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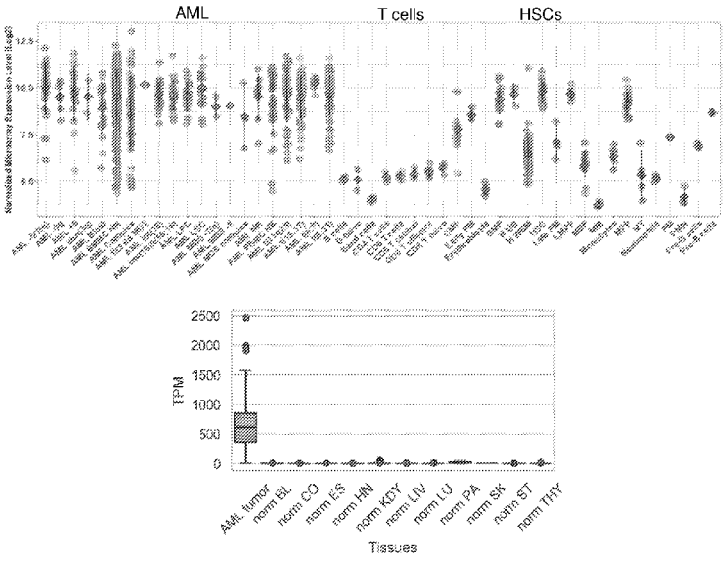
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(54) Title: CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

FIG. 1
FLT3



(57) Abstract: Provided herein are acute myeloid leukemia antigen targets for chimeric receptors and methods of using same.

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[Continued on next page]

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CHIMERIC RECEPTORS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/841,128, filed April 30, 2019; U.S. Provisional Application No. 62/854,151, filed May 29, 2019; and U.S. Provisional Application No. 62/893,106, filed August 28, 2019; each of which are hereby incorporated in its entirety by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Month XX, 20XX, is named XXXXXUS_sequencelisting.txt, and is X,XXX,XXX bytes in size.

BACKGROUND

[0003] Chimeric antigen receptor (CAR) based adoptive cell therapies used to redirect the specificity and function of immunoresponsive cells, such as T cells, have shown efficacy in patients with lymphoid malignancies (Pule et al., *Nat. Med.* (14):1264-1270 (2008); Maude et al., N *Engl J Med.* (371):1507-17 (2014); Brentjens et al., *Sci Transl Med.* (5):177ra38 (2013)). CAR T cells have been shown to induce complete remission in patients with CD19-expressing malignancies for whom chemotherapies have led to drug resistance and tumor progression. The success of CD19 CAR therapy provides optimism for treating other hematological malignancies, such as acute myeloid leukemia (AML). Acute myeloid leukemia is the most common acute leukemia in adults. AML is a cancer of the myeloid line of blood cells and is characterized by the rapid growth of abnormal cells that build up in the bone marrow and blood and interfere with normal blood cells. Sometimes, AML can spread to the brain, skin, or gums. The standard chemotherapy treatments for AML have not changed substantially over the past 40 years (Pulte et al., 2008), and overall survival remains very poor.

[0004] One challenge to developing CAR therapy for AML is the lack of suitable targets. The ability to identify appropriate CAR targets is important to effectively targeting and treating the tumor without damaging normal cells that express the same target antigen. Thus, there remains a need for CAR-T cell-based AML therapies that target AML cells without targeting normal cells or tissues.

SUMMARY

[0005] In one aspect, provided herein are isolated immunoresponsive cells comprising: (a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and (b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen, wherein each antigen is selected from FLT3, CD33, CLEC12A, MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70, and wherein the first antigen is different from the second antigen.

[0006] In some embodiments, the first antigen is FLT3 and the extracellular antigen-binding domain of the first chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from (a) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto; (c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto; (d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto; (e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto; (f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto; (g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and (h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto.

[0007] In some embodiments, the second antigen is CD33 and the extracellular antigen-binding domain of the second chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino

acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto, and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto; or the second antigen is CLEC12A and comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto, (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto, and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

[0008] In some embodiments, the first antigen is CLEC12A and the extracellular antigen-binding domain of the first chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto, optionally wherein the second antigen is CD33 and the extracellular antigen-binding domain of the second chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

[0009] In some embodiments, binding of the first chimeric receptor to the first antigen is capable of activating the immunoresponsive cell and/or binding of the second chimeric

receptor to the second antigen is capable of stimulating the immunoresponsive cell; and/or the immunoresponsive cell exhibits a greater degree of cytolytic activity against target cells that are positive for both the first antigen and the second antigen as compared to cytolytic activity against target cells that are positive for only the first antigen or the second antigen; and/or the first chimeric receptor binds to the first antigen with a binding affinity that is lower than the binding affinity of the second chimeric receptor for the second antigen; and/or the first chimeric receptor binds to the first antigen with a low binding avidity.

[0010] In some embodiments, the first chimeric receptor is a first CAR and the second chimeric receptor is a second CAR and each CAR comprises: a CD3zeta-chain intracellular signaling domain, optionally wherein each CAR further comprises one or more additional intracellular signaling domains, and the one or more additional intracellular signaling domains are selected from a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain; and/or a transmembrane domain, and the transmembrane domain is selected from a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain; and/or a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from SEQ ID NOs: 55-64.

[0011] In some embodiments, the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain, optionally wherein the inhibitory chimeric receptor inhibits one or more activities of the cell.

[0012] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell, optionally wherein the antigen that is expressed on a non-tumor cell is derived from a tissue selected from brain, neuronal tissue, endocrine, bone, bone

marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0013] In some embodiments, the inhibitory chimeric receptor binds an antigen selected from EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

[0014] In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain comprising a single chain variable fragment (scFv), and the scFv is derived from an anti-EMCN antibody.

[0015] In some embodiments, the antigen-binding domain of the first chimeric receptor, the antigen-binding domain of the second chimeric receptor, and/or the antigen-binding domain of the inhibitory chimeric receptor comprises one or more single chain variable fragments (scFvs), wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide linker, and optionally wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

[0016] In some embodiments, each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0017] In some embodiments, each of the one or more scFvs binds to a distinct epitope on the same antigen.

[0018] In some embodiments, each of the one or more scFvs is separated by a peptide linker, optionally wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

[0019] In some embodiments, the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the immunoresponsive cell is allogeneic.

[0020] In another aspect, provided herein are pharmaceutical compositions comprising an effective amount of the isolated immunoresponsive cell of any embodiment herein and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

[0021] In another aspect, provided herein are methods of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the isolated immunoresponsive cells of any embodiment herein or the pharmaceutical composition of any embodiment herein.

[0022] In another aspect, provided herein are methods of treating or preventing a myeloid disorder in a subject, comprising administering to the subject an effective amount of the isolated immunoresponsive cell of any embodiment herein or the pharmaceutical composition of any embodiment herein, optionally wherein the myeloid disorder is myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia (AML), acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

[0023] In another aspect, provided herein are kits for treating and/or preventing a myeloid disorder, comprising the isolated immunoresponsive cell of any embodiment herein or the pharmaceutical composition of any embodiment herein, optionally wherein the kit further comprises written instructions for using the cell for treating and/or preventing a myeloid disorder in a subject .

[0024] In one aspect, provided herein are chimeric receptors comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, and SPNS3.

[0025] In some embodiments, the antigen is MS4A3. In some embodiments, the antigen is VSTM1. In some embodiments, wherein the antigen is LAT2. In some embodiments, the antigen is MLC1. In some embodiments, the antigen is CD131. In some embodiments, the antigen is GAPT. In some embodiments, the antigen is PRAM1. In some embodiments, the antigen is SLC22A16. In some embodiments, the antigen is SLC17A9. In some embodiments, the antigen is SPNS3.

[0026] In some embodiments, the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the chimeric receptor is a CAR.

[0027] In some embodiments, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group

consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0028] In some embodiments, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0029] In some embodiments, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

[0030] In some embodiments, the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0031] In another aspect, provided herein are isolated cells comprising the chimeric receptor of any one of the embodiments.

[0032] In some embodiments, the chimeric receptor is recombinantly expressed. In some embodiments, the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

[0033] In some embodiments, the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

[0034] In some embodiments, the cell is autologous. In some embodiments, the cell is allogeneic.

[0035] In another aspect, provided herein are isolated cells comprising (a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and (b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen, wherein each antigen is selected from the group consisting of the antigens listed in Table 1 or the first and second antigens are selected from the group consisting of the antigen pairs listed in Table 3, and wherein the first antigen is different from the second.

[0036] In another aspect, provided herein are isolated cells comprising (a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and

[0037] (b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen, wherein each antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70, and wherein the first antigen is different from the second.

[0038] In some embodiments, the first antigen is MS4A3 and the second antigen is selected from the group consisting of VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0039] In some embodiments, the first antigen is VSTM1 and the second antigen is selected from the group consisting of MS4A3, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0040] In some embodiments, the first antigen is LAT2 and the second antigen is selected from the group consisting of MS4A3, VSTM1, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0041] In some embodiments, the first antigen is MLC1 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0042] In some embodiments, the first antigen is CD131 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0043] In some embodiments, the first antigen is GAPT and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0044] In some embodiments, the first antigen is PRAM1 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0045] In some embodiments, the first antigen is SLC22A16 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0046] In some embodiments, the first antigen is SLC17A9 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0047] In some embodiments, the first antigen is SPNS3 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0048] In some embodiments, the first antigen is FLT3. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical

thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto; (c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto; (d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto; (e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto; (f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto; (g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and (h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical

thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen is CD33. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the

amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen is CLECL12A. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto. some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto. some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

[0049] In some embodiments, the first antigen is CLECL12A. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of

SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen is CD33. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 19 or a

sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

[0050] In some embodiments, the cell is an immunoresponsive cell. In some embodiments, binding of the first chimeric receptor to the first antigen is capable of activating the immunoresponsive cell. In some embodiments, binding of the second chimeric receptor to the second antigen is capable of stimulating the immunoresponsive cell.

[0051] In some embodiments, binding of the first chimeric receptor to the first antigen and binding of the second chimeric receptor to the second antigen are required for activating the immunoresponsive cell.

[0052] In some embodiments, the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to against cells that are singly positive for the first antigen.

[0053] In some embodiments, binding of the first chimeric receptor to the first antigen or binding of the second chimeric receptor to the second antigen is capable of activating the immunoresponsive cell.

[0054] In some embodiments, the first chimeric receptor binds to the first antigen with a low binding affinity. In some embodiments, the first chimeric receptor binds to the first antigen with a binding affinity that is lower than the binding affinity with which the second chimeric receptor binds to the second antigen. In some embodiments, the first chimeric receptor binds to the first antigen with a low binding avidity.

[0055] In some embodiments, the first chimeric receptor and/or the second chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR). In some embodiments, the first chimeric receptor and/or the second chimeric receptor is a CAR. In some embodiments, the first chimeric receptor is a first CAR and the second chimeric receptor is a second CAR.

[0056] In some embodiments, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10

intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0057] In some embodiments, the one or more intracellular signaling domains of the first CAR are different from the one or more intracellular signaling domains of the second CAR.

[0058] In some embodiments, the first CAR and the second CAR each comprise a CD3zeta-chain intracellular signaling domain.

[0059] In some embodiments, the first CAR and the second CAR each further comprises an additional intracellular signaling domain selected from the group consisting of a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0060] In some embodiments, the additional intracellular signaling domain of the first CAR is different from the additional intracellular signaling domain of the second CAR.

[0061] In some embodiments, each CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0062] In some embodiments, the transmembrane domain of the first CAR is different from the transmembrane domain of the second CAR.

[0063] In some embodiments, each CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

[0064] In some embodiments, the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain

comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0065] In some embodiments, the first chimeric receptor is recombinantly expressed.

[0066] In some embodiments, the first chimeric receptor is expressed from a vector, or a selected locus from the genome of the cell.

[0067] In some embodiments, the second chimeric receptor is recombinantly expressed.

[0068] In some embodiments, the second chimeric receptor is expressed from a vector, or a selected locus from the genome of the cell.

[0069] In some embodiments, the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain.

[0070] In some embodiments, the inhibitory chimeric receptor inhibits one or more activities of the cell.

[0071] In some embodiments, the inhibitory chimeric receptor binds an antigen that is not expressed on a tumor cell.

[0072] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell.

[0073] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0074] In some embodiments, the tumor cell is an acute myeloid leukemia (AML) cell.

[0075] In some embodiments, the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

[0076] In some embodiments, the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

[0077] In some embodiments, the enzymatic inhibitory domain comprises an enzyme catalytic domain.

[0078] In some embodiments, the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

[0079] In some embodiments, the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

[0080] In some embodiments, the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

[0081] In some embodiments, the inhibitory chimeric receptor binds an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, and FFAR2.

[0082] In some embodiments, the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

[0083] In some embodiments, the cell is autologous.

[0084] In some embodiments, the cell is allogeneic.

[0085] In another aspect, provided herein are chimeric receptors comprising two or more antigen-binding domains, wherein each antigen-binding domain binds to an antigen selected from the group consisting of the antigens listed in Table 1 or the two or more antigen-bonding domains bind to an antigen pair selected from the group consisting of the antigen pairs listed in Table 3, wherein each antigen-binding domain binds to a different antigen.

[0086] In another aspect, provided herein are chimeric receptors comprising two or more antigen-binding domains, wherein each antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70, wherein each antigen-binding domain binds to a different antigen.

[0087] In some embodiments, one antigen-binding domain binds MS4A3 and a second antigen-binding domain binds to an antigen selected from the group consisting of VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0088] In some embodiments, one antigen-binding domain binds VSTM1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0089] In some embodiments, one antigen-binding domain binds LAT2 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0090] In some embodiments, one antigen-binding domain binds MLC1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0091] In some embodiments, one antigen-binding domain binds CD131 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0092] In some embodiments, one antigen-binding domain binds GAPT and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0093] In some embodiments, one antigen-binding domain binds PRAM1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0094] In some embodiments, one antigen-binding domain binds SLC22A16 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC17A9, SPNS3, FLT3, CD33,

CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0095] In some embodiments, one antigen-binding domain binds SLC17A9 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0096] In some embodiments, one antigen-binding domain binds SPNS3 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0097] In some embodiments, one antigen binding domain binds FLT3. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto; (c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto; (d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto; (e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto; (f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto; (g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and (h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at

least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, a second antigen-binding domain binds CD33. In some embodiments that may be combined with any

of the preceding embodiments, the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, a second antigen-binding domain binds CLEC12A. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CLECL12A comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CLECL12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto. some embodiments that may be combined with any of the

preceding embodiments, the second antigen-binding domain that binds CLECL12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto. some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CLECL12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

[0098] In some embodiments, one antigen binding domain binds CLEC12A. In some embodiments, the antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto. In some embodiments, the antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, a second antigen-binding domain binds CD33. In some embodiments that may be combined with any of the preceding embodiments, the second

antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto. In some embodiments that may be combined with any of the preceding embodiments, the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a light chain variable domain (VL) comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

[0099] In some embodiments, the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

[0100] In some embodiments, the chimeric receptor is a CAR.

[0101] In some embodiments, the CAR is a bispecific CAR.

[0102] In some embodiments, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3-zeta chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0103] In some embodiments, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane

domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0104] In some embodiments, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

[0105] In some embodiments, each antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0106] In another aspect, provided herein are isolated cells comprising the chimeric receptor of any one of the embodiments.

[0107] In some embodiments, the cell further comprises an additional chimeric receptor comprising an antigen-binding domain.

[0108] In some embodiments, the additional chimeric receptor binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0109] In some embodiments, each of the two chimeric receptors binds to different antigens.

[0110] In some embodiments, the cell is an immunoresponsive cell.

[0111] In some embodiments, binding of the chimeric receptor to either of the two antigens is capable of activating the immunoresponsive cell.

[0112] In some embodiments, binding of the additional chimeric receptor to its cognate antigen is capable of stimulating the immunoresponsive cell.

[0113] In some embodiments, binding of the chimeric receptor to either of the two antigens and binding of the additional chimeric receptor to its cognate antigen are required for activating the immunoresponsive cell.

[0114] In some embodiments, the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for either of the two antigens bound by the chimeric receptor and positive for the antigen bound by the additional chimeric receptor, as compared to against cells that are only positive for a single antigen.

[0115] In some embodiments, the additional chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

[0116] In some embodiments, the additional chimeric receptor is a CAR.

[0117] In some embodiments, the chimeric receptor is a first CAR and the additional chimeric receptor is a second CAR.

[0118] In some embodiments, the second CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0119] In some embodiments, the one or more intracellular signaling domains of the first CAR are different from the one or more intracellular signaling domains of the second CAR.

[0120] In some embodiments, the first CAR and the second CAR each comprise a CD3zeta-chain intracellular signaling domain.

[0121] In some embodiments, the first CAR and the second CAR each further comprises an additional intracellular signaling domain selected from the group consisting of a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30

intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0122] In some embodiments, the additional intracellular signaling domain of the first CAR is different from the additional intracellular signaling domain of the second CAR.

[0123] In some embodiments, the second CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0124] In some embodiments, the transmembrane domain of the first CAR is different from the transmembrane domain of the second CAR.

[0125] In some embodiments, the second CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

[0126] In some embodiments, the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain.

[0127] In some embodiments, the additional chimeric receptor is an inhibitory chimeric receptor comprising an antigen-binding domain.

[0128] In some embodiments, the inhibitory chimeric receptor inhibits one or more activities of the cell.

[0129] In some embodiments, the inhibitory chimeric receptor binds an antigen that is not expressed on a tumor cell.

[0130] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell.

[0131] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0132] In some embodiments, the tumor cell is an acute myeloid leukemia (AML) cell.

[0133] In some embodiments, the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

[0134] In some embodiments, the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

[0135] In some embodiments, wherein the enzymatic inhibitory domain comprises an enzyme catalytic domain.

[0136] In some embodiments, the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

[0137] In some embodiments, the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

[0138] In some embodiments, the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

[0139] In some embodiments, in the inhibitory chimeric receptor binds an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, and FFAR2.

[0140] In some embodiments, the antigen-binding domain of the additional chimeric receptor and/or inhibitory chimeric receptor comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0141] In some embodiments, each chimeric receptor is recombinantly expressed.

[0142] In some embodiments, each chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

[0143] In some embodiments, the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an

ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

[0144] In some embodiments, the cell is autologous.

[0145] In some embodiments, the cell is allogeneic.

[0146] In another aspect, provided herein are chimeric inhibitory receptors comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, and FFAR2.

[0147] In some embodiments, the antigen is EMCN.

[0148] In some embodiments, the antigen is JAM2.

[0149] In some embodiments, the antigen is MS4A15.

[0150] In some embodiments, the antigen is C4BPA.

[0151] In some embodiments, the antigen is TRPM1.

[0152] In some embodiments, the antigen is SCTR.

[0153] In some embodiments, the antigen is SLC2A2.

[0154] In some embodiments, the antigen is KCNQ2.

[0155] In some embodiments, the antigen is PERP.

[0156] In some embodiments, when expressed on a cell the inhibitory chimeric receptor inhibits one or more activities of the cell.

[0157] In some embodiments, the antigen that is not expressed on a tumor cell.

[0158] In some embodiments, the antigen is expressed on a non-tumor cell.

[0159] In some embodiments, the antigen is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0160] In some embodiments, the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

[0161] In some embodiments, the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

[0162] In some embodiments, the enzymatic inhibitory domain comprises an enzyme catalytic domain.

[0163] In some embodiments, the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

[0164] In some embodiments, the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

[0165] In some embodiments, the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

[0166] In some embodiments, the antigen-binding domain comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0167] In another aspect, provided herein are isolated cells comprising the chimeric inhibitory receptor of any one of the embodiments.

[0168] In some embodiments, the chimeric inhibitory receptor is recombinantly expressed.

[0169] In some embodiments, the chimeric inhibitory receptor is expressed from a vector or a selected locus from the genome of the cell.

[0170] In some embodiments, the cell further comprises a chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0171] In another aspect, provided herein are isolated cells comprising (a) a chimeric inhibitory receptor comprising an extracellular antigen-binding domain that binds to a first antigen, wherein the first antigen is selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, and FFAR2, and (b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein

each antigen-binding domain binds to an antigen selected from the group consisting of the antigens listed in Table 1.

[0172] In another aspect, provided herein are isolated cells comprising (a) a chimeric inhibitory receptor comprising an extracellular antigen-binding domain that binds to a first antigen, wherein the first antigen is selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, and FFAR2, and (b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein each antigen-bind domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

[0173] In some embodiments, the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

[0174] In some embodiments, the chimeric receptor is a CAR.

[0175] In some embodiments, the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

[0176] In some embodiments, the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

[0177] In some embodiments, the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

[0178] In some embodiments, the antigen-binding domain of the chimeric inhibitory receptor and/or the chimeric receptor comprises an antibody, an antigen-binding fragment of an antibody, a F(ab) fragment, a F(ab') fragment, a single chain variable fragment (scFv), or a single-domain antibody (sdAb). In some embodiments, the antigen-binding domain comprises a single chain variable fragment (scFv). In some embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the VH and VL are separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27. In some embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0179] In some embodiments, the cell is an immunoresponsive cell.

[0180] In some embodiments, binding of the chimeric inhibitory receptor to the first antigen is capable of inhibiting the immunoresponsive cell.

[0181] In some embodiments, binding of the chimeric receptor to the second antigen is capable of activating the immunoresponsive cell.

[0182] In some embodiments, the chimeric receptor binds to the second antigen with a low binding affinity.

[0183] In some embodiments, the chimeric receptor binds to the second antigen with a binding affinity that is lower than the binding affinity with which the chimeric inhibitory receptor binds to the first antigen.

[0184] In some embodiments, the chimeric receptor binds to the first antigen with a low binding avidity.

[0185] In some embodiments, the chimeric receptor is recombinantly expressed.

[0186] In some embodiments, the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

[0187] In some embodiments, the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

[0188] In some embodiments, the cell is autologous.

[0189] In some embodiments, the cell is allogeneic.

[0190] In another aspect, provided herein are isolated nucleic acids encoding the chimeric receptor of any one of the embodiments.

[0191] In another aspect, provided herein are isolated nucleic acids encoding the chimeric receptor of any one the embodiments

[0192] In another aspect, provided herein are isolated nucleic acid encoding the chimeric inhibitory receptor of any one the embodiments.

[0193] In another aspect, provided herein are vectors comprising the nucleic acid of claim 175.

[0194] In some embodiments, the vector further comprises the nucleic acid of the embodiments.

[0195] In another aspect, provided herein vectors comprising the nucleic acid of the embodiments.

[0196] In another aspect, provided herein are genetically modified cells comprising the nucleic acid of the embodiments.

[0197] In some embodiments, the cell further comprises the nucleic acid of the embodiments.

[0198] In another aspect, provided herein are genetically modified cells comprising the nucleic acid of the embodiments.

[0199] In another aspect, provided herein are genetically engineered cells comprising the vector of any one of the embodiments

[0200] In another aspect, provided herein are methods of reducing tumor burden in a subject, comprising administering to the subject an effective amount of the isolated cell of any one of the embodiments.

[0201] In some embodiments, the method reduces the number of tumor cells.

[0202] In some embodiments, the method reduces tumor size.

[0203] In some embodiments, the method eradicates the tumor in the subject.

[0204] In another aspect, provided herein are methods of treating or preventing a myeloid disorder in a subject, comprising administering to the subject an effective amount of the isolated cell of any one of the embodiments.

[0205] In some embodiments, the myeloid disorder is myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia (AML), acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

[0206] In some embodiments, the myeloid disorder is acute myeloid leukemia (AML).

[0207] In some embodiments, the method reduces or eradicates the tumor burden in the subject.

[0208] In another aspect, provided herein are pharmaceutical compositions comprising an effective amount of the isolated cell of any one of the embodiments and a pharmaceutically acceptable excipient.

[0209] In some embodiment, the pharmaceutical composition is for treating and/or preventing a myeloid disorder.

[0210] In another aspect, provided herein are kits for treating and/or preventing a myeloid disorder, comprising an isolated cell of any one of the embodiments.

[0211] In some embodiments, the kit further comprises written instructions for using the cell for treating and/or preventing a myeloid disorder in a subject .

[0212] In another aspect, provided herein are kits for treating and/or preventing a myeloid disorder, comprising the isolated nucleic acid of any one of the embodiments.

[0213] In some embodiments, the kit further comprises written instructions for using the nucleic acid for producing one or more antigen-specific cells for treating and/or preventing a myeloid disorder in a subject.

[0214] In another aspect, provided herein are kits for treating and/or preventing a myeloid disorder, comprising a vector of any one of the embodiments.

[0215] In some embodiments, the kit further comprises written instructions for using the vector for producing one or more antigen-specific cells for treating and/or preventing a myeloid disorder in a subject.

[0216] In another aspect, provided herein are methods of treating and/or preventing a myeloid disorder, comprising administering an effective amount of at least one antibody that binds to an antigen, wherein the antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, and SPNS3.

[0217] In some embodiments, the myeloid disorder is selected from the group consisting of myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, or acute myeloid leukemia (AML) , acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

[0218] In some embodiments, the myeloid disorder is acute myeloid leukemia (AML).

[0219] In some embodiments, the method reduces or eradicates the tumor burden in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0220] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0221] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings.

[0222] FIG. 1 provides microarray and RNA-Seq data for FLT3 expression in the indicated tissues or cell types.

[0223] FIG. 2 provides microarray and RNA-Seq data for MS4A3 expression in the indicated tissues or cell types.

[0224] FIG. 3 provides microarray and RNA-Seq data for CD33 expression in the indicated tissues or cell types.

[0225] FIG. 4 provides microarray and RNA-Seq data for CLEC12A expression in the indicated tissues or cell types.

[0226] FIG. 5 provides microarray and RNA-Seq data for CD312/ADGRE2 expression in the indicated tissues or cell types.

[0227] FIG. 6 provides microarray and RNA-Seq data for SLC22A16 expression in the indicated tissues or cell types.

[0228] FIG. 7 provides microarray and RNA-Seq data for CD123/ILR3RA expression in the indicated tissues or cell types.

[0229] FIG. 8 provides microarray and RNA-Seq data for LAT2 expression in the indicated tissues or cell types.

[0230] FIG. 9 provides microarray and RNA-Seq data for PIEZO1/FAM38A expression in the indicated tissues or cell types.

[0231] FIG. 10 provides microarray and RNA-Seq data for CD38 expression in the indicated tissues or cell types.

[0232] FIG. 11 provides microarray and RNA-Seq data for EMB expression in the indicated tissues or cell types.

[0233] FIG. 12 provides microarray and RNA-Seq data for CD131/CSF2RB expression in the indicated tissues or cell types.

[0234] FIG. 13 provides microarray and RNA-Seq data for P2RY8 expression in the indicated tissues or cell types.

[0235] FIG. 14 provides microarray and RNA-Seq data for LILRA2/CD85H expression in the indicated tissues or cell types.

[0236] FIG. 15 provides microarray and RNA-Seq data for SLC17A9 expression in the indicated tissues or cell types.

[0237] FIG. 16 provides microarray and RNA-Seq data for MYADM expression in the indicated tissues or cell types.

[0238] FIG. 17 provides microarray and RNA-Seq data for CD300LF expression in the indicated tissues or cell types.

[0239] FIG. 18 provides microarray and RNA-Seq data for CD244/SLAMF4 expression in the indicated tissues or cell types.

[0240] FIG. 19 provides microarray and RNA-Seq data for PLAUR expression in the indicated tissues or cell types.

[0241] FIG. 20 provides microarray and RNA-Seq data for CD93 expression in the indicated tissues or cell types.

[0242] FIG. 21 provides microarray and RNA-Seq data for SPNS3 expression in the indicated tissues or cell types.

[0243] FIG. 22 provides microarray and RNA-Seq data for GAPT expression in the indicated tissues or cell types.

[0244] FIG. 23 provides microarray and RNA-Seq data for RASGRP4 expression in the indicated tissues or cell types.

[0245] FIG. 24 provides microarray and RNA-Seq data for CD117/c-Kit expression in the indicated tissues or cell types.

[0246] FIG. 25 provides microarray and RNA-Seq data for CD123/ILR3RA expression in the indicated tissues or cell types.

[0247] FIG. 26 provides microarray and RNA-Seq data for EMCN (endomucin) expression in the indicated tissues or cell types.

[0248] FIG. 27 provides microarray and RNA-Seq data for JAM2 expression in the indicated tissues or cell types.

[0249] FIG. 28 provides microarray and RNA-Seq data for MS4A15 expression in the indicated tissues or cell types.

[0250] FIG. 29 provides microarray and RNA-Seq data for SLC34A2 expression in the indicated tissues or cell types.

[0251] FIG. 30 provides microarray and RNA-Seq data for SLC2A2 expression in the indicated tissues or cell types.

[0252] FIG. 31 provides microarray and RNA-Seq data for TRPM1 expression in the indicated tissues or cell types.

[0253] FIG. 32 provides microarray and RNA-Seq data for SCTR expression in the indicated tissues or cell types.

[0254] FIG. 33 provides microarray and RNA-Seq data for KCNQ2 expression in the indicated tissues or cell types.

[0255] FIG. 34 provides microarray and RNA-Seq data for PERP expression in the indicated tissues or cell types.

[0256] FIG. 35A shows the % cells expressing YFP and the FLT3 CAR from donor 1 for each of the six FLT3 scFvs tested. **FIG. 35B** shows the mean fluorescent intensity of the YFP positive cells from donor 1 for each of the six FLT3 scFvs tested. **FIG. 35C** shows the % cells expressing YFP and the FLT3 CAR from donor 2 for each of the six FLT3 scFvs tested. **FIG. 35D** shows the mean fluorescent intensity of the YFP positive cells from donor 2 for each of the six FLT3 scFvs tested.

[0257] FIG. 36A shows FLT3 CAR T cells from donor 1 induced killing of SEM cells. **FIG. 36B** shows FLT3 CAR T cells from donor 1 induced killing of MOLM13 cells. **FIG. 36C** shows FLT3 CAR T cells from donor 2 induced killing of SEM cells. **FIG. 36D** shows FLT3 CAR T cells from donor 2 induced killing of MOLM13 cells.

[0258] FIG. 37A show TNFα secretion by FLT3 CAR T cells from donor 1 after incubation with SEM cells. **FIG. 37B** show IFNγ secretion by FLT3 CAR T cells from donor 1 after incubation with SEM cells. **FIG. 37C** show IL-2 secretion by FLT3 CAR T cells from donor 1 after incubation with SEM cells. **FIG. 37D** show TNFα secretion by FLT3 CAR T cells from donor 2 after incubation with SEM cells. **FIG. 37E** show IFNγ secretion by FLT3 CAR T cells from donor 2 after incubation with SEM cells. **FIG. 37F** show IL-2 secretion by FLT3 CAR T cells from donor 1 after incubation with SEM cells.

[0259] FIG. 38A show TNFα secretion by FLT3 CAR T cells from donor 1 after incubation with MOLM-13 cells. **FIG. 38B** show IFNγ secretion by FLT3 CAR T cells from donor 1 after incubation with MOLM-13 cells. **FIG. 38C** show IL-2 secretion by FLT3 CAR T cells from donor 1 after incubation with MOLM-13 cells. **FIG. 38D** show TNFα secretion by FLT3 CAR T cells from donor 2 after incubation with MOLM-13 cells. **FIG. 38E** show IFNγ secretion by FLT3 CAR T cells from donor 2 after incubation with MOLM-13 cells. **FIG. 38F** show IL-2 secretion by FLT3 CAR T cells from donor 1 after incubation with MOLM-13 cells.

[0260] FIG. 39 shows a heat mat of the FLT3, CD33, and CLEC12A microarray expression data in various tissues and/or cell lines.

[0261] FIG. 40A shows the flow cytometry histogram plots showing expression of FLT3 protein expression (x-axis) in the AML cell lines MOLM-13, THP-1, and the ALL cell line SEM. **FIG. 40B** shows that the FLT3 CAR T cells killed MOML-13, THP-1, and SEM human leukemia cells. **FIG. 40C** shows a dose dependent FLT3 CAR T cell killing of MOLM-13 AML cells.

[0262] FIG. 41A shows that FLT3 CAR T cells induced TNF-α, IFN-γ, and IL-2 production after incubation with MOLM-14 cells. **FIG. 41B** shows that FLT3 CAR T cells induced TNF-α, IFN-γ, and IL-2 production after incubation with MOLM-13 cells. **FIG. 41C** shows that FLT3 CAR T cells induced TNF-α, IFN-γ, and IL-2 production after incubation with EOL-1 cells.

[0263] FIG. 42A shows the flow cytometry histogram plots showing expression of CD33 protein expression (x-axis) in the AML cell lines MOLM-13, MV4-1, and THP-1. **FIG. 42B** shows that the CD33 CAR T cells killed MOML-13, MV-11, and THP-1 human leukemia cells. **FIG. 42C** shows a dose dependent CD33 CAR T cell killing of MOLM-13 AML cells with both CD33 CAR T cells.

[0264] FIG. 43A shows that CD33 CAR T cells induced TNF-α, IFN-γ, and IL-2 production after incubation with MOLM-13 cells. **FIG. 43B** shows that CD33 CAR T cells induced TNF-α, IFN-γ, and IL-2 production after incubation with MV4-11 cells. **FIG. 43C** shows that CD33 CAR T cells induced TNF-α, IFN-γ, and IL-2 production in THP-1 cells.

[0265] FIG. 44 shows flow cytometry plots of CLEC12A expression in U937, THP-1, HL-60, MV-14, MOLM-14, MOLM-13, Nalm6, Raji, K562, and SEM cell lines.

[0266] FIG. 45A shows flow cytometry histogram plots showing expression of CLEC12A protein expression in four AML cell lines. **FIG. 45B** shows in vitro co-culture cytotoxicity assays showing that CLEC12A CAR T cells kill human AML cells (E:T ratio of 1:1) with percent killing activity on y-axis. **FIG. 45C** shows CLEC12A CAR T cells have potent cytokine secretion (IL-2) when co-cultured with human acute myeloid leukemia (AML) cell lines, as detected by Luminex assay. CAR #1 = SB01261, CLEC12A(357) CAR T cell; CAR #2 = SB01262, CLEC12A(378) CAR T cells; CAR #3 = SB01263, CLEC12A(161) CAR T cells.

[0267] FIG. 46A shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against MOLM-13 (low CLEC12A expression). **FIG. 46B** shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against MV4-11 (low CLEC12A

expression **FIG. 46C** shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against MOLM-14 (high CLEC12A expression). **FIG. 46D** shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against U937. **FIG. 46E** shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against THP-1. **FIG. 46F** shows CLEC12A CAR T cells cytotoxicity activity and cytokine production against HL-60. Each CLEC12A CAR had a CD8 hinge and transmembrane domain and 4-1BB and CD3ζ co-stimulatory domains. The FLT3(NC7) CAR had a 4-1BB co-stimulatory domain. The CD33(hu195) CAR had a CD28 co-stimulatory domain.

[0268] FIG. 47 provides schematics of CLEC12A CARs synthesized.

[0269] FIG. 48A shows transduction of the indicated CLEC12A CAR in T cells. **FIG. 48B** shows the mean fluorescent intensity (MFI) of the indicated CLEC12A CAR in T cells.

[0270] FIG. 49 shows the cytotoxicity of the indicated CLEC12A CAR T cells against HL-60, MOLM14, MV4-11, and MOLM-13 cells as indicated by the percent killing of the cell lines after incubation with the CAR T cells.

[0271] FIG. 50 shows the IL-2 production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells.

[0272] FIG. 51 shows the IFN-γ production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells.

[0273] FIG. 52 shows the TNF-α production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells.

[0274] FIG. 53A shows the fLuc bioluminescence in non-tumor bearing mice treated with CD33 CAR T cells. **FIG. 53B** shows the fLuc bioluminescence in non-tumor bearing mice treated with FLT3 CAR T cells. **FIG. 53C** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with control unengineered T cells. The endpoint criteria was reached on day 14 and the mice sacrificed. **FIG. 53D** shows the fLuc bioluminescence in non-tumor bearing mice treated with control unengineered T cells. **FIG. 53E** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with FLT3 CAR T cells once on day 5. **FIG. 53F** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with FLT3 CAR T cells once on day 8. The endpoint criteria was reached on day 14 and the mice sacrificed. **FIG. 53G** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with FLT3 CAR T cells twice on days 5 and 12. **FIG. 53H** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with CD33 CAR T cells once on day 5. **FIG. 53I** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with CD33 CAR T cells once on day 8. The endpoint criteria was reached on day 14 and the

mice sacrificed. **FIG. 53J** shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with CD33 CAR T cells twice on days 5 and 12. **FIG. 53K**. provides images of the fLuc bioluminescence *in vivo* in each treatment group.

[0275] FIG. 54A shows the *in vivo* image results of a T cell dose escalation assay with FLT3 CAR T cells and CD33 CAR T cells alone or in combination. **FIG. 54B** shows the shows the fLuc bioluminescence in non-tumor bearing mice treated with non-engineered T cells. **FIG. 54C** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with non-engineered T cells. **FIG. 54D** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $9x10^6$ FLT3 T cells. **FIG. 54E** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $18x10^6$ FLT3 T cells. **FIG. 54F** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $0.625x10^6$ CD33 T cells. **FIG. 54G** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $1.25x10^6$ CD33 T cells. **FIG. 54H** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $2.5x10^6$ CD33 T cells. **FIG. 54I** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $5x10^6$ CD33 T cells. **FIG. 54J** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $10x10^6$ CD33 T cells. **FIG. 54K** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $4.5x10^6$ FLT3 CAR T cells and $2.5x10^6$ CD33 T cells. **FIG. 54L** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $9x10^6$ FLT3 CAR T cells and $5x10^6$ CD33 T cells. **FIG. 54M** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $18x10^6$ FLT3 CAR T cells and $10x10^6$ CD33 T cells.

[0276] FIG. 55A shows the fold change on day 7 in the relative bioluminescence of tumor MOLM-14 cells *in vivo* after treatment with the indicated CAR. **FIG. 55B** shows the fold change on day 11 in the relative bioluminescence of tumor MOLM-14 cells *in vivo* after treatment with the indicated CAR. **FIG. 55C** shows the bioluminescence of tumor MOLM-14 cells *in vivo* after treatment with the indicated CAR.

[0277] FIG. 56A provides schematics of indicated CARs with alternative hinge sequences. **FIG. 56B** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with MOLM-13 cells. **FIG. 56C** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with MOLM-14 cells. **FIG. 56D** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with MV4-11 cells. **FIG. 56E** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with SEM cells.

[0278] FIG. 57A shows the percent killing, as normalized to non-transduced CAR T cells by the indicated CAR T cells after incubation with MOLM-13 cells. **FIG. 57B** shows the IL-2 production of the indicated CAR after incubation with MOLM-13 cells. **FIG. 57C** shows the production of IFN-γ by the indicated CAR T cells after incubation with MOLM-13 cells. **FIG. 57D** shows the production of TNF-α by the indicated CAR T cells after incubation with MOLM-13 cells.

[0279] FIG. 58A shows *in vivo* survival after treatment with the indicated CAR T cells in the MOLM-13 model. **FIG. 58B** shows the bioluminescence of tumor MOLM-13 cells *in vivo* after treatment with the indicated CAR.

[0280] FIG. 59A shows expression of two bicistronic FLT3 and CD33 CARs in T cells, SB01266 and SB01659. **FIG. 59B** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with MOLM-13 cells. **FIG. 59C** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with MV4-11 cells. **FIG. 59D** shows the cytotoxicity and IL-2 production of the indicated CAR after incubation with SEM cells.

[0281] FIG. 60A shows FLT3 and CD33 staining in unengineered K562 cells. **FIG. 60B** shows FLT3 and CD33 staining in K562 cells engineered to express FLT3. **FIG. 60C** shows FLT3 and CD33 staining in K562 cells engineered to express CD33.

[0282] FIG. 61 shows the cytotoxity, IL-2 production, IFN-γ production, and TNF-α production by monovalent FLT3 CAR T cells, monovalent CD33 CAR T cells, and bicistronic FLT3 and CD33 CAR T cells incubated with K562 cells expressing FLT3 (upper panels), CD33 (middle panels), or a 1:1 mix of FLT3 and CD33 expressing cells (bottom panels).

[0283] FIG. 62 shows the cytotoxity, IL-2 production, IFN-γ production, and TNF-α production by monovalent FLT3 CAR T cells, monovalent CD33 CAR T cells, and bicistronic FLT3 and CD33 CAR T cells incubated with MOLM-13 cells (upper panels), MV4-11 cells (middle panels), or SEM cells (bottom panels).

[0284] FIG. 63A shows a schematic of a loop FLT3 bivalent CAR. **FIG. 63B** shows a schematic of a tandem FLT3 bivalent CAR. **FIG. 63C** shows the cytotoxicity of a monovalent FLT3 CAR T cell, the loop bivalent FLT3 CAR T cell, and the tandem FLT3 CAR T cell after incubation with MV4-11 cells (top) or SEM cells (bottom).

[0285] FIG. 64A shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the indicated CAR constructs after incubation with MOLM-13 cells. **FIG. 64B** shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the indicated CAR constructs after incubation with

MV4-11 cells. **FIG. 64C** shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the indicated CAR constructs after incubation with K562 cells expressing CD33.

[0286] **FIG. 65A** shows EMCN expression on the indicated cell types. **FIG. 65B** shows EMCN expression on HSCs and LSCs. **FIG. 65C** shows a summary of EMCN expression on HSCs and LSCs from multiple donors.

[0287] **FIG. 66A** shows FLT3, CD33, and FLT3/CD33 CAR NK cell killing of SEM cells. **FIG. 66B** shows IFN-γ secretion by FLT3 CAR NK cells after incubation with SEM cells. **FIG. 66C** shows TNF-α secretion by FLT3 CAR NK cells after incubation with SEM cells. **FIG. 66D** shows FLT3, CD33, and FLT3/CD33 CAR NK cell killing of PL-21 cells.

DETAILED DESCRIPTION

[0288] The practice of the present disclosure will employ, unless otherwise indicated, conventional methods of molecular biology, chemistry, biochemistry, virology, and immunology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Hepatitis C Viruses: Genomes and Molecular Biology (S.L. Tan ed., Taylor & Francis, 2006); Fundamental Virology, 3rd Edition, vol. I & II (B.N. Fields and D.M. Knipe, eds.); Handbook of Experimental Immunology, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., Blackwell Scientific Publications); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.).

Definitions

[0289] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art. 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 difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodologies by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 4th ed. (2012) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer-defined protocols and conditions unless otherwise noted.

[0290] As used herein, the singular forms "a," "an," and "the" include the plural referents unless the context clearly indicates otherwise. The terms "include," "such as," and the like are intended to convey inclusion without limitation, unless otherwise specifically indicated.

[0291] As used herein, the term "comprising" also specifically includes embodiments "consisting of" and "consisting essentially of" the recited elements, unless specifically indicated otherwise.

[0292] The term "about" indicates and encompasses an indicated value and a range above and below that value. In certain embodiments, the term "about" indicates the designated value ± 10%, ± 5%, or ± 1%. In certain embodiments, where applicable, the term "about" indicates the designated value(s) ± one standard deviation of that value(s).

[0293] As used herein, the term "activating an immunoresponsive cell" refers to the induction of signal transduction or changes in protein expression in the cell that results in the initiation of an immune response. For example, when CD3 chains cluster in response to ligand binding and immunoreceptor tyrosine-based inhibition motifs (ITAMs) a signal transduction cascade is produced. In certain embodiments, when an endogenous TCR or an exogenous CAR binds antigen, a formation of an immunological synapse occurs that includes clustering of many molecules near the bound receptor (e.g. CD4 or CD8, CD3γ/δ/ε/ζ, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated. This phosphorylation in turn initiates a T cell activation pathway ultimately activating transcription factors, such as NF-κB and AP-1. These transcription factors induce global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response.

[0294] As used herein, the term "stimulates an immunoresponsive cell" refers to a signal that results in a robust and sustained immune response. In various embodiments, this occurs after immune cell (e.g., T-cell) activation or concomitantly mediated through receptors including, but not limited to, CD28, CD137 (4-1BB), OX40, CD40 and ICOS. Without being bound to a particular theory, receiving multiple stimulatory signals is important to mount a robust and long-term T cell mediated immune response. Without receiving these stimulatory signals, T cells quickly become inhibited and unresponsive to antigen. While the effects of these co-stimulatory signals vary and remain partially understood, they generally result in increasing gene expression in order to generate long lived, proliferative, and anti-apoptotic T cells that robustly respond to antigen for complete and sustained eradication.

[0295] As used herein, the term "chimeric antigen receptor" or alternatively a "CAR" refers to a recombinant polypeptide construct comprising at least an extracellular antigen-binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as "an intracellular signaling domain") comprising a functional signaling domain derived from a stimulatory molecule as defined below. In some embodiments, the domains in the CAR polypeptide construct are in the same polypeptide chain, e.g., comprise a chimeric fusion protein. In some embodiments, the domains in the CAR polypeptide construct are not contiguous with each other, e.g., are in different polypeptide chains, e.g., as provided in an RCAR as described herein. In one aspect, the stimulatory molecule is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain comprises a primary signaling domain (e.g., a primary signaling domain of CD3-zeta). In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is chosen from 4-1BB (i.e., CD 137), CD27, ICOS, and/or CD28. In one aspect, the CAR. comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more co-stimulatory molecule(s) and a functional signaling domain derived from a stimulator}' molecule. In one aspect the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen-binding domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (e.g., an scFv) during cellular processing and localization of the CAR to the cellular membrane.

[0296] As used herein, the term "intracellular signaling domain" refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers. In some embodiments, the signaling domain of a chimeric receptor of the present disclosure is derived from a stimulatory molecule or co-stimulatory molecule described herein, or is a synthesized or engineered signaling domain.

[0297] The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources. Antibodies can be tetramers of immunoglobulin molecules.

[0298] As used herein, the term "antibody fragment" refers to at least one portion of an intact antibody, or recombinant variants thereof, and refers to the antigen-binding domain, e.g., an antigenic determining variable region of an intact antibody, that is sufficient to confer recognition and specific binding of the antibody fragment to a target, such as an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, and Fv fragments, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, and multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23: 1126-1 136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3)(see U.S. Patent No. : 6,703,199, which describes fibronectin polypeptide minibodies).

[0299] As used herein, the term "single-chain variable fragment" or "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain poly peptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order,

e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL- linker- VH or may comprise VH-linker-VL.

[0300] The term "complementarity determining region" or "CDR," as used herein, refers to the sequences of ammo acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise ammo acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al, (1997) JMB 273,927-948 ("Chothia" numbering scheme), or a combination thereof. Under the Kabat numbering scheme, in some embodiments, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under the Chothia numbering scheme, in some embodiments, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the VL are numbered 26-32 (LCDRl), 50-52 (LCDR2), and 91-96 (LCDR3). In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both. For instance, in some embodiments, the CDRs correspond to amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in a VH, e.g., a mammalian VH, e.g., a human VH; and ammo acid residues 24-34 (LCDRl), 50-56 (LCDR2), and 89-97 (LCDR3) in a VL, e.g., a mammalian VL, e.g., a human VL.

[0301] The portion of the chimeric receptor of the present disclosure comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen-binding domain is expressed as part of a contiguous polypeptide chain including, for example, scFv antibody fragments, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains ,a humanized antibody, a bispecific antibody, an antibody conjugate (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al, 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al, 1988, Science 242:423-426). In one aspect, the antigen-binding domain of a

chimeric receptor of the present disclosure comprises an antibody fragment. In a further aspect, the chimeric receptor comprises an antibody fragment that comprises an scFv.

[0302] As used herein, the term "antibody heavy chain" refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

[0303] As used herein, the term "antibody light chain" refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ) light chains refer to the two major antibody light chain isotypes.

[0304] As used herein, the term "recombinant antibody" refers to an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage or yeast expression system. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DN A molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using recombinant DNA or amino acid sequence technology which is available and well known in the art.

[0305] As used herein, the term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen.

[0306] As used herein, the term "anti-tumor effect" or "anti-tumor activity" refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, decrease in tumor cell proliferation, decrease in tumor ceil survival, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the present disclosure in prevention of the occurrence of tumor in the first place.

[0307] As used herein, the term "autologous" refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

[0308] As used herein, the term "allogeneic" refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more

individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some embodiments, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically.

[0309] As used herein, the term "affinity" refers to a measure of binding strength. Without being bound to theory, affinity depends on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and on the distribution of charged and hydrophobic groups. Affinity also includes the term "avidity," which refers to the strength of the antigen- antibody bond after formation of reversible complexes. Methods for calculating the affinity of an antibody for an antigen are known in the art, including use of binding experiments to calculate affinity. Antibody activity in functional assays (e.g., flow cytometry assay) is also reflective of antibody affinity. Antibodies and affinities can be phenotypically characterized and compared using functional assays (e.g., flow cytometry assay).

[0310] As used herein, the term "immunosuppressive activity" refers to the induction of signal transduction or changes in protein expression in a cell, such as an activated immunoresponsive cell, that results in a decrease in an immune response. Non-limiting examples of polypeptides known to suppress or decrease an immune response via their binding include CD47, PD-1, CTLA-4, and their corresponding ligands, including SIRPa, PD-L1, PD-L2, B7-1, and B7-2. Such polypeptides may be present in the tumor microenvironment and can inhibit immune responses to neoplastic cells. In various embodiments, inhibiting, blocking, or otherwise antagonizing the interaction of immunosuppressive polypeptides and/or their ligands may enhance the immune response of the immunoresponsive cell.

[0311] As used herein, the term “enzymatic inhibitory domain” refers to a protein domain that inhibits an intracellular signal transduction cascade, for example a native T cell activation cascade. In some embodiments, the enzymatic inhibitory domain of a chimeric inhibitory receptor of the present disclosure comprises at least a portion of an extracellular domain, a transmembrane domain, and/or an intracellular domain. In some embodiments, the enzymatic inhibitory domain comprises at least a portion of an enzyme. In some embodiments, the enzyme is selected from CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP (see e.g., Stanford et al., Regulation of TCR signaling by tyrosine phosphatases: from immune homeostasis to autoimmunity, Immunology, 2012 Sep; 137(1): 1-19). In some embodiments, the portion of the enzyme comprises an enzyme domain(s), an enzyme fragment(s), or a mutant(s) thereof.

In some embodiments, the portion of the enzyme is a catalytic domain of the enzyme. In some embodiments, the enzyme domain(s), enzyme fragment(s), or mutants(s) thereof are selected to maximize efficacy and minimize basal inhibition.

[0312] As used herein, the term "immunostimulatory activity" refers to induction of signal transduction or changes in protein expression in a cell, such as an activated immunoresponsive cell, that results in an increase in an immune response. Immunostimulatory activity may include pro-inflammatory activity. Non-limiting examples of polypeptides known to stimulate or increase an immune response via their binding include CD28, OX-40, 4-1BB, and their corresponding ligands, including B7-1, B7-2, OX-40L, and 4-1BBL. Such polypeptides may be present in the tumor microenvironment and can activate immune responses to neoplastic cells. In various embodiments, promoting, stimulating, or otherwise agonizing pro-inflammatory polypeptides and/or their ligands may enhance the immune response of the immunoresponsive cell.

[0313] Isolated nucleic acid molecules of the present disclosure include any nucleic acid molecule that encodes a polypeptide of the present disclosure, or fragment thereof. Such nucleic acid molecules need not be 100% homologous or identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Nucleic acids having "substantial identity" or "substantial homology" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. As used herein, "hybridize" refers to pairing to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. For example, stringent salt concentration may be less than about 750 mM NaCl and 75 mM trisodium citrate, less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide or at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, at least about 37°C, or at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency may be accomplished by combining these various conditions as needed.

[0314] By "substantially identical" or "substantially homologous" is meant a polypeptide or nucleic acid molecule exhibiting at least about 50% homologous or identical to a reference

amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least about 60%, about80%,about 85%, about 90%, about 95%,about 99%, or about 100% homologous or identical at the amino acid level or nucleic acid to the sequence used for comparison. Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e-3 and e-100 indicating a closely related sequence.

[0315] As used herein, the term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of rnRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non- coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0316] As used herein, the term "ligand" refers to a molecule that binds to a receptor. In particular, the ligand binds a receptor on another cell, allowing for cell-to-cell recognition and/or interaction.

[0317] The terms "effective amount" and "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. In some embodiments, an "effective amount" or a "therapeutically effective amount" is an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis of a disease or disorder of interest, e.g., a myeloid disorder.

[0318] As used herein, the term "immunoresponsive cell" refers to a cell that functions in an immune response (e.g., an immune effector response) or a progenitor, or progeny thereof. Examples of immune effector cells include, without limitation, alpha/beta T cells, gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

[0319] As used herein, the term "immune effector response" or "immune effector function" refers to a function or response, e.g., of an immunoresponsive cell, that enhances or promotes an immune attack of a target cell. For example, an immune effector function or response may refer to a property of a T cell or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

[0320] As used herein, the term "flexible polypeptide linker" or "linker" refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is a positive integer equal to or greater than 1. For example, n=l, n=2, n=3, n=4, n=5 and n=6, n=7, n=8, n=9 and n=10. In some embodiments, the flexible polypeptide linkers include, but are not limited to, $(Gly_4Ser)_4$ or $(Gly_4Ser)_3$. In other embodiments, the linkers include multiple repeats of (Gly_2Ser), (GlySer) or (Gly_3Ser). Also included within the scope of the present disclosure are linkers described, for example, in WO2012/138475.

[0321] As used herein, the term "specifically binds" refers to a polypeptide, or fragment thereof, that recognizes and binds a biological molecule of interest (e.g., a polypeptide), but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the present disclosure. In certain embodiments, "specifically binds" refers to binding of, for example, an antibody to an epitope or antigen or antigenic determinant in such a manner that binding can be displaced or

competed with a second preparation of identical or similar epitope, antigen or antigenic determinant.

[0322] As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a CAR of the present disclosure). In some embodiments, the terms "treat," "treatment", and "treating" refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments, the terms "treat", "treatment", and "treating" refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In some embodiments the terms "treat", "treatment", and "treating" refer to the reduction or stabilization of tumor size or cancerous cell count.

[0323] As used herein, the term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

[0324] Other aspects of the present disclosure are described in the following sections and are within the ambit of the claimed invention.

Other interpretational conventions

[0325] Ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0326] Unless otherwise indicated, reference to a compound that has one or more stereocenters intends each stereoisomer, and all combinations of stereoisomers, thereof.

Myeloid antigens

[0327] Certain aspects of the present disclosure relate to chimeric receptors and cells, such as immunoresponsive cells, that have been genetically modified to express one or more of such chimeric receptors that bind to an antigen of interest, and to methods of using such receptors and cells to treat and/or prevent myeloid malignancies, such as AML, and other pathologies where an antigen-specific immune response is desired. Malignant cells have developed a series of mechanisms to protect themselves from immune recognition and elimination. The

present disclosure provides immunogenicity within the tumor microenvironment for treating such malignant cells.

[0328] Certain aspects of the present disclosure related to chimeric receptors that specifically bind one or more antigens expressed on a myeloid cell useful for treating myeloid malignancies, and to immunoresponsive cells genetically modified to express such chimeric receptors. Myeloid malignancies are clonal diseases caused by dysfunction of hematopoietic stem cells or progenitor cells, resulting from genetic and epigenetic alterations that disrupt key processes such as cell proliferation and differentiation. Myeloid malignancies can be chronic or acute. Chronic diseases include myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML). Acute diseases include acute myeloid leukemia (AML).

[0329] AML is characterized by the rapid growth of abnormal leukocytes which accumulate in the bone marrow and disrupt the production of normal blood cells. Symptoms of AML include fatigue, shortness of breath, increased susceptibility of infection, and easy bruising and bleeding. The majority of AML cases occur de novo, but some cases can be secondary to a chronic disease. There are eight different subtypes of AML that are based on the type of cell from which the leukemia is originated and how mature the cells are. The AML subtypes include myeloblastic-undifferentiated (M0), myeloblastic-minimal maturation (M1) , myeloblastic-full maturation (M2), promyeloctic (M3), myelomonocytic (M4), monocytic (M5), erythroleukemia (M6), and megakaryocyte (M7).

[0330] In certain embodiments, the present disclosure relates to AML antigens and combinations of AML antigen that are suitable for use in chimeric receptors (e.g., chimeric TCRs or CARs) to increase efficacy and reduce off-tumor toxicity in the treatment of AML.

[0331] **Table 1** provides AML antigens suitable for use in chimeric receptors described in the methods and compositions presented herein.

Table 1. AML Antigens

Antigen	UniProt Accession No.	Name	Short Description
FLT3	P36888	Fms Related Tyrosine Kinase 3	A receptor tyrosine kinase that regulates hematopoiesis and involved in apoptosis, proliferation, and differentiation of hematopoietic cells in bone marrow.
MS4A3/CD20L	Q96HJ5	Membrane Spanning 4-Domains A3	A member of the membrane-spanning 4A gene family, which display unique expression patterns among meatopoietic cells and nonlymphoid tissues. This protein likely plays a role in signal transduction and may function as a subunit associate with other receptor complexes.
CD33	P20138	CD33 Molecule	A putative adhesion molecule of myelomonocytic-derived cells, and may act as an inhibitory receptor

Table 1. AML Antigens			
Antigen	**UniProt Accession No.**	**Name**	**Short Description**
CLEC12A/CD371	Q5QGZ9	C-Type Lectin Domain Family 12 Member A	A cell surface receptor negative regulator of granulocyte and monocyte function, and modulates signaling cascades and tyrosine phosphorylation of target MAP kinases.
ADGRE2/EMR2	Q9UHX3	Adhesion G Protein-Coupled Receptor E2	A transmembrane receptor that promotes cell-cell adhesion through interaction with chondroitin sulfate chains.
SLC22A16	Q86VW1	Solute Carrier Family 22 Member 16	A carnitine transporter that is partially sodium-ion dependent.
CD123/IL3RA	P26951	Interleukin 3 Receptor Subunit Alpha	The ligand specific alpha subunit of a heterodimeric cytokine receptor for interleukin 3.
MLC1	Q15049	Megalencephalic Leukoencephalopathy With Subcortical Cysts 1	Unknown gene product, but has homology to other proteins suggest it to be an integral membrane transporter.
SPNS3	Q6ZMD2	Sphingolipid Transporter 3 (Putative)	A sphingolipid transporter.
GAPT	Q8N292	GRB2 Binding Adaptor Protein, Transmembrane	A transmembrane protein that has been shown to negatively regulate B-cell proliferation.
LAT2	Q9GZY6	Linker For Activation Of T Cells Family Member 2	Involved in receptor mediated signaling in mast cells, B cells, and myeloid cells through recruitment of GRB2 (Growth Factor Receptor Bound Protein 2)
PIEZO1	Q92508	Piezo Type Mechanosensitive Ion Channel Component 1	Subunit of a mechanosensitive ion channel that links mechanical forces to biological signals.
CD38	P28907	CD38 Molecule	A transmembrane glycoprotein that synthesizes and hydrolyzes cyclic ADP-ribose, which is an intracellular calcium ion mobilizing messenger.
EMB	Q6PCB8	Embigin	A transmembrane glycoprotein that is a member of the immunoglobulin superfamily. May be involved in cell growth and development
CD131/CSF2RB	P32927	Colony Stimulating Factor 2 Receptor Beta Common Subunit	The common beta chain of the high affinity receptor for IL3, IL5, and CSF.
LILRA2/CD85H	Q8N149	Leukocyte Immunoglobulin Like Receptor A2	A receptor that inhibits dendritic cell differentiation and antigen presentation and suppresses innate immune response. Predominantly expressed on monocytes and B cells.
SLC17A9	Q9BYT1	Solute Carrier Family 17 Member 9	Transmembrane protein that is involved in transport of small molecules, participating in vesicular uptake, storage, and secretion of ATP.
MYADM	Q96S97	Myeloid Associated Differentiation Marker	Hematopoietic-associate marker gene expressed in multipotent progenitor cells and up-regulated during myeloid differentiation.
CD300LF	Q8TDQ1	CD300 Molecule Like Family Member F	A cell surface glycoprotein that is involved in regulation of immune response. Acts as inhibitory receptor for myeloid cells and mast cells.
CD244	Q9BZW8	CD244 Molecule	A cell surface receptor expressed on NK cells and some T cells, and mediates non-MHC restricted killing. Ligand is CD48
CD93	Q9NPY3	CD93 Molecule	A cell surface glycoprotein and thought to be involved in intercellular adhesion and clearance of apoptotic cells.

Table 1. AML Antigens			
Antigen	**UniProt Accession No.**	**Name**	**Short Description**
CD117/CKIT	P10721	KIT Proto-Oncogene Receptor Tyrosine Kinase	A tyrosine-protein kinase that acts as a cell-surface receptor for the cytokine KITLG/SCF and plays an essential role in regulation of cell survival and proliferation, hematopoiesis, stem cell maintenance, gametogenesis, mas cell development, migration and function, and in melanogenesis.
CMTM7	Q96FZ5	CKLF Like MARVEL Transmembrane Domain Containing 7	A gene that belongs to the chemokine-like factor gene superfamily, and acts as a tumor suppressor and epidermal growth factor receptor during tumor pathogenesis.
CYBA	P13498	Cytochrome B-245 Alpha Chain	Critical component of membrane-bound oxidase of phagocytes that generates superoxide.
HCK	P08631	HCK Proto-Oncogene, Src Family Tyrosine Kinase	A tyrosine kinase that is primarily hemopoietic, particularly in cells of myeloid and B-lymphoid lineages.
ICAM3	P32942	Intercellular Adhesion Molecule 3	An intercellular adhesion molecule that binds to leukocyte adhesion LFA-1 protein. This protein is constitutively and abundantly expressed on all leukocytes and may also be a potent signaling molecule.
LRRC37A2	A6NM11	Leucine Rich Repeat Containing 37 Member A2	A protein that belongs to the LRRC37 gene family. Contains leucine-rich repeat motifs and predicted to mediate protein-ligand interactions.
ITGAM	P11215	Integrin Subunit Alpha M	The integrin alpha M chain, and is implicated in various adhesive interaction of monocytes, macrophages, and granulocytes.
ITGB2	P05107	Integrin Subunit Beta 2	An integrin beta chain that combines with multiple alpha chains to form multiple heterodimers. Involved in cell adhesion and cell-surface mediated signaling, and plays role in immune response.
LILRA1	O75019	Leukocyte Immunoglobulin Like Receptor A1	This protein is an activating member of the leukocyte immunoglobulin-like receptor (LIR) family and may act as a receptor for class I MHC antigens. Predominantly expressed in B cells and contributes to the regulation of immune responses.
PRTN3	P24158	Proteinase 3	Serine protease that degrades elastin, fibronectin, laminin, vitronectine, and collagen. May play a role in neutrophil transendothelial migration.
CARD9	Q9H257	Caspase Recruitment Domain Family Member 9	An adapter protein that plays a role in innate immune response to a number of intracellular pathogens. CARD is a domain know play a regulatory role in cell apoptosis.
SIGLEC5	O15389	Sialic Acid Binding Ig Like Lectin 5	A cell surface adhesion molecule that mediates sialic-acid dependent binding to cells. A member of CD33-related subset of siglecs and inhibits activation of several cells, including monocytes, macrophages, and neutrophils.
SELL	P14151	Selectin L	A cell surface adhesion molecule required for binding and subsequent rolling of leukocytes on endothelial cells migrating into secondary lymphoid organs and inflammation sites.
MLKL	Q8NB16	Mixed Lineage Kinase Domain Like Pseudokinase	A pseudokinase that plays a role in TNF-induced necroptosis, a programmed cell death process.
INPP5D	Q92835	Inositol Polyphosphate-5-Phosphatase D	A phosphatidylinositol phosphatase that acts overall as a negative regulator of myeloid cell proliferation and survival.
APBB1IP	Q7Z5R6	Amyloid Beta Precursor Protein Binding Family B Member 1	Appears to function in signal transduction from Ras activation to actin cytoskeletal remodeling.

Table 1. AML Antigens			
Antigen	**UniProt Accession No.**	**Name**	**Short Description**
ITGA4	P13612	Integrin Subunit Alpha 4	Integrins are heterodimeric integral membrane proteins and functions in cell surface adhesion and signaling. This protein is the alpha 4 subunit which associates with a beta 1 or beta 7 subunit to form an integrin that may play a role in cell motility and migration.
C3AR1	Q16581	Complement C3a Receptor 1	Receptor for chemotactic and inflammatory peptide anaphylatoxin C3a. This receptor stimulates chemotaxis, granule enzyme release and superoxide anion production.
ITGA5	P08648	Integrin Subunit Alpha 5	Integrins are heterodimeric integral membrane proteins and functions in cell surface adhesion and signaling. This protein is the alpha 5 subunit which associates with a beta 1 subunit, and may play a role in tumor invasion.
FMNL1	O95466	Formin Like 1	A formin-related protein, and is implicated in morphogenesis, cytokinesis, and cell polarity. May play a role in control of cell motility and survival of macrophages.
VSTM1	Q6UX27	V-set and transmembrane domain-containing protein 1	VSTM1 is an inhibitory receptor encoded within the leukocyte receptor complex (LRC) on chromosome 19 that contains 2 immunoreceptor tyrosine-based inhibitory motifs (ITIMs).
PRAM1	Q96QH2	PML-RARA-regulated adapter molecule 1	PRAM1 is similar to FYN binding protein (FYB/SLAP-130), which is an adaptor protein involved in T cell receptor mediated signaling. PRAM1 is expressed and regulated during normal myelopoiesis.
IL1RAP	Q9NPH3	Interleukin-1 receptor accessory protein	IL1RAP induces synthesis of acute phase and proinflammatory proteins during infection, tissue damage, or stress, by forming a complex at the cell membrane with an interleukin 1 receptor and an accessory protein
CCR1/CD191	P32246	C-C chemokine receptor type 1	CCR1 is a member of the beta chemokine receptor family, which belongs to G protein-coupled receptors. Chemokines and their receptors, which mediate signal transduction, are critical for the recruitment of effector immune cells to the site of inflammation.
LILRB2	Q8N423	Leukocyte immunoglobulin-like receptor subfamily B member 2	LILRB2 is a member of the leukocyte immunoglobulin-like receptor (LIR) family that contains two or four extracellular immunoglobulin domains, a transmembrane domain, and two to four cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs). LILRB2 is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response.
CD70	P32970	CD70 Molecule	CD70 is a ligand for CD27 and is expressed on highly activated lymphocytes (e.g., T-cell and B-cell lymphomas).

[0332] In some embodiments, the AML antigen is an FLT3 antigen. In some embodiments, the AML antigen is an MS4A3 antigen. In some embodiments, the AML antigen is a CD33 antigen. In some embodiments, the AML antigen is a CLEC12A antigen. In some embodiments, the AML antigen is a CD312/ADGRE2 antigen. In some embodiments, the AML antigen is an SLC22A16 antigen. In some embodiments, the AML antigen is a CD123/ILR3RA antigen. In some embodiments, the AML antigen is an LAT2 antigen. In some embodiments, the AML antigen is a PIEZO1/FAM38A antigen. In some embodiments, the AML antigen is a CD38 antigen. In some embodiments, the AML antigen is an EMB

antigen. In some embodiments, the AML antigen is a CD131/CSF2RB antigen. In some embodiments, the AML antigen is a P2RY8 antigen. In some embodiments, the AML antigen is a LILRA2/CD85H antigen. In some embodiments, the AML antigen is an SLC17A9 antigen. In some embodiments, the AML antigen is an MYADM antigen. In some embodiments, the AML antigen is a CD300LF antigen. In some embodiments, the AML antigen is a CD244/SLAMF4 antigen. In some embodiments, the AML antigen is a PLAUR antigen. In some embodiments, the AML antigen is a CD93 antigen. In some embodiments, the AML antigen is an SPNS3 antigen. In some embodiments, the AML antigen is a GAPT antigen. In some embodiments, the AML antigen is a RASGRP4 antigen. In some embodiments, the AML antigen is a CD117/c-Kit antigen. In some embodiments, the AML antigen is a CD123/ILR3RA antigen. In some embodiments, the AML antigen is an SLC34A2 antigen. In some embodiments, the AML antigen is a VSTM1 antigen. In some embodiments, the AML antigen is an MLC1 antigen. In some embodiments, the AML antigen is a PRAM1 antigen. In some embodiments, the AML antigen is an HCK antigen. In some embodiments, the AML antigen is an ICAM3 antigen. In some embodiments, the AML antigen is a LRRC37A2 antigen. In some embodiments, the AML antigen is an ITGAM antigen. In some embodiments, the AML antigen is an ITGB2 antigen. In some embodiments, the AML antigen is a LILRA1 antigen. In some embodiments, the AML antigen is a PRTN3 antigen. In some embodiments, AML antigen is a CARD9 antigen. In some embodiments, the AML antigen is an SIGLEC5 antigen. In some embodiments, the AML antigen is a SELL antigen. In some embodiments, the AML antigen is a MLKL antigen. In some embodiments, the AML antigen is an INPP5D antigen. In some embodiments, the AML antigen is an APBB1IP antigen. In some embodiments, the AML antigen is an ITGA4 antigen. In some embodiments, the AML antigen is a C3AR1 antigen. In some embodiments, the AML antigen is an ITGA5 antigen. In some embodiments, the AML antigen is an FMNL1 antigen. In some embodiments, the AML antigen is an IL1RAP antigen. In some embodiments, the AML antigen is a CCR1/CD191 antigen. In some embodiments, the AML antigen is an LILRB2 antigen. In some embodiments, the AML antigen is a CD70 antigen.

Chimeric receptors

[0333] Certain aspects of the present disclosure relate to chimeric receptors and nucleic acids that encode such chimeric receptors that bind to an antigen of interest.

Antibodies and Antigen-binding fragments

[0334] In some embodiments, chimeric receptors comprise one or more of the amino acid sequences listed in Table A1 or Table A2. Table A1 provides the variable domains of an antibody heavy chain or light chain. The CDRs were determined using the Kabat method and are underlined in Table A1 for each variable heavy chain or variable light chain and shown in Table A2. In some embodiments, nucleic acids encoding any of the chimeric receptors of the present disclosure comprise one or more of the nucleic acid sequences listed in Table B.

Table A1

Amino Acid Sequence	SEQ ID NO:	Description
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLE WMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTA VYYCARVVAAAVADYWGQGTLVTVSS	1	Heavy chain variable domain of anti-FLT3 antibody D4-3
DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPG QSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CMQSLQTPFTFGPGTKVDIK	2	Light chain variable domain of anti-FLT3 antibody D4-3
EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLE WMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTA VYYCATFALFGFREQAFDIWGQGTTVTVSS	3	Heavy chain variable domain of anti-FLT3 antibody NC7
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSY STPFTFGPGTKVDIK	4	Light chain variable domain of anti-FLT3 antibody NC7
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLE WMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTA VYYCARGVGAHDAFDIWGQGTTVTVSS	5	Heavy chain variable domain of anti-FLT3 antibody EB10
DVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGNNYLDWYLQKPG QSPQLLIYLGSNRASGVPDRFSGSGSDTDFTLQISRVEAEDVGVYY CMQGTHPAISFGQGTRLEIK	6	Light chain variable domain of anti-FLT3 antibody EB10
QVQLQQPGAELVKPGASLKLSCKSSGYTFTSYWMHWVRQRPGHGLE WIGEIDPSDSYKDYNQKFKDKATLTVDRSSNTAYMHLSSLTSDDSA VYYCARAITTTPFDFWGQGTTLTVSS	7	Heavy chain variable domain of anti-FLT3 antibody 4G8
DIVLTQSPATLSVTPGDSVSLSCRASQSISNNLHWYQQKSHESPRL LIKYASQSISGIPSRFSGSGSGTDFTLSINSVETEDFGVYFCQQSN TWPYTFGGGTKLEIKR	8	Light chain variable domain of anti-FLT3 antibody 4G8
QVTLKESGPTLVKPTETLTLTCTLSGFSLNNARMGVSWIRQPPGKC LEWLAHIFSNDEKSYSTSLKNRLTISKDSSKTQVVLTMTNVDPVDT ATYYCARIVGYGSGWYGFFDYWGQGTLVTVSS	9	Heavy chain variable domain of anti-FLT3 antibody FL_39
DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKR LIYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHN SYPLTFGCGTKVEIK	10	Light chain variable domain of anti-FLT3 antibody FL_39
QVTLKESGPVLVKPTETLTLTCTVSGFSLRNARMAVSWIRQPPGKT LEWLAHIFSNDEKSYSTSLKSRLTISKDTSKSQVVLTMTNMDPVDT ATYYCARIVGYGSGWYGYFDYWGQGTLVTVSS	11	Heavy chain variable domain of anti-FLT3 antibody FL_16
DIQMTQSPSSVSASVGDRVTITCRASQDIRYDLAWYQQKPGKAPKR LIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHN FYPLTFGGGTKVEIK	12	Light chain variable domain of anti-FLT3 antibody FL_16
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLE WVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTA VYYCANLAPWAAYWGQGTLVTVSS	13	Heavy chain variable domain of anti-FLT3 antibody ml0006
EIVLTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPG QSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYY CMQALQTPHTFGQGTKLEIK	14	Light chain variable domain of anti-FLT3 antibody ml0006

Table A1		
Amino Acid Sequence	**SEQ ID NO:**	**Description**
QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGLHWVRQSPGKGLE WLGVIWSGGSTDYNAAFISRLSISKDNSKSQVFFKMNSLQADDTAI YYCARKGGIYYANHYYAMDYWGQGTSVTVSS	15	Heavy chain variable domain of anti-FLT3 antibody BV10
DIVMTQSPSSLSVSAGEKVTMSCKSSQSLLNSGNQKNYMAWYQQKP GQPPKLLIYGASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVY YCQNDHSYPLTFGAGTKLELKR	16	Light chain variable domain of anti-FLT3 antibody BV10
QVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPGQGLE WIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTA VYYCARGRPAMDYWGQGTLVTVSS	17	Heavy chain variable domain of anti-CD33 antibody lintuzumab
DIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQQKPGK APKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYC QQSKEVPWTFGQGTKVEIK	18	Light chain variable domain of anti-CD33 antibody lintuzumab
EVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNIHWVRQAPGQSLE WIGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDTA FYYCVNGNPWLAYWGQGTLVTVSS	19	Heavy chain variable domain of anti-CD33 antibody gemtuzumab
DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQQKPGK APKLLMYAASNQGSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYC QQTKEVPWSFGQGTKVEVKR	20	Light chain variable domain of anti-CD33 antibody gemtuzumab
QVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGL EWIGEIYHSGSPDYNPSLKSRVTISVDKSRNQFSLKLSSVTAADTA VYYCAKVSTGGFFDYWGQGTLVTVSS	21	Heavy chain variable domain of anti-CLEC12A antibody SC02-357
EIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY STPPTFGPGTKVEIK	22	Light chain variable domain of anti-CLEC12A antibody SC02-357
QVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGL EWIGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTA VYYCARSSSGGFFDYWGQGTLVTVSS	23	Heavy chain variable domain of anti-CLEC12A antibody SC02-378
EIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY STPPTFGQGTKVEIK	24	Light chain variable domain of anti-CLEC12A antibody SC02-378
EIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSY STPPTFGQGTKVEIK	25	Heavy chain variable domain of anti-CLEC12A antibody SC02-161
QVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGL EWIGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTA VYYCARQTTAGSFDYWGQGTLVTVSS	26	Light chain variable domain of anti-CLEC12A antibody SC02-161
GGGGSGGGGSGGGGS	27	scFv linker 1
EAAAKEAAAKEAAAKEAAAK	74	scFv linker 2
GGGGSGGGGSGGGGSGGGGSGGGGS	218	scFv linker 3
GGGGSGGGGSGGGGS	219	scFv linker 4

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAGCCAGGCGCTTCCGTCAAGGTGA GTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGGGCACGGCAGGCCCC TGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGCTACGCA CAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGG AATTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGC TGCTGTGGCAGACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGC	28	Heavy chain variable domain of anti-FLT3 antibody D4-3
GACGTAGTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCA TCTCATGTAGATCCTCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTA TTTGCAGAAACCGGGCCAGAGCCCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCT GGTGTCCCTGATAGATTCTCCGGGAGTGGATCAGGTACGGATTTTACACTGAAGATCAGCA	29	Light chain variable domain of anti-FLT3 antibody D4-3

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATGCAATCACTCCAGACCCCGTTTAC CTTTGGGCCTGGAACAAAGGTAGATATTAAA		
GAGGTTCAACTGGTACAAAGCGGAGCCGAGGTAAAGAAACCAGGGAGTAGCGTCAAAGTGT CCTGCAAAGCCTCAGGCGGCACATTCAGTAGCTATGCTATTTCATGGGTACGCCAAGCACC AGGACAGGGGCTGGAGTGGATGGGCGGGATTATCCCCATCTTCGGTACGGCAAACTATGCA CAAAAGTTCCAGGGACGAGTCACCATCACGGCTGATAAGTCCACCTCCACCGCCTATATGG AGCTGAGTTCCCTTCGGAGCGAGGATACTGCTGTGTATTATTGTGCCACGTTCGCACTGTT CGGTTTTCGGGAGCAGGCGTTTGATATTTGGGGACAAGGCACAACGGTCACGGTCAGTTCA	30	Heavy chain variable domain of anti-FLT3 antibody NC7
GACATTCAGATGACCCAGAGTCCCTCTTCATTGAGTGCGAGCGTCGGTGATCGGGTTACGA TAACCTGTAGGGCCTCCCAAAGTATATCATCATATTTGAACTGGTACCAACAGAAACCTGG GAAAGCGCCGAAGCTCCTTATCTATGCTGCCAGCTCTTTGCAAAGCGGTGTGCCCTCACGG TTCTCCGGTAGTGGGTCCGGGACCGACTTCACTTTGACCATCAGCAGCCTTCAGCCAGAGG ATCTTGCCACTTATTACTGCCAGCAATCTTATAGCACACCGTTTACATTCGGTCCAGGCAC AAAGGTAGACATTAAG	31	Light chain variable domain of anti-FLT3 antibody NC7
GAGGTACAGCTTGTGCAGAGTGGAGCAGAAGTTAAAAAACCCGGAGCTTCCGTGAAGGTAA GCTGCAAGGCTTCAGGATATACATTTACTAGCTACTACATGCACTGGGTCCGCCAAGCTCC GGGCCAAGGCCTTGAATGGATGGGCATCATAAATCCCAGTGGAGGCTCAACGAGCTATGCA CAAAAGTTCCAAGGGCGCGTTACCATGACGCGCGACACCAGCACGTCCACCGTCTATATGG AACTCTCAAGTTTGCGATCTGAAGATACGGCTGTCTACTATTGCGCACGAGGGGTCGGAGC GCATGACGCCTTCGACATCTGGGGACAAGGGACTACAGTAACTGTGTCAAGC	32	Heavy chain variable domain of anti-FLT3 antibody EB10
GATGTTGTTATGACACAGTCTCCCCTCTCTTTGCCTGTTACGCCTGGCGAGCCCGCCTCTA TTTCTTGTCGATCTAGTCAGAGCCTGCTGCATTCTAATGGAAACAACTATTTGGACTGGTA CTTGCAAAAGCCGGGTCAAAGTCCC	33	Light chain variable domain of anti-FLT3 antibody EB10
CAAGTCCAACTTCAGCAGCCAGGCGCTGAGTTGGTTAAACCGGGCGCAAGCCTCAAACTTA GTTGCAAGTCATCCGGATATACTTTCACGTCTTATTGGATGCATTGGGTACGACAAAGACC TGGTCACGGCCTCGAATGGATTGGCGAAATCGACCCGTCAGACAGCTACAAGGATTACAAC CAGAAATTCAAAGATAAGGCAACACTTACTGTGGATCGCTCAAGTAACACGGCTTACATGC ACCTCTCTTCACTCACGTCTGACGACAGTGCGGTGTATTATTGCGCCCGCGCTATTACAAC AACCCCTTTCGATTTCTGGGGCCAGGGTACTACGCTCACAGTCTCATCC	34	Heavy chain variable domain of anti-FLT3 antibody 4G8
GATATCGTCCTCACCCAATCCCCGGCTACTTTGAGTGTAACACCAGGCGACAGCGTGTCAC TGTCATGCCGAGCCTCCCAGTCAATCAGCAATAATCTGCATTGGTATCAACAGAAATCACA CGAATCCCCCCGACTTTTGATAAAGTATGCGTCACAGTCCATATCAGGCATTCCCAGTAGG TTTTCAGGCAGTGGTTCAGGTACTGACTTCACCCTCTCCATTAACTCTGTAGAAACAGAGG ACTTTGGCGTCTACTTCTGTCAGCAATCCAACACCTGGCCTTATACATTCGGCGGCGGCAC TAAGCTGGAAATTAAGAGA	35	Light chain variable domain of anti-FLT3 antibody 4G8
CAAGTAACCCTTAAAGAGTCCGGCCCCACTTTGGTTAAACCTACTGAAACACTTACACTCA CATGCACATTGTCCGGCTTTTCACTCAACAACGCAAGGATGGGTGTGTCCTGGATTCGCCA GCCCCCTGGAAAATGTTTGGAATGGCTCGCTCATATATTTAGCAACGACGAGAAAAGTTAC TCAACTTCACTCAAGAACCGCCTCACTATTAGCAAAGATTCCTCCAAAACCCAAGTAGTTC TGACAATGACGAATGTAGACCCAGTCGATACTGCAACTTACTATTGCGCACGAATAGTCGG TTACGGGAGTGGCTGGTATGGGTTTTTCGACTATTGGGGACAGGGCACTCTTGTAACAGTA AGTAGC	36	Heavy chain variable domain of anti-FLT3 antibody FL39
GACATCCAGATGACTCAATCTCCATCTAGCCTCTCAGCGTCTGTGGGCGATCGAGTCACCA TCACCTGTAGGGCTTCCCAGGGTATAAGGAATGATTTGGGCTGGTACCAGCAAAAACCGGG TAAGGCTCCGAAACGACTGATATACGCAGCTTCTACGTTGCAATCCGGGGTGCCATCCAGA TTTAGTGGCAGCGGGAGCGGTACTGAGTTTACGCTGACTATCTCCTCACTTCAGCCAGAGG ATTTCGCCACGTACTATTGTCTGCAACACAACTCCTATCCGCTGACCTTCGGGTGCGGGAC AAAGGTGGAAATTAAA	37	Light chain variable domain of anti-FLT3 antibody FL39
CAAGTGACCTTGAAGGAGTCAGGGCCAGTGTTGGTAAAACCTACTGAGACTCTCACGTTGA CATGCACGGTATCAGGTTTCAGCCTGAGGAACGCTCGGATGGCCGTCAGTTGGATACGCCA GCCGCCAGGCAAAACTCTTGAATGGTTGGCGCACATATTCAGTAACGACGAGAAATCTTAC TCTACATCCCTTAAGTCTCGCCTCACCATTTCTAAAGACACATCCAAATCACAAGTGGTAC TCACGATGACAAACATGGACCCTGTTGACACTGCTACATATTATTGTGCTAGGATAGTGGG CTACGGTAGCGGATGGTACGGTTATTTTGATTACTGGGGACAAGGGACGCTTGTTACGGTG TCCTCA	38	Heavy chain variable domain of anti-FLT3 antibody FL16

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GACATTCAGATGACCCAGTCTCCGTCCAGCGTTAGCGCAAGCGTGGGGGATAGAGTCACTA TTACGTGTAGAGCCAGTCAAGATATACGGTACGATCTTGCTTGGTATCAGCAAAAACCGGG AAAAGCCCCGAAGAGACTTATATATGCAGCTTCCTCCTTGCAAAGCGGGGTCCCATCCCGG TTTAGTGGTAGTGGTTCCGGAACAGAGTTCACGCTGACTATTTCATCACTGCAACCCGAAG ATTTTGCCACCTACTACTGCCTTCAACACAATTTCTATCCTCTTACCTTCGGCGGAGGTAC TAAGGTAGAGATTAAG	39	Light chain variable domain of anti-FLT3 antibody FL16
GAAGTACAGTTGGTTGAGAGTGGTGGAGGACTCGTTCAACCTGGCGGTAGTTTGCGACTCA GCTGCGCGGCTTCCGGTTTCACCTTCTCATCCTATGGGATGCACTGGGTCAGACAAGCCCC TGGAAAGGGCCTCGAATGGGTTGCTGTGATTAGCTATGACGGCTCTAATAAATACTATGCA GATAGTGTAAAGGGGAGATTTACGATTTCTCGCGATAATAGCAAAAATACGCTGTACCTGC AAATGGAAACCAACAGCCTGCGAGCGGAAGATACGGCGGTTTATTACTGCGCGAATCTTGC CCCGTGGGCAGCATACTGGGGACAGGGGACGTTGGTGACGGTAAGCAGT	40	Heavy chain variable domain of anti-FLT3 antibody ml0006
GAGATTGTGCTCACCCAGTCTCCACTCAGCCTTCCTGTAACGCCCGGTGAGCCTGCCTCTA TATCATGCCGAAGTTCCCAAAGCCTTCTGCACTCAAACGGCTATAACTACTTGGACTGGTA CCTCCAGAAGCCCGGCCAAAGTCCTCAACTGTTGATATACCTGGGGTCCAACCGGGCATCA GGAGTACCTGATAGATTCTCAGGAAGTGGGTCAGGAACCGACTTCACGCTGAAAATTAGTC GCGTAGAGGCGGAAGATGTAGGTGTGTATTACTGTATGCAGGCGTTGCAAACACCGCACAC TTTTGGACAGGGAACCAAACTGGAAATAAAGACCAGTAGTGGT	41	Light chain variable domain of anti-FLT3 antibody ml0006
CAGGTCCAACTGAAACAAAGCGGTCCCGGTCTTGTCCAGCCCTCCCAATCTCTCAGTATTA CTTGCACTGTGTCAGGTTTCAGCCTCACGAACTACGGTCTGCATTGGGTCCGCCAGTCTCC AGGAAAAGGCCTGGAGTGGCTCGGTGTTATCTGGAGTGGTGGAAGTACGGATTACAATGCT GCCTTTATCTCTCGGCTCAGTATCTCCAAAGATAACTCTAAGTCCCAAGTCTTTTTCAAAA TGAACTCTTTGCAGGCAGATGATACGGCCATATACTATTGCGCACGCAAGGGTGGGATCTA CTATGCAAACCACTATTACGCGATGGACTACTGGGGCCAAGGCACGAGTGTTACCGTGTCA AGC	42	Heavy chain variable domain of anti-FLT3 antibody BV10
GACATAGTGATGACTCAGTCTCCGTCCTCTCTTTCCGTGAGTGCGGGCGAAAAGGTTACCA TGTCCTGCAAAAGTTCACAGTCACTTCTCAATTCTGGCAACCAAAAAAATTACATGGCATG GTATCAACAGAAACCAGGTCAGCCGCCAAAGCTCCTCATATATGGTGCATCAACGCGAGAG TCAGGCGTACCTGACAGGTTTACCGGATCTGGCAGCGGTACAGACTTTACTCTTACCATAT CAAGTGTGCAGGCAGAGGACTTGGCGGTATACTATTGTCAAAACGATCATAGTTACCCTCT TACATTTGGCGCGGGCACTAAACTGGAGCTGAAACGC	43	Light chain variable domain of anti-FLT3 antibody BV10
CAGGTTCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGT CCTGCAAGGCCAGCGGCTACACCTTTACCGACTACAACATGCACTGGGTCCGACAGGCCCC TGGACAAGGACTTGAGTGGATCGGCTACATCTACCCCTACAATGGCGGCACCGGCTACAAC CAGAAGTTCAAGAGCAAGGCCACCATCACCGCCGACGAGAGCACAAACACCGCCTACATGG AACTGAGCAGCCTGAGAAGCGAGGACACCGCCGTGTACTACTGTGCCAGAGGCAGACCCGC CATGGATTATTGGGGACAGGGCACCCTGGTCACCGTTTCTAGC	44	Heavy chain variable domain of anti-CD33 antibody lintuzumab
GATATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGAGTGACCA TCACCTGTAGAGCCAGCGAGAGCGTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCA GCAGAAGCCCGGCAAGGCCCCTAAGCTGCTGATCTACGCCGCCAGCAATCAAGGCAGCGGA GTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCACCGATTTCACCCTGACAATCTCTAGCC TCCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGCAAAGAGGTGCCCTGGACATT CGGCCAGGGCACAAAGGTGGAAATCAAG	45	Light chain variable domain of anti-CD33 antibody lintuzumab
GAAGTGCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGT CCTGCAAGGCCAGCGGCTACACCATCACCGACAGCAACATCCACTGGGTCCGACAGGCTCC AGGCCAGTCTCTTGAGTGGATCGGCTACATCTACCCCTACAACGGCGGCACCGACTACAAC CAGAAGTTCAAGAACCGGGCCACACTGACCGTGGACAACCCTACCAATACCGCCTACATGG AACTGAGCAGCCTGCGGAGCGAGGACACCGCCTTTTACTACTGCGTGAACGGCAACCCCTG GCTGGCCTATTGGGGACAGGGAACACTGGTCACAGTGTCTAGC	46	Heavy chain variable domain of anti-CD33 antibody gemtuzumab
GATATTCAGCTGACACAGAGCCCCAGCACACTGTCTGCCTCTGTGGGCGACAGAGTGACCA TCACCTGTAGAGCCAGCGAGAGCCTGGACAACTACGGCATCAGATTTCTGACCTGGTTCCA GCAGAAGCCCGGCAAGGCTCCTAAGCTGCTGATGTACGCCGCCAGCAATCAAGGCAGCGGA GTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCACAGAGTTCACCCTGACAATCTCTAGCC TCCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGACCAAAGAGGTGCCCTGGTCCTT TGGACAGGGCACCAAGGTGGAAGTGAAGCGG	47	Light chain variable domain of anti-CD33 antibody gemtuzumab

Table B		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
CAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA CCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCC CCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGCCCCGACTACAAC CCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAGGAACCAGTTCTCCCTGA AGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAAGGTTAGTACTGG TGGTTTCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGT	48	Heavy chain variable domain of anti-CLEC12A antibody SC02-357
GAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCA TCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAACCAGG GAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGG TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCCAGGGAC CAAGGTGGAGATCAAA	49	Light chain variable domain of anti-CLEC12A antibody SC02-357
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA CCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCC CCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAAC CCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAAGAACCAGTTCTCCCTGA AGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAGGTCGTCTTCTGG TGGTTTCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGT	50	Heavy chain variable domain of anti-CLEC12A antibody SC02-378
GAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCA TCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAACCAGG GAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGG TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCAAGGGAC CAAGGTGGAGATCAAA	51	Light chain variable domain of anti-CLEC12A antibody SC02-378
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCA CCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCC CCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAAC CCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAAGAACCAGTTCTCCCTGA AGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAGGCAGACTACTGC TGGGTCCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGT	52	Heavy chain variable domain of anti-CLEC12A antibody SC02-161
GAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCA TCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAACCAGG GAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGG TTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAG ATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCAAGGGAC CAAGGTGGAGATCAAA	53	Light chain variable domain of anti-CLEC12A antibody SC02-161
GGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCT	54	scFv linker 1
GAAGCCGCGGCAAAAGAGGCAGCAGCAAAAGAGGCAGCAGCCAAA	220	scFv linker 2
GGTGGTGGTGGCAGTGGTGGCGGTGGCTCAGGTGGCGGCGGATCAGGCGGTGGTGGTTCTG GCGGCGGTGGATCT	221	scFv linker 3
GGCGGCGGAGGTTCCGGCGGTGGCGGAAGCGGAGGTGGTGGCTCT	223	scFv linker 4

Table A2												
Ab	**SEQ ID NO**	**CDR-H1**	**SEQ ID NO**	**CDR-H2**	**SEQ ID NO**	**CDR-H3**	**SEQ ID NO**	**CDR-L1**	**SEQ ID NO**	**CDR-L2**	**SEQ ID NO**	**CDR-L3**
FLT3 antibody D4-3	75	GYTFTSYYMH	76	IINPSGGSTSYAQKFQG	77	VVAAAVADY	78	RSSQSLLHSNGYNYLD	79	LGSNRA	80	MQSLQTPFT
FLT3 antibody NC7	81	GGTFSSYAIS	82	GIIPIFGTANYAQKFQG	83	FALFGFREQAFDI	84	RASQSISSYLN	85	AASSLQS	86	QQSYSTPFT
FLT3 antibody EB10	87	GYTFTSYYMH	88	IINPSGGSTSYAQKFQG	89	GVGAHDAFDI	90	RSSQSLLHSN	91	LGSNRA	92	MQGTHPAIS

								GNNYLD				
FLT3 antibody 4G8	93	SYWMH	94	EIDPSDSYKDYNQKFKD	95	AITTTPFDF	96	RASQSISNNLH	97	YASQSIS	98	QQSNTWPYT
FLT3 antibody FL_39	99	NARMGVS	100	HIFSNDEKSYSTSLKN	101	IVGYGSGWYGFFDY	102	RASQGIRNDLG	103	AASTLQS	104	LQHNSYPLT
FLT3 antibody FL_16	105	NARMAVS	106	HIFSNDEKSYSTSLKS	107	IVGYGSGWYGYFDY	108	RASQDIRYDLA	109	AASSLQS	110	LQHNFYPLT
FLT3 antibody ml0006	110	GFTFSSYG	112	ISYDGSNK	113	ANLAPWAAYW	114	QSLLHSNGYNY	115	LGS	116	MQALQTPHT
FLT3 antibody BV10	117	NYGLH	118	VIWSGGSTDYNAAFIS	119	KGGIYYANHYYAMDY	120	KSSQSLLNSGNQKNYMA	121	GASTRES	122	QNDHSYPLT
CD33 antibody lintuzumab	123	DYNMH	124	YIYPYNGGTGYNQKFKSKA	125	GRPAMDYWGQ	126	RASESVDNYGISFMN	127	AASNQGS	128	QQSKEVPWT
CD33 antibody gemtuzumab	129	GYTITDSN	130	IYPYNGGT	131	VNGNPWLAY	132	ESLDNYGIRF	133	AAS	134	QQTKEVPWS
CLEC12A antibody SC02-357	135	SSNWWS	136	EIYHSGSPDYNPSLKS	137	VSTGGFFDY	138	RASQSISSYLN	139	AASSLQS	140	QQSYSTPPT
CLEC12A antibody SC02-378	141	SSNWWS	142	EIYHSGSPNYNPSLKS	143	SSSGGFFDY	144	RASQSISSYLN	145	AASSLQS	146	QQSYSTPPT
CLEC12A antibody SC02-161	147	RASQSISSYLN	148	AASSLQ	149	QQSYSTPPT	150	SSNWWS	151	EIYHSGSPNYNPSLKS	152	QTTAGSFDY

[0335] Certain aspects of the present disclosure relate to chimeric receptors (e.g., CAR or chimeric TCR) comprising an extracellular antigen-binding domain that bind to one or more antigens of the present disclosure. In some embodiments, the antigen-binding domain are derived from an antibody, or antigen-binding fragment thereof.

