(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 September 2018 (27.09.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/175733 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 16/28* (2006.01)

(21) International Application Number:
PCT/US2018/023785

(22) International Filing Date:
22 March 2018 (22.03.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/474,991 22 March 2017 (22.03.2017) US
62/621,356 24 January 2018 (24.01.2018) US

(71) Applicants: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, 4056 Basel (CH). **THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA** [US/US]; 3160 Chestnut Street, Suite 200, Philadelphia, PA 19104 (US).

(72) Inventors: **FRAIETTA, Joseph, A.**; 18 Saddle Lane, Cherry Hill, NJ 08002 (US). **MELENHORST, Jan, J.**; 11 Exton Circle, Cherry Hill, NJ 08003 (US).

(74) Agent: **COLLAZO, Diana M.** et al.; Lando & Anastasi, LLP, Riverfront Office Park, One Main Street, Suite 1100, Cambridge, MA 02142 (US).

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

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

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(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.

WO 2018/175733 A1

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, 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 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 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 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

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) 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 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 pathwy 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 modualtor (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 preantepenultimte 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 modualtor (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 modulaotr (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 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 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 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 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 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, 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 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 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, 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.

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 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 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 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, BAFFR, 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 preantepenultimte 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.

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.

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.

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 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 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.

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 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, 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 modualtor (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 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, 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, 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;

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

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 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 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 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-expressing cell comprising a disruption in Tet2, e.g., monoallelic or biallelic discruption 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 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, 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, 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, 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 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 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 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 thereof.

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 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 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.

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/μg 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. TCRVβ 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 TCRVβ usage in a CLL patient who had a clonal expansion of CAR T-cells. In **FIG. 4A,** the average frequency of TCRVβ 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. TCRVβ 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 TCRVβ gene segment is represented by a slice that is proportional to its frequency. The slice representing the proportion of TCRVβ5.1 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 TCRVβ5.1 positive relative to TCRVβ13.1 (negative control). In **FIG. 4C**, the abundance of TCRVβ5.1 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 TCRVβ5.1 clone (CASSLDGSGQGSDYGYTF) is shown as a red dot in each bivariate plot. In **FIG. 4D**, the kinetics of TCRVβ5.1 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 TCRVβ5.1 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; EF1α, 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; EF1α, 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 IFNγ 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; EF1α, 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. Hsp90α/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 ≤ 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 restimualtion 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 IFNγ, TNFα 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 IFNγ (top panel), TNFα (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 cytolysis) 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 (*$P < 0.05$; **$P \leq 0.01$).

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 eachother. 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 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 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 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 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) 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 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 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 connotate 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 is has the required function, namely, the ability to generate a signal under the appropriate conditions. It does not connotate 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 ~~a~~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 simulation. 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 (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, 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 myeloic-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, 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 "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.

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 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 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 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 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 enbodiments, 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 m^7G 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 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 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 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 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 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 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, 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.

The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

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 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.

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 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

"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 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 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 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 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 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 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 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 methlcytosine 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
DTKWHSFKSY YGIPCMKGSQ NSRVSPDFTQ ESRGYSKCLQ NGGIKRTVSE
        110         120         130         140         150
PSLSGLLQIK KLKQDQKANG ERRNFGVSQE RNPGESSQPN VSDLSDKKES
        160         170         180         190         200
VSSVAQENAV KDFTSFSTHN CSGPENPELQ ILNEQEGKSA NYHDKNIVLL
        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
ADNASKLAAM LNTCSFQKPE QLQQQKSVFE ICPSPAENNI QGTTKLASGE
        360         370         380         390         400
EFCSGSSSNL QAPGGSSERY LKQNEMNGAY FKQSSVFTKD SFSATTTPPP
        410         420         430         440         450
PSQLLLSPPP PLPQVPQLPS EGKSTLNGGV LEEHHHYPNQ SNTTLLREVK
        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
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
        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
NVQMGLEEVQ NINRRNSPYS QTMKSSACKI QVSCSNNTHL VSENKEQTTH
        860         870         880         890         900
PELFAGNKTQ NLHHMQYFPN NVIPKQDLLH RCFQEQEQKS QQASVLQGYK
        910         920         930         940         950
NRNQDMSGQQ AAQLAQQRYL IHNHANVFPV PDQGGSHTQT PPQKDTQKHA
        960         970         980         990        1000
```

```
ALRWHLLQKQ EQQQTQQPQT ESCHSQMHRP IKVEPGCKPH ACMHTAPPEN
      1010        1020        1030        1040        1050
KTWKKVTKQE NPPASCDNVQ QKSIIETMEQ HLKQFHAKSL FDHKALTLKS
      1060        1070        1080        1090        1100
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
      1210        1220        1230        1240        1250
RRSSSEEKLL CLVRERAGHT CEAAVIVILI LVWEGIPLSL ADKLYSELTE
      1260        1270        1280        1290        1300
TLRKYGTLTN RRCALNEERT CACQGLDPET CGASFSFGCS WSMYYNGCKF
      1310        1320        1330        1340        1350
ARSKIPRKFK LLGDDPKEEE KLESHLQNLS TLMAPTYKKL APDAYNNQIE
      1360        1370        1380        1390        1400
YEHRAPECRL GLKEGRPFSG VTACLDFCAH AHRDLHNMQN GSTLVCTLTR
      1410        1420        1430        1440        1450
EDNREFGGKP EDEQLHVLPL YKVSDVDEFG SVEAQEEKKR SGAIQVLSSF
      1460        1470        1480        1490        1500
RRKVRMLAEP VKTCRQRKLE AKKAAAEKLS SLENSSNKNE KEKSAPSRTK
      1510        1520        1530        1540        1550
QTENASQAKQ LAELLRLSGP VMQQSQQPQP LQKQPPQPQQ QQRPQQQQPH
      1560        1570        1580        1590        1600
HPQTESVNSY SASGSTNPYM RRPNPVSPYP NSSHTSDIYG STSPMNFYST
      1610        1620        1630        1640        1650
SSQAAGSYLN SSNPMNPYPG LLNQNTQYPS YQCNGNLSVD NCSPYLGSYS
      1660        1670        1680        1690        1700
PQSQPMDLYR YPSQDPLSKL SLPPIHTLYQ PRFGNSQSFT SKYLGYGNQN
      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
LPALNHDRTA CVQGGLHKLS DANGQEKQPL ALVQGVASGA EDNDEVWSDS
      1860        1870        1880        1890        1900
EQSFLDPDIG GVAVAPTHGS ILIECAKREL HATTPLKNPN RNHPTRISLV
      1910        1920        1930        1940        1950
FYQHKSMNEP KHGLALWEAK MAEKAREKEE ECEKYGPDYV PQKSHGKKVK
      1960        1970        1980        1990        2000
REPAEPHETS EPTYLRFIKS LAERTMSVTT DSTVTTSPYA FTRVTGPYNR
 2002
YI
      [SEQ ID NO: 1357]
```

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	GGCAGTGGCAGCGGCGAGAGCTTGGGCGGCCGCCGCCGC CTCCTCGCGAGCGCCGCGCGCCCGGGTCCCG CTCGCATGCAAGTCACGTCCGCCCCCTCGGCGCGGCCGCC CCGAGACGCCGGCCCCGCTGAGTGATGAGA ACAGACGTCAAACTGCCTTATGAATATTGATGCGGAGGC TAGGCTGCTTTCGTAGAGAAGCAGAAGGAAG CAAGATGGCTGCCCTTTAGGATTTGTTAGAAAGGAGACC CGACTGCAACTGCTGGATTGCTGCAAGGCTG AGGGACGAGAACGAGGCTGGCAAACATTCAGCAGCACA CCCTCTCAAGATTGTTTACTTGCCTTTGCTCC TGTTGAGTTACAACGCTTGGAAGCAGGAGATGGGCTCAG CAGCAGCCAATAGGACATGATCCAGGAAGAG CAGTAAGGGACTGAGCTGCTGAATTCAACTAGAGGGCAG CCTTGTGGATGGCCCCGAAGCAAGCCTGATG GAACAGGATAGAACCAACCATGTTGAGGGCAACAGACTA AGTCCATTCCTGATACCATCACCTCCCATTT GCCAGACAGAACCTCTGGCTACAAAGCTCCAGAATGGAA GCCCACTGCCTGAGAGAGCTCATCCAGAAGT AAATGGAGACACCAAGTGGCACTCTTTCAAAAGTTATTA TGGAATACCCTGTATGAAGGGAAGCCAGAAT AGTCGTGTGAGTCCTGACTTTACACAAGAAAGTAGAGGG TATTCCAAGTGTTTGCAAAATGGAGGAATAA AACGCACAGTTAGTGAACCTTCTCTCTCTGGGCTCCTTCA GATCAAGAAATTGAAACAAGACCAAAAGGC TAATGGAGAAAGACGTAACTTCGGGGTAAGCCAAGAAAG AAATCCAGGTGAAAGCAGTCAACCAAATGTC TCCGATTTGAGTGATAAGAAAGAATCTGTGAGTTCTGTAG CCCAAGAAAATGCAGTTAAAGATTTCACCA GTTTTTCAACACATAACTGCAGTGGGCCTGAAAATCCAG AGCTTCAGATTCTGAATGAGCAGGAGGGGAA AAGTGCTAATTACCATGACAAGAACATTGTATTACTTAAA AACAAGGCAGTGCTAATGCCTAATGGTGCT ACAGTTTCTGCCTCTTCCGTGGAACACACACATGGTGAAC TCCTGGAAAAAACACTGTCTCAATATTATC CAGATTGTGTTTCCATTGCGGTGCAGAAAACCACATCTCA CATAAATGCCATTAACAGTCAGGCTACTAA TGAGTTGTCCTGTGAGATCACTCACCCATCGCATACCTCA GGGCAGATCAATTCCGCACAGACCTCTAAC TCTGAGCTGCCTCCAAAGCCAGCTGCAGTGGTGAGTGAG GCCTGTGATGCTGATGATGCTGATAATGCCA GTAAACTAGCTGCAATGCTAAATACCTGTTCCTTTCAGAA ACCAGAACAACTACAACAACAAAAATCAGT TTTTGAGATATGCCCATCTCCTGCAGAAAATAACATCCAG GGAACCACAAAGCTAGCGTCTGGTGAAGAA TTCTGTTCAGGTTCCAGCAGCAATTTGCAAGCTCCTGGTG GCAGCTCTGAACGGTATTTAAAACAAAATG AAATGAATGGTGCTTACTTCAAGCAAAGCTCAGTGTTCAC TAAGGATTCCTTTTCTGCCACTACCACACC ACCACCACCATCACAATTGCTTCTTTCTCCCCCTCCTCCTC TTCCACAGGTTCCTCAGCTTCCTTCAGAA GGAAAAAGCACTCTGAATGGTGGAGTTTTAGAAGAACAC

		CACCACTACCCCAACCAAAGTAACACAACAC TTTTAAGGGAAGTGAAAATAGAGGGTAAACCTGAGGCAC CACCTTCCCAGAGTCCTAATCCATCTACACA TGTATGCAGCCCTTCTCCGATGCTTTCTGAAAGGCCTCAG AATAATTGTGTGAACAGGAATGACATACAG ACTGCAGGGACAATGACTGTTCCATTGTGTTCTGAGAAA ACAAGACCAATGTCAGAACACCTCAAGCATA ACCCACCAATTTTTGGTAGCAGTGGAGAGCTACAGGACA ACTGCCAGCAGTTGATGAGAAACAAAGAGCA AGAGATTCTGAAGGGTCGAGACAAGGAGCAAACACGAG ATCTTGTGCCCCCAACACAGCACTATCTGAAA CCAGGATGGATTGAATTGAAGGCCCCTCGTTTTCACCAAG CGGAATCCCATCTAAAACGTAATGAGGCAT CACTGCCATCAATTCTTCAGTATCAACCCAATCTCTCCAA TCAAATGACCTCCAAACAATACACTGGAAA TTCCAACATGCCTGGGGGGCTCCCAAGGCAAGCTTACAC CCAGAAAACAACACAGCTGGAGCACAAGTCA CAAATGTACCAAGTTGAAATGAATCAAGGGCAGTCCCAA GGTACAGTGGACCAACATCTCCAGTTCCAAA AACCCTCACACCAGGTGCACTTCTCCAAAACAGACCATTT ACCAAAAGCTCATGTGCAGTCACTGTGTGG CACTAGATTTCATTTTCAACAAAGAGCAGATTCCCAAACT GAAAAACTTATGTCCCCAGTGTTGAAACAG CACTTGAATCAACAGGCTTCAGAGACTGAGCCATTTTCAA ACTCACACCTTTTGCAACATAAGCCTCATA AACAGGCAGCACAAACACAACCATCCCAGAGTTCACATC TCCCTCAAAACCAGCAACAGCAGCAAAAATT ACAAATAAAGAATAAAGAGGAAATACTCCAGACTTTTCC TCACCCCCAAAGCAACAATGATCAGCAAAGA GAAGGATCATTCTTTGGCCAGACTAAAGTGGAAGAATGT TTTCATGGTGAAAATCAGTATTCAAAATCAA GCGAGTTCGAGACTCATAATGTCCAAATGGGACTGGAGG AAGTACAGAATATAAATCGTAGAAATTCCCC TTATAGTCAGACCATGAAATCAAGTGCATGCAAAATACA GGTTTCTTGTTCAAACAATACACACCTAGTT TCAGAGAATAAAGAACAGACTACACATCCTGAACTTTTT GCAGGAAACAAGACCCAAAACTTGCATCACA TGCAATATTTTCCAAATAATGTGATCCCAAAGCAAGATCT TCTTCACAGGTGCTTTCAAGAACAGGAGCA GAAGTCACAACAAGCTTCAGTTCTACAGGGATATAAAAA TAGAAACCAAGATATGTCTGGTCAACAAGCT GCGCAACTTGCTCAGCAAAGGTACTTGATACATAACCAT GCAAATGTTTTTCCTGTGCCTGACCAGGGAG GAAGTCACACTCAGACCCCTCCCCAGAAGGACACTCAAA AGCATGCTGCTCTAAGGTGGCATCTCTTACA GAAGCAAGAACAGCAGCAAACACAGCAACCCCAAACTG AGTCTTGCCATAGTCAGATGCACAGGCCAATT AAGGTGGAACCTGGATGCAAGCCACATGCCTGTATGCAC ACAGCACCACCAGAAAACAAAACATGGAAAA AGGTAACTAAGCAAGAGAATCCACCTGCAAGCTGTGATA ATGTGCAGCAAAAGAGCATCATTGAGACCAT GGAGCAGCATCTGAAGCAGTTTCACGCCAAGTCGTTATTT GACCATAAGGCTCTTACTCTCAAATCACAG AAGCAAGTAAAAGTTGAAATGTCAGGGCCAGTCACAGTT

		TTGACTAGACAAACCACTGCTGCAGAACTTG ATAGCCACACCCCAGCTTTAGAGCAGCAAACAACTTCTTC AGAAAAGACACCAACCAAAAGAACAGCTGC TTCTGTTCTCAATAATTTTATAGAGTCACCTTCCAAATTAC TAGATACTCCTATAAAAAATTTATTGGAT ACACCTGTCAAGACTCAATATGATTTCCCATCTTGCAGAT GTGTAGAGCAAATTATTGAAAAAGATGAAG GTCCTTTTTATACCCATCTAGGAGCAGGTCCTAATGTGGC AGCTATTAGAGAAATCATGGAAGAAAGGTT TGGACAGAAGGGTAAAGCTATTAGGATTGAAAGAGTCAT CTATACTGGTAAAGAAGGCAAAAGTTCTCAG GGATGTCCTATTGCTAAGTGGGTGGTTCGCAGAAGCAGC AGTGAAGAGAAGCTACTGTGTTTGGTGCGGG AGCGAGCTGGCCACACCTGTGAGGCTGCAGTGATTGTGA TTCTCATCCTGGTGTGGGAAGGAATCCCGCT GTCTCTGGCTGACAAACTCTACTCGGAGCTTACCGAGACG CTGAGGAAATACGGCACGCTCACCAATCGC CGGTGTGCCTTGAATGAAGAGAGAACTTGCGCCTGTCAG GGGCTGGATCCAGAAACCTGTGGTGCCTCCT TCTCTTTTGGTTGTTCATGGAGCATGTACTACAATGGATG TAAGTTTGCCAGAAGCAAGATCCCAAGGAA GTTTAAGCTGCTTGGGGATGACCCAAAAGAGGAAGAGAA ACTGGAGTCTCATTTGCAAAACCTGTCCACT CTTATGGCACCAACATATAAGAAACTTGCACCTGATGCAT ATAATAATCAGATTGAATATGAACACAGAG CACCAGAGTGCCGTCTGGGTCTGAAGGAAGGCCGTCCAT TCTCAGGGGTCACTGCATGTTTGGACTTCTG TGCTCATGCCCACAGAGACTTGCACAACATGCAGAATGG CAGCACATTGGTATGCACTCTCACTAGAGAA GACAATCGAGAATTTGGAGGAAAACCTGAGGATGAGCAG CTTCACGTTCTGCCTTTATACAAAGTCTCTG ACGTGGATGAGTTTGGGAGTGTGGAAGCTCAGGAGGAGA AAAAACGGAGTGGTGCCATTCAGGTACTGAG TTCTTTTCGGCGAAAAGTCAGGATGTTAGCAGAGCCAGTC AAGACTTGCCGACAAAGGAAACTAGAAGCC AAGAAAGCTGCAGCTGAAAAGCTTTCCTCCCTGGAGAAC AGCTCAAATAAAAATGAAAAGGAAAAGTCAG CCCCATCACGTACAAAACAAACTGAAAACGCAAGCCAGG CTAAACAGTTGGCAGAACTTTTGCGACTTTC AGGACCAGTCATGCAGCAGTCCCAGCAGCCCCAGCCTCT ACAGAAGCAGCCACCACAGCCCCAGCAGCAG CAGAGACCCCAGCAGCAGCAGCCACATCACCCTCAGACA GAGTCTGTCAACTCTTATTCTGCTTCTGGAT CCACCAATCCATACATGAGACGGCCCAATCCAGTTAGTC CTTATCCAAACTCTTCACACACTTCAGATAT CTATGGAAGCACCAGCCCTATGAACTTCTATTCCACCTCA TCTCAAGCTGCAGGTTCATATTTGAATTCT TCTAATCCCATGAACCCTTACCCTGGGCTTTTGAATCAGA ATACCCAATATCCATCATATCAATGCAATG GAAACCTATCAGTGGACAACTGCTCCCCATATCTGGGTTC CTATTCTCCCCAGTCTCAGCCGATGGATCT GTATAGGTATCCAAGCCAAGACCCTCTGTCTAAGCTCAGT CTACCACCCATCCATACACTTTACCAGCCA AGGTTTGGAAATAGCCAGAGTTTTACATCTAAATACTTAG

		GTTATGGAAACCAAAATATGCAGGGAGATG GTTTCAGCAGTTGTACCATTAGACCAAATGTACATCATGT AGGGAAATTGCCTCCTTATCCCACTCATGA GATGGATGGCCACTTCATGGGAGCCACCTCTAGATTACC ACCCAATCTGAGCAATCCAAACATGGACTAT AAAAATGGTGAACATCATTCACCTTCTCACATAATCCATA ACTACAGTGCAGCTCCGGGCATGTTCAACA GCTCTCTTCATGCCCTGCATCTCCAAAACAAGGAGAATGA CATGCTTTCCCACACAGCTAATGGGTTATC AAAGATGCTTCCAGCTCTTAACCATGATAGAACTGCTTGT GTCCAAGGAGGCTTACACAAATTAAGTGAT GCTAATGGTCAGGAAAAGCAGCCATTGGCACTAGTCCAG GGTGTGGCTTCTGGTGCAGAGGACAACGATG AGGTCTGGTCAGACAGCGAGCAGAGCTTTCTGGATCCTG ACATTGGGGGAGTGGCCGTGGCTCCAACTCA TGGGTCAATTCTCATTGAGTGTGCAAAGCGTGAGCTGCAT GCCACAACCCCTTTAAAGAATCCCAATAGG AATCACCCCACCAGGATCTCCCTCGTCTTTTACCAGCATA AGAGCATGAATGAGCCAAAACATGGCTTGG CTCTTTGGGAAGCCAAAATGGCTGAAAAAGCCCGTGAGA AAGAGGAAGAGTGTGAAAAGTATGGCCCAGA CTATGTGCCTCAGAAATCCCATGGCAAAAAAGTGAAACG GGAGCCTGCTGAGCCACATGAAACTTCAGAG CCCACTTACCTGCGTTTCATCAAGTCTCTTGCCGAAAGGA CCATGTCCGTGACCACAGACTCCACAGTAA CTACATCTCCATATGCCTTCACTCGGGTCACAGGGCCTTA CAACAGATATATATGATATCACCCCCTTTT GTTGGTTACCTCACTTGAAAAGACCACAACCAACCTGTCA GTAGTATAGTTCTCATGACGTGGGCAGTGG GGAAAGGTCACAGTATTCATGACAAATGTGGTGGGAAAA ACCTCAGCTCACCAGCAACAAAAGAGGTTAT CTTACCATAGCACTTAATTTTCACTGGCTCCCAAGTGGTC ACAGATGGCATCTAGGAAAAGACCAAAGCA TTCTATGCAAAAAGAAGGTGGGGAAGAAAGTGTTCCGCA ATTTACATTTTTAAACACTGGTTCTATTATT GGACGAGATGATATGTAAATGTGATCCCCCCCCCCCCGCTT ACAACTCTACACATCTGTGACCACTTTTAA TAATATCAAGTTTGCATAGTCATGGAACACAAATCAAAC AAGTACTGTAGTATTACAGTGACAGGAATCT TAAAATACCATCTGGTGCTGAATATATGATGTACTGAAAT ACTGGAATTATGGCTTTTTGAAATGCAGTT TTTACTGTAATCTTAACTTTTATTTATCAAAATAGCTACA GGAAACATGAATAGCAGGAAAACACTGAAT TTGTTTGGATGTTCTAAGAAATGGTGCTAAGAAAATGGTG TCTTTAATAGCTAAAAATTTAATGCCTTTA TATCATCAAGATGCTATCAGTGTACTCCAGTGCCCTTGAA TAATAGGGGTACCTTTTCATTCAAGTTTTT ATCATAATTACCTATTCTTACACAAGCTTAGTTTTTAAAA TGTGGACATTTTAAAGGCCTCTGGATTTTG CTCATCCAGTGAAGTCCTTGTAGGACAATAAACGTATATA TGTACATATATACACAAACATGTATATGTG CACACACATGTATATGTATAAATATTTTAAATGGTGTTTT AGAAGCACTTTGTCTACCTAAGCTTTGACA ACTTGAACAATGCTAAGGTACTGAGATGTTTAAAAAACA

		AGTTTACTTTCATTTTAGAATGCAAAGTTGA TTTTTTTAAGGAAACAAAGAAAGCTTTTAAAATATTTTTG CTTTTAGCCATGCATCTGCTGATGAGCAAT TGTGTCCATTTTTAACACAGCCAGTTAAATCCACCATGGG GCTTACTGGATTCAAGGGAATACGTTAGTC CACAAAACATGTTTTCTGGTGCTCATCTCACATGCTATAC TGTAAAACAGTTTTATACAAAATTGTATGA CAAGTTCATTGCTCAAAAATGTACAGTTTTAAGAATTTTC TATTAACTGCAGGTAATAATTAGCTGCATG CTGCAGACTCAACAAAGCTAGTTCACTGAAGCCTATGCT ATTTTATGGATCATAGGCTCTTCAGAGAACT GAATGGCAGTCTGCCTTTGTGTTGATAATTATGTACATTG TGACGTTGTCATTTCTTAGCTTAAGTGTCC TCTTTAACAAGAGGATTGAGCAGACTGATGCCTGCATAA GATGAATAAACAGGGTTAGTTCCATGTGAAT CTGTCAGTTAAAAAGAAACAAAAACAGGCAGCTGGTTTG CTGTGGTGGTTTTAAATCATTAATTTGTATA AAGAAGTGAAAGAGTTGTATAGTAAATTAAATTGTAAAC AAAACTTTTTTAATGCAATGCTTTAGTATTT TAGTACTGTAAAAAAATTAAATATATACATATATATATAT ATATATATATATATATATATATGAGTTTGAAG CAGAATTCACATCATGATGGTGCTACTCAGCCTGCTACAA ATATATCATAATGTGAGCTAAGAATTCATT AAATGTTTGAGTGATGTTCCTACTTGTCATATACCTCAAC ACTAGTTTGGCAATAGGATATTGAACTGAG AGTGAAAGCATTGTGTACCATCATTTTTTTCCAAGTCCTT TTTTTTATTGTTAAAAAAAAAAGCATACCT TTTTTCAATACTTGATTTCTTAGCAAGTATAACTTGAACTT CAACCTTTTTGTTCTAAAAATTCAGGGAT ATTTCAGCTCATGCTCTCCCTATGCCAACATGTCACCTGT GTTTATGTAAAATTGTTGTAGGTTAATAAA TATATTCTTTGTCAGGGATTTAACCCTTTTATTTTGAATCC CTTCTATTTTACTTGTACATGTGCTGATG TAACTAAAACTAATTTTGTAAATCTGTTGGCTCTTTTTATT GTAAAGAAAAGCATTTTAAAAGTTTGAGG AATCTTTTGACTGTTTCAAGCAGGAAAAAAAAATTACAT GAAAATAGAATGCACTGAGTTGATAAAGGGA AAAATTGTAAGGCAGGAGTTTGGCAAGTGGCTGTTGGCC AGAGACTTACTTGTAACTCTCTAAATGAAGT TTTTTTGATCCTGTAATCACTGAAGGTACATACTCCATGT GGACTTCCCTTAAACAGGCAAACACCTACA GGTATGGTGTGCAACAGATTGTACAATTACATTTTGGCCT AAATACATTTTTGCTTACTAGTATTTAAAA TAAATTCTTAATCAGAGGAGGCCTTTGGGTTTTATTGGTC AAATCTTTGTAAGCTGGCTTTTGTCTTTTT AAAAAATTTCTTGAATTTGTGGTTGTGTCCAATTTGCAAA CATTTCCAAAAATGTTTGCTTTGCTTACAA ACCACATGATTTTAATGTTTTTTGTATACCATAATATCTA GCCCCAAACATTTGATTACTACATGTGCAT TGGTGATTTTGATCATCCATTCTTAATATTTGATTTCTGTG TCACCTACTGTCATTTGTTAAACTGCTGG CCAACAAGAACAGGAAGTATAGTTTGGGGGGTTGGGGAG AGTTTACATAAGGAAGAGAAGAAATTGAGTG GCATATTGTAAATATCAGATCTATAATTGTAAATATAAAA

		CCTGCCTCAGTTAGAATGAATGGAAAGCAG ATCTACAATTTGCTAATATAGGAATATCAGGTTGACTATA TAGCCATACTTGAAAATGCTTCTGAGTGGT GTCAACTTTACTTGAATGAATTTTTCATCTTGATTGACGC ACAGTGATGTACAGTTCACTTCTGAAGCTA GTGGTTAACTTGTGTAGGAAACTTTTGCAGTTTGACACTA AGATAACTTCTGTGTGCATTTTTCTATGCT TTTTTAAAAACTAGTTTCATTTCATTTTCATGAGATGTTTG GTTTATAAGATCTGAGGATGGTTATAAAT ACTGTAAGTATTGTAATGTTATGAATGCAGGTTATTTGAA AGCTGTTTATTATTATATCATTCCTGATAA TGCTATGTGAGTGTTTTTAATAAAATTTATATTTATTTAAT GCACTCTAAAAAAAAAAAAAAAAAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X1, mRNA [SEQ ID NO: 1359]	XM_005263082.1	AAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGGATTTGT TAGAAAGGAGACCCGACTGCAACTGCTGGAT TGCTGCAAGGCTGAGGGACGAGAACGAGAATTCAACTAG AGGGCAGCCTTGTGGATGGCCCCGAAGCAAG CCTGATGGAACAGGATAGAACCAACCATGTTGAGGGCAA CAGACTAAGTCCATTCCTGATACCATCACCT CCCATTTGCCAGACAGAACCTCTGGCTACAAAGCTCCAG AATGGAAGCCCACTGCCTGAGAGAGCTCATC CAGAAGTAAATGGAGACACCAAGTGGCACTCTTTCAAAA GTTATTATGGAATACCCTGTATGAAGGGAAG CCAGAATAGTCGTGTGAGTCCTGACTTTACACAAGAAAG TAGAGGGTATTCCAAGTGTTTGCAAAATGGA GGAATAAAACGCACAGTTAGTGAACCTTCTCTCTCTGGGC TCCTTCAGATCAAGAAATTGAAACAAGACC AAAAGGCTAATGGAGAAAGACGTAACTTCGGGGTAAGCC AAGAAAGAAATCCAGGTGAAAGCAGTCAACC AAATGTCTCCGATTTGAGTGATAAGAAAGAATCTGTGAG TTCTGTAGCCCAAGAAAATGCAGTTAAAGAT TTCACCAGTTTTTCAACACATAACTGCAGTGGGCCTGAAA ATCCAGAGCTTCAGATTCTGAATGAGCAGG AGGGGAAAAGTGCTAATTACCATGACAAGAACATTGTAT TACTTAAAAACAAGGCAGTGCTAATGCCTAA TGGTGCTACAGTTTCTGCCTCTTCCGTGGAACACACACAT GGTGAACTCCTGGAAAAAACACTGTCTCAA TATTATCCAGATTGTGTTTCCATTGCGGTGCAGAAAACCA CATCTCACATAAATGCCATTAACAGTCAGG CTACTAATGAGTTGTCCTGTGAGATCACTCACCCATCGCA TACCTCAGGGCAGATCAATTCCGCACAGAC CTCTAACTCTGAGCTGCCTCCAAAGCCAGCTGCAGTGGTG AGTGAGGCCTGTGATGCTGATGATGCTGAT AATGCCAGTAAACTAGCTGCAATGCTAAATACCTGTTCCT TTCAGAAACCAGAACAACTACAACAACAAA AATCAGTTTTTGAGATATGCCCATCTCCTGCAGAAAATAA CATCCAGGGAACCACAAAGCTAGCGTCTGG TGAAGAATTCTGTTCAGGTTCCAGCAGCAATTTGCAAGCT CCTGGTGGCAGCTCTGAACGGTATTTAAAA CAAAATGAAATGAATGGTGCTTACTTCAAGCAAAGCTCA GTGTTCACTAAGGATTCCTTTTCTGCCACTA CCACACCACCACCACCATCACAATTGCTTCTTTCTCCCCC TCCTCCTCTTCCACAGGTTCCTCAGCTTCC TTCAGAAGGAAAAAGCACTCTGAATGGTGGAGTTTTAGA

		AGAACACCACCACTACCCCAACCAAAGTAAC ACAACACTTTTAAGGGAAGTGAAAATAGAGGGTAAACCT GAGGCACCACCTTCCCAGAGTCCTAATCCAT CTACACATGTATGCAGCCCTTCTCCGATGCTTTCTGAAAG GCCTCAGAATAATTGTGTGAACAGGAATGA CATACAGACTGCAGGGACAATGACTGTTCCATTGTGTTCT GAGAAAACAAGACCAATGTCAGAACACCTC AAGCATAACCCACCAATTTTTGGTAGCAGTGGAGAGCTA CAGGACAACTGCCAGCAGTTGATGAGAAACA AAGAGCAAGAGATTCTGAAGGGTCGAGACAAGGAGCAA ACACGAGATCTTGTGCCCCCAACACAGCACTA TCTGAAACCAGGATGGATTGAATTGAAGGCCCCTCGTTTT CACCAAGCGGAATCCCATCTAAAACGTAAT GAGGCATCACTGCCATCAATTCTTCAGTATCAACCCAATC TCTCCAATCAAATGACCTCCAAACAATACA CTGGAAATTCCAACATGCCTGGGGGGCTCCCAAGGCAAG CTTACACCCAGAAAACAACACAGCTGGAGCA CAAGTCACAAATGTACCAAGTTGAAATGAATCAAGGGCA GTCCCAAGGTACAGTGGACCAACATCTCCAG TTCCAAAAACCCTCACACCAGGTGCACTTCTCCAAAACA GACCATTTACCAAAAGCTCATGTGCAGTCAC TGTGTGGCACTAGATTTCATTTTCAACAAAGAGCAGATTC CCAAACTGAAAAACTTATGTCCCCAGTGTT GAAACAGCACTTGAATCAACAGGCTTCAGAGACTGAGCC ATTTTCAAACTCACACCTTTTGCAACATAAG CCTCATAAACAGGCAGCACAAACACAACCATCCCAGAGT TCACATCTCCCTCAAAACCAGCAACAGCAGC AAAAATTACAAATAAAGAATAAAGAGGAAATACTCCAG ACTTTTCCTCACCCCCAAAGCAACAATGATCA GCAAAGAGAAGGATCATTCTTTGGCCAGACTAAAGTGGA AGAATGTTTTCATGGTGAAAATCAGTATTCA AAATCAAGCGAGTTCGAGACTCATAATGTCCAAATGGGA CTGGAGGAAGTACAGAATATAAATCGTAGAA ATTCCCCTTATAGTCAGACCATGAAATCAAGTGCATGCAA AATACAGGTTTCTTGTTCAAACAATACACA CCTAGTTTCAGAGAATAAAGAACAGACTACACATCCTGA ACTTTTTGCAGGAAACAAGACCCAAAACTTG CATCACATGCAATATTTTCCAAATAATGTGATCCCAAAGC AAGATCTTCTTCACAGGTGCTTTCAAGAAC AGGAGCAGAAGTCACAACAAGCTTCAGTTCTACAGGGAT ATAAAAATAGAAACCAAGATATGTCTGGTCA ACAAGCTGCGCAACTTGCTCAGCAAAGGTACTTGATACA TAACCATGCAAATGTTTTTCCTGTGCCTGAC CAGGGAGGAAGTCACACTCAGACCCCTCCCCAGAAGGAC ACTCAAAAGCATGCTGCTCTAAGGTGGCATC TCTTACAGAAGCAAGAACAGCAGCAAACACAGCAACCCC AAACTGAGTCTTGCCATAGTCAGATGCACAG GCCAATTAAGGTGGAACCTGGATGCAAGCCACATGCCTG TATGCACACAGCACCACCAGAAAACAAAACA TGGAAAAAGGTAACTAAGCAAGAGAATCCACCTGCAAGC TGTGATAATGTGCAGCAAAAGAGCATCATTG AGACCATGGAGCAGCATCTGAAGCAGTTTCACGCCAAGT CGTTATTTGACCATAAGGCTCTTACTCTCAA ATCACAGAAGCAAGTAAAAGTTGAAATGTCAGGGCCAGT

		CACAGTTTTGACTAGACAAACCACTGCTGCA GAACTTGATAGCCACACCCCAGCTTTAGAGCAGCAAACA ACTTCTTCAGAAAAGACACCAACCAAAAGAA CAGCTGCTTCTGTTCTCAATAATTTTATAGAGTCACCTTCC AAATTACTAGATACTCCTATAAAAAATTT ATTGGATACACCTGTCAAGACTCAATATGATTTCCCATCT TGCAGATGTGTAGAGCAAATTATTGAAAAA GATGAAGGTCCTTTTTATACCCATCTAGGAGCAGGTCCTA ATGTGGCAGCTATTAGAGAAATCATGGAAG AAAGGTTTGGACAGAAGGGTAAAGCTATTAGGATTGAAA GAGTCATCTATACTGGTAAAGAAGGCAAAAG TTCTCAGGGATGTCCTATTGCTAAGTGGGTGGTTCGCAGA AGCAGCAGTGAAGAGAAGCTACTGTGTTTG GTGCGGGAGCGAGCTGGCCACACCTGTGAGGCTGCAGTG ATTGTGATTCTCATCCTGGTGTGGGAAGGAA TCCCGCTGTCTCTGGCTGACAAACTCTACTCGGAGCTTAC CGAGACGCTGAGGAAATACGGCACGCTCAC CAATCGCCGGTGTGCCTTGAATGAAGAGAGAACTTGCGC CTGTCAGGGGCTGGATCCAGAAACCTGTGGT GCCTCCTTCTCTTTTGGTTGTTCATGGAGCATGTACTACA ATGGATGTAAGTTTGCCAGAAGCAAGATCC CAAGGAAGTTTAAGCTGCTTGGGGATGACCCAAAAGAGG AAGAGAAACTGGAGTCTCATTTGCAAAACCT GTCCACTCTTATGGCACCAACATATAAGAAACTTGCACCT GATGCATATAATAATCAGATTGAATATGAA CACAGAGCACCAGAGTGCCGTCTGGGTCTGAAGGAAGGC CGTCCATTCTCAGGGGTCACTGCATGTTTGG ACTTCTGTGCTCATGCCCACAGAGACTTGCACAACATGCA GAATGGCAGCACATTGGTATGCACTCTCAC TAGAGAAGACAATCGAGAATTTGGAGGAAAACCTGAGG ATGAGCAGCTTCACGTTCTGCCTTTATACAAA GTCTCTGACGTGGATGAGTTTGGGAGTGTGGAAGCTCAG GAGGAGAAAAAACGGAGTGGTGCCATTCAGG TACTGAGTTCTTTTCGGCGAAAAGTCAGGATGTTAGCAGA GCCAGTCAAGACTTGCCGACAAAGGAAACT AGAAGCCAAGAAAGCTGCAGCTGAAAAGCTTTCCTCCCT GGAGAACAGCTCAAATAAAAATGAAAAGGAA AAGTCAGCCCCATCACGTACAAAACAAACTGAAAACGCA AGCCAGGCTAAACAGTTGGCAGAACTTTTGC GACTTTCAGGACCAGTCATGCAGCAGTCCCAGCAGCCCC AGCCTCTACAGAAGCAGCCACCACAGCCCCA GCAGCAGCAGAGACCCCAGCAGCAGCAGCCACATCACCC TCAGACAGAGTCTGTCAACTCTTATTCTGCT TCTGGATCCACCAATCCATACATGAGACGGCCCAATCCA GTTAGTCCTTATCCAAACTCTTCACACACTT CAGATATCTATGGAAGCACCAGCCCTATGAACTTCTATTC CACCTCATCTCAAGCTGCAGGTTCATATTT GAATTCTTCTAATCCCATGAACCCTTACCCTGGGCTTTTG AATCAGAATACCCAATATCCATCATATCAA TGCAATGGAAACCTATCAGTGGACAACTGCTCCCCATATC TGGGTTCCTATTCTCCCCAGTCTCAGCCGA TGGATCTGTATAGGTATCCAAGCCAAGACCCTCTGTCTAA GCTCAGTCTACCACCCATCCATACACTTTA CCAGCCAAGGTTTGGAAATAGCCAGAGTTTTACATCTAA

		ATACTTAGGTTATGGAAACCAAAATATGCAG GGAGATGGTTTCAGCAGTTGTACCATTAGACCAAATGTA CATCATGTAGGGAAATTGCCTCCTTATCCCA CTCATGAGATGGATGGCCACTTCATGGGAGCCACCTCTA GATTACCACCCAATCTGAGCAATCCAAACAT GGACTATAAAAATGGTGAACATCATTCACCTTCTCACATA ATCCATAACTACAGTGCAGCTCCGGGCATG TTCAACAGCTCTCTTCATGCCCTGCATCTCCAAAACAAGG AGAATGACATGCTTTCCCACACAGCTAATG GGTTATCAAAGATGCTTCCAGCTCTTAACCATGATAGAAC TGCTTGTGTCCAAGGAGGCTTACACAAATT AAGTGATGCTAATGGTCAGGAAAAGCAGCCATTGGCACT AGTCCAGGGTGTGGCTTCTGGTGCAGAGGAC AACGATGAGGTCTGGTCAGACAGCGAGCAGAGCTTTCTG GATCCTGACATTGGGGGAGTGGCCGTGGCTC CAACTCATGGGTCAATTCTCATTGAGTGTGCAAAGCGTGA GCTGCATGCCACAACCCCTTTAAAGAATCC CAATAGGAATCACCCCACCAGGATCTCCCTCGTCTTTTAC CAGCATAAGAGCATGAATGAGCCAAAACAT GGCTTGGCTCTTTGGGAAGCCAAAATGGCTGAAAAAGCC CGTGAGAAAGAGGAAGAGTGTGAAAAGTATG GCCCAGACTATGTGCCTCAGAAATCCCATGGCAAAAAAG TGAAACGGGAGCCTGCTGAGCCACATGAAAC TTCAGAGCCCACTTACCTGCGTTTCATCAAGTCTCTTGCC GAAAGGACCATGTCCGTGACCACAGACTCC ACAGTAACTACATCTCCATATGCCTTCACTCGGGTCACAG GGCCTTACAACAGATATATATGATATCACC CCCTTTTGTTGGTTACCTCACTTGAAAAGACCACAACCAA CCTGTCAGTAGTATAGTTCTCATGACGTGG GCAGTGGGGAAAGGTCACAGTATTCATGACAAATGTGGT GGGAAAAACCTCAGCTCACCAGCAACAAAAG AGGTTATCTTACCATAGCACTTAATTTTCACTGGCTCCCA AGTGGTCACAGATGGCATCTAGGAAAAGAC CAAAGCATTCTATGCAAAAAGAAGGTGGGGAAGAAAGT GTTCCGCAATTTACATTTTTAAACACTGGTTC TATTATTGGACGAGATGATATGTAAATGTGATCCCCCCCC CCCGCTTACAACTCTACACATCTGTGACCA CTTTTAATAATATCAAGTTTGCATAGTCATGGAACACAAA TCAAACAAGTACTGTAGTATTACAGTGACA GGAATCTTAAAATACCATCTGGTGCTGAATATATGATGTA CTGAAATACTGGAATTATGGCTTTTTGAAA TGCAGTTTTTACTGTAATCTTAACTTTTATTTATCAAAATA GCTACAGGAAACATGAATAGCAGGAAAAC ACTGAATTTGTTTGGATGTTCTAAGAAATGGTGCTAAGAA AATGGTGTCTTTAATAGCTAAAAATTTAAT GCCTTTATATCATCAAGATGCTATCAGTGTACTCCAGTGC CCTTGAATAATAGGGGTACCTTTTCATTCA AGTTTTTATCATAATTACCTATTCTTACACAAGCTTAGTTT TTAAAATGTGGACATTTTAAAGGCCTCTG GATTTTGCTCATCCAGTGAAGTCCTTGTAGGACAATAAAC GTATATATGTACATATATACACAAACATGT ATATGTGCACACACATGTATATGTATAAATATTTTAAATG GTGTTTTAGAAGCACTTTGTCTACCTAAGC TTTGACAACTTGAACAATGCTAAGGTACTGAGATGTTTAA

		AAAACAAGTTTACTTTCATTTTAGAATGCA AAGTTGATTTTTTTAAGGAAACAAAGAAAGCTTTTAAAAT ATTTTTGCTTTTAGCCATGCATCTGCTGAT GAGCAATTGTGTCCATTTTTAACACAGCCAGTTAAATCCA CCATGGGGCTTACTGGATTCAAGGGAATAC GTTAGTCCACAAAACATGTTTTCTGGTGCTCATCTCACAT GCTATACTGTAAAACAGTTTTATACAAAAT TGTATGACAAGTTCATTGCTCAAAAATGTACAGTTTTAAG AATTTTCTATTAACTGCAGGTAATAATTAG CTGCATGCTGCAGACTCAACAAAGCTAGTTCACTGAAGC CTATGCTATTTTATGGATCATAGGCTCTTCA GAGAACTGAATGGCAGTCTGCCTTTGTGTTGATAATTATG TACATTGTGACGTTGTCATTTCTTAGCTTA AGTGTCCTCTTTAACAAGAGGATTGAGCAGACTGATGCCT GCATAAGATGAATAAACAGGGTTAGTTCCA TGTGAATCTGTCAGTTAAAAAGAAACAAAAACAGGCAGC TGGTTTGCTGTGGTGGTTTTAAATCATTAAT TTGTATAAAGAAGTGAAAGAGTTGTATAGTAAATTAAAT TGTAAACAAAACTTTTTTAATGCAATGCTTT AGTATTTTAGTACTGTAAAAAAATTAAATATATACATATA TATATATATATATATATATATATATATGAG TTTGAAGCAGAATTCACATCATGATGGTGCTACTCAGCCT GCTACAAATATATCATAATGTGAGCTAAGA ATTCATTAAATGTTTGAGTGATGTTCCTACTTGTCATATA CCTCAACACTAGTTTGGCAATAGGATATTG AACTGAGAGTGAAAGCATTGTGTACCATCATTTTTTTCCA AGTCCTTTTTTTTATTGTTAAAAAAAAAAG CATACCTTTTTTCAATACTTGATTTCTTAGCAAGTATAACT TGAACTTCAACCTTTTTGTTCTAAAAATT CAGGGATATTTCAGCTCATGCTCTCCCTATGCCAACATGT CACCTGTGTTTATGTAAAATTGTTGTAGGT TAATAAATATATTCTTTGTCAGGGATTTAACCCTTTTATTT TGAATCCCTTCTATTTTACTTGTACATGT GCTGATGTAACTAAAACTAATTTTGTAAATCTGTTGGCTC TTTTTATTGTAAAGAAAAGCATTTTAAAAG TTTGAGGAATCTTTTGACTGTTTCAAGCAGGAAAAAAAA ATTACATGAAAATAGAATGCACTGAGTTGAT AAAGGGAAAAATTGTAAGGCAGGAGTTTGGCAAGTGGCT GTTGGCCAGAGACTTACTTGTAACTCTCTAA ATGAAGTTTTTTTGATCCTGTAATCACTGAAGGTACATAC TCCATGTGGACTTCCCTTAAACAGGCAAAC ACCTACAGGTATGGTGTGCAACAGATTGTACAATTACATT TTGGCCTAAATACATTTTTGCTTACTAGTA TTTAAAATAAATTCTTAATCAGAGGAGGCCTTTGGGTTTT ATTGGTCAAATCTTTGTAAGCTGGCTTTTG TCTTTTTAAAAAATTTCTTGAATTTGTGGTTGTGTCCAATT TGCAAACATTTCCAAAAATGTTTGCTTTG CTTACAAACCACATGATTTTAATGTTTTTTGTATACCATA ATATCTAGCCCCAAACATTTGATTACTACA TGTGCATTGGTGATTTTGATCATCCATTCTTAATATTTGAT TTCTGTGTCACCTACTGTCATTTGTTAAA CTGCTGGCCAACAAGAACAGGAAGTATAGTTTGGGGGGT TGGGGAGAGTTTACATAAGGAAGAGAAGAAA TTGAGTGGCATATTGTAAATATCAGATCTATAATTGTAAA

		TATAAAACCTGCCTCAGTTAGAATGAATGG AAAGCAGATCTACAATTTGCTAATATAGGAATATCAGGT TGACTATATAGCCATACTTGAAAATGCTTCT GAGTGGTGTCAACTTTACTTGAATGAATTTTTCATCTTGA TTGACGCACAGTGATGTACAGTTCACTTCT GAAGCTAGTGGTTAACTTGTGTAGGAAACTTTTGCAGTTT GACACTAAGATAACTTCTGTGTGCATTTTT CTATGCTTTTTTAAAAACTAGTTTCATTTCATTTTCATGAG ATGTTTGGTTTATAAGATCTGAGGATGGT TATAAATACTGTAAGTATTGTAATGTTATGAATGCAGGTT ATTTGAAAGCTGTTTATTATTATATCATTC CTGATAATGCTATGTGAGTGTTTTTAATAAAATTTATATT TATTTAATGCACTCTAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X2, mRNA [SEQ ID NO: 1360]	XM_006714242.2	GTAGAGAAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGG ATTTGTTAGAAAGGAGACCCGACTGCAACTG CTGGATTGCTGCAAGGCTGAGGGACGAGAACGAGGCTGG CAAACATTCAGCAGCACACCCTCTCAAGATT GTTTACTTGCCTTTGCTCCTGTTGAGTTACAACGCTTGGA AGCAGGAGATGGGCTCAGCAGCAGCCAATA GGACATGATCCAGGAAGAGCAGTAAGGGACTGAGCTGCT GAATTCAACTAGAGGGCAGCCTTGTGGATGG CCCCGAAGCAAGCCTGATGGAACAGGATAGAACCAACCA TGTTGAGGGCAACAGACTAAGTCCATTCCTG ATACCATCACCTCCCATTTGCCAGACAGAACCTCTGGCTA CAAAGCTCCAGAATGGAAGCCCACTGCCTG AGAGAGCTCATCCAGAAGTAAATGGAGACACCAAGTGGC ACTCTTTCAAAAGTTATTATGGAATACCCTG TATGAAGGGAAGCCAGAATAGTCGTGTGAGTCCTGACTT TACACAAGAAAGTAGAGGGTATTCCAAGTGT TTGCAAAATGGAGGAATAAAACGCACAGTTAGTGAACCT TCTCTCTCTGGGCTCCTTCAGATCAAGAAAT TGAAACAAGACCAAAAGGCTAATGGAGAAAGACGTAAC TTCGGGGTAAGCCAAGAAAGAAATCCAGGTGA AAGCAGTCAACCAAATGTCTCCGATTTGAGTGATAAGAA AGAATCTGTGAGTTCTGTAGCCCAAGAAAAT GCAGTTAAAGATTTCACCAGTTTTTCAACACATAACTGCA GTGGGCCTGAAAATCCAGAGCTTCAGATTC TGAATGAGCAGGAGGGGAAAAGTGCTAATTACCATGACA AGAACATTGTATTACTTAAAAACAAGGCAGT GCTAATGCCTAATGGTGCTACAGTTTCTGCCTCTTCCGTG GAACACACACATGGTGAACTCCTGGAAAAA ACACTGTCTCAATATTATCCAGATTGTGTTTCCATTGCGG TGCAGAAAACCACATCTCACATAAATGCCA TTAACAGTCAGGCTACTAATGAGTTGTCCTGTGAGATCAC TCACCCATCGCATACCTCAGGGCAGATCAA TTCCGCACAGACCTCTAACTCTGAGCTGCCTCCAAAGCCA GCTGCAGTGGTGAGTGAGGCCTGTGATGCT GATGATGCTGATAATGCCAGTAAACTAGCTGCAATGCTA AATACCTGTTCCTTTCAGAAACCAGAACAAC TACAACAACAAAAATCAGTTTTTGAGATATGCCCATCTCC TGCAGAAAATAACATCCAGGGAACCACAAA GCTAGCGTCTGGTGAAGAATTCTGTTCAGGTTCCAGCAGC AATTTGCAAGCTCCTGGTGGCAGCTCTGAA CGGTATTTAAAACAAAATGAAATGAATGGTGCTTACTTC

		AAGCAAAGCTCAGTGTTCACTAAGGATTCCT TTTCTGCCACTACCACACCACCACCACCATCACAATTGCT TCTTTCTCCCCCTCCTCCTCTTCCACAGGT TCCTCAGCTTCCTTCAGAAGGAAAAAGCACTCTGAATGGT GGAGTTTTAGAAGAACACCACCACTACCCC AACCAAAGTAACACAACACTTTTAAGGGAAGTGAAAATA GAGGGTAAACCTGAGGCACCACCTTCCCAGA GTCCTAATCCATCTACACATGTATGCAGCCCTTCTCCGAT GCTTTCTGAAAGGCCTCAGAATAATTGTGT GAACAGGAATGACATACAGACTGCAGGGACAATGACTGT TCCATTGTGTTCTGAGAAAACAAGACCAATG TCAGAACACCTCAAGCATAACCCACCAATTTTTGGTAGCA GTGGAGAGCTACAGGACAACTGCCAGCAGT TGATGAGAAACAAAGAGCAAGAGATTCTGAAGGGTCGA GACAAGGAGCAAACACGAGATCTTGTGCCCCC AACACAGCACTATCTGAAACCAGGATGGATTGAATTGAA GGCCCCTCGTTTTCACCAAGCGGAATCCCAT CTAAAACGTAATGAGGCATCACTGCCATCAATTCTTCAGT ATCAACCCAATCTCTCCAATCAAATGACCT CCAAACAATACACTGGAAATTCCAACATGCCTGGGGGGC TCCCAAGGCAAGCTTACACCCAGAAAACAAC ACAGCTGGAGCACAAGTCACAAATGTACCAAGTTGAAAT GAATCAAGGGCAGTCCCAAGGTACAGTGGAC CAACATCTCCAGTTCCAAAAACCCTCACACCAGGTGCACT TCTCCAAAACAGACCATTTACCAAAAGCTC ATGTGCAGTCACTGTGTGGCACTAGATTTCATTTTCAACA AAGAGCAGATTCCCAAACTGAAAAACTTAT GTCCCCAGTGTTGAAACAGCACTTGAATCAACAGGCTTC AGAGACTGAGCCATTTTCAAACTCACACCTT TTGCAACATAAGCCTCATAAACAGGCAGCACAAACACAA CCATCCCAGAGTTCACATCTCCCTCAAAACC AGCAACAGCAGCAAAAATTACAAATAAAGAATAAAGAG GAAATACTCCAGACTTTTCCTCACCCCCAAAG CAACAATGATCAGCAAAGAGAAGGATCATTCTTTGGCCA GACTAAAGTGGAAGAATGTTTTCATGGTGAA AATCAGTATTCAAAATCAAGCGAGTTCGAGACTCATAAT GTCCAAATGGGACTGGAGGAAGTACAGAATA TAAATCGTAGAAATTCCCCTTATAGTCAGACCATGAAATC AAGTGCATGCAAAATACAGGTTTCTTGTTC AAACAATACACACCTAGTTTCAGAGAATAAAGAACAGAC TACACATCCTGAACTTTTTGCAGGAAACAAG ACCCAAAACTTGCATCACATGCAATATTTTCCAAATAATG TGATCCCAAAGCAAGATCTTCTTCACAGGT GCTTTCAAGAACAGGAGCAGAAGTCACAACAAGCTTCAG TTCTACAGGGATATAAAAATAGAAACCAAGA TATGTCTGGTCAACAAGCTGCGCAACTTGCTCAGCAAAG GTACTTGATACATAACCATGCAAATGTTTTT CCTGTGCCTGACCAGGGAGGAAGTCACACTCAGACCCCT CCCCAGAAGGACACTCAAAAGCATGCTGCTC TAAGGTGGCATCTCTTACAGAAGCAAGAACAGCAGCAAA CACAGCAACCCCAAACTGAGTCTTGCCATAG TCAGATGCACAGGCCAATTAAGGTGGAACCTGGATGCAA GCCACATGCCTGTATGCACACAGCACCACCA GAAAACAAAACATGGAAAAAGGTAACTAAGCAAGAGAA

		TCCACCTGCAAGCTGTGATAATGTGCAGCAAA AGAGCATCATTGAGACCATGGAGCAGCATCTGAAGCAGT TTCACGCCAAGTCGTTATTTGACCATAAGGC TCTTACTCTCAAATCACAGAAGCAAGTAAAAGTTGAAAT GTCAGGGCCAGTCACAGTTTTGACTAGACAA ACCACTGCTGCAGAACTTGATAGCCACACCCCAGCTTTAG AGCAGCAAACAACTTCTTCAGAAAAGACAC CAACCAAAAGAACAGCTGCTTCTGTTCTCAATAATTTTAT AGAGTCACCTTCCAAATTACTAGATACTCC TATAAAAAATTTATTGGATACACCTGTCAAGACTCAATAT GATTTCCCATCTTGCAGATGTGTAGGTTTG GACAGAAGGGTAAAGCTATTAGGATTGAAAGAGTCATCT ATACTGGTAAAGAAGGCAAAAGTTCTCAGGG ATGTCCTATTGCTAAGTGGGAGAACTTGCGCCTGTCAGGG GCTGGATCCAGAAACCTGTGGTGCCTCCTT CTCTTTTGGTTGTTCATGGAGCATGTACTACAATGGATGT AAGTTTGCCAGAAGCAAGATCCCAAGGAAG TTTAAGCTGCTTGGGGATGACCCAAAAGAGGAAGAGAAA CTGGAGTCTCATTTGCAAAACCTGTCCACTC TTATGGCACCAACATATAAGAAACTTGCACCTGATGCAT ATAATAATCAGATTGAATATGAACACAGAGC ACCAGAGTGCCGTCTGGGTCTGAAGGAAGGCCGTCCATT CTCAGGGGTCACTGCATGTTTGGACTTCTGT GCTCATGCCCACAGAGACTTGCACAACATGCAGAATGGC AGCACATTGGTATGCACTCTCACTAGAGAAG ACAATCGAGAATTTGGAGGAAAACCTGAGGATGAGCAGC TTCACGTTCTGCCTTTATACAAAGTCTCTGA CGTGGATGAGTTTGGGAGTGTGGAAGCTCAGGAGGAGAA AAAACGGAGTGGTGCCATTCAGGTACTGAGT TCTTTTCGGCGAAAAGTCAGGATGTTAGCAGAGCCAGTC AAGACTTGCCGACAAAGGAAACTAGAAGCCA AGAAAGCTGCAGCTGAAAAGCTTTCCTCCCTGGAGAACA GCTCAAATAAAAATGAAAAGGAAAAGTCAGC CCCATCACGTACAAAACAAACTGAAAACGCAAGCCAGGC TAAACAGTTGGCAGAACTTTTGCGACTTTCA GGACCAGTCATGCAGCAGTCCCAGCAGCCCCAGCCTCTA CAGAAGCAGCCACCACAGCCCCAGCAGCAGC AGAGACCCCAGCAGCAGCAGCCACATCACCCTCAGACAG AGTCTGTCAACTCTTATTCTGCTTCTGGATC CACCAATCCATACATGAGACGGCCCAATCCAGTTAGTCCT TATCCAAACTCTTCACACACTTCAGATATC TATGGAAGCACCAGCCCTATGAACTTCTATTCCACCTCAT CTCAAGCTGCAGGTTCATATTTGAATTCTT CTAATCCCATGAACCCTTACCCTGGGCTTTTGAATCAGAA TACCCAATATCCATCATATCAATGCAATGG AAACCTATCAGTGGACAACTGCTCCCCATATCTGGGTTCC TATTCTCCCCAGTCTCAGCCGATGGATCTG TATAGGTATCCAAGCCAAGACCCTCTGTCTAAGCTCAGTC TACCACCCATCCATACACTTTACCAGCCAA GGTTTGGAAATAGCCAGAGTTTTACATCTAAATACTTAGG TTATGGAAACCAAAATATGCAGGGAGATGG TTTCAGCAGTTGTACCATTAGACCAAATGTACATCATGTA GGGAAATTGCCTCCTTATCCCACTCATGAG ATGGATGGCCACTTCATGGGAGCCACCTCTAGATTACCAC

		CCAATCTGAGCAATCCAAACATGGACTATA AAAATGGTGAACATCATTCACCTTCTCACATAATCCATAA CTACAGTGCAGCTCCGGGCATGTTCAACAG CTCTCTTCATGCCCTGCATCTCCAAAACAAGGAGAATGAC ATGCTTTCCCACACAGCTAATGGGTTATCA AAGATGCTTCCAGCTCTTAACCATGATAGAACTGCTTGTG TCCAAGGAGGCTTACACAAATTAAGTGATG CTAATGGTCAGGAAAAGCAGCCATTGGCACTAGTCCAGG GTGTGGCTTCTGGTGCAGAGGACAACGATGA GGTCTGGTCAGACAGCGAGCAGAGCTTTCTGGATCCTGA CATTGGGGGAGTGGCCGTGGCTCCAACTCAT GGGTCAATTCTCATTGAGTGTGCAAAGCGTGAGCTGCAT GCCACAACCCCTTTAAAGAATCCCAATAGGA ATCACCCCACCAGGATCTCCCTCGTCTTTTACCAGCATAA GAGCATGAATGAGCCAAAACATGGCTTGGC TCTTTGGGAAGCCAAAATGGCTGAAAAAGCCCGTGAGAA AGAGGAAGAGTGTGAAAAGTATGGCCCAGAC TATGTGCCTCAGAAATCCCATGGCAAAAAAGTGAAACGG GAGCCTGCTGAGCCACATGAAACTTCAGAGC CCACTTACCTGCGTTTCATCAAGTCTCTTGCCGAAAGGAC CATGTCCGTGACCACAGACTCCACAGTAAC TACATCTCCATATGCCTTCACTCGGGTCACAGGGCCTTAC AACAGATATATATGATATCACCCCCTTTTG TTGGTTACCTCACTTGAAAAGACCACAACCAACCTGTCAG TAGTATAGTTCTCATGACGTGGGCAGTGGG GAAAGGTCACAGTATTCATGACAAATGTGGTGGGAAAAA CCTCAGCTCACCAGCAACAAAAGAGGTTATC TTACCATAGCACTTAATTTTCACTGGCTCCCAAGTGGTCA CAGATGGCATCTAGGAAAAGACCAAAGCAT TCTATGCAAAAAGAAGGTGGGGAAGAAAGTGTTCCGCAA TTTACATTTTTAAACACTGGTTCTATTATTG GACGAGATGATATGTAAATGTGATCCCCCCCCCCCGCTTA CAACTCTACACATCTGTGACCACTTTTAAT AATATCAAGTTTGCATAGTCATGGAACACAAATCAAACA AGTACTGTAGTATTACAGTGACAGGAATCTT AAAATACCATCTGGTGCTGAATATATGATGTACTGAAAT ACTGGAATTATGGCTTTTTGAAATGCAGTTT TTACTGTAATCTTAACTTTTATTTATCAAAATAGCTACAG GAAACATGAATAGCAGGAAAACACTGAATT TGTTTGGATGTTCTAAGAAATGGTGCTAAGAAAATGGTGT CTTTAATAGCTAAAAATTTAATGCCTTTAT ATCATCAAGATGCTATCAGTGTACTCCAGTGCCCTTGAAT AATAGGGGTACCTTTTCATTCAAGTTTTTA TCATAATTACCTATTCTTACACAAGCTTAGTTTTTAAAAT GTGGACATTTTAAAGGCCTCTGGATTTTGC TCATCCAGTGAAGTCCTTGTAGGACAATAAACGTATATAT GTACATATATACACAAACATGTATATGTGC ACACACATGTATATGTATAAATATTTTAAATGGTGTTTTA GAAGCACTTTGTCTACCTAAGCTTTGACAA CTTGAACAATGCTAAGGTACTGAGATGTTTAAAAAACAA GTTTACTTTCATTTTAGAATGCAAAGTTGAT TTTTTTAAGGAAACAAAGAAAGCTTTTAAAATATTTTTGC TTTTAGCCATGCATCTGCTGATGAGCAATT GTGTCCATTTTTAACACAGCCAGTTAAATCCACCATGGGG

		CTTACTGGATTCAAGGGAATACGTTAGTCC ACAAAACATGTTTTCTGGTGCTCATCTCACATGCTATACT GTAAAACAGTTTTATACAAAATTGTATGAC AAGTTCATTGCTCAAAAATGTACAGTTTTAAGAATTTTCT ATTAACTGCAGGTAATAATTAGCTGCATGC TGCAGACTCAACAAAGCTAGTTCACTGAAGCCTATGCTAT TTTATGGATCATAGGCTCTTCAGAGAACTG AATGGCAGTCTGCCTTTGTGTTGATAATTATGTACATTGT GACGTTGTCATTTCTTAGCTTAAGTGTCCT CTTTAACAAGAGGATTGAGCAGACTGATGCCTGCATAAG ATGAATAAACAGGGTTAGTTCCATGTGAATC TGTCAGTTAAAAAGAAACAAAAACAGGCAGCTGGTTTGC TGTGGTGGTTTTAAATCATTAATTTGTATAA AGAAGTGAAAGAGTTGTATAGTAAATTAAATTGTAAACA AAACTTTTTTAATGCAATGCTTTAGTATTTT AGTACTGTAAAAAAATTAAATATATACATATATATATATA TATATATATATATATATATGAGTTTGAAGC AGAATTCACATCATGATGGTGCTACTCAGCCTGCTACAAA TATATCATAATGTGAGCTAAGAATTCATTA AATGTTTGAGTGATGTTCCTACTTGTCATATACCTCAACA CTAGTTTGGCAATAGGATATTGAACTGAGA GTGAAAGCATTGTGTACCATCATTTTTTTCCAAGTCCTTTT TTTTATTGTTAAAAAAAAAAGCATACCTT TTTTCAATACTTGATTTCTTAGCAAGTATAACTTGAACTTC AACCTTTTTGTTCTAAAAATTCAGGGATA TTTCAGCTCATGCTCTCCCTATGCCAACATGTCACCTGTG TTTATGTAAAATTGTTGTAGGTTAATAAAT ATATTCTTTGTCAGGGATTTAACCCTTTTATTTTGAATCCC TTCTATTTTACTTGTACATGTGCTGATGT AACTAAAACTAATTTTGTAAATCTGTTGGCTCTTTTTATTG TAAAGAAAAGCATTTTAAAAGTTTGAGGA ATCTTTTGACTGTTTCAAGCAGGAAAAAAAAATTACATG AAAATAGAATGCACTGAGTTGATAAAGGGAA AAATTGTAAGGCAGGAGTTTGGCAAGTGGCTGTTGGCCA GAGACTTACTTGTAACTCTCTAAATGAAGTT TTTTTGATCCTGTAATCACTGAAGGTACATACTCCATGTG GACTTCCCTTAAACAGGCAAACACCTACAG GTATGGTGTGCAACAGATTGTACAATTACATTTTGGCCTA AATACATTTTTGCTTACTAGTATTTAAAAT AAATTCTTAATCAGAGGAGGCCTTTGGGTTTTATTGGTCA AATCTTTGTAAGCTGGCTTTTGTCTTTTTA AAAAATTTCTTGAATTTGTGGTTGTGTCCAATTTGCAAAC ATTTCCAAAAATGTTTGCTTTGCTTACAAA CCACATGATTTTAATGTTTTTTGTATACCATAATATCTAGC CCCAAACATTTGATTACTACATGTGCATT GGTGATTTTGATCATCCATTCTTAATATTTGATTTCTGTGT CACCTACTGTCATTTGTTAAACTGCTGGC CAACAAGAACAGGAAGTATAGTTTGGGGGGTTGGGGAGA GTTTACATAAGGAAGAGAAGAAATTGAGTGG CATATTGTAAATATCAGATCTATAATTGTAAATATAAAAC CTGCCTCAGTTAGAATGAATGGAAAGCAGA TCTACAATTTGCTAATATAGGAATATCAGGTTGACTATAT AGCCATACTTGAAAATGCTTCTGAGTGGTG TCAACTTTACTTGAATGAATTTTTCATCTTGATTGACGCA

		CAGTGATGTACAGTTCACTTCTGAAGCTAG TGGTTAACTTGTGTAGGAAACTTTTGCAGTTTGACACTAA GATAACTTCTGTGTGCATTTTTCTATGCTT TTTTAAAAACTAGTTTCATTTCATTTTCATGAGATGTTTGG TTTATAAGATCTGAGGATGGTTATAAATA CTGTAAGTATTGTAATGTTATGAATGCAGGTTATTTGAAA GCTGTTTATTATTATATCATTCCTGATAAT GCTATGTGAGTGTTTTTAATAAAATTTATATTTATTTAATG CACTCTAA
Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant 2, mRNA [SEQ ID NO: 1361]	NM_017628.4	AAACAGAAGGTGGGCCGGGGCGGGGAGAAACAGAACTC GGTCAATTTCCCAGTTTGTCGGGTCTTTAAAA ATACAGGCCCCTAAAGCACTAAGGGCATGCCCTCGGTGA AACAGGGGAGCGCTTCTGCTGAATGAGATTA AAGCGACAGAAAAGGGAAAGGAGAGCGCGGGCAACGGG ATCTAAAGGGAGATAGAGACGCGGGCCTCTGA GGGCTGGCAAACATTCAGCAGCACACCCTCTCAAGATTG TTTACTTGCCTTTGCTCCTGTTGAGTTACAA CGCTTGGAAGCAGGAGATGGGCTCAGCAGCAGCCAATAG GACATGATCCAGGAAGAGCAGTAAGGGACTG AGCTGCTGAATTCAACTAGAGGGCAGCCTTGTGGATGGC CCCGAAGCAAGCCTGATGGAACAGGATAGAA CCAACCATGTTGAGGGCAACAGACTAAGTCCATTCCTGA TACCATCACCTCCCATTTGCCAGACAGAACC TCTGGCTACAAAGCTCCAGAATGGAAGCCCACTGCCTGA GAGAGCTCATCCAGAAGTAAATGGAGACACC AAGTGGCACTCTTTCAAAAGTTATTATGGAATACCCTGTA TGAAGGGAAGCCAGAATAGTCGTGTGAGTC CTGACTTTACACAAGAAAGTAGAGGGTATTCCAAGTGTTT GCAAAATGGAGGAATAAAACGCACAGTTAG TGAACCTTCTCTCTCTGGGCTCCTTCAGATCAAGAAATTG AAACAAGACCAAAAGGCTAATGGAGAAAGA CGTAACTTCGGGGTAAGCCAAGAAAGAAATCCAGGTGAA AGCAGTCAACCAAATGTCTCCGATTTGAGTG ATAAGAAAGAATCTGTGAGTTCTGTAGCCCAAGAAAATG CAGTTAAAGATTTCACCAGTTTTTCAACACA TAACTGCAGTGGGCCTGAAAATCCAGAGCTTCAGATTCT GAATGAGCAGGAGGGGAAAAGTGCTAATTAC CATGACAAGAACATTGTATTACTTAAAAACAAGGCAGTG CTAATGCCTAATGGTGCTACAGTTTCTGCCT CTTCCGTGGAACACACACATGGTGAACTCCTGGAAAAAA CACTGTCTCAATATTATCCAGATTGTGTTTC CATTGCGGTGCAGAAAACCACATCTCACATAAATGCCAT TAACAGTCAGGCTACTAATGAGTTGTCCTGT GAGATCACTCACCCATCGCATACCTCAGGGCAGATCAAT TCCGCACAGACCTCTAACTCTGAGCTGCCTC CAAAGCCAGCTGCAGTGGTGAGTGAGGCCTGTGATGCTG ATGATGCTGATAATGCCAGTAAACTAGCTGC AATGCTAAATACCTGTTCCTTTCAGAAACCAGAACAACTA CAACAACAAAAATCAGTTTTTGAGATATGC CCATCTCCTGCAGAAAATAACATCCAGGGAACCACAAAG CTAGCGTCTGGTGAAGAATTCTGTTCAGGTT CCAGCAGCAATTTGCAAGCTCCTGGTGGCAGCTCTGAAC GGTATTTAAAACAAAATGAAATGAATGGTGC TTACTTCAAGCAAAGCTCAGTGTTCACTAAGGATTCCTTT

		TCTGCCACTACCACACCACCACCACCATCA CAATTGCTTCTTTCTCCCCCTCCTCCTCTTCCACAGGTTCC TCAGCTTCCTTCAGAAGGAAAAAGCACTC TGAATGGTGGAGTTTTAGAAGAACACCACCACTACCCCA ACCAAAGTAACACAACACTTTTAAGGGAAGT GAAAATAGAGGGTAAACCTGAGGCACCACCTTCCCAGAG TCCTAATCCATCTACACATGTATGCAGCCCT TCTCCGATGCTTTCTGAAAGGCCTCAGAATAATTGTGTGA ACAGGAATGACATACAGACTGCAGGGACAA TGACTGTTCCATTGTGTTCTGAGAAAACAAGACCAATGTC AGAACACCTCAAGCATAACCCACCAATTTT TGGTAGCAGTGGAGAGCTACAGGACAACTGCCAGCAGTT GATGAGAAACAAAGAGCAAGAGATTCTGAAG GGTCGAGACAAGGAGCAAACACGAGATCTTGTGCCCCCA ACACAGCACTATCTGAAACCAGGATGGATTG AATTGAAGGCCCCTCGTTTTCACCAAGCGGAATCCCATCT AAAACGTAATGAGGCATCACTGCCATCAAT TCTTCAGTATCAACCCAATCTCTCCAATCAAATGACCTCC AAACAATACACTGGAAATTCCAACATGCCT GGGGGGCTCCCAAGGCAAGCTTACACCCAGAAAACAACA CAGCTGGAGCACAAGTCACAAATGTACCAAG TTGAAATGAATCAAGGGCAGTCCCAAGGTACAGTGGACC AACATCTCCAGTTCCAAAAACCCTCACACCA GGTGCACTTCTCCAAAACAGACCATTTACCAAAAGCTCAT GTGCAGTCACTGTGTGGCACTAGATTTCAT TTTCAACAAAGAGCAGATTCCCAAACTGAAAAACTTATG TCCCCAGTGTTGAAACAGCACTTGAATCAAC AGGCTTCAGAGACTGAGCCATTTTCAAACTCACACCTTTT GCAACATAAGCCTCATAAACAGGCAGCACA AACACAACCATCCCAGAGTTCACATCTCCCTCAAAACCA GCAACAGCAGCAAAAATTACAAATAAAGAAT AAAGAGGAAATACTCCAGACTTTTCCTCACCCCCAAAGC AACAATGATCAGCAAAGAGAAGGATCATTCT TTGGCCAGACTAAAGTGGAAGAATGTTTTCATGGTGAAA ATCAGTATTCAAAATCAAGCGAGTTCGAGAC TCATAATGTCCAAATGGGACTGGAGGAAGTACAGAATAT AAATCGTAGAAATTCCCCTTATAGTCAGACC ATGAAATCAAGTGCATGCAAAATACAGGTTTCTTGTTCAA ACAATACACACCTAGTTTCAGAGAATAAAG AACAGACTACACATCCTGAACTTTTTGCAGGAAACAAGA CCCAAAACTTGCATCACATGCAATATTTTCC AAATAATGTGATCCCAAAGCAAGATCTTCTTCACAGGTG CTTTCAAGAACAGGAGCAGAAGTCACAACAA GCTTCAGTTCTACAGGGATATAAAAATAGAAACCAAGAT ATGTCTGGTCAACAAGCTGCGCAACTTGCTC AGCAAAGGTACTTGATACATAACCATGCAAATGTTTTTCC TGTGCCTGACCAGGGAGGAAGTCACACTCA GACCCCTCCCCAGAAGGACACTCAAAAGCATGCTGCTCT AAGGTGGCATCTCTTACAGAAGCAAGAACAG CAGCAAACACAGCAACCCCAAACTGAGTCTTGCCATAGT CAGATGCACAGGCCAATTAAGGTGGAACCTG GATGCAAGCCACATGCCTGTATGCACACAGCACCACCAG AAAACAAAACATGGAAAAAGGTAACTAAGCA AGAGAATCCACCTGCAAGCTGTGATAATGTGCAGCAAAA

		GAGCATCATTGAGACCATGGAGCAGCATCTG AAGCAGTTTCACGCCAAGTCGTTATTTGACCATAAGGCTC TTACTCTCAAATCACAGAAGCAAGTAAAAG TTGAAATGTCAGGGCCAGTCACAGTTTTGACTAGACAAA CCACTGCTGCAGAACTTGATAGCCACACCCC AGCTTTAGAGCAGCAAACAACTTCTTCAGAAAAGACACC AACCAAAAGAACAGCTGCTTCTGTTCTCAAT AATTTTATAGAGTCACCTTCCAAATTACTAGATACTCCTA TAAAAAATTTATTGGATACACCTGTCAAGA CTCAATATGATTTCCCATCTTGCAGATGTGTAGGTAAGTG CCAGAAATGTACTGAGACACATGGCGTTTA TCCAGAATTAGCAAATTTATCTTCAGATATGGGATTTTCC TTCTTTTTTTAAATCTTGAGTCTGGCAGCA ATTTGTAAAGGCTCATAAAAATCTGAAGCTTACATTTTTT GTCAAGTTACCGATGCTTGTGTCTTGTGAA AGAGAACTTCACTTACATGCAGTTTTTCCAAAAGAATTAA ATAATCGTGCATGTTTATTTTTCCCTCTCT TCAGATCCTGTAAAATTTGAATGTATCTGTTTTAGATCAA TTCGCCTATTTAGCTCTTTGTATATTATCT CCTGGAGAGACAGCTAGGCAGCAAAAAAACAATCTATTA AAATGAGAAAATAACGACCATAGGCAGTCTA ATGTACGAACTTTAAATATTTTTTAATTCAAGGTAAAATA TATTAGTTTCACAAGATTTCTGGCTAATAG GGAAATTATTATCTTCAGTCTTCATGAGTTGGGGGAAATG ATAATGCTGACACTCTTAGTGCTCCTAAAG TTTCCTTTTCTCCATTTATACATTTGGAATGTTGTGATTTA TATTCATTTTGATTCCCTTTTCTCTAAAA TTTCATCTTTTTGATTAAAAAATATGATACAGGCATACCT CAGAGATATTGTGGGTTTGGCTCCATACCA CAATAAAATGAATATTACAATAAAGCAAGTTGTAAGGAC TTTTTGGTTTCTCACTGTATGTAAAAGTTAT TTATATACTATACTGTAACATACTAAGTGTGCAATAGCAT TGTGTCTAAAAAATATATACTTTAAAAATA ATTTATTGTTAAAAAAATGCCAACAATTATCTGGGCCTTT AGTGAGTGCTAATCTTTTTGCTGGTGGAGG GTCGTGCTTCAGTATTGATCGCTGTGGACTGATCATGGTG GTAGTTGCTGAAGGTTGCTGGGATGGCTGT GTGTGTGGCAATTTCTTAAAATAAGACAACAGTGAAGTG CTGTATCAATTGATTTTTCCATTCACAAAAG ATTTCTCTGTAGCATGCAATGCTGTTTGATAGCATTTAAC CCACAGCAGAATTTCTTTGAAAATTGGACT CAGTCCTCTCAAACTGTGCTGCTGCTTTATCAACTAAGTT TTTGTAATTTTCTGAATCCTTTGTTGTCAT TTCAGCAGTTTACAGCATCTTCATTGGAAGTATATTCCAT CTCAAACATTCTTTGTTCATCCATAAGAAG CAACTTCTTATCAAGTTTTTTCATGACATTGCAGTAACTC AGCCCCATCTTCAGGCTCTACTTCTAATTC TGGTTCTCTTGCTACATCTCCCTCATCTGCAGTGACCTCTC CACGGAAGTCTTGAACTCCTCAAAGTAAT CCATGAGGGTTGGAATCAACTTCTAAACTCCTGTTAATGT TGATATATTGACCCCCTCCCATGAATTATG AATGTTCTTAATAACTTCTAAATGGTGATACCTTTCCAGA AGGCTTTCAATGTACTTTGCCCGGATCCAT CAGAAGACTATCTTGGCAGCTGTAGACTAACAATATATTT

		CTTAAATGATAAGACTTGAAAGTCAAAAGT ACTCCTTAATCCATAGGCTGCAGAATCAATGTTGTATTAA CAGGCACGAAAACAGCATTAATCTTGTGCA TCTCCATCGGAGCTCTTGGGTGACTAGGTGCCTTGAGCAG TAATATTTTGAAAGGAGGTTTTGGTTTTGT TTTTTGTTTTTTTTTTTTGTTTTTTAGCAGTAAGTCTCAACA CTGGGCTTAAAATATTCAGTAAACTATG TTGTAAAAAGATGTGTTATCATCCAGACTTTGTTGTTCCA TTACTCTACACAAGCAGGGTACACTTAGCA TAATTCTTAAGGGCCTTGGAATTTTCAGAATGGTAAATGA GTATGGGCTTCAACTTAAAATCATCAACTG CATTAGCCTGTAACAAGAGAGTCAGCCTGTCCTTTGAAGC AAGGCATTGACTTCTATCTATGAAAGTCTT AGATGGCACCTTGTTTCAATAGTAGGCTGTTTAGTACAGC CACCTTCATCAGTGATCTTAGCTAGATCTT CTGCATAACTTGCTGCAGCTTCTACATCAGCACTTGCTGC CTCACCTTGTCCTTTTATGTTATAGAGACA GCTGCGCTTCTTAAACTTTATAAACCAACTTCTGCTAGCT TCCAACTTCTCTTCTGCAGCTTCCTCATTC TCTTCATAGAACTGAAGGGAGTCAAGGCCTTGCTCTGGAT TAAGCTTTGGCTTAAGGAATGTTGTGGCTG ACGTGATCTTCTATCCAGACCACTAAAGCGCTCTCCATAT CAGCAATAAGGCCGTTTTGCTTTCTTACCT TTCATGTGTTCACTGGAGTAATTTCCTTCAAGAATTTTTCC TTTACATTCACAACTTGGCTAACTGGCAT GCAAGGCCTAGCTTTCAGCCTGTCTTGGCTTTTGACATGC CTTCCTCACTTAGCTCGTCATATCTAGCTT TTGATTTAAAGTGGCAGGCATACAACTCTTCCTTTCACTT GAACACTTAGAGGCCACTGTAGGGTTATTA ATTGGCCTAATTTCAATATTGTTGTGTTTTAGGGAATAGA GAGGCCCAGGGAGAGGGAGAGAGCCCAAAC GGCTGGTTGATAGAGCAGGCAGAATGCACACAACATTTA TCAGATTATGTTTGCACCATTTACCAGATTA TGGGTACGGTTTGTGGCACCCCCCAAAAATTAGAATAGT AACATCAAAGATCACTGATCACAGATCGCCA TAACATAAATAATAATAAACTTTAAAATACTGTGAGAAT TACCAAAATGTGATACAGAGACATGAAGTGA GCACATGCTGTTGAAAAAAATGACACTGATAGACATACT TAACACGTGGGATTGCCACAAACCTTCAGTT TGTAAAAGTCACAGTAACTGTGACTCACAAAAGAACAAA GCACAATAAAACGAGGTATGCCTGTATTTTT AAAAAAAGCTTTTTGTTAAAATTCAGGATATGTAATAGGT CTGTAGGAATAGTGAAATATTTTTGCTGAT GGATGTAGATATATACGTGGATAGAGATGAAGATCTTAA TTATAGCTATGCAGCATAGATTTAGTCAAAG ACATTTGAAAAGACAAATGTTAAATTAGTGTGGCTAATG ACCTACCCGTGCCATGTTTTCCCTCTTGCAA TGAGATACCCCACACTGTGTAGAAGGATGGAGGGAGGAC TCCTACTGTCCCTCTTTGCGTGTGGTTATTA AGTTGCCTCACTGGGCTAAAACACCACACATCTCATAGAT AATATTTGGTAAGTTGTAATCGTCTTCACT CTTCTCTTATCACCCACCCCTATCTTCCCACTTTTCCATCT TTGTTGGTTTGCAACAGCCCCTTCTTTTT GCCTGACTCTCCAGGATTTTCTCTCATCATAAATTGTTCTA

		AAGTACATACTAATATGGGTCTGGATTGA CTATTCTTATTTGCAAAACAGCAATTAAATGTTATAGGGA AGTAGGAAGAAAAAGGGGTATCCTTGACAA TAAACCAAGCAATATTCTGGGGGTGGGATAGAGCAGGAA ATTTTATTTTTAATCTTTTAAAATCCAAGTA ATAGGTAGGCTTCCAGTTAGCTTTAAATGTTTTTTTTTTCC AGCTCAAAAAATTGGATTGTAGTTGATAC TACATATAATACATTCTAATTCCCTCACTGTATTCTTTGTT TAGTTTCATTTATTTGGTTTAAAATAATT TTTTATCCCATATCTGAAATGTAATATATTTTTATCCAACA ACCAGCATGTACATATACTTAATTATGTG GCACATTTTCTAATAGATCAGTCCATCAATCTACTCATTT TAAAGAAAAAAAAATTTTAAAGTCACTTTT AGAGCCCTTAATGTGTAGTTGGGGGTTAAGCTTTGTGGAT GTAGCCTTTATATTTAGTATAATTGAGGTC TAAAATAATAATCTTCTATTATCTCAACAGAGCAAATTAT TGAAAAAGATGAAGGTCCTTTTTATACCCA TCTAGGAGCAGGTCCTAATGTGGCAGCTATTAGAGAAAT CATGGAAGAAAGGTAATTAACGCAAAGGCAC AGGGCAGATTAACGTTTATCCTTTTGTATATGTCAGAATT TTTCCAGCCTTCACACACAAAGCAGTAAAC AATTGTAAATTGAGTAATTATTAGTAGGCTTAGCTATTCT AGGGTTGCCAACACTACACACTGTGCTATT CACCAGAGAGTCACAATATTTGACAGGACTAATAGTCTG CTAGCTGGCACAGGCTGCCCACTTTGCGATG GATGCCAGAAAACCCAGGCATGAACAGGAATCGGCCAGC CAGGCTGCCAGCCACAAGGTACTGGCACAGG CTCCAACGAGAGGTCCCACTCTGGCTTTCCCACCTGATAA TAAAGTGTCAAAGCAGAAAGACTGGTAAAG TGTGGTATAAGAAAAGAACCACTGAATTAAATTCACCTA GTGTTGCAAATGAGTACTTATCTCTAAGTTT TCTTTTACCATAAAAAGAGAGCAAGTGTGATATGTTGAAT AGAAAGAGAAACATACTATTTACAGCTGCC TTTTTTTTTTTTTTTCGCTATCAATCACAGGTATACAAGTA CTTGCCTTTACTCCTGCATGTAGAAGACT CTTATGAGCGAGATAATGCAGAGAAGGCCTTTCATATAA ATTTATACAGCTCTGAGCTGTTCTTCTTCTA GGGTGCCTTTTCATTAAGAGGTAGGCAGTATTATTATTAA AGTACTTAGGATACATTGGGGCAGCTAGGA CATATTCAGTATCATTCTTGCTCCATTTCCAAATTATTCAT TTCTAAATTAGCATGTAGAAGTTCACTAA ATAATCATCTAGTGGCCTGGCAGAAATAGTGAATTTCCCT AAGTGCCTTTTTTTTGTTGTTTTTTTGTTT TGTTTTTTAAACAAGCAGTAGGTGGTGCTTTGGTCATAAG GGAAGATATAGTCTATTTCTAGGACTATTC CATATTTTCCATGTGGCTGGATACTAACTATTTGCCAGCC TCCTTTTCTAAATTGTGAGACATTCTTGGA GGAACAGTTCTAACTAAAATCTATTATGACTCCCCAAGTT TTAAAATAGCTAAATTTAGTAAGGGAAAAA ATAGTTTATGTTTTAGAAGACTGAACTTAGCAAACTAACC TGAATTTTGTGCTTTGTGAAATTTTATATC GAAATGAGCTTTCCCATTTTCACCCACATGTAATTTACAA AATAGTTCATTACAATTATCTGTACATTTT GATATTGAGGAAAAACAAGGCTTAAAAACCATTATCCAG

		TTTGCTTGGCGTAGACCTGTTTAAAAAATAA TAAACCGTTCATTTCTCAGGATGTGGTCATAGAATAAAGT TATGCTCAAATGTTCAAATATTTAAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X9, mRNA [SEQ ID NO: 1362]	XM_011532044.1	TCAGGCTCTACTTCTAATTCTGGTTCTCTTGCTACATCTCC CTCATCTGCAGTGACCTCTCCACGGAAGT CTTGAACTCCTCAAAAGCAAATTATTGAAAAAGATGAAG GTCCTTTTTATACCCATCTAGGAGCAGGTCC TAATGTGGCAGCTATTAGAGAAATCATGGAAGAAAGGTT TGGACAGAAGGGTAAAGCTATTAGGATTGAA AGAGTCATCTATACTGGTAAAGAAGGCAAAAGTTCTCAG GGATGTCCTATTGCTAAGTGGGTGGTTCGCA GAAGCAGCAGTGAAGAGAAGCTACTGTGTTTGGTGCGGG AGCGAGCTGGCCACACCTGTGAGGCTGCAGT GATTGTGATTCTCATCCTGGTGTGGGAAGGAATCCCGCTG TCTCTGGCTGACAAACTCTACTCGGAGCTT ACCGAGACGCTGAGGAAATACGGCACGCTCACCAATCGC CGGTGTGCCTTGAATGAAGAGAGAACTTGCG CCTGTCAGGGGCTGGATCCAGAAACCTGTGGTGCCTCCTT CTCTTTTGGTTGTTCATGGAGCATGTACTA CAATGGATGTAAGTTTGCCAGAAGCAAGATCCCAAGGAA GTTTAAGCTGCTTGGGGATGACCCAAAAGAG GAAGAGAAACTGGAGTCTCATTTGCAAAACCTGTCCACT CTTATGGCACCAACATATAAGAAACTTGCAC CTGATGCATATAATAATCAGATTGAATATGAACACAGAG CACCAGAGTGCCGTCTGGGTCTGAAGGAAGG CCGTCCATTCTCAGGGGTCACTGCATGTTTGGACTTCTGT GCTCATGCCCACAGAGACTTGCACAACATG CAGAATGGCAGCACATTGGTATGCACTCTCACTAGAGAA GACAATCGAGAATTTGGAGGAAAACCTGAGG ATGAGCAGCTTCACGTTCTGCCTTTATACAAAGTCTCTGA CGTGGATGAGTTTGGGAGTGTGGAAGCTCA GGAGGAGAAAAAACGGAGTGGTGCCATTCAGGTACTGAG TTCTTTTCGGCGAAAAGTCAGGATGTTAGCA GAGCCAGTCAAGACTTGCCGACAAAGGAAACTAGAAGCC AAGAAAGCTGCAGCTGAAAAGCTTTCCTCCC TGGAGAACAGCTCAAATAAAAATGAAAAGGAAAAGTCA GCCCCATCACGTACAAAACAAACTGAAAACGC AAGCCAGGCTAAACAGTTGGCAGAACTTTTGCGACTTTC AGGACCAGTCATGCAGCAGTCCCAGCAGCCC CAGCCTCTACAGAAGCAGCCACCACAGCCCCAGCAGCAG CAGAGACCCCAGCAGCAGCAGCCACATCACC CTCAGACAGAGTCTGTCAACTCTTATTCTGCTTCTGGATC CACCAATCCATACATGAGACGGCCCAATCC AGTTAGTCCTTATCCAAACTCTTCACACACTTCAGATATC TATGGAAGCACCAGCCCTATGAACTTCTAT TCCACCTCATCTCAAGCTGCAGGTTCATATTTGAATTCTT CTAATCCCATGAACCCTTACCCTGGGCTTT TGAATCAGAATACCCAATATCCATCATATCAATGCAATG GAAACCTATCAGTGGACAACTGCTCCCCATA TCTGGGTTCCTATTCTCCCCAGTCTCAGCCGATGGATCTG TATAGGTATCCAAGCCAAGACCCTCTGTCT AAGCTCAGTCTACCACCCATCCATACACTTTACCAGCCAA GGTTTGGAAATAGCCAGAGTTTTACATCTA AATACTTAGGTTATGGAAACCAAAATATGCAGGGAGATG

		GTTTCAGCAGTTGTACCATTAGACCAAATGT ACATCATGTAGGGAAATTGCCTCCTTATCCCACTCATGAG ATGGATGGCCACTTCATGGGAGCCACCTCT AGATTACCACCCAATCTGAGCAATCCAAACATGGACTAT AAAAATGGTGAACATCATTCACCTTCTCACA TAATCCATAACTACAGTGCAGCTCCGGGCATGTTCAACA GCTCTCTTCATGCCCTGCATCTCCAAAACAA GGAGAATGACATGCTTTCCCACACAGCTAATGGGTTATC AAAGATGCTTCCAGCTCTTAACCATGATAGA ACTGCTTGTGTCCAAGGAGGCTTACACAAATTAAGTGAT GCTAATGGTCAGGAAAAGCAGCCATTGGCAC TAGTCCAGGGTGTGGCTTCTGGTGCAGAGGACAACGATG AGGTCTGGTCAGACAGCGAGCAGAGCTTTCT GGATCCTGACATTGGGGGAGTGGCCGTGGCTCCAACTCA TGGGTCAATTCTCATTGAGTGTGCAAAGCGT GAGCTGCATGCCACAACCCCTTTAAAGAATCCCAATAGG AATCACCCCACCAGGATCTCCCTCGTCTTTT ACCAGCATAAGAGCATGAATGAGCCAAAACATGGCTTGG CTCTTTGGGAAGCCAAAATGGCTGAAAAAGC CCGTGAGAAAGAGGAAGAGTGTGAAAAGTATGGCCCAG ACTATGTGCCTCAGAAATCCCATGGCAAAAAA GTGAAACGGGAGCCTGCTGAGCCACATGAAACTTCAGAG CCCACTTACCTGCGTTTCATCAAGTCTCTTG CCGAAAGGACCATGTCCGTGACCACAGACTCCACAGTAA CTACATCTCCATATGCCTTCACTCGGGTCAC AGGGCCTTACAACAGATATATATGATATCACCCCCTTTTG TTGGTTACCTCACTTGAAAAGACCACAACC AACCTGTCAGTAGTATAGTTCTCATGACGTGGGCAGTGG GGAAAGGTCACAGTATTCATGACAAATGTGG TGGGAAAAACCTCAGCTCACCAGCAACAAAAGAGGTTAT CTTACCATAGCACTTAATTTTCACTGGCTCC CAAGTGGTCACAGATGGCATCTAGGAAAAGACCAAAGCA TTCTATGCAAAAAGAAGGTGGGGAAGAAAGT GTTCCGCAATTTACATTTTTAAACACTGGTTCTATTATTGG ACGAGATGATATGTAAATGTGATCCCCCC CCCCCGCTTACAACTCTACACATCTGTGACCACTTTTAAT AATATCAAGTTTGCATAGTCATGGAACACA AATCAAACAAGTACTGTAGTATTACAGTGACAGGAATCT TAAAATACCATCTGGTGCTGAATATATGATG TACTGAAATACTGGAATTATGGCTTTTTGAAATGCAGTTT TTACTGTAATCTTAACTTTTATTTATCAAA ATAGCTACAGGAAACATGAATAGCAGGAAAACACTGAAT TTGTTTGGATGTTCTAAGAAATGGTGCTAAG AAAATGGTGTCTTTAATAGCTAAAAATTTAATGCCTTTAT ATCATCAAGATGCTATCAGTGTACTCCAGT GCCCTTGAATAATAGGGGTACCTTTTCATTCAAGTTTTTA TCATAATTACCTATTCTTACACAAGCTTAG TTTTTAAAATGTGGACATTTTAAAGGCCTCTGGATTTTGC TCATCCAGTGAAGTCCTTGTAGGACAATAA ACGTATATATGTACATATATACACAAACATGTATATGTGC ACACACATGTATATGTATAAATATTTTAAA TGGTGTTTTAGAAGCACTTTGTCTACCTAAGCTTTGACAA CTTGAACAATGCTAAGGTACTGAGATGTTT AAAAAACAAGTTTACTTTCATTTTAGAATGCAAAGTTGAT

		TTTTTTAAGGAAACAAAGAAAGCTTTTAAA ATATTTTTGCTTTTAGCCATGCATCTGCTGATGAGCAATT GTGTCCATTTTTAACACAGCCAGTTAAATC CACCATGGGGCTTACTGGATTCAAGGGAATACGTTAGTC CACAAAACATGTTTTCTGGTGCTCATCTCAC ATGCTATACTGTAAAACAGTTTTATACAAAATTGTATGAC AAGTTCATTGCTCAAAAATGTACAGTTTTA AGAATTTTCTATTAACTGCAGGTAATAATTAGCTGCATGC TGCAGACTCAACAAAGCTAGTTCACTGAAG CCTATGCTATTTTATGGATCATAGGCTCTTCAGAGAACTG AATGGCAGTCTGCCTTTGTGTTGATAATTA TGTACATTGTGACGTTGTCATTTCTTAGCTTAAGTGTCCTC TTTAACAAGAGGATTGAGCAGACTGATGC CTGCATAAGATGAATAAACAGGGTTAGTTCCATGTGAAT CTGTCAGTTAAAAAGAAACAAAAACAGGCAG CTGGTTTGCTGTGGTGGTTTTAAATCATTAATTTGTATAA AGAAGTGAAAGAGTTGTATAGTAAATTAAA TTGTAAACAAAACTTTTTTAATGCAATGCTTTAGTATTTT AGTACTGTAAAAAAATTAAATATATACATA TATATATATATATATATATATATATATATGAGTTTGAAGC AGAATTCACATCATGATGGTGCTACTCAGC CTGCTACAAATATATCATAATGTGAGCTAAGAATTCATTA AATGTTTGAGTGATGTTCCTACTTGTCATA TACCTCAACACTAGTTTGGCAATAGGATATTGAACTGAG AGTGAAAGCATTGTGTACCATCATTTTTTTC CAAGTCCTTTTTTTTATTGTTAAAAAAAAAAGCATACCTT TTTTCAATACTTGATTTCTTAGCAAGTATA ACTTGAACTTCAACCTTTTTGTTCTAAAAATTCAGGGATA TTTCAGCTCATGCTCTCCCTATGCCAACAT GTCACCTGTGTTTATGTAAAATTGTTGTAGGTTAATAAAT ATATTCTTTGTCAGGGATTTAACCCTTTTA TTTTGAATCCCTTCTATTTTACTTGTACATGTGCTGATGTA ACTAAAACTAATTTTGTAAATCTGTTGGC TCTTTTTATTGTAAAGAAAAGCATTTTAAAAGTTTGAGGA ATCTTTTGACTGTTTCAAGCAGGAAAAAAA AATTACATGAAAATAGAATGCACTGAGTTGATAAAGGGA AAAATTGTAAGGCAGGAGTTTGGCAAGTGGC TGTTGGCCAGAGACTTACTTGTAACTCTCTAAATGAAGTT TTTTTGATCCTGTAATCACTGAAGGTACAT ACTCCATGTGGACTTCCCTTAAACAGGCAAACACCTACA GGTATGGTGTGCAACAGATTGTACAATTACA TTTTGGCCTAAATACATTTTTGCTTACTAGTATTTAAAATA AATTCTTAATCAGAGGAGGCCTTTGGGTT TTATTGGTCAAATCTTTGTAAGCTGGCTTTTGTCTTTTTAA AAAATTTCTTGAATTTGTGGTTGTGTCCA ATTTGCAAACATTTCCAAAAATGTTTGCTTTGCTTACAAA CCACATGATTTTAATGTTTTTTGTATACCA TAATATCTAGCCCCAAACATTTGATTACTACATGTGCATT GGTGATTTTGATCATCCATTCTTAATATTT GATTTCTGTGTCACCTACTGTCATTTGTTAAACTGCTGGC CAACAAGAACAGGAAGTATAGTTTGGGGGG TTGGGGAGAGTTTACATAAGGAAGAGAAGAAATTGAGTG GCATATTGTAAATATCAGATCTATAATTGTA AATATAAAACCTGCCTCAGTTAGAATGAATGGAAAGCAG

		ATCTACAATTTGCTAATATAGGAATATCAGG TTGACTATATAGCCATACTTGAAAATGCTTCTGAGTGGTG TCAACTTTACTTGAATGAATTTTTCATCTT GATTGACGCACAGTGATGTACAGTTCACTTCTGAAGCTAG TGGTTAACTTGTGTAGGAAACTTTTGCAGT TTGACACTAAGATAACTTCTGTGTGCATTTTTCTATGCTTT TTTAAAAACTAGTTTCATTTCATTTTCAT GAGATGTTTGGTTTATAAGATCTGAGGATGGTTATAAATA CTGTAAGTATTGTAATGTTATGAATGCAGG TTATTTGAAAGCTGTTTATTATTATATCATTCCTGATAATG CTATGTGAGTGTTTTTAATAAAATTTATA TTTATTTAATGCACTCTAA
PREDICTED: Homo sapiens tet methylcytosine dioxygenase 2 (TET2), transcript variant X7, mRNA [SEQ ID NO: 1363]	XM_011532043.1	GTAGAGAAGCAGAAGGAAGCAAGATGGCTGCCCTTTAGG ATTTGTTAGAAAGGAGACCCGACTGCAACTG CTGGATTGCTGCAAGGCTGAGGGACGAGAACGAGGCTGG CAAACATTCAGCAGCACACCCTCTCAAGATT GTTTACTTGCCTTTGCTCCTGTTGAGTTACAACGCTTGGA AGCAGGAGATGGGCTCAGCAGCAGCCAATA GGACATGATCCAGGAAGAGCAGTAAGGGACTGAGCTGCT GAATTCAACTAGAGGGCAGCCTTGTGGATGG CCCCGAAGCAAGCCTGATGGAACAGGATAGAACCAACCA TGTTGAGGGCAACAGACTAAGTCCATTCCTG ATACCATCACCTCCCATTTGCCAGACAGAACCTCTGGCTA CAAAGCTCCAGAATGGAAGCCCACTGCCTG AGAGAGCTCATCCAGAAGTAAATGGAGACACCAAGTGGC ACTCTTTCAAAAGTTATTATGGAATACCCTG TATGAAGGGAAGCCAGAATAGTCGTGTGAGTCCTGACTT TACACAAGAAAGTAGAGGGTATTCCAAGTGT TTGCAAAATGGAGGAATAAAACGCACAGTTAGTGAACCT TCTCTCTCTGGGCTCCTTCAGATCAAGAAAT TGAAACAAGACCAAAAGGCTAATGGAGAAAGACGTAAC TTCGGGGTAAGCCAAGAAAGAAATCCAGGTGA AAGCAGTCAACCAAATGTCTCCGATTTGAGTGATAAGAA AGAATCTGTGAGTTCTGTAGCCCAAGAAAAT GCAGTTAAAGATTTCACCAGTTTTTCAACACATAACTGCA GTGGGCCTGAAAATCCAGAGCTTCAGATTC TGAATGAGCAGGAGGGGAAAAGTGCTAATTACCATGACA AGAACATTGTATTACTTAAAAACAAGGCAGT GCTAATGCCTAATGGTGCTACAGTTTCTGCCTCTTCCGTG GAACACACACATGGTGAACTCCTGGAAAAA ACACTGTCTCAATATTATCCAGATTGTGTTTCCATTGCGG TGCAGAAAACCACATCTCACATAAATGCCA TTAACAGTCAGGCTACTAATGAGTTGTCCTGTGAGATCAC TCACCCATCGCATACCTCAGGGCAGATCAA TTCCGCACAGACCTCTAACTCTGAGCTGCCTCCAAAGCCA GCTGCAGTGGTGAGTGAGGCCTGTGATGCT GATGATGCTGATAATGCCAGTAAACTAGCTGCAATGCTA AATACCTGTTCCTTTCAGAAACCAGAACAAC TACAACAACAAAAATCAGTTTTTGAGATATGCCCATCTCC TGCAGAAAATAACATCCAGGGAACCACAAA GCTAGCGTCTGGTGAAGAATTCTGTTCAGGTTCCAGCAGC AATTTGCAAGCTCCTGGTGGCAGCTCTGAA CGGTATTTAAAACAAAATGAAATGAATGGTGCTTACTTC AAGCAAAGCTCAGTGTTCACTAAGGATTCCT

		TTTCTGCCACTACCACACCACCACCACCATCACAATTGCT TCTTTCTCCCCCTCCTCCTCTTCCACAGGT TCCTCAGCTTCCTTCAGAAGGAAAAAGCACTCTGAATGGT GGAGTTTTAGAAGAACACCACCACTACCCC AACCAAAGTAACACAACACTTTTAAGGGAAGTGAAAATA GAGGGTAAACCTGAGGCACCACCTTCCCAGA GTCCTAATCCATCTACACATGTATGCAGCCCTTCTCCGAT GCTTTCTGAAAGGCCTCAGAATAATTGTGT GAACAGGAATGACATACAGACTGCAGGGACAATGACTGT TCCATTGTGTTCTGAGAAAACAAGACCAATG TCAGAACACCTCAAGCATAACCCACCAATTTTTGGTAGCA GTGGAGAGCTACAGGACAACTGCCAGCAGT TGATGAGAAACAAAGAGCAAGAGATTCTGAAGGGTCGA GACAAGGAGCAAACACGAGATCTTGTGCCCCC AACACAGCACTATCTGAAACCAGGATGGATTGAATTGAA GGCCCCTCGTTTTCACCAAGCGGAATCCCAT CTAAAACGTAATGAGGCATCACTGCCATCAATTCTTCAGT ATCAACCCAATCTCTCCAATCAAATGACCT CCAAACAATACACTGGAAATTCCAACATGCCTGGGGGGC TCCCAAGGCAAGCTTACACCCAGAAAACAAC ACAGCTGGAGCACAAGTCACAAATGTACCAAGTTGAAAT GAATCAAGGGCAGTCCCAAGGTACAGTGGAC CAACATCTCCAGTTCCAAAAACCCTCACACCAGGTGCACT TCTCCAAAACAGACCATTTACCAAAAGCTC ATGTGCAGTCACTGTGTGGCACTAGATTTCATTTTCAACA AAGAGCAGATTCCCAAACTGAAAAACTTAT GTCCCCAGTGTTGAAACAGCACTTGAATCAACAGGCTTC AGAGACTGAGCCATTTTCAAACTCACACCTT TTGCAACATAAGCCTCATAAACAGGCAGCACAAACACAA CCATCCCAGAGTTCACATCTCCCTCAAAACC AGCAACAGCAGCAAAAATTACAAATAAAGAATAAAGAG GAAATACTCCAGACTTTTCCTCACCCCCAAAG CAACAATGATCAGCAAAGAGAAGGATCATTCTTTGGCCA GACTAAAGTGGAAGAATGTTTTCATGGTGAA AATCAGTATTCAAAATCAAGCGAGTTCGAGACTCATAAT GTCCAAATGGGACTGGAGGAAGTACAGAATA TAAATCGTAGAAATTCCCCTTATAGTCAGACCATGAAATC AAGTGCATGCAAAATACAGGTTTCTTGTTC AAACAATACACACCTAGTTTCAGAGAATAAAGAACAGAC TACACATCCTGAACTTTTTGCAGGAAACAAG ACCCAAAACTTGCATCACATGCAATATTTTCCAAATAATG TGATCCCAAAGCAAGATCTTCTTCACAGGT GCTTTCAAGAACAGGAGCAGAAGTCACAACAAGCTTCAG TTCTACAGGGATATAAAAATAGAAACCAAGA TATGTCTGGTCAACAAGCTGCGCAACTTGCTCAGCAAAG GTACTTGATACATAACCATGCAAATGTTTTT CCTGTGCCTGACCAGGGAGGAAGTCACACTCAGACCCCT CCCCAGAAGGACACTCAAAAGCATGCTGCTC TAAGGTGGCATCTCTTACAGAAGCAAGAACAGCAGCAAA CACAGCAACCCCAAACTGAGTCTTGCCATAG TCAGATGCACAGGCCAATTAAGGTGGAACCTGGATGCAA GCCACATGCCTGTATGCACACAGCACCACCA GAAAACAAAACATGGAAAAAGGTAACTAAGCAAGAGAA TCCACCTGCAAGCTGTGATAATGTGCAGCAAA

		AGAGCATCATTGAGACCATGGAGCAGCATCTGAAGCAGT TTCACGCCAAGTCGTTATTTGACCATAAGGC TCTTACTCTCAAATCACAGAAGCAAGTAAAAGTTGAAAT GTCAGGGCCAGTCACAGTTTTGACTAGACAA ACCACTGCTGCAGAACTTGATAGCCACACCCCAGCTTTAG AGCAGCAAACAACTTCTTCAGAAAAGACAC CAACCAAAAGAACAGCTGCTTCTGTTCTCAATAATTTTAT AGAGTCACCTTCCAAATTACTAGATACTCC TATAAAAAATTTATTGGATACACCTGTCAAGACTCAATAT GATTTCCCATCTTGCAGATGTGTAGAGCAA ATTATTGAAAAAGATGAAGGTCCTTTTTATACCCATCTAG GAGCAGGTCCTAATGTGGCAGCTATTAGAG AAATCATGGAAGAAAGGTATACAAGTACTTGCCTTTACT CCTGCATGTAGAAGACTCTTATGAGCGAGAT AATGCAGAGAAGGCCTTTCATATAAATTTATACAGCTCTG AGCTGTTCTTCTTCTAGGGTGCCTTTTCAT TAAGAGGTAGGCAGTATTATTATTAAAGTACTTAGGATA CATTGGGGCAGCTAGGACATATTCAGTATCA TTCTTGCTCCATTTCCAAATTATTCATTTCTAAATTAGCAT GTAGAAGTTCACTAAATAATCATCTAGTG GCCTGGCAGAAATAGTGAATTTCCCTAAGTGCCTTTTTTT TGTTGTTTTTTTGTTTTGTTTTTTAAACAA GCAGTAGGTGGTGCTTTGGTCATAAGGGAAGATATAGTC TATTTCTAGGACTATTCCATATTTTCCATGT GGCTGGATACTAACTATTTGCCAGCCTCCTTTTCTAAATT GTGAGACATTCTTGGAGGAACAGTTCTAAC TAAAATCTATTATGACTCCCCAAGTTTTAAAATAGCTAAA TTTAGTAAGGGAAAAAATAGTTTATGTTTT AGAAGACTGAACTTAGCAAACTAACCTGAATTTTGTGCTT 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.

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, 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.

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.

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.

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 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 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 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 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 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 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 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 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, 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, 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 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) 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) 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 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 NFκB in response to TNF, e.g., a process involing 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 intergrity) 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 αβ 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 involing 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 involigin 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 wising 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. 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 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-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 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 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. (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 used in a system for gene editing. Pennisi (2013) Science 341: 833-836.

The CRISPR/Cas system can thus be used to modify, e.g., delete one or more nucleic acids, 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), or a gene regulatory element 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 introduce a premature stop which thus decreases expression of a functional 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). The CRISPR/Cas system can alternatively be used like RNA interference, turning off the Tet-associated gene (e.g., Tet2-associated gene) and/or a Tet (e.g., Tet1, Tet2, and/or Tet3, e.g., Tet2) in a reversible fashion. In a mammalian cell, for example, the RNA can guide the Cas protein to a promoter 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), sterically blocking RNA polymerases.

CRISPR/Cas systems for gene editing in eukaryotic cells typically involve (1) a guide RNA molecule (gRNA) comprising a targeting sequence (which is capable of hybridizing to the genomic DNA target sequence), and sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, and (2) a Cas, e.g., Cas9, protein. The targeting sequence and the sequence which is capable of binding to a Cas, e.g., Cas9 enzyme, may be disposed on the same or different molecules. If disposed on different molecules, each includes a hybridization domain which allows the molecules to associate, e.g., through hybridization.

An exemplary gRNA molecule of the present invention comprises, e.g., consists of a first nucleic acid having the sequence (where the "n"'s refer to the residues of the targeting sequence (e.g., as described herein, e.g., in Table 3), and may consist of 15-25 nucelotides, e.g., consist of 20 nucleotides):

nnnnnnnnnnnnnnnnnnnnGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 40);

and a second nucleic acid sequence having the sequence:

AACUUACCAAGGAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGGUGC, optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 7) additional U nucleotides at the 3' end (SEQ ID NO: 41).

The second nucleic acid molecule may alternatively consist of a fragment of the sequence above, wherein such fragment is capable of hybridizing to the first nucleic acid. An example of such second nucleic acid molecule is:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGC, optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 7) additional U nucleotides at the 3' end (SEQ ID NO: 42).

Another exemplary gRNA molecule of the present invention comprises, e.g., consists of a first nucleic acid having the sequence (where the "n"'s refer to the residues of the targeting sequence (e.g., as described herein, e.g., in Table 3), and may consist of 15-25 nucelotides, e.g., consist of 20 nucleotides):

nnnnnnnnnnnnnnnnnnGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGU CCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 43), optionally with 1, 2, 3, 4, 5, 6, or 7 (e.g., 4 or 7, e.g., 4) additional U nucleotides at the 3' end. Artificial CRISPR/Cas systems can be generated which inhibit 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, using technology known in the art, e.g., that are described in U.S. Publication No.20140068797, WO2015/048577, and Cong (2013) Science 339: 819-823. Other artificial CRISPR/Cas systems that are known in the art may also be generated which inhibit 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., that described in Tsai (2014) Nature Biotechnol., 32:6 569-576, U.S. Patent No.: 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359, the contents of which are hereby incorporated by reference in their entirety. Such systems can be generated which inhibit 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, engineering a CRISPR/Cas system to include a gRNA molecule comprising a targeting sequence that hybridizes to a sequence of a target gene, 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. In embodiments, the gRNA comprises a targeting sequence which is fully complementarity to 15-25 nucleotides, e.g., 20 nucleotides, of a target gene, 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. In embodiments, the 15-25 nucleotides, e.g., 20 nucleotides, of a target gene, 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, are disposed immediately 5' to a protospacer adjacent motif (PAM) sequence recognized by the Cas protein of the CRISPR/Cas system (e.g., where the system comprises a *S. pyogenes* Cas9 protein, the PAM sequence comprises NGG, where N can be any of A, T, G or C). In embodiments, the targeting sequence of the gRNA comprises, e.g., consists of, a RNA sequence complementary to a sequence listed in Table 2. In embodiments, the gRNA comprises a targeting sequence listed in Table 3.

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 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.

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	GAGAUUAAAGCGACAGAAAA	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	UGCGCCCACCUUACCCUCAG	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	UGCUUUCGUAGAGAAGCAGA	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	CCUCCGCAUCAAUAUUCAUA	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	UUGUAACUCAACAGGAGCAA	167
54790_3_55	TET2	EXON	-	chr4:105190415 -105190435	UCCAAGCGUUGUAACUCAAC	168
54790_3_60	TET2	EXON	-	chr4:105190462 -105190482	CUUCCUGGAUCAUGUCCUAU	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	CACUCUUUCAAAAGUUAUUA	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	CAUUGUAUUACUUAAAAACA	204
54790_4_151	TET2	EXON	+	chr4:105234548 -105234568	AAGGCAGUGCUAAUGCCUAA	205
54790_4_153	TET2	EXON	+	chr4:105234574 -105234594	UACAGUUUCUGCCUCUUCCG	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	CAUAAAUGCCAUUAACAGUC	210
54790_4_177	TET2	EXON	+	chr4:105234734 -105234754	ACUCACCCAUCGCAUACCUC	211
54790_4_178	TET2	EXON	+	chr4:105234735 -105234755	CUCACCCAUCGCAUACCUCA	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	UCCUGCAGAAAAUAACAUCC	215
54790_4_201	TET2	EXON	+	chr4:105234944 -105234964	CCUGCAGAAAAUAACAUCCA	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	UCCCCCUCCUCCUCUUCCAC	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	AAAGUAACACAACACUUUUA	228
54790_4_258	TET2	EXON	+	chr4:105235261 -105235281	AAGUAACACAACACUUUUAA	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	GCAACAUAAGCCUCAUAAAC	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	CAAAGCAAGAUCUUCUUCAC	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	AAUAGAAACCAAGAUAUGUC	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	UACUCCUAUAAAAAAUUUAU	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	AGCAAAUUUAUCUUCAGAUA	300
54790_4_597	TET2	EXON	+	chr4:105237398 -105237418	GCAAAUUUAUCUUCAGAUAU	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	CUUUUCUCCAUUUAUACAUU	315
54790_4_741	TET2	EXON	+	chr4:105240332 -105240352	AAAGCUUUUUGUUAAAAUUC	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	UAGUGAAAUAUUUUUGCUGA	319
54790_4_760	TET2	EXON	+	chr4:105240395 -105240415	GAUGGAUGUAGAUAUAUACG	320
54790_4_770	TET2	EXON	+	chr4:105240478 -105240498	AGACAAAUGUUAAAUUAGUG	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	CUACUGUCCCUCUUUGCGUG	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	UCCCACUUUUCCAUCUUUGU	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	AAACAGCAAUUAAAUGUUAU	336
54790_4_842	TET2	EXON	+	chr4:105240834 -105240854	AACAGCAAUUAAAUGUUAUA	337
54790_4_845	TET2	EXON	+	chr4:105240841 -105240861	AUUAAAUGUUAUAGGGAAGU	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	UUCUGGGGGUGGGAUAGAGC	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	UGUACAUAUACUUAAUUAUG	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	AAGUGUCAAAGCAGAAAGAC	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	GUAUUAUUAUUAAAGUACUU	393
54790_4_1135	TET2	EXON	+	chr4:105242114 -105242134	UUAAAGUACUUAGGAUACAU	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	UUCACUAAAUAAUCAUCUAG	398
54790_4_1156	TET2	EXON	+	chr4:105242215 -105242235	UAAAUAAUCAUCUAGUGGCC	399
54790_4_1162	TET2	EXON	+	chr4:105242287 -105242307	UUGUUUUUUAAACAAGCAGU	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	GGAAGAUAUAGUCUAUUUCU	405
54790_4_1176	TET2	EXON	+	chr4:105242351 -105242371	ACUAUUCCAUAUUUUCCAUG	406
54790_4_1178	TET2	EXON	+	chr4:105242355 -105242375	UUCCAUAUUUUCCAUGUGGC	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	UGUUCCAUCAGGCUUGCUUC	420
54790_4_1281	TET2	EXON	-	chr4:105233931 -105233951	CUGUUCCAUCAGGCUUGCUU	421
54790_4_1285	TET2	EXON	-	chr4:105233941 -105233961	UGGUUCUAUCCUGUUCCAUC	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	UCACUCAAAUCGGAGACAUU	449

54790_4_1399	TET2	EXON	-	chr4:105234369 -105234389	UUCUUUCUUAUCACUCAAAU	450
54790_4_1410	TET2	EXON	-	chr4:105234408 -105234428	AUCUUUAACUGCAUUUUCUU	451
54790_4_1411	TET2	EXON	-	chr4:105234409 -105234429	AAUCUUUAACUGCAUUUUCU	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	UUCACCAUGUGUGUGUUCCA	459
54790_4_1457	TET2	EXON	-	chr4:105234619 -105234639	AUUGAGACAGUGUUUUUUCC	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	AUUAGUAGCCUGACUGUUAA	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	AGAGUGCUUUUUCCUUCUGA	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	GGGCUGCAUACAUGUGUAGA	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	CUUUGUUUCUCAUCAACUGC	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	GCCUUCAAUUCAAUCCAUCC	524
54790_4_1711	TET2	EXON	-	chr4:105235650 -105235670	UUCCGCUUGGUGAAAACGAG	525
54790_4_1712	TET2	EXON	-	chr4:105235651 -105235671	AUUCCGCUUGGUGAAAACGA	526
54790_4_1713	TET2	EXON	-	chr4:105235652 -105235672	GAUUCCGCUUGGUGAAAACG	527
54790_4_1722	TET2	EXON	-	chr4:105235663 -105235683	GUUUUAGAUGGGAUUCCGCU	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	UGGGAGCCCCCCAGGCAUGU	537
54790_4_1758	TET2	EXON	-	chr4:105235778 -105235798	GCUUGCCUUGGGAGCCCCCC	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	GGACAUAAGUUUUUCAGUUU	555
54790_4_1829	TET2	EXON	-	chr4:105236011 -105236031	GGGACAUAAGUUUUUCAGUU	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	AACAUUCUUCCACUUUAGUC	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	CUUGUUGACCAGACAUAUCU	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	UGCUUUUGAGUGUCCUUCUG	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	UUCCAUGUUUUGUUUUCUGG	607
54790_4_2079	TET2	EXON	-	chr4:105236936 -105236956	UUUUUCCAUGUUUUGUUUUC	608
54790_4_2085	TET2	EXON	-	chr4:105236978 -105236998	ACAUUAUCACAGCUUGCAGG	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	CUUAUGGUCAAAUAACGACU	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	GCUAUCAAGUUCUGCAGCAG	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	GUAUCCAAUAAAUUUUUUAU	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	GAAGAUAAAUUUGCUAAUUC	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	GCACGAUUAUUUAAUUCUUU	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	CAAAUGUAUAAAUGGAGAAA	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	GAUGGAAAAGUGGGAAGAUA	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	UAUUGCUUGGUUUAUUGUCA	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	AAAAAACAUUUAAAGCUAAC	674
54790_4_2446	TET2	EXON	-	chr4:105241000 -105241020	UACAAUCCAAUUUUUUGAGC	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	AUAUAUUACAUUUCAGAUAU	678
54790_4_2469	TET2	EXON	-	chr4:105241108 -105241128	AAUAUAUUACAUUUCAGAUA	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	UCUCUAAUAGCUGCCACAUU	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	UGUCAAAUAUUGUGACUCUC	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	AUAGCGAAAAAAAAAAAAAA	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	CUGCCUACCUCUUAAUGAAA	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	UUUCACAAAGCACAAAAUUC	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	CGGUUUAUUAUUUUUUAAAC	739
54790_4_2781	TET2	EXON	-	chr4:105242715 -105242735	ACCACAUCCUGAGAAAUGAA	740
54790_5_3	TET2	EXON	+	chr4:105242816 -105242836	CUGUGGGUUUCUUUAAGGUU	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	ACCGAGACGCUGAGGAAAUA	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	CUUCACUUACUCUUCAUUCA	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	CCUUCUCUUUUGGUUGUUCA	785

54790_7_31	TET2	EXON	+	chr4:105259682 -105259702	UCAUGGAGCAUGUACUACAA	786
54790_7_35	TET2	EXON	+	chr4:105259713 -105259733	UUGCCAGAAGCAAGAUCCCA	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	ACCUGAUGCAUAUAAUAAUC	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	AAGGAAGGCCGUCCAUUCUC	813
54790_9_24	TET2	EXON	+	chr4:105269652 -105269672	AGGAAGGCCGUCCAUUCUCA	814
54790_9_25	TET2	EXON	+	chr4:105269653 -105269673	GGAAGGCCGUCCAUUCUCAG	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	AGCUGAAAAGCUUUCCUCCC	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	CCACCAAUCCAUACAUGAGA	865
54790_11_19	TET2	EXON	+	chr4:105275256-105275276	UCACACACUUCAGAUAUCUA	866
54790_11_24	TET2	EXON	+	chr4:105275304-105275324	UCCACCUCAUCUCAAGCUGC	867
54790_11_34	TET2	EXON	+	chr4:105275346-105275366	AAUCCCAUGAACCCUUACCC	868
54790_11_35	TET2	EXON	+	chr4:105275347-105275367	AUCCCAUGAACCCUUACCCU	869
54790_11_44	TET2	EXON	+	chr4:105275391-105275411	UAUCCAUCAUAUCAAUGCAA	870
54790_11_47	TET2	EXON	+	chr4:105275405-105275425	AUGCAAUGGAAACCUAUCAG	871
54790_11_49	TET2	EXON	+	chr4:105275426-105275446	GGACAACUGCUCCCCAUAUC	872
54790_11_50	TET2	EXON	+	chr4:105275427-105275447	GACAACUGCUCCCCAUAUCU	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	UCCAUACACUUUACCAGCCA	876
54790_11_59	TET2	EXON	+	chr4:105275538-105275558	ACACUUUACCAGCCAAGGUU	877
54790_11_65	TET2	EXON	+	chr4:105275571-105275591	AGUUUUACAUCUAAAUACUU	878
54790_11_68	TET2	EXON	+	chr4:105275577-105275597	ACAUCUAAAUACUUAGGUUA	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	AACCAAAAUAUGCAGGGAGA	882
54790_11_85	TET2	EXON	+	chr4:105275643 -105275663	AGACCAAAUGUACAUCAUGU	883
54790_11_86	TET2	EXON	+	chr4:105275644 -105275664	GACCAAAUGUACAUCAUGUA	884
54790_11_92	TET2	EXON	+	chr4:105275675 -105275695	UCCUUAUCCCACUCAUGAGA	885
54790_11_93	TET2	EXON	+	chr4:105275679 -105275699	UAUCCCACUCAUGAGAUGGA	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	AUGCUUUCCCACACAGCUAA	894
54790_11_121	TET2	EXON	+	chr4:105275875 -105275895	UGCUUUCCCACACAGCUAAU	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	CACAAAUUAAGUGAUGCUAA	898
54790_11_137	TET2	EXON	+	chr4:105275963 -105275983	AUUAAGUGAUGCUAAUGGUC	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	CCCAUGGCAAAAAAGUGAAA	927
54790_11_223	TET2	EXON	+	chr4:105276341 -105276361	CCAUGGCAAAAAAGUGAAAC	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	AUGAUAUCACCCCCUUUUGU	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	CCAUAGCACUUAAUUUUCAC	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	AGGAAUCUUAAAAUACCAUC	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	AGUUAAAAAGAAACAAAAAC	988
54790_11_541	TET2	EXON	+	chr4:105278015 -105278035	AAGAAACAAAAACAGGCAGC	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	CUUUUUGUUCUAAAAAUUCA	996
54790_11_637	TET2	EXON	+	chr4:105278532 -105278552	UGUUUAUGUAAAAUUGUUGU	997
54790_11_643	TET2	EXON	+	chr4:105278556 -105278576	UAAUAAAUAUAUUCUUUGUC	998
54790_11_645	TET2	EXON	+	chr4:105278557 -105278577	AAUAAAUAUAUUCUUUGUCA	999
54790_11_664	TET2	EXON	+	chr4:105278640 -105278660	AACUAAUUUUGUAAAUCUGU	1000
54790_11_679	TET2	EXON	+	chr4:105278680 -105278700	AAAAGCAUUUUAAAAGUUUG	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	CAGAUUGUACAAUUACAUUU	1015
54790_11_748	TET2	EXON	+	chr4:105278978 -105278998	UAAAAUAAAUUCUUAAUCAG	1016
54790_11_751	TET2	EXON	+	chr4:105278981 -105279001	AAUAAAUUCUUAAUCAGAGG	1017
54790_11_753	TET2	EXON	+	chr4:105278988 -105279008	UCUUAAUCAGAGGAGGCCUU	1018
54790_11_754	TET2	EXON	+	chr4:105278989 -105279009	CUUAAUCAGAGGAGGCCUUU	1019
54790_11_757	TET2	EXON	+	chr4:105278998 -105279018	AGGAGGCCUUUGGGUUUUAU	1020
54790_11_762	TET2	EXON	+	chr4:105279017 -105279037	UUGGUCAAAUCUUUGUAAGC	1021
54790_11_772	TET2	EXON	+	chr4:105279052 -105279072	UAAAAAAUUUCUUGAAUUUG	1022
54790_11_799	TET2	EXON	+	chr4:105279173 -105279193	UUUGAUUACUACAUGUGCAU	1023
54790_11_813	TET2	EXON	+	chr4:105279240 -105279260	ACUGUCAUUUGUUAAACUGC	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	AGCUAGUGGUUAACUUGUGU	1041
54790_11_912	TET2	EXON	+	chr4:105279632 -105279652	UUUCAUUUUCAUGAGAUGUU	1042
54790_11_920	TET2	EXON	+	chr4:105279648 -105279668	UGUUUGGUUUAUAAGAUCUG	1043
54790_11_921	TET2	EXON	+	chr4:105279652 -105279672	UGGUUUAUAAGAUCUGAGGA	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	GGAUAAGGACUAACUGGAUU	1054
54790_11_978	TET2	EXON	-	chr4:105275230-105275250	UGGAUAAGGACUAACUGGAU	1055
54790_11_980	TET2	EXON	-	chr4:105275235-105275255	GAGUUUGGAUAAGGACUAAC	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
54790_11_1012	TET2	EXON	-	chr4:105275352-105275372	AGCCCAGGGUAAGGGUUCAU	1064
54790_11_1013	TET2	EXON	-	chr4:105275353-105275373	AAGCCCAGGGUAAGGGUUCA	1065
54790_11_1017	TET2	EXON	-	chr4:105275360-105275380	GAUUCAAAAGCCCAGGGUAA	1066
54790_11_1018	TET2	EXON	-	chr4:105275361-105275381	UGAUUCAAAAGCCCAGGGUA	1067
54790_11_1021	TET2	EXON	-	chr4:105275366-105275386	UAUUCUGAUUCAAAAGCCCA	1068
54790_11_1022	TET2	EXON	-	chr4:105275367-105275387	GUAUUCUGAUUCAAAAGCCC	1069
54790_11_1026	TET2	EXON	-	chr4:105275389-105275409	GCAUUGAUAUGAUGGAUAUU	1070
54790_11_1027	TET2	EXON	-	chr4:105275390-105275410	UGCAUUGAUAUGAUGGAUAU	1071
54790_11_1031	TET2	EXON	-	chr4:105275397-105275417	UUUCCAUUGCAUUGAUAUGA	1072
54790_11_1034	TET2	EXON	-	chr4:105275420-105275440	GGGAGCAGUUGUCCACUGAU	1073

54790_11_1035	TET2	EXON	-	chr4:105275440 -105275460	AGAAUAGGAACCCAGAUAUG	1074
54790_11_1037	TET2	EXON	-	chr4:105275441 -105275461	GAGAAUAGGAACCCAGAUAU	1075
54790_11_1040	TET2	EXON	-	chr4:105275442 -105275462	GGAGAAUAGGAACCCAGAUA	1076
54790_11_1042	TET2	EXON	-	chr4:105275455 -105275475	CGGCUGAGACUGGGGAGAAU	1077
54790_11_1046	TET2	EXON	-	chr4:105275463 -105275483	AGAUCCAUCGGCUGAGACUG	1078
54790_11_1049	TET2	EXON	-	chr4:105275464 -105275484	CAGAUCCAUCGGCUGAGACU	1079
54790_11_1050	TET2	EXON	-	chr4:105275465 -105275485	ACAGAUCCAUCGGCUGAGAC	1080
54790_11_1055	TET2	EXON	-	chr4:105275475 -105275495	GGAUACCUAUACAGAUCCAU	1081
54790_11_1058	TET2	EXON	-	chr4:105275496 -105275516	UUAGACAGAGGGUCUUGGCU	1082
54790_11_1060	TET2	EXON	-	chr4:105275501 -105275521	UGAGCUUAGACAGAGGGUCU	1083
54790_11_1061	TET2	EXON	-	chr4:105275507 -105275527	GUAGACUGAGCUUAGACAGA	1084
54790_11_1062	TET2	EXON	-	chr4:105275508 -105275528	GGUAGACUGAGCUUAGACAG	1085
54790_11_1067	TET2	EXON	-	chr4:105275529 -105275549	UGGUAAAGUGUAUGGAUGGG	1086
54790_11_1068	TET2	EXON	-	chr4:105275532 -105275552	GGCUGGUAAAGUGUAUGGAU	1087
54790_11_1069	TET2	EXON	-	chr4:105275533 -105275553	UGGCUGGUAAAGUGUAUGGA	1088
54790_11_1072	TET2	EXON	-	chr4:105275537 -105275557	ACCUUGGCUGGUAAAGUGUA	1089
54790_11_1075	TET2	EXON	-	chr4:105275549 -105275569	GGCUAUUUCCAAACCUUGGC	1090
54790_11_1076	TET2	EXON	-	chr4:105275553 -105275573	CUCUGGCUAUUUCCAAACCU	1091
54790_11_1079	TET2	EXON	-	chr4:105275570 -105275590	AGUAUUUAGAUGUAAAACUC	1092
54790_11_1085	TET2	EXON	-	chr4:105275606 -105275626	AACCAUCUCCCUGCAUAUUU	1093
54790_11_1089	TET2	EXON	-	chr4:105275641 -105275661	AUGAUGUACAUUUGGUCUAA	1094
54790_11_1092	TET2	EXON	-	chr4:105275649 -105275669	UUCCCUACAUGAUGUACAUU	1095
54790_11_1093	TET2	EXON	-	chr4:105275676 -105275696	AUCUCAUGAGUGGGAUAAGG	1096
54790_11_1095	TET2	EXON	-	chr4:105275679 -105275699	UCCAUCUCAUGAGUGGGAUA	1097

54790_11_1097	TET2	EXON	-	chr4:105275685 -105275705	UGGCCAUCCAUCUCAUGAGU	1098
54790_11_1098	TET2	EXON	-	chr4:105275686 -105275706	GUGGCCAUCCAUCUCAUGAG	1099
54790_11_1102	TET2	EXON	-	chr4:105275705 -105275725	UAGAGGUGGCUCCCAUGAAG	1100
54790_11_1105	TET2	EXON	-	chr4:105275719 -105275739	AUUGGGUGGUAAUCUAGAGG	1101
54790_11_1107	TET2	EXON	-	chr4:105275722 -105275742	CAGAUUGGGUGGUAAUCUAG	1102
54790_11_1111	TET2	EXON	-	chr4:105275733 -105275753	UUUGGAUUGCUCAGAUUGGG	1103
54790_11_1112	TET2	EXON	-	chr4:105275736 -105275756	AUGUUUGGAUUGCUCAGAUU	1104
54790_11_1113	TET2	EXON	-	chr4:105275737 -105275757	CAUGUUUGGAUUGCUCAGAU	1105
54790_11_1120	TET2	EXON	-	chr4:105275751 -105275771	CCAUUUUUAUAGUCCAUGUU	1106
54790_11_1125	TET2	EXON	-	chr4:105275787 -105275807	UAGUUAUGGAUUAUGUGAGA	1107
54790_11_1129	TET2	EXON	-	chr4:105275801 -105275821	CCGGAGCUGCACUGUAGUUA	1108
54790_11_1133	TET2	EXON	-	chr4:105275820 -105275840	AGAGAGCUGUUGAACAUGCC	1109
54790_11_1144	TET2	EXON	-	chr4:105275848 -105275868	CUCCUUGUUUUGGAGAUGCA	1110
54790_11_1145	TET2	EXON	-	chr4:105275849 -105275869	UCUCCUUGUUUUGGAGAUGC	1111
54790_11_1148	TET2	EXON	-	chr4:105275858 -105275878	GCAUGUCAUUCUCCUUGUUU	1112
54790_11_1154	TET2	EXON	-	chr4:105275884 -105275904	UGAUAACCCAUUAGCUGUGU	1113
54790_11_1155	TET2	EXON	-	chr4:105275885 -105275905	UUGAUAACCCAUUAGCUGUG	1114
54790_11_1161	TET2	EXON	-	chr4:105275916 -105275936	GUUCUAUCAUGGUUAAGAGC	1115
54790_11_1165	TET2	EXON	-	chr4:105275927 -105275947	GGACACAAGCAGUUCUAUCA	1116
54790_11_1169	TET2	EXON	-	chr4:105275948 -105275968	UUAAUUUGUGUAAGCCUCCU	1117
54790_11_1175	TET2	EXON	-	chr4:105275997 -105276017	ACACCCUGGACUAGUGCCAA	1118
54790_11_1176	TET2	EXON	-	chr4:105276011 -105276031	CUGCACCAGAAGCCACACCC	1119
54790_11_1182	TET2	EXON	-	chr4:105276081 -105276101	ACGGCCACUCCCCCAAUGUC	1120
54790_11_1186	TET2	EXON	-	chr4:105276100 -105276120	UGACCCAUGAGUUGGAGCCA	1121

54790_11_1188	TET2	EXON	-	chr4:105276108 -105276128	AUGAGAAUUGACCCAUGAGU	1122
54790_11_1200	TET2	EXON	-	chr4:105276157 -105276177	GGGAUUCUUUAAAGGGGUUG	1123
54790_11_1202	TET2	EXON	-	chr4:105276163 -105276183	CCUAUUGGGAUUCUUUAAAG	1124
54790_11_1203	TET2	EXON	-	chr4:105276164 -105276184	UCCUAUUGGGAUUCUUUAAA	1125
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
54790_11_1211	TET2	EXON	-	chr4:105276191 -105276211	AGACGAGGGAGAUCCUGGUG	1129
54790_11_1212	TET2	EXON	-	chr4:105276192 -105276212	AAGACGAGGGAGAUCCUGGU	1130
54790_11_1214	TET2	EXON	-	chr4:105276193 -105276213	AAAGACGAGGGAGAUCCUGG	1131
54790_11_1216	TET2	EXON	-	chr4:105276196 -105276216	GUAAAAGACGAGGGAGAUCC	1132
54790_11_1219	TET2	EXON	-	chr4:105276205 -105276225	CUUAUGCUGGUAAAAGACGA	1133
54790_11_1221	TET2	EXON	-	chr4:105276206 -105276226	UCUUAUGCUGGUAAAAGACG	1134
54790_11_1228	TET2	EXON	-	chr4:105276218 -105276238	GCUCAUUCAUGCUCUUAUGC	1135
54790_11_1230	TET2	EXON	-	chr4:105276240 -105276260	CAAAGAGCCAAGCCAUGUUU	1136
54790_11_1241	TET2	EXON	-	chr4:105276268 -105276288	ACGGGCUUUUUCAGCCAUUU	1137
54790_11_1246	TET2	EXON	-	chr4:105276286 -105276306	ACACUCUUCCUCUUUCUCAC	1138
54790_11_1247	TET2	EXON	-	chr4:105276287 -105276307	CACACUCUUCCUCUUUCUCA	1139
54790_11_1251	TET2	EXON	-	chr4:105276320 -105276340	AUUUCUGAGGCACAUAGUCU	1140
54790_11_1252	TET2	EXON	-	chr4:105276321 -105276341	GAUUUCUGAGGCACAUAGUC	1141
54790_11_1260	TET2	EXON	-	chr4:105276333 -105276353	UUUUUGCCAUGGGAUUUCUG	1142
54790_11_1263	TET2	EXON	-	chr4:105276343 -105276363	CCGUUUCACUUUUUUGCCAU	1143
54790_11_1265	TET2	EXON	-	chr4:105276344 -105276364	CCCGUUUCACUUUUUUGCCA	1144
54790_11_1269	TET2	EXON	-	chr4:105276369 -105276389	GAAGUUUCAUGUGGCUCAGC	1145

54790_11_1270	TET2	EXON	-	chr4:105276378 -105276398	GUGGGCUCUGAAGUUUCAUG	1146
54790_11_1273	TET2	EXON	-	chr4:105276396 -105276416	UUGAUGAAACGCAGGUAAGU	1147
54790_11_1274	TET2	EXON	-	chr4:105276397 -105276417	CUUGAUGAAACGCAGGUAAG	1148
54790_11_1277	TET2	EXON	-	chr4:105276404 -105276424	CAAGAGACUUGAUGAAACGC	1149
54790_11_1281	TET2	EXON	-	chr4:105276427 -105276447	GGUCACGGACAUGGUCCUUU	1150
54790_11_1282	TET2	EXON	-	chr4:105276436 -105276456	GGAGUCUGUGGUCACGGACA	1151
54790_11_1284	TET2	EXON	-	chr4:105276442 -105276462	UACUGUGGAGUCUGUGGUCA	1152
54790_11_1286	TET2	EXON	-	chr4:105276448 -105276468	UGUAGUUACUGUGGAGUCUG	1153
54790_11_1288	TET2	EXON	-	chr4:105276457 -105276477	AUAUGGAGAUGUAGUUACUG	1154
54790_11_1290	TET2	EXON	-	chr4:105276474 -105276494	GUGACCCGAGUGAAGGCAUA	1155
54790_11_1294	TET2	EXON	-	chr4:105276481 -105276501	AGGCCCUGUGACCCGAGUGA	1156
54790_11_1297	TET2	EXON	-	chr4:105276501 -105276521	UAUCAUAUAUAUCUGUUGUA	1157
54790_11_1300	TET2	EXON	-	chr4:105276527 -105276547	GUGAGGUAACCAACAAAAGG	1158
54790_11_1301	TET2	EXON	-	chr4:105276528 -105276548	AGUGAGGUAACCAACAAAAG	1159
54790_11_1303	TET2	EXON	-	chr4:105276529 -105276549	AAGUGAGGUAACCAACAAAA	1160
54790_11_1305	TET2	EXON	-	chr4:105276530 -105276550	CAAGUGAGGUAACCAACAAA	1161
54790_11_1310	TET2	EXON	-	chr4:105276544 -105276564	GGUUGUGGUCUUUUCAAGUG	1162
54790_11_1312	TET2	EXON	-	chr4:105276559 -105276579	UACUACUGACAGGUUGGUUG	1163
54790_11_1313	TET2	EXON	-	chr4:105276565 -105276585	GAACUAUACUACUGACAGGU	1164
54790_11_1314	TET2	EXON	-	chr4:105276569 -105276589	AUGAGAACUAUACUACUGAC	1165
54790_11_1331	TET2	EXON	-	chr4:105276646 -105276666	CUUUUGUUGCUGGUGAGCUG	1166
54790_11_1334	TET2	EXON	-	chr4:105276656 -105276676	AAGAUAACCUCUUUUGUUGC	1167
54790_11_1336	TET2	EXON	-	chr4:105276680 -105276700	CCAGUGAAAAUUAAGUGCUA	1168
54790_11_1339	TET2	EXON	-	chr4:105276705 -105276725	GAUGCCAUCUGUGACCACUU	1169

54790_11_1344	TET2	EXON	-	chr4:105276706 -105276726	AGAUGCCAUCUGUGACCACU	1170
54790_11_1354	TET2	EXON	-	chr4:105276738 -105276758	UCUUUUUGCAUAGAAUGCUU	1171
54790_11_1363	TET2	EXON	-	chr4:105276780 -105276800	GUGUUUAAAAAUGUAAAUUG	1172
54790_11_1370	TET2	EXON	-	chr4:105276841 -105276861	AGAGUUGUAAGCGGGGGGGG	1173
54790_11_1371	TET2	EXON	-	chr4:105276842 -105276862	UAGAGUUGUAAGCGGGGGGG	1174
54790_11_1374	TET2	EXON	-	chr4:105276843 -105276863	GUAGAGUUGUAAGCGGGGGG	1175
54790_11_1376	TET2	EXON	-	chr4:105276844 -105276864	UGUAGAGUUGUAAGCGGGGG	1176
54790_11_1378	TET2	EXON	-	chr4:105276845 -105276865	GUGUAGAGUUGUAAGCGGGG	1177
54790_11_1379	TET2	EXON	-	chr4:105276846 -105276866	UGUGUAGAGUUGUAAGCGGG	1178
54790_11_1382	TET2	EXON	-	chr4:105276847 -105276867	AUGUGUAGAGUUGUAAGCGG	1179
54790_11_1383	TET2	EXON	-	chr4:105276848 -105276868	GAUGUGUAGAGUUGUAAGCG	1180
54790_11_1386	TET2	EXON	-	chr4:105276849 -105276869	AGAUGUGUAGAGUUGUAAGC	1181
54790_11_1388	TET2	EXON	-	chr4:105276850 -105276870	CAGAUGUGUAGAGUUGUAAG	1182
54790_11_1394	TET2	EXON	-	chr4:105276876 -105276896	AAACUUGAUAUUAUUAAAAG	1183
54790_11_1406	TET2	EXON	-	chr4:105276963 -105276983	AUCAUAUAUUCAGCACCAGA	1184
54790_11_1440	TET2	EXON	-	chr4:105277160 -105277180	AUAGCAUCUUGAUGAUAUAA	1185
54790_11_1444	TET2	EXON	-	chr4:105277192 -105277212	CCCCUAUUAUUCAAGGGCAC	1186
54790_11_1446	TET2	EXON	-	chr4:105277198 -105277218	AAGGUACCCCUAUUAUUCAA	1187
54790_11_1447	TET2	EXON	-	chr4:105277199 -105277219	AAAGGUACCCCUAUUAUUCA	1188
54790_11_1451	TET2	EXON	-	chr4:105277217 -105277237	UGAUAAAAACUUGAAUGAAA	1189
54790_11_1457	TET2	EXON	-	chr4:105277246 -105277266	AACUAAGCUUGUGUAAGAAU	1190
54790_11_1463	TET2	EXON	-	chr4:105277293 -105277313	ACUGGAUGAGCAAAAUCCAG	1191
54790_11_1469	TET2	EXON	-	chr4:105277311 -105277331	UUGUCCUACAAGGACUUCAC	1192
54790_11_1471	TET2	EXON	-	chr4:105277321 -105277341	AUAUCGUUUAUUGUCCUACA	1193

54790_11_1478	TET2	EXON	-	chr4:105277432 -105277452	UGUUCAAGUUGUCAAAGCUU	1194
54790_11_1501	TET2	EXON	-	chr4:105277563 -105277583	AUUGCUCAUCAGCAGAUGCA	1195
54790_11_1505	TET2	EXON	-	chr4:105277591 -105277611	UUAACUGGCUGUGUUAAAAA	1196
54790_11_1507	TET2	EXON	-	chr4:105277606 -105277626	AGCCCCAUGGUGGAUUUAAC	1197
54790_11_1509	TET2	EXON	-	chr4:105277616 -105277636	GAAUCCAGUAAGCCCCAUGG	1198
54790_11_1512	TET2	EXON	-	chr4:105277619 -105277639	CUUGAAUCCAGUAAGCCCCA	1199
54790_11_1517	TET2	EXON	-	chr4:105277655 -105277675	GCACCAGAAAACAUGUUUUG	1200
54790_11_1547	TET2	EXON	-	chr4:105277827 -105277847	UAUGAUCCAUAAAAUAGCAU	1201
54790_11_1553	TET2	EXON	-	chr4:105277879 -105277899	GUACAUAAUUAUCAACACAA	1202
54790_11_1558	TET2	EXON	-	chr4:105277934 -105277954	GCUCAAUCCUCUUGUUAAAG	1203
54790_11_1565	TET2	EXON	-	chr4:105277966 -105277986	CCUGUUUAUUCAUCUUAUGC	1204
54790_11_1574	TET2	EXON	-	chr4:105277996 -105278016	UUUUAACUGACAGAUUCACA	1205
54790_11_1613	TET2	EXON	-	chr4:105278246 -105278266	CAUUAUGAUAUAUUUGUAGC	1206
54790_11_1621	TET2	EXON	-	chr4:105278304 -105278324	UGUUGAGGUAUAUGACAAGU	1207
54790_11_1624	TET2	EXON	-	chr4:105278319 -105278339	CUAUUGCCAAACUAGUGUUG	1208
54790_11_1630	TET2	EXON	-	chr4:105278373 -105278393	AAGGACUUGGAAAAAAAUGA	1209
54790_11_1636	TET2	EXON	-	chr4:105278386 -105278406	UAACAAUAAAAAAAAAGGACU	1210
54790_11_1643	TET2	EXON	-	chr4:105278392 -105278412	UUUUUUUAACAAUAAAAAAA	1211
54790_11_1647	TET2	EXON	-	chr4:105278423 -105278443	AGAAAUCAAGUAUUGAAAAA	1212
54790_11_1658	TET2	EXON	-	chr4:105278470 -105278490	CCUGAAUUUUUAGAACAAAA	1213
54790_11_1667	TET2	EXON	-	chr4:105278513 -105278533	ACAGGUGACAUGUUGGCAUA	1214
54790_11_1669	TET2	EXON	-	chr4:105278514 -105278534	CACAGGUGACAUGUUGGCAU	1215
54790_11_1674	TET2	EXON	-	chr4:105278520 -105278540	CAUAAACACAGGUGACAUGU	1216
54790_11_1675	TET2	EXON	-	chr4:105278531 -105278551	CAACAAUUUUACAUAAACAC	1217

54790_11_1682	TET2	EXON	-	chr4:105278589 -105278609	AGAAGGGAUUCAAAAUAAAA	1218
54790_11_1683	TET2	EXON	-	chr4:105278590 -105278610	UAGAAGGGAUUCAAAAUAAA	1219
54790_11_1685	TET2	EXON	-	chr4:105278605 -105278625	CAUGUACAAGUAAAAUAGAA	1220
54790_11_1687	TET2	EXON	-	chr4:105278606 -105278626	ACAUGUACAAGUAAAAUAGA	1221
54790_11_1733	TET2	EXON	-	chr4:105278813 -105278833	GAGAGUUACAAGUAAGUCUC	1222
54790_11_1739	TET2	EXON	-	chr4:105278855 -105278875	UAUGUACCUUCAGUGAUUAC	1223
54790_11_1746	TET2	EXON	-	chr4:105278880 -105278900	CUGUUUAAGGGAAGUCCACA	1224
54790_11_1749	TET2	EXON	-	chr4:105278892 -105278912	GUAGGUGUUUGCCUGUUUAA	1225
54790_11_1751	TET2	EXON	-	chr4:105278893 -105278913	UGUAGGUGUUUGCCUGUUUA	1226
54790_11_1754	TET2	EXON	-	chr4:105278910 -105278930	CUGUUGCACACCAUACCUGU	1227
54790_11_1758	TET2	EXON	-	chr4:105278953 -105278973	UAGUAAGCAAAAAUGUAUUU	1228
54790_11_1768	TET2	EXON	-	chr4:105279007 -105279027	AUUUGACCAAUAAAACCCAA	1229
54790_11_1784	TET2	EXON	-	chr4:105279084 -105279104	UUUUGGAAAUGUUUGCAAAU	1230
54790_11_1789	TET2	EXON	-	chr4:105279101 -105279121	GUAAGCAAAGCAAACAUUUU	1231
54790_11_1792	TET2	EXON	-	chr4:105279127 -105279147	CAAAAAACAUUAAAAUCAUG	1232
54790_11_1796	TET2	EXON	-	chr4:105279154 -105279174	AUGUUUGGGGCUAGAUAUUA	1233
54790_11_1797	TET2	EXON	-	chr4:105279167 -105279187	AUGUAGUAAUCAAAUGUUUG	1234
54790_11_1798	TET2	EXON	-	chr4:105279168 -105279188	CAUGUAGUAAUCAAAUGUUU	1235
54790_11_1800	TET2	EXON	-	chr4:105279169 -105279189	ACAUGUAGUAAUCAAAUGUU	1236
54790_11_1803	TET2	EXON	-	chr4:105279212 -105279232	CAGAAAUCAAAUAUUAAGAA	1237
54790_11_1809	TET2	EXON	-	chr4:105279240 -105279260	GCAGUUUAACAAAUGACAGU	1238
54790_11_1814	TET2	EXON	-	chr4:105279266 -105279286	ACUAUACUUCCUGUUCUUGU	1239
54790_11_1832	TET2	EXON	-	chr4:105279376 -105279396	CCAUUCAUUCUAACUGAGGC	1240
54790_11_1833	TET2	EXON	-	chr4:105279380 -105279400	CUUUCCAUUCAUUCUAACUG	1241

54790_11_1841	TET2	EXON	-	chr4:105279449 -105279469	CUCAGAAGCAUUUUCAAGUA	1242
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 incorproated 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 nucleci 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 continguous 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 continguous 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 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 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) 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 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 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 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., 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 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.

Table 4

	SHRNA_NAME	Target sequence of shRNA	Nucleic Acid encoding Tet2 shRNA
TET2	TET2-3838_76472_insert (TET2 shRNA #1)	CACATGGCGTTTATCCAGAAT (SEQ ID NO: 1244)	CACATGGCGTTTATCCAGAATCTCGAGATTCTGGATAAACGCCATGTGTTTTTTGAATTCGCACCAGCACGCTACGCACACACAGTACACACACTGACGTTTCGCCGTCTTC (SEQ ID NO: 1253)
TET2	TET2_NM_017628.4_25616_concept (TET2 shRNA #2)	CAGATGCACAGGCCAATTAAG (SEQ ID NO: 1245)	GAAGACGCACCGGCAGATGTACAGGCTAATTAAGGTTAATATTCATAGCCTTAATTGGCCTGTGCATCTGTTTTTTGAATTCGCACCAGCACGCTACGCAACACGTCAACCAGTGTCAGTGTTTCGCCGT (SEQ ID NO: 1254)
TET2	TET2_NM_017628.4_25625_concept (TET2 shRNA #3)	GAGCTGCTGAATTCAACTAGA (SEQ ID NO: 1246)	GAAGACGCACCGGGAGCTGCTGAATTCAATTAGAGTTAATATTCATAGCTCTAGTTGAATTCAGCAGCTCTTTTTTGAATTCGCACCAGCACGCTACGCATGCAGTCAACCAGTGTCAACCATTCGCCGT (SEQ ID NO: 1255)
TET2	TET2-6571_76471_target (TET2 shRNA #4)	CAGATCGCCATAACATAAATA (SEQ ID NO: 1247)	CAGATCGCCATAACATAAATACTCGAGTATTTATGTTATGGCGATCTGTTTTTTGAATTCGCACCAGCACGCTACGCATGACCAGTACACACACTGCATGTTCGCCGTCTTC (SEQ ID NO: 1256)
TET2	TET2_NM_017628.4_25619_target (TET2 shRNA #5)	GACCATGGAGCAGCATCTGAA (SEQ ID NO: 1248)	GAAGACGCACCGGGACCATGGAGTAGCATTTGAAGTTAATATTCATAGCTTCAGATGCTGCTCCATGGTCTTTTTTGAATTCGCACCAGCACGCTACGCATGGTGTCAACCAGTGTCAGTTGTTCGCCGT (SEQ ID NO: 1257)
TET2	TET2 shRNA #6	GCCAAGTCATTATTTGACCAT (SEQ ID NO: 1249)	GCCAAGTCATTATTTGACCATCTCGAGATGGTCAAATAATGACTTGGCTTTTTTGA (SEQ ID NO: 1258)
TET2	TET2 shRNA #7	CCTCAGAGATATTGTGGGTTT (SEQ ID NO: 1250)	CCTCAGAGATATTGTGGGTTTCTCGAGAAACCCACAATATCTCTGAGGTTTTTTGA (SEQ ID NO: 1259)

TET2	TET2 shRNA #8	GGGTAAGCCAAGAAAGAAA (SEQ ID NO: 1251)	GGGTAAGCCAAGAAAGAAACTCGAGTTTCTTTCTTGGCTTACCCTTTTTTGA (SEQ ID NO: 1260)
TET2	TET2 8 long (TET2 shRNA #9)	GGGTAAGCCAAGAAAGAAA (SEQ ID NO: 1252)	GAAGACGCACCGGGGGTAAGCCAAGAAAGAAAGTTAATATTCATAGCTTTCTTTCTTGGCTTACCCTTTTTTGAATTCGCACCAGCACGCTACGCAACACGTCAACCAGTGTCAGTGTTTCGCCGT (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 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 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 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 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 moledule modualtors (e.g., inhibitors) are described below.

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 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-1β converting enzyme (ICE). See, e.g., US 5,985,863, herein incorporated by reference in its entirety.

NOTCH2 inhibitors

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-C_{1-3}-alkoxygliotoxin, 6-C_{2-3}-acyloxy-gliotoxin, 6-dihydro-gliotoxin, 6-dihydroxy-gliotoxin, 6-[(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.

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)-acetylamino]-N-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-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

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.

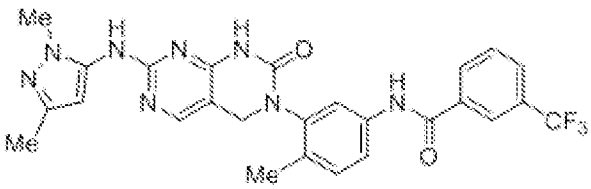
Tet inhibitors

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

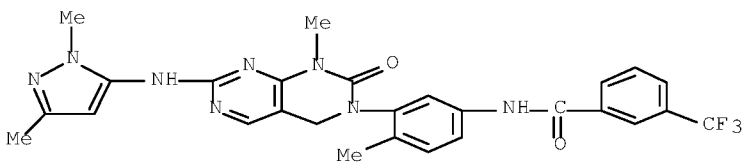
In embodiments, a Tet2 inhibitor is a small molecule that inhibits Tet2 expression and/or function. For example, a Tet2 inhibitor according to the present invention is 2-hydroxyglutarate (CAS #2889-31-8).

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

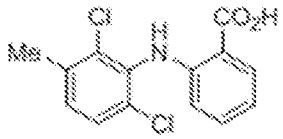


Pluripotin SC1

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

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[(αS,2S)-α-amino-η-oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-L-prolyl] (Cyl-1); Cyclo[(αS,2S)-α-amino-η-oxo-2-oxiraneoctanoyl-O-methyl-D-tyrosyl-L-isoleucyl-(2S)-2-piperidinecarbonyl] (Cyl-2); Cyclic[L-alanyl-D-alanyl-(2S)-η-oxo-L-α-aminooxiraneoctanoyl-D-prolyl] (HC-toxin); Cyclo[(αS,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 (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.

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 modualtors (e.g., inhibitors) are described below.

IFN-γ inhibitors

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 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 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.

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.

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-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

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

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 modualtors (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 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). 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).

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 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

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.

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 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, 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 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.

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) 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 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 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 molecule comprises an antibody fragment 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 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-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 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 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 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 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.

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 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 TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGA GAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTC GCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTG GTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT GCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGAT CCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCT TGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCT GGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACC TTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTA AAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGAT AGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCG GTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAG CGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTG GTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAG ATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAG GACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACAC AAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTG ACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGT TCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGG GGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGA CTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA ATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTC AGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTG 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 PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGKM	
7	Ig4 hinge (na)	GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCC GAGTTCCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCCAAG CCCAAGGACACCCTGATGATCAGCCGGACCCCCGAGGTGACC TGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAG TTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAG ACCAAGCCCCGGGAGGAGCAGTTCAATAGCACCTACCGGGTG GTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGC AAGGAATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCCAG CAGCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCTC GGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAGGAGA TGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCT TCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCC AGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACA AGAGCCGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTGA TGCACGAGGCCCTGCACAACCACTACACCCAGAAGAGCCTGA GCCTGTCCCTGGGCAAGATG	103
8	IgD hinge (aa)	RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEE KKKEKEKEEQEERETKTPECPSHTQPLGVYLLTPAVQDLWLRD KATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNGS QSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQA PVKLSLNLLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREV NTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYTCVVSH EDSRTLLNASRSLEVSYVTDH	47
9	IgD hinge (na)	AGGTGGCCCGAAAGTCCCAAGGCCCAGGCATCTAGTGTTCCT ACTGCACAGCCCCAGGCAGAAGGCAGCCTAGCCAAAGCTACT ACTGCACCTGCCACTACGCGCAATACTGGCCGTGGCGGGGAG GAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGA GGGAGACCAAGACCCCTGAATGTCCATCCCATACCCAGCCGC TGGGCGTCTATCTCTTGACTCCCGCAGTACAGGACTTGTGGCT TAGAGATAAGGCCACCTTTACATGTTTCGTCGTGGGCTCTGAC CTGAAGGATGCCCATTTGACTTGGGAGGTTGCCGGAAAGGTA CCCACAGGGGGGGTTGAGGAAGGGTTGCTGGAGCGCCATTCC AATGGCTCTCAGAGCCAGCACTCAAGACTCACCCTTCCGAGA TCCCTGTGGAACGCCGGGACCTCTGTCACATGTACTCTAAATC ATCCTAGCCTGCCCCCACAGCGTCTGATGGCCCTTAGAGAGC CAGCCGCCCAGGCACCAGTTAAGCTTAGCCTGAATCTGCTCG CCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCG AAGTGTCCGGCTTTAGCCCGCCCAACATCTTGCTCATGTGGCT GGAGGACCAGCGAGAAGTGAACACCAGCGGCTTCGCTCCAG CCCGGCCCCCACCCCAGCCGGGTTCTACCACATTCTGGGCCT GGAGTGTCTTAAGGGTCCCAGCACCACCTAGCCCCCAGCCAG CCACATACACCTGTGTTGTGTCCCATGAAGATAGCAGGACCC TGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCTACGTGACTGA CCATT	48
10	GS hinge/linker (aa)	GGGGSGGGGS	49
11	GS hinge/linker (na)	GGTGGCGGAGGTTCTGGAGGTGGAGGTTCC	50
12	CD8TM (aa)	IYIWAPLAGTCGVLLLSLVITLYC	15
13	CD8 TM (na)	ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTC TCCTGTCACTGGTTATCACCCTTTACTGC	56
14	4-1BB	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	16

	intracellular domain (aa)		
15	4-1BB intracellular domain (na)	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCA TTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGT AGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACT G	60
16	CD27 (aa)	QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPA CSP	51
17	CD27 (na)	AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAA CATGACTCCCCGCCGCCCCGGGCCCACCCGCAAGCATTACCA 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)	Pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedr sqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptah pspsprpagqfqtlv	
25	PD-1 extracellular domain (na)	Cccggatggtttctggactctccggatcgcccgtggaatcccccaaccttctcaccggcactcttggtt gtgactgagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgctgaact ggtaccgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccggaagatcggtcgcaac cgggacaggattgtcggttccgcgtgactcaactgccgaatggcagagacttccacatgagcgtggt ccgcgctaggcgaaacgactccgggacctacctgtgcggagccatctcgctggcgcctaaggccca aatcaaagagagcttgagggccgaactgagagtgaccgagcgcagagctgaggtgccaactgcac atccatccccatcgcctcggcctgcggggcagtttcagaccctggtc	
26	PD-1 CAR (aa) with signal	Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnw yrmspsnqtdklaafpedrsqpgqdcrfrvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqi keslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptiasqplslrpeacrpaagga vhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpe eeeggcelrvkfsrsadapaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeg lynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr	
27	PD-1 CAR (na)	Atggccctccctgtcactgccctgcttctcccccctcgcactcctgctccacgccgctagaccacccgg atggtttctggactctccggatcgcccgtggaatcccccaaccttctcaccggcactcttggttgtgact	

		gagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgctgaactggtac cgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccggaagatcggtcgcaaccgggga caggattgtcggttccgcgtgactcaactgccgaatggcagagacttccacatgagcgtggtccgcgc taggcgaaacgactccgggacctacctgtgcggagccatctcgctggcgcctaaggcccaaatcaa agagagcttgagggccgaactgagagtgaccgagcgcagagctgaggtgccaactgcacatccatc cccatcgcctcggcctgcggggcagtttcagaccctggtcacgaccactccggcgccgcgcccacc gactccggccccaactatcgcgagccagcccctgtcgctgaggccggaagcatgccgccctgccgc cggaggtgctgtgcatacccggggattggacttcgcatgcgacatctacatttgggctcctctcgccgg aacttgtggcgtgctccttctgtccctggtcatcaccctgtactgcaagcggggtcggaaaaagcttctg tacattttcaagcagcccttcatgaggcccgtgcaaaccacccaggaggaggacggttgctcctgccg gttccccgaagaggaagaaggaggttgcgagctgcgcgtgaagttctcccggagcgccgacgccc ccgcctataagcagggccagaaccagctgtacaacgaactgaacctgggacggcgggaagagtac gatgtgctggacaagcggcgcggccgggaccccgaaatgggcgggaagcctagaagaaagaacc ctcaggaaggcctgtataacgagctgcagaaggacaagatggccgaggcctactccgaaattggga tgaagggagagcggcggaggggaaaggggcacgacggcctgtaccaaggactgtccaccgccac caaggacacatacgatgccctgcacatgcaggcccttcccccctcgc	
28	linker	(Gly-Gly-Gly-Ser)n, where n = 1-10	105
29	linker	(Gly4 Ser)4	106
30	linker	(Gly4 Ser)3	107
31	linker	(Gly3Ser)	108
32	polyA	aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

		aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

		aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

		tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt

		aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa

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 intracellar, 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, 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.

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.

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.

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 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 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 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 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.

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 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 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 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 there of, e.g., single chain TCR, and the like. In some instances, it is beneficial for the antigen binding domain to be derived from the same species in which the CAR will ultimately be used in. For example, for use in humans, it may be beneficial for the antigen binding domain of the CAR to comprise human or humanized residues for the antigen binding domain of an antibody or antibody fragment.

In one embodiment, the CD19 CAR is a CD19 CAR described in US Pat. No. 8,399,645; US 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 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 (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 referenc 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 referenc 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 referenc 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., 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, 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, 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, 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, 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 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) from PCT Publication WO2014/089335 (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)

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., 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 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).

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.

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.

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., Gastoenterology 143(4):1095-1107 (2012).

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 (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 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, 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, 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 again IL-11Ra is a peptide, see, e.g., Huang et al., Cancer Res 72(1):271-281 (2012).

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 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).

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.

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; 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.

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 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 (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).

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, NKIbetaB, or an antibody described in WO2013165940, or 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).

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 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 (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 (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., clinicaltrial.gov/show/NCT02054351.

In one embodiment, an antigen binding domain against TSHR is an antigen binding portion, 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, 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).

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 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).

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 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-2009-02-205500. 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 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.

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 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 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.

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.

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 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.

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 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 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 comprises sets of glycine and serine repeats such as $(Gly_4Ser)n$, where n is a positive integer equal to or greater than 1 (SEQ ID NO:22). In one embodiment, the linker can be $(Gly_4Ser)_4$ (SEQ ID NO:29)

or $(Gly_4Ser)_3$(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 Vα and Vβ 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 intracellar, 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) 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 sulfhdryl 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.*, 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; 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 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 bispecifc, 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 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 futher associated with VL domains to form a series of FVs (or scFvs), as described in, *e.g.*, US5864019; and single chain binding polypeptides with 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, 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, 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, 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 VH can be upstream or downstream of the VL. In some embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_1) upstream of its VL (VL_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_2) upstream of its VH (VH_2), such that the overall bispecific antibody molecule has the arrangement VH_1-VL_1-VL_2-VH_2. In other embodiments, the upstream antibody or antibody fragment (e.g., scFv) is arranged with its VL (VL_1) upstream of its VH (VH_1) and the downstream antibody or antibody fragment (e.g., scFv) is arranged with its VH (VH_2) upstream of its VL (VL_2), such that the overall bispecific antibody molecule has the arrangement VL_1-VH_1-VH_2-VL_2. Optionally, a linker is disposed between the two antibodies or antibody fragments (e.g., scFvs), e.g., between VL_1 and VL_2 if the construct is arranged as VH_1-VL_1-VL_2-VH_2, or between VH_1 and VH_2 if the construct is arranged as VL_1-VH_1-VH_2-VL_2. The linker may be a linker as described herein, e.g., a (Gly_4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6, 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 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, 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, Tm can be measured. Methods for measuring Tm 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 Tm, temperature denaturation and temperature aggregation.

The binding capacity of the mutant scFvs can be determined using assays know 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 (Tm) 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, Tm 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, Tm 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, Tm 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 (Cp) 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 Cp is a hallmark of a denatured or inactive protein composition. The change in heat capacity (ΔCp) 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. 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.

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 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 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 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, 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., 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 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 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 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).

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.

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) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossom 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 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.

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.

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, 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 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 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 REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKM (SEQ ID NO:6). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of GAGAGCAAGTACGGCCCTCCCTGCCCCCCTTGCCCTGCCCCCGAGTTCCTGGGCGGACCC AGCGTGTTCCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCCGGACCCCCGAG GTGACCTGTGTGGTGGTGGACGTGTCCCAGGAGGACCCCGAGGTCCAGTTCAACTGGTA CGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGTTCAAT AGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAA GGAATACAAGTGTAAGGTGTCCAACAAGGGCCTGCCCAGCAGCATCGAGAAAACCATCA GCAAGGCCAAGGGCCAGCCTCGGGAGCCCCAGGTGTACACCCTGCCCCCTAGCCAAGAG GAGATGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTACCCCAGCGA CATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCC CTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCCGGCTGACCGTGGACAAGAGC CGGTGGCAGGAGGGCAACGTCTTTAGCTGCTCCGTGATGCACGAGGCCCTGCACAACCA CTACACCCAGAAGAGCCTGAGCCTGTCCCTGGGCAAGATG (SEQ ID NO:7).

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 RWPESPKAQASSVPTAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPE

CPSHTQPLGVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLE
RHSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLLASS
DPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPP
SPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDH (SEQ ID NO:8). In some embodiments, the hinge or spacer comprises a hinge encoded by a nucleotide sequence of
AGGTGGCCCGAAAGTCCCAAGGCCCAGGCATCTAGTGTTCCTACTGCACAGCCCCAGGC
AGAAGGCAGCCTAGCCAAAGCTACTACTGCACCTGCCACTACGCGCAATACTGGCCGTG
GCGGGGAGGAGAAGAAAAAGGAGAAAGAGAAAGAAGAACAGGAAGAGAGGGAGACCA
AGACCCCTGAATGTCCATCCCATACCCAGCCGCTGGGCGTCTATCTCTTGACTCCCGCAG
TACAGGACTTGTGGCTTAGAGATAAGGCCACCTTTACATGTTTCGTCGTGGGCTCTGACC
TGAAGGATGCCCATTTGACTTGGGAGGTTGCCGGAAAGGTACCCACAGGGGGGGTTGAG
GAAGGGTTGCTGGAGCGCCATTCCAATGGCTCTCAGAGCCAGCACTCAAGACTCACCCTT
CCGAGATCCCTGTGGAACGCCGGGACCTCTGTCACATGTACTCTAAATCATCCTAGCCTG
CCCCCACAGCGTCTGATGGCCCTTAGAGAGCCAGCCGCCCAGGCACCAGTTAAGCTTAG
CCTGAATCTGCTCGCCAGTAGTGATCCCCCAGAGGCCGCCAGCTGGCTCTTATGCGAAGT
GTCCGGCTTTAGCCCGCCCAACATCTTGCTCATGTGGCTGGAGGACCAGCGAGAAGTGA
ACACCAGCGGCTTCGCTCCAGCCCGGCCCCCACCCCAGCCGGGTTCTACCACATTCTGGG
CCTGGAGTGTCTTAAGGGTCCCAGCACCACCTAGCCCCCAGCCAGCCACATACACCTGTG
TTGTGTCCCATGAAGATAGCAGGACCCTGCTAAATGCTTCTAGGAGTCTGGAGGTTTCCT
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.

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 (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 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 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 (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, 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 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, 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 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 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 CGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCG 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 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 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 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 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 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 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.

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. 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 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 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, 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).

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 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.

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, 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 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 comprises an scFv, and the other comprises a camelid 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 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 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, 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 a. 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 incombinations 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.

Malpvtalllplalllhaarppgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcr frvtqlpngrdfhmsvvrarrndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptiasqplslr peacrpaaggavhtrgldfacdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsad apaykqgqnqlynelnlgrreeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdt ydalhmqalppr (SEQ ID NO:26).

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

pgwfldspdrpwnpptfspallvvtegdnatftcsfsntsesfvlnwyrmspsnqtdklaafpedrsqpgqdcrfrvtqlpngrdfhmsvvrar rndsgtylcgaislapkaqikeslraelrvterraevptahpspsprpagqfqtlvtttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfa cdiyiwaplagtcgvlllslvitlyckrgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcelrvkfsrsadapaykqgqnqlynelnlgr reeydvldkrrgrdpemggkprrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstatkdtydalhmqalppr (SEQ 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.

atggccctccctgtcactgccctgcttctccccctcgcactcctgctccacgccgctagaccacccggatggtttctggactctccggatcgcccgtg gaatcccccaaccttctcaccggcactcttggttgtgactgagggcgataatgcgaccttcacgtgctcgttctccaacacctccgaatcattcgtgct gaactggtaccgcatgagcccgtcaaaccagaccgacaagctcgccgcgtttccggaagatcggtcgcaaccgggacaggattgtcggttccgc gtgactcaactgccgaatggcagagacttccacatgagcgtggtccgcgctaggcgaaacgactccgggacctacctgtgcggagccatctcgct ggcgcctaaggcccaaatcaaagagagcttgagggccgaactgagagtgaccgagcgcagagctgaggtgccaactgcacatccatccccatc gcctcggcctgcggggcagtttcagaccctggtcacgaccactccggcgccgcgcccaccgactccggccccaactatcgcgagccagccccct gtcgctgaggccggaagcatgccgccctgccgccggaggtgctgtgcatacccggggattggacttcgcatgcgacatctacatttgggctcctct cgccggaacttgtggcgtgctccttctgtccctggtcatcaccctgtactgcaagcggggtcggaaaaagcttctgtacattttcaagcagcccttcat gaggcccgtgcaaaccacccaggaggaggacggttgctcctgccggttccccgaagaggaagaaggaggttgcgagctgcgcgtgaagttctc ccggagcgccgacgcccccgcctataagcagggccagaaccagctgtacaacgaactgaacctgggacggcgggaagagtacgatgtgctgg acaagcggcgcggccgggaccccgaaatgggcgggaagcctagaagaaagaaccctcaggaaggcctgtataacgagctgcagaaggacaa gatggccgaggcctactccgaaattgggatgaagggagagcggcggaggggaaaggggcacgacggcctgtaccaaggactgtccaccgcc accaaggacacatacgatgccctgcacatgcaggcccttcccccctcgc (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 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 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 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 Egnl. 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, 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 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 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 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 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 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 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:

D V P D Y A S L G G P S S P K K K R K V S R G V Q V E T I S P G D G R T F P K R G Q T C V V H Y T G M L E D G K K F D S S R D R N K P F K F M L G K Q E V I R G W E E G V A Q M S V G Q R A K L T I S P D Y A Y G A T G H P G I I P P H A T L V F D V E L L K L E T S Y (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:

V Q V E T I S P G D G R T F P K R G Q T C V V H Y T G M L E D G K K F D S S R D R N K P F K F M L G K Q E V I R G W E E G V A Q M S V G Q R A K L T I S P D Y A Y G A T G H P G I I P P H A T L V F D V E L L K L E T S (SEQ ID NO:55)

The amino acid sequence of FRB is as follows:

ILWHEMWHEG LEEASRLYFG ERNVKGMFEV LEPLHAMMER GPQTLKETSF NQAYGRDLME AQEWCRKYMK SGNVKDLTQA WDLYYHVFRR ISK (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 ETSFNQAYGRDL**X**MEAQEWCRKYMKSGNVKDL**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

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, 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. 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

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 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

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 (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.

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, synthetic DNA sequence or any other appropriate source of DNA. The desired temple 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); 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 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 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 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 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 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 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.

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 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 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 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 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

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

5' caps on 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 (Amaxa Nucleofector-II (Amaxa Biosystems, Cologne, Germany)), (ECM 830 (BTX) (Harvard Instruments, Boston, Mass.) or the Gene Pulser II (BioRad, Denver, Colo.), Multiporator (Eppendort, 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.

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 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, 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, 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 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 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, 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, 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.

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 gens 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 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 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 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, 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 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. 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 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 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-1α, 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 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 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 40 (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-1α promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the 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 metallothionine promoter, a glucocorticoid 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 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 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 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 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 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 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 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 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 PERCOLL™ 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 Miltenyi™. In one embodiment, the ratio of cells to CD25 depletion reagent is 1e7 cells to 20 uL, or 1e7 cells to15 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 10^9 CD25+ T cells. In other aspects, the population of immune effector cells to be depleted include about 1 x 10^9 to 1x 10^{10} CD25+ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2 x 10^9 T regulatory cells, e.g., CD25+ cells, or less (e.g., 1 x 10^9, 5 x 10^8, 1 x 10^8, 5 x 10^7, 1 x 10^7, 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 there, 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 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 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, TNFα, IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other 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 (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 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 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 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 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. 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 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 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 diaglycerol kinase (DGK)-deficient. DGK-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.

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.

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).

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 1 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 mehod 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.

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 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. (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.

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 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 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. (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 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 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., 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 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 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 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
PPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSLRPSLTGARRLVETIFLGSRPW
MPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVTPAAGVCAREKPQ

GSVAAPEEEDTDPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERRFLRNTKK
FISLGKHAKLSLQELTWKMSVRGCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVV
ELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRELSEAEVRQHREARPALL
TSRLRFIPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASV
LGLDDIHRAWRTFVLRVRAQDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRR
YAVVQKAAHGHVRKAFKSHVSTLTDLQPYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSG
LFDVFLRFMCHHAVRIRGKSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGIRRDGLLLRLV
DDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLRKTVVNFPVEDEALGGTAFVQMPAHGLF
PWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRRKLFGVLRLKCHSLFLDLQ
VNSLQTVCTNIYKILLLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAKNAG
MSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTA
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 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 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
 121 tgccgctggc cacgttcgtg cggcgcctgg ggccccaggg ctggcggctg gtgcagcgcg
 181 gggacccggc ggctttccgc gcgctggtgg cccagtgcct ggtgtgcgtg ccctgggacg
 241 cacggccgcc ccccgccgcc ccctccttcc gccaggtgtc ctgcctgaag gagctggtgg
 301 cccgagtgct gcagaggctg tgcgagcgcg gcgcgaagaa cgtgctggcc ttcggcttcg
 361 cgctgctgga cggggcccgc gggggccccc ccgaggcctt caccaccagc gtgcgcagct
 421 acctgcccaa cacggtgacc gacgcactgc gggggagcgg ggcgtggggg ctgctgttgc
 481 gccgcgtggg cgacgacgtg ctggttcacc tgctggcacg ctgcgcgctc tttgtgctgg
 541 tggctcccag ctgcgcctac caggtgtgcg ggccgccgct gtaccagctc ggcgctgcca
 601 ctcaggcccg gcccccgcca cacgctagtg gaccccgaag gcgtctggga tgcgaacggg
 661 cctggaacca tagcgtcagg gaggccgggg tccccctggg cctgccagcc ccgggtgcga
 721 ggaggcgcgg gggcagtgcc agccgaagtc tgccgttgcc caagaggccc aggcgtggcg
 781 ctgcccctga gccggagcgg acgcccgttg ggcaggggtc ctgggcccac ccgggcagga
 841 cgcgtggacc gagtgaccgt ggtttctgtg tggtgtcacc tgccagaccc gccgaagaag
 901 ccacctcttt ggagggtgcg ctctctggca cgcgccactc ccacccatcc gtgggccgcc
 961 agcaccacgc gggccccccа tccacatcgc ggccaccacg tccctgggac acgccttgtc
1021 ccccggtgta cgccgagacc aagcacttcc tctactcctc aggcgacaag gagcagctgc
1081 ggccctcctt cctactcagc tctctgaggc ccagcctgac tggcgctcgg aggctcgtgg
1141 agaccatctt tctgggttcc aggccctgga tgccagggac tccccgcagg ttgccccgcc
1201 tgccccagcg ctactggcaa atgcggcccc tgtttctgga gctgcttggg aaccacgcgc
1261 agtgccccta cggggtgctc ctcaagacgc actgcccgct gcgagctgcg gtcaccccag
1321 cagccggtgt ctgtgcccgg gagaagcccc agggctctgt ggcggccccc gaggaggagg
1381 acacagaccc ccgtcgcctg gtgcagctgc tccgccagca cagcagcccc tggcaggtgt
```

```
1441 acggcttcgt gcgggcctgc ctgcgccggc tggtgccccc aggcctctgg ggctccaggc
1501 acaacgaacg ccgcttcctc aggaacacca agaagttcat ctccctgggg aagcatgcca
1561 agctctcgct gcaggagctg acgtggaaga tgagcgtgcg gggctgcgct tggctgcgca
1621 ggagcccagg ggttggctgt gttccggccg cagagcaccg tctgcgtgag gagatcctgg
1681 ccaagttcct gcactggctg atgagtgtgt acgtcgtcga gctgctcagg tctttctttt
1741 atgtcacgga gaccacgttt caaaagaaca ggctcttttt ctaccggaag agtgtctgga
1801 gcaagttgca aagcattgga atcagacagc acttgaagag ggtgcagctg cgggagctgt
1861 cggaagcaga ggtcaggcag catcgggaag ccaggcccgc cctgctgacg tccagactcc
1921 gcttcatccc caagcctgac gggctgcggc cgattgtgaa catggactac gtcgtgggag
1981 ccagaacgtt ccgcagagaa aagagggccg agcgtctcac ctcgagggtg aaggcactgt
2041 tcagcgtgct caactacgag cgggcgcggc gccccggcct cctgggcgcc tctgtgctgg
2101 gcctggacga tatccacagg gcctggcgca ccttcgtgct gcgtgtgcgg gcccaggacc
2161 cgccgcctga gctgtacttt gtcaaggtgg atgtgacggg cgcgtacgac accatccccc
2221 aggacaggct cacggaggtc atcgccagca tcatcaaacc ccagaacacg tactgcgtgc
2281 gtcggtatgc cgtggtccag aaggccgccc atgggcacgt ccgcaaggcc ttcaagagcc
2341 acgtctctac cttgacagac ctccagccgt acatgcgaca gttcgtggct cacctgcagg
2401 agaccagccc gctgagggat gccgtcgtca tcgagcagag ctcctccctg aatgaggcca
2461 gcagtggcct cttcgacgtc ttcctacgct tcatgtgcca ccacgccgtg cgcatcaggg
2521 gcaagtccta cgtccagtgc caggggatcc cgcagggctc catcctctcc acgctgctct
2581 gcagcctgtg ctacggcgac atggagaaca agctgtttgc ggggattcgg cgggacgggc
2641 tgctcctgcg tttggtggat gatttcttgt tggtgacacc tcacctcacc cacgcgaaaa
2701 ccttcctcag gaccctggtc cgaggtgtcc ctgagtatgg ctgcgtggtg aacttgcgga
2761 agacagtggt gaacttccct gtagaagacg aggccctggg tggcacggct tttgttcaga
2821 tgccggccca cggcctattc ccctggtgcg gcctgctgct ggatacccgg accctggagg
2881 tgcagagcga ctactccagc tatgcccgga cctccatcag agccagtctc accttcaacc
2941 gcggcttcaa ggctgggagg aacatgcgtc gcaaactctt tggggtcttg cggctgaagt
3001 gtcacagcct gtttctggat ttgcaggtga acagcctcca gacggtgtgc accaacatct
3061 acaagatcct cctgctgcag gcgtacaggt ttcacgcatg tgtgctgcag ctcccatttc
3121 atcagcaagt ttggaagaac cccacatttt tcctgcgcgt catctctgac acggcctccc
3181 tctgctactc catcctgaaa gccaagaacg cagggatgtc gctgggggcc aagggcgccg
3241 ccggccctct gccctccgag gccgtgcagt ggctgtgcca ccaagcattc ctgctcaagc
3301 tgactcgaca ccgtgtcacc tacgtgccac tcctggggtc actcaggaca gcccagacgc
3361 agctgagtcg gaagctcccg gggacgacgc tgactgccct ggaggccgca gccaacccgg
3421 cactgccctc agacttcaag accatcctgg actgatggcc acccgcccac agccaggccg
3481 agagcagaca ccagcagccc tgtcacgccg ggctctacgt cccagggagg gaggggcggc
3541 ccacacccag gcccgcaccg ctgggagtct gaggcctgag tgagtgtttg gccgaggcct
3601 gcatgtccgg ctgaaggctg agtgtccggc tgaggcctga gcgagtgtcc agccaagggc
3661 tgagtgtcca gcacacctgc cgtcttcact tccccacagg ctggcgctcg gctccacccc
3721 agggccagct tttcctcacc aggagcccgg cttccactcc ccacatagga atagtccatc
3781 cccagattcg ccattgttca cccctcgccc tgccctcctt tgccttccac ccccaccatc
3841 caggtggaga ccctgagaag gaccctggga gctctgggaa tttggagtga ccaaaggtgt
3901 gccctgtaca caggcgagga ccctgcacct ggatgggggt ccctgtgggt caaattgggg
3961 ggaggtgctg tgggagtaaa atactgaata tatgagtttt tcagttttga aaaaaaaaaa
4021 aaaaaaa (SEQ ID NO: 64)
```

