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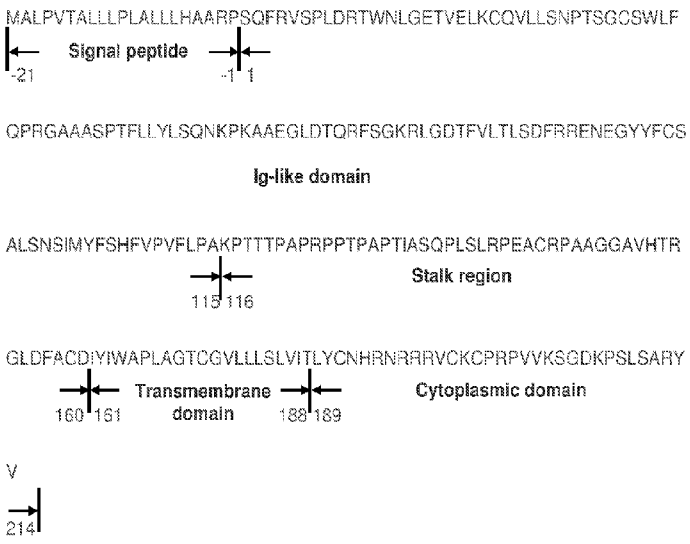

FIG. 1

(57) Abstract: The present disclosure relates to T cells capable of co-expressing T cell receptors ("TCR") together with CD8 polypeptides and the use thereof in adoptive cellular therapy. The present disclosure further provides for modified CD8 sequences, vectors, and associated methods thereof.

[Continued on next page]

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CD8 POLYPEPTIDES, COMPOSITIONS, AND METHODS OF USING THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This is an International Application under the Patent Cooperation Treaty, claiming priority to United States Provisional Patent Application No. 63/132,824, filed December 31, 2020, United States Provisional Patent Application No. 63/247,775, filed September 23, 2021 and German Provisional Patent Application No. 10 2021 100 038.6, filed January 4, 2021, the contents of which are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The official copy of the sequence listing is submitted concurrently via EFS-Web as an ASCII-formatted sequence listing with a file named "3000011-022977_Sequence_Listing_Final.txt" created on December 28, 2021, and having a size of 514,610 bytes, and is filed concurrently with the specification. The sequence listing contained in this ASCII-formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND

Field

[0003] The present disclosure relates to T cells capable of co-expressing T cell receptors ("TCR") together with CD8 polypeptides and the use thereof in adoptive cellular therapy. The present disclosure further provides for modified CD8 sequences, vectors, compositions, transformed T cells, and associated methods thereof.

Background

[0004] CD8 and CD4 are transmembrane glycoproteins characteristic of distinct populations of T lymphocytes whose antigen responses are restricted by class I and class II MHC molecules, respectively. They play major roles both in the differentiation and selection of T cells during thymic development and in the activation of mature T lymphocytes in response to antigen presenting cells. Both CD8 and CD4 are immunoglobulin superfamily proteins. They determine antigen restriction by binding to MHC molecules at an interface distinct from the region presenting the antigenic peptide, but the structural basis for their similar functions appears to be very different. Their sequence similarity is low and, whereas CD4 is expressed on the cell

surface as a monomer, CD8 is expressed as an αα homodimer (e.g., FIG. 55C) or an αβ heterodimer (e.g., FIG. 55A). In humans, this CD8αα homodimer may functionally substitute for the CD8αβ heterodimer. CD8 contacts an acidic loop in the α3 domain of Class I MHC, thereby increasing the avidity of the T cell for its target. CD8 is also involved in the phosphorylation events leading to CTL activation through the association of its α chain cytoplasmic tail with the tyrosine kinase $p56^{lck}$.

[0005] It is desirable to develop methods of manufacturing T cells with enhanced, specific cytotoxic activity for immunotherapy.

BRIEF SUMMARY

[0006] In an embodiment, CD8 polypeptides described herein may comprise a CD8α immunoglobulin (Ig)-like domain, a CD8β region, a CD8α transmembrane domain, and a CD8α cytoplasmic domain. In another embodiment, the CD8β region is a CD8β stalk region or domain.

[0007] In an embodiment, CD8 polypeptides described herein may comprise (a) an immunoglobulin (Ig)-like domain comprising at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1, (b) a CD8β region comprising at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity sequence identity to the amino acid sequence of SEQ ID NO: 2, (c) a transmembrane domain comprising at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, and (d) a cytoplasmic domain comprising at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4.

[0008] In an embodiment, CD8 polypeptides described herein have at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5.

[0009] In an embodiment, CD8 polypeptides described herein have at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 7.

[0010] In an embodiment, the CD8 polypeptides described herein may comprise a signal peptide with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NO: 6, SEQ ID

NO: 293, or SEQ ID NO: 294 fused to the N-terminus or to the C-terminus of CD8 polypeptides described herein.

[0011] In an embodiment, CD8 polypeptides described herein may comprise (a) SEQ ID NO: 1 comprising one, two, three, four, or five amino acid substitutions; (b) SEQ ID NO: 2 comprising one, two, three, four, or five amino acid substitutions; (c) SEQ ID NO: 3 comprising one, two, three, four, or five amino acid substitutions, and (d) SEQ ID NO: 4 comprising one, two, three, four, or five amino acid substitutions.

[0012] In an embodiment, CD8 polypeptides described herein may be CD8α or modified CD8α polypeptides.

[0013] In an embodiment, the disclosure provides for nucleic acids encode polypeptides described herein.

[0014] In an embodiment, a vector may comprise a nucleic acid encoding CD8 polypeptides described herein.

[0015] In an embodiment, the vector may comprise a nucleic acid encoding T cell receptor (TCR) comprising an α chain and a β chain. In another embodiment, the vector may comprise a nucleic acid encoding a CAR-T.

[0016] In an embodiment, TCR α chain and TCR β chain may be selected from SEQ ID NO: 15 and 16; 17 and 18; 19 and 20; 21 and 22; 23 and 24; 25 and 26; 27 and 28; 29 and 30; 31 and 32; 33 and 34; 35 and 36; 37 and 38; 39 and 40; 41 and 42; 43 and 44; 45 and 46; 47 and 48; 49 and 50; 51 and 52; 53 and 54; 55 and 56; 57 and 58; 59 and 60; 61 and 62; 63 and 64; 65 and 66; 67 and 68; 69 and 70; 71 and 303; 304 and 74; 75 and 76; 77 and 78; 79 and 80; 81 and 82; 83 and 84; 85 and 86; 87 and 88; 89 and 90; and 91 and 92.

[0017] In an embodiment, the vector may comprise a nucleic acid encoding a CD8β polypeptide.

[0018] In an embodiment, CD8β polypeptide may comprise the amino acid sequence of any one of SEQ ID NO: 8, 9, 10, 11, 12, 13, or 14.

[0019] In an embodiment, the vector may comprise nucleic acid encoding a 2A peptide or an internal ribosome entry site (IRES) positioned between the nucleic acid encoding the modified CD8α polypeptide and the nucleic acid encoding a CD8β polypeptide.

[0020] In an embodiment, the vector may comprise nucleic acid encoding a 2A peptide positioned between the nucleic acid encoding a TCR α chain and the nucleic acid encoding a TCR β chain.

[0021] In an embodiment, the 2A peptide may be selected from P2A (SEQ ID NO: 93), T2A (SEQ ID NO: 94), E2A (SEQ ID NO: 95), or F2A (SEQ ID NO: 96).

[0022] In an embodiment, the IRES may be selected from the group consisting of IRES from picornavirus, IRES from flavivirus, IRES from pestivirus, IRES from retrovirus, IRES from lentivirus, IRES from insect RNA virus, and IRES from cellular mRNA.

[0023] In an embodiment, the vector may further comprise a post-transcriptional regulatory element (PRE) sequence selected from a Woodchuck PRE (WPRE) and variants thereof, a hepatitis B virus (HBV) PRE (HPRE), or a combination thereof.

[0024] In an embodiment, the vector may further comprise a promoter selected from cytomegalovirus (CMV) promoter, phosphoglycerate kinase (PGK) promoter, myelin basic protein (MBP) promoter, glial fibrillary acidic protein (GFAP) promoter, modified MoMuLV LTR comprising myeloproliferative sarcoma virus enhancer (MNDU3), Ubiqitin C promoter, EF-1 alpha promoter, Murine Stem Cell Virus (MSCV) promoter, or a combination thereof.

[0025] In an embodiment, the vector may be a viral vector or a non-viral vector.

[0026] In an embodiment, the vector may be selected from adenoviruses, poxviruses, alphaviruses, arenaviruses, flaviruses, rhabdoviruses, retroviruses, lentiviruses, herpesviruses, paramyxoviruses, picornaviruses, or a combination thereof.

[0027] In an embodiment, the vector may be pseudotyped with an envelope protein of a virus selected from the native feline endogenous virus (RD114), a chimeric version of RD114 (RD114TR), gibbon ape leukemia virus (GALV), a chimeric version of GALV (GALV-TR), amphotropic murine leukemia virus (MLV 4070A), baculovirus (GP64), vesicular stomatitis virus (VSV-G), fowl plague virus (FPV), Ebola virus (EboV), or baboon retroviral envelope glycoprotein (BaEV), lymphocytic choriomeningitis virus (LCMV), or a combination thereof.

[0028] In an embodiment, the vector may further comprise a nucleic acid encoding a T cell receptor (TCR).

[0029] In another embodiment, the vector may further comprise a nucleic acid encoding a chimeric antigen receptor (CAR).

[0030] In an embodiment, an isolated nucleic acid may comprise a nucleic acid sequence encoding a T-cell receptor comprising an α chain and a β chain and a CD8 polypeptide comprising an α chain and a β chain. The isolated nucleic acid may comprise a nucleic acid at least 80% identical to the nucleic acid sequence of SEQ ID NO: 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301. The isolated nucleic acid may be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic

acid sequence of SEQ ID NO: 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301. In an aspect, sequences described herein may be isolated or recombinant sequences.

[0031] In an embodiment, the isolated nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 267.

[0032] In an embodiment, the isolated nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 279.

[0033] In an embodiment, the isolated polypeptide(s) may be encoded by the nucleic acids described herein.

[0034] In an embodiment, the isolated polypeptide may comprise the amino acid sequence at least about 80% identical to the amino acid sequence of SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302. The amino acid sequence may be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302. In another aspect, SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302 comprise 1, 2, 3, 4, 5, 10, 15, or 20 or more amino acid substitutions or deletions. In yet another aspect, SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302 comprise at most 1, 2, 3, 4, 5, 10, 15, or 20 amino acid substitutions or deletions.

[0035] In an embodiment, the isolated polypeptide may comprise the amino acid sequence of SEQ ID NO: 268.

[0036] In an embodiment, the isolated polypeptide may comprise the amino acid sequence of SEQ ID NO: 280. In an embodiment, a cell may be transduced with the vector.

[0037] In an embodiment, the cell may comprise αβ T cell, γδ T cell, natural killer cell, CD4+ /CD8+ cell, or combinations thereof.

[0038] In an embodiment, αβ T cell may comprise CD4+ T cell and CD8+ T cell.

[0039] In an embodiment, a method of preparing T cells for immunotherapy may comprise isolating T cells from a blood sample of a human subject, activating the isolated T cells, transducing the activated T cells with the vector, and expanding the transduced T cells.

[0040] In an embodiment, the T cell may be CD4+ T cell.

[0041] In an embodiment, the T cell may be CD8+ T cell.

[0042] In an embodiment, the T cell may be γδ T cell.

[0043] In an embodiment, the T cells may be a αβ T cell and express a CD8 polypeptide described herein.

[0044] In an embodiment, the T cells may be a γδ T cell and express a modified CD8 polypeptide described herein, for example, a modified CD8α polypeptide or a modified CD8α polypeptide with a CD8β stalk region, e.g., m1CD8α in Constructs #11 and #12 (FIG. 4) and CD8α* (FIG. 55B).

[0045] In an embodiment, a method of treating a patient who has cancer may comprise administering to the patient a composition comprising the population of expanded T cells, wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide is selected from SEQ ID NO: 98-255, wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, prostate cancer, or a combination thereof.

[0046] In an embodiment, the composition may further comprise an adjuvant.

[0047] In an embodiment, the adjuvant may be selected from anti-CD40 antibody, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, atezolizumab, interferon-alpha, interferon-beta, CpG oligonucleotides and derivatives, poly(I:C) and derivatives, RNA, sildenafil, particulate formulations with poly(lactide co-glycolide) (PLG), virosomes, interleukin (IL)-1, IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-21, IL-23, or combinations thereof.

[0048] In an embodiment, a method of eliciting an immune response in a patient who has cancer may comprise administering to the patient a composition comprising the population of expanded T cells, wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide is selected from SEQ ID NO: 98-255, wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, prostate cancer, or a combination thereof.

[0049] The disclosure further provides for a population of modified T cells that present an exogenous CD8 co-receptor comprising a polypeptide described herein, for example, amino acid

sequences at least 80%, at least 85%, at least 90%, or at least 95%, at least 99%, or 100% to SEQ ID NO: 5, 7, 258, 259, 8, 9, 10, 11, 12, 13, or 14 and a T cell receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] FIG. 1 shows a representative CD8α subunit, *e.g.*, SEQ ID NO: 258 (CD8α1), .In this embodiment, CD8α1 includes five domains: (1) signal peptide, (2) Ig-like domain-1, (3) a stalk region, (4) transmembrane (TM) domain, and (5) a cytoplasmic tail (Cyto) comprising a *lck*-binding motif.

[0051] FIG. 2 shows a sequence alignment between CD8α1 (SEQ ID NO: 258) and m1CD8α (SEQ ID NO: 7).

[0052] FIG. 3 shows a sequence alignment between CD8α2 (SEQ ID NO: 259) and m2CD8α (SEQ ID NO: 262), in which the cysteine substitution at position 112 is indicated by an arrow.

[0053] FIG. 4 shows vectors according to an aspect of the disclosure.

[0054] FIG. 5A shows titers of viral vectors shown in FIG. 4.

[0055] FIG. 5B shows titers of further viral vectors in accordance with an embodiment of the present disclosure. Construct #13; Construct #14; Construct #15; Construct #16; Construct #17; Construct #18; Construct #19; Construct #21; Construct #10n; Construct #11n; and TCR: R11KEA (SEQ ID NO: 15 and SEQ ID NO: 16) (Construct #8), which binds PRAME-004 (SLLQHLIGL) (SEQ ID NO: 147). Note that Constructs #10 and #10n are different batches of the same construct (SEQ ID NO: 291 and 292) and Constructs #11 and #11n are different batches of the same construct (SEQ ID NO: 285 and 286).

[0056] FIG. 6 shows T cell manufacturing.

[0057] FIG. 7A shows expression of activation markers before and after activation in CD3+CD8+ cells.

[0058] FIG. 7B shows expression of activation markers before and after activation in CD3+CD4+ cells .

[0059] FIG. 8A shows fold expansion of cells transduced with various constructs from Donor #1. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control). Note that Constructs #9 and #9b are different batches of the same construct (SEQ ID NO: 287 and 288).

[0060] FIG. 8B shows fold expansion of cells transduced with various constructs from Donor #2. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE) (Construct #8); NT = Non-transduced T cells (as a negative control).

[0061] FIG. 9A shows flow plots of cells transduced with Construct #9 .

[0062] FIG. 9B shows flow plots of cells transduced with Construct #10 in accordance with one embodiment of the present disclosure.

[0063] FIG. 9C shows flow plots of cells transduced with Construct #11.

[0064] FIG. 9D shows flow plots of cells transduced with Construct #12.

[0065] FIG. 10 shows % CD8+CD4+ of cells transduced with various constructs for Donor #1 and Donor #2. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0066] FIG. 11 shows % Tet of CD8+CD4+ of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0067] FIG. 12 shows Tet MFI (CD8+CD4+Tet+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0068] FIG. 13 shows CD8α MFI (CD8+CD4+Tet+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0069] FIG. 14 shows % CD8+CD4 (of CD3+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0070] FIG. 15 shows % CD8+Tet+ (of CD3+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0071] FIG. 16 shows Tet MFI (CD8+Tet+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0072] FIG. 17 shows CD8α MFI (CD8+Tet+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0073] FIG. 18 shows % Tet+ (of CD3+) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0074] FIG. 19 shows VCN (upper panel) and CD3+Tet+/VCN (lower panel) of cells transduced with various constructs. The constructs are as follows: Construct #9b; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; TCR = R11KEA.WPRE^{wt} (TCR with wild type WPRE); NT = Non-transduced T cells (as a negative control).

[0075] FIG. 20A-20C depicts data showing that constructs (#10, #11, & #12) are comparable to TCR-only in mediating cytotoxicity against target positive cells lines expressing antigen at different levels (UACC257 at 1081 copies per cell and A375 at 50 copies per cell).

[0076] FIG. 21A-21B depict data showing that IFNγ secretion in response to UACC257 is comparable among constructs, however with A375, #10 expressing is the highest among all constructs. However, comparing #9 with #11 expressing wild type and modified CD8 coreceptor sequences respectively, T cells transduced with #11 induced stronger cytokine response measured as IFNγ quantified in the supernatants from Incucyte plates. Construct #9; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; Construct #8 = R11KEA TCR only.

[0077] FIG. 22 depicts an exemplary experiment design to assess DC maturation and cytokine secretion by PBMC-derived product in response to UACC257 and A375 targets. N=2.

[0078] FIG. 23A-23B depicts data showing that the IFNγ secretion in response to A375 increases in the presence of iDCs. In the tri-cocultures with iDCs, IFNγ secretion is higher in Construct #10 compared to the other constructs. However, comparing Construct #9 with Construct #11 expressing wild type and modified CD8 coreceptor sequences respectively, T cells transduced with #11 induced stronger cytokine response measured as IFNγ quantified in the

culture supernatants of three-way cocultures using donor D600115, E:T:iDC::1:1/10:1/4. Construct #9; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; Construct #8 = R11KEA TCR only.

[0079] FIG. 24A-24B depicts data showing that IFNγ secretion in response to A375 increases in the presence of iDCs. In the tri-cocultures with iDCs, IFNγ secretion was higher in Construct #10 compared to the other constructs. IFNγ quantified in the culture supernatants of three-way cocultures using donor D150081, E:T:iDC::1:1/10:1/4. Construct #9; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; Construct #8 = R11KEA TCR only.

[0080] FIG. 25A-25B depicts data showing that IFNγ secretion in response to UACC257 increases in the presence of iDCs. In the tri-cocultures with iDCs, IFNγ secretion is higher in Construct #10 compared to the other constructs. However, comparing Construct #9 with Construct #11 expressing wild type and modified CD8 coreceptor sequences respectively, T cells transduced with Construct #11 induced stronger cytokine response measured as IFNγ quantified in the culture supernatants of three-way cocultures using donor D600115, E:T:iDC::1:1/10:1/4. Construct #9; Construct #10; Construct #11; Construct #12; Construct #1; Construct #2; Construct #8 = R11KEA TCR only.

[0081] FIG. 26 shows T cell manufacturing in accordance with one embodiment of the present disclosure.

[0082] FIG. 27A shows expression of activation markers before and after activation in CD3+CD8+ cells.

[0083] FIG. 27B shows expression of activation markers before and after activation in CD3+CD4+ cells in accordance with one embodiment of the present disclosure.

[0084] FIG. 28 shows fold expansion of cells transduced with various constructs.

[0085] FIG. 29A & 29B show % CD8+CD4+ of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0086] FIG. 30A & 30B show % Tet of CD8+CD4+ of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0087] FIG. 31A & 31B show Tet MFI (CD8+CD4+Tet+) of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0088] FIG. 32A & 32B show % CD8+CD4- (of CD3+) of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0089] FIG. 33A & 33B show % CD8+Tet+ (of CD3+) of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0090] FIG. 34A & 34B show Tet MFI (CD8+Tet+) of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0091] FIG. 35A & 35B show % Tet+ (of CD3+) of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0092] FIG. 36A & 36B show VCN of cells transduced with various constructs in accordance with one embodiment of the present disclosure.

[0093] FIG. 37 shows T cell manufacturing in accordance with one embodiment of the present disclosure.

[0094] FIG. 38 shows % Tet of CD8+CD4+ of cells transduced with various constructs.

[0095] FIG. 39 shows Tet MFI of CD8+CD4+Tet+ of cells transduced with various constructs.

[0096] FIG. 40 shows Tet MFI of CD8+Tet+ of cells transduced with various constructs.

[0097] FIG. 41 shows % Tet+ of CD3+ cells transduced with various constructs.

[0098] FIG. 42 shows vector copy number (VCN) of cells transduced with various constructs.

[0099] FIG. 43 shows the % T cell subsets in cells transduced with various constructs .FACS analysis was gated on CD3+TCR+.

[00100] FIG. 44A and FIG. 44B shows % T cell subsets in cells transduced with various constructs .FACS analysis was gated on CD4+CD8+ for FIG. 44A and on CD4-CD8+TCR+ for FIG. 44B.

[00101] FIG. 45A and 45B depicts data showing that Constructs #13 and #10 are comparable to TCR-only in mediating cytotoxicity against UACC257 target positive cells lines expressing high levels of antigen (1081 copies per cell). Construct # 15 was also effective but slower in killing compared to Constructs #13 and #10. The effector:target ratio used to generate these results was 4:1.

[00102] FIG. 46 shows IFNγ secretion in response in UACC257 cell line was higher with Construct #13 compared to Construct #10. IFNγ quantified in the supernatants from Incucyte plates. The effector:target ratio used to generate these results was 4:1.

[0101] FIG. 47 shows ICI marker frequency (2B4, 41BB, LAG3, PD-1, TIGIT, TIM3, CD39+CD69+, and CD39-CD69-).

[0102] FIG. 48A – 48G show increased expression of IFNγ, IL-2, and TNFα with CD4+CD8+ cells transduced with Construct #10 (WT signal peptide, CD8β1) compared to other constructs. FACS analysis was gated on CD3+CD4+CD8+ cells against UACC257, 4:1 E:T.

[0103] FIG. 49A-49G show increased expression of IFNγ, IL-2, MIP-1β, and TNFα with CD4-CD8+ cells transduced with Construct #10 (WT signal peptide, CD8β1) compared to other constructs. FACS analysis was gated on CD3+CD4-CD8+ cells against UACC257, 4:1 E:T.

[0104] FIG. 50A-50G show increased expression of IL-2 and TNFα with CD3+TCR+ cells transduced with Construct #10 (WT signal peptide, CD8β1) compared to other constructs. FACS analysis was gated on CD3+TCR+ cells against UACC257, 4:1 E:T.

[0105] FIG. 51A-51C show results from FACS analysis gated on CD4+CD8+ cells against A375, 4:1 E:T.

[0106] FIG. 52A-52C show results from FACS analysis gated on CD4-CD8+ cells against A375, 4:1 E:T.

[0107] FIG. 53A-53C show results from FACS analysis gated on CD3+TCR+ cells against A375, 4:1 E:T.

[0108] FIG. 54 shows T cell manufacturing in accordance with one embodiment of the present disclosure.

[0109] FIG. 55A-55C show interaction between peptide/MHC complex of antigen-presenting cell (APC) with T cell by binding a complex of TCR and CD8αβ heterodimer (FIG. 55A, e.g., produced by transducing T cells with Constructs #2, #3, #4, #10, #13, #14, #15, #16, #17, #18, or #21), a complex of TCR and homodimer CD8α having its stalk region replaced with CD8β stalk region (CD8αα*) (FIG. 55B, e.g., produced by transducing T cells with Construct #11, #12, or #19), and a complex of TCR and CD8α homodimer (FIG. 55C, e.g., produced by transducing T cells with Constructs #1, #5, #6, #7, or #9).

[0110] FIG. 56 shows the levels of IL-12 secretion by dendritic cells (DC) in the presence of CD4+ T cells transduced with Construct #10 or #11 and immature dendritic cells (iDCs) in accordance with one embodiment of the present disclosure.

[0111] FIG. 57 shows the levels of TNF-α secretion by dendritic cells (DC) in the presence of CD4+ T cells transduced with Construct #10 or #11 and immature dendritic cells (iDCs) in accordance with one embodiment of the present disclosure.

[0112] FIG. 58 shows the levels of IL-6 secretion by dendritic cells (DC) in the presence of CD4+ T cells transduced with Construct #10 or #11 and immature dendritic cells (iDCs) in accordance with one embodiment of the present disclosure.

[0113] FIG. 59 shows a scheme of determining the levels of cytokine secretion by dendritic cells (DC) in the presence of PBMCs transduced with various constructs and target cells in accordance with one embodiment of the present disclosure.

[0114] FIG. 60 shows the levels of IL-12 secretion by dendritic cells (DC) in the presence of PBMCs transduced with various constructs and target cells in accordance with one embodiment of the present disclosure.

[0115] FIG. 61 shows the levels of TNF-α secretion by dendritic cells (DC) in the presence of PBMCs transduced with various constructs and target cells in accordance with one embodiment of the present disclosure

[0116] FIG. 62 shows the levels of IL-6 secretion by dendritic cells (DC) in the presence of PBMCs transduced with various constructs and target cells in accordance with one embodiment of the present disclosure.

[0117] FIG. 63A-63C show IFNγ production from the transduced CD4+ selected T cells obtained from Donor #1 (FIG. 63A), Donor #2 (FIG. 63B), and Donor #3 (FIG. 63C) in accordance to one embodiment of the present disclosure.

[0118] FIG. 63D shows EC50 values (ng/ml) in FIG. 63A-63C.

[0119] FIG. 64A-64C show IFNγ production from the transduced PBMC obtained from Donor #4 (FIG. 64A), Donor #1 (FIG. 64B), and Donor #3 (FIG. 64C) and their respective EC50 values (ng/ml) in accordance to one embodiment of the present disclosure.

[0120] FIG. 64D shows comparison of EC50 values (ng/ml) among different donors in FIG. 64A-64C.

[0121] FIG. 65A-65C show IFNγ production from the transduced PBMC (FIG. 65A), CD8+ selected T cells (FIG. 65B), and CD4+ selected T cells (FIG. 65C) and their respective EC50 values (ng/ml) from a single donor in accordance to one embodiment of the present disclosure.

DETAILED DESCRIPTION

Modified CD8 polypeptides

[0117] CD8 polypeptides described herein may comprise the general structure of a N-terminal signal peptide (optional), CD8α immunoglobulin (Ig)-like domain, CD8□ region (domain), CD8α transmembrane domain, and a CD8α cytoplasmic domain. The modified CD8 polypeptides described herein shown an unexpected improvement in functionality of T cells co-transduced with a vector expressing a TCR and CD8 polypeptide.

[0118] CD8 polypeptides described herein may comprise the general structure of a N-terminal signal peptide (optional), CD8α immunoglobulin (Ig)-like domain, a stalk domain or region, CD8α transmembrane domain, and a CD8α cytoplasmic domain.

[0119] In an embodiment, CD8 polypeptides described herein may comprise (a) an

immunoglobulin (Ig)-like domain comprising at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1; (b) a region comprising at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2; (c) a transmembrane domain comprising at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, and (d) a cytoplasmic domain comprising at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4. The CD8 polypeptides described herein may be co-expressed with a T-cell receptor or CAR-T in a T-cell and used in methods of adoptive cell therapy (ACT). The T-cell may be an αβ T-cell or a γδ T-cell.

[0120] In another embodiment, CD8 polypeptides described herein may comprise (a) at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1; (b) at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2; (c) at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3, and (d) a at least about 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4. The CD8 polypeptides described herein may be co-expressed with a T-cell receptor or CAR-T in a T-cell and used in methods of adoptive cell therapy (ACT). The T-cell may be an αβ T-cell or a γδ T-cell.

[0121] In another embodiment, CD8 polypeptides described herein may comprise (a) SEQ ID NO: 1 comprising one, two, three, four, or five amino acid substitutions; (b) SEQ ID NO: 2 comprising one, two, three, four, or five amino acid substitutions; (c) SEQ ID NO: 3 comprising one, two, three, four, or five amino acid substitutions, and (d) SEQ ID NO: 4 comprising one, two, three, four, or five amino acid substitutions. In an embodiment, the substitutions are conservative amino acid substitutions. The CD8 polypeptides described herein may be co-expressed with a T-cell receptor or CAR-T in a T-cell and used in methods of adoptive cell therapy (ACT). The T-cell may be an γδ T-cell or a γδ T-cell.

[0122] CD8 is a membrane-anchored glycoprotein that functions as a coreceptor for antigen recognition of the peptide/MHC class I complexes by T cell receptors (TCR) and plays an important role in T cell development in the thymus and T cell activation in the periphery.

Functional CD8 is a dimeric protein made of either two α chains (CD8αα) or an α chain and a β chain (CD8αβ), and the surface expression of the β chain may require its association with the coexpressed α chain to form the CD8αβ heterodimer. CD8αα and CD8αβ may be differentially expressed on a variety of lymphocytes. CD8αβ is expressed predominantly on the surface of $\alpha\beta TCR^+$ T cells and thymocytes, and CD8αα on a subset of $\alpha\beta TCR^+$, $\gamma\delta TCR^+$ intestinal intraepithelial lymphocytes, NK cells, dendritic cells, and a small fraction of $CD4^+$ T cells.

[0123] For example, human CD8 gene may express a protein of 235 amino acids. FIG. 1 shows a CD8α protein (CD8α1 – SEQ ID NO: 258), which in an aspect is divided into the following domains (starting at the amino terminal and ending at the carboxy terminal of the polypeptide): (1) signal peptide (amino acids -21 to -1), which may be cleaved off in human cells during the transport of the receptor to the cell surface and thus may not constitute part of the mature, active receptor; (2) immunoglobulin (Ig)-like domain (in this embodiment, amino acids 1-115), which may assume a structure, referred to as the immunoglobulin fold, which is similar to those of many other molecules involved in regulating the immune system, the immunoglobulin family of proteins. The crystal structure of the CD8αα receptor in complex with the human MHC molecule HLA-A2 has demonstrated how the Ig domain of CD8αα receptor binds the ligand; (3) membrane proximal region (in this embodiment, amino acids 116-160), which may be an extended linker region allowing the CD8αα receptor to "reach" from the surface of the T-cell over the top of the MHC to the a3 domain of the MHC where it binds. The stalk region may be glycosylated and may be inflexible; (4) transmembrane domain (in this embodiment, amino acids 161-188), which may anchor the CD8αα receptor in the cell membrane and is therefore not part of the soluble recombinant protein; and (5) cytoplasmic domain (in this embodiment, amino acids 189-214), which can mediate a signaling function in T-cells through its association with $p56^{lck}$, which may be involved in the T cell activation cascade of phosphorylation events.

[0124] CD8α sequences may generally have a sufficient portion of the immunoglobulin domain to be able to bind to MHC. Generally, CD8α molecules may contain all or a substantial part of immunoglobulin domain of CD8α, e.g., SEQ ID NO: 258, but in an aspect may contain at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110 or 115 amino acids of the immunoglobulin domain. The CD8α molecules of the present disclosure may be preferably dimers (*e.g.,* CD8αα or CD8αβ), although CD8α monomer may be included within the scope of the present disclosure. In an aspect, CD8α of the present disclosure may comprise CD8α1 (SEQ ID NO: 258) and CD8α2 (SEQ ID NO: 259).

[0125] CD8α and β subunits may have similar structural motifs, including an Ig-like domain, a stalk region of 30–40 amino acids, a transmembrane region, and a short cytoplasmic domain of about 20 amino acids. CD8α and β chains have two and one *N*-linked glycosylation sites, respectively, in the Ig-like domains where they share < 20% identity in their amino acid sequences. The CD8β stalk region is 10–13 amino acids shorter than the CD8α stalk and is highly glycosylated with *O*-linked carbohydrates. These carbohydrates on the β, but not the α, stalk region appear to be quite heterogeneous due to complex sialylations, which may be differentially regulated during the developmental stages of thymocytes and upon activation of T cells. Glycan adducts have been shown to play regulatory roles in the functions of glycoproteins and in immune responses. Glycans proximal to transmembrane domains can affect the orientation of adjacent motifs. The unique biochemical properties of the CD8β chain stalk region may present a plausible candidate for modulating the coreceptor function.

[0126] The CD8 polypeptide may be modified, in which CD8α region, for example a stalk region, may be replaced by CD8β region. In another aspect, to create a CD8□-CD8□ polypeptide. In an embodiment, the modified CD8 polypeptides described herein may have a region comprising at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2. The modified CD8α polypeptides described herein may have an immunoglobulin (Ig)-like domain having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1. Modified CD8 polypeptides may have a transmembrane domain comprising at least at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 3. Modified CD8 polypeptides described herein may have a cytoplasmic tail comprising at least at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4. The CD8 polypeptides described herein may have at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 5. The CD8 polypeptides described herein may comprise a signal peptide comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 294 fused to the N-terminus or fused to the C-terminus of mCD8α polypeptide. The CD8 polypeptides described herein may have at

least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 7.

T-Cells

[0127] T-cells may express the modified CD8 polypeptides described herein. For example, a T-cell may co-express a T-cell Receptor (TCR) and modified CD8 polypeptides described herein. T-cells may also express a chimeric antigen receptor (CAR), CAR-analogues, or CAR derivatives.

[0128] The T-cell may be a αβ T cell, γδ T cell, natural killer T cell, or a combination thereof if in a population. The T cell may be a CD4+ T cell, CD8+ T cell, or a CD4+/CD8+ T cell.

T-cell Receptors

[0129] A T-cell may co-express a T-cell receptor (TCR), antigen binding protein, or both, with modified CD8 polypeptides described herein, including, but are not limited to, those listed in Table 3 (SEQ ID NOs: 15-92). Further, a T-cell may express a TCRs and antigen binding proteins described in U.S. Patent Application Publication No. 2017/0267738; U.S. Patent Application Publication No. 2017/0312350; U.S. Patent Application Publication No. 2018/0051080; U.S. Patent Application Publication No. 2018/0164315; U.S. Patent Application Publication No. 2018/0161396; U.S. Patent Application Publication No. 2018/0162922; U.S. Patent Application Publication No. 2018/0273602; U.S. Patent Application Publication No. 2019/0016801; U.S. Patent Application Publication No. 2019/0002556; U.S. Patent Application Publication No. 2019/0135914; U.S. Patent 10,538,573; U.S. Patent 10,626,160; U.S. Patent Application Publication No. 2019/0321478; U.S. Patent Application Publication No. 2019/0256572; U.S. Patent 10,550,182; U.S. Patent 10,526,407; U.S. Patent Application Publication No. 2019/0284276; U.S. Patent Application Publication No. 2019/0016802; U.S. Patent Application Publication No. 2019/0016803; U.S. Patent Application Publication No. 2019/0016804; U.S. Patent 10,583,573; U.S. Patent Application Publication No. 2020/0339652; U.S. Patent 10,537,624; U.S. Patent 10,596,242; U.S. Patent Application Publication No. 2020/0188497; U.S. Patent 10,800,845; U.S. Patent Application Publication No. 2020/0385468; U.S. Patent 10,527,623; U.S. Patent 10,725,044; U.S. Patent Application Publication No. 2020/0249233; U.S. Patent 10,702,609; U.S. Patent Application Publication No. 2020/0254106; U.S. Patent 10,800,832; U.S. Patent Application Publication No. 2020/0123221; U.S. Patent 10,590,194; U.S. Patent 10,723,796; U.S. Patent Application Publication No. 2020/0140540; U.S. Patent 10,618,956; U.S. Patent Application Publication No. 2020/0207849; U.S. Patent

Application Publication No. 2020/0088726; and U.S. Patent Application Publication No. 2020/0384028; the contents of each of these publications and sequence listings described therein are herein incorporated by reference in their entireties. The T-cell may be a αβ T cell, γδ T cell, natural killer T cell. Natural killer cell. In an embodiment, TCRs described herein are single-chain TCRs or soluble TCRs.

[0130] Further, the TCRs that may be co-expressed with the modified CD8 polypeptides described herein in a T-cell may be TCRs comprised of an alpha chain (TCR□) and a beta chain (TCR□). The TCRα chains and TCRβ chains that may be used in TCRs may be selected from R11KEA (SEQ ID NO: 15 and 16), R20P1H7 (SEQ ID NO: 17 and 18), R7P1D5 (SEQ ID NO: 19 and 20), R10P2G12 (SEQ ID NO: 21 and 22), R10P1A7 (SEQ ID NO: 23 and 24), R4P1D10 (SEQ ID NO: 25 and 26), R4P3F9 (SEQ ID NO: 27 and 28), R4P3H3 (SEQ ID NO: 29 and 30), R36P3F9 (SEQ ID NO: 31 and 32), R52P2G11 (SEQ ID NO: 33 and 34), R53P2A9 (SEQ ID NO: 35 and 36), R26P1A9 (SEQ ID NO: 37 and 38), R26P2A6 (SEQ ID NO: 39 and 40), R26P3H1 (SEQ ID NO: 41 and 42), R35P3A4 (SEQ ID NO: 43 and 44), R37P1C9 (SEQ ID NO: 45 and 46), R37P1H1 (SEQ ID NO: 47 and 48), R42P3A9 (SEQ ID NO: 49 and 50), R43P3F2 (SEQ ID NO: 51 and 52), R43P3G5 (SEQ ID NO: 53 and 54), R59P2E7 (SEQ ID NO: 55 and 56), R11P3D3 (SEQ ID NO: 57 and 58), R16P1C10 (SEQ ID NO: 59 and 60), R16P1E8 (SEQ ID NO: 61 and 62), R17P1A9 (SEQ ID NO: 63 and 64), R17P1D7 (SEQ ID NO: 65 and 66), R17P1G3 (SEQ ID NO: 67 and 68), R17P2B6 (SEQ ID NO: 69 and 70), R11P3D3KE (SEQ ID NO: 71 and 303), R39P1C12 (SEQ ID NO: 304 and 74), R39P1F5 (SEQ ID NO: 75 and 76), R40P1C2 (SEQ ID NO: 77 and 78), R41P3E6 (SEQ ID NO: 79 and 80), R43P3G4 (SEQ ID NO: 81 and 82), R44P3B3 (SEQ ID NO: 83 and 84), R44P3E7 (SEQ ID NO: 85 and 86), R49P2B7 (SEQ ID NO: 87 and 88), R55P1G7 (SEQ ID NO: 89 and 90), or R59P2A7 (SEQ ID NO: 91 and 92). The T-cell may be a αβ T cell, γδ T cell, or a natural killer T cell.

[0131] Table 1 shows examples of the peptides to which TCRs bind when the peptide is in a complex with an MHC molecule. (MHC molecules in humans may be referred to as HLA, human leukocyte-antigens).

Table 1: T-Cell Receptor and Peptides

TCR name	Peptide (SEQ ID NO:)
R20P1H7, R7P1D5, R10P2G12	KVLEHVVRV (SEQ ID NO: 215)
R10P1A7	KIQEILTQV (SEQ ID NO: 123)
R4P1D10, R4P3F9, R4P3H3	FLLDGSANV (SEQ ID NO: 238)
R36P3F9, R52P2G11, R53P2A9	ILQDGQFLV (SEQ ID NO: 193)

R26P1A9, R26P2A6, R26P3H1, R35P3A4, R37P1C9, R37P1H1, R42P3A9, R43P3F2, R43P3G5, R59P2E7	KVLEYVIKV (SEQ ID NO: 202)
R11KEA, R11P3D3, R16P1C10, R16P1E8, R17P1A9, R17P1D7, R17P1G3, R17P2B6, R11P3D3KE	SLLQHLIGL (SEQ ID NO: 147)
R39P1C12, R39P1F5, R40P1C2, R41P3E6, R43P3G4, R44P3B3, R44P3E7, R49P2B7, R55P1G7, R59P2A7	ALSVLRLAL (SEQ ID NO: 248)

Tumor Associated Antigens (TAA)

[0132] Tumor associated antigen (TAA) peptides may be used with the CD8 polypeptides constructs, methods and embodiments described herein. For example, the T-cell receptors (TCRs) described herein may specifically bind to the TAA peptide when bound to a human leukocyte antigen (HLA). This is also known as a major histocompatibility complex (MHC) molecule. The MHC-molecules of the human are also designated as human leukocyte-antigens (HLA).

[0133] Tumor associated antigen (TAA) peptides that may be used with the CD8 polypeptides described herein include, but are not limited to, those listed in Table 3 and those TAA peptides described in U.S. Patent Application Publication No. 2016/0187351; U.S. Patent Application Publication No. 2017/0165335; U.S. Patent Application Publication No. 2017/0035807; U.S. Patent Application Publication No. 2016/0280759; U.S. Patent Application Publication No. 2016/0287687; U.S. Patent Application Publication No. 2016/0346371; U.S. Patent Application Publication No. 2016/0368965; U.S. Patent Application Publication No. 2017/0022251; U.S. Patent Application Publication No. 2017/0002055; U.S. Patent Application Publication No. 2017/0029486; U.S. Patent Application Publication No. 2017/0037089; U.S. Patent Application Publication No. 2017/0136108; U.S. Patent Application Publication No. 2017/0101473; U.S. Patent Application Publication No. 2017/0096461; U.S. Patent Application Publication No. 2017/0165337; U.S. Patent Application Publication No. 2017/0189505; U.S. Patent Application Publication No. 2017/0173132; U.S. Patent Application Publication No. 2017/0296640; U.S. Patent Application Publication No. 2017/0253633; U.S. Patent Application Publication No. 2017/0260249; U.S. Patent Application Publication No. 2018/0051080; U.S. Patent Application Publication No. 2018/0164315; U.S. Patent Application Publication No.

2018/0291082; U.S. Patent Application Publication No. 2018/0291083; U.S. Patent Application Publication No. 2019/0255110; U.S. Patent No. 9,717,774; U.S. Patent No. 9,895,415; U.S. Patent Application Publication No. 2019/0247433; U.S. Patent Application Publication No. 2019/0292520; U.S. Patent Application Publication No. 2020/0085930; U.S. Patent 10,336,809; U.S. Patent No. 10,131,703; U.S. Patent No. 10,081,664; U.S. Patent No. 10,081,664; U.S. Patent No. 10,093,715; U.S. Patent No. 10,583,573; and U.S. Patent Application Publication No. 2020/00085930; the contents of each of these publications, sequences, and sequence listings described therein are herein incorporated by reference in their entireties. The Tumor associated antigen (TAA) peptides described herein may be bound to an HLA (MHC molecule). The Tumor associated antigen (TAA) peptides bound to an HLA may be recognized by a TCR described herein, optionally co-expressed with CD8 polypeptides described herein.

[0134] T cells may be engineered to express a chimeric antigen receptor (CAR) comprising a ligand binding domain derived from NKG2D, NKG2A, NKG2C, NKG2F, LLT1, AICL, CD26, NKRP1, NKp30, NKp44, NKp46, CD244 (2B4), DNAM-1, and NKp80, or an anti-tumor antibody such as anti-Her2neu or anti-EGFR and a signaling domain obtained from CD3-ζ, Dap 10, CD28, 4-IBB, and CD40L. In some examples, the chimeric receptor binds MICA, MICB, Her2neu, EGFR, mesothelin, CD38, CD20, CD 19, PSA, RON, CD30, CD22, CD37, CD38, CD56, CD33, CD30, CD138, CD123, CD79b, CD70, CD75, CA6, GD2, alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), CEACAM5, CA-125, MUC-16, 5T4, NaPi2b, ROR1, ROR2, 5T4, PLIF, Her2/Neu, EGFRvIII, GPMNB, LIV-1, glycolipidF77, fibroblast activating protein, PSMA, STEAP-1, STEAP-2, c-met, CSPG4, Nectin-4, VEGFR2, PSCA, folate binding protein/receptor, SLC44A4, Cripto, CTAG1B, AXL, IL-13R, IL-3R, SLTRK6, gp100, MART1, Tyrosinase, SSX2, SSX4, NYESO-1, epithelial tumor antigen (ETA), MAGEA family genes (such as MAGE3A. MAGE4A), KKLC1, mutated ras, βraf, p53, MHC class I chain-related molecule A (MICA), or MHC class I chain-related molecule B (MICB), HPV, or CMV. The T-cell may be a αβ T cell, γδ T cell, or a natural killer T cell.

Culturing T-Cells

[0135] Methods for the activation, transduction, and/or expansion of T cells, e.g., tumor-infiltrating lymphocytes, CD8+ T cells, CD4+ T cells, and T cells, that may be used for transgene expression are described herein. T cells may be activated, transduced, and expanded, while depleting α- and/or β-TCR positive cells. The T-cell may be a αβ T cell, γδ T cell, or a natural killer T cell.

[0136] Methods for the *ex vivo* expansion of a population of engineered γδ T-cells for

adoptive transfer therapy are described herein. Engineered γδ T cells of the disclosure may be expanded *ex vivo*. Engineered T cells described herein can be expanded in vitro without activation by APCs, or without co-culture with APCs, and aminophosphates. Methods for transducing T cells are described in U.S. Patent Application No. Patent Application No. 2019/0175650, published on June 13, 2019, the contents of which are incorporated by reference in their entirety. Other methods for transduction and culturing of T-cells may be used.

[0137] T cells, including γδ T cells, may be isolated from a complex sample that is cultured *in vitro*. In an embodiment, whole PBMC population, without prior depletion of specific cell populations, such as monocytes, αβ T-cells, B-cells, and NK cells, can be activated and expanded. In an embodiment, enriched T cell populations can be generated prior to their specific activation and expansion. In an embodiment, activation and expansion of γδ T cells may be performed with or without the presence of native or engineered antigen presenting cells (APCs). In an embodiments, isolation and expansion of T cells from tumor specimens can be performed using immobilized T cell mitogens, including antibodies specific to γδ TCR, and other γδ TCR activating agents, including lectins. In an embodiment, isolation and expansion of γδ T cells from tumor specimens can be performed in the absence of γδ T cell mitogens, including antibodies specific to γδ TCR, and other γδ TCR activating agents, including lectins.

[0138] T cells, including γδ T cells, may be isolated from leukapheresis of a subject, for example, a human subject. In an embodiment, γδ T cells are not isolated from peripheral blood mononuclear cells (PBMC). The T cells may be isolated using anti-CD3 and anti-CD28 antibodies, optionally with recombinant human Interleukin-2 (rhIL-2), *e.g.*, between about 50 and 150 U/mL rhIL-2.

[0139] The isolated T cells can rapidly expand in response to contact with one or more antigens. Some γδ T cells, such as Vγ9Vδ2+ T cells, can rapidly expand in vitro in response to contact with some antigens, like prenyl-pyrophosphates, alkyl amines, and metabolites or microbial extracts during tissue culture. Stimulated T-cells can exhibit numerous antigen-presentation, co-stimulation, and adhesion molecules that can facilitate the isolation of T-cells from a complex sample. T cells within a complex sample can be stimulated in vitro with at least one antigen for 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or another suitable period of time. Stimulation of T cells with a suitable antigen can expand T cell population *in vitro*.

[0140] Activation and expansion of γδ T cells can be performed using activation and co-stimulatory agents described herein to trigger specific γδ T cell proliferation and persistence populations. In an embodiment, activation and expansion of γδ T-cells from different cultures

can achieve distinct clonal or mixed polyclonal population subsets. In an embodiment, different agonist agents can be used to identify agents that provide specific γδ activating signals. In an embodiment, agents that provide specific γδ activating signals can be different monoclonal antibodies (MAbs) directed against the γδ TCRs. In an embodiment, companion co-stimulatory agents to assist in triggering specific γδ T cell proliferation without induction of cell energy and apoptosis can be used. These co-stimulatory agents can include ligands binding to receptors expressed on γδ cells, such as NKG2D, CD161, CD70, JAML, DNAX accessory molecule-1 (DNAM-1), ICOS, CD27, CD137, CD30, HVEM, SLAM, CD122, DAP, and CD28. In an embodiment, co-stimulatory agents can be antibodies specific to unique epitopes on CD2 and CD3 molecules. CD2 and CD3 can have different conformation structures when expressed on αβ or γδ T-cells. In an embodiment, specific antibodies to CD3 and CD2 can lead to distinct activation of γδ T cells.

[0141] Non-limiting examples of antigens that may be used to stimulate the expansion of T cells, including γδ T cells, from a complex sample in vitro may comprise, prenyl-pyrophosphates, such as isopentenyl pyrophosphate (IPP), alkyl-amines, metabolites of human microbial pathogens, metabolites of commensal bacteria, methyl-3-butenyl-1-pyrophosphate (2M3B1PP), (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), ethyl pyrophosphate (EPP), farnesyl pyrophosphate (FPP), dimethylallyl phosphate (DMAP), dimethylallyl pyrophosphate (DMAPP), ethyl-adenosine triphosphate (EPPPA), geranyl pyrophosphate (GPP), geranylgeranyl pyrophosphate (GGPP), isopentenyl-adenosine triphosphate (IPPPA), monoethyl phosphate (MEP), monoethyl pyrophosphate (MEPP), 3-formyl-1-butyl-pyrophosphate (TUBAg 1), X-pyrophosphate (TUBAg 2), 3-formyl-1-butyl-uridine triphosphate (TUBAg 3), 3-formyl-1-butyl-deoxythymidine triphosphate (TUBAg 4), monoethyl alkylamines, allyl pyrophosphate, crotoyl pyrophosphate, dimethylallyl-γ-uridine triphosphate, crotoyl-γ-uridine triphosphate, allyl-γ-uridine triphosphate, ethylamine, isobutylamine, sec-butylamine, iso-amylamine and nitrogen containing bisphosphonates.

[0142] A population of T-cells, including γδ T cells, may be expanded *ex vivo* prior to engineering of the T-cells. Non-limiting example of reagents that can be used to facilitate the expansion of a T-cell population in vitro may comprise anti-CD3 or anti-CD2, anti-CD27, anti-CD30, anti-CD70, anti-OX40 antibodies, IL-2, IL-15, IL-12, IL-9, IL-33, IL-18, or IL-21, CD70 (CD27 ligand), phytohaemagglutinin (PHA), concavalin A (ConA), pokeweed (PWM), protein peanut agglutinin (PNA), soybean agglutinin (SBA), Les Culinaris Agglutinin (LCA), Pisum Sativum Agglutinin (PSA), Helix pomatia agglutinin (HPA), Vicia graminea Lectin (VGA), or

another suitable mitogen capable of stimulating T-cell proliferation. Further, the T-cells may be expanded using MCSF, IL-6, eotaxin, IFN-alpha, IL-7, gamma-induced protein 10, IFN-gamma, IL-1RA, IL-12, MIP-1alpha, IL-2, IL-13, MIP-1beta, IL-2R, IL-15, and combinations thereof.

[0143] The ability of γδ T cells to recognize a broad spectrum of antigens can be enhanced by genetic engineering of the γδ T cells. The γδ T cells can be engineered to provide a universal allogeneic therapy that recognizes an antigen of choice in vivo. Genetic engineering of the γδ T-cells may comprise stably integrating a construct expressing a tumor recognition moiety, such as αβ TCR, γδ TCR, chimeric antigen receptor (CAR), which combines both antigen-binding and T-cell activating functions into a single receptor, an antigen binding fragment thereof, or a lymphocyte activation domain into the genome of the isolated γδ T-cell(s), a cytokine (for example, IL-15, IL-12, IL-2. IL-7. IL-21, IL-18, IL-19, IL-33, IL-4, IL-9, IL-23, or IL1β) to enhance T-cell proliferation, survival, and function ex vivo and in vivo. Genetic engineering of the isolated γδ T-cell may also include deleting or disrupting gene expression from one or more endogenous genes in the genome of the isolated γδ T-cells, such as the MHC locus (loci).

[0144] Engineered (or transduced) T cells, including γδ T cells, can be expanded *ex vivo* without stimulation by an antigen presenting cell or aminobisphosphonate. Antigen reactive engineered T cells of the present disclosure may be expanded ex vivo and in vivo. In an embodiment, an active population of engineered T cells may be expanded *ex vivo* without antigen stimulation by an antigen presenting cell, an antigenic peptide, a non-peptide molecule, or a small molecule compound, such as an aminobisphosphonate but using certain antibodies, cytokines, mitogens, or fusion proteins, such as IL-17 Fc fusion, MICA Fc fusion, and CD70 Fc fusion. Examples of antibodies that can be used in the expansion of a γδ T-cell population include anti-CD3, anti-CD27, anti-CD30, anti-CD70, anti-OX40, anti-NKG2D, or anti-CD2 antibodies, examples of cytokines may comprise IL-2, IL-15, IL-12, IL-21, IL-18, IL-9, IL-7, and/or IL-33, and examples of mitogens may comprise CD70 the ligand for human CD27, phytohaemagglutinin (PHA), concavalin A (ConA), pokeweed mitogen (PWM), protein peanut agglutinin (PNA), soybean agglutinin (SBA), les culinaris agglutinin (LCA), pisum sativum agglutinin (PSA), Helix pomatia agglutinin (HPA), Vicia graminea Lectin (VGA) or another suitable mitogen capable of stimulating T-cell proliferation.

[0145] A population of engineered T cells, including γδ T cells, can be expanded in less than 60 days, less than 48 days, less than 36 days, less than 24 days, less than 12 days, or less than 6 days. In an embodiment, a population of engineered T cells can be expanded from about 7 days to about 49 days, about 7 days to about 42 days, from about 7 days to about 35 days, from about

7 days to about 28 days, from about 7 days to about 21 days, or from about 7 days to about 14 days. The T-cells may be expanded for between about 1 and 21 days. For example, the T-cells may be expanded for about at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days.

[0146] In an embodiment, the same methodology may be used to isolate, activate, and expand αβ T cells.

[0147] In an embodiment, the same methodology may be used to isolate, activate, and expand γδ T cells.

[0148] **Vectors**

[0149] Engineered T-cells may be generated using various methods, including those recognized in the literature. For example, a polynucleotide encoding an expression cassette that comprises a tumor recognition, or another type of recognition moiety, can be stably introduced into the T-cell by a transposon/transposase system or a viral-based gene transfer system, such as a lentiviral or a retroviral system, or another suitable method, such as transfection, electroporation, transduction, lipofection, calcium phosphate ($CaPO_4$), nanoengineered substances, such as Ormosil, viral delivery methods, including adenoviruses, retroviruses, lentiviruses, adeno-associated viruses, or another suitable method. A number of viral methods have been used for human gene therapy, such as the methods described in WO 1993/020221, the content of which is incorporated herein in its entirety. Non-limiting examples of viral methods that can be used to engineer T cells may comprise γ-retroviral, adenoviral, lentiviral, herpes simplex virus, vaccinia virus, pox virus, or adeno-virus associated viral methods. The T cells may be αβ T cells or γδ T cells.

[0150] Viruses used for transfection of T-cells include naturally occurring viruses as well as artificial viruses. Viruses may be either an enveloped or non-enveloped virus. Parvoviruses (such as AAVs) are examples of non-enveloped viruses. The viruses may be enveloped viruses. The viruses used for transfection of T-cells may be retroviruses and in particular lentiviruses. Viral envelope proteins that can promote viral infection of eukaryotic cells may comprise HIV-1 derived lentiviral vectors (LVs) pseudotyped with envelope glycoproteins (GPs) from the vesicular stomatitis virus (VSV-G), the modified feline endogenous retrovirus (RD114TR) (SEQ ID NO: 97), and the modified gibbon ape leukemia virus (GALVTR). These envelope proteins can efficiently promote entry of other viruses, such as parvoviruses, including adeno-associated viruses (AAV), thereby demonstrating their broad efficiency. For example, other viral envelop proteins may be used including Moloney murine leukemia virus (MLV) 4070 env (such as

described in Merten et al., *J. Virol.* 79:834-840, 2005; the content of which is incorporated herein by reference), RD114 env, chimeric envelope protein RD114pro or RDpro (which is an RD114-HIV chimera that was constructed by replacing the R peptide cleavage sequence of RD114 with the HIV-1 matrix/capsid (MA/CA) cleavage sequence, such as described in Bell et al. *Experimental Biology and Medicine* 2010; 235: 1269–1276; the content of which is incorporated herein by reference), baculovirus GP64 env (such as described in Wang et al. *J. Virol.* 81:10869-10878, 2007; the content of which is incorporated herein by reference), or GALV env (such as described in Merten et al., *J. Virol.* 79:834-840, 2005; the content of which is incorporated herein by reference), or derivatives thereof.

[0151] A single lentiviral cassette can be used to create a single lentiviral vector, expressing at least four individual monomer proteins of two distinct dimers from a single multi-cistronic mRNA so as to co-express the dimers on the cell surface. For example, the integration of a single copy of the lentiviral vector was sufficient to transform T cells to co-express TCRαβ and CD8αβ, optionally αβ T cells or γδ T cells.

[0152] Vectors may comprise a multi-cistronic cassette within a single vector capable of expressing more than one, more than two, more than three, more than four genes, more than five genes, or more than six genes, in which the polypeptides encoded by these genes may interact with one another or may form dimers. The dimers may be homodimers, *e.g.*, two identical proteins forming a dimer, or heterodimers, *e.g.*, two structurally different proteins forming a dimer.

[0153] Additionally, multiple vectors may be used to transfect cells with the constructs and sequences described herein. For example, the TCR transgene may be on one vector and the CD8 transgene encoding a polypeptide described herein may be on a second that are transfected either simultaneously or sequentially using recognized methods. A T-cell line may be stably transfected with a CD8 transgene encoding a CD8 polypeptide described herein and then sequentially transfected with a TCR transgene or *visa verse*.

[0154] In some embodiments, the transgene may further include one or more multicistronic element(s) and the multicistronic element(s) may be positioned, for example, between the nucleic acid sequence encoding the TCRα or a portion thereof and the nucleic acid sequence encoding the TCRβ or a portion thereof; between the nucleic acid sequence encoding the CD8α or a portion thereof and the nucleic acid sequence encoding the CD8β or a portion thereof, or between any two nucleic acid sequences encoding of TCRα, TCRβ, CD8α, and CD8β. In some embodiments, the multicistronic element(s) may include a sequence encoding a ribosome skip

element selected from among a T2A, a P2A, a E2A or a F2A or an internal ribosome entry site (IRES).

[00155] As used herein, the term "self-cleaving 2A peptide" refers to relatively short peptides (of the order of 20 amino acids long, depending on the virus of origin) acting co-translationally, by preventing the formation of a normal peptide bond between the glycine and last proline, resulting in the ribosome skipping to the next codon, and the nascent peptide cleaving between the Gly and Pro. After cleavage, the short 2A peptide remains fused to the C-terminus of the 'upstream' protein, while the proline is added to the N-terminus of the 'downstream' protein. Self-cleaving 2A peptide may be selected from porcine teschovirus-1 (P2A), equine rhinitis A virus (E2A), Thosea asigna virus (T2A), foot-and-mouth disease virus (F2A), or any combination thereof (see, e.g., Kim et al., PLOS One 6:e18556, 2011, the content of which including 2A nucleic acid and amino acid sequences are incorporated herein by reference in their entireties). By adding the linker sequences (GSG or SGSG (SEQ ID NO: 266)) before the self-cleaving 2A sequence, this may enable efficient synthesis of biologically active proteins, e.g., TCRs.

[0156] As used herein, the term "internal ribosome entry site (IRES)" refers to a nucleotide sequence located in a messenger RNA (mRNA) sequence, which can initiate translation without relying on the 5′ cap structure. IRES is usually located in the 5′ untranslated region (5′UTR) but may also be located in other positions of the mRNA. In one embodiment IRES may be selected from IRES from viruses, IRES from cellular mRNAs, in particular IRES from picornavirus, such as polio, EMCV and FMDV, flavivirus, such as hepatitis C virus (HCV), pestivirus, such as classical swine fever virus (CSFV), retrovirus, such as murine leukaemia virus (MLV), lentivirus, such as simian immunodeficiency virus (SIV), and insect RNA virus, such as cricket paralysis virus (CRPV), and IRES from cellular mRNAs, e.g. translation initiation factors, such as eIF4G, and DAP5, transcription factors, such as c-Myc, and NF-κB-repressing factor (NRF), growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), platelet-derived growth factor B (PDGF-B), homeotic genes, such as antennapedia, survival proteins, such as X-linked inhibitor of apoptosis (XIAP), and Apaf-1, and other cellular mRNA, such as BiP.

[0157] Constructs and vectors described herein are used with the methodology described in U.S. Patent Application Publication No. 2019/0175650, published on June 13, 2019, the contents of which are incorporated by reference in their entirety.

[0158] Non-viral vectors may also be used with the sequences, constructs, and cells

described herein.

[0159] The cells may be transfected by other means known in the art including lipofection (liposome-based transfection), electroporation, calcium phosphate transfection, biolistic particle delivery (*e.g.*, gene guns), microinjection, or combinations thereof. Various methods of transfecting cells are known in the art. *See, e.g.*, Sambrook & Russell (Eds.) Molecular Cloning: A Laboratory Manual (3rd Ed.) Volumes 1–3 (2001) Cold Spring Harbor Laboratory Press; Ramamoorth & Narvekar "Non Viral Vectors in Gene Therapy- An Overview." J Clin Diagn Res. (2015) 9(1): GE01–GE06.

[0160] **Compositions**

[0161] Compositions may comprise the modified CD8 polypeptides described herein. Further, compositions described herein may comprise a T-cell expressing CD8 polypeptides described herein. The compositions described herein may comprise a T-cell expressing CD8 polypeptides described herein and a T-cell receptor (TCR), optionally a TCR that specifically binds one of the TAA described herein complexed with an antigen presenting protein, *e.g.*, MHC, referred to as HLA in humans, for human leukocyte antigen.

[0162] To facilitate administration, the T cells described herein can be made into a pharmaceutical composition or made into an implant appropriate for administration *in vivo*, with pharmaceutically acceptable carriers or diluents. The means of making such a composition or an implant are described in the art. *See, e.g.*, Remington's Pharmaceutical Sciences, 16th Ed., Mack, ed. (1980).

[0163] The T cells described herein can be formulated into a preparation in semisolid or liquid form, such as a capsule, solution, infusion, or injection. Means known in the art can be utilized to prevent or minimize release and absorption of the composition until it reaches the target tissue or organ, or to ensure timed-release of the composition. Desirably, however, a pharmaceutically acceptable form is employed that does not hinder the cells from expressing the CARs or TCRs. Thus, desirably the T cells described herein can be made into a pharmaceutical composition comprising a carrier. The T cells described herein can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. Preferred carriers include, for example, a balanced salt solution, preferably Hanks' balanced salt solution, or normal saline. The formulation should suit the mode of administration. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (*e.g.*, NaCl), saline, buffered saline, as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, *e.g.*,

lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, that do not deleteriously react with the T-cells. The T-cells may be αβ T cells or γδ T cells that express CD8 polypeptides described herein, optionally a TCR described herein.

[0164] A composition of the present invention can be provided in unit dosage form wherein each dosage unit, *e.g.*, an injection, contains a predetermined amount of the composition, alone or in appropriate combination with other active agents.

[0165] The compositions described herein may be a pharmaceutical composition. Pharmaceutical composition described herein may further comprise an adjuvant selected from the group consisting of colony-stimulating factors, including but not limited to Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim), cyclophosphamide, imiquimod, resiquimod, interferon-alpha, or a combination thereof.

[0166] Pharmaceutical composition described herein may comprise an adjuvant selected from the group consisting of colony-stimulating factors, *e.g.*, Granulocyte Macrophage Colony Stimulating Factor (GM-CSF, sargramostim), cyclophosphamide, imiquimod and resiquimod.

[0167] Preferred adjuvants include but are not limited to cyclophosphamide, imiquimod or resiquimod. Even more preferred adjuvants are Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, poly-ICLC (Hiltonol®) and anti-CD40 mAB, or combinations thereof.

[0168] Other examples for useful adjuvants include, but are not limited to chemically modified CpGs (e.g. CpR, Idera), dsRNA analogues such as Poly(I:C) and derivates thereof (e.g. AmpliGen®, Hiltonol®, poly-(ICLC), poly(IC-R), poly(I:C12U), non-CpG bacterial DNA or RNA as well as immunoactive small molecules and antibodies such as cyclophosphamide, sunitinib, immune checkpoint inhibitors including ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, and cemiplimab, Bevacizumab®, celebrex, NCX-4016, sildenafil, tadalafil, vardenafil, sorafenib, temozolomide, temsirolimus, XL-999, CP-547632, pazopanib, VEGF Trap, ZD2171, AZD2171, anti-CTLA4, other antibodies targeting key structures of the immune system (e.g. anti-CD40, anti-TGFbeta, anti-TNFalpha receptor) and SC58175, which may act therapeutically and/or as an adjuvant. The amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan without undue experimentation.

[0169] Other adjuvants include but are not limited to anti-CD40, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, atezolizumab, interferon-alpha, interferon-beta, CpG oligonucleotides and derivatives, poly-(I:C) and derivatives, RNA,

sildenafil, and particulate formulations with poly(lactide co-glycolide) (PLG), Polyinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose (poly-ICLC), virosomes, and/or interleukin-1 (IL-1), IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-18, IL-21, and IL-23. *See, e.g.*, Narayanan *et al.* J. Med. Chem. (2003) 46(23): 5031–5044; Pohar *et al.* Scientific Reports 7 14598 (2017); Grajkowski *et al.* Nucleic Acids Research (2005) 33(11): 3550–3560; Martins *et al.* Expert Rev Vaccines (2015) 14(3): 447–59.

