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(54) Titre : BIOMARQUEURS ET TRAITEMENTS A BASE DE CELLULES CAR-T AYANT UNE EFFICACITE ACCRUE
(54) Title: BIOMARKERS AND CAR T CELL THERAPIES WITH ENHANCED EFFICACY

(57) **Abrégé/Abstract:**

The invention provides compositions and methods improved CAR T cell therapies. Specifically, the invention provides cells with altered expression and/or function of one or more genes, e.g., associated with Tet2, and methods of use therefore. The invention further provides inhibitors of the one or more genes and methods of use therefore in connection with CAR T cells.

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(54) Title: BIOMARKERS AND CAR T CELL THERAPIES WITH ENHANCED EFFICACY

(57) Abstract: The invention provides compositions and methods improved CAR T cell therapies. Specifically, the invention provides cells with altered expression and/or function of one or more genes, e.g., associated with Tet2, and methods of use therefore. The invention further provides inhibitors of the one or more genes and methods of use therefore in connection with CAR T cells.

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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JUMBO APPLICATIONS/PATENTS

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BIOMARKERS AND CAR T CELL THERAPIES WITH ENHANCED EFFICACY

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 62/474,991, filed March 22,
5 2017, and U.S. Application Serial No. 62/621,356, filed January 24, 2018. The contents of the
aforementioned applications are incorporated herein by reference in their entireties.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically
10 in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on
March 20, 2018, is named N2067-7125WO_SL.txt and is 509,059 bytes in size.

FIELD OF THE INVENTION

The present invention relates generally to the use of immune effector cells (e.g., T cells, NK
15 cells) engineered to express a Chimeric Antigen Receptor (CAR) to treat a disease associated with
expression of a tumor antigen.

BACKGROUND OF THE INVENTION

Adoptive cell transfer (ACT) therapy with autologous T-cells, especially with T-cells
20 transduced with Chimeric Antigen Receptors (CARs), has shown promise in hematologic cancer
trials. There is a medical need for T cell therapies, especially CAR T cell therapies with improved
efficacy.

SUMMARY OF THE INVENTION

25 The present invention provides, at least in part, compositions and methods that disrupt one or
more genes associated with a methylcytosine dioxygenase gene, e.g., Tet2, and uses of such
compositions and methods for increasing the functional activities of engineered cells (e.g., gene-
modified antigen-specific T cells, such as CAR T cells). In particular, the present invention provides
methods and compositions for bolstering the therapeutic efficacy of chimeric antigen receptor (CAR)
30 T cells. While not to be bound by the theory, it is believed that in certain embodiments, alteration of
one or more genes described herein can lead to, e.g., central memory phenotype, and thereby
increases CAR T cell proliferation and/or function.

Accordingly, in an aspect, the present invention provides a cell (e.g., a population of cells),
e.g., an immune effector cell, expressing a chimeric antigen receptor (CAR), wherein the CAR
35 comprises an antigen-binding domain, a transmembrane domain, and an intracellular signaling
domain, and wherein the cell has altered expression and/or function of a Tet2-associated gene (e.g.,
one or more Tet2-associated genes).

In some embodiments, the cell has reduced or eliminated expression and/or function of a Tet2-associated gene. In some embodiments, the cell has increased or activated expression and/or function of a Tet2-associated gene. In some embodiments, the cell has reduced or eliminated expression and/or function of a first Tet2-associated gene, and increased or activated expression and/or function of a second Tet2-associated gene. In some embodiments, the cell further has reduced or eliminated expression and/or function of Tet2.

In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

In one embodiment, the Tet2-associated gene comprises IFNG. In one embodiment, the Tet2-associated gene comprises NOTCH2. In one embodiment, the Tet2-associated gene comprises CD28. In one embodiment, the Tet2-associated gene comprises ICOS. In one embodiment, the Tet2-associated gene comprises IL2RA. In one embodiment, the Tet2-associated gene comprises PRDM1.

In one embodiment, the Tet2-associated gene comprises IFNG and NOTCH2. In one embodiment, the Tet2-associated gene comprises IFNG and CD28. In one embodiment, the Tet2-associated gene comprises IFNG and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2 and CD28. In one embodiment, the Tet2-associated gene comprises NOTCH2 and ICOS. In one embodiment, the Tet2-associated gene comprises NOTCH2 and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2 and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28 and ICOS. In one embodiment, the Tet2-associated gene comprises CD28 and IL2RA. In one embodiment, the Tet2-associated gene comprises CD28 and PRDM1. In one embodiment, the Tet2-associated gene comprises ICOS and IL2RA. In one embodiment, the Tet2-associated gene comprises ICOS and PRDM1. In one embodiment, the Tet2-associated gene comprises IL2RA and PRDM1.

In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and CD28. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, and ICOS. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2,

CD28, and, PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises ICOS, IL2RA, and PRDM1.

In one embodiment, the Tet2-associated gene comprises CD28, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and ICOS.

In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS, and IL2RA. In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, ICOS, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG, CD28, ICOS, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, IL2RA, and PRDM1.

In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS, IL2RA, and PRDM1.

In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8. In some embodiments, the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8, Column B. In some embodiments, the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8, Column A.

In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D. In some embodiments, the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D. In some embodiments, the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D.

In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes in a pathway (e.g., one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pathways) chosen from Table 9, Column A. In some embodiments, the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column A. In some embodiments, the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column A.

In some embodiments, the pathway is chosen from one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all) of: (1) a leukocyte differentiation pathway; (2) a pathway of positive regulation of immune system process; (3) a transmembrane receptor protein tyrosine kinase signaling pathway; (4) a pathway of regulation of anatomical structure morphogenesis; (5) a pathway of TNFA signaling via NFkB; (6) a pathway of positive regulation of hydrolase activity; (7) a wound healing pathway; (8) an alpha-beta T cell activation pathway; (9) a pathway of regulation of cellular component movement; (10) an inflammatory response pathway; (11) a myeloid cell differentiation pathway; (12) a cytokine production pathway; (13) a pathway of downregulation in UV response; (14) a pathway of negative regulation of multicellular organismal process; (15) a blood vessel morphogenesis pathway; (16) a NFAT-dependent transcription pathway; (17) a pathway of positive regulation of apoptotic process; (18) a hypoxia pathway; (19) a pathway of upregulation by KRAS signaling; or (20) a pathway of stress-activated protein kinase signaling cascade.

In some embodiments, the one or more genes associated with a leukocyte differentiation pathway are chosen from Table 9, Row 1. In some embodiments, the one or more genes associated with a pathway of positive regulation of immune system process are chosen from Table 9, Row 56. In some embodiments, the one or more genes associated with a transmembrane receptor protein tyrosine kinase signaling pathway are chosen from Table 9, Row 85. In some embodiments, the one or more genes associated with a pathway of regulation of anatomical structure morphogenesis are chosen from Table 9, Row 128. In some embodiments, the one or more genes associated with a pathway of TNFA signaling via NFkB are chosen from Table 9, Row 134. In some embodiments, the one or more genes associated with a pathway of positive regulation of hydrolase activity are chosen from Table 9, Row 137. In some embodiments, the one or more genes associated with a wound healing pathway are chosen from Table 9, Row 141. In some embodiments, the one or more genes associated with a alpha-beta T cell activation pathway are chosen from Table 9, Row 149. In some embodiments, the one or more genes associated with a pathway of regulation of cellular component movement are

chosen from Table 9, Row 180. In some embodiments, the one or more genes associated with an inflammatory response pathway are chosen from Table 9, Row 197. In some embodiments, the one or more genes associated with a myeloid cell differentiation pathway are chosen from Table 9, Row 206. In some embodiments, the one or more genes associated with a cytokine production pathway are chosen from Table 9, Row 221. In some embodiments, the one or more genes associated with a pathway of downregulation in UV response are chosen from Table 9, Row 233. In some embodiments, the one or more genes associated with a pathway of negative regulation of multicellular organismal process are chosen from Table 9, Row 235. In some embodiments, the one or more genes associated with a blood vessel morphogenesis pathway are chosen from Table 9, Row 237. In some embodiments, the one or more genes associated with a NFAT-dependent transcription pathway are chosen from Table 9, Row 243. In some embodiments, the one or more genes associated with a pathway of positive regulation of apoptotic process are chosen from Table 9, Row 250. In some embodiments, the one or more genes associated with a hypoxia pathway are chosen from Table 9, Row 256. In some embodiments, the one or more genes associated with a pathway of upregulation by KRAS signaling are chosen from Table 9, Row 258. In some embodiments, the one or more genes associated with a pathway of stress-activated protein kinase signaling cascade are chosen from Table 9, Row 260.

In some embodiments, the Tet2-associated gene comprises a gene (e.g., one or more genes) associated with a central memory phenotype. In some embodiments, the central memory phenotype is a central memory T cell phenotype. In some embodiments, the central memory phenotype comprises a higher expression level of CCR7 and/or CD45RO, compared to the expression level of CCR7 and/or CD45RO in a naïve cell (e.g., a naïve T cell). In some embodiments, the central memory phenotype comprises a lower expression level of CD45RA, compared to the expression level of CD45RA in a naïve cell (e.g., a naïve T cell). In some embodiments, the central memory phenotype comprises enhanced antigen-dependent proliferation of the cell. In some embodiments, the central memory phenotype comprises a reduced expression level of IFN- γ and/or CD107a, e.g., when the cell is activated with an anti-CD3 or anti-CD28 antibody.

In some embodiments, the cell comprises a modulator (e.g., an inhibitor or an activator) of the Tet2-associated gene.

In some embodiments, the modulator (e.g., inhibitor or activator) is (1) a gene editing system targeted to one or more sites within the Tet2-associated gene or a regulatory element thereof; (2) a nucleic acid encoding one or more components of said gene editing system; or (3) a combination thereof. In some embodiments, the gene editing system is selected from the group consisting of: a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, and a meganuclease system. In some embodiments, the gene editing system binds to a target sequence in an early exon or intron of the Tet2-associated gene. In some embodiments, the gene editing system binds a target sequence of the Tet2-associated gene, and the target sequence is upstream of exon 4, e.g., in exon 1, exon 2, or

exon 3. In some embodiments, the gene editing system binds to a target sequence in a late exon or intron of the Tet2-associated gene. In some embodiments, the gene editing system binds a target sequence of the Tet2-associated gene, and the target sequence is downstream of a preantepenultimate exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon. In some embodiments, the gene editing system is a CRISPR/Cas system comprising a gRNA molecule comprising a targeting sequence which hybridizes to a target sequence of the Tet2-associated gene.

In some embodiments, the modulator (e.g., inhibitor) is an siRNA or shRNA specific for the Tet2-associated gene, or nucleic acid encoding said siRNA or shRNA. In some embodiments, the siRNA or shRNA comprises a sequence complementary to a sequence of an mRNA of the Tet2-associated gene.

In some embodiments, the modulator (e.g., inhibitor or activator) is a small molecule.

In some embodiments, the modulator (e.g., inhibitor or activator) is a protein. In some embodiments, the modulator (e.g., inhibitor) is a dominant negative binding partner of a protein encoded by the Tet2-associated gene, or a nucleic acid encoding said dominant negative binding partner. In some embodiments, the modulator (e.g., inhibitor) is a dominant negative (e.g., catalytically inactive) variant of a protein encoded by the Tet2-associated gene, or a nucleic acid encoding said dominant negative variant.

In some embodiments, the cell comprises an inhibitor of a first Tet2-associated gene and an activator of a second Tet2-associated gene. In some embodiments, the cell further comprises an inhibitor of Tet2.

In an aspect, the present invention provides a cell (e.g., a population of cells), e.g., an immune effector cell, expressing a chimeric antigen receptor (CAR), e.g., a CAR-expressing cell, wherein the CAR comprises an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, and wherein the CAR-expressing cell has a disruption of Tet2, e.g., altered expression and/or function of Tet2.

In some embodiments, a CAR-expressing cell with a disruption in Tet2, e.g., as described herein, has one, two, three, four or more (e.g., all) of the following characteristics:

(i) increased expansion potential, e.g., at least 1.5, 2, 3, 4, 5, or 6 fold expansion as measured by an assay of Example 1;

(ii) one or more properties of short lived memory T cells, e.g., increased expression of EOMES, decreased expression of KLRG1, increase cytotoxic activity, or increased memory T cell potential as measured by an assay of Example 1;

(iii) increased effector function, e.g., increased degranulation of CD107a, granzyme B and perforin as measured by an assay of Example 1;

(iv) increased cytolytic activity as measured by an assay of Example 1; or

(v) increased proliferative capacity, e.g., as measured by increased Ki67, as measured by an assay of Example 1, compared to an otherwise identical or similar CAR-expressing cell with non-disrupted Tet2, e.g., wild type Tet2.

In some embodiments, the CAR-expressing cell with a disruption of Tet2 has a monoallelic
5 disruption of Tet2, e.g., the cell has one allele of Tet2 that is disrupted (e.g., as described herein), and a wild type Tet2 allele.

In some embodiments, the CAR-expressing cell with a disruption of Tet2 has a biallelic disruption of Tet2, e.g., the cell has two alleles of Tet2 that are disrupted (e.g., as described herein).

In some embodiments, the disruption of Tet2 in the immune effector cell or CAR-expressing
10 cell is produced by a mutation that alters, e.g., reduces, the function of Tet2, e.g., a hypomorphic mutation, e.g., an E1879Q mutation as described herein. In some embodiments, the hypomorphic mutation in Tet2, e.g., E1879Q, results in a Tet2 protein that has reduced function compared to a Tet2 protein produced by a wild type Tet2 allele, as described in an assay of Example 1.

In some embodiments, the disruption of Tet2 in the immune effector cell or CAR-expressing
15 cell is produced by lentiviral integration, e.g., integration of a lentivirus encoding a CAR molecule, in the Tet2 gene, e.g., in the promoter, introns or exons of the Tet2 gene, e.g., as described in Example 1.

In some embodiments, Tet2 disruption, e.g., as described herein, is produced in the immune effector cell population of CAR-expressing cell population by contacting the cell population with a Tet2 inhibitor, e.g., a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a
20 lentivirus encoding a CAR molecule as described herein); a dominant negative Tet2 isoform, or a nucleic acid encoding said dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

In some embodiments, Tet2 disruption produced by any of the methods disclosed herein can be monoallelic or biallelic. In some embodiments, a Tet2 disruption produced in a cell by any of the
25 methods disclosed herein is monoallelic, e.g., the cell has one disrupted Tet2 allele and one wild type Tet2 allele. In some embodiments, a Tet2 disruption produced in a cell by any of the methods disclosed herein is biallelic, e.g., the cell has two disrupted Tet2 alleles, e.g., two different disruptions, e.g., as described herein.

In some embodiments, a Tet2 disruption is present in the immune effector cell population,
30 e.g., prior to expression of a CAR molecule. In some embodiments, an immune effector cell population comprises a Tet2 disrupted allele, e.g., a monoallelic Tet2 disruption as described herein, e.g., a monoallelic hypomorphic Tet2 allele.

In some embodiments, an immune effector cell population comprising a Tet2 disrupted allele, e.g., a hypomorphic Tet2 allele, is contacted with a Tet2 inhibitor, e.g., a small molecule inhibitor of
35 Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein); a dominant negative Tet2 isoform, or a nucleic acid encoding said dominant negative Tet2;

an RNAi agent targeting Tet2; a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2, thereby disrupting the wild type allele of Tet2 resulting in, e.g., biallelic disruption of Tet2.

In some embodiments of any of the compositions disclosed herein, the antigen-binding
 5 domain binds to a tumor antigen selected from a group consisting of: TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII, GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX,
 10 LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, MAGE-A1, legumain, HPV E6,E7, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1,
 15 p53, p53 mutant, prostein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2,
 20 CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, and IGLL1.

In some embodiments of any of the compositions disclosed herein, the tumor antigen is CD19.

In some embodiments of any of the compositions disclosed herein, the antigen-binding domain is an antibody or antibody fragment as described in, e.g., WO2012/079000 or
 25 WO2014/153270. In some embodiments, the transmembrane domain comprises: an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO: 12, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 12; or the sequence of SEQ ID NO: 12. In some embodiments, the antigen binding domain is connected to the transmembrane domain by a hinge region, wherein said hinge
 30 region comprises SEQ ID NO: 2 or SEQ ID NO: 6, or a sequence with 95-99% identity thereof.

In some embodiments of any of the compositions disclosed herein, the intracellular signaling domain comprises a primary signaling domain and/or a costimulatory signaling domain, wherein the primary signaling domain comprises a functional signaling domain of a protein chosen from CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, common FcR gamma (FCER1G), FcR beta (Fc Epsilon
 35 R1b), CD79a, CD79b, Fcgamma RIIa, DAP10, or DAP12.

In some embodiments of any of the compositions disclosed herein, the primary signaling domain comprises: an amino acid sequence having at least one, two or three modifications but not

more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20; or the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20.

In some embodiments of any of the compositions disclosed herein, the intracellular signaling domain comprises a costimulatory signaling domain, or a primary signaling domain and a costimulatory signaling domain, wherein the costimulatory signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFTR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, and NKG2D.

In some embodiments of any of the compositions disclosed herein, the costimulatory signaling domain comprises an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 16, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 16. In some embodiments, the costimulatory signaling domain comprises a sequence of SEQ ID NO: 14 or SEQ ID NO: 16. In some embodiments, the intracellular domain comprises the sequence of SEQ ID NO: 14 or SEQ ID NO: 16, and the sequence of SEQ ID NO: 18 or SEQ ID NO: 20, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In some embodiments, the cell further comprises a leader sequence comprises the sequence of SEQ ID NO: 2.

In some embodiments, the cell is an immune effector cell (e.g., a population of immune effector cells). In some embodiments, the immune effector cell is a T cell or an NK cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the T cell is a CD4+ T cell, a CD8+ T cell, or a combination thereof. In some embodiments, the cell is a human cell.

In some embodiments, the cell comprises an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

In some embodiments, the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is (1) a gene editing system targeted to one or more sites within an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene or a regulatory element thereof; (2) a nucleic acid encoding one or more components of said gene editing system; or (3) a combination thereof. In some embodiments, the

gene editing system is selected from the group consisting of: a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, and a meganuclease system. In some embodiments, the gene editing system binds to a target sequence in an early exon or intron of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene. In some embodiments, the gene editing system binds a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, and the target sequence is upstream of exon 4, e.g., in exon1, exon2, or exon3, e.g. in exon 3. In some embodiments, the gene editing system binds to a target sequence in a late exon or intron of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene. In some embodiments, the gene editing system binds a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, and the target sequence is downstream of a preantepenultimate exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon. In some embodiments, the gene editing system is a CRISPR/Cas system comprising a gRNA molecule comprising a targeting sequence which hybridizes to a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

In some embodiments, the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is an siRNA or shRNA specific for IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1, or nucleic acid encoding said siRNA or shRNA. In some embodiments, the siRNA or shRNA comprises a sequence complementary to a sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 mRNA.

In some embodiments, the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a small molecule.

In some embodiments, the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a protein, e.g., is a dominant negative binding partner of a protein encoded by an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, or a nucleic acid encoding said dominant negative binding partner. In some embodiments, the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a protein, e.g., is a dominant negative (e.g., catalytically inactive) variant of a protein encoded by an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, or a nucleic acid encoding said dominant negative variant.

In another aspect, the present invention provides a method of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell described herein, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of altering (e.g., decreasing or increasing) expression and/or function of a Tet2-associated gene (e.g., one or more Tet2-associated genes) in said cell, wherein the Tet2-associated gene is chosen from one or more (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the method comprises altering (e.g., decreasing) expression and/or function of one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the method further comprises altering (e.g., decreasing) expression and/or function of Tet2.

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In another aspect, the present invention provides a method of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell described herein, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of contacting said cell with a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, said step comprises contacting said cells with an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the inhibitor is selected from the group consisting of: (1) a gene editing system targeted to one or more sites within the Tet2-associated gene, or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the Tet2-associated gene, or a binding partner of a protein encoded by the Tet2-associated gene; (4) a small molecule that inhibits expression and/or function of the Tet2-associated gene; (5) a nucleic acid encoding any of (1)-(3); and (6) any combination of (1) –(5). In some embodiments, the method further comprises contacting said cell with an inhibitor of Tet2.

In some embodiments, said contacting occurs *ex vivo*. In some embodiments, the contacting occurs *in vivo*. In some embodiments, the contacting occurs *in vivo* prior to delivery of nucleic acid encoding a CAR into the cell. In some embodiments, the contacting occurs *in vivo* after the cells have been administered to a subject in need thereof.

In another aspect, the invention provides a method for treating a cancer in a subject, comprising administering to said subject an effective amount of a cell described herein.

In some embodiments, the method further comprises administering to said subject a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from one or more (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the method further comprises administering to said subject an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the method further comprises administering to said subject an inhibitor of Tet2.

5 In another aspect, the present invention provides a method of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell described herein, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of altering (e.g., decreasing) expression and/or function of Tet2 by contacting said cell with a Tet2 inhibitor.

10 In some embodiments, the Tet2 inhibitor is chosen from: a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein); a dominant negative Tet2 isoform, or a nucleic acid encoding said dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

15 In some embodiments, said contacting occurs *ex vivo*. In some embodiments, the contacting occurs *in vivo*. In some embodiments, the contacting occurs *in vivo* prior to delivery of nucleic acid encoding a CAR into the cell. In some embodiments, the contacting occurs *in vivo* after the cells have been administered to a subject in need thereof.

20 In another aspect, the invention provides a method for treating a cancer in a subject, comprising administering to said subject an effective amount of a cell described herein.

In another aspect, the invention provides a cell for use in a method of treating a subject in need thereof, comprising administering to said subject an effective amount of a cell described herein.

25 In some embodiments, the method further comprises administering to said subject a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype. In some
30 embodiments, the method further comprises administering to said subject an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

35 In some embodiments, the method further comprises administering to said subject an inhibitor of Tet2, e.g., a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein); a dominant negative Tet2 isoform, or a nucleic acid encoding said dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

In another aspect, the invention provides a CAR-expressing cell therapy for use in a method of treating a subject in need thereof, comprising administering to said subject the CAR-expressing cell therapy and a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the method further comprises administering to said subject an inhibitor of Tet2.

In another aspect, the invention provides a CAR-expressing cell therapy for use in a method of treating a subject in need thereof, comprising administering to said subject the CAR-expressing cell therapy and an inhibitor of Tet2.

In some embodiments, the Tet2 inhibitor is chosen from: a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein), dominant negative Tet2 isoforms, and nucleic acid encoding said dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

In some embodiments, the subject receives a pre-treatment of the modulator (e.g., inhibitor), prior to the initiation of the CAR-expressing cell therapy. In some embodiments, the subject receives concurrent treatment with the modulator (e.g., inhibitor) and the CAR expressing cell therapy. In some embodiments, the subject receives treatment with the modulator (e.g., inhibitor) post-CAR-expressing cell therapy.

In some embodiments, the subject has a disease associated with expression of a tumor antigen, e.g., a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of the tumor antigen.

In some embodiments, the cancer is a hematologic cancer or a solid tumor. In some embodiments, the cancer is a hematologic cancer chosen from one or more of chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-

Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or pre-leukemia.

In some embodiments, the cancer is selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers.

In another aspect, the invention provides a method of treating a subject, comprising administering to said subject a modulator (e.g., an inhibitor or activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype, wherein said subject has received, is receiving, or is about to receive therapy comprising a CAR-expressing cell.

In some embodiments, the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the method further comprises administering to said subject an inhibitor of Tet2.

In another aspect, the invention provides a method of treating a subject, comprising administering to said subject an inhibitor of Tet2. In some embodiments, the Tet2 inhibitor is chosen from a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein); a dominant negative Tet2 isoform, or a nucleic acid encoding said dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

In another aspect, the invention provides a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) for use in the treatment of a subject, wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or
 5 more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype, and wherein said subject has received, is receiving, or is about to receive therapy comprising a CAR-expressing cell.

In some embodiments, the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS,
 10 IL2RA, or PRDM1. In some embodiments, subject has received, is receiving, or is about to receive an inhibitor of Tet2.

In yet another aspect, the invention provides a Tet2 inhibitor for use in the treatment of a subject, e.g., a subject with a condition or disease disclosed herein, wherein said subject has received,
 15 is receiving, or is about to receive therapy comprising a CAR-expressing cell.

In one aspect, disclosed herein is a method of making a population of Chimeric Antigen Receptor (CAR)-expressing immune effector cells, comprising

- a) providing a population of immune effector cells, e.g., T cells;
- 20 b) contacting the population of immune effector cells with a nucleic acid encoding a CAR polypeptide;
- c) contacting the population of immune effector cells with a Tet2 inhibitor, e.g., as described herein ;
- and
- 25 d) maintaining the cells under conditions that allow expression of the CAR polypeptide, thereby making a population of CAR-expressing immune effector cells.

In some embodiments, the Tet2 inhibitor is chosen from: a Tet2 inhibitor, e.g., a small molecule inhibitor of Tet 2 (e.g., 2-hydroxyglutarate); a lentivirus (e.g., a lentivirus encoding a CAR molecule as described herein) ; a dominant negative Tet2 isoform, or a nucleic acid encoding said
 30 dominant negative Tet2; an RNAi agent targeting Tet2 (e.g., siRNA or shRNA); a CRISPR-Cas9 targeting Tet2; or a ZFN/TALEN targeting Tet2.

In some embodiments, a CAR- expressing cell manufactured with Tet 2 inhibitor as disclosed herein, has one, two, three, four or more (e.g., all) of the following characteristics:

- (i) increased expansion potential, e.g., at least 1.5, 2, 3, 4, 5, or 6 fold expansion as measured
 35 by an assay of Example 1;

(ii) one or more properties of short lived memory T cells, e.g., increased expression of Eomes, decreased expression of KLRG1, increase cytotoxic activity or increased memory T cell potential as measured by an assay of Example 1;

(iii) increased effector function, e.g., increased degranulation of CD107a, granzyme B and perforin as measured by an assay of Example 1;

(iv) increased cytolytic activity as measured by an assay of Example 1; or

(v) increased proliferative capacity, e.g., as measured by increased Ki67, as measured by an assay of Example 1,

compared to an otherwise similar CAR-expressing cell with non-disrupted Tet2, e.g., wild type Tet2.

In some embodiments of a method of manufacturing disclosed herein, a Tet2 disruption is present in the immune effector cell population, e.g., prior to contacting with a nucleic acid encoding a CAR polypeptide. In some embodiments, the immune effector cell population comprises a Tet2 disrupted allele, e.g., a monoallelic Tet2 disruption as described herein, e.g., a monoallelic hypomorphic Tet2 allele. In some embodiments of a method of manufacturing disclosed herein, contacting an immune effector cell population comprising a monoallelic disruption in Tet2 with an inhibitor of Tet2, e.g., as described herein, results in biallelic disruption of Tet2, e.g., disruption of the wild type allele of Tet2.

In some embodiments of a method of manufacturing disclosed herein, a Tet2 disruption is present in the immune effector cell population, e.g., prior to contacting with a nucleic acid encoding a CAR polypeptide. In some embodiments, the immune effector cell population comprises one or more Tet2 disrupted alleles, e.g., biallelic disruption in Tet2.

In some embodiments of a method of manufacturing disclosed herein, a Tet2 disruption is not present in the immune effector cell population, e.g., prior to contacting with a nucleic acid encoding a CAR polypeptide. In some embodiments, contacting an immune effector cell population comprising no disrupted Tet2 alleles, e.g., comprising two wild type Tet2 alleles, with an inhibitor of Tet2, e.g., as described herein, results in biallelic disruption of Tet2, e.g., disruption of the wild type allele of Tet2.

In some embodiments, a CAR-expressing population manufactured with the immune effector population comprising biallelic disruption of Tet2 has one, two, three, four or more (e.g., all) of the following characteristics:

(i) increased expansion potential, e.g., at least 1.5, 2, 3, 4, 5, or 6 fold expansion as measured by an assay of Example 1;

(ii) properties of short lived memory T cells, e.g., increased expression of EOMES, decreased expression of KLRG1, increase cytotoxic activity or increased memory T cell potential as measured by an assay of Example 1;

(iii) increased effector function, e.g., increased degranulation of CD107a, granzyme B and perforin as measured by an assay of Example 1;

(iv) increased cytolytic activity as measured by an assay of Example 1; or

(v) increased proliferative capacity, e.g., as measured by increased Ki67, as measured by an
5 assay of Example 1,

compared to an otherwise similar CAR-expressing cell with non-disrupted Tet2, e.g., wild type Tet2.

In some embodiments of any of the methods or compositions disclosed herein, a CAR-
10 expressing cell comprising a disruption in Tet2, e.g., monoallelic or biallelic disruption in Tet2 (e.g., by any of the methods disclosed herein), can populate, e.g., develop or divide into, a CAR-expressing cell population, e.g., expand into a clonal CAR-expressing cell population. In some embodiments, a CAR-expressing cell population derived from one CAR-expressing cell, e.g., a clonal population of CAR-expressing cells, can be administered to a subject, e.g., for the treatment of a disease or
15 condition, e.g., a cancer, e.g., a cancer associated with expression of an antigen recognized by the CAR-expressing cell. In some embodiments, a clonal population of CAR-expressing cells results in treatment, e.g., as described herein, of said disease.

In another aspect, the invention provides a method of manufacturing a CAR-expressing cell,
20 comprising introducing a nucleic acid encoding a CAR into a cell such that said nucleic acid (or CAR-encoding portion thereof) integrates into the genome of the cell within a Tet2-associated gene (e.g., one or more Tet2-associated genes) (e.g., within an intron or exon of the Tet2-associated gene), such that expression and/or function of the Tet2-associated genes is altered (e.g., reduced or eliminated), wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG,
25 NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the Tet2-associated gene is chosen from IFNG, NOTCH2, CD28,
30 ICOS, IL2RA, or PRDM1.

In another aspect, the invention provides a method of manufacturing a CAR-expressing cell, comprising contacting said CAR-expressing cell *ex vivo* with a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more
35 genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the Tet2-associated gene is chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

In another aspect, the invention provides a vector comprising sequence encoding a CAR and sequence encoding a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the modulator (e.g., inhibitor) is a (1) a gene editing system targeted to one or more sites within the gene, or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the Tet2-associated gene, or a binding partner of a protein encoded by the Tet2-associated gene; and (4) a nucleic acid encoding any of (1)-(3), or combinations thereof.

In some embodiments, the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In some embodiments, the sequence encoding a CAR and the sequence encoding the inhibitor are separated by a 2A site.

In another aspect, the invention provides a gene editing system that is specific for a sequence of a Tet2-associated gene (e.g., one or more Tet2-associated genes) or a regulatory element thereof, wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the gene editing system is specific for a sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

In some embodiments, the gene editing system is a CRISPR/Cas gene editing system, a zinc finger nuclease system, a TALEN system, or a meganuclease system. In some embodiments, the gene editing system is a CRISPR/Cas gene editing system.

In some embodiments, the gene editing system comprises: a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a Cas9 protein; a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a nucleic acid encoding a Cas9 protein; a nucleic acid encoding a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a Cas9 protein; or a nucleic acid encoding a

gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a nucleic acid encoding a Cas9 protein.

In some embodiments, the gene editing system further comprises a template DNA. In some embodiments, the template DNA comprises nucleic acid sequence encoding a CAR, e.g., a CAR as
5 described herein.

In another aspect, the invention provides a composition for the *ex vivo* manufacture of a CAR-expressing cell, comprising a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of: (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1; (ii) one or more genes listed in Table 8; (iii) one
10 or more genes listed in Table 9, Column D; (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or (v) one or more genes associated with a central memory phenotype.

In some embodiments, the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

In some embodiments, the modulator (e.g., inhibitor) is a (1) a gene editing system targeted to one or more sites within the Tet2-associated gene or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the gene, or a binding partner of a protein encoded by the Tet2-associated gene; or (4) a nucleic acid encoding any of (1)-(3), or combinations
15 thereof.
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In some embodiments, the composition further comprises an inhibitor of Tet2.

In another aspect, the invention provides a population of cells comprising one or more cells disclosed herein, wherein the population of cells comprises a higher (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold higher) percentage of Tscm cells (e.g., CD45RA+CD62L+CCR7+ (optionally
25 CD27+CD95+) T cells) than a population of cells which does not comprise one or more cells in which expression and/or function of a Tet2-associated gene (e.g., one or more Tet2-associated genes) in said cell has been reduced or eliminated.

In another aspect, the invention provides a population of cells comprising one or more cells of any of claims 1-89, wherein at least 50% (e.g., at least 60%, 70%, 80%, 85%, 90%, 95%, 97%, or
30 99%) of the population of cells have a central memory T cell phenotype.

In some embodiments, the central memory cell phenotype is a central memory T cell phenotype. In some embodiments, at least 50% (e.g., at least 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99%) of the population of cells express CD45RO and/or CCR7.

BRIEF DESCRIPTION OF THE DRAWINGS

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

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FIGS. 1A-1D depict evaluation of clinical responses following adoptive transfer of CAR T-cells in a CLL patient. **FIG. 1A** shows the *in vivo* expansion and persistence of CTL019 CAR T-cells prior to and following two infusions. The frequency of CTL019 cells is depicted as average transgene copies/ μ g DNA. **FIG. 1B** shows longitudinal measurements of serum cytokines before and after CAR T-cell infusions. An absolute measurement of each cytokine was derived from a standard curve based on recombinant protein concentrations over a threefold eight-point dilution series. Each sample was analyzed in duplicate with average values shown (coefficient of variation less than 10%). **FIG. 1C** shows the total number of circulating CLL cells before and after CTL019 therapy. Calculations were based on absolute lymphocyte counts from complete blood count values assuming a 5-liter volume of peripheral blood. **FIG. 1D** shows sequential computed tomography imaging showing resolution of chemotherapy-refractory lymphadenopathy. Masses were progressively reduced beginning two months following the second infusion of CAR T-cells, as indicated by the arrows, and were resolved by one year and beyond (data not shown).

FIG. 2 depicts that the outgrowth of CAR T-cells in Patient 10 occurs in the CD8 compartment. Kinetics of total CTL019 CAR T-cell expansion (left graph) relative to CD8+ CTL019 cell expansion (right graph) are shown pre- and post-infusion. The number of circulating CTL019 cells was calculated based on frequencies of CD3+ and CD8+ CAR+ populations and absolute cell counts. All observed values were above the limit of detection by flow cytometry (0.1%).

FIG. 3 depicts that CAR T-cells manufactured from Patient 10 exhibit a polyclonal composition. TCRV β distribution in CD8- (left pie chart) and CD8+ (right pie chart) CAR T-cells in the cellular infusion product of Patient 10 is shown.

FIGS. 4A-4D depict distribution of TCRV β usage in a CLL patient who had a clonal expansion of CAR T-cells. In **FIG. 4A**, the average frequency of TCRV β gene segment usage in the peripheral blood of a CLL patient one month (left pie chart) and two months (middle pie chart) following the second infusion of CAR T-cells is depicted. TCRV β clonotype frequencies in sorted CD8+ CAR T-cells at the peak of expansion following the second infusion are shown in the rightmost pie chart. Each TCRV β gene segment is represented by a slice that is proportional to its frequency. The slice representing the proportion of TCRV β 5.1 usage at each time point is indicated in each pie chart. In **FIG. 4B**, flow cytometric analysis of PBMC illustrates the large proportion of CD8+ CAR T-cells that are TCRV β 5.1 positive relative to TCRV β 13.1 (negative control). In **FIG. 4C**, the abundance of TCRV β 5.1 clonotypes in sorted CD8+ CAR+ T-cells at the peak of activity is depicted

in pre-infusion CD8+ CTL019 cells and in whole blood at one as well as two months following the second CAR T-cell treatment as determined by deep repertoire sequencing. The dominant TCRV β 5.1 clone (CASSLDGSGQGSDYGYTF) is shown as a red dot in each bivariate plot. In **FIG. 4D**, the kinetics of TCRV β 5.1 clonal expansion following the second infusion of CAR T-cells are plotted in parallel with CAR proliferation and persistence levels. Levels of the CAR and the dominant TCRV β 5.1 clone (shown as percentage of cells with the detectable clonal sequence) were measured by qPCR on DNA extracted from whole blood.

FIGS. 5A-5B depict analysis of CAR lentiviral integration sites and detection of *TET2* chimeric transcripts in Patient 10. In **FIG. 5A**, the relative abundance of CAR T-cell clones following the second infusion is summarized as a stacked bar graph. Different bars indicate the major cell clones, as marked by integration sites. A key to the sites is shown below the graph. Each integration site is named by the nearest gene. Relative abundance was estimated using the SonicLength method. Estimated relative abundances below 3% are binned as “Low Abundance.” **FIG. 5B** depicts a diagram of the vector at the *TET2* integration site locus illustrating splicing of truncated transcripts into the vector provirus that were detected at the peak of *in vivo* CAR T-cell activity (Day 121). Each of the splicing events recruited ectopic in-frame stop codons (denoted by the small asterisks above the solid black lines), which represent the spliced products. Sequences corresponding to the splice junctions for the three chimeric messages (five total junctions) are listed below the diagram. Underlined regions in the table below the diagram correspond to splice donors and acceptors. LTR, long terminal repeat; cPPT, polypurine tract; EF1 α , elongation factor 1 alpha promoter.

FIGS. 6A-6B depict strategy for detection of *TET2* chimeric transcripts in Patient 10. In **FIG. 6A**, the strategy for detection of polyadenylated RNA corresponding to truncated *TET2* transcripts is depicted. Boxes represent the genomic regions between *TET2* exon 9 and 10 with the integrated vector present. Blue and red arrows indicate general locations of the forward and reverse primers which are listed below the diagram. LTR, long terminal repeat; cPPT, polypurine tract; EF1 α , elongation factor 1 alpha promoter. **FIG. 6B** shows visualization of chimeric *TET2* RT-PCR products. PCR products were separated on a native agarose gel and stained with ethidium bromide. Expected sizes of amplicons are listed above the gel. Truncated transcripts are highlighted by boxes. A key to the RT-PCR reactions is shown below the diagram.

FIGS. 7A-7G depict that *TET2* deficiency alters the epigenetic landscape and T-cell differentiation. In **FIG. 7A**, total 5-hmc levels in CAR+ and CAR- CD8+ T-cells cultured from Patient 10 at the peak of the response to CTL019 therapy are shown. Histograms depict the intensity of intracellular 5-hmc staining as determined by flow cytometry. **FIG. 7B** shows Venn diagrams of differential ATAC-seq regions (left) and enrichment of those peaks in each portion of the diagrams (right) in CAR+ and CAR- CD8+ T-cells cultured from Patient 10. In **FIG. 7C**, genome browser views of ATAC enrichment at the *IFNG* locus corresponding to the patient cells above are shown.

FIG. 7D depicts frequencies of IFN γ and CD107a expressing CD8⁺ CAR⁺ as well as CAR⁻ T-cells expanded from Patient 10 that were unstimulated or stimulated with anti-CD3/CD28 antibody-coated beads. Contour plot insets indicate the frequencies of gated cell populations. In **FIG. 7E**, the *ex vivo* differentiation phenotype of CAR T-cells at the peak of *in vivo* activity is shown in two long-term complete responding CLL patients (Patients 1 and 2) compared to Patient 10. Pie slices represent the relative frequency of each T-cell subset. Naive-like T cells: CCR7⁺CD45RO⁻; central memory T cells: CCR7⁺CD45RO⁺; effector memory T cells: CCR7⁻CD45RO⁺; and effector T cells: CCR7⁻CD45RO⁻. The CTL019 cell level as determined by quantitative PCR and the frequencies of activated CAR T-cells expressing HLA-DR (cell surface activation marker) at the peak of each patient's response are listed below the pie charts. In **FIG. 7F**, *TET2* expression is shown in primary CD8⁺ T-cells derived from healthy donors that were lentivirally transduced with a scrambled shRNA (control) or *TET2* sequences as measured by quantitative PCR. Error bars depict s.e.m. In **FIG. 7G**, the frequencies of central memory (left), effector memory (middle) and effector CD8⁺ T cells from healthy donors following shRNA-mediated knock-down of *TET2* and *in vitro* expansion are depicted. The frequency of each subset is presented relative to its counterpart that was transduced with the scrambled shRNA ($n = 12$). *P* values were determined using a two-tailed, paired student's *t*-test.

FIG. 8 depicts that *TET2*-disrupted CAR T-cells from Patient 10 exhibit a global chromatin profile consistent with suppressed effector differentiation and activity. GO terms associated with chromatin regions that are significantly more closed in *TET2*-disrupted CD8⁺ CAR⁺ T-cells from Patient 10 compared to their matched CD8⁺ CAR⁻ T-cell counterpart are listed.

FIG. 9 depicts the differentiation state of CAR T-cells in Patient 10 over time. Representative contour plots of flow cytometric data depicting the frequency of CAR⁺ and CAR⁻ CD8⁺ T-cells in Patient 10 that express HLA-DR (surface molecule indicative of T-cell activation). The proportions of these cells that express CD45RO and CCR7 as determinants of differentiation status are shown. Contour plot insets indicate the frequencies of the gated cell populations.

FIGS. 10A-10C depict that knock-down of *TET2* increases the frequency of CAR⁺ T cells and reduces effector differentiation. **FIG. 10A** shows representative flow cytometry plots showing the differentiation state of healthy donor CD8⁺ CAR⁺ T cells following transduction with a scrambled shRNA (control) or an shRNA targeting *TET2*. Insets define frequencies of gated populations. **FIG. 10B** and **FIG. 10C** show frequencies of healthy subject CAR⁺ CD8⁺ T cells and CAR⁺ CD4⁺ T-cells, respectively, according to differentiation phenotype following control or *TET2* shRNA transduction ($n = 10$). *P* values were computed using a two-tailed, paired student's *t*-test.

FIGs. 11A-11E depict results of the investigation of CAR lentiviral integration sites and *TET2* deficiency in Patient 10. **FIG. 11A** shows the relative abundance of CAR T-cell clones following the second infusion summarized as a stacked bar graph. Different horizontal bars indicate the major cell clones, as marked by integration sites. A key to the sites is shown below the graph.

Estimated relative abundances below 3% are binned as “Low Abundance.” **FIG. 11B** shows CAR T-cell diversity in Patient 10 over time using the Shannon index, which describes both the number of different unique integration sites and the evenness of distribution of cells sampled among integration sites. **FIG. 11C** shows a diagram of the vector at the *TET2* integration site locus illustrating splicing of truncated transcripts into the vector provirus that were detected at the peak of *in vivo* CAR T-cell activity (Day 121). Each of the splicing events recruited ectopic in-frame stop codons (denoted by the small asterisks above the solid black lines), which represent the spliced products. Sequences corresponding to the splice junctions for the three chimeric messages (five total junctions) are listed below the diagram. Underlined regions in the table below the diagram correspond to splice donors and acceptors. LTR, long terminal repeat; cPPT, polypurine tract; EF1 α , elongation factor 1 alpha promoter. **FIG. 11D** shows a diagram of the TET2-catalyzed sequential oxidations of 5-mC to 5-hmC and to 5-fC and 5-caC is shown (top). Dot blots for 5-mC, 5-hmC, 5-fC and 5-caC in 600 ng of genomic DNA isolated from HEK293T cells transfected with the E1879Q TET2 mutant are shown. Assay controls include an empty vector, wild-type TET2 and catalytically inactive (HxD) TET2 mutant (bottom left). A western blot using anti-FLAG antibody to detect hTET2 in the above cells is also shown. Hsp90 α/β was used as a loading control (bottom right). **FIG. 11E** shows genomic levels of 5-mC, 5-hmC, 5-fC, and 5-caC modifications produced by the E1879Q TET2 mutant and quantified by LC-MS/MS as the percent of total cytosine modifications are depicted. Percentages derived from the mean of independent experiments (n = 3) are shown (**P $\leq$ 0.01 determined using a two-tailed, paired student’s t-test).

FIGs. 12A-12C depicts the effect of TET2 deficiency on the epigenetic landscape of CAR T-cells. **FIG. 12A** shows an enrichment of transcription factor (TF) binding motifs in chromatin regions gained or lost in CAR+ compared to CAR- T-cells from Patient 10. **FIG. 12B** shows the longitudinal differentiation phenotypes of CD8+ CAR+ and CAR- T-cells from Patient 10 (left panel). Differentiation phenotype at the peak of *in vivo* activity is shown in two long-term complete responding CLL patients (Patients 1 and 2) compared to Patient 10 (right panel). Pie slices represent the relative frequency of each T-cell subset. The CTL019 cell level as determined by quantitative PCR and the frequencies of activated CAR T-cells expressing HLA-DR (cell surface activation marker) at the peak of each patient’s response are listed below the pie charts. **FIG. 12C** shows Long-term proliferation of CTL019 cells in response to repetitive stimulation with K562 cells expressing CD19 or mesothelin (negative control). CAR T-cells were transduced to express either a scrambled control or TET2-specific shRNA. Each arrow indicates when cells were exposed to antigen. P values were determined using a two-tailed, paired student’s t-test (*P < 0.05).

FIG. 13 depicts the outgrowth of CAR T-cells in Patient 10 in the CD8 compartment. Pre- and post-infusion kinetics of CAR T-cell expansion (CD3+, CD8+ and CD8-) are shown in Patient 10 compared to other responders. The number of circulating CTL019 cells was calculated based on

frequencies of CD3+, CD8+ and CD8- CAR T-cell populations and absolute cell counts. All observed values were above the limit of detection by flow cytometry (0.1%).

FIGs. 14A-14D depict profiling of immune cell populations and CAR T-cell detection in Patient 10 at a long-term post-infusion time point. **FIG. 14A** shows the flow cytometry gating strategy to identify peripheral blood CAR T-cells in Patient 10. **FIG. 14B** shows relative percentages of CTL019 cells in the CD4 and CD8 compartments of this patient. T-cells from a healthy subject served as a negative control. **FIG. 14C** shows frequencies of circulating B-cells in Patient 10 compared to a healthy subject. Pre-gating was performed to exclude dead cells as well as doublets, and all gating thresholds were based on fluorescence minus one (FMO) controls. **FIG. 14D** shows Enumeration of various immune cell populations in the blood of Patient 10. The frequency of each population is listed in a separate column that corresponds to its phenotypic marker. **FIG. 14E** shows persistence of CAR T-cells in the peripheral blood of Patient 10 as determined by qPCR. The average threshold cycle (Ct) value obtained from three replicates and standard deviation (SD) are listed. Calculations of CAR T-cell abundance are reported as an average marking per cell as well as transgene copies per microgram of genomic DNA.

FIG. 15 depicts global chromatin profiling of TET2-deficient CAR T-cells from Patient 10. Gene ontology (GO) terms associated with chromatin regions that are significantly more open in TET2-disrupted CD8+ CAR+ T-cells from Patient 10 compared to their matched CD8+ CAR- T-cell counterpart are listed.

FIG. 16 depicts differentiation state of CAR T-cells in Patient 10 compared to other responders over time. Example gating strategy used to determine the differentiation phenotype of CD8+ CAR+ and CAR- T-cells from a complete responder (top left panel). Line graphs depict the differentiation state of these cell populations in other responding patients over time and are plotted with corresponding CAR T-cell levels in the blood, as determined by qPCR.

FIG. 17 depicts CAR T-cell viability following TET2 knock-down and serial restimulation with tumour targets. Viability of CAR+ T-cells transduced with a TET2 shRNA or scrambled control and restimulated with K562 cells expressing CD19 (n = 12). Each arrow indicates the time point at which cells were exposed to antigen.

FIGs. 18A-18B depict CAR T-cell cytokine profiles following TET2 inhibition. **FIG. 18A** shows representative flow cytometry of acute intracellular cytokine production by healthy donor CAR T-cells transduced with a TET2 shRNA or scrambled control (left panel). Production of IFN γ , TNF α and IL-2 by total CD3+, CD4+ and CD8+ CAR T-cells is shown. These cells were stimulated with CD3/CD28 (top right panel) or CAR anti-idiotypic antibody (bottom right panel) coated beads. **FIG. 18B** shows production of IFN γ (top panel), TNF α (middle panel) and IL-2 (bottom panel) by TET2-deficient or control CAR T-cells following restimulation with CD19 antigen. Each arrow indicates when CAR T-cells were exposed to CD19.

FIGs. 19A-19C depict Effect of TET2 knock-down on the cytotoxic machinery of CAR T-cells. **FIG. 19A** depicts flow cytometry plots showing the frequency of TET2 knock-out or control CAR T-cells expressing CD107a (a marker of cytotoxicity) following CD3/CD28 and CAR-specific stimulation (left panel). Summarized data from analysis of CAR T-cells manufactured from n = 6 different healthy donors is shown (right panel). **FIG. 19B** shows representative histograms illustrating expression levels of granzyme B and perforin in CAR T-cells in the setting of TET2 inhibition as compared to its counterpart control (left panel). Pooled data from CAR T-cells of n = 5 healthy donors is summarized in the right panels. **FIG. 19C** shows the cytotoxic capacity of CTL019 cells (transduced with a TET2 or scrambled control shRNA) after overnight co-culture with luciferase-expressing OSU-CLL (left panel) or NALM-6 (right panel) cells. Untransduced T-cells were included as an additional group to control for non-specific lysis. P values were determined using a two-tailed, paired student's t-test (*P < 0.05; **P ≤ 0.01).

FIGs. 20A-20B depict effector and memory molecule expression by Patient 10 CAR T-cells compared to other responding subjects. **FIG. 20A** shows expression of granzyme B (left panel) and the frequency of CAR- and CAR+ T-cells co-expressing granzyme B/Ki-67 (right panel) at the peak of in vivo CTL019 expansion in Patient 10 compared to 3 other complete responders. **FIG. 20B** shows representative histograms of intracellular EOMES expression (left panel), and contour plots depicting frequencies of CD27 (middle panels) and KLRG1-expressing (right panels) lymphocytes in the same cell populations of these patients.

DETAILED DESCRIPTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

The term “a” and “an” refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The term “about” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances ±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term “Chimeric Antigen Receptor” or alternatively a “CAR” refers to a set of polypeptides, typically two in the simplest embodiments, which when in an immune effector cell, provides the cell with specificity for a target cell, typically a cancer cell, and with intracellular signal generation. In some embodiments, a CAR comprises at least an extracellular antigen binding domain, a transmembrane domain and a cytoplasmic signaling domain (also referred to herein as “an intracellular signaling domain”) comprising a functional signaling domain derived from a stimulatory

molecule and/or costimulatory molecule as defined below. In some aspects, the set of polypeptides are contiguous with each other. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain. In one aspect, the stimulatory molecule is the zeta chain associated with the T cell receptor complex. In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is chosen from the costimulatory molecules described herein, e.g., 4-1BB (i.e., CD137), CD27 and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising a functional signaling domain derived from a costimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, a transmembrane domain and an intracellular signaling domain comprising at least two functional signaling domains derived from one or more costimulatory molecule(s) and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises an optional leader sequence at the amino-terminus (N-ter) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at the N-terminus of the extracellular antigen binding domain, wherein the leader sequence is optionally cleaved from the antigen binding domain (e.g., a scFv) during cellular processing and localization of the CAR to the cellular membrane.

A CAR that comprises an antigen binding domain (e.g., a scFv, or TCR) that targets a specific tumor maker X, such as those described herein, is also referred to as XCAR. For example, a CAR that comprises an antigen binding domain that targets CD19 is referred to as CD19CAR.

The term “signaling domain” refers to the functional portion of a protein which acts by transmitting information within the cell to regulate cellular activity via defined signaling pathways by generating second messengers or functioning as effectors by responding to such messengers.

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

The term “antibody fragment” refers to at least one portion of an antibody, that retains the ability to specifically interact with (e.g., by binding, steric hinderance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide brudge at the hinge region, and an isolated CDR or other epitope binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, Nature Biotechnology 23:1126-1136, 2005). Antigen binding fragments can also be grafted into scaffolds based on polypeptides such as a fibronectin type III (Fn3) (see U.S. Patent No.: 6,703,199, which describes fibronectin polypeptide minibodies).

The term “scFv” refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked, e.g., via a synthetic linker, e.g., a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The portion of the CAR of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), a humanized antibody or bispecific antibody (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises a scFv. The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), “Sequences of Proteins of Immunological Interest,” 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (“Kabat” numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 (“Chothia” numbering scheme), or a combination thereof.

As used herein, the term “binding domain” or “antibody molecule” refers to a protein, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain

sequence. The term “binding domain” or “antibody molecule” encompasses antibodies and antibody fragments. In an embodiment, an antibody molecule is a multispecific antibody molecule, e.g., it comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second
 5 immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a
 10 second epitope.

The portion of the CAR of the invention comprising an antibody or antibody fragment thereof may exist in a variety of forms where the antigen binding domain is expressed as part of a contiguous polypeptide chain including, for example, a single domain antibody fragment (sdAb), a single chain antibody (scFv), a humanized antibody, or bispecific antibody (Harlow et al., 1999, In: Using
 15 Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426). In one aspect, the antigen binding domain of a CAR composition of the invention comprises an antibody fragment. In a further aspect, the CAR comprises an antibody fragment that comprises a scFv.

20 The term “antibody heavy chain,” refers to the larger of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations, and which normally determines the class to which the antibody belongs.

The term “antibody light chain,” refers to the smaller of the two types of polypeptide chains present in antibody molecules in their naturally occurring conformations. Kappa (κ) and lambda (λ)
 25 light chains refer to the two major antibody light chain isotypes.

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

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

antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an “antigen” as that term is used herein.

Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a “gene” at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a fluid with other biological components.

The term “anti-cancer effect” refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of cancer cells, a decrease in the number of metastases, an increase in life expectancy, decrease in cancer cell proliferation, decrease in cancer cell survival, or amelioration of various physiological symptoms associated with the cancerous condition. An “anti-cancer effect” can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies in prevention of the occurrence of cancer in the first place. The term “anti-tumor effect” refers to a biological effect which can be manifested by various means, including but not limited to, e.g., a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, or a decrease in tumor cell survival.

The term “autologous” refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

The term “allogeneic” refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically

The term “xenogeneic” refers to a graft derived from an animal of a different species.

The term “cancer” refers to a disease characterized by the uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like. The terms “tumor” and “cancer” are used interchangeably herein, e.g., both terms encompass solid and liquid, e.g., diffuse or circulating, tumors. As used herein, the term “cancer” or “tumor” includes premalignant, as well as malignant cancers and tumors.

“Derived from” as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connote or include a process or source limitation on a first molecule that is derived from a second molecule. For example, in the case of an intracellular signaling domain that is derived from a CD3zeta molecule, the intracellular signaling domain retains sufficient CD3zeta structure such that it has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connote or include a limitation to a particular process of producing the intracellular signaling domain, e.g., it does not mean that, to provide the intracellular signaling domain, one must start with a CD3zeta sequence and delete unwanted sequence, or impose mutations, to arrive at the intracellular signaling domain.

The phrase “disease associated with expression of a tumor antigen as described herein” includes, but is not limited to, a disease associated with expression of a tumor antigen as described herein or condition associated with cells which express a tumor antigen as described herein including, e.g., proliferative diseases such as a cancer or malignancy or a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia; or a noncancer related indication associated with cells which express a tumor antigen as described herein. In one aspect, a cancer associated with expression of a tumor antigen as described herein is a hematological cancer. In one aspect, a cancer associated with expression of a tumor antigen as described herein is a solid cancer. Further diseases associated with expression of a tumor antigen described herein include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of a tumor antigen as described herein. Non-cancer related indications associated with expression of a tumor antigen as described herein include, but are not limited to, e.g., autoimmune disease, (e.g., lupus), inflammatory disorders (allergy and asthma) and transplantation. In some embodiments, the tumor antigen-expressing cells express, or at any time expressed, mRNA encoding the tumor antigen. In an embodiment, the tumor antigen-expressing cells produce the tumor antigen protein (e.g., wild-type or mutant), and the tumor antigen protein may be present at normal levels or reduced levels. In an embodiment, the tumor antigen-expressing cells produced detectable levels of a tumor antigen protein at one point, and subsequently produced substantially no detectable tumor antigen protein.

The term “conservative sequence modifications” refers to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody or antibody fragment containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody or antibody fragment of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino

acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested using the functional assays described herein.

The term “stimulation,” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex or CAR) with its cognate ligand (or tumor antigen in the case of a CAR) thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex or signal transduction via the appropriate NK receptor or signaling domains of the CAR. Stimulation can mediate altered expression of certain molecules.

The term “stimulatory molecule,” refers to a molecule expressed by an immune cell (e.g., T cell, NK cell, B cell) that provides the cytoplasmic signaling sequence(s) that regulate activation of the immune cell in a stimulatory way for at least some aspect of the immune cell signaling pathway. In one aspect, the signal is a primary signal that is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a “primary signaling domain”) that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12. In a specific CAR of the invention, the intracellular signaling domain in any one or more CARS of the invention comprises an intracellular signaling sequence, e.g., a primary signaling sequence of CD3-zeta. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence provided as SEQ ID NO:18, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In a specific CAR of the invention, the primary signaling sequence of CD3-zeta is the sequence as provided in SEQ ID NO: 20, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

The term “antigen presenting cell” or “APC” refers to an immune system cell such as an accessory cell (e.g., a B-cell, a dendritic cell, and the like) that displays a foreign antigen complexed with major histocompatibility complexes (MHC's) on its surface. T-cells may recognize these complexes using their T-cell receptors (TCRs). APCs process antigens and present them to T-cells.

An “intracellular signaling domain,” as the term is used herein, refers to an intracellular portion of a molecule. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell. Examples of immune effector function, e.g., in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines.

In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent stimulation. In an embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

A primary intracellular signaling domain can comprise a signaling motif which is known as an immunoreceptor tyrosine-based activation motif or ITAM. Examples of ITAM containing primary cytoplasmic signaling sequences include, but are not limited to, those derived from CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12.

The term “zeta” or alternatively “zeta chain”, “CD3-zeta” or “TCR-zeta” is defined as the protein provided as GenBan Acc. No. BAG36664.1, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, and a “zeta stimulatory domain” or alternatively a “CD3-zeta stimulatory domain” or a “TCR-zeta stimulatory domain” is defined as the amino acid residues from the cytoplasmic domain of the zeta chain, or functional derivatives thereof, that are sufficient to functionally transmit an initial signal necessary for T cell activation. In one aspect the cytoplasmic domain of zeta comprises residues 52 through 164 of GenBank Acc. No. BAG36664.1 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like, that are functional orthologs thereof. In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO: 18. In one aspect, the “zeta stimulatory domain” or a “CD3-zeta stimulatory domain” is the sequence provided as SEQ ID NO: 20.

The term a “costimulatory molecule” refers to a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are contribute to an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor, as well as OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS

(CD278), and 4-1BB (CD137). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRP1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

A costimulatory intracellular signaling domain can be the intracellular portion of a costimulatory molecule. A costimulatory molecule can be represented in the following protein families: TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), and activating NK cell receptors. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3, and a ligand that specifically binds with CD83, and the like.

The intracellular signaling domain can comprise the entire intracellular portion, or the entire native intracellular signaling domain, of the molecule from which it is derived, or a functional fragment or derivative thereof.

The term “4-1BB” refers to a member of the TNFR superfamily with an amino acid sequence provided as GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like; and a “4-1BB costimulatory domain” is defined as amino acid residues 214-255 of GenBank Acc. No. AAA62478.2, or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like. In one aspect, the “4-1BB costimulatory domain” is the sequence provided as SEQ ID NO: 14 or the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

“Immune effector cell,” as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

“Immune effector function or immune effector response,” as that term is used herein, refers to function or response, e.g., of an immune effector cell, that enhances or promotes an immune attack of a target cell. E.g., an immune effector function or response refers a property of a T or NK cell that promotes killing or the inhibition of growth or proliferation, of a target cell. In the case of a T cell, primary stimulation and co-stimulation are examples of immune effector function or response.

The term “encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term “effective amount” or “therapeutically effective amount” are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

The term “endogenous” refers to any material from or produced inside an organism, cell, tissue or system.

The term “exogenous” refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term “expression” refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

The term “transfer vector” refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term “transfer vector” includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term “expression vector” refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed.

An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes)

and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term “lentivirus” refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses.

The term “lentiviral vector” refers to a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., *Mol. Ther.* 17(8): 1453–1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include but are not limited to, e.g., the LENTIVECTOR® gene delivery technology from Oxford BioMedica, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

The term “homologous” or “identity” refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

“Humanized” forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies and antibody fragments thereof are human immunoglobulins (recipient antibody or antibody fragment) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, a humanized antibody/antibody fragment can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications can further refine and optimize antibody or antibody fragment performance. In general, the humanized antibody or antibody fragment thereof will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or a significant portion of the FR regions are those of a human immunoglobulin sequence. The humanized antibody or antibody fragment can also comprise at least a portion of an immunoglobulin constant

region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature*, 321: 522-525, 1986; Reichmann et al., *Nature*, 332: 323-329, 1988; Presta, *Curr. Op. Struct. Biol.*, 2: 593-596, 1992.

“Fully human” refers to an immunoglobulin, such as an antibody or antibody fragment,
 5 where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody or immunoglobulin.

The term “isolated” means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not “isolated,” but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is
 10 “isolated.” An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. “A” refers to adenosine, “C” refers to cytosine, “G” refers to guanosine, “T” refers to thymidine, and “U” refers to uridine.

15 The term “operably linked” or “transcriptional control” refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the
 20 transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term “parenteral” administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or
 25 infusion techniques.

The term “nucleic acid” or “polynucleotide” refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a
 30 manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with
 35 mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); and Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)).

The terms “peptide,” “polypeptide,” and “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein’s or peptide’s sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term “promoter” refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term “promoter/regulatory sequence” refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term “constitutive” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term “inducible” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term “tissue-specific” promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

The terms “cancer associated antigen” or “tumor antigen” interchangeably refers to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cancer cell, either entirely or as a fragment (e.g., MHC/peptide), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some embodiments, a tumor antigen is a marker expressed by both normal cells and cancer cells, e.g., a lineage marker, e.g., CD19 on B cells. In some embodiments, a tumor antigen is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold

overexpression or more in comparison to a normal cell. In some embodiments, a tumor antigen is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a tumor antigen will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (e.g., MHC/peptide), and not synthesized or expressed on the surface of a normal cell. In some embodiments, the CARs of the present invention includes CARs comprising an antigen binding domain (e.g., antibody or antibody fragment) that binds to a MHC presented peptide. Normally, peptides derived from endogenous proteins fill the pockets of Major histocompatibility complex (MHC) class I molecules, and are recognized by T cell receptors (TCRs) on CD8 + T lymphocytes. The MHC class I complexes are constitutively expressed by all nucleated cells. In cancer, virus-specific and/or tumor-specific peptide/MHC complexes represent a unique class of cell surface targets for immunotherapy. TCR-like antibodies targeting peptides derived from viral or tumor antigens in the context of human leukocyte antigen (HLA)-A1 or HLA-A2 have been described (see, e.g., Sastry et al., J Virol. 2011 85(5):1935-1942; Sergeeva et al., Blood, 2011 117(16):4262-4272; Verma et al., J Immunol 2010 184(4):2156-2165; Willemsen et al., Gene Ther 2001 8(21) :1601-1608 ; Dao et al., Sci Transl Med 2013 5(176) :176ra33 ; Tassev et al., Cancer Gene Ther 2012 19(2):84-100). For example, TCR-like antibody can be identified from screening a library, such as a human scFv phage displayed library.

The term “tumor-supporting antigen” or “cancer-supporting antigen” interchangeably refer to a molecule (typically a protein, carbohydrate or lipid) that is expressed on the surface of a cell that is, itself, not cancerous, but supports the cancer cells, e.g., by promoting their growth or survival e.g., resistance to immune cells. Exemplary cells of this type include stromal cells and myeloid-derived suppressor cells (MDSCs). The tumor-supporting antigen itself need not play a role in supporting the tumor cells so long as the antigen is present on a cell that supports cancer cells.

The term “flexible polypeptide linker” or “linker” as used in the context of a scFv refers to a peptide linker that consists of amino acids such as glycine and/or serine residues used alone or in combination, to link variable heavy and variable light chain regions together. In one embodiment, the flexible polypeptide linker is a Gly/Ser linker and comprises the amino acid sequence (Gly-Gly-Gly-Ser)_n, where n is a positive integer equal to or greater than 1. For example, n=1, n=2, n=3, n=4, n=5 and n=6, n=7, n=8, n=9 and n=10 (SEQ ID NO:28). In one embodiment, the flexible polypeptide linkers include, but are not limited to, (Gly₄ Ser)₄ (SEQ ID NO:29) or (Gly₄ Ser)₃ (SEQ ID NO:30). In another embodiment, the linkers include multiple repeats of (Gly₂Ser), (GlySer) or (Gly₃Ser) (SEQ ID NO:31). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference).

As used herein, a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m⁷G cap) is a modified guanine nucleotide that has been added to the “front” or 5' end of a eukaryotic messenger RNA shortly after the start of transcription. The 5' cap consists of a terminal

group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA
 5 polymerase. This enzymatic complex catalyzes the chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, “in vitro transcribed RNA” refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro
 10 transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a “poly(A)” is a series of adenosines attached by polyadenylation to the mRNA. In the preferred embodiment of a construct for transient expression, the polyA is between 50 and 5000 (SEQ ID NO: 34), preferably greater than 64, more preferably greater than 100, most
 15 preferably greater than 300 or 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, “polyadenylation” refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine
 20 nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation
 25 occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, “transient” refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms “treat”, “treatment” and “treating” refer to the reduction or
 35 amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more therapies (e.g., one or more

therapeutic agents such as a CAR of the invention). In specific embodiments, the terms “treat”, “treatment” and “treating” refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms “treat”, “treatment” and “treating” -refer to the inhibition of the progression of a proliferative disorder, either physically by, e.g., stabilization of a discernible symptom,
 5 physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms “treat”, “treatment” and “treating” refer to the reduction or stabilization of tumor size or cancerous cell count.

The term “signal transduction pathway” refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase “cell surface receptor” includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.
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The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).
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The term, a “substantially purified” cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term
 20 refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term “therapeutic” as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term “prophylaxis” as used herein means the prevention of or protective treatment for a disease or disease state.
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In the context of the present invention, “tumor antigen” or “hyperproliferative disorder antigen” or “antigen associated with a hyperproliferative disorder” refers to antigens that are common to specific hyperproliferative disorders. In certain aspects, the hyperproliferative disorder antigens of the present invention are derived from, cancers including but not limited to primary or metastatic
 30 melanoma, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemias, uterine cancer, cervical cancer, bladder cancer, kidney cancer and adenocarcinomas such as breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, and the like.

The term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or
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“transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The term “specifically binds,” refers to an antibody, or a ligand, which recognizes and binds with a binding partner (e.g., a tumor antigen) protein present in a sample, but which antibody or
5 ligand does not substantially recognize or bind other molecules in the sample.

“Regulatable chimeric antigen receptor (RCAR),” as that term is used herein, refers to a set of polypeptides, typically two in the simplest embodiments, which when in a RCARX cell, provides the RCARX cell with specificity for a target cell, typically a cancer cell, and with regulatable intracellular signal generation or proliferation, which can optimize an immune effector property of the RCARX
10 cell. An RCARX cell relies at least in part, on an antigen binding domain to provide specificity to a target cell that comprises the antigen bound by the antigen binding domain. In an embodiment, an RCAR includes a dimerization switch that, upon the presence of a dimerization molecule, can couple an intracellular signaling domain to the antigen binding domain.

“Membrane anchor” or “membrane tethering domain”, as that term is used herein, refers to a
15 polypeptide or moiety, e.g., a myristoyl group, sufficient to anchor an extracellular or intracellular domain to the plasma membrane.

“Switch domain,” as that term is used herein, e.g., when referring to an RCAR, refers to an entity, typically a polypeptide-based entity, that, in the presence of a dimerization molecule, associates with another switch domain. The association results in a functional coupling of a first
20 entity linked to, e.g., fused to, a first switch domain, and a second entity linked to, e.g., fused to, a second switch domain. A first and second switch domain are collectively referred to as a dimerization switch. In embodiments, the first and second switch domains are the same as one another, e.g., they are polypeptides having the same primary amino acid sequence, and are referred to collectively as a homodimerization switch. In embodiments, the first and second switch domains are different from
25 one another, e.g., they are polypeptides having different primary amino acid sequences, and are referred to collectively as a heterodimerization switch. In embodiments, the switch is intracellular. In embodiments, the switch is extracellular. In embodiments, the switch domain is a polypeptide-based entity, e.g., FKBP or FRB-based, and the dimerization molecule is small molecule, e.g., a rapalogue. In embodiments, the switch domain is a polypeptide-based entity, e.g., an scFv that binds a myc
30 peptide, and the dimerization molecule is a polypeptide, a fragment thereof, or a multimer of a polypeptide, e.g., a myc ligand or multimers of a myc ligand that bind to one or more myc scFvs. In embodiments, the switch domain is a polypeptide-based entity, e.g., myc receptor, and the dimerization molecule is an antibody or fragments thereof, e.g., myc antibody.

“Dimerization molecule,” as that term is used herein, e.g., when referring to an RCAR, refers
35 to a molecule that promotes the association of a first switch domain with a second switch domain. In embodiments, the dimerization molecule does not naturally occur in the subject, or does not occur in

concentrations that would result in significant dimerization. In embodiments, the dimerization molecule is a small molecule, e.g., rapamycin or a rapalogue, e.g., RAD001.

The term “bioequivalent” refers to an amount of an agent other than the reference compound (e.g., RAD001), required to produce an effect equivalent to the effect produced by the reference dose or reference amount of the reference compound (e.g., RAD001). In an embodiment the effect is the level of mTOR inhibition, e.g., as measured by P70 S6 kinase inhibition, e.g., as evaluated in an in vivo or in vitro assay, e.g., as measured by an assay described herein, e.g., the Boulay assay. In an embodiment, the effect is alteration of the ratio of PD-1 positive/PD-1 negative T cells, as measured by cell sorting. In an embodiment a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of P70 S6 kinase inhibition as does the reference dose or reference amount of a reference compound. In an embodiment, a bioequivalent amount or dose of an mTOR inhibitor is the amount or dose that achieves the same level of alteration in the ratio of PD-1 positive/PD-1 negative T cells as does the reference dose or reference amount of a reference compound.

The term “low, immune enhancing, dose” when used in conjunction with an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., RAD001 or rapamycin, or a catalytic mTOR inhibitor, refers to a dose of mTOR inhibitor that partially, but not fully, inhibits mTOR activity, e.g., as measured by the inhibition of P70 S6 kinase activity. Methods for evaluating mTOR activity, e.g., by inhibition of P70 S6 kinase, are discussed herein. The dose is insufficient to result in complete immune suppression but is sufficient to enhance the immune response. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in a decrease in the number of PD-1 positive T cells and/or an increase in the number of PD-1 negative T cells, or an increase in the ratio of PD-1 negative T cells/PD-1 positive T cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in an increase in the number of naive T cells. In an embodiment, the low, immune enhancing, dose of mTOR inhibitor results in one or more of the following:

an increase in the expression of one or more of the following markers: CD62L^{high}, CD127^{high}, CD27⁺, and BCL2, e.g., on memory T cells, e.g., memory T cell precursors;

a decrease in the expression of KLRG1, e.g., on memory T cells, e.g., memory T cell precursors; and

an increase in the number of memory T cell precursors, e.g., cells with any one or combination of the following characteristics: increased CD62L^{high}, increased CD127^{high}, increased CD27⁺, decreased KLRG1, and increased BCL2;

wherein any of the changes described above occurs, e.g., at least transiently, e.g., as compared to a non-treated subject.

“Refractory” as used herein refers to a disease, e.g., cancer, that does not respond to a treatment. In embodiments, a refractory cancer can be resistant to a treatment before or at the

beginning of the treatment. In other embodiments, the refractory cancer can become resistant during a treatment. A refractory cancer is also called a resistant cancer.

“Relapsed” as used herein refers to the return of a disease (e.g., cancer) or the signs and symptoms of a disease such as cancer after a period of improvement, e.g., after prior treatment of a therapy, e.g., cancer therapy

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

As used herein, the term “IFNG,” “interferon gamma,” or “IFN- γ ” refers to the gene IFNG and the protein encoded by the gene. In the human genome, IFNG is located on chromosome 12q15. An exemplary IFNG sequence is provided in Genebank number: NM_000619.2.

As used herein, the term “NOTCH2,” “neurogenic locus notch homolog protein 2,” or “hN2” refers to the gene NOTCH2 and the protein encoded by the gene. In the human genome, NOTCH2 is located on chromosome 1p12. Two exemplary Notch2 isoforms are provided in Genebank numbers: NM_001200001.1 and NM_024408.3.

As used herein, the term “IL2RA,” “interleukin-2 receptor subunit alpha,” “IL-2-RA,” or “IL2-RA” refers to the gene IL2RA and the protein encoded by the gene. It is also known as “CD25,” “TAC antigen,” or “p55.” In the human genome, IL2RA is located on chromosome 10p15.1. Three exemplary IL2RA isoforms are provided in Genebank numbers: NM_000417.2, NM_001308242.1, and NM_001308243.1.

As used herein, the term “PRDM1” or “PR domain zinc finger protein 1” refers to the gene PRDM1 and the protein encoded by the gene. It is also known as “BLIMP-1,” “Beta-interferon gene positive regulatory domain I-binding factor,” “PR domain-containing protein 1,” “Positive regulatory domain I-binding factor 1,” “PRDI-BF1,” and “PRDI-binding factor 1.” In the human genome, PRDM1 is located on chromosome 6q21. Four exemplary PRDM1 isoforms are provided in Genebank numbers: NM_001198.3, NM_182907.2, XM_011536063.2, and XM_017011187.1.

“Tet” as the term is used herein, refers to the family of genes, and the proteins encoded by said genes, of the ten-eleven translocation methylcytosine dioxygenase family. Tet includes, for example, Tet1, Tet2 and Tet3.

“Tet2” as the term is used herein, refers to gene, tet methylcytosine dioxygenase 2, and the protein encoded by said gene, the tet2 methylcytosine dioxygenase, which catalyzes the conversion of methylcytosine to 5-hydroxymethylcytosine. It is sometimes also referred to as “KIAA1546,” “FLJ20032” and “tet oncogene family member 2.” The encoded protein is involved in myelopoiesis, and defects in this gene have been associated with several myeloproliferative disorders. In the human genome, TET2 is located on chromosome 4q24. Currently six TET2 isoforms have been described and their Genebank numbers are: NM_001127208.2; XM_005263082.1; XM_006714242.2; NM_017628.4; XM_011532044.1; and XM_011532043.1.

An example of the protein sequence of human Tet2 is provided as UniProt accession number Q6N021:

	10	20	30	40	50
	MEQDRTNHVE	GNRLSPFLIP	SPPICQTEPL	ATKLQNGSPL	PERAHPEVNG
	60	70	80	90	100
15	DTKWHSEFKSY	YGIPCMKGSQ	NSRVSPDFTQ	ESRGYSKCLQ	NGGIKRTVSE
	110	120	130	140	150
	PSLSGLLQIK	KLKQDQKANG	ERRNFGVSQE	RNPGESSQPN	VSDLSDKKES
	160	170	180	190	200
	VSSVAQENAV	KDFTSFSTHN	CSGPENPELQ	ILNEQEGKSA	NYHDKNIVLL
20	210	220	230	240	250
	KNKAVLMPNG	ATVSASSVEH	THGELLEKTL	SQYYPDCVSI	AVQKTTSHIN
	260	270	280	290	300
	AINSQATNEL	SCEITHPSHT	SGQINSAQTS	NSELPPKPAA	VVSEACDADD
	310	320	330	340	350
25	ADNASKLAAM	LNTCSFQKPE	QLQQQKSVFE	ICPSPAENNI	QGTTKLASGE
	360	370	380	390	400
	EFCSGSSSNL	QAPGGSSERY	LKQNEMNAY	FKQSSVFTKD	SFSATTTPPP
	410	420	430	440	450
	PSQLLLSPPP	PLPQVPQLPS	EGKSTLNGGV	LEEHHHYPNQ	SNTTLLREVK
30	460	470	480	490	500
	IEGKPEAPPS	QSPNPSTHVC	SPSPMLSERP	QNNCVNRNDI	QTAGTMTVPL
	510	520	530	540	550
	CSEKTRPMSE	HLKHNPPIFG	SSGELQDNCQ	QLMRNKEQEI	LKGRDKEQTR
	560	570	580	590	600
35	DLVPPTQHYL	KPGWIELKAP	RFHQAESHLK	RNEASLPSIL	QYQPNLSNQM
	610	620	630	640	650
	TSKQYTGNSN	MPGGLPRQAY	TQKTTQLEHK	SQMYQVEMNQ	GQSQGTVDQH
	660	670	680	690	700
	LQFQKPSHQV	HFSKTDHLPK	AHVQSLCGTR	FHFQQRADSQ	TEKLMSPVLK
40	710	720	730	740	750
	QHLNQQASET	EPFSNSHLLQ	HKPHKQAAQT	QPSQSSHLPQ	NQQQQQKLQI
	760	770	780	790	800
	KNKEEILQTF	PHPQSNNDQQ	REGSFFGQTK	VEECFHGENQ	YSKSSEFETH
	810	820	830	840	850
45	NVQMGLEEVQ	NINRRNSPYS	QTMKSSACKI	QVSCSNNTHL	VSENKEQTTH
	860	870	880	890	900
	PELFAGNKTQ	NLHHMQYFPN	NVIPKQDLLH	RCFQEQEQKS	QQASVLQGYK
	910	920	930	940	950
	NRNQDMSGQQ	AAQLAQQRYL	IHNHANVFPV	PDQGGSTQT	PPQKDTQKHA
50	960	970	980	990	1000

	ALRWHLLOKQ	EQQQTQQPQT	ESCHSQMHRP	IKVEPGCKPH	ACMHTAPPEN
	1010	1020	1030	1040	1050
	KTWKKVTKQE	NPPASCDNVQ	QKSIIETMEQ	HLKQFHAKSL	FDHKALTLKS
	1060	1070	1080	1090	1100
5	QKQVKVEMSG	PVTVLTRQTT	AAELDSHTPA	LEQQTTSSEK	TPTKRTAASV
	1110	1120	1130	1140	1150
	LNNFIESPSK	LLDTPIKNLL	DTPVKTQYDF	PSCRCVEQII	EKDEGPFYTH
	1160	1170	1180	1190	1200
	LGAGPNVAAI	REIMEERFGQ	KGKAIRIERV	IYTGKEGKSS	QGCPIAKWVV
10	1210	1220	1230	1240	1250
	RRSSSEKLL	CLVRERAGHT	CEAAVIVILI	LVWEGIPLSL	ADKLYSELTE
	1260	1270	1280	1290	1300
	TLRKYGTLTN	RRCALNEERT	CACQGLDPET	CGASFSGGCS	WSMYNGCKF
	1310	1320	1330	1340	1350
15	ARSKIPRKF	LLGDDPKEEE	KLESHLQNL	TLMAPTYKKL	APDAYNNQIE
	1360	1370	1380	1390	1400
	YEHRAPECRL	GLKEGRPFSG	VTACLDFFCAH	AHRDLHNMQN	GSTLVCTLTR
	1410	1420	1430	1440	1450
	EDNREFGGKP	EDEQLHVLPL	YKVSDVDEFG	SVEAQEEKKR	SGAIQVLSSF
20	1460	1470	1480	1490	1500
	RRKVRMLAEP	VKTCRQRKLE	AKKAAAEKLS	SLNSSNKNE	KEKSAPSRTK
	1510	1520	1530	1540	1550
	QTENASQAKQ	LAELLRLSGP	VMQQSQQPQP	LQKQPPQPQQ	QQRFPQQQPH
	1560	1570	1580	1590	1600
25	HPQTESVNSY	SASGSTNPYM	RRPNPVSPYP	NSSHTSDIYG	STSPMNFYST
	1610	1620	1630	1640	1650
	SSQAAGSYLN	SSNPMNPYPG	LLNQNTQYPS	YQCNGNLSVD	NCSPYLGSYS
	1660	1670	1680	1690	1700
	PQSQPMDLR	YPSQDPLSKL	SLPPIHTLYQ	PRFGNSQSFT	SKYLGYGNNQ
30	1710	1720	1730	1740	1750
	MQGDGFSSCT	IRPNVHHVGK	LPPYPTHEMD	GHFMGATSRL	PPNLSNPNMD
	1760	1770	1780	1790	1800
	YKNGEHHSPS	HIIHNYSAAP	GMFNSSLHAL	HLQNKENDML	SHTANGLSKM
	1810	1820	1830	1840	1850
35	LPALNHDRTA	CVQGGGLHKLS	DANGQEKQPL	ALVQGVASGA	EDNDEVWSDS
	1860	1870	1880	1890	1900
	EQSFLDPDIG	GVAVAPTHGS	ILIECAKREL	HATTPLKNPN	RNHPTRISLV
	1910	1920	1930	1940	1950
	FYQHKSMNEP	KHGLALWEAK	MAEKAREKEE	ECEKYGPDYV	PQKSHGKKVK
40	1960	1970	1980	1990	2000
	REPAEPHETS	EPTYLRFIKS	LAERTMSVTT	DSTVTTSPYA	FTRVTGPYNR
	2002				
	YI				
		[SEQ ID NO: 1357]			

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The tet2 gene is located on chromosome 4, location GRCh38.p2 (GCF_000001405.28) (NC_000004.12 (105145875 to 105279803); Gene ID 54790).

