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(54) Title: MULTI-CHAIN SYNTHETIC RECEPTORS FOR SIMULTANEOUS LIGAND-INDUCED TRANSCRIPTIONAL REGULATION AND MEMBRANE-PROXIMAL SIGNAL TRANSDUCTION

(57) **Abstract:** The present disclosure generally relates to multi-chain chimeric polypeptides or receptors having distinct polypeptide chains that associate post-translationally to enable the simultaneous activation of the signaling domain and the release of a transcriptional regulator upon binding of a ligand. The disclosure also provides nucleic acid constructs, recombinant cells, vectors, pharmaceutical compositions, and methods of treatment including the multi-chain chimeric polypeptides of the disclosure. The disclosure further provides methods for simultaneously inducing T cell signaling and gene regulation in a T cell.

WO 2024/040033 A2

MULTI-CHAIN SYNTHETIC RECEPTORS FOR SIMULTANEOUS LIGAND-INDUCED TRANSCRIPTIONAL REGULATION AND MEMBRANE-PROXIMAL SIGNAL TRANSDUCTION

CROSS REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority to U.S. Provisional Patent Application Serial No. 63/398,185 filed on August 15, 2022, the disclosure of which is incorporated by reference herein in its entirety, including any drawings

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant no. OD025751-01 awarded by The National Institute of Health. The government has certain rights in the invention.

INCORPORATION OF THE SEQUENCE LISTING

[0003] This application contains a Sequence Listing, which is hereby incorporated herein by reference in its entirety. The accompanying Sequence Listing file named "2023-08-14 Sequence_Listing_ST26 048536-727001WO.xml" was created on August 14, 2023 and is 345,882 bytes.

FIELD

[0004] The present disclosure generally relates to the fields of immunology and medicine. More particularly, the present disclosure relates to compositions and methods including multi-chain receptors. The present disclosure also relates to enhanced CAR T Cell therapy including such receptors.

BACKGROUND

[0005] Many important advancements have been made in the development of immunotherapies based on adoptive transfer of lymphocytes (*e.g.*, T cells), foremost of which are T cells expressing chimeric immunoreceptors including chimeric antigen receptors (CAR-T cells). An important problem limiting these therapies, however, the inability to modulate or turn off CAR-T activity when needed. The regulation of CAR-T activity is particularly important to reduce or eliminate interactions causing significant side effects upon the administration of

chimeric antigen receptor T cells.

[0006] Chimeric immunoreceptors such as CARs, unlike T cell receptors (TCR), include molecules in which tumor antigen recognition and intracellular activation are combined. The structure and design of such immunoreceptors minimally includes an extracellular antigen recognition domain linked through a TMD to an intracellular activation domain or domains. Examples of such immunoreceptors include synNotch receptors capable of binding user-defined cell surface displayed ligands, which triggers proteolytic cleavage of the receptor and the release of a transcriptional regulator that induces a custom transcriptional program in the cell. Other examples include receptors that incorporate signaling (e.g., co-stimulation, CD3 zeta) that can initiate activation of T cells concomitant with custom transcriptional regulation. Those examples of immunoreceptors are designed as a single protein chain, with contiguous fusion of costimulatory, CD3 zeta, and transcriptional regulator domains.

[0007] There is also a need for receptors that can enable the simultaneous signal transduction and transcriptional regulation for more efficient and more reliable induction of proximal T cell activation signals and gene regulation in primary T cells.

SUMMARY

[0008] The present disclosure generally relates generally to multi-chain chimeric polypeptides (e.g., chimeric immunoreceptors) having an architecture that enables transcriptional regulation and signal transduction using distinct protein chains. Particularly, the disclosure relates to multi-chain chimeric polypeptides having distinct polypeptide chains that associate with one another post-translationally (via residues located within the transmembrane domain) to enable the simultaneous activation of the signaling domain and the release of a transcriptional regulator upon binding of a ligand.

[0009] Provided herein, *inter alia*, are multi-chain chimeric polypeptides including (a) a first polypeptide having (i) an extracellular ligand-binding domain with a binding affinity for a selected ligand, (ii) a first transmembrane domain (TMD) including a first modified interface and (iii) a first intracellular domain (ICD) including a transcriptional regulator, and (b) a second polypeptide having (i) a second TMD with a second interface and (ii) a second ICD having a signaling domain, wherein the first and the second modified interfaces each include amino acid residues having opposite charges, wherein the first polypeptide is coupled to the second

polypeptide via the first modified interface and the second interface and wherein binding of the selected ligand to the extracellular ligand-binding domain induces activity of the signaling domain and release of the transcriptional regulator. In some embodiments, binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces the activity of the signaling domain and the release of the transcriptional regulator.

[0010] In some embodiments, the first polypeptide includes (i) the extracellular ligand-binding domain, (ii) the first TMD, and (iii) the first intracellular domain, in order from N-terminus to C-terminus of the first polypeptide. In some embodiments, the first TMD includes (i) 10 to 25 contiguous valine residues or (ii) a Notch 1 TMD.

[0011] In some embodiments of the multi-chain chimeric polypeptides, the second polypeptide includes (i) the second TMD, and (ii) the second intracellular domain in order from N-terminus to C-terminus of the second polypeptide.

[0012] In some embodiments of the multi-chain chimeric polypeptides, the first modified interface includes a positively charged residue, the second interface includes a negatively charged residue, and the first polypeptide is coupled to the second polypeptide via the electrostatic force between the first and the second modified interfaces. In some embodiments, the positively charged residue is lysine or arginine. In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the first TMD comprises SEQ ID NO:21 or functional variants thereof and the lysine or arginine residue is at a position selected from positions 10 to 14 of SEQ ID NO:21; or (ii) the first TMD comprises SEQ ID NO:18 and the lysine or arginine is at a position selected from positions 8 to 11 of SEQ ID NO: 18. In some embodiments where the first transmembrane comprises SEQ ID NO:21, the lysine or arginine residue is at position 12 of SEQ ID NO:21. In some embodiments where the first transmembrane comprises SEQ ID NO:18, the lysine or arginine residue is at position 9 relative to SEQ ID NO:18.

[0013] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the extracellular domain includes an antigen-binding moiety that binds (or is capable of binding) to a ligand on the surface of a cell. In some embodiments, the antigen-binding moiety is selected from the group consisting of an antibody, a nanobody, a diabody, a triabody, a minibody, an $F(ab')_2$ fragment, an F(ab) fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and a functional fragment thereof.

[0014] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the

ligand includes a protein or a carbohydrate. In some embodiments, the ligand is a tumor-associated antigen or a tumor-specific antigen. In some embodiments, the ligand includes cell-surface receptors, adhesion proteins, integrins, mucins, lectins, tumor associated antigens, and tumor specific antigens. In some embodiments, the ligand includes CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent Protein (eGFP), or signal regulatory protein a (SIRPα).

[0015] In some embodiments, the ligands are present on the surface of cells. In some embodiments, the cell is a human cell. In some embodiments, the cell is a tumor cell.

[0016] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the transcriptional regulator includes a transcriptional activator or a transcriptional repressor. In some embodiments, the transcriptional regulator includes Ga14-VP16, Ga14-VP64, tetR-VP64, ZFHD1-VP64, Ga14-KRAB, or HAP1-VP16.

[0017] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the transcriptional regulator is a human or a humanized transcriptional regulator. In some embodiments, the transcriptional regulator is HNF1a.

[0018] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the second polypeptide comprises a signaling domain comprising CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, or CD66d, signaling domain.

[0019] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the signaling domain includes DAP12, CD3 zeta, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fc.ε.RI, DAP10, DAP12, or CD66d, signaling domain.

[0020] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the

first polypeptide further includes one or more of the following: a hinge domain, a ligand-inducible proteolytic cleavage site, an autoproteolytic peptide sequence, a nuclear localization signal, a juxtamembrane domain. In some embodiments, the juxtamembrane domain is a polybasic domain. In some embodiments, the polybasic domain includes Notch-1 or Notch-2 juxtamembrane domains. In some embodiments, the autoproteolytic peptide sequence is from a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

[0021] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the first polypeptide further includes a hinge domain from CD8, CD28, OX40, or IgG4. In some embodiments, the hinge domain is derived from CD8. In some embodiments, the hinge domain includes a truncated CD8α hinge domain (also referred to as CD8 Hinge or CD8 Hinge 2).

[0022] In some embodiments of the multi-chain chimeric polypeptides of the disclosure, the ligand-inducible proteolytic cleavage site is cleavable by gamma secretase.

[0023] In an aspect, also provided herein are multi-chain chimeric polypeptides including (a) a first polypeptide having (i) an extracellular ligand-binding domain including CD19scFv, (ii) a first TMD including a contiguous stretch of valine residues including a lysine or arginine residue and (iii) a Notch 2 juxtamembrane domain and (iv) a first ICD having a Gal4VP64 transcriptional regulator; and (b) a second polypeptide having DNAX –activation protein 12 (DAP12); wherein the first polypeptide is coupled to the second polypeptide via a lysine or arginine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, wherein the contiguous stretch of valine residues comprises 5 to 25 valine residues and the lysine residue is flanked by a contiguous stretch of 5 to 15 valine residues. In some embodiments, the contiguous stretch of valine residues include 5 to 25 valine residues. In some embodiments, the first TMD includes 15 to 20 valine residues. In some embodiments, the lysine residue is at position 9 or 10 of the first TMD.

[0024] In an aspect, also provided herein are multi-chain chimeric polypeptides including (a) a first polypeptide having (i) the extracellular ligand-binding domain comprising CD19scFv, the

first TMD compriseing a contiguous stretch of valine residues comprising a lysine or arginine residue, the first polypeptide further comprises a Notch 2 juxtamembrane domain and the first intracellular domain comprising a Gal4VP64 transcriptional regulator, the second polypeptide comprising a CD3z signaling domain, and wherein the first polypeptide is coupled to the second polypeptide via the lysine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator.

[0025] In an aspect, also provided herein are multi-chain chimeric polypeptides including (a) a first polypeptide having (i) an extracellular ligand-binding domain comprises CD19scFv, the first TMD comprising a contiguous stretch of valine residues comprising a lysine or arginine residue, the first polypeptide further comprising a Notch 2 juxtamembrane domain and the first intracellular domain comprising a human or humanized transcriptional regulator, the second polypeptide comprising DNAX –activation protein 12 (DAP12) or CD3z, and wherein the first polypeptide is coupled to the second polypeptide via the lysine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator.

[0026] In some embodiments, the transcriptional regulator is HNF1a.

[0027] In some embodiments, the multi-chain chimeric polypeptides of the disclosure are immunoreceptors. In some embodiments, the immunoreceptors are chimeric antigen receptors.

[0028] In an aspect, provided herein are multi-chain chimeric polypeptides comprising (a) a first polypeptide comprising (i) an extracellular ligand-binding domain having a binding affinity for a selected ligand, (ii) a first transmembrane domain (TMD) comprising a first modified interface and (iii) a first intracellular domain comprising a transcriptional regulator or a signaling domain; and (b) a second polypeptide comprising (i) a second TMD comprising a second interface and (ii) a second intracellular domain comprising a signaling domain; wherein the first and the second modified interfaces each comprise amino acid residues having opposite charges, and wherein the first polypeptide is coupled to the second polypeptide via the first modified interface and the second interface and wherein binding of the selected ligand to the extracellular ligand-binding domain induces activity of the signaling domain and release of the transcriptional regulator.

[0029] In some embodiments, the first intracellular domain comprises a transcriptional

regulator. In some embodiments, the transcriptional regulator comprises Ga14-VP16, Ga14-VP64, tetR-VP64, ZFHD1-VP64, Ga14-KRAB, or HAP1-VP16. In some embodiments, the transcriptional regulator is a human or a humanized transcriptional regulator. In some embodiments, the transcriptional regulator is HNF1a.

[0030] In some embodiments, the first intracellular domain comprises a signaling domain. In some embodiments, the signaling domain comprises one or more of a CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, CD66d, 4-1BB, or common gamma chain signaling domain.

[0031] In some embodiments, the signaling domain of the second polypeptide comprises a signaling domain comprising CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, CD66d, or 4-1BB signaling domain.

[0032] In some embodiments, the signaling domain of the second polypeptide comprises a cytokine signaling domain. In some embodiments, the cytokine signaling domain comprises an IL-2Rb, IL-4Ra, IL-7Ra, IL-9Ra, IL-13R, IL-15R, or IL-21R endodomain.

[0033] In some embodiments, binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces the activity of the signaling domain and the release of the transcriptional regulator.

[0034] In some embodiments, the first polypeptide comprises (i) the extracellular ligand-binding domain, (ii) the first TMD, and (iii) the first intracellular domain, in order from N-terminus to C-terminus of the first polypeptide.

[0035] In some embodiments, the first TMD comprises: (i) 10 to 25 contiguous valine residues, or (ii) a Notch 1 transmembrane domain.

[0036] In some embodiments, the second polypeptide comprises (i) the second TMD, and (ii) the second intracellular domain, in order from N-terminus to C-terminus of the second polypeptide.

[0037] In some embodiments, the first modified interface comprises a positively charged residue, and wherein the second interface comprises a negatively charged residue, and wherein the first polypeptide is coupled to the second polypeptide via the electrostatic force between the first and the second modified interfaces. In some embodiments, the positively charged residue is lysine or arginine.

[0038] In some embodiments, (i) the first TMD comprises SEQ ID NO:21 or functional variants thereof and the lysine or arginine residue is at position selected from positions 10 to 14 of SEQ ID NO:21 or (ii) the first TMD comprises SEQ ID NO:18 and the lysine or arginine is at position selected from positions 8 to 11 of SEQ ID NO: 18. In some embodiments, the lysine or arginine residue is (i) at position 12 of SEQ ID NO:21, or (ii) is at position 9 of SEQ ID NO:18.

[0039] In some embodiments, the extracellular domain comprises an antigen-binding moiety capable of binding to a ligand on the surface of a cell. In some embodiments, the antigen-binding moiety is selected from the group consisting of an antibody, a nanobody, a diabody, a triabody, a minibody, an $F(ab')_2$ fragment, an F(ab) fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and a functional fragment thereof.

[0040] In some embodiments, the ligand comprises a protein or a carbohydrate.

[0041] In some embodiments, the ligand is a tumor-associated antigen or a tumor-specific antigen.

[0042] In some embodiments, the ligand comprises cell-surface receptors, adhesion proteins, integrins, mucins, lectins, tumor associated antigens, or tumor specific antigens.

[0043] In some embodiments, the ligand comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent Protein (eGFP), or signal regulatory protein a (SIRPα).

[0044] In some embodiments, the cell is a human cell.

[0045] In some embodiments, the cell is a tumor cell.

[0046] In some embodiments, the first polypeptide further comprises one or more of the following: a hinge domain, a ligand-inducible proteolytic cleavage site, an autoproteolytic peptide sequence, a nuclear localization signal, a juxtamembrane domain. In some embodiments, the juxtamembrane domain is a polybasic domain. In some embodiments, the polybasic domain

comprises Notch-1 or Notch-2 juxtamembrane domains. In some embodiments, the autoproteolytic peptide sequence is from a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

[0047] In some embodiments, the first polypeptide further comprises a hinge domain from CD8, CD28, OX40, or IgG4. In some embodiments, the hinge domain comprises a truncated CD8α hinge domain.

[0048] In an aspect, provided herein are recombinant nucleic acid constructs including, in the 5' to 3' direction, a first cassette and a second cassette, wherein the first cassette and the second cassette are joined by an autoproteolytic peptide and wherein the first cassette encodes the first polypeptide and the second cassette encodes the second polypeptide of the multi-chain chimeric polypeptides of the disclosure.

[0049] In a further aspect, provided herein are nucleic acid constructs including, in the 5' to 3' direction, a first cassette and a second cassette, wherein the first cassette and the second cassette are joined by an autoproteolytic peptide and wherein the first cassette encodes the second polypeptide and the second cassette encodes the first polypeptide of the multi-chain chimeric polypeptides of the disclosure.

[0050] In a further aspect, provided herein are nucleic acid constructs encoding the first polypeptide of any one of the multi-chain chimeric polypeptide of the present disclosure.

[0051] further aspect, provided herein are nucleic acid constructs encoding the second polypeptide of any one of the multi-chain chimeric polypeptide of the present disclosure.

[0052] In some embodiments, the autoproteolytic peptide is a Thosea asigna virus 2A (T2A) peptide.

[0053] In some embodiments, the nucleic acid construct has a nucleic acid sequence including 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, 3, 4, 5, 6, 7, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58 or functional variants thereof.

[0054] In a further aspect, the disclosure provides vectors including the recombinant nucleic acid molecules of the disclosure. In some embodiments, the vectors are expression vectors. In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is a

retroviral vector. In some embodiments, the viral vector is selected from the group consisting of a lentiviral vector, an adeno virus vector, and an adeno-associated virus vector.

[0055] In another aspect, provided herein are recombinant cells including a) multi-chain chimeric polypeptide of the disclosure, or b) recombinant nucleic acid molecules of the disclosure, or c) vectors of the disclosure. In some embodiments, the recombinant cell is a human cell. In some embodiments, the recombinant cell is tumor cell. In some embodiments, the recombinant cell is an immune cell. In some embodiments the immune cell is a B cell, a monocyte, a natural killer cell, a basophil, an eosinophil, a neutrophil, a dendritic cell, a macrophage, a regulatory T cell, a helper T cell, a cytotoxic T cell, and other T cells. In some embodiments, the T cell is a CD4+ T cell or a CD8+ T cell.

[0056] The present disclosure also provides pharmaceutical compositions including the recombinant cells of the disclosure and a pharmaceutically acceptable excipient.

[0057] Also provided herein, are methods of simultaneously inducing T cell signaling and gene regulation in a T cell including (a) providing a T cell having the multi-chain chimeric polypeptide or an antigen receptor of the disclosure; and (b) exposing the T cell to the selected ligand, wherein binding of a selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator.

[0058] In a further aspect, the disclosure provides methods of simultaneously inducing T cell signaling and gene regulation in a T cell including (a) providing (i) a vector including a multi-chain chimeric polypeptide of the disclosure or (ii) a first vector including a first polypeptide of the disclosure and a second vector including the second polypeptide of the disclosure; and (b) transducing a T cell with the vector or vectors, wherein binding of a selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator. In some embodiments, the induced intracellular signaling of the T cell modulates expression of a selected gene involved in proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and/or cytolytic activity.

[0059] In a further aspect, the disclosure provides methods of inducing enhanced T cell signaling in a T cell, the method comprising (a) providing a T cell comprising the multi-chain chimeric polypeptide of the present disclosure; and (b) exposing the T cell to a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain enhances

intracellular signaling.

[0060] In a further aspect, the disclosure provides, methods of inducing enhanced T cell signaling in a T cell, the method comprising (a) providing a vector comprising the multi-chain chimeric polypeptide of the present disclosure, or a vector comprising the first polypeptide and a second vector comprising the second polypeptide of any one of the multi-chain polypeptides of the present disclosure; and (b) transducing a T cell with the vector or vectors, wherein binding of the selected ligand to the extracellular ligand-binding domain induces enhanced intracellular signaling.

[0061] In some embodiments, the released transcriptional regulator modulates expression of a payload in the T cell. In some embodiments, the payload includes a chemokine, a chemokine receptor, a chimeric antigen receptor, a cytokine, a cytokine receptor, a differentiation factor, a growth factor, a growth factor receptor, a hormone, a metabolic enzyme, a pathogen-derived protein, a proliferation inducer, a receptor, an RNA guided nuclease, a site-specific nuclease, a T cell receptor, a toxin, a toxin derived protein, a transcriptional regulator, a transcriptional activator, a transcriptional repressor, a translational regulator, a translational activator, a translational repressor, an activating immuno-receptor, an antibody, an apoptosis inhibitor, an apoptosis inducer, an engineered T cell receptor, an immuno-activator, an immuno-inhibitor, or an inhibiting immuno-receptor.

[0062] In another aspect, provided herein are methods for the treatment of a health condition in a subject in need thereof including administering to the subject a therapeutically effective amount of the recombinant cells or the pharmaceutical compositions of the disclosure wherein the recombinant cells or the pharmaceutical composition treat the health condition in the subject. In some embodiments, the administered recombinant cell modulates an activity of a target cell in the individual. In some embodiments, the activity of the target cell includes expression of a selected gene, proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and cytolytic activity. In some embodiments, the target cell is a cancer cell. In some embodiments, the cancer cell is a solid tumor cell or a hematological malignancy cell. In some embodiments, the hematological malignancy cell is a multiple myeloma cell.

[0063] Also provided herein, are methods for regulating a T cell activity including (a) providing an effective amount of any of the recombinant cells of the disclosure; and (b)

contacting the cell with a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain (i) induces cleavage of a ligand-inducible proteolytic cleavage site and releases the transcriptional regulator and simultaneously (ii) activates T cell signaling, wherein the released transcriptional regulator modulates an activity of the recombinant cell.

[0064] The foregoing summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative embodiments and features described herein, further aspects, embodiments, objects and features of the disclosure will become fully apparent from the drawings and the detailed description and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0065] **FIG. 1** shows a schematic of a multi-chain chimeric polypeptide according to the disclosure with a first chain/first polypeptide (on the right) delivering transcriptional regulation via a transcription factor and a second chain/second polypeptide (on the left) delivering signaling functions. The first polypeptide includes an extracellular ligand-binding domain having a binding affinity for a selected ligand, a first TMD with a first modified interface (a positively-charged amino acid residue) and a first ICD having a transcriptional regulator. The second polypeptide includes a second TMD having a second interface (a negatively-charged amino acid residue) and a second ICD having a signaling domain. Coupling of the first and second polypeptide is via the interaction between the positively-charged and negatively charged amino acid residues. Activation of the multi-chain chimeric polypeptide by ligand binding (e.g., CD 19) leads to the dissociation of the transcriptional regulator (e.g., Gal4VP64) which then enters the nucleus and activates transcription of a gene (in this example, BFP). A schematic for a transcription regulator response cassette with the BFP gene is also shown.

[0066] **FIGS. 2A-D** schematically summarize the results from experiments performed to evaluate the functionality of various chimeric multi-chain polypeptides/receptors in order to optimize multi-chain chimeric receptors that induce activity of the signaling domain and release of the transcriptional regulator upon ligand binding to the extracellular ligand-binding domain. **FIG. 2A** shows an embodiment of a multi-chain chimeric polypeptide (e.g., immunoreceptor) referred to as 056. The 056 embodiment includes i) a first polypeptide having a CD19scFv binding domain, a TREM2 first TMD with a positively charged first modified interface and a

Gal4VP64 translational regulator, and (ii) a second polypeptide chain comprising DAP12 having a negatively charged modified interface. The nucleic acid construct encoding the 056 embodiment (SEQ ID NO: 2) includes, in the 5' to 3' direction, DAP12, T2A linker, and a cassette encoding CD19scFV-TREM2-Gal4VP64 first polypeptide. **FIG. 2B** shows another design of a multi-chain chimeric polypeptide denoted 056C. The 056C embodiment includes i) a first polypeptide having a CD19scFv binding domain, a truncated CD8α-hinge domain (labeled as CD8 Hinge2), a Notch1 first TMD with a positively charged first modified interface including an L1747K (Uniprot P46531 numbering Uniprot P46531 amino acids 1736 to 1757 with a L1747K mutation) substitution, a Notch2 juxtamembrane domain, a Gal4VP64 translational regulator, and (ii) a second polypeptide chain comprising DAP12. The nucleic acid construct encoding the 056C embodiment (SEQ ID NO: 4) includes, in the 5' to 3' direction, DAP12, T2A linker, and a cassette encoding a first polypeptide having CD19scFV, truncated CD8α hinge domain (CD8 Hinge2), Notch 1 with L1747K substitution (Uniprot P46531 amino acids 1736 to 1757 with a L1747K mutation), Notch 2 juxtamembrane domain and Gal4VP64 transcriptional regulator. **FIG. 2C** shows a different design of a multi-chain chimeric polypeptide (056D) having i) a first polypeptide having a CD19scFv binding domain, TREM2 hinge, a Notch1 first TMD with a positively charged first modified interface including an L1747K substitution (Uniprot P46531 amino acids 1736 to 1757 with a L1747K mutation), a Notch2 juxtamembrane domain, a Gal4VP64 translational regulator, and (ii) a second polypeptide chain including DAP12. The nucleic acid construct encoding the 056D embodiment (SEQ ID NO:5) includes, in the 5' to 3' direction, DAP12, T2A autoproteolytic peptide sequence and a cassette encoding CD19scFV-TREM2-Notch1 with a L1747K substitution (Uniprot P46531 amino acids 1736 to 1757 with a L1747K mutation) -Gal4VP64 first polypeptide. **FIG. 2D** shows a multi-chain chimeric polypeptide, denoted 056B, having i) a first polypeptide having a CD19scFv binding domain, TREM2 hinge, a Notch1 first TMD with a positively charged first modified interface including a gamma secretase active site, a Notch2 juxtamembrane domain, a Gal4VP64 translational regulator, and (ii) a second polypeptide chain including DAP12 (056B). The nucleic acid construct encoding the 056B embodiment (SEQ ID NO:3) includes, in the 5' to 3' direction, DAP12, T2A autoproteolytic peptide sequence, and a cassette encoding CD19scFV-TREM2-Notch1 insert with a gamma-secretase active site-TREM2 intracellular domain sequence-Gal4VP64 first polypeptide. A comparison of the expression levels of the four multi-chain

receptors 056, 056C, 056D and 056B is also shown. Out of the four designs, 056C has the strongest transcriptional activity. The bottom panel in each of FIGS. 2A-2D shows the relative expression of the four receptors, measured by anti-myc-tag staining (denoted the second chain (y-axis), versus the reporter construct expression, measured by GFP (x-axis). Specifically, primary human CD3+ T cells were activated with anti-CD3/anti-CD28 Dynabeads (Gibco) and transduced with one lentiviral construct (two can also be used, where one lentivirus encodes a first polypeptide and the second lentivirus encodes a second polypeptide of a multi-chain chimeric polypeptide). The DAP12/TREM immunoreceptor (056) and the DAP12/Hinge Notch hybrid (056C) show some expression whereas the DAP12/TREM-Notch Hybrid 1 (056D) and the DAP12/TREM-Notch hybrid2 (056B) do not show expression.

[0067] **FIGS. 3A-C** depict a comparison of the design (**FIG. 3A**), activation dynamics (**FIG. 3B**) and killing activity (**FIG. 3C**) between the 056 and 056C multi-chain chimeric polypeptides described in **FIG. 2A**. **FIG. 3B** shows receptor transcriptional activation of an inducible BFP reporter gene (measured using a Fortessa X-50 (BD Sciences) in T cells expressing anti-CD19 receptors with (i) no additional cells (upper trace), (ii) K562 cells (middle trace) or (iii) CD19+ K562 cells (lower trace). **FIG. 3C** also shows a comparison of the killing activity for 056 and 056C receptors. The 056C receptor has a higher transcriptional activity than the 056 receptor but does not give a strong killing response.

[0068] **FIGS. 4A-4D** illustrate additional designs of multi-chain chimeric polypeptides (056, 056E, 056F and 056G). **FIG. 4A** shows receptor 056, the same described in **FIG. 2A**, **FIG. 4B** depicts a design of a multi-chain chimeric polypeptide (056E) in which the second polypeptide includes DAP12 and a Gal4VP64 transcriptional regulator and the first polypeptide includes a CD19scFv ligand binding domain, a TREM2 transmembrane and intracellular domains. 056E receptor is encoded by a nucleic acid construct having a DAP12, a Gal4VP64 cassette that is linked (by a T2A linker) to a cassette having a CD19scFV, a TREM2 transmembrane and intracellular domains. Such a design has limited relative expression. **FIG. 4C** depicts an embodiment of the multi-chain chimeric polypeptide according to the disclosure (056F) in which the first polypeptide includes a CD19scFV ligand binding domain, a truncated CD8α hinge domain (CD8 Hinge 2), a polyvaline TMD with a V9K substitution, a Notch 2 juxtamembrane domain and a Gal4VP64 transcriptional regulator, and the second polypeptide including DAP12 signaling domain. The 056F embodiment is encoded by a nucleic acid construct having, in the 5'

to 3' direction, DAP12 followed by a T2A autoproteolytic peptide sequence, and a cassette including the a) truncated CD8α hinge domain (CD8 Hinge 2), b) a polyvaline TMD with a V9K substitution, c) a Notch 2 juxtamembrane domain and d) a Gal4VP64 transcriptional regulator. **FIG. 4D** shows an embodiment (056G) similar to the 056F embodiment except that it has a polyvaline TMD with a V10K substitution. The 056F amd 056G embodiments having polyvaline TMD exhibit increased expression compared to other embodiments.

[0069] **FIGS. 5A-D** depict a comparison of the killing activity (target clearance) and activation dynamics of the embodiments of multi-chain polypeptides, 056, 056E, 056F and 056G described in **FIGS. 4A-D**. Embodiment 056 and 056E do not exhibit strong transcriptional activation but are able to exhibit killing activity of target cells. Embodiments 056F and 056G show strong transcriptional activation but only 056 F achieves target killing.

[0070] **FIG. 6** shows a schematic of the multi-chain receptor modular engineering strategy, using pRay056F as the prototype.

[0071] **FIG**. 7 shows testing of the multi-chain receptor function with CD3z substitution.

[0072] **FIG. 8** shows testing of the multi-chain receptor function with a human transcription factor.

[0073] **FIG. 9** shows a schematic of the multi-chain receptor with modular engineering strategy using dual vector transduction.

[0074] **FIG. 10** shows the expression profile of each multi-chain receptor pair.

[0075] **FIG. 11** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows T cell counts on days 5, 12, and 20 at an ET ratio of 1:1.

[0076] **FIG. 12** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows T cell counts on days 5, 12, and 20 at an ET ratio of 1:2.

[0077] **FIG. 13** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows T cell counts on days 5, 12, and 20 at an ET ratio of 1:4.

[0078] **FIG. 14** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows K562 cell counts on days 5, 12, and 20 at an ET ratio of 1:1.

[0079] **FIG. 15** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows K562 cell counts on days 5, 12, and 20 at an ET ratio of 1:2.

[0080] **FIG. 16** shows a screen for multi-chain receptors that enhance killing and survival. The graph shows K562 cell counts on days 5, 12, and 20 at an ET ratio of 1:4.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0081] It is widely known in the chimeric polypeptide (e.g., receptor) field that proximity to the membrane when adding a signaling domain is important and does matter in how well the domain signals. The present disclosure provides chimeric polypeptides/receptors that have a different architecture than what is commonly known in the field and that allow more control on where the signaling motifs are located. The relationship of the chimeric polypeptides/receptors of the present disclosure to the membranes is much more controllable than what has been previously known.

[0082] Accordingly, the disclosure provides, *inter alia*, multi-chain chimeric polypeptides having two distinct polypeptides (chains) such that a transcriptional factor is located on the first polypeptide close to the membrane and a signaling domain is located on the second polypeptide. The first polypeptide and the second polypeptide can associate to allow, in a ligand dependent manner, the *simultaneous* modulation of signaling (*e.g.*, T-cell signaling) from one chain and the transcriptional regulation from another chain, upon binding of a select ligand to one of the polypeptides.

[0083] Particularly, the disclosure relates to multi-chain chimeric polypeptides with distinct polypeptide chains that can associate post-translationally via residues located within the TMD of the first and second polypeptide. Ligand binding to the extracellular ligand binding domain (ECD) of the first polypeptide, can activate cell signaling and cleavage release of a transcriptional regulator from the first polypeptide. The cleaved transcriptional regulator can function, therefore, free of any other fused signaling domain and vice versa.

[0084] In some embodiments, the multi-chain chimeric polypeptides presented herein can have the following unique architecture in which the first polypeptide may include (i) an extracellular ligand-binding domain having a binding affinity (specificity) for a selected ligand, (ii) a first TMD with a first modified interface and (iii) a first intracellular domain with a transcriptional regulator. The second polypeptide may include (i) a second TMD having a second interface and (ii) a second intracellular domain with a signaling domain. The first and the second polypeptide can associate via charged residues within the first modified interface and the second interface. Binding of the select ligand to the extracellular ligand-binding domain can

simultaneously induce activity of the signaling domain and release of the transcriptional regulator.

[0085] The disclosure further provides compositions including chimeric antigen receptors and nucleic acid constructs encoding the same, host cells genetically modified with the nucleic acid constructs, pharmaceutical compositions as well as methods for modulating an immune response or an activity of a cell, methods for inducing T cell signaling and methods for the treatment of various health conditions, such as diseases (e.g., cancers).

[0086] The multi-chain chimeric polypeptides of the disclosure constitute a platform for engineering T cells that can, not only detect and activate a response to a tumor, but additionally turn on one or more transcriptional program(s) such as the production of various biologics, cytokines, inflammatory factor, regulatory RNAs, *etc*. The multi-chain chimeric polypeptides of the disclosure allow the coupling of receptor capabilities with defined auxiliary functions that are not normally encoded or triggered through the signaling domains built into the synthetic receptors of the prior art. Accordingly, the multi-chain chimeric polypeptides of the disclosure can enable a broadened approach to positively modify T cell function in different ways than what has been known to date. The multi-chain chimeric polypeptides of the disclosure can also be used for delivering payloads, such as chemokines, to recruit different immune cells for tumor control, or guide T cell fate towards more efficacious subtypes through the expression of transcription factors or other factors controlling differentiation, *etc*.

[0087] The design of the multi-chain polypeptides of the disclosure can facilitate and enable more instant signaling capabilities to receptors that previously has only linked antigen recognition to a transcription response (*i.e.* with no other signaling effects). By incorporating signaling domains in the multi-chain format of the disclosure, a skilled in the art can effectively couple auxiliary functions to a transcriptional response and have both activated at the same time and in response to the same antigen. Auxiliary functions can include, for example, destroying cells (through the use of immunoreceptor tyrosine-based activation motif (ITAM) domains on the signaling chain) or promoting survival signaling through the use of costimulatory domains (such as 41BB, CD28 costimulatory domains, *etc*).

[0088] In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols generally identify similar components, unless context dictates otherwise. The illustrative alternatives described in the

detailed description, drawings, and claims are not meant to be limiting. Other alternatives may be used and other changes may be made without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, and designed in a wide variety of different configurations, all of which are explicitly contemplated and make part of this application.

I. DEFINITIONS

[0089] Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art.

[0090] The singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells, including mixtures thereof. "A and/or B" is used herein to include all of the following alternatives: "A", "B", "A or B", and "A and B".

[0091] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges can independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure. Certain ranges are presented herein with numerical values being preceded by the term "about" which, as used herein, has its ordinary meaning of approximately. The term "about" is used to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining

whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number can be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. If the degree of approximation is not otherwise clear from the context, "about" means either within plus or minus 10% of the provided value, or rounded to the nearest significant figure, in all cases inclusive of the provided value. In some embodiments, the term "about" indicates the designated value ± up to 10%, up to ± 5%, or up to ± 1%.

[0092] The terms "administration" and "administering", as used herein, refer to the delivery of a bioactive composition or formulation by an administration route comprising, but not limited to oral, intranasal, transdermal, intravenous, intra-arterial, intramuscular, intranodal, intraperitoneal, subcutaneous, and intramuscular administration, or combinations thereof. The term includes, but is not limited to, administering by a medical professional and self-administering.

[0093] It is understood that such terms as "cell", "cell culture", and "cell line" refer not only to the particular subject cell, cell culture, or cell line but also to the progeny or potential progeny of such a cell, cell culture, or cell line, without regard to the number of transfers or passages in culture. It should be understood that not all progeny are exactly identical to the parental cell. This is because certain modifications can occur in succeeding generations due to either mutation (e.g., deliberate or inadvertent mutations) or environmental influences (e.g., methylation or other epigenetic modifications), such that progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein, so long as the progeny retain the same functionality as that of the original cell, cell culture, or cell line.

[0094] It is understood that aspects and embodiments of the disclosure described herein include "comprising", "consisting", and "consisting essentially of" aspects and embodiments. As used herein, "comprising" is synonymous with "including", "containing", or "characterized by", and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any elements, steps, or ingredients not specified in the claimed composition or method. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claimed composition or method. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of steps of a method, is

understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or steps.

[0095] The term "cancer" refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Some types of cancer cells can aggregate into a mass, such as a tumor, but some cancer cells can exist alone within a subject. A tumor can be a solid tumor, a soft tissue tumor, or a metastatic lesion. As used herein, the term "cancer" also encompass other types of non-tumor cancers. Non-limiting examples include blood cancers or hematological malignancies, such as leukemia, lymphoma, and myeloma. Cancers can include premalignant, as well as malignant cancers.

[0096] The term "nucleic acid" is used herein in reference to either DNA or RNA, or molecules which contain deoxy- and/or ribonucleotides. Nucleic acids may be naturally occurring or synthetically made, and as such, include analogs of naturally occurring polynucleotides in which one or more nucleotides are modified over naturally occurring nucleotides.

[0097] The term "operably linked", as used herein, denotes a physical or functional linkage between two or more elements, e.g., polypeptide sequences or polynucleotide sequences, which permit them to operate in their intended fashion.

[0098] The term "percent identity," as used herein in the context of two or more nucleic acid sequences or proteins, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acids that are the same (*e.g.*, about 50% sequence identity or higher – e.g., about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using, for example The National Center for Biotechnology's (NCBI) BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a sequence. This definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Sequence identity can be calculated using published techniques and publicly

available computer programs, such as the GCS program package (Devereux et al, Nucleic Acids Res. 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul *et al.*, J Mol Biol 215:403, 1990 (incorporated herein by reference in its entirety). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof. For example, an amino acid sequence that is "substantially identical" to a reference sequence has at least about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%, including all values in between, sequence identity to the reference amino acid sequence including all values in between. For polypeptides, the length of comparison sequences will generally be at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 25, at least 50, at least 75, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, or at least 350 contiguous amino acids (e.g., a full-length sequence) including all values in between. For nucleic acids, the length of comparison sequences will generally be at least 5, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, or 25 contiguous nucleotides including all values in between (e.g., the full-length nucleotide sequence).

[0099] As used herein, a "subject" or an "individual" includes animals, such as human (e.g., human individuals) and non-human animals. In some embodiments, a "subject" or "individual" is a patient under the care of a physician. Thus, the subject can be a human patient or an individual who has, is at risk of having, or is suspected of having a disease of interest (e.g., cancer) and/or one or more symptoms of the disease. The subject can also be an individual who is diagnosed with a risk of the condition of interest at the time of diagnosis or later. The term "non-human animals" includes all vertebrates, e.g., mammals, e.g., rodents, e.g., mice, and non-mammals, such as non-human primates, e.g., sheep, dogs, cows, chickens, amphibians, reptiles, etc.

[0100] The term "recombinant" when used with reference to a nucleic acid, means that the nucleic acid has been altered or produced through human intervention such as, for example, has been modified by or is the result of laboratory methods. Thus, for example, recombinant nucleic acids include viral genomes and nucleic acids that are produced by laboratory methods.

Recombinant proteins or recombinant polypeptides generated by recombinant constructs can include amino acid residues not found within the native (non-recombinant or wild-type) form of the protein or can be include amino acid residues that have been modified, e.g., labeled. The term can include any modifications to the peptide, protein, or nucleic acid sequence. Such modifications may include the following: any chemical modifications of the peptide, protein or nucleic acid sequence, including of one or more amino acids, deoxyribonucleotides, or ribonucleotides; addition, deletion, and/or substitution of one or more of amino acids in the peptide or protein; creation of a fusion protein, e.g., a fusion protein comprising an antibody fragment; and addition, deletion, and/or substitution of one or more of nucleic acids in the nucleic acid sequence.

