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(54) Titre : THERAPIE CONTRE LE CANCER DES CELLULES IMMUNITAIRES ANTI-LIV1
(54) Title: ANTI-LIV1 IMMUNE CELL CANCER THERAPY

(57) Abrégé/Abstract:
Provided herein, in some embodiments, are methods and compositions (e.g., cell compositions) for the treatment of cancer, such as LIV1^{+} malignancies.

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(54) Title: ANTI-LIV1 IMMUNE CELL CANCER THERAPY

(57) Abstract: Provided herein, in some embodiments, are methods and compositions (e.g., cell compositions) for the treatment of cancer, such as $LIV1^+$ malignancies.

ANTI-LIV1 IMMUNE CELL CANCER THERAPY

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/756,723, filed November 7, 2018, the disclosure of which is hereby incorporated by reference in its entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The name of the text file containing the Sequence Listing is " CT113_Seqlisting.txt", which was created on November 7, 2019 and is 191,166 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

BACKGROUND

[0003] Chimeric antigen receptor (CAR) T-cell therapy uses genetically-modified T cells to more specifically and efficiently target and kill cancer cells. After T cells have been collected from the blood, the cells are engineered to include CARs on their surface. The CARs may be introduced into the T cells using CRISPR/Cas9 gene editing technology. When these allogeneic CAR T cells are injected into a patient, the receptors enable the T cells to kill cancer cells.

SUMMARY

[0004] LIV1, a member of the ZIP family of highly conserved transmembrane zinc transporter proteins, is expressed at elevated levels in estrogen receptor-positive breast cancer and tumors of the lymph nodes. Further aberrant expression of zinc transporters such as LIV1 is known to lead to deregulated Zn intake or deficiency, leading to uncontrolled growth such that occur in cancer. Thus, LIV1 is a desirable transmembrane protein for targeting cancer. In fact, the LIV-1 protein has been implicated in breast cancer, prostate cancer, squamous tumors, and neuronal tumors.

[0005] Some aspects of the present disclosure provide an engineered T cell comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprise an ectodomain that binds specifically to LIV1. In some embodiments, the engineered T cell further comprises a disrupted T cell receptor alpha chain constant region (*TRAC*) gene. For example, the *TRAC* gene may be disrupted by insertion of the nucleic acid encoding a CAR. In some embodiments, the engineered T cell further comprises a disrupted beta-2-microglobulin (*β2M*) gene.

[0006] The ectodomain of the CAR, in some embodiments, comprises an anti-LIV1 antibody. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv). The anti-LIV1 scFv, in some embodiments, comprises an amino acid sequence of any one of SEQ ID NO: 54, 70, 83 or 86. In some embodiments, the anti-LIV1 scFv comprises a heavy chain variable region (VH) comprising an amino acid sequence of any one of SEQ ID NO: 55 or 90 and/or a light chain variable region (VL) comprising an amino acid sequence of any one of SEQ ID NO: 56 or 88. In some embodiments, the anti-LIV1 scFv comprises a VH comprising CDR amino acid sequences of SEQ ID NO: 57, SEQ ID NO: 58, and/or SEQ ID NO: 59; and/or the anti-LIV1 scFv comprises a VL sequence comprising CDR amino acid sequences of SEQ ID NO: 60, SEQ ID NO: 61, and/or SEQ ID NO: 62.

[0007] The CAR, in some embodiments, comprises a CD3ζ cytoplasmic signaling domain. In some embodiments, the CAR comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

[0008] In some embodiments, the *TRAC* gene comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111, and/or wherein the CAR comprises the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108. In some embodiments, the disrupted *β2M* gene comprises at least one nucleotide sequence selected from any one of SEQ ID NOs: 9-14.

[0009] Also provided herein, in some aspects, is a population of engineered T cells (e.g., comprising a nucleic acid encoding an anti-LIV1 CAR), wherein at least 25% or at least 50% of engineered T cells of the population express the CAR. In some aspects, at least 15% or at least 50% of engineered T cells of the population express the CAR. For example, at least 70% of engineered T cells of the population express the CAR. In another example, at least 30% of engineered T cells of the population express the CAR.

[0010] In some embodiments, at least 25% of engineered T cells of the population express the CAR following at least 7 or at least 14 days of *in vitro* proliferation.

[0011] In some embodiments, at least 50% of engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein. For example, at least 90% of engineered T cells of the population may not express a detectable level of TCR protein.

[0012] In some embodiments, at least 50% of engineered T cells of the population do not express a detectable level of β2M protein. For example, at least 70% of engineered T cells of the population may not express a detectable level of β2M protein.

[0013] In some embodiments, engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 50% of the cancer cells of the population. For example, engineered T cells of the population may induce cell lysis of at least 70%, at least 80%, or at least 90% of the cancer cells of the population. In some embodiments, engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells, secrete IFNγ. In some embodiments, the ratio of engineered T cells to cancer cells is 1:1 to 2:1. The cancer cells may be, for example, sarcoma cells or breast cancer cells. Other cancer cells may be targeted.

[0014] In some embodiments, proliferative capacity of engineered T cells of the population is within 10% of proliferative capacity of control cells.

[0015] Other aspects of the present disclosure provide a method that comprises administering the population of engineered T cells as described herein. In some embodiments, percent body weight of the subject, following 5-10 days of administration, is within 10% of initial body weight of the subject, wherein initial body weight of the subject is body weight of the subject at the time of administration. In some embodiments, the subject is a human subject. In some embodiments, the subject has a cancer. The cancer may express LIV1, for example.

[0016] Further aspects of the present disclosure provide a method for producing an engineered T cell, the method comprising (a) delivering to a T cell a RNA-guided nuclease, a gRNA targeting a *TRAC* gene, and a vector comprising a donor template that comprises a nucleic acid encoding a CAR that comprise an ectodomain that binds specifically to LIV1, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the *TRAC* gene, and (b) producing an engineered T cell. In some embodiments, the gRNA

targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 18 or 19, or targets the nucleotide sequence of SEQ ID NO: 40.

[0017] In some embodiments, the method further comprises delivering to the T cell a gRNA targeting the *β2M* gene. In some embodiments, the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 20 or 21, or targets the nucleotide sequence of SEQ ID NO: 41.

[0018] In some embodiments, the RNA-guided nuclease is a Cas9 nuclease, optionally a *S. pyogenes* Cas9 nuclease.

[0019] In some embodiments, the donor template comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111.

[0020] In some embodiments, the CAR comprises the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1** shows flow cytometry results to assess TRAC, β2M, and anti-Liv1a CAR expression levels at the cell surface of the edited cell population.

[0022] **FIGS. 2A-2B** show that anti-Liv1a CAR T cells, particularly those expressing the CTX971, CTX975 and CTX976 constructs, exhibited potent cytotoxicity towards the A498 (FIG 2A) and ZR-75-1 (FIG. 2B) cell lines.

[0023] **FIGS. 3A-3D** show cytokine secretion of anti-Liv1a CAR T cells when co-cultured with target cell lines A498 and ZR-75-1. FIGS. 3A and 3B show secretion of the effector cytokine interferon-ɣ (IFN-ɣ) in A498 (FIG. 3A) and ZR-75-1 (FIG. 3B) cell lines. FIGS. 3C and 3D show secretion of the effector cytokine interleukin-2 (IL-2) in A498 (FIG. 3C) and ZR-75-1 (FIG. 3D) cell lines.

[0024] **FIG. 4** shows an alignment of scFV constructs (VL and VH) – 971 (SEQ ID NO: 54); 973 (SEQ ID NO: 82); 975 (SEQ ID NO: 83); 977 (SEQ ID NO: 84)

[0025] **FIG. 5** shows an alignment of scFV constructs (VH and VL) - 979 (SEQ ID NO: 70); 974 (SEQ ID NO: 85); 976 (SEQ ID NO: 86); 978 (SEQ ID NO: 87), 972 (SEQ ID NO: 127)

DETAILED DESCRIPTION

LIV1 Cancer Antigen

[0026] In some embodiments, the T cells of the present disclosure are engineered with a chimeric antigen receptor (CAR) designed to target LIV1. LIV1, also known as Solute Carrier Family 39 Member 6, SLC39A6, ZIP6, and LIV-1, is a member of the ZIP family of highly conserved transmembrane zinc transporter proteins. LIV1 is expressed at elevated levels in breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and neuronal tumors. Notably, LIV1 has a restricted expression in normal tissues, *e.g.*, non-cancerous breast, prostate, and testis. Thus, LIV1 is a desirable transmembrane protein for targeting cancer. In fact, the LIV-1 protein has been implicated in breast cancer, prostate cancer, squamous tumors, and neuronal tumors.

[0027] Thus, in some embodiments, T cells of the present disclosure are engineered to express a CAR comprising an anti-LIV1 antibody (e.g., anti-LIV1 scFv). In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv encoded by the sequence of any one of SEQ ID NOs: 53, 69, 97, 102, 106, 110, 114, or 118. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv comprising the sequence of any one of SEQ ID NOs: 54, 70, 82, 83, 84, 85, 86, or 87. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv comprising a VH comprising an amino acid sequence of any one of SEQ ID NO: 55, 90 or 98. In some embodiments, the anti-LIV1 antibody is an anti-LIV1 scFv comprising a VL comprising an amino acid sequence of any one of SEQ ID NO: 56, 88 or 128.In some embodiments, a CAR comprising an anti-LIV1 antibody is encoded by the sequence of any one of SEQ ID NOs: 49, 51, 65, 67, 95, 100, 104, 108, 112, or 116. In some embodiments, a CAR comprising an anti-LIV7 antibody is encoded by a sequence comprising a nucleic acid that is at least 90% identical to SEQ ID NOs: 49, 51, 65, 67, 95, 100, 104, 108, 112, or 116. In some embodiments, a CAR comprising an anti-LIV1 antibody comprises the sequence of any one of SEQ ID NOs: 49, 51, 65, 67, 95, 100, 104, 108, 112, or 116. In some embodiments, a CAR comprising an anti-LIV1 antibody comprises an anti-LIV1 antibody as described in US 9,228,026.

Multi-Gene Editing

[0028] The engineered T cells of the present disclosure, in some embodiments, include more than one gene edit, for example, in more than one gene. For example, an engineered T cell may comprise a disrupted T cell receptor alpha chain constant region (*TRAC*) gene, a

disrupted beta-2-microglobulin (*β2M*) gene, a disrupted programmed cell death-1 (*PD-1* or *PDCD1*) gene, a disrupted *CD70* gene, or any combination of two or more of the foregoing disrupted genes. In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene, a disrupted *β2M* gene, and a disrupted *CD70* gene. In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene, a disrupted *β2M* gene, and a disrupted *PD-1* gene. In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene, a disrupted *β2M* gene, a disrupted *CD70* gene and a disrupted *PD-1* gene.

[0029] It should be understood that gene disruption encompasses gene modification through gene editing (*e.g.*, using CRISPR/Cas gene editing to insert or delete one or more nucleotides). In some embodiments, a disrupted gene is a gene that does not encode functional protein. In some embodiments, a cell that comprises a disrupted gene does not express (*e.g.*, at the cell surface) a detectable level (*e.g.* by antibody, *e.g.*, by flow cytometry) of the protein encoded by the gene. A cell that does not express a detectable level of the protein may be referred to as a knockout cell. For example, a cell having a *β2M* gene edit may be considered a β2M knockout cell if *β*2M protein cannot be detected at the cell surface using an antibody that specifically binds *β*2M protein.

[0030] Provided herein, in some embodiments, are populations of cells in which a certain percentage of the cells has been edited (*e.g.*, *β2M* gene edited), resulting in a certain percentage of cells not expressing a particular gene and/or protein. In some embodiments, at least 50% (*e.g.*, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 85%) of the cells of a gene-edited population of cells are β2M knockout cells. In some embodiments, at least 50% of the cells (*e.g.* T cells) of the population do not express detectable levels of β2M protein. In some embodiments, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the cells of a gene-edited population of cells may be β2M knockout cells.

[0031] Methods of using CRISPR-Cas gene editing technology to create a genomic deletion in a cell (e.g., to knock out a gene in a cell) are known (Bauer DE et al. Vis. Exp. 2015;95;e52118).

***TRAC* Gene Edit**

[0032] In some embodiments, an engineered T cell comprises a disrupted *TRAC* gene. This disruption leads to loss of function of the TCR and renders the engineered T cell non-alloreactive and suitable for allogeneic transplantation, minimizing the risk of graft versus

host disease. In some embodiments, expression of the endogenous *TRAC* gene is eliminated to prevent a graft-versus-host response. In some embodiments, a disruption in the *TRAC* gene expression is created by knocking a chimeric antigen receptor (CAR) into the *TRAC* gene (*e.g.*, using an adeno-associated viral (AAV) vector and donor template). In some embodiments, a disruption in the *TRAC* gene expression is created by gRNAs targeting the *TRAC* genomic region. In some embodiments, a genomic deletion in the *TRAC* gene is created by knocking a chimeric antigen receptor (CAR) into the *TRAC* gene (*e.g.*, using an AAV vector and donor template). In some embodiments, a disruption in the TRAC gene expression is created by gRNAs targeting the *TRAC* genomic region and knocking a chimeric antigen receptor (CAR) into the *TRAC* gene.

[0033] Non-limiting examples of modified and unmodified TRAC gRNA sequences that may be used as provided herein to create a genomic disruption in the *TRAC* gene are listed in **Table 4** (e.g., SEQ ID NOs: 18 and 19). See also International Application No. PCT/US2018/032334, filed May 11, 2018, incorporated herein by reference. Other gRNA sequences may be designed using the *TRAC* gene sequence located on chromosome 14 (GRCh38: chromosome 14: 22,547,506-22,552,154;. Ensembl; ENSG00000277734). In some embodiments, gRNAs targeting the TRAC genomic region create Indels in the *TRAC* gene disrupting expression of the mRNA or protein.

[0034] In some embodiments, at least 50% of a population of engineered T cells do not express a detectable level of T cell receptor (TCR) surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of a population may not express a detectable level of TCR surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the population of engineered T cells do not express a detectable level of TCR surface protein.

[0035] In some embodiments, gRNAs targeting the *TRAC* genomic region create Indels in the *TRAC* gene comprising at least one nucleotide sequence selected from the following sequences in **Table 1**:

Table 1.

Sequence	SEQ ID NO:
AAGAGCAACAAATCTGACT	1
AAGAGCAACAGTGCTGTGCCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	2
AAGAGCAACAGTGCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	3
AAGAGCAACAGTGCCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	4
AAGAGCAACAGTGCTGACTAAGAGCAACAAATCTGACT	5
AAGAGCAACAGTGCTGTGGGCCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	6
AAGAGCAACAGTGCTGGCCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	7
AAGAGCAACAGTGCTGTGTGCCTGGAGCAACAAATCTGACT AAGAGCAACAAATCTGACT	8

[0036] In some embodiments, an engineered T cell comprises a deletion in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 15-30 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of more than 30 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of 20 base pairs in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of SEQ ID NO: 92 (AGAGCAACAGTGCTGTGGCC) in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion comprising SEQ ID NO: 92 (AGAGCAACAGTGCTGTGGCC) in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion of SEQ ID NO: 40 in the *TRAC* gene relative to unmodified T cells. In some embodiments, an engineered T cell comprises a deletion comprising SEQ ID NO: 40 in the *TRAC* gene relative to unmodified T cells.

***β2M* Gene Edit**

[0037] In some embodiments, an engineered T cell comprises a disrupted *β2M* gene. β2M is a common (invariant) component of MHC I complexes. Disrupting its expression by gene editing will prevent host versus therapeutic allogeneic T cells responses leading to increased

allogeneic T cell persistence. In some embodiments, expression of the endogenous *β2M* gene is eliminated to prevent a host-versus-graft response.

[0038] Non-limiting examples of modified and unmodified β2M gRNA sequences that may Non-limiting examples of modified and unmodified β2M gRNA sequences that may be used as provided herein to create a genomic disruption in the *β2M* gene are listed in **Table 4** (e.g., SEQ ID NOs: 20 and 21). See also International Application No. PCT/US2018/032334, filed May 11, 2018, incorporated herein by reference. Other gRNA sequences may be designed using the *β2M* gene sequence located on Chromosome 15 (GRCh38 coordinates: Chromosome 15: 44,711,477-44,718,877 ; Ensembl: ENSG00000166710).

[0039] In some embodiments, gRNAs targeting the β2M genomic region create Indels in the *β2M* gene disrupting expression of the mRNA or protein.

[0040] In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does not express a detectable level of β2M surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the engineered T cells of a population may not express a detectable level of β2M surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of β2M surface protein.

[0041] In some embodiments, an edited *β2M* gene comprises at least one nucleotide sequence selected from the following sequences in **Table 2**.

Table 2.

Sequences	SEQ ID NO:
CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGCCTGGA GGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	9
CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCGCCTGGAG GCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	10
CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGAGGCT ATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	11
CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGATAGC CTGGAGGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	12
CGTGGCCTTAGCTGTGCTCGCGCTATCCAGCGTGAGTCTCTCCT ACCCTCCCGCT	13
CGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGTGGCCT GGAGGCTATCCAGCGTGAGTCTCTCCTACCCTCCCGCT	14

***PD-1* Gene Edit**

[0042] PD-1 is an immune checkpoint molecule that is upregulated in activated T cells and serves to dampen or stop T cell responses. Disrupting PD-1 by gene editing could lead to more persistent and/or potent therapeutic T cell responses and/or reduce immune suppression in a subject. In some embodiments, an engineered T cell comprises a disrupted PD-*1* gene. In some embodiments, expression of the endogenous *PD-1* gene is eliminated to enhance anti-tumor efficacy of the CAR T cells of the present disclosure.

[0043] Non-limiting examples of modified and unmodified PD-1 gRNA sequences that may be used as provided herein to create a genomic deletion in the PD-1 gene are listed in **Table 4** (e.g., SEQ ID NOs: 22 and 23). See also International Application No. PCT/US2018/032334, filed May 11, 2018, incorporated herein by reference. Other gRNA sequences may be designed using the PD-1 gene sequence located on Chromosome 2 (GRCh38 coordinates: Chromosome 2: 241,849,881-241,858,908; Ensembl: ENSG00000188389).

[0044] In some embodiments, gRNAs targeting the PD-1 genomic region create Indels in the PD-1 gene disrupting expression of the PD-1 mRNA or protein.

[0045] In some embodiments, an engineered T cell comprises a disrupted PD-1 gene. In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does not express a detectable level of PD-1 surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the engineered T cells of a population may not express a detectable level of PD-1 surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-

60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of PD-1 surface protein.

***CD70* Gene Edit**

[0046] Cluster of Differentiation 70 (CD70) is a member of the tumor necrosis factor superfamily and its expression is restricted to activated T and B lymphocytes and mature dendritic cells. CD70 has also been detected on hematological tumors and on carcinomas. CD70 is implicated in tumor cell and regulatory T cell survival through interaction with its ligand, CD27. Disrupting CD70 by gene editing increases cell expansion and reduces cell exhaustion. In some embodiments, an engineered T cell comprises a disrupted *CD70* gene. In some embodiments, expression of the endogenous *CD70* gene is eliminated to enhance anti-tumor efficacy of the CAR T cells of the present disclosure. In some embodiments, gRNAs targeting the *CD70* genomic region create Indels in, or around, the *CD70* gene disrupting expression of the CD70 mRNA and/or protein.

[0047] Non-limiting examples of modified and unmodified CD70 gRNA sequences that may be used as provided herein to create a genomic disruption in the CD70 gene are listed in **Table 4** (e.g., SEQ ID NOs: 24-27). Other gRNA sequences may be designed using the CD70 gene sequence located on Chromosome 19 (GRCh38 coordinates: Chromosome 19: 6,583,183-6,604,103; Ensembl: ENSG00000125726).

[0048] In some embodiments, an engineered T cell comprises a disrupted *CD70* gene. In some embodiments, at least 50% of the engineered T cells of a population of engineered T cells does not express a detectable level of CD70 surface protein. For example, at least 55%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the engineered T cells of a population may not express a detectable level of CD70 surface protein. In some embodiments, 50%-100%, 50%-90%, 50%-80%, 50%-70%, 50%-60%, 60%-100%, 60%-90%, 60%-80%, 60%-70%, 70%-100%, 70%-90%, 70%-80%, 80%-100%, 80%-90%, or 90%-100% of the engineered T cells of a population does not express a detectable level of CD70 surface protein.

Cellular Phenotypes

[0049] In some embodiments, one or more gene edits within a population of cells results in a phenotype associated with changes in cellular proliferative capacity, cellular exhaustion,

cellular viability, cellular lysis capability (*e.g.*, increase cytokine production and/or release), or any combination thereof.

[0050] In some embodiments, engineered T cells of the present disclosure exhibit at least 20% greater cellular proliferative capacity, relative to control T cells. For example, engineered T cells may exhibit at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 90% greater cellular proliferative capacity, relative to control T cells. In some embodiments, engineered T cells of the present disclosure exhibit 20%-100%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-100%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, 30%-50%, 40%-100%, 40%-90%, 40%-80%, 40%-70%, 40%-60%, 40%-50%, 50%-100%, 50%-90%, 50%-80%, 50%-70%, or 50%-60% greater cellular proliferative capacity, relative to control T cells.

[0051] In some embodiments, engineered T cells of the present disclosure exhibit an at least 20% increase in cellular viability, relative to control cells. For example, engineered T cells of the present disclosure may exhibit at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 90% increase in cellular viability, relative to control cells. In some embodiments, engineered T cells of the present disclosure exhibit a 20%-100%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-100%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, 30%-50%, 40%-100%, 40%-90%, 40%-80%, 40%-70%, 40%-60%, 40%-50%, 50%-100%, 50%-90%, 50%-80%, 50%-70%, or 50%-60% increase in cellular viability, relative to control cells.

[0052] In some embodiments, engineered T cells of the present disclosure exhibit an at least 20% increase in cellular lysis capability (kill at least 20% more target cells), relative to control cells. For example, engineered T cells of the present disclosure may exhibit an at least at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 90% increase in cellular lysis capability, relative to control cells. In some embodiments, engineered T cells of the present disclosure exhibit a 20%-100%, 20%-90%, 20%-80%, 20%-70%, 20%-60%, 20%-50%, 30%-100%, 30%-90%, 30%-80%, 30%-70%, 30%-60%, 30%-50%, 40%-100%, 40%-90%, 40%-80%, 40%-70%, 40%-60%, 40%-50%, 50%-100%, 50%-90%, 50%-80%, 50%-70%, or 50%-60% increase in cellular lysis capability, relative to control cells. For example, the level of cytokines (*e.g.*, IL-2 and/or IFN-gamma) secreted by the engineered T

cells may at least 2-fold (*e.g.*, at least 3-fold, at least 4-fold, or at least 5-fold) greater than the level of cytokines secreted by control T cells.

[0053] Control T cells, in some embodiments, are engineered T cells (*e.g.*, gene edited T cells). In some embodiments, control T cells are engineered T cells that comprise a disrupted *TRAC* gene, a nucleic acid encoding a CAR (*e.g.*, an anti-LIV1 CAR) inserted into the *TRAC* gene, and/or a disrupted *β2M* gene. In some embodiments, control T cells are unedited T cells.

Gene Editing Methods

[0054] Gene editing (including genomic editing) is a type of genetic engineering in which nucleotide(s)/nucleic acid(s) is/are inserted, deleted, and/or substituted in a DNA sequence, such as in the genome of a targeted cell. Targeted gene editing enables insertion, deletion, and/or substitution at pre-selected sites in the genome of a targeted cell (*e.g.*, in a targeted gene or targeted DNA sequence). When an sequence of an endogenous gene is edited, for example by deletion, insertion or substitution of nucleotide(s)/nucleic acid(s), the endogenous gene comprising the affected sequence may be knocked-out or knocked-down due to the sequence alteration. Therefore, targeted editing may be used to disrupt endogenous gene expression. "Targeted integration" refers to a process involving insertion of one or more exogenous sequences, with or without deletion of an endogenous sequence at the insertion site. Targeted integration can result from targeted gene editing when a donor template containing an exogenous sequence is present.