[0336] In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto. In some

embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 6 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 8 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 10 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 12 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 14 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 18 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 20 or a sequence at least 90% identical thereto. In some

embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 22 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 24 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto. In some embodiments, the antigen binding domain comprises a VL comprising the amino acid sequence of SEQ ID NO: 26 or a sequence at least 90% identical thereto.

[0337] In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 75, a CDR-H2 sequence as shown in SEQ ID NO: 76, and a CDR-H3 sequence as shown in SEQ ID NO: 77. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 78, a CDR-L2 sequence as shown in SEQ ID NO: 79, and a CDR-L3 sequence as shown in SEQ ID NO: 80. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 81, a CDR-H2 sequence as shown in SEQ ID NO: 82, and a CDR-H3 sequence as shown in SEQ ID NO: 83. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 84, a CDR-L2 sequence as shown in SEQ ID NO: 85, and a CDR-L3 sequence as shown in SEQ ID NO: 86. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 87, a CDR-H2 sequence as shown in SEQ ID NO: 88, and a CDR-H3 sequence as shown in SEQ ID NO: 89. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 90, a CDR-L2 sequence as shown in SEQ ID NO: 91, and a CDR-L3 sequence as shown in SEQ ID NO: 92. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 93, a CDR-H2 sequence as shown in SEQ ID NO: 94, and a CDR-H3 sequence as shown in SEQ ID NO: 95. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 96, a CDR-L2 sequence as shown in SEQ ID NO: 97, and a CDR-L3 sequence as shown in SEQ ID NO: 98. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 99, a CDR-H2 sequence as shown in SEQ ID NO: 100, and a CDR-H3 sequence as shown in SEQ ID NO: 101. In some embodiments, the

antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 102, a CDR-L2 sequence as shown in SEQ ID NO: 103, and a CDR-L3 sequence as shown in SEQ ID NO: 104. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 105, a CDR-H2 sequence as shown in SEQ ID NO: 106, and a CDR-H3 sequence as shown in SEQ ID NO: 107. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 108, a CDR-L2 sequence as shown in SEQ ID NO: 109, and a CDR-L3 sequence as shown in SEQ ID NO: 110. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 111, a CDR-H2 sequence as shown in SEQ ID NO: 112, and a CDR-H3 sequence as shown in SEQ ID NO: 113. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 114, a CDR-L2 sequence as shown in SEQ ID NO: 115, and a CDR-L3 sequence as shown in SEQ ID NO: 116. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 117, a CDR-H2 sequence as shown in SEQ ID NO: 118, and a CDR-H3 sequence as shown in SEQ ID NO: 119. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 120, a CDR-L2 sequence as shown in SEQ ID NO: 121, and a CDR-L3 sequence as shown in SEQ ID NO: 122. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 123, a CDR-H2 sequence as shown in SEQ ID NO: 124, and a CDR-H3 sequence as shown in SEQ ID NO: 125. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 126, a CDR-L2 sequence as shown in SEQ ID NO: 127, and a CDR-L3 sequence as shown in SEQ ID NO: 128. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 129, a CDR-H2 sequence as shown in SEQ ID NO: 130, and a CDR-H3 sequence as shown in SEQ ID NO: 131. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 132, a CDR-L2 sequence as shown in SEQ ID NO: 133, and a CDR-L3 sequence as shown in SEQ ID NO: 134. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 135, a CDR-H2 sequence as shown in SEQ ID NO: 136, and a CDR-H3 sequence as shown in SEQ ID NO: 137. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 138, a CDR-L2 sequence as shown in SEQ ID NO: 139, and a CDR-L3 sequence as shown in SEQ ID NO: 140. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 141, a CDR-H2 sequence as shown in SEQ ID NO: 142, and a CDR-H3 sequence as shown in SEQ ID NO: 143. In some

embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 144, a CDR-L2 sequence as shown in SEQ ID NO: 145, and a CDR-L3 sequence as shown in SEQ ID NO: 146. In some embodiments, the antigen binding domain comprises a CDR-H1 sequence as shown in SEQ ID NO: 147, a CDR-H2 sequence as shown in SEQ ID NO: 148, and a CDR-H3 sequence as shown in SEQ ID NO: 149. In some embodiments, the antigen binding domain comprises a CDR-L1 sequence as shown in SEQ ID NO: 150, a CDR-L2 sequence as shown in SEQ ID NO: 151, and a CDR-L3 sequence as shown in SEQ ID NO: 152.

[0338] Suitable antibodies of the present disclosure include any antibody, whether natural or synthetic, full length or a fragment thereof, monoclonal or polyclonal, that binds sufficiently strongly and specifically to a myeloid (e.g., AML) antigen. In some embodiments, the antibody may have a K_d of at most about at most 10^{-6}M, at most about 10^{-7}M, at most about 10^{-8}M, at most about 10^{-9}M, at most about 10^{-10}M, at most about 10^{-11}M, or at most about 10^{-12}M.

[0339] In some embodiments, antibodies and derivatives thereof that may be used include, without limitation, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, human antibodies, humanized, antibodies primatized (CDR-grafted) antibodies, veneered antibodies, single-chain antibodies, phage-produced antibodies (e.g., from phage display libraries), and functional binding fragments of antibodies. For example, antibody fragments capable of binding to a myeloid (e.g., AML) antigen, or portions thereof, include, without limitation, Fv, Fab, Fab' and $F(ab')_2$ fragments. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For example, and not by way of limitation, papain or pepsin cleavage can generate Fab or $F(ab')_2$ fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab or $F(ab')_2$ fragments. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH, domain and hinge region of the heavy chain.

[0340] Methods of raising an antibody targeting a specific antigen are generally known in the art. Synthetic and engineered antibodies are described in, e.g., US4816567, EP0125023Bl, US4816397, EP0120694Bl, WO 86/01533, EP0194276Bl, US5225539, EP0239400Bl, EP0451216Bl, EP0519596Al and US4946778.

[0341] In some embodiments, commercially available antibodies may be used for binding to a myeloid (e.g., AML) antigen. The CDRs of the commercially available antibodies are

readily accessible by one skilled in the art using conventional sequencing technology. Further, one skilled in the art is able to construct nucleic acids encoding scFvs and chimeric receptors (e.g., CARs and TCRs) based on the CDRs of such commercially available antibodies.

[0342] In some embodiments, a chimeric receptor comprises an antigen-binding domain that specifically binds FLT3. In some embodiments, the FLT3-specific antigen-binding domain is derived from an anti-FLT3 antibody, such as the D4-3, NC7, or EB10 antibody described in U.S. Patent No. 8,071,099. In some embodiments, the FLT3-specific antigen-binding domain is derived from an anti-FLT3 antibody, such as the 4G8 or BV10 antibody described in U.S. Patent No. 9,023,996. In some embodiments, the FLT3-specific antigen-binding domain is derived from an anti-FLT3 antibody, such as the FL_16 or FL_39 antibody described in U.S. Patent Publication No. 2017/0037149, published February 9, 2017. In some embodiments, the FLT3-specific antigen-binding domain is derived from an anti-FLT3 antibody, such as the ml0006 antibody described in International Patent Publication WO 2018/119279, published June 28, 2018. The antigen-binding domain may be an scFv that comprises a light chain variable domain (VL) and a heavy chain variable domain (VH). In some embodiments, the chimeric receptor may have a multispecific antigen-binding domain. For example, the chimeric receptor may be specific for FLT3 and one or more additional antigens, such as CD33 and/or CLEC12A.

[0343] In some embodiments, a chimeric receptor comprises an antigen-binding domain that specifically binds CD33. In some embodiments, the CD33-specific antigen-binding domain is derived from an anti-CD33 antibody, such as lintuzumab described in U.S. Patent Publication No. 2018/0002397, published January 4, 2018. In some embodiments, the CD33-specific antigen-binding domain is derived from an anti-CD33 antibody, such as gemtuzumab described in U.S. Patent No. 5,739,116. The antigen-binding domain may be an scFv that comprises a light chain variable domain (VL) and a heavy chain variable domain (VH). In some embodiments, the chimeric receptor may have a multispecific antigen-binding domain. For example, the chimeric receptor may be specific for CD33 and one or more additional antigens, such as FLT3 and/or CLEC12A.

[0344] In some embodiments, a chimeric receptor comprises an antigen-binding domain that specifically binds CLEC12A. In some embodiments, the CLEC12A-specific antigen-binding domain is derived from an anti-CLEC12A antibody, such as the SC02-357, SC02-378, or SC02-161 antibody described in U.S. Patent No. 7,741,443. The antigen-binding domain may be an scFv that comprises a light chain variable domain (VL) and a heavy chain variable

domain (VH). In some embodiments, the chimeric receptor may have a multispecific antigen-binding domain. For example, the chimeric receptor may be specific for CLEC12A and one or more additional antigens, such as FLT3 and/or CD33.

T cell receptor (TCR)

[0345] Certain aspects of the present disclosure relate to chimeric receptors that specifically bind to an antigen expressed on myeloid cell, such as an AML cell. In some embodiments, the chimeric receptor is a chimeric T cell receptor (TCR). TCRs of the present disclosure are disulfide-linked heterodimeric proteins containing two variable chains expressed as part of a complex with the invariant CD3 chain molecules. TCRs are found on the surface of T cells, and are responsible for recognizing antigens as peptides bound to major histocompatibility complex (MHC) molecules. In certain embodiments, a TCR of the present disclosure comprises an alpha chain encoded by TRA and a beta chain encoded by TRB. In certain embodiments, a TCR comprises a gamma chain and a delta chain (encoded by TRG and TRD, respectively).

[0346] Each chain of a TCR is composed of two extracellular domains: a variable (V) region and a constant (C) region. The constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail. The variable region binds to the peptide/MHC complex. Each of the variable regions has three complementarity determining regions (CDRs).

[0347] In certain embodiments, a TCR can form a receptor complex with three dimeric signaling modules CD3δ/ε, CD3γ/ε, and CD247ζ/ζ or CD247ζ/η. When a TCR complex engages with its antigen and MHC (peptide/MHC), the T cell expressing the TCR complex is activated.

[0348] In some embodiments, a TCR of the present disclosure is a recombinant TCR. In certain embodiments, the TCR is a non-naturally occurring TCR. In certain embodiments, the TCR differs from a naturally occurring TCR by at least one amino acid residue. In some embodiments, the TCR differs from a naturally occurring TCR by at least 2 amino acid residues, at least 3 amino acid residues, at least 4 amino acid residues, at least 5 amino acid residues, at least 6 amino acid residues, at least 7 amino acid residues, at least 8 amino acid residues, at least 9 amino acid residues, at least 10 amino acid residues, at least 11 amino acid residues, at least 12 amino acid residues, at least 13 amino acid residues, at least 14 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 40 amino acid residues, at least

50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, or more amino acid residues. In certain embodiments, the TCR is modified from a naturally occurring TCR by at least one amino acid residue. In some embodiments, the TCR is modified from a naturally occurring TCR by at least 2 amino acid residues, at least 3 amino acid residues, at least 4 amino acid residues, at least 5 amino acid residues, at least 6 amino acid residues, at least 7 amino acid residues, at least 8 amino acid residues, at least 9 amino acid residues, at least 10 amino acid residues, at least 11 amino acid residues, at least 12 amino acid residues, at least 13 amino acid residues, at least 14 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, or more amino acid residues.

Chimeric TCRs

[0349] In some embodiments, a TCR of the present disclosure comprises one or more antigen-binding domains that may be grafted to one or more constant domain of a TCR chain, for example a TCR alpha chain or TCR beta chain, to create a chimeric TCR that binds specifically to a target antigen of the present disclosure (e.g., an AML antigen). Without wishing to be bound by theory, it is believed that chimeric TCRs may signal through the TCR complex upon antigen binding. For example, an antibody or antibody fragment (e.g., scFv) can be grafted to the constant domain, e.g., at least a portion of the extracellular constant domain, the transmembrane domain and the cytoplasmic domain, of a TCR chain, such as the TCR alpha chain and/or the TCR beta chain. As another example, the CDRs of an antibody or antibody fragment may be grafted into a TCR alpha chain and/or beta chain to create a chimeric TCR that binds specifically to an antigen of the present disclosure (e.g., an AML antigen). Such chimeric TCRs may be produced by methods known in the art (e.g., Willemsen RA et al., Gene Therapy 2000; 7:1369-1377; Zhang T et al., Cancer Gene Ther 2004 11: 487-496; and Aggen et al., Gene Ther. 2012 Apr; 19(4): 365-74).

Chimeric antigen receptors (CARs)

[0350] Certain aspects of the present disclosure relate to chimeric receptors that specifically bind to an antigen expressed on myeloid cell, such as an AML cell. In some embodiments, the chimeric receptor is a chimeric antigen receptor (CAR).

[0351] In some embodiments, CARs are engineered receptors that graft or confer a specificity of interest onto an immune effector cell. In certain embodiments, CARs can be used to graft the specificity of an antibody onto an immunoresponsive cell, such as a T cell. In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain (e.g., an scFv) fused to a transmembrane domain, fused to one or more intracellular signaling domains.

[0352] In some embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce activation of the immunoresponsive cell. In some embodiments, binding of the chimeric antigen receptor to its cognate ligand is sufficient to induce stimulation of the immunoresponsive cell. In some embodiments, activation of an immunoresponsive cell results in killing of target cells. In some embodiments, activation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell results in cytokine or chemokine expression and/or secretion by the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces differentiation of the immunoresponsive cell. In some embodiments, stimulation of an immunoresponsive cell induces proliferation of the immunoresponsive cell.

[0353] A CAR of the present disclosure may be a first, second, or third generation CAR. "First generation" CARs comprise a single intracellular signaling domain, generally derived from a T cell receptor chain. "First generation" CARs generally have the intracellular signaling domain from the CD3-zeta (CD3ζ) chain, which is the primary transmitter of signals from endogenous TCRs. "First generation" CARs can provide de novo antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation. "Second generation" CARs add a second intracellular signaling domain from one of various co-stimulatory molecules (e.g., CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs provide both co-stimulation (e.g., CD28 or 4- 1BB) and activation (CD3ζ). Preclinical studies have indicated that "Second Generation" CARs can improve the anti-tumor activity of immunoresponsive cell, such as a T cell. "Third generation" CARs have multiple intracellular co-stimulation signaling domains (e.g., CD28 and 4-1BB) and an intracellular activation signaling domain (CD3ζ).

[0354] In some embodiments, the extracellular antigen-binding domain of a CAR of the present disclosure binds to one or more antigens expressed on a myeloid cell, such as an AML cell, with a dissociation constant (K_d) of about 2 x 10^{-7} M or less, about 1 x 10^{-7} M or less, about 9 x 10^{-8} M or less, about 1 x 10^{-8} M or less, about 9 x 10^{-9} M or less, about 5 x 10^{-9} M or less, about 4 x 10^{-9} M or less, about 3 x 10^{-9} M or less, about 2 x 10^{-9} M or less, or about 1 x 10^{-9} M or less. In some embodiments, the K_d ranges from about is about 2 x 10^{-7} M to about 1 x 10^{-9} M.

[0355] Binding of the extracellular antigen-binding domain of a CAR of the present disclosure can be determined by, for example, an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay (RIA), FACS analysis, a bioassay (e.g., growth inhibition), or a Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (e.g., an antibody or scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in an RIA assay. The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the extracellular antigen-binding domain of the CAR is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (e.g., EBFP, EBFP2, Azurite, and mKalamal), cyan fluorescent protein (e.g., ECFP, Cerulean, and CyPet), and yellow fluorescent protein (e.g., YFP, Citrine, Venus, and YPet).

[0356] In some embodiments, CARs of the present disclosure comprise an extracellular antigen-binding domain that binds to one or more antigens expressed on a myeloid (e.g., an AML) cell, a transmembrane domain, and one or more intracellular signaling domains. In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises a Fab fragment, which may be crosslinked. In certain embodiments, the extracellular binding domain is a $F(ab)_2$ fragment.

Extracellular antigen-binding domain

[0357] In some embodiments, the extracellular antigen-binding domain of a CAR of the present disclosure specifically binds to one or more antigens expressed on a myeloid cell, such as an AML cell. In certain embodiments, the extracellular antigen-binding domain binds to one or more antigens expressed on an AML cell (AML antigens). In some embodiments, the one or more AML antigens are human polypeptides.

[0358] Antigen-binding domains of the present disclosure can include any domain that binds to the antigen including, without limitation, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a bispecific antibody, a conjugated antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody (sdAb) such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen-binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, or a fragment thereof, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen-binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen-binding domain of the CAR to comprise human or humanized residues for the antigen-binding domain of an antibody or antibody fragment.

[0359] In some embodiments, the extracellular antigen-binding domain comprises an antibody. In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is a humanized antibody. In certain embodiments, the antibody is a chimeric antibody. In some embodiments, the extracellular antigen-binding domain comprises an antigen-binding fragment of an antibody.

[0360] In some embodiments, the extracellular antigen-binding domain comprises a F(ab) fragment. In certain embodiments, the extracellular antigen-binding domain comprises a F(ab') fragment.

[0361] In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen. In some embodiments, the extracellular antigen-binding domain comprises a first scFv and a second scFv. In some embodiments, the first scFv and the second scFv bind distinct epitopes on the same antigen. In certain embodiments, the scFv is a human scFv. In certain embodiments, the scFv is a humanized scFv. In certain embodiments, the scFv is a chimeric scFv. In certain embodiments, the scFv comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In certain embodiments, the VH and VL are separated by a peptide linker. In certain embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 27 or SEQ ID NO: 74. In certain embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 218 or 219. In certain embodiments, the peptide linker is encoded by a nucleic acid comprising the sequence of

SEQ ID NO: 54, 220, 221, or 222. In certain embodiments, the scFv comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

[0362] In some embodiments, each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain. When there are two or more scFv linked together, each scFv can be linked to the next scFv with a peptide linked. In some embodiments, each of the one or more scFvs is separated by a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

[0363] In some embodiments, the cell comprises a first chimeric receptor and a second chimeric receptor. The antigen binding domain of the first chimeric receptor and the antigen binding domain of the second chimeric receptor can be an appropriate antigen biding domain described herein or known in the art. For example, the first or second antigen binding domain can be one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs). In some embodiments, the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises two single chain variable fragments (scFvs). In some embodiments, each of the two scFvs binds to a distinct epitope on the same antigen.

[0364] In some embodiments, the extracellular antigen-binding domain comprises a single-domain antibody (sdAb). In certain embodiments, the sdAb is a humanized sdAb. In certain embodiments, the sdAb is a chimeric sdAb.

[0365] In some embodiments, a CAR of the present disclosure may comprise two or more antigen-binding domains, three or more antigen-binding domains, four or more antigen-binding domains, five or more antigen-binding domains, six or more antigen-binding domains, seven or more antigen-binding domains, eight or more antigen-binding domains, nine or more antigen-binding domains, or ten or more antigen-binding domains. In some embodiments, each of the two or more antigen-binding domains binds the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different epitope of the same antigen. In some embodiments, each of the two or more antigen-binding domains binds a different antigen. In some embodiments, the two or more antigen-binding domains provide the CAR with logic gating, such as OR logic gating.

[0366] In some embodiments, the CAR comprises two antigen-binding domains. In some embodiments, the two antigen-binding domains are attached to one another via a flexible

linker. In some embodiments, each of the two-antigen-binding domains may be independently selected from an antibody, an antigen-binding fragment of an antibody, an scFv, a sdAb, a recombinant fibronectin domain, a T cell receptor (TCR), a recombinant TCR with enhanced affinity, and a single chain TCR. In some embodiments, the CAR comprising two antigen-binding domains is a bispecific CAR or a tandem CAR (tanCAR).

[0367] In certain embodiments, the bispecific CAR or tanCAR comprises an antigen-binding domain comprising a bispecific antibody or antibody fragment (e.g., scFv). In some embodiments, within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_1) upstream of its VL (VL_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_2) upstream of its VH (VH_2), such that the overall bispecific antibody molecule has the arrangement VH_1-VL_1-VL_2-VH_2. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_1) upstream of its VH (VH_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_2) upstream of its VL (VL_2), such that the overall bispecific antibody molecule has the arrangement VL_1 VH_1-VH_2-VL_2. In some embodiments, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), for example, between VL_1 and VL_2 if the construct is arranged as VH_1-VL_1-VL_2-VH_2, or between VH_1 and VH_2 if the construct is arranged as VL_1-VH_1-VH_2-VL_2. The linker may be a linker as described herein, e.g., a (Gly_4-Ser)n linker, wherein n is 1 , 2, 3, 4, 5, or 6. In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. In some embodiments, a linker is disposed between the VL and VH of the first scFv. In some embodiments, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers may be the same or different. Accordingly, in some embodiments, a bispecific CAR or tanCAR comprises VLs, VHs, and may further comprise one or more linkers in an arrangement as described herein.

[0368] In some embodiments, chimeric receptors comprise a bivalent CAR. In some embodiments, the bivalent CAR is an FLT3 bivalent CAR. In some embodiments, the bivalent FLT3 CAR comprises an NC7 scFv and a D4-3 scFv. In some embodiments, the bivalent CAR is a CD33 bivalent CAR. In some embodiments, the bivalent CAR is a CLEC12A bivalent CAR.

[0369] In some embodiments, the chimeric receptor comprises a bivalent chimeric antigen receptor. In some embodiments, the bivalent chimeric receptor comprises an FLT3 CAR and

a CD33 CAR. In some embodiments, the bivalent chimeric receptor comprises an FLT3 CAR and a CLEC12A CAR. In some embodiments, the bivalent chimeric receptor comprises an CLEC12A CAR and a CD33 CAR. In some embodiments, the bivalent chimeric receptor comprises an EMCN CAR. In some embodiments, the bivalent chimeric receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 1. In some embodiments, the bivalent chimeric receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 2. In some embodiments, the bivalent chimeric receptor comprises a CAR with two or more antigen binding domains targeting any antigen pair provided in Table 3. In some embodiments, the bivalent chimeric antigen receptor comprises a CAR with any combination of two or more antigen binding domains as described herein.

[0370] In some embodiments, chimeric receptors comprise a bicistronic chimeric antigen receptor. In some embodiments, the bicistronic chimeric antigen receptor comprises an FLT3 CAR and a CD33 CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises an FLT3 CAR and a CLEC12A CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises an CLEC12A CAR and a CD33 CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises an EMCN CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 1. In some embodiments, the bicistronic chimeric antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 2. In some embodiments, the bicistronic chimeric antigen receptor comprises a CAR with two or more antigen binding domains targeting any antigen pair provided in Table 3. In some embodiments, the bicistronic chimeric antigen receptor comprises a CAR with any combination of two or more antigen binding domains as described herein.

Transmembrane domain

[0371] In some embodiments, the transmembrane domain of a CAR of the present disclosure comprises a hydrophobic alpha helix that spans at least a portion of a cell membrane. It has been shown that different transmembrane domains can result in different receptor stability. After antigen recognition, receptors cluster and a signal is transmitted to the cell. In some embodiments, the transmembrane domain of a CAR of the present disclosure can comprise the transmembrane domain of a CD8 polypeptide, a CD28 polypeptide, a CD3-zeta polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS

polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a LIR-1 (LILRB1) polypeptide, or can be a synthetic peptide, or any combination thereof.

[0372] In some embodiments, the transmembrane domain is derived from a CD8 polypeptide. Any suitable CD8 polypeptide may be used. Exemplary CD8 polypeptides include, without limitation, NCBI Reference Nos. NP_001139345 and AAA92533.1. In some embodiments, the transmembrane domain is derived from a CD28 polypeptide. Any suitable CD28 polypeptide may be used. Exemplary CD28 polypeptides include, without limitation, NCBI Reference Nos. NP_006130.1 and NP_031668.3. In some embodiments, the transmembrane domain is derived from a CD3-zeta polypeptide. Any suitable CD3-zeta polypeptide may be used. Exemplary CD3-zeta polypeptides include, without limitation, NCBI Reference Nos. NP_932170.1 and NP_001106862.1. In some embodiments, the transmembrane domain is derived from a CD4 polypeptide. Any suitable CD4 polypeptide may be used. Exemplary CD4 polypeptides include, without limitation, NCBI Reference Nos. NP_000607.1 and NP_038516.1. In some embodiments, the transmembrane domain is derived from a 4-1BB polypeptide. Any suitable 4-1BB polypeptide may be used. Exemplary 4-1BB polypeptides include, without limitation, NCBI Reference Nos. NP_001552.2 and NP_001070977.1. In some embodiments, the transmembrane domain is derived from an OX40 polypeptide. Any suitable OX40 polypeptide may be used. Exemplary OX40 polypeptides include, without limitation, NCBI Reference Nos. NP_003318.1 and NP_035789.1. In some embodiments, the transmembrane domain is derived from an ICOS polypeptide. Any suitable ICOS polypeptide may be used. Exemplary ICOS polypeptides include, without limitation, NCBI Reference Nos. NP_036224 and NP_059508. In some embodiments, the transmembrane domain is derived from a CTLA-4 polypeptide. Any suitable CTLA-4 polypeptide may be used. Exemplary CTLA-4 polypeptides include, without limitation, NCBI Reference Nos. NP_005205.2 and NP_033973.2. In some embodiments, the transmembrane domain is derived from a PD-1 polypeptide. Any suitable PD-1 polypeptide may be used. Exemplary PD-1 polypeptides include, without limitation, NCBI Reference Nos. NP_005009 and NP_032824. In some embodiments, the transmembrane domain is derived from a LAG-3 polypeptide. Any suitable LAG-3 polypeptide may be used. Exemplary LAG-3 polypeptides include, without limitation, NCBI Reference Nos. NP_002277.4 and NP_032505.1. In some embodiments, the transmembrane domain is derived from a 2B4 polypeptide. Any suitable 2B4 polypeptide may be used. Exemplary 2B4 polypeptides include, without limitation, NCBI Reference Nos.

NP_057466.1 and NP_061199.2. In some embodiments, the transmembrane domain is derived from a BTLA polypeptide. Any suitable BTLA polypeptide may be used. Exemplary BTLA polypeptides include, without limitation, NCBI Reference Nos. NP_861445.4 and NP_001032808.2. Any suitable LIR-1 (LILRB1) polypeptide may be used. Exemplary LIR-1 (LILRB1) polypeptides include, without limitation, NCBI Reference Nos. NP_001075106.2 and NP_001075107.2.

[0373] In some embodiments, the transmembrane domain comprises a polypeptide comprising an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_001139345, AAA92533.1, NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2, NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2, or fragments thereof. In some embodiments, the homology may be determined using standard software such as BLAST or FASTA. In some embodiments, the polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative amino acid substitutions. In some embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_001139345, AAA92533.1, NP_006130.1, NP_031668.3, NP_932170.1, NP_001106862.1, NP_000607.1, NP_038516.1, NP_001552.2, NP_001070977.1, NP_003318.1, NP_035789.1, NP_036224, NP_059508, NP_005205.2, NP_033973.2, NP_005009, NP_032824, NP_002277.4, NP_032505.1, NP_057466.1, NP_061199.2, NP_861445.4, or NP_001032808.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, or at least 240 amino acids in length.

[0374] Further examples of suitable polypeptides from which a transmembrane domain may be derived include, without limitation, the transmembrane region(s) of the alpha, beta or zeta chain of the T-cell receptor, CD27, CD3 epsilon, CD45, CD5, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, CD2, CD27, LFA-1 (CD11a, CD18), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7Rα, ITGA1, VLA1 , CD49a,

ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, and NG2C.

[0375] In some embodiments, the transmembrane domain comprises the sequence shown in SEQ ID NO: 209. In some embodiments, the transmembrane domain comprises the sequence shown in SEQ ID NO: 210. In some embodiments, the transmembrane domain comprises the sequence shown in SEQ ID NO: 211.

Spacer region

[0376] In some embodiments, a CAR of the present disclosure can also comprise a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region may be flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition. In some embodiments, the spacer region may be a hinge from a human protein. For example, the hinge may be a human Ig (immunoglobulin) hinge, including without limitation an IgG4 hinge, an IgG2 hinge, a CD8a hinge, or an IgD hinge. In some embodiments, the spacer region may comprise an IgG4 hinge, an IgG2 hinge, an IgD hinge, a CD28 hinge, a KIR2DS2 hinge, an LNGFR hinge, or a PDGFR-beta extracellular linker. In some embodiments, the spacer region is localized between the antigen-binding domain and the transmembrane domain. In some embodiments, a spacer region may comprise any of the amino acid sequences listed in Table C, or an amino acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the amino acid sequences listed in Table C. In some embodiments, nucleic acids encoding any of the spacer regions of the present disclosure may comprise any of the nucleic acid sequences listed in Table D, or a nucleic acid sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the nucleic acid sequences listed in Table D.

Table C

Amino Acid Sequence	SEQ ID NO:	Description
AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	55	CD28 hinge
ESKYGPPCPSCP	56	IgG4 minimal hinge

Table C		
Amino Acid Sequence	**SEQ ID NO:**	**Description**
ESKYGPPAPSAP	57	IgG4 minimal hinge, no disulfides
ESKYGPPCPPCP	58	IgG4 S228P minimal hinge, enhanced disulfide formation
EPKSCDKTHTCP	59	IgG1 minimal hinge
AAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVIT LYCNHRN	60	Extended CD8a hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQTVCEPCL DSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAV CRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQN TVCEECPDGTYSDEADAEC	61	LNGFR hinge
ACPTGLYTHSGECCKACNLGEGVAQPCGANQTVC	62	Truncated LNGFR hinge (TNFR-Cys1)
AVGQDTQEVIVVPHSLPFKV	64	PDGFR-beta extracellular linker

Table D		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACC TGGATAATGAAAAGAGTAATGGGACTATCATTCATGT AAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCG GTCCGTCTAAACCT	65	CD28 hinge
GAA AGC AAG TAC GGT CCA CCT TGC CCT AGC TGT CCG	66	IgG4 minimal hinge
GAA TCC AAG TAC GGC CCC CCA GCG CCT AGT GCC CCA	67	IgG4 minimal hinge, no disulfides
GAA TCT AAA TAT GGC CCG CCA TGC CCG CCT TGC CCA	68	IgG4 S228P minimal hinge, enhanced disulfide formation
GAA CCG AAG TCT TGT GAT AAA ACT CAT ACG TGC CCG	69	IgG1 minimal hinge
GCT GCT GCT TTC GTA CCC GTG TTC CTC CCT GCT AAG CCT ACG ACT ACC CCC GCA CCG AGA CCA CCC ACG CCA GCA CCC ACG ATTGCT AGC CAG CCC CTT AGT TTG CGA CCA GAA GCT TGT CGG CCT GCT GCT GGT GGC GCG GTA CAT ACC CGC GGC CTT GAT TTT GCTTGC GAT ATA TAT ATC TGG GCG CCT CTG GCC GGA ACA TGC GGG GTC CTC CTC CTT TCT CTG GTT ATT ACT CTC TAC TGT AAT CACAGG AAT	70	Extended CD8a hinge
GCC TGC CCG ACC GGG CTC TAC ACT CAT AGC GGG GAA TGT TGT AAG GCA TGT AAC TTG GGT GAG GGC GTC GCA CAG CCC TGC GGAGCT AAC CAA ACA GTG TGC GAA CCC TGC CTC GAT AGT GTG ACG TTC TCT GAT GTT GTA TCA GCT ACA GAG CCT TGC AAA CCA TGTACT GAG TGC GTT GGA CTT CAG TCA ATG AGC GCT CCA TGT GTG GAG GCA GAT GAT GCG GTC TGT CGA TGT GCT TAC GGA TAC TACCAA GAC GAG ACA ACA GGG CGG TGC GAG GCC TGT AGA GTT TGT GAG GCG GGC TCC GGG CTG GTG TTT TCA TGT CAA GAC AAG CAAAAT ACG GTC TGT GAA GAG TGC CCT GAT GGC ACC TAC TCA GAC GAA GCA GAT GCA GAA TGC	71	LNGFR hinge
GCC TGC CCT ACA GGA CTC TAC ACG CAT AGC GGT GAG TGT TGT AAA GCA TGC AAC CTC GGG GAA GGT GTA GCC CAG CCA TGC GGG GCT AAC CAA ACC GTT TGC	72	Truncated LNGFR hinge (TNFR-Cys1)

Table D		
Nucleic Acid Sequence	**SEQ ID NO:**	**Description**
GCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTG CCACACTCCTTGCCCTTTAAGGTG	73	PDGFR-beta extracellular linker

[0377] In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 55. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 56. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 57. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 58. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 59. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 60. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 61. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 62. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 63. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 64. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 206. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 207. In some embodiments, the spacer region comprises the sequence shown in SEQ ID NO: 208.

[0378] In some embodiments, a CAR of the present disclosure may further include a short oligopeptide or polypeptide linker that is between 2 amino acid residues and 10 amino acid residues in length, and that may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A non-limiting example of a suitable linker is a glycine-serine doublet. In some embodiments, the linker comprises the ammo acid sequence of GGCKJSGGCKJS.

Intracellular signaling domains

[0379] In some embodiments, a CAR of the present disclosure comprises one or more cytoplasmic domains or regions. The cytoplasmic domain or region of the CAR may include an intracellular signaling domain. An intracellular signaling domain is typically responsible for activation of one or more effector functions of an immune cell (e.g., a T cell or an NK cell) that has been engineered to express a CAR of the present disclosure. For example, an effector function of a T cell may be cytolytic activity or helper activity, such as the secretion of cytokines. Accordingly, in some embodiments the term "intracellular signaling domain" refers to the portion of a protein which transduces an effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain may be

employed, in many instances it is not necessary to use the entire chain. In embodiments where a truncated portion of the intracellular signaling domain is used, such a truncated portion may be used in place of the corresponding intact chain as long as the truncated portion transduces the effector function signal.

[0380] Examples of suitable intracellular signaling domains that may be used in CARs of the present disclosure include, without limitation, cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

[0381] Without wishing to be bound by theory, it is believed that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is thus also required for full activation. Accordingly, T cell activation may be mediated by two distinct classes of cytoplasmic signaling sequences, those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

[0382] In some embodiments, a primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs (ITAMs). Examples of suitable ITAM-containing primary intracellular signaling domains that that may be used in the CARs of the present disclosure include, without limitation, those of CD3-zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FcεRI, DAP10, DAP12, and CD66d.

[0383] In some embodiments, a CAR of the present disclosure comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta polypeptide. A CD3-zeta polypeptide of the present disclosure may have an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homologous to the sequence of NCBI Reference No. NP_932170 or NP_001106864.2, or fragments thereof. In some embodiments, the CD3-zeta polypeptide may comprise one conservative amino acid substitution, up to two conservative amino acid substitutions, or up to three conservative

amino acid substitutions. In some embodiments, the polypeptide can have an amino acid sequence that is a consecutive portion of NCBI Reference No. NP_932170 or NP_001106864.2 that is at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, or at least 160, at least 170, or at least 180 amino acids in length.

[0384] In other embodiments, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In one embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

[0385] In some embodiments, the intracellular signaling domain of a CAR of the present disclosure can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the present disclosure. For example, the intracellular signaling domain of the CAR can comprise a CD3-zeta chain portion and a costimulatory signaling domain. The costimulatory signaling domain may refer to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule of the present disclosure is a cell surface molecule other than an antigen receptor or its ligands that may be required for an efficient response of lymphocytes to an antigen. Examples of suitable costimulatory molecules include, without limitation, CD97, CD2, ICOS, CD27, CD154, CD8, OX40, 4-1BB, CD28, ZAP40, CD30, GITR, HVEM, DAP10, DAP12, MyD88, 2B4, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, an MHC class I molecule, a TNF receptor protein, an Immunoglobulin-like protein, a cytokine receptor, an integrin, a signaling lymphocytic activation molecule (SLAM protein), an activating NK cell receptor, BTLA, a Toll ligand receptor, CDS, ICAM-1, (CD11a/CD18), BAFFR, KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, ITGB7, NKG2D, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME

(SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and the like.

[0386] In some embodiments, the intracellular signaling sequences within the cytoplasmic portion of a CAR of the present disclosure may be linked to each other in a random or specified order. In some embodiments, a short oligopeptide or polypeptide linker, for example, between 2 amino acids and 10 amino acids (e.g., 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 8 amino acids, 9 amino acids, or 10 amino acids) in length may form the linkage between intracellular signaling sequences. In one embodiment, a glycine-serine doublet can be used as a suitable linker. In one embodiment, a single ammo acid, e.g., an alanine or a glycine, can be used as a suitable linker.

[0387] In some embodiments, the intracellular signaling domain comprises two or more costimulatory signaling domains, e.g., two costimulatory signaling domains, three costimulatory signaling domains, four costimulatory signaling domains, five costimulatory signaling domains, six costimulatory signaling domains, seven costimulatory signaling domains, eight costimulatory signaling domains, nine costimulatory signaling domains, 10 costimulatory signaling domains, or more costimulatory signaling domains. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the two or more costimulatory signaling domains are separated by a linker of the present disclosure. In one embodiment, the linker is a glycine residue. In another embodiment, the linker is an alanine residue.

[0388] In some embodiments, a cell of the present disclosure expresses a CAR that includes an antigen-binding domain that binds a target antigen of the present disclosure, a transmembrane domain of the present disclosure, a primary signaling domain, and one or more costimulatory signaling domains.

[0389] In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 153 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 155 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 157 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 159 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 161 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 163 or a

sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 165 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 167 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 169 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 171 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 173 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 175 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 177 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 179 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 181 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 183 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 185 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 187 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 189 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 191 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 19 or a sequence at least 90% identical thereto 3. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 195 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 197 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 199 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 201 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 203 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 205 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ

ID NO: 212 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 214 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the amino acid sequence as shown in SEQ ID NO: 216 or a sequence at least 90% identical thereto.

[0390] In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 154 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 156 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 158 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 160 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 162 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 164 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 166 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 168 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 170 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 172 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 174 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 176 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 178 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 180 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 182 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 184 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 186 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 188 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 190 or a sequence at least 90% identical thereto. In some

embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 192 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 194 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 196 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 198 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 200 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 202 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 204 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 213 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 215 or a sequence at least 90% identical thereto. In some embodiments, a CAR comprises the nucleotide sequence as shown in SEQ ID NO: 217 or a sequence at least 90% identical thereto.

Natural killer cell receptor (NKR) CARs

[0391] In some embodiments, a CAR of the present disclosure comprises one or more components of a natural killer cell receptor (NKR), thereby forming an NKR-CAR. The NKR component may be a transmembrane domain, a hinge domain, or a cytoplasmic domain from any suitable natural killer cell receptor, including without limitation, a killer cell immunoglobulin-like receptor (KIR),such as KIR2DL1, KIR2DL2/L3, KIR2DL4, KIR2DL5A, KIR2DL5B, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, DIR2DS5, KIR3DL1/S1, KIR3DL2, KIR3DL3, KIR2DP1, and KIRS DPI; a natural cytotoxicity receptor (NCR), such as NKp30, NKp44, NKp46; a signaling lymphocyte activation molecule (SLAM) family of immune cell receptor, such as CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10; an Fc receptor (FcR), such as CD16, and CD64; and an Ly49 receptor, such as LY49A and LY49C. In some embodiments, the NKR-CAR may interact with an adaptor molecule or intracellular signaling domain, such as DAP12. Exemplary configurations and sequences of CARs comprising NKR components are described in International Patent Publication WO2014/145252, published September 18, 2014.

Chimeric inhibitory receptors

[0392] Certain aspects of the present disclosure relate to chimeric inhibitory receptors. Chimeric inhibitory receptors are useful, for example, as NOT logic gates for controlling cell activity, such as immune cell activity. In some embodiments, chimeric inhibitory receptors of the present disclosure specifically bind to one or more antigens that are expressed on normal cells but not on tumor cells.

[0393] In some embodiments, the chimeric inhibitory receptor comprises an antigen-binding domain, a transmembrane domain of the present disclosure (e.g., any suitable transmembrane domain used in conjunction with a chimeric receptor of the present disclosure), and an intracellular domain. In some embodiments, the chimeric inhibitory receptor may inhibit one or more activities of a cell, such as an immunoresponsive cell.

[0394] In some embodiments, the chimeric inhibitory receptor may comprise an enzymatic inhibitory domain. When the chimeric inhibitory receptor is located proximal to a receptor, such as an immune receptor in a cell membrane, binding of a cognate antigen to the antigen-binding domain activates the enzymatic inhibitory domain to inhibit activation of the receptor. As used herein, the term "enzymatic inhibitory domain" refers to a protein domain that inhibits an intracellular signal transduction cascade, for example a native T cell activation cascade. The disclosed chimeric inhibitory receptors thus can be engineered to contain appropriate antigen-binding domains that will reduce, for example, immune responses in the presence of the cognate antigen. Uses of chimeric inhibitory receptors of the present disclosure include, but are not limited to, reducing immune responses, controlling T cell activation, and controlling CAR-T responses.

[0395] In some embodiments, the enzymatic inhibitory domain of a chimeric inhibitory receptor of the present disclosure comprises at least a portion of an extracellular domain, a transmembrane domain, and/or an intracellular domain. In some embodiments, the enzymatic inhibitory domain comprises at least a portion of an enzyme. In some embodiments, the enzyme is selected from CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP (see e.g., Stanford et al., Regulation of TCR signaling by tyrosine phosphatases: from immune homeostasis to autoimmunity, Immunology, 2012 Sep; 137(1): 1-19). In some embodiments, the portion of the enzyme comprises an enzyme domain(s), an enzyme fragment(s), or a mutant(s) thereof. In some embodiments, the portion of the enzyme is a catalytic domain of the enzyme. In some embodiments, the enzyme domain(s), enzyme fragment(s), or mutants(s) thereof are selected to maximize efficacy and minimize basal inhibition.

[0396] In some embodiments, the enzymatic inhibitory domain comprises one or more modifications that modulate basal inhibition. Examples of modifications include, but are not limited to, truncation mutation(s), amino acid substitution(s), introduction of locations for post-translational modification (examples of which are known to those having skill in the art), and addition of new functional groups. In some embodiments, the enzyme domain(s), enzyme fragment(s), or mutants(s) thereof are selected to maximize efficacy and minimize basal inhibition. In some embodiments, the one or more modifications reduce basal inhibition. In other embodiments, the one or more modifications increase basal inhibition.

[0397] In some embodiments, the enzymatic inhibitory domain inhibits, for example, immune receptor activation upon recruitment of a chimeric inhibitory receptor of the present disclosure proximal to an immune receptor. In some embodiments, the immune receptor is a naturally-occurring immune receptor. In some embodiments, the immune receptor is a naturally-occurring antigen receptor. In some embodiments, the immune receptor is selected from a T cell receptor, a pattern recognition receptor (PRR), a NOD-like receptor (NLR), a Toll-like receptor (TLR), a killer activated receptor (KAR), a killer inhibitor receptor (KIR), a complement receptor, an Fc receptor, a B cell receptor, and a cytokine receptor. In some embodiments, the immune receptor is a T cell receptor. In some embodiments, the immune receptor is a chimeric immune receptor. In some embodiments, the chimeric immune receptor is a chimeric TCR or a CAR.

[0398] In some embodiments, a chimeric inhibitory receptor of the present disclosure may also comprise one or more intracellular inhibitory co-signaling domains. In some embodiments, the intracellular inhibitory co-signaling domains comprise an inhibitory domain. In some embodiments, the one or more intracellular inhibitory co-signaling domains comprise one or more ITIM-containing protein, or fragment(s) thereof. ITIMs are conserved amino acid sequences found in cytoplasmic tails of many inhibitory immune receptors. In some embodiments, the one or more ITIM-containing proteins, or fragments thereof, are selected from PD-1, CTLA4, TIGIT, and LAIR1. In some embodiments, the one or more intracellular inhibitory co-signaling domains comprise one or more non-ITIM scaffold proteins, or a fragment(s) thereof. In some embodiments, the one or more non-ITIM scaffold proteins, or fragments thereof, are selected from GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1. Further examples suitable intracellular inhibitory co-signaling domains include, without limitation, PD-L1, TIM3, VISTA, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGF beta.

[0399] In some embodiments, the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell. Exemplary antigens for use in a chimeric inhibitory receptor are described in **Table 2**.

Table 2

Antigen	UniProt Accession No.	Name	Short Description
EMCN	Q9ULC0	Endomucin	A sialoglycoprotein that interferes with assembly of focal adhesion complexes and inhibits interaction between cells and extracellular matrix.
JAM2	P57087	Junctional Adhesion Molecule 2	Localized at tight junctions, acts as an adhesive ligand for interacting with variety of immune cell types
MS4A15	Q8N5U1	Membrane-spanning 4-domains subfamily A member 15	A four-transmembrane spanning protein, member of the CD20-like family.
C4BPA	P04003	Complement Component 4 Binding Protein Alpha	Subunit of the C4b-binding protein that controls activation of the complement cascade via the classical complement activation pathway.
TRPM1	Q7Z4N2	Transient Receptor Potential Cation Channel Subfamily M Member 1	Calcium permeable cation channel expressed in melanocytes and may play role in melanin synthesis. Essential for the depolarizing photoresponse.
SCTR	P47872	Secretin Receptor	Binds secretin, which is most potent regulator of pancreatic bicarbonate, electrolyte and volume secretion
SLC2A2	P11168	Solute Carrier Family 2, facilitated glucose transporter member 2	Glycoprotein of liver, intestine, and kidney, that mediates facilitated bidirectional transfer of glucose across plasma membrane
KCNQ2	O43526	Potassium Voltage-Gated Channel Subfamily Q Member 2	Associates with KCNQ3 and forms a potassium channel. Important in regulation of neuronal excitability.
PERP	Q96FX8	P53 Apoptosis Effector Related To PMP22	Component of intercellular desmosome junctions, plays role in stratified epithelial integrity
WLS	Q5T9L3	Wntless Wnt Ligand Secretion Mediator	Regulates Wnt protein sorting and secretion, plays key role in regulation of expression, subcellular localization, binding and organelle-specific association of Wnt proteins.
FFAR2	O15552	Free Fatty Acid Receptor 2	G protein-coupled receptor activated by short chain fatty acids.
PTPRB	P23467	Protein Tyrosine Phosphatase Receptor Type B	Member of the protein tyrosine phosphatase (PTP) family.The extracellular region of this PTP is composed of multiple fibronectin type_III repeats.
NCKAP1	Q9Y2A7	NCK Associated Protein 1	Part of the WAVE complex that regulates lamellipodia formation. The WAVE complex regulates actin filament reorganization via its interaction with the Arp2/3 complex.
MPZL2	O60487	Myelin Protein Zero Like 2, Epithelial V-like antigen	Epithelial V-like antigen (EVA) is expressed in thymus epithelium and strongly downregulated by thymocyte developmental progression.
PLSCR4	Q9NRQ2	Phospholipid Scramblase 4	May mediate accelerated ATP-independent bidirectional transbilayer migration of

			phospholipids upon binding calcium ions that results in a loss of phospholipid asymmetry in the plasma membrane.
TMEM47	Q9BQJ4	Transmembrane Protein 47	Encodes a member of the PMP22/EMP/claudin protein family. The encoded protein is localized to the ER and the plasma membrane.
ADGRL4	Q9HBW9	Adhesion G Protein-Coupled Receptor L4	Endothelial orphan receptor that acts as a key regulator of angiogenesis.
MET	P08581	MET Proto-Oncogene, Receptor Tyrosine Kinase	Mesenchymal Epithelial Transition MET is a prototypical receptor tyrosine kinase. Its ligand is Hepatocyte Growth Factor (HGF).
BACE2	Q9Y5Z0	Beta-Secretase 2	An integral membrane glycoprotein that functions as an aspartic protease. The encoded protein cleaves amyloid precursor protein into amyloid beta peptide
ATP8B1	O43520	ATPase Phospholipid Transporting 8B1	A member of the P-type cation transport ATPase family, which belongs to the subfamily of aminophospholipid-transporting ATPases.
LIFR	P42702	LIF Receptor Subunit Alpha	a protein that belongs to the type I cytokine receptor family. This protein combines with a high-affinity converter subunit, gp130, to form a receptor complex that mediates the action of the leukemia inhibitory factor
ART4	Q93070	ADP-Ribosyltransferase 4	A member of the ADP-ribosyltransferase gene family
CALCRL	Q16602	Calcitonin Receptor Like Receptor	Receptor for calcitonin-gene-related peptide (CGRP) together with RAMP1 and receptor for adrenomedullin together with RAMP3
CNTNAP3	Q9BZ76	Contactin Associated Protein Family Member 3	NCP proteins mediate neuron-glial interactions in vertebrates and glial-glial contact in invertebrates.
PCDH9	Q9HC56	Protocadherin 9	Mediates cell adhesion in neural tissues in the presence of calcium.
IL18R1	Q13478	Interleukin 18 Receptor 1	Type I transmembrane protein and interleukin 18 (IL-18) cytokine receptor
SLC8A3	P57103	Solute Carrier Family 8 Member A3	Multi-pass (11) transmembrane sodium/calcium exchanger integral membrane protein
CDH26	Q8IXH8	Cadherin 26	Type I transmembrane protein involved in cell interactions, migration, and differentiation
TMEM163	Q8TC26	Transmembrane Protein 163	Multi-pass (6) transmembrane protein that binds divalent cations
ABCA13	Q86UQ4	ATP Binding Cassette Subfamily A Member 13	Member of ATP-binding cassette (ABC) gene subfamily A (ABCA).
CACHD1	Q5VU97	Cache Domain Containing 1	Type I transmembrane voltage-dependent calcium channel
CYYR1	Q96J86	Cysteine And Tyrosine Rich 1	Type I transmembrane protein
ABCB1	P08183	ATP Binding Cassette Subfamily B Member 1	Multi-pass (14) ATP-binding transmembrane protein
ADGRG6	Q86SQ4	Adhesion G Protein-Coupled Receptor G6	G-protein coupled receptor which is activated by type IV collagen. Couples to G(i)-proteins as well as G(s)-proteins

ATP9A	O75110	ATPase Phospholipid Transporting 9A	Gene Ontology (GO) annotations related to this gene include nucleotide binding and cation-transporting ATPase activity.
CALN1	Q9BXU9	Calneuron 1	A protein with high similarity to the calcium-binding proteins of the calmodulin family.
CDCP1	Q9H5V8	CUB Domain Containing Protein 1	A transmembrane protein which contains three extracellular CUB domains and acts as a substrate for Src family kinases.
IL12RB2	Q99665	Interleukin 12 Receptor Subunit Beta 2	A type I transmembrane protein identified as a subunit of the interleukin 12 receptor complex.
SLC16A14	Q7RTX9	Solute Carrier Family 16 Member 14	Proton-linked monocarboxylate transporter. May catalyze the transport of monocarboxylates across the plasma membrane.
TMEM136	Q6ZRR5	Transmembrane Protein 136	Gene Ontology (GO) annotations related to this gene include integral component of membrane
TMEM200A	Q86VY9	Transmembrane Protein 200A	Gene Ontology (GO) annotations related to this gene include integral component of membrane

[0400] In some embodiments, the chimeric inhibitory receptor binds an EMCN antigen. In some embodiments, the chimeric inhibitory receptor binds an JAM2 antigen. In some embodiments, the chimeric inhibitory receptor binds an MS4A15 antigen. In some embodiments, the chimeric inhibitory receptor binds an C4BPA antigen. In some embodiments, the chimeric inhibitory receptor binds an TRPM1 antigen. In some embodiments, the chimeric inhibitory receptor binds an SCTR antigen. In some embodiments, the chimeric inhibitory receptor binds an SLC2A2 antigen. In some embodiments, the chimeric inhibitory receptor binds an KCNQ2 antigen. In some embodiments, the chimeric inhibitory receptor binds a PERP antigen. In some embodiments, the chimeric inhibitory receptor binds an WLS antigen. In some embodiments, the chimeric inhibitory receptor binds an FFAR2 antigen.

[0401] In some embodiments, the chimeric inhibitory receptor is a multispecific receptor comprising two or more antigen-binding domains, such that the chimeric inhibitory receptor can bind two or more antigens. Alternatively, a cell can be edited to express two or more chimeric inhibitory receptors that bind to different antigens.

[0402] In some embodiments, the chimeric inhibitory receptor binds a PTPRB antigen. In some embodiments, the chimeric inhibitory receptor binds an NCKAP1 antigen. In some embodiments, the chimeric inhibitory receptor binds an MPZL2 antigen. In some embodiments, the chimeric inhibitory receptor binds a PLSCR4 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM47 antigen. In some embodiments, the chimeric inhibitory receptor binds an ADGRL4 antigen. In some

embodiments, the chimeric inhibitory receptor binds an MET antigen. In some embodiments, the chimeric inhibitory receptor binds a BACE2 antigen. In some embodiments, the chimeric inhibitory receptor binds an ATP8B1 antigen. In some embodiments, the chimeric inhibitory receptor binds an LIFR antigen. In some embodiments, the chimeric inhibitory receptor binds an ART4 antigen. In some embodiments, the chimeric inhibitory receptor binds a CALCRL antigen. In some embodiments, the chimeric inhibitory receptor binds a CNTNAP3 antigen In some embodiments, the chimeric inhibitory receptor binds a PCDH9 antigen. In some embodiments, the chimeric inhibitory receptor binds an IL18R1 antigen. In some embodiments, the chimeric inhibitory receptor binds an SLC8A3 antigen. In some embodiments, the chimeric inhibitory receptor binds a CDH26 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM163 antigen. In some embodiments, the chimeric inhibitory receptor binds an ABCA13 antigen. In some embodiments, the chimeric inhibitory receptor binds a CACHD1 antigen. In some embodiments, the chimeric inhibitory receptor binds a CYYR1 antigen. In some embodiments, the chimeric inhibitory receptor binds an ABCB1 antigen. In some embodiments, the chimeric inhibitory receptor binds an ADGRG6 antigen. In some embodiments, the chimeric inhibitory receptor binds an ATP9A antigen. In some embodiments, the chimeric inhibitory receptor binds a CALN1 antigen. In some embodiments, the chimeric inhibitory receptor binds a CALN1 antigen. In some embodiments, the chimeric inhibitory receptor binds a CDCP1 antigen. In some embodiments, the chimeric inhibitory receptor binds an IL12RB2 antigen. In some embodiments, the chimeric inhibitory receptor binds a SLC16A14 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM136 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM200A antigen.

Immunoresponsive cells

[0403] Certain aspects of the present disclosure relate to a cell, such as an immunoresponsive cell, that has been genetically engineered to comprise one or more chimeric receptors of the present disclosure or one or more nucleic acids encoding such chimeric receptors , and to methods of using such cells for treating myeloid malignancies (e.g., AML).

[0404] In some embodiments, the cell is a mammalian cell. In some embodiments, the mammalian cell is a primary cell. In some embodiments, the mammalian cell is a cell line. In some embodiments, the mammalian cell a bone marrow cell, a blood cell, a skin cell, bone cell, a muscle cell, a neuronal cell, a fat cell, a liver cell, or a heart cell. In some

embodiments, the cell is a stem cell. Exemplary stem cells include, without limitation embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult stem cells, and tissue-specific stem cells, such as hematopoietic stem cells (blood stem cells), mesenchymal stem cells (MSC), neural stem cells, epithelial stem cells, or skin stem cells. In some embodiments, the cell is a cell that is derived or differentiated from a stem cell of the present disclosure. In some embodiments, the cell is an immune cell. Immune cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immune cells include, without limitation, T cells (*e.g.*, helper T cells, cytotoxic T cells, memory T cells, regulatory T cells, natural killer T cells, alpha beta T cells, and gamma delta T cells), B cells, natural killer (NK) cells, dendritic cells, myeloid cells, macrophages, and monocytes. In some embodiments, the cell is a neuronal cell. Neuronal cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary neuronal cells include, without limitation, neural progenitor cells, neurons (*e.g.*, sensory neurons, motor neurons, cholinergic neurons, GABAergic neurons, glutamatergic neurons, dopaminergic neurons, or serotonergic neurons), astrocytes, oligodendrocytes, and microglia.

[0405] In some embodiments, the cell is an immunoresponsive cell. Immunoresponsive cells of the present disclosure may be isolated or differentiated from a stem cell of the present disclosure (e.g., from an ESC or iPSC). Exemplary immunoresponsive cells of the present disclosure include, without limitation, cells of the lymphoid lineage. The lymphoid lineage, comprising B cells, T cells, and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. Examples of immunoresponsive cells of the lymphoid lineage include, without limitation, T cells, Natural Killer (NK) cells, embryonic stem cells, pluripotent stem cells, and induced pluripotent stem cells (e.g., those from which lymphoid cells may be derived or differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. In some embodiments, T cells of the present disclosure can be any type of T cells, including, without limitation, T helper cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: e.g., T_{EM} cells and T_{EMRA} cells, regulatory T cells (also known as suppressor T cells), natural killer T cells, mucosal associated invariant T cells, and γδ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may

be genetically modified to target specific antigens through the introduction of one or more chimeric receptors, such as a chimeric TCRs or CARs.

[0406] Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

[0407] In some embodiments, an immunoresponsive cell of the present disclosure is a T cell. T cells of the present disclosure may be autologous, allogeneic, or derived in vitro from engineered progenitor or stem cells.

[0408] In some embodiments, an immunoresponsive cell of the present disclosure is a universal T cell with deficient TCR-αβ. Methods of developing universal T cells are described in the art, for example, in Valton et al., Molecular Therapy (2015); 23 9, 1507-1518, and Torikai et al., Blood 2012 119:5697-5705.

[0409] In some embodiments, an immunoresponsive cell of the present disclosure is an isolated immunoresponsive cell comprising one or more chimeric receptors of the present disclosure. In some embodiments, the immunoresponsive cell comprises one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, or ten or more chimeric receptors of the present disclosure.

[0410] In some embodiments, an immunoresponsive cell is a T cell. In some embodiments, an immunoresponsive cell is a Natural Killer (NK) cell.

Cells expressing multiple chimeric receptors

[0411] In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors of the present disclosure. In some embodiments, the cell comprises two or more chimeric receptors, wherein one of the two or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises three or more chimeric receptors, wherein at least one of the three or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises four or more chimeric receptors, wherein at least one of the four or more chimeric receptors is a chimeric inhibitory receptor. In some embodiments, the cell comprises five or more chimeric receptors, wherein at least one of the five or more chimeric receptors is a chimeric inhibitory receptor.

[0412] In some embodiments, each of the two or more chimeric receptors comprise a different antigen-binding domain, e.g., that binds to the same antigen or to a different antigen. In some embodiments each antigen bound by the two or more chimeric receptors are

expressed on the same myeloid cell type (e.g., same AML cell type). In one embodiment, the cell comprises a first chimeric receptor that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second chimeric receptor that targets a second, different, antigen and includes an intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. Without wishing to be bound by theory, it is believed that placement of a costimulatory signaling domain (e.g., 4-1BB, CD28, or OX-40) onto the first chimeric receptor, and placement of a primary signaling domain (e.g., CD3-zeta chain) on the second chimeric receptor may limit chimeric receptor activity to cells where both targets are expressed. Accordingly, in some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) includes: (a) a first chimeric receptor comprising an antigen-binding domain that binds a first antigen, a transmembrane domain, and a costimulatory signaling domain; and (b) a second chimeric receptor comprising an antigen-binding domain that binds a second antigen, a transmembrane domain, and a primary signaling domain. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) includes: (a) a first chimeric receptor comprising an antigen-binding domain that binds a first antigen, a transmembrane domain, and a primary signaling domain; and (b) a second chimeric receptor comprising an antigen-binding domain that binds a second antigen, a transmembrane domain, and a costimulatory signaling domain. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) includes: (a) a first chimeric receptor comprising an antigen-binding domain that binds a first antigen, a transmembrane domain, a primary signaling domain and a costimulatory domain; and (b) a second chimeric receptor comprising an antigen-binding domain that binds a second antigen, a transmembrane domain, a primary signaling domain and a costimulatory domain. In embodiments where both the first chimeric receptor and the second chimeric receptor each comprise a costimulatory signaling domain, the costimulatory signaling domain of the first chimeric receptor and the costimulatory signaling domain of the second chimeric receptor may be derived from the same protein, such as from 4-1BB, CD28, or OX40. Alternatively, the costimulatory signaling domain of the first chimeric receptor may be derived from a different protein than that of the costimulatory signaling domain of the second chimeric receptor.

[0413] In embodiments where a cell of the present disclosure (e.g., an immunoresponsive cell) expresses two or more distinct chimeric receptors, the antigen-binding domain of each of the different chimeric receptors may be designed such that the antigen-binding domains do not interact with one another. For example, a cell of the present disclosure (e.g., an

immunoresponsive cell) expressing a first chimeric receptor and a second chimeric receptor may comprise a first chimeric receptor that comprises an antigen-binding domain that does not form an association with the antigen-binding domain of the second chimeric receptor. For example, the antigen-binding domain of the first chimeric receptor may comprise an antibody fragment, such as an scFv, while the antigen-binding domain of the second chimeric receptor may comprise a VHH.

[0414] Without wishing to be bound by theory, it is believed that in cells having a plurality of chimeric membrane embedded receptors that each comprise an antigen-binding domain, interactions between the antigen-binding domains of each of the receptors can be undesirable, because such interactions may inhibit the ability of one or more of the antigen-binding domains to bind their cognate antigens. Accordingly, in embodiments where cells of the present disclosure (e.g., immunoresponsive cells) express two or more chimeric receptors, the chimeric receptors comprise antigen-binding domains that minimize such inhibitory interactions. In one embodiment, the antigen-binding domain of one chimeric receptor comprises an scFv and the antigen-binding domain of the second chimeric receptor comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

[0415] In some embodiments, when present on the surface of a cell, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen is not substantially reduced by the presence of the second chimeric receptor. In some embodiments, binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the presence of the second chimeric receptor is 85%, 90%, 95%, 96%, 97%, 98%, or 99% of binding of the antigen-binding domain of the first chimeric receptor to its cognate antigen in the absence of the second chimeric receptor. In some embodiments, when present on the surface of a cell, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another less than if both were scFv antigen-binding domains. In some embodiments, the antigen-binding domains of the first chimeric receptor and the second chimeric receptor associate with one another 85%, 90%, 95%, 96%, 97%, 98%, or 99% less than if both were scFv antigen-binding domains.

[0416] In embodiments where a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more distinct chimeric receptors of the present disclosure that bind to different antigens, the two or more chimeric receptor provide the cell with logic gating, such as OR logic gating, AND logic gating, NOT logic gating, or any combination of such logic gating. Accordingly, in certain embodiments, a cell of the present disclosure (e.g., an

immunoresponsive cell) comprises two or more chimeric receptors, and binding of the first chimeric receptor to the first antigen is capable of activating the cell. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors, and binding of the second chimeric receptor to the second antigen is capable of stimulating the cell. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors, and binding of the first chimeric receptor to the first antigen and binding of the second chimeric receptor to the second antigen are required for activating the cell. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors, and binding of the first chimeric receptor to the first antigen and binding of the second chimeric receptor to the second antigen are required for simulating the cell. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors, and the cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to the cytolytic activity against cells that are positive for only the first antigen or only the second antigen. In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises two or more chimeric receptors, and binding of the first chimeric receptor to the first antigen or binding of the second chimeric receptor to the second antigen is capable of activating the immunoresponsive cell.

[0417] In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises a split chimeric receptor system, such as a split CAR system. Exemplary split chimeric receptor systems are described in WO2014/055442 and WO2014/055657. In some embodiments, a split chimeric receptor system comprises a cell expressing a first chimeric receptor having a first antigen-binding domain and a costimulatory domain (e.g., 4-1BB), as well as a second chimeric receptor having a second antigen-binding domain and an intracellular signaling domain (e.g., CD3-zeta). In such embodiments, when the cell encounters the first antigen, the costimulatory domain is activated, and the cell proliferates. Additionally, when the cell encounters the second antigen, the intracellular signaling domain is activated and cell-killing activity is induced. Accordingly, in some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) is only fully activated in the presence of both antigens.

[0418] In certain embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to against cells that are singly positive for

the first antigen. In certain embodiments, the first chimeric receptor binds to a first antigen with a low binding affinity or a low binding avidity. In certain embodiments, the first chimeric receptor binds to the first antigen at an epitope of low accessibility. In certain embodiments, first chimeric receptor binds to the first antigen with a binding affinity that is lower compared to the binding affinity with which the second chimeric receptor binds to the second antigen. In some embodiments, the first chimeric receptor binds to the first antigen with a binding affinity that is at least 5-fold lower compared to the binding affinity with which the second chimeric receptor binds to the second antigen. In some embodiments, the first chimeric receptor binds to the first antigen with a binding affinity that is at least 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 5000-fold, 1000-fold, 5000-fold, or 10000-fold lower compared to the binding affinity with which the second chimeric receptor binds to the second antigen.

[0419] In some embodiments, pairing choices should favor redundant expression of the two target antigens in the tumor in order to minimize the risk of antigen escape. Accordingly, in some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises (i) a first chimeric receptor that binds to a first antigen and (ii) a second chimeric receptor that binds to a second antigen, wherein the combination of both chimeric receptors binding to their targets antigens produces a therapeutic effect. In embodiments, binding to only one target antigen does not achieve a therapeutic effect.

[0420] In some embodiments, the chimeric receptor binds an FLT3 antigen. In some embodiments, the chimeric receptor binds an MS4A3 antigen. In some embodiments, the chimeric receptor binds a CD33 antigen. In some embodiments, the chimeric receptor binds a CLEC12A antigen. In some embodiments, the chimeric receptor binds a CD312/ADGRE2 antigen. In some embodiments, the chimeric receptor binds an SLC22A16 antigen. In some embodiments, the chimeric receptor binds a CD123/ILR3RA antigen. In some embodiments, the chimeric receptor binds an LAT2 antigen. In some embodiments, the chimeric receptor binds a PIEZO1/FAM38A antigen. In some embodiments, the chimeric receptor binds a CD38 antigen. In some embodiments, the chimeric receptor binds an EMB antigen. In some embodiments, the chimeric receptor binds a CD131/CSF2RB antigen. In some embodiments, the chimeric receptor binds a P2RY8 antigen. In some embodiments, the chimeric receptor binds a LILRA2/CD85H antigen. In some embodiments, the chimeric receptor binds an SLC17A9 antigen. In some embodiments, the chimeric receptor binds an MYADM antigen. In some embodiments, the chimeric receptor binds a CD300LF antigen. In some embodiments, the chimeric receptor binds a CD244/SLAMF4 antigen. In some embodiments,

the chimeric receptor binds a PLAUR antigen. In some embodiments, the chimeric receptor binds a CD93 antigen. In some embodiments, the chimeric receptor binds an SPNS3 antigen. In some embodiments, the chimeric receptor binds a GAPT antigen. In some embodiments, the chimeric receptor binds a RASGRP4 antigen. In some embodiments, the chimeric receptor binds a CD117/c-Kit antigen. In some embodiments, the chimeric receptor binds a CD123/ILR3RA antigen.

[0421] In some embodiments, the chimeric receptor binds a VSTM1 antigen. In some embodiments, the chimeric receptor binds an MLC1 antigen. In some embodiments, the chimeric receptor binds a PRAM1 antigen. In some embodiments, the chimeric receptor binds an HCK antigen. In some embodiments, the chimeric receptor binds an ICAM3 antigen. In some embodiments, the chimeric receptor binds an LRRC37A2 antigen. In some embodiments, the chimeric receptor binds an ITGAM antigen. In some embodiments, the chimeric receptor binds an ITGB2 antigen. In some embodiments, the chimeric receptor binds an LILRA1 antigen. In some embodiments, the chimeric receptor binds a PRTN3 antigen. In some embodiments, the chimeric receptor binds a CARD9 antigen. In some embodiments, the chimeric receptor binds a SIGLEC5 antigen. In some embodiments, the chimeric receptor binds a SELL antigen. In some embodiments, the chimeric receptor binds an MLKL antigen. In some embodiments, the chimeric receptor binds an INPP5D antigen. In some embodiments, the chimeric receptor binds an APBB1IP antigen. In some embodiments, the chimeric receptor binds an ITGA4 antigen. In some embodiments, the chimeric receptor binds a C3AR1 antigen. In some embodiments, the chimeric receptor binds an ITGA5 antigen. In some embodiments, the chimeric receptor binds a FMNL1 antigen. In some embodiments, the chimeric receptor binds an IL1RAP antigen. In some embodiments, the chimeric receptor binds a CCR1/CD191 antigen. In some embodiments, the chimeric receptor binds an LILRB2 antigen. In some embodiments, the chimeric receptor binds a CD70 antigen. In some embodiments, the chimeric receptor is a multispecific receptor comprising two or more antigen-binding domains, such that the chimeric receptor can bind two or more antigens.

[0422] In some embodiments, an immunoresponsive cell may comprise one or more more tumor-targeting chimeric receptors and one or more inhibitory chimeric receptors that targets an antigen that is not expressed on the tumor. Combinations of tumor-targeting chimeric receptors and inhibitory chimeric receptors in the same immunoresponsive cell may be used to reduce on-target off-tumor toxicity. For instance, if a healthy cell expresses both an antigen that is recognized by a tumor-targeting chimeric receptor and an antigen that is recognized by

an inhibitory chimeric receptor, an immunoresponsive cell expressing the tumor antigen may bind to the healthy cell. In such a case, the inhibitory chimeric antigen will also bind its cognate ligand on the healthy cell and the inhibitory function of the inhibitory chimeric receptor will reduce, decrese, prevent, or inhibit the activation of the immunoresponsive cell via the tumor-targeting chimeric receptor.

[0423] In some embodiments, the inhibitory chimeric receptor binds an EMCN (Endomucin) antigen. In some embodiments, the inhibitory chimeric receptor binds a JAM2 antigen. In some embodiments, the inhibitory chimeric receptor binds an MS4A15 antigen. In some embodiments, the inhibitory chimeric receptor binds an SLC34A2 antigen. In some embodiments, the inhibitory chimeric receptor binds an SLC2A2 antigen. In some embodiments, the c inhibitory himeric receptor binds a TRPM1 antigen. In some embodiments, the inhibitory chimeric receptor binds an SCTR antigen. In some embodiments, the inhibitory chimeric receptor binds a KCNQ2 antigen. In some embodiments, the inhibitory chimeric receptor binds a PERP antigen. In some embodiments, the inhibitory chimeric receptor binds a WLS antigen. In some embodiments, the inhibitory chimeric receptor binds a FFAR2 antigen. In some embodiments, the inhibitory chimeric receptor binds a PTPRB antigen. In some embodiments, the inhibitory chimeric receptor binds an NCKAP1 antigen. In some embodiments, the inhibitory chimeric receptor binds an MPZL2 antigen. In some embodiments, the inhibitory chimeric receptor binds a PLSCR4 antigen. In some embodiments, the inhibitory chimeric receptor binds a TMEM47 antigen. In some embodiments, the inhibitory chimeric receptor binds an ADGRL4 antigen. In some embodiments, the inhibitory chimeric receptor binds a MET antigen. In some embodiments, the inhibitory chimeric receptor binds a BACE2 antigen. In some embodiments, the inhibitory chimeric receptor binds a ATP8B1 antigen. In some embodiments, the inhibitory chimeric receptor binds an LIFR antigen. In some embodiments, the inhibitory chimeric receptor binds an ART4 antigen. In some embodiments, the inhibitory chimeric receptor binds a CALCRL antigen. In some embodiments, the inhibitory chimeric receptor binds a CNTNAP3 antigen. In some embodiments, the inhibitory chimeric receptor binds a PCDH9 antigen. In some embodiments, the inhibitory chimeric receptor binds an IL18R1 antigen. In some embodiments, the inhibitory chimeric receptor binds an SLC8A3 antigen. In some embodiments, the inhibitory chimeric receptor binds a CDH26 antigen. In some embodiments, the inhibitory chimeric receptor binds an SLC8A3 antigen. In some embodiments, the inhibitory chimeric receptor binds a TMEM163 antigen. In some embodiments, the inhibitory chimeric receptor binds an ABCA13 antigen. In some

embodiments, the inhibitory chimeric receptor binds a CACHD1 antigen. In some embodiments, the inhibitory chimeric receptor binds a CYYR1 antigen. In some embodiments, the inhibitory chimeric receptor binds an ABCB1 antigen. In some embodiments, the inhibitory chimeric receptor binds an ADGRG6 antigen. In some embodiments, the inhibitory chimeric receptor binds an ATP9A antigen. In some embodiments, the inhibitory chimeric receptor binds a CALN1 antigen. In some embodiments, the inhibitory chimeric receptor binds a CDCP1 antigen. In some embodiments, the inhibitory chimeric receptor binds a IL12RB2 antigen. In some embodiments, the inhibitory chimeric receptor binds a SLC16A14 antigen. In some embodiments, the inhibitory chimeric receptor binds a TMEM136 antigen. In some embodiments, the inhibitory chimeric receptor binds a TMEM200A antigen.

[0424] Alternatively, a cell express two or more chimeric receptors that bind to different antigens. Exemplary pairs of antigens are shown in **Table 3**.

Table 3

Antigen 1	Antigen 2		Antigen 1	Antigen 2
ITGA4	SLC17A9		CD244	EMB
ITGA4	LRRC37A2		CD244	MLKL
ITGA4	EMB		CD244	ADGRE2
ITGA4	MLKL		PRTN3	SIGLEC5
ITGA4	MYADM		EMB	MLKL
ITGA4	ADGRE2		EMB	MYADM
ITGA5	ITGAM		EMB	FLT3
ITGA5	LRRC37A2		EMB	ADGRE2
ITGA5	PRTN3		EMB	LILRA2
ITGA5	MLC1		CARD9	MLC1
ITGA5	ITGB2		CARD9	LAT2
ITGA5	LAT2		CARD9	SIGLEC5
ITGA5	MS4A3		CARD9	CD300LF
ITGA5	PIEZO1		CARD9	INPP5D
ITGAM	LRRC37A2		CARD9	MS4A3
ITGAM	PRTN3		CARD9	HCK
ITGAM	MLC1		CARD9	ICAM3
ITGAM	LAT2		CARD9	CD38
ITGAM	MS4A3		CARD9	CD33
ITGAM	PIEZO1		CARD9	PIEZO1
CSF2RB	SLC17A9		CARD9	FMNL1
CSF2RB	LRRC37A2		CARD9	CLEC12A
CSF2RB	EMB		CARD9	CD93
CSF2RB	MLKL		MLC1	SIGLEC5
CSF2RB	ADGRE2		MLC1	MS4A3
SLC17A9	CMTM7		MLC1	PIEZO1
SLC17A9	LRRC37A2		ITGB2	MS4A3
SLC17A9	CD244		ITGB2	PIEZO1
SLC17A9	EMB		LAT2	SIGLEC5
SLC17A9	MLKL		LAT2	MS4A3
SLC17A9	MYADM		LAT2	PIEZO1
SLC17A9	FLT3		CD300LF	MS4A3

Table 3				
Antigen 1	**Antigen 2**		**Antigen 1**	**Antigen 2**
SLC17A9	ADGRE2		CD300LF	PIEZO1
SLC17A9	LILRA2		MLKL	MYADM
SLC17A9	LILRA1		MLKL	FLT3
CYBA	LRRC37A2		MLKL	ADGRE2
CYBA	PRTN3		MLKL	LILRA2
CYBA	MLC1		MLKL	LILRA1
CYBA	LAT2		INPP5D	MS4A3
CYBA	MS4A3		INPP5D	PIEZO1
CYBA	ICAM3		MYADM	FLT3
CYBA	PIEZO1		MS4A3	PIEZO1
CMTM7	LRRC37A2		HCK	PIEZO1
CMTM7	EMB		APBB1IP	C3AR1
CMTM7	MLKL		ICAM3	PIEZO1
CMTM7	MYADM		CD38	PIEZO1
CMTM7	ADGRE2		CD38	CD93
CMTM7	LILRA2		CD33	PIEZO1
CMTM7	LILRA1		FLT3	ADGRE2
LRRC37A2	CD244		ADGRE2	LILRA2
LRRC37A2	PRTN3		ADGRE2	LILRA1
LRRC37A2	EMB		LRRC37A2	HCK
LRRC37A2	CARD9		LRRC37A2	APBB1IP
LRRC37A2	MLC1		LRRC37A2	ICAM3
LRRC37A2	ITGB2		LRRC37A2	CD33
LRRC37A2	LAT2		LRRC37A2	PIEZO1
LRRC37A2	SIGLEC5		LRRC37A2	FLT3
LRRC37A2	CD300LF		LRRC37A2	ADGRE2
LRRC37A2	MLKL		LRRC37A2	FMNL1
LRRC37A2	INPP5D		LRRC37A2	CLEC12A
LRRC37A2	MYADM		LRRC37A2	LILRA2
LRRC37A2	MS4A3		LRRC37A2	LILRA1

[0425] In some embodiments, the two or more antigens are ITGA4 and SLC17A9. In some embodiments, the two or more antigens are ITGA4 and LRRC37A2. In some embodiments, the two or more antigens are ITGA4 and EMB. In some embodiments, the two or more antigens are ITGA4 and MLKL. In some embodiments, the two or more antigens are ITGA4 and MYADM. In some embodiments, the two or more antigens are ITGA4 and ADGRE2.

[0426] In some embodiments, the two or more antigens are ITGA5 and ITGAM. In some embodiments, the two or more antigens are ITGA5 and LRRC37A2. In some embodiments, the two or more antigens are ITGA5 and PRTN3. In some embodiments, the two or more antigens are ITGA5 and MLC1. In some embodiments, the two or more antigens are ITGA5 and ITGB2. some embodiments, the two or more antigens are ITGA5 and LAT2. In some embodiments, the two or more antigens are ITGA5 and MS4A3. In some embodiments, the two or more antigens are ITGA5 and PIEZO1.

[0427] In some embodiments, the two or more antigens are ITGAM and PIEZO1. In some embodiments, the two or more antigens are ITGAM and LRRC37A2. In some embodiments, the two or more antigens are ITGAM and PRTN3. In some embodiments, the two or more

antigens are ITGAM and MLC1. In some embodiments, the two or more antigens are ITGAM and LAT2. In some embodiments, the two or more antigens are ITGAM and MS4A3.

[0428] In some embodiments, the two or more antigens are CSF2RB and SLC17A9. In some embodiments, the two or more antigens are CSF2RB and LRRC37A2. In some embodiments, the two or more antigens are CSF2RB and EMB. In some embodiments, the two or more antigens are CSF2RB and MLKL. In some embodiments, the two or more antigens are CSF2RB and ADGRE2.

[0429] In some embodiments, the two or more antigens are SLC17A9 and CMTM7. In some embodiments, the two or more antigens are SLC17A9 and LRRC37A2. In some embodiments, the two or more antigens are SLC17A9 and CD244. In some embodiments, the two or more antigens are SLC17A9 and EMB. In some embodiments, the two or more antigens are SLC17A9 and MLKL. In some embodiments, the two or more antigens are SLC17A9 and MYADM. In some embodiments, the two or more antigens are SLC17A9 and FLT3. In some embodiments, the two or more antigens are SLC17A9 and ADGRE2. In some embodiments, the two or more antigens are SLC17A9 and LILRA2. In some embodiments, the two or more antigens are SLC17A9 and LILRA1.

[0430] In some embodiments, the two or more antigens are CYBA and LRRC37A2. In some embodiments, the two or more antigens are CYBA and PRTN3. In some embodiments, the two or more antigens are CYBA and MLC1. In some embodiments, the two or more antigens are CYBA and LAT2. In some embodiments, the two or more antigens are CYBA and MS4A3. In some embodiments, the two or more antigens are CYBA and ICAM3. In some embodiments, the two or more antigens are CYBA and PIEZO1.

[0431] In some embodiments, the two or more antigens are CMTM7 and LRRC37A2. In some embodiments, the two or more antigens are CMTM7 and EMB. In some embodiments, the two or more antigens are CMTM7 and MLKL. In some embodiments, the two or more antigens are CMTM7 and MYADM. In some embodiments, the two or more antigens are CMTM7 and ADGRE2. In some embodiments, the two or more antigens are CMTM7 and LILRA2. In some embodiments, the two or more antigens are CMTM7 and LILRA1.

[0432] In some embodiments, the two or more antigens are LRRC37A2 and CD244. In some embodiments, the two or more antigens are LRRC37A2 and PRTN3. In some embodiments, the two or more antigens are LRRC37A2 and EMB. In some embodiments, the two or more antigens are LRRC37A2 and CARD9. In some embodiments, the two or more antigens are LRRC37A2 and MLC1. In some embodiments, the two or more antigens are LRRC37A2 and

ITGB2. In some embodiments, the two or more antigens are LRRC37A2 and LAT2. In some embodiments, the two or more antigens are LRRC37A2 and SIGLEC5. In some embodiments, the two or more antigens are LRRC37A2 and CD300LF. In some embodiments, the two or more antigens are LRRC37A2 and MLKL. In some embodiments, the two or more antigens are LRRC37A2 and INPP5D. In some embodiments, the two or more antigens are LRRC37A2 and MYADM. In some embodiments, the two or more antigens are LRRC37A2 and MS4A3. In some embodiments, the two or more antigens are LRRC37A2 and HCK. In some embodiments, the two or more antigens are LRRC37A2 and APBB1IP. In some embodiments, the two or more antigens are LRRC37A2 and ICAM3. In some embodiments, the two or more antigens are LRRC37A2 and CD33. In some embodiments, the two or more antigens are LRRC37A2 and PIEZO1. In some embodiments, the two or more antigens are LRRC37A2 and FLT3. In some embodiments, the two or more antigens are LRRC37A2 and ADGRE2. In some embodiments, the two or more antigens are LRRC37A2 and FMNL1. In some embodiments, the two or more antigens are LRRC37A2 and CLEC12A. In some embodiments, the two or more antigens are LRRC37A2 and LILRA2. In some embodiments, the two or more antigens are LRRC37A2 and LILRA1. In some embodiments, the two or more antigens are LRRC37A2 and CD93. In some embodiments, the two or more antigens are LRRC37A2 and C3AR1.

[0433] In some embodiments, the two or more antigens are CD244 and EMB. In some embodiments, the two or more antigens are CD244 and MLKL. In some embodiments, the two or more antigens are CD244 and ADGRE2.

[0434] In some embodiments, the two or more antigens are PRTN3 and SIGLEC5.

[0435] In some embodiments, the two or more antigens are EMB and MLKL. In some embodiments, the two or more antigens are EMB and MYADM. In some embodiments, the two or more antigens are EMB and FLT3. In some embodiments, the two or more antigens are EMB and ADGRE2. In some embodiments, the two or more antigens are EMB and LILRA2.

[0436] In some embodiments, the two or more antigens are CARD9 and MLC1. In some embodiments, the two or more antigens are CARD9 and LAT2. In some embodiments, the two or more antigens are CARD9 and SIGLEC5. In some embodiments, the two or more antigens are CARD9 and CD300LF. In some embodiments, the two or more antigens are CARD9 and INPP5D. In some embodiments, the two or more antigens are CARD9 and MS4A3. In some embodiments, the two or more antigens are CARD9 and HCK. In some embodiments, the two or more antigens are CARD9 and ICAM3. In some embodiments, the

two or more antigens are CARD9 and CD38. In some embodiments, the two or more antigens are CARD9 and CD33. In some embodiments, the two or more antigens are CARD9 and PIEZO1. In some embodiments, the two or more antigens are CARD9 and FMNL1. In some embodiments, the two or more antigens are CARD9 and CLEC12A. In some embodiments, the two or more antigens are CARD9 and CD93.

[0437] In some embodiments, the two or more antigens are MLC1 and SIGLEC5. In some embodiments, the two or more antigens are MLC1 and MS4A3. In some embodiments, the two or more antigens are MLC1 and PIEZO1.

[0438] In some embodiments, the two or more antigens are ITGB2 and PIEZO1. In some embodiments, the two or more antigens are ITGB2 and MS4A3.

[0439] In some embodiments, the two or more antigens are LAT2 and MS4A3. In some embodiments, the two or more antigens are LAT2 and SIGLEC5. In some embodiments, the two or more antigens are LAT2 and PIEZO1.

[0440] In some embodiments, the two or more antigens are CD300LF and PIEZO1. In some embodiments, the two or more antigens are CD300LF and MS4A3.

[0441] In some embodiments, the two or more antigens are MLKL and MYADM. In some embodiments, the two or more antigens are MLKL and FLT3. In some embodiments, the two or more antigens are MLKL and ADGRE2. In some embodiments, the two or more antigens are MLKL and LILRA2. In some embodiments, the two or more antigens are MLKL and LILRA1.

[0442] In some embodiments, the two or more antigens are INPP5D and PIEZO1. In some embodiments, the two or more antigens are INPP5D and MS4A3.

[0443] In some embodiments, the two or more antigens are MYADM and FLT3.

[0444] In some embodiments, the two or more antigens are MS4A3 and PIEZO1. In some embodiments, the two or more antigens are HCK and PIEZO1. In some embodiments, the two or more antigens are ICAM3 and PIEZO1. In some embodiments, the two or more antigens are CD38 and PIEZO1. In some embodiments, the two or more antigens are CD93 and CD38. In some embodiments, the two or more antigens are CD33 and PIEZO1.

[0445] In some embodiments, the two or more antigens are APBB1IP and C3AR1.

[0446] In some embodiments, the two or more antigens are FLT3 and ADGRE2. In some embodiments, the two or more antigens are ADGRE2 and LILRA2. In some embodiments, the two or more antigens are ADGRE2 and LILRA1.

[0447] In some embodiments, the two or more antigens are PIEZO1 and P2RY8.

[0448] In some embodiments, the two or more antigens are FLT3 and CD33. In some embodiments, a cell expresses two or more chimeric receptors of the present disclosure, wherein one chimeric receptor binds FLT3 and the second chimeric receptor binds CD33. In some embodiments, a chimeric receptor is a multispecific receptor comprising two or more antigen-binding domains, such that one antigen-binding domain binds FLT3 and a second antigen-binding domain binds CD33. In some embodiments, the chimeric antigen receptor and/or antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto; (c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto; (d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto; (e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto; (f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto; (g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and (h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments, the chimeric antigen receptor and/or or antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical

thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

[0449] In some embodiments, the two or more antigens are FLT3 and CLEC12A. In some embodiments, a cell expresses two or more chimeric receptors of the present disclosure, wherein one chimeric receptor binds FLT3 and the second chimeric receptor binds CLEC12A. In some embodiments, a chimeric receptor is a multispecific receptor comprising two or more antigen-binding domains, such that one antigen-binding domain binds FLT3 and a second antigen-binding domain binds CLEC12A. In some embodiments, a chimeric receptor is a multispecific receptor comprising two or more antigen-binding domains, such that one antigen-binding domain binds FLT3 and a second antigen-binding domain binds CD33. In some embodiments, the chimeric antigen receptor and/or or antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto; (c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto; (d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto; (e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto; (f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto; (g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and (h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto. In some embodiments, the chimeric antigen receptor and/or or antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) and a light chain variable domain

(VL) selected from:(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

[0450] In some embodiments, the two or more antigens are CLEC12A and CD33. In some embodiments, a cell expresses two or more chimeric receptors of the present disclosure, wherein one chimeric receptor binds CLEC12A and the second chimeric receptor binds CD33. In some embodiments, a chimeric receptor is a multispecific receptor comprising two or more antigen-binding domains, such that one antigen-binding domain binds CLEC12A and a second antigen-binding domain binds CD33. In some embodiments, the chimeric antigen receptor and/or or antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from:(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto; (b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and (c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26or a sequence at least 90% identical thereto. In some embodiments, the chimeric antigen receptor and/or or antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from: (a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and (b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

[0451] In some embodiments, the immunoresponsive cell comprises a bicistronic chimeric antigen receptor. In some embodiments, the bicistronic chimeric antigen receptor comprises an FLT3 CAR and a CD33 CAR. In some embodiments, the bicistronic chimeric antigen

receptor comprises an FLT3 CAR and a CLEC12A CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises an CLEC12A CAR and a CD33 CAR. In some embodiments, the bicistronic chimeric antigen receptor comprises any pair of antigens provided in Table 3.

Chimeric inhibitory receptors

[0452] In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric inhibitory receptors of the present disclosure. In some embodiments, each of the one or more chimeric inhibitory receptors comprises an antigen-binding domain that binds an antigen expressed on normal cells but not on tumor cells, such as AML cells. In some embodiments, the one or more chimeric inhibitory receptors bind antigens that are expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

[0453] In some embodiments, a chimeric inhibitory receptor may be used, for example, with one or more chimeric receptors (e.g., chimeric TCRs or CARs) expressed on a cell of the present disclosure (e.g., an immunoresponsive cell) as NOT logic gates to control, modulate, or otherwise inhibit one or more activities of the one or more chimeric receptors. In some embodiments, a chimeric receptor of the present disclosure may inhibit one or more activities of a cell of the present disclosure (e.g., an immunoresponsive cell). In some embodiments, the chimeric inhibitory receptor is combined with one or more chimeric receptors of the present disclosure to combine OR logic gating with NOT logic gating and/or AND logic gating with NOT logic gating.

