43, wherein the method is used to treat an autoimmune disease.

47. The method of claim 42 or 43, wherein the method is used to treat a degenerative disease.

48. The method of claim 42 or 43, wherein the method is used to generate natural or engineered cells, tissues or organs for transplantation.

49. The method of claim 42 or 43, wherein the method is used to prevent or treat graft rejection.

50. The method of claim 42 or 43, wherein the method is used to treat an infectious disease.

51. The method of claim 42 or 43; further comprising administering or providing one or more additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

52. The method of any one of claims 42 - 51, wherein the subject is administered two or more populations of cells each expressing one or both of: (i) a distinct chimeric receptor comprising a G-CSFR ECD or (ii) at least one distinct variant form of G-CSF.

53. The method of claim 42, wherein at least one population(s) of cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR(s).

54. The method of claim 52, wherein one or both of the first and second population(s) of immune cells further expresses one or both of:

(a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and

(b) at least one antigen binding signaling receptor.

55. The method of any one of claims 52 - 54, further comprising one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a distinct variant ECD of G-CSFR, (ii) a distinct variant G-CSF, (iii) a distinct an agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

56. The method of any one of claims 42 - 55, wherein the cells expressing at least one receptor comprising a variant ECD of G-CSFR further express at least one antigen binding signaling receptor(s).

57. The method of claim 56, wherein the antigen binding signaling receptor comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

58. The method of claim 57, wherein the antigen binding signaling receptor comprises a CAR.

59. The method of claim 55, wherein the cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof.

60. The method of claim 59, wherein the cytokine is IL-18.

61. The method of claim 61 or 62, wherein the cytokine is human.

62. A method of treating a subject in need thereof, wherein the method comprises:

i) isolating an immune cell-containing sample; (ii) transducing or transfecting the immune cell(s) with a nucleic acid sequence(s) encoding a receptor comprising a variant ECD of G-CSFR; (iii) administering the immune cell(s) from (ii) to the subject; and (iv) contacting the immune cell(s) with a variant G-CSF of claims 1 – 6 or 10 - 12 that selectively binds the receptor.

63. The method of claim 62, wherein the subject has undergone an immuno-depletion treatment prior to administering the cells to the subject.

64. The method of claim 62, wherein the immune cell-containing sample is isolated from the subject to whom the cells will be administered.

65. The method of claim 62, wherein the immune cell-containing sample is isolated from a subject distinct from a subject to whom the cells will be administered.

66. The method of claim 62, wherein the immune cell-containing sample is generated from cells derived from the subject to whom the cells are administered or from a subject distinct from a subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells.

67. The method of claim 62, wherein the immune cell(s) are contacted with the variant G-CSF *in vitro* prior to administering the cells to the subject.

68. The method of claim 62, wherein the immune cell(s) are contacted with the variant G-CSF that binds the receptor for a sufficient time to activate signaling from the receptor.

69. A kit comprising:

cells encoding a receptor comprising a variant ECD of G-CSFR and instructions for use; and wherein

the kit comprises a variant G-CSF of claims 1 – 6 or 10 - 12; and, optionally, wherein the cells are immune cells.

70. A kit comprising:

(a) one or more nucleic acid sequence(s) encoding a receptor comprising the variant ECD of G-CSFR;

(b) the variant G-CSF of any one of claims 1 – 6 or 10 - 12, the nucleic acid sequence(s) of claim 7 or the one or more expression vector(s) of claim 11; and

(c) instructions for use.

71. The kit of claim 70, further comprising one or more expression vector(s) that encode one or more cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, CCL19, the receptor NKG2D, and combinations thereof.

72. The kit of claim 70, further comprising one or more expression vector(s) that encodes at least one antigen binding receptor(s).

73. The kit of claim 70 or 72, wherein the at least one antigen binding receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

74. The kit of claim 73, further comprising one or more expression vector(s) that encodes a chimeric antigen receptor.

75. The kit of claim 69, wherein the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CCL19, the receptor NKG2D, and combinations thereof.

76. The kit of claim 69 or 75, wherein the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s).

77. The kit of claim 76, wherein the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

78. The kit of claim 77, wherein the cells further comprise one or more expression vector(s) that encode at least one CAR(s), and, optionally, the CAR is a mesothelin CAR.

79. A chimeric receptor, comprising:

(a) an extracellular domain (ECD) operatively linked to at least one second domain; the second domain comprising:

(b) an intracellular domain (ICD) comprising at least one signaling molecule binding site from an intracellular domain of a cytokine receptor; wherein

the at least one signaling molecule binding site is selected from the group consisting of: a SHC binding site of Interleukin (IL)-2Rβ; a STAT5 binding site of IL-2Rβ, an IRS-1 or IRS-2 binding site of IL-4Rα, a STAT6 binding site of IL-4Rα, a SHP-2 binding site of gp130, a STAT3 binding site of gp130, a SHP-1 or SHP-2 binding site of EPOR, a STAT5 binding site of Erythropoietin Receptor (EPOR), a STAT1 or STAT2 binding site of Interferon Alpha And Beta Receptor Subunit 2 (IFNAR2), and a STAT1 binding site of Interferon Gamma Receptor 1 (IFNγR1), or combinations thereof; wherein

the ICD further comprises at least one Box 1 region and at least one Box 2 region of at least one protein selected from the group consisting of G-CSFR, gp130, EPOR, and Interferon Gamma Receptor 2 (IFNγR2), or combinations thereof; and

(c) at least one third domain comprising a transmembrane domain (TMD); wherein

the ECD is N-terminal to the TMD, and the TMD is N-terminal to the ICD.

80. A chimeric receptor, comprising:

an ECD operatively linked to a second domain; the second domain comprising an ICD, wherein the ICD comprises:

(i)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of IL-4Rα; or

(ii)

(a) a Box 1 and a Box 2 region of gp130;

(b) at least one signaling molecule binding site of gp130; or

(iii)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) at least one signaling molecule binding site of EPOR; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(v)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) at least one signaling molecule binding site of Interferon Gamma Receptor 1 (IFN γR1); wherein

the ECD is N-terminal to a TMD, and the TMD is N-terminal to the ICD.

81. The chimeric receptor of claim 79 or 80, wherein the ECD is an ECD of G-CSFR (Granulocyte-Colony Stimulating Factor Receptor), optionally wherein the ECD of G-CSFR comprises the ECD of G-CSFR of any of claims 2 - 10.

82. The chimeric receptor of any one of claims 79 – 81, wherein the TMD is a TMD of G-CSFR and, optionally, the TMD is a wild-type TMD.

83. The chimeric receptor of claim 79 - 82, wherein an activated form of the chimeric receptor forms a homodimer, and, optionally,

activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with a G-CSF, and, optionally,

the G-CSF is a wild-type G-CSF, and, optionally,

the extracellular domain of the G-CSFR is a wild-type extracellular domain.

84. The chimeric receptor of claim 79 - 81, wherein the chimeric receptor is expressed in a cell, and, optionally, an immune cell, and, optionally the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

85. The chimeric receptor of claim 84, wherein the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

86. The chimeric receptor of any one of the above claims, wherein the ICD comprises:

(a) an amino acid sequence of one or both of SEQ ID NO. 90 or 91; or

(b) an amino acid sequence of one or both of SEQ ID NO. 90 or 92; or

(c) an amino acid sequence of SEQ ID NO. 93; or

(d) an amino acid sequence of SEQ ID NO. 94; or

(e) an amino acid sequence of one or both of SEQ ID NO. 95 or 96; or

(f) an amino acid sequence of SEQ ID NO. 97 or 98; or

(g) an amino acid sequence of SEQ ID NO. 99 or 100.

87. The chimeric receptor of any one of the above claims, wherein the transmembrane domain comprises a sequence set forth in SEQ ID NO. 88.

88. A system for selective activation of a receptor expressed on a cell surface, the system comprising:

(a) the variant G-CSF of any one of claims 1 – 6 or 10 - 12; and

(b) the receptor comprising a variant ECD of G-CSFR of any one of claims 79 - 87; wherein

the variant G-CSF preferentially binds the receptor comprising the variant ECD of G-CSFR as compared to an otherwise identical wild type G-CSFR ECD, and the receptor comprising the variant ECD of G-CSFR preferentially binds the variant G-CSF as compared to an otherwise identical wild type G-CSF.

89. One or more nucleic acid sequence(s) encoding a chimeric receptor of any one of the above claims.

90. The nucleic acid sequence(s) of claim 89, wherein the ECD of the G-CSFR is encoded by nucleic acid sequence(s) set forth in any one of SEQ ID NO. 85, 86 or 87.

91. One or more expression vector(s) comprising the nucleic acid sequence(s) of claim 89 or 90.

92. The expression vector(s) of claim 91, wherein the vector(s) are selected from the group consisting of: a retroviral vector, a lentiviral vector, an adenoviral vector and a plasmid.

93. One or more nucleic acid sequence(s) encoding a chimeric receptor; wherein the chimeric receptor comprises:

an ECD operatively linked to a second domain; the second domain comprising:

(i)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of IL-4Rα; or

(ii)

(a) a Box 1 and a Box 2 region of gp130;

(b) at least one signaling molecule binding site of gp130; or

(iii)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) at least one signaling molecule binding site of EPOR; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) at least one signaling molecule binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(v)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) at least one signaling molecule binding site of Interferon Gamma Receptor 1 (IFN γR1).

94. The nucleic acid of claim 93, wherein, the ECD is an ECD of G-CSFR (Granulocyte-Colony Stimulating Factor Receptor).

95. The nucleic acid of claim 93 or 94, wherein the TMD is a TMD of G-CSFR and, optionally, the TMD is a wild-type TMD.

96. The nucleic acid sequence(s) of claim 94 or 95, wherein the ECD of the G-CSFR is encoded by a nucleic acid sequence set forth in any one of SEQ ID NO. 85, 86 or 87.

97. The nucleic acid sequence(s) of any one of claims 93 - 96, comprising:

(a) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 90 or 91; or

(b) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 90 or 92; or

(c) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 93; or

(d) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 94; or

(e) a sequence encoding an ICD comprising an amino acid sequence of one or both of SEQ ID NO. 95 or 96; or

(f) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 97 or 98; or

(g) a sequence encoding an ICD comprising an amino acid sequence of SEQ ID NO. 99 or 100.

98. One or more expression vector(s) comprising the nucleic acid sequence(s) of any one of claims 93 - 97.

99. The expression vector(s) of claim 98, wherein the vector(s) are selected from the group consisting of: a retroviral vector, a lentiviral vector, an adenoviral vector and a plasmid.

100. A cell comprising a nucleic acid sequence(s) encoding a chimeric receptor of any one of claims 79 – 87.

101. The cell of claim 100, wherein the cell is an immune cell, and, optionally the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

102. The cell of claim 101, wherein the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

103. A cell comprising the nucleic acid sequence(s) of any one of claims 93 – 97.

104. A cell comprising the expression vector(s) of claim 98 or 99.

105. A method of selective activation of a chimeric receptor expressed on the surface of a cell, comprising contacting the chimeric receptor of any one of claims 79-87 with a cytokine that selectively binds the chimeric receptor.

106. The method of claim 105, wherein an activated form of the chimeric receptor forms a homodimer, and, optionally,

activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with the cytokine.

107. The method of claim 105 or 106, wherein the cytokine that selectively binds the chimeric receptor is a G-CSF, and, optionally, the chimeric receptor is activated upon contact with a G-CSF, and, optionally,

the G-CSF is a wild-type G-CSF, and, optionally,

the extracellular domain of the G-CSFR is a wild-type extracellular domain.

108. The method of any one of claims 105 – 107, wherein the chimeric receptor is expressed in a cell, and, optionally,

an immune cell, and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

109. The method of claim 108, wherein a first population of immune cells expresses the chimeric receptor and a second population of immune cells express a cytokine that binds the chimeric receptor; optionally, wherein one or both of the first and second population(s) of immune cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR.

110. The method of claim 109, wherein one or both of the first and second population(s) of immune cells further expresses one or both of:

(a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and

(b) at least one antigen binding signaling receptor.

111. The method of claim 109 or 110, wherein each of the first and second populations of immune cells express a distinct chimeric receptor comprising a distinct variant ECD of G-CSFR and a distinct variant G-CSF.

112. The method of any one of claims 109 - 111, further comprising one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a distinct variant ECD of G-CSFR, (ii) a distinct variant G-CSF, (iii) a distinct an agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

113. A method of producing a chimeric receptor in a cell, comprising:

introducing into the cell the nucleic acid sequence(s) of any one of claims 89, 90, or 93-97 or the expression vector(s) of any one of claims 91, 92, 98 or 99; and, optionally, the method comprises gene editing; and, optionally,

the cell is an immune cell, and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

114. A method of treating a subject in need thereof, comprising:

administering to the subject a cell expressing a chimeric receptor of any one of claims 79 – 87, and providing to the subject a cytokine that specifically binds the chimeric receptor.

115. The method of claim 114, wherein an activated form of the chimeric receptor forms a homodimer; and, optionally,

activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor, and, optionally, the chimeric receptor is activated upon contact with the cytokine.

116. The method of claim 114 or 115, wherein the cytokine is G-CSF; and, optionally,

the G-CSF is a wild-type G-CSF, and, optionally,

the extracellular domain of the G-CSFR is a wild-type extracellular domain.

117. The method of any one of claims 114 – 116, wherein

the chimeric receptor is expressed in a cell, and, optionally,

an immune cell, and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

118. The method of any one of claims 106 - 117; further comprising administering or providing at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s).

119. The method of any one of claims 114 - 118, wherein the subject is administered two or more populations of cells each expressing a distinct chimeric receptor and each expressing a distinct variant form of a cytokine.

120. The method of any one of claims 114 - 119, wherein the cells expressing the chimeric receptor further express at least one antigen binding signaling receptor.

121. The method of claim 120, wherein the antigen binding signaling receptor comprises at least one receptor(s) selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

122. The method of claim 121, wherein the antigen binding signaling receptor is a CAR.

123. The method of claim 118, wherein the at least one cytokine(s) or chemokine(s) is selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof.

124. The method of claim 123, wherein the cytokine is IL-18.

125. The method of claim 123 or 124, wherein the cytokine is human.

126. The method of any one of claims 114 - 125, wherein the method is used to treat cancer.

127. The method of any one of claims 114 - 125, wherein the method is used to treat an autoimmune disease.

128. The method of any one of claims 114 - 125, wherein the method is used to treat an inflammatory condition.

129. The method of any one of claims 114 - 125, wherein the method is used to treat a degenerative disease.

130. The method of any one of claims 114 - 125, wherein the method is used to generate natural or engineered cells, tissues or organs for transplantation.

131. The method of any one of claims 114 - 125, wherein the method is used to prevent or treat allograft rejection.

132. The method of claim 114, wherein the method comprises:

i) isolating an immune cell-containing sample; (ii) introducing to the immune cells a nucleic acid sequence encoding the chimeric cytokine receptor; (iii) administering the immune cells from (ii) to the subject; and (iv) contacting the immune cells with the cytokine that binds the chimeric receptor.

133. The method of claim 132; wherein the subject has undergone an immuno-depletion treatment prior to administering or infusing the cells to the subject.

134. The method of claim 132, wherein the immune cell-containing sample is isolated from a subject to whom the cells will be administered.

135. The method of claim 132, wherein the immune cell-containing sample is isolated from a subject distinct from a subject to whom the cells will be administered.

136. The method of any one of claims 132, wherein the immune cell-containing sample is generated from cells derived from a subject to whom the cells will be administered, or from a subject distinct from the subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells.

137. The method of claim 132, wherein the immune cells are contacted with the cytokine *in vitro* prior to administering or infusing the cells to the subject.

138. The method of claim 132, wherein the immune cells are contacted with the cytokine for a sufficient time to activate signaling from the chimeric receptor.

139. The method of any one of claims 132 - 138, wherein the cytokine is G-CSF; and, optionally,

the G-CSF is a wild-type G-CSF, and, optionally,

the extracellular domain of the G-CSFR is a wild-type extracellular domain.

140. A kit comprising:

cells encoding one or more chimeric receptor(s) of any one of claims 79-87,

and, optionally, the cells are immune cells; and

instructions for use;

and, optionally, the kit comprises at least one cytokine(s) that binds the chimeric receptor(s).

141. A kit comprising:

at least one expression vector(s) encoding one or more chimeric receptor(s) of any one of claims 79-87 and instructions for use;

and, optionally, the kit comprises at least one cytokine(s) that binds the chimeric receptor(s).

142. The kit of claim 141, further comprising one or more expression vector(s) that encode one or more cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof.

143. The kit of claim 141 or 142, further comprising one or more expression vector(s) that encodes at least one antigen binding receptor(s).

144. The kit of claim 142, wherein the at least one antigen binding receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

145. The kit of claim 144, further comprising an expression vector that encodes at least one CAR(s), and, optionally, wherein the CAR(s) is a mesothelin CAR.

146. The kit of claim 140, wherein the cells further comprise one or more expression vector(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s).

147. The kit of claim 140, wherein the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1, or CCL19, or the receptor NKG2D, and combinations thereof.

148. The kit of any of claim 142, further comprising one or more expression vector(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s).

149. The kit of claim 148, wherein the cells further comprise one or more expression vector(s) that encode at least one cytokine(s) or chemokine(s) selected from the group consisting of IL-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF-α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), CD40 ligand, B cell activating factor (BAFF), Flt3 ligand, CCL21, CCL5, XCL1,or CCL19, or the receptor NKG2D, and combinations thereof.

150. The kit of claim any one of claims 140, 146 or 147, wherein the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s).

151. The kit of claim 150, wherein the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

152. The kit of any one of claims 140, 146, 147, 150 or 151, wherein the cells further comprise one or more expression vector(s) that encode at least one CAR(s), and, optionally, wherein the CAR is a mesothelin CAR.

153. The kit of any one of claims 140, 146, 147, 150 or 151, wherein the cells further comprise one or more expression vector(s) encoding one or more distinct chimeric receptor(s) of any one of claims 79-87.

154. The kit of any one of claims 141-145, 148 or 149, further comprising one or more expression vector(s) encoding one or more distinct chimeric receptor(s) of any one of claims 79-87.

155. A system for selective activation of a cell, the system comprising:

(i) a receptor comprising a variant extracellular domain (ECD) of Granulocyte Colony-Stimulating Factor Receptor (G-CSFR); and

(ii) a variant G-CSF that selectively binds the receptor of (i); and one or both of:

(a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s);

and

(b) at least one antigen binding signaling receptor(s).

156. The system of claim 155, wherein the variant G-CSF comprises any one of claims 1 – 6 or 10 – 12.

157. The system of claim 155 or 156, wherein the receptor comprises a variant ECD of G-CSFR of any one of claims 79 - 87.

158. The system for selective activation of a cell of any one of claims 155, wherein the at least one additional cytokine(s) or chemokine(s) comprises at least one of interleukin (IL)-18, IL-21, interferon-α, interferon-β, interferon-γ, IL-17, IL-21, TNF- α, CXCL13, CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL19, and the receptor NKG2D, and combinations thereof.

159. The system for selective activation of a cell of claim 158, wherein the at least one additional cytokine(s) comprises IL-18.

160. The system for selective activation of a cell of any one of claims 155- 159, wherein the antigen binding signaling receptor(s) comprises at least one of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

161. The system for selective activation of a cell of claim 160, wherein the antigen binding signaling receptor comprises a CAR; and, optionally, wherein the CAR is a mesothelin CAR.

162. The system for selective activation of a cell of any one of the above claims; wherein the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof.

163. The system for selective activation of a cell of claim 162, wherein

the at least one mutation in the site II interface region is located at an amino acid position of the G-CSFR ECD selected from the group consisting of amino acid position 141,167, 168, 171, 172, 173, 174, 197, 199, 200, 202 and 288 of the sequence shown in SEQ ID NO. 2.

164. The system for selective activation of a cell of claim 162, wherein the at least one mutation in the site II interface region of the G-CSFR ECD is selected from the group consisting of R141E, R167D, K168D, K168E, L171E, L172E, Y173K, Q174E, D197K, D197R, M199D, D200K, D200R, V202D, R288D, and R288E of the sequence shown in SEQ ID NO. 2.

165. The system for selective activation of a cell of claim 162, wherein

the at least one mutation in the site III interface region of the G-CSFR ECD is selected from the group consisting of amino acid position 30, 41, 73, 75, 79, 86, 87, 88, 89, 91, and 93 of amino acids 2-308 of the sequence shown in SEQ ID NO. 2.

166. The system for selective activation of a cell of claim 162, wherein

the at least one mutation in the site III interface region of the G-CSFR ECD is selected from the group consisting of S30D, R41E, Q73W, F75KF, S79D, L86D, Q87D, I88E, L89A, Q91D, Q91K, and E93K of the sequence shown in SEQ ID NO. 2.

167. The system for selective activation of a cell of any one of the above claims, wherein the G-CSFR ECD comprises a combination of a plurality of mutations of a design number shown in Table 4; wherein the mutations correspond to an amino acid position of the sequence shown in SEQ ID NO. 2.

168. The system for selective activation of a cell of any one of the above claims, wherein the G-CSFR ECD comprises the mutations: R41E, R141E, and R167D of the sequence shown in SEQ ID NO. 2.

169. The system for selective activation of a cell of any one of the above claims, wherein the receptor comprising a variant ECD of G-CSFR is a chimeric receptor.

170. The system for selective activation of a cell of claim 169, wherein

the chimeric receptor is operatively linked to at least one second domain; the second domain comprising at least one signaling molecule binding site from an intracellular domain (ICD) of one or more cytokine receptor(s); wherein the at least one signaling molecule binding site is selected from the group consisting of:

a STAT3 binding site of G-CSFR, a STAT3 binding site of glycoprotein 130 (gp130), a SHP-2 binding site of gp130, a SHC binding site of IL-2Rβ, a STAT5 binding site of IL-2Rβ, a STAT3 binding site of IL-2Rβ, a STAT1 binding site of IL-2Rβ, a STAT5 binding site of IL-7Rα, a phosphatidylinositol 3-kinase (PI3K) binding site of IL-7Rα, a STAT4 binding site of IL-12Rβ_2, a STAT5 binding site of IL-12Rβ_2, a STAT3 binding site of IL-12Rβ_2, a STAT5 binding site of IL-21R, a STAT3 binding site of IL-21R a STAT1 binding site of IL-21R, an IRS-1 or IRS-2 binding site of IL-4Rα, a STAT6 binding site of IL-4Rα, a SHP-1 or SHP-2 binding site of Erythropoietin Receptor (EPOR), a STAT5 binding site of EPOR, a STAT1 or STAT2 binding site of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2), and a STAT1 binding site of Interferon Gamma Receptor 1 (IFNγR1), or combinations thereof;

optionally, the ICD comprises a Box 1 region and a Box 2 region of a protein selected from the group consisting of G-CSFR, gp130 EPOR, and Interferon Gamma Receptor 2 (IFNγR2), or combinations thereof; and,

optionally, the chimeric receptor comprises a third domain comprising a transmembrane domain (TMD) of a protein selected from the group consisting of: G-CSFR, gp130 (Glycoprotein 130), and IL-2Rβ, and, optionally, the TMD is a wild-type TMD.

171. The system for selective activation of a cell of claim 169, wherein the chimeric receptor is operatively linked to at least one second domain; the second domain comprising:

(i)

(a) a Box 1 and a Box 2 region of gp130; and

(b) a C-terminal region of IL-2Rβ; or

(ii)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-2Rβ; or

(iii)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-12Rβ_2; or

(iv)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-21R; or

(v)

(a) a Box 1 and a Box 2 region of IL-2Rβ; and

(b) a C-terminal region of IL-2Rβ; or

(vi)

(a) a Box 1 and a Box 2 region of G-CSFR; and

(b) a C-terminal region of IL-7Rα; or

(vii)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) a C-terminal region of IL-4Rα; or

(viii)

(a) a Box 1 and a Box 2 region of gp130;

(b) a C-terminal region of gp130; or

(ix)

(a) a Box 1 and a Box 2 region of Erythropoietin Receptor (EPOR);

(b) a C-terminal region of EPOR; or

(x)

(a) a Box 1 and a Box 2 region of G-CSFR;

(b) a C-terminal region of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2); or

(xi)

(a) a Box 1 and a Box 2 region of Interferon Gamma Receptor 2 (IFNγR2);

(b) a C-terminal region of Interferon Gamma Receptor 1 (IFN γR1).

172. The system for selective activation of a cell of claim 170 or 171, wherein the ECD is N-terminal to the TMD, and TMD N-terminal to the ICD.

173. The system for selective activation of a cell of any one of the above claims, wherein the receptor comprising a variant ECD of G-CSFR is expressed on the cell.

174. The system for selective activation of a cell of any one of the above claims, wherein the cell is an immune cell and, optionally the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

175. The system of claim 174, wherein the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, cytotoxic $CD4^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

176. The system for selective activation of a cell of any one of the above claims, wherein activation of the receptor comprising a variant ECD of G-CSFR by the variant G-CSF causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity,

cytokine secretion, memory, enhanced activity of a cell expressing the receptor, or combinations thereof.

177. The system for selective activation of a cell of any one of the above claims, wherein the variant G-CSF comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof.

178. The system for selective activation of a cell of claim 177, wherein the at least one mutation in the site II interface region of the variant G-CSF is located at an amino acid position selected from the group consisting of amino acid position 12, 16, 19, 20, 104, 108, 109, 112, 115, 116, 118, 119, 122 and 123 of the sequence shown in SEQ ID NO. 1.

179. The system for selective activation of a cell of claim 178, wherein

the at least one mutation in the site II interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of: S12E, S12K, S12R, K16D, L18F, E19K, Q20E, D104K, D104R, L108K, L108R, D109R, D112R, D112K, T115E, T115K, T116D, Q119E, Q119R, E122K, E122R, and E123R.

180. The system for selective activation of a cell of any one of claims 177-179, wherein the at least one mutation in the site III interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of: 38, 39, 40, 41, 46, 47, 48, 49, and 147 of the sequence shown in SEQ ID NO. 1.

181. The system for selective activation of a cell of claim 180, wherein

the at least one mutation in the site III interface region of the variant G-CSF is selected from a group of mutations selected from the group consisting of: T38R, Y39E, K40D, K40F, L41D, L41E, L41K, E46R, L47D, V48K, V48R, L49K, and R147E.

182. The system for selective activation of a cell of any one of the above claims, wherein the cell expresses both the receptor comprising the variant ECD of G-CSFR and the at least one additional cytokine or chemokine.

183. The system for selective activation of a cell of any one of claims 155- 181, wherein two or more populations of cells each express a distinct chimeric receptor comprising a G-CSFR ECD and each express a distinct variant form of G-CSF.

184. The system of claim 183, wherein the first population of immune cells further expresses one or both of:

(a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and

(b) at least one antigen binding signaling receptor.

185. The system for selective activation of a cell of claim 182, wherein the cell is an immune cell; and wherein the immune cell further expresses at least one antigen binding signaling receptor; and wherein the antigen binding signaling receptor selectively binds to an antigen expressed on a second cell.

186. The system for selective activation of a cell of claim 184, wherein the antigen binding signaling receptor comprises a chimeric antigen receptor (CAR), and, optionally, wherein the CAR is a mesothelin CAR.

187. The system for selective activation of a cell of claim 186, wherein the additional cytokine comprises IL-18.

188. The system for selective activation of a cell of any one of claims 184 - 187, wherein the second cell is a cancer cell.

189. One or more nucleic acid sequence(s) encoding the system of any one of the above claims.

190. One or more expression vector(s) comprising the nucleic acid sequence(s) of claim 189.

191. One or more cell(s) engineered to express the system of any one of claims 155-188.

192. The cell(s) of claim 191, wherein the cell(s) is an immune cell and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

193. The cell(s) of claim 192, wherein the T-cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, cytotoxic $CD4^+$ T cell, naïve CD8+T cell, naïve $CD4^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

194. A method of selective activation of a receptor comprising a variant ECD of G-CSFR expressed on the surface of a cell, comprising:

introducing into the cell one or more nucleic acid sequence(s) encoding at least one receptor(s) comprising a variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of

(i)) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of the system of any one of claims 155-188; and

(ii) at least one antigen binding signaling receptor(s) of the system of any one of claims 155-188; and

contacting the receptor(s) comprising the variant ECD of G-CSFR with a variant G-CSF or the variant G-CSF of any one of claims 155-188.

195. The method of claim 194, wherein the receptor(s) is expressed on an immune cell, and, optionally the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

196. The method of claim 195, wherein the T cell is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, cytotoxic $CD4^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

197. The method of claim 195, wherein the selective activation of the receptor(s) expressed on the immune cell causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, enhanced activity of the immune cell, or combinations thereof.

198. The method of claim 195, wherein a first population of immune cells expresses one or more receptor(s) comprising the variant ECD of G-CSFR and a second population of immune cells express one or more variant G-CSF; optionally, wherein one or both of the first and second population(s) of immune cells further express at least one distinct antigen binding signaling receptor(s); and, optionally, wherein the at least one distinct antigen binding signaling receptor(s) comprises at least one CAR.

199. The method of claim 198, wherein one or both of the first and second population(s) of immune cells further expresses one or both of:

(a)) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and

(b) at least one antigen binding signaling receptor.

200. The method of claim 198 or 199, wherein each of the first and second populations of immune cells express at least one distinct receptor(s) comprising a distinct variant ECD of G-CSFR and at least one distinct variant G-CSF.

201. The method of any one of claims 198 - 200, further comprising one or more additional populations of immune cells; wherein each additional population of immune cells expresses at least one of (i) a distinct receptor comprising a distinct variant ECD of G-CSFR, (ii) a

distinct variant G-CSF, (iii) a distinct agonistic or antagonistic signaling protein and (iv) a distinct antigen binding signaling receptor.

202. A method of producing a cell expressing the receptor comprising the variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of:

(i)) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of the system of any one of claims 155-188; and

(ii) at least one antigen binding signaling receptor(s) of the system of any one of claims 155-188;

the method comprising introducing to the cells one or more nucleic acid(s) or expression vector(s) encoding the receptor, and one or both of (i), and (ii).

203. The method of claim 200, wherein a first population of immune cells expresses the receptor(s) comprising the variant ECD of G-CSFR and a second population of immune cells express the variant G-CSF.

204. The method of claim 203, wherein one or both of the first and second population(s) of immune cells further expresses one or both of:

(a) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the at least one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s); and

(b) at least one antigen binding signaling receptor.

205. A method of increasing an immune response in a subject in need thereof, comprising administering to the subject the immune cell(s) of claim 192.

206. A method of treating a disease in a subject in need thereof, comprising:

administering to the subject the immune cell(s) of claim 192.

207. The method of claim 204, further comprising administering or providing at least one variant G-CSF to the subject.

208. The method of claim 204 - 207, wherein the method is used to treat cancer.

209. The method of claim 204 - 207, wherein the method is used to treat an inflammatory condition.

210. The method of claim 204 - 207, wherein the method is used to treat an autoimmune disease or condition.

211. The method of claim 204 - 207, wherein the method is used to treat a degenerative disease.

212. The method of claim 204 - 207, wherein the method is used to generate natural or engineered cells, tissues or organs for transplantation.

213. The method of claim 204 - 207, wherein the method is used to prevent or treat graft rejection.

214. The method of claim 204 - 207, wherein the method is used to treat an infectious disease.

215. The method of claim 204 - 207, further comprising administering or providing at least one additional active agent; optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s).

216. A method of treating a subject in need thereof, wherein the method comprises:

(i) isolating an immune cell-containing sample;

(ii) introducing the immune cells with one or more nucleic acid sequence(s) encoding one or more receptor(s) comprising the variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of:

(a) at least one additional active agent; optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of the system of any one of claims 155-188; and optionally,

(b) one or more antigen binding signaling receptor(s) of the system of any one of claims 155-188;

(iii) administering the immune cell(s) from (ii) to the subject; and

(iv) contacting the immune cell(s) with a variant G-CSF that specifically binds the receptor(s) comprising the variant ECD of G-CSFR.

217. The method of claim 216, wherein the subject has undergone an immuno-depletion treatment prior to administering or infusing the immune cell(s) to the subject.

218. The method of claim 216, wherein the immune cell-containing sample is isolated from the subject to whom the cell(s) are administered.

219. The method of claim 216, wherein the immune cell-containing sample is isolated from a subject distinct from the subject to whom the cell(s) are administered.

220. The method of any one of claims 216 - 219, wherein the immune cell-containing sample is generated from source cells derived from the subject to whom the cell(s) are administered, or from a subject distinct from a subject to whom the cells will be administered, and, optionally, wherein the source cells are stem cells, and, optionally, pluripotent stem cells.

221. The method of claim 216, wherein the immune cell(s) are contacted with the variant G-CSF or additional cytokine or chemokine *in vitro* prior to administering the immune cell(s) to the subject.

222. The method of any one of claims 216 - 220, wherein the immune cell(s) are contacted with the variant G-CSF for a sufficient time to activate signaling from the receptor comprising the variant ECD of G-CSFR of the system of any one of claims 1-32.

223. A kit comprising cells encoding:

the receptor comprising the variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of:

(a) the at least one additional cytokine and chemokine of the system of any one of claims 155-188; and,

(b) the at least one antigen binding signaling receptor of the system of any one of claims 155-188;

and instructions for use; and optionally, wherein the cells are immune cells.

224. A kit comprising:

(i) one or more nucleic acid sequence(s) or expression vector(s) encoding the receptor comprising the variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of:

(a) at least one additional active agent(s); optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of the system of any one of claims 155-188; and,

(b) the at least one antigen binding signaling receptor of the system of any one of claims 155-188; and

(ii) at least one variant G-CSF; and

(iii) instructions for use;

wherein the receptor(s) and one or both of (a) and (b) are located on the same or separate nucleic acid sequence or expression vector.

225. A kit comprising:

(i) cells comprising one or more nucleic acid sequence(s) or expression vector(s) encoding the receptor comprising the variant ECD of G-CSFR of the system of any one of claims 155-188; and one or both of:

(a) at least one additional active agent(s); optionally wherein the at least one additional active agent comprises at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent(s) of the system of any one of claims 155-188; and

(b) at least one antigen binding signaling receptor(s) of the system of any one of claims 155-188; and

(ii) instructions for use;

and optionally wherein the kit comprises a variant G-CSF that specifically binds the receptor comprising the variant ECD of G-CSFR.

226. A chimeric receptor, comprising:

(i) an extracellular domain (ECD) of Interleukin Receptor alpha (IL-7Rα);

(ii) a transmembrane domain (TMD); and

(iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from a wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD.

227. The chimeric receptor of claim 226, wherein the carboxy terminus (C-terminus) of the ECD is linked to the amino terminus (N-terminus) of the TMD, and the C-terminus of TMD is linked to the N-terminus of the ICD.

228. The chimeric receptor of claim 226 or 227, wherein the ECD is the ECD of native human IL-7Rα.

229. The chimeric receptor of any one of claims 226 - 228, wherein the TMD is the TMD of IL-7Rα.

230. The chimeric receptor of claim 229, wherein the TMD is the TMD of native human IL-7Rα.

231. The chimeric receptor of any one of claim 226 - 230, wherein the ICD comprises at least one signaling molecule binding site from an intracellular domain of a cytokine receptor, and, optionally,

the at least one signaling molecule binding site comprises:

(a) a Jak 1 binding site (Box 1 and 2 region) of IL-2Rβ, IL-4Rα, IL-7Ra, IL-21R, or gp130;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ or IL-7Ra;

(d) a STAT3 binding site of IL-21R or gp130;

(e) a STAT4 binding site of IL-12Rβ2;

(f) a STAT6 binding site of IL-4Rα;

(g) an IRS-1 or IRS-2 binding site of IL-4Rα; and

(h) a SHP-2 binding site of gp130;

(i) a PI3K binding site of IL-7Rα;

or combinations thereof.

232. The chimeric receptor of any one of claims 226 - 231, wherein the ICD comprises at least an intracellular signaling domain of a receptor that is activated by heterodimerization with the common gamma chain (gc).

233. The chimeric receptor of any one of claims 226 - 231, wherein the ICD comprises at least an intracellular signaling domain of a cytokine receptor selected from the group consisting of: IL-2Rβ (Interleukin-2 receptor beta), IL-4Rα (Interleukin-4 Receptor alpha), IL-9Rα (Interleukin-9 Receptor alpha), IL-12R (Interleukin-12 Receptor), IL-21R (Interleukin-21 Receptor) and glycoprotein 130 (gp130), and combinations thereof.

234. A chimeric receptor, comprising an ECD of IL-7Rα and a TMD operatively linked to an ICD, the ICD comprising:

(i)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(ii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ; and

(c) a STAT5 binding site of IL-2Rβ; or

(iii)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(iv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT4 binding site of IL-12Rβ2; or

(v)

(a) a Box 1 and a Box 2 region of IL-21R; and

(b) a STAT3 binding site of IL-21R; or

(vi)

(a) a Box 1 and a Box 2 region of IL-7Rα; and

(b) a STAT3 binding site of IL-21R; or

(vii)

(a) a Box 1 and a Box 2 region of IL-21R;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(viii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a STAT3 binding site of IL-21R; and

(c) a STAT5 and PI3 kinase binding site of IL-7Rα;

(ix)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(x)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ; and

(d) a STAT3 binding site of IL-21R; or

(xi)

(a) a Box 1 and a Box 2 region of IL-2Rβ;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xii)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) a SHC binding site of IL-2Rβ;

(c) a STAT5 binding site of IL-2Rβ;

(d) a STAT4 binding site of IL12Rβ2; and

(e) a STAT3 binding site of IL-21R; or

(xiii)

(a) a Box 1 and a Box 2 region of IL-4Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4Rα; and

(c) a STAT6 binding site of IL-4Rα; or

(xiv)

(a) a Box 1 and a Box 2 region of IL-7Rα;

(b) an IRS-1 or IRS-2 binding site of IL-4α; and

(c) a STAT6 binding site of IL-4α; or

(xv)

(a) a Box 1 and a Box 2 region of gp130;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130; or

(xvi)

(a) a Box 1 and a Box 2 region of IL-7α;

(b) a SHP-2 binding site of gp130; and

(c) a STAT3 binding site of gp130.

235. The chimeric receptor of claim 234, wherein

(b) is N-terminal to (c); or wherein

(c) is N-terminal to (b); or wherein

(c) is N-terminal to (d); or wherein

(d) is N-terminal to (c); or wherein

(d) is N-terminal to (e); or wherein

(e) is N-terminal to (d).

236. The chimeric receptor of any one of the above claims, wherein the ICD comprises a sequence at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to a sequence shown in at least one of SEQ ID NO: 85-107.

237. The chimeric receptor of any one of the above claims, wherein an activated form of the chimeric receptor forms a heterodimer and, optionally, the activation of the chimeric receptor causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the chimeric receptor, and, optionally,

the chimeric receptor is activated upon contact with interleukin (IL)-7.

238. The chimeric receptor of claim 237, wherein the IL-7 is a wild-type, human IL-7.

239. The chimeric receptor of claim 237, wherein the IL-7 harbors 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations compared to wild-type IL-7.

240. The chimeric receptor of claim 237, wherein the IL-7 comprises one or more chemical modifications.

241. The chimeric receptor of claim 240, wherein the IL-7 is modified by pegylation.

242. The chimeric receptor of claim 241, wherein the IL-7 is pegylated by chemical addition of polyethylene glycol (PEG) to the N-terminus or C-terminus of the IL-7 protein.

243. The chimeric receptor of any one of the above claims, wherein

the chimeric receptor is expressed on a cell.

244. The chimeric receptor of claim 243, wherein the cell is an immune cell and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

245. The chimeric receptor of claim 244, wherein the T cell, is selected from the group consisting of a $CD8^+$ T cell, cytotoxic $CD8^+$ T cell, naïve $CD4^+$ T cell, naïve $CD8^+$ T cell, helper T cell, regulatory T cell, memory T cell, and γδT cell.

246. The chimeric receptor of any one of the above claims, wherein

activation of the receptor by IL-7 causes a cellular response comprising at least one of proliferation, viability, persistence, cytotoxicity, cytokine secretion, memory, and enhanced activity of a cell expressing the receptor.

247. One or more nucleic acid sequence(s) encoding the receptor of any one of the above claims.

248. One or more expression vector(s) comprising the nucleic acid sequence(s) of claim 20.

249. A cell comprising the nucleic acid sequence(s) of claim 247, or the expression vector(s) of claim 248.

250. The cell of claim 249, wherein the cell is an immune cell and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

251. The cell of claim 250, wherein the T cell, is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD4^+$ T cells, naïve $CD8^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells.

252. A system for activation of a receptor expressed on a cell surface, the system comprising:

(a) the chimeric receptor of any one of claims 226 - 245; and

(b) IL-7.

253. A system for activation of an immune cell, the system comprising:

(a) the chimeric receptor of claims 226 - 245;

(b) IL-7; and

(c) an antigen binding signaling receptor.

254. A system for activation of an immune cell, the system comprising:

(a) the chimeric receptor of claims 226 - 245;

(b) IL-7; and

(c) at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

255. The system of claim 254, further comprising at least one antigen binding signaling receptor.

256. The system of claim 253 or 255, wherein the at least one antigen binding signaling receptor comprises at least one receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

257. The system of claim 256, wherein the at least one antigen binding signaling receptor is a CAR.

258. The system of claim 254, wherein the cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-a, interferon-b, interferon-g, IL-17, IL-21, TNF-a, CXCL13, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL19, NKG2D, and combinations thereof.

259. The system of claim 258, wherein the cytokine is IL-18.

260. The system of claim 258 or 259 wherein the cytokine is human.

261. A method of activation of a chimeric receptor expressed on the surface of a cell, comprising:

contacting the chimeric receptor with IL-7 to activate the chimeric receptor; wherein the chimeric receptor comprises:

(i) an extracellular domain (ECD) of IL-7Rα;

(ii) a transmembrane domain (TMD); and

(iii) an intracellular domain (ICD) of a cytokine receptor that is distinct from the wild-type, human IL-7Rα intracellular signaling domain set forth in SEQ ID NO:109; wherein the ECD and TMD are each operatively linked to the ICD.

262. The method of claim 253, wherein the chimeric receptor is the chimeric receptor of any one of claims 227 - 245.

263. A method of producing a chimeric receptor in a cell, the method comprising: introducing into the cell the nucleic acid sequence(s) of claim 247 or the expression vector(s) of claim 262.

264. The method of claim 262, further comprising editing the sequence(s) or sequence(s) of the vector(s) into the genome of the cell.

265. The method of claim 262 or 263, wherein the cell is an immune cell; and, optionally, the immune cell is:

a T cell, and, optionally,

an NK cell, and, optionally,

an NKT cell, and, optionally,

a B cell, and, optionally,

a plasma cell, and, optionally,

a macrophage, and, optionally,

a dendritic cell, and, optionally,

the cell is a stem cell, and, optionally,

the cell is a primary cell, and, optionally,

the cell is a human cell.

266. The method of claim 265, wherein the T cell, is selected from the group consisting of $CD8^+$ T cells, cytotoxic $CD8^+$ T cells, naïve $CD4^+$ T cells, naïve $CD8^+$ T cells, helper T cells, regulatory T cells, memory T cells, and γδT cells.

267. A method of increasing an immune response in a subject in need thereof, comprising:

administering to the subject cell(s) expressing the chimeric receptor of any one of claims 226 - 245, and administering or providing IL-7 to the subject.

268. A method of treating a subject in need thereof, comprising:

administering to the subject cell(s) expressing the chimeric receptor of any one of claims 226 - 245, and administering or providing IL-7 to the subject.

269. The method of claim 267 or 268, wherein the method is used to treat cancer.

270. The method of claim 267 or 268, wherein the method is used to treat an autoimmune disease.

271. The method of claim 267 or 268, wherein the method is used to treat an inflammatory condition.

272. The method of claim 267 or 268, wherein the method is used to treat a degenerative disease.

273. The method of claim 267 or 268, wherein the method is used to generate natural or engineered cells, tissues or organs for transplantation.

274. The method of claim 267 or 268, wherein the method is used to prevent or treat graft rejection.

275. The method of claim 267 or 268, wherein the method is used to treat an infectious disease.

276. The method of claim 267 or 268; further comprising administering or providing at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

277. The method of claim 267 or 268, wherein the subject is administered cells expressing at least one additional distinct chimeric receptor.

278. The method of claim 277, wherein the at least one additional distinct chimeric receptor is a chimeric receptor comprising a variant ECD of Granulocyte Cell Stimulating Factor Receptor (G-CSFR).

279. The method of claim 278, wherein the cells expressing the at least one additional distinct chimeric receptor comprising a variant ECD of G-CSFR are contacted with one or more variant G-CSF, and optionally, the subject is administered one or more variant G-CSF.

280. The method of any one of claims 276 - 279, wherein the method comprises:

i) isolating an immune cell-containing sample; (ii) transducing or transfecting the immune cell(s) with nucleic acid sequence(s) encoding the chimeric cytokine receptor(s); (iii) administering the immune cell(s) from (ii) to the subject; and (iv) contacting the immune cells with IL-7.

281. The method of claim 277, further comprising introducing to the immune cell(s) with nucleic acid sequence(s) encoding at least one additional agonistic or antagonistic signaling protein(s); and, optionally, the one or more additional agonistic or antagonistic signaling protein(s) comprises one or more cytokine(s), chemokine(s), hormone(s), antibody(ies) or derivative(s) thereof, or other affinity reagent.

282. The method of claim 281, wherein the at least one cytokine or chemokine is selected from the group consisting of IL-18, IL-21, interferon-a, interferon-b, interferon-g, IL-17, IL-21, TNF- a, CXCL13, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL19, NKG2D, and combinations thereof.

283. The method of claim 280, further comprising introducing to the immune cell(s) with nucleic acid sequence(s) encoding at least one antigen binding signaling receptor.

284. The method of claim 283, wherein the at least one antigen binding signaling receptor is selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a native B cell Receptor, an engineered B Cell Receptor (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

285. The method of claim 280, wherein the subject has undergone an immuno-depletion treatment prior to administering the cells to the subject.

286. The method of claim 280, wherein the immune cell-containing sample is isolated from the subject to whom the cells are administered.

287. The method of claim 280, wherein the immune cell-containing sample is isolated from a subject distinct from a subject to whom the cells will be administered.

288. The method of claim 280, wherein the immune cell-containing sample is generated from cells derived from the subject to whom the cells are administered or from a subject

distinct from a subject to whom the cells will be administered, and, optionally, wherein the cells are stem cells, and, optionally, pluripotent stem cells.

289. The method of claim 280, wherein the immune cells are contacted with one or both of IL-7 or a variant G-CSF *in vitro* prior to administering the cells to the subject.

290. The method of claim 280, wherein the immune cells are contacted with one or both of IL-7 or a variant G-CSF for a sufficient time to activate signaling from the chimeric receptor of one of any one of claims 226 - 246.

291. The method of any one of claims 267 -290, wherein the cells administered to the subject further express at least one antigen binding signaling receptor selected from the group consisting of: a native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

292. The method of any one of claims 267 - 291, wherein the cells administered to the subject further express a receptor comprising a variant ECD of G-CSFR.

293. The method of claim 292, wherein the variant ECD of G-CSFR comprises at least one mutation in a site II interface region, at least one mutation in a site III interface region, or combinations thereof.

294. The method of any one of claims 267 - 291, wherein the cells administered to the subject further express IL-18.

295. A kit, comprising:

cells encoding a chimeric receptor of any one of claim 226 - 245, and, optionally, the cells are immune cells; and instructions for use; and, optionally, the kit comprises IL-7 and, optionally, and optionally the kit comprises a variant G-CSF.

296. A kit, comprising:

one or more expression vector(s) comprising the nucleic acid sequence(s) encoding the chimeric receptor of any one of claims 226 - 245 and instructions for use; and, optionally, the kit comprises IL-7 and, optionally, the kit comprises a variant G-CSF.

297. The kit of claim 296, further comprising one or more expression vector(s) that encode a cytokine or chemokine selected from the group consisting of IL-18, IL-21,

interferon-a, interferon-b, interferon-g, IL-17, IL-21, TNF- a, CXCL13, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL19, NKG2D, and combinations thereof.

298. The kit of claim 296, further comprising one or more expression vector(s) that encodes at least one receptor selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor, and combinations thereof.

299. The kit of claim 298, further comprising an expression vector that encodes a chimeric antigen receptor.

300. The kit of claim 295, wherein the cells further comprise one or more expression vector(s) that encode at least one cytokine or chemokine selected from the group consisting of IL-18, IL-21, interferon-a, interferon-b, interferon-g, IL-17, IL-21, TNF- a, CXCL13, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL19, NKG2D, and combinations thereof.

301. The kit of claim 295, wherein the cells further comprise one or more expression vector(s) that encode at least one antigen binding signaling receptor(s).

302. The kit of claim 301, wherein the at least one antigen binding signaling receptor(s) is selected from the group consisting of: native T Cell Receptor, an engineered T Cell Receptor (TCR), a Chimeric Antigen Receptors (CAR), a native B cell Receptor, an engineered B Cell Receptors (BCR), a stress ligand receptor, a pattern recognition receptor and combinations thereof.