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)

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 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 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):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

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 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 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 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 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 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, TGFβ, 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 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., 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% CO_2).

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, 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., 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, 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-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 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 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 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 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 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 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 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-$\gamma^{-/-}$ 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-$\gamma^{-/-}$ 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 pastrointestinal 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.

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.

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

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.

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.

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.

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 isFolate 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.

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.

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.

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.

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.

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.

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.

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.

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.

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 GloboHCAR, 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.

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.

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.

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.

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.

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.

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 caner, 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.

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.

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.

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.

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 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.

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 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 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 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.

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 prostein CAR, wherein the cancer cells express prostein.

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.

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 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.

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 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.

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 ERGCAR, 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 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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 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.

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 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 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.

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.

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.

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.

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.

In one aspect, the present invention relates to treatment of a subject in vivo using an 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 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 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 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 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 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 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 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 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 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 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 ("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 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 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 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 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, 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-redirected 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). 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.

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 hematolical 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 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 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 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 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 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 expessing 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 expessing 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 aCAR 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-expressingT 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 (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (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 (Cytoxan®, 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,

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

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with fludarabine, cyclophosphamide, and rituximab (FCR). In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the fludarabine is administered at a dosage of about 10-50 mg/m^2 (e.g., about 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45, or 45-50 mg/m^2), e.g., intravenously. In embodiments, the cyclophosphamide is administered at a dosage of about 200-300 mg/m^2 (e.g., about 200-225, 225-250, 250-275, or 275-300 mg/m^2), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m2 (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with bendamustine and rituximab. In embodiments, the subject has CLL. For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the bendamustine is administered at a dosage of about 70-110 mg/m2 (e.g., 70-80, 80-90, 90-100, or 100-110 mg/m2), e.g., intravenously. In embodiments, the rituximab is administered at a dosage of about 400-600 mg/m2 (e.g., 400-450, 450-500, 500-550, or 550-600 mg/m^2), e.g., intravenously.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and/or a corticosteroid (e.g., prednisone). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab, cyclophosphamide, doxorubicine, vincristine, and prednisone (R-CHOP). In embodiments, the subject has diffuse large B-cell lymphoma (DLBCL). In embodiments, the subject has nonbulky limited-stage DLBCL (e.g., comprises a tumor having a size/diameter of less than 7 cm). In embodiments, the subject is treated with radiation in combination with the R-CHOP. For example, the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP), followed by radiation. In some cases, the subject is administered R-CHOP (e.g., 1-6 cycles, e.g., 1, 2, 3, 4, 5, or 6 cycles of R-CHOP) following radiation.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with dose-adjusted EPOCH-R (DA-EPOCH-R). In embodiments, the subject has a B cell lymphoma, e.g., a Myc-rearranged aggressive B cell lymphoma.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and/or lenalidomide. Lenalidomide ((*RS*)-3-(4-Amino-1-oxo 1,3-dihydro-2*H*-isoindol- 2-yl)piperidine-2,6-dione) is an immunomodulator. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab and lenalidomide. In embodiments, the subject has follicular lymphoma (FL) or mantle cell lymphoma (MCL). In embodiments, the subject has FL and has not previously been treated with a cancer therapy. In embodiments, lenalidomide is administered at a dosage of about 10-20 mg (e.g., 10-15 or 15-20 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m^2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m^2), e.g., intravenously.

Exemplary mTOR inhibitors include, e.g., temsirolimus; ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.0^{4,9}] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); everolimus (Afinitor® or RAD001); rapamycin (AY22989, Sirolimus®); simapimod (CAS 164301-51-3); emsirolimus, (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-

phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine-, inner salt (SF1126, CAS 936487-67-1) (SEQ ID NO: 1262), and XL765.

Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon γ, CAS 951209-71-5, available from IRX Therapeutics).

Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauorubicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cerubidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idarubicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin.

Exemplary vinca alkaloids include, e.g., vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®).

Exemplary proteosome inhibitors include bortezomib (Velcade®); carfilzomib (PX-171-007, (*S*)-4-Methyl-*N*-((*S*)-1-(((*S*)-4-methyl-1-((*R*)-2-methyloxiran-2-yl)-1-oxopentan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and *O*-Methyl-*N*-[(2-methyl-5-thiazolyl)carbonyl]-L-seryl-*O*-methyl-*N*-[(1*S*)-2-[(2*R*)-2-methyl-2-oxiranyl]-2-oxo-1-(phenylmethyl)ethyl]- L-serinamide (ONX-0912).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab. Brentuximab is an antibody-drug conjugate of anti-CD30 antibody and monomethyl auristatin E. In embodiments, the subject has Hodgkin's lymphoma (HL), e.g., relapsed or refractory HL. In embodiments, the subject comprises CD30+ HL. In embodiments, the subject has undergone an autologous stem cell transplant (ASCT). In embodiments, the subject has not undergone an ASCT. In embodiments, brentuximab is administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with brentuximab and dacarbazine or in combination with brentuximab and bendamustine. Dacarbazine is an alkylating agent with a chemical name of 5-(3,3-Dimethyl-1-triazenyl)imidazole-4-carboxamide. Bendamustine is an alkylating agent with a chemical name of 4-[5-[Bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid. In embodiments, the subject has Hodgkin's lymphoma (HL). In embodiments, the subject has not previously been treated with a cancer therapy. In embodiments, the subject is at least 60 years of age, e.g., 60, 65, 70, 75, 80, 85, or older. In embodiments, dacarbazine is administered at a dosage of about 300-450 mg/m^2 (e.g., about 300-325, 325-350, 350-375, 375-400, 400-425, or 425-450 mg/m^2), e.g., intravenously. In

embodiments, bendamustine is administered at a dosage of about 75-125 mg/m2 (e.g., 75-100 or 100-125 mg/m^2, e.g., about 90 mg/m^2), e.g., intravenously. In embodiments, brentuximab is administered at a dosage of about 1-3 mg/kg (e.g., about 1-1.5, 1.5-2, 2-2.5, or 2.5-3 mg/kg), e.g., intravenously, e.g., every 3 weeks.

In some embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD20 inhibitor, e.g., an anti-CD20 antibody (e.g., an anti-CD20 mono- or bispecific antibody) or a fragment thereof. Exemplary anti-CD20 antibodies include but are not limited to rituximab, ofatumumab, ocrelizumab, veltuzumab, obinutuzumab, TRU-015 (Trubion Pharmaceuticals), ocaratuzumab, and Pro131921 (Genentech). See, e.g., Lim et al. Haematologica. 95.1(2010):135-43.

In some embodiments, the anti-CD20 antibody comprises rituximab. Rituximab is a chimeric mouse/human monoclonal antibody IgG1 kappa that binds to CD20 and causes cytolysis of a CD20 expressing cell, e.g., as described in www.accessdata.fda.gov/drugsatfda_docs/label/2010/103705s5311lbl.pdf. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with rituximab. In embodiments, the subject has CLL or SLL.

In some embodiments, rituximab is administered intravenously, e.g., as an intravenous infusion. For example, each infusion provides about 500-2000 mg (e.g., about 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, or 1900-2000 mg) of rituximab. In some embodiments, rituximab is administered at a dose of 150 mg/m^2 to 750 mg/m^2, e.g., about 150-175 mg/m^2, 175-200 mg/m^2, 200-225 mg/m^2, 225-250 mg/m^2, 250-300 mg/m^2, 300-325 mg/m^2, 325-350 mg/m^2, 350-375 mg/m^2, 375-400 mg/m^2, 400-425 mg/m^2, 425-450 mg/m^2, 450-475 mg/m^2, 475-500 mg/m^2, 500-525 mg/m^2, 525-550 mg/m^2, 550-575 mg/m^2, 575-600 mg/m^2, 600-625 mg/m^2, 625-650 mg/m^2, 650-675 mg/m^2, or 675-700 mg/m^2, where m^2 indicates the body surface area of the subject. In some embodiments, rituximab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, rituximab is administered at a dosing interval of at least 0.5 weeks, e.g., 0.5, 1, 2, 3, 4, 5, 6, 7, 8 weeks, or more. In some embodiments, rituximab is administered at a dose and dosing interval described herein for a period of time, e.g., at least 2 weeks, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 weeks, or greater. For example, rituximab is administered at a dose and dosing interval described herein for a total of at least 4 doses per treatment cycle (e.g., at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or more doses per treatment cycle).

In some embodiments, the anti-CD20 antibody comprises ofatumumab. Ofatumumab is an anti-CD20 IgG1κ human monoclonal antibody with a molecular weight of approximately 149 kDa. For example, ofatumumab is generated using transgenic mouse and hybridoma technology and is expressed and purified from a recombinant murine cell line (NS0). See, e.g.,

www.accessdata.fda.gov/drugsatfda_docs/label/2009/125326lbl.pdf; and Clinical Trial Identifier number NCT01363128, NCT01515176, NCT01626352, and NCT01397591. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with ofatumumab. In embodiments, the subject has CLL or SLL.

In some embodiments, ofatumumab is administered as an intravenous infusion. For example, each infusion provides about 150-3000 mg (e.g., about 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1200, 1200-1400, 1400-1600, 1600-1800, 1800-2000, 2000-2200, 2200-2400, 2400-2600, 2600-2800, or 2800-3000 mg) of ofatumumab. In embodiments, ofatumumab is administered at a starting dosage of about 300 mg, followed by 2000 mg, e.g., for about 11 doses, e.g., for 24 weeks. In some embodiments, ofatumumab is administered at a dosing interval of at least 4 days, e.g., 4, 7, 14, 21, 28, 35 days, or more. For example, ofatumumab is administered at a dosing interval of at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 26, 28, 20, 22, 24, 26, 28, 30 weeks, or more. In some embodiments, ofatumumab is administered at a dose and dosing interval described herein for a period of time, e.g., at least 1 week, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 26, 28, 30, 40, 50, 60 weeks or greater, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or greater, or 1, 2, 3, 4, 5 years or greater. For example, ofatumumab is administered at a dose and dosing interval described herein for a total of at least 2 doses per treatment cycle (e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, or more doses per treatment cycle).

In some cases, the anti-CD20 antibody comprises ocrelizumab. Ocrelizumab is a humanized anti-CD20 monoclonal antibody, e.g., as described in Clinical Trials Identifier Nos. NCT00077870, NCT01412333, NCT00779220, NCT00673920, NCT01194570, and Kappos et al. Lancet. 19.378(2011):1779-87.

In some cases, the anti-CD20 antibody comprises veltuzumab. Veltuzumab is a humanized monoclonal antibody against CD20. See, e.g., Clinical Trial Identifier No. NCT00547066, NCT00546793, NCT01101581, and Goldenberg et al. Leuk Lymphoma. 51(5)(2010):747-55.

In some cases, the anti-CD20 antibody comprises GA101. GA101 (also called obinutuzumab or RO5072759) is a humanized and glyco-engineered anti-CD20 monoclonal antibody. See, e.g., Robak. Curr. Opin. Investig. Drugs. 10.6(2009):588-96; Clinical Trial Identifier Numbers: NCT01995669, NCT01889797, NCT02229422, and NCT01414205; and www.accessdata.fda.gov/drugsatfda_docs/label/2013/125486s000lbl.pdf.

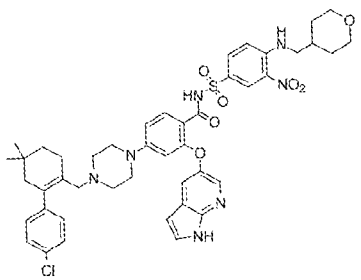
In some cases, the anti-CD20 antibody comprises AME-133v. AME-133v (also called LY2469298 or ocaratuzumab) is a humanized IgG1 monoclonal antibody against CD20 with increased affinity for the FcγRIIIa receptor and an enhanced antibody dependent cellular cytotoxicity (ADCC) activity compared with rituximab. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25; and Forero-Torres et al. Clin Cancer Res. 18.5(2012):1395-403.

In some cases, the anti-CD20 antibody comprises PRO131921. PRO131921 is a humanized anti-CD20 monoclonal antibody engineered to have better binding to FcγRIIIa and enhanced ADCC compared with rituximab. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25; and Casulo et al. Clin Immunol. 154.1(2014):37-46; and Clinical Trial Identifier No. NCT00452127.

In some cases, the anti-CD20 antibody comprises TRU-015. TRU-015 is an anti-CD20 fusion protein derived from domains of an antibody against CD20. TRU-015 is smaller than monoclonal antibodies, but retains Fc-mediated effector functions. See, e.g., Robak et al. BioDrugs 25.1(2011):13-25. TRU-015 contains an anti-CD20 single-chain variable fragment (scFv) linked to human IgG1 hinge, CH2, and CH3 domains but lacks CH1 and CL domains.

In some embodiments, an anti-CD20 antibody described herein is conjugated or otherwise bound to a therapeutic agent, e.g., a chemotherapeutic agent (e.g., cytoxan, fludarabine, histone deacetylase inhibitor, demethylating agent, peptide vaccine, anti-tumor antibiotic, tyrosine kinase inhibitor, alkylating agent, anti-microtubule or anti-mitotic agent), anti-allergic agent, anti-nausea agent (or anti-emetic), pain reliever, or cytoprotective agent described herein.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a B-cell lymphoma 2 (BCL-2) inhibitor (e.g., venetoclax, also called ABT-199 or GDC-0199;) and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with venetoclax and rituximab. Venetoclax is a small molecule that inhibits the anti-apoptotic protein, BCL-2. The structure of venetoclax (4-(4-{[2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-*N*-({3-nitro-4-[(tetrahydro-2*H*-pyran-4-ylmethyl)amino]phenyl}sulfonyl)-2-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yloxy)benzamide) is shown below.



In embodiments, the subject has CLL. In embodiments, the subject has relapsed CLL, e.g., the subject has previously been administered a cancer therapy. In embodiments, venetoclax is administered at a dosage of about 15-600 mg (e.g., 15-20, 20-50, 50-75, 75-100, 100-200, 200-300, 300-400, 400-500, or 500-600 mg), e.g., daily. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m2), e.g., intravenously, e.g., monthly

In an embodiment, cells expressing a CAR described herein are administered to a subject in combination with a molecule that decreases the Treg cell population. Methods that decrease the number of (e.g., deplete) Treg cells are known in the art and include, e.g., CD25 depletion, cyclophosphamide administration, modulating GITR function. Without wishing to be bound by theory, it is believed that reducing the number of Treg cells in a subject prior to apheresis or prior to administration of a CAR-expressing cell described herein reduces the number of unwanted immune cells (e.g., Tregs) in the tumor microenvironment and reduces the subject's risk of relapse. In one embodiment, cells expressing a CAR described herein are administered to a subject in combination with a molecule targeting GITR and/or modulating GITR functions, such as a GITR agonist and/or a GITR antibody that depletes regulatory T cells (Tregs). In embodiments, cells expressing a CAR described herein are administered to a subject in combination with cyclophosphamide. In one embodiment, the GITR binding molecules and/or molecules modulating GITR functions (e.g., GITR agonist and/or Treg depleting GITR antibodies) are administered prior to administration of the CAR-expressing cell. For example, in one embodiment, the GITR agonist can be administered prior to apheresis of the cells. In embodiments, cyclophosphamide is administered to the subject prior to administration (e.g., infusion or re-infusion) of the CAR-expressing cell or prior to aphersis of the cells. In embodiments, cyclophosphamide and an anti-GITR antibody are administered to the subject prior to administration (e.g., infusion or re-infusion) of the CAR-expressing cell or prior to apheresis of the cells. In one embodiment, the subject has cancer (e.g., a solid cancer or a hematological cancer such as ALL or CLL). In an embodiment, the subject has CLL. In embodiments, the subject has ALL. In embodiments, the subject has a solid cancer, e.g., a solid cancer described herein. Exemplary GITR agonists include, e.g., GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies) such as, e.g., a GITR fusion protein described in U.S. Patent No.: 6,111,090, European Patent No.: 090505B1, U.S Patent No.: 8,586,023, PCT Publication Nos.: WO 2010/003118 and 2011/090754, or an anti-GITR antibody described, e.g., in U.S. Patent No.: 7,025,962, European Patent No.: 1947183B1, U.S. Patent No.: 7,812,135, U.S. Patent No.: 8,388,967, U.S. Patent No.: 8,591,886, European Patent No.: EP 1866339, PCT Publication No.: WO 2011/028683, PCT Publication No.:WO 2013/039954, PCT Publication No.: WO2005/007190, PCT Publication No.: WO 2007/133822, PCT Publication No.: WO2005/055808, PCT Publication No.: WO 99/40196, PCT Publication No.: WO 2001/03720, PCT Publication No.: WO99/20758, PCT Publication No.: WO2006/083289, PCT Publication No.: WO 2005/115451, U.S. Patent No.: 7,618,632, and PCT Publication No.: WO 2011/051726.

In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with an mTOR inhibitor, e.g., an mTOR inhibitor described herein, e.g., a rapalog such as everolimus. In one embodiment, the mTOR inhibitor is administered prior to the CAR-expressing cell. For example, in one embodiment, the mTOR inhibitor can be administered prior to apheresis of the cells. In one embodiment, the subject has CLL.

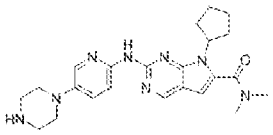
In one embodiment, a CAR expressing cell described herein is administered to a subject in combination with a GITR agonist, e.g., a GITR agonist described herein. In one embodiment, the GITR agonist is administered prior to the CAR-expressing cell. For example, in one embodiment, the GITR agonist can be administered prior to apheresis of the cells. In one embodiment, the subject has CLL.

In one embodiment, a CAR-expressing cell described herein can be used in combination with a kinase inhibitor. In one embodiment, the kinase inhibitor is a CDK4 inhibitor, e.g., a CDK4 inhibitor described herein, e.g., a CD4/6 inhibitor, such as, e.g., 6-Acetyl-8-cyclopentyl-5-methyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one, hydrochloride (also referred to as palbociclib or PD0332991). In one embodiment, the kinase inhibitor is a BTK inhibitor, e.g., a BTK inhibitor described herein, such as, e.g., ibrutinib. In one embodiment, the kinase inhibitor is an mTOR inhibitor, e.g., an mTOR inhibitor described herein, such as, e.g., rapamycin, a rapamycin analog, OSI-027. The mTOR inhibitor can be, e.g., an mTORC1 inhibitor and/or an mTORC2 inhibitor, e.g., an mTORC1 inhibitor and/or mTORC2 inhibitor described herein. In one embodiment, the kinase inhibitor is a MNK inhibitor, e.g., a MNK inhibitor described herein, such as, e.g., 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-*d*] pyrimidine. The MNK inhibitor can be, e.g., a MNK1a, MNK1b, MNK2a and/or MNK2b inhibitor. In one embodiment, the kinase inhibitor is a dual PI3K/mTOR inhibitor described herein, such as, e.g., PF-04695102.