Examples of nucleic acid sequences encoding Tet2 are provided below. There are 6 identified isoforms of human Tet2 have been identified. The mRNA sequences are provided below (In
embodiments, in each sequence, T may be replaced with U). In embodiments, Tet2 includes the
proteins encoded by each of the sequences below:

Name	NCBI Reference Sequence	Sequence
Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant 1, mRNA [SEQ ID NO: 1358]	NM_001127208.2	GGCAGTGGCAGCGGCGAGAGCTTGGGCGGCCGCGCCGCGC CTCCTCGCGAGCGCCGCGCGCCCGGGTCCCG CTCGCATGCAAGTCACGTCCGCCCCCTCGGCGCGGCCGCC CCGAGACGCCGGCCCCGCTGAGTGATGAGA ACAGACGTCAAACCTGCCTTATGAATATTGATGCGGAGGC TAGGCTGCTTTCGTAGAGAAGCAGAAGGAAG CAAGATGGCTGCCCTTTAGGATTTGTTAGAAAGGAGACC CGACTGCAAACCTGCTGGATTGCTGCAAGGCTG AGGGACGAGAACGAGGCTGGCAAACATTCAGCAGCACA CCCTCTCAAGATTGTTTACTTGCCTTTGCTCC TGTTGAGTTACAACGCTTGGAAGCAGGAGATGGGCTCAG CAGCAGCCAATAGGACATGATCCAGGAAGAG CAGTAAGGGACTGAGCTGCTGAATTCAACTAGAGGGCAG CCTTGTTGGATGGCCCCGAAGCAAGCCTGATG GAACAGGATAGAACCAACCATGTTGAGGGCAACAGACTA AGTCCATTCTGATACCATCACCTCCCATT GCCAGACAGAACCTCTGGCTACAAAGCTCCAGAATGGAA GCCCACTGCCTGAGAGAGCTCATCCAGAAGT AAATGGAGACACCAAGTGGCACTCTTTCAAAGTTATTA TGGAATACCCTGTATGAAGGGAAGCCAGAAT AGTCGTGTGAGTCCTGACTTTACACAAGAAAGTAGAGGG TATTCCAAGTGTTTGCAAAATGGAGGAATAA AACGCACAGTTAGTGAACCTTCTCTCTCTGGGCTCCTTCA GATCAAGAAATTGAAACAAGACCAAAAGGC TAATGGAGAAAGACGTAACCTTCGGGGTAAGCCAAGAAAG AAATCCAGGTGAAAGCAGTCAACCAAATGTC TCCGATTTGAGTGATAAGAAAGAATCTGTGAGTTCTGTAG CCCAAGAAAATGCAGTTAAAGATTTACCA GTTTTTCAACACATAACTGCAGTGGGCCTGAAAATCCAG AGCTTCAGATTCTGAATGAGCAGGAGGGGAA AAGTGCTAATTACCATGACAAGAACATTGTATTACTTAAA AACAAGGCAGTGCTAATGCCTAATGGTGCT ACAGTTTCTGCCTCTTCCGTGGAACACACACATGGTGAAC TCCTGGAAAAAACACTGTCTCAATATTATC CAGATTGTGTTTCCATTGCGGTGCAGAAAACCACATCTCA CATAAATGCCATTAACAGTCAGGCTACTAA TGAGTTGTCCTGTGAGATCACTACCCATCGCATACTCA GGGCAGATCAATTCCGCACAGACCTCTAAC TCTGAGCTGCCTCCAAAGCCAGCTGCAGTGGTGAGTGAG GCCTGTGATGCTGATGATGCTGATAATGCCA GTAAACTAGCTGCAATGCTAAATACCTGTTTCCTTTCAGAA ACCAGAACAACCTACAACAACAAAAATCAGT TTTTGAGATATGCCCATCTCCTGCAGAAAATAACATCCAG GGAACCACAAAGCTAGCGTCTGGTGAAGAA TTCTGTTCAAGTTCCAGCAGCAATTTGCAAGCTCCTGGTG GCAGCTCTGAACGGTATTTAAAACAAAATG AAATGAATGGTGCTTACTTCAAGCAAAGCTCAGTGTTTAC TAAGGATTCTTTTTCTGCCACTACCACACC ACCACCACCATCACAATTGCTTCTTTCTCCCCCTCCTCCTC TTCCACAGGTTCTCAGCTTCTTTCAGAA GGAAAAAGCACTCTGAATGGTGGAGTTTTAGAAGAACAC

	CACCCTACCCCAACCAAAGTAACACAACAC TTTTAAGGGAAGTGAAAATAGAGGGTAAACCTGAGGCAC CACCTTCCCAGAGTCCTAATCCATCTACACA TGTATGCAGCCCTTCTCCGATGCTTTCTGAAAGGCCTCAG AATAATTGTGTGAACAGGAATGACATACAG ACTGCAGGGACAATGACTGTTCCATTGTGTTCTGAGAAA ACAAGACCAATGTCAGAACACCTCAAGCATA ACCCACCAATTTTTGGTAGCAGTGGAGAGCTACAGGACA ACTGCCAGCAGTTGATGAGAAACAAAGAGCA AGAGATTCTGAAGGGTCGAGACAAGGAGCAAACACGAG ATCTTGTGCCCCCAACACAGCACTATCTGAAA CCAGGATGGATTGAATTGAAGGCCCTCGTTTTTCACCAAG CGGAATCCCATCTAAAACGTAATGAGGCAT CACTGCCATCAATTCTTCAGTATCAACCCAATCTCTCCAA TCAAATGACCTCCAAACAATACACTGGAAA TTCCAACATGCCTGGGGGGCTCCCAAGGCAAGCTTACAC CCAGAAAACAACACAGCTGGAGCACAAGTCA CAAATGTACCAAGTTGAAATGAATCAAGGGCAGTCCCAA GGTACAGTGGACCAACATCTCCAGTTCCAAA AACCCTCACACCAGGTGCACTTCTCCAAAACAGACCATTT ACCAAAAGCTCATGTGCAGTCACTGTGTGG CACTAGATTTTCATTTTCAACAAAGAGCAGATTCCCAAAC GAAAAACTTATGTCCCCAGTGTTGAAACAG CACTTGAATCAACAGGCTTCAGAGACTGAGCCATTTTCAA ACTCACACCTTTTGCAACATAAGCCTCATA AACAGGCAGCACAAACACAACCATCCCAGAGTTCACATC TCCCTCAAACACAGCAACAGCAGCAAAAATT ACAAATAAAGAATAAAGAGGAAATACTCCAGACTTTTCC TCACCCCCAAAGCAACAATGATCAGCAAAGA GAAGGATCATTCTTTGGCCAGACTAAAGTGGAAGAATGT TTTCATGGTGAAAATCAGTATTCAAATCAA GCGAGTTCGAGACTCATAATGTCCAAATGGGACTGGAGG AAGTACAGAATATAAATCGTAGAAATTCCCC TTATAGTCAGACCATGAAATCAAGTGCATGCAAAATACA GGTTTCTTGTTCAAACAATACACACCTAGTT TCAGAGAATAAAGAACAGACTACACATCCTGAACTTTTT GCAGGAAACAAGACCCCAAACTTGCATCACA TGCAATATTTTCCAAATAATGTGATCCCAAAGCAAGATCT TCTTCACAGGTGCTTTCAAGAACAGGAGCA GAAGTCACAACAAGCTTCAGTTCTACAGGGATATAAAAA TAGAAACCAAGATATGTCTGGTCAACAAGCT GCGCAACTTGCTCAGCAAAGGTACTTGATACATAACCAT GCAAATGTTTTTCTGTGCCTGACCAGGGAG GAAGTCACACTCAGACCCCTCCCCAGAAGGACACTCAA AGCATGCTGCTCTAAGGTGGCATCTCTTACA GAAGCAAGAACAGCAGCAAACACAGCAACCCCCAACTG AGTCTTGCCATAGTCAGATGCACAGGCCAATT AAGGTGGAACCTGGATGCAAGCCACATGCCTGTATGCAC ACAGCACCACCAGAAAACAAAACATGGAAAA AGGTAATAAGCAAGAGAATCCACCTGCAAGCTGTGATA ATGTGCAGCAAAAGAGCATCATTGAGACCAT GGAGCAGCATCTGAAGCAGTTTCACGCCAAGTCGTTATTT GACCATAAGGCTCTTACTCTCAAATCACAG AAGCAAGTAAAAGTTGAAATGTCAGGGCCAGTCACAGTT

	TTGACTAGACAAACCACTGCTGCAGAACTTG ATAGCCACACCCCAGCTTTAGAGCAGCAAACAACTTCTTC AGAAAAGACACCAACCAAAAAGAACAGCTGC TTCTGTTCTCAATAATTTTATAGAGTCACCTTCCAAATTAC TAGATACTCCTATAAAAAAATTTATTGGAT ACACCTGTCAAGACTCAATATGATTTCCCATCTTGCAGAT GTGTAGAGCAAATTATTGAAAAAGATGAAG GTCCTTTTTTATACCCATCTAGGAGCAGGTCCTAATGTGGC AGCTATTAGAGAAATCATGGAAGAAAGGTT TGGACAGAAGGGTAAAGCTATTAGGATTGAAAGAGTCAT CTATACTGGTAAAGAAGGCAAAAAGTTCTCAG GGATGTCCTATTGCTAAGTGGGTGGTTCGCAGAAGCAGC AGTGAAGAGAAGCTACTGTGTTTGGTGCGGG AGCGAGCTGGCCACACCTGTGAGGCTGCAGTGATTGTGA TTCTCATCCTGGTGTGGGAAGGAATCCCGCT GTCTCTGGCTGACAACTCTACTCGGAGCTTACCGAGACG CTGAGGAAATACGGCACGCTCACCAATCGC CGGTGTGCCTTGAATGAAGAGAGAACTTGCGCCTGTCAG GGGCTGGATCCAGAAACCTGTGGTGCCTCCT TCTCTTTTGGTTGTTTCATGGAGCATGTACTACAATGGATG TAAGTTTGCCAGAAGCAAGATCCCAAGGAA GTTTAAGCTGCTTGGGGATGACCCAAAAGAGGAAGAGAA ACTGGAGTCTCATTTGCAAAACCTGTCCACT CTTATGGCACCAACATATAAGAAACTTGCACCTGATGCAT ATAATAATCAGATTGAATATGAACACAGAG CACCAGAGTGCCGTCTGGGTCTGAAGGAAGGCCGTCCAT TCTCAGGGGTCACTGCATGTTTGGACTTCTG TGCTCATGCCCACAGAGACTTGCACAACATGCAGAATGG CAGCACATTGGTATGCACTCTCACTAGAGAA GACAATCGAGAATTTGGAGGAAAACCTGAGGATGAGCAG CTTCACGTTCTGCCTTTATACAAAGTCTCTG ACGTGGATGAGTTTGGGAGTGTGGAAGCTCAGGAGGAGA AAAAACGGAGTGGTGCCATTCAGGTACTGAG TTCTTTTCGGCGAAAAGTCAGGATGTTAGCAGAGCCAGTC AAGACTTGCCGACAAAGGAACTAGAAAGCC AAGAAAGCTGCAGCTGAAAAGCTTTCCTCCCTGGAGAAC AGCTCAAATAAAAAATGAAAAGGAAAAGTCAG CCCCATCACGTACAAAACAACTGAAAACGCAAGCCAGG CTAAACAGTTGGCAGAAGTTTTGCGACTTTC AGGACCAGTCATGCAGCAGTCCCAGCAGCCCCAGCCTCT ACAGAAGCAGCCACCACAGCCCCAGCAGCAG CAGAGACCCCAGCAGCAGCAGCCACATCACCTCAGACA GAGTCTGTCAACTCTTATTCTGCTTCTGGAT CCACCAATCCATACATGAGACGGCCCAATCCAGTTAGTC CTTATCCAACTCTTCACACACTTCAGATAT CTATGGAAGCACCAGCCCTATGAACTTCTATTCCACCTCA TCTCAAGCTGCAGGTTTCATATTTGAATTCT TCTAATCCCATGAACCCTTACCCTGGGCTTTTGAATCAGA ATACCCAATATCCATCATATCAATGCAATG GAAACCTATCAGTGGACAAGTCTCCCCATATCTGGGTTC CTATTCTCCCCAGTCTCAGCCGATGGATCT GTATAGGTATCCAAGCCAAGACCCTCTGTCTAAGCTCAGT CTACCACCCATCCATACACTTTACCAGCCA AGGTTTGGAAATAGCCAGAGTTTTACATCTAAATACTTAG

	GTTATGGAAACCAAAATATGCAGGGAGATG GTTTCAGCAGTTGTACCATTAGACCAAATGTACATCATGT AGGGAAATTGCCTCCTTATCCCACCTCATGA GATGGATGGCCACTTCATGGGAGCCACCTCTAGATTACC ACCCAATCTGAGCAATCCAAACATGGACTAT AAAAATGGTGAACATCATTACCTTCTCACATAATCCATA ACTACAGTGCAGCTCCGGGCATGTTCAACA GCTCTCTTCATGCCCTGCATCTCCAAAACAAGGAGAATGA CATGCTTTCCACACAGCTAATGGGTTATC AAAGATGCTTCCAGCTCTTAACCATGATAGAACTGCTTGT GTCCAAGGAGGCTTACACAAATTAAGTGAT GCTAATGGTCAGGAAAAGCAGCCATTGGCACTAGTCCAG GGTGTGGCTTCTGGTGCAGAGGACAACGATG AGGTCTGGTCAGACAGCGAGCAGAGCTTTCTGGATCCTG ACATTGGGGGAGTGGCCGTGGCTCCAACCTCA TGGGTCAATTCTCATTGAGTGTGCAAAGCGTGAGCTGCAT GCCACAACCCCTTTAAAGAATCCCAATAGG AATCACCCACCAGGATCTCCCTCGTCTTTTACCAGCATA AGAGCATGAATGAGCCAAAACATGGCTTGG CTCTTTGGGAAGCCAAAATGGCTGAAAAAGCCCGTGAGA AAGAGGAAGAGTGTGAAAAGTATGGCCCAGA CTATGTGCCTCAGAAATCCCATGGCAAAAAAGTGAAACG GGAGCCTGCTGAGCCACATGAAACTTCAGAG CCCCTTACCTGCGTTTCATCAAGTCTCTTGCCGAAAGGA CCATGTCCGTGACCACAGACTCCACAGTAA CTACATCTCCATATGCCTTCACTCGGGTCACAGGGCCTTA CAACAGATATATATGATATCACCCCTTTT GTTGGTTACCTCACTTGAAAAGACCACAACCAACCTGTCA GTAGTATAGTTCTCATGACGTGGGCAGTGG GGAAAGGTCACAGTATTCATGACAAATGTGGTGGGAAAA ACCTCAGCTCACCAGCAACAAAAGAGGTTAT CTTACCATAGCACTTAATTTTCACTGGCTCCCAAGTGGTC ACAGATGGCATCTAGGAAAAGACCAAAGCA TTCTATGCAAAAAGAAGGTGGGGAAGAAAGTGTTCCGCA ATTTACATTTTTTAAACACTGGTTCTATTATT GGACGAGATGATATGTAAATGTGATCCCCCCCCCGCTT ACAACCTCTACACATCTGTGACCCTTTTAA TAATATCAAGTTTGCATAGTCATGGAACACAAATCAAAC AAGTACTGTAGTATTACAGTGACAGGAATCT TAAAATACCATCTGGTGCTGAATATATGATGTACTGAAAT ACTGGAATTATGGCTTTTTTGAAATGCAGTT TTTACTGTAATCTTAACTTTTATTTATCAAAATAGCTACA GGAAACATGAATAGCAGGAAAACACTGAAT TTGTTTGGATGTTCTAAGAAATGGTGCTAAGAAAATGGTG TCTTTAATAGCTAAAAATTTAATGCCTTTA TATCATCAAGATGCTATCAGTGTACTCCAGTGCCCTTGAA TAATAGGGGTACCTTTTCATTCAAGTTTTT ATCATAATTACCTATTCTTACACAAGCTTAGTTTTTAAAA TGTGGACATTTTAAAGGCCTCTGGATTTTG CTCATCCAGTGAAGTCCTTGTAAGGACAATAAACGTATATA TGTACATATATACACAAACATGTATATGTG CACACACATGTATATGTATAAATATTTTAAATGGTGTTTT AGAAGCACTTTGTCTACCTAAGCTTTGACA ACTTGAACAATGCTAAGGTACTGAGATGTTTAAAAAACA

	AGTTTACTTTCATTTT TAGAATGCAAAGTTGA TTTTTTTAAGGAAACAAAGAAAGCTTTTAAAATATTTTGTG CTTTTAGCCATGCATCTGCTGATGAGCAAT TGTGTCCATTTT AACACAGCCAGTTAAATCCACCATGGG GCTTACTGGATTCAAGGGAATACGTTAGTC CACAAAACATGTTTTCTGGTGCTCATCTCACATGCTATAC TGTA AACAGTTTTATACAAAATTGTATGA CAAGTTCATTGCTCAAAAATGTACAGTTTTAAGAATTTTC TATTA ACTGCAGGTAATAATTAGCTGCATG CTGCAGACTCAACAAAGCTAGTTCACTGAAGCCTATGCT ATTTTATGGATCATAGGCTCTTCAGAGAACT GAATGGCAGTCTGCCTTTGTGTTGATAATTATGTACATTG TGACGTTGTCATTTCTTAGCTTAAGTGTCC TCTTTAACAAGAGGATTGAGCAGACTGATGCCTGCATAA GATGAATAAACAGGGTTAGTTCCATGTGAAT CTGTCAGTTAAAAAGAAACAAAAACAGGCAGCTGGTTTG CTGTGGTGGTTTTAAATCATTAAATTTGTATA AAGAAGTGAAAGAGTTGTATAGTAAATTAAATTGTAAAC AAAAC TTTTTTAATGCAATGCTTTAGTATTT TAGTACTGTAAAAAAATTAAATATATACATATATATATAT ATATATATATATATATATATAGAGTTTGAAG CAGAATTCACATCATGATGGTGCTACTCAGCCTGCTACAA ATATATCATAATGTGAGCTAAGAATTCATT AAATGTTTGAGTGATGTTCCCTACTTGTCATATACCTCAAC ACTAGTTTGGCAATAGGATATTGAACTGAG AGTGAAAGCATTGTGTACCATCATTTTTTTCCAAGTCCTT TTTTTTATTGTTAAAAAAAAGCATACCT TTTTTCAATACTTGATTTCTTAGCAAGTATAACTTGAACTT CAACCTTTTTGTTCTAAAAATTCAGGGAT ATTCAGCTCATGCTCTCCCTATGCCAACATGTCACCTGT GTTTATGTAAAATTGTTGTAGGTTAATAAA TATATTCTTTGTCAGGGATTTAACCCTTTTATTTTGAATCC CTTCTATTTTACTTGTACATGTGCTGATG TAACTAAACTAATTTTGTAAATCTGTTGGCTCTTTTTATT GTAAAGAAAAGCATTTTAAAAGTTTGAGG AATCTTTTGACTGTTTCAAGCAGGAAAAAAAATTACAT GAAAATAGAATGCACTGAGTTGATAAAGGGA AAAATTGTAAGGCAGGAGTTTGGCAAGTGGCTGTTGGCC AGAGACTTACTTGTA ACTCTCTAAATGAAGT TTTTTTGATCCTGTAATCACTGAAGGTACATACTCCATGT GGACTTCCCTTAAACAGGCAAACACCTACA GGTATGGTGTGCAACAGATTGTACAATTACATTTTGGCCT AAATACATTTTTTGCTTACTAGTATTTAAAA TAAATTCTTAATCAGAGGAGGCCCTTTGGGTTTTATTGGTC AAATCTTTGTAAGCTGGCTTTTGTCTTTTT AAAAAATTTCTTGAATTTGTGGTTGTGTCCAATTTGCAAA CATTTCCAAAAATGTTTGCTTTGCTTACAA ACCACATGATTTTAATGTTTTTTGTATACCATAATATCTA GCCCCAAACATTTGATTACTACATGTGCAT TGGTGATTTTGATCATCCATTCTTAATATTTGATTTCTGTG TCACCTACTGTCATTTGT TAAACTGCTGG CCAACAAGAACAGGAAGTATAGTTTGGGGGGTTGGGGAG AGTTTACATAAGGAAGAGAAGAAATTGAGTG GCATATTGTAAATATCAGATCTATAATTGTAAATATAAAA

		CCTGCCTCAGTTAGAATGAATGGAAAGCAG ATCTACAATTTGCTAATATAGGAATATCAGGTTGACTATA TAGCCATACTTGAAAATGCTTCTGAGTGGT GTCAACTTTACTTGAATGAATTTTTCATCTTGATTGACGC ACAGTGATGTACAGTTCACTTCTGAAGCTA GTGGTTAACTTGTGTAGGAACTTTTGCAGTTTGACACTA AGATAACTTCTGTGTGCATTTTTCTATGCT TTTTTAAAACTAGTTTCATTTTCATTTTCATGAGATGTTTG GTTTATAAGATCTGAGGATGGTTATAAAT ACTGTAAGTATTGTAATGTTATGAATGCAGGTTATTTGAA AGCTGTTTATTATTATATCATTCCTGATAA TGCTATGTGAGTGTTTTTAATAAAATTTATATTTATTTAAT GCACTCTAAAAAAAAAAAAAAAAAAAAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X1, mRNA [SEQ ID NO: 1359]	XM_005263 082.1	AAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGGATTTGT TAGAAAGGAGACCCGACTGCAACTGCTGGAT TGCTGCAAGGCTGAGGGACGAGAACGAGAATTCAACTAG AGGGCAGCCTTGTGGATGGCCCCGAAGCAAG CCTGATGGAACAGGATAGAACCAACCATGTTGAGGGCAA CAGACTAAGTCCATTCTGATACCATCACCT CCCATTGCGCAGACAGAACCTCTGGCTACAAAGCTCCAG AATGGAAGCCCACTGCCTGAGAGAGCTCATC CAGAAGTAAATGGAGACACCAAGTGGCACTCTTTCAAAA GTTATTATGGAATACCCTGTATGAAGGGAAG CCAGAATAGTCGTGTGAGTCCTGACTTTACACAAGAAAG TAGAGGGTATTCCAAGTGTTTGCAAAATGGA GGAATAAAACGCACAGTTAGTGAACCTTCTCTCTCTGGGC TCCTTCAGATCAAGAAATTGAAACAAGACC AAAAGGCTAATGGAGAAAGACGTAACTTCGGGGTAAGCC AAGAAAGAAATCCAGGTGAAAGCAGTCAACC AAATGTCTCCGATTTGAGTGATAAGAAAGAATCTGTGAG TTCTGTAGCCCAAGAAAATGCAGTTAAAGAT TTCACCAGTTTTTCAACACATAACTGCAGTGGGCCTGAAA ATCCAGAGCTTCAGATTCTGAATGAGCAGG AGGGGAAAAGTGCTAATTACCATGACAAGAACATTGTAT TACTTAAAAACAAGGCAGTGCTAATGCCTAA TGGTGCTACAGTTTCTGCCTCTTCCGTGGAACACACACAT GGTGAACCTCCTGGAAAAAACACTGTCTCAA TATTATCCAGATTGTGTTTCCATTGCGGTGCAGAAAACCA CATCTCACATAAATGCCATTAACAGTCAGG CTACTAATGAGTTGTCCTGTGAGATCACTCACCCATCGCA TACCTCAGGGCAGATCAATTCCGCACAGAC CTCTAACTCTGAGCTGCCTCCAAAGCCAGCTGCAGTGGTG AGTGAGGCCTGTGATGCTGATGATGCTGAT AATGCCAGTAACTAGCTGCAATGCTAAATACCTGTTCT TTCAGAAACCAGAACAACTACAACAACAAA AATCAGTTTTTTGAGATATGCCCATCTCCTGCAGAAAATAA CATCCAGGGAACCAAAAGCTAGCGTCTGG TGAAGAATTCTGTTTCAGGTTCCAGCAGCAATTTGCAAGCT CCTGGTGGCAGCTCTGAACGGTATTTAAAA CAAAATGAAATGAATGGTGCTTACTTCAAGCAAAGCTCA GTGTTCACTAAGGATTCTTTTTCTGCCACTA CCACACCACCACCACCATCACAATTGCTTCTTTCTCCCCC TCCTCCTCTTCCACAGGTTCTCAGCTTCC TTCAGAAGGAAAAAGCACTCTGAATGGTGGAGTTTTAGA

	AGAACACCACCACTACCCCAACCAAAGTAAC ACAACACTTTTAAAGGGAAGTGAAAATAGAGGGGTAAACCT GAGGCACCACCTTCCCAGAGTCCTAATCCAT CTACACATGTATGCAGCCCTTCTCCGATGCTTTCTGAAAG GCCTCAGAATAATTGTGTGAACAGGAATGA CATACAGACTGCAGGGACAATGACTGTTCCATTGTGTTCT GAGAAAACAAGACCAATGTCAGAACACCTC AAGCATAACCCACCAATTTTTTGGTAGCAGTGGAGAGCTA CAGGACAACCTGCCAGCAGTTGATGAGAAACA AAGAGCAAGAGATTCTGAAGGGTCGAGACAAGGAGCAA ACACGAGATCTTGTGCCCCCAACACAGCACTA TCTGAAACCAGGATGGATTGAATTGAAGGCCCTCGTTTT CACCAAGCGGAATCCCATCTAAAACGTAAT GAGGCATCACTGCCATCAATTCTTCAGTATCAACCCAATC TCTCCAATCAAATGACCTCCAAACAATACA CTGGAAATTCCAACATGCCTGGGGGGCTCCCAAGGCAAG CTTACACCCAGAAAACAACACAGCTGGAGCA CAAGTCACAAATGTACCAAGTTGAAATGAATCAAGGGCA GTCCCAAGGTACAGTGGACCAACATCTCCAG TTCCAAAAACCCTCACACCAGGTGCACTTCTCCAAAACA GACCATTTACCAAAAGCTCATGTGCAGTCAC TGTGTGGCACTAGATTTTCATTTTCAACAAAGAGCAGATTC CCAAAGTGAACAACTTATGTCCCCAGTGTT GAAACAGCACTTGAATCAACAGGCTTCAGAGACTGAGCC ATTTTCAAACCTCACACCTTTTGCAACATAAG CCTCATAAACAGGCAGCACAAACACAACCATCCCAGAGT TCACATCTCCCTCAAAACCAGCAACAGCAGC AAAAATTACAAATAAAGAATAAAGAGGAAATACTCCAG ACTTTTCCTCACCCCCAAAGCAACAATGATCA GCAAAGAGAAGGATCATTCTTTGGCCAGACTAAAGTGGA AGAATGTTTTTCATGGTGAAAATCAGTATTCA AAATCAAGCGAGTTCGAGACTCATAATGTCCAAATGGGA CTGGAGGAAGTACAGAATATAAATCGTAGAA ATTCCCCTTATAGTCAGACCATGAAATCAAGTGCATGCAA AATACAGGTTTCTTGTTCAAACAATACACA CCTAGTTTCAGAGAATAAAGAACAGACTACACATCCTGA ACTTTTTGCAGGAAACAAGACCCAAAACCTTG CATCACATGCAATATTTTCCAAATAATGTGATCCCAAAGC AAGATCTTCTTCACAGGTGCTTTCAAGAAC AGGAGCAGAAGTCACAACAAGCTTCAGTTCTACAGGGAT ATAAAAATAGAAACCAAGATATGTCTGGTCA ACAAGCTGCGCAACTTGCTCAGCAAAGGTACTTGATACA TAACCATGCAAATGTTTTTCTGTGCCTGAC CAGGGAGGAAGTCACACTCAGACCCCTCCCCAGAAGGAC ACTCAAAGCATGCTGCTCTAAGGTGGCATC TCTTACAGAAGCAAGAACAGCAGCAAACACAGCAACCCC AAAGTGAAGTCTTGCCATAGTCAGATGCACAG GCCAATTAAGGTGGAACCTGGATGCAAGCCACATGCCTG TATGCACACAGCACCACCAGAAAACAAAACA TGGAAAAAGGTAAGCAAGAGAATCCACCTGCAAGC TGTGATAATGTGCAGCAAAAGAGCATCATTG AGACCATGGAGCAGCATCTGAAGCAGTTTCACGCCAAGT CGTTATTTGACCATAAGGCTCTTACTCTCAA ATCACAGAAGCAAGTAAAAGTTGAAATGTCAGGGCCAGT

	CACAGTTTTGACTAGACAAACCACTGCTGCA GAACTTGATAGCCACACCCCAGCTTTAGAGCAGCAAACA ACTTCTTCAGAAAAGACACCAACCAAAAAGAA CAGCTGCTTCTGTTCTCAATAATTTTATAGAGTCACCTTCC AAATTACTAGATACTCCTATAAAAAAATTT ATTGGATACACCTGTCAAGACTCAATATGATTTCCCATCT TGCAGATGTGTAGAGCAAATTATTGAAAAA GATGAAGGTCCTTTTTTATACCCATCTAGGAGCAGGTCCTA ATGTGGCAGCTATTAGAGAAATCATGGAAG AAAGGTTTGGACAGAAGGGTAAAGCTATTAGGATTGAAA GAGTCATCTATACTGGTAAAGAAGGCAAAAAG TTCTCAGGGATGTCCTATTGCTAAGTGGGTGGTTCGCAGA AGCAGCAGTGAAGAGAAGCTACTGTGTTTG GTGCGGGAGCGAGCTGGCCACACCTGTGAGGCTGCAGTG ATTGTGATTCTCATCCTGGTGTGGGAAGGAA TCCCGCTGTCTCTGGCTGACAACTCTACTCGGAGCTTAC CGAGACGCTGAGGAAATACGGCACGCTCAC CAATCGCCGGTGTGCCTTGAATGAAGAGAGAACTTGCGC CTGTCAGGGGCTGGATCCAGAAACCTGTGGT GCCTCCTTCTCTTTTGGTTGTTTCATGGAGCATGTACTACA ATGGATGTAAGTTTGCCAGAAGCAAGATCC CAAGGAAGTTTAAGCTGCTTGGGGATGACCCAAAAGAGG AAGAGAACTGGAGTCTCATTTGCAAAACCT GTCCACTCTTATGGCACCAACATATAAGAACTTGACACCT GATGCATATAATAATCAGATTGAATATGAA CACAGAGCACCAGAGTGCCGTCTGGGTCTGAAGGAAGGC CGTCCATTCTCAGGGGCTCACTGCATGTTTGG ACTTCTGTGCTCATGCCCACAGAGACTTGCACAACATGCA GAATGGCAGCACATTGGTATGCACTCTCAC TAGAGAAGACAATCGAGAATTTGGAGGAAAACCTGAGG ATGAGCAGCTTCACGTTCTGCCTTTATACAAA GTCTCTGACGTGGATGAGTTTGGGAGTGTGGAAGCTCAG GAGGAGAAAAAACGGAGTGGTGCCATTCAGG TACTGAGTTCTTTTCGGCGAAAAGTCAGGATGTTAGCAGA GCCAGTCAAGACTTGCCGACAAAGGAACT AGAAGCCAAGAAAGCTGCAGCTGAAAAGCTTTCCTCCCT GGAGAACAGCTCAAATAAAAAATGAAAAGGAA AAGTCAGCCCCATCACGTACAAAACAACTGAAAACGCA AGCCAGGCTAAACAGTTGGCAGAACTTTTGC GACTTTCAGGACCAGTCATGCAGCAGTCCCAGCAGCCCC AGCCTCTACAGAAGCAGCCACCACAGCCCCA GCAGCAGCAGAGACCCAGCAGCAGCAGCCACATCACCC TCAGACAGAGTCTGTCAACTCTTATTCTGCT TCTGGATCCACCAATCCATACATGAGACGGCCCAATCCA GTTAGTCCTTATCCAACTCTTCACACACTT CAGATATCTATGGAAGCACCAGCCCTATGAACTTCTATTC CACCTCATCTCAAGCTGCAGGTTTCATATT GAATTCTTCTAATCCCATGAACCCTTACCCTGGGCTTTTG AATCAGAATACCCAATATCCATCATATCAA TGCAATGGAAACCTATCAGTGGACAACCTGCTCCCCATATC TGGGTTCCTATTCTCCCCAGTCTCAGCCGA TGGATCTGTATAGGTATCCAAGCCAAGACCCTCTGTCTAA GCTCAGTCTACCACCCATCCATACACTTTA CCAGCCAAGGTTTGGAAATAGCCAGAGTTTACATCTAA

	ATACTTAGGTTATGGAAACCAAAATATGCAG GGAGATGGTTTCAGCAGTTGTACCATTAGACCAAATGTA CATCATGTAGGGAAATTGCCTCCTTATCCCA CTCATGAGATGGATGGCCACTTCATGGGAGCCACCTCTA GATTACCACCCAATCTGAGCAATCCAAACAT GGACTATAAAAATGGTGAACATCATTACCTTCTCACATA ATCCATAACTACAGTGCAGCTCCGGGCATG TTCAACAGCTCTCTTCATGCCCTGCATCTCCAAAACAAGG AGAATGACATGCTTTCCACACAGCTAATG GGTTATCAAAGATGCTTCCAGCTCTTAACCATGATAGAAC TGCTTGTGTCCAAGGAGGCTTACACAAATT AAGTGATGCTAATGGTCAGGAAAAGCAGCCATTGGCACT AGTCCAGGGTGTGGCTTCTGGTGCAGAGGAC AACGATGAGGTCTGGTCAGACAGCGAGCAGAGCTTTCTG GATCCTGACATTGGGGGAGTGGCCGTGGCTC CAACTCATGGGTCAATTCTCATTGAGTGTGCAAAGCGTGA GCTGCATGCCACAACCCCTTTAAAGAATCC CAATAGGAATCACCCACCAGGATCTCCCTCGTCTTTTAC CAGCATAAGAGCATGAATGAGCCAAAACAT GGCTTGGCTCTTTGGGAAGCCAAAATGGCTGAAAAAGCC CGTGAGAAAGAGGAAGAGTGTGAAAAGTATG GCCCAGACTATGTGCCTCAGAAATCCCATGGCAAAAAG TGAAACGGGAGCCTGCTGAGCCACATGAAAC TTCAGAGCCCCTTACCTGCGTTTCATCAAGTCTCTTGCC GAAAGGACCATGTCCGTGACCACAGACTCC ACAGTAACTACATCTCCATATGCCTTCACTCGGGTCACAG GGCTTACAACAGATATATATGATATCACC CCCTTTTGTTGGTTACCTCACTTGAAAAGACCACAACCAA CCTGTCAGTAGTATAGTTCTCATGACGTGG GCAGTGGGGAAAGGTCACAGTATTCATGACAAATGTGGT GGGAAAAACCTCAGCTCACCAGCAACAAAAG AGGTTATCTTACCATAGCACTTAATTTTCACTGGCTCCCA AGTGGTCACAGATGGCATCTAGGAAAAGAC CAAAGCATTCTATGCAAAAAGAAGGTGGGGAAGAAAGT GTTCCGCAATTTACATTTTAAACACTGGTTC TATTATTGGACGAGATGATATGTAAATGTGATCCCCCCCC CCCGCTTACAACCTCTACACATCTGTGACCA CTTTAAATAATATCAAGTTTGCATAGTCATGGAACACAAA TCAAACAAGTACTGTAGTATTACAGTGACA GGAATCTTAAAATACCATCTGGTGCTGAATATATGATGTA CTGAAATACTGGAATTATGGCTTTTTTGAAA TGCAGTTTTTACTGTAATCTTAACTTTTATTTATCAAATA GCTACAGGAAACATGAATAGCAGGAAAAC ACTGAATTTGTTTGGATGTTCTAAGAAATGGTGCTAAGAA AATGGTGTCTTTAATAGCTAAAAATTTAAT GCCTTTATATCATCAAGATGCTATCAGTGTACTCCAGTGC CCTTGAATAATAGGGGTACCTTTTCATTCA AGTTTTTATCATAATTACCTATTCTTACACAAGCTTAGTTT TTAAAATGTGGACATTTTAAAGGCCTCTG GATTTTGCTCATCCAGTGAAGTCCTTGTAGGACAATAAAC GTATATATGTACATATATACACAAACATGT ATATGTGCACACACATGTATATGTATAAATATTTTAAATG GTGTTTTAGAAAGCACTTTGTCTACCTAAGC TTTGACAACCTGAACAATGCTAAGGTACTGAGATGTTTAA

	AAAACAAGTTTACTTTCATTTTAGAATGCA AAGTTGATTTTTTTAAGGAAACAAAGAAAGCTTTTAAAAT ATTTTGTCTTTTAGCCATGCATCTGCTGAT GAGCAATTGTGTCCATTTTAAACACAGCCAGTTAAATCCA CCATGGGGCTTACTGGATTCAAGGGAATAC GTTAGTCCACAAAACATGTTTTCTGGTGCTCATCTCACAT GCTATACTGTAAAACAGTTTTATACAAAAT TGTATGACAAGTTCATTGCTCAAAAATGTACAGTTTTAAG AATTTTCTATTAAGTGCAGGTAATAATTAG CTGCATGCTGCAGACTCAACAAAGCTAGTTCAGTGAAGC CTATGCTATTTTATGGATCATAGGCTCTTCA GAGAACTGAATGGCAGTCTGCCTTTGTGTTGATAATTATG TACATTGTGACGTTGTCATTTCTTAGCTTA AGTGTCCCTCTTTAACAAGAGGATTGAGCAGACTGATGCCT GCATAAGATGAATAAACAGGGTTAGTTCCA TGTGAATCTGTCAGTTAAAAAGAAACAAAAACAGGCAGC TGGTTTGCTGTGGTGGTTTTAAATCATTAAT TTGTATAAAGAAGTGAAAGAGTTGTATAGTAAATTAAAT TGTAACAAAACCTTTTTTAATGCAATGCTTT AGTATTTTAGTACTGTAAAAAATTAAATATATACATATA TATATATATATATATATATATATATATATGAG TTTGAAGCAGAATTCACATCATGATGGTGCTACTCAGCCT GCTACAAATATATCATAATGTGAGCTAAGA ATTCATTAAATGTTTGAGTGATGTTCCCTACTTGTCATATA CCTCAACACTAGTTTGGCAATAGGATATTG AACTGAGAGTGAAAGCATTGTGTACCATCATTTTTTTTCCA AGTCCTTTTTTTTATTGTTAAAAAAG CATACCTTTTTTCAATACTTGATTTCTTAGCAAGTATAACT TGAACCTCAACCTTTTTGTTCTAAAAATT CAGGGATATTTAGCTCATGCTCTCCCTATGCCAACATGT CACCTGTGTTTATGTAAAATTGTTGTAGGT TAATAAATATATTCTTTGTCAGGGATTAAACCCTTTTATTT TGAATCCCTTCTATTTTACTTGTACATGT GCTGATGTAACATAAACTAATTTTGTAATCTGTTGGCTC TTTTTATTGTAAAGAAAAGCATTTTAAAAG TTTGAGGAATCTTTTACTGTTTCAAGCAGGAAAAAAA ATTACATGAAAATAGAATGCACTGAGTTGAT AAAGGGAAAAATTGTAAGGCAGGAGTTTGGCAAGTGGCT GTTGGCCAGAGACTTACTTGTAACCTCTCTAA ATGAAGTTTTTTTGATCCTGTAATCACTGAAGGTACATAC TCCATGTGGACTTCCCTTAAACAGGCAAAC ACCTACAGGTATGGTGTGCAACAGATTGTACAATTACATT TTGGCCTAAATACATTTTTGCTTACTAGTA TTTAAAATAAATTCTTAATCAGAGGAGGCCTTTGGGTTTT ATTGGTCAAATCTTTGTAAGCTGGCTTTTG TCTTTTAAAAAATTTCTTGAATTTGTGGTTGTGTCCAATT TGCAAACATTTCCAAAAATGTTTGCTTTG CTTACAAACCACATGATTTTAATGTTTTTTGTATACCATA ATATCTAGCCCCAACATTTGATTACTACA TGTGCATTGGTGATTTTGATCATCCATTCTTAATATTTGAT TTCTGTGTCACCTACTGTCATTTGTTAAA CTGCTGGCCAACAAGAACAGGAAGTATAGTTTGGGGGGT TGGGGAGAGTTTACATAAGGAAGAGAAGAAA TTGAGTGGCATATTGTAAATATCAGATCTATAATTGTAAA

		TATAAAACCTGCCTCAGTTAGAATGAATGG AAAGCAGATCTACAATTTGCTAATATAGGAATATCAGGT TGACTATATAGCCATACTTGAAAATGCTTCT GAGTGGTGTCAACTTTACTTGAATGAATTTTTTCATCTTGA TTGACGCACAGTGATGTACAGTTCACTTCT GAAGCTAGTGGTTAACTTGTGTAGGAACTTTTGCAGTTT GACACTAAGATAACTTCTGTGTGCATTTTT CTATGCTTTTTTTAAAACTAGTTTCATTTTCATTTTCATGAG ATGTTTGGTTTATAAGATCTGAGGATGGT TATAAATACTGTAAGTATTGTAATGTTATGAATGCAGGTT ATTTGAAAGCTGTTTATTATTATATCATTC CTGATAATGCTATGTGAGTGTTTTTAATAAAATTTATATT TATTTAATGCACTCTAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X2, mRNA [SEQ ID NO: 1360]	XM_006714 242.2	GTAGAGAAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGG ATTTGTTAGAAAGGAGACCCGACTGCAACTG CTGGATTGCTGCAAGGCTGAGGGACGAGAACGAGGCTGG CAAACATTCAGCAGCACACCCTCTCAAGATT GTTTACTTGCCTTTGCTCCTGTTGAGTTACAACGCTTGGA AGCAGGAGATGGGCTCAGCAGCAGCCAATA GGACATGATCCAGGAAGAGCAGTAAGGGACTGAGCTGCT GAATTCAACTAGAGGGCAGCCTTGTGGATGG CCCCGAAGCAAGCCTGATGGAACAGGATAGAACCAACCA TGTTGAGGGGCAACAGACTAAGTCCATTCCTG ATACCATCACCTCCCATTTGCCAGACAGAACCTCTGGCTA CAAAGCTCCAGAATGGAAGCCCACTGCCTG AGAGAGCTCATCCAGAAGTAAATGGAGACACCAAGTGGC ACTCTTTCAAAAGTTATTATGGAATACCCTG TATGAAGGGAAGCCAGAATAGTCGTGTGAGTCCTGACTT TACACAAGAAAGTAGAGGGTATTCCAAGTGT TTGCAAAATGGAGGAATAAAACGCACAGTTAGTGAACCT TCTCTCTCTGGGCTCCTTCAGATCAAGAAAT TGAAACAAGACCAAAAAGGCTAATGGAGAAAGACGTAAC TTCGGGGTAAGCCAAGAAAGAAATCCAGGTGA AAGCAGTCAACCAAATGTCTCCGATTTGAGTGATAAGAA AGAATCTGTGAGTTCTGTAGCCCAAGAAAAT GCAGTTAAAGATTTTACCAGTTTTTCAACACATAACTGCA GTGGGCCTGAAAATCCAGAGCTTCAGATTC TGAATGAGCAGGAGGGGAAAAGTGCTAATTACCATGACA AGAACATTGTATTACTTAAAAACAAGGCAGT GCTAATGCCTAATGGTGCTACAGTTTCTGCCTCTTCCGTG GAACACACACATGGTGAACCTCCTGGAAAAA ACACTGTCTCAATATTATCCAGATTGTGTTTCCATTGCGG TGCAGAAAACCACATCTCACATAAATGCCA TTAACAGTCAGGCTACTAATGAGTTGTCCTGTGAGATCAC TCACCCATCGCATACCTCAGGGCAGATCAA TTCCGCACAGACCTCTAACTCTGAGCTGCCTCCAAAGCCA GCTGCAGTGGTGAGTGAGGCCTGTGATGCT GATGATGCTGATAATGCCAGTAACTAGCTGCAATGCTA AATACCTGTTCTTTTCAGAAACCAGAACAAAC TACAACAACAAAAATCAGTTTTTGAGATATGCCCATCTCC TGCAGAAAATAACATCCAGGGAACCACAAA GCTAGCGTCTGGTGAAGAATTCTGTTTCAGGTTCCAGCAGC AATTTGCAAGCTCCTGGTGGCAGCTCTGAA CGGTATTTAAACAAAATGAAATGAATGGTGCTTACTTC

	AAGCAAAGCTCAGTGTTCACTAAGGATTCCT TTTCTGCCACTACCACACCACCACCACCATCACAATTGCT TCTTTCTCCCCCTCCTCCTCTTCCACAGGT TCCTCAGCTTCCTTCAGAAGGAAAAAGCACTCTGAATGGT GGAGTTTTAGAGAAGAACACCACCCTACCCC AACCAAAGTAACACAACACTTTTAAGGGAAGTGAAAATA GAGGGTAAACCTGAGGCACCACCTTCCCAGA GTCCTAATCCATCTACACATGTATGCAGCCCTTCTCCGAT GCTTTCTGAAAGGCCTCAGAATAATTGTGT GAACAGGAATGACATACAGACTGCAGGGACAATGACTGT TCCATTGTGTTCTGAGAAAACAAGACCAATG TCAGAACACCTCAAGCATAACCCACCAATTTTTGGTAGCA GTGGAGAGCTACAGGACAACCTGCCAGCAGT TGATGAGAAACAAAGAGCAAGAGATTCTGAAGGGTCGA GACAAGGAGCAAACACGAGATCTTGTGCCCCC AACACAGCACTATCTGAAACCAGGATGGATTGAATTGAA GGCCCCCTCGTTTTACCAAGCGGAATCCCAT CTAAAACGTAATGAGGCATCACTGCCATCAATTCTTCAGT ATCAACCCAATCTCTCCAATCAAATGACCT CCAAACAATACACTGGAAATTCCAACATGCCTGGGGGGC TCCCAAGGCAAGCTTACACCCAGAAAACAAC ACAGCTGGAGCACAAGTCACAAATGTACCAAGTTGAAAT GAATCAAGGGCAGTCCCAAGGTACAGTGGAC CAACATCTCCAGTTCCAAAAACCCTCACACCAGGTGCACT TCTCCAAAACAGACCATTTACCAAAAGCTC ATGTGCAGTCACTGTGTGGCACTAGATTTTCATTTTCAACA AAGAGCAGATTCCCAAACCTGAAAAACTTAT GTCCCCAGTGTTGAAACAGCACTTGAATCAACAGGCTTC AGAGACTGAGCCATTTTCAAACCTCACACCTT TTGCAACATAAGCCTCATAAACAGGCAGCACAAACACAA CCATCCCAGAGTTCACATCTCCCTCAAAACC AGCAACAGCAGCAAAAATTACAAATAAAGAATAAAGAG GAAATACTCCAGACTTTTCCTCACCCCCAAAG CAACAATGATCAGCAAAGAGAAGGATCATTCTTTGGCCA GACTAAAGTGGAAGAATGTTTTTCATGGTGAA AATCAGTATTCAAATCAAGCGAGTTCGAGACTCATAAT GTCCAAATGGGACTGGAGGAAGTACAGAATA TAAATCGTAGAAATTCCCCTTATAGTCAGACCATGAAATC AAGTGCAATGCAAAATACAGGTTTCTTGTTT AAACAATACACACCTAGTTTCAGAGAATAAAGAACAGAC TACACATCCTGAACTTTTTGCAGGAAACAAG ACCCAAAACCTTGCATCACATGCAATATTTTCCAAATAATG TGATCCCAAAGCAAGATCTTCTTCACAGGT GCTTTCAAGAACAGGAGCAGAAGTCACAACAAGCTTCAG TTCTACAGGGATATAAAAATAGAAACCAAGA TATGTCTGGTCAACAAGCTGCGCAACTTGCTCAGCAAAG GTAATTGATACATAACCATGCAAATGTTTTT CCTGTGCCTGACCAGGGAGGAAGTCACACTCAGACCCCT CCCCAGAAGGACACTCAAAGCATGCTGCTC TAAGGTGGCATCTCTTACAGAAGCAAGAACAGCAGCAAA CACAGCAACCCCCAAACTGAGTCTTGCCATAG TCAGATGCACAGGCCAATTAAGGTGGAACCTGGATGCAA GCCACATGCCTGTATGCACACAGCACCACCA GAAAACAAAACATGGAAAAAGGTAAGCAAGAGAA

	TCCACCTGCAAGCTGTGATAATGTGCAGCAAA AGAGCATCATTGAGACCATGGAGCAGCATCTGAAGCAGT TTCACGCCAAGTCGTTATTTGACCATAAGGC TCTTACTCTCAAATCACAGAAGCAAGTAAAAGTTGAAAT GTCAGGGCCAGTCACAGTTTTTGACTIONAGACAA ACCACTGCTGCAGAACTTGATAGCCACACCCCAGCTTTAG AGCAGCAAACAACCTTCTTCAGAAAAGACAC CAACCAAAAGAACAGCTGCTTCTGTTCTCAATAATTTTAT AGAGTCACCTTCCAAATTACTAGATACTCC TATAAAAAATTTATTGGATACACCTGTCAAGACTCAATAT GATTTCCCATCTTGCAGATGTGTAGGTTTG GACAGAAGGGTAAAGCTATTAGGATTGAAAGAGTCATCT ATACTGGTAAAGAAGGCAAAAGTTCTCAGGG ATGTCCTATTGCTAAGTGGGAGAACTTGCGCCTGTCAGGG GCTGGATCCAGAAACCTGTGGTGCCTCCTT CTCTTTTGGTTGTTTCATGGAGCATGTACTACAATGGATGT AAGTTTGCCAGAAGCAAGATCCCAAGGAAG TTTAAGCTGCTTGGGGATGACCCAAAAGAGGAAGAGAAA CTGGAGTCTCATTTGCAAAACCTGTCCACTC TTATGGCACCAACATATAAGAACTTGACCTGATGCAT ATAATAATCAGATTGAATATGAACACAGAGC ACCAGAGTGCCGTCTGGGTCTGAAGGAAGGCCGTCCATT CTCAGGGGTCACTGCATGTTTGGACTTCTGT GCTCATGCCCACAGAGACTTGACACAACATGCAGAATGGC AGCACATTGGTATGCACTCTCACTAGAGAAG ACAATCGAGAATTTGGAGGAAAACCTGAGGATGAGCAGC TTCACGTTCTGCCTTTATACAAAGTCTCTGA CGTGGATGAGTTTGGGAGTGTGGAAGCTCAGGAGGAGAA AAAACGGAGTGGTGCCATTCAGGTACTGAGT TCTTTTCGGCGAAAAGTCAGGATGTTAGCAGAGCCAGTC AAGACTTGCCGACAAAGGAACTAGAAAGCCA AGAAAGCTGCAGCTGAAAAGCTTTCCTCCCTGGAGAACA GCTCAAATAAAAATGAAAAGGAAAAGTCAGC CCCATCACGTACAAAACAACTGAAAACGCAAGCCAGGC TAAACAGTTGGCAGAACTTTTGCGACTTTCA GGACCAGTCATGCAGCAGTCCCAGCAGCCCCAGCCTCTA CAGAAGCAGCCACCACAGCCCCAGCAGCAGC AGAGACCCCAGCAGCAGCAGCCACATCACCTCAGACAG AGTCTGTCAACTCTTATTCTGCTTCTGGATC CACCAATCCATACATGAGACGGCCCAATCCAGTTAGTCCT TATCCAAACTCTTCACACACTTCAGATATC TATGGAAGCACCAGCCCTATGAACTTCTATTCCACCTCAT CTCAAGCTGCAGGTTTCATATTTGAATTCTT CTAATCCCATGAACCCTTACCCTGGGCTTTTGAATCAGAA TACCCAATATCCATCATATCAATGCAATGG AAACCTATCAGTGGACAACCTGCTCCCCATATCTGGGTTCC TATTCTCCCCAGTCTCAGCCGATGGATCTG TATAGGTATCCAAGCCAAGACCCTCTGTCTAAGCTCAGTC TACCACCCATCCATACACTTTACCAGCCAA GGTTTGGAAATAGCCAGAGTTTTACATCTAAATACTTAGG TTATGGAAACCAAAAATATGCAGGGAGATGG TTTCAGCAGTTGTACCATTAGACCAAATGTACATCATGTA GGGAAATTGCCTCCTTATCCCACTCATGAG ATGGATGGCCACTTCATGGGAGCCACCTCTAGATTACCAC

	CCAATCTGAGCAATCCAAACATGGACTATA AAAATGGTGAACATCATTCACCTTCTCACATAATCCATAA CTACAGTGCAGCTCCGGGCATGTTCAACAG CTCTCTTCATGCCCTGCATCTCCAAAACAAGGAGAATGAC ATGCTTTCCACACAGCTAATGGGTTATCA AAGATGCTTCCAGCTCTTAACCATGATAGAACTGCTTGTG TCCAAGGAGGCTTACACAAATTAAGTGATG CTAATGGTCAGGAAAAGCAGCCATTGGCACTAGTCCAGG GTGTGGCTTCTGGTGCAGAGGACAACGATGA GGTCTGGTCAGACAGCGAGCAGAGCTTTCTGGATCCTGA CATTGGGGGAGTGGCCGTGGCTCCAACTCAT GGGTCAATTCTCATTGAGTGTGCAAAGCGTGAGCTGCAT GCCACAACCCCTTTAAAGAATCCCAATAGGA ATCACCCACCAGGATCTCCCTCGTCTTTTACCAGCATAA GAGCATGAATGAGCCAAAACATGGCTTGGC TCTTTGGGAAGCCAAAATGGCTGAAAAAGCCCGTGAGAA AGAGGAAGAGTGTGAAAAGTATGGCCCAGAC TATGTGCCTCAGAAATCCCATGGCAAAAAAGTGAAACGG GAGCCTGCTGAGCCACATGAACTTCAGAGC CCACTTACCTGCGTTTCATCAAGTCTCTTGCCGAAAGGAC CATGTCCGTGACCACAGACTCCACAGTAAC TACATCTCCATATGCCTTCACTCGGGTCACAGGGCCTTAC AACAGATATATATGATATCACCCCTTTTG TTGGTTACCTCACTTGAAAAGACCACAACCAACCTGTCAG TAGTATAGTTCTCATGACGTGGGCAGTGGG GAAAGGTCACAGTATTCATGACAAATGTGGTGGGAAAAA CCTCAGCTCACCAGCAACAAAAGAGGTTATC TTACCATAGCACTTAATTTTCACTGGCTCCCAAGTGGTCA CAGATGGCATCTAGGAAAAGACCAAAGCAT TCTATGCAAAAAGAAGGTGGGGAAGAAAGTGTTCCGCAA TTTACATTTTAAACACTGGTTCTATTATTG GACGAGATGATATGTAAATGTGATCCCCCCCCCGCTTA CAACTCTACACATCTGTGACCACTTTTAAT AATATCAAGTTTGCATAGTCATGGAACACAAATCAAACA AGTACTGTAGTATTACAGTGACAGGAATCTT AAAATACCATCTGGTGTGCTGAATATATGATGTACTGAAAT ACTGGAATTATGGCTTTTTTGAATGCAGTTT TACTGTAATCTTAACCTTTTATTTATCAAAATAGCTACAG GAAACATGAATAGCAGGAAAACACTGAATT TGTTTGGATGTTCTAAGAAATGGTGCTAAGAAAATGGTGT CTTTAATAGCTAAAAATTTAATGCCTTTAT ATCATCAAGATGCTATCAGTGTACTCCAGTGCCCTTGAAT AATAGGGGTACCTTTTTCATTCAAGTTTTTA TCATAATTACCTATTCTTACACAAGCTTAGTTTTTAAAAT GTGGACATTTTAAAGGCCTCTGGATTTTGC TCATCCAGTGAAGTCCTTGTAGGACAATAAACGTATATAT GTACATATATACACAAACATGTATATGTGC ACACACATGTATATGTATAAATATTTTAAATGGTGTTTTA GAAGCACTTTGTCTACCTAAGCTTTGACAA CTTGAACAATGCTAAGGTACTGAGATGTTTAAAAAACAA GTTTACTTTTCATTTTAGAAATGCAAAGTTGAT TTTTTTAAGGAAACAAAGAAAGCTTTTAAAATATTTTTGC TTTAGCCATGCATCTGCTGATGAGCAATT GTGTCCATTTTAAACACAGCCAGTTAAATCCACCATGGGG

	CTTACTGGATTCAAGGGAATACGTTAGTCC ACAAAACATGTTTTCTGGTGCTCATCTCACATGCTATACT GTAAAACAGTTTTATACAAAATTGTATGAC AAGTTCATTGCTCAAAAATGTACAGTTTTAAGAATTTTCT ATTAAGTGCAGGTAATAATTAGCTGCATGC TGCAGACTCAACAAAGCTAGTTCCTACTGAAGCCTATGCTAT TTTATGGATCATAGGCTCTTCAGAGAACTG AATGGCAGTCTGCCTTTGTGTTGATAATTATGTACATTGT GACGTTGTCATTTCTTAGCTTAAGTGTCT CTTTAACAAGAGGATTGAGCAGACTGATGCCTGCATAAG ATGAATAAACAGGGTTAGTTCCATGTGAATC TGTCAGTTAAAAAGAAACAAAAACAGGCAGCTGGTTTGC TGTGGTGGTTTTAAATCATTAAATTTGTATAA AGAAGTGAAAGAGTTGTATAGTAAATTAAATTGTAAACA AACTTTTTTAATGCAATGCTTTAGTATTTT AGTACTGTAAAAAAATTAAATATATACATATATATATATA TATATATATATATATATATGAGTTTGAAGC AGAATTCACATCATGATGGTGCTACTCAGCCTGCTACAAA TATATCATAATGTGAGCTAAGAATTCATTA AATGTTTGAGTGATGTTCTACTTGTGCATATACCTCAACA CTAGTTTGGCAATAGGATATTGAACTGAGA GTGAAAGCATTGTGTACCATCATTTTTTTTCCAAGTCCTTTT TTTTATTGTTAAAAAAAAGCATACCTT TTTTCAATACTTGATTTCTTAGCAAGTATAACTTGAACCTC AACCTTTTTGTTCTAAAAATTCAGGGATA TTTCAGCTCATGCTCTCCCTATGCCAACATGTCACCTGTG TTTATGTAAAATTGTTGTAGGTTAATAAAT ATATTCTTTGTCAGGGATTAAACCCTTTTATTTTGAATCCC TTCTATTTTACTTGTACATGTGCTGATGT AACTAAACTAATTTTGTAAATCTGTTGGCTCTTTTTTATTG TAAAGAAAAGCATTTTAAAAGTTTGAGGA ATCTTTTGACTGTTTCAAGCAGGAAAAAAAATTACATG AAAATAGAATGCACTGAGTTGATAAAGGGAA AAATTGTAAGGCAGGAGTTTGGCAAGTGGCTGTTGGCCA GAGACTTACTTGTAACCTCTCTAAATGAAGTT TTTTTGATCCTGTAATCACTGAAGGTACATACTCCATGTG GACTTCCCTTAAACAGGCAAACACCTACAG GTATGGTGTGCAACAGATTGTACAATTACATTTTGGCCTA AATACATTTTGTCTTACTAGTATTTAAAAT AAATTCTTAATCAGAGGAGGCCTTTGGGTTTTATTGGTCA AATCTTTGTAAGCTGGCTTTTGTCTTTTAA AAAAATTTCTTGAATTTGTGGTTGTGTCCAATTTGCAAAC ATTTCCAAAAATGTTTGTCTTTGCTTACAAA CCACATGATTTTAATGTTTTTTGTATACCATAATATCTAGC CCCAAACATTTGATTACTACATGTGCATT GGTGATTTTGATCATCCATTCTTAATATTTGATTTCTGTGT CACCTACTGTCATTTGTTAAACTGCTGGC CAACAAGAACAGGAAGTATAGTTTGGGGGGTTGGGGAGA GTTTACATAAGGAAGAGAAGAAATTGAGTGG CATATTGTAAATATCAGATCTATAATTGTAAATATAAAAC CTGCCTCAGTTAGAATGAATGGAAAGCAGA TCTACAATTTGCTAATATAGGAATATCAGGTTGACTATAT AGCCATACTTGAAAATGCTTCTGAGTGGTG TCAACTTTACTTGAATGAATTTTTTCATCTTGATTGACGCA

		CAGTGATGTACAGTTCACCTTCTGAAGCTAG TGGTAACTTGTGTAGGAACTTTTGCAGTTTGACACTAA GATAACTTCTGTGTGCATTTTTCTATGCTT TTTTAAAACTAGTTTCATTTTCATTTTCATGAGATGTTTGG TTTATAAGATCTGAGGATGGTTATAAATA CTGTAAGTATTGTAATGTTATGAATGCAGGTTATTTGAAA GCTGTTTATTATTATATCATTCTGATAAT GCTATGTGAGTGTTTTTAATAAAATTTATATTTATTTAATG CACTCTAA
Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant 2, mRNA [SEQ ID NO: 1361]	NM_017628. 4	AAACAGAAGGTGGGCCGGGGCGGGGAGAAACAGAAGCTC GGTCAATTTCCCAGTTTGTCTGGGTCTTTAAAA ATACAGGCCCTTAAAGCACTAAGGGCATGCCCTCGGTGA AACAGGGGAGCGCTTCTGCTGAATGAGATTA AAGCGACAGAAAAGGGAAAGGAGAGCGCGGGCAACGGG ATCTAAAGGGAGATAGAGACGCGGGCCTCTGA GGGCTGGCAAACATTCAGCAGCACACCCTCTCAAGATTG TTTACTTGCCTTTGCTCCTGTTGAGTTACAA CGCTTGGGAAGCAGGAGATGGGCTCAGCAGCAGCCAATAG GACATGATCCAGGAAGAGCAGTAAGGGACTG AGCTGCTGAATTCAACTAGAGGGCAGCCTTGTGGATGGC CCCGAAGCAAGCCTGATGGAACAGGATAGAA CCAACCATGTTGAGGGCAACAGACTAAGTCCATTCTCTGA TACCATCACCTCCCATTTGCCAGACAGAACC TCTGGCTACAAAGCTCCAGAATGGAAGCCCAGTGCCTGA GAGAGCTCATCCAGAAGTAAATGGAGACACC AAGTGGCACTCTTTCAAAAGTTATTATGGAATACCCTGTA TGAAGGGAAGCCAGAATAGTCGTGTGAGTC CTGACTTTACACAAGAAAGTAGAGGGTATTCCAAGTGTTT GCAAAATGGAGGAATAAAACGCACAGTTAG TGAACCTTCTCTCTCTGGGCTCCTTCAGATCAAGAAATTG AAACAAGACCAAAAAGGCTAATGGAGAAAGA CGTAACTTCGGGGTAAGCCAAGAAAGAAATCCAGGTGAA AGCAGTCAACCAAATGTCTCCGATTTGAGTG ATAAGAAAGAATCTGTGAGTTCTGTAGCCCAAGAAAATG CAGTTAAAGATTTACCCAGTTTTTTCAACACA TAACTGCAGTGGGCCTGAAAATCCAGAGCTTCAGATTCT GAATGAGCAGGAGGGGAAAAGTGCTAATTAC CATGACAAGAACATTGTATTACTTAAAAACAAGGCAGTG CTAATGCCTAATGGTGCTACAGTTTCTGCCT CTTCCGTGGAACACACACATGGTGAATCCTGGAAAAAA CACTGTCTCAATATTATCCAGATTGTGTTTC CATTGCGGTGCAGAAAACCACATCTCACATAAATGCCAT TAACAGTCAGGCTACTAATGAGTTGTCCTGT GAGATCACTCACCCATCGCATACCTCAGGGCAGATCAAT TCCGCACAGACCTCTAACTCTGAGCTGCCTC CAAAGCCAGCTGCAGTGGTGAGTGAGGCCTGTGATGCTG ATGATGCTGATAATGCCAGTAAACTAGCTGC AATGCTAAATACCTGTTCTTTTCAGAAACCAGAACAACTA CAACAACAAAAATCAGTTTTTTGAGATATGC CCATCTCCTGCAGAAAATAACATCCAGGGAACCACAAAG CTAGCGTCTGGTGAAGAATTCTGTTTCAGGTT CCAGCAGCAATTTGCAAGCTCCTGGTGGCAGCTCTGAAC GGTATTTAAACAAAATGAAATGAATGGTGC TTACTTCAAGCAAAGCTCAGTGTTCACTAAGGATTCCTTT

	TCTGCCACTACCACACCACCACCACCATCA CAATTGCTTCTTTCTCCCCCTCCTCCTCTTCCACAGGTTCC TCAGCTTCCTTCAGAAGGAAAAAGCACTC TGAATGGTGGAGTTTTAGAAGAACACCACCCTACCCCA ACCAAAGTAACACAACACTTTTAAGGGAAGT GAAAATAGAGGGTAAACCTGAGGCACCACCTTCCCAGAG TCCTAATCCATCTACACATGTATGCAGCCCT TCTCCGATGCTTTCTGAAAGGCCTCAGAATAATTGTGTGA ACAGGAATGACATACAGACTGCAGGGACAA TGACTGTTCCATTGTGTTCTGAGAAAACAAGACCAATGTC AGAACACCTCAAGCATAACCCACCAATTTT TGGTAGCAGTGGAGAGCTACAGGACAACCTGCCAGCAGTT GATGAGAAACAAAGAGCAAGAGATTCTGAAG GGTCGAGACAAGGAGCAAACACGAGATCTTGTGCCCCCA ACACAGCACTATCTGAAACCAGGATGGATTG AATTGAAGGCCCTCGTTTTTCACCAAGCGGAATCCCATCT AAAACGTAATGAGGCATCACTGCCATCAAT TCTTCAGTATCAACCCAATCTCTCCAATCAAATGACCTCC AAACAATACACTGGAAATTCCAACATGCCT GGGGGGCTCCCAAGGCAAGCTTACACCCAGAAAACAACA CAGCTGGAGCACAAGTCACAAATGTACCAAG TTGAAATGAATCAAGGGCAGTCCCAAGGTACAGTGGACC AACATCTCCAGTTCCAAAAACCCTCACACCA GGTGCCTTCTCCAAAACAGACCATTTACCAAAAGCTCAT GTGCAGTCACTGTGTGGCACTAGATTTTCAT TTTCAACAAAGAGCAGATTCCCAAACCTGAAAACTTATG TCCCCAGTGTGAAACAGCACTTGAATCAAC AGGCTTCAGAGACTGAGCCATTTTCAAACCTCACACCTTTT GCAACATAAGCCTCATAAACAGGCAGCACA AACACAACCATCCCAGAGTTACATCTCCCTCAAAACCA GCAACAGCAGCAAAAATTACAAATAAAGAAT AAAGAGGAAATACTCCAGACTTTTCCTCACCCCCAAAGC AACAAATGATCAGCAAAGAGAAGGATCATTCT TTGGCCAGACTAAAGTGGAAGAATGTTTTTCATGGTGAAA ATCAGTATTCAAAATCAAGCGAGTTTCGAGAC TCATAATGTCCAAATGGGACTGGAGGAAGTACAGAATAT AAATCGTAGAAATTCCCCTTATAGTCAGACC ATGAAATCAAGTGCATGCAAAATACAGGTTTCTTGTTCAA ACAATACACACCTAGTTTCAGAGAATAAAG AACAGACTACACATCCTGAACTTTTTTGCAAGGAAACAAGA CCCAAACTTGCATCACATGCAATATTTTCC AAATAATGTGATCCCAAAGCAAGATCTTCTTCACAGGTG CTTTCAAGAACAGGAGCAGAAGTCACAACAA GCTTCAGTTCTACAGGGATATAAAAATAGAAACCAAGAT ATGTCTGGTCAACAAGCTGCGCAACTTGCTC AGCAAAGGTACTTGATACATAACCATGCAAATGTTTTTCC TGTGCCTGACCAGGGAGGAAGTCACACTCA GACCCCTCCCCAGAAGGACACTCAAAAGCATGCTGCTCT AAGGTGGCATCTCTTACAGAAGCAAGAACAG CAGCAAACACAGCAACCCCAAACCTGAGTCTTGCCATAGT CAGATGCACAGGCCAATTAAGGTGGAACCTG GATGCAAGCCACATGCCTGTATGCACACAGCACCACCAG AAAACAAAACATGGAAAAAGGTAACCTAAGCA AGAGAATCCACCTGCAAGCTGTGATAATGTGCAGCAAAA

		GAGCATCATTGAGACCATGGAGCAGCATCTG AAGCAGTTTCACGCCAAGTCGTTATTTGACCATAAGGCTC TACTCTCAAATCACAGAAGCAAGTAAAAG TTGAAATGTCAGGGCCAGTCACAGTTTTGACTAGACAAA CCACTGCTGCAGAACTTGATAGCCACACCCC AGCTTTAGAGCAGCAAACAACCTTCTTCAGAAAAGACACC AACCAAAAGAACAGCTGCTTCTGTTCTCAAT AATTTTATAGAGTCACCTTCCAAATTACTAGATACTCCTA TAAAAAATTTATTGGATACACCTGTCAAGA CTCAATATGATTTCCCATCTTGCAGATGTGTAGGTAAGTG CCAGAAATGTACTGAGACACATGGCGTTTA TCCAGAATTAGCAAATTTATCTTCAGATATGGGATTTTCC TTCTTTTTTTAAATCTTGAGTCTGGCAGCA ATTTGTAAAGGCTCATAAAAATCTGAAGCTTACATTTTTT GTCAAGTTACCGATGCTTGTGTCTTGTGAA AGAGAACTTCACTTACATGCAGTTTTTCCAAAAGAATTAA ATAATCGTGCATGTTTATTTTTCCCTCTCT TCAGATCCTGTAAAATTTGAATGTATCTGTTTTAGATCAA TTCGCCTATTTAGCTCTTTGTATATTATCT CCTGGAGAGACAGCTAGGCAGCAAAAAACAATCTATTA AAATGAGAAAATAACGACCATAGGCAGTCTA ATGTACGAACTTTAAATATTTTTTAATTCAAGGTAAAATA TATTAGTTTCACAAGATTTCTGGCTAATAG GGAAATTATTATCTTCAGTCTTCATGAGTTGGGGGAAATG ATAATGCTGACACTCTTAGTGCTCCTAAAG TTTCCTTTTCTCCATTTATACATTTGGAATGTTGTGATTTA TATTCATTTTGATTCCCTTTTCTCTAAAA TTTCATCTTTTTGATTAAAAAATATGATACAGGCATACCT CAGAGATATTGTGGGTTTGGCTCCATACCA CAATAAAATGAATATTACAATAAAGCAAGTTGTAAGGAC TTTTTGGTTTCTCACTGTATGTAAAAGTTAT TTATATACTATACTGTAACATACTAAGTGTGCAATAGCAT TGTGTCTAAAAAATATATACTTTAAAAATA ATTTATTGTTAAAAAAATGCCAACAATTATCTGGGCCTTT AGTGAGTGCTAATCTTTTTTGCTGGTGGAGG GTCGTGCTTCAGTATTGATCGCTGTGGACTGATCATGGTG GTAGTTGCTGAAGGTTGCTGGGATGGCTGT GTGTGTGGCAATTTCTTAAAATAAGACAACAGTGAAGTG CTGTATCAATTGATTTTTCCATTACAAAAG ATTTCTCTGTAGCATGCAATGCTGTTTGATAGCATTTAAC CCACAGCAGAATTTCTTTGAAAATTGGACT CAGTCCTCTCAAACGTGTGCTGCTGCTTTATCAACTAAGTT TTTGTAATTTTCTGAATCCTTTGTTGTCAT TTCAGCAGTTTACAGCATCTTCATTGGAAGTATATTCCAT CTCAAACATTCTTTGTTTCATCCATAAGAAG CAACTTCTTATCAAGTTTTTTCATGACATTGCAGTAACTC AGCCCCATCTTCAGGCTCTACTTCTAATTC TGGTTCTCTTGCTACATCTCCCTCATCTGCAGTGACCTCTC CACGGAAGTCTTGAACCTCCTCAAAGTAAT CCATGAGGGTTGGAATCAACTTCTAAACTCCTGTTAATGT TGATATATTGACCCCCCTCCCATGAATTATG AATGTTCTTAATAACTTCTAAATGGTGATACCTTTCCAGA AGGCTTTCAATGTACTTTGCCCGGATCCAT CAGAAGACTATCTTGGCAGCTGTAGACTAACAATATATTT

	CTTAAATGATAAGACTTGAAAGTCAAAAGT ACTCCTTAATCCATAGGCTGCAGAATCAATGTTGTATTAA CAGGCACGAAAACAGCATTAAATCTTGTGCA TCTCCATCGGAGCTCTTGGGTGACTAGGTGCCTTGAGCAG TAATATTTTGAAAGGAGGTTTTGGTTTTGT TTTTTGTTTTTTTTTTTTTGTTTTTTAGCAGTAAGTCTCAACA CTGGGCTTAAAATATTCAGTAAACTATG TTGTAAAAAGATGTGTTATCATCCAGACTTTGTTGTTCCA TACTCTACACAAGCAGGGTACACTTAGCA TAATTCTTAAGGGCCTTGGAATTTTCAGAATGGTAAATGA GTATGGGCTTCAACTTAAAATCATCAACTG CATTAGCCTGTAACAAGAGAGTCAGCCTGTCCTTTGAAGC AAGGCATTGACTTCTATCTATGAAAGTCTT AGATGGCACCTTGTTTCAATAGTAGGCTGTTTAGTACAGC CACCTTCATCAGTGATCTTAGCTAGATCTT CTGCATAACTTGCTGCAGCTTCTACATCAGCACTTGCTGC CTCACCTTGTCCTTTTATGTTATAGAGACA GCTGCGCTTCTTAAACTTTATAAACCAACTTCTGCTAGCT TCCAACTTCTCTTCTGCAGCTTCCTCATTC TCTTCATAGAACTGAAGGGAGTCAAGGCCTTGCTCTGGAT TAAGCTTTGGCTTAAGGAATGTTGTGGCTG ACGTGATCTTCTATCCAGACCACTAAAGCGCTCTCCATAT CAGCAATAAGGCCGTTTTGCTTTCTTACCT TTCATGTGTTCACTGGAGTAATTTCCCTTCAAGAATTTTTCC TTTACATTCACAACCTTGGCTAACTGGCAT GCAAGGCCTAGCTTTCAGCCTGTCTTGGCTTTTGACATGC CTTCCTCACTTAGCTCGTCATATCTAGCTT TTGATTTAAAGTGGCAGGCATACAACCTTTCCTTTCACTT GAACACTTAGAGGCCACTGTAGGGTTATTA ATTGGCCTAATTTCAATATTGTTGTGTTTTAGGGAATAGA GAGGCCAGGGAGAGGGAGAGAGCCCAAAC GGCTGGTTGATAGAGCAGGCAGAATGCACACAACATTTA TCAGATTATGTTTGCACCATTTACCAGATTA TGGGTACGGTTTGTGGCACCCCCCAAAAATTAGAATAGT AACATCAAAGATCACTGATCACAGATCGCCA TAACATAAATAATAATAAACTTTAAAATACTGTGAGAAT TACCAAAATGTGATACAGAGACATGAAGTGA GCACATGCTGTTGAAAAAAATGACACTGATAGACATACT TAACACGTGGGATTGCCACAAACCTTCAGTT TGTAAGTCAACAGTAACTGTGACTCACAAAAGAACAAA GCACAATAAAACGAGGTATGCCTGTATTTTT AAAAAAAGCTTTTTGTAAATTCAGGATATGTAATAGGT CTGTAGGAATAGTGAAATATTTTTGCTGAT GGATGTAGATATATACGTGGATAGAGATGAAGATCTTAA TTATAGCTATGCAGCATAGATTTAGTCAAAG ACATTTGAAAAGACAAATGTTAAATTAGTGTGGCTAATG ACCTACCCGTGCCATGTTTTCCCTCTTGCAA TGAGATACCCACACTGTGTAGAAGGATGGAGGGAGGAC TCCTACTGTCCCTCTTTGCGTGTGGTTATTA AGTTGCCTCACTGGGCTAAAACACCACACATCTCATAGAT AATATTTGGTAAGTTGTAATCGTCTTCACT CTTCTCTTATCACCCACCCCTATCTTCCCACTTTTCCATCT TTGTTGGTTTGCAACAGCCCCTTCTTTTT GCCTGACTCTCCAGGATTTTCTCTCATCATAAATTGTTCTA

	AAGTACATACTAATATGGGTCTGGATTGA CTATTCTTATTTGCAAAACAGCAATTAAATGTTATAGGGA AGTAGGAAGAAAAAGGGGTATCCTTGACAA TAAACCAAGCAATATTCTGGGGGTGGGATAGAGCAGGAA ATTTTATTTTAAATCTTTTAAAATCCAAGTA ATAGGTAGGCTTCCAGTTAGCTTTAAATGTTTTTTTTTCC AGCTCAAAAAATTGGATTGTAGTTGATAC TACATATAATACATTCTAATTCCCTCACTGTATTCTTTGTT TAGTTTCATTTATTTGGTTTAAAATAATT TTTTATCCCATATCTGAAATGTAATATATTTTATCCAACA ACCAGCATGTACATATACTTAATTATGTG GCACATTTTCTAATAGATCAGTCCATCAATCTACTCATTT TAAAGAAAAAAAATTTTAAAGTCACTTTT AGAGCCCTTAATGTGTAGTTGGGGGTAAAGCTTTGTGGAT GTAGCCTTTATATTTAGTATAATTGAGGTC TAAAATAATAATCTTCTATTATCTCAACAGAGCAAATTAT TGAAAAAGATGAAGGTCCTTTTTATACCCA TCTAGGAGCAGGTCCTAATGTGGCAGCTATTAGAGAAAT CATGGAAGAAAGGTAATTAACGCAAAGGCAC AGGGCAGATTAAACGTTTATCCTTTTGTATATGTCAGAATT TTTCCAGCCTTCACACACAAAGCAGTAAAC AATTGTAAATTGAGTAATTATTAGTAGGCTTAGCTATTCT AGGGTTGCCAACACTACACACTGTGCTATT CACCAGAGAGTCACAATATTTGACAGGACTAATAGTCTG CTAGCTGGCACAGGCTGCCCACCTTTGCGATG GATGCCAGAAAACCCAGGCATGAACAGGAATCGGCCAGC CAGGCTGCCAGCCACAAGGTACTGGCACAGG CTCCAACGAGAGGTCCCCTCTGGCTTTCCCACCTGATAA TAAAGTGTCAAAGCAGAAAGACTGGTAAAG TGTGGTATAAGAAAAGAACCCTGAATTAAATTCACCTA GTGTTGCAAATGAGTACTTATCTCTAAGTTT TCTTTTACCATAAAAAGAGAGCAAGTGTGATATGTTGAAT AGAAAGAGAAACATACTATTTACAGCTGCC TTTTTTTTTTTTTTTCGCTATCAATCACAGGTATACAAGTA CTTGCCTTTACTCCTGCATGTAGAAGACT CTTATGAGCGAGATAATGCAGAGAAGGCCTTTCATATAA ATTTATACAGCTCTGAGCTGTTCTTCTTCTA GGGTGCCTTTTCATTAAGAGGTAGGCAGTATTATTATTAA AGTACTTAGGATACATTGGGGCAGCTAGGA CATATTCAGTATCATTCTTGCTCCATTTCCAAATTATTCAT TTCTAAATTAGCATGTAGAAGTTCCTAA ATAATCATCTAGTGGCCTGGCAGAAATAGTGAATTTCCCT AAGTGCCTTTTTTTTTGTTGTTTTTTTTGTTT TGTTTTTTTAAACAAGCAGTAGGTGGTGCTTTGGTCATAAG GGAAGATATAGTCTATTTCTAGGACTATTC CATATTTTCCATGTGGCTGGATACTAACTATTTGCCAGCC TCCTTTTCTAAATTGTGAGACATTCTTGGA GGAACAGTTCTAACTAAAATCTATTATGACTCCCCAAGTT TTAAAATAGCTAAATTTAGTAAGGGAAAAA ATAGTTTATGTTTTAGAAGACTGAACTTAGCAAATAACC TGAATTTTGTGCTTTGTGAAATTTTATATC GAAATGAGCTTTCCCATTTTCACCCACATGTAATTTACAA AATAGTTCATTACAATTATCTGTACATTTT GATATTGAGGAAAAACAAGGCTTAAAAACCATTATCCAG

		TTTGCTTGGCGTAGACCTGTTTAAAAAATAA TAAACCGTTCATTTCTCAGGATGTGGTCATAGAATAAAGT TATGCTCAAATGTTCAAATATTTAAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X9, mRNA [SEQ ID NO: 1362]	XM_011532 044.1	TCAGGCTCTACTTCTAATTCTGGTTCTCTTGCTACATCTCC CTCATCTGCAGTGACCTCTCCACGGAAGT CTTGAACCTCCTCAAAAGCAAATTATTGAAAAAGATGAAG GTCCTTTTTATACCCATCTAGGAGCAGGTCC TAATGTGGCAGCTATTAGAGAAATCATGGAAGAAAGGTT TGGACAGAAGGGTAAAGCTATTAGGATTGAA AGAGTCATCTATACTGGTAAAGAAGGCAAAAGTTCTCAG GGATGTCCTATTGCTAAGTGGGTGGTTCGCA GAAGCAGCAGTGAAGAGAAGCTACTGTGTTTGGTGCGGG AGCGAGCTGGCCACACCTGTGAGGCTGCAGT GATTGTGATTCTCATCCTGGTGTGGGAAGGAATCCCGCTG TCTCTGGCTGACAACTCTACTCGGAGCTT ACCGAGACGCTGAGGAAATACGGCACGCTCACCAATCGC CGGTGTGCCTTGAATGAAGAGAGAACTTGCG CCTGTCAGGGGCTGGATCCAGAAACCTGTGGTGCCTCCTT CTCTTTTGGTTGTTTCATGGAGCATGTACTA CAATGGATGTAAGTTTGCCAGAAGCAAGATCCCAAGGAA GTTTAAGCTGCTTGGGGATGACCCAAAAGAG GAAGAGAACTGGAGTCTCATTTGCAAAACCTGTCCACT CTTATGGCACCAACATATAAGAACTTGCAC CTGATGCATATAATAATCAGATTGAATATGAACACAGAG CACCAGAGTGCCGTCTGGGTCTGAAGGAAGG CCGTCCATTCTCAGGGGTCACTGCATGTTTGGACTTCTGT GCTCATGCCCACAGAGACTTGCACAACATG CAGAATGGCAGCACATTGGTATGCACTCTCACTAGAGAA GACAATCGAGAATTTGGAGGAAAACCTGAGG ATGAGCAGCTTCACGTTCTGCCTTTATACAAAGTCTCTGA CGTGGATGAGTTTGGGAGTGTGGAAGCTCA GGAGGAGAAAAAACGGAGTGGTGCCATTCAGGTACTGAG TTCTTTTCGGCGAAAAGTCAGGATGTTAGCA GAGCCAGTCAAGACTTGCCGACAAAGGAACTAGAAGCC AAGAAAGCTGCAGCTGAAAAGCTTTCCTCCC TGGAGAACAGCTCAAATAAAAATGAAAAGGAAAAGTCA GCCCCATCACGTACAAAACAACTGAAAACGC AAGCCAGGCTAAACAGTTGGCAGAACTTTTGCGACTTTC AGGACCAGTCATGCAGCAGTCCCAGCAGCCC CAGCCTCTACAGAAGCAGCCACCACAGCCCCAGCAGCAG CAGAGACCCCAGCAGCAGCAGCCACATCACC CTCAGACAGAGTCTGTCAACTCTTATTCTGCTTCTGGATC CACCAATCCATACATGAGACGGCCCAATCC AGTTAGTCCTTATCCAAACTCTTCACACACTTCAGATATC TATGGAAGCACCAGCCCTATGAACTTCTAT TCCACCTCATCTCAAGCTGCAGGTTTCATATTTGAATTCTT CTAATCCCATGAACCCTTACCCTGGGCTTT TGAATCAGAATACCCAATATCCATCATATCAATGCAATG GAAACCTATCAGTGGACAACCTGCTCCCCATA TCTGGGTTCCTATTCTCCCCAGTCTCAGCCGATGGATCTG TATAGGTATCCAAGCCAAGACCCTCTGTCT AAGCTCAGTCTACCACCCATCCATACACTTTACCAGCCAA GGTTTGGAAATAGCCAGAGTTTACATCTA AATACTTAGGTTATGGAAACCAAAATATGCAGGGAGATG

	GTTTCAGCAGTTGTACCATTAGACCAAATGT ACATCATGTAGGGAAATTGCCTCCTTATCCCACATCATGAG ATGGATGGCCACTTCATGGGAGCCACCTCT AGATTACCACCCAATCTGAGCAATCCAAACATGGACTAT AAAAATGGTGAACATCATTACCTTCTCACA TAATCCATAACTACAGTGCAGCTCCGGGCATGTTCAACA GCTCTCTTCATGCCCTGCATCTCCAAAACAA GGAGAATGACATGCTTTCCACACAGCTAATGGGTTATC AAAGATGCTTCCAGCTCTTAACCATGATAGA ACTGCTTGTGTCCAAGGAGGCTTACACAAATTAAGTGAT GCTAATGGTCAGGAAAAGCAGCCATTGGCAC TAGTCCAGGGTGTGGCTTCTGGTGCAGAGGACAACGATG AGGTCTGGTCAGACAGCGAGCAGAGCTTTCT GGATCCTGACATTGGGGGAGTGGCCGTGGCTCCAACTCA TGGGTCAATTCTCATTGAGTGTGCAAAGCGT GAGCTGCATGCCACAACCCCTTTAAAGAATCCCAATAGG AATCACCCCACCAGGATCTCCCTCGTCTTTT ACCAGCATAAGAGCATGAATGAGCCAAAACATGGCTTGG CTCTTTGGGAAGCCAAAATGGCTGAAAAAGC CCGTGAGAAAGAGGAAGAGTGTGAAAAGTATGGCCCAG ACTATGTGCCTCAGAAATCCCATGGCAAAAAA GTGAAACGGGAGCCTGCTGAGCCACATGAACTTCAGAG CCCCTTACCTGCGTTTCATCAAGTCTCTTG CCGAAAGGACCATGTCCGTGACCACAGACTCCACAGTAA CTACATCTCCATATGCCTTCACTCGGGTCAC AGGGCCTTACAACAGATATATATGATATCACCCCCTTTTG TTGGTTACCTCACTTGAAAAGACCACAACC AACCTGTCAGTAGTATAGTTCTCATGACGTGGGCAGTGG GGAAAGGTCACAGTATTCATGACAAATGTGG TGGGAAAAACCTCAGCTCACCAGCAACAAAAGAGGTTAT CTTACCATAGCACTTAATTTTCACTGGCTCC CAAGTGGTCACAGATGGCATCTAGGAAAAGACCAAAGCA TTCTATGCAAAAAGAAGGTGGGGAAGAAAGT GTTCCGCAATTTACATTTTAAACACTGGTTCTATTATTGG ACGAGATGATATGTAAATGTGATCCCCC CCCCCGCTTACAACCTCTACACATCTGTGACCACTTTTAAT AATATCAAGTTTGCATAGTCATGGAACACA AATCAAACAAGTACTGTAGTATTACAGTGACAGGAATCT TAAAATACCATCTGGTGCTGAATATATGATG TACTGAAATACTGGAATTATGGCTTTTTGAAATGCAGTTT TACTGTAATCTTAACCTTTTATTTATCAAA ATAGCTACAGGAAACATGAATAGCAGGAAAACACTGAAT TTGTTTGGATGTTCTAAGAAATGGTGCTAAG AAAATGGTGTCTTTAATAGCTAAAAATTTAATGCCTTTAT ATCATCAAGATGCTATCAGTGTACTCCAGT GCCCTTGAATAATAGGGGTACCTTTTCATTCAAGTTTTTA TCATAATTACCTATTCTTACACAAGCTTAG TTTTTAAAATGTGGACATTTTAAAGGCCTCTGGATTTTGC TCATCCAGTGAAGTCCTTGTAGGACAATAA ACGTATATATGTACATATATACACAAACATGTATATGTGC ACACACATGTATATGTATAAATATTTTAAA TGGTGTTTTAGAAAGCACTTTGTCTACCTAAGCTTTGACAA CTTGAACAATGCTAAGGTACTGAGATGTTT AAAAACAAGTTTACTTTTCAATTTTAGAATGCAAAGTTGAT

	TTTTTTAAGGAAACAAAGAAAGCTTTTAAA ATATTTTGTCTTTTAGCCATGCATCTGCTGATGAGCAATT GTGTCCATTTTTAACACAGCCAGTTAAATC CACCATGGGGCTTACTGGATTCAAGGGAATACGTTAGTC CACAAAACATGTTTTCTGGTGCTCATCTCAC ATGCTATACTGTAAAACAGTTTTATACAAAATTGTATGAC AAGTTCATTGCTCAAAAATGTACAGTTTTA AGAATTTTCTATTAAGTGCAGGTAATAATTAGCTGCATGC TGCAGACTCAACAAAGCTAGTTCAGTGAAG CCTATGCTATTTTATGGATCATAGGCTCTTCAGAGAACTG AATGGCAGTCTGCCTTTGTGTTGATAATTA TGTACATTGTGACGTTGTCATTTCTTAGCTTAAGTGTCTC TTTAACAAGAGGATTGAGCAGACTGATGC CTGCATAAGATGAATAAACAGGGTTAGTTCCATGTGAAT CTGTCAGTTAAAAAGAAACAAAAACAGGCAG CTGGTTTGCTGTGGTGGTTTTAAATCATTAAATTTGTATAA AGAAGTGAAAGAGTTGTATAGTAAATTTAA TTGTAAACAAAACCTTTTTTAATGCAATGCTTTAGTATTTT AGTACTGTAAAAAAATTAAATATATACATA TATATATATATATATATATATATATATATGAGTTTGAAGC AGAATTCACATCATGATGGTGCTACTCAGC CTGCTACAAATATATCATAATGTGAGCTAAGAATTCATTA AATGTTTGAGTGATGTTCCCTACTTGTCATA TACCTCAACACTAGTTTGGCAATAGGATATTGAACTGAG AGTGAAAGCATTGTGTACCATCATTTTTTTTC CAAGTCCTTTTTTTTATTGTTAAAAAAAAGCATACCTT TTTTCAATACTTGATTTCTTAGCAAGTATA ACTTGAACCTCAACCTTTTTGTTCTAAAAATTCAGGGATA TTTCAGCTCATGCTCTCCCTATGCCAACAT GTCACCTGTGTTTATGTAAAATTGTTGTAGGTTAATAAAT ATATTCTTTGTCAGGGATTAAACCCTTTTA TTTTGAATCCCTTCTATTTTACTTGTACATGTGCTGATGTA ACTAAAACATAATTTTGTAATCTGTTGGC TCTTTTTATTGTAAAGAAAAGCATTTTAAAAGTTTGAGGA ATCTTTTGACTGTTTCAAGCAGGAAAAAAA AATTACATGAAAATAGAATGCACTGAGTTGATAAAGGGA AAAATTGTAAGGCAGGAGTTTGGCAAGTGGC TGTTGGCCAGAGACTTACTTGTAACCTCTCTAAATGAAGTT TTTTTGATCCTGTAATCACTGAAGGTACAT ACTCCATGTGGACTTCCCTTAAACAGGCAAACACCTACA GGTATGGTGTGCAACAGATTGTACAATTACA TTTTGGCCTAAATACATTTTGTCTTACTAGTATTTAAAATA AATTCTTAATCAGAGGAGGCCTTTGGGTT TTATTGGTCAAATCTTTGTAAAGCTGGCTTTTGTCTTTTTAA AAAATTTCTTGAATTTGTGGTTGTGTCCA ATTTGCAAACATTTCCAAAAATGTTTGCTTTGCTTACAAA CCACATGATTTTAATGTTTTTTGTATACCA TAATATCTAGCCCCAAACATTTGATTACTACATGTGCATT GGTGATTTTGATCATCCATTCTTAATATTT GATTTCTGTGTCACCTACTGTCATTTGTTAAACTGCTGGC CAACAAGAACAGGAAGTATAGTTTGGGGGG TTGGGGAGAGTTTACATAAGGAAGAGAAGAAATTGAGTG GCATATTGTAAATATCAGATCTATAATTGTA AATATAAAACCTGCCTCAGTTAGAATGAATGGAAAGCAG

		ATCTACAATTTGCTAATATAGGAATATCAGG TTGACTATATAGCCATACTTGAAAATGCTTCTGAGTGGTG TCAACTTTACTTGAATGAATTTTTTCATCTT GATTGACGCACAGTGATGTACAGTTCACCTTCTGAAGCTAG TGGTTAACTTGTGTAGGAAACTTTTGCAGT TTGACACTAAGATAACTTCTGTGTGCATTTTTCTATGCTTT TTTAAAACTAGTTTTCATTTTCATTTTCAT GAGATGTTTGGTTTATAAGATCTGAGGATGGTTATAAATA CTGTAAGTATTGTAATGTTATGAATGCAGG TTATTTGAAAGCTGTTTATTATTATATCATTCCCTGATAATG CTATGTGAGTGTTTTTAATAAAATTTATA TTTATTTAATGCACTCTAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X7, mRNA [SEQ ID NO: 1363]	XM_011532 043.1	GTAGAGAAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGG ATTTGTTAGAAAGGAGACCCGACTGCAACTG CTGGATTGCTGCAAGGCTGAGGGACGAGAACGAGGCTGG CAAACATTCAGCAGCACACCCTCTCAAGATT GTTTACTTGCCTTTGCTCCTGTTGAGTTACAACGCTTGGA AGCAGGAGATGGGCTCAGCAGCAGCCAATA GGACATGATCCAGGAAGAGCAGTAAGGGACTGAGCTGCT GAATTCAACTAGAGGGCAGCCTTGTGGATGG CCCCGAAGCAAGCCTGATGGAACAGGATAGAACCAACCA TGTTGAGGGCAACAGACTAAGTCCATTCCCTG ATACCATCACCTCCCATTTGCCAGACAGAACCTCTGGCTA CAAAGCTCCAGAATGGAAGCCCACTGCCTG AGAGAGCTCATCCAGAAGTAAATGGAGACACCAAGTGGC ACTCTTTCAAAGTTATTATGGAATACCCTG TATGAAGGGAAGCCAGAATAGTCGTGTGAGTCCTGACTT TACACAAGAAAGTAGAGGGTATTCCAAGTGT TTGCAAAATGGAGGAATAAAACGCACAGTTAGTGAACCT TCTCTCTCTGGGCTCCTTCAGATCAAGAAAT TGAAACAAGACCAAAAAGGCTAATGGAGAAAGACGTAAC TTCGGGGTAAGCCAAGAAAGAAATCCAGGTGA AAGCAGTCAACCAAATGTCTCCGATTTGAGTGATAAGAA AGAATCTGTGAGTTCTGTAGCCCAAGAAAAT GCAGTTAAAGATTTTACCAGTTTTTCAACACATAACTGCA GTGGGCCTGAAAATCCAGAGCTTCAGATTC TGAATGAGCAGGAGGGGAAAAGTGCTAATTACCATGACA AGAACATTGTATTACTTAAAAACAAGGCAGT GCTAATGCCTAATGGTGCTACAGTTTCTGCCTCTTCCGTG GAACACACACATGGTGAACCTCCTGGAAAAA ACACTGTCTCAATATTATCCAGATTGTGTTTCCATTGCGG TGCAGAAAACCACATCTCACATAAATGCCA TTAACAGTCAGGCTACTAATGAGTTGTCCTGTGAGATCAC TCACCCATCGCATACCTCAGGGCAGATCAA TTCCGCACAGACCTCTAACTCTGAGCTGCCTCCAAAGCCA GCTGCAGTGGTGAGTGAGGCCTGTGATGCT GATGATGCTGATAATGCCAGTAAACTAGCTGCAATGCTA AATACCTGTTCCTTTTCAGAAACCAGAACAAAC TACAACAACAAAAATCAGTTTTTTGAGATATGCCCATCTCC TGCAGAAAATAACATCCAGGGAACCACAAA GCTAGCGTCTGGTGAAGAATTCTGTTTCAGGTTCCAGCAGC AATTTGCAAGCTCCTGGTGGCAGCTCTGAA CGGTATTTAAAACAAAATGAAATGAATGGTGCTTACTTC AAGCAAAGCTCAGTGTTCACTAAGGATTCCT

	TTTCTGCCACTACCACACCACCACCACCATCACAATTGCT TCTTTCTCCCCCTCCTCCTCTTCCACAGGT TCCTCAGCTTCCTTCAGAAGGAAAAAGCACTCTGAATGGT GGAGTTTTAGAGAAGAACACCACCCTACCCC AACCAAAGTAACACAACACTTTTAAGGGAAGTGAAAATA GAGGGTAAACCTGAGGCACCACCTTCCCAGA GTCCTAATCCATCTACACATGTATGCAGCCCTTCTCCGAT GCTTTCTGAAAGGCCTCAGAATAATTGTGT GAACAGGAATGACATACAGACTGCAGGGACAATGACTGT TCCATTGTGTTCTGAGAAAACAAGACCAATG TCAGAACACCTCAAGCATAACCCACCAATTTTTGGTAGCA GTGGAGAGCTACAGGACAACCTGCCAGCAGT TGATGAGAAACAAAGAGCAAGAGATTCTGAAGGGTCGA GACAAGGAGCAAACACGAGATCTTGTGCCCCC AACACAGCACTATCTGAAACCAGGATGGATTGAATTGAA GGCCCCCTCGTTTTTCACCAAGCGGAATCCCAT CTAAAACGTAATGAGGCATCACTGCCATCAATTCTTCAGT ATCAACCCAATCTCTCCAATCAAATGACCT CCAAACAATACACTGGAAATTCCAACATGCCTGGGGGGC TCCCAAGGCAAGCTTACACCCAGAAAACAAC ACAGCTGGAGCACAAGTCACAAATGTACCAAGTTGAAAT GAATCAAGGGCAGTCCCAAGGTACAGTGGAC CAACATCTCCAGTTCCAAAAACCCTCACACCAGGTGCACT TCTCCAAAACAGACCATTTACCAAAAAGCTC ATGTGCAGTCACTGTGTGGCACTAGATTTTCATTTTCAACA AAGAGCAGATTCCCAAACCTGAAAAACTTAT GTCCCCAGTGTTGAAACAGCACTTGAATCAACAGGCTTC AGAGACTGAGCCATTTTCAAACCTCACACCTT TTGCAACATAAGCCTCATAAACAGGCAGCACAAACACAA CCATCCCAGAGTTTACATCTCCCTCAAAACC AGCAACAGCAGCAAAAATTACAAATAAAGAATAAAGAG GAAATACTCCAGACTTTTCCTCACCCCCAAAG CAACAATGATCAGCAAAGAGAAGGATCATTCTTTGGCCA GACTAAAGTGGAAGAATGTTTTTCATGGTGAA AATCAGTATTCAAATCAAGCGAGTTCGAGACTCATAAT GTCCAAATGGGACTGGAGGAAGTACAGAATA TAAATCGTAGAAATTCCCCTTATAGTCAGACCATGAAATC AAGTGCAATGCAAAATACAGGTTTCTTGTTT AAACAATACACACCTAGTTTCAGAGAATAAAGAACAGAC TACACATCCTGAACTTTTTTGCAGGAAACAAG ACCCAAACTTGCATCACATGCAATATTTTCCAAATAATG TGATCCCAAAGCAAGATCTTCTTCACAGGT GCTTTCAAGAACAGGAGCAGAAGTCACAACAAGCTTCAG TTCTACAGGGATATAAAAATAGAAACCAAGA TATGTCTGGTCAACAAGCTGCGCAACTTGCTCAGCAAAG GACTTGATACATAACCATGCAAATGTTTTT CCTGTGCCTGACCAGGGAGGAAGTCACACTCAGACCCCT CCCCAGAAGGACACTCAAAAGCATGCTGCTC TAAGGTGGCATCTCTTACAGAAGCAAGAACAGCAGCAAA CACAGCAACCCCAAACCTGAGTCTTGCCATAG TCAGATGCACAGGCCAATTAAGGTGGAACCTGGATGCAA GCCACATGCCTGTATGCACACAGCACCACCA GAAAACAAAACATGGAAAAAGGTAAGCAAGAGAA TCCACCTGCAAGCTGTGATAATGTGCAGCAAA

		AGAGCATCATTGAGACCATGGAGCAGCATCTGAAGCAGT TTCACGCCAAGTCGTTATTTGACCATAAGGC TCTTACTCTCAAATCACAGAAGCAAGTAAAAGTTGAAAT GTCAGGGCCAGTCACAGTTTTGACTAGACAA ACCACTGCTGCAGAACTTGATAGCCACACCCCAGCTTTAG AGCAGCAAACAACCTTCTTCAGAAAAGACAC CAACCAAAAAGAACAGCTGCTTCTGTTCTCAATAATTTTAT AGAGTCACCTTCCAAATTACTAGATACTCC TATAAAAAATTTATTGGATACACCTGTCAAGACTCAATAT GATTTCCCATCTTGCAGATGTGTAGAGCAA ATTATTGAAAAAGATGAAGGTCCTTTTTTATACCCATCTAG GAGCAGGTCCTAATGTGGCAGCTATTAGAG AAATCATGGAAGAAAGGTATACAAGTACTTGCCTTTACT CCTGCATGTAGAAGACTCTTATGAGCGAGAT AATGCAGAGAAGGCCTTTCATATAAATTTATACAGCTCTG AGCTGTTCTTCTTCTAGGGTGCCTTTTTCAT TAAGAGGTAGGCAGTATTATTATTAAAGTACTTAGGATA CATTGGGGCAGCTAGGACATATTCAGTATCA TTCTTGCTCCATTTCCAAATTATTCATTTCTAAATTAGCAT GTAGAAGTTCACTAAATAATCATCTAGTG GCCTGGCAGAAATAGTGAATTTCCCTAAGTGCCTTTTTTT TGTTGTTTTTTTGTTTTGTTTTTTTAAACAA GCAGTAGGTGGTGCTTTGGTCATAAGGGAAGATATAGTC TATTTCTAGGACTATTCCATATTTTCCATGT GGCTGGATACTAACTATTTGCCAGCCTCCTTTTCTAAATT GTGAGACATTCTTGGAGGAACAGTTCTAAC TAAAATCTATTATGACTCCCCAAGTTTTAAAATAGCTAAA TTTAGTAAGGGAAAAAATAGTTTATGTTTT AGAAGACTGAACTTAGCAAACCTGAATTTTGTGCTT TGTGAAATTTTATATCGAAATGAGCTTTCC CATTTTCACCCACATGTAATTTACAAAATAGTTCATTACA ATTATCTGTACATTTTGATATTGAGGAAAA ACAAGGCTTAAAAACCATTATCCAGTTTGCTTGGCGTAGA CCTGTTTAAAAAATAATAAACCGTTCATTT CTCAGGATGTGGTCATAGAATAAAGTTATGCTCAAATGTT CAAA

“Tet inhibitor” or “Tet[x] inhibitor” (e.g., “Tet1 inhibitor,” “Tet2 inhibitor”, or “Tet3 inhibitor”) as the terms are used herein, refers to a molecule, or group of molecules (e.g., a system) that reduces or eliminates the function and/or expression of the corresponding Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. In embodiments, a Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitor is a molecule that inhibits the expression of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2, e.g., reduces or eliminates expression of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. In embodiments, the Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitor is a molecule that inhibits the function of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. An example of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitor that inhibits the expression of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 is a gene editing system, e.g., as described herein, that is targeted to nucleic acid within the Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 gene, or its regulatory elements, such that modification of the nucleic acid at or near the gene editing

system binding site(s) is modified to reduce or eliminate expression of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. Another example of a Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitor that inhibits the expression of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 is a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), capable of hybridizing with Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 mRNA and causing a reduction or elimination of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 translation. Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitors also include nucleic acids encoding molecules which inhibit Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 expression (e.g., nucleic acid encoding an anti-Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 shRNA or siRNA, or nucleic acid encoding one or more, e.g., all, components of an anti-Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 gene editing system). An example of a molecule that inhibits the function of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 is a molecule, e.g., a protein or small molecule which inhibits one or more activities of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. An example is a small molecule inhibitor of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. Another example is a dominant negative Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 protein. Another example is a dominant negative version of a Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 binding partner, e.g., an associated histone deacetylase (HDAC). Another example is a molecule, e.g., a small molecule, which inhibits a Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 binding partner, e.g., a Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2-associated HDAC inhibitor. Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 inhibitors also include nucleic acids encoding inhibitors of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 function.

The terms “IFNG inhibitor” and “IFN- γ inhibitor” are used herein interchangeably and refer to a molecule, or group of molecules (e.g., a system), that reduces or eliminates the expression and/or function of IFN- γ . IFN- γ inhibitors include all antagonists or inhibitors of all suitable forms of IFN- γ , IFN- γ receptors (e.g., IFN- γ receptor 1 and/or IFN- γ receptor 2), or IFN- γ effectors (e.g., TNFSF14, TNFRSF3, TNFRSF14, or TNFRSF6B). Exemplary IFN- γ inhibitors include, but are not limited to, a gene editing system targeting the IFN- γ gene or a regulatory element thereof; a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), that reduces IFN- γ translation; and a protein, peptide, or small molecule that inhibits one or more activities of IFN- γ .

A “NOTCH2 inhibitor” as the term is used herein refers to a molecule, or group of molecules (e.g., a system), that reduces or eliminates the expression and/or function of NOTCH2. Exemplary Notch2 inhibitors include, but are not limited to, a gene editing system targeting the NOTCH2 gene or a regulatory element thereof; a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), that reduces NOTCH2 translation; and a protein, peptide, or small molecule that inhibits one or more activities of NOTCH2.

An “IL2RA inhibitor” as the term is used herein refers to a molecule, or group of molecules (e.g., a system), that reduces or eliminates the expression and/or function of IL2RA. Exemplary

IL2RA inhibitors include, but are not limited to, a gene editing system targeting the IL2RA gene or a regulatory element thereof; a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), that reduces IL2RA translation; and a protein, peptide, or small molecule that inhibits one or more activities of IL2RA.

5 A “PRDM1 inhibitor” as the term is used herein refers to a molecule, or group of molecules (e.g., a system), that reduces or eliminates the expression and/or function of PRDM1. Exemplary PRDM1 inhibitors include, but are not limited to, a gene editing system targeting the PRDM1 gene or a regulatory element thereof; a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), that reduces PRDM1 translation; and a protein, peptide,
10 or small molecule that inhibits one or more activities of PRDM1.

A “Tet2-associated gene,” as used herein, refers to a gene whose structure, expression, and/or function, or a gene encoding a gene product (e.g., an mRNA or a polypeptide) whose structure, expression, and/or function, is associated with (e.g., affected or modulated by) Tet2. The Tet2-associated gene does not include a Tet2 gene.

15 In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes described herein. In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes described in Table 8. In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes described in Table 9.