[0055] Targeted editing can be achieved either through a nuclease-independent approach, or through a nuclease-dependent approach. In the nuclease-independent targeted editing approach, homologous recombination is guided by homologous sequences flanking an exogenous polynucleotide to be introduced into an endogenous sequence through the enzymatic machinery of the host cell. The exogenous polynucleotide may introduce deletions, insertions or replacement of nucleotides in the endogenous sequence.

[0056] Alternatively, the nuclease-dependent approach can achieve targeted editing with higher frequency through the specific introduction of double strand breaks (DSBs) by specific rare-cutting nucleases (*e.g.*, endonucleases). Such nuclease-dependent targeted editing also utilizes DNA repair mechanisms, for example, non-homologous end joining (NHEJ), which occurs in response to DSBs. DNA repair by NHEJ often leads to random insertions or

deletions (indels) of a small number of endogenous nucleotides. In contrast to NHEJ mediated repair, repair can also occur by a homology directed repair (HDR). When a donor template containing exogenous genetic material flanked by a pair of homology arms is present, the exogenous genetic material can be introduced into the genome by HDR, which results in targeted integration of the exogenous genetic material.

[0057] Available endonucleases capable of introducing specific and targeted DSBs include, but not limited to, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and RNA-guided CRISPR-Cas9 nuclease (CRISPR/Cas9; Clustered Regular Interspaced Short Palindromic Repeats Associated 9). Additionally, DICE (dual integrase cassette exchange) system utilizing phiC31 and Bxb1 integrases may also be used for targeted integration.

[0058] ZFNs are targeted nucleases comprising a nuclease fused to a zinc finger DNA binding domain (ZFBD), which is a polypeptide domain that binds DNA in a sequence-specific manner through one or more zinc fingers. A zinc finger is a domain of about 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include, but not limited to, C2H2 zinc fingers, C3H zinc fingers, and C4 zinc fingers. A designed zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, *e.g.*, application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. See, for example, U.S. Pat. Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536 and WO 03/016496. A selected zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. ZFNs are described in greater detail in U.S. Pat. No. 7,888,121 and U.S. Pat. No. 7,972,854. The most recognized example of a ZFN is a fusion of the FokI nuclease with a zinc finger DNA binding domain.

[0059] A TALEN is a targeted nuclease comprising a nuclease fused to a TAL effector DNA binding domain. A "transcription activator-like effector DNA binding domain", "TAL effector DNA binding domain", or "TALE DNA binding domain" is a polypeptide domain of TAL effector proteins that is responsible for binding of the TAL effector protein to DNA. TAL effector proteins are secreted by plant pathogens of the genus *Xanthomonas* during infection. These proteins enter the nucleus of the plant cell, bind effector-specific DNA sequences via their DNA binding domain, and activate gene transcription at these sequences

via their transactivation domains. TAL effector DNA binding domain specificity depends on an effector-variable number of imperfect 34 amino acid repeats, which comprise polymorphisms at select repeat positions called repeat variable-diresidues (RVD). TALENs are described in greater detail in US Patent Application No. 2011/0145940. The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

[0060] Additional examples of targeted nucleases suitable for use as provided herein include, but are not limited to, Bxb1, phiC31, R4, PhiBT1, and Wβ/SPBc/TP901-1, whether used individually or in combination.

[0061] Other non-limiting examples of targeted nucleases include naturally-occurring and recombinant nucleases, *e.g.*, CRISPR/Cas9, restriction endonucleases, meganucleases homing endonucleases, and the like.

CRISPR-Cas9 Gene Editing

[0062] The CRISPR-Cas9 system is a naturally-occurring defense mechanism in prokaryotes that has been repurposed as a RNA-guided DNA-targeting platform used for gene editing. It relies on the DNA nuclease Cas9, and two noncoding RNAs-crisprRNA (crRNA) and trans-activating RNA (tracrRNA)—to target the cleavage of DNA.

[0063] crRNA drives sequence recognition and specificity of the CRISPR-Cas9 complex through Watson-Crick base pairing typically with a 20 nucleotide (nt) sequence in the target DNA. Changing the sequence of the 5' 20nt in the crRNA allows targeting of the CRISPR-Cas9 complex to specific loci. The CRISPR-Cas9 complex only binds DNA sequences that contain a sequence match to the first 20 nt of the crRNA, single-guide RNA (sgRNA), if the target sequence is followed by a specific short DNA motif (with the sequence NGG) referred to as a protospacer adjacent motif (PAM).

[0064] TracrRNA hybridizes with the 3' end of crRNA to form an RNA-duplex structure that is bound by the Cas9 endonuclease to form the catalytically active CRISPR-Cas9 complex, which can then cleave the target DNA.

[0065] Once the CRISPR-Cas9 complex is bound to DNA at a target site, two independent nuclease domains within the Cas9 enzyme each cleave one of the DNA strands upstream of the PAM site, leaving a double-strand break (DSB) where both strands of the DNA terminate in a base pair (a blunt end).

[0066] After binding of CRISPR-Cas9 complex to DNA at a specific target site and formation of the site-specific DSB, the next key step is repair of the DSB. Cells use two main DNA repair pathways to repair the DSB: non-homologous end-joining (NHEJ) and homology-directed repair (HDR).

[0067] NHEJ is a robust repair mechanism that appears highly active in the majority of cell types, including non-dividing cells. NHEJ is error-prone and can often result in the removal or addition of between one and several hundred nucleotides at the site of the DSB, though such modifications are typically < 20 nt. The resulting insertions and deletions (indels) can disrupt coding or noncoding regions of genes. Alternatively, HDR uses a long stretch of homologous donor DNA, provided endogenously or exogenously, to repair the DSB with high fidelity. HDR is active only in dividing cells, and occurs at a relatively low frequency in most cell types. In many embodiments of the present disclosure, NHEJ is utilized as the repair operant.

[0068] In some embodiments, the Cas9 (CRISPR associated protein 9) endonuclease is from *Streptococcus pyogenes*, although other Cas9 homologs may be used. It should be understood, that wild-type Cas9 may be used or modified versions of Cas9 may be used (*e.g.*, evolved versions of Cas9, or Cas9 orthologues or variants), as provided herein. In some embodiments, Cas9 may be substituted with another RNA-guided endonuclease, such as Cpf1 (of a class II CRISPR/Cas system).

Guide RNAs

[0069] The present disclosure provides a genome-targeting nucleic acid that can direct the activities of an associated polypeptide (*e.g.*, a site-directed polypeptide) to a specific target sequence within a target nucleic acid. The genome-targeting nucleic acid can be an RNA. A genome-targeting RNA is referred to as a "guide RNA" or "gRNA" herein. A guide RNA comprises at least a spacer sequence that hybridizes to a target nucleic acid sequence of interest, and a CRISPR repeat sequence. In Type II systems, the gRNA also comprises a second RNA called the tracrRNA sequence. In the Type II guide RNA (gRNA), the CRISPR repeat sequence and tracrRNA sequence hybridize to each other to form a duplex. In the Type V guide RNA (gRNA), the crRNA forms a duplex. In both systems, the duplex binds a site-directed polypeptide, such that the guide RNA and site-direct polypeptide form a complex. In some embodiments, the genome-targeting nucleic acid provides target specificity to the complex by virtue of its association with the site-directed polypeptide. The genome-targeting nucleic acid thus directs the activity of the site-directed polypeptide.

[0070] As is understood by the person of ordinary skill in the art, each guide RNA is designed to include a spacer sequence complementary to its genomic target sequence. See Jinek *et al.*, Science, 337, 816-821 (2012) and Deltcheva *et al.*, Nature, 471, 602-607 (2011).

[0071] In some embodiments, the genome-targeting nucleic acid is a double-molecule guide RNA. In some embodiments, the genome-targeting nucleic acid is a single-molecule guide RNA.

[0072] A double-molecule guide RNA comprises two strands of RNA. The first strand comprises in the 5' to 3' direction, an optional spacer extension sequence, a spacer sequence and a minimum CRISPR repeat sequence. The second strand comprises a minimum tracrRNA sequence (complementary to the minimum CRISPR repeat sequence), a 3' tracrRNA sequence and an optional tracrRNA extension sequence.

[0073] A single-molecule guide RNA (sgRNA) in a Type II system comprises, in the 5' to 3' direction, an optional spacer extension sequence, a spacer sequence, a minimum CRISPR repeat sequence, a single-molecule guide linker, a minimum tracrRNA sequence, a 3' tracrRNA sequence and an optional tracrRNA extension sequence. The optional tracrRNA extension may comprise elements that contribute additional functionality (*e.g.*, stability) to the guide RNA. The single-molecule guide linker links the minimum CRISPR repeat and the minimum tracrRNA sequence to form a hairpin structure. The optional tracrRNA extension comprises one or more hairpins.

[0074] A single-molecule guide RNA (referred to as a "sgRNA" or "gRNA") in a Type V system comprises, in the 5' to 3' direction, a minimum CRISPR repeat sequence and a spacer sequence.

[0075] The sgRNA can comprise a 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. The sgRNA can comprise a less than 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. The sgRNA can comprise a more than 20 nucleotide spacer sequence at the 5' end of the sgRNA sequence. The sgRNA can comprise a variable length spacer sequence with 17-30 nucleotides at the 5' end of the sgRNA sequence (see **Table 3**).

[0076] The sgRNA can comprise no uracil at the 3' end of the sgRNA sequence. The sgRNA can comprise one or more uracil at the 3' end of the sgRNA sequence. For example, the sgRNA can comprise 1 uracil (U) at the 3' end of the sgRNA sequence. The sgRNA can comprise 2 uracil (UU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 3 uracil (UUU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 4 uracil

(UUUU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 5 uracil (UUUUU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 6 uracil (UUUUUU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 7 uracil (UUUUUUU) at the 3' end of the sgRNA sequence. The sgRNA can comprise 8 uracil (UUUUUUUU) at the 3' end of the sgRNA sequence.

[0077] The sgRNA can be unmodified or modified. For example, modified sgRNAs can comprise one or more 2'-O-methyl phosphorothioate nucleotides.

Table 3.

SEQ ID NO.	sgRNA sequence
15	nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuuu
16	nnnnnnnnnnnnnnnnnnnnguuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugc
17	$n_{(17-30)}$guuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcu$_{(1-8)}$

[0078] By way of illustration, guide RNAs used in the CRISPR/Cas/Cpf1 system, or other smaller RNAs can be readily synthesized by chemical means, as illustrated below and described in the art. While chemical synthetic procedures are continually expanding, purifications of such RNAs by procedures such as high performance liquid chromatography (HPLC, which avoids the use of gels such as PAGE) tends to become more challenging as polynucleotide lengths increase significantly beyond a hundred or so nucleotides. One approach used for generating RNAs of greater length is to produce two or more molecules that are ligated together. Much longer RNAs, such as those encoding a Cas9 or Cpf1 endonuclease, are more readily generated enzymatically. Various types of RNA modifications can be introduced during or after chemical synthesis and/or enzymatic generation of RNAs, *e.g.*, modifications that enhance stability, reduce the likelihood or degree of innate immune response, and/or enhance other attributes, as described in the art.

Spacer Sequence

[0079] A gRNA comprises a spacer sequence. A spacer sequence is a sequence (e.g., a 20 nucleotide sequence) that defines the target sequence (e.g., a DNA target sequences, such as a genomic target sequence) of a target nucleic acid of interest. In some embodiments, the spacer sequence is 15 to 30 nucleotides. In some embodiments, the spacer sequence is 15, 16, 17, 18, 19, 29, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides. In some embodiments, a spacer sequence is 20 nucleotides.

[0080] The "target sequence" is adjacent to a PAM sequence and is the sequence modified by an RNA-guided nuclease (*e.g.*, Cas9). The "target nucleic acid" is a double-stranded molecule: one strand comprises the target sequence and is referred to as the "PAM strand," and the other complementary strand is referred to as the "non-PAM strand." One of skill in the art recognizes that the gRNA spacer sequence hybridizes to the reverse complement of the target sequence, which is located in the non-PAM strand of the target nucleic acid of interest. Thus, the gRNA spacer sequence is the RNA equivalent of the target sequence. For example, if the target sequence is 5′-AGAGCAACAGTGCTGTGGCC-3′ (SEQ ID NO: 92), then the gRNA spacer sequence is 5′-AGAGCAACAGUGCUGUGGCC-3′ (SEQ ID NO: 93). The spacer of a gRNA interacts with a target nucleic acid of interest in a sequence-specific manner via hybridization (*i.e.*, base pairing). The nucleotide sequence of the spacer thus varies depending on the target sequence of the target nucleic acid of interest.

[0081] In a CRISPR/Cas system herein, the spacer sequence is designed to hybridize to a region of the target nucleic acid that is located 5' of a PAM of the Cas9 enzyme used in the system. The spacer may perfectly match the target sequence or may have mismatches. Each Cas9 enzyme has a particular PAM sequence that it recognizes in a target DNA. For example, *S. pyogenes* recognizes in a target nucleic acid a PAM that comprises the sequence 5'-NRG-3', where R comprises either A or G, where N is any nucleotide and N is immediately 3' of the target nucleic acid sequence targeted by the spacer sequence.

[0082] In some embodiments, the target nucleic acid sequence comprises 20 nucleotides. In some embodiments, the target nucleic acid comprises less than 20 nucleotides. In some embodiments, the target nucleic acid comprises more than 20 nucleotides. In some embodiments, the target nucleic acid comprises at least: 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 or more nucleotides. In some embodiments, the target nucleic acid comprises at most: 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 or more nucleotides. In some embodiments, the target nucleic acid sequence comprises 20 bases immediately 5' of the first nucleotide of the PAM. For example, in a sequence comprising 5'-NNNNNNNNNNNNNNNNNNNNNRG-3' (SEQ ID NO: 130), the target nucleic acid comprises the sequence that corresponds to the Ns, wherein N is any nucleotide, and the underlined NRG sequence is the *S. pyogenes* PAM.

[0083] Non-limiting examples of gRNAs that may be used as provided herein are provided in **Table 4** and PCT/US2018/032334, filed May 11, 2018.

Table 4. gRNA Sequences/Target Sequences

gRNA Sequences		
Name	**Unmodified Sequence**	**Modified Sequence**
TRAC sgRNA	AGAGCAACAGUGCUGUGGC Cguuuuagagcuagaaauagcaaguuaaa auaaggcuaguccguuaucaacuugaaaa aguggcaccgagucggugcUUUU (SEQ ID NO: 18)	A*G*A*GCAACAGUGCUGUG GCCguuuuagagcuagaaauagcaaguu aaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcU*U*U *U (SEQ ID NO: 28)
TRAC sgRNA spacer	AGAGCAACAGUGCUGUGGC C (SEQ ID NO: 19)	A*G*A*GCAACAGUGCUGUG GCC (SEQ ID NO: 29)
β2M sgRNA	GCUACUCUCUCUUUCUGGC Cguuuuagagcuagaaauagcaaguuaaa auaaggcuaguccguuaucaacuugaaaa aguggcaccgagucggugcUUUU (SEQ ID NO: 20)	G*C*U*ACUCUCUCUUUCUG GCCguuuuagagcuagaaauagcaaguu aaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcU*U*U *U (SEQ ID NO: 30)
β2M sgRNA spacer	GCUACUCUCUCUUUCUGGC C (SEQ ID NO: 21)	G*C*U*ACUCUCUCUUUCUG GCC (SEQ ID NO: 31)
PD-1 sgRNA	CUGCAGCUUCUCCAACACA Uguuuuagagcuagaaauagcaaguuaaa auaaggcuaguccguuaucaacuugaaaa aguggcaccgagucggugcUUUU (SEQ ID NO: 22)	C*U*G*CAGCUUCUCCAACA CAUguuuuagagcuagaaauagcaagu uaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucggugcU*U* U*U (SEQ ID NO: 32)
PD-1 sgRNA spacer	CUGCAGCUUCUCCAACACA U (SEQ ID NO: 23)	C*U*G*CAGCUUCUCCAACA CAU (SEQ ID NO: 33)
CD70 sgRNA (E1_T7)	GCUUUGGUCCCAUUGGUCG Cguuuuagagcuagaaauagcaaguuaaa auaaggcuaguccguuaucaacuugaaaa aguggcaccgagucggugcUUUU (SEQ ID NO: 24)	G*C*U*UUGGUCCCAUUGGU CGCguuuuagagcuagaaauagcaaguu aaaauaaggcuaguccguuaucaacuuga aaaaguggcaccgagucggugcU*U*U *U (SEQ ID NO: 34), T7
CD70 sgRNA (E1_T7) spacer	GCUUUGGUCCCAUUGGUCG C (SEQ ID NO: 25)	G*C*U*UUGGUCCCAUUGGU CGC (SEQ ID NO: 35)
CD70 sgRNA (E1_T8)	GCCCGCAGGACGCACCCAUA guuuuagagcuagaaauagcaaguuaaaa uaaggcuaguccguuaucaacuugaaaaa guggcaccgagucggugcUUUU (SEQ ID NO: 26)	G*C*C*CGCAGGACGCACCC AUAguuuuagagcuagaaauagcaagu uaaaauaaggcuaguccguuaucaacuug aaaaaguggcaccgagucggugcU*U* U*U (SEQ ID NO: 36), T8
CD70 sgRNA (E1_T8) spacer	GCCCGCAGGACGCACCCAUA (SEQ ID NO: 27)	G*C*C*CGCAGGACGCACCC AUA (SEQ ID NO: 37)
Target Sequences		
Guide Name	**Target Sequence (PAM)**	
CD70 sgRNA (E1_T7)	GCTTTGGTCCCATTGGTCGC (GGG) (SEQ ID NO: 38)	
CD70 sgRNA (E1_T8)	GCCCGCAGGACGCACCCATA (GGG) (SEQ ID NO: 39)	
TRAC sgRNA	AGAGCAACAGTGCTGTGGCC (TGG) (SEQ ID NO: 40)	
β2M sgRNA	GCTACTCTCTCTTTCTGGCC (TGG) (SEQ ID NO: 41)	
PD-1 sgRNA	CTGCAGCTTCTCCAACACAT (CGG) (SEQ ID NO: 42)	

*: 2'-O-methyl phosphorothioate residue

Chimeric antigen receptor (CAR) T cells

[0084] A chimeric antigen receptor refers to an artificial immune cell receptor that is engineered to recognize and bind to an antigen expressed by tumor cells. Generally, a CAR is designed for a T cell and is a chimera of a signaling domain of the T-cell receptor (TCR) complex and an antigen-recognizing domain (*e.g.*, a single chain fragment (scFv) of an antibody or other antibody fragment) (Enblad et al., Human Gene Therapy. 2015; 26(8):498-505). A T cell that expresses a CAR is referred to as a CAR T cell. CARs have the ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC-restricted manner. The non-MHC-restricted antigen recognition gives T-cells expressing CARs the ability to recognize an antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T-cell receptor (TCR) alpha and beta chains.

[0085] There are four generations of CARs, each of which contains different components. First generation CARs join an antibody-derived scFv to the CD3zeta (ζ or z) intracellular signaling domain of the T-cell receptor through hinge and transmembrane domains. Second generation CARs incorporate an additional domain, *e.g.,* CD28, 4-1BB (41BB), or ICOS, to supply a costimulatory signal. Third-generation CARs contain two costimulatory domains fused with the TCR CD3ζ chain. Third-generation costimulatory domains may include, *e.g.*, a combination of CD3ζ, CD27, CD28, 4-1BB, ICOS, or OX40. CARs, in some embodiments, contain an ectodomain (*e.g.*, CD3ζ), commonly derived from a single chain variable fragment (scFv), a hinge, a transmembrane domain, and an endodomain with one (first generation), two (second generation), or three (third generation) signaling domains derived from CD3Z and/or co-stimulatory molecules (Maude et al., *Blood.* 2015; 125(26):4017-4023; Kakarla and Gottschalk, *Cancer J.* 2014; 20(2):151-155).

[0086] CARs typically differ in their functional properties. The CD3ζ signaling domain of the T-cell receptor, when engaged, will activate and induce proliferation of T-cells but can lead to anergy (a lack of reaction by the body's defense mechanisms, resulting in direct induction of peripheral lymphocyte tolerance). Lymphocytes are considered anergic when they fail to respond to a specific antigen. The addition of a costimulatory domain in second-generation CARs improved replicative capacity and persistence of modified T-cells. Similar antitumor effects are observed *in vitro* with CD28 or 4-1BB CARs, but preclinical *in vivo* studies suggest that 4-1BB CARs may produce superior proliferation and/or persistence. Clinical trials suggest that both of these second-generation CARs are capable of inducing

substantial T-cell proliferation *in vivo*, but CARs containing the 4-1BB costimulatory domain appear to persist longer. Third generation CARs combine multiple signaling domains (costimulatory) to augment potency.

[0087] In some embodiments, a chimeric antigen receptor is a first generation CAR. In other embodiments, a chimeric antigen receptor is a second generation CAR. In yet other embodiments, a chimeric antigen receptor is a third generation CAR.

[0088] A CAR, in some embodiments, comprises an extracellular (ecto) domain comprising an antigen binding domain (*e.g.*, an antibody, such as an scFv), a transmembrane domain, and a cytoplasmic (endo) domain.

[0089] **Ectodomain.** The ectodomain is the region of the CAR that is exposed to the extracellular fluid and, in some embodiments, includes an antigen binding domain, and optionally a signal peptide, a spacer domain, and/or a hinge domain. In some embodiments, the antigen binding domain is a single-chain variable fragment (scFv) that include the light and heavy chains of immunoglobulins connected with a short linker peptide. The linker, in some embodiments, includes hydrophilic residues with stretches of glycine and serine for flexibility as well as stretches of glutamate and lysine for added solubility. A single-chain variable fragment (scFv) is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. Non-limiting examples of VH and VL protein sequences that may be used to create an anti-LIV1 scFv may include the amino acid sequence of SEQ ID NOs: 55, 90 or 98 (VH) and SEQ ID NOs: 56, 88 or 128 (VL). In some embodiments, the scFv of the present disclosure is humanized. In other embodiments, the scFv is fully human. In yet other embodiments, the scFv is a chimera (*e.g.*, of mouse and human sequence). In some embodiments, the scFv is an anti-LIV1 scFv (binds specifically to LIV1). Non-limiting examples of anti-LIV1 scFv proteins that may be used as provided herein may include the amino acid sequence of any one of SEQ ID NOs: 54, 70, 82, 83, 84, 85, 86, or 87. Other scFv proteins may be used.

[0090] The signal peptide can enhance the antigen specificity of CAR binding. Signal peptides can be derived from antibodies, such as, but not limited to, CD8, as well as epitope tags such as, but not limited to, GST or FLAG. Examples of signal peptides include MLLLVTSLLLCELPHPAFLLIP (SEQ ID NO: 94) and MALPVTALLLPLALLLHAARP (SEQ ID NO: 73). Other signal peptides may be used.

[0091] In some embodiments, a spacer domain or hinge domain is located between an extracellular domain (comprising the antigen binding domain) and a transmembrane domain of a CAR, or between a cytoplasmic domain and a transmembrane domain of the CAR. A spacer domain is any oligopeptide or polypeptide that functions to link the transmembrane domain to the extracellular domain and/or the cytoplasmic domain in the polypeptide chain. A hinge domain is any oligopeptide or polypeptide that functions to provide flexibility to the CAR, or domains thereof, or to prevent steric hindrance of the CAR, or domains thereof. In some embodiments, a spacer domain or a hinge domain may comprise up to 300 amino acids (*e.g.*, 10 to 100 amino acids, or 5 to 20 amino acids). In some embodiments, one or more spacer domain(s) may be included in other regions of a CAR. In some embodiments, the hinge domain is a CD8 hinge domain. Other hinge domains may be used.

