

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2019216982 B2**

(54) Title
Non-HLA restricted T cell receptors and uses thereof

(51) International Patent Classification(s)
C07K 14/705 (2006.01) **C12N 5/0735** (2010.01)
A61K 35/15 (2015.01) **C12N 5/0783** (2010.01)
A61K 35/17 (2015.01) **C12N 5/0789** (2010.01)
A61K 35/545 (2015.01) **C12N 5/10** (2006.01)
C07K 14/725 (2006.01)

(21) Application No: **2019216982** (22) Date of Filing: **2019.02.11**

(87) WIPO No: **WO19/157454**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/629,072	2018.02.11	US

(43) Publication Date: **2019.08.15**

(44) Accepted Journal Date: **2024.07.04**

(71) Applicant(s)
Memorial Sloan-Kettering Cancer Center

(72) Inventor(s)
SADELAIN, Michel;MANSILLA-SOTO, Jorge A.;EYQUEM, Justin;DOBRIN, Anton

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2017180989 A2
TORIKAI, H. et al, "A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR", Blood, 2012, vol. 119, no. 24.
EYQUEM, J. et al, "Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection", Nature, 2017, vol. 543, no. 7653, pages 113-117



(51) International Patent Classification:

C07K 14/705 (2006.01) C12N 5/10 (2006.01)
C07K 14/725 (2006.01) C12N 5/0735 (2010.01)
A61K 35/17 (2015.01) C12N 5/0783 (2010.01)
A61K 35/15 (2015.01) C12N 5/0789 (2010.01)
A61K 35/545 (2015.01)

(21) International Application Number:

PCT/US2019/017525

(22) International Filing Date:

11 February 2019 (11.02.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/629,072 11 February 2018 (11.02.2018) US

(71) Applicant: **MEMORIAL SLOAN-KETTERING CANCER CENTER** [US/US]; 1275 York Avenue, New York, NY 10065 (US).

(72) Inventors: **SADELAIN, Michel**; 15 West 84th Street, Apt. 2C, New York, NY 10024 (US). **MANSILLA-SOTO, Jorge, A.**; 72-72 112th Street, Apt. 3A, Forest Hills, NY 11375 (US). **EYQUEM, Justin**; 1275 York Avenue, New

York, NY 10065 (US). **DOBRIN, Anton**; 303 East 71st Street, Apt. 4F, New York, NY 10021 (US).

(74) Agent: **LENDARIS, Steven P.** et al.; Baker Botts LLP, 30 Rockefeller Plaza, New York, NY 10112-4498 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: NON-HLA RESTRICTED T CELL RECEPTORS AND USES THEREOF

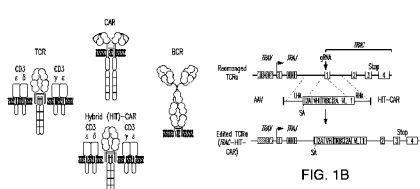


FIG. 1B

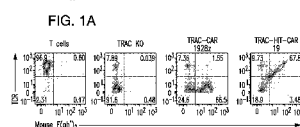


FIG. 1C

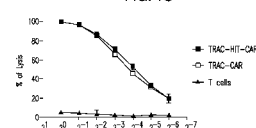


FIG. 1D

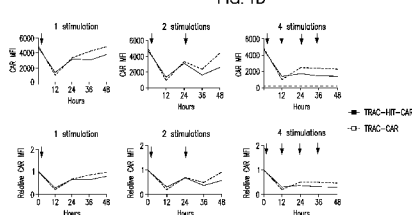


FIG. 1E

(57) Abstract: The presently disclosed subject matter provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to novel designs of T cell receptors (TCRs) and engineered immunoresponsive cells comprising the same. The novel TCR binds to an antigen in an HLA-independent manner. In certain embodiments, the novel TCR provides enhanced sensitivity for a target gene having a low expression level.

Published:

— *with international search report (Art. 21(3))*

NON-HLA RESTRICTED T CELL RECEPTORS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No. 62/629,072, filed February 11, 2018, the content of which is incorporated by reference in its entirety, and to which priority is claimed.

INTRODUCTION

10 The presently disclosed subject matter provides methods and compositions for enhancing the immune response toward cancers and pathogens. It relates to novel designs of T cell receptors (TCRs) and engineered immunoresponsive cells comprising the same. The engineered immunoresponsive cells comprising the novel TCRs are antigen-directed.

BACKGROUND OF THE INVENTION

15 Adoptive immunotherapy using antigen recognizing receptors (e.g., chimeric antigen receptors (CARs)) has shown remarkable clinical results in the treatment of leukemia and is one of the most promising new strategies to treat cancer. To generate CAR therapies, current clinical protocols employ autologous T cells and randomly integrating vectors, including gamma-retroviral, lentiviral and transposons, which all result in semi-random integration and variable expression of the CAR owing to transgene
20 variegation. Altogether, the conjunction of autologous cell sourcing and random vector integration is prone to generating cell products with variable potency. Thus, there is a need of novel designs of antigen recognizing receptors having consistent potency and increased ability to detect low levels of target antigen.

25 SUMMARY OF THE INVENTION

According to a first aspect, the present invention provides a recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises: a) an extracellular antigen-binding domain of an antibody, or antigen-binding fragment thereof; and b) a constant domain that comprises a native TRAC peptide or a native TRBC peptide.

30 According to a second aspect, the present invention provides an immunoresponsive cell comprising a recombinant TCR of the first aspect.

According to a third aspect, the present invention provides a pharmaceutical composition comprising an effective amount of an immunoresponsive cell of the second aspect and a pharmaceutically acceptable excipient.

5 According to a fourth aspect, the present invention provides a method of reducing tumor burden in a subject, treating or preventing a neoplasm, and/or lengthening survival of a subject having a neoplasm, the method comprising administering to the subject an effective amount of the immunoresponsive cells of the second aspect or the pharmaceutical composition of the third aspect.

10 According to a fifth aspect, the present invention provides a method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a nucleic acid sequence encoding a recombinant TCR according to the first aspect, optionally wherein the nucleic acid sequence is comprised in a vector.

According to a sixth aspect, the present invention provides a nucleic acid encoding a recombinant TCR of the first aspect.

15 According to a seventh aspect, the present invention provides a vector comprising the nucleic acid of the sixth aspect.

20 According to an eighth aspect, the present invention provides a kit comprising a recombinant TCR of the first aspect, an immunoresponsive cell of the second aspect, a pharmaceutical composition of third aspect, a nucleic acid composition of the sixth aspect, or a vector of the seventh aspect.

25 According to a ninth aspect, the present invention provides use of the immunoresponsive cells of the second aspect or the pharmaceutical composition of the third aspect for the manufacture of a medicament for use in reducing tumor burden, for treating or preventing a neoplasm, or for lengthening survival in a subject.

30 The presently disclosed subject matter generally provides HLA-independent (or non-HLA restricted) T cell receptors (referred to as "HI-TCRs") that bind to an antigen of interest in an HLA-independent manner and immunoresponsive cells comprising thereof. The presently disclosed subject matter also provides methods of using such cells for inducing and/or enhancing an immune response to a target antigen, and/or treating and/or preventing neoplasia or other diseases/disorders where an increase in an antigen-specific immune response is desired.

The presently disclosed subject matter provides recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises an extracellular antigen-binding domain and a constant domain, wherein the recombinant TCR binds to an antigen in an HLA-independent manner.

5 In certain embodiments, the constant domain comprises a native or modified TRAC peptide, and/or a native or modified TRBC peptide. In certain embodiments, the constant domain is capable of forming a homodimer or a heterodimer with another constant domain.

10 In certain embodiments, the recombinant TCR is expressed from an expression cassette placed in an endogenous *TRAC* locus and/or a *TRBC* locus of an immunoresponsive cell. In certain embodiments, the placement of the recombinant TCR expression cassette disrupts or abolishes the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain embodiments, the placement of the recombinant TCR expression cassette prevents
15 or eliminates mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain embodiments, the antigen binding chain is capable of associating with a CD3 ζ polypeptide. The antigen binding chain, upon binding to an antigen, is capable of activating the CD3 ζ polypeptide associated to the antigen binding chain. The activation of the CD3 ζ polypeptide is
20 capable of activating an immunoresponsive cell. The CD3 ζ polypeptide can be endogenous or exogenous. In certain embodiments, the CD3 ζ polypeptide is endogenous and is endogenous and integrated in the native CD3 complex. In certain embodiments, the CD3 ζ polypeptide is exogenous and optionally integrated with a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an
25 OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof. In certain embodiments, the antigen binding chain further comprises a co-stimulatory region, wherein the recombinant TCR, upon binding to an antigen, is capable of stimulating an immunoresponsive cell. The co-stimulatory region can include a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB
30 polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof. In certain embodiments, the co-stimulatory region comprises a CD28 polypeptide.

In certain embodiments, the recombinant TCR is capable of associating with a CD3 complex. In certain embodiments, the recombinant TCR is capable of integrating

with a CD3 complex and providing HLA-independent antigen recognition. In certain embodiments, the CD3 complex is endogenous. In certain embodiments, the recombinant TCR replaces an endogenous TCR in a CD3/TCR complex.

In certain embodiments, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain. The extracellular antigen-binding domain can include a ligand for a cell-surface receptor, a receptor for a cell surface ligand, an antigen binding portion of an antibody or a fragment thereof or an antigen binding portion of a TCR. In certain embodiments, the extracellular antigen-binding domain comprises a heavy chain variable region (V_H) of an antibody, or a VHH from a camelid V_H -only antibody and/or a light chain variable region (V_L) of an antibody. In certain embodiments, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain comprises a V_H of an antibody, wherein the human, murine, or camelid V_H is capable of dimerizing with another extracellular antigen-binding domain comprising a V_L of the antibody and form a fragment variable (F_v). In certain embodiments, the extracellular antigen-binding domain comprises a V_L of an antibody, wherein the V_L is capable of dimerizing with another extracellular antigen-binding domain comprising a V_H of the antibody and form a fragment variable (F_v).

In certain embodiments, the recombinant TCR binds to a tumor antigen. The tumor antigen can be selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB. In certain embodiments, the tumor antigen is CD19.

In certain embodiments, the recombinant TCR exhibits greater antigen sensitivity than a CAR targeting the same antigen. In certain embodiments, the recombinant TCR is capable of inducing an immune response when binds to an antigen that has a low density

on the surface of a tumor cell. In certain embodiments, the antigen that has a low density on the cell surface has below about 2,000 molecules per cell.

The presently disclosed subject matter further provides immunoresponsive cells comprising a recombinant TCR described herein. In certain embodiments, the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell. In certain embodiments, the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization. The placement of the expression cassette of the recombinant TCR can disrupt or abolish the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell, whereby preventing or eliminating mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. The endogenous gene locus can be a *CD3 δ* locus, a *CD3 ϵ* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus or a *TRGC* locus or a *TRDC* locus. In certain embodiments, the endogenous gene locus is a *TRAC* locus and/or a *TRBC* locus. The endogenous gene locus can include a modified transcription terminator region. In certain embodiments, the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, a SV40 transcription terminator and a P2A element; the P2A element allows the use of the endogenous transcription terminator of the targeted gene. In certain embodiments, when one endogenous T cell receptor locus in a cell is modified to express the at least one antigen binding chain of the recombinant TCR, one or more other endogenous T cell receptor locus in the cell is modified to eliminate the expression of an endogenous TCR chain. In certain embodiments, the one or more other endogenous T cell receptor loci are further modified to express a gene of interest. The gene of interest can be an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene or a suicide gene. In certain embodiments, one or more endogenous TCR loci are further modified to incorporate a sequence encoding co-stimulatory signaling domain(s) to generate a TCR chain containing such signaling domain(s) at the carboxy terminus.

In certain embodiments, the immunoresponsive cell is selected from the group consisting of a T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a human embryonic stem cell, and a pluripotent stem cell from

which lymphoid cells may be differentiated. In certain embodiments, the immunoresponsive cell is autologous.

In certain embodiments, the immunoresponsive cell further comprises at least one exogenous co-stimulatory ligand. In certain embodiments, the co-stimulatory ligand is selected from the group consisting of CD80, CD86, 41BBL, CD275, CD40L, OX40L and any combination thereof. In certain embodiments, the cell further comprises or consists of one exogenous co-stimulatory ligand. In certain embodiments, the one exogenous co-stimulatory ligand is CD80 or 4-1BBL. In certain embodiments, the cell further comprises or consists of two exogenous co-stimulatory ligands. In certain embodiments, In certain embodiments, the two exogenous co-stimulatory ligands are CD80 and 4-1BBL.

In certain embodiments, the immunoresponsive cell further comprises at least one chimeric costimulatory receptor (CCR). In certain embodiments, the CCR comprising a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof.

The presently disclosed subject matter also provides pharmaceutical compositions comprising the immunoresponsive cell(s) disclosed herein and a pharmaceutically acceptable excipient. The pharmaceutical composition can be used for treating a neoplasia.

Further provided are methods of reducing tumor burden in a subject. In addition, the presently disclosed subject matter provides methods of lengthening survival of a subject having a neoplasm (e.g., cancer). In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells described herein or the pharmaceutical composition described herein.

The presently disclosed subject matter also provides methods of treating or preventing a neoplasm. In certain embodiments, the method comprises administering to the subject an effective amount of the immunoresponsive cells described herein or the pharmaceutical composition described herein. The neoplasm can be selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, non-Hodgkin's lymphoma and adenocarcinoma. In certain embodiments, the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19. In

certain embodiments, the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, an adenocarcinoma, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin (MSLN), ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB. In certain embodiments, the neoplasm is CD19+ ALL.

The presently disclosed subject matter further provides methods for producing an antigen-specific immunoresponsive cell. In certain embodiments, the method comprises introducing into an immunoresponsive cell a nucleic acid sequence encoding a recombinant TCR described herein. The nucleic acid sequence can be comprised in a vector. In certain embodiments, the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell. In certain embodiments, the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization. The endogenous gene locus can be a *CD3δ* locus, a *CD3ε* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus, a *TRDC* locus and/or a *TRGC* locus. In certain embodiments, the endogenous gene locus is a *TRAC* locus or a *TRBC* locus. In certain embodiments, the placement of the expression cassette of the recombinant TCR disrupts or abolishes the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell, whereby preventing or eliminating mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain embodiments, the endogenous gene locus comprises a modified transcription terminator region. In certain embodiments, the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription

terminator and a P2A element. In certain embodiments, when one endogenous T cell receptor locus in a cell is modified to express the at least one antigen binding chain of the recombinant TCR, one or more other endogenous T cell receptor locus in the cell is modified to eliminate the expression of an endogenous TCR chain. In certain
5 embodiments, the one or more other endogenous T cell receptor locus are further modified to express a gene of interest. In certain embodiments, the gene of interest is an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene or a suicide gene.

 The presently disclosed subject matter further provides nucleotide acids encoding a recombinant TCR described herein, and nucleic acid compositions comprising a
10 recombinant TCR described herein. In certain embodiments, the nucleic acid sequences are comprised in a vector. The presently disclosed subject matter also provides vectors comprising the nucleic acid composition described herein. Further provided are kits comprising a recombinant TCR described herein, an immunoresponsive cell described herein, a pharmaceutical composition described herein, a nucleic acid composition
15 described herein, or a vector described herein. In certain embodiments, the kit further comprises written instructions for treating and/or preventing a neoplasm, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

 Illustrative neoplasms for which the presently disclosed subject matter can be used include, but are not limited to leukemias (e.g., acute leukemia, acute lymphocytic
20 leukemia, acute myelocytic leukemia a, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as
25 sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma,
30 adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder

carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

5 The presently disclosed subject matter further provides use of any recombinant TCR, any pharmaceutical composition or any immunoresponsive cell disclosed herein for used in a therapy.

BRIEF DESCRIPTION OF THE FIGURES

10 The following Detailed Description, given by way of example, but not intended to limit the presently disclosed subject matter to specific embodiments described, may be understood in conjunction with the accompanying drawings.

15 Figures 1A-1E depict HLA-Independent TCR-based Chimeric Antigen Receptor HIT (HIT-CAR, i.e., HI-TCR or HIT) and gene targeting strategy at the TRAC locus in human T cells. (A) Schematic representation of the T Cell Receptor (TCR), the B Cell Receptor (BCR), a Chimeric Antigen Receptor (CAR) and the HLA-Independent TCR-based Chimeric Antigen Receptor (HIT-CAR, i.e., HI-TCR or HIT). (B) CRISPR/Cas9-targeted integration of the 3 receptors into the TRAC locus. Top: TRAC locus; middle: rAAV6 containing the different receptor cassette flanked by homology arms. (C) Representative TCR/Mouse F(ab')₂ flow cytometry plots 4 days after TRAC targeting. The TCR antibody epitope recognize the constant chain of the TCR alpha and beta. (D) Cytotoxic activity using an 18-hour bioluminescence assay, using firefly luciferase (FFL)-expressing NALM-6 as targets cells (n=3). (E) Relative CAR MFI (1=MFI at 0h) of CAR T cells after 1, 2 or 4 (arrows) stimulations on CD19 positive target cells.

25 Figures 2A-2B depict expression and therapeutic efficacy of HI-TCR. (A) Representative TCR/Mouse F(ab')₂ flow cytometry plots 4 days after TRAC targeting. (B) Kaplan-Meier analysis of the mice survival where NALM-6- bearing mice were treated with 5x10⁵ CAR T cells.

30 Figures 3A-3E depict gene targeting strategy and expression of NYESO TCR. (A) Schematic representation of the NYESO TCR genes integrated into the TCR alpha or beta chain. (B) Representative TCR-V-beta-1 flow cytometry plots 4 days after TRAC or TRBC targeting. (C) Cytotoxic activity using an 18-hour bioluminescence assay, using firefly luciferase (FFL)-expressing PC3 as targets cells (n=3). (D) Schematic representation of a co-targeting into both the TCR alpha and the TCR beta. (E)

Representative TCR-V-beta-1/4-1BBL flow cytometry plots 4 days after TRAC and TRBC co- targeting.

Figures 4A-4C depict strategy of integrating a CAR into a TRAC locus and modulation the expression by various transcriptional termination signals / 3' untranslated regions (3'UTR). (A) Schematic representation of the 1928z CAR gene integrated into the TRAC locus. Poly A (black box) corresponds to the segment of the CAR cassette that was modified to test different viral and mammalian 3' UTRs. (B) Representative CAR flow plots 3 days after TRAC (left panel). and geometric mean fluorescence intensity (gMFI), MFI, and Median values for the CAR-expressing population (right panel). Boxed is the original 3'UTR sequence of bovine growth hormone poly A. (C) Absolute (top) and relative (bottom) CAR MFI (1=MFI at 0h) of CAR T cells after 1, 2 stimulations on CD19 positive target cells (as shown in Fig. 1).

Figures 5A and 5B depict efficacy of genetically integrated CARs with different 3'UTR sequences. (A) FFL-NALM-6-bearing mice were treated with 1×10^5 CAR T cells, where tumor burden is shown as bioluminescent signal quantified per animal 14 days post T-cell injection. n = 6 mice per group. (B) Tumor burden (average radiance) of NALM-6-bearing mice treated with 1×10^5 CAR T cells (n = 6; line = one mouse) quantified at days 7, 14, and 21 post T-cell injection.

Figures 6A-6C depict HIT gene targeting at the TRAC locus in human T cells. (A) CRISPR/Cas9-targeted CAR or HIT gene integration into the TRAC locus. Top, TRAC locus; middle, rAAV6 containing the CAR cassette flanked by homology arms; bottom, rAAV6 containing the HIT cassette flanked by homology arms. (B) Representative CAR/HIT flow plots 4 days after transfection of T cells with Cas9 mRNA and TRAC gRNA and addition of AAV6. CAR and HIT surface protein were detected using a goat anti-mouse IgG. (C) Average CAR/HIT mean fluorescence intensity (MFI) analyzed by FACS 4 days after transduction (n = 6 independent experiments).

Figures 7A-7B depict HIT T cells outperform CAR T cells at killing target cells expressing low antigen levels. Nalm6 cell line (expressing firefly luciferase) was gene edited at the CD19 locus using CRISPR/Cas9, and clones expressing different CD19 levels were generated. (A) FACS analysis of representative Nalm6 clones for each CD19 expression level group (Neg = negative). (B) Cytotoxic activity using an 4h bioluminescence assay, using NALM6 as targets cells expressing different CD19 levels and CAR (red squares) or HIT (blue circles) T cells at 1:1 effector (E): target (T) ratio.

Figures 8A-8C depict HIT T cells outperform CAR T cells at killing target cells expressing low antigen levels. Cytotoxic activity using an 18 h bioluminescence assay, using NALM6 as targets cells expressing different CD19 levels (indicated at the right), which were incubated with untransduced T cells (A), CAR T cells (B), and HIT T cells (C) at different effector (E): target (T) ratios.

Figures 9A-9D depict HIT T cells expressing costimulatory ligands outperform CAR T cells in controlling established B-ALL tumor with very low CD19 levels. NALM-6-bearing mice were treated with 4×10^5 untransduced (NT), CAR, or HIT T cells. Tumor burden_{SEP} was quantified weekly over a 54-day period using BLI. Quantification is the average photon count of ventral and dorsal acquisitions per animal at all given time points. Each line represents one mouse, and $n = 5$ mice per group. (A) Untransduced (black) vs CAR (red) T cells. (B) CAR (red) vs HIT (green) T cells. (C) T cells expressing HIT alone (green), HIT + CD80 co-stimulatory ligand (orange), HIT + 41BBL co-stimulatory ligand (pink), or HIT + CD80 + 41BBL co-stimulatory ligands (blue). (D) Mouse survival analysis.

Figures 10A-10C depict that baseline TRAC-CAR expression can be controlled by distinct 3'UTR sequences without affecting cell surface replenishment kinetic after antigen encounter. (A) CRISPR/Cas9-targeted CAR gene integration into the TRAC locus. The targeting construct (AAV) contains the 1928z CAR coding sequence followed by a 3'UTR sequence, flanked by sequences homologous to the TRAC locus (LHA_{SEP}^[1] and RHA, left and right homology arm). (B) Each 3'UTR sequence provides different CAR surface levels (measured by FACS). TK thymidine kinase (short version); GCSF: human GCSF, derived from pEF-BOS plasmid; TCR α : TCR alpha, exon 4; HBB: human B-globin; RBG: rabbit B-globin; SV40: simian virus 40 poly A; P2A: porcine teschovirus-1 self-cleaving 2A sequence; it allowed the use of the endogenous TRAC poly A sequence. (C) CAR T cells were stimulated once (indicated by a red arrow) with CD19-expressing 3T3 cells, and CAR MFI was measured every 24h over a 3-day period. All CAR T cells show similar CAR expression regulation.

DETAILED DESCRIPTION OF THE INVENTION

The presently disclosed subject matter provides HLA-independent (or non-HLA restricted) T cell receptors (referred to as "HI-TCRs") that bind to an antigen of interest in an HLA-independent manner. In certain embodiments, the HI-TCRs are TCR molecules where the TCR variable domain is replaced by the variable domain from an antibody

(Fv), resulting in a FvTCR. In certain non-limiting embodiments, the HI-TCR can bind to a tumor antigen or a pathogen antigen. The presently disclosed subject matter also provides cells, including genetically modified immunoresponsive cells (e.g., T cells, NKT cells, or CTL cells) comprising the presently disclosed HI-TCR. In certain non-limiting
5 embodiments, binding of the antigen by the HI-TCR is capable of activating the immunoresponsive cell. The presently disclosed subject matter also provides methods of using such cells for inducing and/or enhancing an immune response to a target antigen, and/or treating and/or preventing neoplasia or other diseases/disorders where an increase in an antigen-specific immune response is desired.

10 1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used in the presently disclosed subject matter: Singleton et al., Dictionary of Microbiology and Molecular
15 Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

20 As used herein, the term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to
25 about 20%, e.g., up to about 10%, up to about 5%, or up to about 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, e.g., within about 5-fold or within about 2-fold, of a value.

By “activates an immunoresponsive cell” is meant induction of signal transduction
30 or changes in protein expression in the cell resulting in initiation of an immune response. For example, when CD3 Chains cluster in response to ligand binding and immunoreceptor tyrosine-based inhibition motifs (ITAMs) a signal transduction cascade is produced. In certain embodiments, when an endogenous TCR or an exogenous CAR binds to an antigen, a formation of an immunological synapse occurs that includes

clustering of many molecules near the bound receptor (e.g. CD4 or CD8, CD3 $\gamma/\delta/\epsilon/\zeta$, etc.). This clustering of membrane bound signaling molecules allows for ITAM motifs contained within the CD3 chains to become phosphorylated. This phosphorylation in turn initiates a T cell activation pathway ultimately activating transcription factors, such as

5 NF- κ B and AP-1. These transcription factors induce global gene expression of the T cell to increase IL-2 production for proliferation and expression of master regulator T cell proteins in order to initiate a T cell mediated immune response.

By “stimulates an immunoresponsive cell” is meant a signal that results in a robust and sustained immune response. In various embodiments, this occurs after

10 immune cell (e.g., T-cell) activation or concomitantly mediated through receptors including, but not limited to, CD28, CD137 (4-1BB), OX40, CD40 and ICOS. Receiving multiple stimulatory signals can be important to mount a robust and long-term T cell mediated immune response. T cells can quickly become inhibited and unresponsive to antigen. While the effects of these co-stimulatory signals may vary, they generally result

15 in increased gene expression in order to generate long lived, proliferative, and anti-apoptotic T cells that robustly respond to antigen for complete and sustained eradication.

The term “antigen recognizing receptor” as used herein refers to a receptor that is capable of activating an immune or immunoresponsive cell (e.g., a T-cell) in response to its binding to an antigen. Non-limiting examples of antigen recognizing receptors include

20 native or endogenous T cell receptors (“TCRs”), and chimeric antigen receptors (“CARs”).

As used herein, the term “antibody” means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both *in vitro* and

25 *in vivo*. Accordingly, as used herein, the term “antibody” means not only intact immunoglobulin molecules but also the well-known active fragments F(ab')₂, and Fab. F(ab')₂, and Fab fragments that lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). As used herein, antibodies include whole

30 native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv), fusion polypeptides, and unconventional antibodies. In certain embodiments, an antibody is a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised

of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant (C_H) region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant C_L region. The light chain constant region is comprised of one domain, C_L . The V_H and V_L regions can be further sub-divided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1 q) of the classical complement system.

As used herein, “CDRs” are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. *See, e.g.*, Kabat et al., Sequences of Proteins of Immunological Interest, 4th U. S. Department of Health and Human Services, National Institutes of Health (1987). Generally, antibodies comprise three heavy chain and three light chain CDRs or CDR regions in the variable region. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. In certain embodiments, the CDRs regions are delineated using the Kabat system (Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

As used herein, the term “single-chain variable fragment” or “scFv” is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of an immunoglobulin covalently linked to form a $V_H::V_L$ heterodimer. The V_H and V_L are either joined directly or joined by a peptide-encoding linker (*e.g.*, 10, 15, 20, 25 amino acids), which connects the N-terminus of the V_H with the C-terminus of the V_L , or the C-terminus of the V_H with the N-terminus of the V_L . The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility. Despite removal of the constant regions and the introduction of a linker, scFv proteins retain the specificity of the original immunoglobulin. Single chain Fv polypeptide antibodies can be expressed from a nucleic acid including V_H - and V_L -encoding sequences as described by Huston, et al. (Proc. Nat. Acad. Sci. USA, 85:5879-5883, 1988). *See, also*, U.S. Patent Nos. 5,091,513, 5,132,405

and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754.

Antagonistic scFvs having inhibitory activity have been described (see, e.g., Zhao et al., Hyrbidoma (Larchmt) 2008 27(6):455-51; Peter et al., J Cachexia Sarcopenia Muscle 2012 August 12; Shieh et al., J Immunol 2009 183(4):2277-85; Giomarelli et al., Thromb Haemost 2007 97(6):955-63; Fife et al., J Clin Invest 2006 116(8):2252-61; Brocks et al., Immunotechnology 1997 3(3):173-84; Moosmayer et al., Ther Immunol 1995 2(10):31-40). Agonistic scFvs having stimulatory activity have been described (see, e.g., Peter et al., J Biol Chem 2003 278(38):36740-7; Xie et al., Nat Biotech 1997 15(8):768-71; Ledbetter et al., Crit Rev Immunol 1997 17(5-6):427-55; Ho et al., Biochim Biophys Acta 2003 1638(3):257-66).

As used herein, the term “affinity” is meant a measure of binding strength. Affinity can depend on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and/or on the distribution of charged and hydrophobic groups. As used herein, the term “affinity” also includes “avidity”, which refers to the strength of the antigen-antibody bond after formation of reversible complexes. Methods for calculating the affinity of an antibody for an antigen are known in the art, including, but not limited to, various antigen-binding experiments, e.g., functional assays (e.g., flow cytometry assay).

The term “chimeric antigen receptor” or “CAR” as used herein refers to a molecule comprising an extracellular antigen-binding domain that is fused to an intracellular signaling domain that is capable of activating or stimulating an immunoresponsive cell, and a transmembrane domain. In certain embodiments, the extracellular antigen-binding domain of a CAR comprises a scFv. The scFv can be derived from fusing the variable heavy and light regions of an antibody. Alternatively or additionally, the scFv may be derived from Fab’s (instead of from an antibody, e.g., obtained from Fab libraries). In certain embodiments, the scFv is fused to the transmembrane domain and then to the intracellular signaling domain. In certain embodiments, the CAR has a high binding affinity or avidity for the antigen.

As used herein, the term “nucleic acid molecules” include any nucleic acid molecule that encodes a polypeptide of interest or a fragment thereof. Such nucleic acid molecules need not be 100% homologous or identical with an endogenous nucleic acid sequence, but may exhibit substantial identity. Polynucleotides having “substantial identity” or “substantial homology” to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By

“hybridize” is meant a pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

5 For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, e.g., less than about 500 mM NaCl and 50 mM trisodium citrate, or less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least
10 about 35% formamide, e.g., at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, at least about 37° C, or at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of
15 stringency are accomplished by combining these various conditions as needed. In certain embodiments, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In certain embodiments, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In certain embodiments, hybridization will occur at 42° C
20 in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

 For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by
25 temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps can be less than about 30 mM NaCl and 3 mM trisodium citrate, e.g., less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, of at least about 42° C,
30 or of at least about 68° C. In certain embodiments, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 42° C. in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In certain embodiments, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those

skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Rogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By “substantially identical” or “substantially homologous” is meant a polypeptide or nucleic acid molecule exhibiting at least about 50% homologous or identical to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). In certain embodiments, such a sequence is at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or at least about 100% homologous or identical to the sequence of the amino acid or nucleic acid used for comparison.

Sequence identity can be measured by using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By “analog” is meant a structurally related polypeptide or nucleic acid molecule having the function of a reference polypeptide or nucleic acid molecule.

The term “ligand” as used herein refers to a molecule that binds to a receptor. In certain embodiments, the ligand binds to a receptor on another cell, allowing for cell-to-cell recognition and/or interaction.

The term “constitutive expression” or “constitutively expressed” as used herein refers to expression or expressed under all physiological conditions.

By “disease” is meant any condition, disease or disorder that damages or interferes with the normal function of a cell, tissue, or organ, e.g., neoplasia, and pathogen infection of cell.

By “effective amount” is meant an amount sufficient to have a therapeutic effect.

- 5 In certain embodiments, an “effective amount” is an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis (e.g., invasion, or migration) of a neoplasia.

By “enforcing tolerance” is meant preventing the activity of self-reactive cells or immunoresponsive cells that target transplanted organs or tissues.

- 10 By “endogenous” is meant a nucleic acid molecule or polypeptide that is normally expressed in a cell or tissue.

- By “exogenous” is meant a nucleic acid molecule or polypeptide that is not endogenously present in a cell, or not present at a level sufficient to achieve the functional effects obtained when over-expressed. The term “exogenous” would therefore
15 encompass any recombinant nucleic acid molecule or polypeptide expressed in a cell, such as foreign, heterologous, and over-expressed nucleic acid molecules and polypeptides. By “exogenous” nucleic acid is meant a nucleic acid not present in a native wild-type cell; for example, an exogenous nucleic acid may vary from an endogenous counterpart by sequence, by position/location, or both. For clarity, an exogenous nucleic
20 acid may have the same or different sequence relative to its native endogenous counterpart; it may be introduced by genetic engineering into the cell itself or a progenitor thereof, and may optionally be linked to alternative control sequences, such as a non-native promoter or secretory sequence.

- By a “heterologous nucleic acid molecule or polypeptide” is meant a nucleic acid
25 molecule (e.g., a cDNA, DNA or RNA molecule) or polypeptide that is not normally present in a cell or sample obtained from a cell. This nucleic acid may be from another organism, or it may be, for example, an mRNA molecule that is not normally expressed in a cell or sample.

- By “immunoresponsive cell” is meant a cell that functions in an immune response
30 or a progenitor, or progeny thereof.

By “modulate” is meant positively or negatively alter. Exemplary modulations include a about 1%, about 2%, about 5%, about 10%, about 25%, about 50%, about 75%, or about 100% change.

By “increase” is meant to alter positively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, about 100% or more.

By “reduce” is meant to alter negatively by at least about 5%. An alteration may be by about 5%, about 10%, about 25%, about 30%, about 50%, about 75%, or even by about 100%.

By “isolated cell” is meant a cell that is separated from the molecular and/or cellular components that naturally accompany the cell.

The terms “isolated,” “purified,” or “biologically pure” refer to material that is free to varying degrees from components which normally accompany it as found in its native state. “Isolate” denotes a degree of separation from original source or surroundings. “Purify” denotes a degree of separation that is higher than isolation. A “purified” or “biologically pure” protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term “purified” can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

The term “antigen-binding domain” as used herein refers to a domain capable of specifically binding a particular antigenic determinant or set of antigenic determinants present on a cell.

“Linker”, as used herein, shall mean a functional group (e.g., chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected to one another. As used herein, a “peptide linker” refers to one or more amino acids used to couple two proteins together (e.g., to couple V_H and V_L domains). In certain embodiments, the linker comprises a sequence set forth in GGGGSGGGGSGGGGS [SEQ ID NO: 31].

By “neoplasm” is meant a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other tissues or organs. Neoplasia growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells.

- 5 Neoplasia can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and
 10 vagina, or a tissue or cell type thereof. Neoplasia include cancers, such as sarcomas, carcinomas, or plasmacytomas (malignant tumor of the plasma cells). In certain embodiments, the neoplasm is a solid tumor.

- Illustrative neoplasms for which the presently disclosed subject matter can be used include, but are not limited to leukemias (e.g., acute leukemia, acute lymphocytic
 15 leukemia, acute myelocytic leukemia a, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin’s disease, non-Hodgkin’s disease), Waldenstrom’s macroglobulinemia, heavy chain disease, and solid tumors such as
 20 sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing’s tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma,
 25 adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm’s tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder
 30 carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

By “receptor” is meant a polypeptide, or portion thereof, present on a cell membrane that selectively binds one or more ligand.

By “recognize” is meant selectively binds to a target. A T cell that recognizes a tumor can express a receptor (e.g., a TCR or CAR) that binds to a tumor antigen.

5 By “reference” or “control” is meant a standard of comparison. For example, the level of scFv-antigen binding by a cell expressing a CAR and an scFv may be compared to the level of scFv-antigen binding in a corresponding cell expressing CAR alone.

By “secreted” is meant a polypeptide that is released from a cell via the secretory pathway through the endoplasmic reticulum, Golgi apparatus, and as a vesicle that
10 transiently fuses at the cell plasma membrane, releasing the proteins outside of the cell.

By “signal sequence” or “leader sequence” is meant a peptide sequence (e.g., 5, 10, 15, 20, 25 or 30 amino acids) present at the N-terminus of newly synthesized proteins that directs their entry to the secretory pathway. Exemplary leader sequences include, but is not limited to, the IL-2 signal sequence: MYRMQLLSIALSLALVTNS [SEQ ID NO:
15 12] (human), MYSMQLASCVTLTLLVLLVNS [SEQ ID NO: 13] (mouse); the kappa leader sequence: METPAQLLFLLLLWLPDTTG [SEQ ID NO: 14] (human), METDTLLLWVLLLWVPGSTG [SEQ ID NO: 15] (mouse); the CD8 leader sequence: MALPVTALLPLALLHAARP [SEQ ID NO: 16] (human); the truncated human CD8 signal peptide: MALPVTALLPLALLHA [SEQ ID NO: 28] (human); the albumin
20 signal sequence: MKWVTFISLLFSSAYS [SEQ ID NO: 29] (human); and the prolactin signal sequence: MDSKGSSQKGSRLLLLVSNNLLCQGVVS [SEQ ID NO: 30] (human). By “soluble” is meant a polypeptide that is freely diffusible in an aqueous environment (e.g., not membrane bound).

By “specifically binds” is meant a polypeptide or fragment thereof that recognizes
25 and binds to a biological molecule of interest (e.g., a polypeptide), but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a presently disclosed polypeptide.

The term “tumor antigen” as used herein refers to an antigen (e.g., a polypeptide) that is uniquely or differentially expressed on a tumor cell compared to a normal or non-
30 IS neoplastic cell. In certain embodiments, a tumor antigen includes any polypeptide expressed by a tumor that is capable of activating or inducing an immune response via an antigen recognizing receptor (e.g., CD19, MUC-16) or capable of suppressing an immune response via receptor-ligand binding (e.g., CD47, PD-L1/L2, B7.1/2).

The terms “comprises”, “comprising”, and are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean “includes”, “including” and the like.

As used herein, “treatment” refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

An “individual” or “subject” herein is a vertebrate, such as a human or non-human animal, for example, a mammal. Mammals include, but are not limited to, humans, primates, farm animals, sport animals, rodents and pets. Non-limiting examples of non-human animal subjects include rodents such as mice, rats, hamsters, and guinea pigs; rabbits; dogs; cats; sheep; pigs; goats; cattle; horses; and non-human primates such as apes and monkeys.

The term “immunocompromised” as used herein refers to a subject who has an immunodeficiency. The subject is very vulnerable to opportunistic infections, infections caused by organisms that usually do not cause disease in a person with a healthy immune system, but can affect people with a poorly functioning or suppressed immune system.

Other aspects of the presently disclosed subject matter are described in the following disclosure and are within the ambit of the presently disclosed subject matter.

2. HLA-Independent T Cell Receptor (HI-TCR)

The present disclosure provides an HI-TCR that binds to an antigen of interest in an HLA-independent manner. In certain non-limiting embodiments, binding of the antigen is capable of activating an immunoresponsive cell comprising the HI-TCR. In certain non-limiting embodiments, the HI-TCR comprises an antigen binding chain. In certain embodiments, the antigen binding chain comprises an extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain is derived from a scFv, Fab, or antibody of murine, human or camelid (e.g., lama) origin. In certain embodiments, the antigen binding chain further comprises a constant domain.

2.1. *Antigens*

In certain embodiments, the HI-TCR binds to a tumor antigen. Any tumor antigen (antigenic peptide) can be used in the tumor-related embodiments described herein.

