THERAPEUTIC ANTIBODY EPITOPE-TAGGED CAR T CELLS FOR ENHANCED CONTROL AND SAFETY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/572,671, filed on April 1, 2024, the contents of which are herein incorporated by reference in their entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing XML associated with this application is provided in XML file format and is hereby incorporated by reference into the specification. The name of the XML file containing the Sequence Listing XML is MNBI_003_01WO_SeqList_ST26.xml. The XML file is 665,052 bytes in size and created on March 27, 2025, and is being submitted electronically via USPTO Patent Center.

FIELD

[0003] The present disclosure relates generally to compositions and methods for enhancing the control and safety of engineered T cell therapeutics. The disclosure provides recombinant receptors and engineered immune cells that contain one or more epitope or mimotope tags for a therapeutic antibody, such that, if an adverse event occurs the therapeutic antibody can be administered to remove the engineered immune cells and mitigate the adverse event. Also provided are recombinant nucleic acid constructs, vectors, particles and cells that comprise such recombinant nucleic acids, methods for preparing immune cells for use in cell therapies, as well as methods of mitigating adverse events in such cell therapies.

BACKGROUND

[0004] Engineered immune cell therapies, such as adoptive T cell therapies using chimeric antigen receptor (CAR) T cells, have revolutionized cancer therapy. To date, five CD19 targeted CAR-T cell therapies have been approved by the FDA for use against hematological B cell cancers. Despite this success, meaningful responses have not been achieved for many tumors. Further, severe and sometimes life-threatening adverse events may be associated with engineered immune cell therapies.

[0005] One challenge for improving engineered immune cell thrapies is to ensure its safety while enhancing potency. The associated adverse events may arise due to an uncontrolled proliferation of the engineered cells *in vivo*, and thus depleting the engineered cells on demand is a promising strategy for improving the safety of such cell therapies. To date, a number of approaches have been proposed for depleting engineered immune cells, such as those employing a "safety switch" (*e.g.,* a herpesvirus thymidine kinase (HSV-tk) gene, an Escherichia coli cytosine deaminase (EC-CD) gene, or an inducible Caspase9 (iCasp9) protein). However, the large size of such a separate polypeptide poses problems for AAV delivery of an expression cassette that encodes both the engineered receptor (*e.g.,* CAR) and the separate depletion polypeptide. Further, these approaches often have limited depletion efficiency or relatively slow depletion rate *in vivo*. Thus, there remains significant unmet needs for improved strategies of depleting engineered immune cells to ensure the safety of corresponding cell therapies.

SUMMARY

[0006] This section provides a general summary of the disclosure, and is not comprehensive of its full scope or all of its features.

[0007] In one aspect, the present disclosure provides a recombinant polypeptide comprising **(i)** an extracellular region comprising a binding region for an antigen; and **(ii)** a transmembrane region, wherein the extracellular region comprises a peptide epitope or mimotope capable of being bound by an anti-CCR4 antibody, wherein the recombinant polypeptide is not CCR4.

[0008] In some embodiments, the recombinant polypeptide is a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

[0009] In some embodiments, the anti-CCR4 antibody is mogamulizumab, the peptide epitope consists of SEQ ID NO: 431, and the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the peptide epitope or mimotope.

[0010] In some embodiments, the binding region comprises a heavy chain variable region (VH) and a light chain variable region (VL), and the peptide epitope or mimotope is located between the VH and the VL.

[0011] In one aspect, the present disclosure provides an engineered immune cell expressing a recombinant polypeptide described herein.

[0012] In one aspect, the present disclosure provides a method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells described herein to the subject. In some embodiments, the method further comprises administering the anti-CCR4 antibody to the subject, wherein the anti-CCR4 antibody is mogamulizumab. In some embodiments, the anti-CCR4 antibody is administered after detection or occurrence of an adverse event caused by the engineered cells in the subject, and wherein administration of the anti-CCR4 antibody reduces or eliminates at least one symptom of the adverse event. In some embodiments, the anti-CCR4 antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells in the subject.

[0013] In one aspect, the present disclosure provides modified peptide epitopes or mimotopes, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (substitutions, insertions, and/or deletions) compared to an unmodified peptide epitope or mimotope, and wherein the modified peptide epitope or mimotope is capable of being bound by a monoclonal antibody.

[0014] In some embodiments, the peptide epitope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 430, and wherein the peptide epitope is capable of being bound by mogamulizumab. In some embodiments, the modified peptide epitope or mimotope does not comprise the cysteine that is present at the C-terminus of SEQ ID NO: 430. In some embodiments, the modified peptide epitope comprises or consists of any one of SEQ ID NO: 431-438. In some embodiments, the modified peptide epitope consists of SEQ ID NO: 431.

[0015] In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 475, and wherein the peptide epitope or mimotope is capable of being bound by rituximab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 476, and wherein the peptide epitope or mimotope is capable of being bound by Palivizumab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequenc having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 477, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab. In some embodiments, the peptide epitope or mimotope

comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 478, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 479, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 480, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 481, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 482, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 483, and wherein the peptide epitope or mimotope is capable of being bound by QBend-10. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 484, and wherein the peptide epitope or mimotope is capable of being bound by Alemtuzumab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 469, and wherein the peptide epitope or mimotope is capable of being bound by Sacituzumab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO:470-474, and wherein the peptide epitope or mimotope is capable of being bound by an anti-GUCY2C antibody. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or

deletions) compared to SEQ ID NO: 439, and wherein the peptide epitope or mimotope is capable of being bound by Tarlatamab. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO: 441-468, and wherein the peptide epitope or mimotope is capable of being bound by an anti-DLL3 antibody. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NOs: 519-524, and wherein the peptide epitope or mimotope is capable of being bound by to CAP-100.

[0016] In some embodiments, the mutation(s) are substitutions. In some embodiments, the mutation(s) are conservative substitutions.

[0017] In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted or deleted. In some embodiments, the N-terminus of the unmodified peptide epitope or mimotope contains cysteine. In some embodiments, the C-terminus of the unmodified peptide epitope or mimotope contains cysteine. In some embodiments, the unmodified peptide epitope or mimotope comprises at least one cysteine between the N-terminus and the C-terminus. In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine, glycine, threonine, alanine, or valine. In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine or valine. In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine. In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with valine. In some embodiments, the modified peptide epitope or mimotope does not comprise any sulfur atoms. In some embodiments, the modified peptide epitope or mimotope does not comprise any cysteine(s).

[0018] In some embodiments, the mutation(s) comprise deleting or substituting one or more amino acids that facilitate polypeptide crosslinking in the unmodified peptide epitope or mimotope. In some embodiments, the mutation(s) comprise deleting or substituting one or more cysteine(s), lysine(s), aspartic acid(s), glutamic acid(s), or any combination thereof.

[0019] In some embodiments, the modified peptide epitope or mimotope is capable of being bound by the corresponding antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM. In some embodiments, the Kd is

measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody.

[0020] In some embodiments, the modified peptide epitope or mimotope is capable of being bound by [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab

mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589.

[0021] In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4, or 5 mutations compared to the unmodified peptide epitope or mimotope.

[0022] In some embodiments, the unmodified peptide epitope or mimotope comprises or consists of no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50, consecutive amino acids in an autoimmune-associated antigen or a cancer-associated antigen.

[0023] In one aspect, the present disclosure provides peptide epitopes comprising or consisting of SEQ ID NO: 430, wherein the peptide epitope is capable of being bound by an anti-CCR4 antibody. In some embodiments, the anti-CCR4 antibody is mogamulizumab.

[0024] In one aspect, the present disclosure provides peptide epitopes comprising or consisting of any one of SEQ ID NO: 439-468, wherein the peptide epitope is capable of being bound by an anti-DLL3 antibody. In some embodiments, the anti-DLL3 antibody is tarlatamab.

[0025] In one aspect, the present disclosure provides peptide epitopes comprising or consisting of any one of SEQ ID NO: 519-524, wherein the peptide epitope is capable of being bound by an anti-CCR7 antibody. In some embodiments, the anti-CCR7 antibody is CAP-100.

[0026] In one aspect, the present disclosure provides recombinant polypeptides comprising or consisting of the peptide epitope or mimotope of the disclosure. In some embodiments, the recombinant polypeptide consists of the peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope is expressed on the surface of a cell. In some embodiments, the peptide epitope or mimotope is located in an extracellular region of the recombinant polypeptide. In some embodiments, the recombinant polypeptide is not any of CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, and

CD52. In some embodiments, the recombinant polypeptide is not CCR4. In some embodiments, the recombinant polypeptide is not CCR7.

[0027] In some embodiments, the recombinant polypeptide does not comprise a cysteine at the C-terminus of the modified peptide epitope or mimotope. In some embodiments, the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the modified peptide epitope or mimotope. In some embodiments, the recombinant polypeptide does not comprise a cysteine at the N-terminus of the modified peptide epitope or mimotope. In some embodiments, the recombinant polypeptide does not comprise a cysteine immediately before the N-terminus of the modified peptide epitope or mimotope.

[0028] In some embodiments, the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s), wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the modified peptide epitope or different from the modified peptide epitope.

[0029] In one aspect, the present disclosure provides recombinant polypeptides comprising a tag, wherein the tag comprises or consists of a peptide epitope or a mimotope that mimics an epitope. In some embodiments, the epitope is derived from CCR4, DLL3, or CCR7.

[0030] In some embodiments, the recombinant polypeptide comprises a binding region for an antigen. In some embodiments, the antigen is expressed on the surface of a target cell.

[0031] In some embodiments, the recombinant polypeptide does not comprise a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

[0032] In some embodiments, the recombinant polypeptide comprises or consists of a T cell receptor fusion construct (TRuC) derived from a CD3 protein and comprising the binding region. In some embodiments, the CD3 protein is CD3ε.

[0033] In some embodiments, the recombinant polypeptide comprises or consists of a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

[0034] In some embodiments, the recombinant polypeptide comprises or consists of a receptor comprising: (a) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRalpha, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRbeta; or (b) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRbeta, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRalpha, optionally, wherein at least one of the constant region of TCRalpha or the constant region of TCRbeta

are an endogenous constant region of TCRalpha or an endogenous constant region of TCRbeta, optionally wherein the receptor is a T-body.

[0035] In some embodiments, the peptide epitope or mimotope is located between the VH and the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located between the VL and the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located between the VH and the constant region of TCRbeta. In some embodiments, the peptide epitope or mimotope is located between the VL and the constant region of TCRbeta. In some embodiments, the peptide epitope or mimotope is located N-terminal to the VH. In some embodiments, the peptide epitope or mimotope is located N-terminal to the VL. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region of TCRbeta.

[0036] In some embodiments, an immune cell expressing the recombinant polypeptide exhibits one or more of the following: (a) improved binding to the antigen expressed on the surface of the target cell; (b) reduced recombinant polypeptide -induced antigen-independent signaling and/or activation of the immune cell; and/or (c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell; compared to an immune cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope.

[0037] In some embodiments, an immune cell expressing the recombinant polypeptide exhibits one or more of the following: (a) comparable levels of binding to the antigen expressed on the surface of the target cell; (b) comparable levels of CAR-induced or TCR-induced antigen-independent signaling and/or activation of the immune cell; and/or (c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell; compared to an immune cell expressing a recombinant polypeptide that does not comprise a peptide epitope or mimotope.

[0038] In some embodiments, the recombinant polypeptide comprises the peptide epitope or mimotope located N-terminal to the binding region. In some embodiments, the peptide epitope or mimotope is separated from the N-terminus of the binding region by no more than 15, no more than 10, no more than 5, no more than 4, no more than 3, no more than 2, no more than 1, or zero amino acid(s).

[0039] In some embodiments, the recombinant polypeptide comprises the peptide epitope or mimotope located C-terminal to the binding region. In some embodiments, the peptide epitope or mimotope is separated from the C-terminus of the binding region by zero, no more than 5, no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, or no more than 60 amino acids.

[0040] In some embodiments, the peptide epitope or mimotope is separated from the binding region by no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

[0041] In some embodiments, the peptide epitope or mimotope is located within the binding region.

[0042] In some embodiments, the TCR is TCRalpha, and the binding region comprises a variable region and a constant region. In some embodiments, the TCR is TCRbeta, and the binding region comprises a variable region and a constant region. In some embodiments, the peptide epitope or mimotope is located between the variable region and the constant region. In some embodiments, the peptide epitope or mimotope is located N-terminal to the variable region. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region.

[0043] In some embodiments, the recombinant polypeptide comprises or consists of the CAR.

[0044] In some embodiments, the binding region comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the binding region comprises a single chain variable fragment (scFv) comprising a VH and a VL. In some embodiments, the VH and the VL is separated by a linker.

[0045] In some embodiments, the binding region comprises one or more variable heavy domains of heavy chain (VHHs). In some embodiments, the binding region comprises at least two VHHs separated by a linker.

[0046] In some embodiments, the binding region comprises a first binding domain and a second binding domain, wherein the first binding domain and the second binding domain are separated by a linker.

[0047] In some embodiments, the recombinant polypeptide comprises a second binding region located N-terminal or C-terminal to the binding region. In some embodiments, the binding region and the second binding region are separated by a linker.

[0048] In some embodiments, the peptide epitope or mimotope is located within the linker.

[0049] In some embodiments, the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

[0050] In some embodiments, the peptide epitope or mimotope is located in between the binding region(s) and a transmembrane region. In some embodiments, the peptide epitope or mimotope is located within a hinge region that connects the binding region(s) to a transmembrane region. In some embodiments, the hinge region comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, no more than 60, no more than 65, or no more than 70 amino acids.

[0051] In some embodiments, the CAR or TCR comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids N-terminal to the binding region(s), excluding the signal peptide.

[0052] In some embodiments, the extracellular region of the CAR or TCR comprises no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, or no more than 500 amino acids.

[0053] In some embodiments, the CAR or TCR comprises, from N-terminus to C-terminus, the binding region(s), a hinge region, a transmembrane region.

[0054] In some embodiments, the antigen is an autoimmune-associated antigen or a cancer-associated antigen. In some embodiments, the peptide epitope is derived from an autoimmune-associated antigen or a cancer-associated antigen or wherein the mimotope mimics an epitope of the autoimmune-associated antigen or the cancer-associated antigen.

[0055] In some embodiments, the autoimmune-associated antigen or the cancer-associated antigen is selected from the group consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140

(PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, and any combinations thereof.

[0056] In some embodiments, the cancer-associated antigen is a cancer-associated antigen for a solid tumor. In some embodiments, the cancer is a solid tumor selected from the group consisting of small cell lung cancer, prostate cancer, colorectal cancer, head and neck squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, lung cancer, pancreatic cancer, stomach cancer, cervical cancer, hepatocellular carcinoma, glioma cancer, neuroblastoma cancer, and renal cell carcinoma.

[0057] In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin α5β1, CD51, CD27, CDH17, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, integrin α11β1, DLL3, CD2, SSTR2, TROP2, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA, LY6G6D, IL-1α, CEA, C242 antigen, CanAg (MUC1 glycoform), CTAA16.88, UCY2C, IGF2BP3, p53R175H, NKG2D ligands, PRAME, MAGEA4, vimentin, HER2/neu, CCR4, CCR5, ROPN1, GPA33, ROR1, KK-LC-1, CA-125, folate receptor 1, folate receptor alpha, MUC1, MUC16, MSLN, CLDN6, WT1, ALPPL2, KRASG12D, KRASG12V, CD70, MSLN, CLDN18.2, HPV E6/E7, GPC3, IL-13Rα, EGFRv3, EGFR806, GD2, or CA9. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin a5b1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, or Integrin α11β1. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

[0058] In some embodiments, the cancer is a hematologic cancer selected from B cell lymphoma, T cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.

[0059] In some embodiments, the cancer-associated antigen is or comprises an antigen selected from Table 2. In some embodiments, the antigen is not CD19. In some embodiments, the antigen is not CCR4. In some embodiments, the antigen is not CCR7. In some embodiments, the antigen is DLL3.

[0060] In some embodiments, the epitope is derived from a cancer-associated antigen selected from Table 2. In some embodiments, the epitope is derived from DLL3.

[0061] In some embodiments, the peptide epitope comprises or consists of at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 consecutive amino acids in SEQ ID NO: 486, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of any one of SEQ ID NO: 439-468, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of SEQ ID NO: 439, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

[0062] In some embodiments, the peptide epitope or mimotope is capable of being bound by a monoclonal antibody that binds to the antigen for the binding region. In some

embodiments, the epitope is derived from a cancer-associated antigen of T cell lymphoma. In some embodiments, the epitope is derived from a cancer-associated antigen of adult T-cell lymphoma (ATL) and/or cutaneous T-cell lymphoma (CTCL).

[0063] In some embodiments, the epitope is derived from a cancer-associated antigen of a Th2 cell, a cutaneous lymphocyte antigen-positive skin-homing T cell, and/or a Treg cell.

[0064] In some embodiments, the epitope is derived from CCR4. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NO: 430-438, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 431.

[0065] In some embodiments, the peptide epitope is derived from CCR7. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 519-524, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NOs: 519-524.

[0066] In some embodiments, the peptide epitope or the mimotope is about 5 to about 10, about 10 to about 20, about 15 to about 25, about 20 to about 30, about 25 to about 35, about 30 to about 40, about 35 to about 45, or about 40 to about 50 amino acids in length. In some embodiments, the peptide epitope or the mimotope is no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids in length.

[0067] In some embodiments, the peptide epitope does not comprise any mutation compared to the native epitope derived from the antigen.

[0068] In some embodiments, the peptide epitope or the mimotope is capable of forming an alpha-helical conformation.

[0069] In some embodiments, the peptide epitope or the mimotope is capable of being bound by an antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM. In some embodiments, the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody. In some embodiments, the antibody is [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-

100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589 .

[0070] In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD3. In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; and (ii) CD16. In some embodiments, the antibody is a tri-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; (ii) 41BB or albumin, and (ii) CD3. In some embodiments, the antibody is tarlatamab.

[0071] In some embodiments, the antibody is mogamulizumab.

[0072] In some embodiments, the antibody is CAP-100.

[0073] In some embodiments, the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s). In some embodiments, the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope in the tag. In some embodiments, the one or more additional peptide epitope(s) or mimotope(s) are different from the peptide epitope or mimotope in the tag. In some embodiments, the recombinant polypeptide comprises 1, 2, 3, or 4 additional peptide epitope(s) or mimotope(s).

[0074] In some embodiments, the recombinant polypeptide comprises an extracellular portion comprising the binding region and the peptide epitope or mimotope.

[0075] In some embodiments, the recombinant polypeptide comprises a transmembrane region. In some embodiments, the recombinant polypeptide comprises a glycosylphosphatidylinositol (GPI) anchor.

[0076] In some embodiments, the recombinant polypeptide comprises an intracellular region. In some embodiments, the intracellular domain is located C-terminal to the transmembrane region. In some embodiments, the intracellular region comprises one or more intracellular signaling domains. In some embodiments, the one or more intracellular signaling domains are derived from a protein selected from the group consisting of CD3zeta, 1xxCD3zeta, CD28, 4-1BB, OX40, IL2Rb turbodomains, MYD88/CD40, MYD88/CD40 inducible costimulatory domain, CD2, B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR

Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; 0X40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB- A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thyl; CD96; CD160; CD200; CD300a/LMIRl; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-l; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin- 1/CLEC7A; DPPIV/CD26; EphB6; TIM- 1 /KIM- 1 /HA VCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, an immunoreceptor tyrosine based activation motif (ITAM), CD27, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, and any combinations thereof. In some embodiments, the intracellular region comprises an intracellular signaling domain derived from 4-1BB and/or an intracellular signaling domain derived from CD3zeta. In some embodiments, the intracellular region comprises, from N-term to C-term, the intracellular signaling domain derived from 4-1BB and the intracellular signaling domain derived from CD3zeta. In some embodiments, the intracellular region comprises the intracellular signaling domain derived from 4-1BB comprising a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 422. In some embodiments, the intracellular region comprises the intracellular signaling domain derived from CD3zeta comprising a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 423. In some embodiments, the intracellular region comprises the intracellular signaling domain derived from CD3zeta comprising a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 488 wherein the amino acid corresponding to position 14 of SEQ ID NO: 488 is lysine.

[0077] In some embodiments, the transmembrane region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 421.

[0078] In some embodiments, the hinge region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 420.

[0079] In some embodiments, the linker comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to any one of SEQ ID NO: 416-418.

[0080] In some embodiments, the recombinant polypeptide comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to any one of SEQ ID NO: 401-413.

[0081] In some embodiments, the recombinant polypeptide is no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, no more than 500, no more than 550, no more than 600, no more than 650, no more than 700, no more than 750, or no more than 800 amino acids in length.

[0082] In one aspect, the present disclosure provides recombinant nucleic acids encoding the peptide epitope or mimotope of the disclosure.

[0083] In one aspect, the present disclosure provides recombinant nucleic acids encoding the recombinant polypeptide of the disclosure.

[0084] In some embodiments, the recombinant nucleic acid encodes the TCRalpha comprising the peptide epitope or mimotope and a TCRbeta. In some embodiments, the recombinant nucleic acid encodes a TCRalpha and the TCRbeta comprising the peptide epitope or mimotope.

[0085] In some embodiments, the recombinant nucleic acid encodes a TCRalpha, a TCRbeta, and the peptide epitope or mimotope located in between the TCRalpha and the TCRbeta.

[0086] In some embodiments, the TCRalpha and the TCRbeta are encoded by the same coding region. In some embodiments, the TCRalpha and the TCRbeta are separated by a cleavage site.

[0087] In some embodiments, the recombinant nucleic acid encodes (i) the VH and the constant region of TCRalpha of the receptor, or (ii) the VL and the constant region of TCRbeta of the receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope. In some embodiments, the recombinant nucleic acid encodes (i) the VH and the constant region of TCRbeta of the receptor, or (ii) the VL and the constant region of TCRalpha of the receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope. In some embodiments, (i) and (ii) are encoded by the same coding region; optionally, wherein (i) and (ii) are separated by a cleavage site.

[0088] In one aspect, the present disclosure provides recombinant nucleic acids encoding (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

[0089] In some embodiments, the recombinant nucleic acid is less than 5.0 kb in length. In some embodiments, the recombinant nucleic acid is less than 4.8 kb in length. In some embodiments, the recombinant nucleic acid is less than 4.7 kb in length.

[0090] In some embodiments, the recombinant nucleic acid further comprises a polynucleotide sequence encoding a second recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof. In some embodiments, the CARD containing protein comprises (i) a CARD domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 11-103 of SEQ ID NO: 1 and (ii) a Src Homology region 2 (SH2) domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 622-716 of SEQ ID NO: 1. In some embodiments, the CARD containing protein comprises a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NO: 1-4. In some embodiments, the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

[0091] In some embodiments, the polynucleotide sequence encoding the recombinant polypeptide is operably linked to a promoter. In some embodiments, the promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma

promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the constitutive promoter is or comprises a CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, MND promoter or a short EF1a promoter.

[0092] In some embodiments, the polynucleotide sequence encoding the recombinant polypeptide is not operably linked to a promoter.

[0093] In some embodiments, the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more cleavable linkers. In some embodiments, the one or more cleavable linkers comprise a P2A, E2A, F2A, or T2A self-cleaving peptide.

[0094] In some embodiments, the recombinant nucleic acid further comprises one or more nucleic acid element sequences. In some embodiments, wherein the one or more nucleic acid element sequences is or comprises ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, a 70 bp poly(A) tract, a 100 bp poly(A) tract, a 172 bp poly(A) tract, a 200 bp poly(A) tract, a 300 bp poly(A) tract, a 325 bp poly(A) tract, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, cleavable linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or any combination thereof.

[0095] In some embodiments, the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or a non-coding RNA. In some embodiments, the non-coding RNA comprises a shRNA or a microRNA.

[0096] In some embodiments, the one or more additional polypeptides comprise an additional potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, or any combination thereof. In some embodiments, the additional potency enhancement polypeptide is or comprises a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (*e.g.,* TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, or CD8alpha/beta.

[0097] In some embodiments, the recombinant nucleic acid is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length. In some embodiments, the recombinant nucleic acid is less than about 5.0 kb in length. In some embodiments, the recombinant nucleic acid is less than about 4.7 kb in length.

[0098] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (3) a cleavable linker encoding sequence, and
(4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure.

[0099] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, and (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

[0100] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure,
(5) a cleavable linker encoding sequence, and (6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

[0101] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (5) a cleavable linker encoding sequence, and (6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

[0102] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor; (5) a cleavable linker encoding sequence, and (6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

[0103] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding an

additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (5) a cleavable linker encoding sequence, and (6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

[0104] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a promoter, (2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (5) a cleavable linker encoding sequence, and (6) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure.

[0105] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, and (5) a cleavable linker encoding sequence.

[0106] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and (5) a cleavable linker encoding sequence.

[0107] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (5) a cleavable linker encoding sequence, (6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and (7) a cleavable linker encoding sequence.

[0108] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding an additional

potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (5) a cleavable linker encoding sequence, (6) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (7) a cleavable linker encoding sequence.

[0109] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (5) a cleavable linker encoding sequence, (6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and (7) a cleavable linker encoding sequence.

[0110] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (5) a cleavable linker encoding sequence, (6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and (7) a cleavable linker encoding sequence.

[0111] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (5) a cleavable linker encoding sequence,

(6) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, and (7) a cleavable linker encoding sequence.

[0112] In one aspect, the present disclosure provides recombinant nucleic acids comprising, from 5' to 3': (1) a cleavable linker encoding sequence, (2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, (3) a cleavable linker encoding sequence, (4) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, (5) a cleavable linker encoding sequence, (6) a polynucleotide sequence encoding a CARD-

containing protein or a functional fragment thereof, and (7) a cleavable linker encoding sequence.

[0113] In one aspect, the present disclosure provides recombinant nucleic acids comprising:

(1) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, wherein the recombinant nucleic acid is an RNA construct.

[0114] In one aspect, the present disclosure provides recombinant nucleic acids comprising: (1) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, (2) a polynucleotide sequence encoding the recombinant polypeptide of the disclosure, and (3) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, wherein the nucleic acid is an RNA construct.

[0115] In one aspect, the present disclosure provides combinations of recombinant nucleic acid constructs comprising: (1) a first nucleic acid construct encoding a CARD-containing protein or a functional fragment thereof, and (2) a second nucleic acid construct encoding the recombinant polypeptide of the disclosure, wherein the first and the second nucleic acid constructs are both RNA constructs.

[0116] In one aspect, the present disclosure provides combinations of recombinant nucleic acid constructs comprising: (1) a first nucleic acid construct encoding a CARD-containing protein or a functional fragment thereof, (2) a second nucleic acid construct encoding the recombinant polypeptide of the disclosure, and (3) a third nucleic acid construct encoding a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and wherein the first, second, and third nucleic acid constructs are each RNA constructs.

[0117] In some embodiments, the CARD-containing protein comprises (i) a CARD domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 11-103 of SEQ ID NO: 1 and (ii) a Src Homology region 2 (SH2) domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 622-716 of SEQ ID NO: 1. In some embodiments, the CARD containing protein comprises a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NO: 1-4. In some embodiments, the second recombinant

polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

[0118] In one aspect, the present disclosure provides a vector comprising the recombinant nucleic acid of the disclosure. In some embodiments, the vector is a plasmid. In some embodiments, the vector is a single stranded DNA vector or a double stranded DNA vector. In some embodiments, the vector is a synthetic DNA vector. In some embodiments, the vector is a linear DNA vector. In some embodiments, the vector is a closed linear DNA vector. In some embodiments, the vector is a synthetic RNA vector. In some embodiments, the vector is a phagemid vector.

[0119] In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector. In some embodiments, the retrovirus is a lentivirus. In some embodiments, the lentivirus is a VSV-G pseudotyped lentivirus. In some embodiments, the AAV vector is AAV6 vector. In some embodiments, the AAV vector is AAV9 vector. In some embodiments, the AAV vector is a split-intein dual AAV vector. In some embodiments, the vector is a redirected lentiviral vector.

[0120] In some embodiments, the vector is a fusosome.

[0121] In some embodiments, the vector is a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein.

[0122] In some embodiments, the vector is an enveloped delivery vehicle.

[0123] In some embodiments, the vector is self-replicating RNA virus.

[0124] In some embodiments, the vector is mRNA-packaging virus-like particle.

[0125] In some embodiments, the vector is RNP-packaging virus-like particle.

[0126] In one aspect, the present disclosure provides particles comprising the recombinant nucleic acid of the disclosure.

[0127] In some embodiments, the particle is a lipid nanoparticle (LNP). In some embodiments, the particle is a selective organ targeting (SORT) LNP. In some embodiments, the particle is an antibody targeted LNP. In some embodiments, the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid).

[0128] In some embodiments, the particle is a polymer nanoparticle.

[0129] In some embodiments, the particle is a protein nanoparticle.

[0130] In one aspect, the present disclosure provides engineered cells, wherein the engineered cell (i) comprises the recombinant nucleic acid of the disclosure (ii) is transfected or transduced by the vector of the disclosure, or (iii) is contacted by the particle of the disclosure.

[0131] In one aspect, the present disclosure provides engineered cells expressing the modified peptide epitope of the disclosure, or the recombinant polypeptide of the disclosure.

[0132] In one aspect, the present disclosure provides engineered cells expressing (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

[0133] In one aspect, the present disclosure provides engineered cell comprising the recombinant polypeptide of the disclosure or the recombiant nucleic acid of the disclosure.

[0134] In some embodiments, the engineered cell further expresses a second recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof. In some embodiments, the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

[0135] In some embodiments, the engineered cell comprises one or more additional peptide epitope(s) or mimotope(s) present on the cell surface, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope or different from the peptide epitope or mimotope.

[0136] In some embodiments, activating the engineered cell results in the secretion of IL-2 by the cell at a level that is at least 10%, at least 20%, at least 50%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, least 30-fold, or at least 100-fold higher than the IL-2 secretion level of a control cell lacking the recombinant polypeptide.

[0137] In some embodiments, expression of the recombinant polypeptide is under the control of an endogenous promoter. In some embodiments, the endogenous promoter is a TCRa promoter, a TCRb promoter, a CD3d promoter, a CD3g promoter, a CD3e promoter, a CD3z promoter, a CARD9 promoter, a CARD10 promoter, a CARD11 promoter, a CARD14 promoter, or a PIK3R3 promoter.

[0138] In some embodiments, expression of the recombinant polypeptide is under the control of an exogenous promoter. In some embodiments, the exogenous promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein- I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse

mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of 29oloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

[0139] In some embodiments, the nucleic acid construct is inserted in a T-cell receptor (TCR) locus, a CD3 locus, a B2 microglobulin (B2M) locus, a class II transactivator (CIITA) locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, a PIK3R3 locus, or a safe harbor locus. In some embodiments, the TCR locus is or comprises a TRAC locus, a TRBC1 locus, a TRBC2 locus, or a TRAJ locus. In some embodiments, the TRAJ locus is or comprises a TRAJ intron splice acceptor locus.

[0140] In some embodiments, the cell does not express a functional gene product of an endogenous TRAC locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRBC1 locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRBC2 locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRAJ locus. In some embodiments, the CD3 locus is or comprises: a CD3d locus, a CD3g locus, a CD3e locus, or CD3z locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3d locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3g locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3e locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3z locus. In some embodiments, the safe harbor locus is or comprises an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[0141] In some embodiments, the nucleic acid construct is inserted is an exon, an intron, between an intron and an exon, or a regulatory region.

[0142] In some embodiments, the cell is selected from the group of consisting of a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T

(NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, and a progenitor cell of the lymphoid lineage.

[0143] In some embodiments, the cell is selected from the group consisting of a T cell, a macrophage, a monocyte, and a natural killer (NK) cell. In some embodiments, the cell is a T cell. In some embodiments, the T cell is selected from the group consisting of a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, and an engineered T cell comprising a transcriptional receptor.

[0144] In some embodiments, the cell is an autologous cell.

[0145] In some embodiments, the cell is an allogeneic cell.

[0146] In some embodiments, the cell is a primary cell.

[0147] In some embodiments, the cell is derived from a stem cell.

[0148] In some embodiments, the cell is genetically modified.

[0149] In some embodiments, the cell has reduced or eliminated cell surface expression of an endogenous T cell receptor.

[0150] In some embodiments, the cell is more prone to display central memory phenotype than a control cell lacking the recombinant polypeptide.

[0151] In some embodiments, the cell further comprises one or more modifications that inactivate or disrupt one or more alleles of TGFbeta, Regnase-1, FAS, PTPN2, NR4A3, CD52, PD-1, B2M, CIITA, SOCS1, CBLB, DGKalpha, and/or DGKζ.

[0152] In some embodiments, the cell is a mammalian cell.

[0153] In some embodiments, the cell is selected from the group consisting of a human cell, a mouse cell, and a canine cell.

[0154] In some embodiments, the cell further comprises a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, an RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof. In some embodiments, the RNA-guided

nuclease is a Life Edit nuclease (LEG). In some embodiments, the RNA-guided nuclease is a LEG14 nuclease.

[0155] In some embodiments, the cell further comprises Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional fragment thereof.

[0156] In some embodiments, the engineered cell exhibits one or more of the following: (a) improved binding to the antigen expressed on the surface of a target cell; (b) reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or (c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell; compared to a control cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope. In some embodiments, the engineered cell exhibits one or more of the following: (a) comparable levels of binding to the antigen expressed on the surface of a target cell; (b) comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or (c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell; compared to a control cell expressing a recombinant polypeptide that does not comprise the peptide epitope or mimotope.