[0092] **Transmembrane Domain.** The transmembrane domain is a hydrophobic alpha helix that spans the membrane. The transmembrane domain provides stability of the CAR. In some embodiments, the transmembrane domain of a CAR as provided herein is a CD8 transmembrane domain. In other embodiments, the transmembrane domain is a CD28 transmembrane domain. In yet other embodiments, the transmembrane domain is a chimera of a CD8 and CD28 transmembrane domain. Other transmembrane domains may be used as provided herein. In some embodiments, the transmembrane domain is a CD8a transmembrane domain: FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGG AVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNR (SEQ ID NO: 129). Other transmembrane domains may be used.

[0093] **Endodomain.** The endodomain is the functional end of the receptor. Following antigen recognition, receptors cluster and a signal is transmitted to the cell. The most commonly used endodomain component is CD3-zeta, which contains three (3) immunoreceptor tyrosine-based activation motif (ITAM)s. This transmits an activation signal to the T cell after the antigen is bound. In many cases, CD3-zeta may not provide a fully competent activation signal and, thus, a co-stimulatory signaling is used. For example, CD28 and/or 4-1BB may be used with CD3-zeta (CD3ζ) to transmit a proliferative/survival signal.

Thus, in some embodiments, the co-stimulatory molecule of a CAR as provided herein is a CD28 co-stimulatory molecule. In other embodiments, the co-stimulatory molecule is a 4-1BB co-stimulatory molecule. In some embodiments, a CAR includes CD3ζ and CD28. In other embodiments, a CAR includes CD3-zeta and 4-1BB. In still other embodiments, a CAR includes CD3ζ, CD28, and 4-1BB. **Table 5** provides examples of signaling molecules that may be used as provided herein.

Table 5

Name	Sequence	SEQ ID NO:
4-1BB	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTT ATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGC CGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG	43
	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	44
CD28	TCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTC CTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCC CCCCACGAGACTTCGCTGCGTACAGGTCC	45
	SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	46
CD3-zeta	CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAA GGACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAG GAGTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATG GGGGGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAA TGAACTCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGG TATGAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATGCACTGC ATATGCAGGCCCTGCCTCCCAGA	47
	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR	48

Antibodies

[0094] An antibody (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term “antibody” encompasses not only intact (i.e., full-length) monoclonal antibodies, but also antigen-binding fragments (such as Fab, Fab', F(ab')2, Fv), single chain variable fragment (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, chimeric antibodies, diabodies, linear antibodies, single chain antibodies, single domain antibodies (*e.g.*, camel or llama VHH antibodies), multispecific antibodies (*e.g.*, bispecific antibodies) and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of

the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies.

[0095] A typical antibody molecule comprises a heavy chain variable region (VH) and a light chain variable region (VL), which are usually involved in antigen binding. These regions/residues that are responsible for antigen-binding can be identified from amino acid sequences of the VH/VL sequences of a reference antibody (e.g., an anti-LIV1 antibody as described herein) by methods known in the art. The VH and VL regions can be further subdivided into regions of hypervariability, also known as "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, which are known as "framework regions" ("FR"). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition, all of which are well known in the art. As used herein, a CDR may refer to the CDR defined by any method known in the art. Two antibodies having the same CDR means that the two antibodies have the same amino acid sequence of that CDR as determined by the same method. See, e.g., Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs.

[0096] In some embodiments, an antibody is an scFv, such as an anti-LIV1 scFv. An antibody includes an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0097] The antibodies to be used as provided herein can be murine, rat, human, or any other origin (including chimeric or humanized antibodies). In some examples, the antibody comprises a modified constant region, such as a constant region that is immunologically inert, *e.g.*, does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC).

[0098] In some embodiments, an antibody of the present disclosure is a humanized antibody. Humanized antibodies refer to forms of non-human (*e.g.*, murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or antigen-binding fragments thereof that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) 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, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody 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 substantially all of the FR regions are those of a human immunoglobulin consensus sequence. A humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

[0099] In some embodiments, an antibody of the present disclosure is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (*e.g.*, a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in

antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or the constant region.

[00100] In some embodiments, an antibody of the present disclosure specifically binds a target antigen, such as human LIV1. An antibody that "specifically binds" (used interchangeably herein) to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody "specifically binds" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to a LIV1 epitope is an antibody that binds this LIV1 epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other LIV1 epitopes or non-LIV1 epitopes. It is also understood by reading this definition that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[00101] In some embodiments, the equilibrium dissociation constant (K_D) between the antibody and LIV1 is 100 pM to 1 μM. In some embodiments, the K_D between the antibody and LIV1 is 1 nM to 100 nM.

[00102] Also within the scope of the present disclosure are functional variants of any of the exemplary antibodies as disclosed herein. A functional variant may contain one or more amino acid residue variations in the VH and/or VL, or in one or more of the VH CDRs and/or one or more of the VL CDRs as relative to a reference antibody, while retaining substantially similar binding and biological activities (e.g., substantially similar binding affinity, binding specificity, inhibitory activity, anti-tumor activity, or a combination thereof) as the reference antibody.

[00103] In some examples, an antibody disclosed herein comprises a VH CDR1, a VH CDR2, and a VH CDR3, which collectively contains no more than 10 amino acid variations (e.g., no more than 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH CDR1, VH CDR2, and VH CDR3 of a reference antibody such as in VH: SEQ ID NO: 55 or

90 or 98; VL: SEQ ID NO: 56 or 88 or 128. "Collectively" means that the total number of amino acid variations in all of the three VH CDRs is within the defined range. Alternatively or in addition, antibody may comprise a VL CDR1, a VL CDR2, and a VL CDR3, which collectively contains no more than 10 amino acid variations (e.g., no more than 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid variation) as compared with the VL CDR1, VL CDR2, and VL CDR3 of the reference antibody.

[00104] In some examples, an antibody disclosed herein may comprise a VH CDR1, a VH CDR2, and a VH CDR3, at least one of which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the counterpart VH CDR of a reference antibody such as in VH: SEQ ID NO: 55 or 90 or 98; VL: SEQ ID NO: 56 or 88 or 128. In specific examples, the antibody comprises a VH CDR3, which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the VH CDR3 of a reference antibody such as in VH: SEQ ID NO: 55 or 90 or 98; VL: SEQ ID NO: 56 or 88 or 128. Alternatively or in addition, an antibody may comprise a VL CDR1, a VL CDR2, and a VL CDR3, at least one of which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the counterpart VL CDR of the reference antibody. In specific examples, the antibody comprises a VL CDR3, which contains no more than 5 amino acid variations (e.g., no more than 4, 3, 2, or 1 amino acid variation) as the LC CDR3 of the reference antibody.

[00105] In some instances, the amino acid residue variations can be conservative amino acid residue substitutions. As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) A → G, S; (b) R → K, H; (c) N → Q, H; (d) D → E, N; (e) C → S, A; (f) Q → N; (g) E → D, Q; (h) G → A; (i) H → N, Q; (j) I → L, V; (k) L → I, V; (l) K → R, H; (m) M→ L, I, Y; (n) F → Y, M, L; (o) P → A; (p) S → T; (q) T→ S; (r) W → Y, F; (s) Y → W, F; and (t) V→ I, L.

[00106] In some embodiments, an antibody disclosed herein may comprise VH CDRs that collectively are at least 80% (e.g., 85%, 90%, 95%, or 98%) identical to the VH CDRs of a reference antibody such as Antibody A (VH: SEQ ID NO: 55; VL: SEQ ID NO: 56) or Antibody B (VH: SEQ ID NO: 90; VL: SEQ ID NO: 88). Alternatively or in addition, the antibody may comprise VL CDRs that collectively are at least 80% (e.g., 85%, 90%, 95%, or 98%) identical to the VL CDRs of the reference antibody. In some embodiments, an antibody may comprise a VH that is at least 80% (e.g., 85%, 90%, 95%, or 98%) identical to the VH of a reference antibody such as in VH: SEQ ID NO: 55 or 90 or 98; VL: SEQ ID NO: 56 or 88 or 128 and/or a VL variable region that is at least 80% (e.g., 85%, 90%, 95%, or 98%) identical to the VL variable region of the reference antibody.

Donor Template

[00107] The nucleic acid encoding a CAR may be delivered to a T cell that comprises what is referred to herein as a donor template (also referred to as a donor polynucleotide). A donor template can contain a non-homologous sequence, such as the nucleic acid encoding a CAR, flanked by two regions of homology to allow for efficient HDR at a genomic location of interest. Alternatively, a donor template may have no regions of homology to the targeted location in the DNA and may be integrated by NHEJ-dependent end joining following cleavage at the target site.

[00108] A donor template can be DNA or RNA, single-stranded and/or double-stranded, and can be introduced into a cell in linear or circular form. If introduced in linear form, the ends of the donor sequence can be protected (*e.g.*, from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al., (1987) Proc. Natl. Acad. Sci. USA 84:4959-4963; Nehls et al., (1996) Science 272:886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[00109] A donor template can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, a donor template can be introduced as naked nucleic acid, as

nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (*e.g.*, adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)).

[00110] A donor template, in some embodiments, is inserted so that its expression is driven by the endogenous promoter at the integration site, namely the promoter that drives expression of the endogenous gene into which the donor is inserted. However, in some embodiments, the donor template comprises an exogenous promoter and/or enhancer, for example a constitutive promoter, an inducible promoter, or tissue-specific promoter. In some embodiments, the exogenous promoter is an EF1α promoter comprising a sequence of SEQ ID NO: 79. Other promoters may be used.

[00111] Furthermore, exogenous sequences may also include transcriptional or translational regulatory sequences, for example, promoters, enhancers, insulators, internal ribosome entry sites, sequences encoding 2A peptides and/or polyadenylation signals.

Delivery Methods and Constructs

[00112] Nucleases and/or donor templates may be delivered using a vector system, including, but not limited to, plasmid vectors, DNA minicircles, retroviral vectors, lentiviral vectors, adenovirus vectors, poxvirus vectors; herpesvirus vectors and adeno-associated virus vectors, and combinations thereof.

[00113] Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids encoding nucleases and donor templates in cells (*e.g.*, T cells). Non-viral vector delivery systems include DNA plasmids, DNA minicircles, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome or poloxamer. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.

[00114] Methods of non-viral delivery of nucleic acids include electroporation, lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, naked RNA, capped RNA, artificial virions, and agent-enhanced uptake of DNA. Sonoporation using, *e.g.*, the Sonitron 2000 system (Rich-Mar) can also be used for delivery of nucleic acids.

Adeno-Associated Viral Delivery

[00115] The donor nucleic acid encoding a CAR construct can be delivered to a cell using an adeno-associated virus (AAV). AAVs are small viruses which integrate site-specifically into the host genome and can therefore deliver a transgene, such as CAR. Inverted terminal repeats (ITRs) are present flanking the AAV genome and/or the transgene of interest and serve as origins of replication. Also present in the AAV genome are rep and cap proteins which, when transcribed, form capsids which encapsulate the AAV genome for delivery into target cells. Surface receptors on these capsids which confer AAV serotype, which determines which target organs the capsids will primarily bind and thus what cells the AAV will most efficiently infect. There are twelve currently known human AAV serotypes. In some embodiments, the AAV is AAV serotype 6 (AAV6).

[00116] Adeno-associated viruses are among the most frequently used viruses for gene therapy for several reasons. First, AAVs do not provoke an immune response upon administration to mammals, including humans. Second, AAVs are effectively delivered to target cells, particularly when consideration is given to selecting the appropriate AAV serotype. Finally, AAVs have the ability to infect both dividing and non-dividing cells because the genome can persist in the host cell without integration. This trait makes them an ideal candidate for gene therapy.

Homology-Directed Repair (HDR)

[00117] The donor nucleic acid encoding a CAR is inserted by homology directed repair (HDR) into the target gene locus. Both strands of the DNA at the target locus are cut by a CRISPR Cas9 enzyme. HDR then occurs to repair the double-strand break (DSB) and insert the donor DNA. For this to occur correctly, the donor sequence is designed with flanking residues which are complementary to the sequence surrounding the DSB site in the target gene (hereinafter "homology arms"). These homology arms serve as the template for DSB repair and allow HDR to be an essentially error-free mechanism. The rate of homology directed repair (HDR) is a function of the distance between the mutation and the cut site so choosing overlapping or nearby target sites is important. Templates can include extra sequences flanked by the homologous regions or can contain a sequence that differs from the genomic sequence, thus allowing sequence editing.

[00118] The target gene can be associated with an immune response in a subject, wherein permanently deleting at least a portion of the target gene will modulate the immune response.

For example, to generate a CAR T cell, the target gene can be the TCRα constant region (TRAC). Disruption of TRAC leads to loss of function of the endogenous TCR.

[00119] In some embodiments, the target gene is in a safe harbor locus.

Engineered T cells

[00120] Engineered (gene edited) CAR T cells of the present disclosure may be autologous ("self") or non-autologous ("non-self," *e.g.*, allogeneic, syngeneic or xenogeneic). "Autologous" refers to cells from the same subject. "Allogeneic" refers to cells of the same species as a subject, but that differ genetically to the cells in the subject. In some embodiments, the T cells are obtained from a mammalian subject. In some embodiments, the T cells are obtained from a human subject.

[00121] T cells can be obtained from a number of sources including, but not limited to, peripheral blood mononuclear cells, bone marrow, lymph nodes tissue, cord blood, thymus issue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled person, such as sedimentation, *e.g.*, FICOLL™ separation.

[00122] In some embodiments, an isolated population of T cells is used. In some embodiments, after isolation of peripheral blood mononuclear cells (PBMC), both cytotoxic and helper T lymphocytes can be sorted into naive, memory, and effector T cell subpopulations either before or after activation, expansion, and/or genetic modification.

[00123] A specific subpopulation of T cells, expressing one or more of the following cell surface markers: TCRab, CD3, CD4, CD8, CD27 CD28, CD38 CD45RA, CD45RO, CD62L, CD127, CD122, CD95, CD197, CCR7, KLRG1, MCH-I proteins and/or MCH-II proteins, can be further isolated by positive or negative selection techniques. In some embodiments, a specific subpopulation of T cells, expressing one or more of the markers selected from the group consisting of TCRab, CD4 and/or CD8, is further isolated by positive or negative selection techniques. In some embodiments, the engineered T cell populations do not express or do not substantially express one or more of the following markers: CD70, CD57, CD244, CD160, PD-1, CTLA4, HM3, and LAG3. In some embodiments, subpopulations of T cells may be isolated by positive or negative selection prior to genetic engineering and/or post genetic engineering.

[00124] In some embodiments, an isolated population of T cells expresses one or more of the markers including, but not limited to a CD3+, CD4+, CD8+, or a combination thereof. In some embodiments, the T cells are isolated from a subject and first activated and stimulated to proliferate *in vitro* prior to undergoing gene editing.

[00125] To achieve sufficient therapeutic doses of T cell compositions, T cells are often subjected to one or more rounds of stimulation, activation and/or expansion. T cells can 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; and 6,867,041. In some embodiments, T cells are activated and expanded for about 1 day to about 4 days, about 1 day to about 3 days, about 1 day to about 2 days, about 2 days to about 3 days, about 2 days to about 4 days, about 3 days to about 4 days, or about 1 day, about 2 days, about 3 days, or about 4 days prior to introduction of the genome editing compositions into the T cells.

[00126] In some embodiments, T cells are activated and expanded for about 4 hours, about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 60 hours, or about 72 hours prior to introduction of the gene editing compositions into the T cells.

[00127] In some embodiments, T cells are activated at the same time that genome editing compositions are introduced into the T cells.

Treatment Methods and Compositions

[00128] Provided herein, in some embodiments, are methods for treating cancer (e.g.: breast cancer). Non-limiting examples of cancers that may be treated as provided herein include: breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and neuronal tumors. In some embodiments, the methods comprise delivering the CAR T cells (e.g., anti-LIV1 CAR T cells) of the present disclosure to a subject having cancer, including, breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and/or neuronal tumors.

[00129] The step of administering may include the placement (*e.g.*, transplantation) of cells, *e.g.*, engineered T cells, into a subject, by a method or route that results in at least partial localization of the introduced cells at a desired site, such as tumor, such that a desired effect(s) is produced. Engineered T cells can be administered by any appropriate route that results in delivery to a desired location in the subject where at least a portion of the implanted cells or components of the cells remain viable. The period of viability of the cells after administration to a subject can be as short as a few hours, *e.g.*, twenty-four hours, to a few days, to as long as several years, or even the life time of the subject, *i.e.*, long-term engraftment. For example, in some aspects described herein, an effective amount of engineered T cells is administered via a systemic route of administration, such as an intraperitoneal or intravenous route.

[00130] A subject may be any subject for whom diagnosis, treatment, or therapy is desired. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[00131] A donor is an individual who is not the subject being treated. A donor is an individual who is not the patient. In some embodiments, a donor is an individual who does not have or is not suspected of having the cancer being treated. In some embodiments, multiple donors, *e.g.*, two or more donors, are used.

[00132] In some embodiments, an engineered T cell population being administered according to the methods described herein comprises allogeneic T cells obtained from one or more donors. Allogeneic refers to a cell, cell population, or biological samples comprising cells, obtained from one or more different donors of the same species, where the genes at one or more loci are not identical to the recipient. For example, an engineered T cell population, being administered to a subject can be derived from one or more unrelated donors, or from one or more non-identical siblings. In some embodiments, syngeneic cell populations may be used, such as those obtained from genetically identical donors, (*e.g.*, identical twins). In some embodiments, the cells are autologous cells; that is, the engineered T cells are obtained or isolated from a subject and administered to the same subject, *i.e.*, the donor and recipient are the same.

[00133] In some embodiments, an engineered T cell population being administered according to the methods described herein does not induce toxicity in the subject, e.g., the engineered T cells do not induce toxicity in non-cancer cells. In some embodiments, an

engineered T cell population being administered does not trigger complement mediated lysis, or does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC).

[00134] An effective amount refers to the amount of a population of engineered T cells needed to prevent or alleviate at least one or more signs or symptoms of a medical condition (*e.g.*, cancer), and relates to a sufficient amount of a composition to provide the desired effect, *e.g.*, to treat a subject having a medical condition. An effective amount also includes an amount sufficient to prevent or delay the development of a symptom of the disease, alter the course of a symptom of the disease (for example but not limited to, slow the progression of a symptom of the disease), or reverse a symptom of the disease. It is understood that for any given case, an appropriate effective amount can be determined by one of ordinary skill in the art using routine experimentation.

[00135] For use in the various aspects described herein, an effective amount of cells (*e.g.*, engineered T cells) comprises at least 10^2 cells, at least 5 X 10^2 cells, at least 10^3 cells, at least 5 X 10^3 cells, at least 10^4 cells, at least 5 X 10^4 cells, at least 10^5 cells, at least 2 X 10^5 cells, at least 3 X 10^5 cells, at least 4 X 10^5 cells, at least 5 X 10^5 cells, at least 6 X 10^5 cells, at least 7 X 10^5 cells, at least 8 X 10^5 cells, at least 9 X 10^5 cells, at least 1 X 10^6 cells, at least 2 X 10^6 cells, at least 3 X 10^6 cells, at least 4 X 10^6 cells, at least 5 X 10^6 cells, at least 6 X 10^6 cells, at least 7 X 10^6 cells, at least 8 X 10^6 cells, at least 9 X 10^6 cells, or multiples thereof. The cells are derived from one or more donors, or are obtained from an autologous source. In some examples described herein, the cells are expanded in culture prior to administration to a subject in need thereof.

[00136] Modes of administration include injection, infusion, instillation, or ingestion. Injection includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection and infusion. In some embodiments, the route is intravenous.

[00137] In some embodiments, engineered T cells are administered systemically, which refers to the administration of a population of cells other than directly into a target site, tissue, or organ, such that it enters, instead, the subject's circulatory system and, thus, is subject to metabolism and other like processes.

[00138] The efficacy of a treatment comprising a composition for the treatment of a medical condition can be determined by the skilled clinician. A treatment is considered "effective treatment," if any one or all of the signs or symptoms of, as but one example, levels of functional target are altered in a beneficial manner (*e.g.*, increased by at least 10%), or other clinically accepted symptoms or markers of disease (*e.g.*, cancer) are improved or ameliorated. Efficacy can also be measured by failure of a subject to worsen as assessed by hospitalization or need for medical interventions (*e.g.*, progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in subject and includes: (1) inhibiting the disease, *e.g.*, arresting, or slowing the progression of symptoms; or (2) relieving the disease, *e.g.*, causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of symptoms.

[00139] The present disclosure is exemplified by the following embodiments:

[00140] Embodiment 1. An engineered T cell comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprise an ectodomain that binds specifically to LIV1.

[00141] Embodiment 2. The engineered T cell of embodiment 1 further comprising a disrupted T cell receptor alpha chain constant region (*TRAC*) gene.

[00142] Embodiment 3. The engineered T cell of embodiment 2, wherein the nucleic acid encoding the CAR is inserted into the *TRAC* gene.

[00143] Embodiment 4. The engineered T cell of any one of embodiments 1-3 further comprising a disrupted beta-2-microglobulin (*β2M*) gene.

[00144] Embodiment 5. The engineered T cell of any one of embodiments 1-4, wherein the ectodomain of the CAR comprises an anti-LIV1 antibody.

[00145] Embodiment 6. The engineered T cell of embodiment 5, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

[00146] Embodiment 7. The engineered T cell of embodiment 6, wherein the anti-LIV1 scFv comprises the same heavy chain variable region (VH) complementarity determining regions (CDRs) and the same light chain variable region (VL) CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56, (ii) a VH set forth as SEQ ID NO: 69 and a VL set forth as SEQ ID

NO: 70, (iii) a VH set forth as SEQ ID NO: 76 and a VL set forth as SEQ ID NO: 77, or (iv) a VH set forth as SEQ ID NO: 83 and a VL set forth as SEQ ID NO: 84.

[00147] Embodiment 8. The engineered T cell of embodiment 7, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

[00148] Embodiment 8.1. The engineered T cell of embodiment 7, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 54 or 70.

[00149] Embodiment 9. The engineered T cell of any one of embodiments 1-8.1, wherein the CAR comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

[00150] Embodiment 10. The engineered T cell of embodiment 9, wherein the CAR further comprises a CD3z cytoplasmic signaling domain.

[00151] Embodiment 11. The engineered T cell of any one of embodiments 3-10, wherein the *TRAC* gene comprises the nucleotide sequence of any one of SEQ ID NOS: 63, 64, 71, or 72, and/or wherein the CAR is encoded by the nucleotide sequence of any one of SEQ ID NOS: 49, 51, 65, or 67.

[00152] Embodiment 12. The engineered T cell of any one of embodiments 4-11, wherein the disrupted β2M gene comprises at least one nucleotide sequence selected from any one of SEQ ID NOS: 9-14.

[00153] Embodiment 13. A population of the engineered T cell of any one of embodiments 1-12, wherein at least 25% or at least 50% of engineered T cells of the population express the CAR.

[00154] Embodiment 14. The population of embodiment 14, wherein at least 70% of engineered T cells of the population express the CAR.

[00155] Embodiment 15. The population of embodiment 13, wherein at least 25% of engineered T cells of the population express the CAR following at least 7 or at least 14 days of *in vitro* proliferation.

[00156] Embodiment 16. The population of any one of embodiments 13-15, wherein at least 50% of engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein.

[00157] Embodiment 17. The population of embodiment 16, wherein at least 90% of engineered T cells of the population do not express a detectable level of TCR protein.

[00158] Embodiment 18. The population of any one of embodiments 13-17, wherein at least 50% of engineered T cells of the population do not express a detectable level of β2M protein.

[00159] Embodiment 19. The population of embodiment 18, wherein at least 70% of engineered T cells of the population do not express a detectable level of β2M protein.

[00160] Embodiment 20. The population of any one of embodiments 13-19, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 10%, at least 25%, or at least 50% of the cancer cells of the population.

[00161] Embodiment 21. The population of embodiment 20, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 70%, at least 80%, or at least 90% of the population of cancer cells.

[00162] Embodiment 22. The population of embodiments 20 or 21, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells, secrete IFNγ.

[00163] Embodiment 23. The population of any one of embodiments 20-22, wherein the ratio of engineered T cells to cancer cells is 1:1 to 2:1.

[00164] Embodiment 24. The population of any one of embodiments 20-23, wherein the cancer cells comprise sarcoma cells.