[0454] In some embodiments, the chimeric inhibitory receptor binds one or more antigens selected from EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

[0455] In some embodiments, the chimeric receptor binds an FLT3 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a

PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0456] In some embodiments, the chimeric receptor binds an MS4A3antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0457] In some embodiments, the chimeric receptor binds a CD33 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0458] In some embodiments, the chimeric receptor binds a CLEC12A antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0459] In some embodiments, the chimeric receptor binds an ADGRE2 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0460] In some embodiments, the chimeric receptor binds a SLC22A16 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an

SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0461] In some embodiments, the chimeric receptor binds a CD123/IL3RA antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0462] In some embodiments, the chimeric receptor binds an MLC1 antigen and the chimeric inhibitory receptor an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0463] In some embodiments, the chimeric receptor binds an SPNS3 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0464] In some embodiments, the chimeric receptor binds a GAPT antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0465] In some embodiments, the chimeric receptor binds a LAT2 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0466] In some embodiments, the chimeric receptor binds a PIEZO1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0467] In some embodiments, the chimeric receptor binds a CD38 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0468] In some embodiments, the chimeric receptor binds an EMB antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0469] In some embodiments, the chimeric receptor binds a CD131/CSF2RB antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an

ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0470] In some embodiments, the chimeric receptor binds a LILRA2/CD85H antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0471] In some embodiments, the chimeric receptor binds a SLC17A9 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0472] In some embodiments, the chimeric receptor binds a MYADM antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0473] In some embodiments, the chimeric receptor binds a CD300LF antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0474] In some embodiments, the chimeric receptor binds a CD244 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a

CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0475] In some embodiments, the chimeric receptor binds a CD93 antigen. In some embodiments, the chimeric receptor binds a CD117/CKIT antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0476] In some embodiments, the chimeric receptor binds a CD117/c-KIT antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0477] In some embodiments, the chimeric receptor binds a CMTM7 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0478] In some embodiments, the chimeric receptor binds a CYBA antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0479] In some embodiments, the chimeric receptor binds an HCK antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR,

an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0480] In some embodiments, the chimeric receptor binds an ICAM3 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0481] In some embodiments, the chimeric receptor binds a LRRC37A3 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0482] In some embodiments, the chimeric receptor binds an ITGAM antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0483] In some embodiments, the chimeric receptor binds an ITGB2 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0484] In some embodiments, the chimeric receptor binds a LILRA1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0485] In some embodiments, the chimeric receptor binds a PRTN3 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0486] In some embodiments, the chimeric receptor binds a CARD9 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0487] In some embodiments, the chimeric receptor binds a SIGLEC5 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0488] In some embodiments, the chimeric receptor binds a SELL antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an

ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0489] In some embodiments, the chimeric receptor binds a MLKL antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0490] In some embodiments, the chimeric receptor binds an INPP5D antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0491] In some embodiments, the chimeric receptor binds an APBB1IP antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0492] In some embodiments, the chimeric receptor binds an ITGA4 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0493] In some embodiments, the chimeric receptor binds a C3AR1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a

CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0494] In some embodiments, the chimeric receptor binds an ITGA5 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0495] In some embodiments, the chimeric receptor binds an FMNL1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0496] In some embodiments, the chimeric receptor binds a VSTM1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0497] In some embodiments, the chimeric receptor binds a PRAM1 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0498] In some embodiments, the chimeric receptor binds an IL1RAP antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2,

a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0499] In some embodiments, the chimeric receptor binds a CCR1/CD191 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0500] In some embodiments, the chimeric receptor binds a LILRB2 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0501] In some embodiments, the chimeric receptor binds a CD70 antigen and the chimeric inhibitory receptor binds an EMCN, a JAM2, an MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR, a PTPRB, a NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, a MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, an SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen.

[0502] In some embodiments, the chimeric inhibitory receptor binds an EMCN antigen and the chimeric receptor binds an FLT3 antigen. In some embodiments, the chimeric inhibitory receptor binds an EMCN antigen and the chimeric receptor binds an MLC1 antigen.

[0503] In some embodiments, the chimeric inhibitory receptor binds a JAM2 antigen and the chimeric receptor binds an FLT3 antigen. In some embodiments, the chimeric inhibitory receptors binds a JAM2 antigen and the chimeric receptor binds an MLC1 antigen.

[0504] In some embodiments, the chimeric inhibitory receptor binds an MS4A15 antigen and the chimeric receptor binds a CLEC12A antigen.

[0505] In some embodiments, the chimeric inhibitory receptor binds an SLC34A2 antigen and the chimeric receptor binds a CLEC12A antigen.

[0506] In some embodiments, the chimeric inhibitory receptor binds a C4BPA antigen and the chimeric receptor binds a CD33 antigen.

[0507] In some embodiments, the chimeric inhibitory receptor binds a TRPM1 antigen and the chimeric receptor binds a CD33 antigen.

[0508] In some embodiments, the chimeric inhibitory receptor binds an SCTR antigen and the chimeric receptor binds an SLC22A16 antigen.

[0509] In some embodiments, the chimeric inhibitory receptor binds an SLC2A2 antigen and the chimeric receptor binds an IL1RAP antigen.

[0510] In some embodiments, the chimeric inhibitory receptor binds a KCNQ2 antigen and the chimeric receptor binds a PIEZO1 antigen. In some embodiments, the chimeric inhibitory receptor binds a KCNQ2 antigen and the chimeric receptor binds an IL1RAP antigen.

[0511] In some embodiments, the chimeric inhibitory receptor binds a PERP antigen and the chimeric receptor binds a CD123 antigen. In some embodiments, the chimeric inhibitory receptor binds a PERP antigen and the chimeric receptor binds an IL3RA antigen.

[0512] In some embodiments, the chimeric inhibitory receptor binds a WLS antigen. In some embodiments, the chimeric inhibitory receptor binds a FFAR2 antigen. In some embodiments, the chimeric inhibitory receptor binds an NCKAP1 antigen. In some embodiments, the chimeric inhibitory receptor binds an MPZL2 antigen. In some embodiments, the chimeric inhibitory receptor binds a PLSCR4 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM47 antigen. In some embodiments, the chimeric inhibitory receptor binds an ADGRL4 antigen. In some embodiments, the chimeric inhibitory receptor binds an MET antigen. In some embodiments, the chimeric inhibitory receptor binds a BACE2 antigen. In some embodiments, the chimeric inhibitory receptor binds an ATP8B1 antigen. In some embodiments, the chimeric inhibitory receptor binds an LIFR antigen. In some embodiments, the chimeric inhibitory receptor binds an ART4 antigen. In some embodiments, the chimeric inhibitory receptor binds a CALCRL antigen. In some embodiments, the chimeric inhibitory receptor binds a CNTNAP3 antigen. In some embodiments, the chimeric inhibitory receptor binds a PCDH9 antigen. In some embodiments, the chimeric inhibitory receptor binds an IL18R1 antigen. In some embodiments, the chimeric inhibitory receptor binds an SLC8A3 antigen. In some embodiments, the chimeric inhibitory receptor binds a CDH26 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM163 antigen. In some

embodiments, the chimeric inhibitory receptor binds a ABCA13 antigen. In some embodiments, the chimeric inhibitory receptor binds a CACHD1 antigen. In some embodiments, the chimeric inhibitory receptor binds a CYYR1 antigen. In some embodiments, the chimeric inhibitory receptor binds an ADGRG6 antigen. In some embodiments, the chimeric inhibitory receptor binds an ATP9A antigen. In some embodiments, the chimeric inhibitory receptor binds a CALN1 antigen. In some embodiments, the chimeric inhibitory receptor binds a CDCP1 antigen. In some embodiments, the chimeric inhibitory receptor binds an IL12RB2 antigen. In some embodiments, the chimeric inhibitory receptor binds an SLC16A14 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM136 antigen. In some embodiments, the chimeric inhibitory receptor binds a TMEM200A antigen.

[0513] In some embodiments, the chimeric inhibitory receptor binds an EMCN, a JAM2, a MS4A15, a C4BPA, a TRPM1, an SCTR, an SLC2A2, a KCNQ2, a PERP, a WLS, a FFAR2, a PTPRB, an NCKAP1, an MPZL2, a PLSCR4, a TMEM47, an ADGRL4, an MET, a BACE2, an ATP8B1, a LIFR, an ART4, a CALCRL, a CNTNAP3, a PCDH9, an IL18R1, a SLC8A3, a CDH26, a TMEM163, an ABCA13, a CACHD1, a CYYR1, an ABCB1, an ADGRG6, an ATP9A, a CALN1, a CDCP1, an IL12RB2, an SLC16A14, a TMEM136, or a TMEM200A antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0514] In some embodiments, the chimeric inhibitory receptor binds an EMCN antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0515] In some embodiments, the chimeric inhibitory receptor binds a JAM2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0516] In some embodiments, the chimeric inhibitory receptor binds an MS4A15 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93,

IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0517] In some embodiments, the chimeric inhibitory receptor binds a C4BPA antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0518] In some embodiments, the chimeric inhibitory receptor binds an TRPM1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0519] In some embodiments, the chimeric inhibitory receptor binds an SCTR antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0520] In some embodiments, the chimeric inhibitory receptor binds an SLC2A2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0521] In some embodiments, the chimeric inhibitory receptor binds a KCNQ2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0522] In some embodiments, the chimeric inhibitory receptor binds a PERP antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0523] In some embodiments, the chimeric inhibitory receptor binds a WLS antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1,

SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0524] In some embodiments, the chimeric inhibitory receptor binds a FFAR2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0525] In some embodiments, the chimeric inhibitory receptor binds a PTPRB antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0526] In some embodiments, the chimeric inhibitory receptor binds an NCKAP1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0527] In some embodiments, the chimeric inhibitory receptor binds an MPZL2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0528] In some embodiments, the chimeric inhibitory receptor binds a PLSCR4 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0529] In some embodiments, the chimeric inhibitory receptor binds a TMEM47 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0530] In some embodiments, the chimeric inhibitory receptor binds an ADGRL4 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0531] In some embodiments, the chimeric inhibitory receptor binds an MET antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0532] In some embodiments, the chimeric inhibitory receptor binds a BACE2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0533] In some embodiments, the chimeric inhibitory receptor binds a ATP8B1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0534] In some embodiments, the chimeric inhibitory receptor binds a LIFR antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0535] In some embodiments, the chimeric inhibitory receptor binds an ART4 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0536] In some embodiments, the chimeric inhibitory receptor binds a CALCRL antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93,

IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0537] In some embodiments, the chimeric inhibitory receptor binds a CNTNAP3 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0538] In some embodiments, the chimeric inhibitory receptor binds a PCDH9 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0539] In some embodiments, the chimeric inhibitory receptor binds an IL18R1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0540] In some embodiments, the chimeric inhibitory receptor binds an SLC8A3 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0541] In some embodiments, the chimeric inhibitory receptor binds a CDH26 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0542] In some embodiments, the chimeric inhibitory receptor binds a TMEM163 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0543] In some embodiments, the chimeric inhibitory receptor binds an ABCA13 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1,

SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0544] In some embodiments, the chimeric inhibitory receptor binds a CACHD1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0545] In some embodiments, the chimeric inhibitory receptor binds a CYYR1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0546] In some embodiments, the chimeric inhibitory receptor binds an ABCB1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0547] In some embodiments, the chimeric inhibitory receptor binds an ADGRG6 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0548] In some embodiments, the chimeric inhibitory receptor binds an ATP9A antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0549] In some embodiments, the chimeric inhibitory receptor binds a CALN1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0550] In some embodiments, the chimeric inhibitory receptor binds a CDCP1 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0551] In some embodiments, the chimeric inhibitory receptor binds an IL12RB2 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0552] In some embodiments, the chimeric inhibitory receptor binds an SLC16A14 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0553] In some embodiments, the chimeric inhibitory receptor binds a TMEM136 antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0554] In some embodiments, the chimeric inhibitory receptor binds a TMEM200A antigen and the chimeric receptor binds a MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, or CD70 antigen.

[0555] In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain derived from an anti-EMCN antibody.The EMCN antibody can be any suitable EMCN antibody known, including, but not limited to, the EMCN antibodies CBFYE-0213, V.7.C7.1, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, and L10F12, as described in Samulowitz J, et al, Am. J. Path., 30 Apr 2002, 160(5):1669-1681, hereby incorporated by reference. EMCN antibodies are generally commercially available. For instance, CBFYE-0213 is a rat anti-human EMCN IgG and is available from Creative Biolabs (Cat. # CBMAB-E0461-FY) and V.7.C7.1 is a rat anti-EMCN IgG2a that cross-reacts with human and mouse EMCN and is available from Abcam (Cat. # ab106100).

[0556] In some embodiments, the EMCN antigen binding domain is an antibody selected from CBFYE-0213, V.7.C7.1, L6H10, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, L10F12, 18HCLC, or V.7.C7.

[0557] Antibodies that bind to CALN1, IL12RB2, CDH26, IL18R1, SLC8A3, ABCB1, ATP9A, CDCP1, EMCN, JAM2, PCDH9, TMEM200A, ADGRL4, ART4, BACE2, CALCRL, LIFR, MET, MPZL2, PTPRB, and WLS are generally known and commercially available from a variety of vendors. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain derived from the anti-CALN1 antibodies 2G5, 2G2, or 3H3. In some embodiments, the inhibitory chimeric receptor an antigen binding domain selected from the anti-IL12RB2 antibodies 2H6, S16020B, 305719, 9H1, 11D102, or REA333. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-CDH26 antibodies 6C10 or CH-19. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-IL18R1 antibodies 70625.111, H44, REA947, REA1095, B-E43, 44G6, or 5. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from from the anti-SLC8A3 antibody C2C12. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-ABCB1 antibodies C219, 4E3.16, C494, JSB-1, REA495, UIC2, SN06-42, OTI1A7, OTI5B3, OTI2G6, OTI6H2, or OTI2C7. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain of the anti-ATP9A antibody 3G2. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-CDCP1 antibodies CUB1, REA194, OTI2B2, OTI2C1, OTI2B8, OTI4G5, OTI5B3, CSTEM26, 309116, or 309121. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-JAM2 antibodies 988905, 988901, 988934, EPR2489(2), CBL560, C47B10-2A9S, C47B10-2B11S, C47B10-2B8S, 500A, J1, CBFYC-2851, MM0425-4L28-IgG1, 14L655, 156623, 156624, 1C2, 1G4, 4L28, CBLXJ-018, 2H5, or FQS3590(3). In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-PCDH9 antibodies 7G3A2 or 7G3F7. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-TMEM200A antibodies 4-B3, CBYJT-3509, or CBYJT-3510. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-ADGRL4 antibody CL4164. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-ART4 antibody 8C11A12. In some embodiments, the inhibitory chimeric

receptor comprises an antigen binding domain selected from the anti-BACE2 antibody 391017. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-CALCRL antibody 998820. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-LIFR antibodies 8E5E4D3, 32953. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain selected from the anti-MPZL2 antibodies G9P3-1 or OTI2C7. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain from the anti-PTPRB antibody 2-A2. In some embodiments, the inhibitory chimeric receptor comprises an antigen binding domain from the anti-WLS antibody YJ5.

Co-stimulatory ligands

[0558] In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) can further include one or more recombinant or exogenous co-stimulatory ligands. For example, the cell can be further transduced with one or more co-stimulatory ligands, such that the cell co-expresses or is induced to co-express one or more chimeric receptors of the present disclosure and one or more co-stimulatory ligands. Without wishing to be bound by theory, it is believed that the interaction between the one or more chimeric receptors and the one or more co-stimulatory ligands may provide a non-antigen-specific signal important for full activation of the cell. Examples of suitable co-stimulatory ligands include, without limitation, members of the tumor necrosis factor (TNF) superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Members of TNF superfamily share a number of common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. Examples of suitable TNF superfamily members include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD 154, CD137L/4-1BBL, TNF-a, CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNFP)/lymphotoxin- alpha (LTa), lymphotoxin-beta (LTP), CD257/B cell-activating factor (B AFF)/Bly s/THANK/Tall- 1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF 14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins and possess an immunoglobulin domain (fold). Examples of suitable

immunoglobulin superfamily ligands include, without limitation, CD80 and CD86, both ligands for CD28, PD-L1/(B7-H1) that ligands for PD-1. In certain embodiments, the one or more co-stimulatory ligands are selected from 4-1BBL, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, PD-L1, and combinations thereof.

Chemokine receptor

[0559] In some embodiments, a cell of the present disclosure (e.g., an immunoresponsive cell) comprises one or more chimeric receptors and may further include one or more chemokine receptors. For example, transgenic expression of chemokine receptor CCR2b or CXCR2 in cells, such as T cells, enhances trafficking to CCL2-secreting or CXCL1-secreting solid tumors (Craddock et al, J Immunother. 2010 Oct; 33(8):780-8 and Kershaw et al. Hum Gene Ther. 2002 Nov 1; 13(16): 1971 -80). Without wishing to be bound by theory, it is believed that chemokine receptors expressed on chimeric receptor-expressing cells of the present disclosure may recognize chemokines secreted by tumors and improve targeting of the cell to the tumor, which may facilitate the infiltration of the cell to the tumor and enhance the antitumor efficacy of the cell. Chemokine receptors of the present disclosure may include a naturally occurring chemokine receptor, a recombinant chemokine receptor, or a chemokine-binding fragment thereof. Examples of suitable chemokine receptors that may expressed on a cell of the present disclosure include, without limitation, a CXC chemokine receptor, such as CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, or CXCR7; a CC chemokine receptor, such as CCR1 , CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, or CCR11; a CX3C chemokine receptor, such as CX3CR1; an XC chemokine receptor, such as XCR1; and chemokine-binding fragments thereof. In some embodiments, the chemokine receptor to be expressed on the cell is chosen based on the chemokines secreted by the tumor.

Chimeric receptor regulation

[0560] Some embodiments of the present disclosure relate to regulating one or more chimeric receptor activities of chimeric receptor-expressing cells of the present disclosure. There are several ways chimeric receptor activities can be regulated. In some embodiments, a regulatable chimeric receptor, wherein one or more chimeric receptor activities can be controlled, may be desirable to optimize the safety and/or efficacy of the chimeric receptor therapy. For example, inducing apoptosis using a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18): 1673-1683) can be used as a safety

switch in the chimeric receptor therapy. In some embodiments, a chimeric receptor-expressing cell of the present disclosure can also express an inducible Caspase-9 (iCaspase-9) that, upon administration of a dimerizer drug, such as rimiducid (IUPAC name: [(1R)-3-(3,4-dimethoxyphenyl)-1-[3-[2-[2-[[2-[3-[(1R)-3-(3,4-dimethoxyphenyl)-1-[(2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carbonyl]oxypropyl]phenoxy]acetyl]amino]ethylamino]-2-oxoethoxy]phenyl]propyl] (2S)-1-[(2S)-2-(3,4,5-trimethoxyphenyl)butanoyl]piperidine-2-carboxylate), induces activation of the Caspase-9 and results in apoptosis of the cells. In some embodiments, the iCaspase-9 contains a binding domain that comprises a chemical inducer of dimerization (CID) that mediates dimerization in the presence of the CID, which results in inducible and selective depletion of the chimeric receptor-expressing cells.

[0561] Alternatively, in some embodiments a chimeric receptor of the present disclosure may be regulated by utilizing a small molecule or an antibody that deactivates or otherwise inhibits chimeric receptor activity. For example, an antibody may delete the chimeric receptor-expressing cells by inducing antibody dependent cell-mediated cytotoxicity (ADCC). In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express an antigen that is recognized by a molecule that is capable of inducing cell death by ADCC or complement-induced cell death. For example, a chimeric receptor-expressing cell of the present disclosure may further express a receptor capable of being targeted by an antibody or antibody fragment. Examples of suitable receptors that may be targeted by an antibody or antibody fragment include, without limitation, EpCAM, VEGFR, integrins (e.g., αvβ3, α4, αI¾β3, α4β7, α5β1, αvβ3, αv), members of the TNF receptor superfamily (e.g., TRAIL-R1 and TRAIL-R2), PDGF receptor, interferon receptor, folate receptor, GPNMB, ICAM-1 , HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD11, CD11a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/IgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41 , CD44, CD51, CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof.

[0562] In some embodiments, a chimeric receptor-expressing cell of the present disclosure may also express a truncated epidermal growth factor receptor (EGFR) that lacks signaling capacity but retains an epitope that is recognized by molecules capable of inducing ADCC (e.g., WO2011/056894).

[0563] In some embodiments, a chimeric receptor-expressing cell of the present disclosure further includes a highly expressing compact marker/suicide gene that combines target

epitopes from both CD32 and CD20 antigens in the chimeric receptor-expressing cell, which binds an anti-CD20 antibody (e.g., rituximab) resulting in selective depletion of the chimeric receptor-expressing cell by ADCC. Other methods for depleting chimeric receptor-expressing cells of the present disclosure my include, without limitation, administration of a monoclonal anti-CD52 antibody that selectively binds and targets the chimeric receptor-expressing cell for destruction by inducing ADCC. In some embodiments, the chimeric receptor-expressing cell can be selectively targeted using a chimeric receptor ligand, such as an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, such as ADCC or ADC activity. In some embodiments, the chimeric receptor ligand can be further coupled to an agent that induces cell killing, such as a toxin. In some embodiments, a chimeric receptor-expressing cell of the present disclosure may further express a target protein recognized by a cell depleting agent of the present disclosure. In some embodiments, the target protein is CD20 and the cell depleting agent is an anti-CD20 antibody. In such embodiments, the cell depleting agent is administered once it is desirable to reduce or eliminate the chimeric receptor-expressing cell. In some embodiments, the cell depleting agent is an anti-CD52 antibody.

[0564] In some embodiments, a regulated chimeric receptor comprises a set of polypeptides, in which the components of a chimeric receptor of the present disclosure are partitioned on separate polypeptides or members. For example, the set of polypeptides may include a dimerization switch that, when in the presence of a dimerization molecule, can couple the polypeptides to one another to form a functional chimeric receptor.

Chimeric Receptor-encoding Nucleic Acid Constructs

[0565] Certain aspects of the present disclosure relate to nucleic acids (e.g., isolated nucleic acids) encoding one or more chimeric receptors of the present disclosure. In some embodiments, the nucleic acid is an RNA construct, such as a messenger RNA (mRNA) transcript or a modified RNA. In some embodiments, the nucleic acid is a DNA construct.

[0566] In some embodiments, a nucleic acid of the present disclosure encodes a chimeric receptor that comprises one or more antigen-binding domain, where each domain binds to a target antigen (e.g., an AML antigen), a transmembrane domain, and one or more intracellular signaling domains. In some embodiments, the nucleic acid encodes a chimeric receptor that comprises an antigen-binding domain, a transmembrane domain, a primary signaling domain (e.g., CD3-zeta domain), and one or more costimulatory signaling domains. In some embodiments, the nucleic acid further comprises a nucleotide sequence encoding a

spacer region. In some embodiments, the antigen-binding domain is connected to the transmembrane domain by the spacer region. In some embodiments, the spacer region comprises a nucleic acid sequence selected from any of the nucleic acid sequences listed in Table D. In some embodiments, the nucleic acid further comprises a nucleotide sequence encoding a leader sequence.

[0567] The nucleic acids of the present disclosure may be obtained using any suitable recombinant methods known in the art, including, without limitation, by screening libraries from cells expressing the gene of interest, by deriving the gene of interest from a vector known to include the gene, or by isolating the gene of interest directly from cells and tissues containing the gene using standard techniques. Alternatively, the gene of interest may be produced synthetically.

[0568] In some embodiments, a nucleic acid of the present disclosure in comprised within a vector. In some embodiments, a nucleic acid of the present disclosure is expressed in a cell via transposons, a CRISPR/Cas9 system, a TALEN, or a zinc finger nuclease.

[0569] In some embodiments, expression of a nucleic acid encoding a chimeric receptor of the present disclosure may be achieved by operably linking the nucleic acid to a promoter and incorporating the construct into an expression vector. A suitable vector can replicate and integrate in eukaryotic cells. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulating expression of the desired nucleic acid.

[0570] In some embodiments, expression constructs of the present disclosure may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols (e.g., US5399346, US5580859, and US5589466). In some embodiments, a vector of the present disclosure is a gene therapy vector.

[0571] A nucleic acid of the present disclosure can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, without limitation, a plasmid, a phagemid, a phage derivative, an animal virus, or a cosmid. In some embodiments, the vector may be an expression vector, a replication vector, a probe generation vector, or a sequencing vector.

[0572] In some embodiments, the plasmid vector comprises a transposon/transposase system to incorporate the nucleic acids of the present disclosure into the host cell genome. Methods of expressing proteins in immune cells using a transposon and transposase plasmid system are generally described in Chicaybam L, Hum Gene Ther. 2019 Apr;30(4):511-522. doi: 10.1089/hum.2018.218; and Ptáčková P, Cytotherapy. 2018 Apr;20(4):507-520. doi:

10.1016/j.jcyt.2017.10.001, each of which are hereby incorporated by reference in their entirety. In some embodiments, the transposon system is the Sleeping Beauty transposon/transposase or the piggyBac transposon/transposase.

[0573] In some embodiments, an expression vector of the present disclosure may be provided to a cell in the form of a viral vector. Suitable viral vector systems are well known in the art. For example, viral vectors may be derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In some embodiments, a vector of the present disclosure is a lentiviral vector. Lentiviral vectors are suitable for long-term gene transfer as such vectors allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors are also advantageous over vectors derived from onco-retroviruses (e.g., murine leukemia viruses) in that lentiviral vectors can transduce non-proliferating cells. In some embodiments, a vector of the present disclosure is an adenoviral vector (A5/35). In some embodiments, a vector of the present disclosure contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (e.g., WO01/96584; WO01/29058; and US6326193). A number of viral based systems have been developed for gene transfer into mammalian cells. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to mammalian cells either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art.

[0574] In some embodiments, vectors of the present disclosure include additional promoter elements, such as enhancers that regulate the frequency of transcriptional initiation. Enhancers are typically located in a region that is 30 bp to 110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements may be flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. For example, in the thymidine kinase (tk) promoter the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, individual elements may function either cooperatively or independently to activate transcription. Exemplary promoters may include, without limitation, the SFFV gene promoter, the EFS gene promoter, the CMV IE gene promoter, the EFla promoter, the ubiquitin C promoter, and the phosphoglycerokinase (PGK) promoter.

[0575] In some embodiments, a promoter that is capable of expressing a nucleic acid of the present disclosure in a mammalian cell, such as an immunoresponsive cell of the present

disclosure, is the EF1a promoter. The native EF1a promoter drives expression of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. The EF1a promoter has been widely used in mammalian expression plasmids and has been shown to be effective in driving chimeric receptor expression from nucleic acids cloned into a lentiviral vector.

[0576] In some embodiments, a promoter that is capable of expressing a nucleic acid of the present disclosure in a mammalian cell, such as an immunoresponsive cell of the present disclosure, is a constitutive promoter. For example, a suitable constitutive promoter is the immediate early cytomegalovirus (CMV) promoter. The CMV promoter is a strong constitutive promoter that is capable of driving high levels of expression of any polynucleotide sequence operatively linked to the promoter. Other suitable constitutive promoters include, without limitation, a ubiquitin C (UbiC) promoter, a simian virus 40 (SV40) early promoter, a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, a MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, an actin promoter, a myosin promoter, an elongation factor-la promoter, a hemoglobin promoter, and a creatine kinase promoter.

[0577] In some embodiments, a promoter that is capable of expressing a nucleic acid of the present disclosure in a mammalian cell, such as an immunoresponsive cell of the present disclosure, is an inducible promoter. Use of an inducible promoter may provide a molecular switch that is capable of inducing or repressing expression of a nucleic acid of the present disclosure when the promoter is operatively linked to the nucleic acid. Examples of inducible promoters include, without limitation, a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0578] In some embodiments, a vector of the present disclosure may further comprise a signal sequence to facilitate secretion, a polyadenylation signal and transcription terminator, an element allowing episomal replication, and/or elements allowing for selection.

[0579] In some embodiments, a vector of the present disclosure can further comprise a selectable marker gene and/or reporter gene to facilitate identification and selection of chimeric receptor-expressing cells from a population of cells that have been transduced with the vector. In some embodiments, the selectable marker may be encoded by a nucleic acid that is separate from the vector and used in a co-transfection procedure. Either selectable marker or reporter gene may be flanked with appropriate regulator sequences to allow

expression in host cells. Examples of selectable markers include, without limitation, antibiotic-resistance genes, such as neo and the like.

[0580] In some embodiments, reporter genes may be used for identifying transduced cells and for evaluating the functionality of regulatory sequences. As disclosed herein, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression results in an easily detectable property, such as enzymatic activity. Expression of the reporter gene can be assayed at a suitable time after the nucleic acid has been introduced into the recipient cells. Examples of reporter genes include, without limitation, genes encoding for luciferase, genes encoding for beta- galactosidase, genes encoding for chloramphenicol acetyl transferase, genes encoding for secreted alkaline phosphatase, and genes encoding for green fluorescent protein. Suitable expression systems are well known in the art and may be prepared using known techniques or obtained commercially. In some embodiments, a construct with a minimal 5' flanking region showing the highest level of expression of the reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0581] In some embodiments, a vector comprising a nuclei acid sequence encoding a chimeric receptor of the present disclosure further comprises a second nucleic acid encoding a polypeptide that increases the activity of the chimeric receptor.

[0582] In embodiments where a chimeric receptor-expressing cell comprises two or more chimeric receptors, a single nucleic acid may encode the two or more chimeric receptors under a single regulatory control element (e.g., promoter) or under separate regulatory control elements for each chimeric receptor-encoding nucleotide sequence comprised in the nucleic acid. In some embodiments where a chimeric receptor-expressing cell comprises two or more chimeric receptors, each chimeric receptor may be encoded by separate nucleic acid. In some embodiments, each separate nucleic acid comprises its own control element (e.g., promoter). In some embodiments, a single nucleic acid encodes the two or more chimeric receptors and the chimeric receptor-encoding nucleotide sequences are in the same reading frame and are expressed as a single polypeptide chain. In such embodiments, the two or more chimeric receptors may be separated by one or more peptide cleavage sites, such as auto-cleavage sites or substrates for an intracellular protease. Suitable peptide cleavage sites may include, without limitation, a T2A peptide cleavage site, a P2A peptide cleavage site, an E2A peptide cleavage sire, and an F2A peptide cleavage site. In some embodiments, the two or more chimeric receptors comprise a T2A peptide cleavage site. In some embodiments, the two or

more chimeric receptors comprise an E2A peptide cleavage site. In some embodiments, the two or more chimeric receptors comprise a T2A and an E2A peptide cleavage site.

[0583] Methods of introducing and expressing genes into a cell are well known in the art. For example, in some embodiments, an expression vector can be transferred into a host cell by physical, chemical, or biological means. Examples of physical means for introducing a nucleic acid into a host cell include, without limitation, calcium phosphate precipitation, lipofection, particle bombardment, microinjection, and electroporation. Examples of chemical means for introducing a nucleic acid into a host cell include, without limitation, colloidal dispersion systems, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in- water emulsions, micelles, mixed micelles, and liposomes. Examples of biological means for introducing a nucleic acid into a host cell include, without limitation, the use of DNA and RNA vectors.

[0584] In some embodiments, liposomes may be used as a non-viral delivery system to introduce a nucleic acid or vector of the present disclosure into a host cell *in vitro*, *ex vivo*, or *in vivo*. In some embodiments, the nucleic acid may be associated with a lipid, for example by being encapsulated in the aqueous interior of a liposome, being interspersed within the lipid bilayer of a liposome, being attached to a liposome via a linking molecule that is associated with both the liposome and the nucleic acid, being entrapped in a liposome, being complexed with a liposome, being dispersed in a solution containing a lipid, being mixed with a lipid, being combined with a lipid, being contained as a suspension in a lipid, being contained or complexed with a micelle, or otherwise being associated with a lipid. As disclosed herein, lipid-associated nucleic acid or vector compositions are not limited to any particular structure in solution. In some embodiments, such compositions may be present in a bilayer structure, as micelles or with a "collapsed" structure. Such compositions may also be interspersed in a solution, forming aggregates that are not uniform in size or shape. As disclosed herein, lipids are fatty substances that may be naturally occurring or synthetic. In some embodiments, lipids can include the fatty droplets that naturally occur in the cytoplasm or the class of compounds that contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes. Suitable lipids may be obtained from commercial sources and include, without limitation, dimyristyl phosphatidylcholine ("DMPC"), dicetylphosphate ("DCP"), cholesterol, and dimyristylphosphatidylglycerol ("DMPG"). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about - 20°C. Chloroform is used as the solvent, as it is more readily evaporated than methanol. As used herein, a "liposome" may encompass a

variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. In some embodiments, liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. In some embodiments, multilamellar liposomes may have multiple lipid layers separated by aqueous medium. Multilamellar liposomes can form spontaneously when phospholipids are suspended in an excess of aqueous solution. In some embodiments, lipid components may undergo self-rearrangement before the formation of closed structures and can entrap water and dissolved solutes between the lipid bilayers. In some embodiments, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules.

[0585] In some embodiments, a nucleic acid or vector of the present disclosure is introduced into a mammalian host cell, such as an immunoresponsive cell of the present disclosure. In some embodiments, the presence of a nucleic acid or vector of the present disclosure in a host cell may be confirmed by any suitable assay known in the art, including without limitation Southern blot assays, Northern blot assays, RT-PCR, PCR, ELISA assays, and Western blot assays.

[0586] In some embodiments, a nucleic acid or vector of the present disclosure is stably transduced into an immunoresponsive cell of the present disclosure. In some embodiments, cells that exhibit stable expression of the nucleic acid or vector express the encoded chimeric receptor for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 3 months, at least 6 months, at least 9 months, or at least 12 months after transduction.

[0587] In embodiments where a chimeric receptor of the present disclosure is transiently expressed in a cell, a chimeric receptor-encoding nucleic acid or vector of the present disclosure is transfected into an immunoresponsive cell of the present disclosure. In some embodiments the immunoresponsive cell expresses the chimeric receptor for about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, or about 15 days after transfection.

[0588] In some embodiments, the nucleic acid construct encodes a bicistronic chimeric antigen receptor. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises an FLT3 CAR and a CD33 CAR. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises an FLT3 CAR and a CLEC12A CAR. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises an CLEC12A CAR and a CD33 CAR. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises an EMCN CAR. In some embodiments, the encoded bicistronic chimeric

antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 1. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 2. In some embodiments, the encoded bicistronic chimeric antigen receptor comprises a CAR with two or more antigen binding domains targeting any antigen pair provided in Table 3.

[0589] In some embodiments, the nucleic acid construct encodes a bivalent chimeric antigen receptor. In some embodiments, the encoded bivalent chimeric antigen receptor comprises an FLT3 CAR and a CD33 CAR. In some embodiments, the encoded bivalent chimeric antigen receptor comprises an FLT3 CAR and a CLEC12A CAR. In some embodiments, the encoded bivalent chimeric antigen receptor comprises an CLEC12A CAR and a CD33 CAR. In some embodiments, the encoded bivalent chimeric antigen receptor comprises an EMCN CAR. In some embodiments, the encoded bivalent chimeric antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 1. In some embodiments, the encoded bivalent chimeric antigen receptor comprises a CAR with an antigen binding domain targeting any antigen provided in Table 2. In some embodiments, the encoded bivalent chimeric antigen receptor comprises a CAR with two or more antigen binding domains targeting any antigen pair provided in Table 3.

Pharmaceutical Compositions and Administration

[0590] Certain aspects of the present disclosure relate to compositions (e.g., pharmaceutical compositions) comprising one or more chimeric receptors of the present disclosure or immunoresponsive cells of the present disclosure that express such one or more chimeric receptors. In some embodiments, compositions comprising chimeric receptors or genetically modified immunoresponsive cells that express such chimeric receptors can be provided systemically or directly to a subject for the treatment of a proliferative disorder, such as a myeloid disorder. In certain embodiments, the composition is directly injected into an organ of interest (e.g., an organ affected by a disorder). Alternatively, the composition may be provided indirectly to the organ of interest, for example, by administration into the circulatory system (e.g., the tumor vasculature). Expansion and differentiation agents can be provided prior to, during, or after administration of the composition to increase production of T cells, NK cells, or CTL cells *in vitro* or *in vivo* .

[0591] Compositions comprising genetically modified cells of the present disclosure may be administered in any physiologically acceptable vehicle, for example intravascularly, although they may also be introduced into bone or other convenient sites where the genetically

modified cells may find an appropriate site for regeneration and differentiation (e.g., thymus). In some embodiments, at least $1x10^5$ cells may be administered, eventually reaching $1x10^{10}$ or more cells. Compositions comprising genetically modified cells of the present disclosure can comprise a purified population of cells. Methods for determining the percentage of genetically modified cells in a population of cells are well known in the art and include, without limitation, fluorescence activated cell sorting (FACS). In some embodiments, the purity of genetically modified cells in a population of cells may be about 50%, about 55%, about 60%, or about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 97%, about 98%, about 99%, or more of the cells in the population of cells. Dosages can be readily adjusted by those skilled in the art (e.g., a decrease in purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like. In some embodiments, factors can also be included, for example, IL-2, IL-3, IL-6, IL-11, IL-7, IL-12, IL-15, IL-21, G-CSF, MCSF, GM-CSF, gamma-interferon, and erythropoietin.

[0592] In certain embodiments, the compositions are pharmaceutical compositions comprising genetically modified cells, such as immunoresponsive cells or their progenitors and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, immunoresponsive cells, or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. In some embodiments, immunoresponsive cells of the present disclosure or their progeny may be derived from peripheral blood cells (e.g., *in vivo*, *ex vivo*, or *in vitro* derived) and may be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition of the present disclosure (e.g., a pharmaceutical composition containing a genetically modified cell of the present disclosure), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

Formulations

[0593] Certain aspects of the present disclosure relate to formulations of compositions comprising chimeric receptors of the present disclosure or genetically modified cells (e.g., immunoresponsive cells of the present disclosure) expressing such chimeric receptors. In some embodiments, compositions of the present disclosure comprising genetically modified cells may be provided as sterile liquid preparations, including without limitation isotonic aqueous solutions, suspensions, emulsions, dispersions, and viscous compositions, which

may be buffered to a selected pH. Liquid preparations are typically easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions may be more convenient to administer, especially by injection. In some embodiments, viscous compositions can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, etc.) and suitable mixtures thereof.

[0594] In some embodiments, sterile injectable solutions can be prepared by incorporating genetically modified cells of the present disclosure in a sufficient amount of the appropriate solvent with various amounts of any other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. In some embodiments, the compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing agents, pH buffering agents, and antimicrobials depending upon the route of administration and the preparation desired.

[0595] In some embodiments, compositions of the present disclosure may further include various additives that may enhance the stability and sterility of the compositions. Examples of such additives include, without limitation, antimicrobial preservatives, antioxidants, chelating agents, and buffers. In some embodiments, microbial contamination may be prevented by the inclusions of any of various antibacterial and antifungal agents, including without limitation parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of an injectable pharmaceutical formulation of the ;present disclosure can be brought about by the use of suitable agents that delay absorption, such as aluminum monostearate and gelatin.

[0596] In some embodiments, compositions of the present disclosure can be isotonic, i.e., having the same osmotic pressure as blood and lacrimal fluid. In some embodiments, the desired isotonicity may be achieved using, for example, sodium chloride, dextrose, boric acid, sodium tartrate, propylene glycol, or other inorganic or organic solutes.

[0597] In some embodiments, the components of the formulations of the present disclosure are selected to be chemically inert and to not affect the viability or efficacy of the genetically modified cells of the present disclosure.

[0598] One consideration concerning the therapeutic use of the genetically modified cells of the present disclosure is the quantity of cells needed to achieve optimal efficacy. In some

embodiments, the quantity of cells to be administered will vary for the subject being treated. In certain embodiments, the quantity of genetically modified cells that are administered to a subject in need thereof may range from 1 x 10^4 cells to 1 x 10^{10} cells. In some embodiments, the precise quantity of cells that would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art based on the present disclosure and the knowledge in the art.

Methods of Treatment

[0599] Certain aspects of the present disclosure relate to methods of using the chimeric receptors and genetically modified cells of the present disclosure (e.g., immunoresponsive cells) that express such chimeric receptors to treat subjects in need thereof. In some embodiments, the methods of the present disclosure are useful for treating cancer in a subject, such as a myeloid disorder. In some embodiments, the myeloid disorder is a myelodysplastic syndrome, a myeloproliferative neoplasm, a chronic myelomonocytic leukemia, acute myeloid leukemia (AML), acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, or polycythemia vera. In some embodiments, the myeloid disorder is AML. Other aspects of the present disclosure relate to use of the chimeric receptors and genetically modified cells of the present disclosure (e.g., immunoresponsive cells) that express such chimeric receptors in methods for treating a pathogen infection or other infectious disease in a subject, such as an immunocompromised human subject. In some embodiments, the methods of the present disclosure may comprise administering genetically modified cells of the present disclosure in an amount effective to achieve the desired effect, including without limitation palliation of an existing condition, prevention of a condition, treatment an existing condition, management of an existing condition, or prevention of recurrence or relapse of a condition. In some embodiments, the effective amount can be provided in one or a series of administrations of the genetically modified cells of the present disclosure (e.g., immunoresponsive cells). In some embodiments, an effective amount can be provided in a bolus or by continuous perfusion.

[0600] As disclosed herein, an "effective amount" or "therapeutically effective amount" is an amount sufficient to affect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the disease, or otherwise reduce the pathological consequences of the

disease. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the form and effective concentration of the immunoresponsive cells administered.

[0601] For adoptive immunotherapy using antigen-specific cells (e.g., immunoresponsive cells such as T cells), cell doses in the range of about 1 x 10^6 to 1 x10^{10} cells (e.g., about 1 x 10^9 cells) are typically infused. Upon administration of the cells into the subject and subsequent differentiation, immunoresponsive cells are induced that are specifically directed against the specific antigen. In some embodiments, induction of immunoresponsive cells can include, without limitation, inactivation of antigen-specific cells such as by deletion or anergy. Inactivation is particularly useful to establish or reestablish tolerance such as in autoimmune disorders. The genetically modified cells can be administered by any method known in the art including, but not limited to, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural, intraperitoneal and directly to the thymus.

Therapeutic treatment

[0602] In some embodiments, the methods of the present disclosure increase an immune response in a subject in need thereof. In some embodiments, the methods of the present disclosure include methods for treating and/or preventing a myeloid disorder in a subject. In some embodiments, the subject is a human. In some embodiments, suitable human subjects for therapy may comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor is one that can be detected on the basis of tumor mass (e.g., based on percentage of leukemic cells, by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). In some embodiments, a pharmaceutical composition of the present disclosure is administered to these subjects to elicit an anti-tumor response, with the objective of palliating their condition. In some embodiments, reduction in tumor mass occurs as a result of administration of the pharmaceutical composition, but any clinical improvement will constitute a benefit. In some embodiments, clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor. In some embodiments, a second group of suitable human subjects are "adjuvant group"

subjects. These subjects are individuals who have had a history of a myeloid disorder, but have been responsive to another mode of therapy. The prior therapy may have included, without limitation, surgical resection, radiotherapy, and/or traditional chemotherapy. As a result, these individuals have no clinically measurable tumor. However, they are suspected of being at risk for progression of the disease, either near the original tumor site, or by metastases. In some embodiments, this group can be further subdivided into high-risk and low-risk individuals. The subdivision can be made on the basis of features observed before or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different myeloid disorder. Features typical of high-risk subgroups are those in which the tumor has invaded neighboring tissues, or who show involvement of lymph nodes.

[0603] In any and all aspects of increasing an immune response as described herein, any increase or decrease or alteration of an aspect of characteristic(s) or function(s) is as compared to a cell not contacted with an immunoresponsive cell as described herein.

[0604] Increasing an immune response can be both enhancing an immune response or inducing an immune response. For instance, increasing an immune response encompasses both the start or initiation of an immune response, or ramping up or amplifying an on-going or existing immune response. In some embodiments, the treatment induces an immune response. In some embodiments, the induced immune response is an adaptive immune response. In some embodiments, the induced immune response is an innate immune response. In some embodiments, the treatment enhances an immune response. In some embodiments, the enhanced immune response is an adaptive immune response. In some embodiments, the enhanced immune response is an innate immune response. In some embodiments, the treatment increases an immune response. In some embodiments, the increased immune response is an adaptive immune response. In some embodiments, the increased immune response is an innate immune response.

[0605] In some embodiments, a further group of subjects are those having a genetic predisposition to a myeloid disorder, but that have not yet evidenced clinical signs of the myeloid disorder. For example, women testing positive for a genetic mutation associated with AML, but still of childbearing age, may benefit from receiving one or more of the cells of the present disclosure (e.g., immunoresponsive cells) in treatment prophylactically to prevent the occurrence of AML until it is suitable to perform preventive surgery. In some embodiments, the subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. In

some embodiments, the subjects may have a history of the condition, for which they have already been treated, in which case the therapeutic objective may typically include a decrease or delay in the risk of recurrence.

Combination therapies

[0606] In some embodiments, genetically modified cells of the present disclosure (e.g., immunoresponsive ells) expressing one or more chimeric receptors of the present disclosure may be used in combination with other known agents and therapies. In some embodiments, a combination therapy of the present disclosure comprises a genetically modified cells of the present disclosure that can be administered in combination with one or more additional therapeutic agents. In some embodiments, the genetically modified cell and the one or more additional therapeutic agents can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the genetically modified can be administered first, and the one or more additional agents can be administered second, or the order of administration can be reversed. In some embodiments, the genetically modified cells are further modified to express one or more additional therapeutic agents.

[0607] In some embodiments, a genetically modified cell of the present disclosure may be used in a treatment regimen in combination with surgery, chemotherapy, radiation, immunosuppressive agents (e.g., cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506), antibodies, or other immunoablative agents (e.g., CAMPATH or anti-CD3 antibodies), cytoxin, fludarabme, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, irradiation, and peptide vaccines.

[0608] In some embodiments, a genetically modified cell of the present disclosure may be used in combination with a lymphodepleting agent. Suitable lymphodepleting agents reduce or decrease lymphocytes, e.g., B cell lymphocytes and/or T cell lymphocytes, prior to immunotherapy. Examples of suitable lymphodepleting agents include, without limitation, fludarabine, cyclophosphamide, corticosteroids, alemtuzumab, total body irradiation (TBI), and any combination thereof.

[0609] In some embodiments, a genetically modified cell of the present disclosure may be used in combination with a chemotherapeutic agent. Suitable chemotherapeutic agents include, without limitation, an anthracycline (e.g., doxorubicin), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, tositumomab), an antimetabolite (e.g., folic acid

antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors, such as fludarabine), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

[0610] Examples of general chemotherapeutic agents suitable for use in combination therapies include, without limitation, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5- deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Piatinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5- fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idaniycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

[0611] Examples of suitable alkylating agents include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®. Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Rev immune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation,

Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamme (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Aitretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HC1 (Treanda®).

[0612] Examples of suitable mTOR inhibitors include, without limitation, temsirolimus, ridaforolimus (deferolimus), AP23573, MK8669, everolimus (Afimtor® or RADOOl), rapamycin (AY22989, Sirolmius®), and XL765.

[0613] Examples of suitable immunomodulators include, without limitation, afutuzumab, pegfilgrastim (Neulasta®), lenalidomide (CC-5013, Revlimid®), thalidomide (Thalomid®), actimid (CC4047), and IRX-2.

[0614] Examples of suitable anthracyclines include, without limitation, doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomyem, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PES®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacet lravidomycin.

[0615] Examples of suitable vinca alkaloids include, without limitation, vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbme®).

[0616] Examples of suitable proteosome inhibitors include, without limitation, bortezomib (Velcade®); carfilzomib; marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and ONX-0912.

[0617] In some embodiments, a genetically modified cell of the present disclosure is administered in combination with a CD20 inhibitor, e.g., an anti-CD20 antibody, or fragment thereof. Exemplary anti-CD20 antibodies include, without limitation, rituximab, ofatumumab, ocrelizumab, veltuzumab, obinutuzumab, TRU- 015 (Trubion Pharmaceuticals), ocaratuzumab, and Prol31921.

[0618] In some embodiments, a genetically modified cell of the present disclosure is administered in combination with an oncolytic virus. In some embodiments, oncolytic viruses are capable of selectively replicating in and triggering the death of or slowing the growth of a cancer cell. In some cases, oncolytic viruses have no effect or a minimal effect on non-cancer cells. Suitable oncolytic viruses include, without limitation, an oncolytic adenovirus, oncolytic Herpes Simplex Viruses, oncolytic retrovirus, oncolytic parvovirus, oncolytic vaccinia virus, oncolytic Sinbis virus, oncolytic influenza virus, or oncolytic RNA virus (e.g., oncolytic reovirus, oncolytic Newcastle Disease Virus (NDV), oncolytic measles virus, or oncolytic vesicular stomatitis virus (VSV)). In some embodiments, the oncolytic virus is a recombinant oncolytic virus.

[0619] In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with a protein tyrosine phosphatase inhibitor, e.g., a SHP-I inhibitor or a SHP-2 inhibitor. In one embodiment, a genetically modified cell of the present disclosure can be used in combination with a kinase inhibitor. Examples of suitable kinase inhibitors include, without limitation, CDK4 inhibitors, CDK4/6 inhibitors, BTK inhibitors, phosphatidylinositol 3 -kinase (PI3K) inhibitors, mTOR inhibitors, MNK inhibitors, and anaplastic lymphoma kinase (ALK) inhibitors.

[0620] In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with a modulator of myeloid-derived suppressor cells (MDSCs). MDSCs accumulate in the periphery and at the tumor site of many solid tumors. These cells suppress T cell responses, thereby hindering the efficacy of chimeric receptor-expressing cell therapy. Without being bound by theory, it is believed that administration of a MDSC modulator enhances the efficacy of a genetically modified cell of the present disclosure. Examples of suitable modulators of MDSCs include, without limitation, MCS110 and BLZ945.

[0621] In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with an agent that inhibits or reduces the activity of immunosuppressive plasma cells. Immunosuppressive plasma cells have been shown to impede T cell-dependent immunogenic chemotherapy, such as oxaliplatin (Shalapour et al.,

Nature 2015, 521 :94- 101). In one embodiment, immunosuppressive plasma cells can express one or more of IgA, interleukin (IL)-10, and PD-L1.

[0622] In some embodiments, a genetically modified cell of the present disclosure is administered to a subject in combination with an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-I5Ra) polypeptide, or a combination of both an IL-15 polypeptide and an IL-15Ra polypeptide. In some embodiments, a genetically modified cell of the present disclosure is further modified to express an interleukin-15 (IL-15) polypeptide, an interleukin-15 receptor alpha (IL-I5Ra) polypeptide, or a combination of both an IL-15 polypeptide and an IL-15Ra polypeptide.

[0623] In some embodiments, a subject having a myeloid disorder (e.g., AML) is administered a genetically modified cell of the present disclosure in combination with an agent, e.g., cytotoxic or chemotherapy agent, a biologic therapy (e.g., antibody, e.g., monoclonal antibody, or cellular therapy), or an inhibitor (e.g., kinase inhibitor). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a cytotoxic agent, e.g., CPX-351 (Celator Pharmaceuticals), cytarabme, daunorubicin, vosaroxin (Sunesis Pharmaceuticals), sapacitabine (Cyclacel Pharmaceuticals), idarubicin, or mitoxantrone. CPX-351 is a liposomal formulation comprising cytarabine and daunorubicin at a 5: 1 molar ratio. In some embodiments, the subject is administered a chimeric receptor- expressing cell described herein in combination with a hypomethylating agent, e.g., a DNA methyltransferase inhibitor, e.g., azacytidine or decitabine. In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a biologic therapy, e.g., an antibody or cellular therapy, e.g., 225Ac-lintuzumab (Actimab-A; Actinium Pharmaceuticals), IPH2102 (Innate Pharma/Bristol Myers Squibb), SGN-CD33A (Seattle Genetics), or gemtuzumab ozogamicin (Mylotarg; Pfizer). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination a FLT3 inhibitor, e.g., sorafenib (Bayer), midostaurin (Novartis), quizartinib (Daiichi Sankyo), crenoianib (Arog Pharmaceuticals), PLX3397 (Daiichi Sankyo), AKN-028 (Akinion Pharmaceuticals), or ASP2215 (Astelias). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with an isocitrate dehydrogenase (IDH) inhibitor, e.g., AG-221 (Celgene/ Agios) or AG- 120 (Agios/Celgene). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a cell cycle regulator, e.g., inhibitor of polo-like kinase 1 (Plkl), e.g., volasertib (Boehringer Ingelheim); or an inhibitor of cyclin-dependent kinase 9 (Cdk9), e.g., alvocidib (Tolero Pharmaceuticals/Sanofi

Aventis). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with a B cell receptor signaling network inhibitor, e.g., an inhibitor of B-cell lymphoma 2 (Bcl- 2), e.g., venetoclax (Abbvie/Roche); or an inhibitor of Button's tyrosine kinase (Btk), e.g., ibrutinib (Pharmacyclics/Johnson & Johnson Janssen Pharmaceutical). In some embodiments, the subject is administered a genetically modified cell of the present disclosure in combination with an inhibitor of M1aminopeptidase; an inhibitor of histone deacetylase (HDAC), e.g., pracinostat (MEI Pharma); a multi-kinase inhibitor, e.g., rigosertib (Onconova Therapeutics/Baxter/SymBio); or a peptidic CXCR4 inverse agonist, e.g., BL-8040 (BioLineRx).

[0624] In some embodiments, a subject can be administered an agent which enhances the activity or fitness of a genetically modified cell of the present disclosure. For example, the agent may inhibit a molecule that modulates or regulates, e.g., inhibits, T cell function. In some embodiments, the molecule that modulates or regulates T cell function is an inhibitory molecule. In some embodiments, inhibitory molecules, such as Programmed Death 1 (PD-1) can decrease the ability of the genetically modified cell to mount an immune effector response. Examples of suitable inhibitory molecules include, without limitation, PD-1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD 160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, adenosine, and TGF beta. Inhibition of a molecule that modulates or regulates, e.g., inhibits, T cell function, e.g., by inhibition at the DNA, RNA or protein level, can optimize the performance of genetically modified cells of the present disclosure. In some embodiments, an agent, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the genetically modified cell. In one embodiment, the inhibitor is an shRNA. In some embodiments, a genetically modified cell of the present disclosure may be further modified to express an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), can be used to inhibit expression of an inhibitory molecule in the genetically modified cell.

[0625] In one embodiment, the agent that modulates or regulates, e.g., inhibits, T-cell function is inhibited within a genetically modified cell of the present disclosure. In such embodiments, a dsRNA molecule that inhibits expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function is linked to the nucleic acid that encodes a component, e.g., all of the components, of a chimeric receptor of the present disclosure. In one embodiment, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is operably linked to a promoter, e.g., a HI- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is expressed, e.g., is expressed within a the genetically modified cell. In one embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on the same vector, e.g., a lentiviral vector, that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the chimeric receptor. In such an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is located on the vector, e.g., the lentiviral vector, 5'- or 3'- to the nucleic acid that encodes a component, e.g., all of the components, of the chimeric receptor. The nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function can be transcribed in the same or different direction as the nucleic acid that encodes a component, e.g., all of the components, of the chimeric receptor. In one embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on a vector other than the vector that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the chimeric receptor. In one embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function it transiently expressed within the genetically modified cell. In one embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is stably integrated into the genome of a genetically modified cell of the present disclosure.

[0626] In one embodiment, an agent that modulates or regulates, e.g., inhibits, T-cell function can be an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD-l , PD-Ll, PD-L2 or

CTLA4. In one embodiment, the agent is an antibody or antibody fragment that binds to TIM3. In one embodiment, the agent is an antibody or antibody fragment that binds to LAG3.

[0627] In some embodiments, the agent which enhances the activity of the genetically modified cell is a CEACAM inhibitor (e.g., CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In one embodiment, the inhibitor of CEACAM is an anti-CEACAM antibody molecule. In one embodiment, the agent which enhances activity of a genetically modified cell of the present disclosure is miR-17-92. In some embodiments, the agent which enhances the activity of the genetically modified cell is CD40L. In some embodiments, the agent which enhances the activity of the genetically modified cell is GM-CSF. In some embodiments, a genetically modified cell of the present disclosure is further modified to express an antibody or antibody fragment that binds to an inhibitory molecule of the present disclosure.

[0628] In one embodiment, the agent which enhances activity of a genetically modified cell of the present disclosure is a cytokine. Cytokines have important functions related to immunoresponsive cell expansion, differentiation, survival, and homeostats. Cytokines that can be administered to the subject receiving a genetically modified cell of the present disclosure include, without limitation, IL-2, IL-4, IL-7, IL-9, IIL-12, L-15, IL-18, and IL-21, or a combination thereof. The cytokine can be administered once a day or more than once a day, e.g., twice a day, three times a day, or four times a day. The cytokine can be administered for more than one day, e.g. the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the cytokine is administered once a day for 7 days. In some embodiments, a genetically modified cell of the present disclosure is further modified to express one or more cytokines, such as IL-2, IL-4, IL-7, IL-9, IL-12, L-15, IL-18, and IL-21.

[0629] In some embodiments, the cytokine can be administered simultaneously or concurrently with the genetically modified cells, e.g., administered on the same day. The cytokine may be prepared in the same pharmaceutical composition as the genetically modified cells, or may be prepared in a separate pharmaceutical composition. Alternatively, the cytokine can be administered shortly after administration of the genetically modified cells, e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the genetically modified cells. In some embodiments where the cytokine is administered in a dosing regimen that occurs over more than one day, the first day of the cytokine dosing regimen can be on the same day as administration with the genetically modified cells, or the first day of the cytokine dosing regimen can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days,

or 7 days after administration of the genetically modified cells. In one embodiment, on the first day, the genetically modified cells are administered to the subject, and on the second day, a cytokine is administered once a day for the next 7 days. In some embodiments, the cytokine is administered for a period of time after administration of the genetically modified cells, e.g., at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of the genetically modified cells. In one embodiment, the cytokine is administered after assessment of the subject's response to the genetically modified cells.

Kits

[0630] Certain aspects of the present disclosure relate to kits for the treatment and/or prevention of a myeloid disorder (e.g., AML). In certain embodiments, the kit includes a therapeutic or prophylactic composition comprising an effective amount of one or more chimeric receptors of the present disclosure, isolated nucleic acids of the present disclosure, vectors of the present disclosure, and/or cells of the present disclosure (e.g., immunoresponsive cells). In some embodiments, the kit comprises a sterile container. In some embodiments, such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. The container may be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

[0631] In some embodiments, therapeutic or prophylactic composition is provided together with instructions for administering the therapeutic or prophylactic composition to a subject having or at risk of developing a myeloid disorder (e.g., AML). In some embodiments, the instructions may include information about the use of the composition for the treatment and/or prevention of the disorder. In some embodiments, the instructions include, without limitation, a description of the therapeutic or prophylactic composition, a dosage schedule, an administration schedule for treatment or prevention of the disorder or a symptom thereof, precautions, warnings, indications, counter-indications, over-dosage information, adverse reactions, animal pharmacology, clinical studies, and/or references. In some embodiments, the instructions can be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

ADDITIONAL EMBODIMENTS

[0632] The paragraphs below provide additional enumerated embodiments.

1. A chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, and SPNS3.

2. The chimeric receptor of paragraph 1, wherein the antigen is MS4A3.

3. The chimeric receptor of paragraph 1, wherein the antigen is VSTM1.

4. The chimeric receptor of paragraph 1, wherein the antigen is LAT2.

5. The chimeric receptor of paragraph 1, wherein the antigen is MLC1.

6. The chimeric receptor of paragraph 1, wherein the antigen is CD131.

7. The chimeric receptor of paragraph 1, wherein the antigen is GAPT.

8. The chimeric receptor of paragraph 1, wherein the antigen is PRAM1.

9. The chimeric receptor of paragraph 1, wherein the antigen is SLC22A16.

10. The chimeric receptor of paragraph 1, wherein the antigen is SLC17A9.

11. The chimeric receptor of paragraph 1, wherein the antigen is SPNS3.

12. The chimeric receptor of any one of paragraphs 1-11, wherein the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

13. The chimeric receptor of paragraph 12, wherein the chimeric receptor is a CAR.

14. The chimeric receptor of paragraph 13, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling

domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

15. The chimeric receptor of paragraph 13 or paragraph 14, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

16. The chimeric receptor of any one of paragraphs 13-15, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

17. The chimeric receptor of any one of paragraphs 1-16, wherein the antigen-binding domain comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

18. The chimeric receptor of any one of paragraphs 1-16, wherein the antigen-binding domain comprises one or more single chain variable fragments (scFvs).

19. The chimeric receptor of paragraph 18, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

20. The chimeric receptor of paragraph 19, wherein the VH and VL are separated by a peptide linker.

21. The chimeric receptor of paragraph 20, wherein the peptide linker comprises an amino acid sequence of SEQ ID NO: 27.

22. The chimeric receptor of any one of paragraphs 19-21, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

23. The chimeric receptor of any one of paragraphs 1-22, wherein the antigen-binding domain comprises two single chain variable fragments (scFvs).

24. The chimeric receptor of paragraph 23, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

25. The chimeric receptor of any one of paragraphs 18-24, wherein each of the one or more scFvs is separated by a peptide linker.

26. The chimeric receptor of paragraph 25, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

27. An isolated cell comprising the chimeric receptor of any one of paragraphs 1-26.

28. The isolated cell of paragraph 27, wherein the chimeric receptor is recombinantly expressed.

29. The isolated cell of paragraph 27 or paragraph 28, wherein the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

30. The isolated cell of any one of paragraphs 27-29, wherein the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

31. The isolated cell of any one of paragraphs 27-30, wherein the cell is autologous.

32. The isolated cell of any one of paragraphs 27-30, wherein the cell is allogeneic.

33. An isolated cell comprising:

(a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and

(b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen,

wherein each antigen is selected from the group consisting of the antigens listed in Table 1 or the first and second antigens are selected from the group consisting of the antigen pairs listed in Table 3, and

wherein the first antigen is different from the second antigen.

34. An isolated cell comprising:

(a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and

(b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen,

wherein each antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70, and

wherein the first antigen is different from the second antigen.

35. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is MS4A3 and the second antigen is selected from the group consisting of VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

36. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is VSTM1 and the second antigen is selected from the group consisting of MS4A3, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

37. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is LAT2 and the second antigen is selected from the group consisting of MS4A3, VSTM1, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33,

CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

38. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is MLC1 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

39. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is CD131 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

40. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is GAPT and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

41. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is PRAM1 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

42. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is SLC22A16 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

43. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is SLC17A9 and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SPNS3, FLT3, CD33,

CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

44. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is SPNS3and the second antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

45. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is FLT3.

46. The isolated cell of paragraph 45, wherein the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto;
(c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto;
(d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto;
(e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto;
(f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto;
(g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and

(h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto.

47. The isolated cell of paragraph 45 or paragraph 46, wherein the second antigen is CD33.

48. The isolated cell of paragraph 47, wherein the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and
(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

49. The isolated cell of paragraph 45 or paragraph 46, wherein the second antigen is CLEC12A.

50. The isolated cell of paragraph 49, wherein the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and
(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

51. The isolated cell of paragraph 33 or paragraph 34, wherein the first antigen is CLEC12A.

52. The isolated cell of paragraph 51, wherein the antigen-binding domain that binds to the first antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and
(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

53. The isolated cell of paragraph 51 or paragraph 52, wherein the second antigen is CD33.

54. The isolated cell of paragraph 53, wherein the antigen-binding domain that binds to the second antigen comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and
(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

55. The isolated cell of any one of paragraphs 33-54, wherein the cell is an immunoresponsive cell.

56. The isolated cell of paragraph 55, wherein binding of the first chimeric receptor to the first antigen is capable of activating the immunoresponsive cell.

57. The isolated cell of paragraph 55, wherein binding of the second chimeric receptor to the second antigen is capable of stimulating the immunoresponsive cell.

58. The isolated cell of paragraph 55, wherein binding of the first chimeric receptor to the first antigen and binding of the second chimeric receptor to the second antigen are required for activating the immunoresponsive cell.

59. The isolated cell of paragraph 55, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against target cells that are positive for both the first antigen and the second antigen as compared to cytolytic activity against target cells that are positive for only the first antigen or the second antigen.

60. The isolated cell of paragraph 55, wherein binding of the first chimeric receptor to the first antigen or binding of the second chimeric receptor to the second antigen is capable of activating the immunoresponsive cell.

61. The isolated cell of any one of paragraphs 33-60, wherein the first chimeric receptor binds to the first antigen with a low binding affinity.

62. The isolated cell of any one of paragraphs 33-61, wherein the first chimeric receptor binds to the first antigen with a binding affinity that is lower than the binding affinity with which the second chimeric receptor binds to the second antigen.

63. The isolated cell of any one of paragraphs 33-62, wherein the first chimeric receptor binds to the first antigen with a low binding avidity.

64. The isolated cell of any one of paragraphs 33-63, wherein the first chimeric receptor and/or the second chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

65. The isolated cell of paragraph 64, wherein the first chimeric receptor and/or the second chimeric receptor is a CAR.

66. The isolated cell of paragraph 65, wherein the first chimeric receptor is a first CAR and the second chimeric receptor is a second CAR.

67. The isolated cell of paragraph 65 or paragraph 66, wherein each CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular

signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

68. The isolated cell of paragraph 67, wherein the one or more intracellular signaling domains of the first CAR are different from the one or more intracellular signaling domains of the second CAR.

69. The isolated cell of paragraph 67, wherein the first CAR and the second CAR each comprise a CD3zeta-chain intracellular signaling domain.

70. The isolated cell of paragraph 69, wherein the first CAR and the second CAR each further comprises an additional intracellular signaling domain selected from the group consisting of a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

71. The isolated cell of paragraph 70, wherein the additional intracellular signaling domain of the first CAR is different from the additional intracellular signaling domain of the second CAR.

72. The isolated cell of any one of paragraphs 64-71, wherein each CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group

consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

73. The isolated cell of paragraph 72, wherein the transmembrane domain of the first CAR is different from the transmembrane domain of the second CAR.

74. The isolated cell of any one of paragraphs 64-73, wherein each CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

75. The isolated cell of any one of paragraphs 33-74, wherein the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

76. The isolated cell of any one of paragraphs 33-75, wherein the antigen-binding domain of the first chimeric receptor and the second chimeric receptor comprises one or more single chain variable fragments (scFvs).

77. The isolated cell of paragraph 76, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

78. The isolated cell of paragraph 77, wherein the VH and VL are separated by a peptide linker.

79. The isolated cell of paragraph 78, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

80. The isolated cell of any one of paragraphs 77-79, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

81. The isolated cell of any one of paragraphs 33-80, wherein the antigen-binding domain of the first chimeric receptor and/or the second chimeric receptor comprises two single chain variable fragments (scFvs).

82. The isolated cell of paragraph 81, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

83. The isolated cell of any one of paragraphs 33-80, wherein the first chimeric receptor is recombinantly expressed.

84. The isolated cell of any one of paragraphs 76-83, wherein each of the one or more scFvs is separated by a peptide linker.

85. The isolated cell of paragraph 84, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

86. The isolated cell of any one of paragraphs 33-85, wherein the first chimeric receptor is expressed from a vector, or a selected locus from the genome of the cell.

87. The isolated cell of any one of paragraphs 33-86, wherein the second chimeric receptor is recombinantly expressed.

88. The isolated cell of any one of paragraphs 33-87, wherein the second chimeric receptor is expressed from a vector, or a selected locus from the genome of the cell.

89. The isolated cell of any one of paragraphs 33-88, wherein the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain.

90. The isolated cell of paragraph 89, wherein the inhibitory chimeric receptor inhibits one or more activities of the cell.

91. The isolated cell of paragraph 89 or paragraph 90, wherein the inhibitory chimeric receptor binds an antigen that is not expressed on a tumor cell.

92. The isolated cell of paragraph 89 or paragraph 90, wherein the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell.

93. The isolated cell of paragraph 92, wherein the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

94. The isolated cell of any one of paragraphs 91-93, wherein the tumor cell is an acute myeloid leukemia (AML) cell.

95. The isolated cell of any one of paragraphs 89-94, wherein the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

96. The isolated cell of paragraph 95, wherein the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

97. The isolated cell of paragraph 95 or paragraph 96, wherein the enzymatic inhibitory domain comprises an enzyme catalytic domain.

98. The isolated cell of paragraph 97, wherein the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

99. The isolated cell of any one of paragraphs 89-98, wherein the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

100. The isolated cell of paragraph 99, wherein the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

101. The isolated cell of any one of paragraphs 89-100, where in the inhibitory chimeric receptor binds an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26,

TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

102. The isolated cell of any one of paragraphs 89-101, wherein the inhibitory chimeric receptor comprises an antigen binding domain derived from an anti-EMCN antibody, an anti-CALN1 antibody, an anti-IL12RB2 antibody, an anti-CDH26 antibody, an anti-IL18R1 antibody, an anti-SLC8A3 antibody, an anti-ABCB1 antibody, an anti-ATP9A antibody, an anti-CDCP1 antibody, an anti-JAM2 antibody, an anti-PCDH9 antibody, an anti-TMEM200A antibody, an anti-ADGRL4 antibody, an anti-ART4 antibody, an anti-BACE2 antibody, an anti-CALCRL antibody, an anti-LIFR antibody, an anti-MET antibody, an anti-MPZL2 antibody, an anti-PTPRB antibody, or an anti-WLS antibody.

103. The isolated cell of paragraph 102, wherein the anti-EMCN antibody is a monoclonal antibody selected from the group consisting of CBFYE-0213, V.7.C7.1, L6H10, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, and L10F12.

104. The isolated cell of any one of paragraphs 33-103, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

105. The isolated cell of any one of paragraphs 33-104, wherein the cell is autologous.

106. The isolated cell of any one of paragraphs 33-104, wherein the cell is allogeneic.

107. A chimeric receptor comprising two or more antigen-binding domains, wherein each of the two or more antigen-binding domains binds to an antigen selected from the group consisting of the antigens listed in Table 1, or wherein two antigen-binding domains of the two or more antigen-bonding domains bind to an antigen pair selected from the group consisting of the antigen pairs listed in Table 3,
wherein each antigen-binding domain binds to a distinct antigen.

108. A chimeric receptor comprising two or more antigen-binding domains, wherein each each of the two or more antigen-binding domains binds to an antigen selected from

the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70,

wherein each antigen-binding domain binds to a distinct antigen.

109. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds MS4A3 and a second antigen-binding domain binds to an antigen selected from the group consisting of VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

110. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds VSTM1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

111. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds LAT2 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

112. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds MLC1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

113. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds CD131 and a second antigen-binding domain binds to an

antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

114. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds GAPT and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

115. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds PRAM1 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

116. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds SLC22A16 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

117. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds SLC17A9 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

118. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen-binding domain binds SPNS3 and a second antigen-binding domain binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1,

CD131, GAPT, PRAM1, SLC22A16, SLC17A9, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

119. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen binding domain binds FLT3.

120. The chimeric receptor of paragraph 119, wherein the antigen-binding domain that binds FLT3 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto;
(c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto;
(d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto;
(e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto;
(f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto;
(g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and
(h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto.

121. The chimeric receptor of paragraph 119 or paragraph 120, wherein a second a second antigen-binding domain binds CD33.

122. The chimeric receptor of paragraph 121, wherein the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and
(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

123. The chimeric receptor of paragraph 119 or paragraph 120, wherein a second antigen-binding domain binds CLEC12A.

124. The chimeric receptor of paragraph 123, wherein the second antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and
(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

125. The chimeric receptor of paragraph 107 or paragraph 108, wherein one antigen binding domain binds CLEC12A.

126. The chimeric receptor of paragraph 125, wherein the antigen-binding domain that binds CLEC12A comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at

least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and
(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

127. The chimeric receptor of paragraph 125 or paragraph 126, wherein a second antigen-binding domain binds CD33.

128. The chimeric receptor of paragraph 127, wherein the second antigen-binding domain that binds CD33 comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and
(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

129. The chimeric receptor of any one of paragraphs 107-128, wherein the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

130. The chimeric receptor of paragraph 129, wherein the chimeric receptor is a CAR.

131. The chimeric receptor of paragraph 130, wherein the CAR is a bispecific CAR.

132. The chimeric receptor of paragraph 130 or paragraph 131, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3-zeta chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40

intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

133. The chimeric receptor of any one of paragraphs 130-132, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

134. The chimeric receptor of any one of paragraphs 120-133, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

135. The chimeric receptor of any one of paragraphs 107-134, wherein each antigen-binding domain comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

136. The chimeric receptor of any one of paragraphs 107-135, wherein each antigen-binding domain comprises one or more single chain variable fragments (scFvs).

137. The chimeric receptor of paragraph 136, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

138. The chimeric receptor of paragraph 137, wherein the VH and VL are separated by a peptide linker.

139. The chimeric receptor of paragraph 138, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

140. The chimeric receptor of any one of paragraphs 137-139, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

141. The chimeric receptor of any one of paragraphs 107-140, wherein at least one of the antigen-binding domains comprises two single chain variable fragments (scFvs).

142. The chimeric receptor of paragraph 141, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

143. The chimeric receptor of any one of paragraphs 135-142, wherein each of the one or more scFvs is separated by a peptide linker.

144. The chimeric receptor of paragraph 143, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

145. The chimeric receptor of any one of paragraphs 141-144, wherein at least one of the antigen-binding domains comprises two single chain variable fragments (scFvs), and each of the two scFvs binds to FLT3 and comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto;
(c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto;
(d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO:

8 or a sequence at least 90% identical thereto;
(e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10 or a sequence at least 90% identical thereto;
(f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto;
(g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and
(h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto.

146. The chimeric receptor of any one of paragraphs 141-145, wherein at least one of the antigen-binding domains comprises two single chain variable fragments (scFvs), and each of the two scFvs binds to CD33 and comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and
(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

147. The chimeric receptor of any one of paragraphs 141-146, wherein at least one of the antigen-binding domains comprises two single chain variable fragments (scFvs), and each of the two scFvs binds to CLEC12A and comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:
(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;
(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at

least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and
(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

148. An isolated cell comprising the chimeric receptor of any one of paragraphs 107-147.

149. The isolated cell of paragraph 148, wherein the cell further comprises an additional chimeric receptor comprising an antigen-binding domain.

150. The isolated cell of paragraph 149, wherein the additional chimeric receptor binds to an antigen selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, FLT3, CD33, CLEC12A, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

151. The isolated cell of paragraph 150, wherein each of the two chimeric receptors binds to distinct antigens.

152. The isolated cell of any one of paragraphs 148-151, wherein the cell is an immunoresponsive cell.

153. The isolated cell of paragraph 152, wherein binding of the chimeric receptor to either of the two antigens is capable of activating the immunoresponsive cell.

154. The isolated cell of paragraph 152, wherein binding of the additional chimeric receptor to its cognate antigen is capable of stimulating the immunoresponsive cell.

155. The isolated cell of paragraph 152, wherein binding of the chimeric receptor to either of the two antigens and binding of the additional chimeric receptor to its cognate antigen are required for activating the immunoresponsive cell.

156. The isolated cell of paragraph 152, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against target cells that are positive for either of the two antigens bound by the chimeric receptor and positive for the antigen bound by the

additional chimeric receptor, as compared to cytolytic activity against target cells that are only positive for a single antigen.

157. The isolated cell of any one of paragraphs 149-156, wherein the additional chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

158. The isolated cell of paragraph 157, wherein the additional chimeric receptor is a CAR.

159. The isolated cell of paragraph 157, wherein the chimeric receptor is a first CAR and the additional chimeric receptor is a second CAR.

160. The isolated cell of paragraph 159, wherein the second CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

161. The isolated cell of paragraph 160, wherein the one or more intracellular signaling domains of the first CAR are different from the one or more intracellular signaling domains of the second CAR.

162. The isolated cell of paragraph 161, wherein the first CAR and the second CAR each comprise a CD3zeta-chain intracellular signaling domain.

163. The isolated cell of paragraph 162, wherein the first CAR and the second CAR each further comprises an additional intracellular signaling domain selected from the group consisting of a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular

signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

164. The isolated cell of paragraph 163, wherein the additional intracellular signaling domain of the first CAR is different from the additional intracellular signaling domain of the second CAR.

165. The isolated cell of any one of paragraphs 160-164, wherein the second CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

166. The isolated cell of paragraph 165, wherein the transmembrane domain of the first CAR is different from the transmembrane domain of the second CAR.

167. The isolated cell of any one of paragraphs 143-166, wherein the second CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

168. The isolated cell of any one of paragraphs 146-167, wherein the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain.

169. The isolated cell of paragraph 149, wherein the additional chimeric receptor is an inhibitory chimeric receptor comprising an antigen-binding domain.

170. The isolated cell of paragraph 168 or paragraph 169, wherein the inhibitory chimeric receptor inhibits one or more activities of the cell.

171. The isolated cell of any one of paragraphs 168-170, wherein the inhibitory chimeric receptor binds an antigen that is not expressed on a tumor cell.

172. The isolated cell of any one of paragraphs 168-170, wherein the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell.

173. The isolated cell of any one of paragraphs 168-170, wherein the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

174. The isolated cell of any one of paragraphs 171-173, wherein the tumor cell is an acute myeloid leukemia (AML) cell.

175. The isolated cell of any one of paragraphs 168-174, wherein the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

176. The isolated cell of paragraph 175, wherein the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

177. The isolated cell of paragraph 175 or paragraph 176, wherein the enzymatic inhibitory domain comprises an enzyme catalytic domain.

178. The isolated cell of paragraph 177, wherein the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

179. The isolated cell of any one of paragraphs 168-178, wherein the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

180. The isolated cell of paragraph 179, wherein the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

181. The isolated cell of any one of paragraphs 168-180, where in the inhibitory chimeric receptor binds an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

182. The isolated cell of any one of paragraphs 149-181, wherein the antigen-binding domain of the additional chimeric receptor and/or inhibitory chimeric receptor comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

183. The isolated cell of any one of paragraphs 149-181, wherein the antigen-binding domain of the additional chimeric receptor and/or inhibitory chimeric receptor comprises one or more single chain variable fragments (scFvs).

184. The isolated cell of paragraph 183, wherein the antigen-binding domain of the inhibitory chimeric receptor is derived from an anti-EMCN antibody, an anti-CALN1 antibody, an anti-IL12RB2 antibody, an anti-CDH26 antibody, an anti-IL18R1 antibody, an anti-SLC8A3 antibody, an anti-ABCB1 antibody, an anti-ATP9A antibody, an anti-CDCP1 antibody, an anti-JAM2 antibody, an anti-PCDH9 antibody, an anti-TMEM200A antibody, an anti-ADGRL4 antibody, an anti-ART4 antibody, an anti-BACE2 antibody, an anti-CALCRL antibody, an anti-LIFR antibody, an anti-MET antibody, an anti-MPZL2 antibody, an anti-PTPRB antibody, or an anti-WLS antibody.

185. The isolated cell of paragraph 184, wherein the anti-EMCN antibody is a monoclonal antibody selected from the group consisting of CBFYE-0213, V.7.C7.1, L6H10, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, and L10F12.

186. The isolated cell of any one of paragraphs 183-185, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

187. The isolated cell of paragraph 186, wherein the VH and VL are separated by a peptide linker.

188. The isolated cell of paragraph 187, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

189. The isolated cell of any one of paragraphs 186-188, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

190. The isolated cell of any one of paragraphs 168-189, wherein the antigen-binding domain of the additional chimeric receptor and/or inhibitory chimeric receptor comprises two single chain variable fragments (scFvs).

191. The isolated cell of paragraph 190, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

192. The isolated cell of any one of paragraphs 182-191, wherein each of the one or more scFvs is separated by a peptide linker.

193. The isolated cell of paragraph 192, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

194. The isolated cell of any one of paragraphs 146-189 wherein each chimeric receptor is recombinantly expressed.

195. The isolated cell of any one of paragraphs 146-194 wherein each chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

196. The isolated cell of any one of paragraphs 146-195, wherein the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a

macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

197. The isolated cell of any one of paragraphs 146-196, wherein the cell is autologous.

198. The isolated cell of any one of paragraphs 146-196, wherein the cell is allogeneic.

199. An inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

200. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is EMCN.

201. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is JAM2.

202. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is MS4A15.

203. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is C4BPA.

204. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is TRPM1.

205. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is SCTR.

206. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is SLC2A2.

207. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is KCNQ2.

208. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is PERP.

209. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is WLS.

210. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is FFAR2.

211. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is PTPRB.

212. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is NCKAP1

213. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is MPZL2.

214. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is PLSCR4.

215. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is TMEM47.

216. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ADGRL4.

217. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is MET.

218. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is BACE2.

219. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ATP8B1.

220. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is LIFR.

221. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ART4.

222. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CALCRL.

223. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CNTNAP3.

224. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is PCDH9.

225. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is IL18R1.

226. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is SLC8A3.

227. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CDH26.

228. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is TMEM163.

229. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ABCA13.

230. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CACHD1.

231. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CYYR1.

232. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ABCB1.

233. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ADGRG6.

234. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is ATP9A.

235. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CALN1.

236. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is CDCP1.

237. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is IL12RB2.

238. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is SLC16A14.

239. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is TMEM136.

240. The inhibitory chimeric receptor of paragraph 199, wherein the antigen is TMEM200A.

241. The inhibitory chimeric receptor of any one of paragraphs 199-240, wherein when expressed on a cell the inhibitory chimeric receptor inhibits one or more activities of the cell.

242. The inhibitory chimeric receptor of any one of paragraphs 199-241, wherein the antigen that is not expressed on a tumor cell.

243. The inhibitory chimeric receptor of any one of paragraphs 199-242, wherein the antigen is expressed on a non-tumor cell.

244. The inhibitory chimeric receptor of paragraph 243, wherein the antigen is expressed on a non-tumor cell derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

245. The inhibitory chimeric receptor of any one of paragraphs 199-244, wherein the inhibitory chimeric receptor comprises an enzymatic inhibitory domain.

246. The inhibitory chimeric receptor of paragraph 245, wherein the enzymatic inhibitory domain inhibits immune receptor activation when proximal to an immune receptor.

247. The inhibitory chimeric receptor of paragraph 245 or paragraph 246, wherein the enzymatic inhibitory domain comprises an enzyme catalytic domain.

248. The inhibitory chimeric receptor of paragraph 247, wherein the enzyme catalytic domain is derived from an enzyme selected from the group consisting of CSK, SHP-1, PTEN, CD45, CD148, PTP-MEG1, PTP-PEST, c-CBL, CBL-b, PTPN22, LAR, PTPH1, SHIP-1, and RasGAP.

249. The inhibitory chimeric receptor of any one of paragraphs 199-248, wherein the inhibitory chimeric receptor further comprises one or more intracellular inhibitory co-signaling domains.

250. The inhibitory chimeric receptor of paragraph 249, wherein the one or more intracellular inhibitory co-signaling domains are selected from the group consisting of PD-1, CTLA4, TIGIT, LAIR1, GRB-2, Dok-1, Dok-2, SLAP, LAG3, HAVR, BTLA, GITR, and PD-L1.

251. The inhibitory chimeric receptor of any one of paragraphs 199-250, wherein the antigen-binding domain comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

252. The inhibitory chimeric receptor of any of any one of paragraphs 199-251, wherein the antigen-binding domain comprises one or more single chain variable fragments (scFvs).

253. The inhibitory chimeric receptor of paragraph 252, wherein the antigen-binding domain is derived from an anti-EMCN antibody, an anti-CALN1 antibody, an anti-IL12RB2 antibody, an anti-CDH26 antibody, an anti-IL18R1 antibody, an anti-SLC8A3 antibody, an anti-ABCB1 antibody, an anti-ATP9A antibody, an anti-CDCP1 antibody, an anti-JAM2 antibody, an anti-PCDH9 antibody, an anti-TMEM200A antibody, an anti-ADGRL4 antibody, an anti-ART4 antibody, an anti-BACE2 antibody, an anti-CALCRL antibody, an anti-LIFR antibody, an anti-MET antibody, an anti-MPZL2 antibody, an anti-PTPRB antibody, or an anti-WLS antibody.

254. The inhibitory chimeric receptor of paragraph 253, wherein the anti-EMCN antibody is a monoclonal antibody selected from the group consisting of CBFYE-0213, V.7.C7.1, L6H10, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, and L10F12.

255. The inhibitory chimeric receptor of any one of paragraphs 252-254, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

256. The inhibitory chimeric receptor of paragraph 255, wherein the VH and VL are separated by a peptide linker.

257. The inhibitory chimeric receptor of paragraph 256, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

258. The inhibitory chimeric receptor of any one of paragraphs 255-257, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

259. The inhibitory chimeric receptor of any one of paragraphs 199-258, wherein the antigen-binding domain comprises two single chain variable fragments (scFvs).

260. The inhibitory chimeric receptor of paragraph 259, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

261. The inhibitory chimeric receptor of any one of paragraphs 251-260, wherein each of the one or more scFvs is separated by a peptide linker.

262. The inhibitory chimeric receptor of paragraph 261, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

263. An isolated cell comprising the inhibitory chimeric receptor of any one of paragraphs 199-262.

264. The isolated cell of paragraph 263, wherein the inhibitory chimeric receptor is recombinantly expressed.

265. The isolated cell of paragraph 263 or paragraph 264, wherein the inhibitory chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

266. The isolated cell of any one of paragraphs 263-265, wherein the cell further comprises a chimeric receptor comprising an extracellular antigen-binding domain that binds to an antigen selected from the group consisting of FLT3, CD33, CLEC12A, MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, , ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

267. An isolated cell comprising:
(a) an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, wherein the first antigen is selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A, and
(b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein each antigen-binding domain binds to an antigen selected from the group consisting of the antigens listed in Table 1.

268. An isolated cell comprising:
(a) an inhibitory chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, wherein the first antigen is selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A, and
(b) a chimeric receptor comprising one or more extracellular antigen-binding domains, wherein each antigen-bind domain binds to an antigen selected from the group consisting of FLT3, CD33, CLEC12A, MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, , ADGRE2, IL3RA,

CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70.

269. The isolated cell of any one of paragraphs 266-268, wherein the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

270. The isolated cell of paragraph 269, wherein the chimeric receptor is a CAR.

271. The isolated cell of paragraph 270, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain.

272. The isolated cell of paragraph 270 or paragraph 271, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

273. The isolated cell of any one of paragraphs 208-272, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

274. The isolated cell of any one of paragraphs 266-273, wherein the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

275. The isolated cell of any one of paragraphs 266-273, wherein the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises one or more single chain variable fragments (scFvs).

276. The isolated cell of paragraph 275, wherein the antigen-binding domain of the inhibitory chimeric receptor is derived from an anti-EMCN antibody, an anti-CALN1 antibody, an anti-IL12RB2 antibody, an anti-CDH26 antibody, an anti-IL18R1 antibody, an anti-SLC8A3 antibody, an anti-ABCB1 antibody, an anti-ATP9A antibody, an anti-CDCP1 antibody, an anti-JAM2 antibody, an anti-PCDH9 antibody, an anti-TMEM200A antibody, an anti-ADGRL4 antibody, an anti-ART4 antibody, an anti-BACE2 antibody, an anti-CALCRL antibody, an anti-LIFR antibody, an anti-MET antibody, an anti-MPZL2 antibody, an anti-PTPRB antibody, or an anti-WLS antibody.

277. The inhibitory chimeric receptor of paragraph 276, wherein the anti-EMCN antibody is a monoclonal antibody selected from the group consisting of CBFYE-0213, V.7.C7.1, L6H10, L4B1, L5F12, L10B5, L3F12, L6H3, L9H8, and L10F12..

278. The isolated cell of any one of paragraphs 275-277, wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

279. The isolated cell of paragraph 278, wherein the VH and VL are separated by a peptide linker.

280. The isolated cell of paragraph 279, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

281. The isolated cell of any one of paragraphs 278-280, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

282. The isolated cell of any one of paragraphs 266-281, wherein the antigen-binding domain of the inhibitory chimeric receptor and/or the chimeric receptor comprises two single chain variable fragments (scFvs).

283. The isolated cell of paragraph 282, wherein each of the two scFvs binds to a distinct epitope on the same antigen.

284. The isolated cell of any one of paragraphs 274-283, wherein each of the one or more scFvs is separated by a peptide linker.

285. The isolated cell of paragraph 284, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

286. The isolated cell of any one of paragraphs 267-285, wherein the cell is an immunoresponsive cell.

287. The isolated cell of paragraph 286, wherein binding of the inhibitory chimeric receptor to the first antigen is capable of inhibiting the immunoresponsive cell.

288. The isolated cell of paragraph 286 or paragraph 287, wherein binding of the chimeric receptor to the second antigen is capable of activating the immunoresponsive cell.

289. The isolated cell of any one of paragraphs 267-288, wherein the chimeric receptor binds to the second antigen with a low binding affinity.

290. The isolated cell of any one of paragraphs 267-289, wherein the chimeric receptor binds to the second antigen with a binding affinity that is lower than the binding affinity with which the inhibitory chimeric receptor binds to the first antigen.

291. The isolated cell of any one of paragraphs 267-290, wherein the chimeric receptor binds to the first antigen with a low binding avidity.

292. The isolated cell of any one of paragraphs 266-291, wherein the chimeric receptor is recombinantly expressed.

293. The isolated cell of any one of paragraphs 266-292, wherein the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

294. The isolated cell of any one of paragraphs 263-293, wherein the cell selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell.

295. The isolated cell of any one of paragraphs 263-294, wherein the cell is autologous.

296. The isolated cell of any one of paragraphs 263-294, wherein the cell is allogeneic.

297. An isolated nucleic acid encoding the chimeric receptor of any one of paragraphs 1-22.

298. An isolated nucleic acid encoding the chimeric receptor of any one of paragraphs 107-140.

299. An isolated nucleic acid encoding the inhibitory chimeric receptor of any one of paragraphs 199-258.

300. A vector comprising the nucleic acid of paragraph 297.

301. The vector of paragraph 300, wherein the vector further comprises the nucleic acid of paragraph 298.

302. The vector of paragraph 300 or paragraph 301, wherein the vector further comprises the nucleic acid of paragraph 299.

303. A vector comprising the nucleic acid of paragraph 298.

304. The vector of paragraph 303, wherein the vector further comprises the nucleic acid of paragraph 299.

305. A vector comprising the nucleic acid of paragraph 299.

306. A genetically modified cell comprising the nucleic acid of paragraph 297.

307. The genetically modified cell of paragraph 306, wherein the cell further comprises the nucleic acid of paragraph 298.

308. The genetically modified cell of paragraph 306 or paragraph 307, wherein the cell further comprises the nucleic acid of paragraph 299.

309. A genetically modified cell comprising the nucleic acid of paragraph 298.

310. The genetically modified cell of paragraph 309, wherein the cell further comprises the nucleic acid of paragraph 299.