20 In some embodiments, the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

In one embodiment, the Tet2-associated gene comprises IFNG. In one embodiment, the Tet2-associated gene comprises NOTCH2. In one embodiment, the Tet2-associated gene comprises CD28. In one embodiment, the Tet2-associated gene comprises ICOS. In one embodiment, the Tet2-associated gene comprises IL2RA. In one embodiment, the Tet2-associated gene comprises PRDM1.
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In one embodiment, the Tet2-associated gene comprises IFNG and NOTCH2. In one embodiment, the Tet2-associated gene comprises IFNG and CD28. In one embodiment, the Tet2-associated gene comprises IFNG and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2 and CD28. In one embodiment, the Tet2-associated gene comprises NOTCH2 and ICOS. In one embodiment, the Tet2-associated gene comprises NOTCH2 and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2 and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28 and ICOS. In one embodiment, the Tet2-associated gene comprises CD28 and IL2RA. In one embodiment, the Tet2-associated gene comprises CD28 and PRDM1. In one embodiment, the Tet2-associated gene comprises ICOS and IL2RA. In one embodiment, the Tet2-associated gene comprises ICOS and PRDM1. In one embodiment, the Tet2-associated gene comprises IL2RA and PRDM1.
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In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and CD28. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and ICOS. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, and ICOS. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, and, PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises ICOS, IL2RA, and PRDM1.

In one embodiment, the Tet2-associated gene comprises CD28, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, ICOS, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, CD28, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, IL2RA, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, ICOS, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, ICOS, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and PRDM1. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and IL2RA. In one embodiment, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, and ICOS.

In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS, and IL2RA. In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG,

NOTCH2, ICOS, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises IFNG, CD28, ICOS, IL2RA, and PRDM1. In some embodiments, the Tet2-associated gene comprises NOTCH2, CD28, ICOS, IL2RA, and PRDM1.

In some embodiments, the Tet2-associated gene comprises IFNG, NOTCH2, CD28, ICOS,
5 IL2RA, and PRDM1.

In certain embodiments, expression and/or function of the Tet2-associated gene is altered when expression and/or function of Tet2 is inhibited. In some embodiments, expression and/or function of the Tet2-associated gene is reduced or eliminated when expression and/or function of Tet2 is inhibited. In other embodiments, expression and/or function of the Tet2-associated gene is
10 increased or activated when expression and/or function of Tet2 is inhibited.

In some embodiments, the Tet2-associated gene or gene product is a member of a biological pathway associated with Tet2 (e.g., associated with inhibition of Tet2). In certain embodiments, the Tet2-associated gene or gene product is downstream of Tet2 in the the pathway. In an embodiment, the Tet2-associated gene or gene product is upstream of Tet2 in the the pathway.

In certain embodiments, the Tet2-associated gene encodes a gene product (e.g., a polypeptide) that interacts, directly or indirectly, with Tet2 (e.g., a Tet2 gene or gene product). In other
15 embodiments, the Tet2-associated gene encodes a gene product (e.g., a polypeptide) that does not interact with Tet2 (e.g., a Tet2 gene or gene product).

As used herein, a “modulator” of a “Tet2-associated gene” refers to a molecule, or group of
20 molecules (e.g., a system) that modulates (e.g., reduces or eliminates, or increases or activates) function and/or expression of a Tet2-associated gene. In certain embodiments, the modulator reduces or eliminates expression and/or function of a Tet2-associated gene. In other embodiment, the modulator increases or activates expression and/or function of a Tet2-associated gene. In certain
25 embodiments, the modulator is an inhibitor of a Tet2-associated gene. In other embodiments the modulator is an activator of a Tet2-associated gene. In some embodiments, the modulator is a gene editing system that is targeted to nucleic acid within the Tet2-associated gene or a regulatory element thereof, e.g., such that the nucleic acid is modified at or near the gene editing system binding site(s) to modulate expression and/or function of the Tet2-associated gene. In some embodiments, the
30 modulator is a component of the gene editing system, or a nucleic acid encoding a component of the gene editing system. In other embodiments, the modulator is a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), capable of hybridizing with an mRNA of the Tet2-associated gene, e.g., causing a reduction or elimination of a Tet2-associated gene product. In other embodiments, the modulator is a nucleic acid encoding the
35 RNA molecule, e.g., shRNA or siRNA. In some embodiments, the modulator is a gene product of a Tet2-associated gene, or a nucleic acid encoding the gene product, e.g., for overexpression of the Tet2-associated gene. In other embodiments, the modulator is a small molecule that modulates expression and/or function of the Tet2-associated gene. In other embodiments, the modulator is a

protein that modulates expression and/or function of the Tet2-associated gene. For example, the modulator can be a variant (e.g., a dominant negative variant or a constitutively active variant), or a binding partner, of a gene product of the Tet2-associated gene. In some embodiments, the modulator is a nucleic acid that encodes the aforesaid protein. The modulator can modulate (e.g., inhibit or
 5 activate) expression and/or function of a Tet2-associated gene before, concurrently with, or after transcription of the Tet2-associated gene, and/or before, concurrently with, or after translation of the Tet2-associated gene.

A “Tet-associated gene,” as used herein, refers to a gene whose structure, expression, and/or function, or a gene encoding a gene product (e.g., an mRNA or a polypeptide) whose structure,
 10 expression, and/or function, is associated with (e.g., affected or modulated by) Tet (e.g., Tet1, Tet2 and/or Tet3). The Tet-associated gene does not include a Tet gene (e.g., a Tet1, Tet2 and/or Tet3 gene).

In certain embodiments, expression and/or function of the Tet-associated gene is altered when expression and/or function of a Tet (e.g., Tet1, Tet2 and/or Tet3) is inhibited. In some embodiments,
 15 expression and/or function of the Tet-associated gene is reduced or eliminated when expression and/or function of a Tet (e.g., Tet1, Tet2 and/or Tet3) is inhibited. In other embodiments, expression and/or function of the Tet-associated gene is increased or activated when expression and/or function of a Tet (e.g., Tet1, Tet2 and/or Tet3) is inhibited.

In some embodiments, the Tet-associated gene or gene product is a member of a biological
 20 pathway associated with a Tet (e.g., Tet1, Tet2 and/or Tet3) (e.g., associated with inhibition of a Tet (e.g., Tet1, Tet2 and/or Tet3)). In certain embodiments, the Tet-associated gene or gene product is downstream of a Tet (e.g., Tet1, Tet2 and/or Tet3) in the the pathway. In an embodiment, the Tet-associated gene or gene product is upstream of a Tet (e.g., Tet1, Tet2 and/or Tet3) in the the pathway.

In certain embodiments, the Tet-associated gene encodes a gene product (e.g., a polypeptide)
 25 that interacts, directly or indirectly, with a Tet (e.g., Tet1, Tet2 and/or Tet3) (e.g., a Tet gene or gene product). In other embodiments, the Tet-associated gene encodes a gene product (e.g., a polypeptide) that does not interact with a Tet (e.g., Tet1, Tet2 and/or Tet3) (e.g., a Tet gene or gene product).

As used herein, a “modulator” of a “Tet-associated gene” refers to a molecule, or group of molecules (e.g., a system) that modulates (e.g., reduces or eliminates, or increases or activates)
 30 function and/or expression of a Tet-associated gene (e.g., a gene associated with Tet1, Tet2 and/or Tet3). In certain embodiments, the modulator reduces or eliminates expression and/or function of a Tet-associated gene. In other embodiment, the modulator increases or activates expression and/or function of a Tet-associated gene. In certain embodiments, the modulator is an inhibitor of a Tet-associated gene. In other embodiments the modulator is an activator of a Tet-associated gene. In
 35 some embodiments, the modulator is a gene editing system that is targeted to nucleic acid within the Tet-associated gene or a regulatory element thereof, e.g., such that the nucleic acid is modified at or near the gene editing system binding site(s) to modulate expression and/or function of the Tet-

associated gene. In some embodiments, the modulator is a component of the gene editing system, or a nucleic acid encoding a component of the gene editing system. In other embodiments, the modulator is a nucleic acid molecule, e.g., RNA molecule, e.g., a short hairpin RNA (shRNA) or short interfering RNA (siRNA), capable of hybridizing with an mRNA of the Tet-associated gene, e.g., causing a reduction or elimination of a Tet-associated gene product. In other embodiments, the modulator is a nucleic acid encoding the RNA molecule, e.g., shRNA or siRNA. In some embodiments, the modulator is a gene product of a Tet-associated gene, or a nucleic acid encoding the gene product, e.g., for overexpression of the Tet-associated gene. In other embodiments, the modulator is a small molecule that modulates expression and/or function of the Tet-associated gene. In other embodiments, the modulator is a protein that modulates expression and/or function of the Tet-associated gene. For example, the modulator can be a variant (e.g., a dominant negative variant or a constitutively active variant), or a binding partner, of a gene product of the Tet-associated gene. In some embodiments, the modulator is a nucleic acid that encodes the aforesaid protein. The modulator can modulate (e.g., inhibit or activate) expression and/or function of a Tet-associated gene before, concurrently with, or after transcription of the Tet-associated gene, and/or before, concurrently with, or after translation of the Tet-associated gene.

A “system” as the term is used herein in connection with gene editing or modulation (e.g., inhibition or activation) of a Tet and/or a Tet-associated gene, e.g., Tet2 and/or a Tet2-associated gene, refers to a group of molecules, e.g., one or more molecules, which together act to effect a desired function.

A “gene editing system” as the term is used herein, refers to a system, e.g., one or more molecules, that direct and effect an alteration, e.g., a deletion, of one or more nucleic acids at or near a site of genomic DNA targeted by said system. Gene editing systems are known in the art, and are described more fully below.

A “binding partner” as the term is used herein in the context of a Tet and/or a Tet-associated molecule, e.g., Tet2 and/or a Tet2-associated molecule, refers to a molecule, e.g., a protein, which interacts, e.g., binds to, a Tet and/or a Tet-associated gene product, e.g., Tet2 and/or a Tet2-associated gene product. Without being bound by theory, it is believed that Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 binds to one or more HDAC proteins. Such HDAC proteins are considered examples of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 binding partners.

A “dominant negative” gene product or protein is one that interferes with the function of another gene product or protein. The other gene product affected can be the same or different from the dominant negative protein. Dominant negative gene products can be of many forms, including truncations, full length proteins with point mutations or fragments thereof, or fusions of full length wild type or mutant proteins or fragments thereof with other proteins. The level of inhibition observed can be very low. For example, it may require a large excess of the dominant negative protein compared to the functional protein or proteins involved in a process in order to see an effect. It may

be difficult to see effects under normal biological assay conditions. In one embodiment, a dominant negative variant of a Tet-associated gene product (e.g., a Tet2-associated gene product) is a catalytically inactive gene product encoded by a Tet-associated gene (e.g., a Tet2-associated gene) variant. In another embodiment, a dominant negative binding partner of a Tet-associated gene product (e.g., a Tet2-associated gene product) is a catalytically inactive gene product encoded by a Tet-associated gene (e.g., a Tet2-associated gene) variant. In one embodiment, a dominant negative Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 is a catalytically inactive Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2. In another embodiment, a dominant negative Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2 binding partner is a catalytically inactive Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2-binding HDAC inhibitor.

Without wishing to be bound by theory, a cell having a “central memory T cell (Tcm) phenotype” expresses CCR7 and CD45RO. In one embodiment, a cell having a central memory T cell phenotype expresses CCR7 and CD45RO, and/or does not express or expresses lower levels of CD45RA as compared to a naive T cell. In one embodiment, a cell having a central memory T cell phenotype expresses CD45RO and CD62L, and/or does not express or expresses lower levels of CD45RA, as compared to a naive T cell. In one embodiment, a cell having a central memory T cell phenotype expresses CCR7, CD45RO, and CD62L, and/or does not express or expresses lower levels of CD45RA as compared to a naive T cell.

Without wishing to be bound by theory, a cell having an “effector memory T cell (Tem) phenotype” does not express or expresses lower levels of CCR7, and expresses higher levels of CD45RO, as compared to a naïve T cell.

The pathways described herein are described, e.g., by Gene Ontology Consortium (e.g., Biological Process Ontology) and/or by Gene Set Enrichment Analysis (GSEA) (e.g., Hallmark or Canonical pathway gene sets).

The Biological Process Ontology is described, e.g., in Ashburner et al. Gene ontology: tool for the unification of biology (2000) Nat Genet 25(1):25-9; The Gene Ontology Consortium. Gene Ontology Consortium: going forward. (2015) Nucl Acids Res 43 Database issue D1049–D1056. The Hallmark gene sets and Canonical pathway gene sets are described, e.g., in Tamayo, et al. (2005) PNAS 102, 15545-15550; Mootha, Lindgren, et al. (2003) Nat Genet 34, 267-273.

As used herein, a “leukocyte differentiation pathway” refers to a process in which a relatively unspecialized hemopoietic precursor cell acquires the specialized features of a leukocyte, e.g., one or more processes categorized under GO:0002521 in the Biological Process Ontology.

As used herein, a “pathway of positive regulation of immune system process” refers to a process that activates or increases the frequency, rate, or extent of an immune system process, e.g., one or more processes categorized under GO:0002684 in the Biological Process Ontology.

As used herein, a “transmembrane receptor protein tyrosine kinase signaling pathway” refers to a signaling pathway initiated by the binding of an extracellular ligand to a cell-surface receptor,

where the cell-surface receptor possesses a tyrosine kinase activity, e.g., one or more pathways categorized under GO:0007169 in the Biological Process Ontology.

As used herein, a “pathway of regulation of anatomical structure morphogenesis” refers to a process that modulates the frequency, rate, or extent of anatomical structure morphogenesis, e.g., one or more process categorized under GO:0022603 in the Biological Process Ontology.

As used herein, a “pathway of TNFA signaling via NFkB” refers to a process regulated by NFkB in response to TNF, e.g., a process involving one or more genes categorized under M5890 in the Hallmark gene sets (GSEA).

As used herein, “pathway of positive regulation of hydrolase activity” refers to a process that activates or increases the frequency, rate, and/or extent of a hydrolase activity, e.g., one or more processes categorized under GO:0051345 in the Biological Process Ontology.

As used herein, “wound healing pathway” refers to a process that restores integrity (e.g., partial or complete integrity) to a damaged tissue, following an injury, e.g., one or more processes categorized under GO:0042060 in the Biological Process Ontology.

As used herein, an “alpha-beta T cell activation pathway” refers to a process involving a change in morphology and/or behavior of an $\alpha\beta$ T cell, e.g., resulting from exposure to a mitogen, cytokine, chemokine, cellular ligand, or an antigen for which it is specific, e.g., one or more changes categorized under GO:0046631 in the Biological Process Ontology.

As used herein, a “pathway of regulation of cellular component movement” refers to a process that modulates the frequency, rate, and/or extent of the movement of a cellular component, e.g., one or more processes categorized under GO:0051270 in the Biological Process Ontology.

As used herein, an “inflammatory response pathway” refers to a defensive reaction (e.g., an immediate defensive reaction), e.g., by a vertebrate tissue, to an infection or injury caused by a chemical or physical agent, e.g., one or more reactions categorized under GO:0006954 in the Biological Process Ontology. In some embodiments, this process is characterized by local vasodilation, extravasation of plasma into intercellular spaces, and/or accumulation of white blood cells and macrophages.

As used herein, a “myeloid cell differentiation pathway” refers to a process in which a relatively unspecialized myeloid precursor cell acquires the specialized features of any cell of the myeloid leukocyte, megakaryocyte, thrombocyte, or erythrocyte lineages, e.g., one or more process categorized under GO:0030099 in the Biological Process Ontology.

As used herein, a “cytokine production pathway” refers to a process in which a cytokine is synthesized or secreted following a cellular stimulus, resulting in an increase in its intracellular or extracellular levels, e.g., one or more process categorized under GO:0001816 in the Biological Process Ontology.

As used herein, a “pathway of down-regulation in UV response” refers to a process involving a gene down-regulated in response to ultraviolet (UV) radiation, e.g., one or more genes categorized under M5942 in the Hallmark gene sets.

As used herein, a “pathway of negative regulation of multicellular organismal process” refers to a process that stops, prevents, or reduces the frequency, rate, and/or extent of an organismal process, the processes pertinent to the function of an organism above the cellular level (e.g., the integrated processes of tissues and organs), e.g., one or more processes categorized under GO:0051241 in the Biological Process Ontology.

As used herein, a “blood vessel morphogenesis pathway” refers to a process in which the anatomical structures of blood vessels are generated and organized, e.g., one or more processes categorized under GO:0048514 in the Biological Process Ontology.

As used herein, an “NFAT-dependent transcription pathway” refers to a process relating to a gene involved in calcineurin-regulated NFAT-dependent transcription in lymphocytes, e.g., one or more genes categorized under M60 in the Canonical pathway gene sets.

As used herein, a “pathway of positive regulation of apoptotic process” refers to a process that activates or increases the frequency, rate, and/or extent of apoptosis, e.g., one or more processes categorized under GO:0043065 in the Biological Process Ontology.

As used herein, a “hypoxia pathway” refers to a process involving a gene up-regulated in response to hypoxia, e.g., one or more genes categorized under M5891 in the Hallmark gene sets.

As used herein, a “pathway of upregulation by KRAS signaling” refers to a process involving a gene up-regulated by KRAS activation, e.g., one or more genes categorized under M5953 in the Hallmark gene sets.

As used herein, a “pathway of stress-activated protein kinase signaling cascade” refers to a signaling pathway in which a stress-activated protein kinase (SAPK) cascade relays one or more of the signals, e.g., one or more signaling pathways categorized under GO:0031098 in the Biological Process Ontology.

Description

The present invention provides modulators (e.g., inhibitors or activators) of Tet-associated genes (e.g., Tet2-associated genes), and inhibitors of a Tet (e.g., Tet1, Tet2, and/or Tet3), e.g., Tet2, and methods of use therefore. In particular, the invention provides CAR-expressing T cells comprising inhibitors of one or more genes described herein, and use of the one or more genes in connection with CAR T cells. The inhibitors of the present invention, together with their methods of use, are described in more detail below. CARs, CAR T cells, and methods of use are further described below.

Without wishing to be bound by theory, it is believed that in certain embodiments, cells with modulated expression and/or function of one or more Tet-associated (e.g., Tet2-associated) genes can

exhibit reduced DNA hydroxymethylation and acquisition of an epigenetic profile consistent with altered T-cell differentiation. For example, CAR T-cells with with modulated expression and/or function of one or more Tet-associated (e.g., Tet2-associated) genes can show an early memory phenotype, which may differ from characteristics of late memory differentiation. Accordingly, in certain embodiments, modulation of expression and/or function of one or more genes in TET (e.g., TET2) pathway can promote T-cell proliferation, therefore enhancing treatment with genetically-redIRECTED T-cells.

Modulators of Tet and Tet-Associated Genes

The present invention provides compositions comprising, e.g., modulators of a Tet-associated gene (e.g., a Tet2-associated gene), optionally and inhibitors of a Tet (Tet1, Tet2, and/or Tet3, e.g., Tet2), and methods for enhancing immune effector cell functions, e.g., CAR-expressing cell functions, by using such compositions and/or other means as described herein. Any modulator a Tet-associated gene (e.g., Tet2-associated gene), and any inhibitor of a Tet (e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2), known in the art, can be used according to the present invention. Examples of modulators of Tet-associated genes (e.g., Tet2-associated genes), and exemplary inhibitors of a Tet (e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2), are described below.

In some embodiments, modulation of any of the Tet2-associated genes by any of the methods disclosed herein can be monoallelic or biallelic. In certain embodiments, the modulation is biallelic (e.g., two modulated alleles). In other embodiments, the modulation is monoallelic (e.g., one modulated allele and one wild type allele).

Gene Editing Systems

According to the present invention, gene editing systems can be used as modulators of a Tet-associated gene (e.g., a Tet2-associated gene) and/or inhibitors of a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2). Also contemplated by the present invention are the uses of nucleic acid encoding one or more components of a gene editing system targeting a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2).

In one embodiment, the Tet2-associated gene is one or more (2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. In one embodiment, the Tet2-associated gene is one or more (e.g., a combination or 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8. In one embodiment, the Tet2-associated gene is one or more (e.g., a combination or 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D. In one embodiment, the Tet2-associated gene is one or more (e.g., a combination or 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes associated with one or more (e.g., a combination or 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pathways chosen from Table 9, Column A. In one embodiment, the Tet2-associated gene is one or more genes associated with a central memory T cell phenotype.

CRISPR/Cas9 Gene Editing Systems

Naturally-occurring CRISPR/Cas systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa et al. (2007) BMC Bioinformatics 8: 172.

5 This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou et al. (2007) Science 315: 1709-1712; Marragini et al. (2008) Science 322: 1843-1845.

The CRISPR/Cas system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice or primates. Wiedenheft et al. (2012) Nature
10 482: 331-8. This is accomplished by, for example, introducing into the eukaryotic cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas.

The CRISPR sequence, sometimes called a CRISPR locus, comprises alternating repeats and spacers. In a naturally-occurring CRISPR, the spacers usually comprise sequences foreign to the bacterium such as a plasmid or phage sequence; in an exemplary CRISPR/Cas system targeting a Tet-
15 associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2), the spacers are derived from the gene sequence of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2), or a sequence of its regulatory elements.

RNA from the CRISPR locus is constitutively expressed and processed into small RNAs. These comprise a spacer flanked by a repeat sequence. The RNAs guide other Cas proteins to silence
20 exogenous genetic elements at the RNA or DNA level. Horvath et al. (2010) Science 327: 167-170; Makarova et al. (2006) Biology Direct 1: 7. The spacers thus serve as templates for RNA molecules, analogously to siRNAs. Pennisi (2013) Science 341: 833-836.

As these naturally occur in many different types of bacteria, the exact arrangements of the CRISPR and structure, function and number of Cas genes and their product differ somewhat from
25 species to species. Haft et al. (2005) PLoS Comput. Biol. 1: e60; Kunin et al. (2007) Genome Biol. 8: R61; Mojica et al. (2005) J. Mol. Evol. 60: 174-182; Bolotin et al. (2005) Microbiol. 151: 2551-2561; Pourcel et al. (2005) Microbiol. 151: 653-663; and Stern et al. (2010) Trends. Genet. 28: 335-340. For example, the Cse (Cas subtype, *E. coli*) proteins (e.g., CasA) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. Brouns et al.
30 (2008) Science 321: 960-964. In other prokaryotes, Cas6 processes the CRISPR transcript. The CRISPR-based phage inactivation in *E. coli* requires Cascade and Cas3, but not Cas1 or Cas2. The Cmr (Cas RAMP module) proteins in *Pyrococcus furiosus* and other prokaryotes form a functional complex with small CRISPR RNAs that recognizes and cleaves complementary target RNAs. A simpler CRISPR system relies on the protein Cas9, which is a nuclease with two active cutting sites,
35 one for each strand of the double helix. Combining Cas9 and modified CRISPR locus RNA can be used in a system for gene editing. Pennisi (2013) Science 341: 833-836.

In one embodiment, foreign DNA can be introduced into the cell along with the CRISPR/Cas system, e.g., DNA encoding a CAR, e.g., as described herein; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to integrate the DNA encoding the CAR, e.g., as described herein, at or near the site targeted by the CRISPR/Cas system. As shown
30 herein, in the examples, but without being bound by theory, such integration may lead to the expression of the CAR as well as disruption of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. Such foreign DNA molecule is referred to herein as “template DNA.” In embodiments, the template DNA further comprises homology arms 5’ to, 3’ to, or both 5’ and 3’ to the nucleic acid of the template DNA which encodes the molecule or
35 molecules of interest (e.g., which encodes a CAR described herein), wherein said homology arms are complementary to genomic DNA sequence flanking the target sequence.

In an embodiment, the CRISPR/Cas system of the present invention comprises Cas9, e.g., *S. pyogenes* Cas9, and a gRNA comprising a targeting sequence which hybridizes to a sequence of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In an embodiment, the CRISPR/Cas system comprises nucleic acid encoding a gRNA specific for a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, and a nucleic acid encoding a Cas protein, e.g., Cas9, e.g., *S. pyogenes* Cas9. In an embodiment, the CRISPR/Cas system comprises a gRNA specific for a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, and a nucleic acid encoding a Cas protein, e.g., Cas9, e.g., *S. pyogenes* Cas9.

Examples of genomic target sequences for Tet2, for which gRNAs comprising complementary targeting sequences can be generated for use in the present invention are listed in the Table 2 below. In embodiments, the gRNA comprises an RNA complement of a Target Sequence of the table below (e.g., for sgTET2_1, the gRNA would comprise CCUUGGACACCUUCUCCUCC (SEQ ID NO: 44)). In embodiments, the gRNA comprises the RNA analog of a Target sequence of the table 2 below (e.g., for sgTET2_1, the gRNA would comprise GGAACCUGUGGAAGAGGAGG (SEQ ID NO: 45)). In embodiments, the Tet2 inhibitor is nucleic acid encoding a gRNA molecule specific for Tet2, wherein the nucleic acid comprises the sequence of a Target Sequence from the 2 table below, e.g., under the control of a U6- or H1- promoter:

Table 2

gRNA ID	Gene Symbol	Chromosome	Position	Strand	Target Sequence within the Tet2 gene sequence
sgTET2_1	TET2	chr4	106156327	-	GGAACCTGTGGAAGAGGAGG (SEQ ID NO: 46)
sgTET2_2	TET2	chr4	106156339	-	GAAGGAAGCTGAGGAACCTG (SEQ ID NO: 47)
sgTET2_3	TET2	chr4	106156897	+	ATGACCTCCAAACAATACAC (SEQ ID NO: 48)
sgTET2_4	TET2	chr4	106157189	-	CAAGTGCTGTTTCAACACTG (SEQ ID NO: 49)
sgTET2_5	TET2	chr4	106157296	-	GGGAGATGTGAACTCTGGGA (SEQ ID NO: 50)
sgTET2_6	TET2	chr4	106155148	-	GGAGGTGATGGTATCAGGAA (SEQ ID NO: 51)
sgTET2_7	TET2	chr4	106155166	-	GGTTCTGTCTGGCAAATGGG (SEQ ID NO: 52)
sgTET2_8	TET2	chr4	106155217	-	GGATGAGCTCTCTCAGGCAG (SEQ ID NO: 53)
sgTET2_9	TET2	chr4	106155403	-	TGAAGGAGCCCAGAGAGAGA (SEQ ID NO: 65)
sgTET2_10	TET2	chr4	106155478	+	GTAAGCCAAGAAAGAAATCC (SEQ ID NO: 66)

Examples of gRNA targeting sequences which are useful in the various embodiments of the present invention to inhibit a Tet, e.g., Tet2, are provided below in Table 3. In embodiments a CRISPR/Cas system of the present invention comprises a gRNA molecule comprising a targeting sequence comprising a sequence listed in Table 3. In embodiments, a CRISPR/Cas system of the present invention comprises a gRNA molecule comprising a targeting sequence that is a sequence listed in Table 3.

Table 3

ID	TARGET	TARGET REGION	STRAND	Location of Genomic Target Sequence (hg38)	gRNA Targeting sequence	SEQ ID NO:
54790_1_1	TET2	EXON	+	chr4:105145928-105145948	UGUCGGGUCUUUAAAAAUAC	73
54790_1_3	TET2	EXON	+	chr4:105145945-105145965	UACAGGCCCCUAAAGCACUA	74
54790_1_4	TET2	EXON	+	chr4:105145946-105145966	ACAGGCCCCUAAAGCACUAA	75
54790_1_5	TET2	EXON	+	chr4:105145957-105145977	AAGCACUAAGGGCAUGCCCU	76
54790_1_8	TET2	EXON	+	chr4:105145966-105145986	GGGCAUGCCCUCGGUGAAAC	77
54790_1_10	TET2	EXON	+	chr4:105145967-105145987	GGCAUGCCCUCGGUGAAACA	78
54790_1_12	TET2	EXON	+	chr4:105145968-105145988	GCAUGCCCUCGGUGAAACAG	79
54790_1_20	TET2	EXON	+	chr4:105146006-105146026	UGAGAUUAAAGCGACAGAAA	80
54790_1_23	TET2	EXON	+	chr4:105146007-105146027	GAGAUUAAAGCGACAGAAA	81
54790_1_25	TET2	EXON	+	chr4:105146012-105146032	UAAAGCGACAGAAAAGGGAA	82
54790_1_30	TET2	EXON	+	chr4:105146021-105146041	AGAAAAGGGAAAGGAGAGCG	83
54790_1_31	TET2	EXON	+	chr4:105146022-105146042	GAAAAGGGAAAGGAGAGCGC	84
54790_1_33	TET2	EXON	+	chr4:105146028-105146048	GGAAAGGAGAGCGCGGGCAA	85
54790_1_35	TET2	EXON	+	chr4:105146029-105146049	GAAAGGAGAGCGCGGGCAAC	86
54790_1_38	TET2	EXON	+	chr4:105146038-105146058	GCGCGGGCAACGGGAUCUAA	87
54790_1_39	TET2	EXON	+	chr4:105146039-105146059	CGCGGGCAACGGGAUCUAAA	88
54790_1_43	TET2	EXON	+	chr4:105146053-105146073	UCUAAAGGGAGAUAGAGACG	89

54790_1_44	TET2	EXON	+	chr4:105146054 -105146074	CUAAAGGGAGAUAGAGACGC	90
54790_1_47	TET2	EXON	+	chr4:105146063 -105146083	GAUAGAGACGCGGGCCUCUG	91
54790_1_48	TET2	EXON	+	chr4:105146064 -105146084	AUAGAGACGCGGGCCUCUGA	92
54790_1_49	TET2	EXON	+	chr4:105146069 -105146089	GACGCGGGCCUCUGAGGGUA	93
54790_1_51	TET2	EXON	+	chr4:105146072 -105146092	GCGGGCCUCUGAGGGUAAGG	94
54790_1_52	TET2	EXON	+	chr4:105146073 -105146093	CGGGCCUCUGAGGGUAAGGU	95
54790_1_54	TET2	EXON	+	chr4:105146082 -105146102	GAGGGUAAGGUGGGCGCAAG	96
54790_1_61	TET2	EXON	-	chr4:105145954 -105145974	GCAUGCCCUUAGUGCUUUAG	97
54790_1_62	TET2	EXON	-	chr4:105145955 -105145975	GGCAUGCCCUUAGUGCUUUA	98
54790_1_64	TET2	EXON	-	chr4:105145956 -105145976	GGGCAUGCCCUUAGUGCUUU	99
54790_1_68	TET2	EXON	-	chr4:105145976 -105145996	GCGCUCCCCUGUUUCACCGA	100
54790_1_69	TET2	EXON	-	chr4:105145977 -105145997	AGCGCUCCCCUGUUUCACCG	101
54790_1_87	TET2	EXON	-	chr4:105146080 -105146100	UGC GCC ACCU UACCCUCAG	102
54790_2_1	TET2	EXON	+	chr4:105146669 -105146689	AGAGCCGGCGGUAGCGGCAG	103
54790_2_2	TET2	EXON	+	chr4:105146675 -105146695	GGCGGUAGCGGCAGUGGCAG	104
54790_2_6	TET2	EXON	+	chr4:105146686 -105146706	CAGUGGCAGCGGCGAGAGCU	105
54790_2_7	TET2	EXON	+	chr4:105146687 -105146707	AGUGGCAGCGGCGAGAGCUU	106
54790_2_8	TET2	EXON	+	chr4:105146690 -105146710	GGCAGCGGCGAGAGCUUGGG	107
54790_2_12	TET2	EXON	+	chr4:105146725 -105146745	CCUCGCGAGCGCCGCGCGCC	108
54790_2_13	TET2	EXON	+	chr4:105146726 -105146746	CUCGCGAGCGCCGCGCGCCC	109
54790_2_14	TET2	EXON	+	chr4:105146761 -105146781	GCAAGUCACGUCCGCCCCCU	110
54790_2_15	TET2	EXON	+	chr4:105146766 -105146786	UCACGUCCGCCCCCUCGGCG	111
54790_2_17	TET2	EXON	+	chr4:105146783 -105146803	GCGCGGCCGCCCCGAGACGC	112
54790_2_24	TET2	EXON	+	chr4:105146836 -105146856	CUGCCUUAUGAAUAUUGAUG	113

54790_2_25	TET2	EXON	+	chr4:105146839 -105146859	CCUUAUGAAUAUUGAUGCGG	114
54790_2_27	TET2	EXON	+	chr4:105146844 -105146864	UGAAUAUUGAUGCGGAGGCU	115
54790_2_34	TET2	EXON	+	chr4:105146868 -105146888	UGC UUUCGUAGAGAAGCAGA	116
54790_2_37	TET2	EXON	+	chr4:105146879 -105146899	AGAAGCAGAAGGAAGCAAGA	117
54790_2_39	TET2	EXON	+	chr4:105146891 -105146911	AAGCAAGAUGGCUGCCCUUU	118
54790_2_44	TET2	EXON	+	chr4:105146905 -105146925	CCCUUUAGGAUUUGUUAGAA	119
54790_2_51	TET2	EXON	+	chr4:105146926 -105146946	GGAGACCCGACUGCAACUGC	120
54790_2_52	TET2	EXON	+	chr4:105146938 -105146958	GCAACUGCUGGAUUGCUGCA	121
54790_2_56	TET2	EXON	+	chr4:105146944 -105146964	GCUGGAUUGCUGCAAGGCUG	122
54790_2_57	TET2	EXON	+	chr4:105146945 -105146965	CUGGAUUGCUGCAAGGCUGA	123
54790_2_62	TET2	EXON	+	chr4:105146957 -105146977	AAGGCUGAGGGACGAGAACG	124
54790_2_64	TET2	EXON	-	chr4:105146676 -105146696	GCUGCCACUGCCGCUACCGC	125
54790_2_65	TET2	EXON	-	chr4:105146716 -105146736	CGCUCGCGAGGAGGCGGCGG	126
54790_2_66	TET2	EXON	-	chr4:105146719 -105146739	CGGCGCUCGCGAGGAGGCGG	127
54790_2_67	TET2	EXON	-	chr4:105146722 -105146742	GCGCGGCGCUCGCGAGGAGG	128
54790_2_68	TET2	EXON	-	chr4:105146725 -105146745	GGCGCGCGGCGCUCGCGAGG	129
54790_2_69	TET2	EXON	-	chr4:105146728 -105146748	CCGGGCGCGCGGCGCUCGCG	130
54790_2_74	TET2	EXON	-	chr4:105146739 -105146759	GCGAGCGGGACCCGGGCGCG	131
54790_2_75	TET2	EXON	-	chr4:105146746 -105146766	CUUGCAUGCGAGCGGGACCC	132
54790_2_76	TET2	EXON	-	chr4:105146747 -105146767	ACUUGCAUGCGAGCGGGACC	133
54790_2_78	TET2	EXON	-	chr4:105146753 -105146773	GACGUGACUUGCAUGCGAGC	134
54790_2_79	TET2	EXON	-	chr4:105146754 -105146774	GGACGUGACUUGCAUGCGAG	135
54790_2_83	TET2	EXON	-	chr4:105146775 -105146795	GGGCGGCCGCGCCGAGGGGG	136
54790_2_85	TET2	EXON	-	chr4:105146778 -105146798	UCGGGGCGGCCGCGCCGAGG	137

54790_2_86	TET2	EXON	-	chr4:105146779 -105146799	CUCGGGGCGGCCGCGCCGAG	138
54790_2_88	TET2	EXON	-	chr4:105146780 -105146800	UCUCGGGGCGGCCGCGCCGA	139
54790_2_89	TET2	EXON	-	chr4:105146781 -105146801	GUCUCGGGGCGGCCGCGCCG	140
54790_2_93	TET2	EXON	-	chr4:105146792 -105146812	GCGGGGCCGGCGUCUCGGGG	141
54790_2_94	TET2	EXON	-	chr4:105146795 -105146815	UCAGCGGGGCCGGCGUCUCG	142
54790_2_95	TET2	EXON	-	chr4:105146796 -105146816	CUCAGCGGGGCCGGCGUCUC	143
54790_2_97	TET2	EXON	-	chr4:105146797 -105146817	ACUCAGCGGGGCCGGCGUCU	144
54790_2_100	TET2	EXON	-	chr4:105146805 -105146825	UUCUCAUCACUCAGCGGGGC	145
54790_2_101	TET2	EXON	-	chr4:105146809 -105146829	UCUGUUCUCAUCACUCAGCG	146
54790_2_103	TET2	EXON	-	chr4:105146810 -105146830	GUCUGUUCUCAUCACUCAGC	147
54790_2_106	TET2	EXON	-	chr4:105146811 -105146831	CGUCUGUUCUCAUCACUCAG	148
54790_2_109	TET2	EXON	-	chr4:105146842 -105146862	CCUCCGCAUCAAAUAUUAUA	149
54790_2_117	TET2	EXON	-	chr4:105146908 -105146928	CCUUUCUAACAAAUCCUAAA	150
54790_2_118	TET2	EXON	-	chr4:105146909 -105146929	UCCUUUCUAACAAAUCCUAA	151
54790_2_122	TET2	EXON	-	chr4:105146934 -105146954	GCAAUCCAGCAGUUGCAGUC	152
54790_2_123	TET2	EXON	-	chr4:105146935 -105146955	AGCAAUCCAGCAGUUGCAGU	153
54790_3_1	TET2	EXON	+	chr4:105190341 -105190361	AAACUCUGUCUUCUCUAGGC	154
54790_3_13	TET2	EXON	+	chr4:105190411 -105190431	UCCUGUUGAGUUACAACGCU	155
54790_3_16	TET2	EXON	+	chr4:105190418 -105190438	GAGUUACAACGCUUGGAAGC	156
54790_3_19	TET2	EXON	+	chr4:105190424 -105190444	CAACGCUUGGAAGCAGGAGA	157
54790_3_21	TET2	EXON	+	chr4:105190425 -105190445	AACGCUUGGAAGCAGGAGAU	158
54790_3_24	TET2	EXON	+	chr4:105190444 -105190464	UGGGCUCAGCAGCAGCCAAU	159
54790_3_26	TET2	EXON	+	chr4:105190456 -105190476	CAGCCAAUAGGACAUGAUCC	160
54790_3_30	TET2	EXON	+	chr4:105190469 -105190489	AUGAUCCAGGAAGAGCAGUA	161

54790_3_32	TET2	EXON	+	chr4:105190470 -105190490	UGAUCCAGGAAGAGCAGUAA	162
54790_3_34	TET2	EXON	+	chr4:105190483 -105190503	GCAGUAAGGGACUGAGCUGC	163
54790_3_37	TET2	EXON	+	chr4:105190494 -105190514	CUGAGCUGCUGGUAAGACAG	164
54790_3_46	TET2	EXON	-	chr4:105190385 -105190405	GCAAGUAAACAAUCUUGAGA	165
54790_3_47	TET2	EXON	-	chr4:105190386 -105190406	GGCAAGUAAACAAUCUUGAG	166
54790_3_52	TET2	EXON	-	chr4:105190407 -105190427	UUGUAAACUACAGGAGCAA	167
54790_3_55	TET2	EXON	-	chr4:105190415 -105190435	UCCAAGCGUUGUAAACUCAA	168
54790_3_60	TET2	EXON	-	chr4:105190462 -105190482	CUUCCUGGAUCAUGUCCUAA	169
54790_3_62	TET2	EXON	-	chr4:105190477 -105190497	CAGUCCCUUACUGCUCUUCC	170
54790_4_7	TET2	EXON	+	chr4:105233887 -105233907	GCUCUUUAGAAUUCAACUAG	171
54790_4_8	TET2	EXON	+	chr4:105233888 -105233908	CUCUUUAGAAUUCAACUAGA	172
54790_4_12	TET2	EXON	+	chr4:105233899 -105233919	UCAACUAGAGGGCAGCCUUG	173
54790_4_14	TET2	EXON	+	chr4:105233903 -105233923	CUAGAGGGCAGCCUUGUGGA	174
54790_4_19	TET2	EXON	+	chr4:105233923 -105233943	UGGCCCCGAAGCAAGCCUGA	175
54790_4_21	TET2	EXON	+	chr4:105233929 -105233949	CGAAGCAAGCCUGAUGGAAC	176
54790_4_25	TET2	EXON	+	chr4:105233950 -105233970	GGAUAGAACCAACCAUGUUG	177
54790_4_26	TET2	EXON	+	chr4:105233951 -105233971	GAUAGAACCAACCAUGUUGA	178
54790_4_30	TET2	EXON	+	chr4:105234010 -105234030	CAUUUGCCAGACAGAACCUC	179
54790_4_37	TET2	EXON	+	chr4:105234029 -105234049	CUGGCUACAAAGCUCCAGAA	180
54790_4_44	TET2	EXON	+	chr4:105234068 -105234088	AGAGCUCAUCCAGAAGUAAA	181
54790_4_45	TET2	EXON	+	chr4:105234081 -105234101	AAGUAAAUGGAGACACCAAG	182
54790_4_47	TET2	EXON	+	chr4:105234104 -105234124	CACUCUUUCAAAGUUAUUA	183
54790_4_54	TET2	EXON	+	chr4:105234121 -105234141	UUAUGGAAUACCCUGUAUGA	184
54790_4_57	TET2	EXON	+	chr4:105234122 -105234142	UAUGGAAUACCCUGUAUGAA	185

54790_4_66	TET2	EXON	+	chr4:105234170 -105234190	GACUUUACACAAGAAAGUAG	186
54790_4_67	TET2	EXON	+	chr4:105234171 -105234191	ACUUUACACAAGAAAGUAGA	187
54790_4_72	TET2	EXON	+	chr4:105234194 -105234214	UAUUCCAAGUGUUUGCAAAA	188
54790_4_74	TET2	EXON	+	chr4:105234197 -105234217	UCCAAGUGUUUGCAAAAUGG	189
54790_4_81	TET2	EXON	+	chr4:105234233 -105234253	GUUAGUGAACCUUCUCUCUC	190
54790_4_82	TET2	EXON	+	chr4:105234234 -105234254	UUAGUGAACCUUCUCUCUCU	191
54790_4_89	TET2	EXON	+	chr4:105234271 -105234291	GAAAUUGAAACAAGACCAAA	192
54790_4_93	TET2	EXON	+	chr4:105234278 -105234298	AAACAAGACCAAAAGGCUAA	193
54790_4_97	TET2	EXON	+	chr4:105234296 -105234316	AAUGGAGAAAGACGUAACUU	194
54790_4_99	TET2	EXON	+	chr4:105234297 -105234317	AUGGAGAAAGACGUAACUUC	195
54790_4_100	TET2	EXON	+	chr4:105234298 -105234318	UGGAGAAAGACGUAACUUCG	196
54790_4_106	TET2	EXON	+	chr4:105234320 -105234340	GUAAGCCAAGAAAGAAAUCC	197
54790_4_123	TET2	EXON	+	chr4:105234437 -105234457	UUUUCAACACAUAACUGCAG	198
54790_4_124	TET2	EXON	+	chr4:105234438 -105234458	UUUCAACACAUAACUGCAGU	199
54790_4_134	TET2	EXON	+	chr4:105234475 -105234495	GCUUCAGAUUCUGAAUGAGC	200
54790_4_138	TET2	EXON	+	chr4:105234478 -105234498	UCAGAUUCUGAAUGAGCAGG	201
54790_4_140	TET2	EXON	+	chr4:105234479 -105234499	CAGAUUCUGAAUGAGCAGGA	202
54790_4_141	TET2	EXON	+	chr4:105234480 -105234500	AGAUUCUGAAUGAGCAGGAG	203
54790_4_147	TET2	EXON	+	chr4:105234529 -105234549	CAUUGUAUUACUUA AAAACA	204
54790_4_151	TET2	EXON	+	chr4:105234548 -105234568	AAGGCAGUGCUAAUGCCUAA	205
54790_4_153	TET2	EXON	+	chr4:105234574 -105234594	UACAGUUUCUGCCUCUCCG	206
54790_4_157	TET2	EXON	+	chr4:105234587 -105234607	UCUUCCGUGGAACACACACA	207
54790_4_161	TET2	EXON	+	chr4:105234598 -105234618	ACACACACAUGGUGAACUCC	208
54790_4_163	TET2	EXON	+	chr4:105234643 -105234663	UCCAGAUUGUGUUUCCAUUG	209

54790_4_171	TET2	EXON	+	chr4:105234685 -105234705	CAUAAAUGCCAUAU AACAGUC	210
54790_4_177	TET2	EXON	+	chr4:105234734 -105234754	ACUCACCCAUCGCAU ACCUC	211
54790_4_178	TET2	EXON	+	chr4:105234735 -105234755	CUCACCCAUCGCAU ACCUCA	212
54790_4_181	TET2	EXON	+	chr4:105234793 -105234813	GCCUCCAAAGCCAGCUGCAG	213
54790_4_184	TET2	EXON	+	chr4:105234802 -105234822	GCCAGCUGCAGUGGUGAGUG	214
54790_4_200	TET2	EXON	+	chr4:105234943 -105234963	UCCUGCAGAAAAUAACA UCC	215
54790_4_201	TET2	EXON	+	chr4:105234944 -105234964	CCUGCAGAAAAUAACA UCCA	216
54790_4_203	TET2	EXON	+	chr4:105234965 -105234985	GGAACCACAAAGCUAGCGUC	217
54790_4_207	TET2	EXON	+	chr4:105234983 -105235003	UCUGGUGAAGAAUUCUGUUC	218
54790_4_211	TET2	EXON	+	chr4:105235010 -105235030	AGCAGCAAUUUGCAAGCUCC	219
54790_4_212	TET2	EXON	+	chr4:105235013 -105235033	AGCAAUUUGCAAGCUCCUGG	220
54790_4_216	TET2	EXON	+	chr4:105235026 -105235046	CUCCUGGUGGCAGCUCUGAA	221
54790_4_219	TET2	EXON	+	chr4:105235052 -105235072	UUAAAACAAAAUGAAAUGAA	222
54790_4_225	TET2	EXON	+	chr4:105235087 -105235107	GCAAAGCUCAGUGUUCACUA	223
54790_4_235	TET2	EXON	+	chr4:105235162 -105235182	UCCCCCUCCUCCUCU UCCAC	224
54790_4_240	TET2	EXON	+	chr4:105235184 -105235204	GUUCCUCAGCUUCCUUCAGA	225
54790_4_245	TET2	EXON	+	chr4:105235202 -105235222	GAAGGAAAAAGCACUCUGAA	226
54790_4_247	TET2	EXON	+	chr4:105235205 -105235225	GGAAAAAGCACUCUGAAUGG	227
54790_4_256	TET2	EXON	+	chr4:105235260 -105235280	AAAGUAACACAACACU UUA	228
54790_4_258	TET2	EXON	+	chr4:105235261 -105235281	AAGUAACACAACACU UUA	229
54790_4_262	TET2	EXON	+	chr4:105235276 -105235296	UUUAAGGGAAGUGAAAAUAG	230
54790_4_263	TET2	EXON	+	chr4:105235277 -105235297	UUAAGGGAAGUGAAAAUAGA	231
54790_4_268	TET2	EXON	+	chr4:105235288 -105235308	GAAAAUAGAGGGUAAACCUG	232
54790_4_272	TET2	EXON	+	chr4:105235356 -105235376	CUUCUCCGAUGCUUUCUGAA	233

54790_4_280	TET2	EXON	+	chr4:105235380 -105235400	CUCAGAAUAAUUGUGUGAAC	234
54790_4_284	TET2	EXON	+	chr4:105235400 -105235420	AGGAAUGACAUACAGACUGC	235
54790_4_286	TET2	EXON	+	chr4:105235401 -105235421	GGAAUGACAUACAGACUGCA	236
54790_4_294	TET2	EXON	+	chr4:105235478 -105235498	AAGCAUAACCCACCAAUUUU	237
54790_4_297	TET2	EXON	+	chr4:105235487 -105235507	CCACCAAUUUUUGGUAGCAG	238
54790_4_302	TET2	EXON	+	chr4:105235498 -105235518	UGGUAGCAGUGGAGAGCUAC	239
54790_4_313	TET2	EXON	+	chr4:105235546 -105235566	CAAAGAGCAAGAGAUUCUGA	240
54790_4_314	TET2	EXON	+	chr4:105235547 -105235567	AAAGAGCAAGAGAUUCUGAA	241
54790_4_317	TET2	EXON	+	chr4:105235558 -105235578	GAUUCUGAAGGGUCGAGACA	242
54790_4_324	TET2	EXON	+	chr4:105235607 -105235627	ACACAGCACUAUCUGAAACC	243
54790_4_326	TET2	EXON	+	chr4:105235611 -105235631	AGCACUAUCUGAAACCAGGA	244
54790_4_329	TET2	EXON	+	chr4:105235624 -105235644	ACCAGGAUGGAUUGAAUUGA	245
54790_4_333	TET2	EXON	+	chr4:105235645 -105235665	GGCCCCUCGUUUUCACCAAG	246
54790_4_339	TET2	EXON	+	chr4:105235669 -105235689	AUCCCAUCUAAAACGUAAUG	247
54790_4_343	TET2	EXON	+	chr4:105235739 -105235759	AUGACCUCCAAACAAUACAC	248
54790_4_347	TET2	EXON	+	chr4:105235757 -105235777	ACUGGAAAUUCCAACAUGCC	249
54790_4_349	TET2	EXON	+	chr4:105235758 -105235778	CUGGAAAUUCCAACAUGCCU	250
54790_4_351	TET2	EXON	+	chr4:105235759 -105235779	UGGAAAUUCCAACAUGCCUG	251
54790_4_352	TET2	EXON	+	chr4:105235760 -105235780	GGAAAUUCCAACAUGCCUGG	252
54790_4_353	TET2	EXON	+	chr4:105235761 -105235781	GAAAUUCCAACAUGCCUGGG	253
54790_4_355	TET2	EXON	+	chr4:105235770 -105235790	ACAUGCCUGGGGGGCUCCCA	254
54790_4_360	TET2	EXON	+	chr4:105235801 -105235821	CACCCAGAAAACAACACAGC	255
54790_4_365	TET2	EXON	+	chr4:105235841 -105235861	UACCAAGUUGAAAUGAAUCA	256
54790_4_366	TET2	EXON	+	chr4:105235842 -105235862	ACCAAGUUGAAAUGAAUCAA	257

54790_4_368	TET2	EXON	+	chr4:105235853 -105235873	AUGAAUCAAGGGCAGUCCCA	258
54790_4_370	TET2	EXON	+	chr4:105235861 -105235881	AGGGCAGUCCCAAGGUACAG	259
54790_4_371	TET2	EXON	+	chr4:105235897 -105235917	GUUCCAAAAACCCUCACACC	260
54790_4_376	TET2	EXON	+	chr4:105235952 -105235972	GCUCAUGUGCAGUCACUGUG	261
54790_4_388	TET2	EXON	+	chr4:105236038 -105236058	GAAACAGCACUUGAAUCAAC	262
54790_4_399	TET2	EXON	+	chr4:105236098 -105236118	GCAACAUAAAGCCUCAUAAAC	263
54790_4_407	TET2	EXON	+	chr4:105236182 -105236202	AUUACAAAUAAAGAAUAAAG	264
54790_4_416	TET2	EXON	+	chr4:105236237 -105236257	AACAAUGAUCAGCAAAGAGA	265
54790_4_417	TET2	EXON	+	chr4:105236249 -105236269	CAAAGAGAAGGAUCAUUCUU	266
54790_4_419	TET2	EXON	+	chr4:105236263 -105236283	AUUCUUUGGCCAGACUAAAG	267
54790_4_426	TET2	EXON	+	chr4:105236279 -105236299	AAAGUGGAAGAAUGUUUUCA	268
54790_4_435	TET2	EXON	+	chr4:105236332 -105236352	CGAGACUCAUAAUGUCCAAA	269
54790_4_438	TET2	EXON	+	chr4:105236333 -105236353	GAGACUCAUAAUGUCCAAAU	270
54790_4_440	TET2	EXON	+	chr4:105236338 -105236358	UCAUAAUGUCCAAAUGGGAC	271
54790_4_444	TET2	EXON	+	chr4:105236341 -105236361	UAAUGUCCAAAUGGGACUGG	272
54790_4_452	TET2	EXON	+	chr4:105236413 -105236433	AUCAAGUGCAUGCAAAAUAC	273
54790_4_466	TET2	EXON	+	chr4:105236486 -105236506	ACACAUCCUGAACUUUUUGC	274
54790_4_475	TET2	EXON	+	chr4:105236562 -105236582	CAAAGCAAGAUUCUUCUUCAC	275
54790_4_479	TET2	EXON	+	chr4:105236578 -105236598	UCACAGGUGCUUUCAAGAAC	276
54790_4_486	TET2	EXON	+	chr4:105236611 -105236631	ACAACAAGCUUCAGUUCUAC	277
54790_4_488	TET2	EXON	+	chr4:105236612 -105236632	CAACAAGCUUCAGUUCUACA	278
54790_4_493	TET2	EXON	+	chr4:105236642 -105236662	AAUAGAAACCAAGAU AUGUC	279
54790_4_494	TET2	EXON	+	chr4:105236673 -105236693	CUGCGCAACUUGCUCAGCAA	280
54790_4_498	TET2	EXON	+	chr4:105236719 -105236739	UGUUUUUCCUGUGCCUGACC	281

54790_4_501	TET2	EXON	+	chr4:105236720 -105236740	GUUUUUCCUGUGCCUGACCA	282
54790_4_503	TET2	EXON	+	chr4:105236723 -105236743	UUUCCUGUGCCUGACCAGGG	283
54790_4_511	TET2	EXON	+	chr4:105236752 -105236772	CACUCAGACCCCUCCCCAGA	284
54790_4_512	TET2	EXON	+	chr4:105236778 -105236798	CUCAAAAGCAUGCUGCUCUA	285
54790_4_513	TET2	EXON	+	chr4:105236781 -105236801	AAAAGCAUGCUGCUCUAAGG	286
54790_4_518	TET2	EXON	+	chr4:105236856 -105236876	CUUGCCAUAGUCAGAUGCAC	287
54790_4_520	TET2	EXON	+	chr4:105236866 -105236886	UCAGAUGCACAGGCCAAUUA	288
54790_4_522	TET2	EXON	+	chr4:105236869 -105236889	GAUGCACAGGCCAAUUAAGG	289
54790_4_525	TET2	EXON	+	chr4:105236876 -105236896	AGGCCAAUUAAGGUGGAACC	290
54790_4_531	TET2	EXON	+	chr4:105236928 -105236948	CACCACCAGAAAACAAAACA	291
54790_4_532	TET2	EXON	+	chr4:105236935 -105236955	AGAAAACAAAACAUGGAAAA	292
54790_4_540	TET2	EXON	+	chr4:105237004 -105237024	AAAGAGCAUCAUUGAGACCA	293
54790_4_545	TET2	EXON	+	chr4:105237052 -105237072	CAAGUCGUUAUUUGACCAUA	294
54790_4_553	TET2	EXON	+	chr4:105237098 -105237118	CAAGUAAAAGUUGAAAUGUC	295
54790_4_554	TET2	EXON	+	chr4:105237099 -105237119	AAGUAAAAGUUGAAAUGUCA	296
54790_4_578	TET2	EXON	+	chr4:105237280 -105237300	UACUCCUAUAAAAAAUUAU	297
54790_4_582	TET2	EXON	+	chr4:105237329 -105237349	UUCCCAUCUUGCAGAUGUGU	298
54790_4_589	TET2	EXON	+	chr4:105237359 -105237379	CAGAAAUGUACUGAGACACA	299
54790_4_596	TET2	EXON	+	chr4:105237397 -105237417	AGCAAUUUAUCUUCAGAUUA	300
54790_4_597	TET2	EXON	+	chr4:105237398 -105237418	GCAAUUUAUCUUCAGAUUAU	301
54790_4_606	TET2	EXON	+	chr4:105237430 -105237450	CUUUUUUUAAAUCUUGAGUC	302
54790_4_614	TET2	EXON	+	chr4:105237446 -105237466	AGUCUGGCAGCAAUUUGUAA	303
54790_4_657	TET2	EXON	+	chr4:105237650 -105237670	GCUCUUUGUAUAUUAUCUCC	304
54790_4_662	TET2	EXON	+	chr4:105237663 -105237683	UAUCUCCUGGAGAGACAGCU	305

54790_4_668	TET2	EXON	+	chr4:105237708 -105237728	AAUGAGAAAAUAACGACCAU	306
54790_4_670	TET2	EXON	+	chr4:105237748 -105237768	UUUAAAUAUUUUUUAAUUCA	307
54790_4_679	TET2	EXON	+	chr4:105237778 -105237798	UAUUAGUUUCACAAGAUUUC	308
54790_4_682	TET2	EXON	+	chr4:105237786 -105237806	UCACAAGAUUUCUGGCUAAU	309
54790_4_686	TET2	EXON	+	chr4:105237787 -105237807	CACAAGAUUUCUGGCUAAUA	310
54790_4_693	TET2	EXON	+	chr4:105237817 -105237837	UAUCUUCAGUCUUCAUGAGU	311
54790_4_695	TET2	EXON	+	chr4:105237818 -105237838	AUCUUCAGUCUUCAUGAGUU	312
54790_4_697	TET2	EXON	+	chr4:105237819 -105237839	UCUUCAGUCUUCAUGAGUUG	313
54790_4_700	TET2	EXON	+	chr4:105237820 -105237840	CUUCAGUCUUCAUGAGUUGG	314
54790_4_709	TET2	EXON	+	chr4:105237882 -105237902	CUUUUCUCCAUUUAUACAUI	315
54790_4_741	TET2	EXON	+	chr4:105240332 -105240352	AAAGCUUUUUGUAAAAUUC	316
54790_4_746	TET2	EXON	+	chr4:105240344 -105240364	UAAAAUUCAGGAUAUGUAAU	317
54790_4_750	TET2	EXON	+	chr4:105240352 -105240372	AGGAUAUGUAAUAGGUCUGU	318
54790_4_754	TET2	EXON	+	chr4:105240377 -105240397	UAGUGAAAUUUUUUGCUGA	319
54790_4_760	TET2	EXON	+	chr4:105240395 -105240415	GAUGGAUGUAGAUUAUACG	320
54790_4_770	TET2	EXON	+	chr4:105240478 -105240498	AGACAAAUGUAAAUIUAGUG	321
54790_4_780	TET2	EXON	+	chr4:105240541 -105240561	GAUACCCCACACUGUGUAGA	322
54790_4_783	TET2	EXON	+	chr4:105240545 -105240565	CCCCACACUGUGUAGAAGGA	323
54790_4_785	TET2	EXON	+	chr4:105240548 -105240568	CACACUGUGUAGAAGGAUGG	324
54790_4_787	TET2	EXON	+	chr4:105240549 -105240569	ACACUGUGUAGAAGGAUGGA	325
54790_4_790	TET2	EXON	+	chr4:105240552 -105240572	CUGUGUAGAAGGAUGGAGGG	326
54790_4_791	TET2	EXON	+	chr4:105240579 -105240599	CUACUGUCCCUCUUGCGUG	327
54790_4_795	TET2	EXON	+	chr4:105240599 -105240619	UGGUUAUUAAGUUGCCUCAC	328
54790_4_796	TET2	EXON	+	chr4:105240600 -105240620	GGUUAUUAAGUUGCCUCACU	329

54790_4_800	TET2	EXON	+	chr4:105240634 -105240654	CACAUCUCAUAGAUAAUAUU	330
54790_4_807	TET2	EXON	+	chr4:105240703 -105240723	UCCCACUUUCCAUCUUUGU	331
54790_4_818	TET2	EXON	+	chr4:105240740 -105240760	UUCUUUUUGCCUGACUCUCC	332
54790_4_829	TET2	EXON	+	chr4:105240784 -105240804	UUCUAAAGUACAUACUAAUA	333
54790_4_830	TET2	EXON	+	chr4:105240785 -105240805	UCUAAAGUACAUACUAAUAU	334
54790_4_833	TET2	EXON	+	chr4:105240790 -105240810	AGUACAUACUAAUAUGGGUC	335
54790_4_841	TET2	EXON	+	chr4:105240833 -105240853	AAACAGCAAUAAAUGUUUAU	336
54790_4_842	TET2	EXON	+	chr4:105240834 -105240854	AACAGCAAUAAAUGUUUAUA	337
54790_4_845	TET2	EXON	+	chr4:105240841 -105240861	AUUAAAUGUUUAUAGGGAAGU	338
54790_4_851	TET2	EXON	+	chr4:105240851 -105240871	AUAGGGAAGUAGGAAGAAAA	339
54790_4_853	TET2	EXON	+	chr4:105240852 -105240872	UAGGGAAGUAGGAAGAAAAA	340
54790_4_855	TET2	EXON	+	chr4:105240853 -105240873	AGGGAAGUAGGAAGAAAAAG	341
54790_4_858	TET2	EXON	+	chr4:105240885 -105240905	CAAUAAACCAAGCAAUAUUC	342
54790_4_861	TET2	EXON	+	chr4:105240886 -105240906	AAUAAACCAAGCAAUAUUCU	343
54790_4_862	TET2	EXON	+	chr4:105240887 -105240907	AUAAACCAAGCAAUAUUCUG	344
54790_4_863	TET2	EXON	+	chr4:105240888 -105240908	UAAACCAAGCAAUAUUCUGG	345
54790_4_865	TET2	EXON	+	chr4:105240891 -105240911	ACCAAGCAAUAUUCUGGGGG	346
54790_4_867	TET2	EXON	+	chr4:105240892 -105240912	CCAAGCAAUAUUCUGGGGGU	347
54790_4_870	TET2	EXON	+	chr4:105240902 -105240922	UUCUGGGGGUGGGGAUAGAGC	348
54790_4_880	TET2	EXON	+	chr4:105240940 -105240960	UCUUUUAAAAUCCAAGUAAU	349
54790_4_881	TET2	EXON	+	chr4:105240944 -105240964	UUAAAAUCCAAGUAAUAGGU	350
54790_4_891	TET2	EXON	+	chr4:105240991 -105241011	UUUUUUCCAGCUCAAAAAAU	351
54790_4_905	TET2	EXON	+	chr4:105241063 -105241083	UUUGUUUAGUUUCAUUUAUU	352
54790_4_929	TET2	EXON	+	chr4:105241146 -105241166	UGUACAUAUACUAAUUAUG	353

54790_4_945	TET2	EXON	+	chr4:105241237 -105241257	UAGAGCCCUUAAUGUGUAGU	354
54790_4_949	TET2	EXON	+	chr4:105241238 -105241258	AGAGCCCUUAAUGUGUAGUU	355
54790_4_951	TET2	EXON	+	chr4:105241239 -105241259	GAGCCCUUAAUGUGUAGUUG	356
54790_4_953	TET2	EXON	+	chr4:105241240 -105241260	AGCCCUUAAUGUGUAGUUGG	357
54790_4_956	TET2	EXON	+	chr4:105241253 -105241273	UAGUUGGGGGUUAAGCUUUG	358
54790_4_962	TET2	EXON	+	chr4:105241283 -105241303	CUUUAUAUUUAGUAUAAUUG	359
54790_4_973	TET2	EXON	+	chr4:105241340 -105241360	CAAAUUAUUGAAAAAGAUGA	360
54790_4_977	TET2	EXON	+	chr4:105241361 -105241381	GGUCCUUUUUAUACCCAUCU	361
54790_4_979	TET2	EXON	+	chr4:105241367 -105241387	UUUUAUACCCAUCUAGGAGC	362
54790_4_984	TET2	EXON	+	chr4:105241378 -105241398	UCUAGGAGCAGGUCCUAAUG	363
54790_4_990	TET2	EXON	+	chr4:105241399 -105241419	GGCAGCUAUUAGAGAAAUCA	364
54790_4_993	TET2	EXON	+	chr4:105241407 -105241427	UUAGAGAAAUCAUGGAAGAA	365
54790_4_995	TET2	EXON	+	chr4:105241422 -105241442	AAGAAAGGUAAUUAACGCAA	366
54790_4_997	TET2	EXON	+	chr4:105241428 -105241448	GGUAAUUAACGCAAAGGCAC	367
54790_4_998	TET2	EXON	+	chr4:105241429 -105241449	GUAAUUAACGCAAAGGCACA	368
54790_4_1014	TET2	EXON	+	chr4:105241523 -105241543	UAAAUUGAGUAAUUAUUAGU	369
54790_4_1019	TET2	EXON	+	chr4:105241538 -105241558	UUAGUAGGCUUAGCUAUUCU	370
54790_4_1020	TET2	EXON	+	chr4:105241539 -105241559	UAGUAGGCUUAGCUAUUCUA	371
54790_4_1029	TET2	EXON	+	chr4:105241592 -105241612	AGAGAGUCACAAUAUUUGAC	372
54790_4_1032	TET2	EXON	+	chr4:105241612 -105241632	AGGACUAAUAGUCUGCUAGC	373
54790_4_1033	TET2	EXON	+	chr4:105241618 -105241638	AAUAGUCUGCUAGCUGGCAC	374
54790_4_1035	TET2	EXON	+	chr4:105241636 -105241656	ACAGGCUGCCCACUUUGCGA	375
54790_4_1040	TET2	EXON	+	chr4:105241653 -105241673	CGAUGGAUGCCAGAAAACCC	376
54790_4_1043	TET2	EXON	+	chr4:105241663 -105241683	CAGAAAACCCAGGCAUGAAC	377

54790_4_1045	TET2	EXON	+	chr4:105241669 -105241689	ACCCAGGCAUGAACAGGAAU	378
54790_4_1046	TET2	EXON	+	chr4:105241678 -105241698	UGAACAGGAAUCGGCCAGCC	379
54790_4_1047	TET2	EXON	+	chr4:105241693 -105241713	CAGCCAGGCUGCCAGCCACA	380
54790_4_1048	TET2	EXON	+	chr4:105241699 -105241719	GGCUGCCAGCCACAAGGUAC	381
54790_4_1049	TET2	EXON	+	chr4:105241705 -105241725	CAGCCACAAGGUACUGGCAC	382
54790_4_1052	TET2	EXON	+	chr4:105241718 -105241738	CUGGCACAGGCUCCAACGAG	383
54790_4_1053	TET2	EXON	+	chr4:105241729 -105241749	UCCAACGAGAGGUCCCACUC	384
54790_4_1058	TET2	EXON	+	chr4:105241770 -105241790	AAGUGUCAAGCAGAAAGAC	385
54790_4_1059	TET2	EXON	+	chr4:105241780 -105241800	GCAGAAAGACUGGUAAAGUG	386
54790_4_1092	TET2	EXON	+	chr4:105241946 -105241966	UUUUUUUCGCUAUCAAUCAC	387
54790_4_1109	TET2	EXON	+	chr4:105242012 -105242032	UGAGCGAGAUAAUGCAGAGA	388
54790_4_1117	TET2	EXON	+	chr4:105242057 -105242077	CUCUGAGCUGUUCUUCUUCU	389
54790_4_1118	TET2	EXON	+	chr4:105242058 -105242078	UCUGAGCUGUUCUUCUUCUA	390
54790_4_1123	TET2	EXON	+	chr4:105242076 -105242096	UAGGGUGCCUUUUCAUUAAG	391
54790_4_1124	TET2	EXON	+	chr4:105242080 -105242100	GUGCCUUUUCAUUAAGAGGU	392
54790_4_1130	TET2	EXON	+	chr4:105242105 -105242125	GUAUUAUUAUUAAGUACUU	393
54790_4_1135	TET2	EXON	+	chr4:105242114 -105242134	UUAAGUACUUAGGAUACAU	394
54790_4_1136	TET2	EXON	+	chr4:105242115 -105242135	UAAAGUACUUAGGAUACAUU	395
54790_4_1137	TET2	EXON	+	chr4:105242116 -105242136	AAAGUACUUAGGAUACAUUG	396
54790_4_1140	TET2	EXON	+	chr4:105242124 -105242144	UAGGAUACAUUGGGGCAGCU	397
54790_4_1154	TET2	EXON	+	chr4:105242210 -105242230	UUCACUAAAUAUCAUCUAG	398
54790_4_1156	TET2	EXON	+	chr4:105242215 -105242235	UAAAUAAUCAUCUAGUGGCC	399
54790_4_1162	TET2	EXON	+	chr4:105242287 -105242307	UUGUUUUUUAACAAGCAGU	400
54790_4_1163	TET2	EXON	+	chr4:105242290 -105242310	UUUUUUAAACAAGCAGUAGG	401

54790_4_1164	TET2	EXON	+	chr4:105242298 -105242318	ACAAGCAGUAGGUGGUGCUU	402
54790_4_1167	TET2	EXON	+	chr4:105242306 -105242326	UAGGUGGUGCUUUGGUCAUA	403
54790_4_1169	TET2	EXON	+	chr4:105242307 -105242327	AGGUGGUGCUUUGGUCAUAA	404
54790_4_1173	TET2	EXON	+	chr4:105242328 -105242348	GGAAGAUUAUAGUCUAUUUCU	405
54790_4_1176	TET2	EXON	+	chr4:105242351 -105242371	ACUAUUCCAUAUUUCCAUG	406
54790_4_1178	TET2	EXON	+	chr4:105242355 -105242375	UCCAUAUUUCCAUGUGGC	407
54790_4_1187	TET2	EXON	+	chr4:105242404 -105242424	UCUAAAUUGUGAGACAUUCU	408
54790_4_1193	TET2	EXON	+	chr4:105242407 -105242427	AAAUUGUGAGACAUUCUUGG	409
54790_4_1201	TET2	EXON	+	chr4:105242469 -105242489	UAAAAUAGCUAAAUUUAGUA	410
54790_4_1205	TET2	EXON	+	chr4:105242470 -105242490	AAAAUAGCUAAAUUUAGUAA	411
54790_4_1241	TET2	EXON	+	chr4:105242625 -105242645	AUCUGUACAUUUUGAUAUUG	412
54790_4_1244	TET2	EXON	+	chr4:105242635 -105242655	UUUGAUAUUGAGGAAAAACA	413
54790_4_1250	TET2	EXON	+	chr4:105242663 -105242683	AAACCAUUAUCCAGUUUGCU	414
54790_4_1258	TET2	EXON	+	chr4:105242705 -105242725	UAAUAAACCGUUCAUUUCUC	415
54790_4_1259	TET2	EXON	+	chr4:105242711 -105242731	ACCGUUCAUUUCUCAGGAUG	416
54790_4_1269	TET2	EXON	-	chr4:105233886 -105233906	UAGUUGAAUUCUAAAGAGCA	417
54790_4_1276	TET2	EXON	-	chr4:105233917 -105233937	UUGCUUCGGGGCCAUCCACA	418
54790_4_1278	TET2	EXON	-	chr4:105233929 -105233949	GUUCCAUCAGGCUUGCUUCG	419
54790_4_1279	TET2	EXON	-	chr4:105233930 -105233950	UGUCCAUCAGGCUUGCUUC	420
54790_4_1281	TET2	EXON	-	chr4:105233931 -105233951	CUGUCCAUCAGGCUUGCUU	421
54790_4_1285	TET2	EXON	-	chr4:105233941 -105233961	UGGUUCUAUCCUGUCCAUC	422
54790_4_1288	TET2	EXON	-	chr4:105233961 -105233981	UCUGUUGCCCUCAACAUGGU	423
54790_4_1289	TET2	EXON	-	chr4:105233965 -105233985	UUAGUCUGUUGCCCUCAACA	424
54790_4_1290	TET2	EXON	-	chr4:105233990 -105234010	GGAGGUGAUGGUAUCAGGAA	425

54790_4_1293	TET2	EXON	-	chr4:105233995 -105234015	AAAUGGGAGGUGAUGGUAUC	426
54790_4_1296	TET2	EXON	-	chr4:105234002 -105234022	GUCUGGCAAAUGGGAGGUGA	427
54790_4_1297	TET2	EXON	-	chr4:105234008 -105234028	GGUUCUGUCUGGCAAAUGGG	428
54790_4_1298	TET2	EXON	-	chr4:105234011 -105234031	AGAGGUUCUGUCUGGCAAAU	429
54790_4_1300	TET2	EXON	-	chr4:105234012 -105234032	CAGAGGUUCUGUCUGGCAAA	430
54790_4_1305	TET2	EXON	-	chr4:105234019 -105234039	UUGUAGCCAGAGGUUCUGUC	431
54790_4_1308	TET2	EXON	-	chr4:105234029 -105234049	UUCUGGAGCUUUGUAGCCAG	432
54790_4_1310	TET2	EXON	-	chr4:105234046 -105234066	CAGGCAGUGGGCUUCCAUUC	433
54790_4_1314	TET2	EXON	-	chr4:105234058 -105234078	GAUGAGCUCUCUCAGGCAGU	434
54790_4_1315	TET2	EXON	-	chr4:105234059 -105234079	GGAUGAGCUCUCUCAGGCAG	435
54790_4_1319	TET2	EXON	-	chr4:105234065 -105234085	ACUUCUGGAUGAGCUCUCUC	436
54790_4_1322	TET2	EXON	-	chr4:105234080 -105234100	UUGGUGUCUCCAUUUACUUC	437
54790_4_1327	TET2	EXON	-	chr4:105234099 -105234119	ACUUUUGAAAGAGUGCCACU	438
54790_4_1334	TET2	EXON	-	chr4:105234134 -105234154	UUCUGGCUUCCCUUCAUACA	439
54790_4_1335	TET2	EXON	-	chr4:105234135 -105234155	AUUCUGGCUUCCCUUCAUAC	440
54790_4_1337	TET2	EXON	-	chr4:105234151 -105234171	CAGGACUCACACGACUAUUC	441
54790_4_1341	TET2	EXON	-	chr4:105234170 -105234190	CUACUUUCUUGUGUAAAGUC	442
54790_4_1351	TET2	EXON	-	chr4:105234201 -105234221	UCCUCCAUUUUGCAAACACU	443
54790_4_1355	TET2	EXON	-	chr4:105234245 -105234265	UGAAGGAGCCCAGAGAGAGA	444
54790_4_1367	TET2	EXON	-	chr4:105234262 -105234282	GUUUCAAUUUCUUGAUCUGA	445
54790_4_1378	TET2	EXON	-	chr4:105234289 -105234309	GUCUUUCUCCAUUAGCCUUU	446
54790_4_1388	TET2	EXON	-	chr4:105234328 -105234348	UUUCACCUGGAUUUCUUUCU	447
54790_4_1392	TET2	EXON	-	chr4:105234341 -105234361	UUUGGUUGACUGCUUUCACC	448
54790_4_1396	TET2	EXON	-	chr4:105234359 -105234379	UCACUCAAUUCGGAGACAUU	449

54790_4_1399	TET2	EXON	-	chr4:105234369 -105234389	UUCUUUCUUAUCACUCAAU	450
54790_4_1410	TET2	EXON	-	chr4:105234408 -105234428	AUCUUUAAACUGCAUUUUCUU	451
54790_4_1411	TET2	EXON	-	chr4:105234409 -105234429	AAUCUUUAAACUGCAUUUUCU	452
54790_4_1416	TET2	EXON	-	chr4:105234435 -105234455	GCAGUUAUGUGUUGAAAAAC	453
54790_4_1422	TET2	EXON	-	chr4:105234464 -105234484	AUCUGAAGCUCUGGAUUUUC	454
54790_4_1423	TET2	EXON	-	chr4:105234473 -105234493	UCAUUCAGAAUCUGAAGCUC	455
54790_4_1435	TET2	EXON	-	chr4:105234520 -105234540	GUAAUACAAUGUUCUUGUCA	456
54790_4_1441	TET2	EXON	-	chr4:105234566 -105234586	GCAGAAACUGUAGCACCAUU	457
54790_4_1444	TET2	EXON	-	chr4:105234588 -105234608	AUGUGUGUGUUCCACGGAAG	458
54790_4_1448	TET2	EXON	-	chr4:105234594 -105234614	UUCACCAUGUGUGUGUCCA	459
54790_4_1457	TET2	EXON	-	chr4:105234619 -105234639	AUUGAGACAGUGUUUUUCC	460
54790_4_1461	TET2	EXON	-	chr4:105234647 -105234667	ACCGCAAUGGAAACACAAUC	461
54790_4_1466	TET2	EXON	-	chr4:105234660 -105234680	UGUGGUUUUCUGCACCGCAA	462
54790_4_1471	TET2	EXON	-	chr4:105234678 -105234698	AAUGGCAUUUAUGUGAGAUG	463
54790_4_1474	TET2	EXON	-	chr4:105234696 -105234716	AUUAGUAGCCUGACUGUAA	464
54790_4_1475	TET2	EXON	-	chr4:105234726 -105234746	CGAUGGGUGAGUGAUCUCAC	465
54790_4_1479	TET2	EXON	-	chr4:105234742 -105234762	UCUGCCCUGAGGUAUGCGAU	466
54790_4_1480	TET2	EXON	-	chr4:105234743 -105234763	AUCUGCCCUGAGGUAUGCGA	467
54790_4_1482	TET2	EXON	-	chr4:105234753 -105234773	UGCGGAAUUGAUCUGCCCUG	468
54790_4_1485	TET2	EXON	-	chr4:105234771 -105234791	CUCAGAGUUAGAGGUCUGUG	469
54790_4_1490	TET2	EXON	-	chr4:105234780 -105234800	UGGAGGCAGCUCAGAGUUAG	470
54790_4_1493	TET2	EXON	-	chr4:105234797 -105234817	ACCACUGCAGCUGGCUUUGG	471
54790_4_1495	TET2	EXON	-	chr4:105234800 -105234820	CUCACCACUGCAGCUGGCUU	472
54790_4_1497	TET2	EXON	-	chr4:105234806 -105234826	GCCUCACUCACCACUGCAGC	473

54790_4_1499	TET2	EXON	-	chr4:105234828 -105234848	AUCAGCAUCAUCAGCAUCAC	474
54790_4_1505	TET2	EXON	-	chr4:105234855 -105234875	UAGCAUUGCAGCUAGUUUAC	475
54790_4_1510	TET2	EXON	-	chr4:105234882 -105234902	UUCUGGUUUCUGAAAGGAAC	476
54790_4_1514	TET2	EXON	-	chr4:105234888 -105234908	UAGUUGUUCUGGUUUCUGAA	477
54790_4_1521	TET2	EXON	-	chr4:105234899 -105234919	UUUUGUUGUUGUAGUUGUUC	478
54790_4_1526	TET2	EXON	-	chr4:105234940 -105234960	UGUUAUUUUCUGCAGGAGAU	479
54790_4_1527	TET2	EXON	-	chr4:105234941 -105234961	AUGUUAUUUUCUGCAGGAGA	480
54790_4_1531	TET2	EXON	-	chr4:105234947 -105234967	CCCUGGAUGUUAUUUUCUGC	481
54790_4_1535	TET2	EXON	-	chr4:105234964 -105234984	ACGCUAGCUUUGUGGUUCCC	482
54790_4_1539	TET2	EXON	-	chr4:105234972 -105234992	UUCACCAGACGCUAGCUUUG	483
54790_4_1545	TET2	EXON	-	chr4:105235011 -105235031	AGGAGCUUGCAAAUUGCUGC	484
54790_4_1551	TET2	EXON	-	chr4:105235031 -105235051	UACCGUUCAGAGCUGCCACC	485
54790_4_1569	TET2	EXON	-	chr4:105235116 -105235136	ACCACACCAUCACCCAGAAA	486
54790_4_1577	TET2	EXON	-	chr4:105235166 -105235186	ACCUGUGGAAGAGGAGGAGG	487
54790_4_1579	TET2	EXON	-	chr4:105235167 -105235187	AACCUGUGGAAGAGGAGGAG	488
54790_4_1581	TET2	EXON	-	chr4:105235168 -105235188	GAACCUGUGGAAGAGGAGGA	489
54790_4_1582	TET2	EXON	-	chr4:105235169 -105235189	GGAACCUGUGGAAGAGGAGG	490
54790_4_1586	TET2	EXON	-	chr4:105235172 -105235192	UGAGGAACCUGUGGAAGAGG	491
54790_4_1588	TET2	EXON	-	chr4:105235175 -105235195	AGCUGAGGAACCUGUGGAAG	492
54790_4_1593	TET2	EXON	-	chr4:105235181 -105235201	GAAGGAAGCUGAGGAACCUG	493
54790_4_1600	TET2	EXON	-	chr4:105235190 -105235210	UUUCCUUCUGAAGGAAGCUG	494
54790_4_1606	TET2	EXON	-	chr4:105235199 -105235219	AGAGUGCUUUUCCUUCUGA	495
54790_4_1617	TET2	EXON	-	chr4:105235246 -105235266	UACUUUGGUUGGGGUAGUGG	496
54790_4_1618	TET2	EXON	-	chr4:105235249 -105235269	UGUUACUUUGGUUGGGGUAG	497

54790_4_1620	TET2	EXON	-	chr4:105235255 -105235275	GUGUUGUGUUACUUUGGUUG	498
54790_4_1621	TET2	EXON	-	chr4:105235256 -105235276	AGUGUUGUGUUACUUUGGUU	499
54790_4_1623	TET2	EXON	-	chr4:105235257 -105235277	AAGUGUUGUGUUACUUUGGU	500
54790_4_1626	TET2	EXON	-	chr4:105235261 -105235281	UUAAAAGUGUUGUGUUACUU	501
54790_4_1633	TET2	EXON	-	chr4:105235307 -105235327	CUCUGGGAAGGUGGUGCCUC	502
54790_4_1634	TET2	EXON	-	chr4:105235316 -105235336	GGAUUAGGACUCUGGGAAGG	503
54790_4_1635	TET2	EXON	-	chr4:105235319 -105235339	GAUGGAUUAGGACUCUGGGA	504
54790_4_1636	TET2	EXON	-	chr4:105235323 -105235343	UGUAGAUGGAUUAGGACUCU	505
54790_4_1638	TET2	EXON	-	chr4:105235324 -105235344	GUGUAGAUGGAUUAGGACUC	506
54790_4_1641	TET2	EXON	-	chr4:105235331 -105235351	CAUACAUGUGUAGAUGGAUU	507
54790_4_1643	TET2	EXON	-	chr4:105235337 -105235357	GGGUGCAUACAUGUGUAGA	508
54790_4_1647	TET2	EXON	-	chr4:105235357 -105235377	UUUCAGAAAGCAUCGGAGAA	509
54790_4_1648	TET2	EXON	-	chr4:105235358 -105235378	CUUUCAGAAAGCAUCGGAGA	510
54790_4_1653	TET2	EXON	-	chr4:105235364 -105235384	UGAGGCCUUUCAGAAAGCAU	511
54790_4_1660	TET2	EXON	-	chr4:105235382 -105235402	CUGUUCACACAAUUAUUCUG	512
54790_4_1668	TET2	EXON	-	chr4:105235439 -105235459	CUUGUUUUCUCAGAACACAA	513
54790_4_1676	TET2	EXON	-	chr4:105235463 -105235483	UGCUUGAGGUGUUCUGACAU	514
54790_4_1678	TET2	EXON	-	chr4:105235477 -105235497	AAAUUGGUGGGUUAUGCUUG	515
54790_4_1680	TET2	EXON	-	chr4:105235489 -105235509	CACUGCUACCAAAAAUUGGU	516
54790_4_1681	TET2	EXON	-	chr4:105235490 -105235510	CCACUGCUACCAAAAAUUGG	517
54790_4_1683	TET2	EXON	-	chr4:105235493 -105235513	UCUCCACUGCUACCAAAAAU	518
54790_4_1690	TET2	EXON	-	chr4:105235531 -105235551	CUUUGUUUCUCAUACUGC	519
54790_4_1699	TET2	EXON	-	chr4:105235604 -105235624	UUCAGAUAGUGCUGUGUUGG	520
54790_4_1700	TET2	EXON	-	chr4:105235605 -105235625	UUUCAGAUAGUGCUGUGUUG	521

54790_4_1702	TET2	EXON	-	chr4:105235606 -105235626	GUUUCAGAUAGUGCUGUGUU	522
54790_4_1703	TET2	EXON	-	chr4:105235607 -105235627	GGUUUCAGAUAGUGCUGUGU	523
54790_4_1708	TET2	EXON	-	chr4:105235628 -105235648	GCCUUCAAUCAAUCCAUC	524
54790_4_1711	TET2	EXON	-	chr4:105235650 -105235670	UUCCGCUUGGUGAAAACGAG	525
54790_4_1712	TET2	EXON	-	chr4:105235651 -105235671	AUCCGCUUGGUGAAAACGA	526
54790_4_1713	TET2	EXON	-	chr4:105235652 -105235672	GAUCCGCUUGGUGAAAACG	527
54790_4_1722	TET2	EXON	-	chr4:105235663 -105235683	GUUUUAGAUGGGAUCCGCU	528
54790_4_1723	TET2	EXON	-	chr4:105235674 -105235694	UGCCUCAUUACGUUUUAGAU	529
54790_4_1724	TET2	EXON	-	chr4:105235675 -105235695	AUGCCUCAUUACGUUUUAGA	530
54790_4_1730	TET2	EXON	-	chr4:105235703 -105235723	GGUUGAUACUGAAGAAUUGA	531
54790_4_1737	TET2	EXON	-	chr4:105235724 -105235744	GUCAUUUGAUUGGAGAGAUU	532
54790_4_1738	TET2	EXON	-	chr4:105235725 -105235745	GGUCAUUUGAUUGGAGAGAU	533
54790_4_1743	TET2	EXON	-	chr4:105235734 -105235754	UUGUUUGGAGGUCAUUUGAU	534
54790_4_1749	TET2	EXON	-	chr4:105235746 -105235766	AUUUCCAGUGUAUUGUUUGG	535
54790_4_1751	TET2	EXON	-	chr4:105235749 -105235769	GGAAUUUCCAGUGUAUUGUU	536
54790_4_1756	TET2	EXON	-	chr4:105235770 -105235790	UGGGAGCCCCCAGGCAUGU	537
54790_4_1758	TET2	EXON	-	chr4:105235778 -105235798	GCUUGCCUUGGGAGCCCCC	538
54790_4_1763	TET2	EXON	-	chr4:105235789 -105235809	UCUGGGUGUAAGCUUGCCUU	539
54790_4_1766	TET2	EXON	-	chr4:105235790 -105235810	UUCUGGGUGUAAGCUUGCCU	540
54790_4_1769	TET2	EXON	-	chr4:105235806 -105235826	CUCCAGCUGUGUUGUUUUCU	541
54790_4_1770	TET2	EXON	-	chr4:105235807 -105235827	GCUCCAGCUGUGUUGUUUUC	542
54790_4_1779	TET2	EXON	-	chr4:105235846 -105235866	GCCCUUGAUUCAUUUCAACU	543
54790_4_1782	TET2	EXON	-	chr4:105235872 -105235892	AUGUUGGUCCACUGUACCUU	544
54790_4_1783	TET2	EXON	-	chr4:105235873 -105235893	GAUGUUGGUCCACUGUACCU	545

54790_4_1790	TET2	EXON	-	chr4:105235888 -105235908	GUUUUUGGAACUGGAGAUGU	546
54790_4_1791	TET2	EXON	-	chr4:105235897 -105235917	GGUGUGAGGGUUUUUGGAAC	547
54790_4_1795	TET2	EXON	-	chr4:105235903 -105235923	GCACCUGGUGUGAGGGUUUU	548
54790_4_1800	TET2	EXON	-	chr4:105235910 -105235930	GAGAAGUGCACCUGGUGUGA	549
54790_4_1801	TET2	EXON	-	chr4:105235911 -105235931	GGAGAAGUGCACCUGGUGUG	550
54790_4_1804	TET2	EXON	-	chr4:105235918 -105235938	CUGUUUUGGAGAAGUGCACC	551
54790_4_1811	TET2	EXON	-	chr4:105235932 -105235952	UUUUGGUAAAUGGUCUGUUU	552
54790_4_1813	TET2	EXON	-	chr4:105235942 -105235962	GCACAUGAGCUUUUGGUAAA	553
54790_4_1814	TET2	EXON	-	chr4:105235949 -105235969	AGUGACUGCACAUGAGCUUU	554
54790_4_1828	TET2	EXON	-	chr4:105236010 -105236030	GGACAUAAAGUUUUUCAGUUU	555
54790_4_1829	TET2	EXON	-	chr4:105236011 -105236031	GGGACAUAAAGUUUUUCAGUU	556
54790_4_1836	TET2	EXON	-	chr4:105236031 -105236051	CAAGUGCUGUUUCAACACUG	557
54790_4_1838	TET2	EXON	-	chr4:105236032 -105236052	UCAAGUGCUGUUUCAACACU	558
54790_4_1839	TET2	EXON	-	chr4:105236033 -105236053	UUCAAGUGCUGUUUCAACAC	559
54790_4_1846	TET2	EXON	-	chr4:105236078 -105236098	AAAAGGUGUGAGUUUGAAAA	560
54790_4_1852	TET2	EXON	-	chr4:105236095 -105236115	UAUGAGGCUUAUGUUGCAAA	561
54790_4_1856	TET2	EXON	-	chr4:105236111 -105236131	GUUUGUGCUGCCUGUUUAUG	562
54790_4_1861	TET2	EXON	-	chr4:105236138 -105236158	GGGAGAUGUGAACUCUGGGA	563
54790_4_1862	TET2	EXON	-	chr4:105236142 -105236162	UUGAGGGAGAUGUGAACUCU	564
54790_4_1864	TET2	EXON	-	chr4:105236143 -105236163	UUUGAGGGAGAUGUGAACUC	565
54790_4_1873	TET2	EXON	-	chr4:105236158 -105236178	GCUGCUGUUGCUGGUUUUGA	566
54790_4_1875	TET2	EXON	-	chr4:105236159 -105236179	UGCUGCUGUUGCUGGUUUUG	567
54790_4_1880	TET2	EXON	-	chr4:105236167 -105236187	GUAAUUUUUGCUGCUGUUGC	568
54790_4_1892	TET2	EXON	-	chr4:105236215 -105236235	UUUGGGGGUGAGGAAAAGUC	569

54790_4_1896	TET2	EXON	-	chr4:105236225 -105236245	UCAUUGUUGCUUUGGGGGUG	570
54790_4_1901	TET2	EXON	-	chr4:105236230 -105236250	GCUGAUCAUUGUUGCUUUGG	571
54790_4_1902	TET2	EXON	-	chr4:105236231 -105236251	UGCUGAUCAUUGUUGCUUUG	572
54790_4_1904	TET2	EXON	-	chr4:105236232 -105236252	UUGCUGAUCAUUGUUGCUUU	573
54790_4_1906	TET2	EXON	-	chr4:105236233 -105236253	UUUGCUGAUCAUUGUUGCUU	574
54790_4_1914	TET2	EXON	-	chr4:105236275 -105236295	AACAUUCUCCACUUUAGUC	575
54790_4_1931	TET2	EXON	-	chr4:105236350 -105236370	GUACUUCCUCCAGUCCCAUU	576
54790_4_1941	TET2	EXON	-	chr4:105236394 -105236414	UUUCAUGGUCUGACUAUAAG	577
54790_4_1943	TET2	EXON	-	chr4:105236395 -105236415	AUUUCAUGGUCUGACUAUAA	578
54790_4_1944	TET2	EXON	-	chr4:105236396 -105236416	GAUUUCAUGGUCUGACUAUA	579
54790_4_1950	TET2	EXON	-	chr4:105236409 -105236429	UUUGCAUGCACUUGAUUUCA	580
54790_4_1960	TET2	EXON	-	chr4:105236461 -105236481	GUUCUUUAUUCUCUGAAACU	581
54790_4_1966	TET2	EXON	-	chr4:105236495 -105236515	UUGUUUCCUGCAAAAAGUUC	582
54790_4_1972	TET2	EXON	-	chr4:105236520 -105236540	UUGCAUGUGAUGCAAGUUUU	583
54790_4_1973	TET2	EXON	-	chr4:105236521 -105236541	AUUGCAUGUGAUGCAAGUUU	584
54790_4_1982	TET2	EXON	-	chr4:105236549 -105236569	UGCUUUGGGAUCACAUUAUU	585
54790_4_1984	TET2	EXON	-	chr4:105236563 -105236583	UGUGAAGAAGAUCUUGCUUU	586
54790_4_1985	TET2	EXON	-	chr4:105236564 -105236584	CUGUGAAGAAGAUCUUGCUU	587
54790_4_2009	TET2	EXON	-	chr4:105236653 -105236673	CUUGUUGACCAGACAUUAUCU	588
54790_4_2017	TET2	EXON	-	chr4:105236713 -105236733	GCACAGGAAAAACAUUUGCA	589
54790_4_2019	TET2	EXON	-	chr4:105236729 -105236749	CUUCCUCCCUGGUCAGGCAC	590
54790_4_2022	TET2	EXON	-	chr4:105236735 -105236755	GUGUGACUUCCUCCCUGGUC	591
54790_4_2023	TET2	EXON	-	chr4:105236740 -105236760	UCUGAGUGUGACUUCCUCCC	592
54790_4_2029	TET2	EXON	-	chr4:105236763 -105236783	UUGAGUGUCCUUCUGGGGAG	593