303. The kit of claim 302, wherein the cells further comprise one or more expression vector(s) that encode at least one CAR(s).

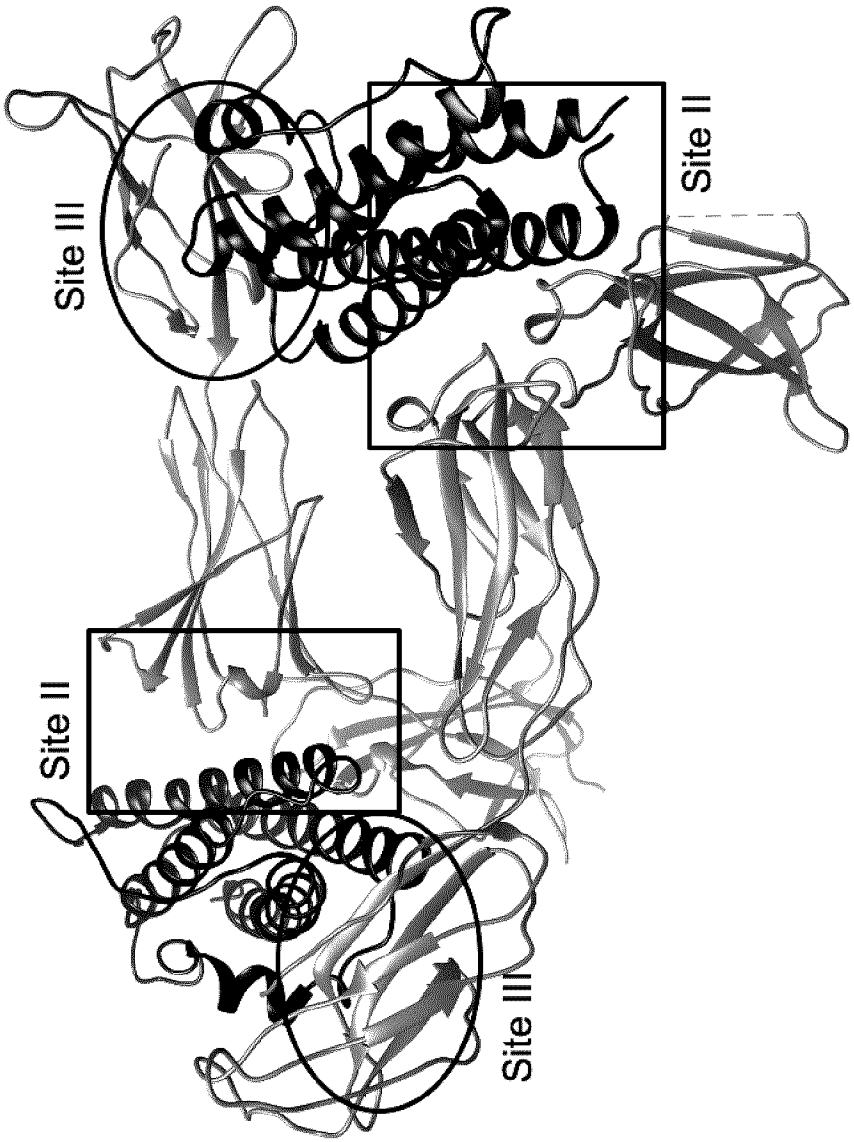


Figure 1

S

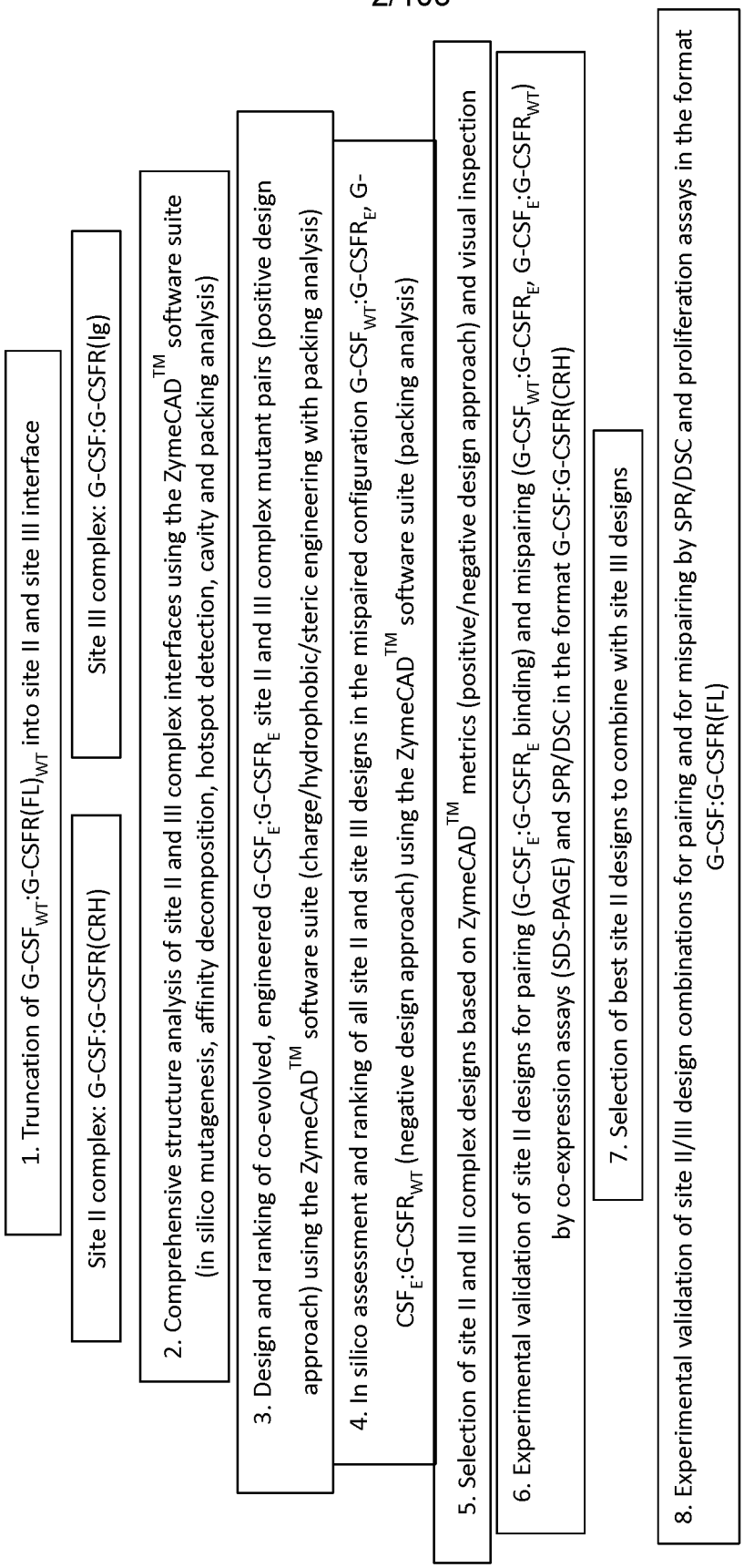

1. Truncation of G-CSF$_{WT}$:G-CSFR(FL)$_{WT}$ into site II and site III interface
Site II complex: G-CSF:G-CSFR(CRH)
Site III complex: G-CSF:G-CSFR(Ig)
2. Comprehensive structure analysis of site II and III complex interfaces using the ZymeCAD™ software suite (in silico mutagenesis, affinity decomposition, hotspot detection, cavity and packing analysis)
3. Design and ranking of co-evolved, engineered G-CSF$_E$:G-CSFR$_E$ site II and III complex mutant pairs (positive design approach) using the ZymeCAD™ software suite (charge/hydrophobic/steric engineering with packing analysis)
4. In silico assessment and ranking of all site II and site III designs in the mispaired configuration G-CSF$_{WT}$:G-CSFR$_E$, G-CSF$_E$:G-CSFR$_{WT}$ (negative design approach) using the ZymeCAD™ software suite (packing analysis)
5. Selection of site II and III complex designs based on ZymeCAD™ metrics (positive/negative design approach) and visual inspection
6. Experimental validation of site II designs for pairing (G-CSF$_E$:G-CSFR$_E$ binding) and mispairing (G-CSF$_{WT}$:G-CSFR$_E$, G-CSF$_E$:G-CSFR$_{WT}$) by co-expression assays (SDS-PAGE) and SPR/DSC in the format G-CSF:G-CSFR(CRH)
7. Selection of best site II designs to combine with site III designs
8. Experimental validation of site II/III design combinations for pairing and for mispairing by SPR/DSC and proliferation assays in the format G-CSF:G-CSFR(FL)

Figure 2

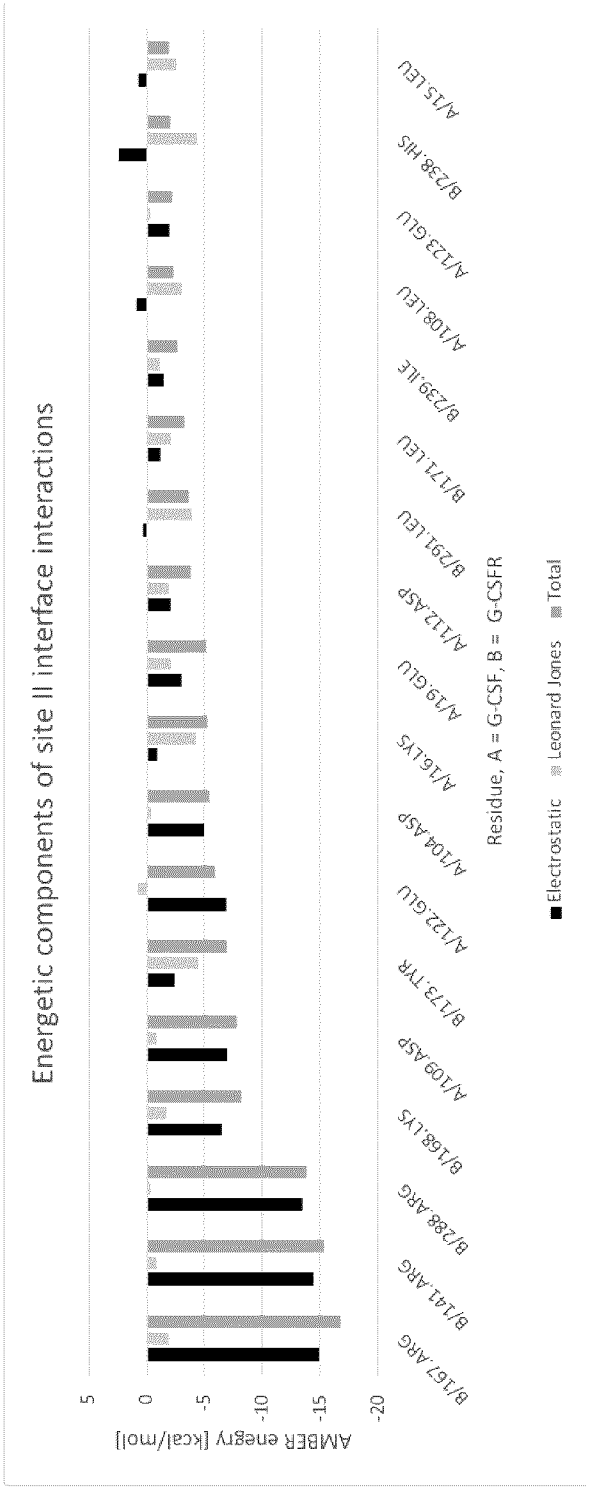

Energetic components of site II interface interactions
AMBER energy [kcal/mol]
5
0
-5
-10
-15
-20
B/167.ARG
B/141.ARG
B/288.ARG
B/168.LYS
A/109.ASP
B/173.TYR
A/122.GLU
A/104.ASP
A/16.LYS
A/19.GLU
A/112.ASP
B/291.LEU
B/171.LEU
B/239.ILE
A/108.LEU
A/123.GLU
B/238.HIS
A/15.LEU
Residue, A = G-CSF, B = G-CSFR
Electrostatic
Leonard Jones
Total

Figure 3

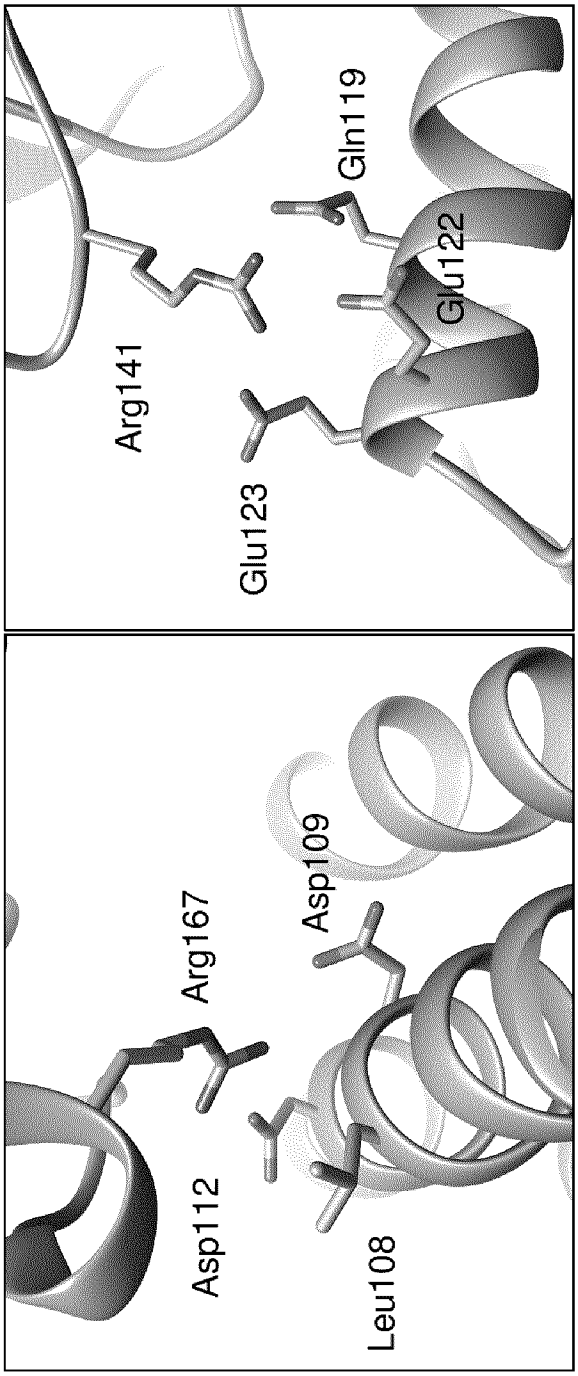


Figure 4

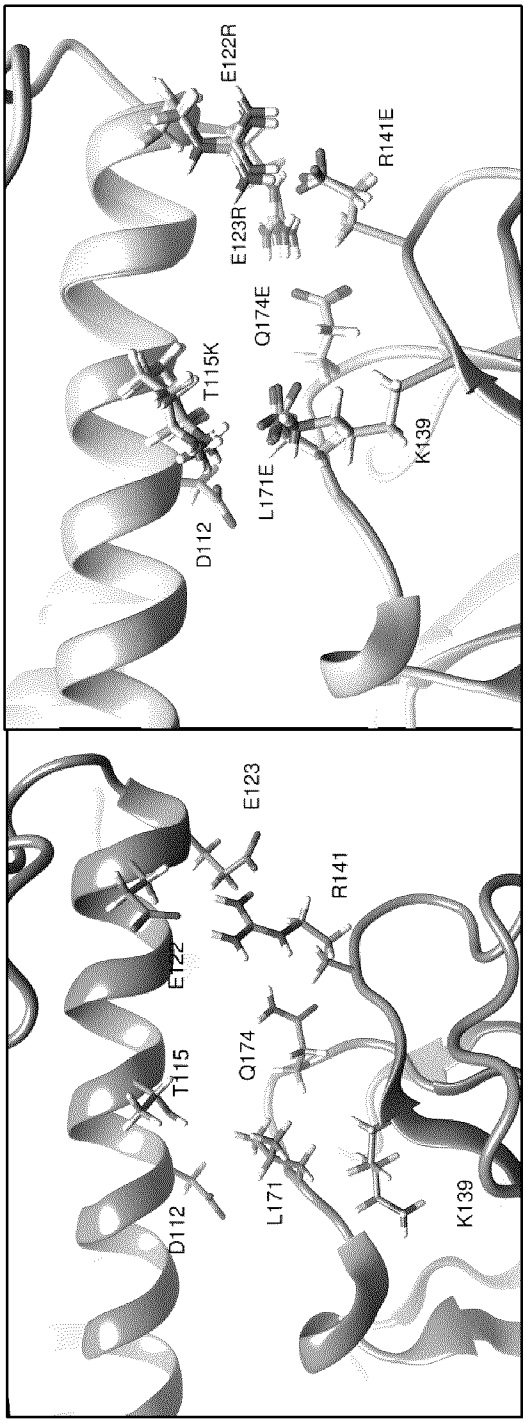
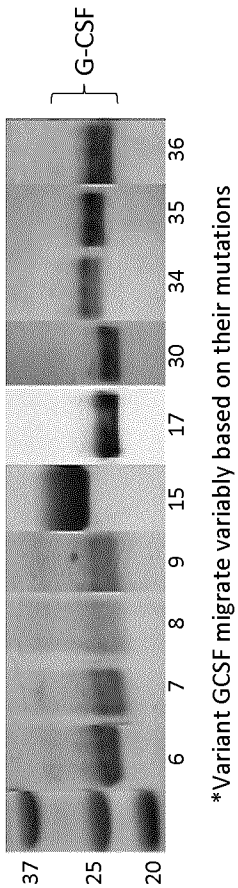

D112
T115
E122
E123
Q174
L171
R141
K139
D112
T115K
E122R
E123R
Q174E
L171E
R141E
K139

Figure 5


Variant site II G-CSF pulldowns
37
25
20
6
7
8
9
15
17
30
34
35
36
G-CSF
*Variant GCSF migrate variably based on their mutations

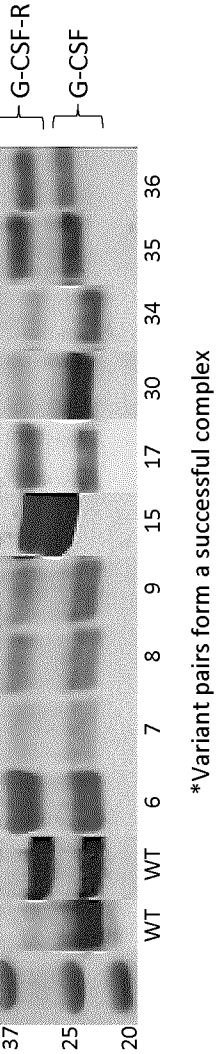
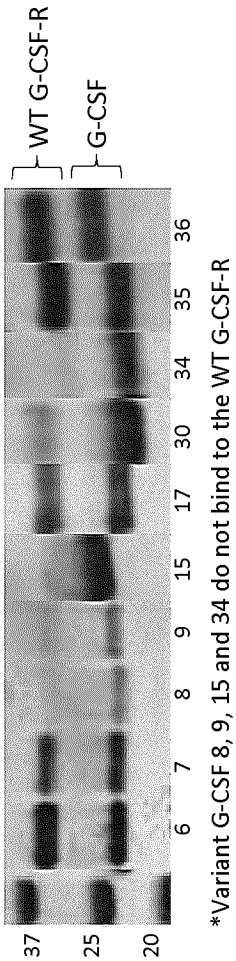
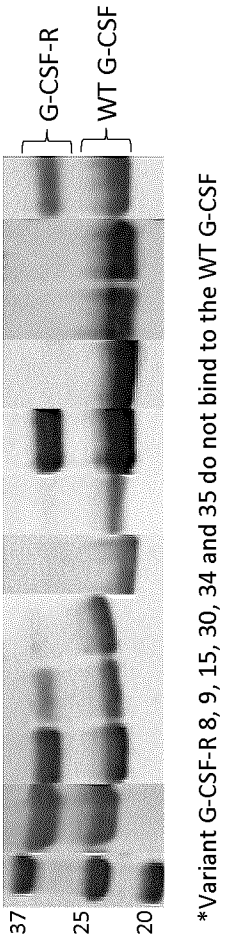

Variant site II co-expressed and purified complexes
37
25
20
WT
WT
WT
6
7
8
9
15
17
30
34
35
36
G-CSF-R
G-CSF
*Variant pairs form a successful complex

Figure 6



Variant site II G-CSF co-expressed with WT G-CSFR



WT G-CSF co-expressed with variant site II G-CSFR

Figure 7

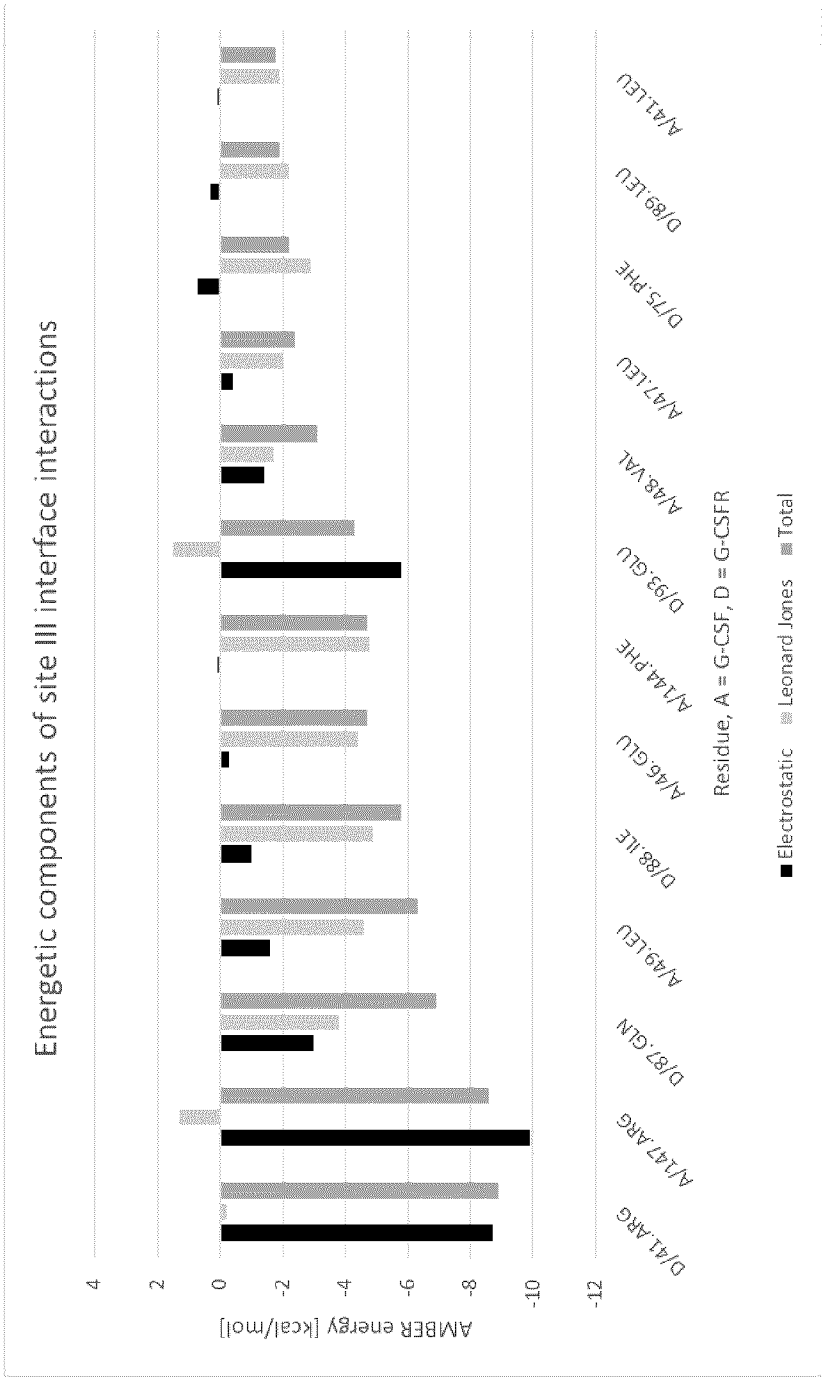

Energetic components of site III interface interactions
AMBER energy [kcal/mol]
4
2
0
-2
-4
-6
-8
-10
-12
D/41.ARG
A/147.ARG
D/87.GLN
A/49.LEU
D/88.ILE
A/46.GLU
A/144.PHE
D/93.GLU
A/48.VAL
A/47.LEU
D/75.PHE
D/89.LEU
A/41.LEU
Residue, A = G-CSF, D = G-CSFR
Electrostatic
Leonard Jones
Total

Figure 8

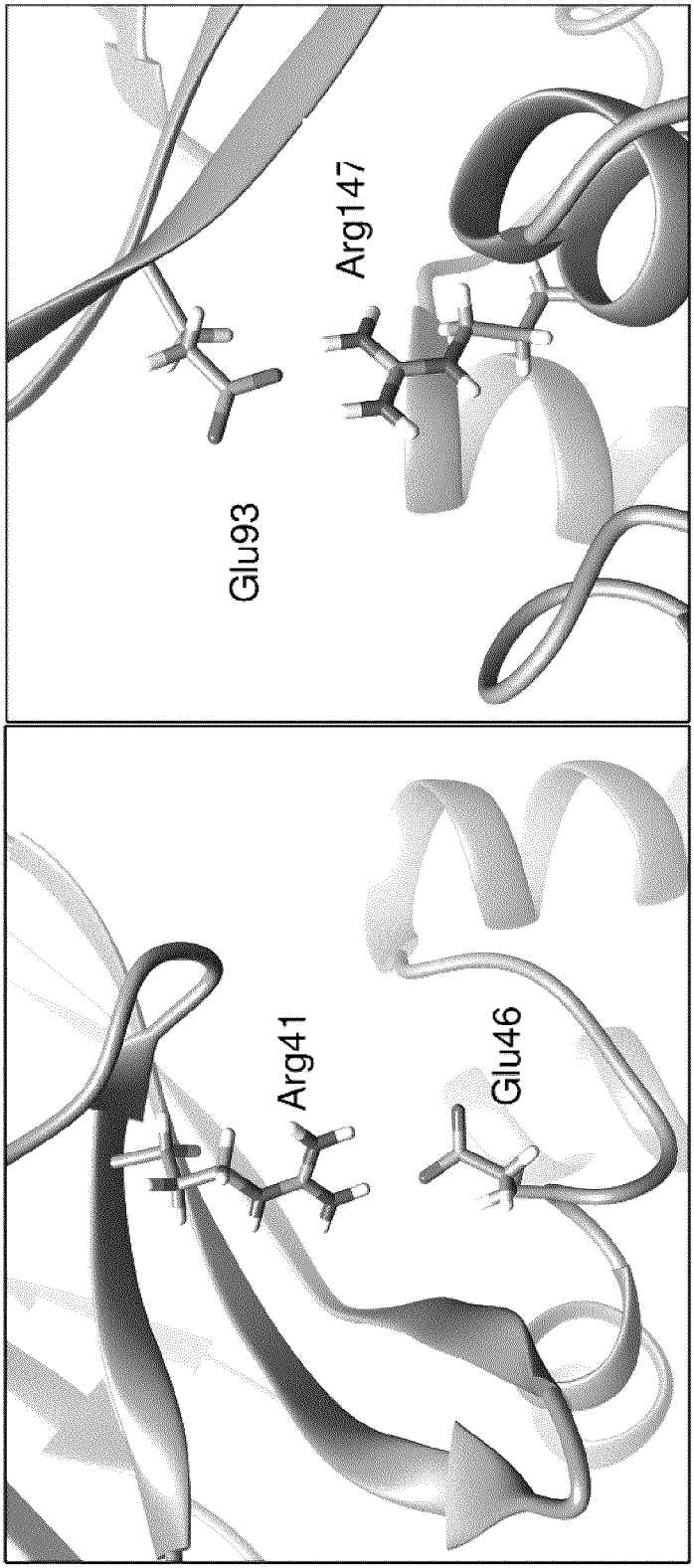


Figure 9

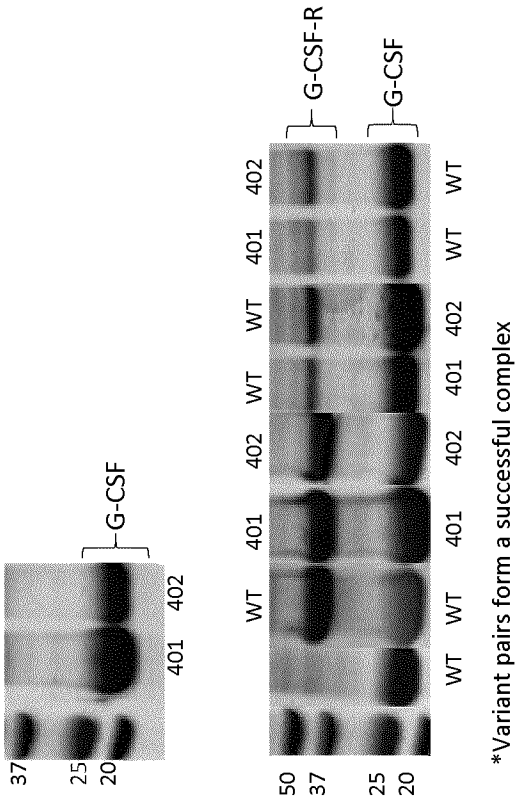

Variant site II/III G-CSF pulldowns
37
25
20
G-CSF
401
402
Variant site II/III co-expressed and purified complexes
50
37
25
20
G-CSF-R
G-CSF
WT
401
402
WT
WT
401
402
401
402
WT
WT
*Variant pairs form a successful complex

Figure 10

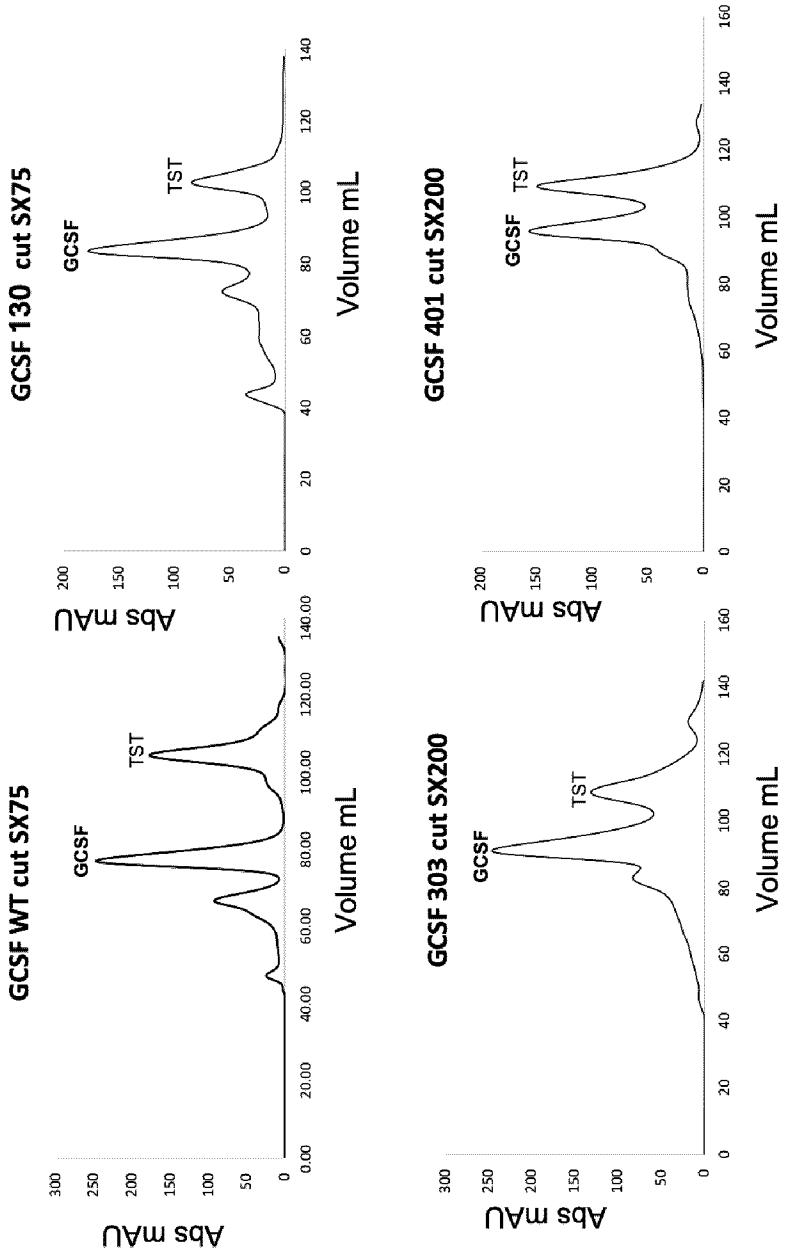

GCSF WT cut SX75
GCSF
TST
Abs mAU
Volume mL
GCSF 130 cut SX75
GCSF
TST
Abs mAU
Volume mL
GCSF 303 cut SX200
GCSF
TST
Abs mAU
Volume mL
GCSF 401 cut SX200
GCSF
TST
Abs mAU
Volume mL

Figure 11

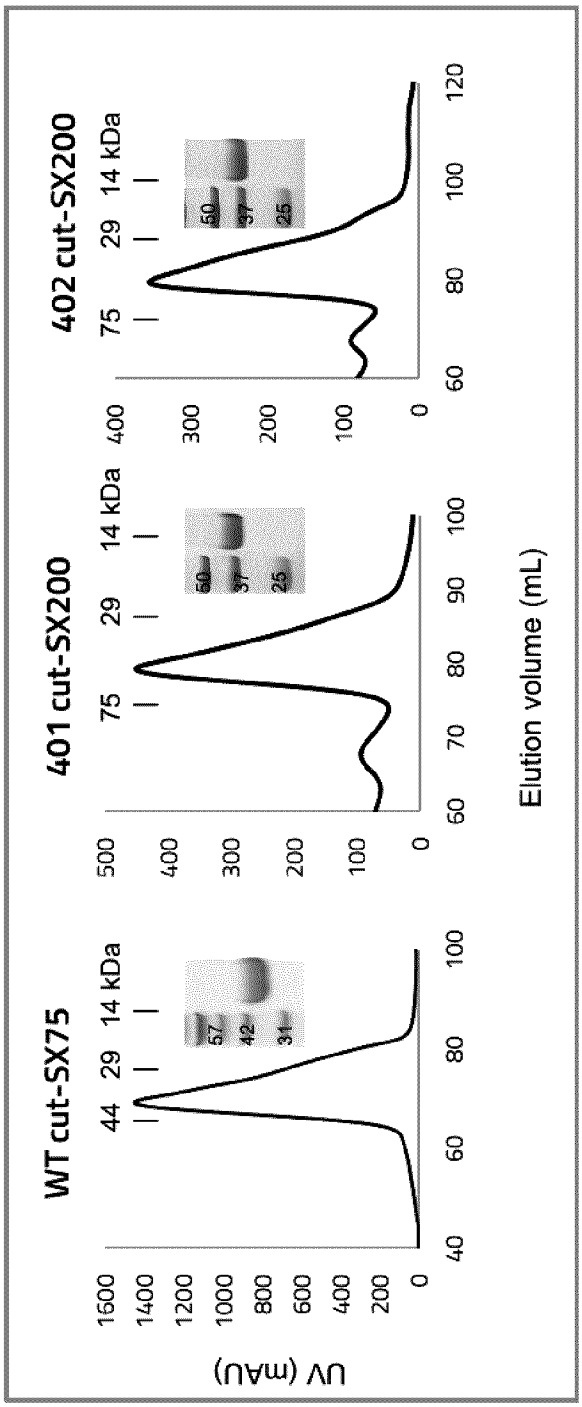

WT cut-SX75
401 cut-SX200
402 cut-SX200
UV (mAU)
1600
1400
1200
1000
800
600
400
200
0
500
400
300
200
100
0
44
29
14 kDa
75
29
14 kDa
75
29
14 kDa
57
42
31
50
37
25
50
37
25
40
60
80
100
60
70
80
90
100
60
80
100
120
Elution volume (mL)

Figure 12

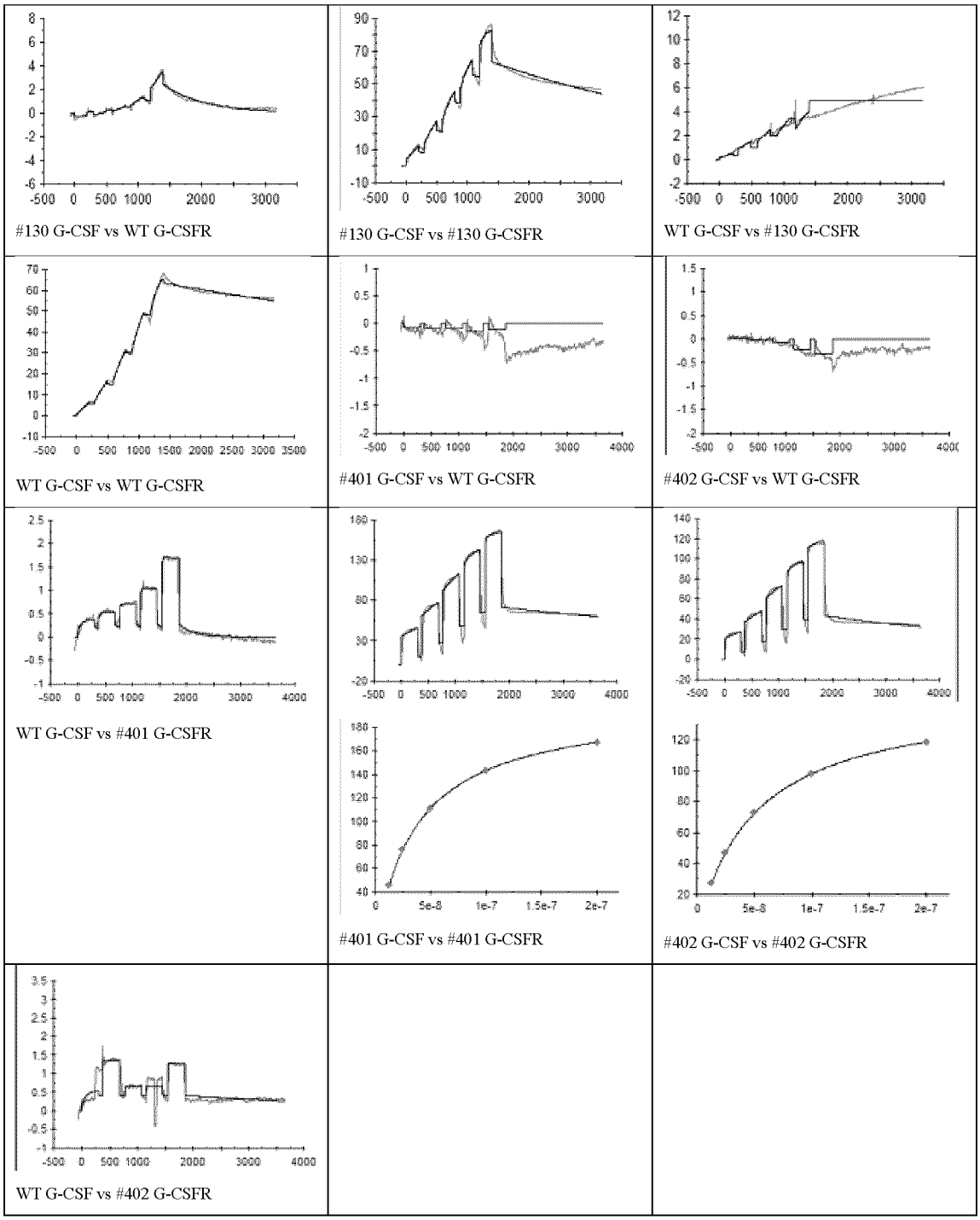

#130 G-CSF vs WT G-CSFR
#130 G-CSF vs #130 G-CSFR
WT G-CSF vs #130 G-CSFR
WT G-CSF vs WT G-CSFR
#401 G-CSF vs WT G-CSFR
#402 G-CSF vs WT G-CSFR
WT G-CSF vs #401 G-CSFR
#401 G-CSF vs #401 G-CSFR
#402 G-CSF vs #402 G-CSFR
WT G-CSF vs #402 G-CSFR

Figure 13

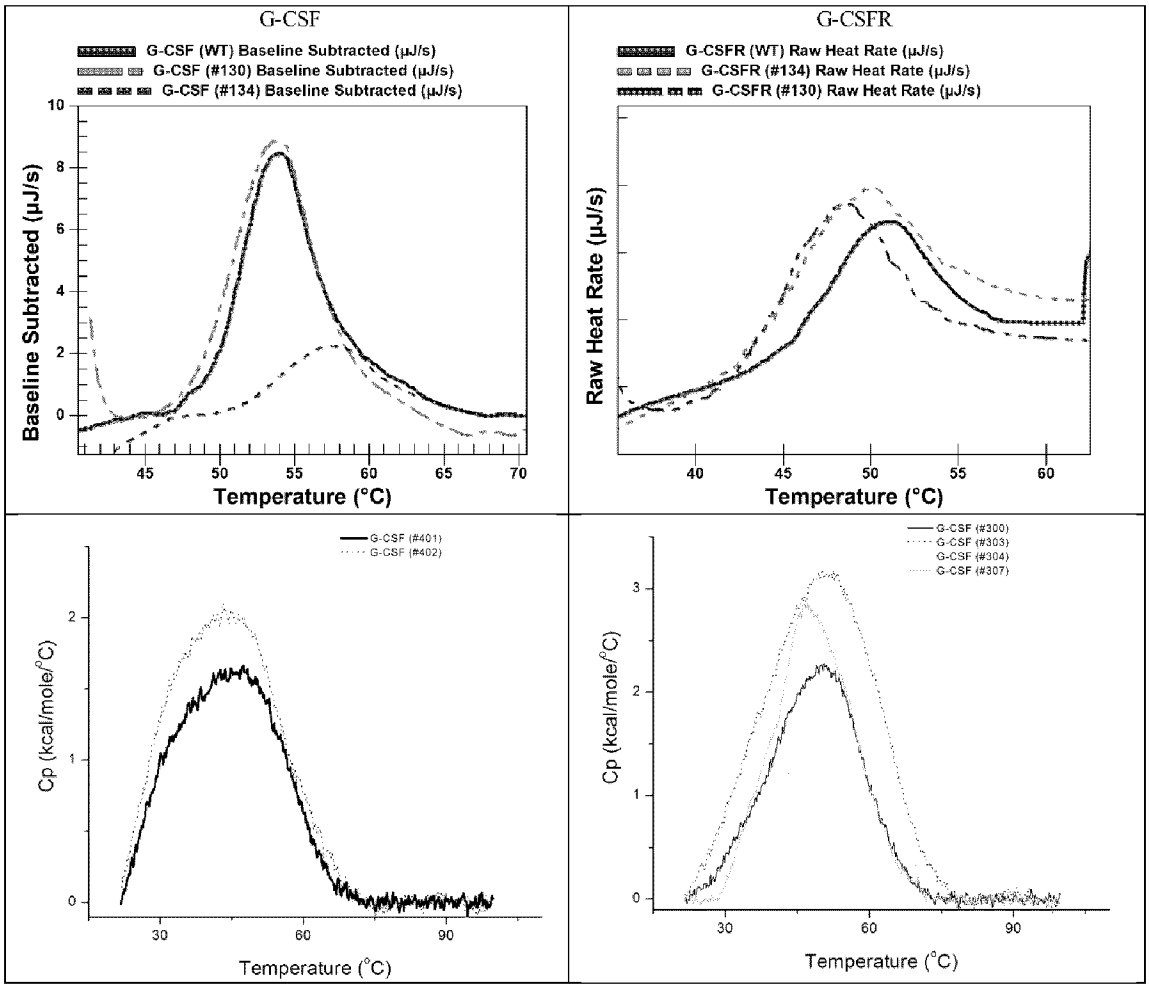

G-CSF
G-CSF (WT) Baseline Subtracted (μJ/s)
G-CSF (#130) Baseline Subtracted (μJ/s)
G-CSF (#134) Baseline Subtracted (μJ/s)
Baseline Subtracted (μJ/s)
Temperature (°C)
G-CSFR
G-CSFR (WT) Raw Heat Rate (μJ/s)
G-CSFR (#134) Raw Heat Rate (μJ/s)
G-CSFR (#130) Raw Heat Rate (μJ/s)
Raw Heat Rate (μJ/s)
Temperature (°C)
G-CSF (#401)
G-CSF (#402)
Cp (kcal/mole/°C)
Temperature (°C)
G-CSF (#300)
G-CSF (#303)
G-CSF (#304)
G-CSF (#307)
Cp (kcal/mole/°C)
Temperature (°C)

Figure 14

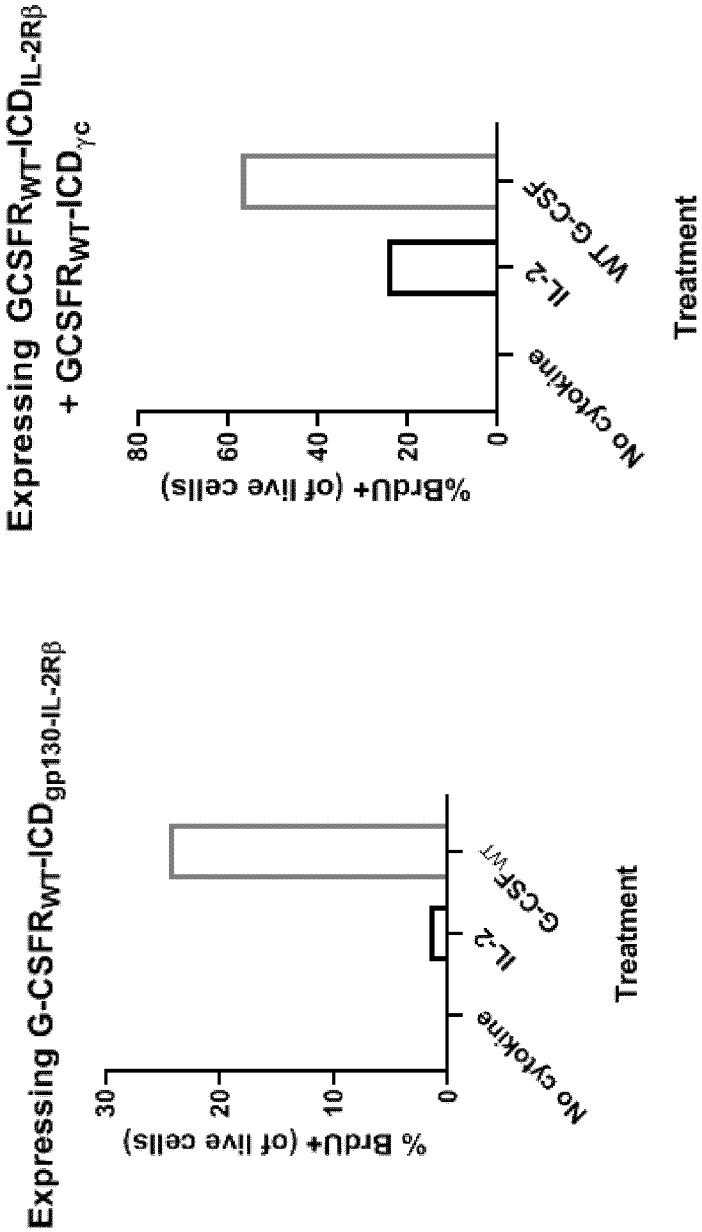

Expressing G-CSFR$_{WT}$-ICD$_{gp130-IL-2R\beta}$
% BrdU+ (of live cells)
30
20
10
0
No cytokine
IL-2
G-CSF$_{WT}$
Treatment
Expressing GCSFR$_{WT}$-ICD$_{IL-2R\beta}$
+ GCSFR$_{WT}$-ICD$_{\gamma c}$
%BrdU+ (of live cells)
80
60
40
20
0
No cytokine
IL-2
WT G-CSF
Treatment