[0101] The term "recombinant" polypeptide as used herein, refers to a polypeptide that has been altered through human intervention. As non-limiting examples, an engineered polypeptide can be one which: 1) has been synthesized or modified *in vitro*, for example, using chemical or enzymatic techniques; 2) includes conjoined polypeptide sequences that are not conjoined in nature; 3) has been engineered using molecular cloning techniques such that it lacks one or more amino acids with respect to the naturally occurring polypeptide sequence; and/or 4) has been manipulated using molecular cloning techniques such that it has one or more sequence changes or rearrangements with respect to the naturally occurring polypeptide.

[0102] As will be understood by one having ordinary skill in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0103] It is appreciated that certain features of the disclosure, which are, for clarity,

described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all subcombinations of the various embodiments and elements thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such subcombination was individually and explicitly disclosed herein.

[0104] Although various features of the disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

II. COMPOSITIONS

A. Multi-Chain Chimeric Polypeptides

[0105] The present disclosure provides, *inter alia*, multi-chain chimeric polypeptides that include two polypeptide chains. A multi-chain polypeptide can be a functional polypeptide that is comprised of two or more discrete polypeptide elements (i.e., "chains"), covalently or non-covalently linked together by molecular association other than by peptide bonding. The chains of a multi-chain polypeptide can be structurally different. The chains of a multi-chain polypeptide can be functionally different. The present disclosure provides multi-chain chimeric polypeptides wherein in one of the polypeptides (first polypeptide) includes (i) an extracellular ligand-binding domain having a binding affinity for a selected ligand, (ii) a first TMD and (iii) a first intracellular domain with a transcriptional regulator. The other polypeptide (second polypeptide) includes (i) a TMD and (ii) an intracellular domain having a signaling domain. The first polypeptide is activated by ligand binding to the extracellular ligand binding domain thereafter leading to the release of the transcriptional regulator from the first polypeptide and to cellular signaling via the signaling domain on the second polypeptide. The two polypeptides are associated together such as, for example, by oppositely charged residues in TMDs.

[0106] The multi-chain chimeric polypeptides disclosed herein can act as receptors and facilitate cellular signaling or amplified activation of cellular pathways under certain cellular and environmental contexts. Such receptor activity can be exploited to enhance and tune the production of therapeutic payloads by cells engineered/transduced to express the multi-chain chimeric polypeptides or receptors of the disclosure.

[0107] Accordingly, in an aspect, provided herein are multi-chain chimeric polypeptides including: (a) a first polypeptide including (i) an extracellular ligand-binding domain having a binding affinity for a selected ligand, (ii) a first TMD having a first modified interface and (iii) a first intracellular domain with a transcriptional regulator; and (b) a second polypeptide including (i) a second TMD having a second interface and (ii) a second intracellular domain having a signaling domain, wherein the first and the second modified interfaces each includes amino acid residues having opposite charges such that the first polypeptide is coupled to the second polypeptide via the first modified interface and the second interface and wherein binding of the selected ligand to the extracellular ligand-binding domain induces activity of the signaling domain and release of the transcriptional regulator. In some embodiments of the multi-chain chimeric polypeptide of the disclosure, the first modified interface comprises a positively charged residue, the second interface comprises a negatively charged residue, and the first polypeptide is coupled to the second polypeptide via the electrostatic force between the first and the second modified interfaces.

[0108] In a further aspect, provided herein are multi-chain chimeric polypeptides that can enable simultaneous transcriptional regulation and cellular activation (e.g., T cell activation), upon binding of a selected ligand to the extracellular-ligand binding domain of the first polypeptide of the multi-chain chimeric polypeptides of the disclosure.

[0109] In some embodiments, the multi-chain chimeric polypeptides of the disclosure can be immunoreceptors. In some embodiments, the immunoreceptors are chimeric antigen receptors (CARS).

1. FIRST POLYPEPTIDES

[0110] The multi-chain polypeptides of the disclosure include a first chimeric polypeptide having an extracellular ligand-binding domain (moiety) with a binding affinity for a selected ligand, a first TMD having a first modified interface, a first intracellular domain and one or more transcriptional regulator. The "interface", as described herein, refers to the outward facing

residues within the transmembrane domain of the first polypeptide that interact with the outward facing residues within the transmembrane domain of another polypeptide. In some embodiments, the outward facing residues within the transmembrane domain of the first polyleptide form a charged surface for interaction with a partner interface from a separate protein, displaying an oppositely charged surface.

[0111] In some embodiments, the first polypeptides of the disclosure include in order from the N-terminus to the C-terminus (i) the extracellular ligand-binding domain, (ii) the first TMD, and (iii) the first TMD.

[0112] In some embodiments, the first polypeptides of the disclosure include an amino acid sequence that is has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO:10 or functional variants thereof.

a. Extracellular Ligand-Binding Domains (ECD)

[0113] In some embodiments, the extracellular domains (ECDs) of the first polypeptide of the multi-chain chimeric polypeptides disclosed herein have a binding affinity for one or more target ligands. The target ligand can be expressed on a cell surface, or otherwise be anchored, immobilized, or restrained so that it can exert a mechanical force on the chimeric receptor. As such, without being bound to any particular theory, binding of the ECD of the fist polypeptide of the multi-chain chimeric polypeptides provided herein to a cell-surface ligand does not necessarily remove the target ligand from the target cell surface, but instead enacts a mechanical pulling force on the chimeric receptor. For example, an otherwise soluble ligand may be targeted if it is bound to a surface, or to a molecule in the extracellular matrix.

[0114] In some embodiments, the target ligand is a cell-surface ligand. Non-limiting examples of suitable ligand types include cell surface receptors; adhesion proteins; carbohydrates, lipids, glycolipids, lipoproteins, and lipopolysaccharides that are surface-bound; integrins, mucins; and lectins. In some embodiments, the ligand is a protein. In some embodiments, the ligands include tumor associated antigens or tumor-specific antigens. In some embodiments, the ligand is a cluster of differentiation ligand (CD) and includes but is not limited to CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181

(CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), or signal regulatory protein a (SIRPα).

[0115] In some embodiments, the extracellular domains of the first polypeptide of the multi-chain chimeric polypeptides of the disclosure includes an antigen-binding moiety. In some embodiments, the antigen-binding moiety can bind to a ligand on the surface of a cell. In some embodiments, the antigen-binding moiety can bind one or more targets. In some embodiments, the antigen-binding moiety includes one or more antigen-binding determinants of an antibody or a functional antigen-binding fragment thereof. One skilled in the art upon reading the present disclosure will readily understand that the term "functional fragment thereof" or "functional variant thereof" refers to a molecule having quantitative and/or qualitative biological activity in common with the wild-type molecule from which the fragment or variant was derived. For example, a functional fragment or a functional variant of an antibody is one which retains essentially the same ability to bind to the same epitope as the antibody from which the functional fragment or functional variant was derived. For instance, an antibody that can bind to an epitope of a cell surface receptor may be truncated at the N-terminus and/or C-terminus, and the retention of its epitope binding activity assessed using assays known to those of skill in the art. The antigen-binding moiety can be, but is not limited to, an antibody, a nanobody, a diabody, a triabody, a minibody, an $F(ab')_2$ fragment, an F(ab) fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and any functional fragment thereof.

[0116] The antigen-binding moiety can include naturally-occurring amino acid sequences or can be engineered, designed, or modified to provide desired and/or improved properties such as, e.g., binding affinity. Generally, the binding affinity of an antigen-binding moiety, e.g., an antibody, for a target antigen (e.g., CD19 antigen) can be calculated by the Scatchard method described by Frankel et al., Mol. Immunol, 16:101-06, 1979. In some embodiments, binding affinity is measured by an antigen/antibody dissociation rate. In some embodiments, binding affinity is measured by a competition radioimmunoassay. In some embodiments, binding affinity is measured by ELISA. In some embodiments, antibody affinity is measured by flow cytometry.

[0117] An antibody that "selectively binds" an antigen (such as CD19) is an antigen-binding

moiety that does not significantly bind other antigens but binds the antigen with high affinity, e.g., with an equilibrium constant (KD) of 100 nM or less, such as 60 nM or less, for example, 30 nM or less, such as, 15 nM or less, or 10 nM or less, or 5 nM or less, or 1 nM or less, or 500 pM or less, or 400 pM or less, or 300 pM or less, or 200 pM or less, or 100 pM or less.

[0118] A skilled artisan can select an ECD based on the desired localization or function of a cell that is genetically modified to express a multi-chain chimeric polypeptide or a first polypeptide of the present disclosure. For example, a first polypeptide with an ECD including an antibody specific for a HER2 antigen can target cells to HER2-expressing breast cancer cells. In some embodiments, the ECD of the disclosed first polypeptide is capable of binding a tumor-associated antigen (TAA) or a tumor-specific antigen (TSA). A skill artisan will understand that TAAs include a molecule, such as e.g., protein, present on tumor cells and on normal cells, or on many normal cells, but at much lower concentration than on tumor cells. In contrast, TSAs generally include a molecule, such as e.g., protein which is present on tumor cells but absent from normal cell.

[0119] In some cases, the antigen-binding moiety is specific for an epitope present in an antigen that is expressed by a tumor cell, i.e., a tumor-associated antigen. The tumor-associated antigen can be an antigen associated with, e.g., a breast cancer cell, a B cell lymphoma, a pancreatic cancer, a Hodgkin lymphoma cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma, a lung cancer cell, a non-Hodgkin B-cell lymphoma (B-NHL) cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma cell, a melanoma cell, a chronic lymphocytic leukemia cell, an acute lymphocytic leukemia cell, a neuroblastoma cell, a glioma, a glioblastoma, a colorectal cancer cell, etc. It will also be understood that a tumor-associated antigen may also be expressed by a non-cancerous cell. In some embodiments, the antigen-binding domain is specific for an epitope present in a tissue-specific antigen. In some embodiments, the antigen-binding domain is specific for an epitope present in a disease-associated antigen.

[0120] Non-limiting examples of suitable target antigens include CD19, B7H3 (CD276), BCMA (CD269), alkaline phosphatase, placental-like 2 (ALPPL2), green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), signal regulatory protein .alpha. (SIRPα), CD123, CD171, CD179.alpha., CD20, CD213A2, CD22, CD24, CD246, CD272, CD30, CD33, CD38, CD44v6, CD46, CD71, CD97, CEA, CLDN6, CLECL1, CS-1, EGFR, EGFRvIII,

ELF2M, EpCAM, EphA2, Ephrin B2, FAP, FLT3, GD2, GD3, GM3, GPRC5D, HER2 (ERBB2/neu), IGLL1, IL-11R.alpha., KIT (CD 117), MUC1, NCAM, PAP, PDGFR-.beta., PRSS21, PSCA, PSMA, ROR1, SSEA-4, TAG72, TEM1/CD248, TEM7R, TSHR, VEGFR2, ALPI, citrullinated vimentin, cMet, and Axl.

[0121] In some embodiments, the target antigen is selected from CD19, B7H3 (CD276), BCMA (CD269), ALPPL2, CD123, CD171, CD179.alpha., CD20, CD213A2, CD22, CD24, CD246, CD272, CD30, CD33, CD38, CD44v6, CD46, CD71, CD97, CEA, CLDN6, CLECL1, CS-1, EGFR, EGFRvIII, ELF2M, EpCAM, EphA2, Ephrin B2, FAP, FLT3, GD2, GD3, GM3, GPRC5D, HER2 (ERBB2/neu), IGLL1, IL-11Ra, KIT (CD117), MUC1, NCAM, PAP, PDGFR-.beta., PRSS21, PSCA, PSMA, ROR1, SSEA-4, TAG72, TEM1/CD248, TEM7R, TSHR, VEGFR2, ALPI, citrullinated vimentin, cMet, Axl, GPC2, human epidermal growth factor receptor 2 (Her2/neu), CD276 (B7H3), IL-13R.alpha.1, IL-13R.alpha.2, .alpha.-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD123, CD93, CD99, CD117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), ALK, DLK1, FAP, NY-ESO, WT1, HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor-1, AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (a subunit of the heterodimeric IL-2 receptor), CD3, CD4, CD5, IFN-.alpha., IFN-.gamma., IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin .alpha.4, integrin .alpha.4.beta.7, LFA-1 (CD11.alpha.), myostatin, OX-40, scleroscin, SOST, TGF.beta.1, TNF-.alpha., VEGF-A, pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD20, CD5, CD7, CD3, TRBC1, TRBC2, BCMA, CD38, CD123, CD93, CD34, CD1a, SLAMF7/CS1, FLT3, CD33, CD123, TALLA-1, CSPG4, DLL3, Kappa light chain, Lamba light chain, CD16/Fc.gamma.RIII, CD64, FITC, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), GD3, EGFRvIII (epidermal growth factor variant III), EGFR and isovariants thereof, TEM-8, sperm protein 17 (Sp17), mesothelin.

[0122] Further non-limiting examples of suitable antigens include PAP (prostatic acid

phosphatase), prostate stem cell antigen (PSCA), prostein, NKG2D, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, an abnormal p53 protein, integrin (33 (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene), Ral-B, GPC2, CD276 (B7H3), or IL-13R.alpha.. In some embodiments, the antigen is Her2. In some embodiments, the antigen is ALPPL2. In some embodiments, the antigen is BCMA. In some embodiments, the antigen-binding moiety of the ECD is specific for a reporter protein, such as GFP and eGFP. Non-limiting examples of such antigen-binding moiety include a LaG17 anti-GFP nanobody. In some embodiments, the antigen-binding moiety of the ECD includes an anti-BCMA fully-humanized VH domain (FHVH). In some embodiments, the antigen is signal regulatory protein.alpha. (SIRP.alpha.).

[0123] Additional antigens suitable for targeting by the first chimeric polypeptide and the multi-chain CARs disclosed herein include, but are not limited to GPC2, human epidermal growth factor receptor 2 (Her2/neu), CD276 (B7H3), IL-13R.alpha.1, IL-13R.alpha.2, .alpha.-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA). Other suitable target antigens include, but are not limited to, tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD123, CD93, CD99, CD117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), ALK, DLK1, FAP, NY-ESO, WT1, HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor-1.

[0124] Additional antigens suitable for targeting by the polypeptides disclosed herein include, but are not limited to, those associated with an inflammatory disease such as, AOC3 (VAP-1), CAM-3001, CCL11 (eotaxin-1), CD125, CD147 (basigin), CD154 (CD40L), CD2, CD20, CD23 (IgE receptor), CD25 (a subunit of the heteromeric of IL-2 receptor), CD3, CD4, CD5, IFN-.alpha., IFN-.gamma., IgE, IgE Fc region, IL-1, IL-12, IL-23, IL-13, IL-17, IL-17A, IL-22, IL-4, IL-5, IL-5, IL-6, IL-6 receptor, integrin .alpha.4, integrin .alpha.4.beta.7, LFA-1 (CD11.alpha.), myostatin, OX-40, scleroscin, SOST, TGF.beta.1, TNF-.alpha., and VEGF-A.

[0125] Further antigens suitable for targeting by the first chimeric polypeptides and the

multi-chain CARs disclosed herein include, but are not limited to the pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD20, CD5, CD7, CD3, TRBC1, TRBC2, BCMA, CD38, CD123, CD93, CD34, CD1a, SLAMF7/CS1, FLT3, CD33, CD123, TALLA-1, CSPG4, DLL3, Kappa light chain, Lamba light chain, CD16/Fc.gamma.RIII, CD64, FITC, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), GD3, EGFRvIII (epidermal growth factor variant III), EGFR and isovariants thereof, TEM-8, sperm protein 17 (Sp17), mesothelin. Further non-limiting examples of suitable antigens include PAP (prostatic acid phosphatase), prostate stem cell antigen (PSCA), prostein, NKG2D, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, an abnormal p53 protein, integrin .beta.3 (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene), and Ral-B. In some embodiments, the antigen is GPC2, CD19, Her2/neu, CD276 (B7H3), IL-13R.alpha.1, or IL-13R.alpha.2. In some embodiments, the antigen is Her2. In some embodiments, the antigen is ALPPL2. In some embodiments, the antigen is BCMA. In some embodiments, the antigen-binding moiety of the ECD is specific for a reporter protein, such as GFP and eGFP. Non-limiting examples of such antigen-binding moiety include a LaG17 anti-GFP nanobody. In some embodiments, the antigen-binding moiety of the ECD includes an anti-BCMA fully-humanized VH domain (FHVH).

[0126] In some embodiments, the antigen can be HER2 produced by HER2-positive breast cancer cells. In some embodiments, the antigen can be CD19 that is expressed on B-cell leukemia. In some embodiments, the antigen can be EGFR that is expressed on glioblastoma multiform (GBM) but much less expressed so on healthy CNS tissue. In some embodiments, the antigen can be CEA that is associated with cancer in adults, for example colon cancer.

[0127] In some embodiments, the antigen-binding moiety of the ECD is specific for a cell surface target, where non-limiting examples of cell surface targets include CD19, CD30, Her2, CD22, ENPP3, EGFR, CD20, CD52, CD11 α, and α -integrin. In some embodiments, the chimeric first polypeptides and the multi-chain CARs disclosed herein include an extracellular domain having an antigen-binding moiety that binds CD19, CEA, HER2, MUC1, CD20, ALPPL2, BCMA, or EGFR. In some embodiments, the multi-chain CARs provided herein include an extracellular domain including an antigen-binding moiety that binds CD19. In some embodiments, the chimeric first polypeptides provided herein include an extracellular domain including an antigen-binding moiety that binds ALPPL2. In some embodiments, the chimeric

first polypeptides provided herein include an extracellular domain including an antigen-binding moiety that binds BCMA. In some embodiments, the chimeric first polypeptides include an extracellular domain including an antigen-binding moiety that binds Her2.

[0128] In some embodiments, antigens suitable for targeting by the chimeric first polypeptides disclosed herein include ligands derived from a pathogen.

b. Linking Sequences between ECD and First Transmembrane domain (TMD)

[0129] The multi-chain chimeric polypeptides and receptors of the disclosure can include a linking sequence disposed between the ECD and the TMD. The linking sequence can be a natural or a synthetic polypeptide. The linking sequence can be a flexible connector that provides structural flexibility and spacing to flanking polypeptide regions. The linking sequence can be a hinge domain inserted N-terminally to the TMD. In some embodiments, the hinge domain is a CD8 hinge domain. In some embodiments, the CD8 hinge domain is a truncated CD8α hinge domain.. In some embodiments, the truncated CD8α is encoded by an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 17. In some embodiments, the truncated CD8α comprises an amino acid sequence comprising SEQ ID NO: 17. In some embodiments, the truncated CD8α is encoded by SEQ ID NO: 17.

[0130] Hinge polypeptide sequences suitable for the compositions and methods of the disclosure can be naturally-occurring hinge polypeptide sequences (e.g., those from naturally-occurring immunoglobulins). Alternatively, a hinge polypeptide sequence can be a synthetic sequence that corresponds to a naturally-occurring hinge polypeptide sequence, or can be an entirely synthetic hinge sequence, or can be engineered, designed, or modified to provide desired and/or improved properties, e.g., modulating transcription. Suitable hinge polypeptide sequences include, but are not limited to, those derived from IgA, IgD, and IgG subclasses, such as IgG1 hinge domain, IgG2 hinge domain, IgG3 hinge domain, and IgG4 hinge domain, or a functional variant thereof. In some embodiments, the hinge polypeptide sequence contains one or more CXXC motifs. In some embodiments, the hinge polypeptide sequence contains one or more CPPC motifs. Additional information in this regard can be found in, for example, a recent review by Vidarsson G. et al., Frontiers Immunol. Oct. 20, 2014, which is hereby incorporated by reference in its entirety.

[0131] Accordingly, in some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgG1 hinge domain or a functional variant thereof. In some

embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgG2 hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgG3 hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgG4 hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgA hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgD hinge domain or a functional variant thereof.

[0132] Additional hinge polypeptide sequences suitable for the compositions and methods disclosed herein include, but are not limited to, hinge polypeptide sequences derived from a CD8α hinge domain, a CD28 hinge domain, a CD152 hinge domain, a PD-1 hinge domain, a CTLA4 hinge domain, an OX40 hinge domain, an Fcγ RIIIα hinge domain, and functional variants thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from a CD8.alpha hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from a CD28 hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an OX40 hinge domain or a functional variant thereof. In some embodiments, the hinge domain includes a hinge polypeptide sequence derived from an IgG4 hinge domain or a functional variant thereof.

[0133] In principle, there are no particular limitations to the length and/or amino acid composition of the hinge domain other than it confers flexibility. However, one skilled in the art will readily appreciate that the length and amino acid composition of the hinge polypeptide sequence can be optimized to vary the orientation and/or proximity of the ECD and the TMD relative to one another, as well as of the first polypeptide and second polypeptide to one another, to achieve a desired activity of the chimeric multi-chain polypeptides of the disclosure. In some embodiments, any arbitrary single-chain peptide including about one to 100 amino acid residues (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc. amino acid residues) can be used as a hinge domain. In some embodiments, the hinge domain includes about 5 to 50, about 10 to 60, about 20 to 70, about 30 to 80, about 40 to 90, about 50 to 100, about 60 to 80, about 70 to 100, about 30 to 60, about 20 to 80, about 30 to 90 amino acid residues. In some embodiments, the hinge domain includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to

25, about 20 to 40, about 30 to 50, about 40 to 60, about 50 to 70 amino acid residues. In some embodiments, the hinge domain includes about 40 to 70, about 50 to 80, about 60 to 80, about 70 to 90, or about 80 to 100 amino acid residues. In some embodiments, the hinge domain includes about 1 to 10, about 5 to 15, about 10 to 20, about 15 to 25 amino acid residues. In some embodiments, the hinge domain includes a sequence having at least 80% sequence identity, such as, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or 99% sequence identity to SEQ ID NO: 17.

c. First Transmembrane Domains

[0134] The multi-chain chimeric polypeptides of the present disclosure include a transmembrane domain located in the first polypeptide (e.g., first TMD). In some embodiments of the first polypeptide of the disclosure, the first TMD is fully synthetic. In some embodiments the first TMD includes a contiguous stretch of valine residues. In some embodiments, the contiguous stretch of valine residues includes 5 to 25 valine residues. In some embodiments, the first TMD includes a contiguous stretch of 5 to 25 valine residues. In some embodiments, the first TMD includes a contiguous stretch of 5, or 6, or 7, or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 consecutive valine residues. Examples of first TMDs having a contiguous stretch of valine residues (referred to as Poly V TMD) are shown in FIGS. 4C and 4D depicting embodiments 056F and 056G respectively of the multi-chain chimeric polypeptides of the disclosure.

[0135] In some embodiments, the first TMD includes a positively charged residue. In some embodiments, the positively charged residue is lysine or arginine. In some embodiments, the positively charged residue is within the contiguous stretch of valine residues. In some embodiments, the positively charged residue is located after the first valine residue from the N-terminus. In some embodiments, the positively charged residue is located after the second or third or fourth or fifth or sixth or seventh or eighth or ninth or tenth or eleventh or twelfth or thirteenth or fourteenth or fifteenth or sixteenth or seventeenth or eighteenth or nineteenth or twentieth or twenty first or twenty second or twenty third valine residue from the N-terminus. In some embodiments, the lysine or arginine residue replaces the sixth or seventh or eighth or ninth or tenth or eleventh or twelfth valine residue from the N-terminus. In some embodiments, the TMD of the first polypeptide includes a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 18. In some embodiments, the TMD is SEQ ID

NO: 18. In some embodiments, the lysine or arginine residue is residue number 8 or residue number 9 or residue number 10 or residue number 11 of SEQ ID NO:18 or at a position corresponding to residue number 8 or residue number 9 or residue number 10 or residue number 11 of SEQ ID NO:18.

[0136] The first TMD of the multi-chain chimeric polypeptides of the disclosure can include a TMD from a Notch receptor. In some embodiments, the TMD includes the Notch 1 receptor (FIGS. 2B-2D depicting embodiments 056C, 056D and 056B of the multi-chain chimeric polypeptides of the disclosure). In some embodiments, the Notch 1 receptor is a human Notch 1 receptor.

[0137] The first TMD can include one or more ligand-inducible proteolytic cleavage sites. Examples of ligand-inducible proteolytic cleavage sites in a Notch receptor (e.g., S2 or S3) are described in US Patent No. 11,202,801, incorporated herein by reference in its entirety. In some embodiments, the ligand-inducible proteolytic cleavage site is cleavable by gamma secretase, a multiprotein enzyme complex as in, for example, embodiment 056B (FIG. 2D.)

[0138] The first polypeptide of the multi-chain chimeric polypeptides of the disclosure can include a juxtamembrane domain (JMD). The JMD can be located C-terminally to the TMD. The JMD can include a highly-charged domain. In some embodiments, the JMD is a Notch 2 JMD.

[0139] In principle, there are no particular limitations to the length and/or amino acid composition of the JMD. Any arbitrary single-chain peptide that includes about 4 to about 40 amino acid residues (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc. amino acid residues) can be used as a JMD. In some embodiments, the JMD includes about 4 to 15, about 6 to 20, about 8 to 25, about 10 to 30, about 12 to 35, about 14 to 40, about 5 to 40, about 10 to 35, about 15 to 30, about 20 to 25, about 20 to 40, about 10 to 30, about 4 to 20, or about 5 to 25 amino acid residues. In some embodiments, the JMD includes about 4 to 10, about 5 to 12, about 6 to 14, about 7 to 18, about 8 to 20, about 9 to 22, about 10 to 24, or about 11 to 26 amino acid residues. In some embodiments, the JMD includes about 4 to 10 residues, such as, 4, 5, 6, 7, 8, 9, or 10 amino acid residues.

[0140] In some embodiments, the juxtamembrane domain is a polybasic domain. In some embodiments, the polybasic domain includes Notch -1 or Notch-2. In some embodiments, the polybasic domain comprises an amino acid sequence where the majority (i.e., at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, or 100%,) of the residues are lysine and/or arginine and/or histidine and/or any combination thereof.

[0141] The JMD can include a sequence having at least 70% sequence identity, such as, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or 99% sequence identity to a JMD sequence from Notch1, Notch2, Notch3, Notch4, CLSTN1, CLSTN2, CSF1R, CXCL16, DAG1, GHR, PTPRF, AGER, KL, NRG1, LRP1B, Jag2, EPCAM, KCNE3, CDH2, CDH5, NRG2, PTPRK, BTC, EPHA3, EPHA4, IL1R2, or PTPRM. In some embodiments, the JMD includes a sequence having only Lys (K) or Arg (R) in the first 4 residues. In some embodiments, the JMD includes one, two, three, four, five, or more basic residues. In some embodiments, the JMD includes five, four, three, two, one, or zero aromatic residues or residues with hydrophobic and/or bulky side chains.

d. First Intracellular Domains and Transcriptional Regulators

[0142] The intracellular domains of the first polypeptides of the multi-chain chimeric polypeptides of the disclosure can include a transcriptional regulator. The transcriptional regulator of the disclosure can be a polypeptide element that acts to activate or inhibit the transcription of a promoter-driven DNA sequence. Transcriptional regulators suitable for the compositions and methods of the disclosure can be naturally-occurring transcriptional regulators or can be engineered, designed, or modified so as to provide desired and/or improved properties, e.g., modulating transcription. As discussed above, the engineered receptors of the present disclosure are advantageous in that they can provide the ability to trigger a custom transcriptional program in engineered cells. In some embodiments, transcriptional regulator of the disclosure is a custom transcriptional regulator that drives transcription off a specific sequence that only appears once in the engineered cell. In some embodiments, the transcriptional regulator of the disclosure includes a human or humanized sequence.

[0143] In some embodiments, the transcriptional regulator directly regulates differentiation of the cell. In some embodiments, the transcriptional regulator indirectly modulates (e.g., regulates) differentiation of the cell by modulating the expression of a second transcription factor. It will be understood by one having ordinary skill in the art that a transcriptional regulator can be a transcriptional activator or a transcriptional repressor. In some embodiments, the transcriptional regulator is a transcriptional repressor. In some embodiments, the transcriptional regulator is a transcriptional activator. In some exemplary embodiments, the transcriptional

regulator of the chimeric receptor of the disclosure includes a transcription factor DNA binding domain (DBD). Exemplary DBDs may include Gal4, tetR, ZFHD1, Zif268, and HAP1. In some exemplary embodiments, at least one transcriptional regulator of the chimeric receptors of the disclosure further includes a transactivation domain (TAD). In some embodiments, the transcriptional regulator can further include a nuclear localization signal.

[0144] In some embodiments, the transcriptional regulator of the chimeric receptor of the disclosure includes a zinc finger-containing transcriptional effector (ZTE) which includes one or more zinc finger proteins or zinc finger motifs (ZFs). For example, a transcriptional regulator of the chimeric receptors of the disclosure may include a DBD containing one or more ZFs. A ZF is a finger-shaped fold in a protein that permits it to interact with nucleic acid sequences such as DNA and RNA. Such finger-shaped fold is well known in the art. The fold is generally created by the binding of specific amino acids in the protein to a zinc atom, and is stabilized by the co-ordination of a zinc ion between four largely invariant (depending on zinc finger framework type) Cys and/or His residues. Exemplary zinc finger proteins may include ZF3, ZF6, ZF10, etc.

[0145] The term "motif" as used herein refers to a structural motif. A ZF motif is a relatively small polypeptide domain having a supersecondary structure, and includes approximately 30 amino acids and folds to form an α-helix adjacent an antiparallel β-sheet (known as a ββα-fold), and is stabilized by a zinc ion. A ZF domain recognizes and binds to a nucleic acid triplet, or an overlapping quadruplet (as explained below), in a double-stranded DNA target sequence. Naturally-occurring zinc finger domains (also known as ZF proteins) have been well studied and described in the literature. Natural ZF proteins can regulate the expression of genes as well as nucleic acid recognition, reverse transcription and virus assembly. Additional information in this regard can be found in, for example, US Patent No. 10,138,493.

[0146] C2H2 zinc fingers (C2H2-ZFs) are among the most prevalent type of vertebrate DNA-binding domain, and generally appear in tandem arrays (ZFAs), with sequential C2H2-ZFs each contacting three (or more) sequential bases. C2H2-ZFs can be assembled in a modular fashion. Given a set of modules with defined three-base specificities, modular assembly also presents a way to construct artificial proteins with specific DNA-binding preferences.

[0147] ZF-containing proteins generally contain strings or chains of ZF motifs, forming an array of ZF (ZFA). Thus, a ZF protein may include two or more ZFs, e.g., a ZFA consisting of 2 or more ZF motifs, which may be directly adjacent one another (e.g., separated by a short linker

sequence), or may be separated by longer, flexible or structured polypeptide sequences. For example, a ZFA can have six ZF motifs (a 6-finger ZFA), seven ZF motifs (a 7-finger ZFA), or eight ZF motifs (an 8-finger ZFA), arranged in tandem. Directly adjacent ZF domains are generally expected to bind to contiguous nucleic acid sequences, e.g., to adjacent trinucleotides/triplets. In some cases, cross-binding may also occur between adjacent ZF and their respective target triplets, which may help to strengthen or enhance the recognition of the target sequence, and leads to the binding of overlapping quadruplet sequences. By comparison, distant ZF domains within the same protein may recognize, and/or bind to, non-contiguous nucleic acid sequences or even to different molecules (e.g., protein rather than nucleic acid).

[0148] In some embodiments, the multi-chain receptors of the disclosure include a zinc finger-containing transcriptional effector (ZTE) having a DNA binding zinc finger protein domain (ZF protein domain) and another domain through which the protein exerts its effect (effector domain). As described in further detail below, exemplary effector domains suitable for the multi-chain receptors of the disclosure include, but are not limited to, transcriptional activating domains (e.g., TADs), epigenetic effector domains, and DNA modifying enzymes.

[0149] In some embodiments, the multi-chain receptors of the disclosure include a transcriptional effector having a non-zinc finger DNA binding domain (e.g., DNA binding domains not having a zinc finger structure) and another domain through which the protein exerts its effect (effector domain). Exemplary non-zinc finger DNA binding domains include the domain derived from PAX6. As described in further detail below, exemplary effector domains suitable for the multi-chain receptors of the disclosure include, but are not limited to, transcriptional activating domains (e.g., TADs), epigenetic effector domains, and DNA modifying enzymes. In some embodiments, the multi-chain receptors of the disclosure include 1 to about 10 DNA binding domains, each of which independently includes a sequence having at least about 90% identity to the sequence of SEQ ID NO: 61 (PAX6). In some embodiments, the DNA binding domain of the transcriptional effector has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 61 (PAX6).

[0150] In some embodiments, the multi-chain receptors of the disclosure include a ZTE with two or more, e.g., 3 or more, for example, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or

more, 18 or more (e.g., up to approximately 30 or 32) ZF motifs arranged adjacent one another in tandem, forming arrays of ZF motifs or ZFA. In some embodiments, the ZTE includes at least 3 ZF motifs, at least 4 ZF motifs, at least 5 ZF motifs, or at least 6 ZF motifs, at least 7 ZF motifs, at least 8 ZF motifs, at least 9 ZF motifs, at least 10 ZF motifs, at least 11 or at least 12 ZF motifs; and in some cases at least 18 ZF motifs. In some embodiments, the ZTE of the engineered Notch receptors disclosed herein contains up to 6, 7, 8, 10, 11, 12, 16, 17, 18, 22, 23, 24, 28, 29, 30, 34, 35, 36, 40, 41, 42, 46, 47, 48, 54, 55, 56, 58, 59, or 60 ZF motifs. In some embodiments, the ZTE of the disclosure bind to orthogonal target nucleic acid binding sites. That is, the ZFs or ZFAs in ZF domain of the ZTE binds orthogonal target nucleic acid sequences. In some embodiments, the orthogonal target nucleic acid binding sites are contiguous. In some embodiments, the ZTE of the engineered Notch receptors disclosed herein binds target orthogonal specific DNA sequences and have, for example, reduced or minimal functional binding potential in a eukaryotic genome.

[0151] In some embodiments of the disclosure, the ZTE includes: (a) a first domain including a DNA-binding zinc finger protein domain (ZF protein domain), and (b) a second domain through which the ZTE exerts its effect (effector domain), wherein the ZTE has the following formula I:

[effector domain]a-[ZF protein domain]-[effector domain]b (Formula I)

wherein a and b are each independently an integer from 0 to 5, and at least one of a and b is not 0; wherein the ZF protein domain includes 1 to about 10 zinc finger arrays (ZFA); wherein the ZFA includes about 1 to 3, about 3 to 6, or about 6 to about 8 zinc finger motifs according to formula II (from N-terminal to C-terminal):

XcCXdCXe-(helix)-HXfH-L2 (Formula II)

wherein L2 is a linker peptide having about 4-6 amino acid residues, C is Cys, H is His, each X is independently any amino acid, c is an integer from 0 to 3, d is an integer from 1 to 5, e is an integer from 2 to 7, f is an integer from 3 to 6, and (helix) is a peptide domain of about 6 amino acids that forms an α-helix, wherein the ZFA is capable of binding a specific nucleic acid sequence.

[0152] In some embodiments, the ZF protein domain of the multi-chain receptors disclosed herein includes 1 to about 10 ZFA, each of which independently includes a sequence having at least about 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 62-67. In some embodiments, the ZFA includes a sequence having at least about 90%, at least about

95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 62-67. In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 62 (ZF3). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 63 (ZF6). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 64 (ZF6/SV40NLS). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 65 (ZF6/Notch1NLS). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 66 (ZF10/SV40NLS). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of SEQ ID NO: 67 (ZF10/Notch1NLS). In some embodiments, the ZFA sequence has at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the sequence of ZF2, ZF4, or ZF11.

[0153] In some embodiments, the ZF protein domain of the multi-chain receptors disclosed herein includes 1 to about 10 ZFA, each of which independently includes a sequence having about 100% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 62-67. In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 62 (ZF3). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO:63 (ZF6). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 64 (ZF6/SV40NLS). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 65 (ZF6/Notch1NLS). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 66 (ZF10/SV40NLS). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 67

(ZF10/Notch1NLS). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of SEQ ID NO: 67 (ZF10/Notch1NLS). In some embodiments, the ZFA sequence has about 100% sequence identity to the sequence of ZF2, ZF4, or ZF11.

[0154] In some embodiments, the ZF protein domain includes multiple ZFAs having the same amino acid sequences. In some embodiments, the ZF protein domain includes multiple ZFAs whose amino acid sequences are different from one another.

[0155] In some embodiments, the ZF protein domain of the multi-chain receptors disclosed herein includes one or more ZFAs that are independently capable of specifically binding to a target nucleic acid sequence selected from the group consisting of SEQ ID NOs: 62-67. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 62. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 63. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 64. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 65. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 66. In some embodiments, at least one ZFA is capable of specifically binding to a target nucleic acid sequence having the sequence of SEQ ID NO: 67. In some embodiments, the ZF protein domain of the engineered Notch receptors disclosed herein includes one or more ZFAs that are independently capable of specifically binding to ZF2, ZF4, or ZF11.

[0156] As described herein, the zinc finger-containing transcriptional effector (ZTE) of the multi-chain receptors disclosed herein includes a second domain through which the ZTE exerts its effect (effector domain). Exemplary effector domains suitable for the multi-chain receptors of the disclosure include, but are not limited to, transcriptional activating domains (e.g., TADs), epigenetic effector domains, and DNA modifying enzymes. Non-limiting examples of transcription-activating domains (TADs) suitable for use in the compositions and methods disclosed herein include herpes simplex virus protein 16 (HSV VP16) activation domain; an activation domain consisting of four tandem copies of VP16 (VP64); a p65 activation domain of NFκB; an Epstein-Barr virus R transactivator activation domain (Rta); a tripartite activator consisting of VP64, and Rta activation domains (VPR); and a histone acetyltransferase core

domain of the human E1A-associated protein p300 (p300 HAT core activation domain). In some embodiments, the effector domain of the ZTE includes a p65 activation domain of NFκB.

[0157] In some embodiments, the transcriptional regulator is from Gal4-VP16, Gal4-VP64, tetR-VP64, ZFHD1-VP64, Gal4-KRAB, and HAP1-VP16. In some embodiments, the transcriptional regulator is Gal4-VP64. In some embodiments, the transcriptional regulator has a sequence derived from or selected from the group consisting of VP64, p65, KRAB transactivating variants, and VP16. In some exemplary embodiments, the transcriptional regulator has a sequence derived from a TAD of human or humanized p65. In some exemplary embodiments, the transcriptional regulator has a sequence derived from a human or humanized p65. In other exemplary embodiments, the transcriptional regulator has a sequence derived from a TAD of human or humanized HNF1α, HSF-1, GATA3, HIF1a, GR Tau1, ATF6, ELF3, p53, MIER3, MLXIPL, NFE2L1, or PTF1A. In other exemplary embodiments, the transcriptional regulator has a sequence derived from human or humanized HNF1α, HSF-1, GATA3, HIF1a, GR Tau1, ATF6, ELF3, p53, MIER3, MLXIPL, NFE2L1, or PTF1A.

[0158] In some embodiments, the human or humanized sequence contains a linear amino acid sequence motif. In some embodiments, the transcriptional regulator described herein is directly fused together, with or without a linker sequence.