[0170] The composition described herein may also include one or more adjuvants. Adjuvants are substances that non-specifically enhance or potentiate the immune response (e.g., immune responses mediated by CD8-positive T cells and helper-T (TH) cells to an antigen and would thus be considered useful in the medicament of the present invention. Suitable adjuvants include, but are not limited to, 1018 ISS, aluminium salts, AMPLIVAX®, AS15, BCG, CP-870,893, CpG7909, CyaA, dSLIM, flagellin or TLR5 ligands derived from flagellin, FLT3 ligand, GM-CSF, IC30, IC31, Imiquimod (ALDARA®), resiquimod, ImuFact IMP321, Interleukins as IL-2, IL-13, IL-21, Interferon-alpha or -beta, or pegylated derivatives thereof, IS Patch, ISS, ISCOMATRIX, ISCOMs, JuvImmune®, LipoVac, MALP2, MF59, monophosphoryl lipid A, Montanide IMS 1312, Montanide ISA 206, Montanide ISA 50V, Montanide ISA-51, water-in-oil and oil-in-water emulsions, OK-432, OM-174, OM-197-MP-EC, ONTAK, OspA, PepTel® vector system, poly(lactide co-glycolide) [PLG]-based and dextran microparticles, talactoferrin SRL172, Virosomes and other Virus-like particles, YF-17D, VEGF trap, R848, beta-glucan, Pam3Cys, Aquila's QS21 stimulon, which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and other proprietary adjuvants such as Ribi's Detox, Quil, or Superfos. Adjuvants such as Freund's or GM-CSF are preferred. Several immunological adjuvants (e.g., MF59) specific for dendritic cells and their preparation have been described previously. Also, cytokines may be used. Several cytokines have been directly linked to influencing dendritic cell migration to lymphoid tissues (e.g., TNF-), accelerating the maturation of dendritic cells into efficient antigen-presenting cells for T-lymphocytes (e.g., GM-CSF, IL-1 and IL-4) (U.S. Pat. No. 5,849,589, incorporated herein by reference in its entirety) and acting as immunoadjuvants (e.g., IL-12, IL-15, IL-23, IL-7, IFN-alpha. IFN-beta).

[0171] CpG immunostimulatory oligonucleotides have also been reported to enhance the effects of adjuvants in a vaccine setting. Without being bound by theory, CpG oligonucleotides act by activating the innate (non-adaptive) immune system via Toll-like receptors (TLR), mainly TLR9. CpG triggered TLR9 activation enhances antigen-specific humoral and cellular responses to a wide variety of antigens, including peptide or protein antigens, live or killed viruses,

dendritic cell vaccines, autologous cellular vaccines and polysaccharide conjugates in both prophylactic and therapeutic vaccines. More importantly it enhances dendritic cell maturation and differentiation, resulting in enhanced activation of TH1 cells and strong cytotoxic T-lymphocyte (CTL) generation, even in the absence of CD4 T cell help. The TH1 bias induced by TLR9 stimulation is maintained even in the presence of vaccine adjuvants such as alum or incomplete Freund's adjuvant (IFA) that normally promote a TH2 bias. CpG oligonucleotides show even greater adjuvant activity when formulated or co-administered with other adjuvants or in formulations such as microparticles, nanoparticles, lipid emulsions or similar formulations, which are especially necessary for inducing a strong response when the antigen is relatively weak. They also accelerate the immune response and enable the antigen doses to be reduced by approximately two orders of magnitude, with comparable antibody responses to the full-dose vaccine without CpG in some experiments (Krieg, 2006). US 6,406,705 B1 describes the combined use of CpG oligonucleotides, non-nucleic acid adjuvants and an antigen to induce an antigen-specific immune response. A CpG TLR9 antagonist is dSLIM (double Stem Loop Immunomodulator) by Mologen (Berlin, Germany) which is a preferred component of the pharmaceutical composition of the present invention. Other TLR binding molecules such as RNA binding TLR 7, TLR 8 and/or TLR 9 may also be used.

[0172] Methods of Treatment and preparing

[0173] Engineered T cells may express modified CD8 polypeptides described herein. Further, the Engineered T cells may express a TCR described herein. The TCR expressed by the engineered T cells may recognize a TAA bound to an HLA as described herein. Engineered T cells of the present disclosure can be used to treat a subject in need of treatment for a condition, for example, a cancer described herein. The T cells may be αβ T cells or γδ T cells that express a modified CD8 polypeptide, optionally a TCR described herein.

[0174] A method of treating a condition (e.g., ailment) in a subject with T cells described herein may comprise administering to the subject a therapeutically effective amount of engineered T cells described herein, optionally γδ T cells. T cells described herein may be administered at various regimens (e.g., timing, concentration, dosage, spacing between treatment, and/or formulation). A subject can also be preconditioned with, for example, chemotherapy, radiation, or a combination of both, prior to receiving engineered T cells of the present disclosure. A population of engineered T cells may also be frozen or cryopreserved prior to being administered to a subject. A population of engineered T cells can include two or more cells that express identical, different, or a combination of identical and different tumor

recognition moieties. For instance, a population of engineered T-cells can include several distinct engineered T cells that are designed to recognize different antigens, or different epitopes of the same antigen. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide described herein, optionally a TCR described herein.

[0175] T cells described herein, including αβ T-cells and γδ T cells, may be used to treat various conditions. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide, optionally a TCR described herein. T cells described herein may be used to treat a cancer, including solid tumors and hematologic malignancies. Non-limiting examples of cancers include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, neuroblastoma, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell

carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, skin carcinoma merkel cell, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrm macroglobulinemia, and Wilms tumor.

[0176] The T cells described herein may be used to treat an infectious disease. The T cells described herein may be used to treat an infectious disease, an infectious disease may be caused a virus. The T cells described herein may be used to treat an immune disease, such as an autoimmune disease. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide, optionally a TCR described herein.

[0177] Treatment with T cells described herein, optionally γδ T cells, may be provided to the subject before, during, and after the clinical onset of the condition. Treatment may be provided to the subject after 1 day, 1 week, 6 months, 12 months, or 2 years after clinical onset of the disease. Treatment may be provided to the subject for more than 1 day, 1 week, 1 month, 6 months, 12 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or more after clinical onset of disease. Treatment may be provided to the subject for less than 1 day, 1 week, 1 month, 6 months, 12 months, or 2 years after clinical onset of the disease. Treatment may also include treating a human in a clinical trial. A treatment can include administering to a subject a pharmaceutical composition comprising engineered T cells described herein. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide, optionally a TCR described herein.

[0178] In an embodiment, administration of engineered T cells of the present disclosure to a subject may modulate the activity of endogenous lymphocytes in a subject's body. In an embodiment, administration of engineered T cells to a subject may provide an antigen to an endogenous T-cell and may boost an immune response. In an embodiment, the memory T cell may be a CD4+ T-cell. In an embodiment, the memory T cell may be a CD8+ T-cell. In an embodiment, administration of engineered T cells of the present disclosure to a subject may activate the cytotoxicity of another immune cell. In an embodiment, the other immune cell may be a CD8+ T-cell. In an embodiment, the other immune cell may be a Natural Killer T-cell. In an embodiment, administration of engineered γδ T-cells of the present disclosure to a subject may suppress a regulatory T-cell. In an embodiment, the regulatory T-cell may be a FOX3+ Treg cell. In an embodiment, the regulatory T-cell may be a FOX3– Treg cell. Non-limiting examples of

cells whose activity can be modulated by engineered T cells of the disclosure may comprise: hematopioietic stem cells; B cells; CD4; CD8; red blood cells; white blood cells; dendritic cells, including dendritic antigen presenting cells; leukocytes; macrophages; memory B cells; memory T-cells; monocytes; natural killer cells; neutrophil granulocytes; T-helper cells; and T-killer cells. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide, optionally a TCR described herein.

[0179] During most bone marrow transplants, a combination of cyclophosphamide with total body irradiation may be conventionally employed to prevent rejection of the hematopietic stem cells (HSC) in the transplant by the subject's immune system. In an embodiment, incubation of donor bone marrow with interleukin-2 (IL-2) ex vivo may be performed to enhance the generation of killer lymphocytes in the donor marrow. Interleukin-2 (IL-2) is a cytokine that may be necessary for the growth, proliferation, and differentiation of wild-type lymphocytes. Current studies of the adoptive transfer of γδ T-cells into humans may require the co-administration of γδ T-cells and interleukin-2. However, both low- and high-dosages of IL-2 can have highly toxic side effects. IL-2 toxicity can manifest in multiple organs/systems, most significantly the heart, lungs, kidneys, and central nervous system. In an embodiment, the disclosure provides a method for administrating engineered T cells to a subject without the co-administration of a native cytokine or modified versions thereof, such as IL-2, IL-15, IL-12, IL-21. In an embodiment, engineered T cells can be administered to a subject without co-administration with IL-2. In an embodiment, engineered T cells may be administered to a subject during a procedure, such as a bone marrow transplant without the co-administration of IL-2.

[0180] In an embodiment, the methods may further comprise administering a chemotherapy agent. The dosage of the chemotherapy agent may be sufficient to deplete the patient's T-cell population. The chemotherapy may be administered about 5–7 days prior to T-cell administration. The chemotherapy agent may be cyclophosphamide, fludarabine, or a combination thereof. The chemotherapy agent may comprise dosing at about 400–600 mg/m^2/day of cyclophosphamide. The chemotherapy agent may comprise dosing at about 10–30 mg/m^2/day of fludarabine.

[0181] In an embodiment, the methods may further comprise pre-treatment of the patient with low-dose radiation prior to administration of the composition comprising T-cells. The low dose radiation may comprise about 1.4 Gy for 1-6 days, preferably about 5 days, prior to administration of the composition comprising T-cells.

[0182] In an embodiment, the patient may be HLA-A*02.

[0183] In an embodiment, the patient may be HLA-A*06.

[0184] In an embodiment, the methods may further comprise administering an anti-PD1 antibody. The anti-PD1 antibody may be a humanized antibody. The anti-PD1 antibody may be pembrolizumab. The dosage of the anti-PD1 antibody may be about 200 mg. The anti-PD1 antibody may be administered every 3 weeks following T-cell administration.

[0185] In an embodiment, the dosage of T-cells may be between about 0.8–1.2 x 10^9 T cells. The dosage of the T cells may be about 0.5 x 10^8 to about 10 x 10^9 T cells. The dosage of T-cells may be about 1.2–3 x 10^9 T cells, about 3–6 x 10^9 T cells, about 10 x 10^9 T cells, about 5 x 10^9 T cells, about 0.1 x 10^9 T cells, about 1 x 10^8 T cells, about 5 x 10^8 T cells, about 1.2–6 x 10^9 T cells, about 1–6 x 10^9 T cells, or about 1–8 x 10^9 T cells.

[0186] In an embodiment, the T cells may be administered in 3 doses. The T-cell doses may escalate with each dose. The T-cells may be administered by intravenous infusion.

[0187] In an embodiment, the CD8 sequences described herein and associated products and compositions may be used autologous or allogenic methods of adoptive cellular therapy. In another embodiment, CD8 sequences, T cells thereof, and compositions may be used in, for example, methods described in U.S. Patent Application Publication 2019/0175650; U.S. Patent Application Publication 2019/0216852; U.S. Patent Application Publication 2019/024743; and U.S. Provisional Patent Application 62/980,844, each of which are incorporated by reference in their entireties.

[0188] The disclosure also provides for a population of modified T cells that present an exogenous CD8 polypeptide described herein and a T cell receptor wherein the population of modified T cells is activated and expanded with a combination of IL-2 and IL-15. In another embodiment, the population of modified T cells are expanded and/or activated with a combination of IL-2, IL-15, and zoledronate. In yet another embodiment, the population of modified T cells are activated with a combination of IL-2, IL-15, and zoledronate while expanded with a combination of IL-2, IL-15, and without zoledronate. The disclosure further provides for use of other interleukins during activation and/or expansion, such as IL-12, IL-18, IL-21, and combinations thereof.

[0189] In an aspect, IL-21, a histone deacetylase inhibitor (HDACi), or combinations thereof may be utilized in the field of cancer treatment, with methods described herein, and/or with ACT processes described herein. In an embodiment, the present disclosure provides methods for re-programming effector T cells to a central memory phenotype comprising culturing the effector T cells with at least one HDACi together with IL-21. Representative HDACi include, for example,

trichostatin A, trapoxin B, phenylbutyrate, valproic acid, vorinostat (suberanilohydroxamic acid), belinostat, panobinostat, dacinostat, entinostat, tacedinaline, and mocetinostat.

[0190] Compositions comprising engineered T cells described herein may be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, pharmaceutical compositions can be administered to a subject already suffering from a disease or condition in an amount sufficient to cure or at least partially arrest the symptoms of the disease or condition. An engineered T-cell can also be administered to lessen a likelihood of developing, contracting, or worsening a condition. Effective amounts of a population of engineered T-cells for therapeutic use can vary based on the severity and course of the disease or condition, previous therapy, the subject's health status, weight, and/or response to the drugs, and/or the judgment of the treating physician. The T cells may be αβ T cells or γδ T cells engineered to express modified CD8 polypeptides described herein and optionally a TCR described herein. T-cell therapy has been successful in treating various cancers. Li *et al*. Signal Transduction and Targeted Therapy 4(35): (2019), the content of which is incorporated by reference in its entirety.

Methods of Administration

[0191] One or multiple engineered T cell populations described herein may be administered to a subject in any order or simultaneously. If simultaneously, the multiple engineered T cell can be provided in a single, unified form, such as an intravenous injection, or in multiple forms, for example, as multiple intravenous infusions, subcutaneous injections or pills. Engineered T-cells can be packed together or separately, in a single package or in a plurality of packages. One or all of the engineered T cells can be given in multiple doses. If not simultaneous, the timing between the multiple doses may vary to as much as about a week, a month, two months, three months, four months, five months, six months, or about a year. In an embodiment, engineered T cells can expand within a subject's body, in vivo, after administration to a subject. Engineered T cells can be frozen to provide cells for multiple treatments with the same cell preparation. Engineered T cells of the present disclosure, and pharmaceutical compositions comprising the same, can be packaged as a kit. A kit may comprise instructions (e.g., written instructions) on the use of engineered T cells and compositions comprising the same.

[0192] A method of treating a cancer may comprise administering to a subject a therapeutically-effective amount of engineered T cells, in which the administration treats the cancer. In an embodiments, the therapeutically-effective amount of engineered γδ T cells may be administered for at least about 10 seconds, 30 seconds, 1 minute, 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6

days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or 1 year. In an embodiment, the therapeutically-effective amount of the engineered T cells may be administered for at least one week. In an embodiment, the therapeutically-effective amount of engineered T cells may be administered for at least two weeks.

[0193] Engineered T-cells described herein, optionally γδ T cells, can be administered before, during, or after the occurrence of a disease or condition, and the timing of administering a pharmaceutical composition comprising an engineered T-cell can vary. For example, engineered T cells can be used as a prophylactic and can be administered continuously to subjects with a propensity to conditions or diseases in order to lessen the likelihood of occurrence of the disease or condition. Engineered T-cells can be administered to a subject during or as soon as possible after the onset of the symptoms. The administration of engineered T cells can be initiated immediately within the onset of symptoms, within the first 3 hours of the onset of the symptoms, within the first 6 hours of the onset of the symptoms, within the first 24 hours of the onset of the symptoms, within 48 hours of the onset of the symptoms, or within any period of time from the onset of symptoms. The initial administration can be via any route practical, such as by any route described herein using any formulation described herein. In an embodiment, the administration of engineered T cells of the present disclosure may be an intravenous administration. One or multiple dosages of engineered T cells can be administered as soon as is practicable after the onset of a cancer, an infectious disease, an immune disease, sepsis, or with a bone marrow transplant, and for a length of time necessary for the treatment of the immune disease, such as, for example, from about 24 hours to about 48 hours, from about 48 hours to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, from about 1 month to about 3 months. For the treatment of cancer, one or multiple dosages of engineered T cells can be administered years after onset of the cancer and before or after other treatments. In an embodiment, engineered γδ T cells can be administered for at least about 10 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 12 hours, 24 hours, at least 48 hours, at least 72 hours, at least 96 hours, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 1 year, at least 2 years at least 3 years, at least 4 years, or at least 5 years. The length of treatment can vary for each subject. The T cells may be αβ T cells or γδ T cells that express a CD8 polypeptide described herein, optionally a TCR described herein.

[0194] Engineered T-cell expressing a CD8 polypeptides described herein, optionally αβ T cells or γδ T cells, may be present in a composition in an amount of at least 1×10^3 cells/ml, at least 2×10^3 cells/ml, at least 3×10^3 cells/ml, at least 4×10^3 cells/ml, at least 5×10^3 cells/ml, at least 6×10^3 cells/ml, at least 7×10^3 cells/ml, at least 8×10^3 cells/ml, at least 9×10^3 cells/ml, at least 1×10^4 cells/ml, at least 2×10^4 cells/ml, at least 3×10^4 cells/ml, at least 4×10^4 cells/ml, at least 5×10^4 cells/ml, at least 6×10^4 cells/ml, at least 7×10^4 cells/ml, at least 8×10^4 cells/ml, at least 9×10^4 cells/ml, at least 1×10^5 cells/ml, at least 2×10^5 cells/ml, at least 3×10^5 cells/ml, at least 4×10^5 cells/ml, at least 5×10^5 cells/ml, at least 6×10^5 cells/ml, at least 7×10^5 cells/ml, at least 8×10^5 cells/ml, at least 9×10^5 cells/ml, at least 1×10^6 cells/ml, at least 2×10^6 cells/ml, at least 3×10^6 cells/ml, at least 4×10^6 cells/ml, at least 5×10^6 cells/ml, at least 6×10^6 cells/ml, at least 7×10^6 cells/ml, at least 8×10^6 cells/ml, at least 9×10^6 cells/ml, at least 1×10^7 cells/ml, at least 2×10^7 cells/ml, at least 3×10^7 cells/ml, at least 4×10^7 cells/ml, at least 5×10^7 cells/ml, at least 6×10^7 cells/ml, at least 7×10^7 cells/ml, at least 8×10^7 cells/ml, at least 9×10^7 cells/ml, at least 1×10^8 cells/ml, at least 2×10^8 cells/ml, at least 3×10^8 cells/ml, at least 4×10^8 cells/ml, at least 5×10^8 cells/ml, at least 6×10^8 cells/ml, at least 7×10^8 cells/ml, at least 8×10^8 cells/ml, at least 9×10^8 cells/ml, at least 1×10^9 cells/ml, or more, from about 1×10^3 cells/ml to about at least 1×10^8 cells/ml, from about 1×10^5 cells/ml to about at least 1×10^8 cells/ml, or from about 1×10^6 cells/ml to about at least 1×10^8 cells/ml.

[0195] **Sequences**

[0196] The sequences described herein may comprise about 80%, about 85%, about 90%, about 85%, about 96%, about 97%, about 98%, or about 99% or 100% identity to the sequence of any of SEQ ID NO: 1 – 97, 256 - 266, 293 and 294. The sequences described herein may comprise at least 80%, at least 85%, at least 90%, at least 85%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to the sequence of any of SEQ ID NO: 1 – 97 and 256 - 266. A sequence "at least 85% identical to a reference sequence" is a sequence having, on its entire length, 85%, or more, in particular 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the entire length of the reference sequence.

[0197] In another embodiment, the disclosure provides for sequences at least 80%, at least 85%, at least 90%, at least 85%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity to WPREmut1 (SEQ ID NO: 256), or WPRE version 2, e.g., WPREmut2 (SEQ ID NO: 257). In another aspect, the disclosure provides for sequences at least 1, 2, 3, 4, 5, 10, 15, or 20 amino acid substitutions in WPREmut1 (SEQ ID NO: 256), or WPRE version 2, e.g., WPREmut2 (SEQ ID NO: 257). In yet another aspect, the disclosure provides for sequences at

most 1, 2, 3, 4, 5, 10, 15, or 20 amino acid substitutions in WPREmut1 (SEQ ID NO: 256), or WPRE version 2, e.g., WPREmut2 (SEQ ID NO: 257). In another aspect, the sequence substitutions are conservative substitutions.

[0198] Percentage of identity may be calculated using a global pairwise alignment (*e.g.,* the two sequences are compared over their entire length). Methods for comparing the identity of two or more sequences are well known in the art. The « needle » program, which uses the Needleman-Wunsch global alignment algorithm (Needleman and Wunsch, 1970 J. Mol. Biol. 48:443-453) to find the optimum alignment (including gaps) of two sequences when considering their entire length, may for example be used. The needle program is for example available on the ebi.ac.uk World Wide Web site and is further described in the following publication *(EMBOSS: The European Molecular Biology Open Software Suite* (2000) Rice, P. Longden, I. and Bleasby, A. Trends in Genetics 16, (6) pp. 276—277). The percentage of identity between two polypeptides, in accordance with the invention, is calculated using the EMBOSS: needle (global) program with a "Gap Open" parameter equal to 10.0, a "Gap Extend" parameter equal to 0.5, and a Blosum62 matrix.

[0199] Proteins consisting of an amino acid sequence "at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical" to a reference sequence may comprise mutations such as deletions, insertions and/or substitutions compared to the reference sequence. In case of substitutions, the protein consisting of an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference sequence may correspond to a homologous sequence derived from another species than the reference sequence.

[0200] Amino acid substitutions may be conservative or non-conservative. Preferably, substitutions are conservative substitutions, in which one amino acid is substituted for another amino acid with similar structural and/or chemical properties.

[0201] Conservative substitutions may comprise those, which are described by Dayhoff in "The Atlas of Protein Sequence and Structure. Vol. 5", Natl. Biomedical Research, the contents of which are incorporated by reference in their entirety. For example, in an embodiment, amino acids, which belong to one of the following groups, can be exchanged for one another, thus, constituting a conservative exchange: Group 1: alanine (A), proline (P), glycine (G), asparagine (N), serine (S), threonine (T); Group 2: cysteine (C), serine (S), tyrosine (Y), threonine (T); Group 3: valine (V), isoleucine (I), leucine (L), methionine (M), alanine (A), phenylalanine (F); Group 4: lysine (K), arginine (R), histidine (H); Group 5: phenylalanine (F), tyrosine (Y), tryptophan (W), histidine (H); and Group 6: aspartic acid (D), glutamic acid (E). In an

embodiment, a conservative amino acid substitution may be selected from the following of T→A, G→A, A→I, T→V, A→M, T→I, A→V, T→G, and/or T→S.

[0202] A conservative amino acid substitution may comprise the substitution of an amino acid by another amino acid of the same class, for example, (1) nonpolar: Ala, Val, Leu, Ile, Pro, Met, Phe, Trp; (2) uncharged polar: Gly, Ser, Thr, Cys, Tyr, Asn, Gln; (3) acidic: Asp, Glu; and (4) basic: Lys, Arg, His. Other conservative amino acid substitutions may also be made as follows: (1) aromatic: Phe, Tyr, His; (2) proton donor: Asn, Gln, Lys, Arg, His, Trp; and (3) proton acceptor: Glu, Asp, Thr, Ser, Tyr, Asn, Gln (see, for example, U.S. Patent No. 10,106,805, the contents of which are incorporated by reference in their entirety).

[0203] Conservative substitutions may be made in accordance with Table A. Methods for predicting tolerance to protein modification may be found in, for example, Guo et al., Proc. Natl. Acad. Sci., USA, 101(25):9205-9210 (2004), the contents of which are incorporated by reference in their entirety.

[0204] **Table A**: Conservative Amino Acid substitution

Conservative Amino Acid Substitutions	
Amino Acid	Substitutions (others are known in the art)
Ala	Ser, Gly, Cys
Arg	Lys, Gln, His
Asn	Gln, His, Glu, Asp
Asp	Glu, Asn, Gln
Cys	Ser, Met, Thr
Gln	Asn, Lys, Glu, Asp, Arg
Glu	Asp, Asn, Gln
Gly	Pro, Ala, Ser
His	Asn, Gln, Lys
Ile	Leu, Val, Met, Ala
Leu	Ile, Val, Met, Ala
Lys	Arg, Gln, His
Met	Leu, Ile, Val, Ala, Phe
Phe	Met, Leu, Tyr, Trp, His
Ser	Thr, Cys, Ala
Thr	Ser, Val, Ala
Trp	Tyr, Phe
Tyr	Trp, Phe, His
Val	Ile, Leu, Met, Ala, Thr

[0205] The sequences described herein may comprise 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 amino acid or nucleotide mutations, substitutions, deletions. Any one of SEQ ID NO: 1 – 97, 256 - 266, 293, and 294 may comprise 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 mutations, substitutions, or deletions. In another aspect, any one of SEQ ID NO: 1 – 97, 256 - 266, 293, and 294 may comprise at most 1, 2, 3, 4, 5, 10, 15, 20, 25, or 30 mutations, substitutions, or deletions. In an aspect, the mutations or substitutions may be conservative amino acid substitutions.

[0206] Conservative substitutions in the polypeptides described herein may be those shown in Table B under the heading of "conservative substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table B, may be introduced and the products screened if needed.

[0207] **Table B:** Amino Acid substitution

Amino Acid Substitutions		
Original Residue (naturally occurring amino acid)	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0208] Unless otherwise indicated, all terms used herein have the same meaning as they would to one skilled in the art.

[0209] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs.

[0210] Activation" as used herein refers broadly to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions. The term "activated T cells" refers to, among other things, T cells that are proliferating.

[0211] "Antibodies" as used herein refer broadly to antibodies or immunoglobulins of any isotype, fragments of antibodies, which retain specific binding to antigen, including, but not limited to, Fab, Fab', Fab'-SH, $(Fab')_2$ Fv, scFv, divalent scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, and fusion proteins including an antigen-specific targeting region of an antibody and a non-antibody protein. Antibodies are organized into five classes—IgG, IgE, IgA, IgD, and IgM.

[0212] "Antigen" or "Antigenic," as used herein, refers broadly to a peptide or a portion of a peptide capable of being bound by an antibody which is additionally capable of inducing an animal to produce an antibody capable of binding to an epitope of that antigen. An antigen may have one epitope or have more than one epitope. The specific reaction referred to herein indicates that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

[0213] "Chimeric antigen receptor" or "CAR" or "CARs" as used herein refers broadly to genetically modified receptors, which graft an antigen specificity onto cells, for example T cells, NK cells, macrophages, and stem cells. CARs can include at least one antigen-specific targeting region (ASTR), a hinge or stalk domain, a transmembrane domain (TM), one or more co-stimulatory domains (CSDs), and an intracellular activating domain (IAD). In certain embodiments, the CSD is optional. In another embodiment, the CAR is a bispecific CAR, which is specific to two different antigens or epitopes. After the ASTR binds specifically to a target antigen, the IAD activates intracellular signaling. For example, the IAD can redirect T cell specificity and reactivity toward a selected target in a non-MHC-restricted manner, exploiting

the antigen-binding properties of antibodies. The non-MHC-restricted antigen recognition gives T cells expressing the CAR the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

[0214] "Cytotoxic T lymphocyte" (CTL) as used herein refers broadly to a T lymphocyte that expresses CD8 on the surface thereof (*e.g.*, a CD8+ T cell). Such cells may be preferably "memory" T cells (T_M cells) that are antigen-experienced.

[0215] "Effective amount", "therapeutically effective amount", or "efficacious amount" as used herein refers broadly to the amount of an agent, or combined amounts of two agents, that, when administered to a mammal or other subject for treating a disease, is sufficient to affect such treatment for the disease. The "therapeutically effective amount" will vary depending on the agent(s), the disease and its severity and the age, weight, etc., of the subject to be treated.

[0216] "Genetically modified" as used herein refers broadly to methods to introduce exogenous nucleic acids into a cell, whether or not the exogenous nucleic acids are integrated into the genome of the cell. "Genetically modified cell" as used herein refers broadly to cells that contain exogenous nucleic acids whether or not the exogenous nucleic acids are integrated into the genome of the cell.

[0217] "Immune cells" as used herein refers broadly to white blood cells (leukocytes) derived from hematopoietic stem cells (HSC) produced in the bone marrow "Immune cells" include, without limitation, lymphocytes (T cells, B cells, natural killer (NK) (CD3-CD56+) cells) and myeloid-derived cells (neutrophil, eosinophil, basophil, monocyte, macrophage, dendritic cells). "T cells" include all types of immune cells expressing CD3 including T-helper cells (CD4+ cells), cytotoxic T-cells (CD8+ cells), T-regulatory cells (Treg) and gamma-delta T cells, and NK T cells (CD3+ and CD56+). A skilled artisan will understand T cells and/or NK cells, as used throughout the disclosure, can include only T cells, only NK cells, or both T cells and NK cells. In certain illustrative embodiments and aspects provided herein, T cells are activated and transduced. Furthermore, T cells are provided in certain illustrative composition embodiments and aspects provided herein. A "cytotoxic cell" includes CD8+ T cells, natural-killer (NK) cells, NK-T cells, γδ T cells, and neutrophils, which are cells capable of mediating cytotoxicity responses.

[0218] "Individual," "subject," "host," and "patient," as used interchangeably herein, refer broadly to a mammal, including, but not limited to, humans, murines (*e.g.*, rats, mice),

lagomorphs (*e.g.*, rabbits), non-human primates, canines, felines, and ungulates (*e.g.*, equines, bovines, ovines, porcines, caprines).

[0219] "Peripheral blood mononuclear cells" or "PBMCs" as used herein refers broadly to any peripheral blood cell having a round nucleus. PBMCs include lymphocytes, such as T cells, B cells, and NK cells, and monocytes.

[0220] "Polynucleotide" and "nucleic acid", as used interchangeably herein, refer broadly to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer including purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0221] "T cell" or "T lymphocyte," as used herein, refer broadly to thymocytes, naïve T lymphocytes, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. Illustrative populations of T cells suitable for use in particular embodiments include, but are not limited to, helper T cells (HTL; CD4+ T cell), a cytotoxic T cell (CTL; CD8+ T cell), CD4+CD8+ T cell, CD4-CD8– T cell, natural killer T cell, T cells expressing αβ TCR (αβ T cells), T cells expressing γδ TCR (γδ T cells), or any other subset of T cells. Other illustrative populations of T cells suitable for use in particular embodiments include, but are not limited to, T cells expressing one or more of the following markers: CD3, CD4, CD8, CD27, CD28, CD45RA, CD45RO, CD62L, CD127, CD197, and HLA-DR and if desired, can be further isolated by positive or negative selection techniques.

[0222] In the present invention, the term "homologous" refers to the degree of identity (see percent identity above) between sequences of two amino acid sequences, *e.g.*, peptide or polypeptide sequences. The aforementioned "homology" is determined by comparing two sequences aligned under optimal conditions over the sequences to be compared. Such a sequence homology can be calculated by creating an alignment using, for example, the ClustalW algorithm. Commonly available sequence analysis software, more specifically, Vector NTI, GENETYX or other tools are provided by public databases.

[0223] The terms "sequence homology" or "sequence identity" are used interchangeably herein. For the purpose of this invention, it is defined here that in order to determine the percentage of sequence homology or sequence identity of two amino acid sequences or of two nucleotide sequences, the sequences are aligned for optimal comparison purposes. In order to optimize the alignment between the two sequences, gaps may be introduced in any of the two

sequences that are compared. Such alignment can be carried out over the full-length of the sequences being compared. Alternatively, the alignment may be carried out over a shorter length, for example over about 5, about 10, about 20, about 50, about 100 or more nucleotides or amino acids. The sequence identity is the percentage of identical matches between the two sequences over the reported aligned region.

[0224] A comparison of sequences and determination of percentage of sequence identity between two sequences can be accomplished using a mathematical algorithm. The skilled person will be aware of the fact that several different computer programs are available to align two sequences and determine the identity between two sequences (Kruskal, J. B. (1983) An overview of sequence comparison. In D. Sankoff and J. B. Kruskal, (ed.), Time warps, string edits and macromolecules: the theory and practice of sequence comparison, Addison Wesley). The percent sequence identity between two amino acid sequences or between two nucleotide sequences may be determined using the Needleman and Wunsch algorithm for the alignment of two sequences. (Needleman, S. B. and Wunsch, C. D. (1970) J. Mal. Biol. 48, 443-453). Both amino acid sequences and nucleotide sequences can be aligned by the algorithm. The Needleman-Wunsch algorithm has been implemented in the computer program NEEDLE. For the purpose of this invention, the NEEDLE program from the EMBOSS package was used (version 2.8.0 or higher, EMBOSS: The European Molecular Biology Open Software Suite (2000) Rice, Longden, and Bleasby, Trends in Genetics 16, (6) 276-277, emboss.bioinformatics.nl/). For amino acid sequences, EBLOSUM62 is used for the substitution matrix. For nucleotide sequence, EDNAFULL is used. The optional parameters used are a gap-open penalty of 10 and a gap extension penalty of 0.5. The skilled person will appreciate that all these different parameters will yield slightly different results but that the overall percentage identity of two sequences is not significantly altered when using different algorithms.

[0225] After alignment by the program NEEDLE as described above the percentage of sequence identity between a query sequence and a sequence of the invention is calculated as follows: Number of corresponding positions in the alignment showing an identical amino acid or identical nucleotide in both sequences divided by the total length of the alignment after subtraction of the total number of gaps in the alignment. The identity defined as herein can be obtained from NEEDLE by using the NOBRIEF option and is labelled in the output of the program as "longest-identity". The nucleotide and amino acid sequences of the present invention can further be used as a "query sequence" to perform a search against sequence databases to, for example, identify other family members or related sequences. Such searches can be performed

using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) J. Mal. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score= 100, word length= 12 to obtain nucleotide sequences homologous to polynucleotides of the invention. BLAST protein searches can be performed with the XBLAST program, score= 50, word length= 3 to obtain amino acid sequences homologous to polypeptides of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) Nucleic Acids Res. 25(17): 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

[0226] "T-cell receptor (TCR)" as used herein refers broadly to a protein receptor on T cells that is composed of a heterodimer of an alpha (α) and beta (β) chain, although in some cells the TCR consists of gamma and delta (γ/δ) chains. The TCR may be modified on any cell comprising a TCR, including a helper T cell, a cytotoxic T cell, a memory T cell, regulatory T cell, natural killer T cell, or a gamma delta T cell.

[0227] The TCR is generally found on the surface of T lymphocytes (or T cells) that is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. It is a heterodimer consisting of an alpha and beta chain in 95% of T cells, while 5% of T cells have TCRs consisting of gamma and delta chains. Engagement of the TCR with antigen and MHC results in activation of its T lymphocyte through a series of biochemical events mediated by associated enzymes, co-receptors, and specialized accessory molecules. In immunology, the CD3 antigen (CD stands for cluster of differentiation) is a protein complex composed of four distinct chains (CD3-γ, CD3δ, and two times CD3ε) in mammals, that associate with molecules known as the T-cell receptor (TCR) and the ζ-chain to generate an activation signal in T lymphocytes. The TCR, ζ-chain, and CD3 molecules together comprise the TCR complex. The CD3-γ, CD3δ, and CD3ε chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The transmembrane region of the CD3 chains is negatively charged, a characteristic that allows these chains to associate with the positively charged TCR chains (TCRα and TCRβ). The intracellular tails of the CD3 molecules contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM for short, which is essential for the signaling capacity of the TCR.

[0228] "Treatment," "treating," and the like, as used herein refer broadly to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of

completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, *e.g.*, in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *e.g.*, arresting its development; and (c) relieving the disease, *e.g.*, causing regression of the disease.

[0229] The ability of dendritic cells (DC) to activate and expand antigen-specific CD8+ T cells may depend on the DC maturation stage and that DCs may need to receive a "licensing" signal, associated with IL-12 production, in order to elicit cytolytic immune response. In particular, the provision of signals through CD40 Ligand (CD40L)-CD40 interactions on CD4+ T cells and DCs, respectively, may be considered important for the DC licensing and induction of cytotoxic CD8+ T cells. DC licensing may result in the upregulation of co-stimulatory molecules, increased survival and better cross-presenting capabilities of DCs. This process may be mediated via CD40/CD40L interaction [S. R. Bennet et al., "Help for cytotoxic T-cell responses is mediated by CD40 signalling," Nature 393(6684):478-480 (1998); S. P. Schoenberger et al., "T-cell help for cytotoxic T-cell help is mediated by CD40-CD40L interactions," Nature 393(6684):480-483 (1998)], but CD40/CD40L-independent mechanisms also exist (CD70, LTβR). In addition, a direct interaction between CD40L expressed on DCs and CD40 on expressed on CD8+ T-cells has also been suggested, providing a possible explanation for the generation of helper-independent CTL responses [S. Johnson et al., "Selected Toll-like receptor ligands and viruses promote helper-independent cytotoxic T-cell priming by upregulating CD40L on dendritic cells," Immunity 30(2):218-227 (2009)].

EXAMPLE 1

Exemplary Nucleic Acid and Amino Acid Sequences

Table 2: CD8-TCR Constructs

Construct #	Nucleic Acid (SEQ ID NO)	Amino Acid (SEQ ID NO)
1	295	296
2	297	298
8	299	300

Construct #	Nucleic Acid (SEQ ID NO)	Amino Acid (SEQ ID NO)
9	287	288
9b	287	288
10	291	292
10n	291	292
11	285	286
11n	285	286
12	301	302
13	267	268
14	269	270
15	271	272
16	273	274
17	275	276
18	277	278
19	279	280
21	281	282
22	283	284
25	289	290

[0230] The inventors found that the various CD8 elements in the vector lead to a surprising increase in expression and activity. For example, despite the observation that Construct #10 has lower viral titers than Constructs #9b, #11, and #12 (FIG. 5A), T cells transduced with Construct #10 expressing CD8αβ heterodimer and TCR at the lowest viral volumetric concentration, e.g., 1.25 µl/10^6 cells, generated higher CD8+CD4+TCR+ cells (56.7%, FIG. 9B) than that of transduced with Construct #9b expressing CD8α and TCR (42.3%, FIG. 9A), Construct #11 expressing CD8αCD8βstalk with CD8α transmembrane and intracellular domain and TCR (51.6%, FIG. 9C), and Construct #12 expressing CD8αCD8βstalk with Neural Cell Adhesion Molecule 1 (NCAM1) transmembrane and intracellular domain and TCR (14.9%, FIG. 9D).

[0231] A vector may comprise any one of nucleic acid sequences of SEQ ID NO: 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301.

[0232] A T-cell may be transduced to express the nucleic acid of SEQ ID NO: 267, 269, 271,

273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301.

[0233] Several of the elements of the constructs in Table 2 are described in Table 3.

Table 3. Representative Protein and DNA sequences

SEQ ID NO:	Description	Sequence
1	CD8α Ig-like domain-1	SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQ PRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDT FVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPA
2	CD8β region	SVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSP
3	CD8α transmembrane domain	IYIWAPLAGTCGVLLLSLVIT
4	CD8α cytoplasmic tail	LYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV
5	m1CD8α (signal-less)	SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQ PRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDT FVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPVFLPAS VVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPIYI WAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVK SGDKPSLSARYV
6	CD8α Signal peptide	MALPVTALLLPLALLLHAARP
7	m1CD8α	MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGET VELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQN KPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYF CSALSNSIMYFSHFVPVFLPASVVDFLPTTAQPTKKSTL KKRVCRLPRPETQKGPLCSPIYIWAPLAGTCGVLLLSLVI TLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV

8	CD8β1	MRPRLWLLLAAQLTVLHGNSVLQQTPAYIKVQTNKMV MLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDS AKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYF CMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRV CRLPRPETQKGPLCSPITLGLLVAGVLVLLVSLGVAIHL CCRRRRARLRFMKQPQGEGISGTFVPQCLHGYYSNTTT SQKLLNPWILKT
9	CD8β2	MRPRLWLLLAAQLTVLHGNSVLQQTPAYIKVQTNKMV MLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDS AKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYF CMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRV CRLPRPETQKGLKGKVYQEPLSPNACMDTTAILQPHRS CLTHGS
10	CD8β3	LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQ APSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASR FILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFL PTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLV AGVLVLLVSLGVAIHLCCRRRRARLRFMKQFYK
11	CD8β4	LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQ APSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASR FILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFL PTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLV AGVLVLLVSLGVAIHLCCRRRRARLRFMKQLRLHPLEK CSRMDY
12	CD8β5	LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQ APSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASR FILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFL PTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLV AGVLVLLVSLGVAIHLCCRRRRARLRFMKQKFNIVCLK ISGFTTCCCFQILQISREYGFGVLLQKDIGQ

13	CD8β6	LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQ APSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASR FILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFL PTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLV AGVLVLLVSLGVAIHLCCRRRRARLRFMKQKFNIVCLK ISGFTTCCCFQILQISREYGFGVLLQKDIGQ
14	CD8β7	LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQ APSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASR FILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFL PTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLV AGVLVLLVSLGVAIHLCCRRRRARLRFMKQPQGEGISG TFVPQCLHGYYSNTTTSQKLLNPWILKT
15	R11KEA alpha chain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGD STNFTCSFPSSNFYALHWYRKETAKSPEALFVMTLNGD EKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYN NNDMRFGAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
16	R11KE beta chain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEV TLRCKPISGHNSLFWYRETMMRGLELLIYFNNNVPIDDS GMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPG STDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHT QKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDSRG

17	R20P1H7 alpha chain	MEKMLECAFIVLWLQLGWLSGEDQVTQSPEALRLQEG ESSSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTLYSAGE EKEKERLKATLTKKESFLHITAPKPEDSATYLCAVQGEN SGYSTLTFGKGTMLLVSPDIQNPDPAVYQLRDSKSSDKS VCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK LVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR LWSS
18	R20P1H7 beta chain	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKL TVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVT DKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASS LGPGLAAYNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPS EAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS GVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRN HFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA DCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVL MAMVKRKDSRG
19	R7P1D5 alpha chain	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEYS SASKIIFGSGTRLSIRPNIQNPDPAVYQLRDSKSSDKSVC LFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLV EKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLW SS

20	R7P1D5 beta chain	MGSWTLCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEV TLRCKPISGHDYLFWYRQTMMRGLELLIYFNNNVPIDD SGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASRA NTGELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHT QKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDSRG
21	R10P2G12 alpha chain	MLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKED VTLDCVYETRDTTYYLFWYKQPPSGELVFLIRRNSFDE QNEISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCALS EGNSGNTPLVFGKGTRLSVIANIQNPDPAVYQLRDSKSS DKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMD FKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCD VKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMT LRLWSS
22	R10P2G12 beta chain	MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKV FLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKE KGDIPEGYSVSREKKERFSLILESASTNQTSMYLCASSLS SGSHQETQYFGPGTRLLVLEDLKNVFPPEVAVFEPSEAE ISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG FTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG

23	R10P1A7 alpha chain	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAESK ETRLMFGDGTQLVVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
24	R10P1A7 beta chain	MLLLLLLLGPGISLLLPGSLAGSGLGAWSQHPSVWICKS GTSVKIECRSLDFQATTMFWYRQFPKQSLMLMATSNEG SKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYI CSARAGGHEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSE AEISHTQKATLVCLATGFYPDHVELSWVVWNGKEVHSG VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD CGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLM AMVKRKDSRG
25	R4P1D10 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVNF HDKIIFGKGTRLHILPNIQNPDPAVYQLRDSKSSDKSVCL FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVE KSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWS S

26	R4P1D10 beta chain	MGFRLLCCVAFCLLGAGPVDSGVTQTPKHLITATGQRV TLRCSPRSGDLSVYWYQQSLDQGLQFLIHYYNGEERAK GNILERFSAQQFPDLHSELNLSSLELGDSALYFCASSVAS AYGYTFGSGTRLTVVEDLNKVFPPEVAVFEPSEAEISHT QKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSV SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDF
27	R4P3F9 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAYS GAGSYQLTFGKGTKLSVIPNIQNPDPAVYQLRDSKSSDK SVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFK SNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTL RLWSS
28	R4P3F9 beta chain	MGFRLLCCVAFCLLGAGPVDSGVTQTPKHLITATGQRV TLRCSPRSGDLSVYWYQQSLDQGLQFLIQYYNGEERAK GNILERFSAQQFPDLHSELNLSSLELGDSALYFCASSVES SYGYTFGSGTRLTVVEDLNKVFPPEVAVFEPSEAEISHT QKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSV SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDF

29	R4P3H3 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVKA GNQFYFGTGTSLTVIPNIQNPDPAVYQLRDSKSSDKSVC LFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLV EKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLW SS
30	R4P3H3 beta chain	MGTRLLCWVVLGFLGTDHTGAGVSQSPRYKVAKRGQ DVALRCDPISGHVSLFWYQQALGQGPEFLTYFQNEAQL DKSGLPSDRFFAERPEGSVSTLKIQRTQQEDSAVYLCAS SLLTSGGDNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPS EAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS GVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRN HFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA DCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVL MAMVKRKDSRG
31	R36P3F9 alpha chain	METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGEN ATMNCSYKTSINNLQWYRQNSGRGLVHLILIRSNEREK HSGRLRVTLDTSKKSSSLLITASRAADTASYFCATVSNY QLIWGAGTKLIIKPDIQNPDPAVYQLRDSKSSDKSVCLF TDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAV AWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKS FETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

32	R36P3F9 beta chain	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKL TVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVT DKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASS STSGGLSGETQYFGPGTRLLVLEDLKNVFPPEVAVFEPS EAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHS GVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRN HFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA DCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVL MAMVKRKDSRG
33	R52P2G11 alpha chain	MKKHLTTFLVILWLYFYRGNGKNQVEQSPQSLIILEGK NCTLQCNYTVSPFSNLRWYKQDTGRGPVSLTIMTFSEN TKSNGRYTATLDADTKQSSLHITASQLSDSASYICVVSA YGKLQFGAGTQVVVTPDIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
34	R52P2G11 beta chain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEV TLRCKPISGHNSLFWYRQTMMRGLELLIYFNNNVPIDDS GMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSLG SPDGNQPQHFGDGTRLSILEDLNKVFPPEVAVFEPSEAEI SHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDF

35	R53P2A9 alpha chain	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAET VTLSCTYDTSESDYYLFWYKQPPSRQMILVIRQEAYKQ QNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAY NSYAGGTSYGKLTFGQGTILTVHPNIQNPDPAVYQLRD SKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDM RSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSP ESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGF NLLMTLRLWSS
36	R53P2A9 beta chain	MGPGLLCWVLLCLLGAGPVDAGVTQSPTHLIKTRGQQ VTLRCSPISGHKSVSWYQQVLGQGPQFIFQYYEKEERG RGNFPDRFSARQFPNYSSELNVNALLLGDSALYLCASSL DGTSEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEIS HTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTD PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQ VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMV KRKDSRG
37	R26P1A9 alpha chain	METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGEN ATMNCSYKTSINNLQWYRQNSGRGLVHLILIRSNEREK HSGRLRVTLDTSKKSSSLLITASRAADTASYFCLIGASGS RLTFGEGTQLTVNPDIQNPDPAVYQLRDSKSSDKSVCLF TDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAV AWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKS FETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

38	R26P1A9 beta chain	MGSWTLCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEV TLRCKPISGHDYLFWYRQTMMRGLELLIYFNNNVPIDD SGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSY FGWNEKLFFGSGTQLSVLEDLNKVFPPEVAVFEPSEAEI SHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDF
39	R26P2A6 alpha chain	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPE GAIVSLNCTYSNSAFQYFMWYRQYSRKGPELLMYTYSS GNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMS DVSGGYNKLIFGAGTRLAVHPYIQNPDPAVYQLRDSKS SDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC DVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLM TLRLWSS
40	R26P2A6 beta chain	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKL TVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVT DKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCAST TPDGTDEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEI SHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDSRG

41	R26P3H1 alpha chain	MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPL TVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGDNLV KGSYGFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRD MNRDDKIIFGKGTRLHILPNIQNPDPAVYQLRDSKSSDK SVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFK SNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTL RLWSS
42	R26P3H1 beta chain	MSNQVLCCVVLCFLGANTVDGGITQSPKYLFRKEGQN VTLSCEQNLNHDAMYWYRQDPGQGLRLIYYSQIVNDF QKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASS RAEGGEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEI SHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDSRG
43	R35P3A4 alpha chain	MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSA VIKCTYSDSASNYFPWYKQELGKRPQLIIDIRSNVGEKK DQRIAVTLNKTAKHFSLHITETQPEDSAVYFCAASPTGG YNKLIFGAGTRLAVHPYIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS

44	R35P3A4 beta chain	MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQS MTLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGI TDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCAS SLGGASQEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEA EISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGV STDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHF RCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADC GFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG
45	R37P1C9 alpha chain	MKLVTSITVLLSLGIMGDAKTTQPNSMESNEEEPVHLPC NHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMA SLAIAEDRKSSTLILHRATLRDAAVYYCILFNFNKFYFGS GTKLNVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETD TNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
46	R37P1C9 beta chain	MGPGLLHWMALCLLGTGHGDAMVIQNPRYQVTQFGK PVTLSCSQTLNHNVMYWYQQKSSQAPKLLFHYYDKDF NNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYLCA TSSGETNEKLFFGSGTQLSVLEDLNKVFPPEVAVFEPSE AEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSG VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD CGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLM AMVKRKDF

47	R37P1H1 alpha chain	MTRVSLLWAVVVSTCLESGMAQTVTQSQPEMSVQEAE TVTLSCTYDTSESNYYLFWYKQPPSRQMILVIRQEAYK QQNATENRFSVNFQKAAKSFSLKISDSQLGDTAMYFCA FGYSGGGADGLTFGKGTHLIIQPYIQNPDPAVYQLRDSK SSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRS MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPES SCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNL LMTLRLWSS
48	R37P1H1 beta chain	MGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQ VTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQ RGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASS NEGQGWEAEAFFGQGTRLTVVEDLNKVFPPEVAVFEPS EAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSG VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD CGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLM AMVKRKDF
49	R42P3A9 alpha chain	MKRILGALLGLLSAQVCCVRGIQVEQSPPDLILQEGANS TLRCNFSDSVNNLQWFHQNPWGQLINLFYIPSGTKQNG RLSATTVATERYSLLYISSSQTTDSGVYFCAVHNFNKFY FGSGTKLNVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDF DSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAW SNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFE TDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

50	R42P3A9 beta chain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPR HLIKEKRETATLKCYPIPRHDTVYWYQQGPGQDPQFLIS FYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDS ALYFCASSLLGQGYNEQFFGPGTRLTVLEDLKNVFPPEV AVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNG KEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFW QNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAE AWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVL VSALVLMAMVKRKDSRG
51	R43P3F2 alpha chain	MLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKED VTLDCVYETRDTTYYLFWYKQPPSGELVFLIRRNSFDE QNEISGRYSWNFQKSTSSFNFTITASQVVDSAVYFCALS NNNAGNMLTFGGGTRLMVKPHIQNPDPAVYQLRDSKS SDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSM DFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC DVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLM TLRLWSS
52	R43P3F2 beta chain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPR HLIKEKRETATLKCYPIPRHDTVYWYQQGPGQDPQFLIS FYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDS ALYFCASSPTGTSGYNEQFFGPGTRLTVLEDLKNVFPPE VAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVN GKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATF WQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSA EAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAV LVSALVLMAMVKRKDSRG

53	R43P3G5 alpha chain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGD STNFTCSFPSSNFYALHWYRWETAKSPEALFVMTLNGD EKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALNR DDKIIFGKGTRLHILPNIQNPDPAVYQLRDSKSSDKSVCL FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVE KSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWS S
54	R43P3G5 beta chain	MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKV FLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKE KGDIPEGYSVSREKKERFSLILESASTNQTSMYLCASRLP SRTYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEIS HTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTD PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQ VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMV KRKDSRG
55	R59P2E7 alpha chain	METLLGLLILWLQLQWVSSKQEVTQIPAALSVPEGENL VLNCSFTDSAIYNLQWFRQDPGKGLTSLLLIQSSQREQT SGRLNASLDKSSGRSTLYIAASQPGDSATYLCAVNSDY KLSFGAGTTVTVRANIQNPDPAVYQLRDSKSSDKSVCL FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVE KSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWS S

56	R59P2E7 beta chain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPR HLIKEKRETATLKCYPIPRHDTVYWYQQGPGQDPQFLIS FYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDS ALYFCASSLGLGTGDYGYTFGSGTRLTVVEDLNKVFPP EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWV NGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSAT FWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVS AEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYA VLVSALVLMAMVKRKDF
57	R11P3D3 alpha chain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGD STNFTCSFPSSNFYALHWYRWETAKSPEALFVMTLNGD EKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYN NNDMRFGAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
58	R11P3D3 beta chain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEV TLRCKPISGHNSLFWYRQTMMRGLELLIYFNNNVPIDDS GMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPG STDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHT QKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRK DSRG

59	R16P1C10 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAAVIS NFGNEKLTFGTGTRLTIIPNIQNPDPAVYQLRDSKSSDKS VCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK LVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR LWSS
60	R16P1C10 beta chain	MGSRLLCWVLLCLLGAGPVKAGVTQTPRYLIKTRGQQ VTLSCSPISGHRSVSWYQQTPGQGLQFLFEYFSETQRNK GNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASSP WDSPNEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEI SHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDSRG
61	R16P1E8 alpha chain	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPE GAIVSLNCTYSNSAFQYFMWYRQYSRKGPELLMYTYSS GNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMS EAAGNKLTFGGGTRVLVKPNIQNPDPAVYQLRDSKSSD KSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDF KSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCD VKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMT LRLWSS
62	R16P1E8 beta chain	MGTRLLCWAALCLLGAELTEAGVAQSPRYKIIEKRQSV AFWCNPISGHATLYWYQQILGQGPKLLIQFQNNGVVDD SQLPKDRFSAERLKGVDSTLKIQPAKLEDSAVYLCASSY TNQGEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAEIS HTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTD PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQ VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT

		SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMV KRKDF
63	R17P1A9 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVLN QAGTALIFGKGTTLSVSSNIQNPDPAVYQLRDSKSSDKS VCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK LVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR LWSS
64	R17P1A9 beta chain	MGFRLLCCVAFCLLGAGPVDSGVTQTPKHLITATGQRV TLRCSPRSGDLSVYWYQQSLDQGLQFLIQYYNGEERAK GNILERFSAQQFPDLHSELNLSSLELGDSALYFCASSAET GPWLGNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAE ISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG FTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG
65	R17P1D7 alpha chain	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAET VTLSCTYDTSESDYYLFWYKQPPSRQMILVIRQEAYKQ QNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAY RWAQGGSEKLVFGKGTKLTVNPYIQKPDPAVYQLRDS KSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMR SMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPE SSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFN LLMTLRLWSS
66	R17P1D7 beta chain	MTIRLLCYMGFYFLGAGLMEADIYQTPRYLVIGTGKKIT LECSQTMGHDKMYWYQQDPGMELHLIHYSYGVNSTE KGDLSSESTVSRIRTEHFPLTLESARPSHTSQYLCATELW SSGGTGELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEI SHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVST

		DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDSRG
67	R17P1G3 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVGPS GTYKYIFGTGTRLKVLANIQNPDPAVYQLRDSKSSDKS VCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKS NSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK LVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLR LWSS
68	R17P1G3 beta chain	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKL TVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVT DKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASS PGGSGNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAE ISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG FTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG
69	R17P2B6 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVVS GGGADGLTFGKGTHLIIQPYIQKPDPAVYQLRDSKSSDK SVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFK SNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDV KLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTL RLWSS
70	R17P2B6 beta chain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPR HLIKEKRETATLKCYPIPRHDTVYWYQQGPGQDPQFLIS FYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDS

		ALYFCASSLGRGGQPQHFGDGTRLSILEDLNKVFPPEVA VFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGK EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQ NPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA WGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLV SALVLMAMVKRKDF
71	R11P3D3KE alpha chain	MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGD STNFTCSFPSSNFYALHWYRKETAKSPEALFVMTLNGD EKKKGRISATLNTKEGYSYLYIKGSQPEDSATYLCALYN NNDMRFGAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
72	R11P3D3KE beta chain	NNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSA VYFCASSPGSTDTQYFGPGTRLTVLEDLKNVFPPEVAVF EPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEV HSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWG RADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSAL VLMAMVKRKDSRG
73	R39P1C12 alpha chain	TYLYWYKQEPGAGLQLLTYIFSNMDMKQDQRLTVLLN KKDKHLSLRIADTQTGDSAIYFCAEIDNQGGKLIFGQGT ELSVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN VSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSD FACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNL NFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
74	R39P1C12 beta chain	MGPGLLCWALLCLLGAGLVDAGVTQSPTHLIKTRGQQ VTLRCSPKSGHDTVSWYQQALGQGPQFIFQYYEEEERQ RGNFPDRFSGHQFPNYSSELNVNALLLGDSALYLCASS QLNTEAFFGQGTRLTVVEDLNKVFPPEVAVFEPSEAEIS HTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTD

		PQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
75	R39P1F5 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVNNARLMFGDGTQLVVKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
76	R39P1F5 beta chain	MDTWLVCWAIFSLLKAGLTEPEVTQTPSHQVTQMGQEVILRCVPISNHLYFYWYRQILGQKVEFLVSFYNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSGQGANEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
77	R40P1C2 alpha chain	MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCTYDTSESDYYLFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCAYLNYQLIWGAGTKLIIKPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
78	R40P1C2 beta chain	MDTWLVCWAIFSLLKAGLTEPEVTQTPSHQVTQMGQEVILRCVPISNHLYFYWYRQILGQKVEFLVSFYNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSEM

		TAVGQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISH TQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDP QPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTS ESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVK RKDSRG
79	R41P3E6 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFT AQLNKASQYVSLLIRDSQPSDSATYLCAAFSGYALNFG KGTSLLVTPHIQNPDPAVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSN KSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETD TNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS
80	R41P3E6 beta chain	MDTWLVCWAIFSLLKAGLTEPEVTQTPSHQVTQMGQE VILRCVPISNHLYFYWYRQILGQKVEFLVSFYNNEISEKS EIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSQY TGELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAEISHTQ KATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQP LKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQF YGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDSRG
81	R43P3G4 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVNG GDMRFGAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSVC LFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLV EKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLW SS

82	R43P3G4 beta chain	MDTWLVCWAIFSLLKAGLTEPEVTQTPSHQVTQMGQE VILRCVPISNHLYFYWYRQILGQKVEFLVSFYNNEISEKS EIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSGQ GALEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISH TQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDP QPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTS ESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVK RKDSRG
83	R44P3B3 alpha chain	MAMLLGASVLILWLQPDWVNSQQKNDDQQVKQNSPS LSVQEGRISILNCDYTNSMFDYFLWYKKYPAEGPTFLISI SSIKDKNEDGRFTVFLNKSAKHLSLHIVPSQPGDSAVYF CAASGLYNQGGKLIFGQGTELSVKPNIQNPDPAVYQLR DSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLD MRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPS PESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGF NLLMTLRLWSS
84	R44P3B3 beta chain	MGCRLLCCVVFCLLQAGPLDTAVSQTPKYLVTQMGND KSIKCEQNLGHDTMYWYKQDSKKFLKIMFSYNNKELII NETVPNRFSPKSPDKAHLNLHINSLELGDSAVYFCASSL GDRGYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEI SHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVST DPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF TSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAM VKRKDSRG
85	R44P3E7 alpha chain	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEINN NARLMFGDGTQLVVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL

		VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS
86	R44P3E7 beta chain	MLSPDLPDSAWNTRLLCHVMLCLLGAVSVAAGVIQSPR HLIKEKRETATLKCYPIPRHDTVYWYQQGPGQDPQFLIS FYEKMQSDKGSIPDRFSAQQFSDYHSELNMSSLELGDS ALYFCASSPPDQNTQYFGPGTRLTVLEDLKNVFPPEVA VFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGK EVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQ NPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA WGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLV SALVLMAMVKRKDSRG
87	R49P2B7 alpha chain	MLLLLVPVLEVIFTLGGTRAQSVTQLGSHVSVSEGALVL LRCNYSSSVPPYLFWYVQYPNQGLQLLLKYTTGATLVK GINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCAVRIFG NEKLTFGTGTRLTIIPNIQNPDPAVYQLRDSKSSDKSVCL FTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNSA VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVE KSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWS S
88	R49P2B7 beta chain	MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKV FLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKE KGDIPEGYSVSREKKERFSLILESASTNQTSMYLCASSL MGELTGELFFGEGSRLTVLEDLKNVFPPEVAVFEPSEAE ISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVS TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCG FTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMA MVKRKDSRG
89	R55P1G7 alpha chain	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPE GAIVSLNCTYSNSAFQYFMWYRQYSRKGPELLMYTYSS GNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAM MGDTGTASKLTFGTGTRLQVTLDIQNPDPAVYQLRDSK

		SSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRS MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPES SCDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNL LMTLRLWSS
90	R55P1G7 beta chain	MGIRLLCRVAFCFLAVGLVDVKVTQSSRYLVKRTGEKV FLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKE KGDIPEGYSVSREKKERFSLILESASTNQTSMYLCASSFG GYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHT QKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQ PLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQ FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDSRG
91	R59P2A7 alpha chain	MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEG AIASLNCTYSDRGSQSFFWYRQYSGKSPELIMSIYSNGD KEDGRFTAQLNKASQYVSLLIRDSQPSDSATYLCAVQP HDMRFGAGTRLTVKPNIQNPDPAVYQLRDSKSSDKSVC LFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLV EKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLW SS
92	R59P2A7 beta chain	MLCSLLALLLGTFFGVRSQTIHQWPATLVQPVGSPLSLE CTVEGTSNPNLYWYRQAAGRGLQLLFYSVGIGQISSEV PQNLSASRPQDRQFILSSKKLLLSDSGFYLCAWSGLVAE QFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKA TLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLK EQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYG LSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQ QGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDS RG
93	P2A	ATNFSLLKQAGDVEENPGP

94	T2A	EGRGSLLTCGDVEENPGP
95	E2A	QCTNYALLKLAGDVESNPGP
96	F2A	VKQTLNFDLLKLAGDVESNPGP
97	RD114TR	MKLPTGMVILCSLIIVRAGFDDPRKAIALVQKQHGKPCE CSGGQVSEAPPNSIQQVTCPGKTAYLMTNQKWKCRVT PKISPSGGELQNCPCNTFQDSMHSSCYTEYRQCRRINKT YYTATLLKIRSGSLNEVQILQNPNQLLQSPCRGSINQPVC WSATAPIHISDGGGPLDTKRVWTVQKRLEQIHKAMTPE LQYHPLALPKVRDDLSLDARTFDILNTTFRLLQMSNFSL AQDCWLCLKLGTPTPLAIPTPSLTYSLADSLANASCQIIP PLLVQPMQFSNSSCLSSPFINDTEQIDLGAVTFTNCTSVA NVSSPLCALNGSVFLCGNNMAYTYLPQNWTRLCVQAS LLPDIDINPGDEPVPIPAIDHYIHRPKRAVQFIPLLAGLGI TAAFTTGATGLGVSVTQYTKLSHQLISDVQVLSGTIQDL QDQVDSLAEVVLQNRRGLDLLTAEQGGICLALQEKCCF YANKSGIVRNKIRTLQEELQKRRESLASNPLWTGLQGFL PYLLPLLGPLLTLLLILTIGPCVFNRLVQFVKDRISVVQA LVLTQQYHQLKPL
256	WPREmut1	cagtctgacgtacgcgtaatcaacctctggattacaaaatttgtgaaagattgactggtatt cttaactatgttgctccttttacgctatgtggatacgctgctttaatgcctttgtatcatgctatt gcttcccgtatggctttcattttctcctccttgtataaatcctggttgctgtctctttatgagga gttgtggcccgttgtcaggcaacgtggcgtggtgtgcactgtgtttgctgacgcaacccc cactggttggggcattgccaccacctgtcagctcctttccgggactttcgctttcccctcc ctattgccacggcggaactcatcgccgcctgccttgcccgctgctggacaggggctcg gctgttgggcactgacaattccgtggtgttgtcggggaaatcatcgtcctttccttggctgc tcgcctgtgttgccacctggattctgcgcgggacgtccttctgctacgtcccttcggccct caatccagcggaccttccttcccgcggcctgctgccggctctgcggcctcttccgcgtct tcgccttcgccctcagacgagtcggatctccctttgggccgcctccccgcc
257	WPREmut2	Gagcatcttaccgccatttatacccatatttgttctgtttttcttgatttgggtatacatttaaat gttaataaaacaaaatggtggggcaatcatttacatttttgggatatgtaattactagttcag gtgtattgccacaagacaaacttgttaagaaactttcccgttatttacgctctgttcctgttaa

		tcaacctctggattacaaaatttgtgaaagattgactgatattcttaactttgttgctcctttac gctgtgtggatttgctgctttattgcctctgtatcttgctattgcttcccgtacggctttcgtttt ctcctccttgtataaatcctggttgctgtctctttttgaggagttgtggcccgttgtccgtcaa cgtggcgtggtgtgctctgtgtttgctgacgcaacccccactggctggggcattgccacc acctgtcaactcctttctgggactttcgctttcccctcccgatcgccacggcagaactcat cgccgcctgccttgcccgctgctggacaggggctaggttgctgggcactgataattccg tggtgttgtc
258	CD8α1	MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGET VELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQN KPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEG**Y**YF CSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLS ARYV
259	CD8α2	MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGET VELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQN KPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEG**C**YF CSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQ PLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLS ARYV
260	CD8α stalk	KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR GLDFACD
261	CD8α Ig-like domain-2	SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQ PRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDT FVLTLSDFRRENEG**C**YFCS2ALSNSIMYFSHFVPVFLPA
262	m2CD8α	MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGET VELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQN KPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEG**C**YF CSALSNSIMYFSHFVPVFLPASVVDFLPTTAQPTKKSTL KKRVCRLPRPETQKGPLCSPIYIWAPLAGTCGVLLLSLVI

		TLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV
263	MSCV promoter	Tgaaagaccccacctgtaggtttggcaagctagcttaagtaacgccattttgcaaggcatggaaaatacataactgagaatagagaagttcagatcaaggttaggaacagagagacagcagaatatgggccaaacaggatatctgtggtaagcagttcctgccccggctcagggccaagaacagatggtccccagatgcggtcccgccctcagcagtttctagagaaccatcagatgtttccagggtgccccaaggacctgaaaatgaccctgtgccttatttgaactaaccaatcagttcgcttctcgcttctgttcgcgcgcttctgctccccgagctcaataaaagagcccacaacccctcact
264	WPRE	cagtctgacgtacgcgtaatcaacctctggattacaaaatttgtgaaagattgactggtattcttaactatgttgctccttttacgctatgtggatacgctgctttaatgcctttgtatcatgctattgcttcccgtatggctttcattttctcctccttgtataaatcctggttgctgtctctttatgaggagttgtggcccgttgtcaggcaacgtggcgtggtgtgcactgtgtttgctgacgcaacccccactggttggggcattgccaccacctgtcagctcctttccgggactttcgctttccccctccctattgccacggcggaactcatcgccgcctgccttgcccgctgctggacaggggctcggctgttgggcactgacaattccgtggtgttgtcggggaagctgacgtcctttccatggctgctcgcctgtgttgccacctggattctgcgcgggacgtccttctgctacgtcccttcggcccctcaatccagcggaccttccttcccgcggcctgctgccggctctgcggcctcttccgcgtcttcgccttcgccctcagacgagtcggatctccctttgggccgcctccccgcc
265	Furin consensus	RXXR
266	Linker	SGSG
293	CD8β Signal peptide	MRPRLWLLLAAQLTVLHGNSV
294	S19 Signal peptide	MEFGLSWLFLVAILKGVQC
303	R11P3D3KE beta chain	MDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRETMMRGLELLIYFNNNVPIDDSGMPEDRFSAKMPNASFSTLKIQPSEPRDSAVYFCASSPGSTDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSES

		YQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKR KDSRG
304	R39P1C12 alpha chain	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDS SVINCTYTDSSSTYLYWYKQEPGAGLQLLTYIFSNMDM KQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEIDN QGGKLIFGQGTELSVKPNIQNPDPAVYQLRDSKSSDKSV CLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL WSS

[0234] The constructs in Table 2 may be assemblages of the individual components described in Table 3. The inventors found that the combination, order, and inclusion of transcription enhancers from Table 3 as described in Table 2 provided unexpected improvements in transfection efficiency, expression levels, and induction of cytotoxic T-cell activities, *e.g.*, IL-12 secretion, IFN-γ secretion, TNF-α secretion, granzyme A secretion, MIP-1a secretion, IP-10 secretion, granzyme B secretion, and combinations thereof.