Figure 15

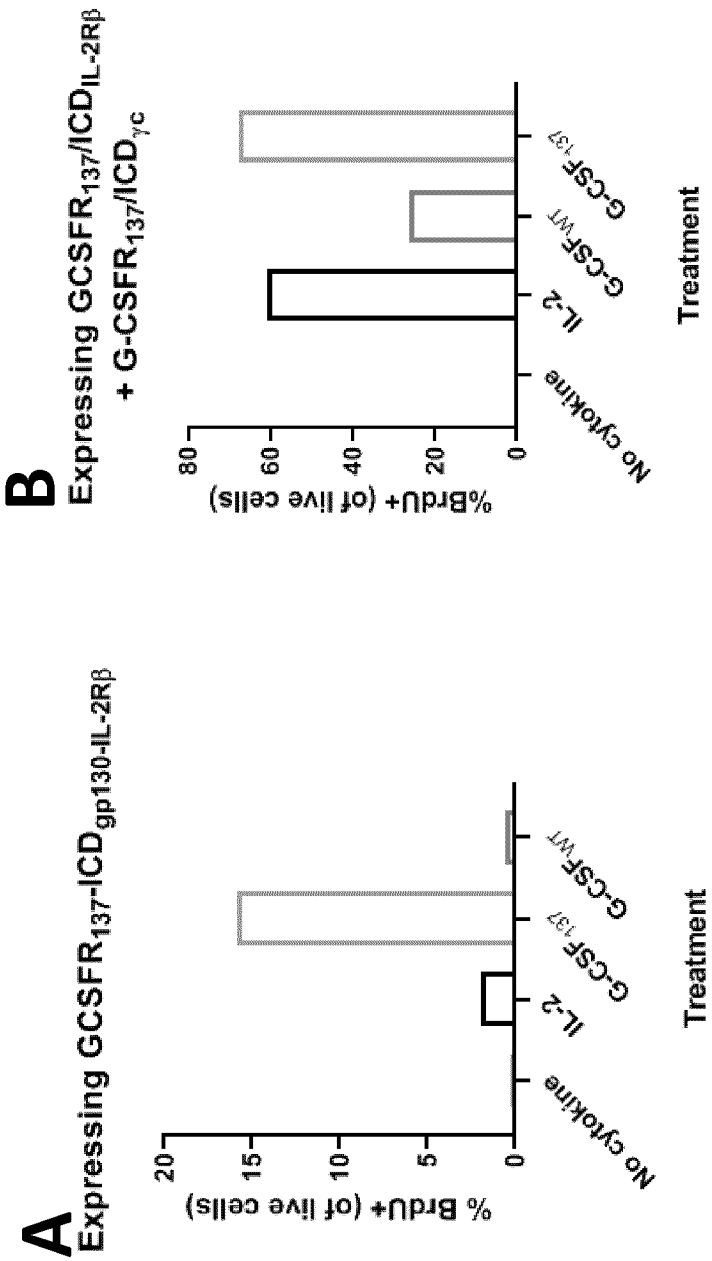

A
Expressing GCSFR137-ICDgp130-IL-2Rβ
% BrdU+ (of live cells)
20
15
10
5
0
No cytokine
IL-2
G-CSF137
G-CSFWT
Treatment
B
Expressing GCSFR137/ICDIL-2Rβ
+ G-CSFR137/ICDγc
%BrdU+ (of live cells)
80
60
40
20
0
No cytokine
IL-2
G-CSFWT
G-CSF137
Treatment

Figure 16

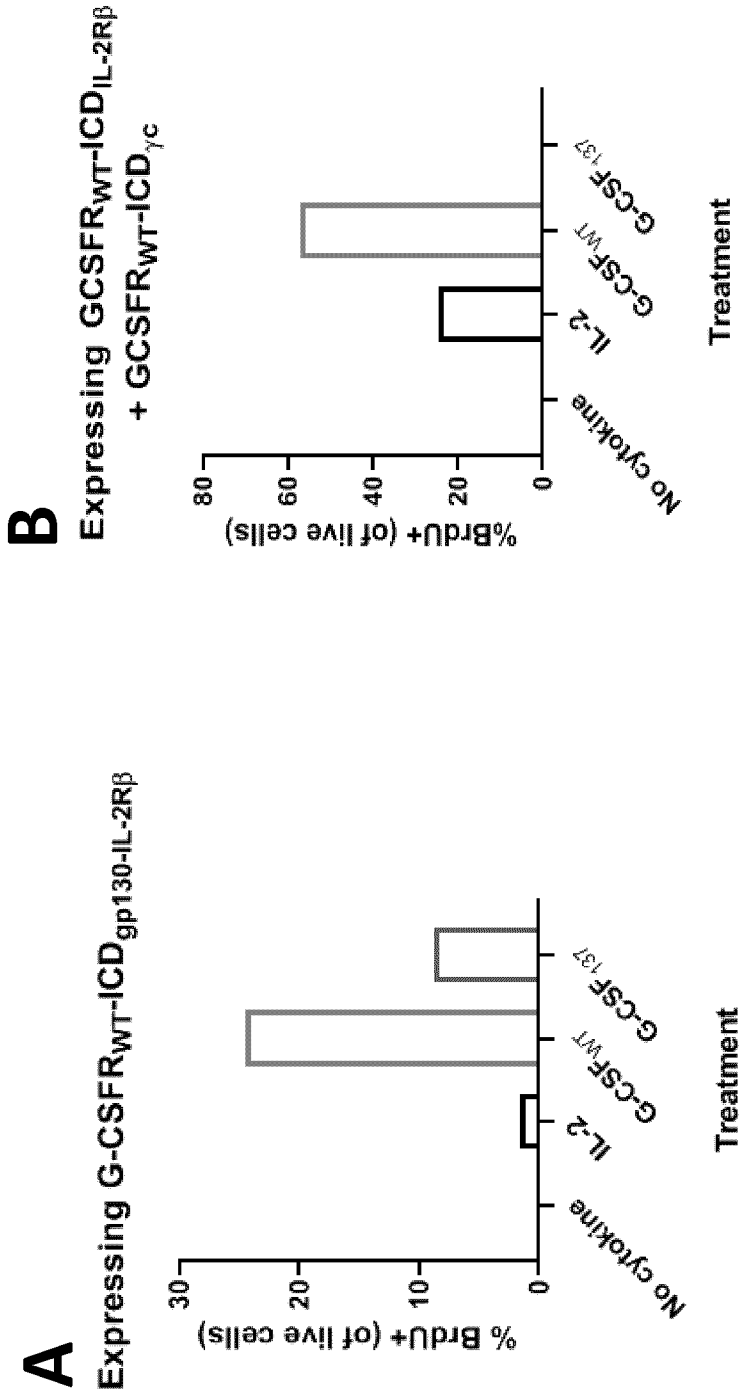

A
Expressing G-CSFR$_{WT}$-ICD$_{gp130-IL-2R\beta}$
% BrdU+ (of live cells)
30
20
10
0
No cytokine
IL-2
G-CSF$_{WT}$
G-CSF$_{137}$
Treatment
B
Expressing GCSFR$_{WT}$-ICD$_{IL-2R\beta}$
+ GCSFR$_{WT}$-ICD$_{\gamma c}$
%BrdU+ (of live cells)
80
60
40
20
0
No cytokine
IL-2
G-CSF$_{WT}$
G-CSF$_{137}$
Treatment

Figure 17

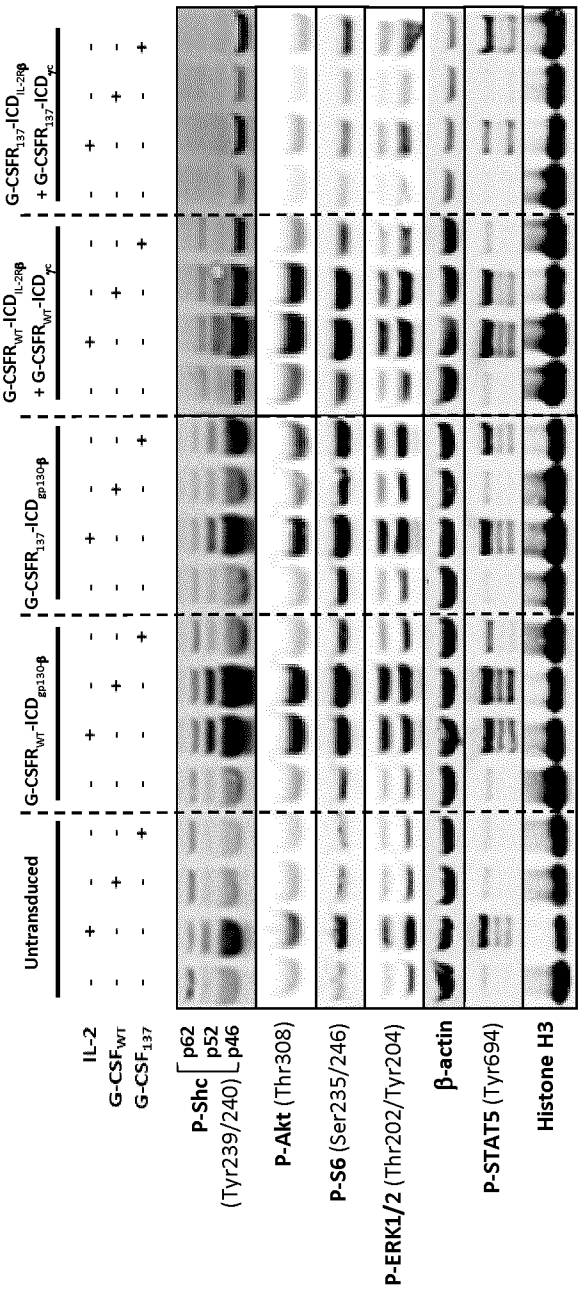

Untransduced
G-CSFR$_{WT}$-ICD$_{gp130}$-β
G-CSFR$_{137}$-ICD$_{gp130}$-β
G-CSFR$_{WT}$-ICD$_{IL-2R}$β + G-CSFR$_{WT}$-ICD$_{γc}$
G-CSFR$_{137}$-ICD$_{IL-2R}$β + G-CSFR$_{137}$-ICD$_{γc}$
IL-2
G-CSF$_{WT}$
G-CSF$_{137}$
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Thr308)
P-S6 (Ser235/246)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT5 (Tyr694)
Histone H3

Figure 18

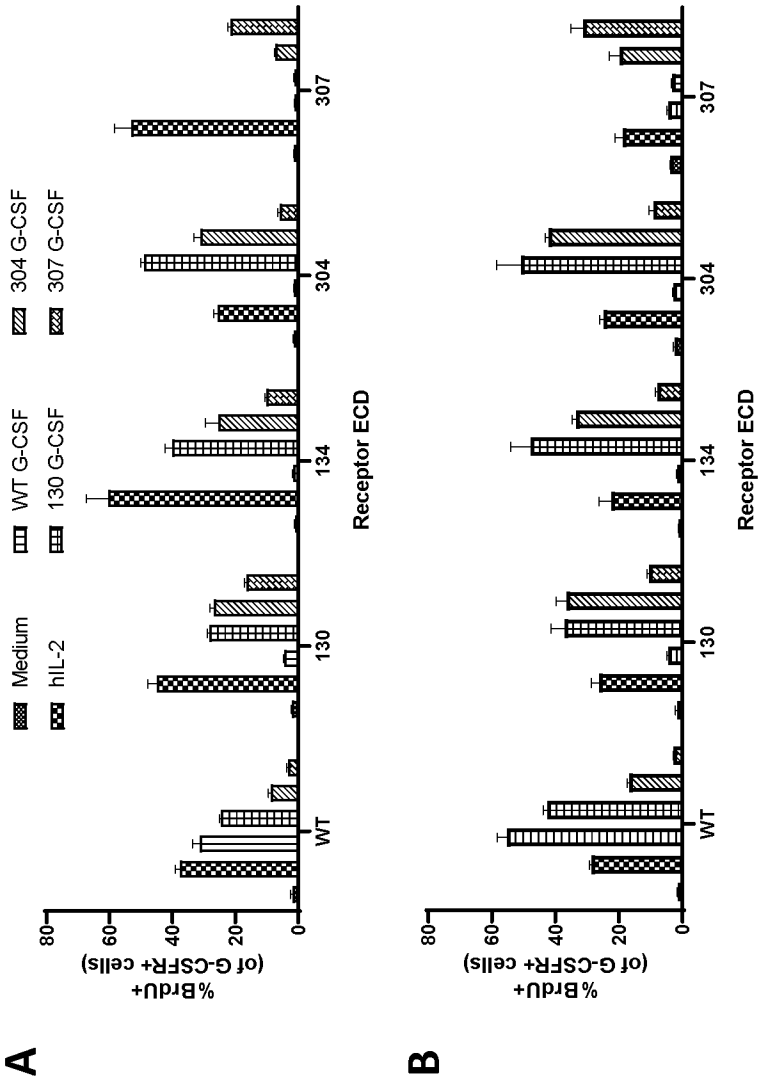

A
B
Medium
hIL-2
WT G-CSF
130 G-CSF
304 G-CSF
307 G-CSF
% BrdU+
(of G-CSFR+ cells)
0
20
40
60
80
WT
130
134
304
307
Receptor ECD

Figure 19

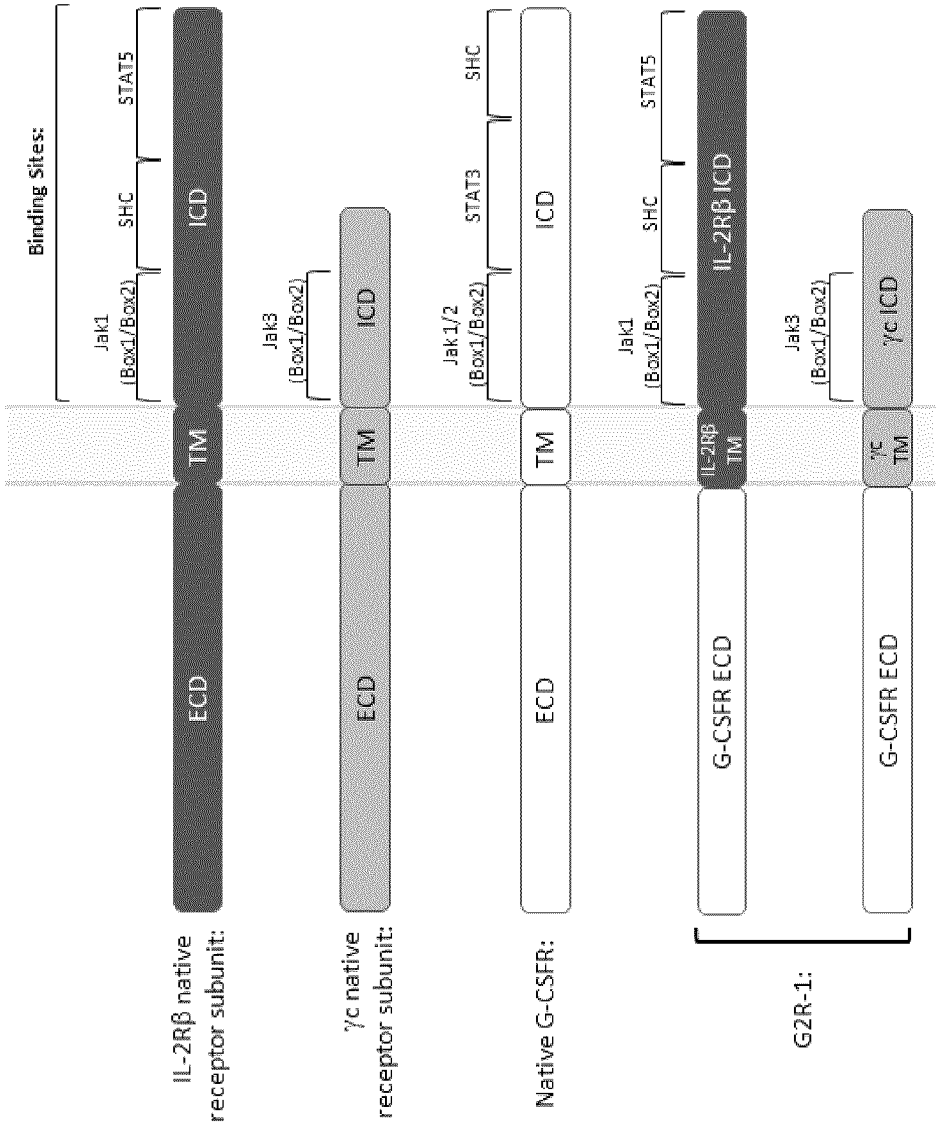

Binding Sites:
Jak1 (Box1/Box2)
SHC
STAT5
ICD
TM
ECD
IL-2Rβ native receptor subunit:
Jak3 (Box1/Box2)
ICD
TM
ECD
γc native receptor subunit:
Jak1/2 (Box1/Box2)
STAT3
SHC
ICD
TM
ECD
Native G-CSFR:
Jak1 (Box1/Box2)
SHC
STAT5
IL-2Rβ ICD
IL-2Rβ TM
G-CSFR ECD
Jak3 (Box1/Box2)
γc ICD
γc TM
G-CSFR ECD
G2R-1:

Figure 20

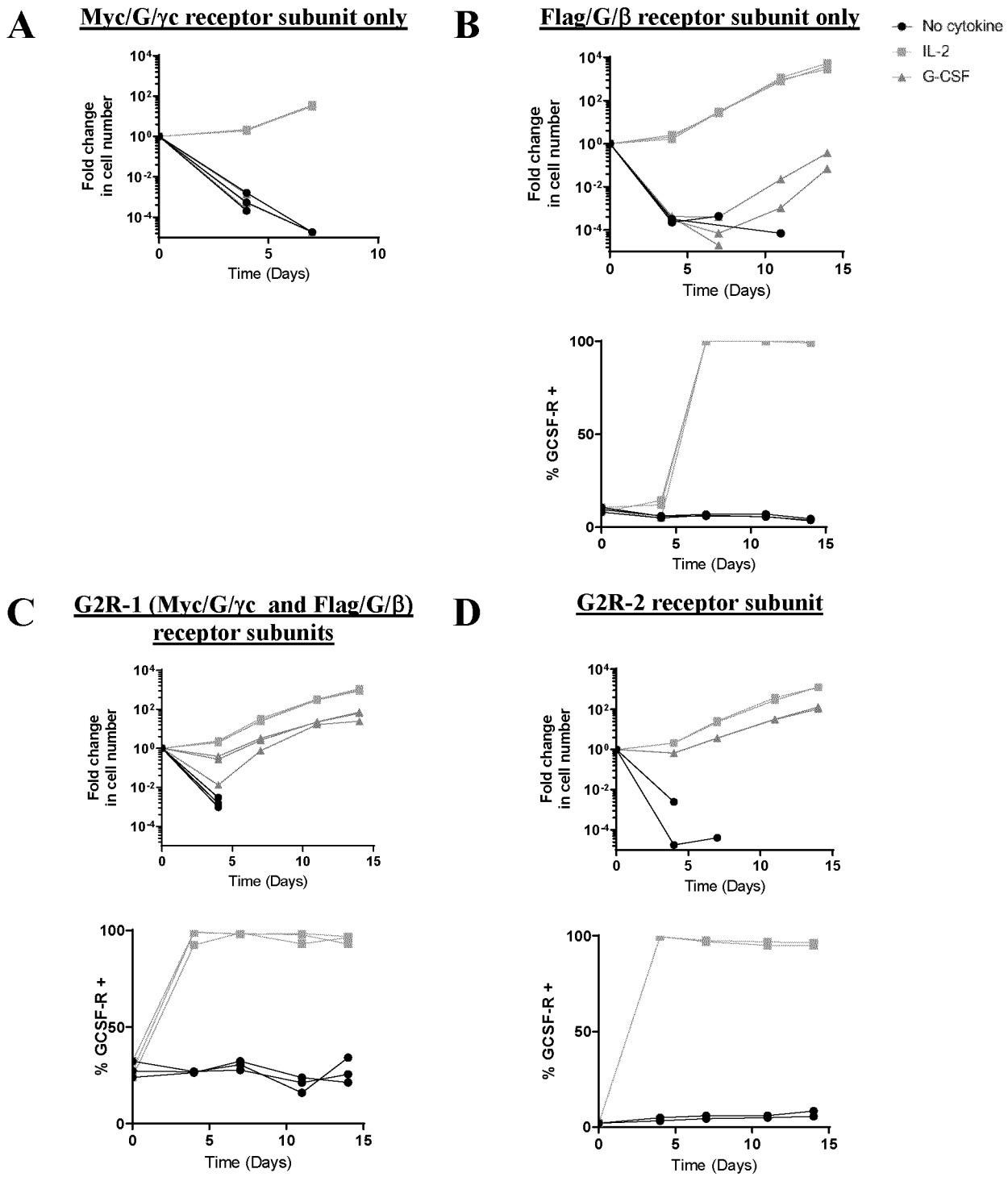

A
Myc/G/γc receptor subunit only
B
Flag/G/β receptor subunit only
No cytokine
IL-2
G-CSF
Fold change in cell number
Time (Days)
% GCSF-R +
C
G2R-1 (Myc/G/γc and Flag/G/β) receptor subunits
D
G2R-2 receptor subunit

Figure 21

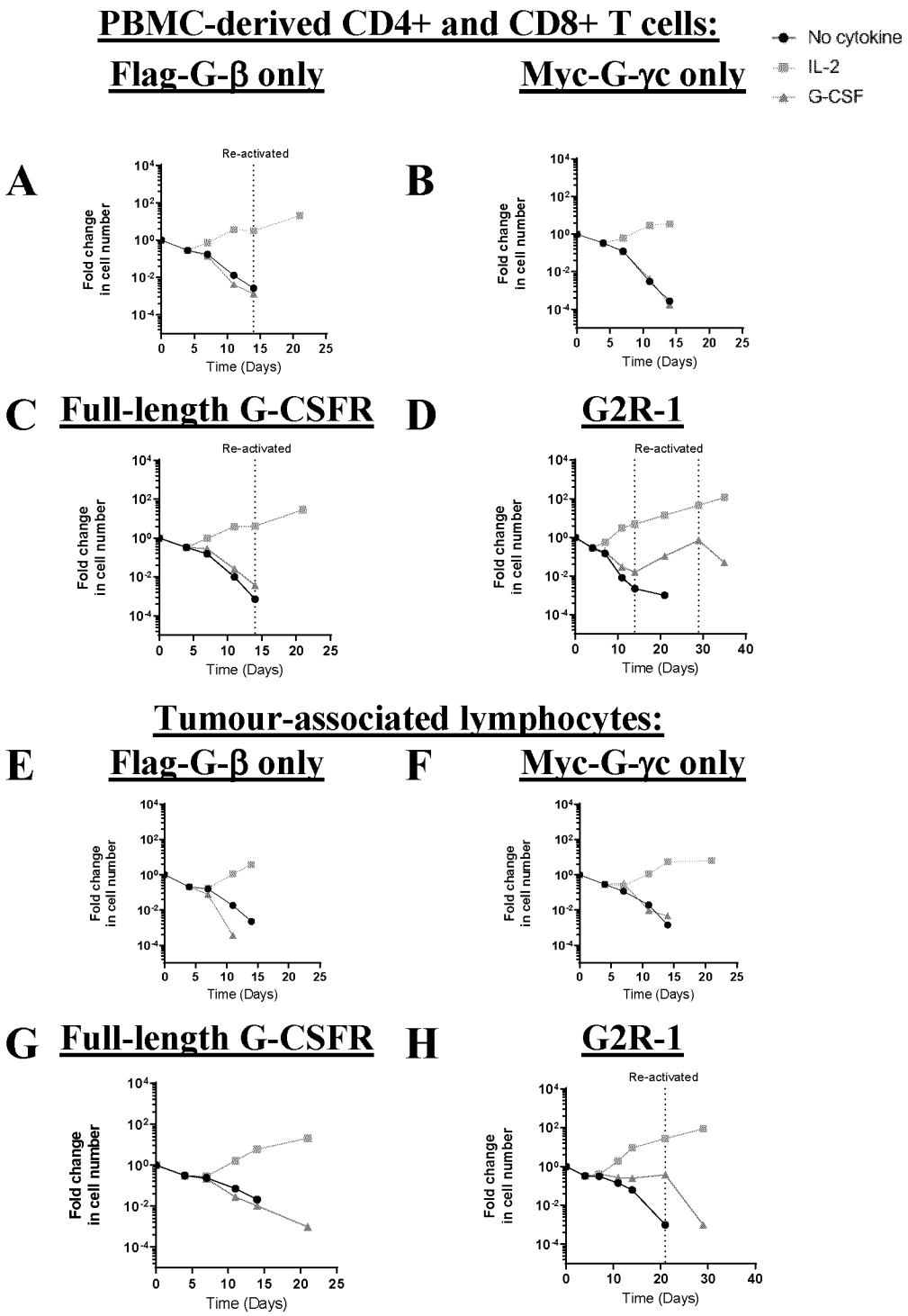

PBMC-derived CD4+ and CD8+ T cells:
Flag-G-β only
Myc-G-γc only
No cytokine
IL-2
G-CSF
A
B
C
Full-length G-CSFR
D
G2R-1
Re-activated
Fold change in cell number
Time (Days)
Tumour-associated lymphocytes:
E
Flag-G-β only
F
Myc-G-γc only
G
Full-length G-CSFR
H
G2R-1

Figure 22

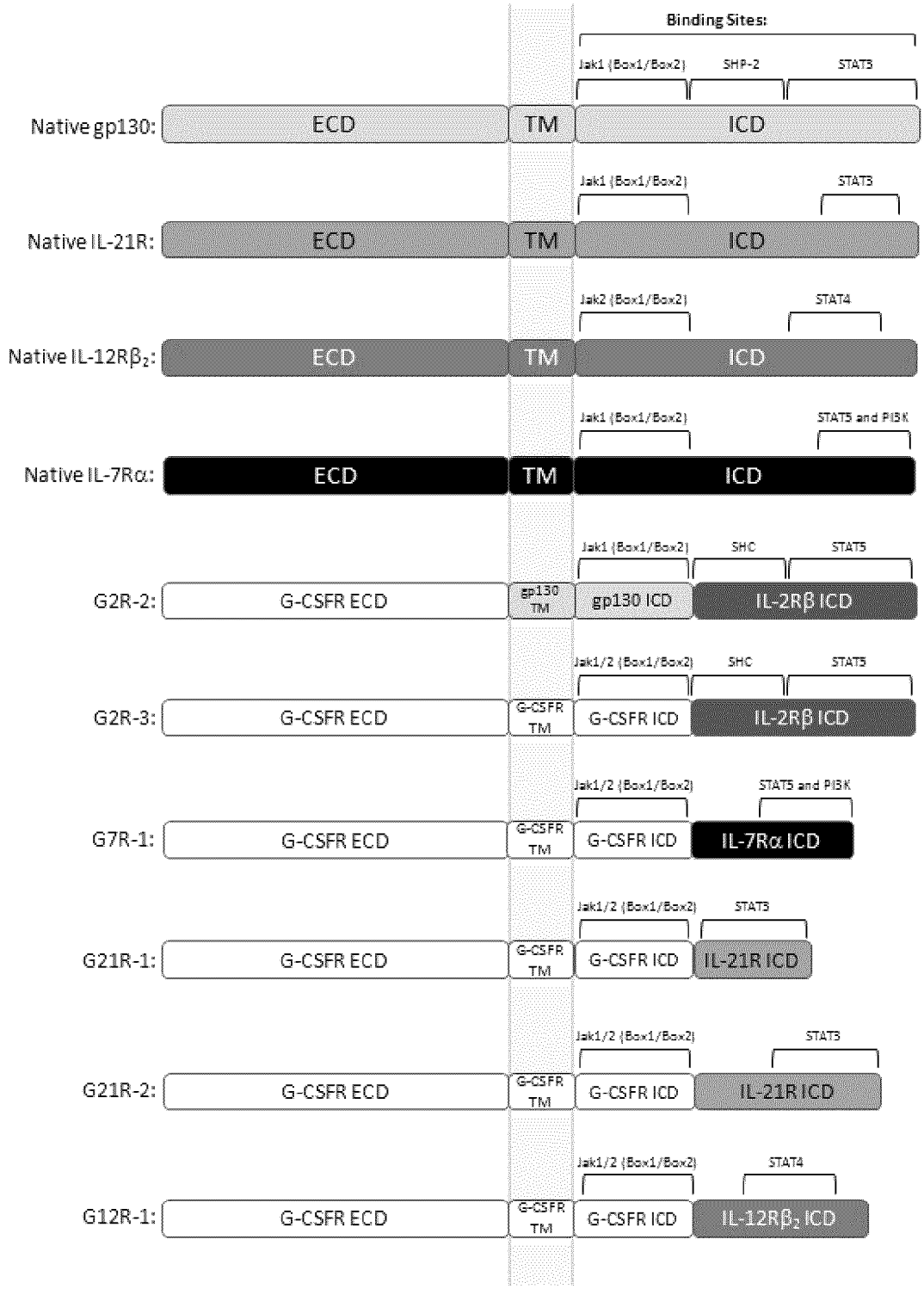

Binding Sites:
Jak1 (Box1/Box2)
SHP-2
STAT3
Native gp130:
ECD
TM
ICD
Jak1 (Box1/Box2)
STAT3
Native IL-21R:
ECD
TM
ICD
Jak2 (Box1/Box2)
STAT4
Native IL-12Rβ2:
ECD
TM
ICD
Jak1 (Box1/Box2)
STAT5 and PI3K
Native IL-7Rα:
ECD
TM
ICD
Jak1 (Box1/Box2)
SHC
STAT5
G2R-2:
G-CSFR ECD
gp130 TM
gp130 ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
SHC
STAT5
G2R-3:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
STAT5 and PI3K
G7R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-7Rα ICD
Jak1/2 (Box1/Box2)
STAT3
G21R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-21R ICD
Jak1/2 (Box1/Box2)
STAT3
G21R-2:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-21R ICD
Jak1/2 (Box1/Box2)
STAT4
G12R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-12Rβ2 ICD

Figure 23

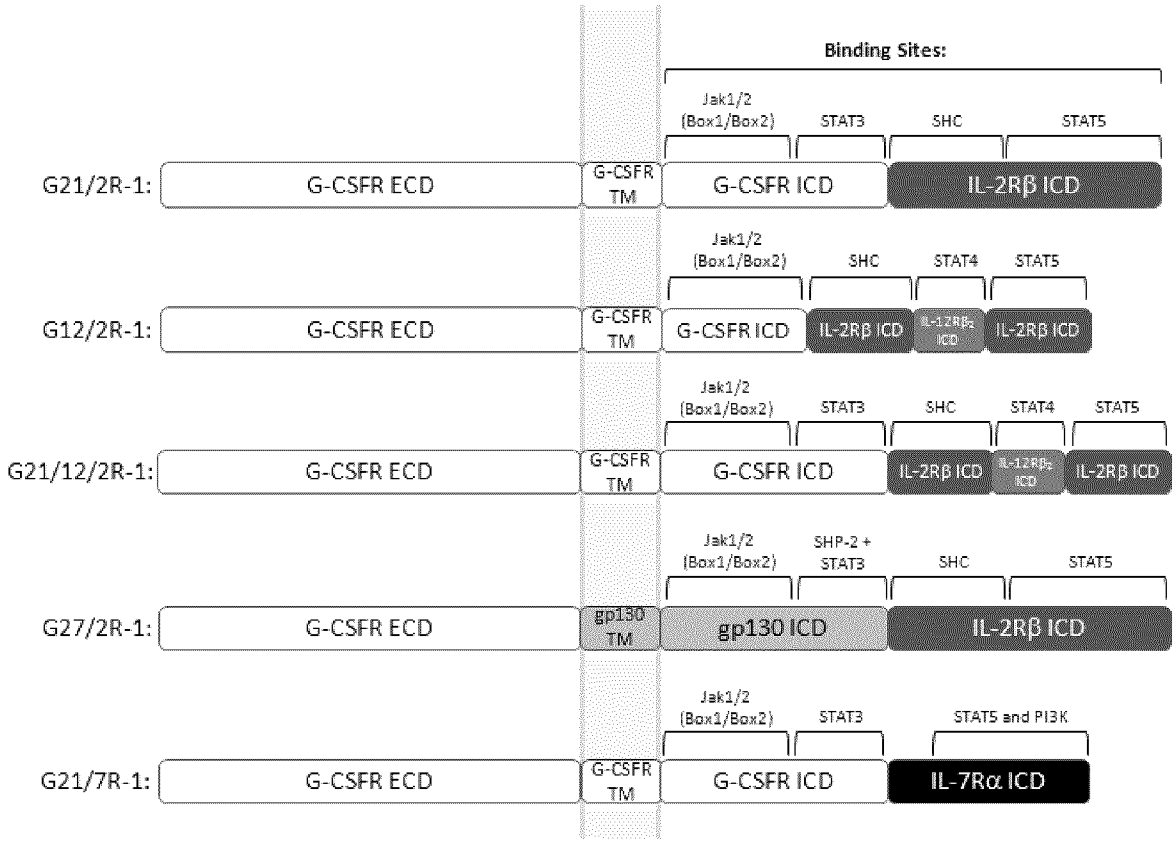

Binding Sites:
Jak1/2 (Box1/Box2)
STAT3
SHC
STAT5
G21/2R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
SHC
STAT4
STAT5
G12/2R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-2Rβ ICD
IL-12Rβ2 ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
STAT3
SHC
STAT4
STAT5
G21/12/2R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-2Rβ ICD
IL-12Rβ2 ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
SHP-2 + STAT3
SHC
STAT5
G27/2R-1:
G-CSFR ECD
gp130 TM
gp130 ICD
IL-2Rβ ICD
Jak1/2 (Box1/Box2)
STAT3
STAT5 and PI3K
G21/7R-1:
G-CSFR ECD
G-CSFR TM
G-CSFR ICD
IL-7Rα ICD

Figure 24

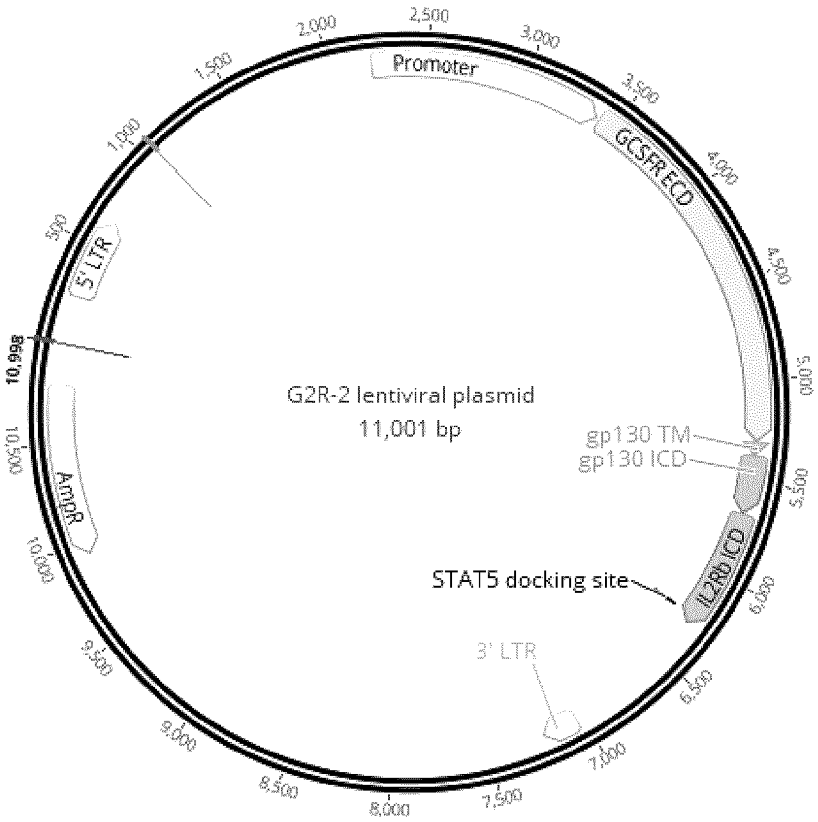

2,500
3,000
3,500
4,000
4,500
5,000
5,500
6,000
6,500
7,000
7,500
8,000
8,500
9,000
9,500
10,000
10,500
10,998
500
1,000
1,500
2,000
Promoter
GCSFR ECD
5' LTR
G2R-2 lentiviral plasmid
11,001 bp
gp130 TM
gp130 ICD
IL2Rβ ICD
STAT5 docking site
3' LTR
AmpR

Figure 25

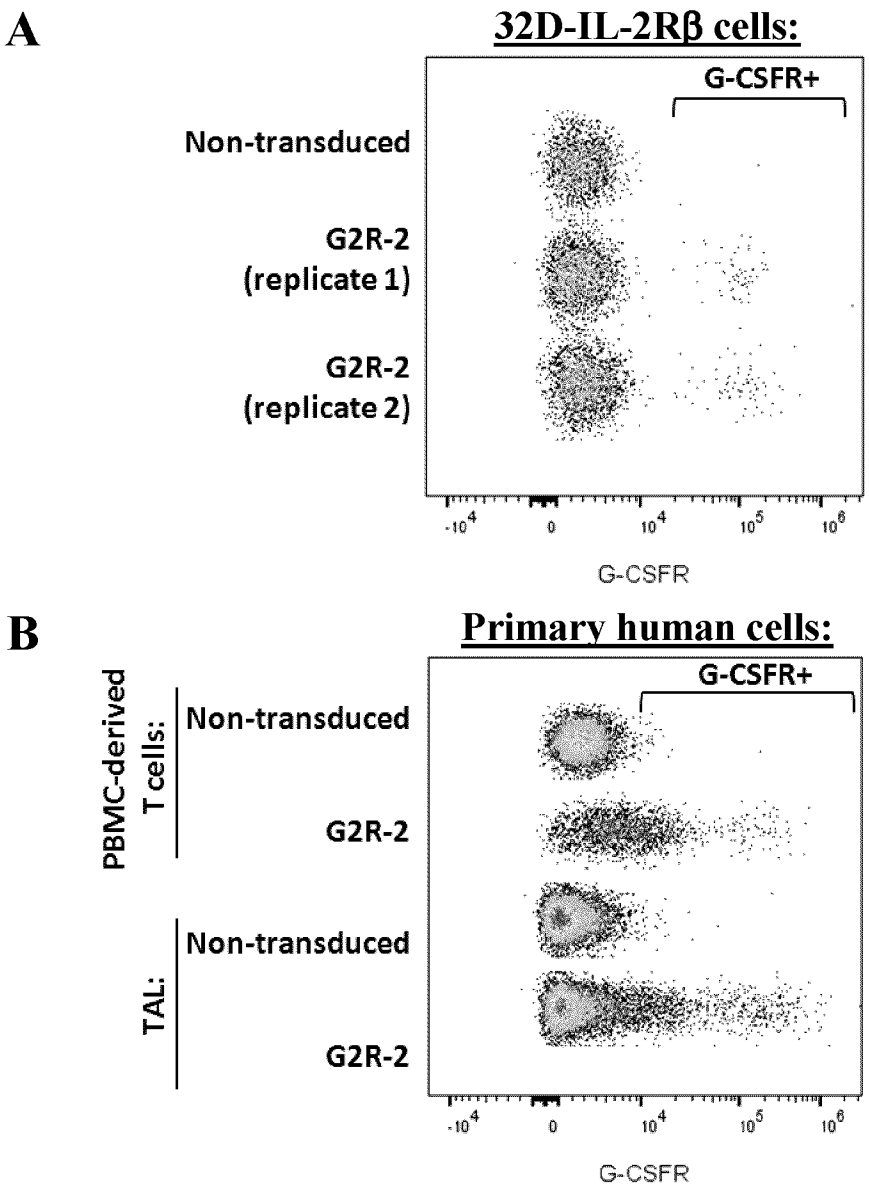

A
32D-IL-2Rβ cells:
G-CSFR+
Non-transduced
G2R-2
(replicate 1)
G2R-2
(replicate 2)
-10^4
0
10^4
10^5
10^6
G-CSFR
B
Primary human cells:
G-CSFR+
PBMC-derived
T cells:
Non-transduced
G2R-2
TAL:
Non-transduced
G2R-2
-10^4
0
10^4
10^5
10^6
G-CSFR

Figure 26

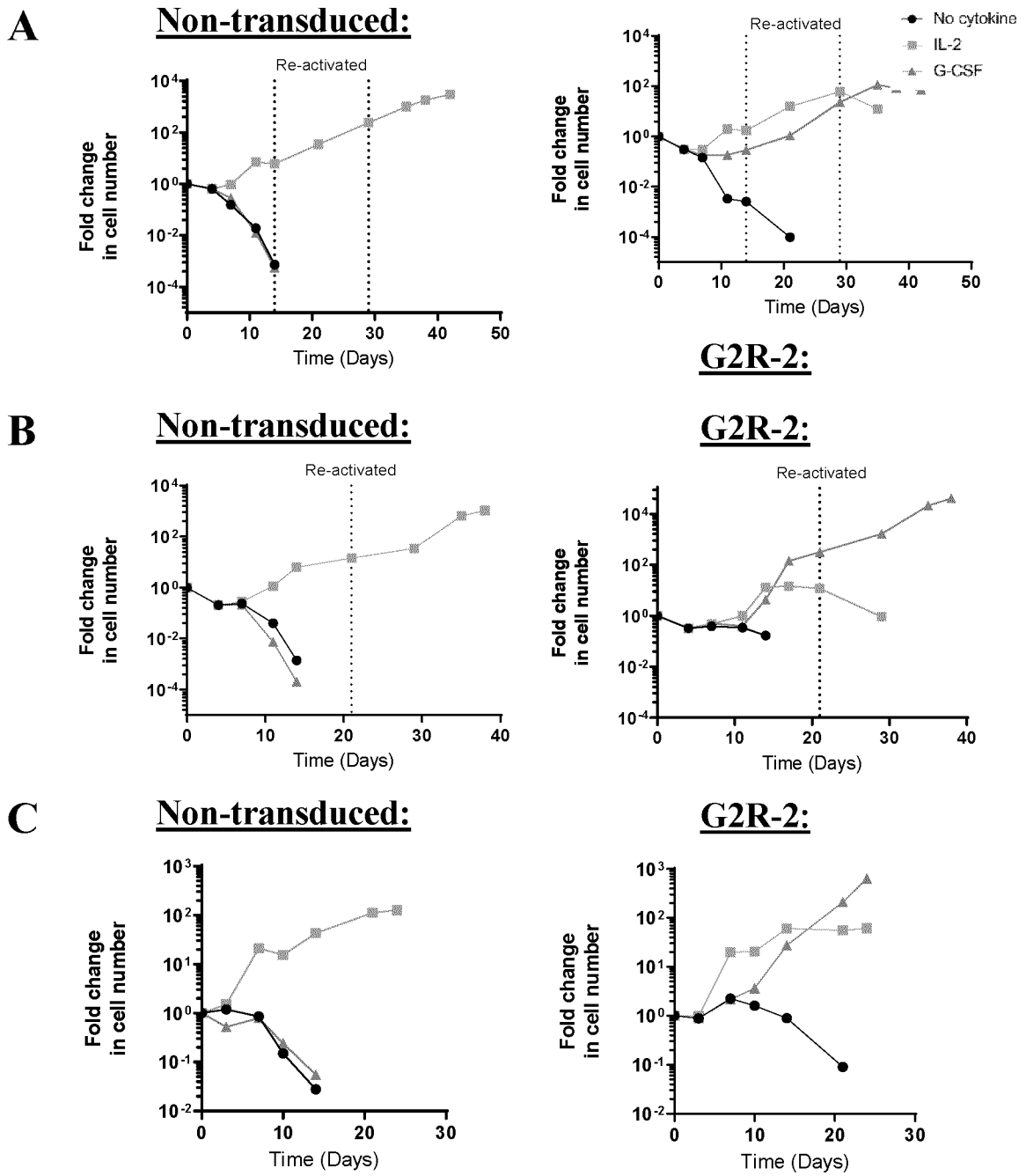

A
Non-transduced:
G2R-2:
Re-activated
No cytokine
IL-2
G-CSF
Fold change in cell number
Time (Days)
B
Non-transduced:
G2R-2:
Re-activated
Fold change in cell number
Time (Days)
C
Non-transduced:
G2R-2:
Fold change in cell number
Time (Days)

Figure 27

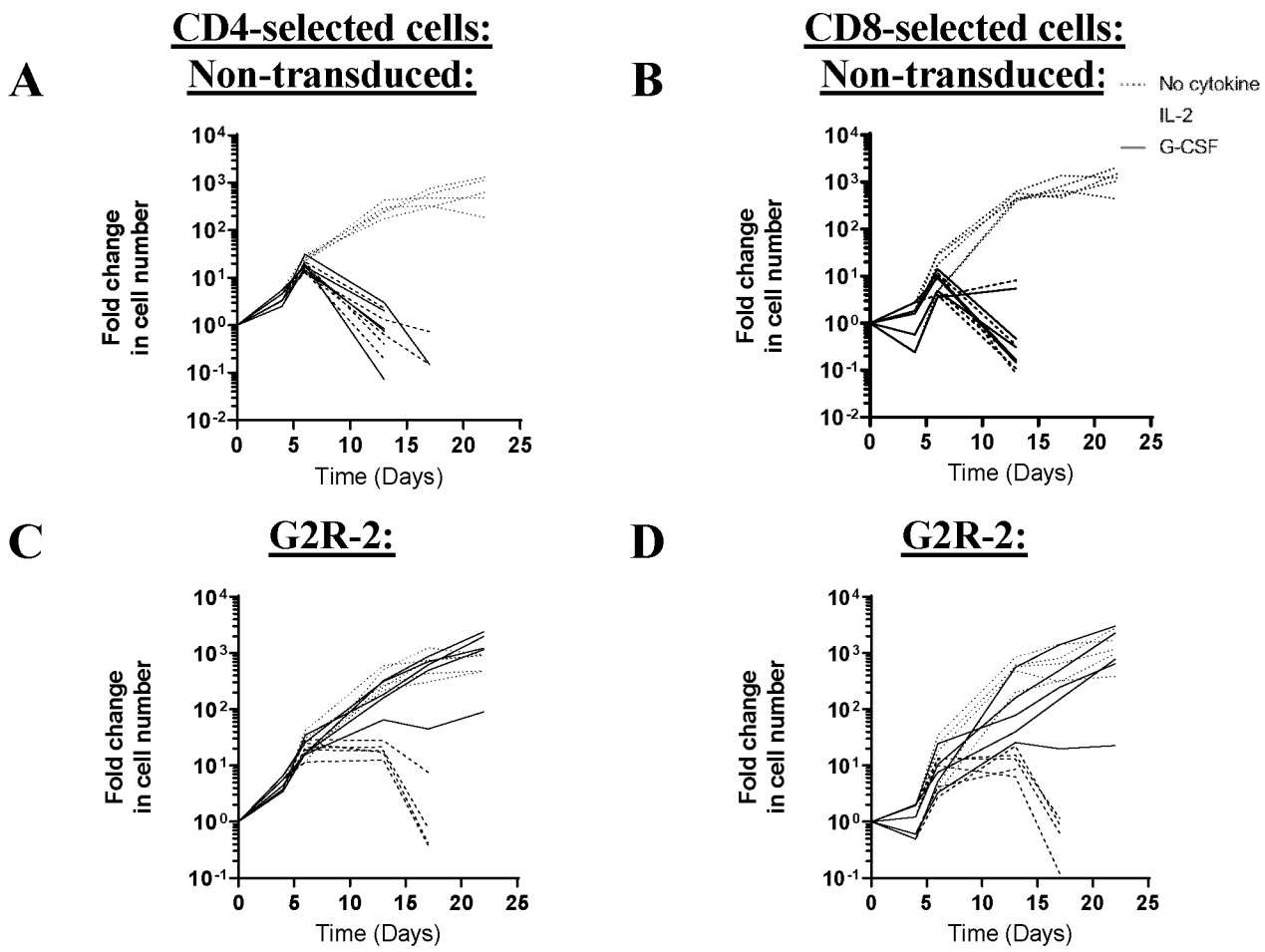

A
CD4-selected cells:
Non-transduced:
B
CD8-selected cells:
Non-transduced:
No cytokine
IL-2
G-CSF
C
G2R-2:
D
G2R-2:
Fold change in cell number
Time (Days)

Figure 28

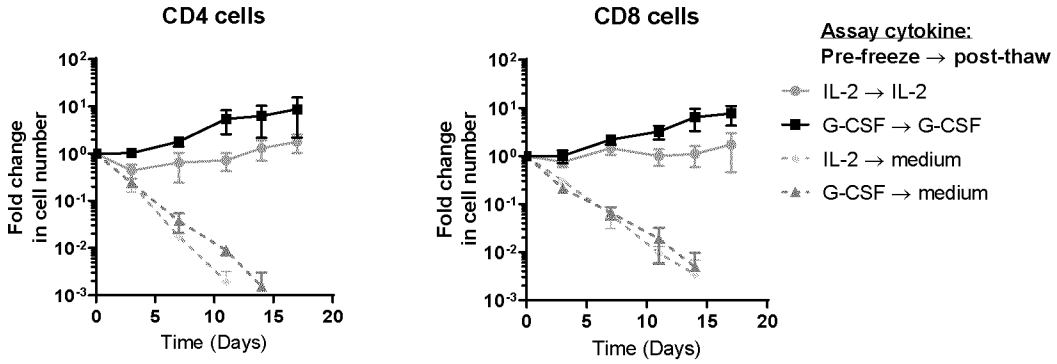

CD4 cells
CD8 cells
Fold change in cell number
Time (Days)
Assay cytokine:
Pre-freeze → post-thaw
IL-2 → IL-2
G-CSF → G-CSF
IL-2 → medium
G-CSF → medium