In one embodiment, the kinase inhibitor is a CDK4 inhibitor selected from aloisine A; flavopiridol or HMR-1275, 2-(2-chlorophenyl)-5,7-dihydroxy-8-[(3S,4R)-3-hydroxy-1-methyl-4-piperidinyl]-4-chromenone; crizotinib (PF-02341066; 2-(2-Chlorophenyl)-5,7-dihydroxy-8-[(2*R*,3*S*)-2-(hydroxymethyl)-1-methyl-3-pyrrolidinyl]- 4*H*-1-benzopyran-4-one, hydrochloride (P276-00); 1-methyl-5-[[2-[5-(trifluoromethyl)-1*H*-imidazol-2-yl]-4-pyridinyl]oxy]-*N*-[4-(trifluoromethyl)phenyl]-1*H*-benzimidazol-2-amine (RAF265); indisulam (E7070); roscovitine (CYC202); palbociclib (PD0332991); dinaciclib (SCH727965); N-[5-[[(5-*tert*-butyloxazol-2-yl)methyl]thio]thiazol-2-yl]piperidine-4-carboxamide (BMS 387032); 4-[[9-chloro-7-(2,6-difluorophenyl)-5*H*-pyrimido[5,4-*d*][2]benzazepin-2-yl]amino]-benzoic acid (MLN8054); 5-[3-(4,6-difluoro-1H-benzimidazol-2-yl)-1H-indazol-5-yl]-N-ethyl-4-methyl-3-pyridinemethanamine (AG-024322); 4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxylic acid N-(piperidin-4-yl)amide (AT7519); 4-[2-methyl-1-(1-methylethyl)-1*H*-imidazol-5-yl]-*N*-[4-(methylsulfonyl)phenyl]- 2-pyrimidinamine (AZD5438); and XL281 (BMS908662).

In one embodiment, the kinase inhibitor is a CDK4 inhibitor, e.g., palbociclib (PD0332991), and the palbociclib is administered at a dose of about 50 mg, 60 mg, 70 mg, 75 mg, 80 mg, 90 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, 130 mg, 135 mg (e.g., 75 mg, 100 mg or 125 mg) daily for a period of time, e.g., daily for 14-21 days of a 28 day cycle, or daily for 7-12 days of a 21 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of palbociclib are administered.

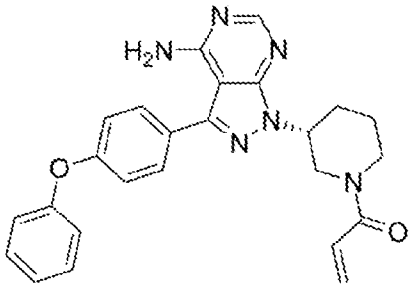
In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a cyclin-dependent kinase (CDK) 4 or 6 inhibitor, e.g., a CDK4 inhibitor or a CDK6 inhibitor described herein. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CDK4/6 inhibitor (e.g., an inhibitor that targets both CDK4 and CDK6), e.g., a CDK4/6 inhibitor described herein. In an embodiment, the subject has MCL. MCL is an aggressive cancer that is poorly responsive to currently available therapies, i.e., essentially incurable. In many cases of MCL, cyclin D1 (a regulator of CDK4/6) is expressed (e.g., due to chromosomal translocation involving immunoglobulin and Cyclin D1 genes) in MCL cells. Thus, without being bound by theory, it is thought that MCL cells are highly sensitive to CDK4/6 inhibition with high specificity (i.e., minimal effect on normal immune cells). CDK4/6 inhibitors alone have had some efficacy in treating MCL, but have only achieved partial remission with a high relapse rate. An exemplary CDK4/6 inhibitor is LEE011 (also called ribociclib), the structure of which is shown below.



Without being bound by theory, it is believed that administration of a CAR-expressing cell described herein with a CDK4/6 inhibitor (e.g., LEE011 or other CDK4/6 inhibitor described herein) can achieve higher responsiveness, e.g., with higher remission rates and/or lower relapse rates, e.g., compared to a CDK4/6 inhibitor alone.

In one embodiment, the kinase inhibitor is a BTK inhibitor selected from ibrutinib (PCI-32765); GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13. In a preferred embodiment, the BTK inhibitor does not reduce or inhibit the kinase activity of interleukin-2-inducible kinase (ITK), and is selected from GDC-0834; RN-486; CGI-560; CGI-1764; HM-71224; CC-292; ONO-4059; CNX-774; and LFM-A13.

In one embodiment, the kinase inhibitor is a BTK inhibitor, e.g., ibrutinib (PCI-32765). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a BTK inhibitor (e.g., ibrutinib). In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with ibrutinib (also called PCI-32765). The structure of ibrutinib (1-[(3*R*)-3-[4-Amino-3-(4-phenoxyphenyl)-1*H*-pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one) is shown below.



In embodiments, the subject has CLL, mantle cell lymphoma (MCL), or small lymphocytic lymphoma (SLL). For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject has relapsed CLL or SLL, e.g., the subject has previously been administered a cancer therapy (e.g., previously been administered one, two, three, or four prior cancer therapies). In embodiments, the subject has refractory CLL or SLL. In other embodiments, the subject has follicular lymphoma, e.g., relapse or refractory follicular lymphoma. In some embodiments, ibrutinib is administered at a dosage of about 300-600 mg/day (e.g., about 300-350, 350-400, 400-450, 450-500, 500-550, or 550-600 mg/day, e.g., about 420 mg/day or about 560 mg/day), e.g., orally. In embodiments, the ibrutinib is administered at a dose of about 250 mg, 300 mg, 350 mg, 400 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600 mg (e.g., 250 mg, 420 mg or 560 mg) daily for a period of time, e.g., daily for 21 day cycle cycle, or daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of ibrutinib are administered. Without being bound by theory, it is thought that the addition of ibrutinib enhances the T cell proliferative response and may shift T cells from a T-helper-2 (Th2) to T-helper-1 (Th1) phenotype. Th1 and Th2 are phenotypes of helper T cells, with Th1 versus Th2 directing different immune response pathways. A Th1 phenotype is associated with proinflammatory responses, e.g., for killing cells, such as intracellular pathogens/viruses or cancerous cells, or perpetuating autoimmune responses. A Th2 phenotype is associated with eosinophil accumulation and anti-inflammatory responses.

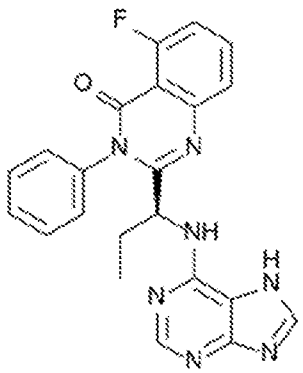
In one embodiment, the kinase inhibitor is an mTOR inhibitor selected from temsirolimus; ridaforolimus (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*, 23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669; everolimus (RAD001); rapamycin (AY22989); simapimod; (5-{2,4-bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502); and N^2-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-

yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine-, inner salt (SF1126) (SEQ ID NO: 1262); and XL765.

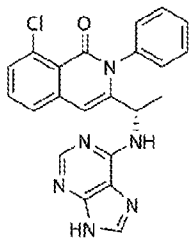
In one embodiment, the kinase inhibitor is an mTOR inhibitor, e.g., rapamycin, and the rapamycin is administered at a dose of about 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg (e.g., 6 mg) daily for a period of time, e.g., daily for 21 day cycle cycle, or daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of rapamycin are administered. In one embodiment, the kinase inhibitor is an mTOR inhibitor, e.g., everolimus and the everolimus is administered at a dose of about 2 mg, 2.5 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg (e.g., 10 mg) daily for a period of time, e.g., daily for 28 day cycle. In one embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more cycles of everolimus are administered.

In one embodiment, the kinase inhibitor is an MNK inhibitor selected from CGP052088; 4-amino-3-(p-fluorophenylamino)-pyrazolo [3,4-*d*] pyrimidine (CGP57380); cercosporamide; ETC-1780445-2; and 4-amino-5-(4-fluoroanilino)-pyrazolo [3,4-*d*] pyrimidine.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a phosphoinositide 3-kinase (PI3K) inhibitor (e.g., a PI3K inhibitor described herein, e.g., idelalisib or duvelisib) and/or rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with idelalisib and rituximab. In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with duvelisib and rituximab. Idelalisib (also called GS-1101 or CAL-101; Gilead) is a small molecule that blocks the delta isoform of PI3K. The structure of idelalisib (5-Fluoro-3-phenyl-2-[(1*S*)-1-(7*H*-purin-6-ylamino)propyl]-4(3*H*)-quinazolinone) is shown below.



Duvelisib (also called IPI-145; Infinity Pharmaceuticals and Abbvie) is a small molecule that blocks PI3K-δ,γ. The structure of duvelisib (8-Chloro-2-phenyl-3-[(1S)-1-(9H-purin-6-ylamino)ethyl]-1(2H)-isoquinolinone) is shown below.



In embodiments, the subject has CLL. In embodiments, the subject has relapsed CLL, e.g., the subject has previously been administered a cancer therapy (e.g., previously been administered an anti-CD20 antibody or previously been administered ibrutinib). For example, the subject has a deletion in the short arm of chromosome 17 (del(17p), e.g., in a leukemic cell). In other examples, the subject does not have a del(17p). In embodiments, the subject comprises a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In other embodiments, the subject does not comprise a leukemic cell comprising a mutation in the immunoglobulin heavy-chain variable-region (IgV_H) gene. In embodiments, the subject has a deletion in the long arm of chromosome 11 (del(11q)). In other embodiments, the subject does not have a del(11q). In embodiments, idelalisib is administered at a dosage of about 100-400 mg (e.g., 100-125, 125-150, 150-175, 175-200, 200-225, 225-250, 250-275, 275-300, 325-350, 350-375, or 375-400 mg), e.g., BID. In embodiments, duvelisib is administered at a dosage of about 15-100 mg (e.g., about 15-25, 25-50, 50-75, or 75-100 mg), e.g., twice a day. In embodiments, rituximab is administered at a dosage of about 350-550 mg/m^2 (e.g., 350-375, 375-400, 400-425, 425-450, 450-475, or 475-500 mg/m^2), e.g., intravenously.

In one embodiment, the kinase inhibitor is a dual phosphatidylinositol 3-kinase (PI3K) and mTOR inhibitor selected from 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF-04691502); *N*-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-*N*'-[4-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)phenyl]urea (PF-05212384, PKI-587); 2-Methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-c]quinolin-1-yl]phenyl}propanenitrile (BEZ-235); apitolisib (GDC-0980, RG7422); 2,4-Difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide (GSK2126458); 8-(6-methoxypyridin-3-yl)-3-methyl-1-(4-(piperazin-1-yl)-3-(trifluoromethyl)phenyl)-1H-imidazo[4,5-c]quinolin-2(3H)-one Maleic acid (NVP-BGT226); 3-[4-(4-Morpholinylpyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol (PI-103); 5-(9-isopropyl-8-methyl-2-morpholino-9H-purin-6-yl)pyrimidin-2-amine (VS-5584, SB2343); and N-[2-[(3,5-Dimethoxyphenyl)amino]quinoxalin-3-yl]-4-[(4-methyl-3-methoxyphenyl)carbonyl]aminophenylsulfonamide (XL765).

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an anaplastic lymphoma kinase (ALK) inhibitor. Exemplary ALK kinases include but are not limited to crizotinib (Pfizer), ceritinib (Novartis), alectinib (Chugai), brigatinib (also called

AP26113; Ariad), entrectinib (Ignyta), PF-06463922 (Pfizer), TSR-011 (Tesaro) (see, e.g., Clinical Trial Identifier No. NCT02048488), CEP-37440 (Teva), and X-396 (Xcovery). In some embodiments, the subject has a solid cancer, e.g., a solid cancer described herein, e.g., lung cancer.

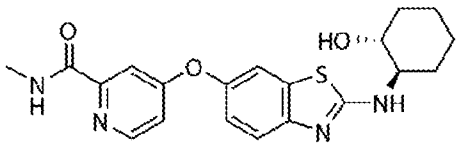
The chemical name of crizotinib is 3-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-ylpyrazol-4-yl)pyridin-2-amine. The chemical name of ceritinib is 5-Chloro-N^2-[2-isopropoxy-5-methyl-4-(4-piperidinyl)phenyl]-N^4-[2-(isopropylsulfonyl)phenyl]-2,4-pyrimidinediamine. The chemical name of alectinib is 9-ethyl-6,6-dimethyl-8-(4-morpholinopiperidin-1-yl)-11-oxo-6,11-dihydro-5H-benzo[b]carbazole-3-carbonitrile. The chemical name of brigatinib is 5-Chloro-N^2-{4-[4-(dimethylamino)-1-piperidinyl]-2-methoxyphenyl}-N^4-[2-(dimethylphosphoryl)phenyl]-2,4-pyrimidinediamine. The chemical name of entrectinib is N-(5-(3,5-difluorobenzyl)-1H-indazol-3-yl)-4-(4-methylpiperazin-1-yl)-2-((tetrahydro-2H-pyran-4-yl)amino)benzamide. The chemical name of PF-06463922 is (10R)-7-Amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3-h][2,5,11]-benzoxadiazacyclotetradecine-3-carbonitrile. The chemical structure of CEP-37440 is (S)-2-((5-chloro-2-((6-(4-(2-hydroxyethyl)piperazin-1-yl)-1-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-2-yl)amino)pyrimidin-4-yl)amino)-N-methylbenzamide. The chemical name of X-396 is (R)-6-amino-5-(1-(2,6-dichloro-3-fluorophenyl)ethoxy)-N-(4-(4-methylpiperazine-1-carbonyl)phenyl)pyridazine-3-carboxamide.

Drugs that inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993) can also be used. In a further aspect, the cell compositions of the present invention may be administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibodies such as OKT3 or CAMPATH. In one aspect, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery.

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with an indoleamine 2,3-dioxygenase (IDO) inhibitor. IDO is an enzyme that catalyzes the degradation of the amino acid, L-tryptophan, to kynurenine. Many cancers overexpress IDO, e.g., prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, and lung cancer. pDCs, macrophages, and dendritic cells (DCs) can express IDO. Without being bound by theory, it is thought that a decrease in L-tryptophan (e.g., catalyzed by IDO) results in an immunosuppressive

milieu by inducing T-cell anergy and apoptosis. Thus, without being bound by theory, it is thought that an IDO inhibitor can enhance the efficacy of a CAR-expressing cell described herein, e.g., by decreasing the suppression or death of a CAR-expressing immune cell. In embodiments, the subject has a solid tumor, e.g., a solid tumor described herein, e.g., prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, or lung cancer. Exemplary inhibitors of IDO include but are not limited to 1-methyl-tryptophan, indoximod (NewLink Genetics) (see, e.g., Clinical Trial Identifier Nos. NCT01191216; NCT01792050), and INCB024360 (Incyte Corp.) (see, e.g., Clinical Trial Identifier Nos. NCT01604889; NCT01685255)

In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a modulator of myeloid-derived suppressor cells (MDSCs). MDSCs accumulate in the periphery and at the tumor site of many solid tumors. These cells suppress T cell responses, thereby hindering the efficacy of CAR-expressing cell therapy. Without being bound by theory, it is thought that administration of a MDSC modulator enhances the efficacy of a CAR-expressing cell described herein. In an embodiment, the subject has a solid tumor, e.g., a solid tumor described herein, e.g., glioblastoma. Exemplary modulators of MDSCs include but are not limited to MCS110 and BLZ945. MCS110 is a monoclonal antibody (mAb) against macrophage colony-stimulating factor (M-CSF). See, e.g., Clinical Trial Identifier No. NCT00757757. BLZ945 is a small molecule inhibitor of colony stimulating factor 1 receptor (CSF1R). See, e.g., Pyonteck et al. Nat. Med. 19(2013):1264-72. The structure of BLZ945 is shown below.



In embodiments, a CAR-expressing cell described herein is administered to a subject in combination with a CD19 CART cell (e.g., CTL019, e.g., as described in WO2012/079000, incorporated herein by reference, or or CTL119). In embodiments, the subject has a CD19+ lymphoma, e.g., a CD19+ Non-Hodgkin's Lymphoma (NHL), a CD19+ FL, or a CD19+ DLBCL. In embodiments, the subject has a relapsed or refractory CD19+ lymphoma. In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of CD19 CART cells. In an example, the lymphodepleting chemotherapy is administered to the subject prior to administration of CD19 CART cells. For example, the lymphodepleting chemotherapy ends 1-4 days (e.g., 1, 2, 3, or 4 days) prior to CD19 CART cell infusion. In embodiments, multiple doses of CD19 CART cells are administered, e.g., as

described herein. For example, a single dose comprises about 5 x 10^8 CD19 CART cells. In embodiments, a lymphodepleting chemotherapy is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of a CAR-expressing cell described herein, e.g., a non-CD19 CAR-expresing cell. In embodiments, a CD19 CART is administered to the subject prior to, concurrently with, or after administration (e.g., infusion) of a non-CD19 CAR-expressing cell, e.g., a non-CD19 CAR-expressing cell described herein.

In some embodiments , a CAR-expressing cell described herein is administered to a subject in combination with a interleukin-15 (IL-15) polypeptide, a interleukin-15 receptor alpha (IL-15Ra) polypeptide, or a combination of both a IL-15 polypeptide and a IL-15Ra polypeptide e.g., hetIL-15 (Admune Therapeutics, LLC). hetIL-15 is a heterodimeric non-covalent complex of IL-15 and IL-15Ra. hetIL-15 is described in, e.g., U.S. 8,124,084, U.S. 2012/0177598, U.S. 2009/0082299, U.S. 2012/0141413, and U.S. 2011/0081311, incorporated herein by reference. In embodiments, het-IL-15 is administered subcutaneously. In embodiments, the subject has a cancer, e.g., solid cancer, e.g., melanoma or colon cancer. In embodiments, the subject has a metastatic cancer.

In one embodiment, the subject can be administered an agent which reduces or ameliorates a side effect associated with the administration of a CAR-expressing cell. Side effects associated with the administration of a CAR-expressing cell include, but are not limited to CRS, and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS). Symptoms of CRS include high fevers, nausea, transient hypotension, hypoxia, and the like. CRS may include clinical constitutional signs and symptoms such as fever, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, and headache. CRS may include clinical skin signs and symptoms such as rash. CRS may include clinical gastrointestinal signs and symsptoms such as nausea, vomiting and diarrhea. CRS may include clinical respiratory signs and symptoms such as tachypnea and hypoxemia. CRS may include clinical cardiovascular signs and symptoms such as tachycardia, widened pulse pressure, hypotension, increased cardac output (early) and potentially diminished cardiac output (late). CRS may include clinical coagulation signs and symptoms such as elevated d-dimer, hypofibrinogenemia with or without bleeding. CRS may include clinical renal signs and symptoms such as azotemia. CRS may include clinical hepatic signs and symptoms such as transaminitis and hyperbilirubinemia. CRS may include clinical neurologic signs and symptoms such as headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, and seizures.

Accordingly, the methods described herein can comprise administering a CAR-expressing cell described herein to a subject and further administering one or more agents to manage elevated levels of a soluble factor resulting from treatment with a CAR-expressing cell. In one embodiment, the soluble factor elevated in the subject is one or more of IFN-γ, TNFα, IL-2 and IL-6. In an embodiment, the factor elevated in the subject is one or more of IL-1, GM-CSF, IL-10, IL-8, IL-5 and

fraktalkine. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors. In one embodiment, the agent that neutralizes one or more of these soluble forms is an antibody or antigen binding fragment thereof. Examples of such agents include, but are not limited to a steroid (e.g., corticosteroid), an inhibitor of TNFα, and an inhibitor of IL-6. An example of a TNFα inhibitor is an anti-TNFα antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab. Another example of a TNFα inhibitor is a fusion protein such as entanercept. Small molecule inhibitors of TNFα include, but are not limited to, xanthine derivatives (e.g. pentoxifylline) and bupropion. An example of an IL-6 inhibitor is an anti-IL-6 antibody molecule or an anti-IL-6 receptor antibody molecule such as tocilizumab (toc), sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In one embodiment, the anti-IL-6 receptor antibody molecule is tocilizumab. An example of an IL-1R based inhibitor is anakinra.

In one embodiment, the subject can be administered 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., Programmed Death 1 (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, CTLA-4, TIM-3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG-3, 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 to inhibit expression of an inhibitory molecule in the CAR-expressing cell. In an embodiment the inhibitor is an shRNA. In an embodiment, the inhibitory molecule is inhibited within a CAR-expressing cell. In these embodiments, a dsRNA molecule that inhibits expression of the inhibitory molecule is linked to the nucleic acid that encodes a component, e.g., all of the components, of the CAR. In one embodiment, the inhibitor of an inhibitory signal can be, e.g., an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD-1, PD-L1, PD-L2 or CTLA4 (e.g., ipilimumab (also referred to as MDX-010 and MDX-101, and marketed as Yervoy®; Bristol-Myers Squibb; Tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206).). In an embodiment, the agent is an antibody or antibody fragment that binds to TIM3. In an embodiment, the agent is an antibody or antibody fragment that binds to CEACAM (CEACAM-1, CEACAM-3, and/or CEACAM-5). In an embodiment, the agent is an antibody or antibody fragment that binds to LAG3.

PD-1 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 PD-1, PD-L1 and PD-L2 have been shown to downregulate T cell activation upon binding to PD-1 (Freeman et a. 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 PD-1 with PD-L1. Antibodies, antibody fragments, and other inhibitors of PD-1, PD-L1 and PD-L2 are available in the art and may be used combination with a cars of the present invention described herein. For example, nivolumab (also referred to as BMS-936558 or MDX1106; Bristol-Myers Squibb) is a fully human IgG4 monoclonal antibody which specifically blocks PD-1. Nivolumab (clone 5C4) and other human monoclonal antibodies that specifically bind to PD-1 are disclosed in US 8,008,449 and WO2006/121168. Pidilizumab (CT-011; Cure Tech) is a humanized IgG1k monoclonal antibody that binds to PD-1. Pidilizumab and other humanized anti-PD-1 monoclonal antibodies are disclosed in WO2009/101611. Pembrolizumab (formerly known as lambrolizumab, and also referred to as MK03475; Merck) is a humanized IgG4 monoclonal antibody that binds to PD-1. Pembrolizumab and other humanized anti-PD-1 antibodies are disclosed in US 8,354,509 and WO2009/114335. MEDI4736 (Medimmune) is a human monoclonal antibody that binds to PDL1, and inhibits interaction of the ligand with PD1. MDPL3280A (Genentech / Roche) is a human Fc optimized IgG1 monoclonal antibody that binds to PD-L1. MDPL3280A and other human monoclonal antibodies to PD-L1 are disclosed in U.S. Patent No.: 7,943,743 and U.S Publication No.: 20120039906. Other anti-PD-L1 binding agents include YW243.55.S70 (heavy and light chain variable regions are shown in SEQ ID NOs 20 and 21 in WO2010/077634) and MDX-1 105 (also referred to as BMS-936559, and, e.g., anti-PD-L1 binding agents disclosed in WO2007/005874). AMP-224 (B7-DCIg; Amplimmune; e.g., disclosed in WO2010/027827 and WO2011/066342), is a PD-L2 Fc fusion soluble receptor that blocks the interaction between PD-1 and B7-H1. Other anti-PD-1 antibodies include AMP 514 (Amplimmune), among others, e.g., anti-PD-1 antibodies disclosed in US 8,609,089, US 2010028330, and/or US 20120114649.

TIM-3 (T cell immunoglobulin-3) also negatively regulates T cell function, particularly in IFN-g-secreting CD4+ T helper 1 and CD8+ T cytotoxic 1 cells, and plays a critical role in T cell exhaustion. Inhibition of the interaction between TIM3 and its ligands, e.g., galectin-9 (Gal9), phosphotidylserine (PS), and HMGB1, can increase immune response. Antibodies, antibody fragments, and other inhibitors of TIM3 and its ligands are available in the art and may be used combination with a CD19 CAR described herein. For example, antibodies, antibody fragments, small molecules, or peptide inhibitors that target TIM3 binds to the IgV domain of TIM3 to inhibit interaction with its ligands. Antibodies and peptides that inhibit TIM3 are disclosed in

WO2013/006490 and US20100247521. Other anti-TIM3 antibodies include humanized versions of RMT3-23 (disclosed in Ngiow et al., 2011, Cancer Res, 71:3540-3551), and clone 8B.2C12 (disclosed in Monney et al., 2002, Nature, 415:536-541). Bi-specific antibodies that inhibit TIM3 and PD-1 are disclosed in US20130156774.

In other embodiments, the agent that enhances the activity of a CAR-expressing cell is a CEACAM inhibitor (*e.g.*, CEACAM-1, CEACAM-3, and/or CEACAM-5 inhibitor). In one embodiment, the inhibitor of CEACAM is an anti-CEACAM antibody molecule. Exemplary anti-CEACAM-1 antibodies are described in WO 2010/125571, WO 2013/082366 WO 2014/059251 and WO 2014/022332, e.g., a monoclonal antibody 34B1, 26H7, and 5F4; or a recombinant form thereof, as described in, *e.g.*, US 2004/0047858, US 7,132,255 and WO 99/052552. In other embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, *e.g.*, Zheng et al. *PLoS One*. 2010 Sep 2;5(9). pii: e12529 (DOI:10:1371/journal.pone.0021146), or crossreacts with CEACAM-1 and CEACAM-5 as described in, *e.g.*, WO 2013/054331 and US 2014/0271618.

Without wishing to be bound by theory, carcinoembryonic antigen cell adhesion molecules (CEACAM), such as CEACAM-1 and CEACAM-5, are believed to mediate, at least in part, inhibition of an anti-tumor immune response (see e.g., Markel et al. J Immunol. 2002 Mar 15;168(6):2803-10; Markel et al. J Immunol. 2006 Nov 1;177(9):6062-71; Markel et al. Immunology. 2009 Feb;126(2):186-200; Markel et al. Cancer Immunol Immunother. 2010 Feb;59(2):215-30; Ortenberg et al. Mol Cancer Ther. 2012 Jun;11(6):1300-10; Stern et al. J Immunol. 2005 Jun 1;174(11):6692-701; Zheng et al. PLoS One. 2010 Sep 2;5(9). pii: e12529). For example, CEACAM-1 has been described as a heterophilic ligand for TIM-3 and as playing a role in TIM-3-mediated T cell tolerance and exhaustion (see e.g., WO 2014/022332; Huang, et al. (2014) Nature doi:10.1038/nature13848). In embodiments, co-blockade of CEACAM-1 and TIM-3 has been shown to enhance an anti-tumor immune response in xenograft colorectal cancer models (see e.g., WO 2014/022332; Huang, et al. (2014), *supra*). In other embodiments, co-blockade of CEACAM-1 and PD-1 reduce T cell tolerance as described, *e.g.*, in WO 2014/059251. Thus, CEACAM inhibitors can be used with the other immunomodulators described herein (e.g., anti-PD-1 and/or anti-TIM-3 inhibitors) to enhance an immune response against a cancer, *e.g.*, a melanoma, a lung cancer (e.g., NSCLC), a bladder cancer, a colon cancer an ovarian cancer, and other cancers as described herein.

LAG-3 (lymphocyte activation gene-3 or CD223) is a cell surface molecule expressed on activated T cells and B cells that has been shown to play a role in CD8+ T cell exhaustion. Antibodies, antibody fragments, and other inhibitors of LAG-3 and its ligands are available in the art and may be used combination with a CD19 CAR described herein. For example, BMS-986016 (Bristol-Myers Squib) is a monoclonal antibody that targets LAG3. IMP701 (Immutep) is an antagonist LAG-3 antibody and IMP731 (Immutep and GlaxoSmithKline) is a depleting LAG-3 antibody. Other LAG-3 inhibitors include IMP321 (Immutep), which is a recombinant fusion protein

of a soluble portion of LAG3 and Ig that binds to MHC class II molecules and activates antigen presenting cells (APC). Other antibodies are disclosed, e.g., in WO2010/019570.

In some embodiments, the agent which enhances the activity of a CAR-expressing cell can be, e.g., a fusion protein comprising a first domain and a second domain, wherein the first domain is an inhibitory molecule, or fragment thereof, and the second domain is a polypeptide that is associated with a positive signal, e.g., a polypeptide comrpsing an antracellular signaling domain as described herein. In some embodiments, the polypeptide that is associated with a positive signal can include a costimulatory domain of CD28, CD27, ICOS, e.g., an intracellular signaling domain of CD28, CD27 and/or ICOS, and/or a primary signaling domain, e.g., of CD3 zeta, e.g., described herein. In one embodiment, the fusion protein is expressed by the same cell that expressed the CAR. In another embodiment, the fusion protein is expressed by a cell, e.g., a T cell that does not express a CAR of the present invention.

In one embodiment, the agent which enhances activity of a CAR-expressing cell described herein is miR-17-92.

In one embodiment, the agent which enhances activity of a CAR-described herein is a cytokine. Cytokines have important functions related to T cell expansion, differentiation, survival, and homeostatis. Cytokines that can be administered to the subject receiving a CAR-expressing cell described herein include: IL-2, IL-4, IL-7, IL-9, IL-15, IL-18, and IL-21, or a combination thereof. In preferred embodiments, the cytokine administered is IL-7, IL-15, or IL-21, or a combination thereof. The cytokine can be administered once a day or more than once a day, e.g., twice a day, three times a day, or four times a day. The cytokine can be administered for more than one day, e.g. the cytokine is administered for 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks. For example, the cytokine is administered once a day for 7 days.

In embodiments, the cytokine is administered in combination with CAR-expressing T cells. The cytokine can be administered simultaneously or concurrently with the CAR-expressing T cells, e.g., administered on the same day. The cytokine may be prepared in the same pharmaceutical composition as the CAR-expressing T cells, or may be prepared in a separate pharmaceutical composition. Alternatively, the cytokine can be administered shortly after administration of the CAR-expressing T cells, e.g., 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the CAR-expressing T cells. In embodiments where the cytokine is administered in a dosing regimen that occurs over more than one day, the first day of the cytokine dosing regimen can be on the same day as administration with the CAR-expressing T cells, or the first day of the cytokine dosing regimen can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, or 7 days after administration of the CAR-expressing T cells. In one embodiment, on the first day, the CAR-expressing T cells are administered to the subject, and on the second day, a cytokine is administered once a day for the next 7 days. In a preferred embodiment, the cytokine to be administered in combination with CAR-expressing T cells is IL-7, IL-15, or IL-21.

In other embodiments, the cytokine is administered a period of time after administration of CAR-expressing cells, e.g., at least 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of CAR-expressing cells. In one embodiment, the cytokine is administered after assessment of the subject's response to the CAR-expressing cells. For example, the subject is administered CAR-expressing cells according to the dosage and regimens described herein. The response of the subject to CAR-expressing cell therapy is assessed at 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 12 weeks, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, or 1 year or more after administration of CAR-expressing cells, using any of the methods described herein, including inhibition of tumor growth, reduction of circulating tumor cells, or tumor regression. Subjects that do not exhibit a sufficient response to CAR-expressing cell therapy can be administered a cytokine. Administration of the cytokine to the subject that has sub-optimal response to the CAR-expressing cell therapy improves CAR-expressing cell efficacy or anti-cancer activity. In a preferred embodiment, the cytokine administered after administration of CAR-expressing cells is IL-7.

Combination with a low dose of an mTOR inhibitor

In one embodiment, the cells expressing a CAR molecule, e.g., a CAR molecule described herein, are administered in combination with a low, immune enhancing dose of an mTOR inhibitor.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 90%, at least 10 but no more than 90%, at least 15, but no more than 90%, at least 20 but no more than 90%, at least 30 but no more than 90%, at least 40 but no more than 90%, at least 50 but no more than 90%, at least 60 but no more than 90%, or at least 70 but no more than 90%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 80%, at least 10 but no more than 80%, at least 15, but no more than 80%, at least 20 but no more than 80%, at least 30 but no more than 80%, at least 40 but no more than 80%, at least 50 but no more than 80%, or at least 60 but no more than 80%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 70%, at least 10 but no more than 70%, at least 15, but no more than 70%, at least 20 but no more than 70%, at least 30 but no more than 70%, at least 40 but no more than 70%, or at least 50 but no more than 70%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 60%, at least 10 but no more than 60%, at least 15, but no more than 60%, at least 20 but no more than 60%, at least 30 but no more than 60%, or at least 40 but no more than 60%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 50%, at least 10 but no more than 50%, at least 15, but no more than 50%, at least 20 but no more than 50%, at least 30 but no more than 50%, or at least 40 but no more than 50%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 40%, at least 10 but no more than 40%, at least 15, but no more than 40%, at least 20 but no more than 40%, at least 30 but no more than 40%, or at least 35 but no more than 40%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 5 but no more than 30%, at least 10 but no more than 30%, at least 15, but no more than 30%, at least 20 but no more than 30%, or at least 25 but no more than 30%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 1, 2, 3, 4 or 5 but no more than 20%, at least 1, 2, 3, 4 or 5 but no more than 30%, at least 1, 2, 3, 4 or 5, but no more than 35, at least 1, 2, 3, 4 or 5 but no more than 40%, or at least 1, 2, 3, 4 or 5 but no more than 45%.

In an embodiment, a dose of an mTOR inhibitor is associated with, or provides, mTOR inhibition of at least 1, 2, 3, 4 or 5 but no more than 90%.

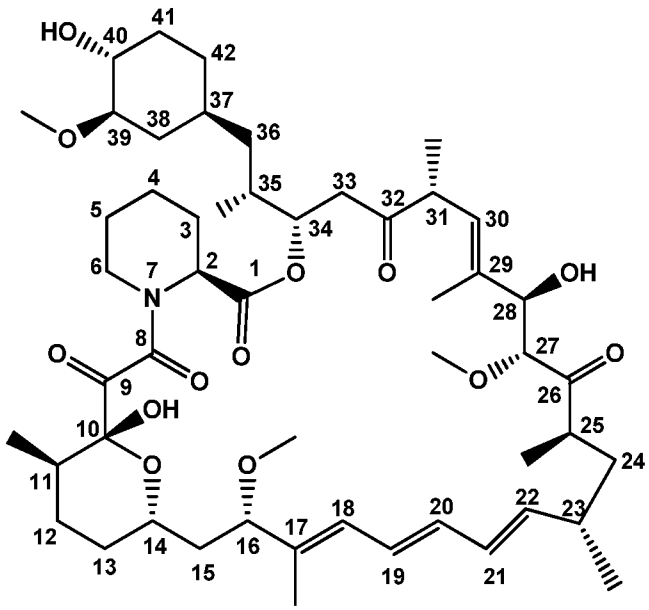
As is discussed herein, the extent of mTOR inhibition can be expressed as the extent of P70 S6 kinase inhibition, e.g., the extent of mTOR inhibition can be determined by the level of decrease in P70 S6 kinase activity, e.g., by the decrease in phosphorylation of a P70 S6 kinase substrate. The level of mTOR inhibition can be evaluated by a method described herein, e.g. by the Boulay assay, or measurement of phosphorylated S6 levels by western blot.

Exemplary mTOR Inhibitors

As used herein, the term "mTOR inhibitor" refers to a compound or ligand, or a pharmaceutically acceptable salt thereof, which inhibits the mTOR kinase in a cell. In an embodiment an mTOR inhibitor is an allosteric inhibitor. In an embodiment an mTOR inhibitor is a catalytic inhibitor.

Allosteric mTOR inhibitors include the neutral tricyclic compound rapamycin (sirolimus), rapamycin-related compounds, that is compounds having structural and functional similarity to rapamycin including, e.g., rapamycin derivatives, rapamycin analogs (also referred to as rapalogs) and other macrolide compounds that inhibit mTOR activity.

Rapamycin is a known macrolide antibiotic produced by Streptomyces hygroscopicus having the structure shown in Formula A.



(A)

See, e.g., McAlpine, J.B., et al., J. Antibiotics (1991) 44: 688; Schreiber, S.L., et al., J. Am. Chem. Soc. (1991) 113: 7433; U.S. Patent No. 3,929,992. There are various numbering schemes proposed for rapamycin. To avoid confusion, when specific rapamycin analogs are named herein, the names are given with reference to rapamycin using the numbering scheme of formula A.

Rapamycin analogs useful in the invention are, for example, O-substituted analogs in which the hydroxyl group on the cyclohexyl ring of rapamycin is replaced by OR_1 in which R_1 is hydroxyalkyl, hydroxyalkoxyalkyl, acylaminoalkyl, or aminoalkyl; e.g. RAD001, also known as, everolimus as described in US 5,665,772 and WO94/09010 the contents of which are incorporated by reference. Other suitable rapamycin analogs include those substituted at the 26- or 28-position. The rapamycin analog may be an epimer of an analog mentioned above, particularly an epimer of an analog substituted in position 40, 28 or 26, and may optionally be further hydrogenated, e.g. as described in US 6,015,815, WO95/14023 and WO99/15530 the contents of which are incorporated by reference, e.g. ABT578 also known as zotarolimus or a rapamycin analog described in US 7,091,213, WO98/02441 and WO01/14387 the contents of which are incorporated by reference, e.g. AP23573 also known as ridaforolimus.

Examples of rapamycin analogs suitable for use in the present invention from US 5,665,772 include, but are not limited to, 40-O-benzyl-rapamycin, 40-O-(4'-hydroxymethyl)benzyl-rapamycin, 40-O-[4'-(1,2-dihydroxyethyl)]benzyl-rapamycin, 40-O-allyl-rapamycin, 40-O-[3'-(2,2-dimethyl-1,3-dioxolan-4(S)-yl)-prop-2'-en-1'-yl]-rapamycin, (2'E,4'S)-40-O-(4',5'-dihydroxypent-2'-en-1'-yl)-rapamycin, 40-O-(2-hydroxy)ethoxycarbonylmethyl-rapamycin, 40-O-(2-hydroxy)ethyl-rapamycin , 40-O-(3-hydroxy)propyl-rapamycin, 40-O-(6-hydroxy)hexyl-rapamycin, 40-O-[2-(2-

hydroxy)ethoxy]ethyl-rapamycin, 40-O-[(3S)-2,2-dimethyldioxolan-3-yl]methyl-rapamycin, 40-O-[(2S)-2,3-dihydroxyprop-1-yl]-rapamycin, 40-O-(2-acetoxy)ethyl-rapamycin, 40-O-(2-nicotinoyloxy)ethyl-rapamycin, 40-O-[2-(N-morpholino)acetoxy]ethyl-rapamycin, 40-O-(2-N-imidazolylacetoxy)ethyl-rapamycin, 40-O-[2-(N-methyl-N'-piperazinyl)acetoxy]ethyl-rapamycin, 39-O-desmethyl-39,40-O,O-ethylene-rapamycin, (26R)-26-dihydro-40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(2-aminoethyl)-rapamycin, 40-O-(2-acetaminoethyl)-rapamycin, 40-O-(2-nicotinamidoethyl)-rapamycin, 40-O-(2-(N-methyl-imidazo-2'-ylcarbethoxamido)ethyl)-rapamycin, 40-O-(2-ethoxycarbonylaminoethyl)-rapamycin, 40-O-(2-tolylsulfonamidoethyl)-rapamycin and 40-O-[2-(4',5'-dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin.

Other rapamycin analogs useful in the present invention are analogs where the hydroxyl group on the cyclohexyl ring of rapamycin and/or the hydroxy group at the 28 position is replaced with an hydroxyester group are known, for example, rapamycin analogs found in US RE44,768, e.g. temsirolimus.

Other rapamycin analogs useful in the preset invention include those wherein the methoxy group at the 16 position is replaced with another substituent, preferably (optionally hydroxy-substituted) alkynyloxy, benzyl, orthomethoxybenzyl or chlorobenzyl and/or wherein the mexthoxy group at the 39 position is deleted together with the 39 carbon so that the cyclohexyl ring of rapamycin becomes a cyclopentyl ring lacking the 39 position methyoxy group; e.g. as described in WO95/16691 and WO96/41807 the contents of which are incorporated by reference. The analogs can be further modified such that the hydroxy at the 40-position of rapamycin is alkylated and/or the 32-carbonyl is reduced.

Rapamycin analogs from WO95/16691 include, but are not limited to, 16-demthoxy-16-(pent-2-ynyl)oxy-rapamycin, 16-demthoxy-16-(but-2-ynyl)oxy-rapamycin, 16-demthoxy-16-(propargyl)oxy-rapamycin, 16-demethoxy-16-(4-hydroxy-but-2-ynyl)oxy-rapamycin, 16-demthoxy-16-benzyloxy-40-O-(2-hydroxyethyl)-rapamycin, 16-demthoxy-16-benzyloxy-rapamycin, 16-demethoxy-16-ortho-methoxybenzyl-rapamycin, 16-demethoxy-40-O-(2-methoxyethyl)-16-pent-2-ynyl)oxy-rapamycin, 39-demethoxy-40-desoxy-39-formyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-hydroxymethyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-carboxy-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-(4-methyl-piperazin-1-yl)carbonyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-(morpholin-4-yl)carbonyl-42-nor-rapamycin, 39-demethoxy-40-desoxy-39-[N-methyl, N-(2-pyridin-2-yl-ethyl)]carbamoyl-42-nor-rapamycin and 39-demethoxy-40-desoxy-39-(p-toluenesulfonylhydrazonomethyl)-42-nor-rapamycin.

Rapamycin analogs from WO96/41807 include, but are not limited to, 32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-rapamycin, 16-O-pent-2-ynyl-32-deoxo-40-O-(2-hydroxy-ethyl)-rapamycin, 16-O-pent-2-ynyl-32-(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin, 32(S)-dihydro-40-O-(2-methoxy)ethyl-rapamycin and 32(S)-dihydro-40-O-(2-hydroxyethyl)-rapamycin.

Another suitable rapamycin analog is umirolimus as described in US2005/0101624 the contents of which are incorporated by reference.

RAD001, otherwise known as everolimus (Afinitor®), has the chemical name (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.04,9]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone

Further examples of allosteric mTOR inhibitors include sirolimus (rapamycin, AY-22989), 40-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin (also called temsirolimus or CCI-779) and ridaforolimus (AP-23573/MK-8669). Other examples of allosteric mTor inhibtors include zotarolimus (ABT578) and umirolimus.

Alternatively or additionally, catalytic, ATP-competitive mTOR inhibitors have been found to target the mTOR kinase domain directly and target both mTORC1 and mTORC2. These are also more effective inhibitors of mTORC1 than such allosteric mTOR inhibitors as rapamycin, because they modulate rapamycin-resistant mTORC1 outputs such as 4EBP1-T37/46 phosphorylation and cap-dependent translation.

Catalytic inhibitors include: BEZ235 or 2-methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-yl-2,3-dihydro-imidazo[4,5-c]quinolin-1-yl)-phenyl]-propionitrile, or the monotosylate salt form. the synthesis of BEZ235 is described in WO2006/122806; CCG168 (otherwise known as AZD-8055, Chresta, C.M., et al., Cancer Res, 2010, 70(1), 288-298) which has the chemical name {5-[2,4-bis-((S)-3-methyl-morpholin-4-yl)-pyrido[2,3d]pyrimidin-7-yl]-2-methoxy-phenyl}-methanol; 3-[2,4-bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl]-N-methylbenzamide (WO09104019); 3-(2-aminobenzo[d]oxazol-5-yl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (WO10051043 and WO2013023184); A N-(3-(N-(3-((3,5-dimethoxyphenyl)amino)quinoxaline-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide (WO07044729 and WO12006552); PKI-587 (Venkatesan, A.M., J. Med.Chem., 2010, 53, 2636-2645) which has the chemical name 1-[4-[4-(dimethylamino)piperidine-1-carbonyl]phenyl]-3-[4-(4,6-dimorpholino-1,3,5-triazin-2-yl)phenyl]urea; GSK-2126458 (ACS Med. Chem. Lett., 2010, 1, 39-43) which has the chemical name 2,4-difluoro-N-{2-methoxy-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide; ; 5-(9-isopropyl-8-methyl-2-morpholino-9H-purin-6-yl)pyrimidin-2-amine (WO10114484); (E)-N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide (WO12007926).

Further examples of catalytic mTOR inhibitors include 8-(6-methoxy-pyridin-3-yl)-3-methyl-1-(4-piperazin-1-yl-3-trifluoromethyl-phenyl)-1,3-dihydro-imidazo[4,5-c]quinolin-2-one (WO2006/122806) and Ku-0063794 (Garcia-Martinez JM, et al.,Biochem J., 2009, 421(1), 29-42. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR).) WYE-354 is another example of a catalytic mTor inhibitor (Yu K, et al. (2009). Biochemical, Cellular, and In vivo

Activity of Novel ATP-Competitive and Selective Inhibitors of the Mammalian Target of Rapamycin. Cancer Res. 69(15): 6232-6240).

mTOR inhibitors useful according to the present invention also include prodrugs, derivatives, pharmaceutically acceptable salts, or analogs thereof of any of the foregoing.

mTOR inhibitors, such as RAD001, may be formulated for delivery based on well-established methods in the art based on the particular dosages described herein. In particular, US Patent 6,004,973 (incorporated herein by reference) provides examples of formulations useable with the mTOR inhibitors described herein.

Evaluation of mTOR Inhibition

mTOR phosphorylates the kinase P70 S6, thereby activating P70 S6 kinase and allowing it to phosphorylate its substrate. The extent of mTOR inhibition can be expressed as the extent of P70 S6 kinase inhibition, e.g., the extent of mTOR inhibition can be determined by the level of decrease in P70 S6 kinase activity, e.g., by the decrease in phosphorylation of a P70 S6 kinase substrate. One can determine the level of mTOR inhibition, by measuring P70 S6 kinase activity (the ability of P70 S6 kinase to phsophorylate a substrate), in the absence of inhibitor, e.g., prior to administration of inhibitor, and in the presences of inhibitor, or after the administration of inhibitor. The level of inhibition of P70 S6 kinase gives the level of mTOR inhibition. Thus, if P70 S6 kinase is inhibited by 40%, mTOR activity, as measured by P70 S6 kinase activity, is inhibited by 40%. The extent or level of inhibition referred to herein is the average level of inhibition over the dosage interval. By way of example, if the inhibitor is given once per week, the level of inhibition is given by the average level of inhibition over that interval, namely a week.

Boulay et al., *Cancer Res*, 2004, 64:252-61, hereby incorporated by reference, teaches an assay that can be used to assess the level of mTOR inhibition (referred to herein as the Boulay assay). In an embodiment, the assay relies on the measurement of P70 S6 kinase activity from biological samples before and after administration of an mTOR inhibitor, e.g., RAD001. Samples can be taken at preselected times after treatment with an mTOR ihibitor, e.g., 24, 48, and 72 hours after treatment. Biological samples, e.g., from skin or peripheral blood mononuclear cells (PBMCs) can be used. Total protein extracts are prepared from the samples. P70 S6 kinase is isolated from the protein extracts by immunoprecipitation using an antibody that specifically recognizes the P70 S6 kinase. Activity of the isolated P70 S6 kinase can be measured in an in vitro kinase assay. The isolated kinase can be incubated with 40S ribosomal subunit substrates (which is an endogenous substrate of P70 S6 kinase) and gamma-^{32}P under conditions that allow phosphorylation of the substrate. Then the reaction mixture can be resolved on an SDS-PAGE gel, and ^{32}P signal analyzed using a PhosphorImager. A ^{32}P signal corresponding to the size of the 40S ribosomal subunit indicates phosphorylated substrate and the activity of P70 S6 kinase. Increases and decreases in kinase activity can be calculated by quantifying the area and intensity of the ^{32}P signal of the phosphorylated

substrate (e.g., using ImageQuant, Molecular Dynamics), assigning arbitrary unit values to the quantified signal, and comparing the values from after administration with values from before administration or with a reference value. For example, percent inhibition of kinase activity can be calculated with the following formula: 1-(value obtained after administration/value obtained before administration) X 100. As described above, the extent or level of inhibition referred to herein is the average level of inhibition over the dosage interval.

Methods for the evaluation of kinase activity, e.g., P70 S6 kinase activity, are also provided in US 7,727,950, hereby incorporated by reference.

The level of mTOR inhibition can also be evaluated by a change in the ration of PD1 negative to PD1 positive T cells. T cells from peripheral blood can be identified as PD1 negative or positive by art-known methods.

Low-Dose mTOR Inhibitors

Methods described herein use low, immune enhancing, dose mTOR inhibitors, doses of mTOR inhibitors, e.g., allosteric mTOR inhibitors, including rapalogs such as RAD001. In contrast, levels of inhibitor that fully or near fully inhibit the mTOR pathway are immunosuppressive and are used, e.g., to prevent organ transplant rejection. In addition, high doses of rapalogs that fully inhibit mTOR also inhibit tumor cell growth and are used to treat a variety of cancers (See, e.g., Antineoplastic effects of mammalian target of rapamycine inhibitors. Salvadori M. World J Transplant. 2012 Oct 24;2(5):74-83; Current and Future Treatment Strategies for Patients with Advanced Hepatocellular Carcinoma: Role of mTOR Inhibition. Finn RS. Liver Cancer. 2012 Nov;1(3-4):247-256; Emerging Signaling Pathways in Hepatocellular Carcinoma. Moeini A, Cornellà H, Villanueva A. Liver Cancer. 2012 Sep;1(2):83-93; Targeted cancer therapy - Are the days of systemic chemotherapy numbered? Joo WD, Visintin I, Mor G. Maturitas. 2013 Sep 20.; *Role of natural and adaptive immunity in renal cell carcinoma response to VEGFR-TKIs and mTOR inhibitor.* Santoni M, Berardi R, Amantini C, Burattini L, Santini D, Santoni G, Cascinu S. Int J Cancer. 2013 Oct 2).

The present invention is based, at least in part, on the surprising finding that doses of mTOR inhibitors well below those used in current clinical settings had a superior effect in increasing an immune response in a subject and increasing the ratio of PD-1 negative T cells/PD-1 positive T cells. It was surprising that low doses of mTOR inhibitors, producing only partial inhibition of mTOR activity, were able to effectively improve immune responses in human human subjects and increase the ratio of PD-1 negative T cells/PD-1 positive T cells.

Alternatively, or in addition, without wishing to be bound by any theory, it is believed that a low, immune enhancing, dose of an mTOR inhibitor can increase naive T cell numbers, e.g., at least transiently, e.g., as compared to a non-treated subject. Alternatively or additionally, again while not

wishing to be bound by theory, it is believed that treatment with an mTOR inhibitor after a sufficient amount of time or sufficient dosing 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;

and wherein any of the changes described above occurs, e.g., at least transiently, e.g., as compared to a non-treated subject (Araki, K et al. (2009) *Nature* 460:108-112). Memory T cell precursors are memory T cells that are early in the differentiation program. For example, memory T cells have one or more of the following characteristics: increased $CD62L^{high}$, increased $CD127^{high}$, increased $CD27^{+}$, decreased KLRG1, and/or increased BCL2.

In an embodiment, the invention relates to a composition, or dosage form, of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., a rapalog, rapamycin, or RAD001, or a catalytic mTOR inhibitor, which, when administered on a selected dosing regimen, e.g., once daily or once weekly, is associated with: a level of mTOR inhibition that is not associated with complete, or significant immune suppression, but is associated with enhancement of the immune response.

An mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., a rapalog, rapamycin, or RAD001, or a catalytic mTOR inhibitor, can be provided in a sustained relase formulation. Any of the compositions or unit dosage forms described herein can be provided in a sustained release formulation. In some embodiments, a sustained release formulation will have lower bioavailability than an immediate release formulation. E.g., in embodiments, to attain a similar therapeutic effect of an immediate release forlation a sustained release formulation will have from about 2 to about 5, about 2.5 to about 3.5, or about 3 times the amount of inhibitor provided in the immediate release formulation.

In an embodiment, immediate release forms, e.g., of RAD001, typically used for one administration per week, having 0.1 to 20, 0.5 to 10, 2.5 to 7.5, 3 to 6, or about 5, mgs per unit dosage form, are provided. For once per week administrations, these immediate release formulations correspond to sustained release forms, having, respectively, 0.3 to 60, 1.5 to 30, 7.5 to 22.5, 9 to 18, or about 15 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001. In embodiments both forms are administered on a once/week basis.

In an embodiment, immediate release forms, e.g., of RAD001, typically used for one administration per day, having having 0.005 to 1.5, 0.01 to 1.5, 0.1 to 1.5, 0.2 to 1.5, 0.3 to 1.5, 0.4 to 1.5, 0.5 to 1.5, 0.6 to 1.5, 0.7 to 1.5, 0.8 to 1.5, 1.0 to 1.5, 0.3 to 0.6, or about 0.5 mgs per unit dosage

form, are provided. For once per day administrations, these immediate release forms correspond to sustained release forms, having, respectively, 0.015 to 4.5, 0.03 to 4.5, 0.3 to 4.5, 0.6 to 4.5, 0.9 to 4.5, 1.2 to 4.5, 1.5 to 4.5, 1.8 to 4.5, 2.1 to 4.5, 2.4 to 4.5, 3.0 to 4.5, 0.9 to 1.8, or about 1.5 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001. For once per week administrations, these immediate release forms correspond to sustained release forms, having, respectively, 0.1 to 30, 0.2 to 30, 2 to 30, 4 to 30, 6 to 30, 8 to 30, 10 to 30, 1.2 to 30, 14 to 30, 16 to 30, 20 to 30, 6 to 12, or about 10 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001.

In an embodiment, immediate release forms, e.g., of RAD001, typically used for one administration per day, having having 0.01 to 1.0 mgs per unit dosage form, are provided. For once per day administrations, these immediate release forms correspond to sustained release forms, having, respectively, 0.03 to 3 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001.For once per week administrations, these immediate release forms correspond to sustained release forms, having, respectively, 0.2 to 20 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001.

In an embodiment, immediate release forms, e.g., of RAD001, typically used for one administration per week, having having 0.5 to 5.0 mgs per unit dosage form, are provided. For once per week administrations, these immediate release forms correspond to sustained release forms, having, respectively, 1.5 to 15 mgs of an mTOR inhibitor, e.g., an allosteric mTOR inhibitor, e.g., rapamycin or RAD001.

As described above, one target of the mTOR pathway is the P70 S6 kinase. Thus, doses of mTOR inhibitors which are useful in the methods and compositions described herein are those which are sufficient to achieve no greater than 80% inhibition of P70 S6 kinase activity relative to the activity of the P70 S6 kinase in the absence of an mTOR inhibitor, e.g., as measured by an assay described herein, e.g., the Boulay assay. In a further aspect, the invention provides an amount of an mTOR inhibitor sufficient to achieve no greater than 38% inhibition of P70 S6 kinase activity relative to P70 S6 kinase activity in the absence of an mTOR inhibitor.

In one aspect the dose of mTOR inhibitor useful in the methods and compositions of the invention is sufficient to achieve, e.g., when administered to a human subject, 90 +/-5 % (i.e., 85-95%), 89+/-5 %, 88+/-5 %, 87+/-5 %, 86+/-5 %, 85+/-5 %, 84+/-5 %, 83+/-5 %, 82+/-5 %, 81+/-5 %, 80+/-5 %, 79+/-5 %, 78+/-5 %, 77+/-5 %, 76+/-5 %, 75+/-5 %, 74+/-5 %, 73+/-5 %, 72 +/-5%, 71 +/-5%, 70 +/-5%, 69 +/-5%, 68 +/-5%, 67 +/-5%, 66 +/-5%, 65 +/-5%, 64 +/-5%, 63 +/-5%, 62 +/-5%, 61 +/-5%, 60 +/-5%, 59 +/-5%, 58 +/-5%, 57 +/-5%, 56 +/-5%, 55 +/-5%, 54 +/-5%, 54 +/-5%, 53 +/-5%, 52 +/-5%, 51 +/-5%, 50 +/-5%, 49 +/-5%, 48 +/-5%, 47 +/-5%, 46 +/-5%, 45 +/-5%, 44 +/-5%, 43 +/-5%, 42 +/-5%, 41 +/-5%, 40 +/-5%, 39 +/-5%, 38 +/-5%, 37 +/-5%, 36 +/-5%, 35 +/-5%, 34 +/-5%, 33 +/-5%, 32 +/-5%, 31 +/-5%, 30 +/-5%, 29 +/-5%, 28 +/-5%, 27 +/-5%, 26 +/-5%, 25 +/-5%, 24 +/-5%, 23 +/-5%, 22 +/-5%, 21 +/-5%, 20 +/-5%, 19 +/-5%, 18 +/-5%, 17 +/-5%, 16 +/-5%, 15 +/-

5%, 14 +/-5%, 13 +/-5%, 12 +/-5%, 11 +/-5%, or 10 +/-5%, inhibition of P70 S6 kinase activity , e.g., as measured by an assay described herein, e.g., the Boulay assay.

P70 S6 kinase activity in a subject may be measured using methods known in the art, such as, for example, according to the methods described in U.S. Pat. 7,727,950, by immunoblot analysis of phosphoP70 S6K levels and/or phosphoP70 S6 levels or by in vitro kinase activity assays.

As used herein, the term "about" in reference to a dose of mTOR inhibitor refers to up to a +/-10% variability in the amount of mTOR inhibitor, but can include no variability around the stated dose.

In some embodiments, the invention provides methods comprising administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage within a target trough level. In some embodiments, the trough level is significantly lower than trough levels associated with dosing regimens used in organ transplant and cancer patients. In an embodiment mTOR inhibitor, e.g., RAD001, or rapamycin, is administerd to result in a trough level that is less than ½, 1/4, 1/10, or 1/20 of the trough level that results in immunosuppression or an anticancer effect. In an embodiment mTOR inhibitor, e.g., RAD001, or rapamycin, is administerd to result in a trough level that is less than ½, 1/4, 1/10, or 1/20 of the trough level provided on the FDA approved packaging insert for use in immunosuppression or an anticancer indications.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 0.1 to 10 ng/ml, 0.1 to 5 ng/ml, 0.1 to 3ng/ml, 0.1 to 2 ng/ml, or 0.1 to 1 ng/ml.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 0.2 to 10 ng/ml, 0.2 to 5 ng/ml, 0.2 to 3ng/ml, 0.2 to 2 ng/ml, or 0.2 to 1 ng/ml.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g. an, allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 0.3 to 10 ng/ml, 0.3 to 5 ng/ml, 0.3 to 3ng/ml, 0.3 to 2 ng/ml, or 0.3 to 1 ng/ml.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 0.4 to 10 ng/ml, 0.4 to 5 ng/ml, 0.4 to 3ng/ml, 0.4 to 2 ng/ml, or 0.4 to 1 ng/ml.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 0.5 to 10 ng/ml, 0.5 to 5 ng/ml, 0.5 to 3ng/ml, 0.5 to 2 ng/ml, or 0.5 to 1 ng/ml.

In an embodiment a method disclosed herein comprises administering to a subject an mTOR inhibitor, e.g., an allosteric inhibitor, e.g., RAD001, at a dosage that provides a target trough level of 1 to 10 ng/ml, 1 to 5 ng/ml, 1 to 3ng/ml, or 1 to 2 ng/ml.

As used herein, the term "trough level" refers to the concentration of a drug in plasma just before the next dose, or the minimum drug conce ntration between two doses.

In some embodiments, a target trough level of RAD001 is in a range of between about 0.1 and 4.9 ng/ml. In an embodiment, the target trough level is below 3ng/ml, e.g., is between 0.3 or less and 3 ng/ml. In an embodiment, the target trough level is below 3ng/ml, e.g., is between 0.3 or less and 1 ng/ml.

In a further aspect, the invention can utilize an mTOR inhibitor other than RAD001 in an amount that is associated with a target trough level that is bioequivalent to the specified target trough level for RAD001. In an embodiment, the target trough level for an mTOR inhibitor other than RAD001, is a level that gives the same level of mTOR inhibition (e.g., as measured by a method described herein, e.g., the inhibition of P70 S6) as does a trough level of RAD001 described herein.

Pharmaceutical compositions: mTOR Inhibitors

In one aspect, the present invention relates to pharmaceutical compositions comprising an mTOR inhibitor, e.g., an mTOR inhibitor as described herein, formulated for use in combination with CAR cells described herein.

In some embodiments, the mTOR inhibitor is formulated for administration in combination with an additional, e.g., as described herein.

In general, compounds of the invention will be administered in therapeutically effective amounts as described above via any of the usual and acceptable modes known in the art, either singly or in combination with one or more therapeutic agents.

The pharmaceutical formulations may be prepared using conventional dissolution and mixing procedures. For example, the bulk drug substance (e.g., an mTOR inhibitor or stabilized form of the compound (e.g., complex with a cyclodextrin derivative or other known complexation agent) is dissolved in a suitable solvent in the presence of one or more of the excipients described herein. The mTOR inhibitor is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the patient an elegant and easily handleable product.

Compounds of the invention can be administered as pharmaceutical compositions by any conventional route, in particular enterally, e.g., orally, e.g., in the form of tablets or capsules, or parenterally, e.g., in the form of injectable solutions or suspensions, topically, e.g., in the form of lotions, gels, ointments or creams, or in a nasal or suppository form. Where an mTOR inhibitor is administered in combination with (either simultaneously with or separately from) another agent as described herein, in one aspect, both components can be administered by the same route (e.g., parenterally). Alternatively, another agent may be administered by a different route relative to the mTOR inhibitor. For example, an mTOR inhibitor may be administered orally and the other agent may be administered parenterally.

SUSTAINED RELEASE

mTOR inhibitors, e.g., allosteric mTOR inhibitors or catalytic mTOR inhibitors, disclosed herein can be provided as pharmaceutical formulations in form of oral solid dosage forms comprising

an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, which satisfy product stability requirements and/or have favorable pharmacokinetic properties over the immediate release (IR) tablets, such as reduced average plasma peak concentrations, reduced inter- and intra-patient variability in the extent of drug absorption and in the plasma peak concentration, reduced C_{max} / C_{min} ratio and/or reduced food effects. Provided pharmaceutical formulations may allow for more precise dose adjustment and/or reduce frequency of adverse events thus providing safer treatments for patients with an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001.

In some embodiments, the present disclosure provides stable extended release formulations of an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, which are multi-particulate systems and may have functional layers and coatings.

The term "extended release, multi-particulate formulation as used herein refers to a formulation which enables release of an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, over an extended period of time e.g. over at least 1, 2, 3, 4, 5 or 6 hours. The extended release formulation may contain matrices and coatings made of special excipients, e.g., as described herein, which are formulated in a manner as to make the active ingredient available over an extended period of time following ingestion.

The term "extended release" can be interchangeably used with the terms "sustained release" (SR) or "prolonged release". The term "extended release" relates to a pharmaceutical formulation that does not release active drug substance immediately after oral dosing but over an extended in accordance with the definition in the pharmacopoeias Ph. Eur. (7^{th} edition) mongraph for tablets and capsules and USP general chapter <1151> for pharmaceutical dosage forms. The term "Immediate Release" (IR) as used herein refers to a pharmaceutical formulation which releases 85% of the active drug substance within less than 60 minutes in accordance with the definition of "Guidance for Industry: "Dissolution Testing of Immediate Release Solid Oral Dosage Forms" (FDA CDER, 1997). In some embodiments, the term "immediate release" means release of everolismus from tablets within the time of 30 minutes, e.g., as measured in the dissolution assay described herein.

Stable extended release formulations of an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, can be characterized by an in-vitro release profile using assays known in the art, such as a dissolution assay as described herein: a dissolution vessel filled with 900 mL phosphate buffer pH 6.8 containing sodium dodecyl sulfate 0.2% at 37°C and the dissolution is performed using a paddle method at 75 rpm according to USP by according to USP testing monograph 711, and Ph.Eur. testing monograph 2.9.3. respectively.

In some embodiments, stable extended release formulations of an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, release the mTOR inhibitor in the in-vitro release assay according to following release specifications:

0.5h: <45%, or <40, e.g., <30%

1h: 20-80%, e.g., 30-60%

2h: >50%, or >70%, e.g., >75%

3h: >60%, or >65%, e.g., >85%, e.g., >90%.

In some embodiments, stable extended release formulations of an mTOR inhibitor disclosed herein, e.g., rapamycin or RAD001, release 50% of the mTOR inhibitor not earlier than 45, 60, 75, 90, 105 min or 120 min in the in-vitro dissolution assay.

Biopolymer delivery methods

In some embodiments, one or more CAR-expressing cells as disclosed herein can be administered or delivered to the subject via a biopolymer scaffold, e.g., a biopolymer implant. Biopolymer scaffolds can support or enhance the delivery, expansion, and/or dispersion of the CAR-expressing cells described herein. A biopolymer scaffold comprises a biocompatible (e.g., does not substantially induce an inflammatory or immune response) and/or a biodegradable polymer that can be naturally occurring or synthetic.

Examples of suitable biopolymers include, but are not limited to, agar, agarose, alginate, alginate/calcium phosphate cement (CPC), beta-galactosidase (β-GAL), (1 ,2,3,4,6-pentaacetyl a-D-galactose), cellulose, chitin, chitosan, collagen, elastin, gelatin, hyaluronic acid collagen, hydroxyapatite, poly(3-hydroxybutyrate-co-3-hydroxy-hexanoate) (PHBHHx), poly(lactide), poly(caprolactone) (PCL), poly(lactide-co-glycolide) (PLG), polyethylene oxide (PEO), poly(lactic-co-glycolic acid) (PLGA), polypropylene oxide (PPO), polyvinyl alcohol) (PVA), silk, soy protein, and soy protein isolate, alone or in combination with any other polymer composition, in any concentration and in any ratio. The biopolymer can be augmented or modified with adhesion- or migration-promoting molecules, e.g., collagen-mimetic peptides that bind to the collagen receptor of lymphocytes, and/or stimulatory molecules to enhance the delivery, expansion, or function, e.g., anti-cancer activity, of the cells to be delivered. The biopolymer scaffold can be an injectable, e.g., a gel or a semi-solid, or a solid composition.

In some embodiments, CAR-expressing cells described herein are seeded onto the biopolymer scaffold prior to delivery to the subject. In embodiments, the biopolymer scaffold further comprises one or more additional therapeutic agents described herein (e.g., another CAR-expressing cell, an antibody, or a small molecule) or agents that enhance the activity of a CAR-expressing cell, e.g., incorporated or conjugated to the biopolymers of the scaffold. In embodiments, the biopolymer scaffold is injected, e.g., intratumorally, or surgically implanted at the tumor or within a proximity of the tumor sufficient to mediate an anti-tumor effect. Additional examples of biopolymer compositions and methods for their delivery are described in Stephan et al., *Nature Biotechnology*, 2015, 33:97-101; and WO2014/110591.

Pharmaceutical Compositions and Treatments

Pharmaceutical compositions of the present invention may comprise a CAR-expressing cell, e.g., a plurality of CAR-expressing cells, as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are in one aspect formulated for intravenous administration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

In one embodiment, the pharmaceutical composition is substantially free of, e.g., there are no detectable levels of a contaminant, e.g., selected from the group consisting of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium and a fungus. In one embodiment, the bacterium is at least one selected from the group consisting of Alcaligenes faecalis, Candida albicans, Escherichia coli, Haemophilus influenza, Neisseria meningitides, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, and Streptococcus pyogenes group A.

When "an immunologically effective amount," "an anti-tumor effective amount," "a tumor-inhibiting effective amount," or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the immune effector cells (e.g., T cells, NK cells) described herein may be administered at a dosage of 10^4 to 10^9 cells/kg body weight, in some instances 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988).

In certain aspects, it may be desired to administer activated immune effector cells (e.g., T cells, NK cells) to a subject and then subsequently redraw blood (or have an apheresis performed), activate immune effector cells (e.g., T cells, NK cells) therefrom according to the present invention, and reinfuse the patient with these activated and expanded immune effector cells (e.g., T cells, NK

cells). This process can be carried out multiple times every few weeks. In certain aspects, immune effector cells (e.g., T cells, NK cells) can be activated from blood draws of from 10cc to 400cc. In certain aspects, immune effector cells (e.g., T cells, NK cells) are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient trans arterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one aspect, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In one aspect, the T cell compositions of the present invention are administered by i.v. injection. The compositions of immune effector cells (e.g., T cells, NK cells) may be injected directly into a tumor, lymph node, or site of infection.

In a particular exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted ex vivo to select and/or isolate the cells of interest, e.g., T cells. These T cell isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the invention may be introduced, thereby creating a CAR T cell of the invention. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR T cells of the present invention. In an additional aspect, expanded cells are administered before or following surgery.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about 100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used (described in U.S. Patent No. 6,120,766).

In one embodiment, the CAR is introduced into immune effector cells (e.g., T cells, NK cells), e.g., using in vitro transcription, and the subject (e.g., human) receives an initial administration of CAR immune effector cells (e.g., T cells, NK cells) of the invention, and one or more subsequent administrations of the CAR immune effector cells (e.g., T cells, NK cells) of the invention, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR immune effector cells (e.g., T cells, NK cells) of the invention are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CAR immune effector cells (e.g., T cells, NK cells) of the invention are administered per week. In one

embodiment, the subject (e.g., human subject) receives more than one administration of the CAR immune effector cells (e.g., T cells, NK cells) per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR immune effector cells (e.g., T cells, NK cells) administrations, and then one or more additional administration of the CAR immune effector cells (e.g., T cells, NK cells) (e.g., more than one administration of the CAR immune effector cells (e.g., T cells, NK cells) per week) is administered to the subject. In another embodiment, the subject (e.g., human subject) receives more than one cycle of CAR immune effector cells (e.g., T cells, NK cells), and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR immune effector cells (e.g., T cells, NK cells) are administered every other day for 3 administrations per week. In one embodiment, the CAR immune effector cells (e.g., T cells, NK cells) of the invention are administered for at least two, three, four, five, six, seven, eight or more weeks.

In one aspect, CAR-expressing cells of the present inventions are generated using lentiviral viral vectors, such as lentivirus. Cells, e.g., CARTs, generated that way will have stable CAR expression.

In one aspect, CAR-expressing cells, e.g., CARTs, are generated using a viral vector such as a gammaretroviral vector, e.g., a gammaretroviral vector described herein. CARTs generated using these vectors can have stable CAR expression.

In one aspect, CARTs transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be effected by RNA CAR vector delivery. In one aspect, the CAR RNA is transduced into the T cell by electroporation.

A potential issue that can arise in patients being treated using transiently expressing CAR immune effector cells (e.g., T cells, NK cells) (particularly with murine scFv bearing CARTs) is anaphylaxis after multiple treatments.

Without being bound by this theory, it is believed that such an anaphylactic response might be caused by a patient developing humoral anti-CAR response, i.e., anti-CAR antibodies having an anti-IgE isotype. It is thought that a patient's antibody producing cells undergo a class switch from IgG isotype (that does not cause anaphylaxis) to IgE isotype when there is a ten to fourteen day break in exposure to antigen.

If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CART infusion breaks should not last more than ten to fourteen days.

EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being

limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1: Disruption of the Methylcytosine Dioxygenase *TET2* Promotes the Therapeutic Efficacy of CD19-targeted T-cells

Summary

Cancer immunotherapy based on genetically redirecting patient T-cells can be used successfully to treat B-cell leukemias and lymphomas. In this approach, the T-cell genome is modified by integration of viral vectors or transposons encoding chimeric antigen receptors (CARs) that direct tumor cell recognition and killing. However, the success of this approach may sometimes be limited by the extent of expansion and persistence of the engineered cells, focusing attention on mechanisms directing CAR T-cell proliferation, effector function and survival. This Example provides mechanistic insights from studies of a patient with chronic lymphocytic leukemia (CLL) who was treated with CAR T-cells that target the CD19 protein on B-cells. Upon infusion of CAR T-cells, anti-tumor activity was evident in the peripheral blood, lymph nodes and bone marrow, and was accompanied by complete remission. Unexpectedly, at the peak of the anti-tumor response, 94% of CAR T-cells originated from a single clone in which lentiviral vector–mediated insertion of the CAR transgene disrupted the gene encoding the methylcytosine dioxygenase TET2. Further analysis revealed a novel hypomorphic mutation in this patient's second TET2 allele. Cells with biallelic TET2 deficiency exhibited reduced DNA hydroxymethylation and an epigenetic profile consistent with altered T-cell differentiation and function. At peak expansion, CAR T-cells with disrupted TET2 showed a central memory phenotype, which differs from typical patients who had long-term durable responses that were instead characterized by late memory/effector differentiation. Experimental knock-down of TET2 recapitulated the effect of TET2 loss on CAR T-lymphocyte fate and anti-tumour potency. The results indicate that TET2 inhibition in this patient promoted T-cell proliferation and enhanced effector function, and that the progeny of a single CAR T-cell induced remission. These data emphasize the importance of TET2 in human T-cell fate determination and suggest that modification of the TET2 pathway can be used to enhance treatment with genetically-redirected T-cells.

Introduction

The human immune system has evolved to recognize and eliminate cells expressing foreign antigens. Although malignant cells may generate neo – "non-self" epitopes that could elicit anti-tumor T-cell immunity, these responses are frequently limited by tolerance of T-cells to the tumor. One approach to overcome tolerance is to genetically re-direct T-lymphocytes to attack cancer cells. T-cells can be transduced with genes that encode chimeric antigen receptors (CARs) composed of

antibody-derived binding moieties linked to the intracellular domain of the CD3ζ chain and optional co-stimulatory endodomains (Gross, G., Waks, T. & Eshhar, Z. *Proceedings of the National Academy of Sciences of the United States of America* 86, 10024-10028 (1989); Irving, B. A. & Weiss, A. *Cell* 64, 891-901 (1991)). One tumor antigen is the CD19 protein, which is found on B-cell derived cancers and has been targeted in successful immunotherapy. For example, autologous anti-CD19 CAR T-cells that incorporate the 4-1BB costimulatory signaling domain (CTL019) can be used to treat B cell malignancies such as CLL and acute lymphocytic leukemia (ALL). In some instances, the success of CAR T-cell therapy may be dependent on achieving sufficient engraftment and survival of adoptively-transferred cells *in vivo*. It was observed that individuals with CLL who respond to CTL019 therapy can have a significant expansion and persistence of CAR-T cells following infusion, whereas in non-responding patients these cells show diminished proliferative capacity. Withoute wishing to be bound by theory, it is believed that the mechanisms involved in the superior anti-tumor potency and long-term survival of CAR T-cells in responding patients include, for example, both T-cell intrinsic and extrinsic factors. Because only a subset of CLL patients experience therapeutic levels of CAR T-cell expansion (Porter, D. L. *et al.*, *Science translational medicine* **7**, 303ra139, doi:10.1126 (2015)), understanding the determinants of successful proliferation and persistence in cases of durable remission is of critical importance.

In this Example, a patient with chronic lymphocytic leukemia (CLL) who was treated with CAR T-cells that target the CD19 protein on B-cells was evaluated. Upon infusion of CAR T-cells, anti-tumor activity was evident in the peripheral blood, lymph nodes and bone marrow, and was accompanied by complete remission. At the peak of the anti-tumor response, 94% of CAR T-cells originated from a single clone in which lentiviral vector–mediated insertion of the CAR transgene disrupted the gene encoding the methylcytosine dioxygenase TET2. Cells harboring this insertion exhibited reduced DNA hydroxymethylation and acquisition of an epigenetic profile consistent with altered T-cell differentiation. At peak expansion, CAR T-cells with the *TET2* insertion showed an early memory phenotype, which differs from typical patients with long-term durable responses that are instead characterized by late memory differentiation. Experimental knockdown of *TET2* in healthy donor T-cells recapitulated the effect of integration-mediated *TET2* disruption on CAR T-lymphocyte fate. It was concluded that reduced activity of TET2, e.g., biallelic disruption, was associated with insertional mutagenesis promoted T-cell proliferation, and that the progeny of a single CAR T-cell induced remission in this patient, i.e., referred to herein as patient 10. These data emphasize the importance of *TET2* in T-cell differentiation and suggest that modification of the TET2 pathway may be useful for enhancing treatment with genetically-redirected T-cells.

Results

A seventy-eight-year-old man with advanced CLL who progressed through multiple chemotherapy and biologic treatment regimens (Patient 10; Table 6) was enrolled in a clinical trial for

CTL019 therapy (NCT01029366). The patient underwent two adoptive transfers of 3.75×10^8 and 5.61×10^8 autologous CTL019 cells, spaced apart by two months. Following the first split-dose infusion of CAR T-cells, he became persistently febrile. No infectious source was identified. Patient 10 was diagnosed with cytokine release syndrome (CRS) and received interleukin (IL)-6 receptor-blocking therapy after which signs and symptoms of CRS rapidly resolved. Patient 10 continued to show progression in his bulky adenopathy and extensive bone marrow infiltration with CLL six weeks after receiving his first dose of CAR T-cells. Most patients who respond to CTL019 treatment exhibit rapid T-cell proliferation concomitant with CRS and a sustained reduction in tumor burden within the first month of infusion, see, e.g., Porter, D. L. et al. Science translational medicine 7, 303ra139, (2015), and this did not occur in Patient 10 (FIGs. 1A-1C).

Table 6. Patient 10 baseline characteristics

Patient	Age/Sex	CLLTumor Characteristics	CLL Involvement at Baseline	Previous Therapies
10	78/Male	ATM deletion (del); del11q; del17p	80% of marrow; bulky adenopathy with the largest mass in the mesentary measuring 13.5 x 6.8 cm	- Rituxan-CVP* ×6 cycles - Rituxan Alone - Fludarabine/Rituxan; Excellent Partial Response - Chlorambucil; Progression - Bendamustine; Progression ×1 cycle - CHOP** ×8 cycles - PCR^ with stable to mild progression on PET/CT# 2 weeks later - Pentastatin/Cytoxan pre-CTL019
*cyclophosphamide, vincristine and prednisolone **cyclophosphamide, doxorubicin, vincristine and prednisone ^pentostatin, rituximab and cyclophosphamide #positron emission tomography–computed tomography				

Because there was a concern that early blockade of IL-6-mediated signaling may have diminished this patient's response to CAR T-cell therapy, a second dose of the cellular product was administered (5.61×10^8 CAR-T cells, 70 days after the first infusion). Infusions were again complicated by CRS manifested by high fevers, hypotension and hypoxia that resolved after several days without intervention. Evaluation of his bone marrow one month later revealed persistent extensive infiltration of CLL and computed tomography (CT) scans showed minimal improvement in extensive adenopathy. Approximately two months following the second infusion, the expansion of CTL019 cells peaked in the peripheral blood, followed by contraction during the ensuing days and weeks (FIG. 1A). The outgrowth of CTL019 cells occurred in the CD8+ T-cell compartment, which is typical in CLL patients who respond to this therapy (FIG. 2 and FIG. 13). This response was accompanied by high-grade CRS that required clinical intervention, with elevated circulating levels of interferon (IFN)-γ, granulocyte-colony stimulating factor (G-CSF), IL-6, IL-8 and IL-10 (FIG. 1B).

At the same time, and coincident with the onset of high fevers, the patient exhibited rapid clearance of CLL cells (FIG. 1C). Next-generation sequencing of rearrangement products at the immunoglobulin heavy chain (IGH) locus, which allows tracking of the leukemic clone, showed a one-log reduction in tumor burden at 51 days following the second infusion, with complete eradication of this clone from the blood one month later (Table 7). CT scans showed dramatic improvement in mediastinal and axillary adenopathy (69% change; FIG. 1D). Six months after the second infusion of CTL019 cells, Patient 10 achieved a complete response with no evidence of CLL in his bone marrow (Table 7) and resolution of all abnormal adenopathy (FIG. 1D). His most recent long-term follow-up evaluation (>4.2 years post-CTL019 cell infusion) revealed the presence of CAR T-cells in the peripheral blood, ongoing B-cell aplasia (FIG. 14A-14C) and no evidence of circulating disease or marrow infiltration. Additional immune cell populations in the blood were normal in frequency, with no observed signs of lymphoproliferative abnormalities (FIG. 14A-14C). Complete remission has been sustained for more than five years at the time of this Example.

Table 7. Tumor burden evaluated by IgH deep sequencing analysis of peripheral blood and bone marrow from Patient 10

Sample Information				IgH Repertoire Assessment				Tumor Clone		
Sample Type	First Infusion[1]	Second Infusion[2]	Entropy[3]	Clonality[4]	Max Frequency (%)[5]	Gene Rearrangements[6]	Fraction of Nucleated[7]	Estimated # of Genomes[8]	Log Reduction (First Infusion)[9]	Log Reduction (Second Infusion)[10]
Peripheral Blood	-29		0.078583	0.990387	99.4	110710	0.343804	109271		
	14		0.067824	0.991416	99.46	157699	0.321995	156371	0.028	
	28		0.164639	0.967638	98.13	10303	0.025852	10034	1.124	
	63	-7	0.154459	0.973519	98.33	60725	0.11781	59601	0.465	
	69	-1	0.149261	0.971558	98.31	47691	0.132377	46809	0.415	
	84	14	0.152248	0.97316	98.34	115697	0.176816	113715	0.289	-0.176
	121	51	0.096938	0.978857	98.93	5126	0.014364	5011	1.379	0.965
	147	77	1.479932	0.260034	15.07	22	0	0	5.499	5.084
	259	189	2.613258	0.175609	14.98	40	0	0	5.532	5.117
	351	281	2.613371	0.213297	15.02	60	0	0	5.532	5.117
	526	456	2.278436	0.240522	16.07	39	0	0	5.503	5.088
	619	549	1.829257	0.348405	13.84	54	0	0	5.491	5.076
	710	640	NA	NA	38.33	12	0	0	5.528	5.113
	1177	1107	2.611971	0.213718	12.09	69	0	0	5.479	5.065
Bone Marrow	101	31	0.015523	0.997597	99.79	139042	0.301654	138663	NA	
	442	372	2.336592	0.167689	11.21	31	0	0	5.458	
	801	731	1.351141	0.32443	17.71	16	0	0	5.247	

[1]Day relative to first infusion; [2]Day relative to second infusion; [3]Shannon's entropy, a measure of the shape of the distribution of the read counts with a low entropy denoting a clonal sample composition; [4]Clonality= 1-(entropy/log2 productive unique rearrangements). Clonality ranges from 0-1 with 1 denoting a clonal and 0 a polyclonal sample; [5]Maximum frequency for each sample; [6]The number of unique IgH rearrangements; [7]The proportion the leukemic clone makes up of the total genomic mass of all input B cells; [8]The estimated number of diploid genomes (or cells) with this leukemia rearrangement; [9]Log10 reduction of the leukemic clonotype relative to the baseline sample; [10]Log10 reduction relative to the second infusion baseline sample; NA, Not available

Next, the clonal architecture of the T-cell repertoire in CTL019 cells was examined before treatment and following adoptive transfer. Deep sequencing of the T-cell receptor beta repertoire revealed that pre-infused CD8+ CTL019 cells and the CD8+ T-cell compartment one month following infusion were polyclonal, with multiple distinct TCRVβ clonotypes similar between the samples (FIG. 3; FIG. 4A). Approximately two months after the second infusion, a TCRVβ5.1+ clone that dominated the CTL019 cell population was present at greater than 50 percent of the total CD8+ T-lymphocytes in the peripheral blood (FIGs. 4A-4B). Subsequent analysis revealed that 94 percent of the CD8+ CAR T-cell repertoire consisted of this single clone that was not detected at the time of transfer or at one month following the second infusion (FIG. 4C). After tumor eradication, the expansion of TCRVβ5.1+ cells declined coincident with CAR T-cell decay kinetics (FIG. 4D). Thus, leukemia was eliminated in this patient primarily by the progeny of a single CAR T-cell that demonstrated massive *in vivo* expansion.

To determine the mechanism underlying the superior proliferative capacity and anti-tumor efficacy of this clonal population, lentiviral integration sites in peripheral blood or CD8+ CAR+ T-cells following adoptive transfer and in the CAR-transduced cell product prior to infusion were examined. Because lentiviral DNA becomes integrated at many sites in the human genome, sequencing of integration acceptor sites can be used to track proliferation of cell clones and investigate potential insertional mutagenesis.

Longitudinal sampling of Patient 10 blood samples revealed a cell clone with an integration site in intron 9 of *TET2*, which was expanded in CAR T-cells at the peak of clinical activity and not at earlier timepoints (FIG. 5A). This large degree of clonal dominance has not been observed in any patient treated with CD19-directed T cells to date. In CLL and ALL, the accumulation of CAR T-cells *in vivo* typically results from the expansion of a diverse poly-clonal or pauci-clonal repertoire within the transduced T-cell population. Cells bearing *TET2* integrants exhibited long-term persistence (FIG. 5A), and were present in the peripheral blood at a relative abundance of 14% at 4.2 years following infusion (FIG.11A). The clonal population contracted over time and appeared to be under homeostatic control, with no signs that insertional oncogenesis had occurred (FIG. 14A-14C).

TET2 is a master regulator of blood cell formation, and haploinsuffciency or deletion of this gene plays a role in normal clonal hematopoiesis (Busque, L. *et al. Nature genetics* 44, 1179-1181, doi:10.1038/ng.2413 (2012)) as well as the initiation of lymphoma and leukemia, including naturally-arising and human T-lymphotropic virus type 1 (HTLV-1)-associated malignancies (Yeh, C. H. *et al. Molecular cancer* 15, 15, doi:10.1186/s12943-016-0500-z (2016)). Although *TET2* inactivation may contribute to increased self-renewal of hematopoietic stem and progenitor cells, it infrequently leads to overt oncogenesis, suggesting that additional gene mutations are required for complete malignant transformation (Zang, S. *et al. The Journal of clinical investigation* 127, 2998-3012, doi:10.1172/JCI92026 (2017)) . Analysis of polyadenylated TET2 RNA populations showed the

appearance of new chimeric RNAs that spliced from *TET2* exon 9 into the vector and terminated, truncating the encoded protein (FIGs. 5B, 6A, and 6B). While it is possible that CAR T-cell specific expression of truncated fusion TET2 mRNA and corresponding protein may have a dominant negative effect on normal TET2 activity, it has been demonstrated that TET2 mutants do not suppress the function of wild-type protein and therefore do not exhibit dominant negative characteristics. Furthermore, assessments of TET2 mutations both *in vitro* and *in vivo* consistently point to a loss-of-function phenotype.

To ascertain whether monoallelic disruption of TET2 by lentiviral integration was primarily responsible for this unprecedented degree of CAR T-cell clonal proliferation, we sequenced CAR+ (TET2-disrupted by lentiviral integration) and CAR- CD8+ T cells from this subject and examined genes involved in hematologic malignancies or precursor lesions. In both samples, a missense variant at amino acid 1879 (exon 11) was found in the catalytic domain of TET2, converting the wild-type residue, glutamic acid, to glutamine (FIG. 11C). Notably, genetic variations in TET2 involving a change of amino acid sequence at position 1879 from glutamic acid to lysine, aspartic acid or alanine have been associated with myelodysplastic-myeloproliferative neoplasms. The c.5635C mutation was present in the allele of TET2 without the integrated CAR transgene, as that chromosome contained the wild-type reference sequence (c.5635G). No other mutations in the panel of 67 additional genes were found.

TET2 encodes methylcytosine dioxygenase, an enzyme that catalyzes the conversion of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), thereby mediating DNA demethylation (Tahiliani, M. et al. Science 324, 930-935 (2009); Iyer, L. M., et al. Cell Cycle 8, 1698-1710 (2009)). Methylation at the C5 position of cytosine normally represses transcription, and therefore, demethylation is expected to activate gene expression. The functional significance of the E1879Q mutation was interrogated using plasmids encoding wild-type TET2 or this TET2 variant that were transiently transfected into HEK293T cells. Analysis of genomic DNA isolated from these cells using dot blotting revealed that E1879Q stalls oxidation at 5-hmC, with a significant reduction in the formation of 5-fC and 5-caC (FIG. 11D). This was in contrast to cells in which oxidation of 5-mC did not occur due to the introduction of a catalytically inactive (HxD) TET2 (FIG. 11D). Uniform overexpression of TET2 protein was verified by western blotting of cell lysates (FIG. 11D). To confirm these findings at a genomic level, DNA from transfected cells was degraded into component nucleosides that were analyzed using liquid chromatography–tandem mass spectrometry. Indeed, cells overexpressing E1879Q exhibited a two-fold reduction in the production of 5-fC and 5-caC compared to their wild-type counterpart (FIG. 11E). These findings support the notion that the TET2 allele not disrupted by lentiviral integration in clonally-expanded CD8+ CAR T-cells of Patient 10 was hypomorphic.

TET2 biallelically disrupted CAR+ Vβ5.1+ CD8+ T-cells *ex vivo* exhibited lower total levels of 5hmC compared to their CAR- Vβ5.1- CD8+ T-cell (*TET2* haploinsufficient) counterpart (FIG. 7A). This was presumably the result of *TET2* deficiency following insertional mutagenesis, as the E1879Q variant did not affect the generation of 5-hmc (FIGs. 11D-11E).

To interrogate the mechanism of *TET2* insertional mutagenesis on CAR T-cell function, ATAC-seq which monitors DNA accessibility was performed (Buenrostro, J. D., et al. Nat Methods 10, 1213-1218 (2013), incorporated by reference herein in its entirety) (Table 10). Although globally the epigenetic changes between *TET2* haploinsufficient (CAR-) and biallelically deficient (CAR+) CD8+ T-cells from this patient were modest (FIG. 7B), gene ontology analysis based on chromatin accessibility profiles did reveal gained accessibility for genes in pathways that regulate the cell cycle and T-cell receptor signaling (FIG. 15 and Table 8), and reduced accessibility for genes in pathways that regulate differentiation, T-cell activation and effector function (FIG. 7C; FIG. 8; Table 8; Table 9 in Appendix). Genes near ATAC-seq reads that were substantially reduced or lost following *TET2* biallelic dysfunction, *e.g.*, insertional mutagenesis, included several regulators of T-cell effector differentiation and function such as *IFNG, NOTCH2, CD28, ICOS, IL2RA* and *PRDM1* (FIG. 7C; Table 8).

To determine whether these changes in the global chromatin landscape of clonally-expanded CAR T-cells could have affected specific transcriptional circuits that are central mediators of T-lymphocyte differentiation and function, we identified transcription factor (TF) motifs gained or lost in CAR+ CD8+ T-cells relative to CAR- CD8+ T-cells (FIG. 12A). TF motifs acquired in CAR+ T-cells included E26 transformation-specific (ETS) (GABPα, ELF1, Elk4) and zinc finger (ZF) TF (Sp1) binding sites that characterize naïve and early memory human CD8+ T-cells44 (FIG. 12A and Table 11). Notably, ETS TFs have been demonstrated to be required for normal T-cell development, activation and survival (Muthusamy, N., Barton, K. & Leiden, J. M. *Nature* 377, 639-642, doi:10.1038/377639a0 (1995)). In contrast, TF motifs that were less accessible (FIG. 12A and Table 11) in Patient 10 CAR+ T-cells (NF-κB, IRF1, NFAT:AP1 and CTCF) are enriched in terminally-differentiated effector and exhausted T-cells and have known key roles in forming the epigenetic landscape that programs their biology. Peaks that closed in the setting of biallelic TET2 loss also included a motif that is recognized by the basic leucine zipper (bZIP) TF, BACH2 (FIG. 12A and Table 11). While BACH2 has been reported to inhibit the expression of effector-related genes to maintain a naïve state in murine CD8+ T cells (Tsukumo, S. *et al. Proceedings of the National Academy of Sciences of the United States of America* 110, 10735-10740, doi:10.1073/pnas.1306691110 (2013), its disruption by lentiviral integration has also been linked to clonal expansion and persistence of HIV-infected T-cells in several studies (Ikeda, T., Shibata, J., Yoshimura, K., Koito, A. & Matsushita, S. *The Journal of infectious diseases* 195, 716-725, doi:10.1086/510915 (2007)). Furthermore, BACH2 is a target of proviral integrations in B-cell

lymphomas induced by murine leukemia virus and its loss is a direct mechanism underlying the clonogenic activity and persistence of immune cells. Overall, the significant differences in chromatin accessibility and associated TF binding motifs of well-defined regulators of T-cell differentiation, development, clonal expansion and persistence support our inference that TET2 deficiency shifted active regulatory programs in the highly potent CAR T-cells found in Patient 10.

In accordance with these findings, functional analysis of *TET2* biallelically deficient, e.g., disrupted, CAR+ T-cells cultured from this patient exhibited a diminished capacity to express IFN-γ and CD107a (surrogate marker for degranulation) when activated (FIG. 7D), consistent with a less differentiated state. Thus, lentiviral integration into *TET2* together with a hypomorphic mutation in the second allele reprogrammed the epigenetic landscape of CAR T-cells cells in a manner that was consistent with altered T-lymphocyte fate.

