

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

04 March 2021 (04.03.2021)



(10) International Publication Number

WO 2021/041806 A1

(51) International Patent Classification:

A61P 43/00 (2006.01)

C07K 14/715 (2006.01)

C07K 14/71 (2006.01)

(21) International Application Number:

PCT/US2020/048402

(22) International Filing Date:

28 August 2020 (28.08.2020)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/894,658

30 August 2019 (30.08.2019)

US

63/053,322

17 July 2020 (17.07.2020)

US

(71) Applicant: ALLOGENE THERAPEUTICS, INC.

[US/US]; 210 E. Grand Avenue, South San Francisco, California 94080 (US).

(72) Inventors: LIN, Regina Junhui; c/o Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, California 94080 (US).

VAN BLARCOM, Thomas, John; c/o Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, California 94080 (US). PANOWSKI, Sil-
er; c/o Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, California 94080 (US). LANG, Shan-
shan; c/o Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, California 94080 (US). SASU,
Barbra Johnson; c/o Allogene Therapeutics, Inc., 210 E. Grand Avenue, South San Francisco, California 94080 (US).

(74) Agent: CHANG, Y., Elaine et al.; Allogene Therapeutics, Inc., Legal IP, 210 East Grand Avenue, South San Francisco, California 94080 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: CHIMERIC CYTOKINE RECEPTORS COMPRISING TGF BETA BINDING DOMAINS

(57) Abstract: Provided herein are chimeric cytokine receptors bearing a binding domain capable of binding a TGF- β ligand or a TGF- β receptor antibody. When present on chimeric antigen receptor (CAR)-bearing immune cells (CAR-T-cells), such receptors allow for increased CAR-T cell expansion, activity and persistence, constitutively and/or through engagement of a TGF- β ligand or a TGF- β receptor antibody. Also provided are methods of making and using the chimeric cytokine receptors described herein.



WO 2021/041806 A1

CHIMERIC CYTOKINE RECEPTORS COMPRISING TGF BETA BINDING DOMAINS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Application No. 62/894,658, filed on August 30, 2019; and U.S. Provisional Application No. 63/053,322, filed on July 17, 2020, the contents of both of which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

[0002] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "AT-030_03WO_SL.txt" created on August 27, 2020, and having a size of 576,780 bytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

BACKGROUND

[0003] Adoptive transfer of immune cells (e.g. T-cells) genetically modified to recognize malignancy-associated antigens is showing promise as a new approach to treating cancer. For example, T-cells can be genetically modified to express chimeric antigen receptors (CARs), which are fusion proteins comprised of an antigen recognition moiety and T-cell activation domains.

[0004] T-cell proliferation, cytotoxic potency and persistence is driven by signal transduction pathways. Conventional CAR designs provide two signals – CD3zeta activation (Signal 1) and co-stimulation (Signal 2, e.g. via 4-1BB, OX40, and/or CD28 expression). In some contexts, a third signal (Signal 3), cytokine-induced cytokine receptor signaling (e.g. cytokine support for immune potentiation), may be desirable. Approaches to provide Signal 3 have however been met with significant limitations.

[0005] One approach to provide cytokine support includes combining CAR-T-cell therapy with systemic infusions of recombinant cytokines/cytokine mimetics, and engineering CAR-T-cells to secrete/express cytokines extracellularly. As cytokines have pleiotropic effects and

can also impact the function of other cell types, the systemic administration or production of immune-potentiating cytokines by CAR-T-cells have at least two major drawbacks: (i) these approaches can cause systemic toxicity in humans, and (ii) in the context of allogeneic CAR-T-cell therapy, these approaches may cause bystander host immune-activation that could accelerate the rejection of allogeneic CAR-T-cells, thereby compromising therapeutic efficacy. Another approach to provide cytokine support was based on introducing a constitutively activated dimerized cytokine receptor, an IL-7Ra – this limits the nature (IL-7 signaling only) and magnitude of signaling output. Yet another approach to provide cytokine support involved incorporating Signal 3 directly into the CAR molecule (Nat Med. 2018 Mar;24(3):352-359.). A limitation of this approach is that the strength of Signal 3 is dependent on the strength of CAR activation. In the absence of target (and CAR activation), Signal 3 would not be transduced.

[0006] Needed are solutions to circumvent these drawbacks by targeting cytokine signals specifically to CAR-T-cells in a context-dependent manner, thus allowing for an improved safety profile and therapeutic efficacy. Provided herein are compositions and methods that address this need.

SUMMARY

[0007] Provided herein are chimeric cytokine receptors comprising TGF- β binding domains. Provided herein are inducible TGF- β -driven chimeric cytokine receptors, active when engaged with a ligand of the transforming growth factor beta cytokine family (TGF- β ligands, e.g., TGF- β 1, TGF- β 2, and TGF- β 3) or activation with an anti-TGF- β -receptor antibody. When present on chimeric antigen receptor (CAR)-bearing immune cells, and engaged with TGF- β ligands and/or activation with an anti-TGF- β R antibody, such receptors allow for increased cytokine receptor signaling (Signal 3), leading to increased immune cell activation, proliferation, persistence, and/or potency of the CAR-bearing immune cells. Accordingly, the chimeric cytokine receptors of the disclosure allow for cytokine signals to be transmitted into the immune cell with endogenous TGF- β ligands, whereby blocking their immune-suppressive signals, and converting them into immune-potentiating signals that can work in concert with, or synergize, CAR-driven activity. Moreover, as clinically approved anti-TGF- β receptor antibodies can cluster and activate the chimeric cytokine receptors of the disclosures, patients treated with anti-TGF- β receptor may benefit not only from the blockage

of the endogenous TGF- β signaling, but from also the activation of cytokine signaling in cells bearing the chimeric cytokine receptors. Also provided herein are constitutively active TGF- β -driven of TGF- β binding domain -containing chimeric cytokine receptors; such receptors continue to signal in the absence of an inducer, but can be further induced or can exhibit further improved properties or activities, for example, in the presence of a TGF- β ligand or an anti-TGF- β R antibody. In some embodiments, the TGF- β R is TGF- β R2, and the antibody is an anti-TGF- β R2 antibody. As used herein, "TGF-beta" is used interchangeably with "TGF- β ."

[0008] Accordingly, in one aspect, provided herein is a chimeric cytokine receptor comprising: (a) a binding domain comprising an extracellular portion of a TGF- β receptor, or a TGF- β antigen binding domain; (b) a transmembrane domain; (c) a Janus Kinase (JAK)-binding domain; and (d) a recruiting domain. As used herein, "extracellular portion" refers to any portion of an extracellular domain of a TGF- β receptor.

[0009] In a related aspect provided herein is a polynucleotide encoding any one of the chimeric cytokine receptors of the disclosure, and an expression vector comprising such a polynucleotide. In some embodiments, the polynucleotide further encodes for a chimeric antigen receptor (CAR), wherein the CAR binds to a target of interest. The target of interest can be any molecule of interest, including, for example, without limitation any one or more of those presented in Table 8.

[0010] In a further aspect, provided herein is an engineered immune cell comprising at least one chimeric cytokine receptor of the disclosure. In another aspect, provided herein is an engineered immune cell comprising at least one chimeric antigen receptor (CAR) and at least one chimeric cytokine receptor of the disclosure. In some embodiments the immune cell is a T-cell. In some embodiments the immune cell is an allogeneic immune cell. In other embodiments, the immune cell is an autologous immune cell. The immune cell may be selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell. In a related aspect, provided herein is a pharmaceutical composition comprising any of the engineered immune cells of the disclosure, and a kit comprising such a pharmaceutical composition. Also provided herein is a method of making the immune cell.

[0011] In another aspect, provided herein is a method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of any of the engineered immune cells described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0012] FIG. 1 shows a schematic of the inducible chimeric cytokine receptor of the disclosure.

[0013] FIG. 2A shows a schematic of the lentiviral vector used to co-express the dominant negative truncations of the TGF β R1 or TGF β R2 cytokine receptor with the 2nd generation EGFRvIII CAR.

10 [0014] FIG. 2B shows the inhibition of the TGF- β signaling by expression of either the TGF β R1 DN or TGF β R2 DN.

[0015] FIG. 3 shows a general schematic of the lentiviral vector used to co-express the TGF β R2 cytokine receptor with the 2nd generation EGFRvIII CAR.

15 [0016] FIG. 4A shows a schematic of the prototypic lentiviral vector used, bearing the IL7R(316-459) and the IL12Rb2(775-825) recruiting domains to mimic IL7 and IL12 signaling in CAR-T-cells.

[0017] FIG. 4B shows TGF- β signaling activity determined by a luciferase reporter assay.

[0018] FIG. 4C shows the activation of the chimeric cytokine receptors of FIG. 4A in the presence of TGF- β .

20 [0019] FIG. 5A shows a schematic of the prototypic lentiviral vector used, having truncations in the binding domain.

[0020] FIG. 5B shows TGF- β signaling activity determined by a luciferase reporter assay.

[0021] FIG. 5C shows the activation of the chimeric cytokine receptors of FIG. 5A in the presence of TGF- β .

25 [0022] FIG. 6A shows a schematic of the modification introduced into the TGF β R2 cassette.

[0023] FIG. 6B shows that the TGF β R2 Δ N25 chimeric cytokine receptors still retained the ability to inhibit TGF- β signaling.

[0024] FIG. 6C shows the activation of the chimeric cytokine receptors of FIG. 6A, evaluated by the STAT reporter activity.

5 [0025] FIG. 7 shows a schematic of the constitutively active chimeric cytokine receptor.

[0026] FIG. 8A shows a schematic of the modification introduced into the TGF β R2 cassette.

[0027] FIG. 8B shows the inhibition of TGF- β signaling by the expression of the chimeric cytokine receptors of FIG. 8A.

10 [0028] FIG. 8C shows the activation of cytokine signaling via the chimeric cytokine receptors of FIG. 8A, measured by the STAT5 reporter activity.

[0029] FIGS. 9A-9B show the amino acid sequences for the wild type TPOR and the various transmembrane deletion or insertion variants. FIGS. 9A-B disclose SEQ ID NOS 235-246, 235, and 247-254, respectively, in order of appearance.

15 [0030] FIG. 10A shows the amino acid sequences for the wild type TPOR and additional transmembrane variants. FIG. 10B shows the inhibition of endogenous TGF- β signaling as determined by luciferase assay by the overexpression of TGF- β -driven chimeric cytokine receptors shown in FIG. 10A, in the presence of different concentrations of TGF- β . FIG. 10C shows the activation of chimeric cytokine receptors in the presence of TGF- β at various
20 concentrations. FIG. 10A discloses SEQ ID NOS 235 and 255-271, respectively, in order of appearance.

[0031] FIG. 11A shows schematics of chimeric cytokine receptor (CCR) CAR expression construct where the expression of the CCR and the EGFRvIII CAR are linked by a P2A peptide. FIGs. 11B-11C are bar graphs depicting the yield of CAR⁺ T cells expressing
25 various CCRs. FIGs. 11D-11E show results of STAT5 phosphorylation in CAR T cells expressing various CCR or controls. FIGs. 11F-11G depict CAR T cells phenotype at Day 14 of production. FIG. 11H shows results of total TGF- β R2 extracellular staining on CAR T

cells. FIG. 11I depicts results of inhibition of TGF- β R2 signaling in CAR T cells expressing different CCRs in the presence of different concentrations of TGF- β .

[0032] FIG. 12A exhibits results of cytotoxicity assay of CAR T cells expressing various CCRs against U87-EGFRvIII cells in the absence of exogenous TGF β . FIGs. 12B-12C show results of cytotoxicity assay of CAR T cells expressing various CCRs at different concentrations of TGF β .

[0033] FIG. 13A shows STAT5 phosphorylation and FIG. 13B depicts the T cell phenotype of CAR T cells expressing CCRs with the S505N/W515K with or without the K553R/K573R substitutions in the TOPR/MPLR and JAK binding domain. All TGF β R2 chimeric cytokine receptor constructs tested in this experiment contain the S505N/W515K substitutions. The constructs labeled RR further contain the additional K553R/K573R substitutions.

[0034] FIGs. 14A-14B show results of long-term cell killing assay of CAR T cells expressing various CCRs in the absence (FIG. 14A) or presence (FIG. 14B) of 5ng/ml TGF β .

[0035] FIGs. 15A-15C show results of activation of STAT5 signaling (FIG. 15A), inhibition of TGF β signaling (FIG. 15B), and long-term cell killing assay (FIG. 15C) of CAR T cells expressing various CCRs, some of which have reduced affinity for TGF β (e.g., D32A, E119A and/or I53A substitutions in the ECD of TGF β R2).

[0036] FIGs. 16A-16B compare the effects of TGF β R2 chimeric cytokine receptors with or without the degradation-resistant K533R/K573R substitutions on STAT5 signaling (FIG. 16A) and long-term cell killing (FIG. 16B).

DETAILED DESCRIPTION

[0037] Provided herein are chimeric cytokine receptors comprising TGF- β binding domains. Provided herein are inducible chimeric cytokine receptors, active when engaged with TGF- β ligands (e.g. TGF- β 1, TGF- β 2, and/or TGF- β 3) or activation with an anti-TGF- β -receptor antibody. Also provided herein are constitutively active chimeric cytokine receptors comprising TGF- β binding domains. Also provided herein are chimeric antigen receptor (CAR)-bearing immune cells (CAR-I-cells, e.g. CAR-T-cells), expressing the chimeric cytokine receptors of the disclosure. In some embodiments, the constitutively active

chimeric cytokine receptors exhibit improved properties or activities when engaged with a TGF- β ligand or activation with an anti-TGF- β -receptor antibody, as compared with constitutively active chimeric cytokine receptors without a TGF- β binding domain. Also provided herein are methods of making and using the chimeric cytokine receptors.

5 I. TGF- β -Bearing Chimeric Cytokine Receptors

[0038] The chimeric cytokine receptors of the disclosure activate signaling upon binding of a TGF- β ligand (for example, TGF- β 1, TGF- β 2, and/or TGF- β 3), or an anti-TGF- β -receptor antibody. These receptors activate signaling when monomers of the receptor cluster and/or dimerize. The chimeric cytokine receptors of the disclosure are dual-function chimeric
10 cytokine receptors which can simultaneously neutralize the immune-suppressive effects of a TGF- β ligand, and mimic the transmission of an immune-potentiating cytokine signal.

[0039] In some embodiments, a monomer of the chimeric cytokine receptor of the disclosure comprises: (a) a binding domain capable of binding a TGF- β ligand or an anti-TGF- β -receptor antibody; (b) a transmembrane domain; (c) a Janus Kinase (JAK)-binding
15 domain; and; (d) a STAT-recruiting domain (e.g. from the cytoplasmic domain of a receptor; e.g. from a cytokine receptor). Each domain can be linked either directly or via one or more peptide linkers. In some embodiments, a monomer of the chimeric cytokine receptor of the disclosure comprises: (a) a binding domain capable of binding a TGF- β ligand or an anti-TGF- β -receptor antibody; (b) a transmembrane domain; (c) a Janus Kinase (JAK)-binding
20 domain; and; (d) a recruiting domain (e.g. from the cytoplasmic domain of a receptor; e.g. from a cytokine receptor). The recruiting domain can be a STAT-recruiting domain, an AP1—recruiting domain, a Myc/Max recruiting domain; or a NFkB-recruiting domain. In some embodiments, the chimeric cytokine receptors are clustered and activated when they bind to TGF- β ligands, and/or are clustered and activated with an anti-TGF- β -receptor
25 antibody. The chimeric cytokine receptors activate signaling upon for example binding a TGF- β ligand, and/or a TGF- β -receptor antibody. In some embodiments, the TGF- β receptor antibody is, without limitation, PF-03446962 or LY3022859. In some embodiments, the chimeric cytokine receptors are constitutively clustered or dimerized.

[0040] As used herein, “TGF- β ligand,” refers to TGF- β 1, TGF- β 2, and TGF- β 3, and isoforms and derivatives thereof. It should be understood that “TGF- β ligand” and “TGF- β ” are used interchangeably herein.

A. Binding Domains

5 [0041] The chimeric cytokine receptors of the disclosure comprise a binding domain capable of binding a TGF- β ligand or an anti-TGF- β -receptor antibody. As referred to herein, a binding domain is the domain of the chimeric cytokine receptor that extends into the extracellular space. The binding domain binds and sequesters TGF- β away from the endogenous TGF- β receptor, thereby preventing or reducing TGF- β -induced immune-
10 suppression. The binding domains of the disclosure bind with TGF- β ligands and anti-TGF- β -receptor antibodies, leading to binding-induced signal transduction.

[0042] In some embodiments, the binding domain comprises an extracellular portion of a TGF- β receptor, for example the extracellular portion of TGF β R1 or TGF β R2.

15 [0043] In some embodiments, the binding domain comprises an extracellular portion of a wild type TGF β receptor. In some embodiments, the TGF- β receptor comprises one or more mutations that enhance or alter the affinity to the binding to the TGF β ligands.

[0044] In some embodiments, the binding domain comprises the extracellular portion of a wild type TGF β R1 or TGF β R2; in some embodiments, the binding domain comprises the extracellular portion of a wild type TGF β R1 or TGF β R2 and comprises the amino acid
20 sequence of SEQ ID NOS: 2 or 3, respectively.

[0045] In some embodiments, the binding domain comprises mutations to the extracellular portion of a wild type TGF- β receptor. In some embodiments, the binding domain comprises mutations to the extracellular portion of a wild type TGF- β receptor, and comprises the amino acid sequences of any one of SEQ ID NO: 4 to SEQ ID NO: 20. In some embodiments, the
25 chimeric cytokine receptor comprises a binding domain that is at least 80%, 85%, 90%, 95%, 98%, or 99%, or 100% identical to any one of the amino acid sequences of SEQ ID NOS: 4-20. In some embodiments, the binding domain does not comprise a signal sequence.

[0046] Table 1 shows exemplary binding domain amino acid sequences of the disclosure. It is noted that the expression and extracellular location of the exemplary binding domain

sequences, such as TGF- β receptor amino acid sequences, can be achieved with the use of a signal sequence. In an exemplary embodiment, a CD8 signal sequence (CD8SS) MALPVTALLLPLALLHAARP (SEQ ID NO: 1) is utilized. In some embodiments, the binding domain comprises the extracellular domain of wild type TGF β R2 comprising the amino acid sequence of SEQ ID NO:159. In some embodiments, the signal sequence is the endogenous signal sequence of human TGF- β R2.

Table 1: Exemplary Binding Domain Sequences

Binding Domain	Amino acid sequence	SEQ ID NO:
TGF β R1 (1-126)	MEAAVAAPRPRLLLLVLAAAAAAAAAALLPGAT ALQCFCHLCTKDNFTCVTDGLCFVSVTETTDKVI HNSMCIAEIDLIPDRPFVCAPSSKTGSVTTTYCC NQDHCNKIELPTTVKSSPGLGPVEL	2
TGF β R2 (1-166) Underlined TGF β R2 signal sequence	<u>MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNN</u> DMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILED AASPKCIMKEKKKPGETFFM CSCSSDECNDNIIFSEEYNTSNPDLLL VIFQ	3
TGF β R2 Δ N25	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS EEYNTSNPDLLL VIFQ	4
TGF β R2 Δ N25FSE-N8	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS ELLLVIFQ	5
TGF β R2 Δ N25FSE-N6	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS EPDLLLVIFQ	6

Binding Domain	Amino acid sequence	SEQ ID NO:
TGFβR2ΔN25FSE-N5	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFS EEDLLLVIFQ	7
TGFβR2ΔN25FSE-N4	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFS EEYDLLLVIFQ	8
TGFβR2ΔN25FSE-N3	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFS EEYNPDLLLVIFQ	9
TGFβR2ΔN25.Glyc33	QLCKFCD <u>NRT</u> STCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFS EEYNTSNPDLLLVIFQ	10
TGFβR2ΔN25.Glyc40	QLCKFCDVRFSTCDN <u>QT</u> SCMSNCSITSICEKPQEV CVAVWRKNDENITLETVCHDPKLPYHDFILED ASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFSE EYNTSNPDLLLVIFQ	11
TGFβR2ΔN25.Glyc56	QLCKFCDVRFSTCDNQKSCMSNCSITSICENPTEV CVAVWRKNDENITLETVCHDPKLPYHDFILED ASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFSE EYNTSNPDLLLVIFQ	12
TGFβR2ΔN25.Glyc58	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPNET CVAVWRKNDENITLETVCHDPKLPYHDFILED ASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIFSE EYNTSNPDLLLVIFQ	13
TGFβR2ΔN25.V33K	QLCKFCD <u>K</u> RSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED	14

Binding Domain	Amino acid sequence	SEQ ID NO:
	AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS EEYNTSNPDLLL VIFQ	
TGFβR2ΔN25.E70K	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDKKNITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS EEYNTSNPDLLL VIFQ	15
TGFβR2ΔN25.Glyc87	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYH <u>NFT</u> LED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF	16
TGFβR2ΔN25.Glyc89	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDF <u>NLTD</u> AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF	17
TGFβR2ΔN25.Glyc102	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMK <u>NKT</u> KPGETFFMCSCSSDECNDNIIF	18
TGFβR2ΔN25.Glyc123	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNITF	19
TGFβR2ΔN25.I89E	QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLPYHDFELED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF	20
TGFβR2 (without the signal sequence)	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDV RFSTCDNQKSCMSNCSITSICEKPQEVAVWRK NDENITLETVCHDPKLPYHDFILED AASPKCIMKE KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD LL VIFQ	159

[0047] In some embodiments, the chimeric cytokine receptor is a dominant negative (DN) wherein the binding domain of the TGF-β receptor is expressed, but the chimeric cytokine receptor does not comprise an intracellular signaling domain - the chimeric cytokine receptor

can bind TGF- β but does not transmit a positive signal (DN chimeric cytokine receptor). In some embodiments, the TGF- β receptor is TGF β R1 (dominant-negative TGF β R1, or TGF β R1 DN) and comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the TGF- β receptor is TGF β R2 (dominant-negative TGF β R2, or TGF β R2 DN) and comprises the amino acid sequence of SEQ ID NO: 3. TGF- β receptor dominant negative sequences may be expressed with the aid of a signal sequence, e.g. a CD8SS signal sequence of SEQ ID NO: 1. Example schematics of a DN chimeric cytokine receptor are shown in FIG. 2A.

[0048] In other embodiments, the binding domain comprises a TGF- β antigen binding domain. Such antigen binding domains include, but are not limited to, a single chain variable fragment (scFv) that can bind the TGF- β ligands, and single domain antibodies (nanobodies). These scFvs and single domain antibodies may include commercially available scFvs and single domain antibodies, and those derived from, for example, camelid and shark antibodies.

[0049] In other embodiments, the binding domain comprises a TGF- β antigen binding domain, wherein the antigen binding domain comprises a Fab fragment.

B. Transmembrane Domains

[0050] The chimeric cytokine receptors of the disclosure comprise transmembrane domains. Such transmembrane domains are coupled to the extracellular binding domain on the N-terminus, and to additional intracellular/cytoplasmic domains on the C-terminus. In some embodiments, the coupling is achieved optionally through a linker.

[0051] As used herein, the transmembrane domains are capable of insertion into the membrane of a cell in which it is expressed. In some embodiments, the transmembrane domains of the disclosure span a cellular membrane, and comprise an extracellular portion, and/or an intracellular portion.

[0052] In some embodiments, the transmembrane domains of the disclosure are engineered and do not resemble any naturally occurring transmembrane domain, e.g. they are non-naturally occurring.

[0053] In other embodiments, the transmembrane domains of the disclosure are derived from naturally occurring receptors.

[0054] In some embodiments, the transmembrane and/or JAK domains of the disclosure are derived from, for example, one or more of the following receptors: erythropoietin receptor (EpoR), Interleukin 6 signal transducer (GP130 or IL6ST), prolactin receptor (PrlR), growth hormone receptor (GHR), granulocyte colony-stimulating factor receptor (GCSFR), and
5 thrombopoietin receptor/ myeloproliferative leukemia protein receptor (TPOR/MPLR).

When derived from naturally occurring receptors, the entire receptor, or the entire transmembrane sequence of the receptor may not be necessary to effectuate constitutive activation and constitutive JAK binding/activation on the intracellular portion. Accordingly fragments of naturally occurring receptors may be utilized. Furthermore, certain mutations
10 may be introduced into the transmembrane domains derived from naturally occurring receptors, to further tune the downstream JAK-dependent signaling. In some embodiments, the chimeric cytokine receptor of the disclosure comprises a portion or a fragment of a naturally occurring receptor, e.g., the transmembrane and/or JAK binding/activation domain of the naturally occurring receptor, optionally comprising one or more mutations therein (e.g.,
15 one or more deletions, insertions and/or substitutions).

[0055] In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring EpoR receptor.

[0056] In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring GP130 receptor.

20 **[0057]** In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring PrlR receptor.

[0058] In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring GHR receptor.

25 **[0059]** In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring GCSF receptor.

[0060] In some embodiments, the transmembrane and/or JAK domains of the disclosure is derived from the naturally occurring TPOR receptor. When the TPOR transmembrane domain assumes a permissive homodimeric conformation, such as in response to a ligand or forced activation resulting from the introduction of engineered modifications, it is capable of

activating downstream cytokine signaling in a JAK2-dependent fashion. The introduction of various modifications to the TPOR transmembrane domain can result in the following: the immune-potentiating cytokine signal may either be (a) quiescent until induced to activate in the presence of extracellular TGF- β , or (b) constitutively active regardless of TGF- β

5 availability.

[0061] Table 2 provides exemplary full length sequences of naturally occurring receptors provided in the disclosure, from which the transmembrane and/or JAK domains are derived.

Table 2: Exemplary Naturally Occurring Receptors

Naturally Occurring Receptor Name	SEQ ID NO:
>AAI12154.1 Erythropoietin receptor [Homo sapiens] MDHLGASLWPQVGSCLLLAGAAWAPPPNLPDPKFESKAALLAARGPEELLCFTER LEDLVCFWEEAASAGVGPGNYSFSYQLEDEPWKLCRLHQAPTARGAVRFWCSLPT ADTSSFVPLELRVTAASGAPRYHRVIHINEVVLLDAPVGLVARLADESGHVVLRLWL PPPETPMTSHIRYEVDVSAGNGAGSVQRVEILEGRTECVLSNLRGRTRYTF AVRAR MAEPSFGGFWSAWSEPVSLLTPSDLDPLILTL SLILVVILVLLTVLALLSHRRALKQKI WPGIPSESEFEGLFTTHKGNFQLWL YQNDGCLWWSPCTPFTEDPPASLEVL SERCW GTMQAVEPGTDDEGPLLEPVGSEHAQDTYL VLDKWLLPRNPPSEDLPGP GGSVDIV AMDEGSEASSCSSALASKPSPEGASAA SFEYTILDPSSQLLRPWTLCP ELPPTPHLK YLYLVVSDSGISTDYSSGDSQGAQGGLSDGPYSNPYENSLIPAAEPLPPSYVACS	21
>AAI17403.1 Interleukin 6 signal transducer (GP130, oncostatin M receptor) [Homo sapiens] MLTLQTWLVQALFIFLTTESTGELLDP CGYISPESPVVQLHSNFTAVCVLKEKCMDY FHVNANYIVWKTNHFTIPKEQYTIINRTASSVTFTDIASLNIQLTCNILTFGQLEQNVY GITIISGLPPEKPKNLSCIVNEGKKMRCEWDRGRETHLETNFTLKSEWATHKFADCK AKRDTPTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNL S VINSEELSSILKLTWTNPSIKSVIILKYNIQYRTKDASTWSQIPPEDTASTRSSFTVQDL KPFTEYVFRIRCMKEDGKGYWSDWSEEASGITYEDRPSKAPSFYKIDPSHTQGYR TVQLVWKTLPPEANGKILDYEVTLTRWKSHLQNYTVNATKLTVNLTNDRYVATL TVRNLVGKSDAAVLTIPACDFQATHPVMDLKAFPKDNMLWVEWTTPRESVKKYIL	22

Naturally Occurring Receptor Name	SEQ ID NO:
EWCVLSDKAPCITDWQQEDGTVHRTYL RGNLAESKCYLITVTPVYADGPGSPESIK AYLKQAPPSKGPTVRTKKVGKNEAVLEWDQLPVDVQNGFIRNYTIFYRT IIGNETA VNV DSSHTEYTLSSLTSDTLYMVRMAAYTDEGGKDGPEFTFTTPKFAQGE IEAIVVPVCLAFLLTLLGVLF CFNKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRH FNSKDQMYSDGNFTDVS VVEIEANDKKPFPEDLKSLDLFKKEKINTEGHSSGIGGSS CMSSSRPSISSSDENESSQNTSSTVQYSTVVHSGYRHQVPSVQVFSRSESTQPLLDSE ERPEDLQLVDHVDGGDGILPRQQYFKQNC SQHESSPDISHFERSKQVSSVNEEDFVR LKQQISDHISQSCGSGQM KMFQEVSAADAFGPGTEGQVERFETVGM EAATDEGMP KSYLPQTVRQGGYMPQ	
>XP_011512371.1 prolactin receptor isoform X2 [Homo sapiens] MKENVASATVFTLLLFLNTCLLNGQLPPGKPEIFKCRSPNKETFTCWWRPGTDGGLP TNYSLTYHREGETLMHECPDYITGGPN SCHFGKQYTSMWRTYIMMVNATNQMGSS FSDELYVDVTYIVQDP PLELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGWFTLLYEI RLKPEKAAEWEIHFAGQQTEFKILSLHPGQKYL VQVRCKPDHGYWSAWSPATFIQIP SDFTMNDTTVWISVAVLSAVICLIIVWAVALKGYSMVTCIFPPVPGPKIKGFD AHL EKGKSEELLSALGCQDFPPTS DYEDLLVEYLEVDDSEDQHLMSVHSKEHPSQGMKP TYLDPD TD SGRGSCDSPSLLSEKCEEPQANSTFYDPEVIEK PENPETHTHTWDPQCIS MEGKIPYFHAGGSKCSTWPLPQPSQHNP RSSYHNITDVCELAVGPAGAPATLLNEA GKDALKSSQTIKSREEGKATQQREVESFHSETDQDTPWLLPQEKTPFGSAKPLDYVE IHKVNKD GAL SLLPKQRENSGKPKKPGTPENNKEYAKVSGVMDNNILVLVPDPHA KNVACFEESA KEAPPSLEQNQA EKALANFTATSSK CRLQLGGLDYLD PACFTHSFH	23
>NP_000154.1 growth hormone receptor isoform 1 precursor [Homo sapiens] MDLWQLLLTLALAGSSDAFSGSEATAAILSRAPWSLQSVNPGLKTNSSKEPKFTKCR SPERETF SCHWTDEVHHGTKNLGPIQLFYTRRNTQEW TQEWKECPDYVSAGENSCY FNSSFTSIWIPYCIKLT SNGGTVDEKCF SVDEIVQDPPIALNW TLLNVSLTGIHADIQ VRWEAPRNADIQKGWMVLEYELQYKEVNETKWKMMDPILTTSPV VYSLKVDKEY EVRVR SKQRNSGNYGEFSEVLYVTLPQMSQFTCEEDFYFPWLLIIIFGIFGLTVMLFV FLFSKQQR IKMLILPPVPV PKIKGIDPDLLKEGKLEEVNTILAIHDSYKPEFHSDDSWV	24