54790_4_2030	TET2	EXON	-	chr4:105236764 -105236784	UUUGAGUGUCCUUCUGGGGA	594
54790_4_2031	TET2	EXON	-	chr4:105236765 -105236785	UUUUGAGUGUCCUUCUGGGG	595
54790_4_2034	TET2	EXON	-	chr4:105236768 -105236788	UGCUIIUUGAGUGUCCUUCUG	596
54790_4_2037	TET2	EXON	-	chr4:105236769 -105236789	AUGCUUUUGAGUGUCCUUCU	597
54790_4_2039	TET2	EXON	-	chr4:105236770 -105236790	CAUGCUUUUGAGUGUCCUUC	598
54790_4_2053	TET2	EXON	-	chr4:105236846 -105236866	CUAUGGCAAGACUCAGUUUG	599
54790_4_2054	TET2	EXON	-	chr4:105236847 -105236867	ACUAUGGCAAGACUCAGUUU	600
54790_4_2055	TET2	EXON	-	chr4:105236848 -105236868	GACUAUGGCAAGACUCAGUU	601
54790_4_2060	TET2	EXON	-	chr4:105236863 -105236883	UUGGCCUGUGCAUCUGACUA	602
54790_4_2063	TET2	EXON	-	chr4:105236882 -105236902	CAUCCAGGUUCCACCUUAAU	603
54790_4_2064	TET2	EXON	-	chr4:105236897 -105236917	CAGGCAUGUGGCUUGCAUCC	604
54790_4_2065	TET2	EXON	-	chr4:105236909 -105236929	GCUGUGUGCAUACAGGCAUG	605
54790_4_2069	TET2	EXON	-	chr4:105236916 -105236936	UGGUGGUGCUGUGUGCAUAC	606
54790_4_2077	TET2	EXON	-	chr4:105236933 -105236953	UCCAUGUUUUGUUUUCUGG	607
54790_4_2079	TET2	EXON	-	chr4:105236936 -105236956	UUUUUCCAUGUUUUGUUUUC	608
54790_4_2085	TET2	EXON	-	chr4:105236978 -105236998	ACAUAUACACAGCUUGCAGG	609
54790_4_2089	TET2	EXON	-	chr4:105236981 -105237001	UGCACAUUAUCACAGCUUGC	610
54790_4_2092	TET2	EXON	-	chr4:105237024 -105237044	CUGCUUCAGAUGCUGCUCCA	611
54790_4_2096	TET2	EXON	-	chr4:105237054 -105237074	CUUAUGGUCAAUAACGACU	612
54790_4_2099	TET2	EXON	-	chr4:105237070 -105237090	AUUUGAGAGUAAGAGCCUUA	613
54790_4_2112	TET2	EXON	-	chr4:105237125 -105237145	UGUCUAGUCAAAACUGUGAC	614
54790_4_2114	TET2	EXON	-	chr4:105237150 -105237170	GCUAUCAGUUCUGCAGCAG	615
54790_4_2118	TET2	EXON	-	chr4:105237172 -105237192	GCUGCUCUAAAGCUGGGGUG	616
54790_4_2119	TET2	EXON	-	chr4:105237177 -105237197	UGUUUGCUGCUCUAAAGCUG	617

54790_4_2120	TET2	EXON	-	chr4:105237178 -105237198	UUGUUUGCUGCUCUAAAGCU	618
54790_4_2122	TET2	EXON	-	chr4:105237179 -105237199	GUUGUUUGCUGCUCUAAAGC	619
54790_4_2135	TET2	EXON	-	chr4:105237218 -105237238	GAAGCAGCUGUUCUUUUGGU	620
54790_4_2137	TET2	EXON	-	chr4:105237222 -105237242	AACAGAAGCAGCUGUUCUUU	621
54790_4_2148	TET2	EXON	-	chr4:105237266 -105237286	GGAGUAUCUAGUAAUUUGGA	622
54790_4_2153	TET2	EXON	-	chr4:105237270 -105237290	UAUAGGAGUAUCUAGUAAUU	623
54790_4_2156	TET2	EXON	-	chr4:105237287 -105237307	GUAUCCAAUAAAUUUUUUUAU	624
54790_4_2160	TET2	EXON	-	chr4:105237311 -105237331	AAAUCAUAUUGAGUCUUGAC	625
54790_4_2163	TET2	EXON	-	chr4:105237334 -105237354	UACCUACACAUCUGCAAGAU	626
54790_4_2165	TET2	EXON	-	chr4:105237335 -105237355	UUACCUACACAUCUGCAAGA	627
54790_4_2170	TET2	EXON	-	chr4:105237361 -105237381	CAUGUGUCUCAGUACAUUUC	628
54790_4_2174	TET2	EXON	-	chr4:105237392 -105237412	GAAGAUAAAUUUGC UAAUUC	629
54790_4_2180	TET2	EXON	-	chr4:105237429 -105237449	ACUCAAGAUUUAAAAAAAGA	630
54790_4_2197	TET2	EXON	-	chr4:105237510 -105237530	CUUUCACAAGACACAAGCAU	631
54790_4_2206	TET2	EXON	-	chr4:105237558 -105237578	GCACGAUUAAUUAAUUCUUU	632
54790_4_2213	TET2	EXON	-	chr4:105237593 -105237613	UUUUACAGGAUCUGAAGAGA	633
54790_4_2215	TET2	EXON	-	chr4:105237594 -105237614	AUUUUACAGGAUCUGAAGAG	634
54790_4_2221	TET2	EXON	-	chr4:105237607 -105237627	CAGAUACAUUCAAAUUUUAC	635
54790_4_2225	TET2	EXON	-	chr4:105237645 -105237665	UAAUAUACAAAGAGCUAAAU	636
54790_4_2233	TET2	EXON	-	chr4:105237671 -105237691	UGCUGCCUAGCUGUCUCUCC	637
54790_4_2247	TET2	EXON	-	chr4:105237727 -105237747	UUCGUACAUUAGACUGCCUA	638
54790_4_2270	TET2	EXON	-	chr4:105237874 -105237894	AAUGGAGAAAAGGAAACUUU	639
54790_4_2274	TET2	EXON	-	chr4:105237884 -105237904	CAA AUGUAUAAAUGGAGAAA	640
54790_4_2277	TET2	EXON	-	chr4:105237892 -105237912	CAACAUUCCAAAUGUAUAAA	641

54790_4_2284	TET2	EXON	-	chr4:105237936 -105237956	AGAUGAAAUUUUAGAGAAAA	642
54790_4_2287	TET2	EXON	-	chr4:105237937 -105237957	AAGAUGAAAUUUUAGAGAAA	643
54790_4_2323	TET2	EXON	-	chr4:105240511 -105240531	AGGGAAAACAUGGCACGGGU	644
54790_4_2325	TET2	EXON	-	chr4:105240515 -105240535	CAAGAGGGAAAACAUGGCAC	645
54790_4_2326	TET2	EXON	-	chr4:105240516 -105240536	GCAAGAGGGAAAACAUGGCA	646
54790_4_2328	TET2	EXON	-	chr4:105240521 -105240541	UCAUUGCAAGAGGGAAAACA	647
54790_4_2330	TET2	EXON	-	chr4:105240530 -105240550	UGGGGUAUCUCAUUGCAAGA	648
54790_4_2331	TET2	EXON	-	chr4:105240531 -105240551	GUGGGGUAUCUCAUUGCAAG	649
54790_4_2336	TET2	EXON	-	chr4:105240548 -105240568	CCAUCCUUCUACACAGUGUG	650
54790_4_2337	TET2	EXON	-	chr4:105240549 -105240569	UCCAUCCUUCUACACAGUGU	651
54790_4_2338	TET2	EXON	-	chr4:105240550 -105240570	CUCCAUCCUUCUACACAGUG	652
54790_4_2342	TET2	EXON	-	chr4:105240581 -105240601	CACACGCAAAGAGGGACAGU	653
54790_4_2345	TET2	EXON	-	chr4:105240589 -105240609	UUAAUAACCACACGCAAAGA	654
54790_4_2347	TET2	EXON	-	chr4:105240590 -105240610	CUUAAUAACCACACGCAAAG	655
54790_4_2353	TET2	EXON	-	chr4:105240616 -105240636	UGUGGUGUUUUAGCCCAGUG	656
54790_4_2357	TET2	EXON	-	chr4:105240634 -105240654	AAUAUUAUCUAUGAGAUGUG	657
54790_4_2365	TET2	EXON	-	chr4:105240693 -105240713	AAAAGUGGGAAGAUAGGGGU	658
54790_4_2366	TET2	EXON	-	chr4:105240694 -105240714	GAAAAGUGGGAAGAUAGGGG	659
54790_4_2368	TET2	EXON	-	chr4:105240697 -105240717	AUGGAAAAGUGGGAAGAUAG	660
54790_4_2369	TET2	EXON	-	chr4:105240698 -105240718	GAUGGAAAAGUGGGAAGAU	661
54790_4_2370	TET2	EXON	-	chr4:105240699 -105240719	AGAUGGAAAAGUGGGAAGAU	662
54790_4_2373	TET2	EXON	-	chr4:105240707 -105240727	ACCAACAAAGAUGGAAAAGU	663
54790_4_2377	TET2	EXON	-	chr4:105240708 -105240728	AACCAACAAAGAUGGAAAAG	664
54790_4_2380	TET2	EXON	-	chr4:105240716 -105240736	CUGUUGCAAACCAACAAAGA	665

54790_4_2382	TET2	EXON	-	chr4:105240739 -105240759	GAGAGUCAGGCAAAAAGAAG	666
54790_4_2383	TET2	EXON	-	chr4:105240740 -105240760	GGAGAGUCAGGCAAAAAGAA	667
54790_4_2384	TET2	EXON	-	chr4:105240741 -105240761	UGGAGAGUCAGGCAAAAAGA	668
54790_4_2389	TET2	EXON	-	chr4:105240752 -105240772	AGAGAAAAUCCUGGAGAGUC	669
54790_4_2393	TET2	EXON	-	chr4:105240761 -105240781	UUUAUGAUGAGAGAAAAUCC	670
54790_4_2422	TET2	EXON	-	chr4:105240882 -105240902	UAUUGCUUGGUUUUAUUGUCA	671
54790_4_2424	TET2	EXON	-	chr4:105240895 -105240915	CCCACCCCCAGAAUAUUGCU	672
54790_4_2434	TET2	EXON	-	chr4:105240954 -105240974	CUGGAAGCCUACCUAUUACU	673
54790_4_2439	TET2	EXON	-	chr4:105240973 -105240993	AAAAACAUUUAAAGCUAAC	674
54790_4_2446	TET2	EXON	-	chr4:105241000 -105241020	UACAAUCCA AUUUUUUGAGC	675
54790_4_2454	TET2	EXON	-	chr4:105241052 -105241072	CUAAACAAAGAAUACAGUGA	676
54790_4_2456	TET2	EXON	-	chr4:105241053 -105241073	ACUAAACAAAGAAUACAGUG	677
54790_4_2468	TET2	EXON	-	chr4:105241107 -105241127	AUAUAUUACA UUCAGAUAU	678
54790_4_2469	TET2	EXON	-	chr4:105241108 -105241128	AAUAUAUUACA UUCAGAUUA	679
54790_4_2475	TET2	EXON	-	chr4:105241136 -105241156	UAUAUGUACAUGCUGGUUGU	680
54790_4_2477	TET2	EXON	-	chr4:105241143 -105241163	AAUUAAGUAUAUGUACAUGC	681
54790_4_2488	TET2	EXON	-	chr4:105241193 -105241213	CUUUAAAAUGAGUAGAUUGA	682
54790_4_2498	TET2	EXON	-	chr4:105241245 -105241265	AACCCCCAACUACACAUUAA	683
54790_4_2499	TET2	EXON	-	chr4:105241246 -105241266	UAACCCCCAACUACACAUUA	684
54790_4_2503	TET2	EXON	-	chr4:105241285 -105241305	CUCAAUUAUACUAAAUAUAA	685
54790_4_2519	TET2	EXON	-	chr4:105241367 -105241387	GCUCCUAGAUGGGUAUAAAA	686
54790_4_2522	TET2	EXON	-	chr4:105241377 -105241397	AUUAGGACCUGCUCCUAGAU	687
54790_4_2523	TET2	EXON	-	chr4:105241378 -105241398	CAUUAGGACCUGCUCCUAGA	688
54790_4_2527	TET2	EXON	-	chr4:105241394 -105241414	UCUCUAAUAGCUGCCACA UU	689

54790_4_2538	TET2	EXON	-	chr4:105241470 -105241490	AAAAUUCUGACAUAUACAAA	690
54790_4_2546	TET2	EXON	-	chr4:105241494 -105241514	ACUGCUUUGUGUGUGAAGGC	691
54790_4_2548	TET2	EXON	-	chr4:105241498 -105241518	GUUUACUGCUUUGUGUGUGA	692
54790_4_2555	TET2	EXON	-	chr4:105241568 -105241588	AAUAGCACAGUGUGUAGUGU	693
54790_4_2558	TET2	EXON	-	chr4:105241593 -105241613	UGUCAAUAUUGUGACUCUC	694
54790_4_2563	TET2	EXON	-	chr4:105241647 -105241667	UCUGGCAUCCAUCGCAAAGU	695
54790_4_2564	TET2	EXON	-	chr4:105241648 -105241668	UUCUGGCAUCCAUCGCAAAG	696
54790_4_2568	TET2	EXON	-	chr4:105241665 -105241685	CUGUUCAUGCCUGGGUUUUC	697
54790_4_2569	TET2	EXON	-	chr4:105241673 -105241693	GCCGAUUCCUGUUCAUGCCU	698
54790_4_2570	TET2	EXON	-	chr4:105241674 -105241694	GGCCGAUUCCUGUUCAUGCC	699
54790_4_2573	TET2	EXON	-	chr4:105241695 -105241715	CUUGUGGCUGGCAGCCUGGC	700
54790_4_2574	TET2	EXON	-	chr4:105241699 -105241719	GUACCUUGUGGCUGGCAGCC	701
54790_4_2575	TET2	EXON	-	chr4:105241707 -105241727	CUGUGCCAGUACCUUGUGGC	702
54790_4_2577	TET2	EXON	-	chr4:105241711 -105241731	GAGCCUGUGCCAGUACCUUG	703
54790_4_2578	TET2	EXON	-	chr4:105241733 -105241753	GCCAGAGUGGGACCUCUCGU	704
54790_4_2582	TET2	EXON	-	chr4:105241745 -105241765	UCAGGUGGGAAAGCCAGAGU	705
54790_4_2585	TET2	EXON	-	chr4:105241746 -105241766	AUCAGGUGGGAAAGCCAGAG	706
54790_4_2591	TET2	EXON	-	chr4:105241759 -105241779	UUGACACUUUAUUAUCAGGU	707
54790_4_2595	TET2	EXON	-	chr4:105241760 -105241780	UUUGACACUUUAUUAUCAGG	708
54790_4_2598	TET2	EXON	-	chr4:105241763 -105241783	UGCUUUGACACUUUAUUAUC	709
54790_4_2609	TET2	EXON	-	chr4:105241819 -105241839	ACUAGGUGAAUUUAAUUCAG	710
54790_4_2613	TET2	EXON	-	chr4:105241836 -105241856	AAGUACUCAUUUGCAACACU	711
54790_4_2622	TET2	EXON	-	chr4:105241878 -105241898	UCACACUUGCUCUCUUUUUA	712
54790_4_2629	TET2	EXON	-	chr4:105241939 -105241959	AUAGCGAAAAAAAAAAAAAAAA	713

54790_4_2633	TET2	EXON	-	chr4:105241986 -105242006	UCUUCUACAUGCAGGAGUAA	714
54790_4_2635	TET2	EXON	-	chr4:105241994 -105242014	CAUAAGAGUCUUCUACAUGC	715
54790_4_2642	TET2	EXON	-	chr4:105242038 -105242058	GCUGUAUAAAUUUAUAUGAA	716
54790_4_2652	TET2	EXON	-	chr4:105242086 -105242106	CUGCCUACCUCUUAUGAAA	717
54790_4_2663	TET2	EXON	-	chr4:105242173 -105242193	AGAAAUGAAUAAUUUGGAAA	718
54790_4_2665	TET2	EXON	-	chr4:105242179 -105242199	UAAUUUAGAAAUGAAUAAUU	719
54790_4_2679	TET2	EXON	-	chr4:105242236 -105242256	GGAAAUUCACUAUUUCUGCC	720
54790_4_2681	TET2	EXON	-	chr4:105242257 -105242277	GUUGUUUUUUUUGGCACUUA	721
54790_4_2683	TET2	EXON	-	chr4:105242258 -105242278	UGUUGUUUUUUUUGGCACUU	722
54790_4_2685	TET2	EXON	-	chr4:105242266 -105242286	UGUUUUUUUGUUGUUUUUUU	723
54790_4_2694	TET2	EXON	-	chr4:105242360 -105242380	AUCCAGCCACAUGGAAAAUA	724
54790_4_2697	TET2	EXON	-	chr4:105242369 -105242389	AUAGUUAGUAUCCAGCCACA	725
54790_4_2701	TET2	EXON	-	chr4:105242395 -105242415	CACAAUUUAGAAAAGGAGGC	726
54790_4_2702	TET2	EXON	-	chr4:105242399 -105242419	GUCUCACAAUUUAGAAAAGG	727
54790_4_2703	TET2	EXON	-	chr4:105242402 -105242422	AAUGUCUCACAAUUUAGAAA	728
54790_4_2721	TET2	EXON	-	chr4:105242462 -105242482	UUUAGCUAUUUUAAAACUUG	729
54790_4_2723	TET2	EXON	-	chr4:105242463 -105242483	AUUUAGCUAUUUUAAAACUU	730
54790_4_2726	TET2	EXON	-	chr4:105242464 -105242484	AAUUUAGCUAUUUUAAAACU	731
54790_4_2742	TET2	EXON	-	chr4:105242539 -105242559	UUUCACAAAGCACAAAUUC	732
54790_4_2749	TET2	EXON	-	chr4:105242583 -105242603	AAUUACAUGUGGGUGAAAAU	733
54790_4_2752	TET2	EXON	-	chr4:105242584 -105242604	AAAUUACAUGUGGGUGAAAA	734
54790_4_2755	TET2	EXON	-	chr4:105242593 -105242613	CUAUUUUGUAAAUUACAUGU	735
54790_4_2756	TET2	EXON	-	chr4:105242594 -105242614	ACUAUUUUGUAAAUUACAUG	736
54790_4_2769	TET2	EXON	-	chr4:105242669 -105242689	ACGCCAAGCAAACUGGAUAA	737

54790_4_2772	TET2	EXON	-	chr4:105242676 -105242696	CAGGUCUACGCCAAGCAAAC	738
54790_4_2780	TET2	EXON	-	chr4:105242695 -105242715	CGGUUUAUUAUUUUUUAAC	739
54790_4_2781	TET2	EXON	-	chr4:105242715 -105242735	ACCACAUCCUGAGAAAUGAA	740
54790_5_3	TET2	EXON	+	chr4:105242816 -105242836	CUGUGGGUUCUUUAAGGUU	741
54790_5_7	TET2	EXON	+	chr4:105242824 -105242844	UUCUUUAAGGUUUGGACAGA	742
54790_5_8	TET2	EXON	+	chr4:105242825 -105242845	UCUUUAAGGUUUGGACAGAA	743
54790_5_15	TET2	EXON	+	chr4:105242838 -105242858	GACAGAAGGGUAAAGCUAUU	744
54790_5_20	TET2	EXON	+	chr4:105242861 -105242881	AUUGAAAGAGUCAUCUAUAC	745
54790_5_23	TET2	EXON	+	chr4:105242870 -105242890	GUCAUCUAUACUGGUAAAGA	746
54790_5_26	TET2	EXON	+	chr4:105242884 -105242904	UAAAGAAGGCAAAAGUUCUC	747
54790_5_27	TET2	EXON	+	chr4:105242885 -105242905	AAAGAAGGCAAAAGUUCUCA	748
54790_5_30	TET2	EXON	+	chr4:105242904 -105242924	AGGGAUGUCCUAUUGCUAAG	749
54790_5_31	TET2	EXON	+	chr4:105242905 -105242925	GGGAUGUCCUAUUGCUAAGU	750
54790_5_51	TET2	EXON	-	chr4:105242915 -105242935	ACACUUACCCACUUAGCAAU	751
54790_6_1	TET2	EXON	+	chr4:105243550 -105243570	GGAAUGGUGAUCCACGCAGG	752
54790_6_7	TET2	EXON	+	chr4:105243589 -105243609	UGAAGAGAAGCUACUGUGUU	753
54790_6_9	TET2	EXON	+	chr4:105243594 -105243614	AGAAGCUACUGUGUUUGGUG	754
54790_6_12	TET2	EXON	+	chr4:105243595 -105243615	GAAGCUACUGUGUUUGGUGC	755
54790_6_14	TET2	EXON	+	chr4:105243605 -105243625	UGUUUGGUGCGGGAGCGAGC	756
54790_6_18	TET2	EXON	+	chr4:105243619 -105243639	GCGAGCUGGCCACACCUGUG	757
54790_6_19	TET2	EXON	+	chr4:105243646 -105243666	AGUGAUUGUGAUUCUCAUCC	758
54790_6_21	TET2	EXON	+	chr4:105243651 -105243671	UUGUGAUUCUCAUCCUGGUG	759
54790_6_24	TET2	EXON	+	chr4:105243652 -105243672	UGUGAUUCUCAUCCUGGUGU	760
54790_6_27	TET2	EXON	+	chr4:105243656 -105243676	AUUCUCAUCCUGGUGUGGGA	761

54790_6_30	TET2	EXON	+	chr4:105243673 -105243693	GGAAGGAAUCCCGCUGUCUC	762
54790_6_32	TET2	EXON	+	chr4:105243691 -105243711	UCUGGCUGACAAACUCUACU	763
54790_6_37	TET2	EXON	+	chr4:105243711 -105243731	CGGAGCUUACCGAGACGCUG	764
54790_6_39	TET2	EXON	+	chr4:105243719 -105243739	ACCGAGACGCUGAGGAAUA	765
54790_6_41	TET2	EXON	+	chr4:105243738 -105243758	ACGGCACGCUCACCAAUCGC	766
54790_6_48	TET2	EXON	+	chr4:105243771 -105243791	AUGAAGAGUAAGUGAAGCCC	767
54790_6_49	TET2	EXON	+	chr4:105243772 -105243792	UGAAGAGUAAGUGAAGCCCA	768
54790_6_51	TET2	EXON	-	chr4:105243564 -105243584	GCUUCUGCGAACCACCUGCG	769
54790_6_56	TET2	EXON	-	chr4:105243631 -105243651	UCACUGCAGCCUCACAGGUG	770
54790_6_57	TET2	EXON	-	chr4:105243636 -105243656	CACAAUCACUGCAGCCUCAC	771
54790_6_62	TET2	EXON	-	chr4:105243667 -105243687	GCGGGAUUCCUUCCCACACC	772
54790_6_66	TET2	EXON	-	chr4:105243685 -105243705	GUUUGUCAGCCAGAGACAGC	773
54790_6_67	TET2	EXON	-	chr4:105243686 -105243706	AGUUUGUCAGCCAGAGACAG	774
54790_6_75	TET2	EXON	-	chr4:105243723 -105243743	GCCGUAUUUCCUCAGCGUCU	775
54790_6_80	TET2	EXON	-	chr4:105243753 -105243773	AUUCAAGGCACACCGGCGAU	776
54790_6_82	TET2	EXON	-	chr4:105243760 -105243780	ACUCUUCAUUCAAGGCACAC	777
54790_6_84	TET2	EXON	-	chr4:105243768 -105243788	CUUCACUUAUCUCUUCAUUA	778
54790_7_10	TET2	EXON	+	chr4:105259615 -105259635	CAGGAGAACUUGCGCCUGUC	779
54790_7_12	TET2	EXON	+	chr4:105259616 -105259636	AGGAGAACUUGCGCCUGUCA	780
54790_7_14	TET2	EXON	+	chr4:105259617 -105259637	GGAGAACUUGCGCCUGUCAG	781
54790_7_16	TET2	EXON	+	chr4:105259621 -105259641	AACUUGCGCCUGUCAGGGGC	782
54790_7_20	TET2	EXON	+	chr4:105259637 -105259657	GGGCUGGAUCCAGAAACCUG	783
54790_7_21	TET2	EXON	+	chr4:105259655 -105259675	UGUGGUGCCUCCUUCUCUUU	784
54790_7_23	TET2	EXON	+	chr4:105259665 -105259685	CCUUCUCUUUUGGUUGUUA	785

54790_7_31	TET2	EXON	+	chr4:105259682 -105259702	UCAUGGAGCAUGUACUACAA	786
54790_7_35	TET2	EXON	+	chr4:105259713 -105259733	UUGCCAGAAGCAAGAUGCCCA	787
54790_7_41	TET2	EXON	+	chr4:105259730 -105259750	CCAAGGAAGUUUAAGCUGCU	788
54790_7_42	TET2	EXON	+	chr4:105259731 -105259751	CAAGGAAGUUUAAGCUGCUU	789
54790_7_44	TET2	EXON	+	chr4:105259732 -105259752	AAGGAAGUUUAAGCUGCUUG	790
54790_7_48	TET2	EXON	+	chr4:105259747 -105259767	GCUUGGGGAUGACCCAAAAG	791
54790_7_53	TET2	EXON	-	chr4:105259632 -105259652	UUCUGGAUCCAGCCCCUGAC	792
54790_7_54	TET2	EXON	-	chr4:105259649 -105259669	AAGGAGGCACCACAGGUUUC	793
54790_7_56	TET2	EXON	-	chr4:105259656 -105259676	AAAAGAGAAGGAGGCACCAC	794
54790_7_57	TET2	EXON	-	chr4:105259665 -105259685	UGAACAACCAAAAGAGAAGG	795
54790_7_58	TET2	EXON	-	chr4:105259668 -105259688	CCAUGAACAACCAAAAGAGA	796
54790_7_72	TET2	EXON	-	chr4:105259719 -105259739	CUUCCUUGGGAUCUUGCUUC	797
54790_7_73	TET2	EXON	-	chr4:105259732 -105259752	CAAGCAGCUUAAACUUCCUU	798
54790_7_74	TET2	EXON	-	chr4:105259733 -105259753	CCAAGCAGCUUAAACUUCCU	799
54790_7_80	TET2	EXON	-	chr4:105259762 -105259782	GAAGUAAACAAACCUCUUUU	800
54790_7_81	TET2	EXON	-	chr4:105259763 -105259783	GGAAGUAAACAAACCUCUUU	801
54790_8_8	TET2	EXON	+	chr4:105261748 -105261768	CUUUAUACAGGAAGAGAAAC	802
54790_8_12	TET2	EXON	+	chr4:105261781 -105261801	GCAAAACCUGUCCACUCUUA	803
54790_8_18	TET2	EXON	+	chr4:105261826 -105261846	ACCUGAUGCAUUAUAAUAUC	804
54790_8_27	TET2	EXON	-	chr4:105261790 -105261810	UUGGUGCCAUAAGAGUGGAC	805
54790_8_30	TET2	EXON	-	chr4:105261795 -105261815	AUAUGUUGGUGCCAUAAGAG	806
54790_8_34	TET2	EXON	-	chr4:105261809 -105261829	GGUGCAAGUUUCUUAUAUGU	807
54790_8_38	TET2	EXON	-	chr4:105261830 -105261850	ACCUGAUUAUUAUAUGCAUC	808
54790_9_14	TET2	EXON	+	chr4:105269623 -105269643	CAGAGCACCAGAGUGCCGUC	809

54790_9_15	TET2	EXON	+	chr4:105269624 -105269644	AGAGCACCAGAGUGCCGUCU	810
54790_9_19	TET2	EXON	+	chr4:105269632 -105269652	AGAGUGCCGUCUGGGUCUGA	811
54790_9_20	TET2	EXON	+	chr4:105269636 -105269656	UGCCGUCUGGGUCUGAAGGA	812
54790_9_22	TET2	EXON	+	chr4:105269651 -105269671	AAGGAAGGCCGUGCAUUCUC	813
54790_9_24	TET2	EXON	+	chr4:105269652 -105269672	AGGAAGGCCGUGCAUUCUCA	814
54790_9_25	TET2	EXON	+	chr4:105269653 -105269673	GGAAGGCCGUGCAUUCUCAG	815
54790_9_27	TET2	EXON	+	chr4:105269668 -105269688	CUCAGGGGUCACUGCAUGUU	816
54790_9_35	TET2	EXON	+	chr4:105269714 -105269734	GACUUGCACAACAUGCAGAA	817
54790_9_37	TET2	EXON	+	chr4:105269725 -105269745	CAUGCAGAAUGGCAGCACAU	818
54790_9_39	TET2	EXON	+	chr4:105269733 -105269753	AUGGCAGCACAUUGGUAAGU	819
54790_9_40	TET2	EXON	+	chr4:105269734 -105269754	UGGCAGCACAUUGGUAAGUU	820
54790_9_43	TET2	EXON	+	chr4:105269740 -105269760	CACAUUGGUAAGUUGGGCUG	821
54790_9_49	TET2	EXON	-	chr4:105269633 -105269653	UUCAGACCCAGACGGCACUC	822
54790_9_50	TET2	EXON	-	chr4:105269641 -105269661	GGCCUUCCUUCAGACCCAGA	823
54790_9_51	TET2	EXON	-	chr4:105269662 -105269682	CAGUGACCCCUGAGAAUGGA	824
54790_9_52	TET2	EXON	-	chr4:105269666 -105269686	CAUGCAGUGACCCCUGAGAA	825
54790_9_61	TET2	EXON	-	chr4:105269709 -105269729	CAUGUUGUGCAAGUCUCUGU	826
54790_9_62	TET2	EXON	-	chr4:105269710 -105269730	GCAUGUUGUGCAAGUCUCUG	827
54790_10_10	TET2	EXON	+	chr4:105272578 -105272598	AGAGAAGACAAUCGAGAAUU	828
54790_10_13	TET2	EXON	+	chr4:105272581 -105272601	GAAGACAAUCGAGAAUUUGG	829
54790_10_16	TET2	EXON	+	chr4:105272592 -105272612	AGAAUUUGGAGGAAAACCUG	830
54790_10_23	TET2	EXON	+	chr4:105272637 -105272657	UUUAUACAAAGUCUCUGACG	831
54790_10_29	TET2	EXON	+	chr4:105272647 -105272667	GUCUCUGACGUGGAUGAGUU	832
54790_10_30	TET2	EXON	+	chr4:105272648 -105272668	UCUCUGACGUGGAUGAGUUU	833

54790_10_33	TET2	EXON	+	chr4:105272655 -105272675	CGUGGAUGAGUUUGGGAGUG	834
54790_10_36	TET2	EXON	+	chr4:105272664 -105272684	GUUUGGGAGUGUGGAAGCUC	835
54790_10_40	TET2	EXON	+	chr4:105272667 -105272687	UGGGAGUGUGGAAGCUCAGG	836
54790_10_46	TET2	EXON	+	chr4:105272678 -105272698	AAGCUCAGGAGGAGAAAAAA	837
54790_10_48	TET2	EXON	+	chr4:105272683 -105272703	CAGGAGGAGAAAAAACGGAG	838
54790_10_49	TET2	EXON	+	chr4:105272694 -105272714	AAAACGGAGUGGUGCCAUUC	839
54790_10_51	TET2	EXON	+	chr4:105272711 -105272731	UUCAGGUACUGAGUUCUUUU	840
54790_10_55	TET2	EXON	+	chr4:105272723 -105272743	GUUCUUUUCGGCGAAAAGUC	841
54790_10_64	TET2	EXON	+	chr4:105272759 -105272779	CAGUCAAGACUUGCCGACAA	842
54790_10_71	TET2	EXON	+	chr4:105272805 -105272825	AGCUGAAAAGCUUCCUCCC	843
54790_10_78	TET2	EXON	+	chr4:105272832 -105272852	CAGCUCAAAUAAAAAUGAAA	844
54790_10_81	TET2	EXON	+	chr4:105272880 -105272900	ACAAACUGAAAACGCAAGCC	845
54790_10_82	TET2	EXON	+	chr4:105272892 -105272912	CGCAAGCCAGGCUAAACAGU	846
54790_10_83	TET2	EXON	+	chr4:105272896 -105272916	AGCCAGGCUAAACAGUUGGC	847
54790_10_85	TET2	EXON	-	chr4:105272557 -105272577	GUGAGAGUGCAUACCUGGUA	848
54790_10_87	TET2	EXON	-	chr4:105272558 -105272578	AGUGAGAGUGCAUACCUGGU	849
54790_10_91	TET2	EXON	-	chr4:105272562 -105272582	CUCUAGUGAGAGUGCAUACC	850
54790_10_99	TET2	EXON	-	chr4:105272611 -105272631	ACGUGAAGCUGCUCAUCCUC	851
54790_10_105	TET2	EXON	-	chr4:105272638 -105272658	ACGUCAGAGACUUUGUAUAA	852
54790_10_114	TET2	EXON	-	chr4:105272711 -105272731	AAAAGAACUCAGUACCUGAA	853
54790_10_127	TET2	EXON	-	chr4:105272761 -105272781	CUUUGUCGGCAAGUCUUGAC	854
54790_10_132	TET2	EXON	-	chr4:105272775 -105272795	UGGCUUCUAGUUUCCUUUGU	855
54790_10_136	TET2	EXON	-	chr4:105272795 -105272815	CUUUUCAGCUGCAGCUUUCU	856
54790_10_145	TET2	EXON	-	chr4:105272822 -105272842	AUUUGAGCUGUUCUCCAGGG	857

54790_10_147	TET2	EXON	-	chr4:105272825 -105272845	UUUAUUUGAGCUGUUCUCCA	858
54790_10_150	TET2	EXON	-	chr4:105272826 -105272846	UUUUAUUUGAGCUGUUCUCC	859
54790_10_167	TET2	EXON	-	chr4:105272867 -105272887	AGUUUGUUUUGUACGUGAUG	860
54790_10_168	TET2	EXON	-	chr4:105272868 -105272888	CAGUUUGUUUUGUACGUGAU	861
54790_10_169	TET2	EXON	-	chr4:105272869 -105272889	UCAGUUUGUUUUGUACGUGA	862
54790_10_177	TET2	EXON	-	chr4:105272901 -105272921	UACCUGCCAACUGUUUAGCC	863
54790_11_9	TET2	EXON	+	chr4:105275178 -105275198	GUCAACUCUUAUUCUGCUUC	864
54790_11_14	TET2	EXON	+	chr4:105275203 -105275223	CCACCAAUCCAUAACAUGAGA	865
54790_11_19	TET2	EXON	+	chr4:105275256 -105275276	UCACACACUUCAGAUUAUCUA	866
54790_11_24	TET2	EXON	+	chr4:105275304 -105275324	UCCACCUCAUCUCAAGCUGC	867
54790_11_34	TET2	EXON	+	chr4:105275346 -105275366	AAUCCCAUGAACCCUUAACCC	868
54790_11_35	TET2	EXON	+	chr4:105275347 -105275367	AUCCCAUGAACCCUUAACCCU	869
54790_11_44	TET2	EXON	+	chr4:105275391 -105275411	UAUCCAUCAUAUCAUAUGCAA	870
54790_11_47	TET2	EXON	+	chr4:105275405 -105275425	AUGCAAUGGAAACCUAUCAG	871
54790_11_49	TET2	EXON	+	chr4:105275426 -105275446	GGACAACUGCUCCCCAUUAUC	872
54790_11_50	TET2	EXON	+	chr4:105275427 -105275447	GACAACUGCUCCCCAUUAUCU	873
54790_11_53	TET2	EXON	+	chr4:105275456 -105275476	UUCUCCCCAGUCUCAGCCGA	874
54790_11_55	TET2	EXON	+	chr4:105275467 -105275487	CUCAGCCGAUGGAUCUGUAU	875
54790_11_56	TET2	EXON	+	chr4:105275533 -105275553	UCCAUAACACUUUACCAGCCA	876
54790_11_59	TET2	EXON	+	chr4:105275538 -105275558	ACACUUUACCAGCCAAGGUU	877
54790_11_65	TET2	EXON	+	chr4:105275571 -105275591	AGUUUUACAUCUAAAUAUCUU	878
54790_11_68	TET2	EXON	+	chr4:105275577 -105275597	ACAUCUAAAUAUCUUAGGUUA	879
54790_11_74	TET2	EXON	+	chr4:105275594 -105275614	UUAUGGAAACCAAAAUAUGC	880
54790_11_77	TET2	EXON	+	chr4:105275595 -105275615	UAUGGAAACCAAAAUAUGCA	881

54790_11_79	TET2	EXON	+	chr4:105275601 -105275621	AACCAAAAU AUGCAGGGAGA	882
54790_11_85	TET2	EXON	+	chr4:105275643 -105275663	AGACCAA AUGUACAUCAUGU	883
54790_11_86	TET2	EXON	+	chr4:105275644 -105275664	GACCAA AUGUACAUCAUGUA	884
54790_11_92	TET2	EXON	+	chr4:105275675 -105275695	UCCUUAU CCCACUCAUGAGA	885
54790_11_93	TET2	EXON	+	chr4:105275679 -105275699	UAU CCCACUCAUGAGAUGGA	886
54790_11_96	TET2	EXON	+	chr4:105275690 -105275710	UGAGAUGGAUGGCCACUUCA	887
54790_11_99	TET2	EXON	+	chr4:105275691 -105275711	GAGAUGGAUGGCCACUUCAU	888
54790_11_104	TET2	EXON	+	chr4:105275735 -105275755	CAAUCUGAGCAAUCCAAACA	889
54790_11_105	TET2	EXON	+	chr4:105275748 -105275768	CCAAACAUGGACUAUAAAAA	890
54790_11_110	TET2	EXON	+	chr4:105275798 -105275818	CCAUAACUACAGUGCAGCUC	891
54790_11_111	TET2	EXON	+	chr4:105275799 -105275819	CAUAACUACAGUGCAGCUCC	892
54790_11_116	TET2	EXON	+	chr4:105275843 -105275863	UGCCCUGCAUCUCCAAAACA	893
54790_11_120	TET2	EXON	+	chr4:105275874 -105275894	AUGCUU UCCCACACAGCUAA	894
54790_11_121	TET2	EXON	+	chr4:105275875 -105275895	UGCUU UCCCACACAGCUAAU	895
54790_11_129	TET2	EXON	+	chr4:105275928 -105275948	GAUAGAACUGCUUGUGUCCA	896
54790_11_131	TET2	EXON	+	chr4:105275931 -105275951	AGAACUGCUUGUGUCCAAGG	897
54790_11_133	TET2	EXON	+	chr4:105275958 -105275978	CACAAAUUAAGUGAUGC UAA	898
54790_11_137	TET2	EXON	+	chr4:105275963 -105275983	AUUAAGUGAUGC UAAUGGUC	899
54790_11_139	TET2	EXON	+	chr4:105275978 -105275998	UGGUCAGGAAAAGCAGCCAU	900
54790_11_141	TET2	EXON	+	chr4:105275990 -105276010	GCAGCCAUUGGCACUAGUCC	901
54790_11_142	TET2	EXON	+	chr4:105275991 -105276011	CAGCCAUUGGCACUAGUCCA	902
54790_11_143	TET2	EXON	+	chr4:105275996 -105276016	AUUGGCACUAGUCCAGGGUG	903
54790_11_145	TET2	EXON	+	chr4:105276003 -105276023	CUAGUCCAGGGUGUGGCUUC	904
54790_11_148	TET2	EXON	+	chr4:105276011 -105276031	GGGUGUGGCUUCUGGUGCAG	905

54790_11_150	TET2	EXON	+	chr4:105276023 -105276043	UGGUGCAGAGGACAACGAUG	906
54790_11_152	TET2	EXON	+	chr4:105276028 -105276048	CAGAGGACAACGAUGAGGUC	907
54790_11_156	TET2	EXON	+	chr4:105276053 -105276073	AGACAGCGAGCAGAGCUUUC	908
54790_11_158	TET2	EXON	+	chr4:105276066 -105276086	AGCUUUCUGGAUCCUGACAU	909
54790_11_160	TET2	EXON	+	chr4:105276067 -105276087	GCUUUCUGGAUCCUGACAUU	910
54790_11_162	TET2	EXON	+	chr4:105276068 -105276088	CUUUCUGGAUCCUGACAUUG	911
54790_11_165	TET2	EXON	+	chr4:105276069 -105276089	UUUCUGGAUCCUGACAUUGG	912
54790_11_168	TET2	EXON	+	chr4:105276074 -105276094	GGAUCCUGACAUUGGGGGAG	913
54790_11_169	TET2	EXON	+	chr4:105276080 -105276100	UGACAUUGGGGGAGUGGCCG	914
54790_11_172	TET2	EXON	+	chr4:105276093 -105276113	GUGGCCGUGGCUCCAACUCA	915
54790_11_173	TET2	EXON	+	chr4:105276094 -105276114	UGGCCGUGGCUCCAACUCAU	916
54790_11_182	TET2	EXON	+	chr4:105276160 -105276180	CCCCUUUAAAGAAUCCCAAU	917
54790_11_186	TET2	EXON	+	chr4:105276175 -105276195	CCAAUAGGAAUCACCCCACC	918
54790_11_193	TET2	EXON	+	chr4:105276225 -105276245	AGCAUGAAUGAGCCAAAACA	919
54790_11_194	TET2	EXON	+	chr4:105276230 -105276250	GAAUGAGCCAAAACAUGGCU	920
54790_11_196	TET2	EXON	+	chr4:105276238 -105276258	CAAAACAUGGCUUGGCUCUU	921
54790_11_199	TET2	EXON	+	chr4:105276239 -105276259	AAAACAUGGCUUGGCUCUUU	922
54790_11_200	TET2	EXON	+	chr4:105276251 -105276271	GGCUCUUUGGGAAGCCAAAA	923
54790_11_210	TET2	EXON	+	chr4:105276275 -105276295	UGAAAAAGCCCGUGAGAAAG	924
54790_11_214	TET2	EXON	+	chr4:105276294 -105276314	GAGGAAGAGUGUGAAAAGUA	925
54790_11_217	TET2	EXON	+	chr4:105276324 -105276344	UAUGUGCCUCAGAAAUCCCA	926
54790_11_221	TET2	EXON	+	chr4:105276340 -105276360	CCCAUGGCAAAAAAAGUGAAA	927
54790_11_223	TET2	EXON	+	chr4:105276341 -105276361	CCAUGGCAAAAAAAGUGAAAC	928
54790_11_231	TET2	EXON	+	chr4:105276409 -105276429	UCAUCAAGUCUCUUGCCGAA	929

54790_11_236	TET2	EXON	+	chr4:105276466 -105276486	CAUCUCCAUAUGCCUUCACU	930
54790_11_237	TET2	EXON	+	chr4:105276467 -105276487	AUCUCCAUAUGCCUUCACUC	931
54790_11_239	TET2	EXON	+	chr4:105276474 -105276494	UAUGCCUUCACUCGGGUCAC	932
54790_11_240	TET2	EXON	+	chr4:105276475 -105276495	AUGCCUUCACUCGGGUCACA	933
54790_11_243	TET2	EXON	+	chr4:105276515 -105276535	AUGAUUACACCCCCUUUUGU	934
54790_11_252	TET2	EXON	+	chr4:105276573 -105276593	GUAGUAUAGUUCUCAUGACG	935
54790_11_253	TET2	EXON	+	chr4:105276574 -105276594	UAGUAUAGUUCUCAUGACGU	936
54790_11_256	TET2	EXON	+	chr4:105276580 -105276600	AGUUCUCAUGACGUGGGCAG	937
54790_11_258	TET2	EXON	+	chr4:105276581 -105276601	GUUCUCAUGACGUGGGCAGU	938
54790_11_259	TET2	EXON	+	chr4:105276582 -105276602	UUCUCAUGACGUGGGCAGUG	939
54790_11_262	TET2	EXON	+	chr4:105276587 -105276607	AUGACGUGGGCAGUGGGGAA	940
54790_11_263	TET2	EXON	+	chr4:105276611 -105276631	CACAGUAUUCAUGACAAAUG	941
54790_11_265	TET2	EXON	+	chr4:105276614 -105276634	AGUAUUCAUGACAAAUGUGG	942
54790_11_267	TET2	EXON	+	chr4:105276615 -105276635	GUAUUCAUGACAAAUGUGGU	943
54790_11_271	TET2	EXON	+	chr4:105276646 -105276666	CAGCUCACCAGCAACAAAAG	944
54790_11_273	TET2	EXON	+	chr4:105276677 -105276697	CCAUAGCACUUAUUUUUCAC	945
54790_11_275	TET2	EXON	+	chr4:105276688 -105276708	AAUUUUCACUGGCUCCCAAG	946
54790_11_280	TET2	EXON	+	chr4:105276698 -105276718	GGCUCCCAAGUGGUCACAGA	947
54790_11_283	TET2	EXON	+	chr4:105276706 -105276726	AGUGGUCACAGAUGGCAUCU	948
54790_11_285	TET2	EXON	+	chr4:105276738 -105276758	AAGCAUUCUAUGCAAAAAGA	949
54790_11_288	TET2	EXON	+	chr4:105276741 -105276761	CAUUCUAUGCAAAAAGAAGG	950
54790_11_289	TET2	EXON	+	chr4:105276742 -105276762	AUUCUAUGCAAAAAGAAGGU	951
54790_11_291	TET2	EXON	+	chr4:105276743 -105276763	UUCUAUGCAAAAAGAAGGUG	952
54790_11_297	TET2	EXON	+	chr4:105276780 -105276800	CAAUUUACAUUUUUAAACAC	953

54790_11_302	TET2	EXON	+	chr4:105276792 -105276812	UUAAACACUGGUUCUAUUAU	954
54790_11_316	TET2	EXON	+	chr4:105276885 -105276905	AUAUCAAGUUUGCAUAGUCA	955
54790_11_321	TET2	EXON	+	chr4:105276925 -105276945	UACUGUAGUAUUACAGUGAC	956
54790_11_323	TET2	EXON	+	chr4:105276945 -105276965	AGGAAUCUAAAAUACCAUC	957
54790_11_329	TET2	EXON	+	chr4:105276975 -105276995	UAUAUGAUGUACUGAAAUAC	958
54790_11_330	TET2	EXON	+	chr4:105276983 -105277003	GUACUGAAAUACUGGAAUUA	959
54790_11_344	TET2	EXON	+	chr4:105277042 -105277062	UUAUUUAUCAAAAUAGCUAC	960
54790_11_352	TET2	EXON	+	chr4:105277058 -105277078	CUACAGGAAACAUGAAUAGC	961
54790_11_356	TET2	EXON	+	chr4:105277078 -105277098	AGGAAAACACUGAAUUUGUU	962
54790_11_359	TET2	EXON	+	chr4:105277094 -105277114	UGUUUGGAUGUUCUAAGAAA	963
54790_11_367	TET2	EXON	+	chr4:105277108 -105277128	AAGAAAUGGUGCUAAGAAAA	964
54790_11_377	TET2	EXON	+	chr4:105277187 -105277207	CUCCAGUGCCCUUGAAUAAU	965
54790_11_378	TET2	EXON	+	chr4:105277188 -105277208	UCCAGUGCCCUUGAAUAAUA	966
54790_11_379	TET2	EXON	+	chr4:105277189 -105277209	CCAGUGCCCUUGAAUAAUAG	967
54790_11_393	TET2	EXON	+	chr4:105277255 -105277275	CAAGCUUAGUUUUUAAAAUG	968
54790_11_395	TET2	EXON	+	chr4:105277267 -105277287	UUAAAAUGUGGACAUUUUAA	969
54790_11_401	TET2	EXON	+	chr4:105277274 -105277294	GUGGACAUUUUAAAGGCCUC	970
54790_11_410	TET2	EXON	+	chr4:105277304 -105277324	UCAUCCAGUGAAGUCCUUGU	971
54790_11_419	TET2	EXON	+	chr4:105277438 -105277458	UGACAACUUGAACAAUGCUA	972
54790_11_437	TET2	EXON	+	chr4:105277501 -105277521	AUGCAAAGUUGAUUUUUUUA	973
54790_11_465	TET2	EXON	+	chr4:105277599 -105277619	ACAGCCAGUUAAAUCCACCA	974
54790_11_466	TET2	EXON	+	chr4:105277600 -105277620	CAGCCAGUUAAAUCCACCAU	975
54790_11_467	TET2	EXON	+	chr4:105277601 -105277621	AGCCAGUUAAAUCCACCAUG	976
54790_11_469	TET2	EXON	+	chr4:105277609 -105277629	AAAUCCACCAUGGGGCUUAC	977

54790_11_472	TET2	EXON	+	chr4:105277617 -105277637	CAUGGGGCUUACUGGAUUCA	978
54790_11_474	TET2	EXON	+	chr4:105277618 -105277638	AUGGGGCUUACUGGAUUCAA	979
54790_11_478	TET2	EXON	+	chr4:105277649 -105277669	AGUCCACAAAACAUGUUUUC	980
54790_11_492	TET2	EXON	+	chr4:105277753 -105277773	AAGAAUUUUCUAUUAACUGC	981
54790_11_503	TET2	EXON	+	chr4:105277818 -105277838	CUGAAGCCUAUGCUAUUUUA	982
54790_11_504	TET2	EXON	+	chr4:105277826 -105277846	UAUGCUAUUUUAUGGAUCAU	983
54790_11_511	TET2	EXON	+	chr4:105277846 -105277866	AGGCUCUUCAGAGAACUGAA	984
54790_11_524	TET2	EXON	+	chr4:105277924 -105277944	UAAGUGUCCUCUUUAACAAG	985
54790_11_532	TET2	EXON	+	chr4:105277963 -105277983	CCUGCAUAAGAUGAAUAAAC	986
54790_11_533	TET2	EXON	+	chr4:105277964 -105277984	CUGCAUAAGAUGAAUAAACA	987
54790_11_539	TET2	EXON	+	chr4:105278008 -105278028	AGUUAAAAAGAAACAAAAC	988
54790_11_541	TET2	EXON	+	chr4:105278015 -105278035	AAGAAACAAAACAGGCAGC	989
54790_11_542	TET2	EXON	+	chr4:105278025 -105278045	AACAGGCAGCUGGUUUGCUG	990
54790_11_543	TET2	EXON	+	chr4:105278028 -105278048	AGGCAGCUGGUUUGCUGUGG	991
54790_11_574	TET2	EXON	+	chr4:105278210 -105278230	AAGCAGAAUUCACAUCAUGA	992
54790_11_587	TET2	EXON	+	chr4:105278310 -105278330	CAUAUACCUCAACACUAGUU	993
54790_11_589	TET2	EXON	+	chr4:105278317 -105278337	CUCAACACUAGUUUGGCAAU	994
54790_11_627	TET2	EXON	+	chr4:105278467 -105278487	CCUUUUUGUUCUAAAAAUUC	995
54790_11_628	TET2	EXON	+	chr4:105278468 -105278488	CUUUUUUGUUCUAAAAAUUCA	996
54790_11_637	TET2	EXON	+	chr4:105278532 -105278552	UGUUUAUGUAAAAUUGUUGU	997
54790_11_643	TET2	EXON	+	chr4:105278556 -105278576	UAAUAAAUUAUUCUUUGUC	998
54790_11_645	TET2	EXON	+	chr4:105278557 -105278577	AAUAAAUUAUUCUUUGUCA	999
54790_11_664	TET2	EXON	+	chr4:105278640 -105278660	AACUAAUUUUGUAAAUCUGU	1000
54790_11_679	TET2	EXON	+	chr4:105278680 -105278700	AAAAGCAUUUAAAAGUUUG	1001

54790_11_686	TET2	EXON	+	chr4:105278704 -105278724	AUCUUUUGACUGUUUCAAGC	1002
54790_11_700	TET2	EXON	+	chr4:105278748 -105278768	AGAAUGCACUGAGUUGAUAA	1003
54790_11_701	TET2	EXON	+	chr4:105278749 -105278769	GAAUGCACUGAGUUGAUAAA	1004
54790_11_703	TET2	EXON	+	chr4:105278762 -105278782	UGAUAAAGGGAAAAAUUGUA	1005
54790_11_707	TET2	EXON	+	chr4:105278766 -105278786	AAAGGGAAAAAUUGUAAGGC	1006
54790_11_708	TET2	EXON	+	chr4:105278773 -105278793	AAAAUUGUAAGGCAGGAGUU	1007
54790_11_710	TET2	EXON	+	chr4:105278780 -105278800	UAAGGCAGGAGUUUGGCAAG	1008
54790_11_711	TET2	EXON	+	chr4:105278787 -105278807	GGAGUUUGGCAAGUGGCUGU	1009
54790_11_721	TET2	EXON	+	chr4:105278846 -105278866	UUUGAUCCUGUAAUCACUGA	1010
54790_11_728	TET2	EXON	+	chr4:105278862 -105278882	CUGAAGGUACAUACUCCAUG	1011
54790_11_729	TET2	EXON	+	chr4:105278878 -105278898	CAUGUGGACUUCCCUUAAAC	1012
54790_11_731	TET2	EXON	+	chr4:105278892 -105278912	UUAAACAGGCAAACACCUAC	1013
54790_11_733	TET2	EXON	+	chr4:105278897 -105278917	CAGGCAAACACCUACAGGUA	1014
54790_11_734	TET2	EXON	+	chr4:105278927 -105278947	CAGAUUGUACAAUUAUUAUU	1015
54790_11_748	TET2	EXON	+	chr4:105278978 -105278998	UAAAAUAAAUUCUUAUCAG	1016
54790_11_751	TET2	EXON	+	chr4:105278981 -105279001	AAUAAAUUCUUAUCAGAGG	1017
54790_11_753	TET2	EXON	+	chr4:105278988 -105279008	UCUUAUCAGAGGAGGCCUU	1018
54790_11_754	TET2	EXON	+	chr4:105278989 -105279009	CUUAAUCAGAGGAGGCCUUU	1019
54790_11_757	TET2	EXON	+	chr4:105278998 -105279018	AGGAGGCCUUUGGGUUUUUAU	1020
54790_11_762	TET2	EXON	+	chr4:105279017 -105279037	UUGGUCAAAUCUUUGUAAGC	1021
54790_11_772	TET2	EXON	+	chr4:105279052 -105279072	UAAAAAAUUCUUGAAUUUG	1022
54790_11_799	TET2	EXON	+	chr4:105279173 -105279193	UUUGAUUACUACAUGUGCAU	1023
54790_11_813	TET2	EXON	+	chr4:105279240 -105279260	ACUGUCAUUUGUUAACUGC	1024
54790_11_818	TET2	EXON	+	chr4:105279254 -105279274	AACUGCUGGCCAACAAGAAC	1025

54790_11_822	TET2	EXON	+	chr4:105279267 -105279287	CAAGAACAGGAAGUAUAGUU	1026
54790_11_825	TET2	EXON	+	chr4:105279268 -105279288	AAGAACAGGAAGUAUAGUUU	1027
54790_11_827	TET2	EXON	+	chr4:105279269 -105279289	AGAACAGGAAGUAUAGUUUG	1028
54790_11_828	TET2	EXON	+	chr4:105279270 -105279290	GAACAGGAAGUAUAGUUUGG	1029
54790_11_829	TET2	EXON	+	chr4:105279271 -105279291	AACAGGAAGUAUAGUUUGGG	1030
54790_11_832	TET2	EXON	+	chr4:105279275 -105279295	GGAAGUAUAGUUUGGGGGGU	1031
54790_11_833	TET2	EXON	+	chr4:105279276 -105279296	GAAGUAUAGUUUGGGGGGUU	1032
54790_11_836	TET2	EXON	+	chr4:105279277 -105279297	AAGUAUAGUUUGGGGGGUUG	1033
54790_11_841	TET2	EXON	+	chr4:105279292 -105279312	GGUUGGGGAGAGUUUACAUA	1034
54790_11_851	TET2	EXON	+	chr4:105279311 -105279331	AAGGAAGAGAAGAAAUUGAG	1035
54790_11_859	TET2	EXON	+	chr4:105279373 -105279393	CCUGCCUCAGUUAGAAUGAA	1036
54790_11_864	TET2	EXON	+	chr4:105279402 -105279422	GAUCUACAAUUUGCUAAUAU	1037
54790_11_865	TET2	EXON	+	chr4:105279411 -105279431	UUUGCUAAUAUAGGAAUAUC	1038
54790_11_871	TET2	EXON	+	chr4:105279449 -105279469	UACUUGAAAAUGCUUCUGAG	1039
54790_11_886	TET2	EXON	+	chr4:105279524 -105279544	CAGUUCACUUCUGAAGCUAG	1040
54790_11_890	TET2	EXON	+	chr4:105279538 -105279558	AGCUAGUGGUUAAACUUGUGU	1041
54790_11_912	TET2	EXON	+	chr4:105279632 -105279652	UUUCAUUUUCAUGAGAUGUU	1042
54790_11_920	TET2	EXON	+	chr4:105279648 -105279668	UGUUUGGUUUUAUAGAUCUG	1043
54790_11_921	TET2	EXON	+	chr4:105279652 -105279672	UGGUUUUAUAGAUCUGAGGA	1044
54790_11_928	TET2	EXON	+	chr4:105279691 -105279711	UAUUGUAAUGUUAUGAAUGC	1045
54790_11_954	TET2	EXON	-	chr4:105275038 -105275058	UCGCAAAAGUUCUGUGGACA	1046
54790_11_955	TET2	EXON	-	chr4:105275039 -105275059	GUCGCAAAAGUUCUGUGGAC	1047
54790_11_957	TET2	EXON	-	chr4:105275044 -105275064	ACAAAGUCGCAAAAGUUCUG	1048
54790_11_960	TET2	EXON	-	chr4:105275165 -105275185	AGUUGACAGACUCUGUCUGA	1049

54790_11_961	TET2	EXON	-	chr4:105275166 -105275186	GAGUUGACAGACUCUGUCUG	1050
54790_11_970	TET2	EXON	-	chr4:105275206 -105275226	CCGUCUCAUGUAUGGAUUGG	1051
54790_11_972	TET2	EXON	-	chr4:105275209 -105275229	GGGCCGUCUCAUGUAUGGAU	1052
54790_11_973	TET2	EXON	-	chr4:105275214 -105275234	GGAUUGGGCCGUCUCAUGUA	1053
54790_11_977	TET2	EXON	-	chr4:105275229 -105275249	GGAUAAGGACUAAACUGGAUU	1054
54790_11_978	TET2	EXON	-	chr4:105275230 -105275250	UGGAUAAGGACUAAACUGGAU	1055
54790_11_980	TET2	EXON	-	chr4:105275235 -105275255	GAGUUUGGAUAAGGACUAAAC	1056
54790_11_982	TET2	EXON	-	chr4:105275244 -105275264	GUGUGUGAAGAGUUUGGAUA	1057
54790_11_984	TET2	EXON	-	chr4:105275250 -105275270	UCUGAAGUGUGUGAAGAGUU	1058
54790_11_991	TET2	EXON	-	chr4:105275287 -105275307	GGAAUAGAAGUUCAUAGGGC	1059
54790_11_992	TET2	EXON	-	chr4:105275291 -105275311	AGGUGGAAUAGAAGUUCAUA	1060
54790_11_993	TET2	EXON	-	chr4:105275292 -105275312	GAGGUGGAAUAGAAGUUCAU	1061
54790_11_999	TET2	EXON	-	chr4:105275308 -105275328	ACCUGCAGCUUGAGAUGAGG	1062
54790_11_1001	TET2	EXON	-	chr4:105275311 -105275331	UGAACCUGCAGCUUGAGAUG	1063
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54790_11_1013	TET2	EXON	-	chr4:105275353 -105275373	AAGCCCAGGGUAAGGGUUCA	1065
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54790_11_1022	TET2	EXON	-	chr4:105275367 -105275387	GUAUUCUGAUUCAAAAAGCCC	1069
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54790_11_1031	TET2	EXON	-	chr4:105275397 -105275417	UUUCCAUGCAUUGAUUAUGA	1072
54790_11_1034	TET2	EXON	-	chr4:105275420 -105275440	GGGAGCAGUUGUCCACUGAU	1073

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54790_11_1205	TET2	EXON	-	chr4:105276165 -105276185	UUCCUAUUGGGAUUCUUUAA	1126
54790_11_1207	TET2	EXON	-	chr4:105276177 -105276197	CUGGUGGGGUGAUUCCUAUU	1127
54790_11_1209	TET2	EXON	-	chr4:105276178 -105276198	CCUGGUGGGGUGAUUCCUAU	1128
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54790_11_1214	TET2	EXON	-	chr4:105276193 -105276213	AAAGACGAGGGAGAUCUGG	1131
54790_11_1216	TET2	EXON	-	chr4:105276196 -105276216	GUAAAAGACGAGGGAGAUC	1132
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54790_11_1221	TET2	EXON	-	chr4:105276206 -105276226	UCUUAUGCUGGUAAAAGACG	1134
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54790_11_1286	TET2	EXON	-	chr4:105276448 -105276468	UGUAGUUACUGUGGAGUCUG	1153
54790_11_1288	TET2	EXON	-	chr4:105276457 -105276477	AUAUGGAGAUGUAGUUACUG	1154
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54790_11_1739	TET2	EXON	-	chr4:105278855 -105278875	UAUGUACCUUCAGUGAUUAC	1223
54790_11_1746	TET2	EXON	-	chr4:105278880 -105278900	CUGUUUAAGGGAAGUCCACA	1224
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54790_11_1751	TET2	EXON	-	chr4:105278893 -105278913	UGUAGGUGUUUGCCUGUUUA	1226
54790_11_1754	TET2	EXON	-	chr4:105278910 -105278930	CUGUUGCACACCAUACCUGU	1227
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54790_11_1768	TET2	EXON	-	chr4:105279007 -105279027	AUUUGACCAAUAAAACCCAA	1229
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54790_11_1789	TET2	EXON	-	chr4:105279101 -105279121	GUAAGCAAAGCAAACAUUUU	1231
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54790_11_1803	TET2	EXON	-	chr4:105279212 -105279232	CAGAAAUCAAUAUUAAGAA	1237
54790_11_1809	TET2	EXON	-	chr4:105279240 -105279260	GCAGUUUAACAAAUGACAGU	1238
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54790_11_1877	TET2	EXON	-	chr4:105279748 -105279768	AACACUCACAUAGCAUUAUC	1243

TALEN Gene Editing Systems

TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effects (TALEs) can be engineered to bind any desired DNA sequence, including a portion of the HLA or TCR gene. By combining an engineered TALE with a DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence, including a HLA or TCR sequence. These can then be introduced into a cell, wherein they can be used for genome editing. Boch (2011) Nature Biotech. 29: 135-6; and Boch et al. (2009) Science 326: 1509-12; Moscou et al. (2009) Science 326: 3501.

TALEs are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12th and 13th amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide recognition. They can thus be engineered to bind to a desired DNA sequence.

To produce a TALEN, a TALE protein is fused to a nuclease (N), which is, for example, a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) Nucl. Acids Res. 39: e82; Miller et al. (2011) Nature Biotech. 29: 143-8; Hockemeyer et al. (2011) Nature Biotech. 29: 731-734; Wood et al. (2011) Science 333: 307; Doyon et al. (2010) Nature Methods 8: 74-79; Szczepek et al. (2007) Nature Biotech. 25: 786-793; and Guo et al. (2010) J. Mol. Biol. 200: 96.

The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) Nature Biotech. 29: 143-8.

A TALEN specific for a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, foreign DNA can be introduced into the cell along with the TALEN, e.g., DNA encoding a CAR, e.g., as described herein; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to integrate the DNA encoding the CAR, e.g., as described herein, at or near the site targeted by the TALEN. As shown herein, in the examples, but

without being bound by theory, such integration may lead to the expression of the CAR as well as disruption of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. Such foreign DNA molecule is referred to herein as “template DNA.” In embodiments, the template DNA further comprises homology arms 5’ to, 3’ to, or both 5’ and 3’ to the nucleic acid of the template DNA which encodes the molecule or molecules of interest (e.g., which encodes a CAR described herein), wherein said homology arms are complementary to genomic DNA sequence flanking the target sequence.

TALENs specific to sequences in a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) Nature Biotech. 29: 149-53; Geibler et al. (2011) PLoS ONE 6: e19509; US 8,420,782 ; US 8,470,973, the contents of which are hereby incorporated by reference in their entirety.

Zinc Finger Nucleases

“ZFN” or “Zinc Finger Nuclease” refer to a zinc finger nuclease, an artificial nuclease which can be used to modify, e.g., delete one or more nucleic acids of, a desired nucleic acid sequence, e.g., a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene.

Like a TALEN, a ZFN comprises a FokI nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) Genetics Society of America 188: 773-782; and Kim et al. (1996) Proc. Natl. Acad. Sci. USA 93: 1156-1160.

A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys2His2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) Proc. Natl. Acad. Sci. USA 95: 10570-5.

Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, in a cell. ZFNs can also be used with homologous recombination to mutate a Tet-associated

gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, or to introduce nucleic acid encoding a CAR at a site at or near the targeted sequence. As discussed above, the nucleic acid encoding a CAR may be introduced as part of a template DNA. In embodiments, the template DNA further comprises homology arms 5' to, 3' to, or both 5' and 3' to the nucleic acid of the template DNA which encodes the molecule or molecules of interest (e.g., which encodes a CAR described herein), wherein said homology arms are complementary to genomic DNA sequence flanking the target sequence.

ZFNs specific to sequences in a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, can be constructed using any method known in the art. See, e.g., Provasi (2011) *Nature Med.* 18: 807-815; Torikai (2013) *Blood* 122: 1341-1349; Cathomen et al. (2008) *Mol. Ther.* 16: 1200-7; and Guo et al. (2010) *J. Mol. Biol.* 400: 96; U.S. Patent Publication 2011/0158957; and U.S. Patent Publication 2012/0060230, the contents of which are hereby incorporated by reference in their entirety. In embodiments, The ZFN gene editing system may also comprise nucleic acid encoding one or more components of the ZFN gene editing system, e.g., a ZFN gene editing system targeted to a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene.

Without being bound by theory, it is believed that use of gene editing systems (e.g., CRISPR/Cas gene editing systems) which target a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, may allow one to modulate (e.g., inhibit) one or more functions of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, by, for example, causing an editing event which results in expression of a truncated Tet-associated gene (e.g., a Tet2-associated gene) and/or a truncated Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. Again, without being bound by theory, such a truncated Tet-associated gene (e.g., a Tet2-associated gene) product and/or truncated Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene product may preserve one or more functions of the Tet-associated gene (e.g., a Tet2-associated gene) product and/or Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene product (e.g., a scaffolding function), while inhibiting one or more other functions of the Tet-associated gene (e.g., a Tet2-associated gene) product and/or Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene product (e.g., a catalytic function), and as such, may be preferable. Gene editing systems which target a late exon or intron of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, may be particularly preferred in this regard. In an aspect, the gene editing system of the invention targets a late exon or intron of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In an aspect, the gene editing system of the invention targets an exon or intron downstream of exon 8. In an aspect, the gene editing system targets exon 8 or exon 9, e.g., exon 9, of a Tet2 gene.

Without being bound by theory, it may also be preferable in other embodiments to target an early exon or intron of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1,

Tet2, and/or Tet3, e.g., Tet2) gene, for example, to introduce a premature stop codon in the targeted gene which results in no expression of the gene product, or expression of a completely non-functional gene product. Gene editing systems which target an early exon or intron of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, may be particularly preferred in this regard. In an aspect, the gene editing system of the invention targets an early exon or intron of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In an aspect, the gene editing system of the invention targets an exon or intron upstream of exon 4. In embodiments, the gene editing system targets exon 1, exon 2, or exon 3, e.g., exon 3, of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene.

Without being bound by theory, it may also be preferable in other embodiments to target a sequence of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, which is specific to one or more isoforms of the gene but does not affect one or more other isoforms of the gene. In embodiments, it may be preferable to specifically target an isoform of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene which contain a catalytic domain.

Double-Stranded RNA, E.g., siRNA or ShRNA, Modulators

According to the present invention, double stranded RNA (“dsRNA”), e.g., siRNA or shRNA can be used as modulators (e.g., inhibitors) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. Also contemplated by the present invention are the uses of nucleic acid encoding said dsRNA modulators (e.g., inhibitors) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene.

In an embodiment, the modulator (e.g., inhibitor) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene is a nucleic acid, e.g., a dsRNA, e.g., a siRNA or shRNA specific for nucleic acid encoding a Tet-associated gene (e.g., a Tet2-associated gene) or gene product and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene or gene product, e.g., genomic DNA or mRNA encoding a Tet-associated gene product (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene product.

An aspect of the invention provides a composition comprising a dsRNA, e.g., a siRNA or shRNA, comprising at least 15 contiguous nucleotides, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 contiguous nucleotides, e.g., 21 contiguous nucleotides, which are complementary (e.g., 100% complementary) to a sequence of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, nucleic acid sequence (e.g., genomic DNA or mRNA encoding a Tet-associated gene (e.g., a Tet2-associated gene) product and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene product). In embodiments, the at least 15 contiguous nucleotides, e.g.,

15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 contiguous nucleotides, e.g., 21 contiguous nucleotides, include contiguous nucleotides of a target sequence of shRNA or nucleic acid encoding Tet2 shRNA listed in Table 4. It is understood that some of the target sequences and/or shRNA molecules are presented as DNA, but the dsRNA agents targeting these sequences or comprising these sequences
 5 can be RNA, or any nucleotide, modified nucleotide or substitute disclosed herein and/or known in the art, provided that the molecule can still mediate RNA interference.

In an embodiment, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is operably linked to a promoter, e.g., a H1- or a U6-derived promoter such that
 10 the dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is expressed within a CAR-expressing cell. See e.g., Tiscornia G., "Development of Lentiviral Vectors Expressing siRNA," Chapter 3, in Gene Transfer: Delivery and Expression of DNA and RNA (eds. Friedmann and Rossi). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2007; Brummelkamp TR, et al. (2002)
 15 Science 296: 550–553; Miyagishi M, et al. (2002) Nat. Biotechnol. 19: 497–500. In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is present on the same vector, e.g., a lentiviral vector, that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the CAR. In such an embodiment, the nucleic
 20 acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is located on the vector, e.g., the lentiviral vector, 5' - or 3' - to the nucleic acid that encodes a component, e.g., all of the components, of the CAR. The nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or
 25 Tet3, e.g., Tet2) gene, can be transcribed in the same or different direction as the nucleic acid that encodes a component, e.g., all of the components, of the CAR. In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is present on a vector other than the vector that comprises a nucleic acid molecule that encodes a component, e.g., all
 30 of the components, of the CAR. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is transiently expressed within a CAR-expressing cell. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g.,
 35 Tet2) gene, is stably integrated into the genome of a CAR-expressing cell.

Examples of nucleic acid sequences that encode shRNA sequences are provided below. The target sequence refers to the sequence within the Tet2 genomic DNA (or surrounding DNA). The

nucleic acid encoding Tet2 shRNA encodes shRNA molecules useful in the present invention. In
embodiments, the Tet2 inhibitor is an siRNA or shRNA specific for a target sequence listed below, or
specific for its mRNA complement. In embodiments, the Tet2 inhibitor is a shRNA encoded by the
Nucleic Acid encoding Tet2 shRNA of the table 4 below. In embodiments, the Tet2 inhibitor is
5 nucleic acid comprising by the nucleic acid encoding Tet2 shRNA of the table 4 below, e.g., which is
under the control of a U6 or H1 promoter such that a Tet2 shRNA is produced. In embodiments, the
invention provides a siRNA or shRNA comprising sequence which is the RNA analog (i.e., all T
nucleic acid residues replaced with U nucleic acid residues) of the target sequence of shRNA, e.g., the
target sequenc of shRNA of any of the shRNAs of Table 4.

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Table 4

	SHRNA_NAME	Target sequence of shRNA	Nucleic Acid encoding Tet2 shRNA
TET2	TET2- 3838_76472_insert (TET2 shRNA #1)	CACATGGCGTTTA TCCAGAAT (SEQ ID NO: 1244)	CACATGGCGTTTATCCAGAAT CTCGAGATTCTGGATAAACGCCATG TGTTTTTTGAATTCGCACCAGCACGC TACGCACACACAGTACACACACTGA CGTTTCGCCGTCTTC (SEQ ID NO: 1253)
TET2	TET2_NM_017628.4_2 5616_concept (TET2 shRNA #2)	CAGATGCACAGGC CAATTAAG (SEQ ID NO: 1245)	GAAGACGCACCGGCAGATGTACAGG CTAATTAAGGTTAATATTCATAGCCT TAATTGGCCTGTGCATCTGTTTTTTG AATTCGCACCAGCACGCTACGCAAC ACGTCAACCAGTGTCAAGTGTTCGCC GT (SEQ ID NO: 1254)
TET2	TET2_NM_017628.4_2 5625_concept (TET2 shRNA #3)	GAGCTGCTGAATT CAACTAGA (SEQ ID NO: 1246)	GAAGACGCACCGGGAGCTGCTGAAT TCAATTAGAGTTAATATTCATAGCTC TAGTTGAATTCAGCAGCTCTTTTTTG AATTCGCACCAGCACGCTACGCATG CAGTCAACCAGTGTCAACCATTTCGC CGT (SEQ ID NO: 1255)
TET2	TET2- 6571_76471_target (TET2 shRNA #4)	CAGATCGCCATAA CATAAATA (SEQ ID NO: 1247)	CAGATCGCCATAACATAAATACTCG AGTATTTATGTTATGGCGATCTGTTT TTTGAATTCGCACCAGCACGCTACGC ATGACCAGTACACACACTGCATGTT CGCCGTCTTC (SEQ ID NO: 1256)
TET2	TET2_NM_017628.4_2 5619_target (TET2 shRNA #5)	GACCATGGAGCAG CATCTGAA (SEQ ID NO: 1248)	GAAGACGCACCGGGACCATGGAGTA GCATTTGAAGTTAATATTCATAGCTT CAGATGCTGCTCCATGGTCTTTTTTG AATTCGCACCAGCACGCTACGCATG GTGTCAACCAGTGTCAAGTTGTTCGCC GT (SEQ ID NO: 1257)
TET2	TET2 shRNA #6	GCCAAGTCATTAT TTGACCAT (SEQ ID NO: 1249)	GCCAAGTCATTATTTGACCATCTCGA GATGGTCAAATAATGACTTGGCTTTT TTGA (SEQ ID NO: 1258)
TET2	TET2 shRNA #7	CCTCAGAGATATT GTGGGTTT (SEQ ID NO: 1250)	CCTCAGAGATATTGTGGGTTTCTCGA GAAACCCACAATATCTCTGAGGTTT TTGA (SEQ ID NO: 1259)

TET2	TET2 shRNA #8	GGGTAAGCCAAGA AAGAAA (SEQ ID NO: 1251)	GGGTAAGCCAAGAAAGAAACTCGAG TTTCTTTCTTGGCTTACCCTTTTTTGA (SEQ ID NO: 1260)
TET2	TET2 8 long (TET2 shRNA #9)	GGGTAAGCCAAGA AAGAAA (SEQ ID NO: 1252)	GAAGACGCACCGGGGGTAAGCCAAG AAAGAAAGTTAATATTCATAGCTTTC TTTCTTGGCTTACCCTTTTTTGAATTC GCACCAGCACGCTACGCAACACGTC AACCAGTGTGAGTGTTCGCCGT (SEQ ID NO: 1261)

Additional dsRNA inhibitor of Tet2, e.g., shRNA and siRNA molecules can be designed and tested using methods known in the art and as described herein. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of SEQ ID NO: 1358. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of SEQ ID NO: 1359. In
5
embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of SEQ ID NO: 1360. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of SEQ ID NO: 1361. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of SEQ ID NO: 1362. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a
10
sequence of SEQ ID NO: 1363. In embodiments, the dsRNA Tet2 inhibitor, e.g., shRNA or siRNA, targets a sequence of an mRNA encoding Tet2.

In some embodiments, the dsRNA inhibitor is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1. For example, exemplary dsRNA inhibitors of PRDM1, e.g., shRNA and siRNA molecules, are known in the art, e.g., as described in WO 2013/070563, incorporated herein by
15
reference in its entirety.

In embodiments, the inhibitor is a nucleic acid, e.g., DNA, encoding a dsRNA inhibitor, e.g., shRNA or siRNA, of any of the above embodiments. In embodiments, the nucleic acid, e.g., DNA, is disposed on a vector, e.g., any conventional expression system, e.g., as described herein, e.g., a lentiviral vector.

Without being bound by theory, a dsRNA inhibitor (e.g., siRNA or shRNA) which targets a sequence of an mRNA of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, which is specific to one or more isoforms of the gene but does not affect one or more other isoforms of the gene (for example, due to targeting a unique splice junction, or targeting a domain which is present in one or more isoforms of the gene, but is not present in one
20
or more other isoforms of the gene). In embodiments, it may be preferable to specifically target an isoform of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene which contain a catalytic domain.

Small Molecules

In some embodiment, the modulator of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene is a small molecule. Exemplary small molecule modulators (e.g., inhibitors) are described below.

5 IFN- γ inhibitors

In embodiments, an IFN- γ inhibitor is a small molecule that inhibits or reduces IFN- γ expression and/or function. In one example, an IFN- γ inhibitor according to the present invention is a small molecule that inhibits or reduces the synthesis of IFN- γ , e.g., a bis phenol or phenoxy compound, or a derivative thereof. See, e.g., US 5,880,146, herein incorporated by reference in its
10 entirety. In another example, an IFN- γ inhibitor according to the present invention is a small molecule that inhibits IFN- γ by decreasing the production of IFN- γ inducing factor (IGIF) or inhibiting interleukin-1 β converting enzyme (ICE). See, e.g., US 5,985,863, herein incorporated by reference in its entirety.

NOTCH2 inhibitors

15 In embodiments, a NOTCH2 inhibitor is a small molecule that inhibits or reduces Notch2 expression and/or function.

In one example, a NOTCH2 inhibitor according to the present invention is gliotoxin or a derivative thereof, e.g., selected from the group consisting of acetylgliotoxin, 6-C₁₋₃-alkoxygliotoxin, 6-C₂₋₃-acyloxy-gliotoxin, 6-dihydro-gliotoxin, 6-dihydroxy-gliotoxin, 6-
20 [(methoxycarbonyl)methoxy]-gliotoxin or 6-cyanomethoxy-gliotoxin, or a salt thereof. See, e.g., US 7,981,878, herein incorporated by reference in its entirety.

In one example, a NOTCH2 inhibitor according to the present invention is 6-4[-(tertbutyl)-phenoxy] pyridine-3-amine, or a derivative thereof, see, e.g., US 9,296,682, herein incorporated by reference in its entirety.

25 In one example, a NOTCH2 inhibitor according to the present invention is a γ -secretase inhibitor, e.g., MK-0752 (Merck & Co.), RO4929097 (Roche), semagacestat (LY-450139; Eli Lilly & Co.), avagacestat (BMS-708163; Bristol-Myers Squib), DAPT (N-[N-(3,5-Difluorophenylacetyl)-L-alanyl]-S-phenylglycine t-Butyl ester), L685,458, compound E ((s,s)-2-(3,5-Difluorophenyl)-acetyl amino]-N-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-
30 propionamide), DBZ (dibenzazepine), JLK6 (7-amino-4-chloro-3-methoxyisocoumarin), or Compound 18 ([11-endo]-N-(5,6,7,8,9,10-hexahydro- 6,9-methano benzo[9][8]annulen-11-yl)-thiophene-2-sulfonamide). See, e.g., Purow B. Adv Exp Med Biol. 2012;727:305-19, herein incorporated by reference in its entirety.

CD28 inhibitors

35 In embodiments, a CD28 inhibitor is a small molecule that inhibits or reduces CD28 expression and/or function.

ICOS inhibitors

In embodiments, an ICOS inhibitor is a small molecule that inhibits or reduces ICOS expression and/or function.

IL2RA inhibitors

In embodiments, an IL2RA inhibitor is a small molecule that inhibits or reduces IL2RA expression and/or function.

In one example, an IL2RA inhibitor according to the present invention is a small molecule that reduces the binding between IL-2 and IL2RA, e.g., acylphenylalanine analogs, e.g., Ro26-4550 (Roche) or a derivative thereof. See, e.g., Thanos et al., Proc Natl Acad Sci U S A. 2006, herein incorporated by reference in its entirety.

PRDM1 inhibitors

In embodiments, a PRDM1 inhibitor is a small molecule that inhibits or reduces PRDM1 expression and/or function.

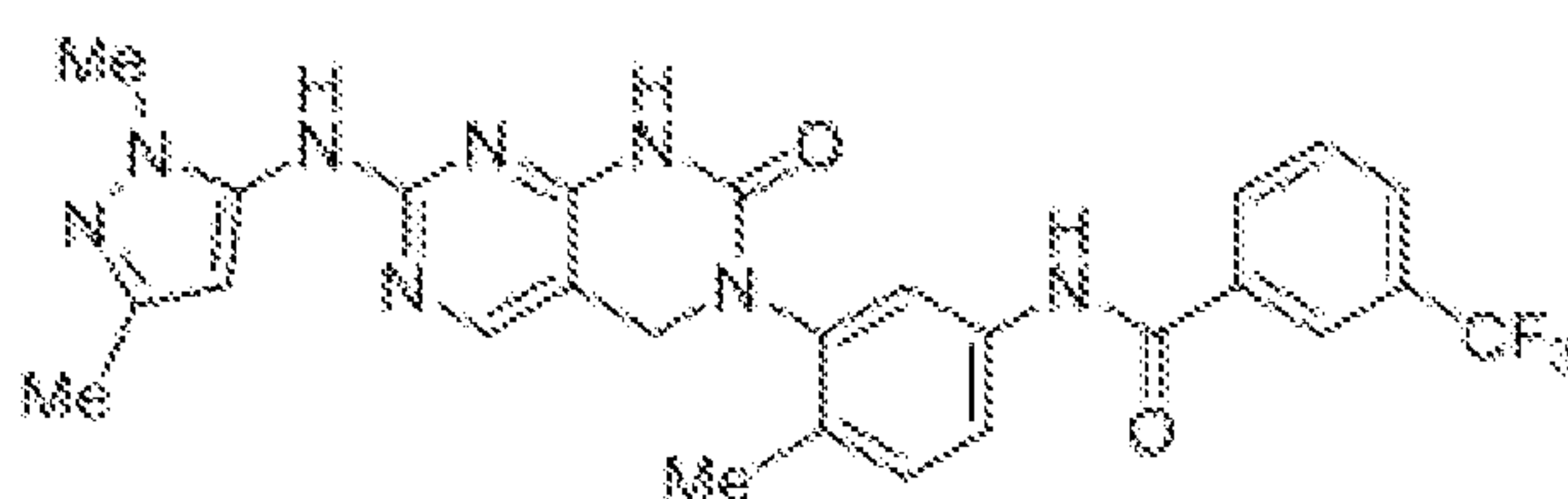
Tet inhibitors

In embodiments, a Tet inhibitor is a small molecule that inhibits expression and/or a function of Tet, e.g., Tet1, Tet2 and/or Tet3, e.g., Tet2.

Tet2 inhibitors

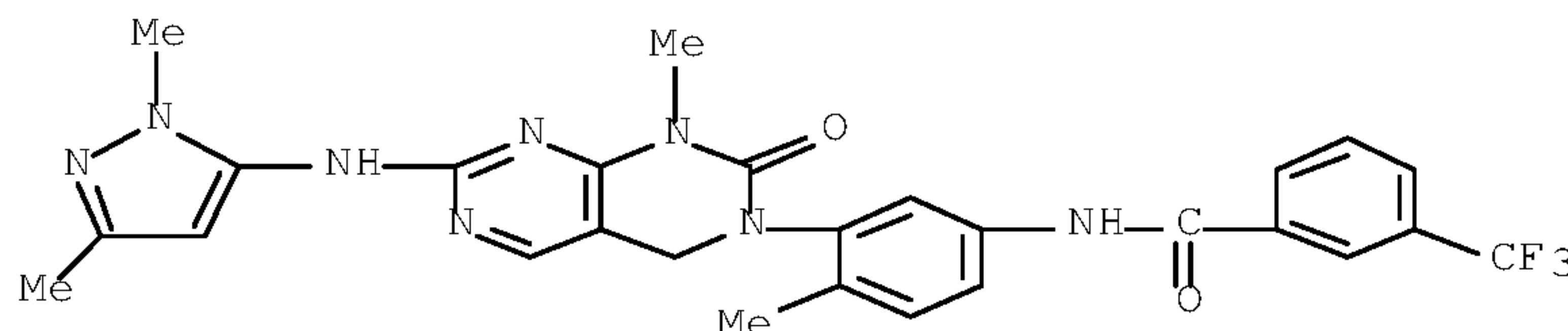
In embodiments, a Tet2 inhibitor is a small molecule that inhibits Tet2 expression and/or function. For example, a Tet2 inhibitor according to the present invention is 2-hydroxyglutarate (CAS #2889-31-8).

In another example, a Tet2 inhibitor according to the present invention has the following structure:

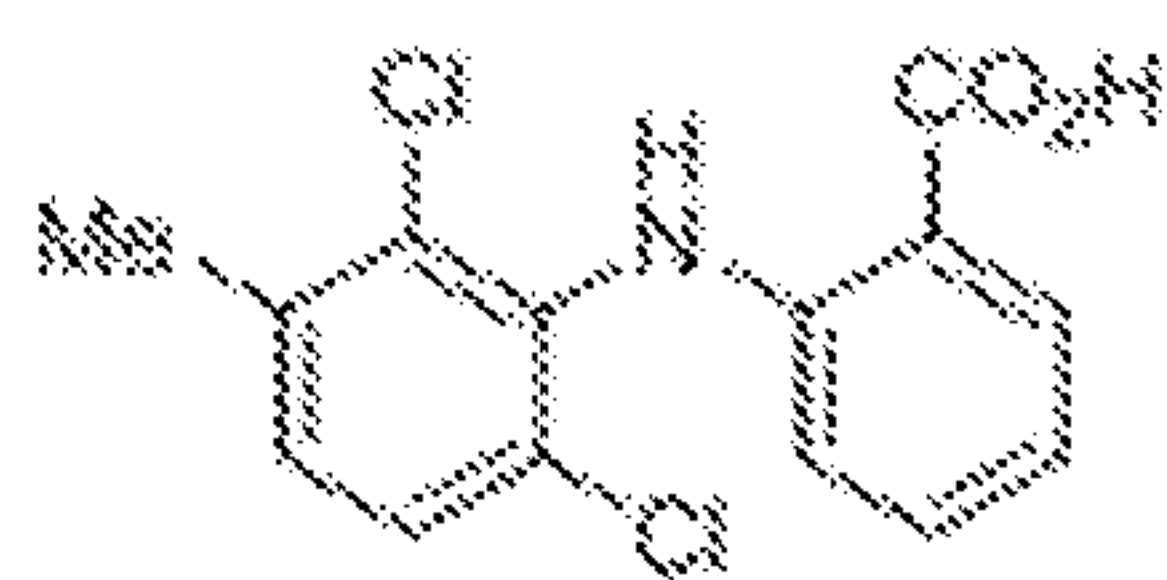


Pluripotin SC1

In another example, a Tet2 inhibitor according to the present invention is N-[3-[7-(2,5-Dimethyl-2H-pyrazol-3-ylamino)-1-methyl-2-oxo-1,4-dihydro-2H-pyrimido[4,5-d]pyrimidin-3-yl]-4-methylphenyl]-3-trifluoromethyl-benzamide (CAS #839707-37-8), and has the following structure:



In another example, a Tet2 inhibitor according to the present invention is 2-[(2,6-dichloro-3-methylphenyl)amino]benzoic acid (CAS # 644-62-2), and has the following structure:



meclofenamic acid

In embodiments, the Tet2 inhibitor of the present invention is a pharmaceutically acceptable salt of any of the foregoing.

HDAC inhibitors

5 Any known HDAC inhibitors can be used according to the present invention. Non-limiting examples of HDAC inhibitors include Voninostat (Zolinza®); Romidepsin (Istodax®); Treichostatin A (TSA); Oxamflatin; Vorinostat (Zolinza®, Suberoylanilide hydroxamic acid); Pyroxamide (syberoyl-3-aminopyridineamide hydroxamic acid); Trapoxin A (RF-1023A); Trapoxin B (RF-10238); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-L-prolyl] (Cyl-1); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-
10 (2S)-2-piperidinecarbonyl] (Cyl-2); Cyclic[L-alanyl-D-alanyl-(2S)- η -oxo-L- α -aminooxiraneoctanoyl-D-prolyl] (HC-toxin); Cyclo[(α S,2S)- α -amino- η -oxo-2-oxiraneoctanoyl-D-phenylalanyl-L-leucyl-(2S)-2-piperidinecarbonyl] (WF-3161); Chlamydocin ((S)-Cyclic(2-methylalanyl-L-phenylalanyl-D-prolyl- η -oxo-L- α -aminooxiraneoctanoyl); Apicidin (Cyclo(8-oxo-L-2-aminodecanoyl-1-methoxy-L-tryptophyl-L-isoleucyl-D-2-piperidinecarbonyl); Romidepsin
15 (Istodax®, FR-901228); 4-Phenylbutyrate; Spiruchostatin A; Mylproin (Valproic acid); Entinostat (MS-275, N-(2-Aminophenyl)-4-[N-(pyridine-3-yl-methoxycarbonyl)-amino-methyl]-benzamide); Depudecin (4,5:8,9-dianhydro-1,2,6,7,11-pentadeoxy- D-threo-D-ido-Undeca-1,6-dienitol); 4-(Acetylamino)-N-(2-aminophenyl)-benzamide (also known as CI-994); N1-(2-Aminophenyl)-N8-phenyl-octanediamide (also known as BML-210); 4-(Dimethylamino)-N-(7-(hydroxyamino)-7-oxoheptyl)benzamide (also known as M344); (E)-3-(4-(((2-(1H-indol-3-yl)ethyl)(2-hydroxyethyl)amino)-methyl)phenyl)-N-hydroxyacrylamide (NVP-LAQ824); Panobinostat (Farydak®); Mocetinostat, and Belinostat.

25 Proteins

In some embodiment, the modulator of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene is a protein. Exemplary protein modulators (e.g., inhibitors) are described below.

IFN- γ inhibitors

30 In embodiments, an IFN- γ inhibitor is a protein that inhibits or reduces IFN- γ expression and/or function. In one example, an IFN- γ inhibitor according to the present invention is an anti-IFN- γ antibody or fragment thereof, or an anti-IFN- γ receptor antibody or fragment thereof. See, e.g., WO

2013/078378, WO 2011/061700, US 6,329,511, US 6,558,661, and US 4,897,264, herein incorporated by reference in their entirety.

In another example, an IFN- γ inhibitor according to the present invention is IFN- γ receptor or fragment thereof, e.g., as described in WO 2011/061700, US 6,558,661, and US 7,608,430, herein
5 incorporated by reference in their entirety.

In another example, an IFN- γ inhibitor according to the present invention is modified or inactivated IFN- γ , or a fragment of IFN- γ , e.g., as described in US 5,451,658 and US 7,973,133, herein incorporated by reference in their entirety.

In another example, an IFN- γ inhibitor according to the present invention is a cytokine which
10 is an antagonist of IFN- γ , e.g., as described in US 5,612,195, herein incorporated by reference in its entirety.

In another example, an IFN- γ inhibitor according to the present invention is a BCRF1 protein that inhibits or reduces production of IFN- γ , e.g., as described in US 5,736,390, herein incorporated by reference in its entirety.

15 **NOTCH2 inhibitors**

In embodiments, a NOTCH2 inhibitor is a protein that inhibits or reduces NOTCH2 expression and/or function. In one example, a Notch2 inhibitor according to the present invention is an anti-NOTCH2 antibody or fragment thereof, see, e.g., WO 2014/141064, WO 2008/091641, US 7,919,092, US 8,226,943, and US 8,404,239, herein incorporated by reference in their entirety.

20 **IL2RA inhibitors**

In embodiments, an IL2RA inhibitor is a protein that inhibits or reduces IL2RA expression and/or function. In one example, an IL2RA inhibitor according to the present invention is an anti-IL2RA antibody or fragment thereof, see, e.g., WO 1990/007861, WO 2000/030679, WO 2014/144935, and US 7,438,907, herein incorporated by reference in their entirety. Exemplary anti-
25 IL2RA antibodies include Daclizumab, Basiliximab, and BT563.

In another example, an IL2RA inhibitor according to the present invention is a peptide antagonist of IL2RA, see, e.g., US 5,635,597 and Emerson et al., Protein Sci. 2003 Apr;12(4):811-22, herein incorporated by reference in their entirety.

PRDM1 inhibitors

30 In embodiments, a PRDM1 inhibitor is a protein or peptide that inhibits or reduces PRDM1 expression and/or function. In one example, a PRDM1 inhibitor according to the present invention is an anti-PRDM1 antibody or fragment thereof. In one example, a PRDM1 inhibitor according to the present invention is a blocking peptide that binds to PRDM1.

Dominant Negative Tet2

35 According to the present invention, dominant negative Tet2 isoforms, and nucleic acid encoding said dominant negative Tet2, can be used as Tet2 inhibitors. In embodiments, the dominant negative Tet2 lacks catalytic function of Tet2. An example of a dominant negative Tet2 is a protein

comprising or consisting of SEQ ID NO: 1357 with the mutation R1261G, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation R1262A, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation S1290A, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation WSMYYN (amino acids 1291-1296 of SEQ ID NO: 1357) to GGSGGS (SEQ ID NNO: 67), according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation M1293A and Y1294A, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation Y1295A, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation S1303N, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation H1382Y, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation D1384A, according to the numbering of SEQ ID NO: 1357. An example of a dominant negative Tet2 is a protein comprising or consisting of SEQ ID NO: 1357 with the mutation D1384V, according to the numbering of SEQ ID NO: 1357. In embodiments, the dominant negative Tet2 may include combinations of any of the aforementioned mutations. Such mutations are additionally described in, for example, Chen et al., Nature, 493:561-564 (2013); Hu et al, Cell, 155:1545-1555 (2013), the contents of which are hereby incorporated by reference in their entirety.

Dominant Negative Tet2 binding partners

Without being bound by theory, it is believed that Tet2 interacts, e.g., binds, with one or more HDAC, e.g., one or more HDAC expressed in immune effector cells, e.g., in T cells, and that such Tet2:HDAC complexes may contribute to Tet2 activity in the cell. In embodiments, a Tet2 inhibitor of the invention is a dominant negative Tet2 binding partner, e.g., a dominant negative Tet2-binding HDAC. In other embodiments, a Tet2 inhibitor of the invention comprises nucleic acid encoding a dominant negative Tet2 binding partner, e.g., a dominant negative Tet2-binding HDAC.

Vectors

As described herein, the invention provides vectors, e.g., as described herein, which encode modulators (e.g., inhibitors) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, such as the gene editing systems, shRNA or siRNA inhibitors, small molecule, peptide, or protein modulators (e.g., inhibitors) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene (e.g., as described herein).

In embodiments further comprising, for example, a CAR, the nucleic acid may further comprise sequence encoding a CAR, e.g., as described herein. In some embodiments, the invention provides a vector comprising a nucleic acid sequence encoding an inhibitor of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, described herein and comprising a nucleic acid sequence encoding a CAR molecule described herein. In
 5 embodiments, nucleic acid sequences are disposed on separate vectors. In other embodiments, the two or more nucleic acid sequences are encoded by a single nucleic molecule in the same frame and as a single polypeptide chain. In this aspect, the two or more CARs can, e.g., be separated by one or more peptide cleavage sites (e.g., an auto-cleavage site or a substrate for an intracellular protease).