[0235] *Tumor Associated Antigens (TAA)*

[0236] In the MHC class I dependent immune reaction, peptides not only have to be able to bind to certain MHC class I molecules expressed by tumor cells, they subsequently also have to be recognized by T cells bearing specific T cell receptors (TCR).

[0237] For proteins to be recognized by T-lymphocytes as tumor-specific or -associated antigens, and to be used in a therapy, particular prerequisites must be fulfilled. The antigen should be expressed mainly by tumor cells and not, or in comparably small amounts, by normal healthy tissues. In a preferred embodiment, the peptide should be over-presented by tumor cells as compared to normal healthy tissues. It is furthermore desirable that the respective antigen is not only present in a type of tumor, but also in high concentrations (*e.g.*, copy numbers of the respective peptide per cell). Tumor-specific and tumor-associated antigens are often derived from proteins directly involved in transformation of a normal cell to a tumor cell due to their function, *e.g.*, in cell cycle control or suppression of apoptosis. Additionally, downstream targets of the proteins directly causative for a transformation may be up-regulated and thus may be indirectly tumor-associated. Such indirect tumor-associated antigens may also be targets of a vaccination approach. Singh-Jasuja *et al*. Cancer Immunol. Immunother. 53 (2004): 187-195. Epitopes are present in the amino acid sequence of the antigen, making the peptide an

"immunogenic peptide", and being derived from a tumor associated antigen, leads to a T-cell-response, both *in vitro* and *in vivo*.

[0238] Any peptide able to bind an MHC molecule may function as a T-cell epitope. For the induction of a T-cell-response, the TAA must be presented a T cell having a corresponding TCR and the host must not have immunological tolerance for this particular epitope. Exemplary Tumor Associated Antigens (TAA) that may be used with the CD8 polypeptides described herein are disclosed herein.

[0239] **Table 4. TAA Peptide sequences**

SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence
98	YLYDSETKNA	151	LLWGHPRVALA	204	SLLNQPKAV
99	HLMDQPLSV	152	VLDGKVAVV	205	KMSELQTYV
100	GLLKKINSV	153	GLLGKVTSV	206	ALLEQTGDMSL
101	FLVDGSSAL	154	KMISAIPTL	207	VIIKGLEEITV
102	FLFDGSANLV	155	GLLETTGLLAT	208	KQFEGTVEI
103	FLYKIIDEL	156	TLNTLDINL	209	KLQEEIPVL
104	FILDSAETTTL	157	VIIKGLEEI	210	GLAEFQENV
105	SVDVSPPKV	158	YLEDGFAYV	211	NVAEIVIHI
106	VADKIHSV	159	KIWEELSVLEV	212	ALAGIVTNV
107	IVDDLTINL	160	LLIPFTIFM	213	NLLIDDKGTIKL
108	GLLEELVTV	161	ISLDEVAVSL	214	VLMQDSRLYL
109	TLDGAAVNQV	162	KISDFGLATV	215	KVLEHVVRV
110	SVLEKEIYSI	163	KLIGNIHGNEV	216	LLWGNLPEI
111	LLDPKTIFL	164	ILLSVLHQL	217	SLMEKNQSL
112	YTFSGDVQL	165	LDSEALLTL	218	KLLAVIHEL
113	YLMDDFSSL	166	VLQENSSDYQSNL	219	ALGDKFLLRV
114	KVWSDVTPL	167	HLLGEGAFAQV	220	FLMKNSDLYGA

115	LLWGHPRVALA	168	SLVENIHVL	221	KLIDHQGLYL
116	KIWEELSVLEV	169	YTFSGDVQL	222	GPGIFPPPPPQP
117	LLIPFTIFM	170	SLSEKSPEV	223	ALNESLVEC
118	FLIENLLAA	171	AMFPDTIPRV	224	GLAALAVHL
119	LLWGHPRVALA	172	FLIENLLAA	225	LLLEAVWHL
120	FLLEREQLL	173	FTAEFLEKV	226	SIIEYLPTL
121	SLAETIFIV	174	ALYGNVQQV	227	TLHDQVHLL
122	TLLEGISRA	175	LFQSRIAGV	228	SLLMWITQC
123	KIQEILTQV	176	ILAEEPIYIRV	229	FLLDKPQDLSI
124	VIFEGEPMYL	177	FLLEREQLL	230	YLLDMPLWYL
125	SLFESLEYL	178	LLLPLELSLA	231	GLLDCPIFL
126	SLLNQPKAV	179	SLAETIFIV	232	VLIEYNFSI
127	GLAEFQENV	180	AILNVDEKNQV	233	TLYNPERTITV
128	KLLAVIHEL	181	RLFEEVLGV	234	AVPPPPSSV
129	TLHDQVHLL	182	YLDEVAFML	235	KLQEELNKV
130	TLYNPERTITV	183	KLIDEDEPLFL	236	KLMDPGSLPPL
131	KLQEKIQEL	184	KLFEKSTGL	237	ALIVSLPYL
132	SVLEKEIYSI	185	SLLEVNEASSV	238	FLLDGSANV
133	RVIDDSLVVGV	186	GVYDGREHTV	239	ALDPSGNQLI
134	VLFGELPAL	187	GLYPVTLVGV	240	ILIKHLVKV
135	GLVDIMVHL	188	ALLSSVAEA	241	VLLDTILQL
136	FLNAIETAL	189	TLLEGISRA	242	HLIAEIHTA
137	ALLQALMEL	190	SLIEESEEL	243	SMNGGVFAV
138	ALSSSQAEV	191	ALYVQAPTV	244	MLAEKLLQA
139	SLITGQDLLSV	192	KLIYKDLVSV	245	YMLDIFHEV

140	QLIEKNWLL	193	ILQDGQFLV	246	ALWLPTDSATV
141	LLDPKTIFL	194	SLLDYEVSI	247	GLASRILDA
142	RLHDENILL	195	LLGDSSFFL	248	ALSVLRLAL
143	YTFSGDVQL	196	VIFEGEPMYL	249	SYVKVLHHL
144	GLPSATTTV	197	ALSYILPYL	250	VYLPKIPSW
145	GLLPSAESIKL	198	FLFVDPELV	251	NYEDHFPLL
146	KTASINQNV	199	SEWGSPHAAVP	252	VYIAELEKI
147	SLLQHLIGL	200	ALSELERVL	253	VHFEDTGKTLLF
148	YLMDDFSSL	201	SLFESLEYL	254	VLSPFILTL
149	LMYPYIYHV	202	KVLEYVIKV	255	HLLEGSVGV
150	KVWSDVTPL	203	VLLNEILEQV		

EXAMPLE 2

CD8α molecules

[0240] CD8α homodimer (CD8αα) may be composed of two α subunits held together by two disulfide bonds at the stalk regions. FIG. 1 shows a CD8α polypeptide, e.g., SEQ ID NO: 258 (CD8α1), that includes five domains: (1) one signal peptide (from -21 to -1), e.g., SEQ ID NO: 6, (2) one Ig-like domain-1 (from 1 to 115), e.g., SEQ ID NO: 1, (3) one stalk region (from 116 to 160), e.g., SEQ ID NO: 260, (4) one transmembrane (TM) domain (from 161-188), e.g., SEQ ID NO: 3, and (5) one cytoplasmic tail (Cyto) comprising a *lck*-binding motif (from 189 to 214), e.g., SEQ ID NO: 4. Another example of CD8α subunit, e.g., CD8α2 (SEQ ID NO: 259), differs from CD8α1 at position 112, at which CD8α2 contains a cysteine (C), whereas CD8α1 contains a tyrosine (Y).

Modified CD8 polypeptides

[0241] Different from CD8α polypeptide, e.g., CD8α1 (SEQ ID NO: 258) and CD8α2 (SEQ ID NO: 259), a modified CD8α polypeptide, e.g., m1CD8α (SEQ ID NO: 7) and m2CD8α (SEQ ID NO: 262), may contain additional regions, such as sequence stretches from a CD8β polypeptide. In an embodiment, SEQ ID NO: 2 or variants thereof are used with a CD8α

polypeptide. In other embodiments, a portion of a CD8α polypeptide, e.g., SEQ ID NO: 260, is removed or not included in modified CD8 polypeptides described herein . FIG. 2 shows a sequence alignment between CD8α1 (SEQ ID NO: 258) and m1CD8α (SEQ ID NO: 7). FIG. 3 shows a sequence alignment between CD8α2 (SEQ ID NO: 259) and m2CD8α (SEQ ID NO: 262), in which the cysteine substitution is indicated by an arrow. The stalk regions are shown within the boxes.

[0242] Modified CD8 expressing cells showed improved functionality in terms of cytotoxicity and cytokine response as compared to original CD8 expressing T cells transduced with the TCR.

EXAMPLE 3

Lentiviral viral vectors

[0243] The lentiviral vectors used herein contain several elements that enhance vector function, including a central polypurine tract (cPPT) for improved replication and nuclear import, a promoter from the murine stem cell virus (MSCV) (SEQ ID NO: 263), which lessens vector silencing in some cell types, a woodchuck hepatitis virus posttranscriptional responsive element (WPRE) (SEQ ID NO: 264) for improved transcriptional termination, and the backbone was a deleted 3'-LTR self-inactivating (SIN) vector design that improves safety, sustained gene expression and anti-silencing properties. Yang *et al. Gene Therapy* (2008) 15, 1411–1423.

[0244] In an embodiment, vectors, constructs, or sequences described herein comprise mutated forms of WPRE. In an embodiment, sequences or vectors described herein comprise mutations in WPRE version 1, e.g., WPREmut1 (SEQ ID NO: 256), or WPRE version 2, e.g., WPREmut2 (SEQ ID NO: 257). Construct #9 and Construct #9b represent two LV production batches with the same construct containing SEQ ID NO: 257 as WPREmut2, with the difference between Construct #9 and Construct #9b being the titer consistent with Table 4. In an embodiment, WPRE mutants comprise at most one mutation, at most two mutations, at most three mutations, at least four mutations, or at most five mutations. In an embodiment, vectors, constructs, or sequences described herein do not comprise WPRE. In an aspect, WPRE sequences described in U.S. 2021/0285011, the content of which is incorporated by reference in its entirety, may be used together with vectors, sequences, or constructs described herein.

[0245] In an embodiment, vectors, constructs, or sequences described herein do not include an X protein promoter. The WPRE mutants described herein do not express an X protein. WPRE promotes accumulation of mRNA, theorized to promote export of mRNA from nucleosome to

cytoplasm to promote translation of the transgene mRNA.

[0246] To obtain optimal co-expression levels of TCRαβ, mCD8α (e.g., m1CD8α (SEQ ID NO: 7) and m2CD8α (SEQ ID NO: 262)) and CD8β (e.g., any one of CD8β1-7 (SEQ ID NO: 8-14)) in the transduced CD4+ T cells, CD8+ T cells, and/or γδ T cells, lentiviral vectors with various designs were generated. T cells may be transduced with two separate lentiviral vectors (2-in-1), e.g., one expressing TCRα and TCRβ and the other expressing mCD8α and CD8β, for co-expression of TCRαβ and CD8αβ heterodimer, or one expressing TCRα and TCRβ and the other expressing mCD8α for co-expression of TCRαβ and mCD8α homodimer. Alternatively, T cells may be transduced with a single lentiviral vector (4-in-1) co-expressing TCRα, TCRβ, mCD8α, and CD8β for co-expression of TCRαβ and CD8αβ heterodimer. In the 4-in-1 vector, the nucleotides encoding TCRα chain, TCRβ chain, mCD8α chain, and CD8β chain may be shuffled in various orders, e.g., from 5' to 3' direction, TCRα-TCRβ-mCD8α-CD8β, TCRα-TCRβ-CD8β-mCD8α, TCRβ-TCRα-mCD8α-CD8β, TCRβ-TCRα-CD8β-mCD8α, mCD8α-CD8β-TCRα-TCRβ, mCD8α-CD8β-TCRβ-TCRα, CD8β-mCD8α-TCRα-TCRβ, and CD8β-mCD8α-TCRβ-TCRα. Various 4-in-1 vectors, thus generated, may be used to transduce CD4+ T cells, CD8+ T cells, and/or γδ T cells, followed by measuring TCRαβ/mCD8α/CD8β co-expression levels of the transduced cells using techniques known in the art, *e.g.*, flow cytometry. Similarly, T cells may be transduced with a single lentiviral vector (3-in-1) co-expressing TCRα, TCRβ, and mCD8α (*e.g.*, m1CD8α and m2CD8α) for co-expression of TCRαβ and mCD8α homodimer. In the 3-in-1 vector, the nucleotides encoding TCRα chain, TCRβ chain, mCD8α chain may be shuffled in various orders, e.g., TCRα-TCRβ-mCD8α, TCRβ-TCRα-mCD8α, mCD8α-TCRα-TCRβ, and mCD8α-TCRβ-TCRα. Various 3-in-1 vectors, thus generated, may be used to transduce CD4+ T cells, CD8+ T cells, and/or γδ T cells, followed by measuring TCRαβ/mCD8α co-expression levels of the transduced cells using techniques known in the art.

[0247] To generate lentiviral vectors co-expressing TCRαβ and mCD8α and/or CD8β, a nucleotide encoding furin-linker (GSG or SGSG (SEQ ID NO: 266))-2A peptide may be positioned between TCRα chain and TCRβ chain, between mCD8α chain and CD8β chain, and between a TCR chain and a CD8 chain to enable highly efficient gene expression. The 2A peptide may be selected from P2A (SEQ ID NO: 93), T2A (SEQ ID NO: 94), E2A (SEQ ID NO: 95), or F2A (SEQ ID NO: 96).

[0248] Lentiviral viral vectors may also contain post-transcriptional regulatory element (PRE), such as WPRE (SEQ ID NO: 264), WPREmut1 (SEQ ID NO: 256), or WPREmut2 (SEQ ID NO: 257), to enhance the expression of the transgene by increasing both nuclear and

cytoplasmic mRNA levels. One or more regulatory elements including mouse RNA transport element (RTE), the constitutive transport element (CTE) of the simian retrovirus type 1 (SRV-1), and the 5′ untranslated region of the human heat shock protein 70 (Hsp70 5′UTR) may also be used and/or in combination with WPRE to increase transgene expression. The WPREmut1 and WPREmut2 do not express an X protein, but still act to enhance translation of the transgene mRNA.

[0249] Lentiviral vectors may be pseudotyped with RD114TR (for example, SEQ ID NO: 97), which is a chimeric glycoprotein comprising an extracellular and transmembrane domain of feline endogenous virus (RD114) fused to cytoplasmic tail (TR) of murine leukemia virus. Other viral envelop proteins, such as VSV-G env, MLV 4070A env, RD114 env, chimeric envelope protein RD114pro, baculovirus GP64 env, or GALV env, or derivatives thereof, may also be used. RD114TR variants comprising at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% to SEQ ID NO: 97 also provided for.

[0250] For example, FIG. 4 shows exemplary vectors, which include two 4-in-1 vectors, e.g., Constructs #10 and #2, co-expressing TCR (TCRα chain and TCRβ chain), CD8α, and CD8β; three 3-in-1 vectors expressing TCR and CD8α, e.g., Constructs #1 and #9, two 3-in-1 vectors expressing TCR and m1CD8α (SEQ ID NO: 7), e.g., Constructs #11 and #12, and Construct #8 expressing TCR only. To improve transcriptional termination, wild type WPRE (WPRE) (SEQ ID NO: 264) is included in Constructs #1, #2, and #8; WPREmut (SEQ ID NO: 257) is included in Constructs #9, #10, #11, and #12.

[0251] Further exemplary constructs (Constructs #13-#19 and #21-#26) are described in Table 2 above. In particular, Constructs #13, #14, and #16 are 4-in-1 constructs co-expressing TCR, CD8α, and CD8β3 with various combinations of signal peptides (SEQ ID NO: 6 [WT CD8α signal peptide]; SEQ ID NO: 293 [WT CD8β signal peptide]; and SEQ ID NO: 294 [S19 signal peptide]) and differing element order. Constructs #15 and #17 are 4-in-1 constructs co-expressing TCR, CD8α, and CD8β5. Construct #15 comprises the WT CD8α signal peptide (SEQ ID NO: 6) and WT CD8β signal peptide (SEQ ID NO: 293), whereas Construct #17 comprises the S19 signal peptide (SEQ ID NO: 294) at the N-terminal end of both CD8α and CD8β5. Construct #21 is a 4-in-1 constructs co-expressing TCR, CD8α, and CD8β2 comprising WT CD8α signal peptide (SEQ ID NO: 6) and WT CD8β signal peptide (SEQ ID NO: 293). Construct #18 is a variant of Construct #10 in which the WT signal peptides for CD8α and CD8β1 (SEQ ID NOs: 6 and 293, respectively) were replaced with S19 signal peptide (SEQ ID NO: 294). Construct #19 is a variant of Construct #11 in which the WT CD8α signal peptide

(SEQ ID NO: 6) was replaced with the S19 signal peptide (SEQ ID NO: 294). Construct #22 is a variant of Construct #11 in which the CD4 transmembrane and intracellular domains are fused to the C-terminus of the CD8β stalk sequence in place of the CD8α transmembrane and intracellular domains. Construct #25 is a variant of Construct #22 in which the CD8β stalk sequence (SEQ ID NO: 2) is replaced with the CD8α stalk sequence (SEQ ID NO: 260).

EXAMPLE 4

Vector screening (Constructs #1, #2, #8, #9, #10, #11, and #12)

Viral titers

[0252] FIG. 5A shows viral titer of Constructs #1, #2, #8, #9, #10, #11, and #12. Table 5 shows viral titers and lentiviral P24 ELISA data for Constructs #9, #10, #11, and #12.

[0253] **Table 5**

Constructs #	Titer	Lentiviral P24
9	5.40×10^9	6556
9b	9.80×10^9	16196
10	6.40×10^9	9525
11	1.30×10^{10}	16797
12	1.20×10^{10}	17996

[0254] For construct 12, NCAMfu refers to NCAMFusion protein expressing modified CD8a extracellular and Neural cell adhesion molecule 1 (CD56) intracellular domain.

[0255] For Table 5, the WPREmut2 portion refers to SEQ ID NO: 257.

T cell manufacturing

Activation

[0256] FIG. 6 shows that, on Day +0, PBMCs (about 9×10^8 cells) obtained from two donors (Donor # 1 and Donor #2) were thawed and rested. Cells were activated in bags (AC290) coated with anti-CD3 and anti-CD28 antibodies in the presence of serum. Activation markers, e.g., CD25, CD69, and human low density lipoprotein receptor (H-LDL-R) are in CD8+ and CD4+

cells, were subsequently measured. FIG. 7A shows that % CD3+CD8+CD25+ cells, % CD3+CD8+CD69+ cells, and % CD3+CD8+H-LDL-R+ cells increase after activation (Post-A) as compared with that before activation (Pre-A). Similarly, FIG. 7B shows that % CD3+CD4+CD25+ cells, % CD3+CD4+CD69+ cells, and % CD3+CD4+H-LDL-R+ cells increase after activation (Post-A) as compared with that before activation (Pre-A). These results support the activation of PBMCs.

[0257] Transduction

[0258] FIG. 6 shows that, on Day +1, activated PBMCs were transduced with viral vectors, *e.g.*, Constructs #1, #2, #8, #9, #10, #11, and #12, in G-Rex® 6 well plates at about 5 x 10^6 cells/well in the absence of serum. The amounts of virus used for transduction are shown in Table 6.

[0259] **Table 6**

Constructs	Virus Volume/1 x 10^6 cells
#9, #10, #11, #12	1.25 µl, 2.5 µl, 5 µl
#1	1.25 µl
#2	5 µl
#8 (TCR)	2.5 µl

[0260] Expansion

[0261] FIG. 6 shows that, on Day +2, transduced PBMCs were expanded in the presence of serum. On Day +6, cells were harvested for subsequent analysis, e.g., FACS-Dextramer and vector copy number (VCN) and were cryopreserved. FIG. 8A and 8B show fold expansion on Day +6 of transduced T cell products obtained from Donor #1 and donor #2, respectively. Viabilities of cells is greater than 90% on Day +6.

[0262] Characterization of T cell products

[0263] Cell counts, FACS-dextramers, and vector copy numbers (VCN) were determined. Tetramer panels may comprise live/dead cells, CD3, CD8α, CD8β, CD4, and peptide/MHC tetramers, e.g., PRAME-004 (SLLQHLIGL) (SEQ ID NO: 147)/MHC tetramers. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by CD4+CD8+Tetramer(Tet)+ and CD8+Tet+.

[0264] FIGS. 9A, 9B, 9C, and 9D show representative flow plots of cells obtained from

Donor #1 indicating % CD8, CD4, and PRAME-004/MHC tetramer (Tet) of cells transduced with Construct #9b, #10, #11, or #12, respectively.

[0265] FIG. 10 shows % CD8+CD4+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show that higher % CD8+CD4+ cells were obtained by transduction with vectors expressing CD8α and TCR with wild type WPRE (Construct #1) and WPREmut2 (Construct #9) than that transduced with Constructs #10, #11, or #12. Construct #8 (TCR only) serves as negative control. FIG. 11 shows % Tet of CD8+CD4+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Constructs #1, #2, #8 (TCR), #9, #10, #11, and #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show that % Tet of CD8+CD4+ cells appear comparable among cells transduced with Constructs #9, #10, and #11, and seems greater than that transduced with Construct #12. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, and followed by CD4+CD8+Tet+.

[0266] FIG. 12 shows Tet MFI of CD8+CD4+Tet+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show that tetramer MFI on CD4+CD8+Tet+ varies among donors. FIG. 13 shows CD8α MFI of CD8+CD4+Tet+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show higher CD8α MFI in cells transduced with vectors expressing CD8α and TCR with wild type WPRE (Construct #1) and WPREmut2 (Construct #9) than that transduced with the other constructs. Transduction volume of 5 µl/10^6 appears to yield better results than 1.25 µl/10^6 and 2.5 µl/10^6. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by CD4+CD8+Tet+, and followed by Tet MFI/CD8α MFI.

[0267] FIG. 14 shows CD8 frequencies (% CD8+CD4- of CD3+) in cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show no difference in the CD8 frequencies among the constructs. Non-transduction (NT) serves as negative control. FIG. 15 shows % CD8+Tet+ (of CD3+) cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show higher frequencies of CD8+Tet+ (of CD3+) in cells transduced with Constructs #9, #11, and #12 than that transduced with Construct #10. FACS analysis was gated on live singlets, followed by CD3+, followed by CD8+CD4-, and followed

by CD8+Tet+.

[0268] FIG. 16 shows Tet MFI of CD8+Tet+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show tetramer MFI of CD8+tet+ cells varies among donors. FIG. 17 shows CD8α MFI of CD8+Tet+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show that CD8α MFI of CD8+Tet+ are comparable among cells transduced with different constructs. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by CD4+CD8+Tet+, and followed by Tet MFI/CD8α MFI.

[0269] FIG. 18 shows % Tet+ of CD3+ cells from Donor #1 (upper panel) and Donor #2 (lower panel) transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show higher frequencies of CD3+Tet+ in cells transduced with Construct #9 or #11 than that transduced with Construct #10 or #12. It appears more % Tet+CD3+ cells in cells transduced with Construct #10 (WPREmut2) than that transduced with Construct #2 (wild type WPRE) at 5 µl per 1 x 10^6 cells. FACS analysis was gated on live singlets, followed by CD3+, followed by CD3+, and followed by Tet+.

[0270] FIG. 19 (upper panel) shows vector copy number (VCN) of cells from Donor #1 transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show higher VCN for cells transduced with Constructs #11 or #12 (may be due to higher titers) than that transduced with Construct #9 or #10. FIG. 19 (lower panel) shows CD3+Tet+/VCN of cells from Donor #1 transduced with Construct #1, #2, #8 (TCR), #9, #10, #11, or #12 at 1.25 µl, 2.5 µl, or 5 µl per 1 x 10^6 cells. These results show higher CD3+Tet+/VCN in cells transduced with Construct #9 than that transduced with Construct #10, #11, or #12.

[0271] In sum, these results show (1) higher % CD8+CD4+ cells obtained by transducing cells with vectors expressing CD8α and TCR with wild type WPRE (Construct #1) and WPREmut2 (Construct #9) than that transduced with Construct #10, #11 or #12; (2) % CD8+CD4+Tet+ cells was comparable among cells transduced with different constructs; (3) dose dependent increase in % tetramer, e.g., 5 µl per 1 x 10^6 cells showed better results than 1.25 µl and 2.5 µl per 1 x 10^6 cells; (4) % CD8+ cells comparable among cells transduced with different constructs; (5) higher frequencies of CD8+Tet+ in cells transduced with Construct #9, #11, or #12 than that transduced with Construct #10; (6) higher frequencies of CD3+Tet+ in cells

transduced with Construct #9 or #11 than that transduced with Construct #10 or #12; (7) higher VCN in cells transduced with Construct #11 or #12 than that transduced with Construct #9 or #10; and (8) higher CD3+tet+/VCN in cells transduced with Construct #9 than that transduced with Construct #10, #11, or #12.

[0272] T cell products transduced with viral vector expressing a transgenic TCR and modified CD8 co-receptor showed superior cytotoxicity and increased cytokine production against target positive cell lines.

EXAMPLE 5

Tumor Death Assay

[0273] FIG. 20A-C depicts data showing that constructs (#10, #11, & #12) are comparable to TCR-only in mediating cytotoxicity against target positive cells lines expressing antigen at different levels (UACC257 at 1081 copies per cell and A375 at 50 copies per cell).

[0274] Table 7

Tumor Cell Line	**Antigen Positivity**
UACC257	High
A375	Low
MCF7	Negative

[0275] Construct #9 loses tumor control over time against the low target antigen expressing A375 cell line.

EXAMPLE 6

IFNγ Secretion Assay

[0276] IFNγ secretion was measured in UACC257 and A375 cells lines. IFNγ secretion in response in UACC257 cell line was comparable among constructs. However, in the A375 cell line, Construct #10 showed higher IFNγ secretion than other constructs. IFNγ quantified in the supernatants from Incucyte plates. FIG. 21A-B.

[0277] FIG. 22 depicts an exemplary experiment design to assess Dendritic Cell (DC) maturation and cytokine secretion by PBMC-derived T cell products in response to exposure to target positive tumor cell lines UACC257 and A375.

[0278] IFNγ secretion in response to A375 increases in the presence of immature DC (iDCs). In the tri-cocultures with iDCs, IFNγsecretion is higher in Construct #10 compared to the other constructs. However, comparing Construct #9 with Construct #11 expressing wild type and modified CD8 coreceptor sequences respectively, T cells transduced with #11 induced stronger

cytokine response measured as IFNγ quantified in the culture supernatants of three-way cocultures using donor D600115, E:T:iDC::1:1/10:1/4. FIG. 23A-B.

[0279] IFNγ secretion in response to A375 increases in the presence of iDCs. In the tri-cocultures with iDCs, IFNγ secretion was higher in Construct #10 compared to the other constructs. IFNγ quantified in the supernatants from DC cocultures D150081, E:T:iDC::1:1/10:1/4. FIG. 24A-B

[0280] IFNγ secretion in response to UACC257 increases in the presence of iDCs. In the tri-cocultures with iDCs, IFNγ secretion is higher in Construct #10 compared to the other constructs. However, comparing Construct #9 with Construct #11 expressing wild type and modified CD8 coreceptor sequences respectively, T cells transduced with Construct #11 induced stronger cytokine response measured as IFNγ quantified in the culture supernatants of three-way cocultures using donor D600115, E:T:iDC::1:1/10:1/4. FIG. 25A-B. These results demonstrate that T cell products co-expressing a transgenic TCR and CD8 co-receptor (αβ heterodimer or modified CD8α homodimer) are able to license DCs in the microenvironment through antigen cross presentation and therefore hold the potential to mount a stronger anti-tumor response and modulate the tumor microenvironment.

EXAMPLE 7

Vector screening (Constructs #13-#21)

Viral titers

[0281] FIG. 5B shows viral titer of Constructs #10, #10n (new batch), #11, #11n (new batch), #13 - #21, and TCR only as a control.

T cell manufacturing

Activation

[0282] FIG. 26 shows that, on Day +0, PBMCs obtained from two HLA-A02+ donors (Donor # 1 and Donor #2) were thawed and rested. Cells were activated in bags (AC290) coated with anti-CD3 and anti-CD28 antibodies in the absence of serum. Activation markers, e.g., CD25, CD69, and human low density lipoprotein receptor (H-LDL-R) are in CD8+ and CD4+ cells, were subsequently measured. FIG. 27A shows that % CD3+CD8+CD25+ cells, % CD3+CD8+CD69+ cells, and % CD3+CD8+H-LDL-R+ cells increase after activation (Post-A) as compared with that before activation (Pre-A). Similarly, FIG. 27B shows that %

CD3+CD4+CD25+ cells, % CD3+CD4+CD69+ cells, and % CD3+CD4+H-LDL-R+ cells increase after activation (Post-A) as compared with that before activation (Pre-A). These results support the activation of PBMCs.

Transduction

[0283] FIG. 26 shows that, on Day +1, activated PBMCs were transduced with viral vectors, *e.g.*, Constructs #8, #10, #10n, #11, #11n, and #13-#21, in G-Rex® 24-well plates at about 2 x 10^6 cells/well in the absence of serum. The amounts of virus used for transduction are shown in Table 8.

[0284] **Table 8**

Constructs	Virus Volume/1 x 10^6 cells
#10n, #11n, #13-#21	0.3 µl, 1.1 µl, 3.3 µl, 10 µl, 30 µl
#8 (TCR), #10	2.5 µl
#11	1.25 µl
NT	-

Expansion

[0285] FIG. 26 shows that, on Day +2, transduced PBMCs were expanded in the absence of serum. On Day +6, cells were harvested for subsequent analysis, e.g., FACS-Tetramer and vector copy number (VCN) and were cryopreserved. FIG. 28 shows fold expansion on Day +6 of transduced T cell products. Viabilities of cells is greater than 90% on Day +6.

Characterization of T cell products

[0286] Cell counts, FACS-dextramers, and vector copy numbers (VCN) were determined. Tetramer panels may comprise live/dead cells, CD3, CD8α, CD8β, CD4, and peptide/MHC tetramers, e.g., PRAME-004 (SLLQHLIGL) (SEQ ID NO: 147)/MHC tetramers. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by CD4+CD8+Tetramer(Tet)+ and CD8+Tet+.

[0287] FIG. 29A and FIG. 29B shows % CD8+CD4+ cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show

comparable frequencies of CD8+CD4+ cells obtained by transduction with all vectors tested. Construct #8 (TCR only) serves as negative control. FIG. 30A and FIG. 30B shows % Tet of CD8+CD4+ cells from transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show that there was a trend towards higher frequencies of CD4+CD8+tet+ in CD8β1 isoforms (Constructs #10 and #18) compared to CD8β3 isoforms (Construct #16) and CD8β5 isoforms (Constructs # 15 and #17). FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, and followed by CD4+CD8+Tet+.

[0288] FIG. 31A and FIG. 31B shows Tet MFI of CD8+CD4+Tet+ cells from transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show a trend towards higher tetramer MFI on CD4+CD8+Tet+ population in CD8β1 isoforms (Constructs #10 and #18) compared to CD8β3 isoforms (Construct #16) and CD8β5 isoforms (Constructs # 15 and #17).

[0289] FIG. 32A and FIG. 32B show CD8 frequencies (% CD8+CD4- of CD3+) in cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show no difference in the CD8 frequencies among the constructs. FIG. 33A and FIG. 33B shows % CD8+Tet+ (of CD3+) cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show slightly higher frequencies of CD8+Tet+ (of CD3+) in cells transduced with Construct #10 than those transduced with the other constructs. FACS analysis was gated on live singlets, followed by CD3+, followed by CD8+CD4-, and followed by Tet+.

[0290] FIG. 34A and FIG. 34B shows Tet MFI of CD8+Tet+ cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show tetramer MFI of CD8+tet+ cells was comparable among CD8β1 (Constructs #18 and #10), CD8β5 (Constructs # 15 and #17), and CD8β3 (Construct #16) isoforms, while Construct #21 expressed lower tetramer MFI.

[0291] FIG. 35A and FIG. 35B shows % Tet+ of CD3+ cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These results show higher frequencies of CD3+Tet+ in cells transduced with Construct #10 (CD8β1) compared to those transduced with CD8β3 (Construct #16) and CD8β5 (Constructs #15 and #17). FACS analysis was gated on live singlets, followed by CD3+, and followed by Tet+.

[0292] FIG. 36A and FIG. 36B shows vector copy number (VCN) of cells transduced with Construct #10, #10n, #11, #13-#21 at 0.3 µl, 1.1 µl, 3.3 µl, 10 µl or 30 µl per 1 x 10^6 cells. These

results show comparable ability of all constructs to integrate and express CD8/TCR genes.

[0293] In sum, these results show (1) viral vectors with CD8β1, CD8β3 and CD8β5 isoforms had good transducing titers; (2) all constructs were capable of successful manufacturing (e.g., high viability, fold expansions in the range of 6-12); (3) frequencies of CD3+tet+ among CD8β isoforms: CD8β1 (Construct #10) was greater than CD8β3 (Construct #16) and CD8β5 (Constructs #15 and #17), with Construct #21 showing the lowest values; (4) frequency of CD3+tet+ in Constructs #11 and #19 (m1CD8α (SEQ ID NO: 7)) showed the highest values; and (5) saturation in %CD3+tet+, %CD8+tet+ and %CD4+CD8+tet+ observed at 10µl/e6. Optimal vector dose ranges between 3.3-10 µl/e6 for all constructs.

EXAMPLE 7

Mid-Scale Vector screening (Constructs #13-#19)

T cell manufacturing

Activation/Transduction

[0294] FIG. 37 shows that, on Day +0, PBMCs obtained from four HLA-A02+ donors were thawed and rested. Cells were activated in bags (AC290) coated with anti-CD3 and anti-CD28 antibodies in the absence of serum. On Day +1, activated PBMCs were transduced with viral vectors, *e.g.*, Constructs #8, #10n, #11n, and #13-#19, in G-Rex® 6-well plates at about 7 x 10^6 cells/well in the absence of serum. The amounts of virus used for transduction are shown in Table 9.

[0295] **Table 9**

Constructs	Virus Volume/1 x 10^6 cells
#13-19	2.5 µl and 5 µl
#10n and #11n	2.5 µl and 5 µl
#8 (TCR)	2.5 µl
NT	-

Expansion

[0296] FIG. 37 shows that, on Day +2, transduced PBMCs were expanded in the absence of serum. On Day +7, cells were harvested for subsequent analysis, e.g., FACS-Tetramer and vector

copy number (VCN) and were cryopreserved. Fold expansion on Day +7 was comparable for all constructs (approximately 30-fold expansion). Viabilities of cells is greater than 90% on Day +7.

Characterization of T cell products

[0297] Cell counts, FACS-dextramers, and vector copy numbers (VCN) were determined. Tetramer panels may comprise live/dead cells, CD3, CD8α, CD8β, CD4, and peptide/MHC tetramers, e.g., PRAME-004 (SLLQHLIGL) (SEQ ID NO: 147)/MHC tetramers. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by CD4+CD8+Tetramer(Tet)+ and CD8+Tet+.

[0298] Similar to results described in Example 6, comparable frequencies of CD8+CD4+ cells were obtained by transduction with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells. Construct #8 (TCR only) serves as negative control. FIG. 38 shows % Tet of CD8+CD4+ cells transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells. Similar to results described in Example 6, these results show that there was a trend towards higher frequencies of CD4+CD8+tet+ in CD8β1 isoforms (Construct #10n) compared to CD8β3 isoforms (Constructs #13, #14, #16) and CD8β5 isoforms (Constructs # 15 and #17). FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, and followed by Tet+.

[0299] FIG. 39 shows Tet MFI of CD8+CD4+Tet+ cells from transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells. These results show higher tetramer MFIs on CD4+CD8+Tet+ population in CD8β1 isoforms (Construct #10n) compared to CD8β3 isoforms (Construct #13) and CD8β5 isoforms (Constructs # 15 and #17).

[0300] Similar to results described in Example 6, results show no difference in the CD8 frequencies (% CD8+CD4- of CD3+) in cells transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells among the constructs (data not shown). Comparable frequencies of CD8+Tet+ (of CD3+) in cells transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells (data not shown). FACS analysis was gated on live singlets, followed by CD3+, followed by CD8+CD4-, and followed by Tet+.

[0301] FIG. 40 shows Tet MFI of CD8+Tet+ cells transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells. These results show tetramer MFI of CD8+tet+ cells was comparable among CD8β1 (Constructs #18 and #10) and CD8β5 (Construct # 15) isoforms, while CD8β3 (Constructs #13, #14, and #16) isoforms expressed lower tetramer MFI.

[0302] FIG. 41 shows % Tet+ of CD3+ cells transduced with Construct #10n, #11n, #13-#19

at 2.5 µl or 5.0 µl per 1 x 10^6 cells. These results show slightly higher frequencies of CD3+Tet+ in cells transduced with Construct #10 (CD8β1) compared to those transduced with CD8β3 (Constructs #13, #14, and #16) and CD8β5 (Construct #15). FACS analysis was gated on live singlets, followed by CD3+, and followed by Tet+. Slightly higher total CD3+tet+ cell counts were observed in PBMC transduced with Construct #10 CD8β1) compared to those transduced with CD8β3 (Constructs #13, #14, and #16) and CD8β5 (Construct #15) (data not shown).

[0303] FIG. 42 shows vector copy number (VCN) of cells transduced with Construct #10n, #11n, #13-#19 at 2.5 µl or 5.0 µl per 1 x 10^6 cells. These results show vector copies per cell remained below 5 in PBMC product derived using each individual construct at vector dose of 2.5 µl or 5.0 µl per 1 x 10^6 cells.

[0304] FIG. 43 shows the % T cell subsets in cells transduced with Construct #10, #11, #13, and #15 for each donor. Construct #8 (TCR only) and non-transduced cells were used as controls. These results show that TCR-only condition has slightly more naïve cells compared to the other constructs, consistent with lower fold-expansion. FIG. 44A and FIG. 44B shows % T cell subsets in cells transduced with Construct #10, #11, #13, and #15 for each donor. Construct #8 (TCR only) and non-transduced cells were used as controls. FACS analysis was gated on CD4+CD8+ for FIG. 44A and on CD4-CD8+TCR+ for FIG. 44B. These results show donor-to-donor variability between frequencies of T cell memory subsets but little difference in the frequencies of T_{naive} and T_{cm} between constructs.

[0305] In sum, these results show (1) viability and fold expansions were comparable among all constructs at day 7; (2) slightly higher frequency of CD3+tet+ observed in CD8β1 (Construct #10) compared to CD8β3 (Constructs # 13, #14, and #16) and CD8β5 (Constructs #15 and #17); (3) vector copies per cell < 5 for majority of the constructs at 2.5-5ul/10^6 dose; and (4) donor-to-donor variability between frequencies of T cell memory subsets but generally, Construct #10 has less naïve but more Tcm cells than the other β isoform constructs.

EXAMPLE 8

Tumor Death Assay – Constructs #10, #11, #13 & #15

[0306] FIG. 45A and 45B depicts data showing that Constructs #13 and #10 are comparable to TCR-only in mediating cytotoxicity against UACC257 target positive cells lines expressing high levels of antigen (1081 copies per cell). Construct # 15 was also effective but slower in killing compared to Constructs #13 and #10. The effector:target ratio used to generate these results was 4:1. Similar results were obtained with a 2:1 effector:target ratio (data not shown).

EXAMPLE 9

IFNγ Secretion Assay – Constructs #10, #11, #13 & #15

[0307] IFNγ secretion was measured in the UACC257 cells line. FIG. 46 shows IFNγ secretion in response in UACC257 cell line was higher with Construct #13 compared to Construct #10. IFNγ quantified in the supernatants from Incucyte plates. The effector:target ratio used to generate these results was 4:1. Similar results were obtained with a 2:1 effector:target ratio (data not shown).

EXAMPLE 10

ICI Marker Expression – Constructs #10, #11, #13 & #15

[0308] ICI marker frequency (2B4, 41BB, LAG3, PD-1, TIGIT, TIM3, CD39+CD69+, and CD39-CD69-) was measured. FIG. 47 shows Construct #15 has higher expression of LAG3, PD-1, and TIGIT compared to other constructs, followed by Construct #10.

EXAMPLE 11

Cytokine Expression – Constructs #10, #11, #13 & #15

[0309] Expression of various cytokines was measured in UACC257 cells co-cultured at a 4:1 E:T ratio with PBMC transduced with Constructs #10, #11, #13, and #15. FIG. 48A – 48G show increased expression of IFNγ, IL-2, and TNFα with CD4+CD8+ cells transduced with construct #10 (WT signal peptide, CD8β1) compared to other constructs. FACS analysis was gated on CD3+CD4+CD8+ cells against UACC257, 4:1 E:T. FIG. 49A-49G show increased expression of IFNγ, IL-2, MIP-1β, and TNFα with CD4-CD8+ cells transduced with construct #10 (WT signal peptide, CD8β1) compared to other constructs. FACS analysis was gated on CD3+CD4-CD8+ cells against UACC257, 4:1 E:T. FIG. 50A-50G show increased expression of IL-2 and TNFα with CD3+TCR+ cells transduced with construct #10 (WT signal peptide, CD8β1) compared to other constructs. MIP-1β expression is highest in Construct #11 (similar results when gated on CD4+CD8+ cells). FACS analysis was gated on CD3+TCR+ cells against UACC257, 4:1 E:T.

[0310] Expression of various cytokines was measured in A375 cells co-cultured at a 4:1 E:T ratio with PBMC transduced with Constructs #10, #11, #13, and #15. FIG. 51A-51C show results from FACS analysis gated on CD4+CD8+ cells against A375, 4:1 E:T. FIG. 52A-52C show results from FACS analysis gated on CD4-CD8+ cells against A375, 4:1 E:T. FIG. 53A-53C show results from FACS analysis gated on CD3+TCR+ cells against A375, 4:1 E:T.

Overall, results were more variable when cells are co-cultured with A375+RFP, but similar trends are observed compared to activation by UACC257+RFP.

EXAMPLE 12

Large-Scale Vector screening (Constructs #10, #11, #13, #16, #18, #19)

T cell manufacturing

Activation/Transduction

[0311] FIG. 54 shows that, on Day +0, PBMCs obtained from three HLA-A02+ donors were thawed and rested. Cells were activated in bags (AC290) coated with anti-CD3 and anti-CD28 antibodies in the absence of serum. On Day +1, activated PBMCs were transduced with viral vectors, *e.g.*, Constructs #8, #10n, #11n, #13, #16, #18, and #19 in G-Rex® 100 cell culture vessels at about 5 x 10^7 cells/vessel in the absence of serum. The amounts of virus used for transduction are shown in Table 10.

[0312] **Table 10**

Constructs	Virus Volume/1 x 10^6 cells
#13, #16, #18, #10n	5 µl
#19 and #11n	2.5 µl
#8 (TCR)	2.5 µl
NT	-

Expansion

[0313] FIG. 54 shows that, on Day +2, transduced PBMCs were expanded in the absence of serum. On Day +7, cells were harvested for subsequent analysis, e.g., FACS-Tetramer and vector copy number (VCN) and were cryopreserved. Fold expansion on Day +7 was comparable for all constructs (approximately 30-fold expansion). Viabilities of cells is greater than 90% on Day +7.

Characterization of T cell products

[0314] Cell counts, FACS-dextramers, and vector copy numbers (VCN) were determined. Tetramer panels may comprise live/dead cells, CD3, CD8α, CD8β, CD4, and peptide/MHC tetramers, e.g., PRAME-004 (SLLQHLIGL) (SEQ ID NO: 147)/MHC tetramers. FACS analysis was gated on live singlets, followed by CD3+, followed by CD4+CD8+, followed by

CD4+CD8+Tetramer(Tet)+ and CD8+Tet+.

[0315] Tumor death assays and cytokine expression in the presence and absence of autologous immature dendritic cells was also measured.

[0316] The results were consistent with the prior examples and are summarized in Table 11.

Table 11

	Parameters	Construct #10	Construct #13	Construct #11	Construct #19	TCR only Construct #8
Manufacturing	Viabilities	>90%	>90%	>90%	>90%	>90%
	Fold Expansion d7	28.7±11%	28.6±11%	31.6±13%	29.6±13%	30.1±11%
	Transgene expression (%CD3+Tet+), mean±SD	46.9±12%	42±9.8%	41±12%	48.2±14%	22.8±8%
	Vector Copy Number	3.3±0.6%	2.6±0.7%	2.0±0.8%	3.1±1.8%	1.7±0.7%
Functionality	Multiple rounds of killing with UACC	+++	+++	+++	+++	+++
	Cytokine secretion (24h, with UACC); IFN-g, TNF-a, IL-2	+++	+++	++	++	++
	Cytokine secretion; CD4+CD8+TCR+ (16h, UACC); ICS	+++	+++	+	+	+/-
	DC licensing assay (PBMC product) IL-12, TNF-a & IL-6	+++	+++	+	+	+
	3D Spheroid Assay	+++	N/A	+++	N/A	++

EXAMPLE 13

DC licensing by CD4 cells expressing Constructs of the Present Disclosure

[0317] FIG. 59 shows a scheme of determining the levels of cytokine secretion by dendritic cells (DC) in the presence of PBMCs transduced with constructs of the present disclosure and in the presence of target cells, e.g., UACC257 cells. Briefly, Day 0, PBMCs (n = 3) were thawed and rested, followed by monocyte isolation and autologous immature DCs (iDC) generation in the presence of IL-4 and GM-CSF; Day 2 and Day 4-5, DC were fed in the presence of IL-4 and GM-CSF; Day 6, iDC (+DC) were co-cultured with PBMC transduced with Construct #13, #16, #10n, #18, #11n, or #19 (Effector) and UACC257 cells (Target) at a ratio of Effector : Target : iDC = 1 : 1/10 : 1/4 or without iDC (-DC), PBMCs transduced with TCR only, PBMCs without transduction (NT), PBMCs treated with iDC and LPS, and iDC only serve as controls; and Day 7

(after co-culturing for 24 hours), supernatants from the co-cultures were harvested, followed by cytokine profiling including, e.g., IL-12, IL-6, and TNF-α, using Multiplex.

[0318] Increased secretion of pro-inflammatory cytokines in tri-cocultures of autologous immature dendritic cells, UACC257 tumor cell line, and CD4+ T cell product expressing CD8αβ heterodimer and TCR (Construct #10) compared with that expressing CD8α* homodimer, in which the stalk region is replaced with CD8β stalk region, and TCR (Construct #11).

[0319] To determine the ability of CD4+ T cells expressing Constructs #10 or #11 to license DC, bulk PBMCs were transduced with Constructs #10 or #11, followed by selection of CD8+ and CD4+ cells from the product. Tri-cocultures of PBMCs, CD8+CD4- selected-product, or CD4+CD8+ selected-product with UACC257 tumor cell line in the presence or absence of autologous immature dendritic cells (iDCs) for 24 h followed by cytokine quantification of IL-12, TNF-α and IL-6 using Multiplex; iDCs alone or with LPS as controls, N = 4-7, mean±SD, P values based on 2way ANOVA.

[0320] In the presence of immature dendritic cells (iDCs) and UACC257 cells, CD4+ T cells expressing Construct #10 (CD4+CD8+ T cells) performed better by inducing higher levels of IL-12 (FIG. 56), TNF-α (FIG. 57), and IL-6 (FIG. 58) secreted by dendritic cells (DC) than CD4+ T cells expressing Construct #11. On the other hand, the levels of IL-12, TNF-α, and IL-6 were comparable between CD8+ T cells expressing Constructs #10 and #11 (CD8+CD4- T cells). These results suggest that CD4+ T cells expressing CD8αβ heterodimer and TCR (Construct #10) may be a better product than CD4+ T cells expressing CD8α* homodimer and TCR (Construct #11) in DC licensing. The negative controls include the cytokine levels obtained (1) in the absence of iDCs (-iDCs), (2) in the presence of non-transduced T cells (NT) + UACC257 cells, and (3) in the presence of T cells transduced with TCR only (TCR) + UACC257 cells. The positive control includes the cytokine levels obtained from iDCs treated with lipopolysaccharide (LPS), which can activate DC.

EXAMPLE 14

Assessment of DC maturation and cytokine secretion by PBMC products in response to UACC257 targets

[0321] FIG. 60 shows IL-12 secretion levels induced by co-culturing PBMCs transduced with constructs of the present disclosure in the presence or absence of iDC and target cells, e.g., UACC257 cells. For example, IL-12 secretion was increased by co-culturing PBMCs transduced with Constructs #10 and 13 in the presence of iDC (+DC) and UACC257, as compared with that

by co-culturing PBMCs transduced with TCR only. Increase of IL-12 secretion suggests (1) polarization towards Th1 cell-mediated immunity including TNF-α production (see, FIG. 61), (2) T cell proliferation, (3) IFN-γ production, and (4) cytolytic activity of cytotoxic T lymphocytes (CTLs).

[0322] FIG. 61 shows TNF-α secretion levels induced by co-culturing PBMCs transduced with constructs of the present disclosure in the presence or absence of iDC and target cells, e.g., UACC257 cells. For example, TNF-α secretion was increased by co-culturing PBMCs transduced with Constructs #10 and 13 in the presence of iDC (+DC) and UACC257, as compared with that by co-culturing PBMCs transduced with TCR only.

[0323] The increased IL-6 secretion (in addition to IL-12, TNF-α) may signify dendritic cell maturation, which may be augmented by CD40-CD40L interactions between CD4+ T cells and DCs. DC maturation and subsequent cytokine secretion may aid in modulation of the proinflammatory environment.

[0324] FIG. 62 shows IL-6 secretion levels induced by co-culturing PBMCs transduced with constructs of the present disclosure in the presence or absence of iDC and target cells, e.g., UACC257 cells. For example, IL-6 secretion was increased by co-culturing PBMCs transduced with Constructs #10 and 13 in the presence of iDC (+DC) and UACC257, as compared with that by co-culturing PBMCs transduced with TCR only.

[0325] These results show that PBMC products containing CD4+ T cells co-expressing transgenic TCR and CD8 co-receptor (CD8αβ heterodimer or CD8α homodimer) may license DCs in the microenvironment through antigen cross presentation to modulate the tumor microenvironment by, e.g., increasing IL-12, IL-6, and TNF-α secretion.

[0326] Table 12 shows comparison between constructs based on manufacturability and functionality.

Table 12

	Parameters	Construct #10	Construct #13	Construct #11	Construct #19	TCR only
Manufacturability	Viabilities	>90%	>90%	>90%	>90%	>90%
	Fold expansion on Day 7	28.7±11%	28.6±11%	31.6±13%	29.6±13%	30.1±11%
	Transgene expression (%CD3+Tet+)	46.9±12%	42±9.8%	41±12%	48.2±14%	22.8±8%

	mean ± SD					
	Vector copy number	3.3±0.6%	2.6±0.7%	2.0±0.8%	3.1±1.8%	1.7±0.7%
Functionality	Multiple rounds of killing with UACC257 cells	+++	+++	+++	+++	+++
	Cytokine secretion (24h, with UACC257 cells); IFN-γ, TNF-α, IL-2	+++	+++	++	++	++
	Cytokine secretion; CD4+CD8+TCR+ (16h with UACC257 cells); ICS	+++	+++	+	+	+/-
	DC licensing assay (PBMC product) IL-12, TNF-α, and IL-6	+++	+++	+	+	+
	3D spheroid assay	+++	N/A	+++	N/A	++

[0327] Notes: "+++" = best response; "++" = good response; "+" = average response; "+/-" = poor response.

[0328] Table 13 shows construct comparison and ranking (the smaller the number the better).

Table 13

Parameters	Construct #10	Construct #13	Construct #11	Construct #19
Manufacturability	1	1	1	1
Functionality PBMC	1	1	2	2
Functionality	1	1	1	1

CD8				
Functionality CD4	1	1	3	3
Time delay*	1	1	1	1
Total	5	5	8	8

* Time delay here refers to any delay from, for example, GMP Vector manufacturing or any delay due to incomplete data set, which may add delay in implementation of constructs in clinical trials.

[0329] In sum, while manufacturability in terms of, e.g., viability, fold expansion, transgene expression, and vector copy number, may be equally good, as ranked 1, among cells transduced with Construct # 10, #11, #13, or #19, functionality in terms of, e.g., cell killing, cytokine secretion, DC licensing, and 3D spheroid forming ability, of cells transduced with Construct #10 and #13 may be better, as ranked 1, than those transduced with Construct #11 and #19, as ranked 1-3.

EXAMPLE 15

EC50 Assays

[0330] To determine the efficacy of T cells transduced with constructs of the present disclosure, e.g., Constructs #10 and #11, against target cells, EC50s were determined based on the levels of IFNγ produced by the transduced cells in the presence of PRAME peptide-pulsed T2 cells.

[0331] For example, to compare EC50s of CD4+ selected T cells transduced with Construct #10 (CD8αβ-TCR), Construct #11 (m1CD8α-TCR), or Construct #8 (TCR only), CD4+ selected products (TCR+ normalized) were co-cultured with PRAME peptide-pulsed T2 cells at defined concentrations at E:T ratio of 1:1 for 24 h. IFNγ levels were quantified in the supernatants after 24 h. FIGS. 63A-63C show IFNγ levels produced by the transduced CD4+ selected T cells obtained from Donor #1, #2, and #3, respectively. In general, CD4+ selected T cells transduced with Construct #10 were more sensitive to PRAME antigen as compared with that transduced with Construct #11 (m1CD8α TCR+ CD4 T cells), as indicated by lower EC50 values (ng/ml) of CD4+ selected T cells transduced with Construct #10 than that transduced with Construct # 11 (FIG. 63D). No response was observed among TCR+ CD4+ cells (FIGS. 63A-63D). These results suggest that CD8αβ heterodimer may impart increased avidity to CD8αβ TCR+ CD4+ T cells as compared to m1CD8a homodimer, leading to better efficacy against target cells.

[0332] Similar experiments were performed using PBMC obtained from Donor #1, #3, and #4. Briefly, PBMC products (TCR+ non-normalized) were co-cultured with PRAME peptide-pulsed T2 cells at defined concentrations at E:T ratio of 1:1 for 24 h. IFNγ levels were quantified in the supernatants after 24 h. FIGS. 64A-64C show IFNγ levels produced by the transduced PBMC obtained from Donor #4, #1, and #3, respectively. Donor-to-donor variability was observed in the EC50 values. For example, while Donor #3 (FIGS. 64C and 64D) shows lower EC50 of PBMC transduced with Construct #10 as compared with that transduced with TCR only, Donors #1 (FIG. 64B) and #4 (FIG. 64A) show comparable EC50s between Construct #10 and TCR only (FIG. 64D). Thus, the increased avidity and efficacy observed in CD4+ selected T cell products expressing TCR and CD8αβ heterodimer as compared with that expressing TCR only may be obtained but to lesser extent when using PBMC products.

[0333] To compare EC50s of different T cell products obtained from the same donor, PBMC products, CD8+ selected products, and CD4+ selected products obtained from a single donor were co-cultured with PRAME peptide-pulsed T2 cells (TCR+ normalized) at defined concentrations at E:T ratio of 1:1 for 24 h. IFNγ levels were quantified in the supernatants after 24 h.. FIGS. 65A-65C show that IFNγ levels produced by PBMC products (FIG. 65A), CD8+ selected products (FIG. 65B), and CD4+ selected products (FIG. 65C), respectively. Consistently, EC50 of CD4+ selected T cells transduced with Construct #10 was lower than that transduced with Construct #11 or TCR only (FIG. 65C), while EC50s of the transduced PBMC and CD8+ selected T cells were comparable between Construct #10 and TCR only transduction. Thus, the increased avidity and efficacy observed in CD4+ selected T cell products expressing TCR and CD8αβ heterodimer as compared with that expressing TCR and m1CD8α homodimer or with that expressing TCR only may be obtained but to lesser extent when using PBMC products or CD8+ selected T cell products.

[0334] All references cited in this specification are herein incorporated by reference as though each reference was specifically and individually indicated to be incorporated by reference. The citation of any reference is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such reference by virtue of prior invention.

[0335] It will be understood that each of the elements described above, or two or more together may also find a useful application in other types of methods differing from the type described above. Without further analysis, the foregoing will so fully reveal the gist of the present disclosure that others can, by applying current knowledge, readily adapt it for various

applications without omitting features that, from the standpoint of prior art, fairly constitute essential characteristics of the generic or specific embodiments of this disclosure set forth in the appended claims. The foregoing embodiments are presented by way of example only; the scope of the present disclosure is to be limited only by the following claims.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid sequence encoding (a) a T-cell receptor (TCR) comprising an α isolated chain and a β chain and a CD8 polypeptide comprising an α chain and a β chain, or (b) a TCR comprising an α chain and a β chain and a CD8 polypeptide comprising an α chain without a β chain, wherein the TCR α chain and the TCR β chain are selected from SEQ ID NO: 15 and 16, 17 and 18, 19 and 20, 21 and 22, 23 and 24, 25 and 26, 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, 47 and 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 and 60, 61 and 62, 63 and 64, 65 and 66, 67 and 68, 69 and 70, 71 and 303, 304 and 74, 75 and 76, 77 and 78, 79 and 80, 81 and 82, 83 and 84, 85 and 86, 87 and 88, 89 and 90, and 91 and 92, wherein the CD8 α chain is SEQ ID NO: 7, 258, 259, 262, or a variant thereof, and wherein the CD8 β chain is SEQ ID NO: 8, 9, 10, 11, 12, 13, or 14.
2. The isolated nucleic acid of claim 1, wherein the TCR α chain and the TCR β chain are selected from SEQ ID NO: 15 and 16, 57 and 58, 59 and 60, 61 and 62, 63 and 64, 65 and 66, 67 and 68, 69 and 70, and 71 and 303.
3. The isolated nucleic acid of claim 1 or 2, wherein the nucleic acid sequence comprises a nucleic acid at least 80% identical to the nucleic acid sequence of SEQ ID NO: 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301.
4. The isolated nucleic acid of claim 3, wherein the nucleic acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the nucleic acid sequence of SEQ ID NO: 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 295, 297, 299, or 301.
5. The isolated nucleic acid of any one of claims 1-4, wherein the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 267.
6. The isolated nucleic acid of any one of claims 1-4, wherein the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 279.
7. An isolated polypeptide encoded by the nucleic acid of any one of claims 1-6.
8. An isolated polypeptide comprising the amino acid sequence at least about 80% identical to the amino acid sequence of SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302.

9. The isolated polypeptide of claim 8, wherein the amino acid sequence is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 296, 298, 300, or 302.

10. The isolated polypeptide of any one of claims 7-9, wherein the amino acid comprises the amino acid sequence of SEQ ID NO: 268.

11. The isolated polypeptide of any one of claims 7-9, wherein the amino acid comprises the amino acid sequence of SEQ ID NO: 280.

12. A vector comprising the nucleic acid of any one of claims 1-6.

13. The vector of claim 12, wherein the vector further comprises a nucleic acid encoding a 2A peptide or an internal ribosome entry site (IRES) positioned between the nucleic acid encoding the CD8 α chain and the nucleic acid encoding the CD8 β chain.

14. The vector of claim 12 or 13, wherein the vector further comprises a nucleic acid encoding a 2A peptide or an IRES positioned between the nucleic acid encoding the TCR α chain and the nucleic acid encoding the TCR β chain.

15. The vector of claim 14, wherein the 2A peptide is P2A (SEQ ID NO: 93), T2A (SEQ ID NO: 94), E2A (SEQ ID NO: 95), or F2A (SEQ ID NO: 96).

16. The vector of any one of claims 12-15, wherein the vector further comprises a post-transcriptional regulatory element (PRE) sequence selected from a Woodchuck PRE (WPRE), Woodchuck PRE (WPRE) mutant 1, Woodchuck PRE (WPRE) mutant 2, or hepatitis B virus (HBV) PRE (HPRE).

17. The vector of claim 16, wherein the post-transcriptional regulatory element (PRE) sequence is Woodchuck PRE (WPRE) mutant 1 comprising the amino acid sequence of SEQ ID NO: 256.

18. The vector of claim 16, wherein the post-transcriptional regulatory element (PRE) sequence is Woodchuck PRE (WPRE) mutant 2 comprising the amino acid sequence of SEQ ID NO: 257.

19. The vector of any one of claims 12-18, wherein the vector further comprises a promoter selected from cytomegalovirus (CMV) promoter, phosphoglycerate kinase (PGK) promoter, myelin basic protein (MBP) promoter, glial fibrillary acidic protein (GFAP) promoter, modified MoMuLV LTR comprising myeloproliferative sarcoma virus enhancer (MNDU3), Ubiqitin C promoter, EF-1 alpha promoter, or Murine Stem Cell Virus (MSCV) promoter.

20. The vector of any one of claims 12-19, wherein the vector is a viral vector or a non-viral vector.

21. The vector of claim 20, wherein the vector is a viral vector.

22. The vector of claim 21, wherein the viral vector is selected from adenoviruses, poxviruses, alphaviruses, arenaviruses, flaviruses, rhabdoviruses, retroviruses, lentiviruses, herpesviruses, paramyxoviruses, picornaviruses, and combinations thereof.

23. The vector of claim 21 or 22, wherein the vector is pseudotyped with an envelope protein of a virus selected from the native feline endogenous virus (RD114), a version of RD114 (RD114TR), gibbon ape leukemia virus (GALV), a version of GALV (GALV-TR), amphotropic murine leukemia virus (MLV 4070A), baculovirus (GP64), vesicular stomatitis virus (VSV-G), fowl plague virus (FPV), Ebola virus (EboV), or baboon retroviral envelope glycoprotein (BaEV), and lymphocytic choriomeningitis virus (LCMV).

24. The vector of any one of claims 12-23, wherein the vector is a lentiviral vector.

25. The vector of any one of claims 12-24, wherein the vector further comprises a nucleic acid encoding a chimeric antigen receptor (CAR).

26. An isolated T cell transduced with the nucleic acid of any one of claims 1-5.

27. An isolated T cell transduced to express the polypeptide of any one of claims 6-10.

28. An isolated T cell transduced with the vector of any one of claims 12-25.

29. The cell of any one of claims 26-28, wherein the cell is an αβ T cell, γδ T cell, and/or natural killer T cell.

30. The cell of claim 29, wherein the αβ T cell is a CD4+ T cell.

31. The cell of claim 29, wherein the αβ T cell is a CD8+ T cell.

32. The cell of claim 29, wherein the γδ T cell is a Vγ9Vδ2+ T cell.

33. A γδ T cell expressing the polypeptide of any one of claims 6-10.

34. A αβ T cell expressing the polypeptide of any one of claims 6-10.

35. A composition comprising the T cell of any one of claims 26-34.

36. The composition of claim 35, wherein the composition is a pharmaceutical composition.

37. The composition of claim 35 or 36, wherein the composition further comprises an adjuvant, excipient, carrier, diluent, buffer, stabilizer, or a combination thereof.

38. The composition of claim 35 or 36, wherein the composition further comprises an adjuvant.

39. The composition of claim 37 or 38, wherein the adjuvant is an anti-CD40 antibody, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, atezolizumab, interferon-alpha, interferon-beta, CpG oligonucleotides and derivatives, poly(I:C) and derivatives, RNA, sildenafil, particulate formulations with poly(lactide co-glycolide) (PLG), virosomes, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-7 (IL-7), interleukin-

12 (IL-12), interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-21 (IL-21), interleukin-23 (IL-23), and combinations thereof.