Naturally Occurring Receptor Name	SEQ ID NO:
EFIELDIDEPDEKTEESDTRLLSSDHEKSHSNLGVKDGDGRTSCCEPDILETDFNA NDIHEGTSEVAQPQRLKGEADLLCLDQKNQNNSPYHDACPATQQPSVIQAEKNKPQ PLPTEGAESTHQA AHIQLSNPSSLNIDFYAQVSDITPAGSVVLSPGQKNKAGMSQC DMHPMEMVSLCQENFLMDNAYFCEADAKKCIPVAPHIKVESHIQPSLNQEDIYITTES LTTAAGRPGTGEHVPVGSEMPVPDYTSIHIVQSPQGLLNATALPLPDKEFLSSCGYVS TDQLNKIMP	
>XP_016855859.1 granulocyte colony-stimulating factor receptor isoform X1 [Homo sapiens] MARLGNC SLTWAALIILLPGSLEECGHISVSAPIVHLGDPITASCIHKQNC SHLDPEP QILWRLGAELQPGGRQQRLSDGTQESIITLPHLNHTQAFLSCCLNWGNSLQILDQVE LRAGYPPAIPHNLSCLMNLTSSSLICQWEPGPETHLPTSFTLKSFKSRGNCQTQGDSI LDCVPKDGQSHCCIPRKHLLLYQNMGIWVQAENALGTSMSPQLCLDPMDVVKLEP PMLRTMDPSPEAAPPQAGCLQLCWEPWQPGLHINQKCELRHKPQRGEASWALVGP LPLEALQYELCGLLPATAYTLQIRCIRWPLPGHWSWSPSLELR TTERAPT VRLDTW WRQRQLDPRTVQLFWKPVPLEEDSGRIQGYVVS WRPSGQAGAILPLCNTTEL SCTF HLPSEAQEVALVAYNSAGTSRPTPVVFSESRGPALTRLHAMARDPHSLWVGWEPPN PWPQGYVIEWGLGPPSASNSNKTWRMEQN GRATGFLLENIRPFQLYEII VTPLYQD TMGPSQHVVAYSQEMAPSHAPELHLKHIGKTWAQLEWVPEPPELGKSPLTHYTIFW TNAQNQSFSAILNASSRGFVLHGLEPASLYHIHLMAASQAGATNSTVLTLMTLTPEG SELHIILGLFGLLLLLLTCLCGTAWLCCSPNRKNPLWPSVPDPAHSSLGSWVPTIMEEL PGPRQGQWL GQTSEMSRALTPHPCVQDAFQLPGLGTPPITKLT VLEEDEKKPVPWE SHNSSETCGLPTLVQTYVLQGDPRAVSTQPQSQSGTSDQVLYGQLLSPTSPGPGHY LRCDSTQPLLAGLTPSPKSYENLWFQASPLGTLVTPAPSQEDDCVFGPLLNFPLLQGI RVHGMEALGSF	25

Naturally Occurring Receptor Name	SEQ ID NO:
>NP_005364.1 thrombopoietin receptor precursor [Homo sapiens] MPSWALFMVTSCLLAPQNLAQVSSQDVSLASDSEPLKCFSRTFEDLTCFWDEEE AAPSGTYQLLYAYPREKPRACPLSSQSMPHFTRYVCQFPDQEEVRLFFPLHLWVK NVFLNQTRTQRVLFVDSVGLPAPPSIIKAMGGSQPGELQISWEEPAPPEISDFLRYELR YGPRDPKNSTGPTVIQLIATETCCPALQRPHSASALDQSPCAQPTMPWQDGPQTSP SREASALTAEGGSCLISGLQPGNSYWLQLRSEPDGISLGGSWGWSLPVTVDLPGDA VALGLQCFTLDLKNVTCQWQQQDHASSQGFFYHSRARCCPRDRYPIWENCEEEEEK TNPGLQTPQFSRCHFCSRNDSEIIHILVEVTTAPGTVHSYLGSPFWIHQAVRLPTPNLH WREISSGHLELEWQHPSSWAAQETCYQLRYTGEGHQDWKVLEPPLGARGGTLELR PRSRYRLQLRARLNGPTYQGPWSSWSDPTRVETATETAWISLVTALHLVLGLSAVL GLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVE PSLLEILPKSSERTPLPLCSSQAQMDYRRLQPSCLGT MPLSVCPPMAESGSCCTTHIA NHSYLPLSYWQQP	26

[0062] In some embodiments, the transmembrane domain of the disclosure is derived from a truncated, or otherwise modified version of the naturally occurring TPOR/MPLR receptor shown in Table 2.

- 5 **[0063]** FIGS. 9A-9B and 10A show the amino acid sequences for the wild type TPOR and the various transmembrane deletion (FIGs. 9A, 10A) or insertion (FIG. 9B) variants.

[0064] Table 3 shows exemplary transmembrane amino acid sequences, coupled to intracellular JAK2 binding domain sequences.

- 10 **[0065]** In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 28. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 29. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 30. In some embodiments, the transmembrane domain
- 15

[illegible]

[illegible]

of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 73. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 74. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 75. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 76. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 77. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 79. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 160. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 217. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises the amino acid sequence of SEQ ID NO: 218, SEQ ID NO: 219, SEQ ID NO: 220, SEQ ID NO: 221, SEQ ID NO: 222, SEQ ID NO: 223, SEQ ID NO: 224, SEQ ID NO: 225, SEQ ID NO: 226, SEQ ID NO: 227, SEQ ID NO: 228, SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO: 233, or SEQ ID NO: 234. In some embodiments, the transmembrane domain of the chimeric cytokine receptor comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NOs: 27-79, 160, and 217-234.

[0066] In some embodiments, the chimeric cytokine receptor (CCR) comprises the binding domain comprising the amino acid sequence of SEQ ID NO: 3, 4 or 159, and the transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 64, 69, or 70. In some embodiments, the CCR is inducible. In some embodiments, the CCR comprises the binding domain comprising the amino acid sequence of SEQ ID NO: 3, 4 or 159, and the transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 38, 39, 40 or 53. In some embodiments, the CCR comprises the binding domain comprising the amino acid sequence of SEQ ID NO: 3, 4 or 159, and the transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 59, 60, 160, or 217. In some embodiments, the CCR is constitutively active. In some

embodiments, the constitutively active CCR of the disclosure dimerizes without a TGF- β ligand.

Table 3: Exemplary Transmembrane + JAK2 Binding Domain Sequences

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
GCSFR(614-710)	LTLMTLTPEGSELHIILGLFGLLLLTCLCGTAWLCCSPNRKNPLWPSVPDPAHSSLSGSWVPTIMEEDAFQLPGLGTPPITKLTVLEEDEKKPVPWE	27
GP130(609-700)	TTPKFAQGEIEAIVVPVCLAFLLTLLGLVLCFNKRDLIKKHIWPNVPDPSKSHIAQWSPHTPPRHNFNNSKDQMYSDGNFTDVSVEIEAND	28
TPOR/MPLR(478-582)	SDPTRVETATETAWISLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	29
TPOR/MPLR(N-1)	SDPTRVETATETWISLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	30
TPOR/MPLR(N-2)	SDPTRVETATETISLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	31
TPOR/MPLR(N-2+1)	SDPTRVETATETLISLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	32
TPOR/MPLR(N-3)	SDPTRVETATETSLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	33
TPOR/MPLR(N-4)	SDPTRVETATETLVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	34
TPOR/MPLR(N-4+1)	SDPTRVETATETILVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	35
TPOR/MPLR(N-5)	SDPTRVETATETVTALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	36
TPOR/MPLR(N-6)	SDPTRVETATETATLHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	37
TPOR/MPLR(N-7)	SDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	38
TPOR/MPLR(N-8)	SDPTRVETATETLHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	39

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(N-9)	SDPTRVETATETHLVGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	40
TPOR/MPLR(N-10)	SDPTRVETATETLVGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	41
TPOR/MPLR(N-11)	SDPTRVETATETVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	42
TPOR/MPLR(N-12)	SDPTRVETATETLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	43
TPOR/MPLR(N-13)	SDPTRVETATETGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	44
TPOR/MPLR(N-14)	SDPTRVETATETLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	45
TPOR/MPLR(N-15)	SDPTRVETATETSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	46
TPOR/MPLR(N-16)	SDPTRVETATETAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	47
TPOR/MPLR(N-17)	SDPTRVETATETVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	48
TPOR/MPLR(N-18)	SDPTRVETATETLGLLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	49
TPOR/MPLR(N+1)	SDPTRVETATETAWLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	50
TPOR/MPLR(N+2)	SDPTRVETATETAWVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	51
TPOR/MPLR(N+3)	SDPTRVETATETAWLVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	52
TPOR/MPLR(N+4)	SDPTRVETATETAWILVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	53
TPOR/MPLR(N+5)	SDPTRVETATETAWLILVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	54
TPOR/MPLR(N+6)	SDPTRVETATETAWLLILVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	55
TPOR/MPLR(N+7)	SDPTRVETATETAWVLLILVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	56
TPOR/MPLR(N+8)	SDPTRVETATETAWLVLLILVLISLVLTALHLVLGLSAVLGLLLLRWQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	57

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(478-582;W515K)	SDPTRVETATETAWISLVTALHLVLGLSAVLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	58
TPOR/MPLR(478-582;H499L,S505N,W515K)	SDPTRVETATETAWISLVTALLVLGLNAVLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	59
TPOR/MPLR(478-582;S505N,W515K)	SDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	60
TPOR/MPLR(N-9-1)	SDPTRVETATEHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	61
TPOR/MPLR(N-9-2)	SDPTRVETATHVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	62
TPOR/MPLR(N-9-3)	SDPTRVETAHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	63
TPOR/MPLR(N-9-4)	SDPTRVETHVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	64
TPOR/MPLR(N-9-5)	SDPTRVEHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	65
TPOR/MPLR(N-9-6)	SDPTRVHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	66
TPOR/MPLR(N-9-7)	SDPTRHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	67
TPOR/MPLR(N-9-8)	SDPTHVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	68
TPOR/MPLR(N-9-9)	SDPHVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	69
TPOR/MPLR(N-9-10)	SDHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	70
TPOR/MPLR(N-9-11)	SHVLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	71
TPOR/MPLR(N-9-12)	HLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	72
TPOR/MPLR(N-C3)	SDPTRVETATETAWISLVHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	73
TPOR/MPLR(N-C5)	SDPTRVETATETAWISHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	74
TPOR/MPLR(N-C5P)	SDPTRVETATETAWISPHVLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	75
TPOR/MPLR(N-C5PP)	SDPTRVETATETAWISPPHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	76

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(N-C6)	SDPTRVETATETAWIHLVLGLSAVLGLLLLRWQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	77
TPOR/MPLR(N-C6P)	SDPTRVETATETAWIPHLVLGLSAVLGLLLLRWQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	78
TPOR/MPLR(N-C6PP)	SDPTRVETATETAWIPPHVLVLGLSAVLGLLLLRWQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	79
TPOR/MPLR(478-582; H499L,S505N,W515K,K553R,K573R)	SDPTRVETATETAWISLVTALLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	217
TPOR/MPLR(478-582) S505N.W515K. K553R.K573R	SDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	160
TPOR/MPLR(478-582) K553R.K573R	SDPTRVETATETAWISLVTALHLVLGLSAVLGLLLLRWQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	218
TPOR/MPLR(N-7) S505N.W515K	SDPTRVETATETALHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	219
TPOR/MPLR(N-7) S505N.W515K. K553R,K573R	SDPTRVETATETALHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	220
TPOR/MPLR(N-8) S505N.W515K	SDPTRVETATETLHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	221
TPOR/MPLR(N-8) S505N.W515K. K553R,K573R	SDPTRVETATETLHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	222
TPOR/MPLR(N-9) S505N.W515K	SDPTRVETATETHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	223
TPOR/MPLR(N-9) S505N.W515K. K553R,K573R	SDPTRVETATETHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	224
TPOR/MPLR(N+4) S505N.W515K	SDPTRVETATETAWILVLISLVTALHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	225

Transmembrane and JAK2 binding domain	Amino acid sequence	SEQ ID NO:
TPOR/MPLR(N+4) S505N.W515K. K553R,K573R	SDPTRVETATETAWILVLISLVTALHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	226
TPOR/MPLR(N-9-1) S505N.W515K	SDPTRVETATEHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	227
TPOR/MPLR(N-9-1) S505N.W515K. K553R,K573R	SDPTRVETATEHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	228
TPOR/MPLR(N-9-4) S505N.W515K	SDPTRVETHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	229
TPOR/MPLR(N-9-4) S505N.W515K. K553R,K573R	SDPTRVETHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	230
TPOR/MPLR(N-9-9) S505N.W515K	SDPHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	231
TPOR/MPLR(N-9-9) S505N.W515K. K553R,K573R	SDPHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	232
TPOR/MPLR(N-9-10) S505N.W515K	SDHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL	233
TPOR/MPLR(N-9-10) S505N.W515K. K553R,K573R	SDHLVLGLNAVLGLLLLRKQFPAHYRRRLHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL	234

C. Janus Kinase (JAK)-Binding Domains

[0067] The chimeric cytokine receptors of the disclosure comprise intracellular JAK-binding domains. The JAK-binding domain is coupled to the C-terminus of the

5 transmembrane domain, either directly, or via a linker. The JAK-binding domain is coupled to the transmembrane domain on the intracellular side of the chimeric cytokine receptor.

[0068] In some embodiments, the JAK-binding domain is a JAK-1-binding domain, a JAK-2 binding domain, a JAK-3 binding domain, or a TYK2 binding domain.

[0069] In some embodiments, the JAK-binding domains of the chimeric cytokine receptors of the disclosure are naturally occurring, and derived from a naturally occurring receptor.

5 [0070] In some embodiments, the JAK-binding domains of the chimeric cytokine receptors of the disclosure are synthetic.

[0071] In some embodiments, the chimeric cytokine receptor comprises a transmembrane and JAK2 binding domain that is at least 80%, 85%, 90%, 95%, 98% or 99%, or 100% identical to any one of the amino acid sequences of SEQ ID NOs: 27-79, 160 and 217-234.

10 [0072] Table 3 provides exemplary amino acid sequences for the transmembrane and JAK2 binding domains of the disclosure. In some embodiments, the transmembrane and JAK2 binding domain comprises one or more mutations, e.g., one or more deletions, insertions and/or substitutions of the wild type sequences. In some embodiments, the transmembrane and JAK2 binding domain comprises one or more substitutions at amino acid positions H499,
15 S505 and W515 of the wild type TPOR/MPLR sequence. See Table 3. In some embodiments, the transmembrane and JAK2 binding domain comprises one or more substitutions at the amino acid positions K533 and K573 of the wild type TPOR/MPLR sequence. In some embodiments, the transmembrane and JAK2 binding domain, e.g., as shown in Table 3, may be combined with a TGF β R2 ectodomain as disclosed herein, e.g., in Table 1, or a PD-1
20 ectodomain (such as a high affinity PD-1 ectodomain, as indicated in SEQ ID NO: 274 or 275 in Table 6) and a recruiting domain to form a chimeric cytokine receptor. In some embodiments, the transmembrane and JAK2 binding domain may be combined with a recruiting domain to form a chimeric cytokine receptor without an ectodomain, see e.g., SEQ
25 ID NOs: 272 or 273. See also USSN 16/804,917, filed on February 28, 2020, and USSN 16/804,545, filed on February 28, 2020, both of which are incorporated herein by reference in their entireties.

D. Recruiting Domains

[0073] The chimeric cytokine receptors of the disclosure comprise cytoplasmic domains comprising recruiting domains (which may also be referred to as “signaling domains”). The

recruiting domain can be a STAT-recruiting domain, an AP1—recruiting domain, a Myc/Max recruiting domain; or an NFkB-recruiting domain. In some embodiments, the recruiting domain is a Signal Transducer and Activator of Transcription (STAT)--recruiting (Stat-activating) domains from receptor tails (cytotails) or from cytokine receptor tails.

- 5 These intracellular recruiting domains of the chimeric cytokine receptors of the disclosure allow for the propagation of Signal 3 in an immune cell comprising a CAR and a chimeric cytokine receptor (e.g. a CAR-T-cell with a chimeric cytokine receptor of the disclosure). Cytokine signaling propagated through the Stat-recruiting domain allows for the cytokine-based immune potentiation of the cell. In some embodiments, the immune-potentiation is homeostatic, e.g. signaling gives rise to increase in immune cells bearing the CAR. In some
10 embodiments, the immune-potentiation is inflammatory, e.g. signaling gives rise to increase in the potency of the immune cells bearing the CAR. In some embodiments, the immune-potentiation prevents exhaustion, e.g. signaling maintains the long-term functionality of immune cells bearing the CAR.

- 15 **[0074]** In some embodiments, the recruiting domains of the disclosure are synthetic, and do not resemble any naturally occurring receptor fragment.

[0075] In some embodiments, the Stat-recruiting domains of the disclosure are synthetic, and do not resemble any naturally occurring receptor fragment.

- [0076]** In other embodiments, the Stat-recruiting domains of the disclosure are derived
20 from cytoplasmic tails of naturally occurring receptors, e.g. derived from naturally occurring cytokine receptors. In some embodiments, the chimeric cytokine receptor comprises a portion or a fragment of a naturally occurring receptor, e.g., the intracellular Stat-recruiting domain of the naturally occurring receptor, optionally with one or more mutations therein (e.g., one or more deletions, insertions and/or substitutions). These cytoplasmic tails of
25 naturally occurring receptors may be the regions downstream of the JAK-activating domains of the transmembrane domain of the receptor. The Stat-recruiting domains of the chimeric cytokine receptors comprise at least one STAT-recruiting domain from at least one receptor. In some embodiments, the Stat-recruiting domain comprises at least one STAT1-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT2-
30 recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT3-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at

least one STAT4-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT5-recruiting domain. In some embodiments, the STAT-recruiting domain comprises at least one STAT6-recruiting domain. In some embodiments, the Stat-recruiting domain comprises at least one STAT7-recruiting domain.

5 **[0077]** In some embodiments, the naturally occurring receptor from which the STAT-recruiting domain is derived, is a not a cytokine receptor.

[0078] In some embodiments, the naturally occurring receptor from which the Stat-recruiting domain is derived, is a cytokine receptor. Exemplary cytokine receptors through which T-cell-immune potentiating cytokines signal include, but are not limited to IL-2
10 receptor, IL-7 receptor, IL-15 receptor, IL12 receptor, and IL-21 receptor. In some embodiments, the cytokine receptor from which the STAT-recruiting domain is derived contains phosphorylatable tyrosine residues downstream of the cognate JAK-binding motifs, and one or more signaling domains of interest may be fused downstream of the transmembrane domain to generate single or multiple signaling outputs. In alternative
15 embodiments, the receptor from which the Stat-recruiting domain is derived, is not a cytokine receptor. By choosing the Stat-recruiting domain of the chimeric cytokine receptor, the receptor can be redirected to signaling of choice. In some embodiments, the chimeric cytokine receptor comprises two or more Stat-recruiting domains from more than one receptor. In some embodiments, the two or more Stat-recruiting domains are linked with or
20 without a peptide linker.

[0079] Table 4 provides exemplary receptors from which Stat-recruiting domains (signaling domains) of the chimeric cytokine receptors of the disclosure are derived. Table 5a provides exemplary amino acid sequences of recruiting domains of the disclosure.

[0080] In some embodiments, the chimeric cytokine receptor of the disclosure comprises
25 the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 80. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 81. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 82. In some embodiments, the chimeric cytokine receptor
30 of the disclosure comprises the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 83. In some embodiments, the chimeric cytokine receptor of the disclosure

5
10
15
20
25
30

5
10
15
20
25
30

the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 122. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of the STAT-recruiting domain of SEQ ID NO: 161. In some embodiments, the chimeric cytokine receptor comprises a recruiting domain that comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99%, or 100% identical to any one of the amino acid sequences of SEQ ID NOs: 80-122 and SEQ ID NO: 161.

Table 4: Recruiting domain sources

Source for recruiting domains
BLNK
IL2RG
EGFR
EpoR
GHR
IFNAR1
IFNAR2
IFNAR1/2
IFNLR1
IL10R1
IL12Rb1
IL12Rb2
IL21R
IL2Rb
IL2small
IL7R
IL7Ra
IL9R
IL15R
IL21R

Table 5a: Recruiting Domain Sequences (Cytotail Sequences)

Recruiting domain	Amino acid sequence	SEQ ID NO:
IL7R(316-459)	ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL SLGTTNSTLPPPFSLQSGILTLPVAGGQPIILSLGSNQEEAYVTM SSFYQNNQ	80
IL2Rb(333-551)	VTQLLLQQDKVPEPASLSSNHSLSCTFNQGYFFHLPDALEIEAC QVYFTYDPYSEEDPDEGVAGAPTGSSPQLQPLSGEDDAYCTFPS RDDLLLFSPSLGGSPSTAPGGSGAGEERMPPSLQERVPRDW DPQPLGPPTPGVPDLVDFQPPPELVREAGEEVPDAGPREGVSFP WSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	81
IFNAR1(508-557)	ISTIATVEETNQTDDEHKKYSSQTSQDSGNYSNEDESESKTSEELQ QDFV	82
IFNAR2(310-515)	KKKVWDYNYDDESDDTEAAPRTSGGGYTMHGLTVRPLGQASA TSTESQLIDPESEEPDLPEVDVELPTMPKDSPPQLELLSGPCERRK SPLQDPFPEEDYSSTEGSGGRITFNVDLNSVFLRVLDDEDSDDLEA PLMLSSHLEEMVDPEDPDNVQSNHLLASGEGTQPTFPSPSEGL WSEDAPSDQSDTSESDDVLDLGDGYIMR	83
IFNAR1/2(IFNAR1 residues 508-557-IFNAR2 residues 310-515)	ISTIATVEETNQTDDEHKKYSSQTSQDSGNYSNEDESESKTSEELQ QDFVKKKVWDYNYDDESDDTEAAPRTSGGGYTMHGLTVRPLG QASATSTESQLIDPESEEPDLPEVDVELPTMPKDSPPQLELLSGP CERRKSPLQDPFPEEDYSSTEGSGGRITFNVDLNSVFLRVLDDEDS DDLEAPLMLSSHLEEMVDPEDPDNVQSNHLLASGEGTQPTFPSP SSEGLWSEDAPSDQSDTSESDDVLDLGDGYIMR	84
IFNLR1(300-520)	RGVRPTPRVRAPATQQTRWKKDLAEDEEEDEEDTEDGVSFQPY IEPPSFLGQEHQAPGHSEAGGVDSGRPRAPLVPSEGSSAWDSSD RSWASTVDSSWDRAAGSSGYLAKEGPGQGPGGDGHQESLPPPEF SKDSGFLEELPEDNLSSWATWGTLPPEPNLVPGGPPVSLQTLTFC WESSPEEEEEARESEIEDSDAGSWGAESTQRTEDRGRRTLGHYMA R	85
Common Gamma Chain(335-369)	IPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPET	86
IL9R(356-521)	TALLTCGPARPWKSVALEEEQEGPGRPLPGLNLSSEDLVLPAGCTEW RVQTLAYLPQEDWAPTSLTRPAPPDSEGRSSSSSSSSNNNNYCA LGCYGGWHLALPGNTQSSGPIPALACGLSCDHQGLTQQGVA WVLAGHCQRPLHEDLQGMILLPSVLSKARSWTF	87
IL21R(322-538)	PRSPAKRLQLTELQEPALVESDGVKPSFWPTAQNSGGSAYSEE RDRPYGLVSIDTVTLDAEGPCTWPCSCEDDGYPALDLDALEPS PGLEDPLLDAGTTVLSCGCVSAGSPGLGGPLGSLDLRLKPLADGE DWAGGLPWGGRSPGGVSESEAGSPLAGLMDTDFDSGFVGSDCS SPVECDFTSPGDEGPSPSYLRQWVVIPLPSSPGPQAS	88
GHR(353-638)	PDEKTEESDTRLLSSDHEKSHSNLGVKDGDSGRTSCCEPDILETD FNANDIHEGTSEVAQPQRLKGEADLLCLDQKNQNNSPYHDACPA TQQPSVIAEKNKPQLPTEGAESTHQAHIQLSNPSSLSNIDFYA QVSDITPAGSVVLSPGQKNKAGMSQCDMHPMEMVSLCQENFLM DNAYFCEADAKKICPVAPHIKVESHIQPSLNQEDIYITTESLTTAAG	89

Recruiting domain	Amino acid sequence	SEQ ID NO:
	RPGTGEHVPGSEMPVPDYTSIHIVQSPQGLILNATALPLPDKEFLS SCGYVSTDQLNKIMP	
EpoR(339-508)	WGTMQAVEPGTDDDEGPLLEPVGSEHAQDTYLVLDKWLLPRNPP SEDLPGPGGSVDIVAMDEGSEASSCSALASKPSPEGASAASFEYT ILDPSQLLRPWTLCPPLPPTPPHLKYLVLVSDSGISTDYSSGDSQ GAQGGLSDGPYSNPYENSLIPAAEPLPPSYVACS	90
murine IL2Rb(337-539)	AVQLLLQKDSAPLPSPSGHSQASCFTNQGYFFFHLPNALEIESCQ VYFTYDPCVEEEVEEDGSRLPEGSPHPPLLPLAGEQDDYCAFPPRD DLLLFPSLSLTPNTAYGGSRAPEERSPLSLHEGLPSLASRDLMLGLQR PLERMPEGDGEGLSANSSGEQASVPEGNLHGQDQDRGQGPILT NTDAYLSLQELQAQDSVHLI	91
murine IL7Ra(316-459)	ARDEVESFLPNDLPAQPEELETQGHRAAVHSANRSPETSVSPPET VRRESPLRCLARNLSTCNAPLLSSRSPDYRDGDRNRPPVYQDLLP NSGNTNVPVPVPQPLPFQSGILIPVSQRQPISTSSVLNQEEAYVTM SSFYQNK	92
EGFR(955-1186)	VIQGDERMHLPSPPTDSNFYRALMDEEDMDDVVDADEYLIPQQG FFSSPSTSRTPLLSSLSATSNNSTVACIDRNLQSCPIKEDSFLQRYS SDPTGALTEDSIDDTFLPVPEYINQSVPKRPAGSVQNPVYHNQPL NPAPSRDPHYQDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHLWA QKGSHQISLDNPDYQQDFFPKAKPNGIFKGSTAENAEYLRVAPQ SSEFIGA	93
EGFR(955-1186;Y974F,d1045-1057)	VIQGDERMHLPSPPTDSNFFRALMDEEDMDDVVDADEYLIPQQG FFSSPSTSRTPLLSSLSATSNNSTVACIDRNLQSCPIKEDSFLQRID DTFLPVPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQD PHSTAVGNPEYLNTVQPTCVNSTFDSPAHLWAQKGSHQISLDNPD YQQDFFPKAKPNGIFKGSTAENAEYLRVAPQSSEFIGA	94
EGFR(955-1009;Y974F)	VIQGDERMHLPSPPTDSNFFRALMDEEDMDDVVDADEYLIPQQG FFSSPSTSRTP	95
EGFR(1019-1085)	NNSTVACIDRNLQSCPIKEDSFLQRIDDTFLPVPEYINQSVPKRPA GSVQNPV	96
EGFR(1037-1103;Y1068/1101F,d1045-1057)	KEDSFLQRIDDTFLPVPEFINQSVPKRPAGSVQNPVYHNQPLNPA PSRDPHFQD	97
EGFR(1066-1118;Y1068/1086F)	VPEFINQSVPKRPAGSVQNPVFNQPLNPAPSRDPHYQDPHSTA VGNPEYLNTV	98
EGFR(1122-1165)	PEYLNTVQPTCVNSTFDSPAHLWAQKGSHQISLDNPDYQQDFFPK EAKPNGIFKG	99
EGFR(1133-1186;Y1148F)	WAQKGSHQISLDNPDFQDFFPKAKPNGIFKGSTAENAEYLRV APQSSEFIGA	100
IL12Rb2(775-825)	SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHEAPLADSL EELEPQ	101
IL7R(376-416)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLP	102
IL7R(424-459)	GILTLPVAQGQPILTSLSNQEEAYVTMSSFYQNK	103

Recruiting domain	Amino acid sequence	SEQ ID NO:
IL7R(376-416,424-459)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSGTTNSTLPQGQPIL TSLGSNQEEAYVTMSSFYQNNQ	104
IL7R(424-459;Y456F)	GILTLPVAAQGGPILSTSLGSNQEEAYVTMSSFFQNNQ	105
IL7R(376-416,424-459,Y456F)	ACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSGTTNSTLPQGQPIL TSLGSNQEEAYVTMSSFFQNNQ	106
IL2Rbsmall(393-433)	DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPS	107
IL2Rbsmall(518-551)	GQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	108
IL2Rbsmall(339-379,393-433)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQDEGV AGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPS	109
IL2Rbsmall(339-379,518-551)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQ GQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	110
IL2Rbsmall(393-433,518-551)	DEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGE FRALNARLPLNTDAYLSLQELQGQDPTHLV	111
IL2Rbsmall(339-379,393-433,518-551)	QQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQDEGV AGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSGQGEFRAL NARLPLNTDAYLSLQELQGQDPTHLV	112
IFNAR2small(310-352)	KKKVWDYNYDDESDDTEAAPRTSGGGYTMHGLTVRPLGQASA	113
IFNAR2small(486-515)	EGLWSEDAPSDQSDTSESDDVLDGDGYIMR	114
IFNAR2small(310-352,486-515)	KKKVWDYNYDDESDDTEAAPRTSGGGYTMHGLTVRPLGQASA EGLWSEDAPSDQSDTSESDDVLDGDGYIMR	115
BLNK(53-208)	ASESPADEEEQWSDDFDSDYENPDEHSDSEMYVMMPAEENADDS YEPPPVEQETRPVHPALPFARGEYIDNRSSQRHSPPFSKTLPSKPS WPSEKARLTSTLPALTALQKPQVPPKPKGLLEADYVVPVEDND ENYIHPTESSPPPEKAPMVNR	116
BLNK(53-208;Y72F)	ASESPADEEEQWSDDFDSDFENPDEHSDSEMYVMMPAEENADDS YEPPPVEQETRPVHPALPFARGEYIDNRSSQRHSPPFSKTLPSKPS WPSEKARLTSTLPALTALQKPQVPPKPKGLLEADYVVPVEDND ENYIHPTESSPPPEKAPMVNR	117
BLNK(53-208;Y72F,Y96F)	ASESPADEEEQWSDDFDSDFENPDEHSDSEMYVMMPAEENADDS FEPPPVEQETRPVHPALPFARGEYIDNRSSQRHSPPFSKTLPSKPS WPSEKARLTSTLPALTALQKPQVPPKPKGLLEADYVVPVEDND ENYIHPTESSPPPEKAPMVNR	118
EpoR(339-508)	WGTMQAVEPGTDDDEGPLLEPVGSEHAQDLYLVLDKWLLPRNPP SEDLPGGPGSVDIVAMDEGSEASSCSALASKPSPEGASAAFEYT ILDPSQLLRPWTLCELPPTPHLKYLYLVSDSGISTDYSSGDSQ GAQGGSLSDGPYSNPYENSLIPAAEPLPPSYVACS	119
IL12Rb2(714-862)	VTPVFRHPPCSNWPQREKGIQGHQASEKDDMMHSASSPPPPRAL QAESRQLVDLYKVLERSGSDPKPENPACPWTVLPAGDLPTHGDL	120

Recruiting domain	Amino acid sequence	SEQ ID NO:
	PSNIDDLPSHEAPLADSLEELEPQHISLSVFPSSSLHPLTFSCGDKLTLDQLKMRCDSLML	
IL12Rb1(622-662)	WDKGERTEPLEKTELPEGAPELALDTELSLEDGDRCKAKM	121
IL10R1(304-578)	VSP ELKNLDLHGSTD SGFGSTKPSLQTEEPQFLLPDPHPQADRTLGNREPPVLGDSCSSGSSNSTDSGICLQEPSPSTGPTWEQQVGSNSRGQDDSGIDL VQNSEGRAGDTQGGSALGHHSPPEPEVPGEEDPAAVAFQGYLRQTRCAEEKATKTGCL EESPLTDGLGPKFGRCLVDEAGLHPPALAKGYLKQDPLEMTLASSGAPTQQWNQPT EEW SLLA LSSCS DLGISDWSFAHDLAPLGCVAAPGGLLGSFNSDLVTLP LISSLQSSE	122
IL7Ra(316-459).IL12Rb2(775-825)	ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD LLLSLGTTNSTLPPFSLQSGILTNPV AQQQPILTSLGSNQEEAYVTMSSFYQNQSRSDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	161

*SR indicates an exemplary peptide linker

[0081] In some embodiments, the Stat-recruiting domain of a chimeric cytokine receptor of the disclosure comprises a STAT-recruiting domain from one receptor.