10 Examples of peptide cleavage sites include the following, wherein the GSG residues are optional:

T2A: (GSG) E G R G S L L T C G D V E E N P G P (SEQ ID NO: 68)

P2A: (GSG) A T N F S L L K Q A G D V E E N P G P (SEQ ID NO: 69)

E2A: (GSG) Q C T N Y A L L K L A G D V E S N P G P (SEQ ID NO: 70)

F2A: (GSG) V K Q T L N F D L L K L A G D V E S N P G P (SEQ ID NO: 71).

15 These peptide cleavage sites are referred to collectively herein as “2A sites.” In embodiments, the vector comprises nucleic acid sequence encoding a CAR described herein and nucleic acid sequence encoding a shRNA or siRNA inhibitor of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, described herein. In
 20 embodiments, the vector comprises nucleic acid sequence encoding a CAR described herein and nucleic acid sequence encoding a genome editing system (e.g., a CRISPR/Cas system) modulator (e.g., inhibitor) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, described herein.

Methods of Use of Modulators

25 The invention provides methods of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell expressing a CAR as described herein, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of altering expression and/or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene.

30 In certain embodiments, the method comprises reducing or eliminating expression and/or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In other embodiments, the method comprises increasing or activating expression and/or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In some embodiments, the method comprises contacting
 35 said cells with a modulator (e.g., an inhibitor) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, as described herein. In some embodiments, the method comprises decreasing the level of 5-hydroxymethylcytosine in said cell.

The invention further provides methods of manufacturing a CAR-expressing cell, e.g., a CAR-expressing cell having improved function (e.g., having improved efficacy, e.g., tumor targeting, or proliferation) comprising the step of altering (e.g., reducing or eliminating, or increasing or activating) the expression or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, in said cell. In embodiments, the method comprises contacting said cells with a modulator (e.g., an inhibitor or activator) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, as described herein. In some embodiments, the contacting is done *ex vivo*. In some embodiments, the contacting is done *in vivo*. In some embodiments, the contacting is done prior to, simultaneously with, or after said cells are modified to express a CAR, e.g., a CAR as described herein.

In embodiments, the invention provides a method for altering (e.g., inhibiting or activating) expression and/or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, in a CAR-expressing cell, e.g., a cell expressing a CAR as described herein, e.g., a CAR19-expressing cell (e.g., CTL019- or CTL119-expressing cell), the method comprising a step of altering (e.g., reducing or eliminating, or increasing or activating) expression and/or function of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In embodiments, the method comprises contacting said cells with a modulator (e.g., an inhibitor or activator) of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, as described herein. In some embodiments, the method comprises decreasing the level of 5-hydroxymethylcytosine in said cell.

In one embodiment, the invention provides a method, e.g., a method described above, comprises introducing nucleic acid encoding a CAR into a cell, e.g., an immune effector cell, e.g., a T cell, at a site within a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, or its regulatory elements, such that expression of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, is disrupted. Integration at a site within a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene may be accomplished, for example, using a gene editing system targeting a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, as described above.

In one embodiment, the invention provides a method, e.g., a method described above, comprising a step of introducing into the cell a gene editing system, e.g., a CRISPR/Cas gene editing system which targets a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene, e.g., a CRISPR/Cas system comprising a gRNA which has a targeting sequence complementary to a target sequence of a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In embodiments, the CRISPR/Cas system is introduced into said cell as a ribonuclear protein complex of gRNA and Cas enzyme, e.g., is introduced via electroporation. In one embodiment, the method comprises

introducing nucleic acid encoding one or more of the components of the CRISPR/Cas system into said cell. In one embodiment, said nucleic acid is disposed on the vector encoding a CAR, e.g., a CAR as described herein.

In one embodiment, the invention provides a method, e.g., a method described above,
 5 comprising a step of introducing into the cell an inhibitory dsRNA, e.g., a shRNA or siRNA, which targets a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In one embodiment, the method comprises introducing into said cell nucleic acid encoding an inhibitory dsRNA, e.g., a shRNA or siRNA, which targets a Tet-associated gene (e.g., a Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) gene. In one
 10 embodiment, said nucleic acid is disposed on the vector encoding a CAR, e.g., a CAR as described herein.

Additional componentents of CARs and CAR T cells, and methods pertaining to the invention are described below.

15 Provided herein are compositions of matter and methods of use for the treatment of a disease such as cancer using immune effector cells (e.g., T cells, NK cells) engineered with CARs of the invention.

In one aspect, the invention provides a number of chimeric antigen receptors (CAR) comprising an antigen binding domain (e.g., antibody or antibody fragment, TCR or TCR fragment)
 20 engineered for specific binding to a tumor antigen, e.g., a tumor antigen described herein. In one aspect, the invention provides an immune effector cell (e.g., T cell, NK cell) engineered to express a CAR, wherein the engineered immune effector cell exhibits an anticancer property. In one aspect, a cell is transformed with the CAR and the CAR is expressed on the cell surface. In some embodiments, the cell (e.g., T cell, NK cell) is transduced with a viral vector encoding a CAR. In some
 25 embodiments, the viral vector is a retroviral vector. In some embodiments, the viral vector is a lentiviral vector. In some such embodiments, the cell may stably express the CAR. In another embodiment, the cell (e.g., T cell, NK cell) is transfected with a nucleic acid, e.g., mRNA, cDNA, DNA, encoding a CAR. In some such embodiments, the cell may transiently express the CAR.

In one aspect, the antigen binding domain of a CAR described herein is a scFv antibody
 30 fragment. In one aspect, such antibody fragments are functional in that they retain the equivalent binding affinity, e.g., they bind the same antigen with comparable affinity, as the IgG antibody from which it is derived. In other embodiments, the antibody fragment has a lower binding affinity, e.g., it binds the same antigen with a lower binding affinity than the antibody from which it is derived, but is functional in that it provides a biological response described herein. In one embodiment, the CAR
 35 molecule comprises an antibody fragment that has a binding affinity K_D of 10^{-4} M to 10^{-8} M, e.g., 10^{-5} M to 10^{-7} M, e.g., 10^{-6} M or 10^{-7} M, for the target antigen. In one embodiment, the antibody fragment

has a binding affinity that is at least five-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold or 1,000-fold less than a reference antibody, e.g., an antibody described herein.

In one aspect such antibody fragments are functional in that they provide a biological response that can include, but is not limited to, activation of an immune response, inhibition of signal-
5 transduction origination from its target antigen, inhibition of kinase activity, and the like, as will be understood by a skilled artisan.

In one aspect, the antigen binding domain of the CAR is a scFv antibody fragment that is humanized compared to the murine sequence of the scFv from which it is derived.

In one aspect, the antigen binding domain of a CAR of the invention (e.g., a scFv) is encoded
10 by a nucleic acid molecule whose sequence has been codon optimized for expression in a mammalian cell. In one aspect, entire CAR construct of the invention is encoded by a nucleic acid molecule whose entire sequence has been codon optimized for expression in a mammalian cell. Codon optimization refers to the discovery that the frequency of occurrence of synonymous codons (i.e., codons that code for the same amino acid) in coding DNA is biased in different species. Such codon
15 degeneracy allows an identical polypeptide to be encoded by a variety of nucleotide sequences. A variety of codon optimization methods is known in the art, and include, e.g., methods disclosed in at least US Patent Numbers 5,786,464 and 6,114,148.

In one aspect, the CARs of the invention combine an antigen binding domain of a specific antibody with an intracellular signaling molecule. For example, in some aspects, the intracellular
20 signaling molecule includes, but is not limited to, CD3-zeta chain, 4-1BB and CD28 signaling modules and combinations thereof. In one aspect, the antigen binding domain binds to a tumor antigen as described herein.

Furthermore, the present invention provides CARs and CAR-expressing cells and their use in medicaments or methods for treating, among other diseases, cancer or any malignancy or autoimmune
25 diseases involving cells or tissues which express a tumor antigen as described herein.

In one aspect, the CAR of the invention can be used to eradicate a normal cell that express a tumor antigen as described herein, thereby applicable for use as a cellular conditioning therapy prior to cell transplantation. In one aspect, the normal cell that expresses a tumor antigen as described herein is a normal stem cell and the cell transplantation is a stem cell transplantation.

30 In one aspect, the invention provides an immune effector cell (e.g., T cell, NK cell) engineered to express a chimeric antigen receptor (CAR), wherein the engineered immune effector cell exhibits an antitumor property. A preferred antigen is a cancer associated antigen (i.e., tumor antigen) described herein. In one aspect, the antigen binding domain of the CAR comprises a partially humanized antibody fragment. In one aspect, the antigen binding domain of the CAR comprises a
35 partially humanized scFv. Accordingly, the invention provides CARs that comprises a humanized antigen binding domain and is engineered into a cell, e.g., a T cell or a NK cell, and methods of their use for adoptive therapy.

In one aspect, the CARs of the invention comprise at least one intracellular domain selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD27 signal domain, a CD3zeta signal domain, and any combination thereof. In one aspect, the CARs of the invention comprise at least one intracellular signaling domain is from one or more costimulatory molecule(s) other than a CD137 (4-1BB) or CD28.

Sequences of some examples of various components of CARs of the instant invention is listed in Table 1, where aa stands for amino acids, and na stands for nucleic acids that encode the corresponding peptide.

Table 1. Sequences of various components of CAR (aa – amino acids, na – nucleic acids that encodes the corresponding protein)

SEQ ID NO	description	Sequence	Corresp. To huCD19
1	EF-1 promoter	CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGC CCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGA ACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAG TGATGTCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGGA GAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTC GCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTG GTTCCCGCGGGCCTGGCCTCTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGAT CCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT TGCGCTTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAGGCCT GGCCTGGGCGCTGGGGCCCGCGCGTGCGAATCTGGTGGCACC TTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTA AAATTTTGTATGACCTGCTGCGACGCTTTTTTTCTGGCAAGAT AGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCG GTTTTTGGGGCCGCGGGCGGCGACGGGGCCCCGTGCGTCCCAG CGCACATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTG GTGCCTGGCCTCGCGCCCGCGTGTATCGCCCCGCCCTGGGCG GCAAGGCTGGCCCGGTGCGCACCAAGTTGCGTGAGCGGAAAG ATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAG GACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACAC AAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTG ACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGT TCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGG GGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGA CTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA ATTTGCCCTTTTGTAGTTTGGATCTTGTTTCATTCTCAAGCCTC AGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAAGGTGTCGTG A	100
2	Leader (aa)	MALPVTALLLPLALLLHAARP	13
3	Leader (na)	ATGGCCCTGCCTGTGACAGCCCTGCTGCTGCCTCTGGCTCTGC TGCTGCATGCCGCTAGACCC	54
4	CD 8 hinge (aa)	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	14
5	CD8 hinge (na)	ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACC ATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGG CCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACTT CGCCTGTGAT	55
6	Ig4 hinge (aa)	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVV	102

		DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP PVLDSGDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGKM	
7	Ig4 hinge (na)	GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCC GAGTTCCTGGGCGGACCCAGCGTGTTCTGTTCCTGTTCCCCCAAG CCCAAGGACACCCTGATGATCAGCCGGACCCCGAGGTGACC TGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAG TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAG ACCAAGCCCCGGGAGGAGCAGTTCAATAGCACCTACCGGGTG GTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGC AAGGAATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCAG CAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTC GGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGA TGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCT TCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCC AGCCCGAGAACAACTACAAGACCACCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCTGTACAGCCGGCTGACCGTGGACA AGAGCCGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTGA TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGA GCCTGTCCCTGGGCAAGATG	103
8	IgD hinge (aa)	RWPESPKAQASSVPTAQPPQAEGSLAKATTAPATTRNTGRGGEE KKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQDLWLRD KATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNGS QSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQA PVKLSLNLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREV NTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVSH EDSRTLNLNASRSLEVSYVTDH	47
9	IgD hinge (na)	AGGTGGCCCGAAAGTCCCAAGGCCAGGCATCTAGTGTTCT ACTGCACAGCCCCAGGCAGAAAGGCAGCCTAGCCAAAGCTACT ACTGCACCTGCCACTACGCGCAATACTGGCCGTGGCGGGGAG GAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGA GGGAGACCAAGACCCCTGAATGTCCATCCCATACCCAGCCGC TGGGCGTCTATCTCTTGACTCCCGCAGTACAGGACTTGTGGCT TAGAGATAAGGCCACCTTTACATGTTTCGTCTGTTGGGCTCTGAC CTGAAGGATGCCCATTTGACTTGGGAGGTTGCCGGAAAGGTA CCCACAGGGGGGGTGTGAGGAAGGGTGTGCTGGAGCGCCATTCC AATGGCTCTCAGAGCCAGCACTCAAGACTCACCTTCCGAGA TCCCTGTGGAACGCCGGGACCTCTGTCACATGTACTCTAAATC ATCCTAGCCTGCCCCACAGCGTCTGATGGCCCTTAGAGAGC CAGCCGCCAGGCACCAGTTAAGCTTAGCCTGAATCTGCTCG CCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCG AAGTGTCCGGCTTTAGCCCGCCCAACATCTTGCTCATGTGGCT GGAGGACCAGCGAGAAGTGAACACCAGCGGCTTCGCTCCAG CCCGGCCCCACCCAGCCGGGTCTACCACATTCTGGGCCT GGAGTGTCTTAAGGGTCCCAGCACCACTAGCCCCCAGCCAG CCACATACACCTGTGTTGTGTCCCATGAAGATAGCAGGACCC TGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCTACGTGACTGA CCATT	48
10	GS hinge/linker (aa)	GGGGS GGGGS	49
11	GS hinge/linker (na)	GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC	50
12	CD8TM (aa)	IYIWAPLAGTCGVLLLSLVITLYC	15
13	CD8 TM (na)	ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTC TCCTGTCACTGGTTATCACCTTTACTGC	56
14	4-1BB	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	16

	intracellular domain (aa)		
15	4-1BB intracellular domain (na)	AAACGGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCA TTTATGAGACCAGTACAACTACTCAAGAGGAAGATGGCTGT AGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACT G	60
16	CD27 (aa)	QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPA CSP	51
17	CD27 (na)	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAA CATGACTCCCCGCCGCCCGGGCCCCACCCGCAAGCATTACCA GCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC	52
18	CD3-zeta (aa)	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	17
19	CD3-zeta (na)	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAA GCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACG AAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGG ACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAG GAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGA GGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGG GCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCA CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCC CTCGC	101
20	CD3-zeta (aa)	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR	43
21	CD3-zeta (na)	AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCA GCAGGGCCAG AACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGA GTACGATGTTT TGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAG CCGAGAAGGA AGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGAT AAGATGGCGG AGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGG GGCAAGGGGC ACGATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACA CCTACGACGC CCTTCACATGCAGGCCCTGCCCCCTCGC	44
22	linker	GGGGS	18
23	linker	GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC	50
24	PD-1 extracellular domain (aa)	Pgwfldspdrpwnpptfspallvvtgednatftcsfntsesevlnwyrmspsnqtdklaafpedr sqpgqdcfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptah ppsprpagqfqlv	
25	PD-1 extracellular domain (na)	Cccggatggtttctggactctccggatgccccgtggaatccccaaaccttctcaccggcactcttggtt gtgactgagggcgataatgcgaccttcacgtgctcgttccaacacctccgaatcattcgtgctgaact ggtaccgcatgagcccgtaaacagaccgacaagctcgccgcgtttccggaagatcggtcgcaac cgggacaggattgtcggttcgcgtgactcaactgccgaatggcagagacttcacatgagcgtggt ccgcgctagggcgaacgactccgggacctacctgtgcggagccatctcgttggcgccaaaggccca aatcaaagagagcttgagggccgaactgagagtgaccgagcgagagctgaggtgccaactgcac atccatccccatcgctcggcctgcggggcagttcagaccctggtc	
26	PD-1 CAR (aa) with signal	Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtgednatftcsfntsesevlnw yrmspsnqtdklaafpedrsqpgqdcfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqi keslraelrvterraevptahpspsprpagqfqlvtttpprptpaptiasqplsrpeacrpaagga vhtgrldfacdiyiwaplagtcgvllslvitlyckrgrklliyfkqpfmrpvqttqeedgcsrfpe eeeggcelrvkfsrsadapaykqqnqlynelnlgrreeydvldkrrgrdpemggkprkrnpqeg lynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdttydalhmqalppr	
27	PD-1 CAR (na)	Atggccctcctgtcactgccctgcttccccctcgactcctgtccacgccgctagaccaccgg atggtttctggactctccggatgccccgtggaatccccaaaccttctcaccggcactcttggttgact	

[illegible]

[illegible]

[illegible]

Cancer Associated Antigens

The present invention provides immune effector cells (e.g., T cells, NK cells) that are engineered to contain one or more CARs that direct the immune effector cells to cancer. This is achieved through an antigen binding domain on the CAR that is specific for a cancer associated antigen. There are two classes of cancer associated antigens (tumor antigens) that can be targeted by the CARs of the instant invention: (1) cancer associated antigens that are expressed on the surface of cancer cells; and (2) cancer associated antigens that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells by MHC (major histocompatibility complex).

Accordingly, the present invention provides CARs that target the following cancer associated antigens (tumor antigens): CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1 (CLECL1), CD33, EGFRvIII, GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, VEGFR2, LewisY, CD24, PDGFR-beta, PRSS21, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, legumain, HPV E6,E7, MAGE-A1, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostatein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, and IGLL1.

Tumor-Supporting Antigens

A CAR described herein can comprise an antigen binding domain (e.g., antibody or antibody fragment, TCR or TCR fragment) that binds to a tumor-supporting antigen (e.g., a tumor-supporting antigen as described herein). In some embodiments, the tumor-supporting antigen is an antigen present on a stromal cell or a myeloid-derived suppressor cell (MDSC). Stromal cells can secrete growth factors to promote cell division in the microenvironment. MDSC cells can inhibit T cell proliferation and activation. Without wishing to be bound by theory, in some embodiments, the CAR-expressing cells destroy the tumor-supporting cells, thereby indirectly inhibiting tumor growth or survival.

In embodiments, the stromal cell antigen is chosen from one or more of: bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) and tenascin. In an embodiment, the FAP-specific antibody is, competes for binding with, or has the same CDRs as, sibrotuzumab. In embodiments, the MDSC antigen is chosen from one or more of: CD33, CD11b, C14, CD15, and CD66b. Accordingly, in some embodiments, the tumor-supporting antigen is chosen from one or more of: bone marrow stromal cell antigen 2 (BST2), fibroblast activation protein (FAP) or tenascin, CD33, CD11b, C14, CD15, and CD66b.

Chimeric Antigen Receptor (CAR)

The present invention encompasses a recombinant DNA construct comprising sequences encoding a CAR, wherein the CAR comprises an antigen binding domain (e.g., antibody or antibody fragment, TCR or TCR fragment) that binds specifically to a cancer associated antigen described herein, wherein the sequence of the antigen binding domain is contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain. The intracellular signaling domain can comprise a costimulatory signaling domain and/or a primary signaling domain, e.g., a zeta chain. The costimulatory signaling domain refers to a portion of the CAR comprising at least a portion of the intracellular domain of a costimulatory molecule.

In specific aspects, a CAR construct of the invention comprises a scFv domain, wherein the scFv may be preceded by an optional leader sequence such as provided in SEQ ID NO: 2, and followed by an optional hinge sequence such as provided in SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:10, a transmembrane region such as provided in SEQ ID NO:12, an intracellular signalling domain that includes SEQ ID NO:14 or SEQ ID NO:16 and a CD3 zeta sequence that includes SEQ ID NO:18 or SEQ ID NO:20, e.g., wherein the domains are contiguous with and in the same reading frame to form a single fusion protein.

In one aspect, an exemplary CAR constructs comprise an optional leader sequence (e.g., a leader sequence described herein), an extracellular antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), and an intracellular stimulatory domain (e.g., an intracellular stimulatory domain described herein). In one aspect, an exemplary CAR construct comprises an optional leader sequence (e.g., a leader sequence described herein), an extracellular antigen binding domain (e.g., an antigen binding domain described herein), a hinge (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein), an intracellular costimulatory signaling domain (e.g., a costimulatory signaling domain described herein) and/or an intracellular primary signaling domain (e.g., a primary signaling domain described herein).

An exemplary leader sequence is provided as SEQ ID NO: 2. An exemplary hinge/spacer sequence is provided as SEQ ID NO: 4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:10. An

exemplary transmembrane domain sequence is provided as SEQ ID NO:12. An exemplary sequence of the intracellular signaling domain of the 4-1BB protein is provided as SEQ ID NO: 14. An exemplary sequence of the intracellular signaling domain of CD27 is provided as SEQ ID NO:16. An exemplary CD3zeta domain sequence is provided as SEQ ID NO: 18 or SEQ ID NO:20.

5 In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises the nucleic acid sequence encoding an antigen binding domain, e.g., described herein, that is contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain.

10 In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding an antigen binding domain, wherein the sequence is contiguous with and in the same reading frame as the nucleic acid sequence encoding an intracellular signaling domain. An exemplary intracellular signaling domain that can be used in the CAR includes, but is not
15 limited to, one or more intracellular signaling domains of, e.g., CD3-zeta, CD28, CD27, 4-1BB, and the like. In some instances, the CAR can comprise any combination of CD3-zeta, CD28, 4-1BB, and the like.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells
20 expressing the nucleic acid molecule, by deriving the nucleic acid molecule from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the nucleic acid of interest can be produced synthetically, rather than cloned.

The present invention includes retroviral and lentiviral vector constructs expressing a CAR
25 that can be directly transduced into a cell.

The present invention also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves *in vitro* transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR") (e.g., a 3' and/or 5' UTR described herein), a 5' cap (e.g., a 5' cap described herein) and/or Internal Ribosome Entry Site (IRES) (e.g., an IRES
30 described herein), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:32). RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the CAR. In an embodiment, an RNA CAR vector is transduced into a cell, e.g., a T cell or a NK cell, by electroporation.

35

Antigen Binding Domain

In one aspect, the CAR of the invention comprises a target-specific binding element otherwise referred to as an antigen binding domain. The choice of moiety depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be
 5 chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus, examples of cell surface markers that may act as ligands for the antigen binding domain in a CAR of the invention include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

In one aspect, the CAR-mediated T-cell response can be directed to an antigen of interest by
 10 way of engineering an antigen binding domain that specifically binds a desired antigen into the CAR.

In one aspect, the portion of the CAR comprising the antigen binding domain comprises an antigen binding domain that targets a tumor antigen, e.g., a tumor antigen described herein.

The antigen binding domain can be any domain that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a
 15 humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, a T cell receptor (TCR), or a fragment thereof, e.g., single chain TCR, and the like. In some instances, it is beneficial
 20 for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

In one embodiment, the CD19 CAR is a CD19 CAR described in US Pat. No. 8,399,645; US
 25 Pat. No. 7,446,190; Xu et al., Leuk Lymphoma. 2013 54(2):255-260(2012); Cruz et al., Blood 122(17):2965-2973 (2013); Brentjens et al., Blood, 118(18):4817-4828 (2011); Kochenderfer et al., Blood 116(20):4099-102 (2010); Kochenderfer et al., Blood 122 (25):4129-39(2013); or 16th Annu Meet Am Soc Gen Cell Ther (ASGCT) (May 15-18, Salt Lake City) 2013, Abst 10 (each of which is herein incorporated by reference in their entirety). In one embodiment, an antigen binding domain
 30 against CD19 is an antigen binding portion, e.g., CDRs, of a CAR, antibody or antigen-binding fragment thereof described in, e.g., PCT publication WO2012/079000 (incorporated herein by reference in its entirety). In one embodiment, an antigen binding domain against CD19 is an antigen binding portion, e.g., CDRs, of a CAR, antibody or antigen-binding fragment thereof described in, e.g., PCT publication WO2014/153270; Kochenderfer, J.N. et al., J. Immunother. 32 (7), 689-702
 35 (2009); Kochenderfer, J.N., et al., Blood, 116 (20), 4099-4102 (2010); PCT publication WO2014/031687; Bejcek, Cancer Research, 55, 2346-2351, 1995; or U.S. Patent No. 7,446,190 (each of which is herein incorporated by reference in their entirety).

In one embodiment, the antigen binding domain against mesothelin is or may be derived from an antigen binding domain, e.g., CDRs, scFv, or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2015/090230 (In one embodiment the CAR is a CAR described in WO2015/090230, the contents of which are incorporated herein in their entirety). In embodiments, the antigen binding domain against mesothelin is or is derived from an antigen binding portion, e.g., CDRs, scFv, or VH and VL, of an antibody, antigen-binding fragment, or CAR described in, e.g., PCT publication WO1997/025068, WO1999/028471, WO2005/014652, WO2006/099141, WO2009/045957, WO2009/068204, WO2013/142034, WO2013/040557, or WO2013/063419 (each of which is herein incorporated by reference in their entirety).

In one embodiment, an antigen binding domain against CD123 is or is derived from an antigen binding portion, e.g., CDRs, scFv or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2014/130635 (incorporated herein by reference in its entirety). In one embodiment, an antigen binding domain against CD123 is or is derived from an antigen binding portion, e.g., CDRs, scFv or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2016/028896 (incorporated herein by reference in its entirety); in embodiments, the CAR is a CAR described in WO2016/028896. In one embodiment, an antigen binding domain against CD123 is or is derived from an antigen binding portion, e.g., CDRs, scFv, or VL and VH, of an antibody, antigen-binding fragment, or CAR described in, e.g., PCT publication WO1997/024373, WO2008/127735 (e.g., a CD123 binding domain of 26292, 32701, 37716 or 32703), WO2014/138805 (e.g., a CD123 binding domain of CSL362), WO2014/138819, WO2013/173820, WO2014/144622, WO2001/66139, WO2010/126066 (e.g., the CD123 binding domain of any of Old4, Old5, Old17, Old19, New102, or Old6), WO2014/144622, or US2009/0252742 (each of which is incorporated herein by reference in its entirety).

In one embodiment, an antigen binding domain against CD22 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Haso et al., Blood, 121(7): 1165-1174 (2013); Wayne et al., Clin Cancer Res 16(6): 1894-1903 (2010); Kato et al., Leuk Res 37(1):83-88 (2013); Creative BioMart (creativebiomart.net): MOM-18047-S(P).

In one embodiment, an antigen binding domain against CS-1 is an antigen binding portion, e.g., CDRs, of Elotuzumab (BMS), see e.g., Tai et al., 2008, Blood 112(4):1329-37; Tai et al., 2007, Blood. 110(5):1656-63.

In one embodiment, an antigen binding domain against CLL-1 is an antigen binding portion, e.g., CDRs or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2016/014535, the contents of which are incorporated herein in their entirety. In one embodiment, an antigen binding domain against CLL-1 is an antigen binding portion, e.g., CDRs, of an antibody available from R&D, ebiosciences, Abcam, for example, PE-CLL1-hu Cat# 353604 (BioLegend); and PE-CLL1 (CLEC12A) Cat# 562566 (BD).

In one embodiment, an antigen binding domain against CD33 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Bross et al., Clin Cancer Res 7(6):1490-1496 (2001) (Gemtuzumab Ozogamicin, hP67.6), Caron et al., Cancer Res 52(24):6761-6767 (1992) (Lintuzumab, HuM195), Lapusan et al., Invest New Drugs 30(3):1121-1131 (2012) (AVE9633), Aigner et al.,
 5 Leukemia 27(5): 1107-1115 (2013) (AMG330, CD33 BiTE), Dutour et al., Adv hematol 2012:683065 (2012), and Pizzitola et al., Leukemia doi:10.1038/Lue.2014.62 (2014). Exemplary CAR molecules that target CD33 are described herein, and are provided in WO2016/014576, e.g., in Table 2 of WO2016/014576 (incorporated by reference in its entirety).

In one embodiment, an antigen binding domain against GD2 is an antigen binding portion,
 10 e.g., CDRs, of an antibody described in, e.g., Mujoo et al., Cancer Res. 47(4):1098-1104 (1987); Cheung et al., Cancer Res 45(6):2642-2649 (1985), Cheung et al., J Clin Oncol 5(9):1430-1440 (1987), Cheung et al., J Clin Oncol 16(9):3053-3060 (1998), Handgretinger et al., Cancer Immunol Immunother 35(3):199-204 (1992). In some embodiments, an antigen binding domain against GD2 is an antigen binding portion of an antibody selected from mAb 14.18, 14G2a, ch14.18, hu14.18, 3F8,
 15 hu3F8, 3G6, 8B6, 60C3, 10B8, ME36.1, and 8H9, see e.g., WO2012033885, WO2013040371, WO2013192294, WO2013061273, WO2013123061, WO2013074916, and WO201385552. In some embodiments, an antigen binding domain against GD2 is an antigen binding portion of an antibody described in US Publication No.: 20100150910 or PCT Publication No.: WO 2011160119.

In one embodiment, an antigen binding domain against BCMA is an antigen binding portion,
 20 e.g., CDRs, of an antibody described in, e.g., WO2012163805, WO200112812, and WO2003062401. In embodiments, additional exemplary BCMA CAR constructs are generated using an antigen binding domain, e.g., CDRs, scFv, or VH and VL sequences from PCT Publication WO2012/0163805 (the contents of which are hereby incorporated by reference in its entirety). In embodiments, additional exemplary BCMA CAR constructs are generated using an antigen binding domain, e.g., CDRs, scFv,
 25 or VH and VL sequences from PCT Publication WO2016/014565 (the contents of which are hereby incorporated by reference in its entirety). In embodiments, additional exemplary BCMA CAR constructs are generated using an antigen binding domain, e.g., CDRs, scFv, or VH and VL sequences from PCT Publication WO2014/122144 (the contents of which are hereby incorporated by reference in its entirety). In embodiments, additional exemplary BCMA CAR constructs are generated using
 30 the CAR molecules, and/or the BCMA binding domains (e.g., CDRs, scFv, or VH and VL sequences) from PCT Publication WO2016/014789 (the contents of which are hereby incorporated by reference in its entirety). In embodiments, additional exemplary BCMA CAR constructs are generated using the CAR molecules, and/or the BCMA binding domains (e.g., CDRs, scFv, or VH and VL sequences)
 35 in its entirety). In embodiments, additional exemplary BCMA CAR constructs are generated using the CAR molecules, and/or the BCMA binding domains (e.g., CDRs, scFv, or VH and VL sequences)

from PCT Publication WO2014/140248 (the contents of which are hereby incorporated by reference in its entirety).

In one embodiment, an antigen binding domain against Tn antigen is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US 2014/0178365, US8,440,798, Brooks et al.,
 5 PNAS 107(22):10056-10061 (2010), and Stone et al., OncoImmunology 1(6):863-873(2012).

In one embodiment, an antigen binding domain against PSMA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Parker et al., Protein Expr Purif 89(2):136-145 (2013), US 20110268656 (J591 ScFv); Frigerio et al, European J Cancer 49(9):2223-2232 (2013) (scFvD2B); WO 2006125481 (mAbs 3/A12, 3/E7 and 3/F11) and single chain antibody fragments (scFv A5 and
 10 D7).

In one embodiment, an antigen binding domain against ROR1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Hudecek et al., Clin Cancer Res 19(12):3153-3164 (2013); WO 2011159847; and US20130101607.

In one embodiment, an antigen binding domain against FLT3 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., WO2011076922, US5777084, EP0754230, US20090297529, and several commercial catalog antibodies (R&D, ebiosciences, Abcam).
 15

In one embodiment, an antigen binding domain against TAG72 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Hombach et al., Gastroenterology 113(4):1163-1170 (1997); and Abcam ab691.

20 In one embodiment, an antigen binding domain against FAP is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Ostermann et al., Clinical Cancer Research 14:4584-4592 (2008) (FAP5), US Pat. Publication No. 2009/0304718; sibrotuzumab (see e.g., Hofheinz et al., Oncology Research and Treatment 26(1), 2003); and Tran et al., J Exp Med 210(6):1125-1135 (2013).

In one embodiment, an antigen binding domain against CD38 is an antigen binding portion, e.g., CDRs, of daratumumab (see, e.g., Groen et al., Blood 116(21):1261-1262 (2010); MOR202 (see, e.g., US8,263,746); or antibodies described in US8,362,211.
 25

In one embodiment, an antigen binding domain against CD44v6 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Casucci et al., Blood 122(20):3461-3472 (2013).

In one embodiment, an antigen binding domain against CEA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Chmielewski et al., Gastroenterology 143(4):1095-1107 (2012).
 30

In one embodiment, an antigen binding domain against EPCAM is an antigen binding portion, e.g., CDRS, of an antibody selected from MT110, EpCAM-CD3 bispecific Ab (see, e.g., clinicaltrials.gov/ct2/show/NCT00635596); Edrecolomab; 3622W94; ING-1; and adecatumumab
 35 (MT201).

In one embodiment, an antigen binding domain against PRSS21 is an antigen binding portion, e.g., CDRs, of an antibody described in US Patent No.: 8,080,650.

In one embodiment, an antigen binding domain against B7H3 is an antigen binding portion, e.g., CDRs, of an antibody MGA271 (Macrogenics).

In one embodiment, an antigen binding domain against KIT is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US7915391, US20120288506 , and several commercial
5 catalog antibodies.

In one embodiment, an antigen binding domain against IL-13Ra2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., WO2008/146911, WO2004087758, several commercial catalog antibodies, and WO2004087758.

In one embodiment, an antigen binding domain against CD30 is an antigen binding portion,
10 e.g., CDRs, of an antibody described in, e.g., US7090843 B1, and EP0805871.

In one embodiment, an antigen binding domain against GD3 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US7253263; US 8,207,308; US 20120276046; EP1013761; WO2005035577; and US6437098.

In one embodiment, an antigen binding domain against CD171 is an antigen binding portion,
15 e.g., CDRs, of an antibody described in, e.g., Hong et al., J Immunother 37(2):93-104 (2014).

In one embodiment, an antigen binding domain against IL-11Ra is an antigen binding portion, e.g., CDRs, of an antibody available from Abcam (cat# ab55262) or Novus Biologicals (cat# EPR5446). In another embodiment, an antigen binding domain against IL-11Ra is a peptide, see, e.g., Huang et al., Cancer Res 72(1):271-281 (2012).

20 In one embodiment, an antigen binding domain against PSCA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Morgenroth et al., Prostate 67(10):1121-1131 (2007) (scFv 7F5); Nejatollahi et al., J of Oncology 2013(2013), article ID 839831 (scFv C5-II); and US Pat Publication No. 20090311181.

In one embodiment, an antigen binding domain against VEGFR2 is an antigen binding
25 portion, e.g., CDRs, of an antibody described in, e.g., Chinnasamy et al., J Clin Invest 120(11):3953-3968 (2010).

In one embodiment, an antigen binding domain against LewisY is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Kelly et al., Cancer Biother Radiopharm 23(4):411-423 (2008) (hu3S193 Ab (scFvs)); Dolezal et al., Protein Engineering 16(1):47-56 (2003) (NC10 scFv).

30 In one embodiment, an antigen binding domain against CD24 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Maliar et al., Gastroenterology 143(5):1375-1384 (2012).

In one embodiment, an antigen binding domain against PDGFR-beta is an antigen binding portion, e.g., CDRs, of an antibody Abcam ab32570.

35 In one embodiment, an antigen binding domain against SSEA-4 is an antigen binding portion, e.g., CDRs, of antibody MC813 (Cell Signaling), or other commercially available antibodies.

In one embodiment, an antigen binding domain against CD20 is an antigen binding portion, e.g., CDRs, of the antibody Rituximab, Ofatumumab, Ocrelizumab, Veltuzumab, or GA101.

In one embodiment, an antigen binding domain against Folate receptor alpha is an antigen binding portion, e.g., CDRs, of the antibody IMGN853, or an antibody described in US20120009181;
5 US4851332, LK26: US5952484.

In one embodiment, an antigen binding domain against ERBB2 (Her2/neu) is an antigen binding portion, e.g., CDRs, of the antibody trastuzumab, or pertuzumab.

In one embodiment, an antigen binding domain against MUC1 is an antigen binding portion, e.g., CDRs, of the antibody SAR566658.

10 In one embodiment, the antigen binding domain against EGFR is antigen binding portion, e.g., CDRs, of the antibody cetuximab, panitumumab, zalutumumab, nimotuzumab, or matuzumab. In one embodiment, the antigen binding domain against EGFRvIII is or may be derived from an antigen binding domain, e.g., CDRs, scFv, or VH and VL, of an antibody, antigen-binding fragment or CAR described in, e.g., PCT publication WO2014/130657 (In one embodiment the CAR is a CAR
15 described in WO2014/130657, the contents of which are incorporated herein in their entirety).

In one embodiment, an antigen binding domain against NCAM is an antigen binding portion, e.g., CDRs, of the antibody clone 2-2B: MAB5324 (EMD Millipore)

In one embodiment, an antigen binding domain against Ephrin B2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Abengozar et al., Blood 119(19):4565-4576
20 (2012).

In one embodiment, an antigen binding domain against IGF-I receptor is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US8344112 B2; EP2322550 A1; WO 2006/138315, or PCT/US2006/022995.

In one embodiment, an antigen binding domain against CAIX is an antigen binding portion, e.g., CDRs, of the antibody clone 303123 (R&D Systems).
25

In one embodiment, an antigen binding domain against LMP2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US7,410,640, or US20050129701.

In one embodiment, an antigen binding domain against gp100 is an antigen binding portion, e.g., CDRs, of the antibody HMB45, NK1betaB, or an antibody described in WO2013165940, or
30 US20130295007.

In one embodiment, an antigen binding domain against tyrosinase is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US5843674; or US19950504048.

In one embodiment, an antigen binding domain against EphA2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Yu et al., Mol Ther 22(1):102-111 (2014).

35 In one embodiment, an antigen binding domain against GD3 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US7253263; US 8,207,308; US 20120276046; EP1013761 A3; 20120276046; WO2005035577; or US6437098.

In one embodiment, an antigen binding domain against fucosyl GM1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., US20100297138; or WO2007/067992.

In one embodiment, an antigen binding domain against sLe is an antigen binding portion, e.g., CDRs, of the antibody G193 (for lewis Y), see Scott AM et al, Cancer Res 60: 3254-61 (2000), also
5 as described in Neeson et al, J Immunol May 2013 190 (Meeting Abstract Supplement) 177.10.

In one embodiment, an antigen binding domain against GM3 is an antigen binding portion, e.g., CDRs, of the antibody CA 2523449 (mAb 14F7).

In one embodiment, an antigen binding domain against HMWMAA is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Kmiecik et al., Oncoimmunology 3(1):e27185
10 (2014) (PMID: 24575382) (mAb9.2.27); US6528481; WO2010033866; or US 20140004124.

In one embodiment, an antigen binding domain against o-acetyl-GD2 is an antigen binding portion, e.g., CDRs, of the antibody 8B6.

In one embodiment, an antigen binding domain against TEM1/CD248 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Marty et al., Cancer Lett 235(2):298-308
15 (2006); Zhao et al., J Immunol Methods 363(2):221-232 (2011).

In one embodiment, an antigen binding domain against CLDN6 is an antigen binding portion, e.g., CDRs, of the antibody IMAB027 (Ganymed Pharmaceuticals), see e.g., clinicaltrials.gov/show/NCT02054351.

In one embodiment, an antigen binding domain against TSHR is an antigen binding portion,
20 e.g., CDRs, of an antibody described in, e.g., US8,603,466; US8,501,415; or US8,309,693.

In one embodiment, an antigen binding domain against GPRC5D is an antigen binding portion, e.g., CDRs, of the antibody FAB6300A (R&D Systems); or LS-A4180 (Lifespan Biosciences).

In one embodiment, an antigen binding domain against CD97 is an antigen binding portion,
25 e.g., CDRs, of an antibody described in, e.g., US6,846,911; de Groot et al., J Immunol 183(6):4127-4134 (2009); or an antibody from R&D:MAB3734.

In one embodiment, an antigen binding domain against ALK is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Mino-Kenudson et al., Clin Cancer Res 16(5):1561-1571 (2010).

30 In one embodiment, an antigen binding domain against polysialic acid is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Nagae et al., J Biol Chem 288(47):33784-33796 (2013).

In one embodiment, an antigen binding domain against PLAC1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Ghods et al., Biotechnol Appl Biochem 2013
35 doi:10.1002/bab.1177.

In one embodiment, an antigen binding domain against GloboH is an antigen binding portion of the antibody VK9; or an antibody described in, e.g., Kudryashov V et al, Glycoconj J.15(3):243-9 (

1998), Lou et al., Proc Natl Acad Sci USA 111(7):2482-2487 (2014) ; MBr1: Bremer E-G et al. J Biol Chem 259:14773-14777 (1984).

In one embodiment, an antigen binding domain against NY-BR-1 is an antigen binding portion, e.g., CDRs of an antibody described in, e.g., Jager et al., Appl Immunohistochem Mol Morphol 15(1):77-83 (2007).

In one embodiment, an antigen binding domain against WT-1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Dao et al., Sci Transl Med 5(176):176ra33 (2013); or WO2012/135854.

In one embodiment, an antigen binding domain against MAGE-A1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Willemsen et al., J Immunol 174(12):7853-7858 (2005) (TCR-like scFv).

In one embodiment, an antigen binding domain against sperm protein 17 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Song et al., Target Oncol 2013 Aug 14 (PMID: 23943313); Song et al., Med Oncol 29(4):2923-2931 (2012).

In one embodiment, an antigen binding domain against Tie 2 is an antigen binding portion, e.g., CDRs, of the antibody AB33 (Cell Signaling Technology).

In one embodiment, an antigen binding domain against MAD-CT-2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., PMID: 2450952; US7635753.

In one embodiment, an antigen binding domain against Fos-related antigen 1 is an antigen binding portion, e.g., CDRs, of the antibody 12F9 (Novus Biologicals).

In one embodiment, an antigen binding domain against MelanA/MART1 is an antigen binding portion, e.g., CDRs, of an antibody described in, EP2514766 A2; or US 7,749,719.

In one embodiment, an antigen binding domain against sarcoma translocation breakpoints is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Luo et al, EMBO Mol. Med. 4(6):453-461 (2012).

In one embodiment, an antigen binding domain against TRP-2 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Wang et al, J Exp Med. 184(6):2207-16 (1996).

In one embodiment, an antigen binding domain against CYP1B1 is an antigen binding portion, e.g., CDRs, of an antibody described in, e.g., Maecker et al, Blood 102 (9): 3287-3294 (2003).

In one embodiment, an antigen binding domain against RAGE-1 is an antigen binding portion, e.g., CDRs, of the antibody MAB5328 (EMD Millipore).

In one embodiment, an antigen binding domain against human telomerase reverse transcriptase is an antigen binding portion, e.g., CDRs, of the antibody cat no: LS-B95-100 (Lifespan Biosciences)

In one embodiment, an antigen binding domain against intestinal carboxyl esterase is an antigen binding portion, e.g., CDRs, of the antibody 4F12: cat no: LS-B6190-50 (Lifespan Biosciences).

5 In one embodiment, an antigen binding domain against mut hsp70-2 is an antigen binding portion, e.g., CDRs, of the antibody Lifespan Biosciences: monoclonal: cat no: LS-C133261-100 (Lifespan Biosciences).

In one embodiment, an antigen binding domain against CD79a is an antigen binding portion, e.g., CDRs, of the antibody Anti-CD79a antibody [HM47/A9] (ab3121), available from Abcam; antibody CD79A Antibody #3351 available from Cell Signalling Technology; or antibody
10 HPA017748 - Anti-CD79A antibody produced in rabbit, available from Sigma Aldrich.

In one embodiment, an antigen binding domain against CD79b is an antigen binding portion, e.g., CDRs, of the antibody polatuzumab vedotin, anti-CD79b described in Dornan et al., “Therapeutic potential of an anti-CD79b antibody-drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma” Blood. 2009 Sep 24;114(13):2721-9. doi: 10.1182/blood-2009-
15 02-205500. Epub 2009 Jul 24, or the bispecific antibody Anti-CD79b/CD3 described in “4507 Pre-Clinical Characterization of T Cell-Dependent Bispecific Antibody Anti-CD79b/CD3 As a Potential Therapy for B Cell Malignancies” Abstracts of 56th ASH Annual Meeting and Exposition, San Francisco, CA December 6-9 2014.

In one embodiment, an antigen binding domain against CD72 is an antigen binding portion, e.g., CDRs, of the antibody J3-109 described in Myers, and Uckun, “An anti-CD72 immunotoxin against therapy-refractory B-lineage acute lymphoblastic leukemia.” Leuk Lymphoma. 1995
20 Jun;18(1-2):119-22, or anti-CD72 (10D6.8.1, mIgG1) described in Polson et al., “Antibody-Drug Conjugates for the Treatment of Non-Hodgkin's Lymphoma: Target and Linker-Drug Selection” Cancer Res March 15, 2009 69; 2358.

25 In one embodiment, an antigen binding domain against LAIR1 is an antigen binding portion, e.g., CDRs, of the antibody ANT-301 LAIR1 antibody, available from ProSpec; or anti-human CD305 (LAIR1) Antibody, available from BioLegend.

In one embodiment, an antigen binding domain against FCAR is an antigen binding portion, e.g., CDRs, of the antibody CD89/FCARAntibody (Catalog#10414-H08H), available from Sino
30 Biological Inc.

In one embodiment, an antigen binding domain against LILRA2 is an antigen binding portion, e.g., CDRs, of the antibody LILRA2 monoclonal antibody (M17), clone 3C7, available from Abnova, or Mouse Anti-LILRA2 antibody, Monoclonal (2D7), available from Lifespan Biosciences.

In one embodiment, an antigen binding domain against CD300LF is an antigen binding
35 portion, e.g., CDRs, of the antibody Mouse Anti-CMRF35-like molecule 1 antibody, Monoclonal[UP-D2], available from BioLegend, or Rat Anti-CMRF35-like molecule 1 antibody, Monoclonal[234903], available from R&D Systems.

In one embodiment, an antigen binding domain against CLEC12A is an antigen binding portion, e.g., CDRs, of the antibody Bispecific T cell Engager (BiTE) scFv-antibody and ADC described in Noordhuis et al., “Targeting of CLEC12A In Acute Myeloid Leukemia by Antibody-Drug-Conjugates and Bispecific CLL-1xCD3 BiTE Antibody” 53rd ASH Annual Meeting and Exposition, December 10-13, 2011, and MCLA-117 (Merus).

In one embodiment, an antigen binding domain against BST2 (also called CD317) is an antigen binding portion, e.g., CDRs, of the antibody Mouse Anti-CD317 antibody, Monoclonal[3H4], available from Antibodies-Online or Mouse Anti-CD317 antibody, Monoclonal[696739], available from R&D Systems.

In one embodiment, an antigen binding domain against EMR2 (also called CD312) is an antigen binding portion, e.g., CDRs, of the antibody Mouse Anti-CD312 antibody, Monoclonal[LS-B8033] available from Lifespan Biosciences, or Mouse Anti-CD312 antibody, Monoclonal[494025] available from R&D Systems.

In one embodiment, an antigen binding domain against LY75 is an antigen binding portion, e.g., CDRs, of the antibody Mouse Anti-Lymphocyte antigen 75 antibody, Monoclonal[HD30] available from EMD Millipore or Mouse Anti-Lymphocyte antigen 75 antibody, Monoclonal[A15797] available from Life Technologies.

In one embodiment, an antigen binding domain against GPC3 is an antigen binding portion, e.g., CDRs, of the antibody hGC33 described in Nakano K, Ishiguro T, Konishi H, et al. Generation of a humanized anti-glypican 3 antibody by CDR grafting and stability optimization. Anticancer Drugs. 2010 Nov;21(10):907–916, or MDX-1414, HN3, or YP7, all three of which are described in Feng et al., “Glypican-3 antibodies: a new therapeutic target for liver cancer.” FEBS Lett. 2014 Jan 21;588(2):377-82.

In one embodiment, an antigen binding domain against FCRL5 is an antigen binding portion, e.g., CDRs, of the anti-FcRL5 antibody described in Elkins et al., “FcRL5 as a target of antibody-drug conjugates for the treatment of multiple myeloma” Mol Cancer Ther. 2012 Oct;11(10):2222-32. .

In one embodiment, an antigen binding domain against IGLL1 is an antigen binding portion, e.g., CDRs, of the antibody Mouse Anti-Immunoglobulin lambda-like polypeptide 1 antibody, Monoclonal[AT1G4] available from Lifespan Biosciences, Mouse Anti-Immunoglobulin lambda-like polypeptide 1 antibody, Monoclonal[HSL11] available from BioLegend.

In one embodiment, the antigen binding domain comprises one, two three (e.g., all three) heavy chain CDRs, HC CDR1, HC CDR2 and HC CDR3, from an antibody listed above, and/or one, two, three (e.g., all three) light chain CDRs, LC CDR1, LC CDR2 and LC CDR3, from an antibody listed above. In one embodiment, the antigen binding domain comprises a heavy chain variable region and/or a variable light chain region of an antibody listed above.

In another aspect, the antigen binding domain comprises a humanized antibody or an antibody fragment. In some aspects, a non-human antibody is humanized, where specific sequences or regions

of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. In one aspect, the antigen binding domain is humanized.

A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology*, 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering*, 7(6):805-814; and Roguska et al., 1994, *PNAS*, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., *J. Immunol.*, 169:1119-25 (2002), Caldas et al., *Protein Eng.*, 13(5):353-60 (2000), Morea et al., *Methods*, 20(3):267-79 (2000), Baca et al., *J. Biol. Chem.*, 272(16):10678-84 (1997), Roguska et al., *Protein Eng.*, 9(10):895-904 (1996), Couto et al., *Cancer Res.*, 55 (23 Supp):5973s-5977s (1995), Couto et al., *Cancer Res.*, 55(8):1717-22 (1995), Sandhu J S, *Gene*, 150(2):409-10 (1994), and Pedersen et al., *J. Mol. Biol.*, 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, *Nature*, 332:323, which are incorporated herein by reference in their entireties.)

A humanized antibody or antibody fragment has one or more amino acid residues remaining in it from a source which is nonhuman. These nonhuman amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. As provided herein, humanized antibodies or antibody fragments comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions wherein the amino acid residues comprising the framework are derived completely or mostly from human germline. Multiple techniques for humanization of antibodies or antibody fragments are well-known in the art and can essentially be performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody, i.e., CDR-grafting (EP 239,400; PCT Publication No. WO 91/09967; and U.S. Pat. Nos. 4,816,567; 6,331,415; 5,225,539; 5,530,101; 5,585,089; 6,548,640, the contents of which are incorporated herein by reference herein in their entirety). In such humanized antibodies and antibody fragments,

substantially less than an intact human variable domain has been substituted by the corresponding sequence from a nonhuman species. Humanized antibodies are often human antibodies in which some CDR residues and possibly some framework (FR) residues are substituted by residues from analogous sites in rodent antibodies. Humanization of antibodies and antibody fragments can also be achieved by veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology*, 28(4/5):489-498; Studnicka et al., *Protein Engineering*, 7(6):805-814 (1994); and Roguska et al., *PNAS*, 91:969-973 (1994)) or chain shuffling (U.S. Pat. No. 5,565,332), the contents of which are incorporated herein by reference herein in their entirety.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987), the contents of which are incorporated herein by reference herein in their entirety). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (see, e.g., Nicholson et al. *Mol. Immun.* 34 (16-17): 1157-1165 (1997); Carter et al., *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993), the contents of which are incorporated herein by reference herein in their entirety). In some embodiments, the framework region, e.g., all four framework regions, of the heavy chain variable region are derived from a VH4_4-59 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g., from the amino acid at the corresponding murine sequence. In one embodiment, the framework region, e.g., all four framework regions of the light chain variable region are derived from a VK3_1.25 germline sequence. In one embodiment, the framework region can comprise, one, two, three, four or five modifications, e.g., substitutions, e.g., from the amino acid at the corresponding murine sequence.

In some aspects, the portion of a CAR composition of the invention that comprises an antibody fragment is humanized with retention of high affinity for the target antigen and other favorable biological properties. According to one aspect of the invention, humanized antibodies and antibody fragments are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate

immunoglobulin to bind the target antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody or antibody fragment characteristic, such as increased affinity for the target antigen, is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

5 A humanized antibody or antibody fragment may retain a similar antigenic specificity as the original antibody, e.g., in the present invention, the ability to bind human a cancer associated antigen as described herein. In some embodiments, a humanized antibody or antibody fragment may have improved affinity and/or specificity of binding to human a cancer associated antigen as described herein.

10 In one aspect, the antigen binding domain of the invention is characterized by particular functional features or properties of an antibody or antibody fragment. For example, in one aspect, the portion of a CAR composition of the invention that comprises an antigen binding domain specifically binds a tumor antigen as described herein.

In one aspect, the anti-cancer associated antigen as described herein binding domain is a
15 fragment, e.g., a single chain variable fragment (scFv). In one aspect, the anti- cancer associated antigen as described herein binding domain is a Fv, a Fab, a (Fab')₂, or a bi-functional (e.g. bi-specific) hybrid antibody (e.g., Lanzavecchia et al., Eur. J. Immunol. 17, 105 (1987)). In one aspect, the antibodies and fragments thereof of the invention binds a cancer associated antigen as described herein protein with wild-type or enhanced affinity.

20 In some instances, scFvs can be prepared according to method known in the art (see, for example, Bird et al., (1988) Science 242:423-426 and Huston et al., (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). ScFv molecules can be produced by linking VH and VL regions together using flexible polypeptide linkers. The scFv molecules comprise a linker (e.g., a Ser-Gly linker) with an optimized length and/or amino acid composition. The linker length can greatly affect how the variable
25 regions of a scFv fold and interact. In fact, if a short polypeptide linker is employed (e.g., between 5-10 amino acids) intrachain folding is prevented. Interchain folding is also required to bring the two variable regions together to form a functional epitope binding site. For examples of linker orientation and size see, e.g., Hollinger et al. 1993 Proc Natl Acad. Sci. U.S.A. 90:6444-6448, U.S. Patent Application Publication Nos. 2005/0100543, 2005/0175606, 2007/0014794, and PCT publication
30 Nos. WO2006/020258 and WO2007/024715, is incorporated herein by reference.

An scFv can comprise a linker of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, or more amino acid residues between its VL and VH regions. The linker sequence may comprise any naturally occurring amino acid. In some embodiments, the linker sequence comprises amino acids glycine and serine. In another embodiment, the linker sequence
35 comprises sets of glycine and serine repeats such as (Gly₄Ser)_n, where n is a positive integer equal to or greater than 1 (SEQ ID NO:22). In one embodiment, the linker can be (Gly₄Ser)₄ (SEQ ID NO:29)

or (Gly₄Ser)₃(SEQ ID NO:30). Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies.

In another aspect, the antigen binding domain is a T cell receptor (“TCR”), or a fragment thereof, for example, a single chain TCR (scTCR). Methods to make such TCRs are known in the art. See, e.g., Willemsen RA et al, Gene Therapy 7: 1369–1377 (2000); Zhang T et al, Cancer Gene Ther 11: 487–496 (2004); Aggen et al, Gene Ther. 19(4):365-74 (2012) (references are incorporated herein by its entirety). For example, scTCR can be engineered that contains the V α and V β genes from a T cell clone linked by a linker (e.g., a flexible peptide). This approach is very useful to cancer associated target that itself is intracellular, however, a fragment of such antigen (peptide) is presented on the surface of the cancer cells by MHC.

Bispecific CARs

In an embodiment a multispecific antibody molecule is a bispecific antibody molecule. A bispecific antibody has specificity for no more than two antigens. A bispecific antibody molecule is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment the first and second epitopes are on the same antigen, e.g., the same protein (or subunit of a multimeric protein). In an embodiment the first and second epitopes overlap. In an embodiment the first and second epitopes do not overlap. In an embodiment the first and second epitopes are on different antigens, e.g., different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody molecule comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody having binding specificity for a first epitope and a half antibody having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment a bispecific antibody molecule comprises a scFv, or fragment thereof, have binding specificity for a first epitope and a scFv, or fragment thereof, have binding specificity for a second epitope.

In certain embodiments, the antibody molecule is a multi-specific (e.g., a bispecific or a trispecific) antibody molecule. Protocols for generating bispecific or heterodimeric antibody molecules are known in the art; including but not limited to, for example, the “knob in a hole” approach described in, e.g., US 5731168; the electrostatic steering Fc pairing as described in, e.g., WO 09/089004, WO 06/106905 and WO 2010/129304; Strand Exchange Engineered Domains (SEED) heterodimer formation as described in, e.g., WO 07/110205; Fab arm exchange as described

in, *e.g.*, WO 08/119353, WO 2011/131746, and WO 2013/060867; double antibody conjugate, *e.g.*, by antibody cross-linking to generate a bi-specific structure using a heterobifunctional reagent having an amine-reactive group and a sulfhydryl reactive group as described in, *e.g.*, US 4433059; bispecific antibody determinants generated by recombining half antibodies (heavy-light chain pairs or Fabs)

5 from different antibodies through cycle of reduction and oxidation of disulfide bonds between the two heavy chains, as described in, *e.g.*, US 4444878; trifunctional antibodies, *e.g.*, three Fab' fragments cross-linked through sulfhydryl reactive groups, as described in, *e.g.*, US5273743; biosynthetic binding proteins, *e.g.*, pair of scFvs cross-linked through C-terminal tails preferably through disulfide or amine-reactive chemical cross-linking, as described in, *e.g.*, US5534254; bifunctional antibodies, *e.g.*,

10 Fab fragments with different binding specificities dimerized through leucine zippers (*e.g.*, c-fos and c-jun) that have replaced the constant domain, as described in, *e.g.*, US5582996; bispecific and oligospecific mono-and oligovalent receptors, *e.g.*, VH-CH1 regions of two antibodies (two Fab fragments) linked through a polypeptide spacer between the CH1 region of one antibody and the VH region of the other antibody typically with associated light chains, as described in, *e.g.*, US5591828;

15 bispecific DNA-antibody conjugates, *e.g.*, crosslinking of antibodies or Fab fragments through a double stranded piece of DNA, as described in, *e.g.*, US5635602; bispecific fusion proteins, *e.g.*, an expression construct containing two scFvs with a hydrophilic helical peptide linker between them and a full constant region, as described in, *e.g.*, US5637481; multivalent and multispecific binding proteins, *e.g.*, dimer of polypeptides having first domain with binding region of Ig heavy chain

20 variable region, and second domain with binding region of Ig light chain variable region, generally termed diabodies (higher order structures are also encompassed creating for bispecific, trispecific, or tetraspecific molecules, as described in, *e.g.*, US5837242; minibody constructs with linked VL and VH chains further connected with peptide spacers to an antibody hinge region and CH3 region, which can be dimerized to form bispecific/multivalent molecules, as described in, *e.g.*, US5837821; VH and

25 VL domains linked with a short peptide linker (*e.g.*, 5 or 10 amino acids) or no linker at all in either orientation, which can form dimers to form bispecific diabodies; trimers and tetramers, as described in, *e.g.*, US5844094; String of VH domains (or VL domains in family members) connected by peptide linkages with crosslinkable groups at the C-terminus further associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with

30 both a VH and a VL domain linked through a peptide linker are combined into multivalent structures through non-covalent or chemical crosslinking to form, *e.g.*, homobivalent, heterobivalent, trivalent, and tetravalent structures using both scFV or diabody type format, as described in, *e.g.*, US5869620. Additional exemplary multispecific and bispecific molecules and methods of making the same are found, for example, in US5910573, US5932448, US5959083, US5989830, US6005079, US6239259,

35 US6294353, US6333396, US6476198, US6511663, US6670453, US6743896, US6809185, US6833441, US7129330, US7183076, US7521056, US7527787, US7534866, US7612181, US2002004587A1, US2002076406A1, US2002103345A1, US2003207346A1, US2003211078A1,

US2004219643A1, US2004220388A1, US2004242847A1, US2005003403A1, US2005004352A1, US2005069552A1, US2005079170A1, US2005100543A1, US2005136049A1, US2005136051A1, US2005163782A1, US2005266425A1, US2006083747A1, US2006120960A1, US2006204493A1, US2006263367A1, US2007004909A1, US2007087381A1, US2007128150A1, US2007141049A1, 5 US2007154901A1, US2007274985A1, US2008050370A1, US2008069820A1, US2008152645A1, US2008171855A1, US2008241884A1, US2008254512A1, US2008260738A1, US2009130106A1, US2009148905A1, US2009155275A1, US2009162359A1, US2009162360A1, US2009175851A1, US2009175867A1, US2009232811A1, US2009234105A1, US2009263392A1, US2009274649A1, EP346087A2, WO0006605A2, WO02072635A2, WO04081051A1, WO06020258A2, 10 WO2007044887A2, WO2007095338A2, WO2007137760A2, WO2008119353A1, WO2009021754A2, WO2009068630A1, WO9103493A1, WO9323537A1, WO9409131A1, WO9412625A2, WO9509917A1, WO9637621A2, WO9964460A1. The contents of the above-referenced applications are incorporated herein by reference in their entireties.

Within each antibody or antibody fragment (e.g., scFv) of a bispecific antibody molecule, the 15 VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH₁) upstream of its VL (VL₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL₂) upstream of its VH (VH₂), such that the overall bispecific antibody molecule has the arrangement VH₁-VL₁-VL₂-VH₂. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with 20 its VL (VL₁) upstream of its VH (VH₁) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH₂) upstream of its VL (VL₂), such that the overall bispecific antibody molecule has the arrangement VL₁-VH₁-VH₂-VL₂. Optionally, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), e.g., between VL₁ and VL₂ if the construct is arranged as VH₁-VL₁-VL₂-VH₂, or between VH₁ and VH₂ if the construct is arranged as VL₁-VH₁- 25 VH₂-VL₂. The linker may be a linker as described herein, e.g., a (Gly₄-Ser)_n linker, wherein n is 1, 2, 3, 4, 5, or 6, preferably 4 (SEQ ID NO: 72). In general, the linker between the two scFvs should be long enough to avoid mispairing between the domains of the two scFvs. Optionally, a linker is disposed between the VL and VH of the first scFv. Optionally, a linker is disposed between the VL and VH of the second scFv. In constructs that have multiple linkers, any two or more of the linkers 30 can be the same or different. Accordingly, in some embodiments, a bispecific CAR comprises VLs, VHs, and optionally one or more linkers in an arrangement as described herein.

Stability and Mutations

The stability of an antigen binding domain to a cancer associated antigen as described herein, 35 e.g., scFv molecules (e.g., soluble scFv), can be evaluated in reference to the biophysical properties (e.g., thermal stability) of a conventional control scFv molecule or a full length antibody. In one embodiment, the humanized scFv has a thermal stability that is greater than about 0.1, about 0.25,

about 0.5, about 0.75, about 1, about 1.25, about 1.5, about 1.75, about 2, about 2.5, about 3, about 3.5, about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10 degrees, about 11 degrees, about 12 degrees, about 13 degrees, about 14 degrees, or about 15 degrees Celsius than a control binding molecule (e.g. a conventional scFv molecule) in the described assays.

The improved thermal stability of the antigen binding domain to a cancer associated antigen described herein, e.g., scFv is subsequently conferred to the entire CAR construct, leading to improved therapeutic properties of the CAR construct. The thermal stability of the antigen binding domain of -a cancer associated antigen described herein, e.g., scFv, can be improved by at least about 2°C or 3°C as compared to a conventional antibody. In one embodiment, the antigen binding domain of -a cancer associated antigen described herein, e.g., scFv, has a 1°C improved thermal stability as compared to a conventional antibody. In another embodiment, the antigen binding domain of a cancer associated antigen described herein, e.g., scFv, has a 2°C improved thermal stability as compared to a conventional antibody. In another embodiment, the scFv has a 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15°C improved thermal stability as compared to a conventional antibody. Comparisons can be made, for example, between the scFv molecules disclosed herein and scFv molecules or Fab fragments of an antibody from which the scFv VH and VL were derived. Thermal stability can be measured using methods known in the art. For example, in one embodiment, T_m can be measured. Methods for measuring T_m and other methods of determining protein stability are described in more detail below.

Mutations in scFv (arising through humanization or direct mutagenesis of the soluble scFv) can alter the stability of the scFv and improve the overall stability of the scFv and the CAR construct. Stability of the humanized scFv is compared against the murine scFv using measurements such as T_m, temperature denaturation and temperature aggregation.

The binding capacity of the mutant scFvs can be determined using assays known in the art and described herein.

In one embodiment, the antigen binding domain of -a cancer associated antigen described herein, e.g., scFv, comprises at least one mutation arising from the humanization process such that the mutated scFv confers improved stability to the CAR construct. In another embodiment, the antigen binding domain of -a cancer associated antigen described herein, e.g., scFv, comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mutations arising from the humanization process such that the mutated scFv confers improved stability to the CAR construct.

Methods of Evaluating Protein Stability

The stability of an antigen binding domain may be assessed using, e.g., the methods described below. Such methods allow for the determination of multiple thermal unfolding transitions where the least stable domain either unfolds first or limits the overall stability threshold of a multidomain unit that unfolds cooperatively (e.g., a multidomain protein which exhibits a single unfolding transition).

The least stable domain can be identified in a number of additional ways. Mutagenesis can be performed to probe which domain limits the overall stability. Additionally, protease resistance of a multidomain protein can be performed under conditions where the least stable domain is known to be intrinsically unfolded via DSC or other spectroscopic methods (Fontana, et al., (1997) Fold. Des., 2: R17-26; Dimasi et al. (2009) J. Mol. Biol. 393: 672-692). Once the least stable domain is identified, the sequence encoding this domain (or a portion thereof) may be employed as a test sequence in the methods.

a) Thermal Stability

The thermal stability of the compositions may be analyzed using a number of non-limiting biophysical or biochemical techniques known in the art. In certain embodiments, thermal stability is evaluated by analytical spectroscopy.

An exemplary analytical spectroscopy method is Differential Scanning Calorimetry (DSC). DSC employs a calorimeter which is sensitive to the heat absorbances that accompany the unfolding of most proteins or protein domains (see, e.g. Sanchez-Ruiz, et al., Biochemistry, 27: 1648-52, 1988). To determine the thermal stability of a protein, a sample of the protein is inserted into the calorimeter and the temperature is raised until the Fab or scFv unfolds. The temperature at which the protein unfolds is indicative of overall protein stability.

Another exemplary analytical spectroscopy method is Circular Dichroism (CD) spectroscopy. CD spectrometry measures the optical activity of a composition as a function of increasing temperature. Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light which arise due to structural asymmetry. A disordered or unfolded structure results in a CD spectrum very different from that of an ordered or folded structure. The CD spectrum reflects the sensitivity of the proteins to the denaturing effects of increasing temperature and is therefore indicative of a protein's thermal stability (see van Mierlo and Steemsma, J. Biotechnol., 79(3):281-98, 2000).

Another exemplary analytical spectroscopy method for measuring thermal stability is Fluorescence Emission Spectroscopy (see van Mierlo and Steemsma, supra). Yet another exemplary analytical spectroscopy method for measuring thermal stability is Nuclear Magnetic Resonance (NMR) spectroscopy (see, e.g. van Mierlo and Steemsma, supra).

The thermal stability of a composition can be measured biochemically. An exemplary biochemical method for assessing thermal stability is a thermal challenge assay. In a "thermal challenge assay", a composition is subjected to a range of elevated temperatures for a set period of time. For example, in one embodiment, test scFv molecules or molecules comprising scFv molecules are subject to a range of increasing temperatures, e.g., for 1-1.5 hours. The activity of the protein is then assayed by a relevant biochemical assay. For example, if the protein is a binding protein (e.g. an scFv or scFv-containing polypeptide) the binding activity of the binding protein may be determined by a functional or quantitative ELISA.

Such an assay may be done in a high-throughput format and those disclosed in the Examples using *E. coli* and high throughput screening. A library of antigen binding domains, e.g., that includes an antigen binding domain to -a cancer associated antigen described herein, e.g., scFv variants, may be created using methods known in the art. Antigen binding domain, e.g., to -a cancer associated antigen described herein, e.g., scFv, expression may be induced and the antigen binding domain, e.g., to -a cancer associated antigen described herein, e.g., scFv, may be subjected to thermal challenge. The challenged test samples may be assayed for binding and those antigen binding domains to -a cancer associated antigen described herein, e.g., scFvs, which are stable may be scaled up and further characterized.

Thermal stability is evaluated by measuring the melting temperature (T_m) of a composition using any of the above techniques (e.g. analytical spectroscopy techniques). The melting temperature is the temperature at the midpoint of a thermal transition curve wherein 50% of molecules of a composition are in a folded state (See e.g., Dimasi et al. (2009) J. Mol Biol. 393: 672-692). In one embodiment, T_m values for an antigen binding domain to -a cancer associated antigen described herein, e.g., scFv, are about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C. In one embodiment, T_m values for an IgG is about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C. In one embodiment, T_m values for an multivalent antibody is about 40°C, 41°C, 42°C, 43°C, 44°C, 45°C, 46°C, 47°C, 48°C, 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, 72°C, 73°C, 74°C, 75°C, 76°C, 77°C, 78°C, 79°C, 80°C, 81°C, 82°C, 83°C, 84°C, 85°C, 86°C, 87°C, 88°C, 89°C, 90°C, 91°C, 92°C, 93°C, 94°C, 95°C, 96°C, 97°C, 98°C, 99°C, 100°C.

Thermal stability is also evaluated by measuring the specific heat or heat capacity (C_p) of a composition using an analytical calorimetric technique (e.g. DSC). The specific heat of a composition is the energy (e.g. in kcal/mol) is required to rise by 1°C, the temperature of 1 mol of water. As large C_p is a hallmark of a denatured or inactive protein composition. The change in heat capacity (ΔC_p) of a composition is measured by determining the specific heat of a composition before and after its thermal transition. Thermal stability may also be evaluated by measuring or determining other parameters of thermodynamic stability including Gibbs free energy of unfolding (ΔG), enthalpy of unfolding (ΔH), or entropy of unfolding (ΔS). One or more of the above biochemical assays (e.g. a

thermal challenge assay) are used to determine the temperature (i.e. the T_C value) at which 50% of the composition retains its activity (e.g. binding activity).

In addition, mutations to the antigen binding domain of a cancer associated antigen described herein, e.g., scFv, can be made to alter the thermal stability of the antigen binding domain of a cancer associated antigen described herein, e.g., scFv, as compared with the unmutated antigen binding domain of a cancer associated antigen described herein, e.g., scFv. When the humanized antigen binding domain of a cancer associated antigen described herein, e.g., scFv, is incorporated into a CAR construct, the antigen binding domain of the cancer associated antigen described herein, e.g., humanized scFv, confers thermal stability to the overall CARs of the present invention. In one embodiment, the antigen binding domain to a cancer associated antigen described herein, e.g., scFv, comprises a single mutation that confers thermal stability to the antigen binding domain of the cancer associated antigen described herein, e.g., scFv. In another embodiment, the antigen binding domain to a cancer associated antigen described herein, e.g., scFv, comprises multiple mutations that confer thermal stability to the antigen binding domain to the cancer associated antigen described herein, e.g., scFv. In one embodiment, the multiple mutations in the antigen binding domain to a cancer associated antigen described herein, e.g., scFv, have an additive effect on thermal stability of the antigen binding domain to the cancer associated antigen described herein binding domain, e.g., scFv.

b) % Aggregation

The stability of a composition can be determined by measuring its propensity to aggregate.

Aggregation can be measured by a number of non-limiting biochemical or biophysical techniques. For example, the aggregation of a composition may be evaluated using chromatography, e.g. Size-Exclusion Chromatography (SEC). SEC separates molecules on the basis of size. A column is filled with semi-solid beads of a polymeric gel that will admit ions and small molecules into their interior but not large ones. When a protein composition is applied to the top of the column, the compact folded proteins (i.e. non-aggregated proteins) are distributed through a larger volume of solvent than is available to the large protein aggregates. Consequently, the large aggregates move more rapidly through the column, and in this way the mixture can be separated or fractionated into its components. Each fraction can be separately quantified (e.g. by light scattering) as it elutes from the gel. Accordingly, the % aggregation of a composition can be determined by comparing the concentration of a fraction with the total concentration of protein applied to the gel. Stable compositions elute from the column as essentially a single fraction and appear as essentially a single peak in the elution profile or chromatogram.

c) Binding Affinity

The stability of a composition can be assessed by determining its target binding affinity. A

wide variety of methods for determining binding affinity are known in the art. An exemplary method for determining binding affinity employs surface plasmon resonance. Surface plasmon resonance is an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of

alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jonsson, U., i (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., et al. (1991) *Anal.*

5 Biochem. 198:268-277.

In one aspect, the antigen binding domain of the CAR comprises an amino acid sequence that is homologous to an antigen binding domain amino acid sequence described herein, and the antigen binding domain retains the desired functional properties of the antigen binding domain described herein.

10 In one specific aspect, the CAR composition of the invention comprises an antibody fragment. In a further aspect, the antibody fragment comprises an scFv.

In various aspects, the antigen binding domain of the CAR is engineered by modifying one or more amino acids within one or both variable regions (e.g., VH and/or VL), for example within one or more CDR regions and/or within one or more framework regions. In one specific aspect, the CAR
15 composition of the invention comprises an antibody fragment. In a further aspect, the antibody fragment comprises an scFv.

It will be understood by one of ordinary skill in the art that the antibody or antibody fragment of the invention may further be modified such that they vary in amino acid sequence (e.g., from wild-type), but not in desired activity. For example, additional nucleotide substitutions leading to amino
20 acid substitutions at “non-essential” amino acid residues may be made to the protein. For example, a nonessential amino acid residue in a molecule may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members, e.g., a conservative substitution, in which an amino acid residue is replaced with an amino acid residue
25 having a similar side chain, may be made.

Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline,
30 phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

Percent identity in the context of two or more nucleic acids or polypeptide sequences, refers to two or more sequences that are the same. Two sequences are “substantially identical” if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (e.g.,
35 60% identity, optionally 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and

aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more
 5 nucleotides (or 20, 50, 200 or more amino acids) in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative
 10 parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of
 15 Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Brent et al., (2003) *Current Protocols in Molecular Biology*).

20 Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977) *Nuc. Acids Res.* 25:3389-3402; and Altschul et al., (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

25 The percent identity between two amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller, (1988) *Comput. Appl. Biosci.* 4:11-17) 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 identity between two amino acid sequences can be determined using the Needleman and Wunsch (1970) *J. Mol. Biol.* 48:444-453)
 30 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.

In one aspect, the present invention contemplates modifications of the starting antibody or fragment (e.g., scFv) amino acid sequence that generate functionally equivalent molecules. For
 35 example, the VH or VL of an antigen binding domain to -a cancer associated antigen described herein, e.g., scFv, comprised in the CAR can be modified to retain at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity of the starting VH or VL framework region of the antigen binding domain to the cancer associated antigen described herein, e.g., scFv.

The present invention contemplates modifications of the entire CAR construct, e.g., modifications in one or more amino acid sequences of the various domains of the CAR construct in order to generate functionally equivalent molecules. The CAR construct can be modified to retain at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity of the starting CAR construct.

10 Transmembrane domain

With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region). In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR e.g., in one embodiment, the transmembrane domain may be from the same protein that the signaling domain, costimulatory domain or the hinge domain is derived from. In another aspect, the transmembrane domain is not derived from the same protein that any other domain of the CAR is derived from. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the cell surface of a CAR-expressing cell. In a different aspect, the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell.

30 The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40,

CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, 5 ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKG2D, NKG2C.

In some instances, the transmembrane domain can be attached to the extracellular region of 10 the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge (e.g., an IgG4 hinge, an IgD hinge), a GS linker (e.g., a GS linker described herein), a KIR2DS2 hinge or a CD8a hinge. In one embodiment, the hinge or spacer comprises (e.g., consists of) the amino acid sequence of SEQ ID NO:4. In one aspect, the transmembrane domain comprises (e.g., consists of) a 15 transmembrane domain of SEQ ID NO: 12.

In one aspect, the hinge or spacer comprises an IgG4 hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence
 ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDG
 VEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP
 20 REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGKM (SEQ ID NO:6). In some
 embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of
 GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCC
 AGCGTGTTCTGTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAG
 25 GTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTA
 CGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAAT
 AGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAA
 GGAATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCA
 GCAAGGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAG
 30 GAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGA
 CATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCC
 CTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGC
 CGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCA
 CTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATG (SEQ ID NO:7).

35 In one aspect, the hinge or spacer comprises an IgD hinge. For example, in one embodiment, the hinge or spacer comprises a hinge of the amino acid sequence
 RWPESPKAQASSVPTAQPPAQEGSLAKATTAPATTRNTGRGGEEKKKKEKEKEEQEERETKTPE

CPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLE
 RHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLLASS
 DPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPP
 SPQPATYTCVVSHEDSRTLNASRSLEVS YVTDH (SEQ ID NO:8). In some embodiments, the

5 hinge or spacer comprises a hinge encoded by a nucleotide sequence of

AGGTGGCCCGAAAGTCCCAAGGCCAGGCATCTAGTGTTCTACTGCACAGCCCCAGGC
 AGAAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACGCGCAATACTGGCCGTG
 GCGGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGAGGGGAGACCA
 AGACCCCTGAATGTCCATCCCATAACCCAGCCGCTGGGCGTCTATCTCTTGACTCCCGCAG
 10 TACAGGACTTGTGGCTTAGAGATAAGGCCACCTTTACATGTTTCGTCGTGGGCTCTGACC
 TGAAGGATGCCCATTGACTTGGGAGGTTGCCGGAAGGTACCCACAGGGGGGGTGTGAG
 GAAGGGTTGCTGGAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAAGACTCACCTT
 CCGAGATCCCTGTGGAACGCCGGGACCTCTGTACATGTACTCTAAATCATCCTAGCCTG
 CCCCCACAGCGTCTGATGGCCCTTAGAGAGCCAGCCGCCCAGGCACCAGTTAAGCTTAG
 15 CCTGAATCTGCTCGCCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCGAAGT
 GTCCGGCTTTAGCCCGCCCAACATCTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGA
 ACACCAGCGGCTTCGCTCCAGCCCGGCCCCCAGCCGGGTTCTACCACATTCTGGG
 CCTGGAGTGTCTTAAGGGTCCCAGCACCACTAGCCCCCAGCCAGCCACATACACCTGTG
 TTGTGTCCCATGAAGATAGCAGGACCCTGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCT
 20 ACGTGACTGACCATT (SEQ ID NO:9).

In one aspect, the transmembrane domain may be recombinant, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In one aspect a triplet of phenylalanine, tryptophan and valine can be found at each end of a recombinant transmembrane domain.

25 Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic region of the CAR. A glycine-serine doublet provides a particularly suitable linker. For example, in one aspect, the linker comprises the amino acid sequence of GGGGSGGGGS (SEQ ID NO: 10). In some embodiments, the linker is encoded by a nucleotide sequence of GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC
 30 (SEQ ID NO: 11).

In one aspect, the hinge or spacer comprises a KIR2DS2 hinge.

Cytoplasmic domain

The cytoplasmic domain or region of the CAR includes an intracellular signaling domain. An
 35 intracellular signaling domain is generally responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been introduced. The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, may be

cytolytic activity or helper activity including the secretion of cytokines. Thus the term “intracellular signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

Examples of intracellular signaling domains for use in the CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any recombinant sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary and/or costimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequences: those that initiate antigen-dependent primary activation through the TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide a secondary or costimulatory signal (secondary cytoplasmic domain, e.g., a costimulatory domain).

A primary signaling domain regulates primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary intracellular signaling domains that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary intracellular signaling domains that are of particular use in the invention include those of CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12. In one embodiment, a CAR of the invention comprises an intracellular signaling domain, e.g., a primary signaling domain of CD3-zeta.

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

The intracellular signalling domain of the CAR can comprise the CD3-zeta signaling domain by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling domain. The costimulatory signaling

domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte
 5 function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM
 10 (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), NKG2D, CEACAM1, CRTAM,
 15 Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a.

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

In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2,
 25 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain
 30 of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In one aspect, the signaling domain of 4-1BB is a signaling domain of SEQ ID NO: 14. In one aspect, the signaling domain of CD3-zeta is a signaling domain of SEQ ID NO: 18.

In one aspect, the intracellular signaling domain is designed to comprise the signaling domain
 35 of CD3-zeta and the signaling domain of CD27. In one aspect, the signaling domain of CD27 comprises an amino acid sequence of

QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP (SEQ ID NO: 16). In one aspect, the signalling domain of CD27 is encoded by a nucleic acid sequence of
 AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCC
 CGGGCCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCG
 5 CTCC (SEQ ID NO: 17).

In one aspect, the CAR-expressing cell described herein can further comprise a second CAR, e.g., a second CAR that includes a different antigen binding domain, e.g., to the same target or a different target (e.g., a target other than a cancer associated antigen described herein or a different cancer associated antigen described herein). In one embodiment, the second CAR includes an antigen
 10 binding domain to a target expressed the same cancer cell type as the cancer associated antigen. In one embodiment, the CAR-expressing cell comprises a first CAR that targets a first antigen and includes an intracellular signaling domain having a costimulatory signaling domain but not a primary signaling domain, and a second CAR that targets a second, different, antigen and includes an
 15 intracellular signaling domain having a primary signaling domain but not a costimulatory signaling domain. While not wishing to be bound by theory, placement of a costimulatory signaling domain, e.g., 4-1BB, CD28, CD27 or OX-40, onto the first CAR, and the primary signaling domain, e.g., CD3 zeta, on the second CAR can limit the CAR activity to cells where both targets are expressed. In one embodiment, the CAR expressing cell comprises a first cancer associated antigen CAR that includes
 20 an antigen binding domain that binds a target antigen described herein, a transmembrane domain and a costimulatory domain and a second CAR that targets a different target antigen (e.g., an antigen expressed on that same cancer cell type as the first target antigen) and includes an antigen binding domain, a transmembrane domain and a primary signaling domain. In another embodiment, the CAR expressing cell comprises a first CAR that includes an antigen binding domain that binds a target
 25 antigen described herein, a transmembrane domain and a primary signaling domain and a second CAR that targets an antigen other than the first target antigen (e.g., an antigen expressed on the same cancer cell type as the first target antigen) and includes an antigen binding domain to the antigen, a transmembrane domain and a costimulatory signaling domain.

In one embodiment, the CAR-expressing cell comprises an XCAR described herein and an inhibitory CAR. In one embodiment, the inhibitory CAR comprises an antigen binding domain that
 30 binds an antigen found on normal cells but not cancer cells, e.g., normal cells that also express CLL. In one embodiment, the inhibitory CAR comprises the antigen binding domain, a transmembrane domain and an intracellular domain of an inhibitory molecule. For example, the intracellular domain of the inhibitory CAR can be an intracellular domain of PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4
 35 or TGF beta.

In one embodiment, when the CAR-expressing cell comprises two or more different CARs, the antigen binding domains of the different CARs can be such that the antigen binding domains do

not interact with one another. For example, a cell expressing a first and second CAR can have an antigen binding domain of the first CAR, e.g., as a fragment, e.g., an scFv, that does not form an association with the antigen binding domain of the second CAR, e.g., the antigen binding domain of the second CAR is a VHH.

5 In some embodiments, the antigen binding domain comprises a single domain antigen binding (SDAB) molecules include molecules whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain variable domains, binding molecules naturally devoid of light chains, single domains derived from conventional 4-chain antibodies, engineered domains and single domain scaffolds other than those derived from antibodies.

10 SDAB molecules may be any of the art, or any future single domain molecules. SDAB molecules may be derived from any species including, but not limited to mouse, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. This term also includes naturally occurring single domain antibody molecules from species other than Camelidae and sharks.

In one aspect, an SDAB molecule can be derived from a variable region of the

15 immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909.

According to another aspect, an SDAB molecule is a naturally occurring single domain

20 antigen binding molecule known as heavy chain devoid of light chains. Such single domain molecules are disclosed in WO 9404678 and Hamers-Casterman, C. et al. (1993) Nature 363:446-448, for example. For clarity reasons, this variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from Camelidae species,

25 for example in camel, llama, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain; such VHHs are within the scope of the invention.

The SDAB molecules can be recombinant, CDR-grafted, humanized, camelized, de-immunized and/or in vitro generated (e.g., selected by phage display).

30 It has also been discovered, that cells having a plurality of chimeric membrane embedded receptors comprising an antigen binding domain that interactions between the antigen binding domain of the receptors can be undesirable, e.g., because it inhibits the ability of one or more of the antigen binding domains to bind its cognate antigen. Accordingly, disclosed herein are cells having a first and a second non-naturally occurring chimeric membrane embedded receptor comprising antigen binding

35 domains that minimize such interactions. Also disclosed herein are nucleic acids encoding a first and a second non-naturally occurring chimeric membrane embedded receptor comprising a antigen binding domains that minimize such interactions, as well as methods of making and using such cells

and nucleic acids. In an embodiment the antigen binding domain of one of said first said second non-naturally occurring chimeric membrane embedded receptor, comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence.

5 In some embodiments, the claimed invention comprises a first and second CAR, wherein the antigen binding domain of one of said first CAR said second CAR does not comprise a variable light domain and a variable heavy domain. In some embodiments, the antigen binding domain of one of said first CAR said second CAR is an scFv, and the other is not an scFv. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a single VH domain,
10 e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a nanobody. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises a camelid VHH domain.

In some embodiments, the antigen binding domain of one of said first CAR said second CAR
15 comprises an scFv, and the other comprises a single VH domain, e.g., a camelid, shark, or lamprey single VH domain, or a single VH domain derived from a human or mouse sequence. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a nanobody. In some embodiments, the antigen binding domain of one of said first CAR said second CAR comprises an scFv, and the other comprises a camelid
20 VHH domain.

In some embodiments, when present on the surface of a cell, binding of the antigen binding domain of said first CAR to its cognate antigen is not substantially reduced by the presence of said second CAR. In some embodiments, binding of the antigen binding domain of said first CAR to its cognate antigen in the presence of said second CAR is 85%, 90%, 95%, 96%, 97%, 98% or 99% of
25 binding of the antigen binding domain of said first CAR to its cognate antigen in the absence of said second CAR.

In some embodiments, when present on the surface of a cell, the antigen binding domains of said first CAR said second CAR, associate with one another less than if both were scFv antigen binding domains. In some embodiments, the antigen binding domains of said first CAR said second
30 CAR, associate with one another 85%, 90%, 95%, 96%, 97%, 98% or 99% less than if both were scFv antigen binding domains.

In another aspect, the CAR-expressing cell described herein can further express another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules,
35 e.g., PD1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT,

LAIR1, CD160, 2B4 and TGF beta. In one embodiment, the agent which inhibits an inhibitory molecule, e.g., is a molecule described herein, e.g., an agent that comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 or TGF beta, or a fragment of any of these (e.g., at least a portion of an extracellular domain of any of these), and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD1 or a fragment thereof (e.g., at least a portion of an extracellular domain of PD1), and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein). PD1 is an inhibitory member of the CD28 family of receptors that also includes CD28, CTLA-4, ICOS, and BTLA. PD-1 is expressed on activated B cells, T cells and myeloid cells (Agata et al. 1996 Int. Immunol 8:765-75). Two ligands for PD1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD1 (Freeman et al. 2000 J Exp Med 192:1027-34; Latchman et al. 2001 Nat Immunol 2:261-8; Carter et al. 2002 Eur J Immunol 32:634-43). PD-L1 is abundant in human cancers (Dong et al. 2003 J Mol Med 81:281-7; Blank et al. 2005 Cancer Immunol. Immunother 54:307-314; Konishi et al. 2004 Clin Cancer Res 10:5094). Immune suppression can be reversed by inhibiting the local interaction of PD1 with PD-L1.

In one embodiment, the agent comprises the extracellular domain (ECD) of an inhibitory molecule, e.g., Programmed Death 1 (PD1), fused to a transmembrane domain and intracellular signaling domains such as 41BB and CD3 zeta (also referred to herein as a PD1 CAR). In one embodiment, the PD1 CAR, when used in combinations with a XCAR described herein, improves the persistence of the T cell. In one embodiment, the CAR is a PD1 CAR comprising the extracellular domain of PD1 indicated as underlined in SEQ ID NO: 26. In one embodiment, the PD1 CAR comprises the amino acid sequence of SEQ ID NO: 26.

MalpvtaIIplalllhaarppgwflsdprpwnpptfspallvvttegdnatftcsfntsfsfvlwyrmspsnqtdklaafpedrsqpgqdc
frvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlytttpparpptpaptiasqplslr
 peacrpaaggavhtrgldfacdiyiwaplagtcgvllslvitlyckrgkrklllyifkqpfmrpvqttqeedgcscrpfpeeeeggcelrvkfsrsad
 apaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdt
 ydalhmqalppr (SEQ ID NO:26).

In one embodiment, the PD1 CAR comprises the amino acid sequence provided below (SEQ ID NO: 39).

pgwfldspdrpwnpptfspallvvt egdnatftcsfntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcfrvtqlpngrdfhmsvvrar
 rndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfa
 cdiyiwaplagtcgvllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcsrfpeeeeggcelrvkfsrsadapaykqqnqlynelnlgr
 reeydvldkrrgrdpemggkprkrnpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdydalhmqalppr (SEQ
 5 ID NO:39).

In one embodiment, the agent comprises a nucleic acid sequence encoding the PD1 CAR,
 e.g., the PD1 CAR described herein. In one embodiment, the nucleic acid sequence for the PD1 CAR
 is shown below, with the PD1 ECD underlined below in SEQ ID NO: 27.

atggccctccctgtcactgcctgtcttccccctgcactcctgtccacgccgctagaccacccggatggttctggactctccggatgccccgtg
 10 gaatcccccaaccttctcaccggcactcttggttgactgagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgct
gaactggtaccgcatgagccccgtcaaaccagaccgacaagctcgccgcgtttccggaagatcggtcgcaaccgggacaggattgctcggtccgc
gtgactcaactgccgaatggcagagacttccacatgagcgtggtccgcgctaggcgaaacgactccgggacctacctgtgcggagccatctcgt
ggcgccctaaggcccaaatcaaagagagcttgagggccgaactgagagtaccgagcgcagagctgaggtgccaactgcacatccatccccatc
gcctcgccctgcggggcagtttcagaccctggtcacgaccactccggcgccgcgcccaccgactccggcccccaactatcgcgagccagcccct
 15 gtcgctgaggccggaagcatgccgccctgccgccggaggtgctgtgcatacccggggattggacttcgcatgcgacatctacattgggctcctct
 cgccggaacttggtgctgctccttctgtccctggtcacaccctgtactgcaagcggggtcggaaaaagcttctgtacatttcaagcagccctcat
 gagggccgtgcaaaccacccaggaggagacgggtgctcctgccggttccccgaagaggaagaaggaggtgcgagctgcgcgtgaagtctc
 ccggagcgcgcgacgccccgcctataagcaggggccagaaccagctgtacaacgaactgaacctgggacggcgggaagagtacgatgtgctgg
 acaagcggcgcggccgggacccccgaaatggcggggaagcctagaagaaagaacctcaggaaggcctgtataacgagctgcagaaggacaa
 20 gatggccgagggcctactccgaaattgggatgaaggagagcggcgagggggaaaggggcacgacggcctgtaccaaggactgtccaccgcc
 accaaggacacatacgatgcctgcacatgcaggccctccccctcgc (SEQ ID NO: 27).

In another aspect, the present invention provides a population of CAR-expressing cells, e.g.,
 CART cells. In some embodiments, the population of CAR-expressing cells comprises a mixture of
 cells expressing different CARs. For example, in one embodiment, the population of CART cells can
 25 include a first cell expressing a CAR having an antigen binding domain to a cancer associated antigen
 described herein, and a second cell expressing a CAR having a different antigen binding domain, e.g.,
 an antigen binding domain to a different a cancer associated antigen described herein, e.g., an antigen
 binding domain to a cancer associated antigen described herein that differs from the cancer associated
 antigen bound by the antigen binding domain of the CAR expressed by the first cell. As another
 30 example, the population of CAR-expressing cells can include a first cell expressing a CAR that
 includes an antigen binding domain to a cancer associated antigen described herein, and a second cell
 expressing a CAR that includes an antigen binding domain to a target other than a cancer associated
 antigen as described herein. In one embodiment, the population of CAR-expressing cells includes,
 e.g., a first cell expressing a CAR that includes a primary intracellular signaling domain, and a second
 35 cell expressing a CAR that includes a secondary signaling domain.

In another aspect, the present invention provides a population of cells wherein at least one cell
 in the population expresses a CAR having an antigen binding domain to a cancer associated antigen

described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Inhibitory molecules, e.g., PD-1, can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD-1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGF beta. In one embodiment, the agent which inhibits an inhibitory molecule, e.g., is a molecule described herein, e.g., an agent that comprises a first polypeptide, e.g., an inhibitory molecule, associated with a second polypeptide that provides a positive signal to the cell, e.g., an intracellular signaling domain described herein. In one embodiment, the agent comprises a first polypeptide, e.g., of an inhibitory molecule such as PD-1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 or TGF beta, or a fragment of any of these, and a second polypeptide which is an intracellular signaling domain described herein (e.g., comprising a costimulatory domain (e.g., 41BB, CD27, OX40 or CD28, e.g., as described herein) and/or a primary signaling domain (e.g., a CD3 zeta signaling domain described herein). In one embodiment, the agent comprises a first polypeptide of PD-1 or a fragment thereof, and a second polypeptide of an intracellular signaling domain described herein (e.g., a CD28 signaling domain described herein and/or a CD3 zeta signaling domain described herein).

In one aspect, the present invention provides methods comprising administering a population of CAR-expressing cells, e.g., CART cells, e.g., a mixture of cells expressing different CARs, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein. In another aspect, the present invention provides methods comprising administering a population of cells wherein at least one cell in the population expresses a CAR having an antigen binding domain of a cancer associated antigen described herein, and a second cell expressing another agent, e.g., an agent which enhances the activity of a CAR-expressing cell, in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein.