Figure 29

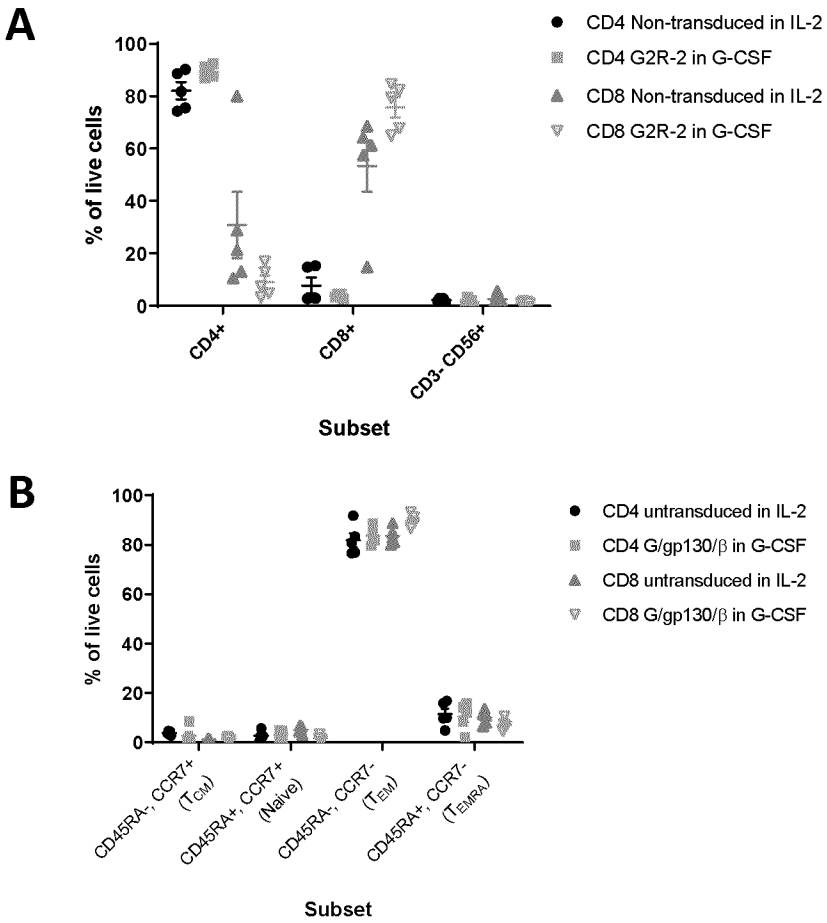

A
100
80
60
40
20
0
% of live cells
CD4+
CD8+
CD3- CD56+
Subset
CD4 Non-transduced in IL-2
CD4 G2R-2 in G-CSF
CD8 Non-transduced in IL-2
CD8 G2R-2 in G-CSF
B
100
80
60
40
20
0
% of live cells
CD45RA-, CCR7+ (TCM)
CD45RA+, CCR7+ (Naive)
CD45RA-, CCR7- (TEM)
CD45RA+, CCR7- (TEMRA)
Subset
CD4 untransduced in IL-2
CD4 G/gp130/β in G-CSF
CD8 untransduced in IL-2
CD8 G/gp130/β in G-CSF

Figure 30

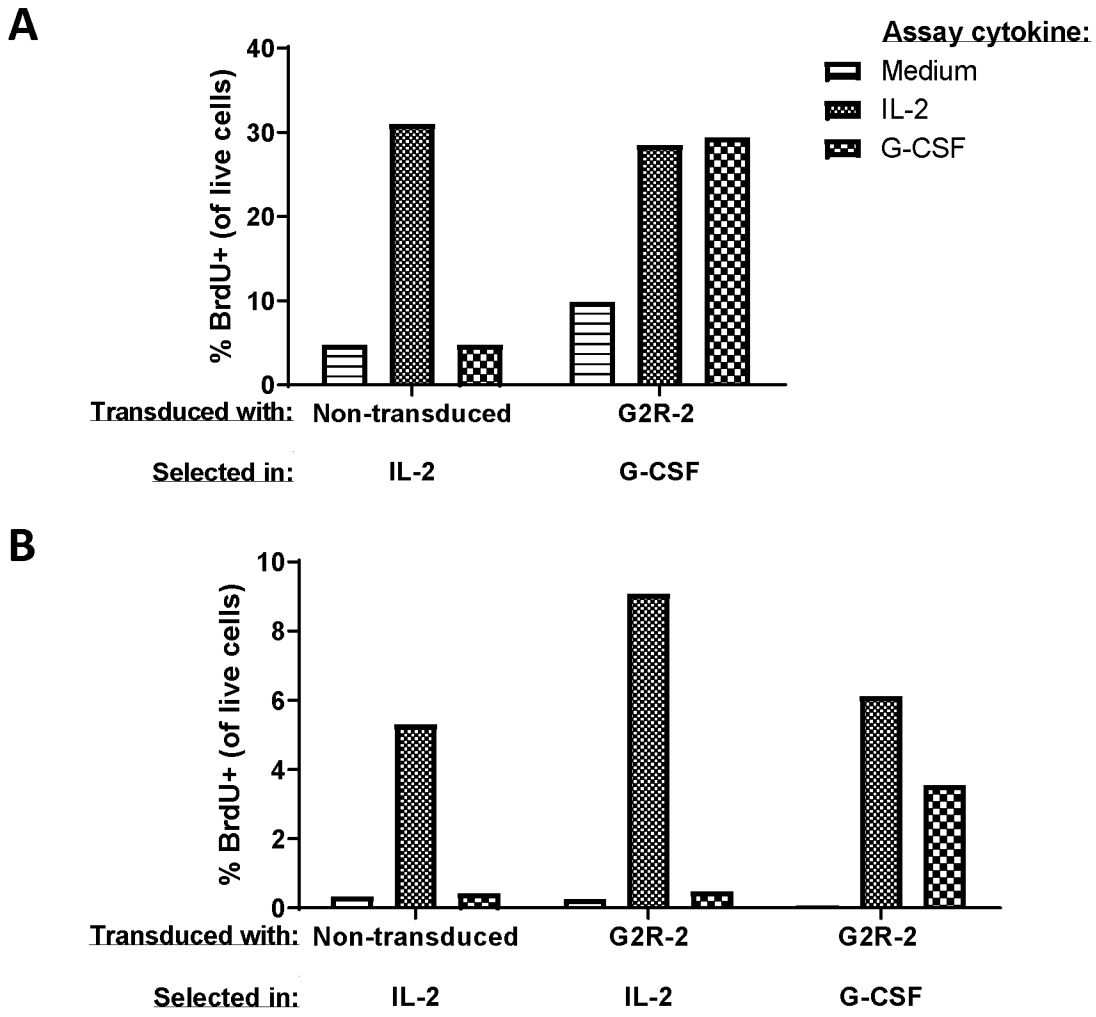

A
Assay cytokine:
Medium
IL-2
G-CSF
% BrdU+ (of live cells)
40
30
20
10
0
Transduced with:
Non-transduced
G2R-2
Selected in:
IL-2
G-CSF
B
% BrdU+ (of live cells)
10
8
6
4
2
0
Transduced with:
Non-transduced
G2R-2
G2R-2
Selected in:
IL-2
IL-2
G-CSF

Figure 31

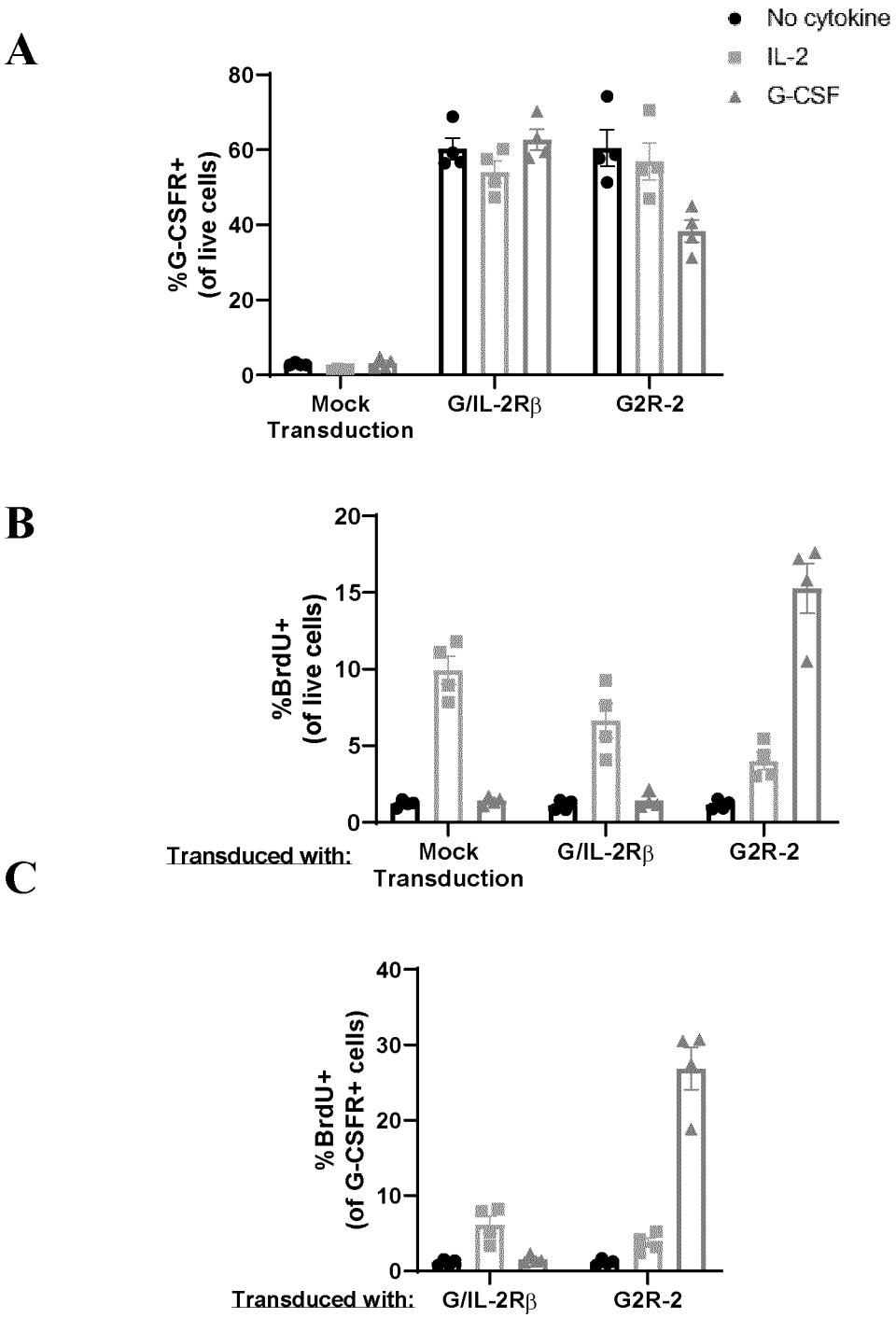

A
No cytokine
IL-2
G-CSF
%G-CSFR+
(of live cells)
80
60
40
20
0
Mock
Transduction
G/IL-2Rβ
G2R-2
B
%BrdU+
(of live cells)
20
15
10
5
0
Transduced with:
Mock
Transduction
G/IL-2Rβ
G2R-2
C
%BrdU+
(of G-CSFR+ cells)
40
30
20
10
0
Transduced with:
G/IL-2Rβ
G2R-2

Figure 32

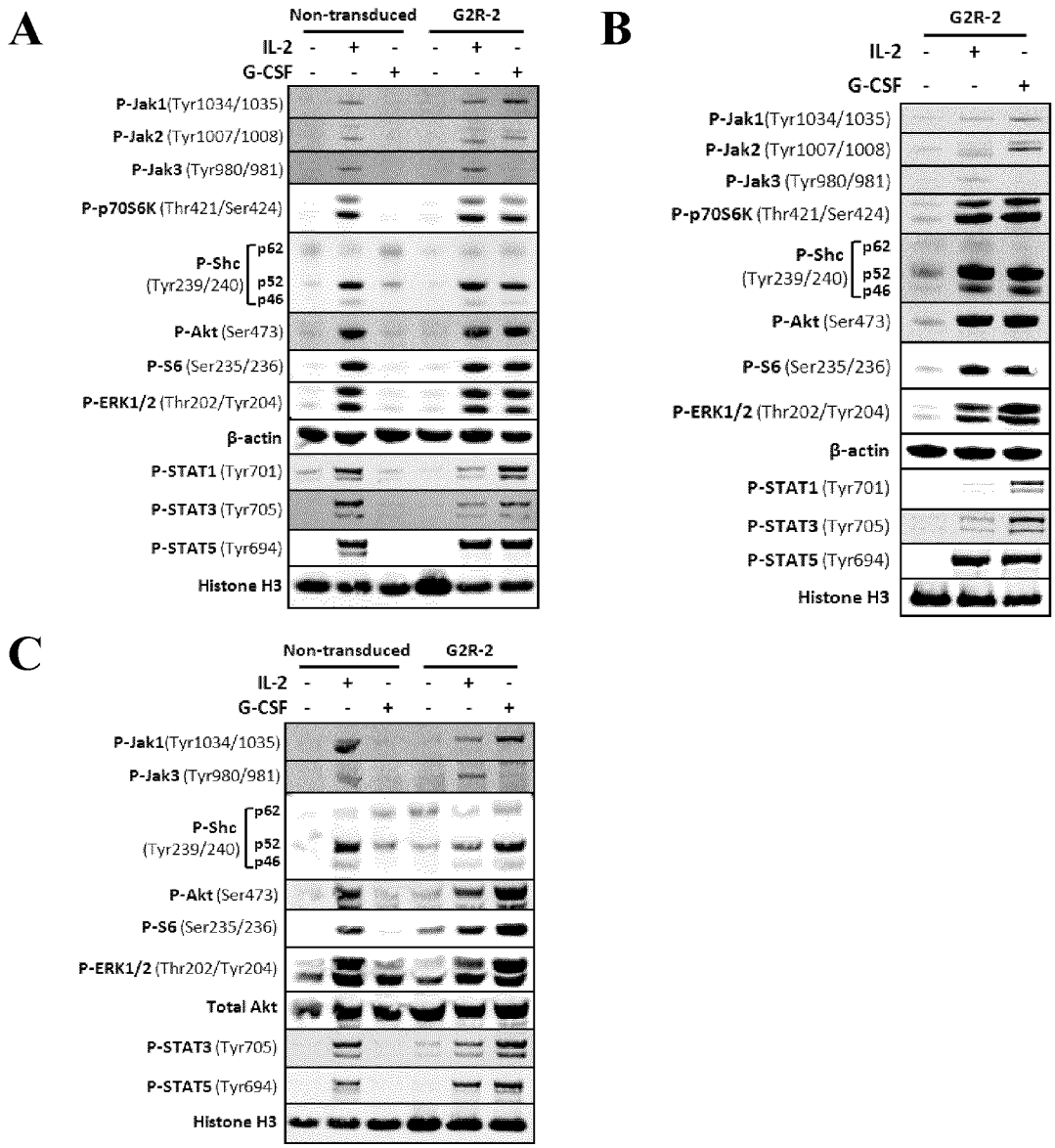

A
Non-transduced
G2R-2
IL-2 - + - - + -
G-CSF - - + - - +
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
P-Jak3 (Tyr980/981)
P-p70S6K (Thr421/Ser424)
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT1 (Tyr701)
P-STAT3 (Tyr705)
P-STAT5 (Tyr694)
Histone H3
B
G2R-2
IL-2 - + -
G-CSF - - +
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
P-Jak3 (Tyr980/981)
P-p70S6K (Thr421/Ser424)
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT1 (Tyr701)
P-STAT3 (Tyr705)
P-STAT5 (Tyr694)
Histone H3
C
Non-transduced
G2R-2
IL-2 - + - - + -
G-CSF - - + - - +
P-Jak1(Tyr1034/1035)
P-Jak3 (Tyr980/981)
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
Total Akt
P-STAT3 (Tyr705)
P-STAT5 (Tyr694)
Histone H3

Figure 33

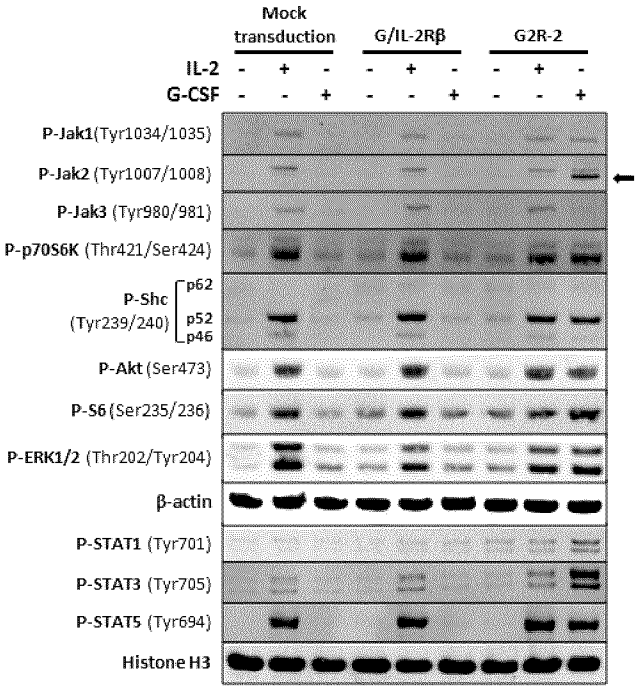

Mock transduction
G/IL-2Rβ
G2R-2
IL-2 - + - - + - - + -
G-CSF - - + - - + - - +
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
P-Jak3 (Tyr980/981)
P-p70S6K (Thr421/Ser424)
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT1 (Tyr701)
P-STAT3 (Tyr705)
P-STAT5 (Tyr694)
Histone H3

Figure 34

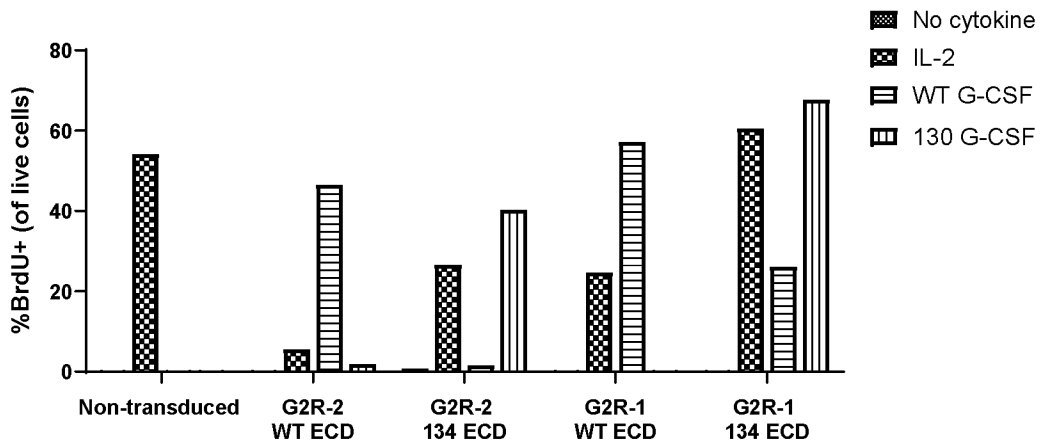

No cytokine
IL-2
WT G-CSF
130 G-CSF
%BrdU+ (of live cells)
80
60
40
20
0
Non-transduced
G2R-2
WT ECD
G2R-2
134 ECD
G2R-1
WT ECD
G2R-1
134 ECD

Figure 35

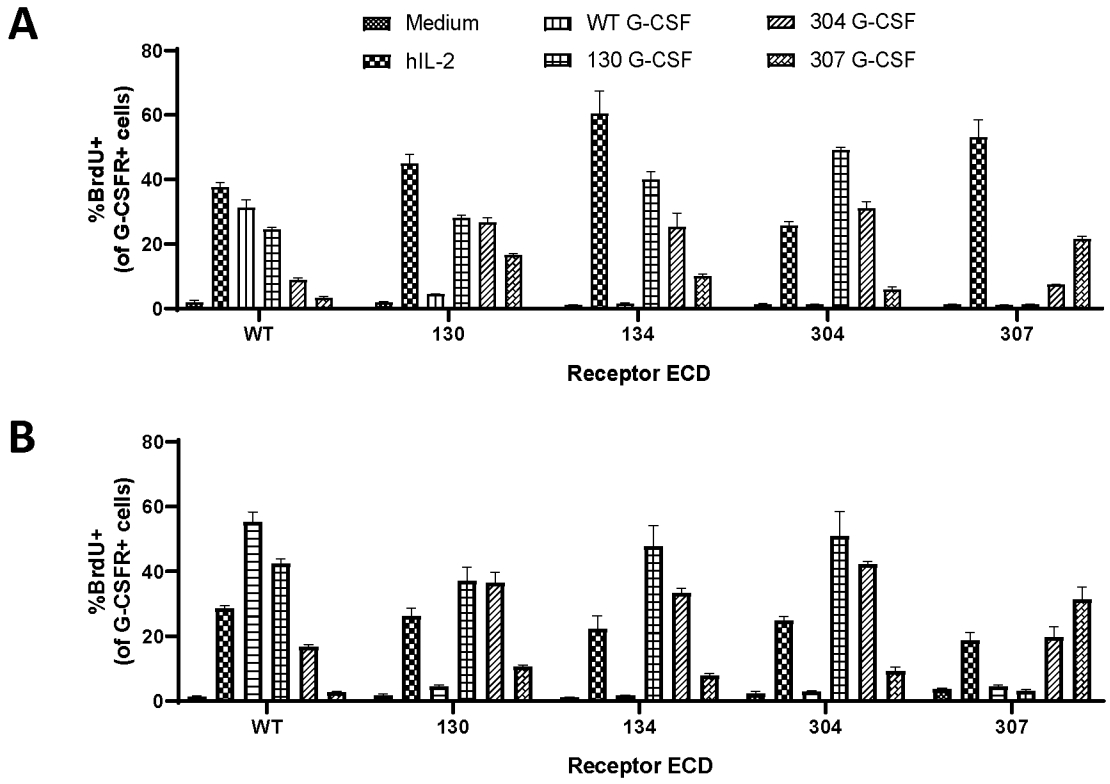

A
Medium
hIL-2
WT G-CSF
130 G-CSF
304 G-CSF
307 G-CSF
%BrdU+
(of G-CSFR+ cells)
80
60
40
20
0
WT
130
134
304
307
Receptor ECD
B
%BrdU+
(of G-CSFR+ cells)
80
60
40
20
0
WT
130
134
304
307
Receptor ECD

Figure 36

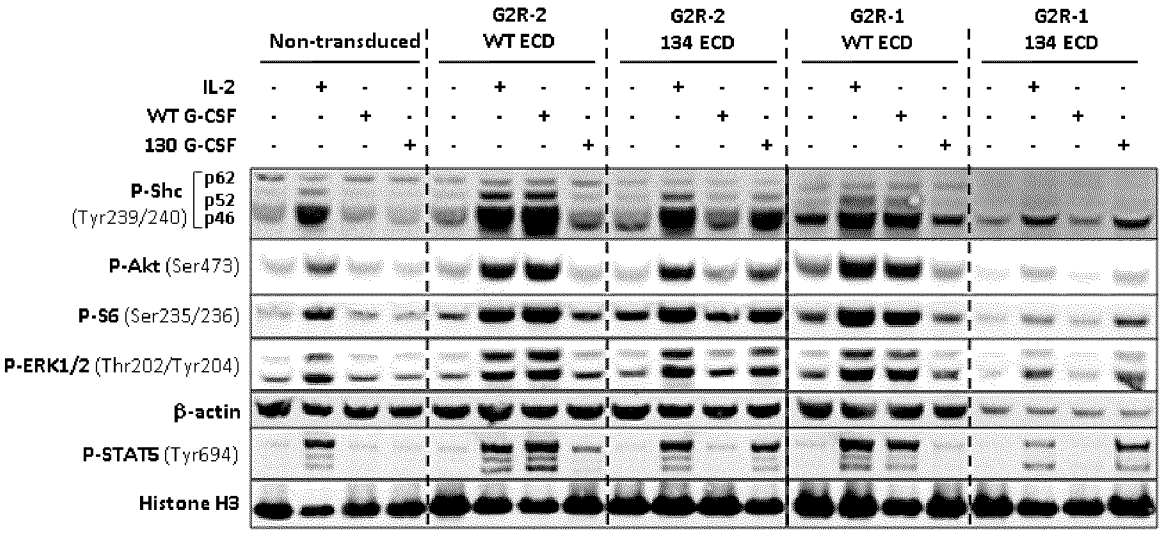
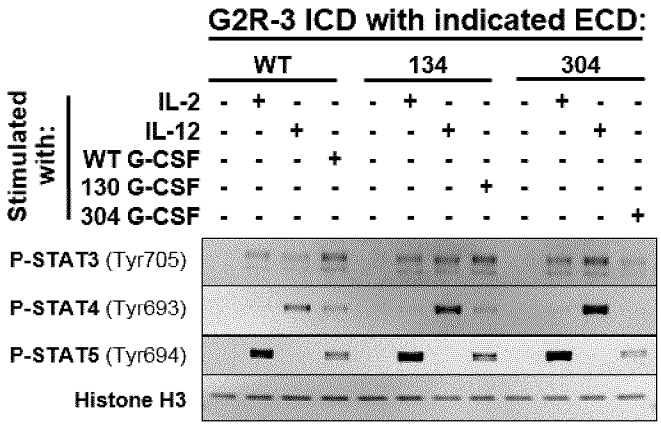

Non-transduced
G2R-2 WT ECD
G2R-2 134 ECD
G2R-1 WT ECD
G2R-1 134 ECD
IL-2
WT G-CSF
130 G-CSF
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT5 (Tyr694)
Histone H3

Figure 37


A
G2R-3 ICD with indicated ECD:
WT
134
304
Stimulated with:
IL-2
IL-12
WT G-CSF
130 G-CSF
304 G-CSF
P-STAT3 (Tyr705)
P-STAT4 (Tyr693)
P-STAT5 (Tyr694)
Histone H3

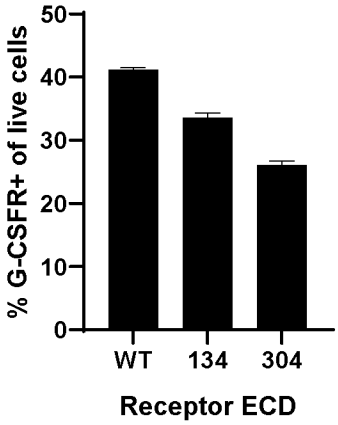

B
% G-CSFR+ of live cells
50
40
30
20
10
0
WT
134
304
Receptor ECD

Figure 38

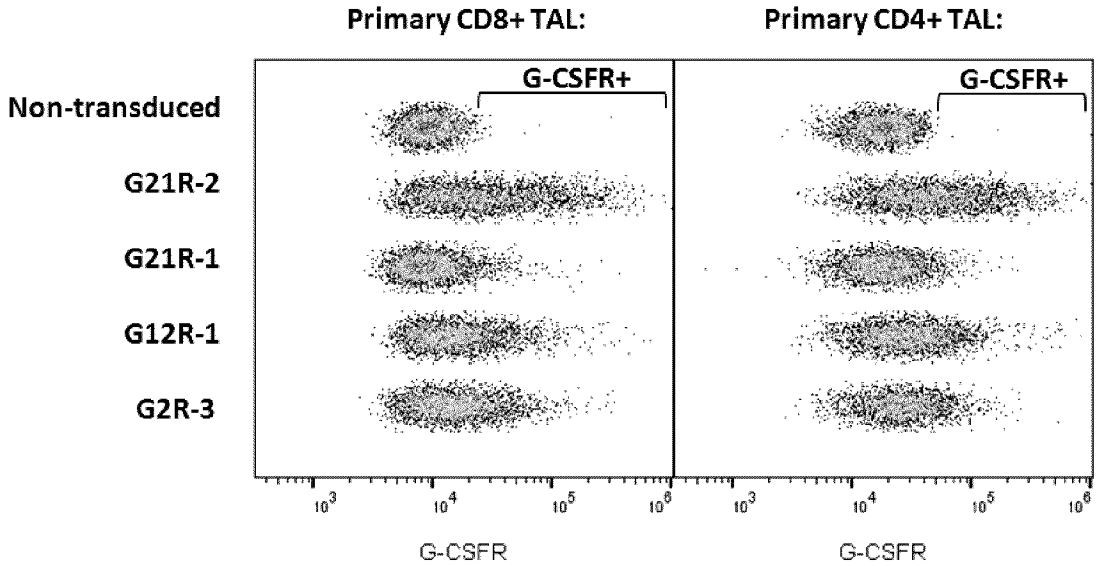

Primary CD8+ TAL:
Primary CD4+ TAL:
G-CSFR+
G-CSFR+
Non-transduced
G21R-2
G21R-1
G12R-1
G2R-3
10^3
10^4
10^5
10^6
10^3
10^4
10^5
10^6
G-CSFR
G-CSFR

Figure 39

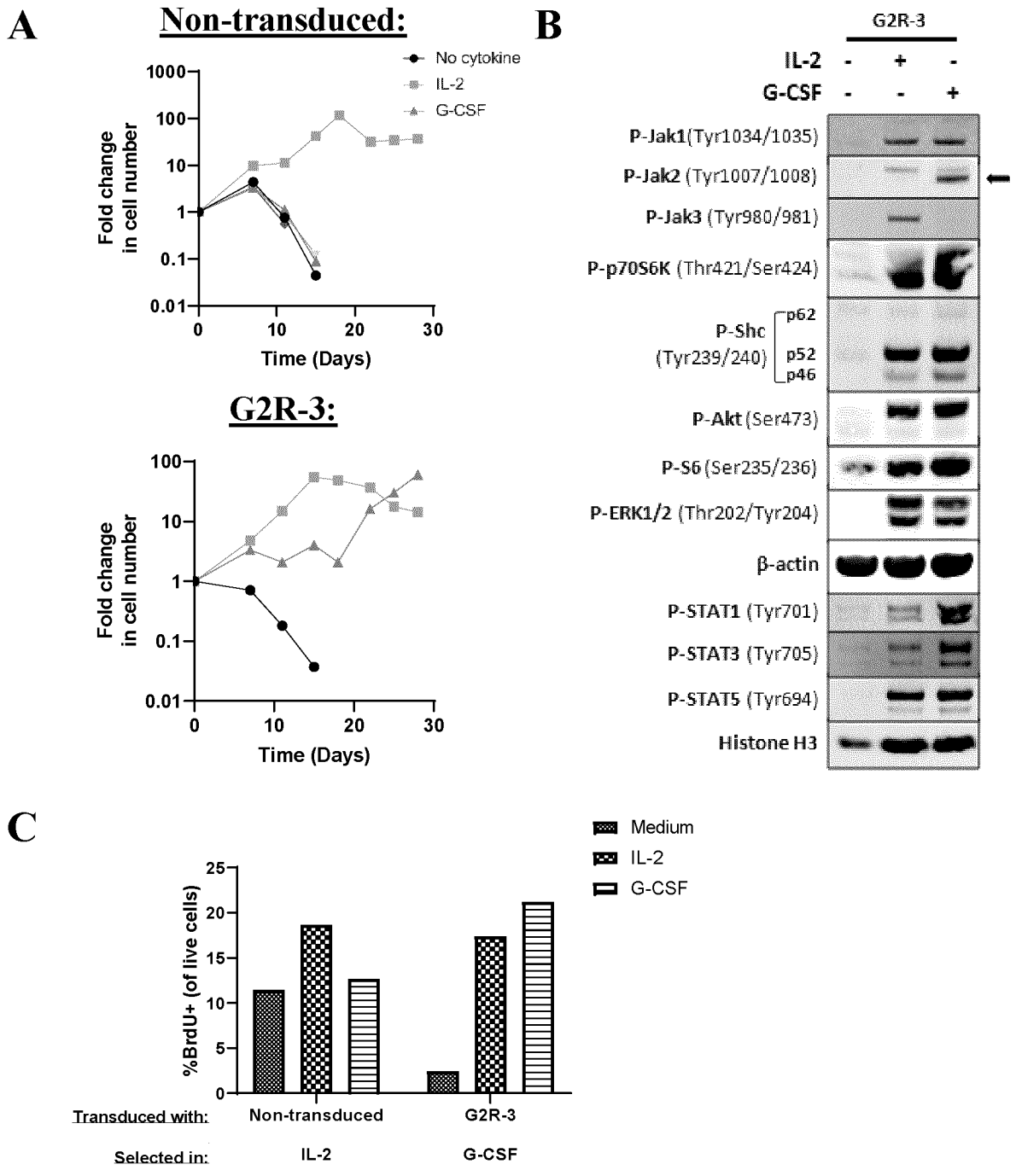
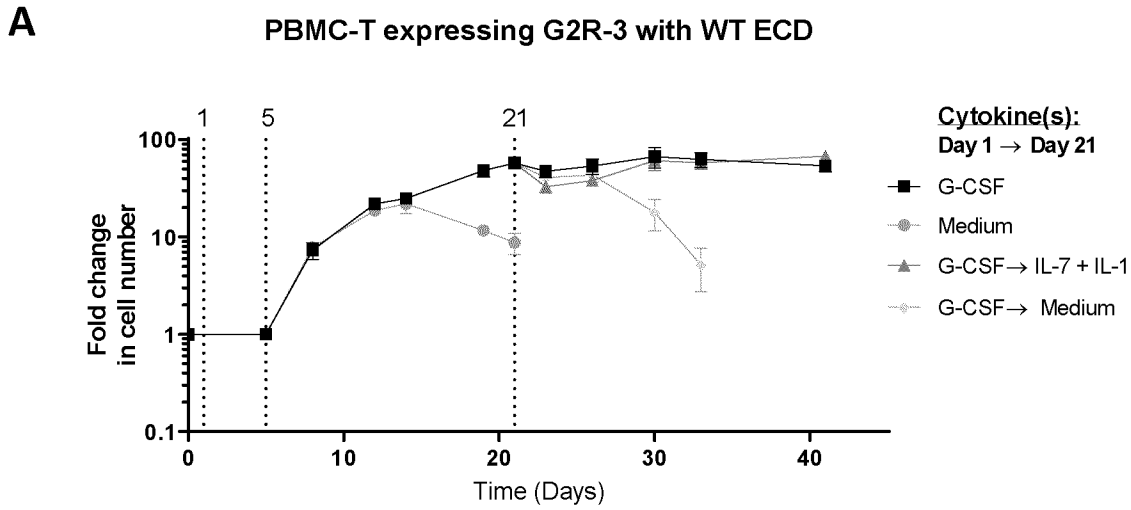

A
Non-transduced:
No cytokine
IL-2
G-CSF
Fold change in cell number
1000
100
10
1
0.1
0.01
0
10
20
30
Time (Days)
G2R-3:
Fold change in cell number
100
10
1
0.1
0.01
0
10
20
30
Time (Days)
B
G2R-3
IL-2 - + -
G-CSF - - +
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
P-Jak3 (Tyr980/981)
P-p70S6K (Thr421/Ser424)
P-Shc (Tyr239/240)
p62
p52
p46
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
P-STAT1 (Tyr701)
P-STAT3 (Tyr705)
P-STAT5 (Tyr694)
Histone H3
C
Medium
IL-2
G-CSF
%BrdU+ (of live cells)
25
20
15
10
5
0
Transduced with:
Non-transduced
G2R-3
Selected in:
IL-2
G-CSF

Figure 40



Day 0: IL-7 added; cells activated with Transact
Day 1: G-CSF added; cells transduced
Day 5-21: G-CSF or medium only added
Day 21: Cells washed 3x and medium changed to G-CSF, IL-7 + IL-15, or medium only

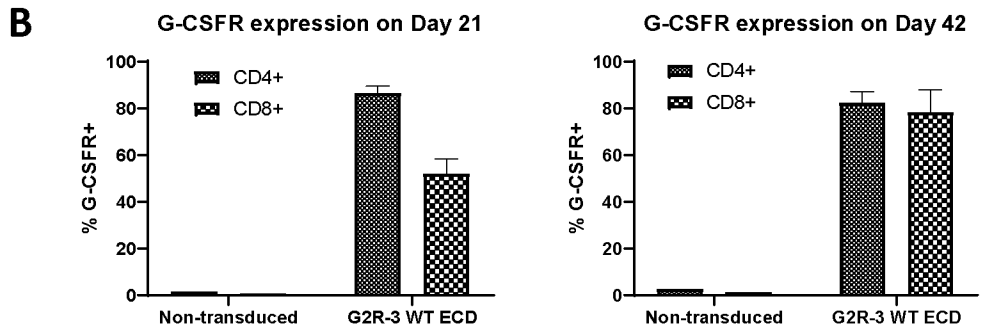


Figure 41

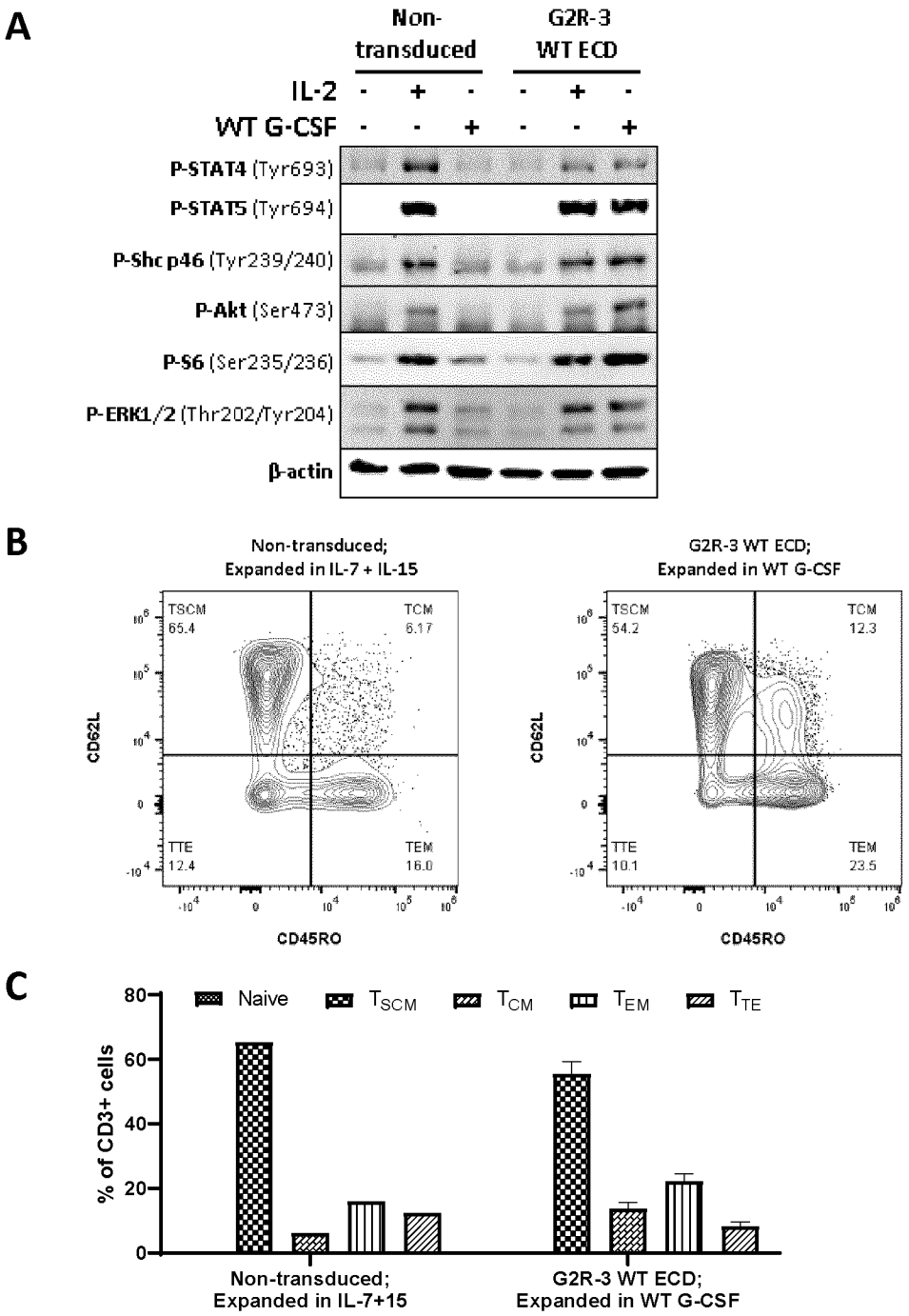

A
Non-transduced
G2R-3 WT ECD
IL-2
WT G-CSF
P-STAT4 (Tyr693)
P-STAT5 (Tyr694)
P-Shc p46 (Tyr239/240)
P-Akt (Ser473)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin
B
Non-transduced; Expanded in IL-7 + IL-15
G2R-3 WT ECD; Expanded in WT G-CSF
TSCM 65.4
TCM 6.17
TTE 12.4
TEM 16.0
TSCM 54.2
TCM 12.3
TTE 10.1
TEM 23.5
CD62L
CD45RO
C
Naive
TSCM
TCM
TEM
TTE
% of CD3+ cells
Non-transduced; Expanded in IL-7+15
G2R-3 WT ECD; Expanded in WT G-CSF

Figure 42

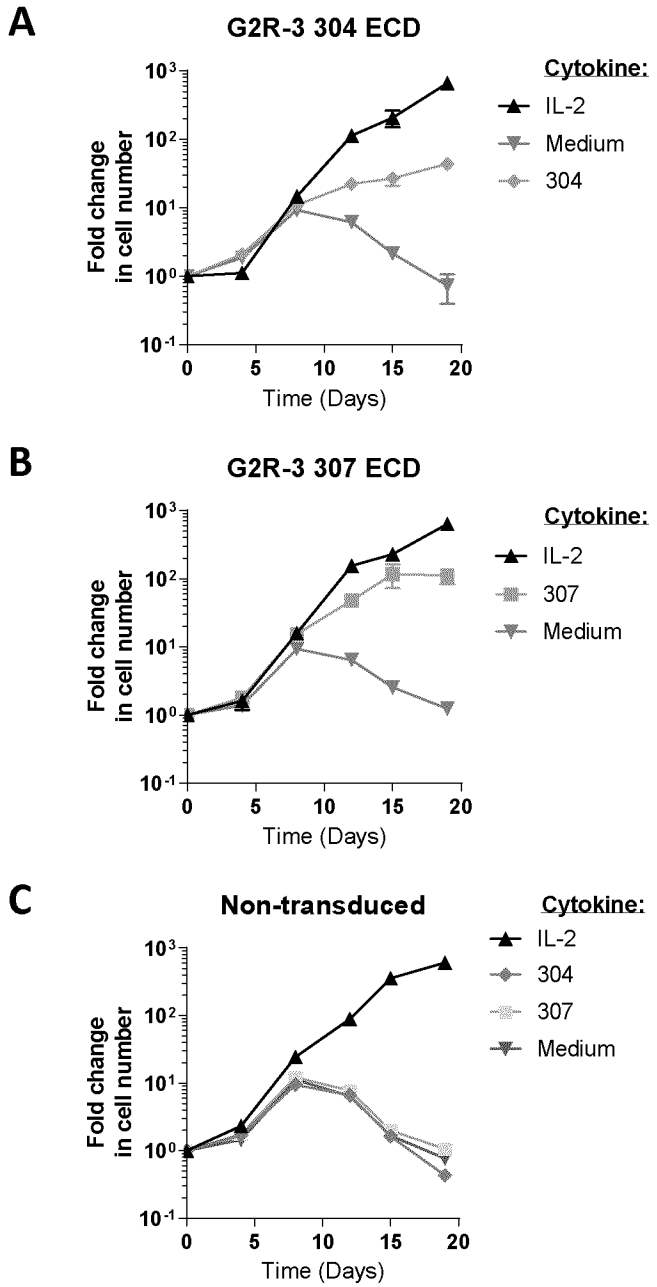

A
G2R-3 304 ECD
Fold change in cell number
Time (Days)
Cytokine:
IL-2
Medium
304
B
G2R-3 307 ECD
Fold change in cell number
Time (Days)
Cytokine:
IL-2
307
Medium
C
Non-transduced
Fold change in cell number
Time (Days)
Cytokine:
IL-2
304
307
Medium

Figure 43

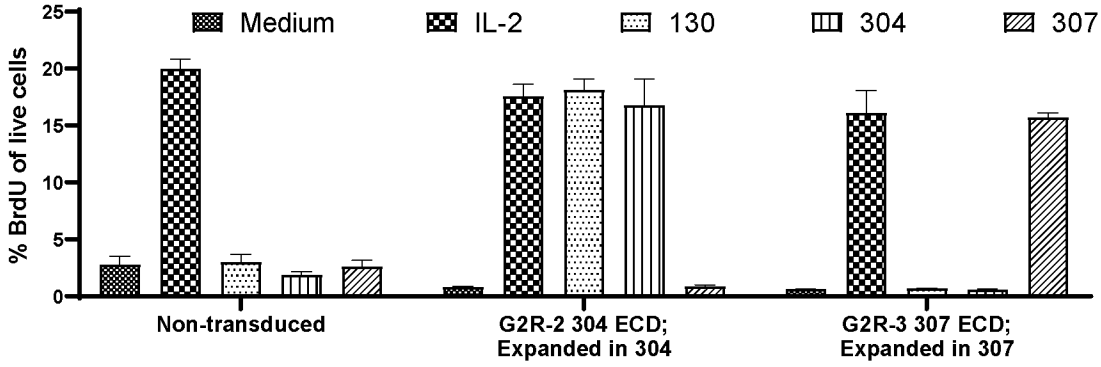

Medium
IL-2
130
304
307
% BrdU of live cells
25
20
15
10
5
0
Non-transduced
G2R-2 304 ECD;
Expanded in 304
G2R-3 307 ECD;
Expanded in 307

Figure 44

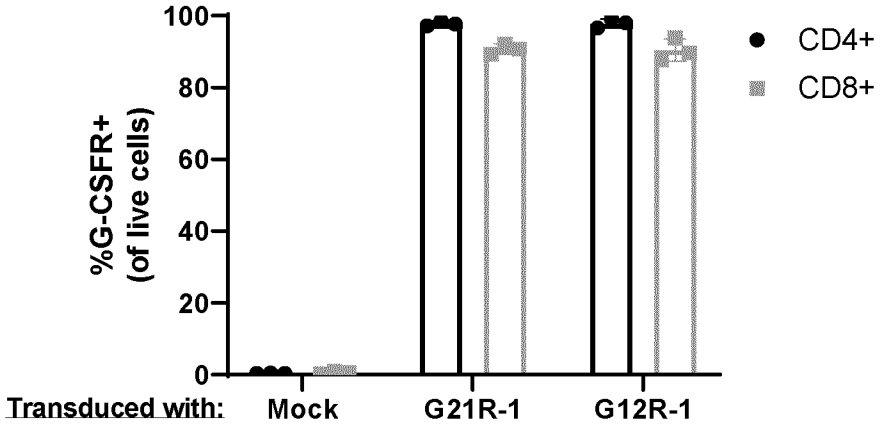

100
80
60
40
20
0
%G-CSFR+
(of live cells)
CD4+
CD8+
Transduced with:
Mock
G21R-1
G12R-1

Figure 45

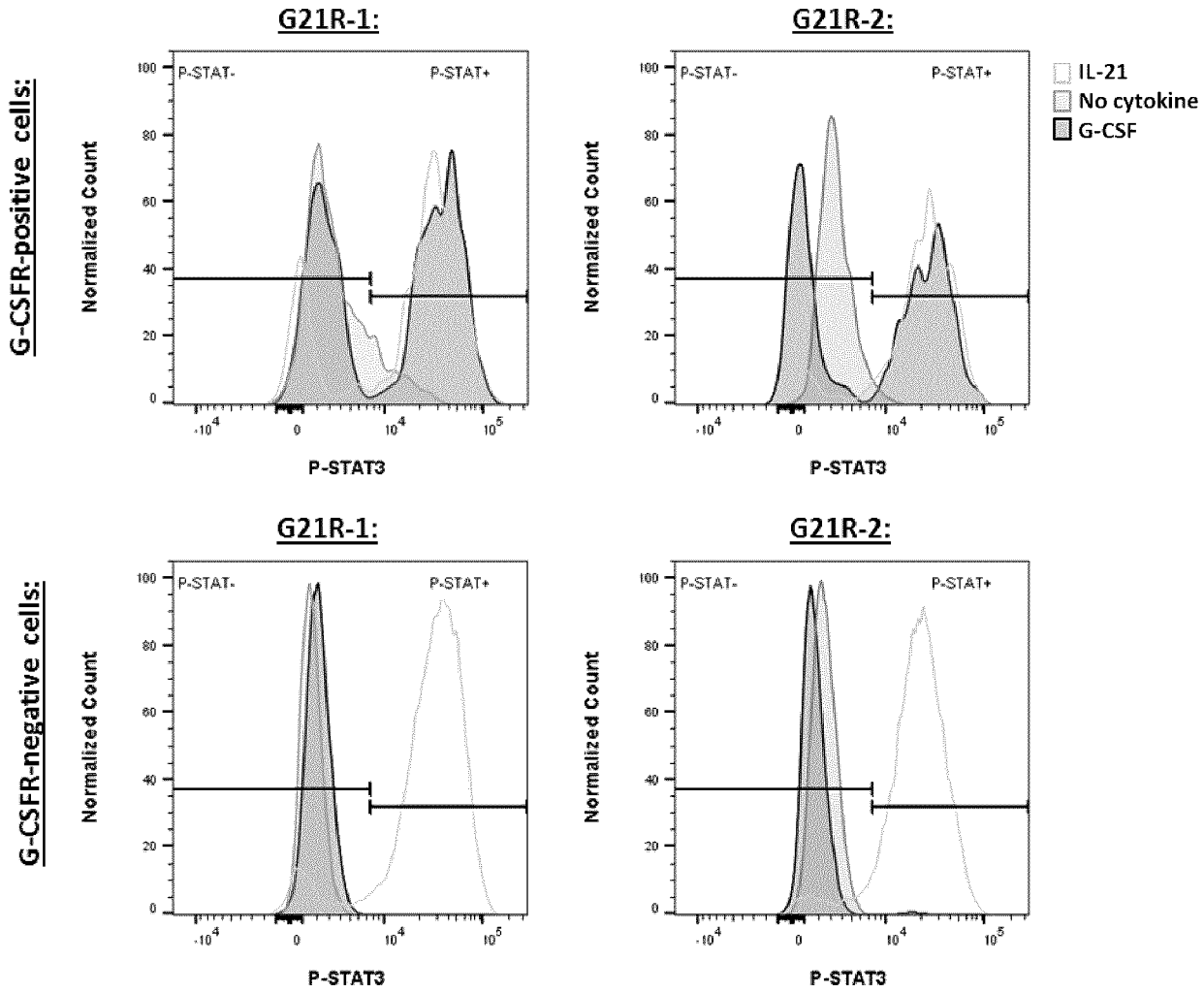

G21R-1:
G21R-2:
G-CSFR-positive cells:
G-CSFR-negative cells:
P-STAT-
P-STAT+
Normalized Count
P-STAT3
IL-21
No cytokine
G-CSF