Table 8. Genes associated with more open or closed ATAC peak regions in CD8+CAR+ (TET2-disrupted) compared to CD8+CAR- T cells from Patient 10

Column A	**Column B**
Chromatin Regions More Open in CD8+CAR+	**Chromatin Regions More Closed in CD8+CAR+**
RPL37A, MIR5092, CBX3P2, CHFR, WASH5P, NCR2, ZNF205-AS1, B2M, KCNQ1OT1, C18orf54, PTGR2, LOC102467080, CCDC152, TUG1, CENPJ, GSS, MIR4489, TOM1L2, PRSS38, SP2, ZNF318, PDPK1, TMEM43, DOCK8, TMEM70, AZIN1, NFIB, MGAT5, ALG10B, ZNF107, TMEM134, EGLN3, PHF20L1, NUPL2, PPCDC, SLC2A11, CCNB1, IRAK1BP1, MCPH1, VWA8, KIAA0040, CES1P1, ITM2C, SOGA1, SHFM1, TEF, IFI27L2, XYLT2, MRPS33, METTL20, GPD1L, LRRC27, RPL36, LOC100507406, SNRNP70, CEBPZOS, PLXNA4, C1orf159, TMEM39A, ZBTB38, IRF7, CYTH2, LINC01030, C21orf91-OT1, API5, MPPE1, IDUA, LINC00426, TRIB1, GABRR3, CCDC80, CSGALNACT1, SLC12A9, LOC101927406, YTHDF2, RNF6, CXCR4, ZNF251, C11orf73, DUSP1, NUDT15, LOC100507316, SLC25A6, PSME1, RCCD1, LOC101927482, PYCR2, TXN2, CARD11, CROCC, F2R, GCNT1, LINC01091, CX3CR1, SHISA5, PYROXD1, TTC22, PML, ANKRD7, TTC27, ACOXL, TTC37, PBX4, SEL1L, MYBL1, SOX6, C1orf21,	MIR4668, LINC01461, TMEM218, KCTD18, KMT2E, NLRP3, NOTCH2, IL1RN, C11orf73, CHGB, NAT10, KLF7, SH3BP5, MIR4464, GALNT2, LINC00332, PTPRG, CSF2, LPP, LOC102659288, RMI2, INPP4B, KLHL29, FOXD2, DLEU1-AS1, MXI1, LINC00865, PTGER4, HSBP1L1, RD3, KCNS2, AQP7P1, NRP2, RGS1, ETS1, SPATA4, SLC22A16, IL13, ARHGAP26-AS1, RUNX1-IT1, LINC01395, NR3C1, MED7, LSMEM1, CD226, DLC1, CAMK2D, LOC100507144, HACE1, SGMS1, RUNX1, KLF6, LOC400997, PHTF2, LINC01257, LOC101927557, SMIM20, ZMYND11, CCDC81, CLIC5, BCL6, NHS, GLRX3, EZH2, C1orf131, INSIG1, SYNE2, ICA1, LOC100506700, MIR1260B, LONRF2, BACH2, MAP3K7, LOC389831, SNORA14B, CCR4, DUSP6, C3orf67-AS1, SYT14, SP140, TMEM131, GPR150, PCSK1, MIR4272, MACF1, GLDC, RAMP3, LOC339862, LOC101927780, ECT2L, ADIPOQ-AS1, BCKDHB, SMARCA2, EVA1A, MTMR2, MIR4697HG, GJA10, SLC4A10, BHLHE40-AS1, AQP7P3, LINC00636, MGME1, HTRA1, C6orf132, MIR5708, KCNQ3, CARS2, MAP7, PDE4D, IMPG2, GNA12, TGFBR3, LINC00673, MIR583, TAGLN3, MAP3K7CL, MIR1208, DUSP5, ICOS, SSBP3-AS1, MTRNR2L1, C21orf91-OT1, LOC286114, SSTR1, VWA8, MIR30B, SLC1A1, PLSCR2, LOC101927560, CCR8, MIR1206, VPS53, LRRC8B, EFHB, GTSF1L, SGMS2, CASC11, CHSY1, PTPN14, LINC01125, ZFAT, TMEM106B, C4orf51, MIR6132, TAF1B, PPP2R2D, CSF1, WWTR1, ZP4, PRDM1, SLC43A2,

SAMD9, LOC101927780, GNPNAT1, TSTD3, BBS10, KIAA1841, C21orf119, FAM73A, ERCC6, USP19, SPA17, KHDC1, MICU2, LINC00501, MYO16-AS1, CH25H, FAM114A2, GGCT, IGF2BP3, MIR21, RSRC1, TOX2, FAM156A, MYB, LYST, ANKRD31, FKTN, COPS7A, DNAJB11, GABRA4, SGCB, IYD, TTC14, ZNF704, RAD54B, GCLC, RAD50, LOC283575, DENND4C, LOC101593348, SLC38A9, APBB1IP, STIM2, CGRRF1, IVNS1ABP, PAK1, NAGA, GTF3C5, PDGFRL, ST3GAL5, RETSAT, PANK2, C5orf51, LOC154449, NEK10, SH2B1, LYSMD4, ZNF688, GAB3, PAIP2B, THYN1, ATXN1L, RAB22A, SATB2, ZC3H10, PRKCH, SEC61A1, PDHB, DBP, WFS1, CCDC141, CDC20, DERL1, RFESD, OR10AD1, CACTIN, SYTL2, GPR146, GPR56, PGAM5, CDKL5, IL12RB2, DCXR, BFSP1, ZNF408, TOMM20L, ZNF891, C1GALT1C1, HUS1, LLGL2, FAM126B, CCDC132, GINS3, NFIA-AS1, DTHD1, ISCA2, PDP2, CMC1, SLC27A1, FAM227B, C15orf41, MDM1, HNRNPA1L2, NUDT9, PGAP2, RNU6-31P, ALG10, PIK3R1, RBM47, TBC1D23, HSD17B7, NUDC, TIGIT, ASPH, TLDC1, ZBED2, YPEL1, CBX7, CMPK2, MIR4465, WDR27, ENPP2, PRSS8, KIAA0408, ARHGAP26-AS1, DHRS9, GJA10, RNU6-2, POLR2D, CTU1, RNF141, EXOC2, MOCS2, EPSTI1, BHLHE22, ZC3H12C, RASL11B, OAF, TRAPPC11, CREG1, IPCEF1, MYPOP, TTC1, ZNF808, PON2, FAM63B, CBLB, SYNE1-AS1, DHRS13, NDUFB5, GNAI3, LINC00924, RPLP1, SLITRK1, CASP3, GCC1, ASPHD2, GUCY1B3, TBC1D32, PC, GNA12, PDE4D, AASDH, SATB1-AS1, SBSPON, ZADH2, ZBTB11-AS1, PRICKLE3, NDUFAF5, ZMPSTE24, RPRD1A, ZNF669, CLIP4, PCNXL3, RCN2, CXorf57, PRDM13, LOC441666, GPALPP1, MRPS14, AAMP, JMJD4, GOT1, N6AMT2, ZC3H7A, NEMF, MIR6871, SAP18, ABCA1, U2AF1, NSDHL, RNF223, NR5A2, RTTN, PLS3, PMM1, HIVEP1, DTX2, PLEC, LOC101929517,	LOC102724550, ATP10A, RAB6B, CARD17, FEM1C, DOCK9-AS1, PCCA-AS1, GFRA2, ARHGEF3-AS1, CLDN18, FAM105A, TMEM261, SNTB2, PDLIM4, STARD8, LCP2, EPAS1, PRKCDBP, PLA1A, MICAL2, MIR5197, VAV3-AS1, NUMB, LOC285762, FDX1, PRKAG2, LINC01031, LTV1, LINC00589, PXYLP1, SLC9A7P1, VKORC1, STAMBPL1, ITPR2, HRH1, GEMIN8, UACA, TLE3, MYCBP2, LRAT, LRRC32, TNFSF18, MAEA, HDAC9, SV2C, PCNX, KIAA1217, ZCCHC5, FYN, ABCA13, CLEC4D, ARHGAP21, CHMP4B, NCALD, HMGB3, PTENP1-AS, LOC101927543, SLC25A51, CA12, PIP5K1C, MIR4637, WARS2, CNIH3, LOC101929715, MIR6876, NIPAL3, LOC101928001, HHLA2, PLB1, SLC25A13, KIF5C, ISM1, MTDH, CCNG2, TNFSF10, SUMF1, DOCK2, EPB41L3, UCP3, TRG-AS1, MOXD1, LINC00581, PPA2, LOC101929551, TNFSF8, ALK, GIMAP5, MIR101-2, CMKLR1, TRABD2A, LOC100129520, THADA, EIF1B-AS1, MIR146A, LINC00607, FAM178B, CHST7, MIR1913, SNX9, DTX2P1-UPK3BP1-PMS2P11, TMPRSS4, LOC101929241, DHRS4, ADAP1, ANKRD11, C4orf32, DNAJC19, LINC00548, C2orf40, PRKY, MIR875, WNT7A, ERVFRD-1, CCR7, NT5E, TIAM2, CXCR4, LOC100128176, THEG, BZW1, LINC01358, LOC100506860, IKZF1, ASXL1, LBH, LOC101927865, LOC286190, APAF1, LOC100506457, MYO3B, THEMIS, NEDD9, FAM53B-AS1, ZNF496, BCL2L14, PLEKHA8P1, SCIN, LINC00892, CETN4P, ZNF292, TNP1, FGF12, ATP6V1B2, LOC101927950, TSEN15, IL20RA, RIPK2, MIR1301, PTPN20B, GAB1, NCK2, ZNF648, PDE4B, USP46, GFI1, OR1K1, ASAP1-IT2, FAM69A, PTEN, BMP1, TNS3, PTPRD, CDCA7L, LMNA, HIVEP2, DUSP10, OTOGL, LMO1, AREG, MED15, STEAP4, GHSR, UPP1, ANXA1, LINCR-0001, SYK, COPS8, SLC38A6, MKKS, MIR6124, CHST2, NRBP1, LOC728730, FLJ21408, 42434, SEMA6A, DOT1L, FLJ27354, RHBDD1, LINC00888, FLJ33360, ZMIZ1-AS1, PLAGL2, MIR92A1, ITGB1, ANXA5, RABGAP1L, RLBP1, TNFAIP8, ASAP1-IT1, ACTN4, DPYSL2, ARHGEF3, RBMS3-AS3, RYBP, LOC100130298, ADRB2, RBPJ, MIR4526, NFATC1, IL5RA, CCDC91, LINC-PINT, ANKRD20A3, LOC101928436, BACH1-IT2, CHMP2B, MIR4729, PTPN3, PRTFDC1, LAPTM4B, CRCP, LINC00423, ANKRD33B, TP63, LOC100507420, GNA14-AS1, TNFSF11, LOC101927243, HIPK2, C1orf106, ARHGAP31-AS1, PSMD14, SPTY2D1, CDH17, LOC441204, SHQ1, CCND2, LINC00824, ITGA2, APOD, ZC3H12C, CD58, C2orf57, SVILP1, BCL9L, MIR1976, LOC152578, KHDC1, LINC00969, SNORA62, AGFG2, LOC101928105, SERTAD2, EDN1, PMEPA1, DSE, C10orf131, LINC00540,

C20orf196, CCDC28A, ZNF648, PEX26, EGR4, RIN3, TMEM167A, HLA-DQA1, NEK2, BAI3, SRP9, YIF1B, CHMP7, ZNF638, ENPP4, BCL10, SCAND2P, RGS14, HLCS, BEND4, SEC61A2, NLK, ARL6IP1, ARHGEF19, LIX1L, CCDC174, CSTF2, ZNF483, CCT6P3, AMN1, MRPL47, PIGV, GBF1, B3GNT5, ZNF862, CNNM4, TSGA10, EBP, RAVER1, EBPL, KIAA0825, LINC00491, TMEM185B, MCFD2, CDC14A, TRIM68, SNRPG, MEF2C-AS1, TOX4, CPNE3, NDUFA12, NEK11, GEMIN5, GALNT11, ARHGAP19, TDG, DIXDC1, APMAP, MCM2, SETBP1, LINC01232, TMEM218, PTK6, AP1G2, APBB1, GAREM, STARD13, ZNF519, TVP23B, DDX56, RAB11FIP1, LOC148696, DUSP10, CRADD, ITGA2, APOA1, ZNF43, CDCA7L, LOC100147773, PARK7, DDIT4, MRPS31, SPATA13, PPIB, CSNK1G1, LUCAT1, MIR210HG, THAP1, ZFP1, PRDM11, RGPD5, PRSS3, CCNC, TXK, PNPO, TRAPPC12, PLCG2, NAPA, EREG, PLAGL1, MINA, TNFAIP1, COPG2, DZANK1, CHST2, TMEM126A, NBEAL1, KHSRP, C10orf99, ANO10, MIR4458HG, FAM175B, TPRKB, USMG5, PTP4A1, ABHD2, ENTPD3-AS1, RAET1K, CAMTA1, CEP131, SPIDR, EIF3D, TMEM161B, NCOA4, MIR4744, SNX19, FAM161A, VWA5A, ABLIM2, DDX10, ZNF84, MOSPD3, ABCB8, GTPBP3, LOC729218, ELP4, SP4, MCM10, SACS, AP2M1, ERCC4, DGCR6L, PSMA6, TIMM10, ZNF692, CRTAM, SLC7A11, FOXK1, COG2, COMMD2, CLU, OSBPL3, CUTA, ASMT, FAM105A, CCL3, ZNF430, MIR4269, SMARCAL1, ST3GAL1, DDX50, TET3, L3MBTL1, DLGAP5, OGG1, THEMIS, ACO2, GNG2, BCOR, GTPBP8, NDUFS8, CPOX, LPAR3, GFPT1, PTPRM, LOC100132529, BTBD6, FAM45B, PPP1R21, LINC01031, CHMP4A, SGMS1, ZSWIM3, RANBP10, FZD6, PRTFDC1, MBD1, SPAG16, TLE3, MAP2K6, RERGL, DUSP11, SMARCAD1, AMFR, RNFT2, COMMD6, MIR4473, CCDC7, YY1, SDHAP3, YIPF5,	LINC00501, ITK, LRRFIP2, LINC01094, MIR548G, ZBED2, CCDC141, LOC102724246, AOAH, RPSAP58, FBXW11, KCNMA1, HSPA4L, DKK2, ARAP3, MIR4435-1HG, MIR573, ARID5B, CD83, FNDC9, OR52K1, CAMK4, C1orf140, MREG, LHFPL2, PAPOLG, RBM26, LOC103352541, MAF, TMC1, NR1I2, POLR2J3, EOMES, TAB2, GATAD2A, RTFDC1, COL6A3, LOC285484, TRIM62, EGOT, CNKSR3, NIFK, GPR55, PTP4A3, MIR4743, SETBP1, WWOX, IGF1, ACSM6, IDH2, B4GALT6, MB21D2, LOC439933, BHLHE40, AKAP11, EED, STX11, MIR4715, MIA2, CASC19, LRRN1, MIR29B1, IL2RA, SORL1, LOC100130992, GNPDA1, PXK, RAMP1, GNAQ, MIS18BP1, MIR623, EGR3, VDR, HMG20B, LOC101928834, ST3GAL1, IRF2, GATB, UTRN, EHD3, PTK2B, HADH, LOC101927653, SLC39A10, ROR2, LRRN3, TGFBR2, FHIT, KIF22, LOC100506585, LOC101060498, INPP5D, UBASH3B, LDLRAD4-AS1, MIR4493, PIK3R1, MAPKAP1, NCOA1, MIR6881, LOC441666, MIR4435-2, TSPAN5, GM2A, METTL6, WI2-2373I1.2, ITPKB-IT1, NBPF25P, TINAG, CCR5, STAB2, C6orf99, TRIB1, P2RY13, DIXDC1, INPP4A, FAM133B, LMNB1, ST8SIA4, OTOL1, SRGAP2D, LOC253573, LTA4H, GPR68, HPSE, SFTPD, C3orf17, DPPA2P3, IER3, CDK5RAP2, CD247, CD44, PIK3AP1, ZADH2, CCDC83, HULC, KLF13, SYTL3, MMP20, LOC100507195, TP53BP2, LOC101927416, SATB1, NRCAM, LOC392452, SMIM3, IL37, CCDC147-AS1, NSUN7, WDR1, SMAP2, LOC102724323, HHAT, MYADM, CTHRC1, SMCR5, NABP1, YWHAQ, ADAM6, MTHFD1L, MALSU1, TRIB2, SFXN4, MIR1200, CSGALNACT2, RSPO3, PKI55, GPR87, IER5, STK39, CD5, NUAK1, CD200R1, RNU5D-1, MIR1343, RANBP9, MIR6743, RNLS, RGS10, LDHD, SYCE1L, GPR171, HECA, WT1, HDAC8, ICAM2, AKAP13, SSPN, SENP6, COPG1, LOC100128993, IL12RB2, ETV1, GBE1, IQSEC2, STON2, DSTN, STK4, UBXN10, TRIM36, RABL2A, TACR3, MIR6729, FLJ20021, FAM196B, ANK1, ZBTB17, SVIL, NBPF20, LINC01132, SNORA72, ISM1-AS1, FAM129A, LINC01359, ELMO1, LRRFIP1, RAB31, ANTXR2, XRCC5, ASIC3, C11orf58, SMKR1, NFKB1, RIMS2, SKI, MIR4786, SLC37A3, SERPINE3, RAD23B, LOC101929153, CTD-2201I18.1, LINC00900, STARD13, MIR604, IRS2, LOC102724467, FLJ36777, ANKRD44-IT1, ACOXL, GOLGB1, SP140L, BTBD11, PTPRE, ANKRD46, CSGALNACT1, PRORY, ABCB4, C12orf42, CYP11A1, EIF1, NAV2-AS5, PIK3CA, G2E3, KIAA0825, R3HCC1, IRF4, LOC101928731, LOC728084, PPP1R3G, MSH4, ADTRP, ATP1A1, TBC1D23, DDX11, ZNF267, DRAM1, DUSP22, C1orf229, MIR6516, CIAO1, NCOA7, RGS2,

FAM175A, ATP5G1, KLHL20, DOPEY1, YIF1A, TTC23L, UBXN2B, TBC1D22B, MTMR9, RPL39L, CYFIP2, KIAA1191, TRIM21, CD34, PHOSPHO2, LOC101929057, INTS3, MCCC2, MORN1, ZFAND2B, UBASH3B, RUNX3, GTF2E1, HNRNPC, BLM, LOH12CR1, EPB41L4A-AS1, CKAP5, MIR548Z, EIF1AY, MAF, USP11, MIR6731, SPHK2, KIAA1462, RAPGEF1, PDE4B, TCEANC, ZDHHC13, SUGP1, DDHD1, ECT2, SPAG1, AKAP13, ACN9, APEX2, KIAA1919, MIR3714, SLC25A3P1, TP53RK, FAM53B-AS1, TMEM5, ACYP2, LINC01395, HSD17B7P2, PGK1, PDHA1, CCNT1, PLRG1, SNRNP48, UBE2C, PITPNC1, NUMB, FOXO1, CALU, PAF1, TMEM2, FKSG29, TSHR, PROSER3, GCSH, CASC21, BCKDHB, COPE, KLHL26, POT1-AS1, CCDC64, PDK3, ELK1, ADIPOR1, CHMP4B, ZNF750, LOC728730, POU4F1, KIZ, GRHPR, ELAC1, COX8A, CCZ1B, CEP164, MPHOSPH6, LINC00595, BHLHE40-AS1, ADRB2, PLXNA1, RSL24D1, MIR4724, MAT1A, FAM122A, SNORA75, DGCR6, RBM27, PSMD8, FLRT3, UTRN, ITGB1, SLC45A4, MIR7854, SNORA26, DCTN4, PROSC, CDKN2D, EOMES, MIR146A, LINC00298, NUPL1, LYRM7, NRL, ANKRD20A11P, RSU1, ZNF286A, PACRGL, TMEM67, TWISTNB, PGPEP1L, EPDR1, AURKAIP1, ZNF736, TBCC, PARP11, EIF2AK1, SDCBP2, NPRL2, LOC102723895, TBC1D4, HMG20B, EPHB1, CD69, TFDP2, AGO2, DAPK2, NAT1, TIMMDC1, STX17, PDXP, SEC22A, PARS2, MAGT1, LINC00536, PREP, HNRNPD, SS18L2, ERMP1, DNAJC19, SNORD50A, EIF3F, GSR, MRPL16, BUB1, LOC101927592, PROSER1, CALCOCO1, IFT27, MALSU1, ATP6V0A4, SLC25A26, ALS2, SLC46A3, C16orf91, BCL2, SNAI3-AS1, RABGGTA, SLC2A8, ZDHHC19, PITRM1, SFMBT2, ALDH4A1, STK39, LOC101928530, NGDN, SSR3, TMEM41A, ABCF2, PCTP, AGGF1, NOC3L, HAVCR1, PEX7, SPRY2, TBL2, C7orf50, MYO9B, GRAMD3,	OR13A1, EDN2, GPR183, LINC01510, LOC100129316, HBS1L, ITGAL, JARID2, ZNF595, LOC646736, TNFRSF18, MIR3194, MIR2355, LINC01140, MIR4435-1, EBF1, TRUB1, MYO1B, LOC100130476, ID3, RUNX3, ZNF365, SLC25A38, LINC01091, APBB1IP, XCR1, TSC22D1, FAM46C, ABHD2, CR2, FAM86B3P, LRRK2, TMPPE, CD55, TAOK3, C1orf105, SAMD3, IFNG, POMT2, MIR4782, SLA, CCNY, MT2A, NFKBIA, SPSB1, CXCR3, SCARB1, UCP2, C9orf72, PSMD5-AS1, GSTO1, LOC101927482, TRANK1, C10orf71, CMC1, EPHA2, MIR1244-1, PRSS55, COL6A4P1, MIR548I3, ADORA3, C10orf128, PEX2, SEC31A, SPATA24, FUT8, PKN2-AS1, GK3P, LOC100288911, REREP3, CD86, MYL4, ANXA6, MBP, RAB32, LINC00152, STK31, MINA, DUSP4, LRRC8C, RFT1, NLRC5, MIR5188, C14orf64, MIR548AN, IQGAP1, PALM2-AKAP2, RFESD, ITPR1, BTBD3, MIR1205, MDFIC, BTD, FAM72B, ZNF254, MAEL, KATNBL1P6, BIRC3, PRDM14, AEBP2, AGPAT9, MEGF10, TNC, KLHL3, PDK4, ADA, PLXNC1, ENAM, HOMER2, PLEKHA7, FRMD4B, PKIA, NFATC2, PVT1, NDFIP1, GNPTAB, MAP6, PGAP1, MBNL1, NT5M, MICU3, ASF1A, SPTSSA, DIABLO, BRD7, GNAI1, MTRNR2L6, RBM20, NDUFV2, BBS10, LYPLAL1, PIM1, LINC01252, ITPKB, LOC100505887, ANKRD20A4, MIR5703, ZBTB10, ARL14EPL, FGGY, TMPRSS11E, TOB2P1, SCML1, CHRNA1, RNASET2, RIN3, GLS, LINC00598, PILRA, ZC3H12D, BATF3, AFF2, KIF13B, TG, LINC01160, LDHA, CLEC16A, RGS9, SPRED2, GALNT18, MMP19, FAM13A, TRIM25, TNFRSF10D, ANKDD1B, IL15RA, SEMA4D, OR4N3P, SH3RF1, ETV6, LSM6, NAB1, GATA3, LOC100506990, CCR3, MIR4681, IDS, ZBTB7A, NOP14-AS1, CELF2, ARL6IP5, SRGAP3, NTPCR, HNRNPA0, LMO7, ACER2, ALG1L2, IL21-AS1, GGA3, ABTB2, BCAR3, LOC728613, AKAP2, RAPGEF1, ATXN1, P2RX4, MIR4461, SMAD3, NFIA-AS1, LOC100505478, FOXO1, SMAD1, MAFIP, CD28, CALCOCO1, MIR648, FAM126A, S1PR1, LOC100288778, HS1BP3-IT1, ANKRD18DP, GOLGA8B, TTLL1, GTF2B, NEB, DYNC2H1, OR52W1, GPI, RASGRF2, ITM2B, EPS8, LOC283788, TATDN2, SPEF2, HIVEP3, LOC151475, CASC23, KANK3, MRPS30, LOC100507406, MBNL2, TGFB2-AS1, MCF2L2, MRPL33, PRKAR1A, SLC26A4, RNF165, DAZAP1, ANKRD6, CCDC64, TMEM200C, ALLC, ZFP36L1, GOLGA8A, LYZ, LOC101928370, ITPRIPL2, CACNA1E, CD59, PTPRC, DLGAP1-AS2, GDNF, FGD2, HNF1A-AS1, SLC8A3, HSP90AB1, CUBN, CTNNA3, GPHN, MIR1207, ILDR1, SLC35G2, LINC01010, PGS1, LINC01250, TLDC1, MIR6723, SERPINB6, PPARG, ZDHHC19, COX17, ODF1,

CARM1, KIAA0141, C5orf64, ZNF32, GHITM, C1orf52, TAOK3, RNGTT, LOC388942, CLCN5, DHTKD1, SPINK2, LOC100132891, SIDT2, ARHGAP22, RFWD3, ELL, MOSPD2, LOC100996286, TMEM115, ARMC1, LINC00598, POC5, NFATC2IP, PER1, LOC100288911, ZNF138, LOC151484, TTC30B, LINC00221, ROBO3, CRB2, RNU6-16P, NFKBIB, GGACT, ZNF133, LINC01222, SRPR, PSMD3, WARS2, TDRKH, CST7, WASH7P, ORMDL2, WNT10A, SLA, LAMTOR4, RPN1, PTK2B, AHR, DHRS4, N6AMT1, PRR5L, TRPS1, OSBPL8, FBXO33, HINT1, SPC25, AP2A1, TAGAP, EVI5L, CDC25C, MIR9-2, LMBR1L, PDCD4-AS1, ABLIM1, ZNF680, LOC439933, ARL13B, CERS5, GLCCI1, MAP9, APOO, CSF1R, MIR8086, TFAP2A-AS1, ZNF124, LOC100130451, LINC00461, MIR7160, ADPRHL1, ACOX3	GYG1, RRAS2, FAM210B, CDV3, GPATCH2, NEK6, LOC101928988, LOC101928730, RFC3, RASGRP1, RASAL2-AS1, HLX, PLAC1, NES, LDLRAD4, B4GALT1-AS1, ST3GAL6, TBL1X, AGK, LOC102724957, MCOLN2, TTPAL, MIR593, LOC100506178, CERS6, PDP2, MIR568, LOC100133050, NAPG, TSC22D2, HSD17B8, SLAMF1, PCSK6, RLIM, MIR548AV, MIR6874, DNAJB5-AS1, CHEK2P2, ZNF592, LOC101928453, MIR3143, PCDH1, EHD1, UNC45A, PLEKHA6, USP25, ARHGDIB, USP3, LMOD3, SFXN1, TSPAN13, CDH13, LOC100507412, FSCN1, LINC00114, SNX13, MIR4316, S100A2, LOC101926963, C9orf24, MIR7848, MEF2D, GOPC, PPP1R16B, ITGB7, KRBA1, AP5M1, ARGLU1, TMEM154, RASGRP3, SMG5, EFHD2, SAMD5, SH3RF2, LINC01366, CAMK1, ZNF815P, C5orf63, P2RY14, CLLU1OS, ZIC3, LHX9, KCNJ1, BPESC1, CD84, IL1R2, LYPD6B, MTRNR2L2, DCAF12L1, ITGAD, EBLN2, MRPL36, FAS-AS1, LOC101928386, PVR, LRRC7, C10orf40, KLF10, FTO, GPR132, GMPR, SOWAHD, STIP1, ZFP36L2, DDAH1, NMBR, SLC4A7, GAP43, MIR1245B, TRPV2, G0S2, APBB2, UQCRBP1, ZC3HAV1, CTSB, PHLDA3, CD2, DUSP2, FTL, SMCO1, LOC100506801, STAT4, NSMCE2, IL10RA, CCDC12, GFOD1, CLCN4, GCNT4, PTPN13, LPGAT1, TMSB10, HPCAL1, LOC101927391, BCL2, PRDX4, TTN-AS1, LOC101927406, POLI, CTNNB1, NPPC, CDK5R1, MCTP2, CCDC102A, STK24, SH3TC2, CFC1, SLED1, GPR174, ZNF827, LAG3, PVRL3-AS1, MARCKS, KCNQ5-IT1, TRERF1, FZD8, BCL2A1, ANK3, JAG1, ASAH2, OR1F1, BCAT1, LRRK1, POLR2J2, PTDSS1, CD3E, MIR3945, RHOH, GNG2, IQGAP2, BTBD19, C1GALT1C1, RGS20, CCDC6, EFTUD1, RBMS1, MIR155, VCAN, GTF2H5, LOC101927651, STX2, IGFBP5, NVL, MSN, RASGRF2-AS1, LOC401585, MGST2, TBX21, MCF2L-AS1, CLDN12, LIMA1, SREBF2, EMC2, AFAP1L2, NOD2, DNM3OS, CLN8, ZNF311, PIK3CG, CP, CCDC85A, ZCRB1, PCDHGC5, FURIN, WNT16, DOCK10, MIR129-1, ZCCHC18, OR2V2, LOC102723703, MEX3C, DNAJC5, PIGB, FAM71B, WIPF1, FNBP1, LINC00603, SH2D1A, GGTLC2, POLR2J, AKNA, AMICA1, PPP1R1C, ST6GAL1, RUNX2, DGKA, CUL4A, BCL11B, MIR155HG, MIR3188, GABRR3, SMAD7, GRAMD1B, CRTAM, AGBL3, CASR, RUNDC3B, DTNA, METTL21EP, LOC727896, TMOD1, SLC4A4, LINC00708, GPR64, ASB4, SETD7, RIF1, SFMBT1, PAX8-AS1, VPS8, FCER1G, TOP1, CPEB2-AS1, TPTE2P6, PYROXD1, IL1RAP, ARHGEF12, CTLA4, STK17B, PPP2R5C, NLRP6, MTRNR2L8, SGK1, MIR4471, BEX5, GRAP2, FIGNL1, MTNR1B, CAPN13, COLEC12

Table 9 is shown in Appendix, which is part of the disclosure, and is incorporated herein by reference in its entirety.

Table 10. Summary statistics for ATAC-seq. of Patient 10 CD8+ CAR+ and CD8+ CAR- T-cells

Sample Name	Nextera Index	Total Reads (paired-end 2 x 75 bp)	Unique Concordant Alignment #	% Aligned Concordantly
CD8+ CAR+ T-cells (Replicate 1)	CAGAGAGG	98,139,264	89,411,351	91.1
CD8+ CAR+ T-cells (Replicate 2)	CTCTCTAC	78,513,260	75,702,498	96.4
CD8+ CAR- T-cells (Replicate 1)	GGACTCCT	220,099,187	204,644,381	93.0
CD8+ CAR- T-cells (Replicate 2)	TAGGCATG	98,975,732	91,325,785	92.3

Table 11. List of uniquely enriched transcription factor binding motifs present in peaks gained in CD8+ CAR+ compared to CD8+ CAR- T-cells from Patient 10

Motif Name	Consensus	P-value	Log P-value	q-value (Benjamini)	# of Target Sequences with Motif (of 817)	% of Target Sequences with Motif	# of Background Sequences with Motif (of 46637)	% of Background Sequences with Motif
NFY(CCAAT)/Promoter/Homer	RGCCAATSRG	1.00E-18	-4.37E+01	0	84	10.28%	1529.7	3.28%
GABPA(ETS)/Jurkat-GABPa-ChIP-Seq(GSE17954)/Homer	RACCGGAAGT	1.00E-15	-3.49E+01	0	106	12.97%	2573.3	5.52%
ELF1(ETS)/Jurkat-ELF1-ChIP-Seq(SRA014231)/Homer	AVCCGGAAGT	1.00E-14	-3.39E+01	0	91	11.14%	2059.7	4.42%
Elk1(ETS)/Hela-Elk1-ChIP-Seq(GSE31477)/Homer	HACTTCCGGY	1.00E-12	-2.77E+01	0	90	11.02%	2265.4	4.86%
Elk4(ETS)/Hela-Elk4-ChIP-Seq(GSE31477)/Homer	NRYTTCCGGY	1.00E-11	-2.55E+01	0	87	10.65%	2243.5	4.81%
Sp1(Zf)/Promoter/Homer	GGCCCCGCCCCC	1.00E-05	-1.18E+01	0.0001	71	8.69%	2341.6	5.02%
ELF5(ETS)/T47D-ELF5-ChIP-Seq(GSE30407)/Homer	ACVAGGAAGT	1.00E-04	-1.14E+01	0.0002	52	6.36%	1557.7	3.34%
ETS:RUNX(ETS,Runt)/Jurkat-RUNX1-ChIP-Seq(GSE17954)/Homer	RCAGGATGTGGT	1.00E-04	-1.12E+01	0.0002	14	1.71%	195.8	0.42%
MYB(HTH)/ERMYB-Myb-	GGCVGTTR	1.00E-04	-	0.0008	99	12.12%	3825	8.20%

ChIPSeq(GSE22095)/Homer			9.52E+00					
KLF5(Zf)/LoVo-KLF5-ChIP-Seq(GSE49402)/Homer	DGGGYGKGGC	1.00E-04	-9.27E+00	0.001	145	17.75%	6109	13.10%
GFY-Staf(?,Zf)/Promoter/Homer	RACTACAATTCCCAGAAKGC	1.00E-03	-8.82E+00	0.0015	13	1.59%	215.9	0.46%
BMYB(HTH)/Hela-BMYB-ChIP-Seq(GSE27030)/Homer	NHAACBGYYV	1.00E-03	-8.36E+00	0.0022	73	8.94%	2714.3	5.82%
AMYB(HTH)/Testes-AMYB-ChIP-Seq(GSE44588)/Homer	TGGCAGTTGG	1.00E-02	-6.74E+00	0.0092	74	9.06%	2928.3	6.28%
GFY(?)/Promoter/Homer	ACTACAATTCCC	1.00E-02	-6.12E+00	0.0161	10	1.22%	189.3	0.41%

Next, the differentiation state of *in vivo* CTL019 cells from Patient 10 was analyzed and compared to CAR T-cells from six other patient who responded to this therapy, including two subjects with CLL (Patients 1 and 2) who had long-term durable remissions (>6 years) and did not have *TET2* integrations. At the peak of *in vivo* engraftment and activation marker expression (FIG. 9), greater than 65% of the CAR T-cells were of a central memory phenotype, which differed from these other complete responders whose repertoires were dominated by CD8+ effector memory and effector CTL019 cells at the height of the response (FIG. 7E). Knockdown of *TET2* recapitulated the effect of its loss in Patient 10, e.g., insertional disruption, on the differentiation state of both total and CAR+ CD8 as well as CD4 human T-cells from healthy subjects (FIGs. 7F-G; FIGs. 10A-10C), implicating TET2 as an epigenetic regulator of T-lymphocyte fate.

TET2-mediated regulation of CD8+ T-cell differentiation may not occur at the transcriptional level, as we did not observe differential TET2 mRNA expression between naïve and memory subsets. These findings may be explained by the observation that although TET2 gene expression is rapidly and transiently increased upon T-cell receptor triggering in a Ca2+-dependent manner, the timing of 5-hmC induction occurs faster than changes in mRNA expression. The antigen receptor signals that regulate TET2 transcription as well as TET enzymatic activity during T-cell activation/differentiation appear to be tightly controlled and may act through multiple independent mechanisms.

To investigate the functional significance of this T-cell differentiation state alteration resulting from TET2 deficiency, an *in vitro* serial re-stimulation assay which is predictive of clinical CAR T-cell potency was performed. Repeated stimulation with CD19-expressing tumour cells allowed TET2-deficient CAR T-cells to continue to expand in an antigen-dependent manner, whereas re-stimulation of CAR T-cells with unaltered TET2 resulted in culture growth arrest (FIG. 12C) without affecting their viability (FIG. 17). These results demonstrate that 2-hydroxyglutarate, an inhibitor of TET2, maintains the survival of murine CD8+ T-cells *ex vivo*, which in turn may mediate the *in vivo* anti-tumor activity of these lymphocytes that would otherwise be diminished by effector differentiation. Although knockout of TET2 in murine CD8+ T-cells shows similar central memory phenotype skewing, their enhanced activity in the context of immunoinflammatory and anti-viral responses is not attributed to a proliferative advantage Carty, S. A. *et al. J Immunol,* doi:10.4049/jimmunol.1700559 (2017)). This underscores the differential effect of TET2 deficiency on the function of human relative to mouse CD8+ T-cells.

To further address to effect of TET2 inhibition on CAR T-cell function, the production of cytokines following acute (FIG. 18A) and chronic (FIG. 18B) *in vitro* antigen stimulation was examined. Consistent with our analysis of CD8+ CAR+ T-cells in Patient 10, IFNγ production following CD3/CD28 activation was diminished in CD8+ as well as CD4+ T-cells with reduced TET2 levels (FIG. 18A). A similar decrease was observed for TNFα generation (FIG. 18B). In contrast, acute production of both TNFα and IL-2 by CD4+ T-cells was increased upon CAR-specific induction (FIG. 18A). While repeated exposure of bulk CTL019 cells to CD19-expressing tumour targets also

led to a decrease in IFNγ elaboration (FIG. 18B), TET2 inhibition resulted in the sustained production of various other stimulatory, inflammatory and regulatory cytokines following multiple rounds of stimulation (FIG. 18B). These observations suggest that TET2 may control human T-cell subset-specific cytokine production in an antigen-receptor and/or co-stimulatory signal-dependent fashion.

In addition to the regulation of cytokine gene loci, DNA methylation is an important, dynamic epigenetic process that influences the formation and maintenance of the effector CD8+ T-cell state. Based on our functional evaluation of TET2-deficient CAR+ T-cells expanded from Patient 10 and findings from other studies, we predicted that knock-down of TET2 would decrease effector molecule expression in CTL019 lymphocytes. CAR-specific, but not CD3/CD28 stimulation increased the expression CD107a (FIG. 19A). This may be attributed, at least in part, to enhanced cytolytic capacity mediated by 4-1BB over CD28 co-stimulation due to NKG2D up-regulation. Because CD8+ T-cell differentiation is accompanied by decreased methylation and upregulated gene expression at effector gene loci, including GZMB (encoding granzyme B) and IFNG, explored whether TET2 inhibition influences critical components of the cytotoxic machinery was subsequently explored. In contrast to IFNγ, TET2 reduction in CD8+ CAR+ T-cells increased granzyme B and perforin expression levels (FIG. 19B). These changes were associated with the heightened cytotoxic activity of TET2 knock-down CAR T-cells upon co-culture with CD19-expressing leukemic targets (FIG. 19C).

The above findings suggest that TET2 deficiency may produce highly potent CAR T-cells with properties of short-lived memory cells that can rapidly expand and elicit robust effector responses, as well as long-lived memory cells that durably persist. Additional effector/memory markers were thus examined in CD8+ CAR+ and CAR- T-cells using retrospective post-infusion samples from Patient 10 and other long-term responding CLL patients. At the height of the response, tumour reactive CAR+ T-cells from Patient 10 possessed higher levels of granzyme B (FIG. 20A) and Eomesodermin (EOMES; transcription factor involved in the formation and maintenance of the CD8+ memory T-cell pool; FIG. 20B, left panel) compared to matched CAR- T-cells, which was distinct from other responders who experienced durable remissions. All clinically active CD8+ CAR+ T-cells in responding patients, including those of Patient 10 expressed CD27, a co-stimulatory receptor involved in the generation of T-cell memory (FIG. 20B, middle panel). The frequency of CTL019 cells expressing KLRG1, a marker of T-lymphocyte senescence that is known to be regulated by DNA methylation, was significantly lower on TET2-deficient Patient 10 CAR T-cells compared to those of other subjects (FIG. 20B, right panel). In accordance with our previous observations, a high frequency of Ki-67 positive CAR+ T-cells was observed at the peak of in vivo expansion in Patient 10 (FIG. 20A, right panel), further suggesting that TET2 is required for CAR-specific CD8+ T-cell proliferation and expansion. These observations collectively support the idea that TET2 loss promotes the development of human memory CAR T-cells capable of eliciting strong anti-tumour effector responses.

In summary, profound clonal expansion of a single CAR-transduced T-cell with biallelic TET2 deficiency, e.g., in which lentiviral integration disrupted *TET2,* transformed a non-curative response into a deep molecular remission in a seventy-eight-year-old CLL patient. Characterization of this T-lymphocyte population revealed that even moderate changes to the epigenetic environment can alter differentiation fate, as well as effector function, and translate into a considerable therapeutic effect. Although the initial studies were based on an extensive analysis of one subject, recapitulation of the effect of *TET2* deficiency on CAR T cell fate, T-lymphocyte differentiation and anti-tumor activity in relevant culture systems involving primary human T-cells from 12 healthy individuals supports the discovery of a modifiable epigenetic pathway that can shape the immune response. Thus, targeting the epigenome using small molecules, highly efficient site-directed transgene integration strategies or other genetic engineering approaches may improve the efficacy and persistence of CAR T-cells in cancer therapy. In addition, this investigation provides the impetus for extending epigenetic landscape mapping to CAR T-cells and a mechanistic framework for determining how TET2 may partially (or fully) regulate their differentiation potential/effector potency through catalytic and/or non-catalytic pathways. Finally, the results indicate that the progeny of a single CAR T-cell are sufficient to mediate potent anti-tumor effects in advanced leukemia.

Methods

Patient samples

Patients were enrolled in institutional review board (IRB)-approved clinical protocol: "Genetically Engineered Lymphocyte Therapy in Treating Patients With B-Cell Leukemia or Lymphoma That is Resistant or Refractory to Chemotherapy" (ClinicalTrials.gov number: NCT01029366) which was designed to evaluate the safety and efficacy of the adoptive transfer of autologous T cells expressing CD19 chimeric antigen receptors that incorporate TCR Zeta and 4-1BB costimulatory domains (CTL019). All participants provided written informed consent in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The current study is a secondary investigation using patient samples collected from existing clinical trials. Therefore, the sample sizes in this Example were determined by the original clinical trial designs and sample availability; no additional inclusion/exclusion criteria were applied.

Cell Lines

The NALM-6 cell line was originally obtained from the American Type Culture Collection (ATCC). OSU-CLL cells were obtained from Ohio State University. Cells were expanded in RPMI media containing 10% fetal bovine serum (FBS), penicillin, and streptomycin at a low passage and tested for mycoplasma using the MycoAlert detection kit as per the manufacturer's (Lonza) instructions. Authentication of cell lines was performed by the University of Arizona (USA) Genetics

Core based on criteria established by the International Cell Line Authentication Committee. Short tandem repeat (STR) profiling revealed that these cell lines were well above the 80% match threshold. NALM-6 and OSU-CLL cells were engineered to constitutively express click beetle green (CBG) luciferase/enhanced GFP (eGFP) and sorted on a FACSAria (BD) to obtain a >99% pure population. Mycoplasma and authentication testing are routinely performed before and after molecular engineering.

CAR T-cell manufacturing and correlative studies

Peripheral blood T cells for CTL019 manufacturing were obtained by leukapheresis as previously described (Fraietta, J. A. et al. Blood 127, 1117-1127 (2016); Kalos, M. et al. Science translational medicine 3, 95ra73, (2011); Porter, D. L. et al. The New England journal of medicine 365, 725-733, (2011), Porter, D. L. et al. Science translational medicine 7, 303ra139, (2015)). The processing, flow cytometric evaluation, quantification of serum cytokines and quantitative PCR analyses of pre- and post-CTL019 infusion samples were conducted as previously reported (Maude, S. L. et al. The New England journal of medicine 371, 1507-1517, (2014)). Next generation sequencing of immunoglobulin heavy chain (IGH) rearrangements was carried out on DNA isolated from blood and marrow samples. Briefly, primers specific for the variable and joining gene segments of the third complementarity-determining region of the IGH were used for amplification and deep sequencing to identify the leukemic clone relative to baseline samples (Adaptive Biotechnologies). The frequency of the leukemic clone in each sample was calculated using the number of total and unique productive reads. These correlative assays were carried out at time points defined by the respective clinical protocols in parallel with disease response evaluations. This clinical trial was a single-treatment study; comparisons between patients in the current study were defined by the observed clinical responses. Investigators were blinded to clinical responses as correlative assays were conducted using de-identified subject samples.

Flow cytometry

Routine assessments of CTL019 expansion and persistence as well as B-CLL burden in the blood and marrow were conducted according to previously published methods using a six-parameter Accuri C6 flow cytometer (BD) (Kalos, M. et al. Science translational medicine 3, 95ra73, (2011); Porter, D. L. et al. Science translational medicine 7, 303ra139, (2015)). T cell immunophenotyping was performed on CTL019 infusion products or post-infusion PBMC samples by surface staining with flow cytometry antibodies immediately following pre-incubation with Aqua Blue dead cell exclusion dye (Invitrogen). The Alexa Fluor 647–conjugated monoclonal antibody that was used to detect the CAR molecule has been described (Jena, B. et al. PLoS One 8, e57838, (2013)). Commercially available flow cytometry antibodies used in the study are as follows: CD3 allophycocyanin (APC) H7, CCR7 PE/CF594 (BD Biosciences); CD107a APC, (BD Biosciences); CD45RO brilliant violet

(BV)570, CD8 BV650, CD4 BV785 (Biolegend); Perforin BV421, Ki-67 Alexa Fluor (AF)700, TNFα BV605 (Biolegend); IFNγ PE, IL-2 PerCP-eFluor 710, Eomes FITC (eBioscience); Granzyme B PE/Cyanine5.5 (Invitrogen) and TCRVβ5.1 APC (eBioscience). The GolgiStop protein transport inhibitor containing monensin and GolgiPlug protein transport inhibitor containing brefeldin A (BD Biosciences) were used when staining for intracellular cytokine production. All flow cytometry reagents were titrated prior to use. Samples were acquired on an LSRFortessa (BD) and data were analyzed using FlowJo software (TreeStar).

TCRVβ deep sequencing

Genomic DNA from pre-infusion T cells, peripheral blood samples or sorted post-infusion T-cells was isolated using the DNeasy Blood and Tissue Kit (Qiagen). TCRVβ deep sequencing was carried out by immunoSEQ (Adaptive Biotechnologies). Only productive TCR rearrangements were used in the assessment of TCR clonotype frequencies.

Integration site analysis

Vector integration sites were detected from genomic DNA as described previously (Brady, T. et al., Nucleic Acids Res 39, e72 (2011); Berry, C. et al., PLoS Comput Biol 2, e157 (2006); Berry, C. C. et al., Bioinformatics 28, 755-762 (2012); Berry, C. C. et al., Bioinformatics 30, 1493-1500 (2014)). Genomic sequences were aligned to the human genome by BLAT (hg18, version 36.1, >95% identity) and statistical methods for analyzing integration site distributions were carried out as previously described (Scholler, J. et al., Science translational medicine 4, 132ra153 (2012)). The SonicAbundance method was used to infer the abundance of cell clones from integration site data (Berry, C. C. et al., Bioinformatics 28, 755-762 (2012)). All samples were analyzed independently in quadruplicate to suppress founder effects in the PCR and stochastics of sampling.

Detection of TET2 chimeric transcripts

Sample RNA was isolated and used as template with the Qiagen One-Step RT-PCR Kit. Primers were designed to target the exon 9 and 10 boundaries of *TET2*, flanking the vector integration site and sequences internal to the anti-CD19BBζ CAR lentiviral vector. These included various regions of the vector sequence (FIG. 6A). Reactions were carried out as per the manufacturer's specifications. Thermocycling temperatures and time for reverse transcription and PCR activation were conducted as per the manufacturer, with the following cycling conditions: 30 seconds at 94°C for melting, 30 seconds at 57°C for primer annealing and 1.5 minutes at 72°C for primer extension (35 cycles). A final extension at 72°C was held for 10 minutes for each sample. PCR products were visualized on ethidium bromide agarose gels (1.5% by weight) via electrophoresis and UV imaging.

Next-generation sequencing of post-infusion CAR T cell samples

CAR+ and CAR- CD8+ T- cells were purified from post-infusion PBMC samples corresponding to the peak of in vivo expansion in Patient 10. T-cells were sorted using a FACSAria (BD) and genomic DNA was isolated from these lymphocytes as described above. A custom targeted next generation sequencing panel of 68 genes associated with hematologic malignancies was then utilized (TruSeq Custom Amplicon, Illumina Inc.) and sequenced on the Illumina MiSeq (Illumina, Inc.). A minimal mean depth of 2110 reads was achieved for the specimens sequenced, with the assay and bioinformatics performed as previously described, e.g., see Daber, R., Sukhadia, S. & Morrissette, J. J. *Cancer Genet* 206, 441-448. The data presented is based on the human reference sequence UCSC build hg 19 (NCBI build 37.1).

Determining the TET2 allele hosting vector integration

A PCR assay was developed to amplify the region of DNA (approximately 4 kB) between the vector integration and the locus of the c.5635G>C mutation. Primers were designed to anneal to the vector sequence (MKL-3: 5'-CTTAAGCCTCAATAAAGCTTGCCTTGAG-3') and multiple locations downstream of the mutation, chr4:105,276,145 (50 bp: 5' GCTGGTAAAAGACGAGGGAGATCCTG-3', 99 bp: 5'-GGCTTCCCAAAGAGCCAAGCCATG-3', 120 bp: 5'-CACGGGCTTTTTCAGCCATTTTGGC-3'). Genomic DNA samples from sorted CAR+ and CAR- CD8+ T cells corresponding to the peak of clonal expansion in Patient 10 were selected for amplification. PCR reactions were carried out with Long Amp Taq polymerase (New England BioLabs) and 100 – 400 ng of DNA from samples, according to manufacturer recommendations. Amplification was conducted as follows: 94°C for 30 seconds, 30 cycles of (94°C for 30 seconds, 60°C for 30 seconds, and 65°C for 3 minutes 20 seconds), and a final extension of 65°C for 10 minutes. Amplified products were separated by electrophoresis on a 1.0% ethidium bromide agarose gel and prominent bands of 4 kb in size were isolated using the QIAquick Gel Extraction Kit (Qiagen). Isolated bands were ligated into pCR2.1 vectors and cloned into TOP-10 chemically competent cells using the TOPO TA Cloning Kit (Invitrogen). Purified plasmids were sequenced using M13 forward and reverse primers using standard Sanger technology. Sequencing results were aligned to vector sequence and reference genome.

Characterization of the TET2 E1879Q mutation

The previously characterized and crystalized human TET2-CS variant (1129-1936 Δ1481-1843) was expressed using a pLEXm expression vector. The E1879Q mutation or mutation of the catalytic H1382Y and D1384A (HxD mutant) were generated by standard means. HEK293T cells were cultured in DMEM with GlutaMAX (ThermoFisher Scientific) and 10% FBS (Sigma). Cells were transfected with wild-type (WT), mutant hTET2-CS or an empty pLEXm vector control using Lipofectamine 2000 (ThermoFisher Scientific) according to the manufacturer's protocol. Media was

changed 24 hours after transfection, and cells were harvested by trypsinization 48 hours after transfection and resuspended in phosphate-buffered saline. Genomic DNA was isolated from four-fifths of the cells using the DNeasy Blood and Tissue Kit (Qiagen) and the remaining one-fifth of the cells were lysed using the CytoBuster Protein Extraction Reagent (EMD Millipore) for western blot analysis.

DNA blots for cytosine modifications were carried out according to established protocols. Purified DNA from HEK293T cells was diluted to 15, 7.5, and 3.5 ng/μL in Tris–EDTA (TE) buffer, pH 8.0 for two-fold dilutions of each sample. One-fourth volume of 2 M NaOH–50 mM EDTA was added to each sample. The DNA was denatured for 10 minutes at 95°C and transferred quickly to ice, followed by the addition of 1:1 ice cold 2 M ammonium acetate. Polyvinylidene Difluoride (PVDF) membranes were cut to size, wet with MeOH and equilibrated in TE buffer and then assembled into the PR 648 Slot Blot Manifold (GE Healthcare Life Sciences). Each well was washed with 400 μL TE drawn through with gentle vacuum, and either 600, 300, or 150 ng of genomic DNA was loaded, followed by another TE wash. Membranes were blocked for 2 hours in 5% milk–TBST, washed thrice with TBST, and blotted at 4°C overnight with primary antibodies against each modified cytosine — 1:5,000 mouse anti-5-mC (Abcam); 1:10,000 rabbit anti-5-hmC (Active Motif); 1:5,000 rabbit anti-5-fC (Active Motif); 1:10,000 rabbit anti-5-caC (Active Motif). Blots were then washed, incubated with a 1:2,000 dilution of a secondary horse anti-mouse- horseradish peroxidase (HRP; Cell Signaling Technology) or 1:5,000 goat anti-rabbit-HRP (Santa Cruz Biotechnology) for 2 hours, washed and imaged using the Immobilon Western Chemiluminescent HRP Substrate (Millipore) and the Amersham Imager 600 (GE Healthcare Life Sciences).

For protein detection, clarified cell lysates were run on 8% sodium dodecyl sulfate polyacrylamide (SDS–PAGE) gels. Gels were transferred together onto a PVDF membrane using the iBlot 2 Gel Transfer Device (ThermoFisher Scientific). Membranes were blocked for 2 hours at room temperature with 5% (w/v) milk in Tris-buffered saline with 0.1% (v/v) Tween-20 (TBST), washed ×3 with TBST and blotted either with primary 1:10,000 anti-FLAG M2 (Sigma) or 1:1,000 anti-Hsp90α/β (Santa Cruz Biotechnology) antibodies at 4°C overnight. Following incubation, membranes were washed and blotted with a 1:5,000 dilution of a secondary goat anti-mouse-HRP (Santa Cruz Biotechnology) for 2 hours, washed and imaged with Immobilon Western Chemiluminescent HRP Substrate (Millipore) on a Amersham Imager 600 (GE Healthcare Life Sciences).

For liquid chromatography tandem-mass spectrometry (LC-MS/MS), 1-2 μg of genomic DNA from each sample was degraded to component nucleosides with 1 U DNA Degradase Plus (Zymo Research Corporation) at 37°C overnight. The nucleoside mixture was diluted ten-fold into 0.1% formic acid, and injected onto an Agilent 1200 Series HPLC with a 5 μm, 2.1 × 250 mm Supelcosil LC-18-S analytical column (Sigma) equilibrated to 45°C in Buffer A (5 mM ammonium formate, pH 4.0). The nucleosides were separated in a gradient of 0–15% Buffer B (4 mM ammonium formate, pH 4.0, 20% (v/v) methanol) over 8 minutes at a flow rate of 0.5 mL/minute. Tandem

MS/MS was performed by positive ion mode ESI on a 6460 triple-quadrupole mass spectrometer (Agilent) with a gas temperature of 250°C, a gas flow of 12 L/minute, a nebulizer pressure of 35 psi, a sheath gas temperature of 300°C, a sheath gas flow of 11 L/minute, a capillary voltage of 3,500 V, a fragmentor voltage of 70 V and a delta EMV of +1,000 V. Collision energies were optimized to 10 V for 5-mC and 5-fC; 15 V for 5-caC; and 25 V for 5-hmC. Multiple reaction monitoring (MRM) mass transitions were 5-mC 242.11 126.066 m/z; 5-hmC 258.11 124.051; 5-fC 256.09 140.046; 5-caC 272.09 156.041; and T 243.10 127.050. Standard curves were generated using standard nucleosides (Berry & Associates, Inc.) ranging from 2.5 μM to 610 pM (12.5 pmol to 3 fmol total). Digested oligonucleotides containing equimolar amounts of each modified cytosine were used as quality control samples. The sample peak areas were fit to the standard curve, as adjusted by the quality control samples to determine the amount of each modified cytosine in the genomic DNA sample. Amounts are expressed as the percentage of total cytosine modifications.

Measurement of total 5-hydroxymethylcytosine levels

CD8+ T-cells were purified from post-infusion PBMC samples using the EasySep Human CD8+ T Cell Immunomagnetic Negative Selection Kit (StemCell Technologies) and expanded *ex vivo* using a previously reported rapid expansion protocol (Jin, J. et al. J Immunother 35, 283-292, (2012)). Following culture, CD8+ CAR+ TCRVβ5.1+ and CD8+ CAR- TCRVβ5.1- T-cells were sorted on a FACSAria (BD). Cells were permeabilized and treated with 300μg/ml DNase I for 60 minutes at 37°C. After washing, samples were incubated with an anti-5hmc monoclonal antibody or an isotype control for 30 minutes, followed by staining with an Alexa Fluor 647-conjugated secondary antibody. Cells were immediately acquired on an LSRFortessa (BD).

Global chromatin profiling by ATAC-seq

Following culture, CD8+ CAR+ TCRVβ5.1+ and CD8+ CAR- TCRVβ5.1- T-cells were sorted on a FACSAria (BD). ATAC-seq was carried out as previously described (Buenrostro, J. D. et al. Nat Methods 10, 1213-1218, (2013); Pauken, K. E. et al. Science 354, 1160-1165, (2016)). Two replicates were performed for each *ex vivo* expanded CD8+ CAR+ TCRVβ5.1+ and CD8+ CAR- TCRVβ5.1- T-cell culture. Briefly, nuclei were isolated from 200,000 sorted CD8+ T-cells for each replicate, followed by the transposition reaction in the presence of Tn5 transposase (Illumina) for 45 minutes at 37°C. Purification of transposed DNA was subsequently completed with a MinElute Kit (Qiagen) and fragments were barcoded with dual indexes (Illumina Nextra). Paired-end sequencing (2 × 75bp reads) was carried out using the Illumina NextSeq 500. Raw sequencing data were processed and aligned to the GRC37h/hg19 reference genome using Bowtie2 and regions of significant enrichment were identified using MACS v1.4.2. Merged peak lists were created using BedTools, sequencing tag enrichment was carried out using HOMER and GO/Pathway analysis was performed

with Metascape. Only high-confidence peaks were used for gene ontology and DNA motif analyses. For these evaluations, peaks with an enrichment score less than 5 were filtered out as previously established.

Intracellular cytokine analysis

Ex vivo expanded CD8+ T-cells were stimulated 3:1 with paramagnetic polystyrene beads coated with anti-CD3 and anti-CD28 monoclonal antibodies for 6 hours in the presence of CD107a monoclonal antibody and the Golgi inhibitors brefeldin A and monensin. Cells were washed, stained with live/dead viability dye, followed by surface staining for CD3, CD8 and TCRVβ5.1+. These lymphocytes were subsequently fixed/permeabilized and intracellularly stained for IFN-γ. Cells were analyzed on an LSRFortessa (BD).

CAR T-cell differentiation and expansion potency assay

Bulk primary human T-cells were activated with paramagnetic polystyrene beads coated with anti-CD3 and anti-CD28 monoclonal antibodies as previously described (Laport, G. G. et al. Blood 102, 2004-2013 (2003)) and transduced with lentiviral vectors encoding the anti-CD19BBζ CAR and shRNA hairpin sequences targeting TET2 or a scrambled control with GFP co-expression (Cellecta). Knockdown efficiency in T-cells following shRNA transduction was determined by real-time quantitative PCR with Taqman gene expression assays (Applied Biosystems) for TET2 (assay Hs00325999_m1) and GAPDH (assay (Hs03929097_g1) as well as GUSB (Hs99999908_m1) which served as loading and normalization controls. Following 14 days of culture, the differentiation phenotype of these cells was determined by flow cytometry. GFP+ CAR+ T-cells were sorted on a FACSAria (BD) and combined 1:1 with irradiated K562 cells engineered to express $CD19^6$ or mesothelin as a negative control. CTL019 cells were serially re-stimulated with irradiated K562 targets for a total of 3 times, with absolute counts and viability assessments taken at regular intervals over 17 days. Cell counts and viability measurements were obtained using the LUNA Automated Cell Counter (Logos Biosystems). Population doublings were calculated using the equation $A_t = A_0 2^n$, where n is the number of population doublings, A_0 is the input number of cells, and A_t is the total number of cells. Supernatants were collected 24 hours after each re-stimulation for longitudinal measurements of cytokine levels in cultures.

Intracellular cytokine, perforin and granzyme B analysis.

CD8+ T-cells from Patient 10 were stimulated 3:1 with paramagnetic polystyrene beads coated with anti-CD3 and anti-CD28 monoclonal antibodies for 6 hours in the presence of CD107a monoclonal antibody and the Golgi inhibitors brefeldin A and monensin. Cells were washed, stained with live/dead viability dye, followed by surface staining for CD3, CD8 and TCRVβ5.1+. These

lymphocytes were subsequently fixed/permeabilized and intracellularly stained for IFNγ. CAR T-cells generated from healthy donors (TET2 knock-down or control) were stimulated in the same way with CD3/CD28 beads or beads coated with an anti-idiotypic antibody against CAR19. Cells were then stained for surface markers (CD3, CD4, CD8 and CAR19). After fixation and permeabilization, intracellular staining for IFNγ, TNFα and IL-2 was performed.

For perforin and granzyme B analysis, CTL019 cells that had been transduced with a TET2 or scrambled control shRNA were expanded for 14 days and cryopreserved. These CAR T-cells were then thawed and rested for 4 hours, followed by live/dead and surface staining for CD3, CD8 as well as CAR19. Intracellular staining for perforin and granzyme was carried out following fixation and permeabilization. Cells were analyzed on an LSRFortessa (BD).

Cytotoxicity assay.

Healthy donor CTL019 cells transduced with a shRNA directed against TET2 or a scrambled control were co-cultured with CBG luciferase-expressing NALM-6 and OSU-CLL cell lines at the indicated ratios for 16 hours. Cell extracts were created using the Bright-Glo Luciferase Assay System (Promega Corporation) and substrate was added according to the manufacturer's instructions. Luciferase measurements were taken on a SpectraMax luminescence microplate reader (Molecular Devices), and specific lysis was calculated.

Analysis of TET2 gene expression levels in T-cell subsets.

TET2 gene expression levels were determined by analyzing a published gene expression dataset for CD8+ T-cell subsets (Naïve, TN; Stem Cell Memory, TSCM; Central Memory, TCM; and Effector Memory, TEM) isolated from 3 different healthy human subjects. Genechip (Affymetrix) data were processed with the Bioconductor Oligo software package (release 3.6, Bioconductor) using the RMA method.

Statistical analyses

Normality was assessed for all data using the D'Agostino-Pearson omnibus test. When the sample size was too small to adequately examine normality, non-parametric statistics were used. For integration site data analysis, genomic feature data comparisons were carried out as previously described using X^2, Fisher's exact tests, or a combination of Bayesian model averaging, conditional logit and regression (Berry, C. et al. PLoS Comput Biol 2, e157 (2006); Brady, T. et al. Genes Dev 23, 633-642, (2009); Berry, C. et al. PLoS Comput Biol 2, e157, (2006); Ocwieja, K. E. et al. PLoS Pathog 7, e1001313, (2011)). Assessment of T-cell differentiation phenotypes in shRNA-mediated *TET2* knockdown experiments was performed using a paired student's *t*-test. With 12 normal donors, 88% power is achieved for detecting a minimum effect size of 1.0 (in the unit of standard deviation) using a two-sided paired student's *t*-test. Estimates of variation within each group of data are

presented as error bars in figures. Analyses were performed with SAS (SAS Institute Inc.), Stata 13.0 (StataCorp) or GraphPad Prism 6 (GraphPad Software). All tests were two-sided. A *P* value < .05 was considered statistically significant.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples specifically point out various aspects of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EQUIVALENTS

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific aspects, it is apparent that other aspects and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such aspects and equivalent variations.

Appendix

Table 9. Gene set enrichment analysis reports showing GO and KEGG pathways and hallmark gene sets associated with ATAC-seq peaks lost in CD8+CAR+ compared to CD8+CAR- Patient 10 T cells *

	Column A	Column B	Column C	Column D
	Group ID Category Term Description	LogP Log(q-value) InTerm_InList	Genes	Symbols
1	1_Summary GO Biological Processes GO:0002521 leukocyte differentiation	-15.06239065 -10.880 93/453	301,302,399,537,558,596,604,639,677,814,841,860,861,914,925,940,942,944,1015,1021,1230,1236,1316,1380,1435,1436,1437,1499,1540,1794,1880,1960,2185,2353,2625,3442,3458,3516,3559,3567,3575,3592,3600,3624,3635,3688,3702,3965,4057,4208,4602,4853,5295,5591,5734,6095,6304,6504,6670,6850,7048,7071,7292,7301,7704,8111,8320,8600,8767,8995,9290,9308,9734,9855,10100,10125,10221,10320,23228,26191,27086,30009,51208,55904,64218,80762,84456,84959,114548,146850,149233,338339,387357,836,911,919,921,960,1525,2534,2796,3037,3077,3479,3481,4004,5063,5290,6441,6885,7356,8440,9402,10507,11148,29851,54518,56940,57162,57829,64581,79109,84174,120425,151888,253260,81,154,335,831,1906,1907,2153,2157,2244,2335,2768,2776,3596,3708,3709,3937,4773,5054,5321,5551,5579,5770,5791,5912,7114,7294,7424,7476,7534,8525,8660,9173,9948,10257,10451,10487,10666,10672,11093,54331,57181,64092,117194,124912,168667,4478,4907,965,1946,1956,3384,3557,3911,3987,4092,4653,4815,4897,5962,6239,6480,9231,9369,10049,10563,22862,23396,23607,26999,29119,55243,9166,255743,27,284,347,3673,4008,5728,5793,5795,5800,5921,6423,7402,8573,9267,9891,10395,23122,23499,25975,54566,79625,375056,103,1030,2034,2113,2296,2672,5305,7049,7520,8621,10296,23335,23543	ANXA1,ANXA2,RHOH,ATP6AP1,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP8,RUNX2,RUNX1,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR1,CCR7,KLF6,CR2,CSF1,CSF1R,CSF2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,PTK2B,FOS,GATA3,IFNA5,IFNG,RBPJ,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,LGALS9,LTF,MEF2C,MYB,NOTCH2,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SLAMF1,SP3,SYK,TGFBR2,KLF10,TNFSF4,TYRO3,ZBTB16,GPR68,EOMES,TNFSF11,RIPK2,TNFSF18,GPR55,CD83,HDAC9,FARP2,TSPAN2,RASGRP1,TRIB1,IKZF1,PLCL2,PTPN22,FOXP1,TBX21,CLDN18,KMT2E,SEMA4A,NDFIP1,L3MBTL3,UBASH3B,NLRP3,PIK3R6,IL23R,CLEC4D,THEMIS,CASP3,CD1C,CD247,CD5,CD44,CXADR,FYN,GNRH1,HAS2,HFE,IGF1,IGF2,LMO1,PAK3,PIK3CA,SFTPD,MAP3K7,SCGB1A1,NCK2,GRAP2,SEMA4D,HHLA2,ICOS,APBB1IP,DUSP22,PELI1,ZP4,CLEC7A,MAPKAP1,SLA2,JAML,BTLA,RICTOR,ACTN4,ADRB2,APOA1,CAST,EDN1,EDN2,F5,F8,FGB,FN1,GNA12,GNAQ,IL13,ITPR1,ITPR2,LCP2,NFATC2,SERPINE1,PLA2G4A,PRF1,PRKCB,PTPN1,PTPRE,RAP2B,TMSB4X,TXK,VEGFC,WNT7A,YWHAZ,DGKZ,IRS2,IL1RL1,WDR1,ABCC4,VAV3,CAP1,CD226,GNA13,ADAMTS13,GNG2,SLC39A10,SAMSN1,MRGPRX2,SPACA3,BMPER,MSN,NT5E,CD58,EFNA5,EGFR,ICAM2,IL1RN,LAMA5,LIMS1,SMAD7,MYOC,NINJ2,NRCAM,RDX,RREB1,ST6GAL1,DLG5,NRXN3,DNAJB6,CXCL13,FNDC3A,PIP5K1C,CD2AP,CYFIP2,CTNNA3,KIRREL,MYADM,NPNT,ABL2,ANGPT1,APOD,ITGA2,LMO7,PTEN,PTPRG,PTPRJ,PTPRO,RASA1,SFRP2,UTRN,CASK,CYTH1,NUAK1,DLC1,CLASP2,MACF1,EGFL6,EPB41L4B,NDNF,MIA3,ADAR,CDKN2B,EPAS1,

Appendix

			,23648,25976,28996,29909,51621,54502,54790,57623,85439,85477,115727,3720,10018,1233,23239,5789,9076,55714,60437	ETS1,FOXC1,GFI1,PIP4K2A,TGFBR3,XRCC5,CDK13,MAEA,WDR7,RBFOX2,SSBP3,TIPARP,HIPK2,GPR171,KLF13,RBM47,TET2,ZFAT,STON2,SCIN,RASGRP4,JARID2,BCL2L11,CCR4,PHLPP1,PTPRD,CLDN1,TENM3,CDH26
2	1_Member GO Biological Processes GO:0002521 leukocyte differentiation	-15.06239065 -10.880 93/453	301,302,399,537,558,596,604,639,677,814,841,860,861,914,925,940,942,944,1015,1021,1230,1236,1316,1380,1435,1436,1437,1499,1540,1794,1880,1960,2185,2353,2625,3442,3458,3516,3559,3567,3575,3592,3600,3624,3635,3688,3702,3965,4057,4208,4602,4853,5295,5591,5734,6095,6304,6504,6670,6850,7048,7071,7292,7301,7704,8111,8320,8600,8767,8995,9290,9308,9734,9855,10100,10125,10221,10320,23228,26191,27086,30009,51208,55904,64218,80762,84456,84959,114548,146850,149233,338339,387357	ANXA1,ANXA2,RHOH,ATP6AP1,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP8,RUNX2,RUNX1,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR1,CCR7,KLF6,CR2,CSF1,CSF1R,CSF2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,PTK2B,FOS,GATA3,IFNA5,IFNG,RBPJ,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,LGALS9,LTF,MEF2C,MYB,NOTCH2,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SLAMF1,SP3,SYK,TGFBR2,KLF10,TNFSF4,TYRO3,ZBTB16,GPR68,EOMES,TNFSF11,RIPK2,TNFSF18,GPR55,CD83,HDAC9,FARP2,TSPAN2,RASGRP1,TRIB1,IKZF1,PLCL2,PTPN22,FOXP1,TBX21,CLDN18,KMT2E,SEMA4A,NDFIP1,L3MBTL3,UBASH3B,NLRP3,PIK3R6,IL23R,CLEC4D,THEMIS
3	1_Member GO Biological Processes GO:0030098 lymphocyte differentiation	-14.43600319 -10.642 70/299	301,399,558,596,604,639,677,814,860,914,925,940,942,944,1015,1021,1236,1316,1380,1499,1540,1794,1880,1960,2185,2625,3442,3458,3516,3559,3575,3592,3600,3624,3635,3688,3702,3965,4602,4853,5295,5591,5734,6095,6304,6504,6670,6850,7048,7292,7301,7704,8320,8767,8995,9308,9734,10125,10320,23228,26191,27086,30009,64218,80762,114548,146850,149233,338339,387357	ANXA1,RHOH,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,RUNX2,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR7,KLF6,CR2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,PTK2B,GATA3,IFNA5,IFNG,RBPJ,IL2RA,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,LGALS9,MYB,NOTCH2,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SLAMF1,SP3,SYK,TGFBR2,TNFSF4,TYRO3,ZBTB16,EOMES,RIPK2,TNFSF18,CD83,HDAC9,RASGRP1,IKZF1,PLCL2,PTPN22,FOXP1,TBX21,SEMA4A,NDFIP1,NLRP3,PIK3R6,IL23R,CLEC4D,THEMIS
4	1_Member GO Biological Processes GO:0070486 leukocyte aggregation	-14.28228891 -10.642 89/436	301,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,960,1021,1236,1499,1525,1540,1794,1960,2534,2625,2796,3037,3077,3442,3458,3479,3481,3559,3575,3592,3600,3702,3965,4004,4602,5063,5290,5295,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7356,7704,8320,8440,8600,8767,8995,9308,9402,10125,10507,11148,26191,27086,29851,30009,54518,56940,57162,57829,64218,64581,79109,80762,84174,114548,120425,1	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CD44,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EGR3,FYN,GATA3,GNRH1,HAS2,HFE,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITK,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,SEMA4D,HHLA2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MA

Appendix

			46850,149233,151888,253260,338339,387357	PKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
5	1_Member GO Biological Processes GO:0001775 cell activation	-14.12538379 -10.642 153/953	81,154,301,335,399,558,596,604,639,677,814,831,836,841,860,911,914,919,921,925,940,942,944,1015,1021,1236,1316,1380,1435,1437,1499,1525,1540,1794,1880,1906,1907,1960,2153,2157,2185,2244,2335,2534,2625,2768,2776,2796,3077,3442,3458,3479,3481,3516,3559,3567,3575,3592,3596,3600,3624,3635,3688,3702,3708,3709,3937,3965,4004,4208,4602,4773,4853,5054,5063,5290,5295,5321,5551,5579,5591,5734,5770,5791,5912,6095,6304,6441,6504,6670,6850,6885,7048,7114,7292,7294,7301,7356,7424,7476,7534,7704,8320,8440,8525,8600,8660,8767,8995,9173,9308,9402,9734,9948,10125,10257,10320,10451,10487,10666,10672,11093,11148,23228,26191,27086,29851,30009,54331,54518,55904,56940,57162,57181,57829,64092,64218,64581,79109,80762,84174,84959,114548,117194,120425,124912,146850,149233,151888,168667,253260,338339,387357	ACTN4,ADRB2,ANXA1,APOA1,RHOH,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CAST,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR7,KLF6,CR2,CSF1,CSF2,CTNNB1,CXADR,CYLD,DOCK2,GPR183,EDN1,EDN2,EGR3,F5,F8,PTK2B,FGB,FN1,FYN,GATA3,GNA12,GNAQ,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,RBPJ,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,ITGB1,ITK,ITPR1,ITPR2,LCP2,LGALS9,LMO1,MEF2C,MYB,NFATC2,NOTCH2,SERPINE1,PAK3,PIK3CA,PIK3R1,PLA2G4A,PRF1,PRKCB,PRKDC,PTGER4,PTPN1,PTPRE,RAP2B,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TMSB4X,TNFSF4,TXK,TYRO3,SCGB1A1,VEGFC,WNT7A,YWHAZ,ZBTB16,EOMES,NCK2,DGKZ,TNFSF11,IRS2,RIPK2,TNFSF18,IL1RL1,CD83,GRAP2,HDAC9,WDR1,RASGRP1,ABCC4,IKZF1,VAV3,CAP1,CD226,GNA13,ADAMTS13,HHLA2,PLCL2,PTPN22,FOXP1,ICOS,TBX21,GNG2,APBB1IP,KMT2E,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,UBASH3B,NLRP3,MRGPRX2,JAML,SPACA3,PIK3R6,IL23R,BTLA,BMPER,RICTOR,CLEC4D,THEMIS
6	1_Member GO Biological Processes GO:0046649 lymphocyte activation	-13.97254737 -10.568 112/618	301,399,558,596,604,639,677,814,836,841,860,911,914,919,921,925,940,942,944,1015,1021,1236,1316,1380,1499,1525,1540,1794,1880,1960,2185,2534,2625,2796,3077,3442,3458,3479,3481,3516,3559,3567,3575,3592,3596,3600,3624,3635,3688,3702,3965,4004,4208,4602,4773,4853,5063,5290,5295,5551,5579,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7301,7356,7704,8320,8440,8600,8660,8767,8995,9308,9402,9734,10125,10320,10451,11148,23228,26191,27086,29851,30009,54518,56940,57162,57181,57829,64092,64218,64581,79109,80762,84174,114548,120425,146850,149233,151888,253260,338339,387357	ANXA1,RHOH,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR7,KLF6,CR2,CTNNB1,CXADR,CYLD,DOCK2,GPR183,EGR3,PTK2B,FYN,GATA3,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,RBPJ,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,ITGB1,ITK,LGALS9,LMO1,MEF2C,MYB,NFATC2,NOTCH2,PAK3,PIK3CA,PIK3R1,PRF1,PRKCB,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,CD83,GRAP2,HDAC9,RASGRP1,IKZF1,VAV3,HHLA2,PLCL2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS

Appendix

7	1_Member GO Biological Processes GO:0007159 leukocyte cell-cell adhesion	-13.64676655 -10.309 92/469	301,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,960,1021,1236,1499,1525,1540,1794,1960,2534,2625,2796,3037,3077,3442,3458,3479,3481,3559,3575,3592,3600,3688,3702,3965,4004,4478,4602,4907,5063,5290,5295,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7356,7704,8320,8440,8600,8767,8995,9308,9402,10125,10507,11148,26191,27086,29851,30009,54518,56940,57162,57829,64218,64581,79109,80762,84174,114548,120425,146850,149233,151888,253260,338339,387357	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CD44,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EGR3,FYN,GATA3,GNRH1,HAS2,HFE,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITGB1,ITK,LGALS9,LMO1,MSN,MYB,NT5E,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,SEMA4D,HHLA2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
8	1_Member GO Biological Processes GO:0042110 T cell activation	-13.40917032 -10.214 86/428	301,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,1021,1236,1499,1525,1540,1794,1960,2534,2625,2796,3077,3442,3458,3479,3481,3559,3575,3592,3600,3702,3965,4004,4602,5063,5290,5295,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7356,7704,8320,8440,8600,8767,8995,9308,9402,10125,11148,26191,27086,29851,30009,54518,56940,57162,57829,64218,64581,79109,80762,84174,114548,120425,146850,149233,151888,253260,338339,387357	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EGR3,FYN,GATA3,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITK,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
9	1_Member GO Biological Processes GO:0070489 T cell aggregation	-13.40917032 -10.214 86/428	301,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,1021,1236,1499,1525,1540,1794,1960,2534,2625,2796,3077,3442,3458,3479,3481,3559,3575,3592,3600,3702,3965,4004,4602,5063,5290,5295,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7356,7704,8320,8440,8600,8767,8995,9308,9402,10125,11148,26191,27086,29851,30009,54518,56940,57162,57829,64218,64581,79109,80762,84174,114548,120425,146850,149233,151888,253260,338339,387357	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EGR3,FYN,GATA3,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITK,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
10	1_Member	-13.39658694	301,399,558,596,604,639,677,814,836,841	ANXA1,RHOH,AXL,BCL2,BCL6,PRDM1,ZFP36L1,C

Appendix

	GO Biological Processes GO:0045321 leukocyte activation	-10.214 123/719	,860,911,914,919,921,925,940,942,944,1015,1021,1236,1316,1380,1435,1437,1499,1525,1540,1794,1880,1906,1907,1960,2185,2534,2625,2796,3077,3442,3458,3479,3481,3516,3559,3567,3575,3592,3596,3600,3624,3635,3688,3702,3937,3965,4004,4208,4602,4773,4853,5063,5290,5295,5551,5579,5591,5734,5791,6095,6304,6441,6504,6670,6850,6885,7048,7292,7301,7356,7704,8320,8440,8600,8660,8767,8995,9173,9308,9402,9734,10125,10320,10451,10666,11148,23228,26191,27086,29851,30009,54518,55904,56940,57162,57181,57829,64092,64218,64581,79109,80762,84174,114548,117194,120425,124912,146850,149233,151888,253260,338339,387357	AMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CCR7,KLF6,CR2,CSF1,CSF2,CTNNB1,CXADR,CYLD,DOCK2,GPR183,EDN1,EDN2,EGR3,PTK2B,FYN,GATA3,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,RBPJ,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,ITGB1,ITK,LCP2,LGALS9,LMO1,MEF2C,MYB,NFATC2,NOTCH2,PAK3,PIK3CA,PIK3R1,PRF1,PRKCB,PRKDC,PTGER4,PTPRE,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,IL1RL1,CD83,GRAP2,HDAC9,RASGRP1,IKZF1,VAV3,CD226,HHLA2,PLCL2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,KMT2E,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,MRGPRX2,JAML,SPACA3,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
11	1_Member GO Biological Processes GO:0071593 lymphocyte aggregation	-13.34885572 -10.208 86/429	301,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,1021,1236,1499,1525,1540,1794,1960,2534,2625,2796,3077,3442,3458,3479,3481,3559,3575,3592,3600,3702,3965,4004,4602,5063,5290,5295,5591,5734,6095,6304,6441,6504,6670,6850,6885,7048,7292,7356,7704,8320,8440,8600,8767,8995,9308,9402,10125,11148,26191,27086,29851,30009,54518,56940,57162,57829,64218,64581,79109,80762,84174,114548,120425,146850,149233,151888,253260,338339,387357	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EGR3,FYN,GATA3,GNRH1,HFE,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITK,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,FOXP1,ICOS,TBX21,APBB1IP,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,CLEC4D,THEMIS
12	1_Member GO Biological Processes GO:0098602 single organism cell adhesion	-13.26473492 -10.161 127/755	81,154,301,335,399,558,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,960,965,1021,1236,1499,1525,1540,1794,1946,1956,1960,2244,2335,2534,2625,2796,3037,3077,3384,3442,3458,3479,3481,3557,3559,3575,3592,3600,3688,3702,3911,3965,3987,4004,4092,4478,4602,4653,4815,4897,4907,5063,5290,5295,5591,5734,5912,5962,6095,6239,6304,6441,6480,6504,6670,6850,6885,7048,7292,7301,73	ACTN4,ADRB2,ANXA1,APOA1,RHOH,AXL,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CD44,CD58,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EFNA5,EGFR,EGR3,FGB,FN1,FYN,GATA3,GNRH1,HAS2,HFE,ICAM2,IFNA5,IFNG,IGF1,IGF2,IL1RN,IL2RA,IL7R,IL12A,IL15,ITGB1,ITK,LAMA5,LGALS9,LIMS1,LMO1,SMAD7,MSN,MYB,MYOC,NINJ2,NRCAM,NT5E,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RAP2B,RDX,RORA,RREB1,SATB1,SFTPD,ST6GAL1,SLAMF

Appendix

			56,7704,8320,8440,8600,8767,8995,9231,9308,9369,9402,10049,10125,10507,10563,11148,22862,23396,23607,26191,26999,27086,29119,29851,30009,54518,55243,56940,57162,57829,64218,64581,79109,80762,84174,84959,91663,114548,120425,146850,149233,151888,253260,255743,338339,387357	1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,DLG5,CD83,NRXN3,GRAP2,DNAJB6,RASGRP1,SEMA4D,CXCL13,HHLA2,FNDC3A,PIP5K1C,CD2AP,PTPN22,CYFIP2,FOXP1,CTNNA3,ICOS,TBX21,APBB1IP,KIRREL,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,UBASH3B,MYADM,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,NPNT,CLEC4D,THEMIS
13	1_Member GO Biological Processes GO:0030155 regulation of cell adhesion	-13.18977525 -10.121 111/626	27,81,154,284,301,335,347,596,604,814,836,914,919,921,940,942,960,1021,1236,1435,1499,1540,1946,1960,2185,2244,2335,2534,2625,2796,3037,3077,3458,3479,3481,3557,3559,3575,3592,3600,3673,3688,3911,3965,3987,4004,4008,4092,4602,4653,5054,5063,5290,5295,5728,5734,5793,5795,5800,5921,5962,6239,6423,6441,6480,6504,6850,6885,7048,7292,7356,7402,7424,7704,8440,8573,8600,8767,8995,9267,9308,9402,9891,10125,10395,10451,10507,10563,11148,23122,23499,25975,26191,29851,54518,54566,56940,57162,57829,79109,79625,80762,84959,91663,114548,146850,149233,151888,253260,255743,375056	ABL2,ACTN4,ADRB2,ANGPT1,ANXA1,APOA1,APOD,BCL2,BCL6,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CD44,CDK6,CCR7,CSF1,CTNNB1,CYLD,EFNA5,EGR3,PTK2B,FGB,FN1,FYN,GATA3,GNRH1,HAS2,HFE,IFNG,IGF1,IGF2,IL1RN,IL2RA,IL7R,IL12A,IL15,ITGA2,ITGB1,LAMA5,LGALS9,LIMS1,LMO1,LMO7,SMAD7,MYB,MYOC,SERPINE1,PAK3,PIK3CA,PIK3R1,PTEN,PTGER4,PTPRG,PTPRJ,PTPRO,RASA1,RDX,RREB1,SFRP2,SFTPD,ST6GAL1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,UTRN,VEGFC,ZBTB16,NCK2,CASK,TNFSF11,RIPK2,TNFSF18,CYTH1,CD83,GRAP2,NUAK1,RASGRP1,DLC1,VAV3,SEMA4D,CXCL13,HHLA2,CLASP2,MACF1,EGFL6,PTPN22,ICOS,APBB1IP,EPB41L4B,DUSP22,PELI1,ZP4,MAPKAP1,NDNF,NDFIP1,UBASH3B,MYADM,NLRP3,PIK3R6,IL23R,BTLA,RICTOR,NPNT,MIA3
14	1_Member GO Biological Processes GO:0030097 hemopoiesis	-13.05577188 -10.019 120/702	103,284,301,302,399,537,558,596,604,639,677,814,836,841,860,861,914,925,940,942,944,1015,1021,1030,1230,1236,1316,1380,1435,1436,1437,1499,1540,1794,1880,1960,2034,2113,2185,2296,2353,2625,2672,3442,3458,3516,3559,3567,3575,3592,3600,3624,3635,3688,3702,3965,4057,4208,4602,4853,5295,5305,5591,5734,6095,6304,6423,6504,6670,6850,7048,7049,7071,7292,7301,7520,7704,8111,8320,8600,8621,8767,8995,9290,9308,9734,9855,10100,10125,10221,10296,10320,23228,23335,23543,23648,25976,26191,27086,28996,29909,30009,51208,51621,54502,54790,55904,57623,64218,80762,84456,84959,8543	ADAR,ANGPT1,ANXA1,ANXA2,RHOH,ATP6AP1,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,RUNX1,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CDKN2B,CCR1,CCR7,KLF6,CR2,CSF1,CSF1R,CSF2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,EPAS1,ETS1,PTK2B,FOXC1,FOS,GATA3,GFI1,IFNA5,IFNG,RBPJ,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,LGALS9,LTF,MEF2C,MYB,NOTCH2,PIK3R1,PIP4K2A,PRKDC,PTGER4,RORA,SATB1,SFRP2,SLAMF1,SP3,SYK,TGFBR2,TGFBR3,KLF10,TNFSF4,TYRO3,XRCC5,ZBTB16,GPR68,EOMES,TNFSF11,CDK13,RIPK2,TNFSF18,GPR55,CD83,HDAC9,FARP2,TSPAN2,RASGRP1,TRIB1,MAEA,IKZF1,PLCL2,WDR7,RBFOX2,SSBP3,TIPARP,PTPN22,FOXP1,HIPK2,GPR171,TBX21,CLDN18,KLF13,RBM47,TET2,KMT2

Appendix

			9,85477,114548,115727,146850,149233,338339,387357	E,ZFAT,SEMA4A,NDFIP1,L3MBTL3,UBASH3B,STON2,SCIN,NLRP3,RASGRP4,PIK3R6,IL23R,CLEC4D,THEMIS
15	1_Member GO Biological Processes GO:0016337 single organismal cell-cell adhesion	-12.56056813 -9.554 119/705	154,301,335,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,960,965,1021,1236,1499,1525,1540,1794,1946,1956,1960,2244,2534,2625,2796,3037,3077,3384,3442,3458,3479,3481,3557,3559,3575,3592,3600,3688,3702,3965,4004,4092,4478,4602,4815,4897,4907,5063,5290,5295,5591,5734,5912,5962,6095,6304,6441,6504,6670,6850,6885,7048,7292,7301,7356,7704,8320,8440,8600,8767,8995,9231,9308,9369,9402,10049,10125,10507,10563,11148,22862,23396,23607,26191,26999,27086,29119,29851,30009,54518,55243,56940,57162,57829,64218,64581,79109,80762,84174,84959,91663,114548,120425,146850,149233,151888,253260,255743,338339,387357	ADRB2,ANXA1,APOA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CD44,CD58,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EFNA5,EGFR,EGR3,FGB,FYN,GATA3,GNRH1,HAS2,HFE,ICAM2,IFNA5,IFNG,IGF1,IGF2,IL1RN,IL2RA,IL7R,IL12A,IL15,ITGB1,ITK,LGALS9,LMO1,SMAD7,MSN,MYB,NINJ2,NRCAM,NT5E,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,RAP2B,RDX,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,DLG5,CD83,NRXN3,GRAP2,DNAJB6,RASGRP1,SEMA4D,CXCL13,HHLA2,FNDC3A,PIP5K1C,CD2AP,PTPN22,CYFIP2,FOXP1,CTNNA3,ICOS,TBX21,APBB1IP,KIRREL,DUSP22,PELI1,ZP4,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,UBASH3B,MYADM,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,NPNT,CLEC4D,THEMIS
16	1_Member GO Biological Processes GO:0048534 hematopoietic or lymphoid organ development	-12.2335602 -9.281 123/746	103,284,301,302,399,537,558,596,604,639,677,814,836,841,860,861,914,925,940,942,944,1015,1021,1030,1230,1236,1316,1380,1435,1436,1437,1499,1540,1794,1880,1960,2034,2113,2185,2296,2353,2625,2672,3442,3458,3516,3559,3567,3575,3592,3600,3624,3635,3688,3702,3720,3965,4057,4208,4602,4853,5295,5305,5591,5734,6095,6304,6423,6504,6670,6850,7048,7049,7071,7292,7301,7520,7704,8111,8320,8600,8621,8767,8995,9290,9308,9734,9855,10018,10100,10125,10221,10296,10320,10563,23228,23335,23543,23648,25976,26191,27086,28996,29909,30009,51208,51621,54502,54790,55904,57623,64218,80762,84456,84959,85439,85477,114548,115727,146850,149233,338339,387357	ADAR,ANGPT1,ANXA1,ANXA2,RHOH,ATP6AP1,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,RUNX1,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CDKN2B,CCR1,CCR7,KLF6,CR2,CSF1,CSF1R,CSF2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,EPAS1,ETS1,PTK2B,FOXC1,FOS,GATA3,GFI1,IFNA5,IFNG,RBPJ,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,JARID2,LGALS9,LTF,MEF2C,MYB,NOTCH2,PIK3R1,PIP4K2A,PRKDC,PTGER4,RORA,SATB1,SFRP2,SLAMF1,SP3,SYK,TGFBR2,TGFBR3,KLF10,TNFSF4,TYRO3,XRCC5,ZBTB16,GPR68,EOMES,TNFSF11,CDK13,RIPK2,TNFSF18,GPR55,CD83,HDAC9,FARP2,BCL2L11,TSPAN2,RASGRP1,TRIB1,MAEA,IKZF1,CXCL13,PLCL2,WDR7,RBFOX2,SSBP3,TIPARP,PTPN22,FOXP1,HIPK2,GPR171,TBX21,CLDN18,KLF13,RBM47,TET2,KMT2E,ZFAT,SEMA4A,NDFIP1,L3MBTL3,UBASH3B,STON2,SCIN,NLRP3,RASGRP4,PIK3R6,IL23R,CLEC4D,THEMIS
17	1_Member	-11.63009899	301,399,596,604,677,814,860,914,925,940	ANXA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,RUNX

Appendix

	GO Biological Processes GO:0030217 T cell differentiation	-8.703 50/200	,942,944,1021,1236,1499,1540,1794,1960,2625,3458,3559,3575,3592,3600,3702,3965,4602,5591,5734,6095,6304,6670,6850,7048,7292,7704,8320,8767,8995,9308,10125,26191,27086,30009,64218,114548,146850,149233,338339,387357	2,CD2,CD8A,CD28,CD86,TNFSF8,CDK6,CCR7,CTNNB1,CYLD,DOCK2,EGR3,GATA3,IFNG,IL2RA,IL7R,IL12A,IL15,ITK,LGALS9,MYB,PRKDC,PTGER4,RORA,SATB1,SP3,SYK,TGFBR2,TNFSF4,ZBTB16,EOMES,RIPK2,TNFSF18,CD83,RASGRP1,PTPN22,FOXP1,TBX21,SEMA4A,NLRP3,PIK3R6,IL23R,CLEC4D,THEMIS
18	1_Member GO Biological Processes GO:0002520 immune system development	-11.37253502 -8.469 126/793	103,284,301,302,399,537,558,596,604,639,677,814,836,841,860,861,914,925,940,942,944,1015,1021,1030,1230,1233,1236,1316,1380,1435,1436,1437,1499,1540,1794,1880,1960,2034,2113,2185,2296,2353,2625,2672,3442,3458,3516,3559,3567,3575,3592,3600,3624,3635,3688,3702,3720,3965,4057,4208,4602,4853,5295,5305,5591,5734,6095,6304,6423,6504,6670,6850,7048,7049,7071,7292,7301,7520,7704,8111,8320,8600,8621,8767,8995,9290,9308,9734,9855,10018,10100,10125,10221,10296,10320,10563,23228,23239,23335,23543,23648,25976,26191,27086,28996,29851,29909,30009,51208,51621,54502,54790,55904,57623,64218,80762,84456,84959,85439,85477,114548,115727,146850,149233,338339,387357	ADAR,ANGPT1,ANXA1,ANXA2,RHOH,ATP6AP1,AXL,BCL2,BCL6,PRDM1,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,RUNX1,CD2,CD8A,CD28,CD86,TNFSF8,CDH17,CDK6,CDKN2B,CCR1,CCR4,CCR7,KLF6,CR2,CSF1,CSF1R,CSF2,CTNNB1,CYLD,DOCK2,GPR183,EGR3,EPAS1,ETS1,PTK2B,FOXC1,FOS,GATA3,GFI1,IFNA5,IFNG,RBPJ,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,ITGB1,ITK,JARID2,LGALS9,LTF,MEF2C,MYB,NOTCH2,PIK3R1,PIP4K2A,PRKDC,PTGER4,RORA,SATB1,SFRP2,SLAMF1,SP3,SYK,TGFBR2,TGFBR3,KLF10,TNFSF4,TYRO3,XRCC5,ZBTB16,GPR68,EOMES,TNFSF11,CDK13,RIPK2,TNFSF18,GPR55,CD83,HDAC9,FARP2,BCL2L11,TSPAN2,RASGRP1,TRIB1,MAEA,IKZF1,CXCL13,PLCL2,PHLPP1,WDR7,RBFOX2,SSBP3,TIPARP,PTPN22,FOXP1,HIPK2,ICOS,GPR171,TBX21,CLDN18,KLF13,RBM47,TET2,KMT2E,ZFAT,SEMA4A,NDFIP1,L3MBTL3,UBASH3B,STON2,SCIN,NLRP3,RASGRP4,PIK3R6,IL23R,CLEC4D,THEMIS
19	1_Member GO Biological Processes GO:0051249 regulation of lymphocyte activation	-10.5788892 -7.697 75/394	301,558,596,604,639,814,836,914,919,921,940,942,1236,1499,1540,1880,1960,2534,2625,2796,3077,3458,3479,3481,3559,3567,3575,3592,3596,3600,3624,3635,3965,4004,4208,4602,4773,5063,5290,5295,6441,6504,6850,6885,7048,7292,7301,7356,7704,8440,8600,8660,8767,8995,9308,9402,10125,10451,11148,26191,29851,30009,56940,57162,57181,57829,64092,79109,80762,84174,114548,146850,149233,151888,253260	ANXA1,AXL,BCL2,BCL6,PRDM1,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CCR7,CTNNB1,CYLD,GPR183,EGR3,FYN,GATA3,GNRH1,HFE,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,LGALS9,LMO1,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,VAV3,HHLA2,PTPN22,ICOS,TBX21,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,MAPKAP1,NDFIP1,SLA2,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
20	1_Member GO Biological Processes GO:0034110	-10.51956331 -7.659 63/305	154,301,604,814,836,914,919,921,940,942,960,1236,1499,1540,1960,2534,2625,2796,3037,3077,3458,3479,3481,3559,3575,3	ADRB2,ANXA1,BCL6,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CD44,CCR7,CTNNB1,CYLD,EGR3,FYN,GATA3,GNRH1,HAS2,HFE,IFNG,IGF1,IGF2,IL2R

Appendix

	regulation of homotypic cell-cell adhesion		592,3600,3965,4004,4602,5063,5290,5295,5962,6441,6504,6850,6885,7048,7292,7356,7704,8440,8600,8767,8995,9308,9402,10125,11148,26191,29851,56940,57162,57829,79109,80762,84959,114548,146850,149233,151888,253260	A,IL7R,IL12A,IL15,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,RDX,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,ICOS,DUSP22,PELI1,ZP4,MAPKAP1,NDFIP1,UBASH3B,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
21	1_Member GO Biological Processes GO:1902107 positive regulation of leukocyte differentiation	-10.4329118 -7.593 37/131	301,537,558,639,841,861,942,1230,1435,1436,1960,2353,2625,3458,3559,3567,3575,3592,3600,3635,3965,4602,6850,7048,7071,7292,7704,8111,8600,8767,9308,10125,10221,55904,114548,146850,149233	ANXA1,ATP6AP1,AXL,PRDM1,CASP8,RUNX1,CD86,CCR1,CSF1,CSF1R,EGR3,FOS,GATA3,IFNG,IL2RA,IL5,IL7R,IL12A,IL15,INPP5D,LGALS9,MYB,SYK,TGFBR2,KLF10,TNFSF4,ZBTB16,GPR68,TNFSF11,RIPK2,CD83,RASGRP1,TRIB1,KMT2E,NLRP3,PIK3R6,IL23R
22	1_Member GO Biological Processes GO:1902105 regulation of leukocyte differentiation	-10.14580798 -7.361 52/232	301,537,558,604,639,814,841,861,914,940,942,1230,1435,1436,1499,1540,1960,2353,2625,3458,3559,3567,3575,3592,3600,3624,3635,3965,4057,4602,5295,6850,7048,7071,7292,7704,8111,8600,8767,8995,9290,9308,10125,10221,27086,51208,55904,80762,84959,114548,146850,149233	ANXA1,ATP6AP1,AXL,BCL6,PRDM1,CAMK4,CASP8,RUNX1,CD2,CD28,CD86,CCR1,CSF1,CSF1R,CTNNB1,CYLD,EGR3,FOS,GATA3,IFNG,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,LGALS9,LTF,MYB,PIK3R1,SYK,TGFBR2,KLF10,TNFSF4,ZBTB16,GPR68,TNFSF11,RIPK2,TNFSF18,GPR55,CD83,RASGRP1,TRIB1,FOXP1,CLDN18,KMT2E,NDFIP1,UBASH3B,NLRP3,PIK3R6,IL23R
23	1_Member GO Biological Processes GO:0045785 positive regulation of cell adhesion	-10.04810315 -7.281 71/373	284,301,335,919,921,940,942,960,1021,1236,1435,1960,2185,2244,2335,2534,2625,3037,3458,3479,3481,3559,3575,3592,3600,3673,3688,3965,3987,4092,4602,4653,5063,5290,5295,5734,5795,6239,6423,6504,6850,6885,7048,7292,7402,7424,7704,8440,8600,8767,8995,9308,9402,10125,10451,10563,11148,25975,29851,54518,54566,57829,79109,79625,91663,114548,146850,149233,151888,253260,255743	ANGPT1,ANXA1,APOA1,CD247,CD5,CD28,CD86,CD44,CDK6,CCR7,CSF1,EGR3,PTK2B,FGB,FN1,FYN,GATA3,HAS2,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,ITGA2,ITGB1,LGALS9,LIMS1,SMAD7,MYB,MYOC,PAK3,PIK3CA,PIK3R1,PTGER4,PTPRJ,RREB1,SFRP2,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,UTRN,VEGFC,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,VAV3,CXCL13,HHLA2,EGFL6,ICOS,APBB1IP,EPB41L4B,ZP4,MAPKAP1,NDNF,MYADM,NLRP3,PIK3R6,IL23R,BTLA,RICTOR,NPNT
24	1_Member GO Biological Processes GO:0002696 positive regulation of leukocyte activation	-9.791600087 -7.040 61/302	301,558,596,604,639,914,919,921,940,942,1236,1880,1960,2534,2625,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4208,4602,4773,5063,5290,5295,6504,6850,6885,7048,7292,7704,8440,8600,8660,8767,9173,9308,9402,10125,10451,10666,11148,29851,30009,57162,57181,57829,79109,114548,124912,146850,149233,151888,253260	ANXA1,AXL,BCL2,BCL6,PRDM1,CD2,CD247,CD5,CD28,CD86,CCR7,GPR183,EGR3,FYN,GATA3,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,IL1RL1,CD83,GRAP2,RASGRP1,VAV3,CD226,HHLA2,ICOS,TBX21,PELI1,SLC39A10,ZP4,MAPKAP1,NLRP3,SPACA3,PIK3R6,IL23R,BTLA,RICTOR
25	1_Member	-9.774174997	154,301,604,814,836,914,919,921,940,942	ADRB2,ANXA1,BCL6,CAMK4,CASP3,CD2,CD247,C

Appendix

	GO Biological Processes GO:0034109 homotypic cell-cell adhesion	-7.039 68/355	,960,1236,1499,1525,1540,1960,2244,2534,2625,2796,3037,3077,3458,3479,3481,3559,3575,3592,3600,3965,4004,4602,5063,5290,5295,5912,5962,6441,6504,6850,6885,7048,7292,7301,7356,7704,8440,8600,8767,8995,9308,9402,10125,11148,26191,29119,29851,56940,57162,57829,79109,80762,84959,114548,146850,149233,151888,253260	D5,CD28,CD86,CD44,CCR7,CTNNB1,CXADR,CYLD,EGR3,FGB,FYN,GATA3,GNRH1,HAS2,HFE,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,RAP2B,RDX,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,CTNNA3,ICOS,DUSP22,PELI1,ZP4,MAPKAP1,NDFIP1,UBASH3B,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
26	1_Member GO Biological Processes GO:1903708 positive regulation of hemopoiesis	-9.750360737 -7.030 41/163	301,537,558,639,841,861,942,1230,1435,1436,1960,2113,2296,2353,2625,3458,3559,3567,3575,3592,3600,3624,3635,3965,4602,6850,7048,7071,7292,7704,8111,8600,8767,9308,10125,10221,55904,85477,114548,146850,149233	ANXA1,ATP6AP1,AXL,PRDM1,CASP8,RUNX1,CD86,CCR1,CSF1,CSF1R,EGR3,ETS1,FOXC1,FOS,GATA3,IFNG,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,LGALS9,MYB,SYK,TGFBR2,KLF10,TNFSF4,ZBTB16,GPR68,TNFSF11,RIPK2,CD83,RASGRP1,TRIB1,KMT2E,SCIN,NLRP3,PIK3R6,IL23R
27	1_Member GO Biological Processes GO:0002694 regulation of leukocyte activation	-9.718261926 -7.013 80/450	301,558,596,604,639,814,836,914,919,921,940,942,1236,1499,1540,1880,1960,2534,2625,2796,3077,3458,3479,3481,3559,3567,3575,3592,3596,3600,3624,3635,3965,4004,4208,4602,4773,5063,5290,5295,5791,6095,6441,6504,6850,6885,7048,7292,7301,7356,7704,8440,8600,8660,8767,8995,9173,9308,9402,10125,10451,10666,11148,26191,29851,30009,56940,57162,57181,57829,64092,79109,80762,84174,114548,124912,146850,149233,151888,253260	ANXA1,AXL,BCL2,BCL6,PRDM1,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CCR7,CTNNB1,CYLD,GPR183,EGR3,FYN,GATA3,GNRH1,HFE,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,LGALS9,LMO1,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,PTPRE,RORA,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,IL1RL1,CD83,GRAP2,RASGRP1,VAV3,CD226,HHLA2,PTPN22,ICOS,TBX21,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,MAPKAP1,NDFIP1,SLA2,NLRP3,SPACA3,PIK3R6,IL23R,BTLA,RICTOR
28	1_Memer GO Biological Processes GO:1903037 regulation of leukocyte cell-cell adhesion	-9.584372588 -6.893 60/298	301,604,814,836,914,919,921,940,942,960,1236,1499,1540,1960,2534,2625,2796,3037,3077,3458,3479,3481,3559,3575,3592,3600,3965,4004,4602,5063,5290,5295,6441,6504,6850,6885,7048,7292,7356,7704,8440,8600,8767,8995,9308,9402,10125,11148,26191,29851,56940,57162,57829,79109,80762,114548,146850,149233,151888,253260	ANXA1,BCL6,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CD44,CCR7,CTNNB1,CYLD,EGR3,FYN,GATA3,GNRH1,HAS2,HFE,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,ICOS,DUSP22,PELI1,ZP4,MAPKAP1,NDFIP1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
29	1_Memer GO Biological Processes GO:0022407 regulation of cell-cell	-9.421583664 -6.788 70/377	154,301,335,604,814,836,914,919,921,940,942,960,1236,1499,1540,1946,1960,2244,2534,2625,2796,3037,3077,3458,3479,3481,3557,3559,3575,3592,3600,3965,4004,4	ADRB2,ANXA1,APOA1,BCL6,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CD44,CCR7,CTNNB1,CYLD,EFNA5,EGR3,FGB,FYN,GATA3,GNRH1,HAS2,HFE,IFNG,IGF1,IGF2,IL1RN,IL2RA,IL7R,IL12A,IL15,LGAL

Appendix

	adhesion		092,4602,5063,5290,5295,5962,6441,6504,6850,6885,7048,7292,7356,7704,8440,8600,8767,8995,9308,9402,10125,10563,11148,26191,29851,56940,57162,57829,79109,80762,84959,91663,114548,146850,149233,151888,253260	S9,LMO1,SMAD7,MYB,PAK3,PIK3CA,PIK3R1,RDX,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,CXCL13,HHLA2,PTPN22,ICOS,DUSP22,PELI1,ZP4,MAPKAP1,NDFIP1,UBASH3B,MYADM,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
30	1_Member GO Biological Processes GO:0050863 regulation of T cell activation	-9.41340847 -6.788 58/286	301,604,814,836,914,919,921,940,942,1236,1499,1540,1960,2534,2625,2796,3077,3458,3479,3481,3559,3575,3592,3600,3965,4004,4602,5063,5290,5295,6441,6504,6850,6885,7048,7292,7356,7704,8440,8600,8767,8995,9308,9402,10125,11148,26191,29851,56940,57162,57829,79109,80762,114548,146850,149233,151888,253260	ANXA1,BCL6,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CCR7,CTNNB1,CYLD,EGR3,FYN,GATA3,GNRH1,HFE,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,LGALS9,LMO1,MYB,PAK3,PIK3CA,PIK3R1,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,SCGB1A1,ZBTB16,NCK2,TNFSF11,RIPK2,TNFSF18,CD83,GRAP2,RASGRP1,HHLA2,PTPN22,ICOS,DUSP22,PELI1,ZP4,MAPKAP1,NDFIP1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
31	1_Member GO Biological Processes GO:0051251 positive regulation of lymphocyte activation	-9.390588338 -6.788 57/279	301,558,596,604,639,919,921,940,942,1236,1880,1960,2534,2625,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4208,4602,4773,5063,5290,5295,6504,6850,6885,7048,7292,7704,8440,8600,8660,8767,9308,9402,10125,10451,11148,29851,30009,57162,57181,57829,79109,114548,146850,149233,151888,253260	ANXA1,AXL,BCL2,BCL6,PRDM1,CD247,CD5,CD28,CD86,CCR7,GPR183,EGR3,FYN,GATA3,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,CD83,GRAP2,RASGRP1,VAV3,HHLA2,ICOS,TBX21,PELI1,SLC39A10,ZP4,MAPKAP1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
32	1_Member GO Biological Processes GO:0050867 positive regulation of cell activation	-9.315701767 -6.730 61/310	301,558,596,604,639,914,919,921,940,942,1236,1880,1960,2534,2625,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4208,4602,4773,5063,5290,5295,6504,6850,6885,7048,7292,7704,8440,8600,8660,8767,9173,9308,9402,10125,10451,10666,11148,29851,30009,57162,57181,57829,79109,114548,124912,146850,149233,151888,253260	ANXA1,AXL,BCL2,BCL6,PRDM1,CD2,CD247,CD5,CD28,CD86,CCR7,GPR183,EGR3,FYN,GATA3,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,IL1RL1,CD83,GRAP2,RASGRP1,VAV3,CD226,HHLA2,ICOS,TBX21,PELI1,SLC39A10,ZP4,MAPKAP1,NLRP3,SPACA3,PIK3R6,IL23R,BTLA,RICTOR
33	1_Member GO Biological Processes GO:0050865 regulation of cell activation	-9.310781523 -6.730 83/483	154,301,558,596,604,639,814,836,914,919,921,940,942,1236,1499,1540,1880,1960,2534,2625,2796,3077,3458,3479,3481,3559,3567,3575,3592,3596,3600,3624,3635,3965,4004,4208,4602,4773,5063,5290,5295,5791,6095,6441,6504,6850,6885,7048,7292,7294,7301,7356,7704,8440,8600,8660,8767,8995,9173,9308,9402,10125,10451,10	ADRB2,ANXA1,AXL,BCL2,BCL6,PRDM1,CAMK4,CASP3,CD2,CD247,CD5,CD28,CD86,CCR7,CTNNB1,CYLD,GPR183,EGR3,FYN,GATA3,GNRH1,HFE,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,LGALS9,LMO1,MEF2C,MYB,NFATC2,PAK3,PIK3CA,PIK3R1,PTPRE,RORA,SFTPD,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,TXK,TYRO3,SCGB1A1,ZBTB16,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,IL1RL1