Regulatable Chimeric Antigen Receptors

In some embodiments, a regulatable CAR (RCAR) where the CAR activity can be controlled is desirable to optimize the safety and efficacy of a CAR therapy. There are many ways CAR activities can be regulated. For example, inducible apoptosis using, e.g., a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J. Med. 2011 Nov. 3; 365(18):1673-1683), can be used as a safety switch in the CAR therapy of the instant invention. In an aspect, a RCAR comprises a set of polypeptides, typically two in the simplest embodiments, in which the components of a standard CAR described herein, e.g., an antigen binding domain and an intracellular signaling domain, are partitioned on separate polypeptides or members. In some embodiments, the set of polypeptides include a dimerization switch that, upon the presence of a dimerization molecule, can

couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain.

In an aspect, an RCAR comprises two polypeptides or members: 1) an intracellular signaling member comprising an intracellular signaling domain, e.g., a primary intracellular signaling domain described herein, and a first switch domain; 2) an antigen binding member comprising an antigen binding domain, e.g., that targets a tumor antigen described herein, as described herein and a second switch domain. Optionally, the RCAR comprises a transmembrane domain described herein. In an embodiment, a transmembrane domain can be disposed on the intracellular signaling member, on the antigen binding member, or on both. (Unless otherwise indicated, when members or elements of an RCAR are described herein, the order can be as provided, but other orders are included as well. In other words, in an embodiment, the order is as set out in the text, but in other embodiments, the order can be different. E.g., the order of elements on one side of a transmembrane region can be different from the example, e.g., the placement of a switch domain relative to a intracellular signaling domain can be different, e.g., reversed).

In an embodiment, the first and second switch domains can form an intracellular or an extracellular dimerization switch. In an embodiment, the dimerization switch can be a homodimerization switch, e.g., where the first and second switch domain are the same, or a heterodimerization switch, e.g., where the first and second switch domain are different from one another.

In embodiments, an RCAR can comprise a “multi switch.” A multi switch can comprise heterodimerization switch domains or homodimerization switch domains. A multi switch comprises a plurality of, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10, switch domains, independently, on a first member, e.g., an antigen binding member, and a second member, e.g., an intracellular signaling member. In an embodiment, the first member can comprise a plurality of first switch domains, e.g., FKBP-based switch domains, and the second member can comprise a plurality of second switch domains, e.g., FRB-based switch domains. In an embodiment, the first member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain, and the second member can comprise a first and a second switch domain, e.g., a FKBP-based switch domain and a FRB-based switch domain.

In an embodiment, the intracellular signaling member comprises one or more intracellular signaling domains, e.g., a primary intracellular signaling domain and one or more costimulatory signaling domains.

In an embodiment, the antigen binding member may comprise one or more intracellular signaling domains, e.g., one or more costimulatory signaling domains. In an embodiment, the antigen binding member comprises a plurality, e.g., 2 or 3 costimulatory signaling domains described herein, e.g., selected from 41BB, CD28, CD27, ICOS, and OX40, and in embodiments, no primary intracellular signaling domain. In an embodiment, the antigen binding member comprises the

following costimulatory signaling domains, from the extracellular to intracellular direction: 41BB-CD27; 41BB-CD27; CD27-41BB; 41BB-CD28; CD28-41BB; OX40-CD28; CD28-OX40; CD28-41BB; or 41BB-CD28. In such embodiments, the intracellular binding member comprises a CD3zeta domain. In one such embodiment the RCAR comprises (1) an antigen binding member comprising,
 5 an antigen binding domain, a transmembrane domain, and two costimulatory domains and a first switch domain; and (2) an intracellular signaling domain comprising a transmembrane domain or membrane tethering domain and at least one primary intracellular signaling domain, and a second switch domain.

An embodiment provides RCARs wherein the antigen binding member is not tethered to the
 10 surface of the CAR cell. This allows a cell having an intracellular signaling member to be conveniently paired with one or more antigen binding domains, without transforming the cell with a sequence that encodes the antigen binding member. In such embodiments, the RCAR comprises: 1) an intracellular signaling member comprising: a first switch domain, a transmembrane domain, an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch
 15 domain; and 2) an antigen binding member comprising: an antigen binding domain, and a second switch domain, wherein the antigen binding member does not comprise a transmembrane domain or membrane tethering domain, and, optionally, does not comprise an intracellular signaling domain. In some embodiments, the RCAR may further comprise 3) a second antigen binding member comprising: a second antigen binding domain, e.g., a second antigen binding domain that binds a
 20 different antigen than is bound by the antigen binding domain; and a second switch domain.

Also provided herein are RCARs wherein the antigen binding member comprises bispecific activation and targeting capacity. In this embodiment, the antigen binding member can comprise a plurality, e.g., 2, 3, 4, or 5 antigen binding domains, e.g., scFvs, wherein each antigen binding domain binds to a target antigen, e.g. different antigens or the same antigen, e.g., the same or different
 25 epitopes on the same antigen. In an embodiment, the plurality of antigen binding domains are in tandem, and optionally, a linker or hinge region is disposed between each of the antigen binding domains. Suitable linkers and hinge regions are described herein.

An embodiment provides RCARs having a configuration that allows switching of proliferation. In this embodiment, the RCAR comprises: 1) an intracellular signaling member
 30 comprising: optionally, a transmembrane domain or membrane tethering domain; one or more co-stimulatory signaling domain, e.g., selected from 41BB, CD28, CD27, ICOS, and OX40, and a switch domain; and 2) an antigen binding member comprising: an antigen binding domain, a transmembrane domain, and a primary intracellular signaling domain, e.g., a CD3zeta domain, wherein the antigen binding member does not comprise a switch domain, or does not comprise a switch domain that
 35 dimerizes with a switch domain on the intracellular signaling member. In an embodiment, the antigen binding member does not comprise a co-stimulatory signaling domain. In an embodiment, the intracellular signaling member comprises a switch domain from a homodimerization switch. In an

embodiment, the intracellular signaling member comprises a first switch domain of a heterodimerization switch and the RCAR comprises a second intracellular signaling member which comprises a second switch domain of the heterodimerization switch. In such embodiments, the second intracellular signaling member comprises the same intracellular signaling domains as the intracellular signaling member. In an embodiment, the dimerization switch is intracellular. In an embodiment, the dimerization switch is extracellular.

In any of the RCAR configurations described here, the first and second switch domains comprise a FKBP-FRB based switch as described herein.

Also provided herein are cells comprising an RCAR described herein. Any cell that is engineered to express a RCAR can be used as a RCARX cell. In an embodiment the RCARX cell is a T cell, and is referred to as a RCART cell. In an embodiment the RCARX cell is an NK cell, and is referred to as a RCARN cell.

Also provided herein are nucleic acids and vectors comprising RCAR encoding sequences. Sequence encoding various elements of an RCAR can be disposed on the same nucleic acid molecule, e.g., the same plasmid or vector, e.g., viral vector, e.g., lentiviral vector. In an embodiment, (i) sequence encoding an antigen binding member and (ii) sequence encoding an intracellular signaling member, can be present on the same nucleic acid, e.g., vector. Production of the corresponding proteins can be achieved, e.g., by the use of separate promoters, or by the use of a bicistronic transcription product (which can result in the production of two proteins by cleavage of a single translation product or by the translation of two separate protein products). In an embodiment, a sequence encoding a cleavable peptide, e.g., a P2A or F2A sequence, is disposed between (i) and (ii). Examples of peptide cleavage sites include the following, wherein the GSG residues are optional:

T2A: (GSG) E G R G S L L T C G D V E E N P G P (SEQ ID NO: 68)

P2A: (GSG) A T N F S L L K Q A G D V E E N P G P (SEQ ID NO: 69)

E2A: (GSG) Q C T N Y A L L K L A G D V E S N P G P (SEQ ID NO: 70)

F2A: (GSG) V K Q T L N F D L L K L A G D V E S N P G P (SEQ ID NO: 71)

In an embodiment, a sequence encoding an IRES, e.g., an EMCV or EV71 IRES, is disposed between (i) and (ii). In these embodiments, (i) and (ii) are transcribed as a single RNA. In an embodiment, a first promoter is operably linked to (i) and a second promoter is operably linked to (ii), such that (i) and (ii) are transcribed as separate mRNAs.

Alternatively, the sequence encoding various elements of an RCAR can be disposed on the different nucleic acid molecules, e.g., different plasmids or vectors, e.g., viral vector, e.g., lentiviral vector. E.g., the (i) sequence encoding an antigen binding member can be present on a first nucleic acid, e.g., a first vector, and the (ii) sequence encoding an intracellular signaling member can be present on the second nucleic acid, e.g., the second vector.

Dimerization switches

Dimerization switches can be non-covalent or covalent. In a non-covalent dimerization switch, the dimerization molecule promotes a non-covalent interaction between the switch domains. In a covalent dimerization switch, the dimerization molecule promotes a covalent interaction between the switch domains.

In an embodiment, the RCAR comprises a FKBP/FRAP, or FKBP/FRB,-based dimerization switch. FKBP12 (FKBP, or FK506 binding protein) is an abundant cytoplasmic protein that serves as the initial intracellular target for the natural product immunosuppressive drug, rapamycin. Rapamycin binds to FKBP and to the large PI3K homolog FRAP (RAFT, mTOR). FRB is a 93 amino acid portion of FRAP, that is sufficient for binding the FKBP-rapamycin complex (Chen, J., Zheng, X. F., Brown, E. J. & Schreiber, S. L. (1995) *Identification of an 11-kDa FKBP12-rapamycin-binding domain within the 289-kDa FKBP12-rapamycin-associated protein and characterization of a critical serine residue*. Proc Natl Acad Sci U S A 92: 4947-51.)

In embodiments, an FKBP/FRAP, e.g., an FKBP/FRB, based switch can use a dimerization molecule, e.g., rapamycin or a rapamycin analog.

The amino acid sequence of FKBP is as follows:

DVPDYASLGGPSSPKKKRKVSRGVQVETISPGDGRTPFKRGQTCV
VHYTGMLDGGKKFDSSRDNRNPKPFKFM LGKQEVIRGWEEGVAQMS
VGQRAKLTISPDIAYGATGHPGIIPPHATLVFDVELLKLETSY (SEQ
ID NO: 54)

In embodiments, an FKBP switch domain can comprise a fragment of FKBP having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., the underlined portion of SEQ ID NO: 54, which is:

VQVETISPGDGRTPFKRGQTCVVHYTGMLDGGKKFDSSRDNRNPKPF
KFM LGKQEVIRGWEEGVAQMSVGQRAKLTISPDIAYGATGHPGII
PPHATLVFDVELLKLETS (SEQ ID NO:55)

The amino acid sequence of FRB is as follows:

ILWHEMWHEG LEEASRLYFG ERNVKGMFEV LEPLHAMMER GPQTLKETSF
NQAYGRDLME AQEWCRKYMK SGNVKDLTQA WDLYYHVFRRI SK (SEQ ID NO: 56)

“FKBP/FRAP, e.g., an FKBP/FRB, based switch” as that term is used herein, refers to a dimerization switch comprising: a first switch domain, which comprises an FKBP fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, e.g., RAD001, and has at least 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identity with, or differs by no more than 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1 amino acid residues from, the FKBP sequence of SEQ ID NO: 54 or 55; and a second switch domain, which comprises an FRB fragment or analog thereof having the ability to bind with FRB, or a fragment or analog thereof, in the presence of rapamycin or a rapalog, and has at least 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identity with, or differs by no more than 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1 amino acid residues from, the FRB

sequence of SEQ ID NO: 56. In an embodiment, a RCAR described herein comprises one switch domain comprises amino acid residues disclosed in SEQ ID NO: 54 (or SEQ ID NO: 55), and one switch domain comprises amino acid residues disclosed in SEQ ID NO: 56.

In embodiments, the FKBP/FRB dimerization switch comprises a modified FRB switch domain that exhibits altered, e.g., enhanced, complex formation between an FRB-based switch domain, e.g., the modified FRB switch domain, a FKBP-based switch domain, and the dimerization molecule, e.g., rapamycin or a rapalogue, e.g., RAD001. In an embodiment, the modified FRB switch domain comprises one or more mutations, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, selected from mutations at amino acid position(s) L2031, E2032, S2035, R2036, F2039, G2040, T2098, W2101, D2102, Y2105, and F2108, where the wild-type amino acid is mutated to any other naturally-occurring amino acid. In an embodiment, a mutant FRB comprises a mutation at E2032, where E2032 is mutated to phenylalanine (E2032F), methionine (E2032M), arginine (E2032R), valine (E2032V), tyrosine (E2032Y), isoleucine (E2032I), e.g., SEQ ID NO: 57, or leucine (E2032L), e.g., SEQ ID NO: 58. In an embodiment, a mutant FRB comprises a mutation at T2098, where T2098 is mutated to phenylalanine (T2098F) or leucine (T2098L), e.g., SEQ ID NO: 59. In an embodiment, a mutant FRB comprises a mutation at E2032 and at T2098, where E2032 is mutated to any amino acid, and where T2098 is mutated to any amino acid, e.g., SEQ ID NO: 60. In an embodiment, a mutant FRB comprises an E2032I and a T2098L mutation, e.g., SEQ ID NO: 61. In an embodiment, a mutant FRB comprises an E2032L and a T2098L mutation, e.g., SEQ ID NO: 62.

Table 10. Exemplary mutant FRB having increased affinity for a dimerization molecule

FRB mutant	Amino Acid Sequence	SEQ ID NO:
E2032I mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRIS KTS	57
E2032L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRIS KTS	58
T2098L mutant	ILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRIS KTS	59
E2032, T2098 mutant	ILWHEMWHEGL ~~X~~ EASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDL ~~X~~ QAWDLYYHVFRRIS KTS	60
E2032I, T2098L mutant	ILWHEMWHEGLIEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRIS KTS	61
E2032L, T2098L mutant	ILWHEMWHEGLLEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLK ETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQAWDLYYHVFRRIS KTS	62

Other suitable dimerization switches include a GyrB-GyrB based dimerization switch, a Gibberellin-based dimerization switch, a tag/binder dimerization switch, and a halo-tag/snap-tag

dimerization switch. Following the guidance provided herein, such switches and relevant dimerization molecules will be apparent to one of ordinary skill.

Dimerization molecule

5 Association between the switch domains is promoted by the dimerization molecule. In the presence of dimerization molecule interaction or association between switch domains allows for signal transduction between a polypeptide associated with, e.g., fused to, a first switch domain, and a polypeptide associated with, e.g., fused to, a second switch domain. In the presence of non-limiting levels of dimerization molecule signal transduction is increased by 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8,
10 1.9, 2, 5, 10, 50, 100 fold, e.g., as measured in a system described herein.

Rapamycin and rapamycin analogs (sometimes referred to as rapalogues), e.g., RAD001, can be used as dimerization molecules in a FKBP/FRB-based dimerization switch described herein. In an embodiment the dimerization molecule can be selected from rapamycin (sirolimus), RAD001 (everolimus), zotarolimus, temsirolimus, AP-23573 (ridaforolimus), biolimus and AP21967.
15 Additional rapamycin analogs suitable for use with FKBP/FRB-based dimerization switches are further described in the section entitled “Combination Therapies”, or in the subsection entitled “Exemplary mTOR inhibitors.”

Split CAR

20 In some embodiments, the CAR-expressing cell uses a split CAR. The split CAR approach is described in more detail in publications WO2014/055442 and WO2014/055657. Briefly, a split CAR system comprises a cell expressing a first CAR having a first antigen binding domain and a costimulatory domain (e.g., 41BB), and the cell also expresses a second CAR having a second antigen binding domain and an intracellular signaling domain (e.g., CD3 zeta). When the cell encounters the
25 first antigen, the costimulatory domain is activated, and the cell proliferates. When the cell encounters the second antigen, the intracellular signaling domain is activated and cell-killing activity begins. Thus, the CAR-expressing cell is only fully activated in the presence of both antigens.

RNA Transfection

30 Disclosed herein are methods for producing an in vitro transcribed RNA CAR. The present invention also includes a CAR encoding RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection can involve in vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence (“UTR”), a 5' cap and/or Internal Ribosome Entry Site
35 (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length (SEQ ID NO:32). RNA so produced can efficiently transfect different kinds of cells. In one aspect, the template includes sequences for the CAR.

In one aspect, a CAR of the present invention is encoded by a messenger RNA (mRNA). In one aspect, the mRNA encoding a CAR described herein is introduced into an immune effector cell, e.g., a T cell or a NK cell, for production of a CAR-expressing cell, e.g., a CART cell or a CAR NK cell.

5 In one embodiment, the *in vitro* transcribed RNA CAR can be introduced to a cell as a form of transient transfection. The RNA is produced by *in vitro* transcription using a polymerase chain reaction (PCR)-generated template. DNA of interest from any source can be directly converted by PCR into a template for *in vitro* mRNA synthesis using appropriate primers and RNA polymerase. The source of the DNA can be, for example, genomic DNA, plasmid DNA, phage DNA, cDNA,
10 synthetic DNA sequence or any other appropriate source of DNA. The desired template for *in vitro* transcription is a CAR described herein. For example, the template for the RNA CAR comprises an extracellular region comprising a single chain variable domain of an antibody to a tumor associated antigen described herein; a hinge region (e.g., a hinge region described herein), a transmembrane domain (e.g., a transmembrane domain described herein such as a transmembrane domain of CD8a);
15 and a cytoplasmic region that includes an intracellular signaling domain, e.g., an intracellular signaling domain described herein, e.g., comprising the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

In one embodiment, the DNA to be used for PCR contains an open reading frame. The DNA can be from a naturally occurring DNA sequence from the genome of an organism. In one
20 embodiment, the nucleic acid can include some or all of the 5' and/or 3' untranslated regions (UTRs). The nucleic acid can include exons and introns. In one embodiment, the DNA to be used for PCR is a human nucleic acid sequence. In another embodiment, the DNA to be used for PCR is a human nucleic acid sequence including the 5' and 3' UTRs. The DNA can alternatively be an artificial DNA sequence that is not normally expressed in a naturally occurring organism. An exemplary artificial
25 DNA sequence is one that contains portions of genes that are ligated together to form an open reading frame that encodes a fusion protein. The portions of DNA that are ligated together can be from a single organism or from more than one organism.

PCR is used to generate a template for *in vitro* transcription of mRNA which is used for transfection. Methods for performing PCR are well known in the art. Primers for use in PCR are
30 designed to have regions that are substantially complementary to regions of the DNA to be used as a template for the PCR. "Substantially complementary," as used herein, refers to sequences of nucleotides where a majority or all of the bases in the primer sequence are complementary, or one or more bases are non-complementary, or mismatched. Substantially complementary sequences are able to anneal or hybridize with the intended DNA target under annealing conditions used for PCR. The
35 primers can be designed to be substantially complementary to any portion of the DNA template. For example, the primers can be designed to amplify the portion of a nucleic acid that is normally transcribed in cells (the open reading frame), including 5' and 3' UTRs. The primers can also be

designed to amplify a portion of a nucleic acid that encodes a particular domain of interest. In one embodiment, the primers are designed to amplify the coding region of a human cDNA, including all or portions of the 5' and 3' UTRs. Primers useful for PCR can be generated by synthetic methods that are well known in the art. "Forward primers" are primers that contain a region of nucleotides that are substantially complementary to nucleotides on the DNA template that are upstream of the DNA sequence that is to be amplified. "Upstream" is used herein to refer to a location 5' to the DNA sequence to be amplified relative to the coding strand. "Reverse primers" are primers that contain a region of nucleotides that are substantially complementary to a double-stranded DNA template that are downstream of the DNA sequence that is to be amplified. "Downstream" is used herein to refer to a location 3' to the DNA sequence to be amplified relative to the coding strand.

Any DNA polymerase useful for PCR can be used in the methods disclosed herein. The reagents and polymerase are commercially available from a number of sources.

Chemical structures with the ability to promote stability and/or translation efficiency may also be used. The RNA preferably has 5' and 3' UTRs. In one embodiment, the 5' UTR is between one and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the nucleic acid of interest. Alternatively, UTR sequences that are not endogenous to the nucleic acid of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the nucleic acid of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of mRNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous nucleic acid. Alternatively, when a 5' UTR that is not endogenous to the nucleic acid of interest is being added by PCR as described above, a consensus Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many mRNAs is known in the art. In other embodiments the 5' UTR can be 5' UTR of an RNA virus whose RNA genome is stable in cells. In other embodiments various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the mRNA.

To enable synthesis of RNA from a DNA template without the need for gene cloning, a promoter of transcription should be attached to the DNA template upstream of the sequence to be transcribed. When a sequence that functions as a promoter for an RNA polymerase is added to the 5' end of the forward primer, the RNA polymerase promoter becomes incorporated into the PCR product
5 upstream of the open reading frame that is to be transcribed. In one preferred embodiment, the promoter is a T7 polymerase promoter, as described elsewhere herein. Other useful promoters include, but are not limited to, T3 and SP6 RNA polymerase promoters. Consensus nucleotide sequences for T7, T3 and SP6 promoters are known in the art.

In a preferred embodiment, the mRNA has both a cap on the 5' end and a 3' poly(A) tail
10 which determine ribosome binding, initiation of translation and stability mRNA in the cell. On a circular DNA template, for instance, plasmid DNA, RNA polymerase produces a long concatameric product which is not suitable for expression in eukaryotic cells. The transcription of plasmid DNA linearized at the end of the 3' UTR results in normal sized mRNA which is not effective in eukaryotic transfection even if it is polyadenylated after transcription.

15 On a linear DNA template, phage T7 RNA polymerase can extend the 3' end of the transcript beyond the last base of the template (Schenborn and Mierendorf, Nuc Acids Res., 13:6223-36 (1985); Nacheva and Berzal-Herranz, Eur. J. Biochem., 270:1485-65 (2003)).

The conventional method of integration of polyA/T stretches into a DNA template is molecular cloning. However polyA/T sequence integrated into plasmid DNA can cause plasmid
20 instability, which is why plasmid DNA templates obtained from bacterial cells are often highly contaminated with deletions and other aberrations. This makes cloning procedures not only laborious and time consuming but often not reliable. That is why a method which allows construction of DNA templates with polyA/T 3' stretch without cloning highly desirable.

The polyA/T segment of the transcriptional DNA template can be produced during PCR by
25 using a reverse primer containing a polyT tail, such as 100T tail (SEQ ID NO: 35) (size can be 50-5000 T (SEQ ID NO: 36)), or after PCR by any other method, including, but not limited to, DNA ligation or in vitro recombination. Poly(A) tails also provide stability to RNAs and reduce their degradation. Generally, the length of a poly(A) tail positively correlates with the stability of the transcribed RNA. In one embodiment, the poly(A) tail is between 100 and 5000 adenosines (SEQ ID
30 NO: 37).

Poly(A) tails of RNAs can be further extended following in vitro transcription with the use of a poly(A) polymerase, such as E. coli polyA polymerase (E-PAP). In one embodiment, increasing the length of a poly(A) tail from 100 nucleotides to between 300 and 400 nucleotides (SEQ ID NO: 38) results in about a two-fold increase in the translation efficiency of the RNA. Additionally, the
35 attachment of different chemical groups to the 3' end can increase mRNA stability. Such attachment can contain modified/artificial nucleotides, aptamers and other compounds. For example, ATP

analogues can be incorporated into the poly(A) tail using poly(A) polymerase. ATP analogues can further increase the stability of the RNA.

5' caps also provide stability to RNA molecules. In a preferred embodiment, RNAs produced by the methods disclosed herein include a 5' cap. The 5' cap is provided using techniques known in the art and described herein (Cougot, et al., Trends in Biochem. Sci., 29:436-444 (2001); Stepinski, et al., RNA, 7:1468-95 (2001); Elango, et al., Biochim. Biophys. Res. Commun., 330:958-966 (2005)).

The RNAs produced by the methods disclosed herein can also contain an internal ribosome entry site (IRES) sequence. The IRES sequence may be any viral, chromosomal or artificially designed sequence which initiates cap-independent ribosome binding to mRNA and facilitates the initiation of translation. Any solutes suitable for cell electroporation, which can contain factors facilitating cellular permeability and viability such as sugars, peptides, lipids, proteins, antioxidants, and surfactants can be included.

RNA can be introduced into target cells using any of a number of different methods, for instance, commercially available methods which include, but are not limited to, electroporation (Amaza Nucleofector-II (Amaza Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendorf, Hamburg Germany), cationic liposome mediated transfection using lipofection, polymer encapsulation, peptide mediated transfection, or biolistic particle delivery systems such as “gene guns” (see, for example, Nishikawa, et al. Hum Gene Ther., 12(8):861-70 (2001)).

Non-Viral Delivery Methods

In some aspects, non-viral methods can be used to deliver a nucleic acid encoding a CAR described herein into a cell or tissue or a subject.

In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced out of a longer nucleic acid and inserted into another place in a genome. For example, a transposon comprises a DNA sequence made up of inverted repeats flanking genes for transposition.

Exemplary methods of nucleic acid delivery using a transposon include a Sleeping Beauty transposon system (SBTS) and a piggyBac (PB) transposon system. See, e.g., Aronovich et al. Hum. Mol. Genet. 20.R1(2011):R14-20; Singh et al. Cancer Res. 15(2008):2961–2971; Huang et al. Mol. Ther. 16(2008):580–589; Grabundzija et al. Mol. Ther. 18(2010):1200–1209; Kebriaei et al. Blood. 122.21(2013):166; Williams. Molecular Therapy 16.9(2008):1515–16; Bell et al. Nat. Protoc. 2.12(2007):3153-65; and Ding et al. Cell. 122.3(2005):473-83, all of which are incorporated herein by reference.

The SBTS includes two components: 1) a transposon containing a transgene and 2) a source of transposase enzyme. The transposase can transpose the transposon from a carrier plasmid (or other donor DNA) to a target DNA, such as a host cell chromosome/genome. For example, the transposase binds to the carrier plasmid/donor DNA, cuts the transposon (including transgene(s)) out of the plasmid, and inserts it into the genome of the host cell. See, e.g., Aronovich et al. *supra*.

Exemplary transposons include a pT2-based transposon. See, e.g., Grabundzija et al. *Nucleic Acids Res.* 41.3(2013):1829-47; and Singh et al. *Cancer Res.* 68.8(2008): 2961–2971, all of which are incorporated herein by reference. Exemplary transposases include a Tc1/mariner-type transposase, e.g., the SB10 transposase or the SB11 transposase (a hyperactive transposase which can be expressed, e.g., from a cytomegalovirus promoter). See, e.g., Aronovich et al.; Kebriaei et al.; and Grabundzija et al., all of which are incorporated herein by reference.

Use of the SBTS permits efficient integration and expression of a transgene, e.g., a nucleic acid encoding a CAR described herein. Provided herein are methods of generating a cell, e.g., T cell or NK cell, that stably expresses a CAR described herein, e.g., using a transposon system such as SBTS.

In accordance with methods described herein, in some embodiments, one or more nucleic acids, e.g., plasmids, containing the SBTS components are delivered to a cell (e.g., T or NK cell). For example, the nucleic acid(s) are delivered by standard methods of nucleic acid (e.g., plasmid DNA) delivery, e.g., methods described herein, e.g., electroporation, transfection, or lipofection. In some embodiments, the nucleic acid contains a transposon comprising a transgene, e.g., a nucleic acid encoding a CAR described herein. In some embodiments, the nucleic acid contains a transposon comprising a transgene (e.g., a nucleic acid encoding a CAR described herein) as well as a nucleic acid sequence encoding a transposase enzyme. In other embodiments, a system with two nucleic acids is provided, e.g., a dual-plasmid system, e.g., where a first plasmid contains a transposon comprising a transgene, and a second plasmid contains a nucleic acid sequence encoding a transposase enzyme. For example, the first and the second nucleic acids are co-delivered into a host cell.

In some embodiments, cells, e.g., T or NK cells, are generated that express a CAR described herein by using a combination of gene insertion using the SBTS and genetic editing using a nuclease (e.g., Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system, or engineered meganuclease re-engineered homing endonucleases).

In some embodiments, use of a non-viral method of delivery permits reprogramming of cells, e.g., T or NK cells, and direct infusion of the cells into a subject. Advantages of non-viral vectors include but are not limited to the ease and relatively low cost of producing sufficient amounts required to meet a patient population, stability during storage, and lack of immunogenicity.

Nucleic Acid Constructs Encoding a CAR

The present invention also provides nucleic acid molecules encoding one or more CAR constructs described herein. In one aspect, the nucleic acid molecule is provided as a messenger RNA transcript. In one aspect, the nucleic acid molecule is provided as a DNA construct.

5 Accordingly, in one aspect, the invention pertains to a nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain that binds to a tumor antigen described herein, a transmembrane domain (e.g., a transmembrane domain described herein), and an intracellular signaling domain (e.g., an intracellular signaling domain described herein) comprising a stimulatory domain, e.g., a costimulatory signaling domain (e.g., a
10 costimulatory signaling domain described herein) and/or a primary signaling domain (e.g., a primary signaling domain described herein, e.g., a zeta chain described herein). In one embodiment, the transmembrane domain is transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In some embodiments,
15 a transmembrane domain may include at least the transmembrane region(s) of, e.g., KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2R beta, IL2R gamma, IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29,
20 ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp.

In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO: 12, or
25 a sequence with 95-99% identity thereof. In one embodiment, the antigen binding domain is connected to the transmembrane domain by a hinge region, e.g., a hinge described herein. In one embodiment, the hinge region comprises SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:10, or a sequence with 95-99% identity thereof. In one embodiment, the isolated nucleic acid molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the
30 costimulatory domain is a functional signaling domain of a protein selected from the group consisting of OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137). Further examples of such costimulatory molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D,
35 ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1,

CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, and PAG/Cbp. In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO:16, or a sequence with 95-99% identity thereof. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of CD3 zeta. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO: 14 or SEQ ID NO:16, or a sequence with 95-99% identity thereof, and the sequence of SEQ ID NO: 18 or SEQ ID NO:20, or a sequence with 95-99% identity thereof, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain.

In another aspect, the invention pertains to an isolated nucleic acid molecule encoding a CAR construct comprising a leader sequence of SEQ ID NO: 2, a scFv domain as described herein, a hinge region of SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:10 (or a sequence with 95-99% identity thereof), a transmembrane domain having a sequence of SEQ ID NO: 12 (or a sequence with 95-99% identity thereof), a 4-1BB costimulatory domain having a sequence of SEQ ID NO:14 or a CD27 costimulatory domain having a sequence of SEQ ID NO:16 (or a sequence with 95-99% identity thereof), and a CD3 zeta stimulatory domain having a sequence of SEQ ID NO:18 or SEQ ID NO:20 (or a sequence with 95-99% identity thereof).

In another aspect, the invention pertains to a nucleic acid molecule encoding a chimeric antigen receptor (CAR) molecule that comprises an antigen binding domain, a transmembrane domain, and an intracellular signaling domain comprising a stimulatory domain, and wherein said antigen binding domain binds to a tumor antigen selected from a group consisting of: CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1 (CLECL1), CD33, EGFRvIII, GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PRSS21, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, TSHR, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, MAGE-A1, legumain, HPV E6,E7, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostatein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a,

CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, and IGLL1.

In one embodiment, the encoded CAR molecule further comprises a sequence encoding a costimulatory domain. In one embodiment, the costimulatory domain is a functional signaling domain of a protein selected from the group consisting of OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137). In one embodiment, the costimulatory domain comprises a sequence of SEQ ID NO: 14. In one embodiment, the transmembrane domain is a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154. In one embodiment, the transmembrane domain comprises a sequence of SEQ ID NO:12. In one embodiment, the intracellular signaling domain comprises a functional signaling domain of 4-1BB and a functional signaling domain of zeta. In one embodiment, the intracellular signaling domain comprises the sequence of SEQ ID NO: 14 and the sequence of SEQ ID NO: 18, wherein the sequences comprising the intracellular signaling domain are expressed in the same frame and as a single polypeptide chain. In one embodiment, the anti-a cancer associated antigen as described herein binding domain is connected to the transmembrane domain by a hinge region. In one embodiment, the hinge region comprises SEQ ID NO:4. In one embodiment, the hinge region comprises SEQ ID NO:6 or SEQ ID NO:8 or SEQ ID NO:10.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

The present invention also provides vectors in which a DNA of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. A retroviral vector may also be, e.g., a gammaretroviral vector. A gammaretroviral vector may include, e.g., a promoter, a packaging signal (ψ), a primer binding site (PBS), one or more (e.g., two) long terminal repeats (LTR), and a transgene of interest, e.g., a gene encoding a CAR. A gammaretroviral vector may lack viral structural genes such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, e.g., in Tobias Maetzig et al., "Gammaretroviral Vectors: Biology, Technology and Application" Viruses. 2011 Jun; 3(6): 677-713.

In another embodiment, the vector comprising the nucleic acid encoding the desired CAR of the invention is an adenoviral vector (A5/35). In another embodiment, the expression of nucleic acids encoding CARs can be accomplished using of transposons such as sleeping beauty, crisper, CAS9, and zinc finger nucleases. See below June et al. 2009*Nature Reviews Immunology* 9.10: 704-716, is
5 incorporated herein by reference.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and
10 translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by
15 reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors,
20 replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., 2012, *MOLECULAR CLONING: A LABORATORY MANUAL*, volumes 1 -4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors
25 include, but are not limited to, retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene transfer into mammalian cells.
30 For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors
35 are used.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a

number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity
 5 begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription. Exemplary promoters include the CMV IE gene, EF-1 α , ubiquitin C, or phosphoglycerokinase (PGK) promoters.

An example of a promoter that is capable of expressing a CAR encoding nucleic acid molecule in a mammalian T cell is the EF1a promoter. The native EF1a promoter drives expression
 10 of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. The EF1a promoter has been extensively used in mammalian expression plasmids and has been shown to be effective in driving CAR expression from nucleic acid molecules cloned into a lentiviral vector. See, e.g., Milone et al., Mol. Ther. 17(8): 1453–1464 (2009). In one aspect, the EF1a promoter comprises the sequence provided as SEQ ID
 15 NO: 1.

Another example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus
 20 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1 α promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the
 25 invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid
 30 promoter, a progesterone promoter, and a tetracycline promoter.

A vector may also include, e.g., a signal sequence to facilitate secretion, a polyadenylation signal and transcription terminator (e.g., from Bovine Growth Hormone (BGH) gene), an element allowing episomal replication and replication in prokaryotes (e.g. SV40 origin and ColE1 or others known in the art) and/or elements to allow selection (e.g., ampicillin resistance gene and/or zeocin
 35 marker).

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter- driven transcription.

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al., 2012, MOLECULAR CLONING: A LABORATORY MANUAL, volumes 1 -4, Cold Spring Harbor Press, NY). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based

systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized
5 delivery system.

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a
10 liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not
15 limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a “collapsed” structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic
20 hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine (“DMPC”) can be obtained from Sigma, St. Louis, MO; dicetyl phosphate (“DCP”) can be obtained from K & K Laboratories (Plainview, NY); cholesterol (“Choi”) can be
25 obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol (“DMPG”) and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. “Liposome” is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or
30 aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991
35 *Glycobiology* 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar

structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

The present invention further provides a vector comprising a CAR encoding nucleic acid molecule. In one aspect, a CAR vector can be directly transduced into a cell, e.g., a T cell or a NK cell. In one aspect, the vector is a cloning or expression vector, e.g., a vector including, but not limited to, one or more plasmids (*e.g.*, expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the CAR construct in mammalian immune effector cells (e.g., T cells, NK cells). In one aspect, the mammalian T cell is a human T cell. In one aspect, the mammalian NK cell is a human NK cell.

Sources of Cells

Prior to expansion and genetic modification or other modification, a source of cells, e.g., T cells or natural killer (NK) cells, can be obtained from a subject. The term “subject” is intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, monkeys, chimpanzees, dogs, cats, mice, rats, and transgenic species thereof. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors.

In certain aspects of the present disclosure, immune effector cells, e.g., T cells, can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and, optionally, to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations.

Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated “flow-through” centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer’s instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media conditions and compositions, for example those described in Smith et al., “Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS Immune Cell Serum Replacement” *Clinical & Translational Immunology* (2015) 4, e31; doi:10.1038/cti.2014.31.

In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLLTM gradient or by counterflow centrifugal elutriation.

The methods described herein can include, e.g., selection of a specific subpopulation of immune effector cells, e.g., T cells, that are a T regulatory cell-depleted population, CD25+ depleted cells, using, e.g., a negative selection technique, e.g., described herein. Preferably, the population of T regulatory depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

In one embodiment, T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. In one embodiment, the anti-CD25 antibody, or fragment thereof, or CD25-binding ligand is conjugated to a substrate, e.g., a bead, or is otherwise coated on a substrate, e.g., a bead. In one embodiment, the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate as described herein.

In one embodiment, the T regulatory cells, e.g., CD25+ T cells, are removed from the population using CD25 depletion reagent from MiltenyiTM. In one embodiment, the ratio of cells to CD25 depletion reagent is 1e7 cells to 20 uL, or 1e7 cells to 15 uL, or 1e7 cells to 10 uL, or 1e7 cells to 5 uL, or 1e7 cells to 2.5 uL, or 1e7 cells to 1.25 uL. In one embodiment, e.g., for T regulatory cells, e.g., CD25+ depletion, greater than 500 million cells/ml is used. In a further aspect, a concentration of cells of 600, 700, 800, or 900 million cells/ml is used.

In one embodiment, the population of immune effector cells to be depleted includes about 6 x 10⁹ CD25+ T cells. In other aspects, the population of immune effector cells to be depleted include about 1 x 10⁹ to 1x 10¹⁰ CD25+ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2 x 10⁹ T regulatory cells, e.g., CD25+ cells, or less (e.g., 1 x 10⁹, 5 x 10⁸, 1 x 10⁸, 5 x 10⁷, 1 x 10⁷, or less CD25+ cells).

In one embodiment, the T regulatory cells, e.g., CD25+ cells, are removed from the population using the CliniMAC system with a depletion tubing set, such as, e.g., tubing 162-01. In one embodiment, the CliniMAC system is run on a depletion setting such as, e.g., DEPLETION2.1.

Without wishing to be bound by a particular theory, decreasing the level of negative regulators of immune cells (e.g., decreasing the number of unwanted immune cells, e.g., T_{REG} cells), in a subject prior to apheresis or during manufacturing of a CAR-expressing cell product can reduce the risk of subject relapse. For example, methods of depleting T_{REG} cells are known in the art. Methods of decreasing T_{REG} cells include, but are not limited to, cyclophosphamide, anti-GITR antibody (an anti-GITR antibody described herein), CD25-depletion, and combinations thereof.

In some embodiments, the manufacturing methods comprise reducing the number of (e.g., depleting) T_{REG} cells prior to manufacturing of the CAR-expressing cell. For example, manufacturing methods comprise contacting the sample, e.g., the apheresis sample, with an anti-GITR antibody and/or an anti-CD25 antibody (or fragment thereof, or a CD25-binding ligand), e.g., to deplete T_{REG} cells prior to manufacturing of the CAR-expressing cell (e.g., T cell, NK cell) product.

In an embodiment, a subject is pre-treated with one or more therapies that reduce T_{REG} cells prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, methods of decreasing T_{REG} cells include, but are not limited to, administration to the subject of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof. Administration of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof, can occur before, during or after an infusion of the CAR-expressing cell product.

In an embodiment, a subject is pre-treated with cyclophosphamide prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, a subject is pre-treated with an anti-GITR antibody prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment.

In one embodiment, the population of cells to be removed are neither the regulatory T cells or tumor cells, but cells that otherwise negatively affect the expansion and/or function of CART cells, e.g. cells expressing CD14, CD11b, CD33, CD15, or other markers expressed by potentially immune suppressive cells. In one embodiment, such cells are envisioned to be removed concurrently with regulatory T cells and/or tumor cells, or following said depletion, or in another order.

The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the

cells negatively selected. For example, to enrich for CD4⁺ cells by negative selection, a monoclonal antibody cocktail can include antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8.

The methods described herein can further include removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CD11b, to thereby provide a population of T regulatory depleted, e.g., CD25⁺ depleted, and tumor antigen depleted cells that are suitable for expression of a CAR, e.g., a CAR described herein. In one embodiment, tumor antigen expressing cells are removed simultaneously with the T regulatory, e.g., CD25⁺ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-tumor antigen antibody, or fragment thereof, can be attached to the same substrate, e.g., bead, which can be used to remove the cells or an anti-CD25 antibody, or fragment thereof, or the anti-tumor antigen antibody, or fragment thereof, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25⁺ cells, and the removal of the tumor antigen expressing cells is sequential, and can occur, e.g., in either order.

Also provided are methods that include removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more of PD1⁺ cells, LAG3⁺ cells, and TIM3⁺ cells, to thereby provide a population of T regulatory depleted, e.g., CD25⁺ depleted cells, and check point inhibitor depleted cells, e.g., PD1⁺, LAG3⁺ and/or TIM3⁺ depleted cells. Exemplary check point inhibitors include B7-H1, B7-1, CD160, P1H, 2B4, PD1, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, TIGIT, CTLA-4, BTLA and LAIR1. In one embodiment, check point inhibitor expressing cells are removed simultaneously with the T regulatory, e.g., CD25⁺ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-check point inhibitor antibody, or fragment thereof, can be attached to the same bead which can be used to remove the cells, or an anti-CD25 antibody, or fragment thereof, and the anti-check point inhibitor antibody, or fragment thereof, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25⁺ cells, and the removal of the check point inhibitor expressing cells is sequential, and can occur, e.g., in either order.

Methods described herein can include a positive selection step. For example, T cells can be isolated by incubation with anti-CD3/anti-CD28 (e.g., 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another embodiment, the time period is 10 to 24 hours, e.g., 24 hours. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals.

Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other
 5 time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points.

In one embodiment, a T cell population can be selected that expresses one or more of IFN- γ , TNF α , IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other
 10 appropriate molecules, e.g., other cytokines. Methods for screening for cell expression can be determined, e.g., by the methods described in PCT Publication No.: WO 2013/126712.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain aspects, it may be desirable to significantly decrease the volume in which beads and cells are mixed together
 15 (e.g., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one aspect, a concentration of 10 billion cells/ml, 9 billion/ml, 8 billion/ml, 7 billion/ml, 6 billion/ml, or 5 billion/ml is used. In one aspect, a concentration of 1 billion cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used.

Using high concentrations can result in increased cell yield, cell activation, and cell
 20 expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (e.g., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration
 25 of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In a related aspect, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more
 30 efficiently captured than CD8+ T cells in dilute concentrations. In one aspect, the concentration of cells used is 5×10^6 /ml. In other aspects, the concentration used can be from about 1×10^5 /ml to 1×10^6 /ml, and any integer value in between.

In other aspects, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by
 35 theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that

removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C or in liquid nitrogen.

In certain aspects, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in immune effector cell therapy for any number of diseases or conditions that would benefit from immune effector cell therapy, such as those described herein. In one aspect a blood sample or an apheresis is taken from a generally healthy subject. In certain aspects, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain aspects, the T cells may be expanded, frozen, and used at a later time. In certain aspects, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further aspect, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

In a further aspect of the present invention, T cells are obtained from a patient directly following treatment that leaves the subject with functional T cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo. Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or

other cells of the hematopoietic lineage, during this recovery phase. Further, in certain aspects, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy.

5 Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

In one embodiment, the immune effector cells expressing a CAR molecule, e.g., a CAR molecule described herein, are obtained from a subject that has received a low, immune enhancing dose of an mTOR inhibitor. In an embodiment, the population of immune effector cells, e.g., T cells, to be engineered to express a CAR, are harvested after a sufficient time, or after sufficient dosing of
10 the low, immune enhancing, dose of an mTOR inhibitor, such that the level of PD1 negative immune effector cells, e.g., T cells, or the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells, in the subject or harvested from the subject has been, at least transiently, increased.

In other embodiments, population of immune effector cells, e.g., T cells, which have, or will
15 be engineered to express a CAR, can be treated ex vivo by contact with an amount of an mTOR inhibitor that increases the number of PD1 negative immune effector cells, e.g., T cells or increases the ratio of PD1 negative immune effector cells, e.g., T cells/ PD1 positive immune effector cells, e.g., T cells.

In one embodiment, a T cell population is diacylglycerol kinase (DGK)-deficient. DGK-
20 deficient cells include cells that do not express DGK RNA or protein, or have reduced or inhibited DGK activity. DGK-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent DGK expression. Alternatively, DGK-deficient cells can be generated by treatment with DGK inhibitors described herein.

25 In one embodiment, a T cell population is Ikaros-deficient. Ikaros-deficient cells include cells that do not express Ikaros RNA or protein, or have reduced or inhibited Ikaros activity, Ikaros-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent Ikaros expression. Alternatively, Ikaros-deficient cells can be generated by treatment with Ikaros inhibitors, e.g., lenalidomide.

30 In embodiments, a T cell population is DGK-deficient and Ikaros-deficient, e.g., does not express DGK and Ikaros, or has reduced or inhibited DGK and Ikaros activity. Such DGK and Ikaros-deficient cells can be generated by any of the methods described herein.

In an embodiment, the NK cells are obtained from the subject. In another embodiment, the NK cells are an NK cell line, e.g., NK-92 cell line (Conkwest).

35

Allogeneic CAR

In embodiments described herein, the immune effector cell can be an allogeneic immune effector cell, e.g., T cell or NK cell. For example, the cell can be an allogeneic T cell, e.g., an allogeneic T cell lacking expression of a functional T cell receptor (TCR) and/or human leukocyte antigen (HLA), e.g., HLA class I and/or HLA class II.

A T cell lacking a functional TCR can be, e.g., engineered such that it does not express any functional TCR on its surface, engineered such that it does not express one or more subunits that comprise a functional TCR or engineered such that it produces very little functional TCR on its surface. Alternatively, the T cell can express a substantially impaired TCR, e.g., by expression of mutated or truncated forms of one or more of the subunits of the TCR. The term “substantially impaired TCR” means that this TCR will not elicit an adverse immune reaction in a host.

A T cell described herein can be, e.g., engineered such that it does not express a functional HLA on its surface. For example, a T cell described herein, can be engineered such that cell surface expression HLA, e.g., HLA class I and/or HLA class II, is downregulated.

In some embodiments, the T cell can lack a functional TCR and a functional HLA, e.g., HLA class I and/or HLA class II.

Modified T cells that lack expression of a functional TCR and/or HLA can be obtained by any suitable means, including a knock out or knock down of one or more subunit of TCR or HLA. For example, the T cell can include a knock down of TCR and/or HLA using siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcription-activator like effector nuclease (TALEN), or zinc finger endonuclease (ZFN).

In some embodiments, the allogeneic cell can be a cell which does not express or expresses at low levels an inhibitory molecule, e.g. by any method described herein. For example, the cell can be a cell that does not express or expresses at low levels an inhibitory molecule, e.g., that can decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4 and TGF beta. Inhibition of an inhibitory molecule, e.g., by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used.

siRNA and shRNA to inhibit TCR or HLA

In some embodiments, TCR expression and/or HLA expression can be inhibited using siRNA or shRNA that targets a nucleic acid encoding a TCR and/or HLA in a T cell.

Expression of siRNA and shRNAs in T cells can be achieved using any conventional expression system, e.g., such as a lentiviral expression system.

Exemplary shRNAs that downregulate expression of components of the TCR are described, e.g., in US Publication No.: 2012/0321667. Exemplary siRNA and shRNA that downregulate expression of HLA class I and/or HLA class II genes are described, e.g., in U.S. publication No.: US 2007/0036773.

5 ***CRISPR to inhibit TCR or HLA***

“CRISPR” or “CRISPR to TCR and/or HLA” or “CRISPR to inhibit TCR and/or HLA” as used herein refers to a set of clustered regularly interspaced short palindromic repeats, or a system comprising such a set of repeats. “Cas”, as used herein, refers to a CRISPR-associated protein. A “CRISPR/Cas” system refers to a system derived from CRISPR and Cas which can be used to silence
10 or mutate a TCR and/or HLA gene.

Naturally-occurring CRISPR/Cas systems are found in approximately 40% of sequenced eubacteria genomes and 90% of sequenced archaea. Grissa et al. (2007) BMC Bioinformatics 8: 172. This system is a type of prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Barrangou et al.
15 (2007) Science 315: 1709-1712; Marragini et al. (2008) Science 322: 1843-1845.

The CRISPR/Cas system has been modified for use in gene editing (silencing, enhancing or changing specific genes) in eukaryotes such as mice or primates. Wiedenheft et al. (2012) Nature 482: 331-8. This is accomplished by introducing into the eukaryotic cell a plasmid containing a specifically designed CRISPR and one or more appropriate Cas.

20 The CRISPR sequence, sometimes called a CRISPR locus, comprises alternating repeats and spacers. In a naturally-occurring CRISPR, the spacers usually comprise sequences foreign to the bacterium such as a plasmid or phage sequence; in the TCR and/or HLA CRISPR/Cas system, the spacers are derived from the TCR or HLA gene sequence.

RNA from the CRISPR locus is constitutively expressed and processed by Cas proteins into
25 small RNAs. These comprise a spacer flanked by a repeat sequence. The RNAs guide other Cas proteins to silence exogenous genetic elements at the RNA or DNA level. Horvath et al. (2010) Science 327: 167-170; Makarova et al. (2006) Biology Direct 1: 7. The spacers thus serve as templates for RNA molecules, analogously to siRNAs. Pennisi (2013) Science 341: 833-836.

As these naturally occur in many different types of bacteria, the exact arrangements of the
30 CRISPR and structure, function and number of Cas genes and their product differ somewhat from species to species. Haft et al. (2005) PLoS Comput. Biol. 1: e60; Kunin et al. (2007) Genome Biol. 8: R61; Mojica et al. (2005) J. Mol. Evol. 60: 174-182; Bolotin et al. (2005) Microbiol. 151: 2551-2561; Pourcel et al. (2005) Microbiol. 151: 653-663; and Stern et al. (2010) Trends. Genet. 28: 335-340. For example, the Cse (Cas subtype, E. coli) proteins (e.g., CasA) form a functional complex, Cascade, that processes CRISPR RNA transcripts into spacer-repeat units that Cascade retains. Brouns et al.
35 (2008) Science 321: 960-964. In other prokaryotes, Cas6 processes the CRISPR transcript. The CRISPR-based phage inactivation in E. coli requires Cascade and Cas3, but not Cas1 or Cas2. The

Cmr (Cas RAMP module) proteins in *Pyrococcus furiosus* and other prokaryotes form a functional complex with small CRISPR RNAs that recognizes and cleaves complementary target RNAs. A simpler CRISPR system relies on the protein Cas9, which is a nuclease with two active cutting sites, one for each strand of the double helix. Combining Cas9 and modified CRISPR locus RNA can be
 5 used in a system for gene editing. Pennisi (2013) *Science* 341: 833-836.

The CRISPR/Cas system can thus be used to edit a TCR and/or HLA gene (adding or deleting a basepair), or introducing a premature stop which thus decreases expression of a TCR and/or HLA. The CRISPR/Cas system can alternatively be used like RNA interference, turning off TCR and/or HLA gene in a reversible fashion. In a mammalian cell, for example, the RNA can guide the Cas
 10 protein to a TCR and/or HLA promoter, sterically blocking RNA polymerases.

Artificial CRISPR/Cas systems can be generated which inhibit TCR and/or HLA, using technology known in the art, e.g., that described in U.S. Publication No. 20140068797, and Cong (2013) *Science* 339: 819-823. Other artificial CRISPR/Cas systems that are known in the art may also be generated which inhibit TCR and/or HLA, e.g., that described in Tsai (2014) *Nature Biotechnol.*,
 15 32:6 569-576, U.S. Patent No.: 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359.

TALEN to inhibit TCR and/or HLA

“TALEN” or “TALEN to HLA and/or TCR” or “TALEN to inhibit HLA and/or TCR” refers to a transcription activator-like effector nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene.

TALENs are produced artificially by fusing a TAL effector DNA binding domain to a DNA cleavage domain. Transcription activator-like effects (TALEs) can be engineered to bind any desired DNA sequence, including a portion of the HLA or TCR gene. By combining an engineered TALE with a DNA cleavage domain, a restriction enzyme can be produced which is specific to any desired DNA sequence, including a HLA or TCR sequence. These can then be introduced into a cell, wherein
 20 they can be used for genome editing. Boch (2011) *Nature Biotech.* 29: 135-6; and Boch et al. (2009) *Science* 326: 1509-12; Moscou et al. (2009) *Science* 326: 3501.

TALEs are proteins secreted by *Xanthomonas* bacteria. The DNA binding domain contains a repeated, highly conserved 33-34 amino acid sequence, with the exception of the 12th and 13th amino acids. These two positions are highly variable, showing a strong correlation with specific nucleotide
 30 recognition. They can thus be engineered to bind to a desired DNA sequence.

To produce a TALEN, a TALE protein is fused to a nuclease (N), which is a wild-type or mutated FokI endonuclease. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. Cermak et al. (2011) *Nucl. Acids Res.* 39: e82; Miller et al. (2011) *Nature Biotech.* 29: 143-8; Hockemeyer et al. (2011) *Nature Biotech.* 29: 731-734;
 35 Wood et al. (2011) *Science* 333: 307; Doyon et al. (2010) *Nature Methods* 8: 74-79; Szczeppek et al. (2007) *Nature Biotech.* 25: 786-793; and Guo et al. (2010) *J. Mol. Biol.* 200: 96.

The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) *Nature Biotech.* 29: 143-8.

A HLA or TCR TALEN can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break via non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, foreign DNA can be introduced into the cell along with the TALEN; depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to correct a defect in the HLA or TCR gene or introduce such a defect into a wt HLA or TCR gene, thus decreasing expression of HLA or TCR.

TALENs specific to sequences in HLA or TCR can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) *Nature Biotech.* 29: 149-53; Geibler et al. (2011) *PLoS ONE* 6: e19509.

Zinc finger nuclease to inhibit HLA and/or TCR

“ZFN” or “Zinc Finger Nuclease” or “ZFN to HLA and/or TCR” or “ZFN to inhibit HLA and/or TCR” refer to a zinc finger nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene.

Like a TALEN, a ZFN comprises a FokI nuclease domain (or derivative thereof) fused to a DNA-binding domain. In the case of a ZFN, the DNA-binding domain comprises one or more zinc fingers. Carroll et al. (2011) *Genetics Society of America* 188: 773-782; and Kim et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 1156-1160.

A zinc finger is a small protein structural motif stabilized by one or more zinc ions. A zinc finger can comprise, for example, Cys2His2, and can recognize an approximately 3-bp sequence. Various zinc fingers of known specificity can be combined to produce multi-finger polypeptides which recognize about 6, 9, 12, 15 or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Like a TALEN, a ZFN must dimerize to cleave DNA. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. Bitinaite et al. (1998) *Proc. Natl. Acad. Sci. USA* 95: 10570-5.

Also like a TALEN, a ZFN can create a double-stranded break in the DNA, which can create a frame-shift mutation if improperly repaired, leading to a decrease in the expression and amount of

HLA and/or TCR in a cell. ZFNs can also be used with homologous recombination to mutate in the HLA or TCR gene.

ZFNs specific to sequences in HLA AND/OR TCR can be constructed using any method known in the art. See, e.g., Provasi (2011) *Nature Med.* 18: 807-815; Torikai (2013) *Blood* 122: 1341-1349; Cathomen et al. (2008) *Mol. Ther.* 16: 1200-7; Guo et al. (2010) *J. Mol. Biol.* 400: 96; U.S. Patent Publication 2011/0158957; and U.S. Patent Publication 2012/0060230.

Telomerase expression

While not wishing to be bound by any particular theory, in some embodiments, a therapeutic T cell has short term persistence in a patient, due to shortened telomeres in the T cell; accordingly, transfection with a telomerase gene can lengthen the telomeres of the T cell and improve persistence of the T cell in the patient. See Carl June, "Adoptive T cell therapy for cancer in the clinic", *Journal of Clinical Investigation*, 117:1466-1476 (2007). Thus, in an embodiment, an immune effector cell, e.g., a T cell, ectopically expresses a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. In some aspects, this disclosure provides a method of producing a CAR-expressing cell, comprising contacting a cell with a nucleic acid encoding a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. The cell may be contacted with the nucleic acid before, simultaneous with, or after being contacted with a construct encoding a CAR.

In one aspect, the disclosure features a method of making a population of immune effector cells (e.g., T cells, NK cells). In an embodiment, the method comprises: providing a population of immune effector cells (e.g., T cells or NK cells), contacting the population of immune effector cells with a nucleic acid encoding a CAR; and contacting the population of immune effector cells with a nucleic acid encoding a telomerase subunit, e.g., hTERT, under conditions that allow for CAR and telomerase expression.

In an embodiment, the nucleic acid encoding the telomerase subunit is DNA. In an embodiment, the nucleic acid encoding the telomerase subunit comprises a promoter capable of driving expression of the telomerase subunit.

In an embodiment, hTERT has the amino acid sequence of GenBank Protein ID AAC51724.1 (Meyerson et al., "hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization" *Cell* Volume 90, Issue 4, 22 August 1997, Pages 785–795) as follows:

MPRAPRCRAVRSLLRSHYREVLPLATFVRRLGPQGWRLVQRGDPAAFRALVAQCLVCVPW
DARPPPAAPSFRQVSCLKELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFTTSVRSY
LPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGAAT
QARPPPHASGPRRRLGCERAWNHSVREAGVPLGLPAPGARRRGGSASRSLPLPKRPRRGAAP
EPERTPVGQGSWAHPGRTRGPSDRGFCVVSPARPAEEATSLEGALSGTRHSHPSVGRQHHAG
PPSTSRRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPW
MPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPKQ

GSVAAP EEEDTDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKK
 FISLGKHAKLSLQELTWKMSVRGCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVV
 ELLRSFFYVTETTFQKNRLFFYRKS VWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALL
 TSRLRFIPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASV
 5 LGLDDIHRAWRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRR
 YAVVQKAAHGHVRKAFKSHVSTLTDLPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSG
 LFDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLV
 DDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFVVEDEALGGTAFVQMPAHGLF
 PWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGLVRLKCHSLFLDLQ
 10 VNSLQTVCTNIYKILLQAYRFHACVLQLPFHQVWKNPTFFLRVISDTASLCYSILKAKNAG
 MSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLP GTTLTA
 LEAAANPALPSDFKTILD (SEQ ID NO: 63)

In an embodiment, the hTERT has a sequence at least 80%, 85%, 90%, 95%, 96[^], 97%, 98%,
 or 99% identical to the sequence of SEQ ID NO: 63. In an embodiment, the hTERT has a sequence
 15 of SEQ ID NO: 63. In an embodiment, the hTERT comprises a deletion (e.g., of no more than 5, 10,
 15, 20, or 30 amino acids) at the N-terminus, the C-terminus, or both. In an embodiment, the hTERT
 comprises a transgenic amino acid sequence (e.g., of no more than 5, 10, 15, 20, or 30 amino acids) at
 the N-terminus, the C-terminus, or both.

In an embodiment, the hTERT is encoded by the nucleic acid sequence of GenBank
 20 Accession No. AF018167 (Meyerson et al., “hEST2, the Putative Human Telomerase Catalytic
 Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization” Cell Volume 90, Issue 4,
 22 August 1997, Pages 785–795):

	1	caggcagcgt	ggtcctgctg	cgcacgtggg	aagccctggc	cccggccacc	cccgcgatgc
	61	cgcgcgctcc	ccgctgccga	gccgtgcgct	ccctgctgcg	cagccactac	cgcgaggtgc
25	121	tgccgctggc	cacgttcgctg	cggcgccctgg	ggccccaggg	ctggcggtctg	gtgcagcgcg
	181	gggacccggc	ggctttccgc	gcgctggtgg	cccagtgcct	ggtgtgcgtg	ccctgggacg
	241	cacggccgcc	ccccgccgcc	ccctccctcc	gccaggtgtc	ctgcctgaag	gagctggtgg
	301	cccagagtgc	gcagaggctg	tgcgagcgcg	gcgcgaagaa	cgtgctggcc	ttcggtctcg
	361	cgtgctgga	cggggcccg	gggggcccc	ccgaggcctt	caccaccagc	gtgcgcagct
30	421	acctgccc	cacggtgacc	gacgcactgc	gggggagcgg	ggcgtggggg	ctgctgttgc
	481	gccgcgtggg	cgacgacgtg	ctggttcacc	tgctggcacg	ctgcgcgctc	tttgtgctgg
	541	tggctcccag	ctgcgcctac	caggtgtgcg	ggccgcgcgt	gtaccagctc	ggcgtgcca
	601	ctcaggcccc	gcccccgcca	cacgctagtg	gacccccgaag	gcgtctggga	tgcgaacggg
	661	cctggaacca	tagcgtcagg	gaggccgggg	tccccctggg	cctgccagcc	ccgggtgcga
35	721	ggaggcgcg	gggcagtgcc	agccgaagtc	tgccgttgcc	caagaggccc	aggcgtggcg
	781	ctgccccctga	gccggagcgg	acgcccgttg	ggcaggggtc	ctgggcccac	ccgggcagga
	841	cgcgtggacc	gagtgaccgt	ggtttctgtg	tgggtgcacc	tgccagacct	gccgaagaag
	901	ccacctcttt	ggaggggtgcg	ctctctggca	cgcgccactc	ccaccatcc	gtgggcccgc
	961	agcaccacgc	gggcccccca	tccacatcgc	ggccaccacg	tccctgggac	acgccttgct
40	1021	ccccggtgta	cgccgagacc	aagcacttcc	tctactcctc	aggcgacaag	gagcagctgc
	1081	ggccctcctt	cctactcagc	tctctgaggc	ccagcctgac	tggcgtcgg	aggctcgtgg
	1141	agaccatctt	tctgggttcc	aggccctgga	tgccagggac	tccccgcagg	ttgccccgcc
	1201	tgccccagcg	ctactggcaa	atgcggcccc	tgtttctgga	gctgcttggg	aaccacgcgc
	1261	agtgccccta	cgggggtgctc	ctcaagacgc	actgcccgcct	gcgagctgcg	gtcacccccag
45	1321	cagccggtgt	ctgtgcccgg	gagaagcccc	agggtctctgt	ggcggcccc	gaggaggagg
	1381	acacagacct	ccgtcgccctg	gtgcagctgc	tccgccagca	cagcagcccc	tggcaggtgt

1441 acggcttcgt gcgggcctgc ctgcgccggc tgggtgcccc aggcctctgg ggctccaggc
 1501 acaacgaacg ccgcttcctc aggaacacca agaagttcat ctccctgggg aagcatgcca
 1561 agctctcgct gcaggagctg acgtggaaga tgagcgtgcg gggctgcgct tggctgcgca
 1621 ggagcccagg ggttggtgtg gtccggcccg cagagcaccg tctgcgtgag gagatcctgg
 5 1681 ccaagttcct gcactggctg atgagtgtgt acgtcgtcga gctgctcagg tctttctttt
 1741 atgtcacgga gaccacgttt caaaagaaca ggctcttttt ctaccggaag agtgtctgga
 1801 gcaagttgca aagcattgga atcagacagc acttgaagag ggtgcagctg cgggagctgt
 1861 cggaagcaga ggtcaggcag catcggaag ccaggcccgc cctgctgacg tccagactcc
 1921 gcttcatccc caagcctgac gggctgcggc cgattgtgaa catggactac gtcgtgggag
 10 1981 ccagaacggt ccgcagagaa aagaggggcg agcgtctcac ctcgagggtg aaggcactgt
 2041 tcagcgtgct caactacgag cgggcgcggc gcccggcct cctgggcgcc tctgtgctgg
 2101 gcctggacga tatccacagg gcctggcgca ccttcgtgct gcgtgtgcgg gcccaggacc
 2161 cgccgcctga gctgtacttt gtcaaggtgg atgtgacggg cgcgtacgac accatcccc
 2221 aggacaggct cacggaggct atcgccagca tcatcaaacc ccagaacacg tactgctgct
 15 2281 gtcggtatgc cgtggctccag aaggccgccc atgggcacgt ccgcaaggcc ttcaagagcc
 2341 acgtctctac cttgacagac ctccagccgt acatgcgaca gttcgtggct cacctgcagg
 2401 agaccagccc gctgagggat gccgtcgtca tcgagcagag ctccctccctg aatgaggcca
 2461 gcagtggcct cttcgacgtc ttctacgct tcatgtgcca ccacgccgtg cgcacaggg
 2521 gcaagtccta cgtccagtgc caggggatcc cgcagggctc catcctctcc acgctgctct
 20 2581 gcagcctgtg ctacggcgac atggagaaca agctgtttgc ggggattcgg cgggacgggc
 2641 tgctcctgcg tttggtggat gatttcttgt tggtgacacc tcacctcacc cacgcgaaaa
 2701 ccttcctcag gaccctggct cgagggtgtcc ctgagtatgg ctgctggtg aacttgcgga
 2761 agacagtggg gaacttcctt gtagaagacg aggcctggg tggcacggct tttgttcaga
 2821 tgccggccca cggcctatcc cctggtgctg gcctgctgct ggatacccgg accctggagg
 25 2881 tgcagagcga ctactccagc tatgcccgga cctccatcag agccagtctc accttcaacc
 2941 gcggcttcaa ggctgggagg aacatgcgtc gcaaactctt tggggctctg cggctgaagt
 3001 gtcacagcct gtttctggat ttgcagggtga acagcctcca gacggtgtgc accaacatct
 3061 acaagatcct cctgctgcag gcgtacaggt ttacgcgatg tgtgctgcag ctcccatctc
 3121 atcagcaagt ttggaagaac cccacatctt tcctgcgcgt catctctgac acggcctccc
 30 3181 tctgctactc catcctgaaa gccaaagaac cagggatgtc gctgggggcc aaggcgccg
 3241 ccggccctct gccctccgag gccgtgcagt ggctgtgcca ccaagcattc ctgctcaagc
 3301 tgactcgaca ccgtgtcacc tacgtgccac tcctggggct actcaggaca gccagacgc
 3361 agctgagtcg gaagctcccg gggacgacgc tgactgccct ggaggccgca gccaacccgg
 3421 cactgccctc agacttcaag accatcctgg actgatggcc acccgcccac agccaggccg
 35 3481 agagcagaca ccagcagccc tgtcacgccg ggctctacgt ccagggaggg gagggcgccg
 3541 ccacacccag gcccgcaccc ctgggagctt gaggcctgag tgagtgtttg gccgaggcct
 3601 gcatgtccgg ctgaaggctg agtgtccggc tgaggcctga gcgagtgtcc agccaagggc
 3661 tgagtgtcca gcacacctgc cgtcttctact tccccacagg ctggcgctcg gctccacccc
 3721 agggccagct tttcctcacc aggagcccgg cttccactcc ccacatagga atagtccatc
 40 3781 cccagattcg ccattgttca cccctcgccc tgccctcctt tgccctccac cccaccatc
 3841 caggtggaga ccctgagaag gaccctggga gctctgggaa tttggagtga ccaaagggtg
 3901 gccctgtaca caggcgagga cctgcacct ggatgggggt ccctgtgggt caaatgggg
 3961 ggagggtgctg tgggagtaaa atactgaata tatgagtttt tcagttttga aaaaaaaaaa
 4021 aaaaaaa (SEQ ID NO: 64)

45 In an embodiment, the hTERT is encoded by a nucleic acid having a sequence at least 80%,
 85%, 90%, 95%, 96, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 64. In an
 embodiment, the hTERT is encoded by a nucleic acid of SEQ ID NO: 64.

Activation and Expansion of Immune Effector Cells (e.g., T Cells)

50 Immune effector cells such as T cells may be activated and expanded generally using methods
 as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358;
 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874;
 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

Generally, a population of immune effector cells e.g., T regulatory cell depleted cells, may be expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a costimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein, such as by contact
 5 with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate
 10 for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody can be used. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):1319-1328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

15 In certain aspects, the primary stimulatory signal and the costimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When coupled to a surface, the agents may be coupled to the same surface (i.e., in “cis” formation) or to separate surfaces (i.e., in “trans” formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one aspect, the agent providing
 20 the costimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain aspects, both agents can be in solution. In one aspect, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial
 25 antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one aspect, the two agents are immobilized on beads, either on the same bead, i.e., “cis,” or to separate beads, i.e., “trans.” By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the
 30 costimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one aspect, a 1:1 ratio of each antibody bound to the beads for CD4+ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio
 35 of 1:1. In one particular aspect an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one aspect, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect, more anti-

CD28 antibody is bound to the particles than anti-CD3 antibody, i.e., the ratio of CD3:CD28 is less than one. In certain aspects, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular aspect, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further aspect, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred aspect, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In one aspect, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet one aspect, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain aspects the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further aspects the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one aspect, a ratio of particles to cells of 1:1 or less is used. In one particular aspect, a preferred particle: cell ratio is 1:5. In further aspects, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one aspect, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular aspect, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In one aspect, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In one aspect, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type. In one aspect, the most typical ratios for use are in the neighborhood of 1:1, 2:1 and 3:1 on the first day.

In further aspects, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative aspect, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further aspect, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one aspect the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, for example PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (i.e., 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain aspects, it may be desirable to significantly decrease the volume in which particles and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one aspect, a concentration of about 10 billion cells/ml, 9 billion/ml, 8 billion/ml, 7 billion/ml, 6 billion/ml, 5 billion/ml, or 2 billion cells/ml is used. In one aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain aspects. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In one embodiment, cells transduced with a nucleic acid encoding a CAR, e.g., a CAR described herein, are expanded, e.g., by a method described herein. In one embodiment, the cells are expanded in culture for a period of several hours (e.g., about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, 21 hours) to about 14 days (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days). In one embodiment, the cells are expanded for a period of 4 to 9 days. In one embodiment, the cells are expanded for a period of 8 days or less, e.g., 7, 6 or 5 days. In one embodiment, the cells, e.g., a CD19 CAR cell described herein, are expanded in culture for 5 days, and the resulting cells are more potent than the same cells expanded in culture for 9 days under the same culture conditions. Potency can be defined, e.g., by various T cell functions, e.g. proliferation, target cell killing, cytokine production, activation, migration, or combinations thereof. In one embodiment, the cells, e.g., a CD19 CAR cell described herein, expanded for 5 days show at least a one, two, three or four fold increase in cells doublings upon antigen stimulation as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., the cells expressing a CD19 CAR described herein, are expanded in culture for 5 days, and the resulting cells exhibit higher proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions. In one embodiment, the cells, e.g., a CD19 CAR

cell described herein, expanded for 5 days show at least a one, two, three, four, five, ten fold or more increase in pg/ml of proinflammatory cytokine production, e.g., IFN- γ and/or GM-CSF levels, as compared to the same cells expanded in culture for 9 days under the same culture conditions.

Several cycles of stimulation may also be desired such that culture time of T cells can be 60
 5 days or more. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN- γ , IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGF β , and TNF- α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are
 10 not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α -MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g.,
 15 penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO₂).

In one embodiment, the cells are expanded in an appropriate media (e.g., media described herein) that includes one or more interleukin that result in at least a 200-fold (e.g., 200-fold, 250-fold,
 20 300-fold, 350-fold) increase in cells over a 14 day expansion period, e.g., as measured by a method described herein such as flow cytometry. In one embodiment, the cells are expanded in the presence of IL-15 and/or IL-7 (e.g., IL-15 and IL-7).

In embodiments, methods described herein, e.g., CAR-expressing cell manufacturing methods, comprise removing T regulatory cells, e.g., CD25+ T cells, from a cell population, e.g.,
 25 using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. Methods of removing T regulatory cells, e.g., CD25+ T cells, from a cell population are described herein. In embodiments, the methods, e.g., manufacturing methods, further comprise contacting a cell population (e.g., a cell population in which T regulatory cells, such as CD25+ T cells, have been depleted; or a cell population that has previously contacted an anti-CD25 antibody, fragment thereof,
 30 or CD25-binding ligand) with IL-15 and/or IL-7. For example, the cell population (e.g., that has previously contacted an anti-CD25 antibody, fragment thereof, or CD25-binding ligand) is expanded in the presence of IL-15 and/or IL-7.

In some embodiments a CAR-expressing cell described herein is contacted with a composition comprising a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-
 35 15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15, during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a IL-15

polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted with a composition comprising a combination of both a IL-15 polypeptide and a IL-15 Ra polypeptide during the manufacturing of the CAR-expressing cell, e.g., ex vivo. In embodiments, a CAR-expressing cell described herein is contacted
 5 with a composition comprising hetIL-15 during the manufacturing of the CAR-expressing cell, e.g., ex vivo.

In one embodiment the CAR-expressing cell described herein is contacted with a composition comprising hetIL-15 during ex vivo expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising an IL-15 polypeptide during ex vivo
 10 expansion. In an embodiment, the CAR-expressing cell described herein is contacted with a composition comprising both an IL-15 polypeptide and an IL-15Ra polypeptide during ex vivo expansion. In one embodiment the contacting results in the survival and proliferation of a lymphocyte subpopulation, e.g., CD8+ T cells.

T cells that have been exposed to varied stimulation times may exhibit different
 15 characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (TH, CD4+) that is greater than the cytotoxic or suppressor T cell population (TC, CD8+). Ex vivo expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of TH cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of TC
 20 cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell population comprising predominately of TH cells may be advantageous. Similarly, if an antigen-specific subset of TC cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such
 25 reproducibility enables the ability to tailor an activated T cell product for specific purposes.

Once a CAR described herein is constructed, various assays can be used to evaluate the activity of the molecule, such as but not limited to, the ability to expand T cells following antigen stimulation, sustain T cell expansion in the absence of re-stimulation, and anti-cancer activities in appropriate in vitro and animal models. Assays to evaluate the effects of a cars of the present
 30 invention are described in further detail below

Western blot analysis of CAR expression in primary T cells can be used to detect the presence of monomers and dimers. See, e.g., Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, T cells (1:1 mixture of CD4⁺ and CD8⁺ T cells) expressing the CARs are expanded *in vitro* for more than 10 days followed by lysis and SDS-PAGE under reducing conditions. CARs containing
 35 the full length TCR- ζ cytoplasmic domain and the endogenous TCR- ζ chain are detected by western blotting using an antibody to the TCR- ζ chain. The same T cell subsets are used for SDS-PAGE analysis under non-reducing conditions to permit evaluation of covalent dimer formation.

In vitro expansion of CAR⁺ T cells following antigen stimulation can be measured by flow cytometry. For example, a mixture of CD4⁺ and CD8⁺ T cells are stimulated with αCD3/αCD28 aAPCs followed by transduction with lentiviral vectors expressing GFP under the control of the promoters to be analyzed. Exemplary promoters include the CMV IE gene, EF-1α, ubiquitin C, or phosphoglycerokinase (PGK) promoters. GFP fluorescence is evaluated on day 6 of culture in the CD4⁺ and/or CD8⁺ T cell subsets by flow cytometry. See, e.g., Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Alternatively, a mixture of CD4⁺ and CD8⁺ T cells are stimulated with αCD3/αCD28 coated magnetic beads on day 0, and transduced with CAR on day 1 using a bicistronic lentiviral vector expressing CAR along with eGFP using a 2A ribosomal skipping sequence. Cultures are re-stimulated with either a cancer associated antigen as described herein⁺ K562 cells (K562 expressing a cancer associated antigen as described herein), wild-type K562 cells (K562 wild type) or K562 cells expressing hCD32 and 4-1BBL in the presence of antiCD3 and anti-CD28 antibody (K562-BBL-3/28) following washing. Exogenous IL-2 is added to the cultures every other day at 100 IU/ml. GFP⁺ T cells are enumerated by flow cytometry using bead-based counting. See, e.g., Milone et al., Molecular Therapy 17(8): 1453-1464 (2009).

Sustained CAR⁺ T cell expansion in the absence of re-stimulation can also be measured. See, e.g., Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Briefly, mean T cell volume (fl) is measured on day 8 of culture using a Coulter Multisizer III particle counter, a Nexcelom Cellometer Vision or Millipore Scepter, following stimulation with αCD3/αCD28 coated magnetic beads on day 0, and transduction with the indicated CAR on day 1.

Animal models can also be used to measure a CART activity. For example, xenograft model using human a cancer associated antigen described herein-specific CAR⁺ T cells to treat a primary human pre-B ALL in immunodeficient mice can be used. See, e.g., Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Very briefly, after establishment of ALL, mice are randomized as to treatment groups. Different numbers of a cancer associated antigen -specific CAREngineered T cells are coinjected at a 1:1 ratio into NOD-SCID-γ^{-/-} mice bearing B-ALL. The number of copies of a cancer associated antigen -specific CAR vector in spleen DNA from mice is evaluated at various times following T cell injection. Animals are assessed for leukemia at weekly intervals. Peripheral blood a cancer associate antigen as described herein⁺ B-ALL blast cell counts are measured in mice that are injected with a cancer associated antigen described herein-ζ CAR⁺ T cells or mock-transduced T cells. Survival curves for the groups are compared using the log-rank test. In addition, absolute peripheral blood CD4⁺ and CD8⁺ T cell counts 4 weeks following T cell injection in NOD-SCID-γ^{-/-} mice can also be analyzed. Mice are injected with leukemic cells and 3 weeks later are injected with T cells engineered to express CAR by a bicistronic lentiviral vector that encodes the CAR linked to eGFP. T cells are normalized to 45–50% input GFP⁺ T cells by mixing with mock-transduced cells prior to injection, and confirmed by flow cytometry. Animals are assessed for leukemia at 1-week intervals. Survival curves for the CAR⁺ T cell groups are compared using the log-rank test.

Dose dependent CAR treatment response can be evaluated. See, *e.g.*, Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). For example, peripheral blood is obtained 35–70 days after establishing leukemia in mice injected on day 21 with CAR T cells, an equivalent number of mock-transduced T cells, or no T cells. Mice from each group are randomly bled for determination of peripheral blood a cancer associate antigen as described herein⁺ ALL blast counts and then killed on days 35 and 49. The remaining animals are evaluated on days 57 and 70.

Assessment of cell proliferation and cytokine production has been previously described, *e.g.*, at Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Briefly, assessment of CAR-mediated proliferation is performed in microtiter plates by mixing washed T cells with K562 cells expressing a cancer associated antigen described herein (K19) or CD32 and CD137 (KT32-BBL) for a final T-cell:K562 ratio of 2:1. K562 cells are irradiated with gamma-radiation prior to use. Anti-CD3 (clone OKT3) and anti- CD28 (clone 9.3) monoclonal antibodies are added to cultures with KT32-BBL cells to serve as a positive control for stimulating T-cell proliferation since these signals support long-term CD8⁺ T cell expansion *ex vivo*. T cells are enumerated in cultures using CountBright™ fluorescent beads (Invitrogen, Carlsbad, CA) and flow cytometry as described by the manufacturer. CAR⁺ T cells are identified by GFP expression using T cells that are engineered with eGFP-2A linked CAR-expressing lentiviral vectors. For CAR⁺ T cells not expressing GFP, the CAR⁺ T cells are detected with biotinylated recombinant a cancer associate antigen as described herein protein and a secondary avidin-PE conjugate. CD4⁺ and CD8⁺ expression on T cells are also simultaneously detected with specific monoclonal antibodies (BD Biosciences). Cytokine measurements are performed on supernatants collected 24 hours following re-stimulation using the human TH1/TH2 cytokine cytometric bead array kit (BD Biosciences, San Diego, CA) according the manufacturer's instructions. Fluorescence is assessed using a FACScalibur flow cytometer, and data is analyzed according to the manufacturer's instructions.

Cytotoxicity can be assessed by a standard 51Cr-release assay. See, *e.g.*, Milone et al., Molecular Therapy 17(8): 1453-1464 (2009). Briefly, target cells (K562 lines and primary pro-B-ALL cells) are loaded with 51Cr (as NaCrO₄, New England Nuclear, Boston, MA) at 37°C for 2 hours with frequent agitation, washed twice in complete RPMI and plated into microtiter plates. Effector T cells are mixed with target cells in the wells in complete RPMI at varying ratios of effector cell:target cell (E:T). Additional wells containing media only (spontaneous release, SR) or a 1% solution of triton-X 100 detergent (total release, TR) are also prepared. After 4 hours of incubation at 37°C, supernatant from each well is harvested. Released 51Cr is then measured using a gamma particle counter (Packard Instrument Co., Waltham, MA). Each condition is performed in at least triplicate, and the percentage of lysis is calculated using the formula: % Lysis = (ER– SR) / (TR – SR), where ER represents the average 51Cr released for each experimental condition.

Imaging technologies can be used to evaluate specific trafficking and proliferation of CARs in tumor-bearing animal models. Such assays have been described, for example, in Barrett et al., Human

Gene Therapy 22:1575-1586 (2011). Briefly, NOD/SCID/ $\gamma c^{-/-}$ (NSG) mice are injected IV with Nalm-6 cells followed 7 days later with T cells 4 hour after electroporation with the CAR constructs. The T cells are stably transfected with a lentiviral construct to express firefly luciferase, and mice are imaged for bioluminescence. Alternatively, therapeutic efficacy and specificity of a single injection of CAR⁺ T cells in Nalm-6 xenograft model can be measured as the following: NSG mice are injected with Nalm-6 transduced to stably express firefly luciferase, followed by a single tail-vein injection of T cells electroporated with cars of the present invention 7 days later. Animals are imaged at various time points post injection. For example, photon-density heat maps of firefly luciferasepositive leukemia in representative mice at day 5 (2 days before treatment) and day 8 (24 hr post CAR⁺ PBLs) can be generated.

Other assays, including those described in the Example section herein as well as those that are known in the art can also be used to evaluate the CARs described herein.

Therapeutic Application

In one aspect, the invention provides methods for treating a disease associated with expression of a cancer associated antigen described herein.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an XCAR, wherein X represents a tumor antigen as described herein, and wherein the cancer cells express said X tumor antigen.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a XCAR described herein, wherein the cancer cells express X. In one embodiment, X is expressed on both normal cells and cancers cells, but is expressed at lower levels on normal cells. In one embodiment, the method further comprises selecting a CAR that binds X with an affinity that allows the XCAR to bind and kill the cancer cells expressing X but less than 30%, 25%, 20%, 15%, 10%, 5% or less of the normal cells expressing X are killed, e.g., as determined by an assay described herein. For example, the assay described in FIGS. 13A and 13B can be used or a killing assay such as flow cytometry based on Cr51 CTL. In one embodiment, the selected CAR has an antigen binding domain that has a binding affinity KD of 10^{-4} M to 10^{-8} M, e.g., 10^{-5} M to 10^{-7} M, e.g., 10^{-6} M or 10^{-7} M, for the target antigen. In one embodiment, the selected antigen binding domain has a binding affinity that is at least five-fold, 10-fold, 20-fold, 30-fold, 50-fold, 100-fold or 1,000-fold less than a reference antibody, e.g., an antibody described herein.

In one embodiment, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express CD19 CAR, wherein the cancer cells express CD19. In one embodiment, the cancer to be

treated is ALL (acute lymphoblastic leukemia), CLL (chronic lymphocytic leukemia), DLBCL (diffuse large B-cell lymphoma), MCL (Mantle cell lymphoma, or MM (multiple myeloma).

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an EGFRvIIICAR, wherein the cancer cells express EGFRvIII. In one embodiment, the cancer to be treated is glioblastoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a mesothelinCAR, wherein the cancer cells express mesothelin. In one embodiment, the cancer to be treated is mesothelioma, pancreatic cancer, or ovarian cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD123CAR, wherein the cancer cells express CD123. In one embodiment, the cancer to be treated is AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD22CAR, wherein the cancer cells express CD22. In one embodiment, the cancer to be treated is B cell malignancies.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CS-1CAR, wherein the cancer cells express CS-1. In one embodiment, the cancer to be treated is multiple myeloma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CLL-1CAR, wherein the cancer cells express CLL-1. In one embodiment, the cancer to be treated is AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD33CAR, wherein the cancer cells express CD33. In one embodiment, the cancer to be treated is AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GD2CAR, wherein the cancer cells express GD2. In one embodiment, the cancer to be treated is neuroblastoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a

BCMACAR, wherein the cancer cells express BCMA. In one embodiment, the cancer to be treated is multiple myeloma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TnCAR, wherein the cancer cells express Tn antigen. In one embodiment, the cancer to be treated is ovarian cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PSMACAR, wherein the cancer cells express PSMA. In one embodiment, the cancer to be treated is prostate cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a ROR1CAR, wherein the cancer cells express ROR1. In one embodiment, the cancer to be treated is B cell malignancies.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a FLT3 CAR, wherein the cancer cells express FLT3. In one embodiment, the cancer to be treated is AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TAG72CAR, wherein the cancer cells express TAG72. In one embodiment, the cancer to be treated is gastrointestinal cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD38CAR, wherein the cancer cells express CD38. In one embodiment, the cancer to be treated is multiple myeloma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD44v6CAR, wherein the cancer cells express CD44v6. In one embodiment, the cancer to be treated is cervical cancer, AML, or MM.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CEACAR, wherein the cancer cells express CEA. In one embodiment, the cancer to be treated is gastrointestinal cancer, or pancreatic cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an

EPCAMCAR, wherein the cancer cells express EPCAM. In one embodiment, the cancer to be treated is gastrointestinal cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a B7H3CAR, wherein the cancer cells express B7H3.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a KITCAR, wherein the cancer cells express KIT. In one embodiment, the cancer to be treated is gastrointestinal cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an IL-13Ra2CAR, wherein the cancer cells express IL-13Ra2. In one embodiment, the cancer to be treated is glioblastoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PRSS21CAR, wherein the cancer cells express PRSS21. In one embodiment, the cancer to be treated is selected from ovarian, pancreatic, lung and breast cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD30CAR, wherein the cancer cells express CD30. In one embodiment, the cancer to be treated is lymphomas, or leukemias.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GD3CAR, wherein the cancer cells express GD3. In one embodiment, the cancer to be treated is melanoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD171CAR, wherein the cancer cells express CD171. In one embodiment, the cancer to be treated is neuroblastoma, ovarian cancer, melanoma, breast cancer, pancreatic cancer, colon cancers, or NSCLC (non-small cell lung cancer).

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an IL-11RaCAR, wherein the cancer cells express IL-11Ra. In one embodiment, the cancer to be treated is osteosarcoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a

PSCACAR, wherein the cancer cells express PSCA. In one embodiment, the cancer to be treated is prostate cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a VEGFR2CAR, wherein the cancer cells express VEGFR2. In one embodiment, the cancer to be treated is a solid tumor.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LewisYCAR, wherein the cancer cells express LewisY. In one embodiment, the cancer to be treated is ovarian cancer, or AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD24CAR, wherein the cancer cells express CD24. In one embodiment, the cancer to be treated is pancreatic cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PDGFR-betaCAR, wherein the cancer cells express PDGFR-beta. In one embodiment, the cancer to be treated is breast cancer, prostate cancer, GIST (gastrointestinal stromal tumor), CML, DFSP (dermatofibrosarcoma protuberans), or glioma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a SSEA-4CAR, wherein the cancer cells express SSEA-4. In one embodiment, the cancer to be treated is glioblastoma, breast cancer, lung cancer, or stem cell cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD20CAR, wherein the cancer cells express CD20. In one embodiment, the cancer to be treated is B cell malignancies.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Folate receptor alphaCAR, wherein the cancer cells express folate receptor alpha. In one embodiment, the cancer to be treated is ovarian cancer, NSCLC, endometrial cancer, renal cancer, or other solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an ERBB2CAR, wherein the cancer cells express ERBB2 (Her2/neu). In one embodiment, the cancer to be treated is breast cancer, gastric cancer, colorectal cancer, lung cancer, or other solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a MUC1CAR, wherein the cancer cells express MUC1. In one embodiment, the cancer to be treated is breast cancer, lung cancer, or other solid tumors.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an EGFRCAR, wherein the cancer cells express EGFR. In one embodiment, the cancer to be treated is glioblastoma, SCLC (small cell lung cancer), SCCHN (squamous cell carcinoma of the head and neck), NSCLC, or other solid tumors.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a NCAMCAR, wherein the cancer cells express NCAM. In one embodiment, the cancer to be treated is neuroblastoma, or other solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CAIXCAR, wherein the cancer cells express CAIX. In one embodiment, the cancer to be treated is renal cancer, CRC, cervical cancer, or other solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an EphA2CAR, wherein the cancer cells express EphA2. In one embodiment, the cancer to be treated is GBM.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GD3CAR, wherein the cancer cells express GD3. In one embodiment, the cancer to be treated is melanoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Fucosyl GM1CAR, wherein the cancer cells express Fucosyl GM

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a sLeCAR, wherein the cancer cells express sLe. In one embodiment, the cancer to be treated is NSCLC, or AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GM3CAR, wherein the cancer cells express GM3.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TGS5CAR, wherein the cancer cells express TGS5.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a HMWMAACAR, wherein the cancer cells express HMWMAA. In one embodiment, the cancer to be treated is melanoma, glioblastoma, or breast cancer.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an o-acetyl-GD2CAR, wherein the cancer cells express o-acetyl-GD2. In one embodiment, the cancer to be treated is neuroblastoma, or melanoma.

15 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD19CAR, wherein the cancer cells express CD19. In one embodiment, the cancer to be treated is Folate receptor beta AML, myeloma

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TEM1/CD248CAR, wherein the cancer cells express TEM1/CD248. In one embodiment, the cancer to be treated is a solid tumor.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TEM7RCAR, wherein the cancer cells express TEM7R. In one embodiment, the cancer to be treated is solid tumor.

25 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CLDN6CAR, wherein the cancer cells express CLDN6. In one embodiment, the cancer to be treated is ovarian cancer, lung cancer, or breast cancer.

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TSHRCAR, wherein the cancer cells express TSHR. In one embodiment, the cancer to be treated is thyroid cancer, or multiple myeloma.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GPRC5DCAR, wherein the cancer cells express GPRC5D. In one embodiment, the cancer to be treated is multiple myeloma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CXORF61CAR, wherein the cancer cells express CXORF61.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD97CAR, wherein the cancer cells express CD97. In one embodiment, the cancer to be treated is B cell malignancies, gastric cancer, pancreatic cancer, esophageal cancer, glioblastoma, breast cancer, or colorectal cancer.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD179aCAR, wherein the cancer cells express CD179a. In one embodiment, the cancer to be treated is B cell malignancies.

15 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an ALK CAR, wherein the cancer cells express ALK. In one embodiment, the cancer to be treated is NSCLC, ALCL (anaplastic large cell lymphoma), IMT (inflammatory myofibroblastic tumor), or neuroblastoma.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Polysialic acid CAR, wherein the cancer cells express Polysialic acid. In one embodiment, the cancer to be treated is small cell lung cancer.

25 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PLAC1CAR, wherein the cancer cells express PLAC1. In one embodiment, the cancer to be treated is HCC (hepatocellular carcinoma).

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GloboH CAR, wherein the cancer cells express GloboH. In one embodiment, the cancer to be treated is ovarian cancer, gastric cancer, prostate cancer, lung cancer, breast cancer, or pancreatic cancer.

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a NY-BR-1CAR, wherein the cancer cells express NY-BR-1. In one embodiment, the cancer to be treated is breast cancer.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a UPK2CAR, wherein the cancer cells express UPK2. In one embodiment, the cancer to be treated is bladder cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a HAVCR1CAR, wherein the cancer cells express HAVCR1. In one embodiment, the cancer to be treated is renal cancer.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a ADRB3CAR, wherein the cancer cells express ADRB3. In one embodiment, the cancer to be treated is Ewing sarcoma.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PANX3CAR, wherein the cancer cells express PANX3. In one embodiment, the cancer to be treated is osteosarcoma.

15 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GPR20CAR, wherein the cancer cells express GPR20. In one embodiment, the cancer to be treated is GIST.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LY6KCAR, wherein the cancer cells express LY6K. In one embodiment, the cancer to be treated is breast cancer, lung cancer, ovary cancer, or cervix cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a OR51E2CAR, wherein the cancer cells express OR51E2. In one embodiment, the cancer to be treated is prostate cancer.

25 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TARPCAR, wherein the cancer cells express TARP. In one embodiment, the cancer to be treated is prostate cancer.

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a WT1CAR, wherein the cancer cells express WT1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a NY-ESO-1CAR, wherein the cancer cells express NY-ESO-1.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LAGE-1a CAR, wherein the cancer cells express LAGE-1a.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a MAGE-A1CAR, wherein the cancer cells express MAGE-A1. In one embodiment, the cancer to be treated is melanoma.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a MAGE A1CAR, wherein the cancer cells express MAGE A1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
10 ETV6-AML CAR, wherein the cancer cells express ETV6-AML.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a sperm protein 17 CAR, wherein the cancer cells express sperm protein 17. In one embodiment, the cancer to be treated is ovarian cancer, HCC, or NSCLC.

15 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a XAGE1CAR, wherein the cancer cells express XAGE1. In one embodiment, the cancer to be treated is Ewings, or rhabdo cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the
20 subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Tie 2 CAR, wherein the cancer cells express Tie 2. In one embodiment, the cancer to be treated is a solid tumor.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
25 MAD-CT-1CAR, wherein the cancer cells express MAD-CT-1. In one embodiment, the cancer to be treated is prostate cancer, or melanoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
30 MAD-CT-2CAR, wherein the cancer cells express MAD-CT-2. In one embodiment, the cancer to be treated is prostate cancer, melanoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Fos-related antigen 1 CAR, wherein the cancer cells express Fos-related antigen 1. In one embodiment, the cancer to be treated is glioma, squamous cell cancer, or pancreatic cancer.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a p53CAR, wherein the cancer cells express p53.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a protein CAR, wherein the cancer cells express protein.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a survivin and telomerase CAR, wherein the cancer cells express survivin and telomerase.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PCTA-1/Galectin 8 CAR, wherein the cancer cells express PCTA-1/Galectin 8.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a MelanA/MART1CAR, wherein the cancer cells express MelanA/MART1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
15 Ras mutant CAR, wherein the cancer cells express Ras mutant.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a p53 mutant CAR, wherein the cancer cells express p53 mutant.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a hTERT CAR, wherein the cancer cells express hTERT.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a sarcoma translocation breakpoints CAR, wherein the cancer cells express sarcoma translocation
25 breakpoints. In one embodiment, the cancer to be treated is sarcoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a ML-IAP CAR, wherein the cancer cells express ML-IAP. In one embodiment, the cancer to be treated is melanoma.

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an ERG CAR, wherein the cancer cells express ERG (TMPRSS2 ETS fusion gene).