Sources of antigen include, but are not limited to, cancer proteins. The antigen can be expressed as a peptide or as an intact protein or portion thereof. The intact protein or a portion thereof can be native or mutagenized. Non-limiting examples of tumor antigens include carbonic anhydrase IX (CAIX), carcinoembryonic antigen (CEA), CD8, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CLL1, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, CD123, CD44V6, an antigen of a cytomegalovirus (CMV) infected cell (e.g., a cell surface antigen), epithelial glycoprotein-2 (EGP-2), epithelial glycoprotein-40 (EGP-40), epithelial cell adhesion molecule (EpCAM), receptor tyrosine-protein kinases erb-B2,3,4 (erb-B2,3,4), folate-binding protein (FBP), fetal acetylcholine receptor (AChR), folate receptor- α , Ganglioside G2 (GD2), Ganglioside G3 (GD3), human Epidermal Growth Factor Receptor 2 (HER-2), human telomerase reverse transcriptase (hTERT), Interleukin-13 receptor subunit alpha-2 (IL-13R α 2), κ -light chain, kinase insert domain receptor (KDR), Lewis Y (LeY), L1 cell adhesion molecule (L1CAM), melanoma antigen family A, 1 (MAGE-A1), Mucin 16 (MUC16), Mucin 1 (MUC1), Mesothelin (MSLN), ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, cancer-testis antigen NY-ESO-1, oncofetal antigen (h5T4), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), ROR1, tumor-associated glycoprotein 72 (TAG-72), vascular endothelial growth factor R2 (VEGF-R2), and Wilms tumor protein (WT-1), BCMA, NKCS1, EGF1R, EGFR-VIII, CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME and ERBB.

In certain embodiments, the HI-TCR binds to CD19. In certain embodiments, the HI-TCR binds to a human CD19 polypeptide. In certain embodiments, the human CD19 polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 11.

PEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLLKLSLGLPGLGIHMRPLAIWLFIFNVSQQM
GGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLGGLGCGLNRSSEGPSSPSGKLMSPKLYVWAKDR
PEIWEGEPPCLPPRDLSLNQSLSQDLTMAPGSTLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRP
ARDMWVMTGLLLPRATAQDAGKYCHRGNTMSFHLEITARPVLWHWLLRTGGWK [SEQ ID NO:
11].

In certain embodiments, the HI-TCR binds to the extracellular domain of a CD19 protein.

In certain embodiments, the HI-TCR binds to a pathogen antigen, e.g., for use in treating and/or preventing a pathogen infection or other infectious disease, for example, in an immunocompromised subject. Non-limiting examples of pathogen includes a virus, bacteria, fungi, parasite and protozoa capable of causing disease.

5 Non-limiting examples of viruses include, *Retroviridae* (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HDTV-III, LAVE or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; *Picornaviridae* (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); *Calciviridae* (e.g. strains that cause gastroenteritis); *Togaviridae* (e.g. equine encephalitis viruses, rubella viruses); *Flaviridae* (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); *Coronaviridae* (e.g. coronaviruses); *Rhabdoviridae* (e.g. vesicular stomatitis viruses, rabies viruses); *Filoviridae* (e.g. ebola viruses); *Paramyxoviridae* (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); *Orthomyxoviridae* (e.g. influenza viruses); *Bungaviridae* (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairoviruses); *Arenaviridae* (hemorrhagic fever viruses); *Reoviridae* (e.g. reoviruses, orbiviruses and rotaviruses); *Birnaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvoviridae* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); *Adenoviridae* (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; *Poxviridae* (variola viruses, vaccinia viruses, pox viruses); and *Iridoviridae* (e.g. African swine fever virus); and unclassified viruses (e.g. the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 =internally transmitted; class 2 =parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

25 Non-limiting examples of bacteria include *Pasteurella*, *Staphylococci*, *Streptococcus*, *Escherichia coli*, *Pseudomonas* species, and *Salmonella* species. Specific examples of infectious bacteria include but are not limited to, *Helicobacter pylori*, *Borrelia burgdorferi*, *Legionella pneumophila*, *Mycobacteria* sps (e.g. *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. goodii*), *Staphylococcus aureus*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Streptococcus pyogenes* (Group A Streptococcus), *Streptococcus agalactiae* (Group B Streptococcus), *Streptococcus* (viridans group), *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*, pathogenic *Campylobacter* sp., *Enterococcus* sp., *Haemophilus influenzae*, *Bacillus anthracis*, *Corynebacterium*

diphtheriae, *corynebacterium sp.*, *Erysipelothrix rhusiopathiae*, *Clostridium perfringers*, *Clostridium tetani*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pasturella multocida*, *Bacteroides sp.*, *Fusobacterium nucleatum*, *Streptobacillus moniliformis*, *Treponema pallidum*, *Treponema pertemue*, *Leptospira*, *Rickettsia*, and *Actinomyces israeli*.

In certain embodiments, the pathogen antigen is a viral antigen present in Cytomegalovirus (CMV), a viral antigen present in Epstein Barr Virus (EBV), a viral antigen present in Human Immunodeficiency Virus (HIV), or a viral antigen present in influenza virus.

2.2. Extracellular Antigen-Binding Domain

In certain embodiments, the antigen binding chain comprises an extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain specifically binds to an antigen, e.g., a tumor antigen or a pathogen antigen, e.g., those disclosed in Section 2.1. In certain embodiments, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain (e.g., forming a fragment variable (Fv)), wherein the dimerized antigen-binding domains (e.g., an Fv) specifically bind to an antigen, e.g., a tumor antigen or a pathogen antigen.

In certain embodiments, the extracellular antigen-binding domain comprises a ligand for a cell-surface receptor. In certain embodiments, the extracellular antigen-binding domain comprises a receptor for a cell surface ligand.

In certain embodiments, the extracellular antigen-binding domain specifically binds to an antigen, e.g., a tumor antigen or a pathogen antigen. In certain embodiments, the antigen binding chain is capable of forming a dimer with another antigen binding chain. In certain embodiments, the HI-TCR comprises a heterodimer comprising two different antigen binding chains. In certain embodiments, the HI-TCR comprises a homodimer comprising two identical antigen binding chains. In certain embodiments, the antigen binding chains dimerize through one or more disulfide-links. In certain embodiments, the antigen binding chain is capable of forming a trimer or oligomer with one or more identical or different antigen binding chains. In certain embodiments, the extracellular antigen-binding domain is capable of dimerizing with another extracellular antigen-binding domain (e.g., forming a fragment variable (Fv)), wherein the dimerized antigen-binding domains (e.g., a Fv) specifically bind to an antigen, e.g., a tumor antigen or a pathogen antigen.

In certain non-limiting embodiments, the extracellular antigen-binding domain of the HI-TCR (for example, an Fv or an analog thereof) binds to an antigen with a dissociation constant (K_d) of about 2×10^{-7} M or less. In certain embodiments, the K_d is about 2×10^{-7} M or less, about 1×10^{-7} M or less, about 9×10^{-8} M or less, about 1×10^{-8} M or less, about 9×10^{-9} M or less, about 5×10^{-9} M or less, about 4×10^{-9} M or less, about 3×10^{-9} or less, about 2×10^{-9} M or less, or about 1×10^{-9} M or less. In certain non-limiting embodiments, the K_d is about 3×10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 1×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 3×10^{-7} M. In certain non-limiting embodiments, the K_d is from about 1.5×10^{-9} M to about 2.7×10^{-7} M.

Binding of the extracellular antigen-binding domain (for example, a Fv or an analog thereof) can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody, or an Fv) specific for the complex of interest. For example, the Fv can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the extracellular antigen-binding domain is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (*e.g.*, EBFP, EBFP2, Azurite, and mKalamal), cyan fluorescent protein (*e.g.*, ECFP, Cerulean, and CyPet), and yellow fluorescent protein (*e.g.*, YFP, Citrine, Venus, and YPet).

In certain embodiments, the extracellular antigen-binding domain comprises an antigen binding portion of a TCR.

In certain embodiments, the extracellular antigen-binding domain comprises an antigen binding portion of an antibody or a fragment thereof. In certain embodiments, the extracellular antigen-binding domain comprises a heavy chain variable region (V_H) and/or a light chain variable region (V_L) of an antibody. In certain embodiments, the extracellular antigen-binding domain comprises a single-chain variable fragment (scFv). In certain embodiments, the extracellular antigen-binding domain comprises a heavy

chain-only antibodies (VHH). In certain embodiments, the extracellular antigen-binding domain comprises a Fab, which is optionally crosslinked. In certain embodiments, the extracellular antigen-binding domain comprises a F(ab)₂. In certain embodiments, any of the foregoing molecules can be comprised in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain.

In certain embodiments, the extracellular antigen-binding domain comprises a heavy chain variable region (V_H) and/or a light chain variable region (V_L) of an antibody, wherein the V_H or the V_L is capable of dimerizing with another extracellular antigen-binding domain comprising a VL or a VH (e.g., forming a fragment variable (Fv)). In certain embodiments, the Fv is a human Fv. In certain embodiments, the Fv is a humanized Fv. In certain embodiments, the Fv is a murine Fv. In certain embodiments, the Fv is identified by screening a Fv phage library with an antigen-Fc fusion protein.

Additional extracellular antigen-binding domains targeting an interested antigen can be obtained by sequencing an existing scFv or a Fab region of an existing antibody targeting the same antigen.

In certain embodiments, the dimerized extracellular antigen-binding domain of a presently disclosed HI-TCR is a murine Fv. In certain embodiments, the dimerized extracellular antigen-binding domain is an Fv that binds to a human CD19 polypeptide. In certain embodiments, the extracellular antigen-binding domain is an Fv, which comprises the amino acid sequence of SEQ ID NO: 9 and specifically binds to a human CD19 polypeptide (e.g., a human CD19 polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 11). In certain embodiments, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 9 is set forth in SEQ ID NO: 10.

In certain embodiments, the Fv comprises a heavy chain variable region (V_H) comprising the amino acid sequence set forth in SEQ ID NO: 7. In certain embodiments, the Fv comprises a light chain variable region (V_L) comprising the amino acid sequence set forth in SEQ ID NO: 8. In certain embodiments, the Fv comprises V_H comprising the amino acid sequence set forth in SEQ ID NO: 7 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 8. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (e.g., at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 7. For example, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about

89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous or identical to SEQ ID NO: 7. In

certain embodiments, the extracellular antigen-binding domain comprises a V_H

comprising the amino sequence set forth in SEQ ID NO: 7. In certain embodiments, the

5 extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 8. For example, the extracellular antigen-binding

domain comprises a V_L comprising an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 10 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous or identical to SEQ ID

NO: 8. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising the amino acid sequence set forth in SEQ ID NO: 8. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid

15 sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 7, and a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 8. In certain embodiments, the

extracellular antigen-binding domain comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 7 and a V_L comprising the amino acid sequence set forth in SEQ 20 ID NO: 8.

In certain embodiments, the Fv comprises a heavy chain variable region (V_H) comprising the amino acid sequence set forth in SEQ ID NO: 44. In certain

embodiments, the Fv comprises a light chain variable region (V_L) comprising the amino

25 acid sequence set forth in SEQ ID NO: 45. In certain embodiments, the Fv comprises V_H comprising the amino acid sequence set forth in SEQ ID NO: 44 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 45. In certain embodiments, the

extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about

30 95%) homologous or identical to SEQ ID NO: 44. For example, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous or identical to SEQ

ID NO: 44. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino sequence set forth in SEQ ID NO: 44. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 45. For example, the extracellular antigen-binding domain comprises a V_L comprising an amino acid sequence that is at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% homologous or identical to SEQ ID NO: 45. In certain embodiments, the extracellular antigen-binding domain comprises a V_L comprising the amino acid sequence set forth in SEQ ID NO: 45. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous to SEQ ID NO: 44, and a V_L comprising an amino acid sequence that is at least about 80% (*e.g.*, at least about 85%, at least about 90%, or at least about 95%) homologous or identical to SEQ ID NO: 45. In certain embodiments, the extracellular antigen-binding domain comprises a V_H comprising the amino acid sequence set forth in SEQ ID NO: 44 and a V_L comprising the amino acid sequence set forth in SEQ ID NO: 45.

In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 1, or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 2 or a conservative modification thereof, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 3, a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 1, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 2, and a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 3. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_L CDR1 comprising the amino acid

sequence set forth in SEQ ID NO: 4, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 1 or a conservative modification thereof, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 2 or a conservative modification thereof, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 3, a conservative modification thereof, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4 or a conservative modification thereof, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5 or a conservative modification thereof, and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6 or a conservative modification thereof. In certain embodiments, the extracellular antigen-binding domain comprises a V_H CDR1 comprising amino acids having the sequence set forth in SEQ ID NO: 1, a V_H CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 2, a V_H CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 3, a V_L CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 4, a V_L CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 5 and a V_L CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 6.

Table 1

		anti-human CD19 scFv (SJ25C1)		
CDRs		1	2	3
V _H	a.a.	GYAFSS [SEQ ID NO: 1]	YPGDGD [SEQ ID NO: 2]	KTISSVVD [SEQ ID NO: 3]
V _L	a.a.	KASQNVGTNVA [SEQ ID NO: 4]	SATYRN [SEQ ID NO: 5]	QQYNRYPYT [SEQ ID NO: 6]
Full V _H		EVKLQQSGAE LVRPGSSVKI SCKASGYAFS SYWMNWVKQR PGQGLEWIGQ IYPGDGDTNY NGKFKGQATL TADKSSSTAY MQLSGLTSED SAVYFCARKT ISSVVDIFYD YWGQGTITVTV SS [SEQ ID NO: 7]		
Full V _L		DIELTQSPKF MSTSVGDRVS VTCKASQNVG TNVAWYQQKPGQSPKPLIYS ATYRNSGVPD RFTGSGSGTD FTLTITNVQS KDLADYFCQQ YNRYPYTSGG GTKLEIKR [SEQ ID NO: 8]		
scFv		MALPVTALLL PLALLLHAEV KLQQSGAELV RPGSSVKISC KASGYAFSSY WMNWVKQRPG QGLEWIGQIY PGDGDNTNYG KFKGQATLTA DKSSSTAYMQ LSGLTSEDSA VYFCARKTIS SVVDIFYFDYW GQGTITVTVSS GGGGSGGGGS GGGGSDIELT QSPKFMSTSV GDRVSVTCKA SQNVGTNVAW YQQKPGQSPK PLIYSATYRN SGVPDRFTGS GSGTDFTLTI TNVQSKDLAD YFCQQYNRYP		

	YTSGGGKLE IKR [SEQ ID NO: 9]
DNA	ATGGCTCTCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCAGAGG TGAAGCTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGTCTCAGTGAAGATTTC CTGCAAGGCTTCTGGCTATGCATTAGTAGCTACTGGATGAACTGGGTGAAGCAGAGG CCTGGACAGGGTCTTGAGTGGATTGGACAGATTTATCCTGGAGATGGTGATACTAACT ACAATGGAAAGTTCAAGGGTCAAGCCACACTGACTGCAGACAAATCCTCCAGCACAGC CTACATGCAGCTCAGCGGCCAATCATCTGAGGACTCTGCGGTCTATTTCTGTGCAAGA AAGACCATTAGTTCGGTAGTAGATTTCTACTTTGACTACTGGGGCCAAGGGACACGG TCACCGTCTCCTCAGGTGGAGGTGGATCAGGTGGAGGTGGATCTGGTGGAGGTGGATC TGACATTGAGCTCACCCAGTCTCCAAAATTCATGTCCACATCAGTAGGAGACAGGGTC AGCGTCACCTGCAAGGCCAGTCAGAATGTGGGTACTAATGTAGCCTGGTATCAACAGA AACCAGGACAATCTCCTAAACCACTGATTTACTCGGCAACCTACCGGAACAGTGGAGT CCCTGATCGCTTACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCACTAAC GTGCAGTCTAAAGACTTGGCAGACTATTTCTGTCAACAATATAACAGGTATCCGTACA CGTCCGAGGGGGGACCAAGCTGGAGATCAAACGG [SEQ ID NO: 10]

As used herein, the term “a conservative sequence modification” refers to an amino acid modification that does not significantly affect or alter the binding characteristics of the presently disclosed HI-TCR comprising the amino acid sequence.

- 5 Conservative modifications can include amino acid substitutions, additions and deletions. Modifications can be introduced into the Fv of the presently disclosed HI-TCR by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Amino acids can be classified into groups according to their physicochemical properties such as charge and polarity. Conservative amino acid
- 10 substitutions are ones in which the amino acid residue is replaced with an amino acid within the same group. For example, amino acids can be classified by charge: positively-charged amino acids include lysine, arginine, histidine, negatively-charged amino acids include aspartic acid, glutamic acid, neutral charge amino acids include alanine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine,
- 15 proline, serine, threonine, tryptophan, tyrosine, and valine. In addition, amino acids can be classified by polarity: polar amino acids include arginine (basic polar), asparagine, aspartic acid (acidic polar), glutamic acid (acidic polar), glutamine, histidine (basic polar), lysine (basic polar), serine, threonine, and tyrosine; non-polar amino acids include alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline,
- 20 tryptophan, and valine. Thus, one or more amino acid residues within a CDR region can be replaced with other amino acid residues from the same group and the altered antibody can be tested for retained function (*i.e.*, the functions set forth in (c) through (l) above) using the functional assays described herein. In certain embodiments, no more than one, no more than two, no more than three, no more than four, no more than five residues
- 25 within a specified sequence or a CDR region are altered.

The V_H and/or V_L amino acid sequences having at least about 80%, at least about 85%, at least about 90%, or at least about 95% (*e.g.*, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99%) homology to a specific sequence (*e.g.*, SEQ ID NOs: 7, and 8) may contain substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the specified sequence(s), but retain the ability to bind to a target antigen (*e.g.*, CD19). In certain embodiments, a total of 1 to 10 amino acids are substituted, inserted and/or deleted in a specific sequence (*e.g.*, SEQ ID NOs: 7, and 8). In certain embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (*e.g.*, in the FRs) of the extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain comprises V_H and/or V_L sequence selected from the group consisting of SEQ ID NOs: 7, and 8, including post-translational modifications of that sequence (SEQ ID NOs: 7 and 8).

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

The percent homology between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent homology between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the amino acids sequences of the presently disclosed subject matter can further be used as a “query sequence” to perform a search against public databases to, for example, identify related sequences. Such searches can be

performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the specified sequences (e.g., heavy and light chain variable region sequences of scFv m903, m904, m905, m906, and m900) disclosed herein. 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.

2.3. Constant Domain

In certain embodiments, the antigen binding chain further comprises a constant domain. In certain embodiments, the constant domain comprises a hinge/spacer region and a transmembrane domain. In certain embodiments, the constant domain is capable of forming a homodimer or a heterodimer with another constant domain. In certain embodiments, the constant domain dimerizes through one or more disulfide-links. In certain embodiments, the antigen binding chain is capable of forming a trimer or oligomer with one or more identical or different constant domains.

In certain non-limiting embodiments, the constant domain comprises a T cell receptor constant region, e.g., T cell receptor alpha constant region (TRAC), T cell receptor beta constant region (TRBC, e.g., TRBC1 or TRBC2), T cell receptor gamma constant region (TRGC, e.g., TRGC1 or TRGC2), T cell receptor delta constant region (TRDC) or any variants or functional fragments thereof.

In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRAC peptide. In certain embodiments, the TRAC polypeptide comprises an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO:38, which is provided below), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

IQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRSMDFKNSAVAWSNKSDFA
 ANAFNNSIIPEDTFFPSPSSCDVKLVKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRLWSS
 [SEQ ID NO:38]

In certain embodiments, the TRAC polypeptide has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% homologous or identical to the amino acid sequence encoded by a transcript expressed by the gene of NCBI Genbank ID: 28755, NG_001332.3, range 925603 to 930229 (SEQ ID NO:29, which is provided below), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

```

1  atatccagaa ccctgaccct gccgtgtacc agctgagaga ctctaaatcc agtgacaagt
10 61 ctgtctgcct attcaccgat ttgtattctc aaacaaatgt gtcacaaagt aaggattctg
121 atgtgtatat cacagacaaa actgtgctag acatgaggtc tatggacttc aagagcaaca
181 gtgctgtggc ctggagcaac aaatctgact ttgcatgtgc aaacgccttc aacaacagca
241 ttattccaga agacaccttc ttccccagcc caggtaaggg cagcttttgg gccttcgcag
301 gctgttttct tgccttcagga atggccaggt tctgcccaga gctctgggtc atgatgtcta
15 361 aaactcctct gattggtggg ctcgccctta tccattgcca ccaaaaccct ctttttacta
421 agaaacagtg agccttggtt tggcagtcca gagaatgaca cgggaaaaaa gcagatgaag
481 agaaggtggc aggagagggc acgtggccca gcctcagtct ctccaactga gttcctgcct
541 gcctgccttt gctcagactg ttgtcccttt actgctcttc taggcctcat tctaagcccc
601 ttctccaagt tgctctctct tattttctccc tgtctgccaa aaaatctttc ccagctcact
20 661 aagtcagtct cacgcagtca ctcatataacc caccaatcac tgattgtgcc ggcacatgaa
721 tgcaccaggt gttgaagtgg aggaattaaa aagtcagatg aggggtgtgc ccagaggaag
781 caccattcta gttgggggag cccatctgtc agctgggaaa agtccaaata acttcagatt
841 ggaatgtgtt ttaactcagg gttgagaaaa cagctacctt caggacaaaa gtcagggaag
901 ggctctctga agaaatgcta cttgaagata ccagccctac caagggcagg gagaggaccc
25 961 tatagaggcc tgggacagga gctcaatgag aaaggagaag agcagcaggc atgagttgaa
1021 tgaaggaggc agggccgggt cacagggcct tctaggccat gagagggtag acagtattct
1081 aaggacgcca gaaagctgtt gatcggtctc aagcagggga gggacaccta atttgctttt
1141 cttttttttt tttttttttt tttttttttt tgagatggag ttttgcctct gttgcccagg
1201 ctggagtgcg atggtgcatc ttggtcact gcaacctccg cctcccagg tcaagtgatt
30 1261 ctctgcctc agcctcccga gtagctgaga ttacaggcac ccgccaccat gcctggctaa
1321 ttttttgtat ttttagtaga gacagggttt cactatgttg gccaggctgg tctcgaactc
1381 ctgacctcag gtgatccacc cgcttcagcc tcccaaagtg ctgggattac aggcgtgagc
1441 caccacaccc ggctgctttt tcttaaagat caatctgagt gctgtacgga gagtgggttg
1501 taagccaaga gtagaagcag aaaggagagc gttgcagcag agagatgatg gaggcctggg
35 1561 caggggtggt gcagggaggt aaccaacacc attcaggttt caaaggtaga accatgcagg
1621 gatgagaaag caaagagggg atcaagggaag gcagctggat tttggcctga gcagctgagt
1681 caatgatagt gccgtttact aagaagaaac caaggaaaaa atttggggtg cagggatcaa
1741 aacttttttg aacatatgaa agtacgtgtt tatactcttt atggcccttg tcactatgta
1801 tgctctgctg cctccattgg actctagaat gaagccaggc aagagcaggg tctatgtgtg
40 1861 atggcacatg tggccagggt catgcaacat gtactttgta caaacagtgt atattgagta
1921 aatagaaatg gtgtccagga gccgaggtat cggctctgcc agggccaggg gctctcccta
1981 gcagggtgctc atatgctgta agttccctcc agatctctcc acaaggaggc atggaaaggc
2041 tgtagttgtt cacctgcccc agaactagga ggtctggggg gggagagtca gcctgctctg
2101 gatgctgaaa gaatgtctgt ttttcctttt agaaagttcc tgtgatgtca agctggtcga
45 2161 gaaaagcttt gaaacaggta agacaggggt ctagcctggg tttgcacagg attgcggaag
2221 tgatgaaccc gcaataaccc tgcttgatg agggagtggg aagaaattag tagatgtggg
2281 aatgaatgat gaggaatgga aacagcggtt caagacctgc ccagagctgg gtgggtctc
2341 tcctgaatcc ctctcaccat ctctgacttt ccattctaag cactttgagg atgagtttct
2401 agcttcaata gaccaaggac tctctcctag gcctctgtat tcctttcaac agctccactg
50 2461 tcaagagagc cagagagagc ttctgggttg cccagctgtg aaatttctga gtcccttagg
2521 gatagcccta aacgaaccag atcatcctga ggacagccaa gaggttttgc cttctttcaa
2581 gacaagcaac agtactcaca taggtgtgtg gcaatggtcc tgtctctcaa gaatcccctg
2641 ccactcctca ccccaccct gggcccatat tcatttccat ttgagttgtt cttattgagt
2701 catccttctt gtggtagcgg aactcactaa ggggcccatc tggaccagag gtattgtgat

```

2761 gataaattct gagcacctac cccatcccca gaagggctca gaaataaaat aagagccaag
 2821 tctagtcggt gtttctgtgc ttgaaacaca atactgttg ccctggaaga atgcacagaa
 2881 tctgtttgta aggggatatg cacagaagct gcaagggaca ggaggtgcag gagctgcagg
 2941 cctccccac ccagcctgct ctgccttggg gaaaaccgtg ggtgtgtcct gcaggccatg
 5 3001 caggcctggg acatgcaagc ccataaccgc tgtggcctct tggttttaca gatacgaacc
 3061 taaactttca aaacctgtca gtgattgggt tccgaatcct cctcctgaaa gtggccgggt
 3121 ttaatctgct catgacgctg cggctgtggg ccagctgagg tgaggggcct tgaagctggg
 3181 agtgggggtt agggacgcgg gtctctgggt gcatcctaag ctctgagagc aaacctccct
 3241 gcagggctct gcttttaagt ccaaagcctg agcccaccaa actctcctac ttcttctgt
 10 3301 tacaaattcc tcttgtgcaa taataatggc ctgaaacgct gtaaaatata ctcatctcag
 3361 ccgcctcagt tgcacttctc ccctatgagg taggaagaac agttgtttag aaacgaagaa
 3421 actgagggcc cacagctaag gagtggagga agagagacac ttgtgtacac cacatgcctt
 3481 gtgttgtagt tctctcaccg tgtaacctcc tcatgtcctc tctccccagt acggctctct
 3541 tagctcagta gaaagaagac attacactca tattacaccc caatcctggc tagagtctcc
 15 3601 gcaccctcct cccccagggt cccagctcgt cttgctgaca actgcaccc gttccatcac
 3661 catcaaaaaa aaactccagg ctgggtgcgg gggctcacac ctgtaatccc agcactttgg
 3721 gaggcagagg caggaggagc acaggagctg gagaccagcc tgggcaacac agggagaccc
 3781 cgctctaca aaaagtga aaattaacca ggtgtggtgc tgcacacctg tagtcccagc
 3841 tacttaagag gctgagatgg gaggatcgct tgagccctgg aatgttgagg ctacaatgag
 20 3901 ctgtgattgc gtcactgcac tccagcctgg aagacaaagc aagatcctgt ctcaaataat
 3961 aaaaaaata agaactccag ggtacatttg ctctagaac tctaccacat agccccaac
 4021 agagccatca ccatcacatc cctaacagtc ctgggtcttc ctgagtgtcc agcctgactt
 4081 ctgttcttcc tcattccaga tctgcaagat tgtaagacag cctgtgctcc ctgctcctt
 4141 cctctgcatt gcccctcttc tccctctcca aacagaggga actctcctac cccaaggag
 25 4201 gtgaaagctg ctaccacctc tgtgcccccc cggcaatgcc accaactgga tcctaccgga
 4261 atttatgatt aagattgctg aagagctgcc aaacactgct gccacccctt ctgttccctt
 4321 attgctgctt gtcactgcct gacattcacg gcagaggcaa ggctgctgca gcctccctg
 4381 gctgtgcaca ttccctcctg ctccccagag actgcctccg ccatcccaca gatgatggat
 4441 cttcagtggt ttctcttggg ctctaggtcc tgcagaatgt tgtgaggggt ttattttttt
 30 4501 ttaatagtgt tcataaagaa atacatagta ttcttcttct caagacgtgg ggggaaatta
 4561 tctcattatc gaggcctgc tatgctgtgt atctgggcgt gttgtatgtc ctgctgccga
 4621 tgccttc [SEQ ID NO:29]

In accordance with the presently disclosed subject matter, an “TRAC nucleic acid molecule” refers to a polynucleotide encoding an TRAC polypeptide.

35 In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRBC peptide. In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRBC2 peptide. In certain embodiments, the TRBC2 polypeptide comprises an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least

40 about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO:39, which is provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

45 DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWVNGKEVHSGVSTDPQPLKEQPALNDSR
 YCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSA
 TILYEILLGKATLYAVLVLSALVLMAMVKRKDSRG [SEQ ID NO:39]

In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRBC1 peptide. In certain embodiments, the TRBC1 polypeptide comprises an amino acid sequence that is at least about 80%, at least about

85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO:40, which is provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

DLNKVFPEEV AVFEPSEAEI SHTQKATLVC LATGFFPDHV ELSWWVNGKE
VHSGVSTDPQ PLKEQPALND SRYCLSSRLR VSATFWQNPR NHFRCQVQFY
GLSENDEWTQ DRAKPVQTQIV SAEAWGRADC GFTSVSYQQG VLSATILYEI
LLGKATLYAV LVSALVLMAM VKRKDF [SEQ ID NO:40]

In certain embodiments, the TRBC polypeptide has an amino acid sequence that is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% homologous or identical to the amino acid sequence encoded by a transcript expressed by a gene of NCBI Genbank ID: 28639, NG_001333.2, range 645749 to 647196 (TRBC1, SEQ ID NO: 30), NCBI Genbank ID: 28638, NG_001333.2 range 655095 to 656583 (TRBC2, SEQ ID NO:31) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

1 aggacctgaa caaggtgttc ccacccgagg tcgctgtgtt tgagccatca gaagcagaga
61 tctccacac ccaaaaggcc aactgggtgt gcctggccac aggtttcttc cccgaccacg
121 tggagctgag ctggtgggtg aatgggaagg aggtgcacag tggggtcagc acagaccgcg
181 agccccctcaa ggagcagccc gccctcaatg actccagata ctgcctgagc agccgcctga
241 ggggtctcggc caccttcttg cagaaccccc gcaaccactt ccgctgtcaa gtccagttct
301 acgggctctc ggagaatgac gagtggaccc aggatagggc caaacccgtc acccagatcg
361 tcagcgccga ggcctggggt agagcagggtg agtggggcct ggggagatgc ctggaggaga
421 ttaggtgaga ccagctacca gggaaaatgg aaagatccag gtagcagaca agactagatc
481 caaaaagaaa ggaaccagcg cacaccatga aggagaattg ggcacctgtg gttcattctt
541 ctcccagatt ctgagcccaa cagagccaag cagctgggtc ccctttctat gtggcctgtg
601 taactctcat ctgggtgtgt cccccatcc ccctcagtgc tgccacatgc catggattgc
661 aaggacaatg tggctgacat ctgcatggca gaagaaagga ggtgctgggc tgtcagagga
721 agctgggtctg ggcctgggag tctgtgcca ctgcaaact gactttactt ttaattgcct
781 atgaaaataa ggtctctcat ttattttcct ctccctgctt tctttcagac tgtggcttta
841 cctcgggtaa gtaagccctt ctttttcctc tccctctctc atggttcttg acctagaacc
901 aaggcatgaa gaaactcacag aactggagg gtggagggtg ggagagacca gagctacctg
961 tgcacaggta cccacctgtc ctctctcctt gccaacagt tctaccagc aaggggtcct
1021 gtctgccacc atcctctatg agatcctgct aggggaaggcc accctgtatg ctgtgctggt
1081 cagcgccctt gtgttgatgg ccatggtaag caggagggca ggatggggcc agcaggctgg
1141 aggtgacaca ctgacaccaa gcacccagaa gtatagagtc cctgccagga ttggagctgg
1201 gcagtaggga gggaagagat ttcattcagg tgcctcagaa gataacttgc acctctgtag
1261 gatcacagtg gaagggatcat gctgggaagg agaagctgga gtcaccagaa aacccaatgg
1321 atgttgtgat gagccttact atttgtgtgg tcaatgggcc ctactacttt ctctcaatcc
1381 tcacaactcc tggctcttaa taacccccaa aactttctct tctgcaggtc aagagaaagg
1441 atttctga [SEQ ID NO:30]

1 aggacctgaa aaacgtgttc ccacccgagg tcgctgtgtt tgagccatca gaagcagaga
61 tctccacac ccaaaaggcc aactgggtat gcctggccac aggtttctac cccgaccacg
121 tggagctgag ctggtgggtg aatgggaagg aggtgcacag tggggtcagc acagaccgcg
181 agccccctcaa ggagcagccc gccctcaatg actccagata ctgcctgagc agccgcctga
241 ggggtctcggc caccttcttg cagaaccccc gcaaccactt ccgctgtcaa gtccagttct

301 acgggctctc ggagaatgac gagtggaccc aggatagggc caaaccctgc acccagatcg
 361 tcagcgccga ggcctggggt agagcaggtg agtggggcct ggggagatgc ctggaggaga
 421 ttaggtgaga ccagctacca gggaaaatgg aaagatccag gtagcggaca agactagatc
 481 cagaagaaag ccagagtggg caaggtggga tgatcaaggt tcacagggtc agcaaagcac
 5 541 ggtgtgcaact tccccacca agaagcatag aggctgaatg gagcacctca agctcattct
 601 tccttcagat cctgacacct tagagctaag ctttcaagtc tccctgagga ccagccatac
 661 agctcagcat ctgagtgggtg tgcattccat tctcttctgg ggtcctggtt tcctaagatc
 721 atagtgacca cttcgctggc actggagcag catgaggagg acagaaccag ggctatcaaa
 781 ggaggctgac tttgtactat ctgatatgca tgtgtttgtg gcctgtgagt ctgtgatgta
 10 841 aggcctcaatg tccttataaa gcagcattct ctcattccatt tttcttcccc tgttttcttt
 901 cagactgtgg cttcacctcc ggtaagttag tctctccttt ttctctctat ctttcgccgt
 961 ctctgctctc gaaccagggc atggagaatc cacggacaca ggggcgtgag ggaggccaga
 1021 gccacctgtg cacaggtgcc tacatgctct gttcttgtca acagagtctt accagcaagg
 1081 ggtcctgtct gccaccatcc tctatgagat cttgctaggg aaggccacct tgtatgccgt
 15 1141 gctggtcagt gccctcgtgc tgatggccat ggtaaggagg aggggtgggag agggcagatg
 1201 atgggggcag gggatggaac atcacacatg gccataaagg aatctcagag ccagagcaca
 1261 gcctaataata tcctatcacc tcaatgaaac cataatgaag ccagactggg gagaaaatgc
 1321 agggaaatata acagaatgca tcatgggagg atggagacaa ccagcgagcc ctactcaaat
 1381 taggcctcag agcccgctc ccctgcccta ctctgctgt gccatagccc ctgaaacct
 20 1441 gaaaatgttc tctcttcac aggtcaagag aaaggattcc agaggctag
 [SEQ ID NO:31]

In accordance with the presently disclosed subject matter, an “TRBC nucleic acid molecule” refers to a polynucleotide encoding an TRBC polypeptide.

25 In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRGC peptide. In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRGC1 peptide. In certain embodiments, the TRGC1 polypeptide has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99%
 30 or 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO: 42, which is provided below.

DKQLDADVSP KPTIFLPSIA ETKLQKAGTY LCLLEKFFPD VIKIHWQEEK
 SNTILGSQEG NTMKTNDTYM KFSWLTVPK SLDKEHRCIV RHENNKNGVD
 QEIIFFPIKT DVITMDPKDN CSKDANDTLL LQLTNTSAYY MYLLLLLKS
 35 VYFAIITCCL LRRTAFCCNG EKS [SEQ ID NO:42]

In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRGC2 peptide. In certain embodiments, the TRGC2 polypeptide has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous or identical to
 40 the amino acid sequence set forth in SEQ ID NO: 43, which is provided below.