5 **[0082]** In order to generate multiple outputs, one or more STAT-recruiting domains may be joined in tandem to mimic signaling from one or more cytokines.

[0083] In some embodiments, the STAT-recruiting domain comprises portions of more than one receptor, e.g. comprising more than one STAT-recruiting domain. In such embodiments, a tandem cytokine signaling domain is provided, allowing for enhanced signaling. Accordingly, in some embodiments, the STAT-recruiting domain of a monomer of the chimeric cytokine receptor of the disclosure comprises the STAT-recruiting domains from more than one receptor, e.g. comprises the STAT-recruiting domains from two, three, four, five, or even six receptors. For example, in some embodiments, STAT-recruiting domains can be linked in tandem to stimulate multiple pathways (e.g., the IL7R(316-459)-
10 IL12Rb2(775-825) fragment fusion for pro-persistence STAT5 and pro-inflammatory STAT4; IL7R(316-459)-IL2Rbsmall(393-433,518-551) for pro-persistence; IL7R(316-459)-EGFR(1122-1165) for pro-persistence and anti-exhaustion; IL2Rbsmall(393-433,518-551)-EGFR(1122-1165) for pro-persistence and anti-exhaustion).

15

[0084] When generating multiple outputs, the proximity of individual STAT-recruiting domains to the cell membrane can influence the strength of their respective signaling outputs. Table 5b shows examples of chimeric cytokine receptors with the dual outputs, where each output can be placed either proximal or distal to the cell membrane.

5 **Table 5b:** Examples of chimeric cytokine receptors with dual outputs

Dual output STAT-recruiting domain	Membrane proximal	Membrane distal
IL2R β small(393-433,518-551)/IL21R(322-538)	IL2R β small(393-433,518-551)	IL21R(322-538)
IL21R(322-538)/IL2R β small(393-433,518-551)	IL21R(322-538)	IL2R β small(393-433,518-551)
IL2R β small(339-379,393-433,518-551)/IL21R(322-538)	IL2R β small(339-379,393-433,518-551)	IL21R(322-538)
IL21R(322-538)/IL2R β small(339-379,393-433,518-551)	IL21R(322-538)	IL2R β small(339-379,393-433,518-551)
IL7R (316-459)/IL21R(322-538)	IL7R (316-459)	IL21R(322-538)
IL7R (316-459)/IL12R β 2(775-825)	IL7R (316-459)	IL12R β 2(775-825)
IL21R(322-538)/IL7R (316-459)	IL21R(322-538)	IL7R (316-459)

[0085] Without being bound to theory or mechanism, in some embodiments, a JAK-protein (JAK1, JAK2, JAK3, or TYK2) is bound to a chimeric cytokine receptor of the disclosure (comprising a binding domain, a transmembrane domain, a JAK-binding domain, and a recruiting domain). In some embodiments, in the presence of (e.g. binding to) a TGF- β ligand or an anti-TGF- β -receptor antibody, the chimeric cytokine receptor clusters and allows for the two bound JAK-proteins to become activated, which in turn phosphorylate tyrosine residues on the recruiting domain of the chimeric receptor. The phosphorylated recruiting domains are then capable of binding the recruited proteins (e.g. a phosphorylated STAT-recruiting domain binds a STAT-protein), which in turn effectuate transcription events in the nucleus.

E. Exemplary TGF- β -Driven Chimeric Cytokine Receptors

[0086] Context-dependent chimeric cytokine receptors of the disclosure may be expressed with a signal sequence, e.g. a CD8SS of SEQ ID NO: 1. Table 6 shows exemplary context-dependent cytokine receptor sequences of the disclosure. The receptors may be expressed with
5 a signal sequence, e.g. a CD8SS of SEQ ID NO: 1.

[0087] In some embodiments, the chimeric cytokine receptor of the disclosure comprises a TGF- β binding domain comprising an amino acid sequence of any one of SEQ ID NOs: 3-20, and 159, a transmembrane and JAK2 binding domain comprising an amino acid sequence of any one of SEQ ID NOs: 27-79, 160 and 217-234, and a recruiting domain comprising an
10 amino acid sequences of any one of SEQ ID NOs: 80-122 and 161. In some embodiments, the chimeric cytokine receptor does not comprise a signal sequence.

[0088] In some embodiments, the chimeric cytokine receptor of the disclosure comprises a TGF- β binding domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, and 159, a TPOR/MPLR transmembrane and JAK2 binding domain
15 comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 39, 40, 53, 59, 60, 61, 64, 69, 70, 160 and 217-234, and a recruiting domain comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 80, 99, 111, 112, and 161. Optionally, the chimeric cytokine receptor comprises a signal sequence that comprises for example the amino acid sequence of SEQ ID NO: 1.

[0089] In some embodiments, the chimeric cytokine receptor comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 3, 4 or 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 64, 69, or 70, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the chimeric cytokine receptor is inducible. In some embodiments,
25 the chimeric cytokine receptor comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 3, 4 or 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 38, 39, 40 or 53, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the chimeric cytokine receptor comprises a binding domain comprising the amino acid sequence
30 of SEQ ID NO: 3, 4 or 159, a transmembrane and JAK2 binding domain comprising the amino

acid sequence of SEQ ID NO: 59, 60, 160, or 217, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the chimeric cytokine receptor is constitutively active. In some embodiments, the constitutively active chimeric cytokine receptor of the disclosure dimerizes without binding to a TGF β ligand or an anti-TGF β R antibody. In some embodiments, the chimeric cytokine receptor of the disclosure inhibits TGF β R-mediated signaling and/or activates STAT-mediated signaling, either constitutively or induced by TGF- β , or an anti-TGF β R antibody. In some embodiments, the chimeric cytokine receptor is constitutively active and/or exhibits further enhanced activities or properties in the presence of a TGF- β R ligand, e.g., TGF- β , or an anti-TGF- β R antibody. In some embodiments, the TGF- β R is TGF- β R2, and the antibody is an anti-TGF- β R2 antibody.

[0090] In some embodiments, the chimeric cytokine receptor does not comprise a signal sequence. In some embodiments, the chimeric cytokine receptor comprises the TGF β R2 endogenous signal sequence or a signal sequence that comprise, e.g., the amino acid sequence of SEQ ID NO:1.

[0091] In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 123. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 124. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 125. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 126. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 127. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 128. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 129. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 130. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 131. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 132. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 133. In some embodiments,

the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 134. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 135. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO: 136.

[illegible]

[illegible]

[illegible]

the disclosure comprises the amino acid sequence of SEQ ID NO:214. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO:215. In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO:216.

- 5 **[0092]** In some embodiments, the chimeric cytokine receptor (CCR) comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 40, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 53, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 38, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 39, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 40, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 53, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 70, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 69, and a recruiting domain comprising the amino acid

sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 64, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 69, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 70, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 160 or 219, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 223, 224, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 159, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 225 or 226, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 60 or 160, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 223, 224, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161. In some embodiments, the CCR comprises a binding domain comprising the amino acid sequence of SEQ ID NO: 4, a transmembrane and JAK2 binding domain comprising the amino acid sequence of SEQ ID NO: 225 or 226, and a recruiting domain comprising the amino acid sequence of SEQ ID NO: 80, 99, 111, 112, or 161.

[0093] In some embodiments, the chimeric cytokine receptor of the disclosure comprises the amino acid sequence of SEQ ID NO:272, SEQ ID NO:273, SEQ ID NO:274, or SEQ ID NO:275. In some embodiments, the chimeric cytokine receptor comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99%, or 100% identical to any one of the amino acid sequences of SEQ ID NOs: 123-216 and SEQ ID NOs: 272-275.

Table 6: Exemplary chimeric cytokine receptor sequences (assembled inducible or constitutively active TGF- β receptor chimeric cytokine receptors):

Receptor	Amino acid sequence	SEQ ID NO:
CD8SS-TGF β R2(1-166).TPOR/MPLR(478-582).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLSAVLGLLLRWQFPA HYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVE PSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLSLGTTNSTLPPPFSLQSGILTLPVAQGGQPIL TSLGSNQEEAYVTMSSFYQNSDPKPENPACPWTVPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	123
CD8SS-TGF β R2(1-166).TPOR/MPLR(478-582;N-1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETWISLVTALHLVLGLSAVLGLLLRWQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQS PNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRES GKNGPHVYQDLLSLGTTNSTLPPPFSLQSGILTLPVAQGGQPILTS LGSNQEEAYVTMSSFYQNSDPKPENPACPWTVPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	124
CD8SS-TGF β R2(1-166).TPOR/MPLR(478-582;N-2).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETISLVTALHLVLGLSAVLGLLLRWQFPAHYR RLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLL EILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESG KNGPHVYQDLLSLGTTNSTLPPPFSLQSGILTLPVAQGGQPILTS GNSNQEEAYVTMSSFYQNSDPKPENPACPWTVPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	125
CD8SS-TGF β R2(1-166).TPOR/MPLR(478-	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED	126

582;N-2+1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLISLVTALHLVLGLSAVLGLLLLRWQFPAHY RRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSL LEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQS PNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRES GKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVAQGGQPILTS LGSNQEEAYVTMSSFYQNSQSDPKPENPACPWTVLPAGDLPTH GYLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-3).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETSLVTALHLVLGLSAVLGLLLLRWQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK NGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVAQGGQPILTSLG SNQEEAYVTMSSFYQNSQSDPKPENPACPWTVLPAGDLPTHG GYLPSNIDDLPSHEAPLADSLEELEPQ	127
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLVTALHLVLGLSAVLGLLLLRWQFPAHYRR RHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK GPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVAQGGQPILTSLG SNQEEAYVTMSSFYQNSQSDPKPENPACPWTVLPAGDLPTHG GYLPSNIDDLPSHEAPLADSLEELEPQ	128
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-4+1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETILVTALHLVLGLSAVLGLLLLRWQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK NGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVAQGGQPILTSLG SNQEEAYVTMSSFYQNSQSDPKPENPACPWTVLPAGDLPTHG GYLPSNIDDLPSHEAPLADSLEELEPQ	129
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-5).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETVTALHLVLGLSAVLGLLLLRWQFPAHYRR RHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK GPHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVAQGGQPILTSLG	130

	NQEEAYVTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHGGLY PSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-6).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLR HALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILP KSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCP SEDDVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNG PHVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVQAQQQPILTSLSGN QEEAYVTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHGGLYPS NIDDLPSHEAPLADSLEELEPQ	131
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-7).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLRH ALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPK SSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCP SEDVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGP HVYQDLLLLSLGTTNSTLPPFSLQSGILTLPVQAQQQPILTSLSGNQ EEAYVTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHGGLYPSN IDDLPSHEAPLADSLEELEPQ	132
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-8).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLHLVLGLSAVLGLLLLRWQFPAHYRRLRHA LWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKS SERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSE DVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGPH VYQDLLLLSLGTTNSTLPPFSLQSGILTLPVQAQQQPILTSLSGNQE EAYVTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHGGLYPSNI DDLPSHEAPLADSLEELEPQ	133
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETHLVLGLSAVLGLLLLRWQFPAHYRRLRHAL WPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSS ERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSE DVVITPESFGRDSSLTCLAGNVSA CDAPILSSSRSLDCRESGKNGPHV YQDLLLLSLGTTNSTLPPFSLQSGILTLPVQAQQQPILTSLSGNQEE AYVTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHGGLYPSNID DLPSHEAPLADSLEELEPQ	134
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-10).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	135

Underlined indicates CD8SS	VIFQSDPTRVETATETVLGLSAVLGLLLLRWQFPAHYRRRLHALW PSLPDLHRVLGQYL RDTAALSPPKATVSDTCEEVEPSLLEILPKSSER TPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVV ITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLLSGTTNSTLPPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAY VTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDL PSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-11).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETVLGLSAVLGLLLLRWQFPAHYRRRLHALW PSLPDLHRVLGQYL RDTAALSPPKATVSDTCEEVEPSLLEILPKSSER TPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVV ITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLLSGTTNSTLPPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAY VTMSSFYQNOQSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDL PSHEAPLADSLEELEPQ	136
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-12).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETVLGLSAVLGLLLLRWQFPAHYRRRLHALWPS LPDLHRVLGQYL RDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTP LPLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVIT PESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD LLLSLGTNSTLPPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAYVT MSSFYQNOQSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPS HEAPLADSLEELEPQ	137
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-13).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETGLSAVLGLLLLRWQFPAHYRRRLHALWPSL PDLHRVLGQYL RDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PLARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITP ESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDL LLSLGTNSTLPPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAYVT MSSFYQNOQSDPKPENPACPWTVLPAGDLPTHDGYLPSNIDDLPS HEAPLADSLEELEPQ	138
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-14).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVC HDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLSAVLGLLLLRWQFPAHYRRRLHALWPSLP DLHRVLGQYL RDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP LARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPE SFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLL LSLGTNSTLPPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAYVTM	139

	SSFYQNNQSDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHE APLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-15).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETSAVLGLLLLRWQFPAHYRRLRHALWPSLPD LHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL ARDEVEGFLQDTPPQLEESEKQRLGGDVQSPNCPSEDVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL SLGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGSNQEEAYVTM SSFYQNNQSDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHE APLADSLEELEPQ	140
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-16).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAVLGLLLLRWQFPAHYRRLRHALWPSLPDL HRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLA RDEVEGFLQDTPPQLEESEKQRLGGDVQSPNCPSEDVVITPESF GRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLS LGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGSNQEEAYVTMS SFYQNNQSDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHE APLADSLEELEPQ	141
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-17).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETVLGLLLLRWQFPAHYRRLRHALWPSLPDLH RVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLAR DEVEGFLQDTPPQLEESEKQRLGGDVQSPNCPSEDVVITPESFG RDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSL GTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGSNQEEAYVTMSS FYQNNQSDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHEA PLADSLEELEPQ	142
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-18).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLGLLLLLRWQFPAHYRRLRHALWPSLPDLHR VLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPLARD EVEGFLQDTPPQLEESEKQRLGGDVQSPNCPSEDVVITPESFGR DSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLGT TNSTLPPFSLQSGILTLPVAQGGQPIILSLGSNQEEAYVTMSSFY QNNQSDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSHEAPL ADSLEELEPQ	143
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+1).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	144

Underlined indicates CD8SS	VIFQSDPTRVETATETAWLISLVTALHLVLGLSAVLGLLLLRWQFPA HYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVE PSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTNPVAQQQPIL TSLGSNQEEAYVTMSSFYQSQSDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+2).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWVLISLVTALHLVLGLSAVLGLLLLRWQFP AHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEV EPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGGD VQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDC RESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTNPVAQQQP ILTSLGSNQEEAYVTMSSFYQSQSDPKPENPACPWTVLPAGDLPT HDGYLPSNIDDLPSHEAPLADSLEELEPQ	145
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+3).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLVLSLVTALHLVLGLSAVLGLLLLRWQF PAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE VEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLGG DVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLD CRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTNPVAQQQ PILTSLGSNQEEAYVTMSSFYQSQSDPKPENPACPWTVLPAGDLP THDGYLPSNIDDLPSHEAPLADSLEELEPQ	146
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWILVLSLVTALHLVLGLSAVLGLLLLRWQ FPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCE EVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLG GDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL DCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTNPVAQQ QPILTSLGSNQEEAYVTMSSFYQSQSDPKPENPACPWTVLPAGD LPTHGYLPSNIDDLPSHEAPLADSLEELEPQ	147
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+5).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLILVLSLVTALHLVLGLSAVLGLLLLRW QFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTC EEVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLG GDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL DCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTNPVAQQ	148

	QPIILTSLSNQEAYVTMSSFYQNSDPKPNPACPWTVLPAGD LPTHGGLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+6).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLLILVLISLVTALHLVLGLSAVLGLLLLRW QFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTC EEVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQRLG GDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL DCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVAAQG QPIILTSLSNQEAYVTMSSFYQNSDPKPNPACPWTVLPAGD LPTHGGLPSNIDDLPSHEAPLADSLEELEPQ	149
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+7).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWVLLILVLISLVTALHLVLGLSAVLGLLLLR WQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSD TCEEVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQR LGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSS RSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVA QGGQPIILTSLSNQEAYVTMSSFYQNSDPKPNPACPWTVLPA GDLPTHGGLPSNIDDLPSHEAPLADSLEELEPQ	150
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+8).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLVLLILVLISLVTALHLVLGLSAVLGLLLLR WQFPAHYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSD TCEEVEPSLLEILPKSSERTPLPLARDEVEGFLQDTFPQQLEESEKQR LGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSS RSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVA QGGQPIILTSLSNQEAYVTMSSFYQNSDPKPNPACPWTVLPA GDLPTHGGLPSNIDDLPSHEAPLADSLEELEPQ	151
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLSAVLGLLLLRWQFPA HYRRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTC EEVE PSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGG DVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLD CRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVAAQGQ PIILTSLSNQEAYVTMSSFYQNS <u>SR</u> SDPKPNPACPWTVLPAG DLPTHGGLPSNIDDLPSHEAPLADSLEELEPQ	162
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582).S505N.W515K.IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	163

Underlined indicates CD8SS	VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAQQQPIL TSLGSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLP THDGYLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582) S505N.W515K.IL2Rbsmall(393-433,518-551) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPM</u> MGRGLLRGLWPLHIVLWTRIASTIP PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPLLEDEGVAGAPTGSSPQPLQPLSGEDDAYCTF PSRDDLLLFSQGGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	164
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582) S505N.W515K. K553R.K573R.IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPM</u> MGRGLLRGLWPLHIVLWTRIASTIP PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPS LLEILPRSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAQQQPIL TSLGSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLP THDGYLPSNIDDLPSHEAPLADSLEELEPQ	165
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582) S505N.W515K. K553R.K573R. IL2Rbsmall(393-433,518-551) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPM</u> MGRGLLRGLWPLHIVLWTRIASTIP PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPS LLEILPRSSERTPLPL <u>LE</u> DEGVAGAPTGSSPQPLQPLSGEDDAYCTF PSRDDLLLFSQGGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	166
TGFbR2(1-166).TPOR/MPLR(478-582) S505N.W515K.IL2Rbsmall(393-433,518-551)	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGA VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR KNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFM CSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISL VTALHLVLGLNAVLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLG QYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> DEGV AGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSQGGQGEFRAL NARLPLNTDAYLSLQELQGQDPHTLV	167
TGFbR2(1-166).TPOR/MPLR(478-582) S505N.W515K. K553R.K573R. IL2Rbsmall(393-433,518-551)	MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGA VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR KNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFM CSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISL VTALHLVLGLNAVLGLLLLRKQFPAHYRRLRHALWPSLPDLHRVLG QYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL <u>LE</u> DEGV AGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSQGGQGEFRAL NARLPLNTDAYLSLQELQGQDPHTLV	168

CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582)S505N.W515K.IL7Ra(316-459) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTINPVAQQQPIL TSLGSNQEEAYVTMSSFYQNNQ	169
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582)S505N.W515K.K553R.K573R. .IL7Ra(316-459) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALHLVLGLNAVLGLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPRATVSDTCEEVEPS LLEILPRSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTINPVAQQQPIL TSLGSNQEEAYVTMSSFYQNNQ	170
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582)S505N.W515K. IL2Rbsmall(339-379,393-433,518-551) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWISLVTALLVLGLNAVLGLLLRKQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPL <u>LE</u> QQDKVPEPASLSSNHSLSCTFNQGYFFH LPDALEIEACQDEGVAGAPTGSSPQPLQPLSGEDDAYCTFSPSRDD LLLFSPSGQGEFRALNARLPLNTDAYLSLQELQGQDPHTLV	171
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETWISLVTALHLVLGLSAVLGLLLRWQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDV QSPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTINPVAQQQPIL TSLGSNQEEAYVTMSSFYQNNQ <u>SR</u> SDPKPENPACPWTVLPAGDLP THDGYLPSNIDDLPSHEAPLADSLEELEPQ	172
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-2).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETISLVTALHLVLGLSAVLGLLLRWQFPAHYR RLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLL EILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQ SPNCPSDEVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE SGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTINPVAQQQPILT	173

	SLGSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPT HDGYLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-2+1).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLISLVTALHLVLGLSAVLGLLLRWQFPAHY RRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSL LEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQ SPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRE SGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPNVAQGGQPILT SLGSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPT HDGYLPSNIDDLPSHEAPLADSLEELEPQ	174
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-3).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETSLVTALHLVLGLSAVLGLLLRWQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESG KNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPNVAQGGQPILTSL GSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	175
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLVLTALHLVLGLSAVLGLLLRWQFPAHYRR RHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEIL PKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESG KNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPNVAQGGQPILTSL GSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	176
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-4+1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETILVTALHLVLGLSAVLGLLLRWQFPAHYRR LRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEI LPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESG KNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPNVAQGGQPILTSL GSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	177
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-5).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	178

	VIFQSDPTRVETATETVTALHLVLGLSAVLGLLLLRWQFPAHYRRL RHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEIL PKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSP NCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESG KNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAGGQPILTS GSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH DGYLPSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-6).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLR HALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILP KSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPN CPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGK NGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAGGQPILTS SNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH GYLPSNIDDLPSHEAPLADSLEELEPQ	179
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-7).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETALHLVLGLSAVLGLLLLRWQFPAHYRRLR ALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPK SSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNC PSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKN GPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAGGQPILTS SNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH GYLPSNIDDLPSHEAPLADSLEELEPQ	180
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-8).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLHLVLGLSAVLGLLLLRWQFPAHYRRLR HALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPK SSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNC PSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKN GPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAGGQPILTS SNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH GYLPSNIDDLPSHEAPLADSLEELEPQ	181
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETHVLVLGLSAVLGLLLLRWQFPAHYRRLR HALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPK SSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNC PSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKN GPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAGGQPILTS SNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTH GYLPSNIDDLPSHEAPLADSLEELEPQ	182

	EEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLP SNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-10).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLVGLSAVLGLLLLRWQFPAHYRRLRHALW PSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSER TPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSED VVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHV YQDLLLSLGTNNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEE AYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSN IDDLPSHEAPLADSLEELEPQ	183
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-11).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLVGLSAVLGLLLLRWQFPAHYRRLRHALW PSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSER TPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSED VVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHV YQDLLLSLGTNNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEE AYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSN IDDLPSHEAPLADSLEELEPQ	184
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-12).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLVGLSAVLGLLLLRWQFPAHYRRLRHALWPS LPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTP LPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVV ITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLSLGTNNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAY VTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNID DLPSHEAPLADSLEELEPQ	185
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-13).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETGLSAVLGLLLLRWQFPAHYRRLRHALWPSL PDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVI TPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLSLGTNNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAY VTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNID DLPSHEAPLADSLEELEPQ	186
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-14).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	187

Underlined indicates CD8SS	VIFQSDPTRVETATETLSAVLGLLLLRWQFPAHYRRRLHALWPSLP DLHRVLGQYLRTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVIT PESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD LLSLGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVT MSSFYQNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHDGYLPSNIDDL PSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-15).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETSAVLGLLLLRWQFPAHYRRRLHALWPSLPD LHRVLGQYLRTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITP ESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDL LLSLGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVT MSSFYQNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHDGYLPSNIDDL PSHEAPLADSLEELEPQ	188
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-16).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAVLGLLLLRWQFPAHYRRRLHALWPSLPDL HRVLGQYLRTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPE SFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLL LSLGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVTM SSFYQNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHDGYLPSNIDDLPS HEAPLADSLEELEPQ	189
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-17).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETVLGLLLLRWQFPAHYRRRLHALWPSLPDLH RVLGQYLRTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL SLGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVTM SSFYQNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHDGYLPSNIDDLPS HEAPLADSLEELEPQ	190
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-18).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETLGLLLLRWQFPAHYRRRLHALWPSLPDLHR VLGQYLRTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESF GRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLS LGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVTMS	191

	SFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSH EAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLISLVTALHLVLGLSAVLGLLLLRWQFPA HYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVE PSLLEILPKSSERTPLPL <u>LEAR</u> DEVEGFLQDTFPQQLEESEKQRLGG DVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLD CRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVAQGGQ PILTSLSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGD LPTHGGLPSNIDDLPSHAPLADSLEELEPQ	192
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+2).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLISLVTALHLVLGLSAVLGLLLLRWQFP AHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEV EPSLLEILPKSSERTPLPL <u>LEAR</u> DEVEGFLQDTFPQQLEESEKQRLGG DVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLD CRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVAQGGQ PILTSLSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGD LPTHGGLPSNIDDLPSHAPLADSLEELEPQ	193
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+3).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLISLVTALHLVLGLSAVLGLLLLRWQF PAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE VEPSLLEILPKSSERTPLPL <u>LEAR</u> DEVEGFLQDTFPQQLEESEKQRLG GDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSL DCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVAQGG QPILTSLSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPA GDLPTHGGLPSNIDDLPSHAPLADSLEELEPQ	194
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWILVLSLVTALHLVLGLSAVLGLLLLRWQ FPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEE EVEPSLLEILPKSSERTPLPL <u>LEAR</u> DEVEGFLQDTFPQQLEESEKQRL GGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSR SLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPFSLQSGILTNPVA QGGQPILTSLSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVL PAGDLPTHGGLPSNIDDLPSHAPLADSLEELEPQ	195
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+5).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	196

Underlined indicates CD8SS	VIFQSDPTRVETATETAWLILVLISLVTALHLVLGLSAVLGLLLLRW QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTC EEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEKQR LGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSS RSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTINPVA QGGQILTSLSGNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVL PAGDLPTHGYPNSNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+6).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLILVLISLVTALHLVLGLSAVLGLLLLRW QFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTC EEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEKQR LGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSS RSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTINPVA QGGQILTSLSGNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVL PAGDLPTHGYPNSNIDDLPSHEAPLADSLEELEPQ	197
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+7).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWVLLILVLISLVTALHLVLGLSAVLGLLLLRW WQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSD TCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEK QRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILS SSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTINP VAQGGQILTSLSGNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWT VLPAGDLPTHGYPNSNIDDLPSHEAPLADSLEELEPQ	198
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N+8).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLVLLILVLISLVTALHLVLGLSAVLGLLLLRW WQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSD TCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEK QRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILS SSRSLDCRESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTINP VAQGGQILTSLSGNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWT VLPAGDLPTHGYPNSNIDDLPSHEAPLADSLEELEPQ	199
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-1).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATEHLVLGLSAVLGLLLLRWQFPAHYRRLRHAL WPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSS ERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEKQRLGGDVQSPNCPS EDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGP HVYQDLLLLSLGTTNSTLPPPFSLQSGILTINPVAQGGQILTSLSGNQ	200

	EEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLP SNIDDLPSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-2).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATHLVGLSAVLGLLLLRWQFPAHYRRRLHALW PSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSER TPLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSED VVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHV YQDLLLLSGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEE AYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSN IDDLPSHEAPLADSLEELEPQ	201
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-3).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETAHLVLGLSAVLGLLLLRWQFPAHYRRRLHALWP SLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERT PLPL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDEV VITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVY QDLLLLSGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEEA YVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNI DDLPSHEAPLADSLEELEPQ	202
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETHLVGLSAVLGLLLLRWQFPAHYRRRLHALWPSL PDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDEVVI TPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLLSGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAY VTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNID DLPSHEAPLADSLEELEPQ	203
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-5).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVEHLVLGLSAVLGLLLLRWQFPAHYRRRLHALWPSLP DLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP L <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDEVVIT PESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD LLLLSGTTNSTLPPFSLQSGILTLPVAQGGQPIILSLGNSQEEAYVT MSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDL PSHEAPLADSLEELEPQ	204
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-6).IL7Ra(316-459).IL12Rb2(775-825)	<u>MALPVTALLPLALLHAARPMGRGLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL	205