Appendix

			666,11148,26191,29851,30009,56940,57162,57181,57829,64092,79109,80762,84174,84959,114548,124912,146850,149233,151888,253260	,CD83,GRAP2,RASGRP1,VAV3,CD226,HHLA2,PTPN22,ICOS,TBX21,DUSP22,PELI1,SLC39A10,ZP4,SAMSN1,MAPKAP1,NDFIP1,SLA2,UBASH3B,NLRP3,SPACA3,PIK3R6,IL23R,BTLA,RICTOR
34	1_Member GO Biological Processes GO:1903706 regulation of hemopoiesis	-9.086034638 -6.527 61/314	301,537,558,604,639,814,841,861,914,940,942,1021,1230,1435,1436,1499,1540,1960,2113,2185,2296,2353,2625,3458,3559,3567,3575,3592,3600,3624,3635,3965,4057,4208,4602,5295,6850,7048,7071,7292,7704,8111,8600,8767,8995,9290,9308,10125,10221,23543,27086,29909,51208,51621,55904,80762,84959,85477,114548,146850,149233	ANXA1,ATP6AP1,AXL,BCL6,PRDM1,CAMK4,CASP8,RUNX1,CD2,CD28,CD86,CDK6,CCR1,CSF1,CSF1R,CTNNB1,CYLD,EGR3,ETS1,PTK2B,FOXC1,FOS,GATA3,IFNG,IL2RA,IL5,IL7R,IL12A,IL15,INHBA,INPP5D,LGALS9,LTF,MEF2C,MYB,PIK3R1,SYK,TGFBR2,KLF10,TNFSF4,ZBTB16,GPR68,TNFSF11,RIPK2,TNFSF18,GPR55,CD83,RASGRP1,TRIB1,RBFOX2,FOXP1,GPR171,CLDN18,KLF13,KMT2E,NDFIP1,UBASH3B,SCIN,NLRP3,PIK3R6,IL23R
35	1_Member GO Biological Processes GO:0098609 cell-cell adhesion	-8.801609801 -6.282 127/876	154,301,335,399,596,604,677,814,836,841,860,911,914,919,921,925,940,942,944,960,965,1015,1021,1236,1499,1525,1540,1794,1946,1956,1960,2244,2534,2625,2796,3037,3077,3384,3442,3458,3479,3481,3557,3559,3575,3592,3600,3688,3702,3965,4004,4092,4478,4602,4815,4897,4907,5063,5290,5295,5591,5734,5789,5793,5912,5962,6095,6304,6441,6504,6670,6850,6885,7048,7292,7301,7356,7704,8320,8440,8600,8767,8995,9076,9231,9308,9369,9402,10049,10125,10507,10563,10666,11148,22862,23396,23607,26191,26999,27086,29119,29851,30009,51208,54518,55243,55714,56940,57162,57829,60437,64218,64581,79109,80762,84174,84959,91663,114548,120425,146850,149233,151888,253260,255743,338339,387357	ADRB2,ANXA1,APOA1,RHOH,BCL2,BCL6,ZFP36L1,CAMK4,CASP3,CASP8,RUNX2,CD1C,CD2,CD247,CD5,CD8A,CD28,CD86,TNFSF8,CD44,CD58,CDH17,CDK6,CCR7,CTNNB1,CXADR,CYLD,DOCK2,EFNA5,EGFR,EGR3,FGB,FYN,GATA3,GNRH1,HAS2,HFE,ICAM2,IFNA5,IFNG,IGF1,IGF2,IL1RN,IL2RA,IL7R,IL12A,IL15,ITGB1,ITK,LGALS9,LMO1,SMAD7,MSN,MYB,NINJ2,NRCAM,NT5E,PAK3,PIK3CA,PIK3R1,PRKDC,PTGER4,PTPRD,PTPRG,RAP2B,RDX,RORA,SATB1,SFTPD,SLAMF1,SP3,SYK,MAP3K7,TGFBR2,TNFSF4,TYRO3,SCGB1A1,ZBTB16,EOMES,NCK2,TNFSF11,RIPK2,TNFSF18,CLDN1,DLG5,CD83,NRXN3,GRAP2,DNAJB6,RASGRP1,SEMA4D,CXCL13,CD226,HHLA2,FNDC3A,PIP5K1C,CD2AP,PTPN22,CYFIP2,FOXP1,CTNNA3,ICOS,TBX21,CLDN18,APBB1IP,KIRREL,TENM3,DUSP22,PELI1,ZP4,CDH26,SEMA4A,CLEC7A,MAPKAP1,NDFIP1,SLA2,UBASH3B,MYADM,NLRP3,JAML,PIK3R6,IL23R,BTLA,RICTOR,NPNT,CLEC4D,THEMIS
36	1_Member GO Biological Processes GO:0034112 positive regulation of homotypic cell-cell adhesion	-7.878425097 -5.488 44/208	301,919,921,940,942,960,1236,1960,2534,2625,3037,3458,3479,3481,3559,3575,3592,3600,3965,4602,5063,5290,5295,6504,6850,6885,7048,7292,7704,8440,8600,8767,9308,9402,10125,11148,29851,57829,79109,114548,146850,149233,151888,253260	ANXA1,CD247,CD5,CD28,CD86,CD44,CCR7,EGR3,FYN,GATA3,HAS2,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,LGALS9,MYB,PAK3,PIK3CA,PIK3R1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TNFSF11,RIPK2,CD83,GRAP2,RASGRP1,HHLA2,ICOS,ZP4,MAPKAP1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
37	1_Member GO Biological Processes	-7.722082654 -5.339	301,596,604,639,836,940,942,1435,1499,1880,2796,3458,3479,3481,3559,3567,3592	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CSF1,CTNNB1,GPR183,GNRH1,IFNG,IGF1,IGF2,IL2RA,I

Appendix

	GO:0032944 regulation of mononuclear cell proliferation	42/196	,3596,3600,3635,3965,4004,4208,4773,64 41,6480,6504,6850,7292,7356,8440,8660, 8767,8995,10451,11148,26191,57162,571 81,57829,80762,149233	L5,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C, NFATC2,SFTPD,ST6GAL1,SLAMF1,SYK,TNFSF4,SC GB1A1,NCK2,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,P TPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R
38	1_Member GO Biological Processes GO:1903039 positive regulation of leukocyte cell-cell adhesion	-7.682428015 -5.306 44/211	301,919,921,940,942,960,1236,1960,2534, 2625,3037,3458,3479,3481,3559,3575,359 2,3600,3965,4602,5063,5290,5295,6504,6 850,6885,7048,7292,7704,8440,8600,8767 ,9308,9402,10125,11148,29851,57829,791 09,114548,146850,149233,151888,253260	ANXA1,CD247,CD5,CD28,CD86,CD44,CCR7,EGR3,F YN,GATA3,HAS2,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12 A,IL15,LGALS9,MYB,PAK3,PIK3CA,PIK3R1,SLAMF 1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TN FSF11,RIPK2,CD83,GRAP2,RASGRP1,HHLA2,ICOS, ZP4,MAPKAP1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
39	1_Member GO Biological Processes GO:0045621 positive regulation of lymphocyte differentiation	-7.564479787 -5.201 24/80	301,558,639,942,1960,2625,3458,3559,35 75,3592,3600,3635,3965,4602,6850,7048, 7292,7704,8767,9308,10125,114548,1468 50,149233	ANXA1,AXL,PRDM1,CD86,EGR3,GATA3,IFNG,IL2R A,IL7R,IL12A,IL15,INPP5D,LGALS9,MYB,SYK,TGF BR2,TNFSF4,ZBTB16,RIPK2,CD83,RASGRP1,NLRP3 ,PIK3R6,IL23R
40	1_Member GO Biological Processes GO:0045619 regulation of lymphocyte differentiation	-7.351281215 -5.019 32/132	301,558,604,639,814,914,940,942,1540,19 60,2625,3458,3559,3575,3592,3600,3624, 3635,3965,4602,6850,7048,7292,7704,876 7,8995,9308,10125,80762,114548,146850, 149233	ANXA1,AXL,BCL6,PRDM1,CAMK4,CD2,CD28,CD86 ,CYLD,EGR3,GATA3,IFNG,IL2RA,IL7R,IL12A,IL15,I NHBA,INPP5D,LGALS9,MYB,SYK,TGFBR2,TNFSF4, ZBTB16,RIPK2,TNFSF18,CD83,RASGRP1,NDFIP1,N LRP3,PIK3R6,IL23R
41	1_Member GO Biological Processes GO:0022409 positive regulation of cell-cell adhesion	-7.210479646 -4.897 47/241	301,919,921,940,942,960,1236,1960,2244, 2534,2625,3037,3458,3479,3481,3559,357 5,3592,3600,3965,4092,4602,5063,5290,5 295,6504,6850,6885,7048,7292,7704,8440 ,8600,8767,9308,9402,10125,10563,11148 ,29851,57829,79109,114548,146850,1492 33,151888,253260	ANXA1,CD247,CD5,CD28,CD86,CD44,CCR7,EGR3,F GB,FYN,GATA3,HAS2,IFNG,IGF1,IGF2,IL2RA,IL7R, IL12A,IL15,LGALS9,SMAD7,MYB,PAK3,PIK3CA,PI K3R1,SLAMF1,SYK,MAP3K7,TGFBR2,TNFSF4,ZBT B16,NCK2,TNFSF11,RIPK2,CD83,GRAP2,RASGRP1, CXCL13,HHLA2,ICOS,ZP4,MAPKAP1,NLRP3,PIK3R 6,IL23R,BTLA,RICTOR
42	1_Member GO Biological Processes GO:0050870 positive regulation of T cell activation	-7.199257814 -4.892 42/204	301,919,921,940,942,1236,1960,2534,262 5,3458,3479,3481,3559,3575,3592,3600,3 965,4602,5063,5290,5295,6504,6850,6885 ,7048,7292,7704,8440,8600,8767,9308,94 02,10125,11148,29851,57829,79109,1145 48,146850,149233,151888,253260	ANXA1,CD247,CD5,CD28,CD86,CCR7,EGR3,FYN,G ATA3,IFNG,IGF1,IGF2,IL2RA,IL7R,IL12A,IL15,LGA LS9,MYB,PAK3,PIK3CA,PIK3R1,SLAMF1,SYK,MAP 3K7,TGFBR2,TNFSF4,ZBTB16,NCK2,TNFSF11,RIPK 2,CD83,GRAP2,RASGRP1,HHLA2,ICOS,ZP4,MAPKA P1,NLRP3,PIK3R6,IL23R,BTLA,RICTOR
43	1_Member GO Biological Processes GO:0070663 regulation of leukocyte proliferation	-7.136431133 -4.848 42/205	301,596,604,639,836,940,942,1435,1499,1 880,2796,3458,3479,3481,3559,3567,3592 ,3596,3600,3635,3965,4004,4208,4773,64 41,6480,6504,6850,7292,7356,8440,8660, 8767,8995,10451,11148,26191,57162,571 81,57829,80762,149233	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CSF 1,CTNNB1,GPR183,GNRH1,IFNG,IGF1,IGF2,IL2RA,I L5,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C, NFATC2,SFTPD,ST6GAL1,SLAMF1,SYK,TNFSF4,SC GB1A1,NCK2,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,P TPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R

Appendix

44	1_Member GO Biological Processes GO:0032946 positive regulation of mononuclear cell proliferation	-7.049664324 -4.776 31/129	301,596,604,940,942,1435,1880,3458,3479,3481,3559,3567,3592,3596,3600,3965,4208,4773,6480,6504,6850,7292,8440,8660,8767,10451,11148,57162,57181,57829,149233	ANXA1,BCL2,BCL6,CD28,CD86,CSF1,GPR183,IFNG,IGF1,IGF2,IL2RA,IL5,IL12A,IL13,IL15,LGALS9,MEF2C,NFATC2,ST6GAL1,SLAMF1,SYK,TNFSF4,NCK2,IRS2,RIPK2,VAV3,HHLA2,PELI1,SLC39A10,ZP4,IL23R
45	1_Member GO Biological Processes GO:0032943 mononuclear cell proliferation	-7.003518618 -4.735 49/260	301,596,604,639,836,940,942,1380,1435,1499,1794,1880,2534,2796,3442,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4004,4208,4773,6304,6441,6480,6504,6850,7292,7356,8440,8660,8767,8995,10451,11148,23228,26191,57162,57181,57829,80762,149233	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CR2,CSF1,CTNNB1,DOCK2,GPR183,FYN,GNRH1,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C,NFATC2,SATB1,SFTPD,ST6GAL1,SLAMF1,SYK,TNFSF4,SCGB1A1,NCK2,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,PLCL2,PTPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R
46	1_Member GO Biological Processes GO:0050670 regulation of lymphocyte proliferation	-6.907221209 -4.649 40/194	301,596,604,639,836,940,942,1499,1880,2796,3458,3479,3481,3559,3567,3592,3596,3600,3635,3965,4004,4208,4773,6441,6504,6850,7292,7356,8440,8660,8767,8995,10451,11148,26191,57162,57181,57829,80762,149233	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CTNNB1,GPR183,GNRH1,IFNG,IGF1,IGF2,IL2RA,IL5,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C,NFATC2,SFTPD,SLAMF1,SYK,TNFSF4,SCGB1A1,NCK2,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,PTPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R
47	1_Member GO Biological Processes GO:0045580 regulation of T cell differentiation	-6.686191889 -4.467 27/107	301,604,814,914,940,942,1540,1960,2625,3458,3559,3575,3592,3600,3965,4602,6850,7048,7292,7704,8767,8995,9308,10125,114548,146850,149233	ANXA1,BCL6,CAMK4,CD2,CD28,CD86,CYLD,EGR3,GATA3,IFNG,IL2RA,IL7R,IL12A,IL15,LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,TNFSF18,CD83,RASGRP1,NLRP3,PIK3R6,IL23R
48	1_Member GO Biological Processes GO:0070661 leukocyte proliferation	-6.679704772 -4.466 50/274	301,596,604,639,836,940,942,1380,1435,1499,1794,1880,2534,2796,3442,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4004,4208,4773,6304,6441,6480,6504,6850,7292,7356,8440,8600,8660,8767,8995,10451,11148,23228,26191,57162,57181,57829,80762,149233	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CR2,CSF1,CTNNB1,DOCK2,GPR183,FYN,GNRH1,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C,NFATC2,SATB1,SFTPD,ST6GAL1,SLAMF1,SYK,TNFSF4,SCGB1A1,NCK2,TNFSF11,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,PLCL2,PTPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R
49	1_Member GO Biological Processes GO:0070665 positive regulation of leukocyte proliferation	-6.574138762 -4.383 31/135	301,596,604,940,942,1435,1880,3458,3479,3481,3559,3567,3592,3596,3600,3965,4208,4773,6480,6504,6850,7292,8440,8660,8767,10451,11148,57162,57181,57829,149233	ANXA1,BCL2,BCL6,CD28,CD86,CSF1,GPR183,IFNG,IGF1,IGF2,IL2RA,IL5,IL12A,IL13,IL15,LGALS9,MEF2C,NFATC2,ST6GAL1,SLAMF1,SYK,TNFSF4,NCK2,IRS2,RIPK2,VAV3,HHLA2,PELI1,SLC39A10,ZP4,IL23R
50	1_Member GO Biological Processes GO:0046651 lymphocyte proliferation	-6.348018927 -4.186 47/257	301,596,604,639,836,940,942,1380,1499,1794,1880,2534,2796,3442,3458,3479,3481,3559,3567,3575,3592,3596,3600,3635,3965,4004,4208,4773,6304,6441,6504,6850,	ANXA1,BCL2,BCL6,PRDM1,CASP3,CD28,CD86,CR2,CTNNB1,DOCK2,GPR183,FYN,GNRH1,IFNA5,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INPP5D,LGALS9,LMO1,MEF2C,NFATC2,SATB1,SFTPD,SLA

Appendix

			7292,7356,8440,8660,8767,8995,10451,11148,23228,26191,57162,57181,57829,80762,149233	MF1,SYK,TNFSF4,SCGB1A1,NCK2,IRS2,RIPK2,TNFSF18,VAV3,HHLA2,PLCL2,PTPN22,PELI1,SLC39A10,ZP4,NDFIP1,IL23R
51	1_Member GO Biological Processes GO:0045582 positive regulation of T cell differentiation	-6.258197119 -4.117 20/68	301,942,1960,2625,3458,3559,3575,3592,3965,4602,6850,7048,7292,7704,8767,9308,10125,114548,146850,149233	ANXA1,CD86,EGR3,GATA3,IFNG,IL2RA,IL7R,IL12A,LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,CD83,RASGRP1,NLRP3,PIK3R6,IL23R
52	1_Member GO Biological Processes GO:0050671 positive regulation of lymphocyte proliferation	-6.13956699 -4.040 29/127	301,596,604,940,942,1880,3458,3479,3481,3559,3567,3592,3596,3600,3965,4208,4773,6504,6850,7292,8440,8660,8767,10451,11148,57162,57181,57829,149233	ANXA1,BCL2,BCL6,CD28,CD86,GPR183,IFNG,IGF1,IGF2,IL2RA,IL5,IL12A,IL13,IL15,LGALS9,MEF2C,NFATC2,SLAMF1,SYK,TNFSF4,NCK2,IRS2,RIPK2,VAV3,HHLA2,PELI1,SLC39A10,ZP4,IL23R
53	1_Member GO Biological Processes GO:0042129 regulation of T cell proliferation	-4.010861497 -2.291 27/146	301,836,940,942,1499,2796,3458,3479,3481,3559,3592,3600,3965,4004,6441,6504,6850,7292,7356,8440,8767,8995,11148,57162,57829,80762,149233	ANXA1,CASP3,CD28,CD86,CTNNB1,GNRH1,IFNG,IGF1,IGF2,IL2RA,IL12A,IL15,LGALS9,LMO1,SFTPD,SLAMF1,SYK,TNFSF4,SCGB1A1,NCK2,RIPK2,TNFSF18,HHLA2,PELI1,ZP4,NDFIP1,IL23R
54	1_Member GO Biological Processes GO:0042098 T cell proliferation	-3.856028459 -2.156 30/173	301,836,940,942,1499,1794,2534,2796,3458,3479,3481,3559,3592,3600,3965,4004,6304,6441,6504,6850,7292,7356,8440,8767,8995,11148,57162,57829,80762,149233	ANXA1,CASP3,CD28,CD86,CTNNB1,DOCK2,FYN,GNRH1,IFNG,IGF1,IGF2,IL2RA,IL12A,IL15,LGALS9,LMO1,SATB1,SFTPD,SLAMF1,SYK,TNFSF4,SCGB1A1,NCK2,RIPK2,TNFSF18,HHLA2,PELI1,ZP4,NDFIP1,IL23R
55	1_Member GO Biological Processes GO:0042102 positive regulation of T cell proliferation	-3.056420025 -1.535 18/94	301,940,942,3458,3479,3481,3559,3592,3600,3965,6504,6850,7292,8440,8767,11148,57829,149233	ANXA1,CD28,CD86,IFNG,IGF1,IGF2,IL2RA,IL12A,IL15,LGALS9,SLAMF1,SYK,TNFSF4,NCK2,RIPK2,HHLA2,ZP4,IL23R
56	2_Summery GO Biological Processes GO:0002684 positive regulation of immune system process	-14.18374272 -10.642 152/943	301,329,330,537,558,596,604,639,841,861,914,919,921,940,942,1230,1235,1236,1240,1378,1380,1435,1436,1540,1690,1846,1848,1880,1907,1960,2113,2185,2209,2296,2353,2534,2625,2672,3093,3320,3384,3458,3479,3481,3559,3567,3575,3592,3596,3600,3624,3635,3673,3702,3708,3709,3783,3937,3965,4057,4068,4208,4214,4313,4602,4772,4773,4790,5054,5063,5142,5144,5289,5290,5295,5567,5579,5591,5641,5707,5716,5728,5734,5795,6197,6504,6850,6885,7048,7071,7097,7292,7294,7301,742	ANXA1,BIRC2,BIRC3,ATP6AP1,AXL,BCL2,BCL6,PRDM1,CASP8,RUNX1,CD2,CD247,CD5,CD28,CD86,CCR1,CCR6,CCR7,CMKLR1,CR1,CR2,CSF1,CSF1R,CYLD,COCH,DUSP4,DUSP6,GPR183,EDN2,EGR3,ETS1,PTK2B,FCGR1A,FOXC1,FOS,FYN,GATA3,GFI1,UBE2K,HSP90AA1,ICAM2,IFNG,IGF1,IGF2,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,ITGA2,ITK,ITPR1,ITPR2,KCNN4,LCP2,LGALS9,LTF,SH2D1A,MEF2C,MAP3K1,MMP2,MYB,NFATC1,NFATC2,NFKB1,SERPINE1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PRKACB,PRKCB,PRKDC,LGMN,PSMD1,PSMD10,PTEN,PTGER4,PTPRJ,RPS6KA3,SLAMF1,SYK,MAP3

Appendix

			4,7456,7704,8111,8440,8600,8660,8767,8 995,9173,9308,9402,9447,9844,10010,101 25,10213,10221,10333,10451,10563,1066 6,11148,23118,23228,23291,23369,23457, 26191,26999,29851,30009,30849,50852,5 4106,55904,56940,57162,57181,57590,57 829,64581,79109,79931,84174,85477,114 548,118788,124912,146850,149233,15188 8,153090,253260,338339,340061,375056, 387357,284,817,1437,1839,1847,1956,224 6,2549,3563,3568,3716,4140,5921,5922,6 711,8452,23239,25780,115727,152559,10 3,154,308,335,347,834,1794,2040,2693,26 97,3442,3554,4142,4907,5321,5654,5740, 5770,6095,6346,6772,6846,7004,7356,855 4,8651,9353,9469,9982,11221,27086,2734 7,51606,55690,60386,80762	K7,TGFBR2,KLF10,TLR2,TNFSF4,TXK,TYRO3,VEG FC,WIPF1,ZBTB16,GPR68,NCK2,TNFSF11,IRS2,RIP K2,TNFSF18,IL1RL1,CD83,GRAP2,AIM2,ELMO1,TA NK,RASGRP1,PSMD14,TRIB1,TLR6,VAV3,CXCL13, CD226,HHLA2,TAB2,PLCL2,FBXW11,PUM2,ABCB9, PTPN22,CYFIP2,ICOS,TBX21,PIK3R4,TRAT1,TLR9, KMT2E,DUSP22,PELI1,SLC39A10,WDFY1,ZP4,CLE C7A,MAPKAP1,TNIP3,SLA2,SCIN,NLRP3,PIK3AP1,S PACA3,PIK3R6,IL23R,BTLA,DAB2IP,RICTOR,CLEC 4D,TMEM173,MIA3,THEMIS,ANGPT1,CAMK2D,CS F2,HBEGF,DUSP5,EGFR,FGF1,GAB1,IL3RA,IL5RA,J AK1,MARK3,RASA1,RASA2,SPTBN1,CUL3,PHLPP1, RASGRP3,RASGRP4,PAQR3,ADAR,ADRB2,ANXA5, APOA1,APOD,CASP1,DOCK2,STOM,GHSR,GJA1,IF NA5,IL1R1,MAS1,NT5E,PLA2G4A,HTRA1,PTGIS,PT PN1,RORA,CCL1,STAT1,XCL2,TEAD4,SCGB1A1,PI AS1,SOCS1,SLIT2,CHST3,FGFBP1,DUSP10,FOXP1,S TK39,ATP6V1H,PACS1,SLC25A19,NDFIP1
57	2_Member GO Biological Processes GO:0002684 positive regulation of immune system process	-14.18374272 -10.642 152/943	301,329,330,537,558,596,604,639,841,861 ,914,919,921,940,942,1230,1235,1236,124 0,1378,1380,1435,1436,1540,1690,1846,1 848,1880,1907,1960,2113,2185,2209,2296 ,2353,2534,2625,2672,3093,3320,3384,34 58,3479,3481,3559,3567,3575,3592,3596, 3600,3624,3635,3673,3702,3708,3709,378 3,3937,3965,4057,4068,4208,4214,4313,4 602,4772,4773,4790,5054,5063,5142,5144 ,5289,5290,5295,5567,5579,5591,5641,57 07,5716,5728,5734,5795,6197,6504,6850, 6885,7048,7071,7097,7292,7294,7301,742 4,7456,7704,8111,8440,8600,8660,8767,8 995,9173,9308,9402,9447,9844,10010,101 25,10213,10221,10333,10451,10563,1066 6,11148,23118,23228,23291,23369,23457, 26191,26999,29851,30009,30849,50852,5 4106,55904,56940,57162,57181,57590,57 829,64581,79109,79931,84174,85477,114 548,118788,124912,146850,149233,15188 8,153090,253260,338339,340061,375056, 387357	ANXA1,BIRC2,BIRC3,ATP6AP1,AXL,BCL2,BCL6,PR DM1,CASP8,RUNX1,CD2,CD247,CD5,CD28,CD86,C CR1,CCR6,CCR7,CMKLR1,CR1,CR2,CSF1,CSF1R,CY LD,COCH,DUSP4,DUSP6,GPR183,EDN2,EGR3,ETS1, PTK2B,FCGR1A,FOXC1,FOS,FYN,GATA3,GFI1,UBE 2K,HSP90AA1,ICAM2,IFNG,IGF1,IGF2,IL2RA,IL5,IL 7R,IL12A,IL13,IL15,INHBA,INPP5D,ITGA2,ITK,ITPR 1,ITPR2,KCNN4,LCP2,LGALS9,LTF,SH2D1A,MEF2C ,MAP3K1,MMP2,MYB,NFATC1,NFATC2,NFKB1,SE RPINE1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R 1,PRKACB,PRKCB,PRKDC,LGMN,PSMD1,PSMD10, PTEN,PTGER4,PTPRJ,RPS6KA3,SLAMF1,SYK,MAP3 K7,TGFBR2,KLF10,TLR2,TNFSF4,TXK,TYRO3,VEG FC,WIPF1,ZBTB16,GPR68,NCK2,TNFSF11,IRS2,RIP K2,TNFSF18,IL1RL1,CD83,GRAP2,AIM2,ELMO1,TA NK,RASGRP1,PSMD14,TRIB1,TLR6,VAV3,CXCL13, CD226,HHLA2,TAB2,PLCL2,FBXW11,PUM2,ABCB9, PTPN22,CYFIP2,ICOS,TBX21,PIK3R4,TRAT1,TLR9, KMT2E,DUSP22,PELI1,SLC39A10,WDFY1,ZP4,CLE C7A,MAPKAP1,TNIP3,SLA2,SCIN,NLRP3,PIK3AP1,S PACA3,PIK3R6,IL23R,BTLA,DAB2IP,RICTOR,CLEC 4D,TMEM173,MIA3,THEMIS

Appendix

58	2_Member GO Biological Processes GO:0002764 immune response-regulating signaling pathway	-10.1545911 -7.361 108/671	284,329,330,596,817,841,919,940,942,1236,1378,1380,1437,1540,1839,1846,1847,1848,1956,2209,2246,2353,2534,2549,2625,2672,3320,3384,3458,3559,3563,3567,3568,3635,3702,3708,3709,3716,3783,3937,3965,4057,4140,4208,4214,4772,4773,4790,5063,5142,5144,5289,5290,5295,5567,5579,5641,5707,5716,5728,5795,5921,5922,6197,6711,6850,6885,7097,7294,7301,7456,8452,8660,8767,9402,9844,10010,10125,10213,10333,10451,10666,23118,23228,23239,23291,23369,25780,26191,26999,30849,50852,54106,56940,57162,57181,57590,64581,79109,79931,84174,115727,118788,152559,153090,253260,338339,387357	ANGPT1,BIRC2,BIRC3,BCL2,CAMK2D,CASP8,CD247,CD28,CD86,CCR7,CR1,CR2,CSF2,CYLD,HBEGF,DUSP4,DUSP5,DUSP6,EGFR,FCGR1A,FGF1,FOS,FYN,GAB1,GATA3,GFI1,HSP90AA1,ICAM2,IFNG,IL2RA,IL3RA,IL5,IL5RA,INPP5D,ITK,ITPR1,ITPR2,JAK1,KCNN4,LCP2,LGALS9,LTF,MARK3,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PRKACB,PRKCB,LGMN,PSMD1,PSMD10,PTEN,PTPRJ,RASA1,RASA2,RPS6KA3,SPTBN1,SYK,MAP3K7,TLR2,TXK,TYRO3,WIPF1,CUL3,IRS2,RIPK2,GRAP2,ELMO1,TANK,RASGRP1,PSMD14,TLR6,VAV3,CD226,TAB2,PLCL2,PHLPP1,FBXW11,PUM2,RASGRP3,PTPN22,CYFIP2,PIK3R4,TRAT1,TLR9,DUSP22,PELI1,SLC39A10,WDFY1,CLEC7A,MAPKAP1,TNIP3,SLA2,RASGRP4,PIK3AP1,PAQR3,DAB2IP,RICTOR,CLEC4D,THEMIS
59	2_Member GO Biological Processes GO:0031347 regulation of defense response	-9.012903094 -6.464 119/798	103,154,301,308,329,330,335,347,604,834,841,919,940,942,1236,1378,1540,1690,1794,1846,1848,2040,2113,2353,2534,2625,2672,2693,2697,3093,3384,3442,3458,3481,3554,3559,3592,3600,3673,3708,3709,3716,3965,4057,4068,4142,4208,4214,4313,4772,4773,4790,4907,5054,5063,5289,5321,5567,5641,5654,5707,5716,5734,5740,5770,6095,6197,6346,6772,6846,6850,6885,7004,7097,7292,7294,7301,7356,8554,8600,8651,8767,8995,9173,9353,9447,9469,9844,9982,10010,10125,10213,10333,10666,11221,23118,23291,23369,26191,27086,27347,30849,51606,54106,55690,57162,57590,60386,64581,79931,80762,114548,118788,146850,149233,153090,253260,338339,340061	ADAR,ADRB2,ANXA1,ANXA5,BIRC2,BIRC3,APOA1,APOD,BCL6,CASP1,CASP8,CD247,CD28,CD86,CCR7,CR1,CYLD,COCH,DOCK2,DUSP4,DUSP6,STOM,ETS1,FOS,FYN,GATA3,GFI1,GHSR,GJA1,UBE2K,ICAM2,IFNA5,IFNG,IGF2,IL1R1,IL2RA,IL12A,IL15,ITGA2,ITPR1,ITPR2,JAK1,LGALS9,LTF,SH2D1A,MAS1,MEF2C,MAP3K1,MMP2,NFATC1,NFATC2,NFKB1,NT5E,SERPINE1,PAK3,PIK3C3,PLA2G4A,PRKACB,LGMN,HTRA1,PSMD1,PSMD10,PTGER4,PTGIS,PTPN1,RORA,RPS6KA3,CCL1,STAT1,XCL2,SYK,MAP3K7,TEAD4,TLR2,TNFSF4,TXK,TYRO3,SCGB1A1,PIAS1,TNFSF11,SOCS1,RIPK2,TNFSF18,IL1RL1,SLIT2,AIM2,CHST3,ELMO1,FGFBP1,TANK,RASGRP1,PSMD14,TLR6,CD226,DUSP10,TAB2,FBXW11,PUM2,PTPN22,FOXP1,STK39,PIK3R4,ATP6V1H,TLR9,PACS1,PELI1,WDFY1,SLC25A19,CLEC7A,TNIP3,NDFIP1,NLRP3,PIK3AP1,PIK3R6,IL23R,DAB2IP,RICTOR,CLEC4D,TMEM173
60	2_Member GO Biological Processes GO:0002768 immune response-regulating cell surface receptor signaling	-8.83665802 -6.307 87/527	284,596,817,841,919,940,942,1236,1378,1380,1437,1839,1847,1848,1956,2209,2246,2353,2534,2549,2625,3320,3384,3458,3559,3563,3567,3568,3635,3702,3708,3709,3716,3783,3937,4140,4208,4214,4772,4773,4790,5063,5142,5144,5290,5295,5567,5	ANGPT1,BCL2,CAMK2D,CASP8,CD247,CD28,CD86,CCR7,CR1,CR2,CSF2,HBEGF,DUSP5,DUSP6,EGFR,FCGR1A,FGF1,FOS,FYN,GAB1,GATA3,HSP90AA1,ICAM2,IFNG,IL2RA,IL3RA,IL5,IL5RA,INPP5D,ITK,ITPR1,ITPR2,JAK1,KCNN4,LCP2,MARK3,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,P

Appendix

	pathway		579,5707,5716,5728,5795,5921,5922,6711,6850,6885,7294,7456,8452,8660,8767,9402,9844,10125,10213,10451,10666,23118,23228,23239,23291,25780,26191,26999,50852,56940,57181,64581,79109,84174,115727,152559,153090,253260,338339,387357	IK3CA,PIK3R1,PRKACB,PRKCB,PSMD1,PSMD10,PTEN,PTPRJ,RASA1,RASA2,SPTBN1,SYK,MAP3K7,TXK,WIPF1,CUL3,IRS2,RIPK2,GRAP2,ELMO1,RASGRP1,PSMD14,VAV3,CD226,TAB2,PLCL2,PHLPP1,FBXW11,RASGRP3,PTPN22,CYFIP2,TRAT1,DUSP22,SLC39A10,CLEC7A,MAPKAP1,SLA2,RASGRP4,PAQR3,DAB2IP,RICTOR,CLEC4D,THEMIS
61	2_Member GO Biological Processes GO:0002757 immune response-activating signal transduction	-8.31109752 -5.861 82/498	329,330,596,841,919,940,942,1236,1378,1380,1540,1846,1848,2209,2353,2534,2625,2672,3320,3384,3458,3635,3702,3708,3709,3783,3937,3965,4057,4208,4214,4772,4773,4790,5063,5142,5144,5289,5290,5295,5567,5579,5641,5707,5716,5728,5795,6197,6850,6885,7097,7294,7301,7456,8767,9402,9844,10010,10213,10333,10451,10666,23118,23228,23291,23369,26191,26999,30849,50852,54106,56940,57162,57181,57590,64581,79931,84174,118788,153090,338339,387357	BIRC2,BIRC3,BCL2,CASP8,CD247,CD28,CD86,CCR7,CR1,CR2,CYLD,DUSP4,DUSP6,FCGR1A,FOS,FYN,GATA3,GFI1,HSP90AA1,ICAM2,IFNG,INPP5D,ITK,ITPR1,ITPR2,KCNN4,LCP2,LGALS9,LTF,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PRKACB,PRKCB,LGMN,PSMD1,PSMD10,PTEN,PTPRJ,RPS6KA3,SYK,MAP3K7,TLR2,TXK,TYRO3,WIPF1,RIPK2,GRAP2,ELMO1,TANK,PSMD14,TLR6,VAV3,CD226,TAB2,PLCL2,FBXW11,PUM2,PTPN22,CYFIP2,PIK3R4,TRAT1,TLR9,DUSP22,PELI1,SLC39A10,WDFY1,CLEC7A,TNIP3,SLA2,PIK3AP1,DAB2IP,CLEC4D,THEMIS
62	2_Member GO Biological Processes GO:0045088 regulation of innate immune response	-8.103811249 -5.694 70/404	103,329,330,841,942,1378,1540,1690,1846,1848,2353,2534,2672,3093,3384,3442,3458,3481,3592,3708,3709,3716,3965,4057,4068,4208,4214,4313,4772,4773,4790,5063,5289,5567,5641,5707,5716,5770,6197,6772,6850,6885,7097,7294,7301,8554,8651,8767,9447,10010,10125,10213,10333,10666,11221,23118,23291,23369,26191,30849,54106,57162,57590,64581,79931,118788,146850,153090,338339,340061	ADAR,BIRC2,BIRC3,CASP8,CD86,CR1,CYLD,COCH,DUSP4,DUSP6,FOS,FYN,GFI1,UBE2K,ICAM2,IFNA5,IFNG,IGF2,IL12A,ITPR1,ITPR2,JAK1,LGALS9,LTF,SH2D1A,MEF2C,MAP3K1,MMP2,NFATC1,NFATC2,NFKB1,PAK3,PIK3C3,PRKACB,LGMN,PSMD1,PSMD10,PTPN1,RPS6KA3,STAT1,SYK,MAP3K7,TLR2,TXK,TYRO3,PIAS1,SOCS1,RIPK2,AIM2,TANK,RASGRP1,PSMD14,TLR6,CD226,DUSP10,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,PIK3R6,DAB2IP,CLEC4D,TMEM173
63	2_Member GO Biological Processes GO:0031349 positive regulation of defense response	-7.142745715 -4.848 72/443	329,330,841,940,942,1236,1540,1690,1846,1848,2353,2534,2672,2697,3093,3384,3592,3600,3673,3708,3709,3965,4057,4068,4208,4214,4313,4772,4773,4790,5054,5063,5289,5321,5567,5641,5707,5716,5734,6197,6346,6846,6850,6885,7097,7292,7294,7301,8600,8767,8995,9173,9447,10010,10125,10213,10333,10666,23118,23291,23369,26191,30849,54106,57162,57590,64581,79931,118788,153090,338339,340061	BIRC2,BIRC3,CASP8,CD28,CD86,CCR7,CYLD,COCH,DUSP4,DUSP6,FOS,FYN,GFI1,GJA1,UBE2K,ICAM2,IL12A,IL15,ITGA2,ITPR1,ITPR2,LGALS9,LTF,SH2D1A,MEF2C,MAP3K1,MMP2,NFATC1,NFATC2,NFKB1,SERPINE1,PAK3,PIK3C3,PLA2G4A,PRKACB,LGMN,PSMD1,PSMD10,PTGER4,RPS6KA3,CCL1,XCL2,SYK,MAP3K7,TLR2,TNFSF4,TXK,TYRO3,TNFSF11,RIPK2,TNFSF18,IL1RL1,AIM2,TANK,RASGRP1,PSMD14,TLR6,CD226,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,DAB2IP,CLEC4D,TMEM173

64	2_Member GO Biological Processes GO:0050778 positive regulation of immune response	-7.133055209 -4.848 99/678	301,329,330,596,841,919,940,942,1236,1378,1380,1540,1690,1846,1848,2209,2353,2534,2625,2672,3093,3320,3384,3458,3592,3596,3600,3635,3702,3708,3709,3783,3937,3965,4057,4068,4208,4214,4313,4602,4772,4773,4790,5063,5142,5144,5289,5290,5295,5567,5579,5641,5707,5716,5728,5795,6197,6850,6885,7097,7292,7294,7301,7456,8767,9402,9447,9844,10010,10125,10213,10333,10451,10666,23118,23228,23291,23369,26191,26999,30009,30849,50852,54106,56940,57162,57181,57590,57829,64581,79931,84174,114548,118788,149233,153090,338339,340061,387357	ANXA1,BIRC2,BIRC3,BCL2,CASP8,CD247,CD28,CD86,CCR7,CR1,CR2,CYLD,COCH,DUSP4,DUSP6,FCGR1A,FOS,FYN,GATA3,GFI1,UBE2K,HSP90AA1,ICAM2,IFNG,IL12A,IL13,IL15,INPP5D,ITK,ITPR1,ITPR2,KCNN4,LCP2,LGALS9,LTF,SH2D1A,MEF2C,MAP3K1,MMP2,MYB,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PRKACB,PRKCB,LGMN,PSMD1,PSMD10,PTEN,PTPRJ,RPS6KA3,SYK,MAP3K7,TLR2,TNFSF4,TXK,TYRO3,WIPF1,RIPK2,GRAP2,AIM2,ELMO1,TANK,RASGRP1,PSMD14,TLR6,VAV3,CD226,TAB2,PLCL2,FBXW11,PUM2,PTPN22,CYFIP2,TBX21,PIK3R4,TRAT1,TLR9,DUSP22,PELI1,SLC39A10,WDFY1,ZP4,CLEC7A,TNIP3,SLA2,NLRP3,PIK3AP1,IL23R,DAB2IP,CLEC4D,TMEM173,THEMIS
65	2_Member GO Biological Processes GO:0002253 activation of immune response	-6.984853506 -4.721 84/550	329,330,596,841,919,940,942,1236,1378,1380,1540,1846,1848,2209,2353,2534,2625,2672,3320,3384,3458,3635,3702,3708,3709,3783,3937,3965,4057,4208,4214,4772,4773,4790,5063,5142,5144,5289,5290,5295,5567,5579,5641,5707,5716,5728,5795,6197,6850,6885,7097,7294,7301,7456,8767,9402,9447,9844,10010,10213,10333,10451,10666,23118,23228,23291,23369,26191,26999,30849,50852,54106,56940,57162,57181,57590,64581,79931,84174,118788,153090,338339,340061,387357	BIRC2,BIRC3,BCL2,CASP8,CD247,CD28,CD86,CCR7,CR1,CR2,CYLD,DUSP4,DUSP6,FCGR1A,FOS,FYN,GATA3,GFI1,HSP90AA1,ICAM2,IFNG,INPP5D,ITK,ITPR1,ITPR2,KCNN4,LCP2,LGALS9,LTF,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PRKACB,PRKCB,LGMN,PSMD1,PSMD10,PTEN,PTPRJ,RPS6KA3,SYK,MAP3K7,TLR2,TXK,TYRO3,WIPF1,RIPK2,GRAP2,AIM2,ELMO1,TANK,PSMD14,TLR6,VAV3,CD226,TAB2,PLCL2,FBXW11,PUM2,PTPN22,CYFIP2,PIK3R4,TRAT1,TLR9,DUSP22,PELI1,SLC39A10,WDFY1,CLEC7A,TNIP3,SLA2,PIK3AP1,DAB2IP,CLEC4D,TMEM173,THEMIS
66	2_Member GO Biological Processes GO:0045089 positive regulation of innate immune response	-6.636562543 -4.427 58/339	329,330,841,942,1540,1690,1846,1848,2353,2534,2672,3093,3384,3592,3708,3709,3965,4057,4068,4208,4214,4313,4772,4773,4790,5063,5289,5567,5641,5707,5716,6197,6850,6885,7097,7294,7301,8767,9447,10010,10125,10213,10333,10666,23118,23291,23369,26191,30849,54106,57162,57590,64581,79931,118788,153090,338339,340061	BIRC2,BIRC3,CASP8,CD86,CYLD,COCH,DUSP4,DUSP6,FOS,FYN,GFI1,UBE2K,ICAM2,IL12A,ITPR1,ITPR2,LGALS9,LTF,SH2D1A,MEF2C,MAP3K1,MMP2,NFATC1,NFATC2,NFKB1,PAK3,PIK3C3,PRKACB,LGMN,PSMD1,PSMD10,RPS6KA3,SYK,MAP3K7,TLR2,TXK,TYRO3,RIPK2,AIM2,TANK,RASGRP1,PSMD14,TLR6,CD226,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,DAB2IP,CLEC4D,TMEM173
67	2_Member GO Biological Processes GO:0002429 immune response-	-5.805251721 -3.775 56/342	596,841,919,940,1236,1378,1380,2209,2534,2625,3320,3384,3458,3635,3702,3708,3709,3783,3937,4208,4772,4773,4790,5063,5142,5144,5290,5295,5567,5579,5707,5	BCL2,CASP8,CD247,CD28,CCR7,CR1,CR2,FCGR1A,FYN,GATA3,HSP90AA1,ICAM2,IFNG,INPP5D,ITK,ITPR1,ITPR2,KCNN4,LCP2,MEF2C,NFATC1,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3CA,PIK3R1,PRKA

WO 2018/175733 PCT/US2018/023785

Appendix

	activating cell surface receptor signaling pathway		716,5728,5795,6850,6885,7294,7456,8767,9402,9844,10213,10451,10666,23118,23228,23291,26191,26999,50852,56940,57181,64581,84174,338339,387357	CB,PRKCB,PSMD1,PSMD10,PTEN,PTPRJ,SYK,MAP3K7,TXK,WIPF1,RIPK2,GRAP2,ELMO1,PSMD14,VAV3,CD226,TAB2,PLCL2,FBXW11,PTPN22,CYFIP2,TRAT1,DUSP22,SLC39A10,CLEC7A,SLA2,CLEC4D,THEMIS
68	2_Member GO Biological Processes GO:0002218 activation of innate immune response	-5.605050038 -3.618 50/297	329,330,841,942,1540,1846,1848,2353,2534,2672,3384,3708,3709,3965,4057,4208,4214,4772,4773,4790,5063,5289,5567,5641,5707,5716,6197,6850,6885,7097,7301,8767,9447,10010,10213,10333,23118,23291,23369,26191,30849,54106,57162,57590,64581,79931,118788,153090,338339,340061	BIRC2,BIRC3,CASP8,CD86,CYLD,DUSP4,DUSP6,FOS,FYN,GFI1,ICAM2,ITPR1,ITPR2,LGALS9,LTF,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PIK3C3,PRKACB,LGMN,PSMD1,PSMD10,RPS6KA3,SYK,MAP3K7,TLR2,TYRO3,RIPK2,AIM2,TANK,PSMD14,TLR6,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,DAB2IP,CLEC4D,TMEM173
69	2_Member GO Biological Processes GO:0002224 toll-like receptor signaling pathway	-5.305184847 -3.351 32/161	329,330,841,942,1846,1848,2353,2672,3965,4057,4208,4214,4790,5289,5641,6197,6885,7097,7301,8767,10010,10333,23118,23291,26191,30849,54106,57162,57590,79931,118788,153090	BIRC2,BIRC3,CASP8,CD86,DUSP4,DUSP6,FOS,GFI1,LGALS9,LTF,MEF2C,MAP3K1,NFKB1,PIK3C3,LGMN,RPS6KA3,MAP3K7,TLR2,TYRO3,RIPK2,TANK,TLR6,TAB2,FBXW11,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,TNIP3,PIK3AP1,DAB2IP
70	2_Member GO Biological Processes GO:0002758 innate immune response-activating signal transduction	-5.200463052 -3.261 48/290	329,330,841,942,1540,1846,1848,2353,2534,2672,3384,3708,3709,3965,4057,4208,4214,4772,4773,4790,5063,5289,5567,5641,5707,5716,6197,6850,6885,7097,7301,8767,10010,10213,10333,23118,23291,23369,26191,30849,54106,57162,57590,64581,79931,118788,153090,338339	BIRC2,BIRC3,CASP8,CD86,CYLD,DUSP4,DUSP6,FOS,FYN,GFI1,ICAM2,ITPR1,ITPR2,LGALS9,LTF,MEF2C,MAP3K1,NFATC1,NFATC2,NFKB1,PAK3,PIK3C3,PRKACB,LGMN,PSMD1,PSMD10,RPS6KA3,SYK,MAP3K7,TLR2,TYRO3,RIPK2,TANK,PSMD14,TLR6,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TLR9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,DAB2IP,CLEC4D
71	2_Member GO Biological Processes GO:0050851 antigen receptor-mediated signaling pathway	-5.192363458 -3.255 40/225	596,919,940,1236,2534,2625,3458,3635,3702,3783,3937,4208,4773,4790,5063,5142,5144,5290,5295,5579,5707,5716,5728,5795,6850,6885,7294,8767,9402,10213,10451,23118,23228,23291,26191,50852,56940,57181,84174,387357	BCL2,CD247,CD28,CCR7,FYN,GATA3,IFNG,INPP5D,ITK,KCNN4,LCP2,MEF2C,NFATC2,NFKB1,PAK3,PDE4B,PDE4D,PIK3CA,PIK3R1,PRKCB,PSMD1,PSMD10,PTEN,PTPRJ,SYK,MAP3K7,TXK,RIPK2,GRAP2,PSMD14,VAV3,TAB2,PLCL2,FBXW11,PTPN22,TRAT1,DUSP22,SLC39A10,SLA2,THEMIS
72	2_Member GO Biological Processes GO:0034142 toll-like receptor 4 signaling pathway	-4.711745438 -2.868 25/119	329,330,841,1846,1848,2353,3965,4057,4208,4214,4790,6197,6885,7097,8767,10010,10333,23118,23291,26191,57162,57590,79931,118788,153090	BIRC2,BIRC3,CASP8,DUSP4,DUSP6,FOS,LGALS9,LTF,MEF2C,MAP3K1,NFKB1,RPS6KA3,MAP3K7,TLR2,RIPK2,TANK,TLR6,TAB2,FBXW11,PTPN22,PELI1,WDFY1,TNIP3,PIK3AP1,DAB2IP
73	2_Member GO Biological Processes GO:0002221 pattern recognition	-4.668281222 -2.830 35/196	329,330,841,942,1540,1846,1848,2353,2672,3965,4057,4208,4214,4790,5289,5641,6197,6885,7097,7301,8767,10010,10333,23118,23291,23369,26191,30849,54106,57	BIRC2,BIRC3,CASP8,CD86,CYLD,DUSP4,DUSP6,FOS,GFI1,LGALS9,LTF,MEF2C,MAP3K1,NFKB1,PIK3C3,LGMN,RPS6KA3,MAP3K7,TLR2,TYRO3,RIPK2,TANK,TLR6,TAB2,FBXW11,PUM2,PTPN22,PIK3R4,TL

Appendix

	receptor signaling pathway		162,57590,64581,79931,118788,153090	R9,PELI1,WDFY1,CLEC7A,TNIP3,PIK3AP1,DAB2IP
74	2_Member GO Biological Processes GO:0050852 T cell receptor signaling pathway	-4.490996007 -2.680 31/168	919,940,1236,2534,2625,3458,3635,3702, 3783,3937,4790,5063,5142,5144,5290,529 5,5707,5716,5728,5795,6885,7294,8767,9 402,10213,23118,23291,26191,50852,569 40,387357	CD247,CD28,CCR7,FYN,GATA3,IFNG,INPP5D,ITK,K CNN4,LCP2,NFKB1,PAK3,PDE4B,PDE4D,PIK3CA,PI K3R1,PSMD1,PSMD10,PTEN,PTPRJ,MAP3K7,TXK,R IPK2,GRAP2,PSMD14,TAB2,FBXW11,PTPN22,TRAT 1,DUSP22,THEMIS
75	2_Member GO Biological Processes GO:0034162 toll-like receptor 9 signaling pathway	-3.051091816 -1.530 16/79	1846,1848,2353,4208,4214,4790,5289,619 7,6885,8767,23118,23291,30849,54106,57 162,118788	DUSP4,DUSP6,FOS,MEF2C,MAP3K1,NFKB1,PIK3C3 ,RPS6KA3,MAP3K7,RIPK2,TAB2,FBXW11,PIK3R4,T LR9,PELI1,PIK3AP1
76	2_Member GO Biological Processes GO:0034138 toll-like receptor 3 signaling pathway	-2.839047488 -1.382 18/98	329,330,841,942,1846,1848,2353,4208,47 90,6197,6885,8767,10010,23118,23291,26 191,57162,57590	BIRC2,BIRC3,CASP8,CD86,DUSP4,DUSP6,FOS,MEF 2C,NFKB1,RPS6KA3,MAP3K7,RIPK2,TANK,TAB2,F BXW11,PTPN22,PELI1,WDFY1
77	2_Member GO Biological Processes GO:0034134 toll-like receptor 2 signaling pathway	-2.75506399 -1.319 16/84	1846,1848,2353,3965,4208,4214,4790,619 7,6885,7097,8767,10333,23118,23291,571 62,118788	DUSP4,DUSP6,FOS,LGALS9,MEF2C,MAP3K1,NFKB 1,RPS6KA3,MAP3K7,TLR2,RIPK2,TLR6,TAB2,FBX W11,PELI1,PIK3AP1
78	2_Member GO Biological Processes GO:0038124 toll-like receptor TLR6:TLR2 signaling pathway	-2.342381479 -1.016 14/76	1846,1848,2353,4208,4214,4790,6197,688 5,7097,8767,10333,23118,23291,57162	DUSP4,DUSP6,FOS,MEF2C,MAP3K1,NFKB1,RPS6K A3,MAP3K7,TLR2,RIPK2,TLR6,TAB2,FBXW11,PELI 1
79	2_Member GO Biological Processes GO:0038123 toll-like receptor TLR1:TLR2 signaling pathway	-2.342381479 -1.016 14/76	1846,1848,2353,4208,4214,4790,6197,688 5,7097,8767,10333,23118,23291,57162	DUSP4,DUSP6,FOS,MEF2C,MAP3K1,NFKB1,RPS6K A3,MAP3K7,TLR2,RIPK2,TLR6,TAB2,FBXW11,PELI 1
80	2_Member GO Biological Processes GO:0002756 MyD88-independent toll-like receptor signaling pathway	-2.338719587 -1.015 15/84	329,330,841,1846,1848,2353,4208,4790,6 197,6885,8767,10010,23118,23291,79931	BIRC2,BIRC3,CASP8,DUSP4,DUSP6,FOS,MEF2C,NF KB1,RPS6KA3,MAP3K7,RIPK2,TANK,TAB2,FBXW1 1,TNIP3

81	2_Member GO Biological Processes GO:0002755 MyD88-dependent toll-like receptor signaling pathway	-2.193138427 -0.915 15/87	1846,1848,2353,4208,4214,4790,6197,6885,7097,8767,10333,23118,23291,54106,57162	DUSP4,DUSP6,FOS,MEF2C,MAP3K1,NFKB1,RPS6KA3,MAP3K7,TLR2,RIPK2,TLR6,TAB2,FBXW11,TLR9,PELI1
82	2_Member GO Biological Processes GO:0002223 stimulatory C-type lectin receptor signaling pathway	-2.097316204 -0.853 19/123	841,2534,3384,3708,3709,4772,4773,4790,5063,5567,5707,5716,6850,6885,10213,23118,23291,64581,338339	CASP8,FYN,ICAM2,ITPR1,ITPR2,NFATC1,NFATC2,NFKB1,PAK3,PRKACB,PSMD1,PSMD10,SYK,MAP3K7,PSMD14,TAB2,FBXW11,CLEC7A,CLEC4D
83	2_Member GO Biological Processes GO:0035666 TRIF-dependent toll-like receptor signaling pathway	-2.091384559 -0.849 14/81	329,330,841,1846,1848,2353,4208,4790,6197,6885,8767,10010,23118,23291	BIRC2,BIRC3,CASP8,DUSP4,DUSP6,FOS,MEF2C,NFKB1,RPS6KA3,MAP3K7,RIPK2,TANK,TAB2,FBXW11
84	2_Member GO Biological Processes GO:0002220 innate immune response activating cell surface receptor signaling pathway	-2.023647181 -0.798 19/125	841,2534,3384,3708,3709,4772,4773,4790,5063,5567,5707,5716,6850,6885,10213,23118,23291,64581,338339	CASP8,FYN,ICAM2,ITPR1,ITPR2,NFATC1,NFATC2,NFKB1,PAK3,PRKACB,PSMD1,PSMD10,SYK,MAP3K7,PSMD14,TAB2,FBXW11,CLEC7A,CLEC4D
85	3_Summary GO Biological Processes GO:0007169 transmembrane receptor protein tyrosine kinase signaling pathway	-12.45003744 -9.472 145/928	27,113,154,238,284,347,356,374,526,528,537,558,814,817,836,860,925,940,942,1435,1436,1437,1499,1536,1759,1839,1846,1847,1848,1946,1956,2060,2099,2185,2246,2257,2296,2534,2549,2625,2675,2692,2693,3320,3479,3481,3516,3559,3563,3567,3568,3672,3702,3708,3709,3716,3937,4140,4145,4208,4313,4653,4790,4920,5063,5137,5289,5290,5295,5494,5564,5567,5579,5584,5641,5707,5716,5728,5739,5770,5791,5793,5795,5921,5922,6197,6314,6383,6461,6711,6720,6772,6850,7074,7294,7424,7498,7531,8440,8452,8569,8651,8660,8767,8828,8997,9146,9844,9982,10018,10125,10126,10213,10252,10451,11082,1115	ABL2,ADCY7,ADRB2,ALK,ANGPT1,APOD,FASLG,AREG,ATP6V1B2,ATP6V1C1,ATP6AP1,AXL,CAMK4,CAMK2D,CASP3,RUNX2,CD8A,CD28,CD86,CSF1,CSF1R,CSF2,CTNNB1,CYBB,DNM1,HBEGF,DUSP4,DUSP5,DUSP6,EFNA5,EGFR,EPS15,ESR1,PTK2B,FGF1,FGF12,FOXC1,FYN,GAB1,GATA3,GFRA2,GHRHR,GHSR,HSP90AA1,IGF1,IGF2,RBPJ,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITK,ITPR1,ITPR2,JAK1,LCP2,MARK3,MATK,MEF2C,MMP2,MYOC,NFKB1,ROR2,PAK3,PDE1C,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,LGMN,PSMD1,PSMD10,PTEN,PTGIR,PTPN1,PTPRE,PTPRG,PTPRJ,RASA1,RASA2,RPS6KA3,ATXN7,SDC2,SHB,SPTBN1,SREBF1,STAT1,SYK,TIAM1,TXK,VEGFC,XDH,YWHAE,NCK2,CUL3,MKNK1,SOCS1,IRS2,RIPK2,NRP2,KALRN,HGS,E

Appendix

		6,11214,23122,23239,23545,25780,25976,	LMO1,FGFBP1,BCL2L11,RASGRP1,DNAL4,PSMD14
		26037,26230,26509,26999,30849,50650,5	,SPRY1,VAV3,ESM1,PTP4A3,AKAP13,CLASP2,PHLP
		0852,51422,51606,54106,54206,54541,55	P1,ATP6V0A2,RASGRP3,TIPARP,SIPA1L1,TIAM2,M
		023,79109,84159,103910,114882,115727,	YOF,CYFIP2,PIK3R4,ARHGEF3,TRAT1,PRKAG2,AT
		121512,152559,153090,253260,81,329,33	P6V1H,TLR9,ERRFI1,DDIT4,PHIP,MAPKAP1,ARID5
		0,335,444,596,834,841,960,1230,1236,164	B,MYL12B,OSBPL8,RASGRP4,FGD4,PAQR3,DAB2I
		7,1687,1880,1896,1906,2074,2146,2244,2	P,RICTOR,ACTN4,BIRC2,BIRC3,APOA1,ASPH,BCL2
		697,3458,3592,3596,3600,3688,3814,3965	,CASP1,CASP8,CD44,CCR1,CCR7,GADD45A,DFNA5
		,4057,4142,4214,4853,5326,5734,6346,65	,GPR183,EDA,EDN1,ERCC6,EZH2,FGB,GJA1,IFNG,I
		04,6754,6846,6885,7097,7476,7852,8600,	L12A,IL13,IL15,ITGB1,KISS1,LGALS9,LTF,MAS1,M
		8743,8995,9064,9218,9290,10333,10507,1	AP3K1,NOTCH2,PLAGL2,PTGER4,CCL1,SLAMF1,S
		0550,10783,10855,10920,11184,23118,23	STR4,XCL2,MAP3K7,TLR2,WNT7A,CXCR4,TNFSF1
		369,26191,28996,29949,53832,54602,552	1,TNFSF10,TNFSF18,MAP3K6,VAPA,GPR55,TLR6,S
		23,56911,56940,57162,57708,60675,7915	EMA4D,ARL6IP5,NEK6,HPSE,COPS8,MAP4K1,TAB
		6,80762,90441,92140,112464,118788,128	2,PUM2,PTPN22,HIPK2,IL19,IL20RA,NDFIP2,TRIM6
		239,146850,149233,168667,200734,25574	2,MAP3K7CL,DUSP22,PELI1,MIER1,PROK2,PLEKH
		3,388121,317,463,649,753,1030,1149,145	F1,NDFIP1,ZNF622,MTDH,PRKCDBP,PIK3AP1,IQG
		3,1960,2335,2353,3037,3077,3397,3554,3	AP3,PIK3R6,IL23R,BMPER,SPRED2,NPNT,TNFAIP8
		975,3987,4092,4435,4488,5054,5654,6262	L3,APAF1,ZFHX3,BMP1,LDLRAD4,CDKN2B,CIDEA
		,6423,6660,7048,7049,7071,7123,7356,82	,CSNK1D,EGR3,FN1,FOS,HAS2,HFE,ID1,IL1R1,LHX
		39,8573,9353,10140,10563,23327,25937,5	1,LIMS1,SMAD7,CITED1,MSX2,SERPINE1,HTRA1,R
		0848,51256,51741,56937,63893,64750,28	YR2,SFRP2,SOX5,TGFBR2,TGFBR3,KLF10,CLEC3B,
		3149,494470,301,308,339,440,476,1393,1	SCGB1A1,USP9X,CASK,SLIT2,TOB1,CXCL13,NEDD
		407,1583,2104,2180,2494,2673,2908,3624	4L,WWTR1,F11R,TBC1D7,WWOX,PMEPA1,UBE2O,
		,3673,3990,4082,4478,5021,5547,5591,59	SMURF2,BCL9L,RNF165,ANXA1,ANXA5,APOBEC1,
		62,6095,6446,6751,7253,7292,7352,7421,	ASNS,ATP1A1,CRHBP,CRY1,CYP11A1,ESRRG,ACS
		7490,7849,8195,8204,8554,8648,8856,906	L1,NR5A2,GFPT1,NR3C1,INHBA,ITGA2,LIPC,MARC
		3,9604,9734,10002,10268,23543,27086,51	KS,MSN,OXTR,PRCP,PRKDC,RDX,RORA,SGK1,SST
		366,5431,55970,64754,84109,90390,255	R1,TSHR,TNFSF4,UCP3,VDR,WT1,PAX8,MKKS,NRI
		738,333,493,540,610,682,1134,1316,1808,	P1,PIAS1,NCOA1,NR1I2,PIAS2,RNF14,HDAC9,NR2E
		1811,1959,2796,3033,3269,3663,4048,425	3,RAMP3,RBFOX2,FOXP1,UBR5,GNG2,GNG12,SMY
		8,5032,5142,5144,5321,6506,6609,8061,9	D3,QRFPR,MED30,PCSK9,APLP1,ATP2B4,ATP7B,H
		173,51141,54434,55328,56729,84152,123	CN2,BSG,CHRNA1,KLF6,DPYSL2,SLC26A3,EGR2,G
		4,1889,3756,6653,7301,8428,9448,10221,	NRH1,HADH,HRH1,IRF5,LTA4H,MGST2,P2RY11,PD
		10771,11221,23291,27347,28951,51765,5	E4B,PDE4D,PLA2G4A,SLC1A2,SMPD1,FOSL1,IL1RL
		4986,57091,83605,131096,154043,286,28	1,INSIG2,SSH1,RNLS,RETN,PPP1R1B,CCR5,ECE1,K
		7,288,323,1232,1233,1235,1237,1240,129	CNH1,SORL1,TYRO3,STK24,MAP4K4,TRIB1,ZMYN
		3,1525,1794,1907,2596,2668,2829,3680,3	D11,DUSP10,FBXW11,STK39,TRIB2,STK26,ULK4,C
		786,3800,3983,4897,5288,5800,6441,6480	ASS4,CCM2,KCNH8,CNKSR3,ANK1,ANK2,ANK3,A
		,7903,8609,8650,9369,9855,10154,10487,	PBB2,CCR3,CCR4,CCR6,CCR8,CMKLR1,COL6A3,C

			10800,22885,23396,55079,57556,64218,120425,284340,302,894,3093,3442,5912,8412,51429,57600,57829,115825,116496,219771,103,900,2776,5569,9467,29982,55697,84417,84959,94032,340061,4772,4773,9402,919,2209,7456,338339	XADR,DOCK2,EDN2,GAP43,GDNF,XCR1,ITGA9,KCNQ3,KIF5C,ABLIM1,NRCAM,PIK3C2G,PTPRO,SFTPD,ST6GAL1,ST8SIA4,KLF7,NUMB,NRXN3,FARP2,PLXNC1,CAP1,CYSLTR1,ABLIM3,PIP5K1C,FEZF2,SEMA6A,SEMA4A,JAML,CXCL17,ANXA2,CCND2,UBE2K,IFNA5,RAP2B,BCAR3,SNX9,FNIP2,ZP4,WDFY2,FAM129A,CCNY,ADAR,CCNG1,GNAQ,PKIA,SH3BP5,NRBF2,VAC14,C2orf40,UBASH3B,CAMK2N2,TMEM173,NFATC1,NFATC2,GRAP2,CD247,FCGR1A,WIPF1,CLEC4D
86	3_Member GO Biological Processes GO:0007169 transmembrane receptor protein tyrosine kinase signaling pathway	-12.45003744 -9.472 145/928	27,113,154,238,284,347,356,374,526,528,537,558,814,817,836,860,925,940,942,1435,1436,1437,1499,1536,1759,1839,1846,1847,1848,1946,1956,2060,2099,2185,2246,2257,2296,2534,2549,2625,2675,2692,2693,3320,3479,3481,3516,3559,3563,3567,3568,3672,3702,3708,3709,3716,3937,4140,4145,4208,4313,4653,4790,4920,5063,5137,5289,5290,5295,5494,5564,5567,5579,5584,5641,5707,5716,5728,5739,5770,5791,5793,5795,5921,5922,6197,6314,6383,6461,6711,6720,6772,6850,7074,7294,7424,7498,7531,8440,8452,8569,8651,8660,8767,8828,8997,9146,9844,9982,10018,10125,10126,10213,10252,10451,11082,11156,11214,23122,23239,23545,25780,25976,26037,26230,26509,26999,30849,50650,50852,51422,51606,54106,54206,54541,55023,79109,84159,103910,114882,115727,121512,152559,153090,253260	ABL2,ADCY7,ADRB2,ALK,ANGPT1,APOD,FASLG,AREG,ATP6V1B2,ATP6V1C1,ATP6AP1,AXL,CAMK4,CAMK2D,CASP3,RUNX2,CD8A,CD28,CD86,CSF1,CSF1R,CSF2,CTNNB1,CYBB,DNM1,HBEGF,DUSP4,DUSP5,DUSP6,EFNA5,EGFR,EPS15,ESR1,PTK2B,FGF1,FGF12,FOXC1,FYN,GAB1,GATA3,GFRA2,GHRHR,GHSR,HSP90AA1,IGF1,IGF2,RBPJ,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITK,ITPR1,ITPR2,JAK1,LCP2,MARK3,MATK,MEF2C,MMP2,MYOC,NFKB1,ROR2,PAK3,PDE1C,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,LGMN,PSMD1,PSMD10,PTEN,PTGIR,PTPN1,PTPRE,PTPRG,PTPRJ,RASA1,RASA2,RPS6KA3,ATXN7,SDC2,SHB,SPTBN1,SREBF1,STAT1,SYK,TIAM1,TXK,VEGFC,XDH,YWHAE,NCK2,CUL3,MKNK1,SOCS1,IRS2,RIPK2,NRP2,KALRN,HGS,ELMO1,FGFBP1,BCL2L11,RASGRP1,DNAL4,PSMD14,SPRY1,VAV3,ESM1,PTP4A3,AKAP13,CLASP2,PHLPP1,ATP6V0A2,RASGRP3,TIPARP,SIPA1L1,TIAM2,MYOF,CYFIP2,PIK3R4,ARHGEF3,TRAT1,PRKAG2,ATP6V1H,TLR9,ERRFI1,DDIT4,PHIP,MAPKAP1,ARID5B,MYL12B,OSBPL8,RASGRP4,FGD4,PAQR3,DAB2IP,RICTOR
87	3_Member GO Biological Processes GO:1902533 positive regulation of intracellular signal transduction	-10.24654239 -7.426 144/983	81,154,238,284,329,330,335,356,444,537,558,596,817,834,841,925,940,960,1230,1236,1435,1436,1437,1499,1647,1687,1839,1847,1848,1880,1896,1906,1956,2074,2099,2146,2185,2244,2246,2534,2549,2625,2697,3458,3479,3481,3559,3563,3567,3568,3592,3596,3600,3672,3688,3716,3814,3965,4057,4140,4142,4214,4653,4853,5063,	ACTN4,ADRB2,ALK,ANGPT1,BIRC2,BIRC3,APOA1,FASLG,ASPH,ATP6AP1,AXL,BCL2,CAMK2D,CASP1,CASP8,CD8A,CD28,CD44,CCR1,CCR7,CSF1,CSF1R,CSF2,CTNNB1,GADD45A,DFNA5,HBEGF,DUSP5,DUSP6,GPR183,EDA,EDN1,EGFR,ERCC6,ESR1,EZH2,PTK2B,FGB,FGF1,FYN,GAB1,GATA3,GJA1,IFNG,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,IL12A,IL13,IL15,ITGA1,ITGB1,JAK1,KISS1,LGALS9,LTF,MARK3,MAS1

WO 2018/175733 PCT/US2018/023785

Appendix

			5326,5494,5579,5707,5716,5728,5734,5739,5770,5795,5921,5922,6346,6504,6711,6754,6846,6850,6885,7074,7097,7476,7498,7852,8440,8452,8600,8651,8660,8743,8767,8995,9064,9218,9290,10018,10125,10213,10333,10507,10550,10783,10855,10920,11184,23118,23369,25780,26191,28996,29949,50852,53832,54106,54602,55223,56911,56940,57162,57708,60675,79156,80762,90441,92140,112464,114882,115727,118788,128239,146850,149233,152559,153090,168667,200734,253260,255743,388121	,MAP3K1,MYOC,NOTCH2,PAK3,PLAGL2,PPM1A,PRKCB,PSMD1,PSMD10,PTEN,PTGER4,PTGIR,PTPN1,PTPRJ,RASA1,RASA2,CCL1,SLAMF1,SPTBN1,SSTR4,XCL2,SYK,MAP3K7,TIAM1,TLR2,WNT7A,XDH,CXCR4,NCK2,CUL3,TNFSF11,SOCS1,IRS2,TNFSF10,RIPK2,TNFSF18,MAP3K6,VAPA,GPR55,BCL2L11,RASGRP1,PSMD14,TLR6,SEMA4D,ARL6IP5,NEK6,HPSE,COPS8,MAP4K1,TAB2,PUM2,RASGRP3,PTPN22,HIPK2,IL19,TRAT1,IL20RA,TLR9,NDFIP2,TRIM62,MAP3K7CL,DUSP22,PELI1,MIER1,PROK2,PLEKHF1,NDFIP1,ZNF622,MTDH,PRKCDBP,OSBPL8,RASGRP4,PIK3AP1,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,BMPER,SPRED2,RICTOR,NPNT,TNFAIP8L3
88	3_Member GO Biological Processes GO:0070848 response to growth factor	-9.39558867 -6.788 136/938	113,284,317,335,356,463,649,753,814,817,836,860,940,942,960,1030,1149,1437,1453,1499,1839,1846,1847,1848,1906,1956,1960,2246,2257,2296,2335,2353,2534,2549,2625,3037,3077,3397,3516,3554,3559,3563,3567,3568,3688,3708,3709,3716,3975,3987,4092,4140,4208,4435,4488,4790,4920,5054,5137,5290,5295,5494,5567,5579,5584,5654,5707,5716,5728,5770,5921,5922,6197,6262,6423,6660,6711,6885,7048,7049,7071,7074,7123,7356,7424,7476,7498,7531,8239,8452,8569,8573,8660,8767,8828,8997,9353,9982,10018,10125,10126,10140,10213,10252,10451,10563,11156,11214,23239,23327,25780,25937,26230,26509,28996,50650,50848,50852,51256,51741,54206,54541,56937,56940,63893,64750,79109,115727,121512,152559,153090,168667,253260,255743,283149,494470	ADCY7,ANGPT1,APAF1,APOA1,FASLG,ZFHX3,BMP1,LDLRAD4,CAMK4,CAMK2D,CASP3,RUNX2,CD28,CD86,CD44,CDKN2B,CIDEA,CSF2,CSNK1D,CTNNB1,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EGFR,EGR3,FGF1,FGF12,FOXC1,FN1,FOS,FYN,GAB1,GATA3,HAS2,HFE,ID1,RBPJ,IL1R1,IL2RA,IL3RA,IL5,IL5RA,ITGB1,ITPR1,ITPR2,JAK1,LHX1,LIMS1,SMAD7,MARK3,MEF2C,CITED1,MSX2,NFKB1,ROR2,SERPINE1,PDE1C,PIK3CA,PIK3R1,PPM1A,PRKACB,PRKCB,PRKCI,HTRA1,PSMD1,PSMD10,PTEN,PTPN1,RASA1,RASA2,RPS6KA3,RYR2,SFRP2,SOX5,SPTBN1,MAP3K7,TGFBR2,TGFBR3,KLF10,TIAM1,CLEC3B,SCGB1A1,VEGFC,WNT7A,XDH,YWHAE,USP9X,CUL3,MKNK1,CASK,IRS2,RIPK2,NRP2,KALRN,SLIT2,FGFBP1,BCL2L11,RASGRP1,DNAL4,TOB1,PSMD14,SPRY1,VAV3,CXCL13,PTP4A3,AKAP13,PHLPP1,NEDD4L,RASGRP3,WWTR1,TIAM2,MYOF,HIPK2,ARHGEF3,F11R,TRAT1,TBC1D7,WWOX,ERRFI1,DDIT4,PMEPA1,DUSP22,UBE2O,SMURF2,MAPKAP1,RASGRP4,FGD4,PAQR3,DAB2IP,BMPER,RICTOR,NPNT,BCL9L,RNF165
89	3_Member GO Biological Processes GO:0032870 cellular response to hormone stimulus	-9.393669113 -6.788 123/822	113,284,301,308,339,440,476,526,528,537,817,1393,1407,1437,1499,1583,1839,1847,1848,1906,1956,2099,2104,2180,2244,2246,2335,2353,2494,2534,2549,2673,2692,2693,2908,3479,3481,3559,3563,3567,3568,3624,3673,3716,3990,4082,4140,4142,	ADCY7,ANGPT1,ANXA1,ANXA5,APOBEC1,ASNS,ATP1A1,ATP6V1B2,ATP6V1C1,ATP6AP1,CAMK2D,CRHBP,CRY1,CSF2,CTNNB1,CYP11A1,HBEGF,DUSP5,DUSP6,EDN1,EGFR,ESR1,ESRRG,ACSL1,FGB,FGF1,FN1,FOS,NR5A2,FYN,GAB1,GFPT1,GHRHR,GHSR,NR3C1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,INHBA,I

Appendix

			4208,4478,4488,4790,5021,5289,5290,5295,5494,5547,5564,5567,5579,5584,5591,5707,5716,5734,5770,5791,5921,5922,5962,6095,6446,6711,6720,6751,6754,6772,7253,7292,7352,7421,7490,7849,8195,8204,8452,8554,8648,8651,8660,8856,9063,9353,9604,9734,10002,10125,10213,10268,11214,23543,23545,25780,27086,30849,51366,51422,51606,54106,54206,54331,55023,55970,56937,64754,84109,90390,114882,115727,152559,153090,255738	TGA2,JAK1,LIPC,MARCKS,MARK3,MAS1,MEF2C,MSN,MSX2,NFKB1,OXTR,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRCP,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTGER4,PTPN1,PTPRE,RASA1,RASA2,RDX,RORA,SGK1,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,TSHR,TNFSF4,UCP3,VDR,WT1,PAX8,MKKS,NRIP1,CUL3,PIAS1,NCOA1,SOCS1,IRS2,NR1I2,PIAS2,SLIT2,RNF14,HDAC9,NR2E3,RASGRP1,PSMD14,RAMP3,AKAP13,RBFOX2,ATP6V0A2,RASGRP3,FOXP1,PIK3R4,UBR5,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,PHIP,GNG12,PMEPA1,SMYD3,QRFPR,MED30,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
90	3_Member GO Biological Processes GO:0010243 response to organonitrogen compound	-9.243229239 -6.673 138/961	113,154,284,301,308,329,333,339,374,440,444,493,526,528,537,540,596,610,682,817,834,836,1134,1316,1393,1407,1437,1499,1583,1808,1811,1839,1847,1848,1906,1956,1959,2185,2246,2353,2534,2549,2673,2692,2693,2697,2796,3033,3269,3397,3479,3481,3559,3563,3567,3568,3596,3663,3673,3709,3716,3990,4048,4082,4140,4142,4208,4258,4313,4435,4790,5021,5032,5142,5144,5289,5290,5295,5321,5494,5564,5567,5579,5584,5591,5707,5716,5728,5734,5770,5791,5921,5922,6262,6383,6506,6609,6711,6720,6751,6754,6772,7071,7074,7352,7490,8061,8452,8651,8660,8767,9173,9353,9734,10125,10213,23545,25780,26191,30849,51141,51422,51606,54106,54206,54331,54434,55023,55328,55970,56729,84109,84152,114882,115727,152559,153090,255738	ADCY7,ADRB2,ANGPT1,ANXA1,ANXA5,BIRC2,APLP1,APOBEC1,AREG,ASNS,ASPH,ATP2B4,ATP6V1B2,ATP6V1C1,ATP6AP1,ATP7B,BCL2,HCN2,BSG,CAMK2D,CASP1,CASP3,CHRNA1,KLF6,CRHBP,CRY1,CSF2,CTNNB1,CYP11A1,DPYSL2,SLC26A3,HBEGF,DUSP5,DUSP6,EDN1,EGFR,EGR2,PTK2B,FGF1,FOS,FYN,GAB1,GFPT1,GHRHR,GHSR,GJA1,GNRH1,HADH,HRH1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,IL13,IRF5,ITGA2,ITPR2,JAK1,LIPC,LTA4H,MARCKS,MARK3,MAS1,MEF2C,MGST2,MMP2,CITED1,NFKB1,OXTR,P2RY11,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PLA2G4A,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTEN,PTGER4,PTPN1,PTPRE,RASA1,RASA2,RYR2,SDC2,SLC1A2,SMPD1,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,KLF10,TIAM1,UCP3,WT1,FOSL1,CUL3,SOCS1,IRS2,RIPK2,IL1RL1,SLIT2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PTPN22,PIK3R4,INSIG2,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,SSH1,PHIP,RNLS,GNG12,RETN,QRFPR,PPP1R1B,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
91	3_Member GO Biological Processes GO:0023014 signal transduction by protein phosphorylation	-8.988894474 -6.450 129/888	154,238,284,335,537,649,817,960,1230,1234,1236,1436,1437,1499,1647,1839,1846,1847,1848,1880,1889,1906,1956,2074,2099,2146,2185,2244,2246,2257,2335,2353,2534,2549,3397,3479,3481,3559,3563,3567,3568,3624,3672,3688,3716,3756,3814,3965,4140,4208,4214,4653,4790,4920,5021,	ADRB2,ALK,ANGPT1,APOA1,ATP6AP1,BMP1,CAMK2D,CD44,CCR1,CCR5,CCR7,CSF1R,CSF2,CTNNB1,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,GPR183,ECE1,EDN1,EGFR,ERCC6,ESR1,EZH2,PTK2B,FGB,FGF1,FGF12,FN1,FOS,FYN,GAB1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,INHBA,ITGA1,ITGB1,JAK1,KCNH1,KISS1,LGALS9,MARK3,MEF2C,MAP3K1,MYOC,N

Appendix

			5063,5707,5716,5728,5734,5770,5795,5921,5922,6197,6346,6423,6504,6609,6653,6711,6754,6846,6850,6885,7048,7074,7301,7476,7498,7852,8428,8452,8600,8660,8767,9064,9146,9290,9448,10125,10213,10221,10252,10333,10550,10771,11184,11221,23118,23239,23291,25780,26191,27347,28951,28996,51765,54106,54206,54986,56911,56940,57091,60675,83605,90441,112464,115727,128239,131096,146850,152559,153090,154043,168667,200734,255743,388121	FKB1,ROR2,OXTR,PAK3,PSMD1,PSMD10,PTEN,PTGER4,PTPN1,PTPRJ,RASA1,RASA2,RPS6KA3,CCL1,SFRP2,SLAMF1,SMPD1,SORL1,SPTBN1,SSTR4,XCL2,SYK,MAP3K7,TGFBR2,TIAM1,TYRO3,WNT7A,XDH,CXCR4,STK24,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,HGS,GPR55,MAP4K4,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,FBXW11,RASGRP3,PTPN22,STK39,TRIB2,HIPK2,STK26,TLR9,ERRFI1,ULK4,MAP3K7CL,DUSP22,CASS4,PROK2,CCM2,ZNF622,PRKCDBP,RASGRP4,IQGAP3,KCNH8,PIK3R6,PAQR3,DAB2IP,CNKSR3,BMPER,SPRED2,NPNT,TNFAIP8L3
92	3_Member GO Biological Processes GO:0071363 cellular response to growth factor stimulus	-8.774215578 -6.264 131/913	113,284,317,335,649,753,814,817,836,860,940,942,960,1030,1149,1437,1453,1499,1839,1846,1847,1848,1906,1956,1960,2246,2257,2296,2335,2353,2534,2549,2625,3037,3077,3397,3516,3559,3563,3567,3568,3688,3708,3709,3716,3975,3987,4092,4140,4208,4435,4488,4790,4920,5054,5137,5290,5295,5494,5567,5579,5584,5654,5707,5716,5728,5770,5921,5922,6197,6262,6423,6660,6711,6885,7048,7049,7071,7074,7123,7424,7476,7498,7531,8239,8452,8569,8573,8660,8767,8828,8997,9353,9982,10018,10125,10126,10140,10213,10252,10451,10563,11156,11214,23239,23327,25780,25937,26230,26509,28996,50650,50848,50852,51741,54206,54541,56937,56940,63893,64750,79109,115727,121512,152559,153090,168667,253260,255743,283149,494470	ADCY7,ANGPT1,APAF1,APOA1,BMP1,LDLRAD4,CAMK4,CAMK2D,CASP3,RUNX2,CD28,CD86,CD44,CDKN2B,CIDEA,CSF2,CSNK1D,CTNNB1,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EGFR,EGR3,FGF1,FGF12,FOXC1,FN1,FOS,FYN,GAB1,GATA3,HAS2,HFE,ID1,RBPJ,IL2RA,IL3RA,IL5,IL5RA,ITGB1,ITPR1,ITPR2,JAK1,LHX1,LIMS1,SMAD7,MARK3,MEF2C,CITED1,MSX2,NFKB1,ROR2,SERPINE1,PDE1C,PIK3CA,PIK3R1,PPM1A,PRKACB,PRKCB,PRKCI,HTRA1,PSMD1,PSMD10,PTEN,PTPN1,RASA1,RASA2,RPS6KA3,RYR2,SFRP2,SOX5,SPTBN1,MAP3K7,TGFBR2,TGFBR3,KLF10,TIAM1,CLEC3B,VEGFC,WNT7A,XDH,YWHAE,USP9X,CUL3,MKNK1,CASK,IRS2,RIPK2,NRP2,KALRN,SLIT2,FGFBP1,BCL2L11,RASGRP1,DNAL4,TOB1,PSMD14,SPRY1,VAV3,CXCL13,PTP4A3,AKAP13,PHLPP1,NEDD4L,RASGRP3,WWTR1,TIAM2,MYOF,HIPK2,ARHGEF3,F11R,TRAT1,WWOX,ERRFI1,DDIT4,PMEPA1,DUSP22,UBE2O,SMURF2,MAPKAP1,RASGRP4,FGD4,PAQR3,DAB2IP,BMPER,RICTOR,NPNT,BCL9L,RNF165
93	3_Member GO Biological Processes GO:1901652 response to peptide	-8.501697735 -6.027 98/629	113,284,301,308,339,374,526,528,537,682,817,1316,1393,1407,1437,1499,1583,1839,1847,1848,1906,1956,1959,2246,2534,2549,2673,2692,2693,2697,2796,3033,3397,3479,3481,3559,3563,3567,3568,3663,3716,3990,4048,4082,4140,4142,4435,4790,5021,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5	ADCY7,ANGPT1,ANXA1,ANXA5,APOBEC1,AREG,ATP6V1B2,ATP6V1C1,ATP6AP1,BSG,CAMK2D,KLF6,CRHBP,CRY1,CSF2,CTNNB1,CYP11A1,HBEGF,DUSP5,DUSP6,EDN1,EGFR,EGR2,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,GJA1,GNRH1,HADH,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,IRF5,JAK1,LIPC,LTA4H,MARCKS,MARK3,MAS1,CITED1,NFKB1,OXTR,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRK

			922,6711,6720,6751,6754,6772,7071,7352,8452,8651,8660,8767,9173,9734,10125,10213,23545,25780,26191,30849,51141,51422,51606,54106,54206,54331,55023,55970,56729,84109,114882,115727,152559,153090,255738	CB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,KLF10,UCP3,CUL3,SOCS1,IRS2,RIPK2,IL1RL1,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PTPN22,PIK3R4,INSIG2,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,PHIP,GNG12,RETN,QRFPR,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
94	3_Member GO Biological Processes GO:0000165 MAPK cascade	-8.244976443 -5.803 123/858	154,238,284,335,537,649,817,960,1230,1234,1236,1436,1437,1499,1647,1839,1846,1847,1848,1880,1889,1906,1956,2074,2099,2146,2185,2244,2246,2257,2335,2353,2534,2549,3397,3479,3481,3559,3563,3567,3568,3624,3672,3688,3716,3814,3965,4140,4208,4214,4653,4790,4920,5021,5063,5707,5716,5728,5734,5770,5795,5921,5922,6197,6346,6423,6504,6609,6653,6711,6754,6846,6850,6885,7074,7476,7498,7852,8428,8452,8600,8660,8767,9064,9146,9290,9448,10125,10213,10221,10252,10333,10550,10771,11184,11221,23118,23239,23291,25780,26191,28951,28996,51765,54106,54206,54986,56911,56940,60675,83605,90441,112464,115727,128239,146850,152559,153090,154043,168667,200734,255743,388121	ADRB2,ALK,ANGPT1,APOA1,ATP6AP1,BMP1,CAMK2D,CD44,CCR1,CCR5,CCR7,CSF1R,CSF2,CTNNB1,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,GPR183,ECE1,EDN1,EGFR,ERCC6,ESR1,EZH2,PTK2B,FGB,FGF1,FGF12,FN1,FOS,FYN,GAB1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,INHBA,ITGA1,ITGB1,JAK1,KISS1,LGALS9,MARK3,MEF2C,MAP3K1,MYOC,NFKB1,ROR2,OXTR,PAK3,PSMD1,PSMD10,PTEN,PTGER4,PTPN1,PTPRJ,RASA1,RASA2,RPS6KA3,CCL1,SFRP2,SLAMF1,SMPD1,SORL1,SPTBN1,SSTR4,XCL2,SYK,MAP3K7,TIAM1,WNT7A,XDH,CXCR4,STK24,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,HGS,GPR55,MAP4K4,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,FBXW11,RASGRP3,PTPN22,TRIB2,HIPK2,STK26,TLR9,ERRFI1,ULK4,MAP3K7CL,DUSP22,PROK2,CCM2,ZNF622,PRKCDBP,RASGRP4,IQGAP3,PIK3R6,PAQR3,DAB2IP,CNKSR3,BMPER,SPRED2,NPNT,TNFAIP8L3
95	3_Member GO Biological Processes GO:0006935 chemotaxis	-8.184503968 -5.758 123/860	27,284,286,287,288,301,323,335,817,1230,1232,1233,1234,1235,1236,1237,1240,1293,1435,1436,1437,1525,1759,1794,1808,1839,1847,1848,1906,1907,1946,1956,1959,1960,2185,2246,2335,2534,2596,2625,2668,2829,3269,3320,3458,3559,3563,3567,3568,3592,3672,3673,3680,3688,3716,3786,3800,3965,3975,3983,4140,4313,4897,5054,5063,5142,5144,5288,5290,5707,5716,5795,5800,5921,5922,6197,6346,6383,6441,6480,6711,6846,6850,7074,7424,7852,7903,8061,8440,8452,8600,8609,8650,8660,8828,8995,8997,9353,9369,9855,10125,10154,10213,10451,10487,10507,10563,10800,22885,23122,23396,25780,55079,57	ABL2,ANGPT1,ANK1,ANK2,ANK3,ANXA1,APBB2,APOA1,CAMK2D,CCR1,CCR3,CCR4,CCR5,CCR6,CCR7,CCR8,CMKLR1,COL6A3,CSF1,CSF1R,CSF2,CXADR,DNM1,DOCK2,DPYSL2,HBEGF,DUSP5,DUSP6,EDN1,EDN2,EFNA5,EGFR,EGR2,EGR3,PTK2B,FGF1,FN1,FYN,GAP43,GATA3,GDNF,XCR1,HRH1,HSP90AA1,IFNG,IL2RA,IL3RA,IL5,IL5RA,IL12A,ITGA1,ITGA2,ITGA9,ITGB1,JAK1,KCNQ3,KIF5C,LGALS9,LHX1,ABLIM1,MARK3,MMP2,NRCAM,SERPINE1,PAK3,PDE4B,PDE4D,PIK3C2G,PIK3CA,PSMD1,PSMD10,PTPRJ,PTPRO,RASA1,RASA2,RPS6KA3,CCL1,SDC2,SFTPD,ST6GAL1,SPTBN1,XCL2,SYK,TIAM1,VEGFC,CXCR4,ST8SIA4,FOSL1,NCK2,CUL3,TNFSF11,KLF7,NUMB,IRS2,NRP2,TNFSF18,KALRN,SLIT2,NRXN3,FARP2,RASGRP1,PLXNC1,PSMD14,VAV3,CAP1,SEM

			556,60675,64218,103910,115727,120425, 152559,153090,284340,494470	A4D,CXCL13,CYSLTR1,ABLIM3,CLASP2,PIP5K1C, RASGRP3,FEZF2,SEMA6A,PROK2,SEMA4A,MYL12 B,RASGRP4,JAML,PAQR3,DAB2IP,CXCL17,RNF165
96	3_Member GO Biological Processes GO:0042330 taxis	-8.154395379 -5.735 123/861	27,284,286,287,288,301,323,335,817,1230 ,1232,1233,1234,1235,1236,1237,1240,12 93,1435,1436,1437,1525,1759,1794,1808, 1839,1847,1848,1906,1907,1946,1956,195 9,1960,2185,2246,2335,2534,2596,2625,2 668,2829,3269,3320,3458,3559,3563,3567 ,3568,3592,3672,3673,3680,3688,3716,37 86,3800,3965,3975,3983,4140,4313,4897, 5054,5063,5142,5144,5288,5290,5707,571 6,5795,5800,5921,5922,6197,6346,6383,6 441,6480,6711,6846,6850,7074,7424,7852 ,7903,8061,8440,8452,8600,8609,8650,86 60,8828,8995,8997,9353,9369,9855,10125 ,10154,10213,10451,10487,10507,10563,1 0800,22885,23122,23396,25780,55079,57 556,60675,64218,103910,115727,120425, 152559,153090,284340,494470	ABL2,ANGPT1,ANK1,ANK2,ANK3,ANXA1,APBB2, APOA1,CAMK2D,CCR1,CCR3,CCR4,CCR5,CCR6,CC R7,CCR8,CMKLR1,COL6A3,CSF1,CSF1R,CSF2,CXA DR,DNM1,DOCK2,DPYSL2,HBEGF,DUSP5,DUSP6,E DN1,EDN2,EFNA5,EGFR,EGR2,EGR3,PTK2B,FGF1,F N1,FYN,GAP43,GATA3,GDNF,XCR1,HRH1,HSP90A A1,IFNG,IL2RA,IL3RA,IL5,IL5RA,IL12A,ITGA1,ITG A2,ITGA9,ITGB1,JAK1,KCNQ3,KIF5C,LGALS9,LHX 1,ABLIM1,MARK3,MMP2,NRCAM,SERPINE1,PAK3, PDE4B,PDE4D,PIK3C2G,PIK3CA,PSMD1,PSMD10,P TPRJ,PTPRO,RASA1,RASA2,RPS6KA3,CCL1,SDC2,S FTPD,ST6GAL1,SPTBN1,XCL2,SYK,TIAM1,VEGFC, CXCR4,ST8SIA4,FOSL1,NCK2,CUL3,TNFSF11,KLF7, NUMB,IRS2,NRP2,TNFSF18,KALRN,SLIT2,NRXN3,F ARP2,RASGRP1,PLXNC1,PSMD14,VAV3,CAP1,SEM A4D,CXCL13,CYSLTR1,ABLIM3,CLASP2,PIP5K1C, RASGRP3,FEZF2,SEMA6A,PROK2,SEMA4A,MYL12 B,RASGRP4,JAML,PAQR3,DAB2IP,CXCL17,RNF165
97	3_Member GO Biological Processes GO:0001934 positive regulation of protein phosphorylation	-7.385129046 -5.041 135/999	113,154,238,284,302,493,537,596,649,817 ,894,960,1230,1236,1435,1436,1437,1453, 1499,1647,1839,1847,1848,1880,1906,194 6,1956,2074,2099,2146,2180,2185,2244,2 246,2534,2549,2675,3077,3093,3442,3458 ,3479,3481,3559,3563,3567,3568,3592,35 96,3600,3624,3672,3688,3716,3814,3937, 3965,4057,4082,4140,4142,4214,5063,529 0,5567,5707,5716,5728,5734,5770,5912,5 921,5922,6346,6423,6504,6711,6754,6846 ,6850,6885,7048,7074,7424,7476,7498,78 52,8412,8428,8452,8600,8651,8660,8767, 8995,9064,9290,10125,10213,10333,1050 7,10550,10920,11184,23118,25780,26191, 27347,28996,51422,51429,51765,54106,5 6911,56940,57600,57829,60675,64754,90 441,112464,114882,115727,115825,11649 6,128239,146850,149233,152559,153090, 168667,219771,253260,255743,388121	ADCY7,ADRB2,ALK,ANGPT1,ANXA2,ATP2B4,ATP 6AP1,BCL2,BMP1,CAMK2D,CCND2,CD44,CCR1,CC R7,CSF1,CSF1R,CSF2,CSNK1D,CTNNB1,GADD45A, HBEGF,DUSP5,DUSP6,GPR183,EDN1,EFNA5,EGFR, ERCC6,ESR1,EZH2,ACSL1,PTK2B,FGB,FGF1,FYN,G AB1,GFRA2,HFE,UBE2K,IFNA5,IFNG,IGF1,IGF2,IL2 RA,IL3RA,IL5,IL5RA,IL12A,IL13,IL15,INHBA,ITGA1 ,ITGB1,JAK1,KISS1,LCP2,LGALS9,LTF,MARCKS,M ARK3,MAS1,MAP3K1,PAK3,PIK3CA,PRKACB,PSM D1,PSMD10,PTEN,PTGER4,PTPN1,RAP2B,RASA1,R ASA2,CCL1,SFRP2,SLAMF1,SPTBN1,SSTR4,XCL2,S YK,MAP3K7,TGFBR2,TIAM1,VEGFC,WNT7A,XDH, CXCR4,BCAR3,STK24,CUL3,TNFSF11,SOCS1,IRS2, RIPK2,TNFSF18,MAP3K6,GPR55,RASGRP1,PSMD14 ,TLR6,SEMA4D,ARL6IP5,COPS8,MAP4K1,TAB2,RA SGRP3,PTPN22,STK39,HIPK2,PRKAG2,SNX9,STK26, TLR9,MAP3K7CL,DUSP22,FNIP2,ZP4,PROK2,SMYD 3,ZNF622,PRKCDBP,OSBPL8,RASGRP4,WDFY2,FA M129A,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,BMP

WO 2018/175733 PCT/US2018/023785

Appendix

				ER,CCNY,RICTOR,NPNT,TNFAIP8L3
98	3_Member GO Biological Processes GO:1901653 cellular response to peptide	-7.350286479 -5.019 81/514	113,284,308,339,526,528,537,817,1316,1393,1437,1499,1583,1839,1847,1848,1906,1956,2246,2534,2549,2673,2692,2693,3397,3479,3481,3559,3563,3567,3568,3716,4082,4140,4142,4790,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5922,6711,6720,6751,6754,6772,7071,8452,8651,8660,8767,9173,9734,10125,10213,23545,25780,26191,30849,51422,51606,54106,54206,54331,55023,55970,114882,115727,152559,153090,255738	ADCY7,ANGPT1,ANXA5,APOBEC1,ATP6V1B2,ATP6V1C1,ATP6AP1,CAMK2D,KLF6,CRHBP,CSF2,CTNNB1,CYP11A1,HBEGF,DUSP5,DUSP6,EDN1,EGFR,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARCKS,MARK3,MAS1,NFKB1,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,KLF10,CUL3,SOCS1,IRS2,RIPK2,IL1RL1,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PTPN22,PIK3R4,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,PHIP,GNG12,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
99	3_Member GO Biological Processes GO:0043434 response to peptide hormone	-7.276231886 -4.951 90/594	113,284,301,308,339,374,526,528,537,682,817,1393,1407,1437,1499,1583,1839,1847,1848,1906,1956,1959,2246,2534,2549,2673,2692,2693,2697,2796,3033,3479,3481,3559,3563,3567,3568,3716,3990,4048,4082,4140,4142,4435,4790,5021,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5922,6711,6720,6751,6754,6772,7352,8452,8651,8660,9734,10125,10213,23545,25780,30849,51141,51422,51606,54106,54206,54331,55023,55970,56729,114882,115727,152559,153090,255738	ADCY7,ANGPT1,ANXA1,ANXA5,APOBEC1,AREG,ATP6V1B2,ATP6V1C1,ATP6AP1,BSG,CAMK2D,CRHBP,CRY1,CSF2,CTNNB1,CYP11A1,HBEGF,DUSP5,DUSP6,EDN1,EGFR,EGR2,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,GJA1,GNRH1,HADH,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,LIPC,LTA4H,MARCKS,MARK3,MAS1,CITED1,NFKB1,OXTR,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,UCP3,CUL3,SOCS1,IRS2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PIK3R4,INSIG2,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,PHIP,GNG12,RETN,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
100	3_Member GO Biological Processes GO:0043408 regulation of MAPK cascade	-7.144146217 -4.848 111/786	154,238,284,537,649,817,960,1230,1236,1436,1437,1499,1647,1839,1846,1847,1848,1880,1889,1906,1956,2074,2099,2146,2185,2244,2246,2335,2534,2549,3397,3479,3481,3559,3563,3567,3568,3624,3672,3688,3716,3814,3965,4140,4214,4653,5063,5707,5716,5728,5770,5795,5921,5922,6346,6423,6504,6609,6653,6711,6754,6846,6850,6885,7074,7476,7498,7852,8428,8452,8600,8660,8767,9064,9146,9290,9448,10125,10213,10221,10252,10333,10550,10771,11184,11221,23118,23239,25780,26191,	ADRB2,ALK,ANGPT1,ATP6AP1,BMP1,CAMK2D,CD44,CCR1,CCR7,CSF1R,CSF2,CTNNB1,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,GPR183,ECE1,EDN1,EGFR,ERCC6,ESR1,EZH2,PTK2B,FGB,FGF1,FN1,FYN,GAB1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,INHBA,ITGA1,ITGB1,JAK1,KISS1,LGALS9,MARK3,MAP3K1,MYOC,PAK3,PSMD1,PSMD10,PTEN,PTPN1,PTPRJ,RASA1,RASA2,CCL1,SFRP2,SLAMF1,SMPD1,SORL1,SPTBN1,SSTR4,XCL2,SYK,MAP3K7,TIAM1,WNT7A,XDH,CXCR4,STK24,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,HGS,GPR55,MAP4K4,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DU