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
35 NA17CAR, wherein the cancer cells express NA17. In one embodiment, the cancer to be treated is melanoma.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PAX3CAR, wherein the cancer cells express PAX3. In one embodiment, the cancer to be treated is alveolar rhabdomyosarcoma.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an androgen receptor CAR, wherein the cancer cells express androgen receptor. In one embodiment, the cancer to be treated is metastatic prostate cancer.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Cyclin B1CAR, wherein the cancer cells express Cyclin B1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a MYCNCAR, wherein the cancer cells express MYCN.

15 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a RhoC CAR, wherein the cancer cells express RhoC.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a TRP-2CAR, wherein the cancer cells express TRP-2. In one embodiment, the cancer to be treated is melanoma.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CYP1B1CAR, wherein the cancer cells express CYP1B1. In one embodiment, the cancer to be treated is breast cancer, colon cancer, lung cancer, esophagus cancer, skin cancer, lymph node cancer, brain cancer, or testis cancer.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a BORIS CAR, wherein the cancer cells express BORIS. In one embodiment, the cancer to be treated is lung cancer.

30 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a SART3CAR, wherein the cancer cells express SART3

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PAX5CAR, wherein the cancer cells express PAX5.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a OY-TES1CAR, wherein the cancer cells express OY-TES1.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LCK CAR, wherein the cancer cells express LCK.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a AKAP-4CAR, wherein the cancer cells express AKAP-4.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a SSX2CAR, wherein the cancer cells express SSX2.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a RAGE-1CAR, wherein the cancer cells express RAGE-1. In one embodiment, the cancer to be treated is RCC (renal cell cancer), or other solid tumors

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a human telomerase reverse transcriptase CAR, wherein the cancer cells express human telomerase reverse transcriptase. In one embodiment, the cancer to be treated is solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a RU1CAR, wherein the cancer cells express RU1.

25 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a RU2CAR, wherein the cancer cells express RU2.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an intestinal carboxyl esterase CAR, wherein the cancer cells express intestinal carboxyl esterase. In one embodiment, the cancer to be treated is thyroid cancer, RCC, CRC (colorectal cancer), breast cancer, or other solid tumors.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a Prostase CAR, wherein the cancer cells express Prostase.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a PAPCAR, wherein the cancer cells express PAP.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an IGF-I receptor CAR, wherein the cancer cells express IGF-I receptor.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a gp100 CAR, wherein the cancer cells express gp100.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a bcr-abl CAR, wherein the cancer cells express bcr-abl.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a tyrosinase CAR, wherein the cancer cells express tyrosinase.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
15 Fucosyl GM1CAR, wherein the cancer cells express Fucosyl GM1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a mut hsp70-2CAR, wherein the cancer cells express mut hsp70-2. In one embodiment, the cancer to be treated is melanoma.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD79a CAR, wherein the cancer cells express CD79a.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a
25 CD79b CAR, wherein the cancer cells express CD79b.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD72 CAR, wherein the cancer cells express CD72.

In one aspect, the present invention provides methods of treating cancer by providing to the
30 subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LAIR1 CAR, wherein the cancer cells express LAIR1.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a FCAR CAR, wherein the cancer cells express FCAR.

35 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LILRA2 CAR, wherein the cancer cells express LILRA2.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CD300LF CAR, wherein the cancer cells express CD300LF.

5 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a CLEC12A CAR, wherein the cancer cells express CLEC12A.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a BST2 CAR, wherein the cancer cells express BST2.

10 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an EMR2 CAR, wherein the cancer cells express EMR2.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a LY75 CAR, wherein the cancer cells express LY75.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a GPC3 CAR, wherein the cancer cells express GPC3.

20 In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express a FCRL5 CAR, wherein the cancer cells express FCRL5.

In one aspect, the present invention provides methods of treating cancer by providing to the subject in need thereof immune effector cells (e.g., T cells, NK cells) that are engineered to express an IGLL1 CAR, wherein the cancer cells express IGLL1.

25 In one aspect, the present invention relates to treatment of a subject in vivo using a PD1 CAR such that growth of cancerous tumors is inhibited. A PD1 CAR may be used alone to inhibit the growth of cancerous tumors. Alternatively, PD1 CAR may be used in conjunction with other CARs, immunogenic agents, standard cancer treatments, or other antibodies. In one embodiment, the subject is treated with a PD1 CAR and an XCAR described herein. In an embodiment, a PD1 CAR is used in
30 conjunction with another CAR, e.g., a CAR described herein, and a kinase inhibitor, e.g., a kinase inhibitor described herein.

In another aspect, a method of treating a subject, e.g., reducing or ameliorating, a hyperproliferative condition or disorder (e.g., a cancer), e.g., solid tumor, a soft tissue tumor, or a metastatic lesion, in a subject is provided. As used herein, the term “cancer” is meant to include all
35 types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various

organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (e.g., colon), genitourinary tract (e.g., renal, urothelial cells), prostate and pharynx. Adenocarcinomas include malignancies such as most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. In one

5 embodiment, the cancer is a melanoma, e.g., an advanced stage melanoma. Metastatic lesions of the aforementioned cancers can also be treated or prevented using the methods and compositions of the invention. Examples of other cancers that can be treated include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine

10 cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin Disease, non-Hodgkin lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic

15 myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those

20 induced by asbestos, and combinations of said cancers. Treatment of metastatic cancers, e.g., metastatic cancers that express PD-L1 (Iwai et al. (2005) *Int. Immunol.* 17:133-144) can be effected using the antibody molecules described herein.

Exemplary cancers whose growth can be inhibited include cancers typically responsive to immunotherapy. Non-limiting examples of cancers for treatment include melanoma (e.g., metastatic

25 malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (e.g. non-small cell lung cancer). Additionally, refractory or recurrent malignancies can be treated using the molecules described herein.

In one aspect, the invention pertains to a vector comprising a CAR operably linked to

30 promoter for expression in mammalian immune effector cells (e.g., T cells, NK cells). In one aspect, the invention provides a recombinant immune effector cell expressing a CAR of the present invention for use in treating cancer expressing a cancer associate antigen as described herein. In one aspect, CAR-expressing cells of the invention is capable of contacting a tumor cell with at least one cancer associated antigen expressed on its surface such that the CAR-expressing cell targets the cancer cell

35 and growth of the cancer is inhibited.

In one aspect, the invention pertains to a method of inhibiting growth of a cancer, comprising contacting the cancer cell with a CAR-expressing cell of the present invention such that the CART is

activated in response to the antigen and targets the cancer cell, wherein the growth of the tumor is inhibited.

In one aspect, the invention pertains to a method of treating cancer in a subject. The method comprises administering to the subject CAR-expressing cell of the present invention such that the cancer is treated in the subject. In one aspect, the cancer associated with expression of a cancer
 5 associate antigen as described herein is a hematological cancer. In one aspect, the hematological cancer is a leukemia or a lymphoma. In one aspect, a cancer associated with expression of a cancer associate antigen as described herein includes cancers and malignancies including, but not limited to, e.g., one or more acute leukemias including but not limited to, e.g., B-cell acute Lymphoid Leukemia
 10 (“BALL”), T-cell acute Lymphoid Leukemia (“TALL”), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), Chronic Lymphoid Leukemia (CLL). Additional cancers or hematologic conditions associated with expression of a cancer associate antigen as described herein include, but are not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse
 15 large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and “preleukemia” which are a diverse collection of hematological conditions
 20 united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further a disease associated with a cancer associate antigen as described herein expression include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases associated with expression of a cancer associate antigen as described herein.

In some embodiments, a cancer that can be treated with CAR-expressing cell of the present
 25 invention is multiple myeloma. Multiple myeloma is a cancer of the blood, characterized by accumulation of a plasma cell clone in the bone marrow. Current therapies for multiple myeloma include, but are not limited to, treatment with lenalidomide, which is an analog of thalidomide. Lenalidomide has activities which include anti-tumor activity, angiogenesis inhibition, and immunomodulation. Generally, myeloma cells are thought to be negative for a cancer associate
 30 antigen as described herein expression by flow cytometry. Thus, in some embodiments, a CD19 CAR, e.g., as described herein, may be used to target myeloma cells. In some embodiments, cars of the present invention therapy can be used in combination with one or more additional therapies, e.g., lenalidomide treatment.

The invention includes a type of cellular therapy where immune effector cells (e.g., T cells,
 35 NK cells) are genetically modified to express a chimeric antigen receptor (CAR) and the CAR-expressing T cell or NK cell is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Unlike antibody therapies, CAR-modified immune effector cells (e.g., T

cells, NK cells) are able to replicate in vivo resulting in long-term persistence that can lead to sustained tumor control. In various aspects, the immune effector cells (e.g., T cells, NK cells) administered to the patient, or their progeny, persist in the patient for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twenty-three months, two years, three years, four years, or five years after administration of the T cell or NK cell to the patient.

The invention also includes a type of cellular therapy where immune effector cells (e.g., T cells, NK cells) are modified, e.g., by in vitro transcribed RNA, to transiently express a chimeric antigen receptor (CAR) and the CAR T cell or NK cell is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Thus, in various aspects, the immune effector cells (e.g., T cells, NK cells) administered to the patient, is present for less than one month, e.g., three weeks, two weeks, one week, after administration of the T cell or NK cell to the patient.

Without wishing to be bound by any particular theory, the anti-tumor immunity response elicited by the CAR-modified immune effector cells (e.g., T cells, NK cells) may be an active or a passive immune response, or alternatively may be due to a direct vs indirect immune response. In one aspect, the CAR transduced immune effector cells (e.g., T cells, NK cells) exhibit specific proinflammatory cytokine secretion and potent cytolytic activity in response to human cancer cells expressing the a cancer associate antigen as described herein, resist soluble a cancer associate antigen as described herein inhibition, mediate bystander killing and mediate regression of an established human tumor. For example, antigen-less tumor cells within a heterogeneous field of a cancer associate antigen as described herein-expressing tumor may be susceptible to indirect destruction by a cancer associate antigen as described herein-redirection immune effector cells (e.g., T cells, NK cells) that has previously reacted against adjacent antigen-positive cancer cells.

In one aspect, the fully-human CAR-modified immune effector cells (e.g., T cells, NK cells) of the invention may be a type of vaccine for ex vivo immunization and/or in vivo therapy in a mammal. In one aspect, the mammal is a human.

With respect to ex vivo immunization, at least one of the following occurs in vitro prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a CAR to the cells or iii) cryopreservation of the cells.

Ex vivo procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (e.g., a human) and genetically modified (i.e., transduced or transfected in vitro) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the CAR-modified cell can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

The procedure for ex vivo expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of ex vivo expansion of the cells. Briefly, ex vivo culture and expansion of immune effector cells (e.g., T cells, NK cells) comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells ex vivo. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

In addition to using a cell-based vaccine in terms of ex vivo immunization, the present invention also provides compositions and methods for in vivo immunization to elicit an immune response directed against an antigen in a patient.

Generally, the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, the CAR-modified immune effector cells (e.g., T cells, NK cells) of the invention are used in the treatment of diseases, disorders and conditions associated with expression of a cancer associate antigen as described herein. In certain aspects, the cells of the invention are used in the treatment of patients at risk for developing diseases, disorders and conditions associated with expression of a cancer associate antigen as described herein. Thus, the present invention provides methods for the treatment or prevention of diseases, disorders and conditions associated with expression of a cancer associate antigen as described herein comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified immune effector cells (e.g., T cells, NK cells) of the invention.

In one aspect the CAR-expressing cells of the inventions may be used to treat a proliferative disease such as a cancer or malignancy or is a precancerous condition such as a myelodysplasia, a myelodysplastic syndrome or a preleukemia. Further a disease associated with a cancer associate antigen as described herein expression include, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing a cancer associated antigen as described herein. Non-cancer related indications associated with expression of a cancer associate antigen as described herein include, but are not limited to, e.g., autoimmune disease, (e.g., lupus), inflammatory disorders (allergy and asthma) and transplantation.

The CAR-modified immune effector cells (e.g., T cells, NK cells) of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations.

Hematologic Cancer

Hematological cancer conditions are the types of cancer such as leukemia, lymphoma, and malignant lymphoproliferative conditions that affect blood, bone marrow and the lymphatic system.

Leukemia can be classified as acute leukemia and chronic leukemia. Acute leukemia can be further classified as acute myelogenous leukemia (AML) and acute lymphoid leukemia (ALL).

5 Chronic leukemia includes chronic myelogenous leukemia (CML) and chronic lymphoid leukemia (CLL). Other related conditions include myelodysplastic syndromes (MDS, formerly known as “preleukemia”) which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

10 Lymphoma is a group of blood cell tumors that develop from lymphocytes. Exemplary lymphomas include non-Hodgkin lymphoma and Hodgkin lymphoma.

The present invention provides for compositions and methods for treating cancer. In one aspect, the cancer is a hematologic cancer including but is not limited to hematological cancer is a leukemia or a lymphoma. In one aspect, the CAR-expressing cells of the invention may be used to treat cancers and malignancies such as, but not limited to, e.g., acute leukemias including but not
 15 limited to, e.g., B-cell acute lymphoid leukemia (“BALL”), T-cell acute lymphoid leukemia (“TALL”), acute lymphoid leukemia (ALL); one or more chronic leukemias including but not limited to, e.g., chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL); additional hematologic cancers or hematologic conditions including, but not limited to, e.g., B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse
 20 large B cell lymphoma, Follicular lymphoma, Hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, Marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, and “preleukemia” which are a diverse collection of hematological conditions
 25 united by ineffective production (or dysplasia) of myeloid blood cells, and the like. Further a disease associated with a cancer associate antigen as described herein expression includes, but not limited to, e.g., atypical and/or non-classical cancers, malignancies, precancerous conditions or proliferative diseases expressing a cancer associate antigen as described herein.

The present invention also provides methods for inhibiting the proliferation or reducing a
 30 cancer associated antigen as described herein-expressing cell population, the methods comprising contacting a population of cells comprising a cancer associated antigen as described herein-expressing cell with a CAR-expressing T cell or NK cell of the invention that binds to the a cancer associate antigen as described herein-expressing cell. In a specific aspect, the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing a cancer
 35 associated antigen as described herein, the methods comprising contacting a cancer associate antigen as described herein-expressing cancer cell population with a CAR-expressing T cell or NK cell of the invention that binds to a cancer associated antigen as described herein-expressing cell. In one aspect,

the present invention provides methods for inhibiting the proliferation or reducing the population of cancer cells expressing a cancer associated antigen as described herein, the methods comprising contacting a cancer associated antigen as described herein-expressing cancer cell population with a CAR-expressing T cell or NK cell of the invention that binds to a cancer associated antigen as described herein-expressing cell. In certain aspects, a CAR-expressing T cell or NK cell of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% in a subject with or animal model for myeloid leukemia or another cancer associated with a cancer associated antigen as described herein-expressing cells relative to a negative control. In one aspect, the subject is a human.

The present invention also provides methods for preventing, treating and/or managing a disease associated with a cancer associated antigen as described herein-expressing cells (e.g., a hematologic cancer or atypical cancer expressing a cancer associated antigen as described herein), the methods comprising administering to a subject in need a CAR T cell or NK cell of the invention that binds to a cancer associated antigen as described herein-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with a cancer associated antigen as described herein-expressing cells include autoimmune disorders (such as lupus), inflammatory disorders (such as allergies and asthma) and cancers (such as hematological cancers or atypical cancers expressing a cancer associated antigen as described herein).

The present invention also provides methods for preventing, treating and/or managing a disease associated with a cancer associated antigen as described herein-expressing cells, the methods comprising administering to a subject in need a CAR T cell or NK cell of the invention that binds to a cancer associated antigen as described herein-expressing cell. In one aspect, the subject is a human.

The present invention provides methods for preventing relapse of cancer associated with a cancer associated antigen as described herein-expressing cells, the methods comprising administering to a subject in need thereof a CAR T cell or NK cell of the invention that binds to a cancer associated antigen as described herein-expressing cell. In one aspect, the methods comprise administering to the subject in need thereof an effective amount of a CAR-expressing T cell or NK cell described herein that binds to a cancer associated antigen as described herein-expressing cell in combination with an effective amount of another therapy.

Combination Therapies

A CAR-expressing cell described herein may be used in combination with other known agents and therapies. Administered “in combination”, as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, e.g., the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the disorder has been cured or eliminated or treatment has ceased for other

reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as “simultaneous” or “concurrent delivery”. In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

A CAR-expressing cell described herein and the at least one additional therapeutic agent can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the CAR-expressing cell described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed.

The CAR therapy and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease.

The CAR therapy can be administered before the other treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

When administered in combination, the CAR therapy and the additional agent (e.g., second or third agent), or all, can be administered in an amount or dose that is higher, lower or the same than the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain embodiments, the administered amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually, e.g., as a monotherapy. In other embodiments, the amount or dosage of the CAR therapy, the additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment of cancer) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent used individually, e.g., as a monotherapy, required to achieve the same therapeutic effect.

In further aspects, a CAR-expressing cell described herein may be used in a treatment regimen in combination with surgery, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. peptide vaccine, such as that described in Izumoto et al. 2008 J Neurosurg 108:963-971.

In one embodiment, a CAR-expressing cell described herein can be used in combination with a chemotherapeutic agent. Exemplary chemotherapeutic agents include an anthracycline (e.g., doxorubicin (e.g., liposomal doxorubicin)), a vinca alkaloid (e.g., vinblastine, vincristine, vindesine, vinorelbine), an alkylating agent (e.g., cyclophosphamide, decarbazine, melphalan, ifosfamide, temozolomide), an immune cell antibody (e.g., alemtuzamab, gemtuzumab, rituximab, ofatumumab, tositumomab, brentuximab), an antimetabolite (including, e.g., folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors (e.g., fludarabine)), an mTOR inhibitor, a TNFR glucocorticoid induced TNFR related protein (GITR) agonist, a proteasome inhibitor (e.g., aclacinomycin A, gliotoxin or bortezomib), an immunomodulator such as thalidomide or a thalidomide derivative (e.g., lenalidomide).

General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytosan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycytidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

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

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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JUMBO APPLICATIONS/PATENTS

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

What is claimed is:

1. A cell (e.g., a population of cells), e.g., an immune effector cell, expressing a chimeric antigen receptor (CAR),

5 wherein the CAR comprises an antigen-binding domain, a transmembrane domain, and an intracellular signaling domain, and

wherein the cell has altered expression and/or function of a Tet2-associated gene (e.g., one or more Tet2-associated genes).

10 2. The cell of claim 1, wherein the cell has reduced or eliminated expression and/or function of a Tet2-associated gene.

3. The cell of claim 1 or 2, wherein the cell has increased or activated expression and/or function of a Tet2-associated gene.

15 4. The cell of any of the preceding claims, wherein the cell has reduced or eliminated expression and/or function of a first Tet2-associated gene, and increased or activated expression and/or function of a second Tet2-associated gene.

20 5. The cell of any of the preceding claims, wherein the cell further has reduced or eliminated expression and/or function of Tet2.

6. The cell of any of the preceding claims, wherein the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

25 7. The cell of claim 6, wherein the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, or all) genes chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

30 8. The cell of any of claims 1-4, wherein the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8.

9. The cell of claim 8, wherein the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8, Column B.

35 10. The cell of claim 8 or 9, wherein the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 8, Column A.

11. The cell of any of claims 1-4, wherein the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D.

5 12. The cell of claim 11, wherein the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D.

13. The cell of claim 11 or 12, wherein the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column D.

10

14. The cell of any of claims 1-4, wherein the Tet2-associated gene comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes in a pathway (e.g., one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pathways) chosen from Table 9, Column A.

15 15. The cell of claim 14, wherein the cell has reduced or eliminated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column A.

16. The cell of claim 14 or 15, wherein the cell has increased or activated expression and/or function of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) genes chosen from Table 9, Column A.

20

17. The cell of any of claims 14-16, wherein the pathway is chosen from one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or all) of:

- (1) a leukocyte differentiation pathway;
- (2) a pathway of positive regulation of immune system process;
- 25 (3) a transmembrane receptor protein tyrosine kinase signaling pathway
- (4) a pathway of regulation of anatomical structure morphogenesis;
- (5) a pathway of TNFA signaling via NFkB;
- (6) a pathway of positive regulation of hydrolase activity;
- (7) a wound healing pathway;
- 30 (8) an alpha-beta T cell activation pathway;
- (9) a pathway of regulation of cellular component movement;
- (10) an inflammatory response pathway;
- (11) a myeloid cell differentiation pathway;
- (12) a cytokine production pathway;
- 35 (13) a pathway of downregulation in UV response;
- (14) a pathway of negative regulation of multicellular organismal process;
- (15) a blood vessel morphogenesis pathway;

- (16) a NFAT-dependent transcription pathway;
- (17) a pathway of positive regulation of apoptotic process;
- (18) a hypoxia pathway;
- (19) a pathway of upregulation by KRAS signaling; or
- 5 (20) a pathway of stress-activated protein kinase signaling cascade.

18. The cell of claim 17, wherein the one or more genes associated with a leukocyte differentiation pathway are chosen from Table 9, Row 1.

10 19. The cell of claim 17, wherein the one or more genes associated with a pathway of positive regulation of immune system process are chosen from Table 9, Row 56.

20. The cell of claim 17, wherein the one or more genes associated with a transmembrane receptor protein tyrosine kinase signaling pathway are chosen from Table 9, Row 85.

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21. The cell of claim 17, wherein the one or more genes associated with a pathway of regulation of anatomical structure morphogenesis are chosen from Table 9, Row 128.

22. The cell of claim 17, wherein the one or more genes associated with a pathway of TNFA signaling via NFKB are chosen from Table 9, Row 134.

20

23. The cell of claim 17, wherein the one or more genes associated with a pathway of positive regulation of hydrolase activity are chosen from Table 9, Row 137.

25 24. The cell of claim 17, wherein the one or more genes associated with a wound healing pathway are chosen from Table 9, Row 141.

25. The cell of claim 17, wherein the one or more genes associated with a alpha-beta T cell activation pathway are chosen from Table 9, Row 149.

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26. The cell of claim 17, wherein the one or more genes associated with a pathway of regulation of cellular component movement are chosen from Table 9, Row 180.

27. The cell of claim 17, wherein the one or more genes associated with an inflammatory response pathway are chosen from Table 9, Row 197.

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28. The cell of claim 17, wherein the one or more genes associated with a myeloid cell differentiation pathway are chosen from Table 9, Row 206.

5 29. The cell of claim 17, wherein the one or more genes associated with a cytokine production pathway are chosen from Table 9, Row 221.

30. The cell of claim 17, wherein the one or more genes associated with a pathway of downregulation in UV response are chosen from Table 9, Row 233.

10 31. The cell of claim 17, wherein the one or more genes associated with a pathway of negative regulation of multicellular organismal process are chosen from Table 9, Row 235.

32. The cell of claim 17, wherein the one or more genes associated with a blood vessel morphogenesis pathway are chosen from Table 9, Row 237.

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33. The cell of claim 17, wherein the one or more genes associated with a NFAT-dependent transcription pathway are chosen from Table 9, Row 243.

34. The cell of claim 17, wherein the one or more genes associated with a pathway of positive regulation of apoptotic process are chosen from Table 9, Row 250.

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35. The cell of claim 17, wherein the one or more genes associated with a hypoxia pathway are chosen from Table 9, Row 256.

25 36. The cell of claim 17, wherein the one or more genes associated with a pathway of upregulation by KRAS signaling are chosen from Table 9, Row 258.

37. The cell of claim 17, wherein the one or more genes associated with a pathway of stress-activated protein kinase signaling cascade are chosen from Table 9, Row 260.

30

38. The cell of claim 1 or 2, wherein the Tet2-associated gene comprises a gene (e.g., one or more genes) associated with a central memory phenotype.

39. The cell of claim 38, wherein the central memory phenotype is a central memory T cell phenotype.

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40. The cell of claim 38 or 39, wherein the central memory phenotype comprises a higher expression level of CCR7 and/or CD45RO, compared to the expression level of CCR7 and/or CD45RO in a naïve cell (e.g., a naïve T cell).

5 41. The cell of any of claims 38-40, wherein the central memory phenotype comprises a lower expression level of CD45RA, compared to the expression level of CD45RA in a naïve cell (e.g., a naïve T cell).

10 42. The cell of any of claims 38-41, wherein the central memory phenotype comprises enhanced antigen-dependent proliferation of the cell.

15 43. The cell of any of claims 38-42, wherein the central memory phenotype comprises a reduced expression level of IFN- γ and/or CD107a, e.g., when the cell is activated with an anti-CD3 or anti-CD28 antibody.

 44. The cell of any of the preceding claims, wherein the cell comprises a modulator (e.g., an inhibitor or an activator) of the Tet2-associated gene.

20 45. The cell of claim 41, wherein the modulator (e.g., inhibitor or activator) is (1) a gene editing system targeted to one or more sites within the Tet2-associated gene or a regulatory element thereof; (2) a nucleic acid encoding one or more components of said gene editing system; or (3) a combination thereof.

25 46. The cell of claim 45, wherein the gene editing system is selected from the group consisting of: a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, and a meganuclease system.

30 47. The cell of claim 45 or 46, wherein the gene editing system binds to a target sequence in an early exon or intron of the Tet2-associated gene.

 48. The cell of any of claims 45-47, wherein the gene editing system binds a target sequence of the Tet2-associated gene, and the target sequence is upstream of exon 4, e.g., in exon 1, exon 2, or exon 3.

35 49. The cell of any of claims 45-48, wherein the gene editing system binds to a target sequence in a late exon or intron of the Tet2-associated gene.

50. The cell of any of claims 45-49, wherein the gene editing system binds a target sequence of the Tet2-associated gene, and the target sequence is downstream of a preantepenultimate exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon.

5 51. The cell of any of claims 45-50, wherein the gene editing system is a CRISPR/Cas system comprising a gRNA molecule comprising a targeting sequence which hybridizes to a target sequence of the Tet2-associated gene.

10 52. The cell of claim 44, wherein the modulator (e.g., inhibitor) is an siRNA or shRNA specific for the Tet2-associated gene, or nucleic acid encoding said siRNA or shRNA.

53. The cell of claim 52, wherein the siRNA or shRNA comprises a sequence complementary to a sequence of an mRNA of the Tet2-associated gene.

15 54. The cell of claim 44, wherein the modulator (e.g., inhibitor or activator) is a small molecule.

55. The cell of claim 44, wherein the modulator (e.g., inhibitor or activator) is a protein.

20 56. The cell of claim 55, wherein the modulator (e.g., inhibitor) is a dominant negative binding partner of a protein encoded by the Tet2-associated gene, or a nucleic acid encoding said dominant negative binding partner.

25 57. The cell of claim 55, wherein the modulator (e.g., inhibitor) is a dominant negative (e.g., catalytically inactive) variant of a protein encoded by the Tet2-associated gene, or a nucleic acid encoding said dominant negative variant.

30 58. The cell of any of the preceding claims, wherein the cell comprises an inhibitor of a first Tet2-associated gene and an activator of a second Tet2-associated gene.

59. The cell of any of the preceding claims, wherein the cell further comprises an inhibitor of Tet2.

35 60. The cell of any of the preceding claims, wherein the antigen-binding domain binds to a tumor antigen is selected from a group consisting of: TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII, GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, PRSS21,

VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, MAGE-A1, legumain, HPV E6,E7, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, and IGLL1.

61. The cell of claim 60, wherein the tumor antigen is CD19.

62. The cell of any of the preceding claims, wherein the antigen-binding domain is an antibody or antibody fragment as described in, e.g., WO2012/079000 or WO2014/153270.

63. The cell of any of the preceding claims, wherein the transmembrane domain comprises: an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO: 12, or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 12; or the sequence of SEQ ID NO: 12.

64. The cell of any of the preceding claims, wherein the antigen binding domain is connected to the transmembrane domain by a hinge region, wherein said hinge region comprises SEQ ID NO: 2 or SEQ ID NO: 6, or a sequence with 95-99% identity thereof.

65. The cell of any of the preceding claims, wherein the intracellular signaling domain comprises a primary signaling domain and/or a costimulatory signaling domain, wherein the primary signaling domain comprises a functional signaling domain of a protein chosen from CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, common FcR gamma (FCER1G), FcR beta (Fc Epsilon R1b), CD79a, CD79b, Fcgamma RIIa, DAP10, or DAP12.

66. The cell of claim 65, wherein the primary signaling domain comprises: an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications of an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20, or a sequence with 95-99% identity

to an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20; or the amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 20.

67. The cell of any of the preceding claims, wherein the intracellular signaling domain
 5 comprises a costimulatory signaling domain, or a primary signaling domain and a costimulatory
 signaling domain, wherein the costimulatory signaling domain comprises a functional signaling
 domain of a protein selected from the group consisting of CD27, CD28, 4-1BB (CD137), OX40,
 CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT,
 NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, HVEM
 10 (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R
 gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD,
 CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29,
 ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244,
 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100
 15 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME
 (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46,
 and NKG2D.

68. The cell of claim 67, wherein the costimulatory signaling domain comprises an amino
 20 acid sequence having at least one, two or three modifications but not more than 20, 10 or 5
 modifications of an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 16, or a sequence with
 95-99% identity to an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 16.

69. The cell of claim 67 or 68, wherein the costimulatory signaling domain comprises a
 25 sequence of SEQ ID NO: 14 or SEQ ID NO: 16.

70. The cell of any of the preceding claims, wherein the intracellular domain comprises the
 sequence of SEQ ID NO: 14 or SEQ ID NO: 16, and the sequence of SEQ ID NO: 18 or SEQ ID NO:
 20, wherein the sequences comprising the intracellular signaling domain are expressed in the same
 30 frame and as a single polypeptide chain.

71. The cell of any of the preceding claims, further comprising a leader sequence comprises
 the sequence of SEQ ID NO: 2.

35 72. The cell of any of the preceding claims, wherein the cell is an immune effector cell (e.g.,
 a population of immune effector cells).

73. The cell of claim 72, wherein the immune effector cell is a T cell or an NK cell.

74. The cell of claim 72 or 73, wherein the immune effector cell is a T cell.

5 75. The cell of claim 73 or 74, wherein the T cell is a CD4+ T cell, a CD8+ T cell, or a combination thereof.

76. The cell of any of the preceding claims, wherein the cell is a human cell.

10 77. The cell of any of the preceding claims, wherein the cell comprises an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

78. The cell of claim 77, wherein the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is (1) a gene editing system targeted to one or more sites within an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene or a regulatory element thereof; (2) a nucleic acid encoding one or more components of said gene editing system; or (3) a combination thereof.

79. The cell of claim 78, wherein the gene editing system is selected from the group consisting of: a CRISPR/Cas9 system, a zinc finger nuclease system, a TALEN system, and a meganuclease system.

80. The cell of claim 78 or 79, wherein the gene editing system binds to a target sequence in an early exon or intron of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

25 81. The cell of any of claims 78-80, wherein the gene editing system binds a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, and the target sequence is upstream of exon 4, e.g., in exon1, exon2, or exon3, e.g. in exon 3.

82. The cell of any of claims 78-81, wherein the gene editing system binds to a target sequence in a late exon or intron of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

83. The cell of any of claims 78-82, wherein the gene editing system binds a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, and the target sequence is downstream of a preantepenultimate exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon.

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84. The cell of any of claims 78-83, wherein the gene editing system is a CRISPR/Cas system comprising a gRNA molecule comprising a targeting sequence which hybridizes to a target sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

5 85. The cell of claim 84, wherein the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is an siRNA or shRNA specific for IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1, or nucleic acid encoding said siRNA or shRNA.

86. The cell of claim 85, wherein the siRNA or shRNA comprises a sequence complementary
10 to a sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 mRNA.

87. The cell of claim 77, wherein the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a small molecule.

15 88. The cell of claim 77, wherein the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a protein, e.g., is a dominant negative binding partner of a protein encoded by an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, or a nucleic acid encoding said dominant negative binding partner.

20 89. The cell of claim 77, wherein the inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 is a protein, e.g., is a dominant negative (e.g., catalytically inactive) variant of a protein encoded by an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene, or a nucleic acid encoding said dominant negative variant.

25 90. A method of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell of any of the preceding claims, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of altering (e.g., decreasing or increasing) expression and/or function of a Tet2-associated gene (e.g., one or more Tet2-associated genes) in said cell, wherein the Tet2-associated gene is chosen from one or more (e.g., 2, 3, 4, or all) of:

- 30 (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
(ii) one or more genes listed in Table 8;
(iii) one or more genes listed in Table 9, Column D;
(iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
(v) one or more genes associated with a central memory phenotype.

35 91. The method of claim 90 or 91, comprising altering (e.g., decreasing) expression and/or function of one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

92. The method of any of claims 90-92, further comprising altering (e.g., decreasing) expression and/or function of Tet2.

93. A method of increasing the therapeutic efficacy of a CAR-expressing cell, e.g., a cell of any of the preceding claims, e.g., a CAR19-expressing cell (e.g., CTL019 or CTL119), comprising a step of contacting said cell with a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

(i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;

(ii) one or more genes listed in Table 8;

(iii) one or more genes listed in Table 9, Column D;

(iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or

(v) one or more genes associated with a central memory phenotype.

94. The method of claim 93, wherein said step comprises contacting said cells with an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

95. The method of claim 93 or 94, wherein the inhibitor is selected from the group consisting of: (1) a gene editing system targeted to one or more sites within the Tet2-associated gene, or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the Tet2-associated gene, or a binding partner of a protein encoded by the Tet2-associated gene; (4) a small molecule that inhibits expression and/or function of the Tet2-associated gene; (5) a nucleic acid encoding any of (1)-(3); and (6) any combination of (1) –(5).

96. The method of any of claims 93-95, further comprising contacting said cell with an inhibitor of Tet2.

97. The method of any of claims 93-96, wherein said contacting occurs *ex vivo*.

98. The method of any of claims 93-97, wherein the contacting occurs *in vivo*.

99. The method of claim 98, wherein the contacting occurs *in vivo* prior to delivery of nucleic acid encoding a CAR into the cell.

100. The method of claim 98, wherein the contacting occurs *in vivo* after the cells have been administered to a subject in need thereof.

101. A method for treating a cancer in a subject, the method comprising administering to said subject an effective amount of the cell of any of claims 1-91.

5 102. The method of claim 101, further comprising administering to said subject a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from one or more (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- 10 (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

15 103. The method of claim 101 or 102, further comprising administering to said subject an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

104. The method of any of claims 101-103, further comprising administering to said subject an inhibitor of Tet2.

20 105. A cell for use in a method of treating a subject in need thereof, the method comprising administering to said subject an effective amount of the cell of any of claims 1-91.

106. The cell for use of claim 105, wherein the method further comprises administering to said subject a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- 30 (v) one or more genes associated with a central memory phenotype.

107. The cell for use of claim 105 or 106, wherein the method further comprises administering to said subject an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

35 108. The cell for use of any of claims 105-107, wherein the method further comprises administering to said subject an inhibitor of Tet2.

109. A CAR-expressing cell therapy for use in a method of treating a subject in need thereof, the method comprising administering to said subject the CAR-expressing cell therapy and a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

110. The CAR-expressing cell therapy for use of claim 109, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

111. The CAR-expressing cell therapy for use of claim 109 or 110, wherein the method further comprises administering to said subject an inhibitor of Tet2.

112. The CAR-expressing cell therapy for use of any of claims 109-111, wherein the subject receives a pre-treatment of the modulator (e.g., inhibitor), prior to the initiation of the CAR-expressing cell therapy.

113. The CAR-expressing cell therapy for use of any of claims 109-112, wherein the subject receives concurrent treatment with the modulator (e.g., inhibitor) and the CAR expressing cell therapy.

114. The CAR-expressing cell therapy for use of any of claims 109-113, wherein the subject receives treatment with the modulator (e.g., inhibitor) post-CAR-expressing cell therapy.

115. The CAR-expressing cell therapy for use of any of claims 109-114, wherein the subject has a disease associated with expression of a tumor antigen, e.g., a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of the tumor antigen.

116. The CAR-expressing cell therapy for use of claim 115, wherein the cancer is a hematologic cancer or a solid tumor.

117. The CAR-expressing cell therapy for use of claim 115 or 116, wherein the cancer is a hematologic cancer chosen from one or more of chronic lymphocytic leukemia (CLL), acute

leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or pre-leukemia.

118. The CAR-expressing cell therapy for use of claim 115 or 116, wherein the cancer is selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers.

119. A method of treating a subject, the method comprising administering to said subject a modulator (e.g., an inhibitor or activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype,

wherein said subject has received, is receiving, or is about to receive therapy comprising a CAR-expressing cell.

120. The method of claim 119, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

121. The method of claim 119 or 120, further comprising administering to said subject an inhibitor of Tet2.

5 122. A modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) for use in the treatment of a subject,
 wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of:
 (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
 (ii) one or more genes listed in Table 8;
 10 (iii) one or more genes listed in Table 9, Column D;
 (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
 (v) one or more genes associated with a central memory phenotype, and
 wherein said subject has received, is receiving, or is about to receive therapy comprising a CAR-expressing cell.

15 123. The modulator for use of claim 122, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

20 124. The modulator for use of claim 122 or 123, wherein subject has received, is receiving, or is about to receive an inhibitor of Tet2.

125. A method of manufacturing a CAR-expressing cell, comprising introducing a nucleic acid encoding a CAR into a cell such that said nucleic acid (or CAR-encoding portion thereof) integrates into the genome of the cell within a Tet2-associated gene (e.g., one or more Tet2-associated genes) (e.g., within an intron or exon of the Tet2-associated gene), such that expression and/or
 25 function of the Tet2-associated genes is altered (e.g., reduced or eliminated),

wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of:

(i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;

(ii) one or more genes listed in Table 8;

30 (iii) one or more genes listed in Table 9, Column D;

(iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or

(v) one or more genes associated with a central memory phenotype.

126. The method of claim 125, wherein the Tet2-associated gene is chosen from IFNG,
 35 NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

127. A method of manufacturing a CAR-expressing cell, comprising contacting said CAR-expressing cell *ex vivo* with a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

128. The method of claim 127, wherein the Tet2-associated gene is chosen from IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

129. A vector comprising sequence encoding a CAR and sequence encoding a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

130. The vector of claim 129, wherein the modulator (e.g., inhibitor) is a (1) a gene editing system targeted to one or more sites within the gene, or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the Tet2-associated gene, or a binding partner of a protein encoded by the Tet2-associated gene; and (4) a nucleic acid encoding any of (1)-(3), or combinations thereof.

131. The vector of claim 129 or 130, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

132. The vector of any of claims 129-131, wherein the sequence encoding a CAR and the sequence encoding the inhibitor are separated by a 2A site.

133. A gene editing system that is specific for a sequence of a Tet2-associated gene (e.g., one or more Tet2-associated genes) or a regulatory element thereof, wherein the Tet2-associated gene is chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;
- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

134. The gene editing system of claim 133, wherein the gene editing system is specific for a sequence of an IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1 gene.

135. The gene editing system of claim 133 or 134, wherein the gene editing system is a CRISPR/Cas gene editing system, a zinc finger nuclease system, a TALEN system, or a meganuclease system.

136. The gene editing system of any of claims 133-135, wherein the gene editing system is a CRISPR/Cas gene editing system.

137. The gene editing system of claim 136, comprising:

a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a Cas9 protein;

a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a nucleic acid encoding a Cas9 protein;

a nucleic acid encoding a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a Cas9 protein; or

a nucleic acid encoding a gRNA molecule comprising a targeting sequence specific to a sequence of the Tet2-associated gene or a regulatory element thereof, and a nucleic acid encoding a Cas9 protein.

138. The gene editing system of any of claims 133-137, further comprising a template DNA.

139. The gene editing system of claim 138, wherein the template DNA comprises nucleic acid sequence encoding a CAR, e.g., a CAR as described herein.

140. A composition for the *ex vivo* manufacture of a CAR-expressing cell, comprising a modulator (e.g., an inhibitor or an activator) of a Tet2-associated gene (e.g., one or more Tet2-associated genes) chosen from (e.g., 2, 3, 4, or all) of:

- (i) one or more of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1;
- (ii) one or more genes listed in Table 8;

- (iii) one or more genes listed in Table 9, Column D;
- (iv) one or more genes associated with one or more pathways listed in Table 9, Column A; or
- (v) one or more genes associated with a central memory phenotype.

5 141. The composition of claim 140, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

10 142. The composition of claim 140 or 141, wherein the modulator (e.g., inhibitor) is a (1) a gene editing system targeted to one or more sites within the Tet2-associated gene or a regulatory element thereof; (2) a nucleic acid (e.g., an siRNA or shRNA) that inhibits expression of the Tet2-associated gene; (3) a protein (e.g., a dominant negative, e.g., catalytically inactive) encoded by the gene, or a binding partner of a protein encoded by the Tet2-associated gene; or (4) a nucleic acid encoding any of (1)-(3), or combinations thereof.

15 143. The composition of claim 142, further comprising an inhibitor of Tet2.

20 144. A population of cells comprising one or more cells of any of claims 1-89, wherein the population of cells comprises a higher (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold higher) percentage of Tscm cells (e.g., CD45RA+CD62L+CCR7+ (optionally CD27+CD95+) T cells) than a population of cells which does not comprise one or more cells in which expression and/or function of a Tet2-associated gene (e.g., one or more Tet2-associated genes) in said cell has been reduced or eliminated.

25 145. A population of cells comprising one or more cells of any of claims 1-89, wherein at least 50% (e.g., at least 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99%) of the population of cells have a central memory T cell phenotype.

30 146. The population of cells of claim 145, wherein the central memory cell phenotype is a central memory T cell phenotype.

 147. The population of cells of claim 145 or 146, wherein at least 50% (e.g., at least 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99%) of the population of cells express CD45RO and/or CCR7.

FIG. 1A

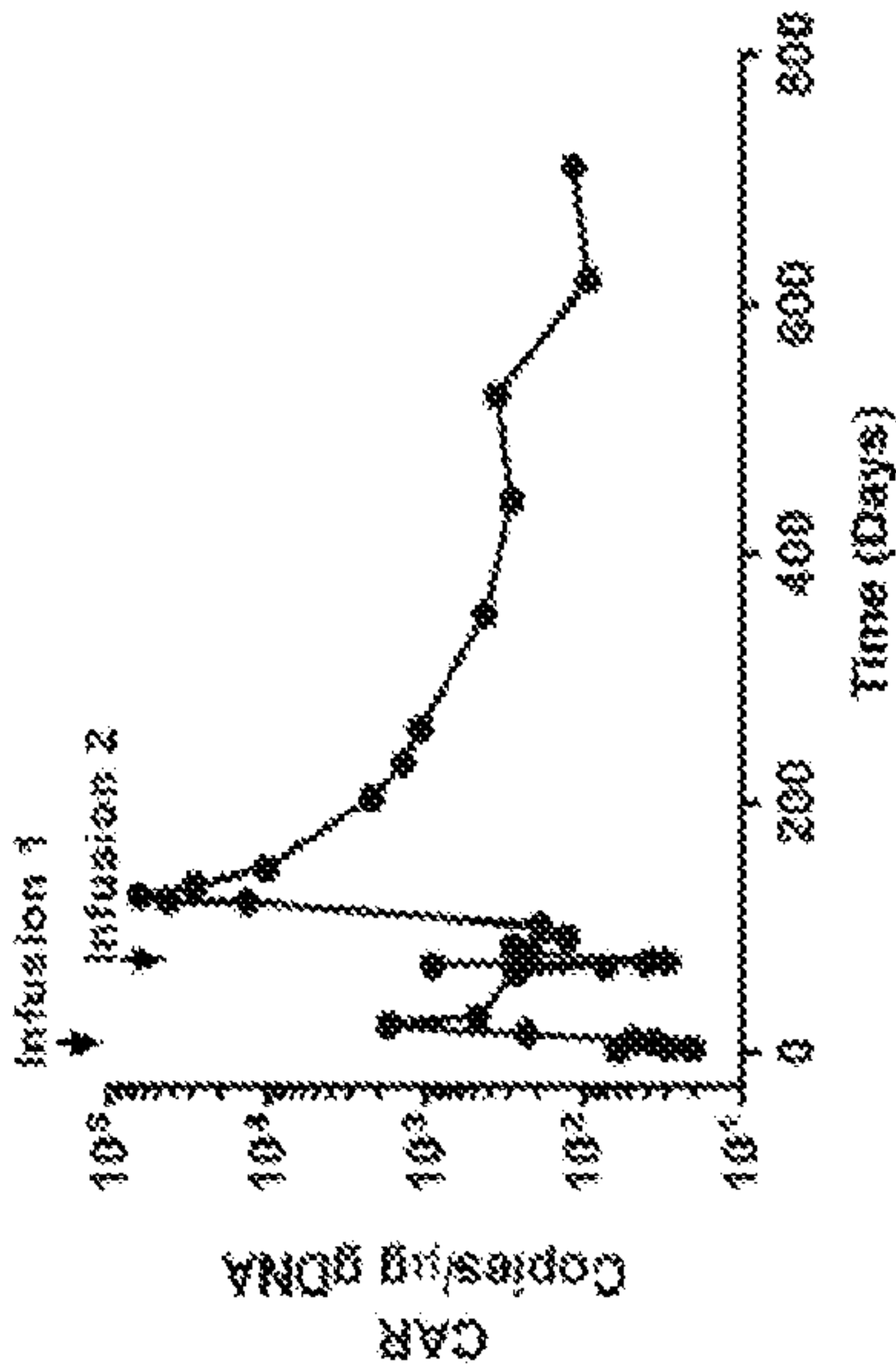


FIG. 1B

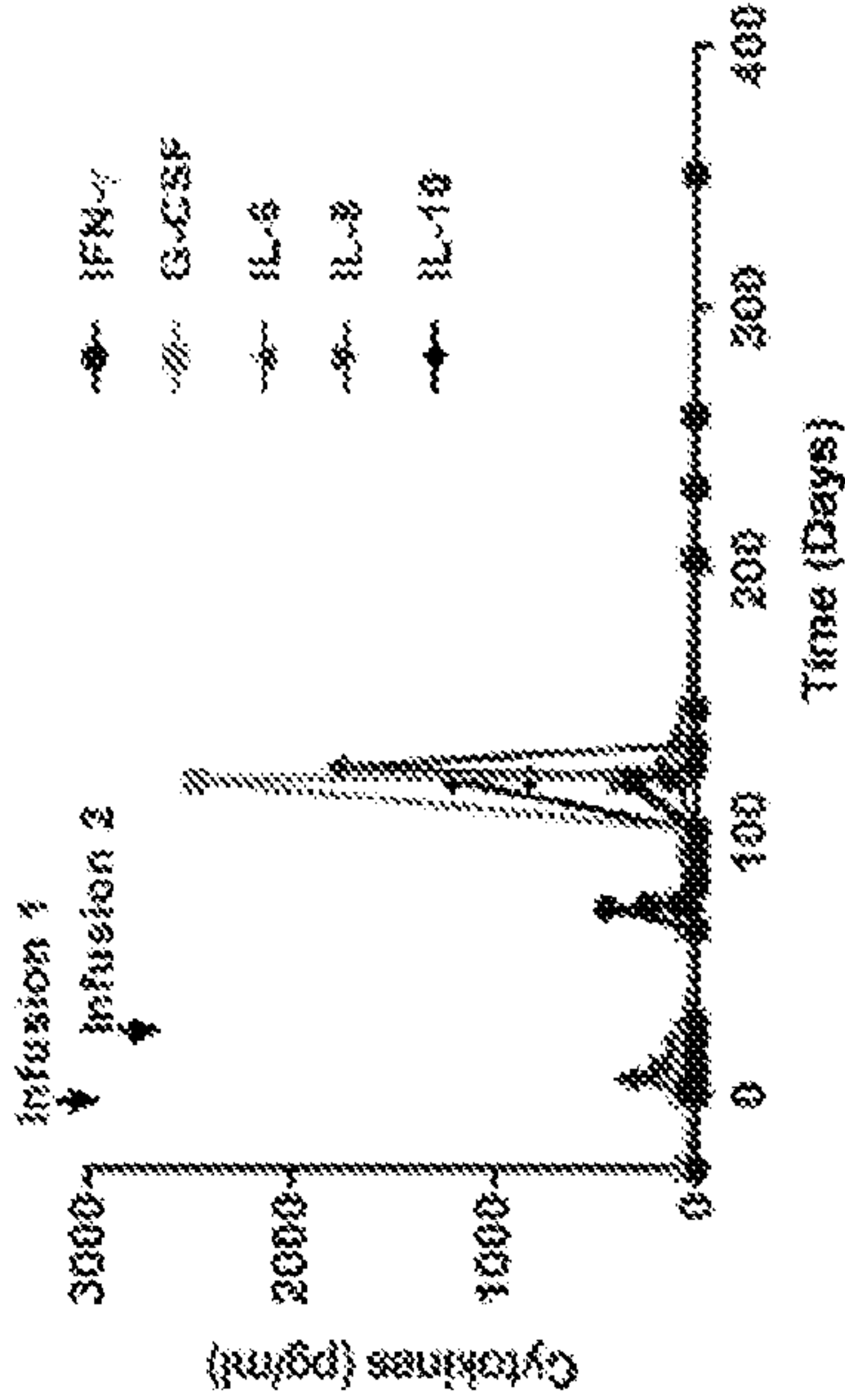


FIG. 1C

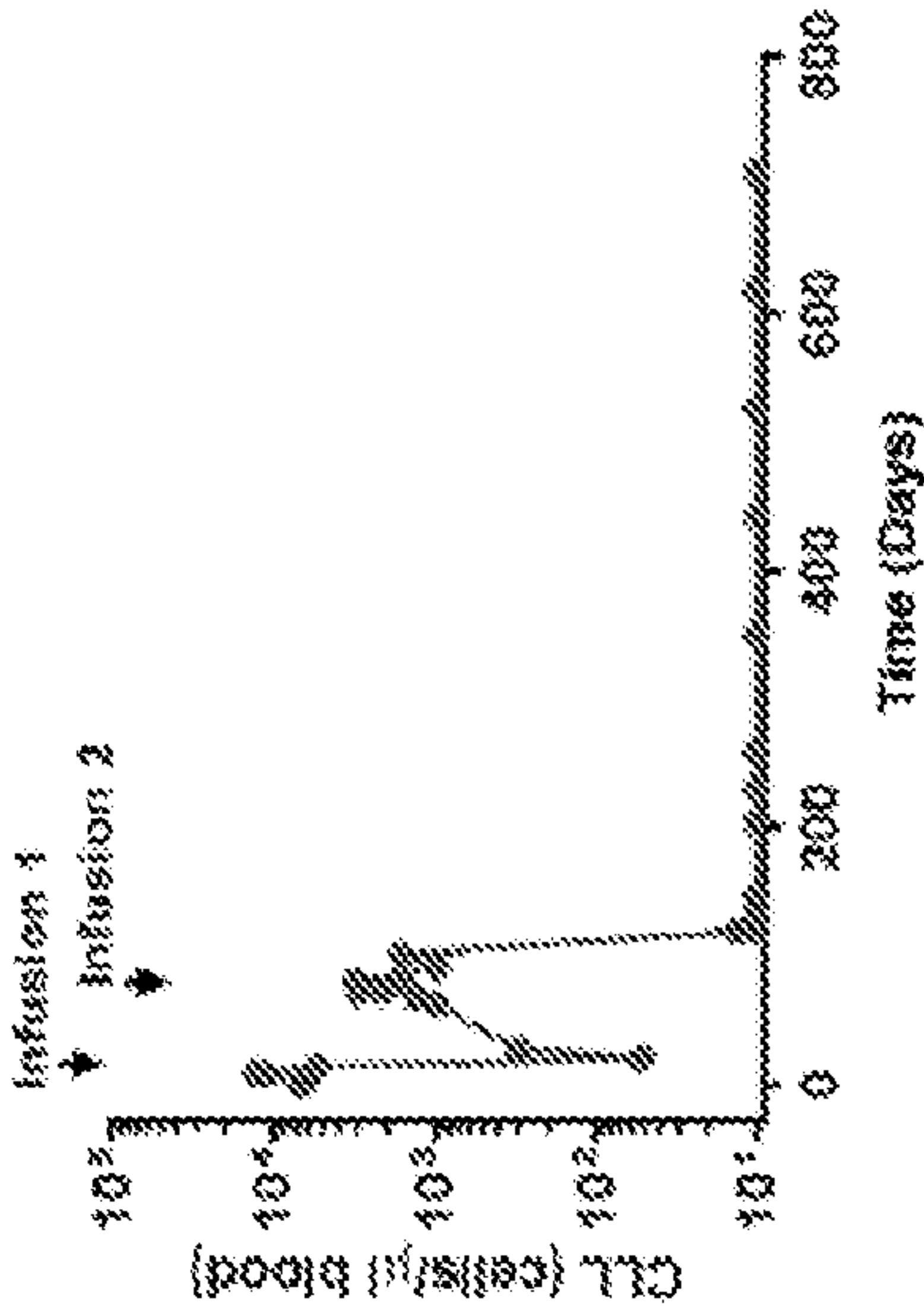


FIG. 1D



FIG. 2

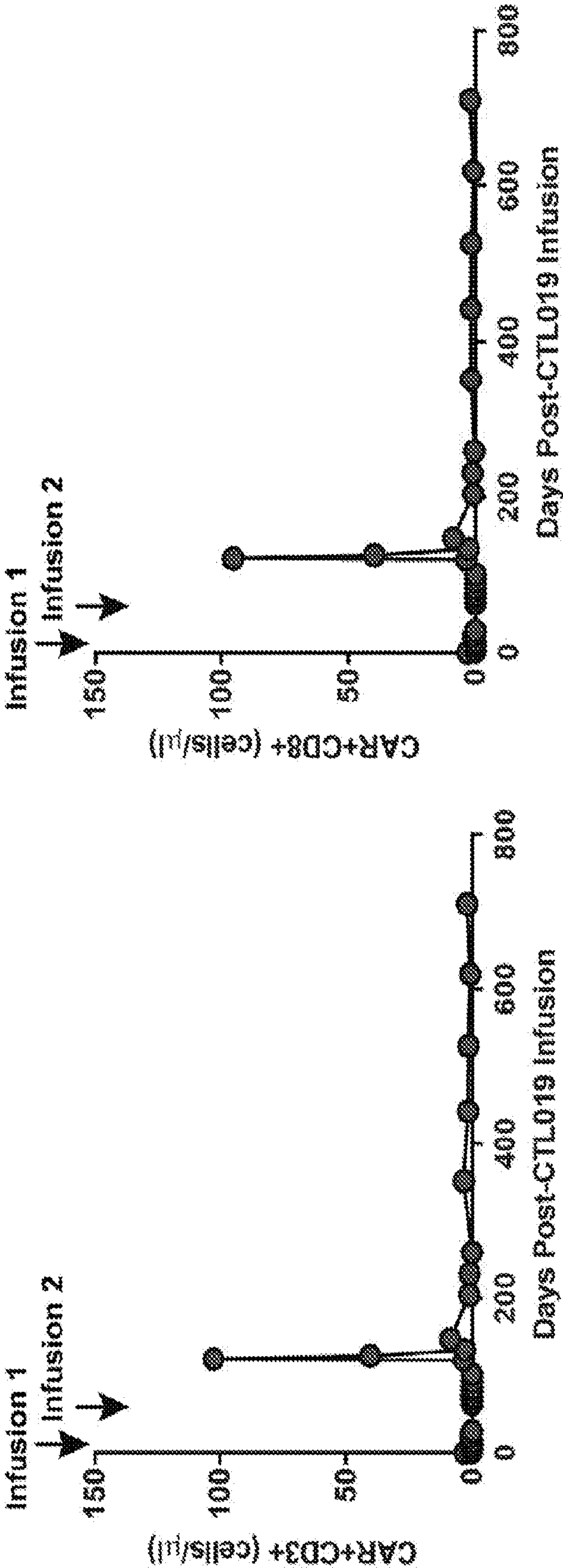


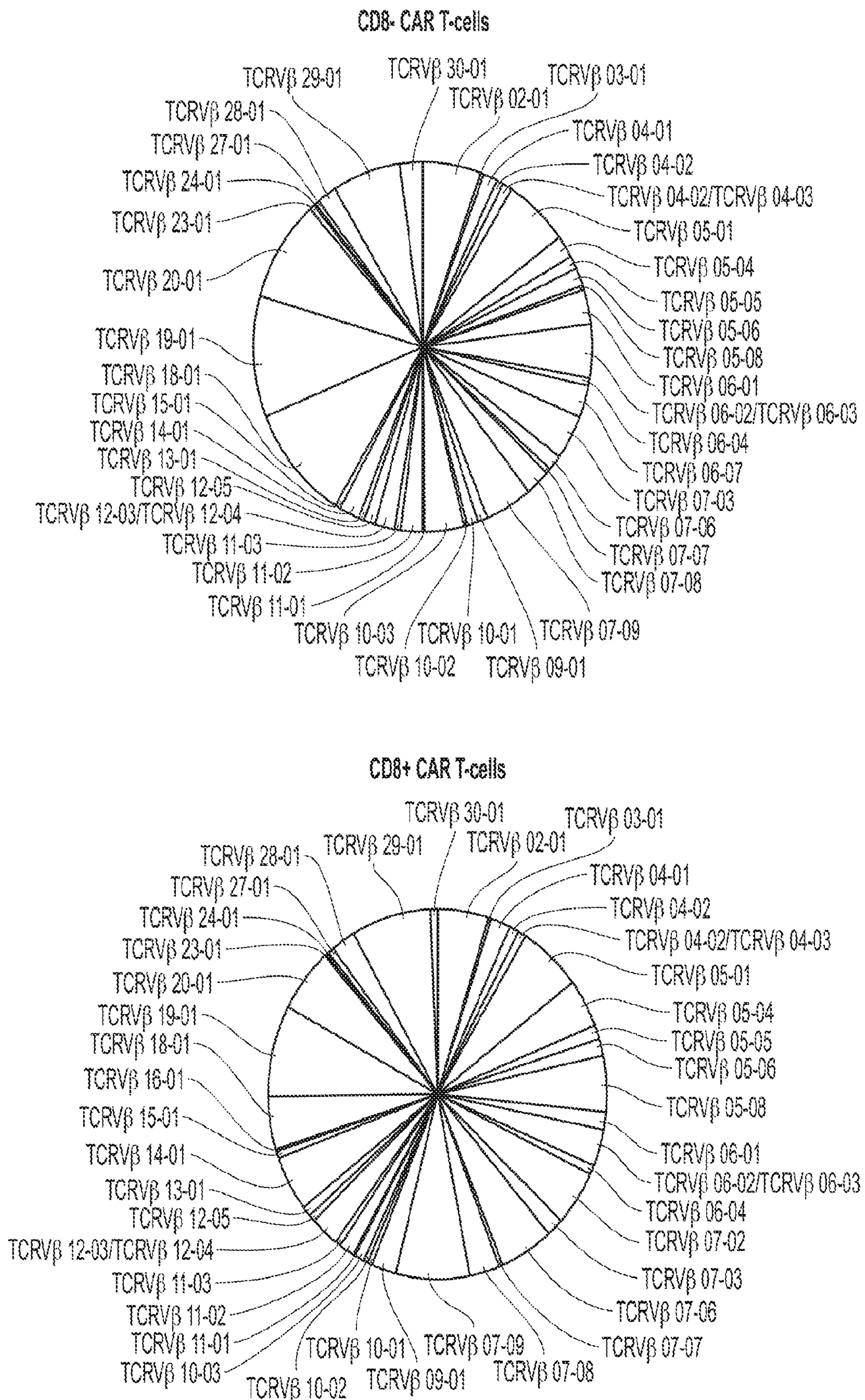
FIG. 3

FIG. 4A

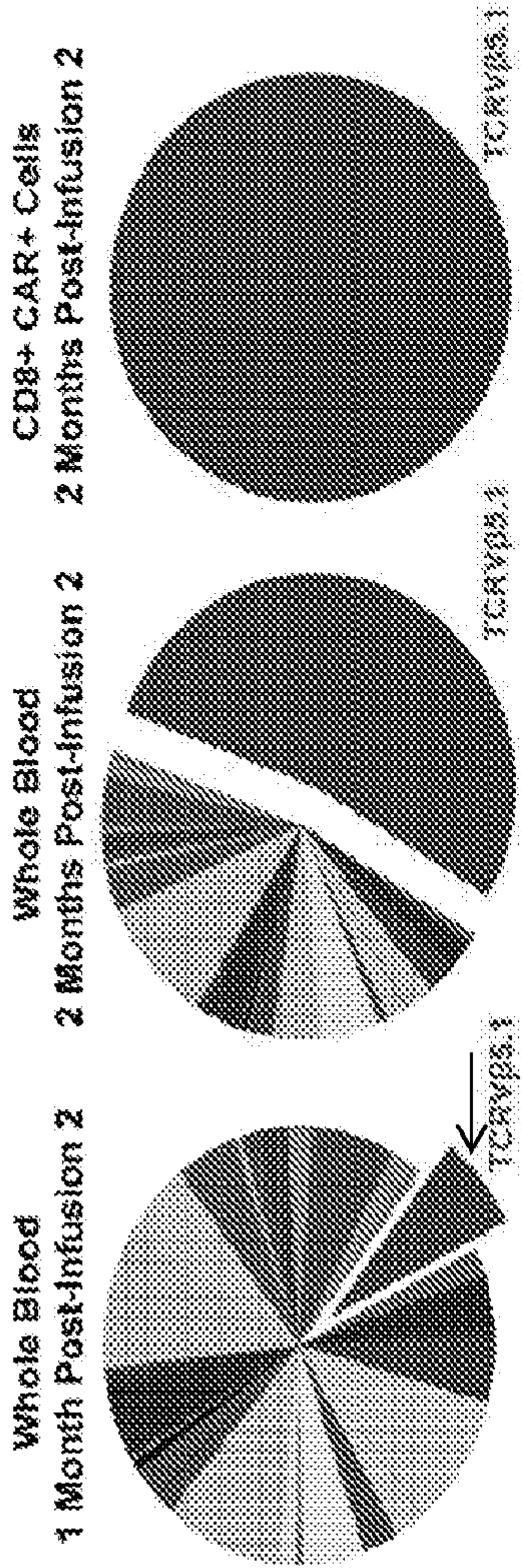


FIG. 4B

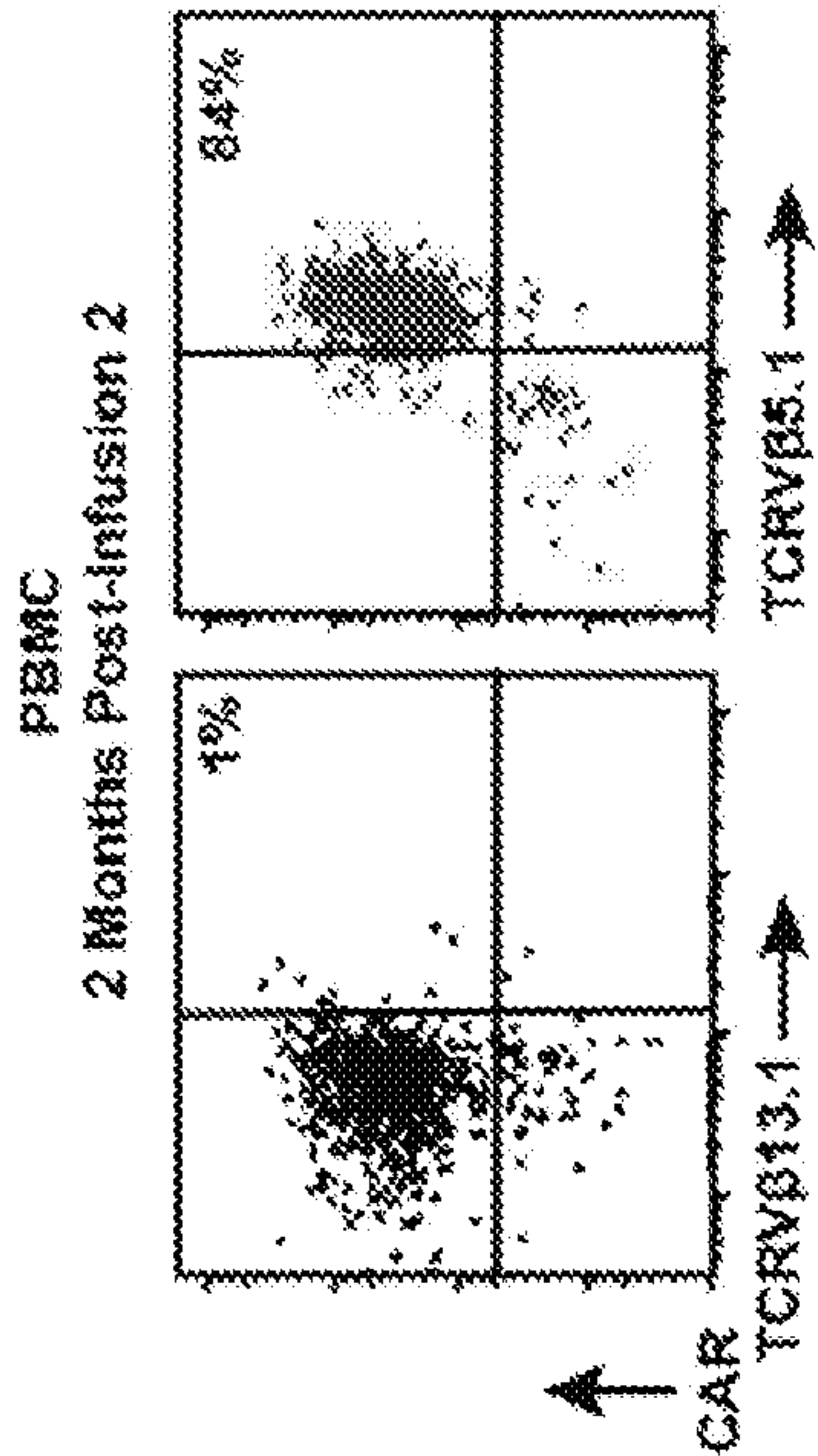


FIG. 4D

FIG. 4C

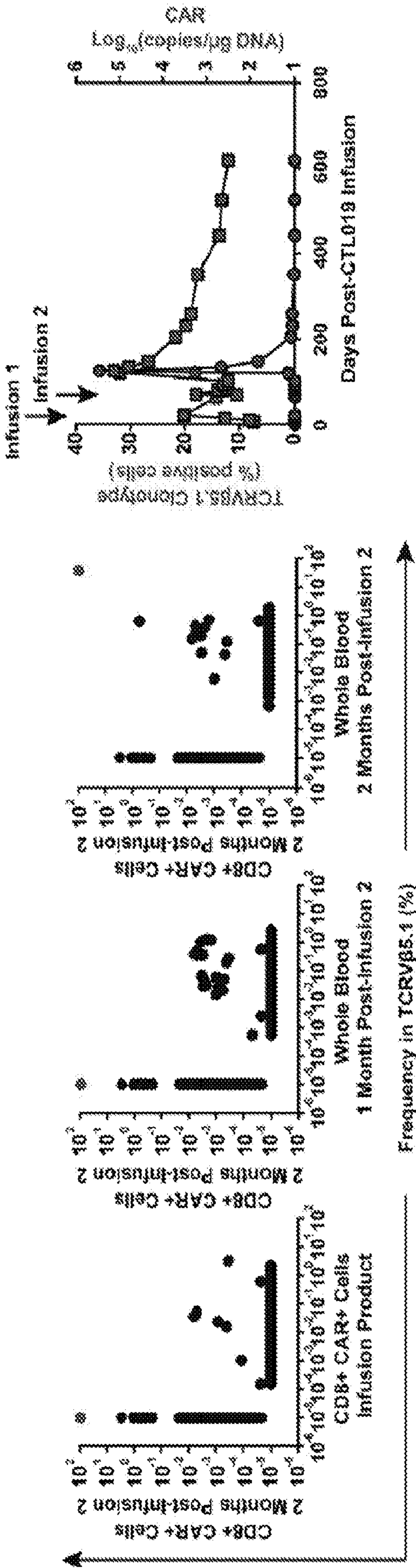


FIG. 5A

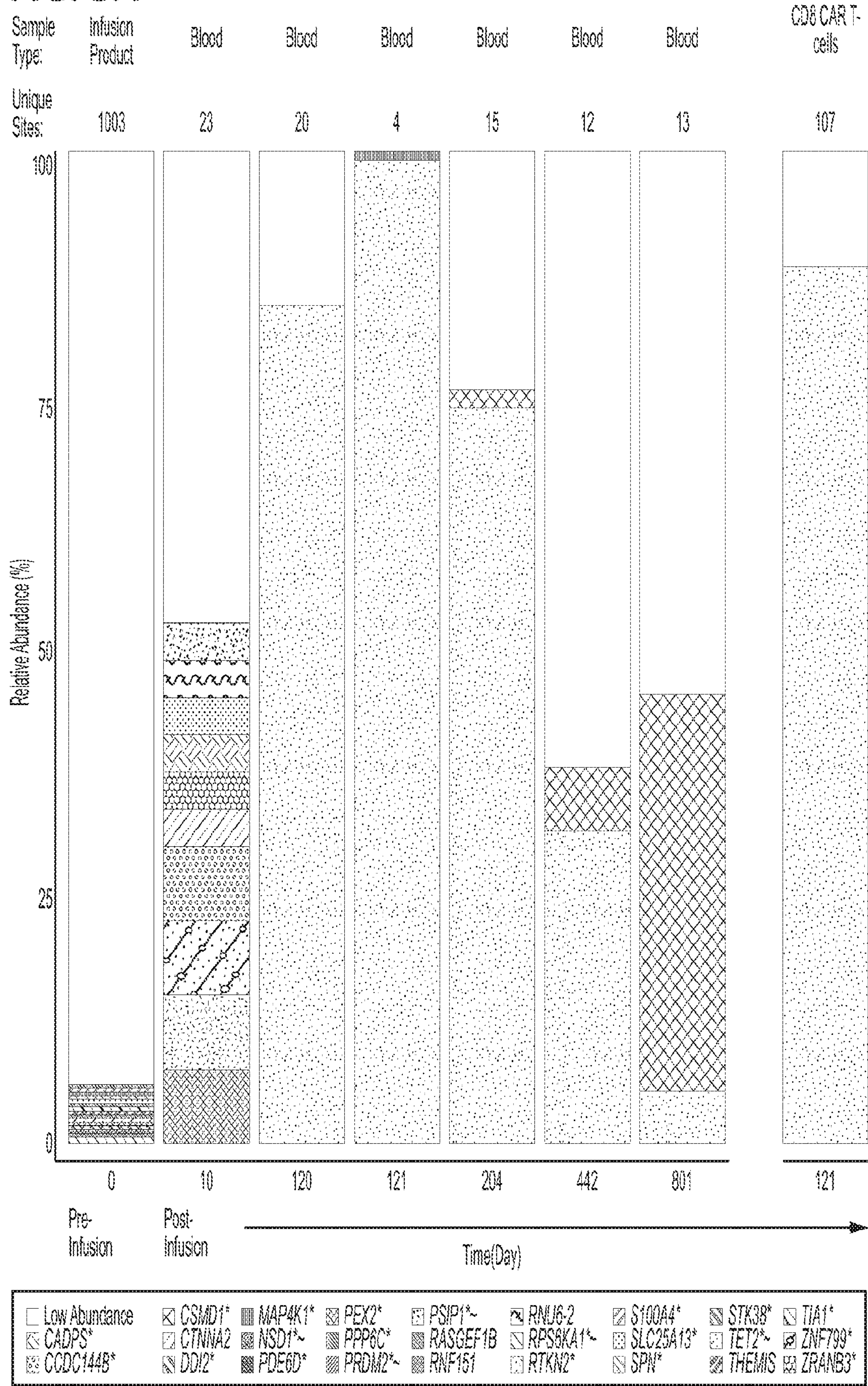


FIG. 5B

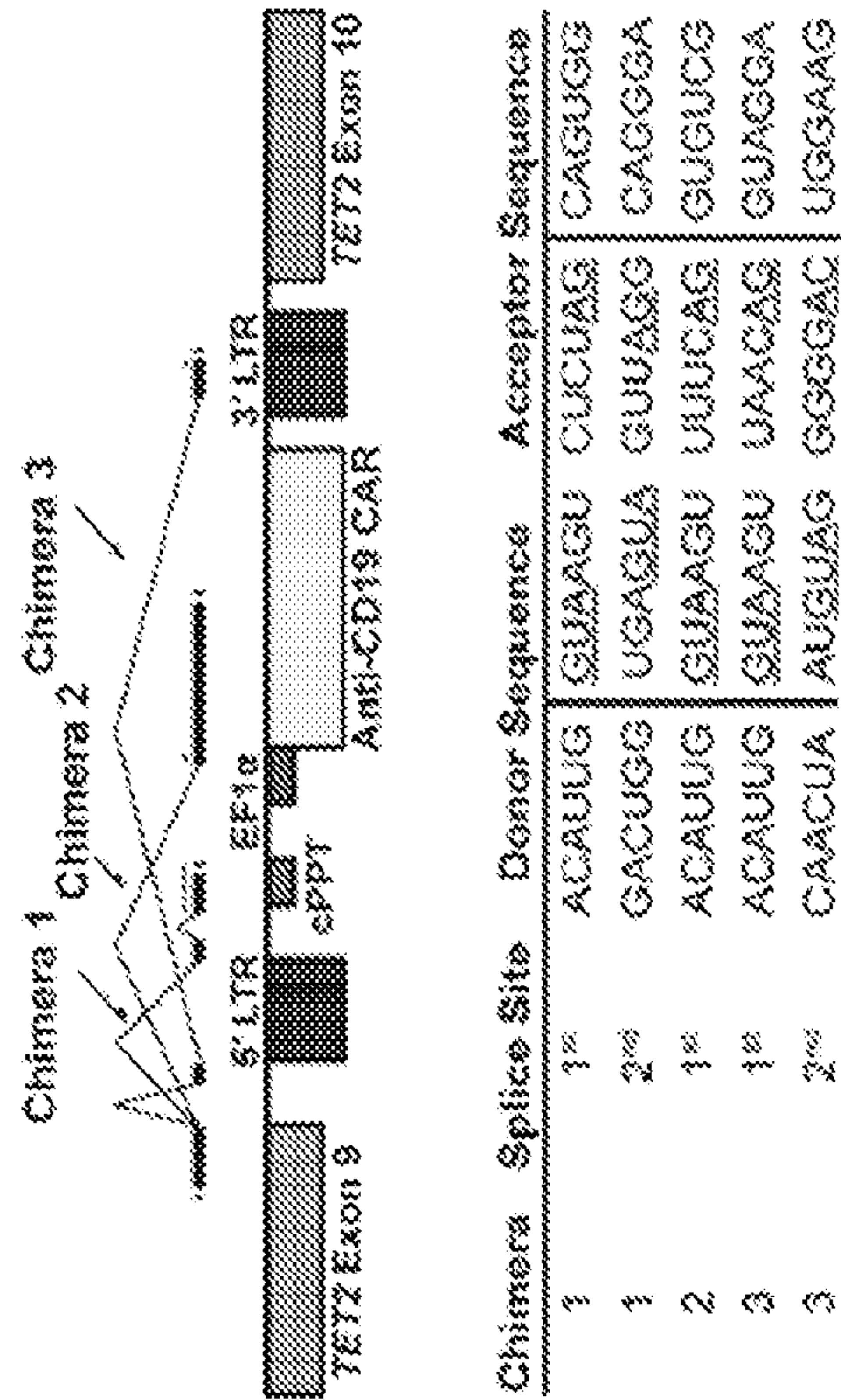


FIG. 6A

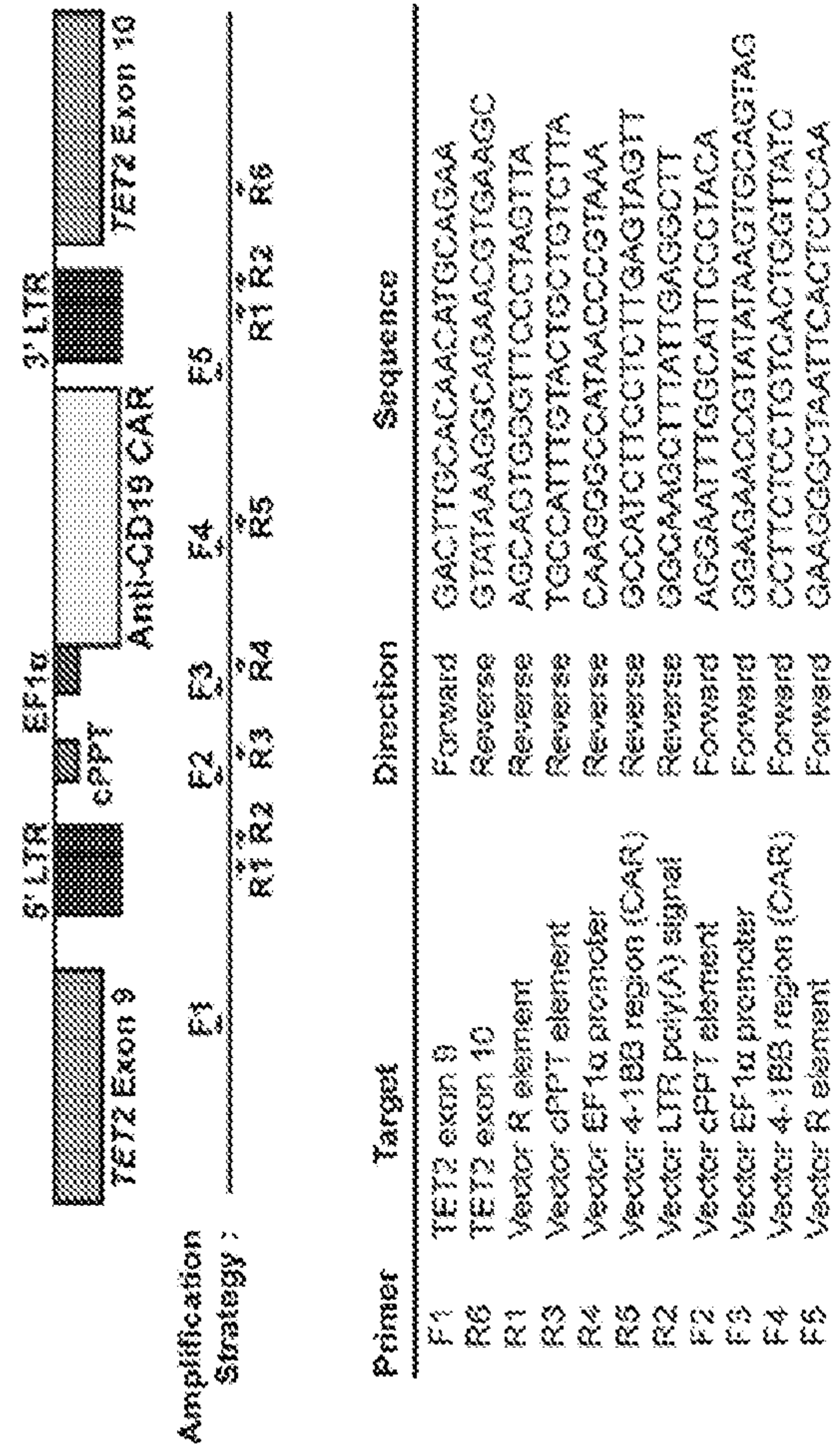


FIG. 6B

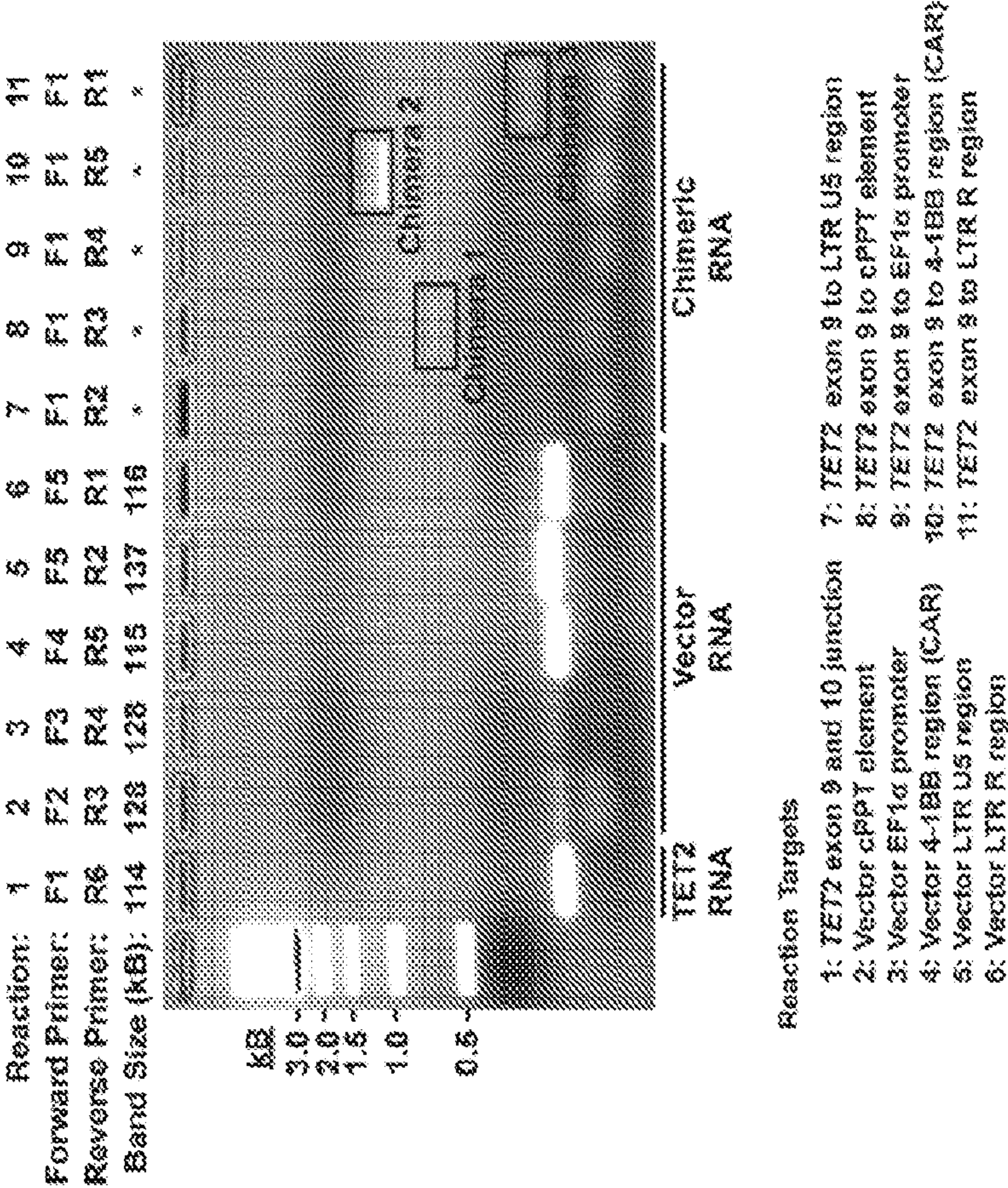


FIG. 7A

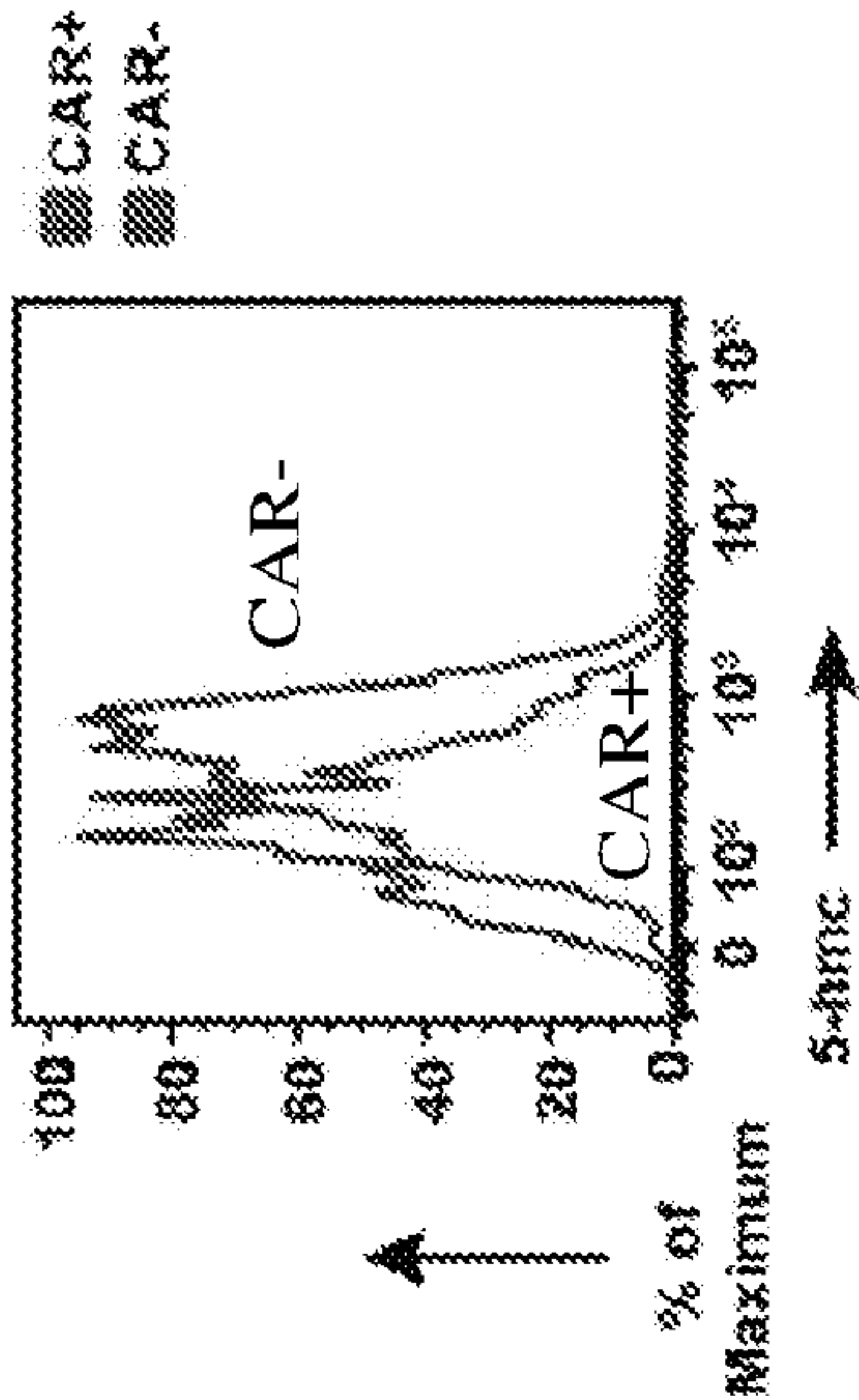


FIG. 7B

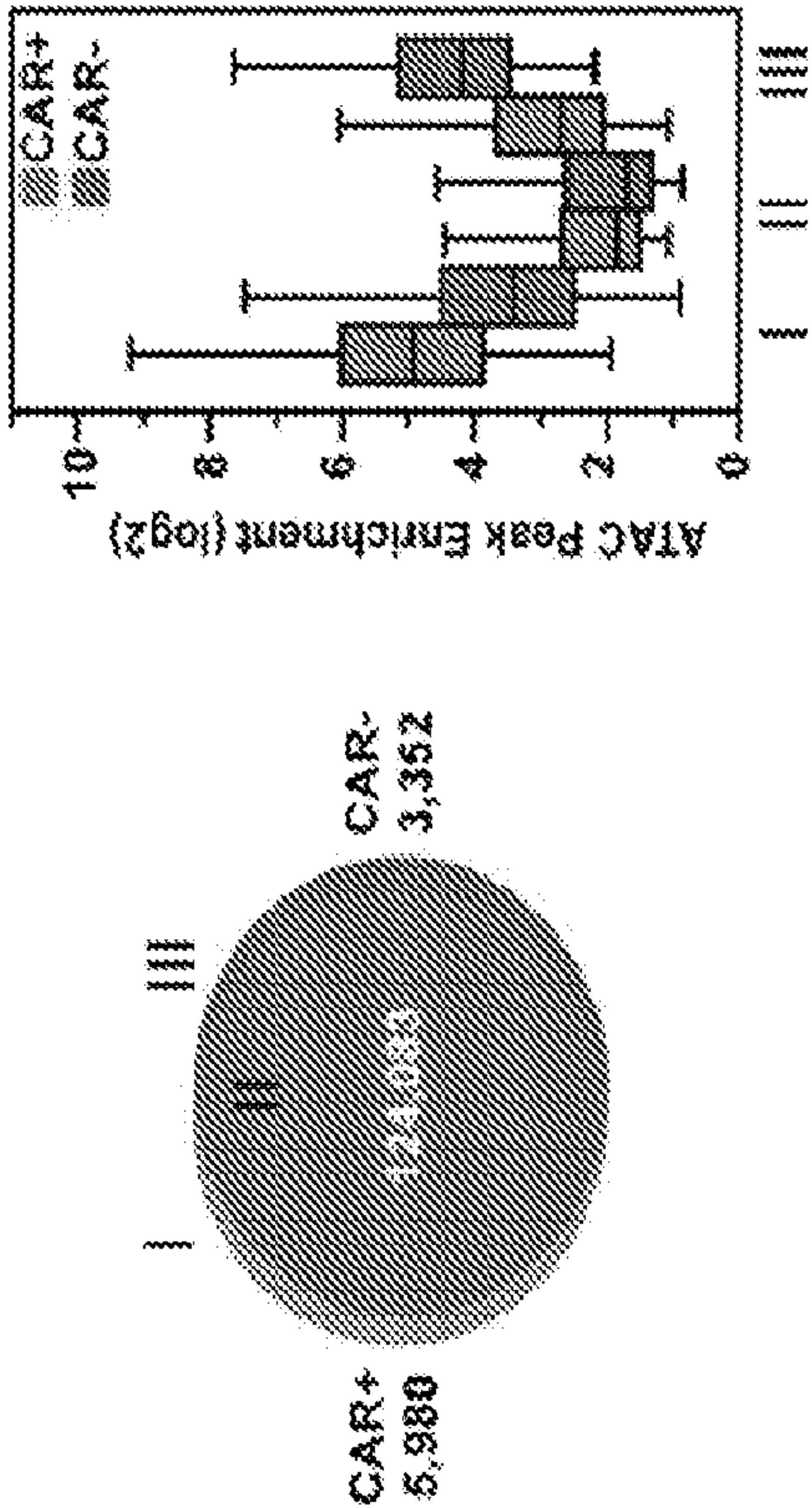


FIG. 7C

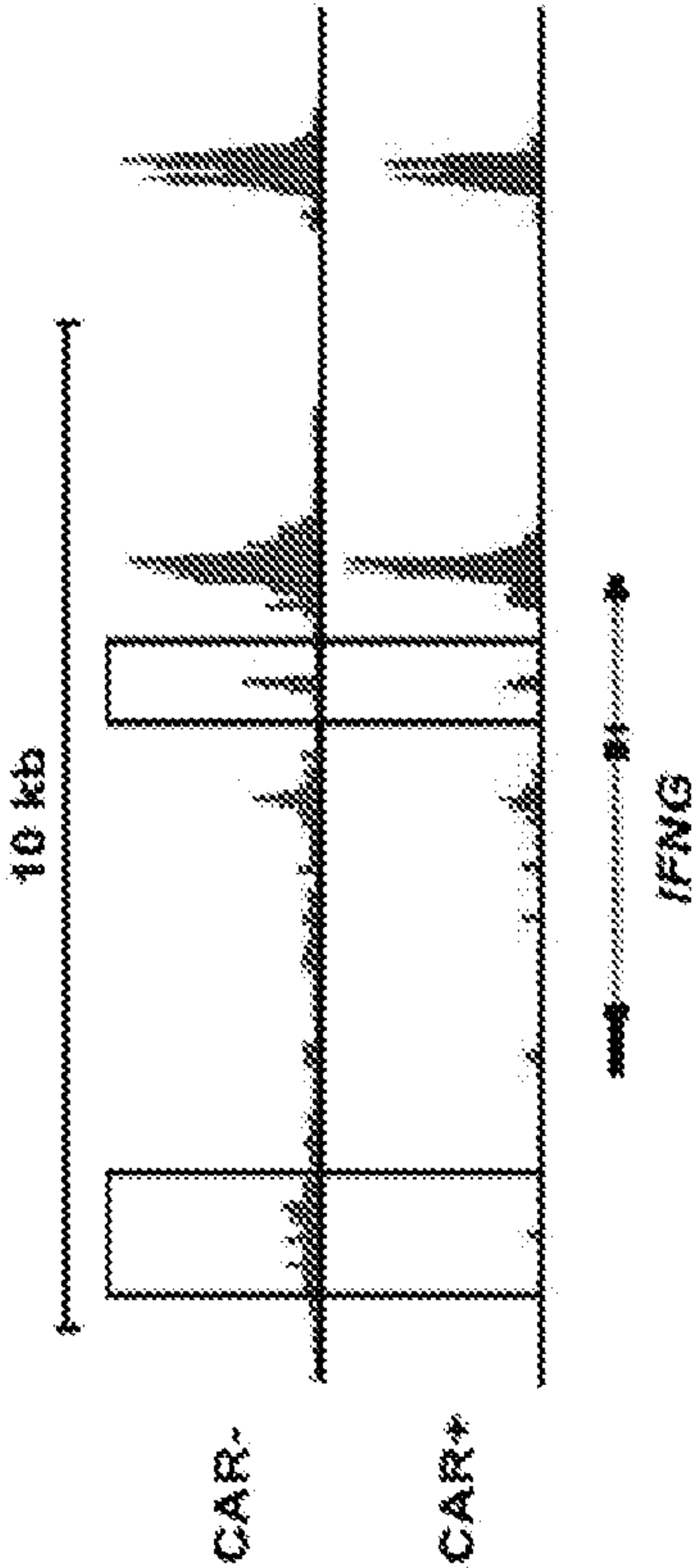
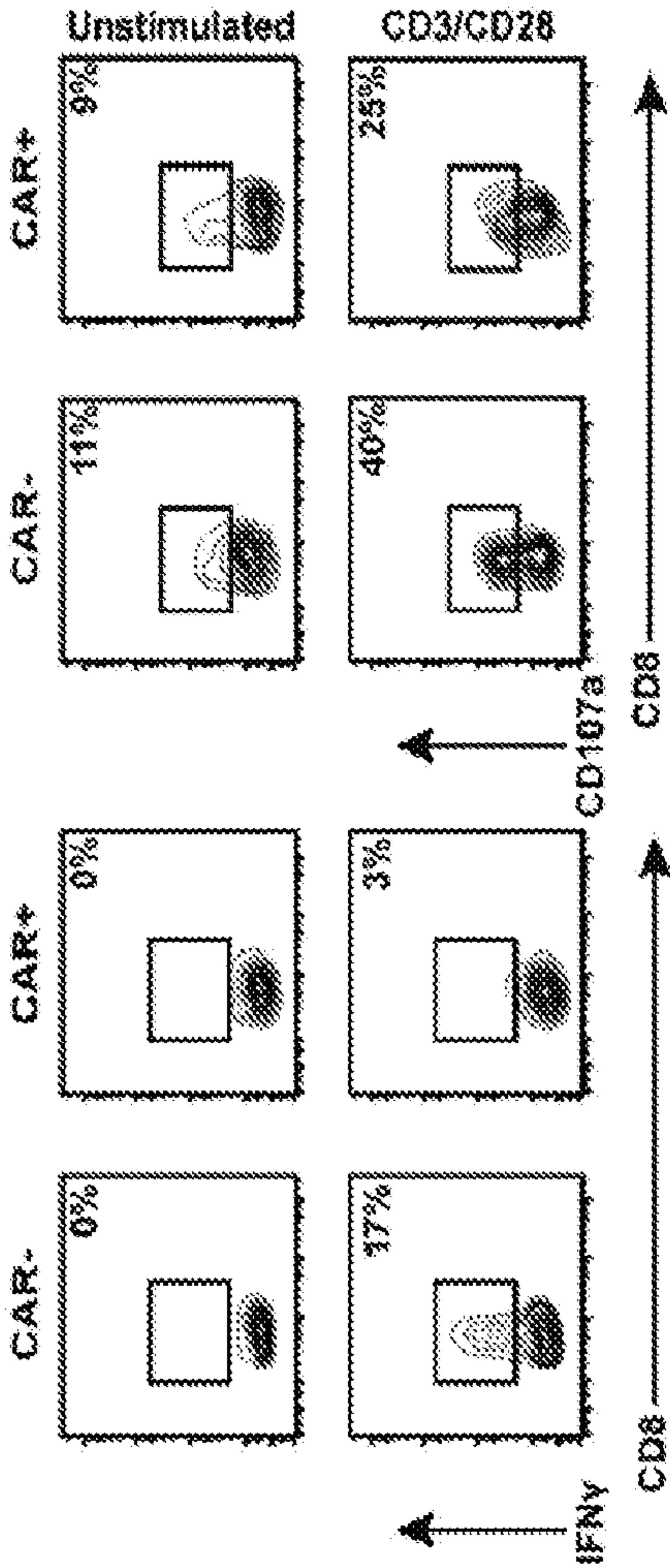