Underlined indicates CD8SS	VIFQSDPTRVHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLP DLHRVLGQYLRLDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLP <u>LE</u> ARDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVIT PESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQD LLLSLGTNSTLPPPFSLQSGILTLPVPAQGGQPIILSLGSNQEEAYVT MSSFYQNNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHGYPNSIDDL PSHEAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-7).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCNDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDL HRVLGQYLRLDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> <u>EA</u> RDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPE SFGDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLL LSLGTNSTLPPPFSLQSGILTLPVPAQGGQPIILSLGSNQEEAYVTM SSFYQNNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHGYPNSIDDLPS HEAPLADSLEELEPQ	206
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-8).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCNDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTHVLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLH RVLGQYLRLDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> <u>EA</u> RDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL SLGTNSTLPPPFSLQSGILTLPVPAQGGQPIILSLGSNQEEAYVTM SSFYQNNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHGYPNSIDDLPS HEAPLADSLEELEPQ	207
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-9).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCNDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPHVLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLH RVLGQYLRLDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> <u>EA</u> RDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPES FGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLL SLGTNSTLPPPFSLQSGILTLPVPAQGGQPIILSLGSNQEEAYVTM SSFYQNNQ <u>SR</u> SDPKPENPACPWTVPAGDLPTHGYPNSIDDLPS HEAPLADSLEELEPQ	208
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-10).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLLRGLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCNDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDHLVLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHR VLGQYLRLDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> <u>EA</u> RDEVEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESF GRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLS LGTNSTLPPPFSLQSGILTLPVPAQGGQPIILSLGSNQEEAYVTMS	209

	SFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSH EAPLADSLEELEPQ	
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-11).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSHLVGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVL GQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARD EVEGFLQDTFPPQLEESEKQRLGGDVQSPNCPSEDVVITPESFGR DSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGT TNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVTMSSFY QNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSH EAPLADSLEELEPQ	210
CD8SS-TGFbR2(1-166).TPOR/MPLR(478-582;N-9-12).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPMGRGLRLWPLHIVLWTRIASTIP</u> PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCM SNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVL GQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARD EVEGFLQDTFPPQLEESEKQRLGGDVQSPNCPSEDVVITPESFGR DSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLLSLGT TNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAYVTMSSFY QNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNIDDLPSH EAPLADSLEELEPQ	211
CD8SS-TGFbR2 ▲ N25.TPOR/MPLR(478-582;N-9).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPQLCKFCDVRFSTCDNQKSCMSNCSI</u> TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP KCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETHLVGLSAVLGLLLLRWQFPAHYRRLRHALWPSL PDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPL PL <u>LE</u> ARDEVEGFLQDTFPPQLEESEKQRLGGDVQSPNCPSEDVVI TPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQ DLLLLSLGTTNSTLPPPFSLQSGILTLPVAAQGPILTSLSNQEEAY VTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLPTHGGLPSNID DLPSHEAPLADSLEELEPQ	212
CD8SS-TGFbR2 ▲ N25.TPOR/MPLR(478-582;N+4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPQLCKFCDVRFSTCDNQKSCMSNCSI</u> TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP KCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETATETAWLVLISLVTALHLVLGLSAVLGLLLLRWQFPAH YRRLRHALWPSLPDLHRVLGQYLRDTAALSPPKATVSDTCEEVEPS LLEILPKSSERTPLPL <u>LE</u> ARDEVEGFLQDTFPPQLEESEKQRLGGDV QSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSSRSLDCR ESGKNGPHVYQDLLLLSLGTTNSTLPPPFSLQSGILTLPVAAQGPIL TSLGSNQEEAYVTMSSFYQNO <u>SR</u> SDPKPENPACPWTVLPAGDLP THDGLPSNIDDLPSHEAPLADSLEELEPQ	213
CD8SS-TGFbR2 ▲ N25.TPOR/MPLR(478-582;N-9-4).IL7Ra(316-459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARPQLCKFCDVRFSTCDNQKSCMSNCSI</u> TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP KCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQSDPTRVETHLVGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDL HRVLGQYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> <u>ARDEVEGFLQDTFPPQLEESEKQRLGGDVQSPNCPSEDVVITPE</u>	214

	SFGRDSSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLL LSLGTNSTLPPFSLQSGILTLPVAQGGPILTSLSGNQEEAYVTM SSFYQNSRSDPKPENPACPWTVPAGDLPHTDGYLPSNIDDLPS HEAPLADSLEELEPQ	
CD8SS- TGFbR2▲N25.TPOR/MPLR(478-582;N-9-9).IL7Ra(316- 459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARP</u> QLCKFCDVRFSTCDNQKSCMSNCISI TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP KCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLLVIFQ SDPHLVGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLG QYLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDE VEGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRD SSLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLGT NSTLPPFSLQSGILTLPVAQGGPILTSLSGNQEEAYVTMSSFYQ NQ <u>SR</u> SDPKPENPACPWTVPAGDLPHTDGYLPSNIDDLPSHEAPL ADSLEELEPQ	215
CD8SS- TGFbR2▲N25.TPOR/MPLR(478-582;N-9-10).IL7Ra(316- 459).IL12Rb2(775-825) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARP</u> QLCKFCDVRFSTCDNQKSCMSNCISI TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASP KCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDLLLVIFQ SDHLVLGLSAVLGLLLLRWQFPAHYRRLRHALWPSLPDLHRVLGQ YLRDTAALSPPKATVSDTCEEVEPSLLEILPKSSERTPLPL <u>LE</u> ARDEV EGFLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDS SLTCLAGNVSACDAPILSSSRSLDCRESGKNGPHVYQDLLLSLGT NSTLPPFSLQSGILTLPVAQGGPILTSLSGNQEEAYVTMSSFYQ NQ <u>SR</u> SDPKPENPACPWTVPAGDLPHTDGYLPSNIDDLPSHEAPL ADSLEELEPQ	216
CD8SS-TPOR/MPLR(478- 582;H499L,S505N,W515K, K553R,K573R).IL2Rb(339- 379,393-433,518-551) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARP</u> SDPTRVETATETAWISLVTA <u>LL</u> VLG LNAVGLLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTAA LSPPRATVSDTCEEVEPSLLEILPRSSERTPLPL <u>LE</u> QQDKVPEPASLS SNHSLTSCFTNQGYFFHLPDALEIEACQDEGVAGAPTGSSPQPL QPLSGEDDAYCTFSPSRDDLLLFSPSGQGEFRALNARLPLNTDAYLS LQELQGQDPHTLV	272
CD8SS-TPOR/MPLR(478- 582;S505N,W515K,K553R,K 573R).IL2Rb(393-433,518- 551) Underlined indicates CD8SS	<u>MALPVTALLPLALLHAARP</u> SDPTRVETATETAWISLVTAHLVL <u>GL</u> NAVGLLLLLRKQFPAHYRRLRHALWPSLPDLHRVLGQYLRDTA <u>AL</u> SPPRATVSDTCEEVEPSLLEILPRSSERTPLPL <u>LE</u> DEGVAGAPTGS SPQPLQPLSGEDDAYCTFSPSRDDLLLFSPSGQGEFRALNARLPLNT DAYLSLQELQGQDPHTLV	273
CD8SS- HAPD1.TPOR/MPLR(478- 582;H499L,S505N,W515K, K553R,K573R).IL2Rb(339- 379,393-433,518-551) Underlined indicates CD8SS Italicized marks high-affinity PD-1 ectodomain	<u>MALPVTALLPLALLHAARP</u> PGWFLDSPDRPWNPPTFSPALLVV TEGDNATFTCSFSNTSESFHVIWHRESPSGQTDTLAAFPEDRSQP GQDCRFRTQLPNGRDFHMSVVRARRNDSGTYVCGVISLAPKIQI KESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVSDPTRVETAT ETAWISLVTA <u>LL</u> VLGLNAVGLLLLLRKQFPAHYRRLRHALWPSLP DLHRVLGQYLRDTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPL PL <u>LE</u> QQDKVPEPASLSNHSLSCTFNQGYFFHLPDALEIEACQD EGVAGAPTGSSPQPLQPLSGEDDAYCTFSPSRDDLLLFSPSGQGEF RALNARLPLNTDAYLSLQELQGQDPHTLV	274

CD8SS-HAPD1.TPOR/MPLR(478-582;S505N,W515K,K553R,K573R).IL2Rb(393-433,518-551) Underlined indicates CD8SS Italicized marks high-affinity PD-1 ectodomain	MALPVTALLPLALLHAARPPGWFLDSPDRPWNPTTFSPALLVV TEGDNATFTCSFSNTSESFHVIWHRESPSGQTDTLAAFPEDRSQP GQDCRFRTVLPLNGRDFHMSVVRARRNDSTGYVCGVISLAPKIQI KESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVSDPTRVETAT ETAWISLVLTALHLVLGLNAVLGLLLLRKQFPAHYRRLRHAWPSLP DLHRVLGQYLRTAALSPPRATVSDTCEEVEPSLLEILPRSSERTPL PLLEDEGVAGAPTGSSPQLQPLSGEDDAYCTFSPSRDDLLLFSPSG QGEFRALNARLPLNTDAYLSLQELQGQDPTHLV	275
---	--	-----

*The underlined *LE* and *SR* indicate exemplary peptide linkers.

F. Expression of chimeric cytokine receptors

[0094] Provided herein are polynucleotides encoding any one of the chimeric cytokine receptors provided herein. Likewise, provided herein are expression vectors comprising such polynucleotides. In some embodiments, the vector is a viral vector. In some embodiments, the vector is not a viral vector.

[0095] In some embodiments, the vector comprises a polynucleotide encoding a chimeric cytokine receptor, and a polynucleotide expressing a chimeric antigen receptor (CAR).

[0096] In some embodiments, expression of the chimeric cytokine receptor and the CAR are expressed as a single polypeptide chain, separated by a linker. FIGS. 2A, 3, 4A, 5A, 6A, 8A, and 11A show schematics of a vector that can be used to co-express the chimeric cytokine receptor and CAR of the disclosure. One or more STAT-recruiting domains may be joined in tandem to mimic signaling from one or more cytokines.

II. CAR-bearing Immune Cells

[0097] Provided herein are engineered immune cells comprising a polynucleotide encoding a chimeric antigen receptor and a chimeric cytokine receptor of the disclosure; and provided herein are engineered immune cells expressing a chimeric antigen receptor (CAR-I cell) and a chimeric cytokine receptor of the disclosure. Examples of immune cells include T-cells, e.g., alpha/beta T-cells and gamma/delta T-cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, invariant NKT cells, mast cells, myeloid-derived phagocytes, dendritic cells, killer dendritic cells, macrophages, and monocytes. Immune cells also refer to cells derived from, for example without limitation, a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood

stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells.

[0098] Accordingly in some embodiments, provided herein are CAR-T cells comprising a chimeric cytokine receptor of the disclosure.

- 5 **[0099]** In some embodiments, a CAR can comprise an extracellular ligand-binding domain (e.g., a single chain variable fragment (scFv)), a transmembrane domain, and an intracellular signaling domain. In some embodiments, the extracellular ligand-binding domain, transmembrane domain, and intracellular signaling domain are in one polypeptide, i.e., in a single chain. Multichain CARs and polypeptides are also provided herein. In some
- 10 embodiments, the multichain CARs comprise: a first polypeptide comprising a transmembrane domain and at least one extracellular ligand-binding domain, and a second polypeptide comprising a transmembrane domain and at least one intracellular signaling domain, wherein the polypeptides assemble together to form a multichain CAR.

- [0100]** The extracellular ligand-binding domain of a CAR specifically binds to a target of interest. The target of interest can be any molecule of interest, including, for example,
- 15 without limitation any one or more of those presented in Table 8.

Table 8: List of targets of interest

BCMA
EGFRvIII
Flt-3
WT-1
CD20
CD23
CD30
CD38
CD70
CD33
CD133
MHC- WT1

TSPAN10
MHC-PRAME
Liv1
ADAM10
CHRNA2
LeY
NKG2D
CS1
CD44v6
ROR1
CD19
Claudin-18.2 (Claudin-18A2 or Claudin18 isoform 2)
DLL3 (Delta-like protein 3, Drosophila Delta homolog 3, Delta3)
Muc17 (Mucin17, Muc3, Muc3)
FAP alpha (Fibroblast Activation Protein alpha)
Ly6G6D (Lymphocyte antigen 6 complex locus protein G6d
c6orf23
G6D
MEGT1
NG25)
RNF43 (E3 ubiquitin-protein ligase RNF43, RING finger protein 43)
ErbB2 (HER2/neu)
carcinoembryonic antigen (CEA)
epithelial cell adhesion molecule (EpCAM)
epidermal growth factor receptor (EGFR)
CD40
disialoganglioside GD2
GD3
C-type lectin-like molecule-1 (CLL-1)
ductal-epithelial mucine
gp36
TAG-72

glycosphingolipids
glioma-associated antigen
β -human chorionic gonadotropin
alphafetoprotein (AFP)
lectin-reactive AFP
thyroglobulin
RAGE-1
MN-CA IX
human telomerase reverse transcriptase
RU1
RU2 (AS)
intestinal carboxyl esterase
mut hsp70-2
M-CSF
prostase
prostase specific antigen (PSA)
PAP
NY-ESO-1
LAGA-Ia
p53
prostein
PSMA
survivin and telomerase
prostate-carcinoma tumor antigen-1 (PCTA-1)
MAGE
ELF2M
neutrophil elastase
ephrin B2
CD22
insulin growth factor (IGF)-I
IGF-II
IGF-I receptor

mesothelin
a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope
5T4
O 1
Nkp30
tumor stromal antigens
the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the AI domain of tenascin-C (TnC AI) and fibroblast associated protein (fap)
LRP6
melamona-associated Chondroitin Sulfate Proteoglycan (MCSP)
MARTI
MUC1
LMP2
Idiotype
NY-ESO-1
Ras mutant
gp100
proteinase 3
bcr-abl
tyrosinase
hTERT
EphA2
ML-TAP
ERG
NA17
PAX3
ALK
Androgen receptor
a lineage-specific or tissue specific antigen such as CD3
CD4
CD8

CD24
CD25
CD34
CD79
CD116
CD117
CD135
CD123
CD138
CTLA-4
B7-1 (CD80)
B7-2 (CD86)
endoglin
a major histocompatibility complex (MHC) molecule
MUC16
PSCA
Trop2
CD171 (L1CAM)
CA9
STEAP1
VEGFR2

[0101] In some embodiments, the extracellular ligand-binding domain of a CAR comprises an scFv comprising the light chain variable (VL) region and the heavy chain variable (VH) region of a target antigen specific monoclonal antibody joined by a flexible linker. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide (Bird et al., Science 242:423-426, 1988) (e.g. glycine-serine containing linkers). In general, linkers can be short, flexible polypeptides and are generally comprised of about 20 or fewer amino acid residues. Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of

scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated
5 using standard protein purification techniques known in the art.

[0102] The intracellular signaling domain of a CAR according to the invention is responsible for intracellular signaling following the binding of extracellular ligand-binding domain to the target resulting in the activation of the immune cell and immune response (Signals 1 and/or 2). The intracellular signaling domain has the ability to activate at least one
10 of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

[0103] In some embodiments, an intracellular signaling domain for use in a CAR can be the cytoplasmic sequences of, for example without limitation, the T cell receptor and co-
15 receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Intracellular signaling domains comprise two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a
20 secondary or co-stimulatory signal. Primary cytoplasmic signaling sequences can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non-limiting examples those derived from
25 TCR ζ , FcR γ , FcR β , FcR ϵ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b and CD66d. In some embodiments, the intracellular signaling domain of the CAR can comprise the CD3 ζ signaling domain. In some embodiments the intracellular signaling domain of the CAR of the invention comprises a domain of a co-stimulatory molecule.

[0104] In some embodiments, the intracellular signaling domain of a CAR of the invention
30 comprises a part of co-stimulatory molecule selected from the group consisting of fragment of 41BB (GenBank: AAA53133.) and CD28 (NP_006130.1).

[0105] CARs are expressed on the surface membrane of the cell. Thus, the CAR comprises a transmembrane domain. Suitable transmembrane domains for a CAR disclosed herein have the ability to (a) be expressed at the surface of a cell, preferably an immune cell such as, for example without limitation, lymphocyte cells or Natural killer (NK) cells, and (b) interact with the ligand-binding domain and intracellular signaling domain for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T cell receptor such as α , β , γ or δ , polypeptide constituting CD3 complex, IL-2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III or CD proteins. Alternatively, the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments said transmembrane domain is derived from the human CD8 α chain (e.g., NP_001139345.1). The transmembrane domain can further comprise a stalk domain between the extracellular ligand-binding domain and said transmembrane domain. A stalk domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4, or CD28, or from all or part of an antibody constant region. Alternatively the stalk domain may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be an entirely synthetic stalk sequence. In some embodiments said stalk domain is a part of human CD8 α chain (e.g., NP_001139345.1). In another particular embodiment, said transmembrane and hinge domains comprise a part of human CD8 α chain. In some embodiments, CARs disclosed herein can comprise an extracellular ligand-binding domain that specifically binds BCMA, CD8 α human hinge and transmembrane domains, the CD3 ζ signaling domain, and 4-1BB signaling domain.

[0106] In some embodiments, a CAR can be introduced into an immune cell as a transgene via a plasmid vector. In some embodiments, the plasmid vector can also contain, for example, a selection marker which provides for identification and/or selection of cells which received the vector.

[0107] Table 7 provides exemplary sequences of CAR components that can be used in the CARs disclosed herein.

Table 7: Exemplary Sequences

Domain	Amino acid sequence	SEQ ID NO:
V5 epitope tag	KPIPNNLLGLDST	152
2173 scFv	EIQLVQSGAEVKKPGESLRISCKGSGFNIEDYYIH WVRQMPGKGLEWMGRIDPENDETKYGPIFQGH VTISADTSINTVYLQWSSLKASDTAMYYCAFRG GVYWGQGTTVTVSSGGGGSGGGGSGGGGSGGG GSDVVMTQSPDSLAVSLGERATINCKSSQSLLDS DGKTYLNLWLQQKPGQPPKRLISLVSKLDSGVPD RFSGSGSGTDFTLTISSLQAEDVAVYYCWQGTHF PGTFGGGGTKVEIK	153
CD8 hinge and transmembrane	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC	154
4-1BB intracellular signaling	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL	155
CD3z intracellular signaling	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR	156
BFP (blue fluorescent protein)	MSELIKENMHMKLYMEGTVDNHHFKCTSEGE KPYEGTQTMRIKVVEGGPLPFAFDILATSFLYGS KTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGG VLTATQDTSLQDGLIYNVKIRGVNFTSNGPVM QKKTLGWEAFTETLYPADGGLEGRNDMALKLV GGSHLIANIKTTYRSKKPAKNLKMPGVYYYVDYR LERIKEANNETYVEQHEVAVARYCDLPSKLGHK LN	157
P2A	GSGATNFSLLKQAGDVEENPGP	158

[0108] In some embodiments, the CAR-immune cell (e.g., CAR-T cell) of the disclosure comprises a polynucleotide encoding a suicide polypeptide, such as for example RQR8. See, 5 e.g., WO2013153391A, which is hereby incorporated by reference in its entirety. In some

embodiments, a suicide polypeptide is expressed on the surface of the cell. In some embodiments, a suicide polypeptide is included in the CAR construct. In some embodiments, a suicide polypeptide is not part of the CAR construct.

[0109] In some embodiments, the extracellular domain of any one of CARs disclosed

5 herein may comprise one or more epitopes specific for (specifically recognized by) a monoclonal antibody. These epitopes are also referred to herein as mAb-specific epitopes. Exemplary mAb-specific epitopes are disclosed in International Patent Publication No. WO 2016/120216, which is incorporated herein in its entirety. In these embodiments, the extracellular domains of the CARs comprise antigen binding domains that specifically bind to
10 a target of interest and one or more epitopes that bind to one or more monoclonal antibodies (mAbs). CARs comprising the mAb-specific epitopes can be single-chain or multi-chain.

[0110] The inclusion of epitopes specific for monoclonal antibodies in the extracellular domain of the CARs described herein allows sorting and depletion of engineered immune cells expressing the CARs. In some embodiments, allowing for depletion provides a safety
15 switch in case of deleterious effects, e.g., upon administration to a subject.

[0111] Methods of preparing immune cells for use in immunotherapy are also provided herein. In some embodiments, the methods comprise introducing a chimeric cytokine receptor and a CAR into immune cells, and expanding the cells. In some embodiments, the invention relates to a method of engineering an immune cell comprising: providing a cell and
20 expressing a chimeric cytokine receptor, and expressing at the surface of the cell at least one CAR. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding a chimeric cytokine receptor, and at least one polynucleotide encoding a CAR, and expressing the polynucleotides in the cell. In some embodiments, the method comprises: transfecting the cell with at least one polynucleotide encoding a chimeric
25 cytokine receptor, at least one polynucleotide encoding a CAR, and expressing the polynucleotides in the cell. In some embodiments, the chimeric cytokine receptor and the CAR reside on one polynucleotide.

[0112] In some embodiments, the one or more polynucleotides encoding the chimeric cytokine receptor and CAR are present in one or more expression vectors for stable
30 expression in the cells. In some embodiments, the polynucleotides are present in viral vectors for stable expression in the cells. In some embodiments, the one or more polynucleotides are

inserted into the cellular genome by random integration, and in other embodiments, inserted into specific locations of the cellular genome by site-specific integration. In some embodiments, the viral vectors may be for example, lentiviral vectors or adenoviral vectors. In some embodiments, the one or more polynucleotides are present in non-viral vectors.

5 **[0113]** In some embodiments, polynucleotides encoding polypeptides according to the present disclosure can be mRNA which is introduced directly into the cells, for example by electroporation. In some embodiments, CytoPulse electroporation technology, such as PulseAgile, can be used to transiently permeabilize living cells for delivery of material into the cells (e.g. US 6,078,490; PCT/US2011/000827; and PCT/US2004/005237). Parameters
10 can be modified in order to determine conditions for high transfection efficiency with minimal mortality.

[0114] Also provided herein are methods of transfecting an immune cell, e.g a T-cell. In some embodiments, the method comprises: contacting a T-cell with RNA and applying to the T-cell an agile pulse sequence. In some embodiments, a method of transfecting an immune
15 cell (e.g. T-cell) comprising contacting the immune cell with RNA and applying to the cell an agile pulse sequence.

[0115] In some embodiments, the method can further comprise a step of genetically modifying a cell by inactivating at least one gene expressing, for example without limitation, a component of the TCR, a target for an immunosuppressive agent, an HLA gene, and/or an
20 immune checkpoint protein such as, for example, PDCD1 or CTLA-4. By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In some embodiments, the gene to be inactivated is selected from the group consisting of, for example without limitation, TCR α , TCR β , CD52, GR, deoxycytidine kinase (DCK), TGF-B, and CTLA-4. In some embodiments the method comprises inactivating one or more genes by
25 introducing into the cells a rare-cutting endonuclease able to selectively inactivate a gene by selective DNA cleavage. In some embodiments the rare-cutting endonuclease can be, for example, a transcription activator-like effector nuclease (TALE-nuclease) or CRISPR-based endonuclease (e.g Cas-9 or Cas12a).

[0116] In another aspect, a step of genetically modifying cells can comprise: modifying
30 immune cells (e.g. T-cells) by inactivating at least one gene expressing a target for an

immunosuppressive agent, and; expanding the cells, optionally in presence of the immunosuppressive agent.

[0117] In some embodiments, the engineered immune cells (e.g. T-cells) provided herein exhibit improved cytotoxicity, increased expansion, and/or increased levels of memory

phenotype markers upon contact with a TGF- β ligand or anti-TGF- β -receptor antibody that binds to the binding domain of the chimeric cytokine receptor relative to engineered immune cells that do not express the chimeric cytokine receptor.

[0118] In some embodiments, the engineered immune cells (e.g. T-cells) provided herein exhibit (i) increased *in vivo* persistence, (ii) increased STAT activation, (iii) increased

cytotoxicity, (iv) increased levels of memory phenotype markers, (v) increased expansion (proliferation), or combinations of these functional features, upon contact with a TGF- β ligand or anti-TGF- β -receptor antibody that binds to the binding domain of the chimeric cytokine receptor relative to engineered immune cells that do not express the chimeric cytokine receptor.

In some embodiments, the improvement in the one or more functional features described herein is dose-dependent, i.e., the functional activity of the immune cell comprising the chimeric cytokine receptors increases upon contact with increasing doses of the PD-L1/PD-L2/TGF-B or an antibody to the respective receptor. In some embodiments, STATs activated by the engineered immune cell comprising one or more chimeric cytokine receptors disclosed are STAT1, STAT2, STAT3, STAT4, STAT5, STAT6, or combinations thereof. In one embodiment, memory phenotype markers that are increased or maintained by the immune cell comprising the chimeric cytokine receptor of the disclosure include stem cell memory (Tscm) markers and central memory (Tcm) markers.

[0119] In some embodiments, the improvement in one or more functional features

exhibited by an engineered immune cell comprising a chimeric cytokine receptor provided herein is at least about 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 25 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 125 fold, 150 fold, 200 fold, 250 fold, 300 fold, 350 fold, 400 fold, 450 fold, or even about 10-500 fold, including values and ranges therebetween, compared to an immune cell that does not express the chimeric cytokine receptor.

[0120] In some embodiments, the improvement in one or more functional features

exhibited by an engineered immune cell comprising a chimeric cytokine receptor provided

herein is at least about 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 90%, 100%, 125%, 150%, 200%, 250%, 300%, 350%, 400%, or even about 80%-500%, including values and ranges therebetween, compared to an engineered immune cell that does not express the chimeric cytokine receptor.

5 **III. Therapeutic Methods**

[0121] Provided herein are pharmaceutical compositions comprising cells bearing the chimeric cytokine receptors and CARs of the disclosure.

[0122] Engineered chimeric cytokine receptor-bearing and CAR-bearing immune cells (e.g. T-cells) obtained by the methods described above, or cell lines derived from such engineered
10 immune cells, can be used as a medicament. In some embodiments, such a medicament can be used for treating a disorder such as for example a viral disease, a bacterial disease, a cancer, an inflammatory disease, an immune disease, or an aging- associated disease. In some embodiments, the cancer is a solid cancer. In some embodiments the cancer is a liquid cancer. The cancer can be selected from the group consisting of gastric cancer, sarcoma,
15 lymphoma, leukemia, head and neck cancer, thymic cancer, epithelial cancer, salivary cancer, liver cancer, stomach cancer, thyroid cancer, lung cancer, small cell lung cancer, ovarian cancer, breast cancer, prostate cancer, esophageal cancer, pancreatic cancer, glioma, glioblastoma, leukemia, multiple myeloma, renal cell carcinoma, bladder cancer, cervical cancer, choriocarcinoma, colon cancer, oral cancer, skin cancer, and melanoma. In some
20 embodiments, the subject is a previously treated adult subject with locally advanced or metastatic melanoma, squamous cell head and neck cancer (SCHNC), ovarian carcinoma, sarcoma, or relapsed or refractory classic Hodgkin's Lymphoma (cHL).

[0123] In some embodiments, engineered immune cells, or cell line derived from the engineered immune cells, can be used in the manufacture of a medicament for treatment of a
25 disorder in a subject in need thereof. In some embodiments, the disorder can be, for example, a cancer, an autoimmune disorder, or an infection.

[0124] Also provided herein are methods for treating subjects in need of such treatment.

[0125] As used herein, the term "subject" refers to any vertebrate including, without limitation, humans and other primates (e.g., chimpanzees, cynomolgous monkeys, and other

apes and monkey species), farm animals (*e.g.*, cattle, sheep, pigs, goats and horses), domestic mammals (*e.g.*, dogs and cats), laboratory animals (*e.g.*, rabbits, rodents such as mice, rats, and guinea pigs), and birds (*e.g.*, domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like). In some embodiments, the subject is
5 a mammal. In exemplary embodiments, the subject is a human.

[0126] In some embodiments the method comprises providing immune cells of the disclosure, bearing the chimeric cytokine receptors and CARs described herein to a subject in need thereof.

[0127] In some embodiments, chimeric cytokine receptor and CAR-bearing T-cells of the
10 invention can undergo robust *in vivo* T-cell expansion and can persist for an extended amount of time.

[0128] Methods of treatment of the invention can be ameliorating, curative or prophylactic. The method of the invention may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment.

[0129] In another aspect, the invention provides a method of inhibiting tumor growth or progression in a subject who has a tumor, comprising administering to the subject an effective amount of chimeric cytokine receptor-expressing and CAR-expressing immune cells as described herein. In another aspect, the invention provides a method of inhibiting or preventing metastasis of cancer cells in a subject, comprising administering to the subject in
20 need thereof an effective amount of engineered immune cells as described herein. In another aspect, the invention provides a method of inducing tumor regression in a subject who has a tumor, comprising administering to the subject an effective amount of engineered immune cells as described herein. In some embodiments, the subject is further administered with an anti-TGF- β R antibody, in particular, an anti-TGF- β R2 antibody.

[0130] In some embodiments, the engineered T-cells herein can be administered
25 parenterally in a subject. In some embodiments, the engineered T-cells disclosed herein can be administered intravenously in a subject.

[0131] Also provided is the use of any of the engineered T-cells provided herein in the manufacture of a medicament for the treatment of cancer or for inhibiting tumor growth or progression in a subject in need thereof.

[0132] In some embodiments, treatment can be administered into subjects undergoing an immunosuppressive treatment. Indeed, the invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T-cells according to the invention within the subject. The administration of the cells or population of cells according to the invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a subject subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. Cells bearing the chimeric cytokine receptors and/or CARs of the disclosure or the pharmaceutical compositions thereof may be administered via one or more of the following routes of administration: intravenous, intraocular, intravitreal, intramuscular, subcutaneous, topical, oral, transdermal, intraperitoneal, intraorbital, by implantation, by inhalation, intrathecal, intraventricular, via the ear, or intranasal.