[0159] In some embodiments, the transcriptional regulator includes a sequence having at least 80% sequence identity, such as, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or 99% sequence identity to one or more of SEQ ID NOs: 68-75. In some embodiments, the transcriptional regulator includes an amino acid sequence having at least 90% sequence identity to one or more of SEQ ID NOs: 68-75. In some embodiments, the transcriptional regulator includes an amino acid sequence having at least 95% sequence identity to one or more of SEQ ID NOs: 68-75. In some embodiments, the transcriptional regulator includes an amino acid sequence having at least 100% sequence identity to one or more of SEQ ID NOs: 68-75. In some embodiments, the transcriptional regulator includes an amino acid sequence of one or more of SEQ ID NOs: 68-75, wherein one, two, three, four, or five of the amino acid residues in one or more of SEQ ID NOs: 68-75 is/are substituted by a different amino acid residue.

[0160] In some embodiments, the transcriptional regulator includes a sequence having at least 80% sequence identity, such as, at least 80%, at least 85%, at least 90%, at least 95%, at least

96%, at least 97%, at least 98%, or 99% sequence identity to one or more of SEQ ID NOs: 76-90. In some embodiments, the transcriptional regulator includes an amino acid sequence having at least 90% sequence identity to one or more of SEQ ID NOs: 76-90. In some embodiments, the transcriptional regulator includes an amino acid sequence having at least 95% sequence identity to one or more of SEQ ID NOs: 76-90. In some embodiments, the transcriptional regulator (includes an amino acid sequence having at least 100% sequence identity to one or more of SEQ ID NOs: 76-90. In some embodiments, the transcriptional regulator includes an amino acid sequence of one or more of SEQ ID NOs: 76-90, wherein one, two, three, four, or five of the amino acid residues in one or more of SEQ ID NOs: 76-90 is/are substituted by a different amino acid residue.

[0161] In some embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include ZF3, a TAD derived from a TAD of a human or humanized p65, and a TAD derived from a TAD of a human or humanized HSF1. In some embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include ZF3, a human or humanized p65, and a human or humanized HSF1. In other exemplary embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include ZF3, a human or humanized p65, and a human or humanized GR Tau1. In some exemplary embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include ZF3, a human or humanized p65, and a human or humanized p53. In other exemplary embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include ZF3, a human or humanized p65, and a human or humanized ATF6.

[0162] In some embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include a TAD derived from a TAD of a human or humanized p65, and a TAD derived from a TAD of a human or humanized HNF1α. In some embodiments, the transcriptional regulators of the multi-chain receptors of the disclosure include a human or humanized p65, and a human or humanized HNF1α.

[0163] In some embodiments, the intracellular domains of the first polypeptides of the disclosure do not include a transcription factor. In some embodiments, the intracellular domain can be responsible for propagation of downstream signaling upon ligand binding by the first polypeptide chain. The intracellular signaling domain can have at least two distinct domains: at least one costimulatory domain and an activation domain.

[0164] In some embodiments, the costimulatory domain comprises a sequence that is derived from a signaling molecule. The signaling molecule can be a protein selected from a class 1 or a class 3 human membrane protein. In some embodiments, the signaling molecule is selected from CD28, 4-1BB, OX40, ICOS, CTLA4, PD1, PD1H, BTLA, B71, B7H1, CD226, CRTAM, TIGIT, CD96, TIM1, TIM2, TIM3, TIM4, CD2, SLAM, 2B4, Ly108, CD84, Ly9, CRACC, BTN1, BTN2, BTN3, LAIR1, LAG3, CD160,, CD27, GITR, CD30, TNFR1, TNFR2, HVEM, LT_R, DR3, DCR3, FAS, CD40, RANK, OPG, TRAILR1, TACI, BAFFR, BCMA, TWEAKR, EDAR, XEDAR, RELT, DR6, TROY, NGFR, CD22, SIGLEC-3, SIGLEC-5, SIGLEC-7, KLRG1, NKR-P1A, ILT2, KIR2DL1, KIR3DL1, CD94-NKG2A, CD300b, CD300e, TREM1, TREM2, ILT7, ILT3, ILT4, TLT-1, CD200R, CD300a, CD300f, DC-SIGN, B7-2, Allergin-1, LAT, BLNK, LAYN, SLP76, EMB-LMP1, HIV-NEF, HVS-TIP, HVS-ORF5, and HVS-stpC. In some exemplary embodiments, the signaling molecule is selected from the list consisting of OX40, ICOS, 4-1BB, CTLA4, CD28, CD30, CD2, CD27, and CD226, and derivatives, mutants, variants, fragments and combinations thereof. In other embodiments, the signaling molecule is selected from the list consisting of OX40, ICOS, 4-1BB, CTLA4, CD28, CD30, CD2, CD27, and CD226, and derivatives, mutants, variants, fragments and combinations thereof. In some embodiments, the signaling molecule is selected from the group consisting of 4-1BB, BAFF-R, BCMA, BTLA, CD2, CD200R, CD244, CD28, CD300a, CD300f, CD40, CD7, CD72, CD96, CRACC, CRTAM, CTLA4, CXADR, DC-SIGN, GITR, HAVCR2, ICOS, ILT2, ILT3, ILT4, KIR2DL1, KIR3DL1, KLRG1, LAG3, LAIR1, NKG2D, NKR-P1A, NTB-A, PD1, Siglec-3, TACI, TIGIT, TLT-1, and TNR8 (CD30), and derivatives, mutants, variants, fragments and combinations thereof. In other embodiments, the signaling molecule is CD28 or 4-1BB. In one exemplary embodiment, the costimulatory domain comprises a sequence that is derived from CD28. In another exemplary embodiment, the costimulatory domain comprises a sequence that is derived from 4-1BB.

[0165] In some embodiments, the activation domain includes one or more conserved amino acid motifs that serve as substrates for phosphorylation such as, for example, immunoreceptor tyrosine-based activation motifs (ITAMs). In some embodiments, the activation domain includes at least 1, at least 2, at least 3, at least 4, or at least 5 specific tyrosine-based motifs selected from ITAM motifs, an ITIM motifs, or related intracellular motifs that serve as a substrate for phosphorylation. In some embodiments of the disclosure, the activation domain of the

intracellular signaling domain includes at least 1, at least 2, at least 3, at least 4, or at least 5 ITAMs. Generally, any activation domain including an ITAM can be suitably used for the construction of the multi-chain receptors as described herein. An ITAM generally includes a conserved protein motif that is often present in the tail portion of signaling molecules expressed in many immune cells. The motif may include two repeats of the amino acid sequence YxxL/I separated by 6-8 amino acids, wherein each x is independently any amino acid, producing the conserved motif YxxL/Ix(6-8)YxxL/I. ITAMs within signaling molecules are important for signal transduction within the cell, which is mediated at least in part by phosphorylation of tyrosine residues in the ITAM following activation of the signaling molecule. ITAMs may also function as docking sites for other proteins involved in signaling pathways

[0166] In some embodiments, the activation domain includes one or more immunoreceptor tyrosine-based activation motifs (ITAMs). In some embodiments, the activation domain is derived from CD3ζ, CD3σ, CD3/, and CD3ε. For instance, in some embodiments, the ITAMs are derived from CD3ζ, CD3σ, CD3/, and CD3ε. In one exemplary embodiment, the ITAM is derived from CD3ζ. In certain embodiments, the ITAM comprises a sequence that is at least about 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical to a CD3ζ ITAM. In some embodiments, the activation domain comprises at least 1, at least 2, at least 3, at least 4, or at least 5 ITAMs independently selected from the ITAMs derived from CD3ζ, FcRγ, and combinations thereof. In some embodiments, the activation domain comprises a CD3ζ ITAM.

[0167] In some embodiments, the intracellular domains of the first polypeptides of the disclosure comprise a signaling chain derived from IL-2 receptor common gamme chain. In some embodiments, the signaling chain derived from IL-2 receptor common gamma chain comprises the amino acid sequence of ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALG EGPGASPCNQHSPYWAPPCYTLKPET (SEQ ID NO: 91).

[0168] Various embodiments of the first polypeptides of the disclosure can include the following non-limiting examples of components. The first polypeptide can include an extracellular domain, a TMD, an intracellular domain and other sequences from known receptors such as, for example, the triggering receptor expressed on myeloid cells 2 (TREM 2; FIGS. 2A, 2B, 2C, 2D, 3A, 4A and 4B). TREM2 can undergo proteolytic cleavage between amino acids H157 and S158 by members of the disintegrin and metalloproteinase (ADAM) family. This

cleavage results in the release of a soluble TREM2 ectodomain (sTREM2) into extracellular fluids, and generation of a membrane tethered C-terminal fragment (CTF). The TREM2 CTF represents a substrate for intramembrane proteolysis by γ-secretase (FIG. 2A). In some embodiments of the disclosure, the first polypeptide can include TREM2 TMD without its cytoplasmic domain. In some embodiments, the first polypeptide can includes a TREM2 extracellular domain, and a TMD with a Notch 1 γ-secretase cleavage site and/or a Notch-2 JMD (FIG 2D).

[0169] The first polypeptide of the multi-chain chimeric polypeptides of the disclosure can include a first modified interface via which the first polypeptide associates with the second polypeptide. The first modified interface can be from a Notch 1 TMD. The first modified interface can have a positively charged residue via which the first polypeptide associates with the second polypeptide of the multiple-chimeric polypeptides of the disclosure. The positively charged residue can be a lysine or arginine. In some embodiments, the lysine or arginine residue can substitute a leucine at location 12 of the Notch 2 JMD (in reference to SEQ ID NO:21 which corresponds to residue 1747 of the whole construct that includes SEQ ID NO:8) In some embodiments, the first polypeptide includes a truncated CD8α hinge domain ECD, a Notch 1 TMD with a L12K mutation, a Notch 2 JMD and a Gal4VP64 transcriptional regulator (FIG. 2B). In some embodiments, the first polypeptide includes a TREM2 ECD, a Notch 1 TMD with a L1747K mutation (Uniprot P46531 amino acids 1736 to 1757 with a L1747K mutation), a Notch 2 JMD and a Gal4VP64 transcriptional regulator (FIG. 2C). In some embodiments, the first polypeptide includes a TREM2 ECD, a Notch 1 TMD with a gamma secretase site, a Notch 2 JMD and a Gal4VP64 transcriptional regulator (FIG. D).

[0170] In other aspects, the first polypeptide can include a truncated CD8α hinge domain (CD8-hinge 2) ECD, a polyvaline TMD with a lysine substitution at position 9 or 10, a Notch 2 JMD and a Gal4VP64 transcriptional regulator (FIG. 4C and 4D).

[0171] In other aspects, the first polypeptide can include a truncated CD8α hinge domain (CD8-hinge 2) ECD, a polyvaline TMD with a lysine substitution at position 9 or 10, a Notch 2 JMD and a human HNF1a transcriptional regulator (FIG. 8A and 8B).

[0172] In other aspects, the first polypeptide can include a truncated CD8α hinge domain (CD8-hinge 2) ECD, a polyvaline TMD with a lysine substitution at position 9 or 10, a Notch 2 JMD and a 4-1BB/CD3 zeta domain (FIG. 10).

[0173] In other aspects, the first polypeptide can include a truncated CD8α hinge domain (CD8-hinge 2) ECD, a polyvaline TMD with a lysine substitution at position 9 or 10, a Notch 2 JMD and a common gamma chain domain (FIG. 10).

[0174] In other aspects, the first polypeptide can include a truncated CD8α hinge domain (CD8-hinge 2) ECD, a polyvaline TMD with a lysine substitution at position 9 or 10, a Notch 2 JMD and a common gamma chain/CD3 zeta domain (FIG. 10). The first polypeptide, can also include various other combinations of components described herein as could be appreciated by the skill in the art.

e. Other components

[0175] In some embodiments of the disclosure, the first polypeptide can further include one or more of the following: an autoproteolytic peptide sequence or a nuclear localization signal.

[0176] In some embodiments, the intracellular domain includes a nuclear localization sequence and a transcriptional regulator such as Gal4-VP16, Gal4-VP64, tetR-VP64, ZFHD1-VP64, Gal4-KRAB, or HAP1-VP16.

[0177] In some embodiments, the autoproteolytic peptide sequence is from a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

[0178] The first polypeptide can further include a proteolytic cleavage site. In some embodiments, the proteolytic cleavage site is cleavable by gamma secretase. In some embodiments, the proteolytic cleavage site is ligand-inducible proteolytic cleavage site. The proteolytic cleavage site can be disposed between the transcriptional regulator and the hinge domain. The proteolytic cleavage site can be ligand-inducible wherein binding of the selected ligand to the extracellular ligand-binding domain induces cleavage at the ligand-inducible proteolytic cleavage site.

2. SECOND POLYPEPTIDES

[0179] The multi-chain chimeric polypeptides of the disclosure include a second polypeptide having (i) a second TMD with a second interface, and (ii) a second intracellular domain in order from N-terminus to C-terminus of the second polypeptide.

[0180] In some embodiments, the first polypeptides of the disclosure include an amino acid

sequence that is has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 9 or SEQ ID NO:11 or functional variants thereof.

a. *Second Transmembrane Domains*

[0181] The second polypeptides of the multi-chain chimeric polypeptides of the disclosure can include a second TMD having a second interface. The second TMD can include any domain that is capable of associating with the first polypeptide of the multi-chain chimeric polypeptides of the disclosure via a charged residue in the TMD and that has a signaling domain that can be activated upon binding of a target ligand to the extracellular ligand-binding domain of the first polypeptide. The second interface is the region in the second polypeptide can include a negatively charged residue via which it associates with a positively charged residue in the TMD of the first polypeptide of the disclosure. In some embodiments, the negatively charged residue can be arginine or lysine.

[0182] In some embodiments, the second polypeptide includes a DAP12 TMD. In some embodiments, the second polypeptide includes a DAP12 domain as well as a DAP12 intracellular signaling domain (*i.e.* cytoplasmic domain) as explained in more details below.

b. *Second Intracellular* Domain

[0183] The second polypeptide of the multi-chain chimeric polypeptides of the disclosure include an intracellular domain. The intracellular domain can be responsible for propagation of downstream signaling upon ligand binding by the first polypeptide chain. The intracellular signaling domain can have at least two distinct domains: at least one costimulatory domain and an activation domain.

[0184] In some embodiments, the costimulatory domain comprises a sequence that is derived from a signaling molecule. The signaling molecule can be a protein selected from a class 1 or a class 3 human membrane protein. In some embodiments, the signaling molecule is selected from CD28, 4-1BB, OX40, ICOS, CTLA4, PD1, PD1H, BTLA, B71, B7H1, CD226, CRTAM, TIGIT, CD96, TIM1, TIM2, TIM3, TIM4, CD2, SLAM, 2B4, Ly108, CD84, Ly9, CRACC, BTN1, BTN2, BTN3, LAIR1, LAG3, CD160,, CD27, GITR, CD30, TNFR1, TNFR2, HVEM, LT_R, DR3, DCR3, FAS, CD40, RANK, OPG, TRAILR1, TACI, BAFFR, BCMA, TWEAKR, EDAR, XEDAR, RELT, DR6, TROY, NGFR, CD22, SIGLEC-3, SIGLEC-5, SIGLEC-7, KLRG1, NKR-P1A, ILT2, KIR2DL1, KIR3DL1, CD94-NKG2A, CD300b, CD300e, TREM1, TREM2, ILT7, ILT3, ILT4, TLT-1, CD200R, CD300a, CD300f, DC-SIGN, B7-2, Allergin-1,

LAT, BLNK, LAYN, SLP76, EMB-LMP1, HIV-NEF, HVS-TIP, HVS-ORF5, and HVS-stpC. In some exemplary embodiments, the signaling molecule is selected from the list consisting of OX40, ICOS, 4-1BB, CTLA4, CD28, CD30, CD2, CD27, and CD226, and derivatives, mutants, variants, fragments and combinations thereof. In other embodiments, the signaling molecule is selected from the list consisting of OX40, ICOS, 4-1BB, CTLA4, CD28, CD30, CD2, CD27, and CD226, and derivatives, mutants, variants, fragments and combinations thereof. In some embodiments, the signaling molecule is selected from the group consisting of 4-1BB, BAFF-R, BCMA, BTLA, CD2, CD200R, CD244, CD28, CD300a, CD300f, CD40, CD7, CD72, CD96, CRACC, CRTAM, CTLA4, CXADR, DC-SIGN, GITR, HAVCR2, ICOS, ILT2, ILT3, ILT4, KIR2DL1, KIR3DL1, KLRG1, LAG3, LAIR1, NKG2D, NKR-P1A, NTB-A, PD1, Siglec-3, TACI, TIGIT, TLT-1, and TNR8 (CD30), and derivatives, mutants, variants, fragments and combinations thereof. In other embodiments, the signaling molecule is CD28 or 4-1BB. In one exemplary embodiment, the costimulatory domain comprises a sequence that is derived from CD28. In another exemplary embodiment, the costimulatory domain comprises a sequence that is derived from 4-1BB.

[0185] In some embodiments, the activation domain includes one or more conserved amino acid motifs that serve as substrates for phosphorylation such as, for example, immunoreceptor tyrosine-based activation motifs (ITAMs). In some embodiments, the activation domain includes at least 1, at least 2, at least 3, at least 4, or at least 5 specific tyrosine-based motifs selected from ITAM motifs, an ITIM motifs, or related intracellular motifs that serve as a substrate for phosphorylation. In some embodiments of the disclosure, the activation domain of the intracellular signaling domain includes at least 1, at least 2, at least 3, at least 4, or at least 5 ITAMs. Generally, any activation domain including an ITAM can be suitably used for the construction of the multi-chain receptors as described herein. An ITAM generally includes a conserved protein motif that is often present in the tail portion of signaling molecules expressed in many immune cells. The motif may include two repeats of the amino acid sequence YxxL/I separated by 6-8 amino acids, wherein each x is independently any amino acid, producing the conserved motif YxxL/Ix(6-8)YxxL/I. ITAMs within signaling molecules are important for signal transduction within the cell, which is mediated at least in part by phosphorylation of tyrosine residues in the ITAM following activation of the signaling molecule. ITAMs may also function as docking sites for other proteins involved in signaling pathways.

[0186] In some embodiments, the activation domain includes one or more immunoreceptor tyrosine-based activation motifs (ITAMs). In some embodiments, the activation domain is derived from CD3ζ, CD3σ, CD3/, and CD3ε. For instance, in some embodiments, the ITAMs are derived from CD3ζ, CD3σ, CD3/, and CD3ε. In one exemplary embodiment, the ITAM is derived from CD3ζ. In certain embodiments, the ITAM comprises a sequence that is at least about 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical to a CD3ζ ITAM. In some embodiments, the activation domain comprises at least 1, at least 2, at least 3, at least 4, or at least 5 ITAMs independently selected from the ITAMs derived from CD3ζ, FcRγ, and combinations thereof. In some embodiments, the activation domain comprises a CD3ζ ITAM.

[0187] In some embodiments, the intracellular domain is from DAP12 (DNAZ activation protein of 12 kDa, TYROBP). The DAP 12 intracellular domain, also known as the cytoplasmic domain of DAP12, includes an ITAM, which gets phosphorylated upon ligand-binding to TREM2, and thereon regulate several intracellular signaling pathways that control cell proliferation and differentiation, survival, phagocytosis, cytoskeletal remodeling, calcium mobilization and/or cytokine production. In some embodiments, the signaling domain can be a CD3 zeta, TCR zeta, FcR γ, FcR β, CD3 γ, CD3 Δ, CD3 ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, or CD66d, signaling domain. In some embodiments, the signaling domain is a CD3 zeta signaling domain.

[0188] In some embodiments, the signaling domain comprises an endodomain of a cytokine receptor.

[0189] In some embodiments, the endodomain is from a type I cytokine receptor. Type I cytokine receptors share a common amino acid motif (WSXWS) in the extracellular portion adjacent to the cell membrane. Type I cytokine receptors include (i) Interleukin receptors, such as the receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL13, IL-15, IL-21, IL-23 and IL-27; (ii) Colony stimulating factor receptors, such as the receptors for erythropoietin, GM-CSF, and G-CSF; and (iii) Hormone receptor/neuropeptide receptor, such as hormone receptor and prolactin receptor. Members of the type I cytokine receptor family comprise different chains, some of which are involved in ligand/cytokine interaction and others that are involved in signal transduction. For example the IL-2 receptor comprises an α-chain, a β-chain and a γ-chain.

[0190] The IL-2 receptor common gamma chain (also known as CD132) is shared between the

IL-2 receptor, IL-4 receptor, IL-7 receptor, IL-9 receptor, IL-13 receptor, IL-15 receptor, and IL-21 receptor.

[0191] In some embodiments, the endomain is from the IL-2 receptor β chain. In some embodiments, the amino acid sequence of the IL-2 receptor beta chain endodomain comprises RNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVL ERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYS EEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSG AGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPDAGPREG VSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHLV SEQ ID NO: 92. In some embodiments, the IL-2 receptor beta chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:92 or functional variants thereof.

[0192] In some embodiments, the endomain is from the IL-4 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-4 receptor alpha chain endodomain comprises KIKKEWWDQIPNPARSRLVAIIIQDAQGSQWEKRSRGQEPAKCPHWKNCLTKLLPCFLE HNMKRDEDPHKAAKEMPFQGSGKSAWCPVEISKTVLWPESISVVRCVELFEAPVECEEE EEVEEEKGSFCASPESSRDDFQEGREGIVARLTESLFLDLLGEENGGFCQQDMGESCLLP PSGSTSAHMPWDEFPSAGPKEAPPWGKEQPLHLEPSPPASPTQSPDNLTCTETPLVIAGN PAYRSFSNSLSQSPCPRELGPDPLLARHLEEVEPEMPCVPQLSEPTTVPQPEPETWEQILR RNVLQHGAAAAPVSAPTSGYQEFVHAVEQGGTQASAVVGLGPPGEAGYKAFSSLLASS AVSPEKCGFGASSGEEGYKPFQDLIPGCPGDPAPVPVPLFTFGLDREPPRSPQSSHLPSSSP EHLGLEPGEKVEDMPKPPLPQEQATDPLVDSLGSGIVYSALTCHLCGHLKQCHGQEDGG QTPVMASPCCGCCCGDRSSPPTTPLRAPDPSPGGVPLEASLCPASLAPSGISEKSKSSSSFH PAPGNAQSSSQTPKIVNFVSVGPTYMRVS SEQ ID NO: 93. In some embodiments, the IL-4 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:93 or functional variants thereof.

[0193] In some embodiments, the endomain is from the IL-7 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-7 receptor alpha chain endodomain comprises KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEGFL QDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACDAPILSSS RSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQPILTSLGSNQE

EAYVTMSSFYQNQ SEQ ID NO: 94. In some embodiments, the IL-7 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:94 or functional variants thereof.

[0194] In some embodiments, the endomain is from the IL-9 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-9 receptor alpha chain endodomain comprises KLSPRVKRIFYQNVPSPAMFFQPLYSVHNGNFQTWMGAHGAGVLLSQDCAGTPQGALE PCVQEATALLTCGPARPWKSVALEEEQEGPGTRLPGNLSSEDVLPAGCTEWRVQTLAYL PQEDWAPTSLTRPAPPDSEGSRSSSSSSSSNNNNYCALGCYGGWHLSALPGNTQSSGPIP ALACGLSCDHQGLETQQGVAWVLAGHCQRPGLHEDLQGMLLPSVLSKARSWTFSEQ ID NO: 95. In some embodiments, the IL-9 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:95 or functional variants thereof.

[0195] In some embodiments, the endomain is from the IL-13 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-13 receptor alpha chain endodomain comprises KRLKIIIFPPIPDPGKIFKEMFGDQNDDTLHWKKYDIYEKQTKEETDSVVLIENLKKASQ SEQ ID NO: 96. In some embodiments, the IL-13 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:96 or functional variants thereof.

[0196] In some embodiments, the endomain is from the IL-15 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-15 receptor alpha chain endodomain comprises KSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL SEQ ID NO: 97. In some embodiments, the IL-15 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:97 or functional variants thereof.

[0197] In some embodiments, the endomain is from the IL-21 receptor alpha chain. In some embodiments, the amino acid sequence of the IL-21 receptor alpha chain endodomain comprises KTHPLWRLWKKIWAVPSPERFFMPLYKGCSGDFKKWVGAPFTGSSLELGPWSPEVPST LEVYSCHPPRSPAKRLQLTELQEPAELVESDGVPKPSFWPTAQNSGGSAYSEERDRPYGL VSIDTVTVLDAEGPCTWPCSCEDDGYPALDLDAGLEPSPGLEDPLLDAGTTVLSCGCVS AGSPGLGGPLGSLLDRLKPPLADGEDWAGGLPWGGRSPGGVSESEAGSPLAGLDMDTF DSGFVGSDCSSPVECDFTSPGDEGPPRSYLRQWVVIPPPLSSPGPQAS SEQ ID NO: 98. In

some embodiments, the IL-21 receptor alpha chain endodomain includes an amino acid sequence that has 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:98 or functional variants thereof.

[0198] In some embodiments, the endodomain is from a type II cytokine receptor. Type II cytokine receptors include those that bind type I and type II interferons, and those that bind members of the interleukin-10 family (interleukin-10, interleukin-20 and interleukin-22).

3. ADDITIONAL EMBODIMENTS OF THE CHIMERIC POLYPEPTIDES

[0199] The multi-chain chimeric polypeptides of the disclosure can include various combinations of the first and second polypeptides described supra. In some embodiments, the multi-chain chimeric polypeptides include (a) a first polypeptide having a CD19scFV as an extracellular ligand-binding domain including (ii) a first TMD having a contiguous stretch of valine residues, and (iii) a Notch 2 juxtamembrane domain and (iv) a first intracellular domain with a Gal4VP64 transcriptional regulator; and (b) a second polypeptide with a DNAX – activation protein 12 (DAP12) wherein the first polypeptide is coupled to the second polypeptide via a lysine residue within the contiguous stretch valine residues and wherein binding of CD19 to the extracellular ligand-binding domain induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, the lysine residue is flanked by a contiguous stretch of 5 to 15 contiguous valine residues. The lysine residue can be at position 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 from the N-terminus of the first TMD.

[0200] In some embodiments, the multi-chain chimeric polypeptides include (a) a first polypeptide having a CD19scFV as an extracellular ligand-binding domain including (ii) a first TMD having a contiguous stretch of valine residues, and (iii) a Notch 2 juxtamembrane domain and (iv) a first intracellular domain with a Gal4VP64 transcriptional regulator; and (b) a second polypeptide with a CD3 zeta signaling domain wherein the first polypeptide is coupled to the second polypeptide via a lysine residue within the contiguous stretch valine residues and wherein binding of CD19 to the extracellular ligand-binding domain induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, the lysine residue is flanked by a contiguous stretch of 5 to 15 contiguous valine residues. The lysine residue can be at position 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 from the N-terminus of the first TMD.

[0201] In some embodiments, the multi-chain chimeric polypeptides include (a) a first polypeptide having a CD19scFV as an extracellular ligand-binding domain including (ii) a first TMD having a contiguous stretch of valine residues, and (iii) a Notch 2 juxtamembrane domain and (iv) a first intracellular domain with a human transcriptional regulator; and (b) a second polypeptide with a DNAX –activation protein 12 (DAP12) wherein the first polypeptide is coupled to the second polypeptide via a lysine residue within the contiguous stretch valine residues and wherein binding of CD19 to the extracellular ligand-binding domain induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, the lysine residue is flanked by a contiguous stretch of 5 to 15 contiguous valine residues. The lysine residue can be at position 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 from the N-terminus of the first TMD. In some embodiments, the human transcriptional regulator is HNF1a.

[0202] In some embodiments, the multi-chain chimeric polypeptides include (a) a first polypeptide having a CD19scFV as an extracellular ligand-binding domain including (ii) a first TMD having a contiguous stretch of valine residues, and (iii) a Notch 2 juxtamembrane domain and (iv) a first intracellular domain with a human transcriptional regulator; and (b) a second polypeptide with a CD3 zeta signaling domain wherein the first polypeptide is coupled to the second polypeptide via a lysine residue within the contiguous stretch valine residues and wherein binding of CD19 to the extracellular ligand-binding domain induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, the lysine residue is flanked by a contiguous stretch of 5 to 15 contiguous valine residues. The lysine residue can be at position 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 from the N-terminus of the first TMD. In some embodiments, the human transcriptional regulator is HNF1a.

[0203] In some embodiments, the multi-chain chimeric polypeptides include (a) a first polypeptide having a CD19scFV as an extracellular ligand-binding domain including (ii) a first TMD having a contiguous stretch of valine residues, and (iii) a Notch 2 juxtamembrane domain and (iv) a first intracellular domain with a human transcriptional regulator; and (b) a second polypeptide with a CD3 zeta signaling domain wherein the first polypeptide is coupled to the second polypeptide via a lysine residue within the contiguous stretch valine residues and wherein binding of CD19 to the extracellular ligand-binding domain induces activity of the signaling domain and releases the transcriptional regulator. In some embodiments, the lysine residue is flanked by a contiguous stretch of 5 to 15 contiguous valine residues. The lysine residue can be

at position 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 from the N-terminus of the first TMD. In some embodiments, the human transcriptional regulator is HNF1a.

4. NUCLEIC ACID CONSTRUCTS ENCODING THE MULTI-CHAIN CHIMERIC POLYPEPTIDES

[0204] The present disclosure also provides recombinant nucleic acid constructs including nucleotide sequences encoding the multi-chain chimeric polypeptides of the disclosure. The present disclosure also provides recombinant nucleic acid constructs having nucleotide sequences that encode only the first polypeptide, or only the second polypeptide. In the case where the recombinant nucleic acid constructs encodes either the first polypeptide or the second polypeptide, a host cell can be transduced with both types of constructs in order to express the multi-chain chimeric polypeptides of the disclosure.

[0205] The recombinant nucleic acid constructs can include a first cassette that encodes the first polypeptide and a second cassette that encodes the second polypeptide of the multi-chain chimeric polypeptides of the disclosure wherein both cassettes are on the same nucleic acid molecule. In some embodiments, the first cassette is 5' to the second cassette. In some embodiments, the first cassette is 3' to the second cassette. In some embodiments, the first and second cassettes are joined by an autoproteolytic peptide. In some embodiments, the autoproteolytic peptide is Thosea asigna virus 2A (T2A) peptide.

[0206] In some embodiments, the recombinant nucleic acid constructs of the disclosure include a nucleotide sequence comprising SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO:7, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58 SEQ ID NO: 99, or any functional variants thereof.

[0207] In some embodiments, the recombinant nucleic acid constructs include a nucleotide sequence having a 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, 3, 4, 5, 6, 7, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 99 or any functional variants thereof.The recombinant nucleic acid constructs of the present disclosure can be of any length, including for example, between about 1.5 Kb and about 50 Kb, between about 5 Kb and about 40 Kb, between about 5 Kb and about 30 Kb, between about 5 Kb and

about 20 Kb, or between about 10 Kb and about 50 Kb, for example between about 15 Kb to 30 Kb, between about 20 Kb and about 50 Kb, between about 20 Kb and about 40 Kb, about 5 Kb and about 25 Kb, or about 30 Kb and about 50 Kb.

[0208] In some embodiments, the recombinant nucleic acid constructs include nucleotide sequences that encode a polypeptide having an amino acid sequence that is at least about 80%, 90%, 95%, 96%, 97, 98%, 99%, or 100% identical to an amino acid sequence encoded by SEQ ID NO:8, SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 60 or functional variants thereof.

[0209] In some embodiments, the nucleotide sequence is incorporated into an expression cassette or an expression vector. It will be understood that an expression cassette generally includes a construct of genetic material that contains coding sequences and enough regulatory information to direct proper transcription and/or translation of the coding sequences in a recipient cell, in vivo and/or ex vivo. Generally, the expression cassette may be inserted into a vector for targeting to a desired host cell and/or into an individual. As such, in some embodiments, an expression cassette of the disclosure include a coding sequence for the chimeric polypeptide as disclosed herein, which is operably linked to expression control elements, such as a promoter, and optionally, any or a combination of other nucleic acid sequences that affect the transcription or translation of the coding sequence.

[0210] The recombinant nucleic acid constructs provided can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide, e.g., first or second polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the recombinant nucleic acid molecules of the disclosure can be double-stranded or single-stranded (e.g., either a sense or an antisense strand).

[0211] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding

sequence (e.g., the coding sequence of a multi-chain chimeric polypeptide or receptor) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction endonucleases, or by performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by in vitro transcription.

B. Vectors

[0212] The recombinant nucleic acid constructs encoding the multi-chain polypeptides of the disclosure can be included in one or more vectors. Accordingly, the present disclosure also provides vectors encoding or expressing the multi-chain chimeric polypeptides of the present disclosure.

[0213] The vector(s) of the disclosure can express either the first or the second polypeptide chains of a multi-chain chimeric polypeptide or both, i.e., the multi-chain polypeptides of the disclosure can be expressed by one vector or by different vectors that are co-transduced into a cell.

[0214] In some embodiments, the multi-chain chimeric polypeptides are incorporated into an expression vector designed for transfer between host cells, and that may be used for the purpose of transformation, e.g., the introduction of heterologous DNA into a host cell. As such, in some embodiments, the vector can be a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. In some embodiments, the expression vector can be an integrating vector.

[0215] In addition to the components of the multi-chain chimeric polypeptides or receptors of the disclosure, the vector or vectors can include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome of a cell.

[0216] In some embodiments, the expression vector can be a viral vector. As will be appreciated by one of skill in the art, the term "viral vector" is widely used to refer either to a nucleic acid molecule (e.g., a transfer plasmid) that includes virus-derived nucleic acid elements that generally facilitate transfer of the nucleic acid molecule or integration into the genome of a

cell or to a viral particle that mediates nucleic acid transfer. Viral particles will generally include various viral components and sometimes also host cell components in addition to nucleic acid(s). The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. Viral vectors and transfer plasmids contain structural and/or functional genetic elements that are primarily derived from a virus.

[0217] The multi-chain chimeric polypeptides or the chimeric antigen receptors of the disclosure can be incorporated into retroviral vectors. The term "retroviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. In some embodiments, the multi-chain chimeric antigen receptors of the disclosure can be incorporated into a lentiviral vector. The lentiviral vector can include structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus, which is a genus of retrovirus.

[0218] Viral vectors that can be used in the disclosure include, for example, adenovirus vectors, and adeno-associated virus vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors, retroviral vectors, lentiviral vectors, (see, for example, Gluzman (Ed.), Eukaryotic Viral Vectors, CSH Laboratory Press, Cold Spring Harbor, N.Y.). For example, a construct as disclosed herein can be produced in a eukaryotic host, such as a mammalian cells (e.g., K562 cells, COS cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, Va.). In selecting an expression system, care should be taken to ensure that the components are compatible with one another. Artisans or ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult P. Jones, "Vectors: Cloning Applications", John Wiley and Sons, New York, N.Y., 2009).

[0219] The multi-chain chimeric polypeptides of the disclosure can be contained within a vector or vectors that is/are capable of directing their expression in, for example, a cell that has been transformed/transduced with the vector/vectors. Suitable vectors for use in eukaryotic and prokaryotic cells are known in the art and are commercially available, or readily prepared by a skilled artisan. In some embodiments, the vector is a lentivirus transfer vector comprising SEQ ID NO: 1 or any functional variants thereof.

[0220] DNA vectors can be introduced into eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be

found in Sambrook et al. (2012, supra) and other standard molecular biology laboratory manuals, such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, nucleoporation, hydrodynamic shock, and infection.

C. Recombinant Cells

[0221] The multi-chain chimeric polypeptides of the disclosure can be introduced or transduced into host cells or recombinant cells such as, for example, a human T lymphocyte, to produce a recombinant cell containing the nucleic acid molecule. Accordingly, some embodiments of the disclosure relate to recombinant cells comprising the multi-chain chimeric polypeptides or CARs or recombinant nucleic acid constructs or the vectors of the disclosure.

[0222] Introduction of the constructs or vectors of the disclosure into cells can be achieved by methods known to those skilled in the art such as, for example, viral infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, nucleofection, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro-injection, nanoparticle-mediated nucleic acid delivery, and the like.

[0223] Accordingly, in some embodiments, the constructs or vectors can be delivered by viral or non-viral delivery vehicles known in the art. For example, the constructs can be stably integrated in the host genome, or can be episomally replicated, or present in the recombinant host cell as a mini-circle expression vector for transient expression. Accordingly, in some embodiments, the constructs are maintained and replicated in the recombinant host cell as an episomal unit. In some embodiments, the constructs are stably integrated into the genome of the recombinant cell. Stable integration can be achieved using classical random genomic recombination techniques or with more precise techniques such as guide RNA-directed CRISPR/Cas9 genome editing, or DNA-guided endonuclease genome editing with NgAgo (Natronobacterium gregoryi Argonaute), or TALENs genome editing (transcription activator-like effector nucleases). In some embodiments, the recombinant nucleic acid molecules of the disclosure are present in the recombinant host cell as mini-circle expression vectors for transient expression.

[0224] The recombinant nucleic acid constructs of the disclosure can be encapsulated in viral

capsids or lipid nanoparticles, or can be delivered by viral or non-viral delivery means and methods known in the art, such as electroporation. For example, introduction of nucleic acids into cells may be achieved by viral transduction. In a non-limiting example, adeno-associated virus (AAV) is engineered to deliver constructs to target cells via viral transduction. Several AAV serotypes have been described, and all of the known serotypes can infect cells from multiple diverse tissue types. AAV is capable of transducing a wide range of species and tissues in vivo with no evidence of toxicity, and it generates relatively mild innate and adaptive immune responses.

[0225] Lentiviral-derived vector systems are also useful for construct delivery and gene therapy via viral transduction. Lentiviral vectors offer several attractive properties as gene-delivery vehicles, including: (i) sustained gene delivery through stable vector integration into host genome; (ii) the capability of infecting both dividing and non-dividing cells; (iii) broad tissue tropisms, including important gene- and cell-therapy-target cell types; (iv) no expression of viral proteins after vector transduction; (v) the ability to deliver complex genetic elements, such as polycistronic or intron-containing sequences; (vi) a potentially safer integration site profile; and (vii) a relatively easy system for vector manipulation and production.

[0226] In some embodiments, the recombinant host cells can be genetically engineered (e.g., transduced or transformed or transfected) with, for example, a vector construct of the present application that can be, for example, a viral vector or a vector for homologous recombination that includes nucleic acid sequences homologous to a portion of the genome of the host cell, or can be an expression vector for the expression of the polypeptides of interest. Host cells can be either untransformed cells or cells that have already been transfected with at least one nucleic acid molecule.

[0227] In some embodiments, the recombinant cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the cell is *in vivo*. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vitro*. In some embodiments, the recombinant cell is a eukaryotic cell. In some embodiments, the recombinant cell is an animal cell. In some embodiments, the animal cell is a mammalian cell. In some embodiments, the animal cell is a human cell. In some embodiments, the cell is a non-human primate cell. In some embodiments, the mammalian cell is an immune cell, or a tumor cell, or a stem cell. In some embodiments, the recombinant cell is an immune system cell, e.g., a lymphocyte (e.g., a T cell or NK cell), or a dendritic cell. In some

embodiments, the immune cell is a B cell, a monocyte, a natural killer (NK) cell, a basophil, an eosinophil, a neutrophil, a dendritic cell, a macrophage, a regulatory T cell, a helper T cell (Tx), a cytotoxic T cell (Tcm), or other T cell. In some embodiments, the immune system cell is a T lymphocyte. In some embodiments, the cell is a CAR expressing reporter T (CAR-T) cell.