40. A method of preparing T cells for immunotherapy comprising

isolating T cells from a blood sample of a human subject,

activating the isolated T cells,

transducing the activated T cells with the nucleic acid of any one of claims 1-6 or the vector of any one of claims 12-25, and

expanding the transduced T cells.

41. The method of claim 40, wherein the blood sample comprises peripheral blood mononuclear cells (PMBC).

42. The method of claim 40 or 41, wherein the activating comprises contacting the T cells with an anti-CD3 and an anti-CD28 antibody.

43. The method of any one of claims 40-42, wherein the T cell is CD4+ T cell.

44. The method of any one of claims 40-42, wherein the T cell is CD8+ T cell.

45. The method of claim 40 or 41, wherein the T cell is γδ T cell or αβ T cell.

46. The method of any one of claims 40-45, wherein the activation and/or expanding steps are in the presence of a combination of IL-2 and IL-15 and optionally with zoledronate.

47. A method of treating a patient who has cancer, comprising administering to the patient the composition of any one of claims 35-39, wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.

48. A method of eliciting an immune response in a patient who has cancer, comprising administering to the patient the composition of any one of claims 35-39, wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.

49. The method of claim 47 or 48, wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide consists of the amino acid sequence of SLLQHLIGL (SEQ ID NO: 147).

50. The isolated nucleic acid of any one of claims 1-4, wherein the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 285 or 301.

51. The isolated polypeptide of any one of claims 7-9, wherein the amino acid comprises the amino acid sequence of SEQ ID NO: 286 or 302.

52. The vector of claim 14, wherein the IRES is selected from the group consisting of IRES from picornavirus, IRES from flavivirus, IRES from pestivirus, IRES from retrovirus, IRES from lentivirus, IRES from insect RNA virus, and IRES from cellular mRNA.

53. The method of claim 40, further comprising isolating CD4+CD8+ T cells from the transduced T cells and expanding the isolated CD4+CD8+ transduced T cells.

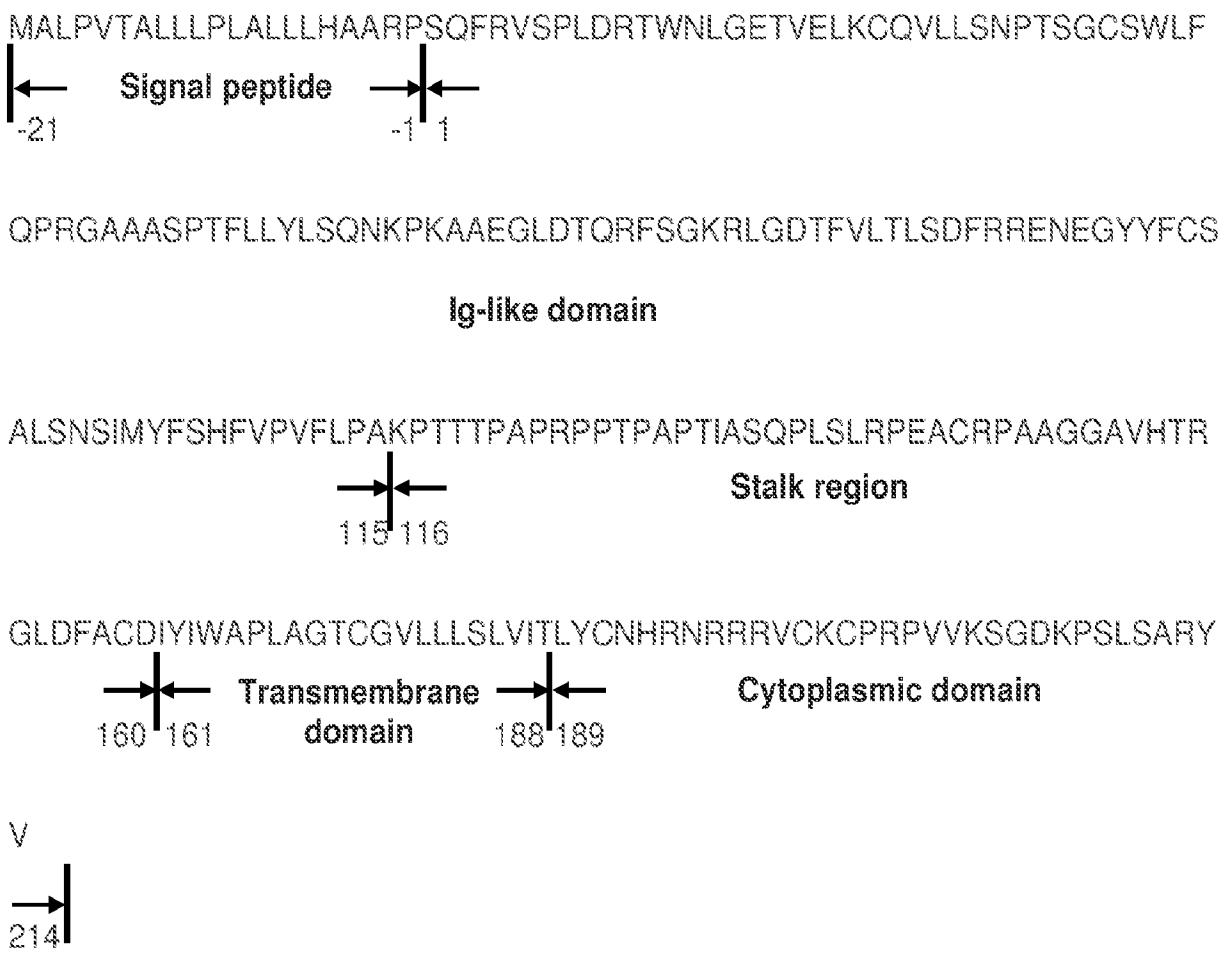

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLF
Signal peptide
-21
-1
1
QPRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCS
Ig-like domain
ALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
Stalk region
115
116
GLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARY
160
161
Transmembrane domain
188
189
Cytoplasmic domain
V
214

FIG. 1

```
CD8α1   1   MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP  60
            MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP
m1CD8α  1   MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP  60

CD8α1   61  RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSN  120
            RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSN
m1CD8α  61  RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALSN  120

CD8α1   121 SIMYFSHFVPVFLPAKPTT-TPAPRPPTPAPTIASQPLSL-RPEACRPAAGGAVHTRGLD  178
            SIMYFSHFVPVFLPA      P    PT   T+  +   L RPE           T+
m1CD8α  121 SIMYFSHFVPVFLPASVVDFLPTTAQPTKKSTLKKRVCRLPRPE----------TQKGP  169

CD8α1   179 FACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV  235
            IYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV
m1CD8α  170 LCSPIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV  226
```

FIG. 2

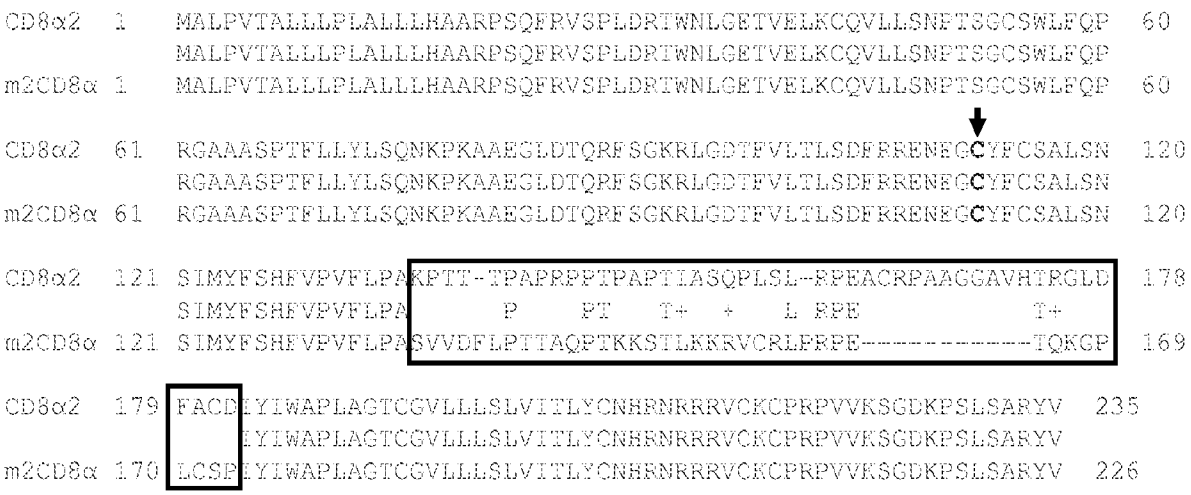

CD8α2 1 MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP 60
MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP
m2CD8α 1 MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQP 60
CD8α2 61 RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGCYFCSALSN 120
RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGCYFCSALSN
m2CD8α 61 RGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGCYFCSALSN 120
CD8α2 121 SIMYFSHFVPVFLPAKPTT-TPAPRPPTPAPTIASQPLSL-RPEACRPAAGGAVHTRGLD 178
SIMYFSHFVPVFLPA P PT T+ + L RPE T+
m2CD8α 121 SIMYFSHFVPVFLPASVVDFLPTTAQPTKKSTLKKRVCRLPRPE----------TQKGP 169
CD8α2 179 FACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV 235
IYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV
m2CD8α 170 LCSPIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSARYV 226

FIG. 3

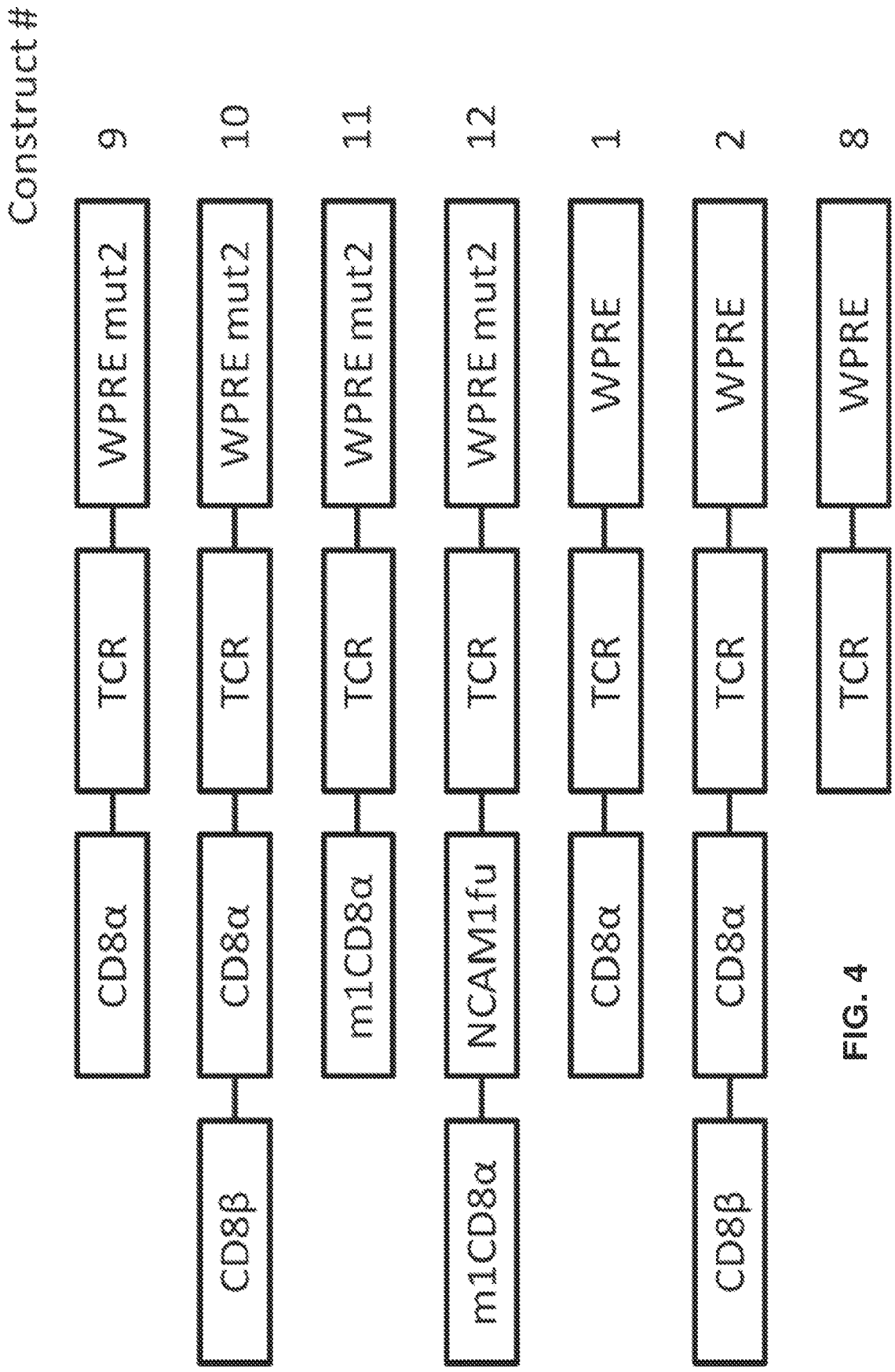

Construct #
9
CD8α
TCR
WPRE mut2
10
CD8β
CD8α
TCR
WPRE mut2
11
m1CD8α
TCR
WPRE mut2
12
m1CD8α
NCAM1fu
TCR
WPRE mut2
1
CD8α
TCR
WPRE
2
CD8β
CD8α
TCR
WPRE
8
TCR
WPRE

FIG. 4

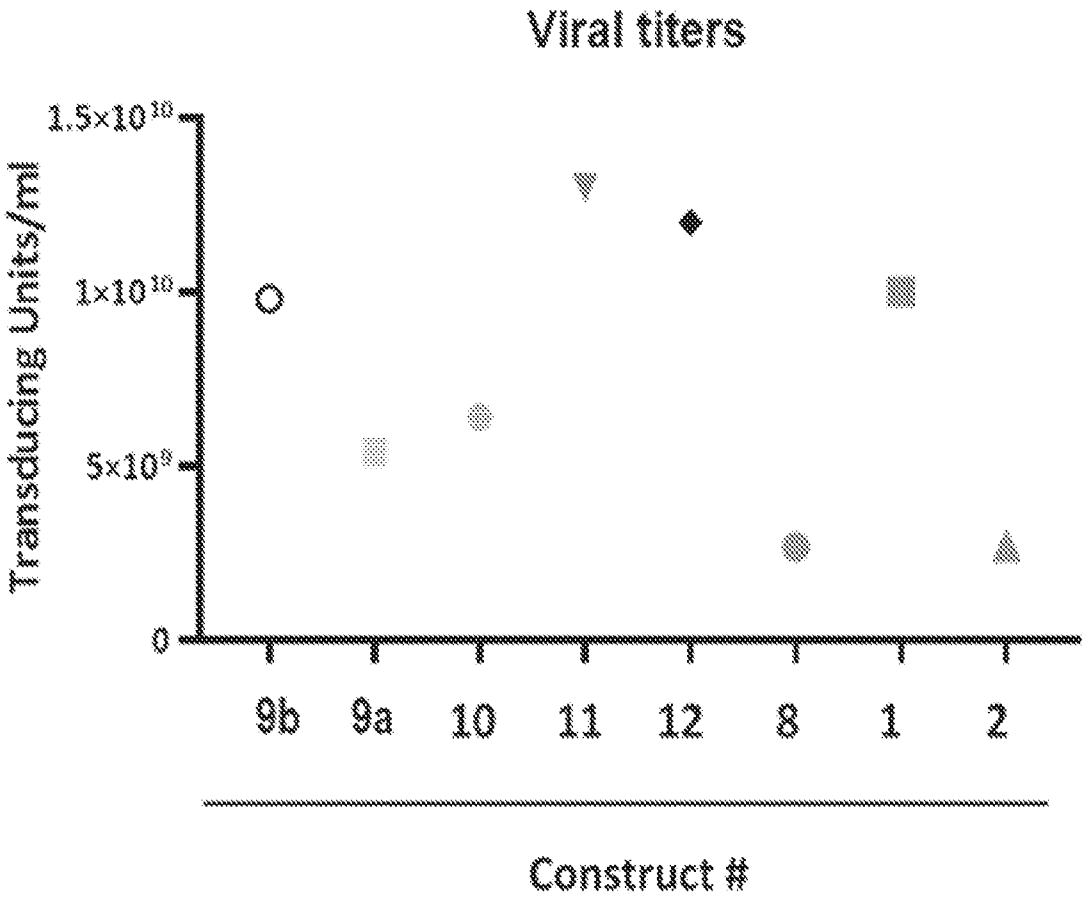

Viral titers
Transducing Units/ml
1.5×10^10
1×10^10
5×10^9
0
9b
9a
10
11
12
8
1
2
Construct #

FIG. 5A

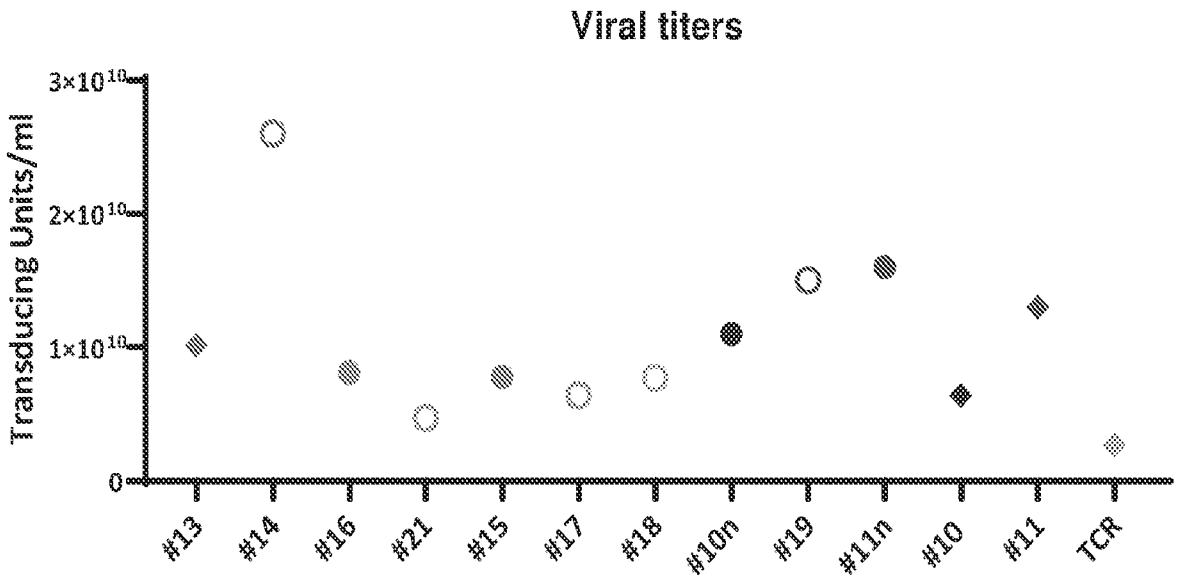

Viral titers
Transducing Units/ml
3×10^10
2×10^10
1×10^10
0
#13
#14
#16
#21
#15
#17
#18
#10n
#19
#11n
#10
#11
TCR

FIG. 5B

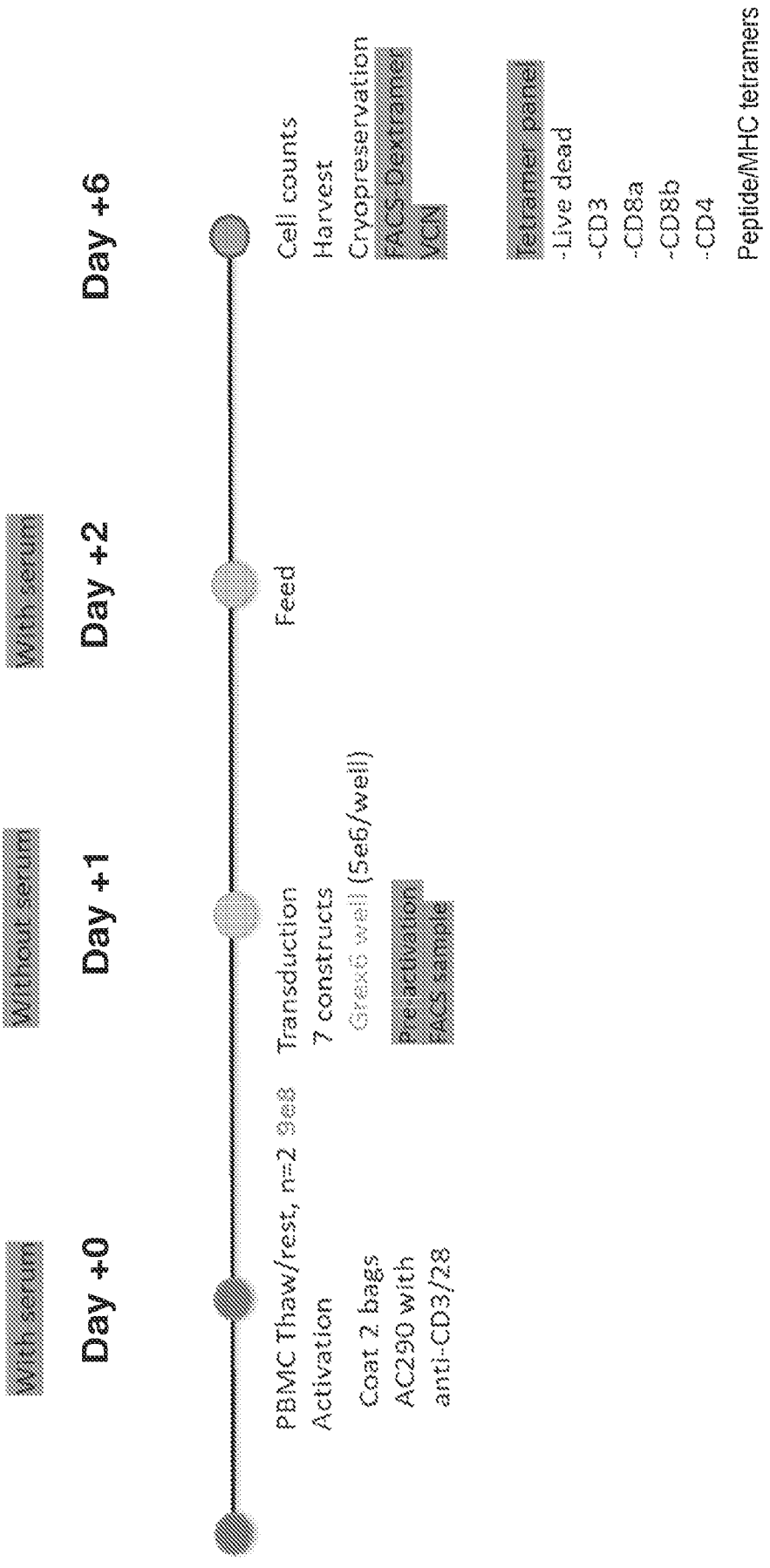

Day +0
Day +1
Day +2
Day +6
With serum
Without serum
With serum
PBMC Thaw/rest, n=2 9e8
Activation
Coat 2 bags
AC290 with
anti-CD3/28
Transduction
7 constructs
Grex6 well (5e6/well)
Pre activation
FACS sample
Feed
Cell counts
Harvest
Cryopreservation
FACS Dextramer
VCN
Dextramer panel
-Live dead
-CD3
-CD8a
-CD8b
-CD4
Peptide/MHC tetramers

FIG. 6

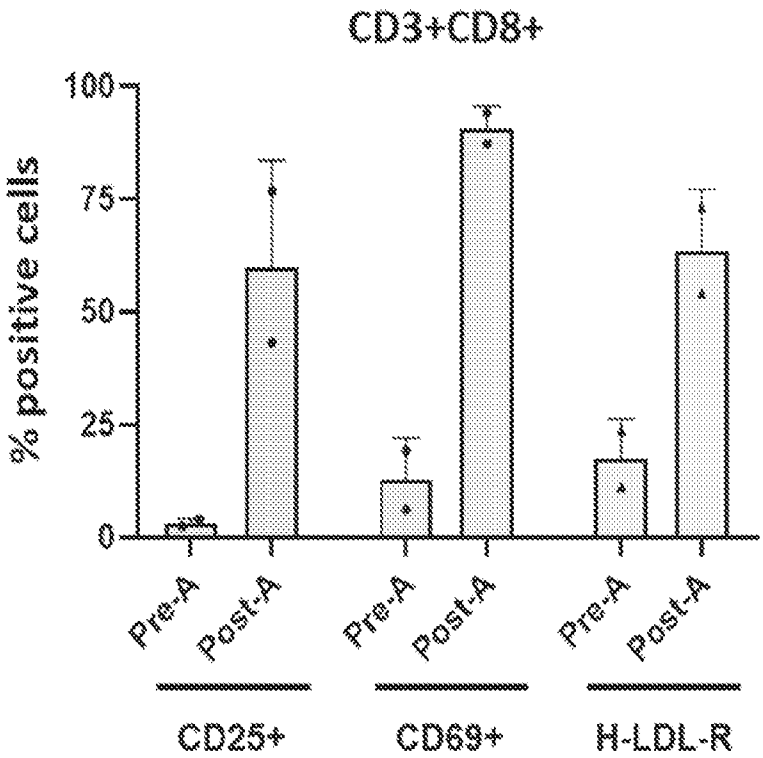

CD3+CD8+
% positive cells
100
75
50
25
0
Pre-A
Post-A
Pre-A
Post-A
Pre-A
Post-A
CD25+
CD69+
H-LDL-R

FIG. 7A

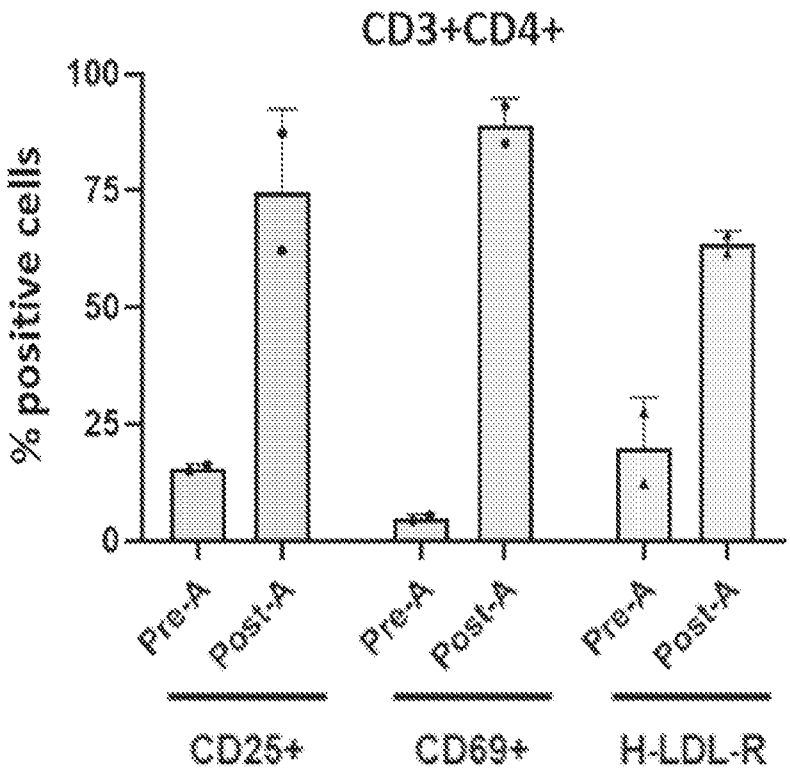

CD3+CD4+
% positive cells
100
75
50
25
0
Pre-A
Post-A
Pre-A
Post-A
Pre-A
Post-A
CD25+
CD69+
H-LDL-R

FIG. 7B

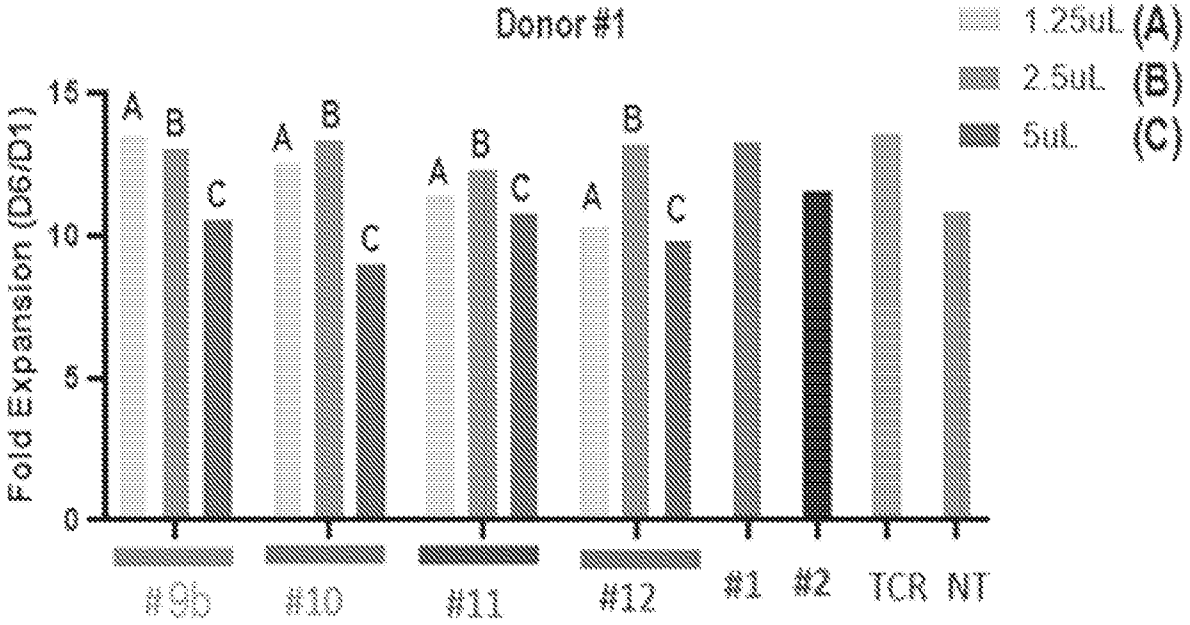

Donor #1
1.25uL (A)
2.5uL (B)
5uL (C)
Fold Expansion (D6/D1)
0
5
10
15
A
B
C
#9b
#10
#11
#12
#1
#2
TCR
NT

FIG. 8A

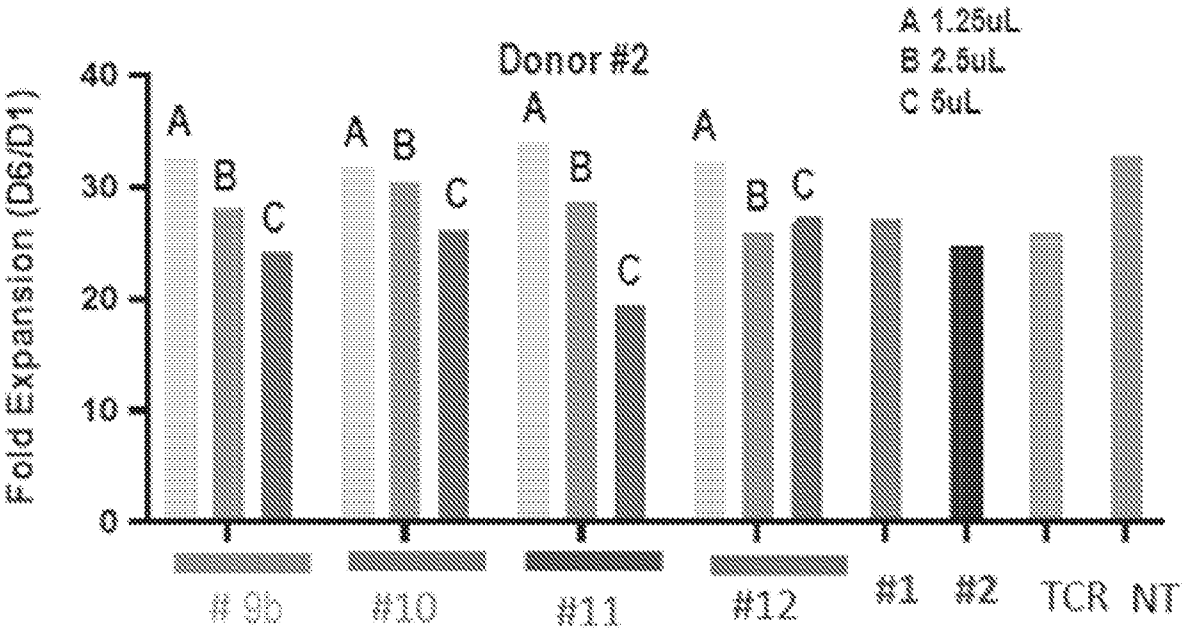

Donor #2
A 1.25uL
B 2.5uL
C 5uL
Fold Expansion (D6/D1)
0
10
20
30
40
A
B
C
#9b
#10
#11
#12
#1
#2
TCR
NT

FIG. 8B

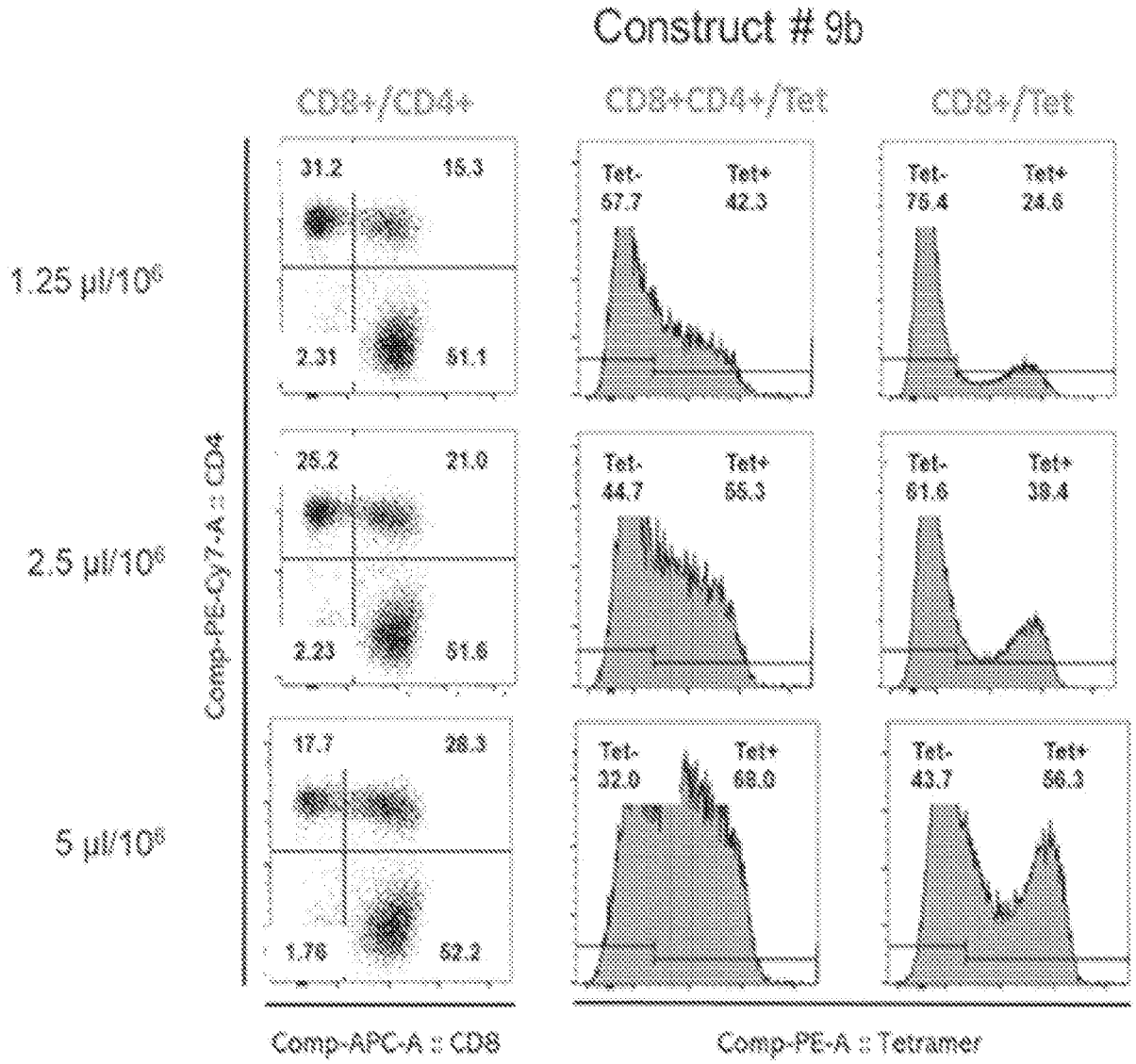

Construct # 9b
CD8+/CD4+
CD8+CD4+/Tet
CD8+/Tet
1.25 µl/10^6
2.5 µl/10^6
5 µl/10^6
31.2
16.3
2.31
51.1
Tet-
57.7
Tet+
42.3
Tet-
75.4
Tet+
24.6
28.2
21.0
2.23
51.6
Tet-
44.7
Tet+
55.3
Tet-
61.6
Tet+
38.4
17.7
28.3
1.76
52.3
Tet-
32.0
Tet+
68.0
Tet-
43.7
Tet+
56.3
Comp-PE-Cy7-A :: CD4
Comp-APC-A :: CD8
Comp-PE-A :: Tetramer

FIG. 9A

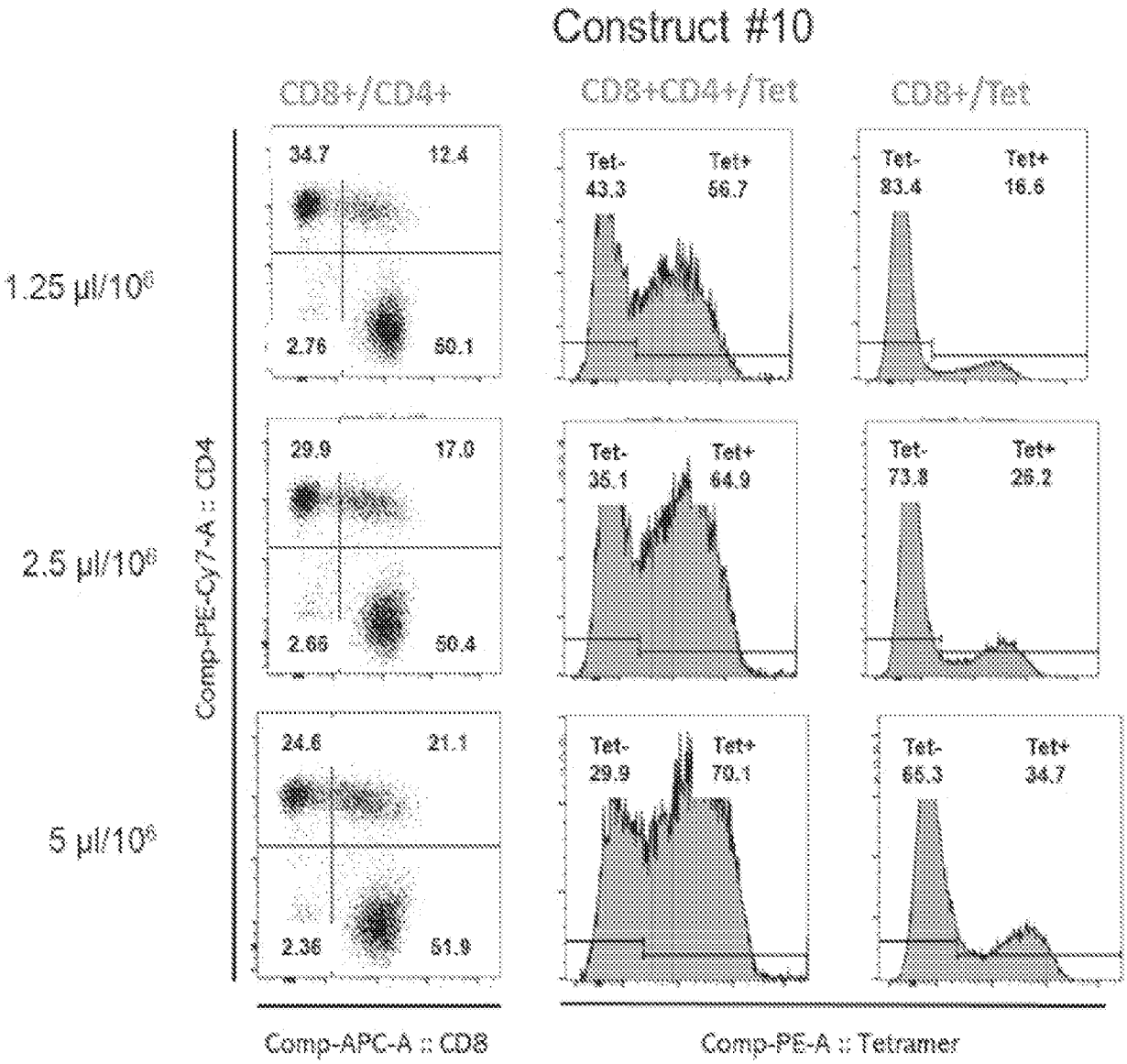

Construct #10
CD8+/CD4+
CD8+CD4+/Tet
CD8+/Tet
1.25 µl/10^6
2.5 µl/10^6
5 µl/10^6
34.7
12.4
2.76
50.1
Tet-
43.3
Tet+
56.7
Tet-
83.4
Tet+
16.6
29.9
17.0
2.66
50.4
Tet-
35.1
Tet+
64.9
Tet-
73.8
Tet+
26.2
24.6
21.1
2.36
51.9
Tet-
29.9
Tet+
70.1
Tet-
65.3
Tet+
34.7
Comp-PE-Cy7-A :: CD4
Comp-APC-A :: CD8
Comp-PE-A :: Tetramer

FIG. 9B

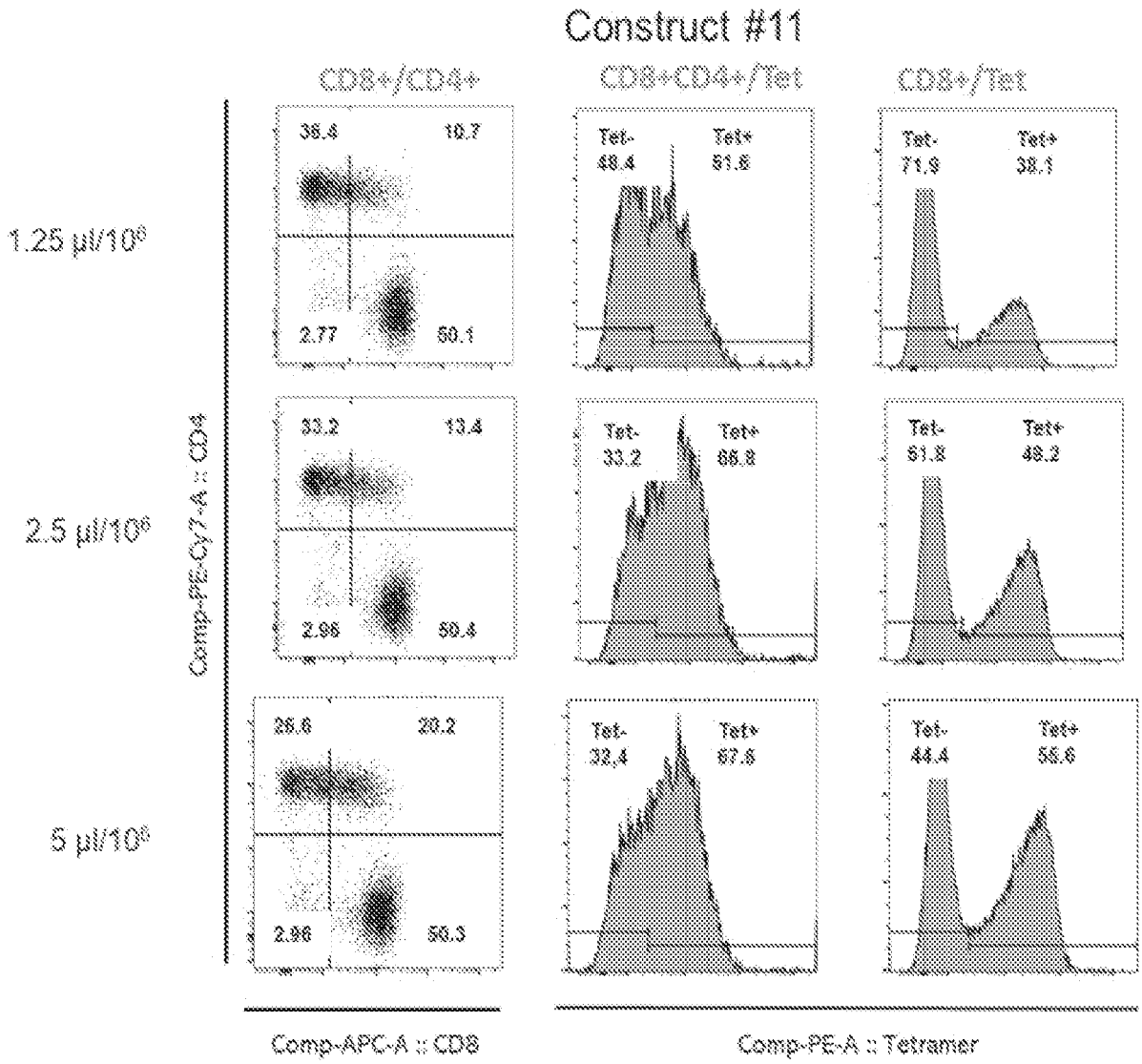

Construct #11
CD8+/CD4+
CD8+CD4+/Tet
CD8+/Tet
1.25 µl/10^6
2.5 µl/10^6
5 µl/10^6
38.4
10.7
2.77
50.1
Tet-
48.4
Tet+
51.6
Tet-
71.9
Tet+
28.1
33.2
13.4
2.96
50.4
Tet-
33.2
Tet+
66.8
Tet-
51.8
Tet+
48.2
25.6
20.2
2.96
50.3
Tet-
32.4
Tet+
67.6
Tet-
44.4
Tet+
55.6
Comp-PE-Cy7-A :: CD4
Comp-APC-A :: CD8
Comp-PE-A :: Tetramer

FIG. 9C

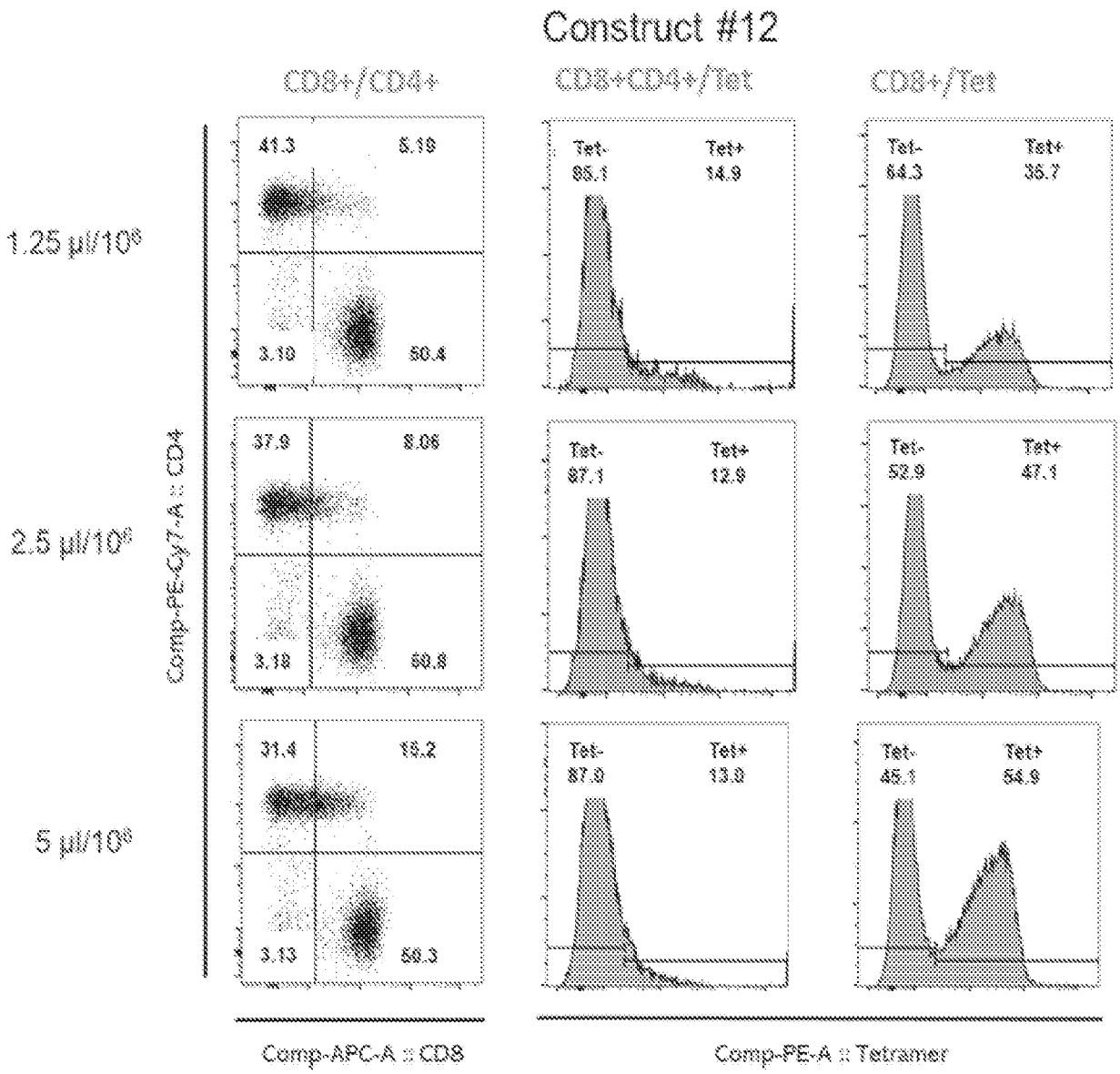

Construct #12
CD8+/CD4+
CD8+CD4+/Tet
CD8+/Tet
1.25 µl/10^6
2.5 µl/10^6
5 µl/10^6
41.3
5.19
3.10
50.4
Tet-
85.1
Tet+
14.9
Tet-
64.3
Tet+
35.7
37.9
8.06
3.18
50.8
Tet-
87.1
Tet+
12.9
Tet-
52.9
Tet+
47.1
31.4
15.2
3.13
50.3
Tet-
87.0
Tet+
13.0
Tet-
45.1
Tet+
54.9
Comp-PE-Cy7-A :: CD4
Comp-APC-A :: CD8
Comp-PE-A :: Tetramer

FIG. 9D

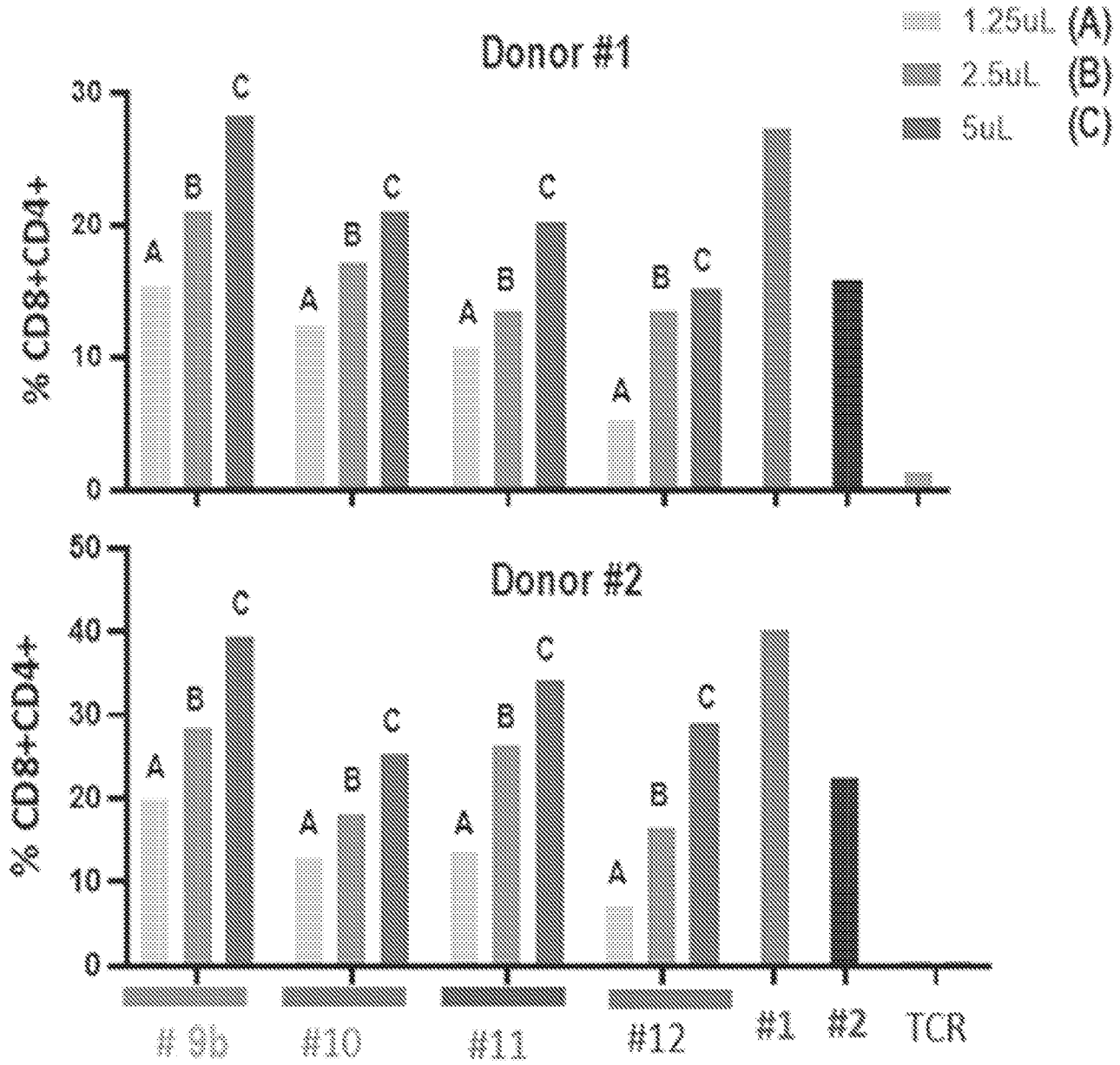

1.25uL (A)
2.5uL (B)
5uL (C)
Donor #1
% CD8+CD4+
30
20
10
0
A
B
C
Donor #2
% CD8+CD4+
50
40
30
20
10
0
9b
#10
#11
#12
#1
#2
TCR

FIG. 10

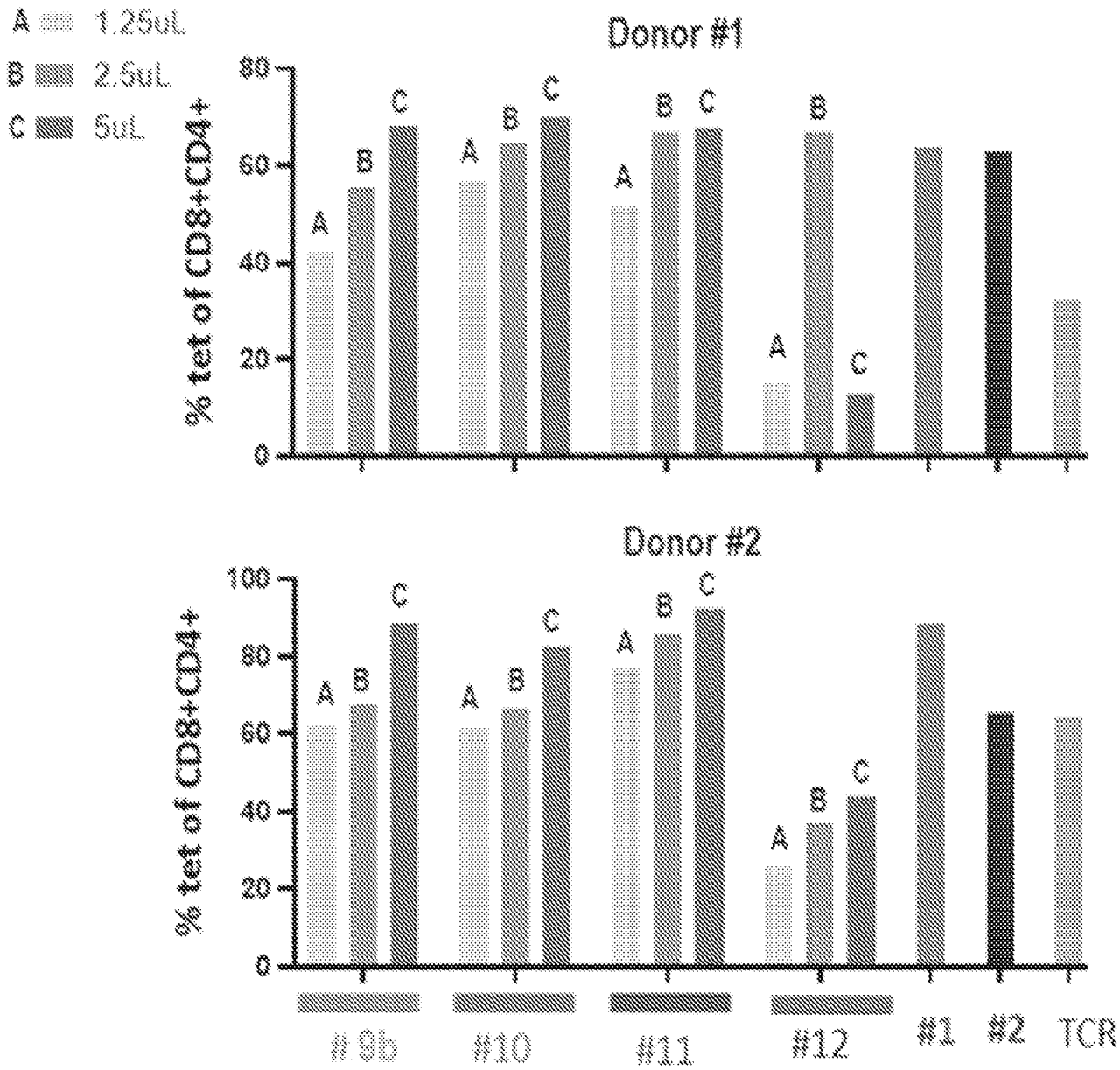

A 1.25uL
B 2.5uL
C 5uL
Donor #1
% tet of CD8+CD4+
0
20
40
60
80
Donor #2
% tet of CD8+CD4+
0
20
40
60
80
100
#9b
#10
#11
#12
#1
#2
TCR

FIG. 11

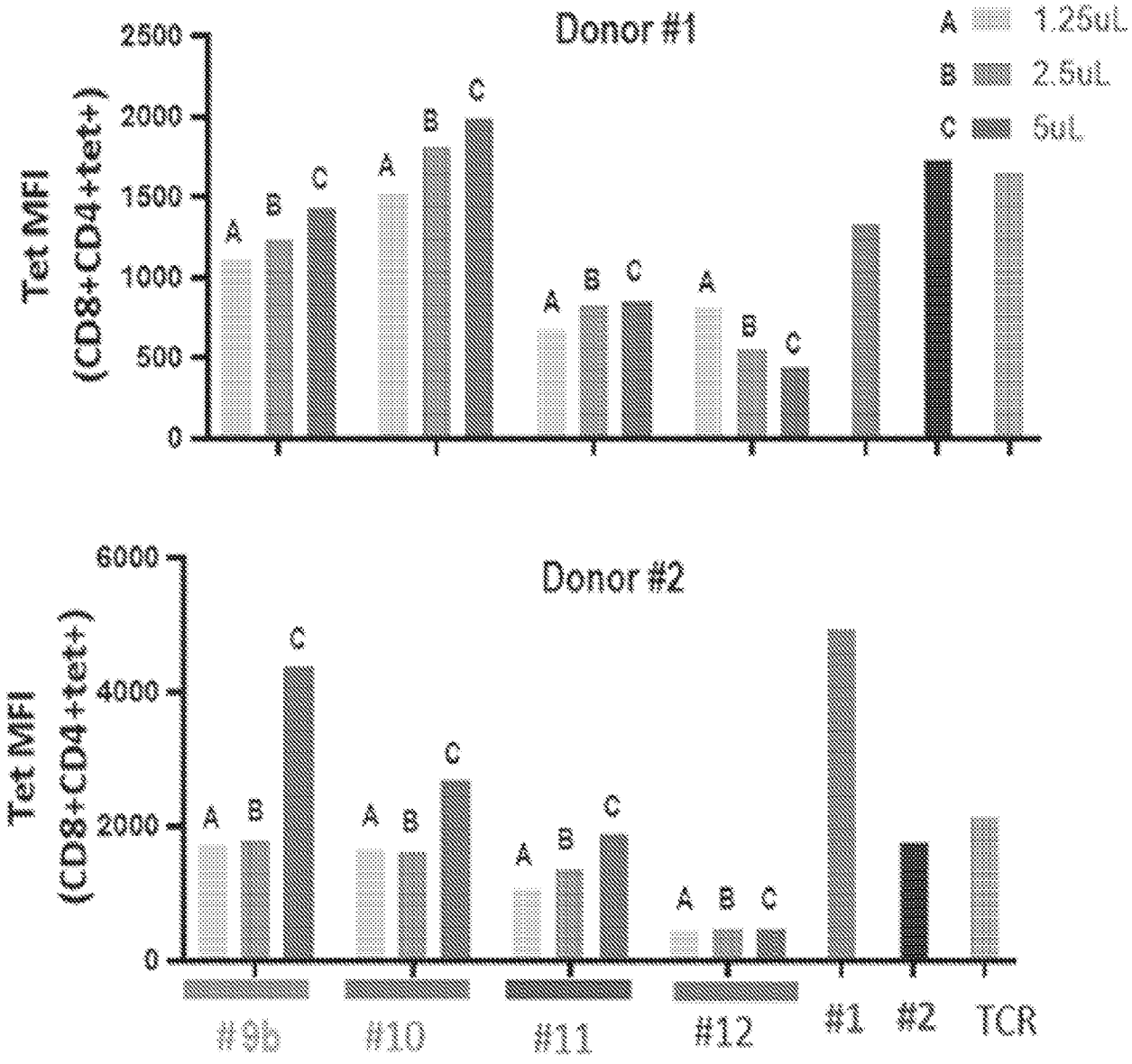

Donor #1
A 1.25uL
B 2.5uL
C 5uL
Tet MFI (CD8+CD4+tet+)
2500
2000
1500
1000
500
0
Donor #2
6000
4000
2000
0
#9b
#10
#11
#12
#1
#2
TCR

FIG. 12

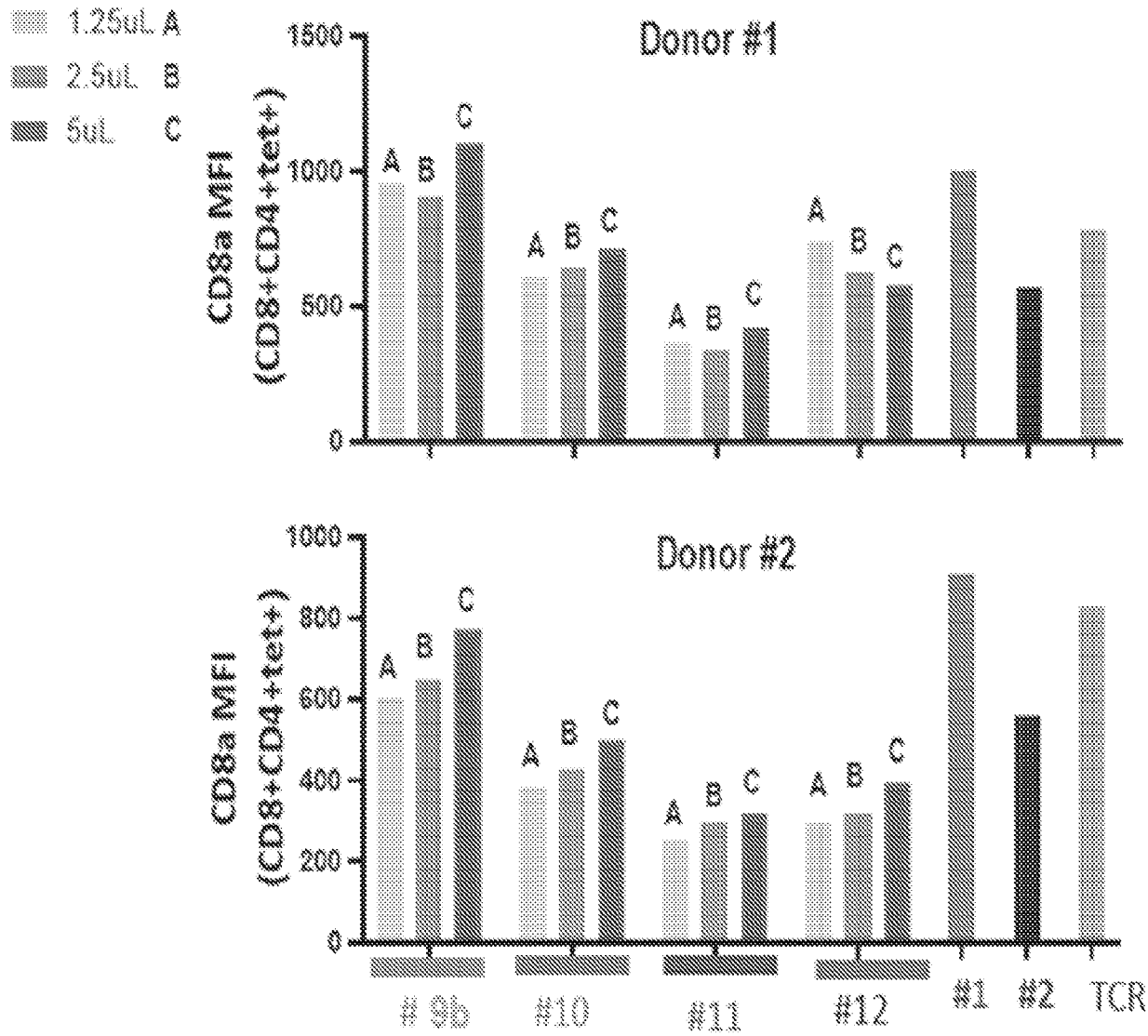

1.25uL A
2.5uL B
5uL C
Donor #1
CD8a MFI
(CD8+CD4+tet+)
1500
1000
500
0
A
B
C
Donor #2
1000
800
600
400
200
0
9b
#10
#11
#12
#1
#2
TCR