DKQLDADVSP KPTIFLPSIA ETKLQKAGTY LCLLEKFFPD IIKIHWQEEK
 SNTILGSQEG NTMKTNDTYM KFSWLTVPK SLDKEHRCIV RHENNKNGID
 QEIIFFPIKT DVTTVDPKYN YSKDANDVIT MDPKDNWSKD ANDTLLLQLT
 45 NTSAYYTYLL LLLKSVVYFA IITCCLLRRT AFCCNGEKS [SEQ ID NO:43]

In certain embodiments, the TRGC polypeptide has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous or identical to the amino acid sequence encoded by a transcript expressed by a gene of NCBI Genbank ID: 6966, NG_001336.2, range 108270 to 113860 (TRGC1, SEQ ID NO: 32), NCBI Genbank ID: 6967, NG_001336.2, range 124376 to 133924 (TRGC2, SEQ ID NO: 33) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

```

1 ataaacaact tgatgcagat gtttccccca agcccactat ttttcttcct tcaattgctg
61 aaacaaagct ccagaaggct ggaacatacc tttgtcttct tgagaaattt ttccctgatg
10 121 ttattaagat acattggcaa gaaaagaaga gcaacacgat tctgggatcc caggagggga
181 acaccatgaa gactaacgac acatacatga aatttagctg gttaacggtg ccagaaaagt
241 cactggacaa agaacacaga tgtatcgtca gacatgagaa taataaaaaac ggagttgatc
301 aagaaattat ctttcctcca ataaagacag gtatgtgttt acgcatatca tctgtcagaa
361 cacttctttg aaagtgaatg ctgcattttt tcctttcagt attaatgaaa aacaaacata
15 421 aatctttctt aaatattgtt acatttaatg gtagcataaa tgccctgcta cttttctata
481 gaattaaaaat ggtatagggt ttggagaaaa caaaattgaa aaagttactg aaggtttgtc
541 agcctcagct ccattatcca aaataagaaa gtcacgtgct ggttttttagg gttgttagat
601 ggattaaaga aacaacatac acagaagcat ctagcaacgt gacacgtggt aaacgctcaa
661 aaagtgttct ccttcttttt gatgacttta cttgatcagg aaataacata tatatgtcctt
20 721 tcaggaatgt tctgccaag caggagagtc actcacctca atcttgctac ccacaaagtt
781 taacctaaaa acaacgggtt cattgttgac aaaatgatgt ttatctgttg ttgacagaat
841 gatgtttatc taaaaacagt tccaattttt tatttctctt gctgagacac aaaggggagg
901 caaatgtgca aagcttgagg gtagtcttac cactgtgctt aagtgttctg atttttctag
961 tgatcagggc aaaataaaaa gtatagtaag ttccaaggca gtgaatatta tacaggagag
25 1021 aagttacagt ttataatgt gttttccttt acactaaatt ctaaaagtaa aaagtctttt
1081 tttttttttg acagagtttc actcttggtg cccaagcagg tgtgctatgg tatgatctca
1141 gctcactgca acctccacct cccgggttca agtgattctc ttacttcagc ctcccgacag
1201 gctgggattg caggcgcttg ccaccacacc tggctaattt ttgtgttttt agtagagatg
1261 gggtttcacc atgttggtcca ggctggtctc aaattcctga cctcaagtga tccatccacc
30 1321 tcggcctcca agtgctggga ttatgggcgt cagccactgt gcccagccta aaagtaaaat
1381 gtctttcatg agcttcccaa ggcagctacg ttaaggagga cacttctctt aatgtcattc
1441 tacagtagat ttctaattgt ctttcttgga agtttgtttt tctgagaaaa gctaaaaata
1501 taacatggaa gtgatcatat tatataatca atgaagtgtt tttcaaggag ataaaaactaa
1561 tctgggtccac acttgcaacc aaccttgatt gagagagaga gagaactcag gatacacttg
35 1621 aagattttat tatggggaac agttacttta ttctttttac ctcaatcaat gcatggaaat
1681 aagtgatagt cattttcatt tatcttttaa taaatgaagt caccatgagg aaaataaaaa
1741 gacattgaaa acccattaaa gtcagccctt aaagatattt ggacatgcag acttgataac
1801 taacgtttgc attcttgaga cttaccctaa acccatacct caagtccaag tttttagaat
1861 tcatgaaata aagatctcag tgaagtgcata aaattgcgca ccagaatcat atccgtatag
40 1921 acaagaacac atctactaga aaaataataa accaacacac caatgcaact gtgttttctt
1981 ctgtttttaa gtatgttggtc tttgtatgca tgtttgcttc ttcttttttt tttttaacat
2041 cacagataaa ttcaactctc acctcagggt ttattgagag aactgtcaat gtgacttggc
2101 ctctgtcttt ctagtcccag aaagaattgc actgaaatct gagctcctgt aataaaaaaca
2161 accatttgct gagagtaatt aacatactga aagagatttt cttagagtac acaatgggtga
45 2221 cattatattg cctctttata aataactttt tatctatttc tgtggattat tcctacaaag
2281 tacttttcat atgtccaatt tcttttcttc ccctacaact actgtctgaa tactggctct
2341 gctatttgct gatatgattc tcggcaagtt gcctgcactt tttaaacttt atttcctcat
2401 tcagaacatg gggccataca taatacaact cacttcagtg ttattgggga attaaacaaa
2461 aaatgcatgg gaagcattta acatagtgcc tgacacaata atgagtactc agtagatgtt
50 2521 agcttttatt aatattgttg ttgttatgtc cagaaacact atacctccag aaaatcatgg
2581 gtacttgctg gggacatttg ggatattgat gatttggaag agaagtactg ctttttttgc
2641 ttagatgaga aatttttcta agccagactc cttcaaatat gtaagattct gttgtggatt
2701 caaggactga aagaattctt ggccgagtggt ggtggcttat ccctgtaatc ccagcatttt
2761 gtgaggacaa ggcaggaaga ttgcttgagt ccaggagttt gaaaccagcc tgcgcaacat
55 2821 ggcgaacccc tgtctctaca aaaaatacaa acattagctc ggagtgtgtg ctgacatgtg

```

2881 cctgtactcc cagctactca gaaggctgag atgggaggat ctcatgagcc tggggagttt
 2941 gaggcttcag tgagccgtga tgacaccgta ctatactcca ctccagcctg ggtgacagtg
 3001 agaccctgcc tcaaaaaaca aacaaacaaa caaacaaaac aaaattaatc tttttgctga
 5 3061 tgtcatgtca gcagtgtgtg ttgaaggctg taaagcagcc atttgttcag tttatttttc
 3121 cattgaacaa gtatttatca aaaacatact ttgtggcagt cactatgcta ggagctatga
 3181 atacagaagg aaaagtaaat gctcttggat actacactcc agttgtgata aaaaagaaaa
 3241 aatgtattct tcaccaactt caacatcttg atgtgcaaaa acataatata tgaattagat
 3301 ctacctaat acacagaatt agaccaattg tttctggaat tgtgggctca tatttttaat
 10 3361 aactgtcctc ctgcctctct gtgcgacagg tttataaata ttcatttaat tacacacaca
 3421 cacacgaaca attgactagt acttgctctc attcttctag atgtcatcac aatggatccc
 3481 aaagacaatt gttcaaaaga tgcaaatggt aagcttttgt gtttttccct tcctcctgat
 3541 ctttttgttt tgaacttctc ttgcttgaaa aatcagggaa tggattttgc taggttggat
 3601 gctgcagaat ggacctagtg atattttaaa ttagtccctc attttctagg agttgtatta
 3661 acaaacctaa ctactgcttt ggggtatgag atgactgtaa attagagagg gtacagtggg
 15 3721 atagtgatat gcttttaatt atttcaaaaa aaagatttta ttcattcatg tgtctttttt
 3781 ctttttcttt tctttttttt ttttttttgg acagagtctt gctctgtcac ccaggctgga
 3841 gtgcggtggc agtatctcag ctccaccaca cctccgcctc ccggcttcaa gtgattctcc
 3901 tgccctcagct tctcgagtag ctgggactac aggcgcgtgc caccatgccc ggctaatttt
 20 3961 tgtattttta gtagagttgg ggtttcacca tgttggccag gatggcctcg aatttgtgac
 4021 ctctgtatct gccccctcgc cctcccgaa tgttgggatt acaggcgtga gtcactgtgc
 4081 ccggcctcct gtctgtctct ttgtttaatg actgggaaaa acatgatacc atgttgcttc
 4141 tgcagttggt ttgttttagt ctttggctct tgctagtagc taataacacg aactagtgtt
 4201 tatcaagtgc tttttacaca gaagggttg ggctgtgttc tgcattttct tgtttaaccc
 25 4261 tcttaaaact cctataaaat ggtacatatt tttctccaa tttacagtcc ctttaagca
 4321 aataattata aaaatcccta tacatgtcac acagctagat ctgggatttc aaatcaggcc
 4381 atcaaacaaa gagtttatgt acttagtaag ttttctgttc ttttctaca atagagtcag
 4441 atagcaagaa attaccaagc caggaacctg aaacaaaacg gacatcatgt ggggctgggt
 4501 ggggtgcatgg gctttgcaga ctggactttc actccagctc ttttaatgat taggtgtaag
 30 4561 tgacctacat tttgtgagca acagttttct catcagccaa caaagaataa ttacaccaga
 4621 ttcacagtta ttgaagagat aaaggcatga atgtgagatg tctggcatag ggcattctcat
 4681 tttagcagaca cagaatgagt acttgtttct ggccttttct ctctacatat gcacaaagaa
 4741 tgcgactaga agcatgggct ctaggcctgc tcaactttcc tctattttcca ataccaaggg
 4801 gctctgactt aggttgccac accaggcaag gagggcagta ccacctcact tgaccaaggg
 4861 caggagtgca cggacacatc acttcttgag atccttttcc acaccaagga ctgatgtttc
 35 4921 tgggaattct actttatgaa gacaaaacat ataaatggaa attttctcag gtagagactc
 4981 actcttgtag ctcatgagt aggcactagt ggtccacccc cactgtcttt acttattcct
 5041 tgacatcaca tatctcttgc aaaacctcaa ataataatga atgcaatcac ccaataatag
 5101 catagccata attagaggca tttaggaaag acaggtgagt gtgccacaac tacctaacac
 40 5161 atcagcaaat ctggattaac cactttcttt gattttccac aatgcaacct tactttttaa
 5221 tagttgggaa tgttctaagt gaatttagca gaggttggtt atcaacttga aagctgaatt
 5281 ctgacttgct tgactcttgg ttgtgctggt agcagtagat gtttactttt aggttttggg
 5341 ggtggtggaa tatcacttca acgtaaatca tcagaaataa gtatttgtga acccctctcg
 5401 cattaatgta tcttattctg taaaaagaac atgtgcaatt tctcttagat acactactgc
 5461 tgcagctcac aaacacctct gcatattaca tgtacctcct cctgtcctc aagagtgtgg
 45 5521 tctattttgc catcatcacc tgctgtctgc ttagaagaac ggctttctgc tgcaatggag
 5581 agaaatcata a [SEQ ID NO:32]
 1 ataaacaact tgatgcagat gtttccccca agccactat ttttcttctc tcgattgctg
 61 aaacaaaact ccagaaggct ggaacatacc tttgtcttct tgagaaattt tttccagata
 50 121 ttattaagat acattggcaa gaaaagaaga gcaacacgat tctgggatcc caggagggga
 181 acaccatgaa gactaacgac acatacatga aatttagctg gttacgggtg ccagaagagt
 241 cactggacaa agaacacaga tgtatcgtca gacatgagaa taataaaaaac ggaattgatc
 301 aagaaattat ctttccctcca ataaagacag gtatgtgttt acacatatca tctgtcagaa
 361 cacttctttg aaagtgaatg ctgcattttt tcctttcagt attaatgaaa aacataaatc
 55 421 tttcttaaaa attgttacat ttaatggtag cgtaaatgcc ctgctacttt tctatagaat
 481 taaaatggta taggttttgg agaaaacaaa attgaaaaag ttgctgaagg tttgtcagcc
 541 tcagctccat tatccaaaat aagaaagtca cgtgctgggt tttagggttg ttagatggat
 601 taaagaacaa acatacacag aagcatctag caacgtgaca cgtggtaaac gctcaaaaag
 661 tgttctccct tcttttgatg actttacttg atcaggaaat aacatatata tgtctttcag
 60 721 gaatgttctg cccaagcagg agagtcactc acctcaatct tgctaccac aaagttaaac
 781 ctaaaaaaca cgggttcatt gttgacaaaa taatgtttat ctgaagataa ctgtagatca

841 tattttatctg tagataatgt ttatctgtgg agtgtggctc tacaaaaacat agaatagtct
 901 tgggtcactgc agttttatag aggccttggg tttttcagag tttcatttta tatatcacca
 961 taaagtaaca tttcataaatt acagggttggg aaggcttaca tgtacaaaaca ttcttccatt
 1021 ttccataata aatgcatttc ctgccattgg tgaatgcagc tcaataaaca tttattgtac
 5 1081 aattatgaca cgccaggctt agtggaatg tggatgaaca gacaaggatg agttactgtc
 1141 ctaaggatga tgcattgacag tgcagagaat atactctctt cctgatcact cagggtcact
 1201 catgattcat gcgcgagggt ccaaaacagt gcctttgatg cagattctgt acatctctag
 1261 acgattggtc caagggtcga atgtgtctctg gccagtggt ccagtcctgtc actatatgtc
 1321 aacatcctga atatgaacat aacagtccaa catctcaaga gtgggcatga aaaggactca
 10 1381 ttttgtgctt tttcctgtgg ttaacaagtc ctttttagcc tgggggaaca agcattaaca
 1441 aaatgtttga agatctttgc cacgtacct tccaaatttc tagggtaagt ctttagcttt
 1501 tcagatcctg agtttctgca atgatcaaat gtgatttggg cagttgcgtt gactttctcc
 1561 tggggctata atggagtga aaggaaacaa tggcaggga aatgcttgct ttcaaaatgg
 1621 tagcatggat gtgttcattc gtgtagttac tgtattagg atagcctttc ctgaaactaa
 15 1681 ctgaagtggg gttataaaaa cagtcccaat tttctatttc ctttgcctag acacaaagag
 1741 gagacaaaag agcaaagctt gagggtagtt ttaccactgt gcttaagtgt tctgattttt
 1801 ccagtgatca gggtgaaata aaaagcatag taagtccag ggcagtgaat accatacagg
 1861 agacaagtta cagttttata atgtgtttta ctttacta aattctaaaa gtaaaatgtc
 1921 tttttttttt tccgagacag agtttctctc ttgtagccca ggcaggagtg ctatgggtgtg
 20 1981 atctcggctc acagcaacct ccacctccca gtttcaagcg attcttctgc ctacgcctcc
 2041 cgagaagttg aaattacagg tgcctggcac catatctcgc taattattct attttagta
 2101 gagatcgggt tttaccatgt tggccaggct ggtctcgaac tcctgacttc aagtgatcca
 2161 cccgcctcag cctcccaaag tgctgggatt acagggtgtg gtcactgtgc cggacctaac
 2221 agtaaaatgt ctttcatgtg cttctcaagg caactacatt aaggaggaca catctcttaa
 25 2281 tgtcattcta cagtagattt ctaatgctct ttcttggag tttgttttc tgagaagagc
 2341 taaaaatata ataacatgga agtgatcata ttatataatc aatgaagtgc tttcaaagga
 2401 gataaaacta acctggtctg catttgcaac cagccttgat tgagagagag agaactcagg
 2461 atacacttag agattttatt atggggaata gttactttat tcattttacc tcaatcaatg
 2521 catggaaaata agtgacagtc attttcattt atctttta atataaaagtc accatgagga
 30 2581 aaatgaaaac ccattaaagt cagtccttaa agatatttgg acatgcagac atgataacta
 2641 acatttccat tcgtgagact tacccaaaac ctatacctca agtccatttc ttagaataca
 2701 tgaaataaag atctcagtga gtgtataaaa ctgcacacca gaatcatatc cgtatagaca
 2761 agaatacatc tactagaaaa atataaaacca aaacaccaag gtgactctgt tttttctgt
 2821 tttaaaatat gttgtctttg tatgcatgtt tgcttcttcc ttttttttt taaacatcgc
 35 2881 agataaattc aactctcacc tcagttgaga gagaactgtc aatgtgactt ggcctctctc
 2941 tttctagtcc cagaaagaat tgcactgaaa tgctgagctc ctgtaataaa aatgaccatt
 3001 tgctgagagt aattaacata ctgaaaagaga ttttcttaga atagtgcaca atggcccaat
 3061 ggtgacatta tattgtctct ttataaatta ttttctatct atttctgtgg attatttcta
 3121 caaagcactt tcatatgtc caattccttt tattccccta caagtactga ctgactactg
 40 3181 gctctgctgt tcaactgat atgacttctg cagtttctgc aagttgcctg cactttttta acgttatttc
 3241 ctcatcaga acatggggcc atacaaaata caactcactt cagtgttatt ggggaattaa
 3301 acaaaataat gcatgggaag catttaacat agtgctgac acaataatga gcactcagta
 3361 gatgttagct tttattaata ttgttgttgc tatgtccaga aacactatac ctccagaaaa
 3421 tcatgggtac ttgctgggga cgttggggat atgcatgatt ttgaaaggag tgactgctct
 45 3481 ttactgctca gatgagaaat ttttctaagc cagactcctt caaacatgta agattctgtt
 3541 gtggattcta ggactgaaag aattccttggc cgagtgtggg ggcttatcct ggtaactca
 3601 tcatttggga ggacaaggca ggaagattgc ttgagcccag gagttggaaa caagcctgga
 3661 caacatggcg aaaccctgtc tctacaaaaa atacaaacat tagctgggtca tgggagtga
 3721 tgccgtgtact ccagctact caggaggcta agataggag atcacctgag cctgggcagt
 50 3781 ttgagggtttc agtgagccgt gatgacacca tactatactc cactccagcc tgggtgacag
 3841 tgacatcctg cctcaaaaaa acccccaaaa ttattctttt tgctgatttc atgtcagcag
 3901 tgtgtgctga aggtgtgaaa gtagccactt gttctgttta tttttccatt gaacaagtat
 3961 ttatcaaaaa cgtactttgt ggaaggcact gtgctaggaa ctatgcatac agaaggaaaa
 4021 ccaaatgttc ttggatacta cactccagtt gtgataaaaa agaaaaaagt attcttcaca
 55 4081 aacttcaaca ttttgatgtg caaaaacata atatatgaat tagatctacc taactacaca
 4141 gaattagacc aattatttct gggattatgg gctcatattt ttaataactg tcctcctacc
 4201 tctctgttga cagggtttat aaatattcat ttaattacac acagtcacag acacactcag
 4261 acacacacac atacacacac acacacacct tgacaaaata tgggcatgaa caattgactg
 4321 gtacttgctc tcattcttct agatgtcacc acagtggatc ccaaatacaa ttattcaaag
 60 4381 gatgcaaatg gtaagttttt gtgtttttta tttcctcctg atcattttta gttttgaact
 4441 tctctggctt gaaaaatcag ggaatggatt ttgctaggtt ggatgctgca gaatggacct

4501 aatcatatattt taaattagtc cctcttttttc taggagttgt attaacaaac ctaactactg
 4561 cttcatgttaa gagatgactg taaattgaag ggtacagtga tatgctttca gttattttcaa
 4621 aaaacagact ttactcatcc atgtgtcttt tttcttttct tttttttctt ttttgagacg
 4681 gagtctcgct ctggtgaaca ggctggattg cagtgcgcgc atctcacctc actacaacct
 5 4741 ccgcctctgg agttcaagcg attctccagc ctcagcttct caagtagctg ggactacagg
 4801 cacatgccac catgtccggg tcatctttgt attttttagca gagaccgggt ttcactatgt
 4861 tggccaggct ggtctagaat tcctgacttc gtgatctgcc cctcagccc tccgaagtgc
 4921 tgggattaca gacgtgagtc actgtgcccg gcctaacagt aaaatgtctt tcatgcgctt
 4981 ctcaaggcaa ctacgttaag gaggacactt ctcttaatgt cattctacag tagattttcta
 10 5041 atgctctttt ttggaagttt gtttttctga gaaaagctaa aaatataaca tgggaagtgt
 5101 catattgtat aatcaatgaa gtgcttttca aggagataaa actaatctgg tccacgtttg
 5161 caaccaacct tgattgagag agagagagaa ctcaggatac acttgagatg tttattatgg
 5221 ggaatagtta ctttattctt ttttcctcaa tcaattcatg gaaaataagt atagtcatat
 5281 tcattttatct ttttaataaat gaagtcacca tgaggaaaat aaaaagacat tgaaaaccca
 15 5341 ttaaagttag cccttaaaga tatttgagca tgcagacttg ataactaacg tttgcattct
 5401 tgagacttac ccaaaaccca tacctcaagt ccatgttttt agaattcatg aaataaagat
 5461 ctcagtgagt gcataaaatt gcgcaccaga atcatatccg tatagacaag aacacatcta
 5521 ctagaaaaat aataaaccaa cacaccaatg caactgtgtt ttcttctgtt ttaaaatatg
 5581 ttgtctttgt atgcatgttt gcttcttcct tttttttttt taacatcaca gataaattca
 20 5641 actctcacct caggttttat tgagagaact gtcaatgtga cttggcctct gtctttctag
 5701 tcccagaaag aatcgactg aaatgctgag ctctgtaat aaaaatgacc atttgctgag
 5761 agtaattaac atactgaaag agattttctt agagtacaca atggtgacat tatattgtct
 5821 ctttataaat aactttctat ctattttctgt ggattattcc taaaagtac ttttcatatg
 5881 tccagtttct tttcttcccc tacaactacc gtctgaatac tggctctgct atttgctgat
 25 5941 atgattctcg gcaagttgcc tgcacttttt aaactttatt tcctcattca gaacatgggg
 6001 ccatgtaata ctcatgtacg tgagtattac gtaataatgc tcacttaagt gttactgggg
 6061 aattaaacaa aaaaatgcat ggcaagcatt taacatagtg cctgacacaa taatgagcac
 6121 tcagtagatg ttagatttta ttaatatgtt tgttgttatg tccggaacaa ctatacctcc
 6181 agaaaatcat gggacttgc ttgggatgtt ggggatatgc atgatttga aaggtatgac
 30 6241 tgcttttttc tgcttagatg agaaattttt ctaagccaga ctcttcaaa tatgtaagat
 6301 tctgttgtgg attctaggac ggaagaattt cttggtcagg tgtggtttct tatccctgta
 6361 atcccagaat tttggggagg caaggcaggga agattgcttg agcccaggag tttgaaacca
 6421 gcctgggcaa caagacgaaa ccctgtctct acaaaaagta ataaattagc ttggcttggg
 6481 ggtgtgtgcc tgtattacca gctattcggg agactgagat gggaggatct cctgaacctg
 35 6541 tgaagtttga ggcttcagt agccgtgatg acaccatact atactcgact ccagcctgtg
 6601 cgacagtgag actctgcgtc aaaaaaaaaa ccccaaaatt attgtttttg ctgatttcag
 6661 gtcagcagtg tgtgctgaag ggtgtaaagt agccacttga tcagtttatt tttccactga
 6721 acaagtattt atcaaaaaca tactttgtgg tctgtttttg ataaataaaa aggcactgtg
 6781 ctaggagcca tgaatacaga aggaaaacca aatgttcttg gatactacac tccagttgtg
 40 6841 ataaaaaaga aaaatgtatt cttcacgaac ttcaacattt tgatatgcaa aaacatagta
 6901 tataaattag atctacctga ttacgtagaa tcagaccaat tatttctgga attgagggct
 6961 catattttta ataactgtcc tcctgcctct ctgttgacag gttttataaa tattcattta
 7021 attacacaca cacacacaca caccttgaca aataatggac atgaacaatt gactagtact
 7081 tgctctcatt cttctagatg tcatcacaat ggatcccaaa gacaattggg caaaagatgc
 45 7141 aaatggtaag cttttgtgtt tttcctttcc tcctgatcat ttaagtttt gaacttctct
 7201 ggcttgaaaa atcaggggaat gggccgggtg cgggtggctca cgctgtaat cccagcaatt
 7261 tgggagggcg aggcgggcgg atcacgaggt caggagatcg agaccatccc ggctaaaacg
 7321 gtgaaacccc gtctctacta aaaatacaaa aaattagccg ggcttagtgg cgggcgcctg
 7381 tagtcccagc tacttgggag gctgaggcag gagaatggcg tgaaccggg aggcggagct
 50 7441 tgcagtgagc cgagattgag ccactgcact ccactccagc ctgggcgaca gagcgagact
 7501 ccgtctcaaa aaaaaaaaaa aaaaaaaaaa aagaaaaatc agggaatgga ttttgctagg
 7561 ttggatgctg cagaatggac ctagtgtat tttaaattag tccctctttt tctaggagtt
 7621 gtattaaaca acctaactac tgcttcgggt atgagatgac tgtaaattag aggggtacagt
 7681 gatatgcttt cagttatttt aaaaaacaga ctttattcat ccgtctgtct tttttttttt
 55 7741 tttttttttt tttttttgag acggaggagt ctcactctat caccaggtct ggagtgcagt
 7801 ggcgcgatct cggctacca taacctccgc cttactgggt caagcgattc tccagcctca
 7861 gcttctcaag tagctgggac tacaggtgca caccaccata cctggctaatt ttttgatatt
 7921 ttaatagaga tgggggtttca ccacgctggc caggatgggt ttgaattctt gacctcgtga
 7981 tctgccccct cgggctccca aacttctggg attataggcg tgagccactg tgcccggcct
 60 8041 tctgtctttt gttataatga ctggggaaaa catgatacca tgttgcttct tgagttgttt
 8101 tgttttagtc tttgggtctt gctagtagct aataacacga actagtgttt atcaagtgtc

8161 ttttacacag aagggccttgt tctgcatttt ctagtttaaat catcttaata ctctataaaa
 8221 gtagtacaat atatcttctc ccattttaca gtccctttaa agtaaataac tataaaaaatc
 8281 ccttatacat gtcacacagc taggtctggc atttcaaatac aggacatcaa acaaagaatt
 8341 cgtgcagtta ctaagtcctc tatttttttct acaatagaaa aaatagcaag aattacagat
 5 8401 agcaagacat tacaaggcag gaatctgaaa cgaaaggac ataatgtggg gctgggtggg
 8461 tgcatgagct ttgcagacta gactttcatt ccagctcttt taatgattag gtgtaagtga
 8521 cctacatttt gtgagtaaca gttttctcat cagccaacta agaataatta caccagattc
 8581 acagttattg aagagataag ggcatgaatg tgagatgtct ggcgtagggt atctcattta
 8641 gcagacacag aatgaatact tgtttctggc tttttctctc tacatatgca caaagaatgt
 10 8701 gactagaagc attggctcta gccctgctca actttctctc atttccaata ccaaggggct
 8761 ctgacttagg ctgccacacc aggcaaggag gggcagtacc acctcacttg accaagggca
 8821 gggagtcacg gacacatcac ttcctgagat cttttccac accaaggact gatgtttctg
 8881 gaattctcac tttatgaaga caaaacatat aaatggaaat ttctgcagga agagactcac
 8941 tcttgtagct cattgagtag gcactagtgg tccaccccca ctgtctttac ttattccttg
 15 9001 acatcacata tctcttgtaa aacctcaaata aatgttaaata gcaatcacc aataatagca
 9061 tagccataat tagaggcatt taggaaagac aggtgagtgt gccacaacta cctaacacat
 9121 cagcaaatac ggattaacca ctttctttga ttttccacaa tgcaacctta ctttttaata
 9181 gttgggaatg ttctaagtga atttagcaga ggttgtaata caactgaaa gctgaattct
 9241 gacttgctctg actcttggtg gtgctggtag cagtagatgt ttacttttag gttttggtgg
 20 9301 tgggtgaata tcacttcaac gtaaatcatc agaaataagt atttgtaac ccctctcgca
 9361 ttaatatatc ttattctgta aaaagaacat gtgcaatttc tcttagatac actactgctg
 9421 cagctcacia acacctctgc atattacacg tacctcctcc tgctcctcaa gagtggtggtc
 9481 tattttgcca tcatcacctg ctgtctgctt agaagaacgg ctttctgctg caatggagag
 9541 aaatcataa [SEQ ID NO:33]
 25

In accordance with the presently disclosed subject matter, an “TRGC nucleic acid molecule” refers to a polynucleotide encoding an TRGC polypeptide.

In certain embodiments, the constant domain of a presently disclosed HI-TCR comprises a native or modified TRDC peptide. In certain embodiments, the TRDC polypeptide has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous or identical to the amino acid sequence set forth in SEQ ID NO: 41, which is provided below.

SQPHTKPSVF VMKNGTNVAC LVKEFYPKDI RINLVSSKKI TEFDP AIVIS
 35 PSGKYNAVKL GKYEDSNSVT CSVQHDNKT V HSTDFEVKTD STDHV KPKET
 ENTQPSKSC HKPKAIVHTE KVNMSLTVL GLRMLFAKT V AVN FLLTAKL
 FFL [SEQ ID NO:41]

In certain non-limiting embodiments, T cell receptor constant region comprises a hinge/spacer region that links the extracellular antigen-binding domain to the constant domain. The hinge/spacer region can be flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition. In certain non-limiting embodiments, the hinge/spacer region can be the hinge region from IgG1, or the CH₂CH₃ region of immunoglobulin and portions of CD3, a portion of a CD28 polypeptide, a portion of a CD8 polypeptide, a variation of any of the foregoing which is at least about 80%, at least about 85%, at least about 90%, or at least about 95% homologous or identical thereto, or a synthetic spacer sequence. In certain non-limiting

embodiments, the hinge/spacer region of the CAR can comprise a native or modified hinge region of a CD3 ζ polypeptide, a CD40 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, a CD166 peptide, a CD166 peptide, a CD8a peptide, a CD8b peptide, an ICOS polypeptide, an ICAM-1 peptide, a CTLA-4 peptide, a synthetic peptide (not based
 5 on a protein associated with the immune response), or a combination thereof.

2.4. Intracellular Signaling Domain

In certain non-limiting embodiments, a presently disclosed HI-TCR comprises an antigen binding chain, which does not comprise an intracellular domain. In certain
 10 embodiments, the antigen binding chain is capable of associating with a CD3 ζ polypeptide. In certain embodiments, the antigen binding chain comprises a constant domain, which is capable of associating with a CD3 ζ polypeptide. In certain
 15 embodiments, the CD3 ζ polypeptide is endogenous. In certain embodiments, the CD3 ζ polypeptide is exogenous. In certain embodiments, binding of the antigen binding chain to an antigen is capable of activating the CD3 ζ polypeptide associated to the antigen
 binding chain. In certain embodiments, the exogenous CD3 ζ polypeptide is fused to or integrated with a costimulatory molecule disclosed herein.

In certain non-limiting embodiments, a presently disclosed HI-TCR comprises an antigen binding chain that comprises an intracellular domain. In certain embodiments,
 20 the intracellular domain comprises a CD3 ζ polypeptide. In certain embodiments, binding of the antigen binding chain to an antigen is capable of activating the CD3 ζ polypeptide of the antigen binding chain.

The activated CD3 ζ polypeptide can activate and/or stimulate an immunoresponsive cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell). CD3 ζ
 25 comprises three immunoreceptor tyrosine-based activation motifs (ITAM1, ITAM2 and ITAM3), three basic-rich stretch (BRS) regions (BRS1, BRS2 and BRS3), and transmits an activation signal to the cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell) after antigen is bound to the antigen binding chain. The intracellular signaling domain of the
 CD3 ζ -chain is the primary transmitter of signals from endogenous TCRs. In certain
 30 embodiments, the CD3 ζ polypeptide comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_932170 (SEQ ID NO: 17), NCBI Reference No: NP_000725.1 (SEQ ID NO: 18) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain non-limiting embodiments, the CD3 ζ polypeptide

comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 17, which is at least 20, or at least 30, or at least 40, or at least 50, and up to 164 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD3 ζ polypeptide comprises or has an amino acid sequence of amino acids 1 to 164, 1 to 50, 50 to 100, 100 to 150, or 150 to 164 of SEQ ID NO: 17. In certain embodiments, the CD3 ζ polypeptide comprises or has an amino acid sequence of amino acids 52 to 164 of SEQ ID NO: 17.

SEQ ID NO: 17 is provided below:

1 MKWKALFTAA ILQAQLPITE AQSFGLLDPK LCYLLDGILF IYGVILTALF LRVKFSRSAD
10 61 APAYQQGQNQ LYNELNLGRRE EYDVLDKRR GRDPEMGGKP QRRKNPQEGLY YNELQKDKMA
121 EAYSEIGMKG EERRGKGHDG LYQGLSTATK DTYDALHMQA LPPR [SEQ ID NO: 17]

In certain embodiments, the intracellular signaling domain comprises a human CD3 ζ polypeptide. The human CD3 ζ polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to SEQ ID NO: 18 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 18 is provided below:

RVKFSRSADA PAYQQGQNQL YNELNLGRRE EYDVLDKRRG RDPEMGGKPR RKNPQEGLYN
20 ELQKDKMAEA YSEIGMKGER RRGKGHDGLY QGLSTATKDT YDALHMQALP PR [SEQ ID NO:
18] .

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 18 is set forth in SEQ ID NO: 19, which is provided below.

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTC
AATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGAAAG
25 CCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGT
GAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCC
ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC [SEQ ID NO: 19]

2.4.1. Co-stimulatory region

In certain non-limiting embodiments, a presently disclosed HI-TCR comprises an antigen binding chain that comprises an intracellular domain, wherein the intracellular domain comprises a co-stimulatory region. In certain embodiments, the intracellular domain comprises a co-stimulatory region and a CD3 ζ polypeptide. In certain embodiments, the intracellular domain comprises a co-stimulatory region and does not comprise a CD3 ζ polypeptide.

In certain embodiments, the co-stimulatory region comprises at least one co-stimulatory molecule, which can provide optimal lymphocyte activation. As used herein, “co-stimulatory molecules” refer to cell surface molecules other than antigen receptors or their ligands that are required for an efficient response of lymphocytes to antigen. The at least one co-stimulatory signaling region can include a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide, or a combination thereof. The co-stimulatory molecule can bind to a co-stimulatory ligand, which is a protein expressed on cell surface that upon binding to its receptor produces a co-stimulatory response, *i.e.*, an intracellular response that effects the stimulation provided when an antigen binds to its CAR molecule. Co-stimulatory ligands, include, but are not limited to CD80, CD86, CD70, OX40L, and 4-1BBL. As one example, a 4-1BB ligand (*i.e.*, 4-1BBL) may bind to 4-1BB (also known as “CD137”) for providing an intracellular signal that in combination with a CAR signal induces an effector cell function of the CAR⁺ T cell. CARs comprising an intracellular signaling domain that comprises a co-stimulatory signaling region comprising 4-1BB, ICOS or DAP-10 are disclosed in U.S. 7,446,190, which is herein incorporated by reference in its entirety.

In certain embodiments, the co-stimulatory region of an antigen binding chain of a HI-TCR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide. The CD28 polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous or identical to the sequence having a NCBI Reference No: P10747 or NP_006130 (SEQ ID NO: 20), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In non-limiting certain embodiments, the CD28 polypeptide comprises or has an amino acid sequence that is a consecutive portion of SEQ ID NO: 20 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. Alternatively or additionally, in non-limiting various embodiments, the CD28 polypeptide comprises or has an amino acid sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, or 200 to 220 of SEQ ID NO: 20. In certain embodiments, the co-stimulatory region comprises a co-stimulatory signaling region that comprises a CD28 polypeptide comprising or having an amino acid sequence of amino acids 180 to 220 of SEQ ID NO: 20.

```

1   MLRLLLALNL FPSIQVTGNK ILVKQSPMLV AYDNAVNLSC KYSYNLFSRE FRASLHKGLD
61  SAVEVCVVYG NYSQQLQVYS KTGFNCDGKL GNESVTFYLQ NLYVNQTDIY FCKIEVMYPP

```

121 PYLDNEKSNG TIIHVKGKHL CPSPLFPGPS KPFWVLVVVG GVLACYSLIV TVAFIIFWVR
 181 SKRSRLLHSD YMNMTPRRPG PTRKHYQPYA PPRDFAAYRS [SEQ ID NO: 20]

In certain embodiments, the co-stimulatory region comprises a human intracellular signaling domain of CD28. The human intracellular signaling domain of CD28 can
 5 comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to SEQ ID NO: 21 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 21 is provided below:

10 RSKRSRLLHS DYMNMTPRRP GPTRKHYQPY APPRDFAAAYR S [SEQ ID NO: 21].

An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 21 is set forth in SEQ ID NO: 22, which is provided below.

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCGGGCCCCACCCGC
 AAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC [SEQ ID NO: 22]

15 In certain embodiments, the co-stimulatory region comprises a co-stimulatory signaling region that comprises two co-stimulatory molecules, e.g., co-stimulatory signaling regions of CD28 and 4-1BB or co-stimulatory signaling regions of CD28 and OX40.

4-1BB can act as a tumor necrosis factor (TNF) ligand and have stimulatory
 20 activity. The 4-1BB polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to the sequence having a NCBI Reference No: P41273 or NP_001552 (SEQ ID NO: 23) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

25 SEQ ID NO: 23 is provided below:

1 MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP NSFSSAGGQR
 61 TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS MCEQDCKQGQ ELTKKGCKDC
 121 CFGTFNDQKR GICRPWTNCS LDGKSVLVNG TKERDVVCGP SPADLSPGAS SVTPPAPARE
 181 PGHSPQIISF FLALTSTALL FLLFFLTLLRF SVVKGGRKKL LYIFKQPFMR PVQTTQEEDG
 30 241 CSCRFPPEEEE GGCEL [SEQ ID NO: 23]

In accordance with the presently disclosed subject matter, a “4-1BB nucleic acid molecule” refers to a polynucleotide encoding a 4-1BB polypeptide.

In certain embodiments, the co-stimulatory region comprises an intracellular signaling domain of 4-1BB. The intracellular signaling domain of 4-1BB can comprise or
 35 have an amino acid sequence that is at least about 85%, about 90%, about 95%, about

96%, about 97%, about 98%, about 99% or about 100% homologous or identical to SEQ ID NO: 24 or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. SEQ ID NO: 24 is provided below:

KRGRKKLLYI FKQPFMRPVQ TTQEEDGCSC RFPEEEEEGGC EL [SEQ ID NO: 24].

5 An exemplary nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 24 is set forth in SEQ ID NO: 27, which is provided below.

AAACGGGGCAGAAAGAACTCCTGTATATATTCAAACAACCATTATGAGACCAGTACAACTACTCA
AGAGGAAGATGGCTGTAGCTGCCGATTTCAGAGAAGAAGAAGGAGGATGTGAACTG [SEQ ID
NO: 27]

10 An OX40 polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to the sequence having a NCBI Reference No: P43489 or NP_003318 (SEQ ID NO: 25), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

15 SEQ ID NO: 25 is provided below:

1 MCVGARRLGR GPCAALLLLG LGLSTVTGLH CVGDTYPSND RCHECRPGN GMVSRCSRSQ
61 NTVCRPCGPG FYNDVVSSKP CKPCTWCNLR SGSERKQLCT ATQDTVCRCR AGTQPLDSYK
121 PGVDCAPCPP GHFSPGDNQA CKPWTNCTLA GKHTLQPASN SSDAICEDRD PPATQPQETQ
181 GPPARPITVQ PTEAWPRTSQ GPSTRPVEVP GGRAVAAILG LGLVLGLLGP LAILLALYLL
20 241 RRDQRLPPDA HKPPGGGSFR TPIQEEQADA HSTLAKI [SEQ ID NO: 25]

In accordance with the presently disclosed subject matter, an “OX40 nucleic acid molecule” refers to a polynucleotide encoding an OX40 polypeptide.

An ICOS polypeptide can comprise or have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or
25 about 100% homologous or identical to the sequence having a NCBI Reference No: NP_036224 (SEQ ID NO: 26) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

SEQ ID NO: 26 is provided below:

1 MKSGLWYFFL FCLRIKVLTG EINGSANYEM FIFHNGGVQI LCKYPDIVQQ FKMQLLKGGQ
30 61 ILCDLTKTKG SGNTVSIKSL KFCHSQLSNN SVSFFLYNLD HSHANYFFCN LSIFDPPPFK
121 VTLTGGYLHI YESQLCCQLK FWLPIGCAAF VVCILGCIL ICWLTKKKYS SSVHDPNGEY
181 MFMRAVNTAK KSRLTDVTL [SEQ ID NO: 26]

In accordance with the presently disclosed subject matter, an “ICOS nucleic acid molecule” refers to a polynucleotide encoding an ICOS polypeptide.

35 In certain embodiments, mutation sites and/or junction between domains/motifs/regions of the CAR derived from different proteins are de-immunized.

Immunogenicity of junctions between different CAR moieties can be predicted using NetMHC 4.0 Server. For each peptide containing at least 1 aa from next moiety, binding affinity to HLA A, B and C, for all alleles, can be predicted. A score of immunogenicity of each peptide can be assigned for each peptide. Immunogenicity score can be calculated using the formula Immunogenicity score=[(50-binding affinity)*HLA frequency]ⁿ. n is the number of prediction for each peptide.

2.5. CD3 Complex

In certain embodiments, a presently disclosed HI-TCR is capable of associating with a CD3 complex (also known as “T-cell co-receptor”). In certain embodiments, the HI-TCR and the CD3 complex form an antigen recognizing receptor complex similar to a native TCR/CD3 complex. In certain embodiments, the CD3 complex is endogenous. In certain embodiments, the CD3 complex is exogenous. In certain embodiments, the presently disclosed HI-TCR replaces a native and/or an endogenous TCR in the CD3/TCR complex. In certain embodiments, the CD3 complex comprises a CD3 γ chain, a CD3 δ chain, and two CD3 ϵ chains.

In certain embodiments, the CD3 γ chain comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to NCBI reference number: NP_000064.1 (SEQ ID NO: 34, which is provided below) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

```
1 meqgkgglavl ilaiillqgt laqsikgnhl vkvydyqedg svlltcdaea knitwfkdgk
61 migfltedkk kwnlgsnakd prgmyqckgs qnkskplqvy yrmcnciel naatisgflf
121 aeivsifvla vgvfyiaqqd gvrqsraskd qtlpndqly qplkdreddq yshlqgnqlr
181 rn [SEQ ID NO:34]
```

In certain embodiments, the CD3 δ chain comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous or identical to NCBI reference numbers: NP_000723.1 (SEQ ID NO: 35, which is provided below), NP_001035741.1 (SEQ ID NO: 36, which is provided below) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

```
1 mehstflsgl vlatllsqvs pfkipieele drvfvcnts itwvegtvgt llsditrldl
61 gkrildprgi yrcngtdiyk dkestvqvhy rmcqscvld patvagiivt dviatlalll
121 gvfcfaghet grlsgaadtd allrndqvyy plrdreddaq shlggnwarn k [SEQ ID NO:
35]
```

```
1 mehstflsgl vlatllsqvs pfkipieele drvfvcnts itwvegtvgt llsditrldl
```

61 gkrildprgi yrcngtdiyk dkestvqvhy rtadtqallr ndqvyqplrd rddaqyshlg
 121 gnwarnk [SEQ ID NO: 36]

In certain embodiments, the CD3ε chain comprises or has an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%,
 5 about 99% or about 100% homologous or identical to NCBI reference number: NP_000724.1 (SEQ ID NO: 37, which is provided below) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

10 1 mqs gthwrvl glcllsvgvw ggdgneemgg itqtpykvsi sgttviltcp qypgseilwq
 61 hndkniggde ddknigsded hlslkefsel eqsgyyvcyp rgskpedanf ylylrarvce
 121 ncmemdvmvsv ativivdici tgglllllvyy wsknrkakak pvtrgagagg rrggqnkerp
 181 ppvpnpdyep irkgqrdlys glnqrri [SEQ ID NO: 37]

In certain embodiments, the recombinant TCR exhibits a greater antigen
 15 sensitivity than a CAR targeting the same antigen. In certain embodiments, the recombinant TCR is capable of inducing an immune response when binding to an antigen that has a low density on the surface of a tumor cell. In certain embodiments, immunoresponsive cells comprising a presently disclosed HI-TCR can be used to treat a
 20 subject having tumor cells with a low expression level of a surface antigen, e.g., from a relapse of a disease, wherein the subject received treatment which leads to residual tumor cells. In certain embodiments, the tumor cells have a low density of a target molecule on the surface of the tumor cells. In certain embodiments, a target molecule having a low
 25 density on the cell surface has a density of less than about 5,000 molecules per cell, less than about 4,000 molecules per cell, less than about 3,000 molecules per cell, less than about 2,000 molecules per cell, less than about 1,500 molecules per cell, less than about 1,000 molecules per cell, less than about 500 molecules per cell, less than about 200 molecules per cell, or less than about 100 molecules per cell. In certain embodiments, a
 30 target molecule having a low density on the cell surface has a density of less than about 2,000 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of less than about 1,500 molecules per cell. In certain
 35 embodiments, a target molecule having a low density on the cell surface has a density of less than about 1,000 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of between about 4,000 molecules per cell and about 2,000 molecules per cell, between about 2,000 molecules per cell and about 1,000 molecules per cell, between about 1,500 molecules per cell and about 1,000 molecules per cell, between about 2,000 molecules per cell and about 500 molecules per

cell, between about 1,000 molecules per cell and about 200 molecules per cell, or between about 1,000 molecules per cell and about 100 molecules per cell.