Appendix

			28951,28996,51765,54106,54206,54986,56911,56940,60675,90441,112464,115727,128239,146850,152559,153090,154043,168667,200734,255743,388121	SP10,TAB2,PHLPP1,RASGRP3,PTPN22,TRIB2,HIPK2,STK26,TLR9,ERRFI1,ULK4,MAP3K7CL,DUSP22,PROK2,ZNF622,PRKCDBP,RASGRP4,IQGAP3,PIK3R6,PAQR3,DAB2IP,CNKSR3,BMPER,SPRED2,NPNT,TNFAIP8L3
101	3_Member GO Biological Processes GO:0071417 cellular response to organonitrogen compound	-6.761409284 -4.533 96/663	113,284,308,333,339,493,526,528,537,610,817,1316,1393,1437,1499,1583,1811,1839,1847,1848,1906,1956,2246,2534,2549,2673,2692,2693,3269,3397,3479,3481,3559,3563,3567,3568,3709,3716,4082,4140,4142,4208,4313,4790,5032,5142,5144,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5922,6262,6711,6720,6751,6754,6772,7071,7490,8452,8651,8660,8767,9173,9353,9734,10125,10213,23545,25780,26191,30849,51422,51606,54106,54206,54331,54434,55023,55970,114882,115727,152559,153090,255738	ADCY7,ANGPT1,ANXA5,APLP1,APOBEC1,ATP2B4,ATP6V1B2,ATP6V1C1,ATP6AP1,HCN2,CAMK2D,KLF6,CRHBP,CSF2,CTNNB1,CYP11A1,SLC26A3,HBEGF,DUSP5,DUSP6,EDN1,EGFR,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,HRH1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITPR2,JAK1,MARCKS,MARK3,MAS1,MEF2C,MMP2,NFKB1,P2RY11,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,RYR2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,KLF10,WT1,CUL3,SOCS1,IRS2,RIPK2,IL1RL1,SLIT2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PTPN22,PIK3R4,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,SSH1,PHIP,GNG12,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
102	3_Member GO Biological Processes GO:0043549 regulation of kinase activity	-6.199216891 -4.081 121/914	103,113,154,238,284,493,817,836,894,900,1030,1236,1435,1436,1437,1647,1839,1846,1847,1848,1906,1946,1956,2074,2146,2180,2185,2246,2534,2549,2776,3481,3559,3563,3567,3568,3672,3716,3937,4057,4082,4140,4142,4214,5063,5290,5295,5564,5567,5569,5707,5716,5728,5770,5795,5912,5921,5922,6423,6609,6653,6711,6850,6885,7048,7074,7852,8428,8440,8452,8600,8651,8660,8767,9064,9146,9467,10125,10213,10221,10252,10333,10451,10920,11184,11214,11221,23118,25780,25937,26191,27347,28951,29982,30849,51422,51429,51765,54106,54206,55697,56911,56940,57829,60675,64754,84152,84417,84959,90441,94032,114882,115727,128239,146850,149233,152559,153090,200734,219771,388121	ADAR,ADCY7,ADRB2,ALK,ANGPT1,ATP2B4,CAMK2D,CASP3,CCND2,CCNG1,CDKN2B,CCR7,CSF1,CSF1R,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EFNA5,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,FYN,GAB1,GNAQ,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,LCP2,LTF,MARCKS,MARK3,MAS1,MAP3K1,PAK3,PIK3CA,PIK3R1,PRKAB1,PRKACB,PKIA,PSMD1,PSMD10,PTEN,PTPN1,PTPRJ,RAP2B,RASA1,RASA2,SFRP2,SMPD1,SORL1,SPTBN1,SYK,MAP3K7,TGFBR2,TIAM1,CXCR4,STK24,NCK2,CUL3,TNFSF11,SOCS1,IRS2,RIPK2,MAP3K6,HGS,SH3BP5,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,VAV3,COPS8,MAP4K1,AKAP13,DUSP10,TAB2,RASGRP3,WWTR1,PTPN22,STK39,TRIB2,NRBF2,PIK3R4,PRKAG2,SNX9,STK26,TLR9,ERRFI1,VAC14,MAP3K7CL,DUSP22,ZP4,PROK2,SMYD3,PPP1R1B,C2orf40,UBASH3B,ZNF622,CAMK2N2,OSBPL8,RASGRP4,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,SPRED2,CCNY,TNFAIP8L3
103	3_Member GO Biological Processes	-6.181387673 -4.067	103,113,284,308,333,339,493,526,528,537,610,817,1316,1393,1437,1499,1583,1811,	ADAR,ADCY7,ANGPT1,ANXA5,APLP1,APOBEC1,ATP2B4,ATP6V1B2,ATP6V1C1,ATP6AP1,HCN2,CAM

Appendix

	GO:1901699 cellular response to nitrogen compound	100/719	1839,1847,1848,1906,1956,2099,2246,2534,2549,2673,2692,2693,3269,3397,3479,3481,3559,3563,3567,3568,3709,3716,4082,4140,4142,4208,4313,4790,5032,5142,5144,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5922,6262,6711,6720,6751,6754,6772,7071,7292,7490,8452,8651,8660,8767,9173,9353,9734,10125,10213,23545,25780,26191,30849,51422,51606,54106,54206,54331,54434,55023,55970,114882,115727,152559,153090,255738,340061	K2D,KLF6,CRHBP,CSF2,CTNNB1,CYP11A1,SLC26A3,HBEGF,DUSP5,DUSP6,EDN1,EGFR,ESR1,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,HRH1,ID1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITPR2,JAK1,MARCKS,MARK3,MAS1,MEF2C,MMP2,NFKB1,P2RY11,PDE4B,PDE4D,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,RYR2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,KLF10,TNFSF4,WT1,CUL3,SOCS1,IRS2,RIPK2,IL1RL1,SLIT2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PTPN22,PIK3R4,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,SSH1,PHIP,GNG12,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9,TMEM173
104	3_Member GO Biological Processes GO:0071375 cellular response to peptide hormone stimulus	-6.065639093 -3.987 75/498	113,284,308,339,526,528,537,817,1393,1437,1499,1583,1839,1847,1848,1906,1956,2246,2534,2549,2673,2692,2693,3479,3481,3559,3563,3567,3568,3716,4082,4140,4142,4790,5289,5290,5295,5494,5564,5567,5579,5584,5591,5707,5716,5770,5791,5921,5922,6711,6720,6751,6754,6772,8452,8651,8660,9734,10125,10213,23545,25780,30849,51422,51606,54106,54206,54331,55023,55970,114882,115727,152559,153090,255738	ADCY7,ANGPT1,ANXA5,APOBEC1,ATP6V1B2,ATP6V1C1,ATP6AP1,CAMK2D,CRHBP,CSF2,CTNNB1,CYP11A1,HBEGF,DUSP5,DUSP6,EDN1,EGFR,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARCKS,MARK3,MAS1,NFKB1,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKACB,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,SSTR1,SSTR4,STAT1,CUL3,SOCS1,IRS2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PIK3R4,PRKAG2,ATP6V1H,TLR9,ERRFI1,GNG2,PHIP,GNG12,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
105	3_Member GO Biological Processes GO:0043410 positive regulation of MAPK cascade	-6.026385014 -3.954 86/597	154,238,284,537,817,960,1230,1236,1436,1437,1499,1647,1839,1847,1848,1880,1906,1956,2074,2099,2146,2185,2244,2246,2534,2549,3479,3481,3559,3563,3567,3568,3672,3688,3716,3814,3965,4140,4214,5063,5707,5716,5728,5770,5921,5922,6346,6504,6711,6754,6846,6850,6885,7074,7476,7498,7852,8452,8600,8660,8767,9064,9290,10125,10213,10333,10550,11184,23118,25780,26191,28996,54106,56911,56940,60675,90441,112464,115727,128239,146850,152559,153090,168667,255743,388121	ADRB2,ALK,ANGPT1,ATP6AP1,CAMK2D,CD44,CCR1,CCR7,CSF1R,CSF2,CTNNB1,GADD45A,HBEGF,DUSP5,DUSP6,GPR183,EDN1,EGFR,ERCC6,ESR1,EZH2,PTK2B,FGB,FGF1,FYN,GAB1,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITGB1,JAK1,KISS1,LGALS9,MARK3,MAP3K1,PAK3,PSMD1,PSMD10,PTEN,PTPN1,RASA1,RASA2,CCL1,SLAMF1,SPTBN1,SSTR4,XCL2,SYK,MAP3K7,TIAM1,WNT7A,XDH,CXCR4,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,GPR55,RASGRP1,PSMD14,TLR6,ARL6IP5,MAP4K1,TAB2,RASGRP3,PTPN22,HIPK2,TLR9,MAP3K7CL,DUSP22,PROK2,ZNF622,PRKCDBP,RASGRP4,IQGAP3,PIK3R6,PAQR3,DAB2IP,BMPER,NPNT,TNFAIP8L3
106	3_Member GO Biological Processes	-5.893467503 -3.838	284,817,940,942,1437,1839,1847,1848,1956,2246,2353,2534,2549,3559,3563,3567,	ANGPT1,CAMK2D,CD28,CD86,CSF2,HBEGF,DUSP5,DUSP6,EGFR,FGF1,FOS,FYN,GAB1,IL2RA,IL3RA,IL

Appendix

	GO:0038095 Fc-epsilon receptor signaling pathway	53/315	3568,3702,3708,3709,3716,3937,4140,4214,4772,4773,4790,5290,5295,5707,5716,5728,5921,5922,6711,6850,6885,8452,8660,9402,10125,10213,10451,23118,23239,23291,25780,50852,79109,115727,152559,153090,253260	5,IL5RA,ITK,ITPR1,ITPR2,JAK1,LCP2,MARK3,MAP3K1,NFATC1,NFATC2,NFKB1,PIK3CA,PIK3R1,PSMD1,PSMD10,PTEN,RASA1,RASA2,SPTBN1,SYK,MAP3K7,CUL3,IRS2,GRAP2,RASGRP1,PSMD14,VAV3,TAB2,PHLPP1,FBXW11,RASGRP3,TRAT1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
107	3_Member GO Biological Processes GO:0071774 response to fibroblast growth factor	-5.805251721 -3.775 56/342	113,284,814,817,860,940,942,960,1437,1499,1839,1847,1848,1956,1960,2246,2257,2534,2549,2625,3559,3563,3567,3568,3708,3709,3716,3975,4140,5137,5290,5295,5567,5707,5716,5728,5921,5922,6711,7356,8452,8569,8660,9982,10125,10213,10252,10563,23239,25780,50852,79109,115727,152559,153090,253260	ADCY7,ANGPT1,CAMK4,CAMK2D,RUNX2,CD28,CD86,CD44,CSF2,CTNNB1,HBEGF,DUSP5,DUSP6,EGFR,EGR3,FGF1,FGF12,FYN,GAB1,GATA3,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,LHX1,MARK3,PDE1C,PIK3CA,PIK3R1,PRKACB,PSMD1,PSMD10,PTEN,RASA1,RASA2,SPTBN1,SCGB1A1,CUL3,MKNK1,IRS2,FGFBP1,RASGRP1,PSMD14,SPRY1,CXCL13,PHLPP1,RASGRP3,TRAT1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
108	3_Member GO Biological Processes GO:0048011 neurotrophin TRK receptor signaling pathway	-5.77785808 -3.751 63/402	113,284,814,817,836,940,942,1437,1839,1846,1847,1848,1956,2246,2534,2549,3559,3563,3567,3568,3708,3709,3716,4140,4208,4790,5137,5290,5295,5567,5584,5707,5716,5728,5921,5922,6197,6711,7074,7531,8452,8660,8767,8997,10018,10125,10126,10213,10252,10451,11214,23239,25780,26230,50650,50852,54541,79109,115727,121512,152559,153090,253260	ADCY7,ANGPT1,CAMK4,CAMK2D,CASP3,CD28,CD86,CSF2,HBEGF,DUSP4,DUSP5,DUSP6,EGFR,FGF1,FYN,GAB1,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,MARK3,MEF2C,NFKB1,PDE1C,PIK3CA,PIK3R1,PRKACB,PRKCI,PSMD1,PSMD10,PTEN,RASA1,RASA2,RPS6KA3,SPTBN1,TIAM1,YWHAE,CUL3,IRS2,RIPK2,KALRN,BCL2L11,RASGRP1,DNAL4,PSMD14,SPRY1,VAV3,AKAP13,PHLPP1,RASGRP3,TIAM2,ARHGEF3,TRAT1,DDIT4,MAPKAP1,RASGRP4,FGD4,PAQR3,DAB2IP,RICTOR
109	3_Member GO Biological Processes GO:0038093 Fc receptor signaling pathway	-5.754873984 -3.734 60/377	284,817,919,940,942,1437,1839,1847,1848,1956,2209,2246,2353,2534,2549,3320,3559,3563,3567,3568,3702,3708,3709,3716,3937,4140,4214,4772,4773,4790,5290,5295,5707,5716,5728,5921,5922,6711,6850,6885,7456,8452,8660,9402,9844,10125,10213,10451,23118,23239,23291,25780,26999,50852,79109,115727,152559,153090,253260,338339	ANGPT1,CAMK2D,CD247,CD28,CD86,CSF2,HBEGF,DUSP5,DUSP6,EGFR,FCGR1A,FGF1,FOS,FYN,GAB1,HSP90AA1,IL2RA,IL3RA,IL5,IL5RA,ITK,ITPR1,ITPR2,JAK1,LCP2,MARK3,MAP3K1,NFATC1,NFATC2,NFKB1,PIK3CA,PIK3R1,PSMD1,PSMD10,PTEN,RASA1,RASA2,SPTBN1,SYK,MAP3K7,WIPF1,CUL3,IRS2,GRAP2,ELMO1,RASGRP1,PSMD14,VAV3,TAB2,PHLPP1,FBXW11,RASGRP3,CYFIP2,TRAT1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR,CLEC4D
110	3_Member GO Biological Processes GO:0045859 regulation of protein kinase activity	-5.728615884 -3.713 114/867	103,113,154,238,284,493,817,836,894,900,1030,1236,1435,1436,1437,1647,1839,1846,1847,1848,1906,1946,1956,2074,2146,2180,2185,2246,2534,2549,2776,3481,3559,3563,3567,3568,3672,3716,3937,4057,4082,4140,4142,4214,5063,5290,5564,5567	ADAR,ADCY7,ADRB2,ALK,ANGPT1,ATP2B4,CAMK2D,CASP3,CCND2,CCNG1,CDKN2B,CCR7,CSF1,CSF1R,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EFNA5,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,FYN,GAB1,GNAQ,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,LCP2,LTF,MARCKS,MARK3,MAS1,MA

Appendix

			,5569,5707,5716,5728,5770,5795,5912,5921,5922,6423,6609,6653,6711,6850,6885,7048,7074,7852,8428,8440,8452,8600,8651,8660,8767,9064,9146,9467,10125,10213,10221,10252,10333,10920,11184,11214,11221,23118,25780,25937,26191,27347,28951,51422,51429,51765,54106,54206,56911,56940,57829,60675,64754,84152,84417,84959,94032,114882,115727,128239,146850,149233,152559,153090,200734,219771	P3K1,PAK3,PIK3CA,PRKAB1,PRKACB,PKIA,PSMD1,PSMD10,PTEN,PTPN1,PTPRJ,RAP2B,RASA1,RASA2,SFRP2,SMPD1,SORL1,SPTBN1,SYK,MAP3K7,TGFBR2,TIAM1,CXCR4,STK24,NCK2,CUL3,TNFSF11,SOCS1,IRS2,RIPK2,MAP3K6,HGS,SH3BP5,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,COPS8,MAP4K1,AKAP13,DUSP10,TAB2,RASGRP3,WWTR1,PTPN22,STK39,TRIB2,PRKAG2,SNX9,STK26,TLR9,ERRFI1,MAP3K7CL,DUSP22,ZP4,PROK2,SMYD3,PPP1R1B,C2orf40,UBASH3B,CAMK2N2,OSBPL8,RASGRP4,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,SPRED2,CCNY
111	3_Member GO Biological Processes GO:0044344 cellular response to fibroblast growth factor stimulus	-5.70910404 -3.697 55/336	113,284,814,817,860,940,942,960,1437,1499,1839,1847,1848,1956,1960,2246,2257,2534,2549,2625,3559,3563,3567,3568,3708,3709,3716,3975,4140,5137,5290,5295,5567,5707,5716,5728,5921,5922,6711,8452,8569,8660,9982,10125,10213,10252,10563,23239,25780,50852,79109,115727,152559,153090,253260	ADCY7,ANGPT1,CAMK4,CAMK2D,RUNX2,CD28,CD86,CD44,CSF2,CTNNB1,HBEGF,DUSP5,DUSP6,EGFR,EGR3,FGF1,FGF12,FYN,GAB1,GATA3,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,LHX1,MARK3,PDE1C,PIK3CA,PIK3R1,PRKACB,PSMD1,PSMD10,PTEN,RASA1,RASA2,SPTBN1,CUL3,MKNK1,IRS2,FGFBP1,RASGRP1,PSMD14,SPRY1,CXCL13,PHLPP1,RASGRP3,TRAT1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
112	3_Member GO Biological Processes GO:0038179 neurotrophin signaling pathway	-5.667543262 -3.661 63/405	113,284,814,817,836,940,942,1437,1839,1846,1847,1848,1956,2246,2534,2549,3559,3563,3567,3568,3708,3709,3716,4140,4208,4790,5137,5290,5295,5567,5584,5707,5716,5728,5921,5922,6197,6711,7074,7531,8452,8660,8767,8997,10018,10125,10126,10213,10252,10451,11214,23239,25780,26230,50650,50852,54541,79109,115727,121512,152559,153090,253260	ADCY7,ANGPT1,CAMK4,CAMK2D,CASP3,CD28,CD86,CSF2,HBEGF,DUSP4,DUSP5,DUSP6,EGFR,FGF1,FYN,GAB1,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,MARK3,MEF2C,NFKB1,PDE1C,PIK3CA,PIK3R1,PRKACB,PRKCI,PSMD1,PSMD10,PTEN,RASA1,RASA2,RPS6KA3,SPTBN1,TIAM1,YWHAE,CUL3,IRS2,RIPK2,KALRN,BCL2L11,RASGRP1,DNAL4,PSMD14,SPRY1,VAV3,AKAP13,PHLPP1,RASGRP3,TIAM2,ARHGEF3,TRAT1,DDIT4,MAPKAP1,RASGRP4,FGD4,PAQR3,DAB2IP,RICTOR
113	3_Member GO Biological Processes GO:0048010 vascular endothelial growth factor receptor signaling pathway	-5.648344545 -3.645 50/296	284,558,817,1437,1536,1839,1847,1848,1956,2185,2246,2296,2534,3320,3559,3563,3567,3568,3708,3709,3716,4140,5063,5290,5295,5579,5707,5716,5770,5921,5922,6461,6711,7424,8440,8452,8660,8828,9844,10125,10213,10451,25780,26509,26999,79109,115727,152559,153090,253260	ANGPT1,AXL,CAMK2D,CSF2,CYBB,HBEGF,DUSP5,DUSP6,EGFR,PTK2B,FGF1,FOXC1,FYN,HSP90AA1,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,MARK3,PAK3,PIK3CA,PIK3R1,PRKCB,PSMD1,PSMD10,PTPN1,RASA1,RASA2,SHB,SPTBN1,VEGFC,NCK2,CUL3,IRS2,NRP2,ELMO1,RASGRP1,PSMD14,VAV3,RASGRP3,MYOF,CYFIP2,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
114	3_Member GO Biological Processes	-5.611458231 -3.619	284,339,526,528,537,817,1407,1437,1839,1847,1848,1956,1959,2246,2534,2549,267	ANGPT1,APOBEC1,ATP6V1B2,ATP6V1C1,ATP6AP1,CAMK2D,CRY1,CSF2,HBEGF,DUSP5,DUSP6,EGFR,

	GO:0032868 response to insulin	68/450	3,2692,2693,3033,3479,3481,3559,3563,3567,3568,3716,4082,4140,4435,5289,5290,5295,5494,5564,5579,5584,5591,5707,5716,5770,5791,5921,5922,6711,6720,7352,8452,8651,8660,9734,10125,10213,23545,25780,30849,51141,51422,51606,54106,54206,55023,56729,114882,115727,152559,153090,255738	EGR2,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,HADH,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARCKS,MARK3,CITED1,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,UCP3,CUL3,SOCS1,IRS2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PIK3R4,INSIG2,PRKAG2,ATP6V1H,TLR9,ERRFI1,PHIP,RETN,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
115	3_Member GO Biological Processes GO:0038127 ERBB signaling pathway	-5.338020633 -3.381 58/371	113,284,356,374,814,817,940,942,1437,1839,1847,1848,1956,2060,2099,2185,2246,2534,2549,3516,3559,3563,3567,3568,3672,3708,3709,3716,4140,4653,5137,5290,5295,5567,5641,5707,5716,5728,5795,5921,5922,6711,8440,8452,8660,9146,10125,10213,10252,23239,25780,50852,54206,79109,115727,152559,153090,253260	ADCY7,ANGPT1,FASLG,AREG,CAMK4,CAMK2D,CD28,CD86,CSF2,HBEGF,DUSP5,DUSP6,EGFR,EPS15,ESR1,PTK2B,FGF1,FYN,GAB1,RBPJ,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITPR1,ITPR2,JAK1,MARK3,MYOC,PDE1C,PIK3CA,PIK3R1,PRKACB,LGMN,PSMD1,PSMD10,PTEN,PTPRJ,RASA1,RASA2,SPTBN1,NCK2,CUL3,IRS2,HGS,RASGRP1,PSMD14,SPRY1,PHLPP1,RASGRP3,TRAT1,ERRFI1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
116	3_Member GO Biological Processes GO:0008543 fibroblast growth factor receptor signaling pathway	-5.243794665 -3.294 51/314	113,284,814,817,860,940,942,1437,1499,1839,1847,1848,1956,2246,2257,2534,2549,2625,3559,3563,3567,3568,3708,3709,3716,4140,5137,5290,5295,5567,5707,5716,5728,5921,5922,6711,8452,8569,8660,9982,10125,10213,10252,23239,25780,50852,79109,115727,152559,153090,253260	ADCY7,ANGPT1,CAMK4,CAMK2D,RUNX2,CD28,CD86,CSF2,CTNNB1,HBEGF,DUSP5,DUSP6,EGFR,FGF1,FGF12,FYN,GAB1,GATA3,IL2RA,IL3RA,IL5,IL5RA,ITPR1,ITPR2,JAK1,MARK3,PDE1C,PIK3CA,PIK3R1,PRKACB,PSMD1,PSMD10,PTEN,RASA1,RASA2,SPTBN1,CUL3,MKNK1,IRS2,FGFBP1,RASGRP1,PSMD14,SPRY1,PHLPP1,RASGRP3,TRAT1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
117	3_Member GO Biological Processes GO:0071900 regulation of protein serine/threonine kinase activity	-5.23276718 -3.286 84/606	238,284,493,817,836,894,900,1030,1436,1437,1647,1839,1846,1847,1848,1906,1956,2074,2146,2180,2185,2246,2534,2549,3481,3559,3563,3567,3568,3672,3716,4057,4082,4140,4142,4214,5063,5569,5707,5716,5728,5770,5795,5921,5922,6423,6609,6653,6711,6850,6885,7074,7852,8452,8600,8660,8767,9064,9146,10125,10213,10221,10252,10333,10920,11184,11221,23118,25780,26191,28951,51422,54106,56911,56940,60675,84417,115727,128239,146850,152559,153090,200734,219771	ALK,ANGPT1,ATP2B4,CAMK2D,CASP3,CCND2,CCNG1,CDKN2B,CSF1R,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,FYN,GAB1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,LTF,MARCKS,MARK3,MAS1,MAP3K1,PAK3,PKIA,PSMD1,PSMD10,PTEN,PTPN1,PTPRJ,RASA1,RASA2,SFRP2,SMPD1,SORL1,SPTBN1,SYK,MAP3K7,TIAM1,CXCR4,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,HGS,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,COPS8,MAP4K1,DUSP10,TAB2,RASGRP3,PTPN22,TRIB2,PRKAG2,TLR9,MAP3K7CL,DUSP22,PROK2,C2orf40,RASGRP4,IQGAP3,PIK3R6,PAQR3,DAB2IP,SPRED2,CCNY
118	3_Member	-5.227915078	284,339,526,528,537,817,1437,1839,1847,	ANGPT1,APOBEC1,ATP6V1B2,ATP6V1C1,ATP6AP1

	GO Biological Processes GO:0032869 cellular response to insulin stimulus	-3.283 61/400	1848,1956,2246,2534,2549,2673,2692,2693,3479,3481,3559,3563,3567,3568,3716,4082,4140,5289,5290,5295,5494,5564,5579,5584,5591,5707,5716,5770,5791,5921,5922,6711,6720,8452,8651,8660,9734,10125,10213,23545,25780,30849,51422,51606,54106,54206,55023,114882,115727,152559,153090,255738	,CAMK2D,CSF2,HBEGF,DUSP5,DUSP6,EGFR,FGF1,FYN,GAB1,GFPT1,GHRHR,GHSR,IGF1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARCKS,MARK3,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKCB,PRKCI,PRKDC,PSMD1,PSMD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,CUL3,SOCS1,IRS2,HDAC9,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PIK3R4,PRKAG2,ATP6V1H,TLR9,ERRFI1,PHIP,OSBPL8,RASGRP4,PAQR3,DAB2IP,PCSK9
119	3_Member GO Biological Processes GO:0033674 positive regulation of kinase activity	-4.92251968 -3.028 86/636	113,154,238,284,493,817,894,1236,1435,1436,1437,1647,1839,1847,1848,1906,1946,1956,2074,2146,2180,2185,2246,2534,2549,3481,3559,3563,3567,3568,3672,3716,3937,4057,4082,4140,4142,4214,5063,5290,5567,5707,5716,5770,5921,5922,6711,6850,6885,7048,7074,7852,8428,8452,8600,8651,8660,8767,9064,10125,10213,10333,10451,10920,11184,23118,25780,27347,30849,51422,51429,51765,54106,56911,57829,60675,90441,114882,115727,128239,146850,149233,152559,153090,219771,388121	ADCY7,ADRB2,ALK,ANGPT1,ATP2B4,CAMK2D,CCND2,CCR7,CSF1,CSF1R,CSF2,GADD45A,HBEGF,DUSP5,DUSP6,EDN1,EFNA5,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,FYN,GAB1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,LCP2,LTF,MARCKS,MARK3,MAS1,MAP3K1,PAK3,PIK3CA,PRKACB,PSMD1,PSMD10,PTPN1,RASA1,RASA2,SPTBN1,SYK,MAP3K7,TGFBR2,TIAM1,CXCR4,STK24,CUL3,TNFSF11,SOCS1,IRS2,RIPK2,MAP3K6,RASGRP1,PSMD14,TLR6,VAV3,COPS8,MAP4K1,TAB2,RASGRP3,STK39,PIK3R4,PRKAG2,SNX9,STK26,TLR9,MAP3K7CL,ZP4,PROK2,ZNF622,OSBPL8,RASGRP4,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,CCNY,TNFAIP8L3
120	3_Member GO Biological Processes GO:0043405 regulation of MAP kinase activity	-4.869043802 -2.988 67/464	238,284,817,1437,1647,1839,1846,1847,1848,1906,1956,2074,2146,2185,2246,2534,2549,3559,3563,3567,3568,3672,3716,4140,4214,5063,5707,5716,5770,5795,5921,5922,6423,6609,6653,6711,6850,6885,7074,7852,8452,8600,8660,8767,9064,9146,10125,10213,10221,10252,10333,11184,11221,23118,25780,26191,28951,54106,56911,56940,60675,115727,128239,146850,152559,153090,200734	ALK,ANGPT1,CAMK2D,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,DUSP6,EDN1,EGFR,ERCC6,EZH2,PTK2B,FGF1,FYN,GAB1,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,MARK3,MAP3K1,PAK3,PSMD1,PSMD10,PTPN1,PTPRJ,RASA1,RASA2,SFRP2,SMPD1,SORL1,SPTBN1,SYK,MAP3K7,TIAM1,CXCR4,CUL3,TNFSF11,IRS2,RIPK2,MAP3K6,HGS,RASGRP1,PSMD14,TRIB1,SPRY1,TLR6,MAP4K1,DUSP10,TAB2,RASGRP3,PTPN22,TRIB2,TLR9,MAP3K7CL,DUSP22,PROK2,RASGRP4,IQGAP3,PIK3R6,PAQR3,DAB2IP,SPRED2
121	3_Member GO Biological Processes GO:0007411 axon guidance	-4.737636492 -2.890 79/578	27,284,286,287,288,323,817,1293,1436,1437,1759,1808,1839,1847,1848,1946,1956,1959,2246,2534,2596,2625,2668,3320,3559,3563,3567,3568,3672,3673,3680,3688,3716,3786,3800,3975,3983,4140,4313,4897,5063,5707,5716,5800,5921,5922,6197,6383,6711,7074,7903,8440,8452,8609,8650,8660,8828,8997,9353,9369,9855,10125,10	ABL2,ANGPT1,ANK1,ANK2,ANK3,APBB2,CAMK2D,COL6A3,CSF1R,CSF2,DNM1,DPYSL2,HBEGF,DUSP5,DUSP6,EFNA5,EGFR,EGR2,FGF1,FYN,GAP43,GATA3,GDNF,HSP90AA1,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITGA2,ITGA9,ITGB1,JAK1,KCNQ3,KIF5C,LHX1,ABLIM1,MARK3,MMP2,NRCAM,PAK3,PSMD1,PSMD10,PTPRO,RASA1,RASA2,RPS6KA3,SDC2,SPTBN1,TIAM1,ST8SIA4,NCK2,CUL3,KLF7,NUMB,IRS2,NRP2

Appendix

			154,10213,10451,10487,10507,22885,23122,23396,25780,55079,57556,64218,103910,115727,152559,153090,494470	,KALRN,SLIT2,NRXN3,FARP2,RASGRP1,PLXNC1,PSMD14,VAV3,CAP1,SEMA4D,ABLIM3,CLASP2,PIP5K1C,RASGRP3,FEZF2,SEMA6A,SEMA4A,MYL12B,RASGRP4,PAQR3,DAB2IP,RNF165
122	3_Member GO Biological Processes GO:0097485 neuron projection guidance	-4.737636492 -2.890 79/578	27,284,286,287,288,323,817,1293,1436,1437,1759,1808,1839,1847,1848,1946,1956,1959,2246,2534,2596,2625,2668,3320,3559,3563,3567,3568,3672,3673,3680,3688,3716,3786,3800,3975,3983,4140,4313,4897,5063,5707,5716,5800,5921,5922,6197,6383,6711,7074,7903,8440,8452,8609,8650,8660,8828,8997,9353,9369,9855,10125,10154,10213,10451,10487,10507,22885,23122,23396,25780,55079,57556,64218,103910,115727,152559,153090,494470	ABL2,ANGPT1,ANK1,ANK2,ANK3,APBB2,CAMK2D,COL6A3,CSF1R,CSF2,DNM1,DPYSL2,HBEGF,DUSP5,DUSP6,EFNA5,EGFR,EGR2,FGF1,FYN,GAP43,GATA3,GDNF,HSP90AA1,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITGA2,ITGA9,ITGB1,JAK1,KCNQ3,KIF5C,LHX1,ABLIM1,MARK3,MMP2,NRCAM,PAK3,PSMD1,PSMD10,PTPRO,RASA1,RASA2,RPS6KA3,SDC2,SPTBN1,TIAM1,ST8SIA4,NCK2,CUL3,KLF7,NUMB,IRS2,NRP2,KALRN,SLIT2,NRXN3,FARP2,RASGRP1,PLXNC1,PSMD14,VAV3,CAP1,SEMA4D,ABLIM3,CLASP2,PIP5K1C,RASGRP3,FEZF2,SEMA6A,SEMA4A,MYL12B,RASGRP4,PAQR3,DAB2IP,RNF165
123	3_Member GO Biological Processes GO:0007173 epidermal growth factor receptor signaling pathway	-4.662140781 -2.830 55/364	113,284,356,374,814,817,940,942,1437,1839,1847,1848,1956,2060,2099,2185,2246,2534,2549,3559,3563,3567,3568,3672,3708,3709,3716,4140,5137,5290,5295,5567,5707,5716,5728,5795,5921,5922,6711,8440,8452,8660,9146,10125,10213,10252,23239,25780,50852,54206,79109,115727,152559,153090,253260	ADCY7,ANGPT1,FASLG,AREG,CAMK4,CAMK2D,CD28,CD86,CSF2,HBEGF,DUSP5,DUSP6,EGFR,EPS15,ESR1,PTK2B,FGF1,FYN,GAB1,IL2RA,IL3RA,IL5,IL5RA,ITGA1,ITPR1,ITPR2,JAK1,MARK3,PDE1C,PIK3CA,PIK3R1,PRKACB,PSMD1,PSMD10,PTEN,PTPRJ,RASA1,RASA2,SPTBN1,NCK2,CUL3,IRS2,HGS,RASGRP1,PSMD14,SPRY1,PHLPP1,RASGRP3,TRAT1,ERRFI1,MAPKAP1,RASGRP4,PAQR3,DAB2IP,RICTOR
124	3_Member GO Biological Processes GO:0045860 positive regulation of protein kinase activity	-4.629446492 -2.801 82/610	113,154,238,284,493,817,894,1236,1435,1436,1437,1647,1839,1847,1848,1906,1946,1956,2074,2146,2180,2185,2246,2534,2549,3481,3559,3563,3567,3568,3672,3716,3937,4057,4082,4140,4142,4214,5063,5290,5567,5707,5716,5770,5921,5922,6711,6850,6885,7048,7074,7852,8428,8452,8600,8651,8660,8767,9064,10125,10213,10333,10920,11184,23118,25780,27347,51422,51429,51765,54106,56911,57829,60675,114882,115727,128239,146850,149233,152559,153090,219771	ADCY7,ADRB2,ALK,ANGPT1,ATP2B4,CAMK2D,CCND2,CCR7,CSF1,CSF1R,CSF2,GADD45A,HBEGF,DUSP5,DUSP6,EDN1,EFNA5,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,FYN,GAB1,IGF2,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,LCP2,LTF,MARCKS,MARK3,MAS1,MAP3K1,PAK3,PIK3CA,PRKACB,PSMD1,PSMD10,PTPN1,RASA1,RASA2,SPTBN1,SYK,MAP3K7,TGFBR2,TIAM1,CXCR4,STK24,CUL3,TNFSF11,SOCS1,IRS2,RIPK2,MAP3K6,RASGRP1,PSMD14,TLR6,COPS8,MAP4K1,TAB2,RASGRP3,STK39,PRKAG2,SNX9,STK26,TLR9,MAP3K7CL,ZP4,PROK2,OSBPL8,RASGRP4,IQGAP3,PIK3R6,IL23R,PAQR3,DAB2IP,CCNY
125	3_Member GO Biological Processes GO:0008286 insulin receptor signaling	-4.192236617 -2.427 50/334	284,526,528,537,817,1437,1839,1847,1848,1956,2246,2534,2549,3481,3559,3563,3567,3568,3716,4140,5289,5290,5295,5494,5564,5579,5707,5716,5770,5791,5921,59	ANGPT1,ATP6V1B2,ATP6V1C1,ATP6AP1,CAMK2D,CSF2,HBEGF,DUSP5,DUSP6,EGFR,FGF1,FYN,GAB1,IGF2,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARK3,PIK3C3,PIK3CA,PIK3R1,PPM1A,PRKAB1,PRKCB,PSMD1,PS

Appendix

	pathway		22,6711,6720,8452,8651,8660,10125,10213,23545,25780,30849,51422,51606,54106,55023,114882,115727,152559,153090	MD10,PTPN1,PTPRE,RASA1,RASA2,SPTBN1,SREBF1,CUL3,SOCS1,IRS2,RASGRP1,PSMD14,ATP6V0A2,RASGRP3,PIK3R4,PRKAG2,ATP6V1H,TLR9,PHIP,OSBPL8,RASGRP4,PAQR3,DAB2IP
126	3_Member GO Biological Processes GO:0032147 activation of protein kinase activity	-4.129069404 -2.379 64/462	113,154,238,284,817,1437,1647,1839,1847,1848,1946,1956,2074,2185,2246,2534,2549,3559,3563,3567,3568,3672,3716,4140,4142,4214,5063,5290,5567,5707,5716,5770,5921,5922,6711,6850,6885,7048,7852,8428,8452,8600,8651,8660,8767,9064,10125,10213,10333,10920,11184,23118,25780,27347,51422,51765,56911,60675,114882,115727,128239,149233,152559,153090	ADCY7,ADRB2,ALK,ANGPT1,CAMK2D,CSF2,GADD45A,HBEGF,DUSP5,DUSP6,EFNA5,EGFR,ERCC6,PTK2B,FGF1,FYN,GAB1,IL2RA,IL3RA,IL5,IL5RA,ITGA1,JAK1,MARK3,MAS1,MAP3K1,PAK3,PIK3CA,PRKACB,PSMD1,PSMD10,PTPN1,RASA1,RASA2,SPTBN1,SYK,MAP3K7,TGFBR2,CXCR4,STK24,CUL3,TNFSF11,SOCS1,IRS2,RIPK2,MAP3K6,RASGRP1,PSMD14,TLR6,COPS8,MAP4K1,TAB2,RASGRP3,STK39,PRKAG2,STK26,MAP3K7CL,PROK2,OSBPL8,RASGRP4,IQGAP3,IL23R,PAQR3,DAB2IP
127	3_Member GO Biological Processes GO:0000186 activation of MAPKK activity	-2.547057501 -1.167 33/234	284,817,1437,1839,1847,1848,1956,2074,2246,2534,3559,3563,3567,3568,3716,4140,4214,5707,5716,5921,5922,6711,6885,8452,8660,9064,10125,10213,25780,56911,115727,152559,153090	ANGPT1,CAMK2D,CSF2,HBEGF,DUSP5,DUSP6,EGFR,ERCC6,FGF1,FYN,IL2RA,IL3RA,IL5,IL5RA,JAK1,MARK3,MAP3K1,PSMD1,PSMD10,RASA1,RASA2,SPTBN1,MAP3K7,CUL3,IRS2,MAP3K6,RASGRP1,PSMD14,RASGRP3,MAP3K7CL,RASGRP4,PAQR3,DAB2IP
128	4_Summary GO Biological Processes GO:0022603 regulation of anatomical structure morphogenesis	-9.489585207 -6.812 137/944	81,154,301,335,356,596,604,753,832,860,861,989,1232,1435,1436,1499,1540,1690,1808,1848,1906,1946,2078,2099,2113,2146,2185,2244,2246,2296,2335,2534,2625,2668,2768,2803,3037,3074,3397,3458,3516,3557,3716,3975,3987,4082,4092,4208,4289,4300,4435,4478,4653,4773,4897,4920,5054,5063,5579,5728,5734,5740,5789,5800,5921,5962,6239,6383,6423,6446,6480,6672,6772,6860,7048,7074,7421,7424,7456,7476,7490,7498,7849,8650,8994,9313,9353,9734,10154,10252,10395,10507,10563,10672,10801,10810,23002,23122,23136,23216,23327,23499,25937,26037,27086,28996,30812,51256,54434,54708,55023,55079,55223,55843,56990,57194,57533,57556,60675,64218,64411,79734,85407,85464,90627,91663,92140,103910,121512,128272,146850,153090,157922,161176,201164,283149,343472,27,322,463,537,558,639,6	ACTN4,ADRB2,ANXA1,APOA1,FASLG,BCL2,BCL6,LDLRAD4,CAPZB,RUNX2,RUNX1,SEPT7,CCR3,CSF1,CSF1R,CTNNB1,CYLD,COCH,DPYSL2,DUSP6,EDN1,EFNA5,ERG,ESR1,ETS1,EZH2,PTK2B,FGB,FGF1,FOXC1,FN1,FYN,GATA3,GDNF,GNA12,GOLGA4,HAS2,HEXB,ID1,IFNG,RBPJ,IL1RN,JAK1,LHX1,LIMS1,MARCKS,SMAD7,MEF2C,MKLN1,MLLT3,CITED1,MSN,MYOC,NFATC2,NRCAM,ROR2,SERPINE1,PAK3,PRKCB,PTEN,PTGER4,PTGIS,PTPRD,PTPRO,RASA1,RDX,RREB1,SDC2,SFRP2,SGK1,ST6GAL1,SP100,STAT1,SYT4,TGFBR2,TIAM1,VDR,VEGFC,WIPF1,WNT7A,WT1,XDH,PAX8,NUMB,LIMD1,MMP20,SLIT2,HDAC9,PLXNC1,SPRY1,DLC1,SEMA4D,CXCL13,GNA13,SEPT9,WASF3,DAAM1,CLASP2,EPB41L3,TBC1D1,NEDD4L,MACF1,WWTR1,SIPA1L1,FOXP1,HIPK2,SOX8,TBC1D7,SSH1,MARCH5,PHIP,FEZF2,TRIM62,ARHGAP15,CDC42SE2,ATP10A,TBC1D14,SEMA6A,PROK2,SEMA4A,ARAP3,KCTD17,NKD1,SSH2,STARD13,MYADM,MTDH,MYL12B,FGD4,ARHGEF19,PIK3R6,DAB2IP,CAMSAP1,SYNE3,PLD6,BCL9L,

Appendix

			88,841,942,1230,1240,1437,1960,2353,26 97,3479,3559,3567,3575,3592,3596,3600, 3624,3635,3642,3688,3965,4000,4057,448 8,4602,4674,4790,5584,6197,6660,6850,6 925,7071,7292,7520,7704,7852,8111,8320 ,8600,8626,8648,8767,9308,10125,10221, 10362,55714,55904,79625,85477,114548, 115825,149233,255743,285590	BARHL2,ABL2,APBB1,ZFHX3,ATP6AP1,AXL,PRDM 1,KLF5,CASP8,CD86,CCR1,CMKLR1,CSF2,EGR3,FO S,GJA1,IGF1,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHB A,INPP5D,INSM1,ITGB1,LGALS9,LMNA,LTF,MSX2, MYB,NAP1L2,NFKB1,PRKCI,RPS6KA3,SOX5,SYK,T CF4,KLF10,TNFSF4,XRCC5,ZBTB16,CXCR4,GPR68, EOMES,TNFSF11,TP63,NCOA1,RIPK2,CD83,RASGR P1,TRIB1,HMG20B,TENM3,KMT2E,NDNF,SCIN,NL RP3,WDFY2,IL23R,NPNT,SH3PXD2B
129	4_Member GO Biological Processes GO:0022603 regulation of anatomical structure morphogenesis	-9.489585207 -6.812 137/944	81,154,301,335,356,596,604,753,832,860, 861,989,1232,1435,1436,1499,1540,1690, 1808,1848,1906,1946,2078,2099,2113,214 6,2185,2244,2246,2296,2335,2534,2625,2 668,2768,2803,3037,3074,3397,3458,3516 ,3557,3716,3975,3987,4082,4092,4208,42 89,4300,4435,4478,4653,4773,4897,4920, 5054,5063,5579,5728,5734,5740,5789,580 0,5921,5962,6239,6383,6423,6446,6480,6 672,6772,6860,7048,7074,7421,7424,7456 ,7476,7490,7498,7849,8650,8994,9313,93 53,9734,10154,10252,10395,10507,10563, 10672,10801,10810,23002,23122,23136,2 3216,23327,23499,25937,26037,27086,28 996,30812,51256,54434,54708,55023,550 79,55223,55843,56990,57194,57533,5755 6,60675,64218,64411,79734,85407,85464, 90627,91663,92140,103910,121512,12827 2,146850,153090,157922,161176,201164, 283149,343472	ACTN4,ADRB2,ANXA1,APOA1,FASLG,BCL2,BCL6, LDLRAD4,CAPZB,RUNX2,RUNX1,SEPT7,CCR3,CSF 1,CSF1R,CTNNB1,CYLD,COCH,DPYSL2,DUSP6,ED N1,EFNA5,ERG,ESR1,ETS1,EZH2,PTK2B,FGB,FGF1, FOXC1,FN1,FYN,GATA3,GDNF,GNA12,GOLGA4,H AS2,HEXB,ID1,IFNG,RBPJ,IL1RN,JAK1,LHX1,LIMS 1,MARCKS,SMAD7,MEF2C,MKLN1,MLLT3,CITED1, MSN,MYOC,NFATC2,NRCAM,ROR2,SERPINE1,PA K3,PRKCB,PTEN,PTGER4,PTGIS,PTPRD,PTPRO,RA SA1,RDX,RREB1,SDC2,SFRP2,SGK1,ST6GAL1,SP10 0,STAT1,SYT4,TGFBR2,TIAM1,VDR,VEGFC,WIPF1, WNT7A,WT1,XDH,PAX8,NUMB,LIMD1,MMP20,SLI T2,HDAC9,PLXNC1,SPRY1,DLC1,SEMA4D,CXCL13, GNA13,SEPT9,WASF3,DAAM1,CLASP2,EPB41L3,TB C1D1,NEDD4L,MACF1,WWTR1,SIPA1L1,FOXP1,HI PK2,SOX8,TBC1D7,SSH1,MARCH5,PHIP,FEZF2,TRI M62,ARHGAP15,CDC42SE2,ATP10A,TBC1D14,SEM A6A,PROK2,SEMA4A,ARAP3,KCTD17,NKD1,SSH2, STARD13,MYADM,MTDH,MYL12B,FGD4,ARHGEF 19,PIK3R6,DAB2IP,CAMSAP1,SYNE3,PLD6,BCL9L, BARHL2
130	4_Member GO Biological Processes GO:0022604 regulation of cell morphogenesis	-8.098516286 -5.694 86/537	81,301,335,753,832,989,1436,1499,1540,1 690,1808,1946,2146,2185,2244,2335,2534 ,2668,2768,2803,3037,3074,3397,3987,40 82,4092,4289,4435,4478,4653,4897,5063, 5728,5789,5800,5921,5962,6239,6383,642 3,6446,6480,6772,6860,7048,7074,7456,7 476,7849,8994,9353,10154,10395,10507,1 0672,10801,10810,23122,23136,23216,23 327,23499,25937,26037,51256,54434,550 23,55079,55223,55843,56990,57194,5753	ACTN4,ANXA1,APOA1,LDLRAD4,CAPZB,SEPT7,CS F1R,CTNNB1,CYLD,COCH,DPYSL2,EFNA5,EZH2,P TK2B,FGB,FN1,FYN,GDNF,GNA12,GOLGA4,HAS2, HEXB,ID1,LIMS1,MARCKS,SMAD7,MKLN1,CITED1 ,MSN,MYOC,NRCAM,PAK3,PTEN,PTPRD,PTPRO,R ASA1,RDX,RREB1,SDC2,SFRP2,SGK1,ST6GAL1,ST AT1,SYT4,TGFBR2,TIAM1,WIPF1,WNT7A,PAX8,LI MD1,SLIT2,PLXNC1,DLC1,SEMA4D,GNA13,SEPT9, WASF3,CLASP2,EPB41L3,TBC1D1,NEDD4L,MACF1 ,WWTR1,SIPA1L1,TBC1D7,SSH1,PHIP,FEZF2,TRIM6

Appendix

			3,57556,64218,64411,79734,85464,91663,103910,121512,153090,157922,161176,283149,343472	2,ARHGAP15,CDC42SE2,ATP10A,TBC1D14,SEMA6A,SEMA4A,ARAP3,KCTD17,SSH2,MYADM,MYL12B,FGD4,DAB2IP,CAMSAP1,SYNE3,BCL9L,BARHL2
131	4_Member GO Biological Processes GO:0045597 positive regulation of cell differentiation	-7.934382345 -5.537 118/823	27,301,322,335,463,537,558,596,604,639,688,841,860,861,942,1230,1240,1435,1436,1437,1499,1906,1946,1960,2113,2146,2185,2244,2296,2335,2353,2534,2625,2668,2697,2803,3037,3458,3479,3516,3559,3567,3575,3592,3596,3600,3624,3635,3642,3688,3965,3975,3987,4000,4057,4082,4208,4488,4602,4653,4674,4773,4790,4897,5063,5584,5734,5789,6197,6239,6423,6446,6660,6850,6860,6925,7048,7071,7074,7292,7421,7424,7520,7704,7849,7852,8111,8320,8600,8626,8648,8650,8767,9308,9353,10125,10221,10362,10507,23327,23499,25937,30812,55079,55714,55904,57556,79625,85477,91663,114548,115825,146850,149233,153090,255743,283149,285590	ABL2,ANXA1,APBB1,APOA1,ZFHX3,ATP6AP1,AXL,BCL2,BCL6,PRDM1,KLF5,CASP8,RUNX2,RUNX1,CD86,CCR1,CMKLR1,CSF1,CSF1R,CSF2,CTNNB1,EDN1,EFNA5,EGR3,ETS1,EZH2,PTK2B,FGB,FOXC1,FN1,FOS,FYN,GATA3,GDNF,GJA1,GOLGA4,HAS2,IFNG,IGF1,RBPJ,IL2RA,IL5,IL7R,IL12A,IL13,IL15,INHBA,INPP5D,INSM1,ITGB1,LGALS9,LHX1,LIMS1,LMNA,LTF,MARCKS,MEF2C,MSX2,MYB,MYOC,NAP1L2,NFATC2,NFKB1,NRCAM,PAK3,PRKCI,PTGER4,PTPRD,RPS6KA3,RREB1,SFRP2,SGK1,SOX5,SYK,SYT4,TCF4,TGFBR2,KLF10,TIAM1,TNFSF4,VDR,VEGFC,XRCC5,ZBTB16,PAX8,CXCR4,GPR68,EOMES,TNFSF11,TP63,NCOA1,NUMB,RIPK2,CD83,SLIT2,RASGRP1,TRIB1,HMG20B,SEMA4D,NEDD4L,MACF1,WWTR1,SOX8,FEZF2,TENM3,KMT2E,SEMA6A,NDNF,SCIN,MYADM,NLRP3,WDFY2,PIK3R6,IL23R,DAB2IP,NPNT,BCL9L,SH3PXD2B
132	4_Member GO Biological Processes GO:0010769 regulation of cell morphogenesis involved in differentiation	-4.092337149 -2.354 50/337	81,335,753,1499,1808,1946,2146,2244,2335,2668,2803,3037,3397,3987,4082,4092,4435,4653,4897,5063,5728,5789,5800,6239,6383,6423,6446,6480,6772,7048,7074,7476,7849,9353,10154,10507,23122,23499,25937,26037,54434,55079,55223,57556,64218,85464,91663,153090,283149,343472	ACTN4,APOA1,LDLRAD4,CTNNB1,DPYSL2,EFNA5,EZH2,FGB,FN1,GDNF,GOLGA4,HAS2,ID1,LIMS1,MARCKS,SMAD7,CITED1,MYOC,NRCAM,PAK3,PTEN,PTPRD,PTPRO,RREB1,SDC2,SFRP2,SGK1,ST6GAL1,STAT1,TGFBR2,TIAM1,WNT7A,PAX8,SLIT2,PLXNC1,SEMA4D,CLASP2,MACF1,WWTR1,SIPA1L1,SSH1,FEZF2,TRIM62,SEMA6A,SEMA4A,SSH2,MYADM,DAB2IP,BCL9L,BARHL2
133	4_Member GO Biological Processes GO:0010770 positive regulation of cell morphogenesis involved in differentiation	-2.577652355 -1.188 25/162	335,1499,1946,2146,2244,2335,2668,2803,3037,3987,4082,4653,5063,5789,6239,6446,7048,7074,7849,9353,10507,23499,25937,91663,283149	APOA1,CTNNB1,EFNA5,EZH2,FGB,FN1,GDNF,GOLGA4,HAS2,LIMS1,MARCKS,MYOC,PAK3,PTPRD,RREB1,SGK1,TGFBR2,TIAM1,PAX8,SLIT2,SEMA4D,MACF1,WWTR1,MYADM,BCL9L
134	5_Summary Hallmark Gene Sets M5890 HALLMARK TNFA SIGNALING VIA	-9.445831962 -6.788 46/200	329,330,374,597,604,960,1316,1435,1437,1647,1839,1846,1847,1880,1906,1959,1960,2353,3575,3624,4082,4783,4790,5054,5055,5142,5734,5791,6446,6648,7071,7097,8061,8553,8660,8767,8848,8870,9308,10	BIRC2,BIRC3,AREG,BCL2A1,BCL6,CD44,KLF6,CSF1,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,GPR183,EDN1,EGR2,EGR3,FOS,IL7R,INHBA,MARCKS,NFIL3,NFKB1,SERPINE1,SERPINB2,PDE4B,PTGER4,PTPRE,SGK1,SOD2,KLF10,TLR2,FOSL1,BHLHE40,IRS2,R

Appendix

	NFKB		010,10221,23258,25816,25976,51278,56937,558,1236,1240,1536,3037,3269,3554,3600,3759,3937,4693,5739,5996,6504,6506,8671,8743,8807,9435,10125,10507,60675,114548,166929	IPK2,TSC22D1,IER3,CD83,TANK,TRIB1,DENND5A,TNFAIP8,TIPARP,IER5,PMEPA1,AXL,CCR7,CMKLR1,CYBB,HAS2,HRH1,IL1R1,IL15,KCNJ2,LCP2,NDP,PTGIR,RGS1,SLAMF1,SLC1A2,SLC4A4,TNFSF10,IL18RAP,CHST2,RASGRP1,SEMA4D,PROK2,NLRP3,SGMS2
135	5_Member Hallmark Gene Sets M5890 HALLMARK TNFA SIGNALING VIA NFKB	-9.445831962 -6.788 46/200	329,330,374,597,604,960,1316,1435,1437,1647,1839,1846,1847,1880,1906,1959,1960,2353,3575,3624,4082,4783,4790,5054,5055,5142,5734,5791,6446,6648,7071,7097,8061,8553,8660,8767,8848,8870,9308,10010,10221,23258,25816,25976,51278,56937	BIRC2,BIRC3,AREG,BCL2A1,BCL6,CD44,KLF6,CSF1,CSF2,GADD45A,HBEGF,DUSP4,DUSP5,GPR183,EDN1,EGR2,EGR3,FOS,IL7R,INHBA,MARCKS,NFIL3,NFKB1,SERPINE1,SERPINB2,PDE4B,PTGER4,PTPRE,SGK1,SOD2,KLF10,TLR2,FOSL1,BHLHE40,IRS2,RIPK2,TSC22D1,IER3,CD83,TANK,TRIB1,DENND5A,TNFAIP8,TIPARP,IER5,PMEPA1
136	5_Member Hallmark Gene Sets M5932 HALLMARK INFLAMMATORY RESPONSE	-5.672152239 -3.663 38/200	558,1236,1240,1316,1435,1536,1839,1880,1906,3037,3269,3554,3575,3600,3624,3759,3937,4693,4790,5054,5142,5734,5739,5791,5996,6504,6506,7097,8671,8743,8767,8807,9435,10125,10507,60675,114548,166929	AXL,CCR7,CMKLR1,KLF6,CSF1,CYBB,HBEGF,GPR183,EDN1,HAS2,HRH1,IL1R1,IL7R,IL15,INHBA,KCNJ2,LCP2,NDP,NFKB1,SERPINE1,PDE4B,PTGER4,PTGIR,PTPRE,RGS1,SLAMF1,SLC1A2,TLR2,SLC4A4,TNFSF10,RIPK2,IL18RAP,CHST2,RASGRP1,SEMA4D,PROK2,NLRP3,SGMS2
137	6_Summary GO Biological Processes GO:0051345 positive regulation of hydrolase activity	-8.709537121 -6.208 123/843	27,113,154,317,335,356,444,483,814,834,836,841,1102,1122,1236,1378,1794,1956,2099,2146,2185,2335,2760,2776,2864,3268,3458,3672,3673,3688,3702,3708,3709,3965,3987,4082,4208,4301,5137,5567,5739,5770,5921,5922,5996,6003,6262,6346,6423,6846,7074,7294,7498,8412,8440,8743,8787,8997,9267,9290,9447,9628,9754,9844,9855,9910,10018,10049,10125,10144,10213,10395,10451,10507,10550,10563,10672,11033,11214,23048,23096,23101,23161,23179,23216,23258,23348,25780,26037,26230,26577,26999,50650,50848,51256,51429,51735,54206,54431,55122,55619,55655,55843,57181,57186,57514,57533,57584,64411,64744,80005,85397,90627,112399,114548,115727,121512,128239,128272,153020,153090,255743,345930,399,604,1946,5962,8195,9353,10252,253260	ABL2,ADCY7,ADRB2,APAF1,APOA1,FASLG,ASPH,ATP1B3,CAMK4,CASP1,CASP3,CASP8,RCBTB2,CHML,CCR7,CR1,DOCK2,EGFR,ESR1,EZH2,PTK2B,FN1,GM2A,GNAQ,FFAR1,AGFG2,IFNG,ITGA1,ITGA2,ITGB1,ITK,ITPR1,ITPR2,LGALS9,LIMS1,MARCKS,MEF2C,MLLT4,PDE1C,PRKACB,PTGIR,PTPN1,RASA1,RASA2,RGS1,RGS13,RYR2,CCL1,SFRP2,XCL2,TIAM1,TXK,XDH,BCAR3,NCK2,TNFSF10,RGS9,KALRN,CYTH1,GPR55,AIM2,RGS6,STARD8,ELMO1,FARP2,RABGAP1L,BCL2L11,DNAJB6,RASGRP1,FAM13A,PSMD14,DLC1,VAV3,SEMA4D,ARL6IP5,CXCL13,GNA13,ADAP1,AKAP13,FNBP1,IQSEC2,MCF2L2,SNX13,RGL1,TBC1D1,DENND5A,DOCK9,RASGRP3,SIPA1L1,TIAM2,PCOLCE2,CYFIP2,ARHGEF3,F11R,TBC1D7,SNX9,RAPGEF6,ERRFI1,DNAJC10,AKIRIN2,DOCK10,NLRP2,ARHGAP15,SLC39A10,RALGAPA2,ARHGAP31,TBC1D14,ARHGAP21,ARAP3,SMAP2,DOCK5,RGS8,STARD13,EGLN3,NLRP3,RASGRP4,FGD4,IQGAP3,ARHGEF19,RASGEF1B,DAB2IP,NPNT,ECT2L,RHOH,BCL6,EFNA5,RDX,MKKS,SLIT2,SPRY1,RICTOR

Appendix

138	6_Member GO Biological Processes GO:0051345 positive regulation of hydrolase activity	-8.709537121 -6.208 123/843	27,113,154,317,335,356,444,483,814,834,836,841,1102,1122,1236,1378,1794,1956,2099,2146,2185,2335,2760,2776,2864,3268,3458,3672,3673,3688,3702,3708,3709,3965,3987,4082,4208,4301,5137,5567,5739,5770,5921,5922,5996,6003,6262,6346,6423,6846,7074,7294,7498,8412,8440,8743,8787,8997,9267,9290,9447,9628,9754,9844,9855,9910,10018,10049,10125,10144,10213,10395,10451,10507,10550,10563,10672,11033,11214,23048,23096,23101,23161,23179,23216,23258,23348,25780,26037,26230,26577,26999,50650,50848,51256,51429,51735,54206,54431,55122,55619,55655,55843,57181,57186,57514,57533,57584,64411,64744,80005,85397,90627,112399,114548,115727,121512,128239,128272,153020,153090,255743,345930	ABL2,ADCY7,ADRB2,APAF1,APOA1,FASLG,ASPH,ATP1B3,CAMK4,CASP1,CASP3,CASP8,RCBTB2,CHML,CCR7,CR1,DOCK2,EGFR,ESR1,EZH2,PTK2B,FN1,GM2A,GNAQ,FFAR1,AGFG2,IFNG,ITGA1,ITGA2,ITGB1,ITK,ITPR1,ITPR2,LGALS9,LIMS1,MARCKS,MEF2C,MLLT4,PDE1C,PRKACB,PTGIR,PTPN1,RASA1,RASA2,RGS1,RGS13,RYR2,CCL1,SFRP2,XCL2,TIAM1,TXK,XDH,BCAR3,NCK2,TNFSF10,RGS9,KALRN,CYTH1,GPR55,AIM2,RGS6,STARD8,ELMO1,FARP2,RABGAP1L,BCL2L11,DNAJB6,RASGRP1,FAM13A,PSMD14,DLC1,VAV3,SEMA4D,ARL6IP5,CXCL13,GNA13,ADAP1,AKAP13,FNBP1,IQSEC2,MCF2L2,SNX13,RGL1,TBC1D1,DENND5A,DOCK9,RASGRP3,SIPA1L1,TIAM2,PCOLCE2,CYFIP2,ARHGEF3,F11R,TBC1D7,SNX9,RAPGEF6,ERRFI1,DNAJC10,AKIRIN2,DOCK10,NLRP2,ARHGAP15,SLC39A10,RALGAPA2,ARHGAP31,TBC1D14,ARHGAP21,ARAP3,SMAP2,DOCK5,RGS8,STARD13,EGLN3,NLRP3,RASGRP4,FGD4,IQGAP3,ARHGEF19,RASGEF1B,DAB2IP,NPNT,ECT2L
139	6_Member GO Biological Processes GO:0043087 regulation of GTPase activity	-5.6299552 -3.632 81/565	399,604,1102,1122,1236,1794,1946,2146,2185,2776,2864,3268,3688,3987,4301,5739,5921,5922,5962,5996,6003,6346,6846,7074,8195,8412,8440,8787,8997,9267,9353,9628,9754,9844,9855,9910,10125,10144,10252,10395,10451,10507,10563,11033,11214,23048,23096,23101,23161,23179,23216,23258,23348,25780,26037,26230,50650,50848,51256,51429,51735,54206,55619,55843,57186,57514,57533,57584,64411,64744,80005,85397,90627,115727,121512,128239,128272,153020,153090,253260,345930	RHOH,BCL6,RCBTB2,CHML,CCR7,DOCK2,EFNA5,EZH2,PTK2B,GNAQ,FFAR1,AGFG2,ITGB1,LIMS1,MLLT4,PTGIR,RASA1,RASA2,RDX,RGS1,RGS13,CCL1,XCL2,TIAM1,MKKS,BCAR3,NCK2,RGS9,KALRN,CYTH1,SLIT2,RGS6,STARD8,ELMO1,FARP2,RABGAP1L,RASGRP1,FAM13A,SPRY1,DLC1,VAV3,SEMA4D,CXCL13,ADAP1,AKAP13,FNBP1,IQSEC2,MCF2L2,SNX13,RGL1,TBC1D1,DENND5A,DOCK9,RASGRP3,SIPA1L1,TIAM2,ARHGEF3,F11R,TBC1D7,SNX9,RAPGEF6,ERRFI1,DOCK10,ARHGAP15,RALGAPA2,ARHGAP31,TBC1D14,ARHGAP21,ARAP3,SMAP2,DOCK5,RGS8,STARD13,RASGRP4,FGD4,IQGAP3,ARHGEF19,RASGEF1B,DAB2IP,RICTOR,ECT2L
140	6_Member GO Biological Processes GO:0043547 positive regulation of GTPase activity	-5.02246638 -3.107 73/513	1102,1122,1236,1794,2146,2185,2776,2864,3268,3688,3987,4301,5739,5921,5922,5996,6003,6346,6846,7074,8412,8440,8787,8997,9267,9628,9754,9844,9855,9910,10125,10144,10395,10451,10507,10563,11033,11214,23048,23096,23101,23161,23179,23216,23258,23348,25780,26037,26230,	RCBTB2,CHML,CCR7,DOCK2,EZH2,PTK2B,GNAQ,FFAR1,AGFG2,ITGB1,LIMS1,MLLT4,PTGIR,RASA1,RASA2,RGS1,RGS13,CCL1,XCL2,TIAM1,BCAR3,NCK2,RGS9,KALRN,CYTH1,RGS6,STARD8,ELMO1,FARP2,RABGAP1L,RASGRP1,FAM13A,DLC1,VAV3,SEMA4D,CXCL13,ADAP1,AKAP13,FNBP1,IQSEC2,MCF2L2,SNX13,RGL1,TBC1D1,DENND5A,DOCK9,RAS

Appendix

			50650,50848,51256,51429,51735,54206,55619,55843,57186,57514,57533,57584,64411,64744,80005,85397,90627,115727,121512,128239,128272,153020,153090,345930	GRP3,SIPA1L1,TIAM2,ARHGEF3,F11R,TBC1D7,SNX9,RAPGEF6,ERRFI1,DOCK10,ARHGAP15,RALGAPA2,ARHGAP31,TBC1D14,ARHGAP21,ARAP3,SMAP2,DOCK5,RGS8,STARD13,RASGRP4,FGD4,IQGAP3,ARHGEF19,RASGEF1B,DAB2IP,ECT2L
141	7_Summary GO Biological Processes GO:0042060 wound healing	-8.560047357 -6.068 110/732	81,154,284,301,302,308,335,347,483,493,558,682,832,836,914,960,965,1316,1525,1839,1906,2054,2113,2146,2153,2157,2244,2335,2534,2596,2625,2697,2768,2776,3442,3479,3481,3635,3660,3672,3673,3680,3688,3708,3709,3778,3796,3835,3937,4488,4602,4815,4853,5054,5055,5290,5295,5321,5547,5567,5579,5734,5739,5770,5890,5912,6239,6383,6850,7035,7048,7114,7294,7301,7424,7476,7534,8140,8291,8525,8573,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23122,23348,23499,26509,27094,30845,50848,54331,54518,54566,55576,79001,79625,81606,84959,120425,128272,146850,375056,113,540,2692,3037,3783,5021,5800,7421,7466,7498,8626,8648,9076,27347,54843	ACTN4,ADRB2,ANGPT1,ANXA1,ANXA2,ANXA5,APOA1,APOD,ATP1B3,ATP2B4,AXL,BSG,CAPZB,CASP3,CD2,CD44,CD58,KLF6,CXADR,HBEGF,EDN1,STX2,ETS1,EZH2,F5,F8,FGB,FN1,FYN,GAP43,GATA3,GJA1,GNA12,GNAQ,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGA9,ITGB1,ITPR1,ITPR2,KCNMA1,KIF2A,KIF22,LCP2,MSX2,MYB,NINJ2,NOTCH2,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGER4,PTGIR,PTPN1,RAD51B,RAP2B,RREB1,SDC2,SYK,TFPI,TGFBR2,TMSB4X,TXK,TYRO3,VEGFC,WNT7A,YWHAZ,SLC7A5,DYSF,DGKZ,CASK,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,CLASP2,DOCK9,MACF1,MYOF,KCNMB3,EHD3,F11R,GNG2,APBB1IP,EPB41L4B,STAB2,VKORC1,NDNF,LBH,UBASH3B,JAML,ARHGEF19,PIK3R6,MIA3,ADCY7,ATP7B,GHRHR,HAS2,KCNN4,OXTR,PTPRO,VDR,WFS1,XDH,TP63,NCOA1,CLDN1,STK39,SYTL2
142	7_Member GO Biological Processes GO:0042060 wound healing	-8.560047357 -6.068 110/732	81,154,284,301,302,308,335,347,483,493,558,682,832,836,914,960,965,1316,1525,1839,1906,2054,2113,2146,2153,2157,2244,2335,2534,2596,2625,2697,2768,2776,3442,3479,3481,3635,3660,3672,3673,3680,3688,3708,3709,3778,3796,3835,3937,4488,4602,4815,4853,5054,5055,5290,5295,5321,5547,5567,5579,5734,5739,5770,5890,5912,6239,6383,6850,7035,7048,7114,7294,7301,7424,7476,7534,8140,8291,8525,8573,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23122,23348,23499,26509,27094,30845,50848,54331,54518,54566,55576,79001,79625,81606,84959,120425,128272,146850,375056	ACTN4,ADRB2,ANGPT1,ANXA1,ANXA2,ANXA5,APOA1,APOD,ATP1B3,ATP2B4,AXL,BSG,CAPZB,CASP3,CD2,CD44,CD58,KLF6,CXADR,HBEGF,EDN1,STX2,ETS1,EZH2,F5,F8,FGB,FN1,FYN,GAP43,GATA3,GJA1,GNA12,GNAQ,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGA9,ITGB1,ITPR1,ITPR2,KCNMA1,KIF2A,KIF22,LCP2,MSX2,MYB,NINJ2,NOTCH2,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGER4,PTGIR,PTPN1,RAD51B,RAP2B,RREB1,SDC2,SYK,TFPI,TGFBR2,TMSB4X,TXK,TYRO3,VEGFC,WNT7A,YWHAZ,SLC7A5,DYSF,DGKZ,CASK,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,CLASP2,DOCK9,MACF1,MYOF,KCNMB3,EHD3,F11R,GNG2,APBB1IP,EPB41L4B,STAB2,VKORC1,NDNF,LBH,UBASH3B,JAML,ARHGEF19,PIK3R6,MIA3
143	7_Member	-6.294468359	81,113,154,284,302,308,335,483,493,540,	ACTN4,ADCY7,ADRB2,ANGPT1,ANXA2,ANXA5,A

Appendix

	GO Biological Processes GO:0050878 regulation of body fluid levels	-4.145 100/715	558,682,832,914,960,965,1525,1906,2054,2153,2157,2244,2335,2534,2625,2692,2697,2768,2776,3037,3442,3479,3481,3635,3660,3672,3673,3688,3708,3709,3778,3783,3796,3835,3937,4602,5021,5054,5055,5290,5295,5321,5547,5567,5579,5734,5739,5770,5800,5890,5912,6850,7035,7114,7294,7301,7421,7424,7466,7498,7534,8140,8525,8626,8648,9076,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23348,27094,27347,30845,50848,54331,54518,54843,55576,79001,81606,84959,120425,146850	POA1,ATP1B3,ATP2B4,ATP7B,AXL,BSG,CAPZB,CD2,CD44,CD58,CXADR,EDN1,STX2,F5,F8,FGB,FN1,FYN,GATA3,GHRHR,GJA1,GNA12,GNAQ,HAS2,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGB1,ITPR1,ITPR2,KCNMA1,KCNN4,KIF2A,KIF22,LCP2,MYB,OXTR,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGER4,PTGIR,PTPN1,PTPRO,RAD51B,RAP2B,SYK,TFPI,TMSB4X,TXK,TYRO3,VDR,VEGFC,WFS1,XDH,YWHAZ,SLC7A5,DGKZ,TP63,NCOA1,CLDN1,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,DOCK9,KCNMB3,STK39,EHD3,F11R,GNG2,APBB1IP,SYTL2,STAB2,VKORC1,LBH,UBASH3B,JAML,PIK3R6
144	7_Member GO Biological Processes GO:0007596 blood coagulation	-6.25191155 -4.115 83/563	81,154,284,302,308,335,483,493,558,682,832,914,960,965,1525,1906,2054,2153,2157,2244,2335,2534,2625,2768,2776,3442,3479,3481,3635,3660,3672,3673,3688,3708,3709,3778,3796,3835,3937,4602,5054,5055,5290,5295,5321,5547,5567,5579,5739,5770,5890,5912,6850,7035,7114,7294,7301,7424,7534,8140,8525,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23348,27094,30845,50848,54331,54518,55576,79001,81606,84959,120425,146850	ACTN4,ADRB2,ANGPT1,ANXA2,ANXA5,APOA1,ATP1B3,ATP2B4,AXL,BSG,CAPZB,CD2,CD44,CD58,CXADR,EDN1,STX2,F5,F8,FGB,FN1,FYN,GATA3,GNA12,GNAQ,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGB1,ITPR1,ITPR2,KCNMA1,KIF2A,KIF22,LCP2,MYB,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGIR,PTPN1,RAD51B,RAP2B,SYK,TFPI,TMSB4X,TXK,TYRO3,VEGFC,YWHAZ,SLC7A5,DGKZ,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,DOCK9,KCNMB3,EHD3,F11R,GNG2,APBB1IP,STAB2,VKORC1,LBH,UBASH3B,JAML,PIK3R6
145	7_Member GO Biological Processes GO:0050817 coagulation	-6.123820721 -4.031 83/567	81,154,284,302,308,335,483,493,558,682,832,914,960,965,1525,1906,2054,2153,2157,2244,2335,2534,2625,2768,2776,3442,3479,3481,3635,3660,3672,3673,3688,3708,3709,3778,3796,3835,3937,4602,5054,5055,5290,5295,5321,5547,5567,5579,5739,5770,5890,5912,6850,7035,7114,7294,7301,7424,7534,8140,8525,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23348,27094,30845,50848,54331,54518,55576,79001,81606,84959,120425,146850	ACTN4,ADRB2,ANGPT1,ANXA2,ANXA5,APOA1,ATP1B3,ATP2B4,AXL,BSG,CAPZB,CD2,CD44,CD58,CXADR,EDN1,STX2,F5,F8,FGB,FN1,FYN,GATA3,GNA12,GNAQ,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGB1,ITPR1,ITPR2,KCNMA1,KIF2A,KIF22,LCP2,MYB,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGIR,PTPN1,RAD51B,RAP2B,SYK,TFPI,TMSB4X,TXK,TYRO3,VEGFC,YWHAZ,SLC7A5,DGKZ,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,DOCK9,KCNMB3,EHD3,F11R,GNG2,APBB1IP,STAB2,VKORC1,LBH,UBASH3B,JAML,PIK3R6
146	7_Member GO Biological Processes	-6.092126737 -4.010	81,154,284,302,308,335,483,493,558,682,832,914,960,965,1525,1906,2054,2153,21	ACTN4,ADRB2,ANGPT1,ANXA2,ANXA5,APOA1,ATP1B3,ATP2B4,AXL,BSG,CAPZB,CD2,CD44,CD58,CX

	GO:0007599 hemostasis	83/568	57,2244,2335,2534,2625,2768,2776,3442,3479,3481,3635,3660,3672,3673,3688,3708,3709,3778,3796,3835,3937,4602,5054,5055,5290,5295,5321,5547,5567,5579,5739,5770,5890,5912,6850,7035,7114,7294,7301,7424,7534,8140,8525,9948,10125,10257,10362,10451,10487,10672,10855,11093,11127,23348,27094,30845,50848,54331,54518,55576,79001,81606,84959,120425,146850	ADR,EDN1,STX2,F5,F8,FGB,FN1,FYN,GATA3,GNA12,GNAQ,IFNA5,IGF1,IGF2,INPP5D,IRF2,ITGA1,ITGA2,ITGB1,ITPR1,ITPR2,KCNMA1,KIF2A,KIF22,LCP2,MYB,SERPINE1,SERPINB2,PIK3CA,PIK3R1,PLA2G4A,PRCP,PRKACB,PRKCB,PTGIR,PTPN1,RAD51B,RAP2B,SYK,TFPI,TMSB4X,TXK,TYRO3,VEGFC,YWHAZ,SLC7A5,DGKZ,WDR1,RASGRP1,ABCC4,HMG20B,VAV3,CAP1,GNA13,HPSE,ADAMTS13,KIF3A,DOCK9,KCNMB3,EHD3,F11R,GNG2,APBB1IP,STAB2,VKORC1,LBH,UBASH3B,JAML,PIK3R6
147	7_Member GO Biological Processes GO:0030168 platelet activation	-4.202860196 -2.435 41/256	81,154,335,558,2153,2157,2244,2335,2534,2768,2776,3479,3481,3708,3709,3937,5054,5290,5295,5321,5579,5770,5912,6850,7114,7294,7301,7424,7534,8525,9948,10125,10257,10451,10487,10672,11093,54331,54518,84959,146850	ACTN4,ADRB2,APOA1,AXL,F5,F8,FGB,FN1,FYN,GNA12,GNAQ,IGF1,IGF2,ITPR1,ITPR2,LCP2,SERPINE1,PIK3CA,PIK3R1,PLA2G4A,PRKCB,PTPN1,RAP2B,SYK,TMSB4X,TXK,TYRO3,VEGFC,YWHAZ,DGKZ,WDR1,RASGRP1,ABCC4,VAV3,CAP1,GNA13,ADAMTS13,GNG2,APBB1IP,UBASH3B,PIK3R6
148	7_Member GO Biological Processes GO:0002576 platelet degranulation	-2.193138427 -0.915 15/87	81,335,2153,2157,2244,2335,3479,3481,5054,6850,7114,7424,9948,10257,10487	ACTN4,APOA1,F5,F8,FGB,FN1,IGF1,IGF2,SERPINE1,SYK,TMSB4X,VEGFC,WDR1,ABCC4,CAP1
149	8_Summary GO Biological Processes GO:0046631 alpha-beta T cell activation	-8.507262902 -6.027 30/107	301,596,604,940,942,944,1794,2625,3077,3458,3592,3600,3702,3965,4602,5734,6095,6304,6850,7048,7292,7704,8320,8767,8995,9308,27086,64218,114548,149233,338339,911,3442,54518,1015,1880,2185,4853,23228,30009,80762,861,5142,5144,6711,6885,3596,6504,10125,64581,117194,6441	ANXA1,BCL2,BCL6,CD28,CD86,TNFSF8,DOCK2,GATA3,HFE,IFNG,IL12A,IL15,ITK,LGALS9,MYB,PTGER4,RORA,SATB1,SYK,TGFBR2,TNFSF4,ZBTB16,EOMES,RIPK2,TNFSF18,CD83,FOXP1,SEMA4A,NLRP3,IL23R,CLEC4D,CD1C,IFNA5,APBB1IP,CDH17,GPR183,PTK2B,NOTCH2,PLCL2,TBX21,NDFIP1,RUNX1,PDE4B,PDE4D,SPTBN1,MAP3K7,IL13,SLAMF1,RASGRP1,CLEC7A,MRGPRX2,SFTPD
150	8_Member GO Biological Processes GO:0046631 alpha-beta T cell activation	-8.507262902 -6.027 30/107	301,596,604,940,942,944,1794,2625,3077,3458,3592,3600,3702,3965,4602,5734,6095,6304,6850,7048,7292,7704,8320,8767,8995,9308,27086,64218,114548,149233	ANXA1,BCL2,BCL6,CD28,CD86,TNFSF8,DOCK2,GATA3,HFE,IFNG,IL12A,IL15,ITK,LGALS9,MYB,PTGER4,RORA,SATB1,SYK,TGFBR2,TNFSF4,ZBTB16,EOMES,RIPK2,TNFSF18,CD83,FOXP1,SEMA4A,NLRP3,IL23R
151	8_Member GO Biological Processes GO:0046632 alpha-beta T cell differentiation	-8.382182698 -5.916 25/79	301,596,604,942,944,2625,3458,3702,3965,4602,5734,6095,6304,6850,7048,7292,7704,8320,8767,8995,9308,27086,64218,114548,149233	ANXA1,BCL2,BCL6,CD86,TNFSF8,GATA3,IFNG,ITK,LGALS9,MYB,PTGER4,RORA,SATB1,SYK,TGFBR2,TNFSF4,ZBTB16,EOMES,RIPK2,TNFSF18,CD83,FOXP1,SEMA4A,NLRP3,IL23R
152	8_Member GO Biological Processes	-6.855267689 -4.622	301,604,942,2625,3458,3965,4602,5734,6095,7292,8320,8767,8995,27086,64218,11	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,TNFSF4,EOMES,RIPK2,TNFSF18,FOXP

Appendix

	GO:0002293 alpha-beta T cell differentiation involved in immune response	17/47	4548,149233	1,SEMA4A,NLRP3,IL23R
153	8_Member GO Biological Processes GO:0002287 alpha-beta T cell activation involved in immune response	-6.855267689 -4.622 17/47	301,604,942,2625,3458,3965,4602,5734,6095,7292,8320,8767,8995,27086,64218,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,TNFSF4,EOMES,RIPK2,TNFSF18,FOXP1,SEMA4A,NLRP3,IL23R
154	8_Member GO Biological Processes GO:0002292 T cell differentiation involved in immune response	-6.599042104 -4.399 18/54	301,604,942,2625,3458,3965,4602,5734,6095,7292,8320,8767,8995,27086,64218,114548,149233,338339	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,TNFSF4,EOMES,RIPK2,TNFSF18,FOXP1,SEMA4A,NLRP3,IL23R,CLEC4D
155	8_Member GO Biological Processes GO:0043367 CD4-positive, alpha-beta T cell differentiation	-6.330216734 -4.173 18/56	301,604,942,2625,3458,3965,4602,5734,6095,6304,7292,8767,8995,9308,27086,64218,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,SATB1,TNFSF4,RIPK2,TNFSF18,CD83,FOXP1,SEMA4A,NLRP3,IL23R
156	8_Member GO Biological Processes GO:0046637 regulation of alpha-beta T cell differentiation	-6.219829628 -4.090 16/46	301,604,942,2625,3458,3965,4602,6850,7048,7292,7704,8767,8995,9308,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,TNFSF18,CD83,NLRP3,IL23R
157	8_Member GO Biological Processes GO:0002294 CD4-positive, alpha-beta T cell differentiation involved in immune response	-6.219829628 -4.090 16/46	301,604,942,2625,3458,3965,4602,5734,6095,7292,8767,8995,27086,64218,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,TNFSF4,RIPK2,TNFSF18,FOXP1,SEMA4A,NLRP3,IL23R
158	8_Member GO Biological Processes GO:0043370 regulation of CD4-positive, alpha-beta T cell differentiation	-5.872464621 -3.830 13/33	301,604,942,2625,3458,3965,4602,7292,8767,8995,9308,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,TNFSF4,RIPK2,TNFSF18,CD83,NLRP3,IL23R
159	8_Member	-5.604775197	301,604,940,942,2625,3077,3458,3592,39	ANXA1,BCL6,CD28,CD86,GATA3,HFE,IFNG,IL12A,

Appendix

	GO Biological Processes GO:0046634 regulation of alpha-beta T cell activation	-3.618 19/68	65,4602,6850,7048,7292,7704,8767,8995,9308,114548,149233	LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,TNFSF18,CD83,NLRP3,IL23R
160	8_Member GO Biological Processes GO:0035710 CD4-positive, alpha-beta T cell activation	-5.49488602 -3.514 18/63	301,604,942,2625,3458,3965,4602,5734,6095,6304,7292,8767,8995,9308,27086,64218,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,PTGER4,RORA,SATB1,TNFSF4,RIPK2,TNFSF18,CD83,FOXP1,SEMA4A,NLRP3,IL23R
161	8_Member GO Biological Processes GO:0046638 positive regulation of alpha-beta T cell differentiation	-5.219746925 -3.278 13/37	301,942,3458,3965,4602,6850,7048,7292,7704,8767,9308,114548,149233	ANXA1,CD86,IFNG,LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,CD83,NLRP3,IL23R
162	8_Member GO Biological Processes GO:0002286 T cell activation involved in immune response	-5.10382991 -3.179 21/86	301,604,911,942,2625,3442,3458,3965,4602,5734,6095,7292,8320,8767,8995,27086,54518,64218,114548,149233,338339	ANXA1,BCL6,CD1C,CD86,GATA3,IFNA5,IFNG,LGALS9,MYB,PTGER4,RORA,TNFSF4,EOMES,RIPK2,TNFSF18,FOXP1,APBB1IP,SEMA4A,NLRP3,IL23R,CLEC4D
163	8_Member GO Biological Processes GO:2000514 regulation of CD4-positive, alpha-beta T cell activation	-5.073044802 -3.153 13/38	301,604,942,2625,3458,3965,4602,7292,8767,8995,9308,114548,149233	ANXA1,BCL6,CD86,GATA3,IFNG,LGALS9,MYB,TNFSF4,RIPK2,TNFSF18,CD83,NLRP3,IL23R
164	8_Member GO Biological Processes GO:0042093 T-helper cell differentiation	-5.000157475 -3.094 14/44	301,604,942,2625,4602,5734,6095,7292,8767,8995,27086,64218,114548,149233	ANXA1,BCL6,CD86,GATA3,MYB,PTGER4,RORA,TNFSF4,RIPK2,TNFSF18,FOXP1,SEMA4A,NLRP3,IL23R
165	8_Member GO Biological Processes GO:0043372 positive regulation of CD4-positive, alpha-beta T cell differentiation	-4.899313681 -3.013 10/24	301,942,3458,3965,4602,7292,8767,9308,114548,149233	ANXA1,CD86,IFNG,LGALS9,MYB,TNFSF4,RIPK2,CD83,NLRP3,IL23R
166	8_Member GO Biological Processes	-4.863955611 -2.985	301,604,911,940,942,1015,1880,2185,2625,3442,3458,3965,4602,4853,5734,6095,7	ANXA1,BCL6,CD1C,CD28,CD86,CDH17,GPR183,PTK2B,GATA3,IFNA5,IFNG,LGALS9,MYB,NOTCH2,PT

Appendix

	GO:0002285 lymphocyte activation involved in immune response	29/146	292,8320,8767,8995,23228,27086,30009,54518,64218,80762,114548,149233,338339	GER4,RORA,TNFSF4,EOMES,RIPK2,TNFSF18,PLCL2,FOXP1,TBX21,APBB1IP,SEMA4A,NDFIP1,NLRP3,IL23R,CLEC4D
167	8_Member GO Biological Processes GO:0046635 positive regulation of alpha-beta T cell activation	-4.847658283 -2.977 15/51	301,940,942,3458,3592,3965,4602,6850,7048,7292,7704,8767,9308,114548,149233	ANXA1,CD28,CD86,IFNG,IL12A,LGALS9,MYB,SYK,TGFBR2,TNFSF4,ZBTB16,RIPK2,CD83,NLRP3,IL23R
168	8_Member GO Biological Processes GO:0032743 positive regulation of interleukin-2 production	-4.548750005 -2.726 11/31	301,861,940,942,5142,5144,6711,6885,7292,8767,9308	ANXA1,RUNX1,CD28,CD86,PDE4B,PDE4D,SPTBN1,MAP3K7,TNFSF4,RIPK2,CD83
169	8_Member GO Biological Processes GO:0002366 leukocyte activation involved in immune response	-4.434879095 -2.633 36/209	301,604,911,940,942,1015,1794,1880,2185,2625,3442,3458,3596,3965,4602,4853,5734,6095,6504,6850,7292,8320,8767,8995,10125,23228,27086,30009,54518,64218,64581,80762,114548,117194,149233,338339	ANXA1,BCL6,CD1C,CD28,CD86,CDH17,DOCK2,GPR183,PTK2B,GATA3,IFNA5,IFNG,IL13,LGALS9,MYB,NOTCH2,PTGER4,RORA,SLAMF1,SYK,TNFSF4,EOMES,RIPK2,TNFSF18,RASGRP1,PLCL2,FOXP1,TBX21,APBB1IP,SEMA4A,CLEC7A,NDFIP1,NLRP3,MRGPRX2,IL23R,CLEC4D
170	8_Member GO Biological Processes GO:2000516 positive regulation of CD4-positive, alpha-beta T cell activation	-4.368990376 -2.579 10/27	301,942,3458,3965,4602,7292,8767,9308,114548,149233	ANXA1,CD86,IFNG,LGALS9,MYB,TNFSF4,RIPK2,CD83,NLRP3,IL23R
171	8_Member GO Biological Processes GO:0002263 cell activation involved in immune response	-4.344896647 -2.559 36/211	301,604,911,940,942,1015,1794,1880,2185,2625,3442,3458,3596,3965,4602,4853,5734,6095,6504,6850,7292,8320,8767,8995,10125,23228,27086,30009,54518,64218,64581,80762,114548,117194,149233,338339	ANXA1,BCL6,CD1C,CD28,CD86,CDH17,DOCK2,GPR183,PTK2B,GATA3,IFNA5,IFNG,IL13,LGALS9,MYB,NOTCH2,PTGER4,RORA,SLAMF1,SYK,TNFSF4,EOMES,RIPK2,TNFSF18,RASGRP1,PLCL2,FOXP1,TBX21,APBB1IP,SEMA4A,CLEC7A,NDFIP1,NLRP3,MRGPRX2,IL23R,CLEC4D
172	8_Member GO Biological Processes GO:0032663 regulation of interleukin-2 production	-3.871571684 -2.168 13/48	301,861,940,942,2625,5142,5144,6441,6711,6885,7292,8767,9308	ANXA1,RUNX1,CD28,CD86,GATA3,PDE4B,PDE4D,SFTPD,SPTBN1,MAP3K7,TNFSF4,RIPK2,CD83
173	8_Member GO Biological Processes	-3.722154279 -2.042	301,604,942,4602,7292,8767,8995,114548,149233	ANXA1,BCL6,CD86,MYB,TNFSF4,RIPK2,TNFSF18,NLRP3,IL23R

Appendix

	GO:0045622 regulation of T-helper cell differentiation	9/26		
174	8_Member GO Biological Processes GO:0045624 positive regulation of T-helper cell differentiation	-3.355799213 -1.763 7/18	301,942,4602,7292,8767,114548,149233	ANXA1,CD86,MYB,TNFSF4,RIPK2,NLRP3,IL23R
175	8_Member GO Biological Processes GO:0032623 interleukin-2 production	-3.321543943 -1.739 13/54	301,861,940,942,2625,5142,5144,6441,6711,6885,7292,8767,9308	ANXA1,RUNX1,CD28,CD86,GATA3,PDE4B,PDE4D,SFTPD,SPTBN1,MAP3K7,TNFSF4,RIPK2,CD83
176	8_Member GO Biological Processes GO:0045064 T-helper 2 cell differentiation	-3.015327845 -1.511 6/15	301,604,942,2625,7292,114548	ANXA1,BCL6,CD86,GATA3,TNFSF4,NLRP3
177	8_Member GO Biological Processes GO:0045628 regulation of T-helper 2 cell differentiation	-2.876220995 -1.405 5/11	301,604,942,7292,114548	ANXA1,BCL6,CD86,TNFSF4,NLRP3
178	8_Member GO Biological Processes GO:0002828 regulation of type 2 immune response	-2.303076406 -0.992 7/26	301,604,942,2625,7292,80762,114548	ANXA1,BCL6,CD86,GATA3,TNFSF4,NDFIP1,NLRP3
179	8_Member GO Biological Processes GO:2000515 negative regulation of CD4-positive, alpha-beta T cell activation	-2.188642457 -0.914 5/15	301,604,3965,7292,8995	ANXA1,BCL6,LGALS9,TNFSF4,TNFSF18
180	9_Summary GO Biological Processes GO:0051270 regulation of cellular component movement	-8.31760893 -5.861 112/757	27,81,284,287,301,308,347,476,596,604,753,817,1021,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2549,2625,2768,2796,3037,3458,3479,3592,3642,3673,3688,3759,3911,3965,4000,4092,4478,4653,4920,5054,5142,5144,5295,5547,5728,5734,5793,5795,5800,5	ABL2,ACTN4,ANGPT1,ANK2,ANXA1,ANXA5,APOD,ATP1A1,BCL2,BCL6,LDLRAD4,CAMK2D,CDK6,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GAB1,GATA3,GNA12,GNRH1,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,KCNJ2,LAMA5,LGALS9,LMNA,SMAD7,MSN,MYOC,ROR2,SERPINE1,PDE4B,PDE4D,PIK3R1,P

Appendix

			912,5962,6239,6262,6423,6446,6653,6672,7048,7049,7074,7114,7424,7476,7852,8195,8428,8650,8660,8828,8995,9353,9469,9734,10154,10221,10395,10396,10507,10563,10672,23122,23224,23499,27086,29119,54566,54986,55079,55619,56729,57111,57531,57556,57688,64218,64411,64750,80005,85407,90627,91663,114882,153090,168667,375056,6480,55223,335,1960,3397,5290,6273,6772,11156,2668,10487,30812,84159	RCP,PTEN,PTGER4,PTPRG,PTPRJ,PTPRO,RAP2B,RDX,RREB1,RYR2,SFRP2,SGK1,SORL1,SP100,TGFBR2,TGFBR3,TIAM1,TMSB4X,VEGFC,WNT7A,CXCR4,MKKS,STK24,NUMB,IRS2,NRP2,TNFSF18,SLIT2,CHST3,HDAC9,PLXNC1,TRIB1,DLC1,ATP8A1,SEMA4D,CXCL13,GNA13,CLASP2,SYNE2,MACF1,FOXP1,CTNNA3,EPB41L4B,ULK4,FEZF2,DOCK10,RETN,RAB25,HACE1,SEMA6A,ZSWIM6,SEMA4A,ARAP3,SMURF2,DOCK5,NKD1,STARD13,MYADM,OSBPL8,DAB2IP,BMPER,MIA3,ST6GAL1,TRIM62,APOA1,EGR3,ID1,PIK3CA,S100A2,STAT1,PTP4A3,GDNF,CAP1,SOX8,ARID5B
181	9_Member GO Biological Processes GO:0051270 regulation of cellular component movement	-8.31760893 -5.861 112/757	27,81,284,287,301,308,347,476,596,604,753,817,1021,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2549,2625,2768,2796,3037,3458,3479,3592,3642,3673,3688,3759,3911,3965,4000,4092,4478,4653,4920,5054,5142,5144,5295,5547,5728,5734,5793,5795,5800,5912,5962,6239,6262,6423,6446,6653,6672,7048,7049,7074,7114,7424,7476,7852,8195,8428,8650,8660,8828,8995,9353,9469,9734,10154,10221,10395,10396,10507,10563,10672,23122,23224,23499,27086,29119,54566,54986,55079,55619,56729,57111,57531,57556,57688,64218,64411,64750,80005,85407,90627,91663,114882,153090,168667,375056	ABL2,ACTN4,ANGPT1,ANK2,ANXA1,ANXA5,APOD,ATP1A1,BCL2,BCL6,LDLRAD4,CAMK2D,CDK6,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GAB1,GATA3,GNA12,GNRH1,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,KCNJ2,LAMA5,LGALS9,LMNA,SMAD7,MSN,MYOC,ROR2,SERPINE1,PDE4B,PDE4D,PIK3R1,PRCP,PTEN,PTGER4,PTPRG,PTPRJ,PTPRO,RAP2B,RDX,RREB1,RYR2,SFRP2,SGK1,SORL1,SP100,TGFBR2,TGFBR3,TIAM1,TMSB4X,VEGFC,WNT7A,CXCR4,MKKS,STK24,NUMB,IRS2,NRP2,TNFSF18,SLIT2,CHST3,HDAC9,PLXNC1,TRIB1,DLC1,ATP8A1,SEMA4D,CXCL13,GNA13,CLASP2,SYNE2,MACF1,FOXP1,CTNNA3,EPB41L4B,ULK4,FEZF2,DOCK10,RETN,RAB25,HACE1,SEMA6A,ZSWIM6,SEMA4A,ARAP3,SMURF2,DOCK5,NKD1,STARD13,MYADM,OSBPL8,DAB2IP,BMPER,MIA3
182	9_Member GO Biological Processes GO:2000145 regulation of cell motility	-6.732777022 -4.509 100/700	27,81,284,301,308,347,596,753,817,1021,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2549,2625,2768,2796,3037,3458,3479,3592,3642,3673,3688,3911,3965,4000,4092,4478,4653,4920,5054,5295,5547,5728,5734,5793,5795,5912,5962,6239,6423,6446,6653,6672,7048,7074,7114,7424,7476,7852,8195,8428,8650,8660,8828,8995,9353,9734,10154,10221,10395,10396,10507,10563,10672,23122,23224,23499,27086,54566,54986,5	ABL2,ACTN4,ANGPT1,ANXA1,ANXA5,APOD,BCL2,LDLRAD4,CAMK2D,CDK6,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GAB1,GATA3,GNA12,GNRH1,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LAMA5,LGALS9,LMNA,SMAD7,MSN,MYOC,ROR2,SERPINE1,PIK3R1,PRCP,PTEN,PTGER4,PTPRG,PTPRJ,RAP2B,RDX,RREB1,SFRP2,SGK1,SORL1,SP100,TGFBR2,TIAM1,TMSB4X,VEGFC,WNT7A,CXCR4,MKKS,STK24,NUMB,IRS2,NRP2,TNFSF18,SLIT2,HDAC9,PLXNC1,TRIB1,DLC1,ATP8A1,SEMA4D,CXCL13,GNA13,C

Appendix

			5619,56729,57111,57531,57556,57688,64218,64411,64750,80005,85407,90627,91663,114882,153090,168667,375056	LASP2,SYNE2,MACF1,FOXP1,EPB41L4B,ULK4,DOCK10,RETN,RAB25,HACE1,SEMA6A,ZSWIM6,SEMA4A,ARAP3,SMURF2,DOCK5,NKD1,STARD13,MYADM,OSBPL8,DAB2IP,BMPER,MIA3
183	9_Member GO Biological Processes GO:0030334 regulation of cell migration	-6.577119816 -4.383 95/660	81,284,301,347,596,753,817,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2549,2625,2768,2796,3037,3458,3479,3592,3642,3673,3688,3911,3965,4000,4092,4478,4653,4920,5054,5295,5547,5728,5734,5793,5795,5912,5962,6239,6423,6446,6653,6672,7048,7074,7114,7424,7476,7852,8428,8650,8660,8828,8995,9353,9734,10154,10221,10395,10396,10507,10563,10672,23122,23224,23499,27086,54566,54986,55619,56729,57111,57531,57556,57688,64218,64411,64750,80005,90627,91663,114882,153090,168667,375056	ACTN4,ANGPT1,ANXA1,APOD,BCL2,LDLRAD4,CAMK2D,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GAB1,GATA3,GNA12,GNRH1,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LAMA5,LGALS9,LMNA,SMAD7,MSN,MYOC,ROR2,SERPINE1,PIK3R1,PRCP,PTEN,PTGER4,PTPRG,PTPRJ,RAP2B,RDX,RREB1,SFRP2,SGK1,SORL1,SP100,TGFBR2,TIAM1,TMSB4X,VEGFC,WNT7A,CXCR4,STK24,NUMB,IRS2,NRP2,TNFSF18,SLIT2,HDAC9,PLXNC1,TRIB1,DLC1,ATP8A1,SEMA4D,CXCL13,GNA13,CLASP2,SYNE2,MACF1,FOXP1,EPB41L4B,ULK4,DOCK10,RETN,RAB25,HACE1,SEMA6A,ZSWIM6,SEMA4A,ARAP3,SMURF2,DOCK5,STARD13,MYADM,OSBPL8,DAB2IP,BMPER,MIA3
184	9_Member GO Biological Processes GO:0040012 regulation of locomotion	-6.149712651 -4.046 104/757	27,81,284,301,308,347,596,753,817,1021,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2549,2625,2768,2796,3037,3458,3479,3592,3642,3673,3688,3911,3965,4000,4092,4478,4653,4920,5054,5295,5547,5728,5734,5793,5795,5800,5912,5962,6239,6423,6446,6480,6653,6672,7048,7074,7114,7424,7476,7852,8195,8428,8650,8660,8828,8995,9353,9734,10154,10221,10395,10396,10507,10563,10672,23122,23224,23499,27086,54566,54986,55079,55223,55619,56729,57111,57531,57556,57688,64218,64411,64750,80005,85407,90627,91663,114882,153090,168667,375056	ABL2,ACTN4,ANGPT1,ANXA1,ANXA5,APOD,BCL2,LDLRAD4,CAMK2D,CDK6,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GAB1,GATA3,GNA12,GNRH1,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LAMA5,LGALS9,LMNA,SMAD7,MSN,MYOC,ROR2,SERPINE1,PIK3R1,PRCP,PTEN,PTGER4,PTPRG,PTPRJ,PTPRO,RAP2B,RDX,RREB1,SFRP2,SGK1,ST6GAL1,SORL1,SP100,TGFBR2,TIAM1,TMSB4X,VEGFC,WNT7A,CXCR4,MKKS,STK24,NUMB,IRS2,NRP2,TNFSF18,SLIT2,HDAC9,PLXNC1,TRIB1,DLC1,ATP8A1,SEMA4D,CXCL13,GNA13,CLASP2,SYNE2,MACF1,FOXP1,EPB41L4B,ULK4,FEZF2,TRIM62,DOCK10,RETN,RAB25,HACE1,SEMA6A,ZSWIM6,SEMA4A,ARAP3,SMURF2,DOCK5,NKD1,STARD13,MYADM,OSBPL8,DAB2IP,BMPER,MIA3
185	9_Member GO Biological Processes GO:0010631 epithelial cell migration	-5.438545724 -3.468 40/220	284,335,1235,1839,1906,1960,2113,2185,2246,2625,3037,3397,3458,3673,3688,5290,5547,5728,5793,6239,6273,6672,6772,7048,7424,7476,8828,9353,9734,10563,11156,23122,23499,27086,54566,57111,64218,90627,153090,168667	ANGPT1,APOA1,CCR6,HBEGF,EDN1,EGR3,ETS1,PTK2B,FGF1,GATA3,HAS2,ID1,IFNG,ITGA2,ITGB1,PIK3CA,PRCP,PTEN,PTPRG,RREB1,S100A2,SP100,STAT1,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,PTP4A3,CLASP2,MACF1,FOXP1,EPB41L4B,RAB25,SEMA4A,STARD13,DAB2IP,BMPER