[0157] In some embodiments, the cell has reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, and/or increase intratumoral presence *in vivo*.

[0158] In some embodiments, the cell has increased or decreased signaling through the CARD11-BCL10-MALT1 complex, NF-κB, AP-1, NFAT, JAK/STAT, and/or MEK/ERK pathways.

[0159] In one aspect, the present disclosure provides compositions comprising the vector of the disclosure, the particle of the disclosure, or the engineered cell of the disclosure. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[0160] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

[0161] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a gene product of an endogenous TRAC locus.

[0162] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide of the disclosure; at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the receptor, or recombinant TCR; and/or at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a gene product of an endogenous TRAC locus.

[0163] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide of the disclosure. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the receptor (*e.g.,* the CAR, T-body, HIT receptor, or TCR). In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus.

[0164] In some embodiments, the composition comprises CD4+ T cells and CD8+ T cells, and the percentage of CD4+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the percentage of CD8+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of

the total cells in the composition; and/or the ratio of CD4+ T cells to CD8+ T cells is from at or about 1:3 to at or about 3:1. In some embodiments, the ratio is at or about 1:1.

[0165] In one aspect, the present disclosure provides methods of preparing an engineered cell, wherein the method comprises contacting the cell with the recombinant nucleic acid of the disclosure, the vector of the disclosure or the particle of the disclosure.

[0166] In one aspect, the present disclosure provides methods of preparing an engineered cell, wherein the method comprises expressing in the cell: (a) the recombinant polypeptide of the disclosure; or (b) at least the following two polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope.

[0167] In some embodiments, the method comprises enriching the engineered cells expressing the recombinant polypeptide using a binding agent that binds to the peptide epitope or mimotope. In some embodiments, the binding agent comprises a monoclonal antibody.

[0168] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

[0169] In some embodiments, the cell is *in vivo*. In some embodiments, the cell is prepared *in vivo*. In some embodiments, the cell is prepared in the body of a subject in need of treatment.

[0170] In some embodiments, the recombinant polypeptide is expressed in the cell, and/or the nucleic acid construct is introduced into the cell, by contacting the cell with a vector or a particle.

[0171] In some embodiments, the vector is a plasmid, a synthetic DNA vector, a single stranded DNA vector, a double stranded DNA vector, a linear DNA vector, a closed linear DNA vector, a synthetic RNA vector, a phagemid vector, an RNA vector, an mRNA vector, or a viral vector. In some embodiments, the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector. In some embodiments, the retrovirus is a lentivirus. In some embodiments, the lentivirus is a VSV-G pseudotyped lentivirus. In some embodiments, the AAV vector is an AAV6 vector or an AAV9 vector.

[0172] In some embodiments, the vector is a fusosome, a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein, or an enveloped delivery vehicle.

[0173] In some embodiments, the vector is a self-replicating RNA virus, an mRNA-packaging virus-like particle, or a ribonucleoprotein (RNP)-packaging virus-like particle.

[0174] In some embodiments, the particle is a lipid nanoparticle (LNP), a selective organ targeting (SORT) LNP, or an antibody targeted LNP. In some embodiments, the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid).

[0175] In some embodiments, the particle is a polymer nanoparticle or a protein nanoparticle.

[0176] In some embodiments, the cell is *ex vivo*.

[0177] In some embodiments, the cell is prepared *ex vivo*.

[0178] In some embodiments, the cell is prepared outside of the body of a subject in need of treatment.

[0179] In some embodiments, the nucleic acid construct is introduced into the cell by electroporation or transfection. In some embodiments, the transfection is lipofection.

[0180] In some embodiments, the nucleic acid construct is a plasmid, a synthetic DNA vector, a single stranded DNA vector, a double stranded DNA vector, a linear DNA vector, a closed linear DNA vector, a synthetic RNA vector, a phagemid vector, an RNA vector, or an mRNA vector.

[0181] In some embodiments, the nucleic acid construct is introduced via a viral vector. In some embodiments, the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector. In some embodiments, the retrovirus is a lentivirus. In some embodiments, the lentivirus is a VSV-G pseudotyped lentivirus. In some embodiments, the AAV vector is an AAV6 vector or an AAV9 vector.

[0182] In one aspect, the present disclosure provides methods of preparing an engineered cell, wherein the method comprises contacting the cell with an AAV vector comprising a recombinant nucleic acid encoding:

(a) the recombinant polypeptide of the disclosure; or

(b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least

one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide.

[0183] In one aspect, the present disclosure provides methods of preparing an engineered cell, wherein the method comprises contacting the cell with a particle comprising a recombinant nucleic acid encoding:

(a) the recombinant polypeptide of the disclosure; or

(b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide. In some embodiments, the particle is a lipid nanoparticle (LNP).

[0184] In some embodiments, the method comprises genetically modifying the cell for expression of the recombinant polypeptide.

[0185] In some embodiments, the method comprises introducing into the cell one or more nucleic acids encoding a sequence specific nuclease or a nucleic acid programmable DNA binding protein. In some embodiments, the method additionally comprises introducing into the cell one or more guide RNAs. In some embodiments, the sequence-specific nuclease is an RNA guided nuclease. In some embodiments, the sequence-specific nuclease or nucleic acid programmable DNA binding domain is a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR-associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof. In some embodiments, the Cas nuclease is Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional portion thereof.

In some embodiments, the RNA-guided nuclease is a Life Edit nuclease (LEG). In some embodiments, the LEG is a LEG14 nuclease.

[0186] In some embodiments, the method comprises introducing into the cell a ribonucleoprotein (RNP) complex comprising the sequence-specific nuclease or nucleic acid programmable DNA binding.

[0187] In some embodiments, introducing the nucleic acid construct comprises contacting the cell with the particle of the disclosure or the vector of the disclosure.

[0188] In some embodiments, the nucleic acid construct is introduced via electroporation. In some embodiments, the nucleic acid construct is introduced via AAV. In some embodiments, the nucleic acid construct is introduced via lentivirus. In some embodiments, the lentivirus is a VSV-G pseudotyped lentivirus. In some embodiments, the nucleic acid construct is introduced via LNP.

[0189] In some embodiments, the nucleic acid construct is inserted into an endogenous locus of the cell. In some embodiments, the endogenous locus is or comprises a TRAC gene locus, a TRBC1 gene locus, a TRBC2 gene locus, a TRAJ locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, or a PIK3R3 locus. In some embodiments, the TRAJ locus is or comprises a TRAJ intron splice acceptor locus. In some embodiments, the endogenous locus is or comprises a TRAC gene locus. In some embodiments, the endogenous locus is or comprises a TRAJ locus. In some embodiments, the TRAJ locus is or comprises a TRAJ intron splice acceptor locus. In some embodiments, the endogenous locus is or comprises a safe harbor locus. In some embodiments, the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

[0190] In some embodiments, the first insertion site is an exon. In some embodiments, the first insertion site is an intron. In some embodiments, the first insertion site is between an intron and an exon. In some embodiments, the first insertion site is in a regulatory region.

[0191] In some embodiments, the first insertion site is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is ngg, nag, ngrrt, ngrm, nnnngatt, nnnnryac, nnagaaw, naaaac, tttv, ttn, attn, tttn, gttn, or yttn and wherein:

(i) r = a or g,

(ii) y = c or t,

(iii) w = a or t,

(iv) v = a or c or g, and

(v) n = a, c, t, or g.

[0192] In some embodiments, the cell comprises a modified TRAC gene locus.

[0193] In some embodiments, the cell has reduced or eliminated surface expression of an endogenous TCR.

[0194] In some embodiments, the method further comprises introducing a gene editing machinery into the cell. In some embodiments, the step of introducing comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery. In some embodiments, the gene editing machinery is CRISPR/Cas9. In some embodiments, the gene editing machinery is introduced via electroporation.

[0195] In some embodiments, the nucleic acid construct is introduced via AAV.

[0196] In some embodiments, the nucleic acid construct is introduced via LNP.

[0197] In some embodiments, the method produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.,* CAR); and (iii) the modified TRAC gene locus. In some embodiments, the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.,* CAR); and (iii) the modified TRAJ locus.

[0198] In some embodiments, the cell is for use in a cell therapy.

[0199] In one aspect, the present disclosure provides methods of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells of the disclosure to the subject. In some embodiments, the peptide epitope or mimotope is bound by an antibody for an antigen associated with the cancer or the autoimmune disease.

[0200] In some embodiments, the method comprises, before the administration, generating engineered cells according to the method of the disclosure.

[0201] In some embodiments, the engineered cells are derived from immune cells: (i) obtained and/or isolated from the subject, (ii) obtained and/or isolated from one or more donors, and/or (iii) derived from one or more stem cells.

[0202] In one aspect, the present disclosure provides methods of treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of the disclosure, the vector of the disclosure, or the particle of the disclosure, to the subject. In some embodiments, the peptide epitope or mimotope is bound by an antibody for an antigen associated with the autoimmune disease. In some

embodiments, the autoimmune disease is lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjogren's syndrome, Hashimoto's thyroiditis, or celiac disease.

[0203] In one aspect, the present disclosure provides methods of treating cancer in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of the disclosure, the vector of the disclosure, or the particle of the disclosure, to the subject. In some embodiments, the peptide epitope or mimotope is capable of being bound by an antibody for an antigen associated with the cancer.

[0204] In some embodiments, the cancer is a hematological cancer. In some embodiments, the cancer is myeloid neoplasm, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blast crisis chronic myelogenous leukemia (bcCML), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, or B-cell lymphoma.

[0205] In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is small cell lung cancer, colorectal cancer, testicular cancer, ovarian cancer, melanoma, lymphoma, leukemia, multiple myeloma, prostate cancer, breast cancer, non-small cell lung cancer, gastric cancer, esophageal cancer, liver cancer, kidney cancer, head & neck cancer, glioblastoma, neuroblastoma, soft tissue sarcoma, uterine cancer, brain cancer, skin cancer, renal cancer, bladder cancer, pancreatic cancer, thyroid cancer, eye cancer, gastrointestinal cancer, carcinoma, or sarcoma.

[0206] In some embodiments, the cancer expresses CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125,

MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, ggcc, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combination thereof.

[0207] In some embodiments, the cancer expresses B7H3, BCMA, CD19, CD20, CD22, CD70, CD79a, CD79b, CLDN6, CLDN18.2, DLL3, GCC, GD2, GD3, GPC3, GPRC5D, KLK3, LY6G6D, p53R175H, PRAME, ROPN1, STEAP1, or STEAP2. In some embodiments, the cancer expresses KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

[0208] In some embodiments, the cancer is selected from Table 2. In some embodiments, the cancer and the corresponding cancer-associated antigen are selected from Table 2.

[0209] In some embodiments, the peptide epitope or mimotope is bound by an antibody for a cancer-associated antigen of T cell lymphoma. In some embodiments, the cancer-associated antigen of T cell lymphoma is CCR4, CCR7, or CD3.

[0210] In some embodiments, the method further comprises administering an antibody to the subject, wherein the antibody binds the tag, the peptide epitope, or the mimotope.

[0211] In some embodiments, the method further comprises monitoring the subject for an adverse event.

[0212] In some embodiments, the method further comprises monitoring the subject for treatment outcomes.

[0213] In one aspect, the present disclosure provides methods of depleting engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

[0214] In one aspect, the present disclosure provides methods of reducing or eliminating at least one symptom of an adverse event caused by engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

[0215] In some embodiments, the subject has been administered the engineered cells within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

[0216] In some embodiments, the subject has been administered the recombinant nucleic acid of the disclosure, the vector of the disclosure, or the particle of the disclosure, within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

[0217] In some embodiments, administration of the antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells expressing the tag, the peptide epitope, or the mimotope in the subject.

[0218] In some embodiments, the antibody is administered after the detection or occurrence of an adverse event, and wherein administration of the antibody reduces or eliminates at least one symptom of the adverse event.

[0219] In some embodiments, the method additionally comprises administration of corticosteroids, tocilizumab, and/or anakyrin.

[0220] In some embodiments, the adverse event is cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), hemophagocytic lymphohistiocytosis (HLH), on-target off-tumor toxicity, tumor lysis syndrome, lymphoproliferative disorder, splenomegaly, lymphadenopathy, an abnormal ratio of

engineered cells to other blood cells, acute toxicity, potency enhancement mediated cytotoxicity, potency enhancement mediated GVHD, uncontrolled expansion of engineered cells, or engineered cell mediated lymphoma.

[0221] In some embodiments, the antibody is administered after the detection or occurrence of a treatment outcome. In some embodiments, the treatment outcome is reduction or elimination of the cancer or the autoimmune disease.

[0222] In some embodiments, the method additionally comprises monitoring the subject for reduction or elimination of at least one symptom of the adverse event. In some embodiments, the monitoring comprises one or more of:

Clinical laboratory tests of ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count;

Imaging, wherein the imaging is PET, CT, MRI, and/or x-ray;

Clinical assessment of body temperature, blood pressure, blood oxygenation, heart rate, cognitive status, and/or organ function; and

Standardized assessment used for detecting or monitoring one or more adverse events. In some embodiments, the standardized assessment comprises the Immune Effector Cell–Associated Encephalopathy (ICE) assessment, the Cornell Assessment, and/or the Pediatric Delirium (CAPD) assessment.

[0223] In some embodiments, the method results in at least one of:

Depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cells in the subject;

Depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of regulatory T cells in the subject;

Clinical laboratory results within the normal range for ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count;

Imaging results indicating a reduction in cancer or reduction in at least one symptoms of an adverse event;

Reduction or absence of fever, hypotension, hypoxia, tachycardia, aphasia, cognitive impairment, coagulopathy, respiratory failure, lymphadenopathy, and/or splenomegaly;

Improved score on a standardized assessment used for detecting or monitoring one or more adverse events; or

Ability to reduce or eliminate administration of corticosteroids, tocilizumab, and/or anakyrin.

[0224] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event. In some embodiments, wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0225] In some embodiments, the method results in higher therapeutic efficacy than a control method to reduce or eliminate the at least one symptom of the adverse event. In some embodiments, the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0226] In some embodiments, the adverse event is an acute adverse event.

[0227] In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to the peptide epitope or the mimotope. In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD3. In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; and (ii) CD16. In some embodiments, the antibody is a tri-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; (ii) 41BB or albumin, and (ii) CD3.

[0228] In some embodiments, the antibody is tarlatamab.

[0229] In some embodiments, the antibody is mogamulizumab.

[0230] In some embodiments, the antibody is CAP-100.

[0231] In some embodiments, a dose of between about 0.003 mg/kg and about 10 mg/kg, between 0.01 mg/kg and 10 mg/kg, between 0.003 mg/kg and 0.3 mg/kg, between 0.05

mg/kg and 5 mg/kg, or between 0.1 mg/kg and 1 mg/kg of the antibody is administered to the subject. In some embodiments, a dose of between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg, of the antibody is administered to the subject.

[0232] In some embodiments, the antibody is administered to the subject once. In some embodiments, the antibody is administered more than once. In some embodiments, the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks.

[0233] In some embodiments, the method comprises (a) administering the engineered cell, the recombinant nucleic acid, the vector, or the particle into a brain of the subject; and (b) administering the antibody outside of the brain of the subject. In some embodiments, the method comprises intraventricular administration of the engineered cells, the recombinant nucleic acid, the vector, or the particle.

[0234] In some embodiments, the method comprises systemic administration of the antibody. In some embodiments, the method comprises intravenous administration of the antibody. In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell that have crossed the blood brain barrier than a control method that reduces or eliminates the at least one symptom of the adverse event. In some embodiments, the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0235] In some embodiments, the antibody is administered more than once. In some embodiments, the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks. In some embodiments, the antibody is administered more than once with an interval of at least 1, 2, 3, or 4 weeks. In some embodiments, the antibody is administered two, three, or four times.

[0236] In some embodiments, both the antibody and the binding region of the recombinant polypeptide can bind the same antigen.

[0237] In some embodiments, the antibody is mogamulizumab, and wherein the method further comprises administering one or more of: (a) at least one retinoid; (b) interferon

alpha; and/or (c) extracorporeal photopheresis (ECP). In some embodiments, wherein the retinoid and/or interferon alpha is delivered systemically.

[0238] In one aspect, the present disclosure provides methods of isolating or enriching the engineered cells of the disclosure, comprising contacting the engineered cells with a binding agent that binds to the epitope or the mimotope.

[0239] In some embodiments, the binding agent comprises an antibody.

[0240] In some embodiments, the binding agent comprises a purification marker. In some embodiments, the purification marker comprises biotin, avidin, a myc tag, an influenza hemagglutinin (HA) tag, a FLAG tag, a glutathione-S transferase (GST) tag, a V5 tag, a poly-Histidine (His) tag, a Fc tag, calmodulin binding protein (CBP), maltose-binding protein (MBP), thioredoxin, or a chitin-binding protein.

[0241] In some embodiments, the binding agent is bound to a solid substrate or a purification bead.

[0242] In some embodiments, the method results in a population of purified engineered cells in which at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cell expresses the recombinant polypeptide.

[0243] In one aspect, the present disclosure provides methods of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering a therapeutically effective amount of engineered cells isolated or enriched by the method of the disclosure.

[0244] In one aspect, the present disclosure provides methods of *ex vivo* or *in vivo* measuring the number of the engineered cells of the disclosure, comprising administering, or contacting the engineered cells with, a binding agent that binds to the epitope or the mimotope. In some embodiments, the binding agent comprises an antibody.

[0245] In one aspect, the present disclosure provides methods of treating cancer or an autoimmune disease in a subject in need thereof, comprising: (a) administering an effective amount of an engineered cell, a recombinant nucleic acid, a vector, or a particle disclosed herein, to the subject, wherein the engineered cell or the recombinant polypeptide comprises two or more of the tags, the peptide epitopes or the mimotopes that are capable of binding to mogamulizumab, and wherein the tags, the peptide epitopes or the mimotopes are the same or different; and (b) administering at least one dose of mogamulizumab to the subject.

[0246] In some embodiments, each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01

mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

[0247] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0248] In some embodiments, the engineered cell, the recombinant nucleic acid, the vector, or the particle is administered at least 1, 2, 3, 4, 5, 6, 7, 8 weeks, or at least 2, 3, 4, 5, 6, 8 or 10 months, or at least 1, 2, 3, 4, or 5 years, before the first dose of mogamulizumab is administered to the subject.

[0249] In one aspect, the present disclosure provides methods of treating cancer in a subject in need thereof, comprising: (a) intraventricularly administering an effective amount of an engineered cell, a recombinant nucleic acid, a vector, or a particle disclosed herein, to the subject; and (b) systemically administering at least one dose of mogamulizumab to the subject.

[0250] In some embodiments, each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

[0251] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cells outside the brain than a control method that reduces or eliminates the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin. In some embodiments, the method comprises administering at least two, at least three, or at least four doses of mogamulizumab to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0252] **FIGS. 1A-1N** Show schematics of non-limiting examples of CAR and TCR construct designs. FIG. 1A: a CAR construct without an epitope tag; FIG. 1B: a CAR construct with an N-terminal epitope tag; FIG. 1C: a CAR construct with an epitope tag in the linker region; FIG. 1D: a CAR construct with an epitope tag in the hinge region; FIG. 1E: a TCRbeta-TCRalpha construct without an epitope tag; FIG. 1F: a TCRbeta-TCRalpha construct with an N-terminal epitope tag; FIG. 1G: a TCRbeta-TCRalpha construct with an epitope tag between the TCRbeta variable and the TCRbeta constant regions; FIG. 1H: a TCRbeta-TCRalpha construct with an epitope tag in the linker region between TCRbeta and TCRalpha; FIG. 1I: a TCRbeta-TCRalpha construct with an epitope tag between the TCRalpha variable and the TCRalpha constant regions; FIG. 1J: a TCRalpha-TCRbeta construct without an epitope tag; FIG. 1K: a TCRalpha-TCRbeta construct with an N-terminal epitope tag; FIG. 1L: a TCRalpha-TCRbeta construct with an epitope tag between the TCRalpha variable and the TCRalpha constant regions; FIG. 1M: a TCRalpha-TCRbeta construct with an epitope tag in the linker region between TCRalpha and TCRbeta; FIG. 1N: a TCRalpha-TCRbeta construct with an epitope tag between the TCRbeta variable and the TCRbeta constant regions.

[0253] **FIGS. 2A-2D** show the expression of CAR constructs tagged with a bi-specific Tarlatamab antibody epitope and functionality of T cells expressing the CAR constructs against antigen (DLL3) positive cell lines. FIG. 2A is a diagram showing the percentage of CAR positive cells. FIG. 2B is a diagram showing the mean fluorescence intensity (MFI) of the cells. FIG. 2C is a diagram showing the killing of a cancer cell line that expresses DLL3 at a low level by the indicated CAR-T cells. FIG. 2D is a diagram showing the secretion of IFNgamma by the indicated CAR-T cells when co-cultured with a cancer cell line that expresses DLL3 at a low level.

[0254] **FIGS. 3A-3B** show the depletion and activation of a CAR-T cells expressing CAR construct that is tagged with a bi-specific tarlatamab antibody epitope. FIG. 3A is a diagram showing the percentage of CAR positive cells. FIG. 3B is a diagram showing the percentage of CD69 positive cells in the indicated cell lines upon tarlatamab treatment.

[0255] **FIGS. 4A-4F** show the expression of CAR constructs with mogamulizumab epitope tags. FIGS. 4A, 4B and 4C are diagrams showing the percentage of CAR positive cells. FIGS. 4D, 4E and 4F are diagrams showing the mean fluorescence intensity (MFI) of cells expressing the CAR constructs.

[0256] **FIGS. 5A-5F** show the ability of different CAR-T cells expressing CAR constructs comprising a mogamulizumab epitope to be bound by mogamulizumab. FIGS. 5A, 5B and 5C are diagrams showing the percentage of T-cells that can be positively stained by mogamulizumab. FIGS. 5D, 5E and 5F are diagrams showing the mean fluorescence intensity (MFI) of the mogamulizumab staining in different CAR-T cells.

[0257] **FIGS. 6A-6F** show the release of cytokines of different CAR-T cells in response to antigen stimulation. FIGS. 6A, 6B and 6C are diagrams showing the production of IL-2 in various CAR-T cells. FIGS. 6D, 6E and 6F are diagrams showing the production of IFNgamma in various CAR-T cells.

[0258] **FIGS. 7A-7D** show the *in vivo* functionality of different CAR-T constructs. FIGS. 7A and 7B are diagrams showing the killing of a cancer cell line with high antigen expression by the indicated CAR-T cells. FIGS. 7C and 7D are diagrams showing the killing of a cancer cell line with low antigen expression by the indicated CAR-T cells.

[0259] **FIGS. 8A-8B** show the inability of a TROP2 tag to be bound by the corresponding antibody (Sacituzumab). FIG. 8A is a diagram showing the percentage of CAR positive cells. FIG. 8B is a diagram showing the percentage of cells positively stained by Sacituzumab.

[0260] **FIGS. 9A-9D** show that T cells expressing a CAR comprising the modified mogamulizumab epitope tag are depleted via ADCC, both *in vitro* and *in vivo*, after treatment with mogamulizumab. FIG. 9A compares the number of GFP-positive (GFP+) T cells for untransduced T cells (UTD) compared to T cells expressing a construct that encodes both the CAR comprising the modified mogamulizumab epitope tag and GFP. FIG. 9B shows the percentage of T cells that are depleted (relative to treatment with an isotype control antibody) after 24 hour *in vitro* co-culture with NK cells at different T cell to NK cell ratios (2:1, 5:1, or 10:1) treated with either 1) 10 ug/mL of an isotype control antibody, 2) 0.1 ug/mL of a mogamulizumab biosimilar antibody, 3) 1.0 ug/mL of a mogamulizumab biosimilar antibody, or 4) 10 ug/mL of a mogamulizumab biosimilar antibody. FIG. 9C shows cell counts in peripheral blood collected from mice treated with either: 1) untransduced T cells (UTD), 2) T cells expressing a CAR comprising the modified mogamulizumab epitope tag but not treated with a mogamulizumab biosimilar antibody (Untreated), 3) T cells expressing a CAR comprising the modified mogamulizumab epitope tag and 1 mg/kg of a mogamulizumab biosimilar antibody on day 7 after T cell infusion (Day 7 Moga Treatment), or 4) T cells expressing a CAR comprising the modified

mogamulizumab epitope tag and 1 mg/kg of a mogamulizumab biosimilar antibody on day 14 after T cell infusion (Day 14 Moga Treatment). FIG. 9D shows cell counts in blood (Blood), bone marrow (BM), or spleen (Spleen) collected from mice 24 hours after treatment with either: 1) untransduced T cells (UTD), 2) T cells expressing a CAR comprising the modified mogamulizumab epitope tag but not treated with a mogamulizumab biosimilar antibody (Untreated), 3) T cells expressing a CAR comprising the modified mogamulizumab epitope tag and a single 1 mg/kg dose of a mogamulizumab biosimilar antibody on day 14 after T cell infusion (Single Moga Dose), or 4) T cells expressing a CAR comprising the modified mogamulizumab epitope tag and four doses of 1 mg/kg mogamulizumab biosimilar antibody, where the mogamulizumab biosimilar antibody was administered on days 5, 8, 11, and 14 after T cell infusion (Repeat Moga Dose).

[0261] **FIGS. 10A-10C** demonstrate that, relative to T cells expressing a CAR comprising an unmodified mogamulizumab epitope tag, T cells expressing a CAR comprising a modified mogamulizumab epitope tag, which modified epitope tag does not comprise a cystine present in the unmodified mogamulizumab epitope tag, exhibit reduced levels of antigen-independent signaling. FIGS. 10A-10B show the level of antigen-independent IFNgamma secretion (FIG. 10A) and T cell proliferation (FIG. 10B) after T cell culture with different concentrations of a mogamulizumab biosimilar (No Moga, 1 ug/mL Moga, 10 ug/mL Moga, or 20 ug/mL Moga). The cells in this assay included T cells expressing a CAR without an epitope tag (CAR), T cells expressing a CAR comprising an unmodified mogamulizumab epitope tag (CAR.Moga), and T cells expressing a CAR comprising a modified mogamulizumab epitope tag that does not comprise a cystine present in the unmodified mogamulizumab epitope tag (CAR.Moga.delCys). FIG. 10C shows the impact on T cell phenotype of T cells expressing a CAR comprising the unmodified mogamulizumab epitope tag or T cells expressing a CAR comprising the modified mogamulizumab epitope tag.

[0262] **FIGS. 11A-11N** demonstrate that a variety of CAR constructs comprising a modified mogamulizumab epitope tag are expressed by T cells at similar levels to matched untagged CAR constructs and, broadly, retain similar levels of functionality. FIGS. 11A-11D show the percentage of CAR positive T cells (FIG. 11A) and CAR MFI (FIG. 11B) based on detection of the CAR and the percentage of CAR positive cells (FIG. 11C) and CAR MFI (FIG. 11D) based on detection of the mogamulizumab epitope tag for T cells expressing a tagged and untagged KLK2 CAR (KLK2.CAR and KLK2.moga-CAR), a

tagged and untagged Claudin-6 CAR (CLDN6.CAR and CLDN6.moga-CAR), a tagged and untagged DLL3 CAR (DLL3.CAR and DLL3.moga-CAR), a tagged and untagged LY6G6D CAR (LY6G6D.CAR and LY6G6D.moga-CAR), and a tagged and untagged CD19 CAR (CD19.CAR and CD19.moga-CAR), FIGS. 11E-11I show IFNgamma secretion after in vitro co-culture with target cells expressing the antigen target for each CAR for T cells expressing both tagged and untagged CARs. FIGS. 11J-N show IL-2 secretion after in vitro co-culture with target cells expressing the antigen target for each CAR for T cells expressing both tagged and untagged CARs.

[0263] **FIGS. 12A-12I** show that different TCR based receptor constructs (transgenic TCRs (tTCRs) and T-bodies) comprising a modified mogamulizumab epitope tag are expressed by T cells at similar levels to matched untagged tTCRs and T-bodies and that T cells expressing tagged T-bodies retain similar levels of functionality to untagged T-bodies. FIGS. 12A-12C show the percentage of T-body positive T cells (FIG. 12A) and T-body MFI (FIG. 12B) based on detection of the T-body and the percentage of T-body positive T cells (FIG. 12C) based on detection of the mogamulizumab epitope tag. FIGS. 12D-12F show the percentage of tTCR positive T cells (FIG. 12D) and tTCR MFI (FIG. 12E) based on detection of the tTCR and the percentage of tTCR positive T cells (FIG. 12F) based on detection of the mogamulizumab epitope tag. FIGS. 12G and 12H show IFNgamma secretion (FIG. 12G) and IL-2 secretion (FIG. 12H) after in vitro co-culture with target cells expressing CD19 for T cells expressing both tagged and untagged anti-CD19 T-body. FIG. 12I shows in vitro NK cell-mediated ADCC depletion of T cells expressing an anti-CD19 T-body comprising the modified mogamulizumab epitope tag.

[0264] In the following detailed description, reference is made to the Figures, which form a part hereof. In the Figures, similar symbols generally identify similar components, unless context dictates otherwise.

[0265] The illustrative alternatives described in the detailed description, drawings, and claims are not meant to be limiting. Other alternatives may be used and other changes may be made without departing from the spirit or scope of the subject matter presented here. It will be readily understood that the aspects and embodiments, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, and designed in a wide variety of different configurations (for example, combined with other aspects and/or embodiments described herein), all of which are explicitly contemplated and make part of this application.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0266] In general, the present disclosure relates, *inter alia*, to compositions and methods for improving adoptive immune cell therapy. The disclosers have discovered ways to improve the control and safety of engineered immune cells by introducing to the surface of the engineered immune cells at least one depletion tag (*e.g.*, an epitope or a mimotope) that is capable of being bound by another molecule (*e.g.*, an antibody) to facilitate the depletion of the engineered immune cells when needed. The depletion tag may be incorporated to the extracellular region of an engineered receptor (*e.g.*, a chimeric antigen receptor or a T cell receptor) or be presented separately on the cell surface. Upon the occurrence of an adverse event in the subject, the engineered cells can be depleted as needed by administering the corresponding tag-binding molecule (*e.g.*, the corresponding antibody). The presence of such tag can also facilitate the sorting and/or enrichment of the engineered cells (*e.g.*, during cell manufacturing).

[0267] At least two classes of depletion tags are disclosed herein. The first class include depletion tags that contain an epitope or mimotope derived from a biomarker of a hematologic cancer (*e.g.*, CCR4). This strategy allows the use of therapeutic antibodies developed for hematologic cancer to effectively deplete the engineered immune cell.

[0268] The second class include depletion tags that contain an epitope or mimotope derived from the same antigen that the engineered receptor binds to (*e.g.*, a DLL3 derived depletion tag for engineered immune cells that bind to DLL3). This strategy allows the use of the corresponding antigen-binding therapeutic antibody to simultaneously deplete the engineered immune cell and at the same time exert its anti-cancer effect.

[0269] Also disclosed are ways to improve the efficacy of the depletion tag in the engineered immune cells context, such as modifications of the epitopes or mimotopes, their placements in the engineered receptor or immune cell, and the delivery of such depletion tags or the antibodies. In some embodiments, the present disclosure relates to exploiting such depletion tags to improve the effectiveness of immune cell therapeutics. In some embodiments, the presence of the depletion tag does not negatively affect, or even improves, the efficacy of the receptor or the engineered cells that it is incorporated into. In particular, in the context of engineered receptors incorporated with the depletion tag, mutating cysteine(s) present in the epitope or mimotope may reduce the level of undesirable antigen-

independent signaling and/or activation (i.e., tonic signaling) and/or improve receptor signaling capability.

I. DEFINITIONS

[0270] Unless otherwise defined, all terms of art, notations, and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this application pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. Many of the techniques and procedures described or referenced herein are well understood and commonly employed using conventional methodology by those skilled in the art. All publications, patent applications, patents, GenBank or other accession numbers and other references mentioned herein are incorporated by reference in their entirety for all purposes.

[0271] The singular form "a", "an", and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes one or more cells, including mixtures thereof. "A and/or B" is used herein to include all of the following alternatives: "A", "B", "A or B", and "A and B."

[0272] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0273] Certain ranges are presented herein with numerical values being preceded by the term "approximately" or "about." The terms "approximately" and "about" are used interchangeably and mean a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by up to 20%, preferably up to 10%, more preferably up to 5%, and more preferably still up to 1%, of a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length, inclusive of the endpoints. When the term "about" is used in conjunction with a numerical range, it

modifies that range by extending the boundaries above and below the numerical values set forth, unless otherwise apparent from context that it is impossible to extend the boundary beyond certain points (*e.g.,* below 0% or above 100% in some cases).

[0274] As used herein, the term "comparable level" refers to a level that is in between 50% and 200%, inclusive of end points, of the reference level in the control set of conditions or circumstances, unless otherwise apparent from context that it is impossible to extend the boundary beyond certain points. In some embodiments, a comparable level may be preferably between 60% and 170%, more preferably between 70% and 140%, even more preferably between 80% and 125%, or more preferably still between 90% and 110%, of the reference level. Those of ordinary skill in the art will appreciate the appropriate control set in obtaining the reference level. In general, the appropriate control set shares identifical features with the test set except the feature(s) of interest, so that the observed results warrant a reasonable conclusion that any changes in the level observed are caused by or indicative of the variation in the feature(s) that are varied.