[0133] In some embodiments the administration of the cells or population of cells (bearing the chimeric cytokine receptors and CARs of the disclosure) can comprise administration of, for example, about 10^4 to about 10^9 cells per kg body weight including all integer values of cell numbers within those ranges. In some embodiments the administration of the cells or population of cells can comprise administration of about 10^4 to 10^5 cells per kg body weight, 10^5 to 10^6 cells per kg body weight, 10^6 to 10^7 cells per kg body weight, 10^7 to 10^8 cells per kg body weight, or 10^8 to 10^9 cells per kg body weight. The cells or population of cells can be administered in one or more doses. In some embodiments, said effective amount of cells can be administered as a single dose. In some embodiments, said effective amount of cells can be administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the subject. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of

a given cell type for a particular disease or condition is within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodiments, an effective amount of cells or composition comprising those cells are administered parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

[0134] The methods can further comprise administering one or more agents to a subject prior to administering the engineered immune cells bearing a CAR and a chimeric cytokine receptor provided herein. In certain embodiments, the agent is a lymphodepleting (preconditioning) regimen. For example, methods of lymphodepleting a subject in need of such therapy comprise administering to the subject specified beneficial doses of cyclophosphamide (between 200 mg/m²/day and 2000 mg/m²/day, about 100 mg/m²/day and about 2000 mg/m²/day; *e.g.*, about 100 mg/m²/day, about 200 mg/m²/day, about 300 mg/m²/day, about 400 mg/m²/day, about 500 mg/m²/day, about 600 mg/m²/day, about 700 mg/m²/day, about 800 mg/m²/day, about 900 mg/m²/day, about 1000 mg/m²/day, about 1500 mg/m²/day or about 2000 mg/m²/day) and specified doses of fludarabine (between 20 mg/m²/day and 900 mg/m²/day, between about 10 mg/m²/day and about 900 mg/m²/day; *e.g.*, about 10 mg/m²/day, about 20 mg/m²/day, about 30 mg/m²/day, about 40 mg/m²/day, about 40 mg/m²/day, about 50 mg/m²/day, about 60 mg/m²/day, about 70 mg/m²/day, about 80 mg/m²/day, about 90 mg/m²/day, about 100 mg/m²/day, about 500 mg/m²/day or about 900 mg/m²/day). An exemplary dosing regimen involves treating a subject comprising administering daily to the patient about 300 mg/m²/day of cyclophosphamide in combination or before or after administering about 30 mg/m²/day of fludarabine for three days prior to administration of a therapeutically effective amount of engineered immune cells to the patient.

[0135] In some embodiments, notably in the case when the engineered cells provided herein have been gene edited to eliminate or minimize surface expression of CD52, lymphodepletion further comprises administration of an anti-CD52 antibody, such as alemtuzumab. In some embodiments, the CD52 antibody is administered at a dose of about 1-20 mg/day IV, *e.g.*, about 13 mg/day IV for 1, 2, 3 or more days. The antibody can be

administered in combination with, before, or after administration of other elements of a lymphodepletion regime (e.g., cyclophosphamide and/or fludarabine).

[0136] In certain embodiments, compositions comprising CAR-expressing immune effector cells disclosed herein may be administered in conjunction with any number of
5 chemotherapeutic agents.

IV. Kits and Articles of Manufacture

[0137] The present disclosure provides kits comprising any one or more of the chimeric cytokine receptors and chimeric cytokine receptor-bearing cells described herein, and pharmaceutical compositions thereof. The present disclosure also provides articles of
10 manufacture comprising any one or more of the chimeric cytokine receptors and chimeric cytokine receptors-bearing CAR-I-cells described herein, pharmaceutical compositions thereof, and kits described herein.

[0138] The following examples are included for illustrative purposes and are not intended to limit the scope of the disclosure.

15 [0139] All patent and non-patent documents referenced throughout this disclosure are incorporated by reference herein in their entirety for all purposes.

EXAMPLES

Example 1: Construction and testing of chimeric cytokine receptor-CAR constructs having a TGFβR1 or TGFβR2 dominant negative truncation

20 [0140] FIG. 1 shows a schematic of the inducible chimeric cytokine receptor of the disclosure. To couple simultaneous TGF-β engagement with cytokine signaling, a chimeric cytokine receptor was constructed, composed of the following modules: (i) a binding domain comprising an extracellular portion of a TGF-β receptor, or a TGF-β antigen binding domain; (ii) a transmembrane domain with an intracellular portion having a JAK2-activating domain
25 and (iii) STAT-recruiting domains comprising STAT-recruiting (STAT-activating) domains from cytokine receptor tails (cytotails). As shown as an example in FIG. 1, the binding domain comprises the extracellular domain of TGFβR2.

[0141] A HEK293T cell reporter assay was used to test the inducibility and magnitude of cytokine signaling using chimeric cytokine receptors for either neutralizing the TGF- β signaling or activating the STAT response, which can be used as a surrogate measurement for the cytokine ICD activation and cytokine signaling. Briefly, 20,000 HEK293T-cells were
5 plated into each well of a poly-L-lysine-coated 96-well flat-bottom plate and cultured overnight at 37 °C with 5% CO₂. A chimeric cytokine receptor-CAR construct (2.5 ng), a TGF- β or STAT-response element that drives Firefly Luciferase (100 ng; Promega), and Renilla Luciferase control reporter vector (1 ng; Promega) were mixed to a final volume of 5 μ l in Opti-MEM (Gibco) (“DNA mix”).

[0142] Cells were transfected with a BFP-EGFRvIII CAR construct where a BFP gene is in place of the chimeric cytokine receptor as a negative control. A dominant negative truncation of TGF β R2 (“TGF β R2 DN”) and a dominant negative truncation of TGF β 1 (“TGF β R1 DN”) were also constructed as additional controls to examine dominant negative effects in the absence of an intracellular cytokine signal. After incubating the DNA mixes with premixed
10 0.3 μ l Lipofectamine 2000 (Invitrogen) and 5 μ l Opti-MEM at room temperature for 20 minutes, the mixture having a total volume of 10 μ l was added to each well containing HEK293T cells. One day after transfection, a commercially available TGF- β 1 ligand (BioLegend, hereinafter in Examples 1-4 referred to as “TGF- β ”) was added to the culture for stimulation, to various final concentrations. After 20-24 hours of stimulation, TGF- β or
15 STAT5 reporter activity was evaluated using the Dual-Glo Luciferase Assay System (Promega). Fold induction of TGF- β or STAT5 reporter activity was normalized to that of HEK293T cells that were transfected with only a reporter vector, and left untreated.

[0143] FIG. 2A shows a schematic of the lentiviral vector used to co-express the dominant negative truncations of the TGF β R1 or TGF β R2 chimeric cytokine receptor with the 2nd
25 generation EGFRvIII CAR.

[0144] FIG. 2B shows the inhibition of the TGF- β signaling by expression of either the TGF β R1 DN or TGF β R2 DN (shown by FIG. 2A, and comprising the amino acid sequences of SEQ ID NOs 2 and 3, respectively). The data shown indicates that the TGF β R2 DN chimeric cytokine receptor inhibits TGF- β signaling induced by the TGF- β ligand (up to 100
30 ng/ml), with higher efficacy than the TGF β R1 DN. This is likely due to the higher affinity of TGF β R2 binding to the TGF- β ligand in comparison to the binding affinity of TGF β R1

binding to the TGF- β ligand (described in Groppe et al., 2008, Mol. Cell, 29(2):157-68). Consequentially, the designs of the following examples focus on the chimeric cytokine receptors having the binding domain of TGF β R2.

Example 2: Designs and testing of inducible chimeric cytokine receptors using TGF β R2

5

[0145] A chimeric cytokine receptor was constructed, as briefly described when referring to FIG. 1, having a binding domain derived from TGF β R2 (“TGF β R2 chimeric cytokine receptor”). To investigate the utility of the TGF β R2 chimeric cytokine receptor in the context of CAR-T cells, variants of TGF β R2 extracellular domains (ECD) and variants of TPOR transmembrane (TM) domains were constructed. Fusions of each TGF β R2 ECD variant, each TPOR TM domain variant, and the intracellular domains (ICD) of desired cytokine receptors were cloned into a lentiviral vector encoding a 2nd generation EGFRvIII-specific CAR (2173scFv; described in *Sci Transl Med.* 2015 Feb 18; 7(275): 275ra22), and the activity of these receptor variants was tested. To permit stoichiometric co-expression of the chimeric cytokine receptor and the CAR, both genes were linked via a P2A peptide (“chimeric cytokine receptor-CAR construct”). To facilitate the detection of transduced cells, a v5 epitope tag (SEQ ID NO: 152) was inserted between the scFv and CD8 hinge domain.

10

15

[0146] FIG. 3 shows a general schematic of the lentiviral vector used to co-express the TGF β R2 chimeric cytokine receptor with the 2nd generation EGFRvIII CAR. One or more cytotaits or recruiting domains may be joined in tandem to mimic signaling from one or more cytokines.

20

[0147] FIGS. 4A-4C show the inhibition of TGF- β signaling by the overexpression of chimeric cytokine receptors constructed using TGF β R2. The lentiviral vectors used were constructed similarly as described in Example 1. FIG. 4A shows a schematic of the prototypic lentiviral vector used, bearing the IL7R(316-459) and the IL12Rb2(775-825) cytotoail or recruiting domains to mimic IL7 and IL12 signaling in CAR-T-cells. A variety of truncations in the transmembrane domain of the TpoR cassette were designed (as shown in Table 3). The capacity of these truncations to regulate cytokine signaling was determined. FIG. 4B shows TGF- β signaling activity determined by a luciferase reporter assay. All the tested chimeric cytokine receptors constructed using TGF β R2 were shown to be able to inhibit TGF- β signaling as they compete for binding via the extracellular domain of TGF β R2 in these

25

30

engineered chimeric receptors. FIG. 4C shows the activation of the chimeric cytokine receptors of FIG. 4A in the presence of TGF- β . The activation of the chimeric cytokine receptor is measured by STAT reporter activity. Several variants were identified to have the ability to induce cytokine signaling by a TGF- β ligand. The amino acid sequences of the transmembrane domains listed in the X-axes of FIGS. 4B-4C are SEQ ID NO: 29 to SEQ ID NO: 40 and SEQ ID NO: 50 to SEQ ID NO: 57, presented in Table 3.

[0148] FIGS. 5A-5C show the inhibition of TGF- β signaling by the overexpression of additional chimeric cytokine receptors constructed using TGF β R2. The lentiviral vectors used were constructed similarly as described when referring to FIGS. 4A-4C, and Example 1. FIG. 5A shows a schematic of the prototypic lentiviral vector used, having truncations in the binding domain.

[0149] Again, additional truncations (N-10, N-11, N-12, etc.) in the TM domain of TpoR cassette were designed (as shown in Table 3), and their capacity to regulate cytokine signaling was determined. FIG. 5B shows TGF- β signaling activity determined by a luciferase reporter assay. Most of the chimeric cytokine receptors tested were shown to be able to inhibit TGF- β signaling (other than the N-12, N-13, N-14 TM truncations, which show less extent of inhibition). FIG. 5C shows the activation of the chimeric cytokine receptors of FIG. 5A in the presence of TGF- β . The activation of the chimeric cytokine receptors is measured by the STAT reporter activity. Several variants were identified to have the ability to induce cytokine signaling by a TGF- β ligand. The amino acid sequences of the transmembrane domains listed in the X-axes of FIGS. 5B-5C are SEQ ID NOs: 29, 38, 53, SEQ ID NO: 40 to SEQ ID NO: 44, and SEQ ID NO: 61 to SEQ ID NO: 72, presented in Table 3.

Example 3: Modifications of the chimeric cytokine receptor binding domain and testing of the constructed chimeric cytokine receptors

[0150] In the absence of TGF β R2, TGF β R1 interacts with the TGF- β ligand with very low affinity. Once the ECD of TGF β R2 binds to the TGF- β ligand, the binary complex has an extended interface to efficiently recruit TGF β R1 to form the ternary complex. The engineered TGF β R2 chimeric cytokine receptor can also engage endogenous TGF β R1, which may sterically intervene the intended signaling through the cytokine receptor ICDs. To abrogate interaction between the TGF β R2 chimeric cytokine receptors and TGF β R1, several variants

for the TGF β R1 cassette were designed, and modifications that can enhance cytokine signaling while inhibiting the TGF- β signaling were identified.

[0151] FIGS. 6A-6C show the inhibition of TGF- β signaling by the expression of chimeric cytokine receptors constructed with TGF β R2 having modifications. FIG. 6A shows a schematic of the modification introduced into the TGF β R2 cassette. The lentiviral vectors used were constructed similarly as described in Example 1. To abolish the engagement of TGF β R1, a truncation (Δ N25) was introduced into the TGF β R2 binding domain based on the previous constructs described when referring to FIGS. 6A-6C (“TGF β R2 Δ N25”), and the inhibition of TGF- β signaling was tested by a TGF- β reporter assay. FIG. 6B shows that the TGF β R2 Δ N25 chimeric cytokine receptors still retained the ability to inhibit TGF- β signaling. FIG. 6C shows the activation of the chimeric cytokine receptors of FIG. 6A, evaluated by the STAT reporter activity. The amino acid sequences of the transmembrane domains listed in the X-axes of FIGS. 6B-6C are SEQ ID NOs: 29, 38, 39, 40, and 53, with a binding domain sequence of SEQ ID NO: 4 or SEQ ID NO: 3, presented in Tables 1 and 3.

[0152] It was determined that the truncation in the TGF β R2 binding domain enhances the cytokine signaling by 5~10 fold, even in the absence of a TGF- β ligand. Interestingly, the Δ N25 truncation was able to enhance the signaling synergistically with the TpoR TM truncations (e.g. N-7, N-8, N-9 and N+4). This combinatorial use of the TGF β R2 binding domain and TpoR TM truncation mutants represents a novel approach for simultaneously inhibiting immunosuppressive TGF- β signaling while transmitting immune-potentiating cytokine signaling.

Example 4: Design and testing of the constitutively active chimeric cytokine receptor

[0153] FIG. 7 shows a schematic of the constitutively active chimeric cytokine receptor. To enable constitutively active signaling of the cytokine intracellular domains, double mutations that inherently dimerize the TPOR transmembrane domain and activate the JAK-STAT pathway were introduced in the TPOR transmembrane domain. As shown as an exemplary construct in FIG. 7, the binding domain comprises the extracellular portion of TGF β R2; exemplary transmembrane domains may comprise the SEQ ID NOs 29, 40, 53 or 60.

[0154] FIGS. 8A-8C show the design and tested function of the constitutively active chimeric cytokine receptors. The lentiviral vectors used were constructed similarly as

described in Example 1. A double mutant (S505N, W515K) was introduced into the TPOR cassette to enforce the dimerization and activation of the receptors, in combination with variants that had shown the most promising functionality (e.g. TGF β R2 Δ N25, N-9 and N+4 truncations in the TPOR cassette). FIG. 8A shows a schematic of the modification introduced into the TGF β R2 cassette. FIG. 8B shows the inhibition of TGF- β signaling by the expression of the chimeric cytokine receptors of FIG. 8A. FIG. 8C shows the activation of cytokine signaling via the chimeric cytokine receptors of FIG. 8A, measured by the STAT5 reporter activity in 293 cells. Compared to the parental designs (TGF β R2_TpoR, TGF β R2_TpoR_N-9, and TGF β R2_TpoR_N+4), receptors with the S505N and W515K mutations display substantial cytokine signaling (e.g. TGF β R2_TpoR.S505.W515K, TGF β R2_TpoR_N-9.S505N.W515K). The sequences of the transmembrane domains listed in the X-axes of FIGS. 8B-8C comprise SEQ ID NOs: 40, 53, 59, and 60 with a binding domain sequence of SEQ ID NO: 4 or SEQ ID NO: 3, presented in Tables 1 and 3.

Example 5 Testing of additional chimeric cytokine receptors

[0155] FIG. 10A shows additional design of inducible TGF- β -driven chimeric cytokine receptors. Constructs with further truncations in the transmembrane domain to decrease the flexibility between the ECD and intracellular signaling domain were made. FIG. 10B shows the inhibition of endogenous TGF- β signaling as determined by luciferase assay by the overexpression of TGF- β -driven chimeric cytokine receptors shown in FIG. 10A in 293 cells in the presence of different concentrations of TGF- β . Some of the constructs also carry the Δ N25 deletion in the ECD domain ("TGF- β R2 Δ N25"). All TGF- β -driven chimeric cytokine receptors in FIG. 10B inhibited the endogenous TGF- β signaling, although clones with the Δ N25 deletion showed slightly decreased inhibition of the TGF- β signaling. The data in FIG. 10C show the activation of chimeric cytokine receptors in the presence of TGF- β at various concentrations. Chimeric receptors with the deletion in the transmembrane domain and Δ N25 deletion induced STAT5 signaling in the presence of TGF- β .

[0156] We next tested the constitutive chimeric receptors in CAR T cells. All constructs tested in FIGs. 11-16 contain the S505N and W515K substitutions in the TPOR/MPLR transmembrane domain. FIG. 11A shows schematics of the lentiviral vectors used to co-express in CAR T cells the TGF- β R2 chimeric cytokine receptors with the EGFRvIII-specific CAR (2173scFv). All chimeric cytokine receptors carry dimerization mutations in the transmembrane domain of TPOR/MPLR (S505N, W515K, see SEQ ID NO:60), and one or

more cytokine receptor recruiting domains joined in tandem to mimic signaling from one or more cytokines. “IL2YY” refers to IL2Rb (393-433, 518-551), and “IL7IL12” refers to IL7R(316-459) and IL12Rb2(775-825) joined in tandem. As controls, CAR T cells were also produced, expressing a dominant negative truncation of TGF- β R2 (“TGF- β R2.DN”),

5 constitutive chimeric cytokine receptor without the TGF- β R2 ECD (“IL7IL12”, “IL2YY”) or a BFP protein. The method to produced CAR T cells were as described in Sommer C, et al. Preclinical Evaluation of Allogeneic CAR T Cells Targeting BCMA for the Treatment of Multiple Myeloma. *Mol Ther.* 2019. doi:10.1016/j.ymthe.2019.04.001 and Sommer C, et al. Allogeneic FLT3 CAR T Cells with an Off-Switch Exhibit Potent Activity against AML and
10 Can Be Depleted to Expedite Bone Marrow Recovery. *Mol Ther.* 2020.

doi:10.1016/j.ymthe.2020.06.022. In brief, primary T cells from a healthy donor were transduced at MOI of 5, with lentiviruses expressing the CAR and each of the TGF β R2 chimeric cytokine receptors or controls. FIG. 11B shows the percentage of CAR⁺ T cells at day 7, 9 and 14 during CAR T cell production. CAR T cells with a constitutive chimeric
15 cytokine receptor as indicated (“TGF- β R2.IL7IL12,” “TGF- β R2.IL2YY,” “IL7IL12,” or “IL2YY”) show higher proliferation and enrichment as compared to BFP alone over the production period. As the result, more EGFRvIII CAR⁺ T cells were obtained over time as compared to CAR T expressing BFP alone without a chimeric cytokine receptor (FIG. 11C).

[0157] FIGs. 11D and 11E show results of assessing STAT 5 phosphorylation in the CAR
20 T cells co-expressing either the various chimeric cytokine receptors or controls. The antibody used for detecting phosphorylated STAT5 was from BD Biosciences (BDB612599). CAR T cells with constitutive chimeric cytokine receptor with an TGF- β R2 ECD, i.e., TGF- β R2.IL7IL12 or TGF- β R2.IL2YY exhibited higher level of STAT5 phosphorylation, even in the absence of TGF β , as compared to IL2YY and IL7IL12, i.e., constitutive chimeric
25 cytokine receptors without a TGF- β R2 ECD, indicating stronger cytokine signaling with a TGF- β R2 ECD domain. The data suggest that the TGF- β R2 ECD improved the intercellular recruiting domain signaling in CAR T cells.

[0158] The data in FIGs. 11F and 11G show that the chimeric cytokine receptors regulated the differentiation of CAR T cells at Day 14 of CAR T production. CD62L and CD45RO
30 staining was conducted using the antibodies from BioLegend (#304822) and BioLegend (#304234), respectively. Notably, CAR T cells expressing the TGF- β R2.IL7IL12 chimeric

cytokine receptor largely differentiated into central memory T cells (CD62L^{hi}CD45RO^{hi}) – a result likely due to strong IL12Rb signaling, while CAR T cells expressing the TGFbR2.IL2YY or IL2YY chimeric cytokine receptor exhibited an enriched population of stem cell-like (stem) memory T cell (CD62L^{hi}CD45RO^{low}), a desirable T cell phenotype that has been associated with better clinical outcome.

[0159] Next, we compared the expression of TGF-βR2 chimeric cytokine receptor to the expression of endogenous TGF-βR2 by measuring total surface ECD by flow cytometry using an anti-human TGF-βR2 polyclonal antibody (R&D Systems, FAB2411A100). The results in FIG. 11H show an MFI of the ECD staining at about 1400-2300 resulting from the endogenous TGF-βR2 in the CAR T cells expressing the IL7IL12 or IL2YY chimeric cytokine receptor and CAR T cells expressing BFP. CAR T cells expressing a TGF-βR2 chimeric cytokine receptor (TGF-βR2.IL7IL12 and TGF-βR2.IL2YY) showed a 2-3 folds excessive MFI value, indicating a 2-3 folds higher levels of the TGF-βR2 ECD staining over the levels from endogenous TGF-βR2 receptor. FIG. 11I shows the level of SMAD phosphorylation within CAR T cells expressing different chimeric cytokine receptors or controls when exposed to various concentrations of TGFβ. The antibody used for detecting phosphorylated SMAD was from BD Biosciences (BDB562586). The results show that CAR T cells expressing the TGF-βR2.IL7IL12 and TGF-βR2.IL2YY chimeric cytokine receptors exhibited decreased SMAD phosphorylation as compared to the CAR T cells expressing the IL7IL12 and IL2YY chimeric cytokine receptors without the TGF-βR2 ectodomain, respectively.

[0160] We next evaluated anti-tumor activities of the EGFRvIII CAR T cells expressing different chimeric cytokine receptors or controls against the target cells U87-EGFRvIII cells. In brief, CAR T cells were incubated with 10,000 target cells at E: T ratio of 1:2 in 200 ul of RPMI medium with 10% of FBS, and TGF-β at various concentrations of 0, 5, and 20 ng/ml. After one week of co-culture with target cells, the CAR T cells in 100 ul supernatant were transferred into new target cells (10,000) with the same TGFβ concentrations as the previous week. The cytotoxicity of the CAR T cells in the second week without added TGF-β was assessed and the results are shown in FIG.12A. In the absence of exogenous TGFβ, CAR T cells expressing TGF-βR2 chimeric cytokine receptors (TGF-βR2.IL7IL12 and TGF-βR2.IL2YY) showed the most persistent cytotoxicity, with most of the target cells killed by

the CAR T cells. CAR T cells expressing chimeric cytokine receptors without the TGF- β R2 ECD (IL7IL12 and IL2YY) also exhibited considerable cytotoxicity and inhibited the growth of the U87 cells till the second week. In comparison, CAR T cells with BFP or TGF- β R2.DN lost the activity and failed to inhibit the growth of the U87 cells (FIG. 12A). The data indicate
 5 that TGF- β R2 chimeric cytokine receptors enable more persistent cytotoxicity against the target cells. In the presence of TGF β , the activity of CAR T cells without expressing a chimeric cytokine receptor with a TGF β R2 ECD was inhibited to various levels by TGF- β , likely the effect of signal transduction via the endogenous TGF- β receptors (FIGs. 12B-12C). In contrast, CAR T cells expressing TGF- β R2 chimeric cytokine receptors were resistant to
 10 TGF- β inhibition and were able to maintain strong cytotoxicity in conditions up to 20 ng/ml of TGF- β (FIGs. 12B, 12C).

[0161] We next evaluated further modified constitutive TGF- β R2 chimeric cytokine receptor in CAR T cells. As shown above constitutive TGF β R2.IL7IL12 chimeric cytokine receptor having the S505N/W515K substitutions in the TPOR/MPLR transmembrane domain
 15 and the IL7Ra/IL12Rb recruiting domains (e.g., SEQ ID NO:163) increased STAT5 phosphorylation and led to substantial differentiation of central memory T cells. As the IL12 cytokine signaling has been implicated in the differentiation of memory T cells, we designed the TGF- β R2.IL7 chimeric cytokine receptor that eliminates the IL12 signaling. In addition, we introduced two mutations in the TpoR JAK-binding domain, K553R and K573R,
 20 (designated as “RR”, e.g., SEQ ID NOS:165 and 170), to decrease ubiquitin-induced receptor degradation. See Saur SJ, Sangkhae V, Geddis AE, Kaushansky K, Hitchcock IS. Ubiquitination and degradation of the thrombopoietin receptor c-Mpl. *Blood*. 2010. doi:10.1182/blood-2009-06-227033. CAR T cells expressing the chimeric cytokine receptor were produced and evaluated for STAT5 phosphorylation and T cell differentiation. Similar
 25 as before, these further modified TGF- β R2 chimeric cytokine receptors showed higher levels of STAT5 signaling as determined by STAT5 phosphorylation than the chimeric cytokine receptors without the TGF- β R2 ECD domain (both the IL7IL12 and IL7 chimeric cytokine receptor constructs contain the S505N/W515K substitutions) (FIG. 13A). In the assessment of T cell differentiation, CAR T cells expressing chimeric cytokine receptors without the
 30 IL12R recruiting domain (TGF- β R2.IL7, TGF- β R2.IL7_RR, and IL7) showed an increased proportion of stem memory T cell than their counterparts with the IL12R recruiting domain

(FIG. 13B). These data demonstrate that by selecting different cytokine receptor motifs, we can modulate the TGF- β R2 chimeric cytokine receptors signaling and T cell differentiation.

[0162] To evaluate how the TGF- β R2 chimeric cytokine receptors affect T cell functions, CAR T cells were evaluated in long-term killing assay. In brief, CAR T cells expressing different chimeric cytokine receptor were mixed with 10,000 U87-EGFRvIII cancer cells at an E:T ratio of 1:1, in 200 μ l RPMI medium with 10% FBS, with or without 5 ng/ml of TGF β . Every two or three days, 100 μ l of the supernatant with CAR T cells were transferred onto 10,000 fresh target cells to the final volume of 200 μ l RPMI medium with 10% FBS and 5 ng/ml of TGF β , and the survival of old target cells were quantified. The long-term cytotoxicity of CAR T cells with different TGF- β R2 chimeric cytokine receptors are summarized in FIGs. 14A-B. In the absence of exogenous TGF β , TGF- β R2 chimeric cytokine receptors with various recruiting domains, for example, TGF- β R2.IL2YY, TGF- β R2.IL7, or TGF- β R2.IL2YYY(IL2Rb(339-379,393-433,518-551)) all conferred more potent and durable cytotoxicity than CAR T cells expressing chimeric cytokine receptors without the TGF- β R2 ECD, confirming the previous observations that the TGF- β R2 ECD domain enhanced the chimeric cytokine receptor signaling (FIG. 14A). In the presence of 5 ng/ml TGF β , CAR T cells expressing the TGF- β R2 chimeric cytokine receptor maintained more potent and persistent cytotoxicity than CAR T cells with or without expressing a chimeric cytokine receptor without a TGF- β R2 ECD (FIG. 14B). The chimeric cytokine receptors tested all contain the S505N/W515K substitutions, and the TGF β R2.IL2YYY and IL2YYY constructs have the additional H499L substitution in the TOPR/MPLR transmembrane domain.

[0163] To further assess the inhibition on TGF β signaling by a TGF- β R2 chimeric cytokine receptor and its influence on the functionality of CAR T cells, we analyzed the TGF- β R2.IL2YY_RR chimeric cytokine receptor (SEQ ID NO: 166), which carries the dimerization mutations in the TM region (S505N, W515K) and the degradation-resistant mutations (K553R and K573R) in the JAK-binding domain. We designed two additional variants bearing mutations in the TGF- β R2 ECD (D32A.E119A and D32A.E119A.I53A) that abolished the receptor's ability to bind the TGF β ligand. CAR T cells expressing the designated chimeric cytokine receptors were produced and evaluated for cytokine signaling (pSTAT5), TGF β signaling (pSMAD), and persistency of cytotoxicity against U87-EGFRvIII

target cells. FIG. 15A shows the analysis of STAT5 signaling in the CAR T cells, in which all the TGF- β R2 chimeric cytokine receptors led to strong STAT5 phosphorylation in CAR T cells, comparing to the T cells expressing only the CAR. When the CAR T cells were exposed to 5 ng/ml of TGF β ligand, various levels of SMAD phosphorylation were observed.

5 Comparing to TGF- β R2 chimeric cytokine receptor with the loss-of-binding mutants, TGF- β R2.IL2YY_RR with the wildtype TGF β binding domain exhibited a decreased level of SMAD phosphorylation, indicating that the endogenous TGF β signaling was successfully inhibited by the TGF- β R2 chimeric cytokine receptor with a wildtype, functional ligand binding domain (FIG. 15B).

10 **[0164]** The long-term cytotoxicity of CAR T cells expressing different TGF- β R2 chimeric cytokine receptor against cancer cells in the presence of 5ng/ml TGF β was shown in FIG. 15C. Comparing to the control CAR T cells, which quickly lost the activity to kill target cells at day 8, CAR T cells with TGF- β R2 chimeric cytokine receptor conferred more durable activity. TGF- β R2 chimeric cytokine receptors with impaired ability to bind to TGF β

15 (D32A.E119A and D32A.E119A.I53A) also showed a faster decline in the cytotoxicity assay than CAR T cells expressing the TGF- β R2.IL2YY_RR chimeric cytokine receptor with a wildtype TGF β binding ECD. These data demonstrate that both the cytokine signaling and the ability to inhibit endogenous TGF β signaling are important for the long-term activity of CAR T cells.

20 **[0165]** Results in FIGs. 16A-B show that the degradation-resistant mutations, K553R and K573R, can further improve the functionality of the constitutive TGF- β R2 chimeric cytokine receptors. In this experiment, CAR T cells expressing TGF- β R2 chimeric cytokine receptors with or without the degradation-resistant mutations (designated as “RR”) were evaluated for the strength of the cytokine signaling as well as the long-term cytotoxicity assay against U87-

25 EGFRvIII in the medium with 5 ng/ml of TGF β ligand. The TGF- β R2 chimeric cytokine receptor with the K553R and K573R substitutions consistently display a stronger STAT5 phosphorylation (FIG. 16A) and a more durable capability to kill the target cells (FIG. 16B).

WHAT IS CLAIMED IS

1. A chimeric cytokine receptor comprising:
 - a. a binding domain comprising an extracellular portion of a TGF- β receptor, or a TGF- β antigen binding domain;
 - 5 b. a transmembrane domain;
 - c. a Janus Kinase (JAK)-binding domain; and
 - d. a recruiting domain.
2. The chimeric cytokine receptor of claim 1, wherein the recruiting domain is a STAT-recruiting domain.
- 10 3. The chimeric cytokine receptor of claim 1 or 2, wherein the recruiting domain is from a receptor, optionally a cytokine receptor.
4. The chimeric cytokine receptor of any one of claims 1 to 3, wherein the chimeric cytokine receptor is clustered.
5. The chimeric cytokine receptor of any one of claims 1 to 3, wherein the chimeric
15 cytokine receptor is dimerized, and each monomer comprises:
 - a. a binding domain comprising an extracellular portion of a TGF- β receptor, or a TGF- β antigen binding domain;
 - b. a transmembrane domain;
 - c. a Janus Kinase (JAK)-binding domain; and
 - 20 d. a recruiting domain.
6. The chimeric cytokine receptor of any one of claims 1 to 5, wherein the TGF- β antigen binding domain is a scFv.
7. The chimeric cytokine receptor of any one of claims 1 to 5, wherein the binding domain comprises an extracellular portion of a wild type TGF- β receptor sequence.