FIG. 13

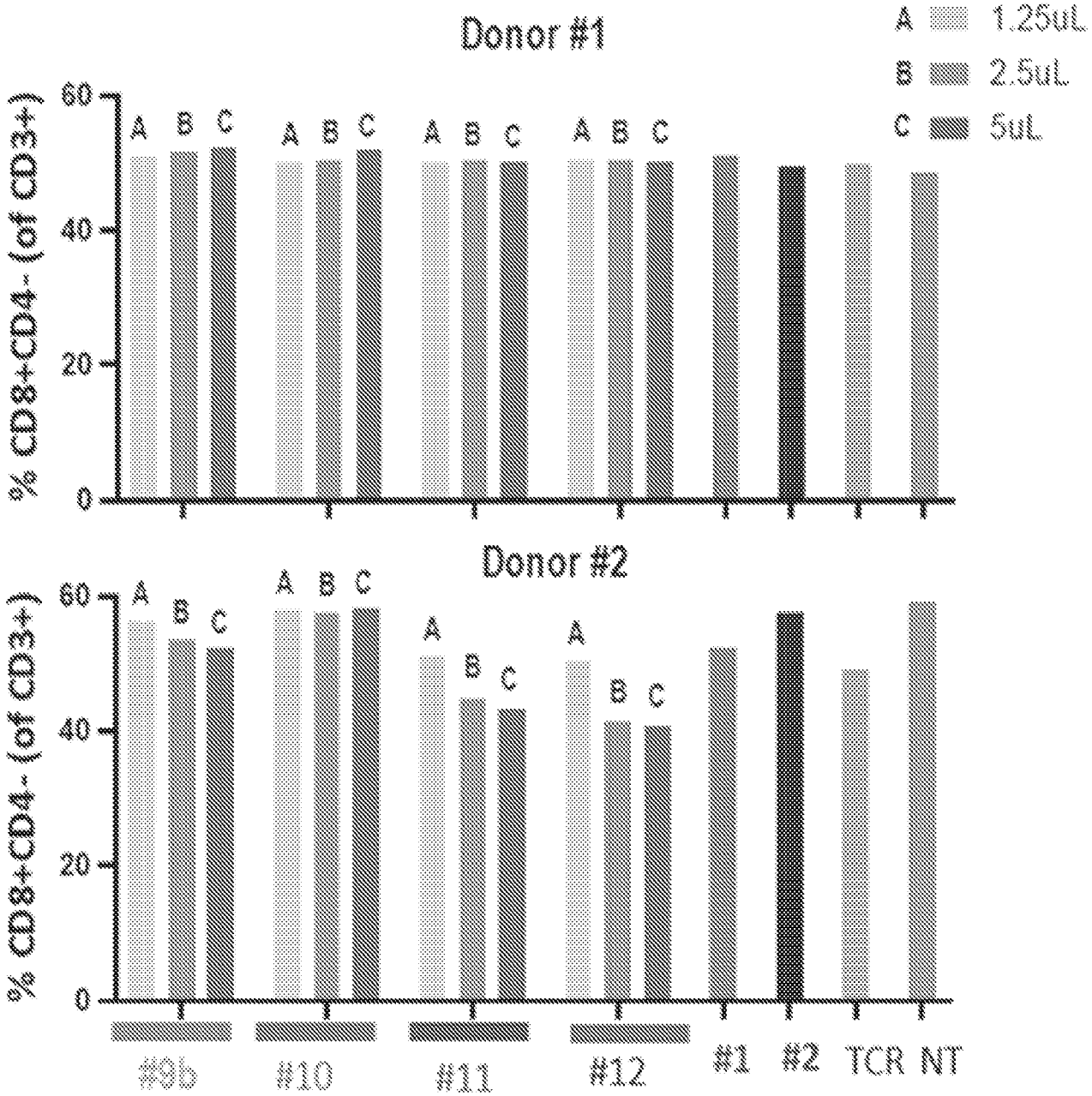

Donor #1
A 1.25uL
B 2.5uL
C 5uL
% CD8+CD4- (of CD3+)
60
40
20
0
A B C A B C A B C A B C
Donor #2
% CD8+CD4- (of CD3+)
60
40
20
0
A B C A B C A B C A B C
#9b
#10
#11
#12
#1
#2
TCR
NT

FIG. 14

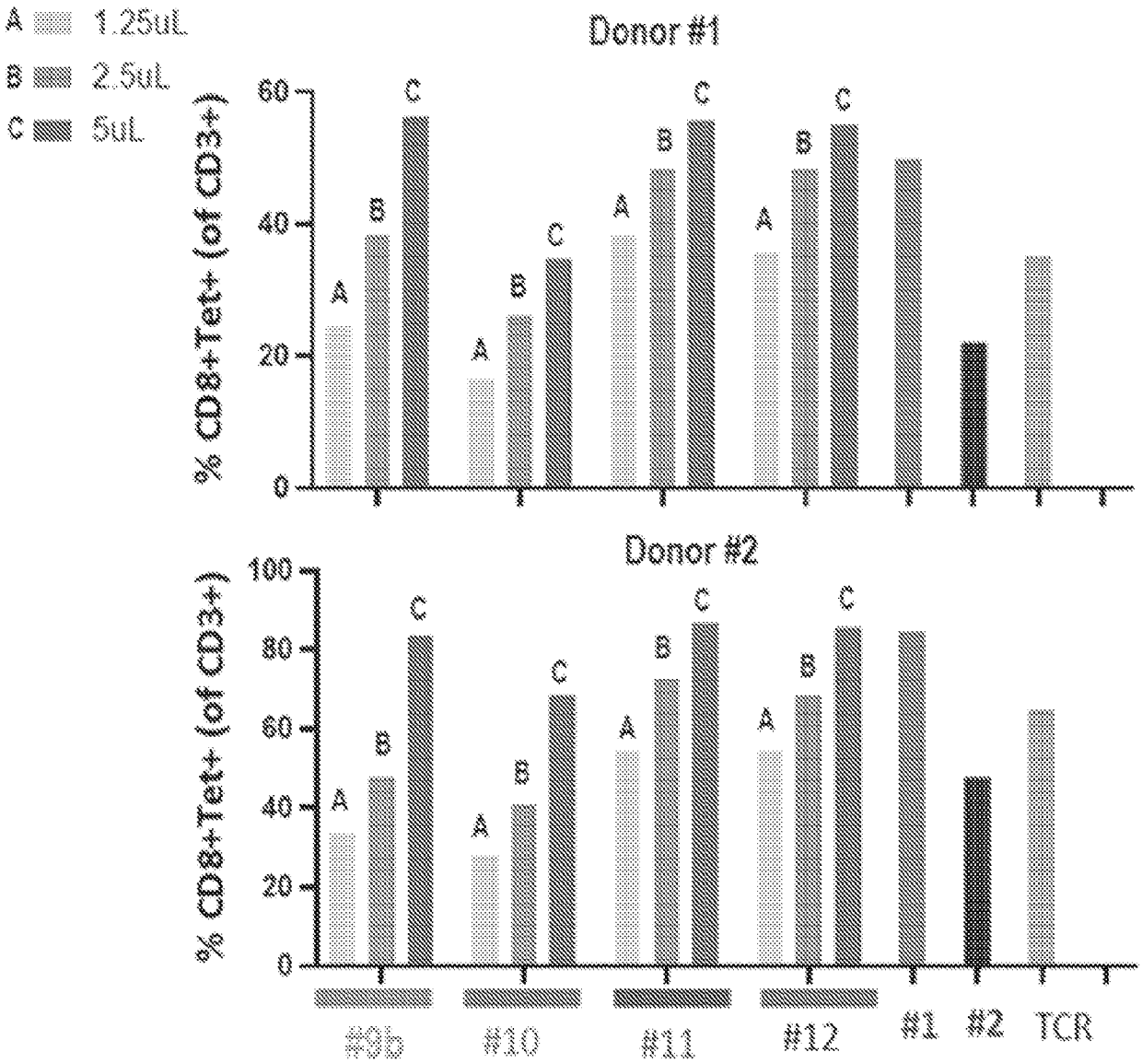

A 1.25uL
B 2.5uL
C 5uL
Donor #1
% CD8+Tet+ (of CD3+)
0
20
40
60
A
B
C
Donor #2
% CD8+Tet+ (of CD3+)
0
20
40
60
80
100
#9b
#10
#11
#12
#1
#2
TCR

FIG. 15

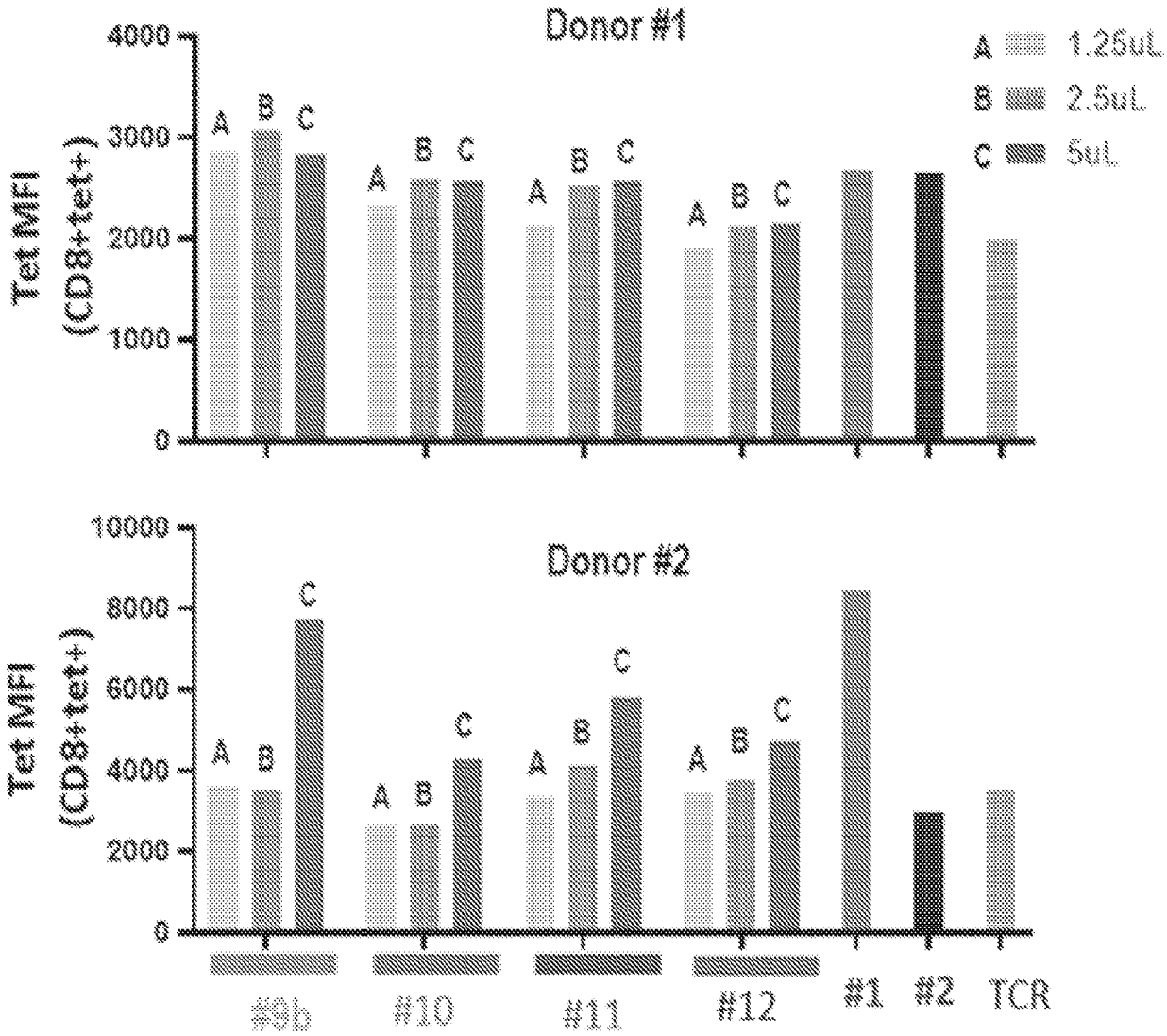

Donor #1
Tet MFI (CD8+tet+)
4000
3000
2000
1000
0
A 1.25uL
B 2.5uL
C 5uL
Donor #2
10000
8000
6000
4000
2000
0
#9b
#10
#11
#12
#1
#2
TCR

FIG. 16

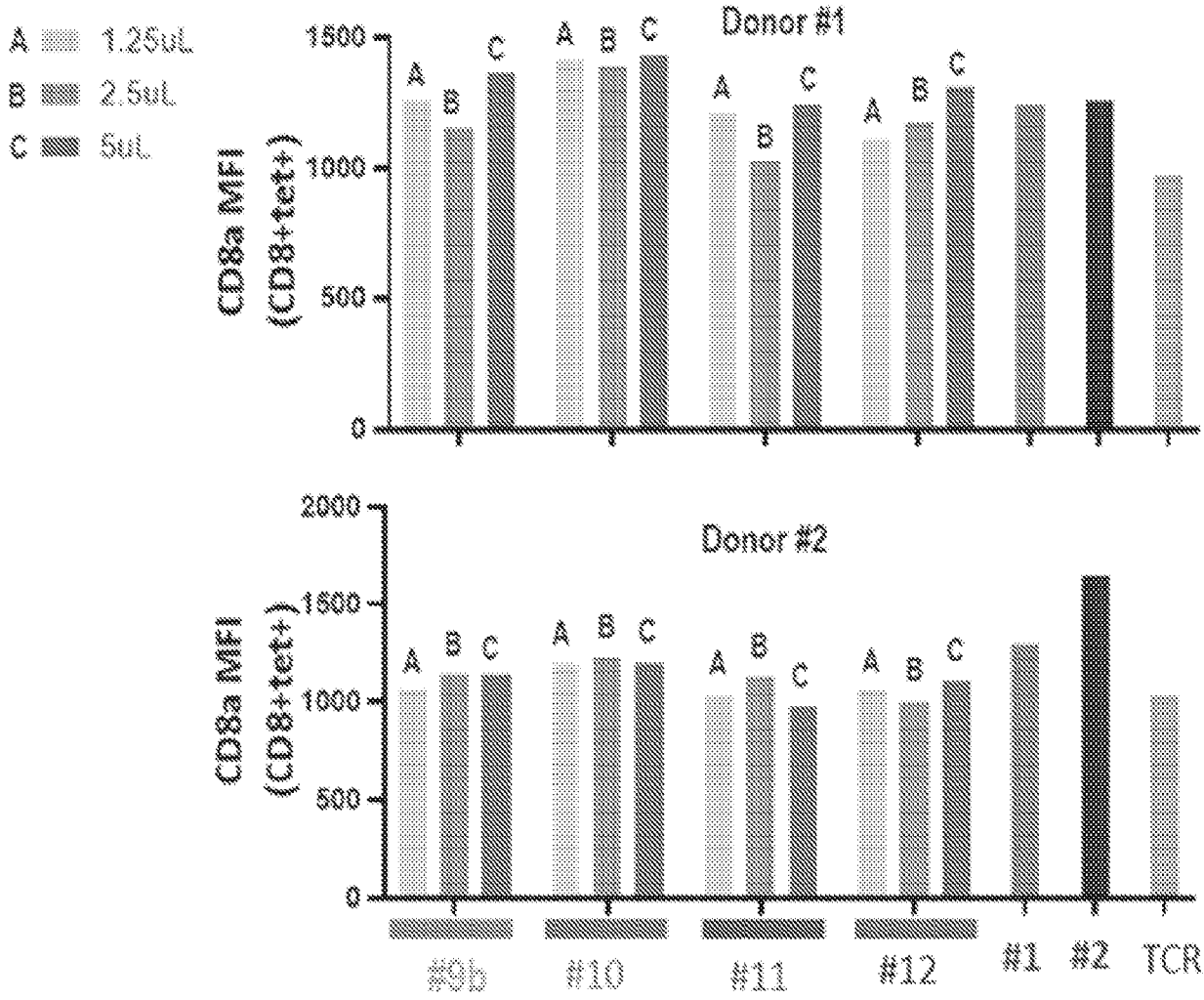

A 1.25uL
B 2.5uL
C 5uL
Donor #1
CD8a MFI (CD8+tet+)
1500
1000
500
0
Donor #2
2000
1500
1000
500
0
#9b
#10
#11
#12
#1
#2
TCR

FIG. 17

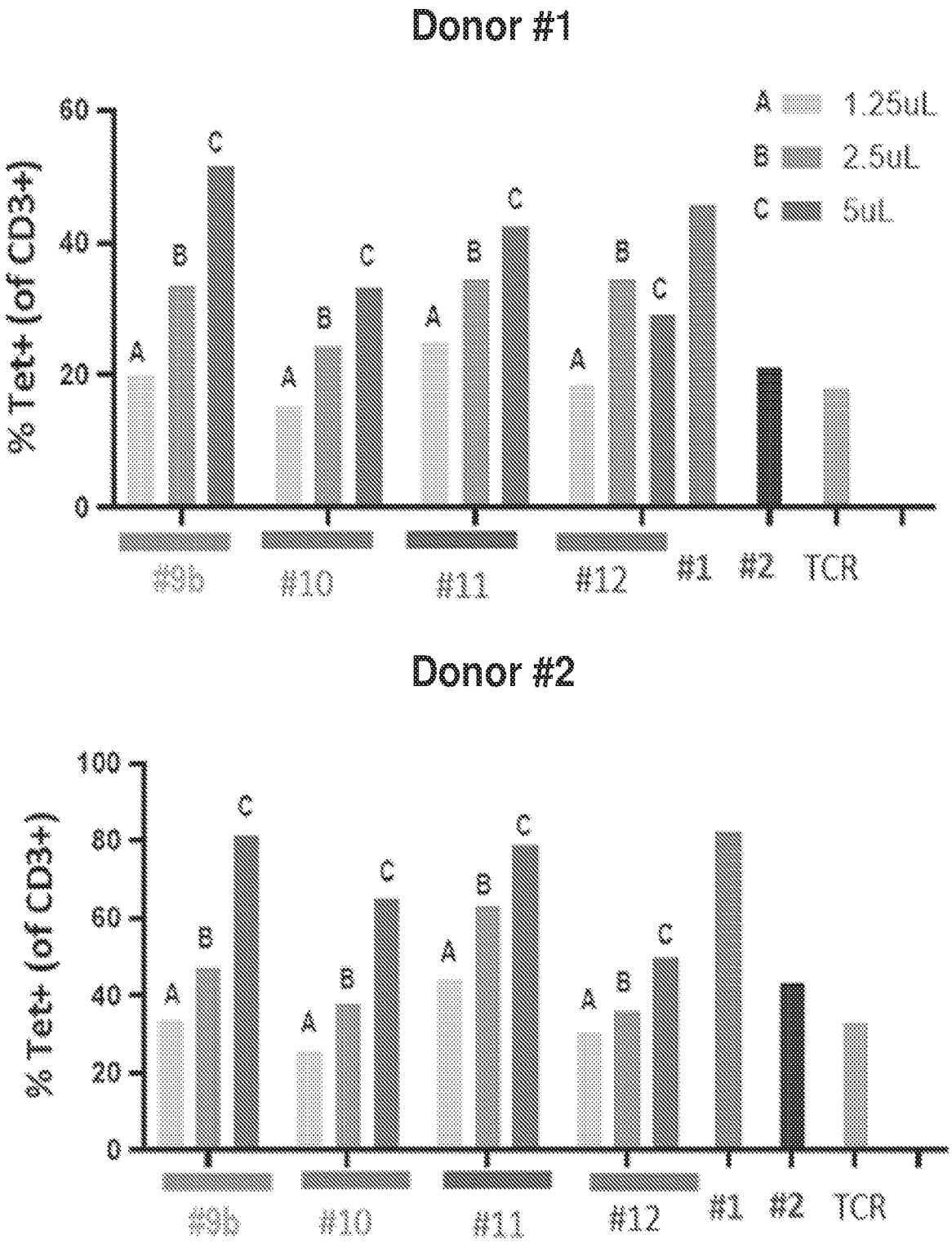

Donor #1
% Tet+ (of CD3+)
60
40
20
0
A 1.25uL
B 2.5uL
C 5uL
A
B
C
#9b
#10
#11
#12
#1
#2
TCR
Donor #2
% Tet+ (of CD3+)
100
80
60
40
20
0
#9b
#10
#11
#12
#1
#2
TCR

FIG. 18

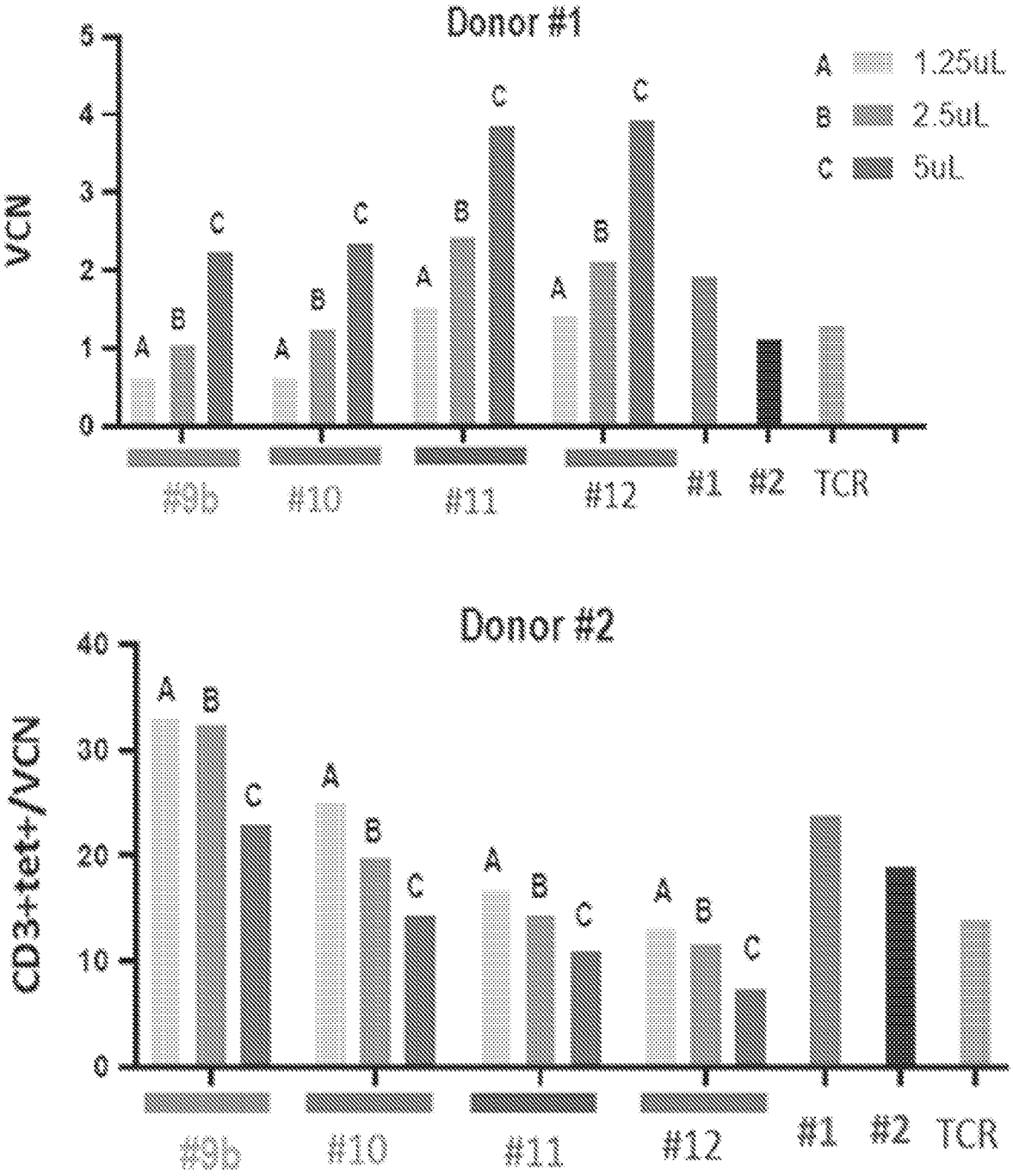

Donor #1
VCN
A 1.25uL
B 2.5uL
C 5uL
#9b
#10
#11
#12
#1
#2
TCR
Donor #2
CD3+tet+/VCN
#9b
#10
#11
#12
#1
#2
TCR

FIG. 19

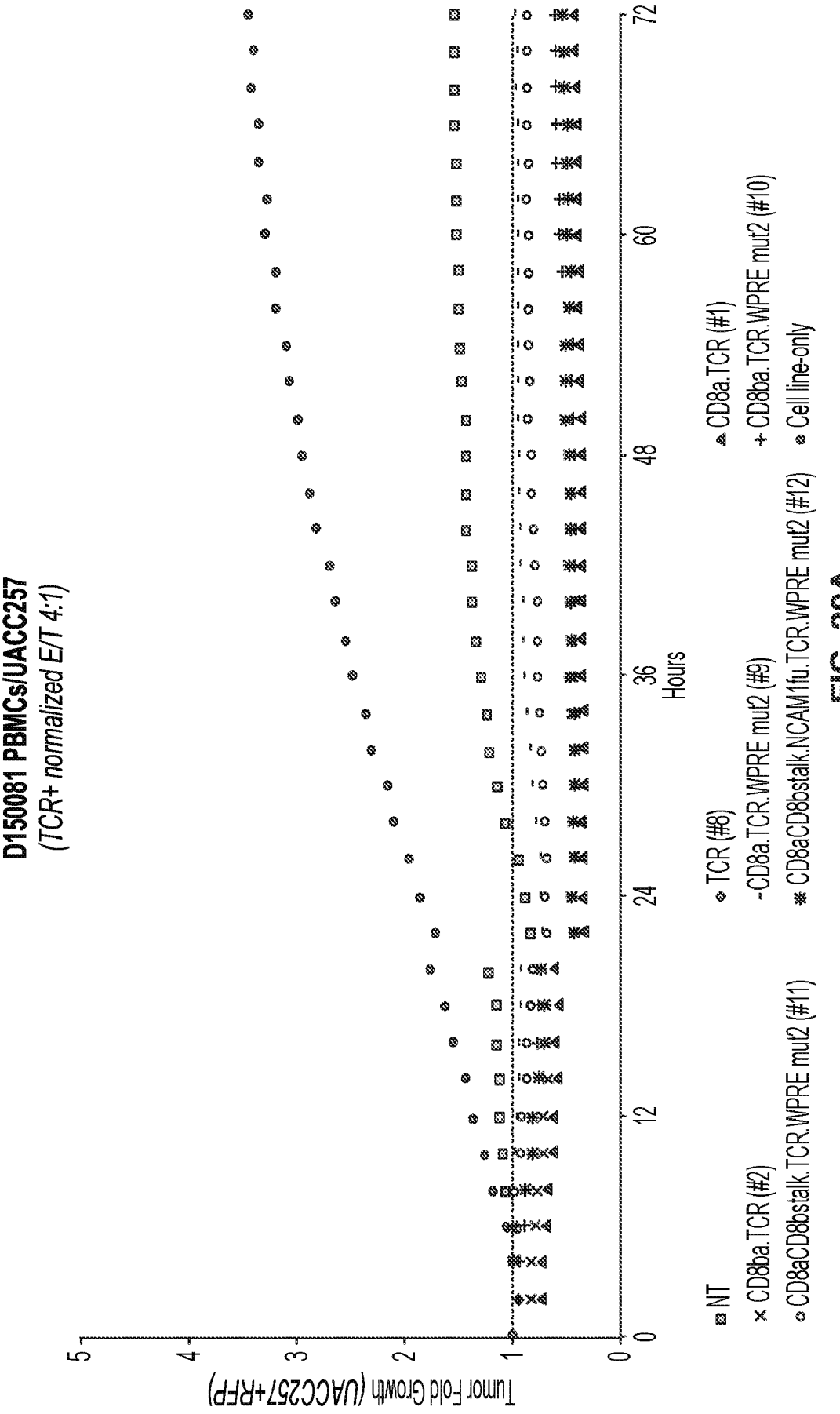

D150081 PBMCs/UACC257
(TCR+ normalized E/T 4:1)
Tumor Fold Growth (UACC257+RFP)
0
1
2
3
4
5
0
12
24
36
48
60
72
Hours
NT
CD8ba.TCR (#2)
CD8aCD8bstalk.TCR.WPRE mut2 (#11)
TCR (#8)
-CD8a.TCR.WPRE mut2 (#9)
CD8aCD8bstalk.NCAM1fu.TCR.WPRE mut2 (#12)
CD8a.TCR (#1)
CD8ba.TCR.WPRE mut2 (#10)
Cell line-only

FIG. 20A

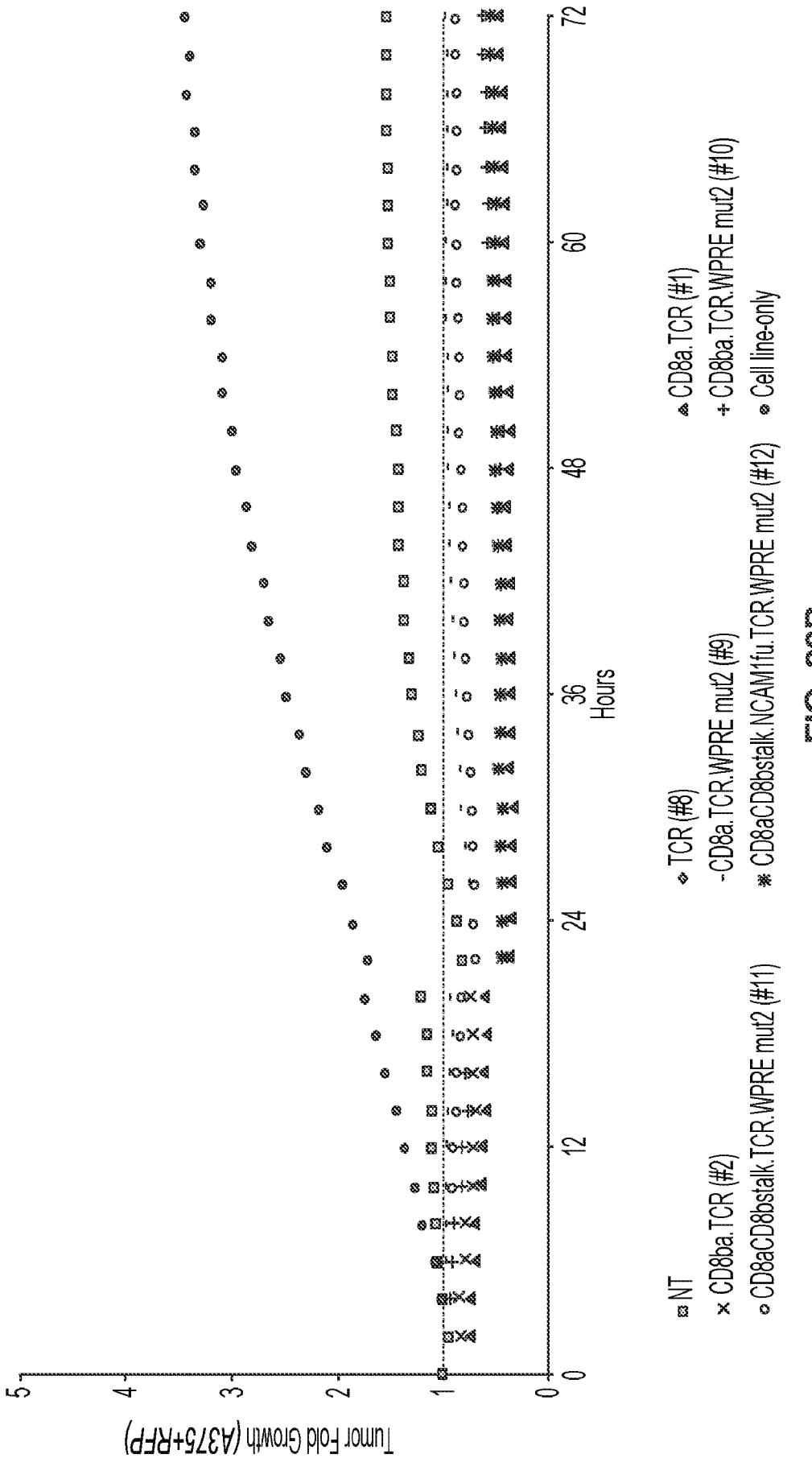

D150081 PBMCs/A375
(TCR+ normalized E/T 4:1)
Tumor Fold Growth (A375+RFP)
0
1
2
3
4
5
0
12
24
36
48
60
72
Hours
NT
CD8ba.TCR (#2)
CD8aCD8bstalk.TCR.WPRE mut2 (#11)
TCR (#8)
-CD8a.TCR.WPRE mut2 (#9)
CD8aCD8bstalk.NCAM1fu.TCR.WPRE mut2 (#12)
CD8a.TCR (#1)
CD8ba.TCR.WPRE mut2 (#10)
Cell line-only

FIG. 20B

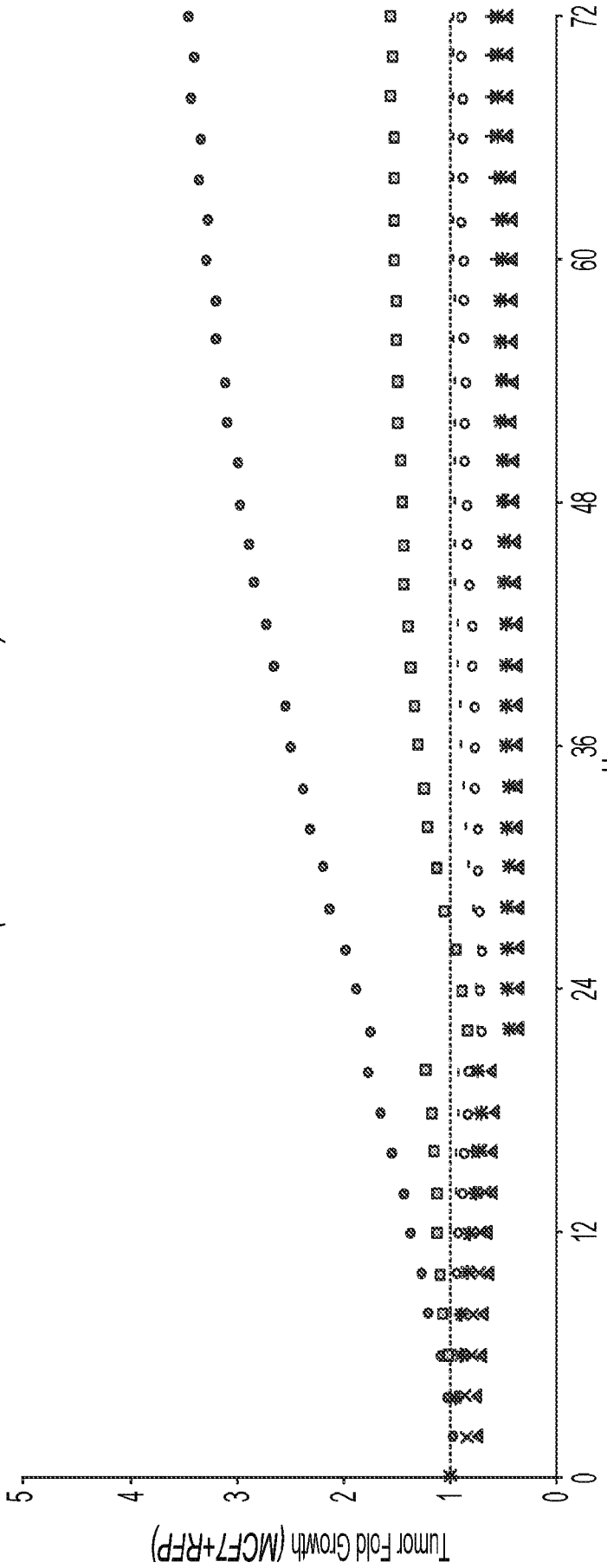

D150081 PBMCs/MCF7
(TCR+ normalized E/T 4:1)
Tumor Fold Growth (MCF7+RFP)
Hours
0
12
24
36
48
60
72
0
1
2
3
4
5
NT
CD8ba.TCR (#2)
CD8aCD8bstalk.TCR.WPRE mut2 (#11)
TCR (#8)
-CD8a.TCR.WPRE mut2 (#9)
CD8aCD8bstalk.NCAM1fu.TCR.WPRE mut2 (#12)
CD8a.TCR (#1)
+ CD8ba.TCR.WPRE mut2 (#10)
Cell line-only

FIG. 20C

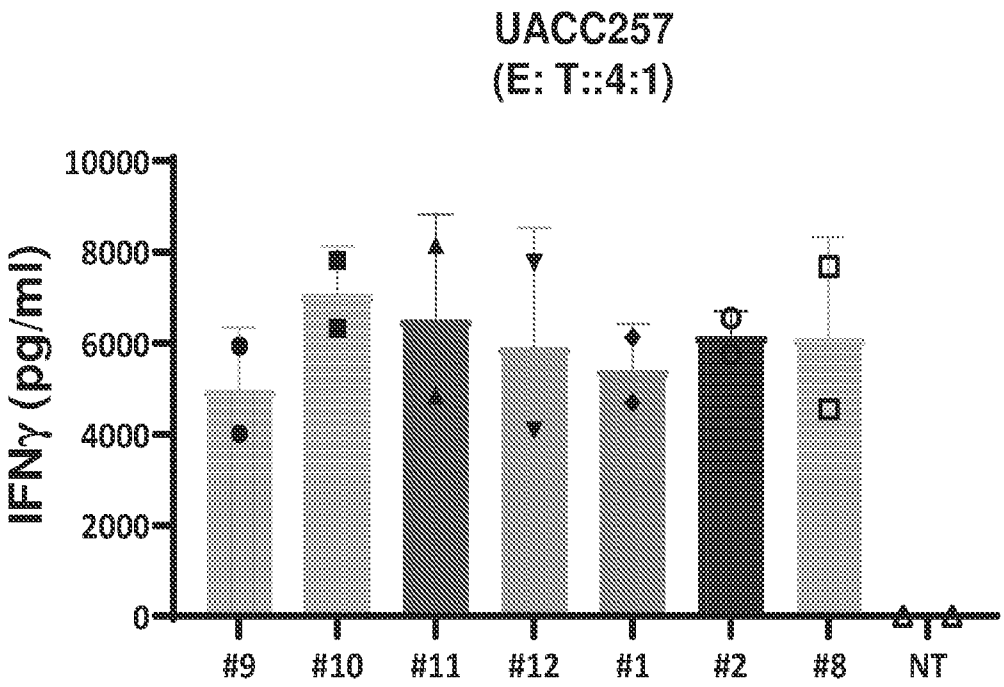


FIG. 21A

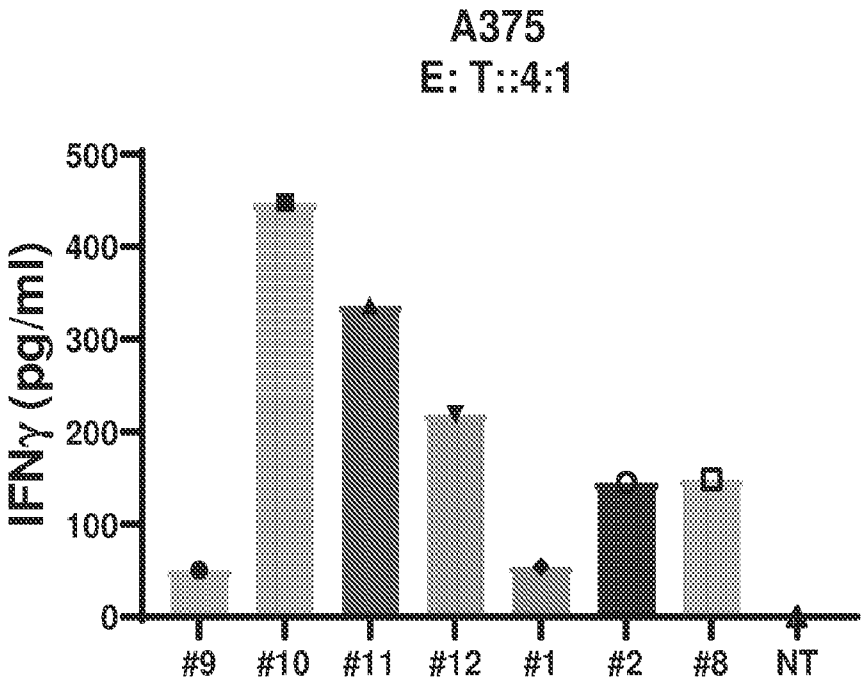
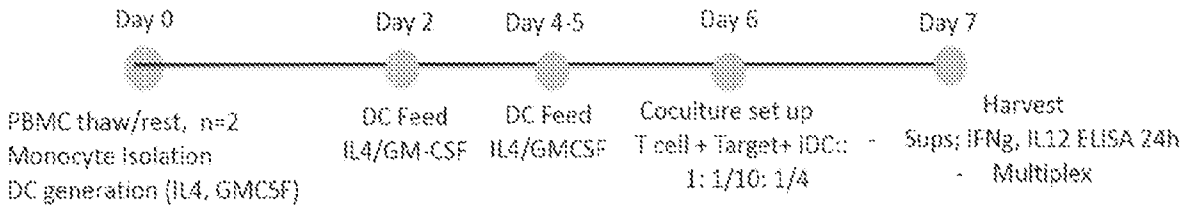


FIG. 21B


Day 0
Day 2
Day 4-5
Day 6
Day 7
PBMC thaw/rest, n=2
Monocyte isolation
DC generation (IL4, GMCSF)
DC Feed
IL4/GM-CSF
DC Feed
IL4/GMCSF
Coculture set up
T cell + Target+ iDC::
1: 1/10: 1/4
Harvest
Sups; IFNg, IL12 ELISA 24h
Multiplex

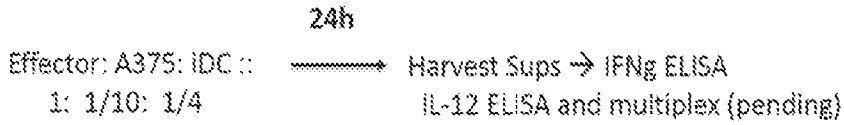

24h
Effector: A375: iDC ::
1: 1/10: 1/4
Harvest Sups → IFNg ELISA
IL-12 ELISA and multiplex (pending)

FIG. 22

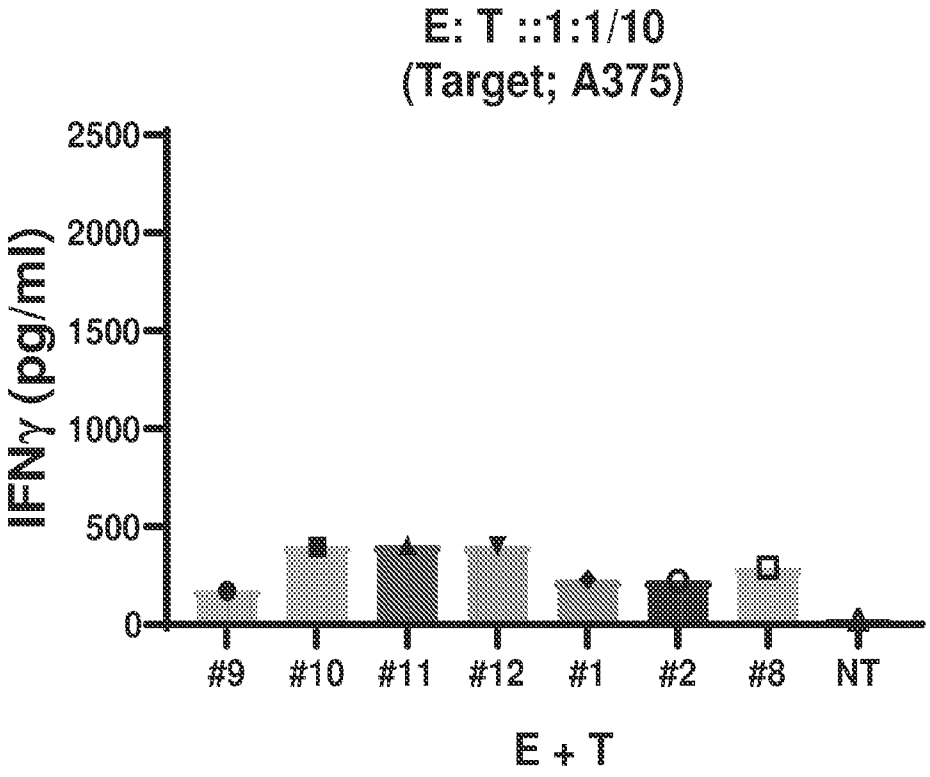


FIG. 23A

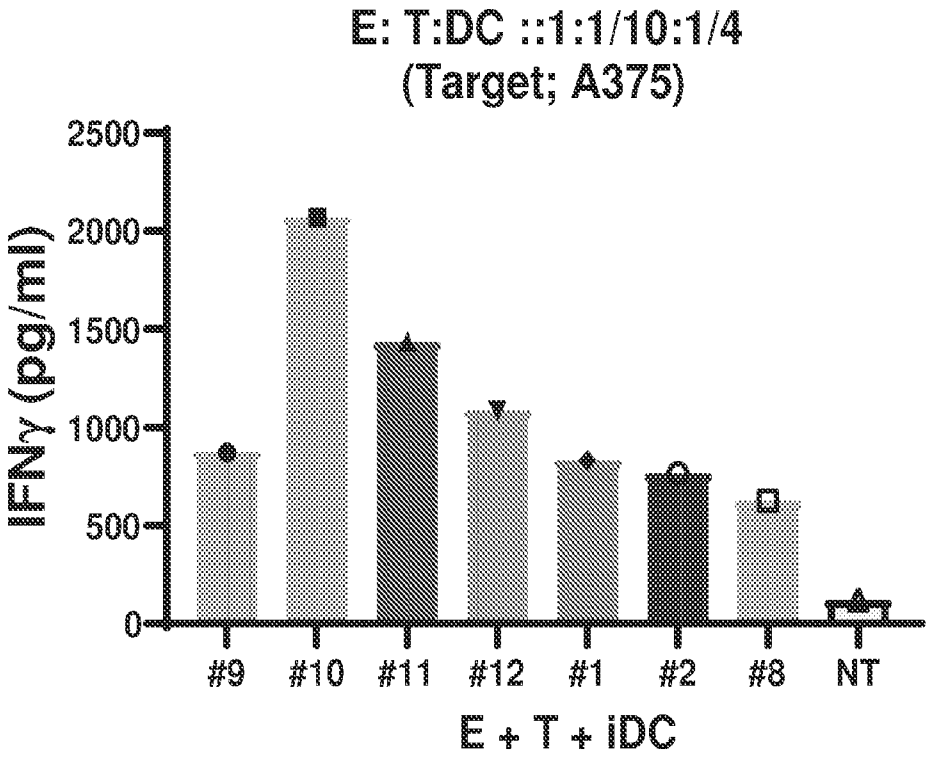


FIG. 23B

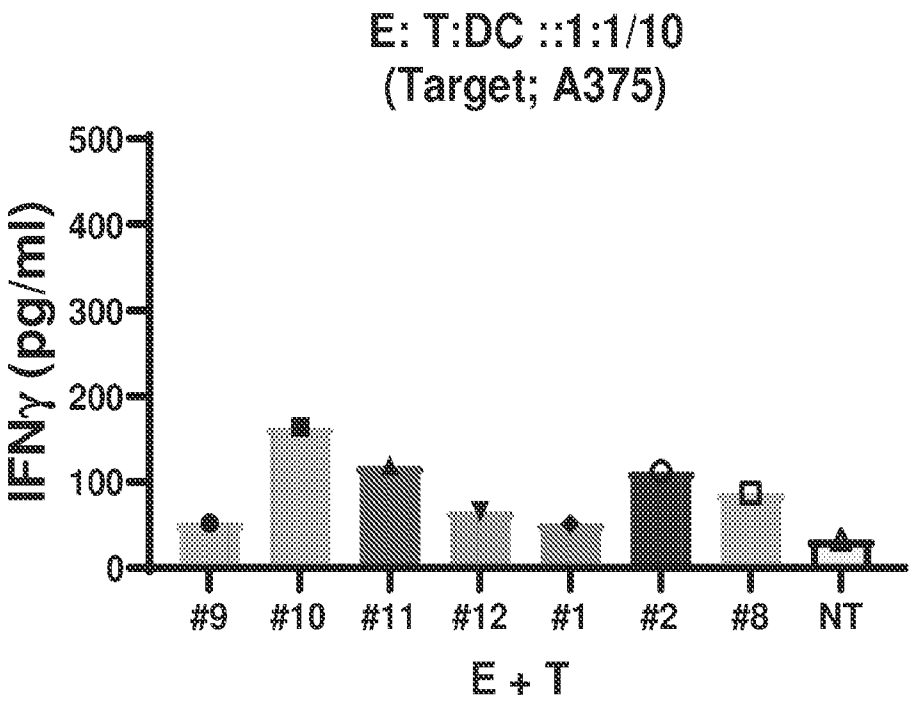


FIG. 24A

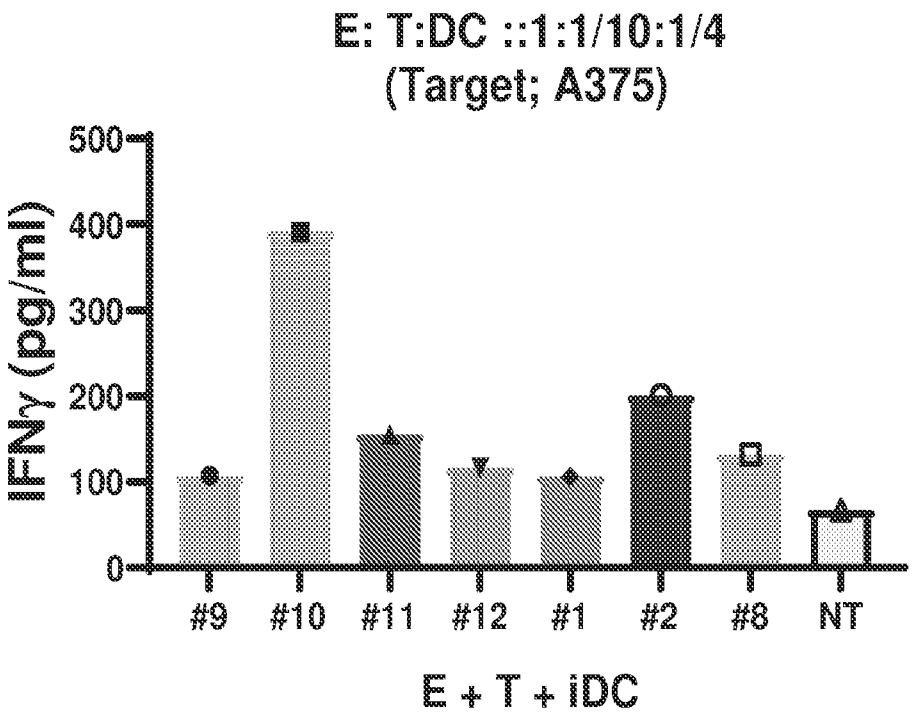


FIG. 24B

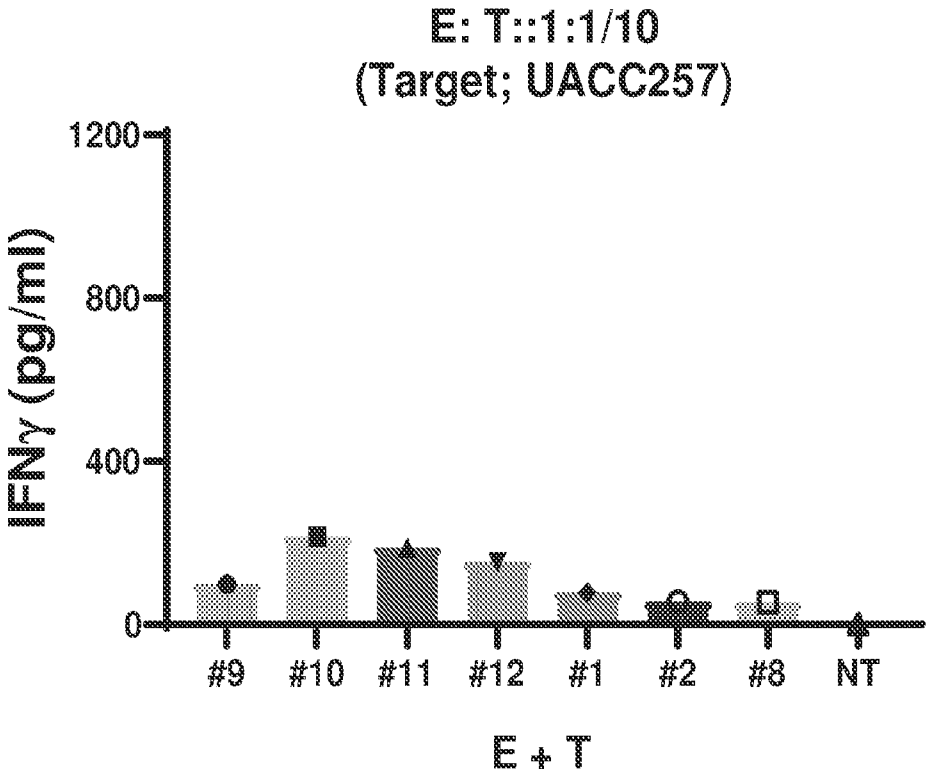


FIG. 25A

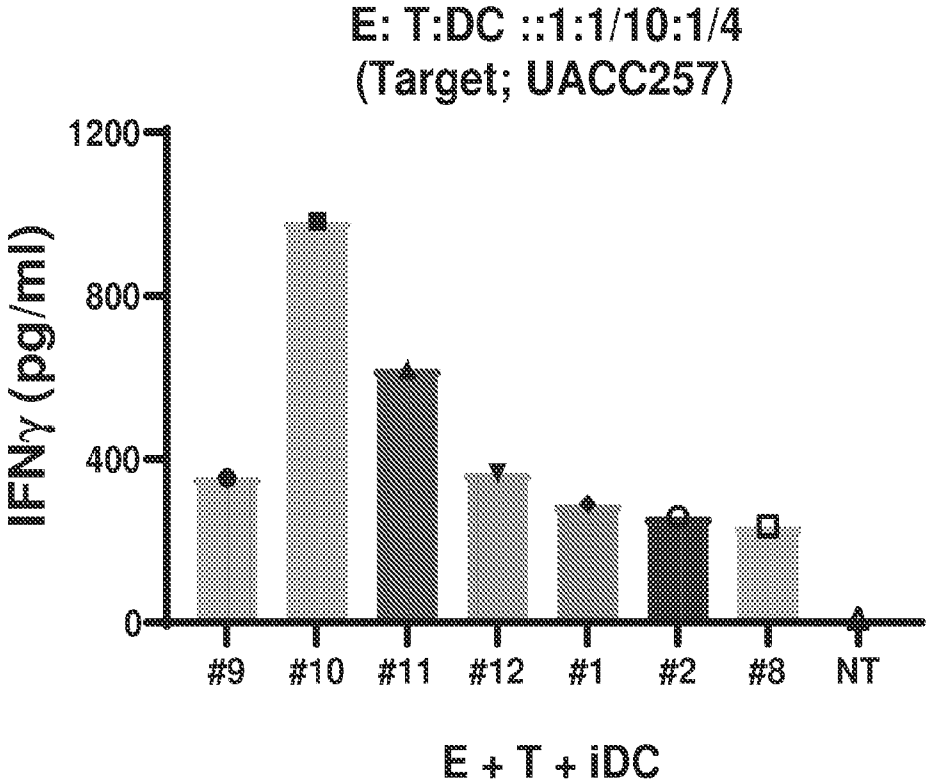


FIG. 25B

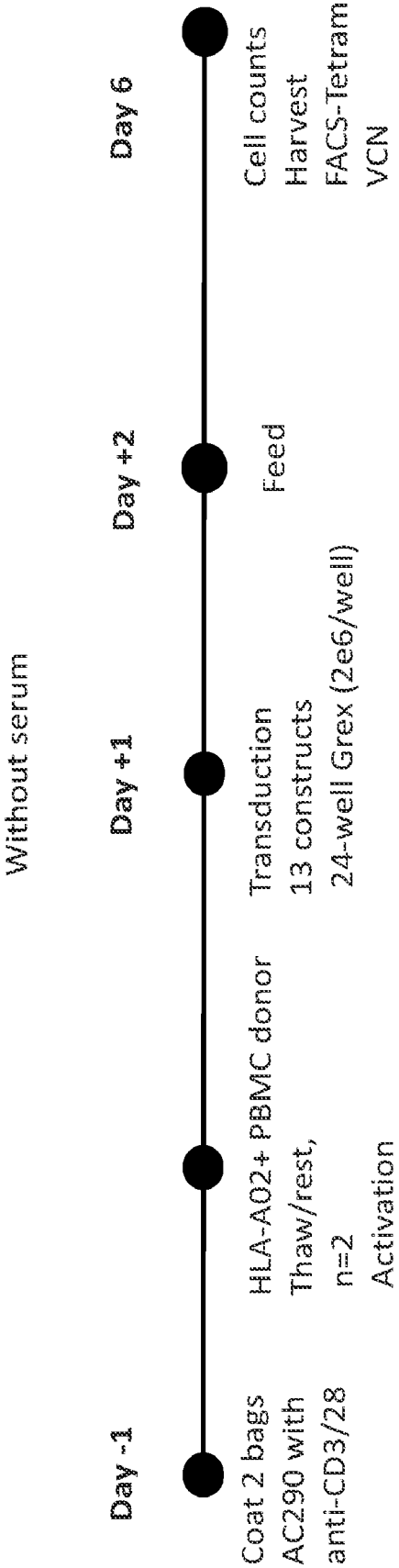

Day -1
Day +1
Day +2
Day 6
Coat 2 bags AC290 with anti-CD3/28
HLA-A02+ PBMC donor Thaw/rest, n=2 Activation
Transduction 13 constructs 24-well Grex (2e6/well)
Feed
Cell counts Harvest FACS-Tetramer VCN
Without serum

FIG. 26

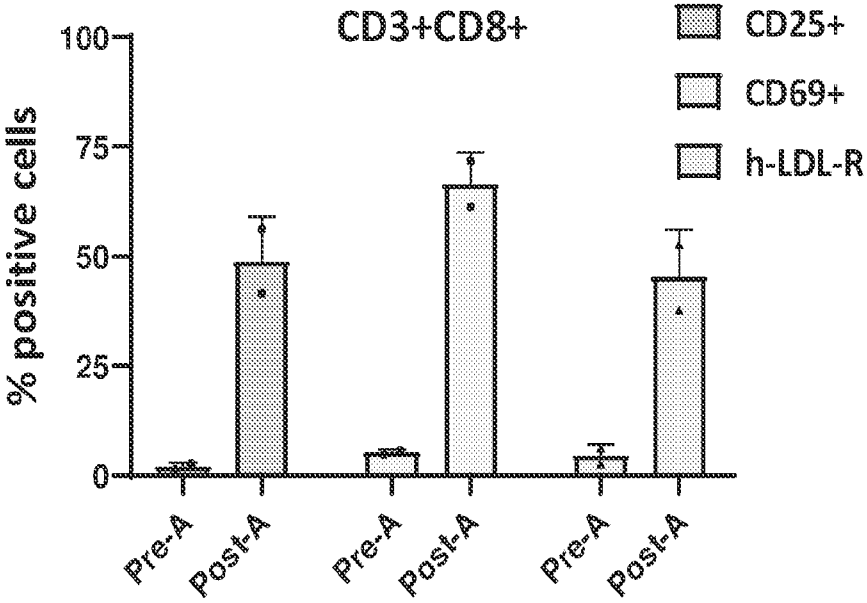


FIG. 27A

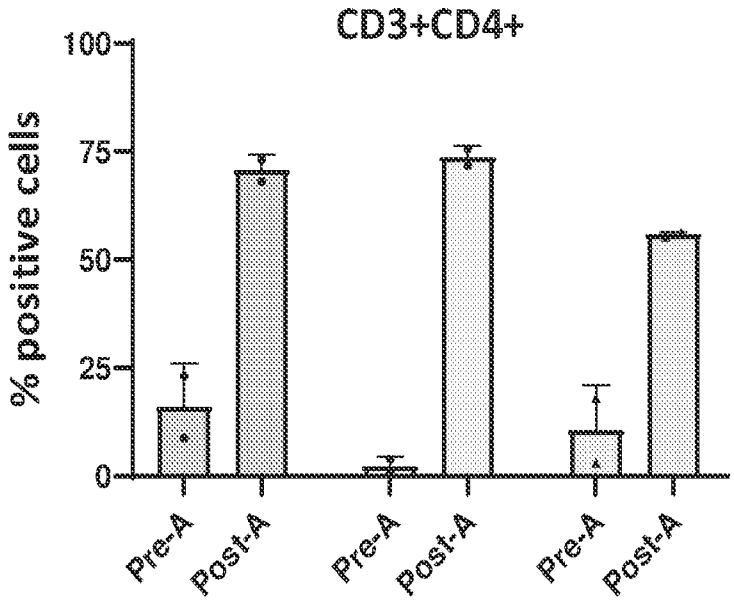


FIG. 27B

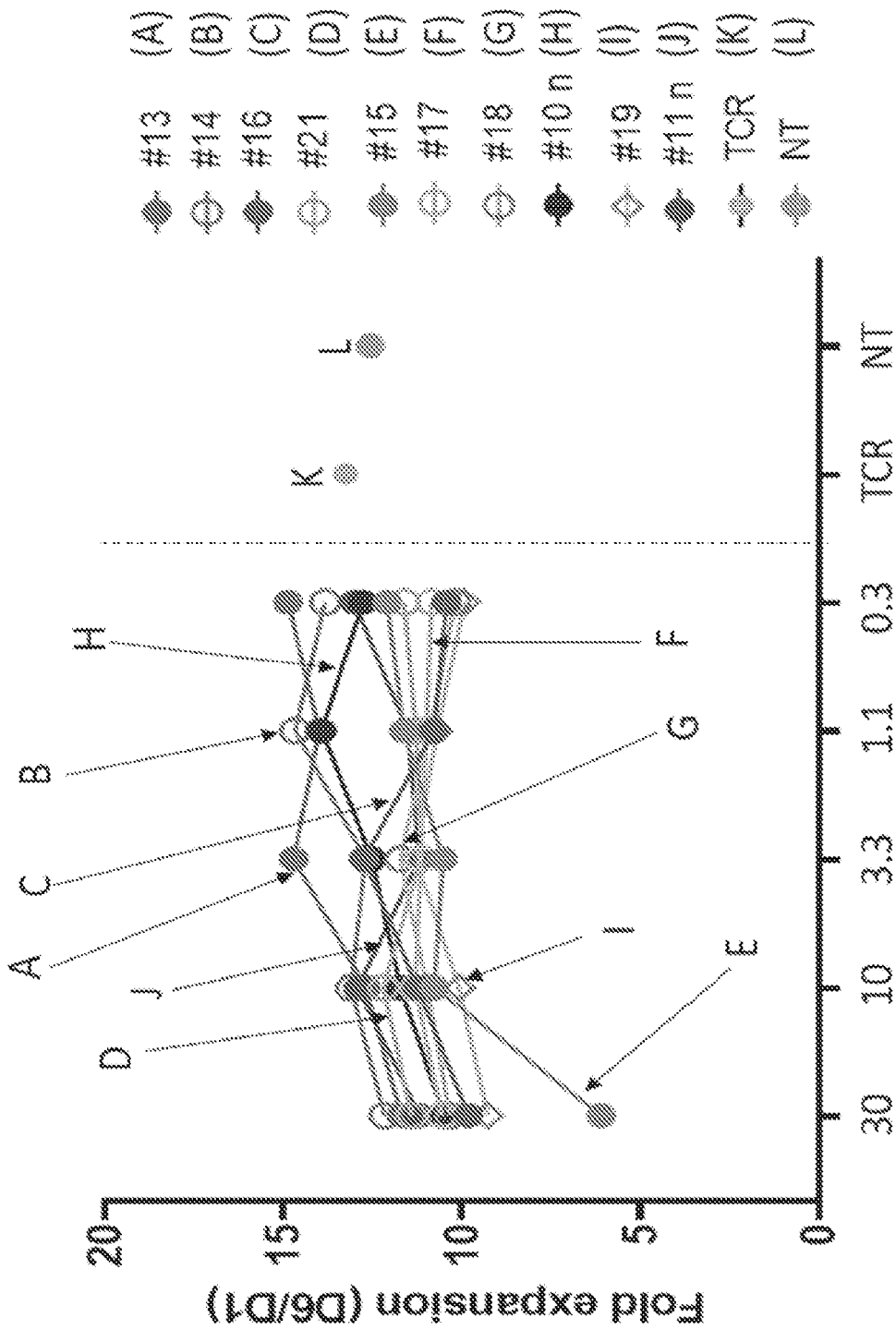

(A) #13
(B) #14
(C) #16
(D) #21
(E) #15
(F) #17
(G) #18
(H) #10 n
(I) #19
(J) #11 n
(K) TCR
(L) NT
Fold expansion (D6/D1)
0
5
10
15
20
30
10
3.3
1.1
0.3
TCR
NT
A
B
C
D
E
F
G
H
I
J
K
L