2.6. HI-TCR 19

In certain embodiments, a presently disclosed HI-TCR comprises two antigen
5 binding chains, e.g., VL-TRAC and VH-TRBC, which are capable of dimerizing, wherein the HI-TCR binds to CD19 (e.g., human CD19).

VL-TRAC

In certain embodiments, a presently disclosed HI-TCR comprises an antigen
binding chain that comprises an extracellular antigen-binding domain of a V_L domain of
10 an antibody and a constant domain of TRAC. In certain embodiments, the antibody binds to CD19 (e.g., human CD19). In certain embodiments, the antigen binding chain is designated as “VL-TRAC”.

VH-TRBC

In certain embodiments, a presently disclosed HI-TCR comprises an antigen
15 binding chain that comprises an extracellular antigen-binding domain of a V_H domain of an antibody and a constant domain of TRBC. In certain embodiments, the antibody binds to CD19 (e.g., human CD19). In certain embodiments, the antigen binding chain is designated as “VH-TRBC”.

3. Immunoresponsive Cells

20 The presently disclosed subject matter provides immunoresponsive cells comprising a presently disclosed HI-TCR. In certain embodiments, the HI-TCR is capable of activating the immunoresponsive cell. Upon binding to the antigen, the immunoresponsive cells exhibit cytolytic effects towards cells bearing the antigen. In certain embodiments, the immunoresponsive cells comprising the HI-TCR exhibits
25 comparable or better therapeutic potency compared to cells comprising a chimeric antigen receptor (CAR) targeting the same antigen. In certain embodiments, the immunoresponsive cells comprising the HI-TCR exhibit comparable or better cytolytic effects compared to cells comprising a chimeric antigen receptor (CAR) targeting the same antigen. In certain embodiments, the immunoresponsive cells comprising the HI-
30 TCR secrete anti-tumor cytokines. The cytokines secreted by the immunoresponsive cells include, but are not limited to, TNF α , IFN γ and IL2.

The immunoresponsive cells of the presently disclosed subject matter can be cells of the lymphoid lineage. The lymphoid lineage, comprising B, T and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system,

detection of foreign agents in the blood, detection of cells foreign to the host, and the like.

Non-limiting examples of immunoresponsive cells of the lymphoid lineage include T cells, Natural Killer T (NKT) cells, and precursors thereof including embryonic stem cells, and pluripotent stem cells (*e.g.*, those from which lymphoid cells may be

5 differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. The T cells of the presently disclosed subject matter can be any type of T cells, including, but not limited to, helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and
10 two types of effector memory T cells: *e.g.*, T_{EM} cells and T_{EMRA} cells, Regulatory T cells (also known as suppressor T cells), Natural killer T cells, Mucosal associated invariant T cells, and $\gamma\delta$ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. A patient's own T cells may be genetically modified to target specific antigens through the
15 introduction of an HI-TCR. In certain embodiments, the immunoresponsive cell is a T cell. The T cell can be a CD4⁺ T cell or a CD8⁺ T cell. In certain embodiments, the T cell is a CD4⁺ T cell. In certain embodiments, the T cell is a CD8⁺ T cell.

In certain embodiments, the immunoresponsive cell comprises an exogenous or a recombinant (*e.g.*, the cell is transduced with) at least one co-stimulatory ligand. In
20 certain embodiments, the immunoresponsive cell co-expresses the HI-TCR and the at least one exogenous co-stimulatory ligand. The interaction between the HI-TCR and at least one exogenous co-stimulatory ligand provides a non-antigen-specific signal important for full activation of an immunoresponsive cell (*e.g.*, T cell). Co-stimulatory ligands include, but are not limited to, members of the tumor necrosis factor (TNF)
25 superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Members of TNF superfamily share a number of common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic
30 segment and a relatively long extracellular region. TNF superfamily members include, but are not limited to, nerve growth factor (NGF), CD40L (CD40L)/CD154, CD137L/4-1BBL, TNF- α , CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNF β)/lymphotoxin-alpha (LT α),

lymphotoxin-beta (LT β), CD257/B cell-activating factor (BAFF)/Blys/THANK/Tall-1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins -- they possess an immunoglobulin domain (fold). Immunoglobulin superfamily ligands include, but are not limited to, CD80 and CD86, both ligands for CD28. In certain embodiments, the at least one co-stimulatory ligand is selected from the group consisting of 4-1BBL, CD275, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, and combinations thereof. In certain embodiments, the immunoresponsive cell comprises or consists of one exogenous or recombinant co-stimulatory ligand. In certain embodiments, the one exogenous or recombinant co-stimulatory ligand is 4-1BBL or CD80. In certain embodiments, the one exogenous or recombinant co-stimulatory ligand is 4-1BBL. In certain embodiments, the immunoresponsive cell comprises or consists of two exogenous or recombinant co-stimulatory ligands. In certain embodiments, the two exogenous or recombinant co-stimulatory ligands are 4-1BBL and CD80.

In certain embodiments, the immunoresponsive cell can comprise or be transduced with at least one chimeric co-stimulatory receptor (CCR). As used herein, the term "chimeric co-stimulatory receptor" or "CCR" refers to a chimeric receptor that binds to an antigen, and, upon its binding to the antigen, provides a co-stimulatory signal to a cell (e.g., a T cell) comprising the CCR, but does not alone provide an activation signal to the cell. CCR is described in Krause, et al., J. Exp. Med. (1998);188(4):619-626, and US20020018783, which is incorporated by reference in its entirety. CCRs mimic co-stimulatory signals, but unlike, CARs, do not provide a T-cell activation signal, e.g., CCRs lack a CD3 ζ polypeptide. CCRs provide co-stimulation, e.g., a CD28-like signal, in the absence of the natural co-stimulatory ligand on the antigen-presenting cell. A combinatorial antigen recognition, i.e., use of a CCR in combination with a CAR, can augment T-cell reactivity against the dual-antigen expressing T cells, thereby improving selective tumor targeting. See WO2014/055668, which is incorporated by reference in its entirety. Kloss et al., describe a strategy that integrates combinatorial antigen recognition, split signaling, and, critically, balanced strength of T-cell activation and co-stimulation to generate T cells that eliminate target cells that express a combination of antigens while sparing cells that express each antigen individually (Kloss et al., Nature Biotechnology (2013);31(1):71-75, the content of which is incorporated by reference in

its entirety). With this approach, T-cell activation requires CAR-mediated recognition of one antigen, whereas co-stimulation is independently mediated by a CCR specific for a second antigen. To achieve tumor selectivity, the combinatorial antigen recognition approach diminishes the efficiency of T-cell activation to a level where it is ineffective without rescue provided by simultaneous CCR recognition of the second antigen.

In certain embodiments, the CCR comprises an extracellular antigen-binding domain that binds to a second antigen, a transmembrane domain, and a co-stimulatory signaling region that comprises at least one co-stimulatory molecule. In certain embodiments, the CCR does not alone deliver an activation signal to the cell. Non-limiting examples of co-stimulatory molecules include CD28, 4-1BB, OX40, ICOS, DAP-10 and any combination thereof. In certain embodiments, the co-stimulatory signaling region of the CCR comprises one co-stimulatory signaling molecule. In certain embodiments, the one co-stimulatory signaling molecule is CD28. In certain embodiments, the one co-stimulatory signaling molecule is 4-1BB. In certain embodiments, the co-stimulatory signaling region of the CCR comprises two co-stimulatory signaling molecules. In certain embodiments, the two co-stimulatory signaling molecules are CD28 and 4-1BB. A second antigen is selected so that expression of both the first antigen and the second antigen is restricted to the targeted cells (e.g., cancerous tissue or cancerous cells). Similar to a CAR, the extracellular antigen-binding domain can be a scFv, a Fab, a F(ab)₂, or a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain.

In certain embodiments, the CCR is co-expressed with a HI-TCR binding to an antigen that is different from the antigen to which the CCR binds, e.g., the HI-TCR binds to a first antigen and the CCR binds to a second antigen.

Types of human lymphocytes of the presently disclosed subject matter include, without limitation, peripheral donor lymphocytes, *e.g.*, those disclosed in Sadelain, M., *et al.* 2003 *Nat Rev Cancer* 3:35-45 (disclosing peripheral donor lymphocytes genetically modified to express CARs), in Morgan, R.A., *et al.* 2006 *Science* 314:126-129 (disclosing peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the α and β heterodimer), in Panelli, M.C., *et al.* 2000 *J Immunol* 164:495-504; Panelli, M.C., *et al.* 2000 *J Immunol* 164:4382-4392 (disclosing lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies), and in Dupont, J., *et al.* 2005 *Cancer Res*

65:5417-5427; Papanicolaou, G.A., *et al.* 2003 *Blood* 102:2498-2505 (disclosing selectively *in vitro*-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells). The immunoresponsive cells (*e.g.*, T cells) can be autologous, non-autologous (*e.g.*,
5 allogeneic), or derived *in vitro* from engineered progenitor or stem cells.

The presently disclosed immunoresponsive cells are capable of modulating the tumor microenvironment. Tumors have a microenvironment that is hostile to the host immune response involving a series of mechanisms by malignant cells to protect themselves from immune recognition and elimination. This “hostile tumor
10 microenvironment” comprises a variety of immune suppressive factors including infiltrating regulatory CD4⁺ T cells (Tregs), myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), immune suppressive cytokines including TGF-β, and expression of ligands targeted to immune suppressive receptors expressed by activated T cells (CTLA-4 and PD-1). These mechanisms of immune suppression play a
15 role in the maintenance of tolerance and suppressing inappropriate immune responses, however within the tumor microenvironment these mechanisms prevent an effective anti-tumor immune response. Collectively these immune suppressive factors can induce either marked anergy or apoptosis of adoptively transferred CAR modified T cells upon encounter with targeted tumor cells.

20 The unpurified source of CTLs may be any known in the art, such as the bone marrow, fetal, neonate or adult or other hematopoietic cell source, *e.g.*, fetal liver, peripheral blood or umbilical cord blood. Various techniques can be employed to separate the cells. For instance, negative selection methods can remove non-CTLs initially. mAbs are particularly useful for identifying markers associated with particular cell lineages
25 and/or stages of differentiation for both positive and negative selections.

A large proportion of terminally differentiated cells can be initially removed by a relatively crude separation. For example, magnetic bead separations can be used initially to remove large numbers of irrelevant cells. In certain embodiments, at least about 80%, usually at least about 70% of the total hematopoietic cells will be removed prior to cell
30 isolation.

Procedures for separation include, but are not limited to, density gradient centrifugation; resetting; coupling to particles that modify cell density; magnetic separation with antibody-coated magnetic beads; affinity chromatography; cytotoxic agents joined to or used in conjunction with a mAb, including, but not limited to,

complement and cytotoxins; and panning with antibody attached to a solid matrix, e.g. plate, chip, elutriation or any other convenient technique.

Techniques for separation and analysis include, but are not limited to, flow cytometry, which can have varying degrees of sophistication, e.g., a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels.

The cells can be selected against dead cells, by employing dyes associated with dead cells such as propidium iodide (PI). In certain embodiments, the cells are collected in a medium comprising 2% fetal calf serum (FCS) or 0.2% bovine serum albumin (BSA) or any other suitable, e.g., sterile, isotonic medium.

4. Vectors

Genetic modification of an immunoresponsive cell (e.g., a T cell or an NKT cell) can be accomplished by transducing a substantially homogeneous cell composition with a recombinant DNA construct. In certain embodiments, a retroviral vector (either gamma-retroviral or lentiviral) is employed for the introduction of the DNA construct into the cell. For example, a polynucleotide encoding an HI-TCR can be cloned into a retroviral vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest. Non-viral vectors may be used as well.

For initial genetic modification of an immunoresponsive cell to include an HI-TCR, a retroviral vector is generally employed for transduction, however any other suitable viral vector or non-viral delivery system can be used. The HI-TCR can be constructed with an auxiliary molecule (e.g., a cytokine) in a single, multicistronic expression cassette, in multiple expression cassettes of a single vector, or in multiple vectors. Examples of elements that create polycistronic expression cassette include, but is not limited to, various viral and non-viral Internal Ribosome Entry Sites (IRES, e.g., FGF-1 IRES, FGF-2 IRES, VEGF IRES, IGF-II IRES, NF- κ B IRES, RUNX1 IRES, p53 IRES, hepatitis A IRES, hepatitis C IRES, pestivirus IRES, aphthovirus IRES, picornavirus IRES, poliovirus IRES and encephalomyocarditis virus IRES) and cleavable linkers (e.g., 2A peptides, e.g., P2A, T2A, E2A and F2A peptides). Combinations of retroviral vector and an appropriate packaging line are also suitable, where the capsid proteins will be functional for infecting human cells. Various amphotropic virus-producing cell lines are known, including, but not limited to, PA12 (Miller, *et al.* (1985) *Mol. Cell. Biol.* 5:431-437); PA317 (Miller, *et al.* (1986) *Mol. Cell. Biol.* 6:2895-2902); and CRIP (Danos, *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464). Non-

amphotropic particles are suitable too, e.g., particles pseudotyped with VSVG, RD114 or GALV envelope and any other known in the art.

Possible methods of transduction also include direct co-culture of the cells with producer cells, e.g., by the method of Bregni, *et al.* (1992) *Blood* 80:1418-1422, or
 5 culturing with viral supernatant alone or concentrated vector stocks with or without appropriate growth factors and polycations, e.g., by the method of Xu, *et al.* (1994) *Exp. Hemat.* 22:223-230; and Hughes, *et al.* (1992) *J. Clin. Invest.* 89:1817.

Other transducing viral vectors can be used to modify an immunoresponsive cell. In certain embodiments, the chosen vector exhibits high efficiency of infection and stable
 10 integration and expression (see, e.g., Cayouette *et al.*, *Human Gene Therapy* 8:423-430, 1997; Kido *et al.*, *Current Eye Research* 15:833-844, 1996; Bloomer *et al.*, *Journal of Virology* 71:6641-6649, 1997; Naldini *et al.*, *Science* 272:263-267, 1996; and Miyoshi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:10319, 1997). Other viral vectors that can be used include, for example, adenoviral, lentiviral, and adena-associated viral vectors, vaccinia
 15 virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, *Human Gene Therapy* 15-14, 1990; Friedman, *Science* 244:1275-1281, 1989; Eglitis *et al.*, *BioTechniques* 6:608-614, 1988; Tolstoshev *et al.*, *Current Opinion in Biotechnology* 1:55-61, 1990; Sharp, *The Lancet* 337:1277-1278, 1991; Cornetta *et al.*, *Nucleic Acid Research and Molecular Biology* 36:311-322, 1987; Anderson, *Science* 226:401-409, 1984; Moen, *Blood Cells* 17:407-416, 1991; Miller *et al.*, *Biotechnology* 7:980-990, 1989; LeGal La Salle *et al.*, *Science* 259:988-990, 1993; and Johnson, *Chest* 107:77S- 83S, 1995). Retroviral vectors are particularly well
 20 developed and have been used in clinical settings (Rosenberg *et al.*, *N. Engl. J. Med* 323:370, 1990; Anderson *et al.*, U.S. Pat. No. 5,399,346).

Non-viral approaches can also be employed for genetic modification of an
 25 immunoresponsive cell. For example, a nucleic acid molecule can be introduced into an immunoresponsive cell by administering the nucleic acid in the presence of lipofection (Feigner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 84:7413, 1987; Ono *et al.*, *Neuroscience Letters* 17:259, 1990; Brigham *et al.*, *Am. J. Med. Sci.* 298:278, 1989; Staubinger *et al.*,
 30 *Methods in Enzymology* 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu *et al.*, *Journal of Biological Chemistry* 263:14621, 1988; Wu *et al.*, *Journal of Biological Chemistry* 264:16985, 1989), or by micro-injection under surgical conditions (Wolff *et al.*, *Science* 247:1465, 1990). Other non-viral means for gene transfer include transfection *in vitro* using calcium phosphate, DEAE dextran, electroporation, and

protoplast fusion. Liposomes can also be potentially beneficial for delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type *ex vivo* (e.g., an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically.

Recombinant receptors can also be derived or obtained using transposases or targeted nucleases (e.g. Zinc finger nucleases, meganucleases, or TALE nucleases, CRISPR).

Transient expression may be obtained by RNA electroporation. In certain embodiments, recombinant receptors can be introduced by a transposon-based vector. In certain embodiments, the transposon-based vector comprises a transposon (a.k.a. a transposable element). In certain embodiments, the transposon can be recognized by a transposase. In certain embodiments, the transposase is a Sleeping Beauty transposase.

Clustered regularly-interspaced short palindromic repeats (CRISPR) system is a genome editing tool discovered in prokaryotic cells. When utilized for genome editing, the system includes Cas9 (a protein able to modify DNA utilizing crRNA as its guide), CRISPR RNA (crRNA, contains the RNA used by Cas9 to guide it to the correct section of host DNA along with a region that binds to tracrRNA (generally in a hairpin loop form) forming an active complex with Cas9), trans-activating crRNA (tracrRNA, binds to crRNA and forms an active complex with Cas9), and an optional section of DNA repair template (DNA that guides the cellular repair process allowing insertion of a specific DNA sequence). CRISPR/Cas9 often employs a plasmid to transfect the target cells. The crRNA needs to be designed for each application as this is the sequence that Cas9 uses to identify and directly bind to the target DNA in a cell. The repair template carrying CAR expression cassette need also be designed for each application, as it must overlap with the sequences on either side of the cut and code for the insertion sequence. Multiple crRNA's and the tracrRNA can be packaged together to form a single-guide RNA (sgRNA). This sgRNA can be joined together with the Cas9 gene and made into a plasmid in order to be transfected into cells.

A zinc-finger nuclease (ZFN) is an artificial restriction enzyme, which is generated by combining a zinc finger DNA-binding domain with a DNA-cleavage domain. A zinc finger domain can be engineered to target specific DNA sequences which allows a zinc-finger nuclease to target desired sequences within genomes. The DNA-binding domains of individual ZFNs typically contain a plurality of individual zinc finger repeats and can each recognize a plurality of basepairs. The most common method to

generate new zinc-finger domain is to combine smaller zinc-finger "modules" of known specificity. The most common cleavage domain in ZFNs is the non-specific cleavage domain from the type II restriction endonuclease FokI. Using the endogenous homologous recombination (HR) machinery and a homologous DNA template carrying CAR expression cassette, ZFNs can be used to insert the CAR expression cassette into genome. When the targeted sequence is cleaved by ZFNs, the HR machinery searches for homology between the damaged chromosome and the homologous DNA template, and then copies the sequence of the template between the two broken ends of the chromosome, whereby the homologous DNA template is integrated into the genome.

Transcription activator-like effector nucleases (TALEN) are restriction enzymes that can be engineered to cut specific sequences of DNA. TALEN system operates on almost the same principle as ZFNs. They are generated by combining a transcription activator-like effectors DNA-binding domain with a DNA cleavage domain. Transcription activator-like effectors (TALEs) are composed of 33-34 amino acid repeating motifs with two variable positions that have a strong recognition for specific nucleotides. By assembling arrays of these TALEs, the TALE DNA-binding domain can be engineered to bind desired DNA sequence, and thereby guide the nuclease to cut at specific locations in genome. cDNA expression for use in polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element or intron (e.g. the elongation factor 1a enhancer/promoter/intron structure). For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

The resulting cells can be grown under conditions similar to those for unmodified cells, whereby the modified cells can be expanded and used for a variety of purposes.

6. Genome Editing Methods

In certain non-limiting embodiments, an HI-TCR, a costimulatory ligand, a CCR or any other molecule/transgene disclosed herein is expressed by an immunoresponsive cell through a modified genomic locus. In certain embodiments, an expression cassette of

the transgene is integrated into a targeted genomic locus of an immunoresponsive cell through targeted genome editing methods. In certain embodiments, the targeted genomic locus can be *CD3δ*, *CD3ε*, *CD247*, *B2M*, *TRAC*, *TRBC1*, *TRBC2*, *TRGC1* and/or *TRGC2* loci.

6.1. Engineering T Cell Receptor Locus

In certain embodiments, an HI-TCR is expressed by an immunoresponsive cell through a modified endogenous T cell receptor locus. In certain embodiments, an HI-TCR expression cassette is integrated at an endogenous T cell receptor locus. In certain embodiments, the HI-TCR expression cassette is integrated within the T cell receptor alpha locus (*TRA*, GenBank ID: 6955). In certain embodiments, the HI-TCR expression cassette is integrated within the T cell receptor beta locus (*TRB*, GenBank ID: 6957). In certain embodiments, the HI-TCR expression cassette is integrated within the T cell receptor gamma locus (*TRG*, GenBank ID: 6965).

In certain embodiments, the HI-TCR expression cassette comprises an extracellular antigen-binding domain that is integrated in the first exon of a TCR constant domain locus, so that the extracellular antigen-binding domain and the TCR constant domain are comprised in one antigen binding chain of the HI-TCR. In certain embodiments, the TCR constant domain locus can be *TRAC*, *TRBC1*, *TRBC2*, *TRGC1*, or *TRGC2*. In certain embodiments, the HI-TCR expression cassette comprises an extracellular antigen-binding domain that is integrated in the first exon of a *TRAC* locus, so that the extracellular antigen-binding domain and a *TRAC* peptide are comprised in a first antigen binding chain of the HI-TCR. In certain embodiments, the HI-TCR expression cassette further comprises a second antigen binding chain, which optionally comprises an extracellular antigen-binding domain and a *TRBC* peptide. In certain embodiments, the HI-TCR expression cassette comprises an extracellular antigen-binding domain that is integrated in the first exon of a *TRBC* locus, so that the extracellular antigen-binding domain and a *TRBC* peptide are comprised in a first antigen binding chain of the HI-TCR. In certain embodiments, the HI-TCR expression cassette further comprises a second antigen binding chain, which optionally comprises an extracellular antigen-binding domain and a *TRAC* peptide. In certain embodiments, the expression cassette comprises elements that create polycistronic expression cassette, e.g., a cleavable peptide, e.g., a 2A peptide.

In certain embodiments, the recombinant TCR is expressed from an expression cassette placed in an endogenous *TRAC* locus and/or a *TRBC* locus of an

immunoresponsive cell. In certain embodiments, the placement of the recombinant TCR expression cassette disrupts or abolishes the endogenous expression of a TCR comprising a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell. In certain embodiments, the placement of the recombinant TCR expression cassette prevents or eliminates mispairing between the recombinant TCR and a native TCR α chain and/or a native TCR β chain in the immunoresponsive cell.

Any suitable genetic editing methods and systems can be used to modify an endogenous T cell receptor locus. The genome editing methods disclosed in Section 4 can be used to modify the endogenous T cell receptor locus. In certain embodiments, a CRISPR system is used to modify T cell receptor locus. In certain embodiments, the CRISPR system targets exon 1 of a human TRAC locus. In certain embodiments, the CRISPR system comprises a guide RNA (gRNA) that targets exon 1 of a human TRAC locus.

In certain embodiments, a zinc-finger nuclease is used to modify an endogenous T cell receptor locus. In certain embodiments, a TALEN system is used to modify an endogenous T cell receptor locus.

In certain embodiments, when one endogenous T cell receptor locus in a cell is modified to express one or more antigen binding chain of an HI-TCR, one or more other endogenous T cell receptor locus in the cell are modified to eliminate the endogenous expression of the endogenous TCR chain. In certain embodiments, the one or more other endogenous T cell receptor locus are further modified to express a gene of interest, e.g., an anti-tumor cytokine (e.g., IL-2, IL-12, TNF α , and INF γ), a co-stimulatory molecule ligand (e.g., 4-1BBL), a tracking gene (e.g., eGFP) or a suicide gene.

6.2. Modifying Gene Expression through Genome Editing of a Promoter or a Transcription Terminator

In certain non-limiting embodiments, the expression of an HI-TCR expression cassette integrated into a targeted genomic locus is driven by an endogenous promoter/enhancer of the genomic locus. In certain embodiments, the expression of an HI-TCR expression cassette integrated into a targeted genomic locus is driven by a modified promoter/enhancer introduced to the genomic locus. Any targeted genome editing methods can be used to modify the promoter/enhancer region of a targeted genomic locus, and thereby enhancing or modifying the expression of an HI-TCR in an immunoresponsive cell. In certain embodiments, the modification comprises replacement of an endogenous promoter with a constitutive promoter or an inducible promoter, or

insertion of a constitutive promoter or inducible promoter to the promoter region of a targeted genomic locus. In certain embodiments, a constitutive promoter is positioned on a targeted genomic locus to drive gene expression of the HI-TCR. Eligible constitutive promoters include, but are not limited to, a CMV promoter, an EF1a promoter, a SV40 promoter, a PGK1 promoter, a Ubc promoter, a beta-actin promoter, and a CAG promoter. Alternatively or additionally, a conditional or inducible promoter is positioned on a targeted genomic locus to drive gene expression of the HI-TCR. Non-limiting examples of conditional promoters include a tetracycline response element (TRE) promoter and an estrogen response element (ERE) promoter. In addition, enhancer elements can be placed in regions other than the promoter region.

In certain non-limiting embodiments, the expression of an HI-TCR expression cassette integrated into a targeted genomic locus is regulated by an endogenous transcription terminator of the genomic locus. In certain embodiments, the expression of an HI-TCR expression cassette integrated into a targeted genomic locus is regulated by a modified transcription terminator introduced to the genomic locus. Any targeted genome editing methods can be used to modify the transcription terminator region of a targeted genomic locus, and thereby modifying the expression of an HI-TCR in an immunoresponsive cell. In certain embodiments, the modification comprises replacement of an endogenous transcription terminator with an alternative transcription terminator, or insertion of an alternative transcription terminator to the transcription terminator region of a targeted genomic locus. In certain embodiments, the alternative transcription terminator comprises a 3'UTR region or a ploy A region of a gene. In certain embodiments, the alternative transcription terminator is endogenous. In certain embodiments, the alternative transcription terminator is exogenous. In certain embodiments, alternative transcription terminators include, but are not limited to, a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription terminator and a P2A element.

Any targeted genome editing methods can be used to modify the promoter/enhancer region and/or the transcription terminator region of a targeted genomic locus. In certain embodiments, a CRISPR system is used to modify the promoter/enhancer region and/or the transcription terminator region of a targeted genomic locus. In certain embodiments, zinc-finger nucleases are used to modify the promoter/enhancer region and/or the transcription terminator region of a targeted genomic

locus. In certain embodiments, a TALEN system is used to modify the promoter/enhancer region and/or the transcription terminator region of a targeted genomic locus.

Methods for delivering the genome editing agents/systems can vary depending on the need. In certain embodiments, the components of a selected genome editing method are delivered as DNA constructs in one or more plasmids. In certain embodiments, the components are delivered via viral vectors. Common delivery methods include but is not limited to, electroporation, microinjection, gene gun, impalefection, hydrostatic pressure, continuous infusion, sonication, magnetofection, adeno-associated viruses, envelope protein pseudotyping of viral vectors, replication-competent vectors cis and trans-acting elements, herpes simplex virus, and chemical vehicles (e.g., oligonucleotides, lipoplexes, polymersomes, polyplexes, dendrimers, inorganic Nanoparticles, and cell-penetrating peptides).

Modification can be made anywhere within a targeted genomic locus, or anywhere that can impact gene expression of a targeted genomic locus. In certain embodiments, the modification occurs upstream of the transcriptional start site of a targeted genomic locus. In certain embodiments, the modification occurs between the transcriptional start site and the protein coding region of a targeted genomic locus. In certain embodiments, the modification occurs downstream of the protein coding region of a targeted genomic locus. In certain embodiments, the modification occurs upstream of the transcriptional start site of a targeted genomic locus, wherein the modification produces a new transcriptional start site.

7. Polypeptides and Analogs

Also included in the presently disclosed subject matter are polypeptides (e.g., CD19, CD8, CD28, CD3 ζ , CD40, 4-1BB, OX40, CD84, CD166, CD8a, CD8b, ICOS, ICAM-1, TRAC, TRBC1, TRBC2, TRGC1, TRGC2, CD3 γ , CD3 δ , CD3 ϵ and CTLA-4) or fragments thereof that are modified in ways that enhance their anti-neoplastic activity when expressed in an immunoresponsive cell. The presently disclosed subject matter provides methods for optimizing an amino acid sequence or nucleic acid sequence by producing an alteration in the sequence. Such alterations may include certain mutations, deletions, insertions, or post-translational modifications. The presently disclosed subject matter further includes analogs of any naturally-occurring polypeptide disclosed herein (including, but not limited to, CD19, CD8, CD28, CD3 ζ , CD40, 4-1BB, OX40, CD84, CD166, CD8a, CD8b, ICOS, ICAM-1, TRAC, TRBC1, TRBC2, TRGC1, TRGC2, CD3 γ , CD3 δ , CD3 ϵ and CTLA-4). Analogs can differ from a naturally-occurring

polypeptide disclosed herein by amino acid sequence differences, by post-translational modifications, or by both. Analogs can exhibit at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more homologous to all or part of a naturally-occurring amino, acid sequence of the presently disclosed subject matter. The length of sequence comparison is at least 5, 10, 15 or 20 amino acid residues, e.g., at least 25, 50, or 75 amino acid residues, or more than 100 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring polypeptides by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual (2d ed.), CSH Press, 1989, or Ausubel et al., supra). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the presently disclosed subject matter also provides fragments of any one of the polypeptides or peptide domains disclosed herein. As used herein, the term “a fragment” means at least 5, 10, 13, or 15 amino acids. In certain embodiments, a fragment comprises at least 20 contiguous amino acids, at least 30 contiguous amino acids, or at least 50 contiguous amino acids. In certain embodiments, a fragment comprises at least 60 to 80, 100, 200, 300 or more contiguous amino acids. Fragments can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Non-protein analogs have a chemical structure designed to mimic the functional activity of a protein disclosed herein. Such analogs may exceed the physiological activity of the original polypeptide. Methods of analog design are well known in the art, and

synthesis of analogs can be carried out according to such methods by modifying the chemical structures such that the resultant analogs increase the anti-neoplastic activity of the original polypeptide when expressed in an immunoresponsive cell. These chemical modifications include, but are not limited to, substituting alternative R groups and varying the degree of saturation at specific carbon atoms of a reference polypeptide. In certain embodiments, the protein analogs are relatively resistant to *in vivo* degradation, resulting in a more prolonged therapeutic effect upon administration. Assays for measuring functional activity include, but are not limited to, those described in the Examples below.

8. Administration

Compositions comprising the presently disclosed immunoresponsive cells can be provided systemically or directly to a subject for inducing and/or enhancing an immune response to an antigen and/or treating and/or preventing a neoplasia, pathogen infection, or infectious disease. In certain embodiments, the presently disclosed immunoresponsive cells or compositions comprising thereof are directly injected into an organ of interest (e.g., an organ affected by a neoplasia). Alternatively, the presently disclosed immunoresponsive cells or compositions comprising thereof are provided indirectly to the organ of interest, for example, by administration into the circulatory system (e.g., the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of the cells or compositions to increase production of T cells, NKT cells, or CTL cells *in vitro* or *in vivo*.

The presently disclosed immunoresponsive cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (e.g., thymus). Usually, at least about 1×10^5 cells will be administered, eventually reaching about 1×10^{10} or more. The presently disclosed immunoresponsive cells can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of the presently disclosed immunoresponsive cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). Suitable ranges of purity in populations comprising the presently disclosed immunoresponsive cells are about 50% to about 55%, about 5% to about 60%, and about 65% to about 70%. In certain embodiments, the purity is about 70% to about 75%, about 75% to about 80%, or about 80% to about 85%. In certain embodiments, the purity is about 85% to about 90%, about 90% to about 95%, and about 95% to about 100%. Dosages can be readily adjusted by those skilled in the art (e.g., a decrease in

purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like.

The presently disclosed compositions can be pharmaceutical compositions comprising the presently disclosed immunoresponsive cells or their progenitors and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, immunoresponsive cells, or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells or their progeny (e.g., *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition of the presently disclosed subject matter (e.g., a pharmaceutical composition comprising a presently disclosed immunoresponsive cell), it can be formulated in a unit dosage injectable form (solution, suspension, emulsion).

9. Formulations

Compositions comprising the presently disclosed immunoresponsive cells can be conveniently provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the genetically modified immunoresponsive cells in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as “REMINGTON’S

PHARMACEUTICAL SCIENCE”, 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the presently disclosed subject matter, however, any vehicle, diluent, or additive used would have to be compatible with the genetically modified immunoresponsive cells or their progenitors.

The compositions can be isotonic, i.e., they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride can be particularly for buffers containing sodium ions.

Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. For example, methylcellulose is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The concentration of the thickener can depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

The quantity of cells to be administered will vary for the subject being treated. In a one embodiment, between about 10^4 and about 10^{10} , between about 10^5 and about 10^9 , or between about 10^6 and about 10^8 of the presently disclosed immunoresponsive cells are administered to a human subject. More effective cells may be administered in even smaller numbers. In certain embodiments, at least about 1×10^8 , about 2×10^8 , about 3×10^8 , about 4×10^8 , or about 5×10^8 of the presently disclosed immunoresponsive cells are administered to a human subject. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size,

age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art.

The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions and to be administered in methods.

5 Typically, any additives (in addition to the active cell(s) and/or agent(s)) are present in an amount of 0.001 to 50% (weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, about 0.0001 to about 1 wt %, about 0.0001 to about 0.05 wt% or about 0.001 to about 20 wt %, about 0.01 to about 10 wt %, or about 0.05 to about 5 wt %. For
10 any composition to be administered to an animal or human, the followings can be determined: toxicity such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse; the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the
15 knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

10. Methods of Treatment

The presently disclosed subject matter provides methods for inducing and/or increasing an immune response in a subject in need thereof. The presently disclosed
20 immunoresponsive cells and compositions comprising thereof can be used for treating and/or preventing a neoplasia in a subject. The presently disclosed immunoresponsive cells and compositions comprising thereof can be used for prolonging the survival of a subject suffering from a neoplasia. The presently disclosed immunoresponsive cells and compositions comprising thereof can also be used for treating and/or preventing a
25 pathogen infection or other infectious disease in a subject, such as an immunocompromised human subject. Such methods comprise administering the presently disclosed immunoresponsive cells in an amount effective or a composition (e.g., pharmaceutical composition) comprising thereof to achieve the desired effect, be it palliation of an existing condition or prevention of recurrence. For treatment, the amount
30 administered is an amount effective in producing the desired effect. An effective amount can be provided in one or a series of administrations. An effective amount can be provided in a bolus or by continuous perfusion.

In certain embodiments, immunoresponsive cells comprising a HI-TCR disclosed herein can be used to treat a subject having tumor cells with a low expression level of a

surface antigen, .e.g, from a relapse of a disease, wherein the subject received treatment which leads to residual tumor cells. In certain embodiments, the tumor cells have low density of a target molecule on the surface of the tumor cells In certain embodiments, a target molecule having a low density on the cell surface has a density of less than about 5,000 molecules per cell, less than about 4,000 molecules per cell, less than about 3,000 molecules per cell, less than about 2,000 molecules per cell, less than about 1,500 molecules per cell, less than about 1,000 molecules per cell, less than about 500 molecules per cell, less than about 200 molecules per cell, or less than about 100 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of less than about 2,000 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of less than about 1,500 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of less than about 1,000 molecules per cell. In certain embodiments, a target molecule having a low density on the cell surface has a density of between about 4,000 molecules per cell and about 2,000 molecules per cell, between about 2,000 molecules per cell and about 1,000 molecules per cell, between about 1,500 molecules per cell and about 1,000 molecules per cell, between about 2,000 molecules per cell and about 500 molecules per cell, between about 1,000 molecules per cell and about 200 molecules per cell, or between about 1,000 molecules per cell and about 100 molecules per cell. In certain embodiments, immunoresponsive cells comprising a HI-TCR disclosed herein can be used to treat a subject having a relapse of a disease, wherein the subject received immunoresponsive cells (e.g., T cells) comprising a CAR comprising an intracellular signaling domain that comprises a co-stimulatory signaling domain comprising a 4-1BB polypeptide (e.g., a 4-1BBz CAR). In certain embodiments, the tumor cells have a low density of a tumor specific antigen on the surface of the tumor cells. In certain embodiments, the disease is CD19⁺ ALL. In certain embodiments, the tumor cells have a low density of CD19 on the tumor cells. Such methods comprise administering the presently disclosed immunoresponsive cells in an amount effective or a composition (e.g., pharmaceutical composition) comprising thereof to achieve the desired effect, alleviation of an existing condition or prevention of recurrence.

An “effective amount” (or, “therapeutically effective amount”) is an amount sufficient to effect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an

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

For adoptive immunotherapy using antigen-specific T cells, cell doses in the range of about 10^6 - 10^{10} (e.g., about 10^9) are typically infused. Upon administration of the presently disclosed cells into the host and subsequent differentiation, T cells are induced that are specifically directed against the specific antigen. The modified cells can be administered by any method known in the art including, but not limited to, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural, intraperitoneal and directly to the thymus.

The presently disclosed subject matter provides methods for treating and/or preventing a neoplasia in a subject. The method can comprise administering an effective amount of the presently disclosed immunoresponsive cells or a composition comprising thereof to a subject having a neoplasia.

Non-limiting examples of neoplasia include blood cancers (e.g. leukemias, lymphomas, and myelomas), ovarian cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, throat cancer, melanoma, neuroblastoma, adenocarcinoma, glioma, soft tissue sarcoma, and various carcinomas (including prostate and small cell lung cancer). Suitable carcinomas further include any known in the field of oncology, including, but not limited to, astrocytoma, fibrosarcoma, myxosarcoma, liposarcoma, oligodendroglioma, ependymoma, medulloblastoma, primitive neural ectodermal tumor (PNET), chondrosarcoma, osteogenic sarcoma, pancreatic ductal adenocarcinoma, small and large cell lung adenocarcinomas, chordoma, angiosarcoma, endotheliosarcoma, squamous cell carcinoma, bronchoalveolarcarcinoma, epithelial adenocarcinoma, and liver metastases thereof, lymphangiosarcoma, lymphangioendotheliosarcoma, hepatoma, cholangiocarcinoma, synovioma, mesothelioma, Ewing's tumor, rhabdomyosarcoma, colon carcinoma, basal cell carcinoma, sweat gland carcinoma, papillary carcinoma, sebaceous gland carcinoma,

papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, breast tumors such as ductal and lobular adenocarcinoma, squamous and adenocarcinomas of the uterine cervix, uterine and ovarian epithelial carcinomas, prostatic adenocarcinomas, transitional squamous cell carcinoma of the bladder, B and T cell lymphomas (nodular and diffuse) plasmacytoma, acute and chronic leukemias, malignant melanoma, soft tissue sarcomas and leiomyosarcomas. In certain embodiments, the neoplasm is selected from the group consisting of blood cancers (e.g. leukemias, lymphomas, and myelomas), ovarian cancer, prostate cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, and throat cancer. In certain embodiments, the presently disclosed immunoresponsive cells and compositions comprising thereof can be used for treating and/or preventing blood cancers (e.g., leukemias, lymphomas, and myelomas) or ovarian cancer, which are not amenable to conventional therapeutic interventions. In certain embodiments, the neoplasm is a solid tumor.

The subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. The subjects can have a history of the condition, for which they have already been treated, in which case the therapeutic objective will typically include a decrease or delay in the risk of recurrence.

Suitable human subjects for therapy typically comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor is one that can be detected on the basis of tumor mass (e.g., by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). A pharmaceutical composition is administered to these subjects to elicit an anti-tumor response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical improvement constitutes a benefit. Clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor.

A second group of suitable subjects is known in the art as the “adjuvant group.” These are individuals who have had a history of neoplasia, but have been responsive to another mode of therapy. The prior therapy can have included, but is not restricted to, surgical resection, radiotherapy, and traditional chemotherapy. As a result, these
5 individuals have no clinically measurable tumor. However, they are suspected of being at risk for progression of the disease, either near the original tumor site, or by metastases. This group can be further subdivided into high-risk and low-risk individuals. The subdivision is made on the basis of features observed before or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different
10 neoplasia. Features typical of high-risk subgroups are those in which the tumor has invaded neighboring tissues, or who show involvement of lymph nodes.

Another group have a genetic predisposition to neoplasia but have not yet evidenced clinical signs of neoplasia. For instance, women testing positive for a genetic mutation associated with breast cancer, but still of childbearing age, can wish to receive
15 one or more of the immunoresponsive cells described herein in treatment prophylactically to prevent the occurrence of neoplasia until it is suitable to perform preventive surgery.

Additionally, the presently disclosed subject matter provides methods for treating and/or preventing a pathogen infection (e.g., viral infection, bacterial infection, fungal infection, parasite infection, or protozoal infection) in a subject, e.g., in an
20 immunocompromised subject. The method can comprise administering an effective amount of the presently disclosed immunoresponsive cells or a composition comprising thereof to a subject having a pathogen infection. Exemplary viral infections susceptible to treatment include, but are not limited to, Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus infections.

Further modification can be introduced to the presently disclosed
25 immunoresponsive cells (e.g., T cells) to avert or minimize the risks of immunological complications (known as “malignant T-cell transformation”), e.g., graft versus-host disease (GvHD), or when healthy tissues express the same target antigens as the tumor cells, leading to outcomes similar to GvHD. A potential solution to this problem is
30 engineering a suicide gene into the presently disclosed immunoresponsive cells. Suitable suicide genes include, but are not limited to, Herpes simplex virus thymidine kinase (hsv-tk), inducible Caspase 9 Suicide gene (iCasp-9), and a truncated human epidermal growth factor receptor (EGFRt) polypeptide. In certain embodiments, the suicide gene is an EGFRt polypeptide. The EGFRt polypeptide can enable T cell elimination by

administering anti-EGFR monoclonal antibody (*e.g.*, cetuximab). EGFRt can be covalently joined to the upstream of the antigen-recognizing receptor of a presently disclosed CAR. The suicide gene can be included within the vector comprising nucleic acids encoding a presently disclosed CAR. In this way, administration of a prodrug
5 designed to activate the suicide gene (*e.g.*, a prodrug (*e.g.*, AP1903 that can activate iCasp-9) during malignant T-cell transformation (*e.g.*, GVHD) triggers apoptosis in the suicide gene-activated CAR-expressing T cells. The incorporation of a suicide gene into the a presently disclosed CAR gives an added level of safety with the ability to eliminate the majority of CAR T cells within a very short time period. A presently disclosed
10 immunoresponsive cell (*e.g.*, a T cell) incorporated with a suicide gene can be pre-emptively eliminated at a given timepoint post CAR T cell infusion, or eradicated at the earliest signs of toxicity.

11. Kits

The presently disclosed subject matter provides kits for inducing and/or enhancing
15 an immune response and/or treating and/or preventing a neoplasia or a pathogen infection in a subject. In certain embodiments, the kit comprises an effective amount of presently disclosed immunoresponsive cells or a pharmaceutical composition comprising thereof. In certain embodiments, the kit comprises a sterile container; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable
20 container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. In certain non-limiting embodiments, the kit includes an isolated nucleic acid molecule encoding an HI-TCR disclosed herein which is directed toward an antigen of interest in expressible form, which may optionally be comprised in one or more vectors.

25 If desired, the immunoresponsive cells and/or nucleic acid molecules are provided together with instructions for administering the cells or nucleic acid molecules to a subject having or at risk of developing a neoplasia or pathogen or immune disorder. The instructions generally include information about the use of the composition for the treatment and/or prevention of neoplasia or a pathogen infection. In certain
30 embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a neoplasia, pathogen infection, or immune disorder or symptoms thereof; precautions; warnings; indications; counter-indications; over-dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed

directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

EXAMPLES

The practice of the present disclosure employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides disclosed herein, and, as such, may be considered in making and practicing the the presently disclosed subject matter. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the presently disclosed cells and compositions, and are not intended to limit the scope of what the inventors regard as their invention.

Example 1 - Targeting T cells with endogenous non-HLA restricted T cell receptor/CD3 complexes

Introduction

Specific modifications were made at a TCR locus designed to alter the antigen specificity of human T cells without disrupting or bypassing their CD3 complex, which physiologically controls and regulates T cell activation. In this approach, the T cell lost its endogenous T cell receptor and acquired the ability to recognize antigen independent of HLA, making use of its CD3 complex, which is otherwise bypassed when using CARs. This was accomplished through the targeted disruption of the endogenous TCR combined with the introduction of antigen binding domains into the TRAC locus in such a way as to preserve all components of the natural CD3 complex in their native structure. The recombinant TCR-like molecule (i.e., HI-TCR, FvTCR or HIT-CAR) can harbor variable domains derived from immunoglobulin genes, or alternative ligands, to direct antigen

recognition. The recombined receptor signals via the CD3 complex, which can either be intact (i.e., in its physiological composition) or augmented through the non-covalent incorporation of costimulatory domains.

Results

5 A novel strategy for one-step generation of TCR and TCR-like T cells was developed by integrating a TCR or TCR-like gene with a distinct variable domain for a predetermined antigen. This resulted in the expression of the new TCR/TCR-like gene under the control of the endogenous TCR promoter (alpha, beta or both) with concomitant disruption of surface expression of the endogenous TCR.

10 A strategy for one-step generation of universal CAR T cells were developed by targeting the integration of a promoter-less CAR gene cassette in the TCR alpha constant chain (TRAC) first exon. This resulted in CAR expression under the control of the endogenous TCR alpha promoter with concomitant disruption of the TCR alpha gene expression, leading to lack of TCR expression at the cell surface. CAR gene targeting at
15 the TRAC locus was accomplished through homologous recombination (HR) by using a site-specific nuclease (e.g. CRISPR/Cas9) and an AAV donor template. Using HR-based gene targeting, the present strategy permitted the generation of T cells with a unique specificity, which was encoded by a chimeric TCR receptor containing a specific antigen-binding domain. The antigen-binding domain was derived from either an
20 Immunoglobulin (i.e. Fv fragment), a ligand for a cell-surface receptor or a TCR ($\alpha\beta$ or $\gamma\delta$). The hybrid Immunoglobulin-TCR chimeric antigen receptor (i.e., HIT-CAR, FvTCR or HI-TCR) or the $\gamma\delta$ TCR allowed the T cell to recognize a target cell in an HLA-independent manner. Inserting a new $\alpha\beta$ TCR could be accomplished in the same way but would result in HLA-restricted antigen recognition.

25 For example, Figure 1A shows schematic representation of the T Cell Receptor (TCR), the B Cell Receptor (BCR), a Chimeric Antigen Receptor (CAR) and the HLA-Independent TCR-based Chimeric Antigen Receptor (i.e., HIT-CAR, FvTCR or HI-TCR). CRISPR/Cas9-targeted integration of the three receptors into the TRAC locus is shown in Figure 1B. The engineered HIT-CAR targets CD19. Cell surface expression of
30 HIT-CAR (i.e., HI-TCR) from TRAC locus is shown in Figure 1C. Cytolytic effects and proliferation of HIT-CAR (i.e., HI-TCR or FvTCR) T cells are shown in Figures 1D and 1E respectively.

Conditions were established to yield up to 65% of HIT-CAR (i.e., HI-TCR or FvTCR) T cells combining target gene disruption and HIT-CAR (i.e., HI-TCR or FvTCR)

targeted insertion in a single step as shown in Figure 2A. These T cells exhibited *in vitro* and *in vivo* tumor lysis activity similar to the previously characterized TRAC-CAR T cells expressing the 1928z CAR as shown in Figure 2B. In addition, antigen interaction induced down-regulation of the HIT-CAR (i.e., HI-TCR or FvTCR) T cells, which was dependent of the number of antigen-dependent stimulations. As endogenous TCR expression is eliminated at the cell surface, these HIT-CAR (i.e., HI-TCR or FvTCR) T cells were useful for the development of off-the-shelf immunotherapy.

Targets other than CD19 can be employed for immunotherapy. Schematic representation of the NYESO TCR genes integrated into the TCR alpha or beta chain is shown in Figure 3A. Cell surface expressions of TRAC-NYESO-TCR and TRBC-NYESO-TCR (i.e., HI-TCRs or FvTCRs) are shown in Figure 3B. Representative TCR-V-beta-1 flow cytometry plots 4 days after TRAC or TRBC targeting are shown in Figure 3B. Cytotoxic activity of the engineered T cells is shown in Figure 3C. Schematic representation of an alternative design of co-targeting into both the TCR alpha and the TCR beta is shown in Figure 3D, where a NYESO-HI-TCR was placed in the TRAC locus and 4-1BBL expression cassette was placed in TRBC locus. Cell surface expressions of the TRAC-NYESO-TCR and TRBC-4-1BBL are shown in Figure 3E.

A variant approach was also provided in which a costimulatory domain is non-covalently inserted into the CD3 complex, which provides supraphysiological antigen sensing and activation. This is achieved by fusing the costimulatory domain to one or both TCR chains. Moreover, two costimulatory dosages are possible, by either fusing the costimulatory domain to one or both modified TCR chains (i.e., antigen binding chains).

Moreover, HI-TCR exhibited greater sensitivity in comparison to CARs. Human T cells edited to replace endogenous TCR with HI-TCR acquired the ability to engage lower antigen densities and kill such cells. For example, Table 2 shows *in vitro* cytotoxic activity of HI-TCR cells. HI-TCR or CD19-CAR was introduced at the endogenous TRAC locus of human peripheral T cells via CRISPR/Cas9-mediated gene editing and AAV6 donor vectors. Five days post-gene targeting, T cells (6, 30, or 150 thousands) were incubated with 250 thousands Nalm6 leukemia cells that express different CD19 levels (from very low to high levels). Cells were incubated for 22h in 500-ul of X-Vivo medium containing serum (without IL2). Co-cultures were analyzed by FACS in the presence of counting beads to determine the total number of Nalm6 cells. Cytotoxic activity is shown as a percentage of Nalm6 killed. E/T: effector (T cells) to target (Nalm6) ratio. The data clearly demonstrate that the HI-TCR can detect lower levels of

CD19 antigen than a CAR. This feature can be very useful for antigens with moderate or low expression such as CD22, BCMA, CCR1, CD70, etc., and can also be useful to treat relapse after CAR therapy for any antigen, as the relapse tumor cells frequently show reduced antigen densities on their surface.

5

Table 2. Tumor Cell Killing Percentage

E/T	HI-TCR	CD19 CAR	CD19 levels
0.024	0	0	very low
	22.53	16.32	low
	29.19	17.18	medium
	38.3	32	high
0.12	15.48	0.4	very low
	51.46	53.31	low
	54.14	63.58	medium
	63.28	63.38	high
0.6	30.36	8.37	very low
	68.32	77.2	low
	77.01	79.38	medium
	78.72	84.37	high

Furthermore, a panel of exogenous and endogenous 3' untranslated regions (3'UTR) were found as capable of regulate precise and predictable gene expression at the TCR locus. For example, Figure 4A shows schematic representation of the CD28z CAR gene integrated into the TRAC locus. Poly A (black box) corresponds to the segment of the CAR cassette that was modified to test different viral and mammalian 3' UTRs. Using TRAC-CAR T cells expressing the CD28z CAR as a model, certain 3'UTRs were shown to lower the CAR expression compared to the bovine growth hormone (bGH) polyA sequence (Figures 4B and 4C), which yielded CAR-T cells with impaired *in vivo* cytotoxic activity (Figures 5A and 5B). Certain other 3'UTRs, including the endogenous TRAC 3'UTR, were shown to increase CAR surface expression levels (Figures 4B and 4C), which produced CAR-T cells with improved *in vivo* cytotoxic activity (Figures 5A and 5B). These 3'UTRs can also be used to regulate precise and predictable gene expression of any HI-TCR disclosed herein.

The above-described genetic modifications at the TRAC locus permit the generation of T cells expressing optimal levels of HI-TCR that like the physiological TCR but unlike CARs, take advantage of the endogenous T cell activating machinery (the CD3 complex and downstream signaling elements). This approach can advance both autologous and allogeneic T cell therapies.

Another relevant area where TCR gene editing has relevant applications is T-iPS-derived T cells. T-iPS cells are pluripotent stem cells obtained through reprogramming of peripheral T lymphocytes. These T-iPS cells therefore contain a rearranged T-cell receptor, either an $\alpha\beta$ or a $\gamma\delta$ TCR, which can be modified using gene-editing technologies. T cells obtained from T-iPS cells through directed differentiation express the rearranged TCR, which can be detected and sequenced. Using this sequence, one can determine the precise location of the rearranged variable domain in the genome of T-iPS cells. Using nucleases that specifically target this rearranged variable domain and a donor DNA containing a TCR variable domain of known specificity, one can replace the endogenous variable domain with the new one. This approach requires two steps of gene editing: one targeting the α (or γ) chain, and the other the β (or δ) chain. The approach can be performed in a single step since only two alleles need to be modified. In addition, using a strategy similar as to the one described for primary human T cells, the TCR chains can be modified to express any HI-TCR disclose herein.

Example 2

CRISPR/Cas9-targeted integration of the three receptors into the TRAC locus is shown in Figure 1A. The engineered HIT-CAR targets CD19. Cell surface expression of HIT-CAR (i.e., HI-TCR) from TRAC locus is shown in Figures 6B and 6C. Cytolytic effects and proliferation of HIT-CAR (i.e., HI-TCR or FvTCR) T cells are shown in Figures 7A, 7B and 8A-8C. In particular, these data show that HIT T cells outperformed CAR-T cells at killing target cells expressing low antigen levels.

Furthermore, as shown in Figures 9A-9D the HI-TCR T cells were engineered to co-express costimulatory ligands. In this specific experiment, the day after targeting the HI-TCR to the TRAC locus, the T cells were transferred with retroviral SFG vectors coding for CD80, 41BBL or both. 5 days after expanding these cells ex vivo, 4×10^5 TRAC-CAR or TRAC-HI-TCR positive T cells were injected into NSG mice bearing NALM6 cells expressing very low level of CD19. The TRAC-HI-TCR T cells were either expressing CD80, 41BBL, both ligand or none. Bioluminescence was used to assess the tumor burden every week and follow the survival of the mice.

It was observed that the TRAC-CAR T cells were not able to control the tumor with very low levels of CD19 and all the mice were terminally ill by day 30. At this low dose, the HI-TCR T cells initially controlled the tumor burden, however the mice relapsed by day 10 and were terminally ill by day 40. The addition of costimulatory ligands to the HI-TCR T cells improved the anti-tumor activity with an optimal response when the HI-TCR T cells co-expressed CD80 and 4-1BBL. This improved activity resulted in a long-term control of NALM6 expressing very low levels of CD19.

Figures 10A-10C further demonstrate that baseline TRAC-CAR expression can be controlled by distinct 3'UTR sequences without affecting cell surface replenishment kinetic after antigen encounter. The TRAC-CAR T cells were engineered the same way as previously described, so that the all the constructs were under the transcriptional control of the TRAC endogenous promoter. It was observed that by modifying the 3'UTR (that includes polyA signal), the baseline expression level were modulated(Figure 10B). When these different TRAC-CAR T cells were cultivated on CD19 expressing tumor cells, a drop in the CAR cell surface expression followed by a replenishment was observed by flow cytometry (Figure 10 C). The different 3'UTR changed the baseline expression level but they retained the same replenishment kinetic and the final expression level was similar to the baseline.

Furthermore, a HI-TCR targeting CD70 and a HI-TCR targeting CD22 were also created. HI-TCR targeting an interested antigen can be created by sequencing an existing scFv or a Fab region of an existing antibody targeting the same antigen to obtain the extracellular antigen-binding domain.

Embodiments of the presently disclosed subject matter

From the foregoing description, it will be apparent that variations and modifications may be made to the presently disclosed subject matter to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or sub-combination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A recombinant T cell receptor (TCR) comprising an antigen binding chain that comprises:
 - a) an extracellular antigen-binding domain of an antibody, or antigen-binding fragment thereof; and
 - b) a constant domain that comprises a native TRAC peptide or a native TRBC peptide.
2. The recombinant TCR of claim 1, wherein the constant domain is capable of forming a homodimer or a heterodimer with another constant domain.
3. The recombinant TCR of claim 1 or 2, wherein the antigen binding chain, upon binding to an antigen, is capable of activating a CD3 ζ polypeptide associated to the antigen binding chain, optionally wherein activation of the CD3 ζ polypeptide is capable of activating an immunoresponsive cell.
4. The recombinant TCR of claim 3, wherein
 - a) the CD3 ζ polypeptide is endogenous and integrated in the native CD3 complex;or
 - b) the CD3 ζ polypeptide is exogenous and optionally integrated with a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof.
5. The recombinant TCR of any one of claims 1-4, wherein the antigen binding chain further comprises a co-stimulatory region, wherein the recombinant TCR, upon binding to an antigen, is capable of stimulating an immunoresponsive cell, optionally wherein the co-stimulatory region comprises a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof.
6. The recombinant TCR of any one of claims 1-5, wherein the recombinant TCR is capable of integrating with a CD3 complex and providing HLA-independent antigen recognition, optionally wherein the CD3 complex is endogenous, optionally wherein the recombinant TCR replaces an endogenous TCR in a CD3/TCR complex.

7. The recombinant TCR of any one of claims 1-6, wherein the antigen-binding chain is capable of dimerizing with another extracellular antigen-binding domain.
8. The recombinant TCR of claim 7, wherein the antigen-binding domain comprises a heavy chain variable region (V_H) of the antibody or a light chain variable region (V_L) of the antibody.
9. The recombinant TCR of claim 8, wherein the antigen-binding chain is capable of dimerizing with a second antigen-binding chain, optionally wherein
 - a) the antigen-binding domain of the first antigen-binding chain comprises a V_H of an antibody, and the second antigen-binding chain comprises an antigen-binding domain comprising a V_L of the antibody; or
 - b) the antigen-binding domain of the first antigen-binding chain comprises a V_L of the antibody, and the second antigen-binding chain comprises an antigen-binding domain comprising a V_H of the antibody.
10. The recombinant TCR of any one of claims 1-9, wherein the recombinant TCR binds to a tumor antigen, optionally wherein the tumor antigen is selected from the group consisting of CD19, MUC16, MUC1, CAIX, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, EGP-2, EGP-40, EpCAM, erb-B2, erb-3, erb-4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, K-light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, Mesothelin, ERBB2, MAGEA3, p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ES0-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD123, CD44V6, NKCS1, EGF1R, EGFR-VIII, and CD99, CD70, ADGRE2, CCR1, LILRB2, LILRB4, PRAME, and ERBB, optionally wherein the tumor antigen is CD19.
11. The recombinant TCR of any one of claims 1-10, wherein
 - a) the recombinant TCR exhibits greater antigen sensitivity than a CAR targeting the same antigen.
 - b) the recombinant TCR is capable of inducing an immune response when binds to an antigen that has a low density on the surface of a tumor cell; and or
 - c) the antigen that has a low density on the cell surface has a density of less than about 10,000 molecules per cell.

12. An immunoresponsive cell comprising a recombinant TCR of any one of claims 1-11.
13. The immunoresponsive cell of claim 12, wherein
 - a) the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell; or
 - b) the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization.
14. The immunoresponsive cell of claim 12 or 13, wherein the endogenous gene locus is a *CD3 δ* locus, a *CD3 ϵ* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus, a *TRDC* locus and/or a *TRGC* locus, or optionally wherein the endogenous gene locus is a *TRAC* locus and/or a *TRBC* locus.
15. The immunoresponsive cell of any one of claims 11-14, wherein the endogenous gene locus comprises a modified transcription terminator region, optionally wherein the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, an HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription terminator and a P2A element.
16. The immunoresponsive cell of claim 15, wherein the immunoresponsive cell further comprises one or more endogenous T cell receptor loci are modified to express a gene of interest, optionally wherein the gene of interest is an anti-tumor cytokine, a co-stimulatory molecule ligand, a tracking gene or a suicide gene.
17. The immunoresponsive cell of any one of claims 12-16, wherein the cell is selected from the group consisting of a T cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.
18. The immunoresponsive cell of any one of claims 12-17, wherein the immunoresponsive cell is autologous.

19. The immunoresponsive cell of any one of claims 12-18, further comprising at least one exogenous co-stimulatory ligand, and optionally wherein the exogenous co-stimulatory ligand is selected from the group consisting of CD80, CD86, 41BBL, CD275, CD40L, OX40L and any combination thereof.
20. The immunoresponsive cell of claim 19, wherein the at least one exogenous co-stimulatory ligand is CD80; wherein the at least one exogenous co-stimulatory ligand is 4-1BBL; or wherein the at least one exogenous co-stimulatory ligand is CD80 and 4-1BBL.
21. The immunoresponsive cell of any one of claims 12-20, further comprising at least one chimeric costimulatory receptor (CCR), optionally wherein the CCR comprises a co-stimulatory molecule selected from the group consisting of a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide and any combination thereof.
22. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 12-21 and a pharmaceutically acceptable excipient.
23. A method of reducing tumor burden in a subject, treating or preventing a neoplasm, and/or lengthening survival of a subject having a neoplasm, the method comprising administering to the subject an effective amount of the immunoresponsive cells of any one of claims 12-21 or the pharmaceutical composition of claim 22.
24. Use of the immunoresponsive cells of any one of claims 12-21 or the pharmaceutical composition of claim 22 for the manufacture of a medicament for reducing tumor burden, for treating or preventing a neoplasm, or for lengthening survival in a subject.
25. The method of claim 23 or the use of claim 24, wherein the neoplasm is selected from the group consisting of blood cancer, B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia, non-Hodgkin's lymphoma adenocarcinomas.
26. The method of claim 23 or 25, or the use of claim 24 or 25 wherein

a) the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to CD19;

b) the neoplasm is B cell leukemia, multiple myeloma, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia, or non-Hodgkin's lymphoma, and the recombinant TCR binds to BCMA, ADGRE2, CCR1, CD22, CD70 or combinations thereof;

c) the neoplasm is CD19⁺ ALL.

27. A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into an immunoresponsive cell a nucleic acid sequence encoding a recombinant TCR according to any one of claims 1-11, optionally wherein the nucleic acid sequence is comprised in a vector.

28. The method of claim 27, wherein

a) the expression cassette of at least one antigen binding chain of the recombinant TCR is placed at an endogenous gene locus of the immunoresponsive cell; and/or

b) the expression cassettes of two antigen binding chains of the recombinant TCR are placed at an endogenous gene locus of the immunoresponsive cell, wherein the two antigen binding chains are capable of dimerization.

29. The method of claim 28, wherein the endogenous gene locus is a *CD3δ* locus, a *CD3ε* locus, a *CD247* locus, a *B2M* locus, a *TRAC* locus, a *TRBC* locus, a *TRDC* locus and/or a *TRGC* locus, optionally wherein the endogenous gene locus is a *TRAC* locus and/or a *TRBC* locus.

30. The method of claim 28 or 29, wherein the endogenous gene locus comprises a modified transcription terminator region, optionally wherein the modified transcription terminator region comprises a genomic element selected from the group consisting of a TK transcription terminator, a GCSF transcription terminator, a TCRA transcription terminator, a HBB transcription terminator, a bovine growth hormone transcription terminator, an SV40 transcription terminator and a P2A element.

31. A nucleic acid encoding a recombinant TCR of any one of claims 1-11.

32. A vector comprising the nucleic acid of claim 31.

33. A kit comprising a recombinant TCR of any one of claims 1-11, an immunoresponsive cell of any one of claims 12-21, a pharmaceutical composition of claim 22, a nucleic acid of claim 31, or a vector of claim 32.
34. The kit of claim 33, wherein the kit further comprises written instructions for treating and/or preventing a neoplasm, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

Memorial Sloan-Kettering Cancer Center
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