[0228] In some embodiments, the cell is a stem cell. In some embodiments, the cell is a hematopoietic stem cell. In some embodiments of the cell, the cell is a lymphocyte. In some embodiments, the cell is a precursor T cell or a T regulatory (Treg) cell. In some embodiments, the cell is a CD34+, CD8+, or a CD4+ cell. In some embodiments, the cell is a CD8+ T cytotoxic lymphocyte cell selected from the group consisting of naive CD8+ T cells, central memory CD8+ T cells, effector memory CD8+ T cells, and bulk CD8+ T cells. In some embodiments of the cell, the cell is a CD4+ T helper lymphocyte cell selected from the group consisting of naive CD4+ T cells, central memory CD4+ T cells, effector memory CD4+ T cells, and bulk CD4+ T cells. In some embodiments, the cell can be obtained by leukapheresis performed on a sample obtained from a subject. In some embodiments, the subject is a human patient.

[0229] In another aspect, provided herein are cell cultures including at least one recombinant cell as disclosed herein, and a culture medium. Generally, the culture medium can be any suitable culture medium for culturing the cells described herein. Techniques for transforming a wide variety of the above-mentioned host cells and species are known in the art and described in the technical and scientific literature. Accordingly, cell cultures including at least one recombinant cell as disclosed herein are also within the scope of this application.

[0230] Methods and systems suitable for generating and maintaining cell cultures are known in the art.

D. Pharmaceutical Compositions

[0231] The disclosure also provides pharmaceutical compositions including a recombinant cell of the disclosure and a pharmaceutically acceptable excipient, e.g. carrier.

[0232] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL.TM.. (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, the composition should be

sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, e.g., sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be generally to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0233] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above.

[0234] In some embodiments, multi-chain CARs and the chimeric polypeptides of the disclosure can also be administered by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey et al. (Nature 418:6893, 2002), Xia et al. (Nature Biotechnol. 20:1006-10, 2002), or Putnam (Am. J. Health Syst. Pharm. 53:151-60, 1996, erratum at Am. J. Health Syst. Pharm. 53:325, 1996).

III. METHODS OF THE DISCLOSURE

A. Methods for Treatment

[0235] The present disclosure provides, *inter alia*, methods for treatment of a health condition in a subject by administering to the subject a therapeutically effective amount of the recombinant cells, the pharmaceutical compositions, the multi-chain chimeric polypeptides or the CARs of the disclosure. The present disclosure also provides, *inter alia*, methods for treating a

health condition in a subject by administering to the subject a vector of the disclosure.

[0236] The present disclosure also provides methods for inducing an immune response in a subject by administering to the subject a therapeutically effective amount of the recombinant cells, the pharmaceutical compositions or the CARs of the disclosure.

[0237] Non-limiting examples of an immune response include cytotoxic T lymphocyte (CTL) response, a B cell response (for example, production of antibodies), an NK cell response or any combinations thereof, when administered to an immunocompetent subject.

[0238] Administration of any one of the vectors or recombinant cells or pharmaceutical compositions described herein can be used to treat patients for relevant health conditions or diseases, such as cancers, or autoimmune diseases or infections (*e.g.* chronic infections). In some embodiments, the vectors or the cells of the disclosure can be incorporated into compositions, e.g., pharmaceutical or therapeutic compositions, for use in methods of treating an individual who has, who is suspected of having, or who may be at high risk for developing one or more autoimmune disorders or diseases associated with checkpoint inhibition. Exemplary autoimmune disorders and diseases can include, without limitation, celiac disease, type I diabetes, Graves' disease, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

[0239] In some embodiments, the methods include administering to the individual an effective number of the recombinant cells disclosed herein, wherein the recombinant cells inhibit an activity of the target cells in the individual. Generally, the target cells of the disclosed methods can be any cell type in an individual and can be, for example a cell from a hematological malignancy, a multiple myeloma cell, a solid tumor cell, an acute myeloma leukemia cell, an anaplastic lymphoma cell, an astrocytoma cell, a B-cell cancer cell, a breast cancer cell, a colon cancer cell, an ependymoma cell, an esophageal cancer cell, a glioblastoma cell, a glioma cell, a leiomyosarcoma cell, a liposarcoma cell, a liver cancer cell, a lung cancer cell, a mantle cell lymphoma cell, a melanoma cell, a neuroblastoma cell, a non-small cell lung cancer cell, an oligodendroglioma cell, an ovarian cancer cell, a pancreatic cancer cell, a peripheral T-cell lymphoma cell, a renal cancer cell, a sarcoma cell, a stomach cancer cell, a carcinoma cell, a mesothelioma cell, or a sarcoma cell. In some embodiments, the target cell is a pathogenic cell.

[0240] In some embodiments, the methods of the disclosure involve administering an

effective amount of the recombinants cells of the disclosure to an individual in need of such treatment. This administering step can be accomplished using any method of implantation delivery in the art. For example, the recombinant cells of the disclosure can be infused directly in the individual's bloodstream or otherwise administered to the individual.

[0241] In some embodiments, the methods disclosed herein include administering, which term is used interchangeably with the terms "introducing," implanting," and "transplanting," recombinant cells into an individual, by a method or route that results in at least partial localization of the introduced cells at a desired site such that a desired effect(s) is/are produced. The recombinant cells or their differentiated progeny can be administered by any appropriate route that results in delivery to a desired location in the individual where at least a portion of the administered cells or components of the cells remain viable. The period of viability of the cells after administration to an individual can be as short as a few hours, e.g., twenty-four hours, to a few days, to as long as several years, or even the lifetime of the individual, i.e., long-term engraftment.

[0242] When provided therapeutically in some embodiments, recombinant cells are provided at (or after) the onset of a symptom or indication of a disease or condition, e.g., upon the onset of disease or condition.

[0243] A therapeutically effective amount includes an amount of recombinant cells that is sufficient to promote a particular beneficial effect when administered to an individual, such as one who has, is suspected of having, or is at risk for a disease. In some embodiments, an effective amount includes an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate effective amount can be determined by one of ordinary skill in the art using routine experimentation.

[0244] For use in the various embodiments described herein, an effective amount of recombinant cells as disclosed herein, can be at least 10^2 cells, at least $5x10^2$ cells, at least 10^3 cells, at least $5x10^3$ cells, at least 10^4 cells, at least $5x10^4$ cells, at least 10^5 cells, at least $2x10^5$ cells, at least $3x10^5$ cells, at least $4x10^5$ cells, at least $5x10^5$ cells, at least $6x10^5$ cells, at least $7x10^5$ cells, at least $8x10^5$ cells, at least $9x10^5$cells, at least $1x10^6$cells, at least $2x10^6$ cells, at least $3x10^6$ cells, at least $4x10^6$ cells, at least $5x10^6$ cells, at least $6x10^6$ cells, at least $7x10^6$ cells,

at least $8x10^6$ cells, at least $9x10^6$ cells, or multiples thereof. The recombinant cells can be derived from one or more donors or can be obtained from an autologous source. In some embodiments, the recombinant cells are expanded in culture prior to administration to an individual in need thereof.

[0245] In some embodiments, the delivery of a recombinant cell composition or a pharmaceutical composition (e.g., a composition including a plurality of recombinant cells according to any of the cells described herein) into an individual by a method or route results in at least partial localization of the cell composition at a desired site. A composition including recombinant cells can be administered by any appropriate route that results in effective treatment in the individual, e.g., administration results in delivery to a desired location in the individual where at least a portion of the composition delivered, e.g., at least $1x10^4$ cells, is delivered to the desired site for a period of time. Modes of administration include injection, infusion, and instillation. "Injection" includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion. In some embodiments, the route is intravenous. For the delivery of cells, delivery by injection or infusion is a preferred mode of administration.

[0246] In some embodiments, the recombinant cells are administered systemically, e.g., via infusion or injection. For example, a population of recombinant cells are administered other than directly into a target site, tissue, or organ, such that it enters, the individual's circulatory system and, thus, is subject to metabolism and other similar biological processes.

[0247] The efficacy of a treatment including any of the compositions provided herein for the treatment of a disease or condition can be determined by a skilled clinician. However, one skilled in the art will appreciate that a treatment is considered effective if any one or all of the signs or symptoms or markers of disease are improved or ameliorated. Efficacy can also be measured by failure of an individual to worsen as assessed by decreased hospitalization or need for medical interventions (e.g., progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the disease, e.g., arresting, or slowing the

progression of symptoms; or (2) relieving the disease, e.g., causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of symptoms.

[0248] The diseases suitable for being treated by the compositions and methods of the disclosure include, but are not limited to, cancers, autoimmune diseases, inflammatory diseases, and infectious diseases. In some embodiments, the disease is a cancer or a chronic infection. In some embodiments, the cancer is a hematological malignancy. In some embodiments, the disease is a solid tumor.

[0249] In some embodiments of the disclosed methods, the individual is a mammal. In some embodiments, the mammal is a human. In some embodiments, the individual has or is suspected of having a disease associated with inhibition of cell signaling mediated by a cell surface ligand or antigen.

[0250] In some embodiments of the methods of the disclosure, the administered recombinant cell modulates an activity of a target cell in the individual. In some embodiments, the activity of the target cell includes expression of a selected gene, proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and cytolytic activity.

[0251] As discussed above, the recombinant cells, and pharmaceutical compositions described herein can be administered in combination with one or more additional therapeutic agents such as, for example, chemotherapeutics or anti-cancer agents or anti-cancer therapies. Administration "in combination with" one or more additional therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order. In some embodiments, the one or more additional therapeutic agents, chemotherapeutics, anti-cancer agents, or anti-cancer therapies is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, and surgery. "Chemotherapy" and "anti-cancer agent" are used interchangeably herein. Various classes of anti-cancer agents can be used. Non-limiting examples include: alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, podophyllotoxin, antibodies (e.g., monoclonal or polyclonal), tyrosine kinase inhibitors (e.g., imatinib mesylate (Gleevec.RTM. or Glivec.RTM.)), hormone treatments, soluble receptors and other antineoplastics.

B. Methods for Simultaneously Inducing T cell Signaling and Gene Regulation or Inducing Enhanced T Cell Signaling

[0252] The present disclosure also provides methods for simultaneously inducing T cell signaling and gene regulation in a T cell including (a) providing a T cell including the multi-chain chimeric polypeptides or the chimeric antigen receptors of the disclosure and (b) exposing the T cell to the selected ligand, wherein binding of a selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator.

[0253] Also provided herein are methods for simultaneously inducing T cell signaling and gene regulation in a T cell, the method including (a) providing a vector comprising a multi-chain chimeric polypeptide or CAR of the disclosure and (b) transducing a T cell with the vector, wherein binding of a selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator.

[0254] Also provided herein are methods of inducing enhanced T cell signaling in a T cell, the method comprising (a) providing a T cell comprising the multi-chain chimeric polypeptide of the present disclosure; and (b) exposing the T cell to a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain enhances intracellular signaling.

[0255] Also provided herein are methods of inducing enhanced T cell signaling in a T cell, the method comprising (a) providing a vector comprising the multi-chain chimeric polypeptide of the present disclosure, or a vector comprising the first polypeptide and a second vector comprising the second polypeptide of any one of the multi-chain polypeptides of the present disclosure; and (b) transducing a T cell with the vector or vectors, wherein binding of the selected ligand to the extracellular ligand-binding domain induces enhanced intracellular signaling.

[0256] In some embodiments, the induced intracellular signaling of the T cell modulates expression of a selected gene involved in proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and/or a cytolytic activity. In some embodiments, the released transcriptional regulator modulates expression of a payload in the T cell. In some embodiments, the payload comprises a chemokine, a chemokine receptor, a chimeric antigen receptor, a cytokine, a cytokine receptor, a differentiation factor, a growth factor, a growth factor receptor, a hormone, a metabolic enzyme, a pathogen-derived protein, a proliferation inducer, a receptor, an RNA guided nuclease, a site-specific nuclease, a T cell receptor, a toxin, a toxin derived protein, a transcriptional regulator, a

transcriptional activator, a transcriptional repressor, a translational regulator, a translational activator, a translational repressor, an activating immuno-receptor, an antibody, an apoptosis inhibitor, an apoptosis inducer, an engineered T cell receptor, an immuno-activator, an immuno-inhibitor, or an inhibiting immuno-receptor.

[0257] In some embodiments, enhanced T cell signaling can be an improvement or enhancement of 1%, 5%, 10%, 25% 50%, 75%, 100%, or greater than 100% in T cell signaling as compared to a reference cell not expressing a chimeric receptor of the disclosure.

C. Methods for Modulating an Activity of a Cell

[0258] In another aspect, provided herein are various methods for modulating an activity of a cell including (a) providing an effective amount of any of the recombinant cells of the disclosure; and (b) contacting the cell with a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain (i) induces cleavage of a ligand-inducible proteolytic cleavage site and releases the transcriptional regulator and simultaneously (ii) activates T cell signaling, wherein the released transcriptional regulator modulates an activity of the recombinant cell. One skilled in the art upon reading the present disclosure will appreciate that the disclosed methods can be carried out i*n vivo, ex vivo,* or *in vitro*.

[0259] Non-limiting exemplary cellular activities that can be modulated using the methods provide herein include, but are not limited to, gene expression, proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a gene product, cellular adhesion, and cytolytic activity.

[0260] In some embodiments, the released transcriptional regulator modulates expression of a gene product of the cell. In some embodiments, the released transcriptional regulator modulates expression of a heterologous gene product in the cell. A heterologous gene product is one that is not normally found in the native cell, e.g., not normally produced by the cell. For example, the cell can be genetically modified with a nucleic acid including a nucleotide sequence encoding the heterologous gene product.

[0261] In some embodiments, the heterologous gene product is a secreted gene product. In some embodiments, the heterologous gene product is a cell surface gene product. In some cases, the heterologous gene product is an intracellular gene product. In some embodiments, the released transcriptional regulator simultaneously modulates expression of two or more heterologous gene products in the cell.

[0262] In some embodiments, the heterologous gene product in the cell is selected from the group consisting of a chemokine, a chemokine receptor, a chimeric antigen receptor, a cytokine, a cytokine receptor, a differentiation factor, a growth factor, a growth factor receptor, a hormone, a metabolic enzyme, a pathogen-derived protein, a proliferation inducer, a receptor, an RNA guided nuclease, a site-specific nuclease, a T-cell receptor (TCR), a chimeric antigen receptor (CAR), a toxin, a toxin-derived protein, a transcriptional regulator, a transcriptional activator, a transcriptional repressor, a translation regulator, a translational activator, a translational repressor, an activating immuno-receptor, an antibody, an apoptosis inhibitor, an apoptosis inducer, an engineered T cell receptor, an immuno-activator, an immuno-inhibitor, and an inhibiting immuno-receptor.

[0263] In some embodiments, the released transcriptional regulator modulates differentiation of the cell, and wherein the cell is an immune cell, a stem cell, a progenitor cell, or a precursor cell.

IV. SYSTEMS AND KITS

[0264] Also provided herein are systems and kits including the multi-chain chimeric polypeptides, CARs, recombinant nucleic acids, recombinant cells, or pharmaceutical compositions provided and described herein as well as written instructions for making and using the same. For example, provided herein, in some embodiments, are systems and/or kits that include one or more of: a multi-chain chimeric polypeptide of the disclosure, a recombinant nucleic acids as described herein, a recombinant cell as described herein, or a pharmaceutical composition as described herein. In some embodiments, the systems and/or kits of the disclosure further include one or more syringes (including pre-filled syringes) and/or catheters (including pre-filled syringes) used to administer one any of the provided recombinant nucleic acids, recombinant cells, or pharmaceutical compositions to an individual. In some embodiments, a kit can have one or more additional therapeutic agents that can be administered simultaneously or sequentially with the other kit components for a desired purpose, e.g., for modulating an activity of a cell, inhibiting a target cancer cell, or treating a health condition (e.g., disease) in an individual in need thereof.

[0265] In some embodiments, a system or kit can further include instructions for using the components of the kit to practice the methods. The instructions for practicing the methods are

generally recorded on a suitable recording medium. For example, the instructions can be printed on a substrate, such as paper or plastic, etc. The instructions can be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging), etc. The instructions can be present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, flash drive, etc. In some instances, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (e.g., via the internet), can be provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions can be recorded on a suitable substrate.

[0266] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

[0267] Throughout this specification, various patents, patent applications and other types of publications (*e.g.*, journal articles, electronic database entries, *etc.*) are referenced. The disclosure of all patents, patent applications, and other publications cited herein are hereby incorporated by reference in their entirety for all purpose.

[0268] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

EXAMPLES

[0269] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art and are explained fully in the literature, such as Sambrook, J., & Russell, D. W. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor, NY: Cold Spring Harbor

Laboratory and Sambrook, J., & Russel, D. W. (2001). *Molecular Cloning: A Laboratory Manual* (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (jointly referred to herein as "Sambrook"); Ausubel, F. M. (1987). *Current Protocols in Molecular Biology*. New York, NY: Wiley (including supplements through 2014); Bollag, D. M. *et al.* (1996). *Protein Methods*. New York, NY: Wiley-Liss; Huang, L. *et al.* (2005). *Nonviral Vectors for Gene Therapy*. San Diego: Academic Press; Kaplitt, M. G. *et al.* (1995). *Viral Vectors: Gene Therapy and Neuroscience Applications*. San Diego, CA: Academic Press; Lefkovits, I. (1997). *The Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*. San Diego, CA: Academic Press; Doyle, A. *et al.* (1998). *Cell and Tissue Culture: Laboratory Procedures in Biotechnology*. New York, NY: Wiley; Mullis, K. B., Ferré, F. & Gibbs, R. (1994). *PCR: The Polymerase Chain Reaction*. Boston: Birkhauser Publisher; Greenfield, E. A. (2014). *Antibodies: A Laboratory Manual* (2nd ed.). New York, NY: Cold Spring Harbor Laboratory Press; Beaucage, S. L. *et al.* (2000). *Current Protocols in Nucleic Acid Chemistry*. New York, NY: Wiley, (including supplements through 2014); and Makrides, S. C. (2003). *Gene Transfer and Expression in Mammalian Cells*. Amsterdam, NL: Elsevier Sciences B.V., the disclosures of which are incorporated herein by reference.

[0270] Additional embodiments are disclosed in further detail in the following examples, which are provided by way of illustration and are not in any way intended to limit the scope of this disclosure or the claims.

EXAMPLE 1: Receptor Expression

[0271] This Example describes flow cytometry data of receptor expression.

[0272] Primary human CD3+ T-cells were activated with anti-CD3/anti-CD28 Dynabeads (Gibco) and transduced with a lentiviral construct expressing a multi-chain receptor construct, and another lentiviral construct containing the transcriptional reporter construct. Receptor expression was measured using an AlexaFluor647-tagged anti-myc antibody (Cell Signaling) against the myc tag on the binder (CD19scFv)-containing chain. Reporter expression was measured through a constitutive mCitrine gene found on the reporter plasmid. Double positive cells were sorted for on Day 5 post initial T-cell stimulation and expanded further for activation testing. Here, receptor designs 056 (FIG. 2A) and 056C (FIG. 2B) showed the highest expression of this cohort.

EXAMPLE 2: Receptor Activation

[0273] This Example describes receptor activation and target killing of two receptor embodiments, 056 and 056C, (FIG. 3A).

[0274] 1E5 double positive T-cells expressing anti-CD19 receptors were co-cultured with: no additions (red, "T cells alone"), 1E5 K562 cells (blue, (+K562)), or 1E5 CD19+ K562 cells (yellow, "+K562-CD19") for 48 hours. Transcriptional activation (FIG. 3B) of an inducible BFP reporter gene was subsequently measured using a Fortessa X-50 (BD). Target killing (FIG. 3C) was measured by DRAQ7 staining and flow cytometry. Receptor 056 showed better killing than receptor 056C, and receptor 056C showed better transcriptional activation.

EXAMPLE 3: Receptor Expression of Receptors 056F and 056G

[0275] This Example describes flow cytometry data of receptor expression of embodiments of receptors 056F and 056G.

[0276] Primary human CD3+ T-cells were activated with anti-CD3/anti-CD28 Dynabeads (Gibco) and transduced with a lentiviral construct expressing a multi-chain receptor construct, and another lentiviral construct containing the transcriptional reporter construct. Receptor expression was measured using an AlexaFluor647-tagged anti-myc antibody (Cell Signaling) against the myc tag on the binder (CD19scFV)-containing chain. Reporter expression was measured through a constitutive mCitrine gene found on the reporter plasmid. Double positive cells are sorted for on Day 5 post initial T-cell stimulation and expanded further for activation testing. Receptor designs 056F (FIG. 4C) and 056G (FIG. 4D) show the highest expression of this cohort.

EXAMPLE 4: Receptor Activation and Performance of Embodiments 056, 056E-G

[0277] This Example describes receptor activation and target killing testing of receptors 056, 056E 056F and 056G.

[0278] 1E5 double positive T-cells expressing anti-CD19 receptors were co-cultured with: no additions (red, "T cells alone"), 1E5 K562 cells (blue, "+K562"), or 1E5 CD19+ K562 cells (yellow, "+K562-CD19") for 5 days. After 24 hrs, transcriptional activation (top row) of an inducible BFP reporter gene was measured using a Fortessa X-50 (BD). Target killing (bottom panel) was measured at 24 and 120 hours by DRAQ7 staining and flow cytometry. Here,

receptors 056F (FIG. 5C) showed both transcriptional activity and killing activity, receptors 056 (FIG. 5A) and 056E (FIG. 5B) show killing, and receptor 056G (FIG. 5D) only shows transcriptional activation.

EXAMPLE 5: Multi-chain Receptor Modular Engineering Strategy

[0279] This Example describes a multi-chain receptor modular engineering strategy using pRay056F as the prototype. As shown in FIG. 6, one chain (DAP12-chain) having the DAP12 signaling domain was replaced with ITAM and signaling domains from the TCR, costimulatory proteins, and cytotokine receptors. The other chain (Main-chain, containing the transcriptional factor Gal4-VP64) will have the transcription factor (TF) replaced with a human transcription factor, or with ITAM and signaling domains from TCR, costimulatory, and cytokine receptors.

EXAMPLE 6: Multi-chain Receptor Function with CD3z Substitution

[0280] This Example describes the testing of multi-chain receptor with CD3z substitution. Here, the DAP12 ITAM signaling domain of the DAP12 multi-chain receptor (056F, FIG. 7A) is replaced with the ITAM signaling domain from CD3z, which contains 3 ITAMs (056I, FIG 7B). 1E5 double positive T-cells expressing anti-CD19 receptors were co-cultured with: no additions (red, "T cells alone"), 1E5 K562 cells (blue, "+K562"), or 1E5 CD19+ K562 cells (yellow, "+K562-CD19") for 5 days. After 24 hrs, transcriptional activation (top row) of an inducible BFP reporter gene was measured using a Fortessa X-50 (BD). Target killing (bottom panel) was measured at 24 and 120 hours by DRAQ7 staining and flow cytometry.This substitution appears to increase killing of target cells (FIG. 7B, bottom row). Transcriptional activation is slightly reduced, but remains strong (FIG. 7B, top row). Transcriptional activation and killing from a SNIPR receptor (FIG. 7C) and standard BBz CAR (FIG. 7D) are included as positive controls for transcriptional activation and killing, respectively.

EXAMPLE 7: Multi-chain receptor function with a Human Transcription Factor

[0281] This Example describes the testing of multi-chain receptor with a human transcription

factor. Here, the Gal4-VP64, which contains nonhuman parts, is substituted with a transcription factor comprised of human parts, in both the DAP12 (056H, FIG. 8A) and CD3z (FIG. 8B) context. These receptor designs are compared against a receptor designed with transcriptional and signaling domains constructed linearly on a single chain receptor (FIG. 8C). 1E5 double positive T-cells expressing anti-CD19 receptors were co-cultured with: no additions (red, "T cells alone"), 1E5 K562 cells (blue, "+K562"), or 1E5 CD19+ K562 cells (yellow, "+K562-CD19") for 5 days. After 24 hrs, transcriptional activation (top row) of an inducible BFP reporter gene was measured using a Fortessa X-50 (BD). Target killing (bottom panel) was measured at 24 and 120 hours by DRAQ7 staining and flow cytometry. Both multi-chain receptors are able to mediate transcriptional activation and killing of target cells (FIGs. 8A and 8B, top row and bottom row), whereas the single chain design does not (FIG. 8C, top row and bottom row). Transcriptional activation and killing from a standard BBz CAR (FIG. 8D) is included as positive control for killing.

EXAMPLE 8: Multi-chain receptor with Modular Engineering Strategy Using Dual Vector Transduction

[0282] This Example describes the modular engineering strategy for multi-chain receptors using dual vector transduction. Here, each chain is expressed from individual promoters introduced by transduction of the DAP12-Chain and Main-Chain constructs listed (FIG. 9). Each Side-chain is combined pairwise with each Main-chain to assess the effect of pairing the two signaling chains together. Three of the main-chain variants contain signaling components in lieu of a transcription factor, designed to deliver additional signaling ability rather than transcriptional activation.

EXAMPLE 9: Expression Profiles of Multi-chain Receptor Pairs

[0283] This Examples describes the expression profile of each multi-chain receptor pair. Cells containing the DAP-12 chain are detected by the T2A-mCherry signal, while cells containing the Main-chain receptor are detected by Myc-tag staining. Myc-tag staining was used to assess receptor expression levels. All receptors show some level of expression, with some

pairs showing better expression than others, as highlight by the higher double-positive populations (FIG. 10).

EXAMPLE 10: Screen for Multi-chain Receptors that Enhance Killing and Survival

[0284] This Example describes a screen for multi-chain receptors that enhance killing and survival. Primary human CD3+ T cells expressing multi-chain receptors were co-cultured with 100k K562 target cells at 1:4, 1:2, and 1:1 T cell to target ratios, and followed over time. Co-cultures were maintained in human T cell media with no exogenous cytokines and fed every 5 days. On days 5, 12, and 20, half of the co-cultures were removed (with media replacement), stained for T cell markers, and analyzed by flow cytometry. Counts showing T cell (FIGs. 11-13) and target cell (FIGs. 14-16
") survival are quantitated. A standard BBz CAR T cell was included as a control (last column of each plot). Several multi-chain receptors appear to mediate superior T cell survival and target cell killing over BBz CAR by the last timepoint (Day 20).

EXAMPLE 11: Receptor and Response Element Construct Design

[0285] This Example describes the design and construction of a family of multi-chain chimeric polypeptides (e.g., receptors) that were built by fusing the CD19 scFv {Porter:2011gr} to the corresponding receptor scaffold and Gal4 DBD VP64. The receptors contained an n-terminal CD8α signal peptide (MALPVTALLLPLALLLHAARP) for membrane targeting and a myc-tag (EQKLISEEDL) for easy determination of surface expression with α-myc A647 (cell-signaling #2233). The receptors were cloned into a modified pHR'SIN:CSW vector containing a PGK promoter for all primary T cell experiments (SEQ ID NO:1).

EXAMPLE 12: Primary Human T cell Isolation and Culture

[0286] This Example describes the isolation and culture of primary human T cells that were subsequently used in various cell transduction experiments described in Example 3 below. In these experiments, primary CD4+ and CD8+ T cells were isolated from anonymous donor blood after apheresis by negative selection (STEMCELL Technologies #15062 & 15063). Blood was obtained from Blood Centers of the Pacific (San Francisco, CA) as approved by the University

Institutional Review Board. T cells were cryopreserved in RPMI-1640 (UCSF cell culture core) with 20% human AB serum (Valley Biomedical Inc., #HP1022) and 10% DMSO. After thawing, T cells were cultured in human T cell medium consisting of X-VIVO 15 (Lonza #04-418Q), 5% Human AB serum and 10 mM neutralized N-acetyl L-Cysteine (Sigma-Aldrich #A9165) supplemented with 30 units/mL IL-2 (NCI BRB Preclinical Repository) for all experiments.

EXAMPLE 13: Lentiviral Transduction of Human T cells

[0287] This Examples describes a general protocol used for lentiviral transduction of human T cells. Pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells (Clontech #11131D) with a pHR'SIN:CSW transgene expression vector and the viral packaging plasmids pCMVdR8.91 and pMD2.G using Mirus TransIT-Lenti (Mirus #MIR 6606). Primary T cells were thawed the same day, and after 24 hours in culture, were stimulated with Human T-Activator CD3/CD28 Dynabeads (Life Technologies #11131D) at a 1:3 cell:bead ratio. At 48 hours, viral supernatant was harvested and the primary T cells were exposed to the virus for 24 hours. At day 5 post T cell stimulation, the Dynabeads were removed, and the T cells expanded until day 14 when they were rested and could be used in assays. T cells were sorted for assays with a Beckton Dickinson (BD) FACs ARIA II.

EXAMPLE 14: Cancer Cell Lines

[0288] This Example describes the generation of myelogenous leukemia cell expressing CD19 at equivalent levels as Daudi tumors. The cancer cell lines used were K562 myelogenous leukemia cells (ATCC #CCL-243). K562s were lentivirally transduced to stably express human CD19 at equivalent levels as Daudi tumors, or to express HER2 via a doxycycline-inducible system. CD19 levels were determined by staining the cells with α-CD19 APC (Biolegend #302212), and HER2 levels were determined by staining the cells with α-HER2 AF647 (Biolegend # 324412). All cell lines were sorted for expression of the transgenes.

EXAMPLE 15: *In vitro* Stimulation of primary T cells

[0289] For all *in vitro* T cell stimulations, 1×10^5 T cells were co-cultured with target cells at a 1:1 ratio in U-bottom 96-well tissue culture plates. The cultures were analyzed at 24 hours or as indicated for reporter activation and/or target cell killing with a BD Fortessa X-50. All flow cytometry analysis was performed in FlowJo software (TreeStar).

EXAMPLE 16: Recombinant Constructs

[0290] The following are examples of recombinant nucleic acid constructs or cassettes encoding examples of the multi-chain chimeric polypeptides of the disclosure. As described herein above, the constructs included two cassettes. In this example, the cassette encoding the second polypeptide is 5' to the cassette encoding the first polypeptide. A T2A autoproteolytic peptide sequence is located between the second and the first polypeptide. Constructs with the first polypeptide 5' to the second polypeptide can be constructed in a similar manner.

Construct	**Construct components in embodiments in which the second polypeptide is N-terminal to the first polypeptide.**
pRay056	DAP12-T2A-CD19scFv-TREM2-Gal4VP64
pRay056B	DAP12-T2A-CD19scFv-TREM2-Notch1TMD(L312K)-Notch2JMD-TREM2-Gal4VP64
pRay056C	DAP12-T2A-CD19scFv-CD8Hinge-Notch1TMD(L312K)-Notch2JMD-Gal4VP64
pRay056D	DAP12-T2A-CD19scFv-TREM2-Notch1TMD(L312K)-Notch2JMD-Gal4VP64
pRay056F	DAP12-T2A-CD19scFv-CD8Hinge-PolyVTMD-V9K-Notch2JMD-Gal4VP64
pRay056G	DAP12-T2A-CD19scFv-CD8Hinge-PolyVTMD-V10K-Notch2JMD-Gal4VP64
pRay056H	CD19scFv_DAP12-T2A-K9-HNF1a
pRay056I	DAP12-CD3z-t2a-CD19scFv-K9-Gal4VP64
pRay056J	DAP12-CD3z-t2a-CD19scFv-K9-HNF1a-p65(361)

CLAIMS

WHAT IS CLAIMED IS:

1. A multi-chain chimeric polypeptide comprising:

(a) a first polypeptide comprising (i) an extracellular ligand-binding domain having a binding affinity for a selected ligand, (ii) a first transmembrane domain (TMD) comprising a first modified interface and (iii) a first intracellular domain comprising a transcriptional regulator; and

(b) a second polypeptide comprising (i) a second TMD comprising a second interface and (ii) a second intracellular domain comprising a signaling domain;

wherein the first and the second modified interfaces each comprise amino acid residues having opposite charges,

and wherein the first polypeptide is coupled to the second polypeptide via the first modified interface and the second interface and wherein binding of the selected ligand to the extracellular ligand-binding domain induces activity of the signaling domain and release of the transcriptional regulator.

2. The multi-chain chimeric polypeptide of claim 1, wherein binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces the activity of the signaling domain and the release of the transcriptional regulator.

3. The multi-chain chimeric polypeptide of claim 1 or 2, wherein the first polypeptide comprises (i) the extracellular ligand-binding domain, (ii) the first TMD, and (iii) the first intracellular domain, in order from N-terminus to C-terminus of the first polypeptide.

4. The multi-chain chimeric polypeptide of any one of claims 1 to 3, wherein the first TMD comprises: (i) 10 to 25 contiguous valine residues, or (ii) a Notch 1 transmembrane domain.

5. The multi-chain chimeric polypeptide of any one of claims 1 to 4, wherein the second polypeptide comprises (i) the second TMD, and (ii) the second intracellular domain, in order from N-terminus to C-terminus of the second polypeptide.

6. The multi-chain chimeric polypeptide of any one of claims 1 to 5, wherein the first modified interface comprises a positively charged residue, and wherein the second interface comprises a negatively charged residue, and wherein the first polypeptide is coupled to the second polypeptide via the electrostatic force between the first and the second modified interfaces.

7. The multi-chain chimeric polypeptide of claim 6, wherein the positively charged residue is lysine or arginine.

8. The multi-chain chimeric polypeptide of claim 7, wherein (i) the first TMD comprises SEQ ID NO:21 or functional variants thereof and the lysine or arginine residue is at position selected from positions 10 to 14 of SEQ ID NO:21 or (ii) the first TMD comprises SEQ ID NO:18 and the lysine or arginine is at position selected from positions 8 to 11 of SEQ ID NO: 18.

9. The multi-chain chimeric polypeptide of claim 8, wherein the lysine or arginine residue is (i) at position 12 of SEQ ID NO:21, or (ii) is at position 9 of SEQ ID NO:18.

10. The multi-chain chimeric polypeptide of any one of claims 1 to 9, wherein the extracellular domain comprises an antigen-binding moiety capable of binding to a ligand on the surface of a cell.

11. The multi-chain chimeric polypeptide of claim 10, wherein the antigen-binding moiety is selected from the group consisting of an antibody, a nanobody, a diabody, a triabody, a minibody, an $F(ab')_2$ fragment, an F(ab) fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and a functional fragment thereof.

12. The multi-chain chimeric polypeptide of any one of claims 1 to 11, wherein the ligand comprises a protein or a carbohydrate.

13. The multi-chain chimeric polypeptide of any one of claims 1 to 12, wherein the ligand is a tumor-associated antigen or a tumor-specific antigen.

14. The multi-chain chimeric polypeptide of any one of claims 1 to 13, wherein the ligand comprises cell-surface receptors, adhesion proteins, integrins, mucins, lectins, tumor associated antigens, or tumor specific antigens.

15. The multi-chain chimeric polypeptide of claim 14, wherein the ligand comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent Protein (eGFP), or signal regulatory protein a (SIRPα).

16. The multi-chain chimeric polypeptide of any one of claims 10 to 15, wherein the cell is a human cell.

17. The multi-chain chimeric polypeptide of any one of claims 10 to 16, wherein the cell is a tumor cell.

18. The multi-chain chimeric polypeptide of any one of claims 1 to 17, wherein the transcriptional regulator comprises a transcriptional activator or a transcriptional repressor.

19. The multi-chain chimeric polypeptide of any one of claims 1 to 18, wherein the transcriptional regulator comprises Ga14-VP16, Ga14-VP64, tetR-VP64, ZFHD1-VP64, Ga14-KRAB, or HAP1-VP16.

20. The multi-chain chimeric polypeptide of any one of claims 1 to 18, wherein the transcriptional regulator is a human or a humanized transcriptional regulator.

21. The multi-chain chimeric polypeptide of any one of claims 1 to 18, wherein the transcriptional regulator is HNF1a.

22. The multi-chain chimeric polypeptide of any one of claims 1 to 21, wherein the second polypeptide comprises a signaling domain comprising CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, or CD66d, signaling domain.

23. The multi-chain chimeric polypeptide of any one of claims 1 to 22, wherein the first polypeptide further comprises one or more of the following: a hinge domain, a ligand-inducible proteolytic cleavage site, an autoproteolytic peptide sequence, a nuclear localization signal, a juxtamembrane domain.

24. The multi-chain chimeric polypeptide of claim 23, wherein the juxtamembrane domain is a polybasic domain.

25. The multi-chain chimeric polypeptide of claim 24, wherein the polybasic domain comprises Notch-1 or Notch-2 juxtamembrane domains.

26. The multi-chain chimeric polypeptide of any one of claims 23 to 25, wherein the autoproteolytic peptide sequence is from a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

27. The multi-chain chimeric polypeptide of any one of claims 23 to 26, wherein the first polypeptide further comprises a hinge domain from CD8, CD28, OX40, or IgG4.

28. The multi-chain chimeric polypeptide of claim 27, wherein the hinge domain comprises a truncated CD8α hinge domain.

29. The multi-chain chimeric polypeptide of any one of claims 24 to 28, wherein the ligand-inducible proteolytic cleavage site is cleavable by gamma secretase.

30. The multi-chain chimeric polypeptide of claim 1, wherein the extracellular ligand-binding domain comprises CD19scFv, the first TMD comprises a contiguous stretch of valine residues comprising a lysine or arginine residue, the first polypeptide further comprises a Notch 2 juxtamembrane domain and the first intracellular domain comprises a Gal4VP64 transcriptional regulator, the second polypeptide comprises DNAX –activation protein 12 (DAP12), and wherein the first polypeptide is coupled to the second polypeptide via the lysine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator.

31. The multi-chain chimeric polypeptide of claim 1, wherein the extracellular ligand-binding domain comprises CD19scFv, the first TMD comprises a contiguous stretch of valine residues comprising a lysine or arginine residue, the first polypeptide further comprises a Notch 2 juxtamembrane domain and the first intracellular domain comprises a Gal4VP64 transcriptional regulator, the second polypeptide comprises a CD3z signaling domain, and wherein the first polypeptide is coupled to the second polypeptide via the lysine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator.

32. The multi-chain chimeric polypeptide of claim 1, wherein the extracellular ligand-binding domain comprises CD19scFv, the first TMD comprises a contiguous stretch of valine residues comprising a lysine or arginine residue, the first polypeptide further comprises a Notch 2 juxtamembrane domain and the first intracellular domain comprises a human or humanized transcriptional regulator, the second polypeptide comprises DNAX –activation protein 12

(DAP12) or CD3z, and wherein the first polypeptide is coupled to the second polypeptide via the lysine residue within the contiguous stretch of valine residues and wherein binding of CD19 to the extracellular ligand-binding domain simultaneously induces activity of the signaling domain and releases the transcriptional regulator.

33. The multi-chain chimeric polypeptide of claim 32, wherein the transcriptional regulator is HNF1a.

34 The multi-chain chimeric polypeptide of claim 30, wherein the contiguous stretch of valine residues comprises 5 to 25 valine residues and wherein the lysine or arginine residue is flanked by a contiguous stretch of 5 to 15 valine residues.

35. The multi-chain chimeric polypeptide of any one of claims 1 to 34, wherein the multi-chain chimeric polypeptide is an immunoreceptor.

36. The multi-chain chimeric polypeptide of claim 35, wherein the immunoreceptor is a chimeric antigen receptor.

37. A multi-chain chimeric polypeptide comprising:

(a) a first polypeptide comprising (i) an extracellular ligand-binding domain having a binding affinity for a selected ligand, (ii) a first transmembrane domain (TMD) comprising a first modified interface and (iii) a first intracellular domain comprising a transcriptional regulator or a signaling domain; and

(b) a second polypeptide comprising (i) a second TMD comprising a second interface and (ii) a second intracellular domain comprising a signaling domain;

wherein the first and the second modified interfaces each comprise amino acid residues having opposite charges,

and wherein the first polypeptide is coupled to the second polypeptide via the first modified interface and the second interface and wherein binding of the selected ligand to the extracellular ligand-binding domain induces activity of the signaling domain and release of the transcriptional regulator.