[00165] Embodiment 25. The population of any one of embodiments 20-23, wherein the cancer cells comprise breast cancer cells.

[00166] Embodiment 27. The population of any one of embodiments 13-26, when administered *in vivo* to a subject, does not induce toxicity in the subject.

[00167] Embodiment 26. A method comprising administering the population of engineered T cells any one of embodiments 13-27 to a subject.

[00168] Embodiment 27. The method of embodiment 26, wherein the subject is a human subject.

[00169] Embodiment 28. The method of embodiment 27, wherein the subject has a cancer.

[00170] Embodiment 29. The method of embodiment 28, wherein the cancer is selected from the group consisting of: breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and/or neuronal tumors.

[00171] Embodiment 30. The method of embodiments 28 or 31, wherein the cancer comprises cancer cells expressing LIV1.

[00172] Embodiment 31. A method for producing an engineered T cell, the method comprising (a) delivering to a T cell, a RNA-guided nuclease, a gRNA targeting a *TRAC* gene, and a vector comprising a donor template that comprises a nucleic acid encoding a CAR that comprise an ectodomain that binds specifically to LIV1; and (b) producing an engineered T cell having a disrupted TRAC gene and expressing the CAR.

[00173] Embodiment 32. The method of embodiment 31, wherein the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 18 or 19, or targets the nucleotide sequence of SEQ ID NO: 40.

[00174] Embodiment 33. The method of embodiments 31 or 32, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the TRAC gene.

[00175] Embodiment 34. The method of any one of embodiments 31-33 further comprising delivering to the T cell a gRNA targeting the *β2M* gene.

[00176] Embodiment 35. The method of embodiment 34, wherein the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 20 or 21, or targets the nucleotide sequence of SEQ ID NO: 41.

[00177] Embodiment 36. The method of any one of embodiments 31-35, wherein the RNA-guided nuclease is a Cas9 nuclease, optionally a *S. pyogenes* Cas9 nuclease.

[00178] Embodiment 37. The method of any one of embodiments 31-38, wherein the ectodomain of the CAR is an anti-LIV1 antibody.

[00179] Embodiment 38. The method of embodiment 37, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

[00180] Embodiment 39. The method of embodiment 38, wherein the anti-LIV1 scFv comprises the same VH complementarity determining regions (CDRs) and the same VL CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56.

[00181] Embodiment 40. The method of embodiment 39, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

[00182] Embodiment 41. The method of embodiment 39, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs : 54 or 70.

[00183] Embodiment 42. The method of any one of embodiments 31-41, wherein the CAR comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

[00184] Embodiment 43. The method of embodiment 42, wherein the CAR further comprises a CD3z cytoplasmic signaling domain.

[00185] Embodiment 44. The method of any one of embodiments 31-43, wherein the donor template comprises the nucleotide sequence of any one of SEQ ID NOS: 63, 64, 71, or 72.

[00186] Embodiment 45. The method of any one of embodiments 31-44, wherein the CAR is encoded by a nucleotide sequence of any one of SEQ ID NOS: 49, 51, 65, or 67.

[00187] The present disclosure is further exemplified by the following embodiments:

[00188] Embodiment A1. An engineered T cell comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an ectodomain that binds specifically to LIV1.

[00189] Embodiment A2. The engineered T cell of embodiment A1 further comprising a disrupted T cell receptor alpha chain constant region (*TRAC*) gene.

[00190] Embodiment A3. The engineered T cell of embodiment A1 or A2 further comprising a disrupted beta-2-microglobulin (*β2M*) gene.

[00191] Embodiment A4. The engineered T cell of any one of embodiments A1-3, wherein the ectodomain of the CAR comprises an anti-LIV1 antibody.

[00192] Embodiment A5. The engineered T cell of embodiment A4, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

[00193] Embodiment A6. The engineered T cell of embodiment A5, wherein the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56 or (ii) a VH set forth as SEQ ID NO: 90 and a VL set forth as SEQ ID NO: 88.

[00194] Embodiment A7. The engineered T cell of embodiment A6, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

[00195] Embodiment A8. The engineered T cell of embodiment A6, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 54, 70, 83, or 86.

[00196] Embodiment A9. The engineered T cell of any one of embodiments A1-A8, wherein the CAR further comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

[00197] Embodiment A10. The engineered T cell of embodiment A9, wherein the CAR further comprises a CD3ζ cytoplasmic signaling domain.

[00198] Embodiment A11. The engineered T cell of any one of embodiments A1-A10, wherein the CAR is encoded by the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108 or a nucleotide sequence comprising a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108.

[00199] Embodiment A12. The engineered T cell of any one of embodiments A1-A11, wherein the nucleic acid encoding the CAR is inserted into the disrupted *TRAC* gene.

[00200] Embodiment A13. The engineered T cell of any one of embodiments A2-A12, wherein the disrupted *TRAC* gene comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111, and/or the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108.

[00201] Embodiment A14. The engineered T cell of any one of embodiments A4-A13, wherein the disrupted *β2M* gene comprises at least one nucleotide sequence selected from any one of SEQ ID NOs: 9-14.

[00202] Embodiment A15. An engineered T cell comprising: (i) a disrupted *TRAC* gene; (ii) a disrupted *β2M* gene; and (iii) a nucleic acid encoding a CAR comprising an anti-LIV1 antigen-binding fragment.

[00203] Embodiment A16. The engineered T cell of embodiment A15, wherein the CAR comprises (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain.

[00204] Embodiment A17. The engineered T cell of embodiments A15 or A16, wherein the disrupted *TRAC* gene comprises the nucleic acid encoding the CAR.

[00205] Embodiment A18. An engineered T cell comprising: (i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain; and (ii) a disrupted *β2M* gene.

[00206] Embodiment A19. An engineered T cell comprising: (i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and (ii) a disrupted *β2M* gene.

[00207] Embodiment A20. An engineered T cell comprising: (i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108 and/or encodes a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and (ii) a disrupted *β2M* gene.

[00208] Embodiment A21. The engineered T cell of any one of embodiments A1-A20, wherein the T cell is a human T cell.

[00209] Embodiment A22. A population of cells comprising the engineered T cell of any one of embodiments A1-A21, wherein at least 15% or at least 50% of engineered T cells of the population express the CAR.

[00210] Embodiment A23. The population of embodiment A22, wherein at least 30% of engineered T cells of the population express the CAR.

[00211] Embodiment A24. The population of embodiment A22, wherein at least 70% of engineered T cells of the population express the CAR.

[00212] Embodiment A25. The population of embodiment A22, wherein at least 25% of engineered T cells of the population express the CAR following at least 7 days or at least 14 days of *in vitro* proliferation.

[00213] Embodiment A26. The population of any one of embodiments A22-A25, wherein at least 50% of engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein.

[00214] Embodiment A27. The population of embodiments A26, wherein at least 90% of engineered T cells of the population do not express a detectable level of TCR protein.

[00215] Embodiment A28. The population of any one of embodiments A22-A27, wherein at least 50% of engineered T cells of the population do not express a detectable level of β2M protein.

[00216] Embodiment A29. The population of embodiment A28, wherein at least 70% of engineered T cells of the population do not express a detectable level of β2M protein.

[00217] Embodiment A30. The population of any one of embodiments A22-A29, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 10%, at least 25%, or at least 50% of the cancer cells of the population.

[00218] Embodiment A31. The population of embodiment A30, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 70%, at least 80%, or at least 90% of the population of cancer cells.

[00219] Embodiment A32. The population of embodiments A30 or A31, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells, secrete IFNγ.

[00220] Embodiment A33. The population of any one of embodiments A30-A32, wherein the ratio of engineered T cells to cancer cells is 1:1 to 2:1.

[00221] Embodiment A34. The population of any one of embodiments A30-A33, wherein the cancer cells comprise sarcoma cells.

[00222] Embodiment A35. The population of any one of embodiments A30-A33, wherein the cancer cells comprise breast cancer cells.

[00223] Embodiment A36. The population of any one of embodiments A22-A35, when administered *in vivo* to a subject, does not induce toxicity in the subject.

[00224] Embodiment A37. A population of cells comprising engineered T cells, wherein the engineered T cells comprise: (i) a disrupted *TRAC* gene; (ii) a disrupted *β2M* gene; and (iii) a nucleic acid encoding a CAR comprising an anti-LIV1 antigen-binding fragment.

[00225] Embodiment A38. The population of cells of embodiment A37, wherein the CAR comprises (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain.

[00226] Embodiment A39. The population of cells of embodiments A37 or A38, wherein the disrupted *TRAC* gene comprises the nucleic acid encoding the CAR.

[00227] Embodiment A40. A population of cells comprising engineered T cells, wherein the engineered T cells comprise: (i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain; and (ii) a disrupted *β2M* gene.

[00228] Embodiment A41. A population of cells comprising engineered T cells, wherein the engineered T cells comprise: (i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108 and/or encodes the CAR of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and (ii) a disrupted *β2M* gene.

[00229] Embodiment A42. A method comprising administering the population of engineered T cells any one of embodiments A22-A41 to a subject.

[00230] Embodiment A43. The method of embodiment A42, wherein the subject is a human subject.

[00231] Embodiment A44. The method of embodiment A43, wherein the subject has a cancer.

[00232] Embodiment A45. The method of embodiment A44, wherein the cancer is selected from the group consisting of: breast cancer, *e.g.*, estrogen receptor-positive breast cancer,

prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and/or neuronal tumors.

[00233] Embodiment A46. The method of embodiments A44 or A45, wherein the cancer comprises cancer cells expressing LIV1.

[00234] Embodiment A47. A method for producing an engineered T cell, the method comprising (a) delivering to a T cell (i) a RNA-guided nuclease, (ii) a gRNA targeting a *TRAC* gene, and (iii) a vector comprising a donor template that comprises a nucleic acid encoding a CAR that comprise an ectodomain that binds specifically to LIV1; and (b) producing an engineered T cell having a disrupted *TRAC* gene and expressing the CAR.

[00235] Embodiment A48. The method of embodiments A47, wherein the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 18 or 19, or targets the nucleotide sequence of SEQ ID NO: 40.

[00236] Embodiment A49. The method of embodiments A47 or A48 further comprising delivering to the T cell a gRNA targeting the *β2M* gene.

[00237] Embodiment A50. The method of embodiments A49, wherein the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 20 or 21, or targets the nucleotide sequence of SEQ ID NO: 41.

[00238] Embodiment A51. The method of any one of embodiments A47-A50, wherein the ectodomain of the CAR comprises an anti-LIV1 antibody.

[00239] Embodiment A52. The method of embodiment A51, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

[00240] Embodiment A53. The method of embodiment A52, wherein the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56, or (ii) a VH set forth as SEQ ID NO: 90 and a VL set forth as SEQ ID NO: 88.

[00241] Embodiment A54. The method of embodiment A53, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

[00242] Embodiment A55. The method of embodiment A53, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 54, 83, 86 or 70.

[00243] Embodiment A56. The method of any one of embodiments A47-A56, wherein the CAR further comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

[00244] Embodiment A57. The method of embodiment A56, wherein the CAR further comprises a CD3ζ cytoplasmic signaling domain.

[00245] Embodiment A58. The method of any one of embodiments A47-A57, wherein the CAR is encoded by a nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108 or a nucleotide sequence comprising a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108.

[00246] Embodiment A59. The method of any one of embodiments A47-A58, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the *TRAC* gene.

[00247] Embodiment A60. The method of any one of embodiments A47-A59, wherein the donor template comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111.

[00248] Embodiment A61. The method of any one of embodiments A47-A60, wherein the RNA-guided nuclease is a Cas9 nuclease, optionally a *S. pyogenes* Cas9 nuclease.

[00249] Embodiment A62. An engineered T cell produced by the method of any one of embodiments A47-A61.

[00250] Embodiment A63. A population of cells comprising the engineered T cell of embodiment A62.

[00251] Embodiment A64. A method of treating cancer in a subject, comprising administering to the subject the population of cells of any one of embodiments A22-A41 or A63.

[00252] Embodiment A65. The method of embodiment A64, wherein the cancer is selected from the group consisting of: pancreatic cancer, gastric cancer, ovarian cancer, uterine cancer, breast cancer, prostate cancer, testicular cancer, thyroid cancer, nasopharyngeal cancer, non-small cell lung (NSCLC), glioblastoma, neuronal, soft tissue sarcomas, leukemia, lymphoma, melanoma, colon cancer, colon adenocarcinoma, brain glioblastoma, hepatocellular carcinoma, liver hepatocholangiocarcinoma, osteosarcoma, gastric cancer, esophagus squamous cell carcinoma, advanced stage pancreas cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell cancer, renal carcinoma, and intrahepatic biliary cancer.

[00253] Embodiment A66. The method of embodiments A64 or A65, wherein the cancer comprises cancer cells expressing LIV1.

EXAMPLES

Example 1. CAR T cell generation and CAR expression

[00254] Activated primary human T cells were electroporated with Cas9:gRNA RNP complexes and adeno-associated adenoviral vectors (AAVs) to generate TRAC$^-$/β2M$^-$/anti-Liv1a CAR$^+$ T cells. Recombinant AAV serotype 6 (AAV6) comprising one of the nucleotide sequences encoding an anti-Liv1a CAR (971 (SEQ ID NO:49), 972 (SEQ ID NO: 65), 972b (SEQ ID NO: 67), 973 (SEQ ID NO: 95), 974 (SEQ ID NO: 100), 975 (SEQ ID NO: 104), and 976 (SEQ ID NO: 108), were delivered with Cas9:sgRNA RNPs (1 μM Cas9, 5 μM gRNA) to activated allogeneic human T cells. The following sgRNAs were used: TRAC (SEQ ID NO: 28) and β2M (SEQ ID NO: 30). The unmodified versions (or other modified versions) of the gRNAs may also be used (e.g., SEQ ID NO: 18 or 20).

[00255] About one (1) week post electroporation, cells were processed for flow cytometry to assess TRAC, β2M, and anti-Liv1a CAR expression levels at the cell surface of the edited cell population (FIG. 1). For all anti-Liv1a CAR T cells and TRAC$^-$/β2M$^-$ control cells, >90% of viable cells lacked expression of TCR and >60% lacked expression of β2M. The cells treated with the construct encoding the 975 and 976 Liv1a CAR had the highest percentage of viable cells expressing an anti-Liv1a CAR$^+$ (>30%).

Example 2. Cytotoxicity

[00256] **Cell Kill Assay**. A cell killing (cytotoxicity) assay was used to assess the ability of the TRAC-/β2M-/anti-Liv1a CAR+ T cells to cause cellular lysis in adherent kidney carcinoma and breast cancer cell lines (A498 and ZR-75-1, respectively). Adherent cells were seeded in 96-well plates at 50,000 cells per well and left overnight at 37°C. During the following day, T cells were added to the wells containing target cells at ratios of 8:1, 4:1, 2:1 or 1:1 T cell:target cell. TRAC-/β2M- T cells were used as a negative control. After approximately 24 hours, 100 μLs of supernatant was removed for cytokine quantification (see below) and T cells were removed from the culture by aspiration and 100 μL CellTiter-Glo® (Promega) was added to each well of the plate to assess the number of remaining viable cells. The amount of light emitted from each well was then quantified using a plate reader. The anti-Liv1a CAR T cells, particularly those expressing the CTX971, CTX975 and CTX976

constructs, exhibited potent cytotoxicity towards the A498 (FIG 2A) and ZR-75-1 (FIG. 2B) cell lines.

Example 3. Effector cytokine secretion

[00257] The MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay kit (Millipore, catalog # HCYTOMAG-60K) using magnetic microspheres, anti-human IFNγ bead (Millipore, catalog # HCYIFNG-MAG) and anti-human IL-2 bead (Millipore, catalog # HIL2-MAG), respectively, was used to quantify IFN-γ and IL-2 secretion in samples from the cytotoxicity assay. The assay was conducted following manufacturer's protocol. MILLIPLEX® standard and quality control (QC) samples were reconstituted, and serial dilutions of the working standards from 10,000 pg/mL to 3.2 pg/mL were prepared. MILLIPLEX® standards, QCs and cell supernatants were added to each plate, and assay media was used to dilute the supernatants. All samples were incubated with anti-human IFNγ and anti-human IL-2 beads for 2 hours. After incubation, the plate was washed using an automated magnetic plate washer. Human cytokine/chemokine detection antibody solution was added to each well and incubated for 1 hour followed by incubation with Streptavidin-Phycoerythrin for 30 minutes. The plate was subsequently washed, samples were resuspended with 150 µL Sheath Fluid, and agitated on a plate shaker for 5 minutes. The samples were read using the Luminex® 100/200™ instrument with xPONENT® software and data acquisition and analysis was completed using MILLIPLEX® Analyst software. The Median Fluorescent Intensity (MFI) data was automatically analyzed using a 5-parameter logistic curve-fitting method for calculating the cytokine concentration measured in the unknown samples.

[00258] As shown in FIGS. 3A-3D, allogeneic T cells containing the CTX971, 975, or 976 CARs secreted the effector cytokines interferon-ɣ (3A, B) and interleukin-2 (3C, D) when co-cultured with the target cells lines A498 and ZR-75-1 at levels significantly above background (2KO/AAV neg T cells co-cultured with the target cell lines).

Table 6.

CAR	CAR structure	SEQ ID NO:
CTX-971 CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ	49, 50
CTX-971b CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	51, 52
CTX-972 CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ	65, 125
CTX-972b CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]- 41BB[co-stimulatory domain]-CD3ζ	67, 126

Table 7. CAR Components

CAR Structure:

CD8[signal peptide]-anti-LIV1[scFV]-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ; or

CD8[signal peptide]-anti-LIV1[scFV]-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ

Name	Sequence	SEQ ID NO:
CTX-971 CAR CD28 co-stim	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTG TTGCTCCACGCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCAC TTTCCTTGCCCGTGACTCTCGGACAACCGGCTTCAATATCTTGCCGC TCATCACAGTCCCTGCTGCATAGCAGTGGTAACACTTATCTTGAGTG GTACCAACAGCGGCCCGGCCAATCTCCTAGGCCCCTGATATATAAG ATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTCTGG GAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAA GACGTGGGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATAC GTTTGGTGGGGGCACAAAAGTCGAGATTAAGGGAGGCGGAGGATC AGGAGGTGGGGGAAGTGGAGGTGGTGGGTCACAAGTACAGCTCGT GCAATCAGGGGCGGAGGTGAAGAAACCAGGGGCGTCTGTGAAGGT AAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACATGCAT TGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGA TAGATCCTGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGG CAGAGTTACGATGACTCGAGATACTAGCATTAATACGGCCTACATG GAGCTTAGCCGCCTGCGGTCCGATGACACGGCCGTTTATTATTGCGC CGTACACAATGCGCACTACGGGACATGGTTCGCGTATTGGGGTCAA GGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTGTCCCGGT ATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGA CACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAG GCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGG ACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGTGC GGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAGG	49

Name	Sequence	SEQ ID NO:
	AATCGCTCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATAT GACTCCTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTAT GCCCCCCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTC CCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTG TATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTG ATAAACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAA GAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAA GATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACG ACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCA ACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCA GATAAT	
CTX-971 CAR CD28 co-stim	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPASISCRSSQS LLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTL KISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGG GSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGL EWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTA VYYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVPVFLPAKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	50
CTX-971b CAR 41BB co-stim	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTG TTGCTCCACGCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCAC TTTCCTTGCCCGTGACTCTCGGACAACCGGCTTCAATATCTTGCCGC TCATCACAGTCCCTGCTGCATAGCAGTGGTAACACTTATCTTGAGTG GTACCAACAGCGGCCCGGCCAATCTCCTAGGCCCCTGATATATAAG ATAAGTACTCGCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTCTGG GAGTGGTACAGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAA GACGTGGGTGTTTATTACTGCTTCCAAGGATCTCACGTGCCATATAC GTTTGGTGGGGGCACAAAAGTCGAGATTAAGGGAGGCGGAGGATC AGGAGGTGGGGGAAGTGGAGGTGGTGGGTCACAAGTACAGCTCGT GCAATCAGGGGCGGAGGTGAAGAAACCAGGGGCGTCTGTGAAGGT AAGCTGTAAGGCATCCGGATTGACAATCGAGGATTATTACATGCAT TGGGTCCGCCAGGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGA TAGATCCTGAAAATGGGGATACAGAGTATGGCCCTAAGTTCCAGGG CAGAGTTACGATGACTCGAGATACTAGCATTAATACGGCCTACATG GAGCTTAGCCGCCTGCGGTCCGATGACACGGCCGTTTATTATTGCGC CGTACACAATGCGCACTACGGGACATGGTTCGCGTATTGGGGTCAA GGAACGCTCGTTACTGTCTCAAGTAGTGCTGCTGCCTTTGTCCCGGT ATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGA CACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAG GCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGG ACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGTGC GGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAGG AATCGCAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAAC CATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAG CTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTG	51

Name	Sequence	SEQ ID NO:
	AAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGA ATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGA CGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATGGGGGGTAA ACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAG AAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGC GAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGA GTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCT GCCTCCCAGATAAT	
CTX-971b CAR 41BB co-stim	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPASISCRSSQS LLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTL KISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSGGG GSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGL EWMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTA VYYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVPVFLPAKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	52
CTX-971 and CTX-971b scFv	GACGTGGTCATGACTCAAAGCCCACTTTCCTTGCCCGTGACTCTCGG ACAACCGGCTTCAATATCTTGCCGCTCATCACAGTCCCTGCTGCATA GCAGTGGTAACACTTATCTTGAGTGGTACCAACAGCGGCCCGGCCA ATCTCCTAGGCCCCTGATATATAAGATAAGTACTCGCTTTTCCGGGG TCCCGGACCGGTTCAGCGGGTCTGGGAGTGGTACAGACTTCACATT GAAGATTTCACGAGTAGAAGCCGAAGACGTGGGTGTTTATTACTGC TTCCAAGGATCTCACGTGCCATATACGTTTGGTGGGGGCACAAAAG TCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGGGGGAAGTGGAG GTGGTGGGTCACAAGTACAGCTCGTGCAATCAGGGGCGGAGGTGAA GAAACCAGGGGCGTCTGTGAAGGTAAGCTGTAAGGCATCCGGATTG ACAATCGAGGATTATTACATGCATTGGGTCCGCCAGGCACCAGGGC AGGGATTGGAGTGGATGGGGTGGATAGATCCTGAAAATGGGGATAC AGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGATGACTCGAGAT ACTAGCATTAATACGGCCTACATGGAGCTTAGCCGCCTGCGGTCCG ATGACACGGCCGTTTATTATTGCGCCGTACACAATGCGCACTACGGG ACATGGTTCGCGTATTGGGGTCAAGGAACGCTCGTTACTGTCTCAAG T	53
CTX-971 and CTX-971b scFv	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSP RPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHV PYTFGGGTKVEIK**GGGGSGGGGSGGGGS**QVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKF	54

Name	Sequence	SEQ ID NO:
(linker underlined)	QGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYW GQGTLVTVSS	
CTX-971 and CTX-971b scFv VH CDRs- in bold	QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPGQGLE WMG**WIDPENGDTEYGPKFQG**RVTMTRDTSINTAYMELSRLRSDDTA VYYCAV**HNAHYGTWFAY**WGQGTLVTVSS	55
CTX-971 and CTX-971b scFv VL CDRs – in bold	DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQRPGQS PRPLIY**KISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGS HVPYT**FGGGTKVEIK	56
CTX-971 and CTX-971b VH CDR1	DYYMH	57
CTX-971 and CTX-971b VH CDR2	WIDPENGDTEYGPKFQG	58
CTX-971 and CTX-971b VH CDR3	HNAHYGTWFAY	59
CTX-971 and CTX-971b VL CDR1	RSSQSLLHSSGNTYLE	60
CTX-971 and CTX-971b VL CDR2	KISTRFS	61
CTX-971 and CTX-971b VL CDR3	FQGSHVPYT	62