186	9_Member GO Biological Processes GO:0090132 epithelium migration	-5.289571105 -3.337 40/223	284,335,1235,1839,1906,1960,2113,2185,2246,2625,3037,3397,3458,3673,3688,5290,5547,5728,5793,6239,6273,6672,6772,7048,7424,7476,8828,9353,9734,10563,11156,23122,23499,27086,54566,57111,64218,90627,153090,168667	ANGPT1,APOA1,CCR6,HBEGF,EDN1,EGR3,ETS1,PTK2B,FGF1,GATA3,HAS2,ID1,IFNG,ITGA2,ITGB1,PIK3CA,PRCP,PTEN,PTPRG,RREB1,S100A2,SP100,STAT1,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,PTP4A3,CLASP2,MACF1,FOXP1,EPB41L4B,RAB25,SEMA4A,STARD13,DAB2IP,BMPER
187	9_Member GO Biological Processes GO:0030335 positive regulation of cell migration	-5.004472773 -3.094 59/389	81,284,596,817,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2625,3037,3458,3479,3592,3642,3673,3688,3965,4653,4920,5054,5295,5734,5962,6239,7048,7074,7424,7476,8650,8660,8828,8995,9734,10396,10507,10563,23122,23224,27086,54566,56729,57111,57556,64218,64750,80005,91663,153090,375056	ACTN4,ANGPT1,BCL2,CAMK2D,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GATA3,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LGALS9,MYOC,ROR2,SERPINE1,PIK3R1,PTGER4,RDX,RREB1,TGFBR2,TIAM1,VEGFC,WNT7A,NUMB,IRS2,NRP2,TNFSF18,HDAC9,ATP8A1,SEMA4D,CXCL13,CLASP2,SYNE2,FOXP1,EPB41L4B,RETN,RAB25,SEMA6A,SEMA4A,SMURF2,DOCK5,MYADM,DAB2IP,MIA3
188	9_Member GO Biological Processes GO:0090130 tissue migration	-5.00285956 -3.094 40/229	284,335,1235,1839,1906,1960,2113,2185,2246,2625,3037,3397,3458,3673,3688,5290,5547,5728,5793,6239,6273,6672,6772,7048,7424,7476,8828,9353,9734,10563,11156,23122,23499,27086,54566,57111,64218,90627,153090,168667	ANGPT1,APOA1,CCR6,HBEGF,EDN1,EGR3,ETS1,PTK2B,FGF1,GATA3,HAS2,ID1,IFNG,ITGA2,ITGB1,PIK3CA,PRCP,PTEN,PTPRG,RREB1,S100A2,SP100,STAT1,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,PTP4A3,CLASP2,MACF1,FOXP1,EPB41L4B,RAB25,SEMA4A,STARD13,DAB2IP,BMPER
189	9_Member GO Biological Processes GO:0051272 positive regulation of cellular component movement	-4.822349659 -2.958 61/412	81,284,596,604,817,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2625,3037,3458,3479,3592,3642,3673,3688,3965,4653,4920,5054,5295,5734,5962,6239,7048,7074,7424,7476,8650,8660,8828,8995,9469,9734,10396,10507,10563,23122,23224,27086,54566,56729,57111,57556,64218,64750,80005,91663,153090,375056	ACTN4,ANGPT1,BCL2,BCL6,CAMK2D,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GATA3,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LGALS9,MYOC,ROR2,SERPINE1,PIK3R1,PTGER4,RDX,RREB1,TGFBR2,TIAM1,VEGFC,WNT7A,NUMB,IRS2,NRP2,TNFSF18,CHST3,HDAC9,ATP8A1,SEMA4D,CXCL13,CLASP2,SYNE2,FOXP1,EPB41L4B,RETN,RAB25,SEMA6A,SEMA4A,SMURF2,DOCK5,MYADM,DAB2IP,MIA3
190	9_Member GO Biological Processes GO:0001667 ameboidal-type cell migration	-4.797907418 -2.935 50/317	284,335,1235,1839,1906,1960,2113,2185,2246,2625,2668,2768,3037,3397,3458,3673,3688,3911,5290,5547,5728,5793,6239,6273,6672,6772,7048,7424,7476,8828,9353,9734,10487,10507,10563,11156,23122,23224,23499,27086,30812,54566,57111,57556,64218,64750,84159,90627,153090,168667	ANGPT1,APOA1,CCR6,HBEGF,EDN1,EGR3,ETS1,PTK2B,FGF1,GATA3,GDNF,GNA12,HAS2,ID1,IFNG,ITGA2,ITGB1,LAMA5,PIK3CA,PRCP,PTEN,PTPRG,RREB1,S100A2,SP100,STAT1,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CAP1,SEMA4D,CXCL13,PTP4A3,CLASP2,SYNE2,MACF1,FOXP1,SOX8,EPB41L4B,RAB25,SEMA6A,SEMA4A,SMURF2,ARID5B,STARD13,DAB2IP,BMPER
191	9_Member GO Biological Processes	-4.604043219 -2.778	81,284,596,817,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2	ACTN4,ANGPT1,BCL2,CAMK2D,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ET

Appendix

	GO:2000147 positive regulation of cell motility	59/401	246,2335,2625,3037,3458,3479,3592,3642,3673,3688,3965,4653,4920,5054,5295,5734,5962,6239,7048,7074,7424,7476,8650,8660,8828,8995,9734,10396,10507,10563,23122,23224,27086,54566,56729,57111,57556,64218,64750,80005,91663,153090,375056	S1,PTK2B,FGF1,FN1,GATA3,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LGALS9,MYOC,ROR2,SERPINE1,PIK3R1,PTGER4,RDX,RREB1,TGFBR2,TIAM1,VEGFC,WNT7A,NUMB,IRS2,NRP2,TNFSF18,HDAC9,ATP8A1,SEMA4D,CXCL13,CLASP2,SYNE2,FOXP1,EPB41L4B,RETN,RAB25,SEMA6A,SEMA4A,SMURF2,DOCK5,MYADM,DAB2IP,MIA3
192	9_Member GO Biological Processes GO:0010632 regulation of epithelial cell migration	-4.596064542 -2.771 31/166	284,1235,1839,1906,2113,2185,2246,2625,3037,3458,3673,5547,5793,6239,6672,7048,7424,7476,8828,9353,9734,10563,23122,23499,27086,54566,57111,64218,90627,153090,168667	ANGPT1,CCR6,HBEGF,EDN1,ETS1,PTK2B,FGF1,GATA3,HAS2,IFNG,ITGA2,PRCP,PTPRG,RREB1,SP100,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,CLASP2,MACF1,FOXP1,EPB41L4B,RAB25,SEMA4A,STARD13,DAB2IP,BMPER
193	9_Member GO Biological Processes GO:0040017 positive regulation of locomotion	-4.351574096 -2.563 60/418	81,284,596,817,1230,1235,1236,1240,1435,1436,1839,1906,1907,1956,2113,2185,2246,2335,2625,3037,3458,3479,3592,3642,3673,3688,3965,4653,4920,5054,5295,5734,5962,6239,7048,7074,7424,7476,8650,8660,8828,8995,9353,9734,10396,10507,10563,23122,23224,27086,54566,56729,57111,57556,64218,64750,80005,91663,153090,375056	ACTN4,ANGPT1,BCL2,CAMK2D,CCR1,CCR6,CCR7,CMKLR1,CSF1,CSF1R,HBEGF,EDN1,EDN2,EGFR,ETS1,PTK2B,FGF1,FN1,GATA3,HAS2,IFNG,IGF1,IL12A,INSM1,ITGA2,ITGB1,LGALS9,MYOC,ROR2,SERPINE1,PIK3R1,PTGER4,RDX,RREB1,TGFBR2,TIAM1,VEGFC,WNT7A,NUMB,IRS2,NRP2,TNFSF18,SLIT2,HDAC9,ATP8A1,SEMA4D,CXCL13,CLASP2,SYNE2,FOXP1,EPB41L4B,RETN,RAB25,SEMA6A,SEMA4A,SMURF2,DOCK5,MYADM,DAB2IP,MIA3
194	9_Member GO Biological Processes GO:0043542 endothelial cell migration	-4.053476657 -2.329 28/153	284,335,1906,1960,2113,2185,2246,2625,3397,3688,5290,5547,5728,6273,6672,6772,7424,7476,8828,9353,9734,10563,11156,27086,64218,90627,153090,168667	ANGPT1,APOA1,EDN1,EGR3,ETS1,PTK2B,FGF1,GATA3,ID1,ITGB1,PIK3CA,PRCP,PTEN,S100A2,SP100,STAT1,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,PTP4A3,FOXP1,SEMA4A,STARD13,DAB2IP,BMPER
195	9_Member GO Biological Processes GO:0010634 positive regulation of epithelial cell migration	-3.413016187 -1.807 20/103	284,1235,1839,1906,2113,2185,2625,3037,3458,3673,6239,7048,7424,7476,8828,9734,23122,27086,54566,57111	ANGPT1,CCR6,HBEGF,EDN1,ETS1,PTK2B,GATA3,HAS2,IFNG,ITGA2,RREB1,TGFBR2,VEGFC,WNT7A,NRP2,HDAC9,CLASP2,FOXP1,EPB41L4B,RAB25
196	9_Member GO Biological Processes GO:0010594 regulation of endothelial cell migration	-2.460981437 -1.105 19/114	284,1906,2113,2185,2246,2625,5547,6672,7424,7476,8828,9353,9734,10563,27086,64218,90627,153090,168667	ANGPT1,EDN1,ETS1,PTK2B,FGF1,GATA3,PRCP,SP100,VEGFC,WNT7A,NRP2,SLIT2,HDAC9,CXCL13,FOXP1,SEMA4A,STARD13,DAB2IP,BMPER
197	10_Summary GO Biological Processes GO:0006954 inflammatory response	-8.202184271 -5.768 99/646	154,301,313,329,330,335,347,558,604,814,834,922,940,943,1230,1232,1233,1234,1236,1378,1393,1435,1436,1536,2113,2157,2335,2353,2625,2693,2697,2829,3077,326	ADRB2,ANXA1,AOAH,BIRC2,BIRC3,APOA1,APOD,AXL,BCL6,CAMK4,CASP1,CD5L,CD28,TNFRSF8,CCR1,CCR3,CCR4,CCR5,CCR7,CR1,CRHBP,CSF1,CSF1R,CYBB,ETS1,F8,FN1,FOS,GATA3,GHSR,GJA1,XCR

Appendix

			9,3516,3554,3557,3559,3567,3568,3596,3600,3673,3965,4048,4142,4790,4907,5004,5054,5290,5321,5547,5734,5740,6095,6346,6672,6846,6850,7097,7292,7301,7356,7852,8111,8600,8767,8807,8995,9051,9173,9353,9435,9447,9734,10100,10125,10331,10563,11221,27086,27178,27347,29949,50848,54106,54899,55655,56300,60675,64581,79931,80762,114548,118788,149233,153090,253260,302,308,1839,1906,2054,2244,5055,5728,6239,7048,7294,8428,8573,8600,10855,23122,55576,81606,84959,526,528639,665,836,919,1235,1240,1794,1907,2040,2185,2246,2534,3592,4057,4534,5145,5305,5654,5800,6480,6720,7004,7074,7424,7709,8195,8787,9469,9844,9982,10154,10221,10507,23369,23545,51606,55079,55690,55763,57533,57556,60386,64218,80763,128866,340061,103,3481	1,HFE,HRH1,RBPJ,IL1R1,IL1RN,IL2RA,IL5,IL5RA,IL13,IL15,ITGA2,LGALS9,LTA4H,MAS1,NFKB1,NT5E,ORM1,SERPINE1,PIK3CA,PLA2G4A,PRCP,PTGER4,PTGIS,RORA,CCL1,SP100,XCL2,SYK,TLR2,TNFSF4,TYRO3,SCGB1A1,CXCR4,GPR68,TNFSF11,RIPK2,IL18RAP,TNFSF18,PSTPIP1,IL1RL1,SLIT2,CHST2,AIM2,HDAC9,TSPAN2,RASGRP1,TLR6,CXCL13,DUSP10,FOXP1,IL37,STK39,IL19,F11R,TLR9,PXK,NLRP2,IL36G,PROK2,CLEC7A,TNIP3,NDFIP1,NLRP3,PIK3AP1,IL23R,DAB2IP,RICTOR,ANXA2,ANXA5,HBEGF,EDN1,STX2,FGB,SERPINB2,PTEN,RREB1,TGFBR2,TXK,STK24,CASK,HPSE,CLASP2,STAB2,LBH,UBASH3B,ATP6V1B2,ATP6V1C1,PRDM1,BNIP3L,CASP3,CD247,CCR6,CMKLR1,DOCK2,EDN2,STOM,PTK2B,FGF1,FYN,IL12A,LTF,MTM1,PDE6A,PIP4K2A,HTRA1,PTPRO,ST6GAL1,SREBF1,TEAD4,TIAM1,VEGFC,ZBTB17,MKKS,RGS9,CHST3,ELMO1,FGFBP1,PLXNC1,TRIB1,SEMA4D,PUM2,ATP6V0A2,ATP6V1H,FEZF2,PACS1,EXOC1,TBC1D14,SEMA6A,SLC25A19,SEMA4A,SPX,CHMP4B,TMEM173,ADAR,IGF2
198	10_Member GO Biological Processes GO:0006954 inflammatory response	-8.202184271 -5.768 99/646	154,301,313,329,330,335,347,558,604,814,834,922,940,943,1230,1232,1233,1234,1236,1378,1393,1435,1436,1536,2113,2157,2335,2353,2625,2693,2697,2829,3077,3269,3516,3554,3557,3559,3567,3568,3596,3600,3673,3965,4048,4142,4790,4907,5004,5054,5290,5321,5547,5734,5740,6095,6346,6672,6846,6850,7097,7292,7301,7356,7852,8111,8600,8767,8807,8995,9051,9173,9353,9435,9447,9734,10100,10125,10331,10563,11221,27086,27178,27347,29949,50848,54106,54899,55655,56300,60675,64581,79931,80762,114548,118788,149233,153090,253260	ADRB2,ANXA1,AOAH,BIRC2,BIRC3,APOA1,APOD,AXL,BCL6,CAMK4,CASP1,CD5L,CD28,TNFRSF8,CCR1,CCR3,CCR4,CCR5,CCR7,CR1,CRHBP,CSF1,CSF1R,CYBB,ETS1,F8,FN1,FOS,GATA3,GHSR,GJA1,XCR1,HFE,HRH1,RBPJ,IL1R1,IL1RN,IL2RA,IL5,IL5RA,IL13,IL15,ITGA2,LGALS9,LTA4H,MAS1,NFKB1,NT5E,ORM1,SERPINE1,PIK3CA,PLA2G4A,PRCP,PTGER4,PTGIS,RORA,CCL1,SP100,XCL2,SYK,TLR2,TNFSF4,TYRO3,SCGB1A1,CXCR4,GPR68,TNFSF11,RIPK2,IL18RAP,TNFSF18,PSTPIP1,IL1RL1,SLIT2,CHST2,AIM2,HDAC9,TSPAN2,RASGRP1,TLR6,CXCL13,DUSP10,FOXP1,IL37,STK39,IL19,F11R,TLR9,PXK,NLRP2,IL36G,PROK2,CLEC7A,TNIP3,NDFIP1,NLRP3,PIK3AP1,IL23R,DAB2IP,RICTOR
199	10_Member GO Biological Processes GO:1903034 regulation of response to wounding	-5.873285265 -3.830 64/408	154,301,302,308,329,330,335,347,604,834,940,1236,1378,1839,1906,2054,2113,2244,2625,2693,2697,3554,3559,3600,3673,4142,4790,4907,5054,5055,5321,5728,5734,5740,6095,6239,6346,6846,6850,7048,7097,7292,7294,7301,7356,8428,8573,8600,	ADRB2,ANXA1,ANXA2,ANXA5,BIRC2,BIRC3,APOA1,APOD,BCL6,CASP1,CD28,CCR7,CR1,HBEGF,EDN1,STX2,ETS1,FGB,GATA3,GHSR,GJA1,IL1R1,IL2RA,IL15,ITGA2,MAS1,NFKB1,NT5E,SERPINE1,SERPINB2,PLA2G4A,PTEN,PTGER4,PTGIS,RORA,RREB1,CCL1,XCL2,SYK,TGFBR2,TLR2,TNFSF4,TXK,TYRO

Appendix

			8995,9173,9353,10855,11221,23122,27086,27347,54106,55576,80762,81606,84959,114548,118788,253260	3,SCGB1A1,STK24,CASK,TNFSF11,TNFSF18,IL1RL1,SLIT2,HPSE,DUSP10,CLASP2,FOXP1,STK39,TLR9,STAB2,NDFIP1,LBH,UBASH3B,NLRP3,PIK3AP1,RICTOR
200	10_Member GO Biological Processes GO:0032101 regulation of response to external stimulus	-5.439136068 -3.468 119/927	154,301,302,308,329,330,335,347,526,528,604,639,665,834,836,919,940,1230,1235,1236,1240,1378,1435,1794,1906,1907,2040,2054,2113,2185,2244,2246,2335,2534,2625,2693,2697,3554,3559,3592,3600,3673,3965,4057,4142,4534,4790,4907,5054,5055,5145,5305,5321,5654,5728,5734,5740,5800,6095,6346,6480,6720,6846,6850,7004,7048,7074,7097,7292,7294,7301,7356,7424,7709,7852,8195,8428,8573,8600,8767,8787,8995,9173,9353,9447,9469,9844,9982,10154,10221,10507,10563,10855,11221,23122,23369,23545,27086,27347,51606,54106,55079,55576,55690,55763,57533,57556,60386,64218,80762,80763,81606,84959,114548,118788,128866,149233,253260,340061	ADRB2,ANXA1,ANXA2,ANXA5,BIRC2,BIRC3,APOA1,APOD,ATP6V1B2,ATP6V1C1,BCL6,PRDM1,BNIP3L,CASP1,CASP3,CD247,CD28,CCR1,CCR6,CCR7,CMKLR1,CR1,CSF1,DOCK2,EDN1,EDN2,STOM,STX2,ETS1,PTK2B,FGB,FGF1,FN1,FYN,GATA3,GHSR,GJA1,IL1R1,IL2RA,IL12A,IL15,ITGA2,LGALS9,LTF,MAS1,MTM1,NFKB1,NT5E,SERPINE1,SERPINB2,PDE6A,PIP4K2A,PLA2G4A,HTRA1,PTEN,PTGER4,PTGIS,PTPRO,RORA,CCL1,ST6GAL1,SREBF1,XCL2,SYK,TEAD4,TGFBR2,TIAM1,TLR2,TNFSF4,TXK,TYRO3,SCGB1A1,VEGFC,ZBTB17,CXCR4,MKKS,STK24,CASK,TNFSF11,RIPK2,RGS9,TNFSF18,IL1RL1,SLIT2,AIM2,CHST3,ELMO1,FGFBP1,PLXNC1,TRIB1,SEMA4D,CXCL13,HPSE,DUSP10,CLASP2,PUM2,ATP6V0A2,FOXP1,STK39,ATP6V1H,TLR9,FEZF2,STAB2,PACS1,EXOC1,TBC1D14,SEMA6A,SLC25A19,SEMA4A,NDFIP1,SPX,LBH,UBASH3B,NLRP3,PIK3AP1,CHMP4B,IL23R,RICTOR,TMEM173
201	10_Member GO Biological Processes GO:0050727 regulation of inflammatory response	-3.865579656 -2.164 44/291	154,301,329,330,335,347,604,834,940,1236,1378,2113,2625,2693,3554,3559,3600,3673,4142,4790,4907,5054,5321,5734,5740,6095,6346,6846,7097,7292,7301,7356,8600,8995,9173,9353,11221,27086,27347,54106,80762,114548,118788,253260	ADRB2,ANXA1,BIRC2,BIRC3,APOA1,APOD,BCL6,CASP1,CD28,CCR7,CR1,ETS1,GATA3,GHSR,IL1R1,IL2RA,IL15,ITGA2,MAS1,NFKB1,NT5E,SERPINE1,PLA2G4A,PTGER4,PTGIS,RORA,CCL1,XCL2,TLR2,TNFSF4,TYRO3,SCGB1A1,TNFSF11,TNFSF18,IL1RL1,SLIT2,DUSP10,FOXP1,STK39,TLR9,NDFIP1,NLRP3,PIK3AP1,RICTOR
202	10_Member GO Biological Processes GO:0032102 negative regulation of response to external stimulus	-3.851059669 -2.153 42/274	154,302,308,335,347,639,1906,2113,2244,2625,2693,2697,3559,4057,4534,4790,4907,5054,5055,5654,5728,5734,5740,5800,6095,6480,7301,8195,8573,9353,10221,10507,10563,11221,23122,57556,64218,80762,80763,84959,114548,128866	ADRB2,ANXA2,ANXA5,APOA1,APOD,PRDM1,EDN1,ETS1,FGB,GATA3,GHSR,GJA1,IL2RA,LTF,MTM1,NFKB1,NT5E,SERPINE1,SERPINB2,HTRA1,PTEN,PTGER4,PTGIS,PTPRO,RORA,ST6GAL1,TYRO3,MKKS,CASK,SLIT2,TRIB1,SEMA4D,CXCL13,DUSP10,CLASP2,SEMA6A,SEMA4A,NDFIP1,SPX,UBASH3B,NLRP3,CHMP4B
203	10_Member GO Biological Processes GO:1903035 negative regulation of	-3.4277688 -1.813 27/158	154,302,308,335,347,1906,2113,2244,2625,2693,2697,3559,4790,4907,5054,5055,5734,5740,6095,7301,8573,9353,11221,23122,80762,84959,114548	ADRB2,ANXA2,ANXA5,APOA1,APOD,EDN1,ETS1,FGB,GATA3,GHSR,GJA1,IL2RA,NFKB1,NT5E,SERPINE1,SERPINB2,PTGER4,PTGIS,RORA,TYRO3,CASK,SLIT2,DUSP10,CLASP2,NDFIP1,UBASH3B,NLRP3

Appendix

	response to wounding			
204	10_Member GO Biological Processes GO:0050728 negative regulation of inflammatory response	-2.349077257 -1.020 17/100	154,335,347,2113,2625,2693,3559,4790,4907,5734,5740,6095,7301,9353,11221,80762,114548	ADRB2,APOA1,APOD,ETS1,GATA3,GHSR,IL2RA,NFKB1,NT5E,PTGER4,PTGIS,RORA,TYRO3,SLIT2,DUSP10,NDFIP1,NLRP3
205	10_Member GO Biological Processes GO:0031348 negative regulation of defense response	-2.283759191 -0.976 22/144	103,154,335,347,1378,2113,2625,2693,3481,3559,3965,4790,4907,5654,5734,5740,6095,7301,9353,11221,80762,114548	ADAR,ADRB2,APOA1,APOD,CR1,ETS1,GATA3,GHSR,IGF2,IL2RA,LGALS9,NFKB1,NT5E,HTRA1,PTGER4,PTGIS,RORA,TYRO3,SLIT2,DUSP10,NDFIP1,NLRP3
206	11_Summary GO Biological Processes GO:0030099 myeloid cell differentiation	-7.670783128 -5.301 60/333	103,302,537,604,814,836,841,861,942,1021,1030,1230,1236,1435,1436,1437,1499,2034,2113,2185,2353,2625,2672,3458,3516,3567,3624,3635,3965,4057,4208,5295,5305,6670,7048,7049,7071,7704,8111,8600,9290,9855,10100,10221,10296,10320,23543,27086,28996,29909,51208,51621,54790,55904,80762,84456,84959,85477,115727,149233,301,1540,7292,8995	ADAR,ANXA2,ATP6AP1,BCL6,CAMK4,CASP3,CASP8,RUNX1,CD86,CDK6,CDKN2B,CCR1,CCR7,CSF1,CSF1R,CSF2,CTNNB1,EPAS1,ETS1,PTK2B,FOS,GATA3,GFI1,IFNG,RBPJ,IL5,INHBA,INPP5D,LGALS9,LTF,MEF2C,PIK3R1,PIP4K2A,SP3,TGFBR2,TGFBR3,KLF10,ZBTB16,GPR68,TNFSF11,GPR55,FARP2,TSPAN2,TRIB1,MAEA,IKZF1,RBFOX2,FOXP1,HIPK2,GPR171,CLDN18,KLF13,TET2,KMT2E,NDFIP1,L3MBTL3,UBASH3B,SCIN,RASGRP4,IL23R,ANXA1,CYLD,TNFSF4,TNFSF18
207	11_Member GO Biological Processes GO:0030099 myeloid cell differentiation	-7.670783128 -5.301 60/333	103,302,537,604,814,836,841,861,942,1021,1030,1230,1236,1435,1436,1437,1499,2034,2113,2185,2353,2625,2672,3458,3516,3567,3624,3635,3965,4057,4208,5295,5305,6670,7048,7049,7071,7704,8111,8600,9290,9855,10100,10221,10296,10320,23543,27086,28996,29909,51208,51621,54790,55904,80762,84456,84959,85477,115727,149233	ADAR,ANXA2,ATP6AP1,BCL6,CAMK4,CASP3,CASP8,RUNX1,CD86,CDK6,CDKN2B,CCR1,CCR7,CSF1,CSF1R,CSF2,CTNNB1,EPAS1,ETS1,PTK2B,FOS,GATA3,GFI1,IFNG,RBPJ,IL5,INHBA,INPP5D,LGALS9,LTF,MEF2C,PIK3R1,PIP4K2A,SP3,TGFBR2,TGFBR3,KLF10,ZBTB16,GPR68,TNFSF11,GPR55,FARP2,TSPAN2,TRIB1,MAEA,IKZF1,RBFOX2,FOXP1,HIPK2,GPR171,CLDN18,KLF13,TET2,KMT2E,NDFIP1,L3MBTL3,UBASH3B,SCIN,RASGRP4,IL23R
208	11_Member GO Biological Processes GO:0002573 myeloid leukocyte differentiation	-6.889155356 -4.636 39/187	302,537,814,841,861,942,1230,1236,1435,1436,1437,1499,2353,2625,3458,3516,3567,3624,3635,3965,4057,4208,5295,6670,7048,7071,8111,8600,9290,9855,10100,10221,27086,51208,55904,80762,84456,84959,149233	ANXA2,ATP6AP1,CAMK4,CASP8,RUNX1,CD86,CCR1,CCR7,CSF1,CSF1R,CSF2,CTNNB1,FOS,GATA3,IFNG,RBPJ,IL5,INHBA,INPP5D,LGALS9,LTF,MEF2C,PIK3R1,SP3,TGFBR2,KLF10,GPR68,TNFSF11,GPR55,FARP2,TSPAN2,TRIB1,FOXP1,CLDN18,KMT2E,NDFIP1,L3MBTL3,UBASH3B,IL23R
209	11_Member GO Biological Processes GO:0002761 regulation of myeloid	-6.037012096 -3.962 26/108	537,814,841,861,1230,1435,1436,1499,2353,3458,3567,3624,3635,4057,5295,7071,8111,8600,9290,10221,27086,51208,55904,80762,84959,149233	ATP6AP1,CAMK4,CASP8,RUNX1,CCR1,CSF1,CSF1R,CTNNB1,FOS,IFNG,IL5,INHBA,INPP5D,LTF,PIK3R1,KLF10,GPR68,TNFSF11,GPR55,TRIB1,FOXP1,CLDN18,KMT2E,NDFIP1,UBASH3B,IL23R

Appendix

	leukocyte differentiation			
210	11_Member GO Biological Processes GO:0045670 regulation of osteoclast differentiation	-5.49488602 -3.514 18/63	537,814,1230,1435,1436,1499,2353,3458,3635,4057,5295,7071,8111,8600,9290,51208,84959,149233	ATP6AP1,CAMK4,CCR1,CSF1,CSF1R,CTNNB1,FOS,IFNG,INPP5D,LTF,PIK3R1,KLF10,GPR68,TNFSF11,GPR55,CLDN18,UBASH3B,IL23R
211	11_Member GO Biological Processes GO:0045637 regulation of myeloid cell differentiation	-5.343829348 -3.384 35/183	537,814,841,861,1021,1230,1435,1436,1499,2113,2185,2353,3458,3567,3624,3635,4057,4208,5295,7071,7704,8111,8600,9290,10221,23543,27086,29909,51208,51621,55904,80762,84959,85477,149233	ATP6AP1,CAMK4,CASP8,RUNX1,CDK6,CCR1,CSF1,CSF1R,CTNNB1,ETS1,PTK2B,FOS,IFNG,IL5,INHBA,INPP5D,LTF,MEF2C,PIK3R1,KLF10,ZBTB16,GPR68,TNFSF11,GPR55,TRIB1,RBFOX2,FOXP1,GPR171,CLDN18,KLF13,KMT2E,NDFIP1,UBASH3B,SCIN,IL23R
212	11_Member GO Biological Processes GO:0030316 osteoclast differentiation	-5.10382991 -3.179 21/86	302,537,814,1230,1435,1436,1499,2353,3458,3635,4057,5295,7071,8111,8600,9290,9855,27086,51208,84959,149233	ANXA2,ATP6AP1,CAMK4,CCR1,CSF1,CSF1R,CTNNB1,FOS,IFNG,INPP5D,LTF,PIK3R1,KLF10,GPR68,TNFSF11,GPR55,FARP2,FOXP1,CLDN18,UBASH3B,IL23R
213	11_Member GO Biological Processes GO:0002763 positive regulation of myeloid leukocyte differentiation	-4.963529231 -3.062 15/50	537,841,861,1230,1435,1436,2353,3458,3567,7071,8111,8600,10221,55904,149233	ATP6AP1,CASP8,RUNX1,CCR1,CSF1,CSF1R,FOS,IFNG,IL5,KLF10,GPR68,TNFSF11,TRIB1,KMT2E,IL23R
214	11_Member GO Biological Processes GO:0045672 positive regulation of osteoclast differentiation	-4.899313681 -3.013 10/24	537,1230,1435,1436,2353,3458,7071,8111,8600,149233	ATP6AP1,CCR1,CSF1,CSF1R,FOS,IFNG,KLF10,GPR68,TNFSF11,IL23R
215	11_Member GO Biological Processes GO:0045639 positive regulation of myeloid cell differentiation	-4.415259056 -2.620 19/81	537,841,861,1230,1435,1436,2113,2353,3458,3567,3624,3635,7071,8111,8600,10221,55904,85477,149233	ATP6AP1,CASP8,RUNX1,CCR1,CSF1,CSF1R,ETS1,FOS,IFNG,IL5,INHBA,INPP5D,KLF10,GPR68,TNFSF11,TRIB1,KMT2E,SCIN,IL23R
216	11_Member GO Biological Processes GO:0002762 negative regulation of myeloid leukocyte differentiation	-3.040178303 -1.521 11/44	861,1499,3624,3635,4057,5295,8111,9290,10221,51208,84959	RUNX1,CTNNB1,INHBA,INPP5D,LTF,PIK3R1,GPR68,GPR55,TRIB1,CLDN18,UBASH3B
217	11_Member GO Biological Processes	-2.869695413 -1.403	301,604,861,1499,1540,3624,3635,4057,5295,7292,8111,8995,9290,10221,51208,84	ANXA1,BCL6,RUNX1,CTNNB1,CYLD,INHBA,INPP5D,LTF,PIK3R1,TNFSF4,GPR68,TNFSF18,GPR55,TRIB

Appendix

	GO:1902106 negative regulation of leukocyte differentiation	16/82	959	1,CLDN18,UBASH3B
218	11_Member GO Biological Processes GO:0045638 negative regulation of myeloid cell differentiation	-2.75506399 -1.319 16/84	861,1021,1499,2185,3624,3635,4057,5295,7704,8111,9290,10221,29909,51208,51621,84959	RUNX1,CDK6,CTNNB1,PTK2B,INHBA,INPP5D,LTF,PIK3R1,ZBTB16,GPR68,GPR55,TRIB1,GPR171,CLDN18,KLF13,UBASH3B
219	11_Member GO Biological Processes GO:1903707 negative regulation of hemopoiesis	-2.575107107 -1.188 21/128	301,604,861,1021,1499,1540,2185,3624,3635,4057,5295,7292,7704,8111,8995,9290,10221,29909,51208,51621,84959	ANXA1,BCL6,RUNX1,CDK6,CTNNB1,CYLD,PTK2B,INHBA,INPP5D,LTF,PIK3R1,TNFSF4,ZBTB16,GPR68,TNFSF18,GPR55,TRIB1,GPR171,CLDN18,KLF13,UBASH3B
220	11_Member GO Biological Processes GO:0045671 negative regulation of osteoclast differentiation	-2.406654493 -1.065 7/25	1499,3635,4057,5295,9290,51208,84959	CTNNB1,INPP5D,LTF,PIK3R1,GPR55,CLDN18,UBASH3B
221	12_Summary GO Biological Processes GO:0001816 cytokine production	-7.544437793 -5.188 95/630	284,301,329,330,335,347,558,604,814,834,861,914,940,942,943,965,1149,1236,1240,1436,1437,1499,1540,2335,2625,2693,3077,3458,3516,3568,3592,3595,3596,3600,3624,3635,3663,3702,3937,3965,4057,4094,4773,4790,5004,5054,5142,5144,5295,5591,5734,6095,6441,6504,6711,6850,6885,7097,7292,7294,7356,7520,8320,8651,8710,8767,9173,9208,9308,9447,10125,10333,10666,10855,11148,23369,23607,26137,26191,26287,27086,27297,28951,29949,50848,54106,54206,55122,55655,57162,80762,114548,149233,192683,340061,308,322,777,1946,2244,2697,2864,3033,3382,3479,3567,3708,3709,3783,4082,5122,5289,5567,6720,7074,7424,8111,8660,55763,80024,399687,537,1808,1906,2668,2692,3814,5021,8600,9146,23122,56729,57829,151056,54843,84236,85477	ANGPT1,ANXA1,BIRC2,BIRC3,APOA1,APOD,AXL,BCL6,CAMK4,CASP1,RUNX1,CD2,CD28,CD86,TNFRSF8,CD58,CIDEA,CCR7,CMKLR1,CSF1R,CSF2,CTNNB1,CYLD,FN1,GATA3,GHSR,HFE,IFNG,RBPJ,IL5RA,IL12A,IL12RB2,IL13,IL15,INHBA,INPP5D,IRF5,ITK,LCP2,LGALS9,LTF,MAF,NFATC2,NFKB1,ORM1,SERPINE1,PDE4B,PDE4D,PIK3R1,PRKDC,PTGER4,RORA,SFTPD,SLAMF1,SPTBN1,SYK,MAP3K7,TLR2,TNFSF4,TXK,SCGB1A1,XRCC5,EOMES,SOCS1,SERPINB7,RIPK2,IL1RL1,LRRFIP1,CD83,AIM2,RASGRP1,TLR6,CD226,HPSE,HHLA2,PUM2,CD2AP,ZBTB20,PTPN22,ANKRD2,FOXP1,CRCP,TRIB2,IL19,F11R,TLR9,ERRFI1,AKIRIN2,NLRP2,PELI1,NDFIP1,NLRP3,IL23R,SCAMP5,TMEM173,ANXA5,APBB1,CACNA1E,EFNA5,FGB,GJA1,FFAR1,HADH,ICA1,IGF1,IL5,ITPR1,ITPR2,KCNN4,MARCKS,PCSK1,PIK3C3,PRKACB,SREBF1,TIAM1,VEGFC,GPR68,IRS2,EXOC1,SLC8B1,MYO18A,ATP6AP1,DPYSL2,EDN1,GDNF,GHRHR,KISS1,OXTR,TNFSF11,HGS,CLASP2,RETN,ZP4,PLB1,SYTL2,RHBDD1,SCIN
222	12_Member	-7.544437793	284,301,329,330,335,347,558,604,814,834	ANGPT1,ANXA1,BIRC2,BIRC3,APOA1,APOD,AXL,

Appendix

	GO Biological Processes GO:0001816 cytokine production	-5.188 95/630	,861,914,940,942,943,965,1149,1236,1240,1436,1437,1499,1540,2335,2625,2693,3077,3458,3516,3568,3592,3595,3596,3600,3624,3635,3663,3702,3937,3965,4057,4094,4773,4790,5004,5054,5142,5144,5295,5591,5734,6095,6441,6504,6711,6850,6885,7097,7292,7294,7356,7520,8320,8651,8710,8767,9173,9208,9308,9447,10125,10333,10666,10855,11148,23369,23607,26137,26191,26287,27086,27297,28951,29949,50848,54106,54206,55122,55655,57162,80762,114548,149233,192683,340061	BCL6,CAMK4,CASP1,RUNX1,CD2,CD28,CD86,TNFRSF8,CD58,CIDEA,CCR7,CMKLR1,CSF1R,CSF2,CTNNB1,CYLD,FN1,GATA3,GHSR,HFE,IFNG,RBPJ,IL5RA,IL12A,IL12RB2,IL13,IL15,INHBA,INPP5D,IRF5,ITK,LCP2,LGALS9,LTF,MAF,NFATC2,NFKB1,ORM1,SERPINE1,PDE4B,PDE4D,PIK3R1,PRKDC,PTGER4,RORA,SFTPD,SLAMF1,SPTBN1,SYK,MAP3K7,TLR2,TNFSF4,TXK,SCGB1A1,XRCC5,EOMES,SOCS1,SERPINB7,RIPK2,IL1RL1,LRRFIP1,CD83,AIM2,RASGRP1,TLR6,CD226,HPSE,HHLA2,PUM2,CD2AP,ZBTB20,PTPN22,ANKRD2,FOXP1,CRCP,TRIB2,IL19,F11R,TLR9,ERRFI1,AKIRIN2,NLRP2,PELI1,NDFIP1,NLRP3,IL23R,SCAMP5,TMEM173
223	12_Member GO Biological Processes GO:0001817 regulation of cytokine production	-6.864047795 -4.622 86/571	284,301,329,330,335,347,558,604,834,861,914,940,942,943,965,1149,1236,1240,1436,1437,1499,1540,2335,2625,2693,3077,3458,3568,3592,3595,3596,3600,3624,3635,3663,3965,4057,4790,5004,5054,5142,5144,5295,5591,5734,6095,6441,6504,6711,6850,6885,7097,7292,7294,7356,7520,8651,8710,8767,9173,9208,9308,9447,10125,10333,10855,11148,23369,23607,26137,26191,26287,27086,27297,28951,50848,54106,54206,55122,55655,57162,80762,114548,149233,192683,340061	ANGPT1,ANXA1,BIRC2,BIRC3,APOA1,APOD,AXL,BCL6,CASP1,RUNX1,CD2,CD28,CD86,TNFRSF8,CD58,CIDEA,CCR7,CMKLR1,CSF1R,CSF2,CTNNB1,CYLD,FN1,GATA3,GHSR,HFE,IFNG,IL5RA,IL12A,IL12RB2,IL13,IL15,INHBA,INPP5D,IRF5,LGALS9,LTF,NFKB1,ORM1,SERPINE1,PDE4B,PDE4D,PIK3R1,PRKDC,PTGER4,RORA,SFTPD,SLAMF1,SPTBN1,SYK,MAP3K7,TLR2,TNFSF4,TXK,SCGB1A1,XRCC5,SOCS1,SERPINB7,RIPK2,IL1RL1,LRRFIP1,CD83,AIM2,RASGRP1,TLR6,HPSE,HHLA2,PUM2,CD2AP,ZBTB20,PTPN22,ANKRD2,FOXP1,CRCP,TRIB2,F11R,TLR9,ERRFI1,AKIRIN2,NLRP2,PELI1,NDFIP1,NLRP3,IL23R,SCAMP5,TMEM173
224	12_Member GO Biological Processes GO:0050707 regulation of cytokine secretion	-5.131517228 -3.199 30/149	284,301,335,834,914,965,1149,1236,1436,2335,2625,3458,3965,5734,6504,6711,6850,7097,7292,8651,9173,9447,10125,10333,26191,27086,54106,55655,114548,192683	ANGPT1,ANXA1,APOA1,CASP1,CD2,CD58,CIDEA,CCR7,CSF1R,FN1,GATA3,IFNG,LGALS9,PTGER4,SLAMF1,SPTBN1,SYK,TLR2,TNFSF4,SOCS1,IL1RL1,AIM2,RASGRP1,TLR6,PTPN22,FOXP1,TLR9,NLRP2,NLRP3,SCAMP5
225	12_Member GO Biological Processes GO:0001819 positive regulation of cytokine production	-5.029864207 -3.112 60/397	301,329,330,834,861,914,940,942,943,965,1236,1436,1437,1499,2625,3458,3592,3595,3596,3600,3663,3965,4790,5054,5142,5144,5295,5591,5734,6095,6504,6711,6850,6885,7097,7292,7294,7520,8710,8767,9173,9208,9308,9447,10125,10333,10855,11148,23369,26137,26191,27297,54106,55122,55655,57162,114548,149233,192683,340061	ANXA1,BIRC2,BIRC3,CASP1,RUNX1,CD2,CD28,CD86,TNFRSF8,CD58,CCR7,CSF1R,CSF2,CTNNB1,GATA3,IFNG,IL12A,IL12RB2,IL13,IL15,IRF5,LGALS9,NFKB1,SERPINE1,PDE4B,PDE4D,PIK3R1,PRKDC,PTGER4,RORA,SLAMF1,SPTBN1,SYK,MAP3K7,TLR2,TNFSF4,TXK,XRCC5,SERPINB7,RIPK2,IL1RL1,LRRFIP1,CD83,AIM2,RASGRP1,TLR6,HPSE,HHLA2,PUM2,ZBTB20,PTPN22,CRCP,TLR9,AKIRIN2,NLRP2,PELI1,NLRP3,IL23R,SCAMP5,TMEM173

Appendix

226	12_Member GO Biological Processes GO:0050663 cytokine secretion	-4.849911096 -2.977 32/169	284,301,335,834,914,965,1149,1236,1436,2335,2625,3458,3516,3937,3965,5734,6504,6711,6850,7097,7292,8651,9173,9447,10125,10333,26191,27086,54106,55655,114548,192683	ANGPT1,ANXA1,APOA1,CASP1,CD2,CD58,CIDEA,CCR7,CSF1R,FN1,GATA3,IFNG,RBPJ,LCP2,LGALS9,PTGER4,SLAMF1,SPTBN1,SYK,TLR2,TNFSF4,SOCS1,IL1RL1,AIM2,RASGRP1,TLR6,PTPN22,FOXP1,TLR9,NLRP2,NLRP3,SCAMP5
227	12_Member GO Biological Processes GO:0001818 negative regulation of cytokine production	-4.531145115 -2.712 37/215	284,301,335,347,558,604,1149,1240,1540,2335,2625,2693,3077,3458,3592,3596,3624,3635,3965,4057,4790,5004,5734,6441,6504,7292,7356,9173,9308,23607,26191,28951,54106,54206,80762,114548,149233	ANGPT1,ANXA1,APOA1,APOD,AXL,BCL6,CIDEA,CMKLR1,CYLD,FN1,GATA3,GHSR,HFE,IFNG,IL12A,IL13,INHBA,INPP5D,LGALS9,LTF,NFKB1,ORM1,PTGER4,SFTPD,SLAMF1,TNFSF4,SCGB1A1,IL1RL1,CD83,CD2AP,PTPN22,TRIB2,TLR9,ERRFI1,NDFIP1,NLRP3,IL23R
228	12_Member GO Biological Processes GO:0050708 regulation of protein secretion	-4.169348012 -2.412 58/406	284,301,308,322,335,777,834,914,965,1149,1236,1436,1946,2244,2335,2625,2693,2697,2864,3033,3382,3458,3479,3567,3596,3708,3709,3783,3965,4082,5122,5289,5567,5734,6504,6711,6720,6850,7074,7097,7292,7424,8111,8651,8660,9173,9447,10125,10333,26191,27086,54106,55655,55763,80024,114548,192683,399687	ANGPT1,ANXA1,ANXA5,APBB1,APOA1,CACNA1E,CASP1,CD2,CD58,CIDEA,CCR7,CSF1R,EFNA5,FGB,FN1,GATA3,GHSR,GJA1,FFAR1,HADH,ICA1,IFNG,IGF1,IL5,IL13,ITPR1,ITPR2,KCNN4,LGALS9,MARCKS,PCSK1,PIK3C3,PRKACB,PTGER4,SLAMF1,SPTBN1,SREBF1,SYK,TIAM1,TLR2,TNFSF4,VEGFC,GPR68,SOCS1,IRS2,IL1RL1,AIM2,RASGRP1,TLR6,PTPN22,FOXP1,TLR9,NLRP2,EXOC1,SLC8B1,NLRP3,SCAMP5,MYO18A
229	12_Member GO Biological Processes GO:1903532 positive regulation of secretion by cell	-3.23490392 -1.664 47/338	322,537,834,914,965,1436,1808,1906,2244,2625,2668,2692,2697,2864,3077,3458,3479,3567,3596,3624,3783,3814,3965,5021,5734,6504,6711,6850,7292,7424,8111,8600,8660,9146,9173,9447,10125,23122,26191,55655,55763,56729,57829,114548,151056,192683,399687	APBB1,ATP6AP1,CASP1,CD2,CD58,CSF1R,DPYSL2,EDN1,FGB,GATA3,GDNF,GHRHR,GJA1,FFAR1,HFE,IFNG,IGF1,IL5,IL13,INHBA,KCNN4,KISS1,LGALS9,OXTR,PTGER4,SLAMF1,SPTBN1,SYK,TNFSF4,VEGFC,GPR68,TNFSF11,IRS2,HGS,IL1RL1,AIM2,RASGRP1,CLASP2,PTPN22,NLRP2,EXOC1,RETN,ZP4,NLRP3,PLB1,SCAMP5,MYO18A
230	12_Member GO Biological Processes GO:0051047 positive regulation of secretion	-3.196622615 -1.640 50/367	322,537,834,914,965,1436,1808,1906,2244,2625,2668,2692,2697,2864,3077,3458,3479,3567,3596,3624,3783,3814,3965,5021,5734,6504,6711,6850,7292,7424,8111,8600,8660,9146,9173,9447,10125,23122,26191,54843,55655,55763,56729,57829,84236,85477,114548,151056,192683,399687	APBB1,ATP6AP1,CASP1,CD2,CD58,CSF1R,DPYSL2,EDN1,FGB,GATA3,GDNF,GHRHR,GJA1,FFAR1,HFE,IFNG,IGF1,IL5,IL13,INHBA,KCNN4,KISS1,LGALS9,OXTR,PTGER4,SLAMF1,SPTBN1,SYK,TNFSF4,VEGFC,GPR68,TNFSF11,IRS2,HGS,IL1RL1,AIM2,RASGRP1,CLASP2,PTPN22,SYTL2,NLRP2,EXOC1,RETN,ZP4,RHBDD1,SCIN,NLRP3,PLB1,SCAMP5,MYO18A
231	12_Member GO Biological Processes GO:0050714 positive regulation of protein secretion	-3.073927265 -1.549 32/209	322,834,914,965,1436,2244,2625,2697,2864,3458,3479,3567,3596,3783,3965,5734,6504,6711,6850,7292,7424,8111,8660,9173,9447,10125,26191,55655,55763,114548,192683,399687	APBB1,CASP1,CD2,CD58,CSF1R,FGB,GATA3,GJA1,FFAR1,IFNG,IGF1,IL5,IL13,KCNN4,LGALS9,PTGER4,SLAMF1,SPTBN1,SYK,TNFSF4,VEGFC,GPR68,IRS2,IL1RL1,AIM2,RASGRP1,PTPN22,NLRP2,EXOC1,NLRP3,SCAMP5,MYO18A
232	12_Member	-2.945701662	834,914,965,1436,2625,3458,3965,5734,6	CASP1,CD2,CD58,CSF1R,GATA3,IFNG,LGALS9,PTG

Appendix

	GO Biological Processes GO:0050715 positive regulation of cytokine secretion	-1.455 18/96	504,6711,6850,9173,9447,10125,26191,55655,114548,192683	ER4,SLAMF1,SPTBN1,SYK,IL1RL1,AIM2,RASGRP1,PTPN22,NLRP2,NLRP3,SCAMP5
233	13_Summary Hallmark Gene Sets M5942 HALLMARK UV RESPONSE DN	-7.461587051 -5.112 34/144	302,323,493,594,861,2534,2697,2908,3037,3382,3397,3638,3778,4092,4790,4853,5054,5332,5728,5922,6310,6383,7035,7048,7049,8553,8621,8821,8997,10000,10395,10659,23266,26037	ANXA2,APBB2,ATP2B4,BCKDHB,RUNX1,FYN,GJA1,NR3C1,HAS2,ICA1,ID1,INSIG1,KCNMA1,SMAD7,NFKB1,NOTCH2,SERPINE1,PLCB4,PTEN,RASA2,ATXN1,SDC2,TFPI,TGFBR2,TGFBR3,BHLHE40,CDK13,INPP4B,KALRN,AKT3,DLC1,CELF2,ADGRL2,SIPA1L1
234	13_Member Hallmark Gene Sets M5942 HALLMARK UV RESPONSE DN	-7.461587051 -5.112 34/144	302,323,493,594,861,2534,2697,2908,3037,3382,3397,3638,3778,4092,4790,4853,5054,5332,5728,5922,6310,6383,7035,7048,7049,8553,8621,8821,8997,10000,10395,10659,23266,26037	ANXA2,APBB2,ATP2B4,BCKDHB,RUNX1,FYN,GJA1,NR3C1,HAS2,ICA1,ID1,INSIG1,KCNMA1,SMAD7,NFKB1,NOTCH2,SERPINE1,PLCB4,PTEN,RASA2,ATXN1,SDC2,TFPI,TGFBR2,TGFBR3,BHLHE40,CDK13,INPP4B,KALRN,AKT3,DLC1,CELF2,ADGRL2,SIPA1L1
235	14_Summary GO Biological Processes GO:0051241 negative regulation of multicellular organismal process	-7.262007047 -4.943 133/985	154,284,301,302,308,335,347,356,374,476,493,558,596,604,753,861,942,1021,1149,1230,1240,1393,1407,1499,1540,1831,1906,2146,2185,2244,2296,2335,2625,2672,2693,2697,2796,3077,3397,3458,3479,3516,3592,3596,3624,3635,3688,3965,4000,4057,4092,4208,4435,4488,4790,5004,5021,5054,5055,5142,5144,5295,5567,5641,5728,5734,5793,5800,6423,6441,6504,6653,6672,6772,7048,7292,7356,7476,7490,7498,7704,7849,8111,8626,8898,8994,8995,9173,9290,9308,9353,10140,10221,10507,10539,10549,10563,11221,22856,22882,23607,25871,26191,27347,28951,29116,29909,30812,51208,51316,51621,54106,54206,55079,55223,55755,57534,57556,64218,80005,80014,80762,80763,81618,84959,85458,90627,114548,149233,152559,153090,162514,255738	ADRB2,ANGPT1,ANXA1,ANXA2,ANXA5,APOA1,APOD,FASLG,AREG,ATP1A1,ATP2B4,AXL,BCL2,BCL6,LDLRAD4,RUNX1,CD86,CDK6,CIDEA,CCR1,CMKLR1,CRHBP,CRY1,CTNNB1,CYLD,TSC22D3,EDN1,EZH2,PTK2B,FGB,FOXC1,FN1,GATA3,GFI1,GHSR,GJA1,GNRH1,HFE,ID1,IFNG,IGF1,RBPJ,IL12A,IL13,INHBA,INPP5D,ITGB1,LGALS9,LMNA,LTF,SMAD7,MEF2C,CITED1,MSX2,NFKB1,ORM1,OXTR,SERPINE1,SERPINB2,PDE4B,PDE4D,PIK3R1,PRKACB,LGMN,PTEN,PTGER4,PTPRG,PTPRO,SFRP2,SFTPD,SLAMF1,SORL1,SP100,STAT1,TGFBR2,TNFSF4,SCGB1A1,WNT7A,WT1,XDH,ZBTB16,PAX8,GPR68,TP63,MTMR2,LIMD1,TNFSF18,IL1RL1,GPR55,CD83,SLIT2,TOB1,TRIB1,SEMA4D,GLRX3,PRDX4,CXCL13,DUSP10,CHSY1,ZHX2,CD2AP,C3orf17,PTPN22,STK39,TRIB2,MYLIP,GPR171,SOX8,CLDN18,PLAC8,KLF13,TLR9,ERRFI1,FEZF2,TRIM62,CDK5RAP2,MIB1,SEMA6A,SEMA4A,DOCK5,WWC2,NDFIP1,SPX,ITM2C,UBASH3B,DIXDC1,STARD13,NLRP3,IL23R,PAQR3,DAB2IP,TRPV3,PCSK9
236	14_Member GO Biological Processes GO:0051241 negative regulation of	-7.262007047 -4.943 133/985	154,284,301,302,308,335,347,356,374,476,493,558,596,604,753,861,942,1021,1149,1230,1240,1393,1407,1499,1540,1831,1906,2146,2185,2244,2296,2335,2625,2672,2	ADRB2,ANGPT1,ANXA1,ANXA2,ANXA5,APOA1,APOD,FASLG,AREG,ATP1A1,ATP2B4,AXL,BCL2,BCL6,LDLRAD4,RUNX1,CD86,CDK6,CIDEA,CCR1,CMKLR1,CRHBP,CRY1,CTNNB1,CYLD,TSC22D3,EDN1

Appendix

	multicellular organismal process		693,2697,2796,3077,3397,3458,3479,3516,3592,3596,3624,3635,3688,3965,4000,4057,4092,4208,4435,4488,4790,5004,5021,5054,5055,5142,5144,5295,5567,5641,5728,5734,5793,5800,6423,6441,6504,6653,6672,6772,7048,7292,7356,7476,7490,7498,7704,7849,8111,8626,8898,8994,8995,9173,9290,9308,9353,10140,10221,10507,10539,10549,10563,11221,22856,22882,23607,25871,26191,27347,28951,29116,29909,30812,51208,51316,51621,54106,54206,55079,55223,55755,57534,57556,64218,80005,80014,80762,80763,81618,84959,85458,90627,114548,149233,152559,153090,162514,255738	,EZH2,PTK2B,FGB,FOXC1,FN1,GATA3,GFI1,GHSR,GJA1,GNRH1,HFE,ID1,IFNG,IGF1,RBPJ,IL12A,IL13,INHBA,INPP5D,ITGB1,LGALS9,LMNA,LTF,SMAD7,MEF2C,CITED1,MSX2,NFKB1,ORM1,OXTR,SERPINE1,SERPINB2,PDE4B,PDE4D,PIK3R1,PRKACB,LGMN,PTEN,PTGER4,PTPRG,PTPRO,SFRP2,SFTPD,SLAMF1,SORL1,SP100,STAT1,TGFBR2,TNFSF4,SCGB1A1,WNT7A,WT1,XDH,ZBTB16,PAX8,GPR68,TP63,MTMR2,LIMD1,TNFSF18,IL1RL1,GPR55,CD83,SLIT2,TOB1,TRIB1,SEMA4D,GLRX3,PRDX4,CXCL13,DUSP10,CHSY1,ZHX2,CD2AP,C3orf17,PTPN22,STK39,TRIB2,MYLIP,GPR171,SOX8,CLDN18,PLAC8,KLF13,TLR9,ERRFI1,FEZF2,TRIM62,CDK5RAP2,MIB1,SEMA6A,SEMA4A,DOCK5,WWC2,NDFIP1,SPX,ITM2C,UBASH3B,DIXDC1,STARD13,NLRP3,IL23R,PAQR3,DAB2IP,TRPV3,PCSK9
237	15_Summary GO Biological Processes GO:0048514 blood vessel morphogenesis	-7.117296743 -4.838 77/486	154,284,302,347,356,639,677,688,861,1232,1499,1906,1960,2028,2034,2113,2185,2246,2296,2335,2697,3037,3397,3516,3688,3716,3911,4092,4313,4435,4897,5054,5290,5547,5579,5728,5734,5740,5921,6095,6423,6461,6672,6733,6772,6850,7048,7424,7476,7490,7498,8828,9353,9369,9394,9734,10267,10352,10451,10563,10672,10855,10908,11082,25976,28996,55576,60675,64218,79625,83605,90627,92140,146850,153090,168667,284340,4208,4693,5784,7798,8061,8710,54206,57534,79659	ADRB2,ANGPT1,ANXA2,APOD,FASLG,PRDM1,ZFP36L1,KLF5,RUNX1,CCR3,CTNNB1,EDN1,EGR3,ENPEP,EPAS1,ETS1,PTK2B,FGF1,FOXC1,FN1,GJA1,HAS2,ID1,RBPJ,ITGB1,JAK1,LAMA5,SMAD7,MMP2,CITED1,NRCAM,SERPINE1,PIK3CA,PRCP,PRKCB,PTEN,PTGER4,PTGIS,RASA1,RORA,SFRP2,SHB,SP100,SRPK2,STAT1,SYK,TGFBR2,VEGFC,WNT7A,WT1,XDH,NRP2,SLIT2,NRXN3,HS6ST1,HDAC9,RAMP1,WARS2,VAV3,CXCL13,GNA13,HPSE,PNPLA6,ESM1,TIPARP,HIPK2,STAB2,PROK2,SEMA4A,NDNF,CCM2,STARD13,MTDH,PIK3R6,DAB2IP,BMPER,CXCL17,MEF2C,NDP,PTPN14,LUZP1,FOSL1,SERPINB7,ERRFI1,MIB1,DYNC2H1
238	15_Member GO Biological Processes GO:0048514 blood vessel morphogenesis	-7.117296743 -4.838 77/486	154,284,302,347,356,639,677,688,861,1232,1499,1906,1960,2028,2034,2113,2185,2246,2296,2335,2697,3037,3397,3516,3688,3716,3911,4092,4313,4435,4897,5054,5290,5547,5579,5728,5734,5740,5921,6095,6423,6461,6672,6733,6772,6850,7048,7424,7476,7490,7498,8828,9353,9369,9394,9734,10267,10352,10451,10563,10672,10855,10908,11082,25976,28996,55576,60675,64218,79625,83605,90627,92140,146850,153090,168667,284340	ADRB2,ANGPT1,ANXA2,APOD,FASLG,PRDM1,ZFP36L1,KLF5,RUNX1,CCR3,CTNNB1,EDN1,EGR3,ENPEP,EPAS1,ETS1,PTK2B,FGF1,FOXC1,FN1,GJA1,HAS2,ID1,RBPJ,ITGB1,JAK1,LAMA5,SMAD7,MMP2,CITED1,NRCAM,SERPINE1,PIK3CA,PRCP,PRKCB,PTEN,PTGER4,PTGIS,RASA1,RORA,SFRP2,SHB,SP100,SRPK2,STAT1,SYK,TGFBR2,VEGFC,WNT7A,WT1,XDH,NRP2,SLIT2,NRXN3,HS6ST1,HDAC9,RAMP1,WARS2,VAV3,CXCL13,GNA13,HPSE,PNPLA6,ESM1,TIPARP,HIPK2,STAB2,PROK2,SEMA4A,NDNF,CCM2,STARD13,MTDH,PIK3R6,DAB2IP,BMPER,CXCL17

Appendix

239	15_Member GO Biological Processes GO:0001944 vasculature development	-6.212064154 -4.090 86/591	154,284,302,347,356,639,677,688,861,1232,1499,1906,1960,2028,2034,2113,2185,2246,2296,2335,2697,3037,3397,3516,3688,3716,3911,4092,4208,4313,4435,4693,4897,5054,5290,5547,5579,5728,5734,5740,5784,5921,6095,6423,6461,6672,6733,6772,6850,7048,7424,7476,7490,7498,7798,8061,8710,8828,9353,9369,9394,9734,10267,10352,10451,10563,10672,10855,10908,11082,25976,28996,54206,55576,57534,60675,64218,79625,79659,83605,90627,92140,146850,153090,168667,284340	ADRB2,ANGPT1,ANXA2,APOD,FASLG,PRDM1,ZFP36L1,KLF5,RUNX1,CCR3,CTNNB1,EDN1,EGR3,ENPEP,EPAS1,ETS1,PTK2B,FGF1,FOXC1,FN1,GJA1,HAS2,ID1,RBPJ,ITGB1,JAK1,LAMA5,SMAD7,MEF2C,MMP2,CITED1,NDP,NRCAM,SERPINE1,PIK3CA,PRCP,PRKCB,PTEN,PTGER4,PTGIS,PTPN14,RASA1,RORA,SFRP2,SHB,SP100,SRPK2,STAT1,SYK,TGFBR2,VEGFC,WNT7A,WT1,XDH,LUZP1,FOSL1,SERPINB7,NRP2,SLIT2,NRXN3,HS6ST1,HDAC9,RAMP1,WARS2,VAV3,CXCL13,GNA13,HPSE,PNPLA6,ESM1,TIPARP,HIPK2,ERRFI1,STAB2,MIB1,PROK2,SEMA4A,NDNF,DYNC2H1,CCM2,STARD13,MTDH,PIK3R6,DAB2IP,BMPER,CXCL17
240	15_Member GO Biological Processes GO:0001568 blood vessel development	-5.997825585 -3.936 83/571	154,284,302,347,356,639,677,688,861,1232,1499,1906,1960,2028,2034,2113,2185,2246,2296,2335,2697,3037,3397,3516,3688,3716,3911,4092,4208,4313,4435,4897,5054,5290,5547,5579,5728,5734,5740,5921,6095,6423,6461,6672,6733,6772,6850,7048,7424,7476,7490,7498,7798,8061,8710,8828,9353,9369,9394,9734,10267,10352,10451,10563,10672,10855,10908,11082,25976,28996,55576,57534,60675,64218,79625,79659,83605,90627,92140,146850,153090,168667,284340	ADRB2,ANGPT1,ANXA2,APOD,FASLG,PRDM1,ZFP36L1,KLF5,RUNX1,CCR3,CTNNB1,EDN1,EGR3,ENPEP,EPAS1,ETS1,PTK2B,FGF1,FOXC1,FN1,GJA1,HAS2,ID1,RBPJ,ITGB1,JAK1,LAMA5,SMAD7,MEF2C,MMP2,CITED1,NRCAM,SERPINE1,PIK3CA,PRCP,PRKCB,PTEN,PTGER4,PTGIS,RASA1,RORA,SFRP2,SHB,SP100,SRPK2,STAT1,SYK,TGFBR2,VEGFC,WNT7A,WT1,XDH,LUZP1,FOSL1,SERPINB7,NRP2,SLIT2,NRXN3,HS6ST1,HDAC9,RAMP1,WARS2,VAV3,CXCL13,GNA13,HPSE,PNPLA6,ESM1,TIPARP,HIPK2,STAB2,MIB1,PROK2,SEMA4A,NDNF,DYNC2H1,CCM2,STARD13,MTDH,PIK3R6,DAB2IP,BMPER,CXCL17
241	15_Member GO Biological Processes GO:0001525 angiogenesis	-5.618070657 -3.623 64/415	154,284,302,347,356,688,861,1232,1499,1906,1960,2028,2034,2113,2185,2246,2296,2335,3397,3516,3688,3716,3911,4313,4897,5054,5290,5547,5579,5728,5740,6095,6423,6461,6672,6733,6772,6850,7048,7424,7476,8828,9353,9369,9394,9734,10267,10451,10563,10672,10855,10908,11082,28996,55576,60675,64218,79625,90627,92140,146850,153090,168667,284340	ADRB2,ANGPT1,ANXA2,APOD,FASLG,KLF5,RUNX1,CCR3,CTNNB1,EDN1,EGR3,ENPEP,EPAS1,ETS1,PTK2B,FGF1,FOXC1,FN1,ID1,RBPJ,ITGB1,JAK1,LAMA5,MMP2,NRCAM,SERPINE1,PIK3CA,PRCP,PRKCB,PTEN,PTGIS,RORA,SFRP2,SHB,SP100,SRPK2,STAT1,SYK,TGFBR2,VEGFC,WNT7A,NRP2,SLIT2,NRXN3,HS6ST1,HDAC9,RAMP1,VAV3,CXCL13,GNA13,HPSE,PNPLA6,ESM1,HIPK2,STAB2,PROK2,SEMA4A,NDNF,STARD13,MTDH,PIK3R6,DAB2IP,BMPER,CXCL17
242	15_Member GO Biological Processes GO:1901342 regulation of vasculature development	-2.339487575 -1.015 32/232	154,356,861,1232,1499,2113,2185,2246,2296,3397,3716,5054,5579,5734,5740,6423,6672,6772,7048,7424,7490,7498,8710,9734,10563,28996,60675,64218,90627,92140,146850,153090	ADRB2,FASLG,RUNX1,CCR3,CTNNB1,ETS1,PTK2B,FGF1,FOXC1,ID1,JAK1,SERPINE1,PRKCB,PTGER4,PTGIS,SFRP2,SP100,STAT1,TGFBR2,VEGFC,WT1,XDH,SERPINB7,HDAC9,CXCL13,HIPK2,PROK2,SEMA4A,STARD13,MTDH,PIK3R6,DAB2IP

Appendix

243	16_Summary Canonical Pathways M60 PID NFAT TFPATHWAY	-6.855267689 -4.622 17/47	356,836,1437,1959,1960,2353,2625,3458,3559,3567,4094,4772,4773,5770,8061,10320,30009,919,925,940,2534,3702,3937,4790,5063,5290,5295,6885,8440,9402,10000,10125,10451,29851,1499,1906,2099,2113,2697,2908,4602,5728,10018,3442,5551,5579,8320,2185,3384,3824,4068,6850,8743	FASLG,CASP3,CSF2,EGR2,EGR3,FOS,GATA3,IFNG,IL2RA,IL5,MAF,NFATC1,NFATC2,PTPN1,FOSL1,IKZF1,TBX21,CD247,CD8A,CD28,FYN,ITK,LCP2,NFKB1,PAK3,PIK3CA,PIK3R1,MAP3K7,NCK2,GRAP2,AKT3,RASGRP1,VAV3,ICOS,CTNNB1,EDN1,ESR1,ETS1,GJA1,NR3C1,MYB,PTEN,BCL2L11,IFNA5,PRF1,PRKCB,EOMES,PTK2B,ICAM2,KLRD1,SH2D1A,SYK,TNFSF10
244	16_Member Canonical Pathways M60 PID NFAT TFPATHWAY	-6.855267689 -4.622 17/47	356,836,1437,1959,1960,2353,2625,3458,3559,3567,4094,4772,4773,5770,8061,10320,30009	FASLG,CASP3,CSF2,EGR2,EGR3,FOS,GATA3,IFNG,IL2RA,IL5,MAF,NFATC1,NFATC2,PTPN1,FOSL1,IKZF1,TBX21
245	16_Member KEGG Pathway hsa04660 T cell receptor signaling pathway	-4.762988786 -2.907 23/104	919,925,940,1437,2353,2534,3458,3567,3702,3937,4772,4773,4790,5063,5290,5295,6885,8440,9402,10000,10125,10451,29851	CD247,CD8A,CD28,CSF2,FOS,FYN,IFNG,IL5,ITK,LCP2,NFATC1,NFATC2,NFKB1,PAK3,PIK3CA,PIK3R1,MAP3K7,NCK2,GRAP2,AKT3,RASGRP1,VAV3,ICOS
246	16_Member Canonical Pathways M167 PID AP1 PATHWAY	-4.217178207 -2.446 17/70	1437,1499,1906,2099,2113,2353,2697,2908,3458,3567,4094,4602,4772,4773,5728,8061,10018	CSF2,CTNNB1,EDN1,ESR1,ETS1,FOS,GJA1,NR3C1,IFNG,IL5,MAF,MYB,NFATC1,NFATC2,PTEN,FOSL1,BCL2L11
247	16_Member Canonical Pathways M235 PID TCR CALCIUM PATHWAY	-2.638008071 -1.235 8/29	356,1437,2353,3458,3559,4772,4773,8061	FASLG,CSF2,FOS,IFNG,IL2RA,NFATC1,NFATC2,FOSL1
248	16_Member Canonical Pathways M272 PID CD8 TCR DOWNSTREAM PATHWAY	-2.53207231 -1.158 13/65	356,919,925,2353,3442,3458,3559,4772,4773,5551,5579,8061,8320	FASLG,CD247,CD8A,FOS,IFNA5,IFNG,IL2RA,NFATC1,NFATC2,PRF1,PRKCB,FOSL1,EOMES
249	16_Member KEGG Pathway hsa04650 Natural killer cell mediated cytotoxicity	-2.334180243 -1.015 21/134	356,836,919,1437,2185,2534,3384,3442,3458,3824,3937,4068,4772,4773,5290,5295,5551,5579,6850,8743,10451	FASLG,CASP3,CD247,CSF2,PTK2B,FYN,ICAM2,IFNA5,IFNG,KLRD1,LCP2,SH2D1A,NFATC1,NFATC2,PIK3CA,PIK3R1,PRF1,PRKCB,SYK,TNFSF10,VAV3
250	17_Summary GO Biological Processes	-6.60235266 -4.399	154,301,308,317,322,323,356,596,597,604,665,817,834,836,841,943,1499,1540,1647	ADRB2,ANXA1,ANXA5,APAF1,APBB1,APBB2,FASLG,BCL2,BCL2A1,BCL6,BNIP3L,CAMK2D,CASP1,C

Appendix

	GO:0043065 positive regulation of apoptotic process	85/570	,1687,1848,3458,3592,3624,3635,3663,3672,3688,3778,3965,4208,4488,5063,5321,5326,5584,5591,5728,5740,6423,6609,6733,7074,7159,7421,7490,7498,7531,7534,7704,8061,8439,8440,8626,8648,8743,8767,8997,9353,10018,10395,10451,10550,10971,11214,26230,29124,29949,50650,51741,53832,55164,55655,56891,79156,79370,81618,85477,90441,112399,114548,121512,153090,255738,400410,54541,921,940,960,1437,2074,2244,2272,2534,2668,3093,3479,3708,4000,5054,5295,5716,5770,6326,6648,6672,7466,8428,8452,9531,10449,10771,26287,28996,51202,54431,55023,55075,57600,64782,79698,90993,246330	ASP3,CASP8,TNFRSF8,CTNNB1,CYLD,GADD45A,DFNA5,DUSP6,IFNG,IL12A,INHBA,INPP5D,IRF5,ITGA1,ITGB1,KCNMA1,LGALS9,MEF2C,MSX2,PAK3,PLA2G4A,PLAGL2,PRKCI,PRKDC,PTEN,PTGIS,SFRP2,SMPD1,SRPK2,TIAM1,TP53BP2,VDR,WT1,XDH,YWHAE,YWHAZ,ZBTB16,FOSL1,NSMAF,NCK2,TP63,NCOA1,TNFSF10,RIPK2,KALRN,SLIT2,BCL2L11,DLC1,VAV3,ARL6IP5,YWHAQ,AKAP13,TIAM2,LGALS13,IL19,ARHGEF3,WWOX,IL20RA,SHQ1,NLRP2,LGALS14,PLEKHF1,BCL2L14,ITM2C,SCIN,ZNF622,EGLN3,NLRP3,FGD4,DAB2IP,PCSK9,ST20,DDIT4,CD5,CD28,CD44,CSF2,ERCC6,FGB,FHIT,FYN,GDNF,UBE2K,IGF1,ITPR1,LMNA,SERPINE1,PIK3R1,PSMD10,PTPN1,SCN2A,SOD2,SP100,WFS1,STK24,CUL3,BAG3,ACAA2,ZMYND11,ANKRD2,HIPK2,DDX47,DNAJC10,PHIP,UACA,FNIP2,AEN,ZMAT4,CREB3L1,PELI3
251	17_Member GO Biological Processes GO:0043065 positive regulation of apoptotic process	-6.60235266 -4.399 85/570	154,301,308,317,322,323,356,596,597,604,665,817,834,836,841,943,1499,1540,1647,1687,1848,3458,3592,3624,3635,3663,3672,3688,3778,3965,4208,4488,5063,5321,5326,5584,5591,5728,5740,6423,6609,6733,7074,7159,7421,7490,7498,7531,7534,7704,8061,8439,8440,8626,8648,8743,8767,8997,9353,10018,10395,10451,10550,10971,11214,26230,29124,29949,50650,51741,53832,55164,55655,56891,79156,79370,81618,85477,90441,112399,114548,121512,153090,255738,400410	ADRB2,ANXA1,ANXA5,APAF1,APBB1,APBB2,FASLG,BCL2,BCL2A1,BCL6,BNIP3L,CAMK2D,CASP1,CASP3,CASP8,TNFRSF8,CTNNB1,CYLD,GADD45A,DFNA5,DUSP6,IFNG,IL12A,INHBA,INPP5D,IRF5,ITGA1,ITGB1,KCNMA1,LGALS9,MEF2C,MSX2,PAK3,PLA2G4A,PLAGL2,PRKCI,PRKDC,PTEN,PTGIS,SFRP2,SMPD1,SRPK2,TIAM1,TP53BP2,VDR,WT1,XDH,YWHAE,YWHAZ,ZBTB16,FOSL1,NSMAF,NCK2,TP63,NCOA1,TNFSF10,RIPK2,KALRN,SLIT2,BCL2L11,DLC1,VAV3,ARL6IP5,YWHAQ,AKAP13,TIAM2,LGALS13,IL19,ARHGEF3,WWOX,IL20RA,SHQ1,NLRP2,LGALS14,PLEKHF1,BCL2L14,ITM2C,SCIN,ZNF622,EGLN3,NLRP3,FGD4,DAB2IP,PCSK9,ST20
252	17_Member GO Biological Processes GO:0043068 positive regulation of programmed cell death	-6.438249527 -4.269 85/575	154,301,308,317,322,323,356,596,597,604,665,817,834,836,841,943,1499,1540,1647,1687,1848,3458,3592,3624,3635,3663,3672,3688,3778,3965,4208,4488,5063,5321,5326,5584,5591,5728,5740,6423,6609,6733,7074,7159,7421,7490,7498,7531,7534,7704,8061,8439,8440,8626,8648,8743,8767,8997,9353,10018,10395,10451,10550,10971,11214,26230,29124,29949,50650,51741,53832,55164,55655,56891,79156,79370,81618,85477,90441,112399,114548,12151	ADRB2,ANXA1,ANXA5,APAF1,APBB1,APBB2,FASLG,BCL2,BCL2A1,BCL6,BNIP3L,CAMK2D,CASP1,CASP3,CASP8,TNFRSF8,CTNNB1,CYLD,GADD45A,DFNA5,DUSP6,IFNG,IL12A,INHBA,INPP5D,IRF5,ITGA1,ITGB1,KCNMA1,LGALS9,MEF2C,MSX2,PAK3,PLA2G4A,PLAGL2,PRKCI,PRKDC,PTEN,PTGIS,SFRP2,SMPD1,SRPK2,TIAM1,TP53BP2,VDR,WT1,XDH,YWHAE,YWHAZ,ZBTB16,FOSL1,NSMAF,NCK2,TP63,NCOA1,TNFSF10,RIPK2,KALRN,SLIT2,BCL2L11,DLC1,VAV3,ARL6IP5,YWHAQ,AKAP13,TIAM2,LGALS13,IL19,ARHGEF3,WWOX,IL20RA,SHQ1,NLRP2,LG

Appendix

			2,153090,255738,400410	ALS14,PLEKHF1,BCL2L14,ITM2C,SCIN,ZNF622,EGLN3,NLRP3,FGD4,DAB2IP,PCSK9,ST20
253	17_Member GO Biological Processes GO:0010942 positive regulation of cell death	-5.639038051 -3.638 86/610	154,301,308,317,322,323,356,596,597,604,665,817,834,836,841,943,1499,1540,1647,1687,1848,3458,3592,3624,3635,3663,3672,3688,3778,3965,4208,4488,5063,5321,5326,5584,5591,5728,5740,6423,6609,6733,7074,7159,7421,7490,7498,7531,7534,7704,8061,8439,8440,8626,8648,8743,8767,8997,9353,10018,10395,10451,10550,10971,11214,26230,29124,29949,50650,51741,53832,54541,55164,55655,56891,79156,79370,81618,85477,90441,112399,114548,121512,153090,255738,400410	ADRB2,ANXA1,ANXA5,APAF1,APBB1,APBB2,FASLG,BCL2,BCL2A1,BCL6,BNIP3L,CAMK2D,CASP1,CASP3,CASP8,TNFRSF8,CTNNB1,CYLD,GADD45A,DFNA5,DUSP6,IFNG,IL12A,INHBA,INPP5D,IRF5,ITGA1,ITGB1,KCNMA1,LGALS9,MEF2C,MSX2,PAK3,PLA2G4A,PLAGL2,PRKCI,PRKDC,PTEN,PTGIS,SFRP2,SMPD1,SRPK2,TIAM1,TP53BP2,VDR,WT1,XDH,YWHAE,YWHAZ,ZBTB16,FOSL1,NSMAF,NCK2,TP63,NCOA1,TNFSF10,RIPK2,KALRN,SLIT2,BCL2L11,DLC1,VAV3,ARL6IP5,YWHAQ,AKAP13,TIAM2,LGALS13,IL19,ARHGEF3,WWOX,IL20RA,DDIT4,SHQ1,NLRP2,LGALS14,PLEKHF1,BCL2L14,ITM2C,SCIN,ZNF622,EGLN3,NLRP3,FGD4,DAB2IP,PCSK9,ST20
254	17_Member GO Biological Processes GO:0097190 apoptotic signaling pathway	-4.638286662 -2.808 83/619	317,356,596,597,665,836,841,921,940,943,960,1437,1499,1540,1687,2074,2244,2272,2534,2668,3093,3458,3479,3592,3624,3708,3965,4000,5054,5295,5326,5591,5716,5728,5740,5770,6326,6423,6648,6672,7074,7159,7466,7531,7534,8428,8440,8452,8626,8743,8997,9531,10018,10449,10451,10550,10771,10971,11214,26230,26287,28996,29949,50650,51202,51741,53832,54431,54541,55023,55075,57600,64782,79156,79370,79698,81618,90441,90993,121512,153090,246330,400410	APAF1,FASLG,BCL2,BCL2A1,BNIP3L,CASP3,CASP8,CD5,CD28,TNFRSF8,CD44,CSF2,CTNNB1,CYLD,DFNA5,ERCC6,FGB,FHIT,FYN,GDNF,UBE2K,IFNG,IGF1,IL12A,INHBA,ITPR1,LGALS9,LMNA,SERPINE1,PIK3R1,PLAGL2,PRKDC,PSMD10,PTEN,PTGIS,PTPN1,SCN2A,SFRP2,SOD2,SP100,TIAM1,TP53BP2,WFS1,YWHAE,YWHAZ,STK24,NCK2,CUL3,TP63,TNFSF10,KALRN,BAG3,BCL2L11,ACAA2,VAV3,ARL6IP5,ZMYND11,YWHAQ,AKAP13,TIAM2,ANKRD2,HIPK2,IL19,ARHGEF3,DDX47,WWOX,IL20RA,DNAJC10,DDIT4,PHIP,UACA,FNIP2,AEN,PLEKHF1,BCL2L14,ZMAT4,ITM2C,ZNF622,CREB3L1,FGD4,DAB2IP,PELI3,ST20
255	17_Member GO Biological Processes GO:0097193 intrinsic apoptotic signaling pathway	-4.136547507 -2.385 45/292	317,596,597,836,841,960,1540,1687,2074,2272,3093,3708,5295,5326,5591,5770,6326,6423,6648,7159,7466,7531,7534,8428,8440,8452,8626,10018,10550,10971,26287,28996,29949,51741,53832,54431,54541,57600,64782,79156,79698,90441,90993,153090,400410	APAF1,BCL2,BCL2A1,CASP3,CASP8,CD44,CYLD,DFNA5,ERCC6,FHIT,UBE2K,ITPR1,PIK3R1,PLAGL2,PRKDC,PTPN1,SCN2A,SFRP2,SOD2,TP53BP2,WFS1,YWHAE,YWHAZ,STK24,NCK2,CUL3,TP63,BCL2L11,ARL6IP5,YWHAQ,ANKRD2,HIPK2,IL19,WWOX,IL20RA,DNAJC10,DDIT4,FNIP2,AEN,PLEKHF1,ZMAT4,ZNF622,CREB3L1,DAB2IP,ST20
256	18_Summary Hallmark Gene Sets M5891 HALLMARK HYPOXIA	-6.537619658 -4.355 40/200	272,302,596,665,771,901,1316,1907,1956,2113,2353,2908,3516,4214,4601,4783,5054,5214,5236,6095,6383,6533,7511,7852,8277,8497,8553,8609,8660,8870,9435,9469,23036,23327,25976,26136,51316,54206,5	AMPD3,ANXA2,BCL2,BNIP3L,CA12,CCNG2,KLF6,EDN2,EGFR,ETS1,FOS,NR3C1,RBPJ,MAP3K1,MXI1,NFIL3,SERPINE1,PFKP,PGM1,RORA,SDC2,SLC6A6,XPNPEP1,CXCR4,TKTL1,PPFIA4,BHLHE40,KLF7,IRS2,IER3,CHST2,CHST3,ZNF292,NEDD4L,TIPARP,TES

Appendix

			4541,112464	,PLAC8,ERRFI1,DDIT4,PRKCDBP
257	18_Member Hallmark Gene Sets M5891 HALLMARK HYPOXIA	-6.537619658 -4.355 40/200	272,302,596,665,771,901,1316,1907,1956,2113,2353,2908,3516,4214,4601,4783,5054,5214,5236,6095,6383,6533,7511,7852,8277,8497,8553,8609,8660,8870,9435,9469,23036,23327,25976,26136,51316,54206,54541,112464	AMPD3,ANXA2,BCL2,BNIP3L,CA12,CCNG2,KLF6,EDN2,EGFR,ETS1,FOS,NR3C1,RBPJ,MAP3K1,MXI1,NFIL3,SERPINE1,PFKP,PGM1,RORA,SDC2,SLC6A6,XPNPEP1,CXCR4,TKTL1,PPFIA4,BHLHE40,KLF7,IRS2,IER3,CHST2,CHST3,ZNF292,NEDD4L,TIPARP,TES,PLAC8,ERRFI1,DDIT4,PRKCDBP
258	19_Summary Hallmark Gene Sets M5953 HALLMARK KRAS SIGNALING UP	-6.537619658 -4.355 40/200	330,347,639,894,1149,1240,1437,1794,1839,1848,2113,3481,3575,3624,3673,3783,3800,4214,4674,6304,6480,7035,7476,7805,7852,8404,9053,9358,9734,9910,10221,10320,11184,22903,23136,27075,28951,54462,56729,83734	BIRC3,APOD,PRDM1,CCND2,CIDEA,CMKLR1,CSF2,DOCK2,HBEGF,DUSP6,ETS1,IGF2,IL7R,INHBA,ITGA2,KCNN4,KIF5C,MAP3K1,NAP1L2,SATB1,ST6GAL1,TFPI,WNT7A,LAPTM5,CXCR4,SPARCL1,MAP7,ITGBL1,HDAC9,RABGAP1L,TRIB1,IKZF1,MAP4K1,BTBD3,EPB41L3,TSPAN13,TRIB2,CCSER2,RETN,ATG10
259	19_Member Hallmark Gene Sets M5953 HALLMARK KRAS SIGNALING UP	-6.537619658 -4.355 40/200	330,347,639,894,1149,1240,1437,1794,1839,1848,2113,3481,3575,3624,3673,3783,3800,4214,4674,6304,6480,7035,7476,7805,7852,8404,9053,9358,9734,9910,10221,10320,11184,22903,23136,27075,28951,54462,56729,83734	BIRC3,APOD,PRDM1,CCND2,CIDEA,CMKLR1,CSF2,DOCK2,HBEGF,DUSP6,ETS1,IGF2,IL7R,INHBA,ITGA2,KCNN4,KIF5C,MAP3K1,NAP1L2,SATB1,ST6GAL1,TFPI,WNT7A,LAPTM5,CXCR4,SPARCL1,MAP7,ITGBL1,HDAC9,RABGAP1L,TRIB1,IKZF1,MAP4K1,BTBD3,EPB41L3,TSPAN13,TRIB2,CCSER2,RETN,ATG10
260	20_Summary GO Biological Processes GO:0031098 stress-activated protein kinase signaling cascade	-6.52604254 -4.348 51/285	1236,1647,1846,1848,1906,2074,2185,2257,2353,2549,3965,4208,4214,4790,4920,5063,5734,5770,6197,6423,6504,6850,6885,7074,7476,7498,8428,8600,8767,9064,9448,10125,10221,10333,10550,10771,11184,11221,23118,23239,23291,26191,27347,28996,51765,54106,54986,56940,83605,90441,153090,238,493,894,1436,1847,1956,2146,2180,2246,3481,3672,4057,4082,4142,5716,7852,10920,60675,128239,146850,219771	CCR7,GADD45A,DUSP4,DUSP6,EDN1,ERCC6,PTK2B,FGF12,FOS,GAB1,LGALS9,MEF2C,MAP3K1,NFKB1,ROR2,PAK3,PTGER4,PTPN1,RPS6KA3,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,STK24,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TRIB1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,FBXW11,PTPN22,STK39,HIPK2,STK26,TLR9,ULK4,DUSP22,CCM2,ZNF622,DAB2IP,ALK,ATP2B4,CCND2,CSF1R,DUSP5,EGFR,EZH2,ACSL1,FGF1,IGF2,ITGA1,LTF,MARCKS,MAS1,PSMD10,CXCR4,COPS8,PROK2,IQGAP3,PIK3R6,CCNY
261	20_Member GO Biological Processes GO:0031098 stress-activated protein kinase signaling cascade	-6.52604254 -4.348 51/285	1236,1647,1846,1848,1906,2074,2185,2257,2353,2549,3965,4208,4214,4790,4920,5063,5734,5770,6197,6423,6504,6850,6885,7074,7476,7498,8428,8600,8767,9064,9448,10125,10221,10333,10550,10771,11184,11221,23118,23239,23291,26191,27347,28996,51765,54106,54986,56940,83605,90441,153090	CCR7,GADD45A,DUSP4,DUSP6,EDN1,ERCC6,PTK2B,FGF12,FOS,GAB1,LGALS9,MEF2C,MAP3K1,NFKB1,ROR2,PAK3,PTGER4,PTPN1,RPS6KA3,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,STK24,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TRIB1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,FBXW11,PTPN22,STK39,HIPK2,STK26,TLR9,ULK4,DUSP22,CCM2,ZNF622,DAB2IP

Appendix

262	20_Member GO Biological Processes GO:0051403 stress-activated MAPK cascade	-5.763370917 -3.739 46/261	1236,1647,1846,1848,1906,2074,2185,2257,2353,2549,3965,4208,4790,4920,5734,5770,6197,6423,6504,6850,6885,7074,7476,7498,8600,8767,9064,9448,10125,10221,10333,10550,10771,11184,11221,23118,23239,23291,26191,28996,54106,54986,56940,83605,90441,153090	CCR7,GADD45A,DUSP4,DUSP6,EDN1,ERCC6,PTK2B,FGF12,FOS,GAB1,LGALS9,MEF2C,NFKB1,ROR2,PTGER4,PTPN1,RPS6KA3,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TRIB1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,FBXW11,PTPN22,HIPK2,TLR9,ULK4,DUSP22,CCM2,ZNF622,DAB2IP
263	20_Member GO Biological Processes GO:0007254 JNK cascade	-5.126657286 -3.197 35/187	1236,1647,1906,2074,2185,2257,2549,4920,5734,5770,6423,6504,6850,6885,7074,7476,8600,8767,9064,9448,10125,10221,10333,10771,11184,11221,23118,23239,26191,28996,54106,54986,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,FGF12,GAB1,ROR2,PTGER4,PTPN1,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TRIB1,TLR6,ZMYND11,MAP4K1,DUSP10,TAB2,PHLPP1,PTPN22,HIPK2,TLR9,ULK4,DUSP22,ZNF622,DAB2IP
264	20_Member GO Biological Processes GO:0046328 regulation of JNK cascade	-4.835856823 -2.969 30/154	1236,1647,1906,2074,2185,2549,5770,6423,6504,6850,6885,7074,7476,8600,8767,9064,9448,10125,10333,10771,11184,11221,23239,26191,28996,54106,54986,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,PTPN1,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TLR6,ZMYND11,MAP4K1,DUSP10,PHLPP1,PTPN22,HIPK2,TLR9,ULK4,DUSP22,ZNF622,DAB2IP
265	20_Member GO Biological Processes GO:0046330 positive regulation of JNK cascade	-4.417457118 -2.620 23/109	1236,1647,1906,2074,2185,2549,5770,6504,6850,6885,7074,7476,8600,8767,9064,10125,10333,11184,28996,54106,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,PTPN1,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,TNFSF11,RIPK2,MAP3K6,RASGRP1,TLR6,MAP4K1,HIPK2,TLR9,DUSP22,ZNF622,DAB2IP
266	20_Member GO Biological Processes GO:0032872 regulation of stress-activated MAPK cascade	-4.145758917 -2.390 33/191	1236,1647,1906,2074,2185,2549,3965,5770,6423,6504,6850,6885,7074,7476,7498,8600,8767,9064,9448,10125,10333,10550,10771,11184,11221,23239,26191,28996,54106,54986,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,LGALS9,PTPN1,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,PHLPP1,PTPN22,HIPK2,TLR9,ULK4,DUSP22,ZNF622,DAB2IP
267	20_Member GO Biological Processes GO:0070302 regulation of stress-activated protein kinase signaling cascade	-4.100214039 -2.356 33/192	1236,1647,1906,2074,2185,2549,3965,5770,6423,6504,6850,6885,7074,7476,7498,8600,8767,9064,9448,10125,10333,10550,10771,11184,11221,23239,26191,28996,54106,54986,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,LGALS9,PTPN1,SFRP2,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,TNFSF11,RIPK2,MAP3K6,MAP4K4,RASGRP1,TLR6,ARL6IP5,ZMYND11,MAP4K1,DUSP10,PHLPP1,PTPN22,HIPK2,TLR9,ULK4,DUSP22,ZNF622,DAB2IP
268	20_Member GO Biological Processes GO:0032874 positive regulation of stress-activated MAPK	-4.098623854 -2.356 25/129	1236,1647,1906,2074,2185,2549,5770,6504,6850,6885,7074,7476,7498,8600,8767,9064,10125,10333,10550,11184,28996,54106,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,PTPN1,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,TNFSF11,RIPK2,MAP3K6,RASGRP1,TLR6,ARL6IP5,MAP4K1,HIPK2,TLR9,DUSP22,ZNF622,DAB2IP

Appendix

	cascade			
269	20_Member GO Biological Processes GO:0070304 positive regulation of stress-activated protein kinase signaling cascade	-4.041923889 -2.319 25/130	1236,1647,1906,2074,2185,2549,5770,6504,6850,6885,7074,7476,7498,8600,8767,9064,10125,10333,10550,11184,28996,54106,56940,90441,153090	CCR7,GADD45A,EDN1,ERCC6,PTK2B,GAB1,PTPN1,SLAMF1,SYK,MAP3K7,TIAM1,WNT7A,XDH,TNFSF11,RIPK2,MAP3K6,RASGRP1,TLR6,ARL6IP5,MAP4K1,HIPK2,TLR9,DUSP22,ZNF622,DAB2IP
270	20_Member GO Biological Processes GO:0043506 regulation of JUN kinase activity	-3.599813354 -1.951 17/78	1906,2074,2185,2549,5770,6423,6850,6885,7074,8600,9064,10333,11184,11221,26191,54106,153090	EDN1,ERCC6,PTK2B,GAB1,PTPN1,SFRP2,SYK,MAP3K7,TIAM1,TNFSF11,MAP3K6,TLR6,MAP4K1,DUSP10,PTPN22,TLR9,DAB2IP
271	20_Member GO Biological Processes GO:0043507 positive regulation of JUN kinase activity	-3.383551195 -1.784 14/60	1906,2074,2185,2549,5770,6850,6885,7074,8600,9064,10333,11184,54106,153090	EDN1,ERCC6,PTK2B,GAB1,PTPN1,SYK,MAP3K7,TIAM1,TNFSF11,MAP3K6,TLR6,MAP4K1,TLR9,DAB2IP
272	20_Member GO Biological Processes GO:0071902 positive regulation of protein serine/threonine kinase activity	-3.192651173 -1.640 41/285	238,493,894,1436,1647,1847,1848,1906,1956,2074,2146,2180,2185,2246,2549,3481,3672,4057,4082,4142,5063,5716,5770,6850,6885,7074,7852,8600,8767,9064,10125,10333,10920,11184,23118,54106,60675,128239,146850,153090,219771	ALK,ATP2B4,CCND2,CSF1R,GADD45A,DUSP5,DUSP6,EDN1,EGFR,ERCC6,EZH2,ACSL1,PTK2B,FGF1,GAB1,IGF2,ITGA1,LTF,MARCKS,MAS1,PAK3,PSMD10,PTPN1,SYK,MAP3K7,TIAM1,CXCR4,TNFSF11,RIPK2,MAP3K6,RASGRP1,TLR6,COPS8,MAP4K1,TAB2,TLR9,PROK2,IQGAP3,PIK3R6,DAB2IP,CCNY
273	20_Member GO Biological Processes GO:0043406 positive regulation of MAP kinase activity	-2.388538528 -1.052 29/203	238,1847,1848,1906,1956,2074,2146,2185,2246,2549,3672,5063,5770,6850,6885,7074,7852,8600,8767,9064,10125,10333,11184,23118,54106,60675,128239,146850,153090	ALK,DUSP5,DUSP6,EDN1,EGFR,ERCC6,EZH2,PTK2B,FGF1,GAB1,ITGA1,PAK3,PTPN1,SYK,MAP3K7,TIAM1,CXCR4,TNFSF11,RIPK2,MAP3K6,RASGRP1,TLR6,MAP4K1,TAB2,TLR9,PROK2,IQGAP3,PIK3R6,DAB2IP
274	20_Member GO Biological Processes GO:0007257 activation of JUN kinase activity	-2.348244098 -1.020 8/32	2074,2549,5770,6850,8600,9064,11184,153090	ERCC6,GAB1,PTPN1,SYK,TNFSF11,MAP3K6,MAP4K1,DAB2IP

*: Column A provides in each cell, from top to bottom: Group ID, Category, Term, and Description. Column B provides in each cell, from top to bottom: LogP, Log(q-value), and InTerm_InList.

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),

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).

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.

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.

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.

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.

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.

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.

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.

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. 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.

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;

(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.

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.

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.

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 pathwy of TNFA signaling via NFKB are chosen from Table 9, Row 134.

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.

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.

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.

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.

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.

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.

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.

35. The cell of claim 17, wherein the one or more genes associated with a hypoxia pathway are chosen from Table 9, Row 256.

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.

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.

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).

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).

42. The cell of any of claims 38-41, wherein the central memory phenotype comprises enhanced antigen-dependent proliferation of the cell.

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.

45. The cell of claim 41, wherein the modualtor (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.

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.

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.

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 preantepenultimte exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon.

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.

52. The cell of claim 44, wherein the modualtor (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.

54. The cell of claim 44, wherein the modualtor (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.

56. The cell of claim 55, wherein the modualtor (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.

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.

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.

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 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 (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.

68. The cell of claim 67, wherein 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.

69. The cell of claim 67 or 68, wherein the costimulatory signaling domain comprises a 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 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.

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.

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.

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.

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 preantepenultimte exon, e.g., is in an antepenultimate exon, a penultimate exon, or a last exon.

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.

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 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.

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.

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.

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:

(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.

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.

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;

(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.

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.

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

(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.

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 modualtor (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.

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;

(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.

123. The modulator for use of claim 122, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

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 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;

(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, 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.

141. The composition of claim 140, wherein the modulator is an inhibitor of IFNG, NOTCH2, CD28, ICOS, IL2RA, or PRDM1.

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.

143. The composition of claim 142, further comprising an inhibitor of Tet2.

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.

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.