38. The multi-chain chimeric polypeptide of claim 37, wherein the first intracellular domain comprises a transcriptional regulator.

39. The multi-chain chimeric polypeptide of claim 38, wherein the wherein the transcriptional regulator comprises Ga14-VP16, Ga14-VP64, tetR-VP64, ZFHD1-VP64, Ga14-KRAB, or HAP1-VP16.

40. The multi-chain chimeric polypeptide of any one of claims 37 to 38, wherein the transcriptional regulator is a human or a humanized transcriptional regulator.

41. The multi-chain chimeric polypeptide of claim 40, wherein the transcriptional regulator is HNF1a.

42. The multi-chain chimeric polypeptide of claim 37, wherein the first intracellular domain comprises a signaling domain.

43. The multi-chain chimeric polypeptide of claim 42, wherein the signaling domain comprises one or more of a CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, CD66d, 4-1BB, or common gamma chain signaling domain.

44. The multi-chain chimeric polypeptide of any one of claims 37 to 43, wherein the signaling domain of the second polypeptide comprises a signaling domain comprising CD3 zeta, TCR zeta, FcR γ, FcRβ, CD3γ, CD3Δ, CD3ε, CD5, CD22, CD79a, CD79b, CD278 (ICOS), Fcε RI, DAP10, DAP12, CD66d, or 4-1BB signaling domain.

45. The multi-chain chimeric polypeptide of any one of claims 37 to 43, wherein the signaling domain of the second polypeptide comprises a cytokine signaling domain.

46. The multi-chain chimeric polypeptide of claim 45, wherein the cytokine signaling domain comprises an IL-2Rb, IL-4Ra, IL-7Ra, IL-9Ra, IL-13R, IL-15R, or IL-21R endodomain.

47. The multi-chain chimeric polypeptide of any one of claims 37 to 46, wherein binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces the activity of the signaling domain and the release of the transcriptional regulator.

48. The multi-chain chimeric polypeptide of any one of claims 37 to 47, wherein the first polypeptide comprises (i) the extracellular ligand-binding domain, (ii) the first TMD, and (iii) the first intracellular domain, in order from N-terminus to C-terminus of the first polypeptide.

49. The multi-chain chimeric polypeptide of any one of claims 37 to 48, wherein the first TMD comprises: (i) 10 to 25 contiguous valine residues, or (ii) a Notch 1 transmembrane domain.

50. The multi-chain chimeric polypeptide of any one of claims 37 to 49, wherein the second polypeptide comprises (i) the second TMD, and (ii) the second intracellular domain, in order from N-terminus to C-terminus of the second polypeptide.

51. The multi-chain chimeric polypeptide of any one of claims 37 to 50, wherein the first modified interface comprises a positively charged residue, and wherein the second interface comprises a negatively charged residue, and wherein the first polypeptide is coupled to the second polypeptide via the electrostatic force between the first and the second modified interfaces.

52. The multi-chain chimeric polypeptide of claim 51, wherein the positively charged residue is lysine or arginine.

53. The multi-chain chimeric polypeptide of claim 52, wherein (i) the first TMD comprises SEQ ID NO:21 or functional variants thereof and the lysine or arginine residue is at position selected from positions 10 to 14 of SEQ ID NO:21 or (ii) the first TMD comprises SEQ ID NO:18 and the lysine or arginine is at position selected from positions 8 to 11 of SEQ ID NO: 18.

54. The multi-chain chimeric polypeptide of claim 53, wherein the lysine or arginine residue is (i) at position 12 of SEQ ID NO:21, or (ii) is at position 9 of SEQ ID NO:18.

55. The multi-chain chimeric polypeptide of any one of claims 37 to 54, wherein the extracellular domain comprises an antigen-binding moiety capable of binding to a ligand on the surface of a cell.

56. The multi-chain chimeric polypeptide of claim 55, wherein the antigen-binding moiety is selected from the group consisting of an antibody, a nanobody, a diabody, a triabody, a minibody, an $F(ab')_2$ fragment, an F(ab) fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and a functional fragment thereof.

57. The multi-chain chimeric polypeptide of any one of claims 37 to 56, wherein the ligand comprises a protein or a carbohydrate.

58. The multi-chain chimeric polypeptide of any one of claims 37 to 57, wherein the ligand is a tumor-associated antigen or a tumor-specific antigen.

59. The multi-chain chimeric polypeptide of any one of claims 37 to 58, wherein the ligand comprises cell-surface receptors, adhesion proteins, integrins, mucins, lectins, tumor associated antigens, or tumor specific antigens.

60. The multi-chain chimeric polypeptide of claim 59, wherein the ligand comprises CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2

(ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent Protein (eGFP), or signal regulatory protein a (SIRPα).

61. The multi-chain chimeric polypeptide of any one of claims 55 to 60, wherein the cell is a human cell.

62. The multi-chain chimeric polypeptide of any one of claims 55 to 61, wherein the cell is a tumor cell.

63. The multi-chain chimeric polypeptide of any one of claims 37 to 62, wherein the first polypeptide further comprises one or more of the following: a hinge domain, a ligand-inducible proteolytic cleavage site, an autoproteolytic peptide sequence, a nuclear localization signal, a juxtamembrane domain.

64. The multi-chain chimeric polypeptide of claim 63, wherein the juxtamembrane domain is a polybasic domain.

65. The multi-chain chimeric polypeptide of claim 64, wherein the polybasic domain comprises Notch-1 or Notch-2 juxtamembrane domains.

66. The multi-chain chimeric polypeptide of any one of claims 63 to 65, wherein the autoproteolytic peptide sequence is from a porcine teschovirus-1 2A (P2A), a foot-and-mouth disease virus (FMDV) 2A (F2A), an Equine Rhinitis A Virus (ERAV) 2A (E2A), a Thosea asigna virus 2A (T2A), a cytoplasmic polyhedrosis virus 2A (BmCPV2A), a Flacherie Virus 2A (BmIFV2A), or a combination thereof.

67. The multi-chain chimeric polypeptide of any one of claims 63 to 66, wherein the first polypeptide further comprises a hinge domain from CD8, CD28, OX40, or IgG4.

68. The multi-chain chimeric polypeptide of claim 67, wherein the hinge domain comprises a truncated CD8α hinge domain.

69. A recombinant nucleic acid construct comprising, in the 5' to 3' direction, a first cassette and a second cassette, wherein the first cassette and the second cassette are joined by an autoproteolytic peptide and wherein the first cassette encodes the first polypeptide of any one of the multi-chain chimeric polypeptides of claim 1 to claim 68, and the second cassette encodes the second polypeptide of any one of the multi-chain chimeric polypeptides of claims 1 to 68.

70. A recombinant nucleic acid construct comprising, in the 5' to 3' direction, a first cassette and a second cassette, wherein the first cassette and the second cassette are joined by an autoproteolytic peptide and wherein the first cassette encodes the second polypeptide of any one of the multi-chain chimeric polypeptides of claim 1 to claim 68, and the second cassette encodes the first polypeptide of any one of the multi-chain chimeric polypeptide of claims 1 to 68.

71. A recombinant nucleic acid construct comprising a nucleic acid sequence encoding the first polypeptide of any one of the multi-chain chimeric polypeptide of claims 1 to 68.

72. A recombinant nucleic acid construct comprising a nucleic acid sequence encoding the second polypeptide of any one of the multi-chain chimeric polypeptide of claims 1 to 68.

73. The nucleic acid construct of any one of claims 69 to 72, wherein the nucleic acid construct comprises a nucleic acid sequence comprising 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2, 3, 4, 5, 6 7, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58 or any functional variants thereof.

74. The nucleic acid construct of claim 69 or claim 70, wherein the autoproteolytic peptide is a Thosea asigna virus 2A (T2A) peptide.

75. A vector comprising the nucleic acid construct of claims 69 to 74.

76. The vector of claim 75, wherein the vector is an expression vector.

77. The vector of claim 75 or 76, wherein the vector is a viral vector.

78. The vector of any one of claims 75 to 77, wherein the viral vector comprises a retroviral, lentiviral vector, an adenovirus vector, and an adeno-associated virus vector.

79. A recombinant cell comprising a) a multi-chain chimeric polypeptide according to any one of claims 1 to 68, b) a nucleic acid construct according to any one of claims 69 to 74, and/or c) a vector according to any one of claims 75 to 78.

80. The recombinant cell of claim 79, wherein the recombinant cell is a human cell.

81. The recombinant cell of claim 79 or 80, wherein the recombinant cell is a tumor cell.

82. The recombinant cell of claim 80, wherein the recombinant cell is an immune cell.

83. The recombinant cell of claim 82, wherein the immune cell is a B cell, a monocyte, a natural killer cell, a basophil, an eosinophil, a neutrophil, a dendritic cell, a macrophage, a regulatory T cell, a helper T cell, a cytotoxic T cell, or other T cells.

84. The recombinant cell of claim 83, wherein the T cell is a CD4+ T cell or a CD8+ T cell.

85. A pharmaceutical composition comprising a recombinant cell according to any one of claims 79 to 84 and a pharmaceutically acceptable excipient.

86. A method of simultaneously inducing T cell signaling and gene regulation in a T cell, the method comprising: (a) providing a T cell comprising the multi-chain chimeric polypeptide of any one of claims 1 to 68; and (b) exposing the T cell to a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator.

87. A method of simultaneously inducing T cell signaling and gene regulation in a T cell, the method comprising:
(a) providing a vector comprising the multi-chain chimeric polypeptide of any one of claims 1 to 68, or a vector comprising the first polypeptide and a second vector comprising the second polypeptide of any one of the multi-chain polypeptides of claims 1 to 68; and
(b) transducing a T cell with the vector or vectors,

wherein binding of the selected ligand to the extracellular ligand-binding domain simultaneously induces intracellular signaling and release of the transcriptional regulator.

88. A method of inducing enhanced T cell signaling in a T cell, the method comprising: (a) providing a T cell comprising the multi-chain chimeric polypeptide of any one of claims 1 to 68; and (b) exposing the T cell to a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain enhances intracellular signaling.

89. A method of inducing enhanced T cell signaling in a T cell, the method comprising:
(a) providing a vector comprising the multi-chain chimeric polypeptide of any one of claims 1 to 68, or a vector comprising the first polypeptide and a second vector comprising the second polypeptide of any one of the multi-chain polypeptides of claims 1 to 68; and
(b) transducing a T cell with the vector or vectors,
wherein binding of the selected ligand to the extracellular ligand-binding domain induces enhanced intracellular signaling.

90. The method of any one of claims 86 -89, wherein the induced intracellular signaling of the T cell modulates expression of a selected gene involved in proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and/or a cytolytic activity.

91. The method of claim 87, wherein the released transcriptional regulator modulates expression of a payload in the T cell.

92. The method of claim 91, wherein the payload comprises a chemokine, a chemokine receptor, a chimeric antigen receptor, a cytokine, a cytokine receptor, a differentiation factor, a growth factor, a growth factor receptor, a hormone, a metabolic enzyme, a pathogen-derived protein, a proliferation inducer, a receptor, an RNA guided nuclease, a site-specific nuclease, a T cell receptor, a toxin, a toxin derived protein, a transcriptional regulator, a transcriptional activator, a transcriptional repressor, a translational regulator, a translational activator, a translational repressor, an activating immuno-receptor, an antibody, an apoptosis inhibitor, an

apoptosis inducer, an engineered T cell receptor, an immuno-activator, an immuno-inhibitor, or an inhibiting immuno-receptor.

93. A method for the treatment of a health condition in a subject in need thereof, the method comprising: administering to the subject a therapeutically effective amount of the recombinant cell of any one of claims 79 to claim 84 or the pharmaceutical composition of claim 85, wherein the recombinant cells or the pharmaceutical composition treat the health condition in the subject.

94. The method of claim 91, wherein the administered recombinant cell modulates an activity of a target cell in the individual.

95. The method of claim 92, wherein the activity of the target cell comprises expression of a selected gene involved in proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and cytolytic activity.

96. The method of claim 93, wherein the target cell is a cancer cell.

97. The method of claim 94, wherein the cancer cell is a solid tumor cell or a hematological malignancy cell.

98. The method of claim 95, wherein the hematological malignancy cell is a multiple myeloma cell.

99. A method for regulating a T cell activity, the method comprises:
(a) providing an effective amount of any of the recombinant cell of claims 79 to 84; and
(b) contacting the cell with a selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain induces cleavage of a ligand-inducible proteolytic cleavage site and (i) releases the transcriptional regulator, wherein the released transcriptional regulator modulates an activity of the recombinant cell; and simultaneously (ii) activates T cell signaling.

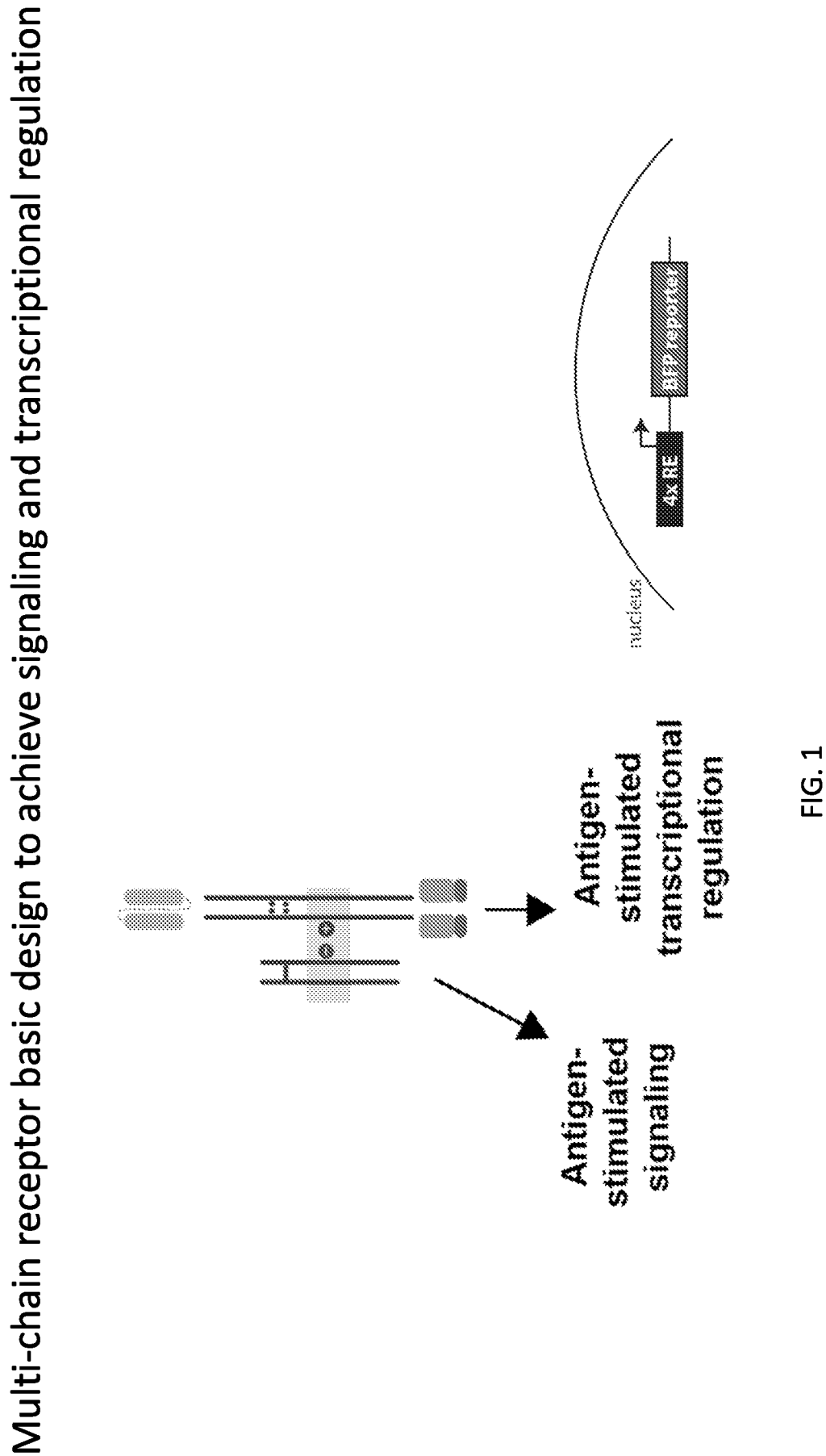

Multi-chain receptor basic design to achieve signaling and transcriptional regulation
Antigen-stimulated signaling
Antigen-stimulated transcriptional regulation
Nucleus
4x RE
GFP reporter

FIG. 1

Primary human CD3+ T cells

056

DAP12/TREM immunoreceptor

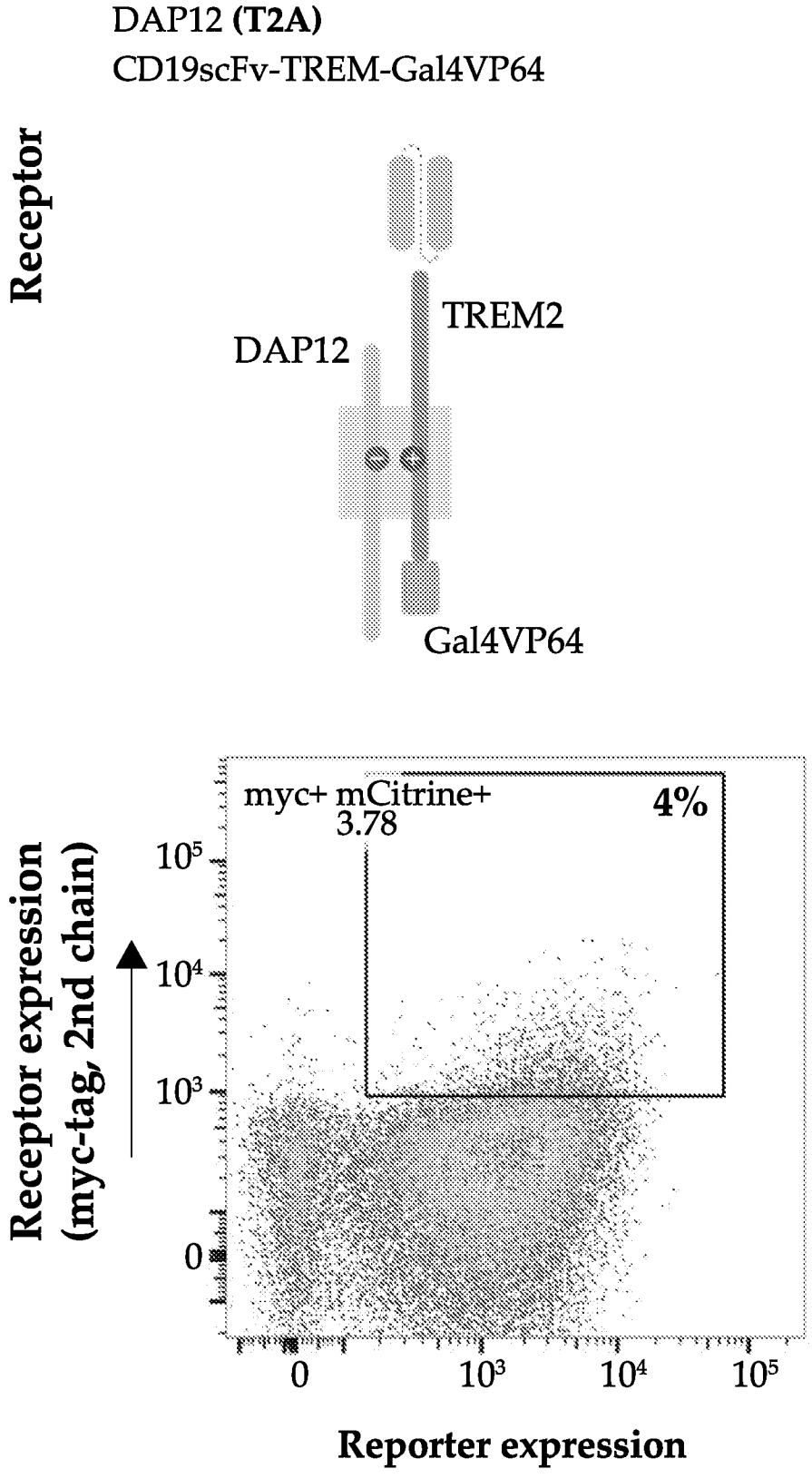
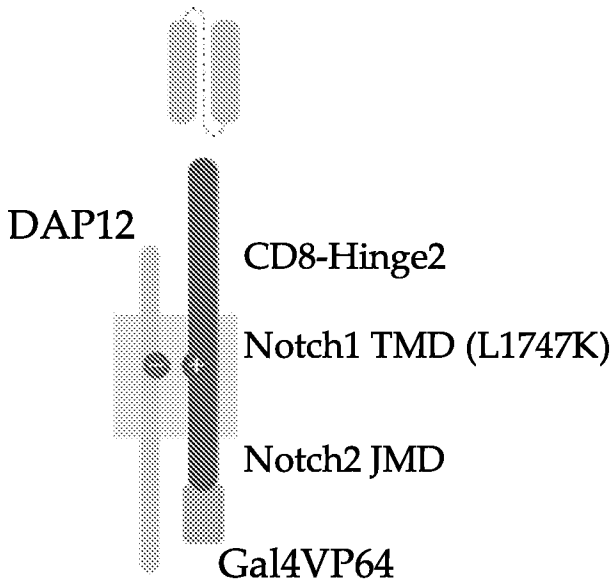


FIG. 2A

056C

DAP12/HingeNotch hybrid

DAP12 **(T2A)**
CD19scFv-CD8-hinge2-Notch1-L1747K-Gal4VP64



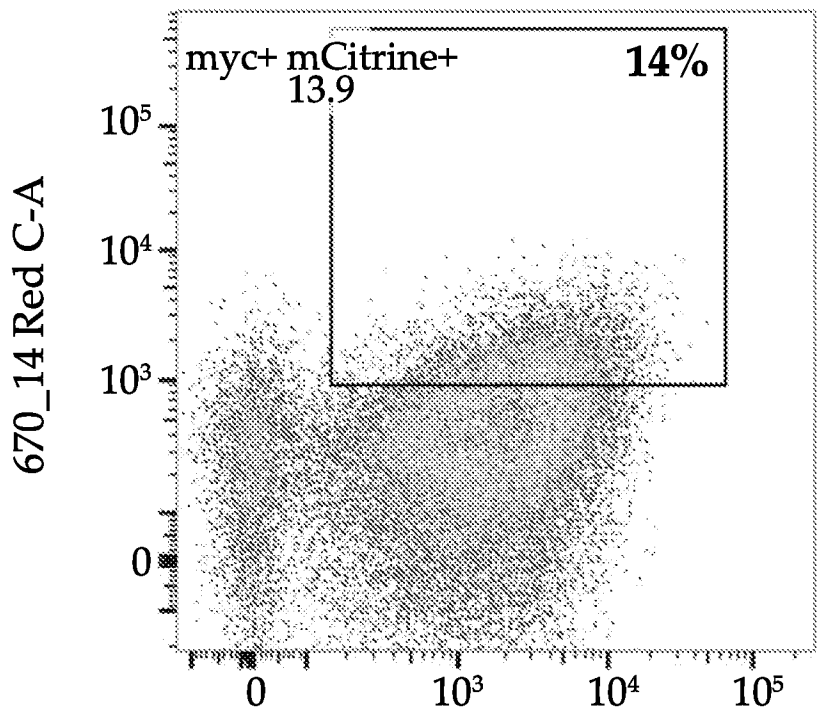
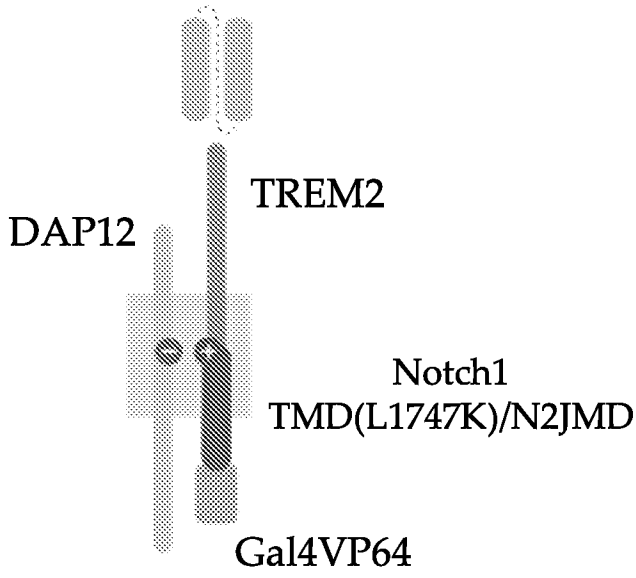


FIG. 2B

056D

DAP12/TREM-Notch hybrid1

DAP12 **(T2A)**
CD19scFv-TREM2-Notch1-L1747K-Gal4VP64



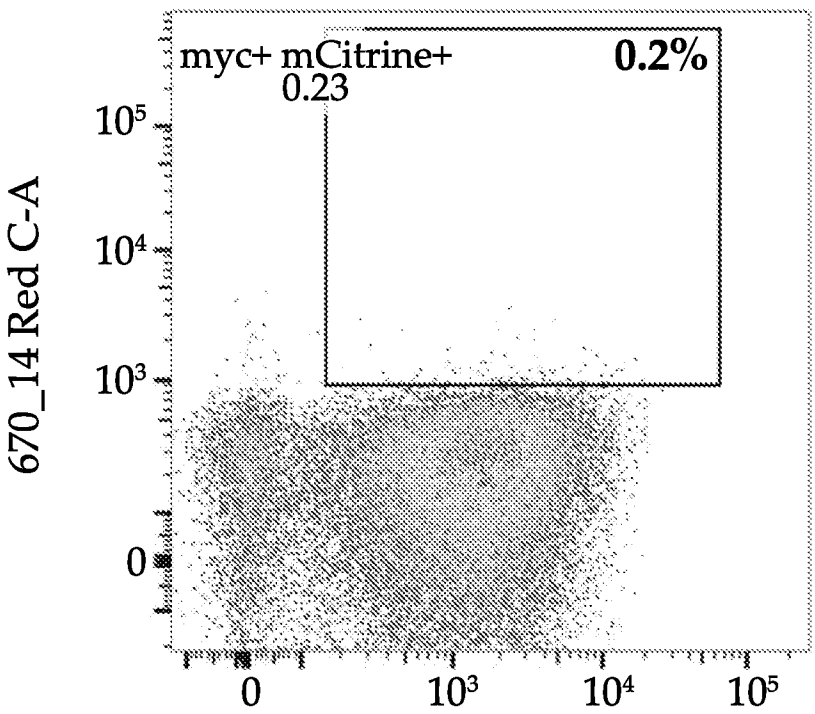
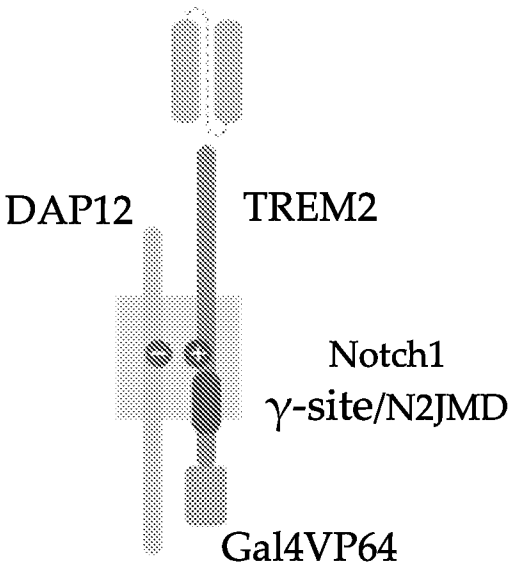


FIG. 2C

056B

DAP12/TREM-Notch hybrid2

DAP12 **(T2A)**
CD19scFv-TREM2-(Notch1insert)-TREM2-Gal4VP64



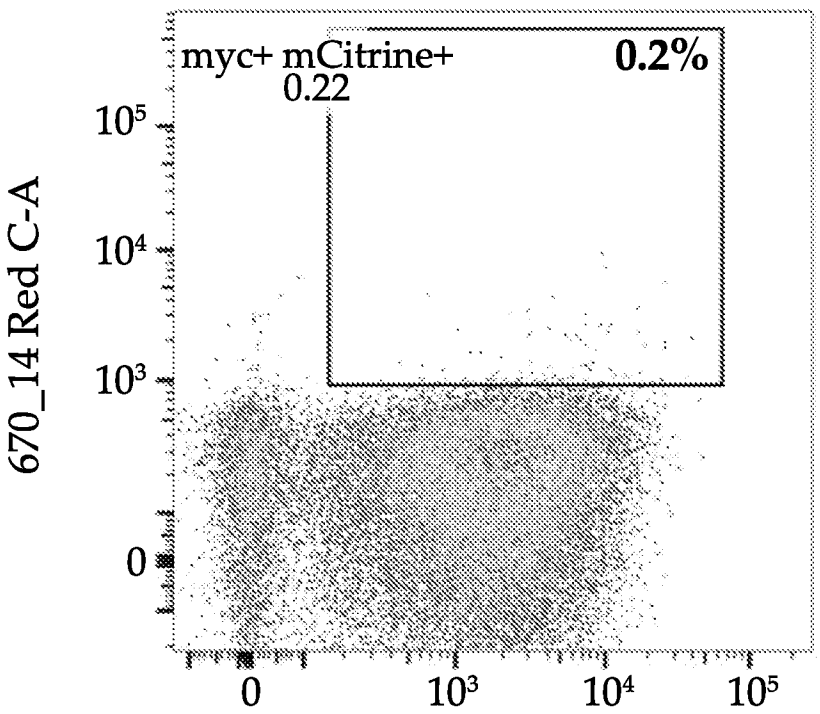


FIG. 2D

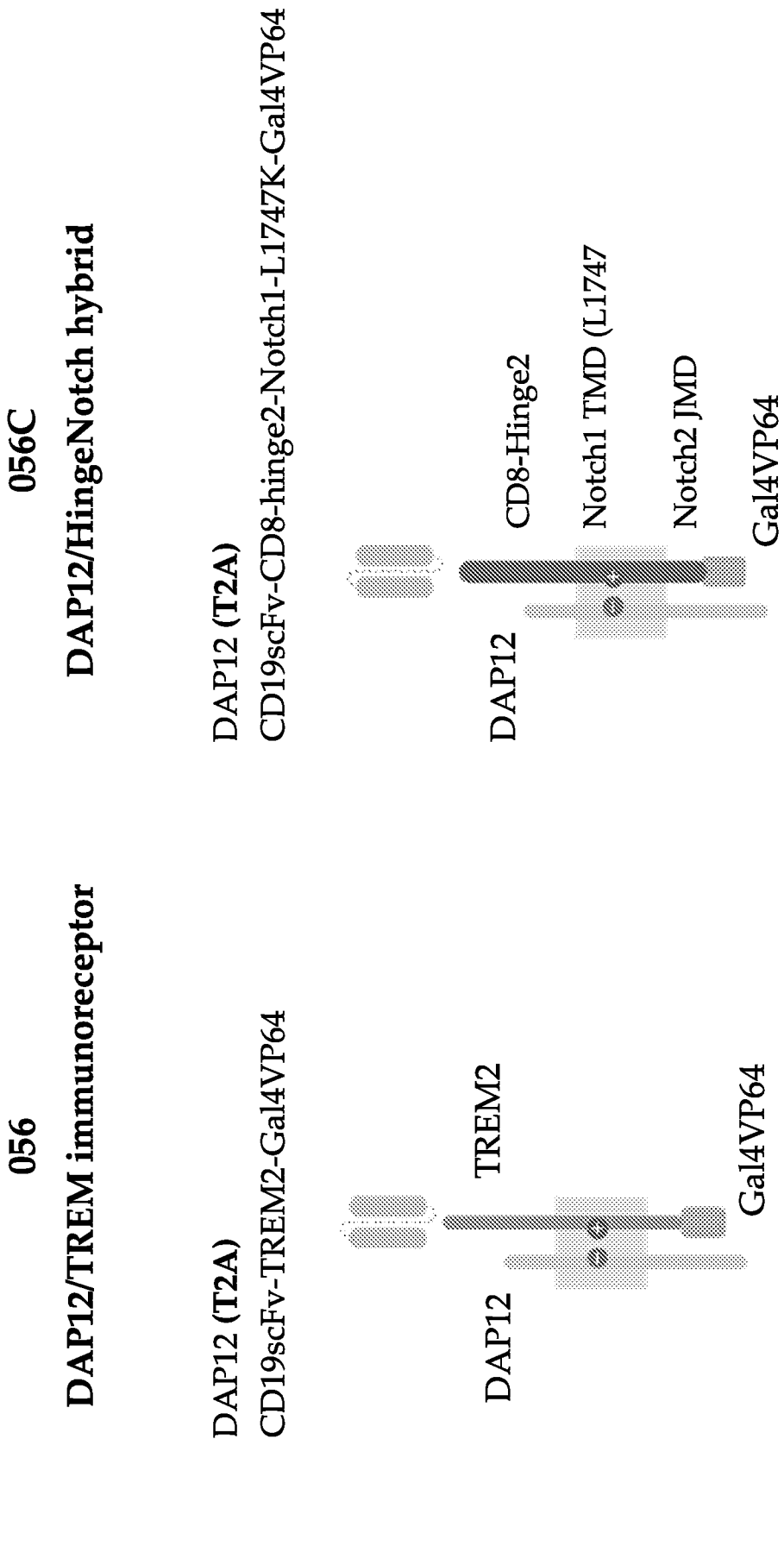

056
DAP12/TREM immunoreceptor
DAP12 (T2A)
CD19scFv-TREM2-Gal4VP64
DAP12
TREM2
Gal4VP64
056C
DAP12/HingeNotch hybrid
DAP12 (T2A)
CD19scFv-CD8-hinge2-Notch1-L1747K-Gal4VP64
DAP12
CD8-Hinge2
Notch1 TMD (L1747
Notch2 JMD
Gal4VP64
Receptor

FIG. 3A

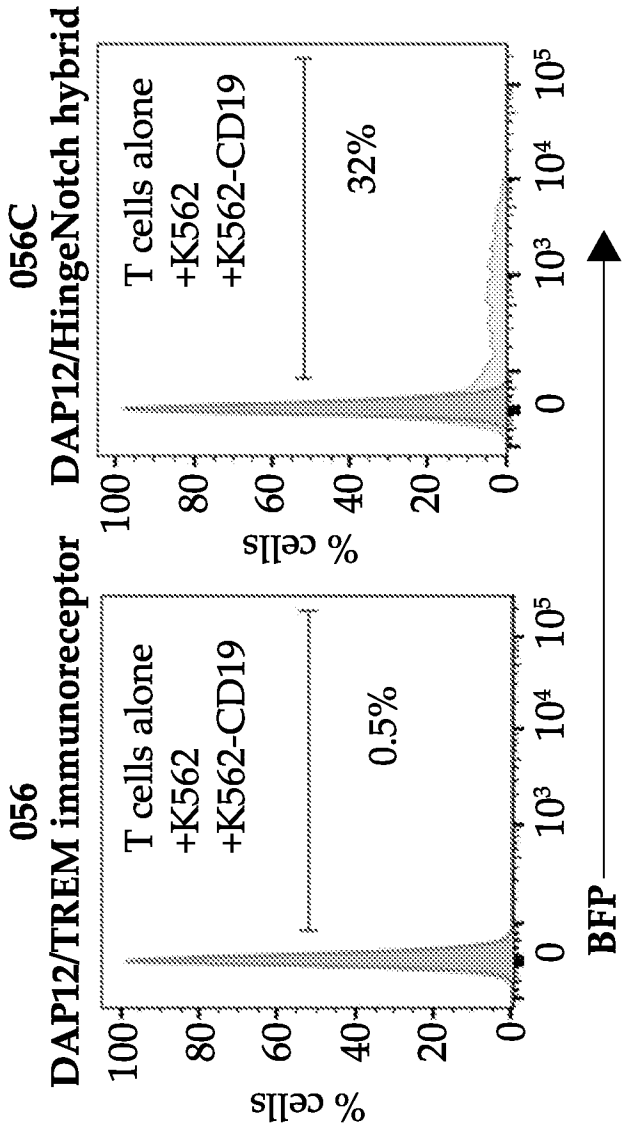

Transcriptional activation
(48 hours)
056
DAP12/TREM immunoreceptor
056C
DAP12/HingeNotch hybrid
% cells
T cells alone
+K562
+K562-CD19
0.5%
32%
BFP

FIG. 3B

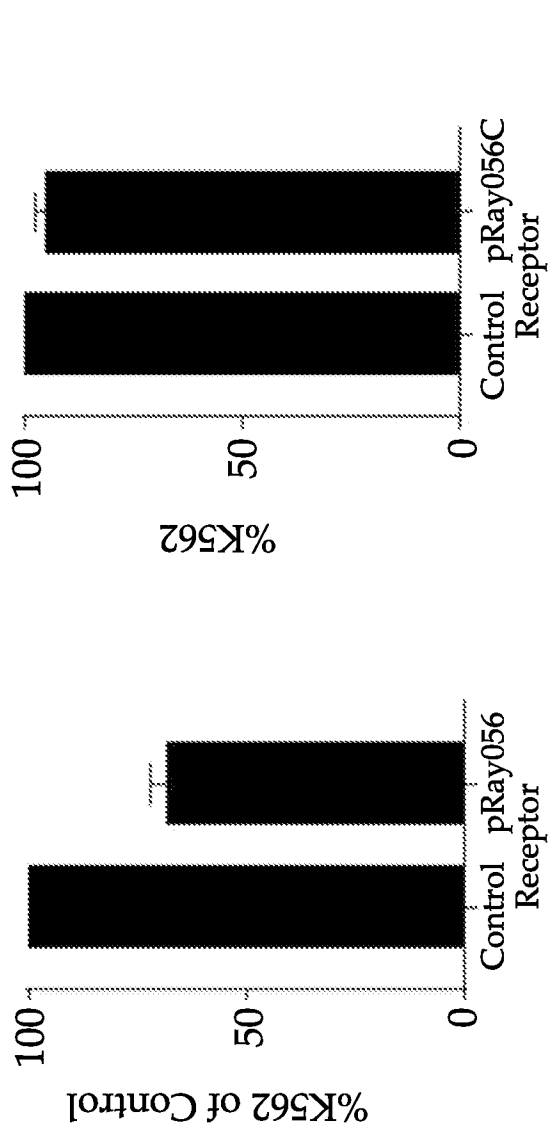

Killing activity
(48 hours)
%K562 of Control
%K562
Control
pRay056
pRay056C
Receptor