311. A genetically modified cell comprising the nucleic acid of paragraph 299.

312. A genetically modified cell comprising the vector of any one of paragraphs 300-305.

313. A method of treating a subject in need thereof, the method comprising administering a therapeutically effective dose of any of the isolated cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

314. A method of stimulating a cell-mediated immune response to a tumor cell in a subject, the method comprising administering to a subject having a tumor a therapeutically effective dose of any of the isolated cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

315. A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the isolated cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

316. A method of reducing tumor burden in a subject, comprising administering to the subject an effective amount of any of the isolated cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

317. The method of paragraph 316, wherein the method reduces the number of tumor cells.

318. The method of paragraph 316, wherein the method reduces tumor size.

319. The method of paragraph 316, wherein the method reduces tumor volume.

320. The method of paragraph 316, wherein the method eradicates the tumor in the subject.

321. A method of treating a subject having a tumor, the method comprising administering a therapeutically effective dose of any of the cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

322. A method of treating or preventing a myeloid disorder in a subject, comprising administering to the subject an effective amount of any of the isolated cells of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

323. The method of paragraph 322, wherein the myeloid disorder is myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia (AML), acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

324. The method of paragraph 322, wherein the myeloid disorder is acute myeloid leukemia (AML).

325. The method of any one of paragraphs 321-324, wherein the method reduces or eradicates the tumor burden in the subject.

326. A pharmaceutical composition comprising an effective amount of the cell of any one of paragraphs 24-106, 146-198, 263-296, or 306-312 and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

327. The pharmaceutical composition of paragraph 326, which is for treating and/or preventing a myeloid disorder.

328. A kit for treating and/or preventing a myeloid disorder, comprising the cell of any one of paragraphs 24-106, 146-198, 263-296, or 306-312.

329. The kit of paragraph 328, wherein the kit further comprises written instructions for using the cell for treating and/or preventing a myeloid disorder in a subject .

330. A kit for treating and/or preventing a myeloid disorder, comprising the isolated nucleic acid of any one of paragraphs 297-299.

331. The kit of paragraph 330, wherein the kit further comprises written instructions for using the nucleic acid for producing one or more antigen-specific cells for treating and/or preventing a myeloid disorder in a subject.

332. A kit for treating and/or preventing a myeloid disorder, comprising the vector of any one of paragraphs 300-305.

333. The kit of paragraph 332, wherein the kit further comprises written instructions for using the vector for producing one or more antigen-specific cells for treating and/or preventing a myeloid disorder in a subject.

334. A method of treating and/or preventing a myeloid disorder, comprising administering an effective amount of at least one antibody that binds to an antigen, wherein the antigen is selected from the group consisting of MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, and SPNS3.

335. The method of paragraph 334, wherein the myeloid disorder is selected from the group consisting of myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, or acute myeloid leukemia (AML) , acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

336. The method of paragraph 335, wherein the myeloid disorder is acute myeloid leukemia (AML).

337. The method of any one of paragraphs 334-336, wherein the method reduces or eradicates the tumor burden in the subject.

338. A chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular antigen-binding domain, one or more intracellular signaling domains, a transmembrane domain, and a spacer region between the antigen-binding domain and the transmembrane domain, wherein the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

339. A chimeric receptor comprising an extracellular antigen-binding domain, wherein the antigen-binding domain comprises two or more antibodies, antigen-binding fragments

of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs).

340. The chimeric receptor of paragraph 339, wherein each of the two or more antibodies, antigen-binding fragments of an antibody, F(ab) fragments, F(ab') fragments, single chain variable fragments (scFvs), or single-domain antibodies (sdAbs) binds to a distinct epitope on the same antigen.

341. The chimeric receptor of paragraph 339 or paragraph 340, wherein the antigen-binding domain comprises two or more single chain variable fragments (scFvs).

342. The chimeric receptor of paragraph 341, wherein each of the two or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

343. The chimeric receptor of paragraph 342, wherein each VH and VL are separated by a peptide linker.

344. The chimeric receptor of paragraph 343, wherein the peptide linker comprises an amino acid sequence of SEQ ID NO: 27.

345. The chimeric receptor of any one of paragraphs 341-344, wherein each of the two or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

346. The chimeric receptor of any one of paragraphs 341-345, wherein each of the two or more scFvs is separated by a peptide linker.

347. The chimeric receptor of paragraph 346, wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

348. The chimeric receptor of any one of paragraphs 339-347, wherein the chimeric receptor is a chimeric T cell receptor or a chimeric antigen receptor (CAR).

349. The chimeric receptor of paragraph 348, wherein the chimeric receptor is a CAR.

350. The chimeric receptor of paragraph 349, wherein the CAR comprises one or more intracellular signaling domains, and the one or more intracellular signaling domains are selected from the group consisting of a CD3zeta-chain intracellular signaling domain, a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, and a MyD88 intracellular signaling domain.

351. The chimeric receptor of paragraph 349 or paragraph 350, wherein the CAR comprises a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain.

352. The chimeric receptor of any one of paragraphs 348-351, wherein the CAR comprises a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

353. An isolated cell comprising the chimeric receptor of any one of paragraphs 339-352.

354. The isolated cell of paragraph 353, wherein the chimeric receptor is recombinantly expressed.

355. The isolated cell of paragraph 353 or paragraph 354, wherein the chimeric receptor is expressed from a vector or a selected locus from the genome of the cell.

356. A pharmaceutical composition comprising the isolated cell of any one of paragraphs 353-355 and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

357. An isolated nucleic acid encoding the chimeric receptor of any one of paragraphs 339-352.

358. A vector comprising the nucleic acid of paragraph 357.

359. A genetically modified cell comprising the nucleic acid of paragraph 357.

EXAMPLES

[0633] The following are examples of methods and compositions of the present disclosure. It is understood that various other embodiments may be practiced, given the general description provided herein.

[0634] Below are examples of specific embodiments for carrying out the claimed subject matter of the present disclosure. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1: Bioinformatics Screen for AML targets

Bioinfomatics Screen Analysis

[0635] Microarray data, RNA-seq data and proteomics data from AML and normal hematopoiesis samples as well as data from off-target tissues were analyzed for potential targets associated with AML. Datasets for microarray were obtained from GEO, datasets for RNA-seq were obtained from The Cancer Genome Atlas (TCGA), and datasets for proteomics were obtained from the Human Protein Atlas and the Human Proteome Map. Each data set was initially analyzed for genes and protein targets associated with AML. Data from the initial individual microarray, RNA-seq and proteomics bioinformatics analysis for AML targets was then compared to provide a refined list of AML targets. Putative AML targets were validated in AML cell lines via antibody staining and flow cytometry, and in primary AML samples via antibody staining and flow cytometry. All data was analyzed to provide a final list of AML targets. Bioinformatics analysis combining microarray expression, RNA-seq expression, and HPA toxicity simultaneously allows a more robust pipeline to identify and validate AML antigen targets.

[0636] Genes identified in the initial analysis with subcellular localization GO Annotation were removed first. 7806 genes were identified as membrane or cell surface localized. Next, genes with expressed in healthy erythroblasts and T cells as compared to AML hematopoietic cells were removed. Genes with "Supported" or "Enhanced" high immunohistochemistry protein expression in tissues other than bone marrow, spleen, lymph node, appendix, or tonsils were removed. Genes were next positively sorted for those with at least 2-fold higher gene expression in AML tissues as compared to non-hematopoietic tissue expression, based on the RNA-seq expression data. The remaining targets were then screened again with a more stringent requirement to remove genes with medium expression in multiple tissues and only retain genes with at least a 4-fold increase in the putative AML gene, as shown in the RNA-seq dataset, across all AML samples and in FLT3-ITD subpopulations. The remaining genes were then manually filtered for genes with monoclonal human-reactive flow cytometry antibodies availability, known biology, and LSC expression.

Example 2: Logic Gating Strategies

[0637] The novel AML antigens identified in **Example 1** were then paired for AND and NOT gating.

[0638] AND pairs with overlapping tissue toxicities according to either the RNA-seq or HPA databases were removed. Potential targets that could pair with a NOT target were determined by any gene with at least two toxic tissues.

[0639] NOT targets were determined using the TCGA RNAseq database using the following criteria: a NOT target had low expression on an AML tissue and a high expression on a desired tissue.

[0640] Single or "OR" gating targets determined by this strategy include FLT3, MS4A3, CD33, CLEC12A, ADGRE2, SLC22A16, CD123/IL3RA, MLC1, SPNS3, and GAPT. Additional AML antigens are described in **Table 1**. Microarray, RNA-seq and protein expression data for AML antigens are shown in **FIGS. 1-25**.

[0641] "AND/NOT" gating targets determined by this strategy include LAT2, PIEZO1, CD38, EMB, CD131/CSF2RB, LILRA2, CD85H, SLC17A9, MYADM, CD300LF, CD244, CD93, and CD117/c-Kit.

[0642] Paired "AND" gating targets are described in **Table 3**.

[0643] "NOT" gating targets determined by this strategy include EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PEPR, WLS, and FFAR. "NOT" are also

described in **Table 2**. Microarray, RNA-seq and protein expression data for NOT antigen gating targets are shown in **FIGS. 26-34**.

Example 3: T Cells Expressing an FLT3 Chimeric Antigen Receptor

[0644] T cells from two different donors were genetically modified to express CARs comprised of six different FLT3 scFv polypeptides. The CARS also included a YFP tag as a reporter gene. Expression of the FLT3 CAR in each donor T cell line was quantified via flow cytometry, as determined by expression of the YFP reporter tag. **FIG. 35A** shows the % cells expressing YFP and the FLT3 CAR from donor 1 for each of the six FLT3 scFvs tested, **FIG. 35B** shows the mean fluorescent intensity of the YFP positive cells from donor 1 for each of the six FLT3 scFvs tested. **FIG. 35C** shows the % cells expressing YFP and the FLT3 CAR from donor 2 for each of the six FLT3 scFvs tested, **FIG. 35D** shows the mean fluorescent intensity of the YFP positive cells from donor 2 for each of the six FLT3 scFvs tested. NV stands for No Vector and is the negative control.

[0645] Next, the FLT3 CAR T cells were assessed for killing activity *in vitro*. FLT3 CARs were mixed at a 1:1 ratio with MOLM13, an AML cell line; SEM, an FLT3+ expression line, and K562, an FLT3-negative cell line as control. T cells and cell lines were co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency was assessed via flow cytometry.

[0646] As shown in **FIG. 36A-D**, the FLT3 CAR T cells from both donors killed FLT3 expressing cells. **FIGS. 36A** and **C** show killing of SEM cells, **FIGS. 36B** and **D** show killing of MOLM13 cells. **FIGS. 36A** and **B** are T cells from donor 1, **FIG. 36C** and **D** are T cells from donor 2.

[0647] Cytokine secretion by the FLT3 CAR T cells after incubation with the SEM or MOLM13 cells was also quantified via ELISA (**FIG. 37A-F** and **FIG. 38A-F**). **FIGS. 37A-C** show the TNF-α, IFN-γ and IL-2 secretion induced in each FLT3 CAR T cell line from donor 1 after incubation with the SEM cells. **FIGS. 37D-F** show the TNF-α, IFN-γ and IL-2 secretion induced in each FLT3 CAR T cell line from donor 2 after incubation with the SEM cells. **FIGS. 38A-C** show the TNF-α, IFN-γ and IL-2 secretion induced in each FLT3 CAR T cell line from donor 1 after incubation with the MOLM13 cells. **FIGS. 38D-F** show the TNF-α, IFN-γ and IL-2 secretion induced in each FLT3 CAR T cell line from donor 2 after incubation with the MOLM13 cells.

Example 4: *In vivo* characterization of T Cells Expressing an FLT3 Chimeric Antigen Receptor

[0648] Next, FLT3 CAR T cells generated in Example 3 are characterized in vivo in a murine model. Patient-derived tumor cells expressing FLT3, such as MOLM13 and MV411, are xenografted into a mouse. FLT3 CAR T cells are then infused into the xenografted mice. Blood is drawn during the course of the experiment, and spleen, bone marrow, and peripheral blood is collected at the end of the time course. Untreated mice are used as a control. Infusion of FLT3 CAR T cells reduces xenografted tumor cell burden and growth in mice treated with FLT3 CAR T cells as compared to untreated mice.

Example 5: T Cells Expressing FLT3 Chimeric Antigen Receptor and EMCN Chimeric Inhibitory Receptor

[0649] A T cell is genetically modified to express a CAR that recognizes the AML antigen FLT3 and an inhibitory CAR that recognizes the non AML-antigen EMCN (endomucin). To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate FLT3 antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, FLT3 CARs are mixed at a 1:1 ratio with MOLM13, an AML cell line; SEM, an FLT3+ expression line, or K562, an FLT3- negative cell line as control. Unmodified T cells are used as a negative control. Dual modified T cells expressing an FLT3 CAR proliferate and produce effector cytokines in response to FLT3 expressing cells.

[0650] Next, the dual modified T cells are co-incubated with cells expressing the cognate EMCN antigen and cells expressing the cognate FLT3 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate FLT3 antigen are killed, while the cells expressing EMCN antigen only are not killed.

[0651] The dual modified FLT3 and EMCN CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of FLT3 and EMCN CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 6: T Cells Expressing FLT3 Chimeric Antigen Receptor and JAM2 Chimeric Inhibitory Receptor

[0652] A T cell is genetically modified to express a CAR that recognizes the AML antigen FLT3 and an inhibitory CAR that recognizes the non AML-antigen JAM2. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate FLT3 antigen and T cell activation is characterized via killing and cytokine production assays

as described in **Example 3**. Briefly, FLT3 CARs are mixed at a 1:1 ratio with MOLM13, an AML cell line; SEM, an FLT3+ expression line, or K562, an FLT3-negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an FLT3 CAR proliferate and produce effector cytokines in response to FLT3 antigen alone.

[0653] Next, the dual modified T cells are co-incubated with cells expressing the cognate JAM2 antigen and cells expressing the cognate FLT3 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate FLT3 antigen are killed, while the cells expressing JAM2 antigen only are not killed.

[0654] The dual modified FLT3 and JAM2 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of FLT3 and JAM2 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 7: T Cells Expressing CLEC12A Chimeric Antigen Receptor and MS4A15 Chimeric Inhibitory Receptor

[0655] A T cell is genetically modified to express a CAR that recognizes the AML antigen CLEC12A and an inhibitory CAR that recognizes the non AML-antigen MS4A15. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate CLEC12A antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, CLEC12A CARs are mixed at a 1:1 ratio with an AML cell line expressing CLEC12A; a MS4A15+ expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an CLEC12A CAR proliferate and produce effector cytokines in response to CLEC12A antigen alone.

[0656] Next, the dual modified T cells are co-incubated with cells expressing the cognate CLEC12A antigen and cells expressing the cognate MS4A15 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate CLEC12A antigen are killed, while the cells expressing MS4A15 antigen only are not killed.

[0657] The dual modified CLEC12A and MS4A15 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of CLEC12A and MS4A15 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 8: T Cells Expressing CLEC12A Chimeric Antigen Receptor and SLC34A2 Chimeric Inhibitory Receptor

[0658] A T cell is genetically modified to express a CAR that recognizes the AML antigen CLEC12A and an inhibitory CAR that recognizes the non AML-antigen SLC34A2. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate CLEC12A antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, CLEC12A CARs are mixed at a 1:1 ratio with an AML cell line expressing CLEC12A; a SLC34A2+ expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an CLEC12A CAR proliferate and produce effector cytokines in response to CLEC12A antigen alone.

[0659] Next, the dual modified T cells are co-incubated with cells expressing the cognate CLEC12A antigen and cells expressing the cognate SLC34A2 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate CLEC12A antigen are killed, while the cells expressing SLC34A2 antigen only are not killed.

[0660] The dual modified CLEC12A and SLC34A2 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of CLEC12A and SLC34A2 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 9: T Cells Expressing IL1RAP Chimeric Antigen Receptor and SLC2A2 Chimeric Inhibitory Receptor

[0661] A T cell is genetically modified to express a CAR that recognizes the AML antigen IL1RAP and an inhibitory CAR that recognizes the non AML-antigen SLC2A2. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate , IL1RAP antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, IL1RAP CARs are mixed at a 1:1 ratio with an AML cell line expressing IL1RAP; a SLC2A2+ expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an IL1RAP CAR proliferate and produce effector cytokines in response to IL1RAP antigen alone.

[0662] Next, the dual modified T cells are co-incubated with cells expressing the cognate IL1RAP antigen and cells expressing the cognate SLC2A2 antigen. T cell activation is

characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate IL1RAP antigen are killed, while the cells expressing SLC2A2 antigen only are not killed.

[0663] The dual modified IL1RAP and SLC2A2 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of IL1RAP and SLC2A2 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 10: T Cells Expressing CD33 Chimeric Antigen Receptor and TRPM1 Chimeric Inhibitory Receptor

[0664] A T cell is genetically modified to express a CAR that recognizes the AML antigen CD33 and an inhibitory CAR that recognizes the non AML-antigen TRPM1. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate CD33 antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, CD33 CARs are mixed at a 1:1 ratio with an AML cell line expressing CD33; a TRPM1+ expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an CD33 CAR proliferate and produce effector cytokines in response to CD33 antigen alone.

[0665] Next, the dual modified T cells are co-incubated with cells expressing the cognate CD33 antigen and cells expressing the cognate TRPM1 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate CD33 antigen are killed, while the cells expressing TRPM1 antigen only are not killed.

[0666] The dual modified CD33 and TRPM1 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of CD33 and TRPM1 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 11: T Cells Expressing SLC22A16 Chimeric Antigen Receptor and SCTR Chimeric Inhibitory Receptor

[0667] A T cell is genetically modified to express a CAR that recognizes the AML antigen SLC22A16 and an inhibitory CAR that recognizes the non AML-antigen SCTR. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate SLC22A16 antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, SLC22A16 CARs are mixed at a 1:1 ratio with an AML cell line expressing SLC22A16; a SCTR + expression line; or a negative cell line as

control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an SLC22A16 CAR proliferate and produce effector cytokines in response to SLC22A16 antigen alone.

[0668] Next, the dual modified T cells are co-incubated with cells expressing the cognate SLC22A16 antigen and cells expressing the cognate SCTR antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate SLC22A16 antigen are killed, while the cells expressing SCTR antigen only are not killed.

[0669] The dual modified SLC22A16 and SCTR CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of SLC22A16 and SCTR CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 12: T Cells Expressing PIEZO1 Chimeric Antigen Receptor and KCNQ2 Chimeric Inhibitory Receptor

[0670] A T cell is genetically modified to express a CAR that recognizes the AML antigen PIEZO1 and an inhibitory CAR that recognizes the non AML-antigen KCNQ2. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate PIEZO16 antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, PIEZO1 CARs are mixed at a 1:1 ratio with an AML cell line expressing PIEZO1; a KCNQ2+ expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an PIEZO1 CAR proliferate and produce effector cytokines in response to PIEZO1 antigen alone.

[0671] Next, the dual modified T cells are co-incubated with cells expressing the cognate PIEZO1 antigen and cells expressing the cognate KCNQ2 antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate PIEZO1 antigen are killed, while the cells expressing KCNQ2 antigen only are not killed.

[0672] The dual modified PIEZO1 and KCNQ2 CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of PIEZO1 and KCNQ2 CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 13: T Cells Expressing IL3RA Chimeric Antigen Receptor and PERP Chimeric Inhibitory Receptor

[0673] A T cell is genetically modified to express a CAR that recognizes the AML antigen IL3RA and an inhibitory CAR that recognizes the non AML-antigen PERP. To confirm that the dual modified T cell is functional, T cells are incubated with cells expressing cognate IL3RA antigen and T cell activation is characterized via killing and cytokine production assays as described in **Example 3**. Briefly, IL3RA CARs are mixed at a 1:1 ratio with an AML cell line expressing IL3RA; a PERP + expression line; or a negative cell line as control. T cells and cell lines are co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency is assessed via flow cytometry. Unmodified T cells are used as a negative control. Dual modified T cells expressing an IL3RA CAR proliferate and produce effector cytokines in response to IL3RA antigen alone.

[0674] Next, the dual modified T cells are co-incubated with cells expressing the cognate IL3RA antigen and cells expressing the cognate PERP antigen. T cell activation is characterized via the killing and cytokine production assays as described above. The cells expressing only the cognate IL3RA antigen are killed, while the cells expressing PERP antigen only are not killed.

[0675] The dual modified IL3RA and PERP CAR T cells are also characterized i*n vivo* as described in **Example 4**. Infusion of IL3RA and PERP CAR T cells reduces xenografted tumor cell burden and growth in treated mice as compared to untreated mice.

Example 14: Expression of FLT3, CD33, and CLEC12A in AML cells

[0676] *Materials and Methods*

[0677] Bioinformatics analyses identified FLT3, CD33, and CLEC12A as highly expressed in AML. A microarray database was constructed by downloading and normalizing >1100 AML patient-derived and healthy hematopoietic cell samples from NCBI GEO. Using this database, genes with high transcriptional expression in AML cells as compared to healthy hematopoietic cells were selectively identified.

[0678] The bioinformatics expression results were confirmed using flow cytometry to analyze FLT3, CD33, and CLEC12A protein levels on the cell surface of AML cells. Cryopreserved AML patient-derived bone marrow mononuclear cells (BMMCs), peripheral blood mononuclear cells (PBMCs), as well as healthy BMMCs and PBMCs (as controls) were thawed on ice and then stained with antibodies recognizing human FLT3, CD33, and CLEC12A proteins. Cells were washed, stained to differentiate between live/dead cells, and analyzed using a Cytoflex flow cytometer and FlowJo software.

[0679] *Results*

[0680] FLT3, CD33, and CLEC12A are highly expressed in AML patient samples as compared to healthy hematopoietic cells. **FIG. 39** shows a heatmap of microarray-based gene expression database containing >1100 healthy and AML patient samples. Cell population in order from top to tottom are: AML -5/7(q), AML -9q, AML+8, AML abn(3q), AML Blast, AML BMMC NK, AML Complex, AML flt3 itd MUT, AML itd(16), AML inv(16)/t(16;16), AML LPC (leukemic progenitor cell), AML LSC (leukemic stem cell), AML MDS -7(q), AML MDS -Y, AML MDS complex, AML NK, AML PBMC NK, AML t(11q23), AML t(15;17), AML t(6;9), AML t(8;21), B cell, B naïve, Band cell, CD4+ T cell, CD8+ T cell, CD8+ T central cell, CD8+ T effector cell, CD8+ T naïve cell, CMP (common myeloid progenitor), Early PM (early promyelocyte), Erythroblasts, GMP (granulocyte macrophage progenitor), H UB (healthy umbilical cord blood), H WBM (healthy whole bone marrow), HSC (hematopoietic stem cell), Late PM (late promyelocyte), LMPP (lymphoid-primed multipotent progenitor), MEP (megakaryocyte erythrocyte progenitor), MM (metamyelocyte), Monocytes, MPP (multipotent progenitor), MY (myelocyte), Neutrophils, PM (promyelocyte), PMN (polymorphonuclear cell), Pre-B cells, Pro-B cells.

[0681] The average normalized expression (log2 expression scale) of FLT3, CD33, and CLEC12A in the indicated primary cells and cell lines are also shown in **Table 4**. The numbers in brackes indicate the number of samples per group.

Table 4

	AML Samples																	
Gene Symbol	AML +8 [19]	AML -5/7(q) [28]	AML -9q [6]	AML BMMC NK [399]	AML Blast [21]	AML Complex [58]	AML LPC [23]	AML LSC [28]	AML NK [9]	AML PBMC NK [32]	AML abn(3q) [2]	AML flt3 itd MUT [1]	AML idt(16) [33]	AML inv(16)/t(16;16) [28]	AML t(11q23) [48]	AML t(15;17) [58]	AML t(6;9) [6]	AML t(8;21) [77]
FLT3	10.0	10.3	9.7	9.6	9.1	8.8	9.8	10.3	9.7	9.3	9.8	10.5	9.9	9.7	9.8	9.6	10.5	9.8
CD33	8.4	8.1	8.5	9.0	8.5	8.5	8.4	8.5	8.6	9.0	9.1	9.5	9.0	9.1	9.2	9.3	8.9	7.9
CLEC12A	7.4	7.5	8.4	8.6	9.3	7.4	8.9	7.6	6.8	7.9	8.1	9.0	8.9	9.2	8.2	7.7	8.3	9.2
Healthy Hematopoietic Cells																		

	Hematopoietic stem cell (HSC) [22]	Lymphoid-primed multipotent progenitor (LMPP) [7]	Late promyelocyte (Late PM) [3]	Megakaryocyte erythrocyte progenitor (MEP) [15]	Metamyelocyte (MM) [3]	Multipotent progenitor (MPP) [17]	Myelocyte (MY) [4]	Monocyte [8]	Neutrophil [3]	Promyelocyte (PM) [2]	Polymorphonuclear cell (PMN) [3]	Pre-B cell [2]	Pro-B cell [2]	Common myeloid progenitor (CMP) [16]	Early promyelocyte (Early PM) [3]	Erythroblast [7]	Granulocyte macrophage progenitor (GMP) [18]	Healthy umbilical cord blood (H UB) [3]
FLT3	10.0	9.9	7.3	6.0	4.1	9.4	5.8	6.5	5.3	7.6	4.6	7.0	8.9	7.9	8.7	4.7	9.6	9.8
CD33	7.2	8.1	7.9	5.8	7.7	7.1	8.5	10.0	6.6	9.1	6.6	9.4	6.3	7.4	8.0	5.2	8.2	8.0
CLEC12A	4.7	8.3	11.1	5.7	12.0	5.6	11.0	10.6	8.4	10.4	9.0	9.3	5.7	7.6	10.6	4.6	8.6	4.7
	Healthy whole bone marrow (H WBM)	B cell [4]	B naïve [2]	Band cell [4]	CD4 T cell [3]	CD8 T cell [6]	CD8 T central [3]	CD8 T effector [8]	CD8 T naïve [4]	Healthy whole bone marrow (H WBM)	B cell [4]	B naïve [2]	Band cell [4]	CD4 T cell [3]	CD8 T cell [6]	CD8 T central [3]	CD8 T effector [8]	CD8 T naïve [4]
FLT3	6.7	5.2	5.4	4.3	5.4	5.3	5.3	5.4	5.7	6.7	5.2	5.4	4.3	5.4	5.3	5.3	5.4	5.7
CD33	8.6	6.2	5.4	6.4	7.4	6.0	6.1	6.5	6.6	8.6	6.2	5.4	6.4	7.4	6.0	6.1	6.5	6.6
CLEC12A	9.7	5.7	5.6	11.7	7.2	4.9	4.7	4.6	4.7	9.7	5.7	5.6	11.7	7.2	4.9	4.7	4.6	4.7
Endothelial Cells																		
	Human aortic endothelial cell (HAEC) [2]	Human coronary artery endothelial cell (HCAEC) [2]	Human Microvascular Endothelial Cells (HMVEC)_qui [3]	Human Umbilical Artery Endothelial Cell (HUAEC) [5]	Human umbilical vein endothelial cell (HUVEC) [5]	Human Pulmonary Artery Endothelial Cell (PAEC) [6]	Pulmonary microvascular endothelial cell (PMVEC) [6]	Human Umbilical Artery Endothelial Cell (HUAEC)_fresh [4]	Human umbilical vein endothelial cell (HUVEC)_fresh [4]	Human umbilical vein endothelial cell (HUVEC)_primary [2]								
FLT3	4.4	4.6	4.4	4.4	5.1	4.6	4.6	4.3	4.7	4.9								
CD33	5.6	5.4	5.6	5.7	6.0	5.2	5.5	5.5	5.6	5.4								
CLEC12A	4.6	4.5	4.1	3.9	4.3	4.3	4.2	4.1	4.1	4.4								

[0682] Flow cytometry analysis of 4 representative AML patient bone marrow mononuclear cells (BMMC) samples demonstrates that a majority of AML blasts express FLT3 and/or CD33 protein (data not shown). Thus, FLT3 "OR" CD33 logic gated CARs can broadly target AML blast populations. Flow cytometry analysis of 4 representative AML patient bone marrow mononuclear cells (BMMC) samples demonstrates that a majority of AML blasts express FLT3 and/or CLEC12A protein (data not shown). Thus, FLT3 "OR" CLEC12A logic

gated CARs can broadly target AML blast populations. Flow cytometry analysis of 2 representative AML patient bone marrow mononuclear cells (BMMC) samples demonstrates that a majority of AML leukemic stem cell (LSC) - enriched population expresses FLT3, or, FLT3 and CD33 protein. Thus, FLT3 "OR" CD33 logic gated CAR target can target AML LSC-enriched populations.

Example 15: *In vitro* characterization of FLT3 CAR T Cells

[0683] *Materials and methods*

[0684] *Cell Surface Expression*

[0685] Cell surface expression of FLT3 in MOLM-13, THP-1, and SEM cells was determined by flow cytometry. Cells were stained with a human FLT3 antibody (conjugated to PE-Cy7, clone: BV10A4H2; Biolegend). An isotype control was used as a negative control for background antibody staining. A ratio was determined between the negative and FLT3+ population, confirming MOLM-13, THP-1, and SEM all robustly express the FLT3 receptor.

[0686] *T cell Assays*

[0687] T cells from 1 donor were genetically modified to express CARs with three different FLT3 scFv polypeptides. The FLT3 scFvs used were ml0006, NC7 (SB00819), and D4-3 (SB00816). The CARs had a CD8 signal sequence, a CD8 hinge, a CD8 transmembrane domain, a 4-1BB co-stimulatory domain and a CD3ζ signaling domain. Each also had a YFP tag on the C terminus.

[0688] Primary T cells were iolated from PBMCs and frozen. T cells were thawed and activated with Human T-Activator CD3/CD28 Dynabeads and cultured in CTS OpTmizer T Cell Expansion medium with IL-2 overnight. Next, T cells were transduced with a CAR lentivirus containing a selected CAR vector. CAR expression was assessed via antibody staining and flow cytometry. On day 9 after transduction, T cells and target cells were col-cultured for a cytotoxicity assay (ET ratio: 1:1, 96-well plate, 200ul total medium volume). The target cells were stained with CellTrace Violet dye to distinguish with T cells.

[0689] For cytokine production assays, supernatant was collected after a 5 or 18-hour co-culture and stored at -80 degree for Luminex assay.

[0690] For cytotoxicity assays, cells were collected after a 18-20 hour incubation and stained with propidium iodide cell viability dye to distinguish live/dead target cells. Cytotoxicity was assessed by flow cytometry and the data analyzed by FlowJo. The cytotoxicity activity was normalized to a no virus T cell control. In certain cases, data was also normalized with a K562 cytotoxicity control..

[0691] The protein and nucleotide sequences of the D4-3 (SB00816) and NC7 (SB00819) FLT3 CARs made are shown in **Table 5**.

Table 5

SEQ ID NO	Name	Sequence
153	CD8ss_FLT3(D4-3)_CD8hinge_CD8TM_41BB ICD_CD3z_YFP SB00816	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSP QLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGSGG GGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTSYAQ KFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSTTTPAPRPPTPAPT IALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSG TGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYG LQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNIL GHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSAL SKDPNEKRDHMVLLEFVTAAGITLGMDELYK
154	CD8ss_FLT3(D4-3)_CD8hinge_CD8TM_41BB ICD_CD3z_YFP SB00816	ACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAATTGTCT AGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCAGACTACT GGGGTCAGGGTACACTTGTGACGGTAAGCAGCACCACGACGCCGGCGCCCCGGCCTCCCACCCCCGCACC AACGATAGCCCTTCAGCCCTTGAGCCTCCGGCCAGAAGCATGCCGCCCGGCAGCCGGAGGTGCAGTCCAT ACGCGCGGACTGGACTTTGCATGTGACATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGC TTTTGTCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCG GCCTGTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGT GAACTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATA ACGAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAAT GGGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGG GACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAG TGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGAC GGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGA CCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTA CGGCCTcCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCC GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGA AGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACAT CCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAAC GGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACC AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGC CCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATC ACTCTCGGCATGGACGAGCTGTACAAG
155	CD8ss_FLT3(NC7)_CD8hinge_CD8TM_41BB ICD_CD3z_YFP SB00819	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVT VSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAS SLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKTTTPAPRPPTPAPTI ALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPV QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGT GMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGL QCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILG HKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALS KDPNEKRDHMVLLEFVTAAGITLGMDELYK
156	CD8ss_FLT3(NC7)_CD8hinge_CD8TM_41BB ICD_CD3z_YFP	CAGAAACCTGGGAAAGCGCCGAAGCTCCTTATCTATGCTGCCAGCTCTTTGCAAAGCGGTGTGCCCTCAC GGTTCTCCGGTAGTGGGTCCGGGACCGACTTCACTTTGACCATCAGCAGCCTTCAGCCAGAGGATCTTGC CACTTATTACTGCCAGCAATCTTATAGCACACCGTTTACATTCGGTCCAGGCACAAAGGTAGACATTAAG ACCACGACGCCGGCGCCCCGGCCTCCCACCCCCGCACCAACGATAGCCCTTCAGCCCTTGAGCCTCCGGC CAGAAGCATGCCGCCCGGCAGCCGGAGGTGCAGTCCATACGCGCGGACTGGACTTTGCATGTGACATCTA CATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACGAAGCGCGGTCGA AAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAAGAAGAGGACGGGT GTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTTAGCAGGTCAGCGGA CGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCAGGGAAGAATAC

	nucleotide	GATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGGAAAAACCCACAGG AGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATAGGAATGAAGGGTGA ACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACGAAGGATACTTATGAT GCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGC TGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTC CGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTG CCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAGTGCTTCGCCCGCTACCCCGACC ACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACA GCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAA CATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTG CTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATC ACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG

[0692] The FLT3 CAR T cells were assessed for killing activity *in vitro*. FLT3 CAR with the NC7 or D4-3 FLT3 scFvs were mixed at a 1:1 ratio with MOLM-13, THP-1, or SEM cells. T cells and cell lines were co-cultured for 18 hours, and then stained with live/dead stain. Unmodified T cells were used as a negative control. Killing efficiency was assessed via flow cytometry. Final killing efficiency was calculated by normalizing killing to an FLT3-negative K562 cell line. A dose response assay was also performed. The NC7 FLT3 CAR was mixed with MOLM-13 cells at increasing effector to target cell ratios (1:9, 1:3, 1:1, 3:1, and 9:1 E:T ratios) and the percent killing efficiency was assessed via flow cytometry as previously described.

[0693] The FLT3 CAR T cells were assessed for cytokine production *in vitro*. All three FLT3 CARs (ml006, NC7, and D4-3) were individually mixed at a 1:1 ratio with the AML cell lines MOML-13, MOLM-14, or Eol 1. T cells and cell lines were co-cultured for 18 hours and the supernatants collected for cytokine quantification using the Luminex multiplexed assays. In all cases the cytokine levels were compared to those of an unengineered T cell negative control.

[0694] *Results*

[0695] NC7 (SB00819) and D4-3 (SB00816) FLT3 CAR T cells show potent cytotoxicity activity against human leukemia cell lines MOLM-13, THP-1, and SEM. **FIG. 40A** shows the flow cytometry histogram plots showing expression of FLT3 protein expression (x-axis) in the leukemia cell lines. Two FLT3 CAR T cells killed MOML-13 and THP-1 human leukemia cells (E:T ratio of 1:1), as well as SEM cells expressing FLT3 (**FIG. 40B**).

[0696] **FIG. 40C** shows a dose dependent FLT3 CAR T cell killing of MOLM-13 AML cells. As the ratio of FLT3 T cells increased (E, effector cells) as compared to MOLM-13 cells (T, target cells), the percent killing increased. Thus, FLT3 CAR T cells demonstrate potent in vitro killing activity against human leukemia cells.

[0697] In addition, the two of the three FLT3 CAR T cells secreted cytokines when incubated with various FLT3 expressing AML cell lines. The NC7 FLT3 CAR and D4-3 FLT3 CAR both secreted TNFα, IFN-γ, and IL-2 when incubated with MOLM-14 cells (**FIG. 41A**), MOLM-13 cells (**FIG. 41B**), and Eol 1 cells (**FIG. 41C**). Thus, FLT3 CAR T cells demonstrated potent reactivity against various human AML tumor cell lines.

Example 16: *In vitro* characterization of CD33 CAR T Cells

[0698] *Materials and methods*

[0699] *Cell surface expression*

[0700] Expression of the CD33 receptor was confirmed MOLM-13, MV4-11, and THP-1 cells using flow cytometry. An isotype control was used as a negative control for background antibody staining, and cells were stained with a human CD33 antibody (conjugated to BV421, clone: WM53; Biolegend), and a ratio was determined between the negative and CD33+ population.

[0701] *T cell assays*

[0702] T cells from 1 donor were genetically modified to express CARs with two different CD33 scFv polypeptides. The CD33 scFvs used were derived from the hu195 (lintuzumab) and Mylo (gemtuzumab) CD33 antibodies. The CARs had an IgK signal sequence, a FLAG tag, a CD28 hinge, a CD28 transmembrane domain, a CD28 intracellular co-stimulatory domain, and a CD3ζ signaling domain. The protein and nucleotide sequences of the hu195 and Mylo CD33 CARs made are shown in Table 6.

Table 6

SEQ ID NO	Name	Sequence
157	IgKss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z SB01052	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNM HWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAM DYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNWFQ QKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFGQGTKVEI KSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFII FWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELN LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR
158	IgKss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z SB01052	ATGGAAACGGATACTCTGCTGCTGTGGGTCCTCTTGCTTTGGGTACCTGGGAGTACCGGCGCTGGCGGG TCCGATTACAAGGACGATGACGACAAAGGGGGTTCTCAGGTTCAGCTGGTTCAGTCTGGCGCCGAAGTG AAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGCGGCTACACCTTTACCGACTACAACATG CACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGATCGGCTACATCTACCCCTACAATGGCGGC ACCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCATCACCGCCGACGAGAGCACAAACACCGCCTAC ATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCCGTGTACTACTGTGCCAGAGGCAGACCCGCCATG GATTATTGGGGACAGGGCACCCTGGTCACCGTTTCTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGT GGCGGAGGCGGTTCTGATATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGA GTGACCATCACCTGTAGAGCCAGCGAGAGCGTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCAG CAGAAGCCCGGCAAGGCCCCTAAGCTGCTGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGC AGATTTTCCGGCTCTGGCAGCGGCACCGATTTCACCCTGACAATCTCTAGCCTCCAGCCTGACGACTTC GCCACCTACTACTGCCAGCAGAGCAAAGAGGTGCCCTGGACATTCGGCCAGGGCACAAAGGTGGAAATC

		AAGAGCGGAGCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGG ACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCTTTC TGGGTGCTTGTGGTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCCTTCATCATT TTCTGGGTCAGGAGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCTCGCAGACCC GGACCTACGCGGAAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGAAGTAGGGTC AAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAAT TTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAG CCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATAC TCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCA ACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC
159	IgKss_Flag_CD33(Mylo)_CD28 hinge_CD28 TM_CD28 ICD_CD3z SB01056	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSEVQLVQSGAEVKKPGSSVKVSCKASGYTITDSNI HWVRQAPGQSLEWIGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDTAFYYCVNGNPWL AYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWFQ QKPGKAPKLLMYAASNQGSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQTKEVPWSFGQGTKVEV KRTSSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVA FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR
160	IgKss_Flag_CD33(Mylo)_CD28 hinge_CD28 TM_CD28 ICD_CD3z SB01056	ATGGAAACGGATACTCTGCTGCTGTGGGTCCTCTTGCTTTGGGTACCTGGGAGTACCGGCGCTGGCGGG TCCGATTACAAGGACGATGACGACAAAGGGGGTTCTGAAGTGCAGCTGGTTCAGTCTGGCGCCGAAGTG AAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGCGGCTACACCATCACCGACAGCAACATC CACTGGGTCCGACAGGCTCCAGGCCAGTCTCTTGAGTGGATCGGCTACATCTACCCCTACAACGGCGGC ACCGACTACAACCAGAAGTTCAAGAACCGGGCCACACTGACCGTGGACAACCCTACCAATACCGCCTAC ATGGAACTGAGCAGCCTGCGGAGCGAGGACACCGCCTTTTACTACTGCGTGAACGGCAACCCCTGGCTG GCCTATTGGGGACAGGGAACACTGGTCACAGTGTCTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGT GGCGGAGGCGGTTCTGATATTCAGCTGACACAGAGCCCCAGCACACTGTCTGCCTCTGTGGGCGACAGA GTGACCATCACCTGTAGAGCCAGCGAGAGCCTGGACAACTACGGCATCAGATTTCTGACCTGGTTCCAG CAGAAGCCCGGCAAGGCTCCTAAGCTGCTGATGTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGC AGATTTTCCGGCTCTGGCAGCGGCACAGAGTTCACCCTGACAATCTCTAGCCTCCAGCCTGACGACTTC GCCACCTACTACTGCCAGCAGACCAAAGAGGTGCCCTGGTCCTTTGGACAGGGCACCAAGGTGGAAGTG AAGCGGACTAGCTCCGGAGCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAG AGTAATGGGACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCT AAACCTTTCTGGGTGCTTGTGGTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCC TTCATCATTTTCTGGGTCAGGAGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCT CGCAGACCCGGACCTACGCGGAAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGA AGTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAAC GAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATG GGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAG GGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC

[0703] The CD33 CAR T cells were assessed for killing activity *in vitro*. CD33 CARs with the hu195 (SB01052) or Mylo (SB01056) CD33 scFvs were mixed at a 1:1 ratio with MOLM-13, MV4-11, or THP-1 cells. T cells and cell lines were co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency was assessed via flow cytometry. Unmodified T cells were used as a negative control. A dose response assay was also performed with both CD33 CARs in the MOLM-13 and MV4-11 cell lines. The CD33 CARs were mixed with MOLM-13 or MV4-11 cells cells at increasing effector to target cell ratios (1:3, 1:2, 1:1, 2:1, and 3:1 E:T ratios) and the percent killing efficiency was assessed via flow

cytometry as previously described. Final killing efficiency was calculated by normalizing killing to a CD33-negative K562 cell line

[0704] The CD33 CAR T cells were assessed for cytokine production *in vitro*. Both CD33 CARs were individually mixed at a 1:1 ratio with the AML cell lines MOML-13, MOLM-14, or Eol 1. T cells and cell lines were co-cultured for 18 hours and the supernatants collected for cytokine quantification using the Luminex multiplex assay. In all cases the cytokine levels were compared to those of an unengineered T cell negative control.

[0705] *Results*

[0706] CD33 CAR T cells show potent cytotoxicity activity in vitro against multiple human acute myeloid leukemia (AML) cell lines. **FIG. 42A** shows flow cytometry histogram plots of cell surface expression of CD33 protein expression in the AML cell lines MOLM-13, MV4-11, and THP-1. **FIG. 42B** shows two different CD33 CAR T cells kill human AML cells in *in vitro* co-culture cytotoxicity assays. In addition, both CD33 antibodies induced cytotoxicity in a dose dependent manner (**FIG. 42C**). In vitro cytotoxicity assay showing increased CD33 CAR T cell killing of MOLM-13 and MV4-11 AML cell lines at higher effector to target cell (E:T) ratios.

[0707] CD33 CAR T cells demonstrates potent reactivity against multiple AML tumor cell lines. CD33 CAR T cells show potent cytokine secretion (IL-2, IFN-ɣ, TNF-α) when co-cultured with human acute myeloid leukemia (AML) cell lines, as detected by Luminex assay. **FIG. 43A** shows that the two different CD33 CAR T cells (SB01052 and SB01056) secreted IL-2, IFN-ɣ, and TNF-α after incubation with MOLM-13 cells. **FIG. 43B** shows that the two different CD33 CAR T cells (SB01052 and SB01056) secreted IL-2, IFN-ɣ, and TNF-α after incubation with MV4-11 AML cells. **FIG. 43C** shows two that the two different CD33 CAR T cells (SB01052 and SB01056) secreted IL-2, IFN-ɣ, and TNF-α after incubation with THP-1 AML cells.

Example 17: *In vitro* characterization of CLEC12A CAR T Cells

[0708] *Materials and methods*

[0709] *Cell Surface Expression*

[0710] Expression of CLEC12A in various AML, leukemia, or lymphoma cell lines (U937, THP-1, HL-60, MV-14, MOLM-14, MOLM-13, Nalm6, Raji, K562, and SEM) was determined by flow cytometry using a PE anti-human CD371 (CLEC12A) antibody (BioLegend, Clone 50C1), and a ratio was determined between the negative and CLEC12A+ population.

[0711] *T cell assays*

[0712] T cells from 1 donor were genetically modified to express CARs with three different CLEC12A scFv polypeptides. The CLEC12A scFvs used were clones 357, 378, and 161. The CARs had a CD8 signal sequence (CD8ss), a CD8 hinge, a CD8 transmembrane domain, an intracellular CD3ζ signaling domain, and a 4-1BB co-stimulatory domain (4-1BB co-stim). A YFP tag was fused to the C terminus of the CD3ζ signaling domain. The protein and nucleotide sequences of the CLEC12A CARs made are shown in Table 7.

Table 7

SEQ ID NO	Name	Sequence
162	CD8ss_Clec12A(357)_CD8hinge_CD8TM_41BBICD_CD3z_YFP SB01261	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPDYNPSLKSRVTISVDKSRNQFSLKLSSVTAADTAVYYCAKVSTGGFFDYWGQGTLVTVS SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGPGTKVEIKTTTPAPRPPTPAPTI ALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGS SGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTL GYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLS YQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
163	CD8ss_Clec12A(357)_CD8hinge_CD8TM_41BBICD_CD3z_YFP SB01261	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCGACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAGGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAAGGTTAGTACTGGTGGTTTCTTTGACTACTGGGGGCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CCAGGGACCAAGGTGGAGATCAAAACCACGACGCCGGCGCCCCGGCCTCCCACCCCCGCACCAACGATA GCCCTTCAGCCCTTGAGCCTCCGGCCAGAAGCATGCCGCCCGGCAGCCGGAGGTGCAGTCCATACGCGC GGACTGGACTTTGCATGTGACATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTG TCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCT GTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAA CTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAAC GAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATG GGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAG GGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCG AGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAG CTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG GGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC GGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAG CAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGC TACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
164	CD8ss_Clec12A(378)	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVS

	_CD8 hinge_CD8 TM_41BB ICD_CD3z _YFP SB01262	SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTI ALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGS SGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTL GYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLS YQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
165	CD8ss_Cle c12A(378) _CD8 hinge_CD8 TM_41BB ICD_CD3z _YFP SB01262	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAGGTCGTCTTCTGGTGGTTTCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CAAGGGACCAAGGTGGAGATCAAAACCACGACGCCGGCGCCCCGGCCTCCCACCCCCGCACCAACGATA GCCCTTCAGCCCTTGAGCCTCCGGCCAGAAGCATGCCGCCCGGCAGCCGGAGGTGCAGTCCATACGCGC GGACTGGACTTTGCATGTGACATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTG TCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCT GTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAA CTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAAC GAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATG GGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAG GGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCG AGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAG CTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG GGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC GGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAG CAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGC TACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
166	CD8ss_Cle c12A(161) _CD8 hinge_CD8 TM_41BB ICD_CD3z _YFP SB01263	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVS SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTI ALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGS SGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTL GYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKED GNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLS YQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
167	CD8ss_Cle c12A(161) _CD8 hinge_CD8 TM_41BB ICD_CD3z _YFP SB01263	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAGGCAGACTACTGCTGGGTCCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT

		TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CAAGGGACCAAGGTGGAGATCAAAACCACGACGCCGGCGCCCCGGCCTCCCACCCCCGCACCAACGATA GCCCTTCAGCCCTTGAGCCTCCGGCCAGAAGCATGCCGCCCGGCAGCCGGAGGTGCAGTCCATACGCGC GGACTGGACTTTGCATGTGACATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTG TCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCT GTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAA CTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAAC GAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATG GGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAG GGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCG AGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAG CTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG GGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC GGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAG CAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGC TACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

[0713] The CLEC12A CAR T cells were assessed for killing activity *in vitro*. CLEC12A CARs were mixed at a 1:1 ratio with U937, THP-1, HL-60, or MOLM-14 cells. T cells and cell lines were co-cultured for 18 hours, and then stained with live/dead stain. Killing efficiency was assessed via flow cytometry. FLT3 CAR T cells expressing SB00819 were used as a comparative benchmark for CAR T cell cytotoxicity and cytokine production. Final killing efficiency was calculated by normalizing killing to an untransduced control T cell sample and unengineered K562 basal killing control using the following formula: % killing = (1 – RatioNE) * 100%; RatioT:C = % live target cells / % live control cells; RatioNLv = Target/Control of no Lentivirus sample; RatioNE (normalized experimental) = RatioTC / RatioNLv.

[0714] The CLEC12A CAR T cells were assessed for cytokine production *in vitro*. All CLEC12A CARs were individually mixed at a 1:1 ratio with the AML cell lines U937, THP-1, HL-60, orMOLM-14. T cells and cell lines were co-cultured for 18 hours and the supernatants collected for IL-2 cytokine quantification using the Luminex multiplex assay. In all cases the cytokine levels were compared to those of an unengineered T cell negative control

[0715] *Results*

[0716] CLEC12A was expressed on the AML, ALL, lymphoma, and leukemia cell lines U937, THP-1, HL-60, MV-14, MOLM-14, MOLM-13, Nalm6, Raji, K562, and SEM. **FIG.**

44 shows flow cytometry plots of CLEC12A expression in all cell lines tested. The ratio of CLEC12A stained cells to unstained in each cell population are indicated on each graph.

[0717] SB01261 has an anti-CLEC12A scFv domain derived from the 357 CLEC12A antibody clone. SB01262 has an anti-CLEC12A scFv domain derived from the 378 CLEC12A antibody clone. SB01263 had an anti-CLEC12A scFv domain derived from the 161 CLEC12A antibody clone.

[0718] **FIG. 45A** shows flow cytometry histogram plots of cell surface expression of CLEC12A protein expression in the AML cell lines U937, THP-1, HL-60, and MOLM-14. **FIG. 45B** shows three different CLEC12A CAR T cells kill human AML cells in *in vitro* co-culture cytotoxicity assays in four different AML cell lines, U937, THP-1, HL-60, or MOLM-14. Thus, CLEC12A CAR T cells showed potent cytotoxicity activity in vitro against multiple human acute myeloid leukemia (AML) cell lines.

[0719] CLEC12A CAR T cells show potent IL-2 cytokine secretion when co-cultured with human acute myeloid leukemia (AML) cell lines, as detected by Luminex assay. **FIG. 45C** shows that three different CLEC12A CAR T cells secreted IL-2 after incubation with U937, THP-1, HL-60, or MOLM-14 cells. Thus, CLEC12A CAR T cells demonstrates potent reactivity against multiple AML tumor cell lines.

[0720] An additional CLEC12A CAR using the 378-derived scFv with a FLAG tag in place of the YFP tag (SB01161) was also made and characterized for cytotoxicity and cytokine secretion activity. The FLT3 CAR with an NC-7 scFv and YFP tag (SB00819) and the CD33 CAR with the hu195 scFv and a FLAG tag (SB01052) were used as controls. All six CARs (four CLEC12A CARs, the FLT3 CAR, and the CD33 CAR) were tested against AML cell lines with low CLEC12A expression, MOLM-13 and MV4-11; and AML cell lines with high CLEC12A expression, MOLM-14, U937, THP-1, and HL-60.

[0721] The CLEC12A CARs showed cytotoxity and IL-2 production after incubation with cell lines with low CLEC12A expression, MOLM-13 (**FIG. 46A**) and MV4-11 (**FIG. 46B**) and high CLEC12A expression, MOLM-14 (**FIG. 45C**), U937 (**FIG. 45D**), THP-1 (**FIG. 46E**), and HL-60 (**FIG. 46F**). In each figure, the number refers to the sample indicated in the figure legend.

Example 18: *In vitro* characterization of CLEC12A CAR T Cells with alternative co-stimulatory domains or secretion signal peptide

[0722] *Materials and methods*

[0723] Three CLEC12A CARs were made with a CD28 co-stimulatory domain, in replacement of the 4-1BB co-stimulatory domain as previously described and shown in Table

6. In addition, three CLEC12A CARs were made with an IgK signal sequence peptide, instead of a CD8 signal sequence peptides. Diagrams of the nine CARs with the various co-stimulatory domains or signal sequence peptides are shown in FIG. 47. The CARs with a CD8 signal sequence included a YFP tag at the C terminus. The CARs with an IgK signal sequence included a FLAG tag between the signal sequence and scFv domains.

[0724] Cells were transduced with the three previously made CLEC12A CARs and the six new CLEC12A CARs. The transduction efficiency was determined via flow cytometry after staining with FLAG antibodies (for the IgK signal sequence CARs), or via the YFP expression signal (for the CD8 signal sequence CARs). An FLT3 CAR (SB00819) and a CD33 CAR (SB01052) were used as controls.

[0725] T cell cytotoxicity and cytokine production after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells were determined as previously described in Example 17.

[0726] The protein and nucleotide sequences of the six additional CLEC12A CARs made are shown in **Table 8**.

Table 8

SEQ ID NO	Name	Sequence
168	CD8ss_Clec12A(357)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_YFP SB01561	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPDYNPSLKSRVTISVDKSRNQFSLKLSSVTAADTAVYYCAKVSTGGFFDYWGQGTLVTVS SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGPGTKVEIKAAAIEVMYPPPYLDN EKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM TPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR GSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVT TLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHY LSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
169	CD8ss_Clec12A(357)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_YFP SB01561	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCGACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAGGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAAGGTTAGTACTGGTGGTTTCTTTGACTACTGGGGGCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CCAGGGACCAAGGTGGAGATCAAAGCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAAT GAAAAGAGTAATGGGACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGT CCGTCTAAACCTTTCTGGGTGCTTGTGGTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACC GTCGCCTTCATCATTTTCTGGGTCAGGAGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATG ACTCCTCGCAGACCCGGACCTACGCGGAAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCG TACAGAAGTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTT TATAACGAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCG GAAATGGGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAG

		ATGGCGGAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTG TACCAGGGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC GGATCGAGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTC GAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC ACCCTGGGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCC GACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAG CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
170	CD8ss_Clec12A(378)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_YFP SB01562	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVS SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKAAAIEVMYPPPYLDN EKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM TPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR GSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVT TLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHY LSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
171	CD8ss_Clec12A(378)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_YFP SB01562	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAGGTCGTCTTCTGGTGGTTTCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CAAGGGACCAAGGTGGAGATCAAAGCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAAT GAAAAGAGTAATGGGACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGT CCGTCTAAACCTTTCTGGGTGCTTGTGGTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACC GTCGCCTTCATCATTTTCTGGGTCAGGAGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATG ACTCCTCGCAGACCCGGACCTACGCGGAAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCG TACAGAAGTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTT TATAACGAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCG GAAATGGGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAG ATGGCGGAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTG TACCAGGGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC GGATCGAGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTC GAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC ACCCTGGGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCC GACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAG CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
172	CD8ss_Clec12A(161)_CD28 hinge_CD28	MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEW IGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVS SGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKAAAIEVMYPPPYLDN EKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNM

	TM_CD28 ICD_CD3z _YFP SB01563	TPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR GSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVT TLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK EDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHY LSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
173	CD8ss_Cle c12A(161) _CD28 hinge_CD2 8 TM_CD28 ICD_CD3z _YFP SB01563	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGCAGGTG CAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCT GGTGGCTCCATCAGCAGTAGTAACTGGTGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGG ATTGGGGAAATCTATCATAGTGGGAGCCCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCA GTAGACAAGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTAT TACTGTGCAAGGCAGACTACTGCTGGGTCCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCG AGTGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCT CCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGC AGCTACTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGC CAAGGGACCAAGGTGGAGATCAAAGCAGCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAAT GAAAAGAGTAATGGGACTATCATTCATGTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGT CCGTCTAAACCTTTCTGGGTGCTTGTGGTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACC GTCGCCTTCATCATTTTCTGGGTCAGGAGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATG ACTCCTCGCAGACCCGGACCTACGCGGAAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCG TACAGAAGTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTT TATAACGAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCG GAAATGGGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAG ATGGCGGAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTG TACCAGGGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC GGATCGAGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTC GAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACC ACCCTGGGCTACGGCCTCCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAG TCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCC GACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAG CTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTC GTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
174	IgKss_Flag _Clec12A(357)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01168	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNW WSWVRQPPGKGLEWIGEIYHSGSPDYNPSLKSRVTISVDKSRNQFSLKLSSVTAADTAVYYCAKVSTGG FFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGPGTKVEIKA AAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
175	IgKss_Flag _Clec12A(357)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01168	ATGGAAACGGATACTCTGCTGCTGTGGGTCCTCTTGCTTTGGGTACCTGGGAGTACCGGCGCTGGCGGG TCCGATTACAAGGACGATGACGACAAAGGGGGTTCTCAGGTGCAGCTGCAGGAGTCGGGGCCAGGACTG GTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGG TGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGC CCCGACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAGGAACCAGTTCTCC CTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAAGGTTAGTACTGGTGGT TTCTTTGACTACTGGGGGCAAGGTACCCTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGT GGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA GACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAA CCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTC AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACT TACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCCAGGGACCAAGGTGGAGATCAAAGCA GCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGGACTATCATTCAT GTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCTTTCTGGGTGCTTGTG

		GTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCCTTCATCATTTTCTGGGTCAGG AGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCTCGCAGACCCGGACCTACGCGG AAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGAAGTAGGGTCAAGTTTAGCAGG TCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCAGG GAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGGAAA AACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATAGGA ATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACGAAG GATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC
176	IgKss_Flag _Clec12A(378)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01169	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNW WSWVRQPPGKGLEWIGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGG FFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKA AAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
177	IgKss_Flag _Clec12A(378)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01169	ATGGAAACGGATACTCTGCTGCTGTGGGTCCTCTTGCTTTGGGTACCTGGGAGTACCGGCGCTGGCGGG TCCGATTACAAGGACGATGACGACAAAGGGGGTTCTCAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTG GTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGG TGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGC CCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAAGAACCAGTTCTCC CTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAGGTCGTCTTCTGGTGGT TTCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGT GGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA GACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAA CCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTC AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACT TACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCAAGGGACCAAGGTGGAGATCAAAGCA GCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGGACTATCATTCAT GTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCTTTCTGGGTGCTTGTG GTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCCTTCATCATTTTCTGGGTCAGG AGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCTCGCAGACCCGGACCTACGCGG AAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGAAGTAGGGTCAAGTTTAGCAGG TCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCAGG GAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGGAAA AACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATAGGA ATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACGAAG GATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC
178	IgKss_Flag _Clec12A(161)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01170	METDTLLLWVLLLWVPGSTGAGGSDYKDDDDKGGSQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNW WSWVRQPPGKGLEWIGEIYHSGSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAG SFDYWGQGTLVTVSSGGGGSGGGGSGGGGSEIELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIKA AAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRR EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
179	IgKss_Flag _Clec12A(161)_CD28 hinge_CD2 8 TM_CD28 ICD_CD3z SB01170	ATGGAAACGGATACTCTGCTGCTGTGGGTCCTCTTGCTTTGGGTACCTGGGAGTACCGGCGCTGGCGGG TCCGATTACAAGGACGATGACGACAAAGGGGGTTCTCAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTG GTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCGTTGTCTCTGGTGGCTCCATCAGCAGTAGTAACTGG TGGAGCTGGGTCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCTATCATAGTGGGAGC CCCAACTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACAAGTCCAAGAACCAGTTCTCC CTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCCGTGTATTACTGTGCAAGGCAGACTACTGCTGGG TCCTTTGACTACTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGT GGCTCTGGCGGTGGCGGATCGGAAATTGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA GACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTACTTAAATTGGTATCAGCAGAAA CCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTC AGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACT TACTACTGTCAACAGAGTTACAGTACCCCTCCAACGTTCGGCCAAGGGACCAAGGTGGAGATCAAAGCA GCAGCTATCGAGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGGACTATCATTCAT

		GTAAAAGGGAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCTTTCTGGGTGCTTGTG GTCGTGGGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCCTTCATCATTTTCTGGGTCAGG AGCAAACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCTCGCAGACCCGGACCTACGCGG AAACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGAAGTAGGGTCAAGTTTAGCAGG TCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCAGG GAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGGAAA AACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATAGGA ATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACGAAG GATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGC

[0727] *Results*

[0728] As shown in **FIG. 48A**, all nine CLEC12A CARs were efficiently transduced into T cells. **FIG. 48B** shows the mean fluorescent intensity (MFI) of the various CARs.

[0729] The CLEC12A-CD28 CAR T cells showed higher cytotoxicity and cytokine production compared to 4-1BB versions in the various cell lines tested (**FIG. 49, 50, 51,** and **52**). In each case, the CAR construct is refered to by the shortened name, e.g, SB01561 is indicated as 1561. The CLEC12A CARs with a CD28 co-stimulatory domain (SB01561, SB01562, SB01563) demonstrated better cytotoxicity against multiple AML and leukemia cell lines, and increased cytokine secretion (IL-2, IFNg, and TNFa) as compared to the CARs with a 41BB co-stimulatory domain (SB01261, SB01262, SB01263). In additional, the IgGk signaling peptide resulted in similar or better cytotoxity and cytokine production as compared to the CD8 constructs (SB011638, SB01169, SB01170). **FIG. 49** shows the cytotoxicity of the indicated CLEC12A CAR T cells against HL-60, MOLM14, MV4-11, and MOLM-13 cells as indicated by the percent killing of the cell lines after incubation with the CAR T cells. **FIG. 50** shows the IL-2 production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells. **FIG. 51** shows the IFN-γ production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells. **FIG. 52** shows the TNF-α production of the indicated CLEC12A CAR T cells after incubation with HL-60, MOLM14, MV4-11, MOLM-13, and K562 cells.

Example 19: *In vivo* characterization of anti-FLT3 and anti-CD33 CAR T cells

[0730] *Materials and Methods*

[0731] *CAR structure*

[0732] The FLT3 CAR (SB00819) had a CD8 signal sequence, the FLT3 scFv, a CD8 hinge, a CD8 transmembrane domain, a 4-1BB co-stimulatory domain, a CD3ζ signaling domain, and a YFP tag. The FLT3 scFv was derived from FLT3 antibody NC7. The CD33 CAR (SB01052) had an IgGk signal sequence, a FLAG tag, the CD33 scFv, a CD28 hinge, a CD28

transmembrane domain, a CD28 co-stimulatory domain, and a CD3ζ signaling domain. The CD33 scFv was derived from CD33 antibody hu195 (Lintuzumab; hu195; SGN-33; humanized anti-CD33 antibody).

[0733] *In Vivo Single Dose Experiment Protocol:*

[0734] The human AML cell line MOLM-13 was chosen as the in vivo AML cell target for testing FLT3 and CD33 CAR T cells due to its robust FLT3 and CD33 surface protein expression. The human AML cell line MOLM-13 was engineered to express a firefly luciferase (fLuc or Luc) reporter (engineered cells denoted MOLM-13-Luc). After injection of these cells into NSG immunocompromised mice, MOLM-13-Luc *in vivo* tumor engraftment and growth was monitored by injecting the mice with an fLuc substrate (e.g. luciferin) and using a bioluminescence imaging system (AMY HT, by Spectral Instruments Imaging) to quantify the amount of light produced from the luciferase enzyme within the MOLM-13 cells.

[0735] $1.0x10^6$ MOLM-13-Luc AML cells were injected (IV) at Day 0. Post- MOLM-13-Luc injection, $10x10^6$ CAR+ T cells /mouse were injected (IV) at day 5, or day 8, or day 5+day12 ($10x10^6$ cells/mouse on each day). MOLM-13-Luc bioluminescence and mouse weight were assessed twice a week for up to 4 weeks. Total survival was assessed for up to 4 weeks.

[0736] The dosing regime and CAR T cells are shown in **Table 9** below.

Table 9

Group	n	AML Cell Line	T cell	T cell dose	CAR T regimen
1	3	none	Non-engineered T	10e6 T cells	5d
2	5	none	FLT3(NC7) CART(SB00819)	10e6 CAR+T cells	5d
3	5	none	CD33(hu195) CART (SB01052)	10e6 CAR+T cells	5d
4	5	MOLM-13-Luc	Non-engineered T	10e6 T cells	5d
5	5	MOLM-13-Luc	FLT3(NC7) CART (SB00819)	10e6 CAR+T cells	5d
6	5	MOLM-13-Luc	FLT3(NC7) CART(SB00819)	10e6 CAR+T cells	8d
7	5	MOLM-13-Luc	FLT3(NC7) CART(SB00819)	10e6 CAR+T cells	5d + 12d
8	5	MOLM-13-Luc	CD33(hu195) CART(SB01052)	10e6 CAR+T cells	5d
9	5	MOLM-13-Luc	CD33(hu195) CART(SB01052)	10e6 CAR+T cells	8d
10	5	MOLM-13-Luc	CD33(hu195) CART (SB01052)	10e6 CAR+T cells	5d + 12d

[0737] *Dose Escalation Materials and Methods*

[0738] For the in vivo dose escalation experiment, on day 0, NSG mice were injected (IV) with human MOLM-13-Luc AML cells (described above). On days 5, mice were injected (IV) with various concentrations of FLT3 CAR T cells or CD33 CAR T cells alone or in combination: $9x10^6$ or $18x10^6$ FLT3 CAR T cells; $0.625x10^6$, $1.25x10^6$, $2.5x10^6$, $5x10^6$, or $10x10^6$ CD33 CAR T cells; or a combination of $4.5x10^6$ FLT3 CAR T cells and $2.5x10^6$

CD33 CAR T cells, $9x10^6$ FLT3 CAR T cells and $5x10^6$ CD33 CAR T cells, or $18x10^6$ FLT3 CAR T cells and $10x10^6$ CD33 CAR T cells. fLuc bioluminescence, weight, and survival were assessed approximately twicea week for 4 weeks. Mice were sacrificed when endpoint criteria were reached. Two control groups were used: 1. Mice not injected with MOLM-13-Luc or T cells, and 2. Mice injected with $1.0x10^6$ MOLM-13-Luc cells and $10.0x10^6$ unengineered T cells.

[0739] *Results*

[0740] **FIG. 53A-J** provide quantification of the fLuc bioluminescence signal in each mouse group after treatment with FLT3 or CD33 CARs on day 5, day 8, or days 5 and 12. **FIG. 53A** shows the background fLuc bioluminescence in non-tumor bearing mice treated with CD33 CAR T cells on day 5. **FIG. 53B** shows the background fLuc bioluminescence in non-tumor bearing mice treated with FLT3 CAR T cells on day 5. **FIG. 53C** shows the fLuc bioluminescence in untreated MOLM-13-Luc tumor bearing mice. The endpoint criteria was reached on day 14 and the mice sacrificed. **FIG. 53D** shows background fLuc bioluminescence in non-tumor bearing mice treated with PBS only. **FIG. 53E** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with FLT3 CAR T cells once on day 5. **FIG. 53F** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with FLT3 CAR T cells once on day 8. The endpoint criteria was reached on day 14 and the mice sacrificed. **FIG. 53G** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with FLT3 CAR T cells twice on days 5 and 12. **FIG. 53H** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with CD33 CAR T cells once on day 5. **FIG. 53I** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with CD33 CAR T cells once on day 8. The endpoint criteria was reached on day 14 and the mice sacrificed. **FIG. 53J** shows the fLuc bioluminescence in MOLM-13-Luc tumor bearing mice treated with CD33 CAR T cells twice on days 5 and 12. Images of the fLuc bioluminsence *in vivo* in each treatment group is shown in **FIG. 53K**.

[0741] Mice treated with FLT3 CAR T cells on day 5 or on day 5 and 7 showed lower tumor burden and survived longer than mice treated with untransduced T cells. Mice treated with CD33 CAR T cells on day 5 or on day 5 and 7 showed lower tumor burden and survived longer than mice treated with untransduced T cells. Thus, both FLT3 and CD33 CAR T cells showed potent *in vivo* efficacy against MOLM-13-Luc AML cells when injected on day 5 or on days 5 + 12 after tumor initiation.

[0742] The results of the CAR T cell dose escalation study are shown in **FIG. 54**. Images of the fLuc bioluminsence *in vivo* in each treatment group are shown. The mice injected with

MOLM-13-Luc cells and unengineered T cells were sacrificed on day 16. Mice treated with $9x10^6$ FLT3 CAR T cells were sacrificed on day 23. No other treatment groups were sacrificed before the end of the time course on day 26 (**FIG. 54A**). **FIG. 54B-M** provide quantification of the fLuc bioluminescence signal in each mouse group. **FIG. 54B** shows the shows the fLuc bioluminescence in non-tumor bearing mice treated with non-engineered T cells. **FIG. 54C** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with non-engineered T cells. **FIG. 54D** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $9x10^6$ FLT3 T cells. **FIG. 54E** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $18x10^6$ FLT3 T cells. **FIG. 54F** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $0.625x10^6$ CD33 T cells. **FIG. 54G** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $1.25x10^6$ CD33 T cells. **FIG. 54H** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $2.5x10^6$ CD33 T cells. **FIG. 54I** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $5x10^6$ CD33 T cells. **FIG. 54J** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $10x10^6$ CD33 T cells. **FIG. 54K** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $4.5x10^6$ FLT3 CAR T cells and $2.5x10^6$ CD33 T cells. **FIG. 54L** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $9x10^6$ FLT3 CAR T cells and $5x10^6$ CD33 T cells. **FIG. 54M** shows the shows the fLuc bioluminescence in MOLM-13 tumor bearing mice treated with $18x10^6$ FLT3 CAR T cells and $10x10^6$ CD33 T cells.

[0743]). Mice treated with CD33 CAR T cells alone or in combination with FLT3 CAR T cells showed a significant reduction in the MOLM-13-Luc bioluminsence signal. Thus, FLT3 and CD33 CAR T cells showed potent *in vivo* activity against MOLM-13-Luc AML cells at different doses.

Example 20: *In vivo* characterization of anti-CLEC12A CAR T cells

[0744] *Materials and methods*

[0745] *CAR structure*

[0746] Six CLEC12A CARs were made using the three CLEC12A binders derived from the 357, 378, and 161 CLEC12A antibodies and either a CD28 (SB01561, SB01562, SB01563) or a 4-1BB co-stimulatory domain (SB01261, SB01262, SB01263). The CARs also had a CD8 signal sequence, a CD8 hinge, a CD8 transmembrane domain, a CD3ζ signaling

domain, and a YFP tag. The CLEC12A CARs are listed in Table 5 below. 28z refers to a CD28 co-stimulatory domain, BBz refers for a 4-1BB co-stimulatory domain. FLT3 CAR T cells with an NC7 scFv and CD33 CAR T cells with a hu195 scFv were used as controls.

[0747] *Single Dose Experiment Protocol:*

[0748] The human AML cell line MOLM-14 was chosen as the in vivo AML cell target for testing CLEC12A CAR T cells due to its robust CLEC12A surface expression (See FIG. 44-45A). MOLM-14 AML cells were engineered to express a firefly luciferase (referred to as fLuc or Luc) reporter (engineered cells denoted MOLM-14-Luc or MOLM-14-fLuc After injection of these cells into NSG immunocompromised mice, MOLM-14-Luc in vivo tumor engraftment and growth was monitored by injecting the mice with an fLuc substrate (e.g. luciferin) and using a bioluminescence imaging system (AMY HT, by Spectral Instruments Imaging) to quantify the amount of light produced from the luciferase enzyme within the MOLM-13 cells.

[0749] $1.0x10^6$ MOLM-14-Luc AML cells were injected (IV) at Day 0. Post- MOLM-14-Luc injection, $10x10^6$ CAR+ T cells /mouse were injected (IV) at day 5. MOLM-14-Luc bioluminescence and mouse weight were assessed twice a week for up to 4 weeks. Total survival was assessed for up to 4 weeks.

[0750] The dosing regime and CAR T cells used are shown in the **Table 10** below.

Table 10

Group	n	AML Cells	CAR T cell	T cell dose	CAR T regimen
1	5	MOLM-14-Luc	Non-engineered T	10e6 T cells	5d
2	5	MOLM-14-Luc	Clec12a-28z (357) CAR T (SB01561)	10.0e6 CAR+ T cells	5d
3	5	MOLM-14-Luc	Clec12a-28z (378) CAR T (SB01562)	10.0e6 CAR+ T cells	5d
4	5	MOLM-14-Luc	Clec12a-28z (161) CAR T (SB01563)	10.0e6 CAR+ T cells	5d
5	5	MOLM-14-Luc	Clec12a-BBz (357) CAR T (SB01261)	10.0e6 CAR+ T cells	5d
6	5	MOLM-14-Luc	Clec12a-BBz (378) CAR T (SB01262)	10.0e6 CAR+ T cells	5d
7	5	MOLM-14-Luc	Clec12a-BBz (161) CAR T (SB01263)	10.0e6 CAR+ T cells	5d
8	5	MOLM-14-Luc	FLT3(NC7) CAR T (SB00819)	10.0e6 CAR+ T cells	5d
9	5	MOLM-14-Luc	CD33(hu195) CAR T (SB01052)	10.0e6 CAR+ T cells	5d

[0751] *Results*

[0752] The results of the in vivo efficacy studay are shown in **FIG. 55A, 55B, and 55C**. The relative tumor bioluminsence of each mouse group are shown as a comparison of Day 7/Day5 (**FIG. 55A**) and Day 11/Day 5 (**FIG. 55B**). The fold change in the MOLM-14 tumor cell bioluminescent signal (fLuc) relative to the day 5 fLuc is shown on the y-axis. Thus, increases in tumor bioluminescence from Day 5 to Day 7 or Day 11 are shown as an increase in the luminescence signal, while decreases in tumor bioluminescence from Day 5 to Day 7 or Day 11 are shown as a decrease of the bioluminescence signal. Treatment with the CD33 and FLT3 CAR T cells reduced the tumor burden, as did the CLEC12A CAR T cells with the

CD28 co-stimulatory domain on Day 7 and Day 11 (**FIG. 55A** and **55B**). In most case, the CARs with the CD28 co-stimulatory domain were more efficacious in reducing tumor burden or extending mouse survival time than the CARs with the 4-1BB co-stimulatory domain. The 357 CLEC12A CAR with a 4-1BB co-stimulatory domain (SB01261) also resulted in reduced tumor burden on Day 7 as compared to Day 5 (**FIG. 55A**). Images of the bioluminsence *in vivo* in each treatment group is shown in **FIG. 55C**.

[0753] Thus, CLEC12A CARs with the CD28 co-stimulatory domains were more effective than the CARS with the 4-1BBz configuration at limiting MOLM-14 tumor growth.

Example 21: *In vitro* and *in vivo* FLT3 CAR hinge optimization

[0754] *Materials and Methods*

[0755] *In vitro Assays*

[0756] To optimize and increase FLT3 (D4-3) CAR T cell activity, an in vitro and in vivo functional screen of alternate hinges was performed. Efforts to optimize the FLT3 (D4-3) hinge region included: a D4-3 FLT3 CAR with a CD8 hinge (standard D4-3 hinge) (SB00816), an NC7 FLT3 CAR with a CD8 hinge (positive control for robust CAR activity) (SB00819), a D4-3 FLT3 CAR with an LNGFR (low-affinity nerve growth factor receptor) hinge (SB1076), a D4-3 FLT3 CAR with a truncated LNGFR (tLNGFR) hinge (SB1077), and a D4-3 FLT3 CAR with a PDGFR (platelet-derived growth factor receptor) hinge (SB01078) were synthesized. A diagram of the D4-3 FLT3 CARs with different hinges is shown in **FIG. 56A**.

[0757] Additional hinges were also assessed in the D4-3 FLT3 CAR. CARs with an IgG4 minimal linker (SB01071), an IgG4 minimal linker without disulfides (SB01072), an IgG4 minimal linker with enhanced disulfides (SB01073), and IgG1 minimal linker (SB01074) were synthesized. These CARs were also assessed for T cell cytotoxity and IL2, IFN-γ, and TNF-α production and compared to the original D4-3 Car sequence and the CARs with the LNGFR and PDGFR hinge sequences.

[0758] T cell cytotoxicity and cytokine production after incubation with MOLM14, MV4-11, MOLM-13, and SEM cells were determined as previously described in **Example 15**.

[0759] The protein and nucleotide sequences of the FLT3 CARs produced are shown in **Table 11**.

Table 11

SEQ ID NO	Name	Sequence
180	CD8ss_FLT3(D4-	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS

	3)_LNGFR hingeCD8 TM_41BB ICD_CD3z _YFP SB01076	GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSACPTGLYTHS GECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEPCKPCTECVGLQSMSAPCVEADDAVCRC AYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEADAECIYIWAPLAGTCGVLLL SLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGK LTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLA DHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK
181	CD8ss_FL T3(D4- 3)_LNGFR hingeCD8 TM_41BB ICD_CD3z _YFP SB01076	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGCCTGCCCGACCGGGCTCTACACTCATAGC GGGGAATGTTGTAAGGCATGTAACTTGGGTGAGGGCGTCGCACAGCCCTGCGGAGCTAACCAAACAGTG TGCGAACCCTGCCTCGATAGTGTGACGTTCTCTGATGTTGTATCAGCTACAGAGCCTTGCAAACCATGT ACTGAGTGCGTTGGACTTCAGTCAATGAGCGCTCCATGTGTGGAGGCAGATGATGCGGTCTGTCGATGT GCTTACGGATACTACCAAGACGAGACAACAGGGCGGTGCGAGGCCTGTAGAGTTTGTGAGGCGGGCTCC GGGCTGGTGTTTTCATGTCAAGACAAGCAAAATACGGTCTGTGAAGAGTGCCCTGATGGCACCTACTCA GACGAAGCAGATGCAGAATGCATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTG TCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCT GTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAA CTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAAC GAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATG GGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCG GAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAG GGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCG AGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAG CTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTG GGCTACGGCCTcCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC ATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC GGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAG CAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCC GACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGC TACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACC GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
182	CD8ss_FL T3(D4- 3)_trunc LNGFR hinge_CD 8 TM_41BB ICD_CD3z _YFP SB01077	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSACPTGLYTHS GECCKACNLGEGVAQPCGANQTVCIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQE EDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMV SKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQC FARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGH KLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALS KDPNEKRDHMVLLEFVTAAGITLGMDELYK
183	CD8ss_FL T3(D4- 3)_trunc LNGFR	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA

	hinge_CD8 TM_41BB ICD_CD3z _YFP SB01077	TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGCCTGCCCTACAGGACTCTACACGCATAGC GGTGAGTGTTGTAAAGCATGCAACCTCGGGGAAGGTGTAGCCCAGCCATGCGGGGCTAACCAAACCGTT TGCATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACGAAG CGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAAGAA GAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTTAGC AGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGC AGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGG AAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATA GGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACG AAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATGGTG AGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC CACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATC TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAGTGC TTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTC CAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAC AAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAG GCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAAC ACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGC AAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTC GGCATGGACGAGCTGTACAAG
184	CD8ss_FLT3(D4-3)_PDGFRb hinge_CD8 TM_41BB ICD_CD3z _YFP SB01078	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSAVGQDTQEVI VVPHSLPFKVIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPIL VELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFF KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIT ADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLE FVTAAGITLGMDELYK
185	CD8ss_FLT3(D4-3)_PDGFRb hinge_CD8 TM_41BB ICD_CD3z _YFP SB01078	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGCTGTGGGCCAGGACACGCAGGAGGTCATC GTGGTGCCACACTCCTTGCCCTTTAAGGTGATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTT TTGCTTTTGTCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTC ATGCGGCCTGTCCAAACAACTCAAGAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGT GGCTGTGAACTTAGGGTCAAGTTTAGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAG CTTTATAACGAATTGAATTTGGGACGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGAC CCGGAAATGGGAGGAAAGCCAAGGCGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGAT AAGATGGCGGAAGCATACTCCGAAATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGC CTGTACCAGGGACTCTCAACTGCTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCG CGCGGATCGAGTGGCACCGGTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTG GTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACC

		TACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTG ACCACCCTGGGCTACGGCCTcCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTC AAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAG ACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTC AAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACC GCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTG CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC TACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAG TTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
186	CD8ss_FL T3(D4- 3)_IgG4 min hinge_CD 8 TM_41BB ICD_CD3z _YFP SB01071	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSESKYGPPCPS CPIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVN GHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGI KANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYK
187	CD8ss_FL T3(D4- 3)_IgG4 min hinge_CD 8 TM_41BB ICD_CD3z _YFP SB01071	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGAAAGCAAGTACGGTCCACCTTGCCCTAGC TGTCCGATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACG AAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAA GAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTT AGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGA CGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGG CGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAA ATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCT ACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATG GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTG ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAG TGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATC AAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTG AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT CTCGGCATGGACGAGCTGTACAAG
188	CD8ss_FL T3(D4- 3)_IgG4 min hinge no disulfid es_CD8 TM_41BB ICD_CD3z _YFP	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSESKYGPPAPS APIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVN GHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGI

	SB01072	KANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYK
189	CD8ss_FL T3(D4-3)_IgG4 min hinge no disulfides_CD8 TM_41BB ICD_CD3z _YFP SB01072	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGAATCCAAGTACGGCCCCCCAGCGCCTAGT GCCCCAATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACG AAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAA GAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTT AGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGA CGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGG CGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAA ATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCT ACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATG GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTG ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAG TGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATC AAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTG AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT CTCGGCATGGACGAGCTGTACAAG
190	CD8ss_FL T3(D4-3)_IgG4 S228P min hinge_CD8 TM_41BB ICD_CD3z _YFP SB01073	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSESKYGPPCPP CPIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVN GHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGI KANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYK
191	CD8ss_FL T3(D4-3)_IgG4 S228P min hinge_CD8 TM_41BB ICD_CD3z _YFP SB01073	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGAATCTAAATATGGCCCGCCATGCCCGCCT TGCCCAATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACG AAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAA GAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTT

		AGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGA CGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGG CGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAA ATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCT ACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATG GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTG ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAG TGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATC AAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTG AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT CTCGGCATGGACGAGCTGTACAAG
192	CD8ss_FL T3(D4- 3)_IgG1 min hinge_CD 8 TM_41BB ICD_CD3z _YFP SB01074	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQS PQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGS GGGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSEPKSCDKTHT CPIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVN GHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGY VQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGI KANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGIT LGMDELYK
193	CD8ss_FL T3(D4- 3)_IgG1 min hinge_CD 8 TM_41BB ICD_CD3z _YFP SB01074	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACGTA GTTATGACACAGTCTCCACTGTCATTGCCAGTAACACCAGGTGAGCCCGCCTCCATCTCATGTAGATCC TCCCAATCTCTCCTTCATTCAAACGGGTATAATTATCTCGACTGGTATTTGCAGAAACCGGGCCAGAGC CCTCAACTGCTCATCTATTTGGGGAGCAACCGGGCCTCTGGTGTCCCTGATAGATTCTCCGGGAGTGGA TCAGGTACGGATTTTACACTGAAGATCAGCAGGGTGGAAGCAGAAGATGTTGGTGTGTATTACTGTATG CAATCACTCCAGACCCCGTTTACCTTTGGGCCTGGAACAAAGGTAGATATTAAAGGCGGAGGGGGATCA GGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGAAGTGCAACTTGTTCAGAGCGGGGCAGAAGTTAAGAAG CCAGGCGCTTCCGTCAAGGTGAGTTGCAAGGCAAGTGGATACACCTTTACGAGTTATTATATGCACTGG GCACGGCAGGCCCCTGGTCAGGGCCTCGAATGGATGGGGATTATAAATCCTTCTGGCGGGTCAACCAGC TACGCACAAAAATTTCAAGGTCGGGTGACAATGACGCGCGACACGTCAACGAGTACAGTGTATATGGAA TTGTCTAGCCTGAGGTCCGAGGATACTGCTGTCTATTATTGTGCTCGCGTGGTCGCTGCTGCTGTGGCA GACTACTGGGGTCAGGGTACACTTGTGACGGTAAGCAGCGAACCGAAGTCTTGTGATAAAACTCATACG TGCCCGATCTACATATGGGCCCCCCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACG AAGCGCGGTCGAAAAAAACTCCTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAA GAAGAGGACGGGTGTTCATGCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTT AGCAGGTCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGA CGCAGGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGG CGGAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAA ATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCT ACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATG GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTG ATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTcCAG TGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTAC GTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATC AAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAG AACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTG AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACT CTCGGCATGGACGAGCTGTACAAG

[0760] *In Vivo Assays*

[0761] *Mouse materials and methods*

[0762] The MOLM-13 cell line expressing firefly luciferase (fLuc or Luc) was used in the in vivo experiments as described in **Example 19**.

[0763] $1.0x10^6$ MOLM-13-Luc AML cells were injected (IV) at Day 0. Post- MOLM-13-Luc injection, $10x10^6$ CAR+ T cells /mouse were injected (IV) at day 5 ($10x10^6$ cells/mouse on each day). MOLM-13-Luc bioluminescence and mouse weight were assessed twice a week for up to 4 weeks. Total survival was assessed for up to 4 weeks.

[0764] The dosing regime and CAR T cells used are shown in the **Table 12** below.

Table 12

Group	n	AML	T cell	T cell dose	CAR T regimen
1	3	none	none	N/A	N/A
2	3	none	Non-engineered T	20e6 T cells	5d
3	5	MOLM-13-Luc	none	N/A	N/A
3	5	MOLM-13-Luc	Non-engineered T	20e6 T cells	5d
4	5	MOLM-13-Luc	FLT3(NC7 + CD8hinge) CAR T (SB00819)	19.2e6 CAR+ T cells	5d
5	5	MOLM-13-Luc	FLT3(D4-3 + CD8hinge) CAR T (SB00816)	17.2e6 CAR+ T cells	5d
6	5	MOLM-13-Luc	FLT3(D4-3 + LNGFRhinge) CAR T (SB01077)	12.4e6 CAR+ T cells	5d
7	5	MOLM-13-Luc	FLT3(D4-3 + PDGFRhinge) CAR T (SB01078)	14.4e6 CAR+ T cells	5d

[0765] *Results*

[0766] *In vitro assays*

[0767] The FLT3 hinge optimization resulted in statistically significant increase in FLT3 CAR activity when using LNGFR and PDGFR hinges (SB1077 and SB1078, respectively). The use of an LNGFR or PDGFR hinge in the D4-3 CAR resulted in increased cytotoxity and IL-2 production after incubation with MOLM-13 cells (FIG. 56B), MOLM-14 cells (FIG. 56C) MV4-11 cells (FIG. 56D), and SEM cells (FIG. 56E) as compared to the CD8 hinge. In each of FIG. 56B-E, the top panel shows the cytotoxity of each CAR after incubation with the indicated cell line and the bottom panel shows the IL-2 production of each CAR after incubation with the indicated cell line.

[0768] Similar results were observed with the other hinge sequences. **FIG. 57A** shows the percent killing, as normalized to non-transduced CAR T cells. **FIG. 57B-D** show the

production of IL-2 (**FIG. 57B**), IFN-γ (**FIG. 57C**), and TNF-α (**FIG. 57D**) by the indicated CAR T cells after incubation with MOLM-13 cells.

[0769] *In vitro assays*

[0770] FLT3 (D4-3) CARs constructed with LNGFR or PDGFR hinges demonstrated increased *in vivo* potency of the CAR T cell against MOLM-13 AML cells compared to the D4-3 FLT3 CAR with CD8 hinge, as shown by the increased mouse survival (**FIG. 58A**). **FIG. 58B** shows the bioluminescence of tumor MOLM-13 cells *in vivo* after treatment with the indicated CAR. Thus, D4-3 FLT3 CARs with LNGFR or PDGFR hinges were more effective in both *in vitro* and *in vivo* AML models than D4-3 FLT3 CAR with CD8 hinge.

Example 22: *In vitro* characterization of bicistronic FLT3 OR CD33 CAR T Cells

[0771] *Materials and methods*

[0772] Four different bicistronic FLT3 and CD33 CARs were made using a CD33 hu195 scFv and a FLT3 NC7 scFv, SB01658, SB01659, SB01530, and SB01266.

[0773] The SB01658 CAR is a CD33 "OR" FLT3 bicistronic CAR with the structure: CD8 signal sequence-FLAG-CD33(hu195)scFv-CD28 hinge-CD28 transmembrane-CD28 co-stimulatory domain-CD3ζ stimulatory domain--T2A-E2A--CD8 signal sequence-FLT3(NC7)scFv-CD8 hinge-CD8 transmembrane-4-1BB co-stimulatory domain-CD3ζ signaling domain-YFP.

[0774] The SB01659 CAR is a CD33 "OR" FLT3 bicistronic CAR with the structure: CD8 signal sequence-FLAG-CD33(hu195)scFv-CD28 hinge-CD28 transmembrane-CD28 co-stimulatory domain-CD3ζ stimulatory domain--E2A-T2A-- CD8 signal sequence-FLT3(NC7)scFv-CD8 hinge-CD8 transmembrane-4-1BB co-stimulatory domain-CD3ζ signaling domain -YFP.

[0775] The SB01530 CAR is a CD33 "OR" FLT3 bicistronic CAR with the structure: CD8 signal sequence-FLAG-CD33(hu195)scFv-CD28 hinge-CD28 transmembrane-CD28 co-stimulatory domain-CD3ζ stimulatory domain--T2A--CD8 signal sequence-FLT3(NC7)scFv-CD8 hinge-CD8 transmembrane-4-1BB co-stimulatory domain-CD3ζ signaling domain -YFP.

[0776] The SB01266 CAR is a CD33 "OR" FLT3 bicistronic CAR with the structure: CD8 signal sequence-FLAG-CD33(hu195)scFv-CD28 hinge-CD28 transmembrane-CD28 co-stimulatory domain-CD3ζ stimulatory domain--T2A--CD8 signal sequence-FLT3(NC7)scFv-CD8 hinge-CD8 transmembrane-4-1BB co-stimulatory domain-CD3ζ signaling domain -YFP SB01530 is a codon optimized version of SB01266.

[0777] The protein and nucleotide sequences of the bicistronic FLT3 and CD33 CARs produced are shown in Table 13.