Name	Sequence	SEQ ID NO:
CTX-971 Donor LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGT AAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTC AAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGAT TTCCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATG CCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGT TTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGT TATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAA GCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAG GCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCA AGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAC TTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGA CTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAA CCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGT GTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTAT TCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCT GATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGG ACTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttgggggga ggggtcggcaattgaaccggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggc tccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgg gtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgc gtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagt tcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcctgggcgctggggccgccg cgtgcgaatctggtggcaccttcgcgcctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacct gctgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttgggg ccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggcc accgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccgg ccctgctgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggtgagtcacccacacaaa ggaaaagggcctttccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctc gattagttctcgagcttttggagtacgtcgtctttaggttgggggggagggttttatgcgatggagtttccccacact gagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggat cttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttcaggtgtcgtgaCCACCATGG CGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCAC GCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGC CCGTGACTCTCGGACAACCGGCTTCAATATCTTGCCGCTCATCACAG TCCCTGCTGCATAGCAGTGGTAACACTTATCTTGAGTGGTACCAACA GCGGCCCGGCCAATCTCCTAGGCCCCTGATATATAAGATAAGTACTC GCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTCTGGGAGTGGTAC AGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACGTGGGT GTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGG GGGCACAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGG GGGAAGTGGAGGTGGTGGGTCACAAGTACAGCTCGTGCAATCAGGG GCGGAGGTGAAGAAACCAGGGGCGTCTGTGAAGGTAAGCTGTAAG GCATCCGGATTGACAATCGAGGATTATTACATGCATTGGGTCCGCCA GGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGATCCTGAA AATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGA TGACTCGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCG CCTGCGGTCCGATGACACGGCCGTTTATTATTGCGCCGTACACAATG	63

Name	Sequence	SEQ ID NO:
	CGCACTACGGGACATGGTTCGCGTATTGGGGTCAAGGAACGCTCGT TACTGTCTCAAGTAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGC CAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCC ACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTG ATATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTG TTGTCACTCGTTATTACTTTGTATTGTAATCACAGGAATCGCTCAAA GCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTCCTCGCC GGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCCCCCCACG AGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAAGCGCA GACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAAC TGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCG GGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCC CCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGA GGCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAA AGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGAT ACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAAT AAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGT GGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAG CATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCT TTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTC TGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGG TCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAAC AGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGC AGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAG TCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGC CCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGC CTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTA AGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTG CCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGT CAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAG CCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATG TGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGT CAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCT ACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCT CAATGAGAAAGG	
CTX-971b Donor LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGT AAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTC AAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGAT TTCCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATG CCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGT TTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGT TATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAA GCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAG GCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCA AGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAC TTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGA CTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAA	64

Name	Sequence	SEQ ID NO:
	CCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGT GTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTAT TCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCT GATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGG ACTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttgggggga ggggtcggcaattgaaccggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggc tccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgg gtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgc gtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagt tcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcctgggcgctggggccgccg cgtgcgaatctggtggcaccttcgcgcctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacct gctgcgacgcttttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggttttgggg ccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggcc accgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccgg ccctgctgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggtgagtcacccacacaaa ggaaaagggcctttccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctc gattagttctcgagcttttggagtacgtcgtctttaggttgggggggagggttttatgcgatggagtttccccacact gagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggat cttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttcaggtgtcgtgaCCACCATGG CGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCAC GCAGCAAGGCCGGACGTGGTCATGACTCAAAGCCCACTTTCCTTGC CCGTGACTCTCGGACAACCGGCTTCAATATCTTGCCGCTCATCACAG TCCCTGCTGCATAGCAGTGGTAACACTTATCTTGAGTGGTACCAACA GCGGCCCGGCCAATCTCCTAGGCCCCTGATATATAAGATAAGTACTC GCTTTTCCGGGGTCCCGGACCGGTTCAGCGGGTCTGGGAGTGGTAC AGACTTCACATTGAAGATTTCACGAGTAGAAGCCGAAGACGTGGGT GTTTATTACTGCTTCCAAGGATCTCACGTGCCATATACGTTTGGTGG GGGCACAAAAGTCGAGATTAAGGGAGGCGGAGGATCAGGAGGTGG GGGAAGTGGAGGTGGTGGGTCACAAGTACAGCTCGTGCAATCAGGG GCGGAGGTGAAGAAACCAGGGGCGTCTGTGAAGGTAAGCTGTAAG GCATCCGGATTGACAATCGAGGATTATTACATGCATTGGGTCCGCCA GGCACCAGGGCAGGGATTGGAGTGGATGGGGTGGATAGATCCTGAA AATGGGGATACAGAGTATGGCCCTAAGTTCCAGGGCAGAGTTACGA TGACTCGAGATACTAGCATTAATACGGCCTACATGGAGCTTAGCCG CCTGCGGTCCGATGACACGGCCGTTTATTATTGCGCCGTACACAATG CGCACTACGGGACATGGTTCGCGTATTGGGGTCAAGGAACGCTCGT TACTGTCTCAAGTAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGC CAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCC ACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACC CGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTG ATATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTG TTGTCACTCGTTATTACTTTGTATTGTAATCACAGGAATCGCAAACG GGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTC CAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCG AAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTAT AACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATA AACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAA AGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGAT GGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACG	

Name	Sequence	SEQ ID NO:
	GGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACC AAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGAT AATAATAAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTT GTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAAC AACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGG GCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCC AGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGAT TGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTA AGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAA AAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAG CCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACT GTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCA AGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGC TCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTG ATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTG GGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATT GGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGAC AAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACC AGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACA GGAGCTCAATGAGAAAGG	
CTX-972 CAR CD28 co-stim	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTG TTGCTCCACGCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCG CTGAGGTCAAAAAGCCCGGCGCGAGCGTAAAAGTCTCCTGCAAGGC GTCAGGGTTGACGATAGAAGATTATTACATGCATTGGGTCAGACAG GCACCCGGACAGGGATTGGAGTGGATGGGTTGGATCGACCCGGAAA ACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAAT GACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGC TTCGCTCTGATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCC CATTACGGAACCTGGTTCGCGTACTGGGGCCAAGGGACCCTGGTTA CGGTTAGCTCTGGTGGGGGTGGAAGCGGGGGAGGGGGTAGCGGAG GTGGCGGAAGTGATGTTGTTATGACACAGAGTCCCCTGTCATTGCCC GTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAGTCAAA GCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACA ACGGCCAGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACAC GCTTCTCAGGTGTTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACA GACTTTACCTTGAAAATAAGCAGGGTTGAAGCTGAGGACGTAGGGG TATATTATTGTTTTCAGGGCAGTCACGTGCCGTACACTGGGGGCGGA ACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCT CCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATG CCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTC GCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGT CCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAGGAATCG CTCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTC CTCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCCC CCCACGAGACTTCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAA GCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAA CGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAA	65

Name	Sequence	SEQ ID NO:
	CGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAG AATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGG CGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACGGG GAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAA AGATACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAA T	
CTX-972 CAR CD28 co-stim	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASG LTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTR DTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTV SSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCRSSQSLLHS SGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISR VEAEDVGVYYCFQGSHVPYTGGGTKVEIKSAAAFVPVFLPAKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	125
CTX-972b CAR 41BB co-stim	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTG TTGCTCCACGCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCG CTGAGGTCAAAAAGCCCGGCGCGAGCGTAAAAGTCTCCTGCAAGGC GTCAGGGTTGACGATAGAAGATTATTACATGCATTGGGTCAGACAG GCACCCGGACAGGGATTGGAGTGGATGGGTTGGATCGACCCGGAAA ACGGTGACACGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAAT GACACGAGATACGTCCATAAATACCGCTTACATGGAACTTTCTCGGC TTCGCTCTGATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCC CATTACGGAACCTGGTTCGCGTACTGGGGCCAAGGGACCCTGGTTA CGGTTAGCTCTGGTGGGGGTGGAAGCGGGGGAGGGGGTAGCGGAG GTGGCGGAAGTGATGTTGTTATGACACAGAGTCCCCTGTCATTGCCC GTCACCCTCGGACAACCAGCTAGCATTTCATGCAGGTCTAGTCAAA GCCTCCTTCACAGTAGCGGCAACACCTACCTCGAATGGTATCAACA ACGGCCAGGGCAATCTCCTCGCCCACTCATATACAAAATCTCTACAC GCTTCTCAGGTGTTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACA GACTTTACCTTGAAAATAAGCAGGGTTGAAGCTGAGGACGTAGGGG TATATTATTGTTTTCAGGGCAGTCACGTGCCGTACACTGGGGGCGGA ACCAAAGTCGAGATAAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCT CCCAGCCAAACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCC GCTCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATG CCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTC GCTTGTGATATTTACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGT CCTTTTGTTGTCACTCGTTATTACTTTGTATTGTAATCACAGGAATCG CAAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTT ATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCC GATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTT TTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAGGACAGAATCAG CTGTATAACGAACTGAATTTGGGACGCCGCGAGGAGTATGACGTGC TTGATAAACGCCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCC GAAGAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGG ATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAAC	67

Name	Sequence	SEQ ID NO:
	GACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGGGTTGAGTAC GGCAACCAAAGATACGTACGATGCACTGCATATGCAGGCCCTGCCT CCCAGATAAT	
CTX-972b CAR 41BB co-stim	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASVKVSCKASG LTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTR DTSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTV SSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISCRSSQSLLHS SGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISR VEAEDVGVYYCFQGSHVPYTGGGTKVEIKSAAAFVPVFLPAKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	126
CTX-972 and CTX-972b scFv	CAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCAAAAAGCCCGGCG CGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTTGACGATAGAAGA TTATTACATGCATTGGGTCAGACAGGCACCCGGACAGGGATTGGAG TGGATGGGTTGGATCGACCCGGAAAACGGTGACACGGAGTATGGGC CGAAGTTTCAGGGGAGGGTCACAATGACACGAGATACGTCCATAAA TACCGCTTACATGGAACTTTCTCGGCTTCGCTCTGATGATACAGCAG TTTACTACTGCGCTGTTCATAATGCCCATTACGGAACCTGGTTCGCG TACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTCTGGTGGGGGTG GAAGCGGGGGAGGGGGTAGCGGAGGTGGCGGAAGTGATGTTGTTA TGACACAGAGTCCCCTGTCATTGCCCGTCACCCTCGGACAACCAGCT AGCATTTCATGCAGGTCTAGTCAAAGCCTCCTTCACAGTAGCGGCAA CACCTACCTCGAATGGTATCAACAACGGCCAGGGCAATCTCCTCGC CCACTCATATACAAAATCTCTACACGCTTCTCAGGTGTTCCCGACCG CTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTTGAAAATAAGCA GGGTTGAAGCTGAGGACGTAGGGGTATATTATTGTTTTCAGGGCAG TCACGTGCCGTACACTGGGGGCGGAACCAAAGTCGAGATAAAG	69
CTX-972 and CTX-972b scFv (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLE WMGWIDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAV YYCAVHNAHYGTWFAYWGQGTLVTVSS**GGGGSGGGGSGGGGS**DVV MTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLI YKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT GGGTKVEIK	127
CTX-972 and CTX-972b scFv VH CDRs- in bold	QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPGQGLE WMG**WIDPENGDTEYGPKFQG**RVTMTRDTSINTAYMELSRLRSDDTA VYYCAV**HNAHYGTWFAY**WGQGTLVTVSS	55

Name	Sequence	SEQ ID NO:
CTX-972 and CTX-972b scFv VL CDRs – in bold	DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQRPGQS PRPLIY**KISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**FQGS HVPYT**GGGTKVEIK	128
CTX-972 and CTX-972b VH CDR1	DYYMH	57
CTX-972 and CTX-972b VH CDR2	WIDPENGDTEYGPKFQG	58
CTX-972 and CTX-972b VH CDR3	HNAHYGTWFAY	59
CTX-972 and CTX-972b VL CDR1	RSSQSLLHSSGNTYLE	60
CTX-972 and CTX-972b VL CDR2	KISTRFS	61
CTX-972 and CTX-972b VL CDR3	FQGSHVPYT	62
CTX-972 Donor LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGT AAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTC AAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGAT TTCCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATG CCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGT TTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGT TATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAA GCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAG	71

Name	Sequence	SEQ ID NO:
	GCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCA AGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAC TTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGA CTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAA CCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGT GTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTAT TCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCT GATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGG ACTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttgggggga ggggtcggcaattgaaccggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggc tccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgg gtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgc gtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagt tcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcctgggcgctggggccgccg cgtgcgaatctggtggcaccttcgcgcctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacct gctgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttgggg ccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggcc accgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccgg ccctgctgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggtgagtcacccacacaaa ggaaaagggcctttccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctc gattagttctcgagcttttggagtacgtcgtctttaggttggggggagggggttttatgcgatggagtttccccacact gagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggat cttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttcaggtgtcgtgaCCACCATGG CGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCAC GCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCA AAAAGCCCGGCGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTT GACGATAGAAGATTATTACATGCATTGGGTCAGACAGGCACCCGGA CAGGGATTGGAGTGGATGGGTTGGATCGACCCGGAAAACGGTGACA CGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAATGACACGAGA TACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCTCTG ATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGA ACCTGGTTCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTC TGGTGGGGGTGGAAGCGGGGGAGGGGGTAGCGGAGGTGGCGGAAG TGATGTTGTTATGACACAGAGTCCCCTGTCATTGCCCGTCACCCTCG GACAACCAGCTAGCATTTCATGCAGGTCTAGTCAAAGCCTCCTTCAC AGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGGCCAGGGC AATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGT GTTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTT GAAAATAAGCAGGGTTGAAGCTGAGGACGTAGGGGTATATTATTGT TTTCAGGGCAGTCACGTGCCGTACACTGGGGGCGGAACCAAAGTCG AGATAAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCAT CGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCG CCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATT TACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTC ACTCGTTATTACTTTGTATTGTAATCACAGGAATCGCTCAAAGCGGA GTAGGTTGTTGCATTCCGATTACATGAATATGACTCCTCGCCGGCCT GGGCCGACAAGAAAACATTACCAACCCTATGCCCCCCCACGAGACT TCGCTGCGTACAGGTCCCGAGTGAAGTTTTCCCGAAGCGCAGACGC	

Name	Sequence	SEQ ID NO:
	TCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAAT TTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGA GAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCCCCAAG AAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGGAGGCCTA CTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAAAAGGTCA CGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTAC GATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAAATC GCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGTGGAGCA ACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATT CCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTG CCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCA GAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGG CCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAG CCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGA AGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCC AACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTAC TGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCT TATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAG TCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCA CATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATG AGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATC TGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTA ACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAA GGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGG GCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAG AAAGG	
CTX-972b Donor LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGT AAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTTC AAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGAT TTCCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAATG CCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGT TTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGT TATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAATAA GCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGGCAG GCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTGGCCA AGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAC TTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGCATCTGGA CTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAA CCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGT GTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTAT TCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCT GATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATGG ACTTCAggctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttgggggga ggggtcggcaattgaaccggtgcctagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggc tccgccttttcccgagggtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgg gtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgc gtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagt tcgaggccttgcgcttaaggagcccttcgcctcgtgcttgagttgaggcctggcctgggcgctggggccgccg	72

Name	Sequence	SEQ ID NO:
	cgtgcgaatctggtggcaccttcgcgcctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacct gctgcgacgctttttttctggcaagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttgggg ccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgcggcc accgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatc gccccgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccgg ccctgctgcagggagctcaaaatggaggacgcggcgctcgggagagcgggcgggtgagtcacccacacaaa ggaaaagggcctttccgtcctcagccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctc gattagttctcgagcttttggagtacgtcgtctttaggttgggggggagggttttatgcgatggagtttccccacact gagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggat cttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttcaggtgtcgtgaCCACCATGG CGCTTCCGGTGACAGCACTGCTCCTCCCCTTGGCGCTGTTGCTCCAC GCAGCAAGGCCGCAAGTTCAACTGGTCCAGTCAGGCGCTGAGGTCA AAAAGCCCGGCGCGAGCGTAAAAGTCTCCTGCAAGGCGTCAGGGTT GACGATAGAAGATTATTACATGCATTGGGTCAGACAGGCACCCGGA CAGGGATTGGAGTGGATGGGTTGGATCGACCCGGAAAACGGTGACA CGGAGTATGGGCCGAAGTTTCAGGGGAGGGTCACAATGACACGAGA TACGTCCATAAATACCGCTTACATGGAACTTTCTCGGCTTCGCTCTG ATGATACAGCAGTTTACTACTGCGCTGTTCATAATGCCCATTACGGA ACCTGGTTCGCGTACTGGGGCCAAGGGACCCTGGTTACGGTTAGCTC TGGTGGGGGTGGAAGCGGGGGAGGGGGTAGCGGAGGTGGCGGAAG TGATGTTGTTATGACACAGAGTCCCCTGTCATTGCCCGTCACCCTCG GACAACCAGCTAGCATTTCATGCAGGTCTAGTCAAAGCCTCCTTCAC AGTAGCGGCAACACCTACCTCGAATGGTATCAACAACGGCCAGGGC AATCTCCTCGCCCACTCATATACAAAATCTCTACACGCTTCTCAGGT GTTCCCGACCGCTTCAGCGGTTCCGGCTCTGGGACAGACTTTACCTT GAAAATAAGCAGGGTTGAAGCTGAGGACGTAGGGGTATATTATTGT TTTCAGGGCAGTCACGTGCCGTACACTGGGGGCGGAACCAAAGTCG AGATAAAGAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCAT CGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCG CCGGGGGTGCTGTTCATACGAGGGGCTTGGACTTCGCTTGTGATATT TACATTTGGGCTCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTC ACTCGTTATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCA GAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGT ACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAA GAAGAAGAAGGAGGATGTGAACTGCGAGTGAAGTTTTCCCGAAGCG CAGACGCTCCGGCATATCAGCAAGGACAGAATCAGCTGTATAACGA ACTGAATTTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGC CGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAAT CCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGATGGCGG AGGCCTACTCAGAAATAGGTATGAAGGGCGAACGACGACGGGGAA AAGGTCACGATGGCCTCTACCAAGGGTTGAGTACGGCAACCAAAGA TACGTACGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAAT AAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTGT GGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAG CATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCT TTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTC TGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGG TCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAAC AGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGC AGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAG	

Name	Sequence	SEQ ID NO:
	TCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGC CCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGC CTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTA AGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTG CCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGT CAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAG CCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATG TGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGT CAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCT ACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCT CAATGAGAAAGG	
CD8 signal peptide	MALPVTALLLPLALLLHAARP	73
CD8a transmembrane + 5' Linker (underlined)	GCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGAC TCCCGCCCCGCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAAC CTCTTAGTCTTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCT GTTCATACGAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGC TCCGTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTAC TTTGTATTGTAATCACAGGAATCGC	74
CD8a transmembrane + 5' Linker (underlined)	SAAAFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNR	75
CD8a transmembrane (without linker)	TTTGTCCCGGTATTTCTCCCAGCCAAACCGACCACGACTCCCGCCCC GCGCCCTCCGACACCCGCTCCCACCATCGCCTCTCAACCTCTTAGTC TTCGCCCCGAGGCATGCCGACCCGCCGCCGGGGGTGCTGTTCATAC GAGGGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGG CGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTGTATT GTAATCACAGGAATCGC	76
CD8a transmembrane (without linker)	FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNR	77
CD28 co-stimulatory	TCAAAGCGGAGTAGGTTGTTGCATTCCGATTACATGAATATGACTCC TCGCCGGCCTGGGCCGACAAGAAAACATTACCAACCCTATGCCCCC CCACGAGACTTCGCTGCGTACAGGTCC	45
CD28 co-stimulatory	SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	46

Name	Sequence	SEQ ID NO:
41BB co-stimulatory	AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTA TGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCG ATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG	43
41BB co-stimulatory	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	44
CD3ζ	CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAG GACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCCGCGAGGA GTATGACGTGCTTGATAAACGCCGGGGGAGAGACCCGGAAATGGGG GGTAAACCCCGAAGAAAGAATCCCCAAGAAGGACTCTACAATGAAC TCCAGAAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAA GGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGG GTTGAGTACGGCAACCAAAGATACGTACGATGCACTGCATATGCAG GCCCTGCCTCCCAGA	47
CD3ζ	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR	48

Table 8. Donor Components

Donor structure: TRAC[LHA]-EF1a[promoter]-CAR-polyA–TRAC[RHA]

Name	Sequence	SEQ ID NO:
TRAC-LHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGT AAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTATAGTT CAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGA TTTCCCAACTTAATGCCAACATACCATAAACCTCCCATTCTGCTAAT GCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAG TTTGCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGA GTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAAAGAAT AAGCAGTATTATTAAGTAGCCCTGCATTTCAGGTTTCCTTGAGTGG CAGGCCAGGCCTGGCCGTGAACGTTCACTGAAATCATGGCCTCTTG GCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCAC GAGCAGCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACTGGC ATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCA TGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGAC CCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTG TCTGCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGT AAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGA GGTCTATGGACTTCA	78
EF1α promoter	GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC CCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGA GAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGC TCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGT AGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA CAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGG GTTATGGCCCTTGCGTGCCTTGAATTACTTCCACTGGCTGCAGTACG TGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTC GAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAG GCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACC TTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAAT TTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCC GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCG AGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAG TCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGT GTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGT TGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAG CTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTC ACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCA TGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGT TCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTT TTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTT AGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTT TGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA AGTTTTTTTCTTCCATTTCAGGTGTCGTGA	79

Name	Sequence	SEQ ID NO:
Synthetic poly(A) signal	AATAAAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGTGTG	80
TRAC–RHA	TGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGG	81

Table 9.