[0275] As will be understood by one having ordinary skill in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0276] It is understood that aspects and embodiments of the disclosure described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments. As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any elements, steps, or ingredients not specified in the claimed composition or method. As used herein,

"consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claimed composition or method. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of steps of a method, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or step.

[0277] The term "percent identity," as used herein in the context of two or more nucleic acids or proteins, refers to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acids that are the same (*e.g.,* about 60% sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. See *e.g.,* the NCBI web site at ncbi.nlm.nih.gov/BLAST. Such sequences are then the to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. This definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. Generally, sequence identity can exist over a region that is at least about 20 amino acids or nucleotides in length, or over a region that is 10-100 amino acids or nucleotides in length, or over the entire length of a given sequence

[0278] If necessary, sequence identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux et al, Nucleic Acids Res. 12:387, 1984), BLASTP, BLASTN, FASTA (Atschul et al., J. Molecular Biol. 215:403, 1990). Sequence identity can be measured using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group at the University of Wisconsin Biotechnology Center (1710 University Avenue, Madison, Wis. 53705), with the default parameters thereof.

[0279] As used herein, the term "mutation" refers to a point mutation, a gene fusion, a substitution, a gain-of-function mutation, a stop-gain mutation, an insertion mutation, a deletion mutation, a duplication mutation and/or a translocation. The mutation may be in one or more genes. The mutation may be naturally occurring. Alternatively, the mutation may be induced or engineered.

[0280] As used herein, the term "vector" refers to a recombinant nucleic acid designed for transfer between host cells, and that may be used for the purpose of transformation, *e.g.*, the introduction of heterologous DNA into a host cell. As such, in some embodiments, the vector can be a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. In some embodiments, the expression vector can be an integrating vector.

[0281] As used herein, the term "viral vector" refers either to a nucleic acid molecule (*e.g.*, a transfer plasmid) that includes virus-derived nucleic acid elements that generally facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will generally include various viral components and sometimes also host cell components in addition to nucleic acid(s). The term viral vector may refer either to a virus or viral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. Viral vectors and transfer plasmids contain structural and/or functional genetic elements that are primarily derived from a virus. Viral vectors that can be used in the disclosure include, for example, retrovirus vectors, adenovirus vectors, and adeno-associated virus vectors, lentivirus vectors, herpes virus, simian virus 40 (SV40), and bovine papilloma virus vectors (see, for example, Gluzman (Ed.), Eukaryotic Viral Vectors, CSH Laboratory Press, Cold Spring Harbor, N.Y.). For example, a recombinant polypeptide as disclosed herein can be produced in a eukaryotic host, such as a mammalian cells (*e.g.*, COS cells, NIH 3T3 cells, or HeLa cells). These cells are available from many sources, including the American Type Culture Collection (Manassas, VA). In selecting an expression system, care should be taken to ensure that the components are compatible with one another. Artisans or ordinary skill are able to make such a determination. Furthermore, if guidance is required in selecting an expression system, skilled artisans may consult P. Jones, "Vectors: Cloning Applications", John Wiley and Sons, New York, N.Y., 2009).

[0282] As used herein, the term "retroviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus. The retroviral vector can be a lentiviral vector.

[0283] As used herein, the term "lentiviral vector" refers to a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus, which is a genus of retrovirus. Lentiviral vectors offer several attractive properties as gene-delivery vehicles, including: (i) sustained gene

delivery through stable vector integration into host genome; (ii) the capability of infecting both dividing and non-dividing cells; (iii) broad tissue tropisms, including important gene- and cell-therapy-target cell types; (iv) no expression of viral proteins after vector transduction; (v) the ability to deliver complex genetic elements, such as polycistronic or intron-containing sequences; (vi) a potentially safer integration site profile; and (vii) a relatively easy system for vector manipulation and production.

[0284] As used herein, the term "pharmaceutically acceptable carrier" as used herein means any suitable carriers, diluents or excipients. These include all aqueous and non-aqueous isotonic sterile injection solutions, which may contain anti-oxidants, buffers and solutes, which render the composition isotonic with the blood of the intended recipient; aqueous and non-aqueous sterile suspensions, which may include suspending agents and thickening agents, dispersion media, antifungal and antibacterial agents, isotonic and absorption agents and the like. It will be understood that compositions of the present disclosure may also include other supplementary physiologically active agents. The carrier must be pharmaceutically "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the subject.

[0285] As used herein, the term "PEGylation" refers to modifying a protein by covalently attaching polyethylene glycol (PEG) to the protein, with "PEGylated" referring to a protein having a PEG attached. A range of PEG, or PEG derivative sizes with optional ranges of from about 10,000 Daltons to about 40,000 Daltons may be attached to the recombinant polypeptides of the disclosure using a variety of chemistries. In some embodiments, the average molecular weight of the PEG, or PEG derivative, is about 1 kD to about 200 kD such as, *e.g.,* about 10 kD to about 150 kD, about 50 kD to about 100 kD, about 5 kD to about 100 kD, about 20 kD to about 80 kD, about 30 kD to about 70 kD, about 40 kD to about 60 kD, about 50 kD to about 100 kD, about 100 kD to about 200 kD, or about 150 kD to about 200 kD. In some embodiments, the average molecular weight of the PEG, or PEG derivative, is about 5 kD, about 10 kD, about 20 kD, about 30 kD, about 40 kD, about 50 kD, about 60 kD, about 70 kD, or about 80 kD. In some embodiments, the average molecular weight of the PEG, or PEG derivative, is about 40 kD.

[0286] As used herein, the terms "administration" and "administering" refer to the delivery of a bioactive composition or formulation by an administration route including, but not limited to, oral, intravenous, intra-arterial, intramuscular, intraperitoneal, subcutaneous,

intramuscular, and topical administration, or combinations thereof. The term includes, but is not limited to, administering by a medical professional and self-administering.

[0287] As used herein, the term "injection" includes intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion.

[0288] The term "cancer" generally refers to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, and certain characteristic morphological features. Cancer cells can be in the form of a tumor, but such cells can exist alone within an animal subject, or can be a non-tumorigenic cancer cell, such as a leukemia cell. These terms include a solid tumor, a soft tissue tumor, or a metastatic lesion. As used herein, the term "cancer" includes premalignant, as well as malignant cancers. In some embodiments, the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

[0289] As used herein, and unless otherwise specified, a "therapeutically effective" or "pharmaceutically effective" amount or number of a subject construct, nucleic acid, cell, or composition of the disclosure generally refer to an amount or number sufficient for a construct, nucleic acid, cell, or composition to accomplish a stated purpose relative to the absence of the composition, *e.g.,* to provide a therapeutic benefit in the treatment or management of the cancer, or to delay or minimize one or more symptoms associated with the cancer. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapeutic agents, which provides a therapeutic benefit in the treatment or management of the cancer. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of the cancer, or enhances the therapeutic efficacy of another therapeutic agent. An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a "therapeutically effective amount." A "reduction" of a symptom means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The exact amount of a composition including a "therapeutically effective amount" will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, *e.g.,* Lieberman, Pharmaceutical Dosage

Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Pickar, Dosage Calculations (1999); and Remington: The Science and Practice of Pharmacy, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins)

[0290] As used herein, a "subject" or an "individual" includes animals, such as human (*e.g.,* human subjects) and non-human animals. In some embodiments, a "subject" or "individual" is a patient under the care of a physician. Thus, the subject can be a human patient or an individual who has, is at risk of having, or is suspected of having a disease of interest (*e.g.,* cancer) and/or one or more symptoms of the disease. The subject can also be an individual who is diagnosed with a risk of the condition of interest at the time of diagnosis or later. The term "non-human animals" includes all vertebrates, *e.g.,* mammals, *e.g.,* rodents, *e.g.,* mice, e.g. non-human primates, and non- mammals, *e.g.,* sheep, dogs, cows, chickens, amphibians, reptiles, etc.

[0291] The term "antibody" herein is used in the broadest sense and includes polyclonal and monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments, including fragment antigen binding (Fab) fragments, F(ab')2 fragments, Fab' fragments, Fv fragments, recombinant IgG (rlgG) fragments, heavy chain variable (VH) regions capable of binding the antigen, single chain antibody fragments, including single chain variable fragments (scFv), and single domain antibodies (e.g ., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, fully human antibodies, humanized antibodies, and heteroconjugate antibodies, multispecific, *e.g.,* bispecific or trispecific, antibodies, diabodies, triabodies, and tetrabodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof also referred to herein as "antigen-binding fragments." The term also encompasses intact or full-length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD.

[0292] The terms "complementarity determining region," and "CDR," synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen *e.g.,*specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR- H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR- L3). "Framework regions" and "FR" are known in the art to refer to the

non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs in each full- length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4).

[0293] The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme); Al-Lazikani et al, (1997) JMB 273,927-948 ("Chothia" numbering scheme); MacCallum et al, J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme); Lefranc MP el ah, "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(l):55-77 ("IMGT" numbering scheme); Honegger A and Pliickthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657- 70, ("Aho" numbering scheme); and Martin el ah, "Modeling antibody hypervariable loops: a combined algorithm," PNAS, 1989, 86(23):9268-9272, ("AbM" numbering scheme).

[0294] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

[0295] The AbM scheme is a compromise between Kabat and Chothia definitions based on that used by Oxford Molecular's AbM antibody modeling software.

[0296] **Table 1**, below, lists exemplary position boundaries of CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3 as identified by Kabat, Chothia, AbM, and Contact schemes, respectively. For CDR-H1, residue numbering is listed using both the Kabat and Chothia numbering schemes. FRs are located between CDRs, for example, with FR-L1

located before CDR-L1, FR-L2 located between CDR-L1 and CDR-L2, FR-L3 located between CDR-L2 and CDR-L3 and so forth. It is noted that because the shown Kabat numbering scheme places insertions at H35A and H35B, the end of the Chothia CDR-H1 loop when numbered using the shown Kabat numbering convention varies between H32 and H34, depending on the length of the loop.

Table 1 Boundaries of CDRs according to various numbering schemes

Table 1. Boundaries of CDRs according to various numbering schemes.				
CDR	**Kabat**	**Chothia**	**AbM**	**Contact**
CDR-L1	L24--L34	L24--L34	L24--L34	L30--L36
CDR-L2	L50--L56	L50--L56	L50--L56	L46--L55
CDR-L3	L89--L97	L89--L97	L89--L97	L89--L96
CDR-H1 (Kabat Numbering[1])	H31--H35B	H26--H32.34	H26--H35B	H30--H35B
CDR-H1	H31--H35	H26--H32	H26--H35	H30--H35
(Chothia Numbering[2])				
CDR-H2	H50--H65	H52--H56	H50--H58	H47--H58
CDR-H3	H95--H102	H95--H102	H95--H102	H93--H101

1 - Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD 2 - Al-Lazikani et al, (1997) JMB 273,927-948

[0297] Thus, unless otherwise specified, a "CDR" or "complementary determining region," or individual specified CDRs (e.g ., CDR-H1, CDR-H2, CDR-H3), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) complementary determining region as defined by any of the aforementioned schemes, or other known schemes. For example, where it is stated that a particular CDR (*e.g.,* a CDR-H3) contains the amino acid sequence of a corresponding CDR in a given VH or VL region amino acid sequence, it is understood that such a CDR has a sequence of the corresponding CDR (*e.g.,* CDR-H3) within the variable region, as defined by any of the aforementioned schemes, or other known schemes. In some embodiments, specific CDR sequences are specified. Exemplary CDR sequences of provided antibodies are described using various numbering schemes, although it is understood that a provided antibody can include CDRs as described according to any of the other aforementioned numbering schemes or other numbering schemes known to a skilled artisan.

[0298] Likewise, unless otherwise specified, a FR or individual specified FR(s) (*e.g.,* FR-Hl, FR-H2, FR-H3, FR-H4), of a given antibody or region thereof, such as a variable region thereof, should be understood to encompass a (or the specific) framework region as defined by any of the known schemes. In some instances, the scheme for identification of a

particular CDR, FR, or FRs or CDRs is specified, such as the CDR as defined by the Kabat, Chothia, AbM, IMGT or Contact method, or other known schemes. In other cases, the particular amino acid sequence of a CDR or FR is given.

[0299] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable regions of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, *e.g.,* Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer a capability for binding an antigen. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, *e.g.,* Portolano et ah, J. Immunol. 150:880-887 (1993); Clarkson et al, Nature 352:624-628 (1991).

[0300] An "antibody fragment" or "antigen-binding fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that is capable of binding the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; heavy chain variable (VH) regions, single-chain antibody molecules such as scFvs and single-domain antibodies comprising only the VH region; and multispecific antibodies formed from antibody fragments. In particular embodiments, the antibodies from which the CARs are derived are single-chain antibody fragments comprising a heavy chain variable (VH) region and/or a light chain variable (VL) region, such as scFvs.

[0301] Single-domain antibodies (sdAbs) are antibody fragments comprising all or a portion of the heavy chain variable region or all or a portion of the light chain variable region of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody.

[0302] A "humanized" antibody is an antibody in which all or substantially all CDR amino acid residues are derived from non-human CDRs and all or substantially all FR amino acid residues are derived from human FRs. A humanized antibody optionally may include at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of a non-human antibody, refers to a variant of the non-human antibody that has undergone humanization, typically to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. In some embodiments, some FR

residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g ., the antibody from which the CDR residues are derived), *e.g.,* to restore or improve antibody specificity or affinity.

[0303] A "human antibody" is an antibody with an amino acid sequence corresponding to that of an antibody produced by a human or a human cell, or non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, including human antibody libraries. The term excludes humanized forms of non-human antibodies comprising non-human antigen-binding regions, such as those in which all or substantially all CDRs are non-human. The term includes antigen-binding fragments of human antibodies. Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. Human antibodies also may be derived from human antibody libraries, including phage display and cell-free libraries, containing antibody-encoding sequences derived from a human repertoire.

[0304] The term "monoclonal antibody" as used herein refers to an antibody obtained from or within a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical, except for possible variants containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. The term is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be made by a variety of techniques, including but not limited to generation from a hybridoma, recombinant DNA methods, phage-display and other antibody display methods.

[0305] In some embodiments, the binding (*e.g.,* antibody-antigen binding or receptor-antigen binding) is "specific." For example, in some embodiments, the monoclonal antibody of the disclosure is capable of specifically binding to the peptide epitope or mimotope. In some embodiments, the receptor or the binding region of the disclosure is capable of

specifically binding to the antigen. As used herein, the terms "specific" or "specifically," when used with reference to binding, refer to measurable and reproducible interactions, such as binding between a target and an antibody or scFv or binding region or receptor, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody, scFv, binding region, or receptor that specifically binds to a target (which can be an epitope or an antigen) is an antibody, scFv, binding region, or receptor that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other molecules (*e.g.,* the extent of binding of the antibody, scFv, binding region, or receptor to an unrelated molecule is less than about 10% of the binding of the antibody, scFv, binding region, or receptor to the target as measured, *e.g.,* by surface plasmon resonance (SPR)). In some embodiments, a specific binding has a dissociation constant (Kd) of ≤ 1 μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In some embodiments, specific binding can include, but does not require, exclusive binding. In some embodiments, the specific binding is exclusive binding.

[0306] The term "engineered cell" as used herein refers to a cell that has been altered in at least some way by human intervention, including, for example, by genetic alterations or modifications such that the engineered cell differs from a wild-type cell.

[0307] The terms "decrease," "reduced," "reduction," and "decreased" are all used herein generally to mean a lowering by a statistically significant amount. However, for avoidance of doubt, "decrease," "reduced," "reduction," "decreased" means a lowering by at least 10% as compared to a reference level, for example a lowering by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90%, or up to and including a 100% lowering (i.e. absent level as compared to a reference sample), or any lowering between 10-100% as compared to a reference level. In some embodiments, the cells are engineered to have reduced expression of one or more targets relative to an unaltered or unmodified wild-type cell.

[0308] The terms "increase" and "increased" are all used herein generally to mean an increase by a statistically significant amount. However, for avoidance of doubt, "increase" and "increased" mean an increase by at least 10% as compared to a reference level, for example an increase by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least 1-fold, at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 20-fold, at

least 30-fold, at least 50-fold, at least 100-fold, at least 200-fold, at least 500-fold, or at least 1000-fold, or any increase between 10% and 1000-fold as compared to a reference level. In some embodiments, the cells are engineered to have increased expression of one or more targets relative to an unaltered or unmodified wild-type cell.

[0309] As used herein, the term "mimotope" refers to a molecule, or a part of a biomolecule, that mimics the structure of an epitope and is capable of being bound by the same antigen-binding region of an antibody that recognize the epitope. The mimotope may act as a competitor for the corresponding epitope in *in vitro* assays (*e.g.,* ELISA) and/or elicit an immunological response in a host that is reactive to the epitope of which it is a mimic. In some embodiments, the mimotope of the disclosure is a peptide.

[0310] As used herein, the terms "conservative amino acid substitution" and "conservative substitution" refer to amino acid substitutions in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.,* lysine, arginine, histidine), acidic side chains (*e.g.,* aspartic acid, glutamic acid), uncharged polar side chains (*e.g.,* glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.,* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.,* threonine, valine, isoleucine) and aromatic side chains (*e.g.,* tyrosine, phenylalanine, tryptophan, histidine).

[0311] Headings, *e.g.,* (a), (b), (i) etc., are presented merely for ease of reading the specification and claims. The use of headings in the specification or claims does not require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.It is appreciated that certain features of the disclosure, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosure, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the disclosure are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements

thereof are also specifically embraced by the present disclosure and are disclosed herein just as if each and every such sub- combination was individually and explicitly disclosed herein.

II. Epitope or Mimitope Tag

[0312] In one aspect, the disclosure provides an epitope or mimotope. In some embodiments, the epitope or mimotope of the disclosure can be used as depletion tag on the surface of engineered immune cells (*e.g.,* CAR-T cells) to improve the clinical safety of those cells.

[0313] In some embodiments, the epitope or mimotope is comprised within a recombinant polypeptide of the disclosure. In some embodiments, the epitope or mimotope is located in the extracellular region of the recombinant polypeptide. In some embodiments, the recombinant polypeptide can be expressed on the surface of an engineered cell of the disclosure. In some embodiments, the recombinant polypeptide comprises a transmembrane region. In some embodiments, the recombinant polypeptide comprises a glycosylphosphatidylinositol (GPI) anchor. In some embodiments, the recombinant polypeptide comprises a signal peptide during protein translation that enables its expression on the cell surface. In some embodiments, such cell surface expression presents the epitope or mimotope tag so that it is capable of being bound by a corresponding antibody for the epitope or mimotope.

[0314] In some embodiments, the epitope or a mimotope tag is comprised within a recombinant receptor of the disclosure.

[0315] In some embodiments, the peptide epitope is derived from an autoimmune-associated antigen. In some embodiments, the peptide epitope is derived from a cancer-associated antigen.

[0316] In some embodiments, the mimotope mimics an epitope of an autoimmune-associated antigen that is bound by a monoclonal antibody. In some embodiments, the mimotope mimics an epitope of a cancer-associated antigen that is bound by a monoclonal antibody.

[0317] In some embodiments, the autoimmune-associated antigen or cancer-associated antigen is selected from the group consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A,

CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, and any combinations thereof.

[0318] In some embodiments, the cancer-associated antigen is a cancer-ssociated antigen for a hematologic cancer. In some embodiments, the cancer is selected from B cell lymphoma, T cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.

[0319] In some embodiments, the cancer-associated antigen is a cancer-associated antigen for a solid tumor. In some embodiments, the cancer is a solid tumor selected from the group consisting of small cell lung cancer, prostate cancer, colorectal cancer, head and neck

squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, lung cancer, pancreatic cancer, stomach cancer, cervical cancer, hepatocellular carcinoma, glioma cancer, neuroblastoma cancer, and renal cell carcinoma. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin α5β1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, integrin α11β1, DLL3, CD2, SSTR2, TROP2, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA, LY6G6D, IL-1α, CEA, C242 antigen, CanAg (MUC1 glycoform), CTAA16.88, UCY2C, IGF2BP3, p53R175H, NKG2D ligands, PRAME, MAGEA4, vimentin, HER2/neu, CCR4, CCR5, ROPN1, ROR1, KK-LC-1, CA-125, folate receptor 1, folate receptor alpha, MUC1, MUC16, MSLN, CLDN6, WT1, ALPPL2, KRASG12D, KRASG12V, CD70, MSLN, CLDN18.2, HPV E6/E7, GPC3, IL-13Rα, EGFRv3, EGFR806, GD2, or CA9. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin a5b1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, or Integrin α11β1. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

[0320] In some embodiments, the cancer-associated antigen is selected from one listed in Table 2 below.

Table 2. Cancers and Associated Antigens

Cancer	Exemplary Cancer-Associated Antigen(s)	Cancer Type
small cell lung cancer	DLL3, CD2, SSTR2, TROP2	solid tumor
prostate cancer	EpCAM, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA	solid tumor
colorectal cancer	LY6G6D, IL-1alpha, CEA, C242 antigen, EGFR, CanAg (MUC1 glycoform), EpCAM, CTAA16.88, UCY2C, IGF2BP3, p53 R175H mutant, CEA, a NKG2D ligand	solid tumor
head and neck squamous cell carcinoma	IGF2BP3, PRAME, MAGEA4	solid tumor
breast cancer	vimentin, HER2/neu, CCR5, TROP-2, IGF2BP3, P53 R175H mutant, ROPN1, ROR1, KK-LC-1	solid tumor
melanoma	PRAME, ROPN1	solid tumor
ovarian cancer	CA-125, folate receptor 1, folate receptor alpha, MUC1, EpCAM, PRAME, MUC16, CA125, MSLN, CLDN6, WT1, ALPPL2	solid tumor

lung cancer	PRAME, KRAS G12D mutant, KRAS G12V mutant, ROR1, MAGEA4	solid tumor
pancreatic cancer	KRAS G12D mutant, KRAS G12V mutant, CD70, MSLN, CEA	solid tumor
stomach cancer	CLDN18.2, p53 R175H mutant	solid tumor
cervical cancer	HPV E6/E7, MSLN	solid tumor
hepatocellular carcinoma	GPC3	solid tumor
glioma cancer	IL-13Ra, EGFRv3, EGFR806, EGFR, GD2	solid tumor
neuroblastoma cancer	CD2	solid tumor
renal cell carcinoma	PSMA, CA9, CD70	solid tumor
B cell lymphoma	CD19, CD20, CD22, BCMA, GPRC5D, CD38, SLAMF7, CD74, CD40, BAFF, CD25, CD80, IGF-2, CD79b, CD137	hematologic cancer
T cell lymphoma	CCR4, CD3	hematologic cancer
chronic lymphocytic leukemia	CD20, ROR1, CD23, CD28	hematologic cancer
acute myeloid leukemia	WT1, CD33, CLEC12A, FLT3, CD123, CD70, p53 R175H mutant, CD44v6, ADGRE2, LILRB2	hematologic cancer

[0321] In some embodiments, the antigen is not CD19. In some embodiments, the antigen is not CCR4. In some embodiments, the antigen is not CCR7.

[0322] In some embodiments, the antigen is Delta-like protein 3 (DLL3). Human DLL3 (Uniprot Accession No. Q9NYJ7) is a biomarker for small cell lung cancer. In some embodiments, the DLL3 protein comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 486. In some embodiments, the epitope comprises at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50, consecutive amino acids in SEQ ID NO: 486, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the epitope comprises about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, consecutive amino acids in SEQ ID NO: 486, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of any one of SEQ ID NO: 439-468, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of SEQ ID NO: 439, or an amino acid sequence having at most 1, at

most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of SEQ ID NO: 439.

[0323] In some embodiments, the epitope is derived from a cancer-associated antigen of adult T-cell lymphoma (ATL) and/or cutaneous T-cell lymphoma (CTCL). In some embodiments, the epitope is derived from a cancer-associated antigen of a Th2 cell, a cutaneous lymphocyte antigen-positive skin-homing T cell, and/or a Treg cell.

[0324] In some embodiments, the epitope is derived from C-C chemokine receptor type 4 (CCR4) (i.e., the cancer associated antigen is CCR4). C-C chemokine receptor type 4 (CCR4), also known as CD194, is a biomarker for T cell lymphoma. Further information of human CCR4 protein is described in Uniprot Accession No. P51679. In some embodiments, the CCR4 protein comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 485. In some embodiments, the epitope comprises at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50, consecutive amino acids in SEQ ID NO: 485, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the epitope comprises about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, consecutive amino acids in SEQ ID NO: 485, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NO: 430-438, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 430. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 431.

[0325] In some embodiments, the epitope is derived from C-C chemokine receptor type 7 (CCR7) (i.e., the cancer associated antigen is CCR7). C-C chemokine receptor type 7 (CCR7), also known as CD197, is a biomarker for types of cancer including breast, lung, pancreatic, squamous cell carcinoma, and gastric cancer. CCR7 is also associated with cancer progression and poor outcomes, including metastasis. Further information of human CCR7 protein is described in Uniprot Accession No. P32248. In some embodiments, the CCR7 protein comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at

least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 557. In some embodiments, the epitope comprises at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50, consecutive amino acids in SEQ ID NO: 557, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the epitope comprises about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, consecutive amino acids in SEQ ID NO: 557, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NO: 519-524, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 519. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 520. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 521. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 522. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 523. In some embodiments, the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 524.

[0326] In some embodiments, the peptide epitope or the mimotope is about 5 to about 10, about 10 to about 20, about 15 to about 25, about 20 to about 30, about 25 to about 35, about 30 to about 40, about 35 to about 45, or about 40 to about 50 amino acids in length. In some embodiments, the peptide epitope or the mimotope is no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids in length. In some embodiments, the peptide epitope does not comprise any mutation compared to the native epitope derived from the antigen. In some embodiments, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the peptide epitope or the mimotope is capable of forming an alpha-helical conformation.

[0327] In some embodiments, the epitope or mimotope is capable of being bound by the antigen-binding molecule (*e.g.,* antibody) with a dissociation constant (Kd) of less than 100 μM, less than 10 μM, less than 1 μM, less than 100 nM, less than 10 nM, less than 1 nM,

less than 100 pM, less than 10 pM, or less than 1 pM, optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized the antigen-binding molecule (*e.g.,* antibody). In some embodiments, the modified peptide epitope or mimotope is capable of being bound by the antigen-binding molecule (*e.g.,* antibody) with a dissociation constant (Kd) of about 100 μM to about 10 μM, about 10 μM to about 1 μM, about 1 μM to about 100 nM, about 100 nM to about 10 nM, about 10 nM to about 1 nM, about 1 nM to about 100 pM, about 100 pM to about 10 pM, or about 10 pM to about 1 pM, optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antigen-binding molecule (*e.g.,* antibody).

[0328] In some embodiments, the epitope or mimotope is that of an antibody. In some embodiments, the antibody is [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin,

Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589. In some embodiments, the antibody is tarlatamab. In some embodiments, the antibody is mogamulizumab. In some embodiments, the antibody is CAP-100.

Modified Epitope or Mimotope

[0329] In some embodiments, the peptide epitope or mimotope of the disclosure comprises or consists of an amino acid sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations compared to an unmodified peptide epitope or mimotope and is capable of being bound by the corresponding monoclonal antibody that the unmodified epitope or mimotope binds to. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4, or 5 mutations compared to the unmodified peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 5 mutations compared to the unmodified peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 4 mutations compared to the unmodified peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 3 mutations compared to the unmodified peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope comprises or consists of an amino acid sequence having 2 mutations compared to the unmodified peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope comprises or consists

of an amino acid sequence having 1 mutation compared to the unmodified peptide epitope or mimotope.

[0330] In some embodiments, the antibody binding affinity of the modified epitope or mimotope is at a comparable level as the antibody binding affinity of the unmodified epitope or mimotope. In some embodiments, the modified peptide epitope or mimotope is capable of being bound by the corresponding antibody with a dissociation constant (Kd) of less than 100 μM, less than 10 μM, less than 1 μM, less than 100 nM, less than 10 nM, less than 1 nM, less than 100 pM, less than 10 pM, or less than 1 pM, optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody. In some embodiments, the modified peptide epitope or mimotope is capable of being bound by the corresponding antibody with a dissociation constant (Kd) of about 100 μM to about 10 μM, about 10 μM to about 1 μM, about 1 μM to about 100 nM, about 100 nM to about 10 nM, about 10 nM to about 1 nM, about 1 nM to about 100 pM, about 100 pM to about 10 pM, or about 10 pM to about 1 pM, optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody.

[0331] In some embodiments, the peptide epitope or mimotope of the disclosure comprises or consists of an amino acid sequence having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations compared to an unmodified peptide epitope or mimotope and is capable of being bound by the corresponding monoclonal antibody that the unmodified epitope or mimotope binds to.

[0332] In some embodiments, the peptide epitope or mimotope of the disclosure comprises or consists of an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations compared to an unmodified peptide epitope or mimotope and is capable of being bound by the corresponding monoclonal antibody that the unmodified epitope or mimotope binds to. In some embodiments, the peptide epitope or mimotope of the disclosure comprises or consists of an amino acid sequence having 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, or 9-10 mutations compared to an unmodified peptide epitope or mimotope and is capable of being bound by the corresponding monoclonal antibody that the unmodified epitope or mimotope binds to.

[0333] In some embodiments, the one or more mutations in the modified epitope or mimotope comprise or consist of substitutions, insertions, deletions, or any combination

thereof. In some embodiments, the one or more mutations in the modified epitope or mimotope comprise or consist of substitutions and/or deletions.

[0334] In some embodiments, the one or more mutations in the modified epitope or mimotope comprise or consist of substitutions. In some embodiments, the substitution(s) are conservative amino acid substitutions.

[0335] In some embodiments, the mutation comprises addition and/or deletion of one or more amino acids at the N-term or C-term of the unmodified epitope or mimotope. In some embodiments, the mutation comprises deleting 1, 2, 3, 4, or 5, amino acids at the N-terminus of the unmodified epitope or mimotope. In some embodiments, the mutation comprises deleting 1, 2, 3, 4, or 5, amino acids at the C-terminus of the unmodified epitope or mimotope. In some embodiments, the mutation comprises inserting 1, 2, 3, 4, or 5, amino acids at the N-terminus of the unmodified epitope or mimotope. In some embodiments, the mutation comprises inserting 1, 2, 3, 4, or 5, amino acids at the C-terminus of the unmodified epitope or mimotope. In some embodiments, the mutation comprises deleting 1, 2, 3, 4, or 5, amino acids at the N-terminus of the unmodified epitope or mimotope and inserting 1, 2, 3, 4, or 5, amino acids at the C-terminus of the unmodified epitope or mimotope. In some embodiments, the mutation comprises inserting 1, 2, 3, 4, or 5, amino acids at the N-terminus of the unmodified epitope or mimotope and deleting 1, 2, 3, 4, or 5, amino acids at the C-terminus of the unmodified epitope or mimotope.

[0336] In some embodiments, the cysteine(s) in the unmodified peptide epitope or mimotope are substituted. In some embodiments, the N-terminus of the unmodified peptide epitope or mimotope contains cysteine. In some embodiments, the C-terminus of the unmodified peptide epitope or mimotope contains cysteine. In some embodiments, the unmodified peptide epitope or mimotope comprises at least one cysteine between the N-terminus and the C-terminus. In some embodiments, the cysteine(s) are substituted with serine, glycine, threonine, alanine, valine, or any combination thereof. In some embodiments, the cysteine(s) are substituted with serine and/or valine. In some embodiments, the cysteine(s) are substituted with serine(s). In some embodiments, the cysteine(s) are substituted with valine(s). In some embodiments, the modified epitope or mimotope does not comprise any cysteine(s).

[0337] In some embodiments, the mutation(s) comprise deleting or substituting one or more amino acids that facilitate polypeptide crosslinking in the unmodified peptide epitope. In some embodiments, the mutation(s) comprise deleting and/or substituting one or more

cysteine(s), lysine(s), aspartic acid(s), glutamic acid(s), or any combination thereof, in the unmodified peptide epitope or mimotope.

[0338] In some embodiments, the unmodified peptide epitope or mimotope comprises or consists of no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50, consecutive amino acids in an autoimmune-associated antigen or a cancer-associated antigen. In some embodiments, the unmodified peptide epitope or mimotope comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, consecutive amino acids in an autoimmune-associated antigen or a cancer-associated antigen.

[0339] In some embodiments, the epitope or mimotope is capable of being bound by a monoclonal antibody that targets a biomarker for a hematologic cancer. In some embodiments, the epitope or mimotope is capable of being bound by an anti-CCR4 monoclonal antibody. In some embodiments, the CCR4 protein comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 485. In some embodiments, the unmodified epitope or mimotope comprises at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, or at least 50, consecutive amino acids in SEQ ID NO: 485. In some embodiments, the unmodified epitope or mimotope comprises about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, consecutive amino acids in SEQ ID NO: 485.

[0340] In some embodiments, the epitope or mimotope is capable of being bound by mogamulizumab, a humanized, afucosylated monoclonal antibody targeting CCR4. The unmodified epitope of mogamulizumab has the amino acid sequence of DESIYSNYYLYESIPKPC (SEQ ID NO: 430). In some embodiments, the modified epitope comprising a substitution or deletion of the cysteine at the C-term of SEQ ID NO: 430. In some embodiments, the modified epitope comprises a serine, glycine, threonine, alanine, or valine substitution of the cysteine at the C-term of SEQ ID NO: 430. In some embodiments, the modified epitope comprises or consists of any one of SEQ ID NO: 432-436. In some embodiments, the modified epitope comprises the deletion of 1, 2, 3, 4, or 5 amino acids at the C-term of SEQ ID NO: 430. In some embodiments, the modified epitope comprises or

consists of SEQ ID NO: 431. . In some embodiments, the modified epitope comprises or consists of SEQ ID NO: 438. In some embodiments, the modified epitope comprises the insertion of 1, 2, 3, 4, or 5, amino acid from the corresponding position of CCR4 (SEQ ID NO: 485) before the N-term of SEQ ID NO: 430. In some embodiments, the modified epitope comprises or consists of SEQ ID NO: 437.