Figure 46

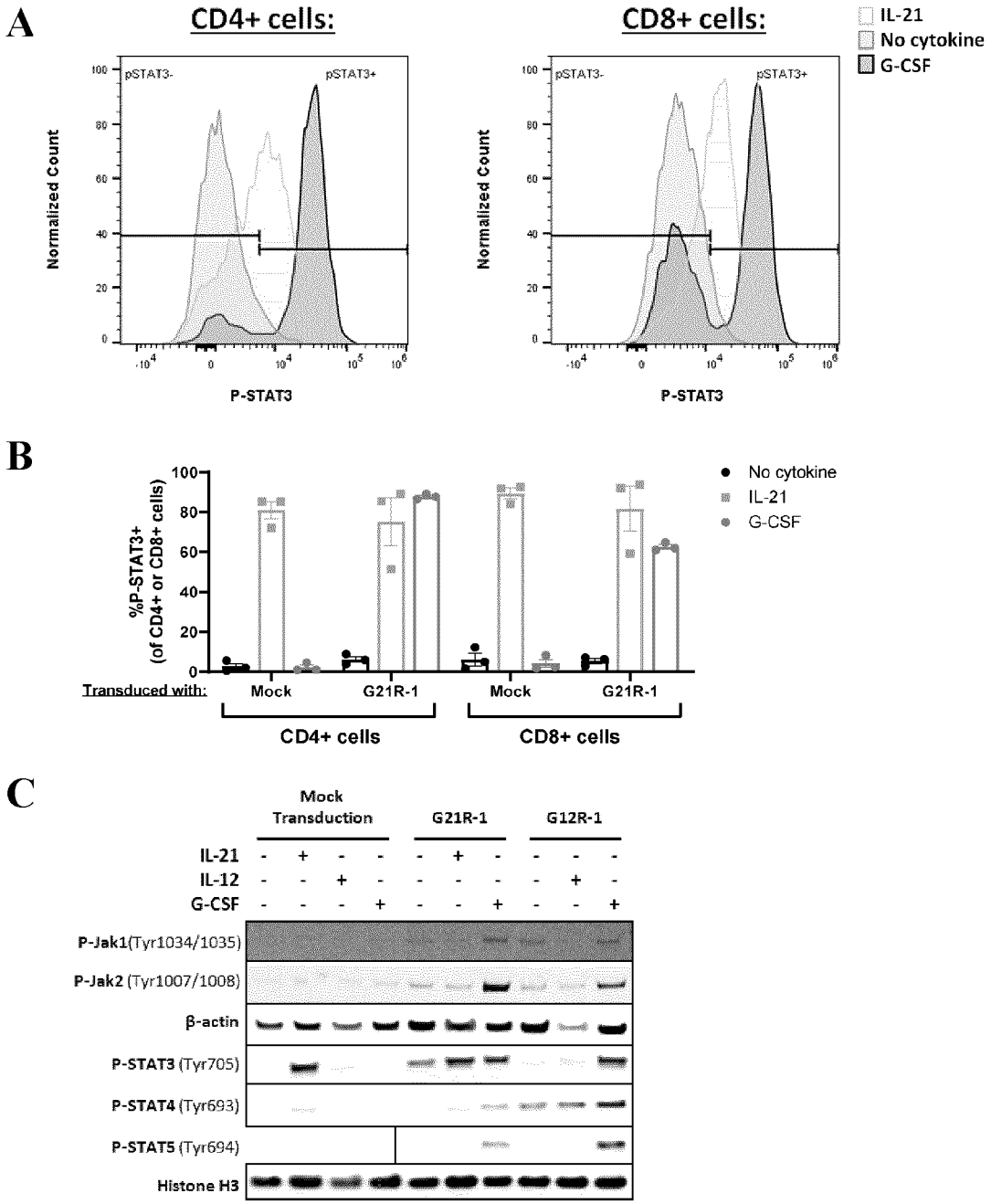

A
CD4+ cells:
CD8+ cells:
IL-21
No cytokine
G-CSF
pSTAT3-
pSTAT3+
Normalized Count
P-STAT3
B
%P-STAT3+
(of CD4+ or CD8+ cells)
No cytokine
IL-21
G-CSF
Transduced with:
Mock
G21R-1
Mock
G21R-1
CD4+ cells
CD8+ cells
C
Mock Transduction
G21R-1
G12R-1
IL-21
IL-12
G-CSF
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
β-actin
P-STAT3 (Tyr705)
P-STAT4 (Tyr693)
P-STAT5 (Tyr694)
Histone H3

Figure 47

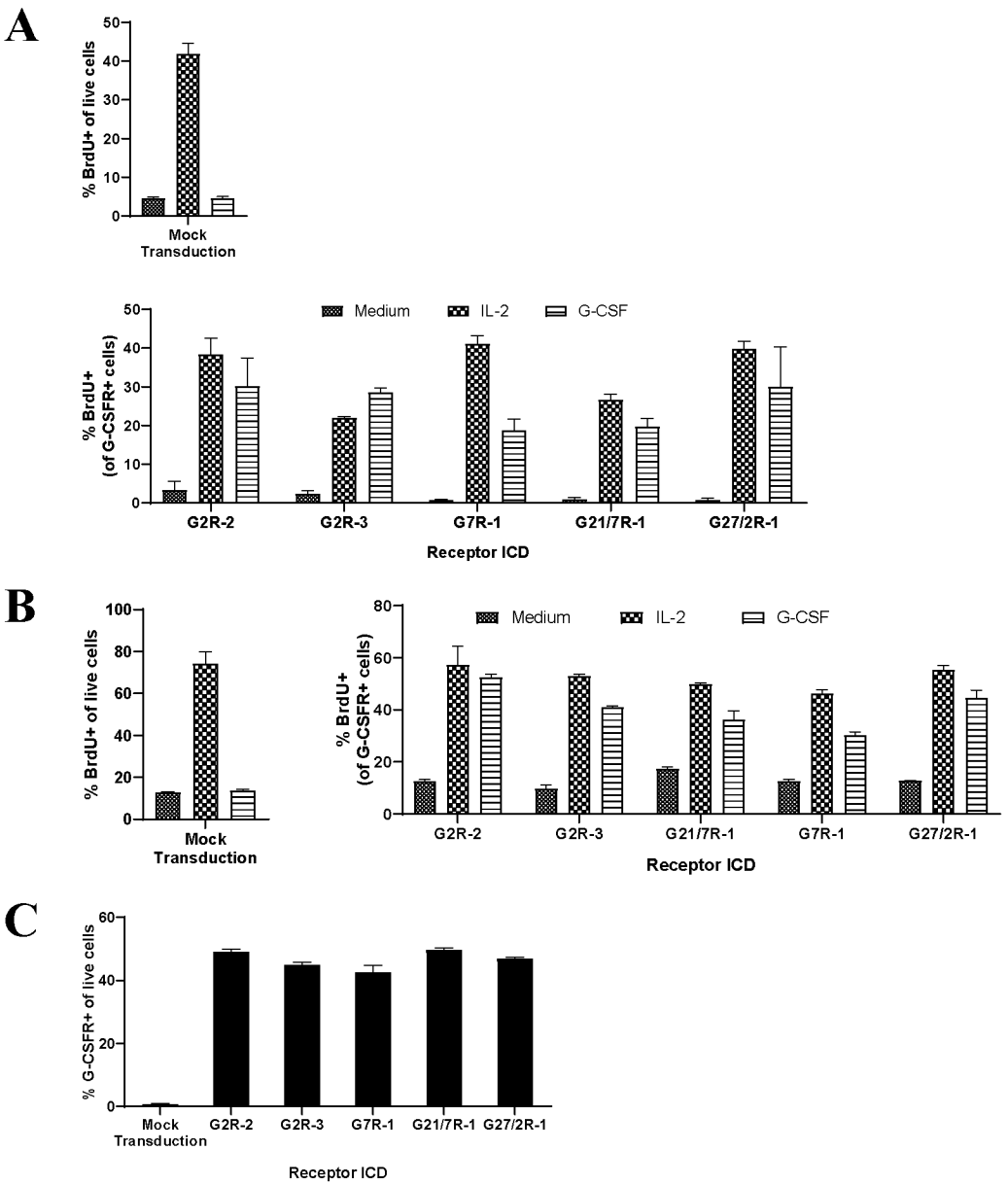

A
% BrdU+ of live cells
Mock Transduction
Medium
IL-2
G-CSF
% BrdU+ (of G-CSFR+ cells)
G2R-2
G2R-3
G7R-1
G21/7R-1
G27/2R-1
Receptor ICD
B
% BrdU+ of live cells
Mock Transduction
Medium
IL-2
G-CSF
% BrdU+ (of G-CSFR+ cells)
G2R-2
G2R-3
G21/7R-1
G7R-1
G27/2R-1
Receptor ICD
C
% G-CSFR+ of live cells
Mock Transduction
G2R-2
G2R-3
G7R-1
G21/7R-1
G27/2R-1
Receptor ICD

Figure 48

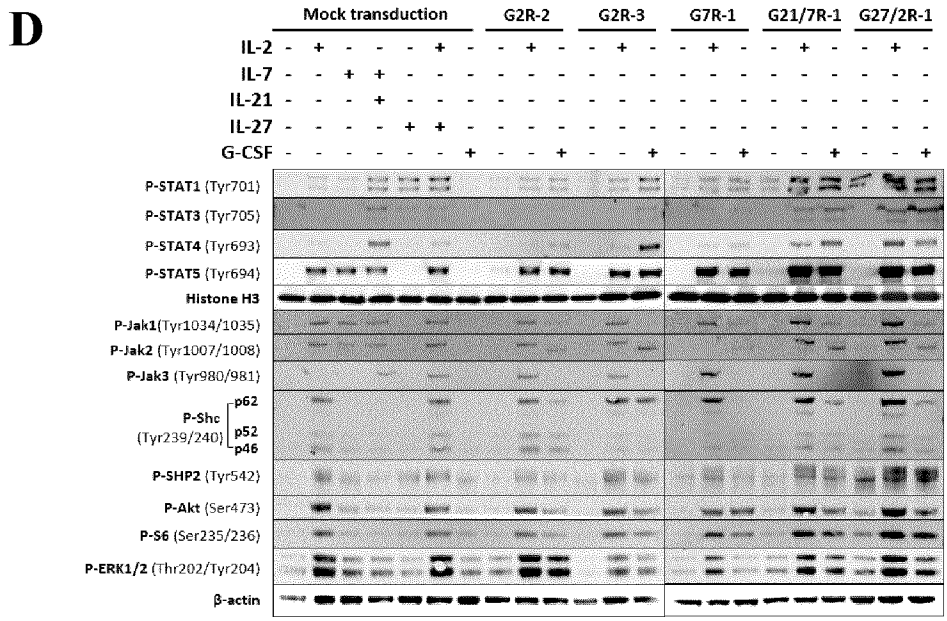


Figure 48 Continued

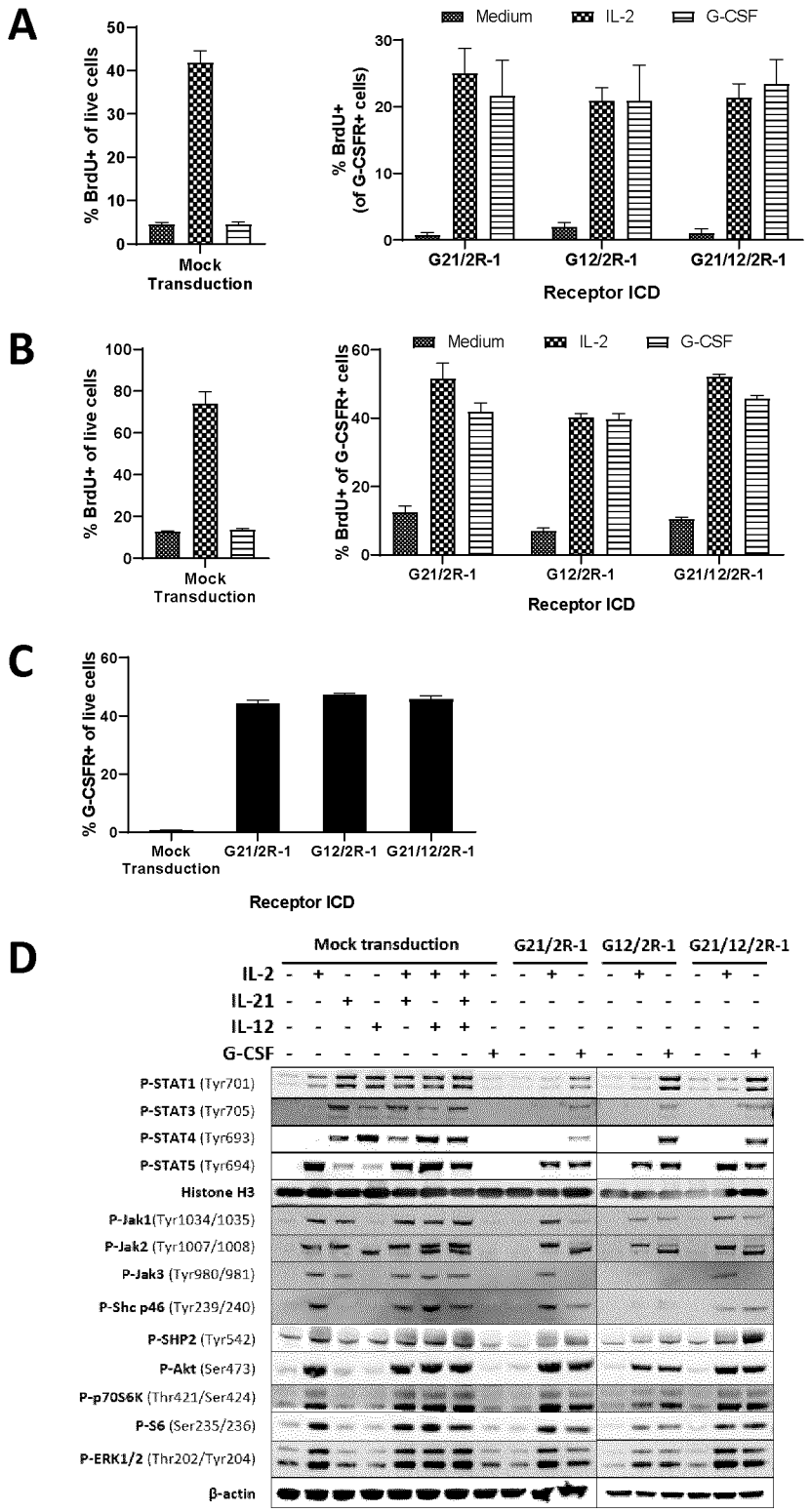

A
% BrdU+ of live cells
Mock Transduction
Medium
IL-2
G-CSF
% BrdU+ (of G-CSFR+ cells)
G21/2R-1
G12/2R-1
G21/12/2R-1
Receptor ICD
B
% BrdU+ of live cells
Mock Transduction
Medium
IL-2
G-CSF
% BrdU+ of G-CSFR+ cells
G21/2R-1
G12/2R-1
G21/12/2R-1
Receptor ICD
C
% G-CSFR+ of live cells
Mock Transduction
G21/2R-1
G12/2R-1
G21/12/2R-1
Receptor ICD
D
Mock transduction
G21/2R-1
G12/2R-1
G21/12/2R-1
IL-2
IL-21
IL-12
G-CSF
P-STAT1 (Tyr701)
P-STAT3 (Tyr705)
P-STAT4 (Tyr693)
P-STAT5 (Tyr694)
Histone H3
P-Jak1(Tyr1034/1035)
P-Jak2 (Tyr1007/1008)
P-Jak3 (Tyr980/981)
P-Shc p46 (Tyr239/240)
P-SHP2 (Tyr542)
P-Akt (Ser473)
P-p70S6K (Thr421/Ser424)
P-S6 (Ser235/236)
P-ERK1/2 (Thr202/Tyr204)
β-actin

Figure 49

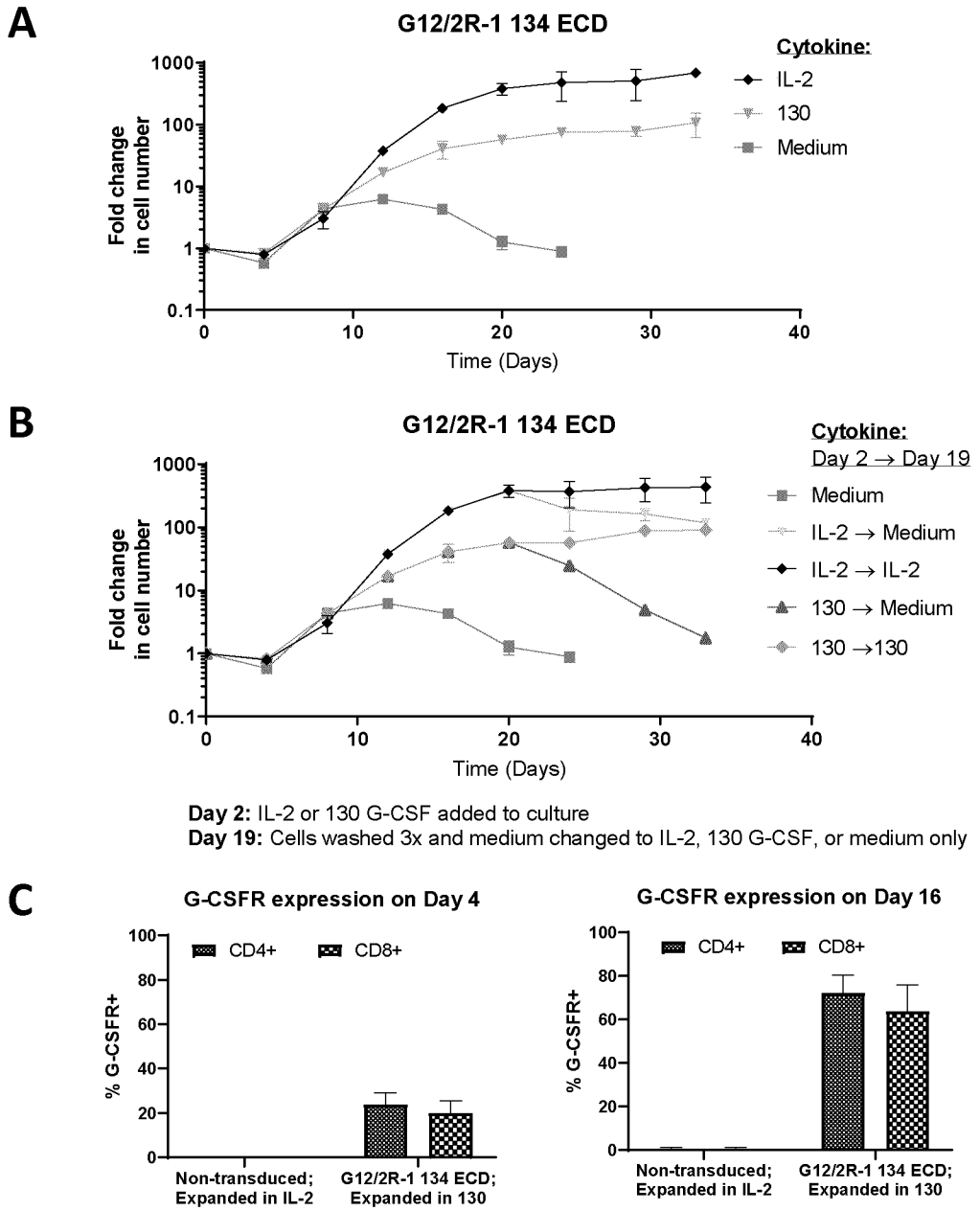

A
G12/2R-1 134 ECD
Cytokine:
IL-2
130
Medium
Fold change in cell number
1000
100
10
1
0.1
0
10
20
30
40
Time (Days)
B
G12/2R-1 134 ECD
Cytokine:
Day 2 → Day 19
Medium
IL-2 → Medium
IL-2 → IL-2
130 → Medium
130 →130
Fold change in cell number
Time (Days)
Day 2: IL-2 or 130 G-CSF added to culture
Day 19: Cells washed 3x and medium changed to IL-2, 130 G-CSF, or medium only
C
G-CSFR expression on Day 4
CD4+
CD8+
% G-CSFR+
100
80
60
40
20
0
Non-transduced; Expanded in IL-2
G12/2R-1 134 ECD; Expanded in 130
G-CSFR expression on Day 16
CD4+
CD8+
% G-CSFR+
Non-transduced; Expanded in IL-2
G12/2R-1 134 ECD; Expanded in 130

Figure 50

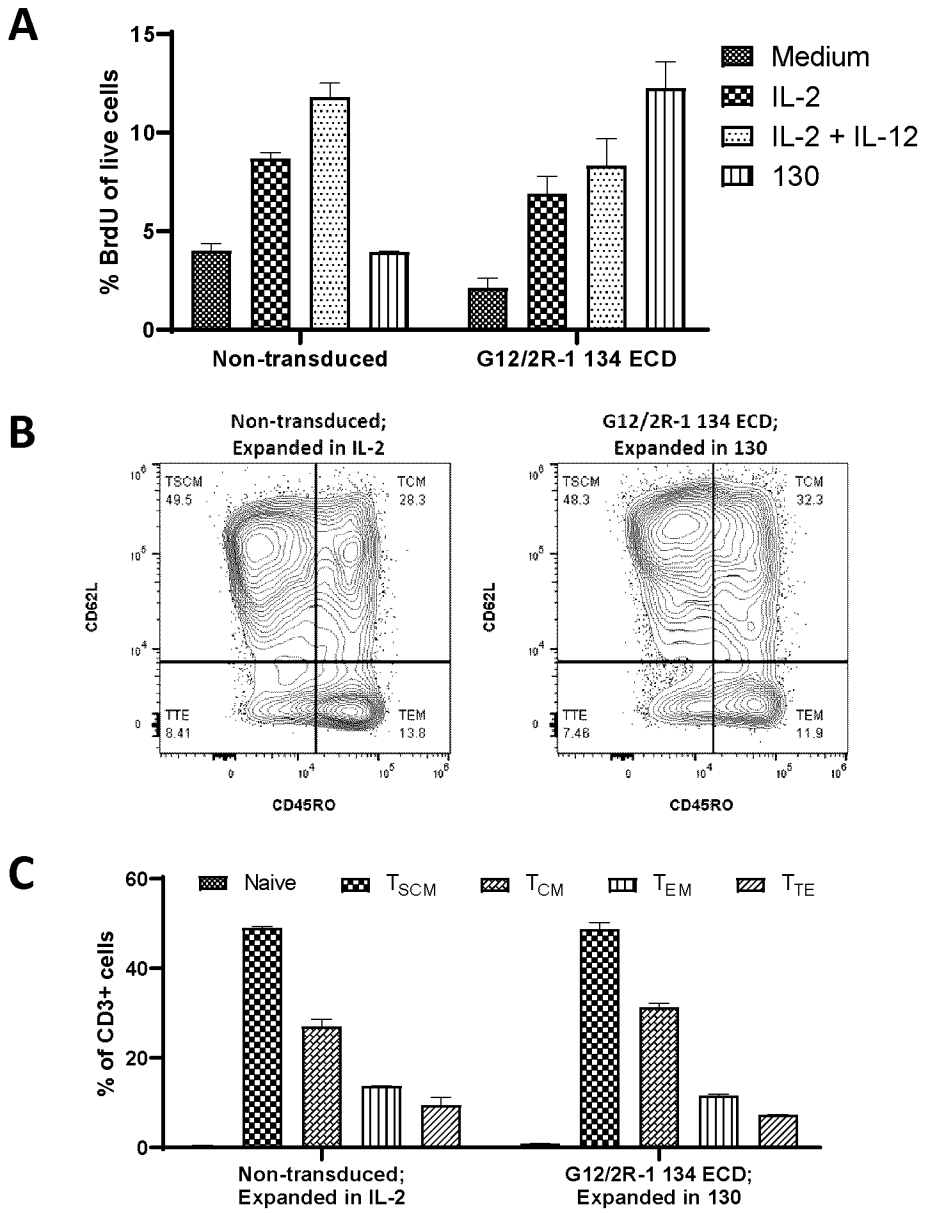

A
% BrdU of live cells
15
10
5
0
Medium
IL-2
IL-2 + IL-12
130
Non-transduced
G12/2R-1 134 ECD
B
Non-transduced;
Expanded in IL-2
G12/2R-1 134 ECD;
Expanded in 130
TSCM
49.5
TCM
28.3
TTE
8.41
TEM
13.8
TSCM
48.3
TCM
32.3
TTE
7.46
TEM
11.9
CD62L
CD45RO
C
% of CD3+ cells
60
40
20
0
Naive
TSCM
TCM
TEM
TTE
Non-transduced;
Expanded in IL-2
G12/2R-1 134 ECD;
Expanded in 130

Figure 51

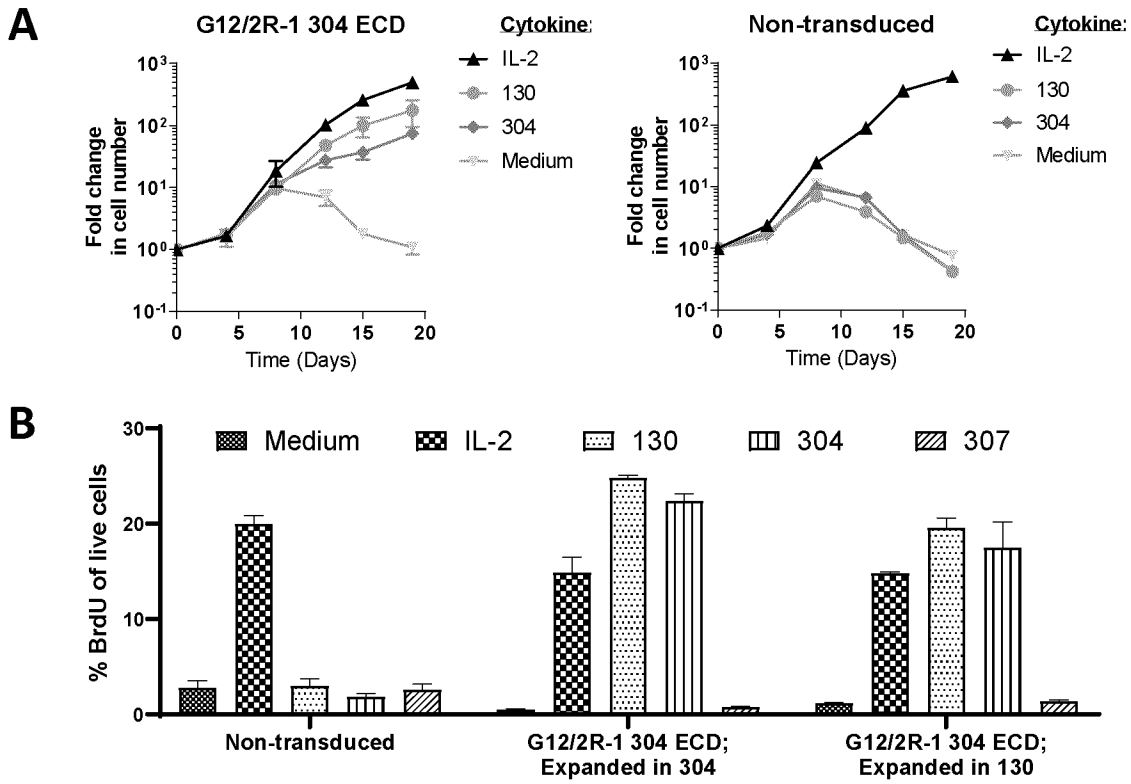


Figure 52

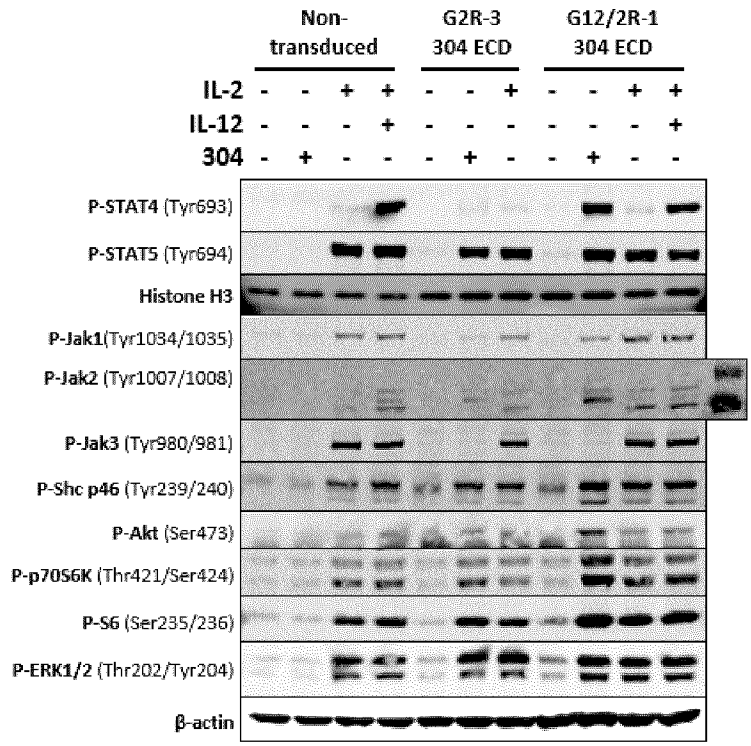


Figure 53

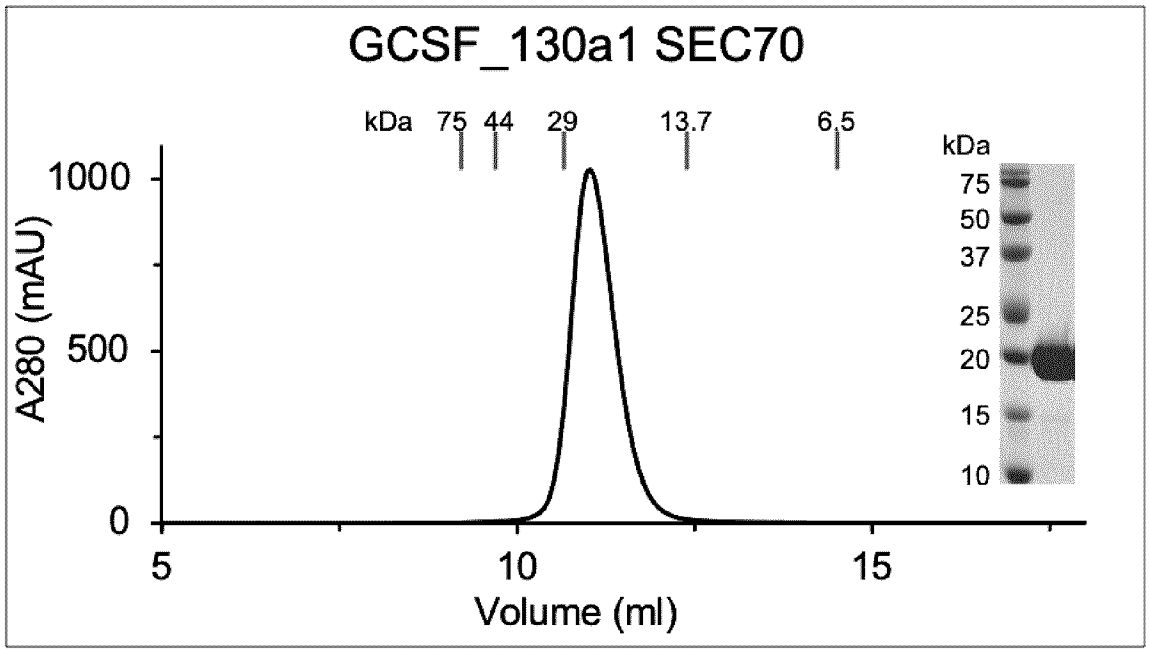

GCSF_130a1 SEC70
kDa 75 44 29 13.7 6.5
kDa
75
50
37
25
20
15
10
1000
500
0
A280 (mAU)
5
10
15
Volume (ml)

Figure 54.

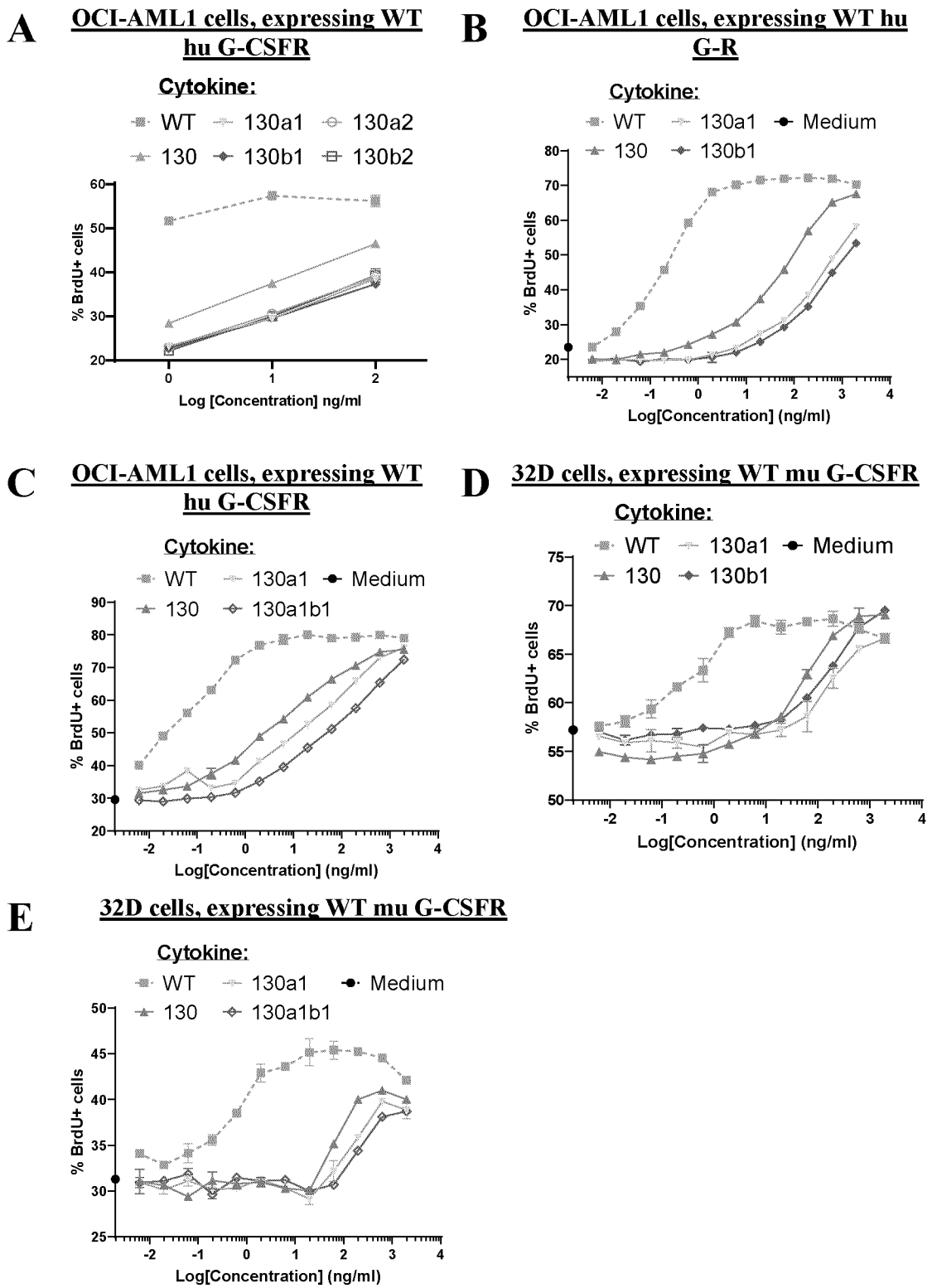

A
OCI-AML1 cells, expressing WT hu G-CSFR
Cytokine:
WT
130a1
130a2
130
130b1
130b2
% BrdU+ cells
Log [Concentration] ng/ml
B
OCI-AML1 cells, expressing WT hu G-R
Cytokine:
WT
130a1
Medium
130
130b1
% BrdU+ cells
Log[Concentration] (ng/ml)
C
OCI-AML1 cells, expressing WT hu G-CSFR
Cytokine:
WT
130a1
Medium
130
130a1b1
% BrdU+ cells
Log[Concentration] (ng/ml)
D
32D cells, expressing WT mu G-CSFR
Cytokine:
WT
130a1
Medium
130
130b1
% BrdU+ cells
Log[Concentration] (ng/ml)
E
32D cells, expressing WT mu G-CSFR
Cytokine:
WT
130a1
Medium
130
130a1b1
% BrdU+ cells
Log[Concentration] (ng/ml)

Figure 55.

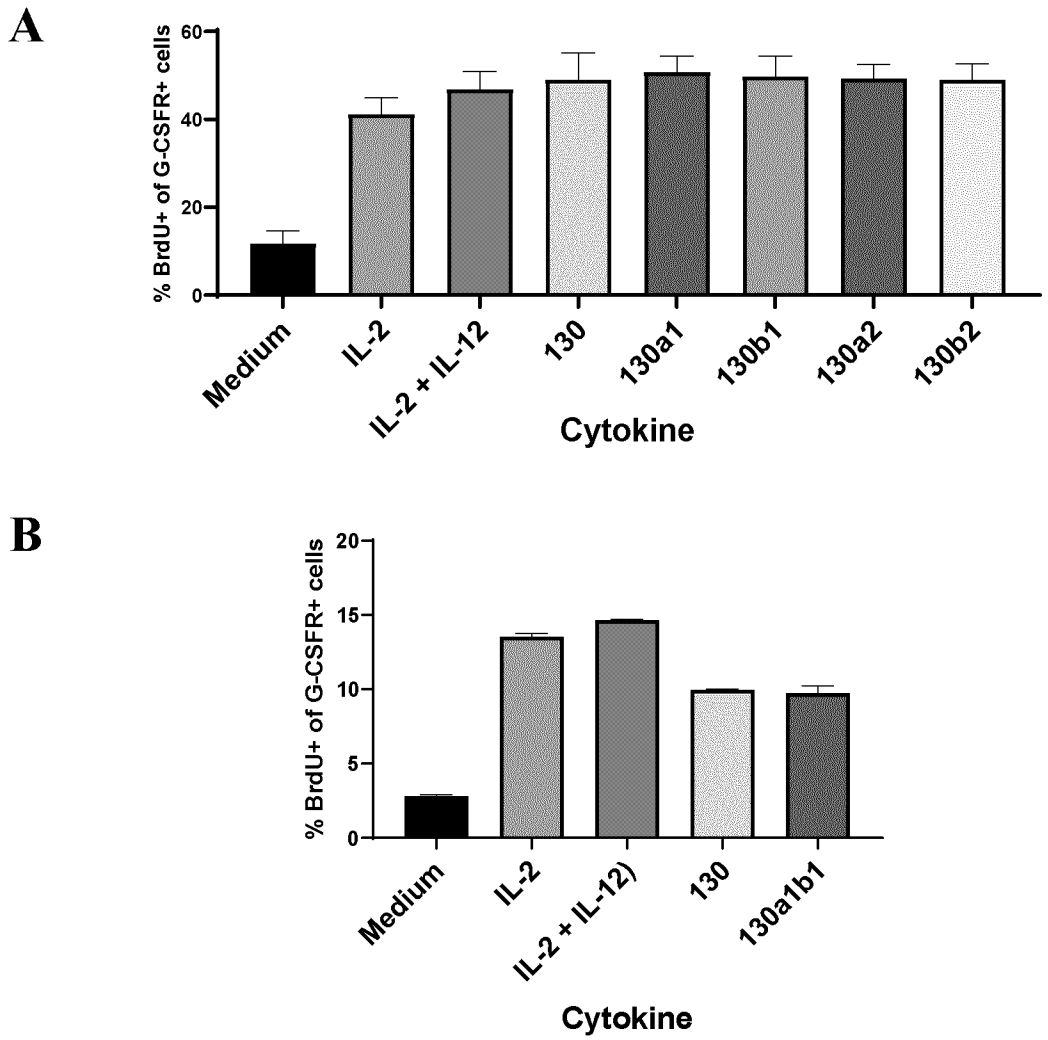

A
% BrdU+ of G-CSFR+ cells
60
40
20
0
Medium
IL-2
IL-2 + IL-12
130
130a1
130b1
130a2
130b2
Cytokine
B
% BrdU+ of G-CSFR+ cells
20
15
10
5
0
Medium
IL-2
IL-2 + IL-12)
130
130a1b1
Cytokine

Figure 56.

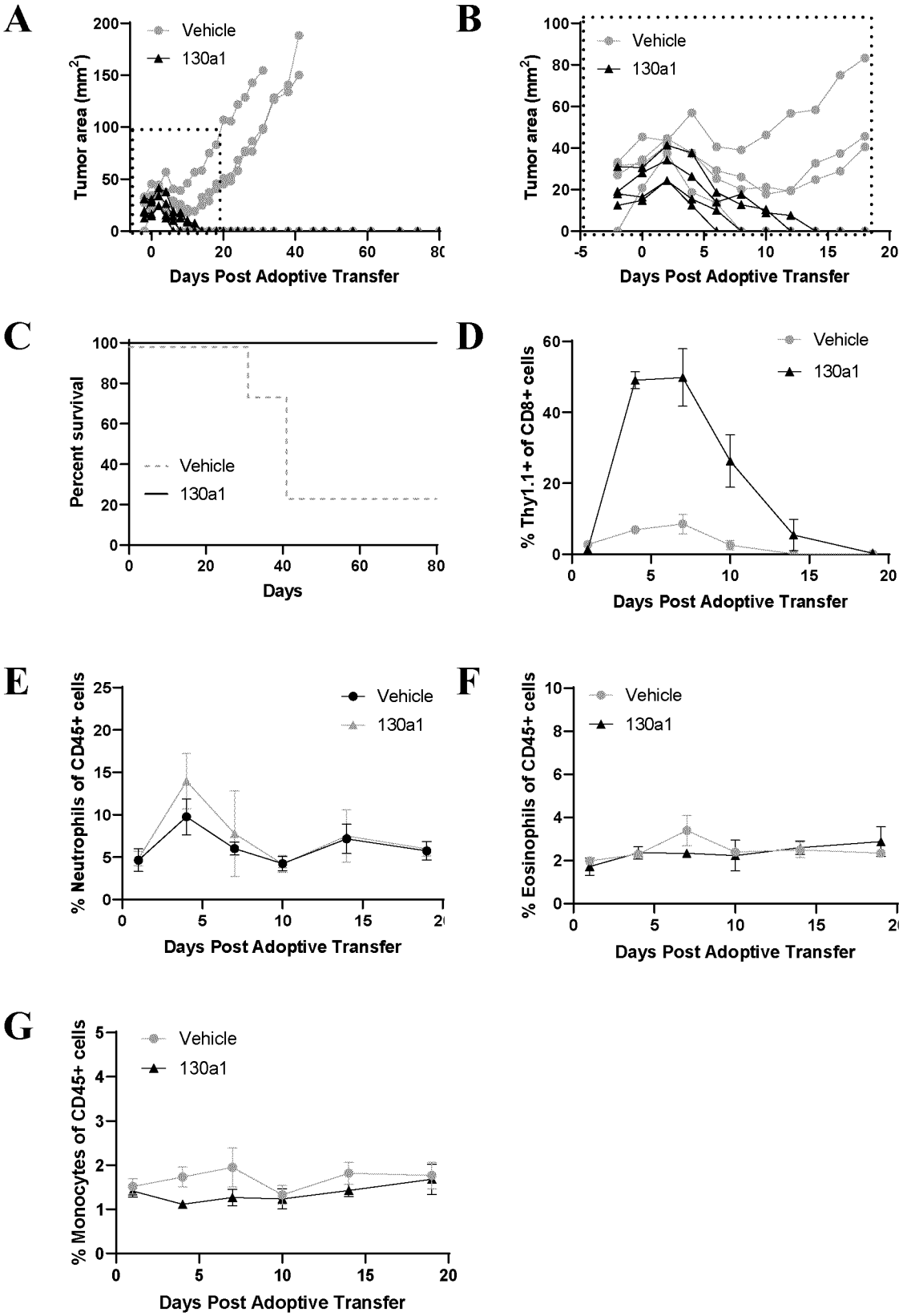

A
Vehicle
130a1
Tumor area (mm2)
Days Post Adoptive Transfer
B
Vehicle
130a1
Tumor area (mm2)
Days Post Adoptive Transfer
C
Percent survival
Vehicle
130a1
Days
D
Vehicle
130a1
% Thy1.1+ of CD8+ cells
Days Post Adoptive Transfer
E
Vehicle
130a1
% Neutrophils of CD45+ cells
Days Post Adoptive Transfer
F
Vehicle
130a1
% Eosinophils of CD45+ cells
Days Post Adoptive Transfer
G
Vehicle
130a1
% Monocytes of CD45+ cells
Days Post Adoptive Transfer

Figure 57

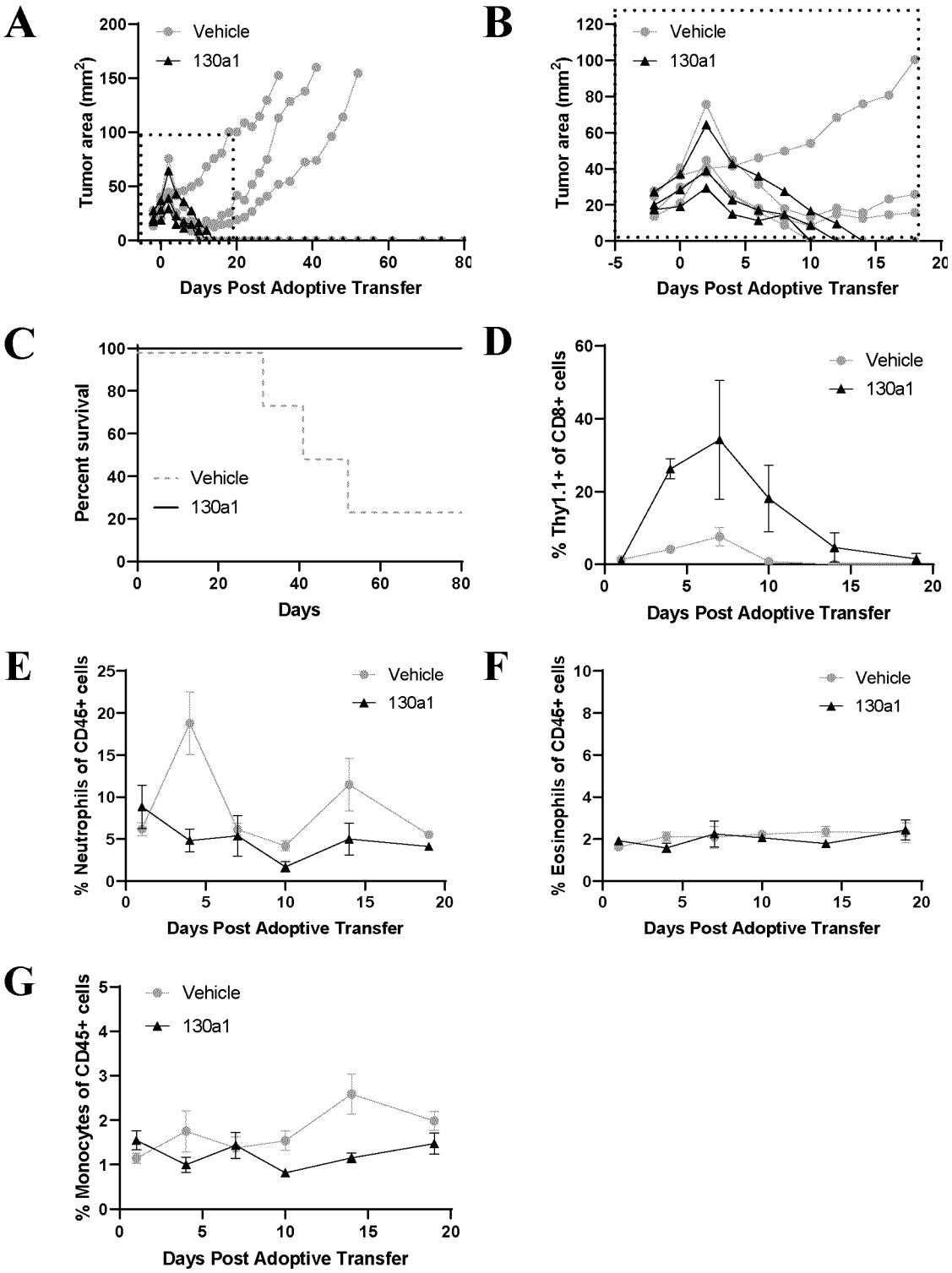

A
Vehicle
130a1
Tumor area (mm2)
Days Post Adoptive Transfer
B
Vehicle
130a1
Tumor area (mm2)
Days Post Adoptive Transfer
C
Vehicle
130a1
Percent survival
Days
D
Vehicle
130a1
% Thy1.1+ of CD8+ cells
Days Post Adoptive Transfer
E
Vehicle
130a1
% Neutrophils of CD45+ cells
Days Post Adoptive Transfer
F
Vehicle
130a1
% Eosinophils of CD45+ cells
Days Post Adoptive Transfer
G
Vehicle
130a1
% Monocytes of CD45+ cells
Days Post Adoptive Transfer

Figure 58.