CAR	CAR structure	SEQ ID NO:
CTX-973 CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	95, 96
CTX-974 CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	100, 101
CTX-975 CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	104, 105
CTX-976 CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	108, 109
CTX-977 CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	112, 113
CTX-978 CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	116, 117
CTX-979 CAR	CD8[signal peptide]-VL-linker-VH-CD8[tm]-41BB[co-stimulatory domain]-CD3ζ	68
CTX-979b CAR	CD8[signal peptide]-VH-linker-VL-CD8[tm]-CD28[co-stimulatory domain]-CD3ζ	66

Table 10. CAR Components

Name	Sequence	SEQ ID NO:
CTX-973 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGGATGTCGTTA TGACACAATCTCCCTTGAGTTTGCCGGTTACCTTGGGAC AACCTGCTAGTATTTCATGTAGGAGTTCTCAAAGTCTCTT GCACTCCTCAGGGAACACCTACCTCGAATGGTACCAACA ACGCCCTGGCCAAAGCCCGCGGCCCTTGATATACAAAAT ATCAACAAGATTTAGCGGGGTACCCGATAGATTCAGCGG CTCTGGCAGCGGGACGGATTTTACCCTGAAAATTAGTCG CGTAGAAGCTGAAGACGTTGGTGTGTATTACTGCTTTCA AGGGAGCCATGTGCCTTACACATTTGGAGGAGGCACCA AGGTCGAGATTAAGGGAGGGGGTGGATCAGGTGGGGGT GGGTCCGGAGGCGGCGGCAGTCAAGTGCAGTTGGTTCA ATCAGGAGCTGAAGTTAAAAAGCCAGGAGCTTCAGTCA AGGTTTCATGCAAGGCGTCCGGTCTCACTATAGAGGATT ACTACATGCACTGGGTGCGGCAAGCTCCAGGCCAGGGG CTGGAGTGGATGGGATGGATTGATCCGGAAAACGGGGA CACAGAGTATGGGCCCAAATTCCAAGGCCGGGTGACAA TGACCAGAGATACTAGTATTTCAACAGCATACATGGAGC TGTCACGGCTGAGGTCAGACGATACGGCAGTCTACTATT GTGCAGTACATAACGCACATTATGGTACGTGGTTCGCTT ATTGGGGTCAAGGTACCCTGGTCACGGTAAGTTCAAGTG CTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGAC CACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCAC CATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGC CGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTG GACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGG GTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTT GTATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGA AACTCCTGTATATATTCAAACAACCATTTATGAGACCAG TACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGAT TTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTG AAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAA GGACAGAATCAGCTGTATAACGAACTGAATTTGGGACG CCGCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGA GAGACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAAT CCCCAAGAAGGACTCTACAATGAACTCCAGAAGGATAA GATGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCG AACGACGACGGGGAAAAGGTCACGATGGCCTCTACCAA GGGTTGAGTACGGCAACCAAAGATACGTACGATGCACT GCATATGCAGGCCCTGCCTCCCAGATAAT	95
CTX-973 CAR 41BB co-stim (aa)	ALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPASIS CRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVP DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGG GTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGAS	96

Name	Sequence	SEQ ID NO:
	VKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPEN GDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYY CAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVPVFLPAKP TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF ACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKKLLY IFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGH DGLYQGLSTATKDTYDALHMQALPPR	
CTX-973 scFv (nt)	GATGTCGTTATGACACAATCTCCCTTGAGTTTGCCGGTTA CCTTGGGACAACCTGCTAGTATTTCATGTAGGAGTTCTC AAAGTCTCTTGCACTCCTCAGGGAACACCTACCTCGAAT GGTACCAACAACGCCCTGGCCAAAGCCCGCGGCCCTTGA TATACAAAATATCAACAAGATTTAGCGGGGTACCCGATA GATTCAGCGGCTCTGGCAGCGGGACGGATTTTACCCTGA AAATTAGTCGCGTAGAAGCTGAAGACGTTGGTGTGTATT ACTGCTTTCAAGGGAGCCATGTGCCTTACACATTTGGAG GAGGCACCAAGGTCGAGATTAAGGGAGGGGGTGGATCA GGTGGGGGTGGGTCCGGAGGCGGCGGCAGTCAAGTGCA GTTGGTTCAATCAGGAGCTGAAGTTAAAAAGCCAGGAG CTTCAGTCAAGGTTTCATGCAAGGCGTCCGGTCTCACTA TAGAGGATTACTACATGCACTGGGTGCGGCAAGCTCCAG GCCAGGGGCTGGAGTGGATGGGATGGATTGATCCGGAA AACGGGGACACAGAGTATGGGCCCAAATTCCAAGGCCG GGTGACAATGACCAGAGATACTAGTATTTCAACAGCATA CATGGAGCTGTCACGGCTGAGGTCAGACGATACGGCAG TCTACTATTGTGCAGTACATAACGCACATTATGGTACGT GGTTCGCTTATTGGGGTCAAGGTACCCTGGTCACGGTAA GTTCA	97
CTX-973 scFv (aa) (linker underlined)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY QQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSG GGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMH WVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQ GTLVTVSS	82
CTX-973 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSIST AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	98
CTX-973 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY QQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	56
CTX-973 Donor (nt)	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT	99

Name	Sequence	SEQ ID NO:
LHA to RHA	TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggagggggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg ccccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctgggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc tcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgctttttttctggc aagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttgggggccgcggg cggcgacgggggcccgtgcgtcccagcgcacatgttcggcgaggcgggggcctgcgagcgc ggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcct cgcgccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgc gtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcg gcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggccttccgtcctc agccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgattagttctc gagcttttggagtacgtcgtctttaggttgggggggagggggttttatgcgatggagtttccccaca ctgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgcc cttttgagttggatcttggttcattctcaagcctcagacagtggttcaaagttttttcttccattc aggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTC CTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGAT GTCGTTATGACACAATCTCCCTTGAGTTTGCCGGTTACCT TGGGACAACCTGCTAGTATTTCATGTAGGAGTTCTCAAA GTCTCTTGCACTCCTCAGGGAACACCTACCTCGAATGGT ACCAACAACGCCCTGGCCAAAGCCCGCGGCCCTTGATAT ACAAAATATCAACAAGATTTAGCGGGGTACCCGATAGA TTCAGCGGCTCTGGCAGCGGGACGGATTTTACCCTGAAA ATTAGTCGCGTAGAAGCTGAAGACGTTGGTGTGTATTAC TGCTTTCAAGGGAGCCATGTGCCTTACACATTTGGAGGA	

Name	Sequence	SEQ ID NO:
	GGCACCAAGGTCGAGATTAAGGGAGGGGGTGGATCAGG TGGGGGTGGGTCCGGAGGCGGCGGCAGTCAAGTGCAGT TGGTTCAATCAGGAGCTGAAGTTAAAAAGCCAGGAGCTT CAGTCAAGGTTTCATGCAAGGCGTCCGGTCTCACTATAG AGGATTACTACATGCACTGGGTGCGGCAAGCTCCAGGCC AGGGGCTGGAGTGGATGGGATGGATTGATCCGGAAAAC GGGGACACAGAGTATGGGCCCAAATTCCAAGGCCGGGT GACAATGACCAGAGATACTAGTATTTCAACAGCATACAT GGAGCTGTCACGGCTGAGGTCAGACGATACGGCAGTCT ACTATTGTGCAGTACATAACGCACATTATGGTACGTGGT TCGCTTATTGGGGTCAAGGTACCCTGGTCACGGTAAGTT CAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAA ACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGC TCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAG GCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGG GGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGT TGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTAT TACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAG AAAGAAACTCCTGTATATATTCAAACAACCATTTATGAG ACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATAT CAGCAAGGACAGAATCAGCTGTATAACGAACTGAATTT GGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCC GGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGA AAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAA GGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGA AGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTC TACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGA TGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATA AAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTT GTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACG CCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCC CCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCT GTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGC TCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCT CGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAG AAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC GGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGG GCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTG CCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTC TTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCT CCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTC ACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAA TCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGA AGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG	

Name	Sequence	SEQ ID NO:
	CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTT TAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAA AGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAG ATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAG AGGCCTGGGACAGGAGCTCAATGAGAAAGG	
CTX-974 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTGCAGC TGGTCCAAAGCGGCGCCGAGGTTAAGAAACCAGGCGCA TCCGTCAAGGTTTCATGTAAAGCAAGTGGCTTGACTATA GAAGACTACTACATGCATTGGGTACGGCAAGCCCCTGGG CAGGGGCTGGAATGGATGGGGTGGATCGACCCGGAGAA TGGTGATACAGAGTACGGACCTAAGTTCCAGGGACGAG TTACCATGACGCGAGATACATCCATCTCCACGGCATACA TGGAGCTGAGTCGACTGCGGAGCGATGATACAGCTGTCT ATTATTGTGCTGTCCACAATGCGCACTACGGCACCTGGT TCGCTTATTGGGGACAAGGTACCCTGGTCACAGTCAGCT CTGGGGGTGGCGGCAGTGGAGGGGGTGGTTCTGGTGGC GGGGGTTCCGATGTTGTAATGACTCAAAGCCCTCTTTCTT TGCCAGTCACTCTCGGACAACCCGCGAGCATATCTTGCA GGTCTTCACAATCACTCCTTCACAGTAGCGGGAATACTT ACTTGGAGTGGTATCAGCAGCGGCCTGGTCAGTCCCCTA GACCGCTTATATATAAGATCTCCACTAGGTTCAGTGGAG TGCCGGACCGCTTTTCAGGCTCAGGTTCCGGGACGGACT TTACATTGAAAATATCCAGGGTGGAGGCGGAGGACGTC GGAGTCTACTATTGCTTCCAAGGCTCCCACGTCCCATAC ACTTTCGGTGGCGGTACAAAAGTGGAAATAAAAAGTGC TGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGACC ACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCACC ATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCATGCC GACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGCTTGG ACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGGCGGG TACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTACTTTG TATTGTAATCACAGGAATCGCAAACGGGGCAGAAAGAA ACTCCTGTATATATTCAAACAACCATTTATGAGACCAGT ACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATT TCCAGAAGAAGAAGAAGGAGGATGTGAACTGCGAGTGA AGTTTTCCCGAAGCGCAGACGCTCCGGCATATCAGCAAG GACAGAATCAGCTGTATAACGAACTGAATTTGGGACGCC GCGAGGAGTATGACGTGCTTGATAAACGCCGGGGGAGA GACCCGGAAATGGGGGGTAAACCCCGAAGAAAGAATCC CCAAGAAGGACTCTACAATGAACTCCAGAAGGATAAGA TGGCGGAGGCCTACTCAGAAATAGGTATGAAGGGCGAA CGACGACGGGGAAAAGGTCACGATGGCCTCTACCAAGG GTTGAGTACGGCAACCAAAGATACGTACGATGCACTGC ATATGCAGGCCCTGCCTCCCAGATAAT	100

Name	Sequence	SEQ ID NO:
CTX-974 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENG DTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC AVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGG SDVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEW YQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGSHVPYTFGGGTKVEIKSAAAFVPVFLP AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	101
CTX-974 scFv (nt)	CAGGTGCAGCTGGTCCAAAGCGGCGCCGAGGTTAAGAA ACCAGGCGCATCCGTCAAGGTTTCATGTAAAGCAAGTGG CTTGACTATAGAAGACTACTACATGCATTGGGTACGGCA AGCCCCTGGGCAGGGGCTGGAATGGATGGGGTGGATCG ACCCGGAGAATGGTGATACAGAGTACGGACCTAAGTTC CAGGGACGAGTTACCATGACGCGAGATACATCCATCTCC ACGGCATACATGGAGCTGAGTCGACTGCGGAGCGATGA TACAGCTGTCTATTATTGTGCTGTCCACAATGCGCACTAC GGCACCTGGTTCGCTTATTGGGGACAAGGTACCCTGGTC ACAGTCAGCTCTGGGGGTGGCGGCAGTGGAGGGGGTGG TTCTGGTGGCGGGGGTTCCGATGTTGTAATGACTCAAAG CCCTCTTTCTTTGCCAGTCACTCTCGGACAACCCGCGAGC ATATCTTGCAGGTCTTCACAATCACTCCTTCACAGTAGC GGGAATACTTACTTGGAGTGGTATCAGCAGCGGCCTGGT CAGTCCCCTAGACCGCTTATATATAAGATCTCCACTAGG TTCAGTGGAGTGCCGGACCGCTTTTCAGGCTCAGGTTCC GGGACGGACTTTACATTGAAAATATCCAGGGTGGAGGC GGAGGACGTCGGAGTCTACTATTGCTTCCAAGGCTCCCA CGTCCCATACACTTTCGGTGGCGGTACAAAAGTGGAAAT AAAA	102
CTX-974 scFv (aa) (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTA YMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVT VSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISC RSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPD RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG TKVEIK	85
CTX-974 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSIST AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	98

Name	Sequence	SEQ ID NO:
CTX-974 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY QQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	56
CTX-974 Donor (nt) LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggaggggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgcctttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg cccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc tcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgctttttttctggc aagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggttttggggccgcggg cggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgc ggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcct cgcgccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgc gtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcg gcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctc agccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgattagttctc gagcttttggagtacgtcgtctttaggttgggggggagggttttatgcgatggagtttccccaca ctgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgcc ctttttgagtttggatcttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttc aggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTC CTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAG GTGCAGCTGGTCCAAAGCGGCGCCGAGGTTAAGAAACC AGGCGCATCCGTCAAGGTTTCATGTAAAGCAAGTGGCTT GACTATAGAAGACTACTACATGCATTGGGTACGGCAAGC	103

Name	Sequence	SEQ ID NO:
	CCCTGGGCAGGGGCTGGAATGGATGGGGTGGATCGACC CGGAGAATGGTGATACAGAGTACGGACCTAAGTTCCAG GGACGAGTTACCATGACGCGAGATACATCCATCTCCACG GCATACATGGAGCTGAGTCGACTGCGGAGCGATGATAC AGCTGTCTATTATTGTGCTGTCCACAATGCGCACTACGG CACCTGGTTCGCTTATTGGGGACAAGGTACCCTGGTCAC AGTCAGCTCTGGGGGTGGCGGCAGTGGAGGGGGTGGTT CTGGTGGCGGGGGTTCCGATGTTGTAATGACTCAAAGCC CTCTTTCTTTGCCAGTCACTCTCGGACAACCCGCGAGCAT ATCTTGCAGGTCTTCACAATCACTCCTTCACAGTAGCGG GAATACTTACTTGGAGTGGTATCAGCAGCGGCCTGGTCA GTCCCCTAGACCGCTTATATATAAGATCTCCACTAGGTT CAGTGGAGTGCCGGACCGCTTTTCAGGCTCAGGTTCCGG GACGGACTTTACATTGAAAATATCCAGGGTGGAGGCGG AGGACGTCGGAGTCTACTATTGCTTCCAAGGCTCCCACG TCCCATACACTTTCGGTGGCGGTACAAAAGTGGAAATAA AAAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAA ACCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGC TCCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAG GCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGG GGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGT TGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTAT TACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAG AAAGAAACTCCTGTATATATTCAAACAACCATTTATGAG ACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATAT CAGCAAGGACAGAATCAGCTGTATAACGAACTGAATTT GGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCC GGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGA AAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAA GGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGA AGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTC TACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGA TGCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATA AAATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTT GTGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACG CCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCC CCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCT GTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGC TCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCT CGGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAG AAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACAC GGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGG GCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTG CCTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTC TTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCT	

Name	Sequence	SEQ ID NO:
	CCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTC ACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAA TCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGA AGTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCA GAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAG CTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTT TAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAA AGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAG ATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAG AGGCCTGGGACAGGAGCTCAATGAGAAAGG	
CTX-975 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGGATGTAGTTA TGACCCAGAGTCCGCTCTCTTTGCCGGTGACGCTCGGCC AACCGGCGTCTATTTCTTGCAGAAGTAGTCAATCACTTC TGCACTCTAGCGGTAACACTTATTTGGAGTGGTATCTCC AACGACCAGGGCAAAGCCCCAAGCCGTTGATTTATAAG ATCTCTACAAGATTCAGCGGAGTGCCCGACAGATTTTCC GGGAGTGGGTCCGGTACTGATTTCACTTTGAAAATTTCC CGCGTCGAGGCTGAAGATGTTGGTGTCTACTACTGCTTT CAGGGGAGCCATGTTCCATATACCTTTGGAGGTGGGACT AAGGTAGAAATTAAAGGTGGGGGTGGATCAGGGGGTGG CGGCAGCGGGGGAGGGGGCTCACAAGTGCAACTTGTGC AAAGTGGGGCCGAGGTGAAAAAACCCGGTGCAAGTGTA AAGGTCTCATGCAAAGCGTCTGGTTTGACAATTGAAGAC TATTATATGCATTGGGTGAGACAGGCCCCGGGCCAAGG CTTGGAATGGATGGGATGGATAGACCCCGAAAACGGTG ACACGGAGTACGGACCTAAATTTCAAGGAAGAGTGACA ATGACACGCGATACATCTATTAACACGGCTTATATGGAA CTGAGCCGACTTCGGAGTGATGACACTGCTGTATATTAT TGCGCCGTCCACAACGCACATTATGGCACCTGGTTTGCG TACTGGGGACAGGGAACTTTGGTTACAGTATCAAGCAGT GCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCG ACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCC ACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCA TGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGG CTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTA CTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGA AAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTG CCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGC GAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATC AGCAAGGACAGAATCAGCTGTATAACGAACTGAATTTG GGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCG GGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGA AAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAA	104

Name	Sequence	SEQ ID NO:
	GGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGA AGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTC TACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGA TGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT	
CTX-975 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPA SISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTF GGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPG ASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP ENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAV YYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVPVFLPA KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	105
CTX-975 scFv (nt)	GATGTAGTTATGACCCAGAGTCCGCTCTCTTTGCCGGTG ACGCTCGGCCAACCGGCGTCTATTTCTTGCAGAAGTAGT CAATCACTTCTGCACTCTAGCGGTAACACTTATTTGGAG TGGTATCTCCAACGACCAGGGCAAAGCCCCAAGCCGTTG ATTTATAAGATCTCTACAAGATTCAGCGGAGTGCCCGAC AGATTTTCCGGGAGTGGGTCCGGTACTGATTTCACTTTG AAAATTTCCCGCGTCGAGGCTGAAGATGTTGGTGTCTAC TACTGCTTTCAGGGGAGCCATGTTCCATATACCTTTGGA GGTGGGACTAAGGTAGAAATTAAAGGTGGGGGTGGATC AGGGGGTGGCGGCAGCGGGGGAGGGGGCTCACAAGTGC AACTTGTGCAAAGTGGGGCCGAGGTGAAAAAACCCGGT GCAAGTGTAAAGGTCTCATGCAAAGCGTCTGGTTTGACA ATTGAAGACTATTATATGCATTGGGTGAGACAGGCCCCG GGCCAAGGCTTGGAATGGATGGGATGGATAGACCCCGA AAACGGTGACACGGAGTACGGACCTAAATTTCAAGGAA GAGTGACAATGACACGCGATACATCTATTAACACGGCTT ATATGGAACTGAGCCGACTTCGGAGTGATGACACTGCTG TATATTATTGCGCCGTCCACAACGCACATTATGGCACCT GGTTTGCGTACTGGGGACAGGGAACTTTGGTTACAGTAT CAAGC	106
CTX-975 scFv (aa) (linker underlined)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSG GGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMH WVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD TSINTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQ GTLVTVSS	83

Name	Sequence	SEQ ID NO:
CTX-975 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINT AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	90
CTX-975 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	88
CTX-975 Donor (nt) LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttctttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg cccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc tcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgctttttttctggc aagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggttttggggccgcggg cggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgc ggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcct cgcgccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgc gtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcg gcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctc agccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgattagttctc gagcttttggagtacgtcgtctttaggttgggggggaggggttttatgcgatggagtttccccaca ctgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgcc ctttttgagtttggatcttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttc	107

Name	Sequence	SEQ ID NO:
	aggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTC CTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGAT GTAGTTATGACCCAGAGTCCGCTCTCTTTGCCGGTGACG CTCGGCCAACCGGCGTCTATTTCTTGCAGAAGTAGTCAA TCACTTCTGCACTCTAGCGGTAACACTTATTTGGAGTGGT ATCTCCAACGACCAGGGCAAAGCCCCAAGCCGTTGATTT ATAAGATCTCTACAAGATTCAGCGGAGTGCCCGACAGAT TTTCCGGGAGTGGGTCCGGTACTGATTTCACTTTGAAAA TTTCCCGCGTCGAGGCTGAAGATGTTGGTGTCTACTACT GCTTTCAGGGGAGCCATGTTCCATATACCTTTGGAGGTG GGACTAAGGTAGAAATTAAAGGTGGGGGTGGATCAGGG GGTGGCGGCAGCGGGGGAGGGGGCTCACAAGTGCAACT TGTGCAAAGTGGGGCCGAGGTGAAAAAACCCGGTGCAA GTGTAAAGGTCTCATGCAAAGCGTCTGGTTTGACAATTG AAGACTATTATATGCATTGGGTGAGACAGGCCCCGGGCC AAGGCTTGGAATGGATGGGATGGATAGACCCCGAAAAC GGTGACACGGAGTACGGACCTAAATTTCAAGGAAGAGT GACAATGACACGCGATACATCTATTAACACGGCTTATAT GGAACTGAGCCGACTTCGGAGTGATGACACTGCTGTATA TTATTGCGCCGTCCACAACGCACATTATGGCACCTGGTT TGCGTACTGGGGACAGGGAACTTTGGTTACAGTATCAAG CAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCT CCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGG CATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGG GCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTT GGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATT ACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGA AAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTG CCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGC GAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATC AGCAAGGACAGAATCAGCTGTATAACGAACTGAATTTG GGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCG GGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAA AGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAG GATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAA GGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGAT GCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAA AATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTG TGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGC CTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCC CAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTG TTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCT CTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTC GGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGA	

Name	Sequence	SEQ ID NO:
	AACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACG GGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGG CACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGC CTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCT TCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTC CTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCA CTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAAT CACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAA GTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCAG AGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGC TGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTT AACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAA GTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGAT ACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAG GCCTGGGACAGGAGCTCAATGAGAAAGG	
CTX-976 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTTCAAC TGGTTCAGAGTGGAGCAGAGGTAAAAAAGCCCGGAGCG TCCGTCAAAGTGTCATGTAAAGCCTCTGGACTTACTATC GAAGACTACTACATGCACTGGGTGAGGCAGGCGCCTGG CCAAGGTCTCGAGTGGATGGGTTGGATTGACCCTGAAA ATGGAGATACAGAATACGGCCCTAAGTTTCAAGGGCGA GTAACTATGACTCGAGATACGTCAATTAATACGGCATAC ATGGAGTTGTCTCGGCTCCGATCTGATGACACTGCAGTT TACTATTGTGCCGTCCACAATGCTCATTACGGGACATGG TTCGCTTACTGGGGGCAAGGGACACTCGTAACGGTTAGC TCTGGGGGAGGAGGGTCTGGTGGAGGGGGCTCAGGAGG GGGTGGTAGCGACGTAGTAATGACCCAGTCACCTCTGTC TTTGCCGGTCACGTTGGGCCAGCCTGCATCCATATCCTG CAGATCCAGCCAGAGCCTCCTGCACAGTAGTGGCAACA CGTATTTGGAATGGTACCTGCAGAGGCCGGGTCAAAGTC CAAAACCGCTGATCTATAAGATATCTACGCGATTTTCAG GGGTGCCGGACCGATTTAGCGGATCAGGAAGTGGAACC GACTTTACGCTCAAGATCAGCCGGGTTGAAGCCGAAGA TGTCGGCGTTTACTACTGTTTCCAAGGAAGCCACGTACC CTATACGTTTGGTGGCGGCACGAAGGTCGAGATAAAGA GTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACC GACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCC CACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGC ATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGG GCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTT GGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATT ACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAG AAAGAAACTCCTGTATATATTCAAACAACCATTTATGAG ACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCT GCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTG	108

Name	Sequence	SEQ ID NO:
	CGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATAT CAGCAAGGACAGAATCAGCTGTATAACGAACTGAATTT GGGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCC GGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAG AAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAGA AGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTATG AAGGGCGAACGACGACGGGGAAAAGGTCACGATGGCCT CTACCAAGGGTTGAGTACGGCAACCAAAGATACGTACG ATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT	
CTX-976 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENG DTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYC AVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGG SDVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEW YLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGSHVPYTFGGGTKVEIKSAAAFVPVFLP AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	109
CTX-976 scFv (nt)	CAGGTTCAACTGGTTCAGAGTGGAGCAGAGGTAAAAAA GCCCGGAGCGTCCGTCAAAGTGTCATGTAAAGCCTCTGG ACTTACTATCGAAGACTACTACATGCACTGGGTGAGGCA GGCGCCTGGCCAAGGTCTCGAGTGGATGGGTTGGATTGA CCCTGAAAATGGAGATACAGAATACGGCCCTAAGTTTCA AGGGCGAGTAACTATGACTCGAGATACGTCAATTAATAC GGCATACATGGAGTTGTCTCGGCTCCGATCTGATGACAC TGCAGTTTACTATTGTGCCGTCCACAATGCTCATTACGG GACATGGTTCGCTTACTGGGGGCAAGGGACACTCGTAAC GGTTAGCTCTGGGGGAGGAGGGTCTGGTGGAGGGGGCT CAGGAGGGGGTGGTAGCGACGTAGTAATGACCCAGTCA CCTCTGTCTTTGCCGGTCACGTTGGGCCAGCCTGCATCCA TATCCTGCAGATCCAGCCAGAGCCTCCTGCACAGTAGTG GCAACACGTATTTGGAATGGTACCTGCAGAGGCCGGGTC AAAGTCCAAAACCGCTGATCTATAAGATATCTACGCGAT TTTCAGGGGTGCCGGACCGATTTAGCGGATCAGGAAGTG GAACCGACTTTACGCTCAAGATCAGCCGGGTTGAAGCCG AAGATGTCGGCGTTTACTACTGTTTCCAAGGAAGCCACG TACCCTATACGTTTGGTGGCGGCACGAAGGTCGAGATAA AG	110
CTX-976 scFv (aa) (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINT AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASIS	86