[0341] In some embodiments, the modified peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations to any one of the unmodified epitopes and mimotopes listed in **Table 3** below and is capable of being bound by the corresponding monoclonal antibody that binds to that unmodified epitope or mimotope.

Table 3. Epitopes and Mimotopes

Epitope or Mimotope	Sequence of the Unmodified Epitope or Mimotope	SEQ ID NO
Rituximab mimotope	CPYSNPSLC	475
Palivizumab epitope	SELLSLINDMPITNDQKKLMSNN	476
Cetuximab mimotope #1	CQFDLSTRRLKC	477
Cetuximab mimotope #2	CQYNLSSRALKC	478
Cetuximab mimotope #3	CVWQRWQKSYVC	479
Cetuximab mimotope #4	CMWDRFSRWYKC	480
Nivolumab epitope #1	SFVLNWYRMSPSNQTDKLAAFPEDR	481
Nivolumab epitope #2	SGTYLCGAISLAPKAQIKE	482
QBend-10	ELPTQGTFSNVSTNVSPAKPTTTA	483
Alemtuzumab	GQNDTSQTSSPS	484
Sacituzumab TROP2 Epitope	QGRGGLDLRV RGEPLQ	469
anti-GUCY2C Epitope 1	NSGDCRSSTCEGLDLLRKIS	470
anti-GUCY2C Epitope 2	RSSTCEGLDLLRKISNAQRM	471
anti-GUCY2C Epitope 3	NSGDCRSSTCEGLDLLRKISNAQRM	472
anti-GUCY2C Epitope 4	RSSTCEGLDLLRKIS	473
anti-GUCY2C Epitope 5	RSSTCEGLDLLRKI	474
Tarlatamab DLL3 (EGF3) Epitope	SGVTCADGPCFNGGLCVGGADPDS AYICHCPPGFQGSNCE	439
DLL3 EGF6	RADPCAARPCAHGGRCYAHFSGLV CACAPGYMGARCE	440
DLL3 EGF6 Epitope 1	RADPCAARPC	441
DLL3 EGF6 Epitope 2	ADPCAARPCA	442
DLL3 EGF6 Epitope 3	DPCAARPCAH	443
DLL3 EGF6 Epitope 4	PCAARPCAHG	444
DLL3 EGF6 Epitope 5	CAARPCAHGG	445
DLL3 EGF6 Epitope 6	AARPCAHGGR	446

DLL3 EGF6 Epitope 7	ARPCAHGGRC	447
DLL3 EGF6 Epitope 8	RPCAHGGRCY	448
DLL3 EGF6 Epitope 9	PCAHGGRCYA	449
DLL3 EGF6 Epitope 10	CAHGGRCYAH	450
DLL3 EGF6 Epitope 11	AHGGRCYAHF	451
DLL3 EGF6 Epitope 12	HGGRCYAHFS	452
DLL3 EGF6 Epitope 13	GGRCYAHFSG	453
DLL3 EGF6 Epitope 14	GRCYAHFSGL	454
DLL3 EGF6 Epitope 15	RCYAHFSGLV	455
DLL3 EGF6 Epitope 16	CYAHFSGLVC	456
DLL3 EGF6 Epitope 17	YAHFSGLVCA	457
DLL3 EGF6 Epitope 18	AHFSGLVCAC	458
DLL3 EGF6 Epitope 19	HFSGLVCACA	459
DLL3 EGF6 Epitope 20	FSGLVCACAP	460
DLL3 EGF6 Epitope 21	SGLVCACAPG	461
DLL3 EGF6 Epitope 22	GLVCACAPGY	462
DLL3 EGF6 Epitope 23	LVCACAPGYM	463
DLL3 EGF6 Epitope 24	VCACAPGYMG	464
DLL3 EGF6 Epitope 25	CACAPGYMGA	465
DLL3 EGF6 Epitope 26	ACAPGYMGAR	466
DLL3 EGF6 Epitope 27	CAPGYMGARC	467
DLL3 EGF6 Epitope 28	APGYMGARCE	468
CCR7 tag unmodified	NTTVDYTLFESLCSKK	519
CCR7 tag C to S	NTTVDYTLFESLSSKK	520
CCR7 tag C to G	NTTVDYTLFESLGSKK	521
CCR7 tag C to T	NTTVDYTLFESLTSKK	522
CCR7 tag C to A	NTTVDYTLFESLASKK	523
CCR7 tag C to V	NTTVDYTLFESLVSKK	524

[0342] In some embodiments, the modified peptide epitope or mimotope is capable of being bound by [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab,

Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589. In some embodiments, the modified peptide epitope or mimotope comprises or consists of an amino acid sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (substitutions, insertions, and/or deletions) compared to an unmodified peptide epitope or mimotope of one of these antibodies but is capable of being bound by that antibody.

[0343] In some embodiments, the modified epitope or mimotope is capable of being bound by an anti-DLL3 monoclonal antibody. In some embodiments, the modified epitope or mimotope comprises 1, 2, 3, 4, or 5, mutations compared to the epitope or mimotope of the anti-DLL3 monoclonal antibody. In some embodiments, the anti-DLL3 monoclonal antibody is tarlatamab. In some embodiments, the unmodified epitope or mimotope comprises or consists of SEQ ID NO: 439.

III. Recombinant Polypeptide

[0344] In one aspect, the disclosure provides recombinant polypeptide comprising or consisting of the epitope or mimotope of the disclosure. In some embodiments, the epitope or mimotope is comprised within a tag. That is, in some embodimetns, the disclosure provides recombinant polypeptides comprising a tag, wherein the tag comprises or consists of the peptide epitope or the mimotope of the disclosure.

[0345] In some embodimetns, the epitope or mimotope is a modified epitope or mimotope of the disclosure.

[0346] In some embodiments, the peptide epitope or mimotope is expressed on the surface of a cell. In some embodiments, the recombinant polypeptide comprises an extracellular region that comprises the peptide epitope or mimotope. In some embodiments, the peptide epitope or mimotope within the recombinant polypeptide is capable of being bound by the corresponding binder (*e.g.,* an antibody) when expressed on the surface of an engineered cell.

[0347] In some embodiments, the epitope or mimotope is derived from the group consisting of CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, and CD52, or is capable of being bound by an antigen-binding molecule (*e.g.,* antibody) that binds to one of these proteins. In some embodiments, the recombinant polypeptide comprises or consists of CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, or CD52, or a functional fragment thereof that comprises the epitope or mimotope. In some embodiments, the epitope or mimotope is derived from CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, or CD52 but incorporated into a different polypeptide to form the recombinant polypeptide; that is, other than the epitope or mimotope, the recombinant polypeptide is not derived from that protein. In some embodiments, the recombinant polypeptide comprises an epitope or

mimotope that is derived from capable of being bound by an anti-CCR4 antibody but is otherwise not derived from CCR4.

[0348] In some embodiments, the epitope or mimotope within the recombinant polypeptide does not comprise a cysteine at its N-terminus. In some embodiments, the recombinant polypeptide does not comprise a cysteine immediately before the N-terminus of the modified peptide epitope or mimotope.

[0349] In some embodiments, the epitope or mimotope within the recombinant polypeptide does not comprise a cysteine at its C-terminus. In some embodiments, the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the modified peptide epitope or mimotope.

[0350] In some embodiments, the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s). In some embodimetns, the one or more additional peptide epitope(s) or mimotope(s) are the same as the modified peptide epitope. In some embodimetns, the one or more additional peptide epitope(s) or mimotope(s) are different from the modified peptide epitope. In some embodiments, the recombinant polypeptide comprises 1, 2, 3, or 4 additional peptide epitopes or mimotopes.

[0351] In some embodiments, the recombinant polypeptide comprises a binding region for an antigen. In some embodiments, the antigen is expressed on the surface of a target cell. In some embodiments, the antigen is an autoimmune-associated antigen. In some embodiments, the antigen is a cancer-associated antigen.

[0352] In some embodiments, the antigen for the binding region is selected from the group consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2,

signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, and any combinations thereof.

[0353] In some embodiments, the cancer-associated antigen for the binding region is a cancer-ssociated antigen for a hematologic cancer. In some embodiments, the cancer is selected from B cell lymphoma, T cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.

[0354] In some embodiments, the cancer-associated antigen for the binding region is a cancer-associated antigen for a solid tumor. In some embodiments, the cancer is a solid tumor selected from the group consisting of small cell lung cancer, prostate cancer, colorectal cancer, head and neck squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, lung cancer, pancreatic cancer, stomach cancer, cervical cancer, hepatocellular carcinoma, glioma cancer, neuroblastoma cancer, and renal cell carcinoma. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin α5β1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, integrin α11β1, DLL3, CD2, SSTR2, TROP2, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA, LY6G6D, IL-1α, CEA, C242 antigen, CanAg (MUC1 glycoform), CTAA16.88, UCY2C, IGF2BP3, p53R175H,

NKG2D ligands, PRAME, MAGEA4, vimentin, HER2/neu, CCR4, CCR5, ROPN1, ROR1, KK-LC-1, CA-125, folate receptor 1, folate receptor alpha, MUC1, MUC16, MSLN, CLDN6, WT1, ALPPL2, KRASG12D, KRASG12V, CD70, MSLN, CLDN18.2, HPV E6/E7, GPC3, IL-13Rα, EGFRv3, EGFR806, GD2, or CA9. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin a5b1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, or Integrin α11β1. In some embodiments, the cancer-associated antigen for the solid tumor comprises or consists of KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

[0355] In some embodiments, the cancer-associated antigen for the binding region is selected from one listed in **Table 2** *supra*.

[0356] In some embodiments, the antigen for the binding region is not CD19. In some embodiments, the antigen for the binding region is not CCR4. In some embodiments, the antigen is not CCR7.

[0357] In some embodiments, the antigen for the binding region is Delta-like protein 3 (DLL3).

[0358] In some embodiments, the antigen for the binding region is a cancer-associated antigen of adult T-cell lymphoma (ATL) and/or cutaneous T-cell lymphoma (CTCL). In some embodiments, In some embodiments, the antigen for the binding region is a cancer-associated antigen of a Th2 cell, a cutaneous lymphocyte antigen-positive skin-homing T cell, and/or a Treg cell. In some embodiments, the antigen for the binding region is C-C chemokine receptor type 4 (CCR4).

[0359] In some embodiments, the binding region is derived from an antibody. For example, the binding region may comprise or consist of an scFv comprising the VH and/or VL derived from the antibody. In some embodiments, the antibody is [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan,

Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589.

[0360] In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises the peptide epitope or mimotope that is capable of being bound by a monoclonal antibody that binds to the same antigen (*e.g.,* an autoimmune associated antigen or cancer associated antigen of the disclosure) as the binding region. For example, in some embodiments, the

binding region is for binding to DLL3 antigen, and the recombinant polypeptide comprises an epitope or mimotope that is capable of being bound by an anti-DLL3 monoclonal antibody.

[0361] In some embodiments, the peptide epitope or mimotope is separated from the binding region by no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

[0362] In some embodiments, the binding region is a single-domain antibody fragment, single-chain variable fragment, antibody variable fragment, complementary determining region 3 fragment, constrained FR3-CDR3-FR4 polypeptide, Fd fragment, antigen-binding fragment, fibronectin-derived 10th fibronectin type III domain, tenascin type III domain, ankyrin repeat motif domain, low-density-lipoprotein-receptor-derived A-domain, lipocalin, Kunitz domain, Protein-A-derived Z domain, gamma-B crystallin-derived domain, ubiquitin-derived domain, Sac7d-derived polypeptide, Fyn-derived SH2 domain, miniprotein, C-type lectin-like domain scaffold, a heavy-chain antibody domain derived from a camelid V_HH fragment, heavy-chain antibody domain derived from cartilaginous fish, immunoglobulin new antigen receptor (IgNAR), V_{NAR} fragment, diabody, triabody, tetrabody, bivalent minibody, bispecific tandem scFv, bispecific tandem V_HH, or bispecific minibody.

[0363] In some embodiments, the peptide epitope or mimotope is located N-terminal to the binding region in the recombinant polypeptide. In some embodiments, the peptide epitope or mimotope is separated from the N-terminus of the binding region by no more than 40, no more than 35, no more than 30, no more than 25, no more than 20, no more than 15, no more than 10, no more than 5, no more than 4, no more than 3, no more than 2, no more than 1, or zero amino acid(s).

[0364] In some embodiments, the recombinant polypeptide comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids N-terminal to the binding region(s), excluding the signal peptide. In some embodiments, the recombinant polypeptide comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids N-terminal to the binding region(s), excluding the signal peptide.

[0365] In some embodiments, the peptide epitope or mimotope is located C-terminal to the binding region in the recombinant polypeptide. In some embodiments, the peptide epitope or mimotope is separated from the C-terminus of the binding region by zero, no more than 5, no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, or no more than 60 amino acids.

[0366] In some embodiments, the recombinant polypeptide comprises no more than 10, no more than 15, no more than 20, the recombinant polypeptide comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, no more than 60, no more than 65, or no more than 70 amino acids after the C-terminus of the binding region.

[0367] In some embodiments, the peptide epitope or mimotope is located within the binding region.

[0368] In some embodiments, the binding region comprises a heavy chain variable domain (VH) and a light chain variable domain (VL). In some embodiments, the binding region comprises a single chain variable fragment (scFv) comprising a VH and a VL. In some embodiments, the VH and the VL is separated by a linker. In some embodiments, the peptide epitope or mimotope is located within the linker. In some embodiments, the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids. In some embodiments, the linker comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids.

[0369] In some embodiments, the binding region comprises one or more variable heavy domains of heavy chain (VHHs). In some embodiments, the binding region comprises at least two VHHs separated by a linker. In some embodiments, the peptide epitope or mimotope is located within the linker. In some embodiments, the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids. In some embodiments, the linker comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids.

[0370] In some embodiments, the binding region comprises a first binding domain and a second binding domain, wherein the first binding domain and the second binding domain are separated by a linker. In some embodiments, the peptide epitope or mimotope is located within the linker. In some embodiments, the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids. In some embodiments, the linker comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids.

[0371] In some embodiments, the recombinant polypeptide comprises a second binding region located N-terminal or C-terminal to the binding region; optionally, wherein the binding region and the second binding region are separated by a linker. In some embodiments, the peptide epitope or mimotope is located within the linker. In some embodiments, the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids. In some embodiments, the linker comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids.

[0372] In some embodiments, the peptide epitope or mimotope is located in between the binding region(s) and a transmembrane region. In some embodiments, the peptide epitope or mimotope is located within a hinge region that connects the binding region(s) to a transmembrane region. In some embodiments, the hinge region comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, no more than 60, no more than 65, or no more than 70 amino acids. In some embodiments, the hinge region comprises or consists of about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, about 45 to about 50, about 50 to about 55, about 55 to about 60, about 60 to about 65, or about 65 to about 70, amino acids.

[0373] In some embodiments, the extracellular region of the recombinant polypeptide (*e.g.,* CAR or TCR) comprises no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, or no more than 500, amino acids.

[0374] In some embodiments, the extracellular region of the recombinant polypeptide (*e.g.,* CAR or TCR) comprises about 200 to about 250, about 250 to about 300, about 300 to about 350, about 350 to about 400, about 400 to about 450, or about 450 to about 500, amino acids. In some embodiments, the extracellular region of the recombinant polypeptide (*e.g.,* CAR or TCR) comprises no more than 400 amino acids.

[0375] In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, no more than 500, no more than 550, no more than 600, no more than 650, no more than 700, no more than 750, or no more than 800, amino acids. In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises about 200 to about 250, about 250 to about 300, about 300 to about 350, about 350 to about 400, about 400 to about 450, about 450 to about 500, about 500 to about 550, about 550 to about 600, about 600 to about 650, about 650 to about 700, about 700 to about 750, or about 750 to about 800, amino acids. In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises no more than 600 amino acids. In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises no more than 550 amino acids.

[0376] In some embodiments, the recombinant polypeptide comprises or consists of an engineered receptor.

[0377] In some embodiments, the recombinant polypeptide does not comprise a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

[0378] In some embodiments, the recombinant polypeptide comprises or consists of a T cell receptor fusion construct (TRuC). In some embodiments, the TRuC is derived from a CD3 protein and comprises a binding region. In some embodiments, the CD3 protein is CD3ε.

[0379] In some embodiments, the recombinant polypeptide comprises or consists of a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

[0380] In some embodiments, the recombinant polypeptide comprises or consists of a receptor comprising: (a) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRalpha, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRbeta; or (b) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRbeta, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRalpha. In some embodiments, , at least one of the constant region of TCRalpha or the constant region of

TCRbeta are an endogenous constant region of TCRalpha or an endogenous constant region of TCRbeta. In some embodiments, the receptor is a T-body.

[0381] In some embodiments, the peptide epitope or mimotope is located between the VH and the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located between the VL and the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located between the VH and the constant region of TCRbeta. In some embodiments, the peptide epitope or mimotope is located between the VL and the constant region of TCRbeta. In some embodiments, the peptide epitope or mimotope is located N-terminal to the VH. In some embodiments, the peptide epitope or mimotope is located N-terminal to the VL. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region of TCRalpha. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region of TCRbeta.

[0382] In some embodiments, the recombinant polypeptide comprises an extracellular portion comprising the binding region and the peptide epitope or mimotope.

[0383] In some embodiments, the recombinant polypeptide comprises a transmembrane region. In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) comprises, from N-terminus to C-terminus, the binding region(s), a hinge region, a transmembrane region. In some embodiments, the transmembrane region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 421. In some embodiments, the hinge region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 420.

[0384] In some embodiments, the recombinant polypeptide (*e.g.,* CAR or TCR) further comprises an intracellular region. In some embodiments, the intracellular region comprises one or more intracellular signaling domains. In some embodiments, the intracellular domain is located C-terminal to the transmembrane region.

[0385] In some embodiments, the one or more intracellular signaling domains are derived from a protein selected from the group consisting of CD3zeta, 1xxCD3zeta, CD28, 4-1BB, OX40, IL2Rb turbodomains, MYD88/CD40, MYD88/CD40 inducible costimulatory domain, CD2, B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B;

BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; 0X40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB- A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thyl; CD96; CD160; CD200; CD300a/LMIRl; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-l; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin- 1/CLEC7A; DPPIV/CD26; EphB6; TIM- 1 /KIM- 1 /HA VCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, an immunoreceptor tyrosine based activation motif (ITAM), CD27, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, and any combinations thereof.

[0386] In some embodiments, the one or more intracellular signaling domains comprises singaling domain(s) derived from 4-1BB and/or CD3zeta. In some embodiments, the intracellular region comprises the signaling domain derived from 4-1BB and the signaling domain derived from CD3zeta. In some embodiments, the intracellular region comprises, from N-term to C-term, (i) the signaling domain derived from 4-1BB and (ii) the signaling domain derived from CD3zeta. In some embodiments, the signaling domain derived from 4-1BB comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 422. In some embodiments, the signaling domain derived from CD3zeta comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 423. In some embodiments, the signaling domain derived from CD3zeta comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 488, wherein the amino acid corresponding to position 14 of SEQ ID NO: 488 is lysine.

[0387] In some embodiments, the recombinant polypeptide comprises a glycosylphosphatidylinositol (GPI) anchor.

[0388] In some embodiments, the TCR is TCRalpha. In some embodiments, the TCR is TCRbeta. In some embodiments, the binding region of the TCR comprises a variable region and a constant region. In some embodiments, the peptide epitope or mimotope is located between the variable region and the constant region. In some embodiments, the variable region and the constant region in the TCR are separated by no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids. In some embodiments, the variable region and the constant region in the TCR are separated by about 10 to about 15, about 15 to about 20, about 20 to about 25, about 25 to about 30, about 30 to about 35, about 35 to about 40, about 40 to about 45, or about 45 to about 50, amino acids. In some embodiments, the peptide epitope or mimotope is located N-terminal to the variable region of the TCR. In some embodiments, the peptide epitope or mimotope is located C-terminal to the constant region of the TCR.

[0389] In some embodiments, an immune cell expressing the recombinant polypeptide (*e.g.,* receptor) of the disclosure exhibits one or more of the following: (a) improved binding to the antigen expressed on the surface of the target cell; (b) reduced recombinant polypeptide mediated antigen-independent signaling and/or activation of the immune cell; and/or (c) reduced cross-linking between the recombinant polypeptide expressed on the cell surface of the immune cell, compared to a control immune cell expressing a control recombinant polypeptide that does not comprise the epitope or mimotope.

[0390] In some embodiments, an immune cell expressing the recombinant polypeptide (*e.g.,* receptor) of the disclosure exhibits one or more of the following: (a) comparable level of binding to the antigen expressed on the surface of the target cell; (b) comparable level of recombinant polypeptide mediated antigen-independent signaling and/or activation of the immune cell; and/or (c) comparable level of cross-linking between the recombinant polypeptide expressed on the cell surface of the immune cell, compared to a control immune cell expressing a control recombinant polypeptide that does not comprise the epitope or mimotope.

In some embodiments, the recombinant polypeptide comprises a modified epitope or mimotope of the disclosure. In some embodiments, an immune cell expressing the recombinant polypeptide (*e.g.,* receptor) comprising the modified epitope or mimotope exhibits one or more of the following: (a) improved binding to the antigen expressed on the surface of the target cell; (b) reduced recombinant polypeptide mediated antigen-

independent signaling and/or activation of the immune cell; and/or (c) reduced cross-linking between the recombinant polypeptide expressed on the cell surface of the immune cell, compared to a control immune cell expressing a control recombinant polypeptide that comprise the corresponding unmodified epitope or mimotope. In some embodiments, an immune cell expressing the recombinant polypeptide (*e.g.,* receptor) comprising the modified epitope or mimotope exhibits one or more of the following: (a) comparable level of binding to the antigen expressed on the surface of the target cell; (b) comparable level of recombinant polypeptide mediated antigen-independent signaling and/or activation of the immune cell; and/or (c) comparable level of cross-linking between the recombinant polypeptide expressed on the cell surface of the immune cell, compared to a control immune cell expressing a control recombinant polypeptide that comprises the corresponding unmodified epitope or mimotope.

IV. Engineered Receptors and Components Thereof

[0391] In one aspect, the disclosure provides engineered receptors, such as Chimeric Antigen Receptors (CARs), T Cell Receptors (TCRs), T cell receptor fusion constructs (TRuCs), and other synthetic Receptors. In some embodiments, the engineered receptor is expressed in a cell. In some embodiments, the engineered receptor comprises the epitope or mimotope of the disclosure. In some embodiments, the engineered receptor is co-expressed with a separate recombinant polypeptide comprising the epitope or mimotope.

[0392] In some embodiments, the engineered receptor is selected form a chimeric antigen receptor (CAR), a T cell receptor (TCR) such as a recombinant TCR, a T cell receptor fusion construct (TRuC), a chimeric auto antigen receptor (CAAR), a B-cell autoantibody receptor (BAR), a chimeric cytokine receptor (CCR), a chimeric chemokine receptor, a synthetic RIP family receptor, a T-body, a synthetic notch receptor (synNotch), a synthetic intramembrane proteolysis receptor (SNIPR), a synthetic Robo receptor (SynRobo receptor), a synthetic RoboNotch receptor (SynRobo receptor), a Modular Extracellular Sensor Architecture (MESA) receptor, a Tango receptor, a ChaCha receptor, a generalized extracellular molecule sensor (GEMS) receptor, a growth factor receptor, a cytokine receptor, a chemokine receptor, a switch receptor, an adhesion molecule, an integrin, an inhibitory receptor, a stimulatory receptor, an immunoreceptor tyrosine-based activation motif (ITAM)-containing receptor, an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor, a hormone receptor, and a receptor tyrosine kinase.

[0393] In some embodiments, the engineered receptor comprises one or more domains that combine an antigen -binding domain (*e.g.,* derived from an antibody or antibody fragment) that provides a capability for binding a desired antigen (*e.g.,* tumor antigen) with intracellular signaling domains. In some embodiments, the intracellular signaling domain is an activating intracellular domain portion, such as a T cell activating domain, providing a primary activation signal. In some embodiments, the intracellular signaling domain contains or additionally contains a costimulatory signaling domain to facilitate effector functions. Upon binding to the molecule, *e.g.,* antigen, the receptor generally delivers an immunostimulatory signal, such as an ITAM-transduced signal, into the cell, thereby promoting an immune response targeted to the disease or condition. In some embodiments, such receptors upon introduction into immune cells can modulate the cell activity, and, in some cases, can modulate cell differentiation or homeostasis, thereby resulting in genetically engineered cells with improved longevity, survival and/or persistence in vivo, such as for use in adoptive cell therapy methods.

[0394] In some embodiments, the engineered receptor is constructed with a capability for binding a particular antigen (or marker or ligand), such as an antigen expressed in a particular cell type to be targeted by adoptive therapy, *e.g.,* a cancer marker, and/or an antigen intended to induce a dampening response, such as an antigen expressed on a normal or non-diseased cell type. Thus, the engineered receptor typically includes in its extracellular portion one or more antigen binding molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody-derived variable domains, and/or antibody-derived molecules.

[0395] In some embodiments, an engineered cell provided herein (*e.g.,* primary or iPSC-derived T cell or primary or iPSC-derived NK cell) includes a recombinant nucleic acid encoding the engineered receptor, wherein the recombinant nucleic acid is inserted in a genomic locus. In some embodiments, the recombinant nucleic acid is inserted into a safe harbor locus, such as but not limited to, an AAVS1 locus, CCR5 locus, CLYBL locus, ROSA26 locus, SHS231 locus, F3 (also known as CD142) locus, MICA locus, MICB locus, LRP1 (also known as CD91) locus, HMGB1 locus, ABO locus, RHD locus, FUT1 locus, or KDM5D locus. In some embodiments, the recombinant nucleic acid is inserted in a B2M locus, CIITA locus, CARD9 locus, CARD10 locus, CARD14 locus, TRAC locus, TRBC1 locus, TRBC2 locus, CARD11 locus, PIK3R3 locus, PD-1 locus, or CTLA-4 locus. Any

suitable method can be used to insert the recombinant nucleic acidinto the genomic locus of the cell including the gene editing methods described herein (*e.g.,* a CRISPR/Cas system).

[0396] In accordance with the present disclosure, on-off or other types of control switch techniques may be incorporated herein. These techniques may comprise use of dimerization domains and optional activators of such domain dimerization, *e.g.,* as disclosed by Wu et al., Science 2015 Oct; 350(6258):aab4077 utilizing FKBP/Rapalog dimerization systems in certain cells, the contents of which are incorporated by reference herein in their entirety. Additional dimerization technology is described in, *e.g.,* Fegan et al. Chem. Rev. 2010, 110, 3315-3336 as well as U.S. Pat. Nos. 5,830,462; 5,834,266; 5,869,337; and 6,165,787, the contents of each of which is also incorporated by reference herein with respect to dimerization technology. Additional dimerization pairs may comprise cyclosporine-A/cyclophilin, receptor, estrogen/estrogen receptor (optionally using tamoxifen, 4-hydroxytamoxifen, or endoxifen), glucocorticoids/glucocorticoid receptor, tetracycline/tetracycline receptor, and/or vitamin D/vitamin D receptor. Further examples of dimerization technology may be found in *e.g.,* WO 2014/127261, WO 2015/090229, US 2014/0286987, US 2015/0266973, US 2016/0046700, U.S. Pat. No. 8,486,693, US 2014/0171649, and US 2012/0130076, the contents of which are further incorporated by reference herein in their entirety.

a. Chimeric Antigen Receptors

[0397] In some embodiments, the CAR is selected from the group consisting of a first generation CAR, a second generation CAR, a third generation CAR, and a fourth generation CAR. In some embodiments, the CAR is or comprises a first generation CAR comprising an antigen binding region, a transmembrane domain, and at least one signaling domain (*e.g.,* one, two or three signaling domains). In some embodiments, the CAR is or comprises a second generation CAR comprising an antigen binding region, a transmembrane domain, and at least two signaling domains. In some embodiments, the CAR is or comprises a third generation CAR comprising an antigen binding region, a transmembrane domain, and at least three signaling domains. In some embodiments, the CAR is or comprises a fourth generation CAR comprising an antigen binding region, a transmembrane domain, three or four signaling domains, and a domain which upon successful signaling of the CAR induces expression of a cytokine gene.

[0398] In some embodiments, the CAR is or comprises a first generation CAR. In some embodiments, a first generation CAR comprises an antigen binding region, a transmembrane domain, and signaling domain. In some embodiments, a signaling domain mediates downstream signaling during T cell activation.

[0399] In some embodiments, the CAR is or comprises a second generation CAR. In some embodiments, a second generation CAR comprises an antigen binding region, a transmembrane domain, and two signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and/or CAR T cell persistence during T cell activation.

[0400] In some embodiments, the CAR is or comprises a third generation CAR. In some embodiments, a third generation CAR comprises an antigen binding region, a transmembrane domain, and at least three signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and or CAR T cell persistence during T cell activation. In some embodiments, a third generation CAR comprises at least two costimulatory domains. In some embodiments, the at least two costimulatory domains are not the same.

[0401] In some embodiments, the CAR is or comprises a fourth generation CAR. In some embodiments, a fourth generation CAR comprises an antigen binding region, a transmembrane domain, and at least two, three, or four signaling domains. In some embodiments, a signaling domain mediates downstream signaling during T cell activation. In some embodiments, a signaling domain is a costimulatory domain. In some embodiments, a costimulatory domain enhances cytokine production, CAR T cell proliferation, and or CAR T cell persistence during T cell activation.

[0402] In some embodiments, the CAR comprises an antigen binding region and a transmembrane domain, but does not comprise any signaling domain. In some embodiments, the transmembrane domain of such CARs binds to another signaling protein. In some embodiments, such CARs comprise a domain that interacts with another signaling protein. In some embodiments, such CARs are capable of activating the signaling protein it interacts with in the presence of the antigen.

[0403] In some embodiments, the antigen binding region of the CAR is or comprises an antigen-binding fragment of an antibody. In some embodiments, the antibody is a human antibody or humanized antibody. In some embodiment, the antibody is a monoclonal antibody.

[0404] In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody (mAh), or a single domain antibody (sdAb), such as sdFv, nanobody, VHH and VNAR. In some embodiments, an antigen binding fragment comprises antibody-derived variable regions joined by a flexible linker.

[0405] In some embodiments, the antibody or antigen-binding fragment thereof is a single chain antibody fragment, such as a single chain variable fragment (scFv) or a diabody or a single domain antibody (sdAb). In some embodiments, the antibody or antigen-binding fragment is a single domain antibody comprising only the VH region. In some embodiments, the antibody or antigen binding fragment is an scFv comprising a heavy chain variable (VH) region and a light chain variable (VL) region.

[0406] In some embodiments, the CAR includes a binding portion or portions of an antibody molecule, such as a heavy chain variable (VH) region and/or light chain variable (VL) region of the antibody, *e.g.,* an scFv antibody fragment. The chimeric receptors, such as CARs, generally include an extracellular antigen binding region, such as a portion of an antibody molecule, generally a variable heavy (VH) chain region and/or variable light (VL) chain region of the antibody, *e.g.,* an scFv antibody fragment.

[0407] In some embodiments, the antibody or antigen binding fragment, contains a heavy and/or light chain variable (VH or VL) region sequence as described, or a sufficient antigen-binding portion thereof. In some embodiments, the antigen-binding fragment, contains a VH region sequence or sufficient antigen -binding portion thereof that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described. In some embodiments, the antigen-binding fragment, contains a VL region sequence or sufficient antigen-binding portion that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. In some embodiments, the antigen-binding fragment, contains a VH region sequence that contains a CDR-H1, CDR-H2 and/or CDR-H3 as described and contains a VL region sequence that contains a CDR-L1, CDR-L2 and/or CDR-L3 as described. Also among the antibodies are those having sequences at least at or

about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to such a sequence.

[0408] In some embodiments, the antibody is a single domain antibody (sdAb) comprising only a VH region sequence or a sufficient antigen-binding portion thereof, such as any of the above described VH sequences (*e.g.,* a CDR-H1, a CDR-H2, a CDR-H3 and/or a CDR-H4).

[0409] In some embodiments, an antibody provided herein or antigen-binding fragment thereof comprising a VH region further comprises a light chain or a sufficient antigen binding portion thereof. For example, in some embodiments, the antibody or antigen - binding fragment thereof contains a VH region and a VL region, or a sufficient antigen binding portion of a VH and VL region. In such embodiments, a VH region sequence can be any of the above described VH sequence. In some such embodiments, the antibody is an antigen binding fragment, such as a Fab or an scFv. In some such embodiments, the antibody is a full-length antibody that also contains a constant region.