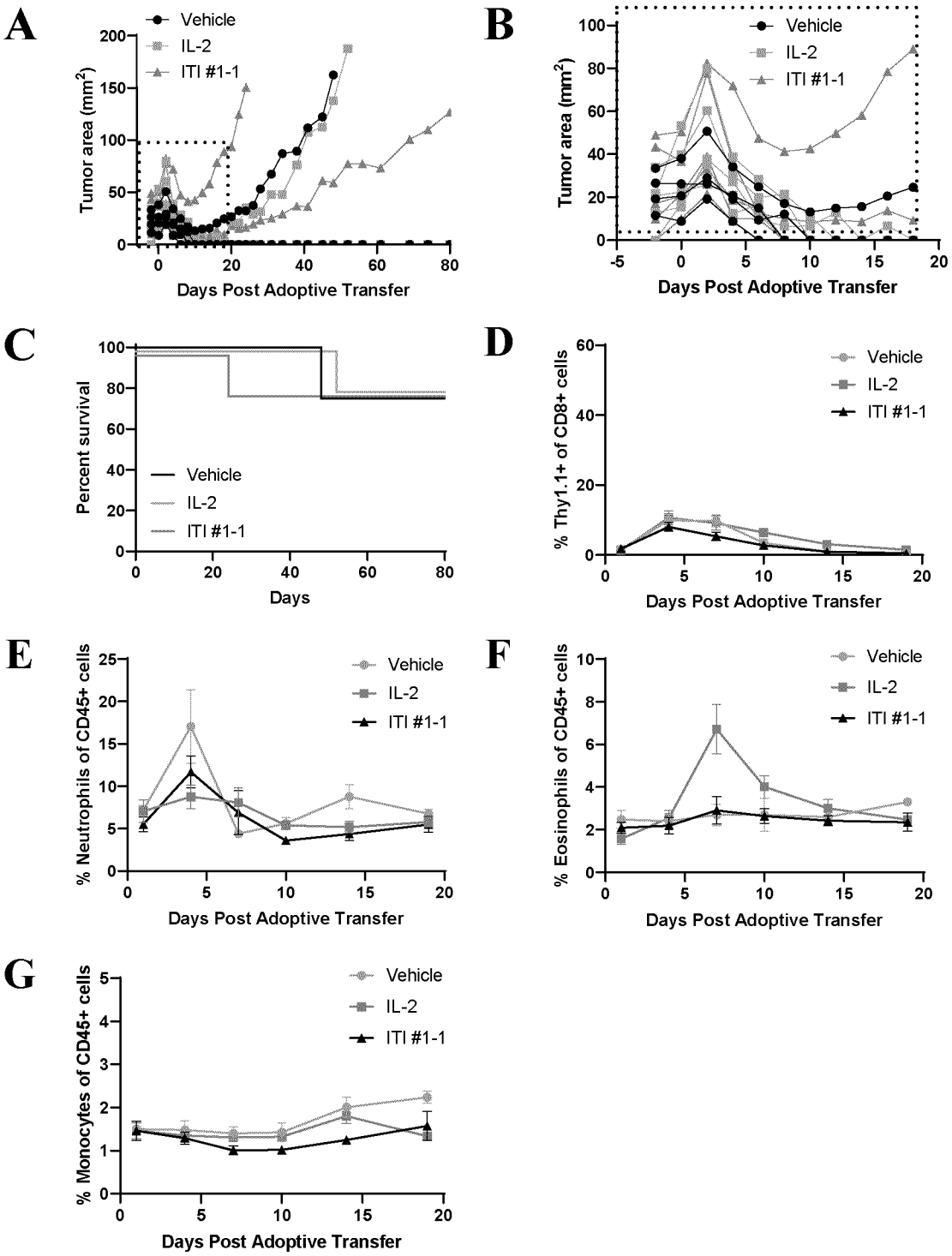

A
Vehicle
IL-2
ITI #1-1
Tumor area (mm²)
Days Post Adoptive Transfer
B
Vehicle
IL-2
ITI #1-1
Tumor area (mm²)
Days Post Adoptive Transfer
C
Percent survival
Vehicle
IL-2
ITI #1-1
Days
D
% Thy1.1+ of CD8+ cells
Vehicle
IL-2
ITI #1-1
Days Post Adoptive Transfer
E
% Neutrophils of CD45+ cells
Vehicle
IL-2
ITI #1-1
Days Post Adoptive Transfer
F
% Eosinophils of CD45+ cells
Vehicle
IL-2
ITI #1-1
Days Post Adoptive Transfer
G
% Monocytes of CD45+ cells
Vehicle
IL-2
ITI #1-1
Days Post Adoptive Transfer

Figure 59.

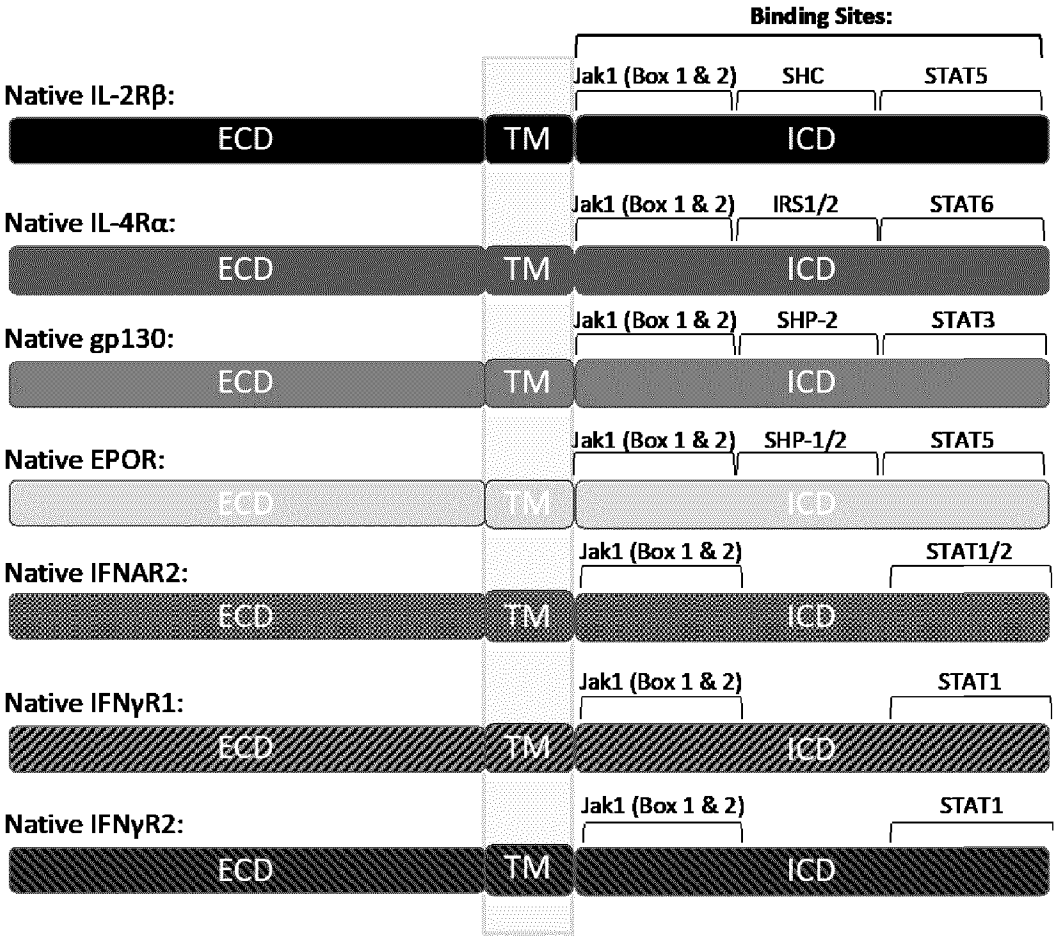


Figure 60. Receptor schematics.

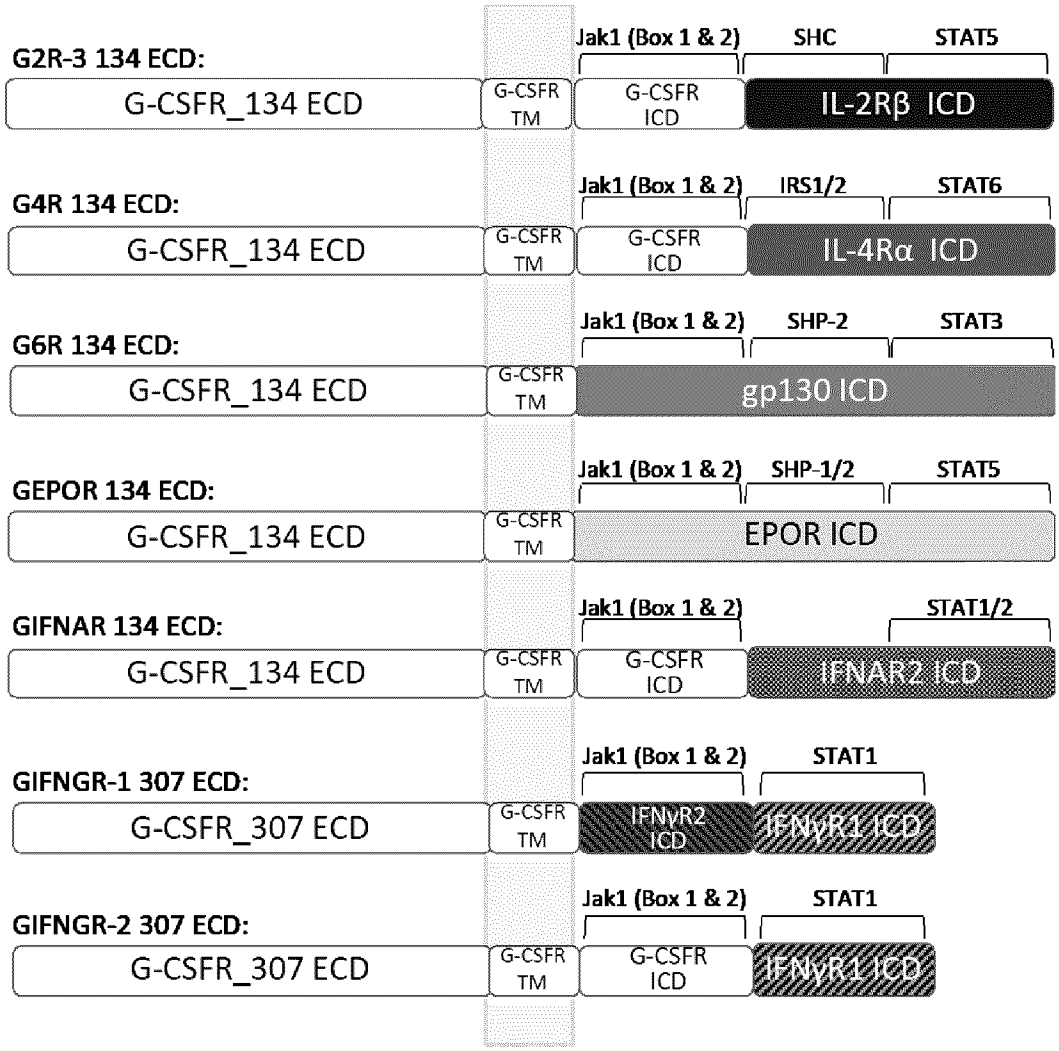


Figure 60 continued. Receptor schematics.

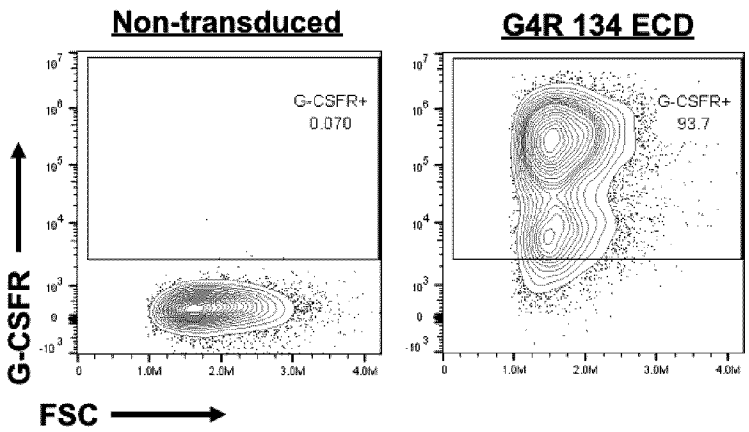


Figure 61. G4R 134 ECD expression on day 8 of expansion.

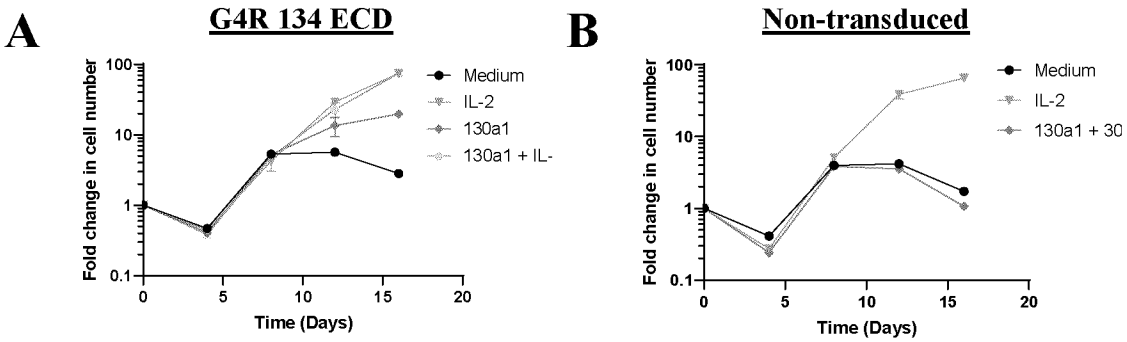


Figure 62. Expansion of human T cells expressing G4R 134 ECD.

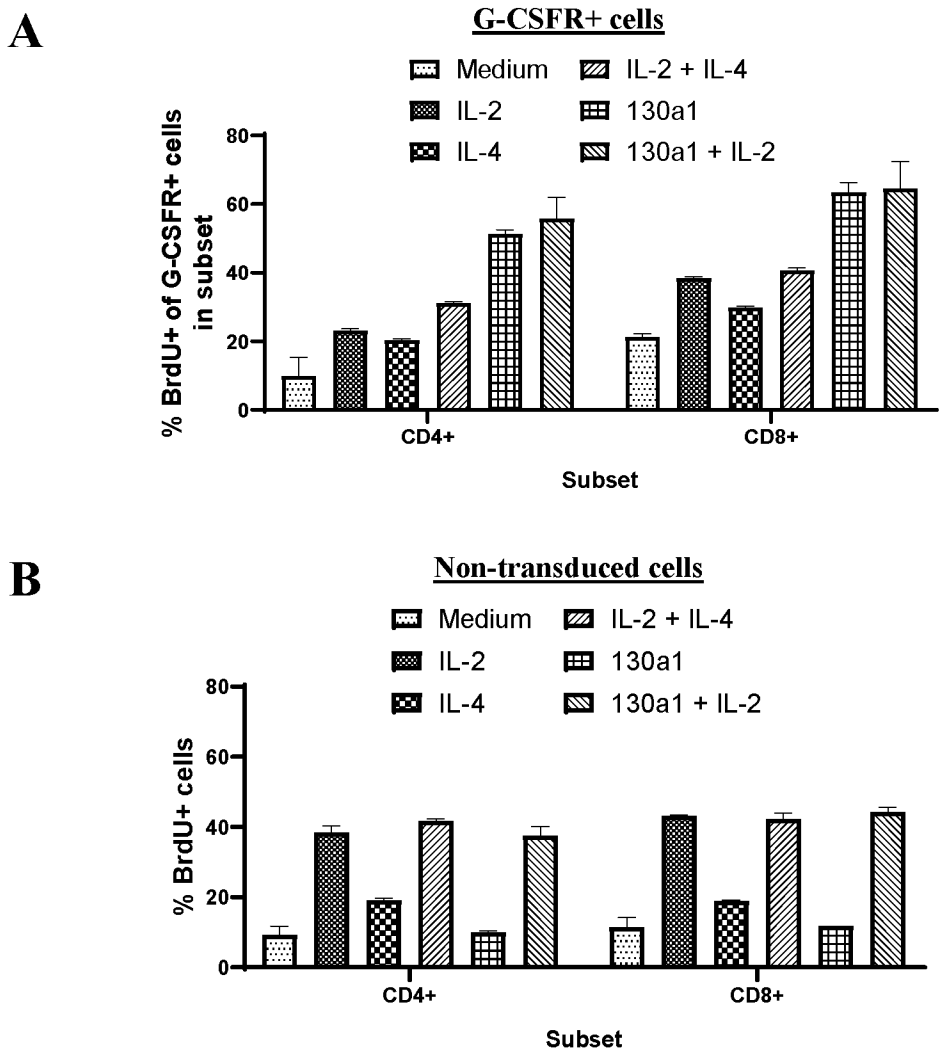


Figure 63. Proliferation of human T cells expressing G4R 134 ECD.

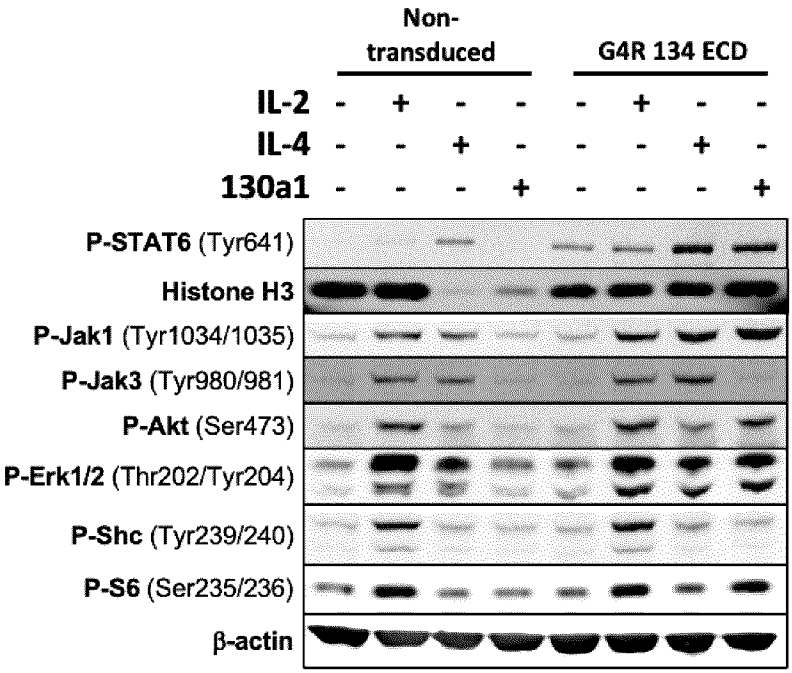


Figure 64. Western blots of human T cells non-transduced or expressing G4R 134 ECD.

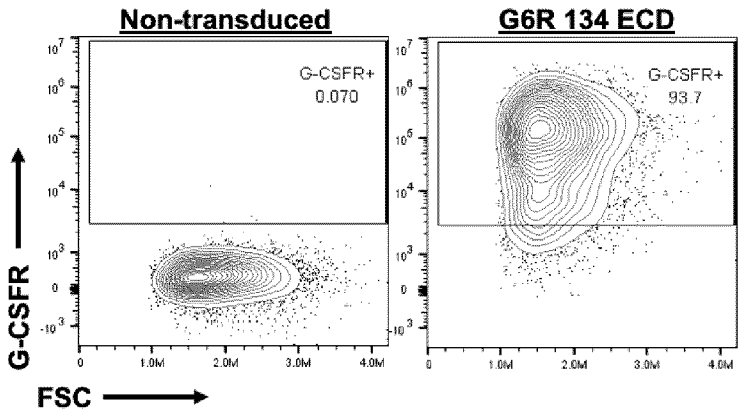


Figure 65. G6R 134 ECD expression on day 8 of expansion.

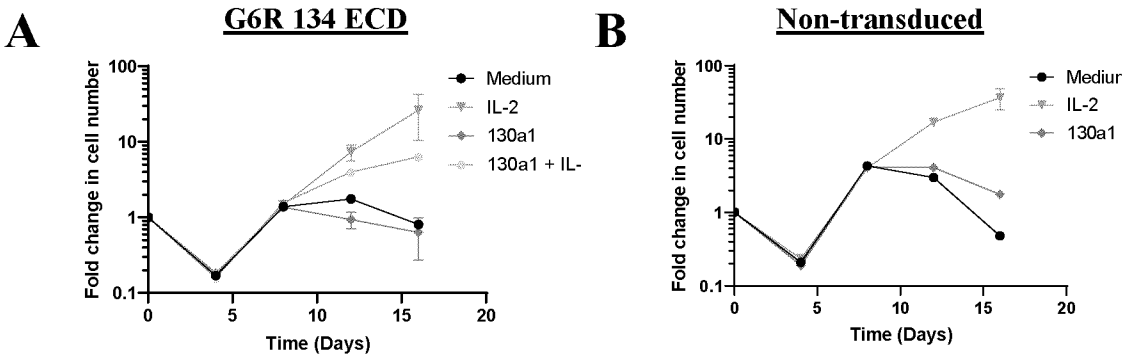


Figure 66. Expansion of human T cells expressing G6R 134 ECD.

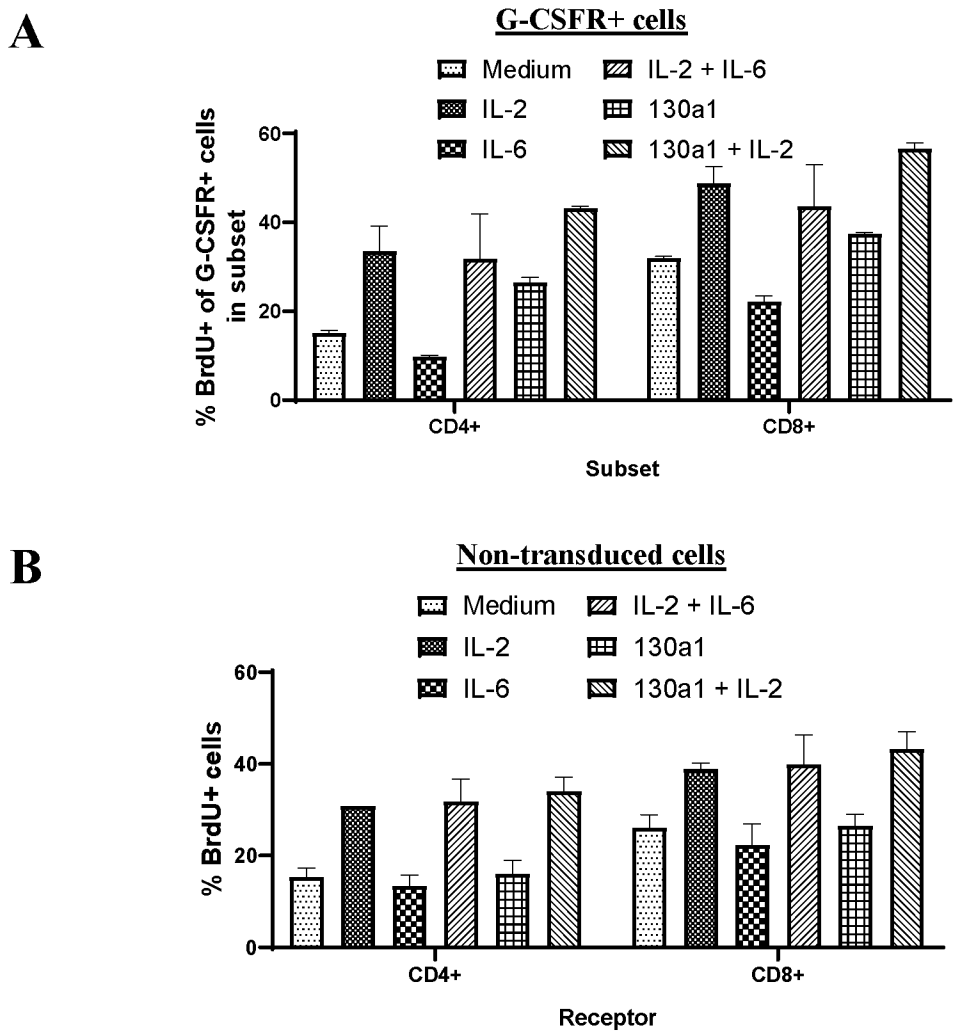


Figure 67. Proliferation of human T cells expressing G6R 134 ECD.

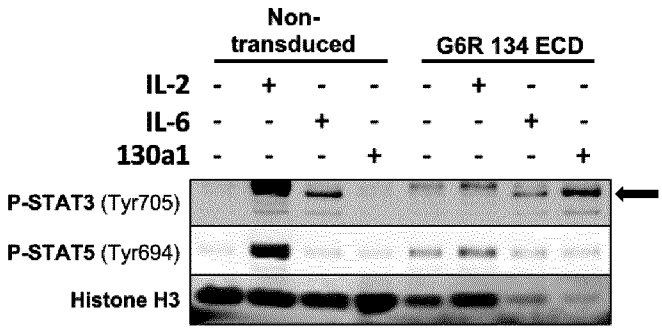


Figure 68. Western blots of human T cells non-transduced or expressing G6R 134 ECD.

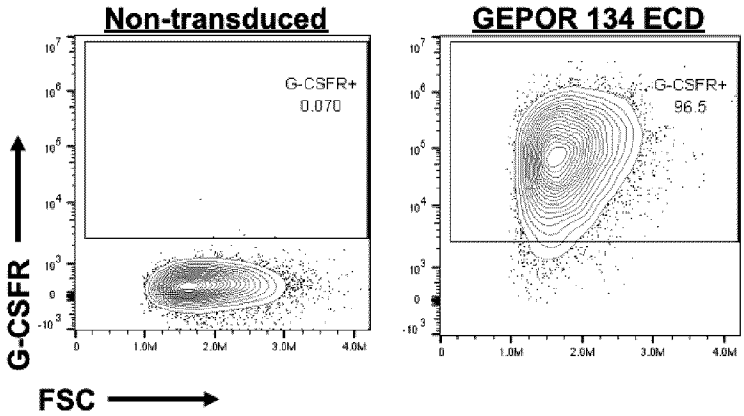


Figure 69. GEPOR 134 ECD expression on day 8 of expansion.

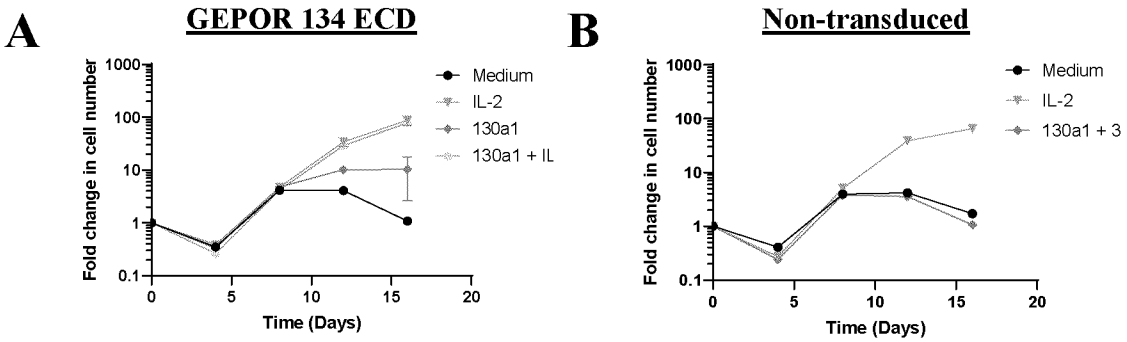


Figure 70. Expansion of human T cells expressing GEPOR 134 ECD.

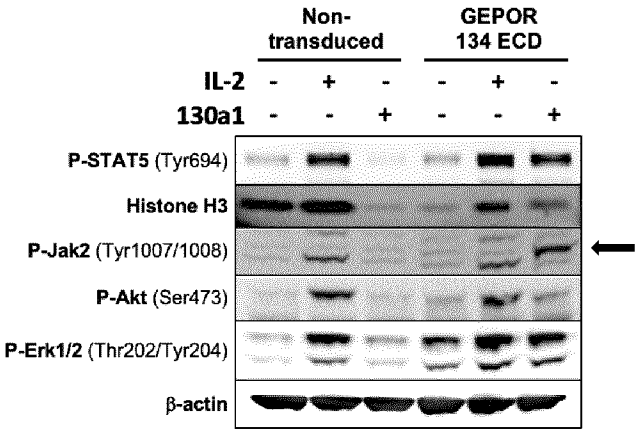


Figure 71. Western blots of human T cells non-transduced or expressing GEPOR 134 ECD.

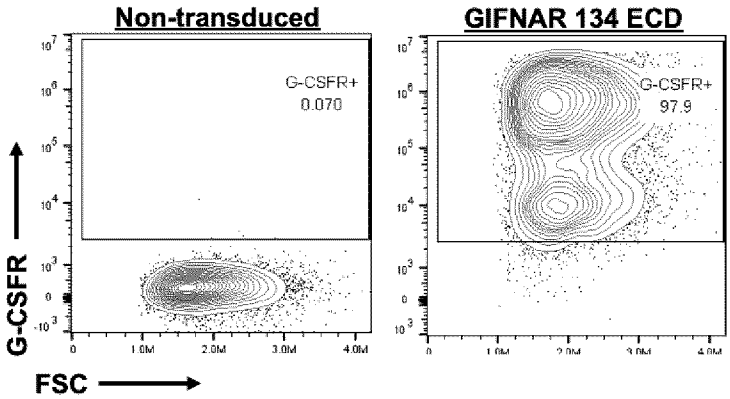


Figure 72. GIFNAR 134 ECD expression on day 8 of expansion

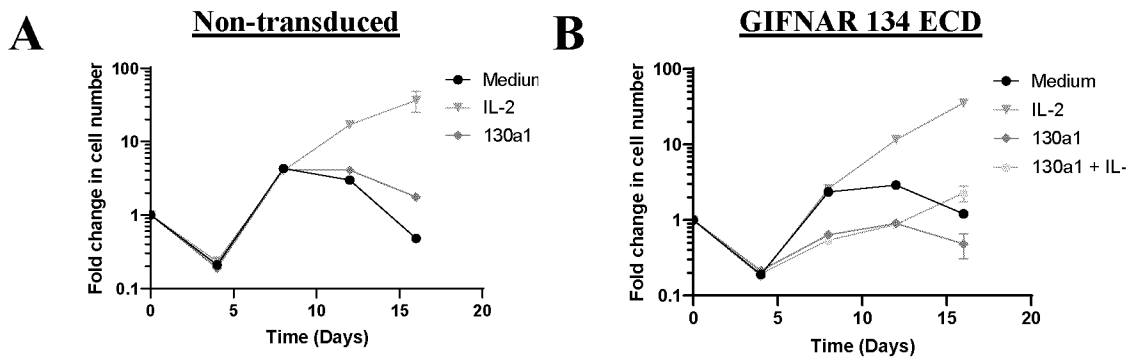


Figure 73. Expansion of human T cells expressing GIFNAR 134 ECD.

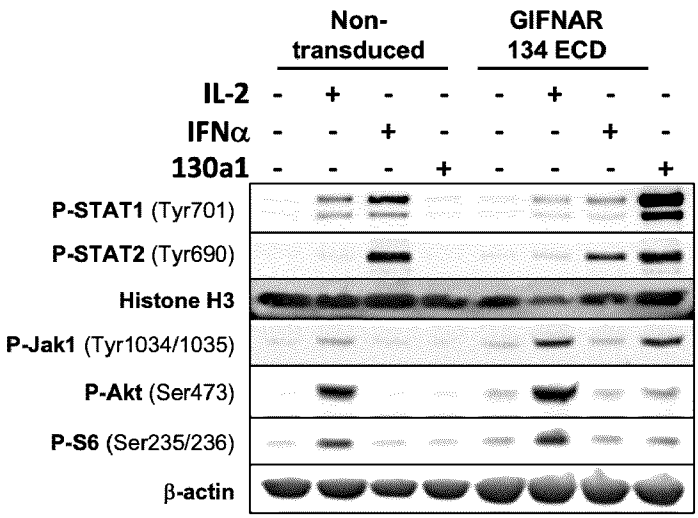


Figure 74. Western blots of human T cells non-transduced or expressing GIFNAR 134 ECD.

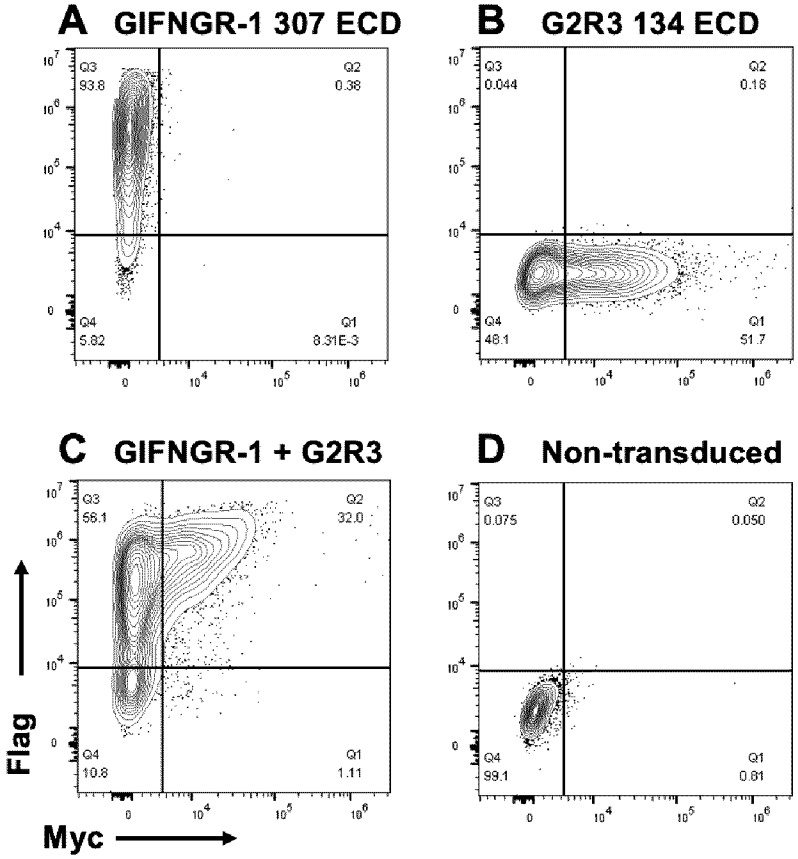


Figure 75. Expression of GIFNGR-1 307 ECD (Flag-tagged) and G2R3 (Myc-tagged) on day 8 of expansion. A) Transduced with GIFNGR-1 307 ECD only; B) transduced with G2R3 134 ECD only; C) co-transduced with GIFNGR-1 307 ECD and G2R3 134 ECD; and D) non-transduced.

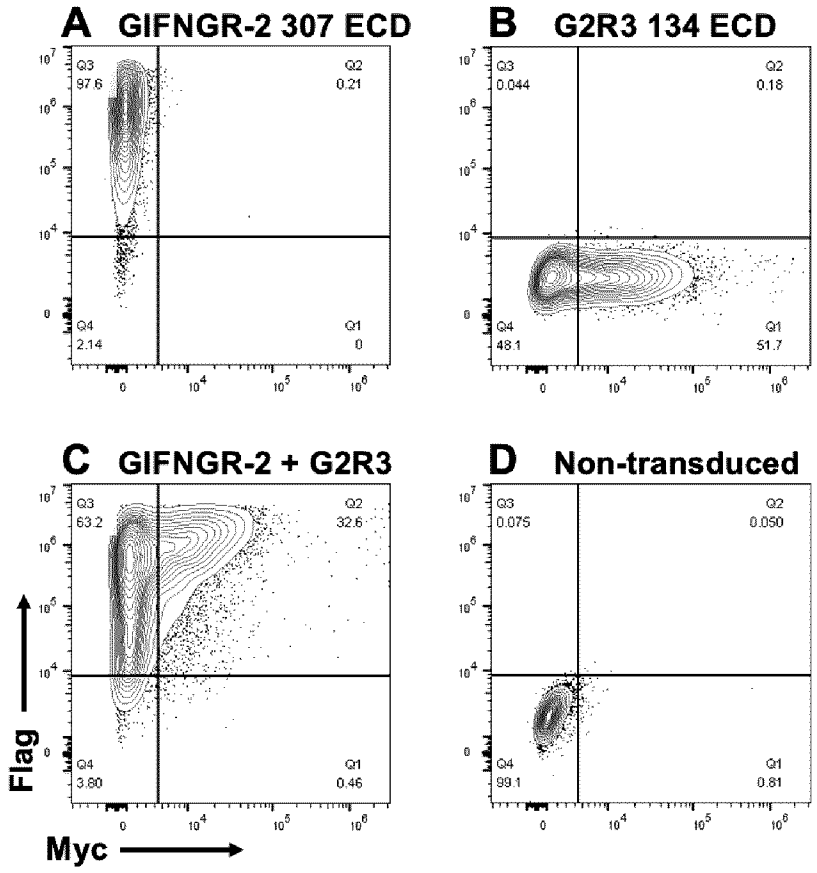


Figure 76. Expression of GIFNGR-2 307 ECD (Flag-tagged) and G2R3 (Myc-tagged) on day 8 of expansion. A) Transduced with GIFNGR-2 307 ECD only; B) transduced with G2R3 134 ECD only; C) co-transduced with GIFNGR-2 307 ECD and G2R3 134 ECD; and D) non-transduced.

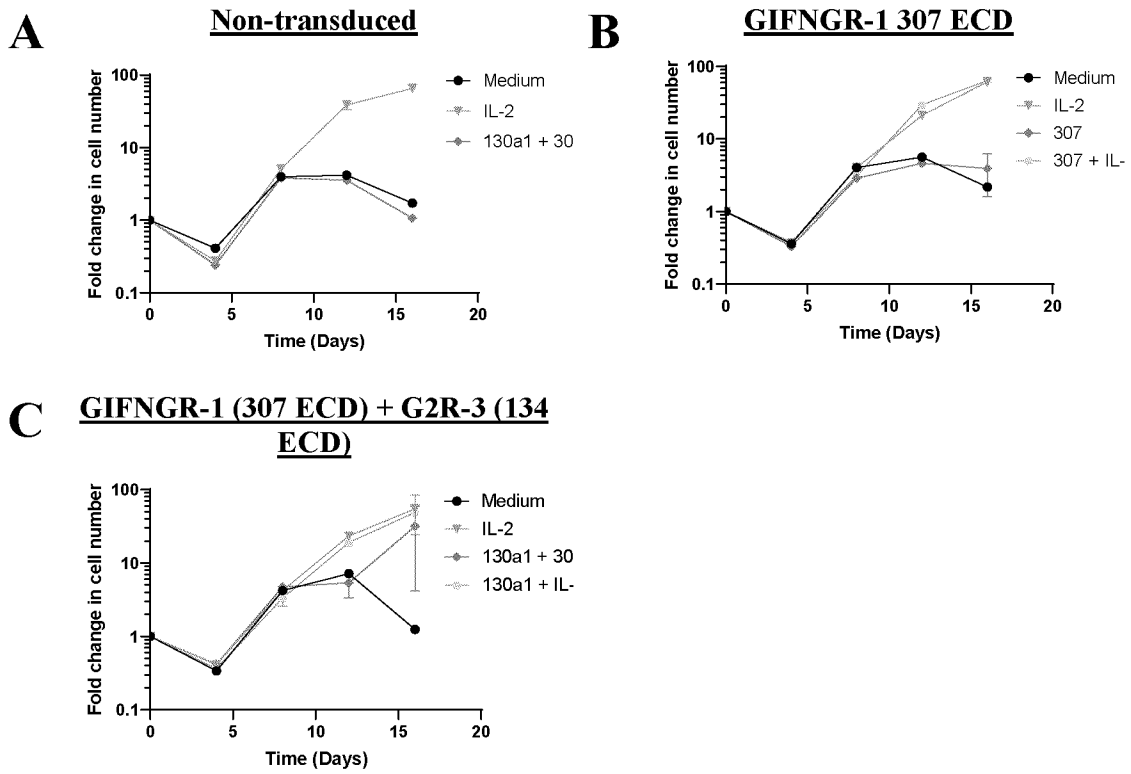


Figure 77. Expansion of human T cells expressing GIFNGR-1 307 ECD.

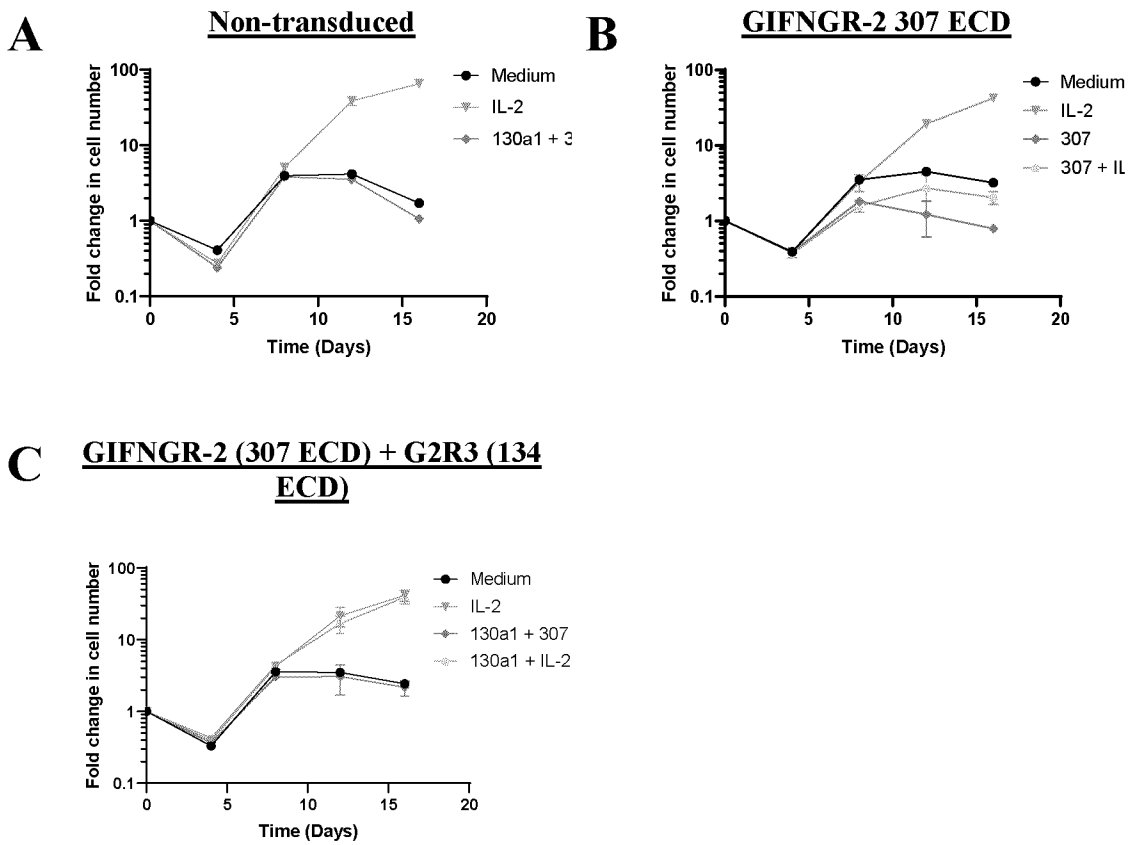


Figure 78. Expansion of human T cells expressing GIFNGR-2 307 ECD.

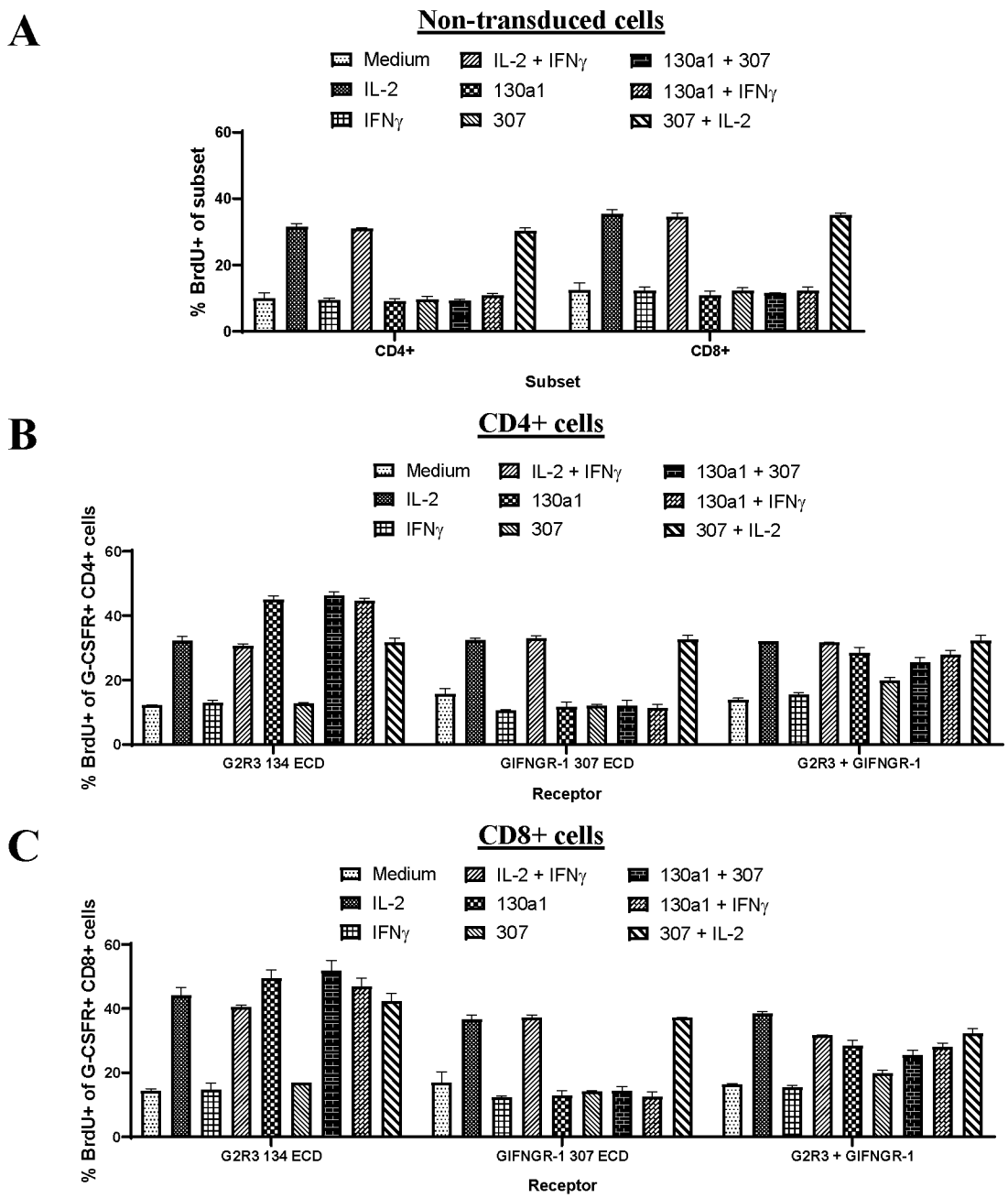


Figure 79. Proliferation of human T cells expressing GIFNGR-1 307 ECD and/or G2R-3 134 ECD.