8. The chimeric cytokine receptor of any one of claims 1 to 5, wherein the binding domain comprises one or more mutations to the extracellular portion of a wild type TGF- β receptor sequence.
9. The chimeric cytokine receptor of any one of claims 1-5, wherein the binding domain
5 comprises the extracellular portion of TGF β R2.
10. The chimeric cytokine receptor of any one of claims 1-5, wherein the binding domain comprises an amino acid sequence of any one of SEQ ID NO: 2 to SEQ ID NO: 20 and SEQ ID NO:159.
11. The chimeric cytokine receptor of any one of claims 1 to 10, wherein the chimeric
10 cytokine receptor is constitutively active or is activated when the binding domain is bound to a TGF- β ligand.
12. The chimeric cytokine receptor of claim 11, wherein the TGF- β ligand is any one of TGF β -1, TGF- β 2, or TGF- β 3.
13. The chimeric cytokine receptor of any one of claims 1 to 10, wherein the chimeric
15 cytokine receptor is constitutively active or is activated when the binding domain is bound to an anti-TGF- β -receptor antibody.
14. The chimeric cytokine receptor of any one of claims 1 to 13, wherein the JAK-binding domain is a JAK1-binding domain, a JAK2-binding domain, a JAK3-binding domain or a TYK2-binding domain.
15. The chimeric cytokine receptor of any one of claims 1 to 14, wherein the
20 transmembrane domain is derived from EpoR, GP130, PrlR, GHR, GCSFR, or TPOR/MPLR receptor.
16. The chimeric cytokine receptor of any one of claims 1 to 15, wherein the transmembrane domain is derived from TPOR/MPLR receptor.
17. The chimeric cytokine receptor of claim 16, wherein the transmembrane domain and
25 JAK binding domain comprises amino acids 478 – 582 of the naturally occurring TPOR/MPLR receptor of SEQ ID NO: 26. The chimeric cytokine receptor of claim 17, wherein the transmembrane domain and JAK binding domain further comprises at

least one substitution at amino acid position H499, S505, W515, K553, or K573 of the TPOR/MPLR receptor.

18. The chimeric cytokine receptor of claim 17, wherein the transmembrane domain and JAK binding domain comprises at least one amino acid substitution selected from H499L, S505N, W515K, K553R, and K573R.
19. The chimeric cytokine receptor of claim 18, wherein the transmembrane domain and JAK binding domain of the TPOR/MPLR receptor comprises (a) the H499L substitution; (b) the S505N substitution; (c) the W515K substitution; (d) the S505N, W515K substitutions; or (e) the H499L, S505N, W515K substitutions.
20. The chimeric cytokine receptor of any one of claims 17-19, wherein the transmembrane domain and JAK binding domain of the TPOR/MPLR receptor further comprises the K553R and/or K573R substitution.
21. The chimeric cytokine receptor of any one of claims 1 to 20, wherein the transmembrane domain and JAK-binding domain comprises an amino acid sequence selected from SEQ ID NO: 27 to SEQ ID NO: 79, SEQ ID NO: 160 and SEQ ID NO: 217 to SEQ ID NO: 234.
22. The chimeric cytokine receptor of any one of claims 2 to 21, wherein the STAT-recruiting domain is from a receptor selected from the receptors presented in Table 4.
23. The chimeric cytokine receptor of any one of claims 2 to 22, wherein the recruiting domain comprises the STAT-recruiting domain from one or more receptors of IL7Ra, IL12Rb2, EGFR, IL-21R or IL2Rb.
24. The chimeric cytokine receptor of any one of claims 2 to 23, wherein the STAT-recruiting domain comprises any one of the amino acid sequences of SEQ ID NO: 80 – SEQ ID NO: 122 and SEQ ID NO: 161.
25. The chimeric cytokine receptor of any one of claims 2-24, wherein the STAT-recruiting domain comprises IL7Ra(316-459) (SEQ ID NO: 80), IL2Rb(339-379, 393-433, 518-551) (SEQ ID NO: 112), IL2Rb(393-433, 518-551) (SEQ ID NO: 111), IL12Rb2(775-825) (SEQ ID NO: 101), IL12Rb2(714-862) (SEQ ID NO: 120),

EGFR(1122-1165)(SEQ ID NO: 99), or IL7Ra(316-459).IL12Rb2(775-825)(SEQ ID NO:161).

26. The chimeric cytokine receptor of any one of claims 1 to 25, wherein the chimeric cytokine receptor comprises an amino acid sequence selected from SEQ ID NOs: 123-151 and SEQ ID NOs: 162-216.
27. The chimeric cytokine receptor of any one of claims 1-26, wherein the chimeric cytokine receptor is inducible or is constitutively active.
28. The chimeric cytokine receptor of claim 27, wherein the chimeric cytokine receptor can be induced by TGF β or an anti-TGF β R antibody.
29. The chimeric cytokine receptor of claim 28, wherein the chimeric cytokine receptor is constitutively active and can be further induced or exhibits further improved activities in the presence of TGF β or an anti-TGF β R antibody.
30. The chimeric cytokine receptor of any one of claims 1-29, wherein the chimeric cytokine receptor is capable of inhibiting TGF β R2-mediated signal transduction and/or enhancing STAT-mediated signal transduction when expressed in a cell.
31. The chimeric cytokine receptor of claim 30, wherein the cell is an immune cell.
32. A polynucleotide encoding the chimeric cytokine receptors of any one of claims 1 to 31.
33. An expression vector comprising the polynucleotide of claim 32.
34. The expression vector of claim 33, further comprising a polynucleotide expressing a chimeric antigen receptor (CAR).
35. The expression vector of claim 34, wherein the CAR binds to any one or more of the targets of Table 8.
36. The expression vector of any one of claims 33-35, wherein the vector is a lentiviral vector.
37. An engineered immune cell comprising the vector of any one of claims 33-36.

38. An engineered immune cell expressing the chimeric cytokine receptor of any one of claims 1-31.
39. The engineered immune cell of claim 37 or 38, further expressing at least one CAR.
40. The engineered immune cell of claim 39, wherein the CAR and the chimeric cytokine
5 receptor are expressed in stoichiometrically equal amounts.
41. The engineered immune cell of claim 39 or 40, wherein the CAR binds to any one or more of the targets of Table 8.
42. The engineered immune cell of any one of claims 37-41, wherein the immune cell is an allogeneic immune cell.
- 10 43. The engineered immune cell of any one of claims 37-41, wherein the immune cell is an autologous immune cell.
44. The engineered immune cell of any one of claims 37-43, wherein the immune cell is selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem
15 cell.
45. The engineered immune cell of claim 44, wherein the immune cell is a T-cell.
46. The engineered immune cell of any one of claims 37-45, wherein the immune cell exhibits reduced TGF- β R-mediated signal transduction and/or enhanced Stat-mediated signal transduction as compared to an immune cell without expressing a chimeric
20 cytokine receptor.
47. The engineered immune cell of any one of claims 37-46, wherein the immune cell exhibits reduced TGF- β R mediated signal transduction and/or enhanced Stat-mediated signal transduction when engaged with TGF- β or an anti-TGF- β R antibody.
48. A method of modulating an activity of the engineered immune cell of any one of
25 claims 37-47, comprising contacting the immune cell with TGF- β or with an anti-TGF- β receptor antibody.

49. A method of preparing an engineered immune cell, the method comprising introducing the polynucleotide of claim 32 or an expression vector of any one of claims 33-36 into an immune cell.
50. The method of claim 48 or 49, wherein the immune cell is selected from the group consisting of: T-cell, dendritic cell, killer dendritic cell, mast cell, NK-cell, macrophage, monocyte, B-cell and an immune cell derived from a stem cell.
51. A pharmaceutical composition comprising the immune cells of any one of claims 37-47.
52. A kit comprising the immune cells of any one of claims 37-47, or the pharmaceutical composition of claim 51.
53. A method of treating a cancer in a subject, comprising administering to the subject a therapeutically effective amount of the engineered immune cells of any one of claims 37-47 or the pharmaceutical composition of claim 51.
54. The method of claim 53, wherein the cancer comprises a solid tumor.
55. The method of claim 53, wherein the cancer comprises a liquid tumor.
56. The method of any one of claims 53-55, wherein the tumor is TGF β positive tumor.
57. The method of any one of claims 53-56, wherein the subject is treated with an anti-TGF- β -receptor antibody.

FIG. 1

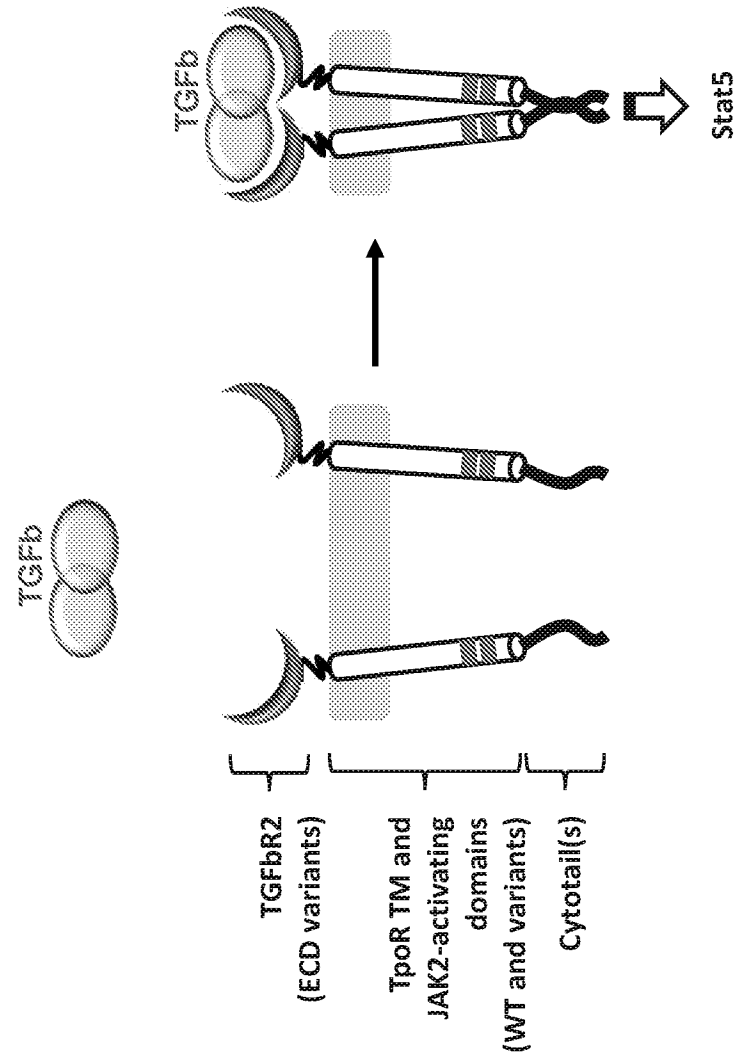


FIG. 2A

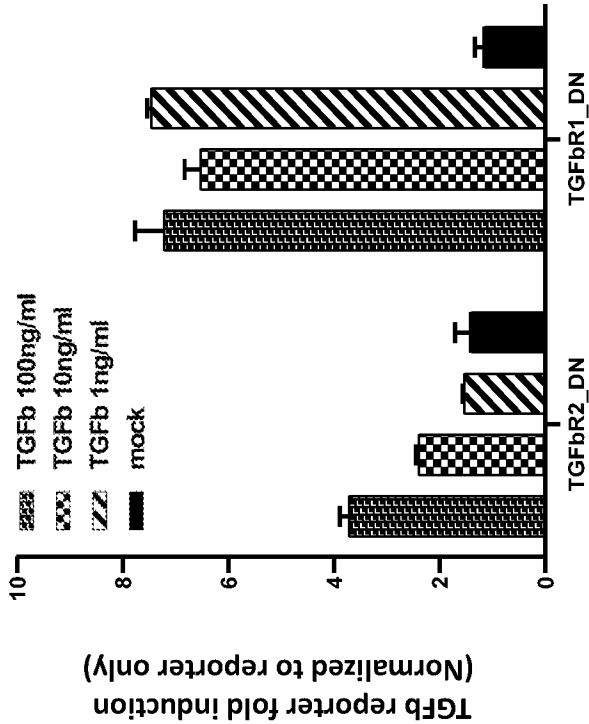
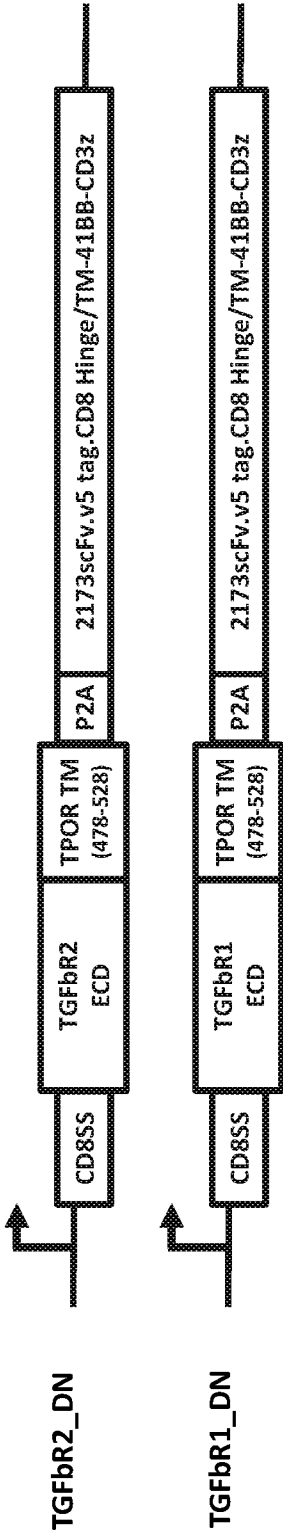


FIG. 2B

FIG. 3

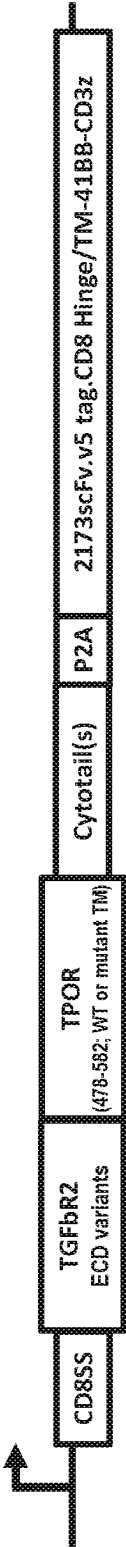


FIG. 4A

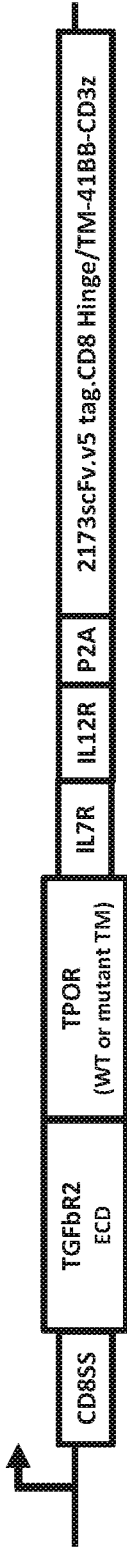
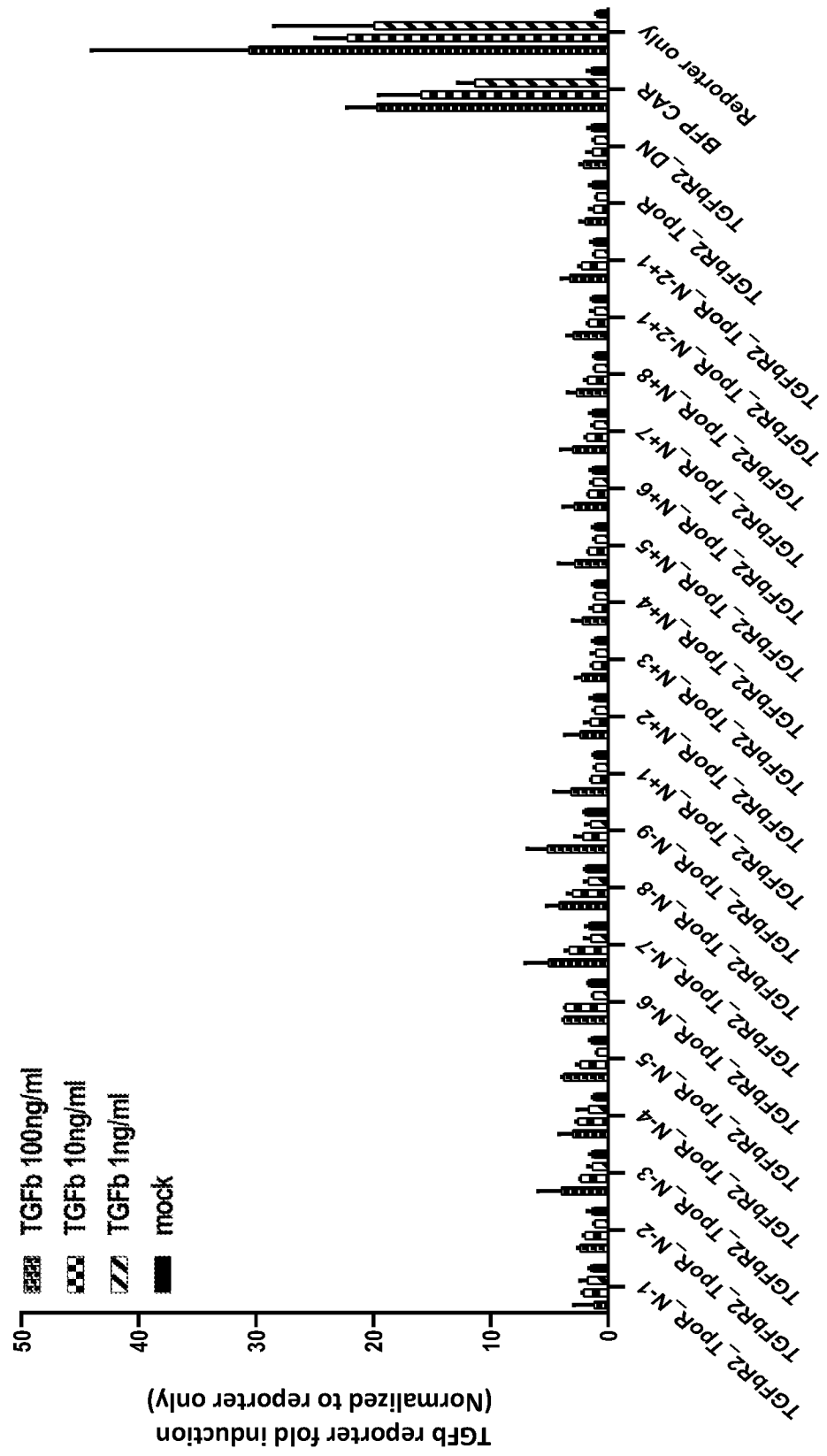