FIG. 3C

Primary human CD3+ T cells

056

DAP12/TREM immunoreceptor

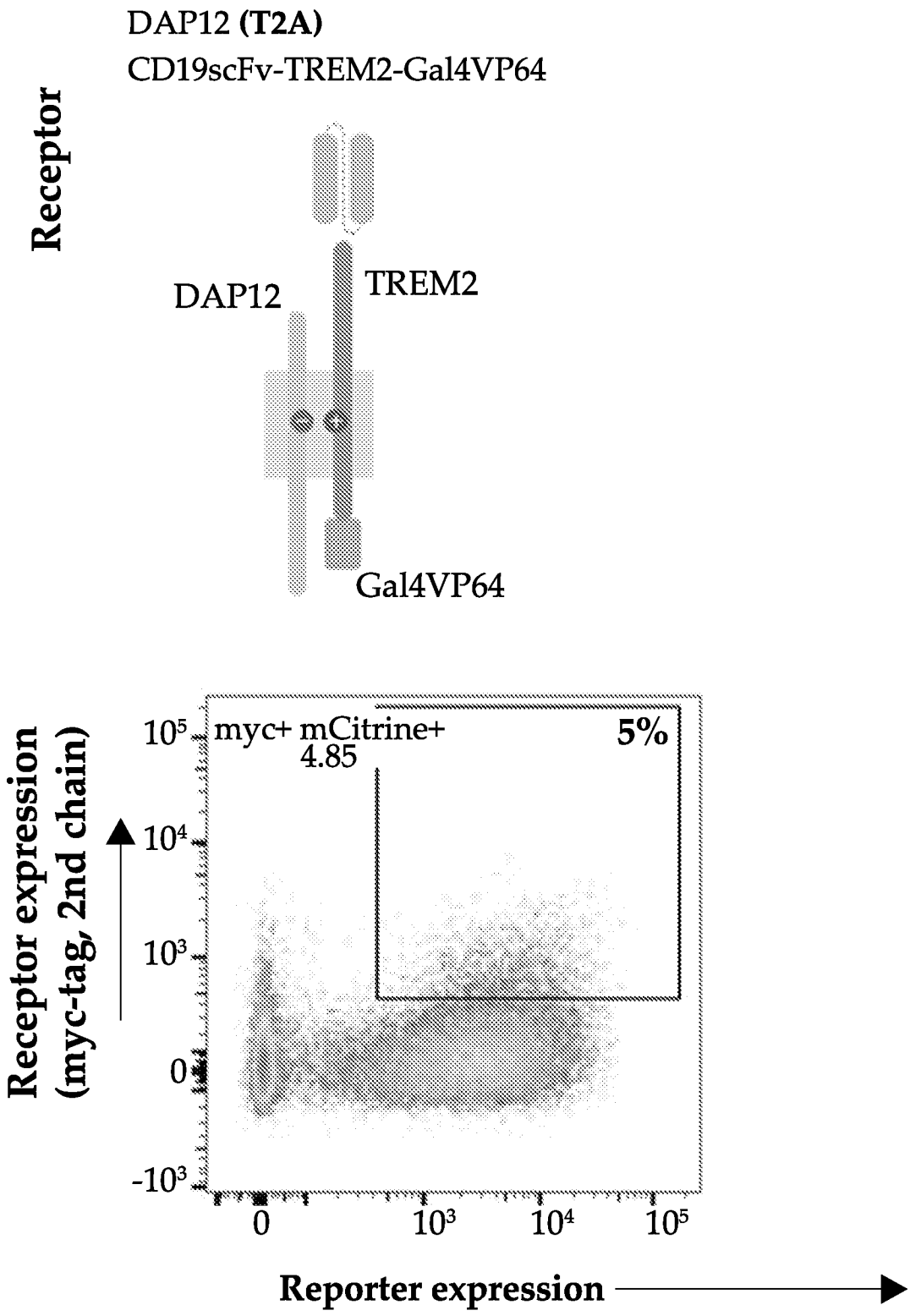


FIG. 4A

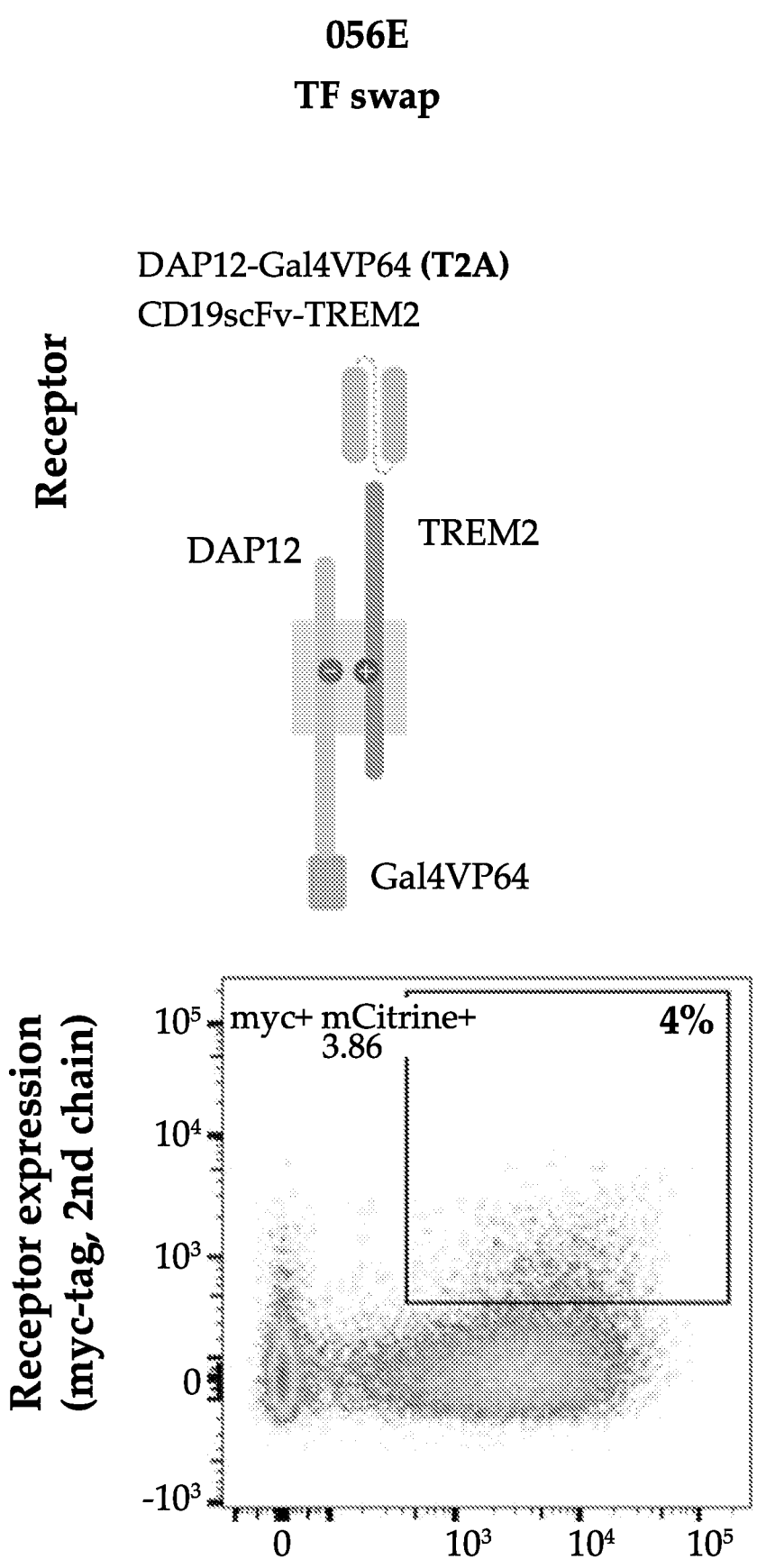
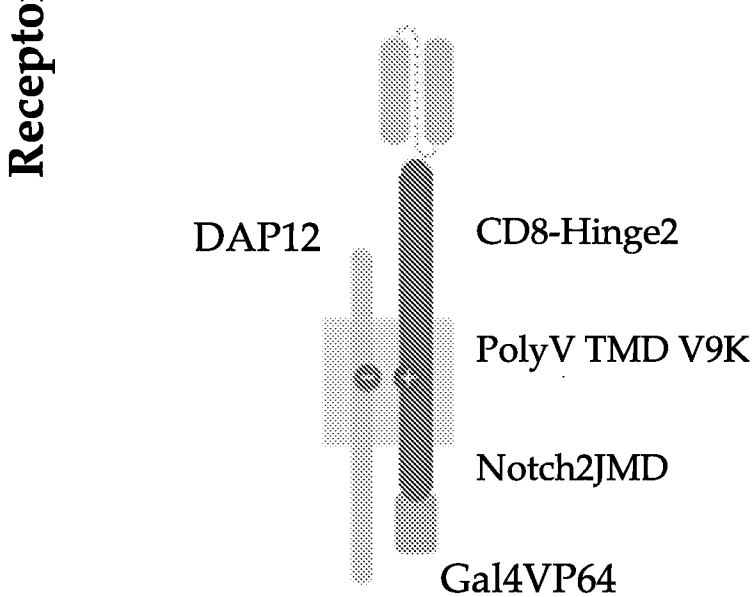

056E
TF swap
DAP12-Gal4VP64 (T2A)
CD19scFv-TREM2
Receptor
DAP12
TREM2
Gal4VP64
Receptor expression
(myc-tag, 2nd chain)
myc+ mCitrine+
3.86
4%
10^5
10^4
10^3
0
-10^3
0
10^3
10^4
10^5

FIG. 4B

056F

DAP12/Hinge-synTMD-Notch2JMD

DAP12 **(T2A)**
CD19scFv
CD8-Hinge2-PolyV-V9K-Notch2JMD
Gal4VP64



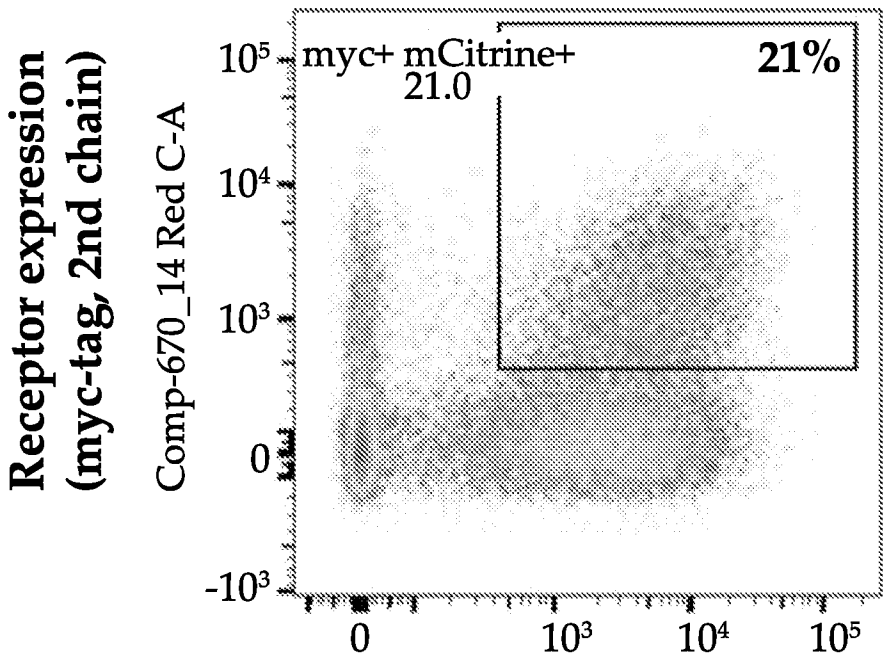


FIG. 4C

056G

DAP12/Hinge-synTMD-Notch2JMD

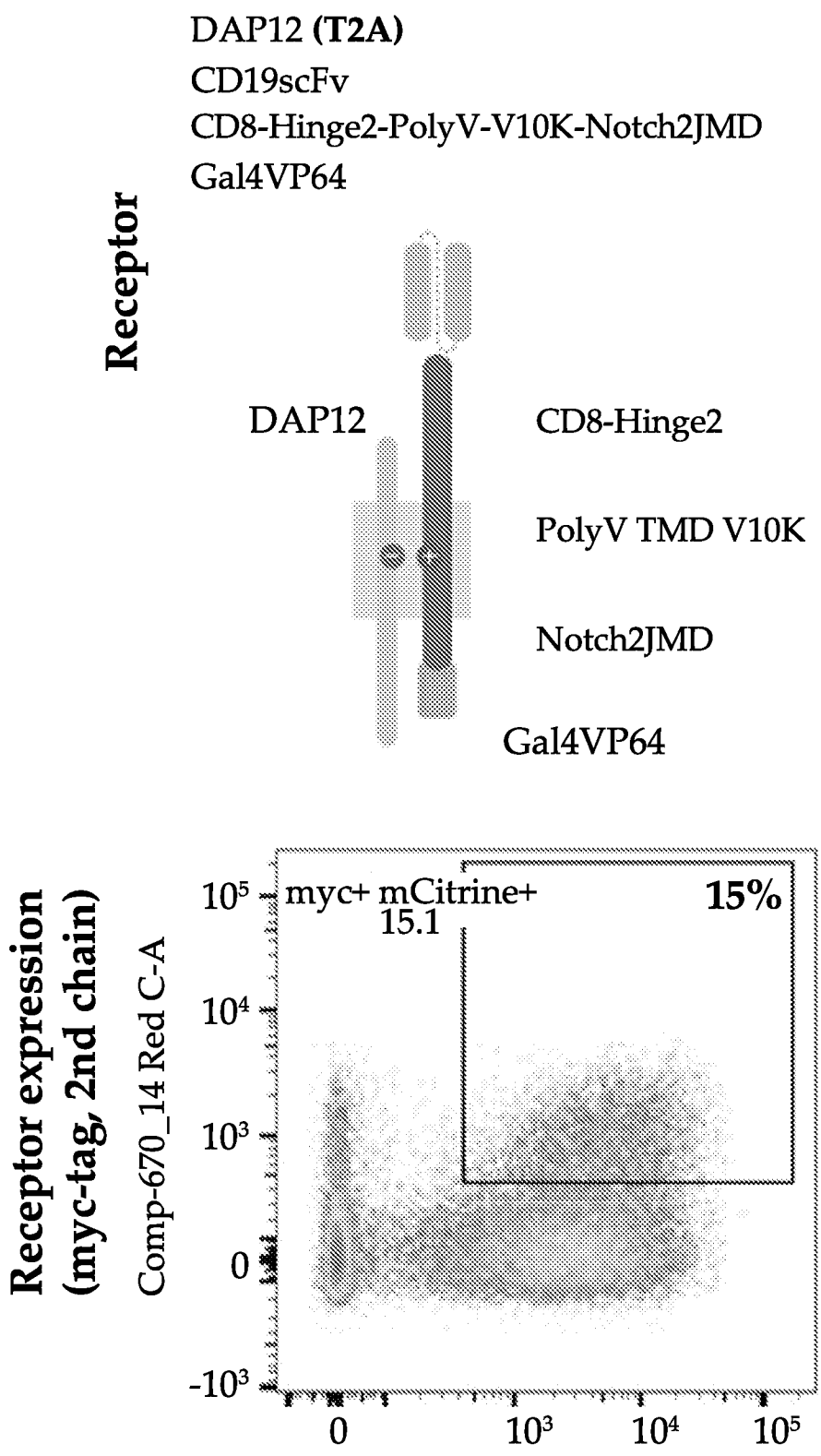


FIG. 4D

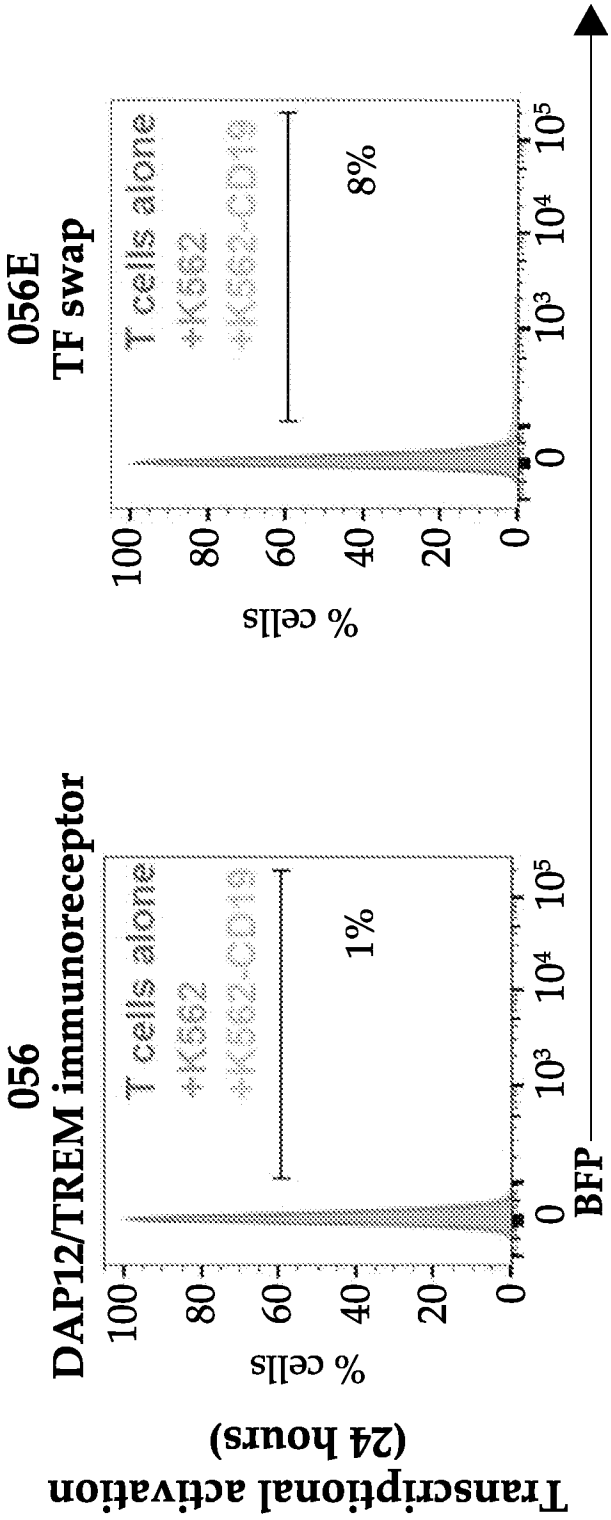

Transcriptional activation
(24 hours)
056
DAP12/TREM immunoreceptor
T cells alone
+K562
+K562-CD19
1%
% cells
BFP
056E
TF swap
T cells alone
+K562
+K562-CD19
8%
% cells

FIG. 5A

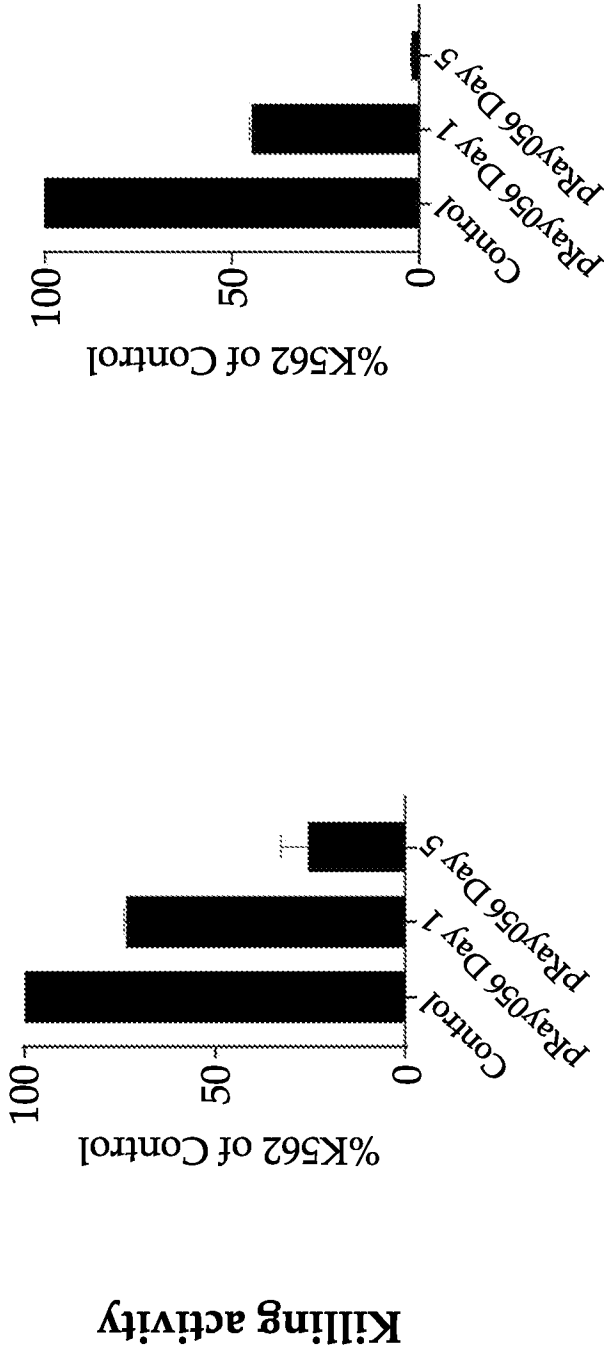

Killing activity
%K562 of Control
Control
PRay056 Day 1
PRay056 Day 5
%K562 of Control
Control
PRay056 Day 1
PRay056 Day 5

FIG. 5B

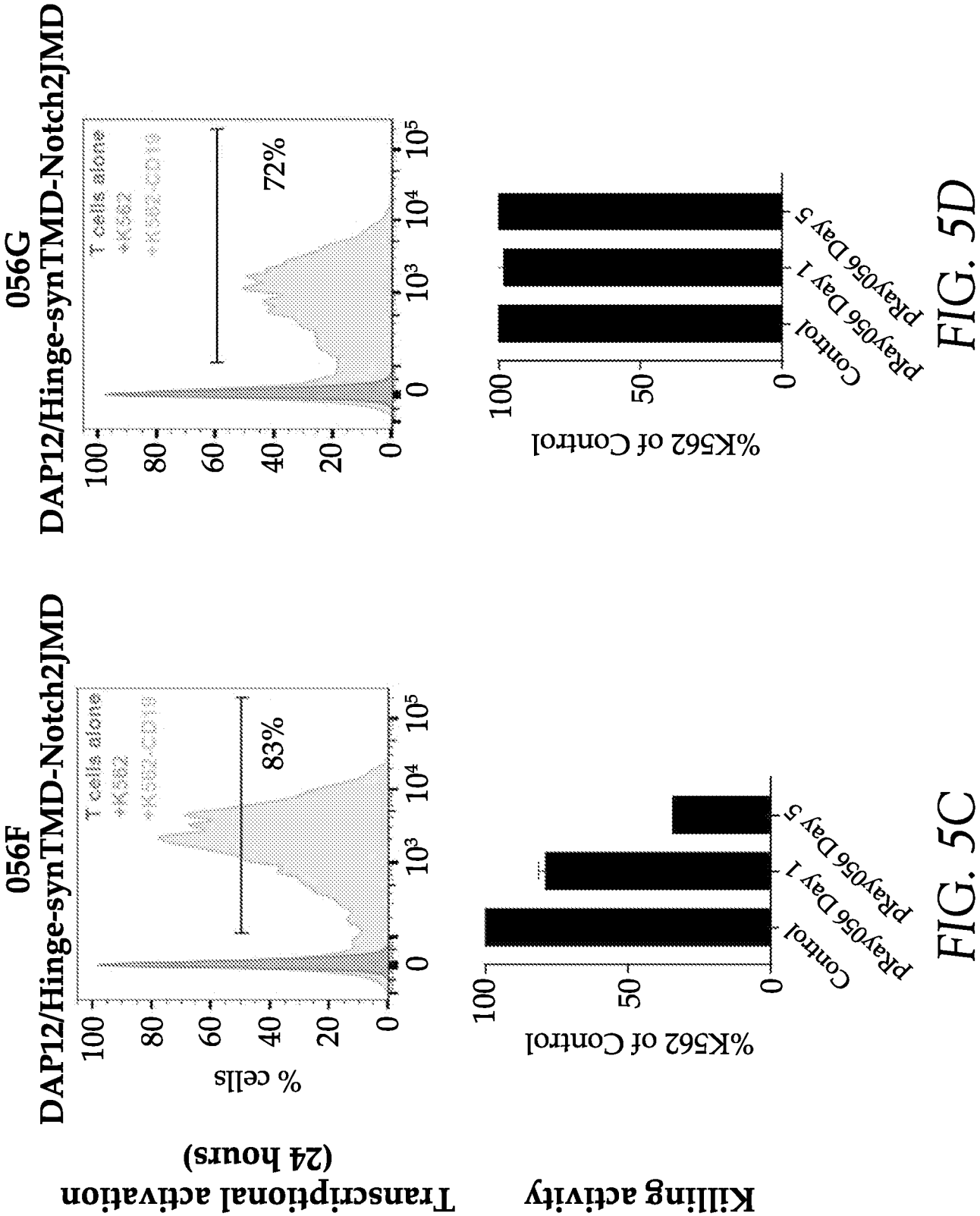

Transcriptional activation
(24 hours)
056F
DAP12/Hinge-synTMD-Notch2JMD
% cells
T cells alone
+K562
+K562-CD19
83%
056G
DAP12/Hinge-synTMD-Notch2JMD
72%
Killing activity
%K562 of Control
Control
PRay056 Day 1
PRay056 Day 5

FIG. 5C FIG. 5D

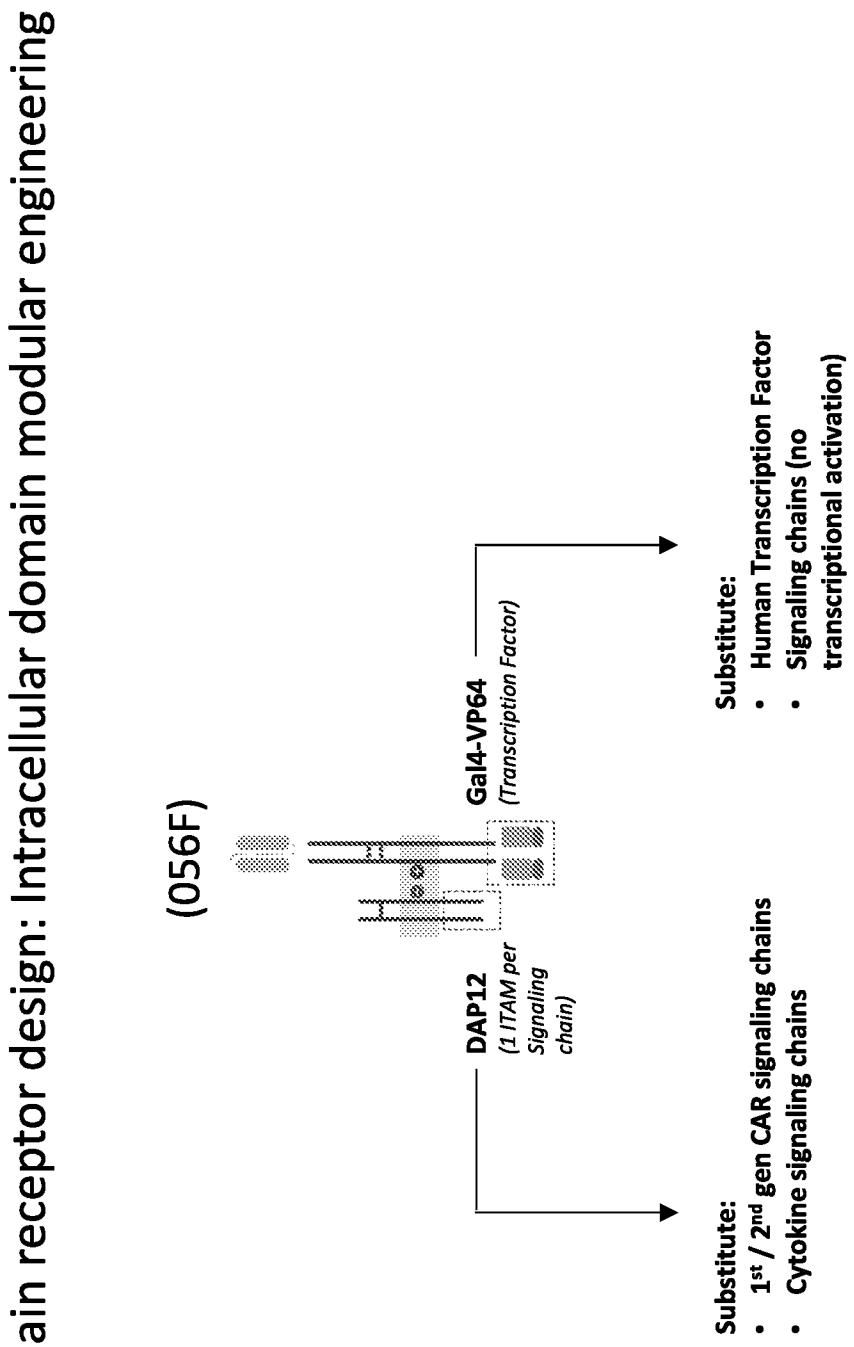

Multi-chain receptor design: Intracellular domain modular engineering
(056F)
Gal4-VP64
(Transcription Factor)
DAP12
(1 ITAM per Signaling chain)
Substitute:
• 1st / 2nd gen CAR signaling chains
• Cytokine signaling chains
Substitute:
• Human Transcription Factor
• Signaling chains (no transcriptional activation)

FIG. 6

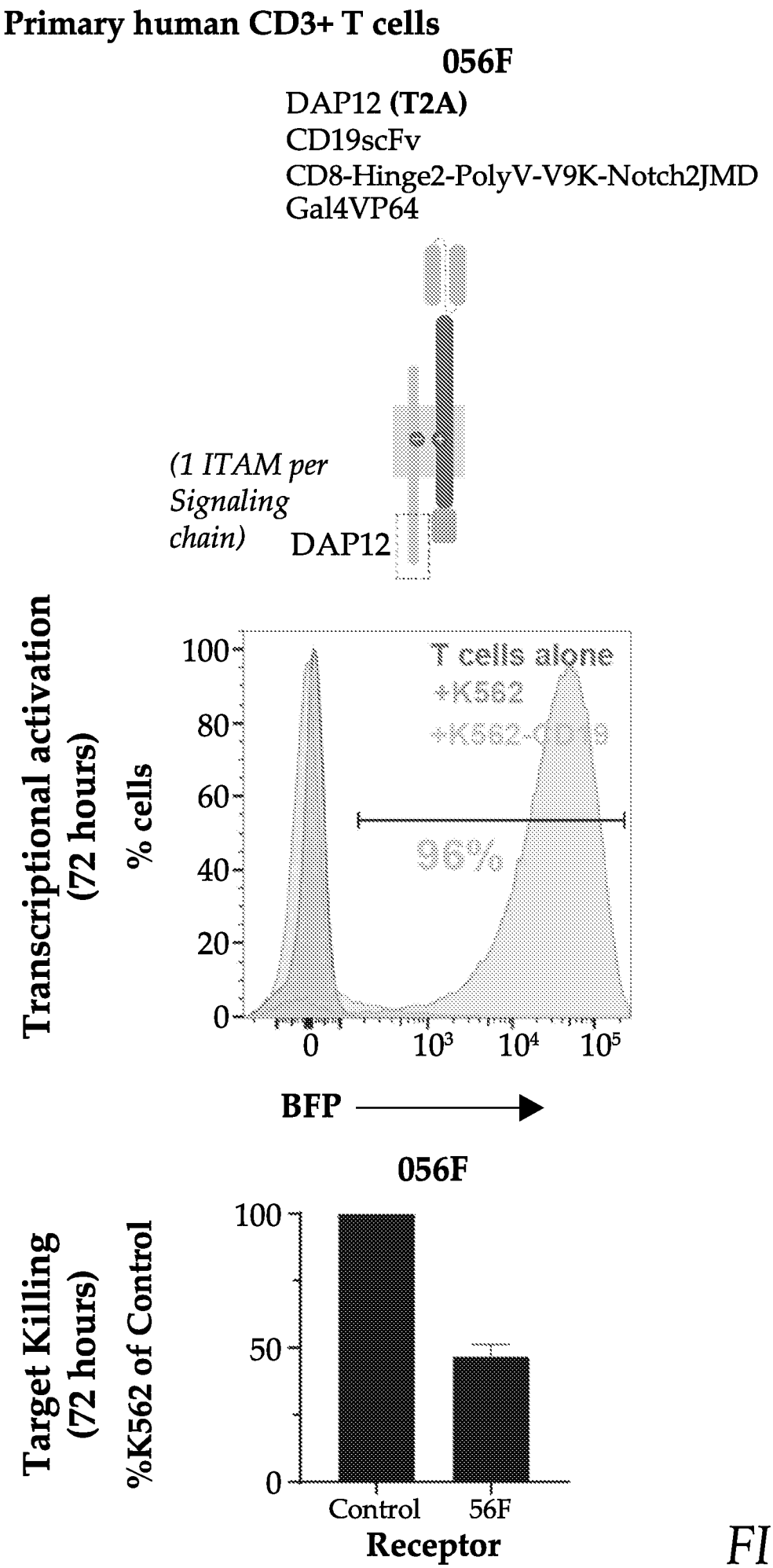

Primary human CD3+ T cells
056F
DAP12 (T2A)
CD19scFv
CD8-Hinge2-PolyV-V9K-Notch2JMD
Gal4VP64
(1 ITAM per Signaling chain)
DAP12
Transcriptional activation (72 hours)
% cells
T cells alone
+K562
96%
BFP
056F
Target Killing (72 hours)
%K562 of Control
Control
56F
Receptor

FIG. 7A

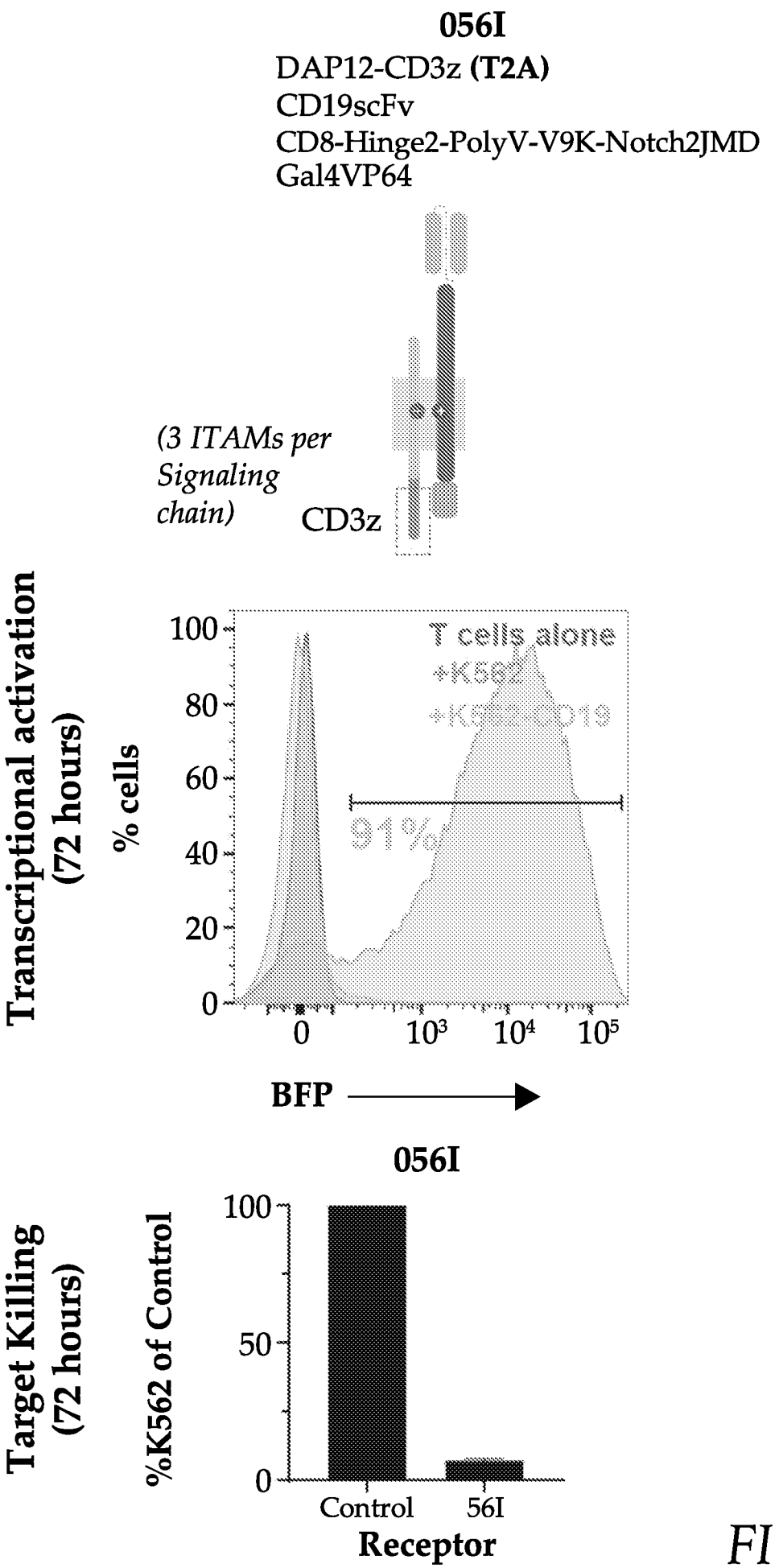

056I
DAP12-CD3z (T2A)
CD19scFv
CD8-Hinge2-PolyV-V9K-Notch2JMD
Gal4VP64
(3 ITAMs per Signaling chain)
CD3z
Transcriptional activation (72 hours)
% cells
T cells alone
+K562
+K562-CD19
91%
BFP
056I
Target Killing (72 hours)
%K562 of Control
Control
56I
Receptor

FIG. 7B

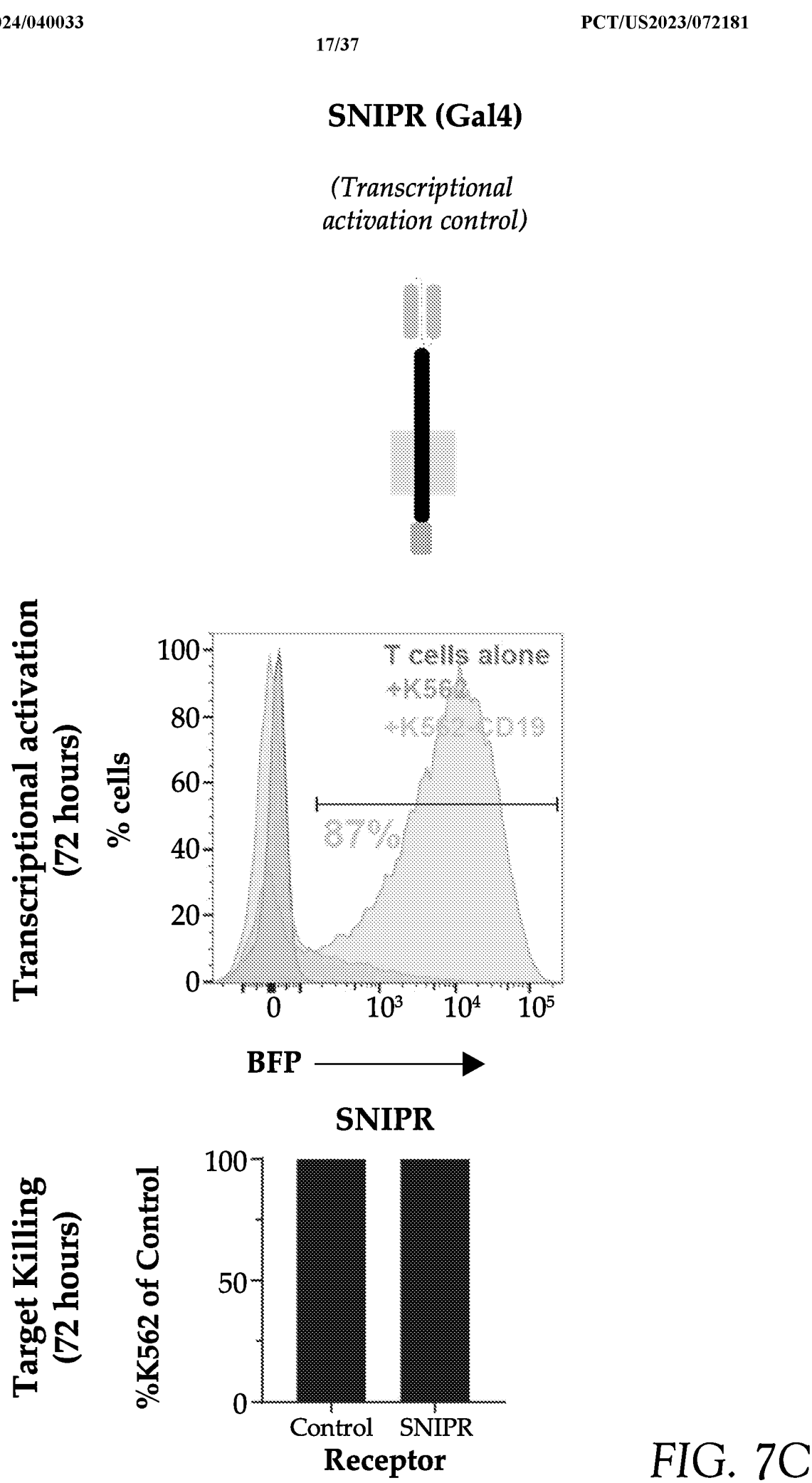

SNIPR (Gal4)
(Transcriptional activation control)
Transcriptional activation (72 hours)
% cells
100
80
60
40
20
0
T cells alone
+K562
+K562-CD19
87%
0
10³
10⁴
10⁵
BFP
SNIPR
Target Killing (72 hours)
%K562 of Control
100
50
0
Control
SNIPR
Receptor