Table 13

SEQ ID NO	Name	Sequence
194	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_E2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01658	MALPVTALLLPLALLLHAARPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKAS GYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTA VYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASES VDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQS KEVPWTFGQGTKVEIKSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSR SADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGEGRGSLLTCGDVEENPGPGSGQCTN YALLKLAGDVESNPGPMALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCA TFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYST PFTFGPGTKVDIKTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFS VSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQE RTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIK ANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGI TLGMDELYK*
195	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_E2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01658	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCTGCTAGACCTGCCG GCGGAAGCGACTACAAGGACGACGATGACAAAGGCGGCGGAGGATCTGGTGGCGGAGGACAGGTTCA GCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGC GGCTACACCTTTACCGACTACAACATGCACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGA TCGGCTACATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCAT CACCGCCGACGAGAGCACAAACACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCC GTGTACTACTGCGCTAGAGGCAGACCCGCCATGGATTATTGGGGCCAGGGAACCCTGGTCACCGTTT CTAGCGGAGGCGGAGGTAGTGGTGGTGGCGGTAGTGGCGGAGGTGGAAGCGATATCCAGATGACACA GAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGAGTGACCATCACCTGTAGAGCCAGCGAGAGC GTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCTAAGCTGC TGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCAC CGATTTCACCCTGACCATCAGTAGCCTGCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGC AAAGAGGTGCCCTGGACATTTGGACAGGGCACCAAGGTGGAAATCAAGAGCGGAGCCGCCGCTATCG AAGTGATGTACCCTCCTCCTTACCTGGACAACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTT GGCGGCGTGCTGGCCTGTTACTCTCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCA AGCGGAGCAGACTGCTGCACTCCGACTACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAA GCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAGA TCCGCCGATGCTCCCGCCTATAAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAATCTGGGGCGCA GAGAAGAGTACGACGTGCTGGATAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACG GAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAG ATCGGAATGAAGGGCGAACGCAGAAGAGGCAAGGGCCACGATGGACTGTATCAGGGCCTGAGCACCG CCACAAAGGACACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGAGGTTCTGGCGAGGGACG CGGGAGTCTACTGACGTGTGGAGACGTGGAGGAAAACCCTGGACCTGGCTCTGGCCAGTGCACCAAT TATGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAACCCAGGACCTATGGCACTGCCCGTCACTG CACTGCTGCTTCCGCTCGCACTTCTGCTGCATGCCGCAAGACCAGAAGTGCAGCTCGTCCAGTCAGG GGCTGAAGTGAAAAAGCCAGGCTCCTCCGTGAAAGTGTCTTGTAAAGCCTCCGGCGGCACCTTCAGC AGCTACGCCATTTCTTGGGTTCGACAAGCTCCAGGCCAGGGCCTCGAATGGATGGGAGGAATCATCC CCATCTTCGGCACCGCCAACTACGCCCAGAAATTCCAGGGACGCGTGACAATCACAGCCGACAAGTC TACCAGCACAGCTTATATGGAACTGTCTAGCCTGCGCTCCGAGGATACAGCTGTGTACTATTGTGCC ACATTCGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCTGGGGCCAAGGCACCACAGTGACAG TGTCCTCTGGCGGTGGTGGATCTGGCGGAGGCGGTTCTGGCGGCGGTGGCAGTGATATTCAAATGAC CCAGTCTCCATCCAGCCTGAGCGCCTCTGTTGGCGACAGAGTGACAATTACATGCCGGGCCAGCCAG AGCATCAGCTCCTACCTGAATTGGTATCAGCAGAAACCAGGCAAAGCTCCCAAACTCCTGATCTATG

		CTGCCTCCAGCCTGCAGAGTGGCGTGCCCTCTAGATTTTCTGGAAGCGGCTCCGGCACCGACTTTAC ACTCACCATAAGCTCCCTGCAGCCAGAAGATCTGGCCACATATTACTGTCAGCAGTCCTACAGCACC CCTTTCACATTCGGCCCAGGCACAAAAGTGGACATTAAGACCACCACACCAGCTCCTCGGCCTCCAA CTCCTGCTCCTACAATTGCTCTGCAGCCCCTGTCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGG CGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCCGGA ACATGCGGAGTGTTGCTGCTGAGCCTGGTCATCACCAAGCGGGGCAGAAAGAAGCTGCTGTACATCT TCAAGCAGCCCTTCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCC CGAGGAAGAAGAAGGCGGCTGCGAGCTGCGCGTGAAGTTTTCTAGAAGCGCTGACGCCCCTGCCTAC AAACAGGGACAAAACCAGCTCTACAATGAACTGAACCTCGGCAGACGCGAGGAATATGATGTGCTGG ACAAAAGACGCGGCAGGGACCCTGAAATGGGAGGGAAGCCTCGGCGGAAAAACCCACAAGAAGGACT GTATAACGAACTCCAAAAGGATAAGATGGCAGAAGCCTATTCCGAGATTGGCATGAAGGGCGAGCGT CGGAGAGGAAAAGGACACGACGGCCTCTACCAGGGCCTGTCTACAGCCACCAAGGATACTTACGACG CACTCCATATGCAGGCTCTCCCACCTAGAGGCTCTAGCGGCACTGGCATGGTGTCCAAGGGCGAAGA ACTGTTCACAGGCGTGGTGCCCATCCTGGTGGAACTGGACGGGGATGTGAACGGCCACAAGTTTAGC GTTAGCGGCGAAGGCGAAGGGGATGCCACATACGGAAAGCTGACACTGAAACTGATCTGCACCACCG GCAAGCTGCCTGTGCCATGGCCTACACTGGTTACCACACTCGGCTACGGCCTGCAGTGCTTCGCCAG ATATCCCGACCATATGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGCTACGTGCAAGAG AGAACCATCTTCTTCAAAGACGACGGCAACTACAAGACCCGGGCAGAAGTGAAGTTTGAGGGCGACA CCCTCGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGATGGAAACATCCTGGGCCACAA GCTCGAGTACAACTACAACAGCCACAACGTGTACATTACCGCCGACAAGCAGAAGAACGGCATCAAG GCCAACTTCAAGATCCGGCACAACATCGAGGATGGCGGGGTGCAGCTGGCCGATCATTACCAGCAGA ATACCCCTATCGGCGACGGCCCTGTTCTGCTGCCCGATAATCACTACCTGAGCTACCAGAGCGCCCT GAGCAAGGACCCCAATGAGAAGAGGGACCACATGGTGCTGCTGGAATTCGTGACAGCCGCCGGAATC ACCCTCGGCATGGACGAACTGTACAAGTGA
196	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_E2A_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01659	MALPVTALLLPLALLLHAARPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKAS GYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTA VYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASES VDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQS KEVPWTFGQGTKVEIKSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSR SADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGQCTNYALLKLAGDVESNPGPGSGEG RGSLLTCGDVEENPGPMALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFS SYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCA TFALFGFREQAFDIWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ SISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYST PFTFGPGTKVDIKTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY KQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFS VSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQE RTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIK ANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGI TLGMDELYK*
197	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_E2A_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01659	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCTGCTAGACCTGCCG GCGGAAGCGACTACAAGGACGACGATGACAAAGGCGGCGGAGGATCTGGTGGCGGAGGACAGGTTCA GCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGC GGCTACACCTTTACCGACTACAACATGCACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGA TCGGCTACATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCAT CACCGCCGACGAGAGCACAAACACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCC GTGTACTACTGCGCTAGAGGCAGACCCGCCATGGATTATTGGGGCCAGGGAACCCTGGTCACCGTTT CTAGCGGAGGCGGAGGTAGTGGTGGTGGCGGTAGTGGCGGAGGTGGAAGCGATATCCAGATGACACA GAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGAGTGACCATCACCTGTAGAGCCAGCGAGAGC GTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCTAAGCTGC TGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCAC CGATTTCACCCTGACCATCAGTAGCCTGCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGC AAAGAGGTGCCCTGGACATTTGGACAGGGCACCAAGGTGGAAATCAAGAGCGGAGCCGCCGCTATCG AAGTGATGTACCCTCCTCCTTACCTGGACAACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTT GGCGGCGTGCTGGCCTGTTACTCTCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCA

		AGCGGAGCAGACTGCTGCACTCCGACTACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAA GCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAGA TCCGCCGATGCTCCCGCCTATAAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAATCTGGGGCGCA GAGAAGAGTACGACGTGCTGGATAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACG GAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAG ATCGGAATGAAGGGCGAACGCAGAAGAGGCAAGGGCCACGATGGACTGTATCAGGGCCTGAGCACCG CCACAAAGGACACCTATGATGCCCTGCACATGCAGGCCCTTCCACCTAGAGGTAGCGGCCAGTGTAC CAACTACGCCCTGCTGAAACTGGCCGGCGACGTGGAATCTAATCCTGGACCTGGATCTGGCGAGGGA CGCGGGAGTCTACTGACGTGTGGAGACGTGGAGGAAAACCCTGGACCTATGGCACTGCCAGTCACTG CCCTGCTGCTTCCACTTGCACTGTTGCTGCACGCCGCTAGACCAGAAGTGCAGCTCGTTCAAAGCGG AGCTGAAGTGAAAAAGCCCGGCTCCTCCGTGAAAGTGTCTTGTAAAGCCTCCGGCGGCACCTTCAGC AGCTACGCCATTTCTTGGGTTCGACAAGCTCCAGGCCAGGGCCTCGAATGGATGGGAGGAATCATCC CCATCTTCGGCACCGCCAATTACGCCCAGAAATTCCAGGGACGCGTGACAATCACAGCCGACAAGTC TACCAGCACAGCTTATATGGAACTGTCTAGCCTGCGCTCCGAGGATACAGCTGTGTACTATTGTGCC ACATTCGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCTGGGGCCAAGGCACCACAGTGACAG TGTCCTCTGGCGGTGGTGGTTCAGGTGGCGGTGGCTCTGGCGGAGGCGGTTCTGATATTCAGATGAC CCAGTCTCCATCCAGCCTGAGCGCCTCTGTTGGCGACAGAGTGACAATTACATGCCGGGCCAGCCAG AGCATCAGCTCCTACCTGAATTGGTATCAGCAGAAACCAGGCAAAGCTCCCAAACTCCTGATCTATG CTGCCTCCAGCCTGCAGAGTGGCGTGCCCTCTAGATTTTCTGGAAGCGGCTCCGGCACCGACTTTAC ACTCACCATAAGCTCCCTGCAGCCAGAAGATCTGGCCACATATTACTGTCAGCAGTCCTACAGCACC CCTTTCACATTCGGCCCAGGCACAAAAGTGGACATTAAGACCACCACACCAGCTCCTCGGCCTCCAA CTCCTGCTCCTACAATTGCTCTGCAGCCCCTGTCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGG CGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCCGGA ACATGCGGAGTGTTGCTGCTGAGCCTGGTCATCACCAAGCGGGGCAGAAAGAAGCTGCTGTACATCT TCAAGCAGCCCTTCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCC CGAGGAAGAAGAAGGCGGCTGCGAACTGCGCGTGAAGTTCTCTAGAAGCGCTGACGCCCCTGCCTAC AAACAGGGACAAAACCAGCTCTACAATGAACTGAACCTCGGCAGACGCGAGGAATATGATGTGCTGG ACAAAAGACGCGGCAGGGACCCTGAAATGGGAGGGAAGCCTCGGCGGAAAAACCCACAAGAAGGACT GTATAACGAACTCCAAAAGGATAAGATGGCAGAAGCCTATTCCGAGATTGGCATGAAGGGCGAGCGT CGGAGAGGAAAAGGACACGACGGCCTCTACCAGGGCCTGTCTACAGCCACCAAGGATACTTACGACG CACTCCATATGCAGGCTCTGCCACCACGAGGCAGCTCTGGAACTGGCATGGTGTCCAAGGGCGAAGA ACTGTTCACAGGCGTGGTGCCCATCCTGGTTGAACTGGATGGCGACGTGAACGGCCACAAGTTTAGC GTTAGCGGAGAAGGCGAAGGCGACGCCACATACGGAAAGCTGACACTGAAACTGATCTGCACCACCG GCAAGCTGCCTGTGCCATGGCCTACACTGGTTACCACACTCGGCTACGGCCTGCAGTGCTTCGCCAG ATATCCCGACCATATGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGCTACGTGCAAGAG AGAACCATCTTCTTCAAAGACGACGGCAACTACAAGACCCGGGCAGAAGTGAAGTTTGAGGGCGACA CCCTCGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGATGGAAACATCCTGGGCCACAA GCTCGAGTACAACTACAACAGCCACAACGTGTACATTACCGCCGACAAGCAGAAGAACGGCATCAAG GCCAACTTCAAGATCCGGCACAACATCGAGGATGGCGGGGTGCAGCTGGCCGATCATTACCAGCAGA ATACCCCTATCGGCGACGGCCCTGTTCTGCTGCCCGATAATCACTACCTGAGCTACCAGAGCGCCCT GAGCAAGGACCCCAATGAGAAGAGGGACCACATGGTGCTGCTGGAATTCGTGACAGCCGCCGGAATC ACCCTCGGCATGGACGAGCTGTATAAGTGA
198	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01266	MALPVTALLLPLALLLHAARPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKAS GYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTA VYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASES VDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQS KEVPWTFGQGTKVEIKSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSR SADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRRKRGSGEGRGSLLTCGDVEENPGPMAL PVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGT TVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKTTTPAP RPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLI CTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKF

		EGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADH YQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
199	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01266	ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGCTG GCGGGTCCGATTACAAGGACGATGACGACAAAGGTGGCGGAGGAAGCGGGGGAGGCGGCCAGGTTCA GCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGC GGCTACACCTTTACCGACTACAACATGCACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGA TCGGCTACATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCAT CACCGCCGACGAGAGCACAAACACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCC GTGTACTACTGTGCCAGAGGCAGACCCGCCATGGATTATTGGGGACAGGGCACCCTGGTCACCGTTT CTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGATATCCAGATGACACA GAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGAGTGACCATCACCTGTAGAGCCAGCGAGAGC GTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCTAAGCTGC TGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCAC CGATTTCACCCTGACAATCTCTAGCCTCCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGC AAAGAGGTGCCCTGGACATTCGGCCAGGGCACAAAGGTGGAAATCAAGAGCGGAGCAGCAGCTATCG AGGTGATGTATCCTCCGCCCTACCTGGATAATGAAAAGAGTAATGGGACTATCATTCATGTAAAAGG GAAGCATCTTTGTCCTTCTCCCCTTTTCCCCGGTCCGTCTAAACCTTTCTGGGTGCTTGTGGTCGTG GGTGGAGTGCTTGCGTGTTACTCCCTGCTGGTGACCGTCGCCTTCATCATTTTCTGGGTCAGGAGCA AACGATCTCGCCTCCTCCATTCTGACTATATGAACATGACTCCTCGCAGACCCGGACCTACGCGGAA ACATTACCAACCGTACGCGCCTCCGAGAGACTTCGCCGCGTACAGAAGTAGGGTCAAGTTTAGCAGG TCAGCGGACGCACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCA GGGAAGAATACGATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCG GAAAAACCCACAGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAA ATAGGAATGAAGGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTG CTACGAAGGATACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCCGCAGGAAAAGAGGAAG CGGCGAAGGTCGAGGCTCTTTGCTCACATGCGGCGATGTGGAAGAAAATCCGGGCCCAATGGCGCTC CCGGTGACAGCACTTCTCTTGCCTCTTGCCCTGCTGTTGCATGCCGCGCGCCCAGAGGTTCAACTGG TACAAAGCGGAGCCGAGGTAAAGAAACCAGGGAGTAGCGTCAAAGTGTCCTGCAAAGCCTCAGGCGG CACATTCAGTAGCTATGCTATTTCATGGGTACGCCAAGCACCAGGACAGGGGCTGGAGTGGATGGGC GGGATTATCCCCATCTTCGGTACGGCAAACTATGCACAAAAGTTCCAGGGACGAGTCACCATCACGG CTGATAAGTCCACCTCCACCGCCTATATGGAGCTGAGTTCCCTTCGGAGCGAGGATACTGCTGTGTA TTATTGTGCCACGTTCGCACTGTTCGGTTTTCGGGAGCAGGCGTTTGATATTTGGGGACAAGGCACA ACGGTCACGGTCAGTTCAGGCGGAGGGGGATCAGGGGGTGGGGGGTCAGGTGGCGGTGGAAGTGACA TTCAGATGACCCAGAGTCCCTCTTCATTGAGTGCGAGCGTCGGTGATCGGGTTACGATAACCTGTAG GGCCTCCCAAAGTATATCATCATATTTGAACTGGTACCAACAGAAACCTGGGAAAGCGCCGAAGCTC CTTATCTATGCTGCCAGCTCTTTGCAAAGCGGTGTGCCCTCACGGTTCTCCGGTAGTGGGTCCGGGA CCGACTTCACTTTGACCATCAGCAGCCTTCAGCCAGAGGATCTTGCCACTTATTACTGCCAGCAATC TTATAGCACACCGTTTACATTCGGTCCAGGCACAAAGGTAGACATTAAGACCACGACGCCGGCGCCC CGGCCTCCCACCCCCGCACCAACGATAGCCCTTCAGCCCTTGAGCCTCCGGCCAGAAGCATGCCGCC CGGCAGCCGGAGGTGCAGTCCATACGCGCGGACTGGACTTTGCATGTGACATCTACATATGGGCCCC CCTCGCCGGTACTTGCGGTGTTTTGCTTTTGTCACTGGTGATTACGAAGCGCGGTCGAAAAAAACTC CTCTACATCTTCAAACAACCTTTCATGCGGCCTGTCCAAACAACTCAAGAAGAGGACGGGTGTTCAT GCCGCTTTCCAGAGGAAGAGGAAGGTGGCTGTGAACTTAGGGTCAAGTTTAGCAGGTCAGCGGACGC ACCAGCTTACAAGCAAGGCCAAAACCAGCTTTATAACGAATTGAATTTGGGACGCAGGGAAGAATAC GATGTGCTCGATAAACGCAGAGGGAGGGACCCGGAAATGGGAGGAAAGCCAAGGCGGAAAAACCCAC AGGAGGGGTTGTACAACGAGCTTCAAAAAGATAAGATGGCGGAAGCATACTCCGAAATAGGAATGAA GGGTGAACGGAGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGACTCTCAACTGCTACGAAGGAT ACTTATGATGCTCTTCACATGCAAGCTCTGCCGCCGCGCGGATCGAGTGGCACCGGTATGGTGAGCA AGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCA CAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATC TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTCCAGT GCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTA CGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTC GAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCC TGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAA CGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCAC TACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACC AGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC CGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

200	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01530	MALPVTALLLPLALLLHAARPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSCKAS GYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTA VYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASES VDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQS KEVPWTFGQGTKVEIKSGAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVV GGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSR SADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSE IGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRRKRGSGEGRGSLLTCGDVEENPGPMAL PVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG GIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGT TVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKTTTPAP RPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKL LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLI CTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKF EGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADH YQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
201	CD8ss_Flag_CD33(hu195)_CD28 hinge_CD28 TM_CD28 ICD_CD3z_T2A_CD8ss_NC7_CD8 hinge_CD8 TM_41BB ICD_CD3z_YFP SB01530	ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCTGCTAGACCTGCCG GCGGAAGCGACTACAAGGACGACGATGACAAAGGCGGCGGAGGATCTGGTGGCGGAGGACAGGTTCA GCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAGGCCAGC GGCTACACCTTTACCGACTACAACATGCACTGGGTCCGACAGGCCCCTGGACAAGGACTTGAGTGGGA TCGGCTACATCTACCCCTACAATGGCGGCACCGGCTACAACCAGAAGTTCAAGAGCAAGGCCACCAT CACCGCCGACGAGAGCACAAACACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCC GTGTACTACTGCGCTAGAGGCAGACCCGCCATGGATTATTGGGGCCAGGGAACCCTGGTCACCGTTT CTAGCGGAGGCGGAGGTAGTGGTGGTGGCGGTAGTGGCGGAGGTGGAAGCGATATCCAGATGACACA GAGCCCCAGCAGCCTGTCTGCCAGCGTGGGAGATAGAGTGACCATCACCTGTAGAGCCAGCGAGAGC GTGGACAACTACGGCATCAGCTTCATGAACTGGTTCCAGCAGAAGCCCGGCAAGGCCCCTAAGCTGC TGATCTACGCCGCCAGCAATCAAGGCAGCGGAGTGCCTAGCAGATTTTCCGGCTCTGGCAGCGGCAC CGATTTCACCCTGACCATCAGTAGCCTGCAGCCTGACGACTTCGCCACCTACTACTGCCAGCAGAGC AAAGAGGTGCCCTGGACATTTGGACAGGGCACCAAGGTGGAAATCAAGAGCGGAGCCGCCGCTATCG AAGTGATGTACCCTCCTCCTTACCTGGACAACGAGAAGTCCAACGGCACCATCATCCACGTGAAGGG CAAGCACCTGTGTCCTTCTCCACTGTTCCCCGGACCTAGCAAGCCTTTCTGGGTGCTCGTTGTTGTT GGCGGCGTGCTGGCCTGTTACTCTCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCA AGCGGAGCAGACTGCTGCACTCCGACTACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAA GCACTACCAGCCTTACGCTCCTCCTAGAGATTTCGCCGCCTACCGGTCCAGAGTGAAGTTCAGCAGA TCCGCCGATGCTCCCGCCTATAAGCAGGGCCAGAATCAGCTGTACAACGAGCTGAATCTGGGGCGCA GAGAAGAGTACGACGTGCTGGATAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACG GAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAG ATCGGAATGAAGGGCGAACGCAGAAGAGGCAAGGGCCACGATGGACTGTATCAGGGCCTGAGCACCG CCACAAAGGACACCTATGATGCCCTGCACATGCAGGCCCTGCCACCTCGGAGAAGAAAAAGAGGCTC TGGCGAAGGCAGAGGCTCCCTGCTTACATGTGGCGACGTGGAAGAGAACCCTGGACCTATGGCACTG CCAGTCACTGCCCTGCTGCTTCCACTTGCACTGTTGCTGCACGCCGCTAGACCAGAAGTGCAGCTCG TTCAAAGCGGAGCTGAAGTGAAAAAGCCCGGCTCCTCCGTGAAAGTGTCTTGTAAAGCCTCCGGCGG CACCTTCAGCAGCTACGCCATTTCTTGGGTTCGACAAGCTCCAGGCCAGGGCCTCGAATGGATGGGA GGAATCATCCCCATCTTCGGCACCGCCAACTACGCCCAGAAATTCCAGGGACGCGTGACAATCACAG CCGACAAGTCTACCAGCACAGCTTATATGGAACTGTCTAGCCTGCGCTCCGAGGATACAGCTGTGTA CTATTGTGCCACATTCGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCTGGGGCCAAGGCACC ACAGTGACAGTGTCCTCTGGCGGTGGTGGATCTGGCGGAGGCGGTTCTGGCGGCGGTGGCAGTGATA TTCAAATGACCCAGTCTCCATCCAGCCTGAGCGCCTCTGTTGGCGACAGAGTGACAATTACATGCCG GGCCAGCCAGAGCATCAGCTCCTACCTGAATTGGTATCAGCAGAAACCAGGCAAAGCTCCCAAACTC CTGATCTATGCTGCCTCCAGCCTGCAGAGTGGCGTGCCCTCTAGATTTTCTGGAAGCGGCTCCGGCA CCGACTTTACACTCACCATAAGCTCCCTGCAGCCAGAAGATCTGGCCACATATTACTGTCAGCAGTC CTACAGCACCCCTTTCACATTCGGCCCAGGCACAAAAGTGGACATTAAGACCACCACACCAGCTCCT CGGCCTCCAACTCCTGCTCCTACAATTGCTCTGCAGCCCCTGTCTCTGAGGCCCGAAGCTTGTAGAC CTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCTCC TCTGGCCGGAACATGCGGAGTGTTGCTGCTGAGCCTGGTCATCACCAAGCGGGGCAGAAAGAAGCTG CTGTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCT GCAGATTCCCCGAGGAAGAAGAAGGCGGCTGCGAACTGCGCGTGAAGTTCTCTAGAAGCGCTGACGC CCCTGCCTACAAACAGGGACAAAACCAGCTCTACAATGAACTGAACCTCGGCAGACGCGAGGAATAT

		GATGTGCTGGACAAAAGACGCGGCAGGGACCCTGAAATGGGAGGGAAGCCTAGAAGAAAGAACCCAC AAGAAGGCCTTTACAACGAACTGCAAAAGGATAAGATGGCAGAAGCTTACTCCGAGATTGGCATGAA GGGCGAGCGTCGGAGAGGAAAAGGACACGACGGCCTCTACCAGGGCCTGTCTACAGCCACCAAGGAT ACTTACGACGCACTCCATATGCAGGCTCTCCCACCAAGAGGCAGCTCTGGCACTGGCATGGTGTCCA AGGGCGAAGAACTGTTCACAGGCGTGGTGCCCATCCTGGTGGAACTGGACGGGGATGTGAACGGCCA CAAGTTTAGCGTTAGCGGAGAAGGCGAAGGCGACGCCACATACGGAAAGCTGACACTGAAACTGATC TGCACCACCGGCAAGCTGCCTGTGCCATGGCCTACACTGGTTACCACACTCGGCTACGGCCTGCAGT GCTTCGCTAGATACCCCGACCATATGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGCTA CGTGCAAGAGAGAACCATCTTCTTCAAAGACGACGGCAACTACAAGACCCGGGCAGAAGTGAAGTTT GAGGGCGACACCCTCGTGAACCGGATCGAGCTGAAGGGCATCGACTTCAAAGAGGATGGAAACATCC TGGGCCACAAGCTCGAGTACAACTACAACAGCCACAACGTGTACATTACCGCCGACAAGCAGAAGAA CGGCATCAAGGCCAACTTCAAGATCCGGCACAACATCGAGGATGGCGGGGTGCAGCTGGCCGATCAT TACCAGCAGAATACCCCTATCGGCGACGGCCCTGTTCTGCTGCCCGATAATCACTACCTGAGCTACC AGAGCGCCCTGAGCAAGGACCCCAATGAGAAGAGGGACCACATGGTGCTGCTGGAATTCGTGACAGC CGCCGGAATCACCCTCGGCATGGACGAGCTGTATAAGTGA

[0778] *K562 Cell Engineering*

[0779] $1x10^6$ K5672 target cellswere transduced with an FLT3- or CD33-expressing lentivirus with a puromycin resistant gene. FLT3 or CD33 expression was assessed 72 hours after transduction using a Cytoflex flow cytometer. Transduced cells were selected with 4ug/ml puromycin for 3 weeks. FLT3 and CD33 expression was determeined using flow cytometry after 3-weeks of puromycin selection. The engineered cells were cultured for one week in complete medium without puromycin. Engineered cells were frozen in cryopreservation medium and stored in liquid nitrogen.

[0780] *Bicistronic CAR T Cell assay with engineered K526 cells*

[0781] T cell cytotoxicity and cytokine production after incubation with MV4-11, MOLM-13, and SEM cells were determined as previously described in **Examples 15-17**.

[0782] The monocistronic FLT3 CAR had an NC7 scFv and a 4-1BB co-stimulatory domain (SB00819). The monocistronic CD33 CAR had a hu195 scFv with a CD28 co-stimulatory domain (SB01052). The bicistronic FLT3 and CD33 CAR was SB01659 as described above.

[0783] *Results*

[0784] The SB01659 bicistronic CAR had better expression in T cells as compared to the SB01266 bicistronic CAR (**FIG. 59A**). The SB01659 bicistronic CAR also demonstrated superior cytotoxity and IL-2 production after incubation with MOLM-13 cells (**FIG. 59B**), MV4-11 cells (**FIG. 59C**), and SEM cells (**FIG. 59D**) as compared to the CD33 monocistronic Car (SB01052) and other bicistronic CARs (SB01658, SB01266, and SB01530, **FIG. 59B**, **59C**, and **59D**). In each case, the CAR construct is refered to by the shortened name, e.g, SB01659 is indicated as 1659. NoT indicates samples without added T cells, NV indicates samples with untransduced T cells. SB01266H and SB01266G were two separate preparations of the SB01266 CAR.

[0785] The K562 cells were engineered to over express either FLT3 or CD33. Unengineered K562 cells expressing neither FLT3 or CD33 are shown in **FIG. 60A**. K562 cells expressing FLT3 are shown in **FIG. 60B**. K562 cells expressing CD33 are shown in **FIG. 60C**.

[0786] The CD33 "OR" FLT3 bicistronic CAR SB01659 outperformed the CD33 and FLT3 monocistronic CARs against engineered K562 cells (**FIG. 61**). The top row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α in the FLT+, CD33- K562 cells. The middle row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α in FLT-, CD33+ K562 cells. The bottom row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α in a 1:1 mixture of FLT3+, CD33- and FLT-, CD33+ K562 cells. The negative control (-) are non-transduced T cells. In each condition, the FLT3/CD33 bicistronic CAR T cells demonstrated better activation and stimulation than the monocistronic FLT3 or CD33 CAR T cells.

[0787] The CD33 "OR" FLT3 bicistronic CAR SB01659 outperformed the CD33 and FLT3 monocistronic CARs against leukemia cell lines (**FIG. 62**). The top row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α after incubation with the MOLM-13 cells, which have high FLT3 and high CD33 expression. The middle row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α after incubation with MV4-11 cells, which have lower FLT3 expression and higher CD33 expression. The bottom row panels show the T cell cytotoxicity, IL-2 production, IFN-γ, TNF-α after incubation with SEM cells, which have higher FLT3 expression and lower CD33 expression. The negative control (-) are non-transduced T cells. In most cell lines, the FLT3/CD33 bicistronic CAR T cells demonstrated better activation and stimulation than the monocistronic FLT3 or CD33 CAR T cells, with the exception of cytotoxicity and IL-2 production in the SEM cell line. However, the biscistronic FLT3/CD33 CAR T cells still induced significant cytotoxicity in the SEM cells (greater than 50%, and greater than the monocistronic CD33 CAR T cells) and superior IFN-γ and TNF-α production in the SEM cells.

Example 23: In vitro characterization of bivalent FLT3 CAR CAR T Cells

[0788] *Materials and methods*

[0789] Two bivalent FLT3 CARs were synthesized, with either a loop scFv (SB01861) or a tandem scFv (SB01862) with the NC7 and D4-3 scFvs. The structure of the loop scFv is shown in **FIG. 63A**. The structure of the tandem scFv is shown in **FIG. 63B**.

[0790] The monovalent FLT3 CAR used as a control was SB00819 with the NC7 scFv.

[0791] T cell cytotoxicity and cytokine production after incubation with MV4-11 and SEM cells were determined as previously described in **Examples 15-17**.

[0792] The protein and nucleotide sequences of the FLT3 CARs produced are shown in **Table 14**.

Table 14

SEQ ID NO	Name	Sequence
202	CD8ss_D43 VL_linker _NC7 VH_linker _NC7 VL_linker _D43 VH_CD8 hinge_CD8 TM_41BB ICD_CD3z ICD_YFP SB01861	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQ SPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGG GSEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQG RVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQGTTVTVSSGSTSGSGKPGSG EGSTKGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKGGGGSEVQLVQSGAEVKKPGASV KVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSL RSEDTAVYYCARVVAAAVADYWGQGTLVTVSSTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVP ILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQH DFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN VYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPNEKRDH MVLLEFVTAAGITLGMDELYK*
203	CD8ss_D43 VL_linker _NC7 VH_linker _NC7 VL_linker _D43 VH_CD8 hinge_CD8 TM_41BB ICD_CD3z ICD_YFP SB01861	ATGGCTCTGCCTGTTACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCCAGACCTGACGT GGTCATGACACAGTCTCCACTGAGCCTGCCTGTGACACCTGGCGAACCTGCCAGCATCAGCTGTAGAA GCAGCCAGAGCCTGCTGCACAGCAACGGCTACAACTACCTGGACTGGTATCTGCAGAAGCCCGGCCAG TCTCCTCAGCTGCTGATCTACCTGGGCTCCAATAGAGCCAGCGGCGTGCCCGATAGATTTTCTGGCAG CGGCAGCGGAACCGACTTCACCCTGAAGATCTCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTACT GTATGCAGTCCCTGCAGACCCCTTTCACCTTCGGACCTGGCACCAAGGTGGACATCAAAGGCGGCGGA GGATCTGAGGTGCAGCTGGTTCAATCTGGCGCCGAAGTGAAGAAACCCGGCAGCTCTGTGAAGGTGTC CTGCAAAGCTAGCGGCGGCACCTTTAGCAGCTACGCCATCTCTTGGGTCCGACAGGCTCCTGGACAAG GCCTGGAATGGATGGGCGGCATCATCCCTATCTTCGGCACCGCCAATTACGCCCAGAAATTCCAGGGC AGAGTGACCATCACCGCCGACAAGAGCACAAGCACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGA GGACACCGCCGTGTACTATTGCGCCACATTCGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCT GGGGCCAGGGCACAACCGTGACAGTGTCTAGCGGCAGCACAAGCGGCTCTGGCAAACCTGGATCTGGC GAGGGAAGCACCAAGGGCGATATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGA CAGAGTGACAATTACCTGCCGGGCCAGCCAGTCCATCAGCTCCTACCTGAATTGGTATCAGCAAAAAC CTGGCAAGGCCCCTAAGCTGCTCATCTATGCCGCTAGCAGTCTGCAGAGCGGAGTGCCCTCAAGATTC AGCGGATCTGGATCCGGCACCGATTTCACACTGACCATAAGCTCACTGCAGCCCGAGGACCTGGCCAC CTACTATTGTCAGCAGTCCTACAGCACCCCGTTCACATTTGGCCCAGGCACAAAAGTCGATATCAAAG GTGGTGGCGGCAGCGAAGTCCAGCTGGTGCAAAGCGGAGCTGAAGTGAAAAAGCCAGGCGCCAGCGTG AAAGTGTCTTGCAAGGCCTCCGGCTACACATTCACCAGCTACTACATGCACTGGGCCAGACAGGCACC AGGACAGGGACTTGAGTGGATGGGCATCATCAATCCTTCCGGCGGCTCCACAAGCTACGCCCAAAAGT TTCAAGGCCGCGTGACCATGACCAGAGACACCAGCACCTCCACCGTGTATATGGAACTGTCTAGCCTG CGCTCCGAGGATACAGCCGTCTACTACTGTGCCAGAGTGGTGGCTGCTGCCGTGGCCGATTATTGGGG ACAGGGAACACTGGTCACCGTGTCCAGCACAACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCAA CCATTGCTCTGCAGCCCCTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCAT ACAAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCCCCTCTGGCTGGAACATGTGGCGTGTT GCTGCTGAGCCTGGTCATCACCAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCA TGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGC GGCTGCGAACTGAGAGTGAAGTTCAGCAGAAGCGCCGACGCTCCCGCCTATAAGCAGGGACAGAACCA GCTCTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGATAAGCGGAGAGGCAGAG ATCCTGAGATGGGCGGAAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTACAATGAGCTGCAGAAA GACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGA TGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGC CTCCAAGAGGATCTAGCGGAACAGGCATGGTGTCCAAAGGCGAGGAACTGTTCACAGGCGTGGTGCCC ATTCTGGTGGAACTGGATGGCGACGTGAACGGCCACAAGTTTAGCGTTAGCGGAGAAGGCGAAGGCGA

		CGCCACATACGGAAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAACTGCCTGTGCCTTGGCCTA CACTCGTGACCACACTCGGCTATGGCCTGCAGTGCTTCGCCAGATATCCCGACCATATGAAGCAGCAC GACTTCTTCAAGAGCGCCATGCCTGAGGGCTACGTGCAAGAGCGGACCATCTTCTTTAAGGACGACGG CAACTACAAGACCCGGGCAGAAGTGAAGTTTGAGGGCGACACCCTGGTCAACCGGATCGAGCTGAAGG GCATCGACTTCAAAGAGGACGGCAACATCCTGGGCCACAAGCTCGAGTACAACTACAACAGCCACAAC GTGTACATCACGGCCGATAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGA GGATGGCGGCGTTCAGCTGGCCGATCACTACCAGCAGAATACCCCTATCGGCGACGGACCTGTGCTGC TCCCCGATAATCACTACCTGAGCTACCAGAGCGCCCTGAGCAAGGACCCCAACGAGAAGAGGGATCAC ATGGTGCTGCTGGAATTCGTGACCGCTGCCGGCATCACCCTCGGCATGGATGAACTGTACAAGTGA
204	CD8ss_NC7 VH_linker _NC7 VL_linker _D43 VL_linker _D43 VH_CD8 hinge_CD8 TM_41BB ICD_CD3z ICD_YFP SB01862	MALPVTALLLPLALLLHAARPEVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEW MGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCATFALFGFREQAFDIWGQG TTVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKL LIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQSYSTPFTFGPGTKVDIKGGGGSGG GGSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKPGQSPQ LLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQSLQTPFTFGPGTKVDIKGGGGSG GGGSGGGGSEVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWARQAPGQGLEWMGIINPSGGSTS YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARVVAAAVADYWGQGTLVTVSSTTTPAPRPP TPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITKRGRKKLLYIF KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDK RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPRGSSGTGMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLP VPWPTLVTTLGYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNR IELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGD GPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*
205	CD8ss_NC7 VH_linker _NC7 VL_linker _D43 VL_linker _D43 VH_CD8 hinge_CD8 TM_41BB ICD_CD3z ICD_YFP SB01862	ATGGCTCTGCCTGTTACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGCTAGACCTGAAGT GCAGCTGGTTCAGTCTGGCGCCGAAGTGAAGAAACCTGGCAGCAGCGTGAAGGTGTCCTGCAAAGCTT CTGGCGGCACCTTCAGCAGCTACGCCATCTCTTGGGTTCGACAGGCCCCTGGACAAGGCCTGGAATGG ATGGGAGGCATCATCCCCATCTTCGGCACCGCCAATTACGCCCAGAAATTCCAGGGCAGAGTGACCAT CACCGCCGACAAGAGCACAAGCACCGCCTACATGGAACTGAGCAGCCTGAGAAGCGAGGACACCGCCG TGTACTACTGCGCCACATTTGCCCTGTTCGGCTTCAGAGAGCAGGCCTTCGATATCTGGGGCCAGGGC ACAACCGTGACCGTTTCTAGCGGAGGCGGAGGATCTGGTGGCGGAGGAAGTGGCGGAGGCGGTTCTGA TATCCAGATGACACAGAGCCCCAGCAGCCTGTCTGCCTCTGTGGGAGACAGAGTGACAATTACCTGCC GGGCCAGCCAGAGCATCAGCTCCTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCCTAAGCTG CTGATCTATGCTGCCTCCAGTCTGCAGAGCGGCGTGCCATCTAGATTTTCTGGCAGCGGCTCCGGCAC CGACTTCACCCTGACAATATCTAGCCTGCAGCCAGAGGACCTGGCCACCTACTACTGTCAGCAGTCCT ACAGCACCCCTTTCACCTTCGGACCTGGCACCAAGGTGGACATCAAAGGTGGTGGTGGCAGTGGTGGC GGTGGCTCAGGTGGCGGCGGATCAGGCGGTGGTGGTTCTGGCGGCGGTGGATCTGATGTGGTTATGAC CCAGTCTCCTCTGAGCCTGCCTGTGACACCTGGCGAACCTGCCAGCATCTCCTGTAGAAGCAGCCAGT CTCTGCTGCACAGCAACGGCTACAACTACCTGGATTGGTATCTCCAGAAACCAGGACAGTCCCCTCAG CTCCTCATCTACCTGGGCAGCAATAGAGCCTCTGGCGTGCCCGATAGATTCAGCGGCTCTGGAAGCGG CACAGATTTCACACTGAAGATCTCCAGAGTGGAAGCCGAGGACGTGGGCGTGTACTATTGCATGCAGA GCCTGCAGACCCCATTCACATTTGGCCCAGGCACAAAAGTCGATATCAAAGGCGGCGGAGGTTCCGGC GGTGGCGGAAGCGGAGGTGGTGGCTCTGAAGTTCAGCTCGTGCAAAGCGGAGCTGAAGTGAAAAAGCC AGGCGCCTCCGTGAAAGTGTCTTGTAAAGCCAGCGGCTACACCTTTACCAGCTACTACATGCACTGGG CCAGACAGGCACCAGGCCAGGGACTTGAGTGGATGGGCATCATCAATCCTAGCGGCGGCAGCACAAGC TACGCCCAAAAGTTTCAAGGCCGCGTGACCATGACCAGAGACACCAGCACCTCCACCGTGTATATGGA ACTGTCCTCTCTGCGGAGCGAAGATACAGCCGTGTATTATTGTGCCAGAGTGGTGGCCGCTGCCGTGG CCGATTATTGGGGACAGGGAACACTGGTCACCGTGTCCAGCACAACAACCCCTGCTCCTAGACCTCCT ACACCAGCTCCAACCATTGCTCTGCAGCCCCTGTCTCTGAGGCCAGAGGCCTGTAGACCTGCTGCTGG CGGAGCTGTGCATACAAGAGGCCTGGATTTCGCCTGCGACATCTACATCTGGGCTCCTCTGGCCGGAA CATGCGGAGTGTTGCTGCTGAGCCTGGTCATCACCAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTC AAGCAGCCCTTCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGA GGAAGAAGAAGGCGGCTGCGAACTGAGAGTGAAGTTTAGCAGAAGCGCCGACGCTCCCGCCTATAAGC AGGGACAGAATCAGCTGTACAATGAGCTGAACCTGGGGCGCAGAGAAGAGTACGACGTGCTGGATAAG CGGAGAGGCAGAGATCCTGAGATGGGCGGCAAGCCCAGACGGAAGAATCCTCAAGAGGGCCTGTATAA CGAGCTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAACGCAGAAGAG GCAAGGGCCACGATGGACTGTATCAGGGCCTGTCCACAGCCACCAAGGACACCTATGATGCCCTGCAC ATGCAGGCCCTGCCTCCAAGAGGATCTTCTGGCACAGGCATGGTGTCCAAGGGCGAAGAACTGTTCAC AGGCGTGGTGCCCATCCTGGTGGAACTGGACGGGGATGTGAACGGCCACAAGTTTAGCGTTAGCGGCG AAGGCGAAGGGGATGCCACATACGGAAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAACTGCCA GTGCCTTGGCCTACACTCGTGACCACACTCGGCTATGGCCTGCAGTGCTTCGCCAGATATCCCGACCA

		TATGAAGCAGCACGACTTCTTCAAGAGCGCCATGCCTGAGGGCTACGTGCAAGAGAGAACCATCTTCT TTAAGGACGACGGCAACTACAAGACCCGGGCAGAAGTGAAGTTCGAGGGCGACACCCTGGTCAACCGG ATCGAGCTGAAGGGCATCGACTTCAAAGAGGACGGCAACATCCTGGGCCACAAGCTCGAGTACAACTA CAACAGCCACAACGTGTACATCACGGCCGATAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCC GGCACAACATCGAGGATGGCGGCGTTCAGCTGGCCGATCACTACCAGCAGAATACCCCTATCGGCGAC GGACCTGTCCTGCTGCCTGACAATCACTACCTGAGCTACCAGAGCGCCCTGAGCAAGGACCCCAACGA GAAGAGGGATCACATGGTGCTGCTGGAATTCGTGACCGCCGCTGGCATCACCCTCGGCATGGATGAGC TGTATAAGTGA

[0793] *Results*

[0794] The novel bivalent CAR designs significantly improved killing of low target antigen density cells. The FLT3 bivalent CARs SB01861 and SB01862 killed MV4-11 cells (low FLT3 expression) at 2 to 2.5-fold greater efficiencies than the monovalent FLT3 CAR SB00819 (**FIG. 63C** upper panel).

[0795] In addition, the FLT3 bivalent CARs killed SEM cells (high FLT3 expression) with similar efficiencies as the monovalent FLT3 CAR (**FIG. 63C** lower panel). Thus, bivalent CAR design architecture can be exploited to build more potent CAR circuits with the greater capacity to respond to low target densities than monovalent CARs.

Example 24: Optimization of hinge and transmembrane domains for use with a CD28 co-stimulatory domain

[0796] *Materials and methods*

[0797] CD33 CARs with the hu195 scFv and CD8 hinge and transmembrane sequences were synthesized. The CARs also comprised a CD28 co-stimulatory domain and a CD3ζ stimulatory domain.

[0798] T cell cytotoxicity and cytokine production after incubation with MV4-11, MOLM-13, and K562 CD33 expressing cells were determined as previously described in **Examples 15-17**.

[0799] CD8 hinge and transmemebrane protein and nucleotide sequences are shown in Table 15. The protein and nucleotide sequences for CAR constructs SB01364, SB01373, and SB01374 are also provided.

Table 15

SEQ ID NO	Name	Sequence
206	CD8 Hinge Sequence 1	TTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD
207	CD8 Hinge Sequence 2 Fitzer-Attas	ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
208	CD8 Hinge Sequence 3	FVPVFLPAKPTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACD
209	CD8 TM Sequence 1	IYIWAPLAGTCGVLLLSLVIT

210	CD8 TM Sequence 2 Fitzer-Attas	IYIWAPLAGTCGVLLLSLVITLYCNHR
211	CD8 TM Sequence 3	IYIWAPLAGTCGVLLLSLVITLYCNHRN
212	GMCSFss_CD33(hu195)_CD8 hinge_CD8 TM_CD28 ICD_CD3z_YFP SB01364	MLLLVTSLLLCELPHPAFLLIPQVQLVQSGAEVKKPGSSVKVSCKASGYTFTDYNMHWVRQAPG QGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSLRSEDTAVYYCARGRPAMDYW GQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASESVDNYGISFMNW FQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQPDDFATYYCQQSKEVPWTFG QGTKVEIKSGTTTPAPRPPTPAPTIALQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP AYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGTGMVSKGEELFTGVVPILVELDG DVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPDHMKQHDFF KSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH NVYITADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSALSKDPN EKRDHMVLLEFVTAAGITLGMDELYK*
213	GMCSFss_CD33(hu195)_CD8 hinge_CD8 TM_CD28 ICD_CD3z_YFP SB01364	ATGCTCCTTCTCGTGACCTCTTTGCTTCTTTGTGAACTCCCACACCCAGCATTCCTCTTGATCC CCCAAGTACAACTCGTCCAATCCGGGGCAGAGGTCAAGAAGCCCGGGAGTTCAGTCAAAGTCTC ATGTAAAGCGTCTGGATATACTTTCACTGATTATAATATGCATTGGGTGAGACAAGCACCCGGC CAGGGGCTGGAATGGATTGGGTATATTTATCCATATAACGGTGGAACAGGGTATAATCAGAAAT TCAAATCTAAAGCTACTATTACAGCGGATGAAAGTACGAATACAGCTTATATGGAGTTGTCCTC ACTCAGGTCCGAAGATACAGCAGTATATTATTGCGCTAGGGGTCGGCCTGCAATGGACTACTGG GGCCAAGGTACTCTCGTGACTGTGTCAAGTGGCGGTGGCGGCAGCGGCGGCGGCGGCTCCGGTG GTGGTGGAAGTGACATTCAAATGACGCAATCCCCATCTAGTCTCAGCGCTTCCGTTGGGGACCG CGTAACAATTACTTGCAGGGCATCAGAATCCGTTGATAATTATGGGATTTCCTTTATGAATTGG TTTCAACAAAAGCCGGGGAAAGCACCAAAACTCCTCATTTATGCAGCTTCCAACCAGGGGTCAG GGGTCCCGTCCCGTTTCAGCGGTTCAGGGAGTGGTACAGACTTTACGCTTACAATTTCCAGTCT CCAACCCGATGATTTTGCAACATATTATTGTCAACAATCCAAGGAAGTTCCTTGGACGTTTGGG CAAGGTACCAAAGTTGAGATAAAATCTGGAACAACAACCCCTGCACCACGGCCCCCTACCCCCG CACCAACCATTGCACTCCAACCCTTGTCCTTGCGCCCCGAGGCCTGCAGGCCCGCCGCCGGTGG TGCAGTTCACACTAGGGGCTTGGACTTTGCTTGTGATATATATATATGGGCTCCACTCGCAGGG ACTTGCGGAGTCCTTTTGCTGTCACTTGTGATTACACGAAGTAAAAGATCTCGGCTTTTGCATT CAGATTATATGAATATGACTCCACGCAGGCCTGGGCCCACACGAAAACATTATCAACCGTATGC ACCCCCACGCGACTTTGCTGCTTATAGGAGCCGGGTCAAATTTTCCCGGAGCGCAGACGCCCCA GCTTACAAACAAGGTCAAAATCAACTTTATAATGAACTCAATTTGGGCCGGCGGGAAGAATATG ATGTCCTTGATAAAAGACGTGGGCGCGACCCGGAAATGGGCGGGAAACCACGTCGCAAGAACCC GCAGGAAGGTTTGTACAACGAACTCCAAAAGGATAAAATGGCTGAAGCTTATTCCGAAATAGGG ATGAAAGGTGAACGGCGCCGCGGTAAAGGCCATGACGGCTTGTATCAAGGTCTTAGTACAGCAA CAAAAGACACATACGACGCTCTCCATATGCAAGCACTCCCACCGCGCGGATCGAGTGGCACCGG TATGGTTTCTAAAGGAGAGGAGCTCTTTACTGGTGTCGTCCCTATATTGGTCGAGCTCGATGGC GACGTTAATGGTCATAAATTCAGTGTGTCAGGAGAGGGAGAAGGCGACGCTACGTATGGCAAAT TGACATTGAAGTTGATATGTACAACGGGTAAACTCCCAGTTCCCTGGCCGACGCTCGTAACAAC GCTGGGTTATGGACTTCAATGTTTTGCTCGTTACCCTGATCACATGAAACAACATGATTTCTTT AAATCTGCTATGCCCGAAGGGTATGTCCAGGAACGGACTATCTTCTTCAAAGATGATGGAAATT ATAAAACTCGCGCAGAGGTGAAATTCGAAGGGGATACTCTTGTGAATCGAATTGAACTTAAAGG TATTGATTTCAAGGAAGATGGGAACATACTCGGGCATAAACTTGAATATAATTATAACAGCCAT AATGTTTACATCACCGCAGATAAACAGAAGAATGGAATTAAAGCGAATTTTAAAATTCGCCATA ATATTGAAGACGGCGGGGTGCAACTCGCTGATCACTATCAACAAAACACTCCAATTGGAGATGG TCCGGTCCTGCTCCCGGACAACCATTATCTTTCTTATCAATCCGCTCTCTCCAAAGATCCTAAT GAAAAGCGGGACCATATGGTCTTGCTTGAGTTTGTCACTGCAGCCGGGATAACTCTGGGGATGG ATGAACTCTACAAATAA
214	GMCSFss_Flag_CD33(hu195)_Fitzer-Attas CD8 hinge_Fitzer-Attas CD8 TM_CD28 ICD_CD3z	MLLLVTSLLLCELPHPAFLLIPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVS CKASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSS LRSEDTAVYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSL QPDDFATYYCQQSKEVPWTFGQGTKVEIKSGALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPA PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRRSKR SRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR

	SB01373	
215	GMCSFss_Flag_CD33(hu195)_Fitzer-Attas CD8 hinge_Fitzer-Attas CD8 TM_CD28 ICD_CD3z SB01373	ATGTTGCTGCTGGTTACCTCTCTCCTCTTGTGTGAGCTTCCGCACCCTGCCTTTCTCCTGATAC CGGCTGGCGGATCTGACTACAAGGACGACGATGACAAAGGCGGCGGCGGGTCCGGAGGAGGTGG ACAGGTCCAGCTCGTACAGTCTGGTGCAGAAGTGAAGAAACCCGGTTCATCAGTTAAGGTTTCT TGCAAAGCCAGTGGCTACACATTTACCGACTACAACATGCACTGGGTCAGACAGGCCCCCGGCC AAGGCTTGGAGTGGATCGGGTACATATACCCTTACAATGGCGGTACTGGATACAACCAGAAATT CAAGAGCAAGGCCACGATTACCGCGGATGAGAGCACAAACACAGCCTATATGGAACTGTCATCT TTGCGAAGCGAGGACACCGCCGTTTATTATTGTGCCAGAGGACGTCCCGCGATGGATTATTGGG GTCAGGGGACACTCGTCACAGTGAGCAGCGGCGGCGGTGGCAGTGGCGGAGGAGGCTCAGGCGG CGGCGGATCTGATATTCAAATGACCCAATCACCATCTTCCCTTTCTGCTAGTGTGGGAGATAGG GTGACTATCACATGTAGAGCTAGCGAATCCGTAGACAACTACGGCATCAGCTTCATGAACTGGT TCCAGCAAAAGCCTGGCAAGGCCCCAAAGTTGCTCATTTACGCGGCCAGCAATCAAGGCAGTGG TGTGCCCAGCAGATTTTCCGGATCAGGCAGCGGAACCGATTTCACCTTGACCATTTCTTCTCTG CAGCCTGACGACTTTGCCACGTACTACTGCCAACAGTCTAAAGAGGTTCCTTGGACTTTTGGGC AGGGAACAAAAGTCGAAATAAAGTCCGGCGCCTTGAGCAACTCTATCATGTACTTTAGCCACTT CGTGCCGGTGTTTCTTCCTGCCAAGCCTACAACTACACCAGCCCCAAGACCCCCAACTCCAGCG CCAACAATCGCGTCCCAGCCCTTGTCTCTGAGACCAGAAGCCTGTAGACCCGCTGCAGGCGGAG CCGTTCATACTCGGGGACTGGATTTCGCATGCGACATTTACATCTGGGCCCCACTGGCTGGCAC GTGTGGGGTCCTGCTTCTGTCTCTGGTAATCACCCTTTATTGCAACCACAGGAGATCCAAGAGG AGCCGCCTGTTGCACTCAGACTACATGAACATGACACCTAGGCGGCCAGGTCCTACTCGAAAAC ACTATCAACCTTACGCTCCCCCTCGGGATTTCGCGGCTTACCGAAGCAGAGTGAAATTCAGCAG ATCCGCTGATGCACCGGCTTATAAGCAGGGCCAAAATCAACTGTACAACGAGCTGAATCTGGGG AGACGGGAAGAGTACGACGTCCTGGACAAGCGCAGGGGAAGAGACCCTGAGATGGGCGGGAAGC CACGTAGGAAGAACCCACAAGAGGGCCTGTATAATGAGCTGCAGAAAGACAAGATGGCAGAGGC TTACAGTGAGATTGGAATGAAGGGTGAAAGGCGGCGGGGAAAGGGCCATGACGGCCTCTATCAG GGACTGTCCACAGCAACTAAGGACACCTATGATGCACTCCACATGCAGGCCCTGCCCCCGAGA
216	CD8ss_Flag_CD33(hu195)_Fitzer-Attas CD8 hinge_Fitzer-Attas CD8 TM_CD28 ICD_CD3z SB01374	MALPVTALLLPLALLLHAARPAGGSDYKDDDDKGGGGSGGGGQVQLVQSGAEVKKPGSSVKVSC KASGYTFTDYNMHWVRQAPGQGLEWIGYIYPYNGGTGYNQKFKSKATITADESTNTAYMELSSL RSEDTAVYYCARGRPAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRV TITCRASESVDNYGISFMNWFQQKPGKAPKLLIYAASNQGSGVPSRFSGSGSGTDFTLTISSLQ PDDFATYYCQQSKEVPWTFGQGTKVEIKSGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRRSKRSRLLHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRSRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
217	CD8ss_Flag_CD33(hu195)_Fitzer-Attas CD8 hinge_Fitzer-Attas CD8 TM_CD28 ICD_CD3z SB01374	ATGGCCCTGCCCGTCACCGCATTGTTGCTCCCCCTCGCGTTGCTGCTCCACGCCGCGCGTCCCG CTGGTGGCTCTGATTATAAAGATGATGACGATAAAGGGGGGGGGCGGTAGCGGCGGTGGTGGTCA AGTCCAACTCGTGCAAAGCGGTGCTGAGGTCAAGAAGCCCGGATCAAGTGTAAAAGTAAGCTGT AAAGCTTCCGGGTATACATTCACTGATTATAATATGCATTGGGTGCGTCAAGCTCCCGGGCAGG GTCTCGAATGGATTGGGTATATATATCCTTATAACGGCGGGACAGGGTATAATCAGAAATTTAA ATCTAAAGCAACAATAACGGCGGATGAATCTACCAATACTGCTTATATGGAGCTTTCCTCTCTC CGCAGTGAAGATACTGCTGTCTATTATTGTGCACGGGGACGCCCTGCAATGGACTACTGGGGAC AAGGCACACTTGTGACAGTCAGCTCTGGTGGTGGTGGATCCGGCGGAGGAGGCTCAGGTGGTGG CGGGAGTGACATTCAAATGACTCAAAGTCCTTCCTCTCTTAGCGCAAGTGTCGGTGACCGCGTC ACAATTACGTGCCGGGCAAGTGAATCAGTCGATAATTATGGTATTTCATTTATGAATTGGTTTC AACAAAAGCCCGGAAAAGCTCCAAAACTGTTGATATATGCAGCTTCAAACCAGGGAAGTGGCGT CCCCTCACGCTTCTCTGGAAGCGGGTCTGGTACTGACTTTACTCTCACAATTTCCTCTCTCCAA CCCGATGATTTTGCAACGTATTATTGTCAACAATCCAAGGAAGTACCTTGGACCTTCGGCCAAG GGACAAAAGTTGAGATTAAATCCGGGACAACAACCCCCGCTCCGCGCCCACCCACACCAGCACC AACAATTGCATCCCAACCATTGAGTCTCAGACCCGAGGCATGCCGACCAGCCGCAGGCGGTGCA GTTCACACTCGCGGTCTCGACTTTGCGTGTGATATATATATTTGGGCACCCCTCGCCGGCACCT GCGGTGTTCTTTTGCTCTCCCTCGTGATTACTCTTTATTGTAATCATCGTAGAAGTAAAAGGTC CCGTTTGCTTCATTCTGATTATATGAATATGACTCCAAGGCGCCCTGGCCCCACACGGAAACAT TATCAACCATATGCCCCACCCCGGGACTTTGCTGCATATAGAAGCCGCGTCAAATTTTCACGGT CAGCAGACGCACCTGCTTACAAACAAGGACAAAACCAATTGTATAATGAACTTAACCTCGGAAG GCGCGAGGAATATGATGTCTTGGATAAAAGGCGCGGGCGGGATCCCGAAATGGGCGGGAAACCT CGGCGCAAGAATCCCCAGGAAGGTCTTTACAACGAACTCCAAAAGGATAAAATGGCAGAAGCTT ATTCAGAAATTGGGATGAAAGGGGAGCGGCGCCGAGGCAAAGGGCATGATGGTCTCTACCAAGG ACTTTCCACCGCTACAAAAGATACATACGACGCATTGCATATGCAAGCTTTGCCACCCCGT

[0800] Results

[0801] CD8 hinge and transmembrane domains were tested in combination wth CARs comprising CD28 co-stimulatory domains. The CD8 hinge and transmembrane sequences were used in place of the CD28 hinge and transmemebrane domains used in previous CAR constructs. CAR constructs SB01364, SB01372, SB01373, and SB01374 demonstated high expression in T cells (data not shown). SB01364 has CD8 hinge sequence 1 (SEQ ID NO: 206), CD8 transmembrane sequence 1 (SEQ ID NO: 209), and a GMCSF signal sequence; SB01372 has CD8 hinge sequence 2 (SEQ ID NO: 207), CD8 transmembrane sequence 2 (SEQ ID NO: 210), and a CD8 signal sequence; SB01373 has CD8 hinge sequence 2 (SEQ ID NO: 207), CD8 transmembrane sequence 2 (SEQ ID NO: 210), and a GMCSF signal sequence; SB01374 has CD8 hinge sequence 1 (SEQ ID NO: 206), CD8 transmembrane sequence 2 (SEQ ID NO: 210), and a CD8 signal sequence.

[0802] **FIG. 64A** shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the indicated CAR constructs after incubation with MOLM-13 cells. **FIG. 64B** shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the indicated CAR constructs after incubation with MV4-11 cells. **FIG. 64A** shows the cytotoxity and IL-2, IFN-γ, and TNF-α secretion of the CAR indicated constructs after incubation with K563 CD33 expressing cells. The CD33 CAR SB01052 was used as a control. Untransduced T cells were used as a negative control (NV). In each case, the CAR construct is refered to by the shortened name, e.g, SB01372 is indicated as 1372.

[0803] Killing and cytokine assays (**FIG. 64A-C**) demonstrate that the CD8 hinge 2 and transmembrane 2, used together in SB01373, was successfully used in place of a CD28 hinge and CD28 transmembrane configuration in the CAR and preserved the CAR activity.

Example 25: Identification of inhibitory chimeric receptor targets on HSCs

[0804] Additional NOT targets expressed on hematopoietic stem cells were identified using the bioinformatics screening described in Examples 1 and 2. In short, 7,860 HSC membrane-associated genes were identified via annotation as “cell surface” or “membrane” genes according to GO and Human Protein Atlas. Expression of the identified HSC proteins was determined in healthy HSCs and AML cells via microarray data and 115 genes were identified. Genes that were confirmed to be highly expressed on HSCs were then confirmed to be lowly expressed in AML cells via RNA-seq, resulting in 15 genes with high expression on HSCs and low expression on AML cells. The resulting hits were confirmed for protein

expression in HSCs. 10 genes with confirmed membrane expression were identified and shown in **Table 16**.

Table 16

Gene symbol	Gene name	Function
(1.) EMCN	Endomucin	Type I transmembrane mucin-like sialoglycoprotein that interferes with cell-cell and -ECM interactions
(2.) PCDH9	Protocadherin 9	Type I transmembrane protein that mediates cell adhesion in presence of calcium
(3.) JAM2	Junctional adhesion molecule 2	Type I transmembrane protein that mediates cell-cell interactions
(4.) IL18R1	Interleukin 18 receptor 1	Type I transmembrane protein and interleukin 18 (IL-18) cytokine receptor
(5.) SLC8A3	Solute carrier family 8 member A3	Multi-pass (11) transmembrane sodium/calcium exchanger integral membrane protein
(6.) CDH26	Cadherin 26	Type I transmembrane protein involved in cell interactions, migration, and differentiation
(7.) TMEM163	Transmembrane protein 163	Multi-pass (6) transmembrane protein that binds divalent cations
(8.) ABCA13	ATP Binding Cassette Transporter A13	Multi-pass (14) ATP-binding transmembrane protein
(9.) CACHD1	Cache Domain Containing 1	Type I transmembrane voltage-dependent calcium channel
(10.) CYYR1	Cysteine And Tyrosine Rich 1	Type I transmembrane protein

[0805] EMCN is highly expressed in the hematopoietic stem cell (HSC) subset and lowly expressed in leukemia stem cells (**FIG. 64A-C**). Bone Marrow Mononuclear Cells from a healthy donor and an AML patient were enriched for CD34+ cells. The CD34 fraction was separated via flow cytometry for CD90 and CD45RA positive cells. The HSCs (CD45+, Lin-, CD34+, CD38-, CD90+, CD45RA-), MPP (CD45+, Lin-, CD34+, CD38-, CD90-, CD45RA-), and LMPP (CD45+, Lin-, CD34+, CD38-, CD90-, CD45RA+) were stained with anti-EMCN antibody (L6H10) and expression of EMCN on the various cell populations was determined. As shown in **FIG. 65A**, EMCN is expressed on HSCs.

[0806] HSCs and LSCs also showed differential expression of EMCN. Bone Marrow Mononuclear Cells from a healthy donor and an AML patient were enriched for CD34+ cells. The CD34 fraction was separated via flow cytometry for CD90 and CD45RA positive cells. The HSCs (CD45+, Lin-, CD34+, CD38-, CD90+, CD45RA-) and LSCs (CD45+, Lin-, CD34+ CD38-, CD90-, CD45RA+) were stained with anti-EMCN antibody and expression of EMCN on the two cell types was compared. As shown in **FIG. 65B**, EMCN is expressed on HSCs but lowly expressed on LSCs. A summary of the EMCN expression on HSCs from 11 donors and LSCs from 7 AML patients is shown in **FIG. 65C**.

[0807] JAM2 was also determined to be expressed on HSCs and progenitor cells and only lowly on AML cells. JAM2 was expressed on 8.78% of HSCs, 10.4% on MPPs, and 9.52 on LMPP cells (data not shown).

[0808] A second screening approach was also used to identify NOT targets. 298 genes with expression profiles similar to EMCN were identified using the Gene Expression Commons platform (Seita J, Sahoo D, Rossi DJ, Bhattacharya D, Serwold T, Inlay MA, et al. (2012) Gene Expression Commons: An Open Platform for Absolute Gene Expression Profiling. PLoS ONE 7(7): e40321. doi.org/10.1371/journal.pone.0040321). 17 genes were predicted to have cell surface expression after manual curation. 9 genes were confirmed to have high expression in HSCs and low expression in AML cells. The nine additional genes are shown in **Table 17.**

Table 17	
Gene Symbol	**Gene Name**
(11.) ABCB1	ATP Binding Cassette Subfamily B Mem.1
(12.) ADGRG6	Adhesion G Protein-Coupled Receptor G6
(13.) ATP9A	ATPase Phospholipid Transporting 9A
(14.) CALN1	Calneuron 1
(15.) CDCP1	CUB Domain Containing Protein 1
(16.) IL12RB2	Interleukin 12 Receptor Subunit Beta 2
(17.) SLC16A14	Solute Carrier Family 16 Member 14
(18.) TMEM136	Transmembrane Protein 136
(19.) TMEM200A	Transmembrane Protein 200A

Example 26: Identification of endothelial safety target antigens

[0809] Endothelial safety targets were identified using the bioinformatics screening described in Examples 1 and 2 on the U133 blood samples microarray dataset. The U133 dataset includes 39 endothelial cell samples (10 fresh sample and 29 from cultured endothelial cells) and 876 AML samples. Briefly, genes with GO-annotated or Affy-annotated surface expression (9885) were identified. Genes with strong evidence for non-surface or membrane localization in Human Protein Atlas were removed, with 7860 genes remaining. These genes were assessed for higher expression in endothelial samples as compared to AML samples, resulting in 32 genes.

[0810] Next, the protein expression of each gene was confirmed in the Human Protein Atlas, surface expression in Genecards, and the size of extracellular domain (ECD) in Uniprot, leaving 16 potential target genes.

[0811] Table 18 provides a list of the endothelial safety target antigens and their fold change (log2 expression scale) in endothelial cells as compared to AML cells.

Table 18	
Gene	**Fold change in endothelial/AML cells**
ADGRG6	7.263917592
PTPRB	7.224113221
NCKAP1	6.568049829
MPZL2	6.543630634
PLSCR4	6.540126087
TMEM47	6.476813823
WLS	6.406909834
ADGRL4	6.277799777
MET	6.132032353
BACE2	5.685994648
ATP8B1	5.636851301
LIFR (225575)	5.521001536
EMCN	5.43255665
ART4	5.314938365
CALCRL	5.312163738
LIFR (225571)	5.083770824
CNTNAP3 /// CNTNAP3B	5.031224128

[0812] Next, expression of previously identified HSC NOT target antigens was assessed on endothelial cells. Such dual targets can be used to protect both HSCs and endothelial cells from AML targeting CARs. The 19 genes identified in **Example 25** were assessed for endothelial expression. 12 genes with dual expression were identified: EMCN, PCDH9, JAM2, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CDCP1, SLC16A14, TMEM136, and TMEM200A. Thus, the endothelial targets identified can be used to protect healthy endothelial cells from CAR immune cells that may possess on-target off-tissue toxicity against endothelial cells. In addition, the twelve identified dual endothelial and HSCs target antigens an be used as single "NOT" target antigens to concurrently protect healthy endothelial cells and hematopoietic stem cells (HSCs) from CAR immune cells that might possess on-target off-tissue toxicity against against healthy HSCs or endothelial cells.

[0813] Gene expression quantification of the identified AML, hematopoietic, and endothelial targets in various cell lines and sample tissues are provided in Tables 18-20. Table 18 shows gene expression in AML samples. Table 19 shows gene expression in healthy hematapoietic samples. Table 20 shows gene expression in endothelial cells.

[0814] CALN1, IL12RB2, ABCA13, CDH26, IL18R1, SLC8A3, TMEM163 are highly expressed in healthy hemaopoietic cells and thus can be used as HSC safety NOT targets.

ADGRL4, ART4, ATP8B1, BACE2, CALCRL, CNTNAP3, LIFR, MET, MPZL2, NCKAP1, PLSCR4, PTPRB, TMEM47, and WLS are highly expressed in endothelial cells and thus can be used as endothelial safety NOT targets. ABCB1, ADGRG6, ATP9A, CACHD1, CDCP1, CYYR1, EMCN, JAM2, PCDH9, SLC16A14, TMEM136, and TMEM200A are highly expressed in healthy hemaopoietic cells and endothelial cells and thus can be used as HSC and endothelial safety NOT targets.

[0815] Table 19. Gene Expression in AML Samples

	Table 19. Gene expression in AML Samples																	
Gene Symbol	AML +8 [19]	AML -5/7(q) [28]	AML -9q [6]	AML BMMC NK [399]	AML Blast [21]	AML Complex [58]	AML LPC [23]	AML LSC [28]	AML NK [9]	AML PBMC NK [32]	AML abn(3q) [2]	AML flt3 itd MUT [1]	AML idt(16) [33]	AML inv(16)/t(16;16) [28]	AML t(11q23) [48]	AML t(15;17) [58]	AML t(6;9) [6]	AML t(8;21) [77]
CALN1	3.5	4.1	3.8	3.9	3.6	4.4	3.8	4.2	3.5	4.0	3.3	3.3	3.6	4.2	3.8	3.5	3.8	3.8
IL12RB2	4.2	4.5	4.2	4.3	4.5	4.6	4.5	4.5	4.1	4.3	4.3	3.9	4.3	4.2	5.4	4.1	4.2	4.1
ABCA13	3.2	3.2	3.2	4.3	3.6	4.2	3.5	3.6	4.0	3.9	3.5	3.2	3.2	3.8	3.5	3.7	3.6	3.9
CDH26	4.1	4.1	4.3	5.2	4.9	5.0	5.1	5.1	4.9	4.9	3.8	4.5	4.3	5.0	4.6	6.3	4.3	6.0
IL18R1	4.6	4.6	4.9	5.2	4.9	5.5	4.8	5.0	5.2	5.5	4.9	4.4	4.5	5.1	4.9	5.1	4.6	5.2
SLC8A3	5.8	5.8	5.8	5.0	5.6	5.2	5.8	5.9	5.2	5.2	5.8	5.6	5.7	5.3	5.1	5.1	5.6	5.2
TMEM163	5.0	5.1	4.5	4.8	4.7	4.9	5.1	5.5	5.4	5.1	5.8	4.7	4.9	5.0	4.6	4.5	5.0	4.5
ABCB1	5.3	5.6	5.6	4.8	5.0	5.0	5.2	5.4	4.7	4.8	5.2	4.8	4.9	4.7	4.6	4.7	4.8	5.1
ADGRG6	4.3	3.7	3.7	3.9	4.3	4.2	4.5	4.8	4.8	3.8	4.0	3.5	3.7	3.5	5.8	3.6	3.6	3.6
ATP9A	5.5	6.1	5.7	5.7	5.9	6.4	6.1	6.6	7.1	6.1	5.2	6.8	5.1	5.2	5.6	6.7	5.1	5.7
CACHD1	4.8	4.9	4.8	5.0	4.8	4.7	5.1	5.3	6.0	5.2	4.8	4.6	4.6	4.4	5.1	4.5	4.7	4.9
CDCP1	5.4	5.7	5.4	5.6	6.0	5.6	6.5	6.7	5.7	5.8	5.3	5.7	5.3	5.4	5.5	5.3	5.2	5.3
CYYR1	3.9	4.2	4.9	4.0	4.0	5.1	4.3	4.4	4.1	4.0	4.1	3.9	3.8	3.7	3.8	3.9	3.8	3.8
EMCN	4.3	4.4	4.3	4.3	4.3	4.3	4.4	4.6	4.4	4.3	4.5	4.0	4.3	4.2	4.3	4.2	4.2	4.3
JAM2	5.3	5.4	5.4	5.2	5.1	5.3	5.2	5.4	5.7	5.2	5.4	5.0	5.1	5.0	5.2	5.2	5.1	5.2
PCDH9	4.7	5.1	4.7	5.2	4.6	5.1	4.8	5.5	6.5	5.4	4.5	4.3	4.7	4.6	6.0	4.7	4.7	4.6
SLC16A14	4.2	4.3	4.2	4.6	3.9	5.0	4.0	4.2	4.3	4.3	4.4	3.9	4.0	4.2	4.1	4.2	4.1	4.0
TMEM136	3.8	3.8	3.7	4.0	4.1	4.0	4.1	4.2	4.2	4.0	3.6	3.4	3.7	3.9	4.0	3.8	3.6	4.0
TMEM200A	3.5	3.8	3.4	3.7	3.9	3.7	3.8	4.6	4.0	3.9	3.4	3.4	3.4	3.4	3.6	3.5	3.4	3.4
ADGRL4	3.5	3.5	3.4	3.8	3.4	3.8	3.3	3.4	3.6	3.6	3.6	3.3	3.5	3.6	3.6	3.6	3.5	3.6
ART4	3.9	3.8	3.7	3.9	4.0	4.1	3.6	3.6	3.8	3.7	4.0	3.6	3.6	3.7	3.7	3.8	3.7	3.7
ATP8B1	3.6	3.8	3.7	3.6	3.5	3.5	3.6	3.7	3.4	3.5	3.5	3.4	3.9	3.8	3.5	3.7	3.6	3.5
BACE2	3.1	3.2	3.1	3.3	3.9	3.4	3.7	3.6	4.2	3.6	3.1	3.0	3.2	3.3	3.3	3.2	3.2	3.3
CALCRL	4.8	5.3	4.6	4.4	4.1	4.6	4.2	4.4	4.2	4.6	4.7	4.2	4.7	4.2	4.2	4.1	4.8	4.2
CNTNAP3	3.8	3.7	3.7	3.7	3.4	3.7	3.5	3.6	3.7	3.6	3.9	3.6	3.6	3.5	3.8	3.8	4.3	3.7
LIFR	3.5	3.5	3.5	3.7	3.5	3.6	3.6	3.6	3.7	3.6	3.5	3.2	3.4	3.5	3.6	3.6	3.5	3.5

MET	3.8	3.8	3.8	4.0	3.9	4.0	4.0	3.9	3.9	3.8	3.8	3.6	3.8	3.8	3.9	3.9	3.8	3.9
MPZL2	3.4	3.5	3.2	3.3	4.0	3.3	3.8	4.1	3.9	3.7	3.5	3.2	3.3	3.2	3.4	3.2	3.2	3.1
NCKAP1	3.6	3.6	3.5	3.8	3.7	4.0	3.7	3.7	3.7	3.8	3.7	3.4	3.5	3.6	3.6	3.6	3.5	3.6
PLSCR4	3.6	4.3	4.0	4.0	4.2	4.5	3.9	4.1	3.8	4.1	4.1	3.4	3.5	3.6	3.6	3.7	3.5	3.6
PTPRB	3.4	3.4	3.4	3.5	3.4	3.5	3.4	3.5	3.5	3.5	3.4	3.3	3.4	3.4	3.4	3.4	3.4	3.4
TMEM47	3.3	3.3	3.4	3.5	3.6	3.5	3.5	3.6	3.7	3.5	3.4	3.4	3.4	3.4	3.4	3.5	3.3	3.4
WLS	4.3	4.2	4.1	4.0	4.0	4.2	4.0	3.9	3.9	4.0	4.5	3.9	4.1	3.8	4.0	4.4	4.1	4.0

Table 20. Gene expression in Healthy Hematopoietic Cells

	Table 20. Gene expression in Healthy Hematopoietic Cells																								
Gene Symbol	Hematopoietic stem cell (HSC) [22]	Lymphoid-primed multipotent progenitor (LMPP) [7]	Late promyelocyte (PM) [3]	Megakaryocyte erythrocyte progenitor (MEP) [15]	Metamyelocyte (MM) [3]	Multipotent progenitor (MPP) [17]	Myelocyte (MY) [4]	Monocyte [8]	Neutrophil [3]	Promyelocyte (PM) [2]	Polymorphonuclear cell (PMN) [3]	Pre-B cell [2]	Pro-B cell [2]	Common myeloid progenitor (CMP) [16]	Early promyelocyte (PM) [3]	Erythroblast [7]	Granulocyte macrophage progenitor (GMP) [18]	Healthy umbilical cord blood (H UB) [3]	Healthy whole bone marrow (H WBM) [74]	B cell [4]	B naïve [2]	Band cell [4]	CD4 T cell [3]	CD8 T cell [5]	CD8 T central [3]	CD8 T effector [8]	CD8 T naïve [3]
CALN1	8.4	5.7	3.6	4.3	3.5	7.7	3.5	3.5	3.6	3.5	3.4	3.7	4.1	5.4	3.7	3.5	4.9	6.8	3.6	3.6	3.5	3.8	3.5	3.5	3.4	3.4	3.5
IL12RB2	7.3	6.3	4.0	4.9	3.7	6.8	3.9	4.2	4.3	4.1	3.8	4.5	6.9	5.6	4.6	4.3	5.5	6.2	4.3	4.3	4.1	3.9	5.0	4.7	4.5	4.4	4.6
ABCA13	6.6	4.2	7.0	3.4	9.2	5.6	9.5	3.3	3.3	8.4	3.7	5.7	3.5	3.8	4.8	3.1	3.6	8.9	7.8	3.2	4.1	3.9	3.2	3.3	3.2	3.2	3.2
CDH26	6.4	7.0	5.9	5.0	5.3	6.4	5.0	4.6	4.8	6.6	6.0	5.3	4.5	6.0	6.0	3.9	6.5	7.2	5.2	4.3	4.5	5.5	4.4	4.5	4.2	4.3	4.4
IL18R1	6.4	5.0	4.1	4.2	6.9	5.9	6.4	4.5	7.6	4.1	9.6	7.0	4.1	4.7	3.8	4.4	5.3	5.0	6.3	4.7	4.9	9.7	5.6	5.9	5.8	5.3	5.2
SLC8A3	7.9	6.2	4.1	6.3	3.7	7.6	4.5	5.4	5.7	5.4	4.0	4.9	4.9	6.5	3.8	5.3	5.2	6.7	5.0	5.9	4.9	3.8	5.4	5.8	5.3	5.3	5.5
TMEM163	7.6	5.9	4.2	4.7	4.3	7.0	4.0	4.6	4.4	4.3	4.2	4.1	4.7	5.8	4.5	4.4	5.1	5.5	4.6	4.5	4.9	4.2	4.6	4.8	4.5	4.7	4.6
ABCB1	7.8	6.6	4.6	5.0	4.8	7.2	4.5	4.7	4.6	4.3	4.7	4.9	4.4	5.5	4.4	4.5	4.9	7.3	4.7	5.1	4.7	4.8	4.9	5.7	6.7	7.3	6.3
ADGRG6	9.9	7.9	4.0	4.3	3.4	9.0	3.6	3.8	3.5	3.6	3.6	3.9	5.5	6.2	5.0	3.7	6.2	9.0	3.9	3.8	3.6	3.5	4.0	4.0	3.7	3.7	3.6
ATP9A	9.5	7.4	6.8	5.7	9.7	8.8	7.9	5.2	5.6	6.2	8.5	5.9	5.9	6.8	7.1	5.5	6.8	9.6	6.7	5.7	5.1	10.5	5.4	5.7	5.4	5.2	5.0

CACHD1	7.1	6.4	4.2	4.6	4.3	6.9	4.4	4.5	4.5	4.4	5.1	4.5	4.8	5.4	4.2	4.3	5.2	5.9	4.5	4.6	4.2	6.0	4.7	4.9	4.7	4.9	4.8
CDCP1	7.8	7.8	4.9	5.7	4.8	7.7	5.0	5.2	5.6	5.6	4.7	5.1	6.0	6.2	4.5	5.1	6.4	8.4	5.3	5.2	5.4	5.1	5.2	5.2	5.2	5.2	5.1
CYYR1	7.5	7.0	8.6	6.5	7.8	7.0	7.2	3.8	4.2	7.6	4.8	4.0	5.8	6.2	8.0	3.8	6.2	5.9	5.1	4.2	3.7	6.8	3.9	3.9	4.1	4.1	4.3
EMCN	8.7	6.0	4.0	4.4	4.2	6.9	4.1	4.6	4.6	4.2	4.0	4.6	5.1	4.8	4.0	4.5	4.5	7.4	4.3	4.7	4.3	4.1	4.9	4.9	4.6	4.5	4.5
JAM2	8.5	8.2	5.6	5.5	5.0	7.7	4.9	4.9	5.0	5.0	5.4	5.1	6.5	6.6	5.1	4.8	7.1	8.2	5.2	5.3	6.6	5.0	5.0	5.1	5.0	5.3	5.3
PCDH9	10.5	7.8	4.4	7.0	4.1	10.0	4.4	4.7	4.7	5.0	4.6	7.5	8.4	8.9	4.2	4.6	6.1	7.7	6.1	9.0	9.9	4.2	4.8	5.5	4.5	4.6	4.5
SLC16A14	7.0	7.6	5.1	4.3	8.4	6.4	8.0	4.2	5.2	5.1	4.1	5.9	7.3	5.0	4.3	4.1	5.9	8.1	6.7	4.7	5.7	4.5	4.7	4.5	4.2	3.9	4.0
TMEM136	6.2	4.6	3.7	4.2	3.8	5.4	3.7	3.8	3.9	3.7	3.9	4.1	4.0	4.8	3.7	3.8	4.7	4.9	3.9	4.0	4.0	4.0	3.9	3.9	3.8	3.7	3.8
TMEM200A	8.0	7.4	3.9	3.8	3.6	7.1	3.5	3.3	3.5	3.5	3.5	4.6	5.6	4.0	3.6	3.4	5.0	10.0	3.7	3.5	3.7	3.4	4.0	3.9	4.2	3.9	3.7
ADGRL4	3.4	3.4	3.3	3.5	3.4	3.4	3.4	3.5	3.6	3.4	3.4	3.4	3.5	3.4	3.3	3.5	3.6	3.4	3.8	3.6	3.3	3.6	3.5	3.8	3.5	3.4	3.4
ART4	3.5	3.6	3.4	3.7	3.7	3.6	3.5	3.6	3.8	3.8	3.5	3.2	3.4	3.7	3.3	5.7	3.7	3.7	4.9	3.8	3.5	3.6	3.6	3.7	3.8	3.8	3.7
ATP8B1	3.8	4.0	4.0	3.6	3.6	3.7	3.7	3.5	3.6	3.3	3.7	3.3	3.5	3.6	4.2	3.7	3.7	3.4	3.4	3.6	3.4	3.8	3.6	3.7	3.5	3.4	3.4
BACE2	3.8	3.3	3.5	4.1	3.3	3.9	3.4	3.1	3.6	3.6	3.2	3.4	3.2	5.3	3.3	3.3	3.4	3.2	3.4	3.8	5.7	3.3	3.1	3.2	3.3	3.2	3.2
CALCRL	4.5	4.1	3.9	4.3	4.1	4.3	4.0	4.9	4.2	4.2	3.9	4.7	4.1	4.3	4.2	4.3	4.5	4.7	4.0	4.1	3.9	4.0	4.5	4.4	4.1	4.2	4.2
CNTNAP3	3.5	3.5	3.4	3.4	6.4	3.5	5.2	3.7	7.8	3.6	7.2	3.7	3.6	3.6	3.4	3.8	3.6	3.6	4.1	3.7	3.5	7.4	3.5	3.7	3.9	3.9	3.8
LIFR	3.6	3.6	3.7	3.7	3.5	3.7	3.6	3.4	3.5	3.6	3.6	3.5	3.6	3.7	3.7	3.4	3.7	3.6	3.7	3.7	3.3	3.6	3.6	3.7	3.5	3.5	3.6
MET	3.9	3.9	3.6	4.0	4.0	3.8	3.7	3.7	3.8	4.1	3.9	3.8	3.8	4.0	3.8	3.8	4.1	3.8	3.9	4.0	3.7	3.9	4.0	4.0	3.8	3.8	3.7
MPZL2	4.7	3.8	3.1	3.3	3.5	3.9	3.7	4.9	5.1	3.2	5.6	4.8	3.2	3.4	3.1	3.1	3.8	4.8	3.4	3.1	3.2	4.7	3.3	3.3	3.1	3.1	3.1
NCKAP1	4.7	3.7	3.5	4.4	3.6	4.4	3.5	3.4	3.4	3.7	3.6	3.7	3.7	3.9	3.3	4.1	3.7	3.5	4.1	3.6	3.5	3.4	3.8	3.7	4.9	4.3	4.4
PLSCR4	7.6	5.7	4.6	5.8	3.2	7.1	3.8	3.5	3.5	4.2	3.8	3.5	4.2	5.7	4.5	6.7	5.4	6.3	4.8	3.5	3.3	3.5	3.5	3.6	3.6	3.5	3.5
PTPRB	3.4	3.4	3.3	3.4	3.3	3.5	3.4	3.5	3.5	3.3	3.3	3.5	3.4	3.5	3.3	3.3	3.5	3.4	3.4	3.5	3.5	3.4	3.5	3.5	3.4	3.4	3.5
TMEM47	3.6	3.5	3.5	3.5	3.3	3.6	3.3	3.5	3.4	3.4	3.4	3.4	3.3	3.6	3.3	3.4	3.7	3.8	3.4	3.5	3.4	3.4	3.4	3.6	3.5	3.6	3.6
WLS	3.9	3.8	4.5	4.0	6.3	3.8	6.5	7.2	8.2	4.0	9.3	6.8	3.8	4.0	4.7	4.2	4.1	3.9	4.9	4.5	4.1	7.7	4.3	4.2	4.3	4.2	4.3

Table 21. Gene Expression in Endothelial Cells

Table 21. Gene Expression in Endothelial Cells										
Gene Symbol	Human aortic endothelial cell (HAEC) [2]	Human coronary artery endothelial cell (HCAEC) [2]	Human Microvascular Endothelial Cells (HMVEC)_qui [3]	Human Umbilical Artery Endothelial Cell (HUAEC) [5]	Human umbilical vein endothelial cell (HUVEC) [5]	Human Pulmonary Artery Endothelial Cell (PAEC) [6]	Pulmonary microvascular endothelial cell	Human Umbilical Artery Endothelial Cell (HUAEC)_fresh	Human umbilical vein endothelial cell (HUVEC)_fresh [4]	Human umbilical vein endothelial cell (HUVEC)_primary [2]
CALN1	3.3	3.5	3.3	3.3	3.4	3.4	3.5	3.3	3.4	3.4
IL12RB2	3.8	4.0	3.8	4.0	4.1	3.9	3.9	4.0	4.0	4.6
ABCA13	3.2	3.2	3.2	3.4	3.7	3.2	3.2	3.2	3.3	3.2
CDH26	4.0	3.9	3.8	3.7	4.0	3.9	3.8	4.1	4.5	3.7
IL18R1	5.3	6.4	3.9	5.5	5.1	4.2	4.3	6.7	8.3	6.4
SLC8A3	4.8	5.0	4.7	4.9	5.2	4.9	4.9	4.8	4.9	4.6
TMEM163	5.3	5.7	5.0	4.3	4.6	4.4	4.4	4.2	4.4	4.4
ABCB1	7.0	5.2	4.3	5.6	5.0	4.3	4.3	4.6	4.4	4.6
ADGRG6	6.0	8.6	6.4	8.8	7.7	5.8	5.4	12.2	11.2	9.5
ATP9A	9.9	10.2	9.0	10.3	9.6	9.5	9.7	10.0	9.4	8.1
CACHD1	6.4	6.6	7.3	6.7	6.6	7.9	7.8	6.8	7.6	6.9
CDCP1	5.4	6.9	5.4	6.7	6.8	5.2	5.4	5.2	5.2	5.8
CYYR1	8.7	7.2	4.9	6.4	6.7	7.5	7.6	9.2	9.9	3.9
EMCN	11.2	9.8	10.4	10.3	9.6	10.9	10.9	9.5	11.6	6.8
JAM2	5.7	6.2	5.0	6.8	7.0	4.9	5.0	10.1	9.5	4.6
PCDH9	9.0	7.3	8.5	6.6	6.4	5.6	5.3	5.5	6.4	5.6
SLC16A14	6.8	3.9	3.9	4.5	4.3	3.7	4.0	9.1	7.7	3.9
TMEM136	5.8	6.0	5.5	6.1	5.8	5.2	5.3	5.9	5.7	4.8
TMEM200A	3.6	6.3	3.6	8.3	7.9	3.4	3.4	8.0	3.7	5.5
ADGRL4	10.8	10.6	10.3	9.7	8.9	10.7	10.8	10.6	9.6	9.2
ART4	9.5	7.1	7.0	9.2	6.1	9.3	9.3	9.8	10.1	6.0
ATP8B1	10.6	9.8	9.0	10.6	9.1	7.2	6.9	9.8	8.9	8.8
BACE2	9.2	8.2	8.0	8.8	6.6	10.8	11.0	10.6	8.9	6.3
CALCRL	8.0	7.8	9.1	5.6	4.7	9.7	9.8	9.6	10.0	6.5
CNTNAP3	9.7	8.9	7.8	7.3	7.2	8.9	9.0	9.1	9.4	6.6
LIFR	7.5	7.7	7.2	7.2	5.8	8.3	8.3	9.1	10.5	6.7
MET	9.3	9.9	9.2	9.6	7.9	9.1	9.1	9.4	10.6	10.4
MPZL2	8.7	9.3	9.1	5.9	4.9	6.1	5.7	10.3	10.2	8.5
NCKAP1	10.6	10.2	9.7	10.5	9.3	9.4	9.5	10.8	10.1	9.7
PLSCR4	11.1	10.2	10.6	11.0	9.4	12.5	12.4	11.2	11.6	6.9
PTPRB	10.8	9.5	10.1	9.4	7.7	8.9	9.0	12.7	11.7	4.7

TMEM47	9.7	10.0	9.0	10.6	8.9	6.5	6.3	10.9	10.5	7.0
WLS	9.0	8.6	9.2	7.8	7.8	8.1	7.9	8.7	8.5	8.3

Example 27: Characterization of monovalent and bicistronic FTL3 and CD33 CAR Natural Killer (NK) Cells

[0816] *Materials and methods*

[0817] *CAR expression in NK cells*

[0818] Primary NK cells were isolated from PBMCs and frozen. Frozen NK cells were thawed and activated with CD335 (NKp46)-Biotin, CD2-Biotin, and MACS Anti-Biotin MACSiBead Particles. Activated NK cells were expanded in NK MACS Medium with IL-2 and frozen on day 15. When ready, the expanded NK cells were thawed and rested in complete medium overnight. NK cells were transduced with selected CAR lentivirus, to produce FLT3 CAR (SB00819) NK cells, CD33 CAR (SB01052) NK cells, or bicistronic FLT3 and CD33 CAR (SB01659) NK cells. CAR expression was assessed on day 6 after transfection.

[0819] *NK cell cytotoxicity assay*

[0820] An NK cell cytotoxicity assay and cytokine production assay were performed as described in **Examples 15-18**, using SEM cells and an ET ration of 1:2. CAR NK cells were incubated with the SEM target cells for 20 hours and the cell supernatant was collected for cytokine production. Cytotoxicity and cytokine production was assessed by flow cytometry and Luminex assay as previously described. Cytotoxicity percentage was normalized to a target cell only control. Non-engineered NK cells expressing EGFP were used as a negative control.

[0821] An NK cell cytotoxicity assay was performed as described in **Examples 15-18**, using PL-21 AML cells. PL-21 AML cells are known to express FLT3 and CD33 (Reiter K, et al, Leukemia (2018) 32, 313–322 shows FLT3 expression in PL-21 cells and Kearney CJ, et al, OncoImmunology, 5:8, e1196308, DOI: 10.1080/2162402X.2016.1196308 shows CD33 expression in PL-21 cells, both of which are incorporated by reference).

[0822] *Results*

[0823] NK cells show up to 45% CAR expression at day 6 after lentivirus transduction. The FLT3 CAR NK cells were 28.8% positive for the FLT3 CAR (data not shown). The CD33 CAR NK cells were 25.8% positive for the CD33 CAR (data not shown). The bicistronic FLT3/CD33 CAR NK cells were 3.9% positive for the FLT3 CAR and 26.9% positive for CD33 (data not shown).

[0824] CAR NK cells demonstrated significant cytotoxicity and cytokine production against SEM cells. The FLT3 CAR NK cells (SB00819), the CD33 CAR NK cells (SB01052), and the FLT3 OR CD33 bicistronic CAR NK cells (SB01659) demonstrated statistically significant killing activity of SEM cells as compared to the EGFP negative control NK cells (**FIG. 66A**). The FLT3 CAR NK cells showed significantly greater IFN-ɣ (**FIG. 66B**) and TNF-α (**FIG. 66C**) cytokine secretion compared to EGFP negative control NK cells after co-culture with SEM cells.

[0825] The FLT3, CD33, and FLT3/CD33 CAR NK cells also demonstrated significant cytotoxicity against PL-21 cells. The CD33 CAR NK cells (SB01052), and the FLT3 OR CD33 bicistronic CAR NK cells (SB01659) show significant cytotoxicity against PL-21 AML cells as compared to EGFP control NK cells (**FIG. 66D**). The FLT3 CAR NK cells (SB00819) showed a trend towards increased killing of PL-21 cells compared to EGFP control NK cells.