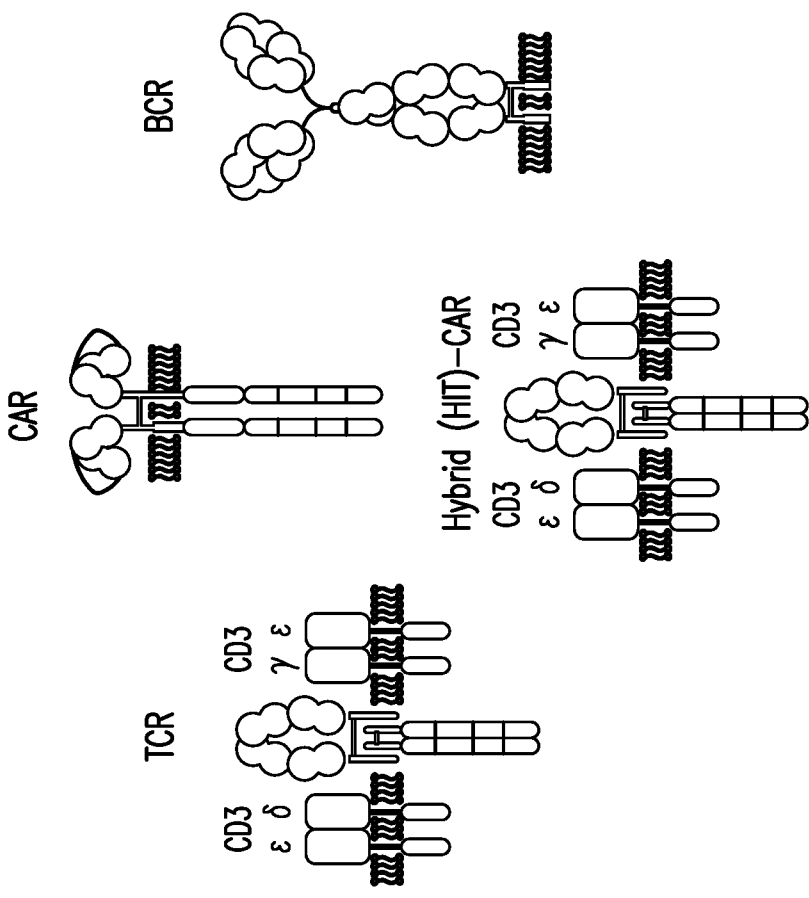


FIG. 1A

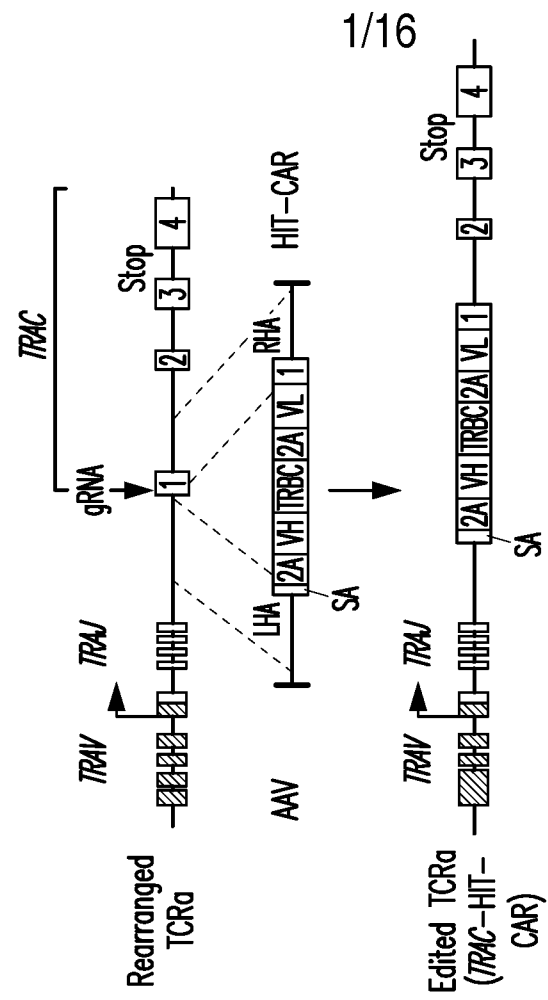


FIG. 1B

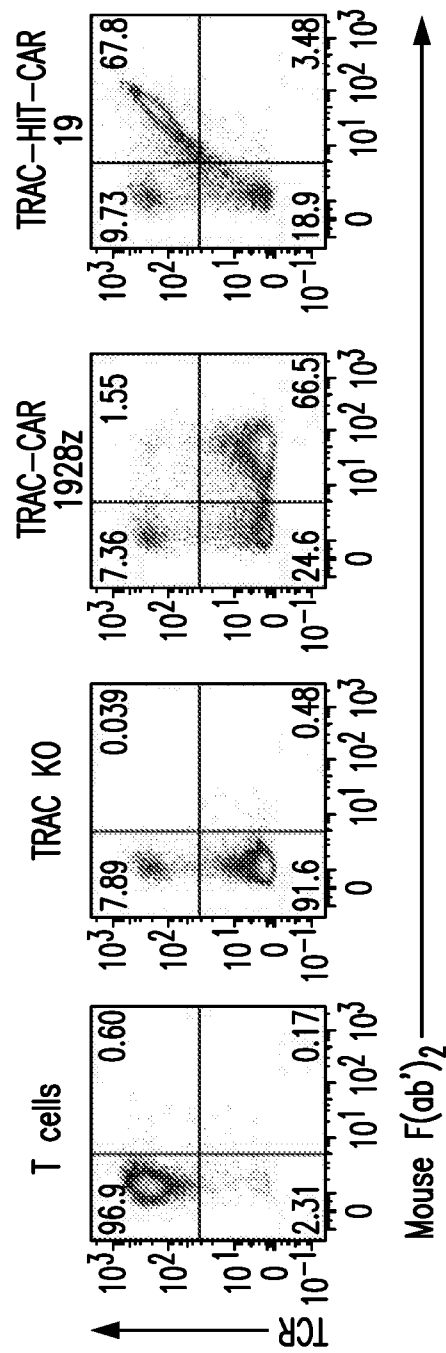


FIG. 1C

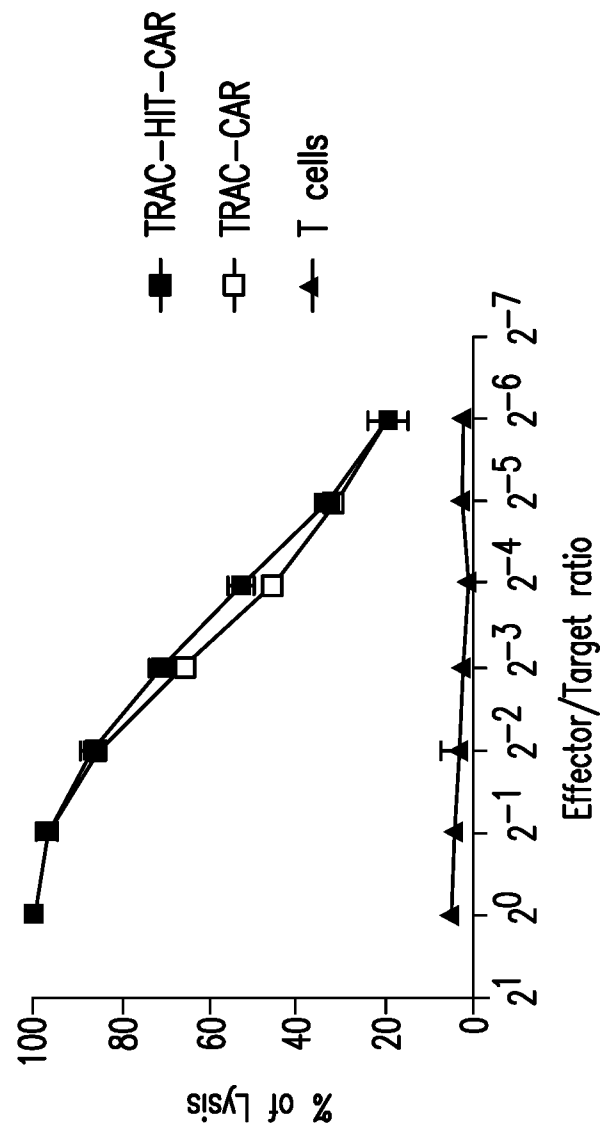


FIG. 1D

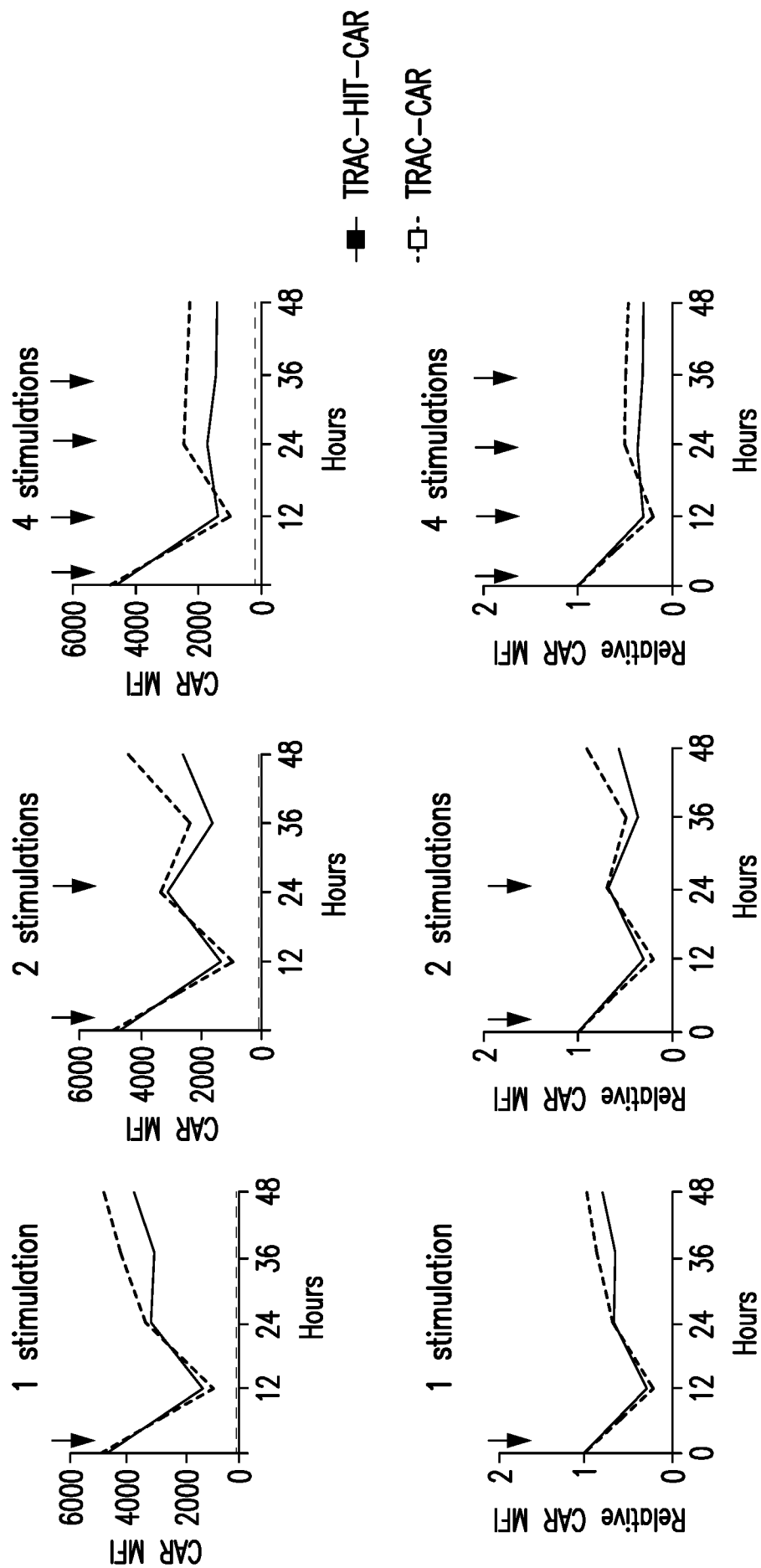


FIG. 1E

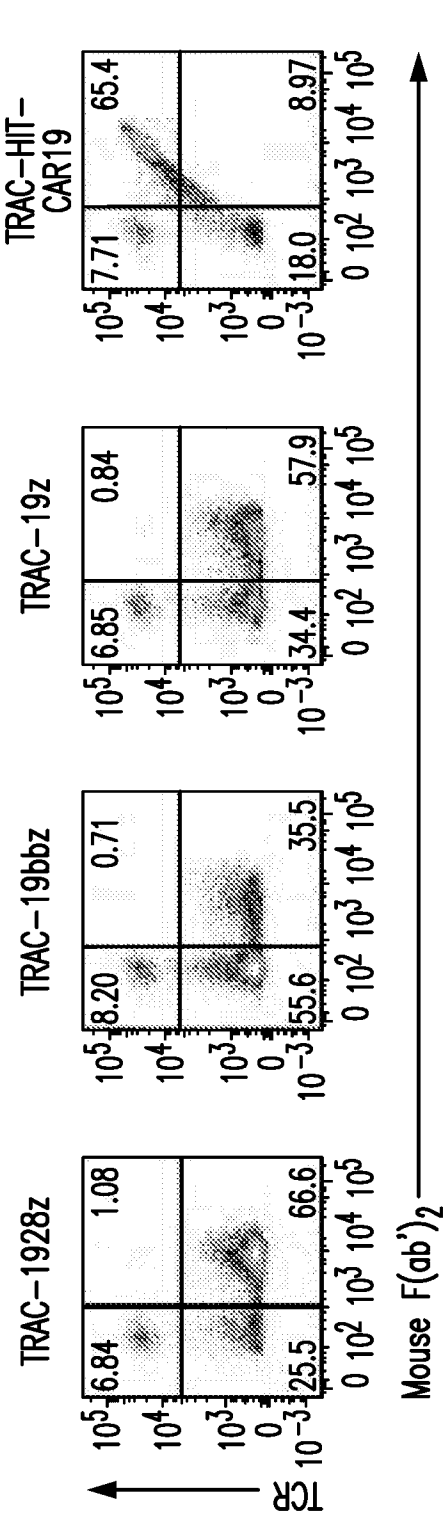


FIG. 2A

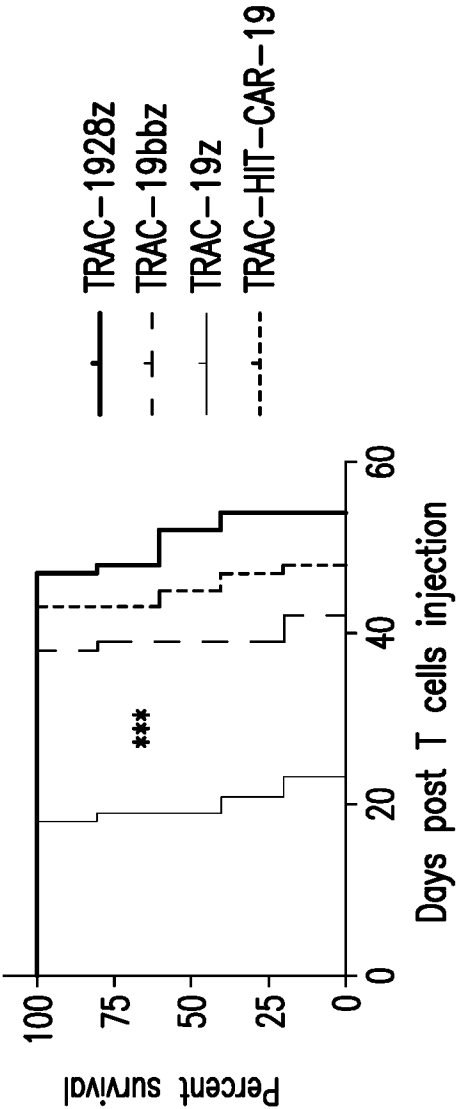


FIG. 2B

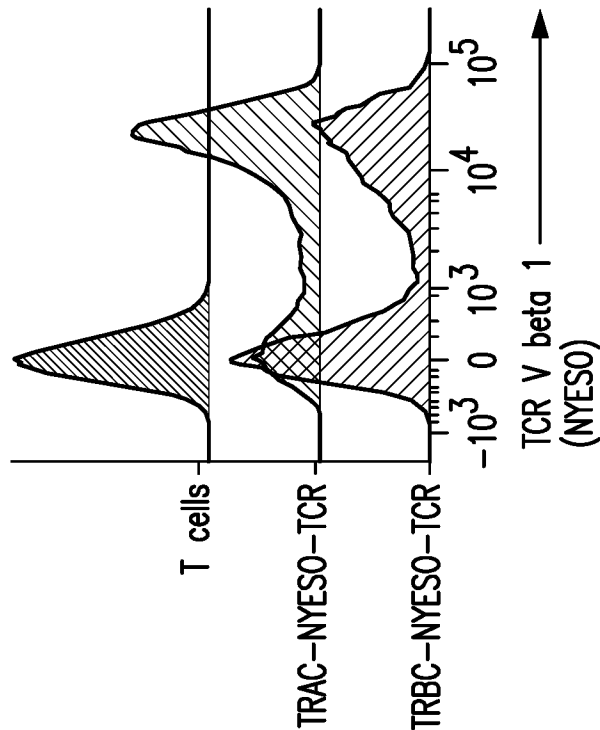


FIG. 3B

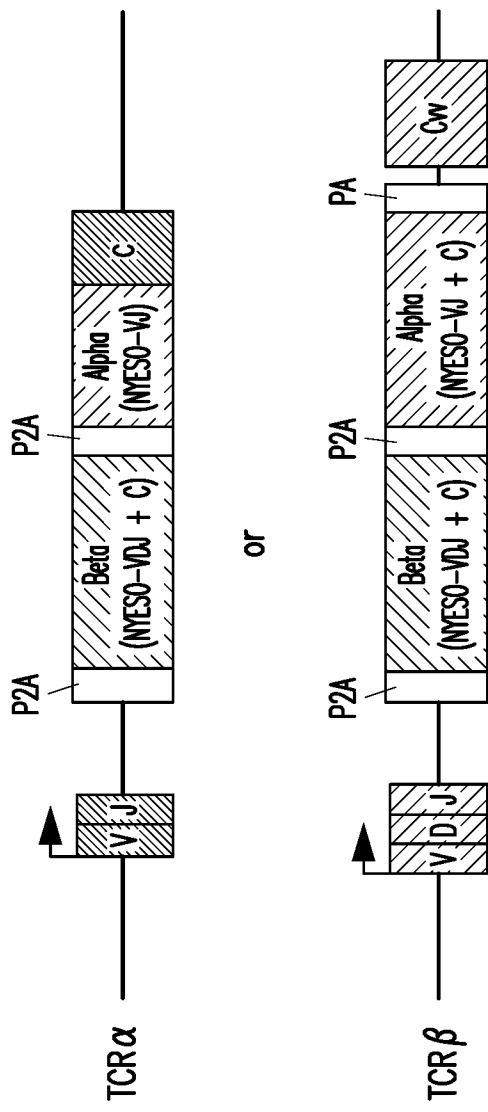


FIG. 3A

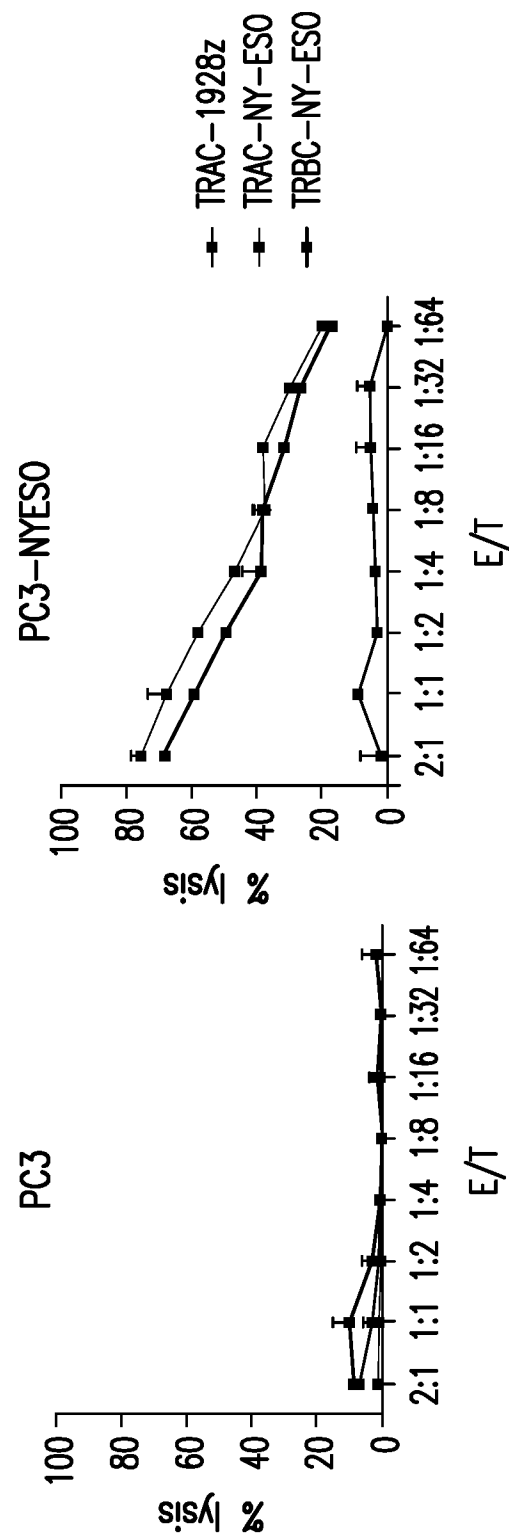
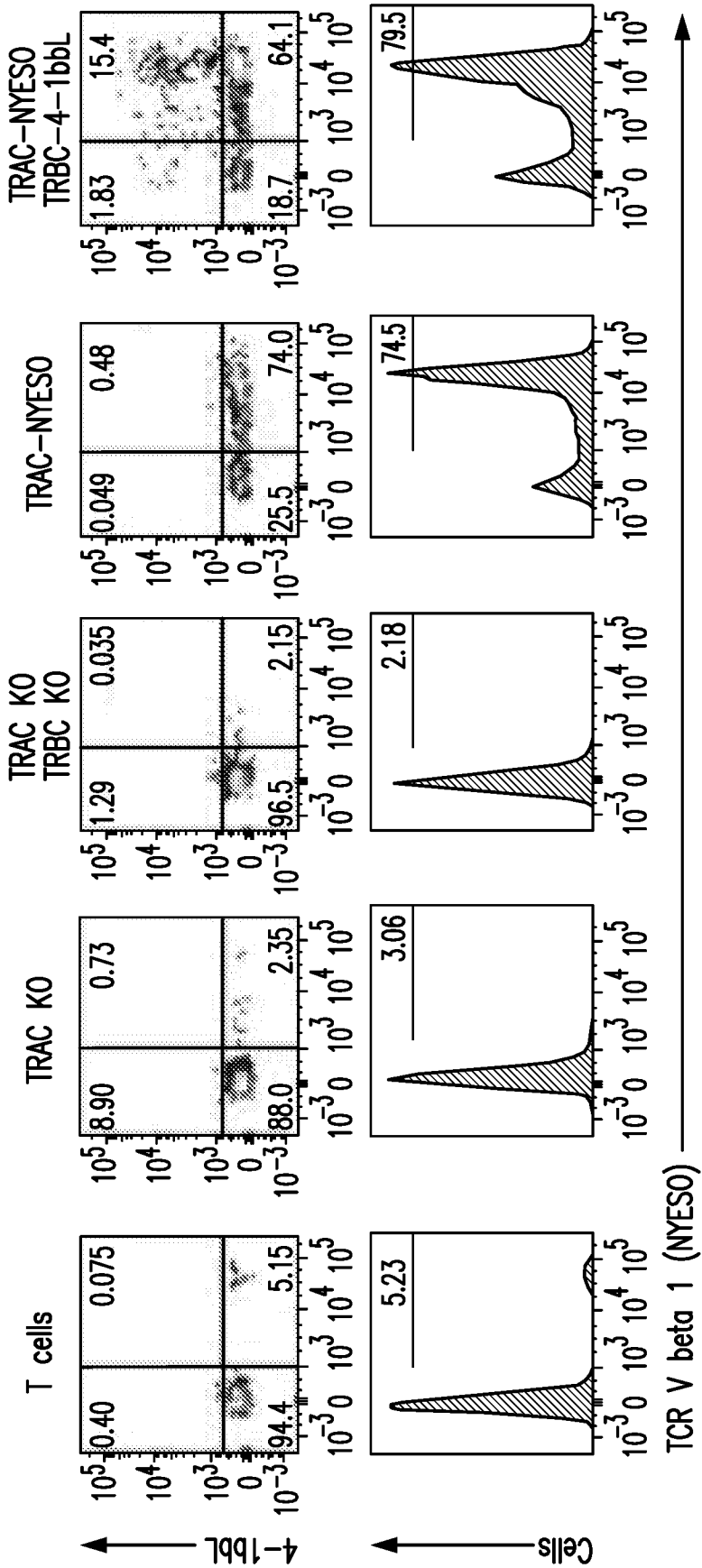
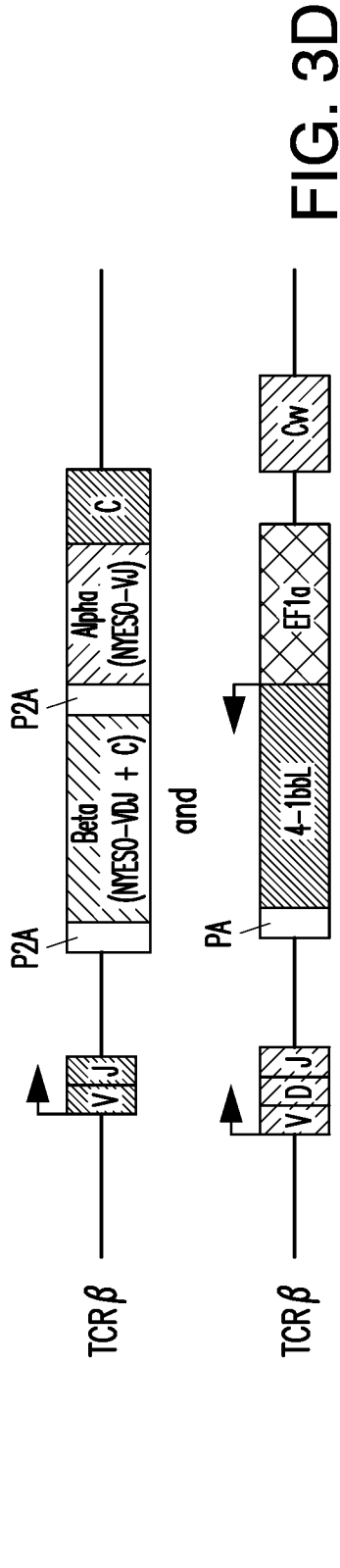


FIG. 3C



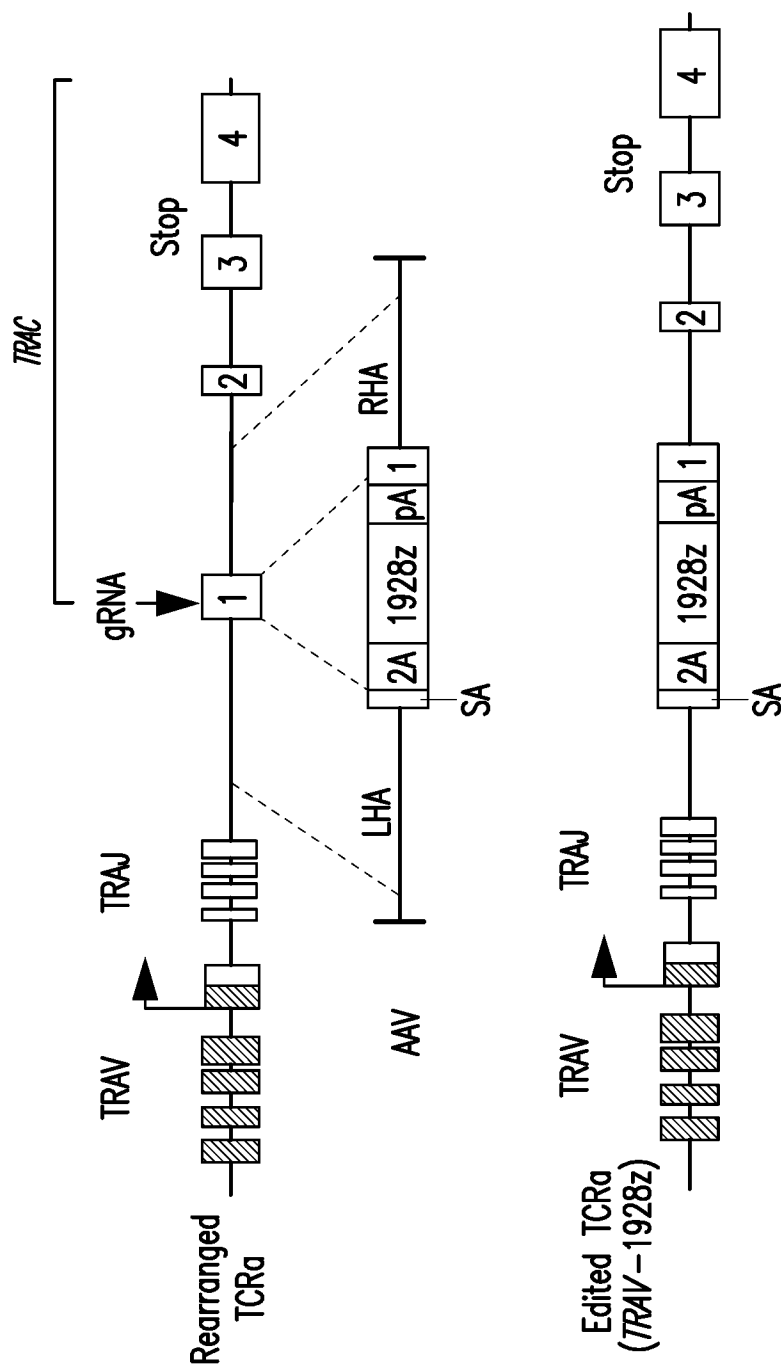


FIG. 4A

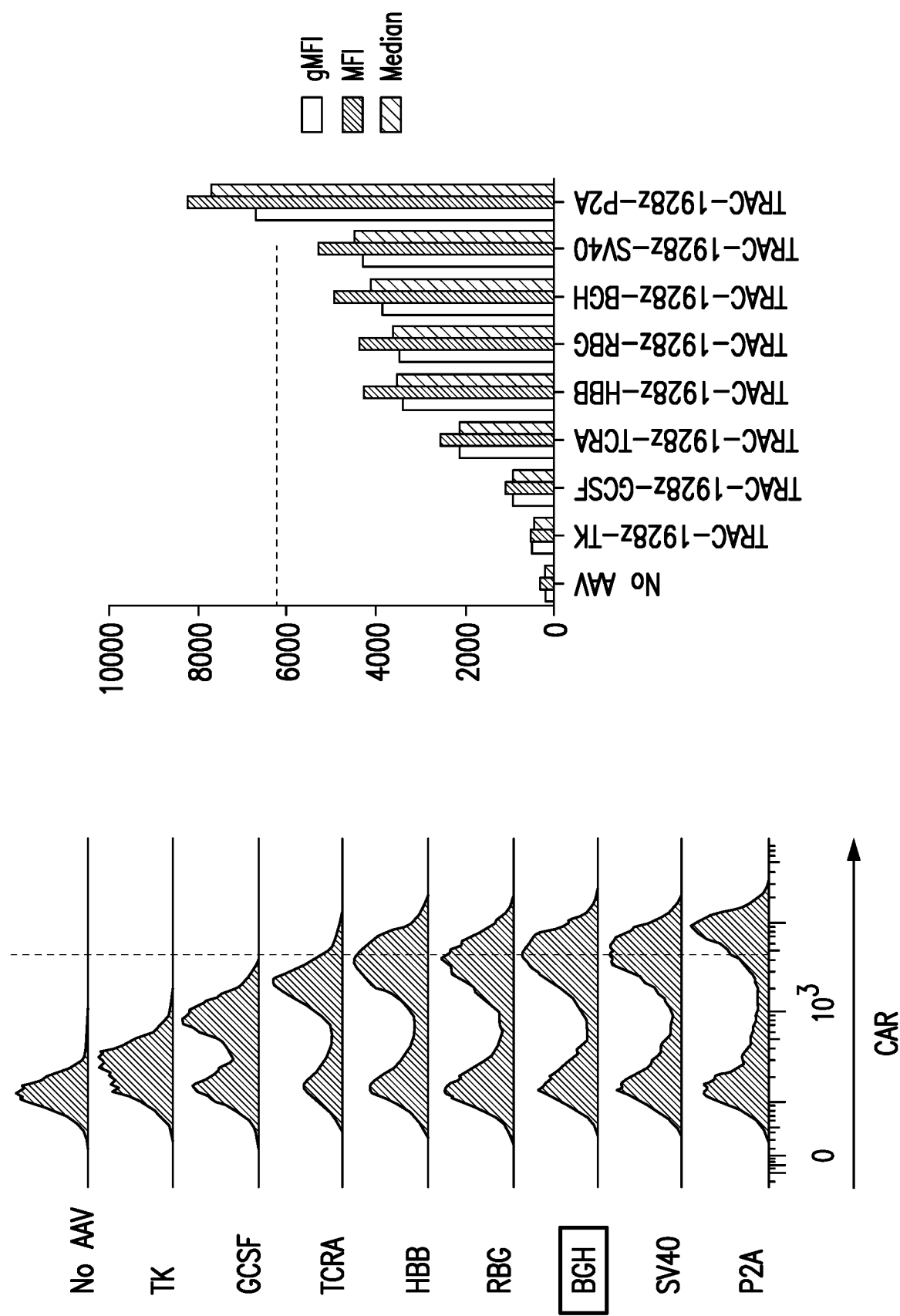


FIG. 4B

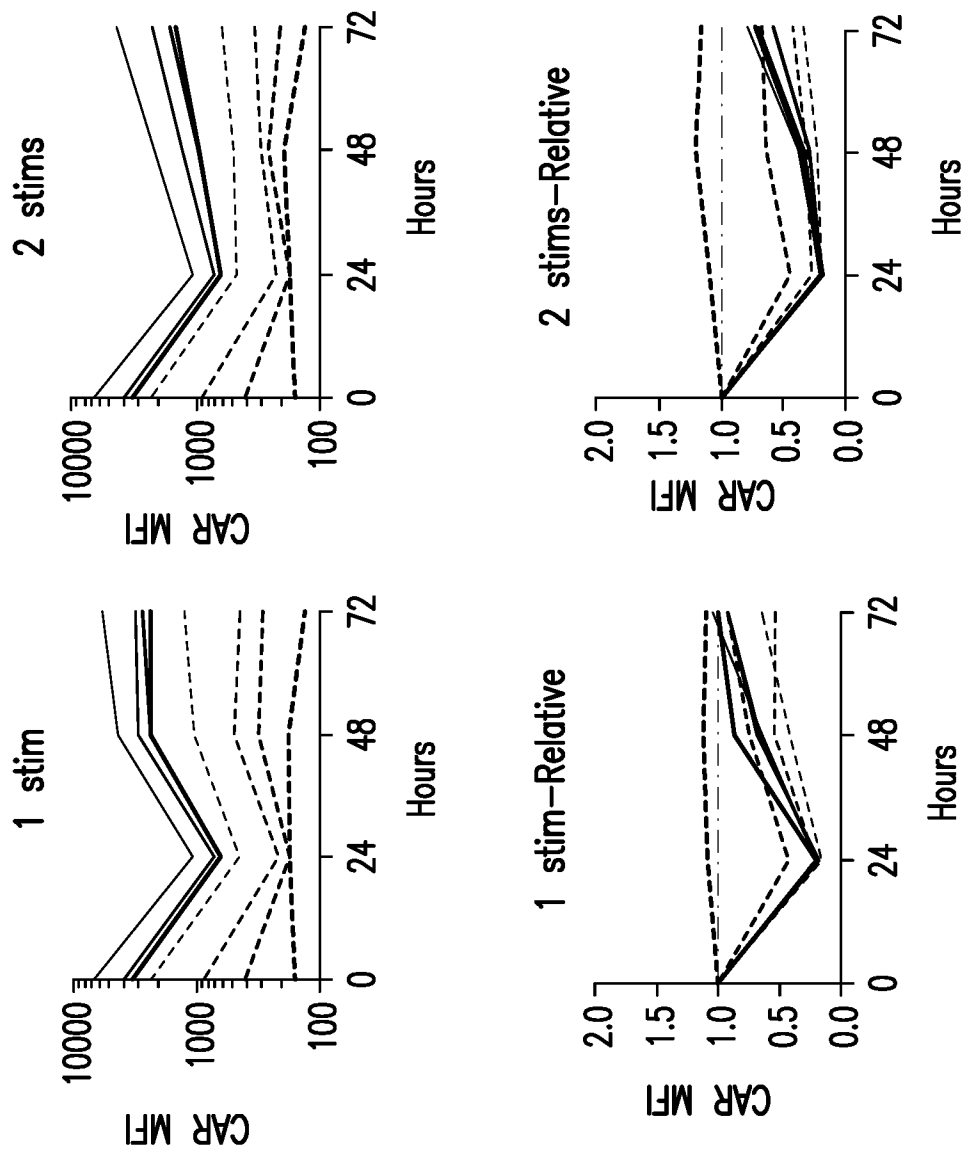


FIG. 4C

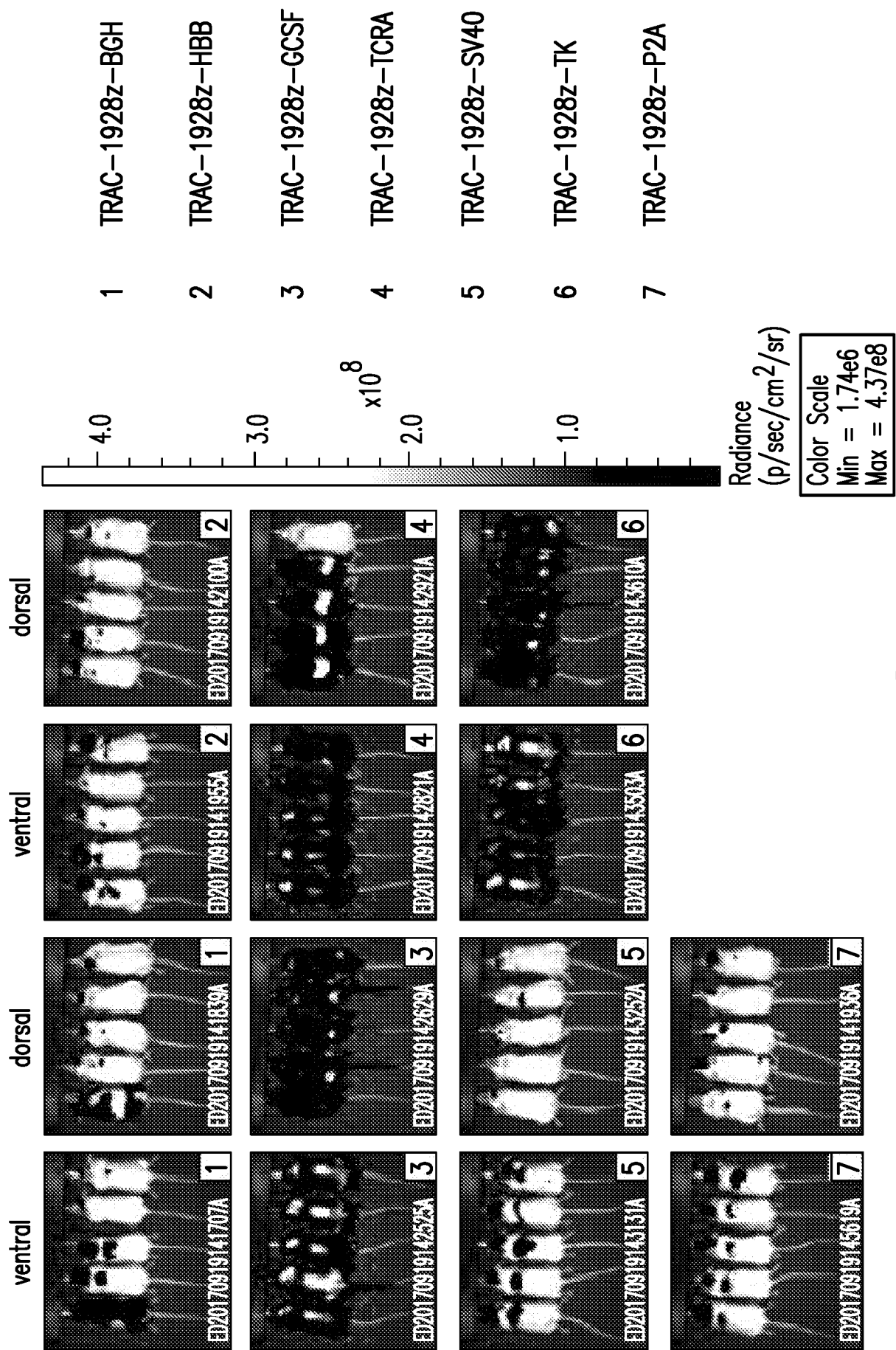


FIG. 5A

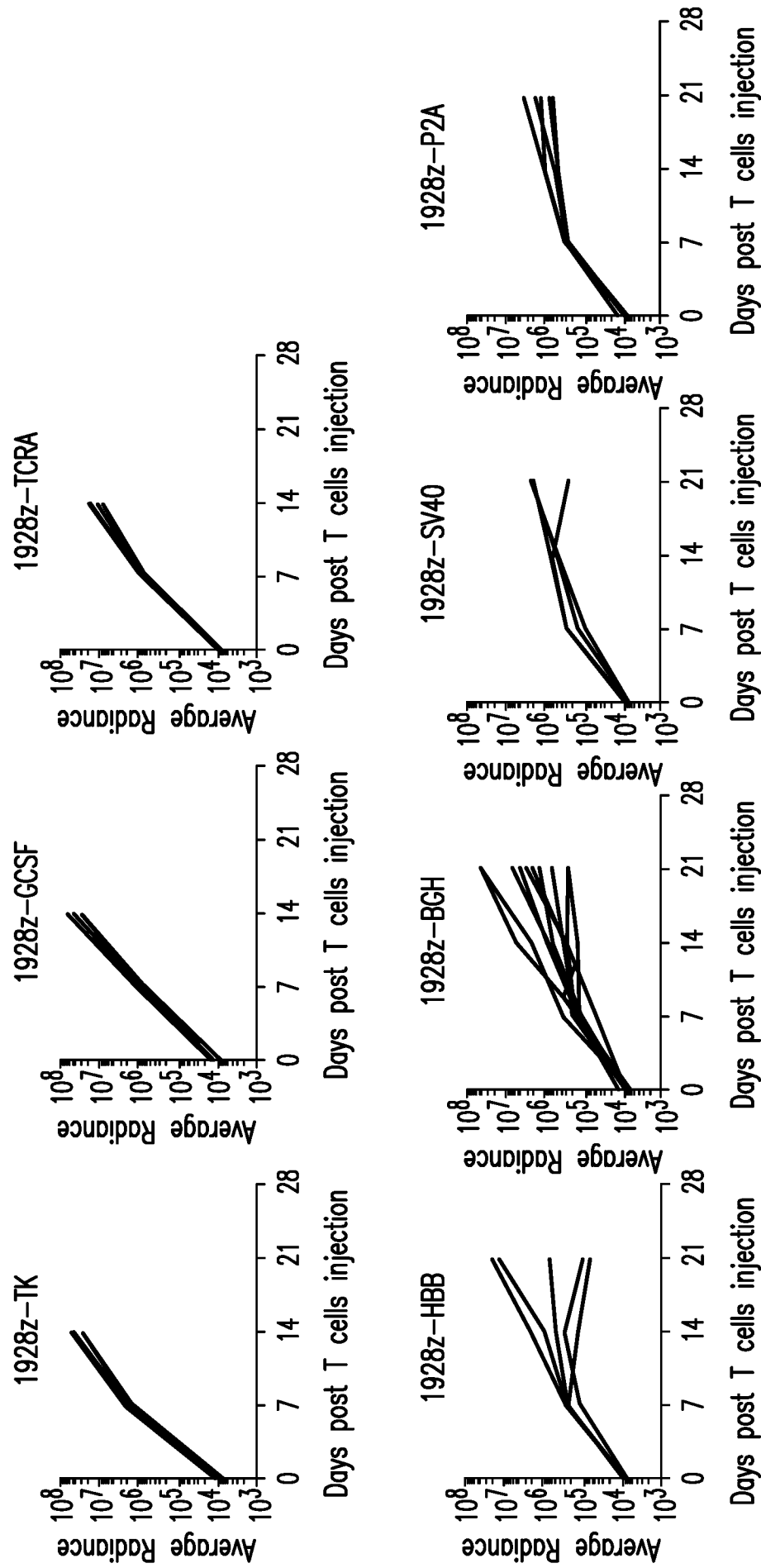


FIG. 5B

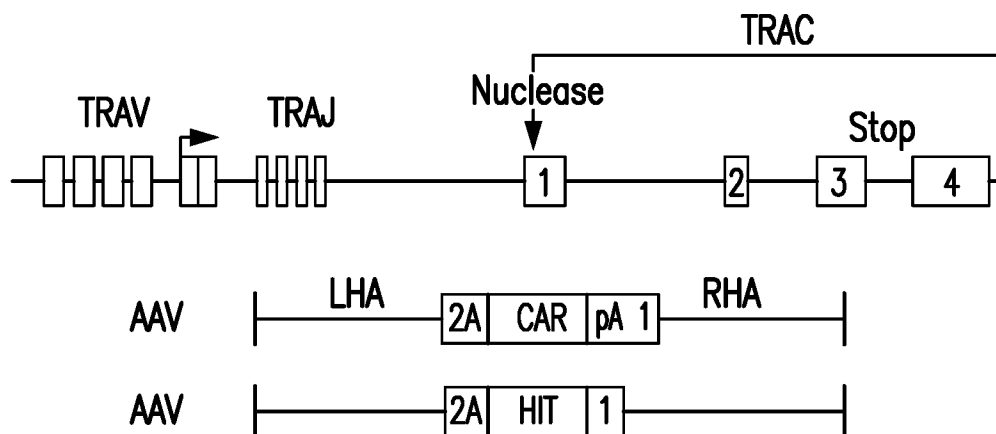


FIG. 6A

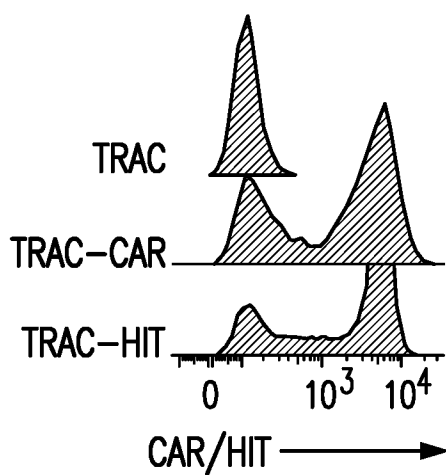


FIG. 6B

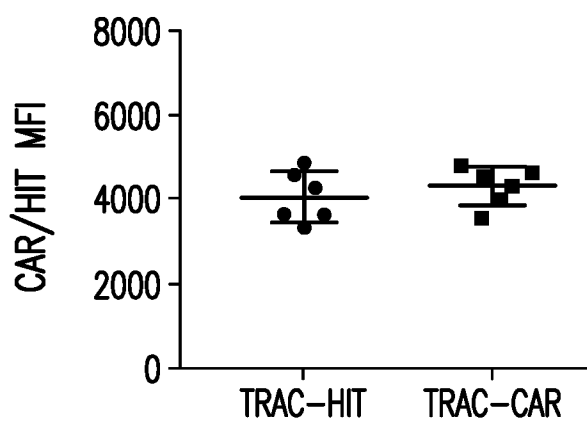


FIG. 6C

13/16

Nalm-6 CD19 locus editing

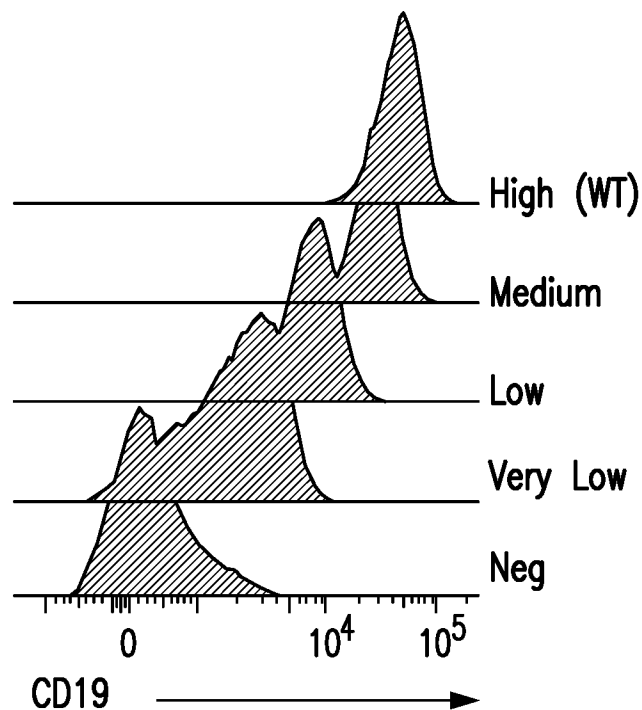


FIG. 7A

4h - Nalm6/T cells incubation (1:1-E:T)