Name	Sequence	SEQ ID NO:
	CRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVP DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGG GTKVEIK	
CTX-976 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINT AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	90
CTX-976 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	88
CTX-976 Donor (nt) LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgcctttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg cccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc tcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgcttttttctggc aagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggttttggggccgcggg cggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgc ggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcct cgcgccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgc gtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcg	111

Name	Sequence	SEQ ID NO:
	gcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctc agccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgattagttctc gagcttttggagtacgtcgtctttaggttgggggggagggggttttatgcgatggagtttccccaca ctgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgcc ctttttgagtttggatcttggttcattctcaagcctcagacagtggttcaaagtttttttcttccatttc aggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTC CTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGCAG GTTCAACTGGTTCAGAGTGGAGCAGAGGTAAAAAAGCC CGGAGCGTCCGTCAAAGTGTCATGTAAAGCCTCTGGACT TACTATCGAAGACTACTACATGCACTGGGTGAGGCAGGC GCCTGGCCAAGGTCTCGAGTGGATGGGTTGGATTGACCC TGAAAATGGAGATACAGAATACGGCCCTAAGTTTCAAG GGCGAGTAACTATGACTCGAGATACGTCAATTAATACGG CATACATGGAGTTGTCTCGGCTCCGATCTGATGACACTG CAGTTTACTATTGTGCCGTCCACAATGCTCATTACGGGA CATGGTTCGCTTACTGGGGGCAAGGGACACTCGTAACGG TTAGCTCTGGGGGAGGAGGGTCTGGTGGAGGGGGCTCA GGAGGGGGTGGTAGCGACGTAGTAATGACCCAGTCACC TCTGTCTTTGCCGGTCACGTTGGGCCAGCCTGCATCCATA TCCTGCAGATCCAGCCAGAGCCTCCTGCACAGTAGTGGC AACACGTATTTGGAATGGTACCTGCAGAGGCCGGGTCAA AGTCCAAAACCGCTGATCTATAAGATATCTACGCGATTT TCAGGGGTGCCGGACCGATTTAGCGGATCAGGAAGTGG AACCGACTTTACGCTCAAGATCAGCCGGGTTGAAGCCGA AGATGTCGGCGTTTACTACTGTTTCCAAGGAAGCCACGT ACCCTATACGTTTGGTGGCGGCACGAAGGTCGAGATAAA GAGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCT CCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGG CATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGG GCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTT GGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATT ACTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGA AAGAAACTCCTGTATATATTCAAACAACCATTTATGAGA CCAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTG CCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGC GAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATC AGCAAGGACAGAATCAGCTGTATAACGAACTGAATTTG GGACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCG GGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAA AGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAG GATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAA GGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGAT GCACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAA AATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTG TGTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGC	

Name	Sequence	SEQ ID NO:
	CTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCC CAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTG TTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCT CTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTC GGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGA AACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACG GGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGG CACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGC CTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCT TCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTC CTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCA CTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAAT CACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAA GTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCAG AGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGC TGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTT AACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAA GTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGAT ACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAG GCCTGGGACAGGAGCTCAATGAGAAAGG	
CTX-977 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGGACGTTGTGA TGACGCAGTCTCCTCTGAGCCTGCCAGTTACGTTGGGGC AACCCGCATCAATATCTTGTAGGTCCAGTCAGAGCCTGC TTCACAGCTCTGGCAACACTTACTTGGAATGGTACCTCC AGAGACCTGGACAGAGTCCCAAGCCATTGATTTACAAG ATTTCAACGCGATTTAGTGGAGTGCCCGATCGATTCTCT GGGAGTGGCTCTGGGACTGATTTCACACTTAAAATAAGT AGGGTGGAGGCTGAAGATGTGGGTGTATATTATTGTTTT CAAGGGTCCCATGTCCCTTACACTTTCGGCGGCGGCACC AAAGTTGAGATCAAAGGTGGTGGTGGGTCCGGCGGTGG AGGCAGTGGGGGTGGCGGGTCACAAGTTCAACTTGTCC AGTCAGGGGCTGAAGTAAAAAAGCCTGGTGCATCAGTT AAAGTTTCATGTAAGGCTTCCGGCCTTACCATTGAAGAT TACTATATGCACTGGGTTAGACAAGCTCCTGGACAAGGT CTGGAGTGGATGGGCTGGATAGACCCCGAGAATGGTGA CACAGAATACGGGCCTAAGTTCCAGGGTAGGGTAACAA TGACGCGGGATACATCCATTTCCACAGCTTACATGGAAC TGAGTAGACTCAGATCTGACGACACTGCTGTCTACTATT GTGCCGTCCATAACGCGCATTATGGCACTTGGTTCGCAT ATTGGGGGCAAGGCACTCTTGTTACAGTGTCCTCAAGTG CTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAACCGA CCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTCCCA CCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGCAT GCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGGC TTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTGG	112

Name	Sequence	SEQ ID NO:
	CGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTAC TTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAA AGAAACTCCTGTATATATTCAAACAACCATTTATGAGAC CAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGC CGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCG AGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCA GCAAGGACAGAATCAGCTGTATAACGAACTGAATTTGG GACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGG GGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAA AGAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAG GATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAA GGGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCT ACCAAGGGTTGAGTACGGCAACCAAAGATACGTACGAT GCACTGCATATGCAGGCCCTGCCTCCCAGATAAT	
CTX-977 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPDVVMTQSPLSLPVTLGQPA SISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSG VPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTF GGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPG ASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDP ENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAV YYCAVHNAHYGTWFAYWGQGTLVTVSSSAAAFVPVFLPA KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRKK LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR	113
CTX-977 scFv (nt)	GACGTTGTGATGACGCAGTCTCCTCTGAGCCTGCCAGTT ACGTTGGGGCAACCCGCATCAATATCTTGTAGGTCCAGT CAGAGCCTGCTTCACAGCTCTGGCAACACTTACTTGGAA TGGTACCTCCAGAGACCTGGACAGAGTCCCAAGCCATTG ATTTACAAGATTTCAACGCGATTTAGTGGAGTGCCCGAT CGATTCTCTGGGAGTGGCTCTGGGACTGATTTCACACTT AAAATAAGTAGGGTGGAGGCTGAAGATGTGGGTGTATA TTATTGTTTTCAAGGGTCCCATGTCCCTTACACTTTCGGC GGCGGCACCAAAGTTGAGATCAAAGGTGGTGGTGGGTC CGGCGGTGGAGGCAGTGGGGGTGGCGGGTCACAAGTTC AACTTGTCCAGTCAGGGGCTGAAGTAAAAAAGCCTGGT GCATCAGTTAAAGTTTCATGTAAGGCTTCCGGCCTTACC ATTGAAGATTACTATATGCACTGGGTTAGACAAGCTCCT GGACAAGGTCTGGAGTGGATGGGCTGGATAGACCCCGA GAATGGTGACACAGAATACGGGCCTAAGTTCCAGGGTA GGGTAACAATGACGCGGGATACATCCATTTCCACAGCTT ACATGGAACTGAGTAGACTCAGATCTGACGACACTGCTG TCTACTATTGTGCCGTCCATAACGCGCATTATGGCACTTG GTTCGCATATTGGGGGCAAGGCACTCTTGTTACAGTGTC CTCA	114

Name	Sequence	SEQ ID NO:
CTX-977 scFv (aa) (linker underlined)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIKGGGGSGGGGSG GGGSQVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMH WVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQ GTLVTVSS	84
CTX-977 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSIST AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	98
CTX-977 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	88
CTX-977 Donor (nt) LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgcctttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg cccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc tcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgcttttttctggc aagatagtcttgtaaatgcgggccaagatctgcacactggtatttcggttttggggccgcggg cggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcctgcgagcgc	115

Name	Sequence	SEQ ID NO:
	ggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcct cgcgccgccgtgtatcgccccgccctgggcggcaaggctggcccggtcggcaccagttgc gtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcg gcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctc agccgtcgcttcatgtgactccacggagtaccgggcgccgtccaggcacctcgattagttctc gagcttttggagtacgtcgtctttaggttgggggggagggttttatgcgatggagtttccccaca ctgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgcc ctttttgagtttggatcttggttcattctcaagcctcagacagtggttcaaagttttttttcttccatttc aggtgtcgtgaCCACCATGGCGCTTCCGGTGACAGCACTGCTC CTCCCCTTGGCGCTGTTGCTCCACGCAGCAAGGCCGGAC GTTGTGATGACGCAGTCTCCTCTGAGCCTGCCAGTTACG TTGGGGCAACCCGCATCAATATCTTGTAGGTCCAGTCAG AGCCTGCTTCACAGCTCTGGCAACACTTACTTGGAATGG TACCTCCAGAGACCTGGACAGAGTCCCAAGCCATTGATT TACAAGATTTCAACGCGATTTAGTGGAGTGCCCGATCGA TTCTCTGGGAGTGGCTCTGGGACTGATTTCACACTTAAA ATAAGTAGGGTGGAGGCTGAAGATGTGGGTGTATATTAT TGTTTTCAAGGGTCCCATGTCCCTTACACTTTCGGCGGCG GCACCAAAGTTGAGATCAAAGGTGGTGGTGGGTCCGGC GGTGGAGGCAGTGGGGGTGGCGGGTCACAAGTTCAACT TGTCCAGTCAGGGGCTGAAGTAAAAAAGCCTGGTGCATC AGTTAAAGTTTCATGTAAGGCTTCCGGCCTTACCATTGA AGATTACTATATGCACTGGGTTAGACAAGCTCCTGGACA AGGTCTGGAGTGGATGGGCTGGATAGACCCCGAGAATG GTGACACAGAATACGGGCCTAAGTTCCAGGGTAGGGTA ACAATGACGCGGGATACATCCATTTCCACAGCTTACATG GAACTGAGTAGACTCAGATCTGACGACACTGCTGTCTAC TATTGTGCCGTCCATAACGCGCATTATGGCACTTGGTTC GCATATTGGGGGCAAGGCACTCTTGTTACAGTGTCCTCA AGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAAC CGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCTC CCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAGGC ATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAGGGG CTTGGACTTCGCTTGTGATATTTACATTTGGGCTCCGTTG GCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTTATTA CTTTGTATTGTAATCACAGGAATCGCAAACGGGGCAGAA AGAAACTCCTGTATATATTCAAACAACCATTTATGAGAC CAGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGC CGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGCG AGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCATATCA GCAAGGACAGAATCAGCTGTATAACGAACTGAATTTGG GACGCCGCGAGGAGTATGACGTGCTTGATAAACGCCGG GGGAGAGACCCGGAAATGGGGGGTAAACCCCGAAGAAA GAATCCCCAAGAAGGACTCTACAATGAACTCCAGAAGG ATAAGATGGCGGAGGCCTACTCAGAAATAGGTATGAAG GGCGAACGACGACGGGGAAAAGGTCACGATGGCCTCTA CCAAGGGTTGAGTACGGCAACCAAAGATACGTACGATG	

Name	Sequence	SEQ ID NO:
	CACTGCATATGCAGGCCCTGCCTCCCAGATAATAATAAA ATCGCTATCCATCGAAGATGGATGTGTGTTGGTTTTTTGT GTGTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCC TTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCC AGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGT TTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTC TGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTC GGCCTTATCCATTGCCACCAAAACCCTCTTTTTACTAAGA AACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACG GGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGG CACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTCCTGC CTGCCTGCCTTTGCTCAGACTGTTTGCCCCTTACTGCTCT TCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTC CTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCA CTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAAT CACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAA GTGGAGGAATTAAAAAGTCAGATGAGGGGTGTGCCCAG AGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGC TGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTT AACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAA GTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGAT ACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAG GCCTGGGACAGGAGCTCAATGAGAAAGG	
CTX-978 CAR 41BB co-stim (nt)	CCACCATGGCGCTTCCGGTGACAGCACTGCTCCTCCCCT TGGCGCTGTTGCTCCACGCAGCAAGGCCGCAGGTACAA CTCGTTCAGAGCGGTGCAGAGGTTAAGAAACCGGGCGC CAGTGTCAAAGTATCATGCAAGGCGAGTGGTCTGACCAT CGAAGATTATTATATGCATTGGGTGAGACAAGCACCGG GGCAGGGGCTCGAATGGATGGGTTGGATCGACCCCGAA AATGGTGATACGGAGTATGGCCCGAAATTTCAGGGTCG GGTCACGATGACCCGCGATACAAGCATCAGTACTGCAT ACATGGAGCTCTCTCGCTTGCGGAGTGATGATACCGCCG TTTATTATTGCGCGGTTCACAACGCTCATTATGGCACTTG GTTCGCGTATTGGGGCCAAGGAACACTGGTTACAGTGA GCAGTGGAGGGGGTGGCTCTGGTGGCGGCGGGAGCGGC GGAGGGGGCAGTGATGTTGTGATGACACAGTCACCCCT GAGTCTCCCGGTCACTCTTGGGCAACCAGCCAGCATAAG CTGTCGCAGTTCTCAGAGCTTGCTCCATAGCTCCGGGAA TACCTACCTCGAATGGTATCTCCAAAGACCCGGTCAATC TCCAAAGCCTTTGATTTACAAGATTAGTACACGATTTAG TGGGGTCCCAGATAGATTTTCAGGTAGTGGATCTGGTAC AGATTTCACATTGAAAATATCACGCGTCGAGGCGGAGG ATGTCGGGGTCTACTATTGCTTTCAAGGTAGTCACGTGC CCTACACGTTTGGTGGCGGTACGAAGGTCGAAATCAAG AGTGCTGCTGCCTTTGTCCCGGTATTTCTCCCAGCCAAA CCGACCACGACTCCCGCCCCGCGCCCTCCGACACCCGCT	116

Name	Sequence	SEQ ID NO:
	CCCACCATCGCCTCTCAACCTCTTAGTCTTCGCCCCGAG GCATGCCGACCCGCCGCCGGGGGTGCTGTTCATACGAG GGGCTTGGACTTCGCTTGTGATATTTACATTTGGGCTCC GTTGGCGGGTACGTGCGGCGTCCTTTTGTTGTCACTCGTT ATTACTTTGTATTGTAATCACAGGAATCGCAAACGGGGC AGAAAGAAACTCCTGTATATATTCAAACAACCATTTATG AGACCAGTACAAACTACTCAAGAGGAAGATGGCTGTAG CTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAAC TGCGAGTGAAGTTTTCCCGAAGCGCAGACGCTCCGGCAT ATCAGCAAGGACAGAATCAGCTGTATAACGAACTGAAT TTGGGACGCCGCGAGGAGTATGACGTGCTTGATAAACG CCGGGGGAGAGACCCGGAAATGGGGGGTAAACCCCGAA GAAAGAATCCCCAAGAAGGACTCTACAATGAACTCCAG AAGGATAAGATGGCGGAGGCCTACTCAGAAATAGGTAT GAAGGGCGAACGACGACGGGGAAAAGGTCACGATGGC CTCTACCAAGGGTTGAGTACGGCAACCAAAGATACGTA CGATGCACTGCATATGCAGGCCCTGCCTCCCAGATAAT	
CTX-978 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENG DTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC AVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGG SDVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEW YLQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGSHVPYTFGGGTKVEIKSAAAFVPVFLP AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	117
CTX-978 scFv (nt)	CAGGTACAACTCGTTCAGAGCGGTGCAGAGGTTAAGAA ACCGGGCGCCAGTGTCAAAGTATCATGCAAGGCGAGTG GTCTGACCATCGAAGATTATTATATGCATTGGGTGAGAC AAGCACCGGGGCAGGGGCTCGAATGGATGGGTTGGATC GACCCCGAAAATGGTGATACGGAGTATGGCCCGAAATTT CAGGGTCGGGTCACGATGACCCGCGATACAAGCATCAG TACTGCATACATGGAGCTCTCTCGCTTGCGGAGTGATGA TACCGCCGTTTATTATTGCGCGGTTCACAACGCTCATTAT GGCACTTGGTTCGCGTATTGGGGCCAAGGAACACTGGTT ACAGTGAGCAGTGGAGGGGGTGGCTCTGGTGGCGGCGG GAGCGGCGGAGGGGGCAGTGATGTTGTGATGACACAGT CACCCCTGAGTCTCCCGGTCACTCTTGGGCAACCAGCCA GCATAAGCTGTCGCAGTTCTCAGAGCTTGCTCCATAGCT CCGGGAATACCTACCTCGAATGGTATCTCCAAAGACCCG GTCAATCTCCAAAGCCTTTGATTTACAAGATTAGTACAC GATTTAGTGGGGTCCCAGATAGATTTTCAGGTAGTGGAT CTGGTACAGATTTCACATTGAAAATATCACGCGTCGAGG	118

Name	Sequence	SEQ ID NO:
	CGGAGGATGTCGGGGTCTACTATTGCTTTCAAGGTAGTC ACGTGCCCTACACGTTTGGTGGCGGTACGAAGGTCGAAA TCAAG	
CTX-978 scFv (aa) (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSISTA YMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVT VSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASISC RSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPD RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGG TKVEIK	87
CTX-978 scFv VH (aa)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSIST AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSS	98
CTX-978 scFv VL (aa)	DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWY LQRPGQSPKPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCFQGSHVPYTFGGGTKVEIK	88
CTX-978 Donor (nt) LHA to RHA	GAGATGTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTAT ATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGT TCTGATTTATAGTTCAAAACCTCTATCAATGAGAGAGCA ATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCA ACATACCATAAACCTCCCATTCTGCTAATGCCCAGCCTA AGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTT GCTTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGC CAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATT TCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGAA CGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAG CTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGC TGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGAC CGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCAT CACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAG GGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCC ACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTG AGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAG GATTCTGATGTGTATATCACAGACAAAACTGTGCTAGAC ATGAGGTCTATGGACTTCAggctccggtgcccgtcagtgggcagagcgca catcgcccacagtccccgagaagttgggggggagggtcggcaattgaaccggtgcctaga gaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagg gtgggggagaaccgtatataagtgcagtagtcgccgtgaacgttcttttcgcaacgggtttgc cgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacgggttatgg cccttgcgtgccttgaattacttccactggctgcagtacgtgattcttgatcccgagcttcgggtt ggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagtt gaggcctggcctgggcgctggggccgccgcgtgcgaatctggtggcaccttcgcgcctgtc	119

Name	Sequence	SEQ ID NO:
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Name	Sequence	SEQ ID NO:
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CTX-979 CAR 41BB co-stim (aa)	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENG DTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYC AVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGG SDVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEW YQQRPGQSPRPLIYKISTRFSGVPDRFSGSGSGTDFTLKISRV EAEDVGVYYCFQGSHVPYTFGGGTKVEIKSAAAFVPVFLP AKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKF SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALHMQALPPR	68
CTX-979b CAR CD28 co-stim (aa)	MALPVTALLLPLALLLHAARPQVQLVQSGAEVKKPGASV KVSCKASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENG DTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYC AVHNAHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGG SDVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEW	66

Name	Sequence	SEQ ID NO:
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CTX-979 and CTX-979b scFv (aa) (linker underlined)	QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQ APGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRDTSINT AYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLV TVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTLGQPASIS CRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVP DRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGG GTKVEIK	70
CTX-979 and CTX-979b scFv VH (aa) CDRs - in bold	QVQLVQSGAEVKKPGASVKVSCKASGLTIE**DYYMH**WVRQAPG QGLEWMG**WIDPENGDTEYGPKFQG**RVTMTRDTSINTAYMELS RLRSDDTAVYYCAV**HNAHYGTWFAY**WGQGTLVTVSS	55
CTX-979 and CTX-979b scFv VL (aa) CDRs - in bold	DVVMTQSPLSLPVTLGQPASISC**RSSQSLLHSSGNTYLE**WYQQR PGQSPRPLIY**KISTRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGV YYC**FQGSHVPYT**FGGGTKVEIK	56

[00259] All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

[00260] The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

[00261] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

[00262] In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of”

shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

[00263] The terms "about" and "substantially" preceding a numerical value mean ±10% of the recited numerical value.

[00264] Where a range of values is provided, each value between the upper and lower ends of the range are specifically contemplated and described herein.

What is claimed is:

CLAIMS

1. An engineered T cell comprising a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an ectodomain that binds specifically to LIV1.

2. The engineered T cell of claim 1 further comprising a disrupted T cell receptor alpha chain constant region (*TRAC*) gene.

3. The engineered T cell of claim 1 or 2 further comprising a disrupted beta-2-microglobulin (*β2M*) gene.

4. The engineered T cell of any one of claims 1-3, wherein the ectodomain of the CAR comprises an anti-LIV1 antibody.

5. The engineered T cell of claim 4, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

6. The engineered T cell of claim 5, wherein the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56 or (ii) a VH set forth as SEQ ID NO: 90 and a VL set forth as SEQ ID NO: 88.

7. The engineered T cell of claim 6, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

8. The engineered T cell of claim 6, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 54, 70, 83, or 86.

9. The engineered T cell of any one of claims 1-8, wherein the CAR further comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

10. The engineered T cell of claim 9, wherein the CAR further comprises a CD3ζ cytoplasmic signaling domain.

11. The engineered T cell of any one of claims 1-10, wherein the CAR is encoded by the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108 or a nucleotide sequence comprising a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108.

12. The engineered T cell of any one of claims 1-11, wherein the nucleic acid encoding the CAR is inserted into the disrupted *TRAC* gene.

13. The engineered T cell of any one of claims 2-12, wherein the disrupted *TRAC* gene comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111, and/or the nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108.

14. The engineered T cell of any one of claims 4-13, wherein the disrupted *β2M* gene comprises at least one nucleotide sequence selected from any one of SEQ ID NOs: 9-14.

15. An engineered T cell comprising:

(i) a disrupted *TRAC* gene;

(ii) a disrupted *β2M* gene; and

(iii) a nucleic acid encoding a CAR comprising an anti-LIV1 antigen-binding fragment.

16. The engineered T cell of claim 15, wherein the CAR comprises (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain.

17. The engineered T cell of claim 15 or 16, wherein the disrupted *TRAC* gene comprises the nucleic acid encoding the CAR.

18. An engineered T cell comprising:

(i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain; and

(ii) a disrupted *β2M* gene.

19. An engineered T cell comprising:

(i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and

(ii) a disrupted *β2M* gene.

20. An engineered T cell comprising:

(i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108 and/or encodes a CAR comprising an amino acid sequence of any one of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and

(ii) a disrupted *β2M* gene.

21. The engineered T cell of any one of claims 1-20, wherein the T cell is a human T cell.

22. A population of cells comprising the engineered T cell of any one of claims 1-21, wherein at least 15% or at least 50% of engineered T cells of the population express the CAR.

23. The population of claim 22, wherein at least 30% of engineered T cells of the population express the CAR.

24. The population of claim 22, wherein at least 70% of engineered T cells of the population express the CAR.

25. The population of claim 22, wherein at least 25% of engineered T cells of the population express the CAR following at least 7 days or at least 14 days of *in vitro* proliferation.

26. The population of any one of claims 22-25, wherein at least 50% of engineered T cells of the population do not express a detectable level of T cell receptor (TCR) protein.

27. The population of claim 26, wherein at least 90% of engineered T cells of the population do not express a detectable level of TCR protein.

28. The population of any one of claims 22-27, wherein at least 50% of engineered T cells of the population do not express a detectable level of β2M protein.

29. The population of claim 28, wherein at least 70% of engineered T cells of the population do not express a detectable level of β2M protein.

30. The population of any one of claims 22-29, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 10%, at least 25%, or at least 50% of the cancer cells of the population.

31. The population of claim 30, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells that express LIV1, induce cell lysis of at least 70%, at least 80%, or at least 90% of the population of cancer cells.

32. The population of claim 30 or 31, wherein engineered T cells of the population, when co-cultured *in vitro* with a population of cancer cells, secrete IFNγ.