[0410] In some embodiments, the nucleotide sequence encoding a CAR may be derived from a mammalian sequence, for example, a mouse sequence, a primate sequence, a human sequence, or combinations thereof. In the cases where the nucleotide sequence encoding a CAR is non-human, the sequence of the CAR may be humanized. The nucleotide sequence encoding a CAR may also be codon-optimized for expression in a mammalian cell, for example, a human cell. In any of these embodiments, the nucleotide sequence encoding a CAR may be at least 80% identical (*e.g.,* at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any of the nucleotide sequences disclosed herein. The sequence variations may be due to codon-optimalization, humanization, restriction enzymebased cloning scars, and/or additional amino acid residues linking the functional domains, etc.

[0411] In certain embodiments, the CAR can be re-engineered as a chimeric autoantibody receptor (CAAR) to selectively deplete autoreactive immune cells. In certain embodiments, CAARs are engineered to target autoantibodies present on immune cells. Exemplary target antigens for CAARs include, but are not limited to, DSG3 (associated with pemphigus volgaris); factor VIII (FVIII)(associated with haemophilia). In any of these embodiments, the extracellular binding domain of the CAR can be codon-optimized for expression in a host cell or have variant sequences to increase functions of the extracellular binding domain.

[0412] In some embodiments, the CAR is a bicistronic CAR. In some embodiments, the immune cell of the disclosure comprises a bicistronic CAR. Bicistronic CARs can comprise

two CARs that bind different targets and are encoded by a single vector. A bicistronic CAR may comprise a first CAR sequence and a second CAR sequence expressed as a single polypeptide comprising a cleavable linker between the first and second CARs. Non-limiting examples of first and/or second CAR sequences include CD19,CD20, BCMA, CD22, CD70, DLL3, LY6G6D, Claudin 6, GCC, p53R175H, and PRAME. An exemplary cleavable linker is Furin-GSG-T2A (see, *e.g.,* Chng et al. MAbs. 2015 Mar-Apr; 7(2): 403-412, which is herein incorporated by reference with respect to cleavable linkers; see also Guedan et al. Mol Ther Methods Clin Dev. 2019 Mar 15; 12: 145-156, which is incorporated herein by reference with respsect to bicistronic CAR design).

[0413] In some embodiments, the CAR is a bispecific CAR. In some embodiments, the immune cell of the disclosure comprises a bispecific CAR. In some embodiments, a first binding motif and a second binding motif (*e.g.,* distinct anti-CD20 and anti-CD19 binding motifs) are both comprised in single bispecific CAR. In such bispecific CARs, a CAR molecule itself may be engineered to recognize more than one antigen. In tandem bispecific CARs, the first and second binding motifs are extracellular and may be characterized as a membrane-proximal binding motif and a membrane-distal binding motif.

[0414] In some embodiments, the CAR further comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene is endogenous or exogenous to a target cell comprising a CAR which comprises a domain which upon successful signaling of the CAR induces expression of a cytokine gene. In some embodiments, a cytokine gene encodes a pro-inflammatory cytokine. In some embodiments, a cytokine gene encodes IL-1, IL-2, IL-9, IL-12, IL-18, TNF, or IFN-gamma, or functional fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a domain which upon successful signaling of the CAR induces expression of a cytokine gene is or comprises a transcription factor or functional domain or fragment thereof. In some embodiments, a transcription factor or functional domain or fragment thereof is or comprises a nuclear factor of activated T cells (NFAT), an NF-kB, or functional domain or fragment thereof. See, *e.g.,* Zhang. C. et al., Engineering CAR-T cells. Biomarker Research. 5:22 (2017); WO 2016126608; Sha, H. et al. Chimaeric antigen receptor T-cell therapy for tumour immunotherapy. Bioscience Reports Jan 27, 2017, 37 (1).

[0415] A skilled artisan is familiar with CARs and different components and configurations of CARs. Any known CAR can be employed in connection with the provided embodiments. In addition to the CARs described herein, various CARs and nucleotide sequences encoding the same are known in the art and would be suitable for engineering cells as described herein. See, *e.g.,* W02013040557; W02012079000; W02016030414; Smith T, et al., Nature Nanotechnology. 2017. DOI: 10.1038/NNAN0.2017.57, the disclosures of which are herein incorporated by reference. Exemplary features and components of a CAR are described in the following subsections.

1. Antigen Binding Region

[0416] In some embodiments, a CAR antigen binding region is or comprises an antibody or antigen-binding portion thereof. In some embodiments, a CAR antigen binding region is or comprises an scFv or Fab.

[0417] In some embodiments, an antigen binding region binds to a cell surface antigen of a cell. In some embodiments, a cell surface antigen is characteristic of (*e.g.,* expressed by) a particular or specific cell type. In some embodiments, a cell surface antigen is characteristic of more than one type of cell.

[0418] In some embodiments, the antigen may be an antigen that is expressed on tumor cells, or an antigen that is characteristic of an autoimmune or inflammatory disease. In some embodiments, the antigen may be an antigen that is exclusively or preferentially expressed on tumor cells, or an antigen that is characteristic of an autoimmune or inflammatory disease. In some embodiments, the antigen binding region targets an antigen characteristic of a neoplastic cell. For instance, the antigen binding region targets an antigen expressed by a neoplastic or cancer cell. In some embodiments, the antigen binding region binds a tumor associated antigen. In some embodiments, the antigen characteristic of a neoplastic cell (*e.g.,* antigen associated with a neoplastic or cancer cell) or a tumor associated antigen is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor.

[0419] In some embodiments, the target antigen is an antigen that includes, but is not limited to, ALPPL2, Epidermal Growth Factor Receptors (EGFR), EGFRvIII, ErbBl/EGFR, ErbB2/HER2, ErbB3/HER3, ErbB4/HER4, Fibroblast Growth Factor Receptors (FGFR), FGFR1, FGFR2, FGFR3, FGFR4, FGFR5, FGFR6, FGFR7, FGFR18, FGFR21, Vascular

Endothelial Growth Factor Receptors (VEGFR), VEGFR1, VEGFR2, VEGR3, VEGF-A, VEGF-B, VEGF-C, VEGF-D, PIGF, RET Receptor, Eph Receptor Family, EphAl, EphA2, Eph A3, EphA4, EphA5, EphA6, EphA7, EphA8, EphA9, EphAlO, EphBl, EphB2. EphB3, EphB4, EphB6, ELF2M, CXCR1, CXCR2, CXCR3, CXCR4, CXCR6, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CFTR, CIC-1, CIC-2, CIC-4, CIC-5, CIC-7, CIC-Ka, CIC-Kb, Bestrophins, TMEM16A, GAB A receptor, glycin receptor, ABC transporters, NAV1.1, NAVI.2, NAVI.3, NAVI.4, NAVI.5, NAVI.6, NAVI.7, NAVI.8, NAVI.9, sphingosine-1 -phosphate receptor (S1P1R), NMDA channel, transmembrane protein, multispan transmembrane protein, T-cell receptor motifs; T-cell alpha chains; T-cell beta chains; T-cell y chains; T-cell 6 chains, GCC, CCR7, CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD3, CD4, CD5, CD7, CD8a, CD8b, CD8, CD1lb, CD11c, CD16, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD35, CD38, CD40, CD45RA, CD45RO, CD46, CD47, CD48, CD52, CD56, CD59, CD62L, CD66, CD68, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD117, CD123, CD127, CD133, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD134, CD137 (4-1 BB), CD163, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, F4/80, IL-4Ra, Sca-1 , CTLA-4, GITR, GARP, LAP, granzyme B, LFA-1, transferrin receptor, NKp46, perforin, CD4+, Thl, Th2, Thl7, Th40, Th22, Th9, Tfh, Canonical Treg, FoxP3+, Tri, Th3, Tregl7, TREG, CDCP, NT5E, EpCAM, CEA, gpA33, Mucins, Carbonic anhydrase IX, Folate binding protein, Gangliosides (*e.g.,* CD2, CD3, GM2), Lewis-y2, aVp3, a5pl, ErbBl/HER2, ErB3, c-MET, IGF1R, TRAIL-R1, TRAIL-R2, RANKL, FAP, Tenascin, PDL-1, BAFF, HDAC, ABL, FLT3, KIT, MET, RET, IL- Ip, ALK, RANKL, mTOR, CTLA-4, IL-6, IL-6R, JAK3, BRAF, PTCH, Smoothened, PIGF, ANPEP, TIMP1, PLAUR, PTPRJ, LTBR, or ANTXR1, Folate receptor alpha (Fra), ERBB2 (Her2/neu), IL-13Ra2, Mesothelin, TSHR, CD171, CD179a, CLECL1, CS-1, CLL-1, GD2, GD3, BCMA (CD269), MUC16 (CA125), L1CAM, LeY, MSLN, IL13Ral, Ll-CAM, Tn Ag, prostate specific membrane antigen (PSMA), ROR1, GM3, CD44v6, B7H3 (CD276), KIT, interleukin- 11 receptor a (IL-l lRa), PSCA, PRSS21, LewisY, platelet-derived growth factor receptor-beta (PDGFR-beta), SSEA-4, DLL3, MUC1, NCAM, Prostase, PAP, Ephrin B2, IGF-1 receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, Fucosyl GM1, sLe, TGS5, HMWMAA, o-acetyl-

GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, CLDN18.2, CX0RF61, CD97, CD 179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO- 1, LAGE-la, MAGE-A1, legumain, HPV E6, E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Major histocompatibility complex class I-related gene protein (MR1), urokinase-type plasminogen activator receptor (uPAR), Fos-related antigen 1, p53, p53 mutant, prostein, survivin, telomerase, PCTA-l/Galectin 8, MelanA/MARTl, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin Bl, MYCN, RhoC, TRP-2, CYPIB I, BORIS, SART3, PAX5, OY- TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, a neoantigen, CD15, CD184, CD56, CD26, CD29, CD44, HLA-A, HLA-B, HLA-C, (HLA-A,B,C) CD49f, CD151 CD340, CD200, tkrA, trkB, trkC, GPRC5D, IGLL1, IL-11Rα, KLK2, KLK3, LY6G6D, p53, p53R175H, KRAS, KRAS G12D, PRAME, SIRPα, TAG72, ALPI, citrullinated vimentin, Axl, or an antigenic fragment or antigenic portion thereof.

[0420] In some embodiments, exemplary target antigens include, but are not limited to, CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD35, CD38, CD40, CD44v6, CD45, CD45RA, CD45RO, CD46, CD47, CD48, CD52, CD56, CD59, CD62L, CD66, CD68, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD117, CD123, CD213A2, CD127, CD133, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLL-1, CS1, EGFR, FGFR2, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), Claudin18.2, PSMA, Mesothelin, IL13Ra2, FAP, signal regulatory protein α (SIRPα), TSHR, EGFRvIII, GD2, GD3, Tn Ag, ROR1, ROR2, GPC1, GPC2, FLT3, FAP, TAG72, CEA, EPCAM, B7H3, KIT, IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFR-beta, SSEA-4, , folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, EGFR, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-1

receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, AFP, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, EGFR, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, , ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, the extracellular portion of the APRIL protein, or any combinations thereof.

[0421] In some embodiments, the target antigen is an antigen that includes, but is not limited to, DLL3, LY6G6D, CLDN6, GCC, p53R175H, KRAS G12D, PRAME, KLK2, KLK3, CD70, STEAP1, STEAP2, GPC3, TROP2, CLDN18.2, ROR1, ROPN1, IGF2BP3, CD19, BCMA, GPRC5D, CD20, CD22, CD79a, CD79b, GD2, GD3, and B7H3.

[0422] In some aspects, the target antigen is AFP, CD19, TRAC, TCRβ, BCMA, CLL-1, CS1, CD38, CD19, TSHR, CD123, CD22, CD30, CD171, CD33, EGFRvIII, GD2, GD3, Tn Ag, PSMA, ROR1, ROR2, GPC1, GPC2, FLT3, FAP, TAG72, CD44v6, CEA, EPCAM,

B7H3, KIT, IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, EGFR, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, the extracellular portion of the APRIL protein, or any combinations thereof.

[0423] In some aspects, the CAR or TCR targets AFP, CD19, TRAC, TCRβ, BCMA, CLL-1, CS1, CD38, CD19, TSHR, CD123, CD22, CD30, CD171, CD33, EGFRvIII, GD2, GD3, Tn Ag, PSMA, ROR1, ROR2, GPC1, GPC2, FLT3, FAP, TAG72, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, EGFR, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72,

LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, IGLL1, the extracellular portion of the APRIL protein, or any combinations thereof.

[0424] In some embodiments, the CAR is a DLL3 CAR. In some embodiments, the extracellular binding domain of the DLL3 CAR comprises an antibody that binds to DLL3, for example, human DLL3. In some embodiments, the DLL3 CAR comprises any one of SEQ ID NOs: 401-413. In some embodiments, the extracellular binding domain of the DLL3 CAR comprises an antibody light chain sequence comprising SEQ ID NO: 415. In some embodiments, the extracellular binding domain of the DLL3 CAR comprises a antibody heavy chain sequence comprising SEQ ID NO: 417. In some embodiments, the extracellular binding domain of the DLL3 CAR comprises a light chain comprising a CDR1 comprising SEQ ID NO: 424, a CDR2 comprising SEQ ID NO: 425, and a CDR3 comprising SEQ ID NOs: 426. In some embodiments, the extracellular binding domain of the DLL3 CAR comprises a heavy chain comprising a CDR1 comprising SEQ ID NO: 427, a CDR2 comprising SEQ ID NO: 428, and a CDR3 comprising SEQ ID NOs: 429.

[0425] In some embodiments, the CAR is a LY6G6D CAR. In some embodiments, the extracellular binding domain of the LY6G6D CAR comprises an antibody that binds to LY6G6D, for example, human LY6G6D.

[0426] In some embodiments, the CAR is a CLDN6 CAR. In some embodiments, the extracellular binding domain of the CLDN6 CAR comprises an antibody that binds to CLDN6, for example, human CLDN6.

[0427] In some embodiments, the CAR is a CLDN18.2 CAR. In some embodiments, the extracellular binding domain of the CLDN18.2 CAR comprises an antibody that binds to CLDN18.2, for example, human CLDN18.2.

[0428] In some embodiments, the CAR is a GCC CAR. In some embodiments, the extracellular binding domain of the GCC CAR comprises an antibody that binds to GCC, for example, human GCC.

[0429] In some embodiments, the CAR is a PRAME CAR. In some embodiments, the extracellular binding domain of the PRAME CAR comprises an antibody that binds to PRAME, for example, human PRAME.

[0430] In some embodiments, the CAR is a KLK2 CAR. In some embodiments, the extracellular binding domain of the KLK2 CAR comprises an antibody that binds to KLK2, for example, human KLK2.

[0431] In some embodiments, the CAR is a KLK3 CAR. In some embodiments, the extracellular binding domain of the KLK3 CAR comprises an antibody that binds to KLK3, for example, human KLK3.

[0432] In some embodiments, the CAR is a CD70 CAR. In some embodiments, the extracellular binding domain of the CD70 CAR comprises an antibody that binds to CD70, for example, human CD70.

[0433] In some embodiments, the CAR is a STEAP1 CAR. In some embodiments, the extracellular binding domain of the STEAP1 CAR comprises an antibody that binds to STEAP1, for example, human STEAP1.

[0434] In some embodiments, the CAR is a STEAP2 CAR. In some embodiments, the extracellular binding domain of the STEAP2 CAR comprises an antibody that binds to STEAP2, for example, human STEAP2.

[0435] In some embodiments, the CAR is a GPC3 CAR. In some embodiments, the extracellular binding domain of the GPC3 CAR comprises an antibody that binds to GPC3, for example, human GPC3.

[0436] In some embodiments, the CAR is a TROP2 CAR. In some embodiments, the extracellular binding domain of the TROP2 CAR comprises an antibody that binds to TROP2, for example, human TROP2.

[0437] In some embodiments, the CAR is a ROPN1 CAR. In some embodiments, the extracellular binding domain of the ROPN1 CAR comprises an antibody that binds to ROPN1, for example, human ROPN1.

[0438] In some embodiments, the CAR is a ROR1 CAR. In some embodiments, the extracellular binding domain of the ROR1 CAR comprises an antibody that binds to ROR1, for example, human ROR1.

[0439] In some embodiments, the CAR is a IGF2BP3 CAR. In some embodiments, the extracellular binding domain of the IGF2BP3 CAR comprises an antibody that binds to IGF2BP3, for example, human IGF2BP3.

[0440] In some embodiments, the CAR is a CD79a CAR. In some embodiments, the extracellular binding domain of the CD79a CAR comprises an antibody that binds to CD79a, for example, human CD79a.

[0441] In some embodiments, the CAR is a CD79b CAR. In some embodiments, the extracellular binding domain of the CD79b CAR comprises an antibody that binds to CD79b, for example, human CD79b.

[0442] In some embodiments, the CAR is a GD2 CAR. In some embodiments, the extracellular binding domain of the GD2 CAR comprises an antibody that binds to GD2 for example, human GD2.

[0443] In some embodiments, the CAR is a GD3 CAR. In some embodiments, the extracellular binding domain of the GD3 CAR comprises an antibody that binds to GD3 for example, human GD3.

[0444] In some embodiments, the CAR is a B7H3 CAR. In some embodiments, the extracellular binding domain of the B7H3 CAR comprises an antibody that sbinds to B7H3 for example, human B7H3.

[0445] In some embodiments, the CAR is a CD19 CAR. In some embodiments, the extracellular binding domain of the CD19 CAR comprises an antibody that binds to CD19, for example, human CD19. In some embodiments, the extracellular binding domain of the CD19 CAR comprises an scFv antibody fragment derived from the FMC63 monoclonal antibody (FMC63), which comprises the heavy chain variable region (VH) and the light chain variable region (VL) of FMC63 connected by a linker peptide. In some embodiments, the linker peptide is a "Whitlow" linker peptide. FMC63 and the derived scFv have been described in Nicholson et al., Mal. lmmun. 34(16-17): 1157-1165 (1997) and PCT Application Publication No. WO2018/213337 A 1, the entire content of each of which is incorporated by reference herein. In some embodiments, the extracellular binding domain of the CD19 CAR comprises an antibody derived from one of the CD19-specific antibodies including, for example, SJ25C1 (Bejcek et al., Cancer Res. 55:2346-2351 (1995)), HD37 (Pezutto et al., J. Immunol. 138(9):2793-2799 (1987)), 4G7 (Meeker et al., Hybridoma 3:305-320 (1984)), B43 (Bejcek (1995)), BLY3 (Bejcek (1995)), B4 (Freedman et al., 70:418-427 (1987)), B4 HB12b (Kansas & Tedder, J. Immunol. 147:4094-4102 (1991); Yazawa et al., Proc. Natl. Acad. Sci. USA 102: 15178- 15183 (2005); Herbst et al., J. Pharmacol. Exp. Ther. 335:213-222 (2010)), BU12 (Gallard et al., J. Immunology, 148(10): 2983-2987 (1992)), and CLB-CD19 (De Rie Cell. Immunol. 118:368- 381(1989)).

[0446] In some embodiments, the CAR is CD22 CAR. CD22, which is a transmembrane protein found mostly on the surface of mature B cells that functions as an inhibitory receptor for B cell receptor (BCR) signaling. CD22 is expressed in 60-70% of B cell lymphomas and leukemias (*e.g.,* B-chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma) and is not present on the cell surface in early stages of B cell development or on stem cells. In some embodiments, the CD22 CAR

comprises an extracellular binding domain that binds CD22, a transmembrane domain, an intracellular signaling domain, and/or an intracellular costimulatory domain. In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv antibody fragment derived from the m971 monoclonal antibody (m971), which comprises the heavy chain variable region (VH) and the light chain variable region (VL) of m971 connected by a linker. In some embodiments, the extracellular binding domain of the CD22 CAR comprises an scFv antibody fragment derived from m971-L7, which an affinity matured variant of m971 with significantly improved CD22 binding affinity compared to the parental antibody m971 (improved from about 2 nM to less than 50 pM). In some embodiments, the scFv antibody fragment derived from m971-L7 comprises the VH and the VL of m971-L7 connected by a 3xG4S linker. In some embodiments, the extracellular binding domain of the CD22 CAR comprises immunotoxins HA22 or BL22. Immunotoxins BL22 and HA22 are therapeutic agents that comprise an scFv that binds CD22 fused to a bacterial toxin, and thus can bind to the surface of the cancer cells that express CD22 and kill the cancer cells. BL22 comprises a dsFv of an anti-CD22 antibody, RFB4, fused to a 38-kDa truncated form of Pseudomonas exotoxin A (Bang et al., Clin. Cancer Res., 11 : 1545-50 (2005)). HA22 (CAT8015, moxetumomab pasudotox) is a mutated, higher affinity version of BL22 (Ho et al., J. Biol. Chem., 280(1): 607-17 (2005)). Suitable sequences of antigen binding regions of HA22 and BL22 that bind CD22 are disclosed in, for example, U.S. Patent Nos. 7,541,034; 7,355,012; and 7,982,011, which are hereby incorporated by reference in their entirety.

[0447] In some embodiments, the CAR is BCMA CAR. BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B cell lineage, with the highest expression on terminally differentiated B cells or mature B lymphocytes. BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma. In some embodiments, the BCMA CAR comprises an extracellular binding domain that binds BCMA, a transmembrane domain, an intracellular signaling domain, and/or an intracellular costimulatory domain. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an antibody that binds to BCMA, for example, human BCMA. CARs directed to BCMA have been described in PCT Application Publication Nos. WO2016/014789, WO2016/014565, WO2013/154760, and WO 2015/128653. BCMA-

binding antibodies are also disclosed in PCT Application Publication Nos. WO2015/166073 and W02014/068079. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv antibody fragment derived from a murine monoclonal antibody as described in Carpenter et al., Clin. Cancer Res. 19(8):2048-2060 (2013). In some embodiments, the scFv antibody fragment is a humanized version of the murine monoclonal antibody (Sommermeyer et al., Leukemia 31 :2191-2199 (2017)). In some embodiments, the extracellular binding domain of the BCMA CAR comprises single variable fragments of two heavy chains (VHH) that can bind to two epitopes of BCMA as described in Zhao et al., J. Hematol. Oneal. 11(1): 141 (2018). In some embodiments, the extracellular binding domain of the BCMA CAR comprises a fully human heavy-chain variable domain (FHVH) as described in Lam et al., Nat. Commun. 11 (1) :283 (2020).

[0448] In some embodiments, the CAR is a GPRC5D CAR. In some embodiments, the extracellular binding domain of the GPRC5D CAR comprises an antibody thatbinds to GPRC5D, for example, human GPRC5D.

[0449] In some embodiments, the CAR is a CD20 CAR. In some embodiments, the extracellular binding domain of the CD20 CAR comprises an antibody that binds to CD20, for example, human CD20.

[0450] In some embodiments, the antigen binding region targets an antigen characteristic of an autoimmune or inflammatory disorder. In some embodiments, the antigen binding region binds an antigen associated with an autoimmune or inflammatory disorder. In some instances, the antigen is expressed by a cell associated with an autoimmune or inflammatory disorder. In some embodiments, the autoimmune or inflammatory disorder is selected from chronic graft-vs-host disease (GVHD), lupus, arthritis, immune complex glomerulonephritis, goodpasture, uveitis, hepatitis, systemic sclerosis or scleroderma, type I diabetes, multiple sclerosis, cold agglutinin disease, Pemphigus vulgaris, Grave's disease, autoimmune hemolytic anemia, Hemophilia A, Primary Sjogren's Syndrome, thrombotic thrombocytopenia purrpura, neuromyelits optica, Evan's syndrome, IgM mediated neuropathy, cyroglobulinemia, dermatomyositis, idiopathic thrombocytopenia, ankylosing spondylitis, bullous pemphigoid, acquired angioedema, chronic urticarial, antiphospholipid demyelinating polyneuropathy, and autoimmune thrombocytopenia or neutropenia or pure red cell aplasias, while exemplary non-limiting examples of alloimmune diseases include allosensitization (see, for example, Blazar et al., 2015, Am. J. Transplant, 15(4):931-41) or xenosensitization from hematopoietic or solid organ transplantation, blood transfusions,

pregnancy with fetal allosensitization, neonatal alloimmune thrombocytopenia, hemolytic disease of the newborn, sensitization to foreign antigens such as can occur with replacement of inherited or acquired deficiency disorders treated with enzyme or protein replacement therapy, blood products, and gene therapy. Allosensitization, in some instances, refers to the development of an immune response (such as circulating antibodies) against human leukocyte antigens that the immune system of the recipient subject or pregnant subject considers to be non-self antigens. In some embodiments, the antigen characteristic of an autoimmune or inflammatory disorder is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, or histidine kinase associated receptor.

[0451] In some embodiments, the antigen binding region targets an antigen characteristic of an infectious disease. In some embodiments, the antigen binding region binds an antigen associated with an infectious disease. In some instances, the antigen is expressed by a cell affected by an infectious disease. In some embodiments, wherein the infectious disease is selected from HIV, hepatitis B virus, hepatitis C virus, Human herpes virus, Human herpes virus 8 (HHV-8, Kaposi sarcoma- associated herpes virus (KSHV)), Human T-lymphotrophic virus-1 (HTLV-1), Merkel cell polyomavirus (MCV), Simian virus 40 (SV40), Epstein-Barr virus, CMV, human papillomavirus. In some embodiments, the antigen characteristic of an infectious disease is selected from a cell surface receptor, an ion channel-linked receptor, an enzyme-linked receptor, a G protein-coupled receptor, receptor tyrosine kinase, tyrosine kinase associated receptor, receptor-like tyrosine phosphatase, receptor serine/ threonine kinase, receptor guanylyl cyclase, histidine kinase associated receptor, HIV Env, gpl20, or CD4-induced epitope on HIV-1 Env.

[0452] In any of these embodiments, the extracellular binding domain of the CAR can be codon-optimized for expression in a host cell or have variant sequences to increase functions of the extracellular binding domain.

[0453] In some embodiments, the CAR is bispecific to two target antigens (*e.g.,* comprising binding region(s) for two target antigens). In some embodiments, the target antigens are different target antigens. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19

and CD20, (ii) CD20 and LI -CAM, (iii) LI -CAM and GD2, (iv) EGFR and LI -CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1. In some embodiments, each of the two different antigen binding regions is an scFv. In some embodiments, the C-terminus of one variable domain (VH or VL) of a first scFv is tethered to the N-terminus of the second scFv (VL or VH, respectively) via a polypeptide linker. In some embodiments, the linker connects the N-terminus of the VH with the C-terminus of VL or the C-terminus of VH with the N-terminus of VL. These scFvs lack the constant regions (Fc) present in the heavy and light chains of the native antibody. The scFvs, with capability for binding at least two different antigens, are arranged in tandem and linked to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In some embodiments, an extracelluar spacer domain may be linked between the antigen-binding region and the transmembrane domain.

[0454] In some embodiments, each antigen-binding region of the CAR comprises a divalent (or bivalent) single-chain variable fragment (di-scFvs, bi-scFvs). In CARs comprising di-scFVs, two scFvs with capability for binding each antigen are linked together by producing a single peptide chain with two VH and two VL regions, yielding tandem scFvs. (Xiong, Cheng- Yi; Natarajan, A; Shi, X B; Denardo, G L; Denardo, S J (2006). "Development of tumor targeting anti-MUC-1 multimer: effects of di-scFv unpaired cysteine location on PEGylation and tumor binding". Protein Engineering Design and Selection 19 (8): 359-367; Kufer, Peter; Lutterbiise, Ralf; Baeuerle, Patrick A. (2004). "A revival of bispecific antibodies". Trends in Biotechnology 22 (5): 238-244). CARs comprising at least two antigen-binding regions would express two scFvs with capability for binding each of the two antigens. The resulting antigen-binding region, with capability for binding at least two different antigens, is joined to the co-stimulatory domain and the intracellular signaling domain via a transmembrane domain. In some embodiments, an extracelluar spacer domain may be linked between the antigen binding domain and the transmembrane domain.

[0455] In some embodiments, each antigen-binding region of the CAR comprises a diabody. In a diabody, the scFvs are created with linker peptides that are too short for the two variable regions to fold together, driving the scFvs to dimerize. Still shorter linkers (one or two amino acids) lead to the formation of trimers, the so-called triabodies or tribodies. Tetrabodies may also be used.

[0456] In some embodiments, the cell is engineered to express more than one CAR, such as two different CARs, in which each CAR has an antigen binding region directed to a different target antigen. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19 and CD20, (ii) CD20 and LI -CAM, (iii) LI -CAM and GD2, (iv) EGFR and LI -CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1.

[0457] In some embodiments, two different engineered cells are prepared that contain the provided modifications with each engineered with a different CAR. In some embodiments, each of the two different CARs has an antigen binding region directed to a different target antigen. In some of any such embodiments, the two different target antigens are any two different antigens described above. In some embodiments, the extracellular binding domains are different and bind two different antigens from (i) CD19 and CD20, (ii) CD20 and LI -CAM, (iii) LI -CAM and GD2, (iv) EGFR and LI -CAM, (v) CD19 and CD22, (vi) EGFR and C-MET, (vii) EGFR and HER2, (viii) C-MET and HER2, or (ix) EGFR and ROR1. In some embodiments, a population of engineered cells expressing a first CAR directed against a first target antigen and a population of engineered cells expressing a second CAR directed against a second target antigen are separately administered to the subject. In some embodiments, the first and second population of cells are administered sequentially in any order. For instance, the population of cells expressing the second CAR is administered a after administration of the population of cells expressing the first CAR.

2. Linker Sequences

[0458] In some embodiments, the antigen binding region of the CAR may comprise one or more linker sequences. In some embodiments, the antigen binding region of the CAR may comprise one or more linker sequences connecting a heavy chain variable region (VH) and a light chain variable region (VL). The VH and the VL may be connected in either order, i.e., VH-linker-VL or VL-linker-VH. Nonlimiting examples of linkers include Whitlow linker, (G4S)n (n can be a positive integer, *e.g.,* 1, 2, 3, 4, 5, 6, etc.) linker, and variants thereof.

[0459] Examples of exemplary linker sequences are described in **Table 4**. In provided aspects, the sequences of each linker in **Table 4** can be combined with any of the other CAR component sequences described here.

Table 4. Exemplary Linker Sequences

Component	Sequence	SEQ ID NO:
G4Sx3 Linker – amino acid	GGGGSGGGGS GGGGS	416
G4S Linker – amino acid	GGTGGAGGCG GTTCT	417
SG4S Linker – amino acid	SGGGGS	418
G4Sx3 Linker – nucleotide	ggcggtggag gatctggagg tggcggaagt ggaggaggtg gttct	504
G4S Linker – nucleotide	GGTGGAGGCG GTTCT	505
SG4S Linker – nucleotide	AGCGGAGGCG GAGGCTCT	506

3. Spacer (Hinge) Sequences

[0460] In some embodiments, the CAR further comprises one or more spacers, *e.g.,* wherein the spacer is a first spacer between the antigen binding region and the transmembrane domain. In some embodiments, the first spacer includes at least a portion of an immunoglobulin constant region or variant or modified version thereof. In some embodiments, the spacer is a second spacer between the transmembrane domain and a signaling domain. In some embodiments, the second spacer is an oligopeptide, *e.g.,* wherein the oligopeptide comprises glycine and serine residues such as but not limited to glycine-serine doublets. In some embodiments, the CAR comprises two or more spacers, *e.g.,* a spacer between the antigen binding region and the transmembrane domain and a spacer between the transmembrane domain and a signaling domain. The terms “hinge” and “spacer” may be used interchangeably in the present disclosure. Non-limiting examples of hinge domains include CD8a hinge domain, CD28 hinge domain, IgG4 hinge domain, IgG4 hinge-CH2-CH3 domain, and variants thereof.

[0461] Examples of exemplary spacer (hinge) sequences are described in **Table 5**. In provided aspects, the sequences of each spacer (hinge) in **Table 5** can be combined with any of the other CAR component sequences described here.

Table 5. Exemplary Spacer (Hinge) Sequences

Component	Sequence	SEQ ID NO:
CD8 Hinge – amino acid	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CD	420
CD8 Hinge, truncated – amino acid	PEACRPAAGGAVHTRGLDFACD	487
CD8 Hinge – nucleotide	accacaacac ctgctccaag gccacccaca ccagctccca ccatcgccag tcagccactg agcctgagac ctgaagcctg tagaccagcc gcaggaggag ccgtgcatac cagaggactg gactttgcct gcgac	508
IgG4 Hinge 1	ESKYGPPCPPCP	355
IgG4 Hinge 2	ESKYGPPCPSCP	356
IgG4 hing-CH2-CH3 domain	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVM HEALHNHYTQKSLSLSLGK	357
CD8alpha Hinge	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA CD	358
CD28 Hinge	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	359
CD28 Hinge	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP	360

4. Transmembrane Domain

[0462] In some embodiments, the CAR transmembrane domain comprises at least a transmembrane region of the TCR alpha chain, TCR beta chain, TCR zeta chain, CD28, CD3 epsilon, CD3 zeta, 1.xxCD3 zeta, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD64, CD80, CD86, CD134, CD 137, CD 154, or functional variant thereof. In some embodiments, the transmembrane domain comprises at least a transmembrane region(s) of CD8a, CD8P, 4-1BB/CD137, CD28, CD34, CD4, FcsRIy, CD16, OX40/CD134, CD3< CD3s, CD3y, CD38, TCRa, TCRb, TCR^, CD32, CD64, CD64, CD45, CD5, CD9, CD22, CD37, CD80, CD86, CD40, CD40L/CD154, VEGFR2, FAS, and FGFR2B, or functional variant thereof.

[0463] Examples of exemplary transmembrane domain sequences are described in **Table 6**. In provided aspects, the sequences of each transmembrane domain in **Table 6** can be combined with any of the other CAR component sequences described here.