FIG. 7C

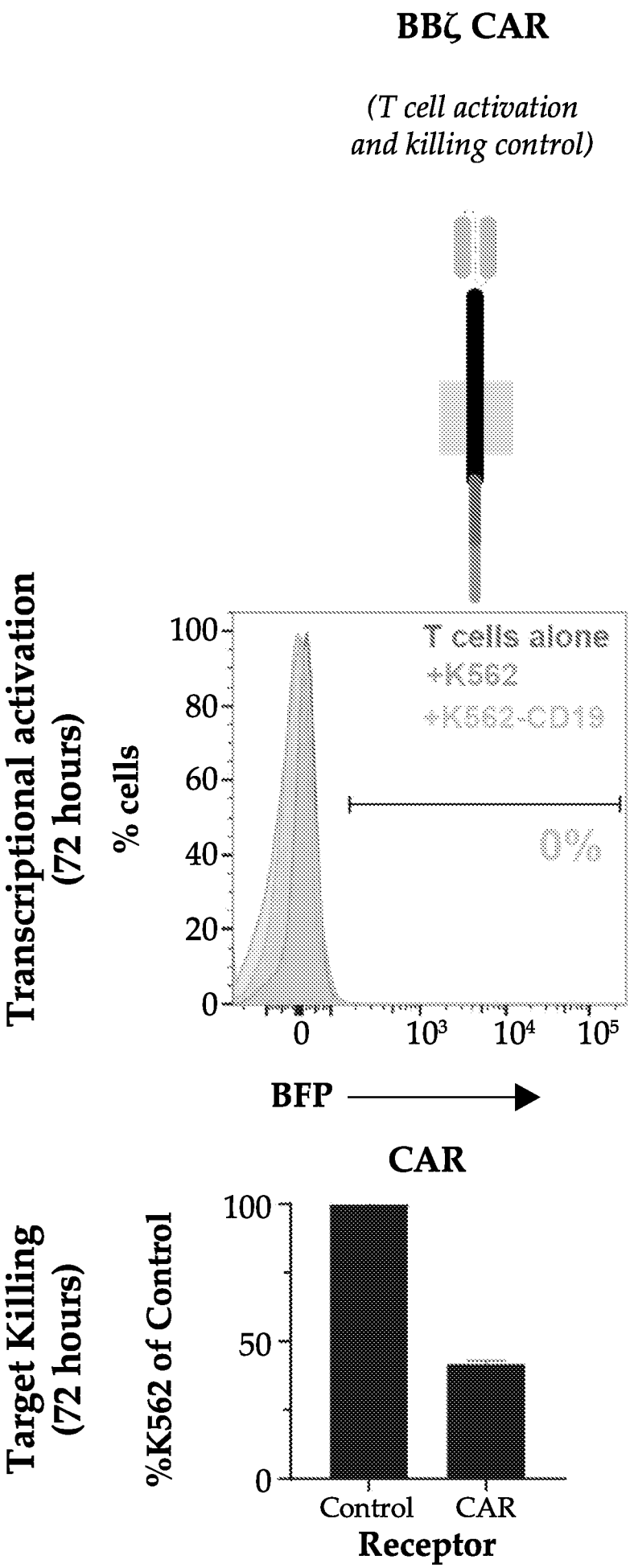

BBζ CAR
(T cell activation and killing control)
Transcriptional activation (72 hours)
% cells
100
80
60
40
20
0
T cells alone
+K562
+K562-CD19
0%
0
10³
10⁴
10⁵
BFP
CAR
Target Killing (72 hours)
%K562 of Control
100
50
0
Control
CAR
Receptor

FIG. 7D

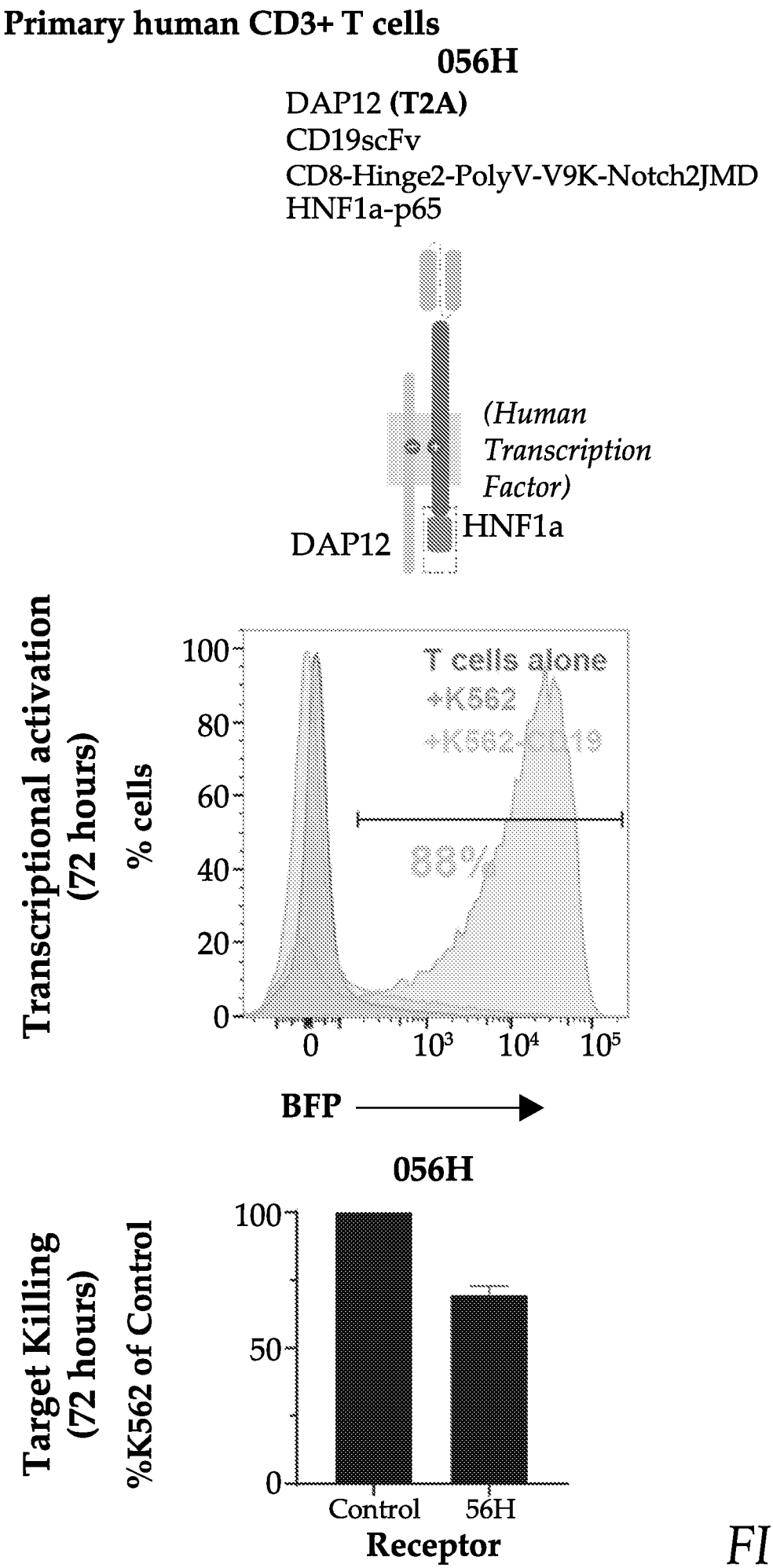

Primary human CD3+ T cells
056H
DAP12 (T2A)
CD19scFv
CD8-Hinge2-PolyV-V9K-Notch2JMD
HNF1a-p65
(Human Transcription Factor)
DAP12
HNF1a
Transcriptional activation (72 hours)
% cells
T cells alone
+K562
88%
BFP
056H
Target Killing (72 hours)
%K562 of Control
Control
56H
Receptor

FIG. 8A

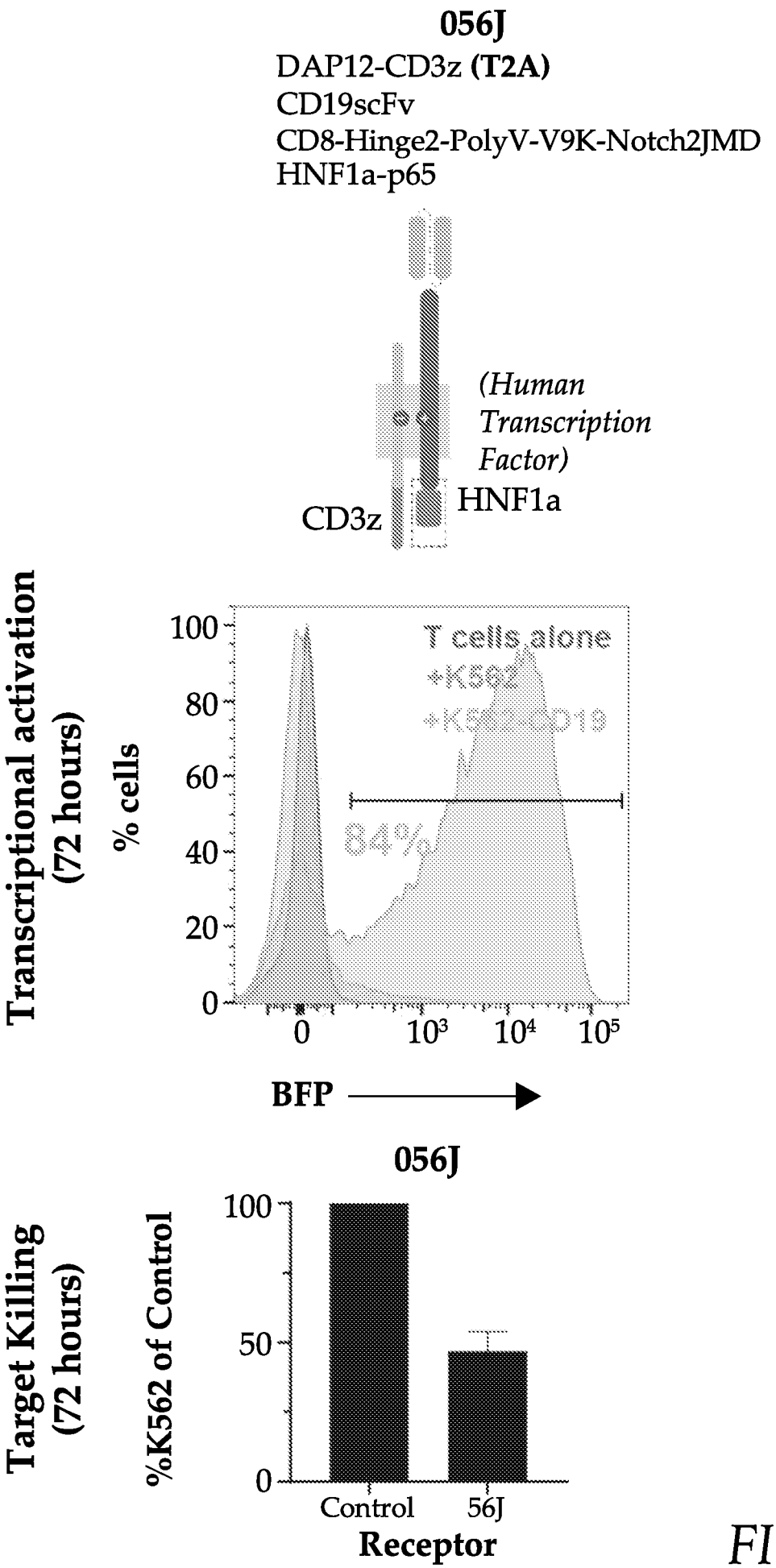

056J
DAP12-CD3z (T2A)
CD19scFv
CD8-Hinge2-PolyV-V9K-Notch2JMD
HNF1a-p65
(Human Transcription Factor)
CD3z
HNF1a
Transcriptional activation (72 hours)
% cells
T cells alone
+K562
84%
BFP
056J
Target Killing (72 hours)
%K562 of Control
Control
56J
Receptor

FIG. 8B

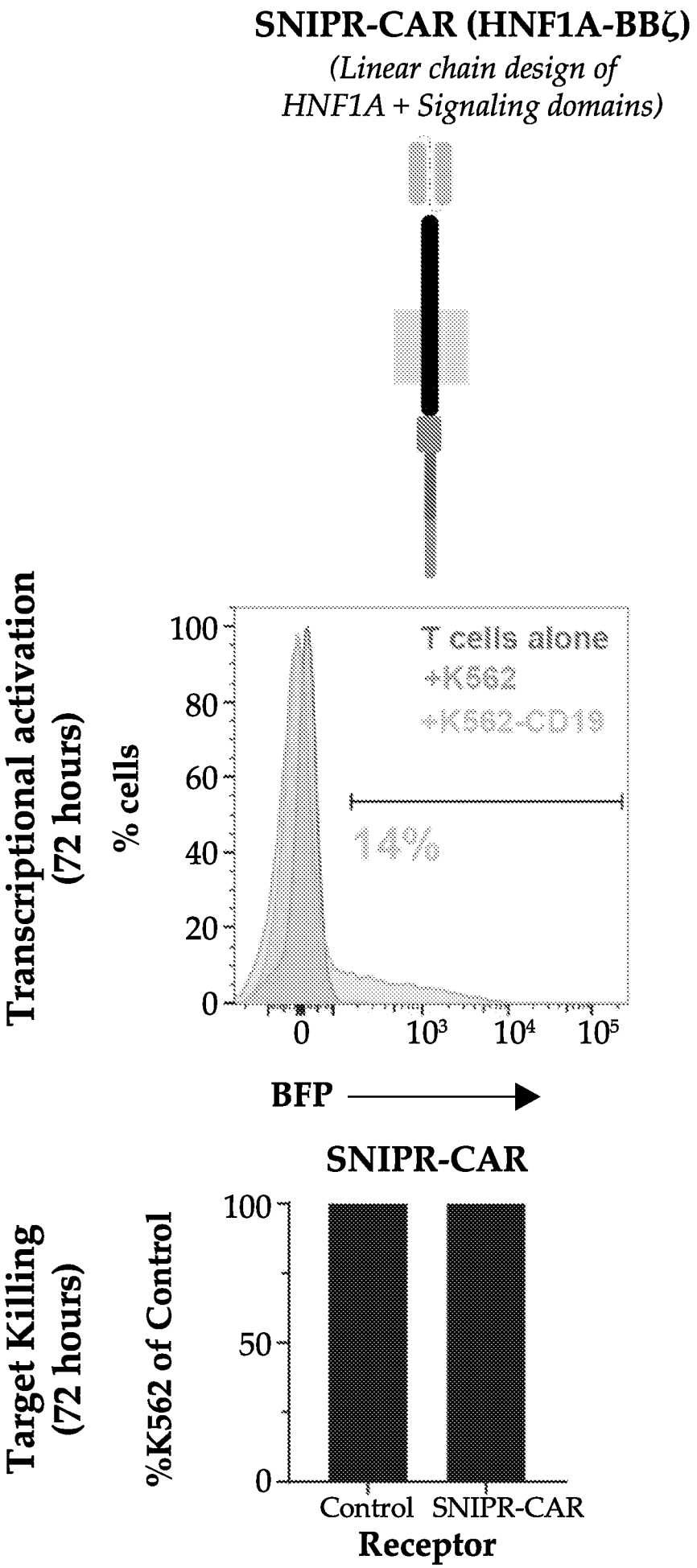

SNIPR-CAR (HNF1A-BBζ)
(Linear chain design of
HNF1A + Signaling domains)
Transcriptional activation
(72 hours)
% cells
100
80
60
40
20
0
T cells alone
+K562
+K562-CD19
14%
0
10³
10⁴
10⁵
BFP
SNIPR-CAR
Target Killing
(72 hours)
%K562 of Control
100
50
0
Control
SNIPR-CAR
Receptor

FIG. 8C

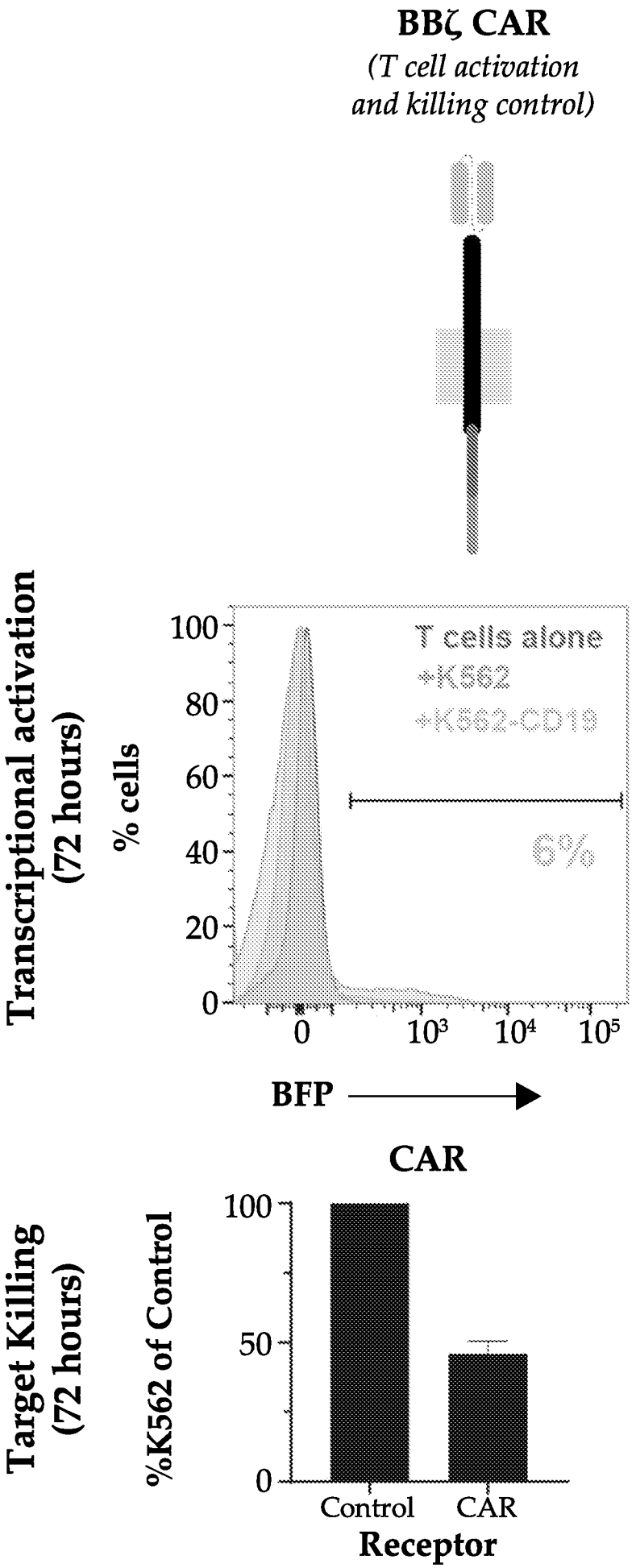

BBζ CAR
(T cell activation
and killing control)
Transcriptional activation
(72 hours)
% cells
100
80
60
40
20
0
T cells alone
+K562
+K562-CD19
6%
0
10³
10⁴
10⁵
BFP
CAR
Target Killing
(72 hours)
%K562 of Control
100
50
0
Control
CAR
Receptor

FIG. 8D

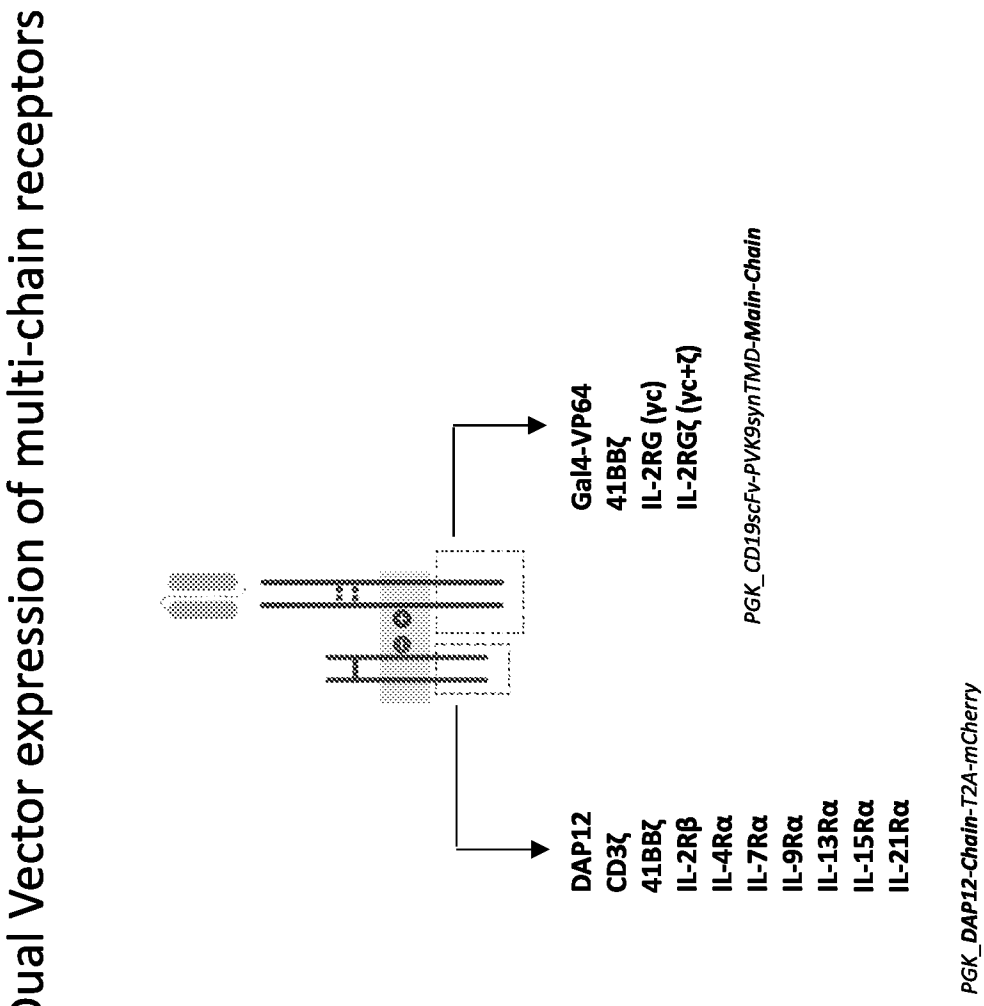

Dual Vector expression of multi-chain receptors
Gal4-VP64
41BBζ
IL-2RG (γc)
IL-2RGζ (γc+ζ)
PGK_CD19scFv-PVK9synTMD-Main-Chain
DAP12
CD3ζ
41BBζ
IL-2Rβ
IL-4Rα
IL-7Rα
IL-9Rα
IL-13Rα
IL-15Rα
IL-21Rα
PGK_DAP12-Chain-T2A-mCherry

FIG. 9

Primary human CD3+ T cells

Expression profile of multi-chain receptors with co-transduced component chains (dual transduction)

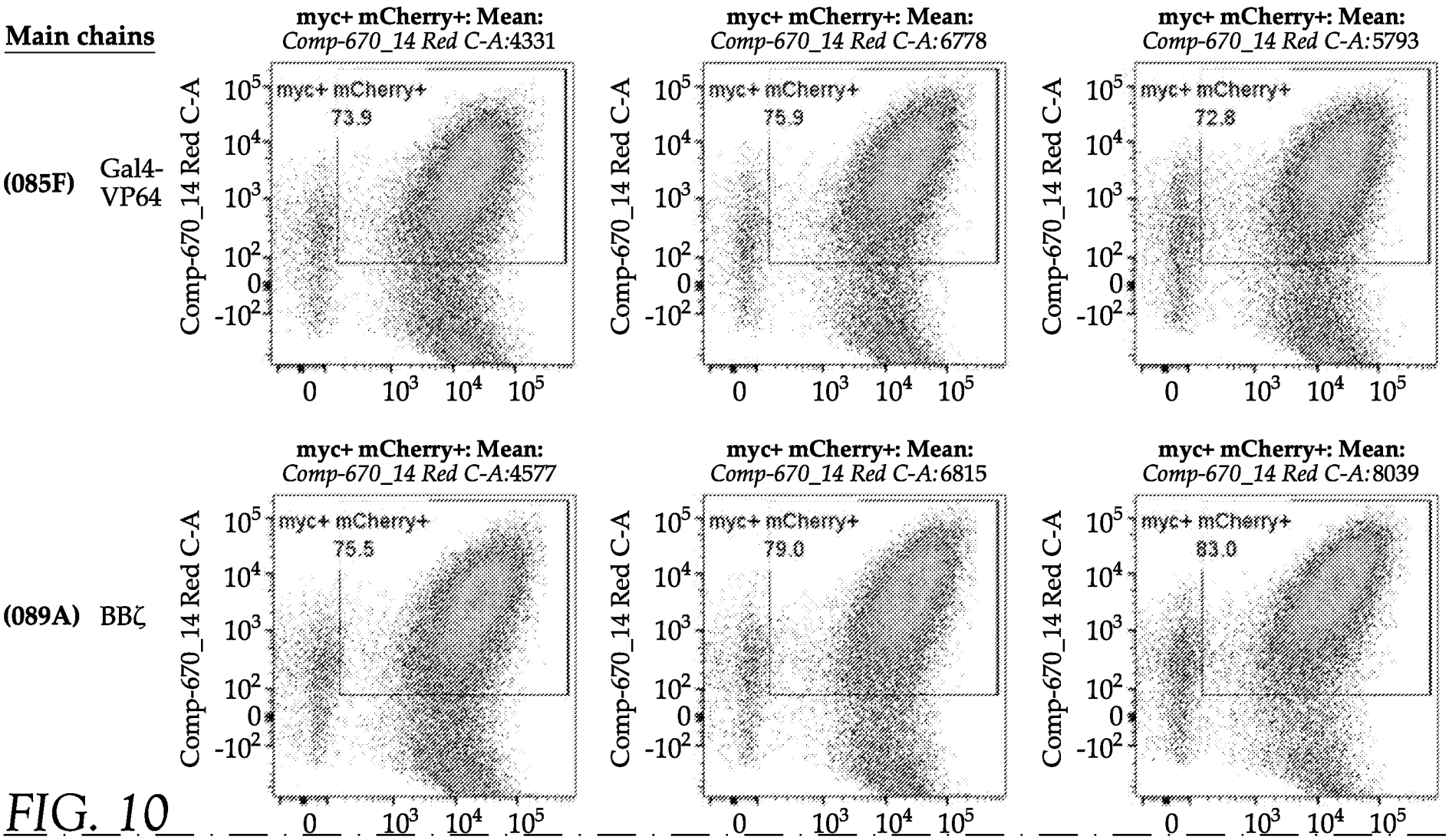


FIG. 10

SUBSTITUTE SHEET (RULE 26)

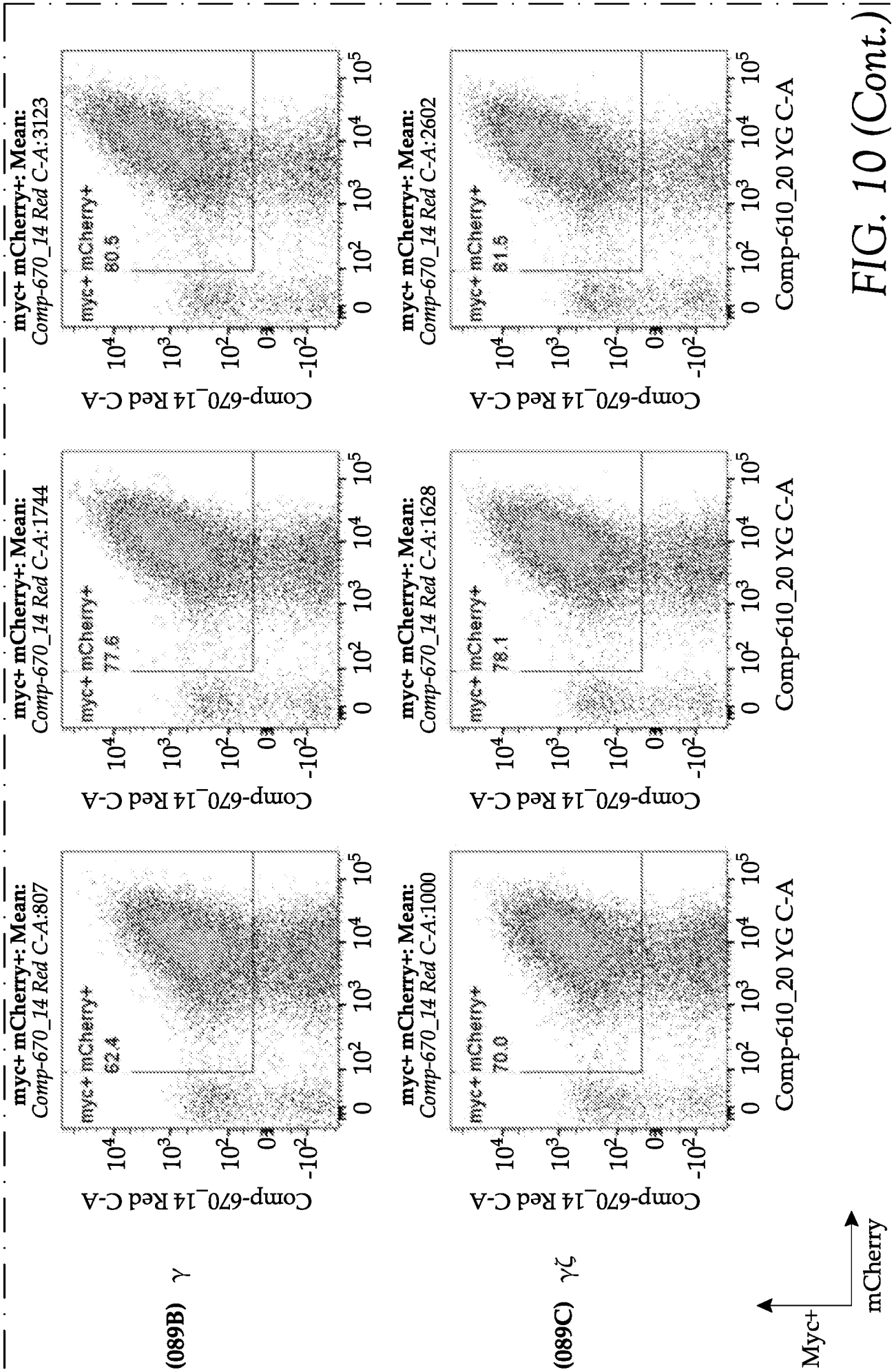
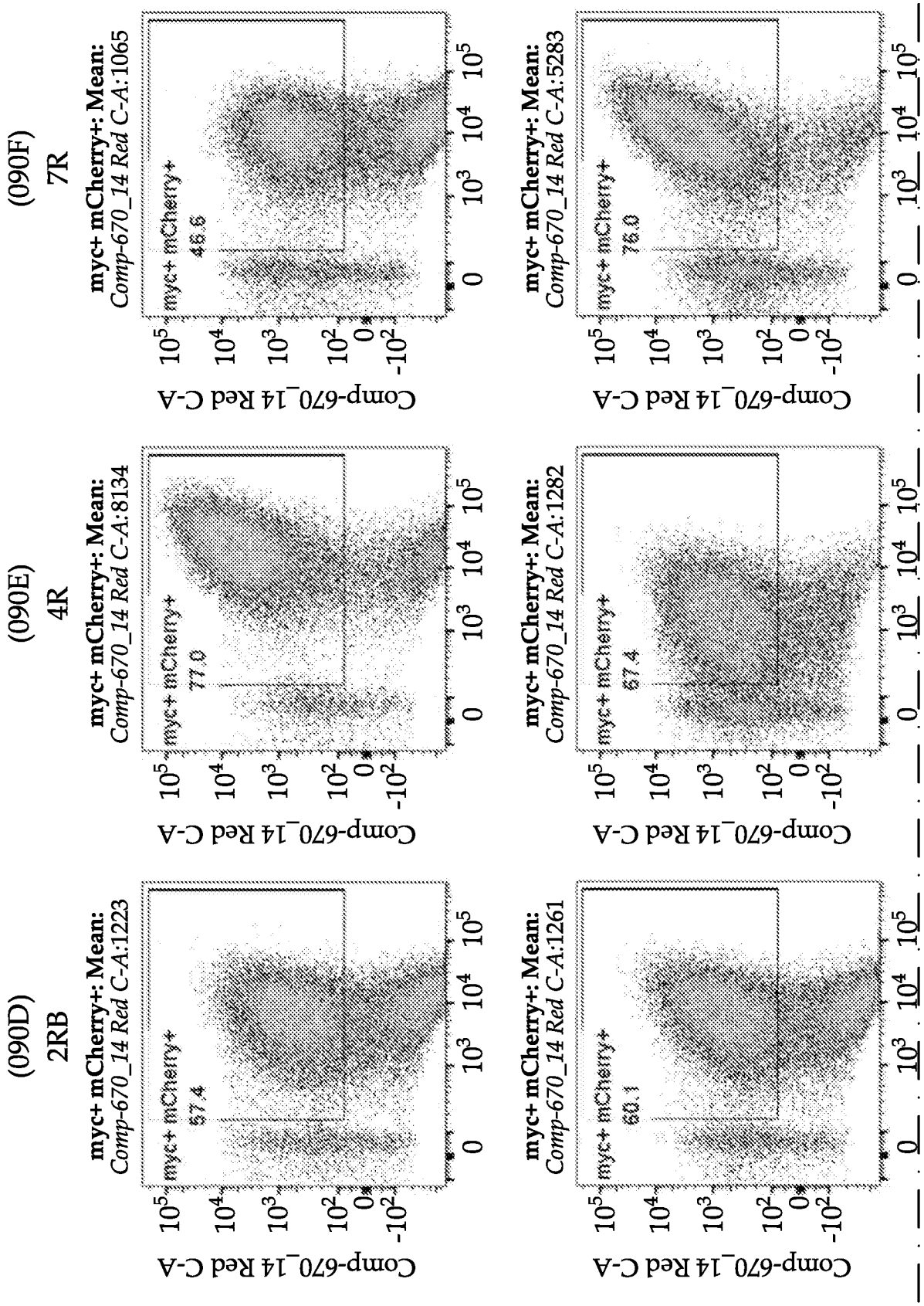

(089B) γ
(089C) γζ
myc+ mCherry+: Mean: Comp-670_14 Red C-A:807
myc+ mCherry+: Mean: Comp-670_14 Red C-A:1744
myc+ mCherry+: Mean: Comp-670_14 Red C-A:3123
myc+ mCherry+: Mean: Comp-670_14 Red C-A:1000
myc+ mCherry+: Mean: Comp-670_14 Red C-A:1628
myc+ mCherry+: Mean: Comp-670_14 Red C-A:2602
myc+ mCherry+
62.4
77.6
80.5
70.0
78.1
81.5
Comp-670_14 Red C-A
Comp-610_20 YG C-A
Myc+
mCherry

FIG. 10 (Cont.)

FIG. 10 (Cont.)