FIG. 4B



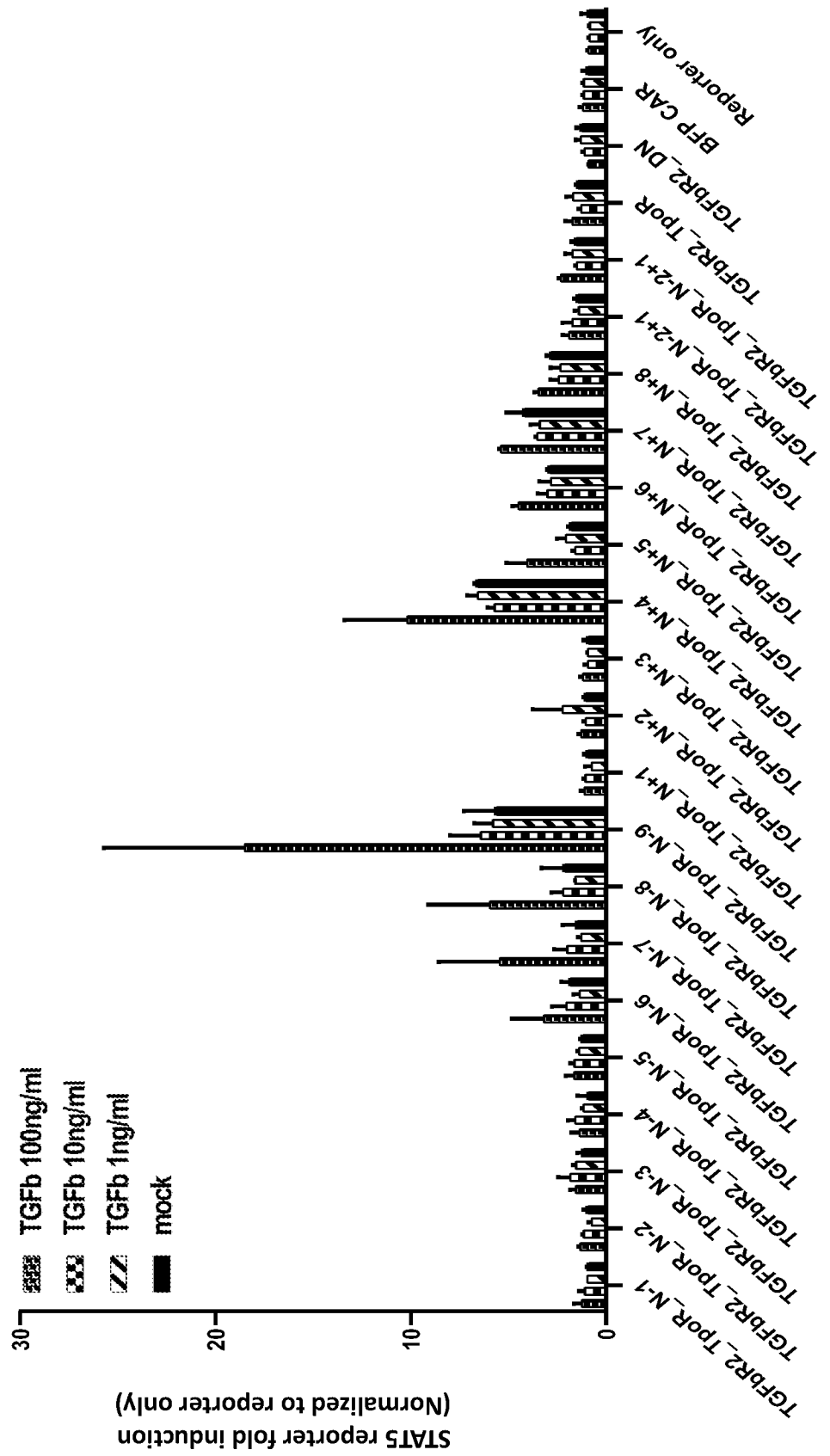


FIG. 5A

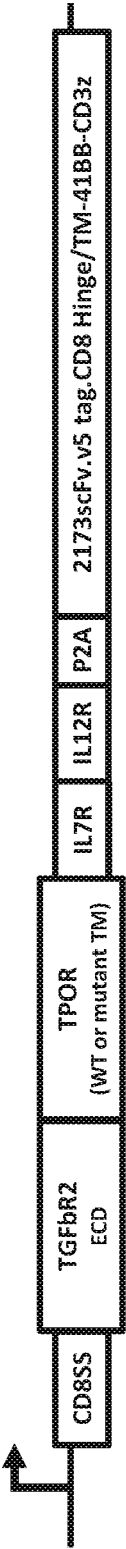


FIG. 5B

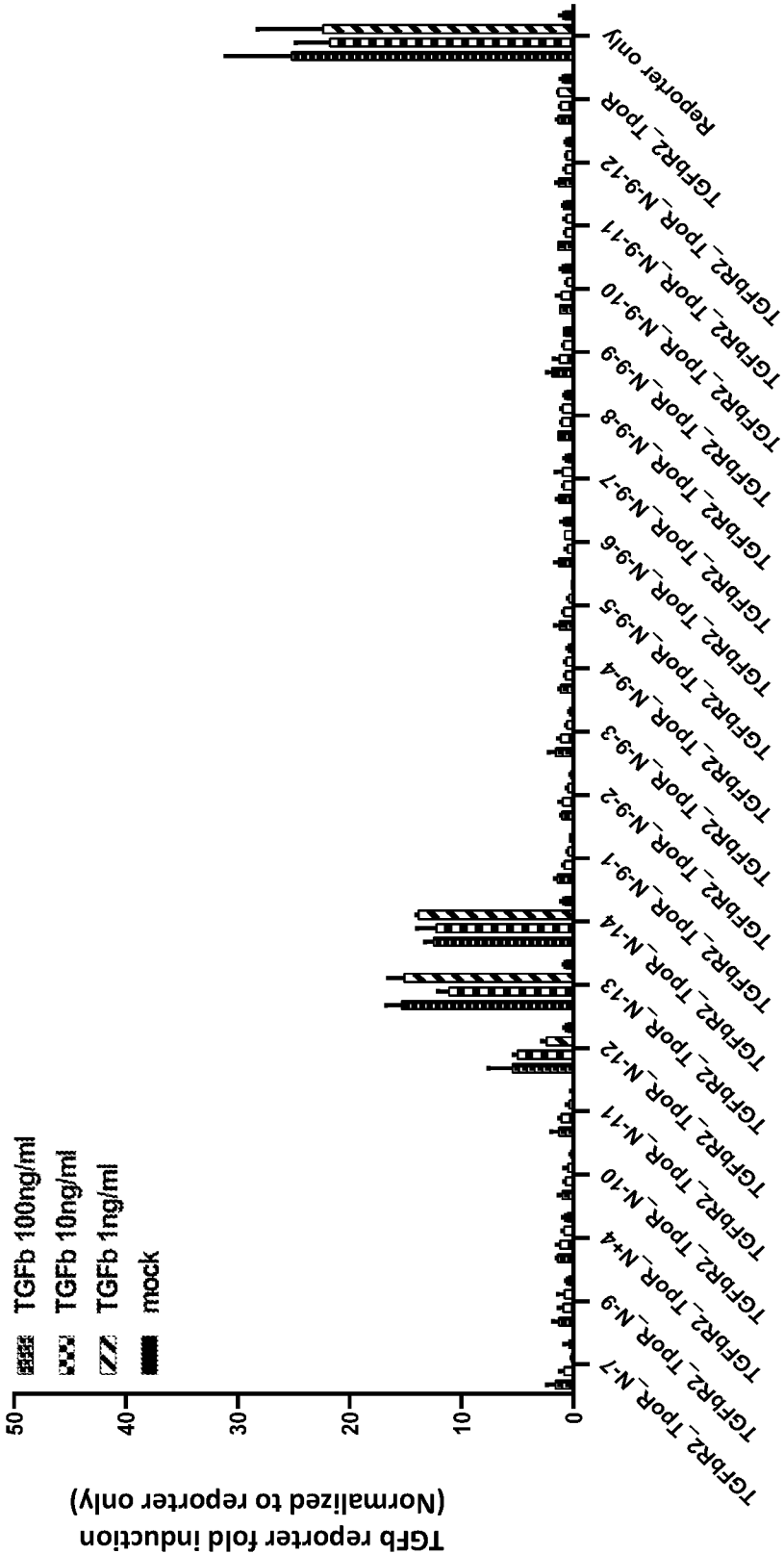


FIG. 5C

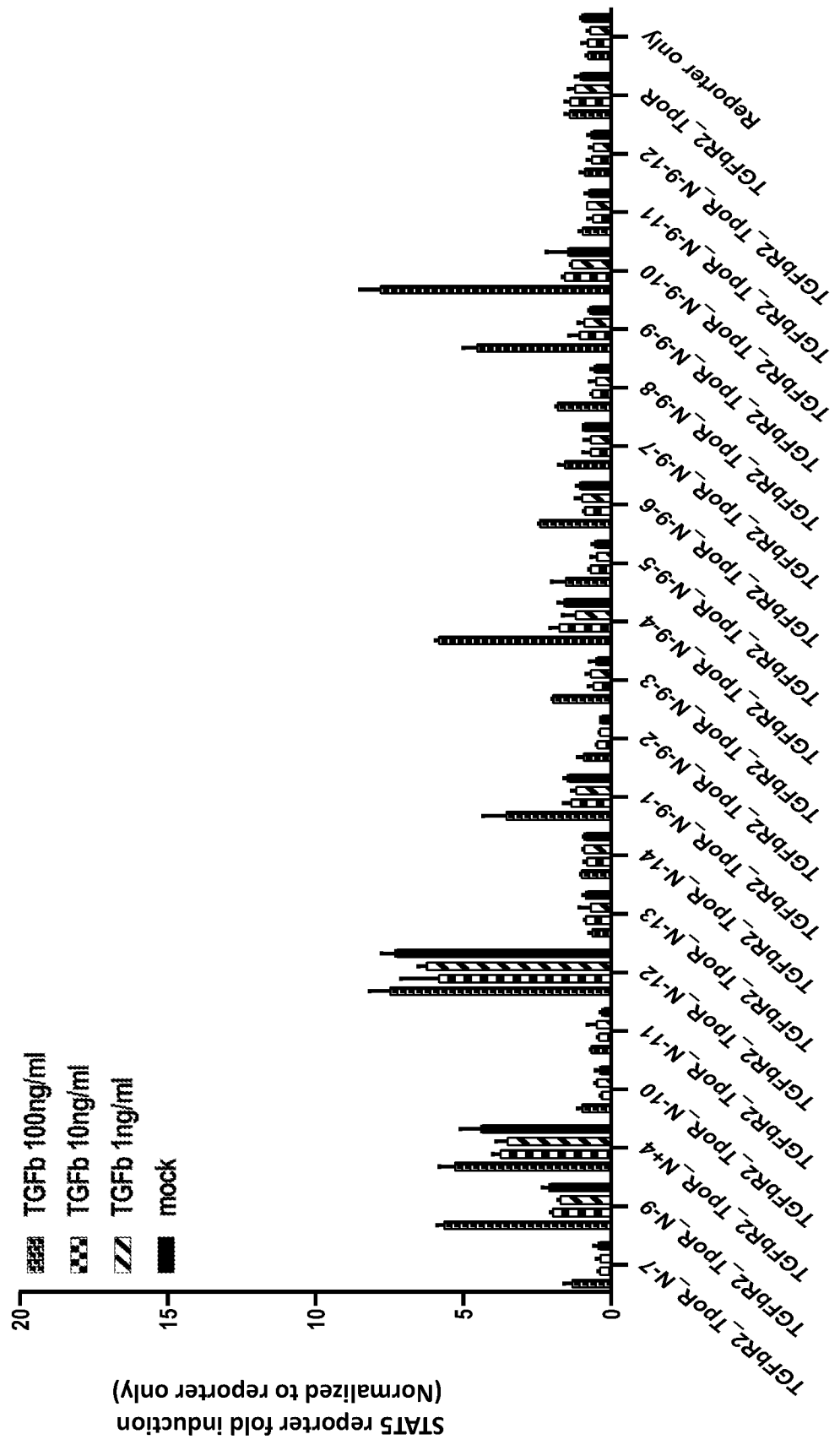


FIG. 6A

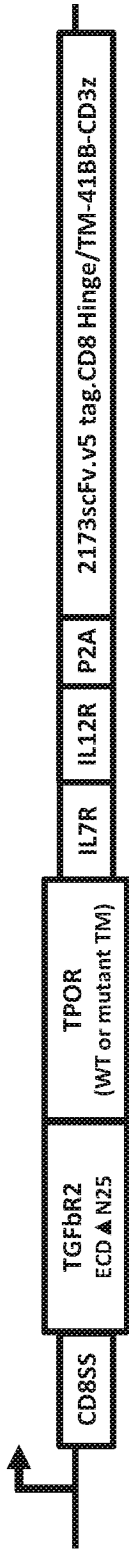


FIG. 6B

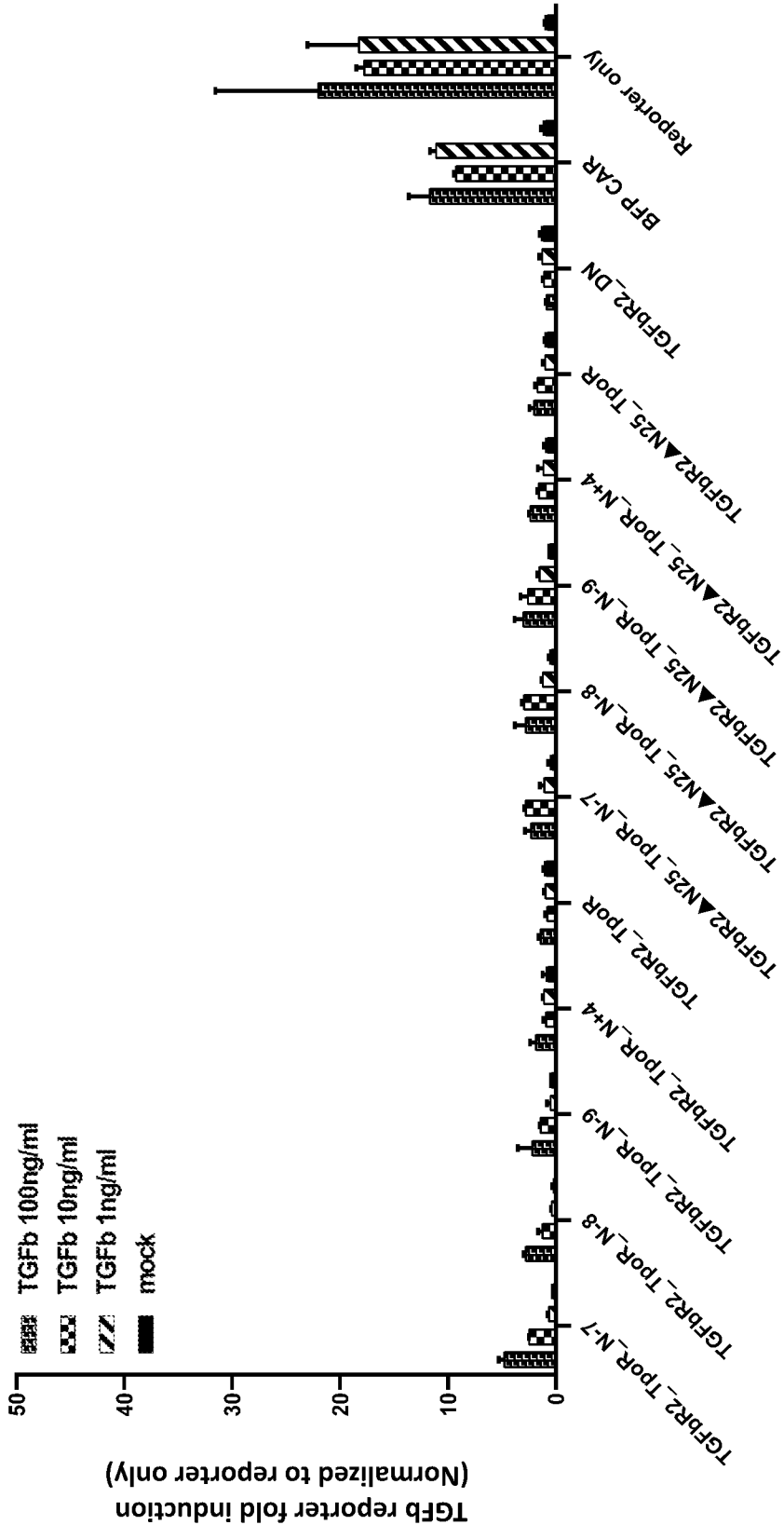


FIG. 6C

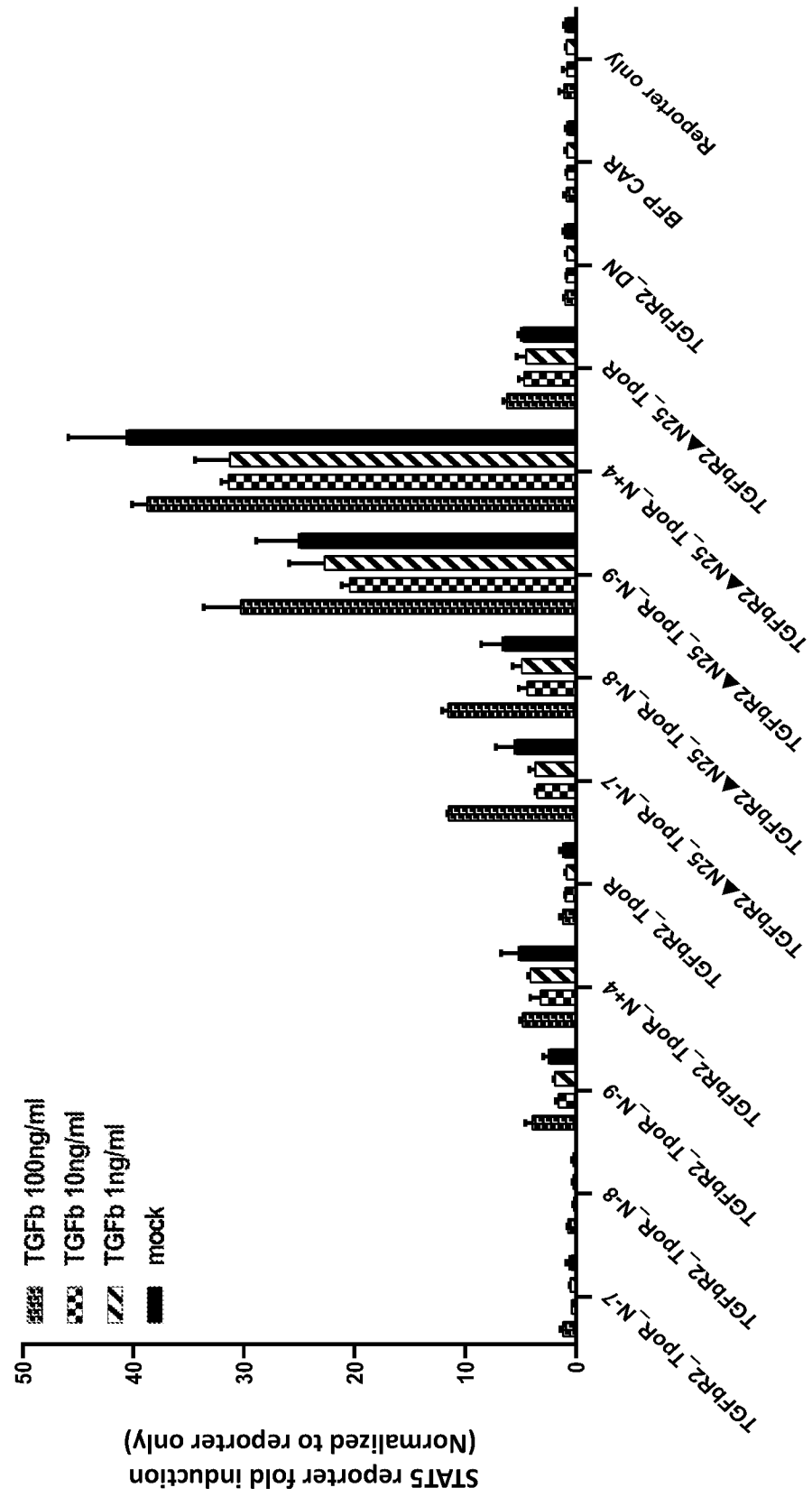


FIG. 7

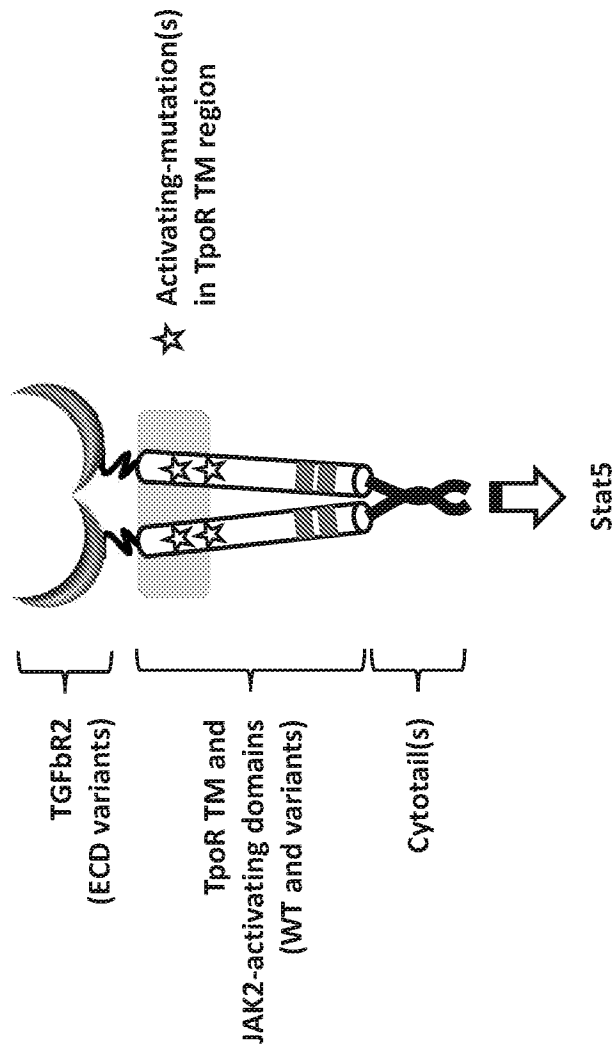


FIG. 8A

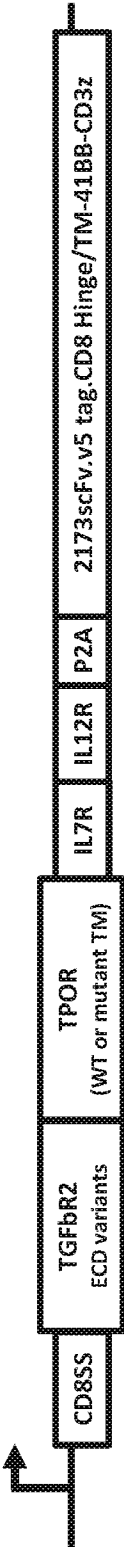


FIG. 8B

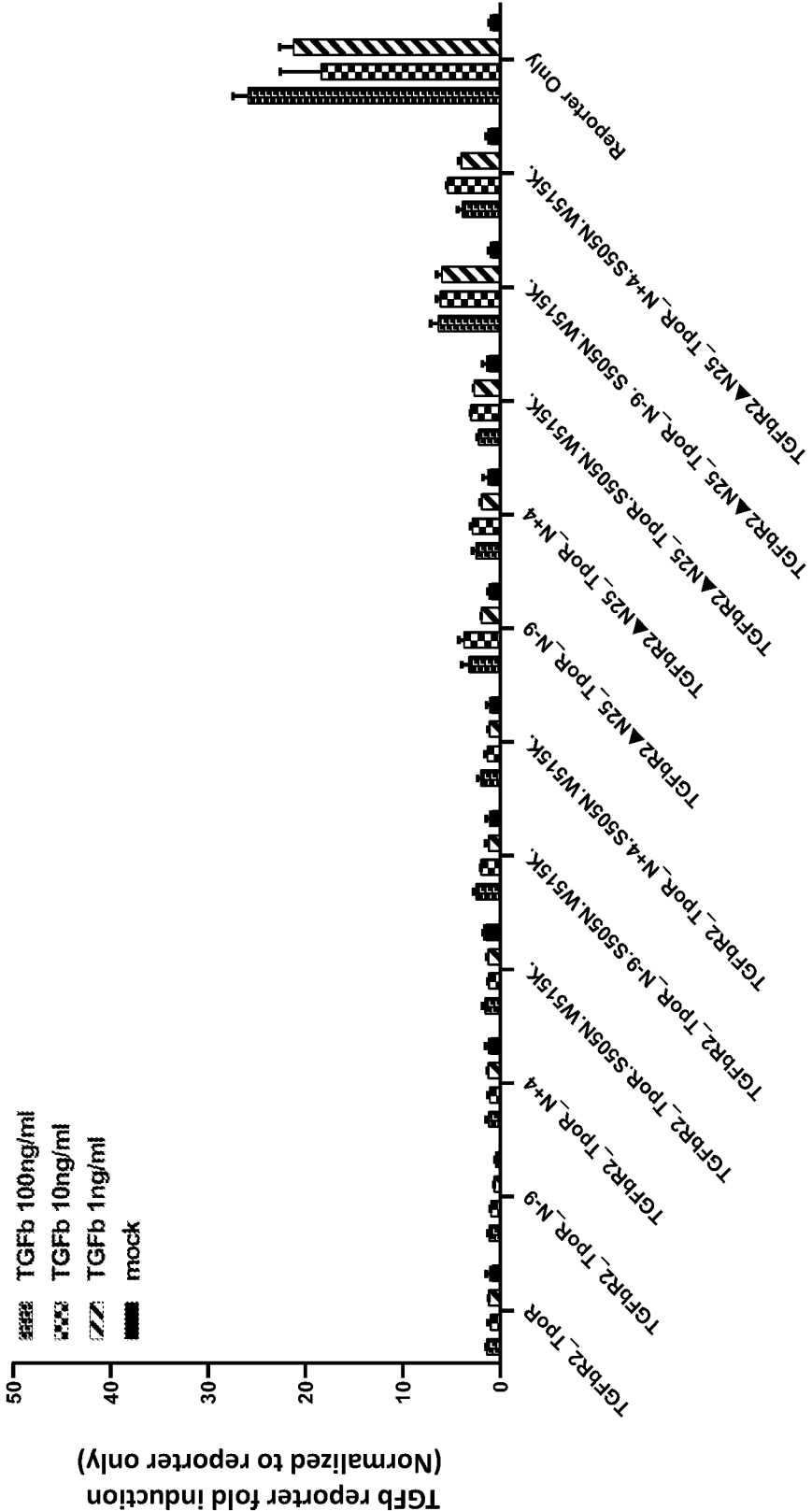
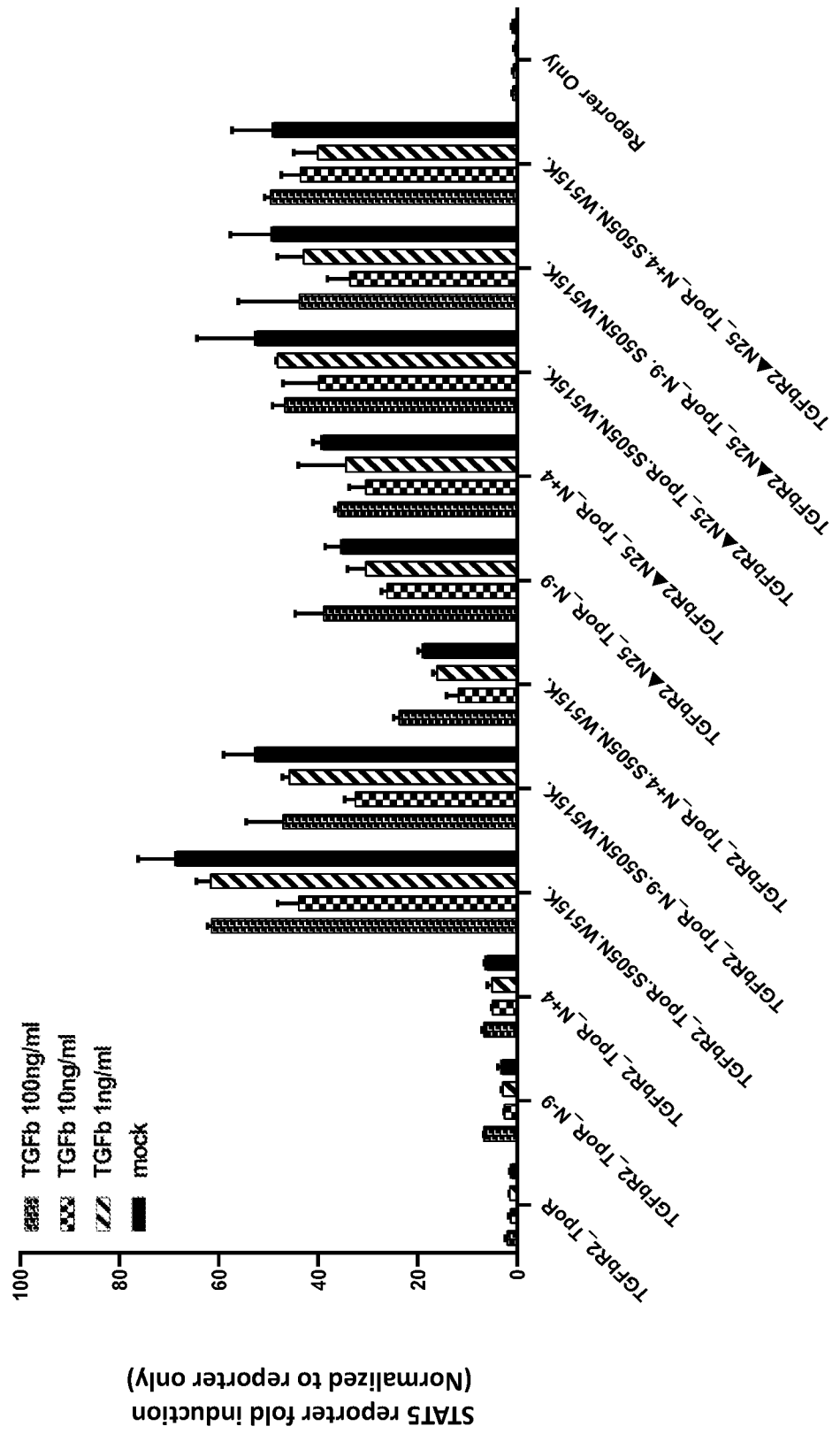


FIG. 8C



Underlined: wildtype TpoR™ region
Bold: means insertion

FIG. 9A

Template:	SDPTRVETATETAWISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 235
N-1:	SDPTRVETATET-WISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 236
N-2:	SDPTRVETATET--ISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 237
N-2+1:	SDPTRVETATET-LISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 238
N-3:	SDPTRVETATET---SLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 239
N-4:	SDPTRVETATET----LV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 240
N-4+1:	SDPTRVETATET--- I LV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 241
N-5:	SDPTRVETATET-----V	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 242
N-6:	SDPTRVETATET-----T	ALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 243
N-7:	SDPTRVETATET-----A	LHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 244
N-8:	SDPTRVETATET-----L	HVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 245
N-9:	SDPTRVETATET-----H	VLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 246

FIG. 9B

Template:	SDPTRVETATETAW-----ISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 235
N+1:	SDPTRVETATETAW-----LISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 247
N+2:	SDPTRVETATETAW----- V LISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 248
N+3:	SDPTRVETATETAW----- L VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 249
N+4:	SDPTRVETATETAW----- IL VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 250
N+5:	SDPTRVETATETAW----- LIL VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 251
N+6:	SDPTRVETATETAW----- LLIL VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 252
N+7:	SDPTRVETATETAW----- VLLIL VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 253
N+8:	SDPTRVETATETAW L VL LIL VLISLV	TALHLVLGLSAVLG	LLLLLRWQFPAHYRRLRHALWPSL	SEQ ID NO. 254

FIG. 10A

		(4114 - 4278 bp)
Template:	SDPTRVETATETAWISLVTAHLVGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 235
N-10:	SDPTRVETATET-----LVGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 255
N-11:	SDPTRVETATET-----VLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 256
N-12:	SDPTRVETATET-----LGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 257
N-13:	SDPTRVETATET-----GLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 258
N-14:	SDPTRVETATET-----LSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 259
N-9-1:	SDPTRVETATE-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 260
N-9-2:	SDPTRVETAT-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 261
N-9-3:	SDPTRVETA-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 262
N-9-4:	SDPTRVET-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 263
N-9-5:	SDPTRVE-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 264
N-9-6:	SDPTRV-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 265
N-9-7:	SDPTR-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 266
N-9-8:	SDPT-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 267
N-9-9:	SDP-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 268
N-9-10:	SD-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 269
N-9-11:	S-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 270
N-9-12:	-----HLVLGLSAVLGGLLLLRWQFFPAHYRRRLRHALWPSL	SEQ ID NO. 271

Fold induction of TGF β reporter

TGF β Concentration	WT	N-9-10	N-9-4 Δ N25	N-9-9 Δ N25	N-9-10 Δ N25	Reporter
TGF β 600 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
TGF β 200 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
TGF β 100 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
TGF β 50 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
TGF β 25 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
TGF β 12.5 ng/ml	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5
mock	~8.5	~7.5	~7.5	~7.5	~7.5	~12.5

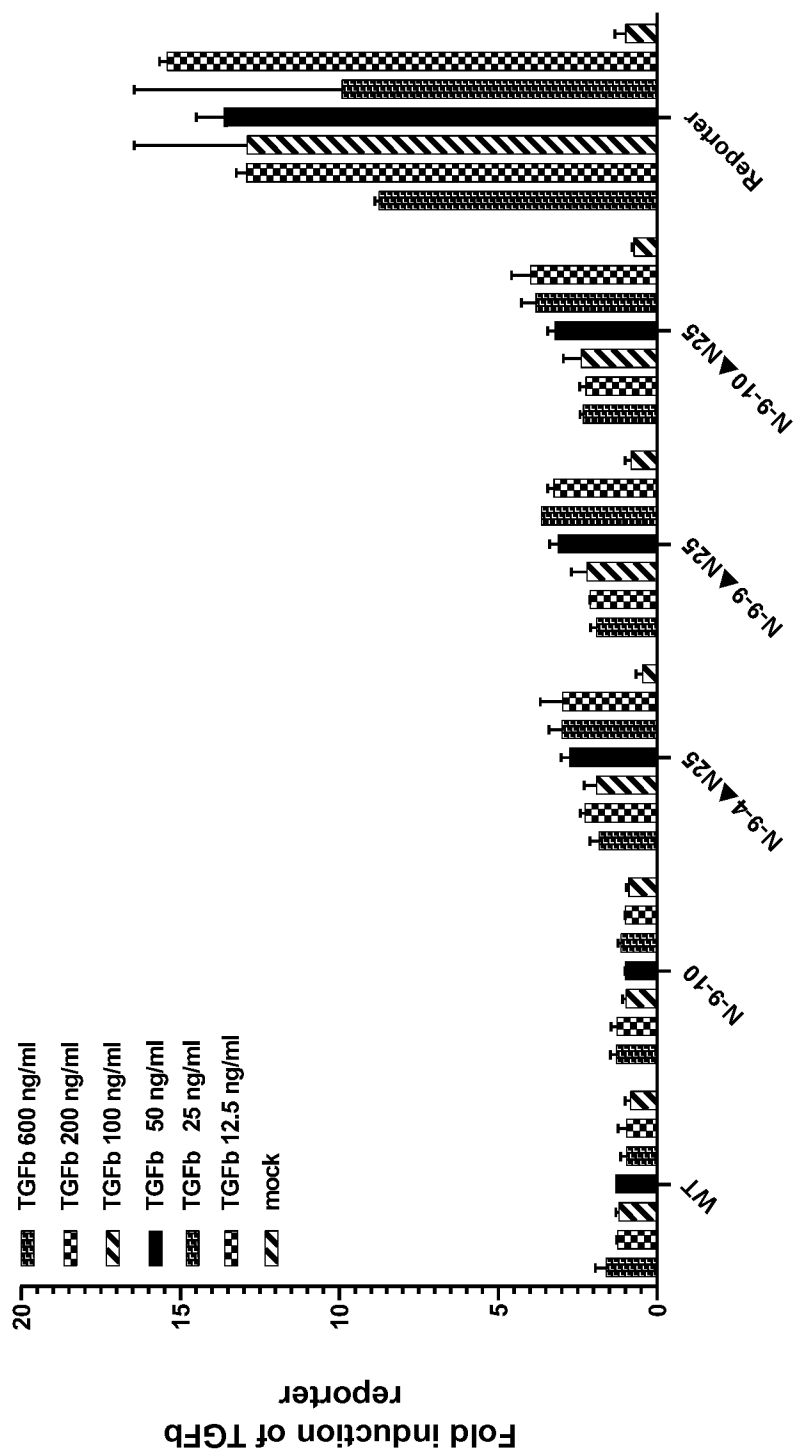


FIG. 10C

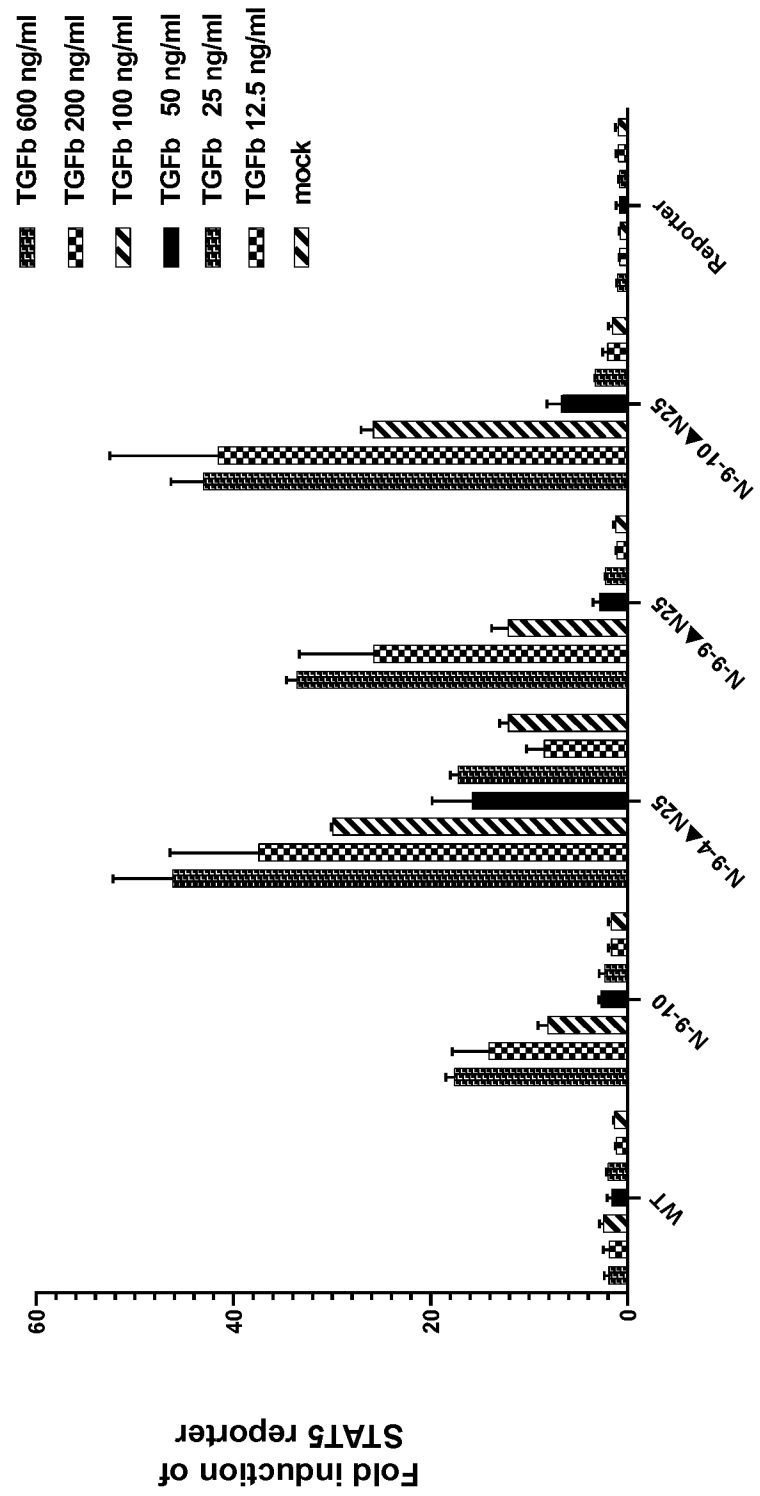


FIG. 11A

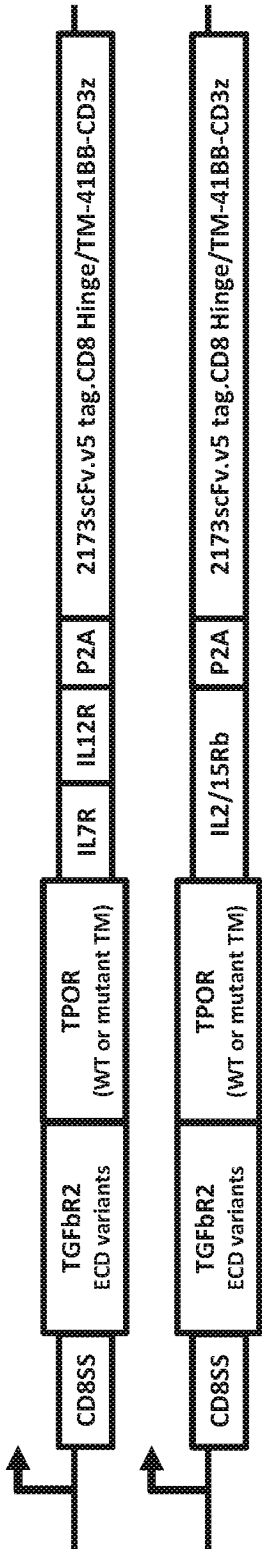


FIG. 11B

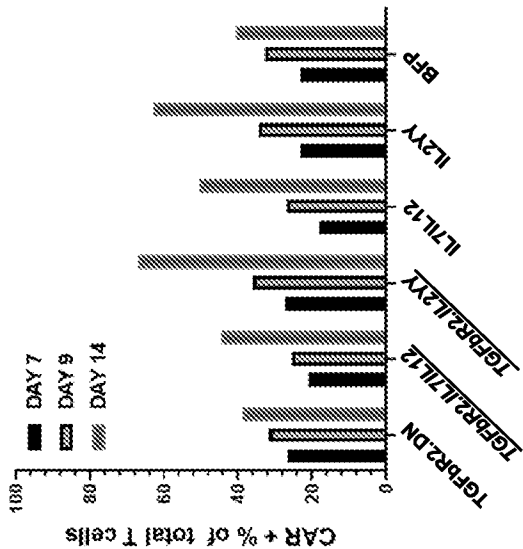


FIG. 11C

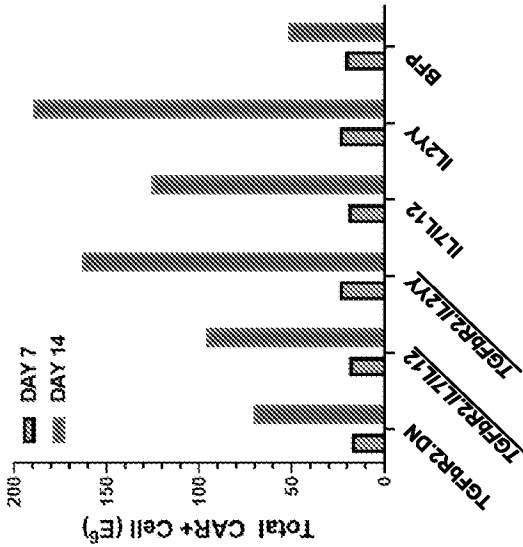


FIG. 11E

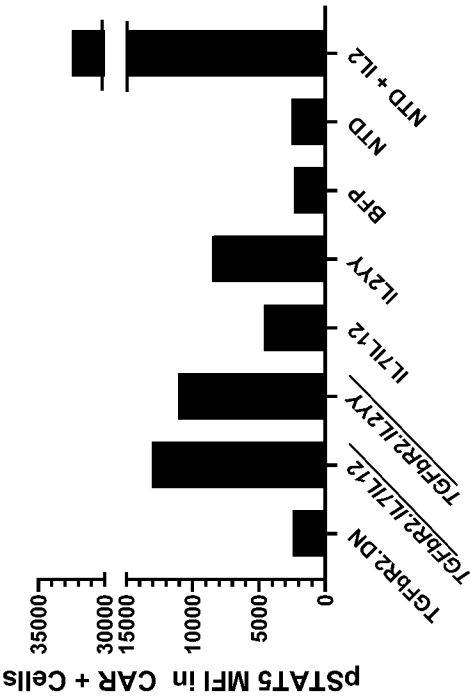
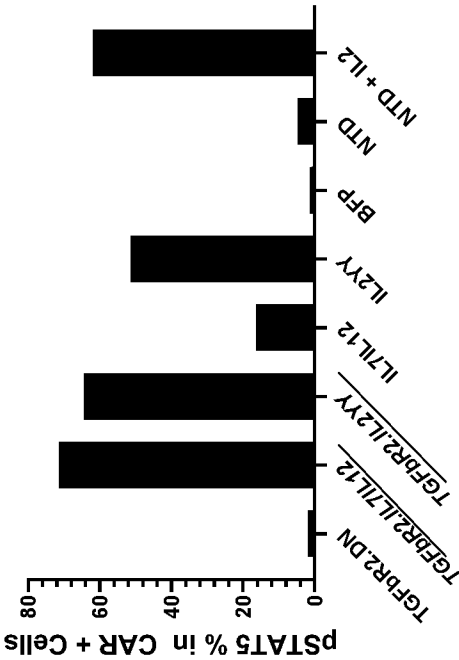