[0826] Thus, monovalent FLT3 CAR NK cells, monovalent CD33 CAR NK cells, and bicistronic FLT3/CD33 CAR NK cells were effective in killing two different AML target cells and secreting cytokines.

INCORPORATION BY REFERENCE

[0827] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

EQUIVALENTS

[0828] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the present disclosure(s). Many variations will become apparent to those skilled in the art upon review of this specification.

CLAIMS

What is claimed is:

1. An isolated immunoresponsive cell comprising:

(a) a first chimeric receptor comprising an extracellular antigen-binding domain that binds to a first antigen, and

(b) a second chimeric receptor comprising an extracellular antigen-binding domain that binds to a second antigen,

wherein each antigen is selected from the group consisting of FLT3, CD33, CLEC12A, MS4A3, VSTM1, LAT2, MLC1, CD131, GAPT, PRAM1, SLC22A16, SLC17A9, SPNS3, ADGRE2, IL3RA, CD117, CD93, IL1RAP, CD244, CCR1, LILRB2, PIEZO1, CD38, EMB, MYADM, LILRA2, CD300LF, and CD70, and

wherein the first antigen is different from the second antigen.

2. The isolated immunoresponsive cell of claim 1, wherein the first antigen is FLT3 and the extracellular antigen-binding domain of the first chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

(a) a VH comprising the amino acid sequence of SEQ ID NO: 3 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 4 or a sequence at least 90% identical thereto;

(b) a VH comprising the amino acid sequence of SEQ ID NO: 1 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 2 or a sequence at least 90% identical thereto;

(c) a VH comprising the amino acid sequence of SEQ ID NO: 5 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 6 or a sequence at least 90% identical thereto;

(d) a VH comprising the amino acid sequence of SEQ ID NO: 7 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 8 or a sequence at least 90% identical thereto;

(e) a VH comprising the amino acid sequence of SEQ ID NO: 9 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 10

or a sequence at least 90% identical thereto;

(f) a VH comprising the amino acid sequence of SEQ ID NO: 11 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 12 or a sequence at least 90% identical thereto;

(g) a VH comprising the amino acid sequence of SEQ ID NO: 13 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 14 or a sequence at least 90% identical thereto; and

(h) a VH comprising the amino acid sequence of SEQ ID NO: 15 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 16 or a sequence at least 90% identical thereto.

3. The isolated immunoresponsive cell of claim 2, wherein:

i) the second antigen is CD33 and the extracellular antigen-binding domain of the second chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto, and

(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto; or

ii) the second antigen is CLEC12A and comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto,

(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto, and

(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto.

4. The isolated immunoresponsive cell of claim 1, wherein the first antigen is CLEC12A and the extracellular antigen-binding domain of the first chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

(a) a VH comprising the amino acid sequence of SEQ ID NO: 21 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 22 or a sequence at least 90% identical thereto;

(b) a VH comprising the amino acid sequence of SEQ ID NO: 23 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 24 or a sequence at least 90% identical thereto; and

(c) a VH comprising the amino acid sequence of SEQ ID NO: 25 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 26 or a sequence at least 90% identical thereto,

optionally wherein the second antigen is CD33 and the extracellular antigen-binding domain of the second chimeric receptor comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the group consisting of:

(a) a VH comprising the amino acid sequence of SEQ ID NO: 17 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 18 or a sequence at least 90% identical thereto; and

(b) a VH comprising the amino acid sequence of SEQ ID NO: 19 or a sequence at least 90% identical thereto and a VL comprising the amino acid sequence of SEQ. ID NO: 20 or a sequence at least 90% identical thereto.

5. The isolated immunoresponsive cell of any one of claims 1-4, wherein:

i) binding of the first chimeric receptor to the first antigen is capable of activating the immunoresponsive cell and/or binding of the second chimeric receptor to the second antigen is capable of stimulating the immunoresponsive cell; and/or

ii) the immunoresponsive cell exhibits a greater degree of cytolytic activity against target cells that are positive for both the first antigen and the second antigen as compared to cytolytic activity against target cells that are positive for only the first antigen or the second antigen; and/or

iii) the first chimeric receptor binds to the first antigen with a binding affinity that is lower than the binding affinity of the second chimeric receptor for the second antigen; and/or

iv) the first chimeric receptor binds to the first antigen with a low binding avidity.

6. The isolated immunoresponsive cell of any one of claims 1-5, wherein the first chimeric receptor is a first CAR and the second chimeric receptor is a second CAR and each CAR comprises:

i) a CD3zeta-chain intracellular signaling domain, optionally wherein each CAR further comprises one or more additional intracellular signaling domains, and the one or more additional intracellular signaling domains are selected from the group consisting of a CD97 intracellular signaling domain, a CD11a-CD18 intracellular signaling domain, a CD2 intracellular signaling domain, an ICOS intracellular signaling domain, a CD27 intracellular signaling domain, a CD154 intracellular signaling domain, a CD8 intracellular signaling domain, an OX40 intracellular signaling domain, a 4-1BB intracellular signaling domain, a CD28 intracellular signaling domain, a ZAP40 intracellular signaling domain, a CD30 intracellular signaling domain, a GITR intracellular signaling domain, an HVEM intracellular signaling domain, a DAP10 intracellular signaling domain, a DAP12 intracellular signaling domain, a MyD88 intracellular signaling domain, and a 2B4 intracellular signaling domain; and/or

ii) a transmembrane domain, and the transmembrane domain is selected from the group consisting of a CD8 transmembrane domain, a CD28 transmembrane

domain a CD3zeta-chain transmembrane domain, a CD4 transmembrane domain, a 4-1BB transmembrane domain, an OX40 transmembrane domain, an ICOS transmembrane domain, a CTLA-4 transmembrane domain, a PD-1 transmembrane domain, a LAG-3 transmembrane domain, a 2B4 transmembrane domain, and a BTLA transmembrane domain; and/or

iii) a spacer region between the antigen-binding domain and the transmembrane domain, and the spacer region has an amino acid sequence selected from the group consisting of SEQ ID NOs: 55-64.

7. The isolated immunoresponsive cell of any one of claims 1-6, wherein the cell further comprises an inhibitory chimeric receptor comprising an antigen-binding domain, optionally wherein the inhibitory chimeric receptor inhibits one or more activities of the cell.

8. The isolated immunoresponsive cell of claim 7, wherein the inhibitory chimeric receptor binds an antigen that is expressed on a non-tumor cell, optionally wherein the antigen that is expressed on a non-tumor cell is derived from a tissue selected from the group consisting of brain, neuronal tissue, endocrine, bone, bone marrow, immune system, endothelial tissue, muscle, lung, liver, gallbladder, pancreas, gastrointestinal tract, kidney, urinary bladder, male reproductive organs, female reproductive organs, adipose, soft tissue, and skin.

9. The isolated immunoresponsive cell of claim 7 or claim 8, where in the inhibitory chimeric receptor binds an antigen selected from the group consisting of EMCN, JAM2, MS4A15, C4BPA, TRPM1, SCTR, SLC2A2, KCNQ2, PERP, WLS, FFAR2, PTPRB, NCKAP1, MPZL2, PLSCR4, TMEM47, ADGRL4, MET, BACE2, ATP8B1, LIFR, ART4, CALCRL, CNTNAP3, PCDH9, IL18R1, SLC8A3, CDH26, TMEM163, ABCA13, CACHD1, CYYR1, ABCB1, ADGRG6, ATP9A, CALN1, CDCP1, IL12RB2, SLC16A14, TMEM136, and TMEM200A.

10. The isolated immunoresponsive cell of any one of claims 7-9, wherein the inhibitory chimeric receptor comprises an antigen binding domain comprising a single chain variable fragment (scFv), and the scFv is derived from an anti-EMCN antibody.

11. The isolated immunoresponsive cell of any one of claims 7-10, wherein the antigen-binding domain of the first chimeric receptor, the antigen-binding domain of the second chimeric receptor, and/or the antigen-binding domain of the inhibitory chimeric receptor comprises one or more single chain variable fragments (scFvs), wherein each of the one or more scFvs comprises a heavy chain variable domain (VH) and a light chain variable domain (VL), optionally wherein the VH and VL are separated by a peptide linker, and optionally wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 27.

12. The isolated immunoresponsive cell of claim 11, wherein each of the one or more scFvs comprises the structure VH-L-VL or VL-L-VH, wherein VH is the heavy chain variable domain, L is the peptide linker, and VL is the light chain variable domain.

13. The isolated immunoresponsive cell of claim 11 or claim 12, wherein each of the one or more scFvs binds to a distinct epitope on the same antigen.

14. The isolated immunoresponsive cell of any one of claims 11-13, wherein each of the one or more scFvs is separated by a peptide linker, optionally wherein the peptide linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 27) or EAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 74).

15. The isolated immunoresponsive cell of any one of claims 1-14, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a myeloid cell, a macrophage, a human embryonic stem cell (ESC), an ESC-derived cell, a pluripotent stem cell, and induced pluripotent stem cell (iPSC), and an iPSC-derived cell, optionally wherein the immunoresponsive cell is allogeneic.

16. A pharmaceutical composition comprising an effective amount of the isolated immunoresponsive cell of any one of 1-15 and a pharmaceutically acceptable carrier, pharmaceutically acceptable excipient, or a combination thereof.

17. A method of providing an anti-tumor immunity in a subject, the method comprising administering to a subject in need thereof a therapeutically effective dose of any of the

isolated immunoresponsive cells of any one of claims 1-15 or the pharmaceutical composition of claim 16.

18. A method of treating or preventing a myeloid disorder in a subject, comprising administering to the subject an effective amount of the isolated immunoresponsive cell of any one of claims 1-15 or the pharmaceutical composition of claim 16, optionally wherein the myeloid disorder is myelodysplastic syndromes, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia (AML), acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, chronic myelocytic leukemia, and polycythemia vera.

19. A kit for treating and/or preventing a myeloid disorder, comprising the isolated immunoresponsive cell of any one of claims 1-15 or the pharmaceutical composition of claim 16, optionally wherein the kit further comprises written instructions for using the cell for treating and/or preventing a myeloid disorder in a subject .

FIG. 1
FLT3

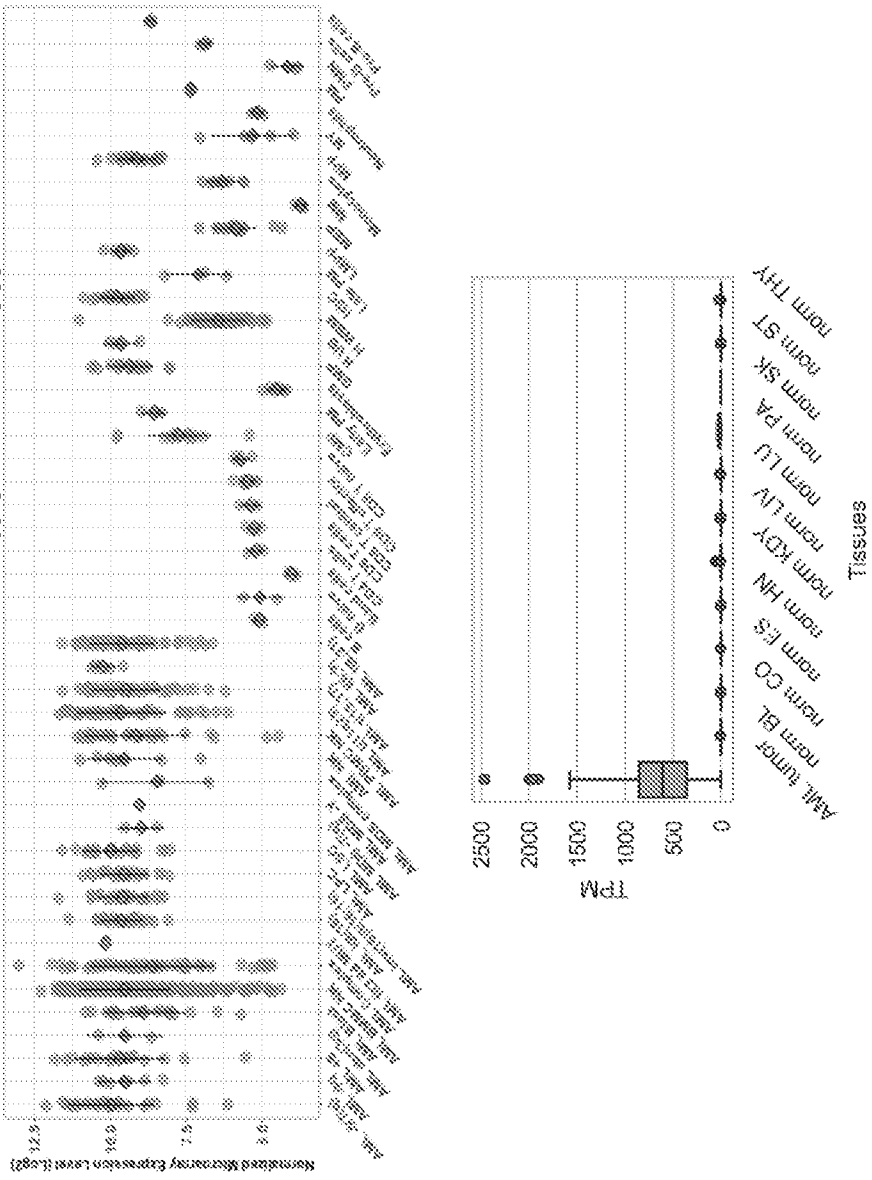

AML
T cells
HSCs
TPM
2500
2000
1500
1000
500
0
AML tumor
norm BL
norm CD
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues

FIG. 2

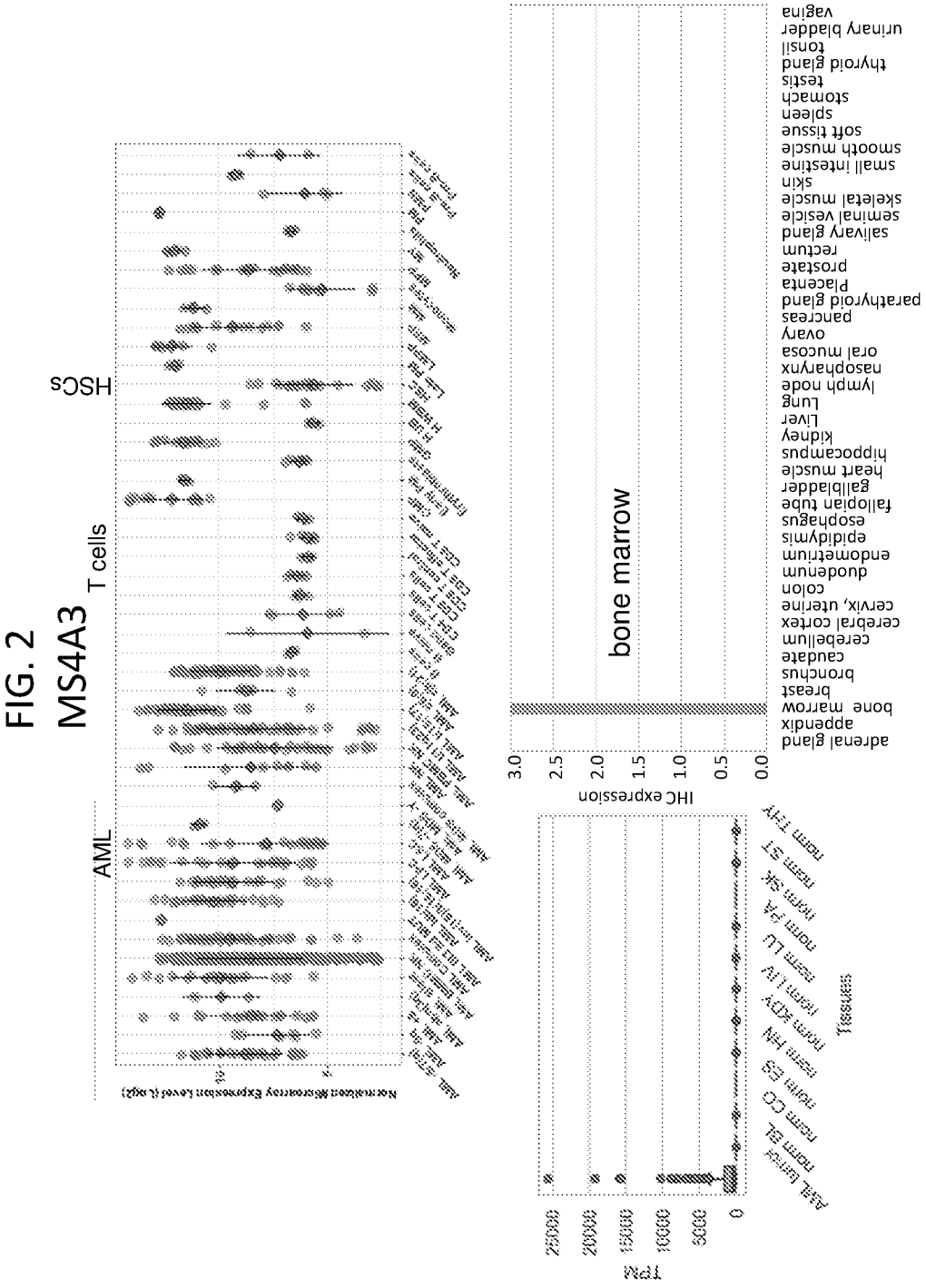

MS4A3
AML
T cells
HSCs
bone marrow
IHC expression
TPM
adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
rectum
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina

FIG. 3
CD33

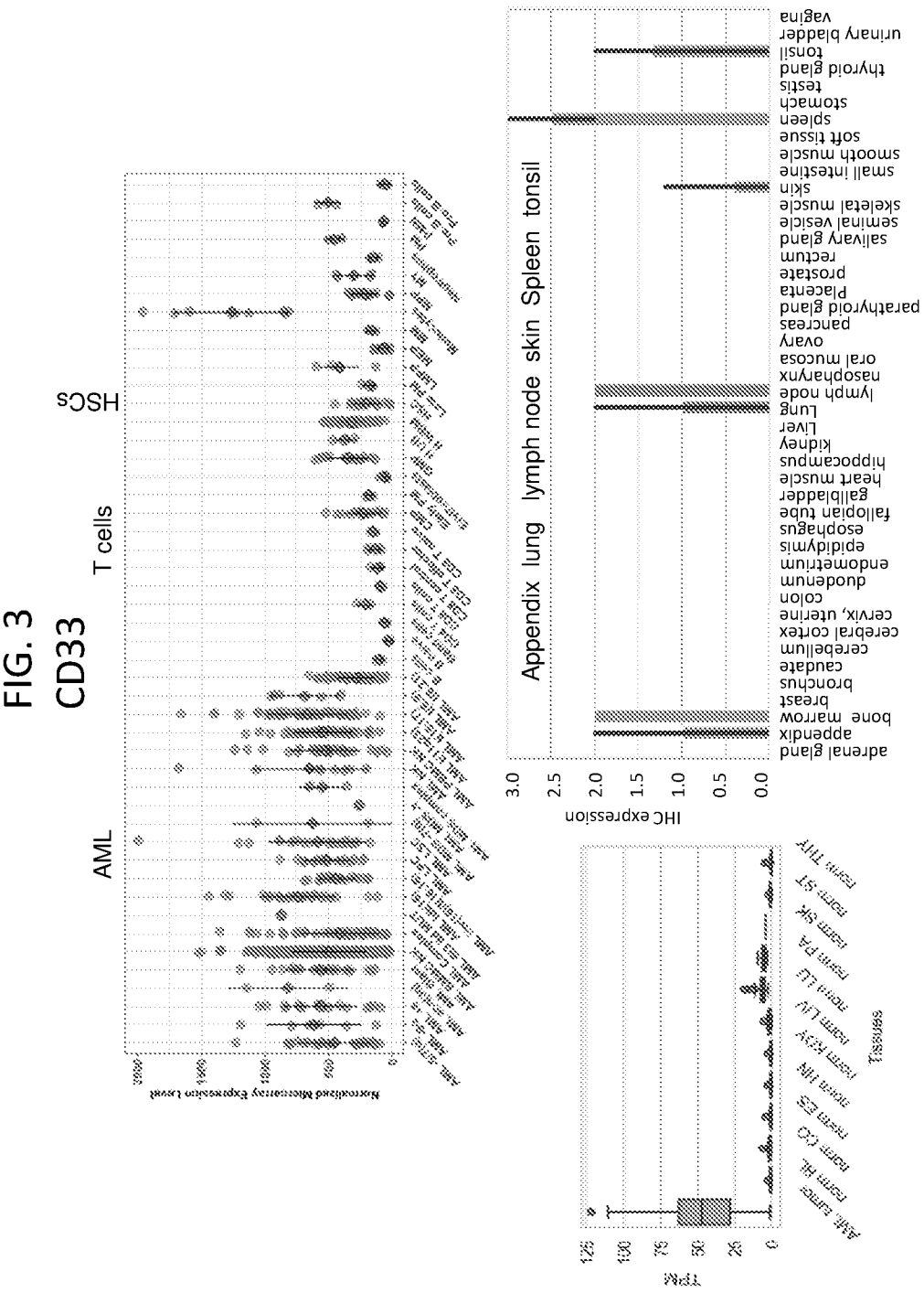

AML
T cells
HSCs
Normalized Microarray Expression Level
Appendix lung lymph node skin Spleen tonsil
IHC expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
rectum
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina
TPM
125
100
75
50
25
0
Tissues

FIG. 4
CLEC12A

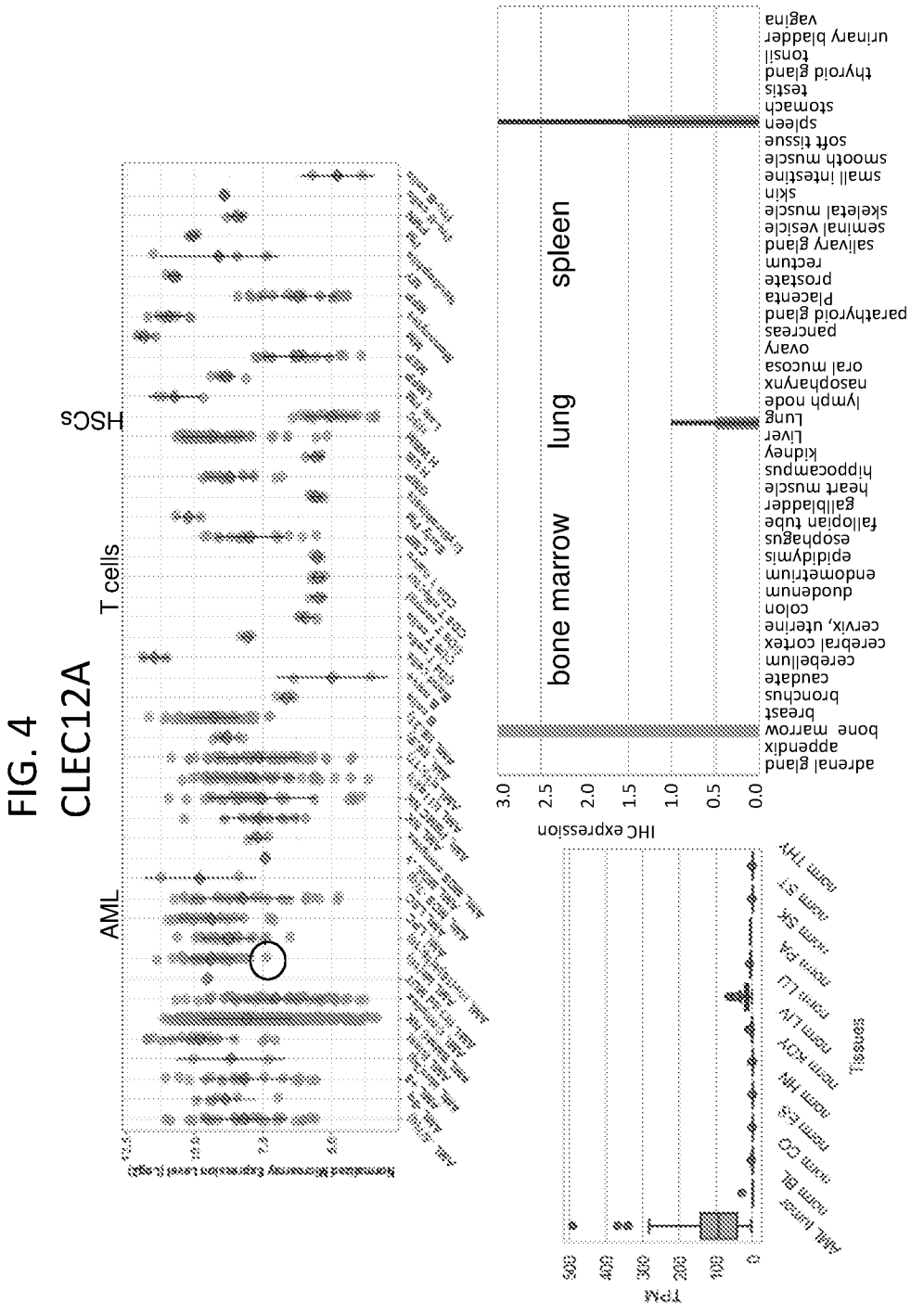


FIG. 5
CD312/ADGRE2

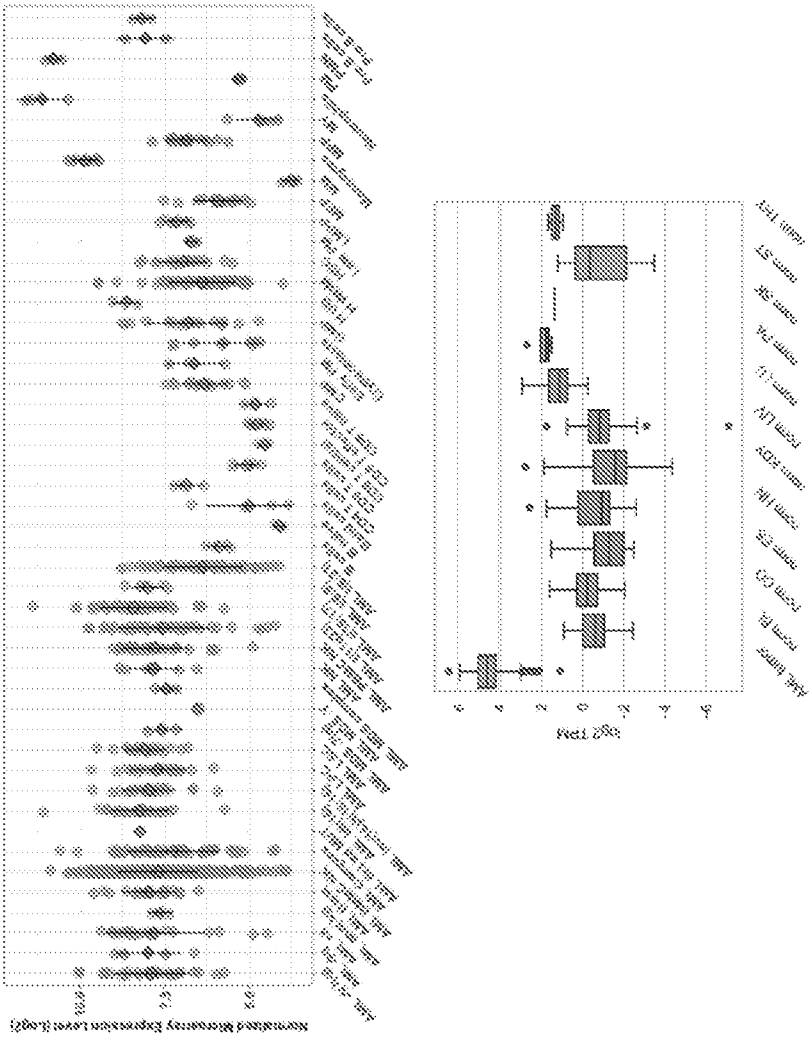


FIG. 6
SLC22A16

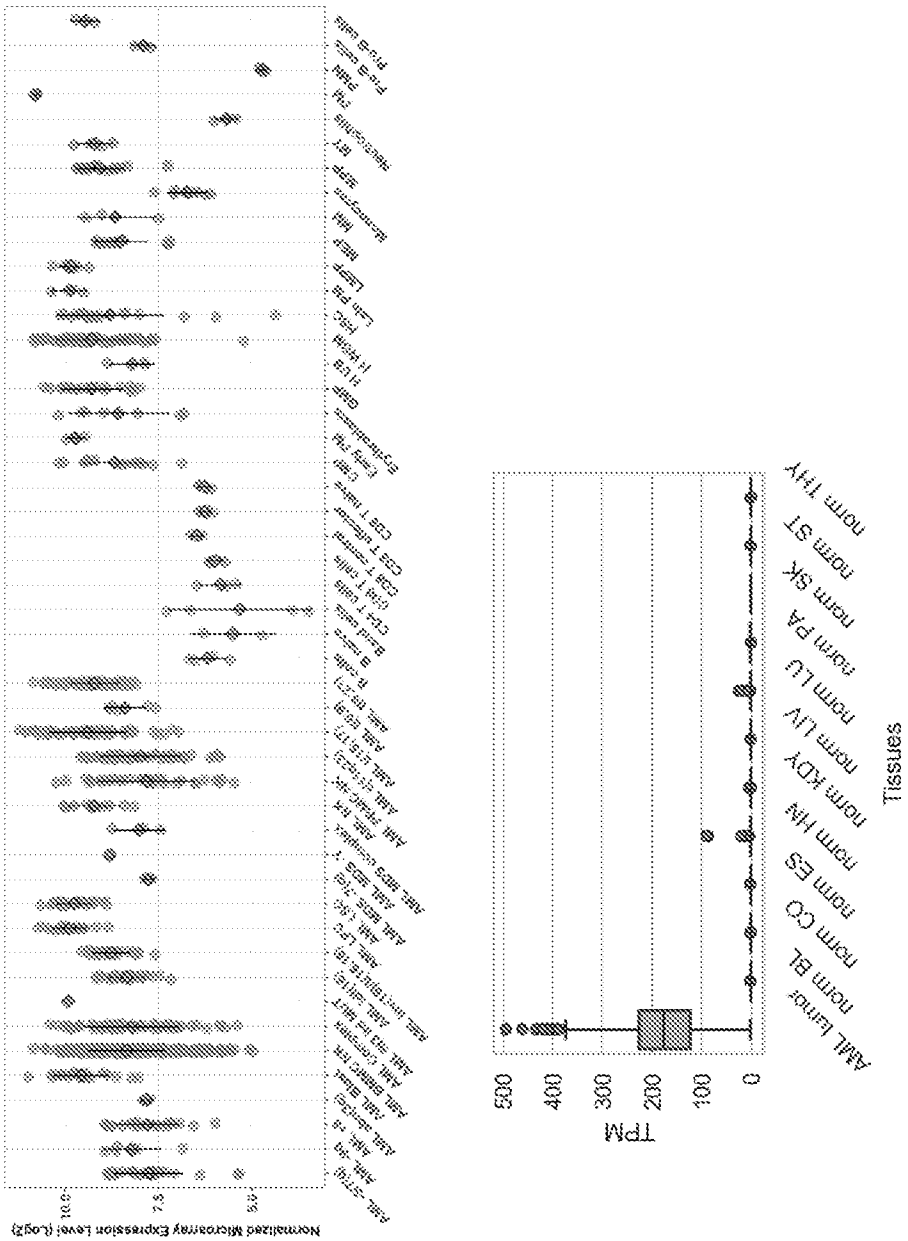


FIG. 7
CD123/ILR3RA

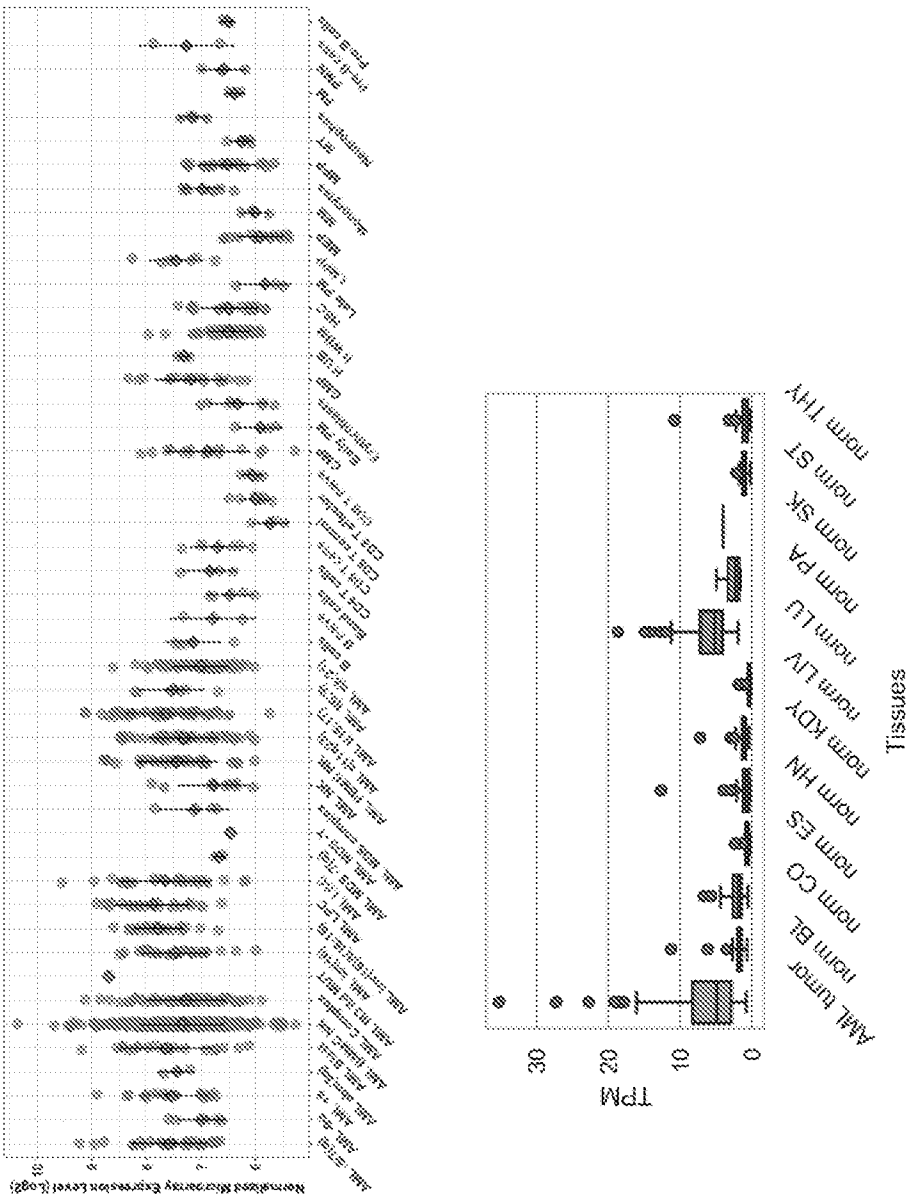


FIG. 8
LAT2

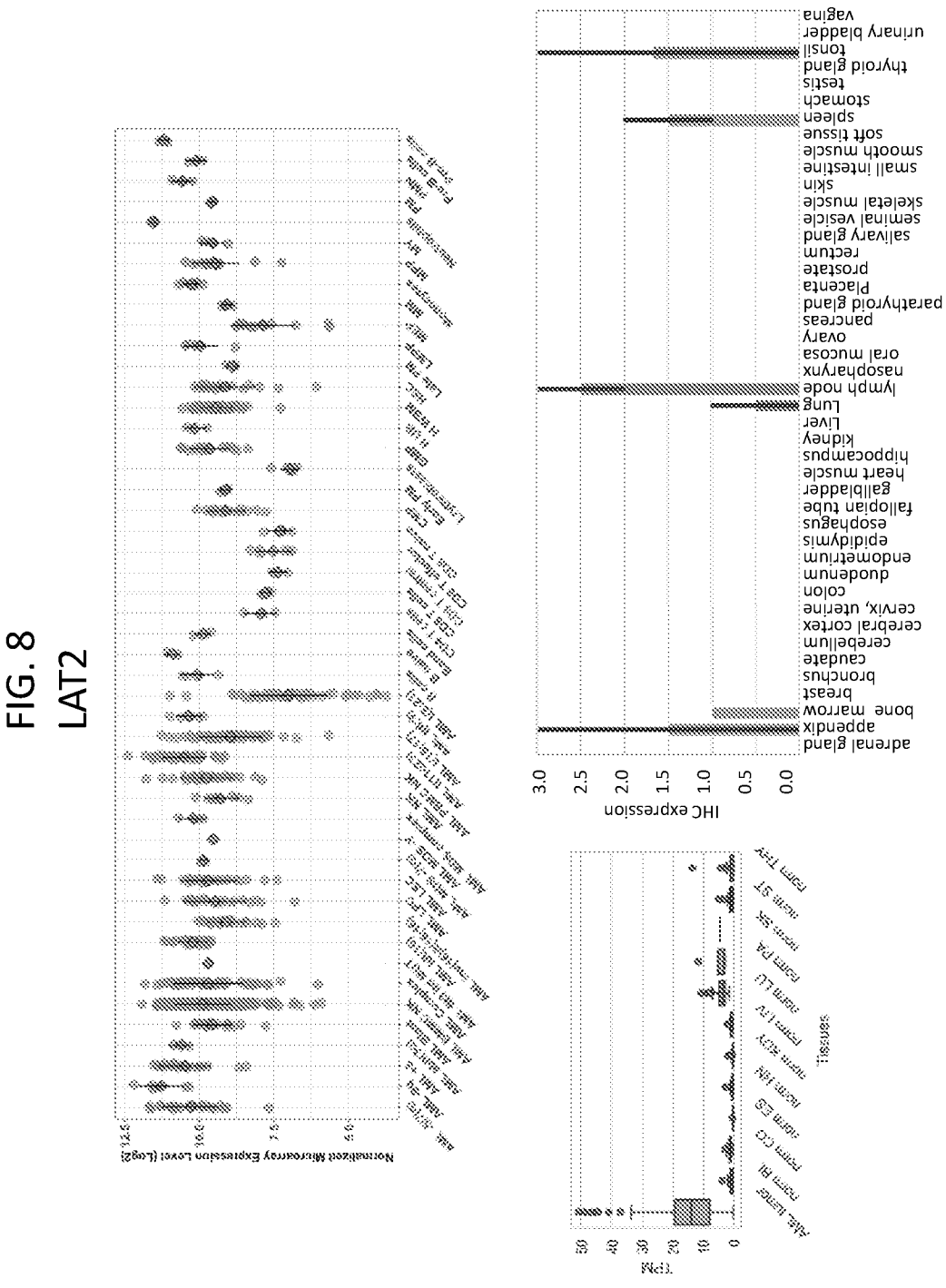

adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
rectum
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina
IHC expression
0.0
0.5
1.0
1.5
2.0
2.5
3.0
TPM
0
10
20
30
40
50
tissues

FIG. 9
PIEZO1/FAM38A

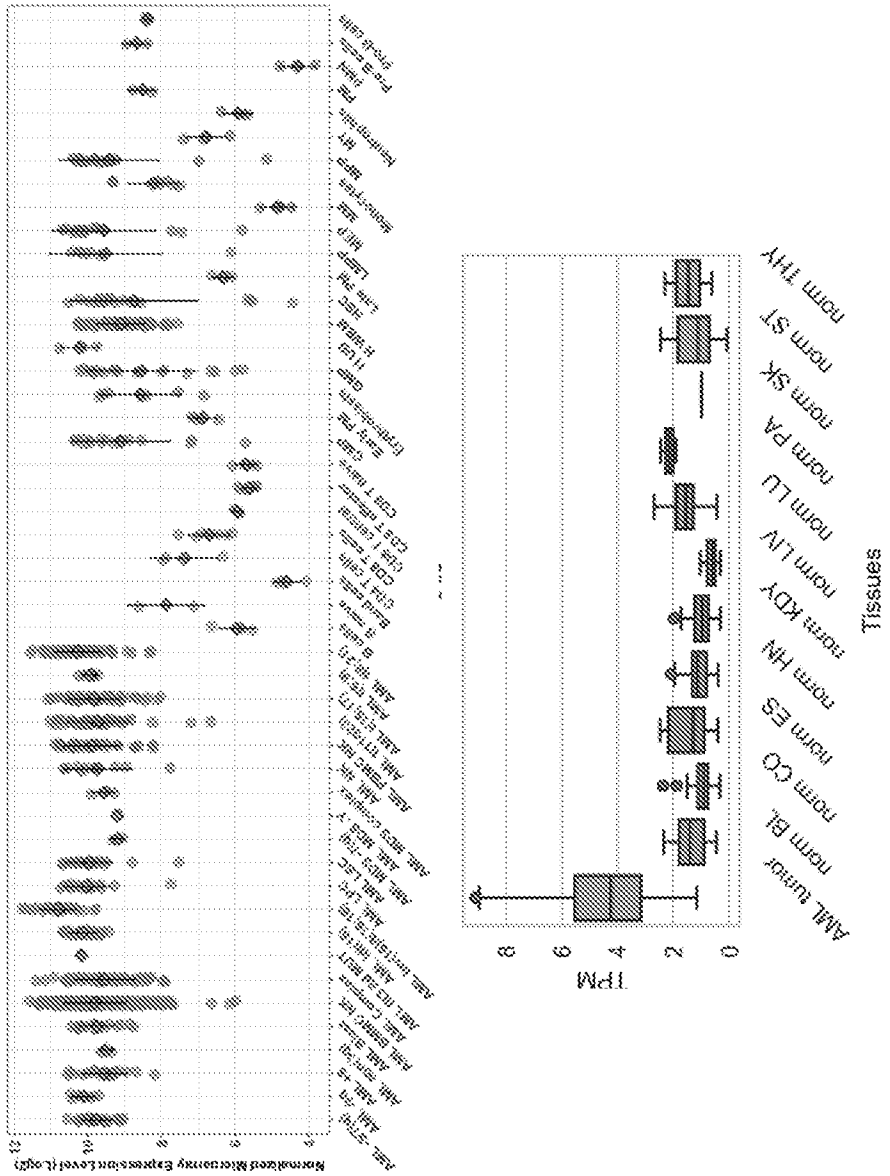


FIG. 10
CD38

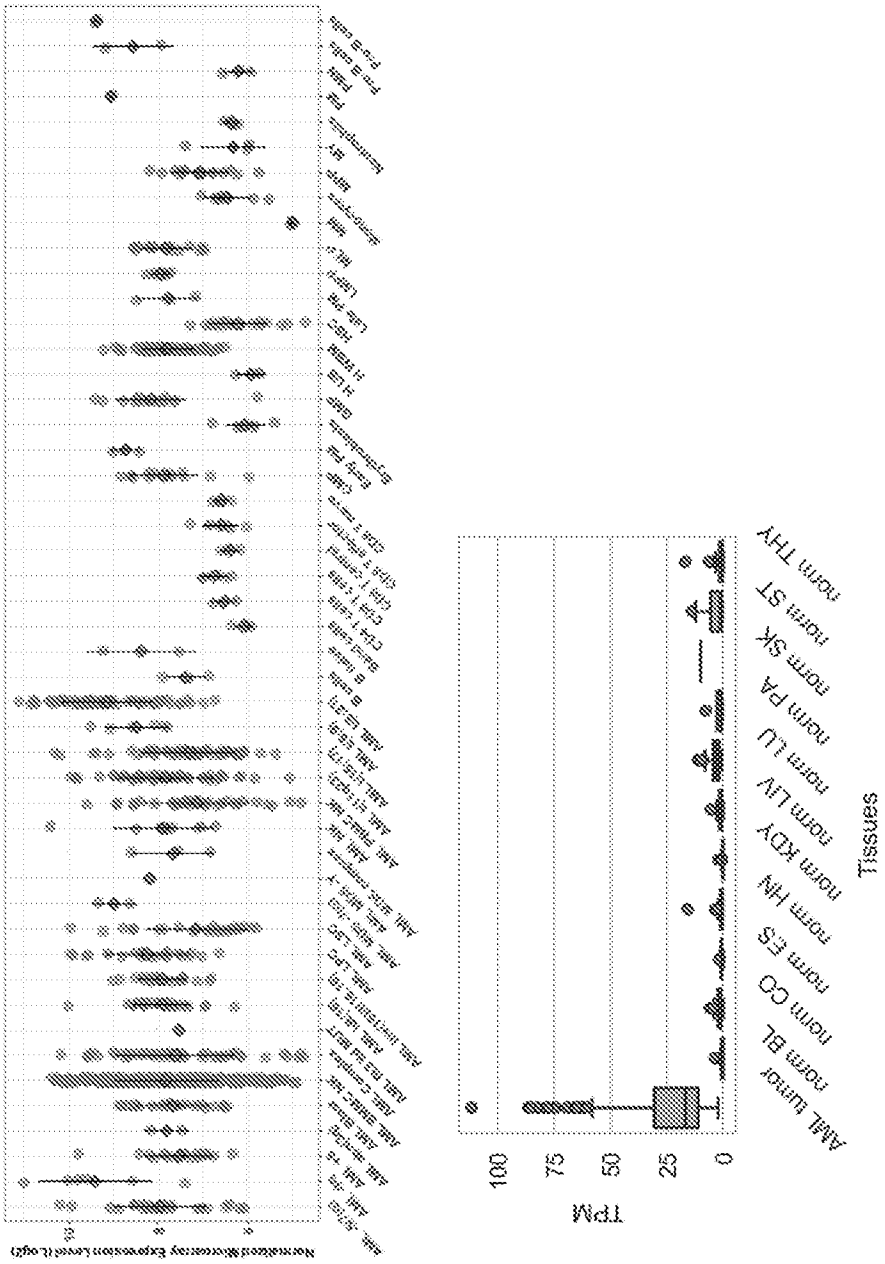

TPM
100
75
50
25
0
AML tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues

FIG. 11
EMB

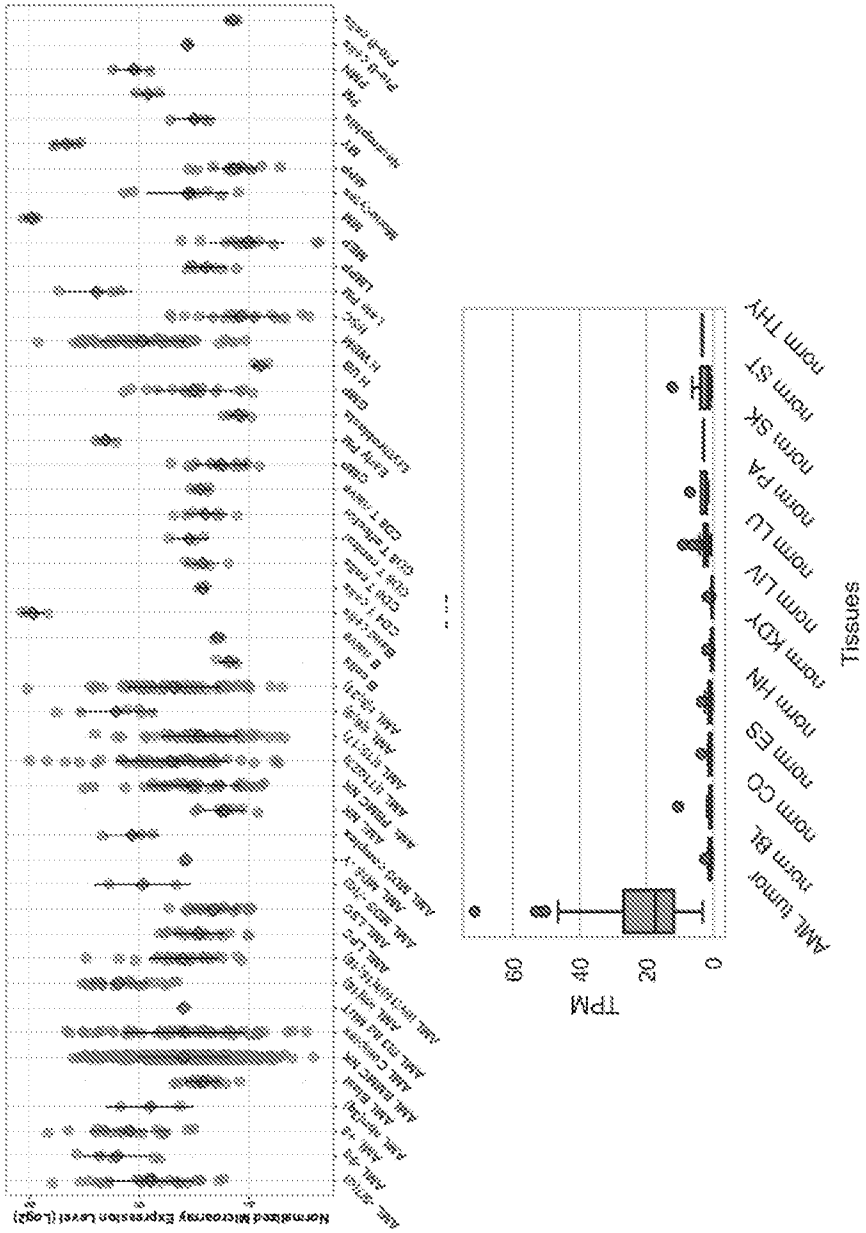

Normalized Microarray Expression Level (Log2)
TPM
0
20
40
60
AML tumor
norm BL
norm CD
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues

FIG. 12
CD131/CSF2RB

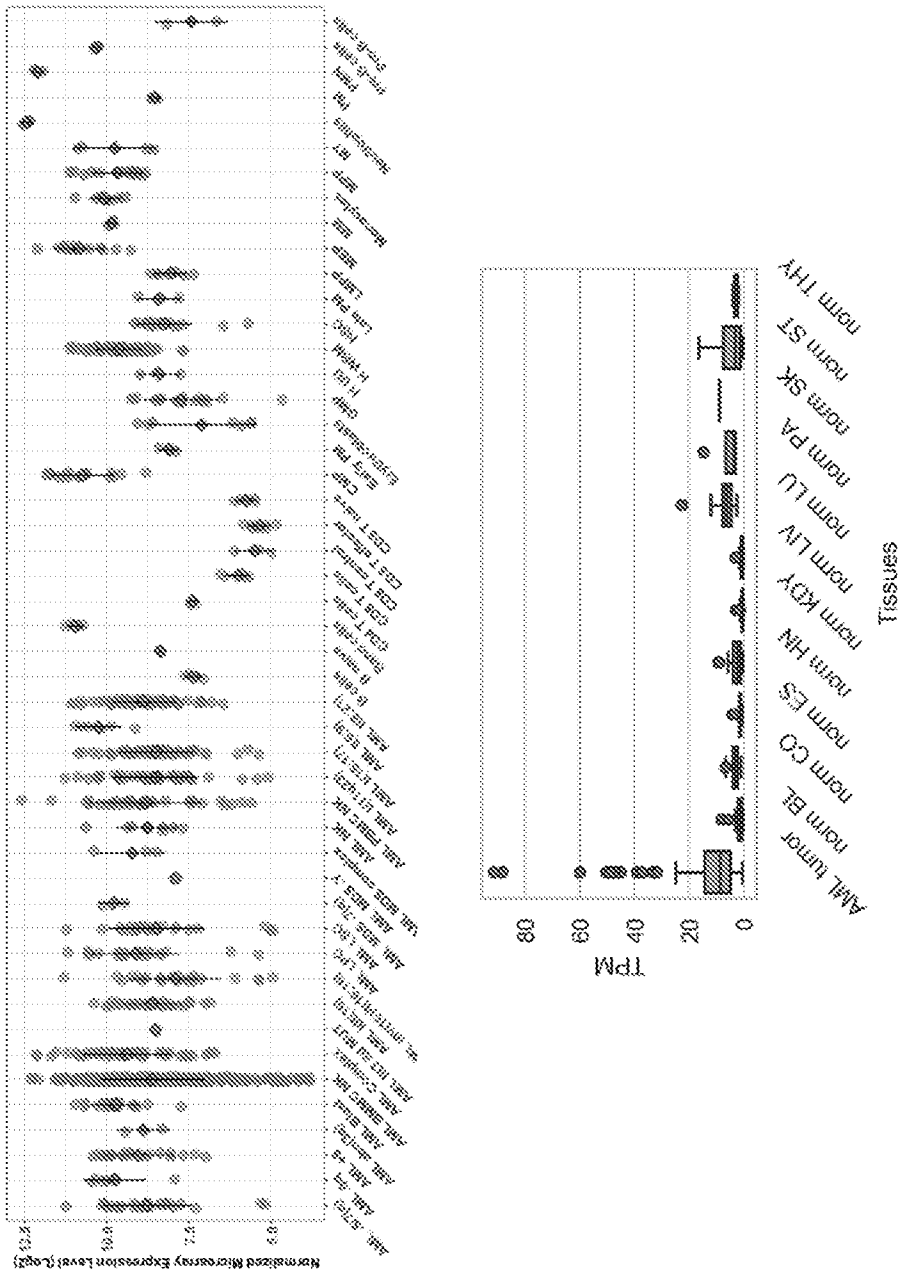


FIG. 13
P2RY8

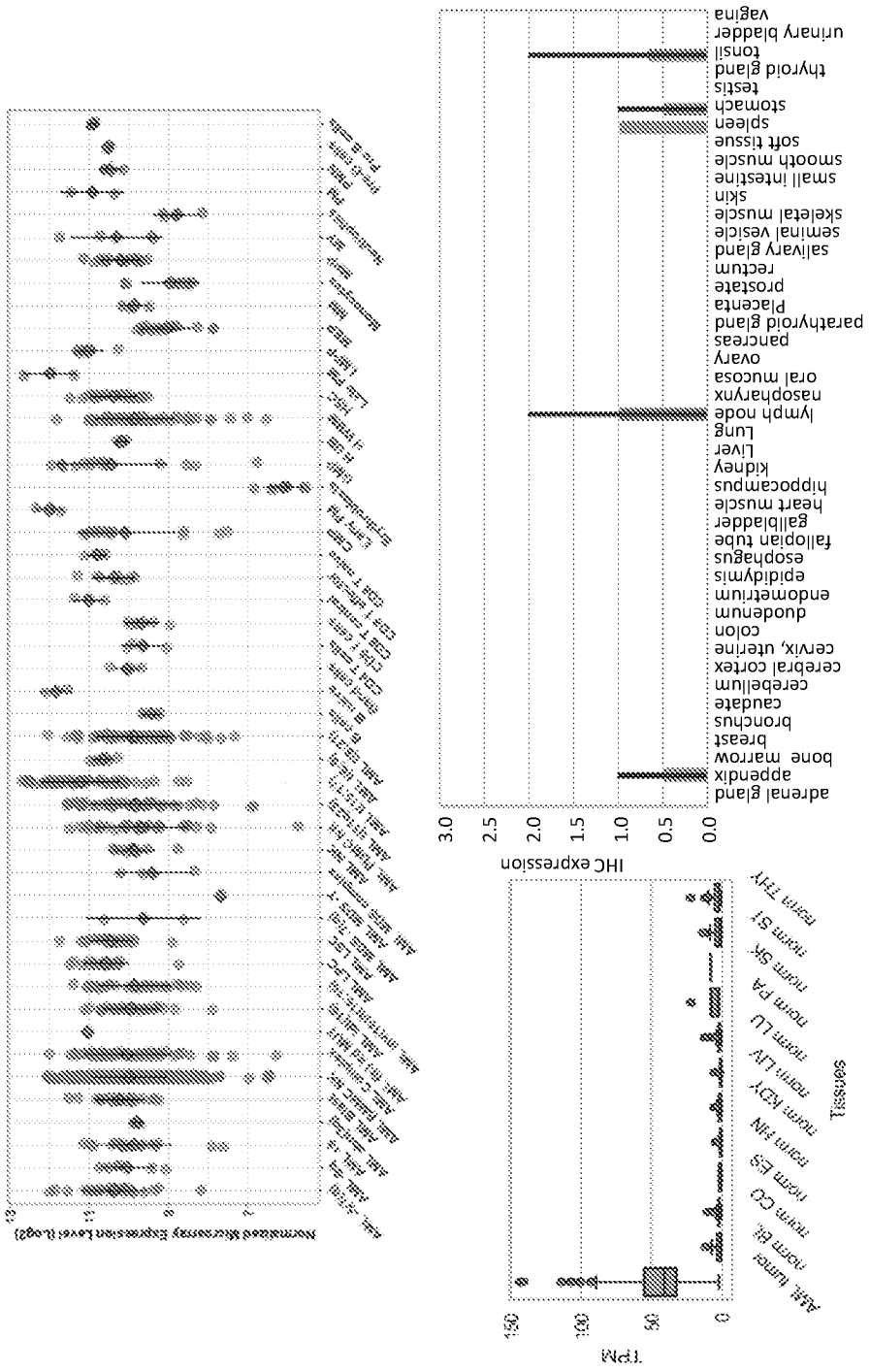

Normalized Microarray Expression Level (Log2)
TPM
IHC expression
Tissues
adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
rectum
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina

FIG. 14
LILRA2/CD85H

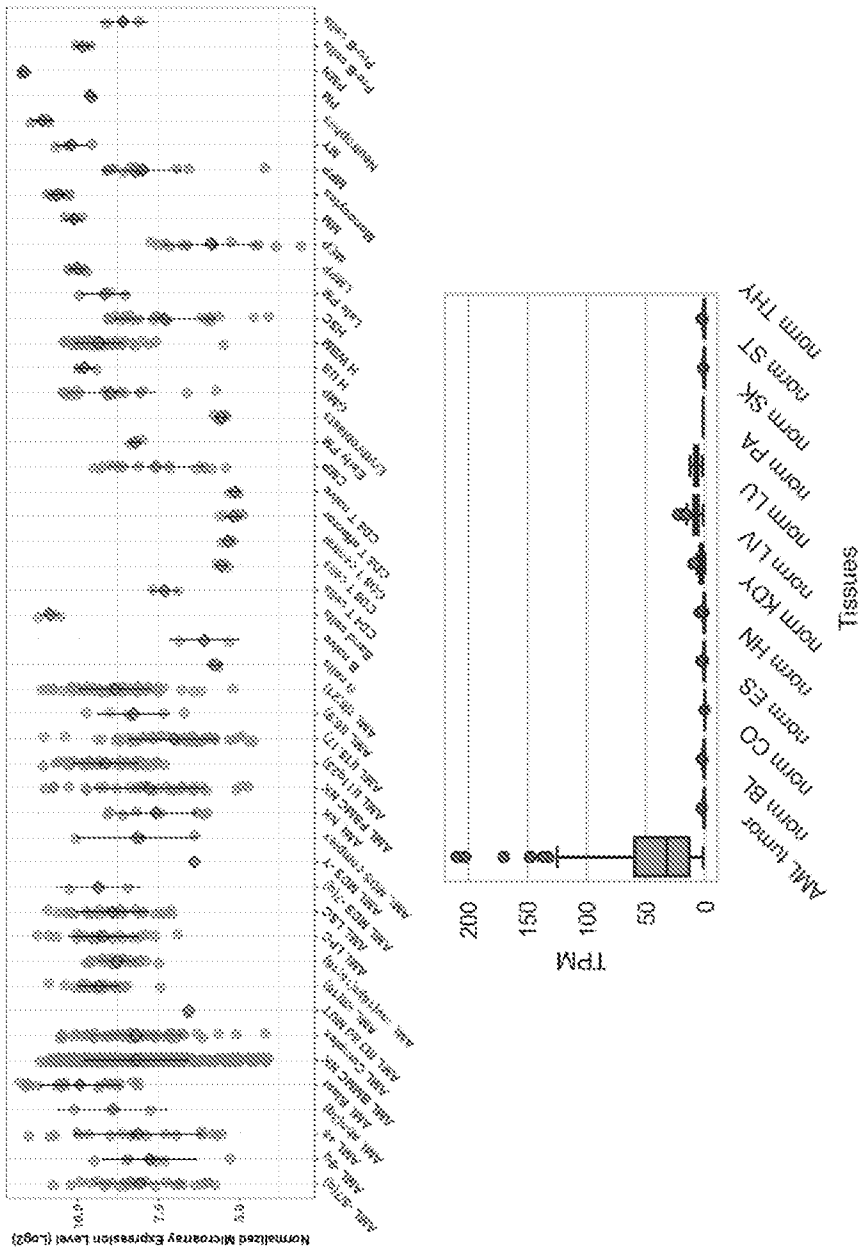


FIG. 15
SLC17A9

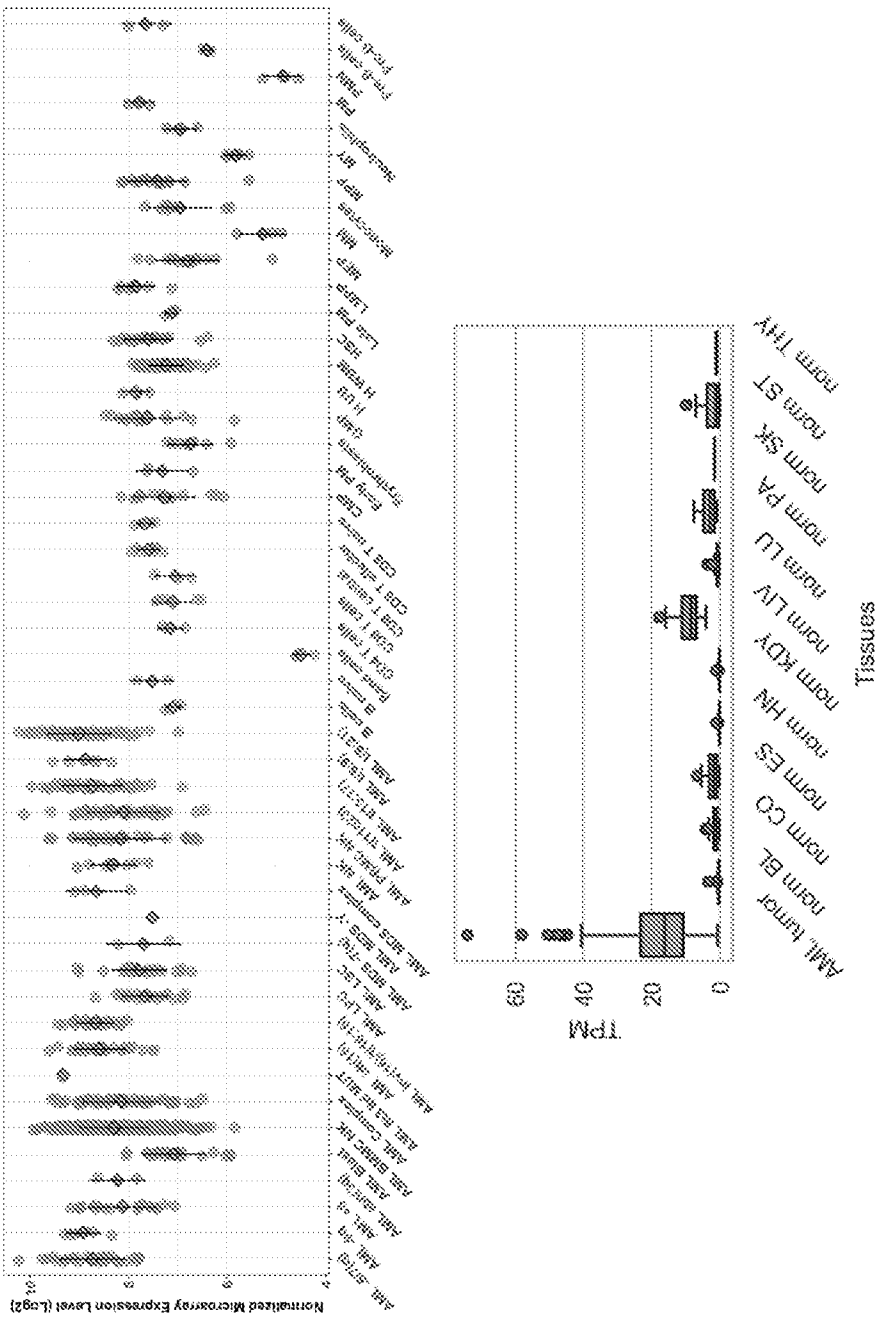


FIG. 16
MYADM

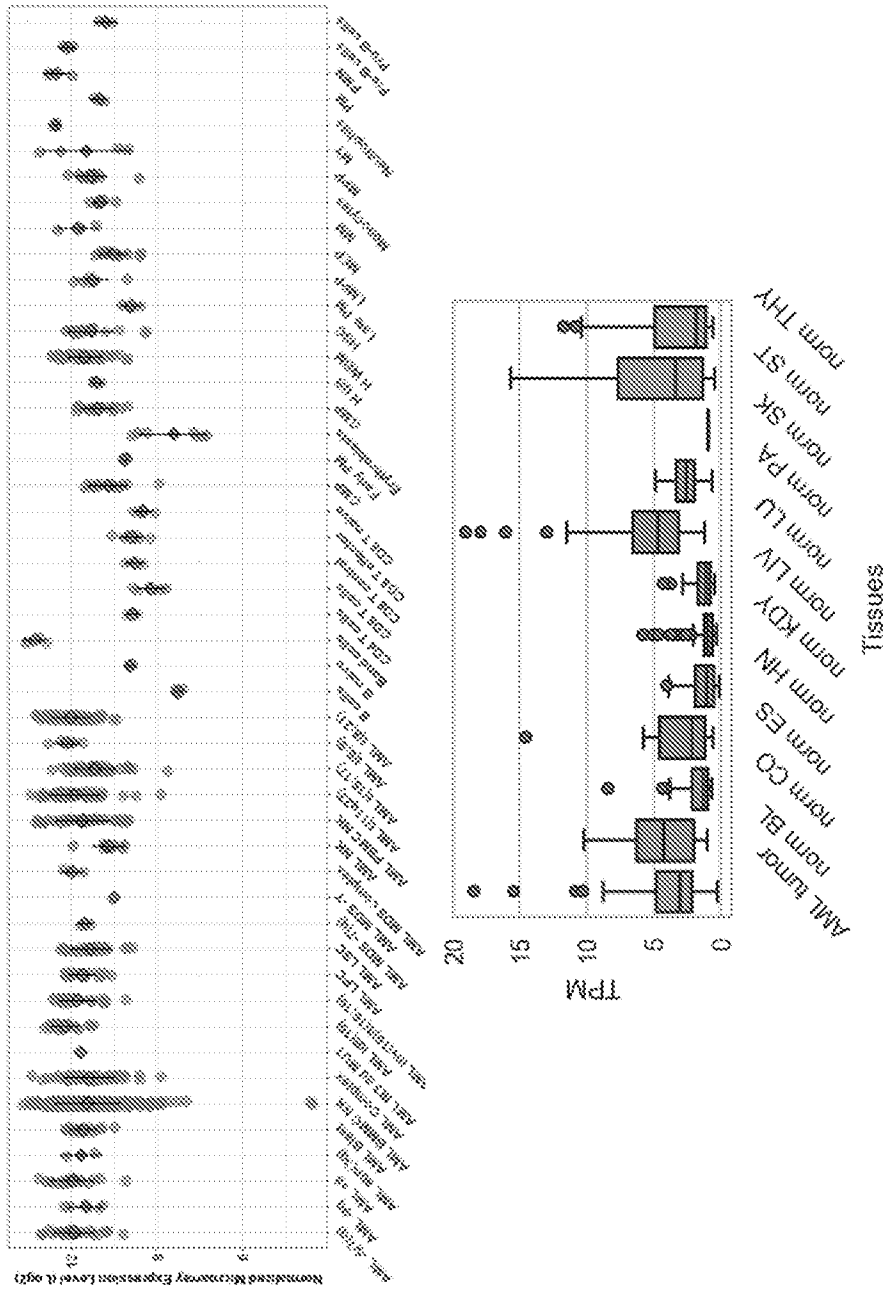

TPM
0
5
10
15
20
AML tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues

FIG. 17
CD300LF

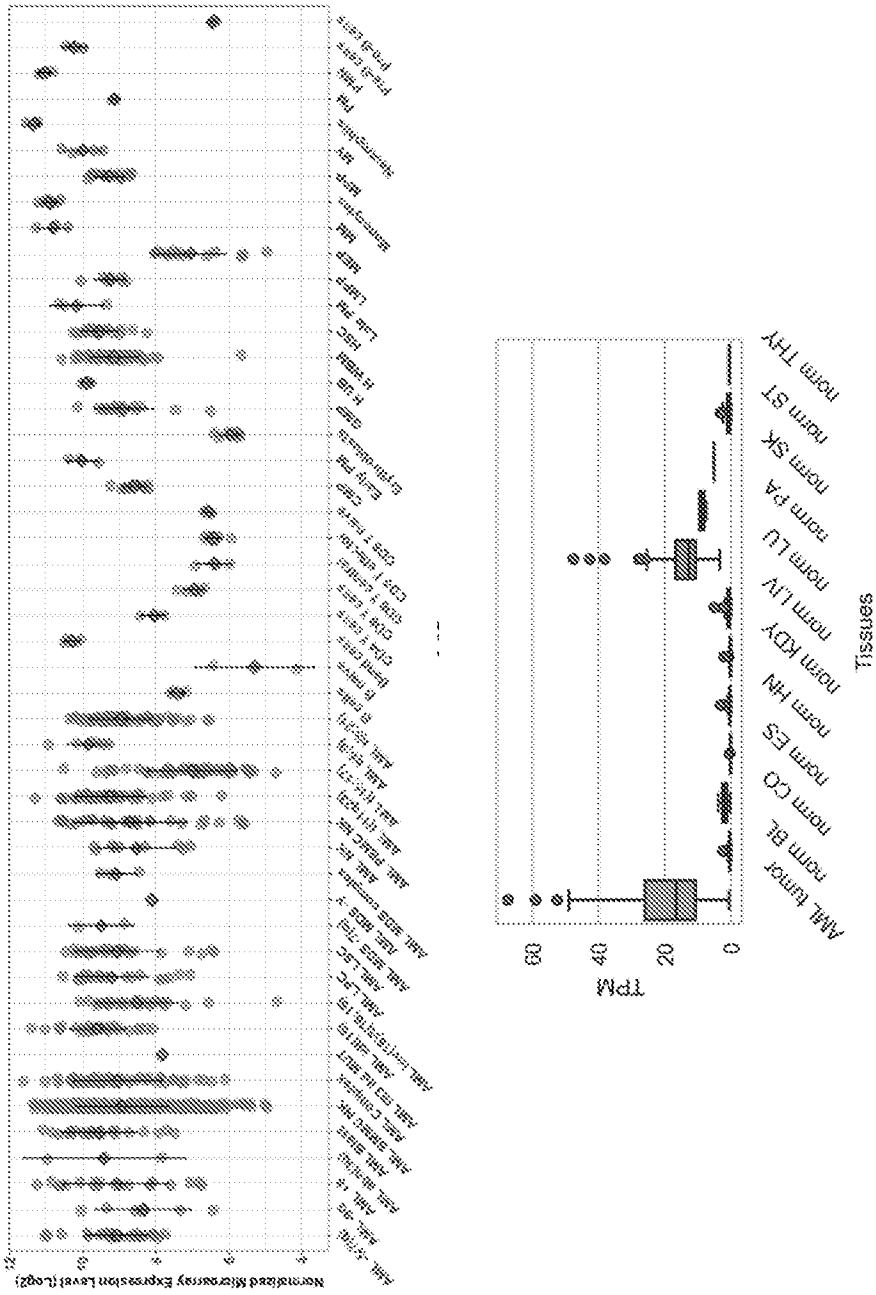


FIG. 18
CD244/SLAMF4

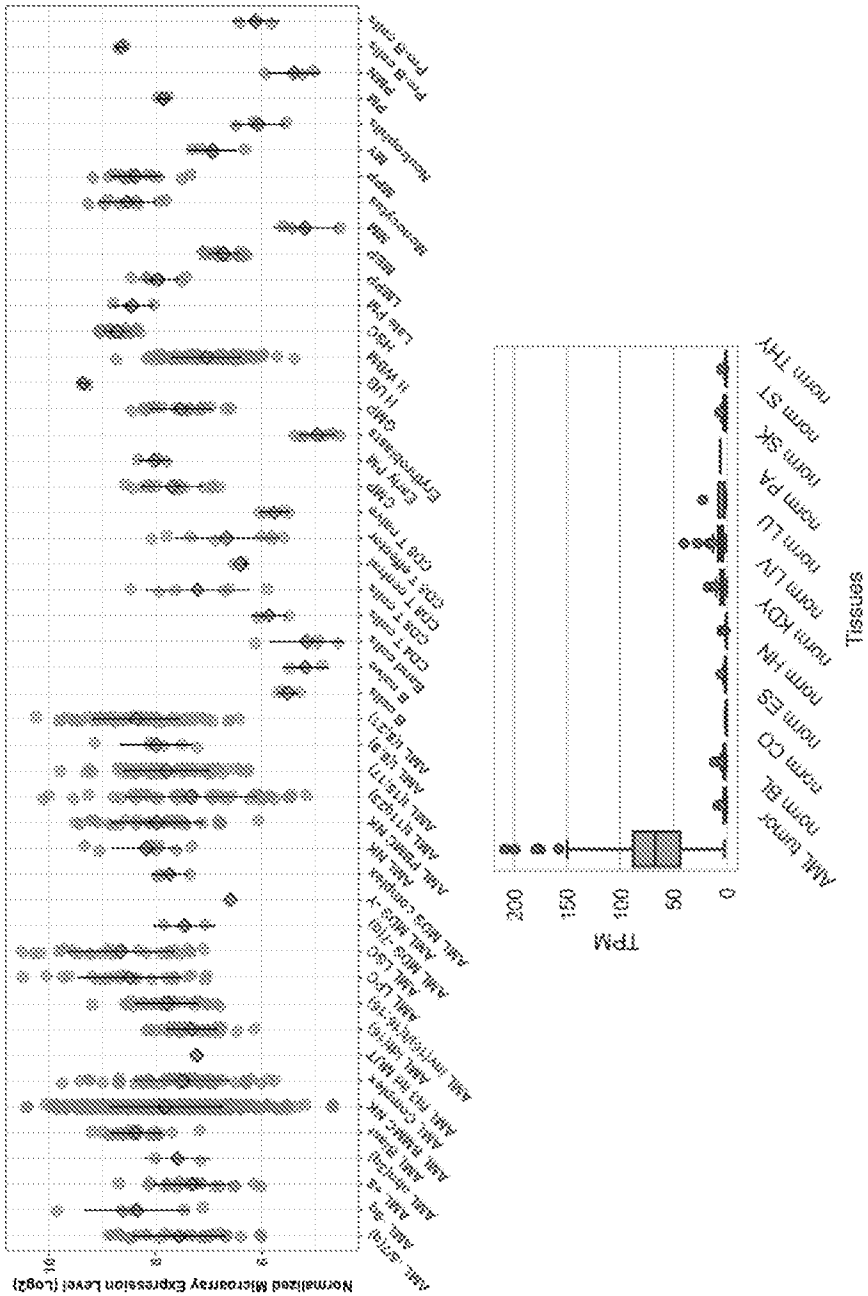


FIG. 19
PLAUR

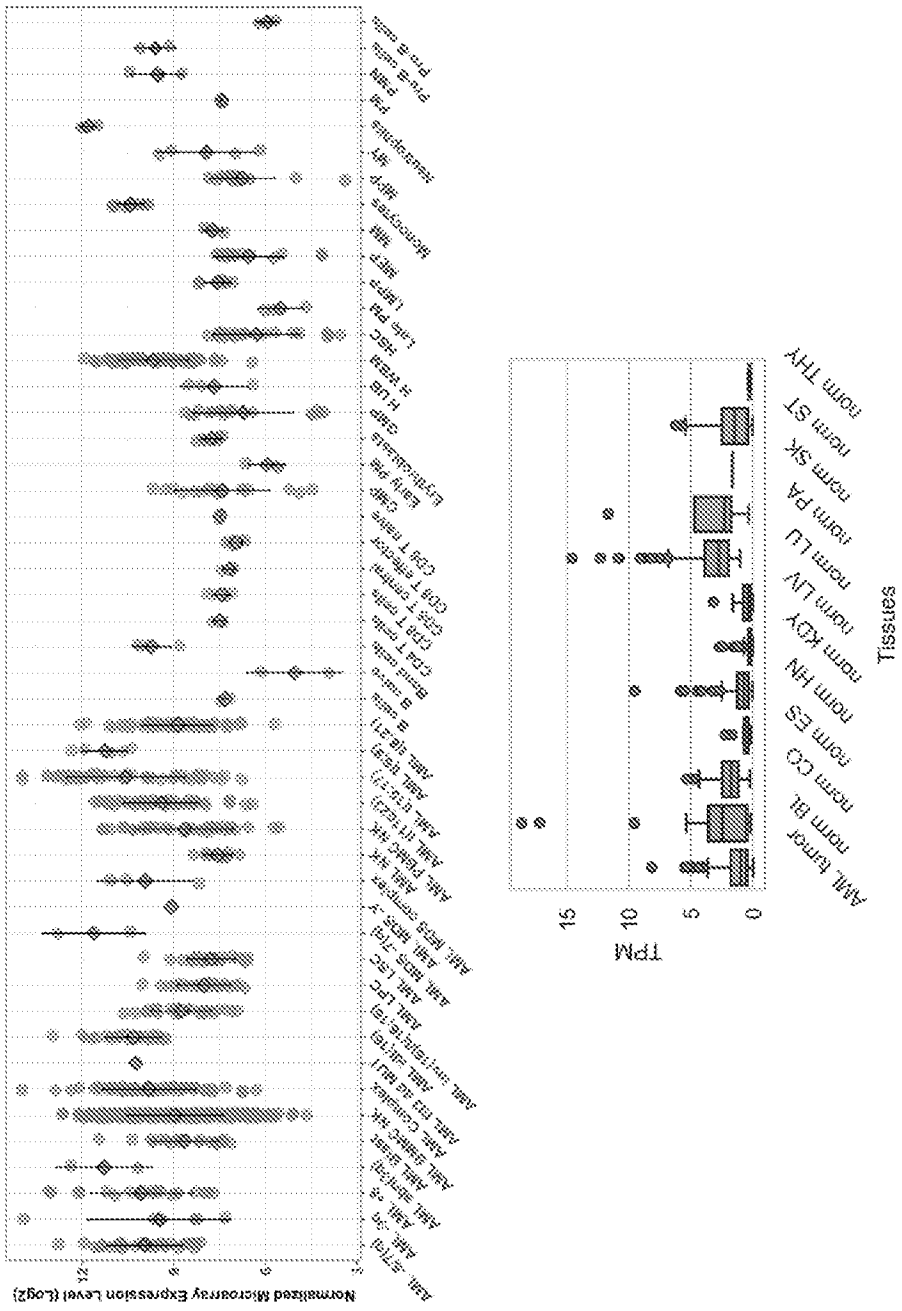

AML Tumor
norm Bl
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues
TPM
0
5
10
15
Normalized Microarray Expression Level (Log2)
0
5
10
15

FIG. 20
CD93

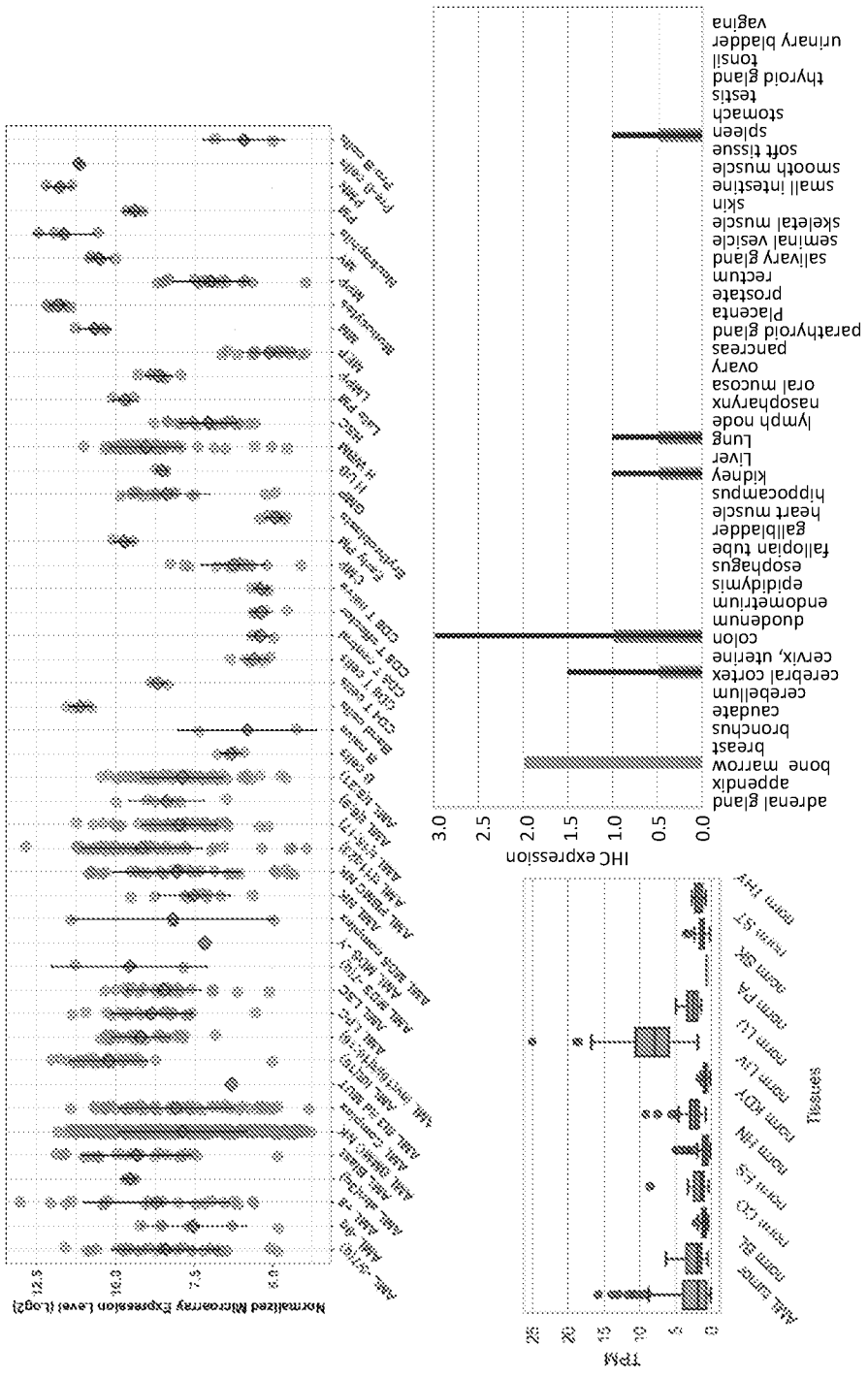

Normalized Microarray Expression Level (Log2)
TPM
IHC expression
Tissues
adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
rectum
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina

FIG. 21
SPNS3

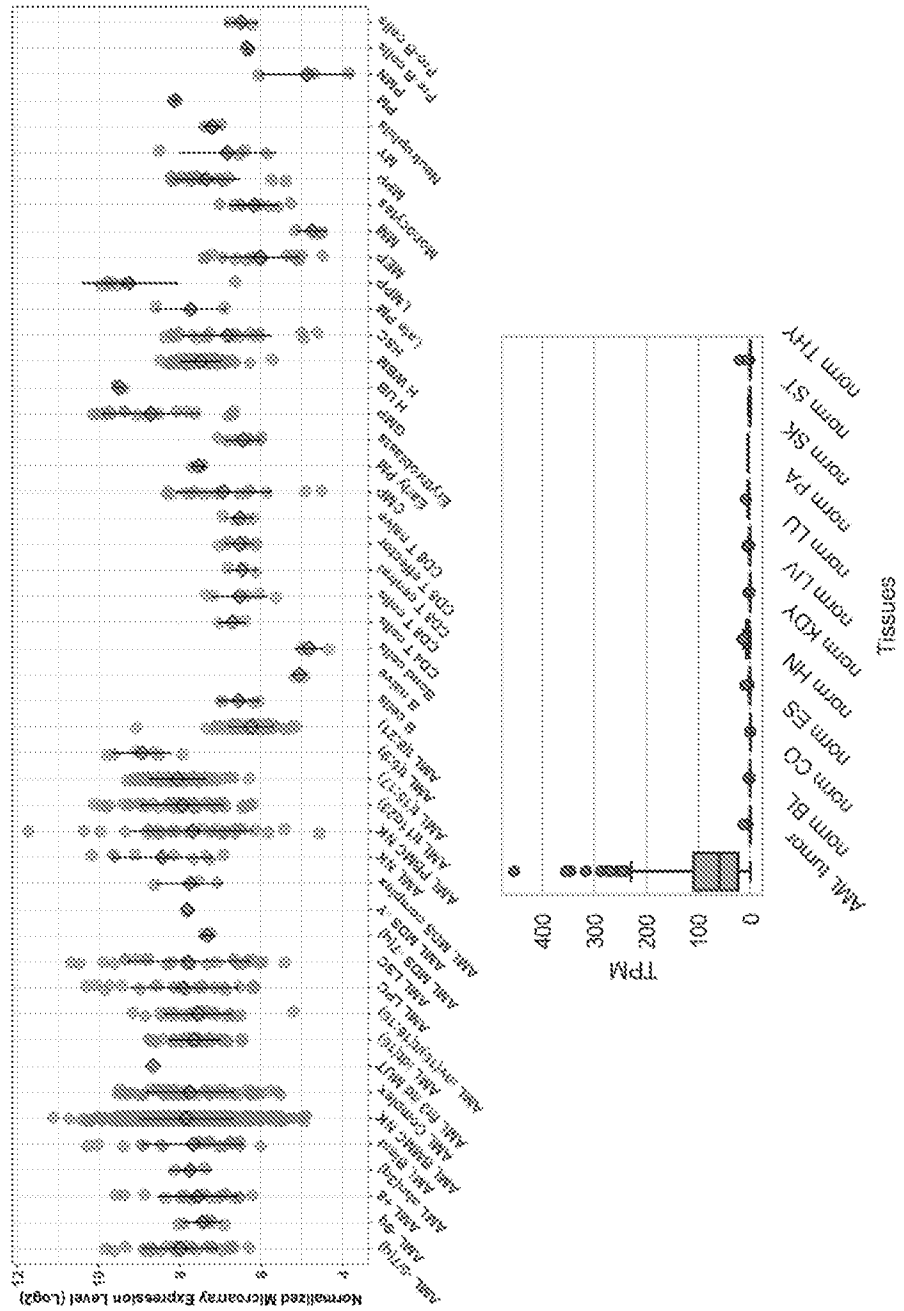

Normalized Microarray Expression Level (Log2)
TPM
Tissues
AML tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY

FIG. 22
GAPT

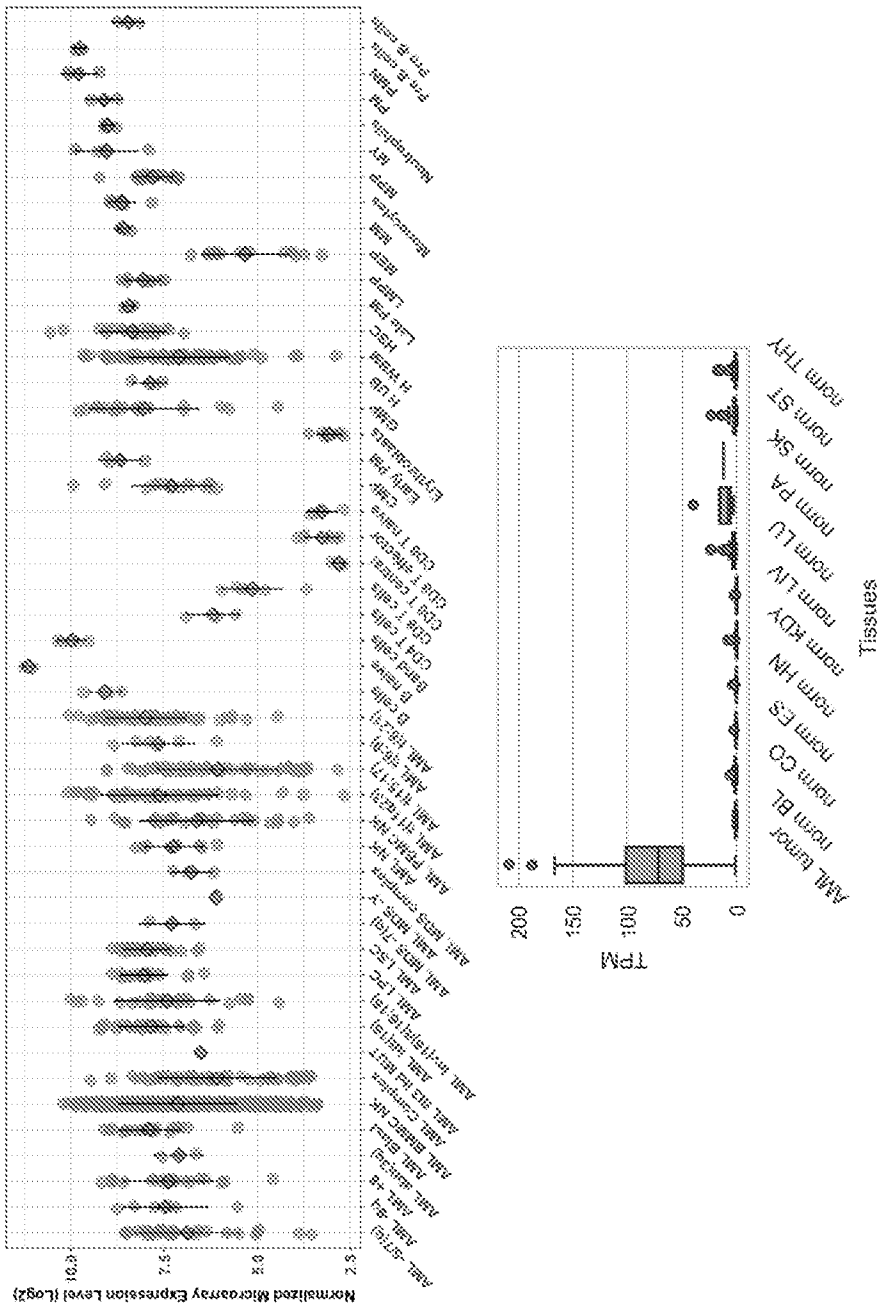


FIG. 23
RASGRP4

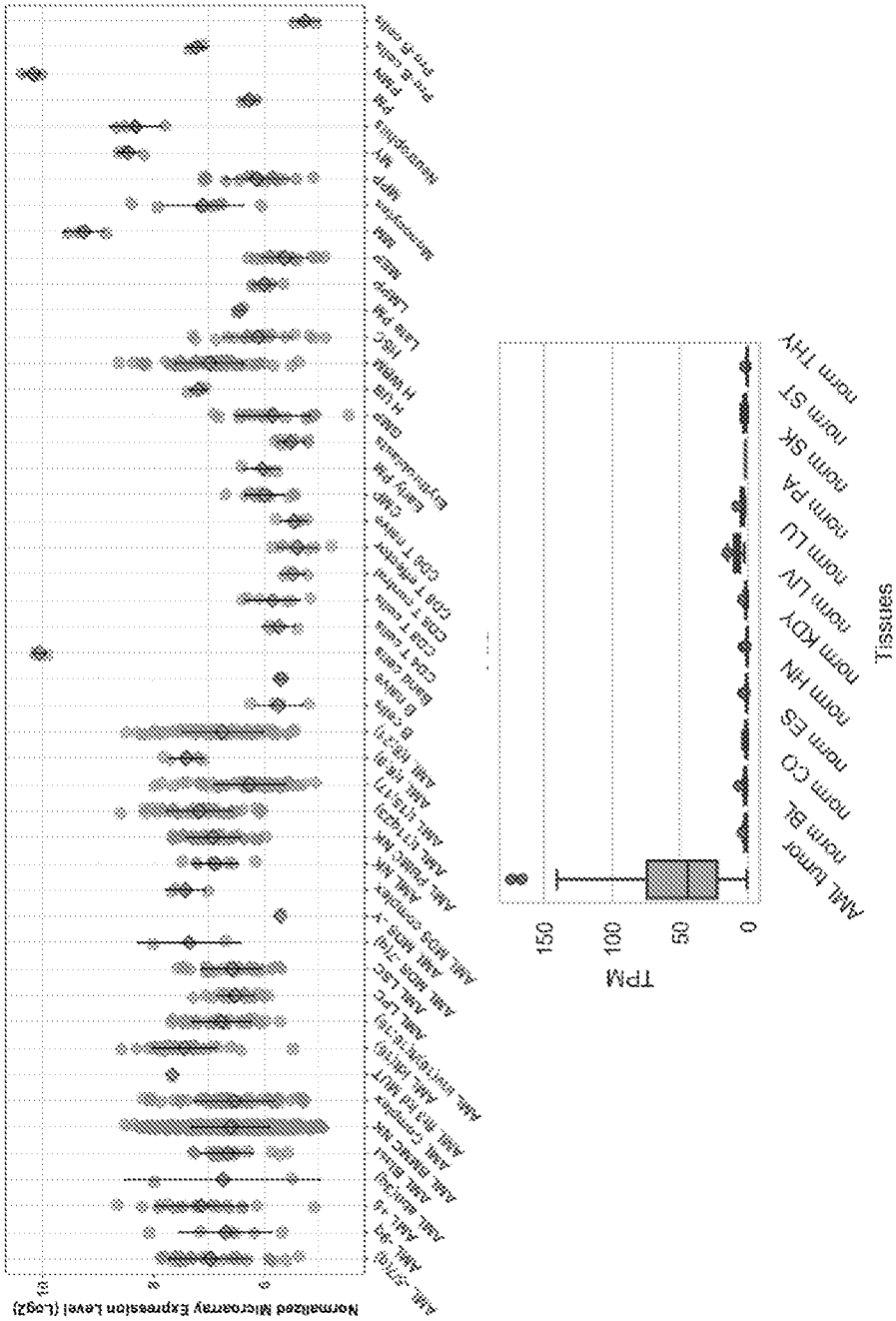


FIG. 24
CD117/C-Kit

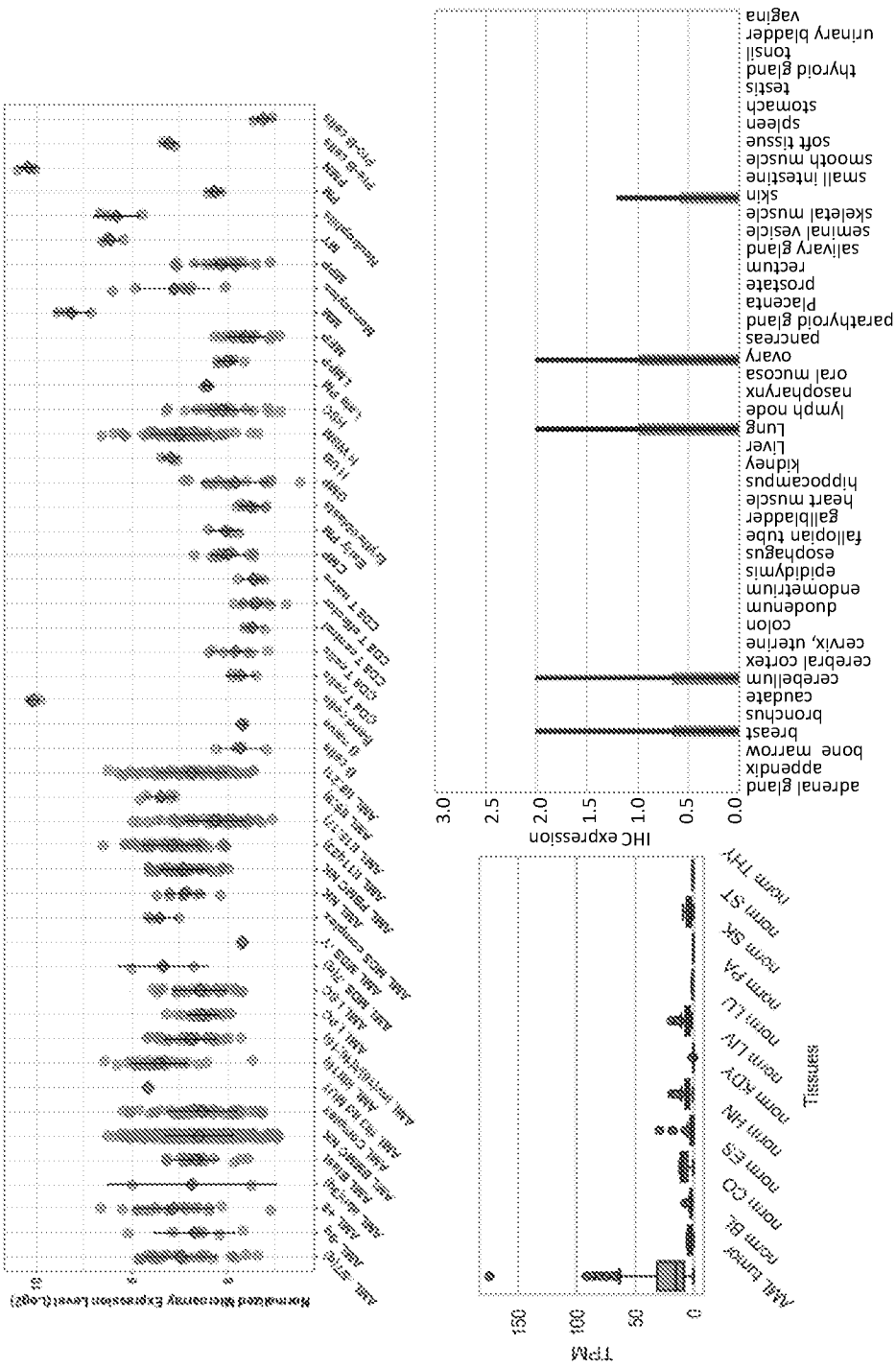


FIG. 25
CD123/ILR3RA

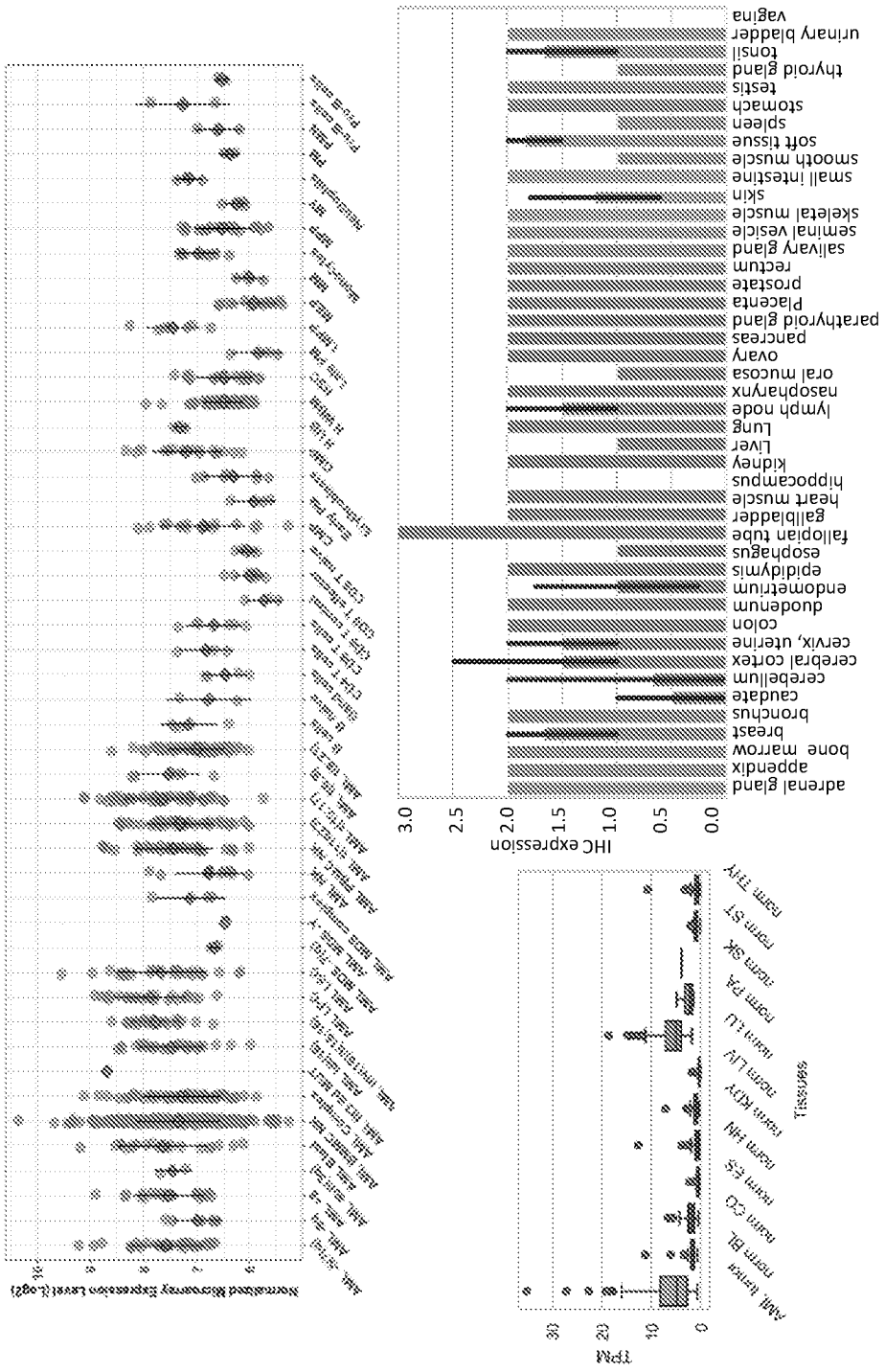


FIG. 26
EMCN (Endomucin)

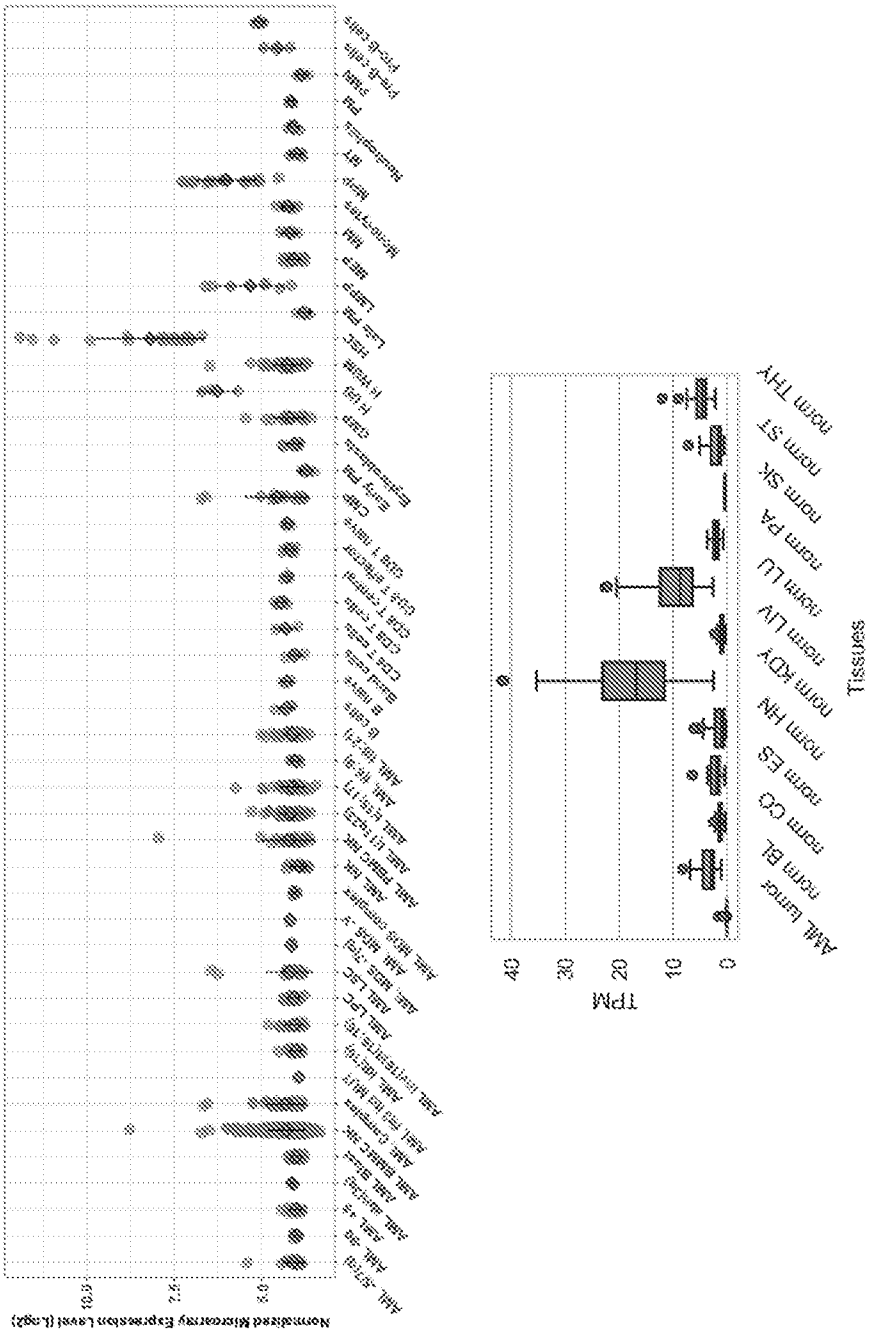


FIG. 27
JAM2

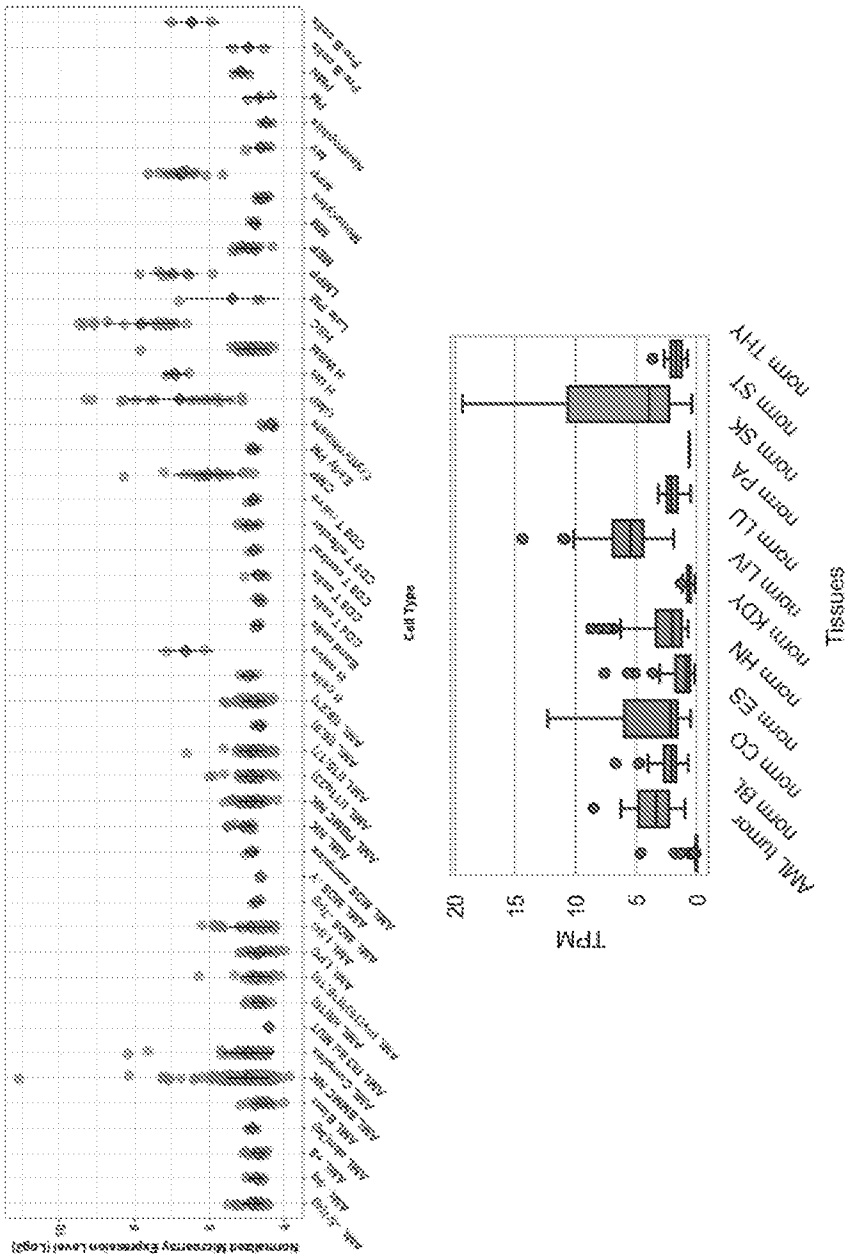

Cell Type
TPM
20
15
10
5
0
AML tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY
Tissues

FIG. 28
MS4A15

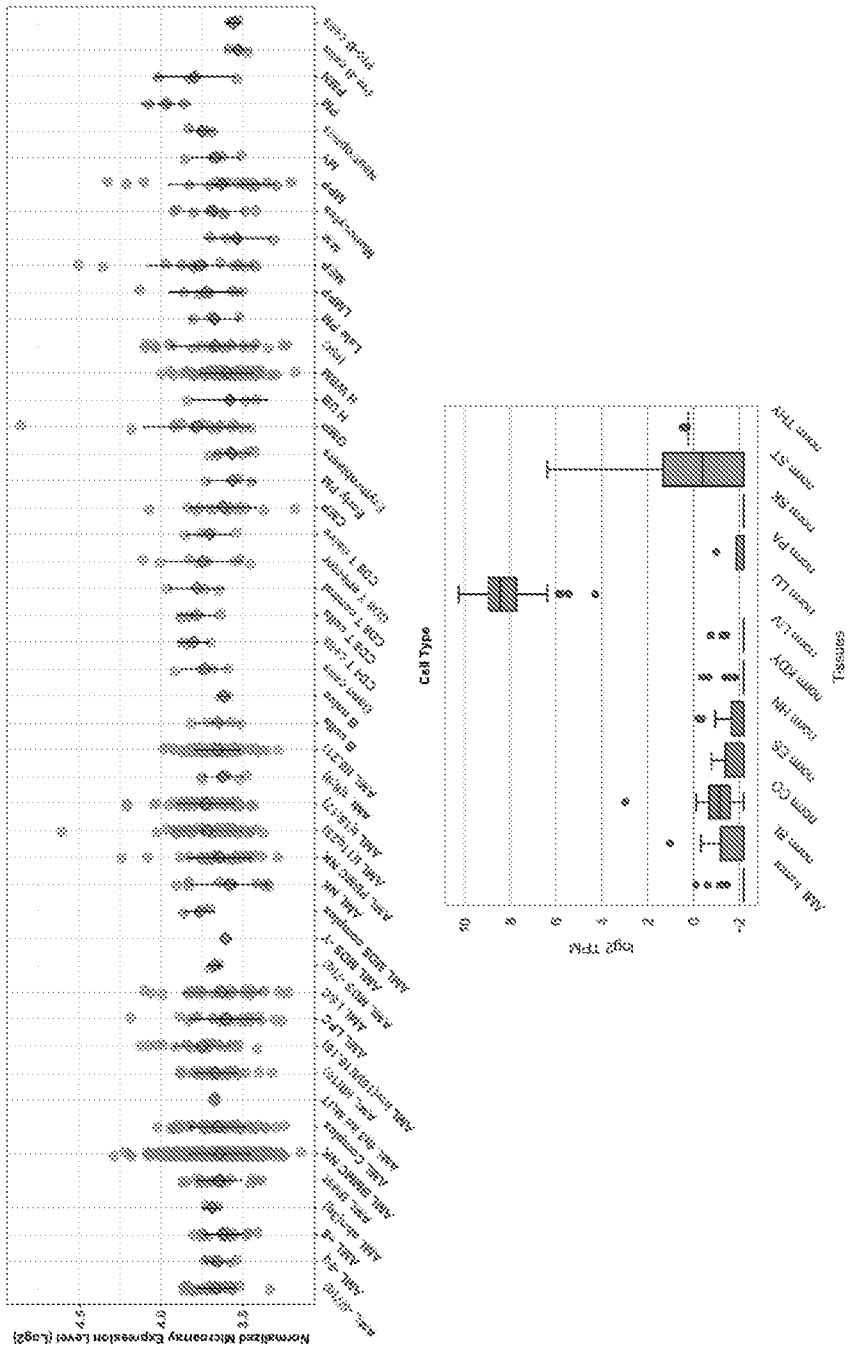


FIG. 29
SLC34A2

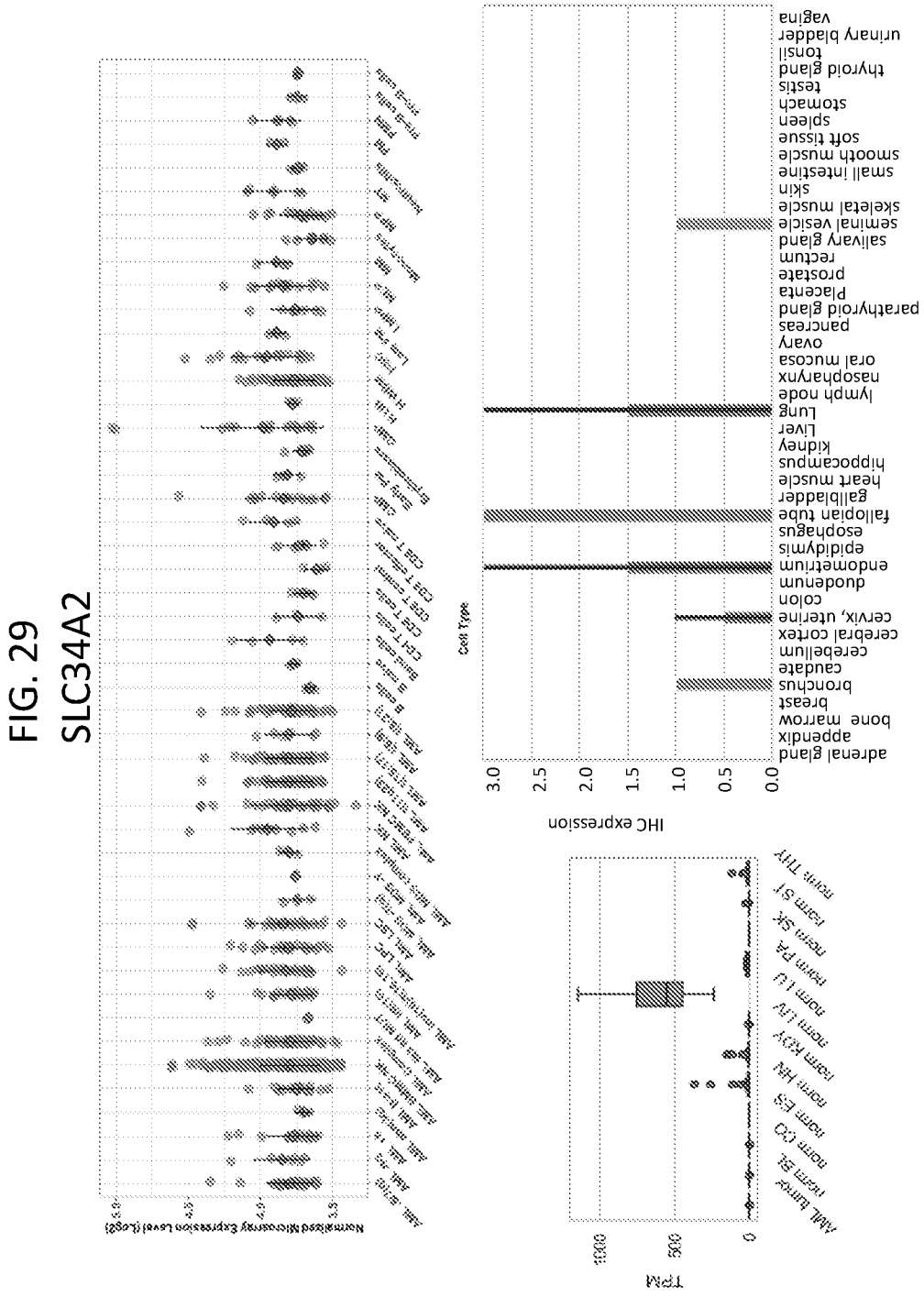


FIG. 30
SLC2A2

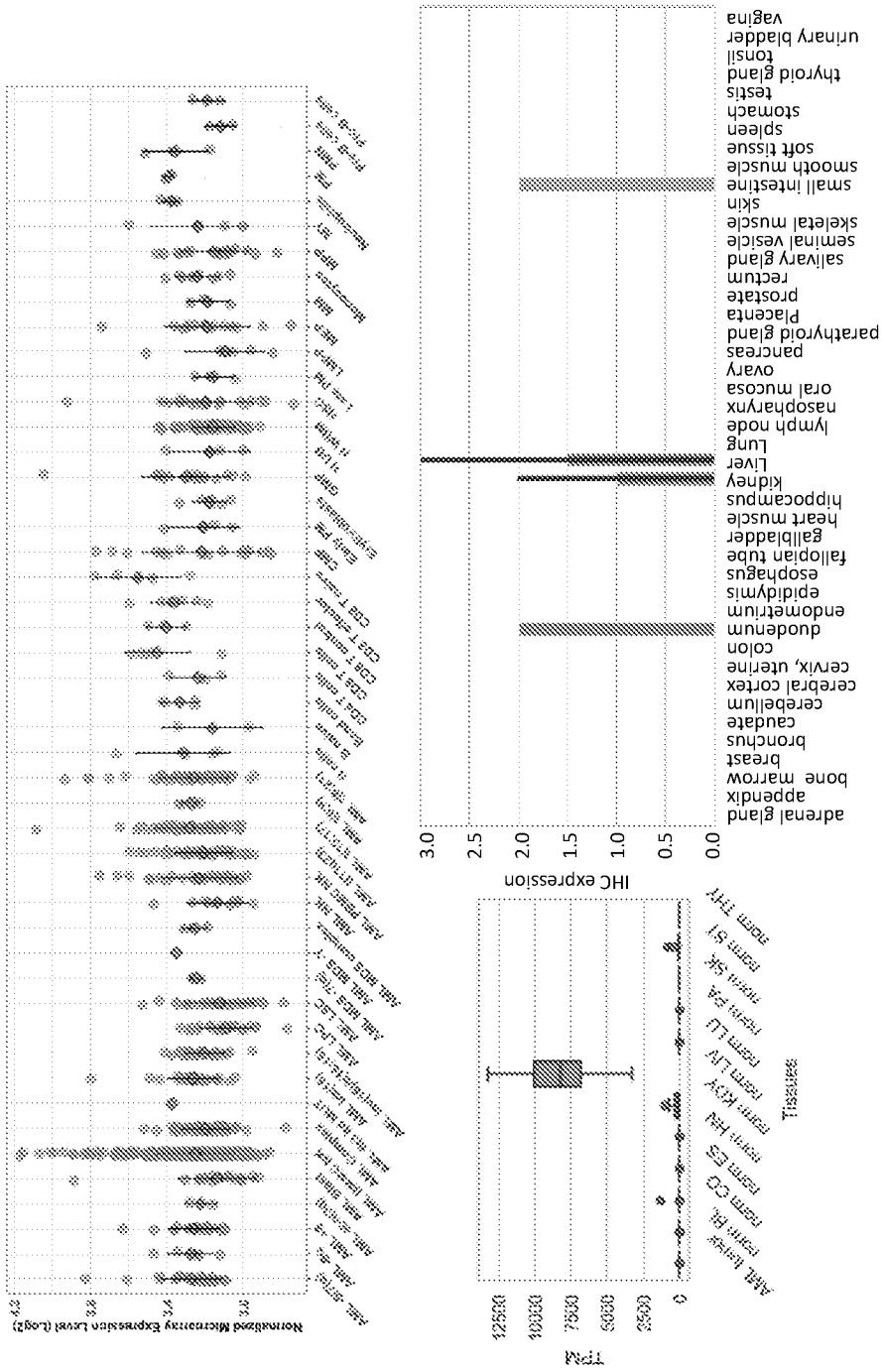


FIG. 31
TRPM1

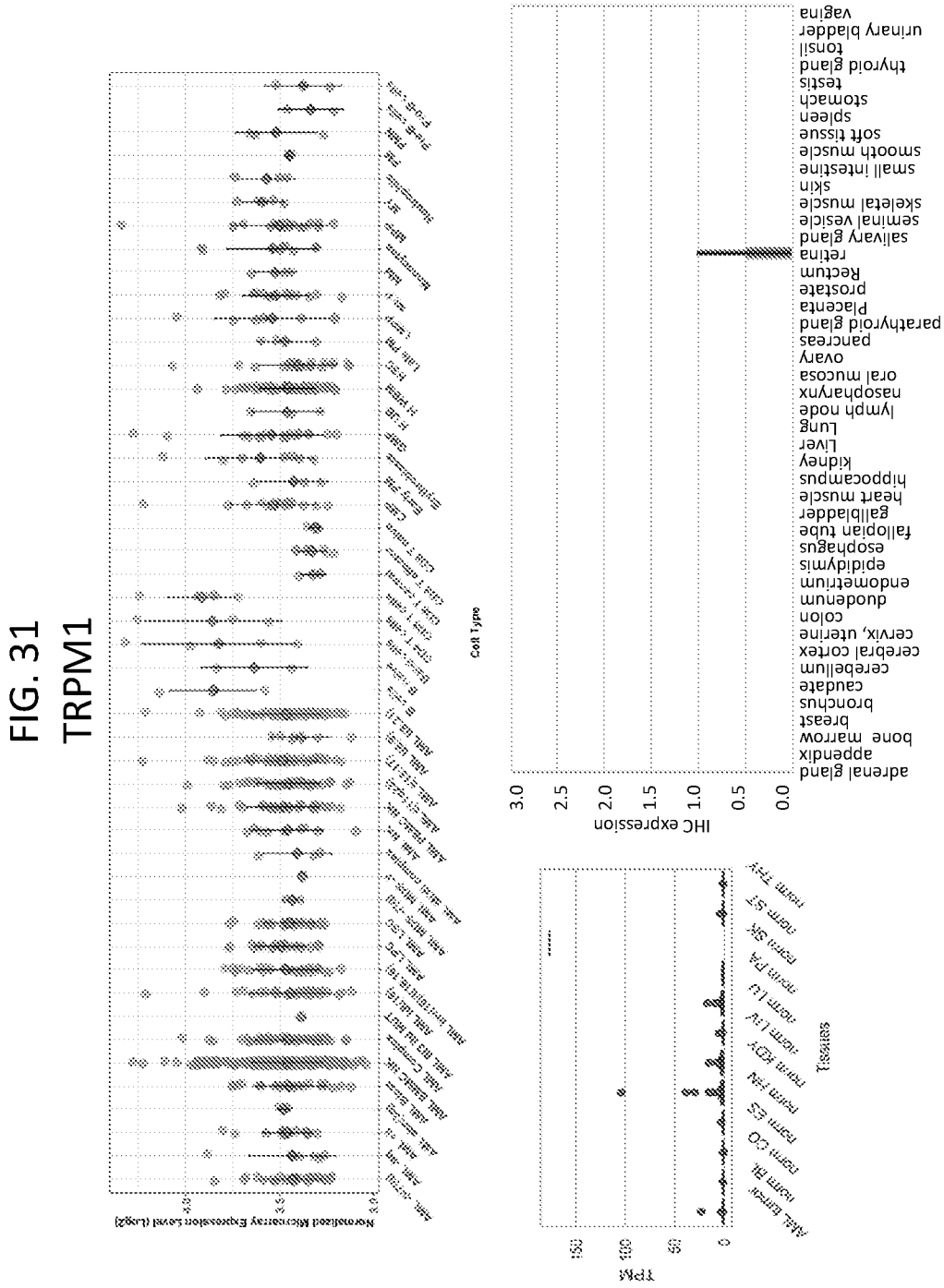

Normalized Microarray Expression Level (Log2)
Cell Type
IHC expression
adrenal gland
appendix
bone marrow
breast
bronchus
caudate
cerebellum
cerebral cortex
cervix, uterine
colon
duodenum
endometrium
epididymis
esophagus
fallopian tube
gallbladder
heart muscle
hippocampus
kidney
Liver
Lung
lymph node
nasopharynx
oral mucosa
ovary
pancreas
parathyroid gland
Placenta
prostate
Rectum
retina
salivary gland
seminal vesicle
skeletal muscle
skin
small intestine
smooth muscle
soft tissue
spleen
stomach
testis
thyroid gland
tonsil
urinary bladder
vagina
TPM
Tissues

FIG. 32
SCTR

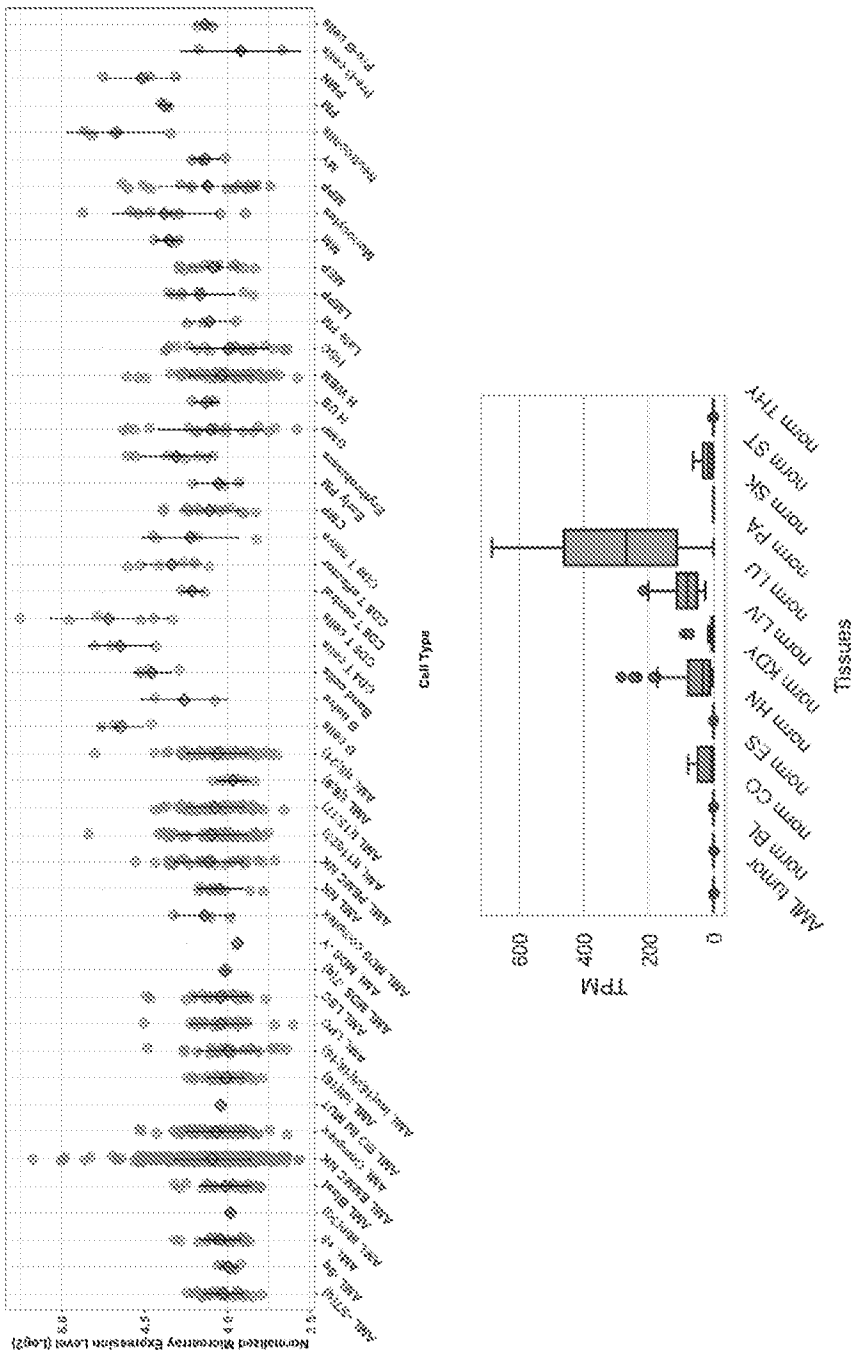

Normalized Microarray Expression Level (Log2)
Cell Type
TPM
Tissues
All tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY

FIG. 33
KCNQ2

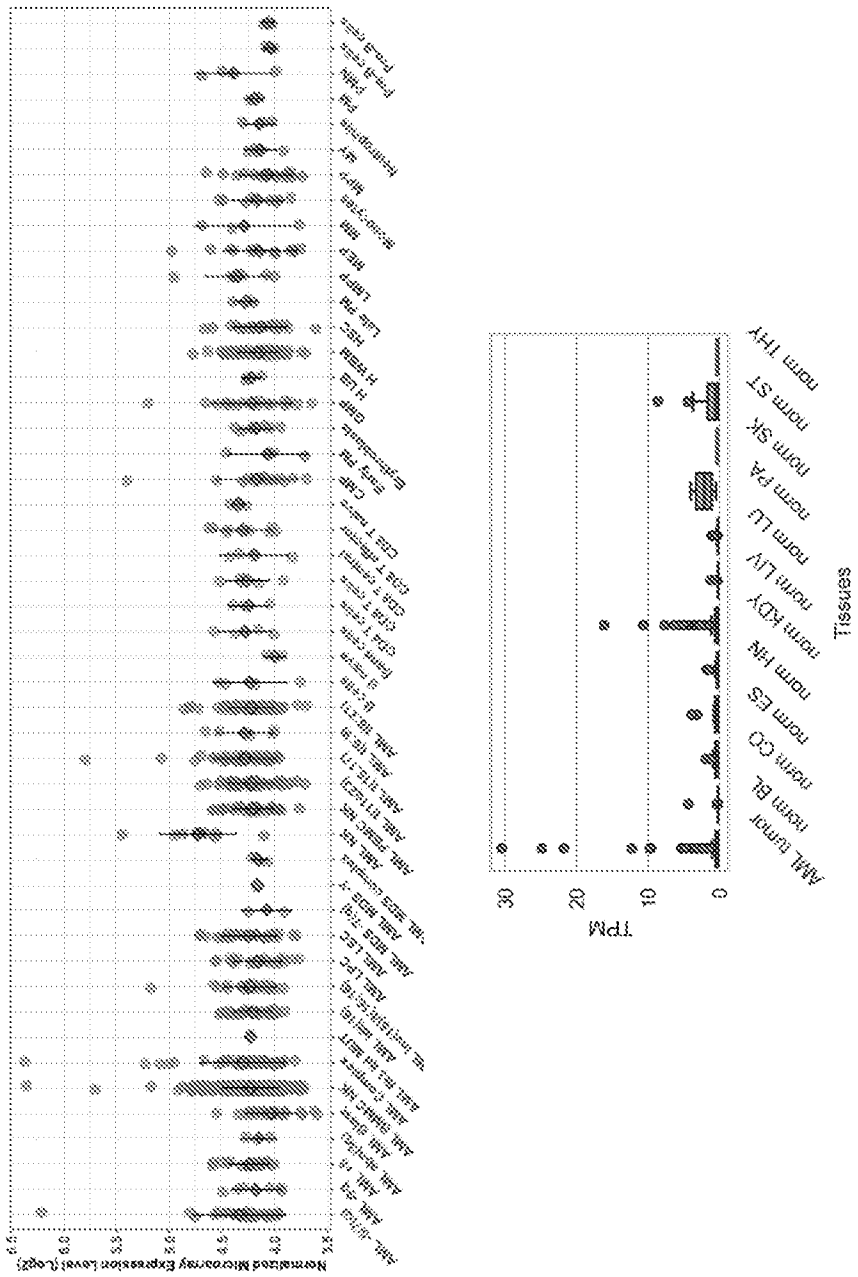

Normalized Microarray Expression Level (Log2)
TPM
Tissues
All_tumor
norm_BL
norm_CO
norm_ES
norm_HN
norm_KDY
norm_LIV
norm_LU
norm_PA
norm_SK
norm_ST
norm_THY

FIG. 34
PERP

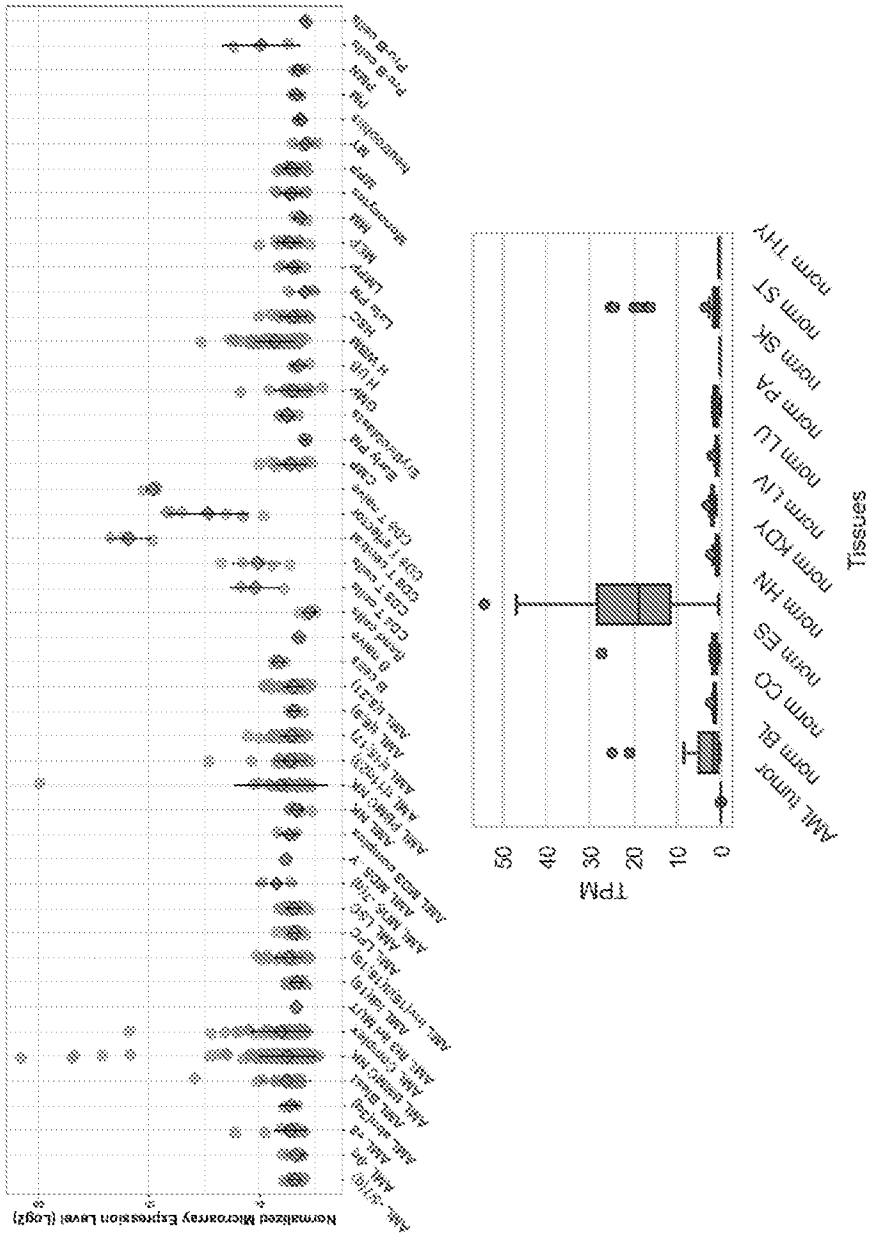

Normalized Microarray Expression Level (Log2)
TPM
Tissues
AML tumor
norm BL
norm CO
norm ES
norm HN
norm KDY
norm LIV
norm LU
norm PA
norm SK
norm ST
norm THY

FIG. 35A

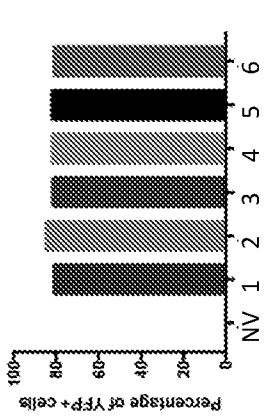


FIG. 35B

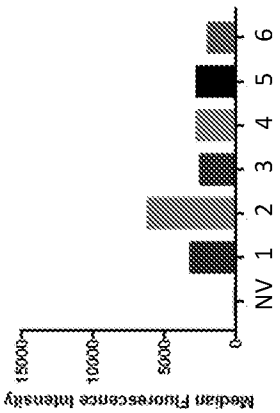


FIG. 35C

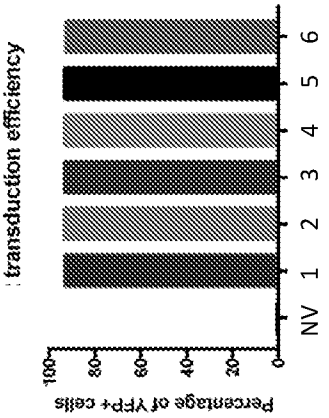


FIG. 35D

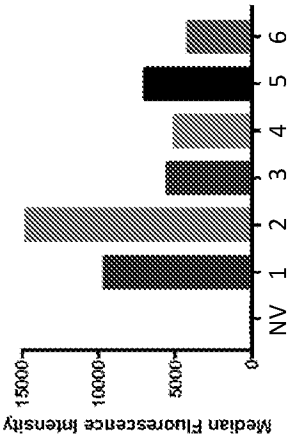


FIG. 36A

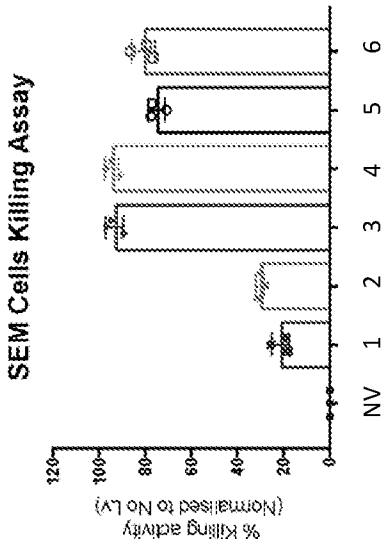


FIG. 36B

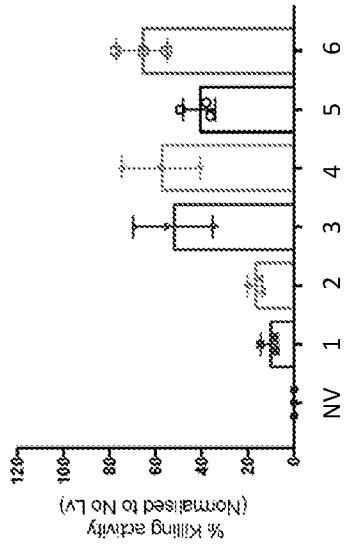


FIG. 36C

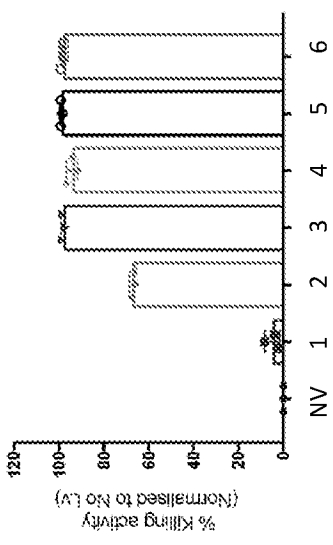
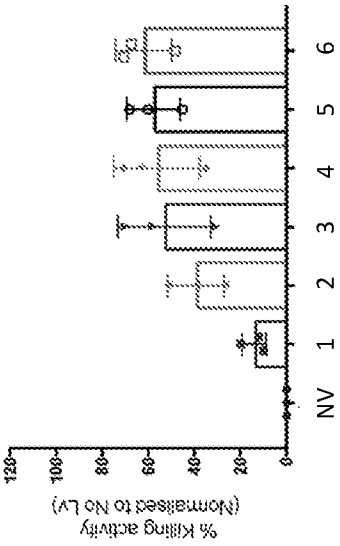


FIG. 36D



36

FIG. 37A
TNF-α

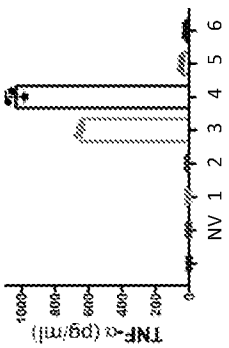


FIG. 37B
IFN-γ

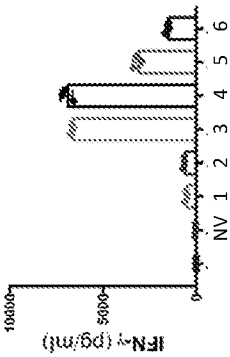


FIG. 37C
IL-2

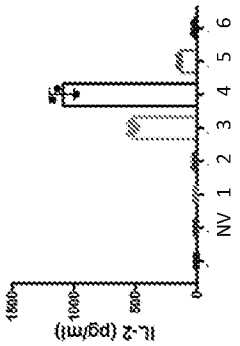


FIG. 37D
TNF-α

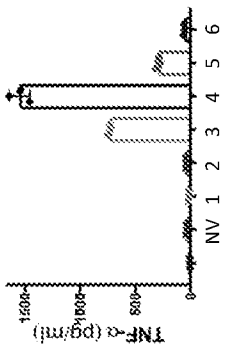


FIG. 37E
IFN-γ

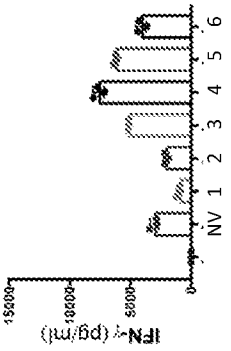
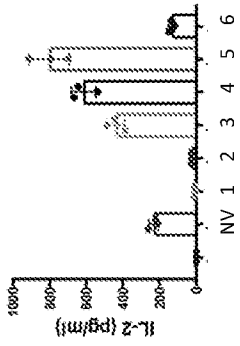


FIG. 37F
IL-2



37

FIG. 38A
TNF-α

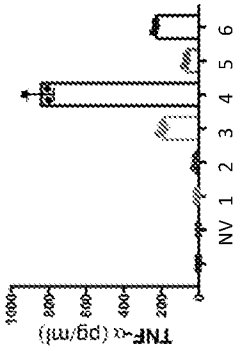


FIG. 38B
IFN-γ

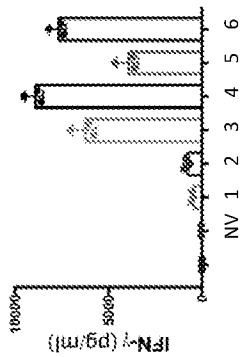


FIG. 38C
IL-2

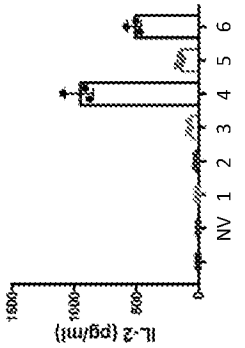


FIG. 38D
TNF-α

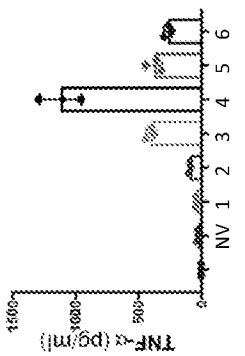


FIG. 38E
IFN-γ

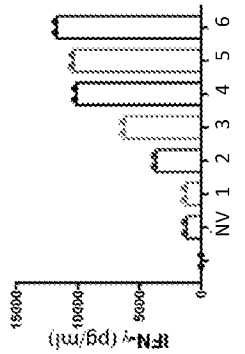
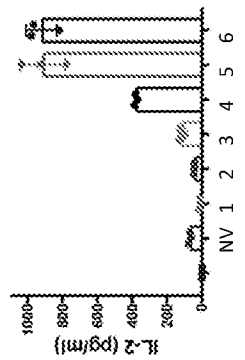


FIG. 38F
IL-2



38

FIG. 39

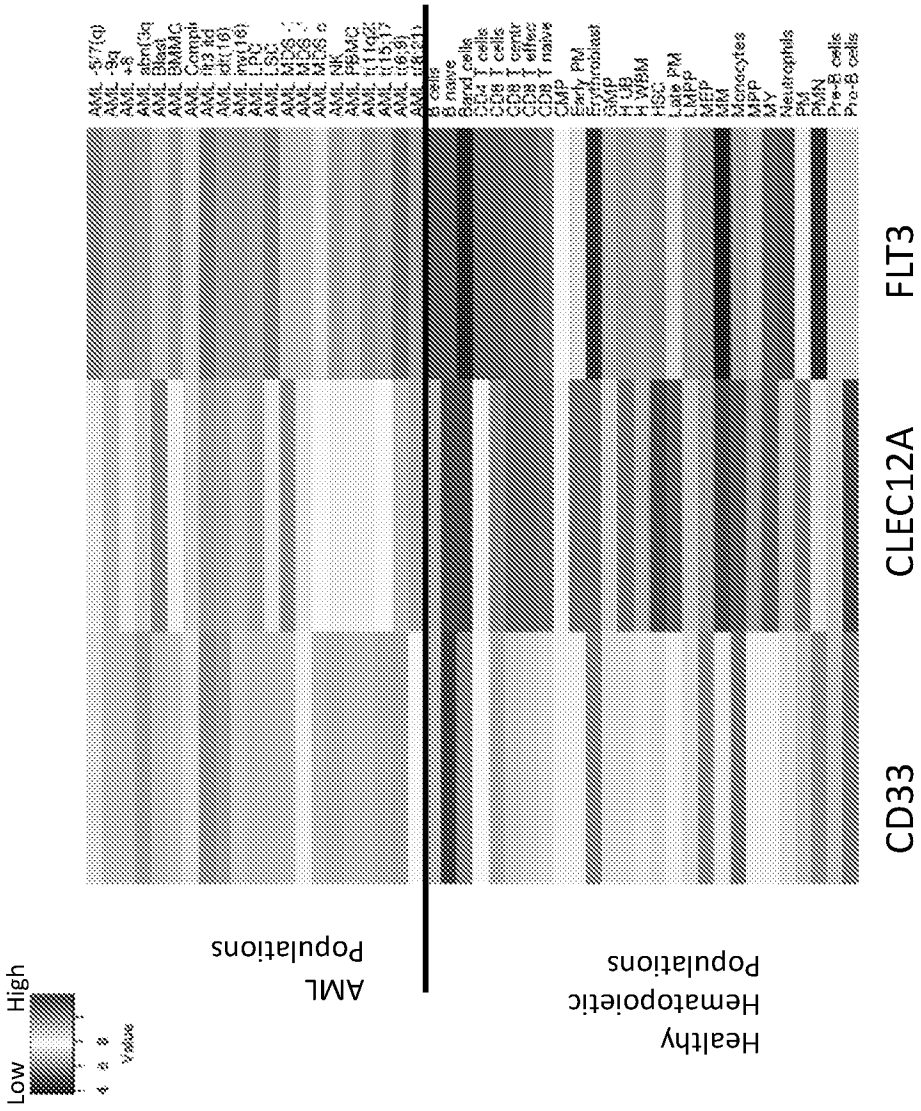

CD33
CLEC12A
FLT3
AML
Populations
Healthy
Hematopoietic
Populations
Low
High
Value

FIG. 40A

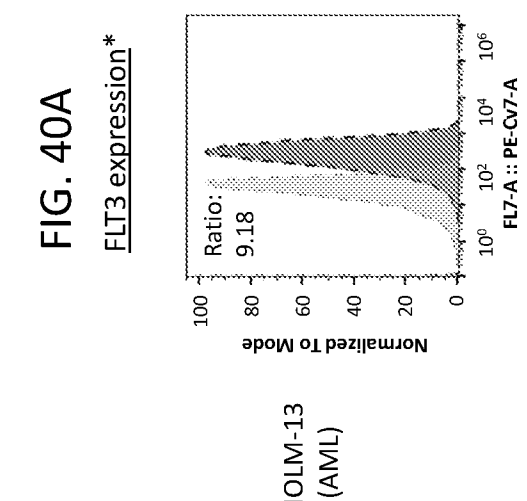


FIG. 40B

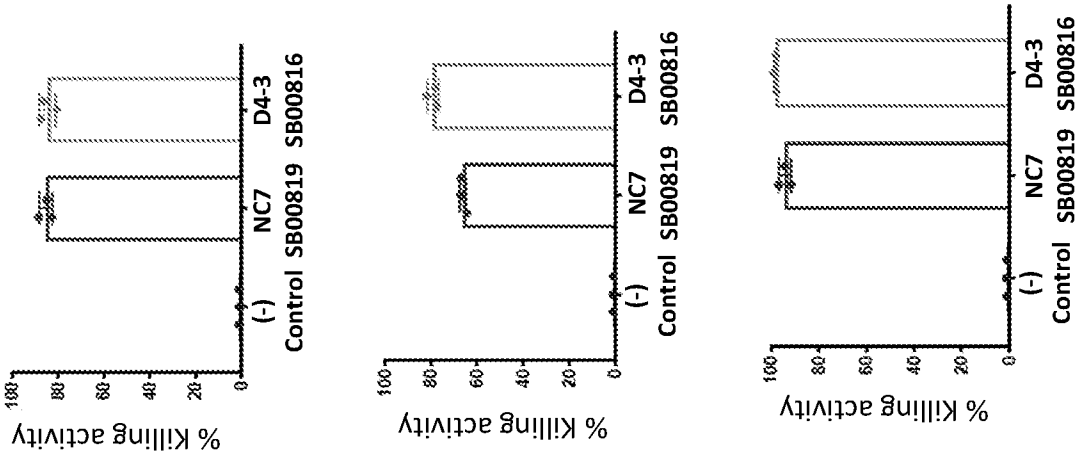


FIG. 40C

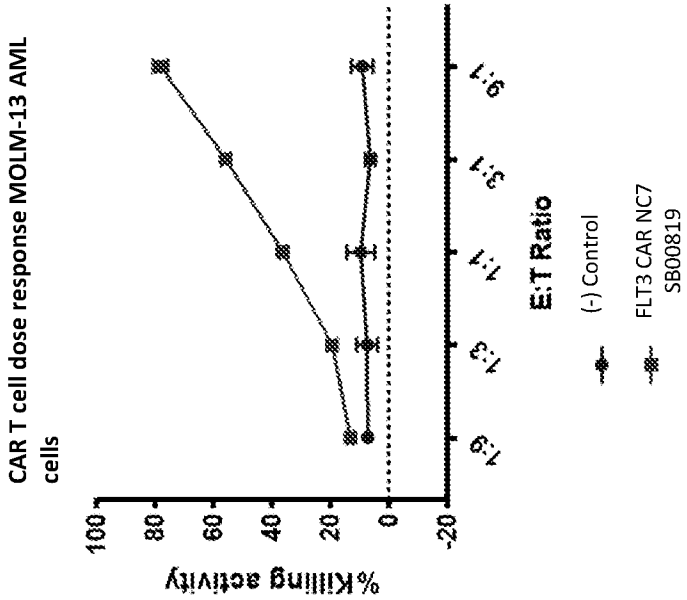


FIG. 41A

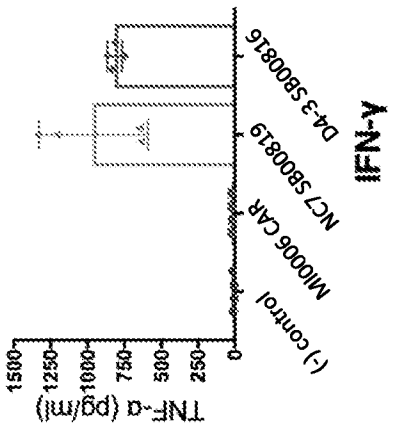


FIG. 41B

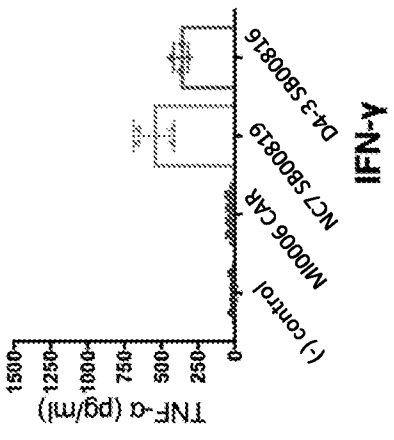


FIG. 41C

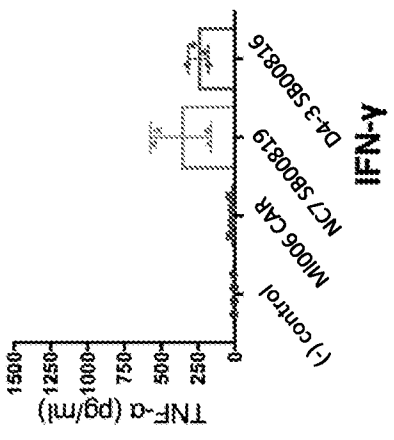


FIG. 42A

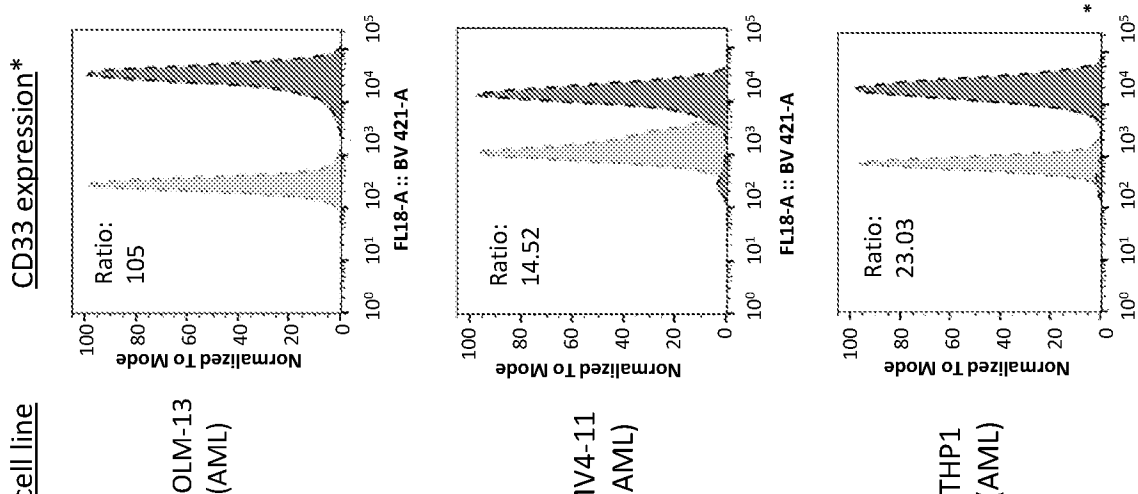


FIG. 42B

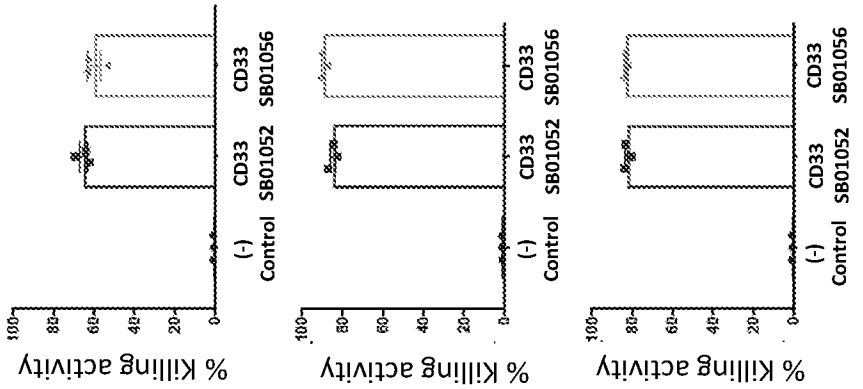


FIG. 42C

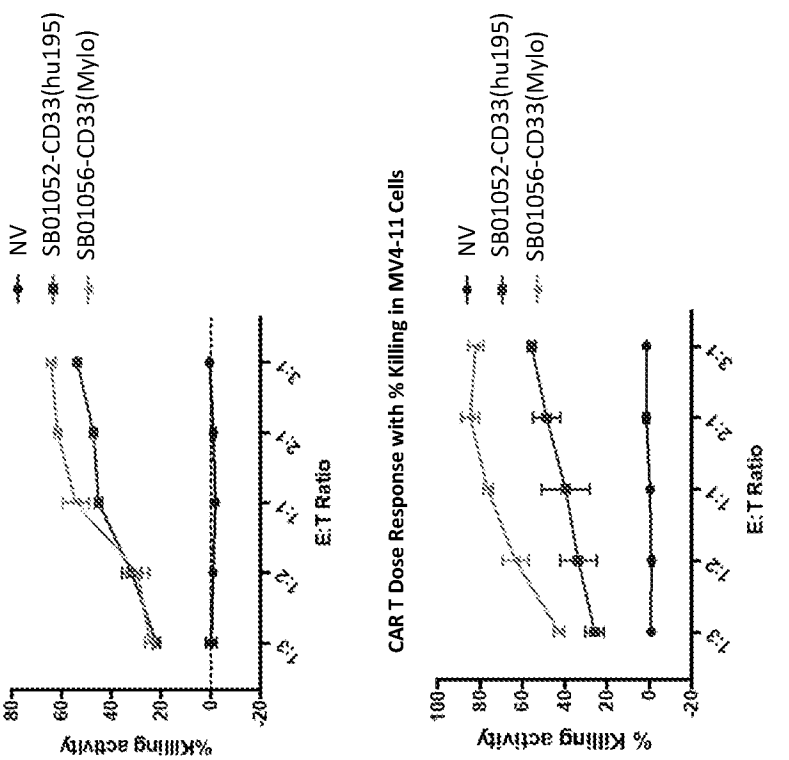


FIG. 43A

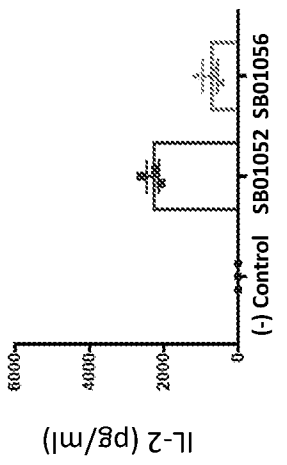


FIG. 43B

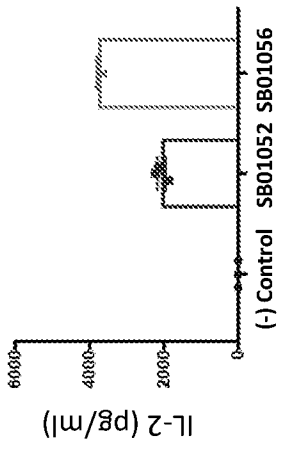


FIG. 43C

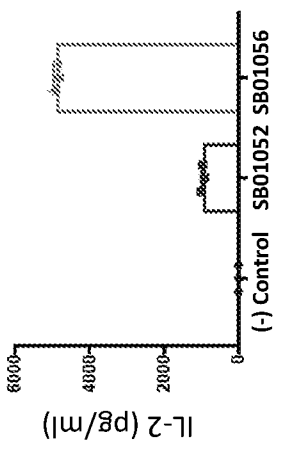


FIG. 44

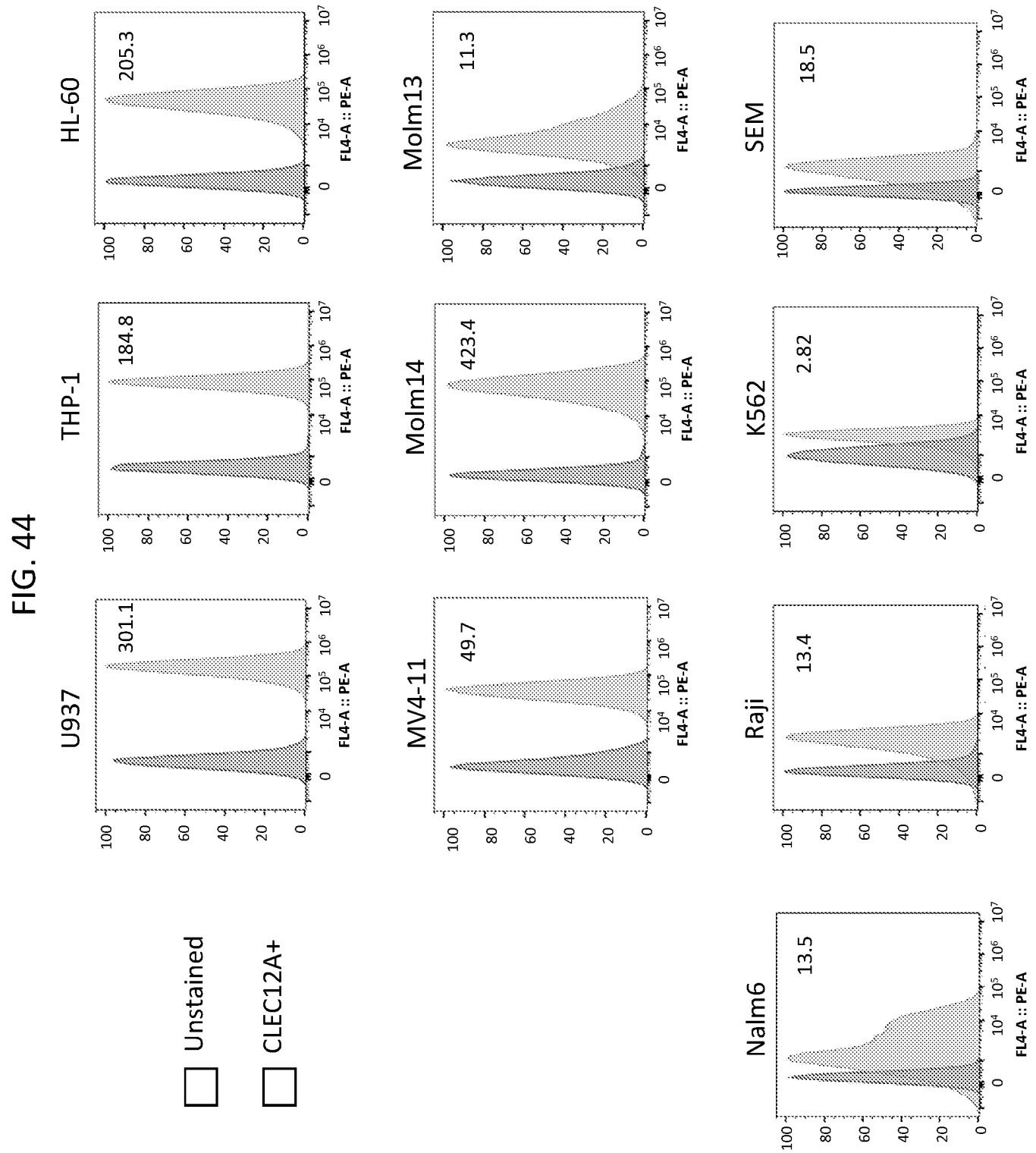
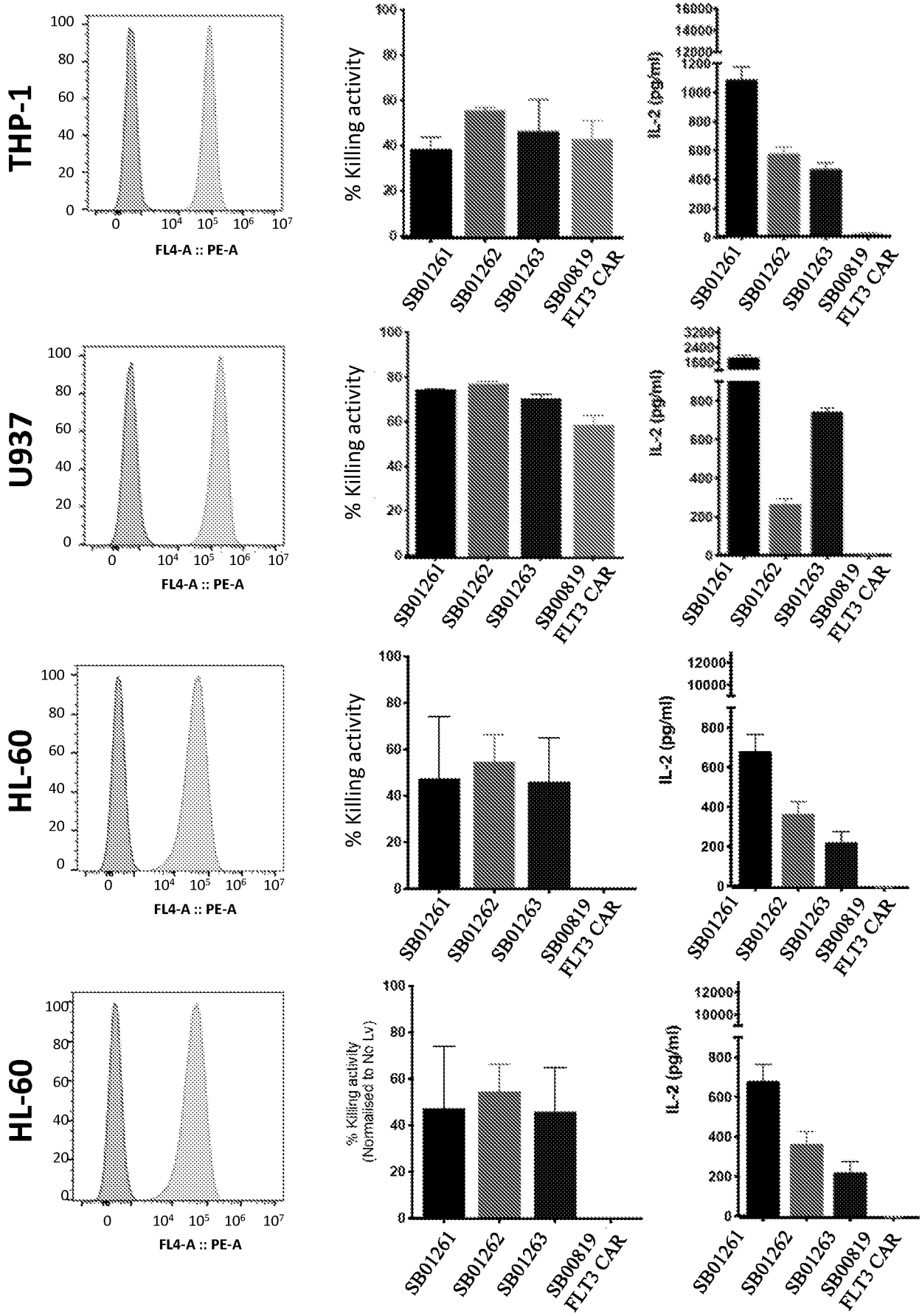

Unstained
CLEC12A+
U937
301.1
THP-1
184.8
HL-60
205.3
MV4-11
49.7
Molm14
423.4
Molm13
11.3
Raji
13.4
K562
2.82
SEM
18.5
Nalm6
13.5
FL4-A :: PE-A

FIG. 45A

FIG. 45B

FIG. 45C



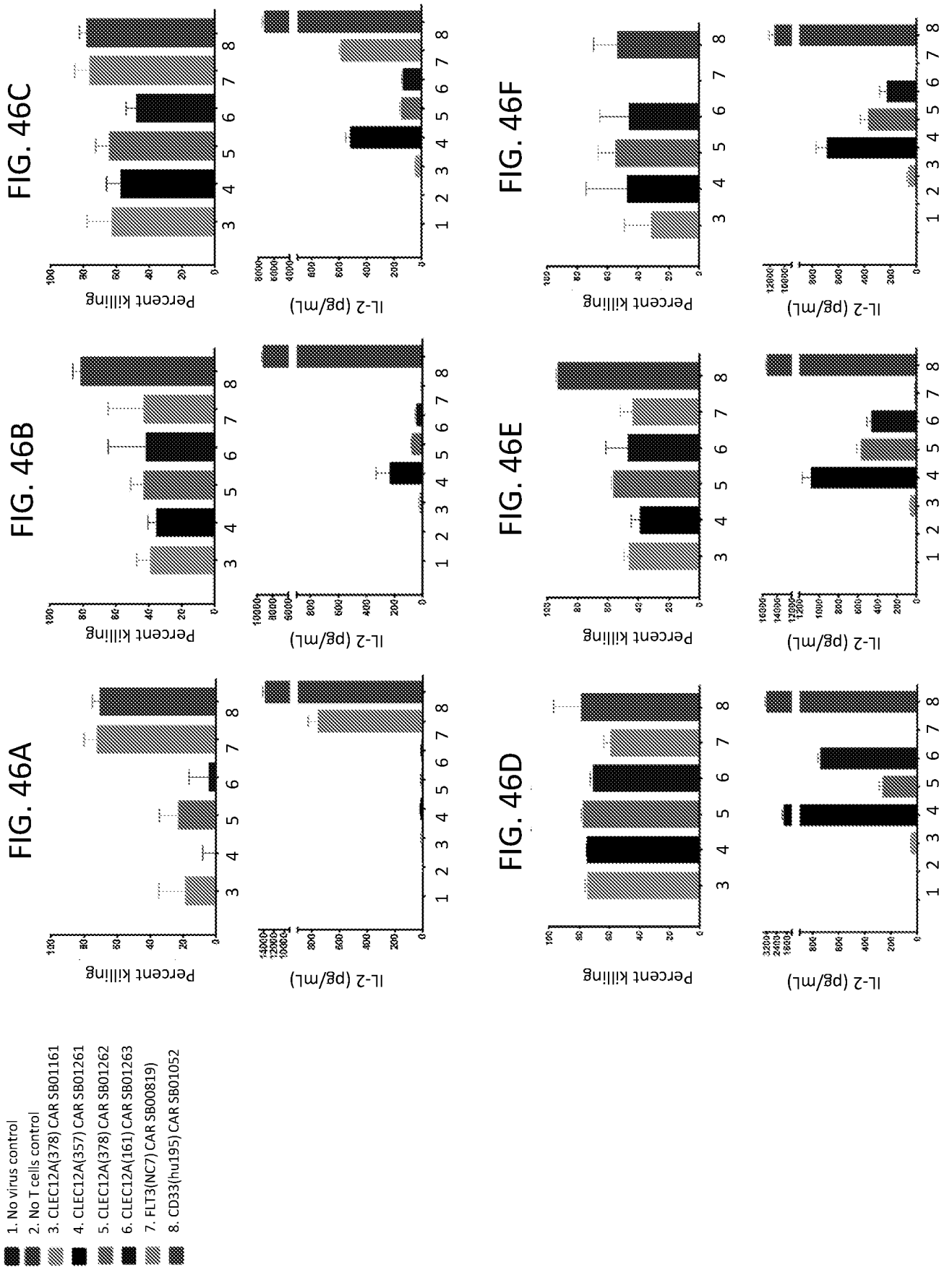
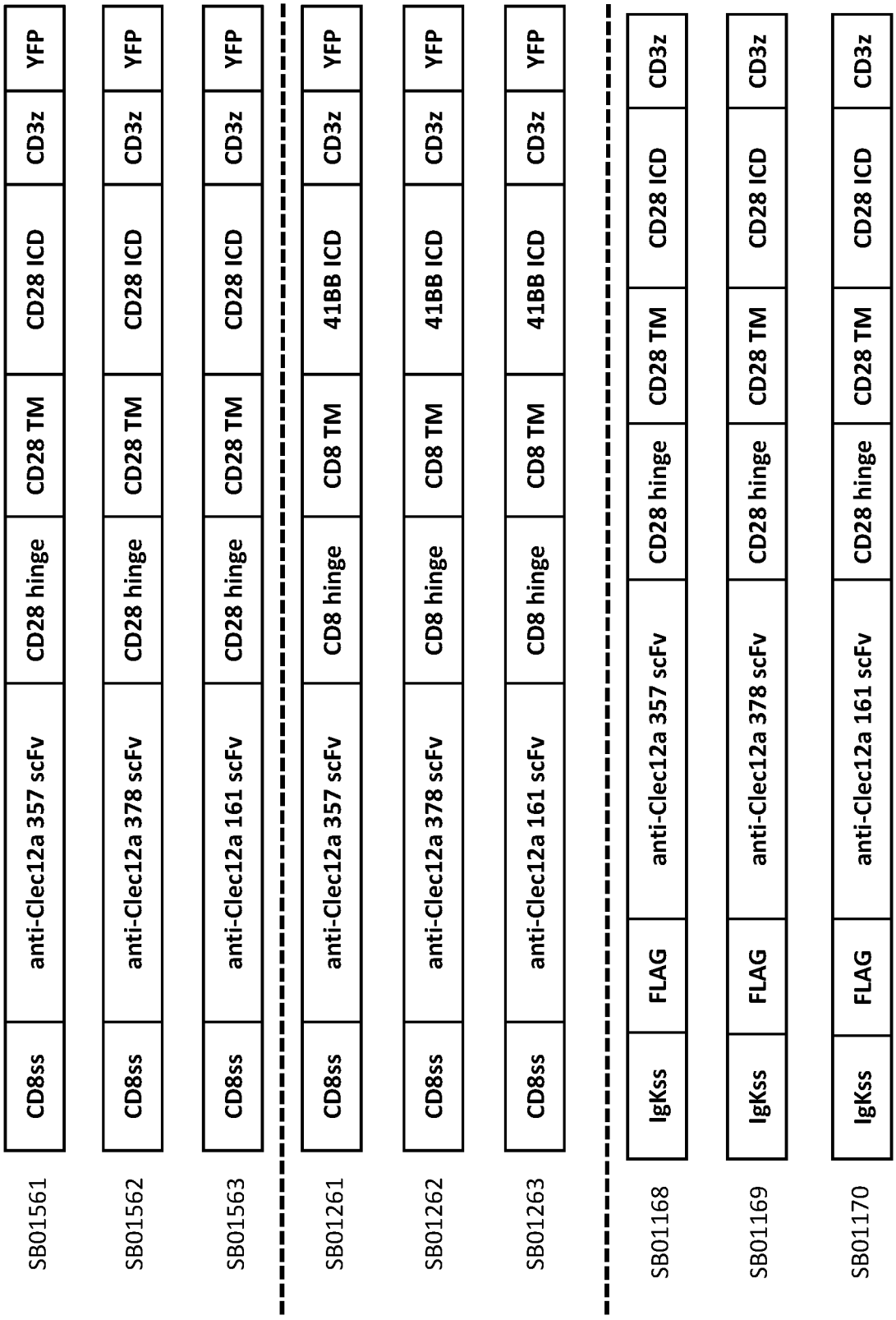

FIG. 46A
FIG. 46B
FIG. 46C
FIG. 46D
FIG. 46E
FIG. 46F
Percent killing
IL-2 (pg/mL)
1. No virus control
2. No T cells control
3. CLEC12A(378) CAR SB01161
4. CLEC12A(357) CAR SB01261
5. CLEC12A(378) CAR SB01262
6. CLEC12A(161) CAR SB01263
7. FLT3(NC7) CAR SB00819)
8. CD33(hu195) CAR SB01052

FIG. 47


SB01561
CD8ss
anti-Clec12a 357 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z
YFP
SB01562
CD8ss
anti-Clec12a 378 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z
YFP
SB01563
CD8ss
anti-Clec12a 161 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z
YFP
SB01261
CD8ss
anti-Clec12a 357 scFv
CD8 hinge
CD8 TM
41BB ICD
CD3z
YFP
SB01262
CD8ss
anti-Clec12a 378 scFv
CD8 hinge
CD8 TM
41BB ICD
CD3z
YFP
SB01263
CD8ss
anti-Clec12a 161 scFv
CD8 hinge
CD8 TM
41BB ICD
CD3z
YFP
SB01168
IgKss
FLAG
anti-Clec12a 357 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z
SB01169
IgKss
FLAG
anti-Clec12a 378 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z
SB01170
IgKss
FLAG
anti-Clec12a 161 scFv
CD28 hinge
CD28 TM
CD28 ICD
CD3z

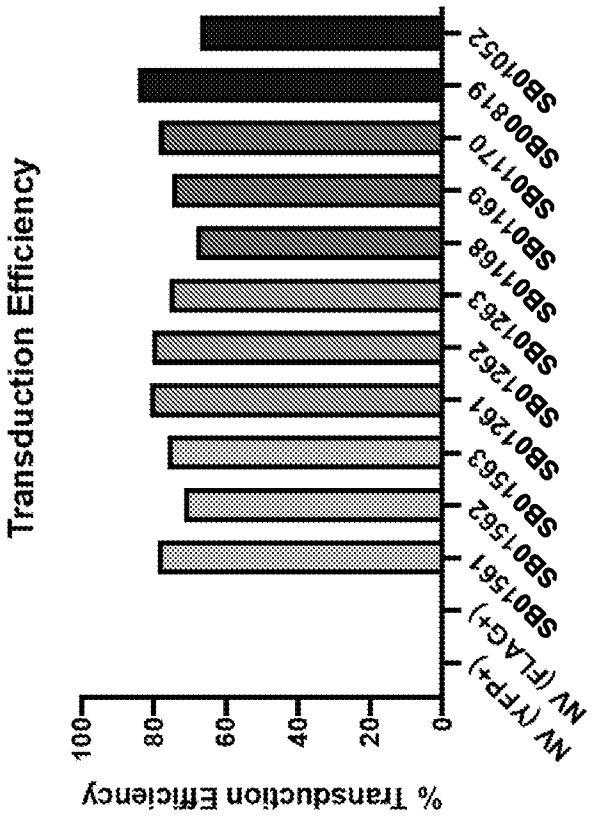


FIG. 48A

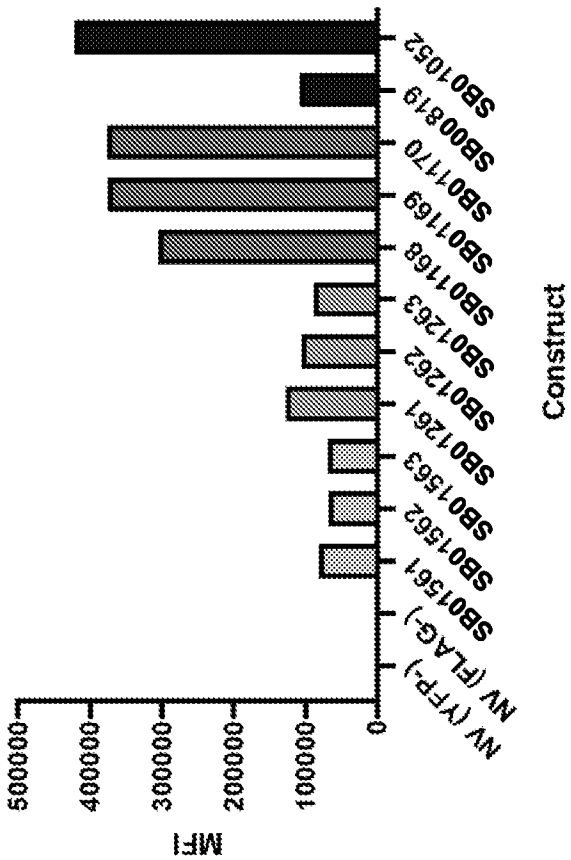


FIG. 48B

FIG. 49

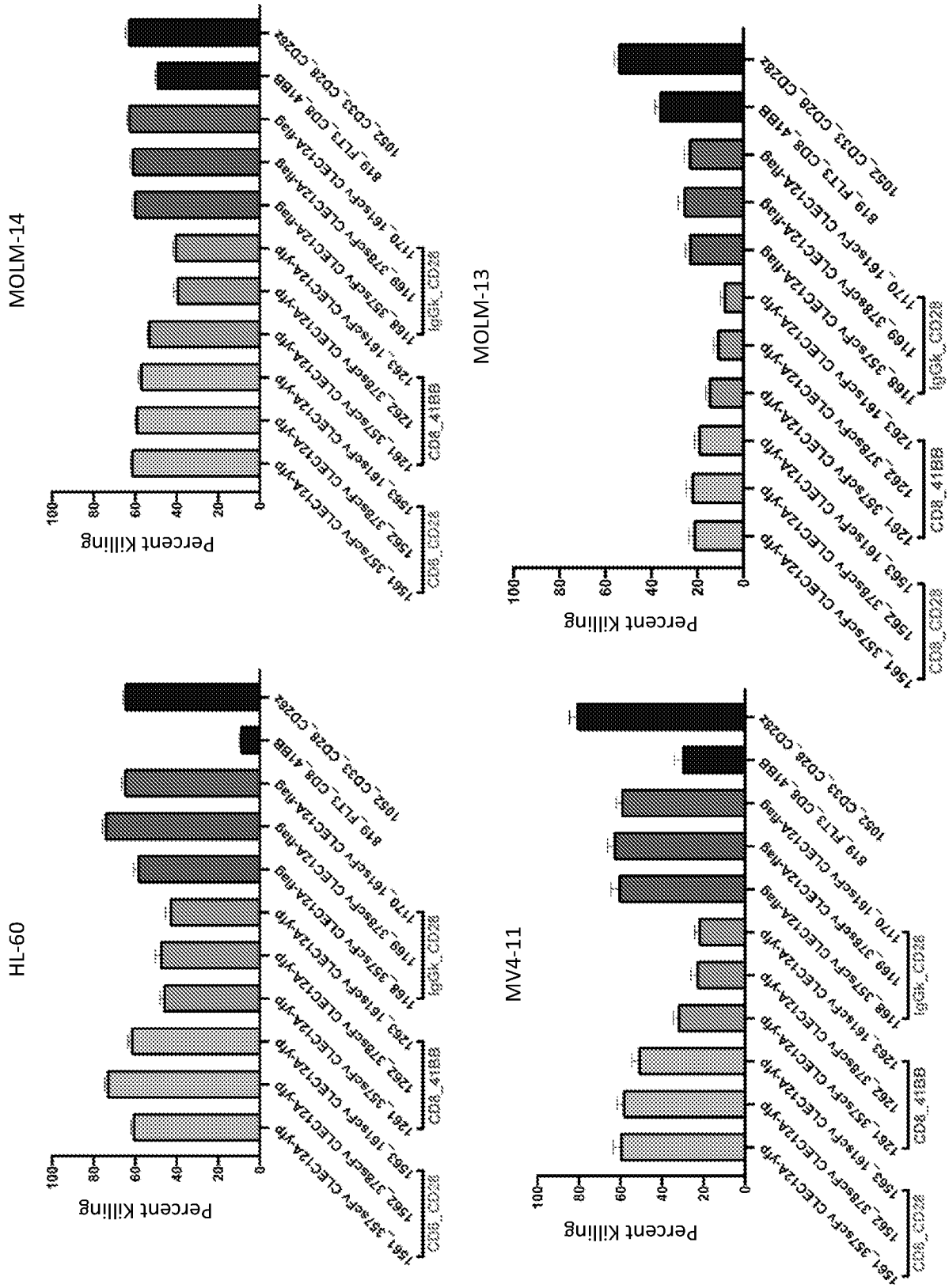

MOLM-14
MOLM-13
HL-60
MV4-11
Percent Killing
0
20
40
60
80
100
1561_357scFv CLEC12A-yfp
1562_378scFv CLEC12A-yfp
1563_161scFv CLEC12A-yfp
1261_357scFv CLEC12A-yfp
1262_378scFv CLEC12A-yfp
1263_161scFv CLEC12A-yfp
1168_357scFv CLEC12A-hlag
1169_378scFv CLEC12A-hlag
1170_161scFv CLEC12A-hlag
819_FLT3_CD8_41BB
1052_CD33_CD28_CD28z
CD8_CD28
CD8_41BB
IgGk_CD28