33. The population of any one of claims 30-32, wherein the ratio of engineered T cells to cancer cells is 1:1 to 2:1.

34. The population of any one of claims 30-33, wherein the cancer cells comprise sarcoma cells.

35. The population of any one of claims 30-33, wherein the cancer cells comprise breast cancer cells.

36. The population of any one of claims 22-35, when administered *in vivo* to a subject, does not induce toxicity in the subject.

37. A population of cells comprising engineered T cells, wherein the engineered T cells comprise:

(i) a disrupted *TRAC* gene;

(ii) a disrupted *β2M* gene; and

(iii) a nucleic acid encoding a CAR comprising an anti-LIV1 antigen-binding fragment.

38. The population of cells of claim 37, wherein the CAR comprises (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain.

39. The population of cells of claim 37 or 38, wherein the disrupted *TRAC* gene comprises the nucleic acid encoding the CAR.

40. A population of cells comprising engineered T cells, wherein the engineered T cells comprise:

(i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR comprising (a) an ectodomain that comprises an anti-LIV1 antigen-binding fragment, (b) a CD8 transmembrane domain, and (c) an endodomain that comprises a 41BB co-stimulatory domain and a CD3ζ cytoplasmic signaling domain; and

(ii) a disrupted *β2M* gene.

41. A population of cells comprising engineered T cells, wherein the engineered T cells comprise:

(i) a disrupted *TRAC* gene, wherein the disrupted *TRAC* gene comprises a nucleic acid encoding a CAR, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108 and/or encodes the CAR of SEQ ID NOs: 50, 52, 105, 109, 68 or 66; and

(ii) a disrupted *β2M* gene.

42. A method comprising administering the population of engineered T cells any one of claims 22-41 to a subject.

43. The method of claim 42, wherein the subject is a human subject.

44. The method of claim 43, wherein the subject has a cancer.

45. The method of claim 44, wherein the cancer is selected from the group consisting of: breast cancer, *e.g.*, estrogen receptor-positive breast cancer, prostate cancer, squamous tumors, *e.g.*, of the skin, bladder, lung, cervix, endometrium, head neck, and biliary tract, and/or neuronal tumors.

46. The method of claim 44 or 45, wherein the cancer comprises cancer cells expressing LIV1.

47. A method for producing an engineered T cell, the method comprising

(a) delivering to a T cell

(i) a RNA-guided nuclease,

(ii) a gRNA targeting a *TRAC* gene, and

(iii) a vector comprising a donor template that comprises a nucleic acid encoding a CAR that comprise an ectodomain that binds specifically to LIV1; and

(b) producing an engineered T cell having a disrupted *TRAC* gene and expressing the CAR.

48. The method of claim 47, wherein the gRNA targeting the *TRAC* gene comprises the nucleotide sequence of SEQ ID NO: 18 or 19, or targets the nucleotide sequence of SEQ ID NO: 40.

49. The method of claim 47 or 48 further comprising delivering to the T cell a gRNA targeting the *β2M* gene.

50. The method of claim 49, wherein the gRNA targeting the *β2M* gene comprises the nucleotide sequence of SEQ ID NO: 20 or 21, or targets the nucleotide sequence of SEQ ID NO: 41.

51. The method of any one of claims 47-50, wherein the ectodomain of the CAR comprises an anti-LIV1 antibody.

52. The method of claim 51, wherein the anti-LIV1 antibody is an anti-LIV1 single-chain variable fragment (scFv).

53. The method of claim 52, wherein the anti-LIV1 scFv comprises the same heavy chain variable domain (VH) complementarity determining regions (CDRs) and the same light chain variable domain (VL) CDRs as a reference antibody, wherein the reference antibody comprises (i) a VH set forth as SEQ ID NO: 55 and a VL set forth as SEQ ID NO: 56, or (ii) a VH set forth as SEQ ID NO: 90 and a VL set forth as SEQ ID NO: 88.

54. The method of claim 53, wherein the anti-LIV1 scFv comprises the same VH and VL chains as the reference antibody.

55. The method of claim 53, wherein the anti-LIV1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 54, 83, 86 or 70.

56. The method of any one of claims 47-56, wherein the CAR further comprises a CD28 co-stimulatory domain or a 41BB co-stimulatory domain.

57. The method of claim 56, wherein the CAR further comprises a CD3ζ cytoplasmic signaling domain.

58. The method of any one of claims 47-57, wherein the CAR is encoded by a nucleotide sequence of any one of SEQ ID NOs: 49, 51, 104, or 108 or a nucleotide sequence comprising a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 49, 51, 104, or 108.

59. The method of any one of claims 47-58, wherein the nucleic acid encoding the CAR is flanked by left and right homology arms to the *TRAC* gene.

60. The method of any one of claims 47-59, wherein the donor template comprises the nucleotide sequence of any one of SEQ ID NOs: 63, 64, 107, or 111.

61. The method of any one of claims 47-60, wherein the RNA-guided nuclease is a Cas9 nuclease, optionally a *S. pyogenes* Cas9 nuclease.

62. An engineered T cell produced by the method of any one of claims 47-61.

63. A population of cells comprising the engineered T cell of claim 62.

64. A method of treating cancer in a subject, comprising administering to the subject the population of cells of any one of claims 22-41 or 63.

65. The method of claim 64, wherein the cancer is selected from the group consisting of: pancreatic cancer, gastric cancer, ovarian cancer, uterine cancer, breast cancer, prostate cancer, testicular cancer, thyroid cancer, nasopharyngeal cancer, non-small cell lung (NSCLC), glioblastoma, neuronal, soft tissue sarcomas, leukemia, lymphoma, melanoma, colon cancer, colon adenocarcinoma, brain glioblastoma, hepatocellular carcinoma, liver hepatocholangiocarcinoma, osteosarcoma, gastric cancer, esophagus squamous cell carcinoma, advanced stage pancreas cancer, lung adenocarcinoma, lung squamous cell carcinoma, lung small cell cancer, renal carcinoma, and intrahepatic biliary cancer.

66. The method of claim 64 or 65, wherein the cancer comprises cancer cells expressing LIV1.

FIG. 1

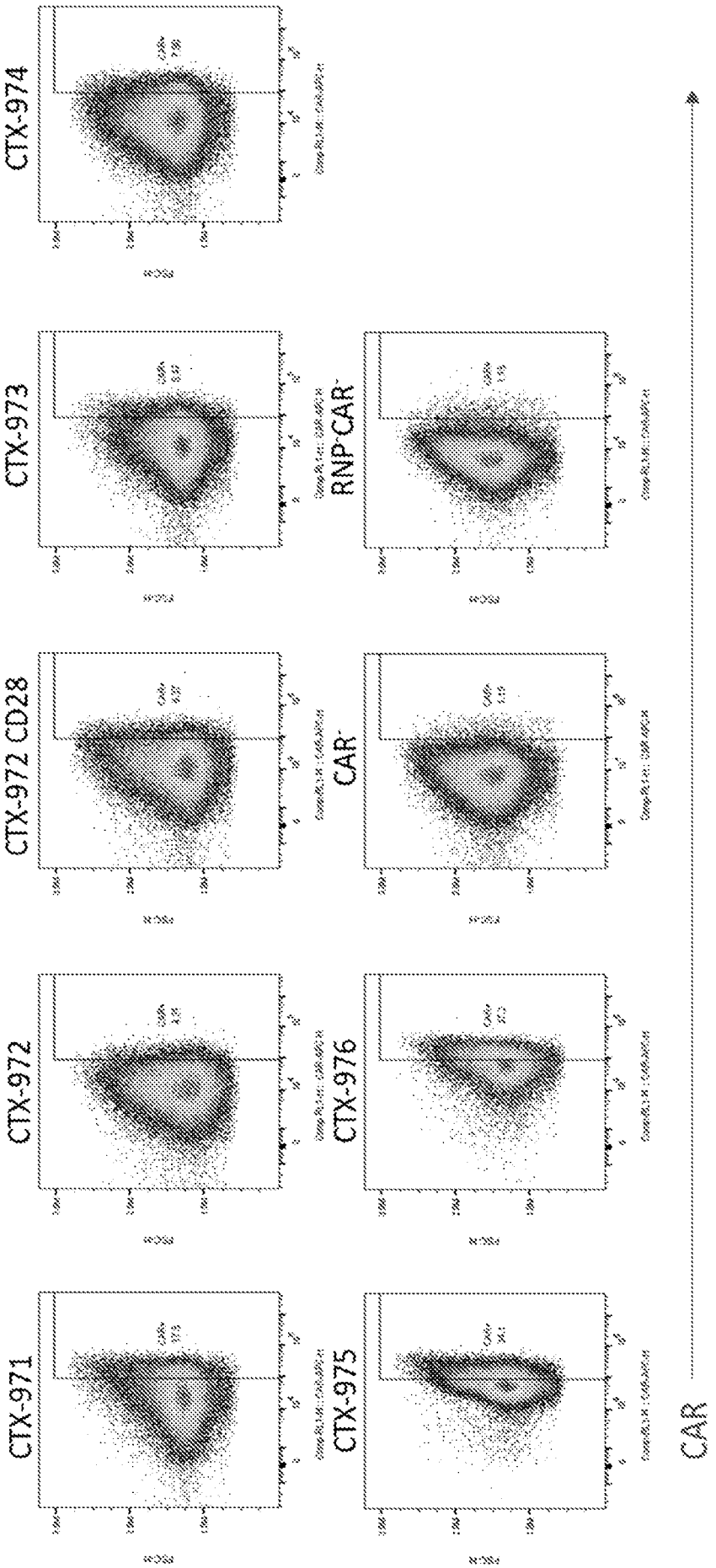
CTX-974
CTX-973
RNP-CAR-
CTX-972 CD28
CAR-
CTX-972
CTX-976
CTX-971
CTX-975
CAR

FIG. 2A

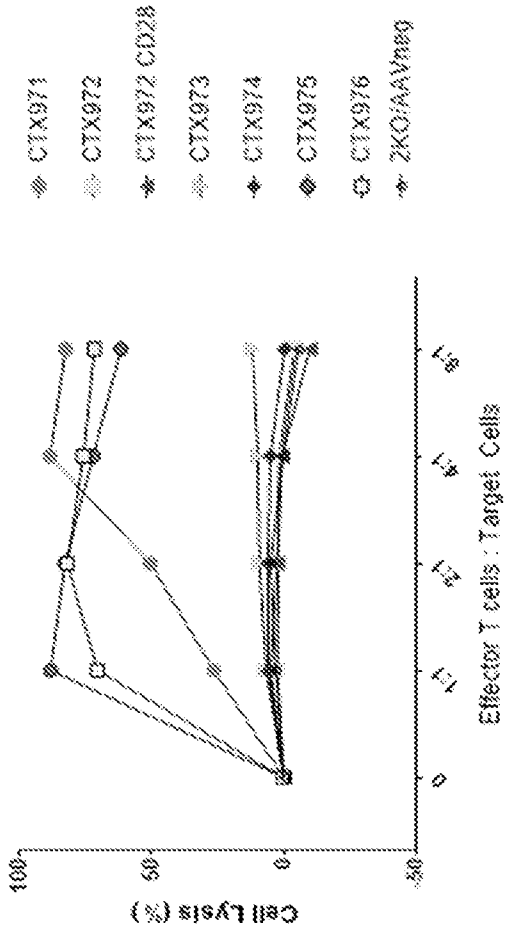
24hrs Cell Kill with anti-LIV1a CAR-T Cells & A498 Target Cells
CTX971
CTX972
CTX972 CD28
CTX973
CTX974
CTX975
CTX976
2KO/AAVneg
Cell Lysis (%)
100
50
0
-50
1/9
1/3
1/1
0
Effector T cells : Target Cells

FIG. 2B

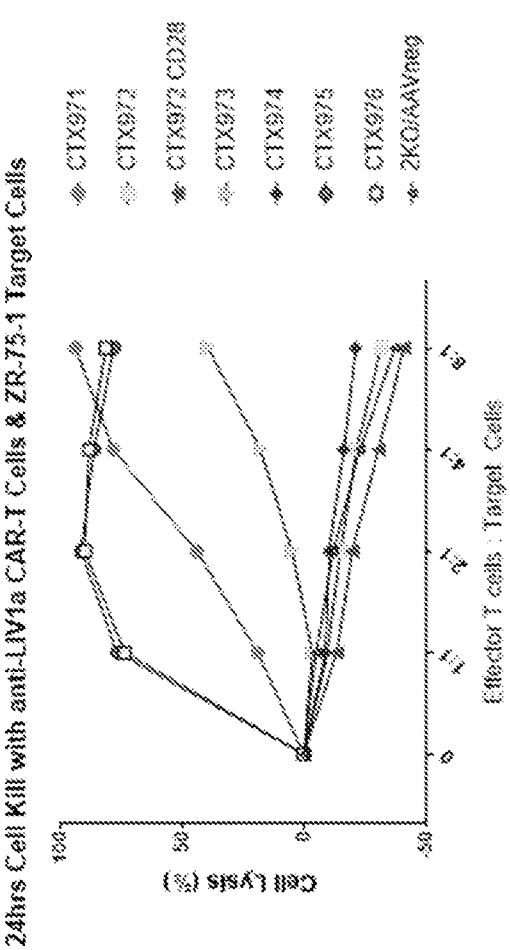
24hrs Cell Kill with anti-LIV1a CAR-T Cells & ZR-75-1 Target Cells
CTX971
CTX972
CTX972 CD28
CTX973
CTX974
CTX975
CTX976
2KO/AAVneg
Cell Lysis (%)
100
50
0
-50
1/9
1/3
1/1
0
Effector T cells : Target Cells

FIG. 3A

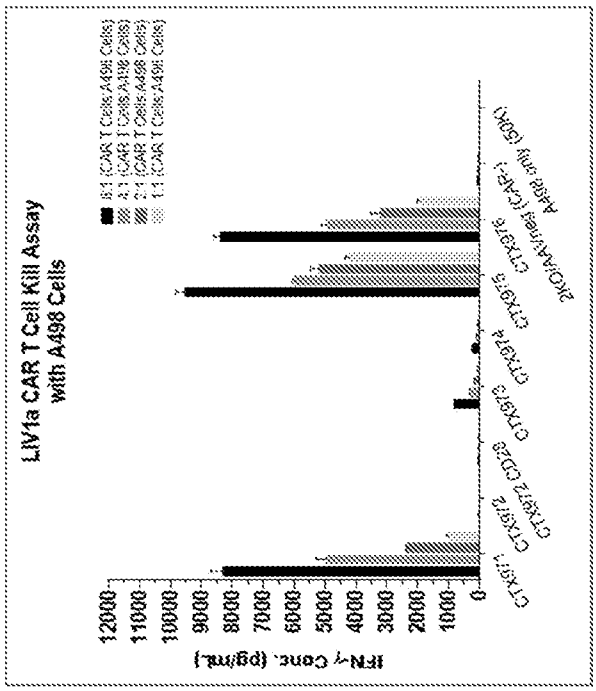

FIG. 3B

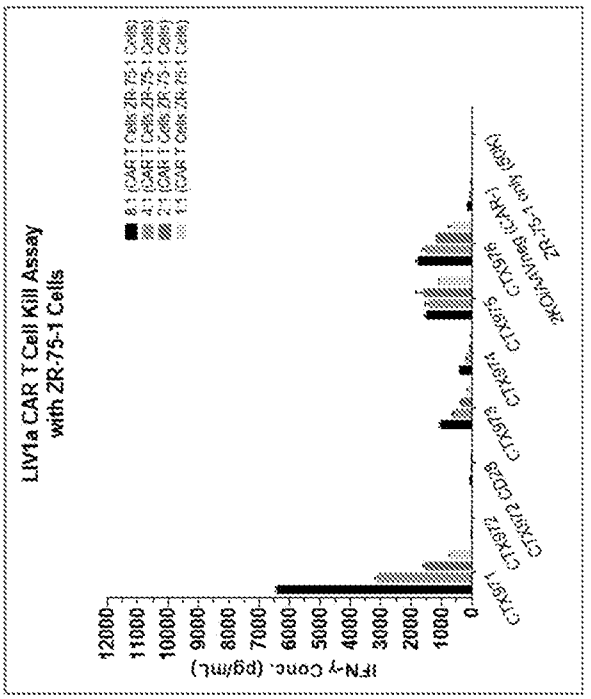

FIG. 3C

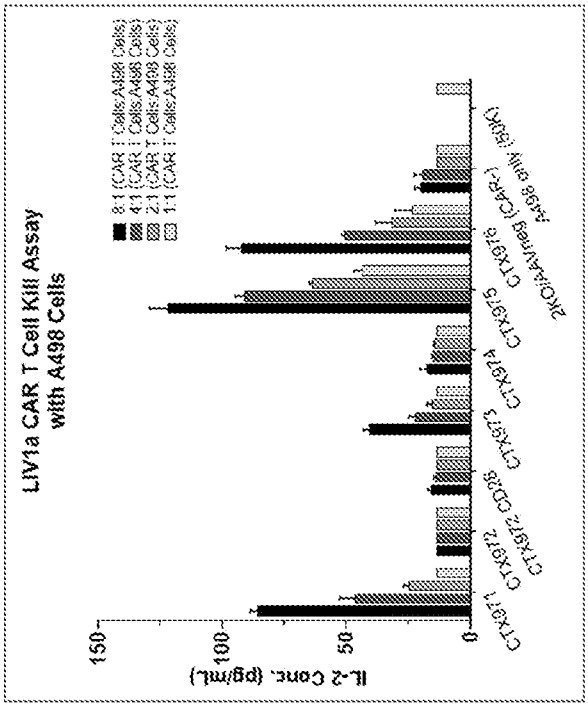

FIG. 3D

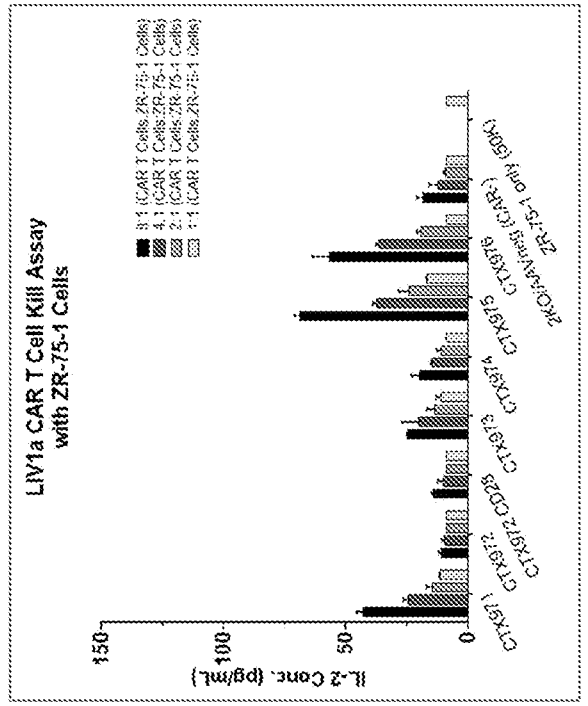

FIG. 4

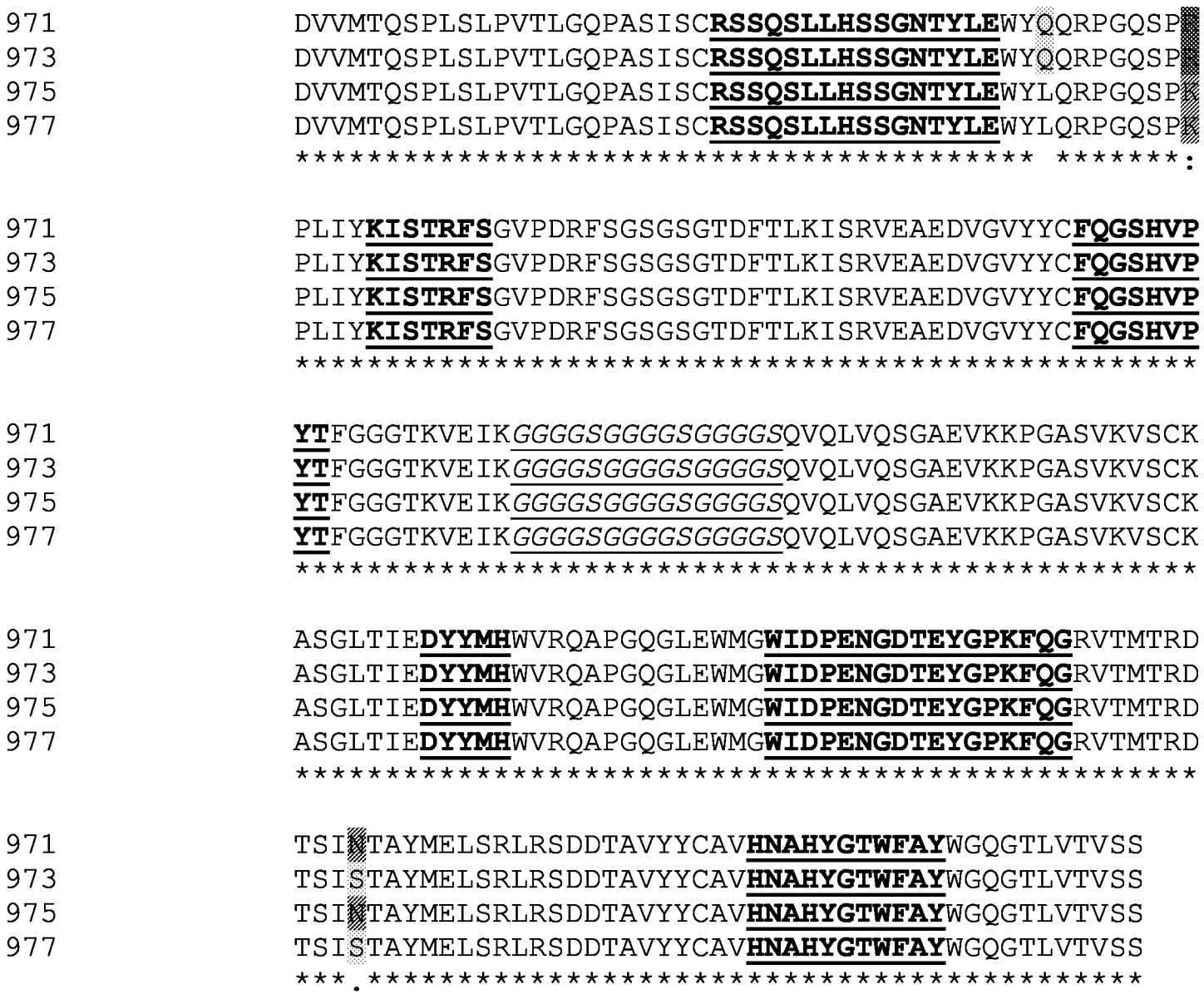
971 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSP
973 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSP
975 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSP
977 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSP
971 PLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP
973 PLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP
975 PLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP
977 PLIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP
971 YTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCK
973 YTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCK
975 YTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCK
977 YTFGGGTKVEIKGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCK
971 ASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD
973 ASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD
975 ASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD
977 ASGLTIEDYYMHWVRQAPGQGLEWMGWIDPENGDTEYGPKFQGRVTMTRD
971 TSITTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS
973 TSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS
975 TSITTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS
977 TSISTAYMELSRLRSDDTAVYYCAVHNAHYGTWFAYWGQGTLVTVSS

FIG. 5

```
979  QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
974  QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
976  QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
978  QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
972  QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYYMHWVRQAPGQGLEWMGW
     **************************************************

979  IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
974  IDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHN
976  IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
978  IDPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAVHN
972  IDPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRLRSDDTAVYYCAVHN
     **************************.************************

979  AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
974  AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
976  AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
978  AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
972  AHYGTWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDVVMTQSPLSLPVTL
     **************************************************

979  GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPD
974  GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPD
976  GQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPD
978  GQPASISCRSSQSLLHSSGNTYLEWYLQRPGQSPKPLIYKISTRFSGVPD
972  GQPASISCRSSQSLLHSSGNTYLEWYQQRPGQSPRPLIYKISTRFSGVPD
     ************************ *******:***************

979  RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
974  RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
976  RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
978  RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGGGTKVEIK
972  RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT-GGGTKVEIK
     ************************************.*********
```