FIG. 11D



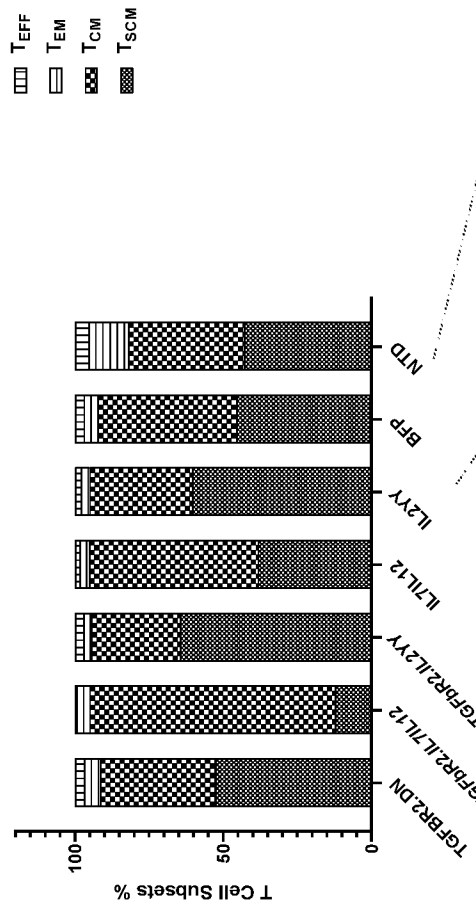


FIG. 11F

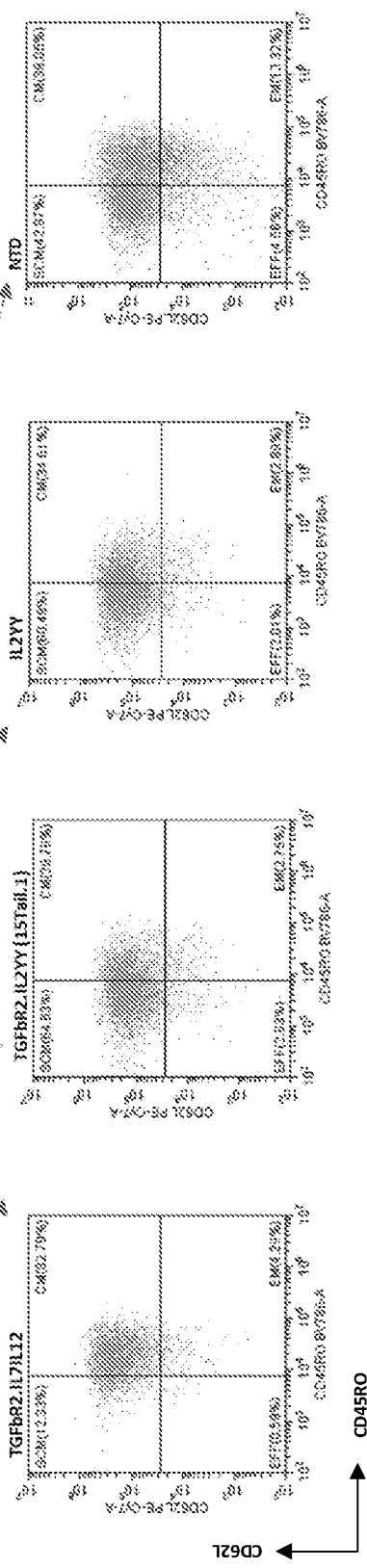


FIG. 11G

FIG. 11I

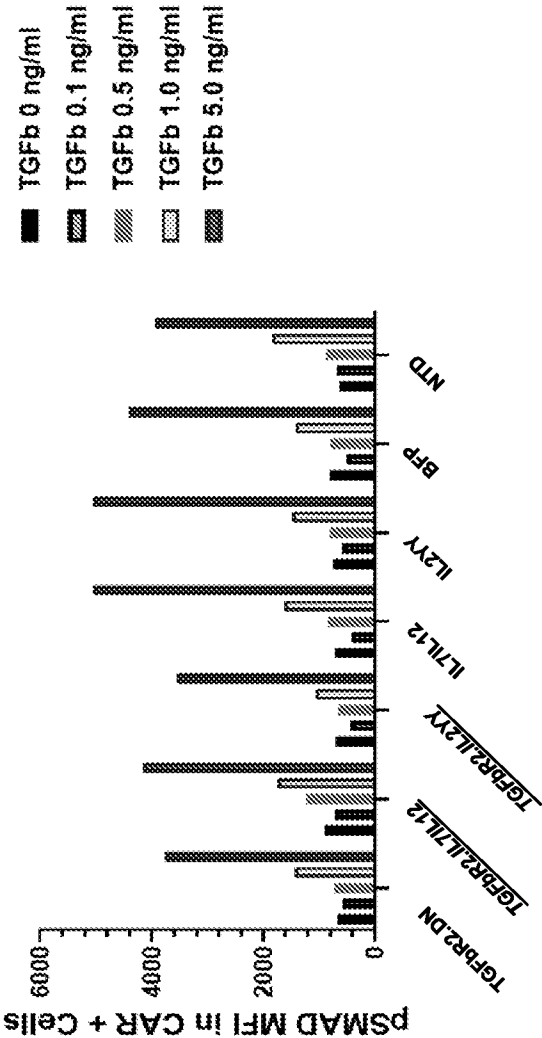


FIG. 11H

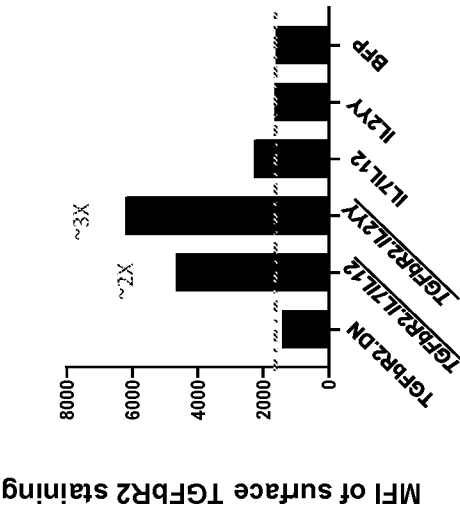


FIG. 12A

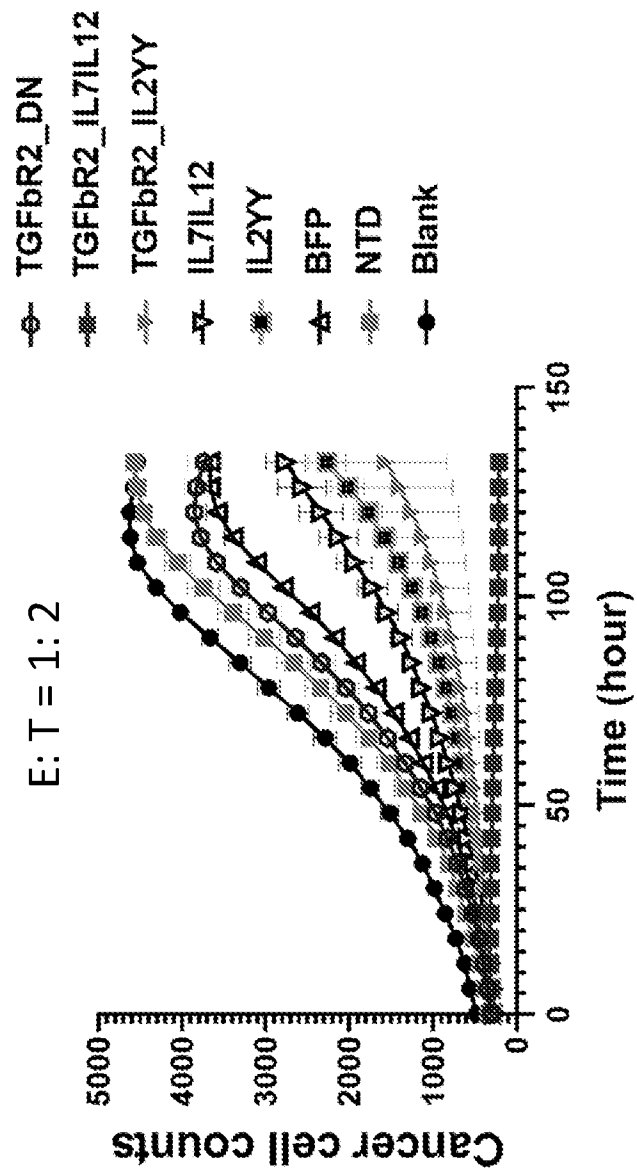


FIG. 12B

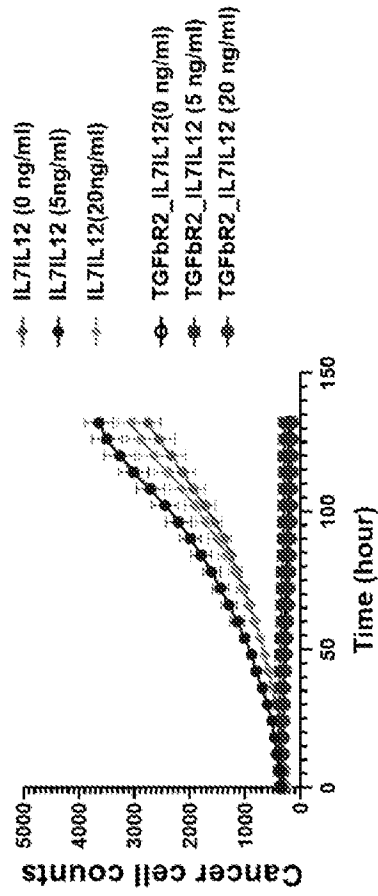


FIG. 12C

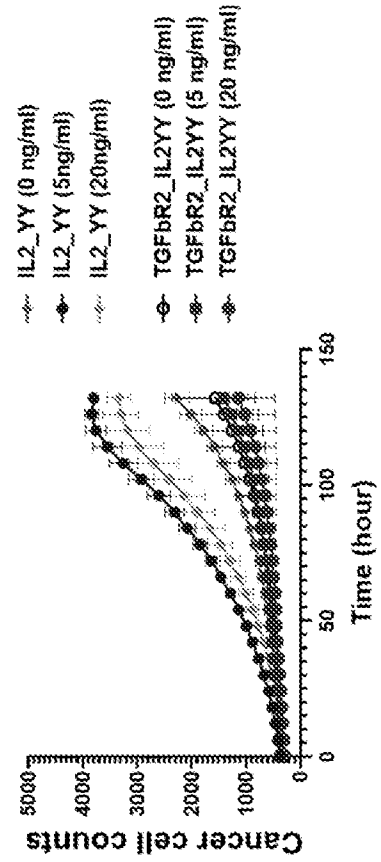


FIG. 13B

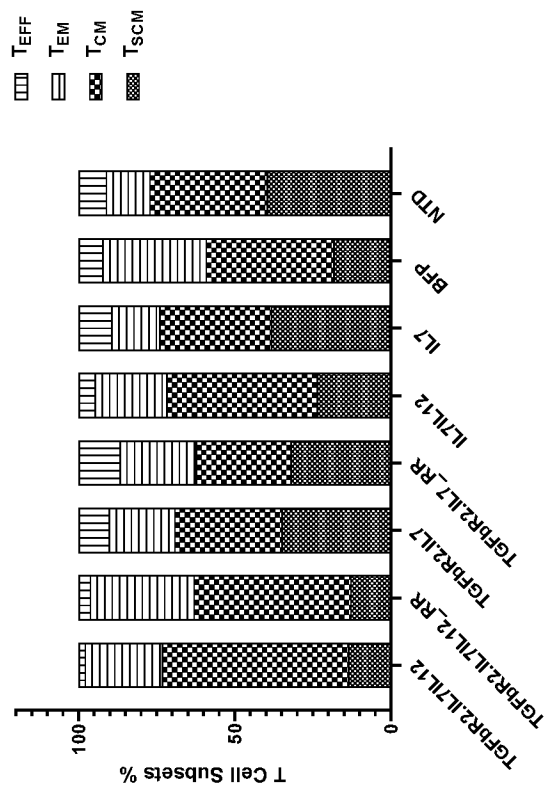
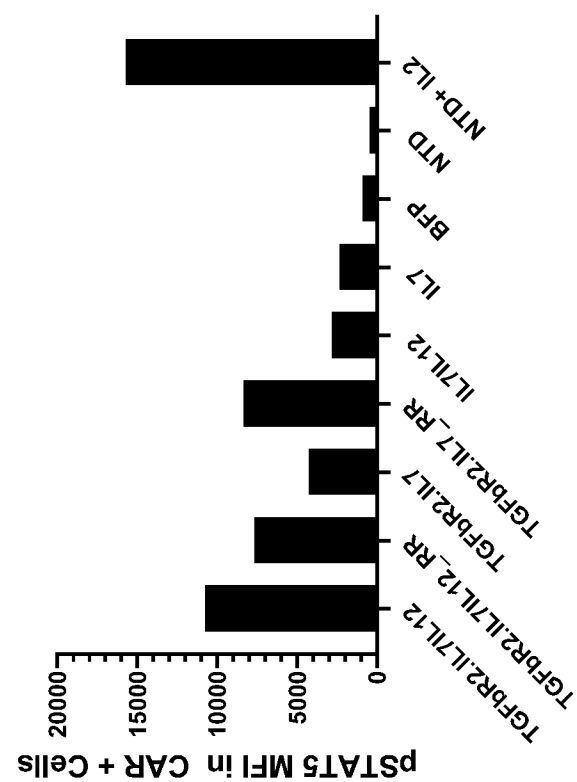
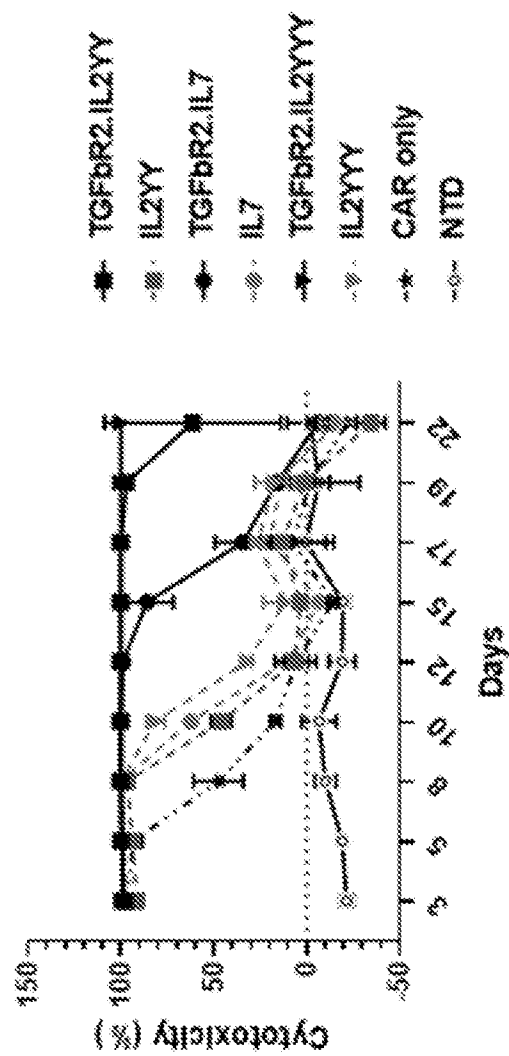


FIG. 13A



[illegible]

Days	TGFβR2:IL2YY (filled squares)	IL2YY (open squares)	TGFβR2:IL7 (filled circles)	IL7 (open circles)	TGFβR2:IL2YY (filled triangles)	IL2YY (open triangles)	CAR only (filled diamonds)	NTD (open diamonds)
0	100	100	100	100	100	100	100	100
5	100	100	100	100	100	100	100	100
10	100	-50	100	100	100	100	-25	-25
15	100	-25	100	100	100	100	-25	-25
17	100	-25	100	100	100	100	-25	-25
19	100	-25	100	100	100	100	-25	-25
22	100	-25	100	100	100	100	-25	-25

FIG. 15A

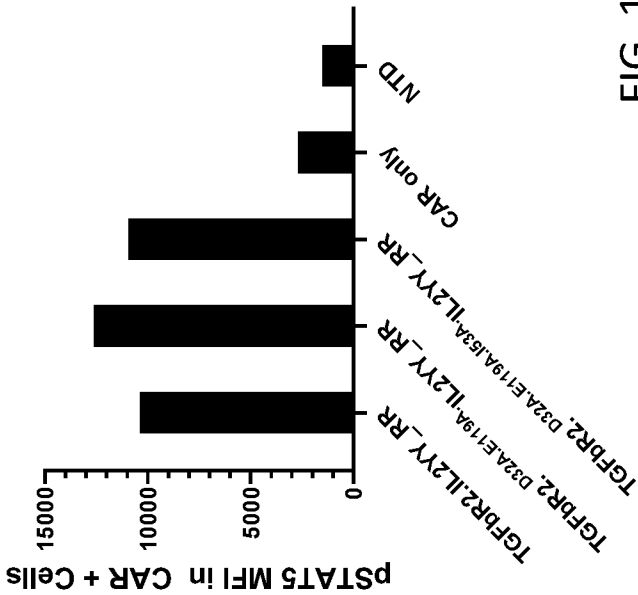


FIG. 15B

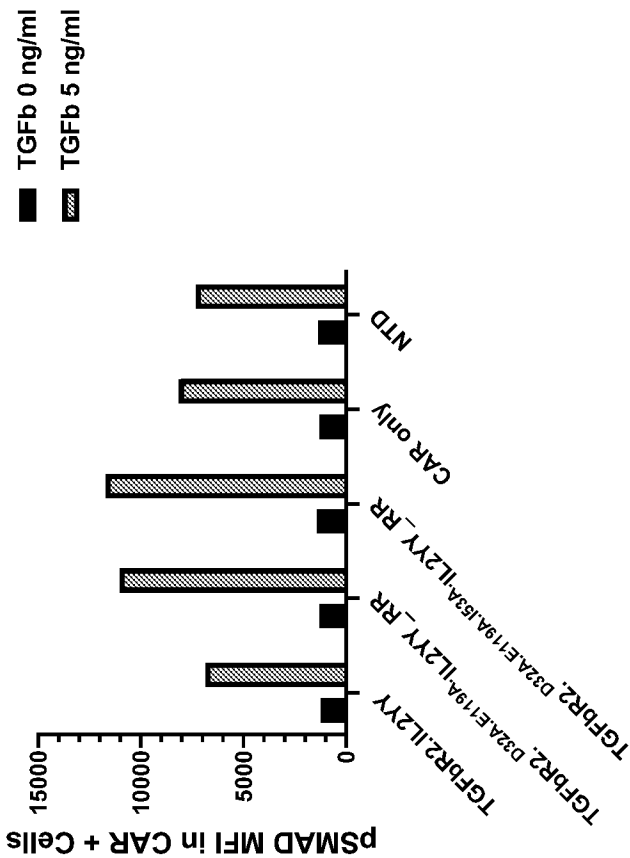


FIG. 15C

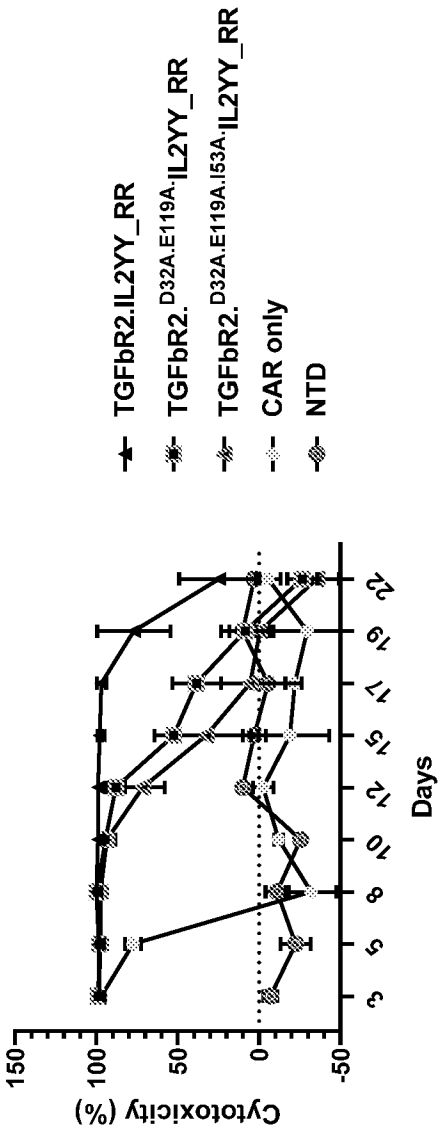


FIG. 16A

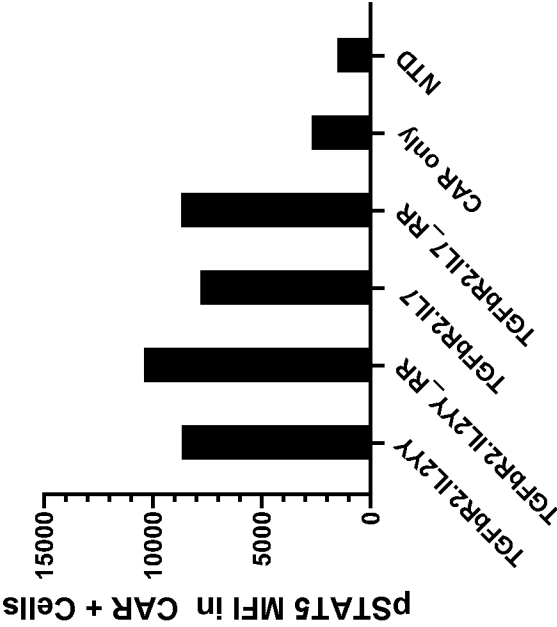
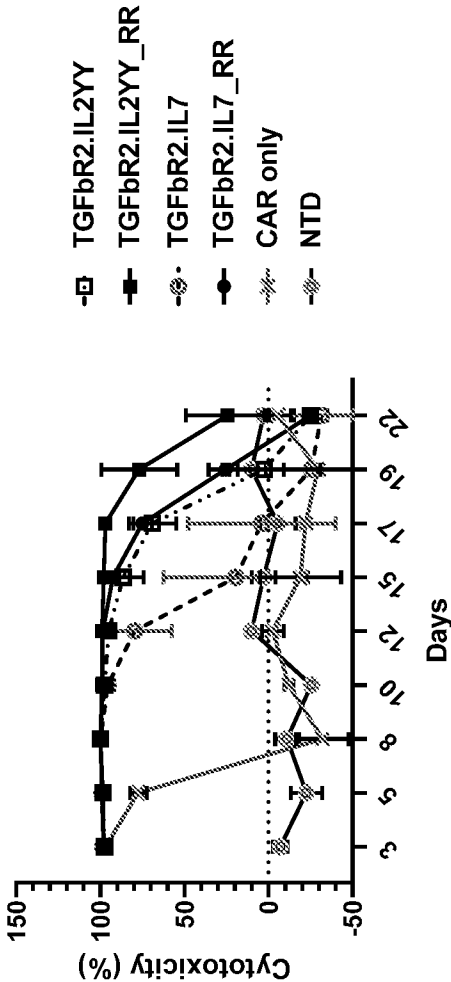


FIG. 16B



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/048402

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P43/00 C07K14/71 C07K14/715
ADD.

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)

A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/038945 A1 (BAYLOR COLLEGE MEDICINE [US]) 1 March 2018 (2018-03-01)	1-5,7,8, 11,14, 22,23, 27-57
Y	abstract paragraph [0003] paragraph [0008] - paragraph [0011] paragraph [0014] - paragraph [0015] paragraph [0018] - paragraph [0020] paragraph [0063] paragraph [0066] paragraph [0077] paragraph [0081] paragraph [0088] paragraph [0090] paragraph [0097] paragraph [0167] paragraph [0132] - paragraph [0133] paragraph [0169] - paragraph [0173] -/-	15-19, 21,26



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

19 November 2020

Date of mailing of the international search report

27/11/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Gurdjian, Didier

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/048402

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	paragraph [0184] paragraph [0188] - paragraph [0204] paragraph [0193] paragraph [0197] paragraph [0203] - paragraph [0204] paragraph [0224]; claims 1,24,26,42,44,49; figures 1,14,20 -----	
X	W0 2018/094244 A1 (BLUEBIRD BIO INC [US]) 24 May 2018 (2018-05-24)	1-5, 8-12, 22-25, 27-56
Y	abstract page 53, paragraph 1 page 55, paragraph 2 page 57, paragraph 3; claims 85-87,113,114,117,121-123,131-135; figures 1,20; examples 5,6,17; sequence 354 -----	15-19, 21,26
Y	W0 2017/103596 A1 (CELLULAR THERAPEUTICS LTD [GB]) 22 June 2017 (2017-06-22) abstract page 8, line 10 - page 9, line 2; claims 1,8-13,32 -----	15-19, 21,26
Y	J.-P. DEFOUR ET AL: "Tryptophan at the transmembrane-cytosolic junction modulates thrombopoietin receptor dimerization and activation", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 15-19, 21110, no. 7, 28 January 2013 (2013-01-28), pages 2540-2545, XP055696776, ISSN: 0027-8424, DOI: 10.1073/pnas.1211560110 abstract page 2542, right-hand column, paragraph 6 page 2543, right-hand column, paragraph 1 -----	15-19, 21,26
A	W0 2019/118508 A1 (UNIV PENNSYLVANIA [US]) 20 June 2019 (2019-06-20) abstract page 6, lines 12-20; claims 21.22,26,27; sequence 18 -----	1-5,7, 11,22, 23,25, 27-57

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/048402

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018038945 A1	01-03-2018	AU 2017317022 A1 CA 3034873 A1 CN 109952309 A EP 3504223 A1 JP 2019528078 A KR 20190039597 A SG 11201901642X A US 2019183936 A1 WO 2018038945 A1	14-03-2019 01-03-2018 28-06-2019 03-07-2019 10-10-2019 12-04-2019 28-03-2019 20-06-2019 01-03-2018
WO 2018094244 A1	24-05-2018	AU 2017362484 A1 BR 112019009904 A2 CA 3043888 A1 CN 110072885 A EP 3541833 A1 JP 2019535275 A KR 20190077562 A US 2019350974 A1 WO 2018094244 A1	30-05-2019 13-08-2019 24-05-2018 30-07-2019 25-09-2019 12-12-2019 03-07-2019 21-11-2019 24-05-2018
WO 2017103596 A1	22-06-2017	EP 3390436 A1 US 2020263130 A1 WO 2017103596 A1	24-10-2018 20-08-2020 22-06-2017
WO 2019118508 A1	20-06-2019	TW 201928052 A US 2019247432 A1 WO 2019118508 A1	16-07-2019 15-08-2019 20-06-2019