FIG. 50

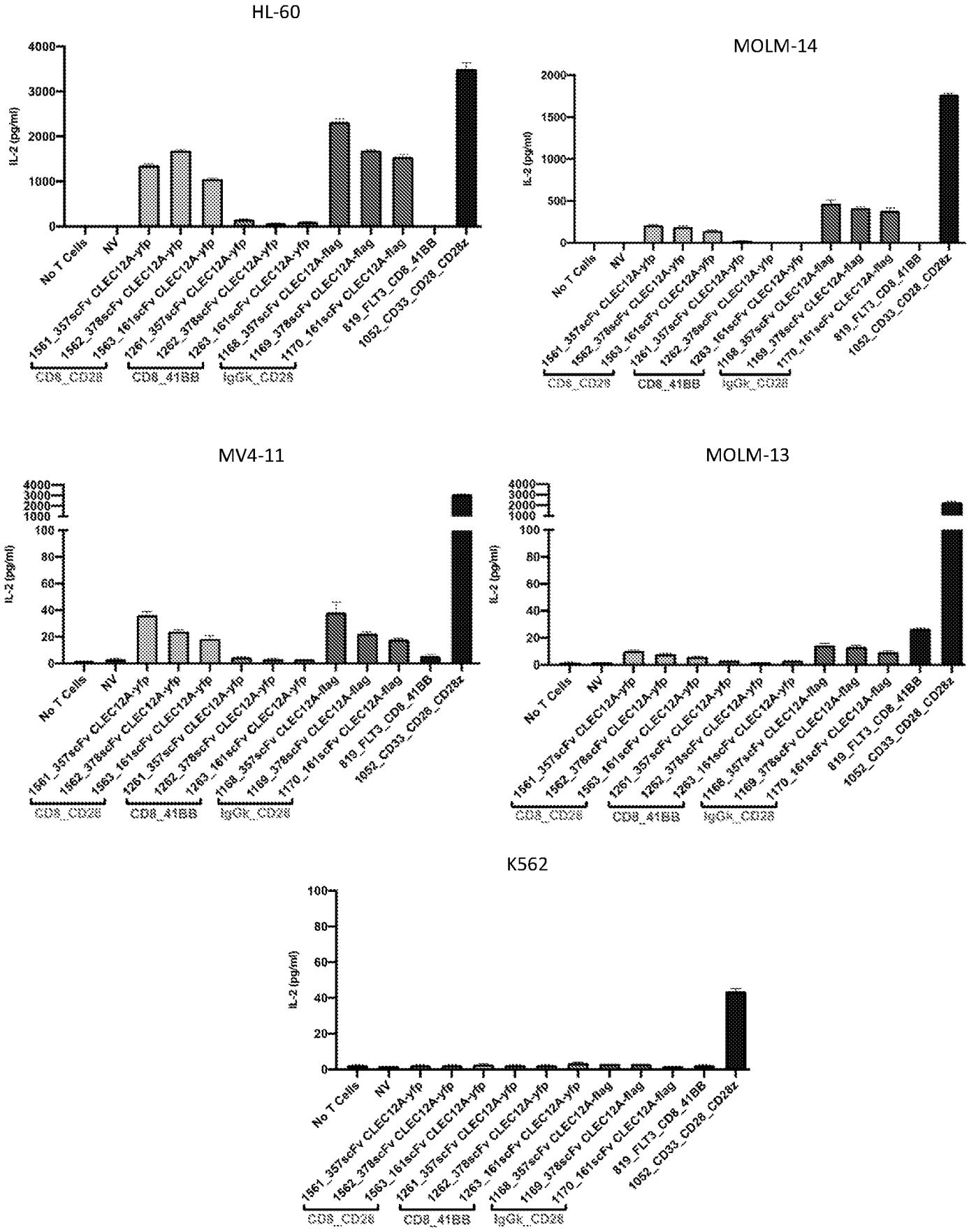

HL-60
MOLM-14
MV4-11
MOLM-13
K562
IL-2 (pg/ml)
No T Cells
NV
1561_357scFv CLEC12A-yfp
1562_378scFv CLEC12A-yfp
1563_161scFv CLEC12A-yfp
1261_357scFv CLEC12A-yfp
1262_378scFv CLEC12A-yfp
1263_161scFv CLEC12A-yfp
1168_357scFv CLEC12A-flag
1169_378scFv CLEC12A-flag
1170_161scFv CLEC12A-flag
819_FLT3_CD8_41BB
1052_CD33_CD28_CD28z
CD8_CD28
CD8_41BB
IgGk_CD28

FIG. 51

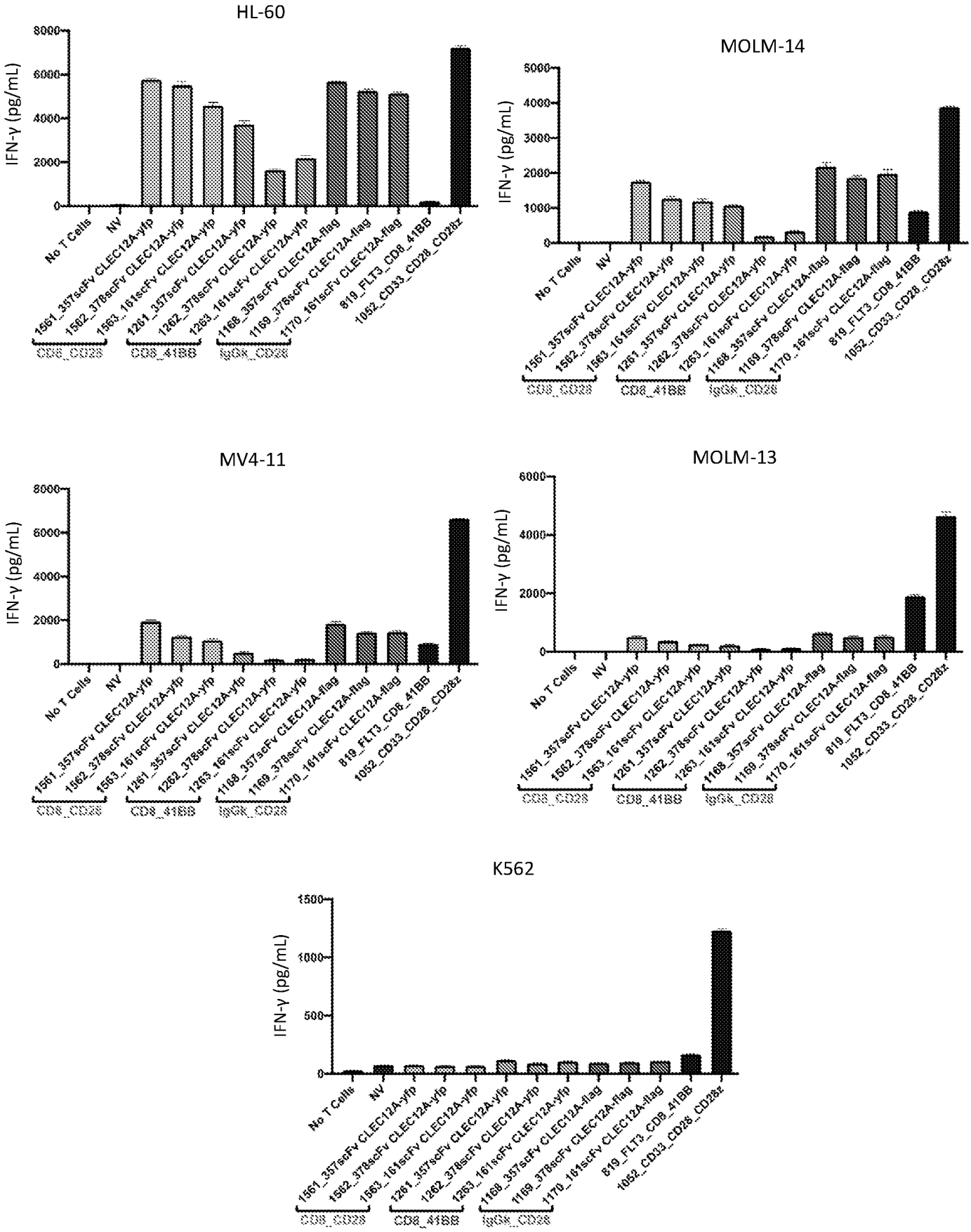

HL-60
MOLM-14
MV4-11
MOLM-13
K562
IFN-γ (pg/mL)
No T Cells
NV
1561_357scFv CLEC12A-yfp
1562_378scFv CLEC12A-yfp
1563_161scFv CLEC12A-yfp
1261_357scFv CLEC12A-yfp
1262_378scFv CLEC12A-yfp
1263_161scFv CLEC12A-yfp
1168_357scFv CLEC12A-flag
1169_378scFv CLEC12A-flag
1170_161scFv CLEC12A-flag
819_FLT3_CD8_41BB
1052_CD33_CD28_CD28z
CD8_CD28
CD8_41BB
IgGk_CD28

FIG. 52

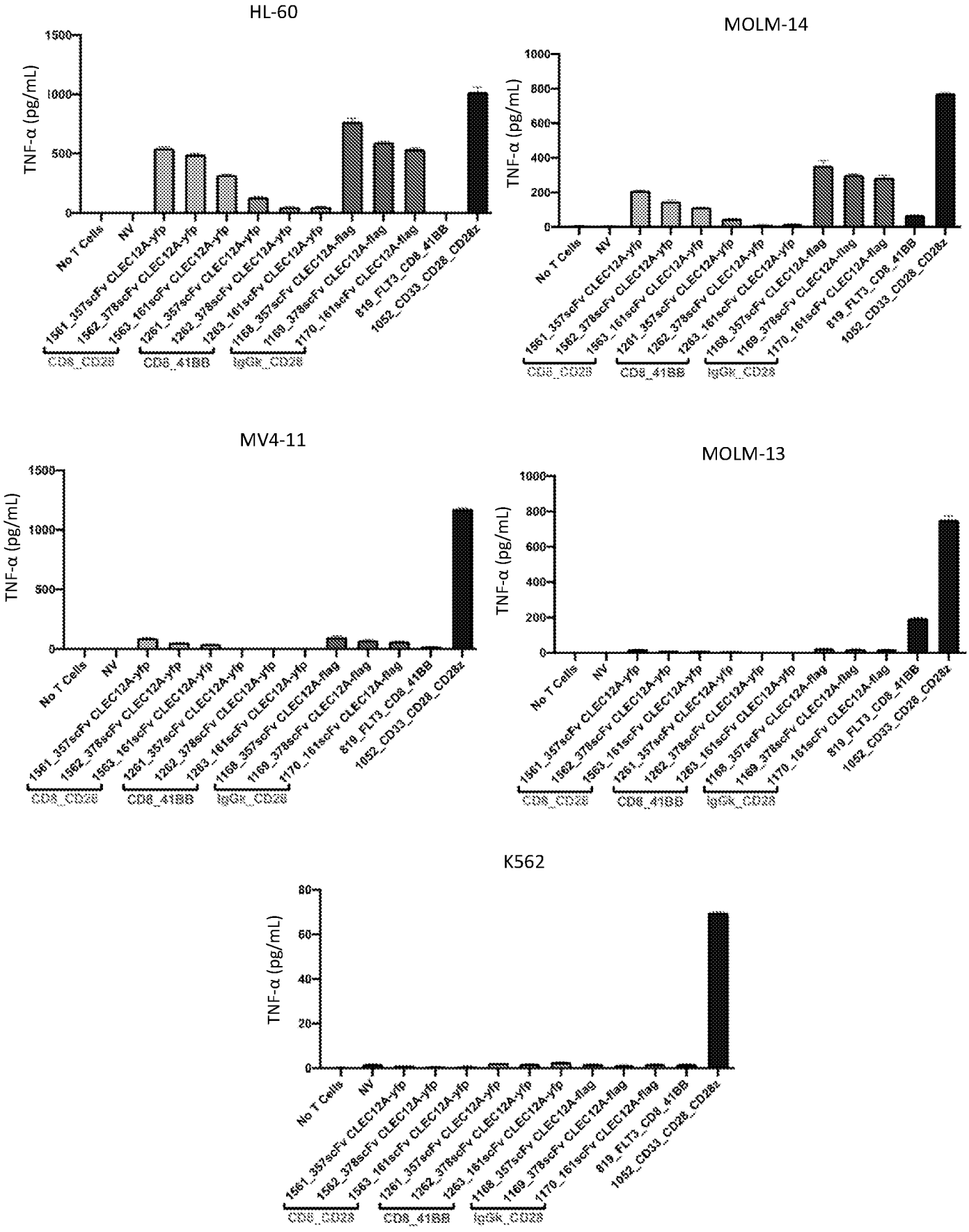

HL-60
MOLM-14
MV4-11
MOLM-13
K562
TNF-α (pg/mL)
No T Cells
NV
1561_357scFv CLEC12A-yfp
1562_378scFv CLEC12A-yfp
1563_161scFv CLEC12A-yfp
1261_357scFv CLEC12A-yfp
1262_378scFv CLEC12A-yfp
1263_161scFv CLEC12A-yfp
1168_357scFv CLEC12A-flag
1169_378scFv CLEC12A-flag
1170_161scFv CLEC12A-flag
819_FLT3_CD8_41BB
1052_CD33_CD28_CD28z
CD8_CD28
CD8_41BB
IgGk_CD28

FIG. 53A

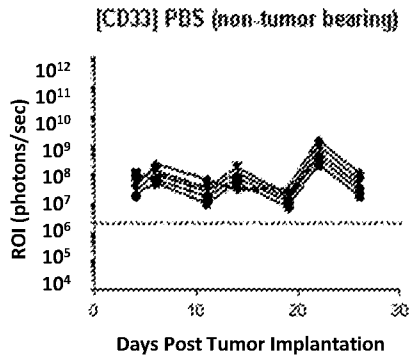


FIG. 53B

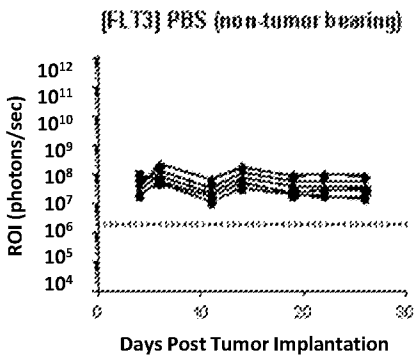


FIG. 53C

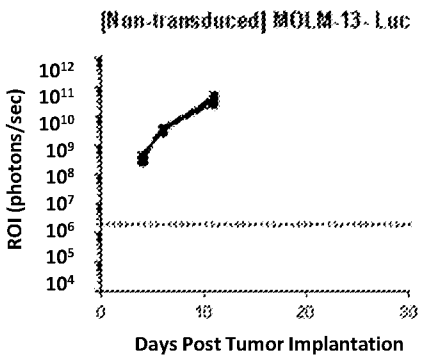


FIG. 53D

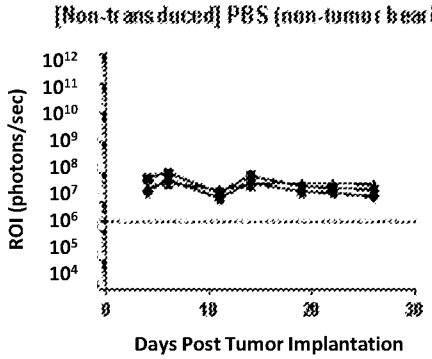


FIG. 53E

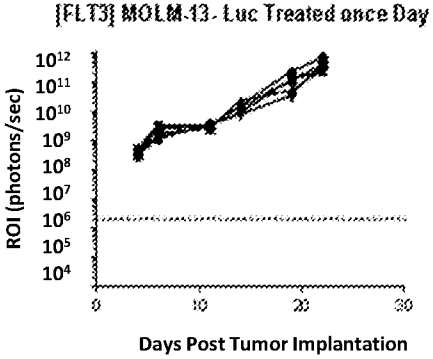


FIG. 53F

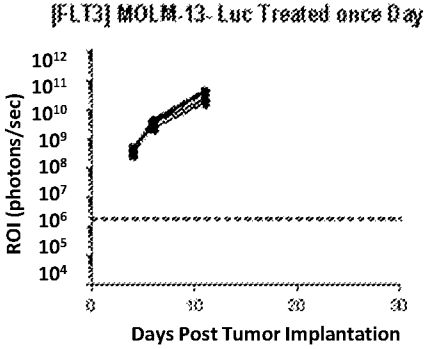


FIG. 53G

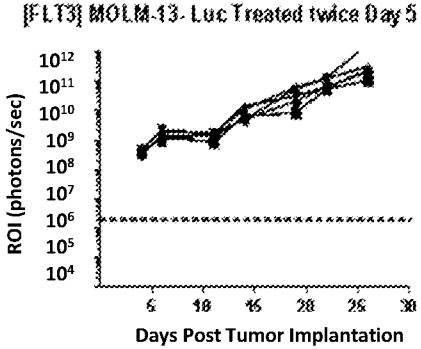


FIG. 53H

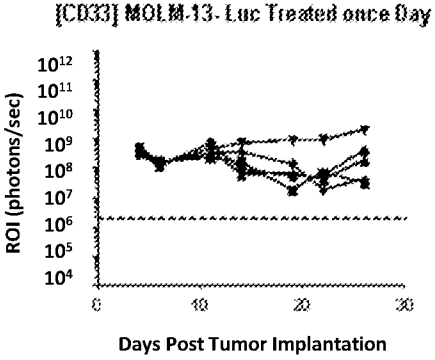


FIG. 53I

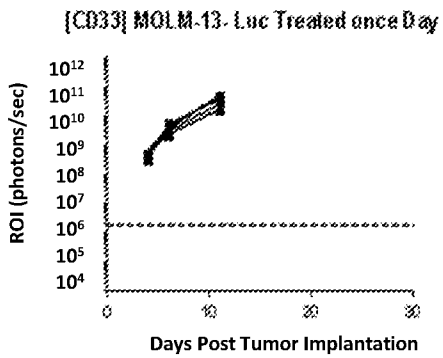
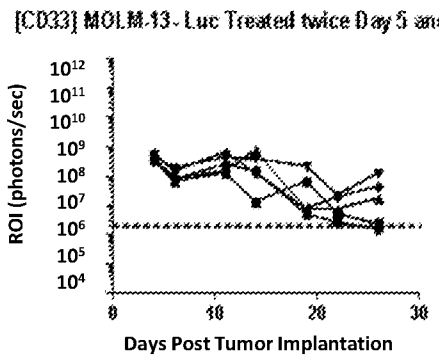


FIG. 53J



SUBSTITUTE SHEET (RULE 26)

FIG. 53K

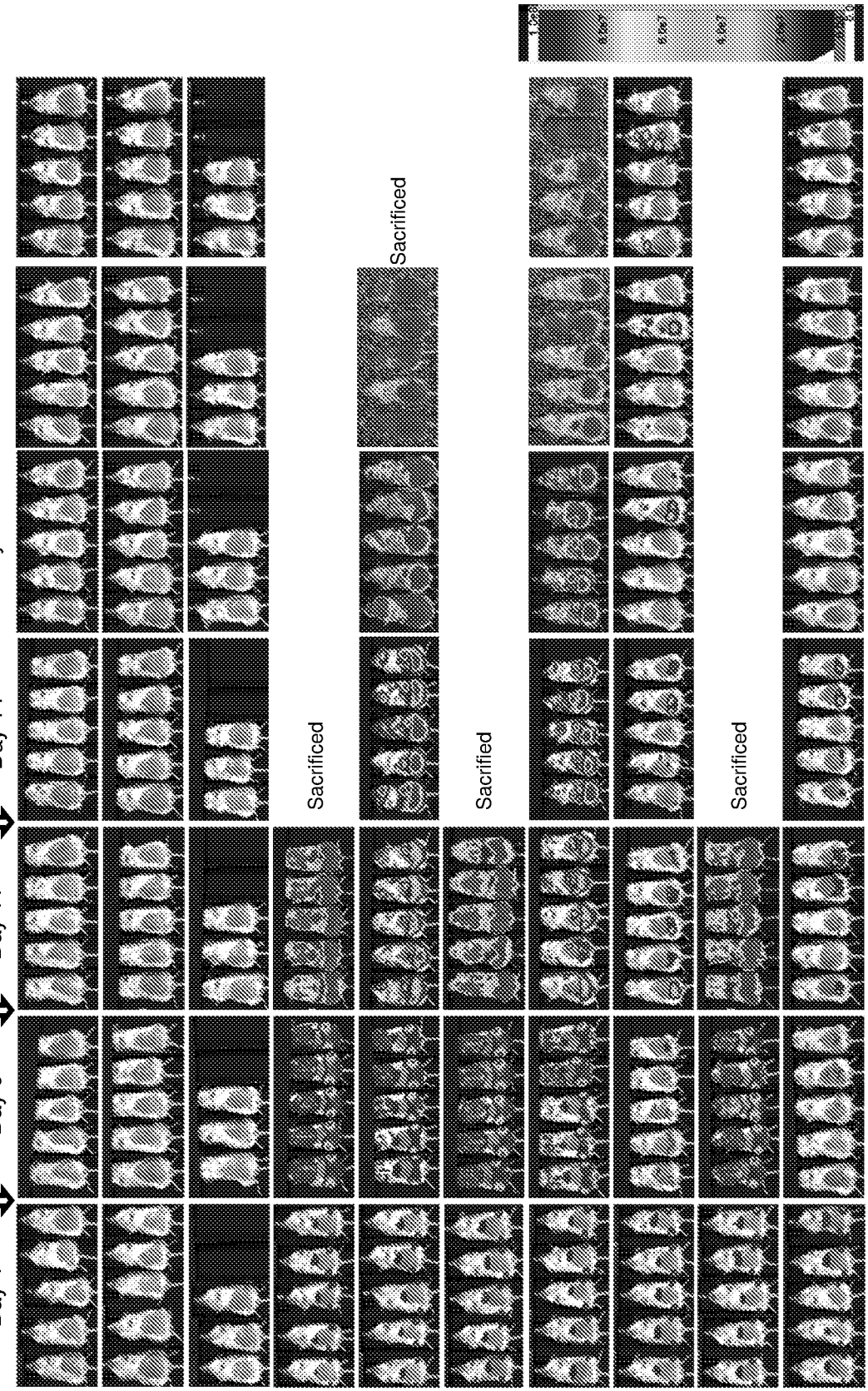

Day 4
Day 6
Day 11
Day 14
Day 19
Day 22
Day 26
Sacrificed
Sacrificed
Sacrificed
Sacrificed
non-tumor bearing mice and CD33+ CAR-T cells
non-tumor bearing mice and FLT3+ CAR-T cells
non-tumor bearing mice and Non-transduced CAR-T cells
MOLM-13 implanted mice and **Non-transduced CAR-T cells**
MOLM-13 implanted mice and **FLT3+** CAR-T cells **[Day5]**
MOLM-13 implanted mice and **FLT3+** CAR-T cells **[Day8]**
MOLM-13 implanted mice and **FLT3+** CAR-T cells **[Day5 and Day 12]**
MOLM-13 implanted mice and **CD33+** CAR-T cells **[Day5]**
MOLM-13 implanted mice and **CD33+ CAR-T cells [Day8]**
MOLM-13 implanted mice and **CD33+** CAR-T cells **[Day5 and Day12]**

FIG. 54A

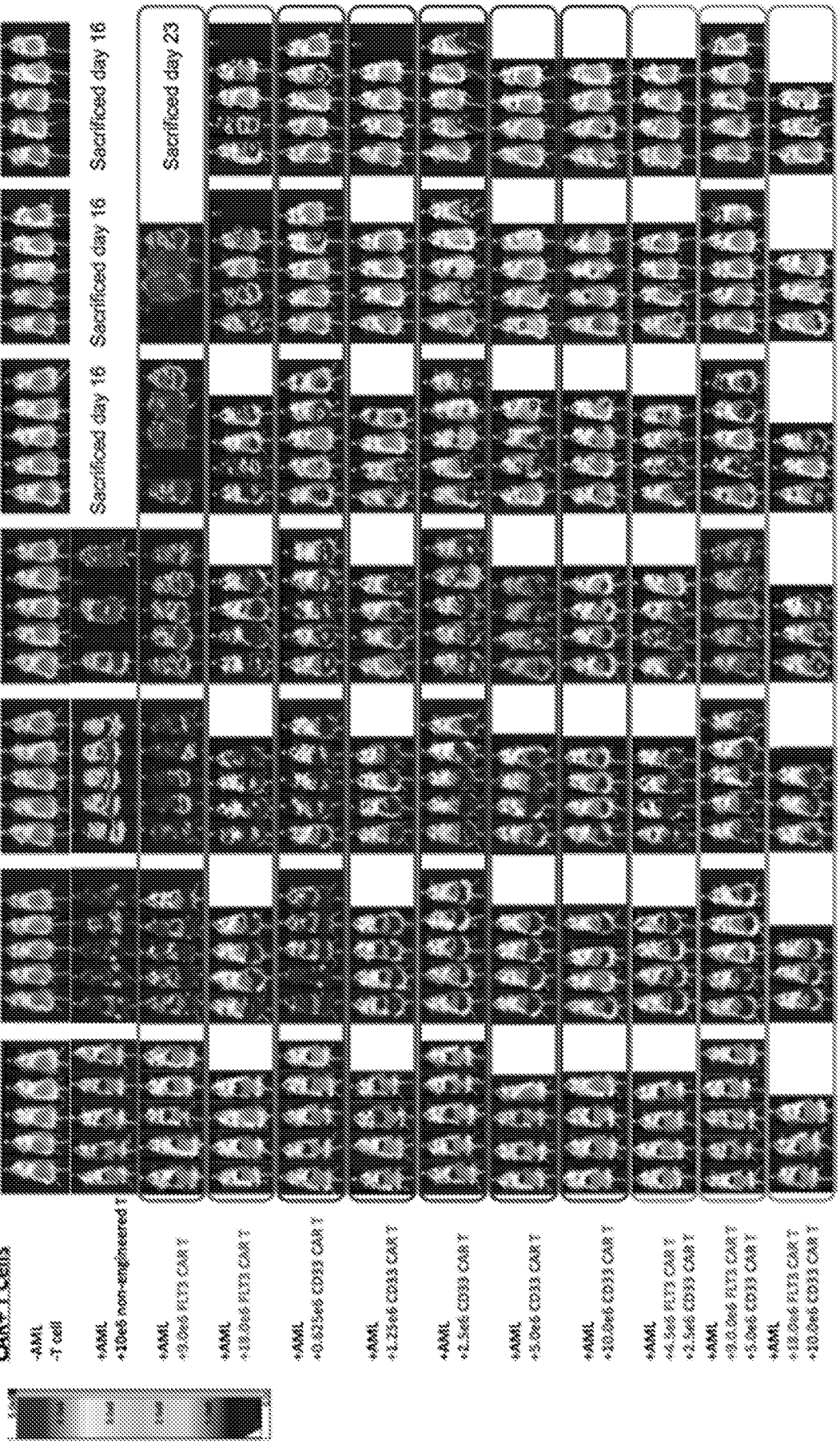

Group &
CAR+ T Cells
-AML
-T cell
+AML
+10e6 non-engineered T
+AML
+3.0e6 FLT3 CAR T
+AML
+10.0e6 FLT3 CAR T
+AML
+0.822e6 CD33 CAR T
+AML
+1.23e6 CD33 CAR T
+AML
+2.5e6 CD33 CAR T
+AML
+5.0e6 CD33 CAR T
+AML
+10.0e6 CD33 CAR T
+AML
+4.5e6 FLT3 CAR T
+2.5e6 CD33 CAR T
+AML
+3.0e6 FLT3 CAR T
+5.0e6 CD33 CAR T
+AML
+10.0e6 FLT3 CAR T
+10.0e6 CD33 CAR T
Day 4
Day 7
Day 13
Day 16
Day 20
Day 23
Day 26
Sacrificed day 16
Sacrificed day 16
Sacrificed day 16
Sacrificed day 23

FIG. 54B

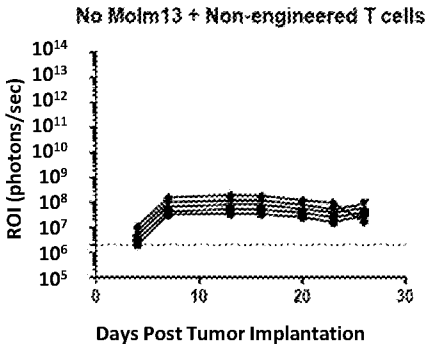


FIG. 54C

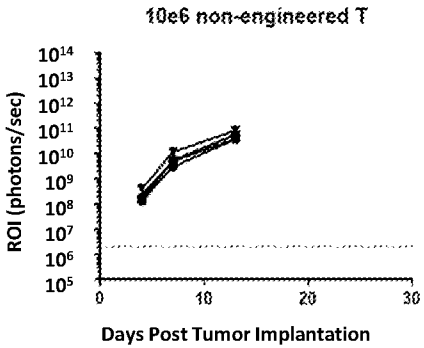


FIG. 54D

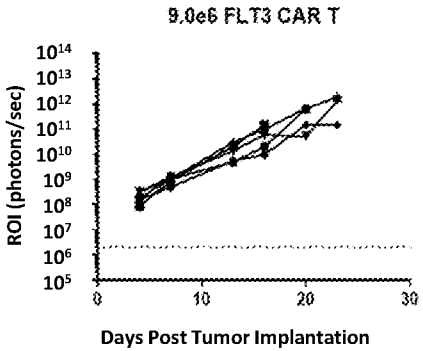


FIG. 54E

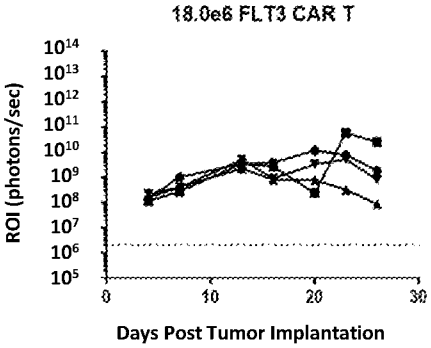


FIG. 54F

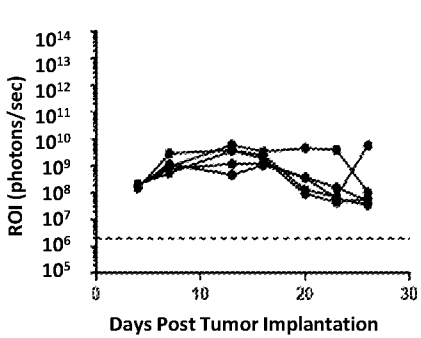


FIG. 54G

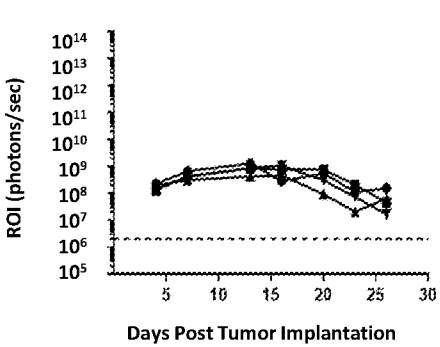


FIG. 54H

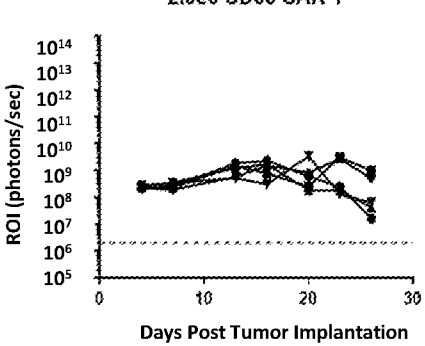


FIG. 54I

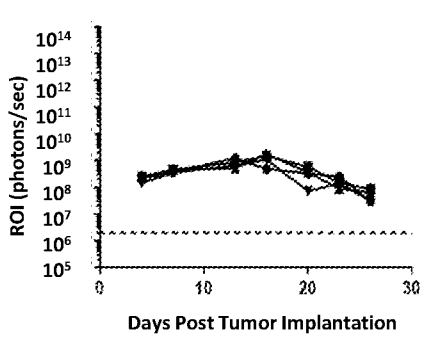


FIG. 54J

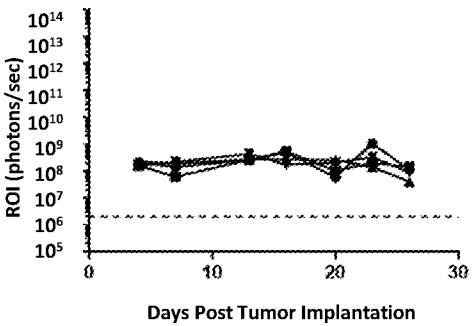


FIG. 54K

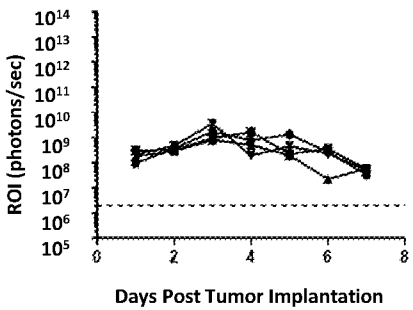


FIG. 54L

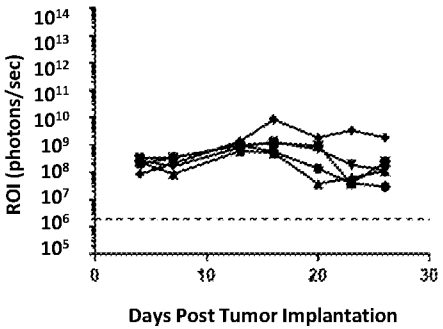
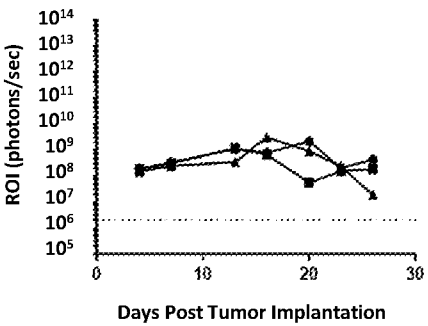


FIG. 54M



SUBSTITUTE SHEET (RULE 26)

FIG. 55B

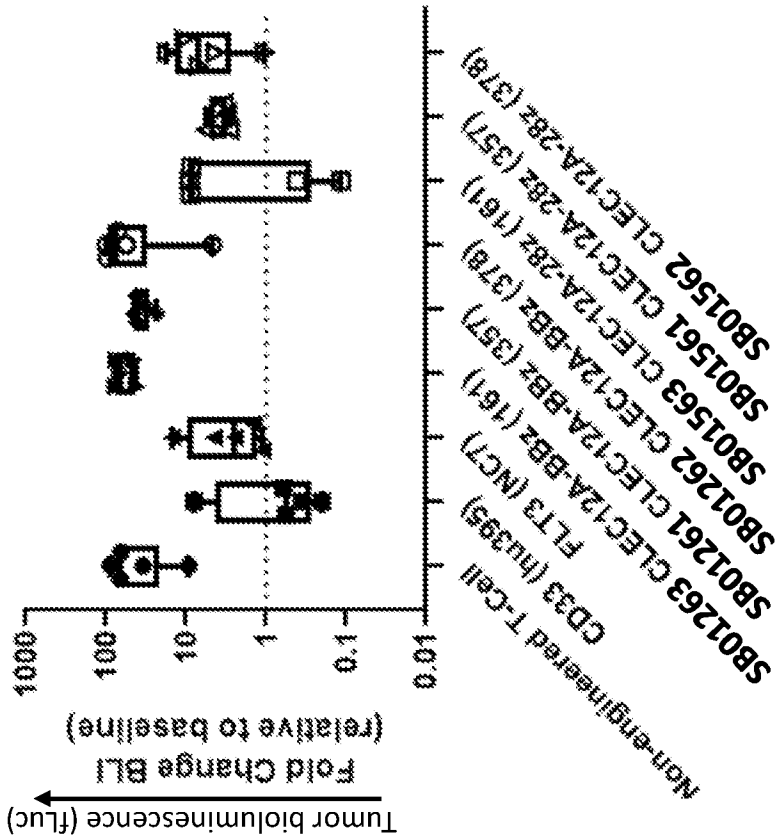


FIG. 55A

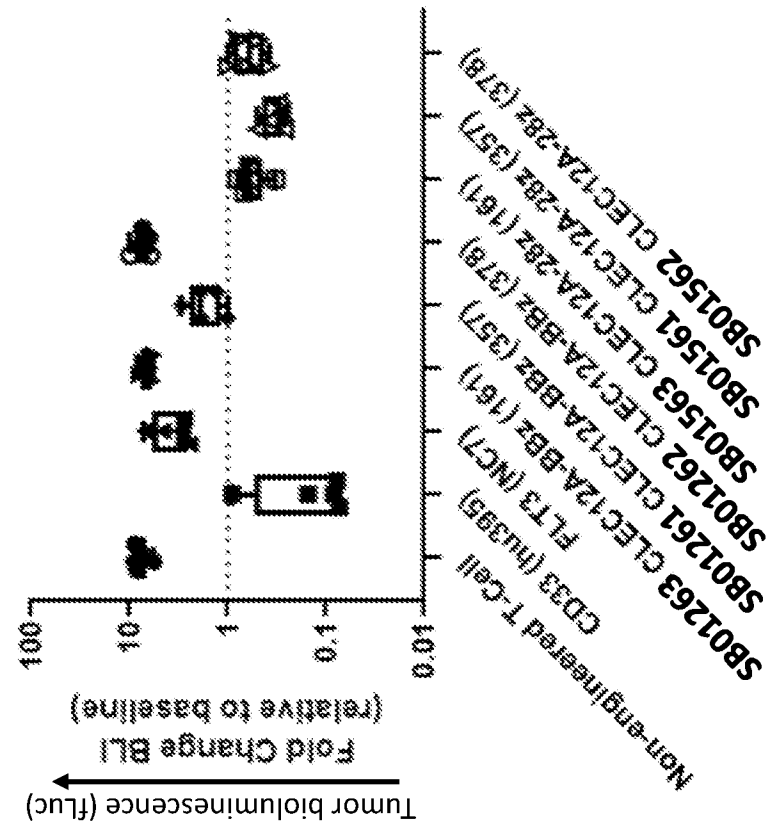


FIG. 55C

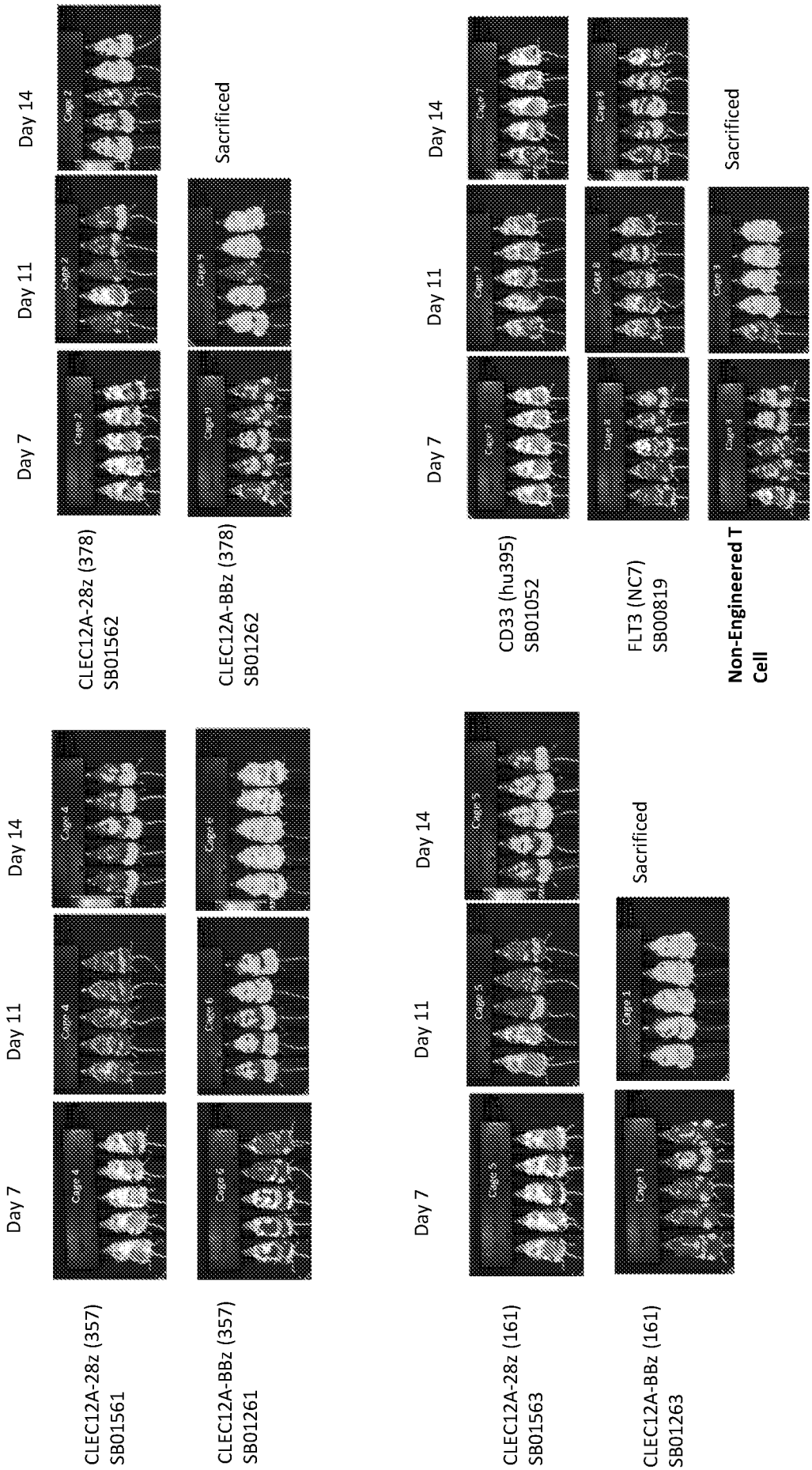

Day 7
Day 11
Day 14
Sacrificed
CLEC12A-28z (378) SB01562
CLEC12A-BBz (378) SB01262
CD33 (hu395) SB01052
FLT3 (NC7) SB00819
Non-Engineered T Cell
CLEC12A-28z (357) SB01561
CLEC12A-BBz (357) SB01261
CLEC12A-28z (161) SB01563
CLEC12A-BBz (161) SB01263

FIG. 56A

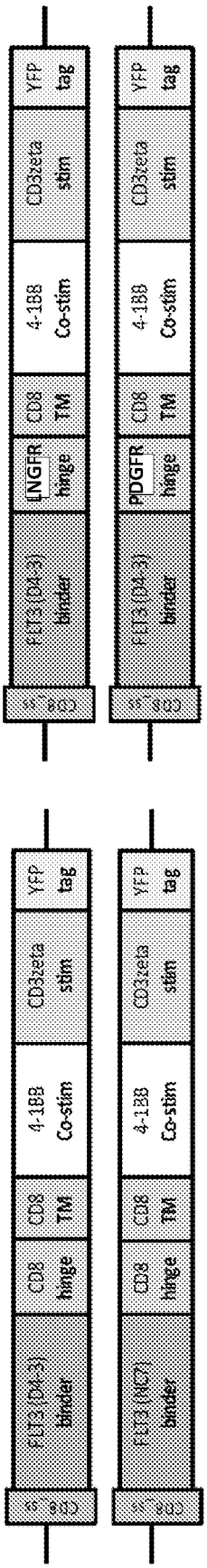


FIG. 56B

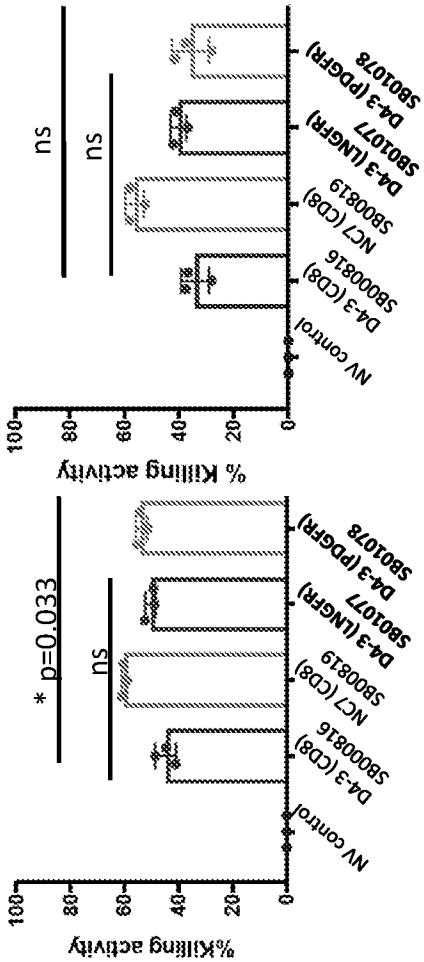


FIG. 56C

FIG. 56D

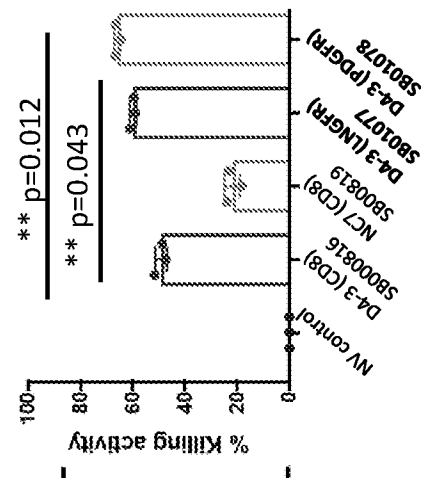
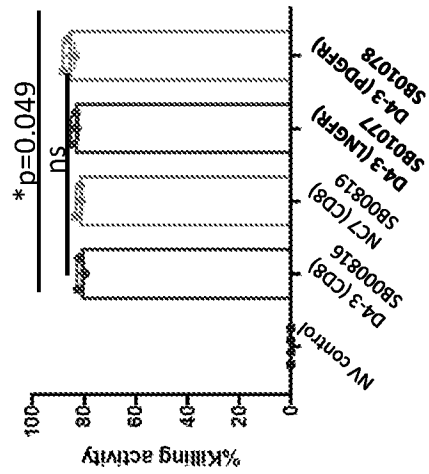
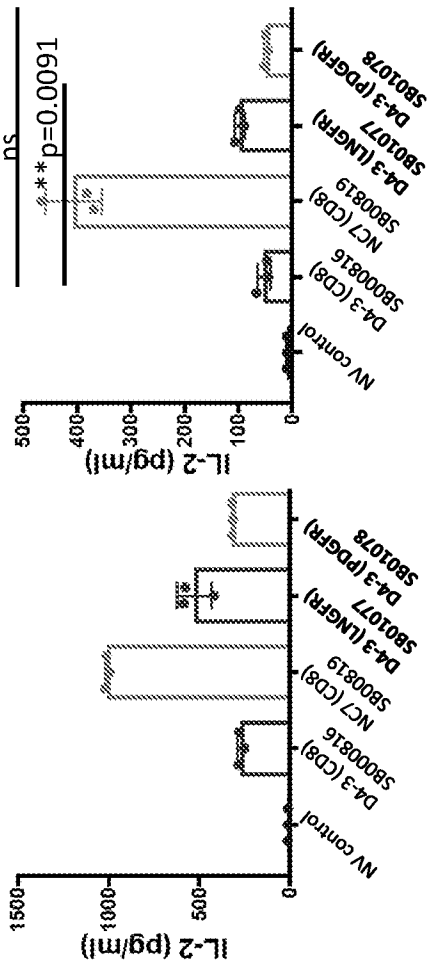
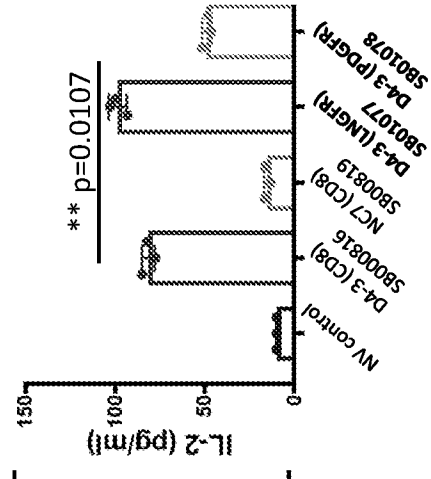


FIG. 56E







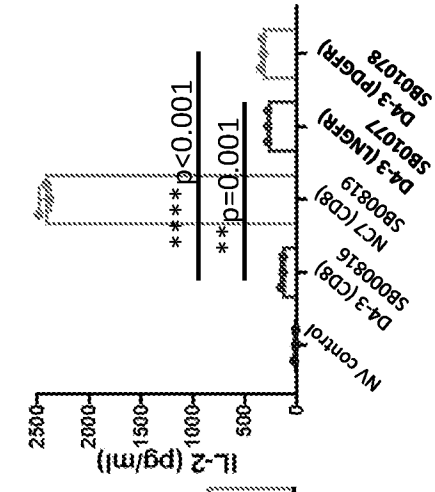


FIG. 57A

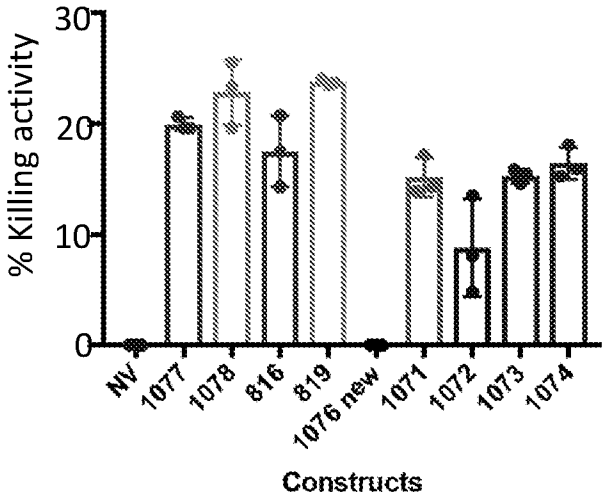


FIG. 57B

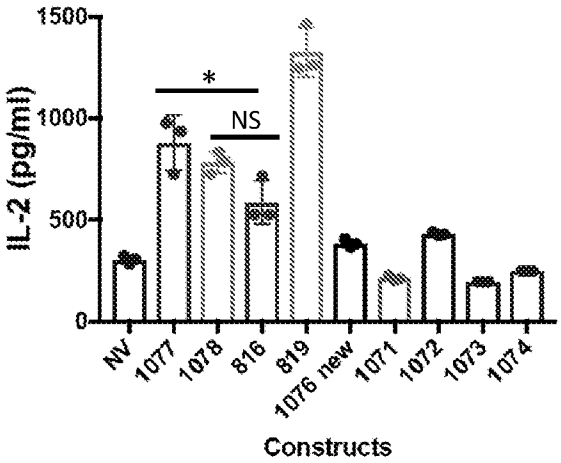


FIG. 57C

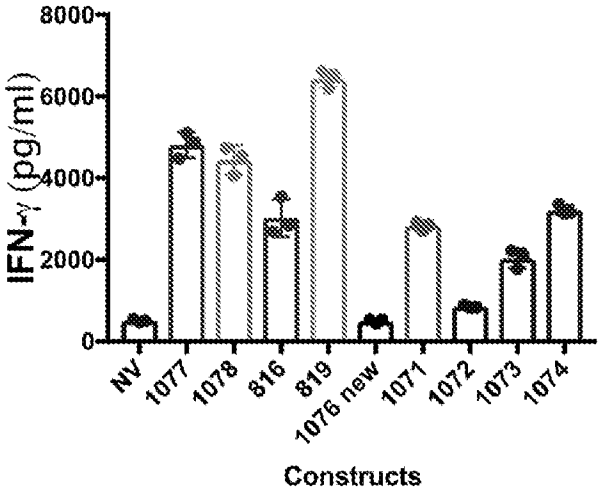
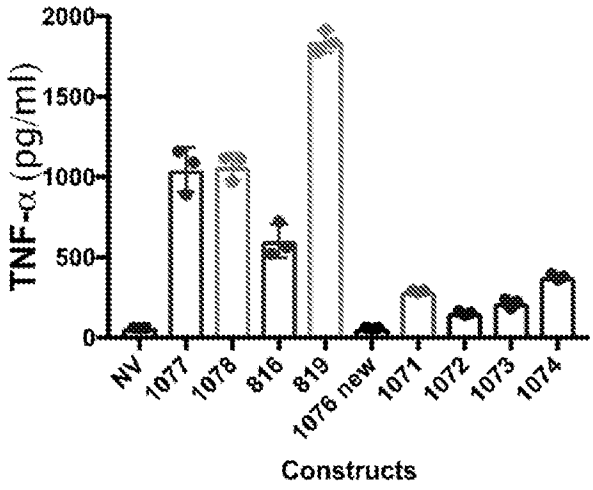


FIG. 57D



SB0#	Hinge Source
1071	IgG4 minimal
1072	IgG4 minimal; No disulfides
1073	IgG4 minimal; enhanced disulfides
1074	IgG1 minimal hinge
1078	PDGFR
1077	LNGFR (truncated)
1076	LNGFR
816	Original D4 hinge
819	NC7 binder

FIG. 58A

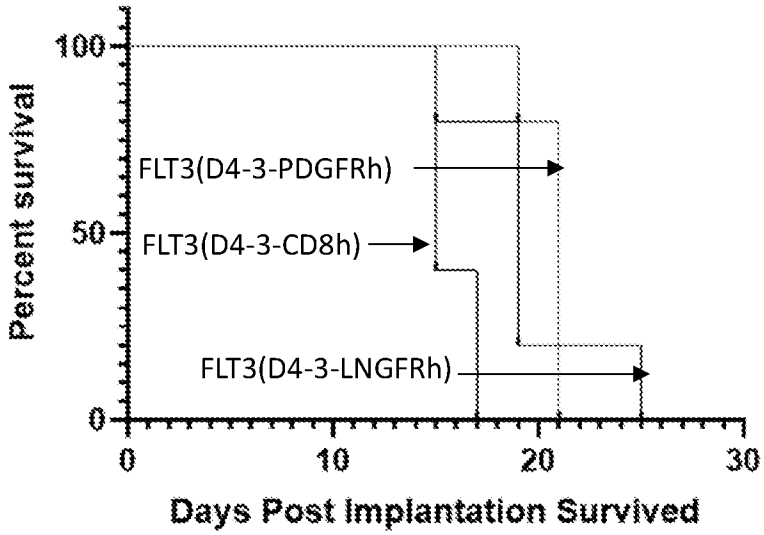


FIG. 58B

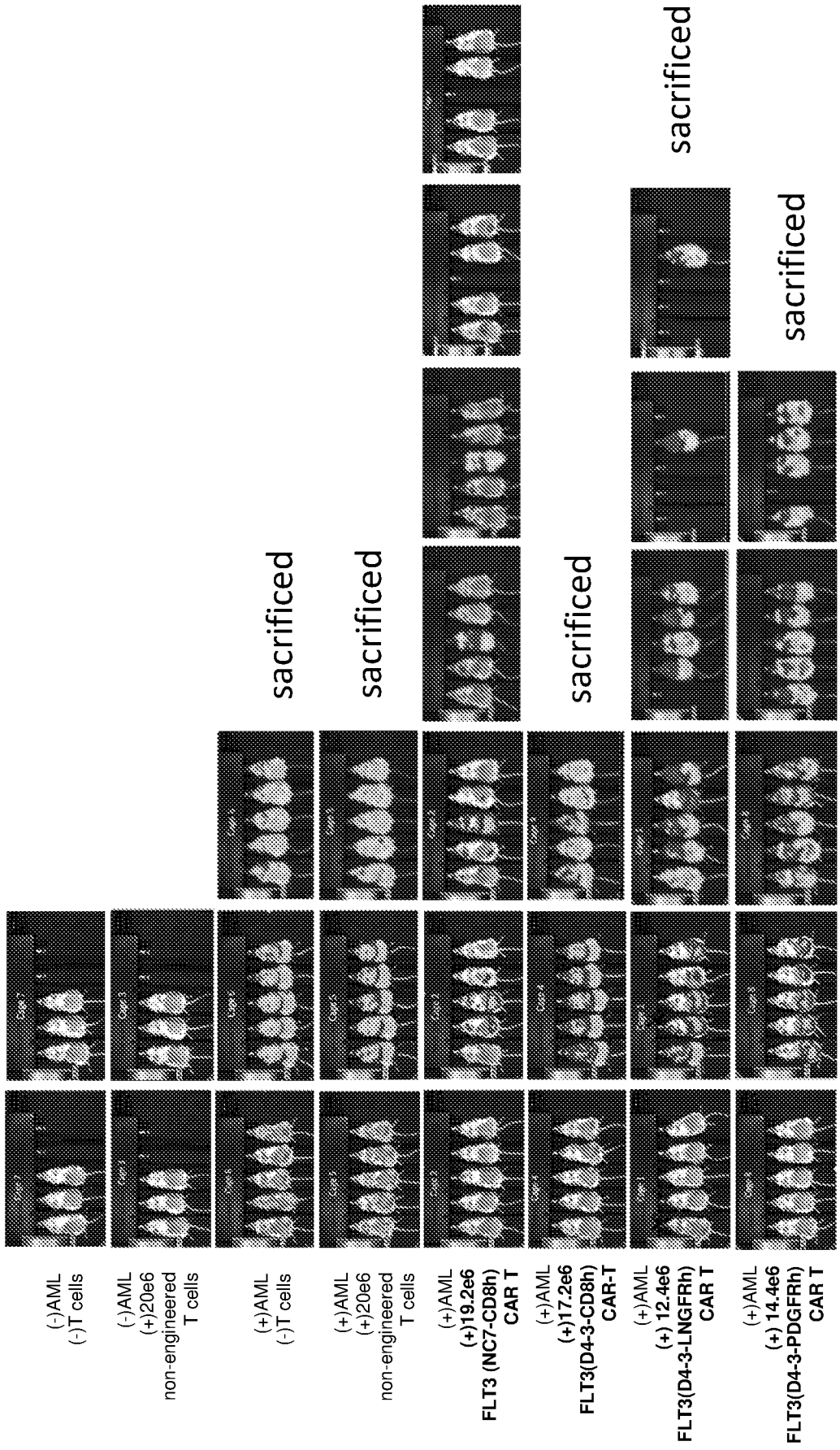

Group & CAR+ T
Day 7
Day 11
Day 14
Day 18
Day 21
Day 25
Day 28
(-)AML (-)T cells
(-)AML (+)20e6 non-engineered T cells
(+)AML (-)T cells
(+)AML (+)20e6 non-engineered T cells
(+)AML (+)19.2e6 FLT3 (NC7-CD8h) CAR T
(+)AML (+)17.2e6 FLT3(D4-3-CD8h) CAR-T
(+)AML (+) 12.4e6 FLT3(D4-3-LNGFRh) CAR T
(+)AML (+) 14.4e6 FLT3(D4-3-PDGFRh) CAR T
sacrificed
sacrificed
sacrificed
sacrificed
sacrificed
23

FIG. 59A

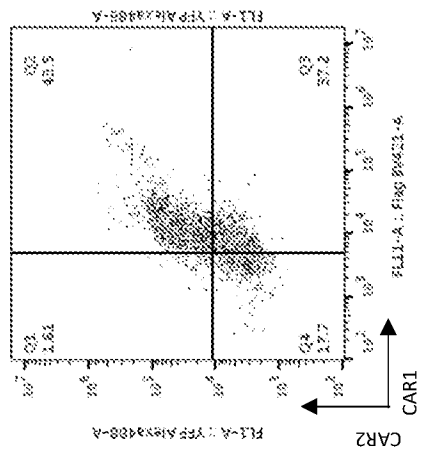
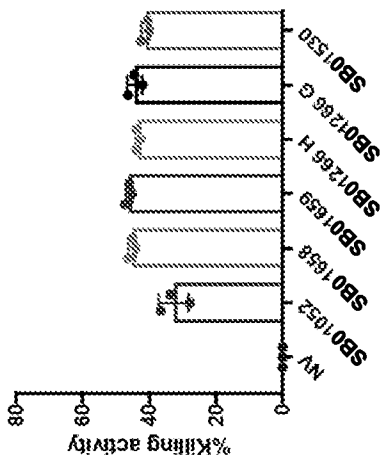


FIG. 59B



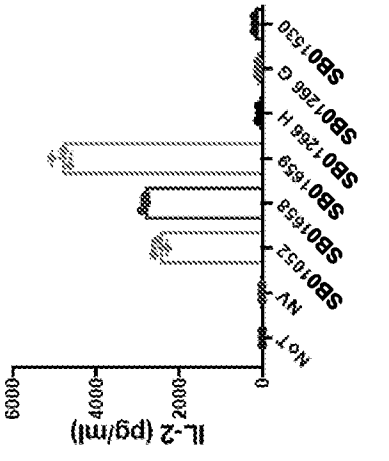
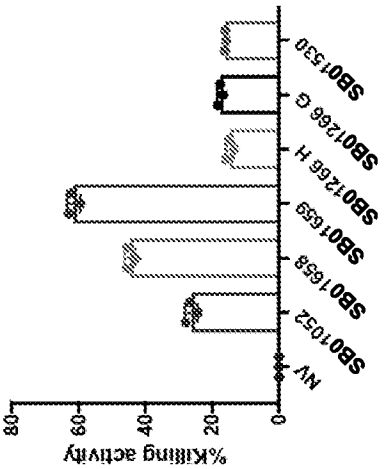


FIG. 59C



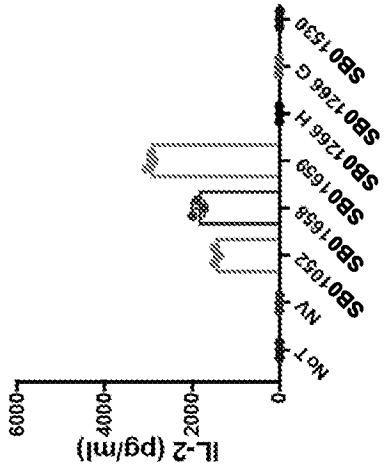
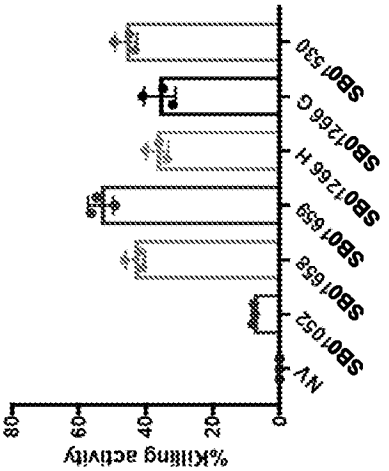
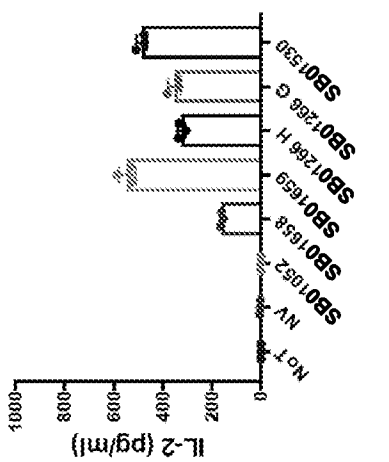


FIG. 59D





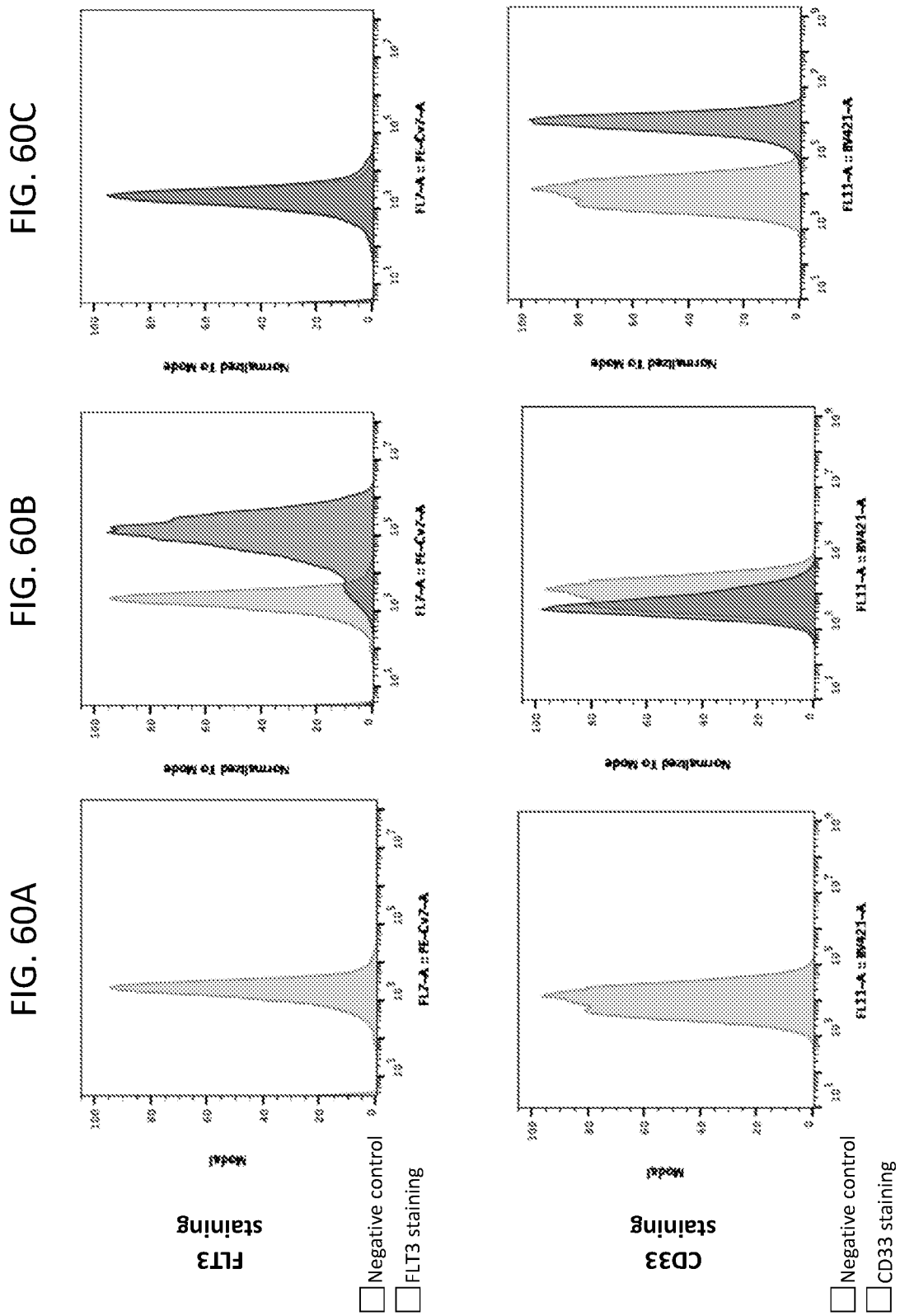

FIG. 60A
FIG. 60B
FIG. 60C
FLT3 staining
CD33 staining
Negative control
FLT3 staining
CD33 staining
Modal
Normalized to Mode
25

FIG. 61

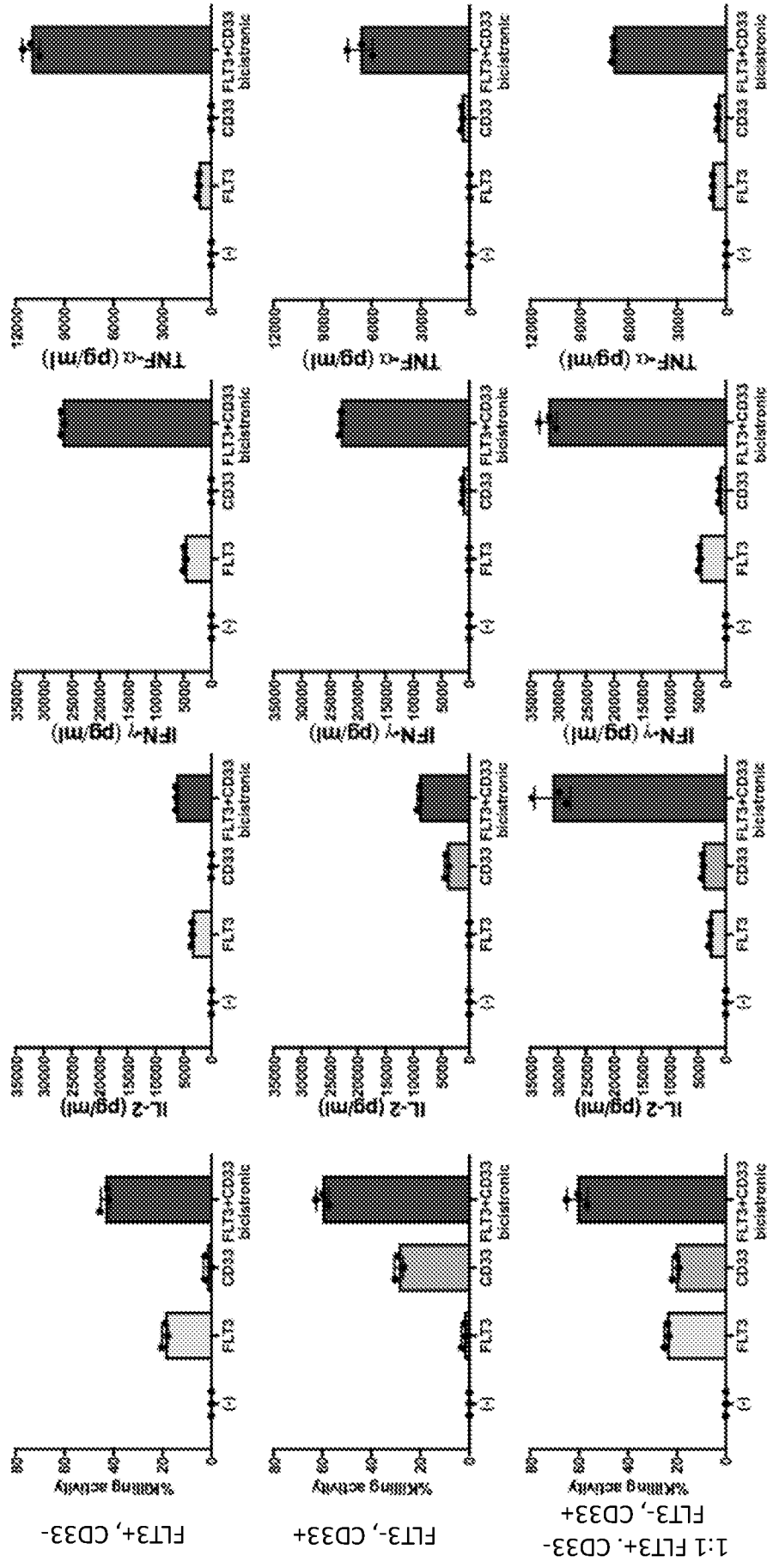

1:1 FLT3+. CD33-
FLT3-, CD33+
FLT3-, CD33+
FLT3+, CD33-
%Killing activity
IL-2 (pg/ml)
IFN-γ (pg/ml)
TNF-α (pg/ml)
(-)
FLT3
CD33 FLT3+CD33 bicistronic

FIG. 62

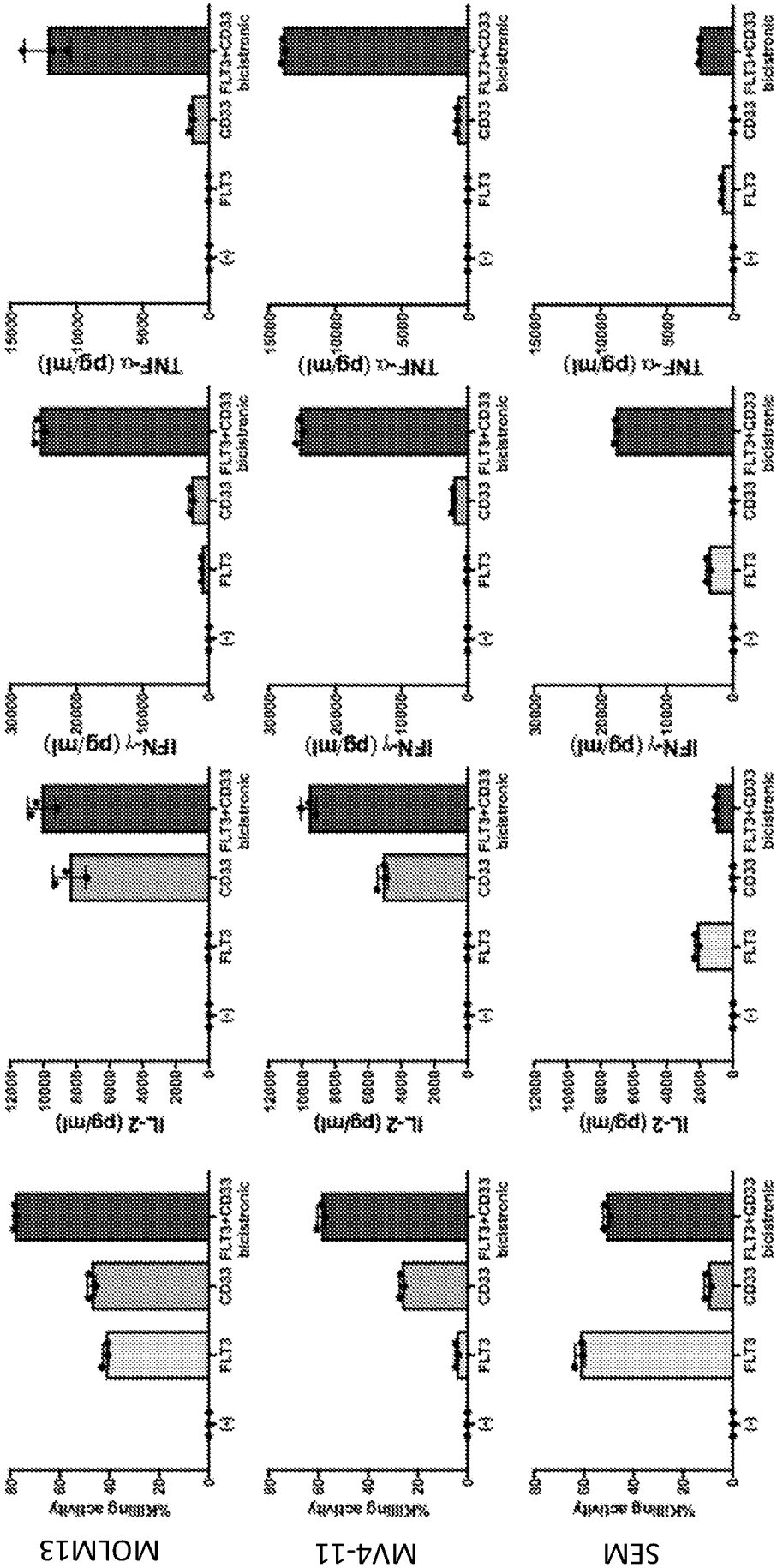

MOLM13
MV4-11
SEM
%Killing activity
IL-2 (pg/ml)
IFN-γ (pg/ml)
TNF-α (pg/ml)
(-)
FLT3
CD33
FLT3+CD33 bicistronic

FIG. 63C

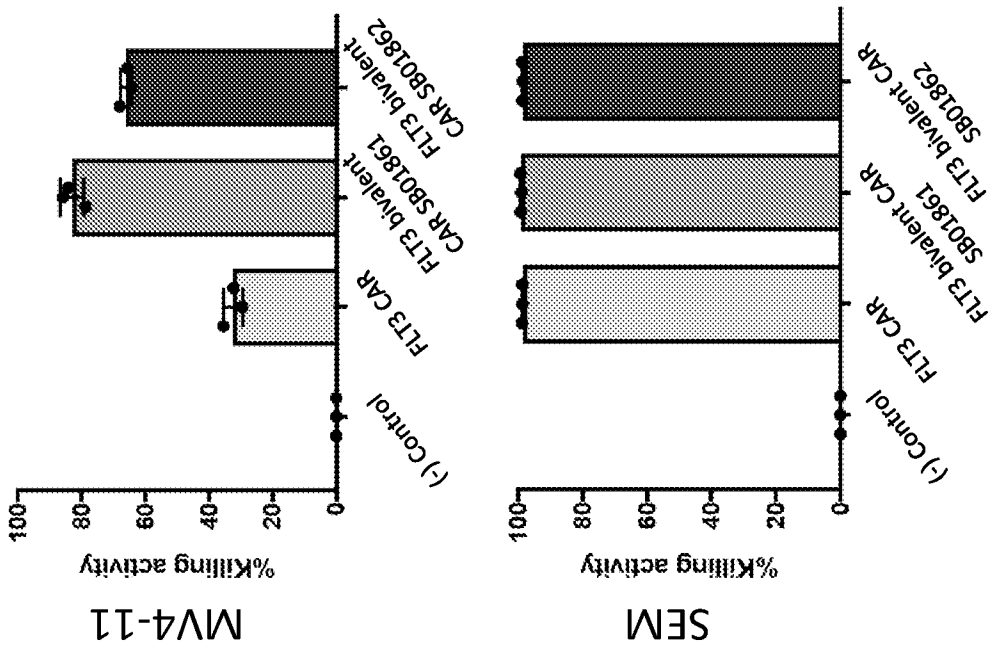


FIG. 63B

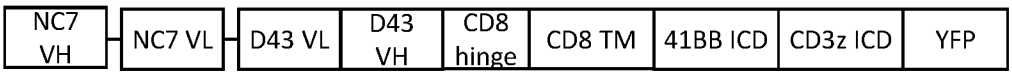


FIG. 63A

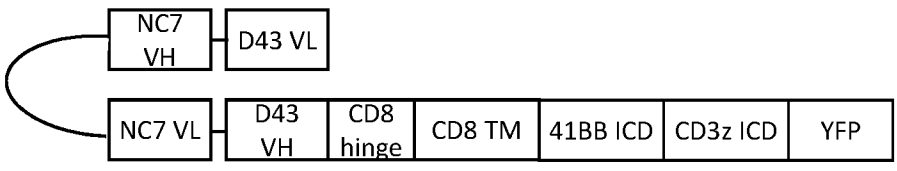


FIG. 64A

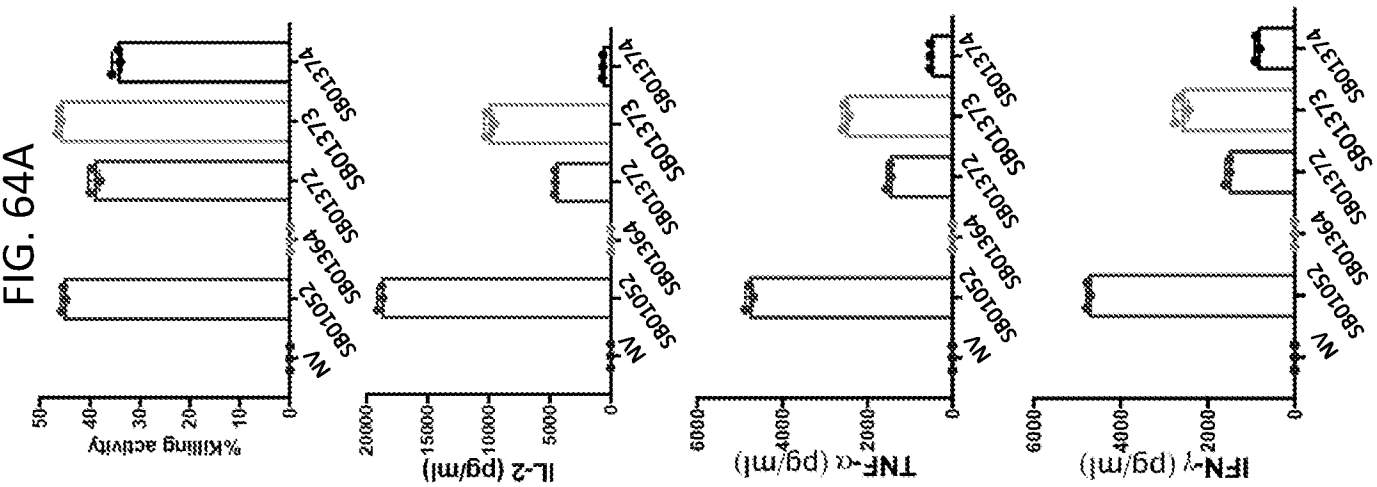


FIG. 64B

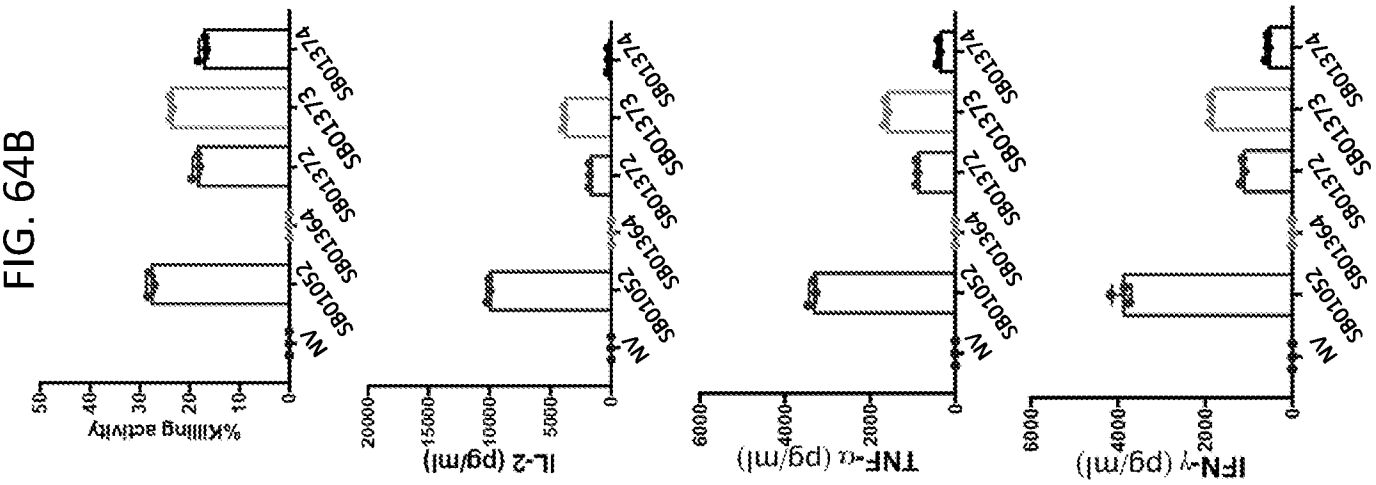


FIG. 64C

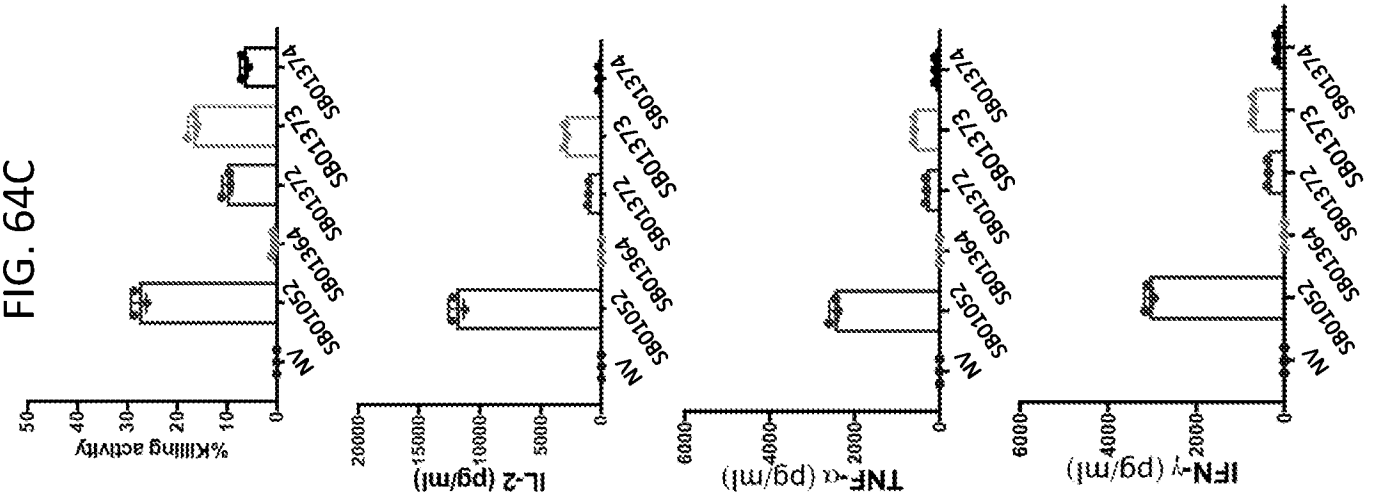


FIG. 65B

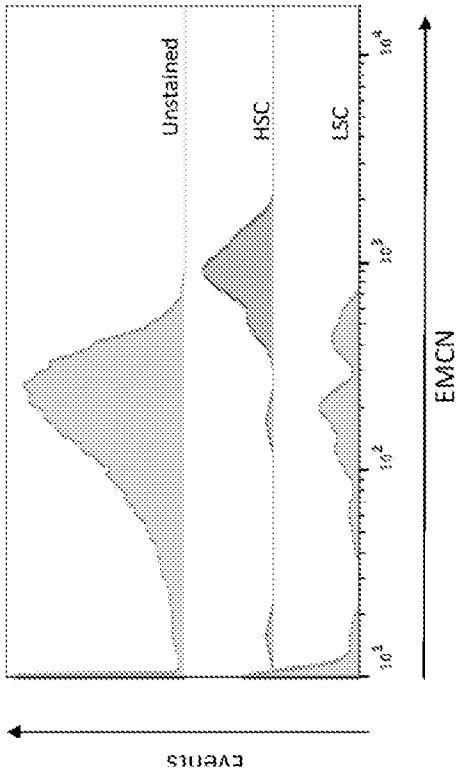


FIG. 65A

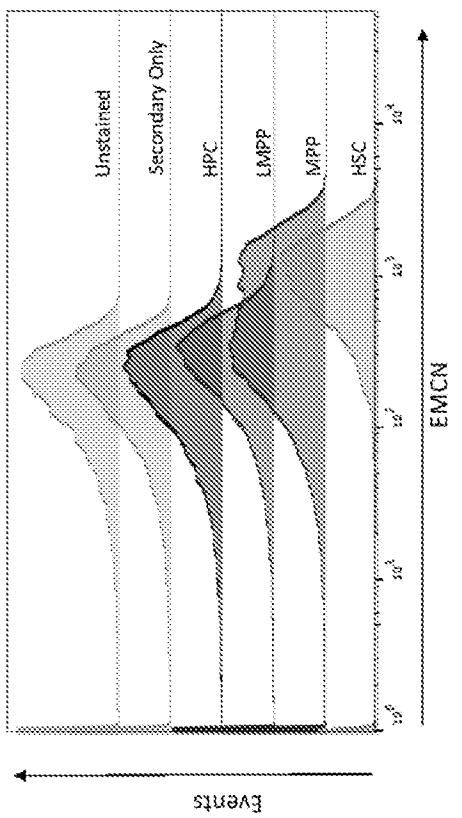


FIG. 65C

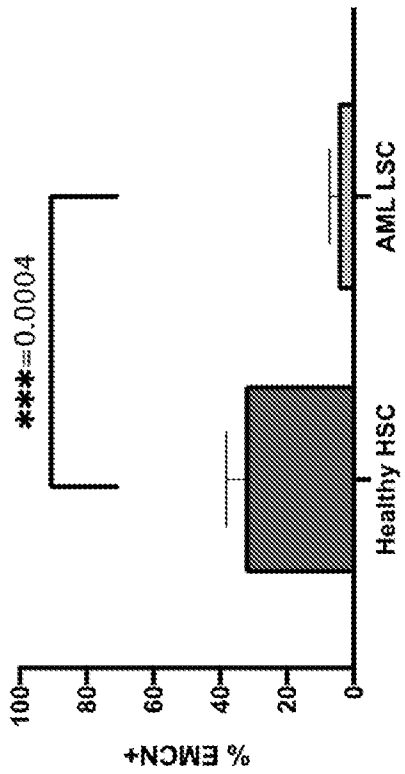


FIG. 66A

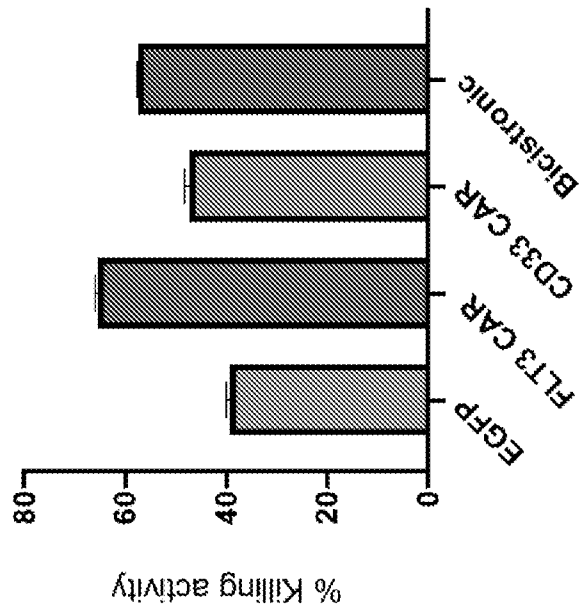


FIG. 66B

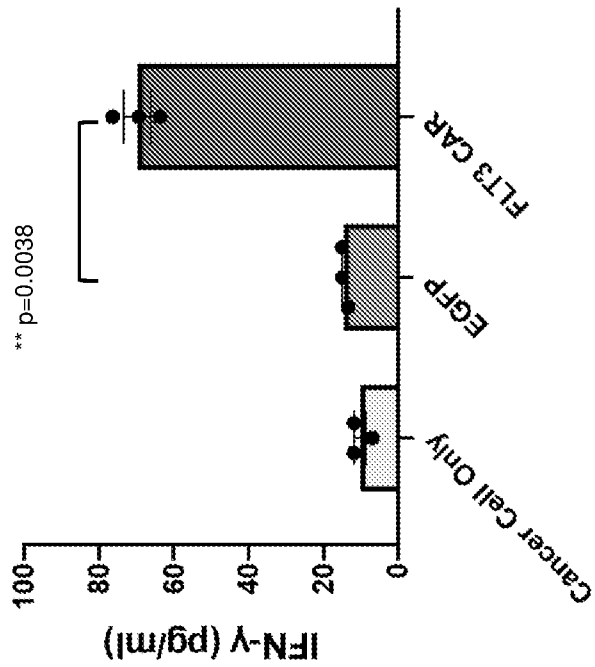


FIG. 66C

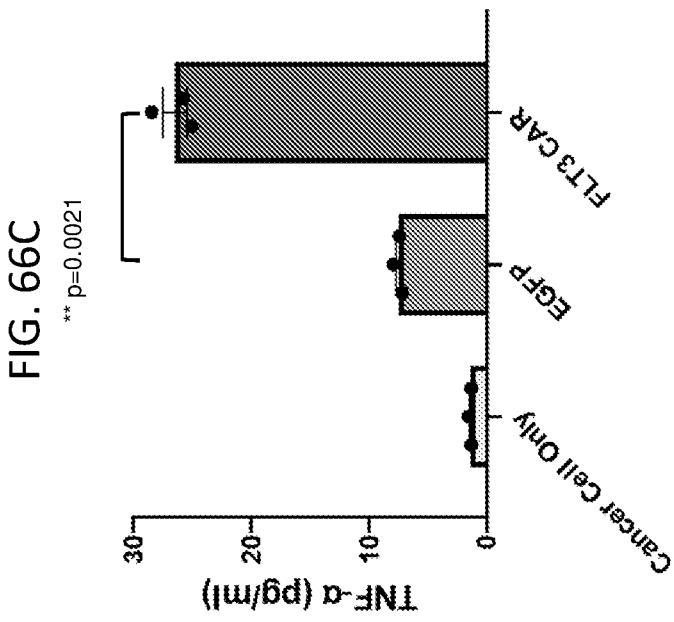


FIG. 66D