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

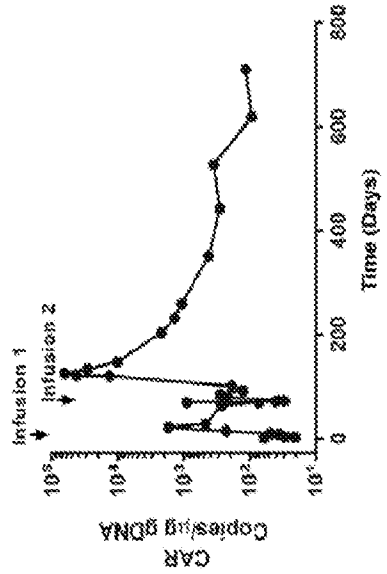


FIG. 1B

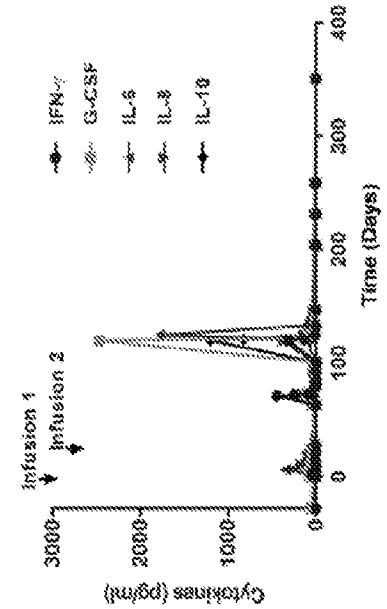


FIG. 1C

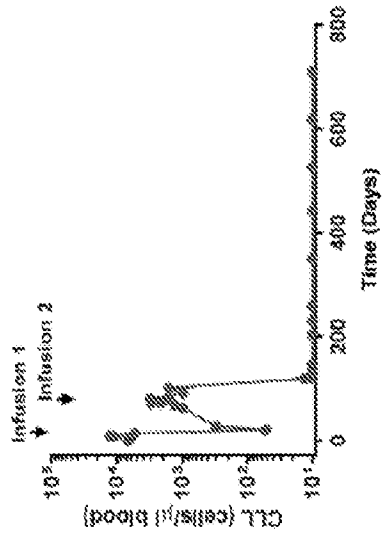


FIG. 1D

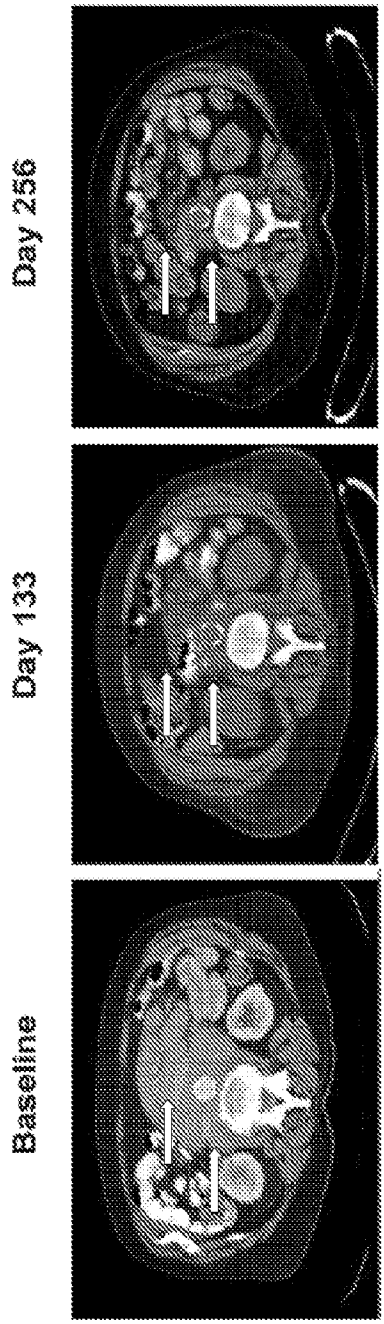


FIG. 2

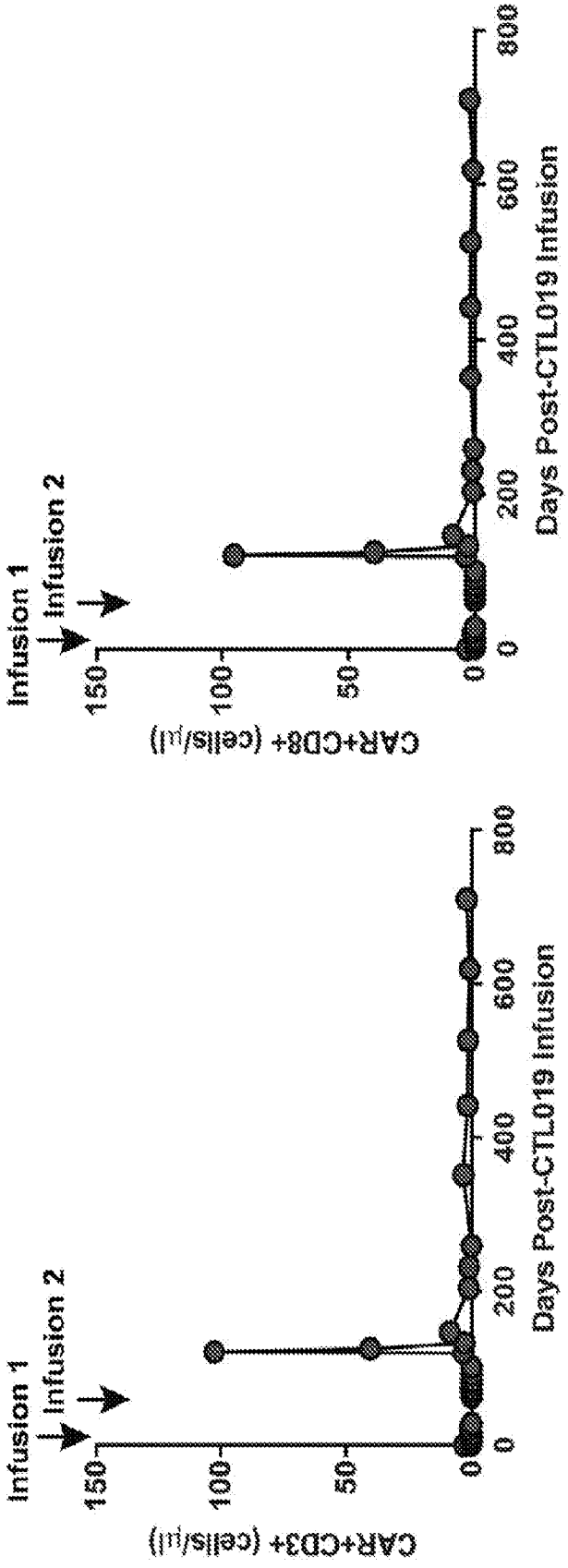

Infusion 1
Infusion 2
150
100
50
0
CAR+CD3+ (cells/μl)
0
200
400
600
800
Days Post-CTL019 Infusion
Infusion 1
Infusion 2
150
100
50
0
CAR+CD8+ (cells/μl)
0
200
400
600
800
Days Post-CTL019 Infusion

FIG. 3

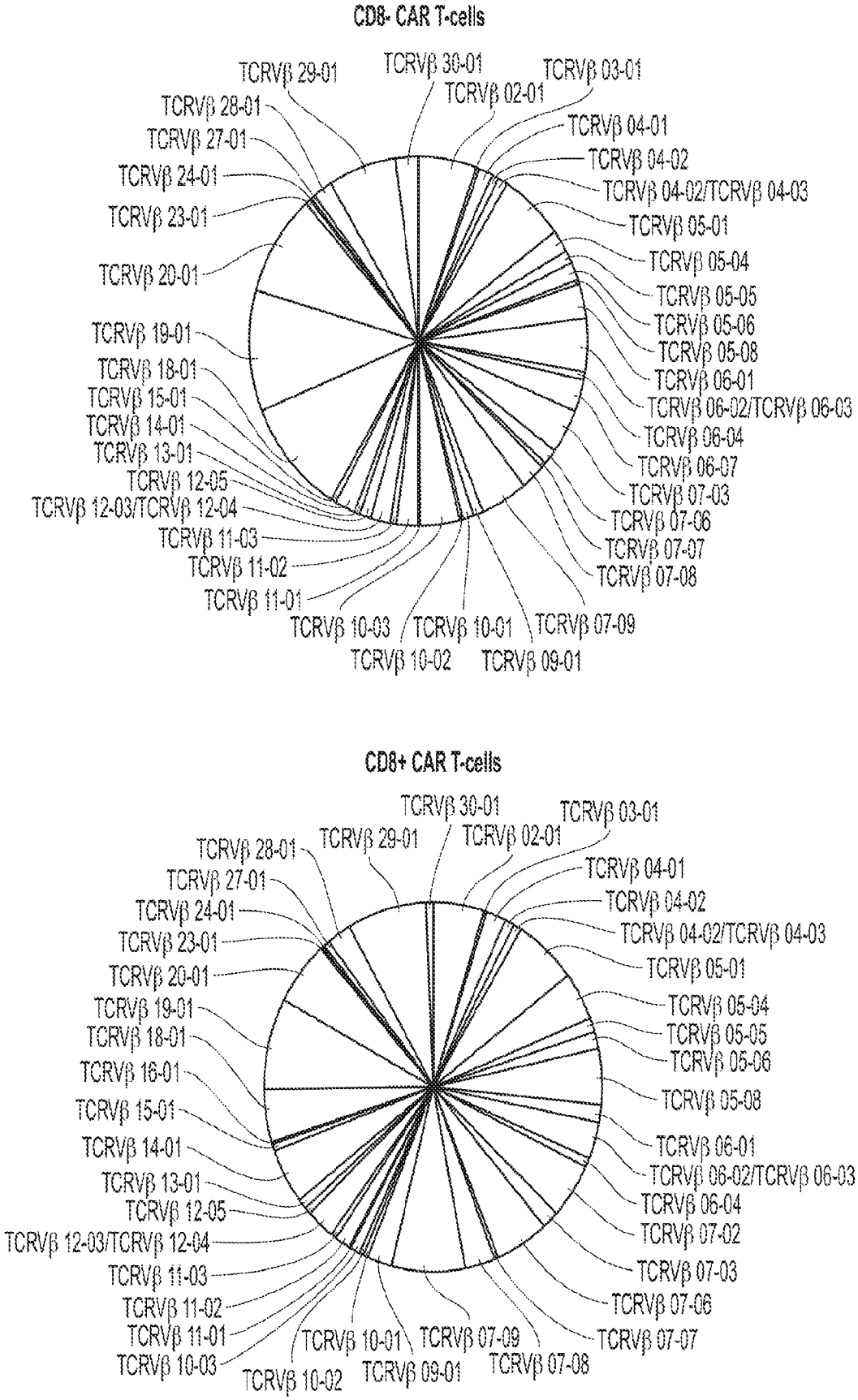

CD8- CAR T-cells
TCRVβ 30-01
TCRVβ 03-01
TCRVβ 29-01
TCRVβ 02-01
TCRVβ 28-01
TCRVβ 04-01
TCRVβ 27-01
TCRVβ 04-02
TCRVβ 24-01
TCRVβ 04-02/TCRVβ 04-03
TCRVβ 23-01
TCRVβ 05-01
TCRVβ 20-01
TCRVβ 05-04
TCRVβ 05-05
TCRVβ 19-01
TCRVβ 05-06
TCRVβ 18-01
TCRVβ 05-08
TCRVβ 15-01
TCRVβ 06-01
TCRVβ 14-01
TCRVβ 06-02/TCRVβ 06-03
TCRVβ 13-01
TCRVβ 06-04
TCRVβ 12-05
TCRVβ 06-07
TCRVβ 12-03/TCRVβ 12-04
TCRVβ 07-03
TCRVβ 11-03
TCRVβ 07-06
TCRVβ 11-02
TCRVβ 07-07
TCRVβ 11-01
TCRVβ 07-08
TCRVβ 10-03
TCRVβ 10-01
TCRVβ 07-09
TCRVβ 10-02
TCRVβ 09-01
CD8+ CAR T-cells
TCRVβ 30-01
TCRVβ 03-01
TCRVβ 28-01
TCRVβ 29-01
TCRVβ 02-01
TCRVβ 27-01
TCRVβ 04-01
TCRVβ 24-01
TCRVβ 04-02
TCRVβ 23-01
TCRVβ 04-02/TCRVβ 04-03
TCRVβ 20-01
TCRVβ 05-01
TCRVβ 19-01
TCRVβ 05-04
TCRVβ 18-01
TCRVβ 05-05
TCRVβ 16-01
TCRVβ 05-06
TCRVβ 15-01
TCRVβ 05-08
TCRVβ 14-01
TCRVβ 06-01
TCRVβ 13-01
TCRVβ 06-02/TCRVβ 06-03
TCRVβ 12-05
TCRVβ 06-04
TCRVβ 12-03/TCRVβ 12-04
TCRVβ 07-02
TCRVβ 11-03
TCRVβ 07-03
TCRVβ 11-02
TCRVβ 07-06
TCRVβ 11-01
TCRVβ 07-07
TCRVβ 10-03
TCRVβ 10-01
TCRVβ 07-09
TCRVβ 10-02
TCRVβ 09-01
TCRVβ 07-08

FIG. 4A

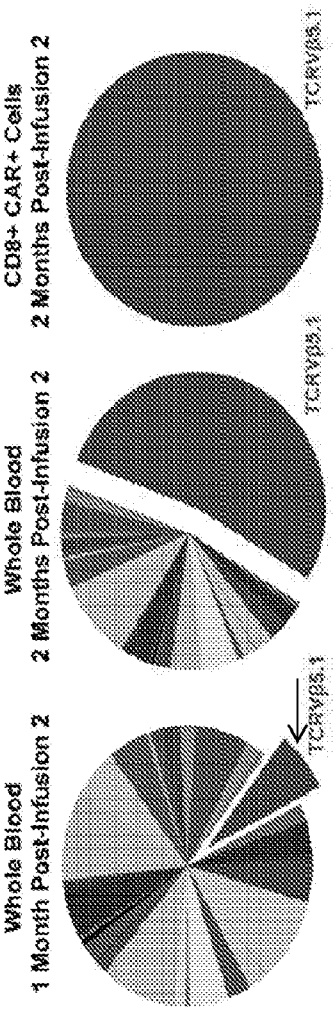

Whole Blood
1 Month Post-Infusion 2
TCRVβ5.1
Whole Blood
2 Months Post-Infusion 2
TCRVβ5.1
CD8+ CAR+ Cells
2 Months Post-Infusion 2
TCRVβ5.1

FIG. 4B

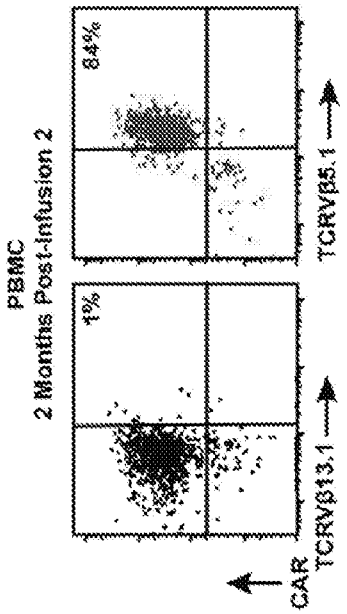

PBMC
2 Months Post-Infusion 2
1%
8.4%
CAR
TCRVβ13.1
TCRVβ5.1

FIG. 4C

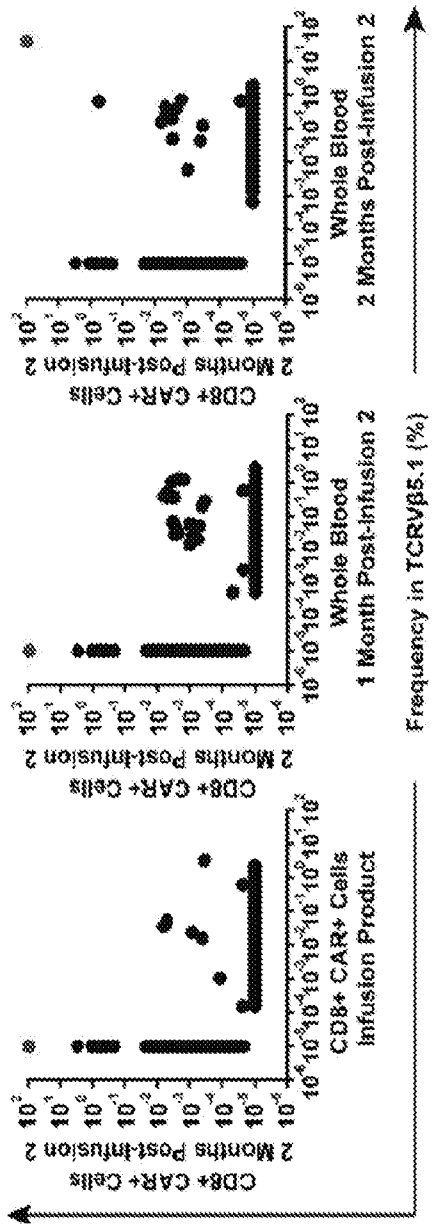

CD8+ CAR+ Cells
2 Months Post-Infusion 2
CD8+ CAR+ Cells
Infusion Product
Whole Blood
1 Month Post-Infusion 2
Whole Blood
2 Months Post-Infusion 2
Frequency in TCRVβ5.1 (%)

FIG. 4D

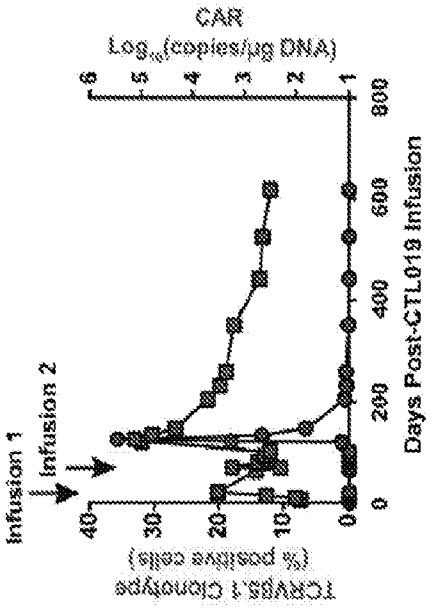

Infusion 1
Infusion 2
TCRVβ5.1 clonotype
(% positive cells)
CAR
Log10(copies/µg DNA)
Days Post-CTL019 Infusion

FIG. 5A

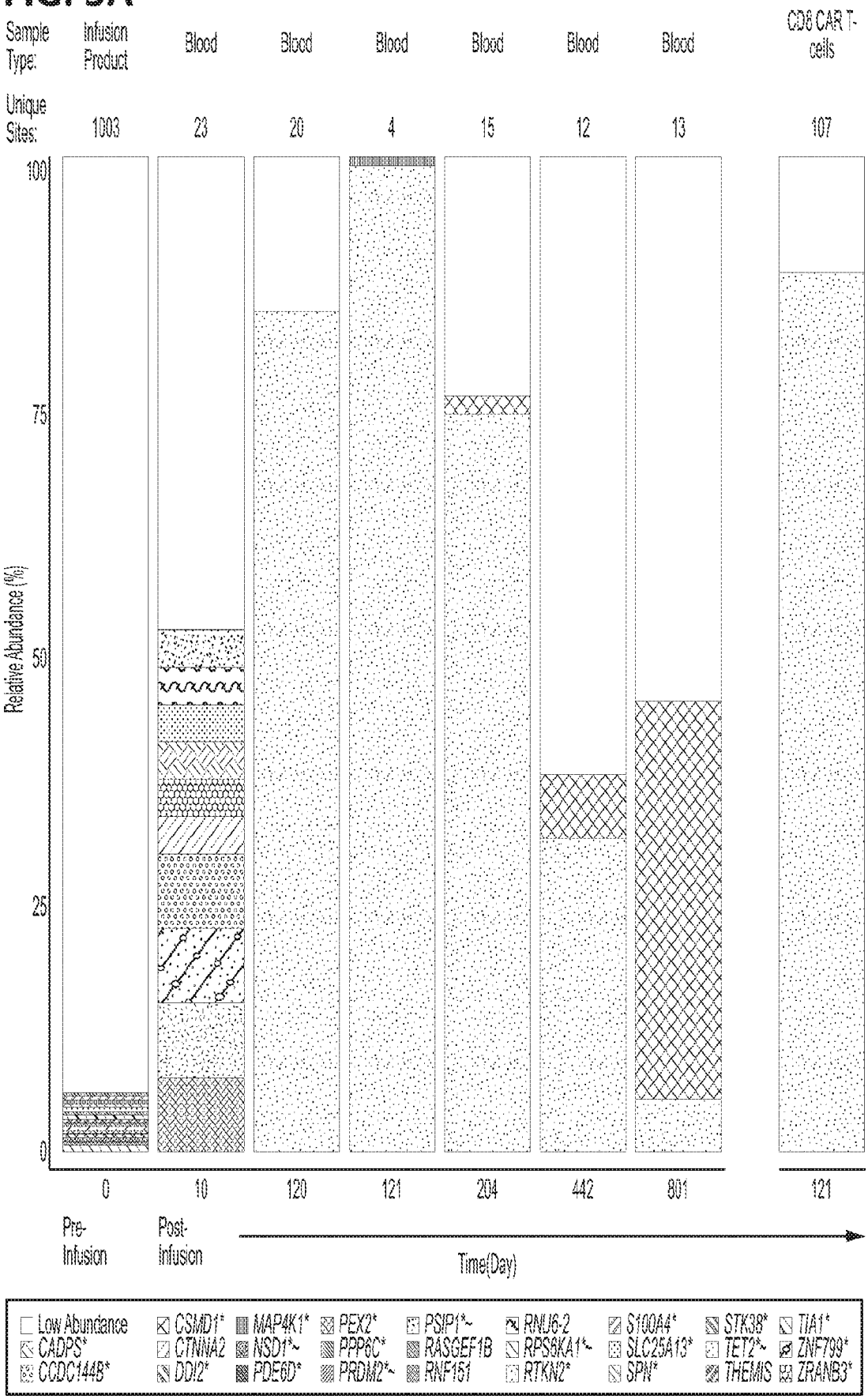
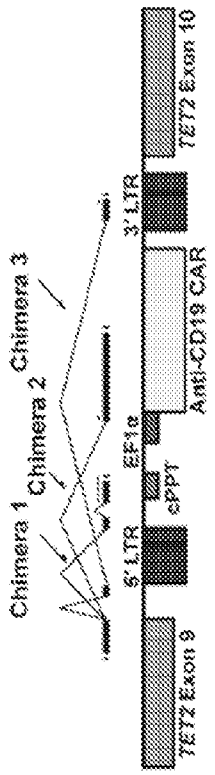

Sample Type:
Infusion Product
Blood
Blood
Blood
Blood
Blood
Blood
CD6 CAR T-cells
Unique Sites:
1003
23
20
4
15
12
13
107
100
75
50
25
0
Relative Abundance (%)
0
10
120
121
204
442
801
121
Pre-Infusion
Post-Infusion
Time(Day)
Low Abundance
CADPS*
CCDC1448*
CSMD1*
CTNNA2
DDI2*
MAP4K1*
NSD1*~
PDE6D*
PEX2*
PPP6C*
PRDM2*~
PSIP1*~
RASGEF1B
RNF151
RNU6-2
RPS6KA1*~
RTKN2*
S100A4*
SLC25A13*
SPN*
STK38*
TET2*~
THEMIS
TIA1*
ZNF799*
ZRANB3*

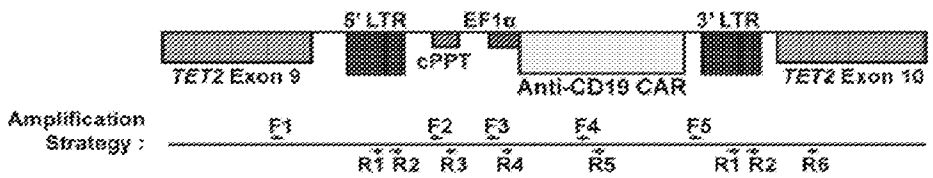
FIG. 5B



Chimera	Splice Site	Donor Sequence	Acceptor Sequence
1	1st	ACAUUG	GUAAGU CUCUAG CAGUGG
1	2nd	GACUGG	UGAGUA GUUAGG CAGGGA
2	1st	ACAUUG	GUAAGU UUUCAG GUGUCG
3	1st	ACAUUG	GUAAGU UAACAG GUAGGA
3	2nd	CAACUA	AUGUAG GGGGAC UGGAAG

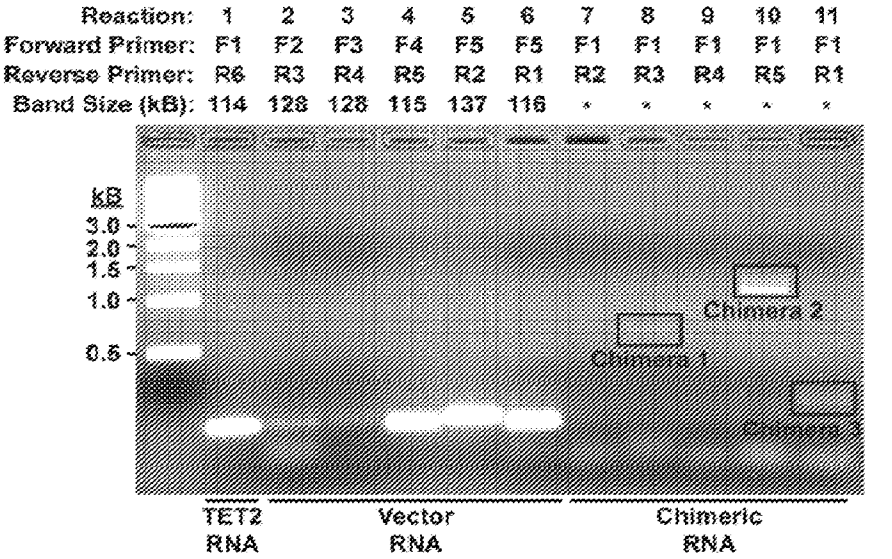
SUBSTITUTE SHEET (RULE 26)

FIG. 6A



Primer	Target	Direction	Sequence
F1	TET2 exon 9	Forward	GACTTGCACAACATGCAGAA
R6	TET2 exon 10	Reverse	GTATAAAGGCAGAACGTGAAGC
R1	Vector R element	Reverse	AGCAGTGGGTTCCCTAGTTA
R3	Vector cPPT element	Reverse	TGCCATTTGTACTGCTGTCTTA
R4	Vector EF1α promoter	Reverse	CAAGGGCCATAACCCGTAAA
R5	Vector 4-1BB region (CAR)	Reverse	GCCATCTTCCTCTTGAGTAGTT
R2	Vector LTR poly(A) signal	Reverse	GGCAAGCTTTATTGAGGCTT
F2	Vector cPPT element	Forward	AGGAATTTGGCATTCCCTACA
F3	Vector EF1α promoter	Forward	GGAGAACCGTATATAAGTGCAGTAG
F4	Vector 4-1BB region (CAR)	Forward	CCTTCTCCTGTCACTGGTTATC
F5	Vector R element	Forward	GAAGGGCTAATTCACTCCCAA

FIG. 6B



Reaction Targets

1: *TET2* exon 9 and 10 junction
2: Vector cPPT element
3: Vector EF1α promoter
4: Vector 4-1BB region (CAR)
5: Vector LTR U5 region
6: Vector LTR R region
7: *TET2* exon 9 to LTR U5 region
8: *TET2* exon 9 to cPPT element
9: *TET2* exon 9 to EF1α promoter
10: *TET2* exon 9 to 4-1BB region (CAR)
11: *TET2* exon 9 to LTR R region

FIG. 7A

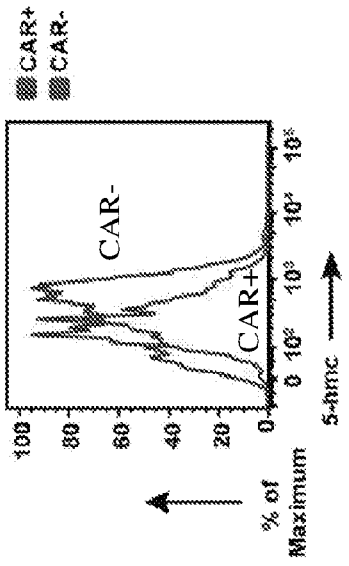


FIG. 7B

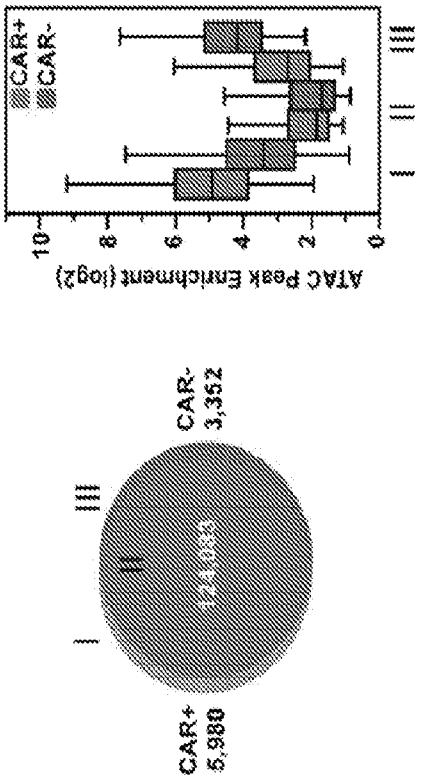


FIG. 7C

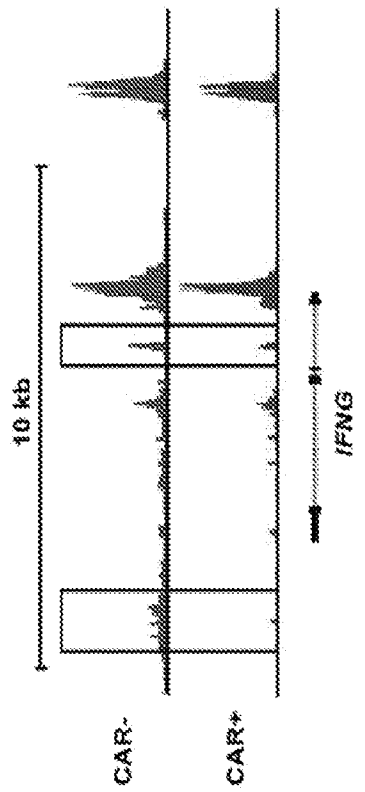
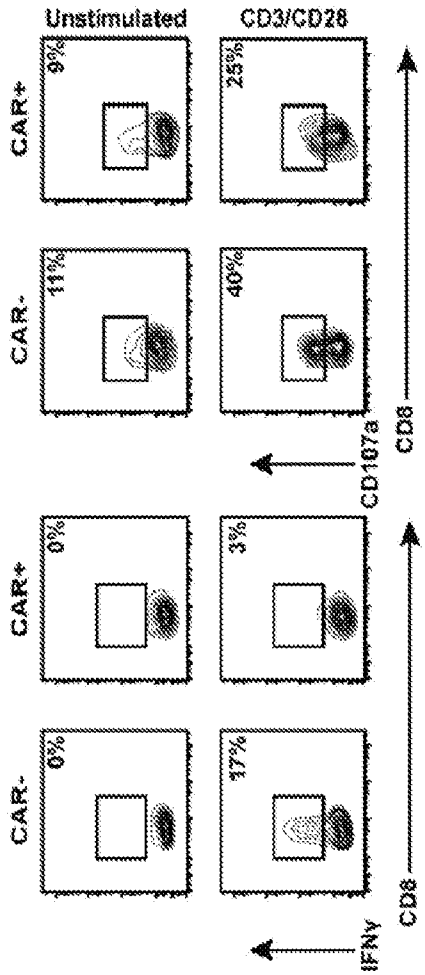


FIG. 7D



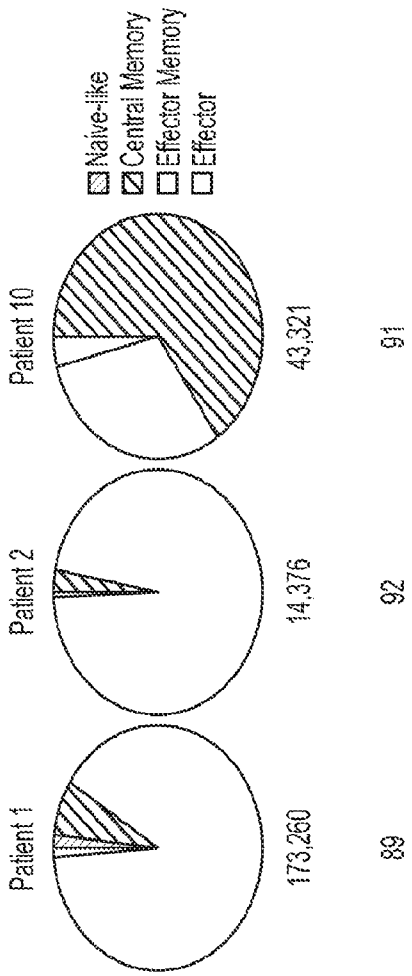

Naive-like
Central Memory
Effector Memory
Effector
Patient 1
Patient 2
Patient 10
CAR T-cell Level (copies/µg DNA)
173,260
14,376
43,321
CD8+ CAR+ HLA-DR+ (%)
89
92
91

FIG. 7E

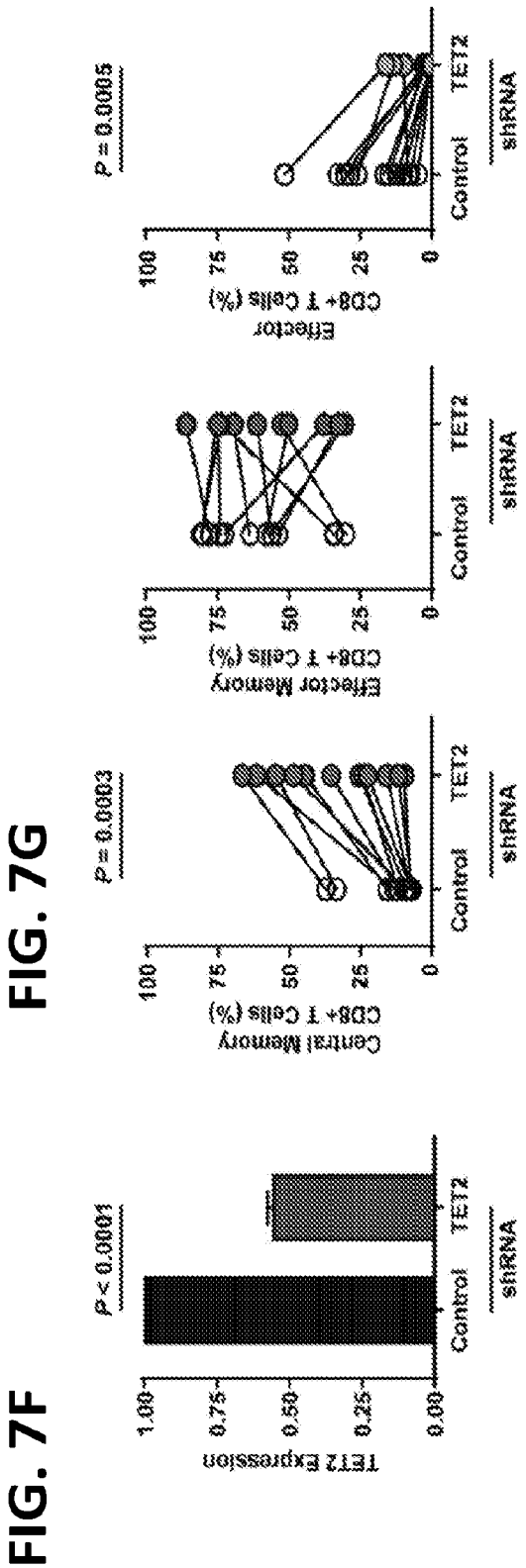

FIG. 7F
TET2 Expression
1.00
0.75
0.50
0.25
0.00
P < 0.0001
Control
TET2
shRNA
FIG. 7G
Central Memory
CD8+ T Cells (%)
P = 0.0003
Effector Memory
CD8+ T Cells (%)
Effector
CD8+ T Cells (%)
P = 0.0005
100
75
50
25
0

FIG. 8

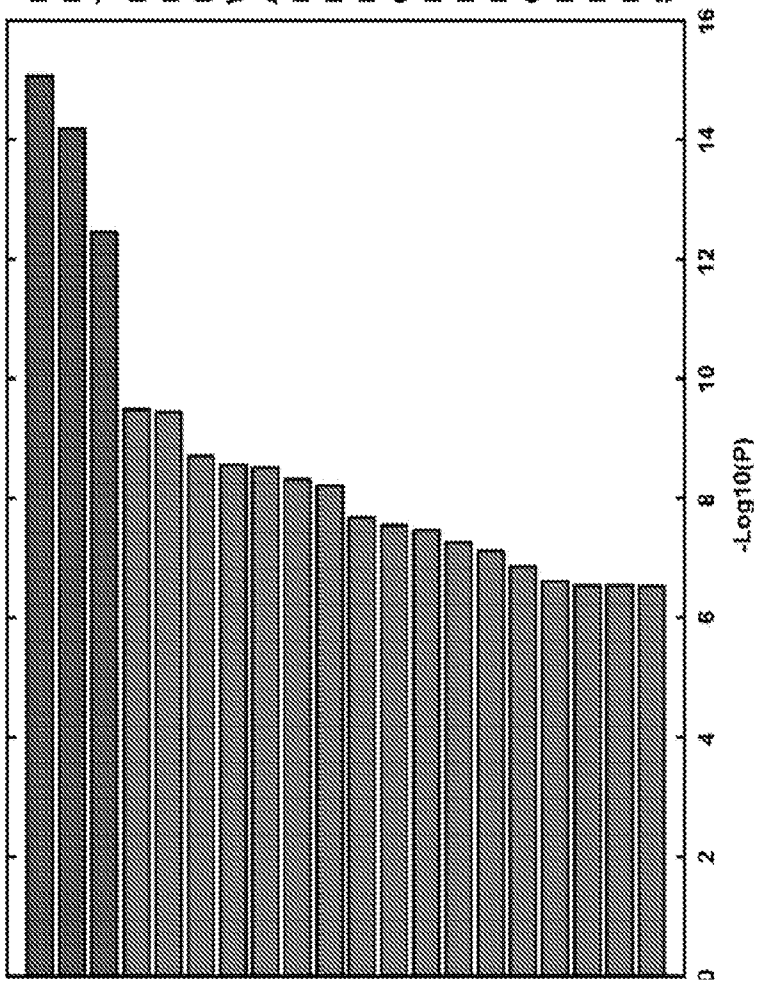

Leukocyte differentiation
Positive regulation of immune system process
Transmembrane receptor protein tyrosine kinase signaling pathway
Regulation of anatomical structure morphogenesis
Hallmark TNFα Signaling via NFκB
Positive regulation of hydrolase activity
Wound healing
Alpha-beta T-cell activation
Regulation of cellular component movement
Inflammatory response
Myeloid cell differentiation
Cytokine production
Hallmark ultraviolet (UV) radiation reponse down-regulated
Negative regulation of multicellular organismal process
Blood vessel morphogenesis
Calcineurin-regulated NFAT-dependent transcription in lymphocytes
Positive regulation of apoptotic process
Hallmark Hypoxia
Hallmark KRAS signaling upregulated
Stress-activated protein kinase signaling cascade
0
2
4
6
8
10
12
14
16
-Log10(P)

FIG. 9

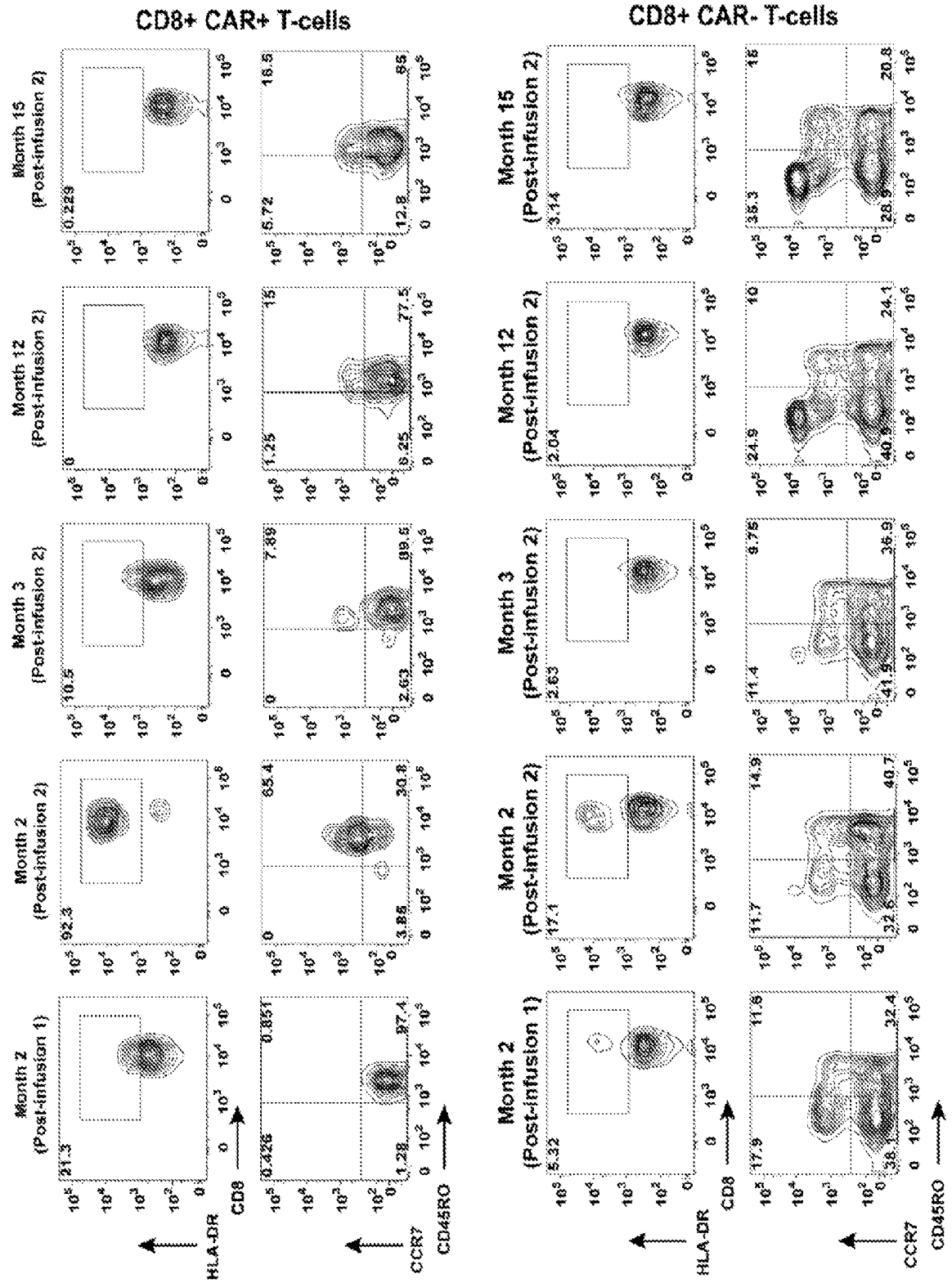

CD8+ CAR+ T-cells
CD8+ CAR- T-cells
Month 2 (Post-infusion 1)
Month 2 (Post-infusion 2)
Month 3 (Post-infusion 2)
Month 12 (Post-infusion 2)
Month 15 (Post-infusion 2)
HLA-DR
CD8
CCR7
CD45RO

FIG. 10A

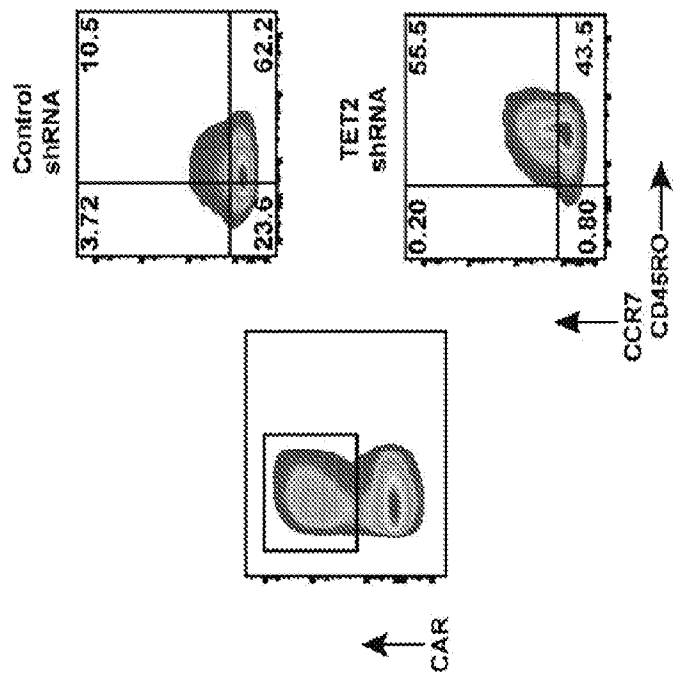


FIG. 10B

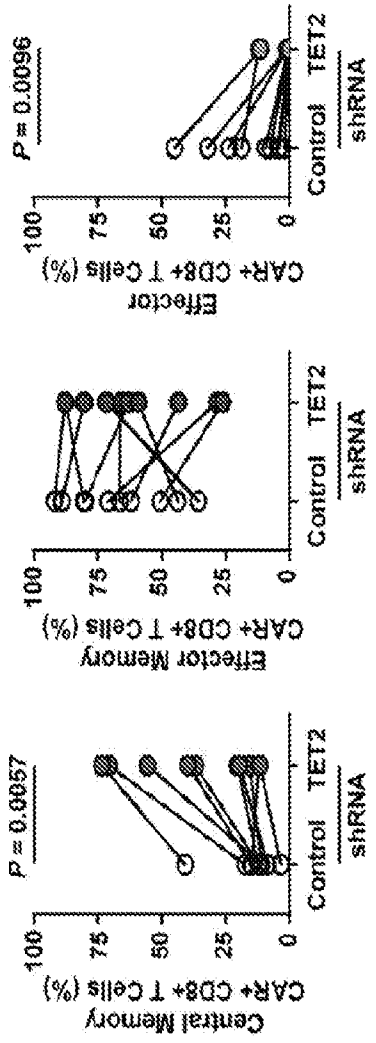


FIG. 10C

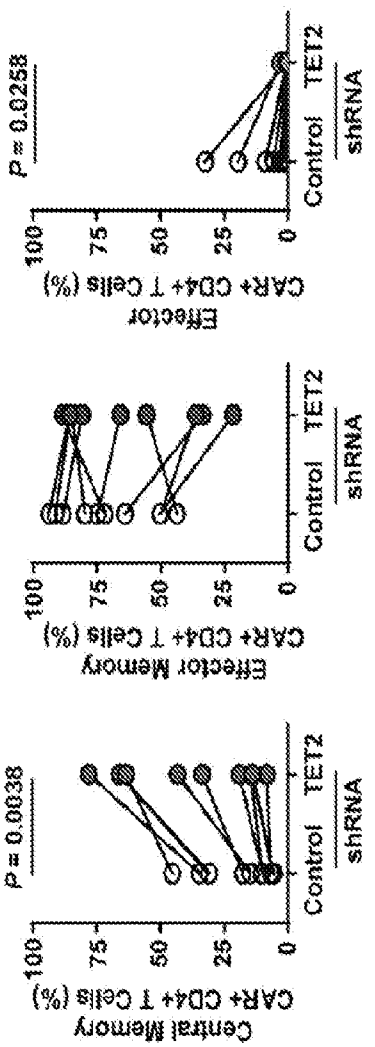


FIG. 11A

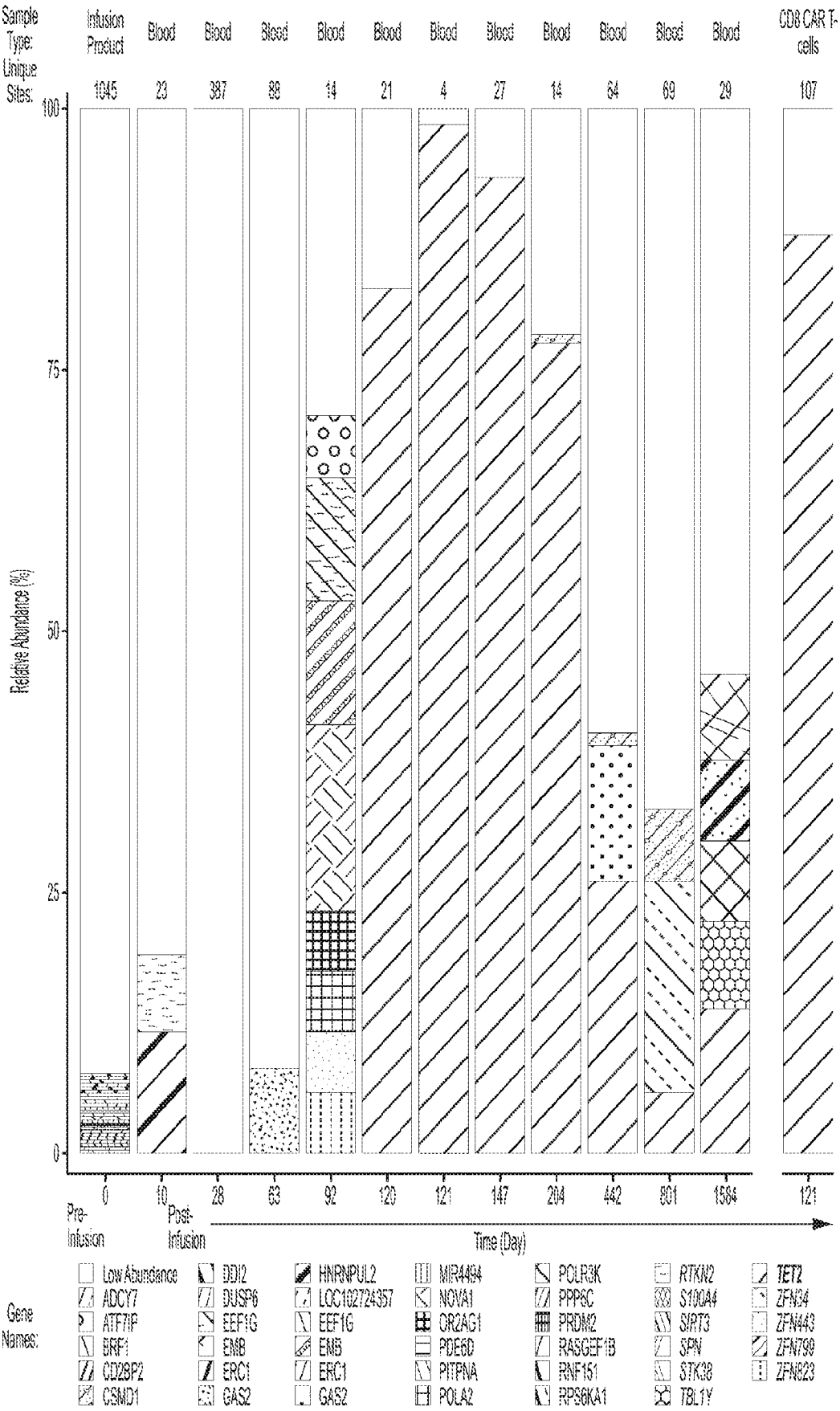

Sample Type:
Infusion Product
Blood
CD8 CAR T-cells
Unique Sites:
1945
23
387
88
14
21
4
27
14
64
69
29
107
Relative Abundance (%)
100
75
50
25
0
0
10
28
63
92
120
121
147
204
442
801
1564
121
Pre-Infusion
Post-Infusion
Time (Day)
Gene Names:
Low Abundance
ADCY7
ATF7IP
BRF1
CD28P2
CSMD1
DDI2
DUSP6
EEF1G
EMB
ERC1
GAS2
HNRNPUL2
LOC102724357
EEF1G
EMB
ERC1
GAS2
MIR4494
NOVA1
OR2AG1
PDE6D
PITPNA
POLA2
POLR3K
PPP6C
PRDM2
RASGEF1B
RNF151
RPS6KA1
RTKN2
S100A4
SIRT3
SPN
STK38
TBL1Y
TET2
ZFN64
ZFN443
ZFN799
ZFN823

FIG. 11B

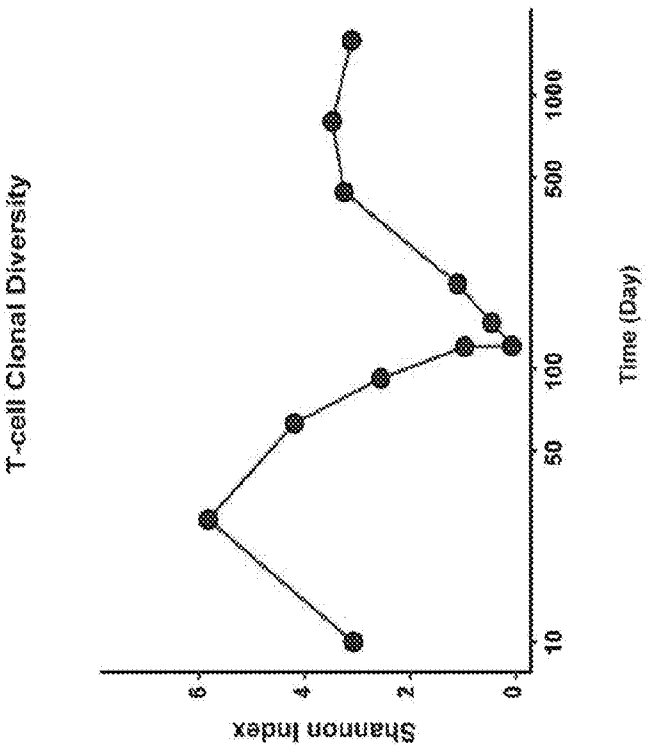


FIG. 11C

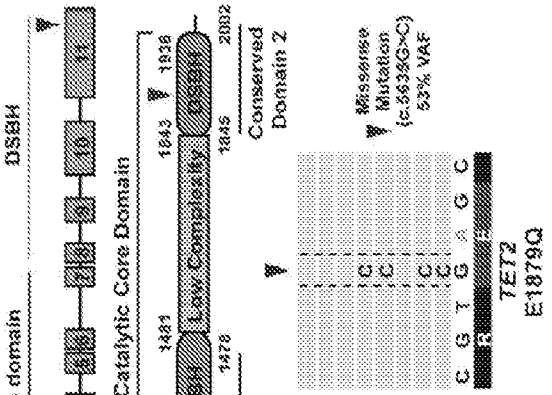


FIG. 11D

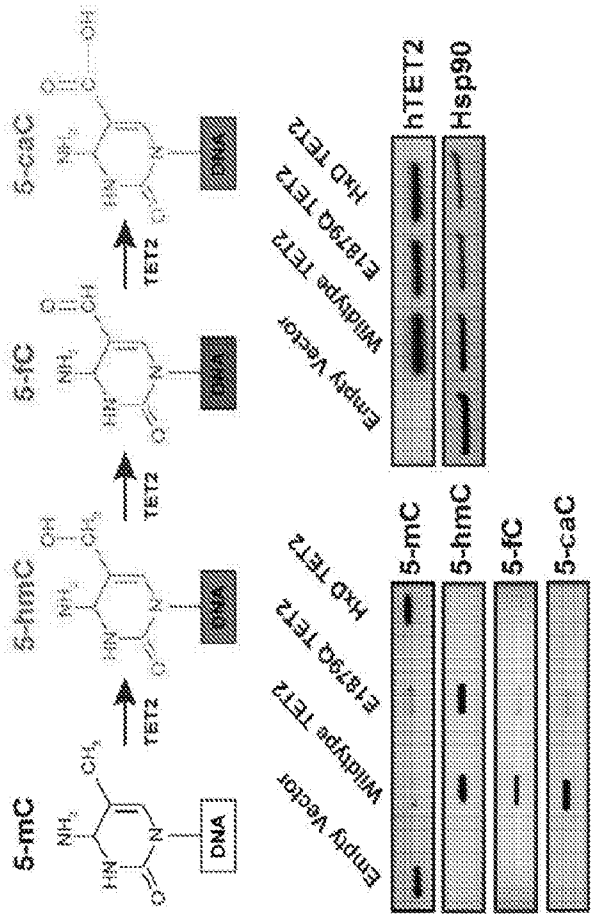

5-mC
5-hmC
5-fC
5-caC
DNA
TET1
TET2
Empty Vector
Wildtype TET2
E1879Q TET2
H/D TET2
hTET2
Hsp90

FIG. 11E

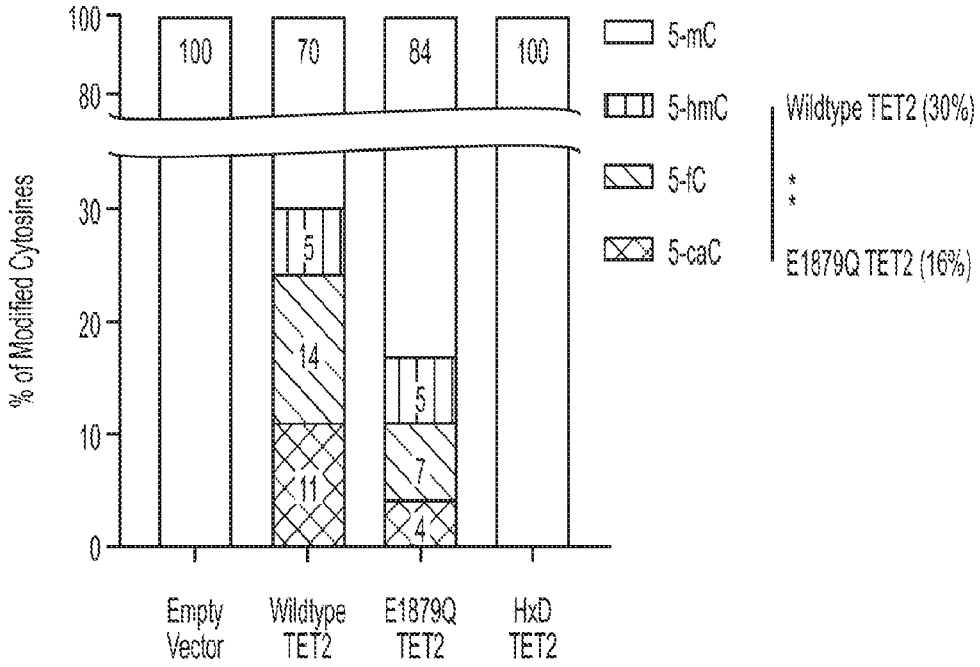

100
80
30
20
10
0
% of Modified Cytosines
100
70
84
100
5
14
11
5
7
4
5-mC
5-hmC
5-fC
5-caC
Wildtype TET2 (30%)
*
*
E1879Q TET2 (16%)
Empty Vector
Wildtype TET2
E1879Q TET2
HxD TET2

FIG. 12A

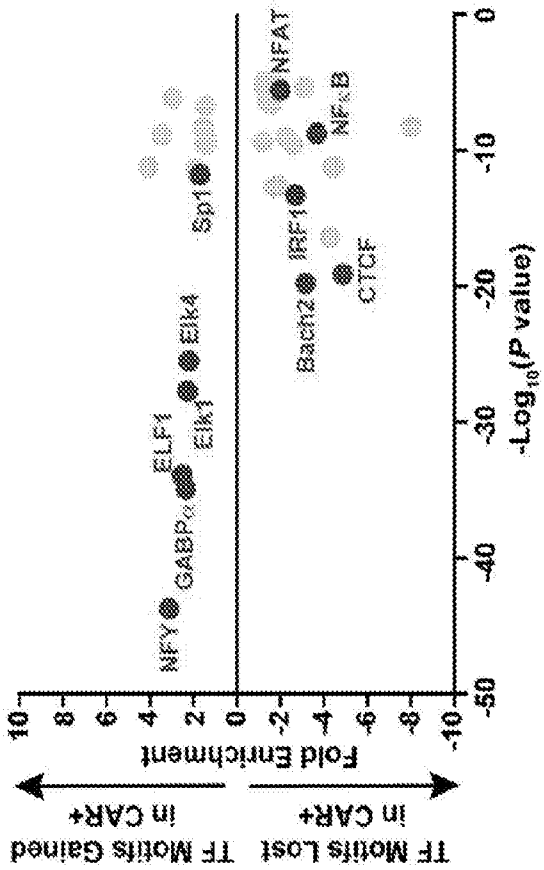

TF Motifs Gained in CAR+
TF Motifs Lost in CAR+
Fold Enrichment
10
8
6
4
2
0
-2
-4
-6
-8
-10
-50
-40
-30
-20
-10
0
-Log10(P value)
NFY
GABPα
ELF1
Elk1
Elk4
Sp1
Bach2
IRF1
CTCF
NF-κB
NFAT

FIG. 12B

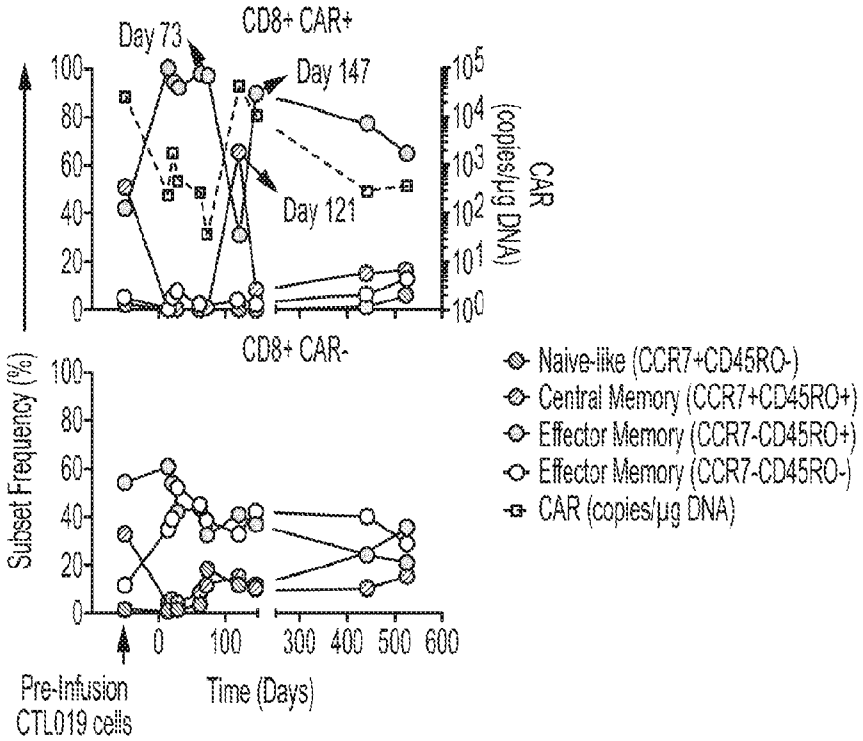

CD8+ CAR+
Day 73
Day 147
Day 121
CD8+ CAR-
Subset Frequency (%)
CAR (copies/µg DNA)
Pre-infusion CTL019 cells
Time (Days)
Naive-like (CCR7+CD45RO-)
Central Memory (CCR7+CD45RO+)
Effector Memory (CCR7-CD45RO+)
Effector Memory (CCR7-CD45RO-)
CAR (copies/µg DNA)

FIG. 12C

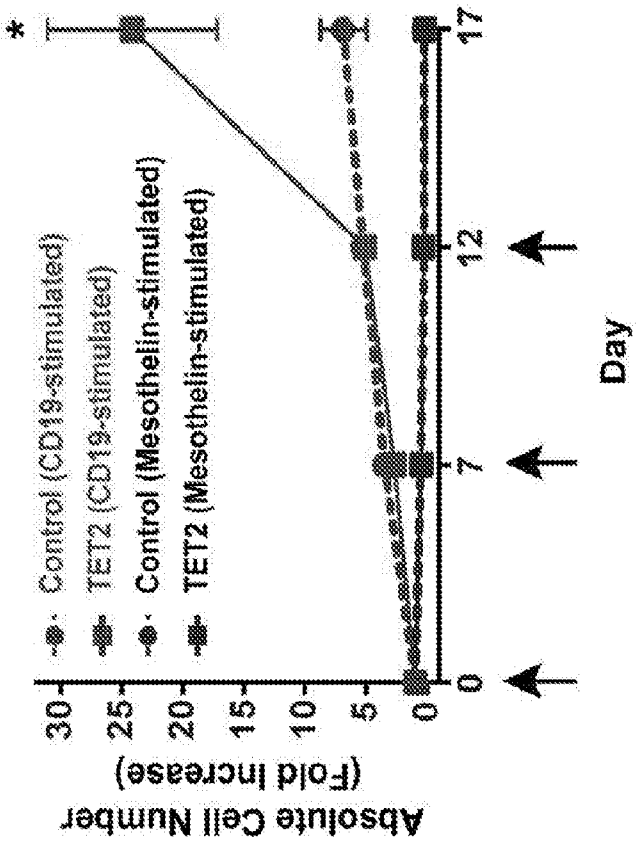

Absolute Cell Number
(Fold Increase)
30
25
20
15
10
5
0
0
7
12
17
Day
Control (CD19-stimulated)
TET2 (CD19-stimulated)
Control (Mesothelin-stimulated)
TET2 (Mesothelin-stimulated)
*

FIG. 13

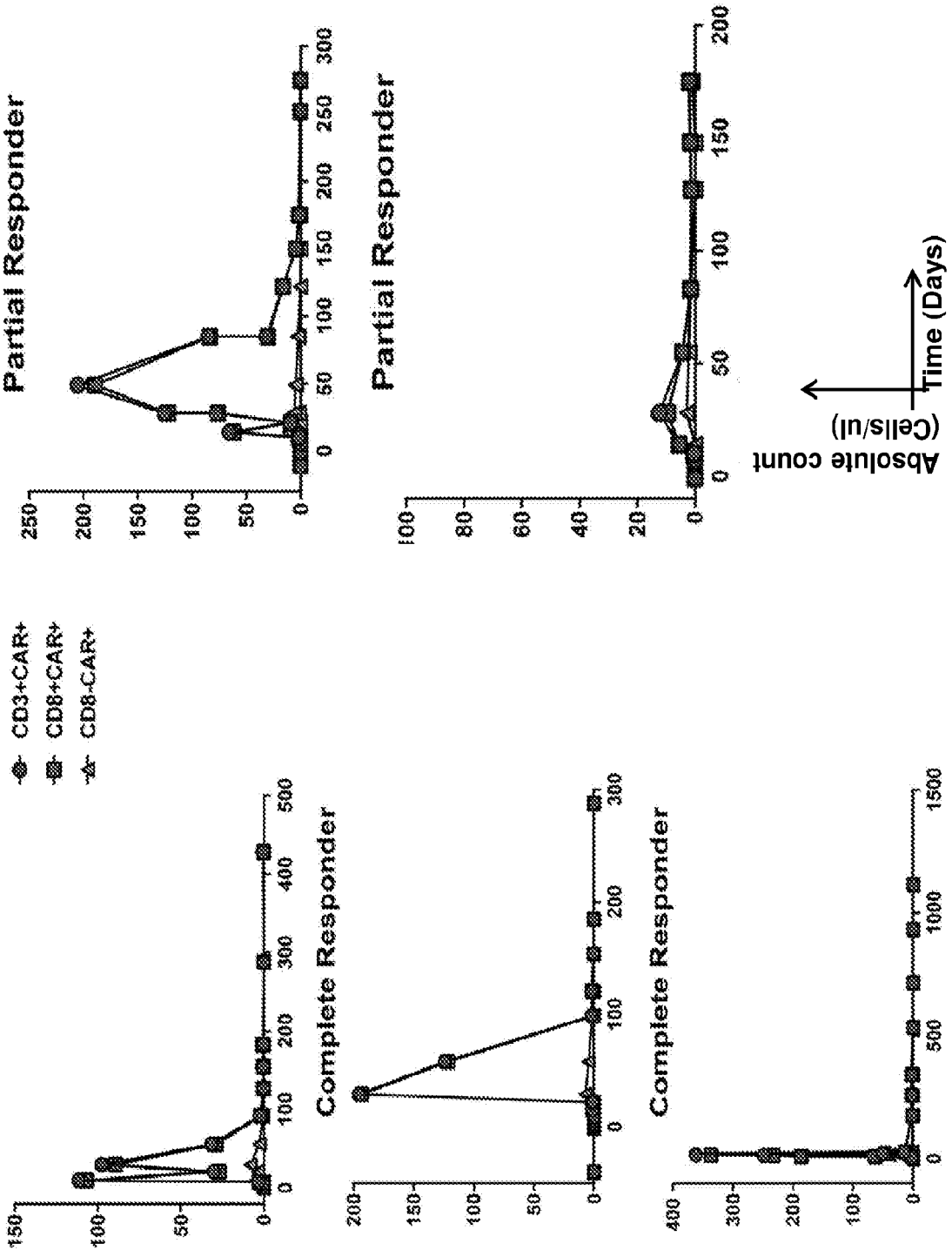

Complete Responder
Partial Responder
Complete Responder
Partial Responder
Complete Responder
CD3+CAR+
CD8+CAR+
CD8-CAR+
Absolute count (Cells/ul)
Time (Days)

FIG. 14A

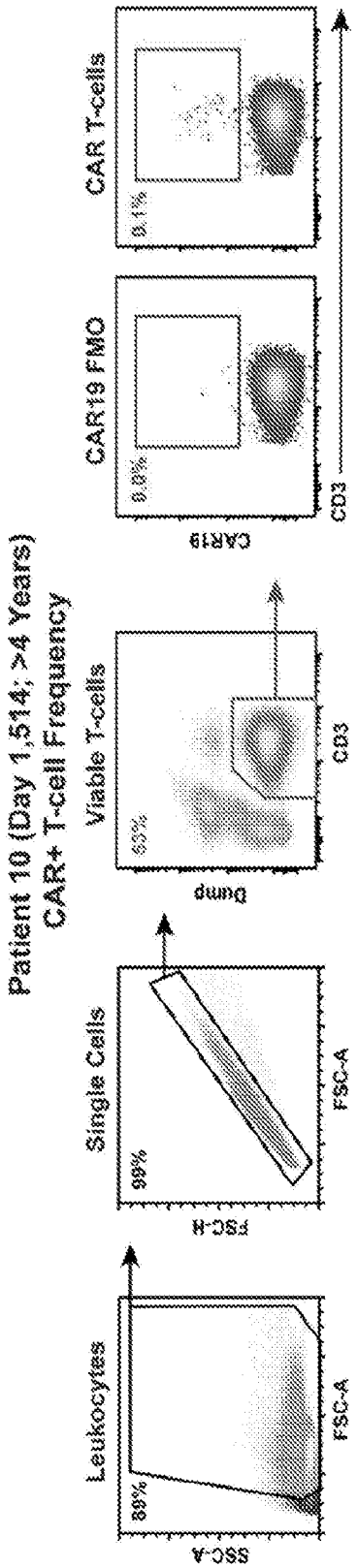


FIG. 14B

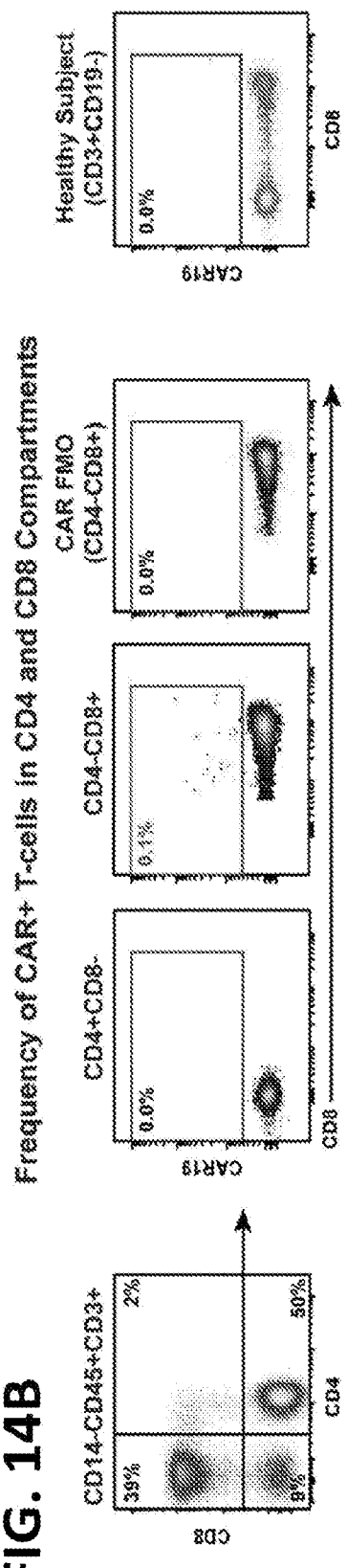


FIG. 14C

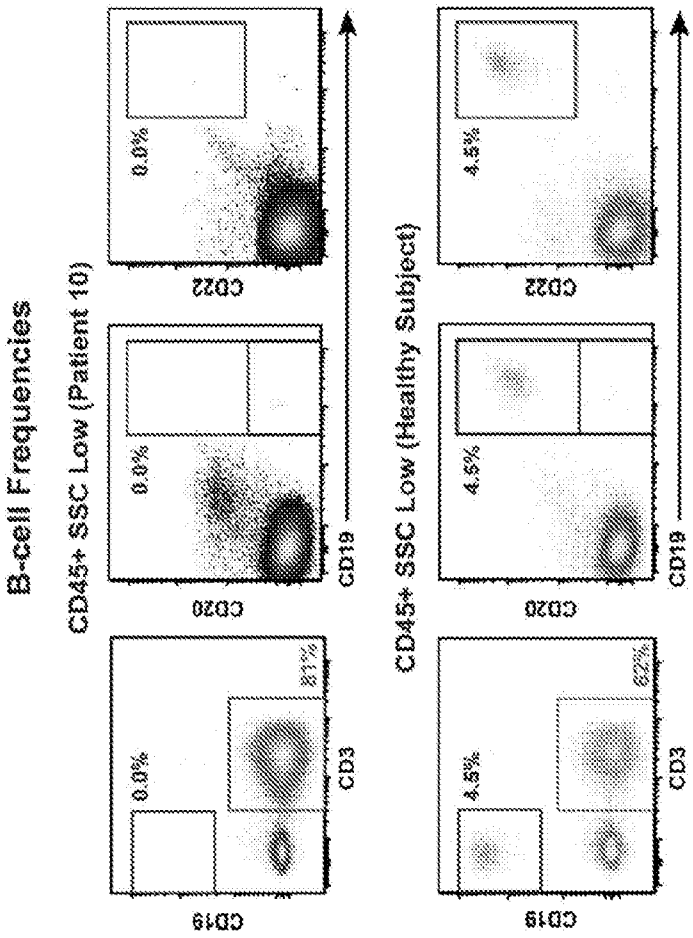


FIG. 14D

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	Average Marking/Cell (%)	Copies/µg DNA
33.56	0.09	0.0860	136.54

FIG. 15

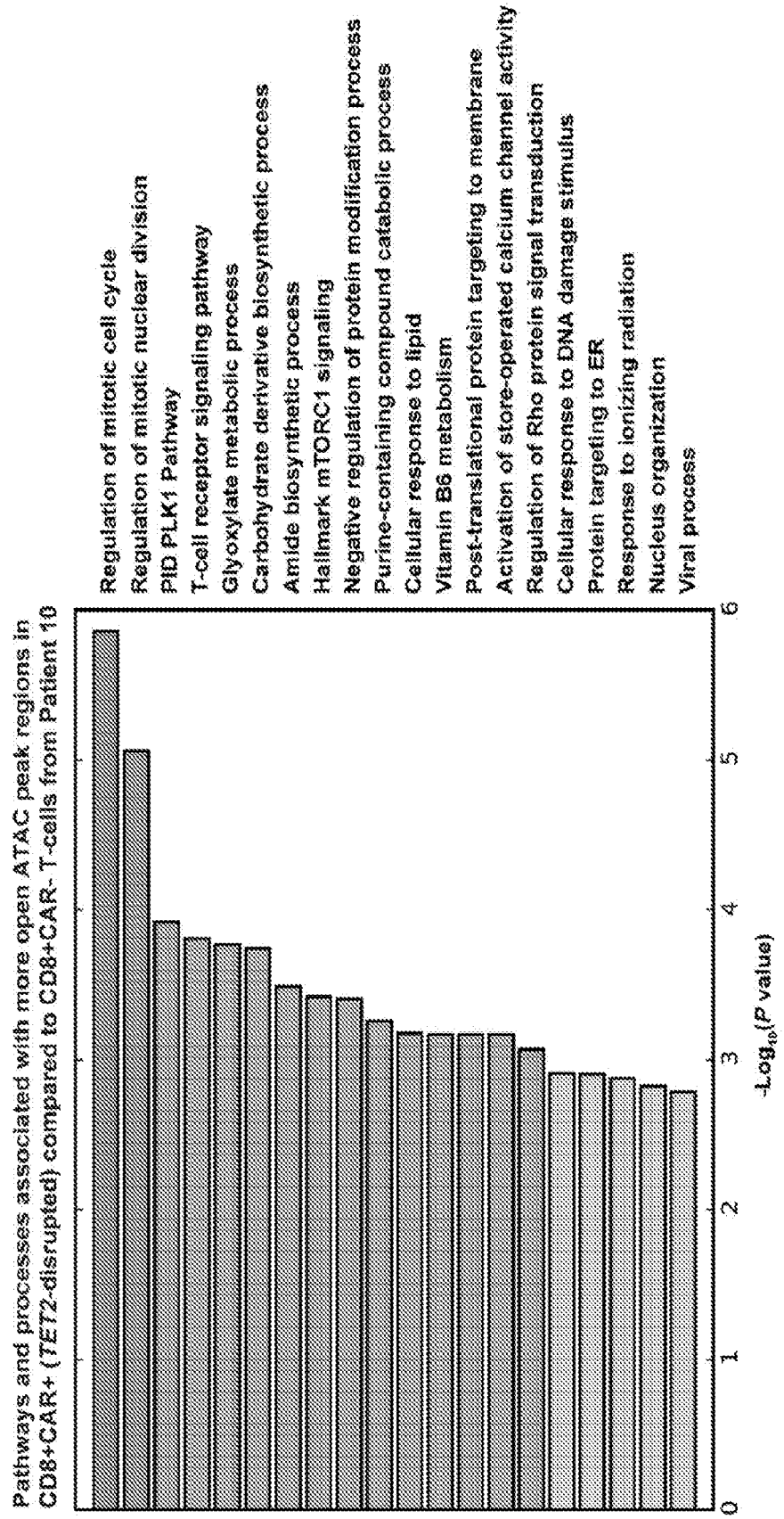
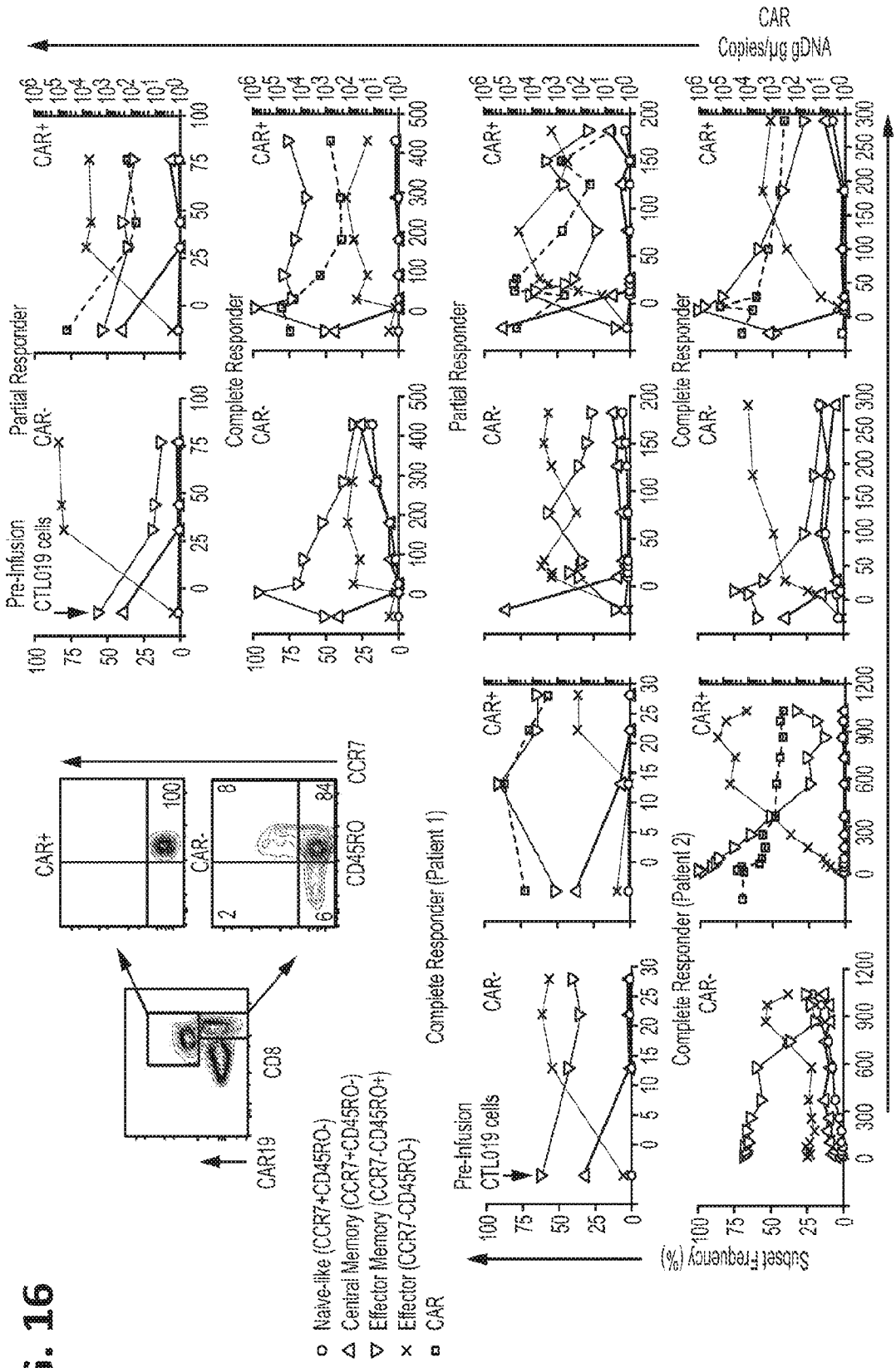

Pathways and processes associated with more open ATAC peak regions in CD8+CAR+ (TET2-disrupted) compared to CD8+CAR- T-cells from Patient 10
Regulation of mitotic cell cycle
Regulation of mitotic nuclear division
PID PLK1 Pathway
T-cell receptor signaling pathway
Glyoxylate metabolic process
Carbohydrate derivative biosynthetic process
Amide biosynthetic process
Hallmark mTORC1 signaling
Negative regulation of protein modification process
Purine-containing compound catabolic process
Cellular response to lipid
Vitamin B6 metabolism
Post-translational protein targeting to membrane
Activation of store-operated calcium channel activity
Regulation of Rho protein signal transduction
Cellular response to DNA damage stimulus
Protein targeting to ER
Response to ionizing radiation
Nucleus organization
Viral process
0
1
2
3
4
5
6
-Log10(P value)

FIG. 16


CAR
Copies/µg gDNA
Partial Responder
Complete Responder
Partial Responder
Complete Responder
CAR+
CAR-
Pre-Infusion
CTL019 cells
Complete Responder (Patient 1)
Complete Responder (Patient 2)
Subset Frequency (%)
CAR+
CAR-
CAR19
CD8
CD45RO
CCR7
Naive-like (CCR7+CD45RO-)
Central Memory (CCR7+CD45RO+)
Effector Memory (CCR7-CD45RO+)
Effector (CCR7-CD45RO-)
CAR

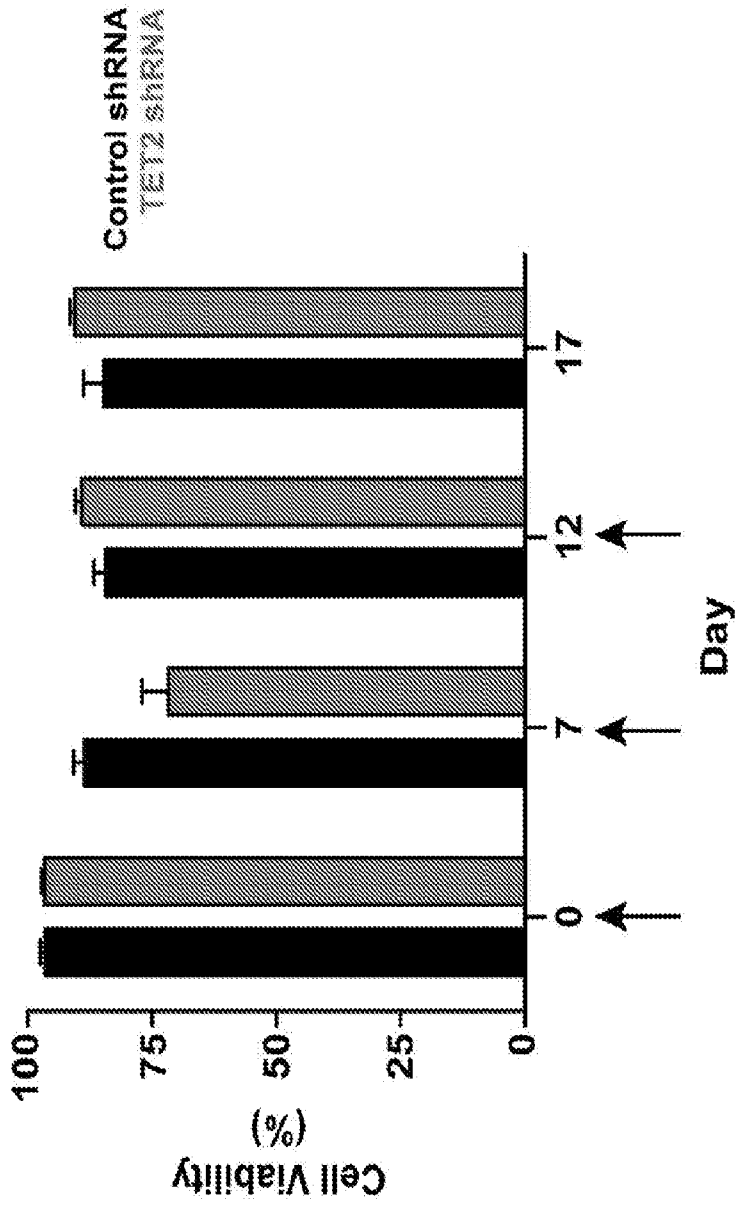

Control shRNA
TET2 shRNA
Cell Viability
(%)
100
75
50
25
0
0
7
12
17
Day

FIG. 17

FIG. 18A

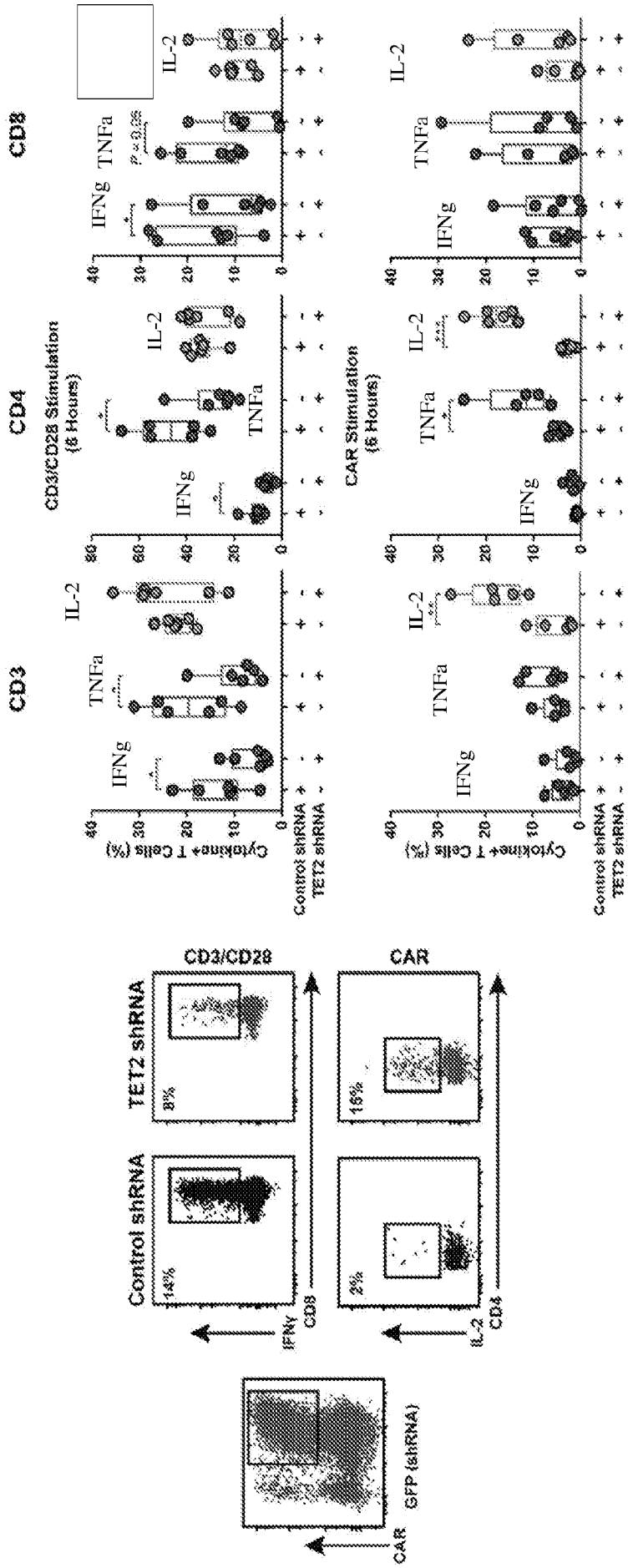

CD3
CD4
CD8
CD3/CD28 Stimulation (6 Hours)
CAR Stimulation (6 Hours)
IFNg
TNFa
IL-2
Cytokine+ T Cells (%)
Control shRNA
TET2 shRNA
CD3/CD28
CAR
14%
8%
2%
16%
IFNγ
IL-2
CD8
GFP (shRNA)

FIG. 18B

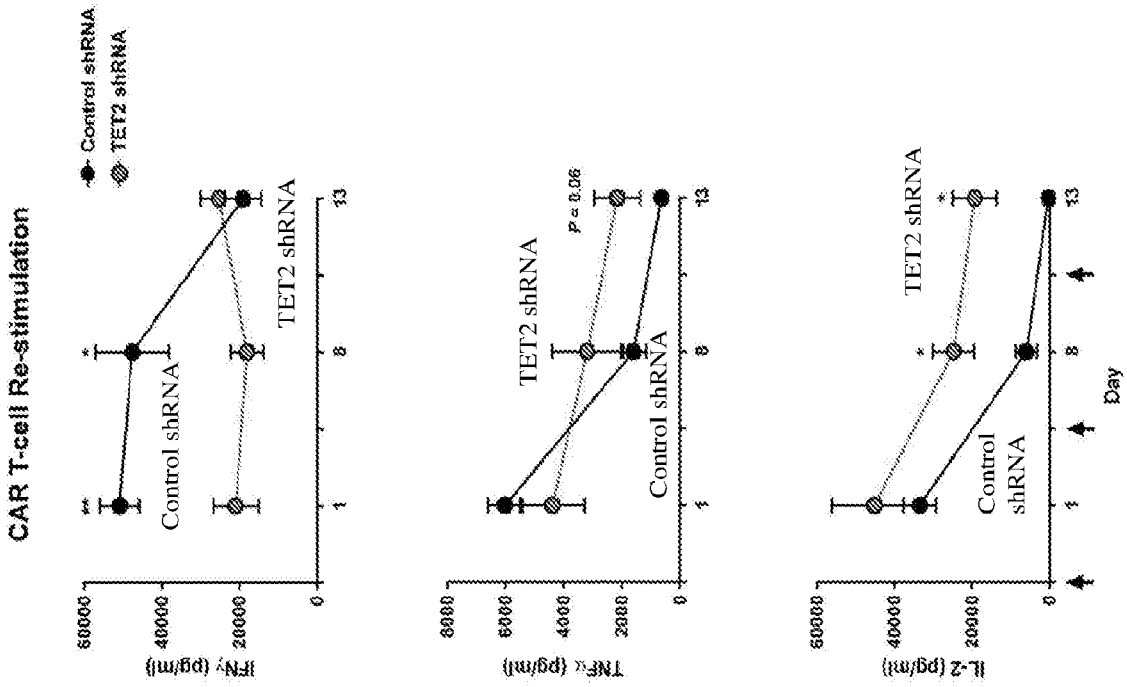

CAR T-cell Re-stimulation
Control shRNA
TET2 shRNA
IFNγ (pg/ml)
TNFα (pg/ml)
IL-2 (pg/ml)
P = 0.06
Day

FIG. 19A

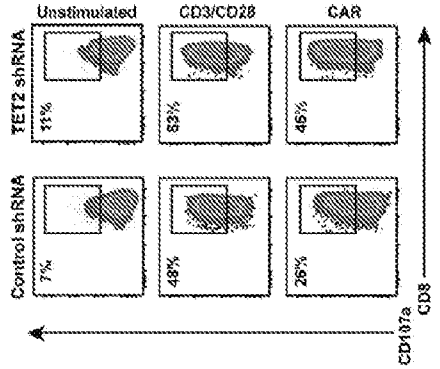


FIG. 19B

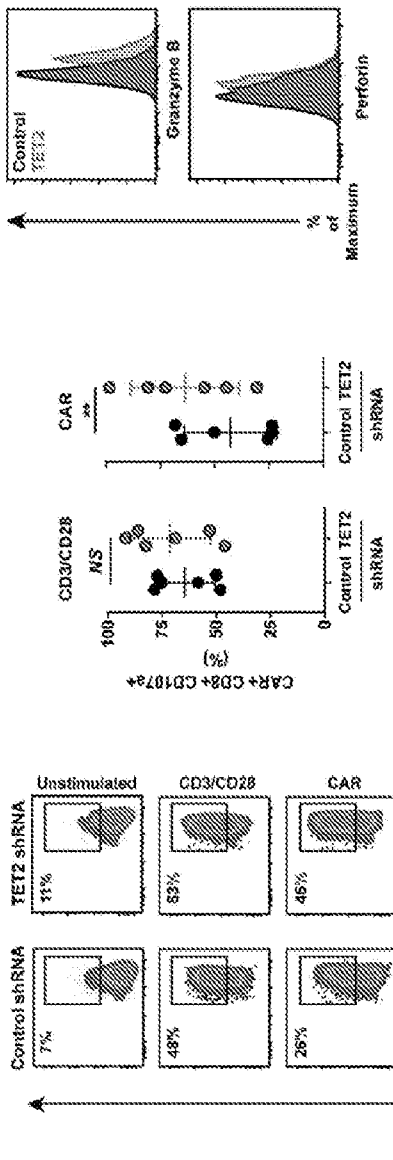


FIG. 19C

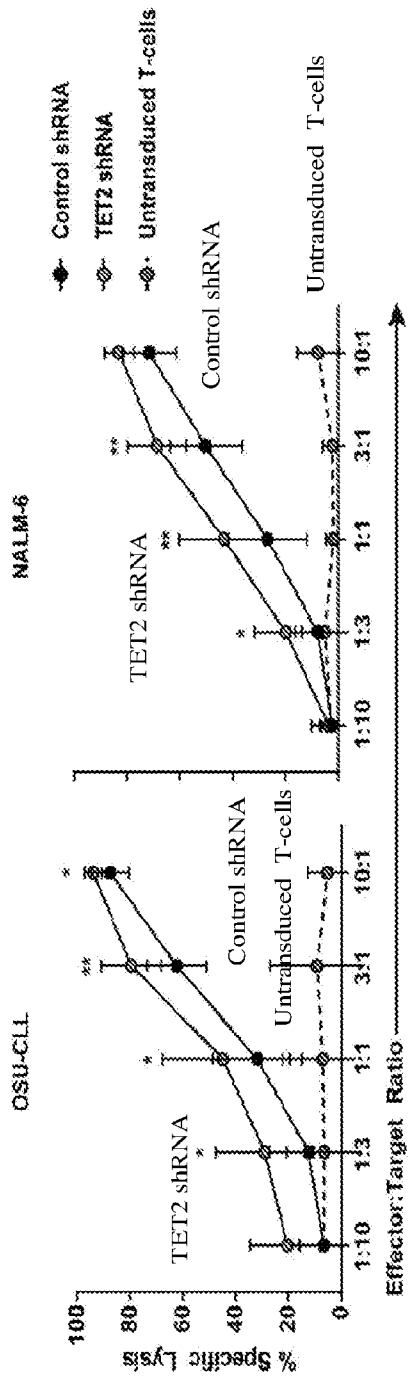


FIG. 20A

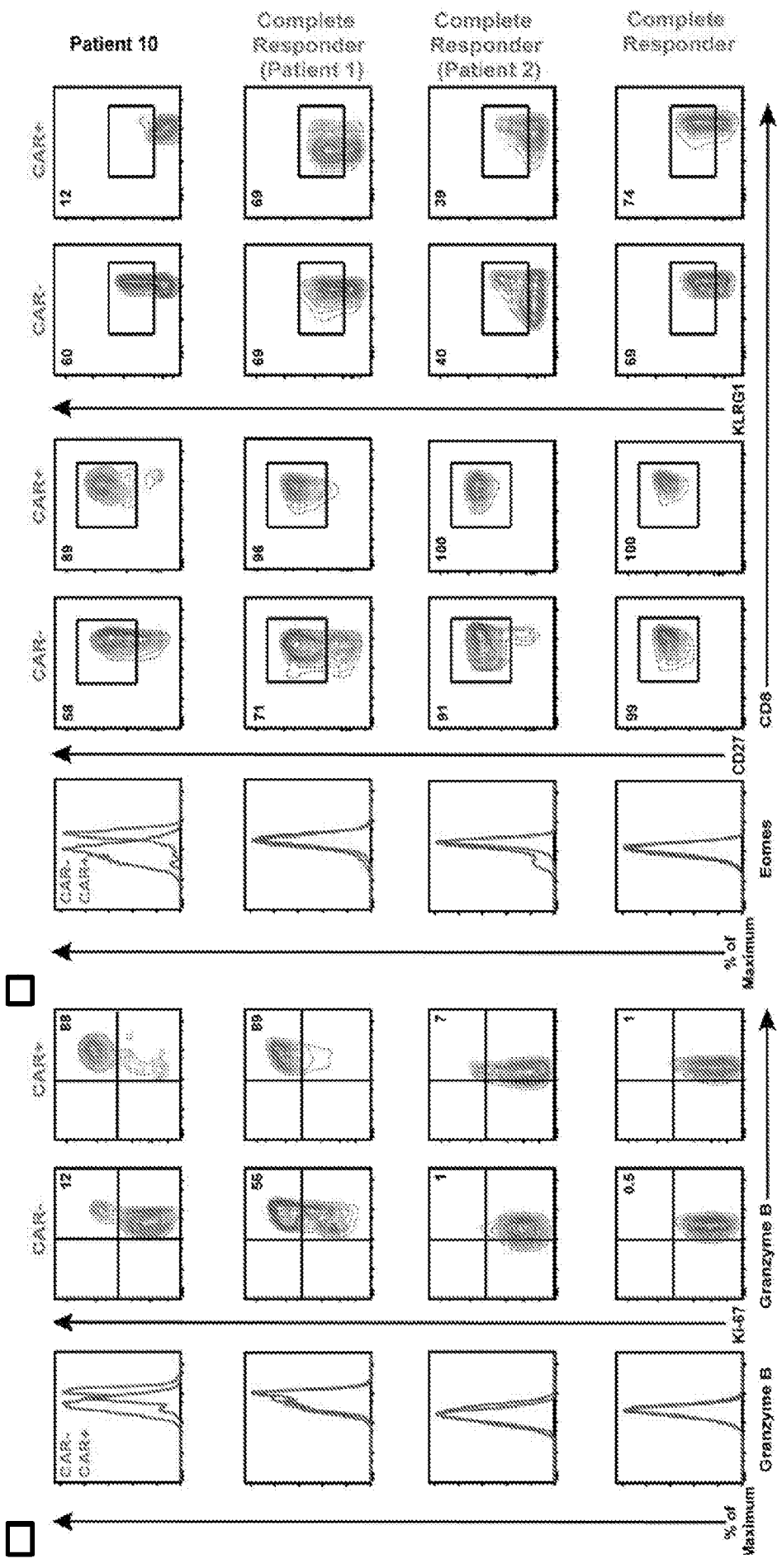


FIG. 20B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/023785

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RYAN URAK ET AL: "Ex vivo Akt inhibition promotes the generation of potent CD19CAR T cells for adoptive immunotherapy", JOURNAL FOR IMMUNOTHERAPY OF CANCER, BIOMED CENTRAL LTD, LONDON, UK, vol. 5, no. 1, 21 March 2017 (2017-03-21), pages 1-13, XP021242928, DOI: 10.1186/S40425-017-0227-4 the whole document ----- -/--	1-147

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2018	06/09/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Manu, Dominique

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/023785

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2 December 2016 (2016-12-02), AVANZI MAURO P ET AL: "IL-18 Secreting CAR T Cells Enhance Cell Persistence, Induce Prolonged B Cell Aplasia and Eradicate CD19+Tumor Cells without Need for Prior Conditioning", XP002782357, Database accession no. PREV201700298608 abstract & BLOOD, vol. 128, no. 22, 2 December 2016 (2016-12-02), page 816, 58TH ANNUAL MEETING AND EXPOSITION OF THE AMERICAN-SOCIETY-OF-HEMATOLOGY (ASH); SAN DIEGO, CA, USA; DECEMBER 03 -06, 2016 ISSN: 0006-4971(print) -----	1-147
X	NINOMIYA SORANOBU ET AL: "Tumor indoleamine 2,3-dioxygenase (IDO) inhibits CD19-CAR T cells and is downregulated by lymphodepleting drugs", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, UNITED STATES, vol. 125, no. 25, 18 June 2015 (2015-06-18), pages 3905-3916, XP002779636, ISSN: 1528-0020 the whole document -----	1-47
X,P	WO 2017/049166 A1 (NOVARTIS AG [CH]; UNIV PENNSYLVANIA [US]; MOTZ GREGORY [US]; BUSHMAN F) 23 March 2017 (2017-03-23) the whole document -----	1-147
T	JOSEPH A. FRAIETTA ET AL: "Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells", NATURE, vol. 558, no. 7709, 30 May 2018 (2018-05-30), pages 307-312, XP055486057, GB ISSN: 0028-0836, DOI: 10.1038/s41586-018-0178-z -----	1-147

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/023785

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-147(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-147(partially)

A cell expressing a CAR wherein the cell has altered expression and/or function of IFN-gamma and a population of cells comprising said cells. A method of increasing th therapeutic efficacy of a CAR-expressing cell comprising altering the expression or function of IFN-gamma or comprising the step of contacting the cell with a modulator of IFN-gamma. A method of treating cancer with the cell or the cell for use in a method of treating a subject. The combined use of a CAR-expressing cell and a modulator of IFN-gamma to treat a subject in need thereof. A method of manufacturing a CAR-expressing cell comprising introducing a nucleic acid encoding a CAR into the genome of the cell within the IFN-gamma gene. A vector comprising sequence encoding a CAR nd a sequence encoding a modulator of IFN-gamma. A gene editing system specific for IFN-gamma. A composition comprising a modulator of IFN-gamma.

2-500. claims: 1-147(partially)

same as invention 1 with the gene listed in table 8 and 9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/023785

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017049166 A1	23-03-2017	AU 2016323985 A1	22-03-2018
		CA 2999070 A1	23-03-2017
		EP 3350320 A1	25-07-2018
		KR 20180051625 A	16-05-2018
		WO 2017049166 A1	23-03-2017