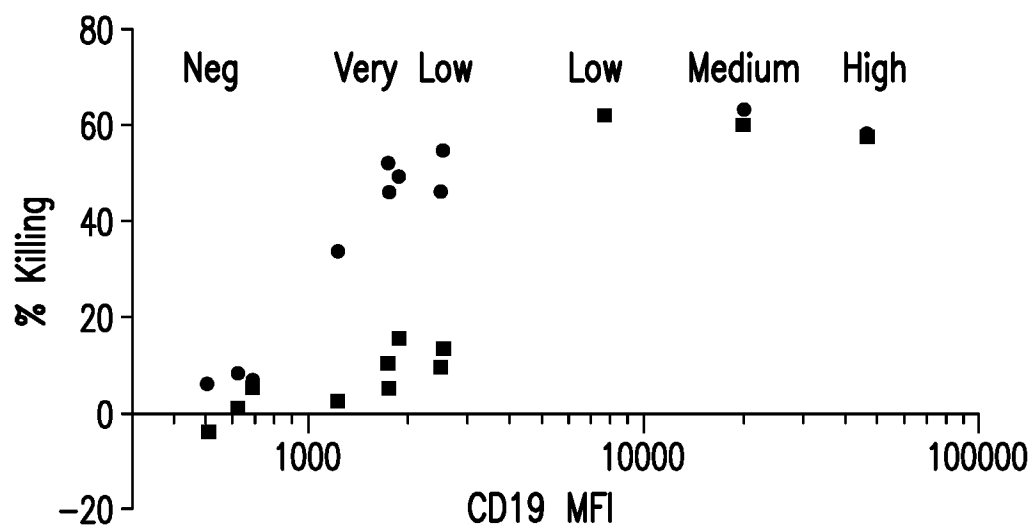


FIG. 7B

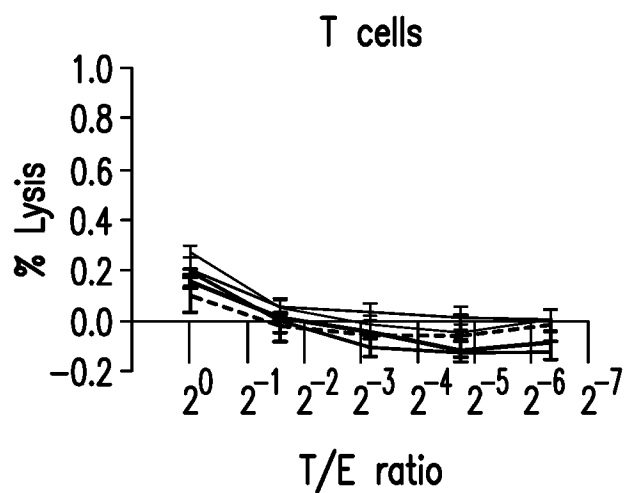


FIG. 8A

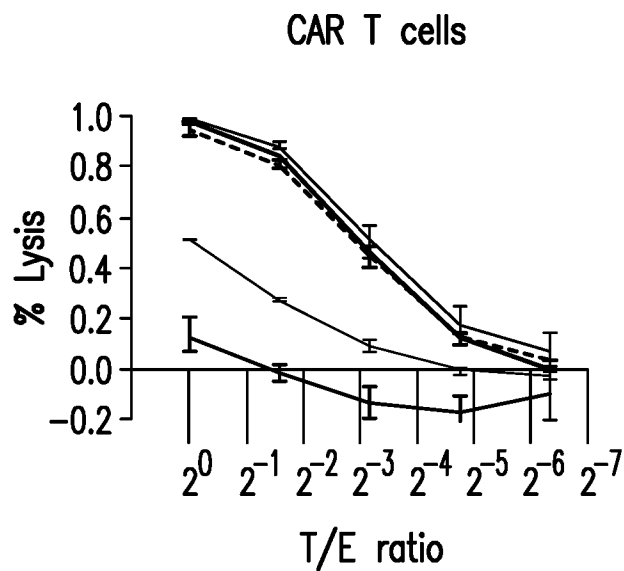


FIG. 8B

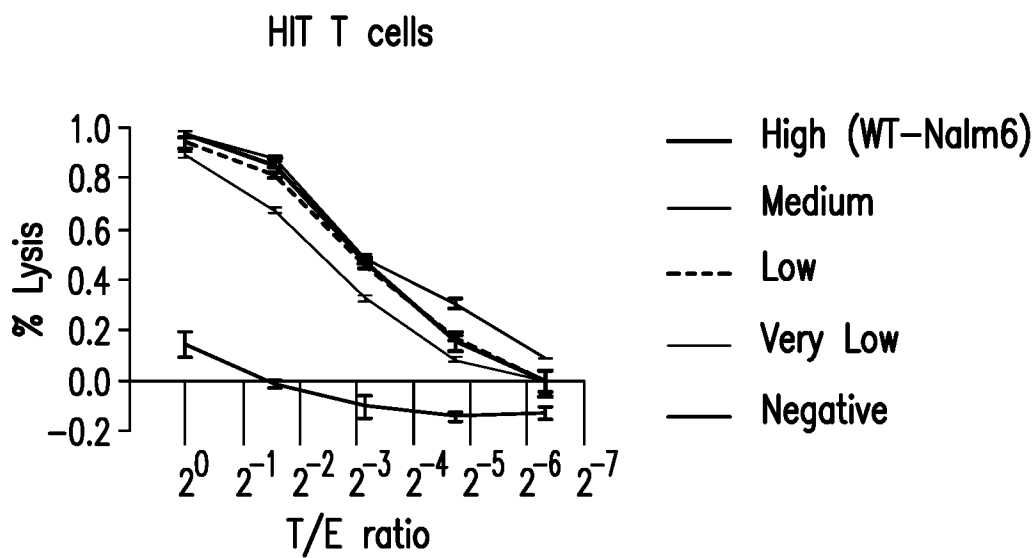


FIG. 8C

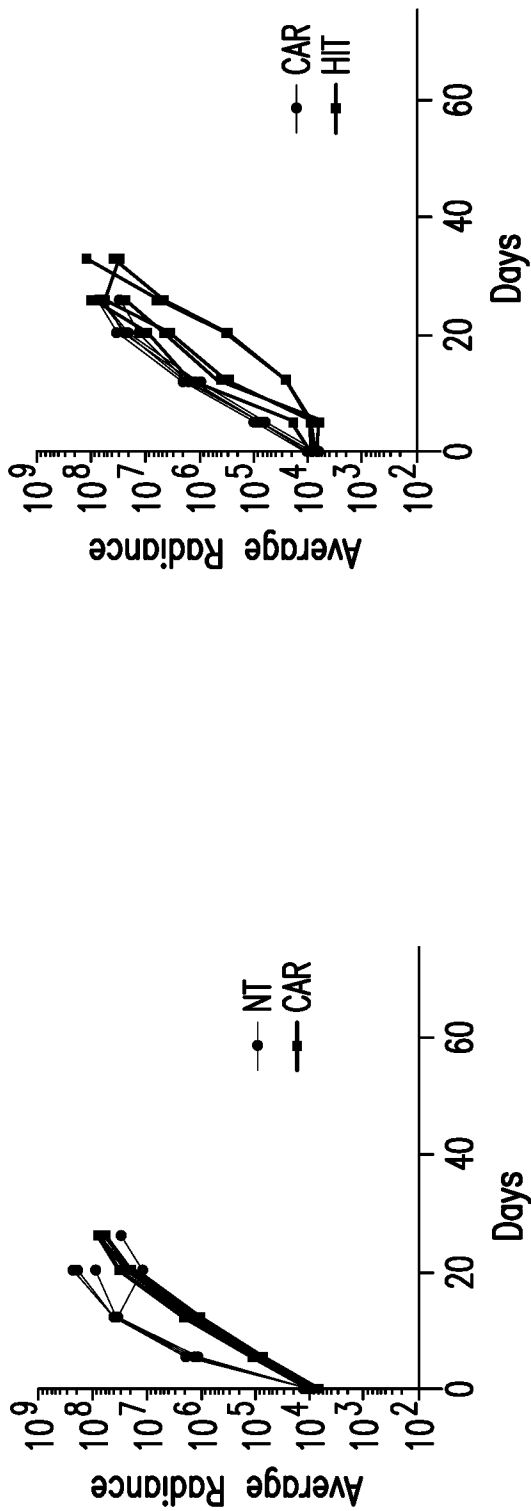


FIG. 9A

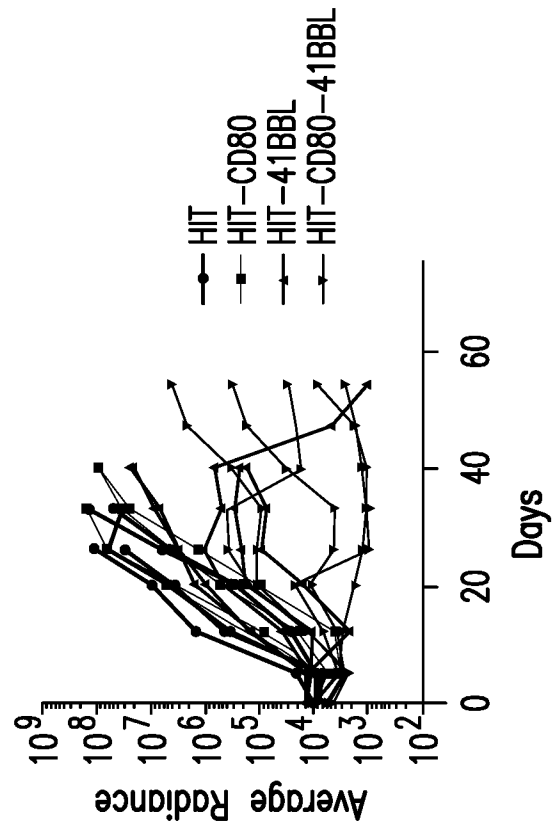


FIG. 9C

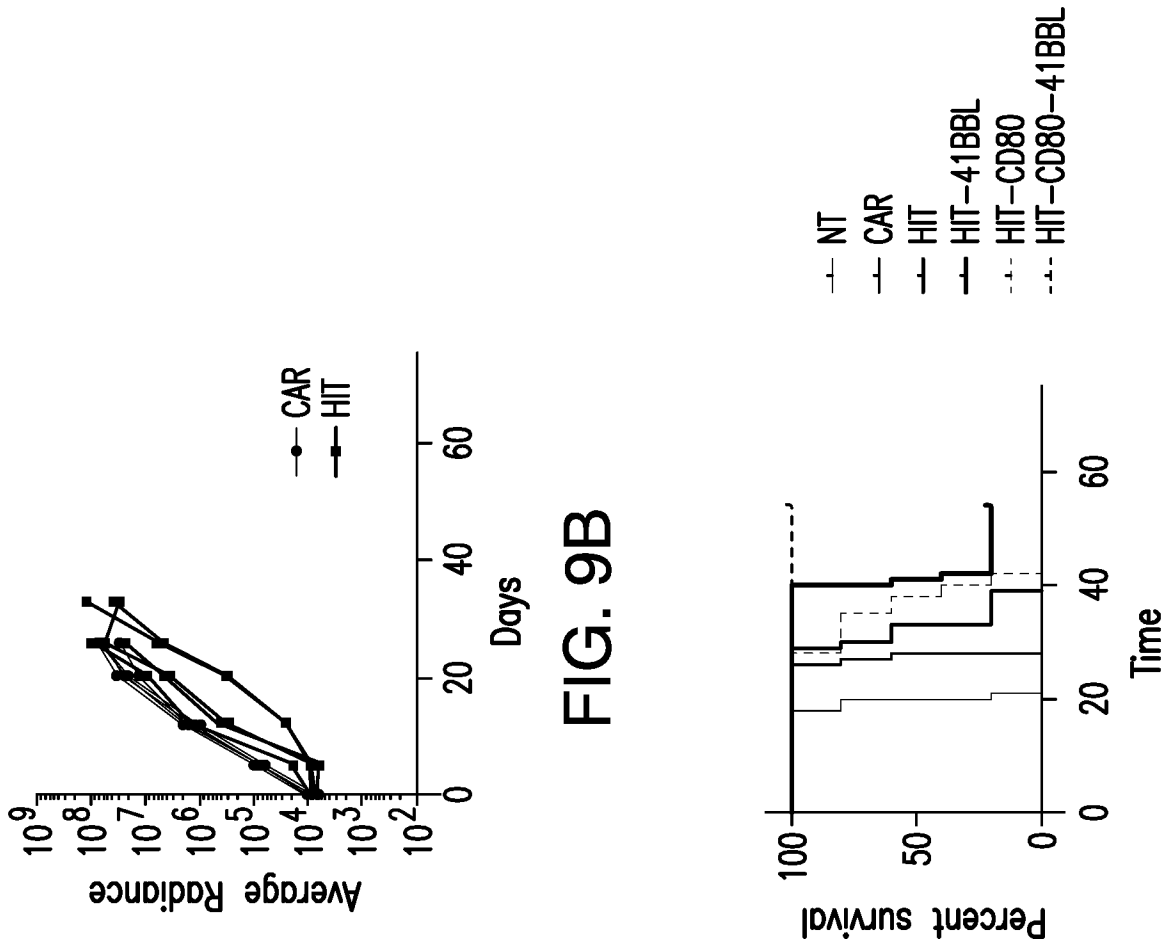


FIG. 9B

FIG. 9D

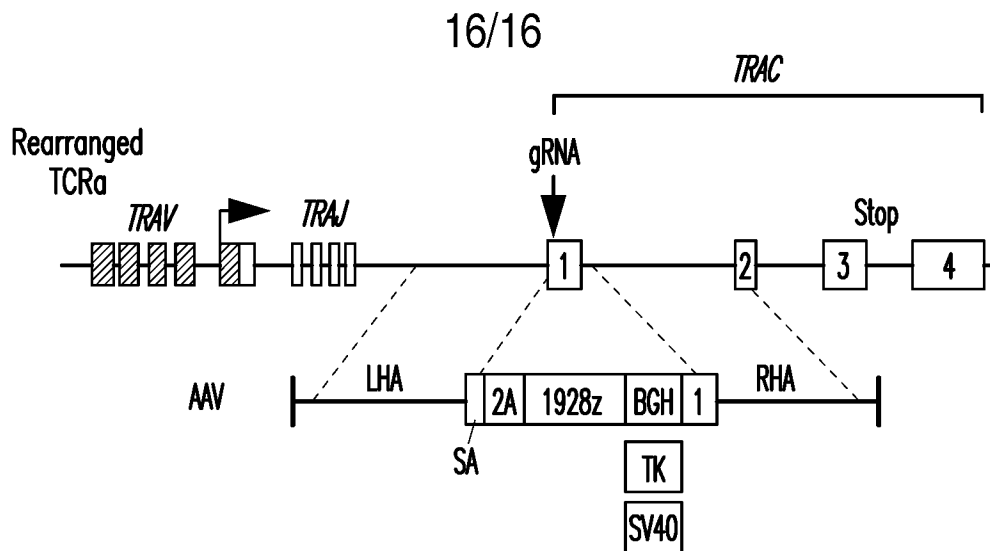


FIG. 10A

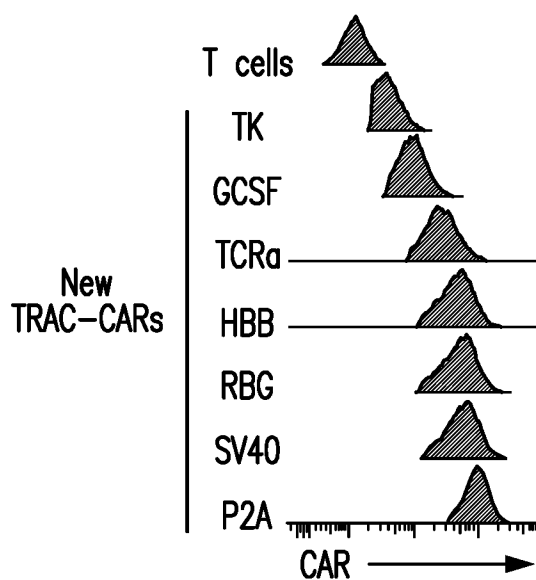


FIG. 10B

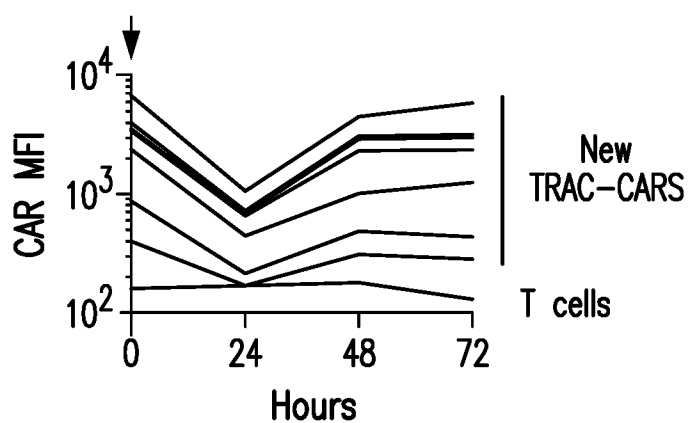


FIG. 10C

SEQUENCE LISTING

<110> MEMORIAL SLOAN-KETTERING CANCER CENTER

<120> NON-HLA RESTRICTED T CELL RECEPTORS AND USES THEREOF

<130> 072734.0845

<140> PCT/US2019/017525

<141> 2019-02-11

<150> 62/629,072

<151> 2018-02-11

<160> 48

<170> PatentIn version 3.5

<210> 1

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 1

Gly Tyr Ala Phe Ser Ser
1 5

<210> 2

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 2

Tyr Pro Gly Asp Gly Asp
1 5

<210> 3
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 3
Lys Thr Ile Ser Ser Val Val Asp Phe
1 5

<210> 4
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 4
Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala
1 5 10

<210> 5
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 5
Ser Ala Thr Tyr Arg Asn
1 5

<210> 6
<211> 9

<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic peptide

<400> 6

Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr
1 5

<210> 7

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 7

Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr
20 25 30

Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 8

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 8

Asp Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
35 40 45

Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80

Lys Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr
85 90 95

Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 9
<211> 263
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polypeptide

<400> 9
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ser Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser
35 40 45

Ser Tyr Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly
65 70 75 80

Lys Phe Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr
85 90 95

Ala Tyr Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

Phe Cys Ala Arg Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly

130

135

140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Glu Leu Thr
145 150 155 160

Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Val
165 170 175

Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn Val Ala Trp Tyr Gln
180 185 190

Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile Tyr Ser Ala Thr Tyr
195 200 205

Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
210 215 220

Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser Lys Asp Leu Ala Asp
225 230 235 240

Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr Thr Ser Gly Gly Gly
245 250 255

Thr Lys Leu Glu Ile Lys Arg
260

<210> 10

<211> 789

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 10

atggctctcc cagtgactgc cctactgctt cccctagcgc ttctcctgca tgcagaggtg
60

aagctgcagc agtctggggc tgagctggtg aggcctgggt cctcagtga gatttcctgc
120

aaggcttctg gctatgcatt cagtagctac tggatgaact gggatgaagca gaggcctgga
180

cagggctcttg agtggattgg acagatttat cctggagatg gtgatactaa ctacaatgga
240

aagttcaagg gtcaagccac actgactgca gacaaatcct ccagcacagc ctacatgcag
300

ctcagcggcc taacatctga ggactctgcg gtctatttct gtgcaagaaa gaccattagt
360

tcggtagtag atttctactt tgactactgg ggccaaggga ccacggtcac cgtctcctca
420

ggatggaggtg gatcagggtg aggtggatct ggtggaggtg gatctgacat tgagctcacc
480

cagtctccaa aattcatgtc cacatcagta ggagacaggg tcagcgtcac ctgcaaggcc
540

agtcagaatg tgggtactaa tgtagcctgg tatcaacaga aaccaggaca atctcctaaa
600

ccactgattt actcggcaac ctaccggaac agtggagtcc ctgatcgctt cacaggcagt
660

ggatctggga cagatttcac tctcaccatc actaacgtgc agtctaaaga cttggcagac
720

tatttctgtc aacaatataa caggtatccg tacacgtccg gaggggggac caagctggag
780

a t c a a a c g g
789

<210> 11

<211> 272

<212> PRT

<213> Homo sapiens

<400> 11

Pro Glu Glu Pro Leu Val Val Lys Val Glu Glu Gly Asp Asn Ala Val
1 5 10 15

Leu Gln Cys Leu Lys Gly Thr Ser Asp Gly Pro Thr Gln Gln Leu Thr
20 25 30

Trp Ser Arg Glu Ser Pro Leu Lys Pro Phe Leu Lys Leu Ser Leu Gly
35 40 45

Leu Pro Gly Leu Gly Ile His Met Arg Pro Leu Ala Ile Trp Leu Phe
50 55 60

Ile Phe Asn Val Ser Gln Gln Met Gly Gly Phe Tyr Leu Cys Gln Pro
65 70 75 80

Gly Pro Pro Ser Glu Lys Ala Trp Gln Pro Gly Trp Thr Val Asn Val
85 90 95

Glu Gly Ser Gly Glu Leu Phe Arg Trp Asn Val Ser Asp Leu Gly Gly
100 105 110

Leu Gly Cys Gly Leu Lys Asn Arg Ser Ser Glu Gly Pro Ser Ser Pro
115 120 125

Ser Gly Lys Leu Met Ser Pro Lys Leu Tyr Val Trp Ala Lys Asp Arg
130 135 140

Pro Glu Ile Trp Glu Gly Glu Pro Pro Cys Leu Pro Pro Arg Asp Ser
145 150 155 160

Leu Asn Gln Ser Leu Ser Gln Asp Leu Thr Met Ala Pro Gly Ser Thr

165

170

175

Leu Trp Leu Ser Cys Gly Val Pro Pro Asp Ser Val Ser Arg Gly Pro
 180 185 190

Leu Ser Trp Thr His Val His Pro Lys Gly Pro Lys Ser Leu Leu Ser
 195 200 205

Leu Glu Leu Lys Asp Asp Arg Pro Ala Arg Asp Met Trp Val Met Glu
 210 215 220

Thr Gly Leu Leu Leu Pro Arg Ala Thr Ala Gln Asp Ala Gly Lys Tyr
 225 230 235 240

Tyr Cys His Arg Gly Asn Leu Thr Met Ser Phe His Leu Glu Ile Thr
 245 250 255

Ala Arg Pro Val Leu Trp His Trp Leu Leu Arg Thr Gly Gly Trp Lys
 260 265 270

<210> 12

<211> 20

<212> PRT

<213> Homo sapiens

<400> 12

Met Tyr Arg Met Gln Leu Leu Ser Cys Ile Ala Leu Ser Leu Ala Leu
 1 5 10 15

Val Thr Asn Ser
 20

<210> 13

<211> 20

<212> PRT

<213> Mus sp.

<400> 13

Met Tyr Ser Met Gln Leu Ala Ser Cys Val Thr Leu Thr Leu Val Leu
1 5 10 15

Leu Val Asn Ser
20

<210> 14

<211> 20

<212> PRT

<213> Homo sapiens

<400> 14

Met Glu Thr Pro Ala Gln Leu Leu Phe Leu Leu Leu Leu Trp Leu Pro
1 5 10 15

Asp Thr Thr Gly
20

<210> 15

<211> 20

<212> PRT

<213> Mus sp.

<400> 15

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly
20

<210> 16

<211> 21

<212> PRT

<213> Homo sapiens

<400> 16

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu

1 5 10 15

His Ala Ala Arg Pro
20

<210> 17

<211> 164

<212> PRT

<213> Homo sapiens

<400> 17

Met Lys Trp Lys Ala Leu Phe Thr Ala Ala Ile Leu Gln Ala Gln Leu
1 5 10 15

Pro Ile Thr Glu Ala Gln Ser Phe Gly Leu Leu Asp Pro Lys Leu Cys
20 25 30

Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr Ala
35 40 45

Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
50 55 60

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
65 70 75 80

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
85 90 95

Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
100 105 110

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
115 120 125

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
 130 135 140

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
 145 150 155 160

Leu Pro Pro Arg

<210> 18
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 18
 Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
 1 5 10 15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
 20 25 30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
 35 40 45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
 50 55 60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
 65 70 75 80

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
 85 90 95

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
 100 105 110

<210> 19

<211> 336

<212> DNA

<213> Homo sapiens

<400> 19

agagtgaagt tcagcaggag cgcagacgcc cccgcgtacc agcagggcca gaaccagctc
60

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc
120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat
180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc
240

cggaggggca aggggcacga tggcctttac caggggtctca gtacagccac caaggacacc
300

t a c g a c g c c c t t c a c a t g c a g g c c c t g c c c c c t c g c
336

<210> 20

<211> 220

<212> PRT

<213> Homo sapiens

<400> 20

Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
1 5 10 15

Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
20 25 30

Asp Asn Ala Val Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
35 40 45

Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu

50

55

60

Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
65 70 75 80

Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
85 90 95

Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
100 105 110

Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
115 120 125

Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
130 135 140

Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
145 150 155 160

Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile
165 170 175

Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
180 185 190

Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
195 200 205

Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
210 215 220

<210> 21

<211> 41

<212> PRT
<213> Homo sapiens

<400> 21
Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser
35 40

<210> 22
<211> 123
<212> DNA
<213> Homo sapiens

<400> 22
aggagtaaga ggagcaggct cctgcacagt gactacatga acatgactcc ccgccgcccc
60

gggcccaccc gcaagcatta ccagccctat gccccaccac gcgacttcgc agcctatcgc
120

t c c
123

<210> 23
<211> 255
<212> PRT
<213> Homo sapiens

<400> 23
Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val Leu
1 5 10 15

Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
20 25 30

Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
35 40 45

Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
50 55 60

Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65 70 75 80

Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
85 90 95

Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
100 105 110

Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
115 120 125

Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
130 135 140

Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
145 150 155 160

Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
165 170 175

Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
180 185 190

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
195 200 205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
210 215 220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
225 230 235 240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
245 250 255

<210> 24

<211> 42

<212> PRT

<213> Homo sapiens

<400> 24

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1 5 10 15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
20 25 30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
35 40

<210> 25

<211> 277

<212> PRT

<213> Homo sapiens

<400> 25

Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu
1 5 10 15

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val
20 25 30

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro

35

40

45

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys
50 55 60

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro
65 70 75 80

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys
85 90 95

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
100 105 110

Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
115 120 125

Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
130 135 140

Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
145 150 155 160

Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
165 170 175

Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
180 185 190

Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
195 200 205

Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
210 215 220

Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
225 230 235 240

Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
245 250 255

Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
260 265 270

Thr Leu Ala Lys Ile
275

<210> 26

<211> 199

<212> PRT

<213> Homo sapiens

<400> 26

Met Lys Ser Gly Leu Trp Tyr Phe Phe Leu Phe Cys Leu Arg Ile Lys
1 5 10 15

Val Leu Thr Gly Glu Ile Asn Gly Ser Ala Asn Tyr Glu Met Phe Ile
20 25 30

Phe His Asn Gly Gly Val Gln Ile Leu Cys Lys Tyr Pro Asp Ile Val
35 40 45

Gln Gln Phe Lys Met Gln Leu Leu Lys Gly Gly Gln Ile Leu Cys Asp
50 55 60

Leu Thr Lys Thr Lys Gly Ser Gly Asn Thr Val Ser Ile Lys Ser Leu
65 70 75 80

Lys Phe Cys His Ser Gln Leu Ser Asn Asn Ser Val Ser Phe Phe Leu

85

90

95

Tyr Asn Leu Asp His Ser His Ala Asn Tyr Tyr Phe Cys Asn Leu Ser
100 105 110

Ile Phe Asp Pro Pro Pro Phe Lys Val Thr Leu Thr Gly Gly Tyr Leu
115 120 125

His Ile Tyr Glu Ser Gln Leu Cys Cys Gln Leu Lys Phe Trp Leu Pro
130 135 140

Ile Gly Cys Ala Ala Phe Val Val Val Cys Ile Leu Gly Cys Ile Leu
145 150 155 160

Ile Cys Trp Leu Thr Lys Lys Lys Tyr Ser Ser Ser Val His Asp Pro
165 170 175

Asn Gly Glu Tyr Met Phe Met Arg Ala Val Asn Thr Ala Lys Lys Ser
180 185 190

Arg Leu Thr Asp Val Thr Leu
195

<210> 27

<211> 126

<212> DNA

<213> Homo sapiens

<400> 27

aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa
60

actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt
120

g a a c t g
126

<210> 28
<211> 18
<212> PRT
<213> Homo sapiens

<400> 28
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala

<210> 29
<211> 4627
<212> DNA
<213> Homo sapiens

<400> 29
atatccagaa ccctgaccct gccgtgtacc agctgagaga ctctaaatcc agtgacaagt
60

ctgtctgcct attcacccgat ttgtattctc aaacaaatgt gtcacaaagt aaggattctg
120

atgtgtatat cacagacaaa actgtgctag acatgaggtc tatggacttc aagagcaaca
180

gtgctgtggc ctggagcaac aaatctgact ttgcatgtgc aaacgccttc aacaacagca
240

ttattccaga agacaccttc ttccccagcc caggtaaggg cagctttggg gccttcgcag
300

gctgtttcct tgcttcagga atggccaggc tctgcccaga gctctgggtca atgatgtcta
360

aaactcctct gattgggtggc ctcggcctta tccattgcca ccaaaaccct ctttttacta
420

agaaacagtg agccttggtc tggcagtgcca gagaatgaca cgggaaaaaa gcagatgaag
480

agaaggtggc aggagagggc acgtggccca gcctcagtct ctccaactga gttcctgcct
540

gcctgccttt gctcagactg tttgcccctt actgctcttc taggcctcat tctaagcccc
600

ttctccaagt tgcctctcct tatttctccc tgtctgccaa aaaatctttc ccagctcact
660

aagtcagtct cacgcagtca ctcattaacc caccaatcac tgattgtgcc ggcacatgaa
720

tgcaccaggt gttgaagtgg aggaattaaa aagtcagatg aggggtgtgc ccagaggaag
780

caccattcta gttgggggag cccatctgtc agctgggaaa agtccaaata acttcagatt
840

ggaatgtgtt ttaactcagg gttgagaaaa cagctacctt caggacaaaa gtcagggaag
900

ggctctctga agaaatgcta cttgaagata ccagccctac caagggcagg gagaggaccc
960

tatagaggcc tgggacagga gctcaatgag aaaggagaag agcagcaggc atgagttgaa
1020

tgaaggaggc agggccgggt cacagggcct tctaggccat gagagggtag acagtattct
1080

aaggacgcca gaaagctgtt gatcggcttc aagcagggga gggacaccta atttgctttt
1140

cttttttttt tttttttttt tttttttttt tgagatggag ttttgctctt gttgcccagg
1200

ctggagtgca atggtgcatc ttggctcact gcaacctccg cctcccagg tcaagtgatt
1260

ctcctgcctc agcctcccga gtagctgaga ttacaggcac ccgccaccat gcctggctaa
1320

ttttttgtat ttttagtaga gacaggggtt cactatgttg gccaggctgg tctcgaactc
1380

ctgacctcag gtgatccacc cgcttcagcc tcccaaagtg ctgggattac aggcgtgagc
1440

caccacaccc ggcctgcttt tcttaaagat caatctgagt gctgtacgga gagtgggttg
1500

taagccaaga gtagaagcag aaagggagca gttgcagcag agagatgatg gaggcctggg
1560

cagggtggtg gcagggaggt aaccaacacc attcaggttt caaaggtaga accatgcagg
1620

gatgagaaag caaagagggg atcaaggaag gcagctggat tttggcctga gcagctgagt
1680

caatgatagt gccgtttact aagaagaaac caaggaaaaa atttgggggtg cagggatcaa
1740

aacttttttg aacatatgaa agtacgtggt tatactcttt atggcccttg tcactatgta
1800

tgccctcgctg cctccattgg actctagaat gaagccaggc aagagcaggg tctatgtgtg
1860

atggcacatg tggccagggt catgcaacat gtactttgta caaacagtgt atattgagta
1920

aatagaaatg gtgtccagga gccgaggtat cggtcctgcc agggccaggg gctctcccta
1980

gcaggtgctc atatgctgta agttccctcc agatctctcc acaaggaggc atggaaaggc
2040

tgtagttggt cacctgcccc agaactagga ggtctggggg gggagagtca gcctgctctg
2100

gatgctgaaa gaatgtctgt ttttcctttt agaaagttcc tgtgatgtca agctggtcga
2160

gaaaagcttt gaaacaggta agacaggggt ctagcctggg tttgcacagg attgcggaag
2220

tgatgaaccc gcaataaccc tgccctggatg agggagtggt aagaaattag tagatgtggg
2280

aatgaatgat gaggaatgga aacagcgggtt caagacctgc ccagagctgg gtggggtctc
2340

tcctgaatcc ctctcaccat ctctgacttt ccattctaag cactttgagg atgagtttct
2400

agcttcaata gaccaaggac tctctcctag gcctctgtat tcctttcaac agtccactg
2460

tcaagagagc cagagagagc ttctgggtgg cccagctgtg aaattttctga gtcccttagg
2520

gatagcccta aacgaaccag atcatcctga ggacagccaa gaggttttgc cttctttcaa
2580

gacaagcaac agtactcaca taggctgtgg gcaatgggcc tgtctctcaa gaatcccctg
2640

ccactcctca caccaccctt gggcccatat tcattttccat ttgagttggt cttattgagt
2700

catccttcct gtggtagcgg aactcactaa ggggcccatac tggacccgag gtattgtgat
2760

gataaattct gagcacctac cccatcccca gaagggctca gaaataaaat aagagccaag
2820

tctagtcggt gtttcctgtc ttgaaacaca atactgttgg ccctggaaga atgcacagaa
2880

tctgtttgta aggggatatg cacagaagct gcaagggaca ggaggtgcag gagctgcagg
2940

cctccccac ccagcctgct ctgccttggg gaaaaccgtg ggtgtgtcct gcaggccatg
3000

caggcctggg acatgcaagc ccataaccgc tgtggcctct tggttttaca gatacgaacc
3060

taaactttca aaacctgtca gtgattgggt tccgaatcct cctcctgaaa gtggccgggt
3120

ttaatctgct catgacgctg cggctgtggt ccagctgagg tgaggggcct tgaagctggg
3180

agtggggttt agggacgcgg gtctctgggt gcatcctaag ctctgagagc aaacctccct
3240

gcagggtctt gctttttaagt ccaaagcctg agcccaccaa actctcctac ttcttcctgt
3300

tacaaattcc tcttgtgcaa taataatggc ctgaaacgct gtaaaatata ctcatttcag
3360

ccgcctcagt tgcacttctc ccctatgagg taggaagaac agttgttttag aaacgaagaa
3420

actgaggccc cacagctaata gagtggagga agagagacac ttgtgtacac cacatgcctt
3480

gtgttgtact tctctcaccg tgtaacctcc tcatgtcctc tctccccagt acggctctct
3540

tagctcagta gaaagaagac attacactca tattacaccc caatcctggc tagagtctcc
3600

gcaccctcct cccccagggg cccagtcgt cttgctgaca actgcacctt gttccatcac
3660

catcaaaaaa aaactccagg ctgggtgcgg gggctcacac ctgtaatccc agcactttgg
3720

gaggcagagg caggaggagc acaggagctg gagaccagcc tgggcaacac agggagaccc
3780

cgcctctaca aaaagtgaaa aaattaacca ggtgtggtgc tgcacacctg tagtcccagc
3840

tacttaagag gctgagatgg gaggatcgct tgagccctgg aatgttgagg ctacaatgag
3900

ctgtgattgc gtcactgcac tccagcctgg aagacaaagc aagatcctgt ctcaaataat
3960

aaaaaaaaata agaactccag ggtacatttg ctccatagaac tctaccacat agccccaaac
4020

agagccatca ccatcacatc cctaacagtc ctgggtcttc ctcagtgtcc agcctgactt
4080

ctgtttcttcc tcattccaga tctgcaagat tgtaagacag cctgtgctcc ctgctcctt
4140

cctctgcatt gcccctcttc tccctctcca aacagaggga actctcctac cccaaggag
4200

gtgaaagctg ctaccacctc tgtgcccccc cggcaatgcc accaactgga tcctaccga
4260

atztatgatt aagattgctg aagagctgcc aaacactgct gccaccccct ctgttccctt
4320

attgctgctt gtcactgcct gacattcacg gcagaggcaa ggctgctgca gcctcccctg
4380

gctgtgcaca ttccctcctg ctccccagag actgcctccg ccatcccaca gatgatggat
4440

cttcagtggg ttctcttggg ctctaggtcc tgcagaatgt tgtgaggggt ttatTTTTTT
4500

ttaatagtgt tcataaagaa atacatagta ttcttcttct caagacgtgg ggggaaatta
4560

tctcattatc gaggccctgc tatgctgtgt atctgggcgt gttgtatgtc ctgctgccga
4620

t g c c t t c
4627

<210> 30

<211> 1448

<212> DNA

<213> Homo sapiens

<400> 30

aggacctgaa caagggtgtc ccacccgagg tcgctgtggt tgagccatca gaagcagaga
60

tctcccacac caaaaggcc aactgggtgt gcctggccac aggcttcttc cccgaccacg
120

tggagctgag ctggtgggtg aatgggaagg aggtgcacag tggggtcagc acagacccgc
180

agccccctcaa ggagcagccc gccctcaatg actccagata ctgcctgagc agccgcctga
240

gggtctcggc caccttctgg cagaaccccc gcaaccactt ccgctgtcaa gtccagttct
300

acgggctctc ggagaatgac gagtggaccc aggatagggc caaaccgctc acccagatcg
360

tcagcgccga ggcctgggggt agagcaggtg agtggggcct ggggagatgc ctggaggaga
420

ttaggtgaga ccagctacca gggaaaatgg aaagatccag gtagcagaca agactagatc
480

caaaaagaaa ggaaccagcg cacaccatga aggagaattg ggcacctgtg gttcattctt
540

ctcccagatt ctcagcccaa cagagccaag cagctgggtc ccctttctat gtggcctgtg
600

taactctcat ctgggtggtg ccccccatcc ccctcagtgc tgccacatgc catggattgc
660

aaggacaatg tggctgacat ctgcatggca gaagaaagga ggtgctgggc tgtcagagga
720

agctggtctg ggcctgggag tctgtgccaa ctgcaaactc gactttactt ttaattgcct
780

atgaaaataa ggtctctcat ttattttcct ctccctgctt tctttcagac tgtggcctta
840

cctcgggtaa gtaagccctt ccttttcctc tccctctctc atggttcttg acctagaacc
900

aaggcatgaa gaactcacag acactggagg gtggaggggtg ggagagacca gagctacctg
960

tgcacaggta cccacctgtc cttcctccgt gccaacagtg tcctaccagc aaggggtcct
1020

gtctgccacc atcctctatg agatcctgct aggggaaggcc accctgtatg ctgtgctggt
1080

cagcgcctt gtgttgatgg ccatggtaag caggagggca ggatggggcc agcaggctgg
1140

aggtgacaca ctgacaccaa gcaccagaa gtatagagtc cctgccagga ttggagctgg
1200

gcagtaggga gggaagagat ttcattcagg tgcctcagaa gataacttgc acctctgtag
1260

gatcacagtg gaaggggtcat gctgggaagg agaagctgga gtcaccagaa aaccaatgg
1320

atgtttgtgat gagccttact atttgtgtgg tcaatgggcc ctactacttt ctctcaatcc
1380

tcacaactcc tggctcttaa taacccccaa aactttctct tctgcaggtc aagagaaagg
1440

a t t t c t g a
1448

<210> 31

<211> 1489

<212> DNA

<213> Homo sapiens

<400> 31

aggacctgaa aaacgtgttc ccacccgagg tcgctgtgtt tgagccatca gaagcagaga
60

tctccacac ccaaaaggcc aactgggtat gcctggccac aggcttctac cccgaccacg
120

tggagctgag ctggtgggtg aatgggaagg aggtgcacag tggggtcagc acagaccgcg
180

agcccctcaa ggagcagccc gccctcaatg actccagata ctgcctgagc agccgcctga
240

gggtctcggc caccttctgg cagaaccccc gcaaccactt ccgctgtcaa gtccagttct
300

acgggctctc ggagaatgac gagtggaccc aggatagggc caaacccgtc acccagatcg
360

tcagcgccga 420	ggcctggggt	agagcaggtg	agtggggcct	ggggagatgc	ctggaggaga
ttaggtgaga 480	ccagctacca	gggaaaatgg	aaagatccag	gtagcggaca	agactagatc
cagaagaaag 540	ccagagtgga	caaggtggga	tgatcaaggt	tcacagggtc	agcaaagcac
ggtgtgcact 600	tccccacca	agaagcatag	aggctgaatg	gagcacctca	agctcattct
tccttcagat 660	cctgacacct	tagagctaag	ctttcaagtc	tccctgagga	ccagccatac
agctcagcat 720	ctgagtgggt	tgcattccat	tctcttctgg	ggtcctgggt	tcctaagatc
atagtgacca 780	cttcgctggc	actggagcag	catgaggagg	acagaaccag	ggctatcaaa
ggaggctgac 840	tttgtactat	ctgatatgca	tgtgtttgtg	gcctgtgagt	ctgtgatgta
aggctcaatg 900	tccttacaaa	gcagcattct	ctcatccatt	tttcttcccc	tgttttcttt
cagactgtgg 960	cttcacctcc	ggtaagtgg	tctctccttt	ttctctctat	ctttcgccgt
ctctgctctc 1020	gaaccagggc	atggagaatc	cacggacaca	ggggcgtag	ggaggccaga
gccacctgtg 1080	cacaggtgcc	tacatgctct	gttcttgta	acagagtctt	accagcaagg
ggtcctgtct 1140	gccaccatcc	tctatgagat	cttgctaggg	aaggccacct	tgtatgccgt
gctggtcagt 1200	gccctcgtgc	tgatggccat	ggtaaggagg	aggggtgggat	agggcagatg
atgggggacg 1260	gggatggaac	atcacacatg	ggcataaagg	aatctcagag	ccagagcaca