FIG. 28

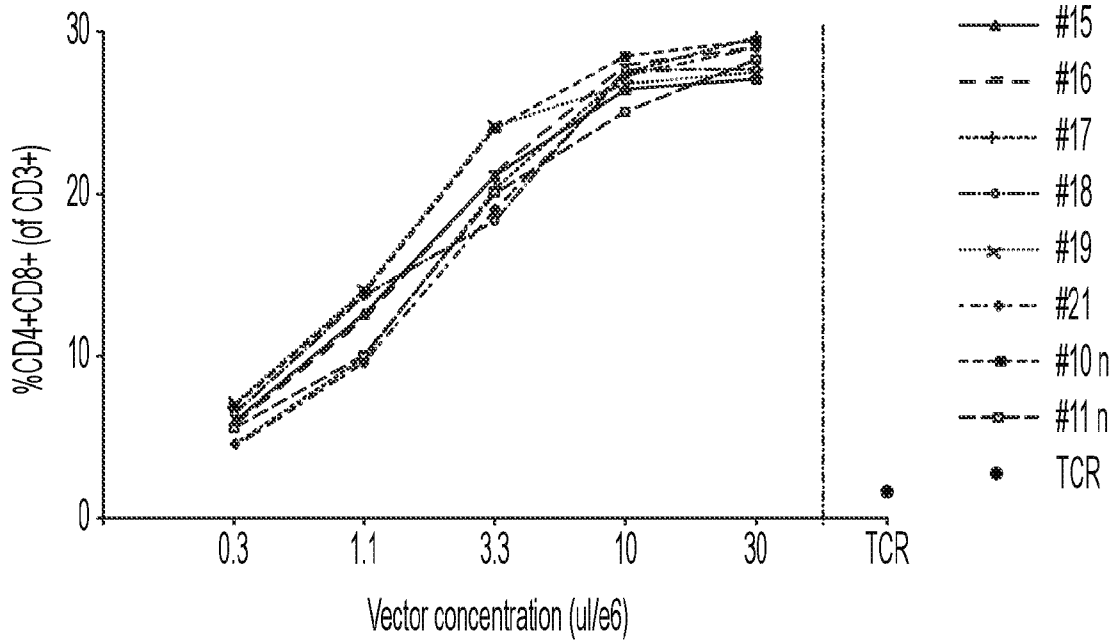

30
20
10
0
%CD4+CD8+ (of CD3+)
0.3
1.1
3.3
10
30
TCR
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR

FIG. 29A

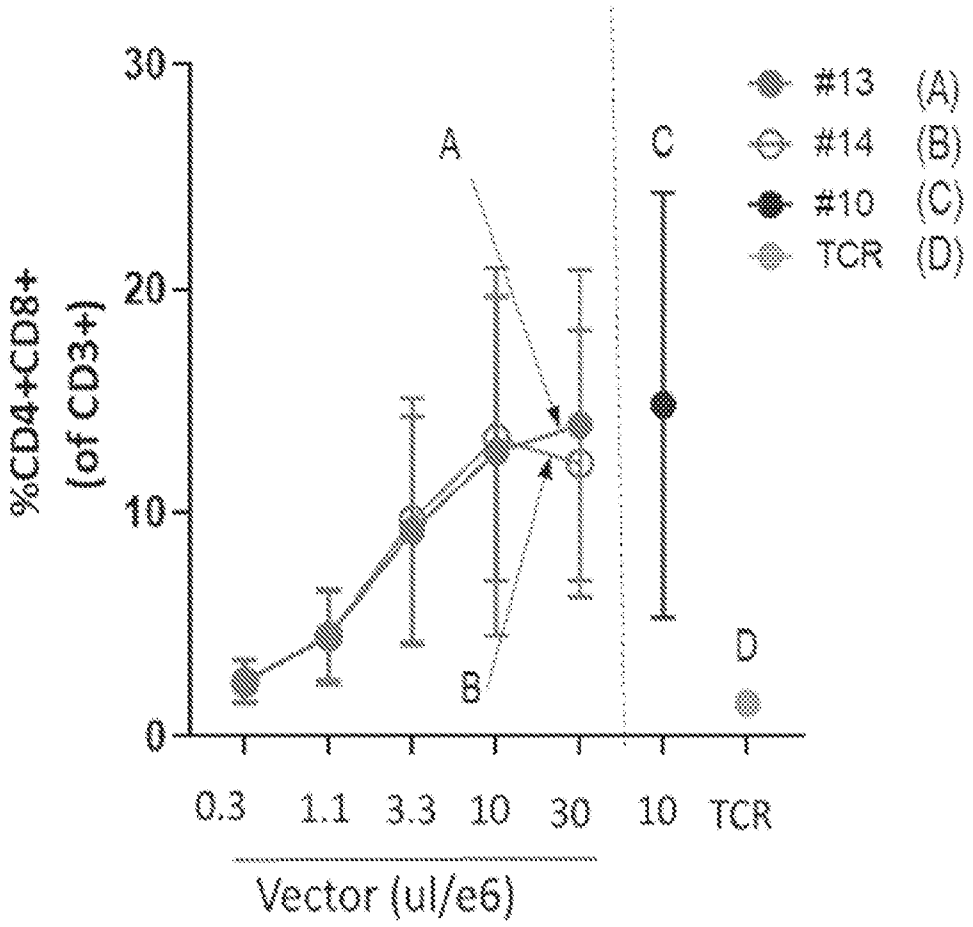

30
20
10
0
%CD4+CD8+
(of CD3+)
A
B
C
D
#13 (A)
#14 (B)
#10 (C)
TCR (D)
0.3
1.1
3.3
10
30
10
TCR
Vector (ul/e6)

FIG. 29B

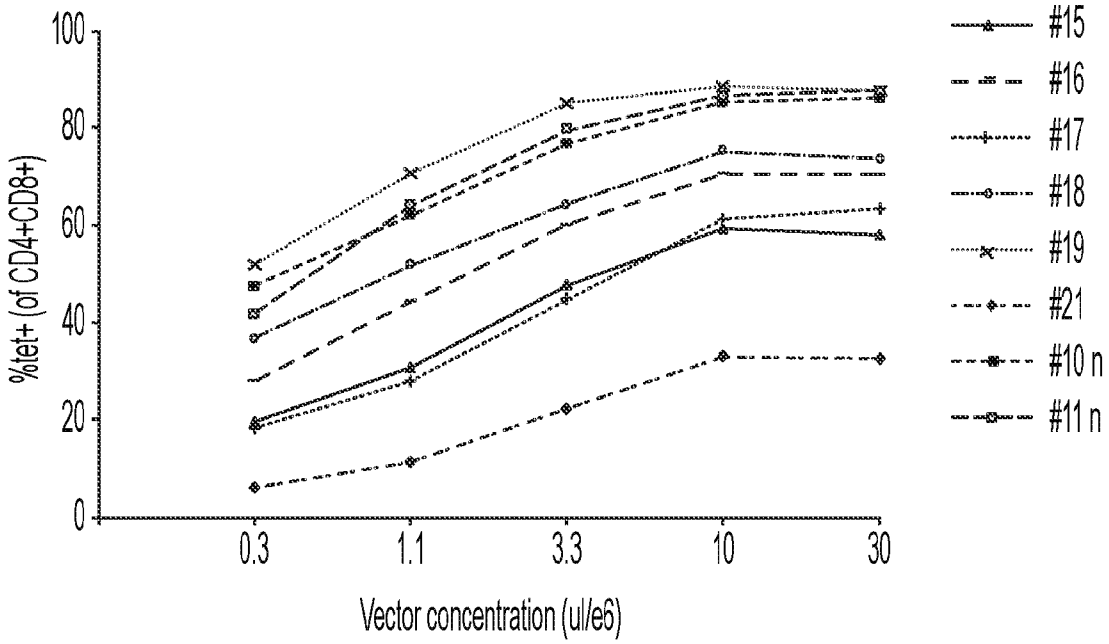

100
80
60
40
20
0
%tet+ (of CD4+CD8+)
0.3
1.1
3.3
10
30
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n

FIG. 30A

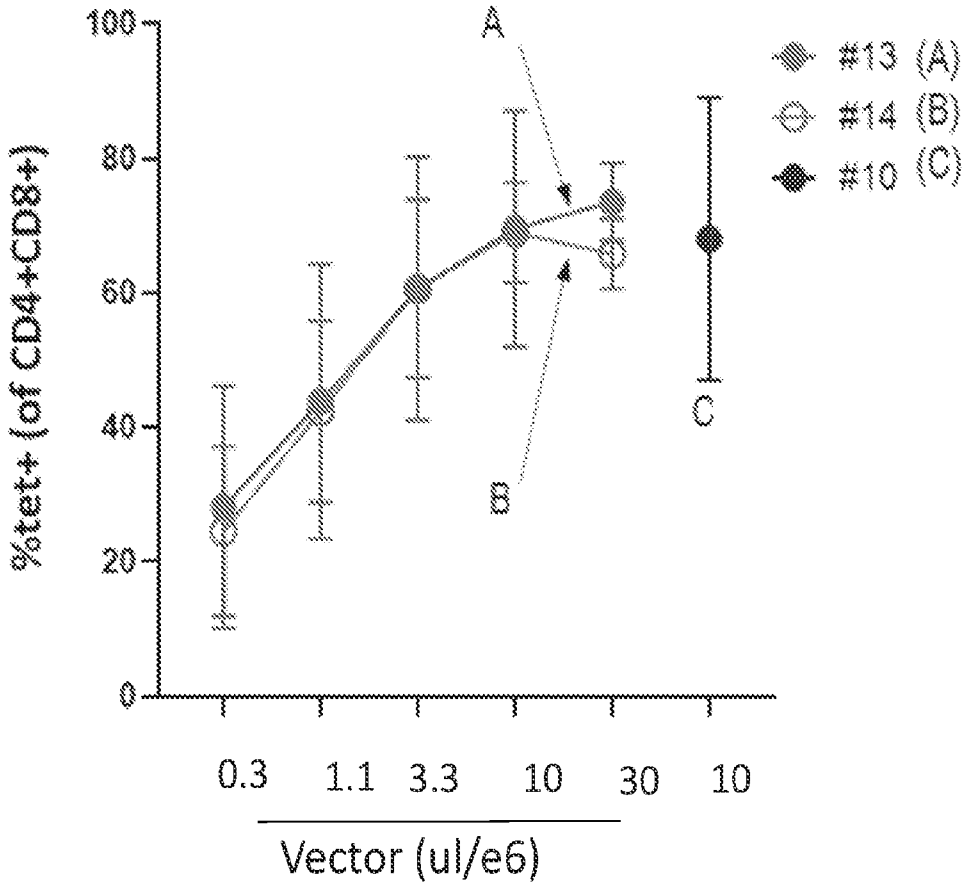

100
80
60
40
20
0
%tet+ (of CD4+CD8+)
A
B
C
#13 (A)
#14 (B)
#10 (C)
0.3
1.1
3.3
10
30
10
Vector (ul/e6)

FIG. 30B

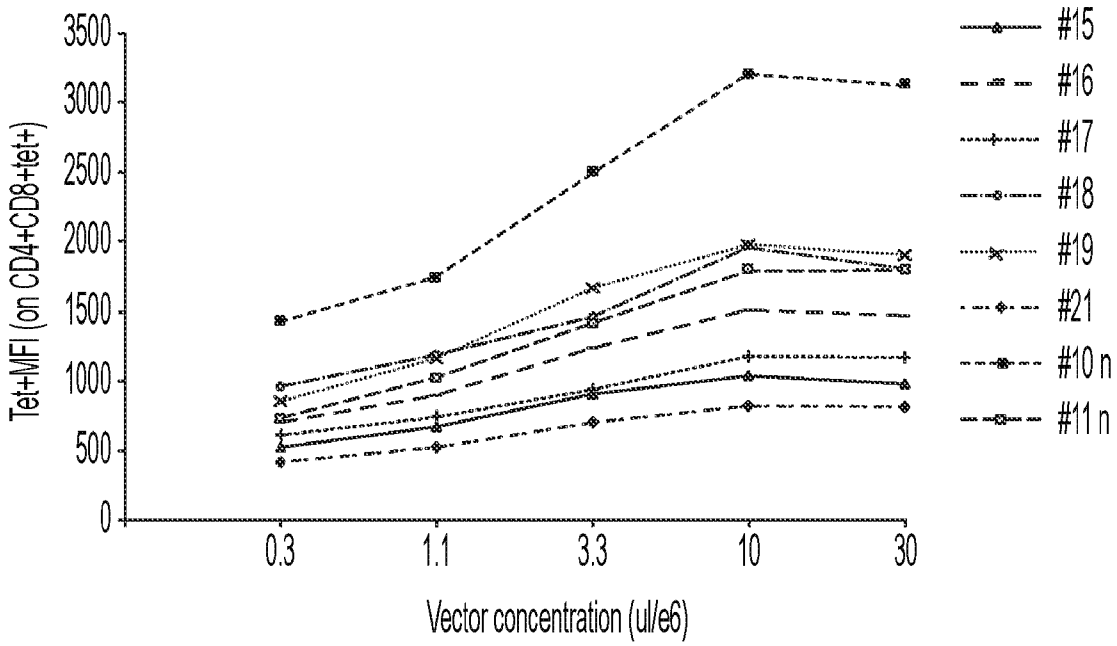

3500
3000
2500
2000
1500
1000
500
0
Tet+MFI (on CD4+CD8+tet+)
0.3
1.1
3.3
10
30
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n

FIG. 31A

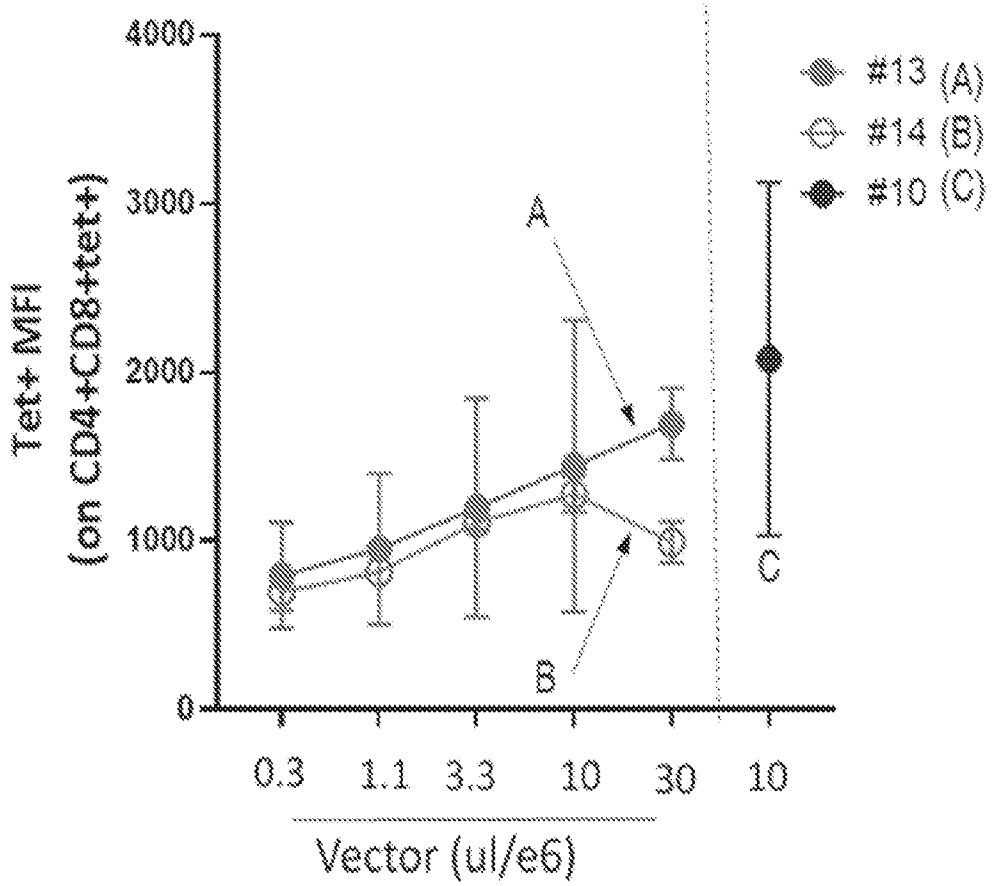

4000
3000
2000
1000
0
Tet+ MFI
(on CD4+CD8+tet+)
#13 (A)
#14 (B)
#10 (C)
A
B
C
0.3
1.1
3.3
10
30
10
Vector (ul/e6)

FIG. 31B

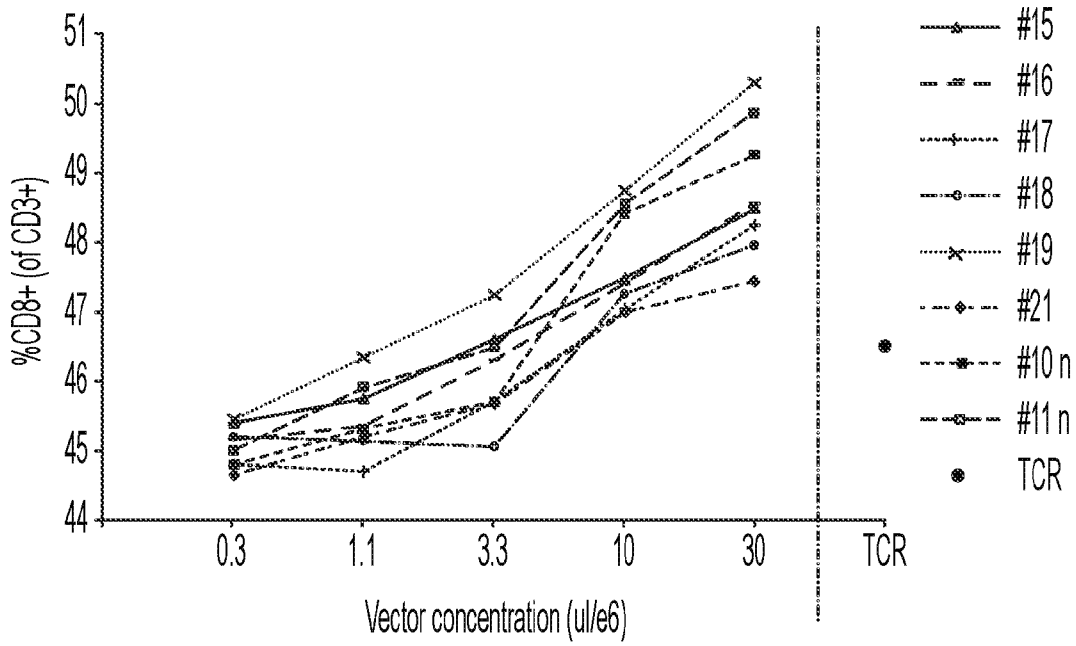

51
50
49
48
47
46
45
44
%CD8+ (of CD3+)
0.3
1.1
3.3
10
30
TCR
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR

FIG. 32A

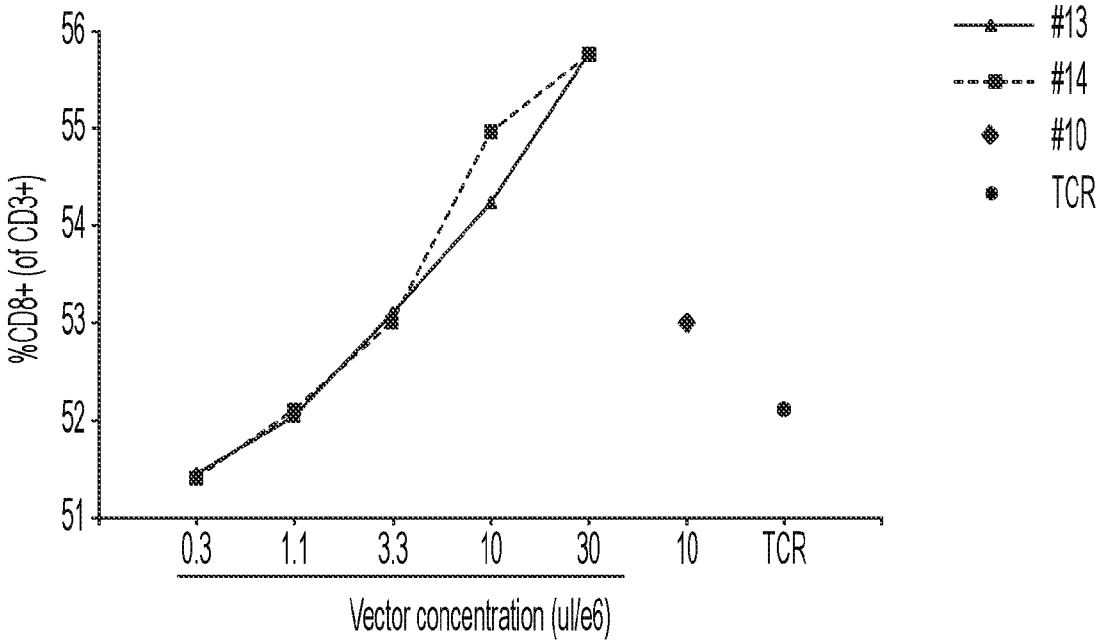

56
55
54
53
52
51
%CD8+ (of CD3+)
0.3
1.1
3.3
10
30
10
TCR
Vector concentration (ul/e6)
#13
#14
#10
TCR

FIG. 32B

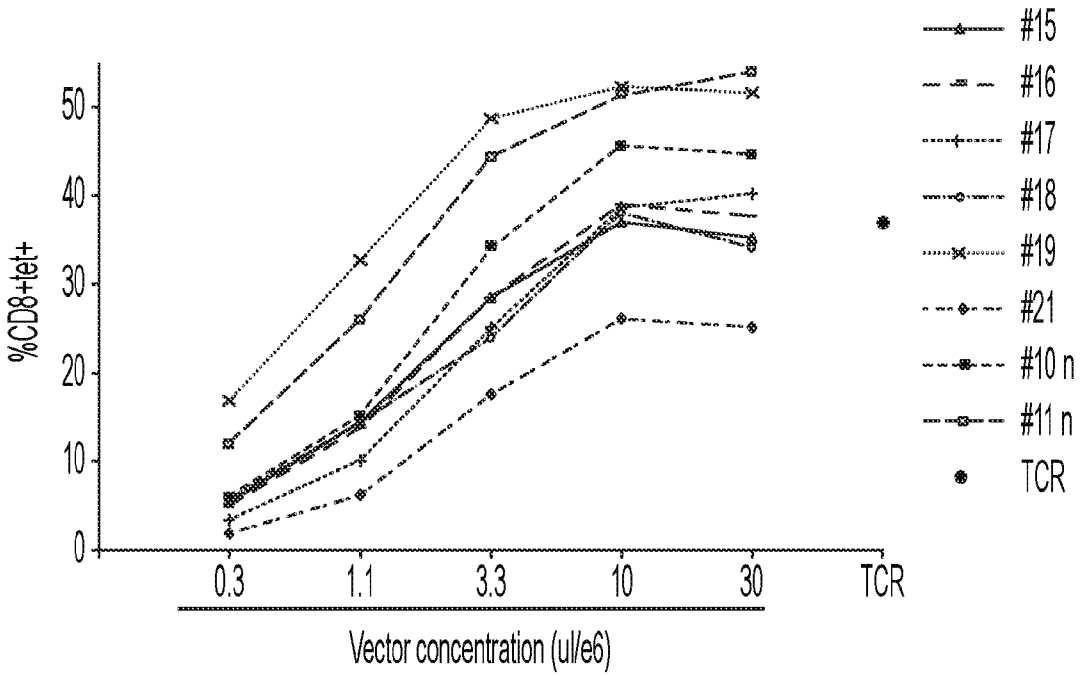

%CD8+tet+
0
10
20
30
40
50
0.3
1.1
3.3
10
30
TCR
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR

FIG. 33A

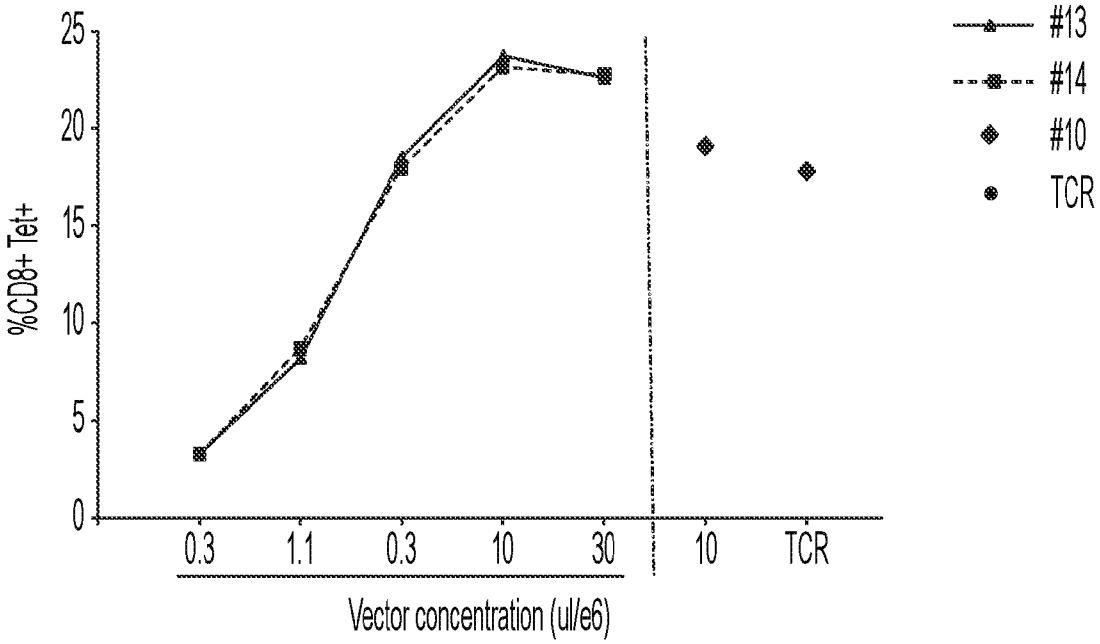

25
20
15
10
5
0
%CD8+ Tet+
0.3
1.1
0.3
10
30
10
TCR
Vector concentration (ul/e6)
#13
#14
#10
TCR

FIG. 33B

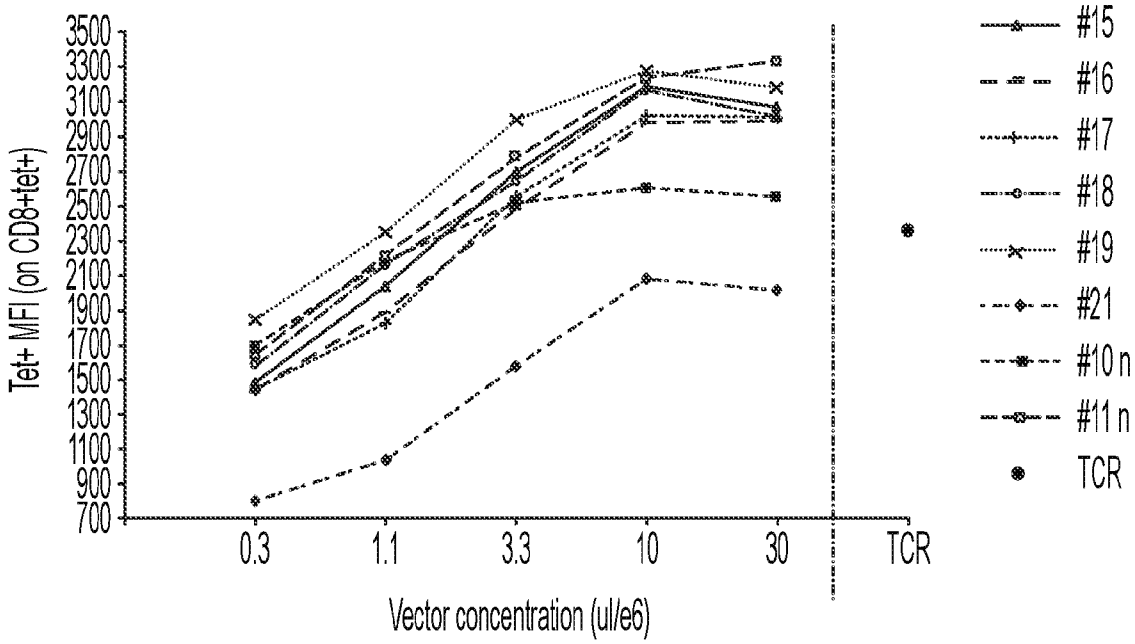

Tet+ MFI (on CD8+tet+)
3500
3300
3100
2900
2700
2500
2300
2100
1900
1700
1500
1300
1100
900
700
0.3
1.1
3.3
10
30
TCR
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR

FIG. 34A

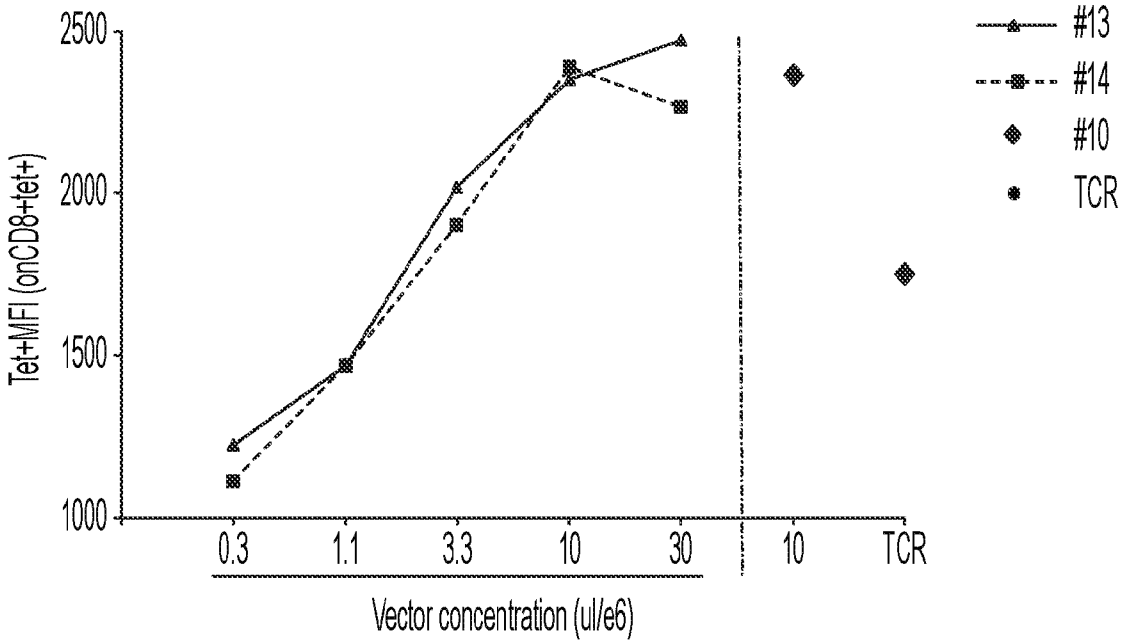

2500
2000
1500
1000
Tet+MFI (onCD8+tet+)
0.3
1.1
3.3
10
30
10
TCR
Vector concentration (ul/e6)
#13
#14
#10
TCR

FIG. 34B

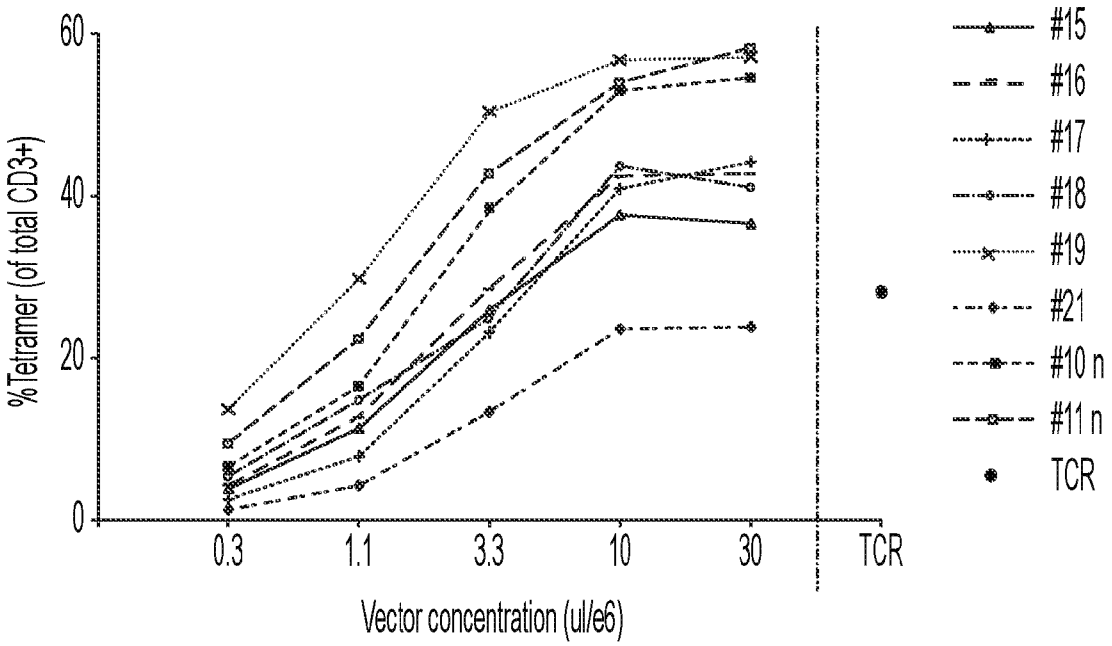

60
40
20
0
% Tetramer (of total CD3+)
0.3
1.1
3.3
10
30
TCR
Vector concentration (ul/e6)
#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR

FIG. 35A

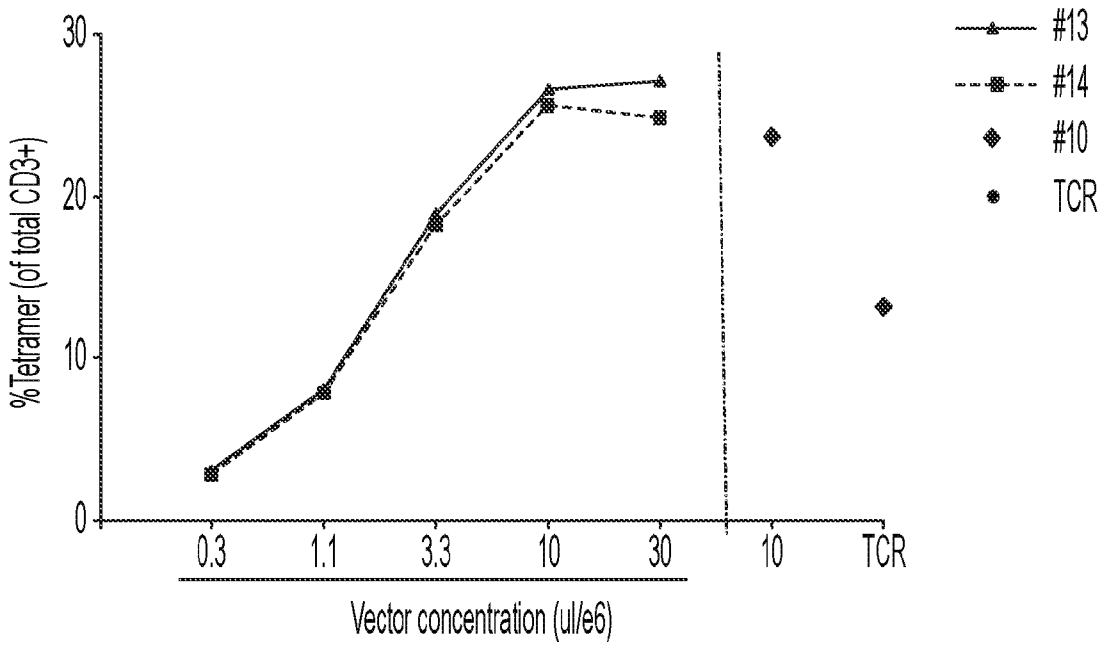

30
20
10
0
% Tetramer (of total CD3+)
0.3
1.1
3.3
10
30
10
TCR
Vector concentration (ul/e6)
#13
#14
#10
TCR

FIG. 35B

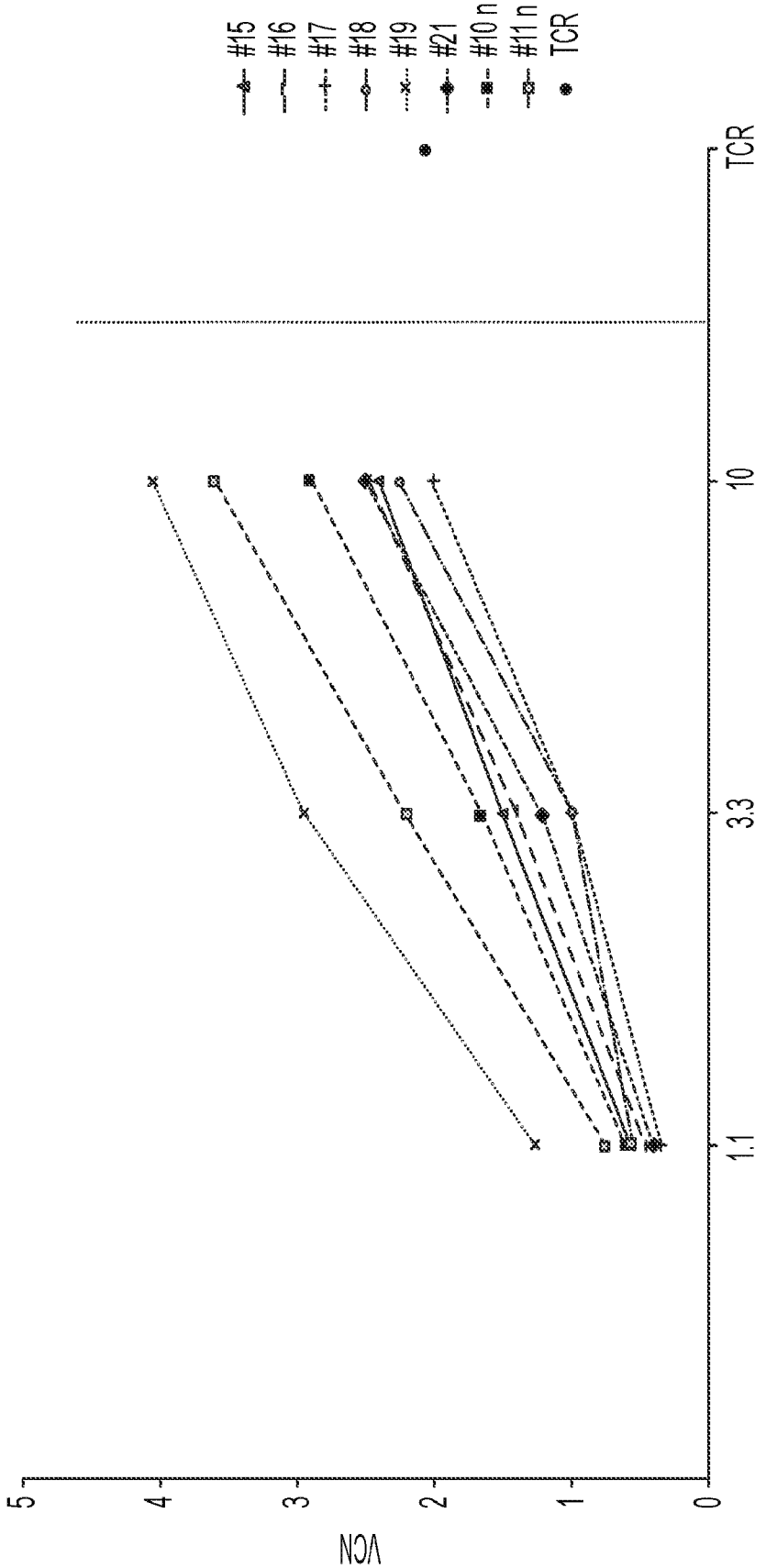

#15
#16
#17
#18
#19
#21
#10 n
#11 n
TCR
VCN
0
1
2
3
4
5
1.1
3.3
10
TCR
Vector concentration (ul/e6)

FIG. 36A

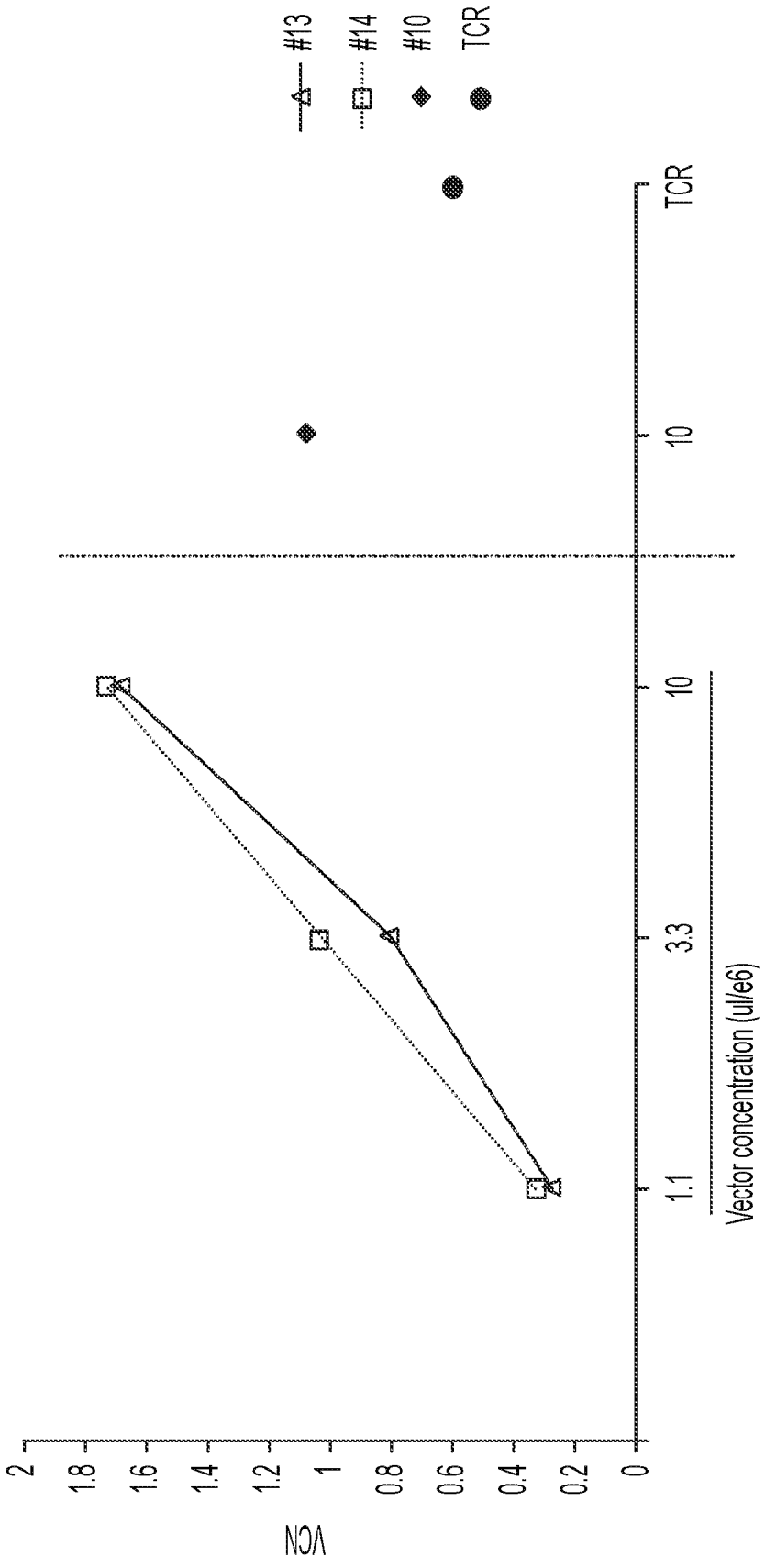

#13
#14
#10
TCR
TCR
10
10
3.3
1.1
Vector concentration (ul/e6)
VCN
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0

FIG. 36B

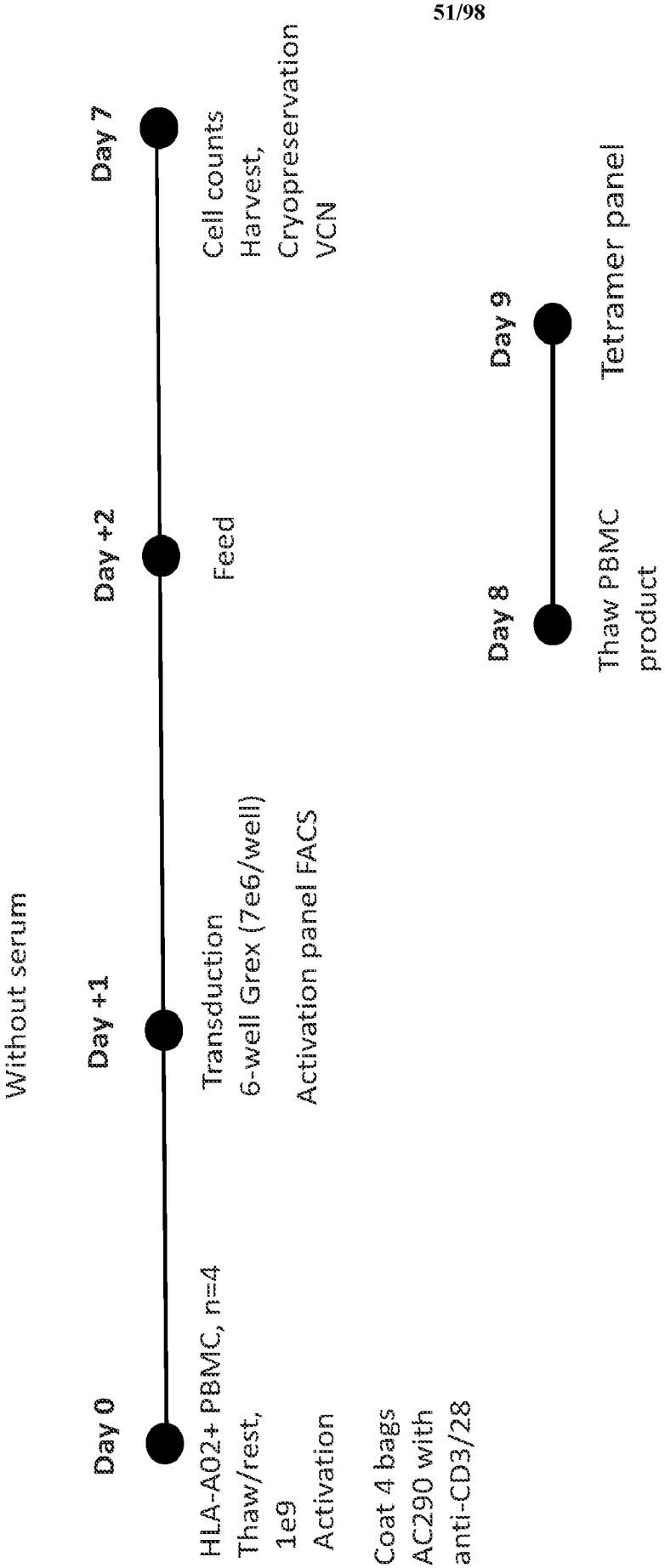

Day 0
Day +1
Day +2
Day 7
Day 8
Day 9
HLA-A02+ PBMC, n=4
Thaw/rest,
1e9
Activation
Coat 4 bags
AC290 with
anti-CD3/28
Transduction
6-well Grex (7e6/well)
Activation panel FACS
Feed
Cell counts
Harvest,
Cryopreservation
VCN
Thaw PBMC
product
Tetramer panel
Without serum

FIG. 37

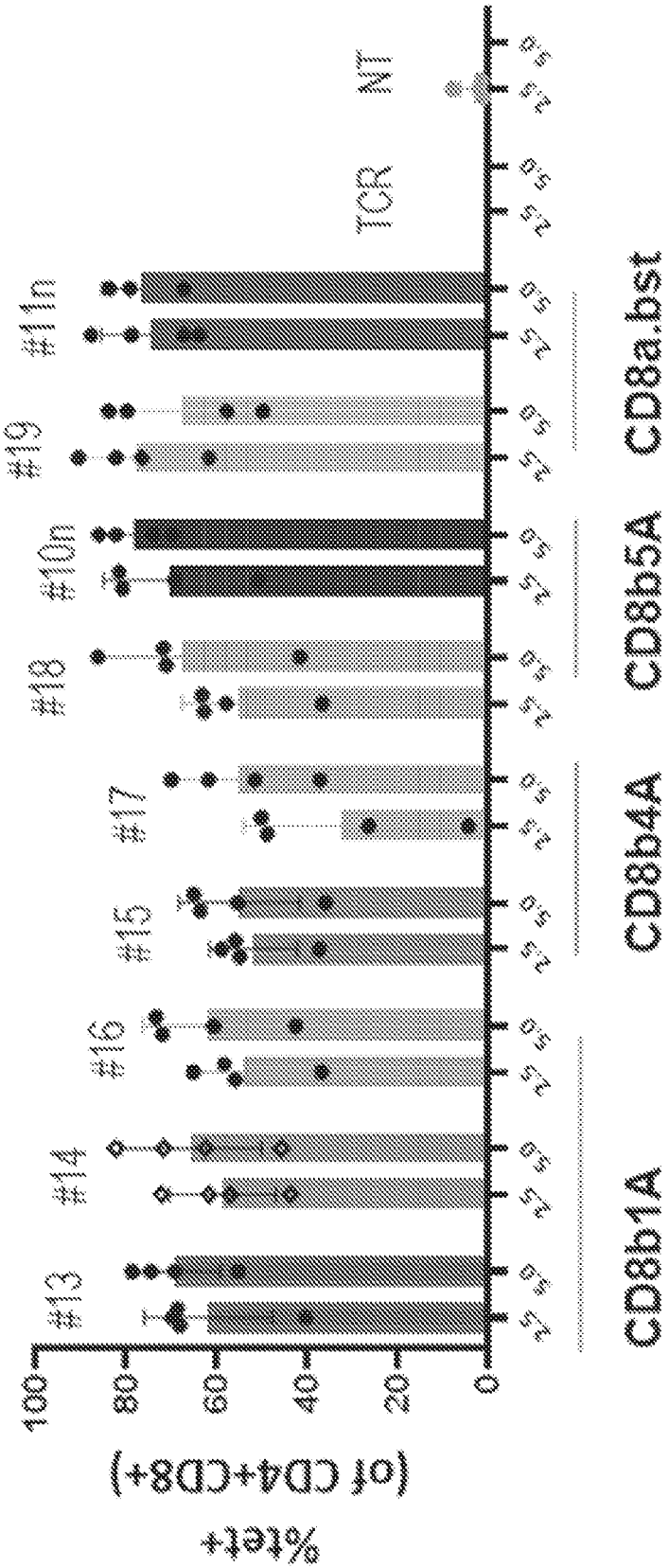

%tet+
(of CD4+CD8+)
100
80
60
40
20
0
#13
#14
#16
#15
#17
#18
#10n
#19
#11n
TCR
NT
CD8b1A
CD8b4A
CD8b5A
CD8a.bst

FIG. 38

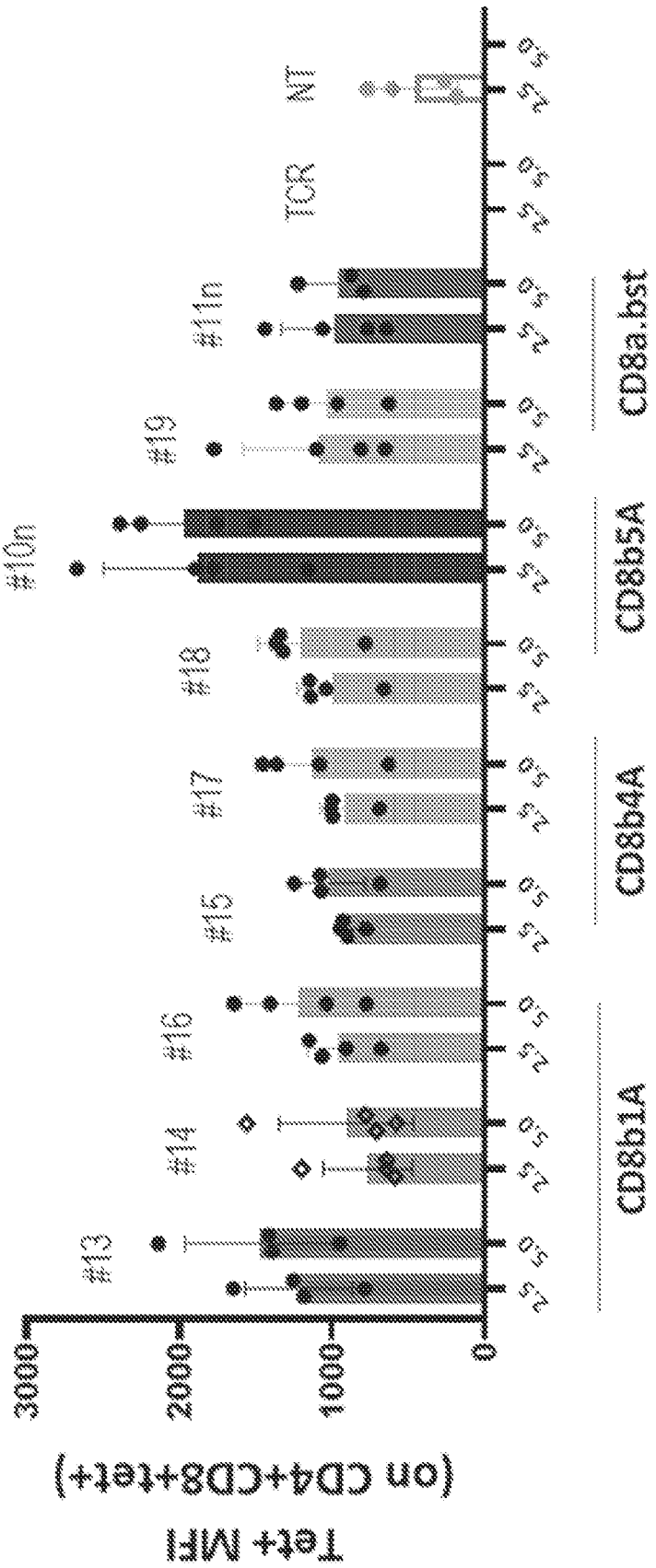

Tet+ MFI
(on CD4+CD8+tet+)
3000
2000
1000
0
#13
#14
#16
#15
#17
#18
#10n
#19
#11n
TCR
NT
CD8b1A
CD8b4A
CD8b5A
CD8a.bst

FIG. 39

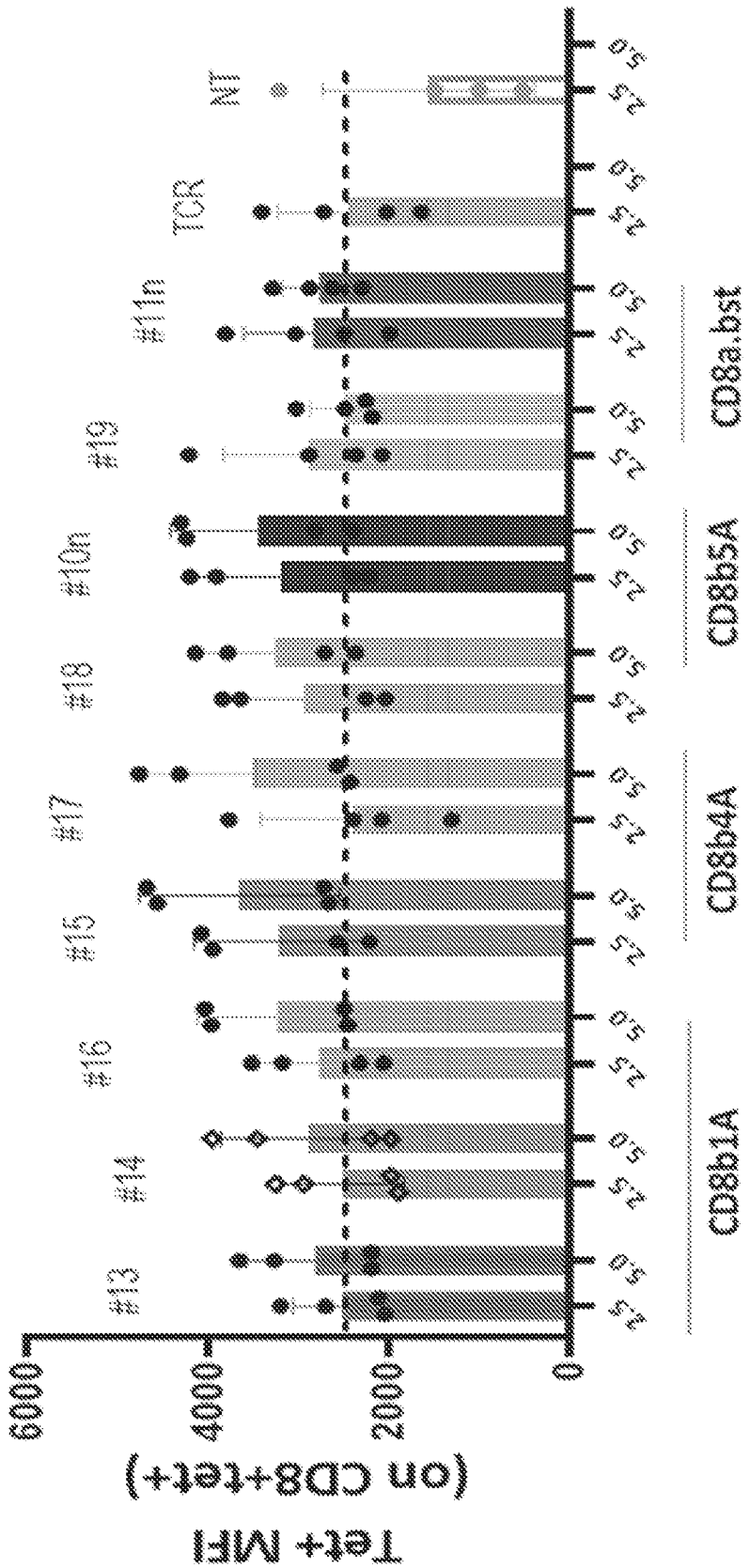

Tet+ MFI
(on CD8+tet+)
6000
4000
2000
0
#13
#14
#16
#15
#17
#18
#10m
#19
#11m
TCR
MT
2.5
5.0
CD8b1A
CD8b4A
CD8b5A
CD8a.bst

FIG. 40

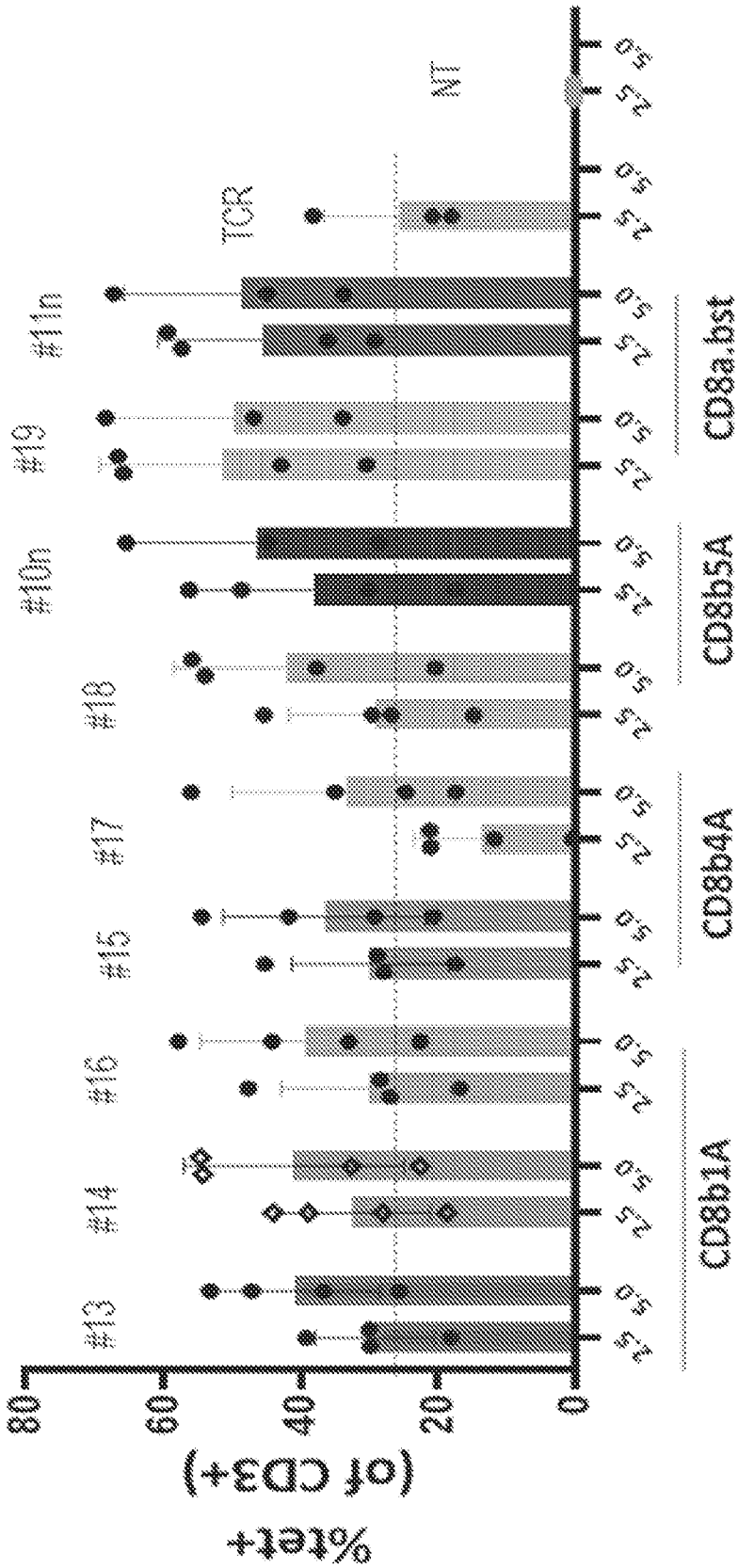

%tet+ (of CD3+)
80
60
40
20
0
#13
#14
#16
#15
#17
#18
#10n
#19
#11n
TCR
MT
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
0.5
2.5
CD8b1A
CD8b4A
CD8b5A
CD8a.bst

FIG. 41

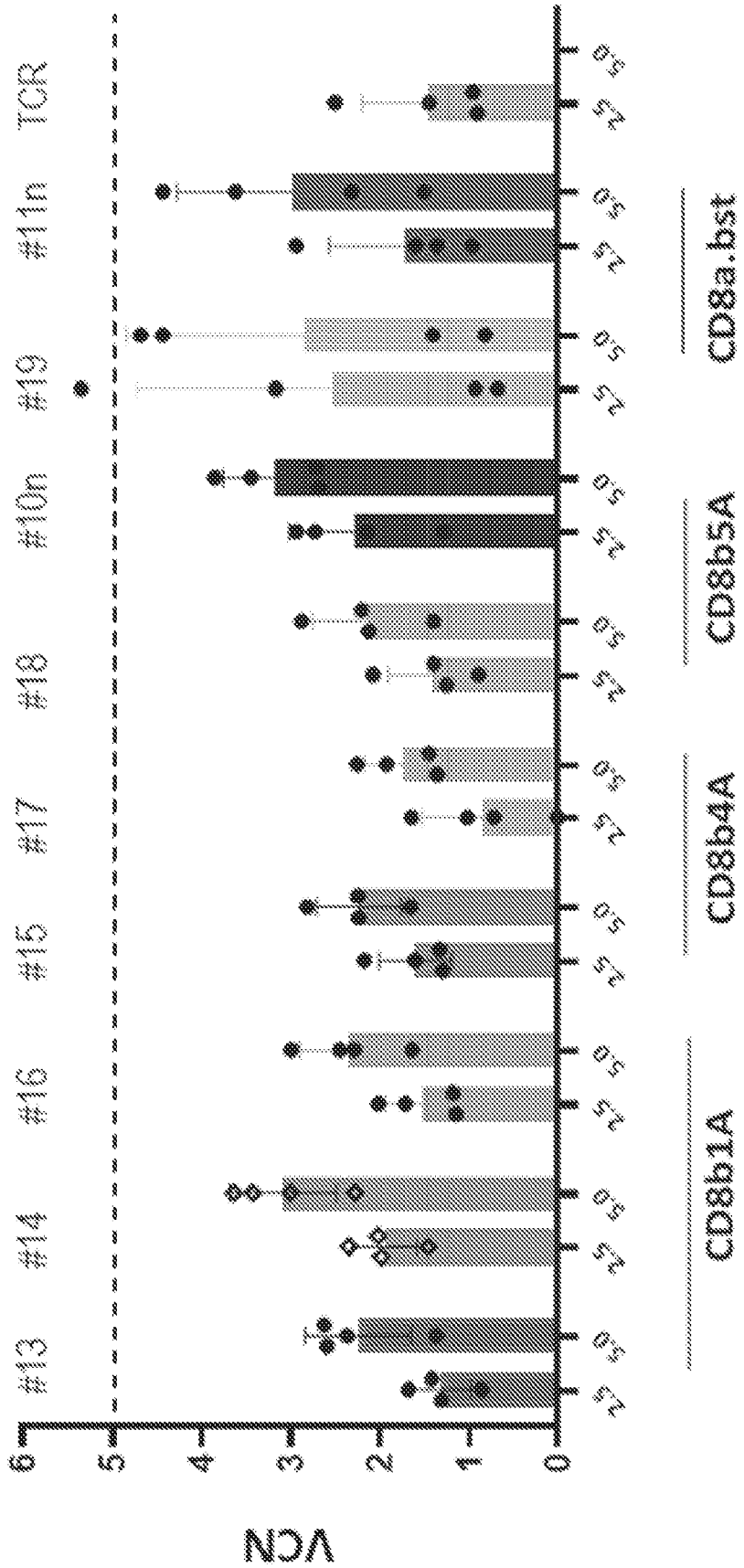

VCN
0
1
2
3
4
5
6
#13
#14
#16
#15
#17
#18
#10n
#19
#11n
TCR
CD8b1A
CD8b4A
CD8b5A
CD8a.bst