Table 6. Exemplary Transmembrane Domain Sequences

Component	Sequence	SEQ ID NO:
CD8 TM – amino acid	IYIWAPLAGTCGVLLLSLVI TLYC	421
CD8 TM – nucleotide	atctacatctgggctcccttggctggaacttgtggagtgctgctcctttctcttgtcattacactgtactgc	509

5. Signaling Domain(s)

[0464] In some embodiments, a CAR described herein comprises one or more signaling domains. A CAR may comprise a costimulatory signaling domain, *e.g.,* to increase signaling potency. See U.S. Pat. Nos. 7,741,465, and 6,319,494, as well as Krause et al. and Finney et al. (supra), Song et al., Blood 119:696-706 (2012); Kalos et al, Sci Transl. Med. 3:95 (2011); Porter et al, N. Engl. J. Med. 365:725-33 (2011), and Gross et al, Annu. Rev. Pharmacol. Toxicol. 56:59-83 (2016). Signals generated through a TCR alone may be insufficient for full activation of a T cell and a secondary or co-stimulatory signal may increase activation. Thus, in some embodiments, a signaling domain further comprises one or more additional signaling domains (*e.g.,* costimulatory signaling domains) that activate one or more immune cell effector functions (*e.g.*, a native immune cell effector function described herein). In some embodiments, a portion of such costimulatory signaling domains may be used, as long as the portion transduces the effector function signal. In some embodiments, a cytoplasmic domain described herein comprises one or more cytoplasmic sequences of a T cell co-receptor (or fragment thereof). Non-limiting examples of such T cell co-receptors comprise CD27, CD28, 4-1BB (CD137), 0X40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA- 1), MYD88, CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that binds with CD83. An exemplary costimulatory protein has the amino acid sequence of a

costimulatory protein found naturally on T cells, the complete native amino acid sequence of which costimulatory protein is described in NCBI Reference Sequence: NP_006130.1.

[0465] In some embodiments, a CAR described herein comprises one or more signaling domain selected from one or more of B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14; LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; 0X40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB- A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thyl; CD96; CD160; CD200; CD300a/LMIRl; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-l; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin- 1/CLEC7A; DPPIV/CD26; EphB6; TIM- 1 /KIM- 1 /HA VCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, a CD3 zeta domain, a 1.xxCD3 zeta domain,an immunoreceptor tyrosinebased activation motif (ITAM), CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that binds CD83, or functional fragment thereof.

[0466] In some embodiments, a CAR comprises a signaling domain which is a costimulatory domain. In some embodiments, a CAR comprises a second costimulatory domain. In some embodiments, a CAR comprises at least two costimulatory domains. In some embodiments, a CAR comprises at least three costimulatory domains. In some embodiments, a CAR comprises a costimulatory domain selected from one or more of CD3zeta, 1.xxCD3zeta, CD27, CD28, 4-1BB, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that binds CD83. In some embodiments, if a CAR comprises two or more costimulatory domains, two costimulatory domains are different. In some embodiments, if a

CAR comprises two or more costimulatory domains, two costimulatory domains are the same.

[0467] In other embodiments, the at least one signaling domain comprises (i) a 4-1BB domain, or functional variant thereof; and (ii) a CD3 zeta domain, or functional variant thereof. In other embodiments, the at least one signaling domain comprises (i) a CD28 domain, or functional variant thereof; and (ii) a CD3 zeta domain, or functional variant thereof. In other embodiments, the at least one signaling domain comprises (i) a CD28 domain, or functional variant thereof; and (ii) a 1.xxCD3 zeta domain, or functional variant thereof.

[0468] In some embodiments, the CAR comprises an extracellular antigen binding region (*e.g.,* antibody or antibody fragment, such as an scFv) that binds to an antigen (*e.g.,* tumor antigen), a spacer (*e.g.,* containing a hinge domain, such as any as described herein), a transmembrane domain (*e.g.,* any as described herein), and one or more intracellular signaling domains (*e.g.,* any intracellular signaling domain, such as a primary signaling domain or costimulatory signaling domain as described herein). In some embodiments, the one or more intracellular signaling domains is or comprises a primary cytoplasmic signaling domain. In some embodiments, the one or more intracellular signaling domains additionally comprises an intracellular signaling domain of a costimulatory molecule (*e.g.,* a costimulatory domain). Any of such components can be any as described above.

[0469] Examples of exemplary signaling domain sequences are described in **Table 7**. In provided aspects, the sequences of each signaling domain in **Table 7** can be combined with any of the other CAR component sequences described here.

Table 7. Exemplary Signaling Domain Sequences

Component	Sequence	SEQ ID NO:
4-1BB costimulatory domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCEL	422
CD3zeta costimulatory domain	RVKFSRSADAPAYQQGQNQLYNELNGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQ KDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR	423
CD3zeta (Q to K mutation)	RVKFSRSADAPAYKQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL	488

	QKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR	
CD28 costimulatory domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRS	361

6. Signal Peptide

[0470] In certain embodiments, the CAR may further comprise one or more signal peptides. In certain embodiments, the CAR may comprise a signal peptide at the N-terminus. Non-limiting examples of signal peptides include CD8a signal peptide, IgK signal peptide, and granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GMCSFR-a, also known as colony stimulating factor 2 receptor subunit alpha (CSF2RA)) signal peptide, and variants thereof, the amino acid sequences of which are provided in **Table 8** below.

[0471] Examples of exemplary signal peptide sequences are described in **Table 8.** In provided aspects, the sequences of each signal peptide in **Table 8** can be combined with any of the other CAR component sequences described here.

Table 8. Exemplary Signal Peptide Sequences

Component	Sequence	SEQ ID NO:
CD8alpha signal peptide	MALPVTALLLPLALLLHAARP	414
IgK signal peptide	METDTLLL WVLLLWVPGSTG	362
GMCSFR-alpha (CSF2RA) signal peptide	MLLL VTSLLLCELPHP AFLLIP	363

b. T Cell Receptors (TCRs)

[0472] In some embodiments, the receptor of the disclosure is a T cell receptor (TCR). A TCR as known by the skilled in the art can be composed of two different, an alpha chain and a beta chain, each consisting of a constant region that anchors the chain inside the T cell surface membrane, and a variable region which can recognize and bind an antigen presented by MHCs. The TCR complex can be associated with six polypeptides forming two heterodimers, CD3γε and CD3δε, and one homodimer, CD3ζ, which together forms the CD3 complex. TCRs can be engineered to utilize the modification of T cells that retain these complexes to bind the antigens expressed by particular tumor cells. As used herein, a TCR can be a naturally-occurring or an engineered TCR.

[0473] TCRs are disulfide-linked membrane anchored heterodimeric proteins, typically comprising highly variable alpha (a) and beta (β) chains expressed as a complex with invariant CD3 chain molecules. T cells expressing these types of TCRs are referred to as α:β (or αβ) T cells. A minority of T cells express an alternative TCR comprising variable gamma (γ) and delta (δ) chains and are referred to as γδ T cells. TCR is not able to mediate signal transduction itself due to its short cytoplasmic tail, so TCR still requires CD3 and zeta to carry out the signal transduction in its place. A TCR receptor complex is an octomeric complex of variable TCR receptor α and β chains with three dimeric signaling modules CD3δ/ε, CD3γ/ε and CD247 ζ/ζ or ζ/η.

[0474] According to embodiments of the application, suitable TCRs are capable of binding a major histocompatibility complex (MHC) on the surface of cancer cells that displays a peptide fragment of a tumor antigen. An MHC is a set of cell-surface proteins which allow the acquired immune system to recognize 'foreign' molecules. Proteins are intracellularly degraded and presented on the surface of cells by the MHC. MHCs displaying "foreign" peptides, such a viral or cancer associated peptides, are recognized by T cells with the appropriate TCRs, prompting cell destruction pathways. MHCs on the surface of cancer cells can display peptide fragments of tumor antigen i.e. an antigen which is present on a cancer cell but not the corresponding non-cancerous cell. T cells which recognize these peptide fragments can exert a cytotoxic effect on the cancer cell.

[0475] According to embodiments of the application, the T cells can be modified to express a heterologous TCR that is capable of binding to MHCs displaying peptide fragments of a tumor antigen expressed by the cancer cells in a specific cancer patient. Tumor antigens expressed by cancer cells in the cancer patient may be identified using standard techniques. For example, a polypeptide, such as an FSH fragment, which is capable of binding to an FSHR, is fused to CD3 epsilon, CD3 gamma or CD3 delta chain. The fusion protein forms a TCR complex on T cells through the interaction of CD3 with TCR α/β chains. The TCR complex is capable of binding FSHR on tumor cells via the FSH fragment, and the binding initiates TCR signaling against the tumor cells. According to yet another embodiment of the invention, the heterologous TCR is expressed together with a CAR that is capable of binding mesothelin. Heterologous TCRs can include αβTCR heterodimers.

[0476] The TCR can be engineered to increase its affinity or avidity for a tumor antigen (i.e., an affinity enhanced TCR). The affinity enhanced TCR can comprise one or more

mutations relative to a naturally occurring TCR, for example, one or more mutations in the hypervariable complementarity determining regions (CDRs) of the variable regions of the TCR α and β chains. These mutations increase the affinity of the TCR for MHCs that display a peptide fragment of a tumor antigen expressed by cancer cells. Suitable methods of generated affinity enhanced TCRs include screening libraries of TCR mutants using phage or yeast display and are well known in the art (see for example Robbins et al J Immunol (2008) 180 (9) : 6116; San Miguel et al (2015) Cancer Cell 28 (3) 281-283; Schmitt et al (2013) Blood 122 348-256; Jiang et al (2015) Cancer Discovery 5 901).

[0477] In some of embodiments, the cell comprises a recombinant nucleic acid encoding a TCRa and/or TCRb chain or a portion thereof that has been codon-optimized. In some embodiments, the transgene encodes a portion of a TCRa and/or TCRb chain with less than 100% amino acid sequence identity to a corresponding portion of a native or endogenous TCRa and/or TCRb chain. In some embodiments, the encoded TCRa and/or TCRb chain contains an amino acid sequence with, with about, or with at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or greater than 99% identity but less than 100% identity to a corresponding native or endogenous TCRa and/or TCRb chain. In particular embodiments, the transgene encodes a TCRa and/or TCRb constant domain or portion thereof with less than 100% amino acid sequence identity to a corresponding native or endogenous TCRa and/or TCRb constant domain. In some embodiments, the TCRa and/or TCRb constant domain contains an amino acid sequence with, with about, or with at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or greater than 99% identity but less than 100% identity to a corresponding native or endogenous TCRa and/or TCRb chain.

[0478] In some embodiments, the recombinant nucleic acid contains one or more modifications(s) to introduce one or more cysteine residues that are capable of forming one or more non-native disulfide bridges between the TCRa chain and TCRB chain. In some embodiments, the one or more non-native cysteine residues are capable of forming non-native disulfide bonds, *e.g.,* with a TCRa chain encoded by the transgene.

[0479] In some embodiments, the transgene encodes all or a portion of a TCRa constant domain (Ca) and/or a TCRb constant domain (Cb) with one or more modifications to remove or prevent a native disulfide bond, *e.g.,* between the TCRb chain encoded by the transgene and the endogenous TCRa chain. In some embodiments, one or more native cysteines that form and/or are capable of forming a native interchain disulfide bond are substituted to another residue, *e.g.,* serine or alanine. In some embodiments, the portion of a

TCRa constant domain (Ca) and/or a TCRb constant domain (Cb) is modified to replace one or more non-cysteine residues to a cysteine. Exemplary of modified encoded TCRa constant domain (Ca) and/or TCRb constant domain (Cb) include any of those described herein, for example, in Section III.A, and those described in W02006/000830, WO 2006/037960 and Kuball et al. (2007) Blood, 109:2331- 2338.

[0480] Among such recombinant TCRs or antigen-binding fragments thereof are TCRs or antigen-binding fragments thereof that contain any of the variable alpha (Va) regions and/or a variable beta (Vb) regions as described, individually, or a sufficient antigen-binding portion of such chain(s). In some embodiment, the provided recombinant TCRs comprise a TCR alpha (TCRa) chain comprising a variable alpha (Va) region, and a TCR beta (TCRb) chain comprising a variable beta (Vb) region.

[0481] In some embodiments, the TCR or antigen-binding fragment thereof is isolated or purified or is recombinant. In particular embodiments, any of the provided TCR or antigenbinding fragment thereof is recombinant. In some aspects, the TCR or antigen-binding fragment thereof is human. In some embodiments, the recombinant TCR is monoclonal. In some aspects, the recombinant TCR is a single chain. In other embodiments, the recombinant TCR contains two chains. In some embodiments, the TCR or antigen-binding fragment thereof is expressed on the surface of a cell.

[0482] Unless otherwise stated, the term "TCR" should be understood to encompass full TCRs as well as antigen-binding portions or antigen-binding fragments thereof. In some embodiments, the TCR is an intact or full-length TCR, such as a TCR containing the a chain and b chain. In some embodiments, the TCR is an antigen-binding portion that is less than a full- length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR can contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable a (Va) chain and variable b (Vb) chain of a TCR, or antigen-binding fragments thereof sufficient to form a binding site for binding to a specific MHC-peptide complex.

[0483] In some embodiments, the variable domains of the TCR contain complementarity determining regions (CDRs), which generally are the primary contributors to antigen recognition and binding capabilities and specificity of the peptide, MHC and/or MHC-

peptide complex. In some embodiments, a CDR of a TCR or combination thereof forms all or substantially all of the antigen-binding site of a given TCR molecule. The various CDRs within a variable region of a TCR chain generally are separated by framework regions (FRs), which generally display less variability among TCR molecules as compared to the CDRs (see, *e.g.,* lores et al., Proc. Nat'l Acad. Sci. U.S.A. 57:9138, 1990; Chothia et al., EMBO J. 737^5, 1988; see also Lefranc et al., Dev. Comp. Immunol. 27:55, 2003). In some embodiments, CDR3 is the main CDR responsible for antigen binding or specificity, or is the most important among the three CDRs on a given TCR variable region for antigen recognition, and/or for interaction with the processed peptide portion of the peptide-MHC complex. In some contexts, the CDR1 of the alpha chain can interact with the N-terminal part of certain antigenic peptides. In some contexts, CDR1 of the beta chain can interact with the C-terminal part of the peptide. In some contexts, CDR2 contributes most strongly to or is the primary CDR responsible for the interaction with or recognition of the MHC portion of the MHC-peptide complex. In some embodiments, the variable region of the b-chain can contain a further hypervariable region (CDR4 or HVR4), which generally is involved in superantigen binding and not antigen recognition (Kotb (1995) Clinical Microbiology Reviews, 8:411-426).

[0484] In some embodiments, the TCRa chain and/or TCRb chain also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, *e.g.,* Janeway et al., Immunobiology: The Immune System in Health and Disease, 3rd Ed., Current Biology Publications, p. 4:33, 1997). In some aspects, each chain (e.g. alpha or beta) of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR, for example via the cytoplasmic tail, is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. In some cases, the structure allows the TCR to associate with other molecules like CD3 and subunits thereof. For example, a TCR containing constant domains with a transmembrane region may anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex. The intracellular tails of CD3 signaling subunits (e.g. CD3y, CD36, CD3s and CD3(^ chains) contain one or more immunoreceptor tyrosine-based activation motif or IT AM and generally are involved in the signaling capacity of the TCR complex.

[0485] The various domains or regions of a TCR can be identified. In some cases, the exact locus of a domain or region can vary depending on the particular structural or homology modeling or other features used to describe a particular domain. It is understood that reference to amino acids, including to a specific sequence set forth as a SEQ ID NO used to describe domain organization of a TCR are for illustrative purposes and are not meant to limit the scope of the embodiments provided. In some cases, the specific domain (e.g. variable or constant) can be several amino acids (such as one, two, three or four) longer or shorter. In some aspects, residues of a TCR are known or can be identified according to the International Immunogenetics Information System (IMGT) numbering system (see e.g. www.imgt.org; see also, Lefranc et al. (2003) Developmental and Comparative Immunology, 27(l);55-77; and The T Cell Factsbook 2nd Edition, Lefranc and LeFranc Academic Press 2001).

[0486] In some embodiments, the TCRa chain and/or TCRb chain each further contain a constant domain. In some embodiments, the a chain constant domain (Ca) and b chain constant domain (Cb) individually are mammalian, such as is a human or murine constant domain. In some embodiments, the constant domain is adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable domains, which variable domains each contain CDRs.

[0487] In some aspects, provided herein are TCRs that contains a human constant region, such as an alpha chain containing a human Ca region and a beta chain containing a human Cb. In some embodiments, the provided TCRs are fully human. Among the provided TCRs are TCRs containing a human constant region, such as fully human TCRs, whose expression and/or activity, such as when expressed in human cells, e.g. human T cells, such as primary human T cells, are not impacted by or are not substantially impacted by the presence of an endogenous human TCR.

[0488] In some embodiments, the TCR may be a heterodimer of two chains TCRa and TCRb that are linked, such as by a disulfide bond or disulfide bonds. In some embodiments, the constant domain of the TCR may contain short connecting sequences in which a cysteine residue forms a disulfide bond, thereby linking the two chains of the TCR. In some embodiments, a TCR may have an additional cysteine residue in each of the TCRa and TCRb chains, such that the TCR contains two disulfide bonds in the constant domains. In

some embodiments, each of the constant and variable domains contains disulfide bonds formed by cysteine residues.

[0489] In some embodiments, the TCR can contain an introduced disulfide bond or bonds. In some embodiments, the native disulfide bonds are not present. In some embodiments, the one or more of the native cysteines (e.g. in the constant domain of the a chain and b chain) that form a native interchain disulfide bond are substituted to another residue, such as to a serine or alanine. In some embodiments, an introduced disulfide bond can be formed by mutating noncysteine residues on the alpha and beta chains, such as in the constant domain of the a chain and b chain, to cysteine. Opposing cysteines in the TCRa and TCRb chains provide a disulfide bond that links the constant regions of TCRa and TCRb chains of the substituted TCR to one another and which is not present in a TCR comprising the unsubstituted constant region in which the native disulfide bonds are present, such as unsubstituted native human constant region or the unsubstituted native mouse constant region. In some embodiments, the presence of non-native cysteine residues (e.g. resulting in one or more non-native disulfide bonds) in a recombinant TCR can favor production of the desired recombinant TCR in a cell in which it is introduced over expression of a mismatched TCR pair containing a native TCR chain. In some embodiments, the TCRa and/or TCRb chain and/or a TCRa and/or TCRb chain constant domains are modified to replace one or more non-cysteine residues to a cysteine.

c. T cell receptor fusion constructs (TRuCs)

[0490] In some embodiments, the receptor of the disclosure is a T cell receptor fusion construct (TRuC) such as those described in WO2016187349A1, which is herein incorporated by reference in its entirety. The TRuC may comprise a TCR subunit and an antigen binding region. In some embodiments, the TRuC is able to associate with one or more endogenous (or alternatively, one or more exogenous, or a combination of endogenous and exogenous) TCR subunits in order to form a functional TCR complex. In some embodiments, the TRuC-mediated T-cell response is directed to the antigen that may be bound by the antigen binding region.

[0491] In some embodiments, the TCR subunit comprises a TCR extracellular domain. In other embodiments, the TCR subunit comprises a TCR transmembrane domain. In yet other embodiments, the TCR subunit comprises a TCR intracellular domain. In further embodiments, the TCR subunit comprises (i) a TCR extracellular domain, (ii) a TCR

transmembrane domain, and (iii) a TCR intracellular domain, wherein at least two of (i), (ii), and (iii) are from the same TCR subunit.

[0492] In some embodiments, the TRuC comprises a TCR extracellular domain or portion thereof of a protein selected from the group consisting of the TCR alpha chain, TCR beta chain, CD3 delta, CD3 epsilon, CD3 gamma, or a functional fragment thereof, or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications thereto.

[0493] In some embodiments, the TRuC comprises a transmembrane domain or portion thereof of a protein selected from the group consisting of the TCR alpha chain, TCR beta chain, TCR zeta chain, CD3 epsilon, CD3 gamma, CD3 delta, CD45, CD2, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD28, CD37, CD64, CD80, CD86, CD134, CD137, and CD154, or a functional fragment thereof, or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications thereto.

[0494] In some embodiments, the TRuC comprises an intracellular domain comprising a stimulatory domain selected from an intracellular signaling domain of CD3 epsilon, CD3 gamma, CD3 delta, 4-1BB, and CD3 zeta, or an amino acid sequence having at least one, two or three modifications thereto.

[0495] In some embodiments, the TRuC further comprises a costimulatory domain or portion thereof of a protein selected from the group consisting of DAP10, DAP12, CD30, LIGHT, OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), or an amino acid sequence having at least one, two or three modifications but not more than 20, 10 or 5 modifications thereto.

[0496] In some embodiments, a TRuC antigen binding region is or comprises an scFv or single domain antibody. The scFv and/or single domain antibody may be any of those described herein. In some embodiments, the binds to a cell surface antigen of a cell. In some embodiments, the scFv and/or the antigen are as described herein.

[0497] In some embodiments, the TRuC or antigen-binding fragment thereof is isolated or purified or is recombinant. In particular embodiments, the TRuC or antigen binding fragment thereof is recombinant. In some embodiments, the TRuC or antigen-binding fragment thereof is human. In some embodiments, the TRuC or antigen-binding fragment thereof is humanized. In some embodiments, the TRuC or antigen-binding fragment thereof is expressed on the surface of a cell.

d. T-bodies and HLA-independent T-cell (HIT) receptors

[0498] In some embodiments, the receptor of the disclosure is a T-body. As used herein, the term "T-body" refers to a receptor that merges the design of a CAR with a TCR. Spefically, in a T-body, the heavy chain variable region (VH) and light chain variable region (VL) domains of an scFv (typically used to construct the antigen-binding portion of a CAR) replace (i) the variable a (Va) chain and variable b (Vb) chain of a TCR, or (ii) the Vb chain and the Va chain of a TCR, respectively. The resulting "T-body" has an antigen-binding region that behaves like that of a CAR, but its intracellular signaling domain utilizes endogenous TCR components (instead of, e.g., the CD3z that is commonly used for a CAR).

[0499] In some embodiments, the T-body may comprise a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRalpha, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRbeta. In some embodiments, the T-body may comprise a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRbeta, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRalpha. In some embodiments, neither the constant region of TCRalpha nor the constant region of TCRbeta are an endogenous constant region of TCRalpha or an endogenous constant region of TCRbeta. In some embodiments, the nucleic acid(s) encoding the constant region(s) of TCRalpha and/or TCRbeta are not the endogenous TCR encoding region(s) of the cell genome.

[0500] In some embodiments, the receptor of the disclosure is an HLA-independent T-cell (HIT) receptor. In some embodiments, the constant region of TCRalpha and/or the constant region of TCRbeta are an endogenous constant region of TCRalpha and/or an endogenous constant region of TCRbeta.

[0501] In some embodiments, the T-body or HIT receptor comprises a transmembrane domain or portion thereof disclosed herein (*e.g.,* as disclosed for TCRs). In some embodiments, the T-body or HIT receptor comprises an intracellular domain comprising a stimulatory domain disclosed herein (*e.g.,* as disclosed for TCRs).

[0502] In some embodiments, the T-body or HIT receptor is isolated or purified or is recombinant. In particular embodiments, the T-body or HIT receptor is recombinant. In some embodiments, the T-body or HIT receptor is human. In some embodiments, the T-body or HIT receptor is humanized. In some embodiments, the T-body or HIT receptor is expressed on the surface of a cell.

e. Synthetic Receptors

[0503] In some embodiments, the engineered receptor is a synthetic receptor comprising an extracellular antigen recognition domain that recognises an antigen on a target cell and a transmembrane domain. In some embodiments, the synthetic receptor further comprises an intracellular signalling domain that activates expression of a response element in the cell when the extracellular antigen recognition domain binds the target cell. The synthetic receptors allow the cells to recognise and bind specific target cells and to activate immune responses upon binding.

[0504] In some embodiments, the synthetic receptor is a synthetic RIP family receptor, a synthetic notch receptor (synNotch), a synthetic intramembrane proteolysis receptor (SNIPR), a synthetic Robo receptor (SynRobo receptor), a synthetic RoboNotch receptor (SynRobo receptor).

[0505] SNIPRs comprise an extracellular antigen binding region, which may be an scFv capable of binding a particular cell surface antigen, such as a tumour antigen, a transmembrane domain, which controls proteolysis and cleavage, and an intracellular signalling domain, which may be a transcription factor. SNIPRs comprise a transmembrane domain from the regulated intracellular proteolysis (RIP) family of proteins. For example, a SNIPR may comprise a transmembrane domain selected from the group consisting of Notchl, Notch2 and Robol. In some embodiments, the synthetic receptor is a SynNotch receptor. Binding of the antigen recognition domain releases a transcription factor. In some embodiments, the SNIPR may further comprise a juxtamembrane domain, such as a human NOTCH2 juxtamembrane domain.

[0506] In some embodiments, the synthetic receptor is a transcriptional regulator, such as the synthetic notch receptors described in US 11,202,801 or other synthetic receptors, such as, for example, those described in provisional application nos: 62/905,251, 63/15,428, 62/905,268, 62/905,263, 62/935,024 and 62/905,248, which are herein incorporated by reference in their entirety.

[0507] In some embodiments, the synthetic receptor comprises a Notch domain encoded by a Notch gene. A preferred Notch gene is NOTCH1. Alternative Notch genes include NOTCH2, NOTCH3 and NOTCH4. In other embodiments, the synthetic receptor comprises a Robol domain.

[0508] In certain embodiments, the synthetic receptor system is multiplexed with multiple transcription factor domains and/or response elements. In certain embodiments, the engineered cell expresses on its surface a synthetic receptor, optionally a SynNotch receptor, comprising an extracellular antigen binding region that recognises an antigen on a target cell, a transmembrane domain, and two or more intracellular signalling domains that activate expression of a two or more response elements in the cell when the extracellular antigen binding region binds the target cell, wherein at least one response element encodes a gene that activates an immune response. In certain embodiments, the engineered cell expresses on its surface a synthetic receptor, optionally a SynNotch receptor, comprising an extracellular antigen binding region that recognises an antigen on a target cell, a transmembrane domain, and two or more intracellular signalling domains that activate expression of a two or more response elements in the cell when the extracellular antigen binding region binds the target cell, wherein at least one response element encodes a transcription factor.

V. T CELL SIGNALING

a. TCR Signaling

[0509] Upon engagement of a peptide-MHC complex, the immunoreceptor tyrosine-based activation motifs (ITAMs) contained within the intracellular tails of the TCR complex become phosphorylated and recruit Zap70. Zap70 then recruits and activates the downstream LAT signalosome containing LAT, PLCy, Slp76. This signalosome causes the activation of the Ras/MAPK/ERK signaling leading to AP-1 transcriptional activity, the activation of PKCθ leading to NF-κB transcriptional activity and finally the influx of calcium which is responsible for NFAT transcriptional activity. Additionally, signaling from costimulatory receptors, such as 41BB, and cytokine support from IL-2 enhances these signaling pathways, and is required for T cells to undergo full activation and avoid anergic cell death. When fully activated, T cells rapidly proliferate and differentiate, secrete inflammatory cytokines, and cytotoxic cells begin performing target killing through release of cytotoxic granules. After resolution of this acute response, activated T cells undergo contraction to a form a small population of long-lived memory T cells that surveil the body, ready to respond to future encounters with the antigen.

b. T Cell Signaling

[0510] T cells become fully activated through T cell receptor (TCR) engagement, costimulatory signaling and cytokine support. TCR activation induces NFAT, NF-κB and AP-1 transcription factors signaling, IL-2 and other inflammatory cytokine secretion.

c. T Cell Exhaustion

[0511] T cell exhaustion is a state of hypo-responsiveness induced in effector T cell populations after chronic exposure to antigen. Exhaustion is marked by the upregulation of surface inhibitory receptors such as PD-1, TIM-3, LAG-3 and CTLA-4 among others, and the inability to proliferate long term, secrete inflammatory cytokines and kill target cells effectively. Exhausted T cells exist in a distinct transcriptional and epigenetic landscape. While exhaustion has been well characterized in chronic viral infections, it is also implicated in cancer, in which tumor infiltrating T cells, and engineered T cell therapies exhibit signs of exhaustion and fail to control tumor growth. To better treat chronic infections or cancer, T cell exhaustion must be prevented or overcome. In fact, checkpoint therapies which block signaling from the PD-1 or CTLA-4 axis of inhibition have proven somewhat effective in reinvigorating T cell responses in certain cancers leading to limited but effective tumor control.

d. NFAT Signaling

[0512] The transcription factor nuclear factor of activated T cells (NFAT) is involved in T cell activation as well as programs of T cell exhaustion. At resting state, NFAT transcription factors are found in the cytoplasm in a phosphorylated state, unable to translocate to the nucleus to induce signaling. The extracellular influx of Ca^{2+} that occurs during T cell activation activates the calcium dependent phosphatase calcineurin, resulting in dephosphorylation of NFAT, and its subsequent translocation and signaling in the nucleus. NFAT binds DNA in multiple ways, as a monomer, dimer or as a complex with other transcription factors. In particular NFAT is known to cooperatively bind with the transcription factor AP-1, inducing transcription of activation associated genes critical for effector T cell function. In contrast, when NFAT is not bound by AP-1, but is instead partnerless, it has been found to induce the expression of exhaustion associated genes, such as the inhibitory receptor PD-1. Additionally, studies have determined that exhaustion induced by partnerless NFAT can be remedied in CAR T cells by over-expressing the AP-1 family member, cJun. NFAT induces the expression of transcription factors TOX and

NR4A1/2/3, which have been associated with T cell exhaustion and deletion or knockdown of these transcription factors has improved T cell phenotype and *in vivo* tumor control.

[0513] As NFAT signaling is key to successful T cell activation, but also plays a role in exhaustion, particularly in the absence of AP-1, it can be used to determine how genetic modifications in T cells influences the dynamics of NFAT signaling. Targeting the NFAT transcriptional pathway can be beneficial for T cell therapeutics.

e. NF-κB Signaling

[0514] NF-κB (nuclear factor kappa light chain enhancer of B cells) is a family of transcription factors that induces transcriptional programs critical for T cell activation and effector function. NF-κB signaling occurs in many cell types. In T cells, NF-κB signaling induces a wide range of transcriptional programs responsible for proliferation and memory formation, resistance to apoptosis, cytokine secretion, and the production of a robust effector T cell response. NF-κB signaling is induced through two pathways, the canonical and noncanonical pathways.

[0515] In the canonical pathway NFKB1 is bound in the cytoplasm by IkBa and IkB-like molecule p105, forming a complex that prevents the nuclear translocation of NKFB1. When activated, TCR signaling induces activation of PKCθ, which in turn phosphorylates and activates CARD11. Activated CARD11 forms a complex with BCL10 and MALT1, and ultimately causes the phosphorylation and degradation of IkBa and IkB-like molecule p105, releasing NF-κB to translocate to the nucleus.

[0516] The non-canonical pathway is triggered by signaling through tumor necrosis factor receptor (TNFR) family members, which includes the costimulatory domain 41BB. At rest, the NFKB2 is bound by p100, during activation NF-κB -inducing kinase (NIK) activates IKKa, which in turn phosphorylates p100 and leads to the release and translocation of NKFB2 to the nucleus.

[0517] In syngeneic models of solid tumors, endogenous T cells that can respond to the tumors required NF-κB signaling to mediate tumor clearance. While NF-κB is induced through TCR signaling, it is also stimulated through the costimulatory domain 41BB (a TNFRS superfamily member), which is used clinically in FDA approved chimeric antigen receptor (CARs) therapies. CAR therapies using 41BB costimulatory domains have been found to persist longer in patients as compared to CAR therapies using other costimulatory domains, such as CD28. The persistence of 41BB CARs is directly linked to NF-κB

signaling, which improves CAR T resistance to apoptosis through suppression of apoptotic proteins such as Bim.

f. AP-1 Signaling

[0518] AP-1 transcription factors are a family of homo or hetero-dimeric proteins formed from complexes of JUN, FOS, ATF or MAF proteins. AP-1 signaling is induced by the phosphorylation cascade called the Mitogen Activated Protein Kinase (MAPK) pathway, triggered by TCR, cytokine/chemokine, or growth factor signaling. AP-1 often signals in complex with NFAT, and as indicated in the NFAT section, the loss of AP-1 causes partnerless NFAT to induce exhaustion programs in T cells, however this exhaustion can be remedied by overexpression of the AP-1 family member c-Jun. Related to this, loss of AP-1 is also known to induce an anergic cell state, where the T cell has partially activated through the TCR, but does not have enough costimulatory and cytokine signaling to induce full activation.

[0519] AP-1 dimers are activated by a plethora of physiological and pathological stimuli. Studies have reported that AP-1 proteins, mostly those that belong to the Jun group, control cell life and death through their ability to regulate the expression and function of cell cycle regulators such as Cyclin D1, p53, p21(cip1/waf1), p19(ARF) and p16. Amongst the Jun proteins, c-Jun is unique in its ability to positively regulate cell proliferation through the repression of tumor suppressor gene expression and function, and induction of cyclin D1 transcription. These actions are antagonized by JunB, which upregulates tumor suppressor genes and represses cyclin D1. An important target for AP-1 effects on cell life and death is the tumor suppressor p53, whose expression as well as transcriptional activity, are modulated by AP-1 proteins.