gcctaataata tcctatcacc tcaatgaaac cataatgaag ccagactggg gagaaaatgc
1320

agggaatatc acagaatgca tcatgggagg atggagacaa ccagcgagcc ctactcaaat
1380

taggcctcag agcccgccctc ccctgcccta ctcttgctgt gccatagccc ctgaaaccct
1440

gaaaatgttc tctcttccac aggtcaagag aaaggattcc agaggctag
1489

<210> 32

<211> 5591

<212> DNA

<213> Homo sapiens

<400> 32

ataaacaact tgatgcagat gtttcccca agcccactat ttttcttcct tcaattgctg
60

aaacaaagct ccagaaggct ggaacatacc tttgtcttct tgagaaattt ttccctgatg
120

ttattaagat acattggcaa gaaaagaaga gcaacacgat tctgggatcc caggagggga
180

acaccatgaa gactaacgac acatacatga aatttagctg gttaacggtg ccagaaaagt
240

cactggacaa agaacacaga tgtatcgtca gacatgagaa taataaaaac ggagttgatc
300

aagaaattat ctttcctcca ataaagacag gtatgtgttt acgcatatca tctgtcagaa
360

cacttctttg aaagtgaatg ctgcattttt tcctttcagt attaatgaaa aacaaacata
420

aatctttctt aaatattggt acatttaatg gtagcataaa tgccctgcta cttttctata
480

gaattaaaat ggtatagggt ttggagaaaa caaaattgaa aaagttactg aaggtttgtc
540

agcctcagct ccattatcca aaataagaaa gtcacgtgct ggTTTTtagg gttgtttagat
600

ggattaaaga aacaacatac acagaagcat ctagcaacgt gacacgtggg aaacgctcaa
660

aaagtgttct cccttctttt gatgacttta cttgatcagg aaataacata tatatgtctt
720

tcaggaatgt tctgccaag caggagagtc actcacctca atcttgctac ccacaaagtt
780

taacctaaaa acaacggggt cattgttgac aaaatgatgt ttatctgttg ttgacagaat
840

gatgtttatc taaaaacagt tccaattttc tatttccttt gctgagacac aaaggggagg
900

caaatgtgca aagcttgagg gtagtcttac cactgtgctt aagtgttctg atttttctag
960

tgatcagggc aaaataaaaa gtatagtaag ttccaaggca gtgaatatta tacaggagag
1020

aagttacagt ttataaatgt gttttccttt acactaaatt ctaaaagtaa aaagtctttt
1080

tttttttttg acagagtttc actcttggtg cccaagcagg tgtgctatgg tatgatctca
1140

gctcactgca acctccacct cccgggttca agtgattctc ttacttcagc ctcccgacag
1200

gctgggattg caggcgcctg ccaccacacc tggctaattt ttgtgttttt agtagagatg
1260

gggtttcacc atgttgggca ggctggtctc aaattcctga cctcaagtga tccatccacc
1320

tcggcctcca agtgctggga ttatgggcgt cagccactgt gccagccta aaagtaaaat
1380

gtctttcatg agcttcccaa ggcagctacg ttaaggagga cacttctctt aatgtcattc
1440

tacagtagat ttctaattgct ctttcttgga agtttgtttt tctgagaaaa gctaaaaata
1500

taacatggaa gtgatcatat tatataatca atgaagtgct tttcaaggag ataaaactaa
1560

tctggtccac acttgcaacc aaccttgatt gagagagaga gagaactcag gatacacttg
1620

aagattttat tatggggaac agttacttta ttctttttac ctcaatcaat gcatggaaat
1680

aagtgatagt catttttcatt tatcttttaa taaatgaagt caccatgagg aaaataaaaa
1740

gacattgaaa acccattaaa gtcagccctt aaagatattt ggacatgcag acttgataac
1800

taacgtttgc attcttgaga cttacccaaa acccatacct caagtccaag tttttagaat
1860

tcatgaaata aagatctcag tgagtgcata aaattgcgca ccagaatcat atccgtatag
1920

acaagaacac atctactaga aaaataataa accaacacac caatgcaact gtgttttctt
1980

ctgttttaaa gtatgttgtc tttgtatgca tgtttgcttc ttcctttttt tttttaacat
2040

cacagataaa ttcaactctc acctcagggt ttattgagag aactgtcaat gtgacttggc
2100

ctctgtcttt ctagtcccag aaagaattgc actgaaatct gagctcctgt aataaaaaca
2160

accatttgct gagagtaatt aacatactga aagagatttt cttagagtac acaatggtga
2220

cattatattg cctctttata aataactttc tatctatttc tgtggattat tcctacaaag
2280

tacttttcat atgtccaatt tcttttcttc ccctacaact actgtctgaa tactggctct
2340

gctatttgct gatatgattc tcggcaagtt gcctgcactt tttaaacttt atttcctcat
2400

tcagaacatg gggccataca taatacaact cacttcagtg ttattgggga attaaacaaa
2460

aaatgcatgg gaagcattta acatagtgcc tgacacaata atgagtactc agtagatggt
2520

agcttttatt aatattgttg ttgttatgtc cagaaacact atacctccag aaaatcatgg
2580

gtacttgctg gggacattgg ggatatgcat gatttggaaa agaatgactg ctttttttgc
2640

ttagatgaga aatttttcta agccagactc cttcaaatat gtaagattct gttgtggatt
2700

caaggactga aagaattctt ggccgagtggt ggtggcttat ccctgtaatc ccagcatttt
2760

gtgaggacaa ggcaggaaga ttgcttgagt ccaggagttt gaaaccagcc tgcgcaacat
2820

ggcgaaaccc tgtctctaca aaaaatacaa acattagctc ggagtgagtg ctgacatgtg
2880

cctgtactcc cagctactca gaaggctgag atgggaggat ctcagtagcc tggggagttt
2940

gaggcttcag tgagccgtga tgacaccgta ctatactcca ctccagcctg ggtgacagtg
3000

agaccctgcc tcaaaaaaca aacaaacaaa caaacaaaac aaaattaatc tttttgctga
3060

tgtcatgtca gcagtgtgtg ttgaaggctg taaagcagcc atttgttcag tttatttttc
3120

cattgaacaa gtatttatca aaacatact ttgtggcagt cactatgcta ggagctatga
3180

atacagaagg aaaagtaaata gctcttggat actacactcc agttgtgata aaaaagaaaa
3240

aatgtattct tcaccaactt caacatcttg atgtgcaaaa acataataca tgaattagat
3300

ctacctaatt acacagaatt agaccaattg tttctggaat tgtgggctca tatttttaat
3360

aactgtcctc ctgcctctct gtcgacaggt ttataaata ttcatttaat tacacacaca
3420

cacacgaaca attgactagt acttgctctc attcttctag atgtcatcac aatggatccc
3480

aaagacaatt gttcaaaaga tgcaaatggg aagcttttgt gtttttccct tcctcctgat
3540

cattttgttt tgaacttctc tggcttgaaa aatcagggaa tggattttgc taggttggat
3600

gctgcagaat ggacctagtg atattttaaa ttagtccctc attttctagg agttgtatta
3660

acaaacctaa ctactgcttt ggggtatgag atgactgtaa attagagagg gtacagtggg
3720

atagtatat gcttttaatt atttcaaaaa aaagatttta ttcattcatg tgtctttttt
3780

ctttttcttt tctttttttt ttttttttgg acagagtctt gctctgtcac ccaggctgga
3840

gtgcggtggc agtatctcag ctcaccacaa cctccgcctc ccggcttcaa gtgattctcc
3900

tgctcagct tctcgagtag ctgggactac aggcgcgtgc caccatgccc ggctaatttt
3960

tgtattttta gtagagttgg ggtttcacca tgttggccag gatggcctcg aatttgtgac
4020

ctcgtgatct gccccctcgc cctcccgaac tgttgggatt acaggcgtga gtcactgtgc
4080

ccggcctcct gtcctgtctt ttgtttaatg actgggaaaa acatgatacc atgttgcttc
4140

tcgagttggtt ttgttttagt ctttggtctt tgctagtagc taataacacg aactagtgtt
4200

tatcaagtgc tttttacaca gaagggcttg ggctgtgttc tgcattttct tgtttaaccc
4260

tcttaaaact cctataaaat ggtacatatt tttctcccaa tttacagtcc ctttaaagca
4320

aataattata aaaatcccta tacatgtcac acagctagat ctgggatttc aaatcaggcc
4380

atcaaacaaa gagtttatgt acttagtaag ttttctgttc tttttctaca atagagtcag
4440

atagcaagaa attaccaagc caggaacctg aaacaaaacg gacatcatgt ggggctgggt
4500

gggtgcatgg gctttgcaga ctggactttc actccagctc ttttaatgat taggtgtaag
4560

tgacctacat tttgtgagca acagttttct catcagccaa caaagaataa ttacaccaga
4620

ttcacagtta ttgaagagat aaaggcatga atgtgagatg tctggcatag ggcattctcat
4680

ttagcagaca cagaatgagt acttgtttct ggctttttct ctctacatat gcacaaagaa
4740

tgcgactaga agcatgggct ctagccctgc tcaactttcc tctattttcca ataccaaggg
4800

gctctgactt aggctgccac accaggcaag gagggcagta ccacctcact tgaccaaggg
4860

cagggagtca cggacacatc acttcttgag atccttttcc acaccaagga ctgatgtttc
4920

tggaattctc actttatgaa gacaaaacat ataaatggaa attttctcag gtagagactc
4980

actcttgtag ctcatagagt aggcactagt ggtccacccc cactgtcttt acttattcct
5040

tgacatcaca tatctcttgc aaaacctcaa ataatatata atgcaatcac ccaataatag
5100

catagccata attagaggca tttaggaaag acagggtgagt gtgccacaac tacctaacac
5160

atcagcaaata ctggattaac cacttttcttt gattttccac aatgcaacct tactttttta
5220

tagttgggaa tgttctaagt gaatttagca gaggttggtta atcaacttga aagctgaatt
5280

ctgacttgtc tgactcttgg tgggtgctggt agcagtagat gtttactttt aggttttggt
5340

ggtggtggaa tatcacttca acgtaaata tcagaaataa gtatttgtga acccctctcg
5400

cattaatgta tcttattctg taaaaagaac atgtgcaatt tctcttagat acactactgc
5460

tgcagctcac aaacacctct gcatattaca tgtacctcct cctgctcctc aagagtgtgg
5520

tctattttgc catcatcacc tgctgtctgc ttagaagaac ggctttctgc tgcaatggag
5580

a g a a a t c a t a a
5591

<210> 33

<211> 9549

<212> DNA

<213> Homo sapiens

<400> 33

ataaacaact tgatgcagat gtttccccca agcccactat ttttcttctt tcgattgctg
60

aaacaaaact ccagaaggct ggaacatacc tttgtcttct tgagaaattt ttcccagata
120

ttattaagat acattggcaa gaaaagaaga gcaacacgat tctgggatcc caggagggga
180

acaccatgaa gactaacgac acatacatga aatttagctg gttaacggtg ccagaagagt
240

cactggacaa agaacacaga tgtatcgtca gacatgagaa taataaaaac ggaattgatc
300

aagaaattat ctttcctcca ataaagacag gtatgtgttt acacatatca tctgtcagaa
360

cacttctttg aaagtgaatg ctgcattttt tcctttcagt attaataaaa aacataaatc
420

tttcttaaaa attgttacat ttaatggtag cgtaaatacc ctgctacttt tctatagaat
480

taaaatggta taggttttgg agaaaacaaa attgaaaaag ttgctgaagg tttgtcagcc
540

tcagctccat tatccaaaat aagaaagtca cgtgctggtt tttagggttg ttagatggat
600

taaagaaaca acatacacag aagcatctag caacgtgaca cgtggtaaac gctcaaaaag
660

tgttctccct tcttttgatg actttacttg atcaggaaat aacatatata tgtctttcag
720

gaatgttctg cccaagcagg agagtcactc acctcaatct tgctaccac aaagtttaac
780

ctaaaaacaa cgggttcatt gttgacaaaa taatgtttat ctgaagataa ctgtagatca
840

tatttatctg tagataatgt ttatctgtgg agtgtggctc tacaaaacat agaatagtct
900

tggtcactgc agttttatag aggccttggg tttttcagag tttcatttta tatatcacca
960

taaagtaaca tttcataatt acaggttggg aaggcttaca tgtacaaaca ttcttccatt
1020

ttccataata aatgcatttc ctgccattgg tgaatgcagc tcaataaaca tttattgtac
1080

aattatgaca cgccaggctt agtggaaatg tggatgaaca gacaaggatg agttactgtc
1140

ctaaggatga tgcacgacag tgcagagaat atactctctt cctgatcact cagggtcact
1200

catgattcat gcgcgaggtc ccaaaacagt gcctttgatg cagattctgt acatctctag
1260

acgattggtc caagggctga atgtgctctg gcccagtggg ccagtctgtc actatatgtc
1320

aacatcctga atatgaacat aacagtccaa catctcaaga gtgggcatga aaaggactca
1380

ttttgtgctt tttcctgtgg ttaacaagtc ctttttagcc tgggggaaca agcattaaca
1440

aaatgtttga agatctttgc cacgtaccat tccaaatttc tagggtaagt ctttagcttt
1500

tcagatcctg agtttctgca atgatcaa atgtatttga cagttgcgtt gactttctcc
1560

tggggctata atggagtgca aaggaaacaa tggcagggaa aatgcttgct ttcaaaatgg
1620

tagcatggat gtgttcattc gtgtagttac tgtattaggt atagcctttc ctgaaactaa
1680

ctgaagtggg gttataaaaa cagtcccaat tttctatttc ctttgctgag acacaaagag
1740

gagacaaaag agcaaagctt gagggtagtt ttaccactgt gcttaagtgt tctgattttt
1800

ccagtgatca ggggtgaaata aaaagcatag taagttccag ggcagtgaat accatacagg
1860

agacaagtta cagttttata atgtgtttta ctttacacta aattctaaaa gtaaaatgtc
1920

tttttttttt tccgagacag agtttcactc ttgtagccca ggcaggagtg ctatggtgtg
1980

atctcggctc acagcaacct ccacctccca gtttcaagcg attcttctgc ctcagcctcc
2040

cgagaagttg aaattacagg tgcctggcac catactctgc taattattct attttttagta
2100

gagatcgggt tttacctatg tggccaggct ggtctcgaac tcctgacttc aagtgatcca
2160

cccgccctcag cctcccaaag tgctgggatt acagggtgtga gtcactgtgc cggacctaac
2220

agtaaaatgt ctttcatgtg cttctcaagg caactacatt aaggaggaca catctcttaa
2280

tgtcattcta cagtagattt ctaatgctct ttcttggaag tttgtttttc tgagaagagc
2340

taaaaatata ataacatgga agtgatcata ttatataatc aatgaagtgc tttcaaagga
2400

gataaaacta acctggtctg catttgcaac cagccttgat tgagagagag agaactcagg
2460

atacacttag agattttatt atggggaata gttactttat tcattttacc tcaatcaatg
2520

catggaaata agtgacagtc attttcatth atcttttaat aaataaagtc accatgagga
2580

aaatgaaaac ccattaaagt cagtccttaa agatatttgg acatgcagac atgataacta
2640

acatttccat tcgtgagact tacccaaaac ctatacctca agtccatttc ttagaataca
2700

tgaaataaag atctcagtga gtgtataaaa ctgcacacca gaatcatatc cgtatagaca
2760

agaatacatc tactagaaaa atataaacca aaacaccaag gtgactctgt ttttttctgt
2820

tttaaaatat gttgtctttg tatgcatggt tgcttcttcc tttttttttt taaacatcgc
2880

agataaattc aactctcacc tcagttgaga gagaactgtc aatgtgactt ggcctctctc
2940

tttctagtcc cagaaagaat tgcactgaaa tgctgagctc ctgtaataaa aatgaccatt
3000

tgctgagagt aattaacata ctgaaagaga ttttcttaga atagtgcaca atggcccaat
3060

ggtgacatta tattgtctct ttataaatta ttttctatct atttctgtgg attatttcta
3120

caaagcactt ttcatatgtc caattccttt tattccccta caagtactga ctgactactg
3180

gctctgctgt tcactgatat gactttcggc aagttgcctg cactttttaa acgttatttc
3240

ctcattcaga acatggggcc atacaaaata caactcactt cagtgttatt ggggaattaa
3300

acaaataaat gcatgggaag catttaacat agtgcctgac acaataatga gcactcagta
3360

gatgttagct tttattaata ttgttggtgc tatgtccaga aacactatac ctccagaaaa
3420

tcattgggtac ttgctgggga cgttggggat atgcatgatt ttgaaaggag tgactgctct
3480

ttactgctca gatgagaaat ttttctaagc cagactcctt caaacatgta agattctggt
3540

gtggattcta ggactgaaag aattcttggc cgagtgtggt ggcttatcct ggtaatctca
3600

tcatttggga ggacaaggca ggaagattgc ttgagcccag gagttggaaa caagcctgga
3660

caacatggcg aaaccctgtc tctacaaaaa atacaaacat tagctgggtca tgggagtgag
3720

tgacctgtact cccagctact caggaggcta agataggagg atcacctgag cctgggcagt
3780

ttgaggtttc agtgagccgt gatgacacca tactatactc cactccagcc tgggtgacag
3840

tgacatcctg cctcaaaaaa acccccaaaa ttattctttt tgctgatttc atgtcagcag
3900

tgtgtgctga aggctgtaaa gtagccactt gttctgttta tttttccatt gaacaagtat
3960

ttatcaaaaa cgtactttgt ggaaggcact gtgctaggaa ctatgcatac agaaggaaaa
4020

ccaaatgttc ttggatacta cactccagtt gtgataaaaa agaaaaaagt attcttcaca
4080

aacttcaaca ttttgatgtg caaaaacata atatatgaat tagatctacc taactacaca
4140

gaattagacc aattatttct gggattatgg gctcatattt ttaataactg tcctcctacc
4200

tctctgttga caggttttat aaatattcat ttaattacac acagtcacag acacactcag
4260

acacacacac atacacacac acacacacct tgacaaataa tgggcatgaa caattgactg
4320

gtacttgctc tcattcttct agatgtcacc acagtggatc ccaaatacaa ttattcaaag
4380

gatgcaaatg gtaagttttt gtgtttttta tttcctcctg atcattttta gttttgaact
4440

tctctggcctt gaaaaatcag ggaatggatt ttgctaggtt ggatgctgca gaatggacct
4500

aatcatattt taaattagtc cctctttttc taggagttgt attaacaac ctaactactg
4560

cttcatgtaa gagatgactg taaattgaag ggtacagtga tatgctttca gttatttcaa
4620

aaaacagact ttactcatcc atgtgtcttt tttcttttct tttttttctt ttttgagacg
4680

gagtctcgct ctgttgaaca ggctggattg cagtgacgcg atctcacctc actacaacct
4740

ccgcctcttg agttcaagcg attctccagc ctcagcttct caagtagctg ggactacagg
4800

cacatgccac catgtccggg tcattcttgt attttttagca gagaccgggt ttcactatgt
4860

tggccaggct ggtctagaat tcctgacttc gtgatctgcc ccctcagccc tccgaagtgc
4920

tgggattaca gacgtgagtc actgtgcccg gcctaacagt aaaatgtctt tcatgcgctt
4980

ctcaaggcaa ctacgttaag gaggacactt ctcttaatgt cattctacag tagatttcta
5040

atgctctttc ttggaagttt gtttttctga gaaaagctaa aaatataaca tggaagtgat
5100

catattgtat aatcaatgaa gtgcttttca aggagataaa actaatctgg tccacgtttg
5160

caaccaacct tgattgagag agagagagaa ctcaggatac acttggagat tttattatgg
5220

ggaatagtta ctttattctt ttttctcaa tcaattcatg gaaataagtg atagtcatat
5280

tcatttatct ttttaataaat gaagtcacca tgaggaaaat aaaaagacat tgaaaaccca
5340

ttaaagttag cccttaaaga tatttggaca tgcagacttg ataactaacg tttgcattct
5400

tgagacttac ccaaaaccca tacctcaagt ccatgttttt agaattcatg aaataaagat
5460

ctcagtgagt gcataaaatt gcgcaccaga atcatatccg tatagacaag aacacatcta
5520

ctagaaaaat aataaaccaa cacaccaatg caactgtgtt ttcttctgtt ttaaaatatg
5580

ttgtctttgt atgcatgttt gcttcttcct tttttttttt taacatcaca gataaattca
5640

actctcacct caggttttat tgagagaact gtcaatgtga cttggcctct gtctttctag
5700

tcccagaaag aatcgcaactg aaatgctgag ctcctgtaat aaaaatgacc atttgctgag
5760

agtaattaac atactgaaag agattttctt agagtacaca atggtgacat tatattgtct
5820

ctttataaat aacttttctat ctattttctgt ggattattcc taaaaagtac ttttcatatg
5880

tccagtttct tttcttcccc tacaactacc gtctgaatac tggctctgct atttgctgat
5940

atgattctcg gcaagttgcc tgcacttttt aaactttatt tcctcattca gaacatgggg
6000

ccatgtaata ctcatgtacg tgagtattac gtaataatgc tcacttaagt gttactgggg
6060

aattaaacaa aaaaatgcat ggcaagcatt taacatagtg cctgacacaa taatgagcac
6120

tcagtagatg ttagatttta ttaatattgt tgttgttatg tccggaaaca ctatacctcc
6180

agaaaatcat gggtagttgc ttgggatggt ggggatatgc atgatttgga aaggatatgac
6240

tgcttttttc tgcttagatg agaaattttt ctaagccaga ctccttcaaa tatgtaagat
6300

tctgttgtgg attctaggac ggaaagaatt cttggtcagg tgtggtttct tatccctgta
6360

atcccagaat tttgggagga caaggcagga agattgcttg agcccaggag tttgaaacca
6420

gcctgggcaa caagacgaaa ccctgtctct acaaaagtac ataaattagc ttggcttggt
6480

gggtgtgtgcc tgtattacca gctattcggg agactgagat gggaggatct cctgaacctg
6540

tgaagtttga ggcttcagtg agccgtgatg acaccatact atactcgact ccagcctgtg
6600

cgacagtgag actctgcgtc aaaaaaaaaa ccccaaaatt attgtttttg ctgatttcag
6660

gtcagcagtg tgtgctgaag ggtgtaaagt agccacttga tcagttttatt tttccactga
6720

acaagtattt atcaaaaaca tactttgtgg tctgtttttg ataaataaaa aggcactgtg
6780

ctaggagcca tgaatacaga aggaaaacca aatgttcttg gatactacac tccagttgtg
6840

ataaaaaaga aaaatgtatt cttcacgaac ttcaacattt tgatatgcaa aaacatagta
6900

tataaattag atctacctga ttacgtagaa tcagaccaat tattttctgga attgagggct
6960

catattttta ataactgtcc tcctgcctct ctgttgacag gttttataaa tattcattta
7020

attacacaca cacacacaca caccttgaca aataatggac atgaacaatt gactagtact
7080

tgctctcatt cttctagatg tcatcacaat ggatcccaaa gacaattggg caaaagatgc
7140

aaatggtaag cttttgtgtt tttcctttcc tcctgatcat ttttaagtttt gaacttctct
7200

ggcttgaaaa atcagggaat gggccgggtg cgggtggctca cgcttgtaat ccagcactt
7260

tgggaggccg aggcgggcg atcacgaggt caggagatcg agaccatccc ggctaaaacg
7320

gtgaaacccc gtctctacta aaaatacaaa aaattagccg ggcttagtgg cgggcgcctg
7380

tagtcccagc tacttgggag gctgaggcag gagaatggcg tgaacccggg aggcggagct
7440

tgcagtgagc cgagattgcg ccactgcact ccactccagc ctgggcgaca gagcgagact
7500

ccgtctcaaa aaaaaaaaaa aaaaaaaaaa aagaaaaatc agggaatgga ttttgctagg
7560

ttggatgctg cagaatggac ctagtgatat tttaaattag tccctctttt tctaggagtt
7620

gtattaacaa acctaactac tgcttcgggt atgagatgac tgtaaattag agggtagagt
7680

gatatgcttt cagttatttc aaaaaacaga ctttattcat ccgtctgtct tttttttttt
7740

tttttttttt tttttttgag acggaggagt ctcactctat caccaggct ggagtgcagt
7800

ggcgcgatct cggctcacca taacctccgc cttactgggt caagcgattc tccagcctca
7860

gcttctcaag tagctgggac tacagggtgca caccaccata cctggctaata ttttgtattt
7920

ttaatagaga tgggggtttca ccacgctggc caggatggtc ttgaattctt gacctcgtga
7980

tctgccccct cgggctccca aacttctggg attataggcg tgagccactg tgcccggcct
8040

tctgtctttt gttataatga ctggggaaaa catgatacca tgttgcttct tgagttgttt
8100

tgtttttagtc tttggtcttt gctagtagct aataacacga actagtgttt atcaagtgct
8160

ttttacacag aagggttgt tctgcatttt ctagtttaata catcttaata ctccataaaa
8220

gtagtacaat atattttctc ccattttaca gtccctttaa agtaaataac tataaaaatc
8280

ccttatacat gtcacacagc taggtctggc atttcaaadc aggacatcaa acaaagaatt
8340

cgtgcagtta ctaagtcctc tattttttct acaatagaaa aaatagcaag aattacagat
8400

agcaagacat tacaaggcag gaatctgaaa cgaaagggac ataatgtggg gctgggtggg
8460

tgcattgagct ttgcagacta gactttcatt ccagctcttt taatgattag gtgtaagtga
8520

cctacatttt gtgagtaaca gttttctcat cagccaacta agaataatta caccagattc
8580

acagttattg aagagataag ggcatgaatg tgagatgtct ggcgtagggt atctcattta
8640

gcagacacag aatgaatact tgtttctggc tttttctctc tacatatgca caaagaatgt
8700

gactagaagc attggctcta gccctgctca actttcctct atttccaata ccaaggggct
8760

ctgacttagg ctgccacacc aggcaaggag gggcagtacc acctcacttg accaagggca
8820

gggagtcacg gacacatcac ttcttgagat ccttttccac accaaggact gatgtttctg
8880

gaattctcac tttatgaaga caaaacatat aaatggaaat ttctgcagga agagactcac
8940

tcttgtagct cattgagtag gcactagtgg tccaccccca ctgtctttac ttattccttg
9000

acatcacata tctcttgtaa aacctcaaat aatgttaaata gcaatcacc aataatagca
9060

tagccataat tagaggcatt taggaaagac aggtgagtgt gccacaacta cctaacacat
9120

cagcaaattc ggattaacca ctttctttga ttttccacaa tgcaacctta ctttttaata
9180

gttggaatg ttctaagtga atttagcaga gggtgttaat caacttgaaa gctgaattct
9240

gacttgtctg actcttggtg gtgctggtag cagtagatgt ttacttttag gttttggtgg
9300

tggtggaata tcacttcaac gtaaatacat agaaataagt atttgtgaac ccctctcgca
9360

ttaatatatc ttattctgta aaaagaacat gtgcaatttc tcttagatac actactgctg
9420

cagctcacia acacctctgc atattacacg tacctcctcc tgctcctcaa gagtggtggtc
9480

tattttgcca tcatcacctg ctgtctgctt agaagaacgg ctttctgctg caatggagag
9540

a a a t c a t a a
9549

<210> 34

<211> 182

<212> PRT

<213> Homo sapiens

<400> 34

Met Glu Gln Gly Lys Gly Leu Ala Val Leu Ile Leu Ala Ile Ile Leu
1 5 10 15

Leu Gln Gly Thr Leu Ala Gln Ser Ile Lys Gly Asn His Leu Val Lys
20 25 30

Val Tyr Asp Tyr Gln Glu Asp Gly Ser Val Leu Leu Thr Cys Asp Ala
35 40 45

Glu Ala Lys Asn Ile Thr Trp Phe Lys Asp Gly Lys Met Ile Gly Phe
50 55 60

Leu Thr Glu Asp Lys Lys Lys Trp Asn Leu Gly Ser Asn Ala Lys Asp
65 70 75 80

Pro Arg Gly Met Tyr Gln Cys Lys Gly Ser Gln Asn Lys Ser Lys Pro
85 90 95

Leu Gln Val Tyr Tyr Arg Met Cys Gln Asn Cys Ile Glu Leu Asn Ala
100 105 110

Ala Thr Ile Ser Gly Phe Leu Phe Ala Glu Ile Val Ser Ile Phe Val
115 120 125

Leu Ala Val Gly Val Tyr Phe Ile Ala Gly Gln Asp Gly Val Arg Gln
130 135 140

Ser Arg Ala Ser Asp Lys Gln Thr Leu Leu Pro Asn Asp Gln Leu Tyr
145 150 155 160

Gln Pro Leu Lys Asp Arg Glu Asp Asp Gln Tyr Ser His Leu Gln Gly
165 170 175

Asn Gln Leu Arg Arg Asn
180

<210> 35

<211> 171

<212> PRT

<213> Homo sapiens

<400> 35

Met Glu His Ser Thr Phe Leu Ser Gly Leu Val Leu Ala Thr Leu Leu
1 5 10 15

Ser Gln Val Ser Pro Phe Lys Ile Pro Ile Glu Glu Leu Glu Asp Arg
20 25 30

Val Phe Val Asn Cys Asn Thr Ser Ile Thr Trp Val Glu Gly Thr Val
35 40 45

Gly Thr Leu Leu Ser Asp Ile Thr Arg Leu Asp Leu Gly Lys Arg Ile
50 55 60

Leu Asp Pro Arg Gly Ile Tyr Arg Cys Asn Gly Thr Asp Ile Tyr Lys
65 70 75 80

Asp Lys Glu Ser Thr Val Gln Val His Tyr Arg Met Cys Gln Ser Cys
85 90 95

Val Glu Leu Asp Pro Ala Thr Val Ala Gly Ile Ile Val Thr Asp Val
100 105 110

Ile Ala Thr Leu Leu Leu Ala Leu Gly Val Phe Cys Phe Ala Gly His
115 120 125

Glu Thr Gly Arg Leu Ser Gly Ala Ala Asp Thr Gln Ala Leu Leu Arg
130 135 140

Asn Asp Gln Val Tyr Gln Pro Leu Arg Asp Arg Asp Asp Ala Gln Tyr
145 150 155 160

Ser His Leu Gly Gly Asn Trp Ala Arg Asn Lys
165 170

<210> 36

<211> 127

<212> PRT

<213> Homo sapiens

<400> 36

Met Glu His Ser Thr Phe Leu Ser Gly Leu Val Leu Ala Thr Leu Leu
1 5 10 15

Ser Gln Val Ser Pro Phe Lys Ile Pro Ile Glu Glu Leu Glu Asp Arg
20 25 30

Val Phe Val Asn Cys Asn Thr Ser Ile Thr Trp Val Glu Gly Thr Val
35 40 45

Gly Thr Leu Leu Ser Asp Ile Thr Arg Leu Asp Leu Gly Lys Arg Ile
50 55 60

Leu Asp Pro Arg Gly Ile Tyr Arg Cys Asn Gly Thr Asp Ile Tyr Lys
65 70 75 80

Asp Lys Glu Ser Thr Val Gln Val His Tyr Arg Thr Ala Asp Thr Gln
85 90 95

Ala Leu Leu Arg Asn Asp Gln Val Tyr Gln Pro Leu Arg Asp Arg Asp
100 105 110

Asp Ala Gln Tyr Ser His Leu Gly Gly Asn Trp Ala Arg Asn Lys
115 120 125

<210> 37

<211> 207

<212> PRT

<213> Homo sapiens

<400> 37

Met Gln Ser Gly Thr His Trp Arg Val Leu Gly Leu Cys Leu Leu Ser
1 5 10 15

Val Gly Val Trp Gly Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr
20 25 30

Gln Thr Pro Tyr Lys Val Ser Ile Ser Gly Thr Thr Val Ile Leu Thr

35

40

45

Cys Pro Gln Tyr Pro Gly Ser Glu Ile Leu Trp Gln His Asn Asp Lys
50 55 60

Asn Ile Gly Gly Asp Glu Asp Asp Lys Asn Ile Gly Ser Asp Glu Asp
65 70 75 80

His Leu Ser Leu Lys Glu Phe Ser Glu Leu Glu Gln Ser Gly Tyr Tyr
85 90 95

Val Cys Tyr Pro Arg Gly Ser Lys Pro Glu Asp Ala Asn Phe Tyr Leu
100 105 110

Tyr Leu Arg Ala Arg Val Cys Glu Asn Cys Met Glu Met Asp Val Met
115 120 125

Ser Val Ala Thr Ile Val Ile Val Asp Ile Cys Ile Thr Gly Gly Leu
130 135 140

Leu Leu Leu Val Tyr Tyr Trp Ser Lys Asn Arg Lys Ala Lys Ala Lys
145 150 155 160

Pro Val Thr Arg Gly Ala Gly Ala Gly Gly Arg Gln Arg Gly Gln Asn
165 170 175

Lys Glu Arg Pro Pro Pro Val Pro Asn Pro Asp Tyr Glu Pro Ile Arg
180 185 190

Lys Gly Gln Arg Asp Leu Tyr Ser Gly Leu Asn Gln Arg Arg Ile
195 200 205

<210> 38

<211> 140

<212> PRT

<213> Homo sapiens

<400> 38

Ile Gln Asn Pro Asp Pro Ala Val Tyr Gln Leu Arg Asp Ser Lys Ser
1 5 10 15

Ser Asp Lys Ser Val Cys Leu Phe Thr Asp Phe Asp Ser Gln Thr Asn
20 25 30

Val Ser Gln Ser Lys Asp Ser Asp Val Tyr Ile Thr Asp Lys Thr Val
35 40 45

Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn Ser Ala Val Ala Trp
50 55 60

Ser Asn Lys Ser Asp Phe Ala Cys Ala Asn Ala Phe Asn Asn Ser Ile
65 70 75 80

Ile Pro Glu Asp Thr Phe Phe Pro Ser Pro Glu Ser Ser Cys Asp Val
85 90 95

Lys Leu Val Glu Lys Ser Phe Glu Thr Asp Thr Asn Leu Asn Phe Gln
100 105 110

Asn Leu Ser Val Ile Gly Phe Arg Ile Leu Leu Leu Lys Val Ala Gly
115 120 125

Phe Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
130 135 140

<210> 39

<211> 178

<212> PRT

<213> Homo sapiens

<400> 39

Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser
1 5 10 15

Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala
20 25 30

Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly
35 40 45

Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys Glu
50 55 60

Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg
65 70 75 80

Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln
85 90 95

Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg
100 105 110

Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala
115 120 125

Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln Gly Val Leu Ser Ala
130 135 140

Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val
145 150 155 160

Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Ser
165 170 175

Arg Gly

<210> 40

<211> 176

<212> PRT

<213> Homo sapiens

<400> 40

Asp Leu Asn Lys Val Phe Pro Pro Glu Val Ala Val Phe Glu Pro Ser
1 5 10 15

Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr Leu Val Cys Leu Ala
20 25 30

Thr Gly Phe Phe Pro Asp His Val Glu Leu Ser Trp Trp Val Asn Gly
35 40 45

Lys Glu Val His Ser Gly Val Ser Thr Asp Pro Gln Pro Leu Lys Glu
50 55 60

Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu Ser Ser Arg Leu Arg
65 70 75 80

Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn His Phe Arg Cys Gln
85 90 95

Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu Trp Thr Gln Asp Arg
100 105 110

Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu Ala Trp Gly Arg Ala
115 120 125

Asp Cys Gly Phe Thr Ser Val Ser Tyr Gln Gln Gly Val Leu Ser Ala
130 135 140

Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala Thr Leu Tyr Ala Val
145 150 155 160

Leu Val Ser Ala Leu Val Leu Met Ala Met Val Lys Arg Lys Asp Phe
165 170 175

<210> 41

<211> 153

<212> PRT

<213> Homo sapiens

<400> 41

Ser Gln Pro His Thr Lys Pro Ser Val Phe Val Met Lys Asn Gly Thr
1 5 10 15

Asn Val Ala Cys Leu Val Lys Glu Phe Tyr Pro Lys Asp Ile Arg Ile
20 25 30

Asn Leu Val Ser Ser Lys Lys Ile Thr Glu Phe Asp Pro Ala Ile Val
35 40 45

Ile Ser Pro Ser Gly Lys Tyr Asn Ala Val Lys Leu Gly Lys Tyr Glu
50 55 60

Asp Ser Asn Ser Val Thr Cys Ser Val Gln His Asp Asn Lys Thr Val
65 70 75 80

His Ser Thr Asp Phe Glu Val Lys Thr Asp Ser Thr Asp His Val Lys
85 90 95

Pro Lys Glu Thr Glu Asn Thr Lys Gln Pro Ser Lys Ser Cys His Lys
100 105 110

Pro Lys Ala Ile Val His Thr Glu Lys Val Asn Met Met Ser Leu Thr
115 120 125

Val Leu Gly Leu Arg Met Leu Phe Ala Lys Thr Val Ala Val Asn Phe
130 135 140

Leu Leu Thr Ala Lys Leu Phe Phe Leu
145 150

<210> 42

<211> 173

<212> PRT

<213> Homo sapiens

<400> 42

Asp Lys Gln Leu Asp Ala Asp Val Ser Pro Lys Pro Thr Ile Phe Leu
1 5 10 15

Pro Ser Ile Ala Glu Thr Lys Leu Gln Lys Ala Gly Thr Tyr Leu Cys
20 25 30

Leu Leu Glu Lys Phe Phe Pro Asp Val Ile Lys Ile His Trp Gln Glu
35 40 45

Lys Lys Ser Asn Thr Ile Leu Gly Ser Gln Glu Gly Asn Thr Met Lys
50 55 60

Thr Asn Asp Thr Tyr Met Lys Phe Ser Trp Leu Thr Val Pro Glu Lys
65 70 75 80

Ser Leu Asp Lys Glu His Arg Cys Ile Val Arg His Glu Asn Asn Lys
85 90 95

Asn Gly Val Asp Gln Glu Ile Ile Phe Pro Pro Ile Lys Thr Asp Val
100 105 110

Ile Thr Met Asp Pro Lys Asp Asn Cys Ser Lys Asp Ala Asn Asp Thr

115

120

125

Leu Leu Leu Gln Leu Thr Asn Thr Ser Ala Tyr Tyr Met Tyr Leu Leu
 130 135 140

Leu Leu Leu Lys Ser Val Val Tyr Phe Ala Ile Ile Thr Cys Cys Leu
 145 150 155 160

Leu Arg Arg Thr Ala Phe Cys Cys Asn Gly Glu Lys Ser
 165 170

<210> 43

<211> 189

<212> PRT

<213> Homo sapiens

<400> 43

Asp Lys Gln Leu Asp Ala Asp Val Ser Pro Lys Pro Thr Ile Phe Leu
 1 5 10 15

Pro Ser Ile Ala Glu Thr Lys Leu Gln Lys Ala Gly Thr Tyr Leu Cys
 20 25 30

Leu Leu Glu Lys Phe Phe Pro Asp Ile Ile Lys Ile His Trp Gln Glu
 35 40 45

Lys Lys Ser Asn Thr Ile Leu Gly Ser Gln Glu Gly Asn Thr Met Lys
 50 55 60

Thr Asn Asp Thr Tyr Met Lys Phe Ser Trp Leu Thr Val Pro Glu Glu
 65 70 75 80

Ser Leu Asp Lys Glu His Arg Cys Ile Val Arg His Glu Asn Asn Lys
 85 90 95

Asn	Gly	Ile	Asp	Gln	Glu	Ile	Ile	Phe	Pro	Pro	Ile	Lys	Thr	Asp	Val
			100					105					110		
Thr	Thr	Val	Asp	Pro	Lys	Tyr	Asn	Tyr	Ser	Lys	Asp	Ala	Asn	Asp	Val
		115					120					125			
Ile	Thr	Met	Asp	Pro	Lys	Asp	Asn	Trp	Ser	Lys	Asp	Ala	Asn	Asp	Thr
	130					135					140				
Leu	Leu	Leu	Gln	Leu	Thr	Asn	Thr	Ser	Ala	Tyr	Tyr	Thr	Tyr	Leu	Leu
145					150					155				160	
Leu	Leu	Leu	Lys	Ser	Val	Val	Tyr	Phe	Ala	Ile	Ile	Thr	Cys	Cys	Leu
			165					170					175		
Leu	Arg	Arg	Thr	Ala	Phe	Cys	Cys	Asn	Gly	Glu	Lys	Ser			
			180					185							
<210> 44															
<211> 120															
<212> PRT															
<213> Artificial Sequence															
<220>															
<223> Description of Artificial Sequence: Synthetic polypeptide															
<400> 44															
Glu	Val	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Arg	Pro	Gly	Ser
1				5					10					15	
Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ala	Phe	Ser	Ser	Tyr
			20					25					30		
Trp	Met	Asn	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
	35					40						45			

Gly Gln Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
50 55 60

Lys Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Gly Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Lys Thr Ile Ser Ser Val Val Asp Phe Tyr Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val
115 120

<210> 45

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 45

Asp Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Pro Leu Ile
35 40 45

Tyr Ser Ala Thr Tyr Arg Asn Ser Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65 70 75 80

Lys Asp Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Arg Tyr Pro Tyr
85 90 95

Thr Ser Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 46

<211> 16

<212> PRT

<213> Homo sapiens

<400> 46

Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Ser Ser Ala Tyr Ser
1 5 10 15

<210> 47

<211> 30

<212> PRT

<213> Homo sapiens

<400> 47

Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu
1 5 10 15

Leu Val Val Ser Asn Leu Leu Leu Cys Gln Gly Val Val Ser
20 25 30

<210> 48

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

peptide

<400> 48

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15