FIG. 42

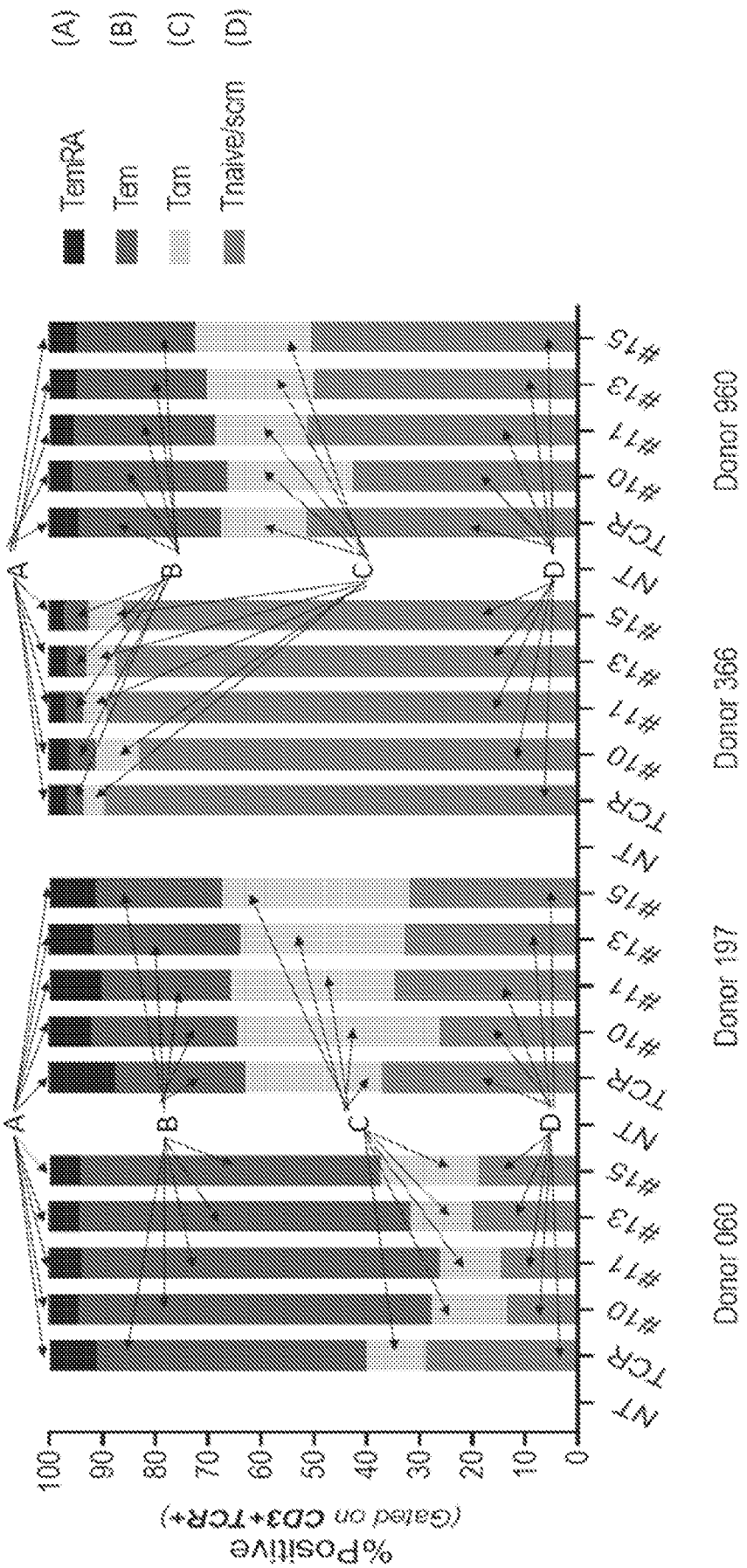

(A)
(B)
(C)
(D)
TemRA
Tem
Tcm
Tnaive/scm
%Positive
(Gated on CD3+TCR+)
100
90
80
70
60
50
40
30
20
10
0
NT
TCR
#10
#11
#13
#15
Donor 060
Donor 197
Donor 366
Donor 960

FIG. 43

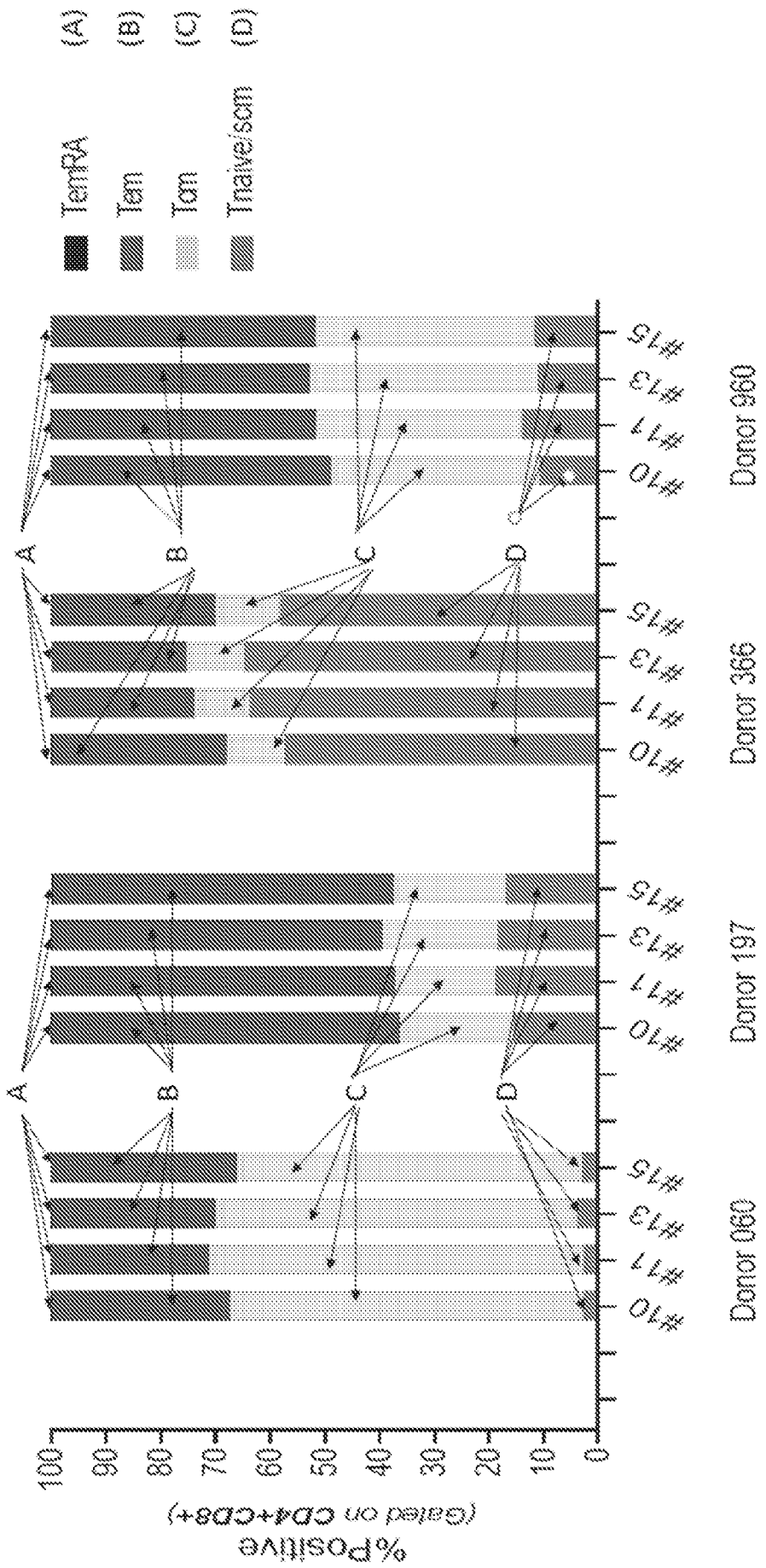

(A)
(B)
(C)
(D)
TemRA
Tem
Tcm
Tnaive/scm
% Positive
(Gated on CD4+CD8+)
100
90
80
70
60
50
40
30
20
10
0
Donor 060
Donor 197
Donor 366
Donor 960
#10
#11
#13
#15
A
B
C
D

FIG. 44A

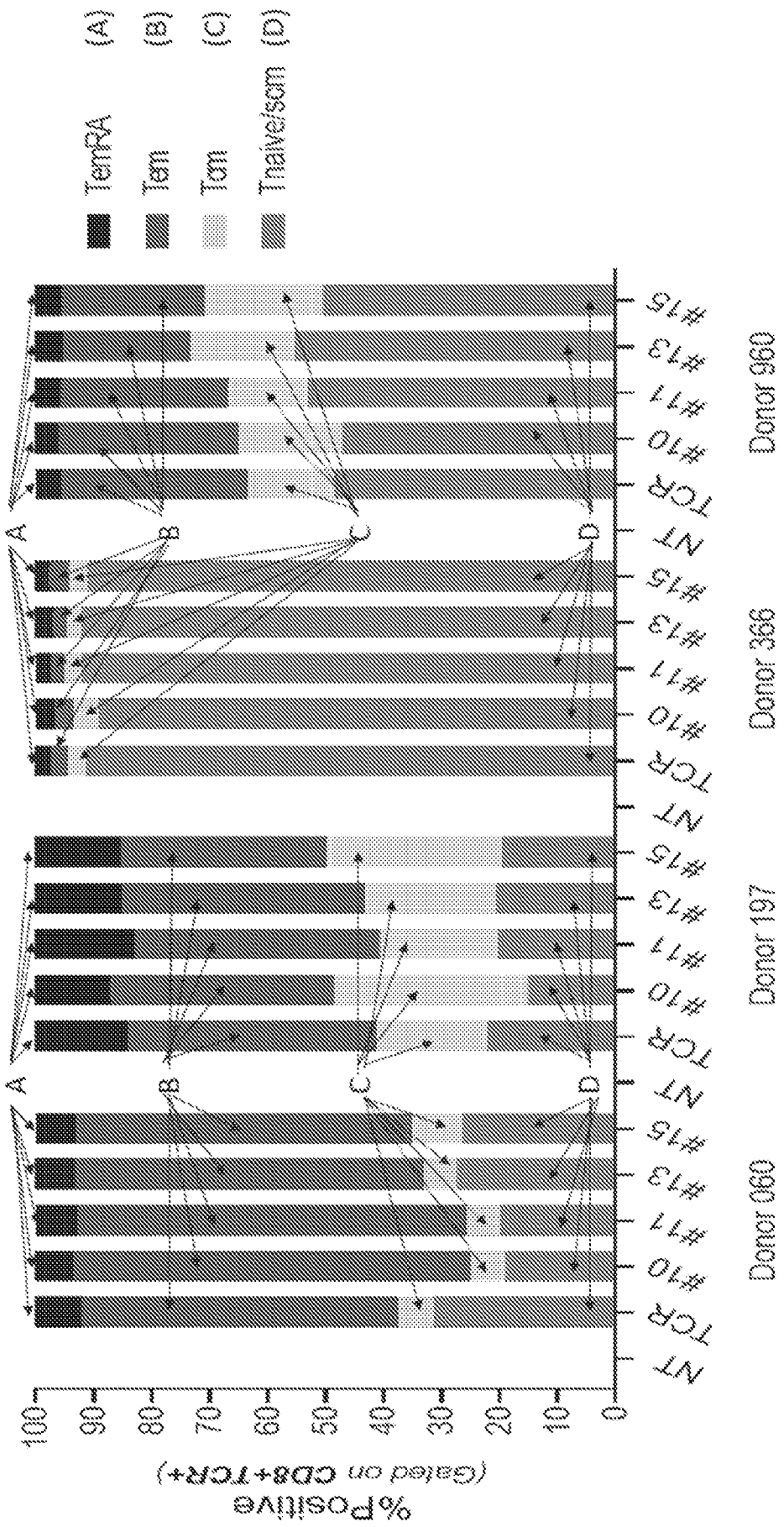

TemRA (A)
Tem (B)
Tcm (C)
Tnaive/scm (D)
% Positive
(Gated on CD8+TCR+)
100
90
80
70
60
50
40
30
20
10
0
NT
TCR
#10
#11
#13
#15
Donor 060
Donor 197
Donor 366
Donor 960

FIG. 44B

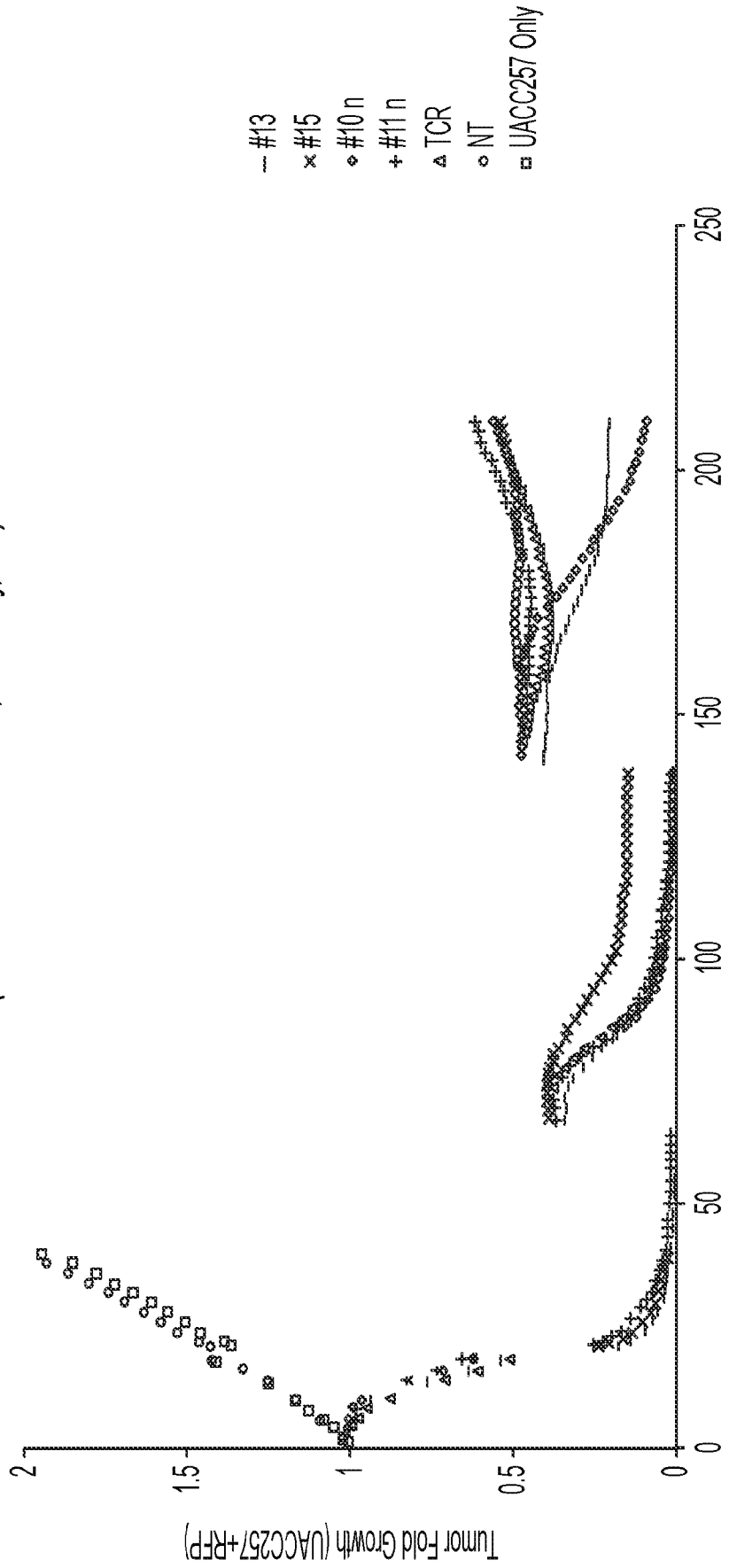

IMA-RND-21-020
(Normalized TCR+ 4:1 E/T, Summary, n=4)
Tumor Fold Growth (UACC257+RFP)
Hours after Co-culture
0
0.5
1
1.5
2
0
50
100
150
200
250
#13
#15
#10 n
#11 n
TCR
NT
UACC257 Only

FIG. 45A

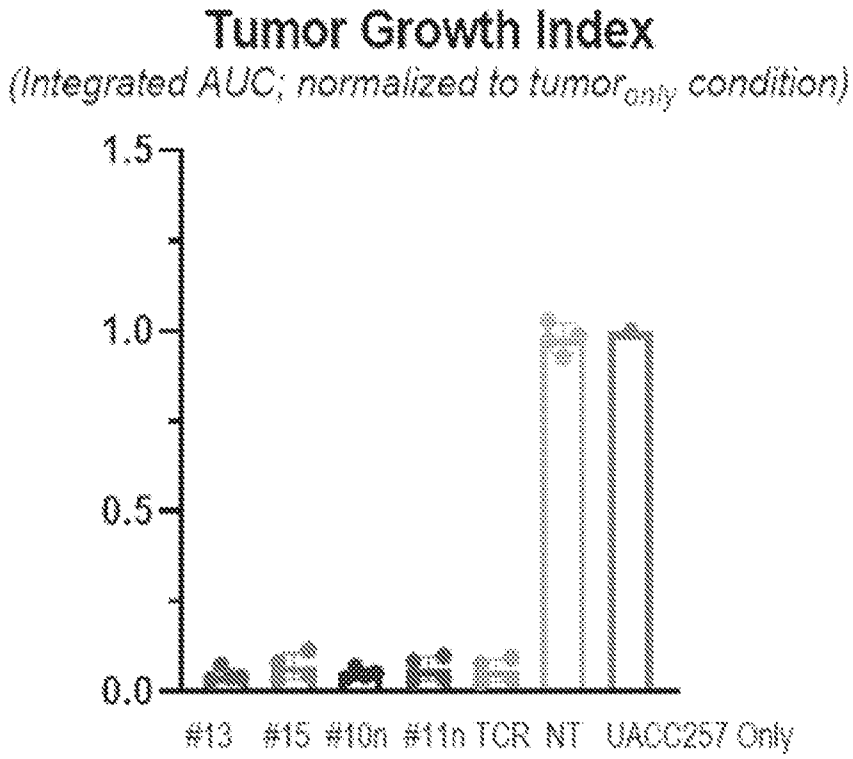

Tumor Growth Index
(Integrated AUC; normalized to tumor$_{only}$ condition)
1.5
1.0
0.5
0.0
#13
#15
#10n
#11n
TCR NT
UACC257 Only

FIG. 45B

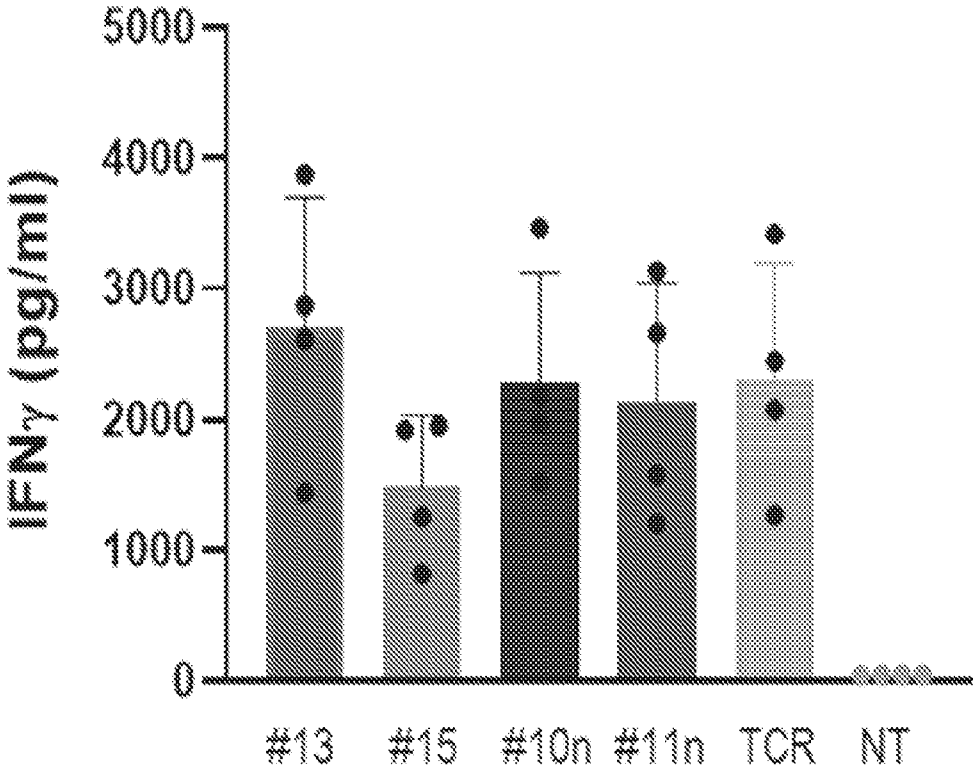


FIG. 46

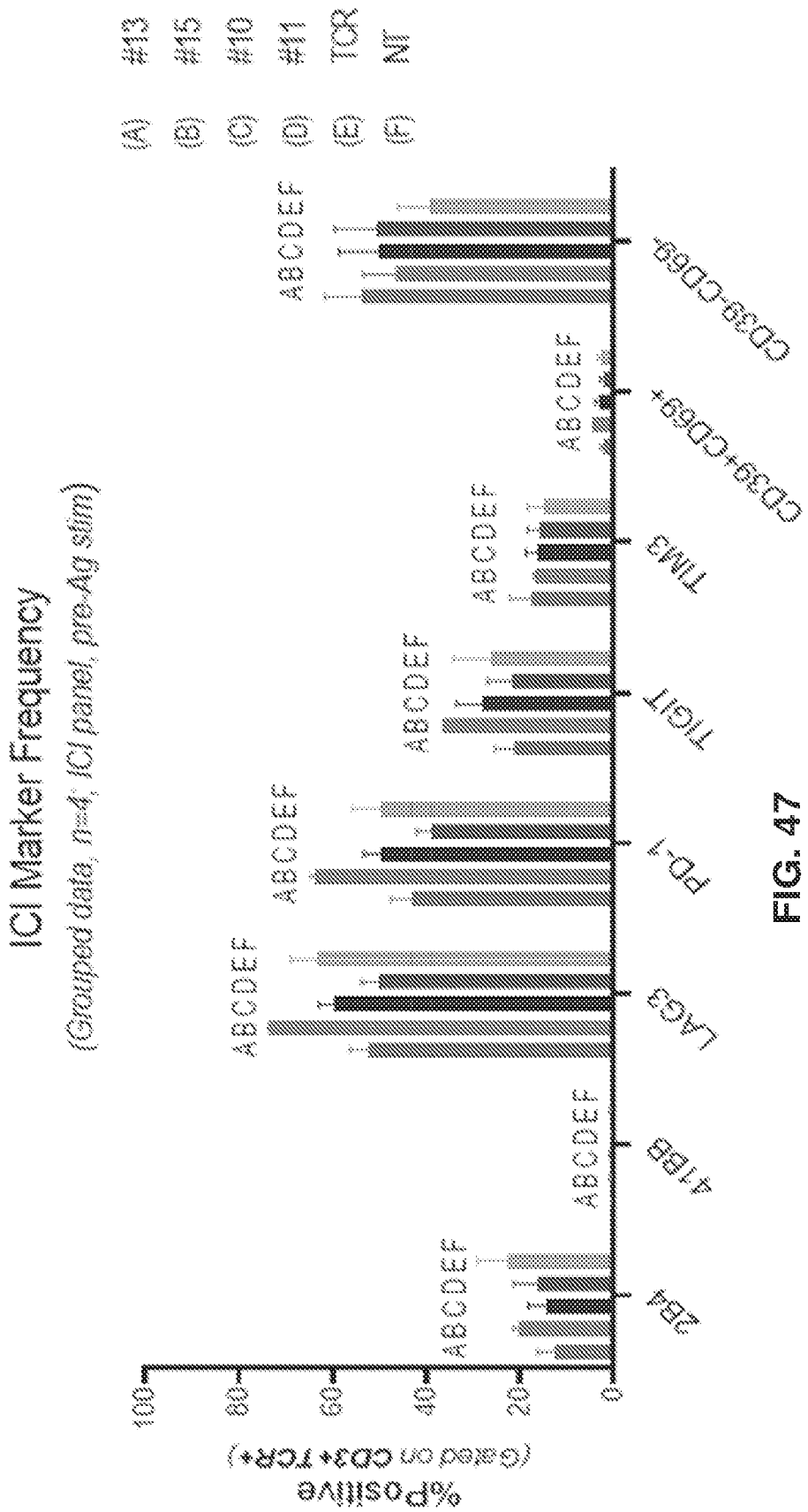

ICI Marker Frequency
(Grouped data, n=4, ICI panel, pre-Ag stim)
%Positive
(Gated on CD3+TCR+)
100
80
60
40
20
0
(A) #13
(B) #15
(C) #10
(D) #11
(E) TCR
(F) NT
2B4
4-1BB
LAG3
PD-1
TIGIT
TIM3
CD39+CD69+
CD39-CD69+
ABCDEF

FIG. 47

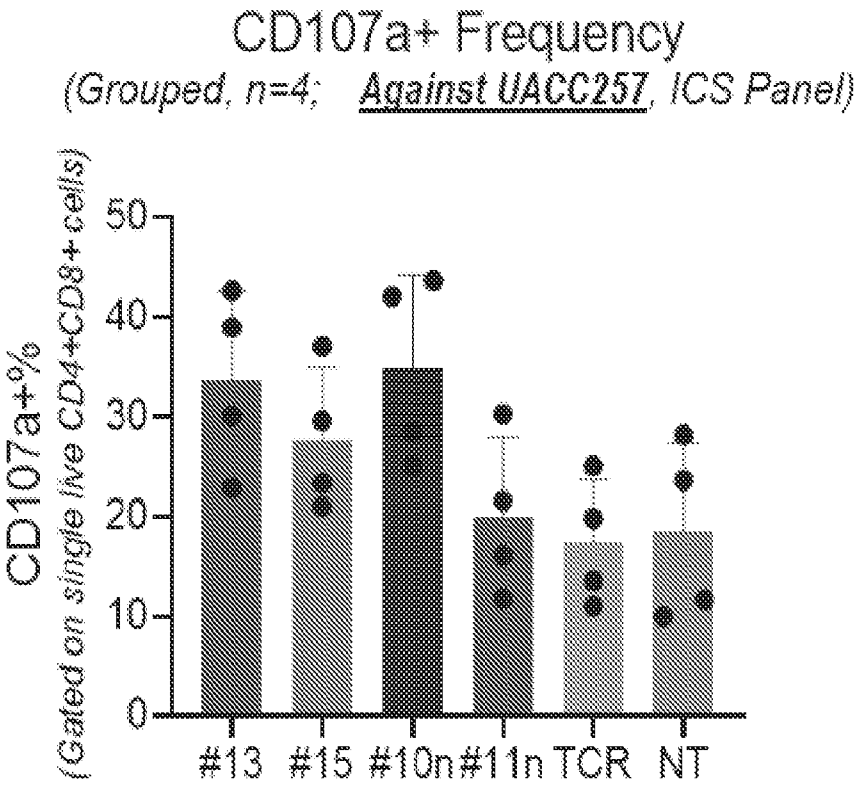


FIG. 48A

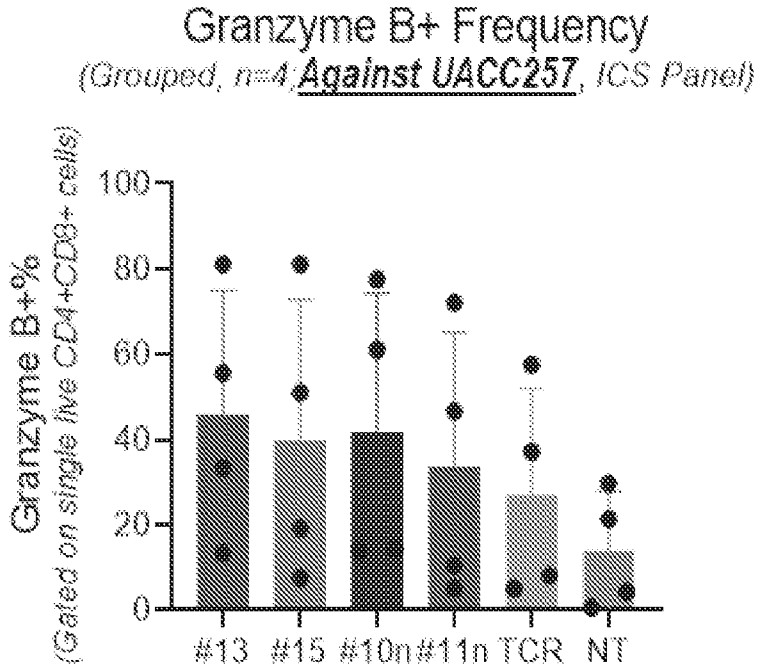


FIG. 48B

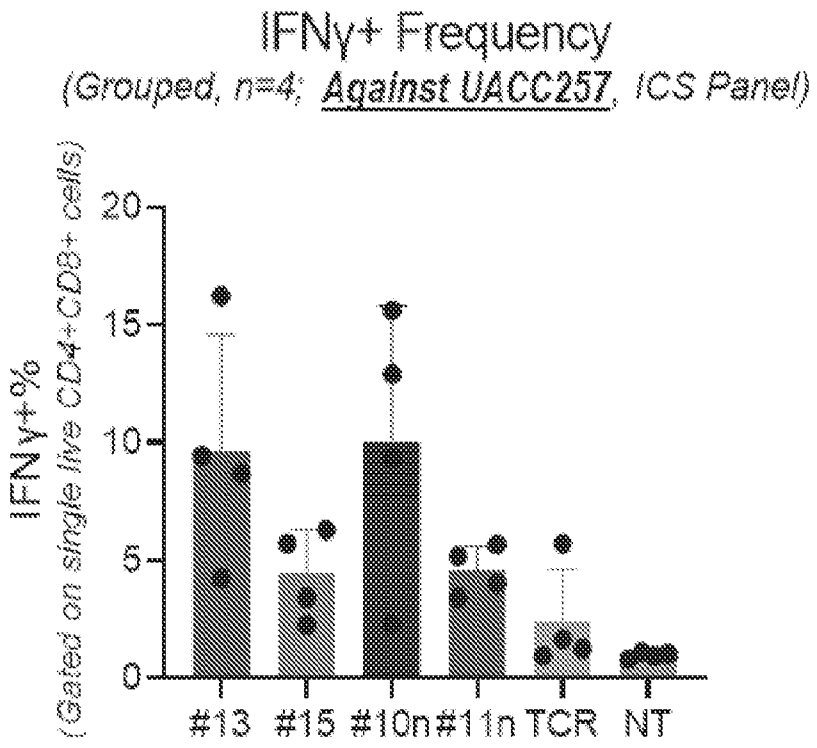


FIG. 48C

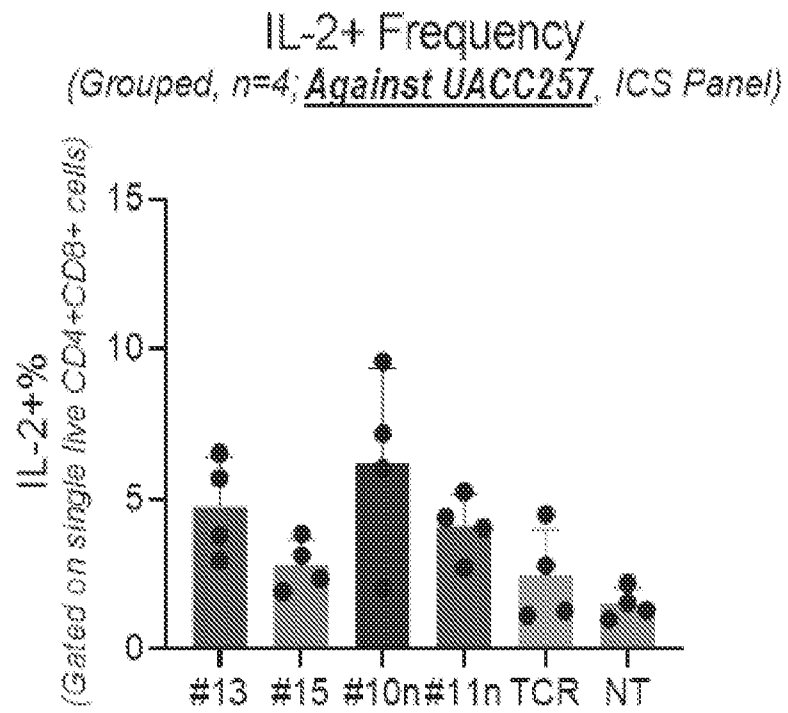


FIG. 48D

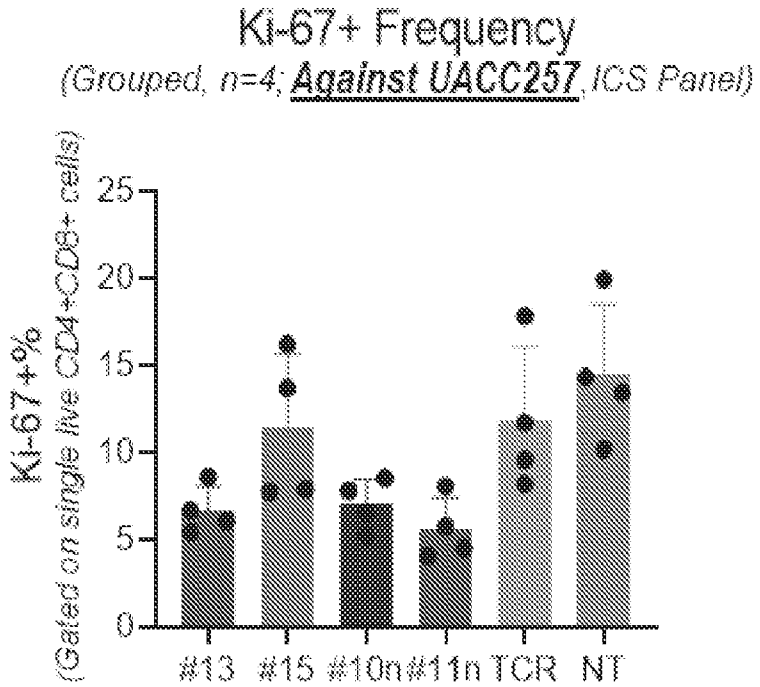


FIG. 48E

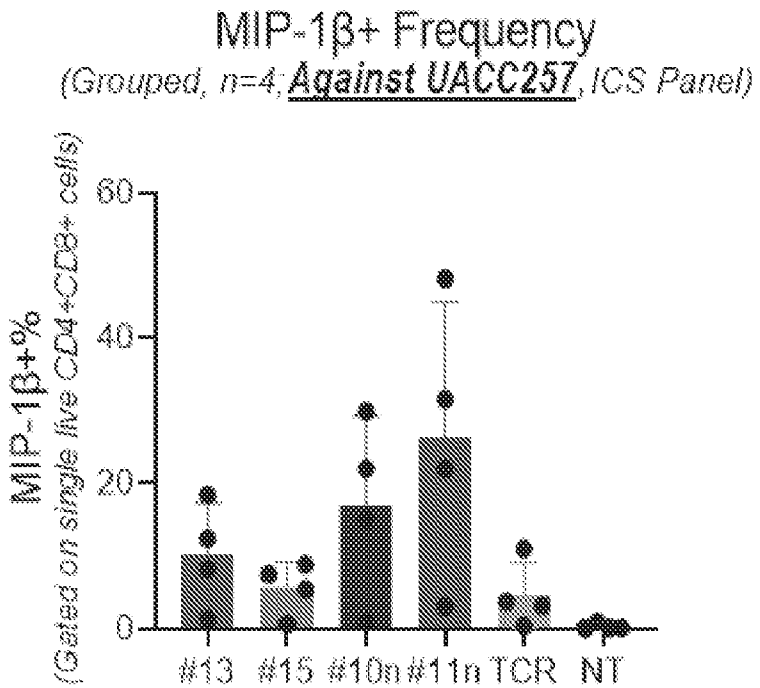


FIG. 48F

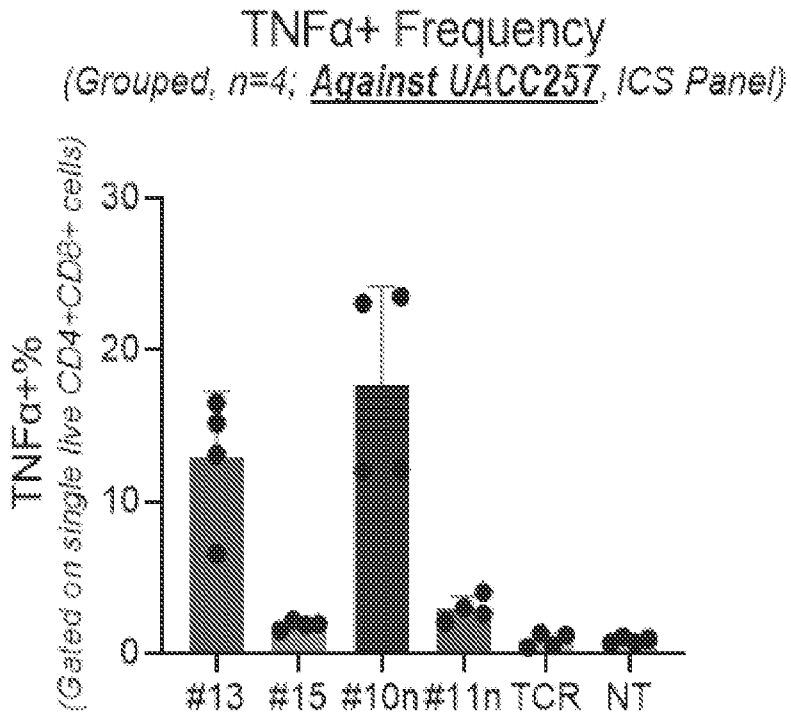

TNFα+ Frequency
(Grouped, n=4; Against UACC257, ICS Panel)
TNFα+%
(Gated on single live CD4+CD8+ cells)
30
20
10
0
#13
#15
#10n
#11n
TCR
NT

FIG. 48G

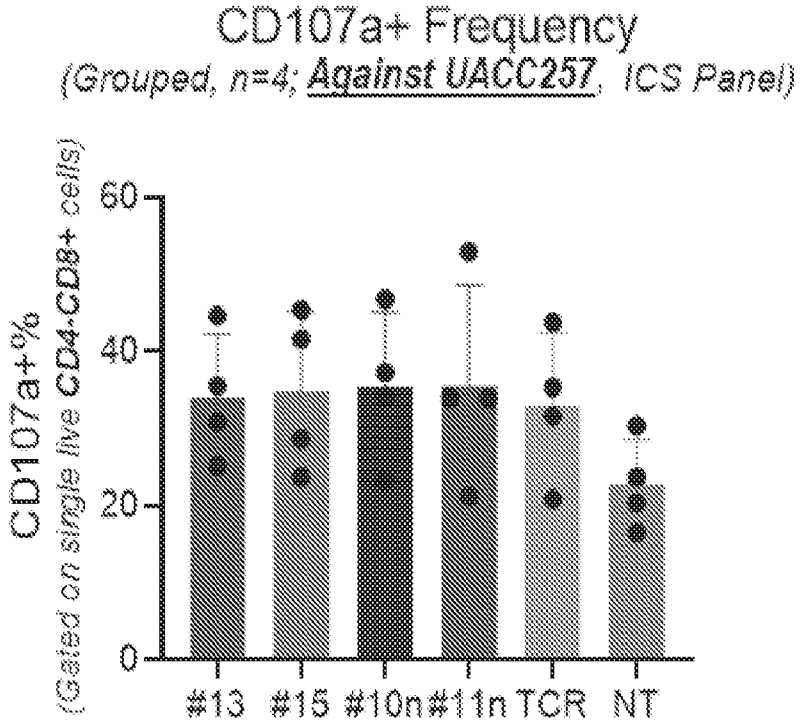


FIG. 49A

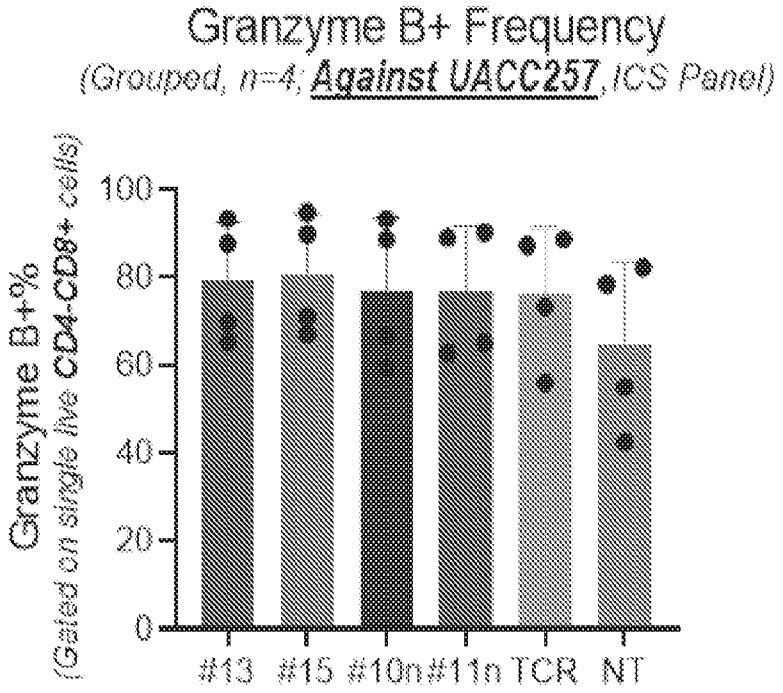


FIG. 49B

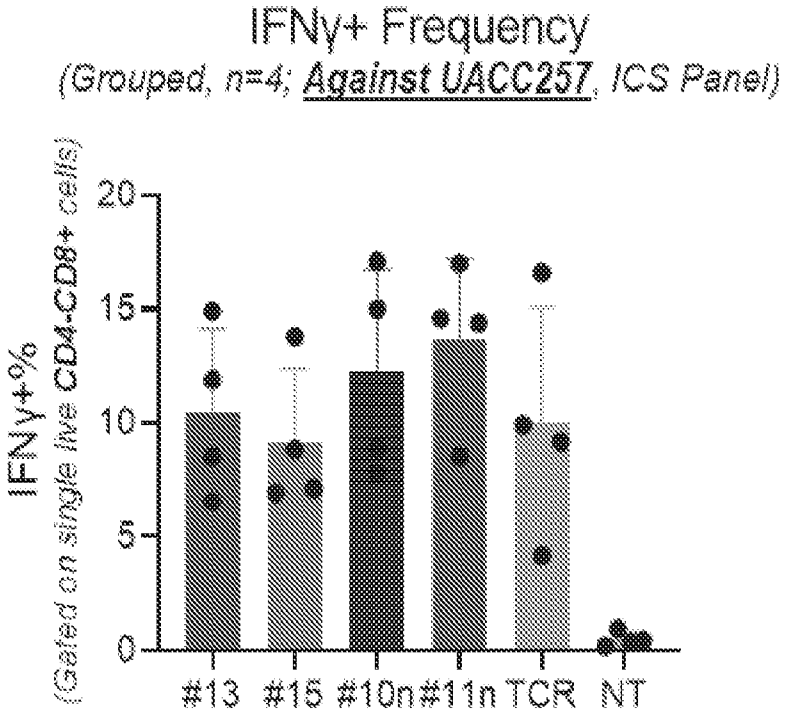


FIG. 49C

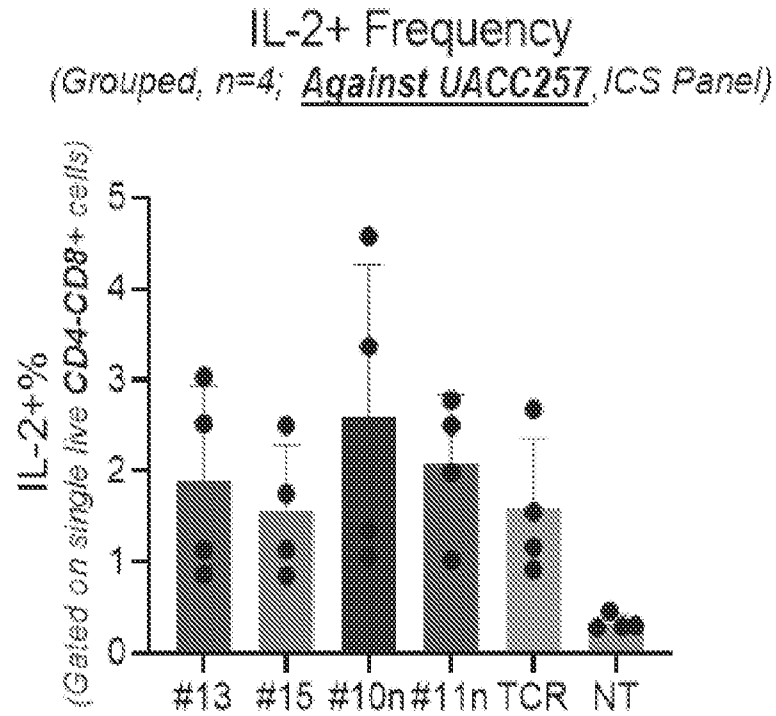


FIG. 49D

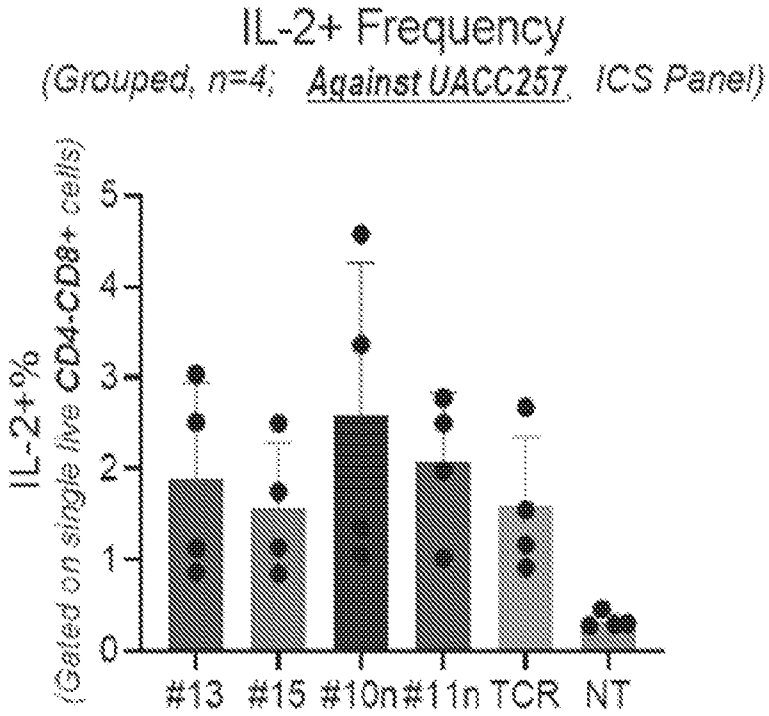


FIG. 49E

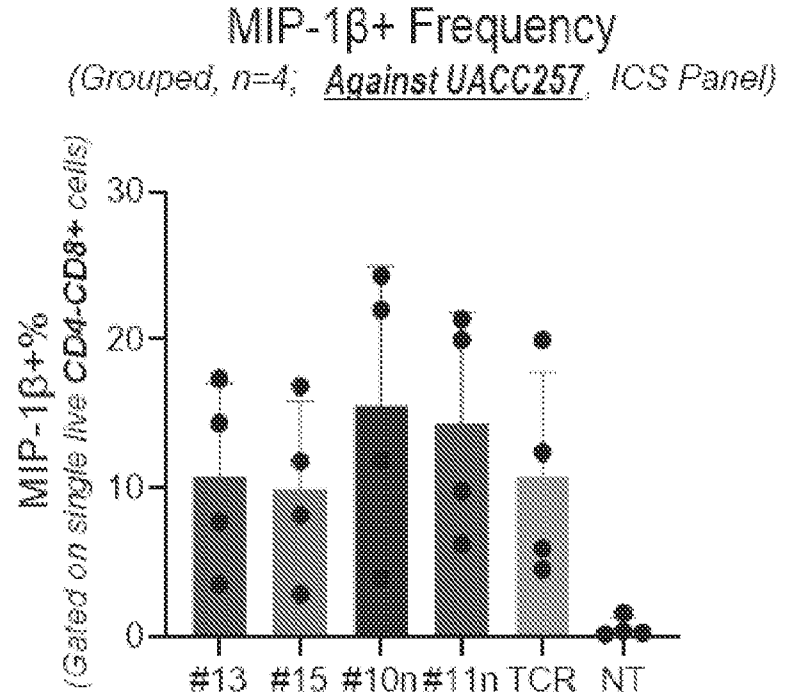


FIG. 49F

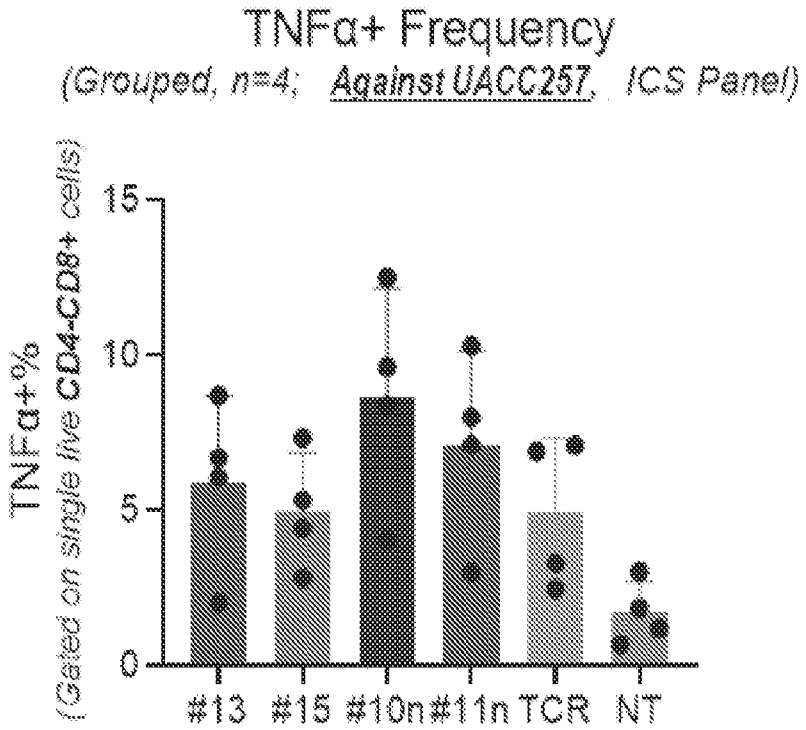

TNFα+ Frequency
(Grouped, n=4; Against UACC257, ICS Panel)
TNFα+%
(Gated on single live CD4-CD8+ cells)
15
10
5
0
#13
#15
#10n
#11n
TCR
NT

FIG. 49G

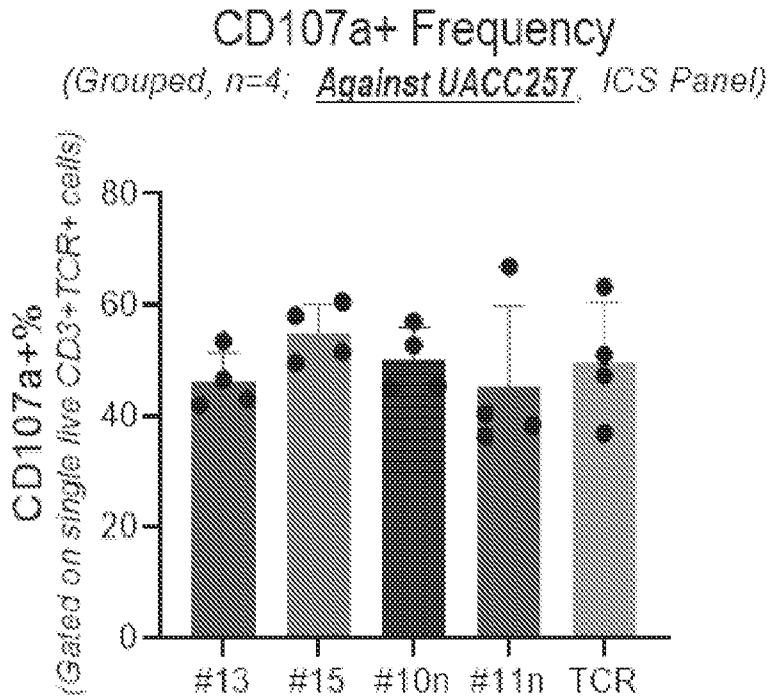


FIG. 50A

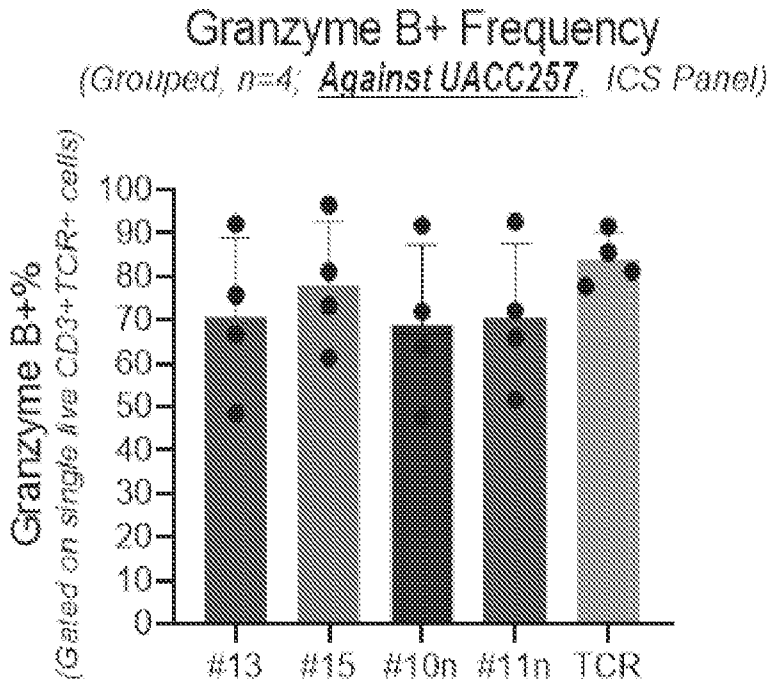


FIG. 50B

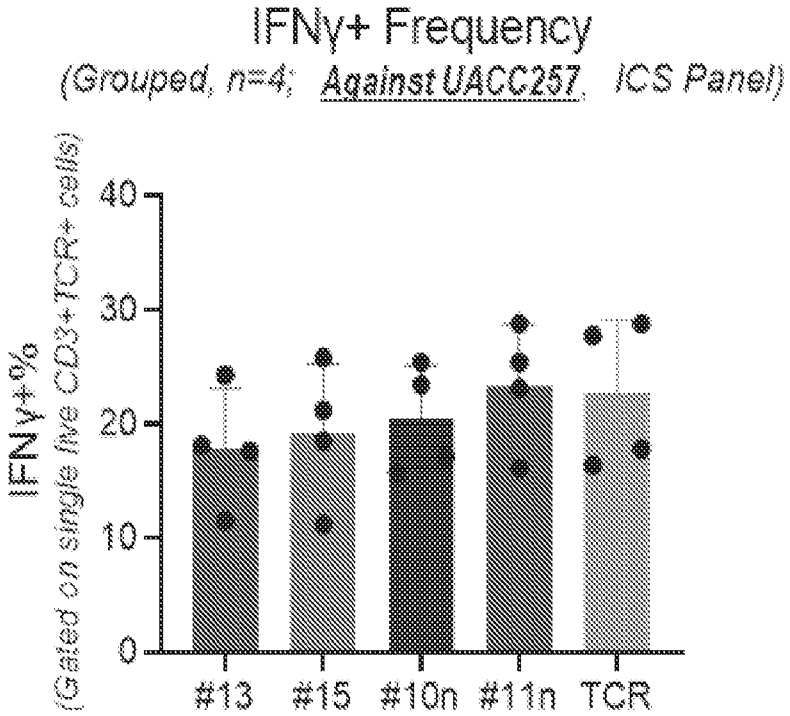


FIG. 50C

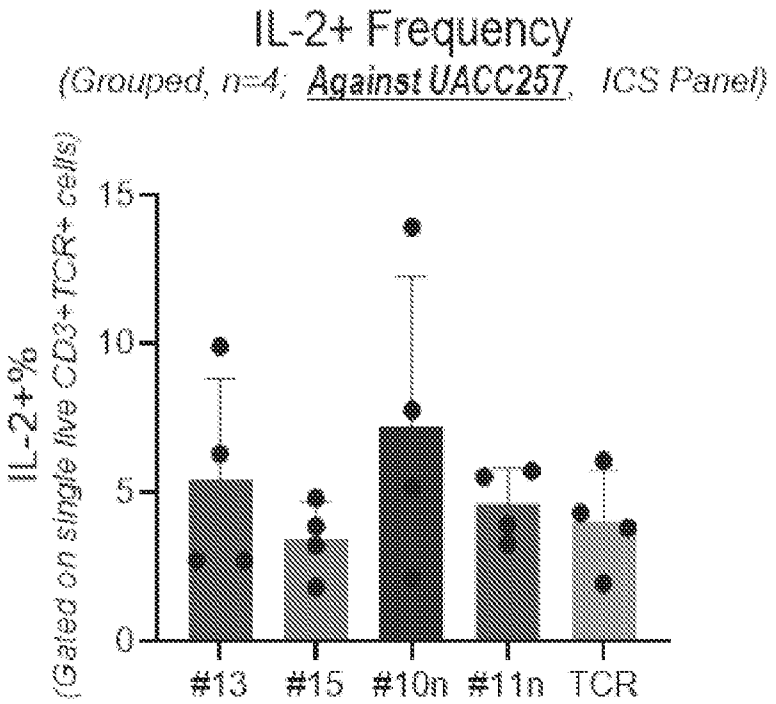


FIG. 50D

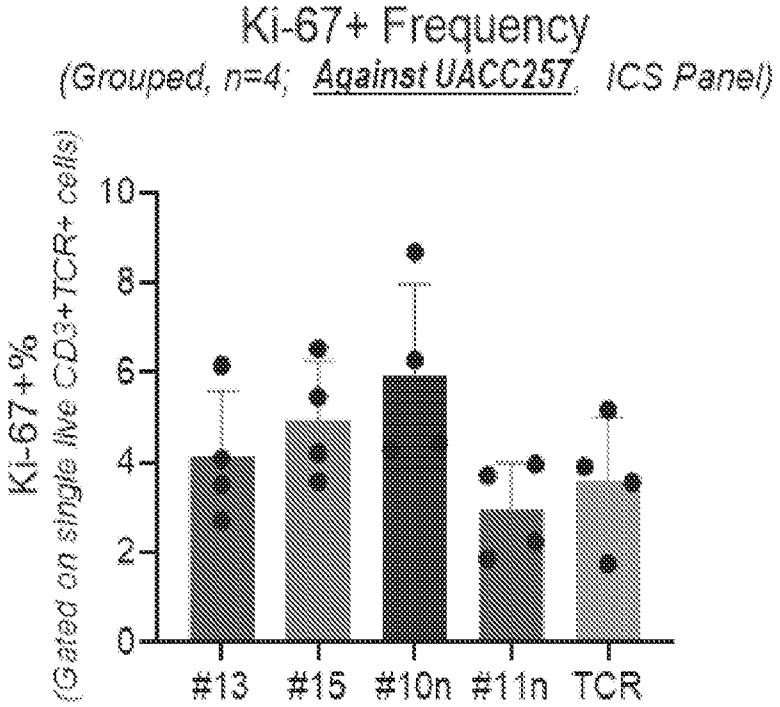


FIG. 50E

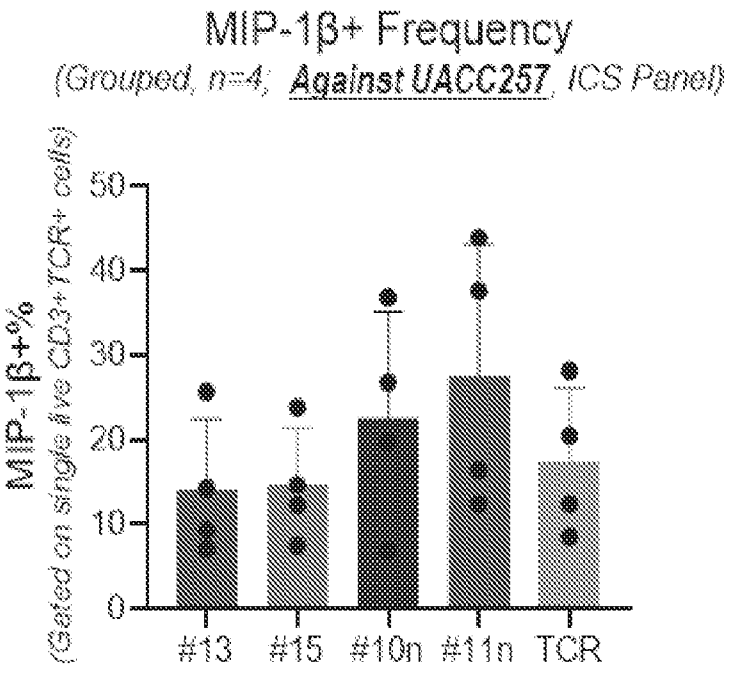


FIG. 50F

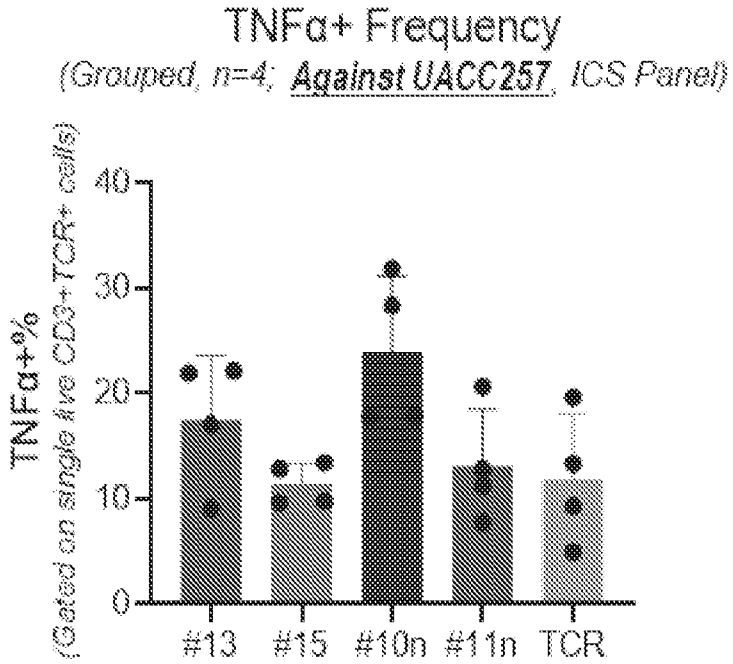

TNFα+ Frequency
(Grouped, n=4; Against UACC257, ICS Panel)
TNFα+%
(Gated on single live CD3+TCR+ cells)
40
30
20
10
0
#13
#15
#10n
#11n
TCR