[0520] The concomitant induction of NFAT and AP-1 takes concerted activation of two different signaling pathways: calcium/calcineurin, which promotes NFAT dephosphorylation, nuclear translocation and activation; and protein kinase C (PKC)/Ras, which promotes the synthesis, phosphorylation, and activation of members of the Fos and Jun families of transcription factors. (Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol 2002 45. 2002;4(5):E131-E136.).

g. IL-2 Signaling

[0521] IL-2 is a pleiotropic cytokine required for activation, proliferation differentiation, and maintenance of T cells. Naïve T cells express a low affinity IL-2 receptor requiring high volumes of IL-2 to initiate activation, while memory and regulatory T cells express a high

affinity IL-2 receptor requiring much lower amounts of IL-2 for effective signaling. The IL-2 receptor makes use of the JAK/STAT signaling cascade, resulting in wide ranging transcriptional changes. Though CD8+ T cells are responsible for the cytotoxic effector response to foreign antigens, CD8+ T cells themselves cannot effectively produce IL-2 and rely instead on helper CD4+ T cells to produce IL-2 and other cytokine support.

[0522] IL-2 is so critical for T cell persistence and proliferation that high dose IL-2 therapy has been FDA approved for certain cancers, with the hopes of expanding endogenous cytotoxic T cell populations to induce tumor rejection. In metastatic melanoma and renal cell carcinoma trials, high dose IL-2 therapy induces limited (7%) long term response rates.

[0523] However, this therapy is not well tolerated by patients, causing multiple deaths, and likely contributes to the expansion of CD4+CD25+FoxP3+ regulatory T cells. Regulatory T cells are known to be pro-tumorigenic and high numbers of regulatory T cells (Tregs) are correlated with poor prognosis in solid tumor settings. Toxicity and the expansion of pro-tumor Treg populations present so much of a challenge to IL-2 therapy that synthetic orthogonal IL-2 cytokine therapies have recently been developed to target the effects of IL-2 cytokine therapy to cytotoxic T cells and avoid expansion of Tregs.

[0524] IL-2 secretion is highly relevant for the persistence and proliferation of T cell therapeutic products, therefore the inventors/disclosers assessed IL-2 secretion capacity of each mutation when expressed in CAR Jurkat cell lines and after co-culture with target cells. As a follow up for relevant hits, the inventors/disclosers assessed the capacity for mutations to improve CAR T cell *in vitro* target killing when cultured without supplemental IL-2. This setting, where IL-2 is withheld, better reflects the challenges of the tumor microenvironment, where IL-2 is scarce and autocrine secretion of IL-2 would be beneficial for maintenance of proliferation and long-term killing capabilities.

h. CARD11-BCL10-MALT Signalosome

[0525] Formation of a CARD11-BCL10-MALT (CBM) signaling complex is a key event in T- and B cell receptor-induced gene expression. After exposure to distinct immune triggers, these molecules form self-organizing filaments with MALT1 protease activity to regulate canonical nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways and the degradation of mRNA-binding proteins, which provides two layers of control of inflammatory gene expression. Deregulation of CARD11, BCL10 or MALT1 expression or CBM signaling have been associated with cancer, immunodeficiency,

and autoimmunity (J. Ruland, L. Hartjes, CARD-BCL-10-MALT1 signalling in protective and pathological immunity. Nat Rev Immunol 19, 118-134 (2019).

[0526] In normal T cells, T cell receptor (TCR) signaling activates PKCθ, which in turn promotes the assembly of the CARD11-BCL10-MALT1 (CBM) signalosome. The CBM complex subsequently has three major outputs: NF-κB transcriptional activity, AP-1 transcriptional activity and MALT1 proteolytic activity. The assembly of the CARD11-BCL10-MALT1 signalosome complex is an essential step in regulating NF-κB in lymphoid immune cells.

[0527] An inhibitory domain present in CARD11 may allow for intramolecular autoinhibition which can prevent CARD11 binding to BCL10 in the absence of upstream signals. Upon phosphorylation of the inhibitory domain in normal T cells, CARD11 autoinhibition can be relieved and the CARD11 protein can oligomerize and promote prion-like assembly of the CBM complex with recruitment of BCL10-MALT1 filaments, which then can allow for CBM complex signaling.

[0528] Genes involved in CBM signaling include but are not limited to caspase recruitment domain family member 11 (CARD11), capping protein regulator and myosin 1 linker 2 (CARMIL2), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), B-cell lymphoma 10 (BCL10). CARD11 can be involved in both the innate and adaptive immune systems. CARD11 is implicated in the activation of NF-kB by the TCR complex.

i. JAK/STAT signaling

[0529] The biochemistry of JAK/STAT signaling is well known to a person of skill in the art. Briefly, signaling begins with extracellular association of cytokines or growth factors with their corresponding transmembrane receptors. This facilitates trans-activation of receptor-bound Janus kinases (JAKS) by putting them in spatial proximity and by prompting conformational changes that distance their kinase domains from inhibitory pseudokinase domains. Activated JAKS then phosphorylate latent STAT monomers, leading to dimerization, nuclear translocation, and DNA binding. In mammals, 4 JAKS (JAK1, JAK2, JAK3, TYK2) and 7 STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) are employed by more than 50 cytokines and growth factors. (Villarino A V., Kanno Y, O'Shea JJ. Mechanisms and consequences of JAK–STAT signaling in the immune system. Nat Immunol 2017 184. 2017;18(4):374-384).

j. Co-Stimulatory Molecule Ssignaling

[0530] Co-stimulatory and co-inhibitory molecules are cell surface receptors and ligands that are classified into various families on the basis of their structure and functions.

[0531] Co-stimulatory and co-inhibitory receptors determine the functional outcome of T cell receptor (TCR) signaling. The specific recognition of cognate antigenic peptides presented by MHC molecules triggers T cell receptor signaling, but it is co-stimulatory and co-inhibitory receptors on T cells that direct T cell function and determine T cell fate. T cell co-signaling receptors have been broadly defined as cell-surface molecules that can transduce signals into T cells to positively (co-stimulatory receptors) or negatively (co-inhibitory receptors) modulate TCR signaling. Examples of co-stimulatory or co-inhibitory receptors includes CD28 and CTLA-4, both of which bind to ligands B7-1 and 7-2. Other genes involved in co-stimulatory molecule signaling include TNFR2, TNFRS1B, and ICOS (Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol 2013 134. 2013;13(4):227-242.)

k. RAS/MEK/ERK Signaling

[0532] As is known by the skilled in the art, the sarcoma/mitogen-activated protein kinase kinase/extracellular receptor kinase (RAS/MEK/ERK) is a conserved signaling pathway that plays pivotal roles in cell proliferation, survival and differentiation. The aberrant activation of the RAS/MEK/ERK signaling pathway induces tumors. Efforts have been dedicated to targeting this signaling pathway for cancer treatment. The aberrant activation of the signaling pathway contributes to tumorigenesis and tumor development.

[0533] It is also known that triggering of a T-cell receptor by its cognate antigen results in nearly immediate activation of downstream signaling cascades, including the RAS/MEK/ERK pathway. Studies have also shown that RAS/MEK/ERK signaling is memory stage-dependent in human T cells, conferring susceptibility to alloreactive T-cell selective inhibition.

[0534] As an important upstream molecular marker of the RAS–RAF–MEK–ERK pathway, RAS serves as a molecular switch by binding GTP/GDP, and it includes four isoforms:HRAS, KRAS4A, KRAS4B, and NRAS. KRAS is the most frequent isoform in all human cancers. KRAS4A and KRAS4B are the different splicing isoforms of the same gene. When the transmembrane receptors (receptor tyrosine kinase, RTKs) are activated, the complexes of growth-factor-receptor bound protein 2 (GRB2) and son of sevenless (SOS) in the cytoplasm are recruited to the inner surface of cell membrane. (McCubrey JA, Steelman

LS, Bäsecke J, Martelli AM. Raf/mek/erk signaling. Target Ther Acute Myeloid Leuk. January 2015:275-305.)

[0535] Several mutations identified by the inventors/disclosers are in genes having various roles in this signaling pathway.

1. Phospholipase C Gamma Signaling

[0536] Phospholipase C (PLC) is an essential mediator of cellular signaling. PLC regulates multiple cellular processes by generating bioactive molecules such as inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). These products propagate and regulate cellular signaling via calcium (Ca2+) mobilization and activation of protein kinase C (PKC), other kinases, and ion channels. PLCγ1, one of the primary subtypes of PLC, is directly activated by membrane receptors, including receptor tyrosine kinases (RTKs), and adhesion receptors such as integrin. PLCγ1 mediates signaling through direct interactions with other signaling molecules via SH domains, as well as its lipase activity. PLCγ1 is frequently enriched and mutated in various cancers, and is involved in the processes of tumorigenesis, including proliferation, migration, and invasion. (Jang HJ, Suh PG, Lee YJ, Shin KJ, Cocco L, Chae YC. PLCγ1: Potential arbitrator of cancer progression. Adv Biol Regul. 2018;67:179-189, and Patterson RL, Van Rossum DB, Nikolaidis N, Gill DL, Snyder SH. Phospholipase C-γ: diverse roles in receptor-mediated calcium signaling. Trends Biochem Sci. 2005;30(12):688-697

[0537] The phospholipase C gamma signaling pathway has been implicated in T cell lymphomas and primarily in cutaneous T cell lymphomas (CTCL). Nine *PLCG1* mutations (p.R48W, p.S312L, p.D342N, p.S345F, p.S520F, p.R1158H, p.E1163K, p.D1165H, and the in-frame indel p.VYEEDM1161V) have been identified in Sézary Syndrome, the leukemic variant of CTCL. (V. M. Patel et al., Frequent and Persistent PLCG1 Mutations in Sézary Cells Directly Enhance PLCγ1 Activity and Stimulate NFκB, AP-1, and NFAT Signaling. J Invest Dermatol 140, 380-389.e384 (2020).

VI. CARD-CONTAINING PROTEINS

CARD11

[0538] Caspase-associated recruitment domain (CARD) is a conserved homology domain comprising a 6-helix bundle or 5-helix bundle. CARDs can mediate protein-protein interactions between key apoptotic signaling molecules. Non-limiting examples of CARDs include CARD6, CARD8, CARD9, CARD10, CARD11, CARD14, CARD16, CARD18,

and CARD19. Non-limiting examples of CARD-containing proteins include those described in Boyle and Monie *bioRxiv* 087908; doi: https://doi.org/10.1101/087908; and/or Park. *In J Mol Med.* 2019 Mar; 43(3):1119-1127, such as human caspase-1, -2, -4, and -5, mouse caspase-1, -2, -11, and -12, ASC, NOD1, NOD2, Apaf-1, BCL-10, and RIG-I. In some embodiments, the CARD containing protein, or functional fragment thereof, is derived from a CARD protein selected from CARD9 (UniProt # Q9H257), CARD10 (UniProt # Q9BWT7) CARD11 (UniProt # Q9BXL7), or CARD14 (UniProt # Q9H257) (Wang et al. J Biol Chem. 2001 Jun 15;276(24):21405-9; Bertin et al. J Biol Chem. 2001 Apr 13;276(15):11877-82.). In some embodiments, the CARD containing protein, or functional fragment thereof, is derived from a CARD11 protein (UniProt #Q9BXL7), or a functional fragment thereof. In some embodiments, the CARD containing protein comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 293-323. In some embodiments, the functional fragment of the CARD containing protein is derived from CARD11 and comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 295.

[0539] In some embodiments, the function of the CARD containing protein or the functional fragment thereof is to bind to a CARD domain on BCL10 (Bertin et al. J. Biol Chem. 201 Apr; 276(15):11877-11882). In some embodiments, the functional fragment thereof comprises at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 300, at least 400, or at least 500 amino acids. Non-limiting examples of a cell include a T cell, a macrophage, a monocyte, and a natural killer (NK) cell. In some embodiments, activation of the cell produces the substrate localized to the intracellular side of the plasma membrane. In some embodiments, the substrate localized to the intracellular side of the plasma membrane of a cell is a phosphoinositide. Non-limiting examples of phosphoinosities include those described in Posor et al. Nat Rev Mol Cell Biol. 2022 Dec;23(12):797-816. In some embodiments, the phosphoinositide is selected from phosphatidylinositol (3,4,5)-trisphosphate (PIP3), phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) (Hawse and Cattley J. Bio. Chem. 2019 Mar; 294(13):4793-4805; Sun et al. PLoS ONE. 2011 Nov; 6(11): e27227). In some embodiments, the polypeptide binds to the phosphoinositide with a Kd of less than 100 μM, 50 μM, 10 μM, 5 μM, 1μM, 0.5 μM, 0.1 μM, 0.05 μM, or 0.01 μM, optionally wherein the

Kd is analyzed using SPR as described in Yu et al. *Molecular Cell.* 2004 March; 13(5):p677-688.

[0540] CARD11, also known as Caspase Recruitment Domain Family Member 11, CARMA1, Carma 1, BIMP3, Caspase Recruitment Domain-Containing Protein 11, BCL10-Interacting Maguk Protein 3, CARD-Containing MAGUK Protein 1, Card-Maguk Protein 1, IMD11A, BENTA, IMD11, or PPBL, is encoded by the human CARD11 gene. CARD11 is a membrane-associated guanylate kinase (MAGUK) family protein, a class of proteins that functions as molecular scaffolds for the assembly of multiprotein complexes at specialized regions of the plasma membrane. CARD11 is also a member of the CARD protein family, defined by the presence of a caspase-associated recruitment domain (CARD). CARD11 plays a role in various immune responses including, amoung others, lymphocyte activation, proliferation, and differentiation, and plays a role as a positive regulator of NF-kB activation.

[0541] CARD11 is ~130 kDa and exhibits a modular structure comprising a CARD domain, a LATCH domain, a coiled-coil domain, an inhibitory domain, and a MAGUK domain (which itself is made up of a PDZ domain, SH3 domain, and GUK domain). CARD11 is expressed in immune cells, with variations in expression levels observed across different cell types and states (Lu et al. The CBM-opathies-A Rapidly Expanding Spectrum of Human Inborn Errors of Immunity Caused by Mutations in the CARD11-BCL10-MALT1 Complex. *Front Immunol.* 2018 Sep 19;9:2078).

[0542] CARD11 is a member of the CARD11-BCL10-MALT1 (CBM) signalosome, which is essential for T cell activation and function upon antigen stimulation (Ruland et al. 2019. *Nat. Rev. Immunol.* 19,118-134). The CBM complex is known to have three important outputs: NF-kB transcriptional activity, AP-1 transcriptional activity, and MALT1 proteolytic activity (Kutzner et al. 2022. *Sci Signaling.* 15: eabk3083). In the absence of signaling from the TCR, the CARD11 inhibitory domain blocks CARD11 from forming a complex with BCL10 and MALT1. However, after TCR signaling, the inhibitory domain is phosphorylated and the CARD11 protein is able to oligimerize and recruite BCL10 and MALT1 to form the CBM complex.

[0543] Human CARD11 (isoform 203) has a CARD domain (SEQ ID NO: 295), a LATCH domain (SEQ ID NO: 342), a coiled-coil domain (SEQ ID NO: 343), an inhibitory domain (SEQ ID NO: 344), and a MAGUK domain (SEQ ID NO: 345). The MAGUK

domain is made up of a PDZ domain (SEQ ID NO: 346), SH3 domain (SEQ ID NO: 347), and GUK domain (SEQ ID NO: 348).

[0544] As used herein, a human CARD11 CARD domain refers to the CARD domain near the N-terminus of the human CARD11 protein. In some embodiments, the human CARD11 CARD domain has an amino acid sequence that corresponds to amino acids 11-103 of of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 11-103 of SEQ ID NO: 1. In some embodiments, the human CARD11 CARD domain has an amino acid sequence corresponding to amino acids 11-103 of SEQ ID NO: 1, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 11-103 of SEQ ID NO: 1.

[0545] As used herein, a human CARD11 LATCH domain refers to the LATCH domain between the N-terminal CARD domain and the C-terminal coiled-coil domain of the human CARD11 protein. In some embodiments, the human CARD11 LATCH domain has an amino acid sequence that corresponds to amino acids 104-122 of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 104-122 of SEQ ID NO: 1. In some embodiments, the human CARD11 LATCH domain has an amino acid sequence corresponding to amino acids 104-122 of SEQ ID NO: 1, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 104-122 of SEQ ID NO: 1.

[0546] As used herein, a human CARD11 coiled-coil domain refers to the coiled-coil domain between the N-terminal LATCH domain and the C-terminal inhibitory domain of the human CARD11 protein. In some embodiments, the human CARD11 coiled-coil domain has an amino acid sequence that corresponds to amino acids 123-442 of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 123-442 of SEQ ID NO: 1. In some embodiments, the human CARD11 coiled-coil domain has an amino acid sequence corresponding to amino acids 123-442 of SEQ ID NO: 1, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 123-442 of SEQ ID NO: 1.

[0547] As used herein, a human CARD11 inhibitory domain refers to the inhibitory domain between the N-terminal coiled-coil domain and the C-terminal MAGUK domain of

the human CARD11 protein. In some embodiments, the human CARD11 inhibitory domain has an amino acid sequence that corresponds to amino acids 443-577 of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 443-577 of SEQ ID NO: 1. In some embodiments, the human CARD11 inhibitory domain has an amino acid sequence corresponding to amino acids 443-577 of SEQ ID NO: 1:, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 443-577 of SEQ ID NO: 1.

[0548] As used herein, a human CARD11 MAGUK domain refers to the MAGUK domain near the C-terminus of the human CARD11 protein. In some embodiments, the human CARD11 MAGUK domain has an amino acid sequence that corresponds to amino acids 667-1140 of SEQ ID NO: 518, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 667-1140 of SEQ ID NO: 518. In some embodiments, the human CARD11 MAGUK domain has an amino acid sequence corresponding to amino acids 667-1140 of SEQ ID NO: 518, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids X-X of SEQ ID NO: 518.

[0549] The human CARD11 gene has eight transcripts, three of which encode a protein isoform of CARD11 (Ensemble, Gene: CARD11). The eight transcripts are named CARD11-201, CARD11-202, CARD11-203, CARD11-204, CARD11-205, CARD11-206, CARD11-201, and CARD11-208 (Ensembl, Gene: CARD11, ENSG00000198286). The coding DNA sequence (CDS) of the six transcripts are as set forth in SEQ ID NO: 349-354, respectively. The amino acid sequences of the three protein isoforms are as set forth in SEQ ID NO: 516-518, respectively (**Table 9**).

[0550] Transcript CARD11-203 (SEQ ID NO: 351; Ensembl Transcript ID: ENST00000396946.9; NCBI Reference Sequence: NM_001324281.3) encodes isoform CARD11-203 (SEQ ID NO: 518; Uniprot Accession No. Q9BXL7; NCBI Reference Sequence: NP_001311210.1), which has 1154 amino acids. CARD11-203 is the longest transcript of the human CARD11 gene. It is designated as the representative transcript in the Ensembl database. In identifying the representative transcript, Ensembl aims to identity the transcript that, on balance, has the highest coverage of conserved exons, highest expression, longest coding sequence and is represented in other key resources, such as NCBI and

UniProt. All splice junctions of the CARD11-203 transcript are supported by at least one non-suspect mRNA.

[0551] Transcript CARD11-201 (SEQ ID NO: 349; Ensembl Transcript ID: ENST00000355508.3; NCBI Reference Sequence: NM_001324281.3) encodes isoform CARD11-201 (SEQ ID NO: 516; Uniprot Accession No. H7BY05), which has 271 amino acids.

[0552] Transcript CARD11-202 (SEQ ID NO: 350; Ensembl Transcript ID: ENST00000356408.3) encodes isoform CARD11-202 (SEQ ID NO: 517; Uniprot Accession No. E2QRC0), which has 73 amino acids. The amino acid sequences of the three isoforms are listed in **Table 9**. The amino acids corresponding to the various domains in the human CARD11 protein are also identified in **Table 9**. "Beginning Sequence" refers to the region N-terminal to the CARD domain. "First connection" refers to the region connecting the CARD domain and the LATCH domain. "Second connection" refers to the region connecting the LATCH domain and the Coiled-Coil domain. "Third connection" refers to the region connecting the Coiled-Coil domain and the Inhibitory domain. "Fourth connection" refers to the region connecting the Inhibitory Domain domain and the MAGUK domain. "End Sequeunce" refers to the region C-terminal to the MAGUK domain.

Table 9. Amino Acid SEQ ID NOs for CARD11 Domains

	CARD11-201	CARD11-202	CARD11-203
Beginning Sequence	N/A	1-17	1-17
CARD Domain	N/A	18-73	18-110
First Connection	N/A	N/A	111
LATCH Domain	N/A	N/A	112-130
Coiled-Coil Domain	N/A	N/A	130-449
Third Connection	N/A	N/A	450
Inhibitory Domain	1-197	N/A	450-666
MAGUK Domain	198-271	N/A	667-1140
End Sequence	N/A	N/A	1141-1154

[0553] As used herein, a human CARD11 PDZ domain refers to the PDZ domain near the N-terminus of the human CARD11 MAGUK domain. In some embodiments, the human CARD11 PDZ domain has an amino acid sequence that corresponds to amino acids 667-755

of SEQ ID NO: 518, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 667-755 of SEQ ID NO. In some embodiments, the human CARD11 PDZ domain has an amino acid sequence corresponding to amino acids 667-755 of SEQ ID NO: 518, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 667-755 of SEQ ID NO: 518.

[0554] As used herein, a human CARD11 SH3 domain refers to the SH3 domain between the N-terminal PDZ domain and the C-terminal GUK domain of the human CARD11 MAGUK domain. In some embodiments, the human CARD11 SH3 domain has an amino acid sequence that corresponds to amino acids 773-806 of SEQ ID NO: 518, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 773-806 of SEQ ID NO: 518. In some embodiments, the human CARD11 SH3 domain has an amino acid sequence corresponding to amino acids 773-806 of SEQ ID NO: 518, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 773-806 of SEQ ID NO: 518.

[0555] As used herein, a human CARD11 GUK domain refers to the GUK domain near the C-terminus of the human CARD11 MAGUK domain. In some embodiments, the human CARD11 GUK domain has an amino acid sequence that corresponds to amino acids 973-1140 of SEQ ID NO: 518, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 973-1140 of SEQ ID NO: 518. In some embodiments, the human CARD11 GUK domain has an amino acid sequence corresponding to amino acids 973-1140 of SEQ ID NO: 518, or an amino acid sequence that has at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with amino acids 973-1140 of SEQ ID NO: 518.

[0556] The amino acid sequences of the CARD11-203 PDZ, SH3, and GUK domains in **Table 10**. "PDZ to SH3 Connection" refers to the region connecting the PDZ domain and the SH3 domain. "SH3 to GUK Connection" refers to the region connecting the SH3 domain and the GUK domain.

Table 10. Amino Acid SEQ ID NOs for CARD11 Domains

	CARD11-203
PDZ Domain	667-755

PDZ to SH3 Connection	756-772
SH3 Domain	773-806
SH3 to GUK Connection	807-972
GUK Domain	973-1140

PIK3R3

[0557] PIK3R3, also known as phosphatidylinositol 3-kinase regulatory subunit gamma (UniProt Q92569), is encoded by the human PIK3R3 gene (NCBI gene ID 8503).

[0558] PIK3R3, also known as phosphatidylinositol 3-kinase regulatory subunit gamma, p55, p55-GAMMA, p55PIK, phosphoinositide-3-kinase regulatory subunit 3, is encoded by the human PIK3R3 gene. PIK3R3 is a regulatory subunit of PI3K and phosphorylates phosphatidylinositol (and similar compounds).

[0559] PIK3R3 is ~62kDa and comprises both a catalytic and regulatory subunit including. PIK3R3 comprises two SH2 domains through which it binds activated (phosphorylated) protein-tyrosine kinases to regulate their activity.

[0560] The human PIK3R3 gene has seven transcripts, five of which encode a protein isoform of PIK3R3 (Ensemble, Gene: PIK3R3, ENSG00000117461). The seven transcripts are named PIK3R3-201, PIK3R3-202, PIK3R3-203, PIK3R3-204, PIK3R3-205, PIK3R3-206, and PIK3R3-207 (Ensemble, Gene: PIK3R3, ENSG00000117461).

[0561] In some embodiments, the disclosure relates to a second polypeptide portion derived from PIK3R3. Non-limiting examples of polypeptides derived from PIK3R3 include either of two SH2 domains, a catalytic subunit, and a regulatory subunit. In some embodiments, the polypeptide does not comprise a Coiled-coil domain or a portion thereof. In some embodiments, the polypeptide comprises a Coiled-coil domain or a portion thereof.

CARD11-PIK3R3 Fusion Protein

[0562] A CARD11-PIK3R3 fusion protein has previously been derived from a patient with CD4+ T cell lymphoma (Wang et al., Genomic profiling of Sézary syndrome identifies alterations of key T cell signaling and differentiation genes. *Nature Genetics* 47, 1426-1434 (2015)). Thereapeutic T cells expressing this CARD11-PIK3R3 fusion protein demonstrate augmented CARD11–BCL10–MALT1 complex signalling and anti-tumour efficacy in

several immunotherapy-refractory models in an antigen-dependent manner (Garcia et al., Naturally occurring T cell mutations enhance engineered T cell therapies. *Nature*. 626, 626-634 (2024)). Therapeutic T cells expressing the CARD11-PIK3R3 fusion protein showed no evidence of malignant transformation up to 418 days after T-cell transfer, underscoring the safety of such an approach. These references are herein incorporated by reference in their entirety.

[0563] The present disclosure provides recombinant nucleic acids encoding a modified peptide epitope or recombinant polypeptide as described herein, wherein the recombinant nucleic acid additionally encodes a CARD-containing protein (*e.g.,* a CARD11-PIK3R3 fusion protein) or a functional fragment thereof, for example those described in PCT/US2023/075738, which is herein incorporated by reference in its entirety.

Engineered CARD11-PIK3R3 Fusion Proteins

[0564] The present disclosure provides CARD-containing proteins. The CARD-containing protein may be, or may comprise, a polypeptide comprising a caspase-associated recruitment domain (CARD) or a functional fragment thereof. A functional fragment of a CARD containing protein can be, for example, a fragment that provides NF-κB transcriptional activity, AP-1 transcriptional activity, and/or MALT1 proteolytic activity activity at a level at least 70%, 75%, 80%, 85%, 90%, or 95% of that of a full-length protein, as determined by the *in vitro* CAR Jurkat assay.

[0565] The disclosure further relates to CARD-containing proteins, or to recombinant nucleic acids encoding polypeptides, comprising a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a phosphorylated tyrosine (pTyr). In some embodiments, the domain is capable of binding to a substrate indirectly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. In some embodiments, the domain is capable of binding to a substrate directly localized to the intracellular side of the plasma membrane. Direct localization can refer to

localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to the intracellular side of the plasma membrane itself. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with the intracellular side of the plasma membrane itself.

[0566] Non-limiting examples of domains capable of binding to (i) and/or (ii) include Src Homology region 2 (SH2) domains, Src Homology region 3 (SH3) domains, pleckstrin homology (PH) domains, and phosphytyrosine-binding (PTB) domains. SH2 and PTB domains mediate protein-protein interactions involved in many signal transduction pathways. SH2, SH3, and PTB domains that can be used in the disclosure include, for example, those disclosed in Schlessinger et al. Sci STKE. 2003 Jul 15;2003(191):RE12. SH2 containing proteins can bind to plasma membrane lipids through a different binding pocket than the pTyr binding pocket; most SH2 domains bind plasma membrane lipids and many have high phosphoinositide specificity (Park et al., Cell. 2016 Apr 7;62(1):7-20). In some embodiments, the domain is, or comprises, an SH3 domain. In some embodiments, the domain is, or comprises, a PTB domain. In some embodiments, the domain is, or comprises, a PH domain. In some embodiments, the domain is, or comprises, an SH2 domain. In some embodiments, the SH2 domain comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 305 and 307-437. In some embodiments, the SH2 domain is from a PIK3R3 protein (UniProt # Q92569). In some embodiments, the SH2 domain comprises the motif of a conserved arginine residue in the FLVR motif (Arg βB5 or Arg175 in the v-Src SH2 domain). Most conserved resides are clustered on the βB strand, and the conserved arginine residue in the FLVR motif plays the central role in forming a double hydrogen bond with the phosphate group of pTyr. Additional residues that are key for phosphopeptide binding are His βD4, Lys βD6, and Arg αA2, which coordinate and anchor the aromatic ring of the phospho-tyrosine (Diop et al. Int J Mol Sci. 2022 Dec 15;23(24):15944). In some embodiments, the SH2 domain is an engineered SH2 domain with an enhanced affinity for phosphotyrosine (*e.g.,* Veggiani et al. Protein Sci. 2019 Feb;28(2):403-413).

[0567] In some embodiments, the CARD containing protein or functional fragment thereof comprises a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a domain capable

of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a pTyr.

[0568] Non-limiting examples of target polypeptides comprising a pTyr include P110α/PIK3CA, P110β/PIK3CB, P110δ/PIK3CD, IGF-1R, ErbB2, CTLA-4, and CD28. In some embodiments, the target polypeptide is derived from IGF-1R, CTLA-4, or CD28. In some embodiments, the target polypeptide comprises or consists of a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 298-304. In some embodiments, the pTyr is located at the position corresponding to pY1346 of SEQ ID NO: 325. In some embodiments, the pTyr is located at the position corresponding to pY1221 of SEQ ID NO: 324. In some embodiments, the polypeptide binds to the target polypeptide with a Kd of less than 100 μM, 50 μM, 10 μM, 5 μM, 1μM, 0.5 μM, 0.1 μM, 0.05 μM, or 0.01 μM, wherein the Kd is analyzed by flourescence polarization assay as described in Hause et al. PLoS One. 2012;7(9):e44471. In some embodiments, the polypeptide has a higher affinity for the target polypeptide comprising the pTyr than a control polypeptide without phosphorylation at the corresponding tyrosine position. In some embodiments, the polypeptide has at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, or at least 100-fold, higher affinity (lower Kd) for the target polypeptide comprising the phosphorylated tyrosine than a control polypeptide without phosphorylation at the corresponding tyrosine position.

[0569] In some embodiments, the domain is capable of binding to a substrate indirectly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. In some embodiments, the domain is capable of binding to a substrate directly localized to the intracellular side of the plasma membrane. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to the intracellular side of the plasma membrane itself. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with the intracellular side of the plasma membrane itself.

[0570] Furthermore, the disclosure relates to polypeptides comprising a CARD containing protein or a functional fragment thereof, and a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a phosphorylated tyrosine (also called a CARD fusion polypeptide), as disclosed herein. In some embodiments, the polypeptide comprises a CARD containing protein, or a functional fragment thereof, and an SH2 domain (also called a CARD-SH2 fusion polypeptide). In some embodiments, the polypeptide is a CARD11-PIK3R3 fusion polypeptide. In some embodiments, the recombinant nucleic acid constructs and/or recombinant nucleic acids encode a CARD11-PIK3R3 fusion polypeptide or any truncations thereof.

[0571] In some embodiments, the disclosure relates to a polypeptide comprising a functional fragment of a CARD containing protein derived from a CARD11 protein and an SH2 domain from a PIK3R3 protein (also called a CARD11-PIK3R3 fusion polypeptide).

[0572] The present disclosure further relates to polypeptides wherein the domain capable of binding to the target polypeptide comprising the phosphorylated tyrosine, or the SH2 domain, or the second polypeptide portion, is located at the N-terminus of the CARD containing protein or functional fragment thereof, between the CARD containing protein and the Coiled-coil domain, or at the C-terminus of the CARD containing protein and/or the Coiled-coil domain. In some embodiments, the polypeptide comprises the CARD domain derived from a CARD11 protein followed by the Coiled-coil domain derived from the CARD11 protein. The present disclosure further relates to polypeptides wherein the domain capable of binding to the target polypeptide comprising the phosphorylated tyrosine, or the SH2 domain, or the second polypeptide portion, is located close to the C-terminus of the polypeptide, wherein the polypeptide has no more than 50, 40, 30, 20, 15, 10, or 5 amino acids at the C-terminus of the domain in (b), or the SH2 domain, or the second polypeptide portion.

[0573] In some embodiments, the polypeptide does not comprise an inhibitory domain (ID) or a portion thereof. In other embodiments, the polypeptide comprises an ID or a portion thereof.

[0574] In some embodiments, the CARD11-PIK3R3 fusion polypeptide includes a CARD domain (i.e., containing protein or functional fragment thereof), a CARD inhibitory domain (ID), a coiled-coil domain, and an SH2 domain from PIK3R3. In some embodiments, the CARD11-PIK3R3 fusion polypeptide includes a functional fragment of a CARD containing

protein derived from CARD11domain, a coiled-coil domain, and an SH2 domain from PIK3R3.

[0575] In some embodiments, the polypeptide of the disclosure has a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 3-292 and functional variants thereof, optionally comprising at least one mutation listed in **Table 11**.

[0576] In some embodiments, the polypeptide of the disclosure has a sequence selected from the group consisting of SEQ ID NO: 3-292 and functional variants thereof, optionally comprising at least one mutation listed in **Table 11**.

[0577] In some embodiments, the polynucleotide of the disclosure has a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 3-292 and functional variants thereof, optionally comprising at least one mutation listed in **Table 11**.

[0578] In some embodiments, the polynucleotide of the disclosure has a sequence selected from the group consisting of SEQ ID NO: 3-292 and functional variants thereof, optionally comprising at least one mutation listed in **Table 11**.