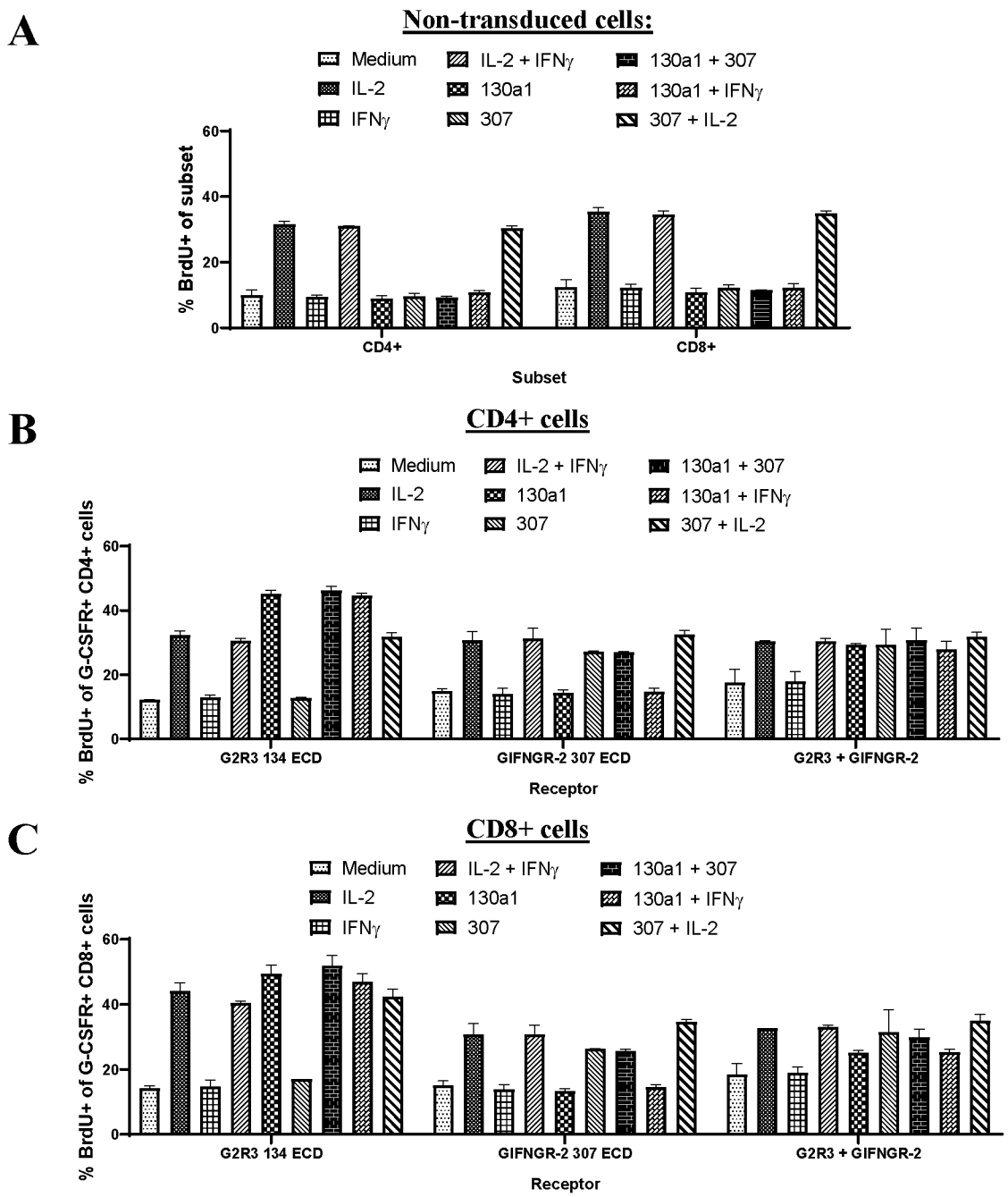


Figure 80. Proliferation of human T cells expressing GIFNGR-2 307 ECD and/or G2R-3 134 ECD.

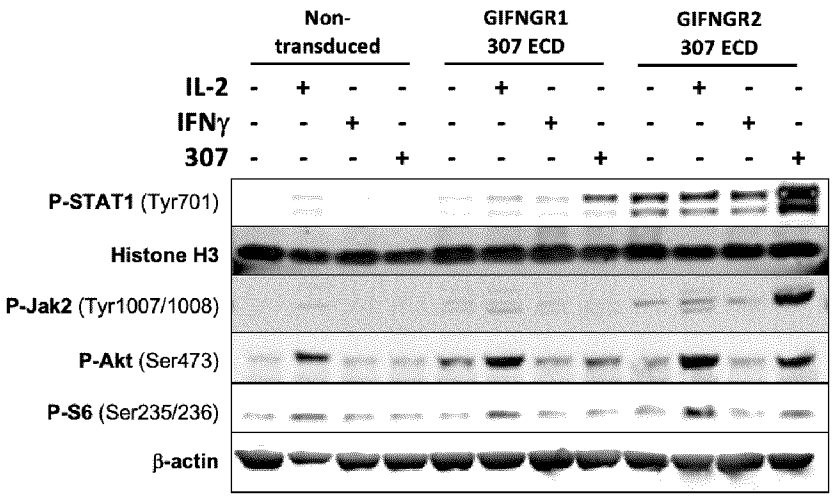


Figure 81. Western blots of human T cells non-transduced or expressing GIFNGR-1 307 ECD or GIFNGR-2 307 ECD.

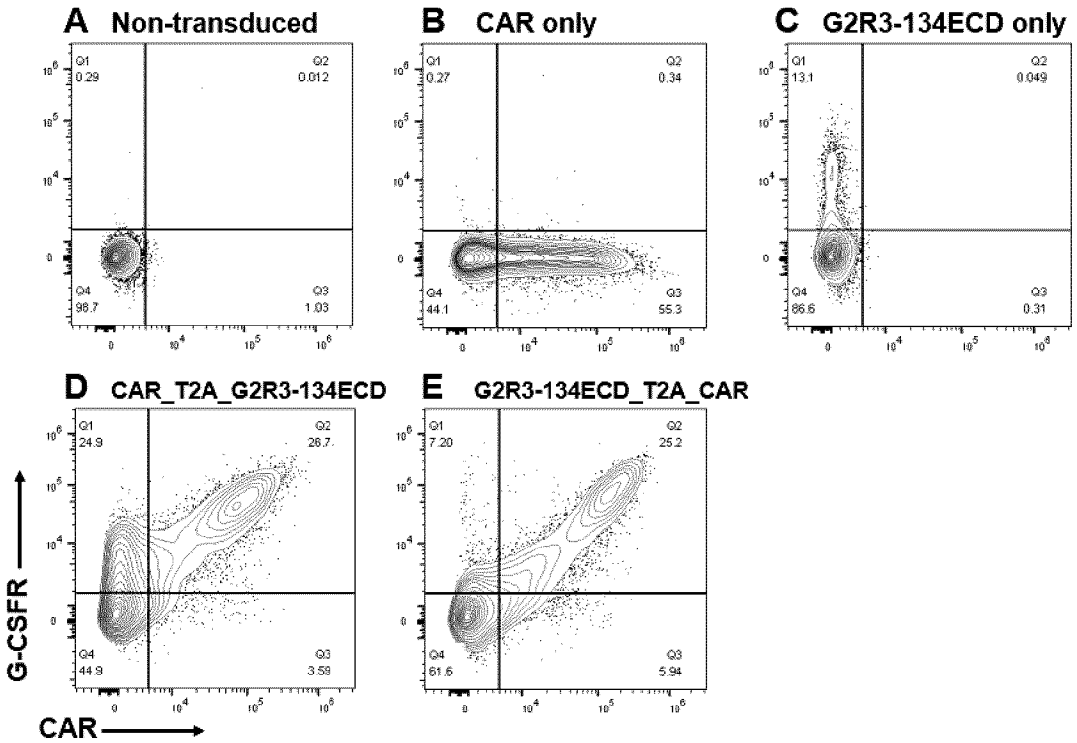


Figure 82. Expression of bicistronic CAR and G2R3 134 ECD constructs in human T cells.

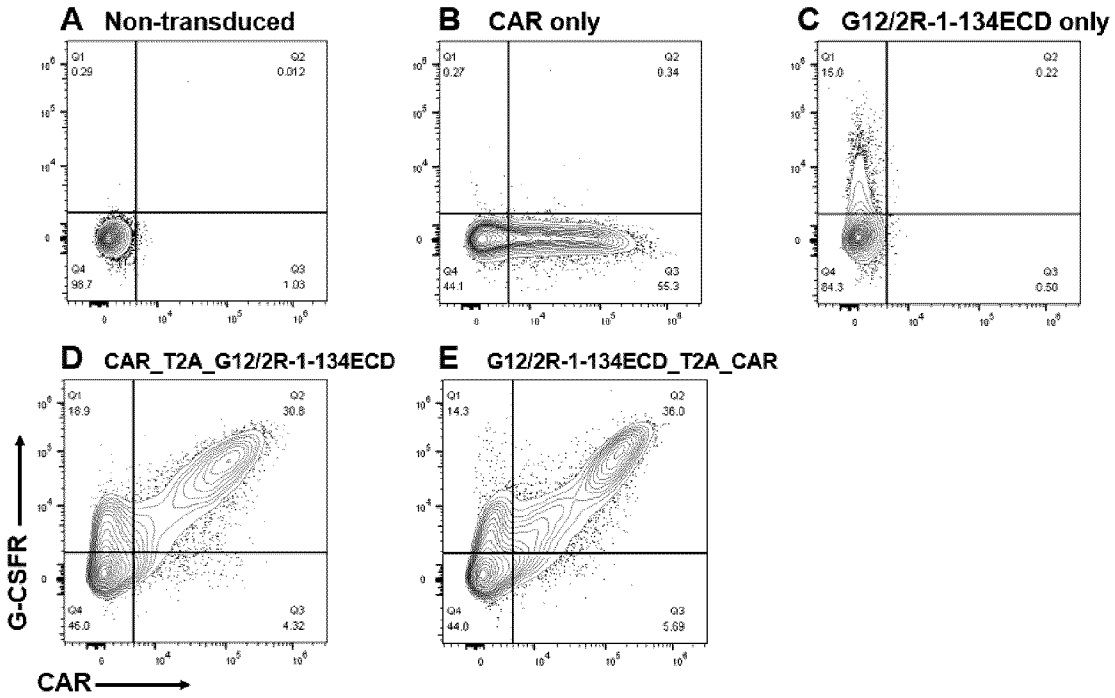


Figure 83. Expression of bicistronic CAR and G12/2R-1-134 ECD constructs in human T cells.

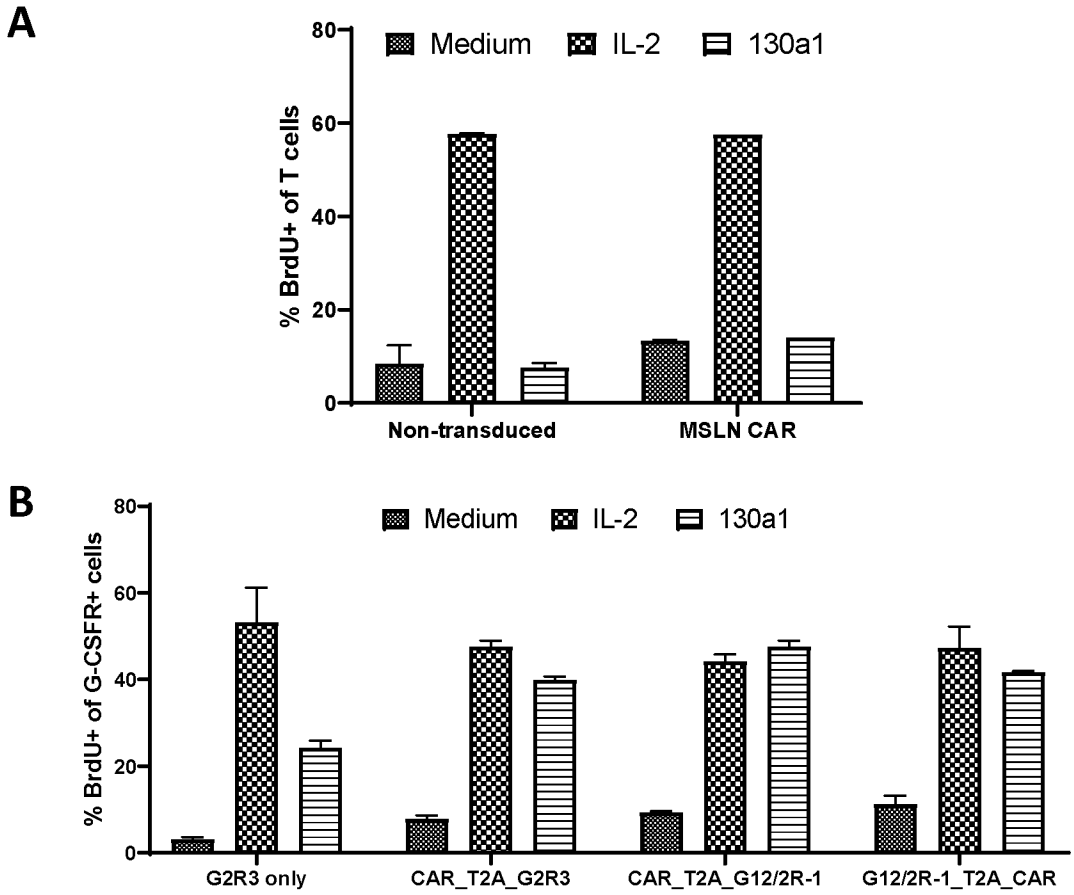


Figure 84. Proliferation of CD3+ T cells expressing mesothelin CAR with or without G2R3 134 ECD, or G12/2R-1 134 ECD.

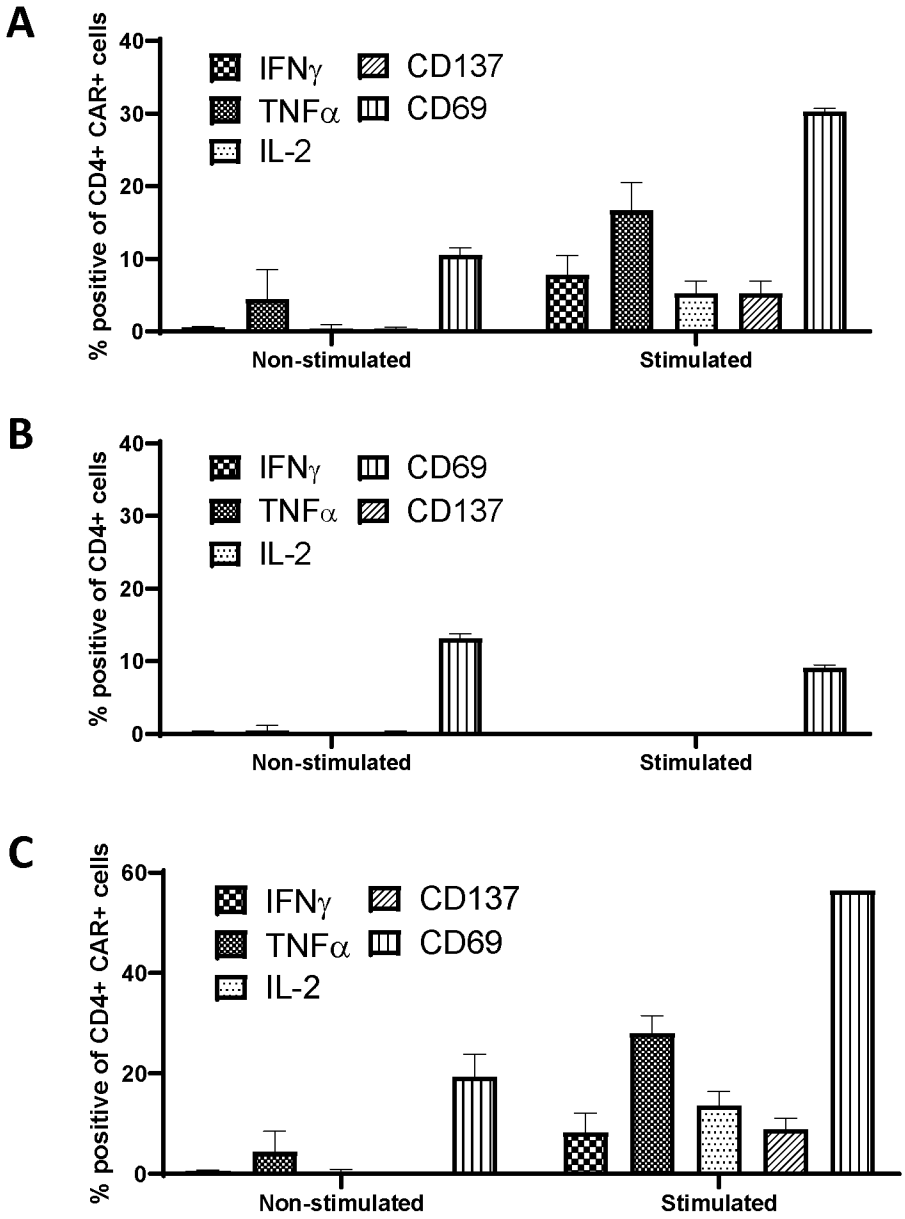


Figure 85. Expression of IFNγ, TNFα, IL-2, CD137 and CD69 by CD4+ T cells expressing bicistronic mesothelin CAR constructs.

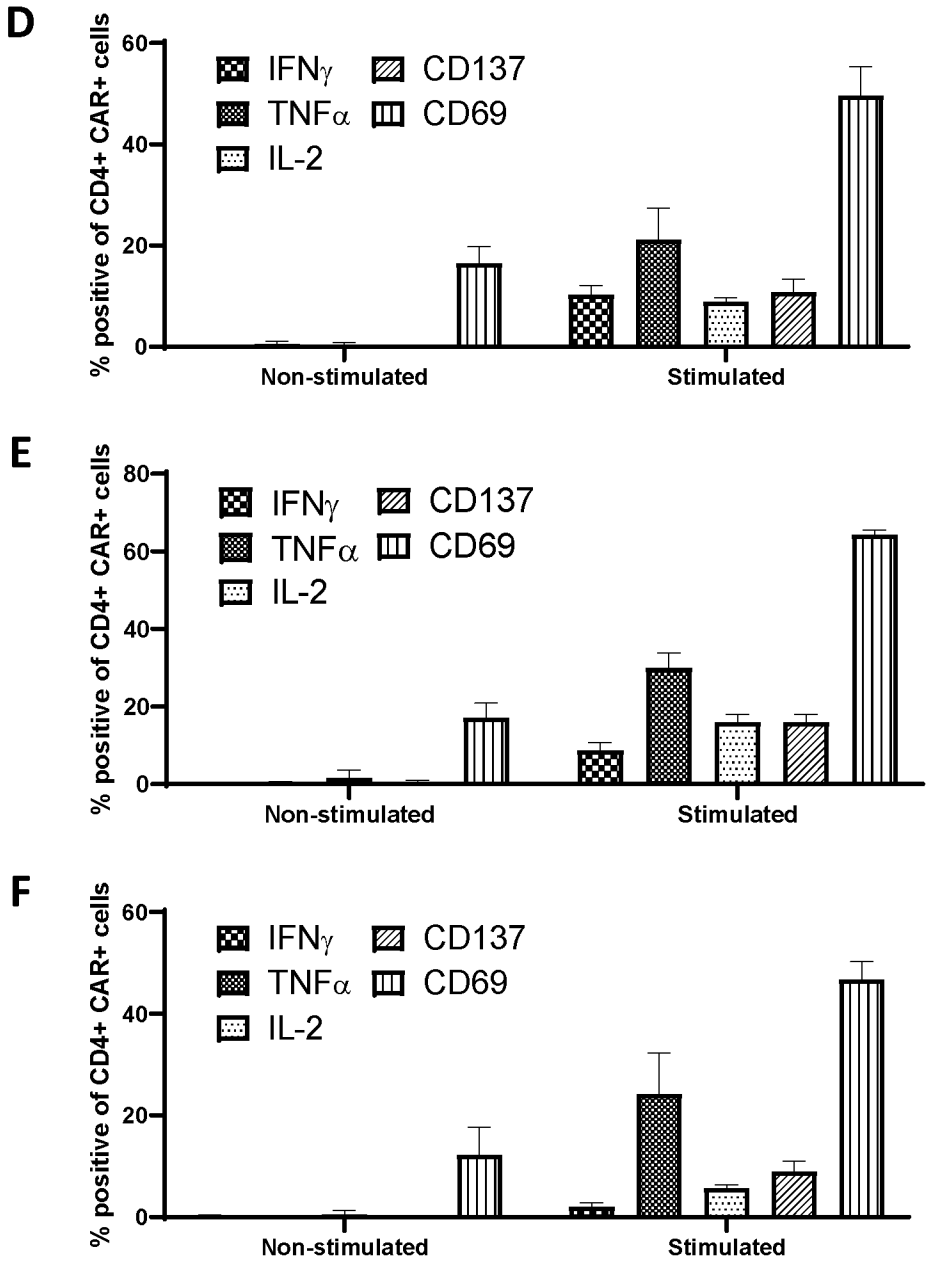


Figure 85 continued. Expression of IFNγ, TNFα, IL-2, CD137 and CD69 by CD4+ T cells expressing bicistronic mesothelin CAR constructs.

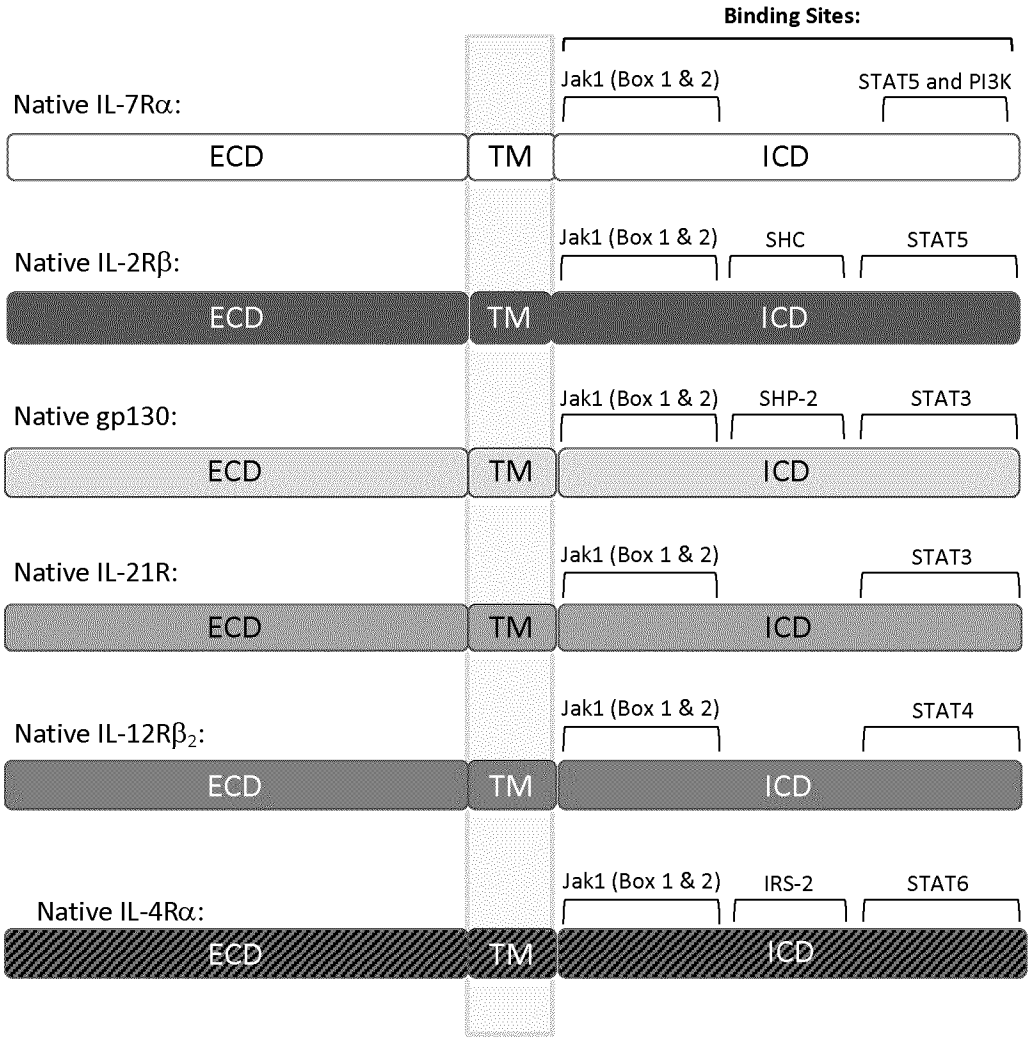


Figure 86. Native cytokine receptor schematics.

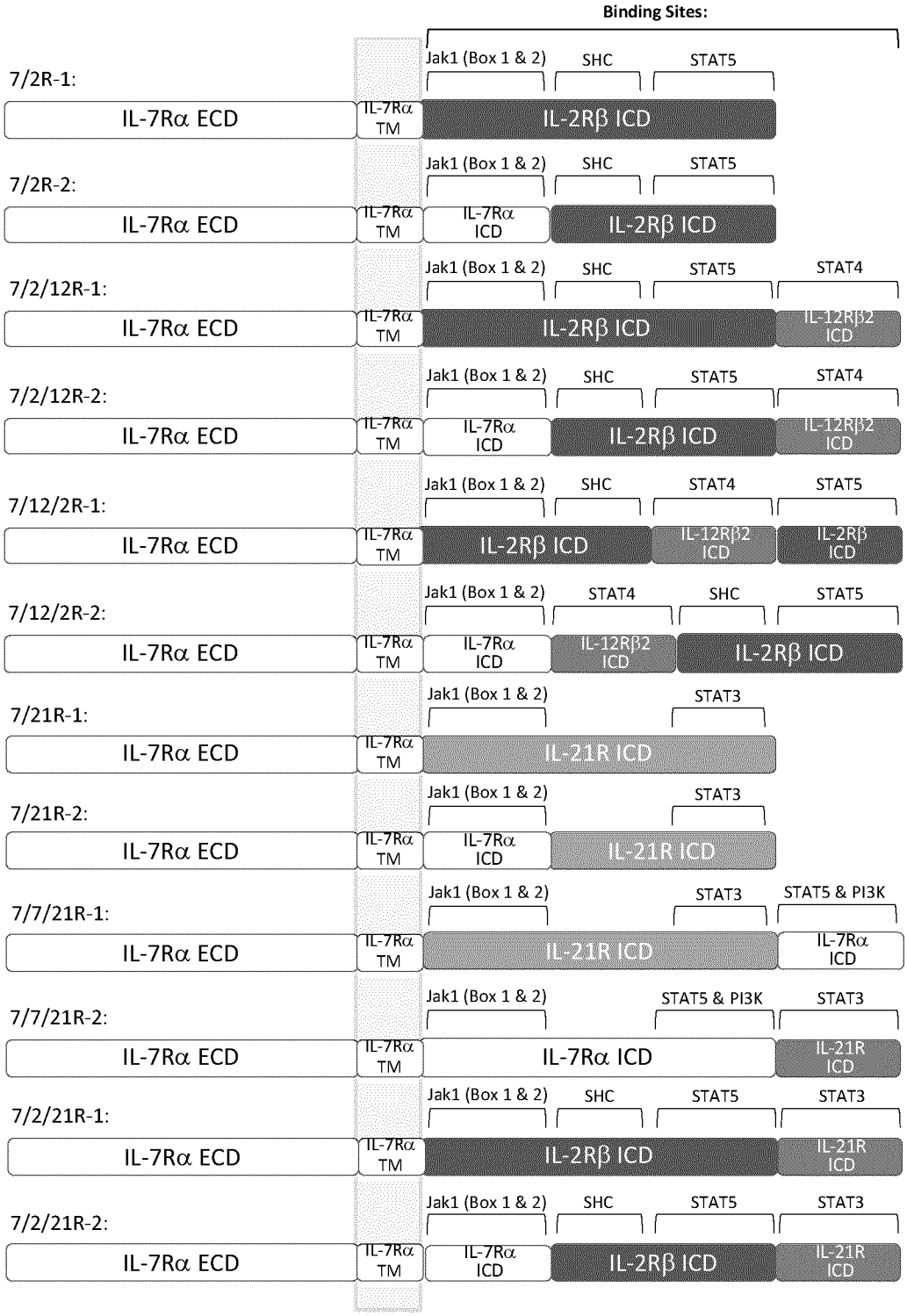


Figure 87. Chimeric receptor schematics.

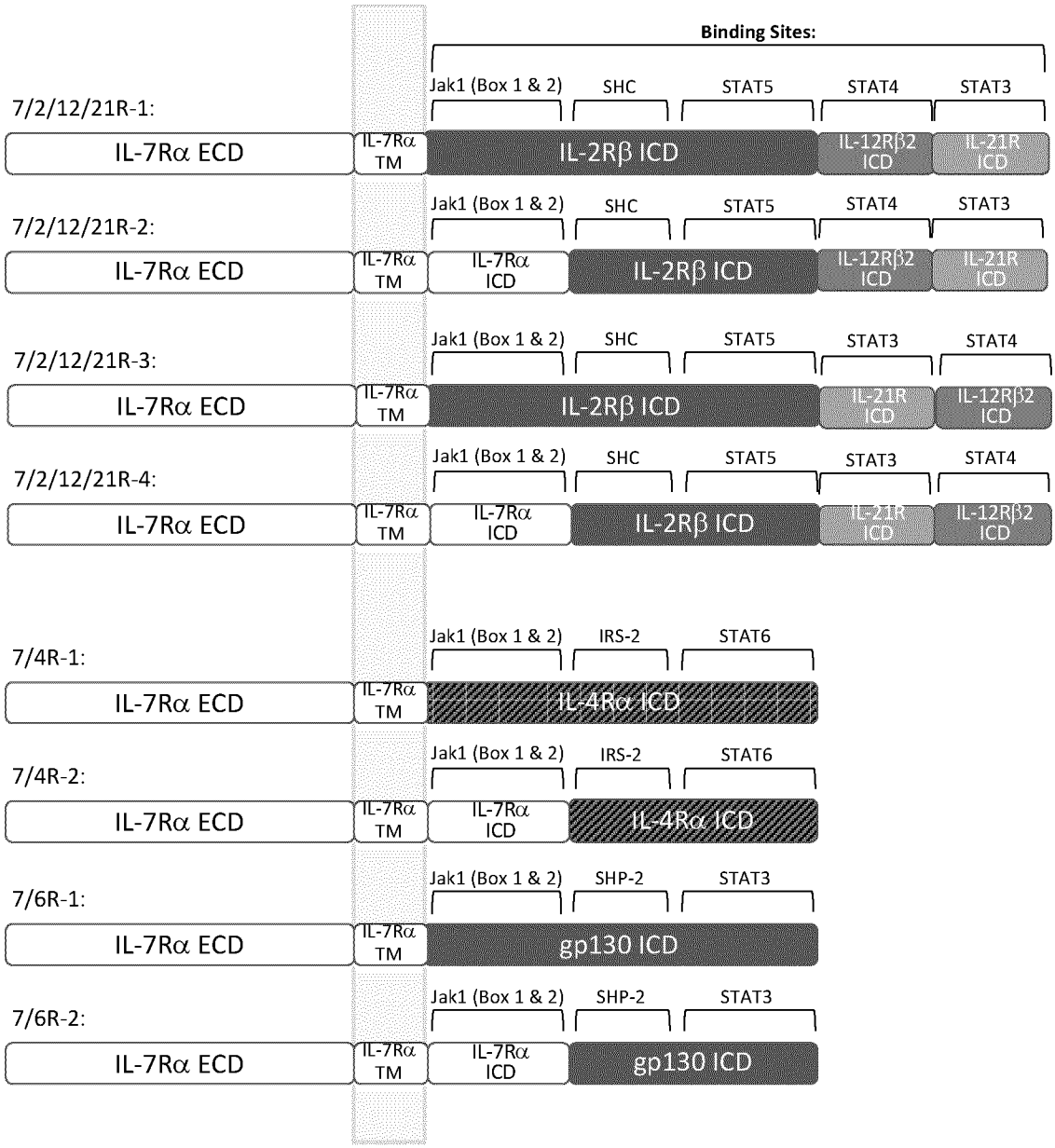


Figure 87 continued. Chimeric receptor schematics.

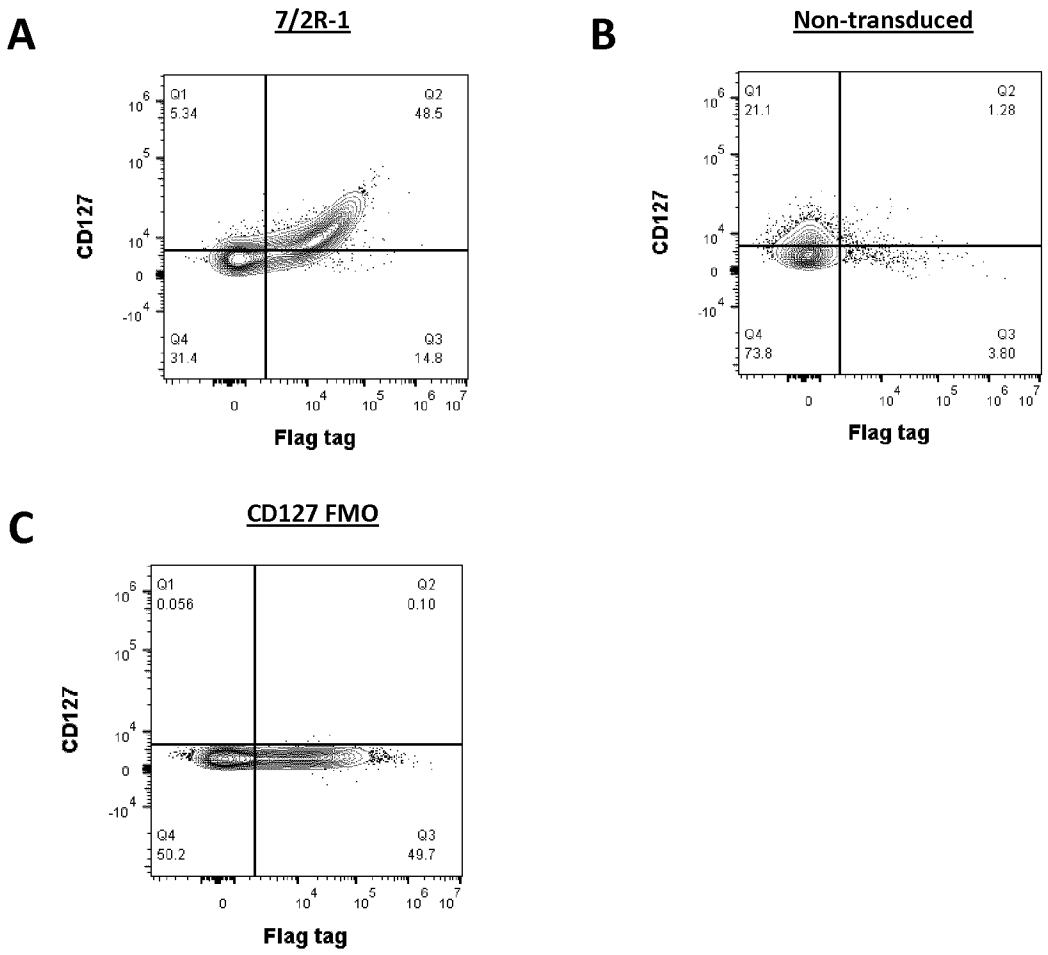


Figure 88. 7/2R-1 receptor expression on day 12 of expansion.

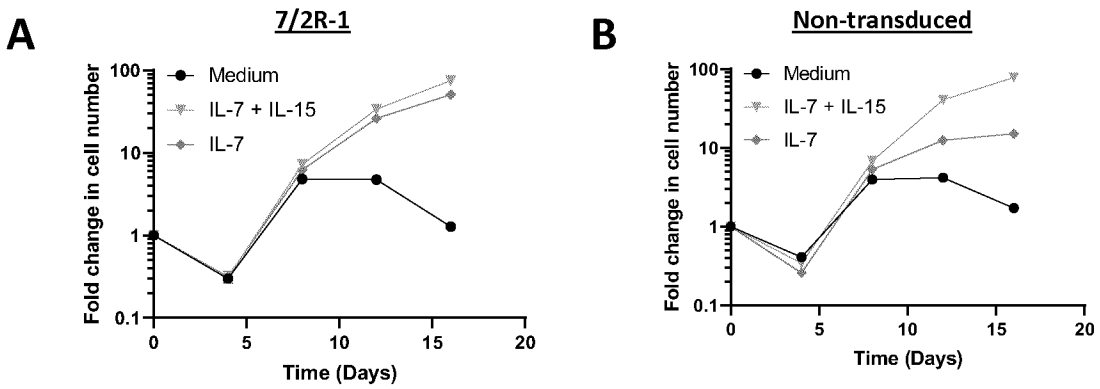


Figure 89. Expansion of human T cells expressing 7/2R-1.

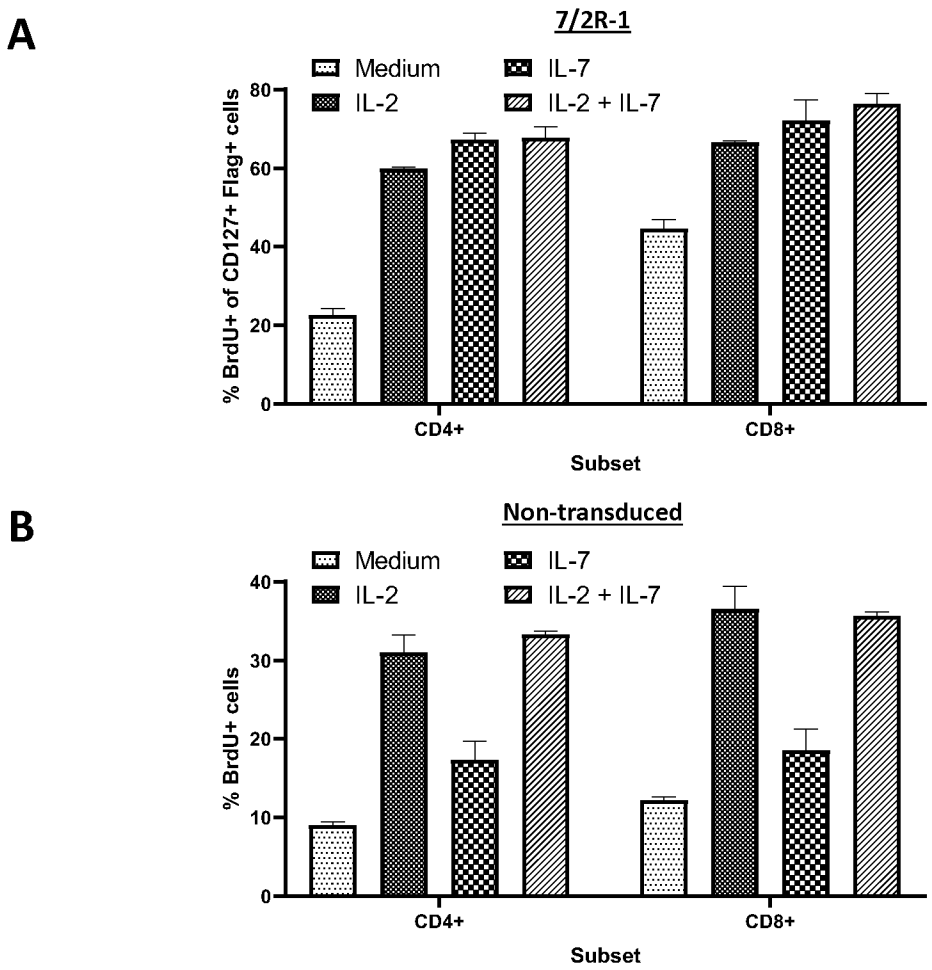


Figure 90. Proliferation of human T cells expressing 7/2R-1.

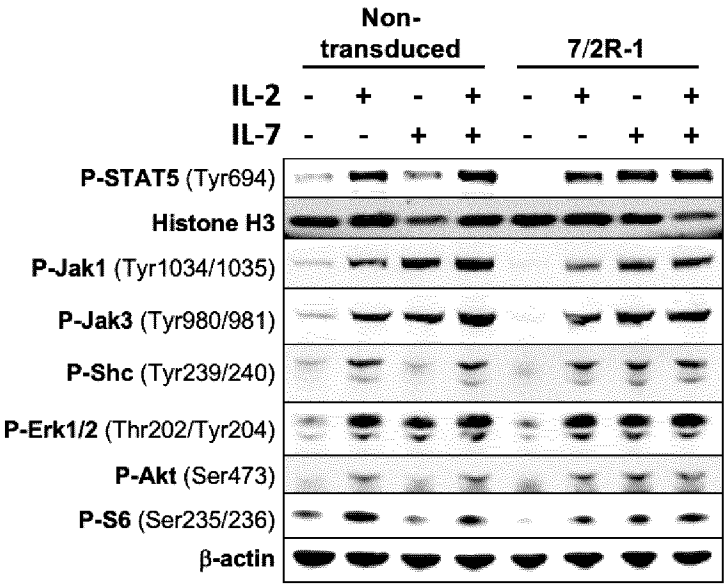
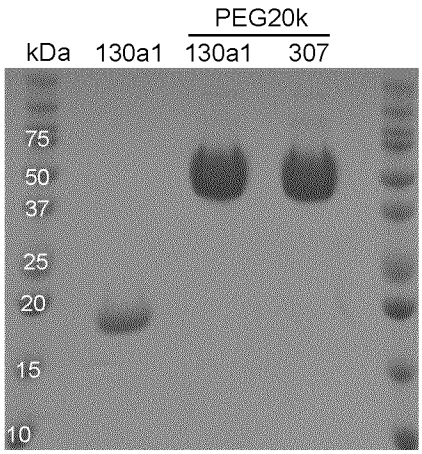


Figure 91. Western blots of human T cells non-transduced or expressing 7/2R-1.