(090D)
2RB
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1223
myc+ mCherry+
67.4
(090E)
4R
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:8134
myc+ mCherry+
77.0
(090F)
7R
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1065
myc+ mCherry+
46.6
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1261
myc+ mCherry+
60.1
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1282
myc+ mCherry+
67.4
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:5283
myc+ mCherry+
76.0
Comp-670_14 Red C-A
0
10^3
10^4
10^5
10^2
-10^2

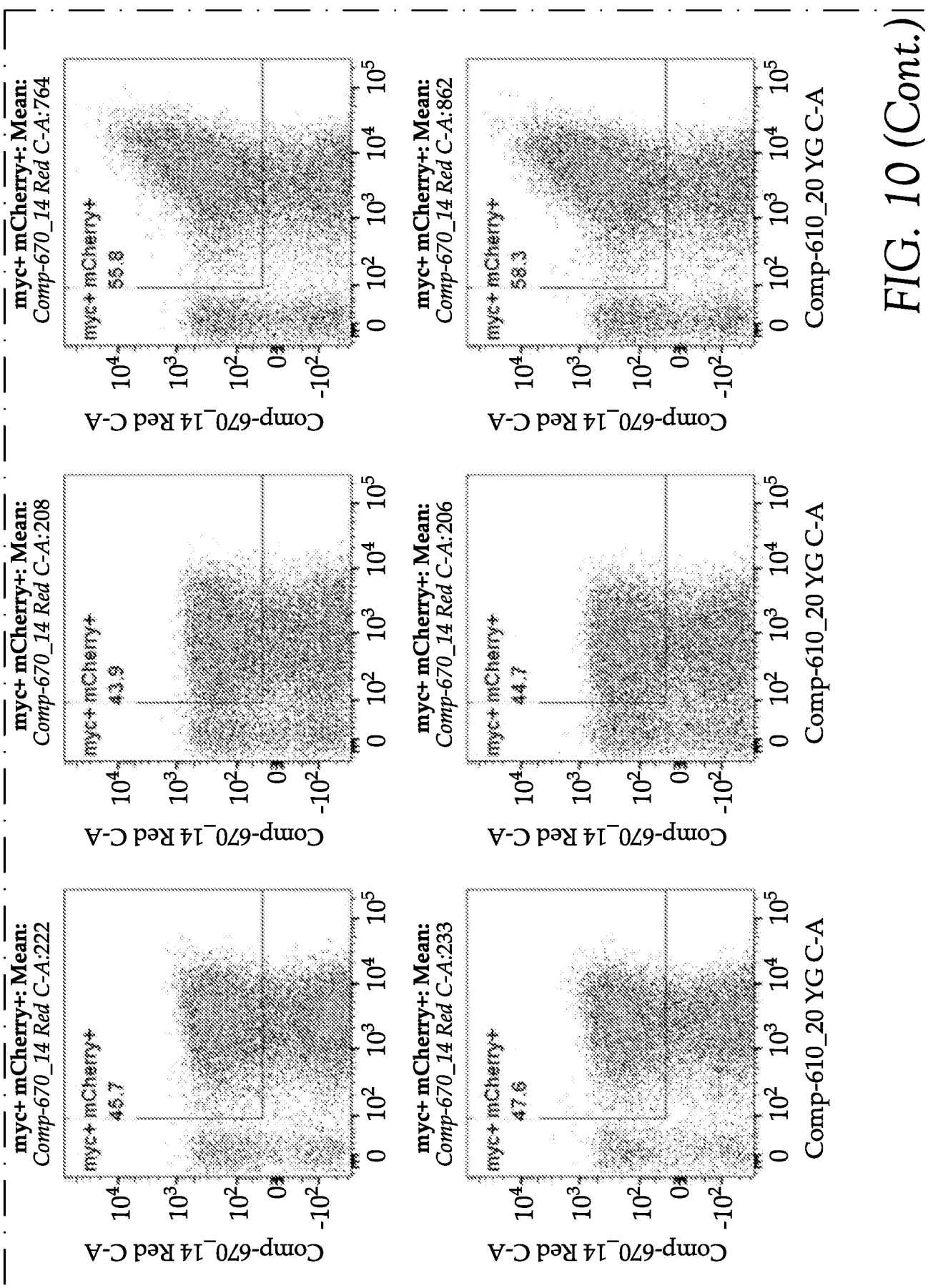
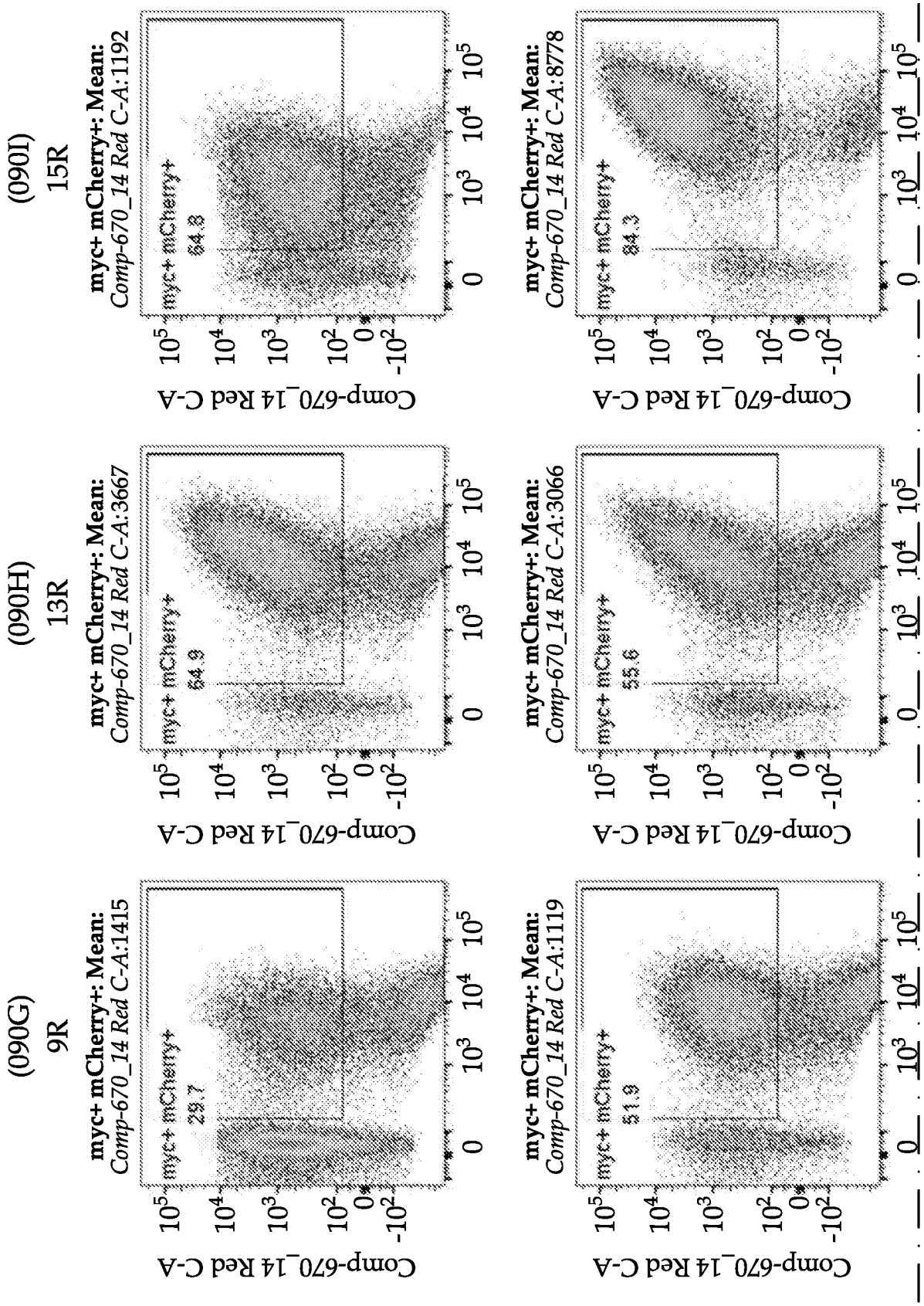

myc+ mCherry+: Mean: Comp-670_14 Red C-A:764
myc+ mCherry+
55.8
myc+ mCherry+: Mean: Comp-670_14 Red C-A:862
myc+ mCherry+
58.3
myc+ mCherry+: Mean: Comp-670_14 Red C-A:208
myc+ mCherry+
43.9
myc+ mCherry+: Mean: Comp-670_14 Red C-A:206
myc+ mCherry+
44.7
myc+ mCherry+: Mean: Comp-670_14 Red C-A:222
myc+ mCherry+
45.7
myc+ mCherry+: Mean: Comp-670_14 Red C-A:233
myc+ mCherry+
47.6
Comp-670_14 Red C-A
Comp-610_20 YG C-A
0
10^2
10^3
10^4
10^5
-10^2

FIG. 10 (Cont.)

FIG. 10 (Cont.)


(090G)
9R
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1415
myc+ mCherry+
29.7
(090H)
13R
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:3667
myc+ mCherry+
64.9
(090I)
15R
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1192
myc+ mCherry+
64.8
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1119
myc+ mCherry+
51.9
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:3066
myc+ mCherry+
55.6
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:8778
myc+ mCherry+
84.3
Comp-670_14 Red C-A
SSC-A
0
10^3
10^4
10^5
-10^2
10^2

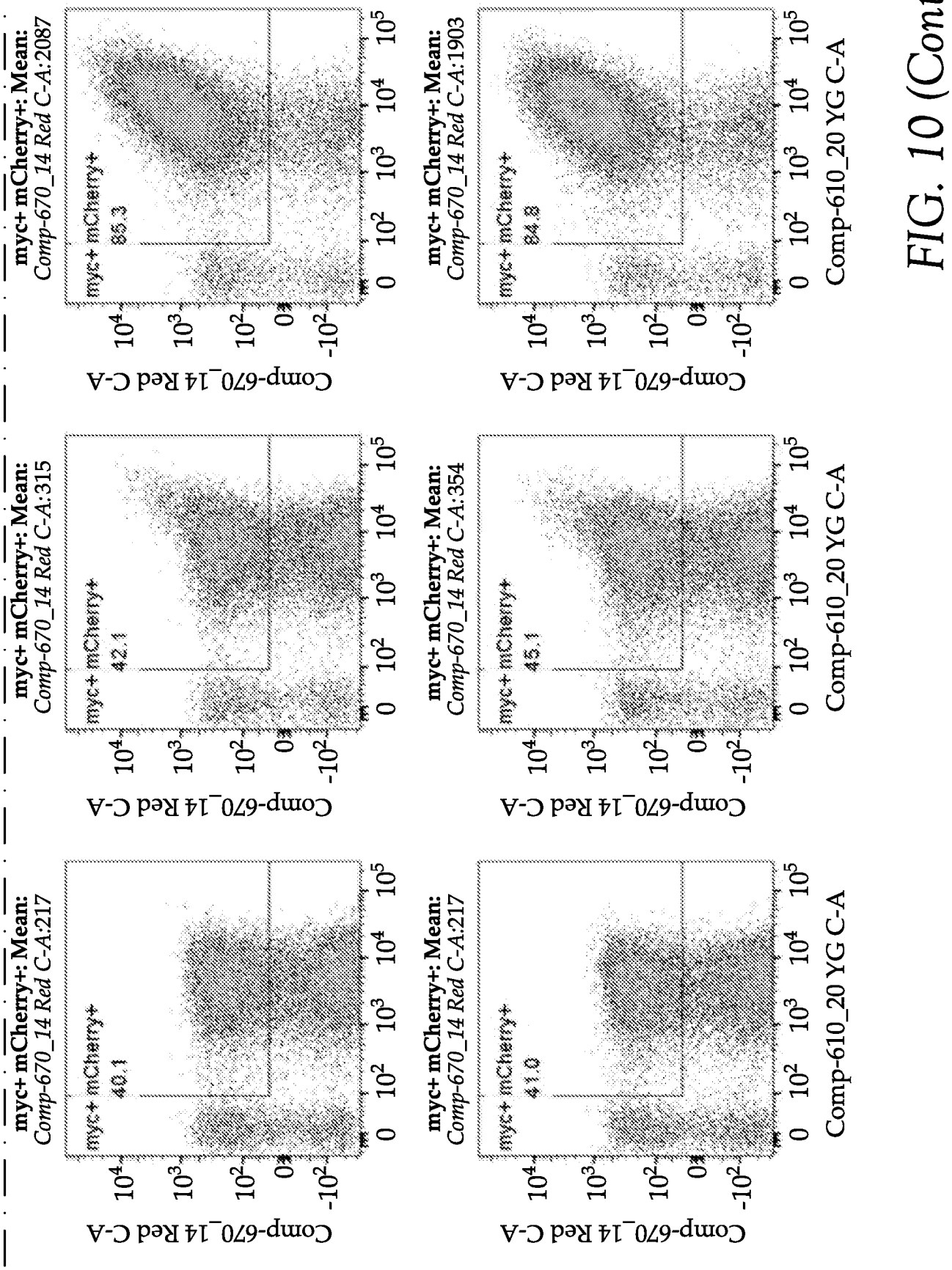
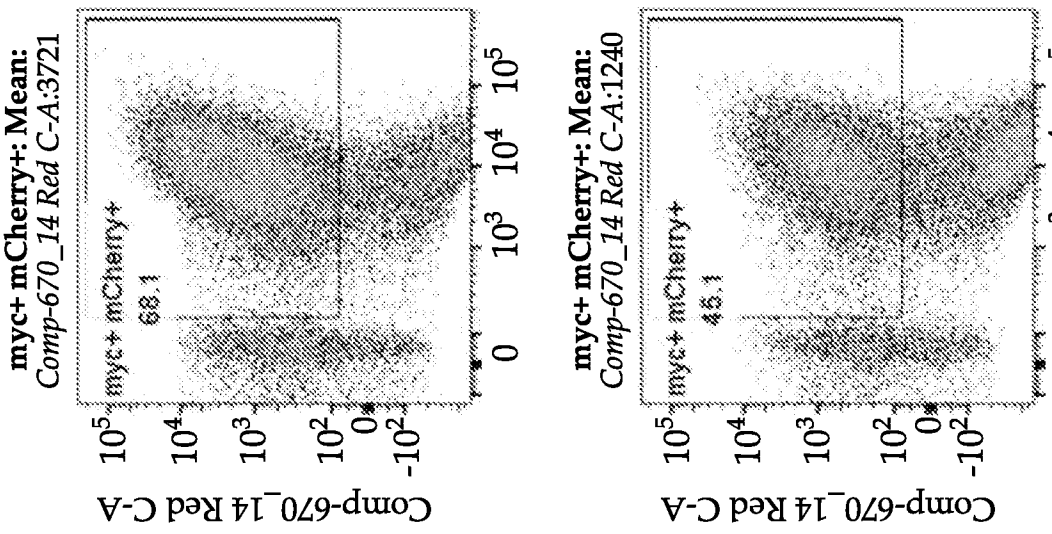

myc+ mCherry+: Mean:
Comp-670_14 Red C-A:2087
myc+ mCherry+
85.3
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:1903
myc+ mCherry+
84.8
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:315
myc+ mCherry+
42.1
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:354
myc+ mCherry+
45.1
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:217
myc+ mCherry+
40.1
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:217
myc+ mCherry+
41.0
Comp-670_14 Red C-A
Comp-610_20 YG C-A

FIG. 10 (Cont.)

FIG. 10 (Cont.)

(090J)
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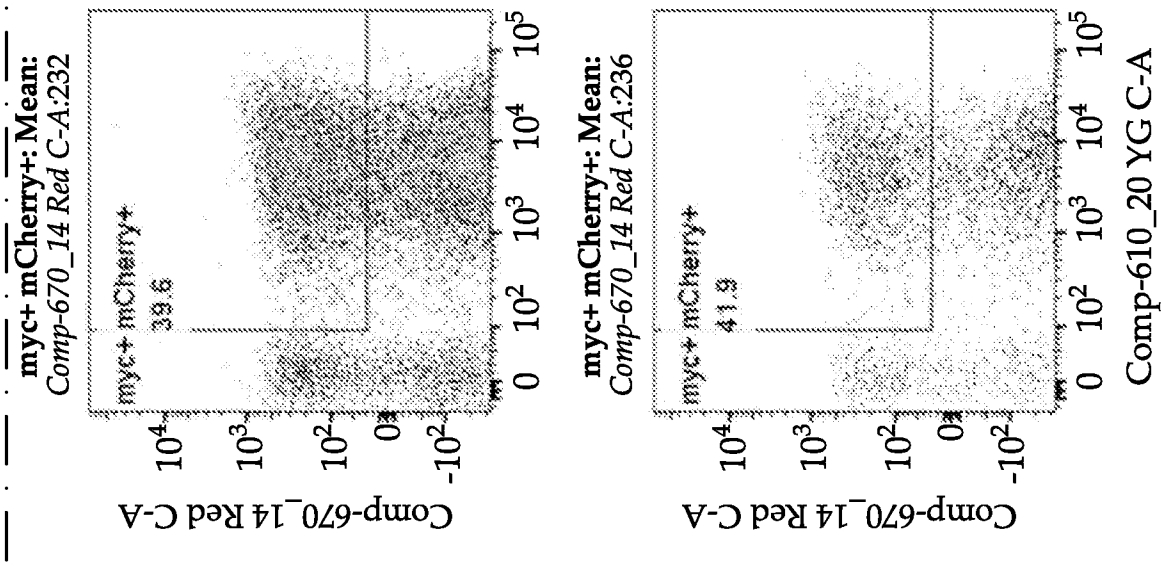

myc+ mCherry+: Mean:
Comp-670_14 Red C-A:232
myc+ mCherry+
39.6
Comp-670_14 Red C-A
myc+ mCherry+: Mean:
Comp-670_14 Red C-A:236
myc+ mCherry+
41.9
Comp-670_14 Red C-A
Comp-610_20 YG C-A

FIG. 10 (Cont.)

FIG. 11

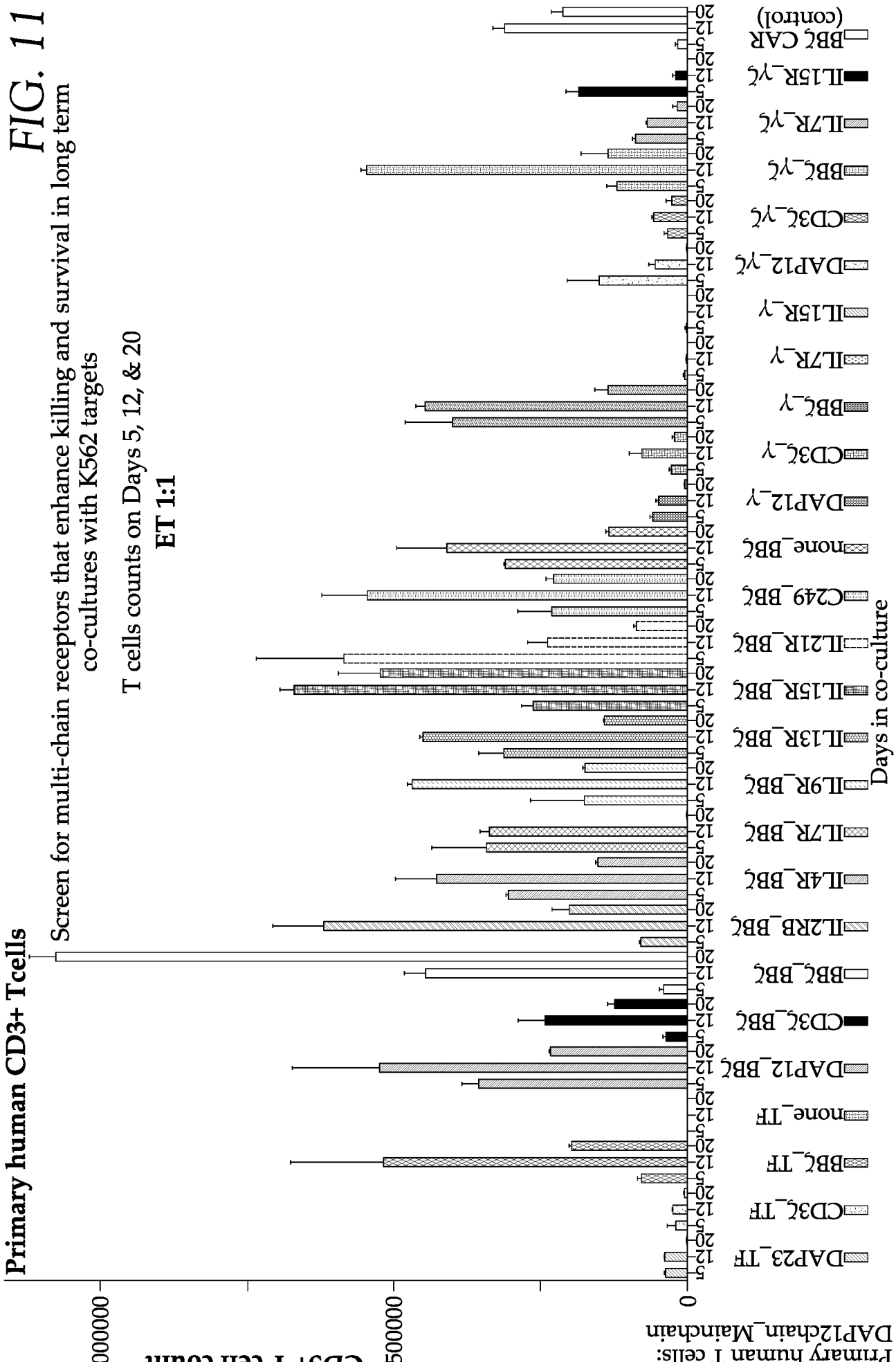

Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
T cells counts on Days 5, 12, & 20
ET 1:1
CD3+ T cell count
1000000
500000
0
Primary human T cells:
DAP12chain_Mainchain
Days in co-culture
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)

FIG. 12

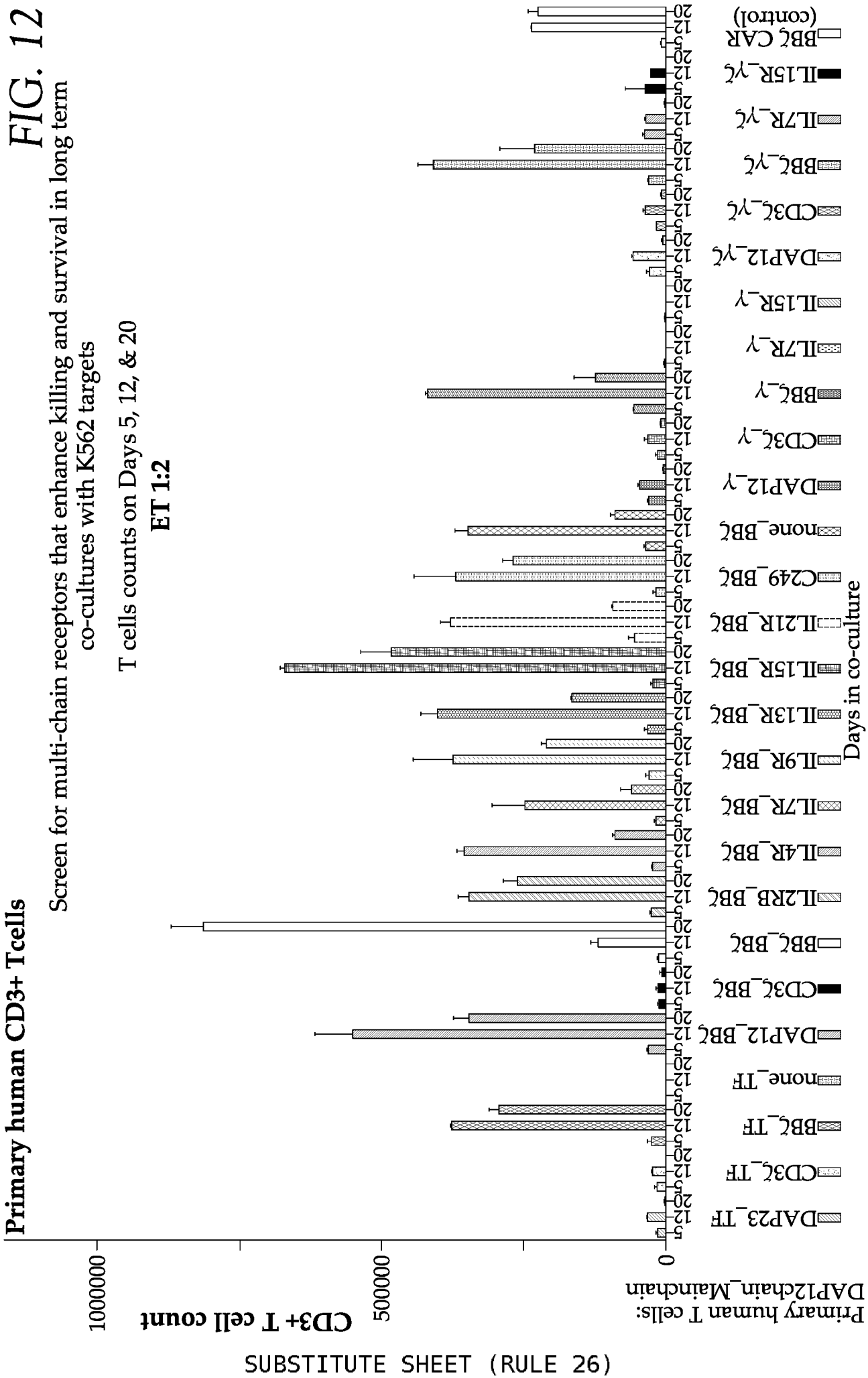

Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
T cells counts on Days 5, 12, & 20
ET 1:2
CD3+ T cell count
1000000
500000
0
Primary human T cells: DAP12chain_Mainchain
Days in co-culture
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)

FIG. 13

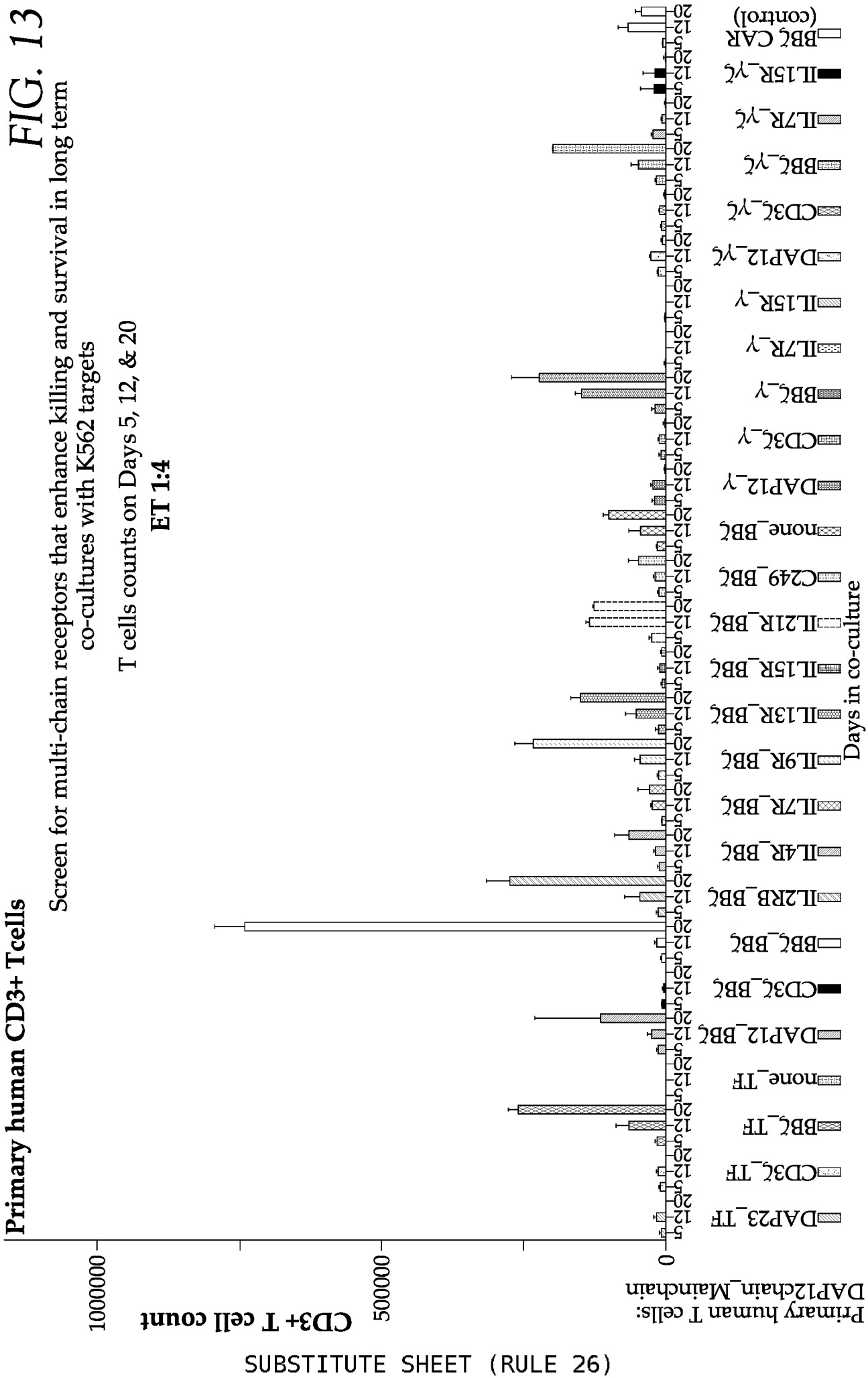

Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
T cells counts on Days 5, 12, & 20
ET 1:4
CD3+ T cell count
1000000
500000
0
Primary human T cells: DAP12chain_Mainchain
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)
5
12
20
Days in co-culture

FIG. 14

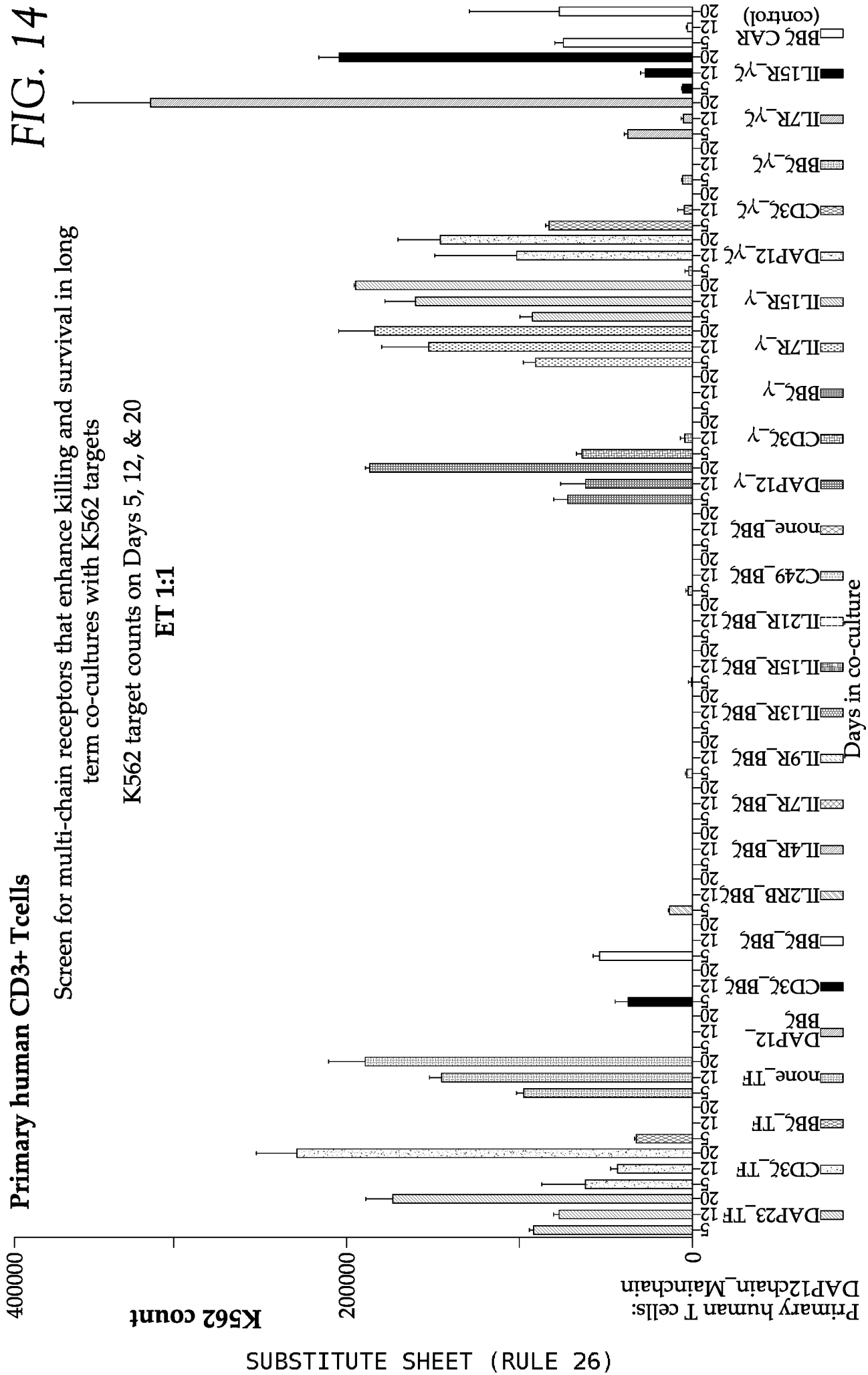

Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
K562 target counts on Days 5, 12, & 20
ET 1:1
K562 count
400000
200000
0
Primary human T cells:
DAP12chain_Mainchain
Days in co-culture
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)
5
12
20

FIG. 15

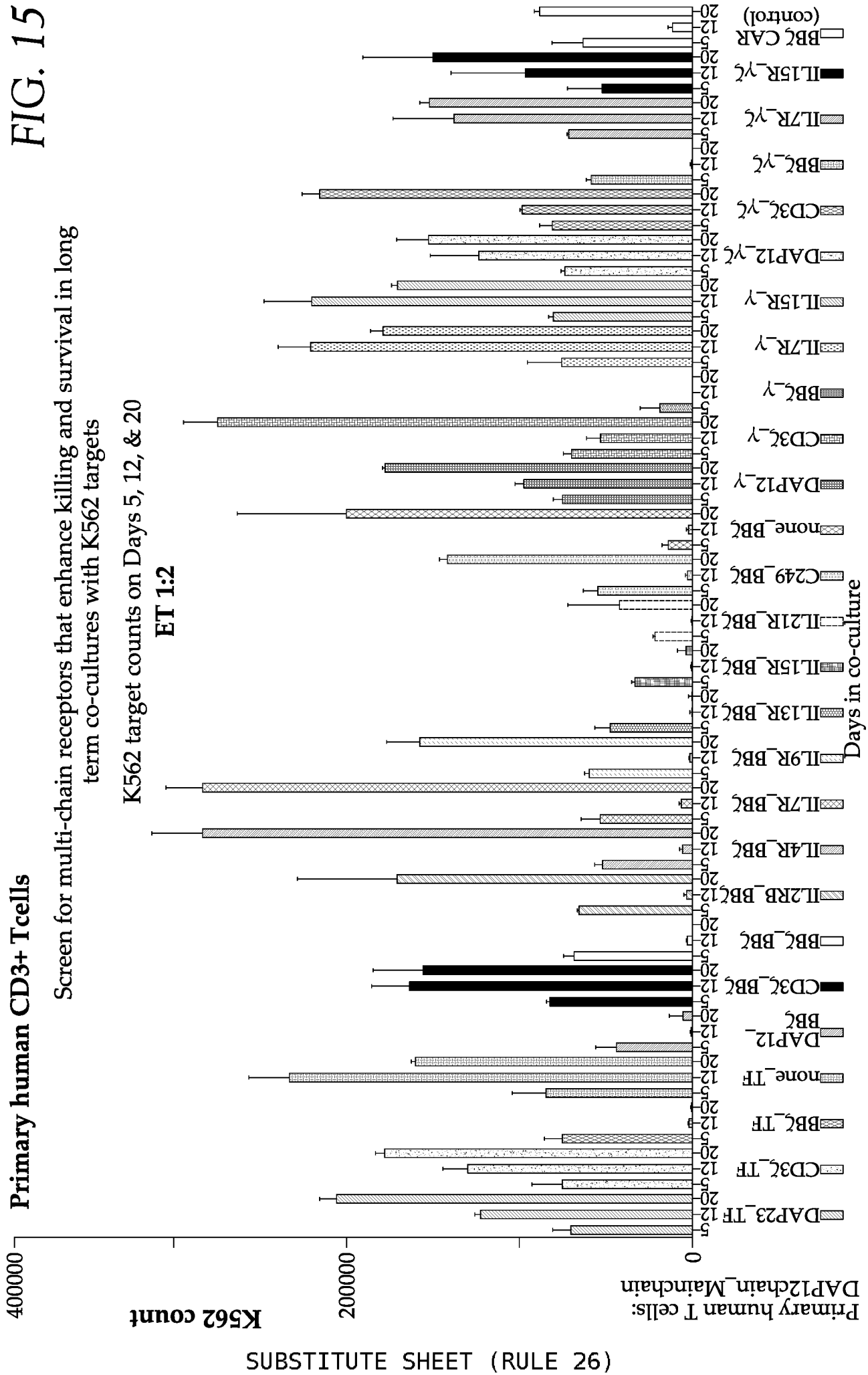
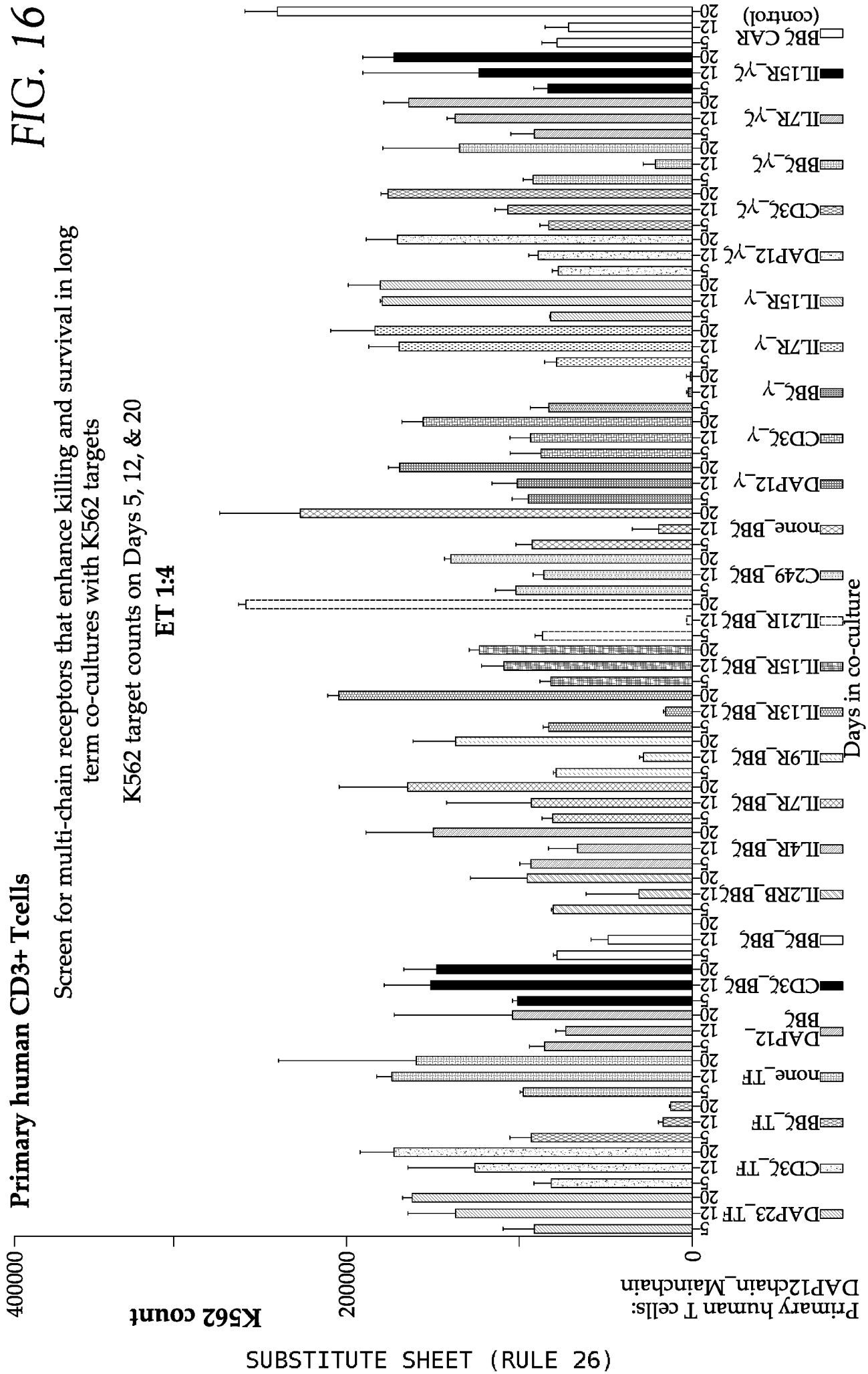

Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
K562 target counts on Days 5, 12, & 20
ET 1:2
K562 count
400000
200000
0
Primary human T cells:
DAP12chain_Mainchain
Days in co-culture
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)
5
12
20

FIG. 16


Primary human CD3+ Tcells
Screen for multi-chain receptors that enhance killing and survival in long term co-cultures with K562 targets
K562 target counts on Days 5, 12, & 20
ET 1:4
K562 count
400000
200000
0
Primary human T cells:
DAP12chain_Mainchain
Days in co-culture
DAP23_TF
CD3ζ_TF
BBζ_TF
none_TF
DAP12_BBζ
CD3ζ_BBζ
BBζ_BBζ
IL2RB_BBζ
IL4R_BBζ
IL7R_BBζ
IL9R_BBζ
IL13R_BBζ
IL15R_BBζ
IL21R_BBζ
C249_BBζ
none_BBζ
DAP12_γ
CD3ζ_γ
BBζ_γ
IL7R_γ
IL15R_γ
DAP12_γζ
CD3ζ_γζ
BBζ_γζ
IL7R_γζ
IL15R_γζ
BBζ CAR (control)
5
12
20