FIG. 7D



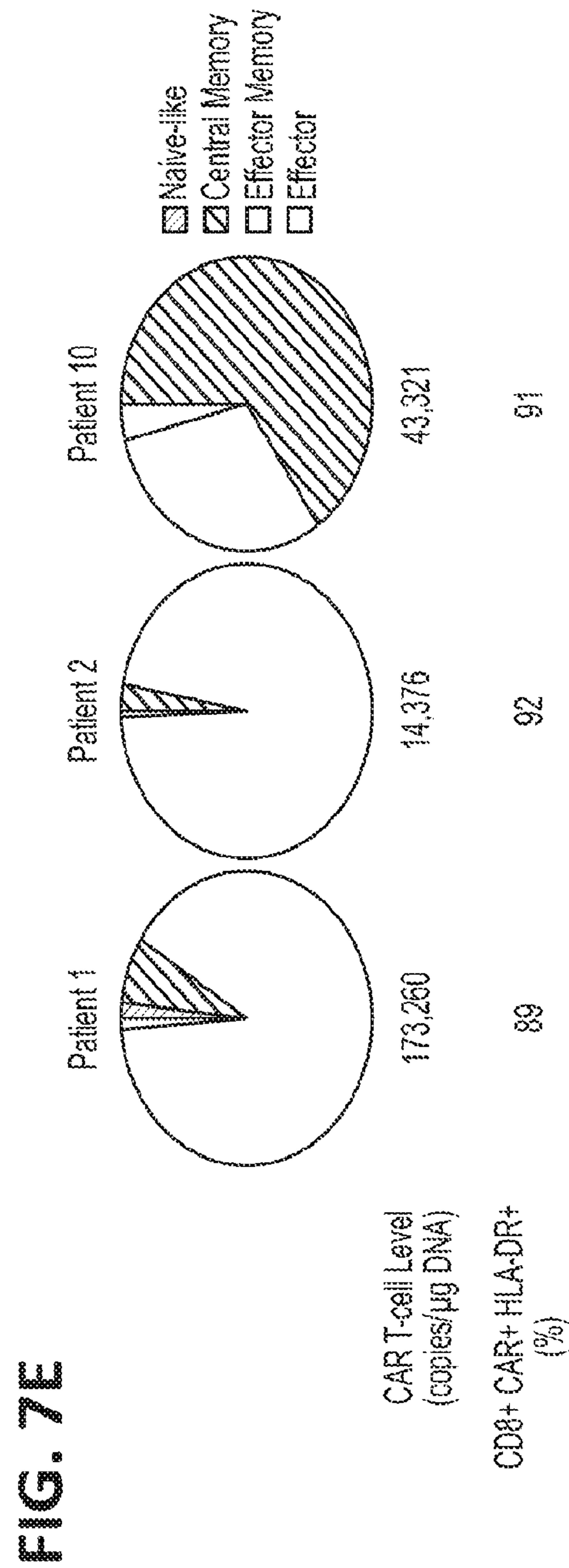


FIG. 7F

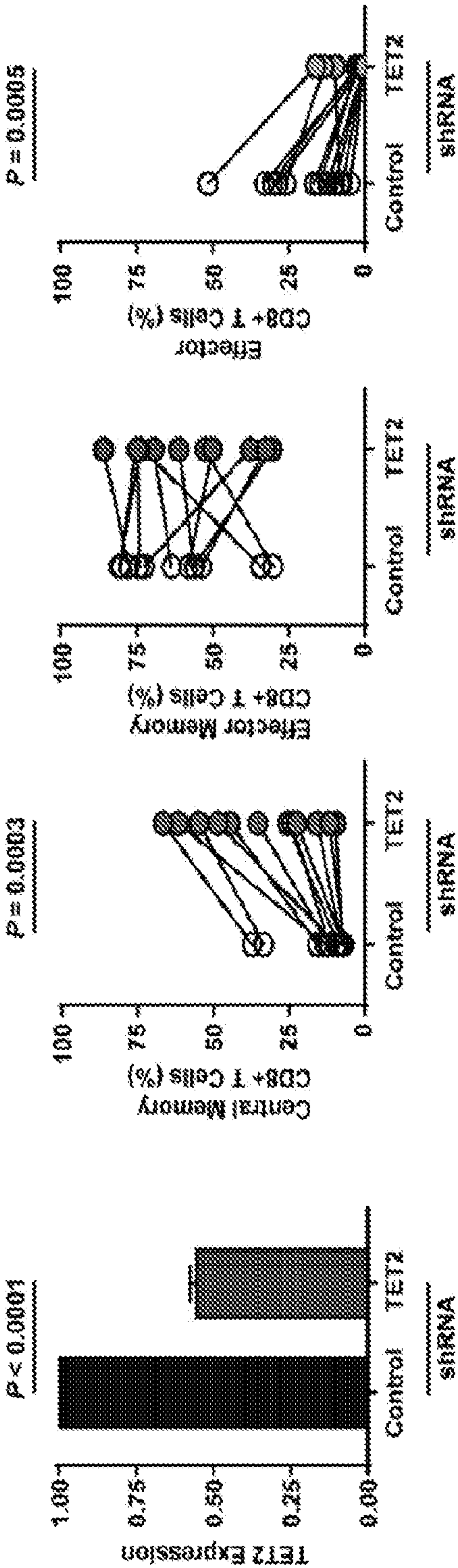


FIG. 8

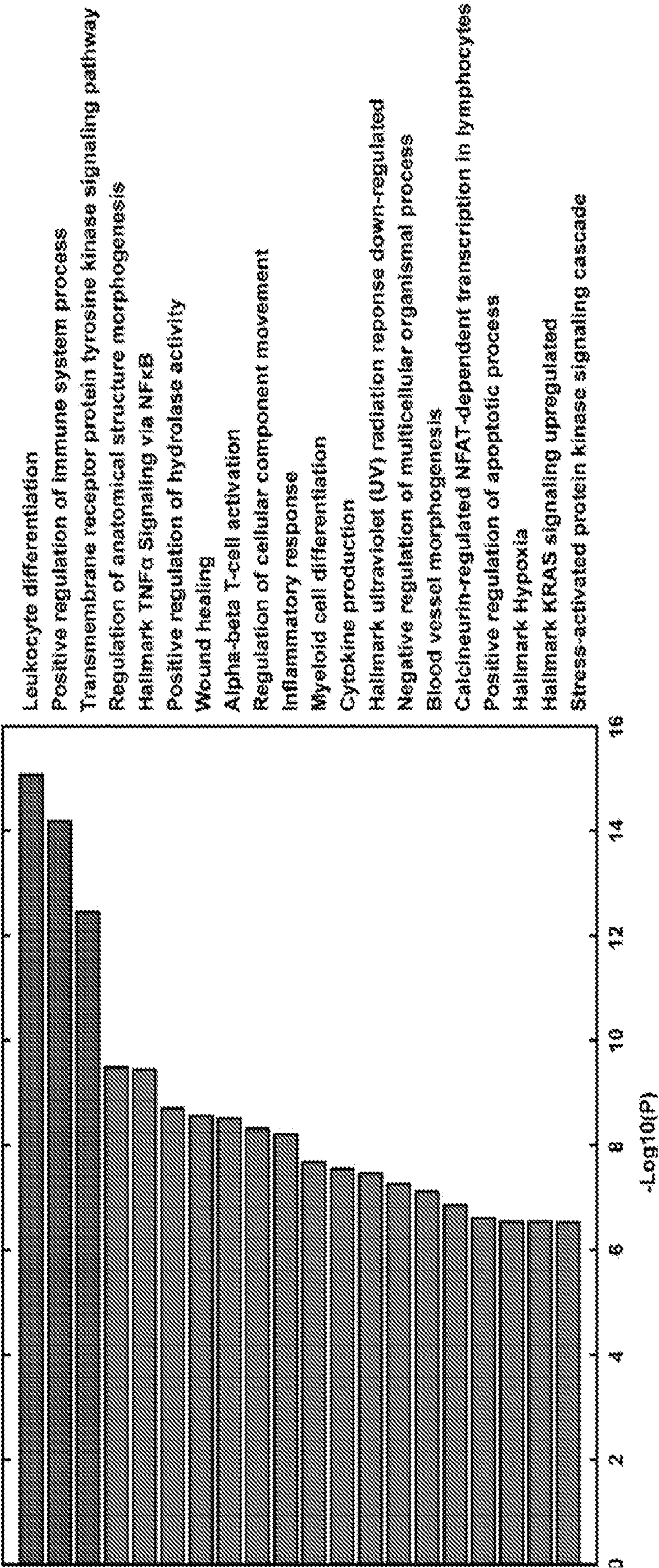


FIG. 9

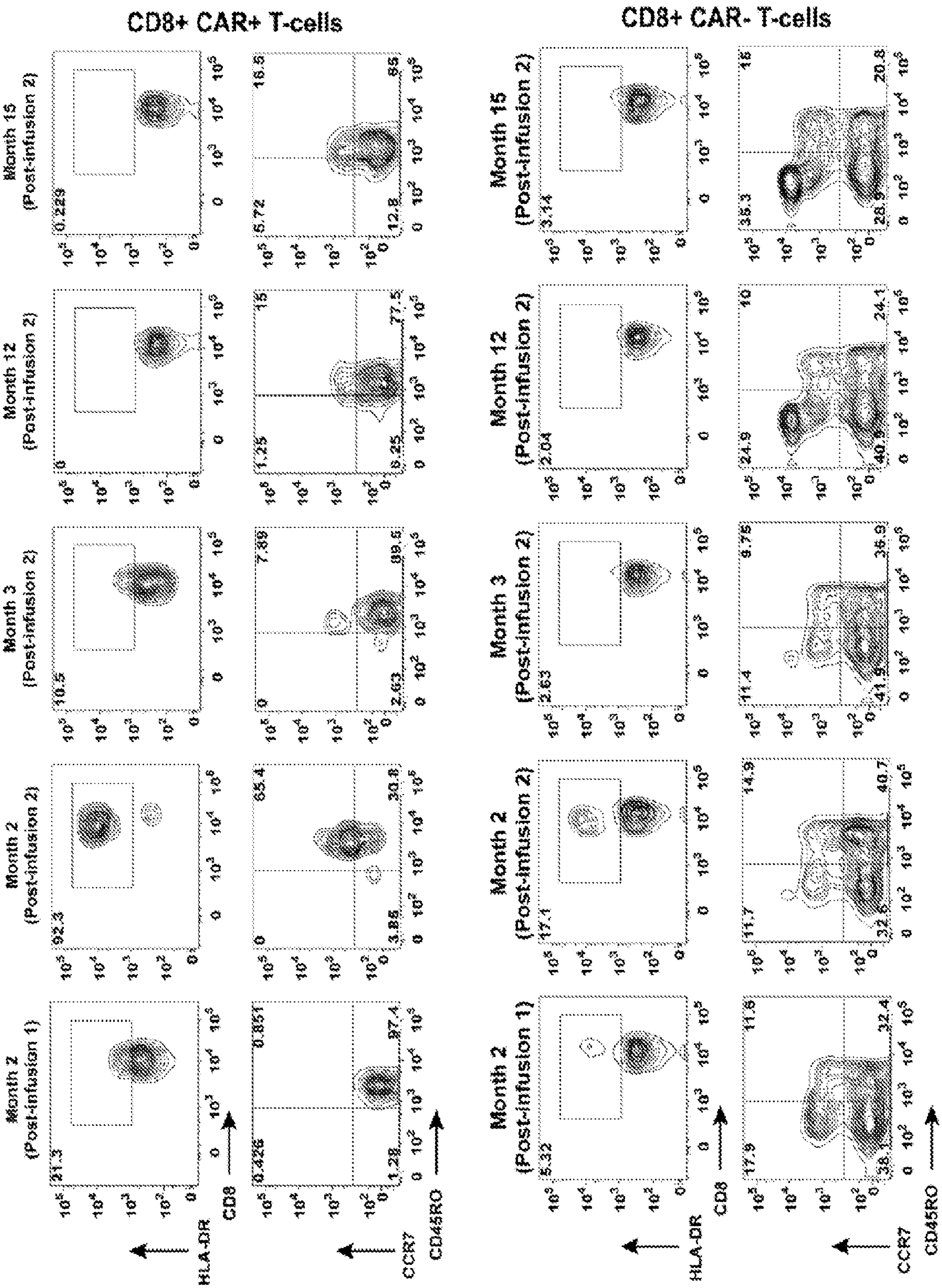


FIG. 10A

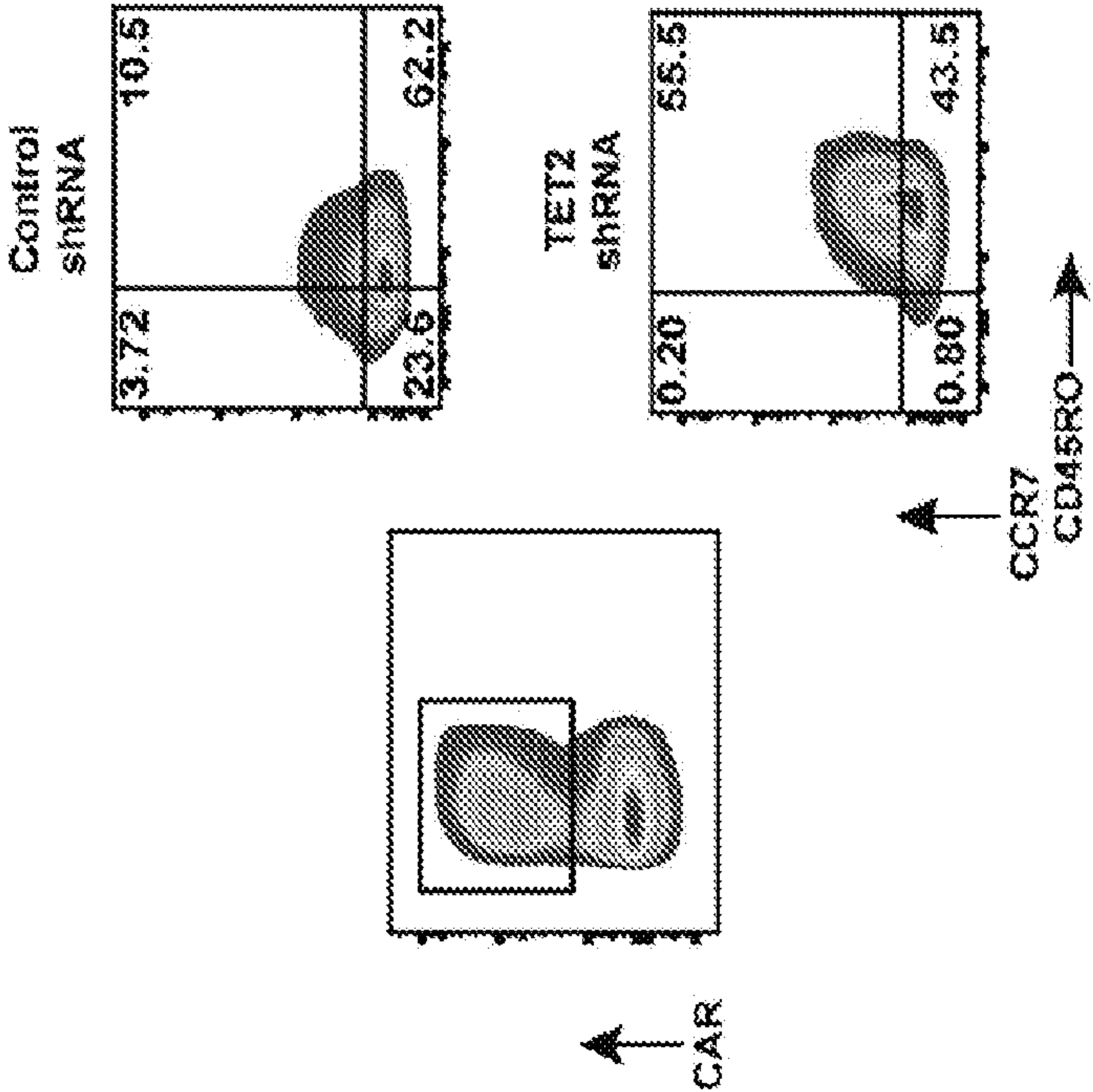


FIG. 10B

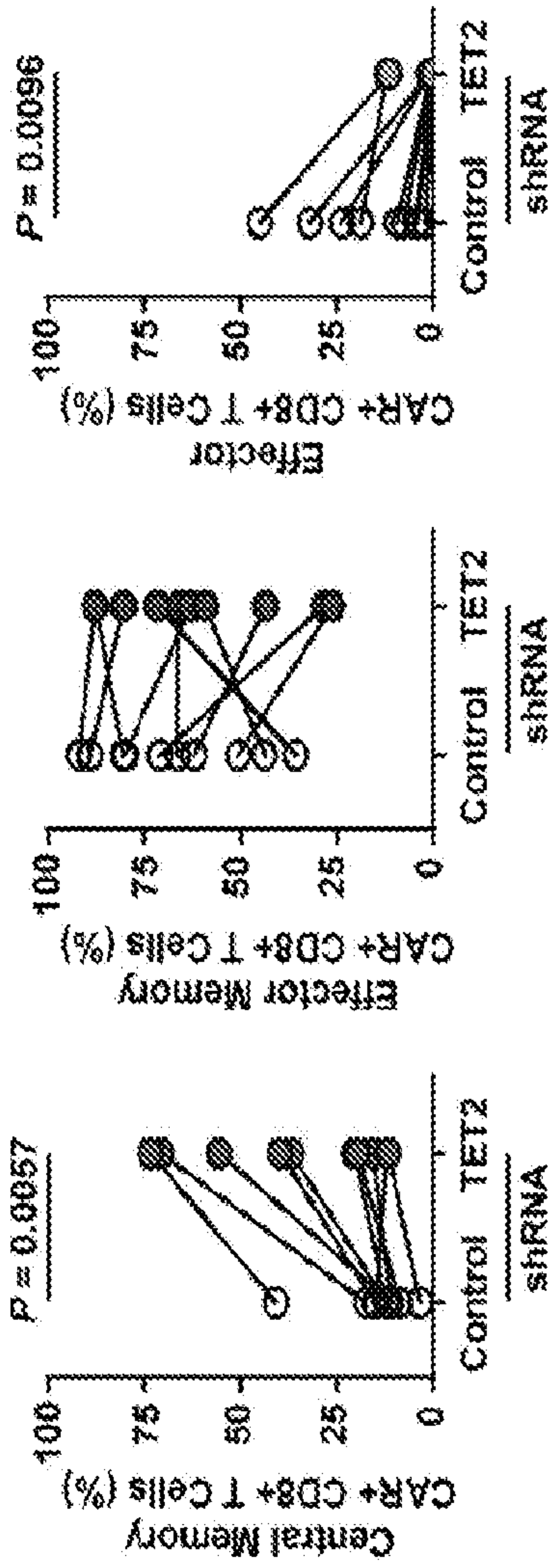


FIG. 10C

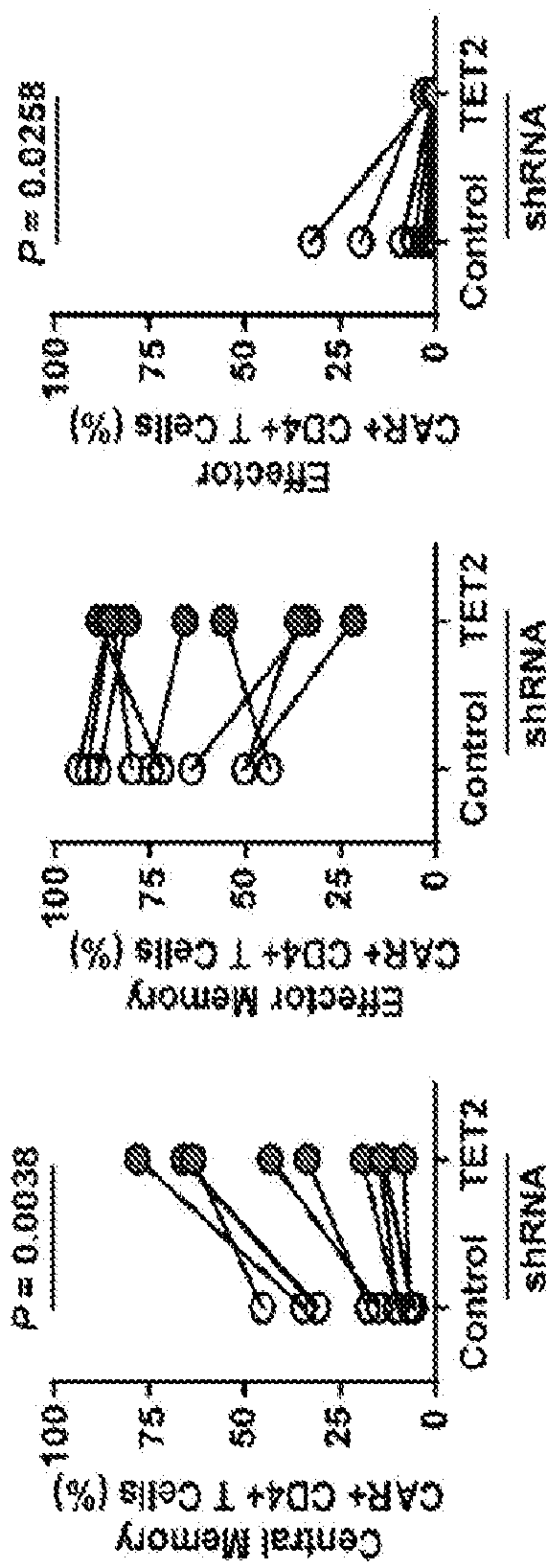


FIG. 11A

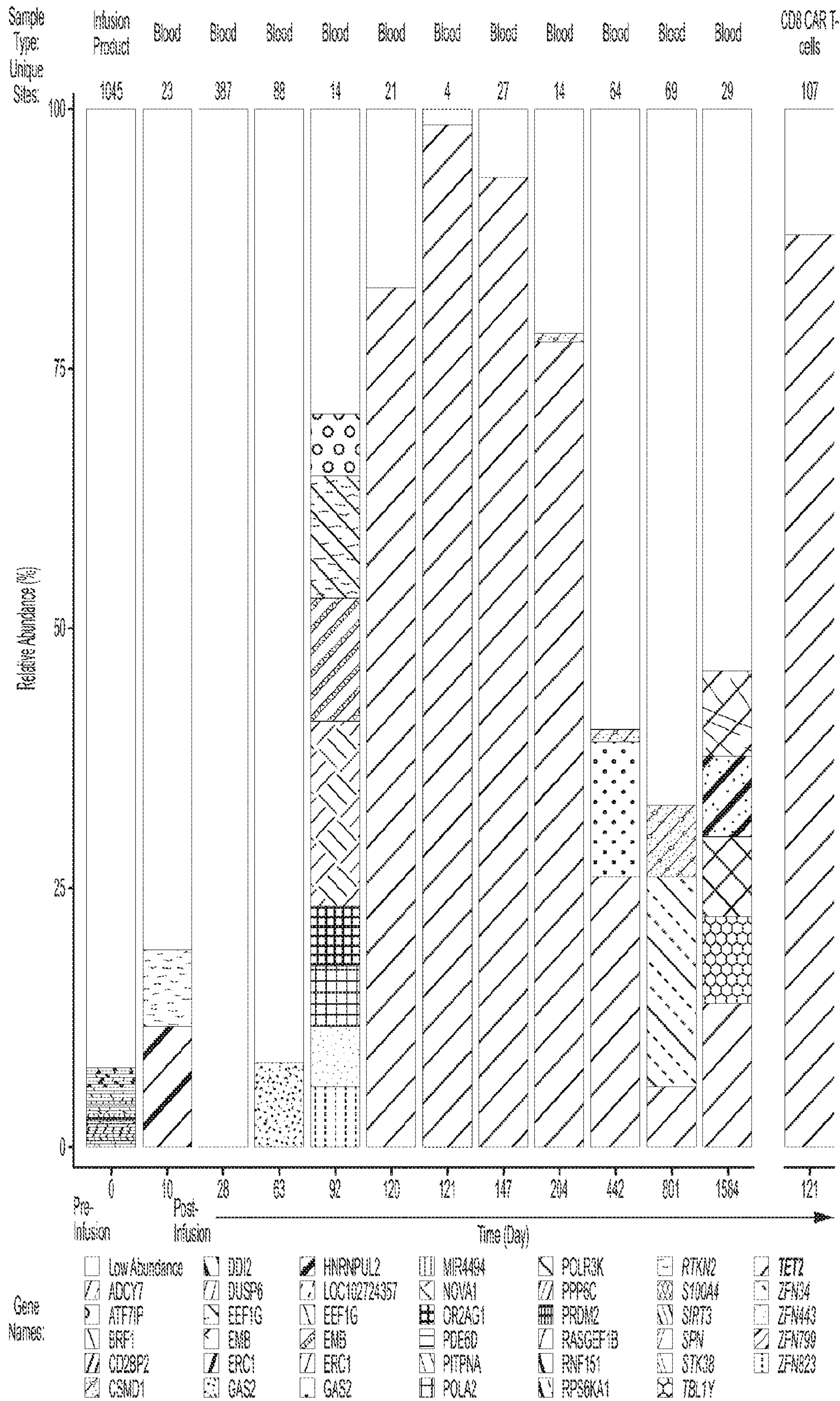


FIG. 11B

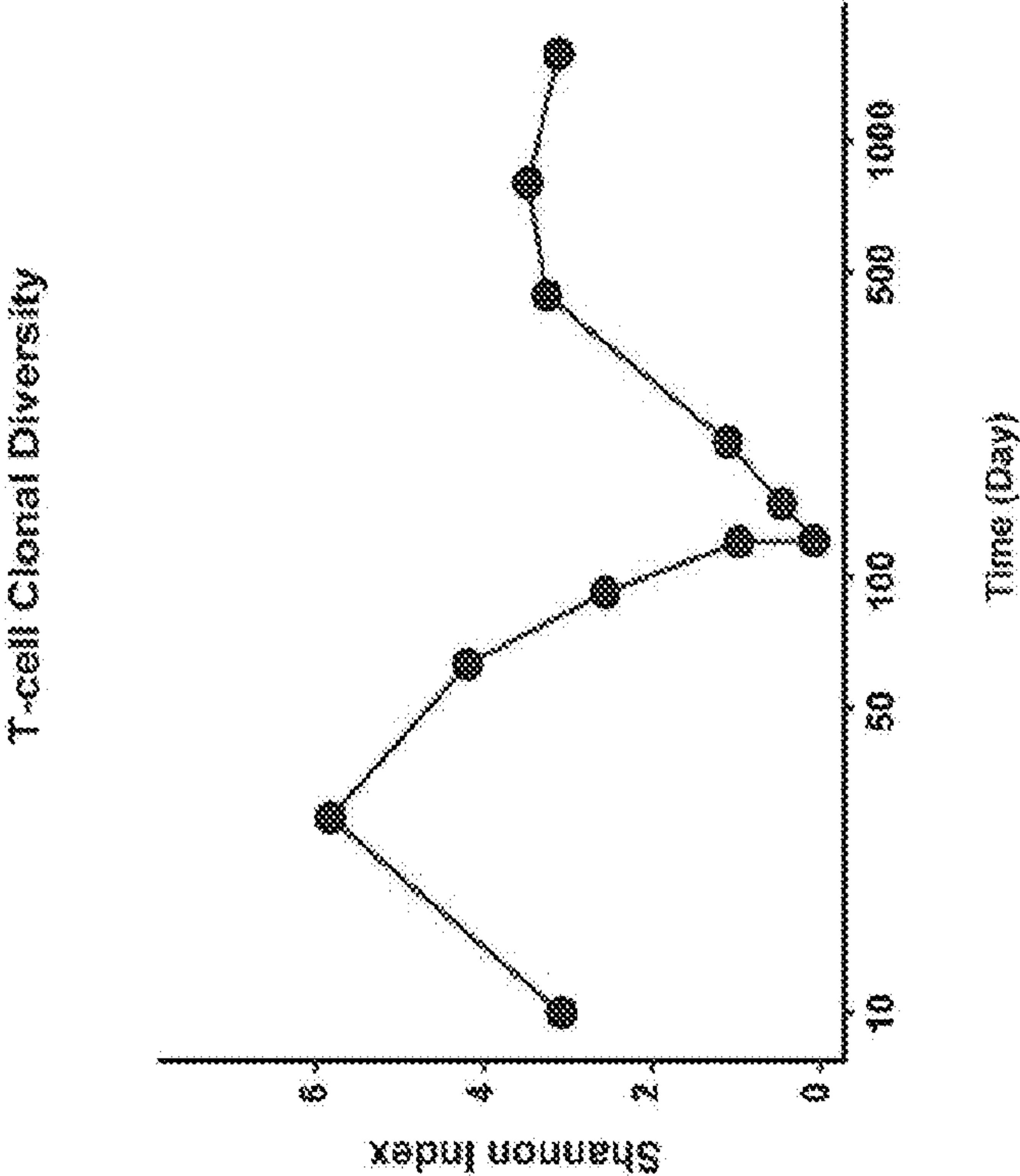


FIG. 11C

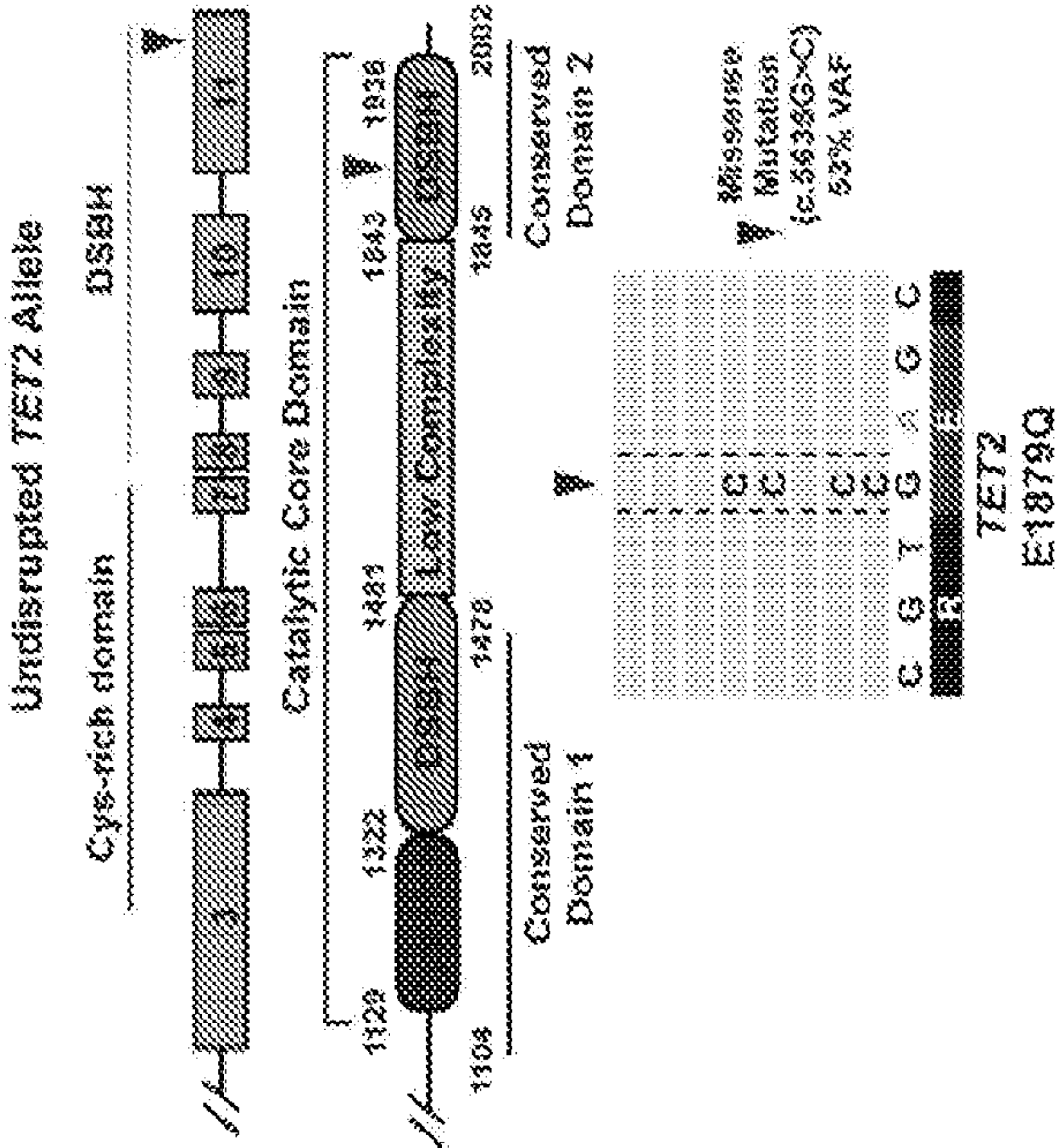


FIG. 11D

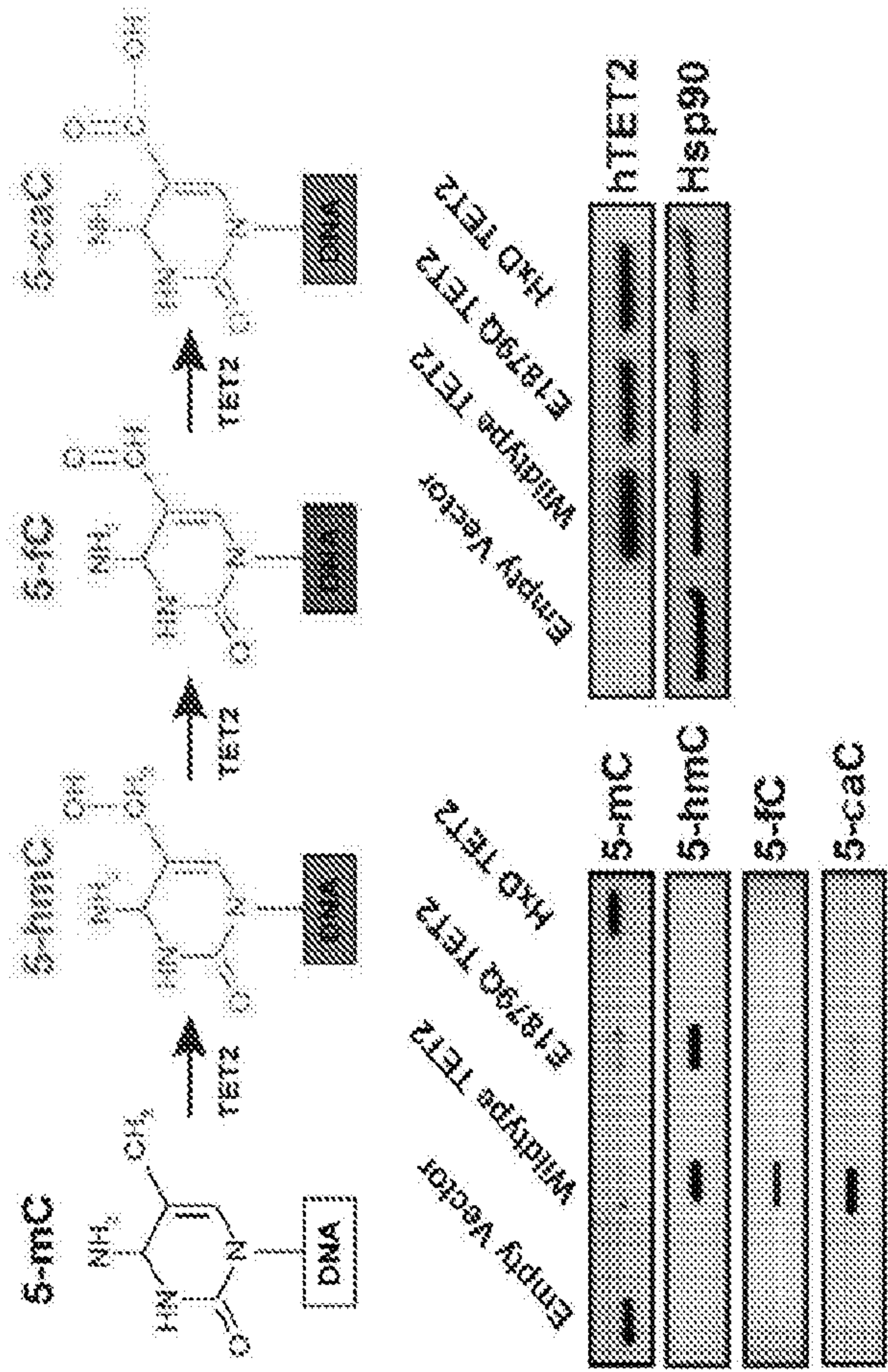


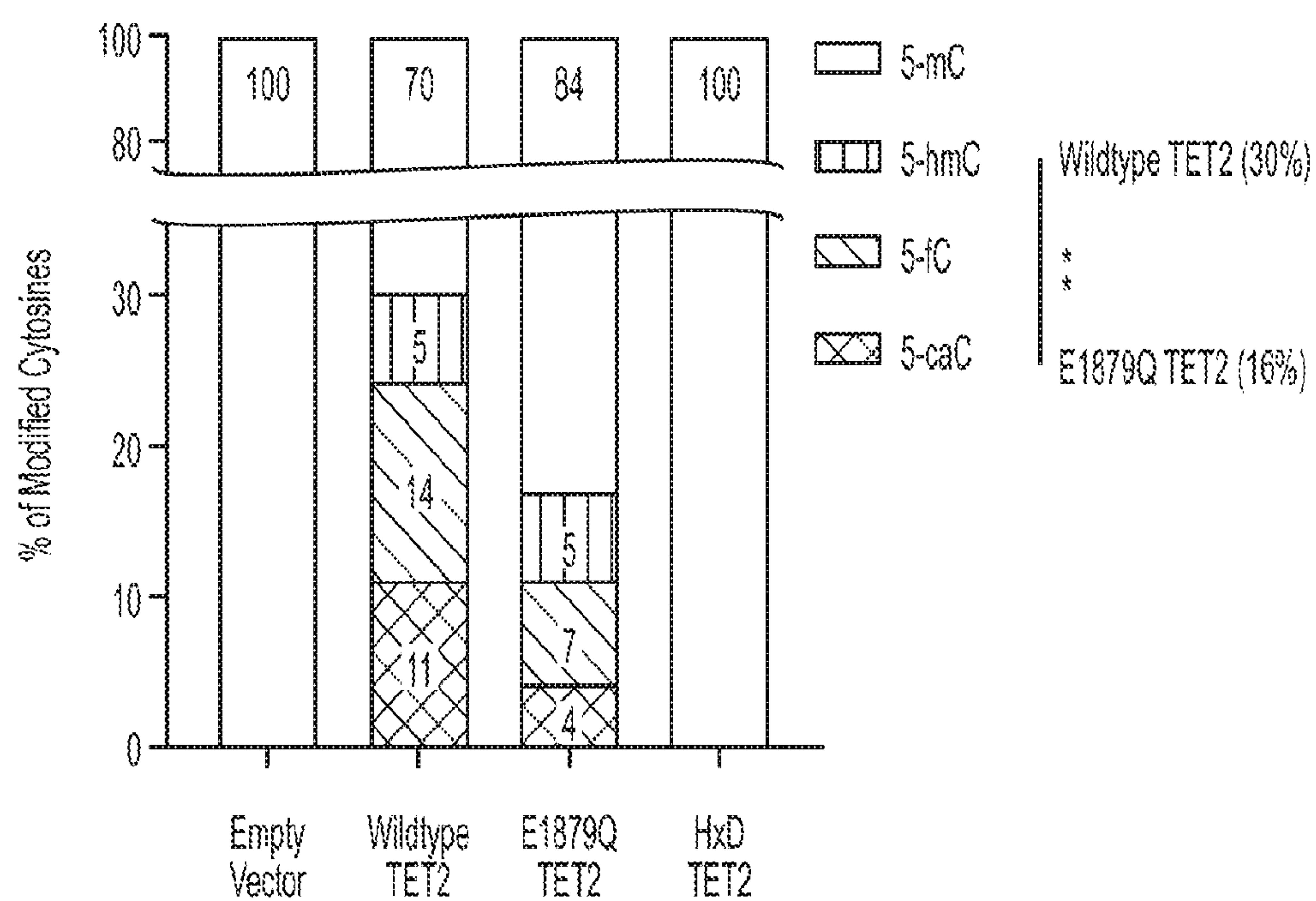
FIG. 11E

FIG. 12A

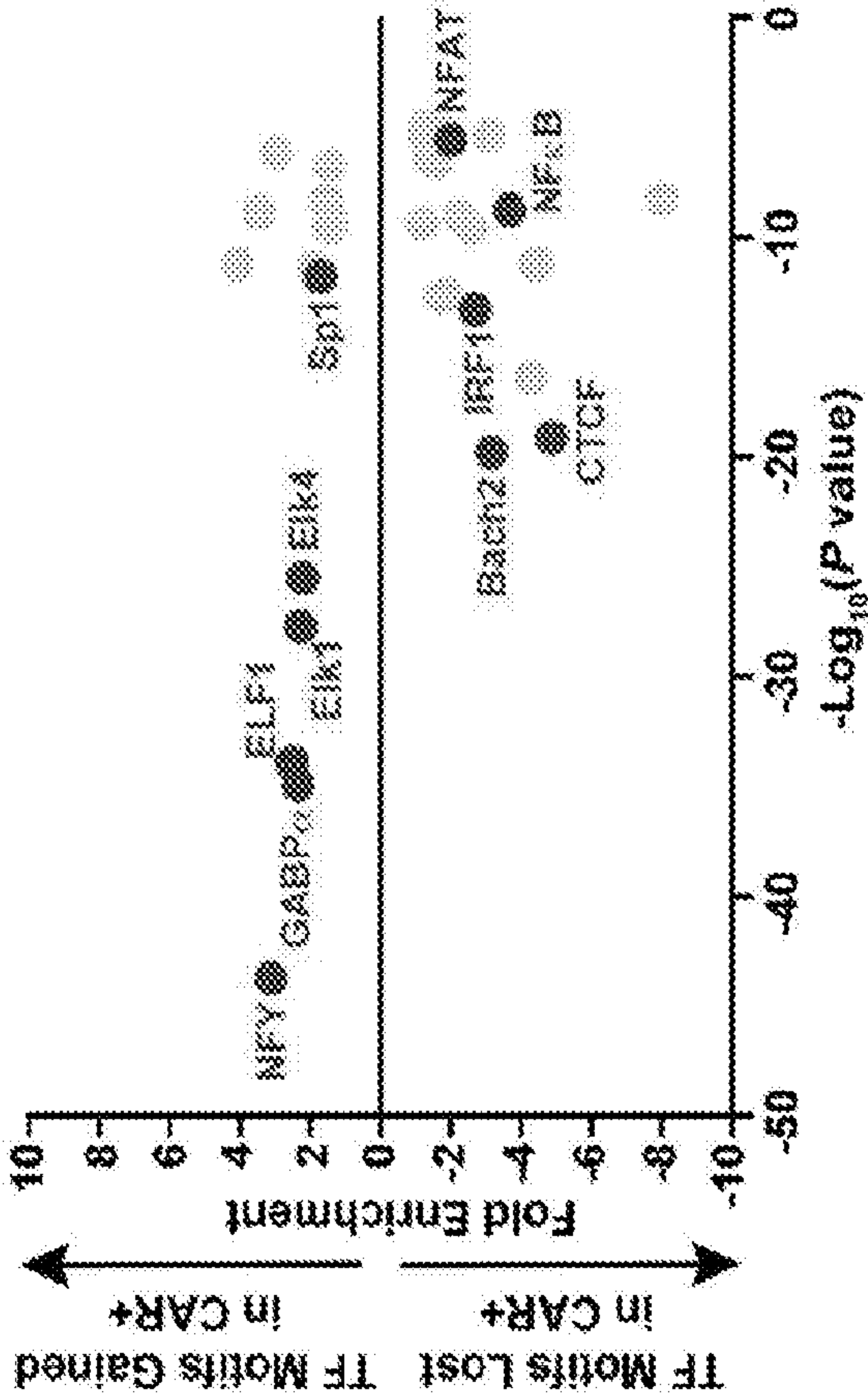


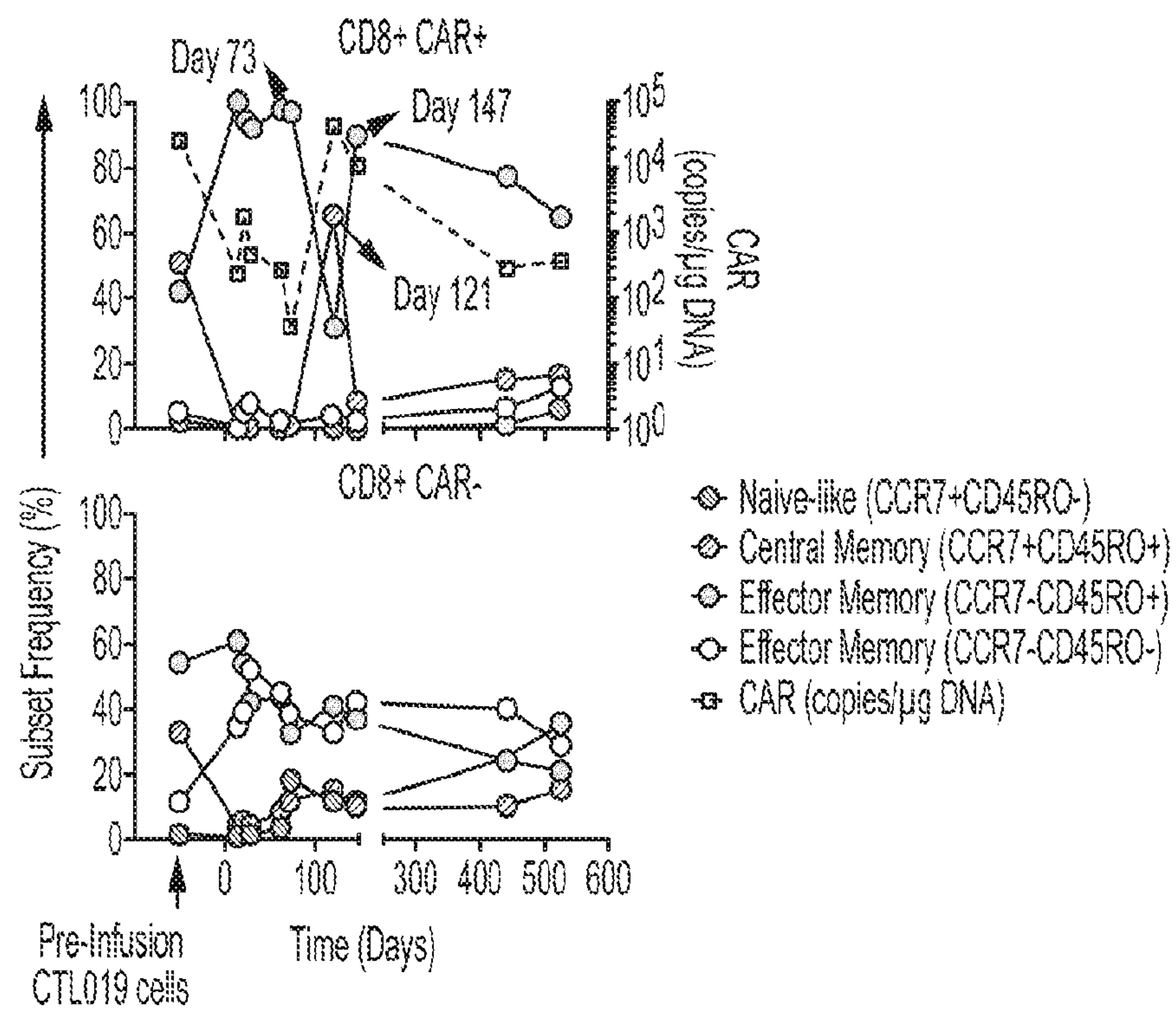
FIG. 12B

FIG. 12C

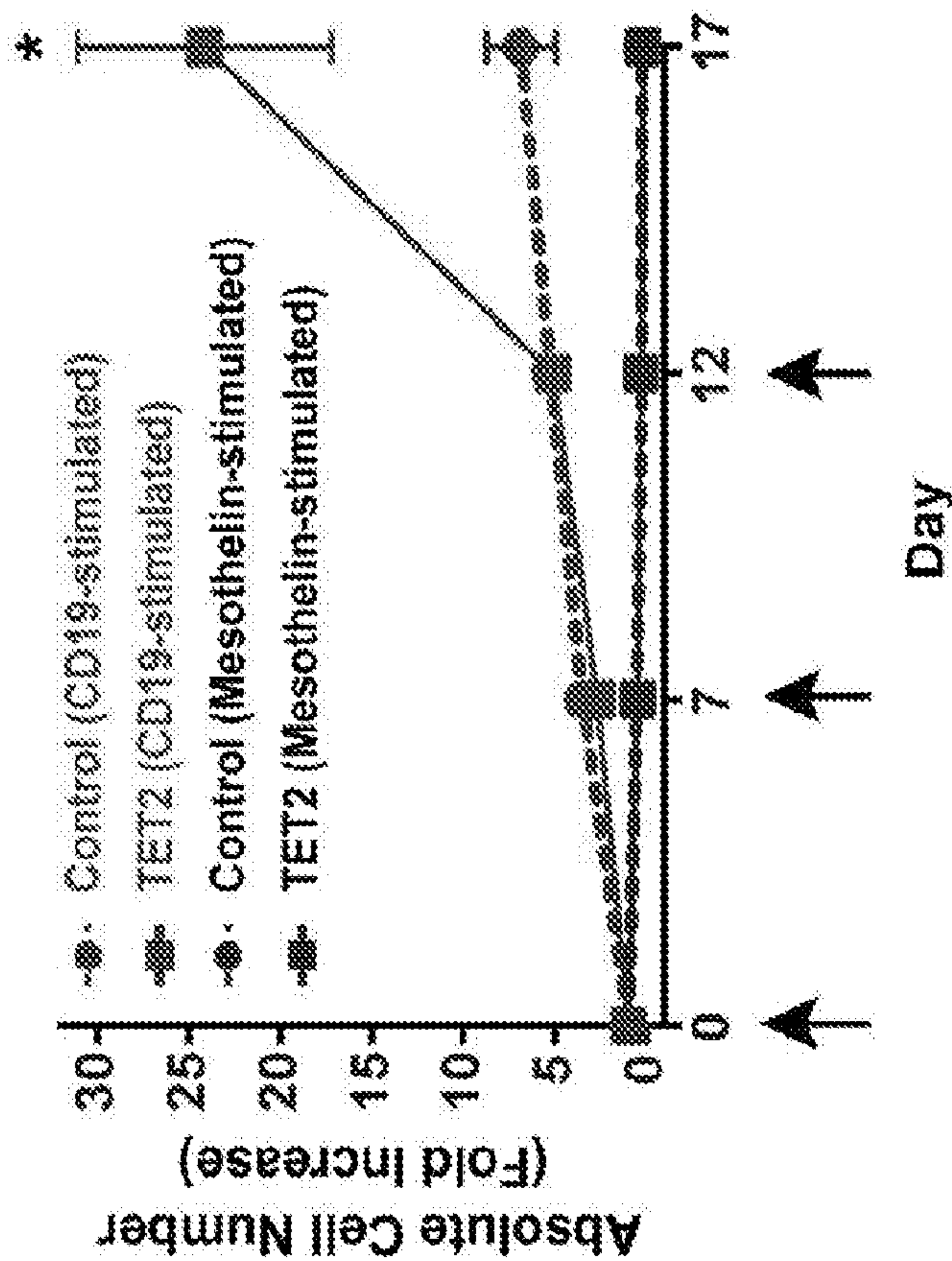


FIG. 13

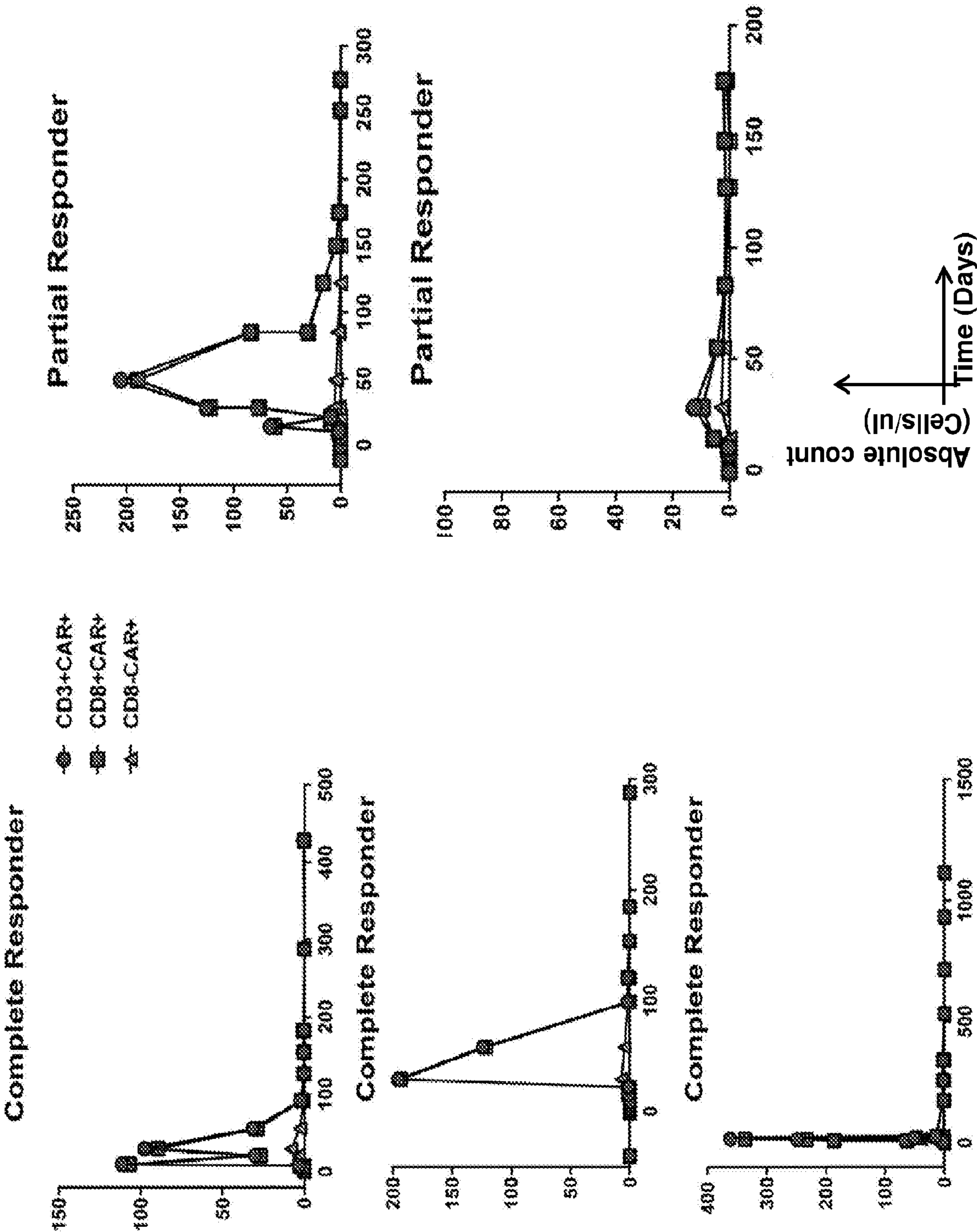


FIG. 14A

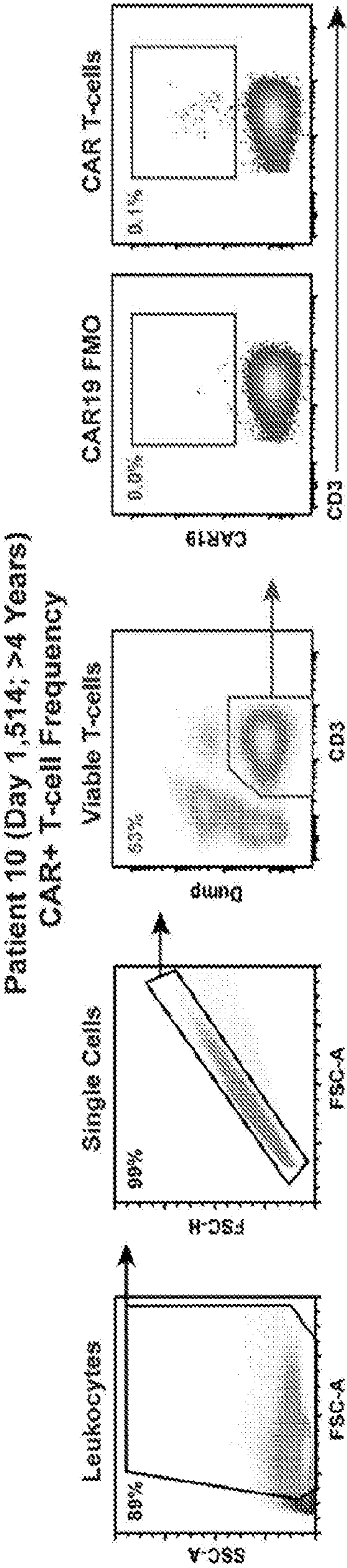


FIG. 14B

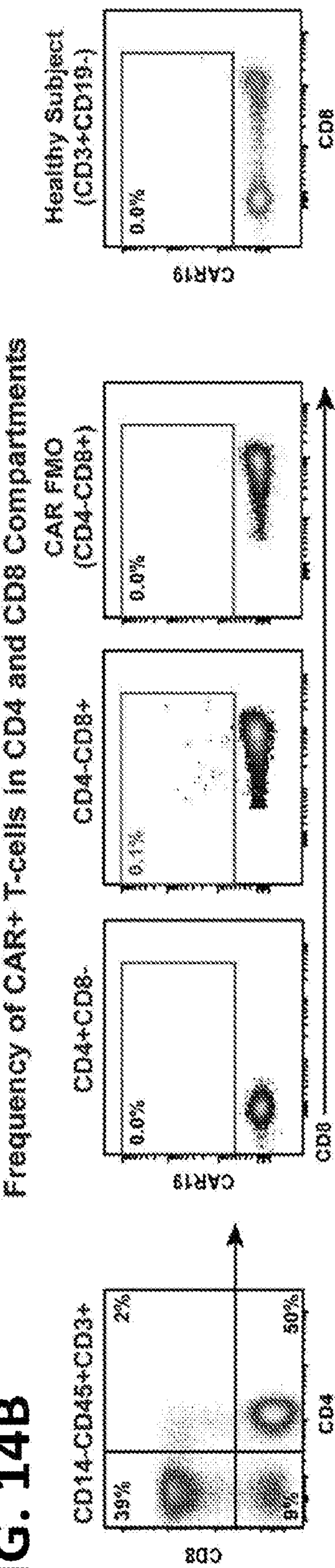


FIG. 14C

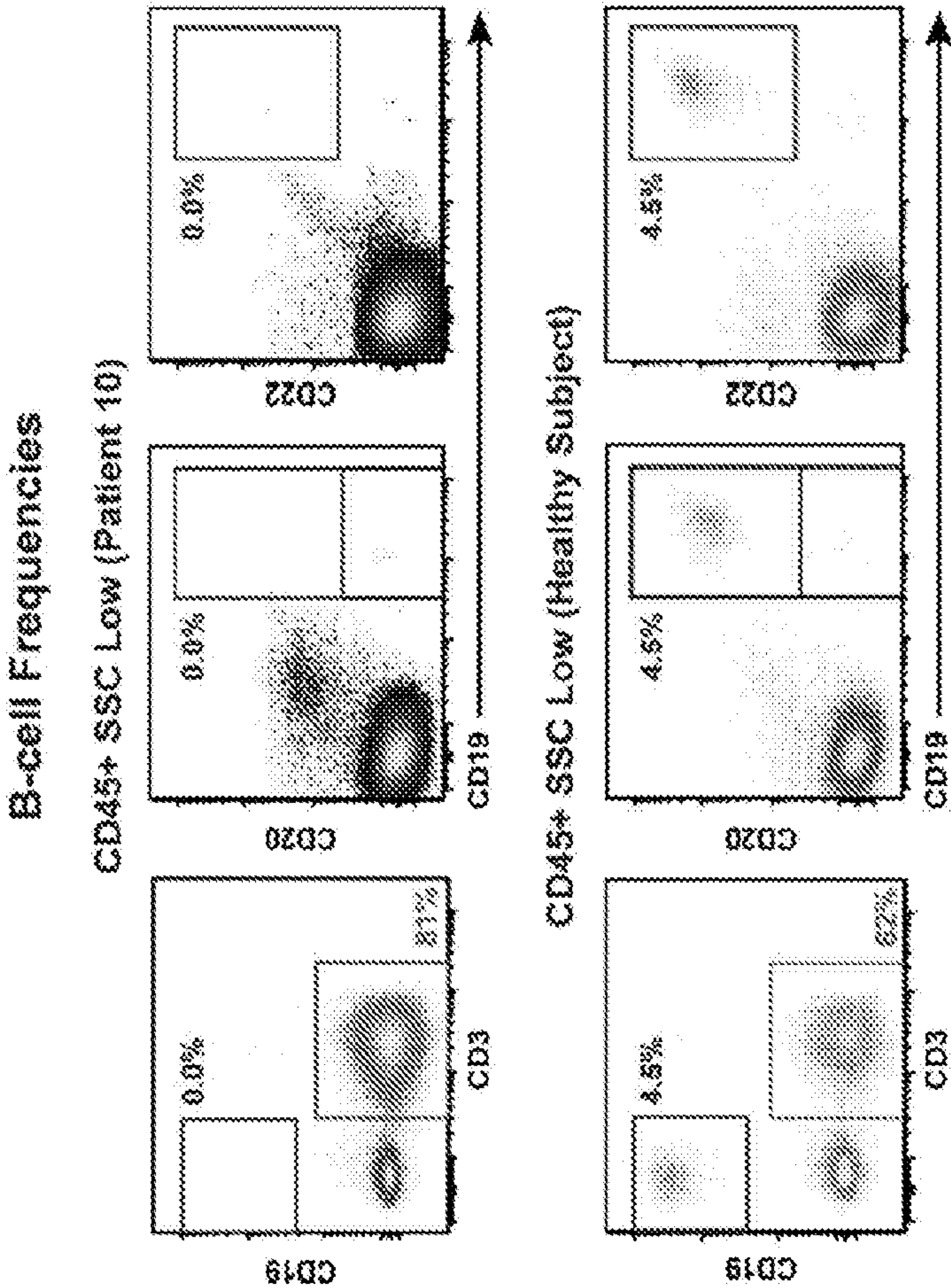


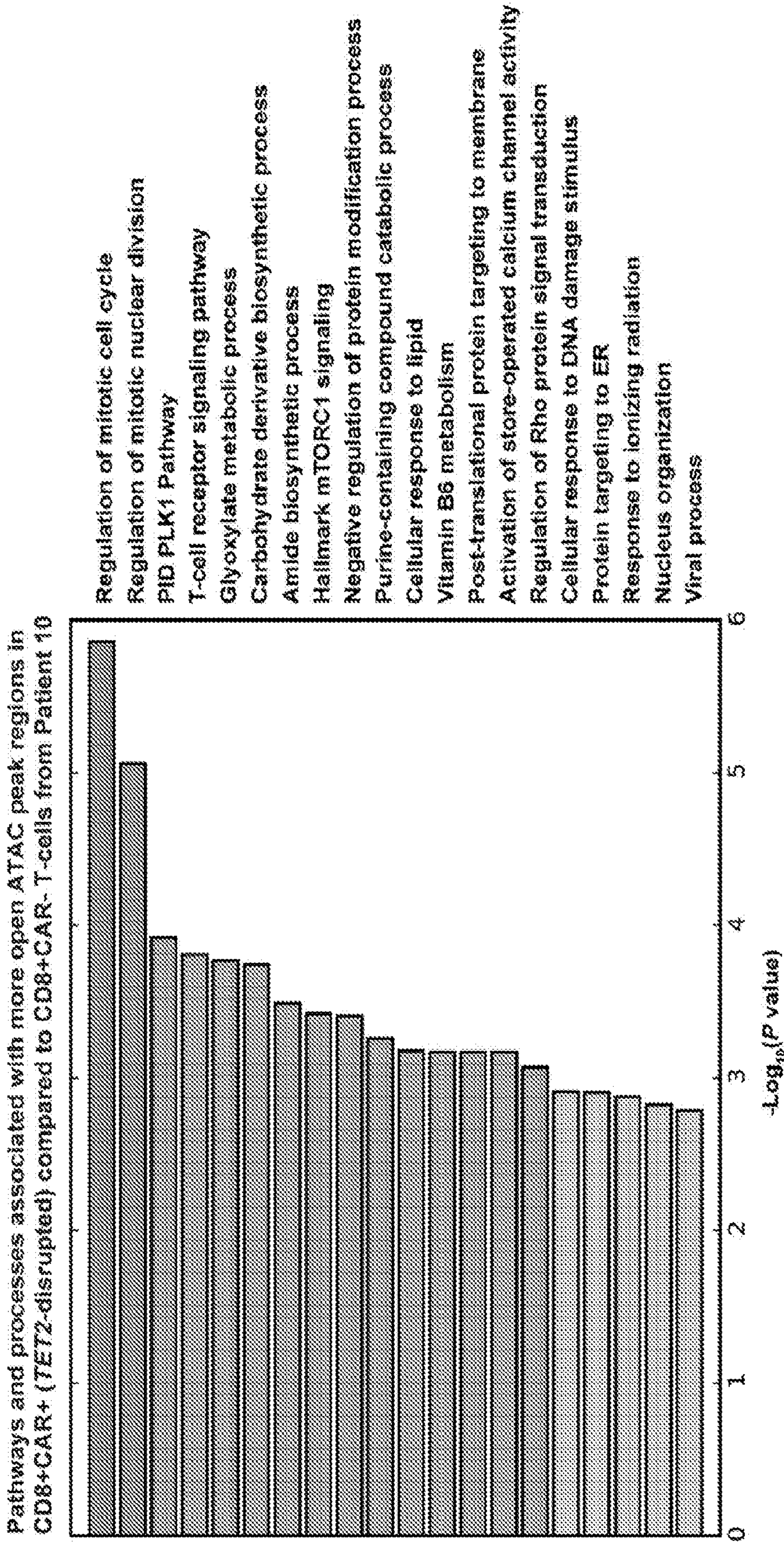
FIG. 14D

Enumeration of Peripheral Blood Cells	
Cell Population	Frequency (%)
CD45+CD14-	65
CD3+ (Live/Dead Dye-)	53
CD3+CD8+	39
CD3+CD4+	51
CD3+CD4+CD8+	2
CD3-CD56+	16
CD3+CD56+	2
CD3+HLA-DR+	5
CD3+CD4+CD8+HLA-DR+	6
CD3+CD4-CD8+HLA-DR+	3
γδ TCR+	9
γδ TCR+CD4+	0.75
γδ TCR+CD8+	8
CD3+CAR19+	0.1
CD3+CD4+CAR19+	0
CD3+CD8+CAR19+	0.1
CD3+HLA-DR+CAR19+	0
γδ TCR+CAR19+	0
CD19+	0

FIG. 14E

qPCR-Based Detection of CAR19 in the Peripheral Blood		
Average Ct	SD	Copies/μg DNA
33.56	0.09	136.54

FIG. 15



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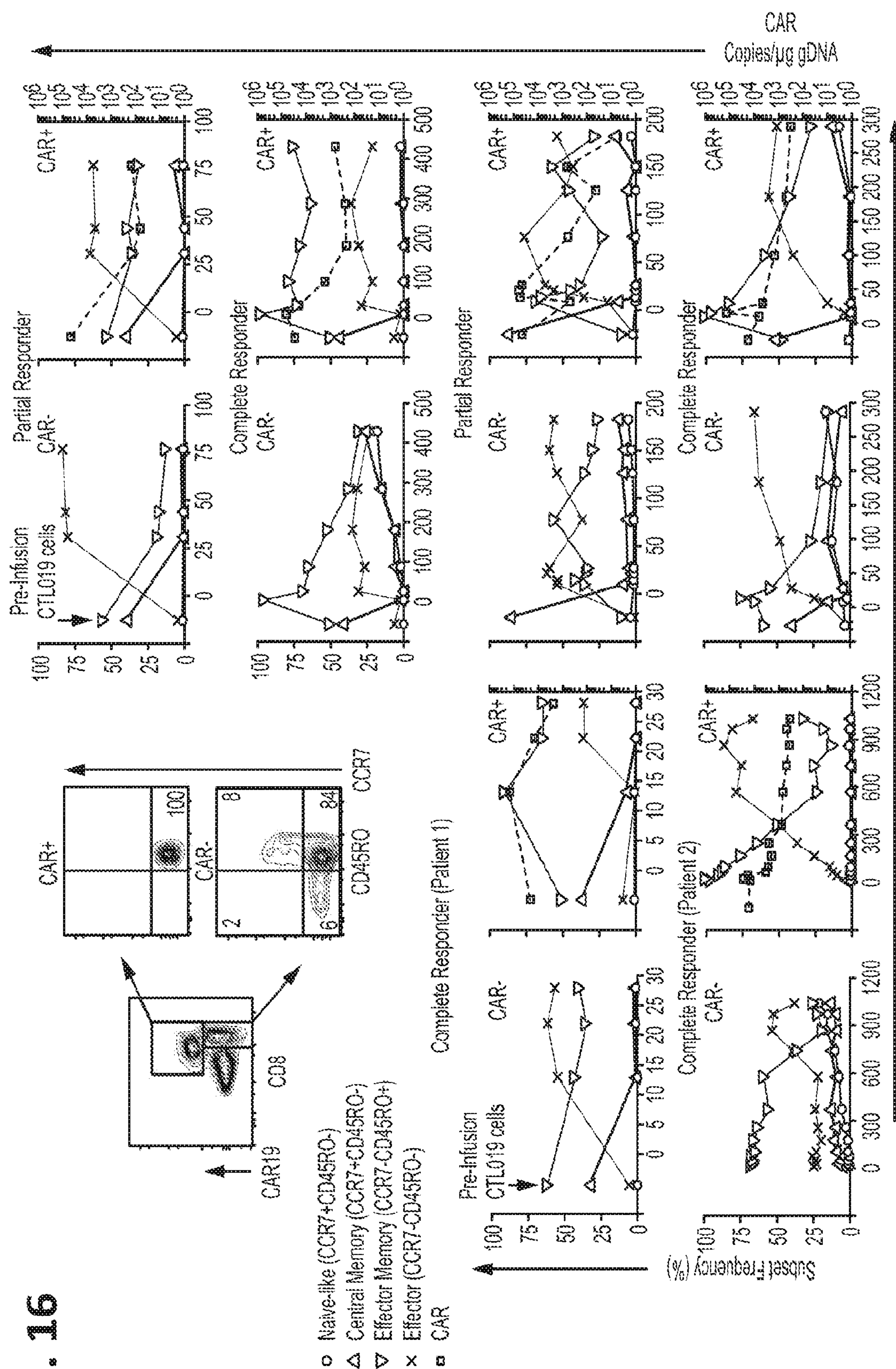


FIG. 17

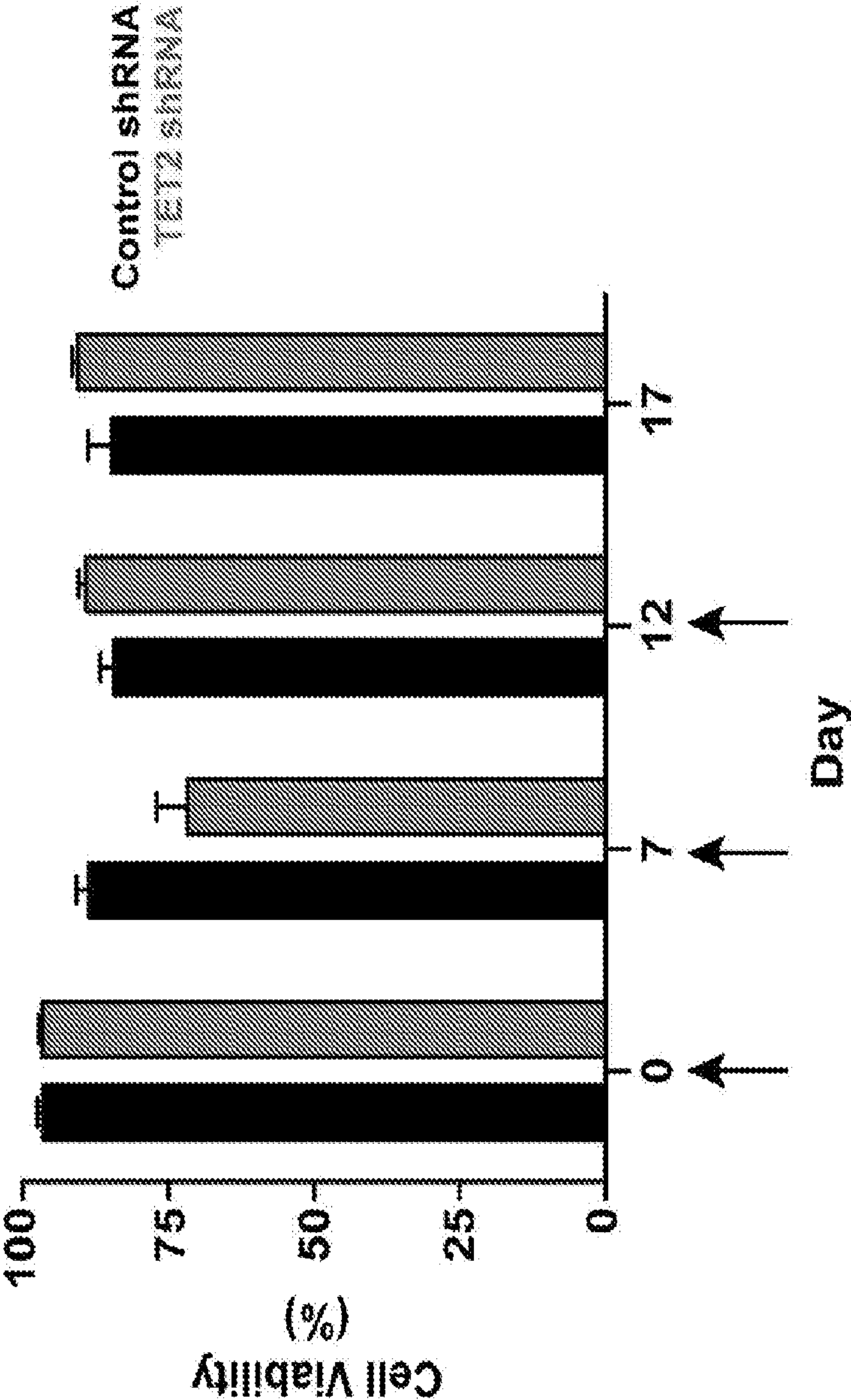


FIG. 18A

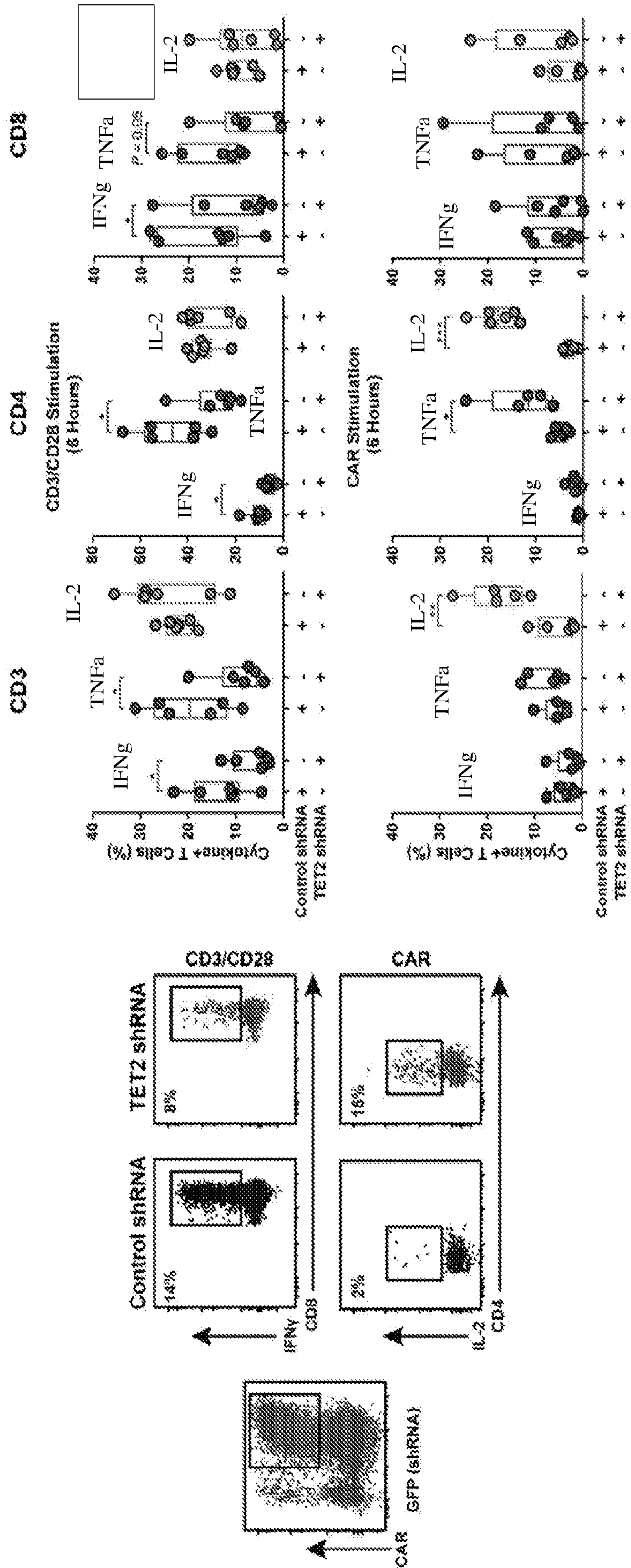


FIG. 18B

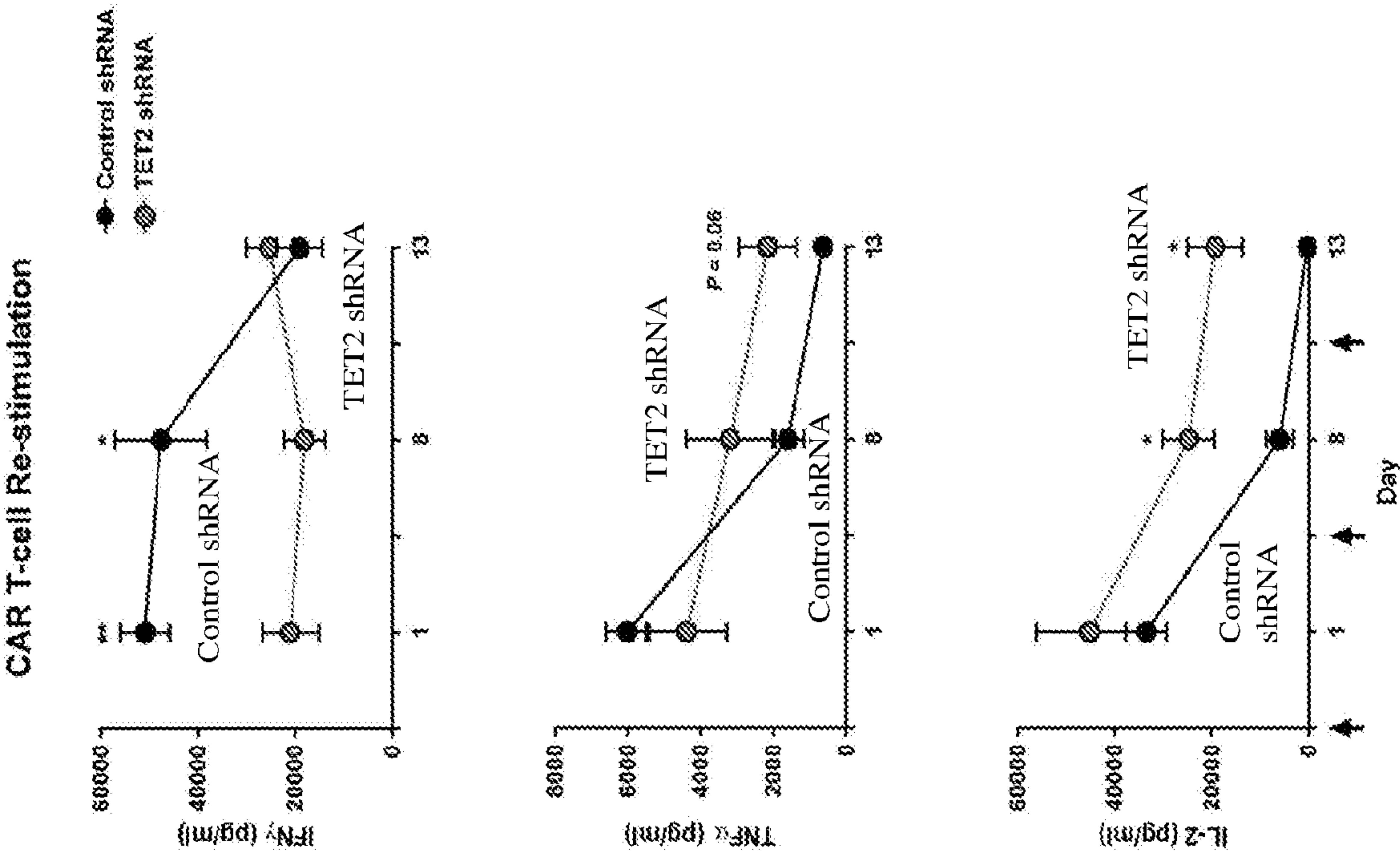


FIG. 19A

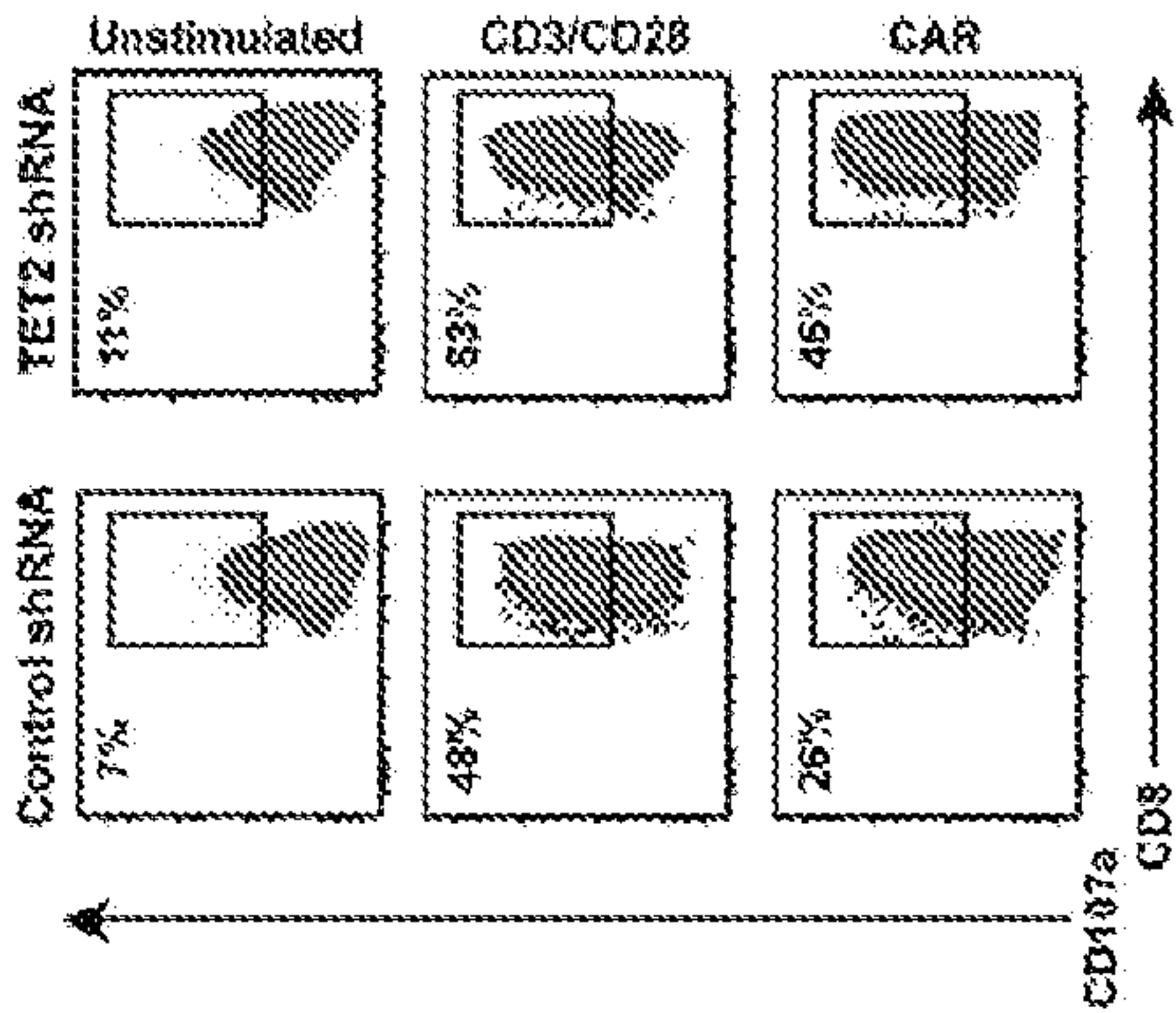


FIG. 19B

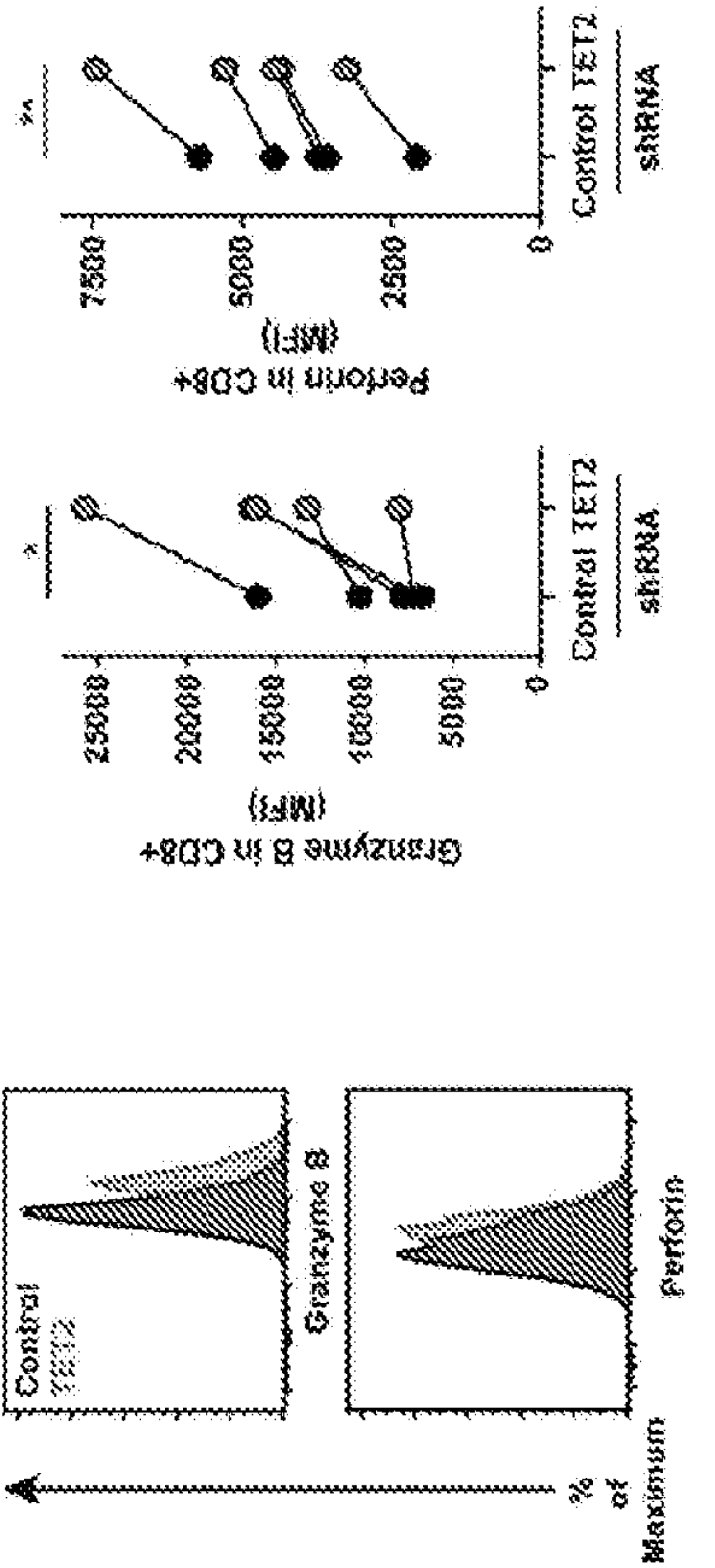


FIG. 19C

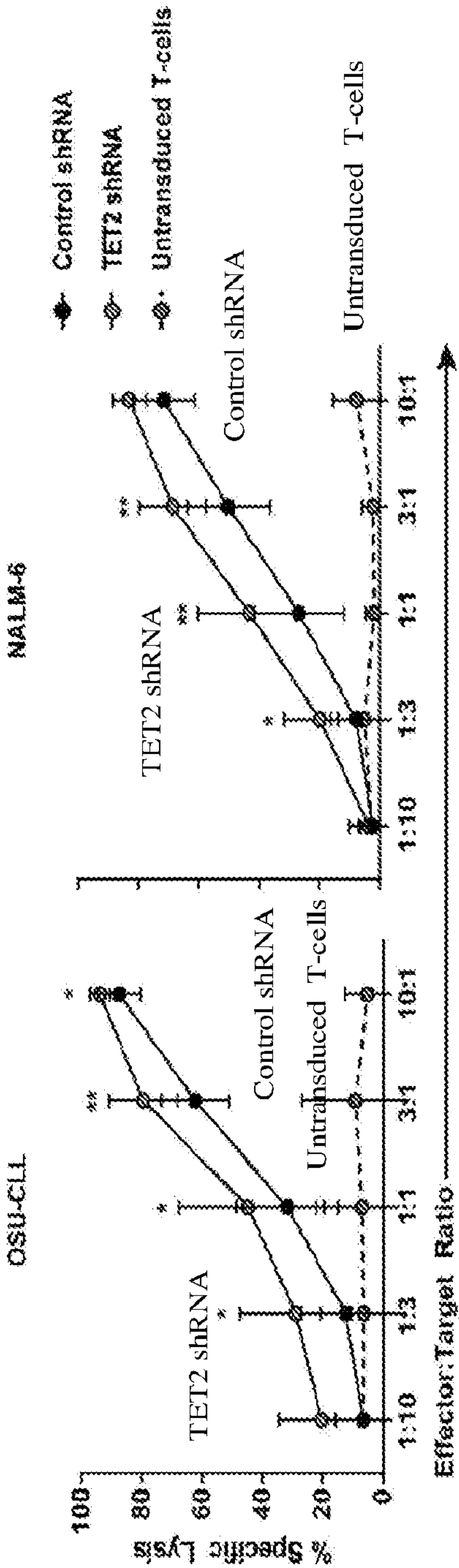


FIG. 20B

FIG. 20A