PEG20k
kDa
130a1
130a1
307
75
50
37
25
20
15
10

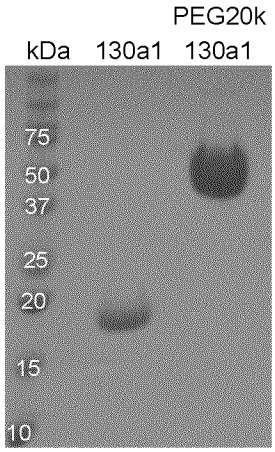

PEG20k
kDa
130a1
130a1
75
50
37
25
20
15
10

Fig. 92

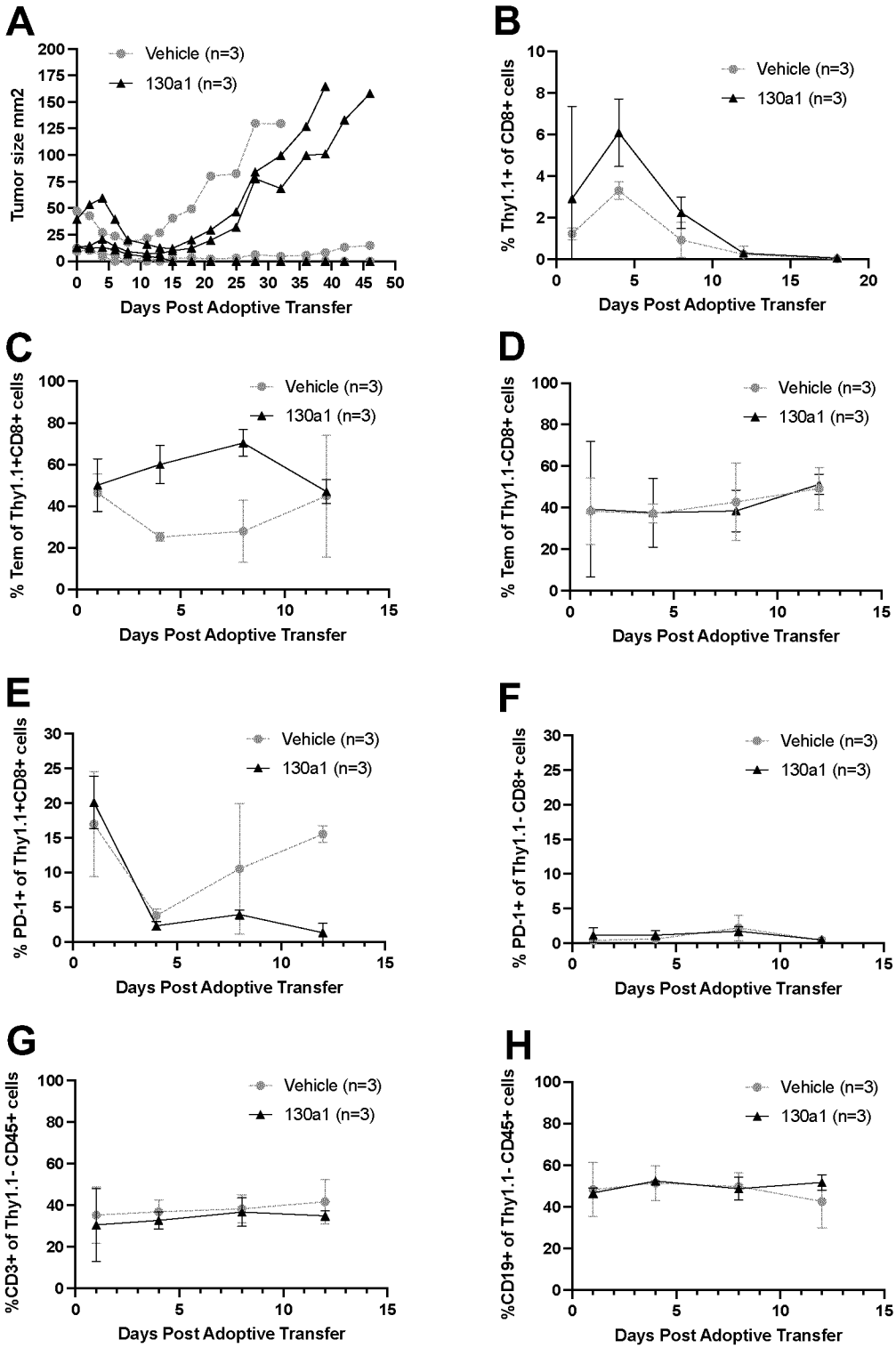

A
Tumor size mm2
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
B
% Thy1.1+ of CD8+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
C
% Tem of Thy1.1+CD8+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
D
% Tem of Thy1.1-CD8+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
E
% PD-1+ of Thy1.1+CD8+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
F
% PD-1+ of Thy1.1- CD8+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
G
%CD3+ of Thy1.1- CD45+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer
H
%CD19+ of Thy1.1- CD45+ cells
Vehicle (n=3)
130a1 (n=3)
Days Post Adoptive Transfer

Fig. 93

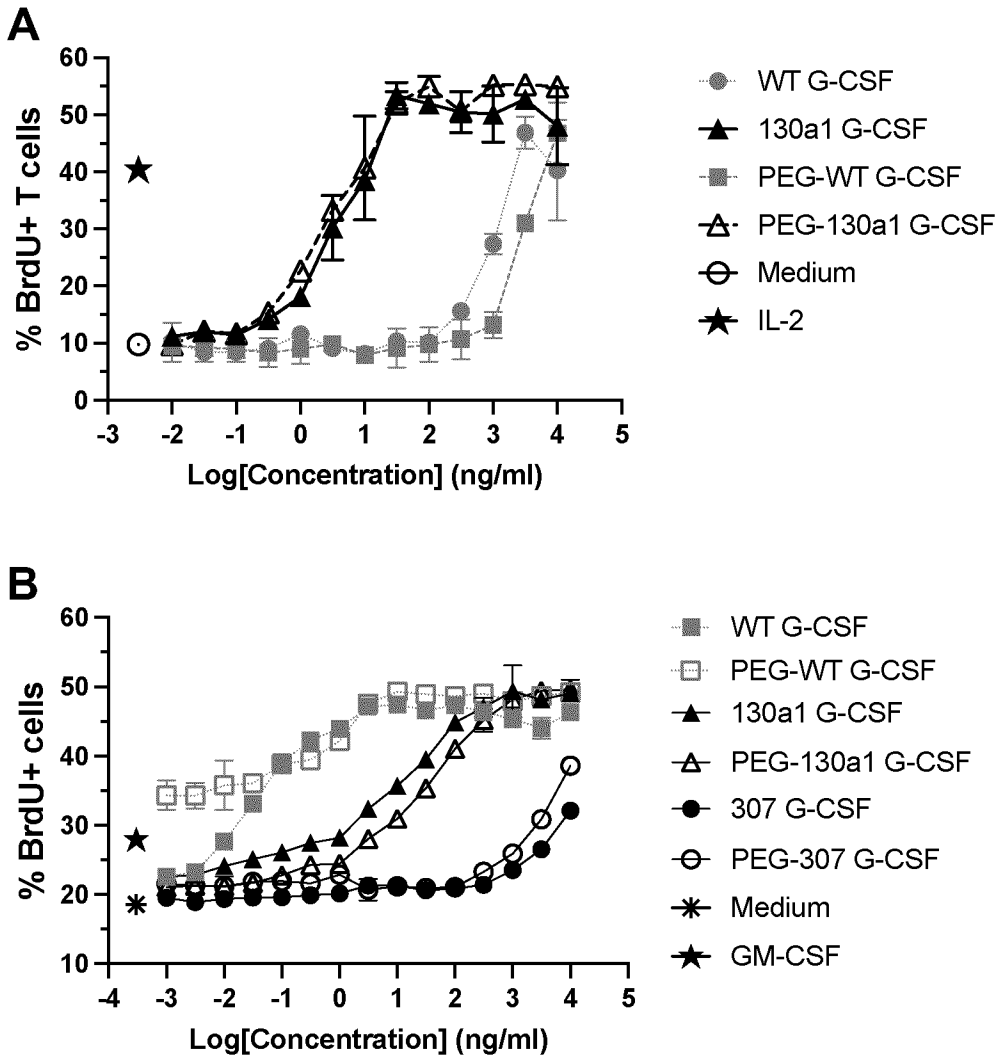

A
% BrdU+ T cells
60
50
40
30
20
10
0
-3
-2
-1
0
1
2
3
4
5
Log[Concentration] (ng/ml)
WT G-CSF
130a1 G-CSF
PEG-WT G-CSF
PEG-130a1 G-CSF
Medium
IL-2
B
% BrdU+ cells
60
50
40
30
20
10
-4
-3
-2
-1
0
1
2
3
4
5
Log[Concentration] (ng/ml)
WT G-CSF
PEG-WT G-CSF
130a1 G-CSF
PEG-130a1 G-CSF
307 G-CSF
PEG-307 G-CSF
Medium
GM-CSF

Fig. 94

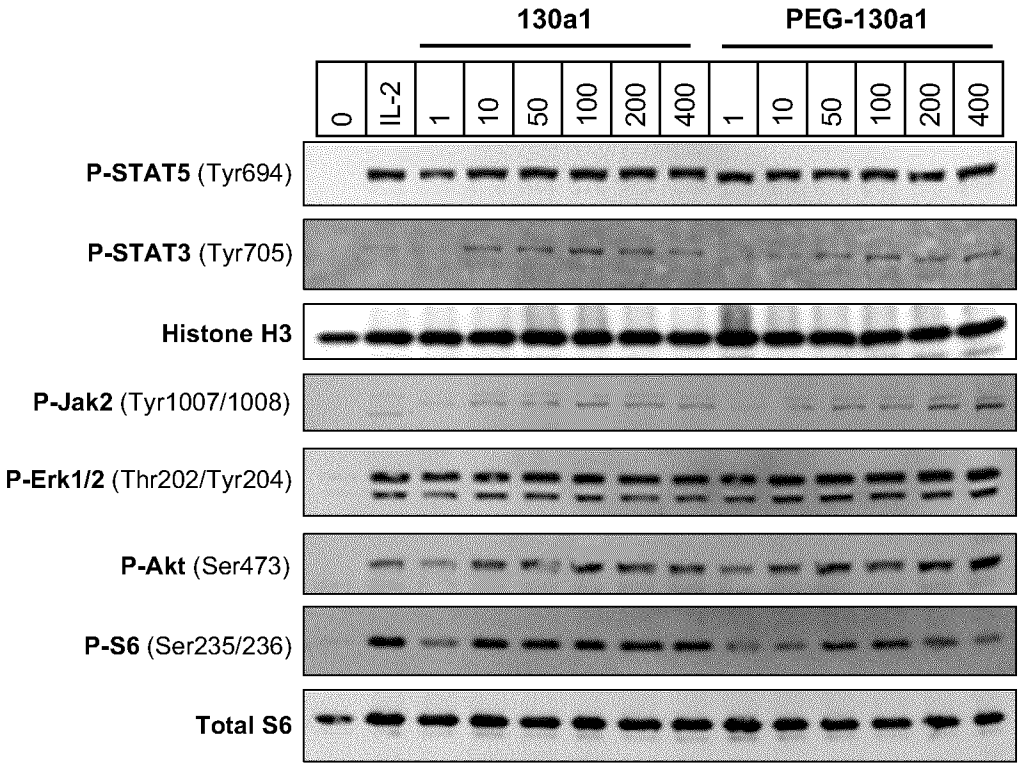

130a1
PEG-130a1
0
IL-2
1
10
50
100
200
400
1
10
50
100
200
400
P-STAT5 (Tyr694)
P-STAT3 (Tyr705)
Histone H3
P-Jak2 (Tyr1007/1008)
P-Erk1/2 (Thr202/Tyr204)
P-Akt (Ser473)
P-S6 (Ser235/236)
Total S6

Fig. 95

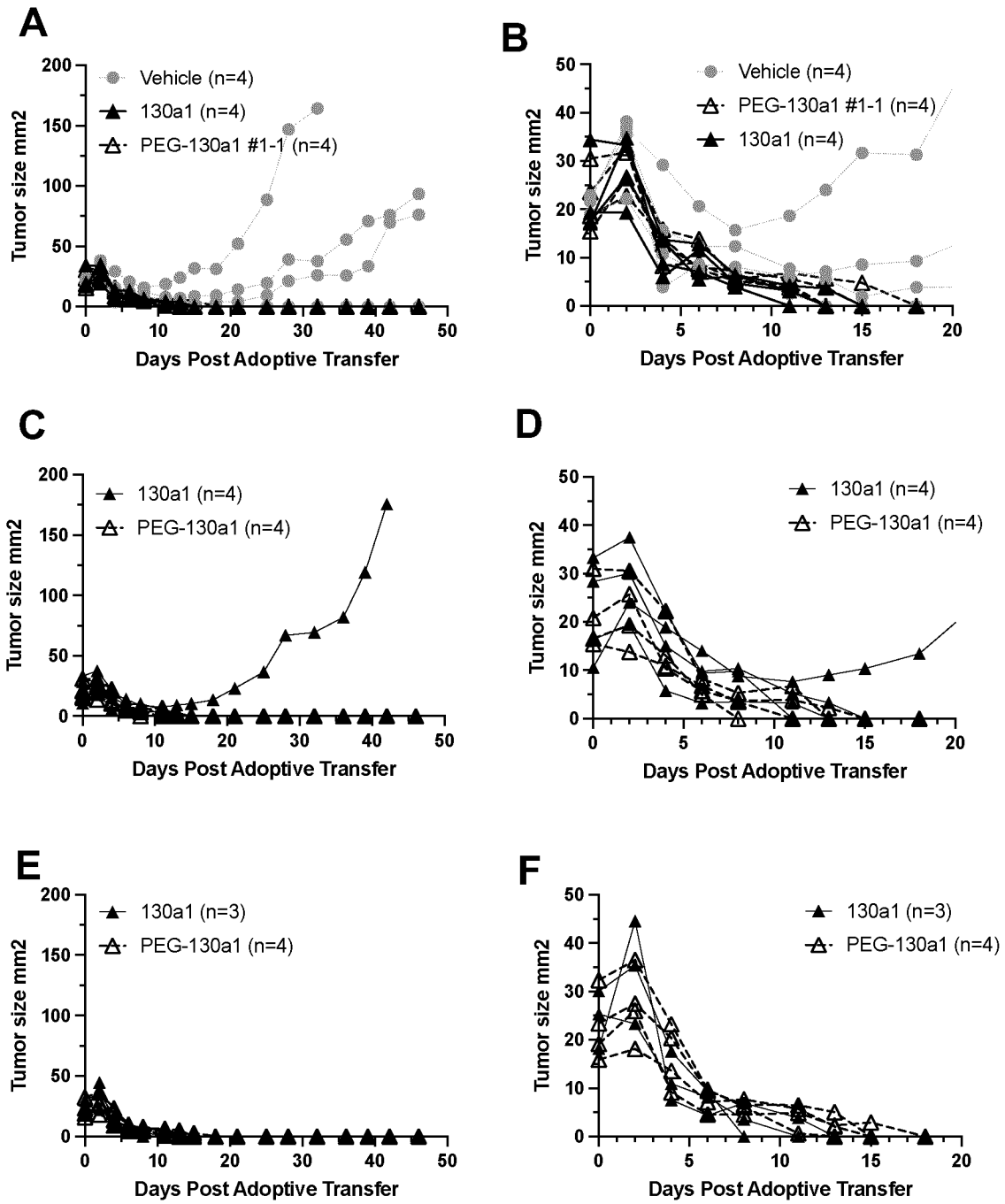

A
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 #1-1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
B
Vehicle (n=4)
PEG-130a1 #1-1 (n=4)
130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
C
130a1 (n=4)
PEG-130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
D
130a1 (n=4)
PEG-130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
E
130a1 (n=3)
PEG-130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
F
130a1 (n=3)
PEG-130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer

Fig. 96

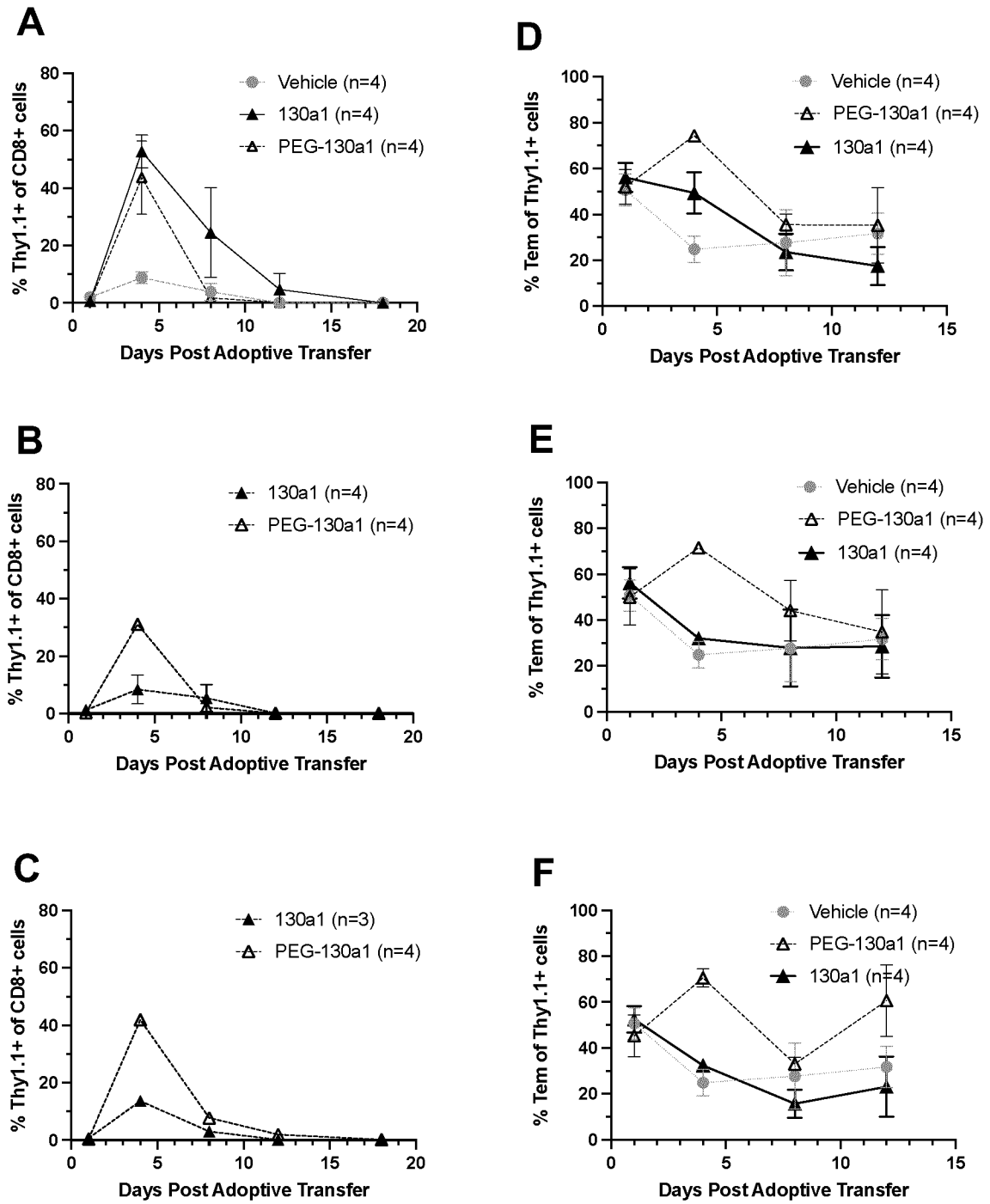

A
% Thy1.1+ of CD8+ cells
Days Post Adoptive Transfer
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
B
% Thy1.1+ of CD8+ cells
Days Post Adoptive Transfer
130a1 (n=4)
PEG-130a1 (n=4)
C
% Thy1.1+ of CD8+ cells
Days Post Adoptive Transfer
130a1 (n=3)
PEG-130a1 (n=4)
D
% Tem of Thy1.1+ cells
Days Post Adoptive Transfer
Vehicle (n=4)
PEG-130a1 (n=4)
130a1 (n=4)
E
% Tem of Thy1.1+ cells
Days Post Adoptive Transfer
Vehicle (n=4)
PEG-130a1 (n=4)
130a1 (n=4)
F
% Tem of Thy1.1+ cells
Days Post Adoptive Transfer
Vehicle (n=4)
PEG-130a1 (n=4)
130a1 (n=4)

Fig. 97

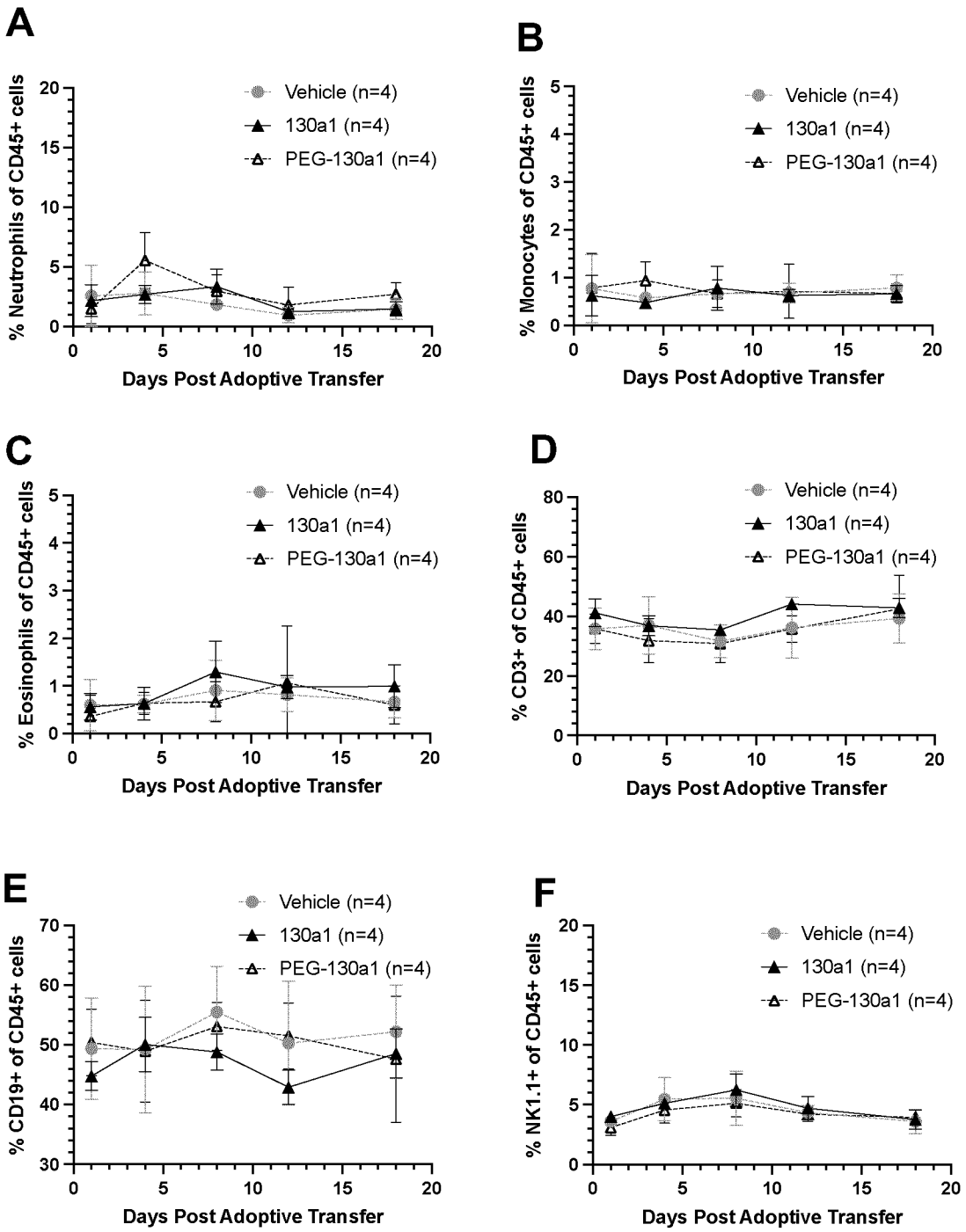

A
% Neutrophils of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer
B
% Monocytes of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer
C
% Eosinophils of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer
D
% CD3+ of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer
E
% CD19+ of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer
F
% NK1.1+ of CD45+ cells
Vehicle (n=4)
130a1 (n=4)
PEG-130a1 (n=4)
Days Post Adoptive Transfer

Fig. 98

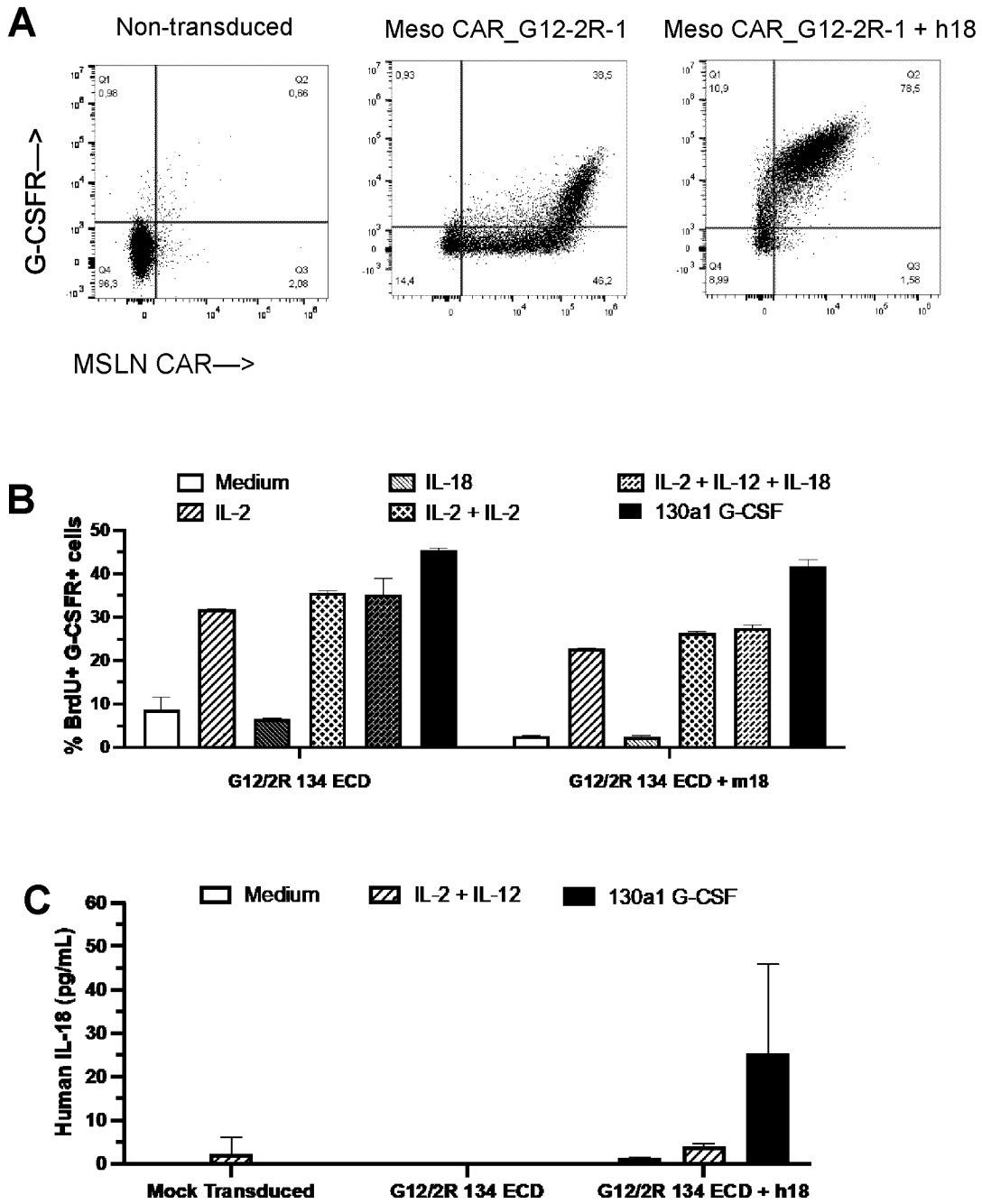

A
Non-transduced
Meso CAR_G12-2R-1
Meso CAR_G12-2R-1 + h18
G-CSFR—>
MSLN CAR—>
B
Medium
IL-2
IL-18
IL-2 + IL-2
IL-2 + IL-12 + IL-18
130a1 G-CSF
% BrdU+ G-CSFR+ cells
G12/2R 134 ECD
G12/2R 134 ECD + m18
C
Medium
IL-2 + IL-12
130a1 G-CSF
Human IL-18 (pg/mL)
Mock Transduced
G12/2R 134 ECD
G12/2R 134 ECD + h18

Figure 99

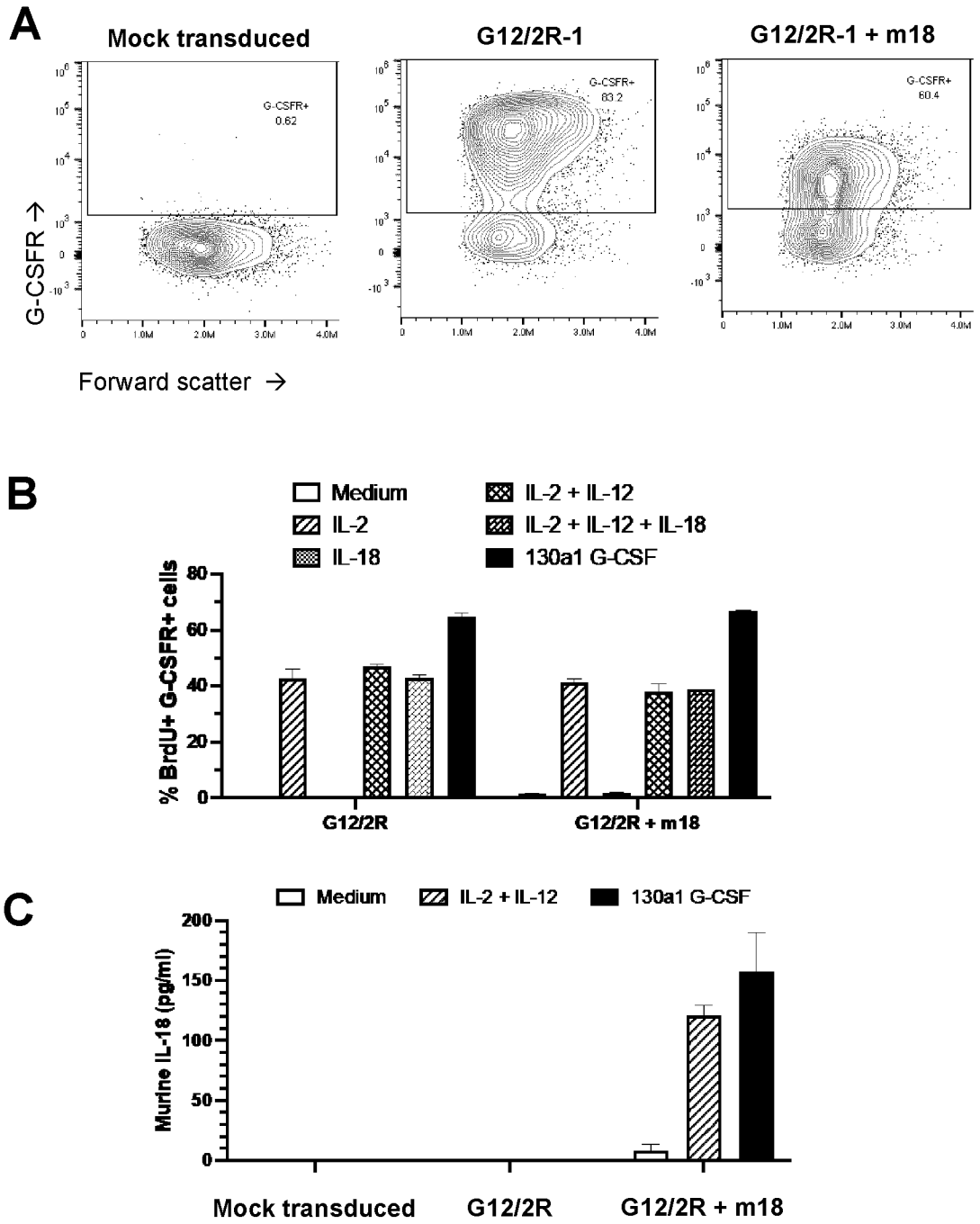
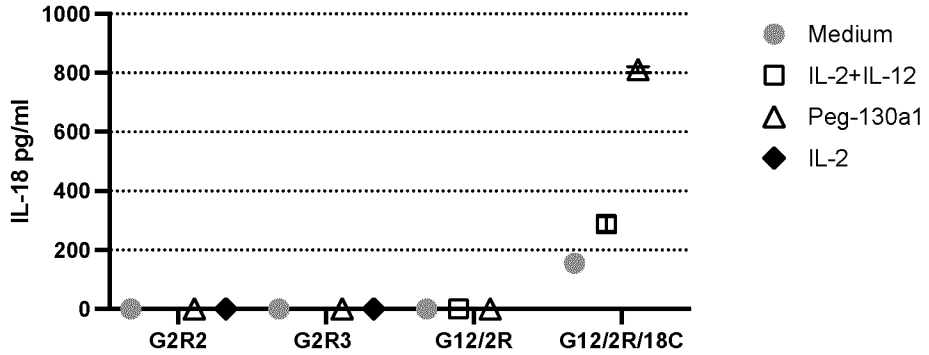

A
Mock transduced
G12/2R-1
G12/2R-1 + m18
G-CSFR+
0.62
G-CSFR+
83.2
G-CSFR+
60.4
G-CSFR →
Forward scatter →
B
Medium
IL-2
IL-18
IL-2 + IL-12
IL-2 + IL-12 + IL-18
130a1 G-CSF
% BrdU+ G-CSFR+ cells
G12/2R
G12/2R + m18
C
Medium
IL-2 + IL-12
130a1 G-CSF
Murine IL-18 (pg/ml)
Mock transduced
G12/2R
G12/2R + m18

Figure 100


1000
800
600
400
200
0
IL-18 pg/ml
G2R2
G2R3
G12/2R
G12/2R/18C
Medium
IL-2+IL-12
Peg-130a1
IL-2

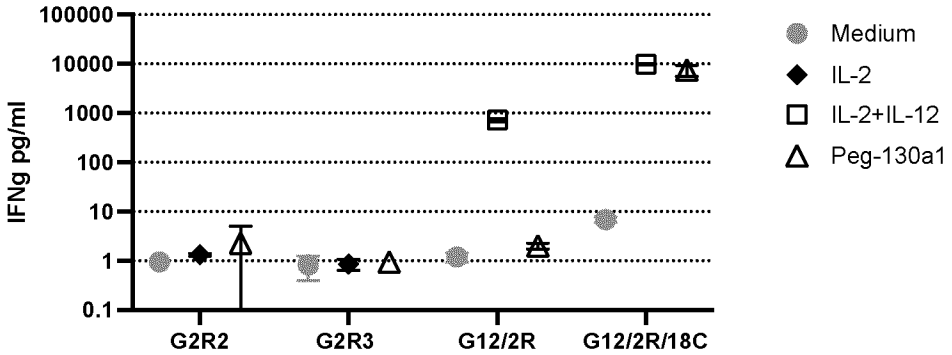

100000
10000
1000
100
10
1
0.1
IFNg pg/ml
G2R2
G2R3
G12/2R
G12/2R/18C
Medium
IL-2
IL-2+IL-12
Peg-130a1

Fig. 101

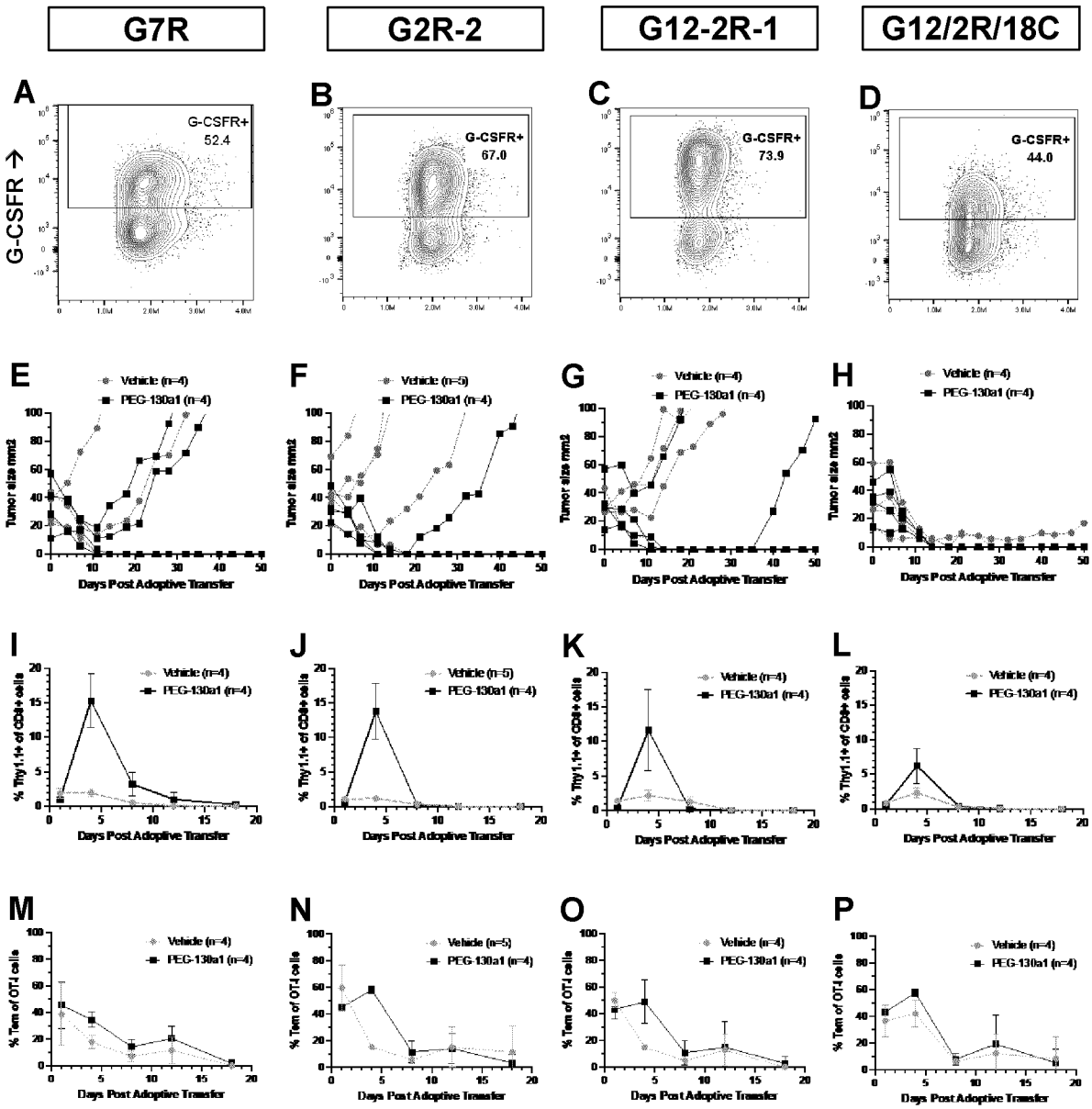

G7R
G2R-2
G12-2R-1
G12/2R/18C
A
B
C
D
G-CSFR →
G-CSFR+
52.4
G-CSFR+
67.0
G-CSFR+
73.9
G-CSFR+
44.0
E
F
G
H
Vehicle (n=4)
PEG-130a1 (n=4)
Vehicle (n=5)
PEG-130a1 (n=4)
Tumor size mm2
Days Post Adoptive Transfer
I
J
K
L
% Thy1.1+ of CD8+ cells
M
N
O
P
% Tem of OT-I cells

Figure 102

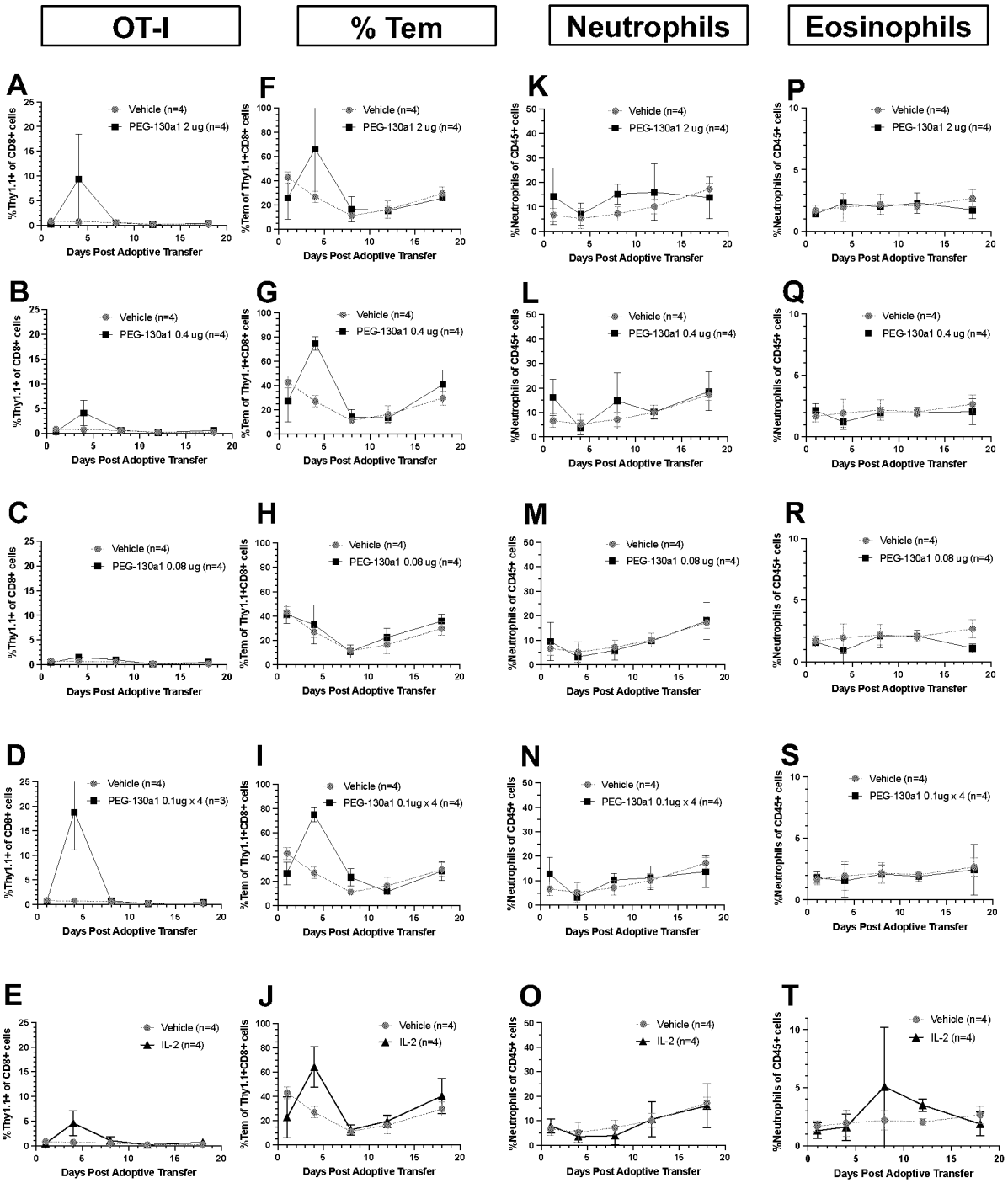

OT-I
% Tem
Neutrophils
Eosinophils
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
Vehicle (n=4)
PEG-130a1 2 ug (n=4)
PEG-130a1 0.4 ug (n=4)
PEG-130a1 0.08 ug (n=4)
PEG-130a1 0.1ug x 4 (n=3)
PEG-130a1 0.1ug x 4 (n=4)
IL-2 (n=4)
%Thy1.1+ of CD8+ cells
%Tem of Thy1.1+CD8+ cells
%Neutrophils of CD45+ cells
Days Post Adoptive Transfer

Fig. 103

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2022/050540

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 19/00*** (2006.01) , ***A61K 35/14*** (2015.01) , ***A61K 38/19*** (2006.01) , ***A61K 38/20*** (2006.01) , ***A61P 25/28*** (2006.01) , ***A61P 35/00*** (2006.01) , ***A61P 37/04*** (2006.01) , ***A61P 37/06*** (2006.01) , ***C07K 14/535*** (2006.01) , ***C07K 14/705*** (2006.01) , ***C07K 14/715*** (2006.01) , ***C12N 5/07*** (2010.01) , ***C12N 5/10*** (2006.01) , ***C12N 15/27*** (2006.01) , ***C12N 15/62*** (2006.01).

CPC: A61K 35/14 (2020.01) , A61K 38/193 (2020.01) , A61K 38/2046 (2020.01) , A61P 25/28 (2020.01) , A61P 35/00 (2020.01) , A61P 37/04 (2020.01) , A61P 37/06 (2020.01) , C07K 14/535 (2020.01) , C07K 14/705 (2020.01) , C07K 14/715 (2020.01) , C07K 14/7153 (2020.01) , C12N 5/10 (2020.05) , C07K 2319/32 (2020.01).

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07K 19/00, A61K 35/14, A61K 38/19, A61K 38/20, A61P 25/28, A61P 35/00, A61P 37/04, A61P 37/06, C07K 14/535, C07K 14/705, C07K 14/715, C12N 5/07, C12N 5/10, C12N 15/27, C12N 15/62.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent Database, Scopus, CAPlus, MEDLINE, BIOSIS, GenomeQuest, Genbank, Protein Data Bank, Genpept.
Keywords: granulocyte colony stimulating factor, g-csf, variant, mutant, chimeric receptor, site II interface, T cell, Provincial Health Services, UVIC Industry Partnerships, Martin Boulanger, Brad Nelson, Cameron Powell, Megan Fuller, SEQ ID NO: 1, L108R.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	WO 2021/068068 A1 (NELSON et al.) 15 April 2021 (15-04-2021)	81-92, 100-102, 105-162, 169-177 & 182-225
P, X/ P, Y	WO 2021/068074 A1 (VOLKERS et al.) 15 April 2021 (15-04-2021)	1, 3-8, 10-20, 28-78, 81-92, 100-102, 105-164, 169-179, 182-225, 278, 279 & 289-303
A	US 5747292 (GREENBERG & NELSON) 05 May 1998 (05-05-1998)	31-78, 81-92, 100-102, 105-158, 160, 161, 169-176, 184-186, 188-193, 195-199 & 202-225

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D"	document cited by the applicant in the international application	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E"	earlier application or patent but published on or after the international filing date	"&"	document member of the same patent family
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 June 2022 (13-06-2022)	Date of mailing of the international search report 16 June 2022 (16-06-2022)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Serisha Moodley (819) 353-9056

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAYTON et al., "*Interaction of Granulocyte Colony-stimulating Factor (G-CSF) with Its Receptor*". The Journal of Biological Chemistry, 18 June 1999 (18-06-1999), Vol. 274(25), pp. 17445-17451, [online] [retrieved on 20 April 2022 (20-04-2022)]. Retrieved from the Internet: <doi: 10.1074/jbc.274.25.17445.>	1, 3-8, 10-20, 28-78, 81-92, 100-102, 105-164, 169-179 & 182-225
A	KOAY et al., "*Distinct region of the granulocyte colony-stimulating factor receptor mediates proliferative signaling through activation of Janus kinase 2 and p44/42 mitogen-activated protein kinase*". Cellular Signalling, 21 January 2002 (21-01-2002), Vol. 14(3), pp. 239-247, [online] [retrieved on 13 May 2022 (13-05-2022)]. Retrieved from the Internet: <https://doi.org/10.1016/S0898-6568(01)00237-6>	1, 3-8, 10-20, 28-78, 81-92, 100-102, 105-164, 169-179 & 182-225
A	WO 2019/169290 A1 (NAGER et al.) 06 September 2019 (06-09-2019)	31-78, 81-92, 100-102, 105-158, 160, 161, 169-176, 184-186, 188-193, 195-199 & 202-225

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
The claims are directed to a plurality of inventive concepts as follows:

Continued on extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

1, 3-8, 10-20, 28-78, 81-92, 100-102, 105-164, 169-179, 182-225, 278, 279 and 289-303 with respect to the first invention directed to a variant G-CSF comprising the site II mutant L108R, which binds to a specific chimeric receptor comprising a corresponding ECD variant of G-CSFR and forms a system for selective activation of a T cell with IL-18.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of: Box No. III

Reference is made to the following documents:

D1 US 5747292 (GREENBERG & NELSON) 5 May 1998 (05-05-1998).

D2 LAYTON et al., THE JOURNAL OF BIOLOGICAL CHEMISTRY, 18 June 1999 (18-06-1999), Vol. 274(25), pp. 17445-17451, [online] [retrieved on 20 April 2022 (20-04-2022)].

D1 discloses chimeric receptors comprised of an extracellular domain (ECD) from an alternative cytokine joined to a transmembrane domain (TM) and an intracellular domain (ICD) of a cytokine that enable activation of cytotoxic T lymphocytes in response to the alternative cytokine rather than the normally required cytokine (columns 4-6). Cytokines include both Granulocyte Colony-Stimulating Factor receptor (G-CSFR) and interleukin 7 receptor (IL-7R) (columns 9-10).

D2 discloses G-CSF variants comprised of mutations at E46 in the site III interface region and/ or D 112 in the site II interface region and a GCSFR R288A variant, as well as chimeric receptors comprised of said G-CSFR variant to determine binding of G-CSF variants to the cytokine receptor homologous module (CRHM) of the receptor (pages 17445-17446 and 17449-17450).

Lack of Unity
The claims are directed to a plurality of inventive concepts as follows:

Group A+ - Claims 1-78 and 155-225 (wholly) and 81-92, 100-102, 105-154, 278,279 and 289-303 (partially) are directed to a variant Granulocyte Colony-Stimulating Factor (G-CSF) comprising at least one mutation in a site II interface region selected from Ll08R, Dll2R, El22R, El22K, El23K and El23R and combinations thereof relative to the amino acid sequence of SEQ ID NO: 1 or at least one mutation in a site III interface region selected from E46R relative to the amino acid sequence of SEQ ID NO: 1 or combinations of the site II and site III interface region mutations recited above, and wherein the variant G-CSF binds selectively to a chimeric receptor comprising a variant extracellular domain (ECD) of G-CSF receptor (G-CSFR), wherein the variant G-CSF and chimeric receptor form a system with at least one additional agonistic or antagonistic signaling protein(s) as recited in claim 158 and/or at least one antigen binding signaling receptor(s) as recited in claim 160 for selective activation of a cell, as well as related subject matter;

Group B+ - Claims 79, 80, 93-99, 103 and 104 (wholly) and 81-92, 100-102 and 105-154 (partially) are directed to a chimeric receptor comprising an ECD of G-CSFR, gp130, EPOR or Interferon Gamma Receptor 2 (IFNyR2) linked to an intracellular domain (ICD), comprising at least one intracellular domain signaling molecule binding site of a cytokine receptor selected from the group as recited in claim 79, and a transmembrane domain (TMD) that is C-terminal to the ECD and N-terminal to the ICD, wherein the chimeric receptor is not comprised of a variant ECD of G-CSFR, as well as related subject matter;

Group C+ - Claims 226-247, 249-277 and 280-288 (wholly) and 278,279 and 289-303 (partially) are directed to a chimeric receptor comprising an ECD and TMD of interleukin-7 receptor alpha (IL-7Ra) and an ICD comprised of a intracellular signaling domain of any one cytokine receptor as recited in claim 23 3 that is distinct from the IL-7Ra intracellular signaling domain as set forth in SEQ ID NO: 109, wherein the ECD and TMD are each operatively linked to the ICD, as well as related subject matter.

(+)=plurality of groups.

Please note that the use of the expression "any one of the above claims" is understood to refer to the first independent claim immediately prior to the dependent claim which features the expression.

Each group of Group A+ relates to a single G-CSF variant that is comprised of either one site II mutant as recited in claim 1 or the site III mutant E46R or a combination of site II and site III mutants forming 127 possible combinations, wherein each G-CSF variant binds to a chimeric receptor comprising a corresponding variant ECD of G-CSFR. Further, each group relates to a system comprised of the G-CSF variant that binds to the chimeric receptor comprising the corresponding variant ECD of G-CSFR and at least one additional signaling protein of claim 158 and/or at least one antigen binding signaling receptor of claim 160. As the claims refer to "combinations thereof', at least 133 169 025 different combinations of systems for selective activation of a cell are possible. It should be noted that group A+ relates to a larger number of claims than some inventions encompassed by group A+ due to the plurality of possible combinations.

Further, the ISA206 (lack of unity) was mailed before receipt of a sequence listing (SL, requested by ISA225). Upon receipt of the SL, it was determined that group A1 does not relate to all of the claims of group A+. Consequently, group A1 relates to claims 1, 3-8, 10-20, 28-78, 81-92, 100-102, 105-164, 169-179, 182-225, 278, 279 and 289-303 (partially) featuring a variant G-CSF comprising the site II mutant L108R, which binds to a specific chimeric receptor comprising a corresponding ECD variant of G-CSFR and forms a system for selective activation of a T cell with IL-18.

Continued on next page.

Continuation of: Box No. III

Each group of Group B+ relates to a chimeric receptor comprised of an ECD and a TMD of any one cytokine linked to an ICD of any one intracellular domain signaling molecule binding site of a cytokine receptor as recited in claim 79, wherein the chimeric receptor is not comprised of a variant ECD of G-CSFR. As the claims refer to "combinations thereof', at least 122 865 different combinations of chimeric receptors are possible. Thus, group Bl would feature a chimeric receptor comprised of the ECD and TM of wildtype G-CSFR and an ICD comprised of a SHC binding site of interleukin (IL)-2Rβ and the Box 1 and Box 2 region of G-CSFR.

Each group of Group C+ relates to a chimeric receptor comprising an ECD and TMD of interleukin-7 receptor alpha (IL-7Ra) and an ICD comprised of a intracellular signaling domain of any one cytokine receptor as recited in claim 233 that is distinct from the IL-7Ra intracellular signaling domain as set forth in SEQ ID NO: 109. As the claims refer to "combinations thereof', at least 63 different combinations of systems for selective activation of a cell are possible. Thus, group C1 would feature a chimeric receptor comprising an ECD and TMD of interleukin-7 receptor alpha (IL-7Ra) and an ICD comprised of a intracellular signaling domain of IL-2Rβ.

Groups A-C relate to structurally distinct cytokine variants, chimeric cytokine receptors and/or systems comprised of said cytokine variants and chimeric receptors for selective activation of a cell. Further cytokine variants and chimeric receptors comprised of ECDs and ICDs of various cytokine receptors and/or variants, including G-CSFR and IL-7Ra, for selective activation of cytokine signalling in cells appear to be known (see D1 and D2), and the subject-matter of the claims is not linked as to form a single general inventive concept that defines a contribution over the prior art. Therefore the claims lack unity *a priori* and *a posteriori.*

Please note that there does not appear to be support for all the possible embodiments.

The claims must be limited to one inventive concept as set out in PCT Rule 13.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2022/050540

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
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