FIG. 50G

Gated on CD4+CD8+

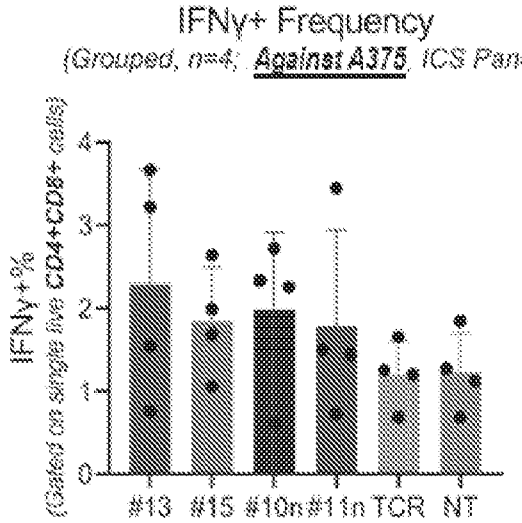


FIG. 51A

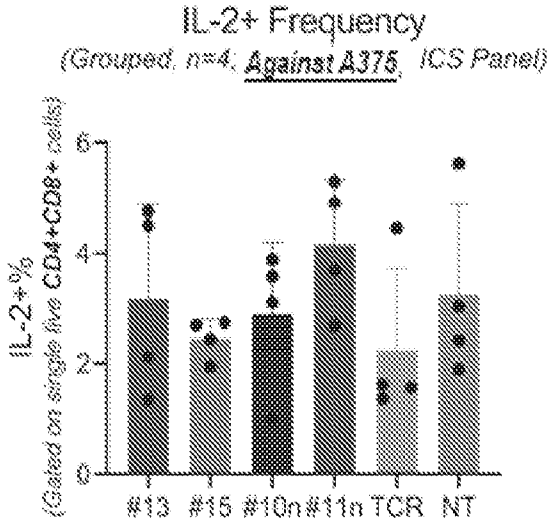


FIG. 51B

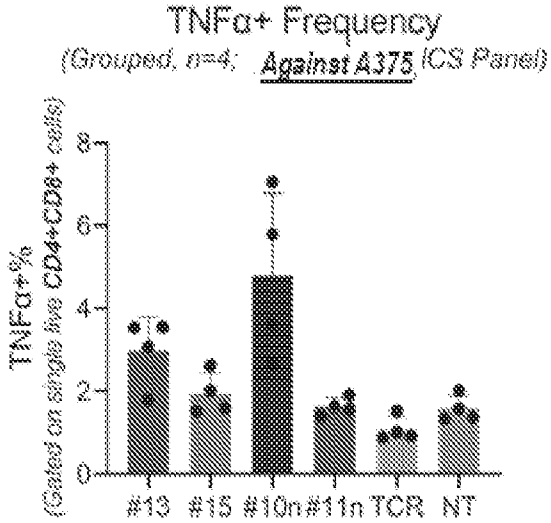


FIG. 51C

Gated on CD4-CD8+

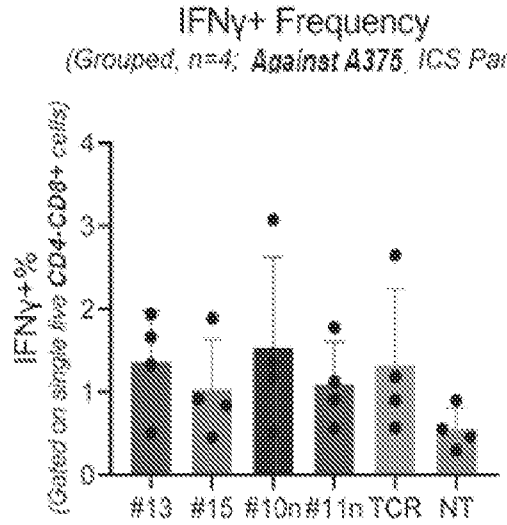


FIG. 52A

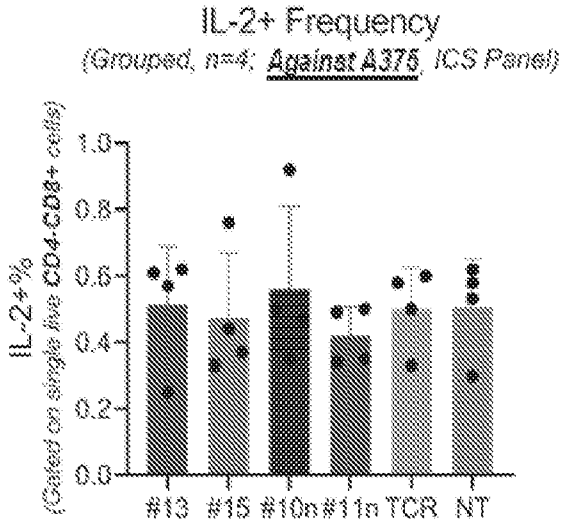


FIG. 52B

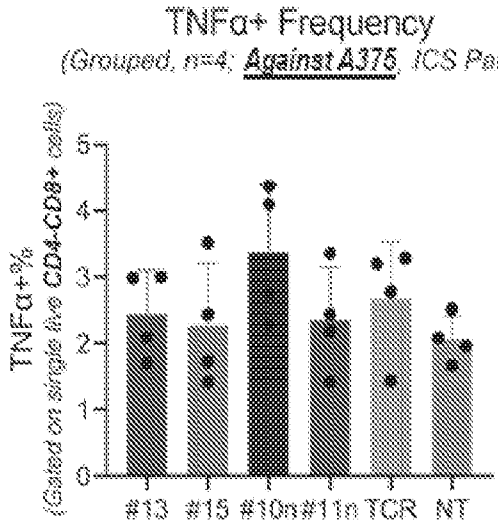


FIG. 52C

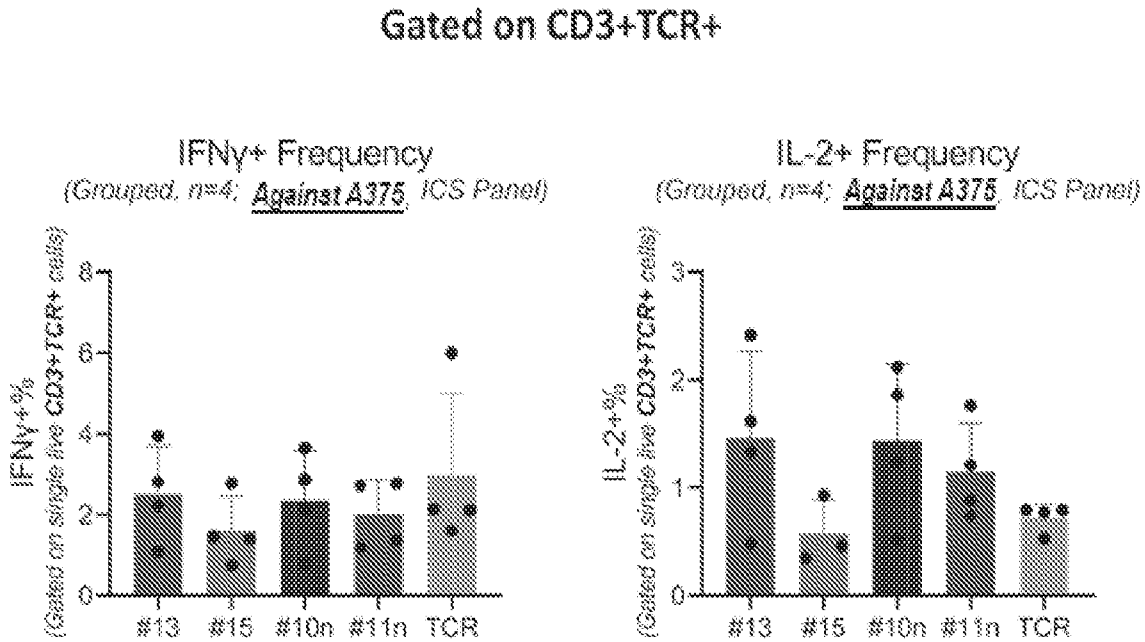


FIG. 53A

FIG. 53B

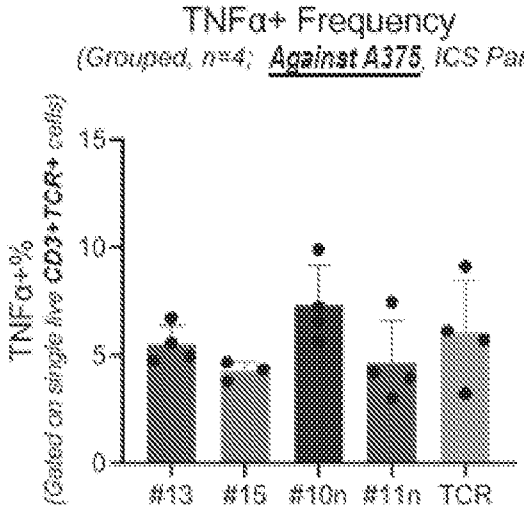


FIG. 53C

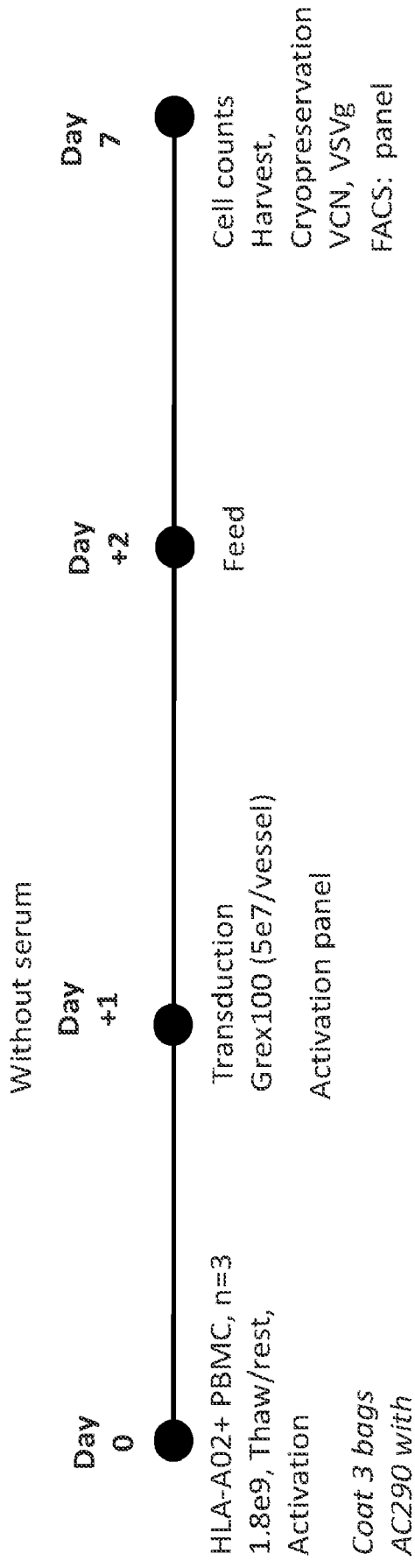

Day 0
HLA-A02+ PBMC, n=3
1.8e9, Thaw/rest, Activation
Coat 3 bags AC290 with anti-CD3/28
Day +1
Without serum
Transduction Grex100 (5e7/vessel)
Activation panel
Day +2
Feed
Day 7
Cell counts Harvest, Cryopreservation
VCN, VSVg
FACS: panel

FIG. 54

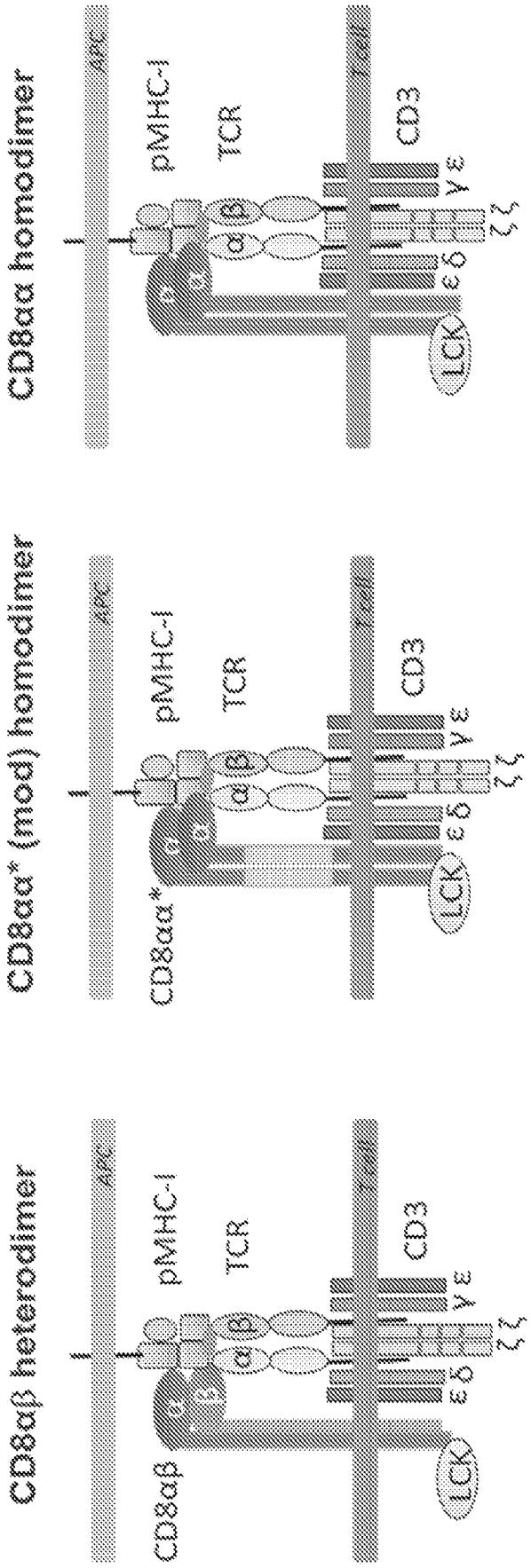


FIG. 55A

FIG. 55B

FIG. 55C

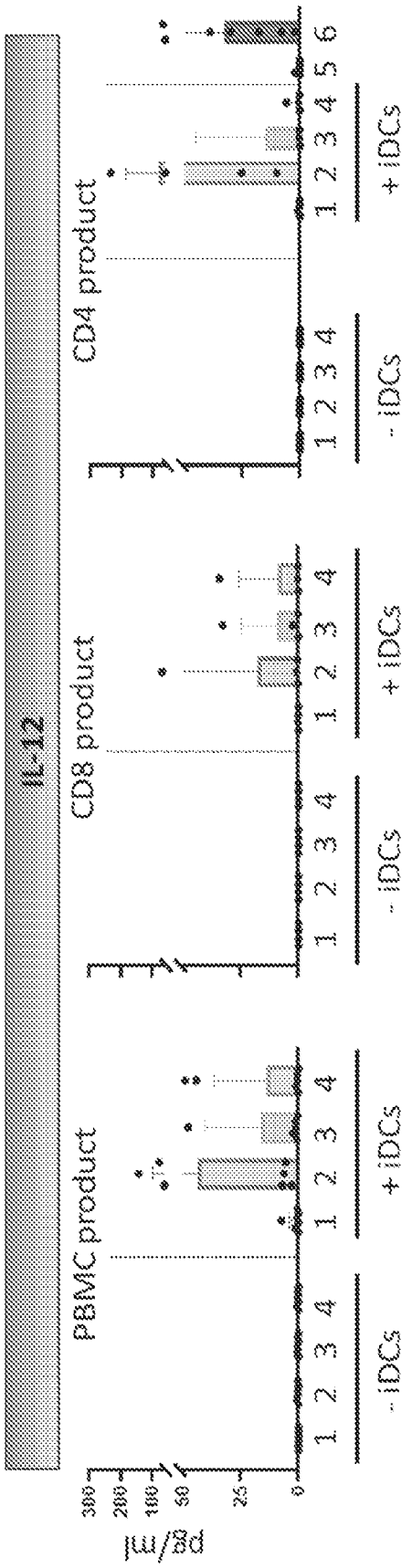

IL-12
PBMC product
CD8 product
CD4 product
pg/ml
300
200
100
50
25
0
1 2 3 4
- iDCs
+ iDCs
1 2 3 4 5 6
1 = NT
2 = Construct #10 + UACC257
3 = Construct #11 + UACC257
4 = TCR + UACC257
5 = iDCs only
6 = iDCs + LPS

FIG. 56

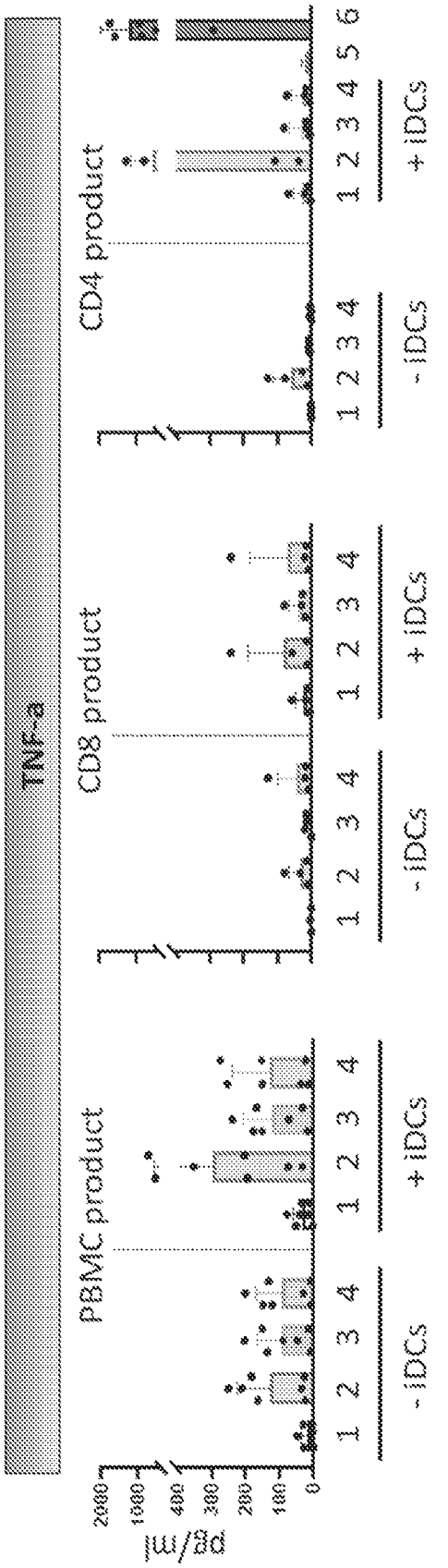

TNF-a
PBMC product
CD8 product
CD4 product
pg/ml
2000
1000
400
300
200
100
0
1 2 3 4
- iDCs
+ iDCs
1 2 3 4 5 6
1 = NT
2 = Construct #10 + UACC257
3 = Construct #11 + UACC257
4 = TCR + UACC257
5 = iDCs only
6 = iDCs + LPS

FIG. 57

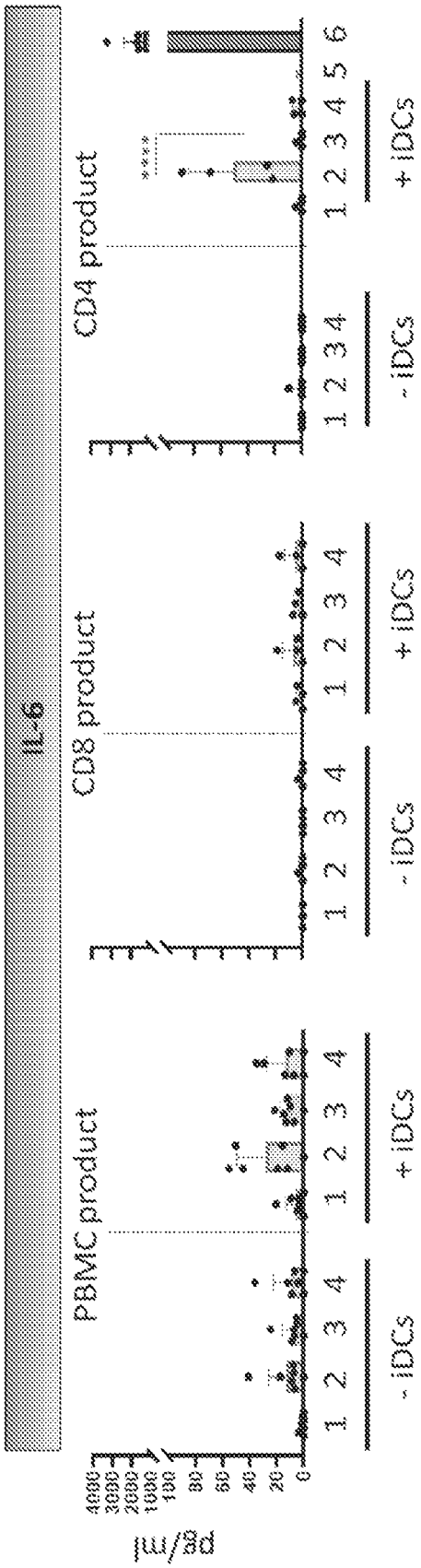

IL-6
PBMC product
CD8 product
CD4 product
pg/ml
4000
3000
2000
1000
100
80
60
40
20
0
1 2 3 4
- iDCs
+ iDCs
****
1 = NT
2 = Construct #10 + UACC257
3 = Construct #11 + UACC257
4 = TCR + UACC257
5 = iDCs only
6 = iDCs + LPS

FIG. 58

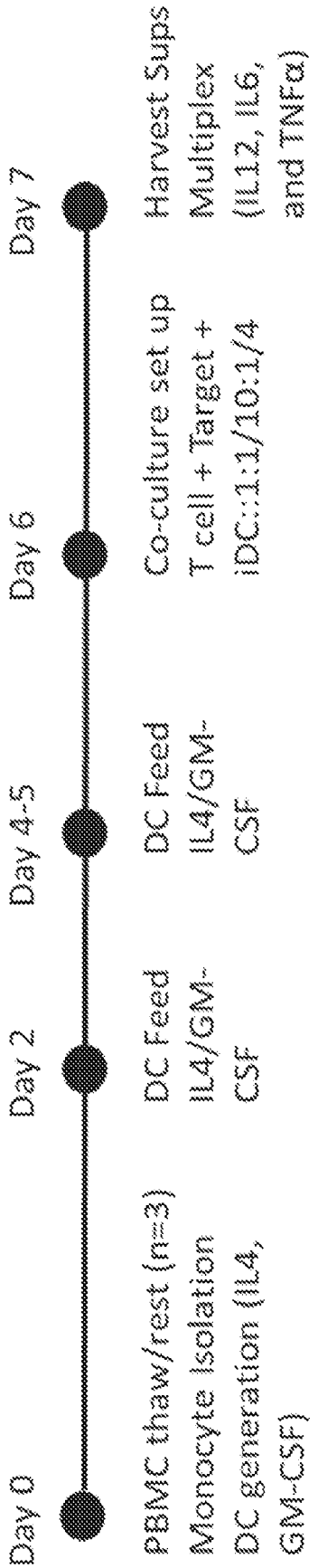

Day 0
PBMC thaw/rest (n=3)
Monocyte Isolation
DC generation (IL4, GM-CSF)
Day 2
DC Feed
IL4/GM-CSF
Day 4-5
DC Feed
IL4/GM-CSF
Day 6
Co-culture set up
T cell + Target + iDC::1:1/10:1/4
Day 7
Harvest Sups
Multiplex (IL12, IL6, and TNFα)

FIG. 59

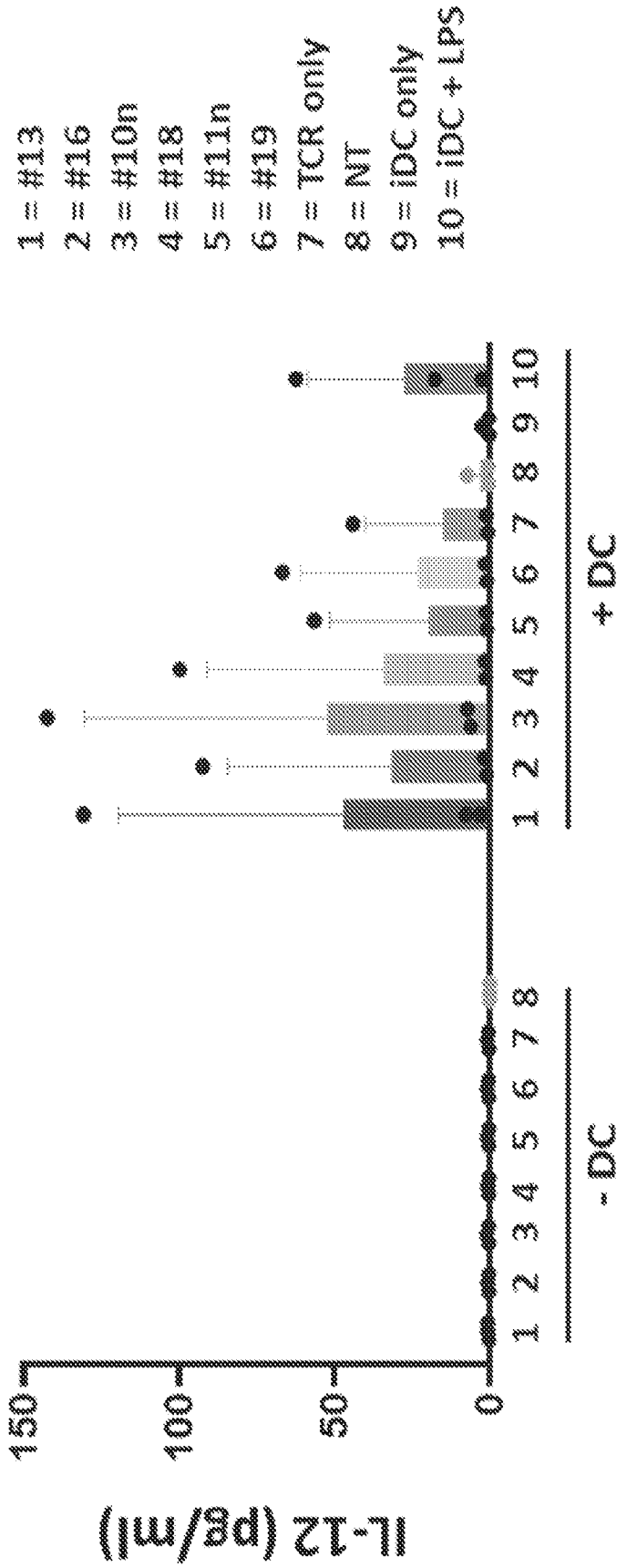

1 = #13
2 = #16
3 = #10n
4 = #18
5 = #11n
6 = #19
7 = TCR only
8 = NT
9 = iDC only
10 = iDC + LPS
IL-12 (pg/ml)
150
100
50
0
1 2 3 4 5 6 7 8
- DC
1 2 3 4 5 6 7 8 9 10
+ DC

FIG. 60

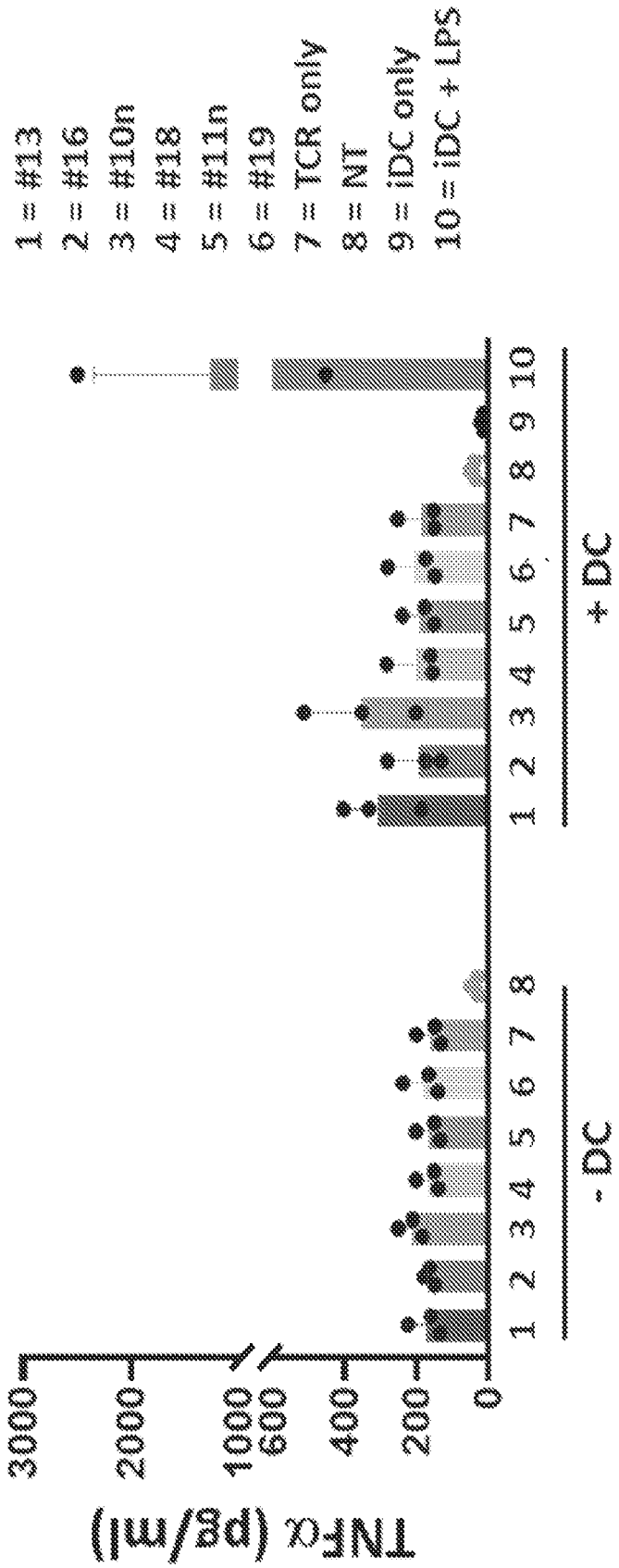

1 = #13
2 = #16
3 = #10n
4 = #18
5 = #11n
6 = #19
7 = TCR only
8 = NT
9 = iDC only
10 = iDC + LPS
TNFα (pg/ml)
3000
2000
1000
600
400
200
0
1 2 3 4 5 6 7 8
- DC
1 2 3 4 5 6 7 8 9 10
+ DC

FIG. 61

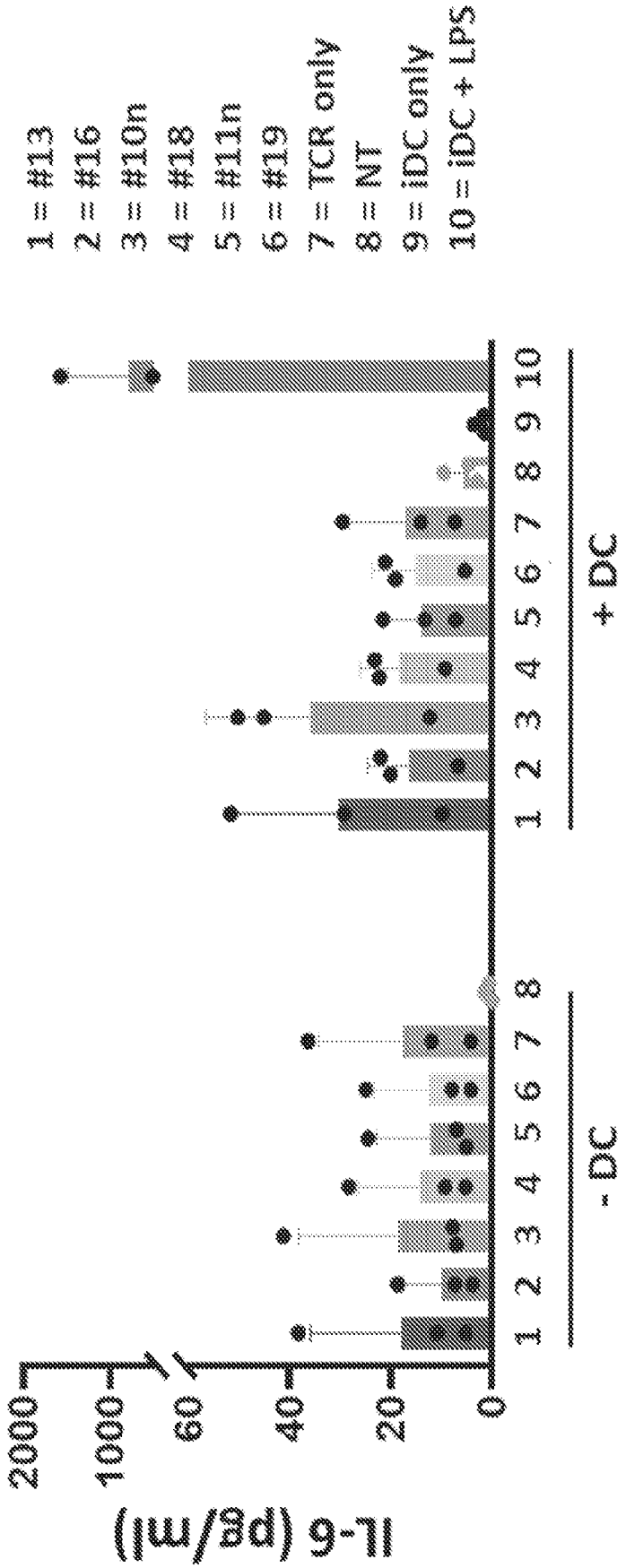

1 = #13
2 = #16
3 = #10n
4 = #18
5 = #11n
6 = #19
7 = TCR only
8 = NT
9 = iDC only
10 = iDC + LPS
IL-6 (pg/ml)
2000
1000
60
40
20
0
1 2 3 4 5 6 7 8
- DC
1 2 3 4 5 6 7 8 9 10
+ DC

FIG. 62

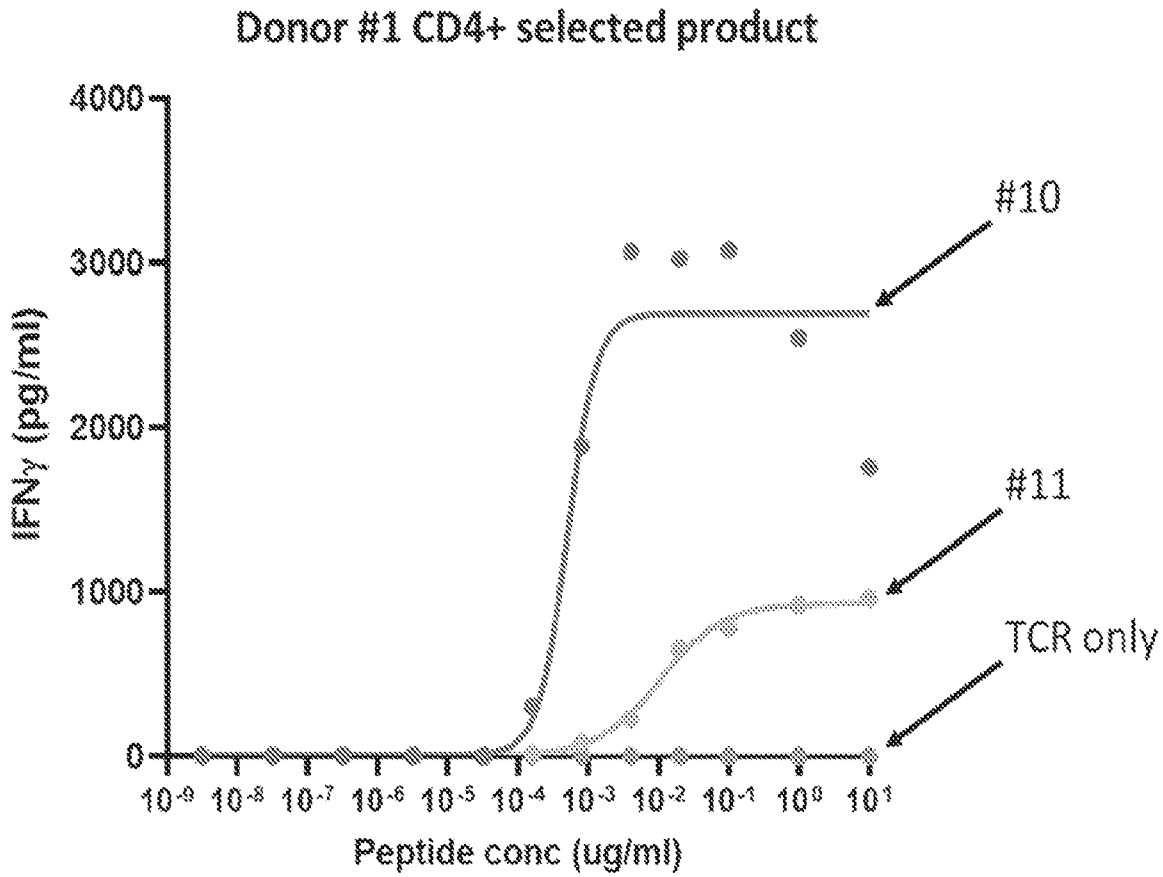

Donor #1 CD4+ selected product
4000
3000
2000
1000
0
IFNγ (pg/ml)
#10
#11
TCR only
10-9 10-8 10-7 10-6 10-5 10-4 10-3 10-2 10-1 100 101
Peptide conc (ug/ml)

FIG. 63A

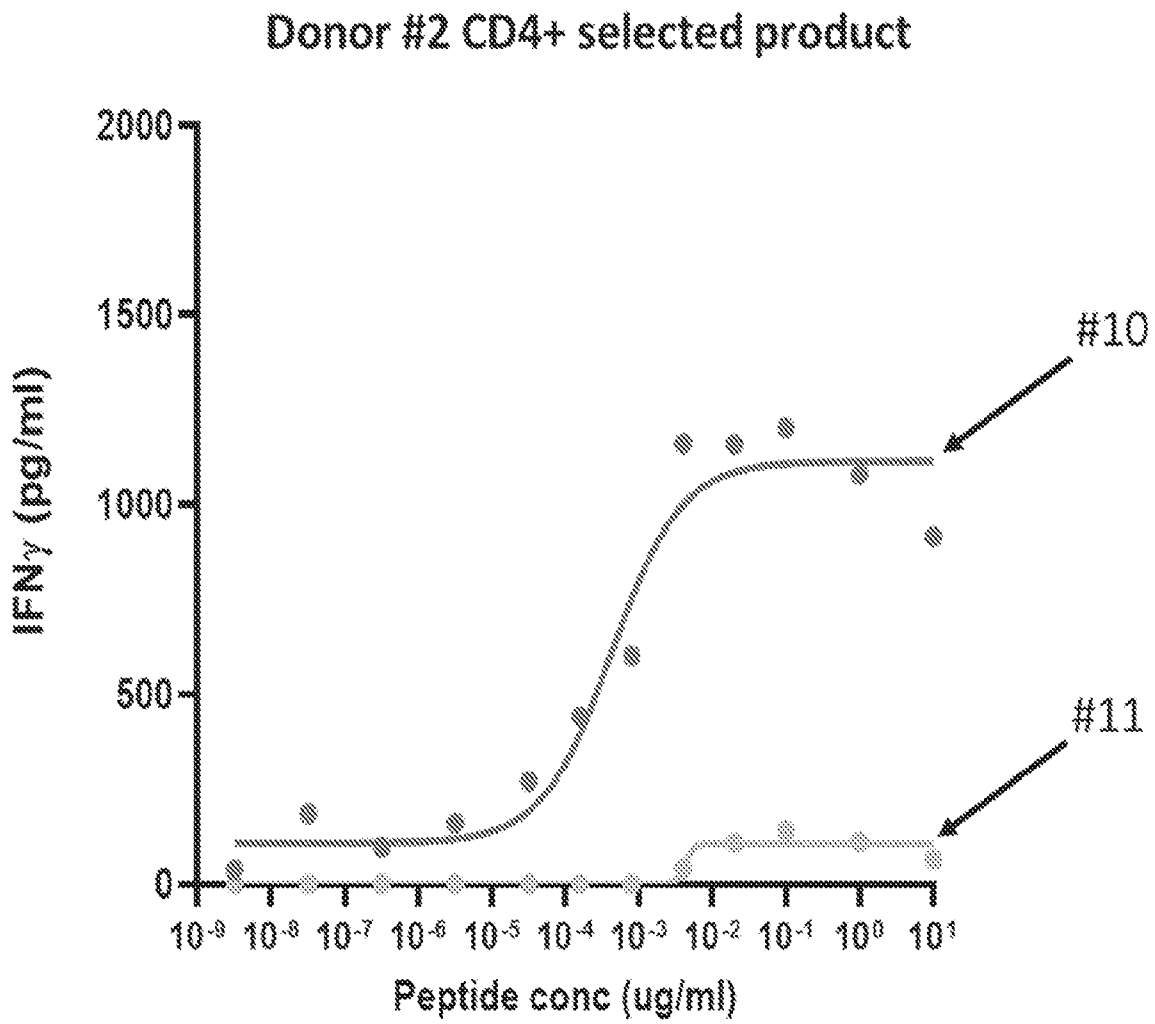

Donor #2 CD4+ selected product
2000
1500
1000
500
0
IFNγ (pg/ml)
#10
#11
10-9 10-8 10-7 10-6 10-5 10-4 10-3 10-2 10-1 100 101
Peptide conc (ug/ml)

FIG. 63B

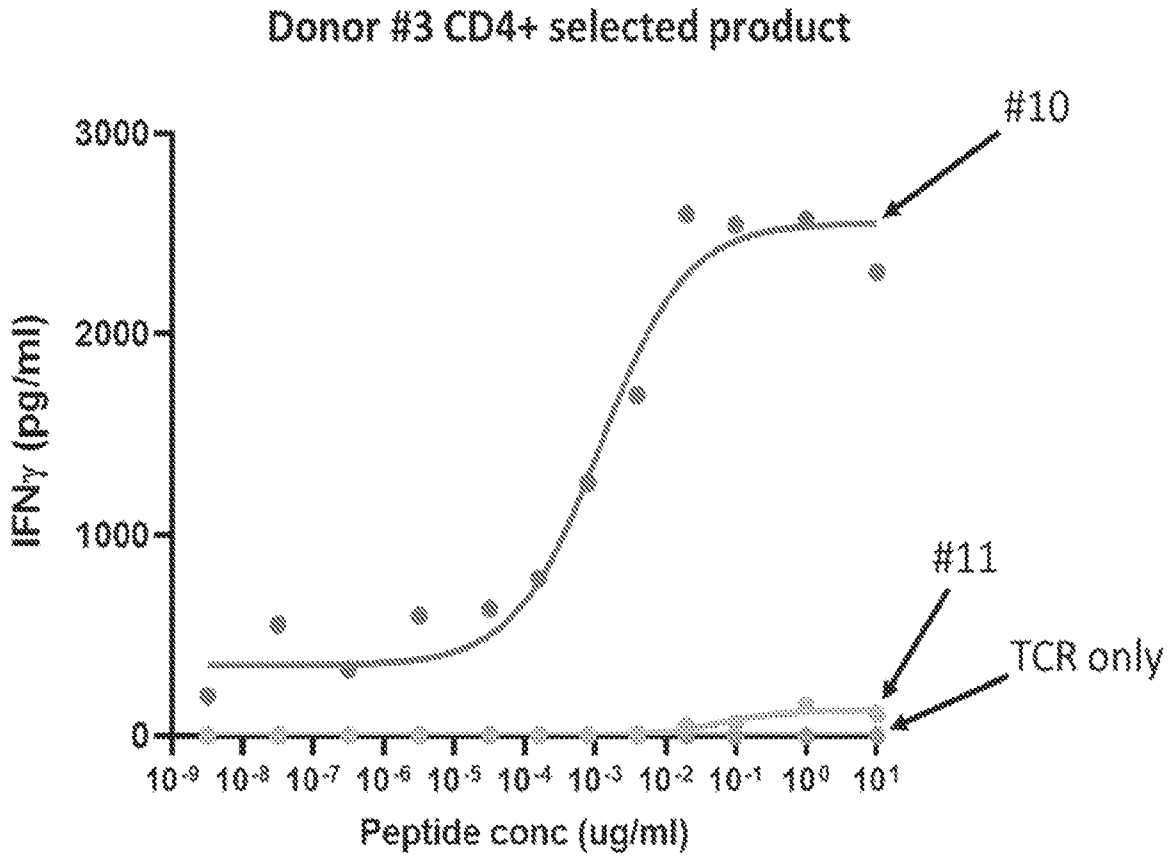
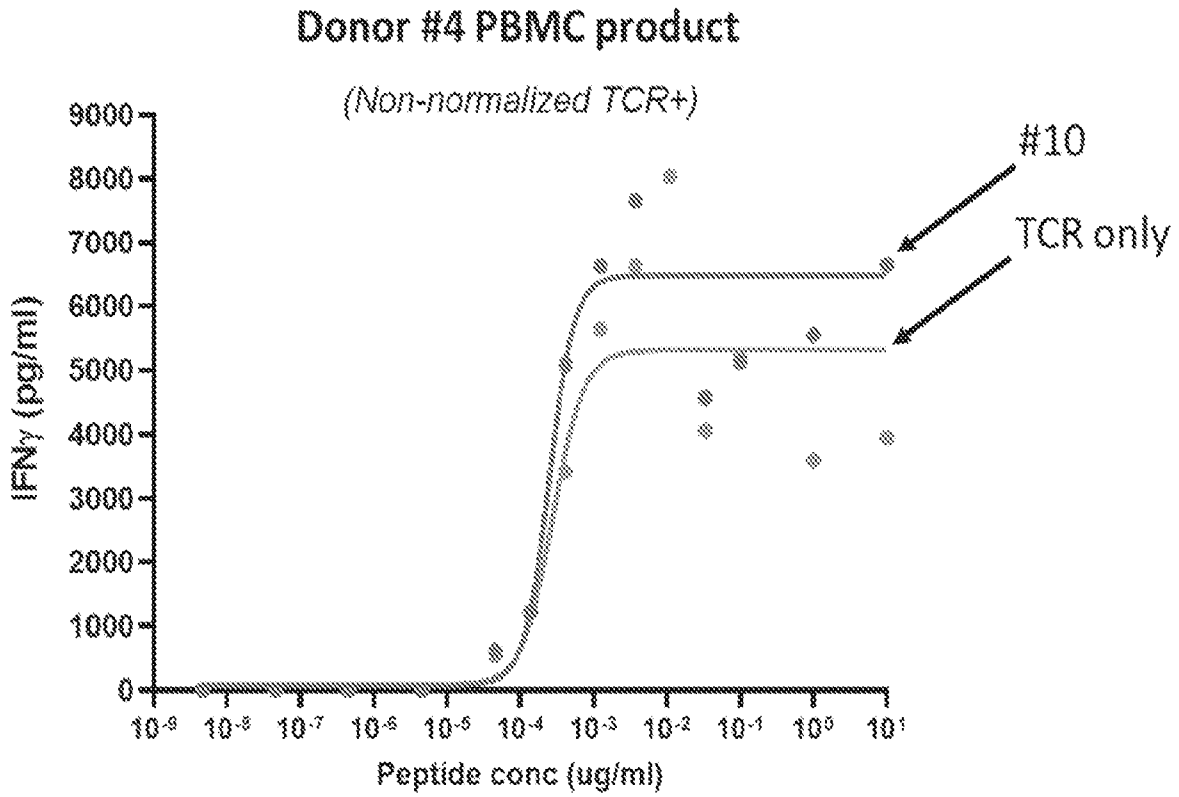

Donor #3 CD4+ selected product
#10
#11
TCR only
IFNγ (pg/ml)
3000
2000
1000
0
10-9 10-8 10-7 10-6 10-5 10-4 10-3 10-2 10-1 100 101
Peptide conc (ug/ml)

FIG. 63C

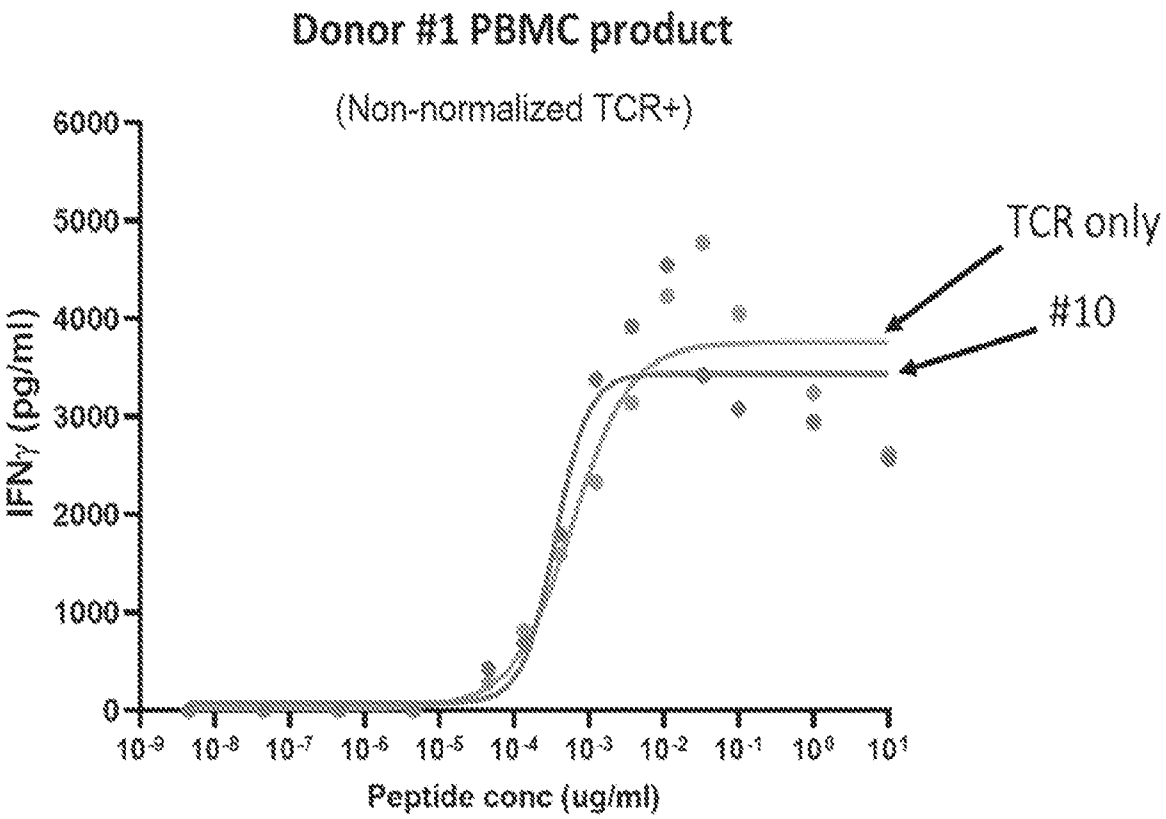
CD4+ selected	CD8βα TCR	CD8α* TCR	TCR
Donor #1	0.51	10.6	-
Donor #2	0.43	4.0	-
Donor #3	1.21	60.0	-

FIG. 63D



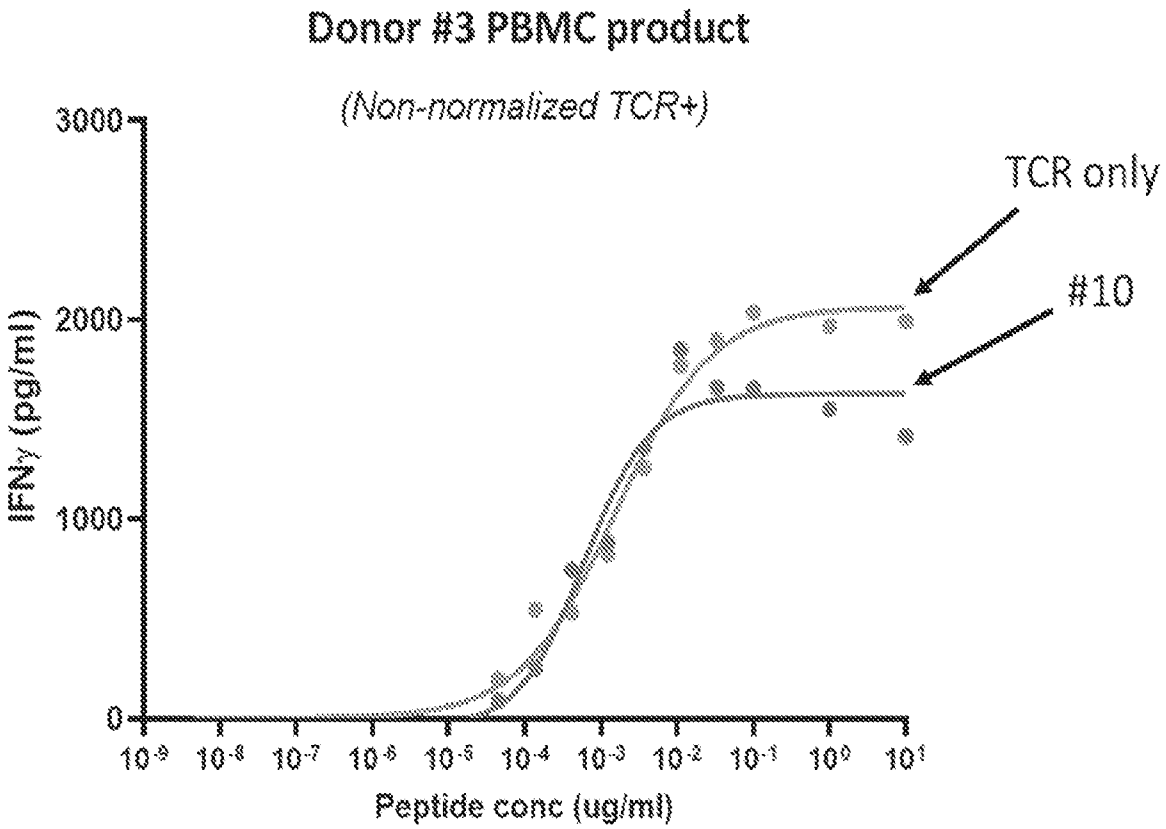
	#10	TCR
EC50 (ng/ml)	0.246	0.276
%CD3+tet+	42.2	17.1

FIG. 64A



	#10	TCR
EC50 (ng/ml)	0.360	0.591
%CD3+tet+	54.7	24.2

FIG. 64B



	#10	TCR
EC50 (ng/ml)	0.608	1.549
%CD3+tet+	45.5	16.6

FIG. 64C

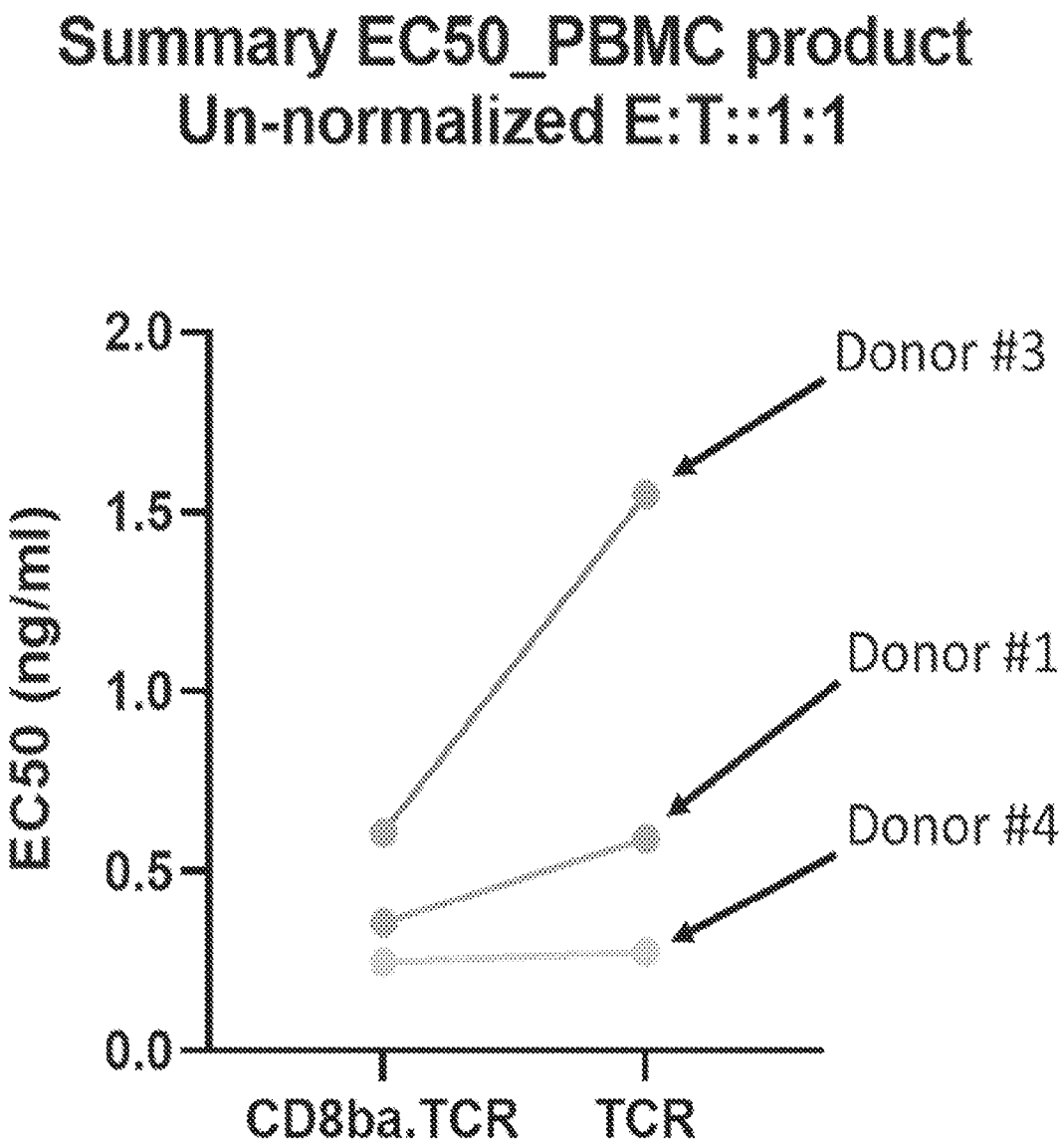
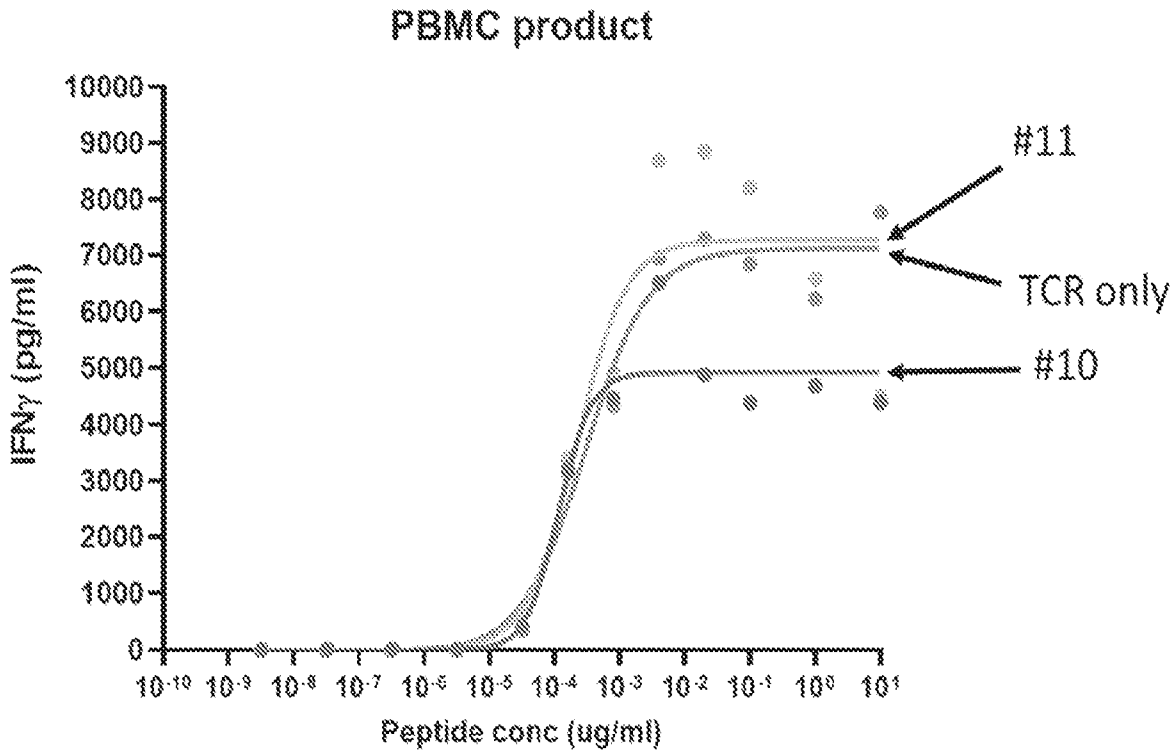
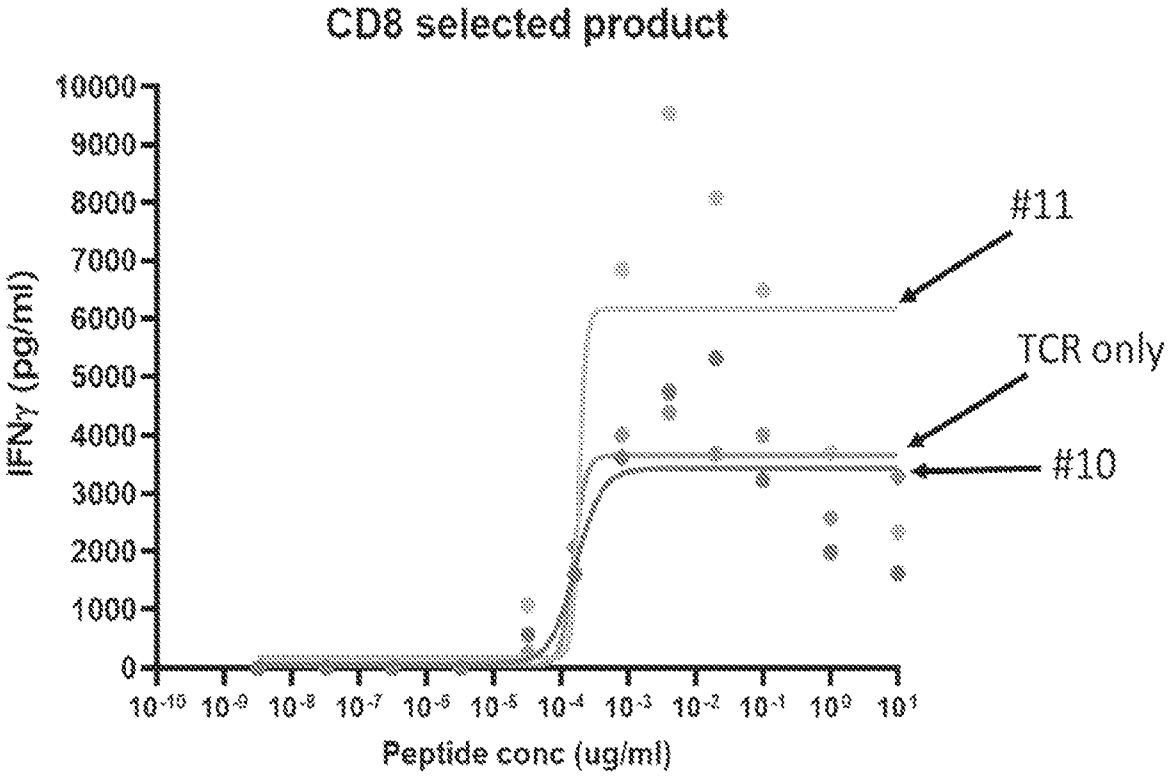

Summary EC50_PBMC product
Un-normalized E:T::1:1
EC50 (ng/ml)
2.0
1.5
1.0
0.5
0.0
CD8ba.TCR
TCR
Donor #3
Donor #1
Donor #4

FIG. 64D



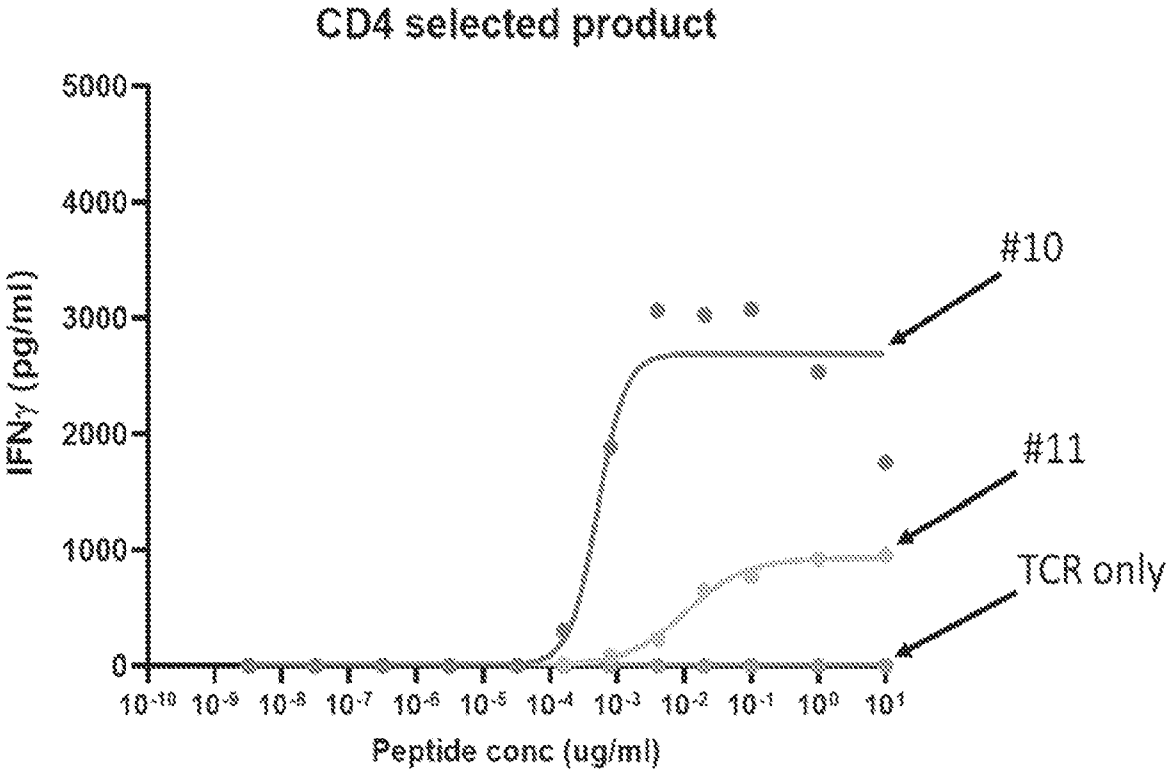
	#10	TCR
EC50 (ng/ml)	0.116	0.295

FIG. 65A



	#10	TCR
EC50 (ng/ml)	0.166	0.153

FIG. 65B



	#10	TCR
EC50 (ng/ml)	0.51	-

FIG. 65C