[0579] The present disclosure provides engineered CARD11-PIK3R3 proteins that have fewer amino acids than the patient derived full-length human CARD11-PIK3R3 fusion. In some embodiments, the engineered CARD11-PIK3R3 protein is a CARD-containing polypeptide or a functional fragment thereof, wherein the CARD-containing polypeptide comprises no more than 724 amino acids. In some embodiments, the CARD-containing polypeptide does not comprise a full length CARD containing protein.

[0580] In some embodiments, the CARD-containing polypeptide exhibits decreased signaling and/or elicits decreased IFNg and/or IL-2 production compared to an immune cell expressing a control polypeptide consisting of SEQ ID NO: 1. In some embodiments, the CARD-containing polypeptide exhibits equivalent or enhanced signaling and/or elicits equivalent or enhanced IFNg and/or IL-2 production compared to an immune cell expressing a control polypeptide consisting of SEQ ID NO: 1. In some embodiments, such engineered proteins afford certain beneficial technical effects, including delivery via integrating vectors.

[0581] In some embodiments, the cell comprises at least one copy, or at least two copies, of endogenous nucleic acid sequence encoding a CARD11 protein, or a protein comprising a CARD domain from CARD11 without any SH2 domain. In some embodiments, the

recombinant nucleic acid of the cell is located at the endogenous CARD11-encoding gene locus, or comprises at least a portion of the endogenous CARD11-encoding gene, of the cell.

[0582] In some embodiments, the cell comprises at least one copy, or at least two copies, of endogenous nucleic acid sequence encoding a PIK3R3 protein, or a protein comprising an SH2 domain from PIK3R3 without any CARD domain. In some embodiments, the recombinant nucleic acid of the cell is located at the endogenous PIK3R3-encoding gene locus, or comprises at least a portion of the endogenous PIK3R3-encoding gene, of the cell.

TABLE 11. CARD11-PIK3R3 Truncations

Description	Sequence	SEQ ID NO
C11P3tr1 amino acid sequence	MDDYMETLKDEEDALWENVECNRHMLSRYINPAKLTPYLRQCKVIDEQD EDEVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVT GKEPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLR QLEDEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLA MRYAQLSEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLK NDIENRPKKEQVLELERENEMLKTKNQELQSIIQAGKRSLPDSDKAILDILE HDRKEALEDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGK DCEMYKHRMNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRK QIRELEEKNDEMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTII SQDFGDASPRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQR AKSPISLKRTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSIMSI TAEPPGNDSIVRRYKEDAPHRSTVEEDENLPHYDEKTWFVEDINRVQAEDL LYGKPDGAFLIRESSKKGCYACSVVADGEVKHCVIYSTARGYGFAEPYNL YSSLKELVLHYQQTSLVQHNDSLNVRLAYPVHAQMPSLCR	3
C11P3tr2 amino acid sequence	MDDYMETLKDEEDALWENVECNRHMLSRYINPAKLTPYLRQCKVIDEQD EDEVLNAPMLPSKINRAGRLLDILHTKGQRGYVVFLESLEFYYPELYKLVT GKEPTRRFSTIVVEEGHEGLTHFLMNEVIKLQQQMKAKDLQRCELLARLR QLEDEKKQMTLTRVELLTFQERYYKMKEERDSYNDELVKVKDDNYNLA MRYAQLSEEKNMAVMRSRDLQLEIDQLKHRLNKMEEECKLERNQSLKLK NDIENRPKKEQVLELERENEMLKTKNQELQSIIQAGKRSLPDSDKAILDILE HDRKEALEDRQELVNRIYNLQEEARQAEELRDKYLEEKEDLELKCSTLGK DCEMYKHRMNTVMLQLEEVERERDQAFHSRDEAQTQYSQCLIEKDKYRK QIRELEEKNDEMRIEMVRREACIVNLESKLRRLSKDSNNLDQSLPRNLPVTII SQDFGDASPRTNGQEADDSSTSEESPEDSKYFLPYHPPQRRMNLKGIQLQR AKSPISLKRTSDFQAKGHEEEGTDASPSSCGSLPITNSFTKMQPPRSRSSIMSI TAEPPGNDSIVRRYKEDADENYFINEEDENLPHYDEKTWFVEDINRVQAED LLYGKPDGAFLIRESSKKGCYACSVVADGEVKHCVIYSTARGYGFAEPYNL YSSLKELVLHYQQTSLVQHNDSLNVRLAYPVHAQMPSLCR	4
C11P3tr1 nucleic acid sequence	atggacgactacatggagaccctgaaagacgaggaggacgccctgtgggagaacgtggagtgcaacaggcacatg ctgagcagatacatcaaccctgccaagctgacaccctacctgagacagtgcaaggtgatcgacgagcaggacgagg acgaagtgctgaacgctcccatgctgccctctaaaatcaacagagccggcagactgctggacatcctgcacaccaag ggccagaggggctacgtggtgttcctggagagcctggagttctactatcccgagctctacaagctcgtgaccggcaag gagcccaccagaagattcagcaccattgtggtggaggagggacacgaaggcctgacccacttcctgatgaacgaggt gatcaagctgcaacagcaaatgaaggccaaagacctgcaacggtgcgagctgctggccagactgaggcagctgga ggacgagaagaagcagatgaccctgaccagagtggaacttctgaccttccaagagagatactacaagatgaaagag gaacgcgatagctacaacgacgaacttgtcaaagtgaaggacgacaactacaacctggccatgagatacgcccagct gagcgaagagaagaacatggccgtgatgaggagcagggatctgcaactggagatcgaccagctgaagcacaggct gaacaagatggaagaggagtgtaagctggaacggaaccagagcctgaagcttaagaacgacatcgagaacagacc caagaaggagcaagtgctggagctggagcgcgaaaacgagatgctcaagaccaagaatcaggagttgcagagcat catccaagccggaaagagaagcctgcccgacagcgacaaggccatcctggatattctggagcacgataggaaggag	328

	gccctggaagacagacaagaactggtgaacagaatctacaatcttcaagaggaagccagacaggcagaagagctca gggacaaatatccttgaggaaaaggaggacctggaactgaagtgctccaccctgggaaaggactgcgagatgtacaa gcacagaatgaacaccgtgatgctgcagctggaagaggtcgagagggagcgcgaccaggccttccacagcaggga cgaagcccagacacagtacagccagtgcctgatcgagaaggacaagtacaggaagcagatcagagaactggagga gaagaatgacgagatgaggatcgagatggtcagaagggaggcctgcatcgtgaacctggaaagcaaactgagaag gctttccaaggacagcaacaatctggatcagagtctgcccagaaacctgcccgtgaccatcattagccaggacttcgg cgacgccagtccaaggaccaacggacaggaggccgacgacagctctaccagcgaagagtctcccgaggactctaa atactttctgccctaccatcctccacaaagaaggatgaacctgaagggcattcaactccaaagagccaagagcccaatc agtctgaagagaaccagcgacttccaagccaaaggccacgaggaagaaggaacagacgcctctccaagctcctgcg gctctctgcccatcaccaactccttcaccaagatgcagccacccaggagtagaagcagcatcatgtccatcacagccg aaccacctggcaacgactccatcgtgagaagatacaaggaagacgctccacataggtccaccgtggaagaagacga gaacctgccacactacgacgaaaagacttggttcgtggaggacatcaataggttcaagctgaggatctgctgtacgg caagcccgacggagcctttctgattcgggagagcagcaagaagggctgctacgcctgctctgtggtggccgacggc gaagtgaagcactgcgtcatctacagcacagctagaggatacggcttcgccgagccatacaacctgtacagcagcctc aaagagctggttcttcactaccagcagaccagcctggtgcagcacaacgattccctcaacgtgagactggcctaccct gtgcacgcccagatgcccagtctgtgcagatga	
C11P3tr2 nucleic acid sequence	atggacgactacatggagaccctgaaagacgaggaggacgccctgtgggagaacgtggagtgcaacaggcacatg ctgagcagatacatcaaccctgccaagctgacaccctacctgagacagtgcaaggtgatcgacgagcaggacgagg acgaagtgctgaacgctcccatgctgccctctaaaatcaacagagccggcagactgctggacatcctgcacaccaag ggccagaggggctacgtggtgttcctggagagcctggagttctactatcccgagctctacaagctcgtgaccggcaag gagcccaccagaagattcagcaccattgtggtggaggagggacacgaaggcctgacccacttcctgatgaacgaggt gatcaagctgcaacagcaaatgaaggccaaagacctgcaacggtgcgagctgctggccagactgaggcagctgga ggacgagaagaagcagatgaccctgaccagagtggaacttctgaccttccaagagagatactacaagatgaaagag gaacgcgatagctacaacgacgaacttgtcaaagtgaaggacgacaactacaacctggccatgagatacgcccagct gagcgaagagaagaacatggccgtgatgaggagcagggatctgcaactggagatcgaccagctgaagcacaggct gaacaagatggaagaggagtgtaagctggaacggaaccagagcctgaagcttaagaacgacatcgagaacagacc caagaaggagcaagtgctggagctggagcgcgaaaacgagatgctcaagaccaagaatcaggagttgcagagcat catccaagccggaaagagaagcctgcccgacagcgacaaggccatcctggatattctggagcacgataggaaggag gccctggaagacagacaagaactggtgaacagaatctacaatcttcaagaggaagccagacaggcagaagagctca gggacaaatatccttgaggaaaaggaggacctggaactgaagtgctccaccctgggaaaggactgcgagatgtacaa gcacagaatgaacaccgtgatgctgcagctggaagaggtcgagagggagcgcgaccaggccttccacagcaggga cgaagcccagacacagtacagccagtgcctgatcgagaaggacaagtacaggaagcagatcagagaactggagga gaagaatgacgagatgaggatcgagatggtcagaagggaggcctgcatcgtgaacctggaaagcaaactgagaag gctttccaaggacagcaacaatctggatcagagtctgcccagaaacctgcccgtgaccatcattagccaggacttcgg cgacgccagtccaaggaccaacggacaggaggccgacgacagctctaccagcgaagagtctcccgaggactctaa atactttctgccctaccatcctccacaaagaaggatgaacctgaagggcattcaactccaaagagccaagagcccaatc agtctgaagagaaccagcgacttccaagccaaaggccacgaggaagaaggaacagacgcctctccaagctcctgcg gctctctgcccatcaccaactccttcaccaagatgcagccacccaggagtagaagcagcatcatgtccatcacagccg aaccacctggcaacgactccatcgtgagaagatacaaggaagacgctgacgagaactacttcatcaacgaagaagac gagaacctgccacactacgacgaaaagacttggttcgtggaggacatcaataggttcaagctgaggatctgctgtac ggcaagcccgacggagcctttctgattcgggagagcagcaagaagggctgctacgcctgctctgtggtggccgacg gcgaagtgaagcactgcgtcatctacagcacagctagaggatacggcttcgccgagccatacaacctgtacagcagc ctcaaagagctggttcttcactaccagcagaccagcctggtgcagcacaacgattccctcaacgtgagactggcctacc ctgtgcacgcccagatgcccagtctgtgcagatga	329

Biological Activity of Engineered CARD11-PIK3R3 Fusion Proteins

[0583] In some embodiments the engineered CARD11-PIK3R3 fusion protein is functionally equivalent to a patient derived full length CARD11-PIK3R3 fusion protein. As used herein, "functionally equivalent" refers to having at least one type of biological activity of a patient-derived human CARD11-PIK3R3 fusion protein.

[0584] In some embodiments an engineered cell comprising the engineered CARD11-PIK3R3 fusion protein is functionally equivalent to an engineered cell comprising a patient derived full length CARD11-PIK3R3 fusion protein. As used herein, "functionally

equivalent" refers to having at least one type of biological activity of an engineered cell comprising a patient-derived human CARD11-PIK3R3 fusion protein.

[0585] The polypeptides of the disclsoure, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, can alter T cell signaling in various ways. In some embodiments, the altering of the T cell signaling can be by enhancing, promoting, improving, reducing, regulating, or modulating the signaling pathway within a T cell. In some embodiments, the altering of the T cell signaling can be by activating, increasing, suppressing, inhibiting, or other means of changing the signaling.

[0586] The polypeptides of the disclsoure, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, can alter T cell signaling through one or more T cell signaling pathways. In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter T cell signaling by increasing one or more pathways and/or reducing one or more pathways, or by a combination of enhancing and/or reducing various pathways. In some embodiments, the mutation can alter cytokine production. The cytokine can be, without limitation, IL-2, IL-4, IL-5, TNF alpha, IFN- gamma, IL-13 and/or other cytokines. In some embodiments, the cytokine is IL-2. In some embodiments, the cytokine is IL-2, and the production of IL-2 is increased.

[0587] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter T cell signaling through one or more T cell signaling pathways. Signaling pathways can be, without limitation, the NFAT pathway, NF-κB pathway, AP-1 pathway, JAK/STAT pathway, RAS/MEK/ERK, and/or phospholipase gamma signaling, or described elsewhere herein.

[0588] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter CARD11-BCL10-MALT1 complex signaling, co-stimulatory molecule signaling, cytokine production and/or transcription factor activity in T cells.

[0589] The disclosure also relates to polypeptides having one or more mutations that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increase intratumoral presence of therapeutic T cells. The disclosure further relates to recombinant nucleic acids encoding polypeptides having one or more mutations, that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell

fitness, enhance *in vivo* persistence, and/or increse intratumoral presence of therapeutic T cells.

[0590] T cell fitness can refer to the ability of a T cell to generate an immune response. The ability of a T cell to perform T cell functions such as signaling, cytokine production, survival, and persistence in a tumor, may contribute to the fitness of a T cell. T cell exhaustion may contribute to reducing its fitness.

[0591] In some embodiments, polypeptides of the disclosure, or the recombinant nucleicacid constructs and/or recombinant nucleic acids of the disclosure can alter *in vivo* persistence in tumors of therapeutic T cells including the mutation. The *in vivo* persistence of therapeutic T cells can refer to the length of time that the therapeutic T cells exist within the tumor of the host after infusion. In some embodiments, *in vivo* persistence of therapeutic T cells is enhanced. Enhanced *in vivo* persistence of therapeutic T cells can include at least a positive log2 fold change in therapeutic T cells as compared to the input total.

[0592] In some embodiments, the polypeptides of the disclosure, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter the therapeutic efficacy of engineered T cells. Alteration of therapeutic efficacy can include, without limitation, a decrease in T cell exhaustion, increased proliferative capacity, enhanced anti-tumor effect, enhanced replicative lifespan, decreased replicative senescence, enhanced ability to kill, enhanced fitness of engineered T cells and/or other functions or activities of T cells. In some embodiments, the nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising an amino acid sequence having at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 3-150 or a variant thereof.

[0593] In some embodiments, the nucleic acids constructs and/or recombinant nucleic acids of the disclosure include a nucleic acid sequence encoding a polypeptide with at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 3-150 or a variant thereof.

VII. RECOMBINANT NUCLEIC ACIDS

[0594] In some embodiments, the engineered polypeptides disclosed herein are encoded by a recombinant nucleic acid. In some embodiments, the recombinant nucleic acid is or

comprises DNA. In some embodiments, the recombinant nucleic acid is or comprises cDNA. In some embodiments, the recombinant nucleic acid is or comprises RNA. In some embodiments, the recombinant nucleic acid is or comprises mRNA.

[0595] A recombinant nucleic acid encoding the engineered polypeptides disclosed herein can be obtained by methods known in the art. For example, the recombinant nucleic acid can be obtained from cloned DNA (*e.g.,* from a DNA library), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA or fragments thereof, purified from the desired cell. When the polynucleotides are produced by recombinant means, any method known to those skilled in the art for identification of nucleic acids that encode desired genes can be used. Polynucleotides produced by recombinant means may also be referred to as "recombinant nucleic acids" herein. Any method available in the art can be used to obtain a full length (i.e. encompassing the entire coding region) cDNA or genomic DNA of interest, such as from a cell or tissue source. Modified or variant polynucleotides can be engineered from a patient-derived polynucleotide using standard recombinant DNA methods. Polynucleotides can be cloned or isolated using any available methods known in the art for cloning and isolating nucleic acid molecules. Such methods include PCR amplification of nucleic acids and screening of libraries, including nucleic acid hybridization screening, antibody-based screening, and activity-based screening.

[0596] Methods for amplification of polynucleotides can be used to isolate polynucleotides encoding a desired protein, including for example, polymerase chain reaction (PCR) methods. PCR can be carried out using any known methods or procedures in the art. Exemplary methods include use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp). A nucleic acid containing gene of interest can be used as a source material from which a desired polypeptide-encoding nucleic acid molecule can be amplified. For example, DNA and mRNA preparations, cell extracts, tissue extracts from an appropriate source (e.g. testis, prostate, breast), fluid samples (e.g. blood, serum, saliva), samples from healthy and/or diseased subjects can be used in amplification methods. The source can be from any eukaryotic species including, but not limited to, vertebrate, mammalian, human, porcine, bovine, feline, avian, equine, canine, and other primate sources. Nucleic acid libraries also can be used as a source material. Primers can be designed to amplify a desired polynucleotide. For example, primers can be designed based on expressed sequences from which a desired polynucleotide is generated. Primers can be designed based on back-translation of a polypeptide amino acid sequence. If desired,

degenerate primers can be used for amplification. Oligonucleotide primers that hybridize to sequences at the 3' and 5' termini of the desired sequence can be uses as primers to amplify by PCR from a nucleic acid sample. Primers can be used to amplify the entire full-length polynucleotide, or a truncated sequence thereof. Nucleic acid molecules generated by amplification can be sequenced and confirmed to encode a desired polypeptide.

[0597] In some embodiments, the recombinant nucleic acid encoding a modified peptide epitope or recombinant polypeptide as described herein additionally encodes at least one additional polypeptide (*e.g.,* a second recombinant polypeptide).

[0598] In some embodimetns, the polynucleotide sequence encoding the recombinant polypeptide and the polynucleotide sequence encoding the at least one additional polypeptide are separated by a polynucleotide sequence encoding one or more cleavable linkers. In some embodiments, all these polynucleotide sequences form a continuous open reading frame. In some embodiments, the one or more cleavable linkers comprise a P2A, E2A, F2A, or T2A self-cleaving peptide.

[0599] In some embodiments, the polynucleotide sequence encoding the recombinant polypeptide and the polynucleotide sequence encoding at least one additional polypeptide are separated by an internal ribozyme entry site (IRES).

[0600] In some embodiments, the recombinant nucleic acid is a DNA. In some embodiments, the recombinant nucleic acid is an RNA. In some embodiments, the recombinant nucleic acid is a mRNA.

[0601] In some embodiments, the recombinant nucleic acid comprises one or more modified nucleotides. In some embodiments, the one or more modified nucleotides are or comprise pseudouridine, N1-methylpseudouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl- pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, or any combination thereof. In some embodiments,the one or more modified nucleotides are selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, and a combination thereof. In some embodiments, the one or more modified nucleotides are N1-methylpseudouridines.

[0602] In some embodiments, the recombinant nucleic acid encodes a TCRalpha, a TCRbeta and a peptide epitope or mimotope. The encoded TCRalpha and/or the TCRbeta

may comprise the encoded peptide epitope or mimotope. In some embodiments, the recombinant nucleic acid encodes the TCRalpha comprising the peptide epitope or mimotope and a TCRbeta. In some embodiments, the recombinant nucleic acid encodes a TCRalpha and the TCRbeta comprising the peptide epitope or mimotope. In some embodiments, the recombinant nucleic acid encodes a TCRalpha, a TCRbeta, and the peptide epitope or mimotope located in between the TCRalpha and the TCRbeta. In some embodiments, the TCRalpha and the TCRbeta are encoded by the same coding region; optionally, wherein the TCRalpha and the TCRbeta are separated by a cleavage site.

[0603] In some embodiments, the recombinant nucleic acid encodes (i) the VH and the constant region of TCRalpha of the receptor, or (ii) the VL and the constant region of TCRbeta of the receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope. In some embodiments, the recombinant nucleic acid encodes (i) the VH and the constant region of TCRbeta of the receptor, or (ii) the VL and the constant region of TCRalpha of the receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope. In some embodiments, (i) and (ii) are encoded by the same coding region; optionally, wherein (i) and (ii) are separated by a cleavage site.

[0604] In some embodiments, the recombinant nucleic acid encodes (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope. In some embodiments, the recombinant nucleic acid is less than 5.0 kb in length. In some embodiments, the recombinant nucleic acid is less than 4.8 kb in length. In some embodiments, the recombinant nucleic acid is less than 4.7 kb in length.

[0605] In some embodiments, the recombinant nucleic acid encoding a modified peptide epitope or recombinant polypeptide as described herein further comprises a polynucleotide sequence encoding a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof disclosed herein. In some embodiments, the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

[0606] Furthermore, the disclosure relates to recombinant nucleic acids encoding polypeptides comprising a CARD containing protein or a functional fragment thereof and a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a phosphorylated tyrosine, as

disclosed herein. In some embodiments, the recombinant nucleic acid encodes a polypeptide comprising a CARD containing protein or a functional fragment thereof and an SH2 domain. In some embodiments, the recombinant nucleic acid encodes a functional fragment of a CARD containing protein derived from a CARD11 protein and an SH2 domain from a PIK3R3 protein. In some embodiments, the recombinant nucleic acid encodes a functional fragment of a CARD containing protein derived from a CARD9 protein and an SH2 domain from a PIK3R3 protein.

[0607] In some embodiments, the recombinant nucleic acid is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length. In some embodiments, the recombinant nucleic acid is less than about 4.7kb in length.

[0608] In some embodiments, the recombinant nucleic acid encoding the polypeptide disclosed herein is comprised with a plasmid. In some embodiments, the plasmid is between about 2 kilobases (kb) and about 10 kb in size, or between about 3 kb and about 8 kb in size. In some embodiments, the plasmid is less than about 8 kb, less than about 7 kb, less than about 6 kb, less than about 5 kb, or less than about 4.5 kb in size. In some embodiments, the plasmid is less than about 8 kb in size. In some embodiments, the plasmid is less than about 7.5 kb in size. In some embodiments, the plasmid is less than about 7 kb in size. In some embodiments, the plasmid is less than about 6.5 kb in size. In some embodiments, the plasmid is less than about 6 kb in size. In some embodiments, the plasmid is less than about 5.5 kb in size. In some embodiments, the plasmid is less than about 5 kb in size. In some embodiments, the plasmid is less than about 4.5 kb in size. In some embodiments, the plasmid is about 4.5 kb in size. In some embodiments, the plasmid is about 5 kb in size. In some embodiments, the plasmid is about 5.5 kb in size. In some embodiments, the plasmid is about 6.5 kb in size. In some embodiments, the plasmid is about 7 kb in size. In some embodiments, the plasmid is about 6 kb in size. In some embodiments, the plasmid is about 7.5 kb in size. In some embodiments, the plasmid is about 8 kb in size.

[0609] In some embodiments, the recombinant nucleic acid encoding the polypeptide disclosed herein contains at least one promoter that is operatively linked to control expression of the polypeptide. In some examples, the recombinant nucleic acid contains two, three, or more promoters operatively linked to control expression of the polypeptide. In some embodiments, recombinant nucleic acid can contain regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type

of host (*e.g.,* bacterium, fungus, plant, or animal) into which the recombinant nucleic acid is to be introduced, as appropriate and taking into consideration whether the recombinant nucleic acid is DNA- or RNA-based. In some embodiments, the recombinant nucleic acid can contain regulatory/control elements, such as a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, internal ribosome entry sites (IRES), a 2A sequence, and splice acceptor or donor. In some embodiments, the recombinant nucleic acid can contain a non-native promoter operably linked to the polypeptide and/or one or more additional polypeptide(s). In some embodiments, the promoter is selected from among an RNA pol I, pol II or pol III promoter. In some embodiments, the promoter is recognized by RNA polymerase II (*e.g.,* a CMV, SV40 early region or adenovirus major late promoter). In another embodiment, the promoter is recognized by RNA polymerase III (*e.g.,* a U6 or Hl promoter). In some embodiments, the promoter can be a non-viral promoter or a viral promoter, such as a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus. Other known promoters also are contemplated.

[0610] In some embodiments, the promoter is or comprises a constitutive promoter. Exemplary constitutive promoters include, MND promoter, EF1a promoter, sEF1 promoter, gamma retroviral LTR promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, MND promoter or a short EF1a promoter.

[0611] In some embodiments, the promoter is a minimal TATA promoter, a pGK, actin promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter,CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1

promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NF-κB promoter, AP-1 promoter, TNF-alpha promoter, and CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter. Exemplary constitutive promoters include, *e.g.,* simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV), human Ubiquitin C promoter (UBC), human elongation factor la promoter (EFla), mouse phosphoglycerate kinase 1 promoter (PGK), and chicken P-Actin promoter coupled with CMV early enhancer (CAG). In some embodiments, the constitutive promoter is a synthetic or modified promoter. In some embodiments, the promoter is or comprises an MND promoter, a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer (see Challita et al. (1995) J. Virol. 69(2):748-755). In some embodiments, the promoter is a tissue-specific promoter. In another embodiment, the promoter is a viral promoter. In another embodiment, the promoter is a non-viral promoter. In some embodiments, exemplary promoters can include, but are not limited to, human elongation factor 1 alpha (EFla) promoter or a modified form thereof or the MND promoter.

[0612] In another embodiment, the promoter is a regulated promoter (*e.g.,* inducible promoter). In some embodiments, the promoter is an inducible promoter or a repressible

promoter. In some embodiments, the promoter comprises a Lac operator sequence, a tetracycline operator sequence, a galactose operator sequence or a doxycycline operator sequence, or is an analog thereof or is capable of being bound by or recognized by a Lac repressor or a tetracycline repressor, or an analog thereof. In some embodiments, the recombinant nucleic acid does not include a regulatory element, e.g. promoter.
In some embodiments, the polynucleotide sequence encoding the recombinant polypeptide is not operably linked to a promoter.

[0613] In some embodiments, the recombinant nucleic acid further comprises one or more additional nucleic acid element sequences. Non-limiting examples of nucleic acid element sequences are ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, a 70 bp poly(A) tract, a 100 bp poly(A) tract, a 172 bp poly(A) tract, a 200 bp poly(A) tract, a 300 bp poly(A) tract, a 325 bp poly(A) tract, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, cleavable linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or any combination thereof.

[0614] In some cases, the nucleic acid sequence encoding the polypeptide disclosed herein contains a signal sequence that encodes a signal peptide. In some aspects, the signal sequence may encode a signal peptide derived from a native polypeptide. In other aspects, the signal sequence may encode a heterologous or non-native signal peptide, such as the exemplary signal peptide of a GMCSFR alpha chain. In some cases, the signal peptide is comprised within the polypeptide.

[0615] In some embodiments, the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or a non-coding RNA, optionally wherein the non-coding RNA comprises a shRNA or a microRNA. In some embodiments, the one or more additional polypeptides comprise an additional potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, or any combination thereof. Non-limiting examples of additional potency enhancement polypeptides include a dominant negative form of an inhibitor of a cell-mediated immune response of the immune

cell (*e.g.,* TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, or CD8alpha/beta.

[0616] In some embodiments, the recombinant nucleic acid contains a nucleic acid sequence encoding one or more additional polypeptides, *e.g.,* one or more marker(s) and/or one or more effector molecules. In some embodiments, the disclosure provides a combination of recombinant nucleic acids, in which the polypeptides are encoded by separate recombinant nucleic acids. In some embodiments, the one or more marker(s) includes a transduction marker, a surrogate marker and/or a selection marker. Among additional nucleic acid sequences introduced, *e.g.,* encoding for one or more additional polypeptide(s), include nucleic acid sequences that can improve the efficacy of therapy, such as by promoting viability and/or function of transferred cells; nucleic acid sequences to provide a genetic marker for selection and/or evaluation of the cells, such as to assess in vivo survival or localization; nucleic acid sequences to improve safety, for example, by making the cell susceptible to negative selection in vivo as described by Lupton S. D. et al., Mol. and Cell Biol., 11:6 (1991); and Riddell et al., Human Gene Therapy 3:319-338 (1992); see also WO 1992008796 and WO 1994028143 describing the use of bifunctional selectable fusion genes derived from fusing a dominant positive selectable marker with a negative selectable marker, and US Patent No. 6,040,177.

[0617] In some embodiments, recombinant nucleic acids can be engineered as a bicistronic unit containing an IRES, which allows coexpression of gene products (e.g. encoding the recombinant receptor and the additional polypeptide) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (e.g. encoding the marker and encoding the recombinant receptor) separated from one another by sequences encoding a self-cleavage peptide (*e.g.,* 2A sequences) or a protease recognition site (*e.g.,* furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A) or after translation, is processed into the individual proteins. In some cases, the peptide, such as a T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (see, *e.g.,* de Felipe, Genetic Vaccines and Ther. 2:13 (2004) and de Felipe et al. Traffic 5:616-626 (2004)). Various 2A elements are known. Examples of 2A sequences that can be used in the methods and system disclosed herein, without

limitation, 2A sequences from the foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), Thosea asigna virus (T2A), and porcine teschovirus-1 (P2A) as described in U.S. Patent Pub. No. 20070116690.

[0618] In some embodiments, the recombinant nucleic acid comprises one or more modified nucleotides. Non-limiting examples of modified nucleotides include pseudouridine, N1-methylpseudouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-l-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl- pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine. In some embodiments, the one or more modified nucleotides are selected from the group consisting of pseudouridine, N1-methylpseudouridine, 5-methylcytosine, 5- methoxyuridine, and a combination thereof. In some embodiments, the one or more modified nucleotides are N1- methylpseudouridines.

[0619] In some aspects, nucleic acid sequences of interest, including coding and/or noncoding sequences and/or partial coding sequences, that are inserted or integrated at the target location in the genome can also be referred to as "transgene," "transgene sequences," "exogenous nucleic acids sequences," "heterologous sequences" or "donor sequences." In some aspects, the transgene is a nucleic acid sequence that is exogenous or heterologous to an endogenous genomic sequences, such as the endogenous genomic sequences at a specific target locus or target location in the genome, of a target cell, *e.g.,* a human T cell. In some aspects, the transgene is a sequence that is modified or different compared to an endogenous genomic sequence at a target locus or target location of a target cell, *e.g.,* a human T cell. In some aspects, the transgene is a nucleic acid sequence that originates from or is modified compared to nucleic acid sequences from different genes, species and/or origins. In some aspects, the transgene is a sequence that is derived from a sequence from a different locus, *e.g.,* a different genomic region or a different gene, of the same species.

[0620] Polynucleotides for insertion can also be referred to as "transgene" or "exogenous sequences" or "donor" polynucleotides or molecules. The template polynucleotide can be DNA, single-stranded and/or double-stranded and can be introduced into a cell in linear or circular form. The template polynucleotide can be RNA single-stranded and/or double-stranded and can be introduced as a RNA molecule (*e.g.,* part of an RNA virus). See also, U.S. Patent Publication Nos. 20100047805 and 20110207221. The template polynucleotide

can also be introduced in DNA form, which may be introduced into the cell in circular or linear form. If introduced in linear form, the ends of the template polynucleotide can be protected (*e.g.,* from exonucleolytic degradation) by known methods. 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. If introduced in double-stranded form, the template polynucleotide may include one or more nuclease target site(s), for example, nuclease target sites flanking the transgene to be integrated into the cell's genome. See, *e.g.,* U.S. Patent Publication No. 20130326645.

VIII. VECTORS

[0621] In another aspect, the present disclosure provides a vector comprising the polynucleotide that encodes the engineered polypeptides disclosed herein. In some embodiments, the vector can be used to transform or transfect a host cell, *e.g.,* a cell for engineering. Exemplary vectors include vectors designed for introduction, propagation and expansion or for expression or both, such as DNA vectors (*e.g.,* a plasmid or closed-end vector) and viral vectors. In some aspects, the vector is an expression vector, *e.g.,* a recombinant expression vector. In some embodiments, the recombinant expression vectors can be prepared using standard recombinant DNA techniques.

[0622] The nucleic acid molecules can be contained within a vector that is capable of directing their expression in, for example, a cell that has been transformed/transduced with the vector. Suitable vectors for use in eukaryotic and prokaryotic cells are known in the art and are commercially available, or readily prepared by a skilled artisan. See for example, Sambrook, J., & Russell, D. W. (2012). Molecular Cloning: A Laboratory Manual (4th ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory and Sambrook, J., & Russel, D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (jointly referred to herein as "Sambrook"); Ausubel, F. M. (1987). Current Protocols in Molecular Biology. New York, NY: Wiley (including supplements through 2014); Bollag, D. M. et al. (1996). Protein Methods. New York, NY:

Wiley-Liss; Huang, L. et al. (2005). Nonviral Vectors for Gene Therapy. San Diego: Academic Press; Kaplitt, M. G. et al. (1995). Viral Vectors: Gene Therapy and Neuroscience Applications. San Diego, CA: Academic Press; Lefkovits, I. (1997). The Immunology Methods Manual: The Comprehensive Sourcebook of Techniques. San Diego, CA: Academic Press; Doyle, A. et al. (1998). Cell and Tissue Culture: Laboratory Procedures in Biotechnology. New York, NY: Wiley; Mullis, K. B., Ferré, F. & Gibbs, R. (1994). PCR: The Polymerase Chain Reaction. Boston: Birkhauser Publisher; Greenfield, E. A. (2014). Antibodies: A Laboratory Manual (2nd ed.). New York, NY: Cold Spring Harbor Laboratory Press; Beaucage, S. L. et al. (2000). Current Protocols in Nucleic Acid Chemistry. New York, NY: Wiley, (including supplements through 2014); and Makrides, S. C. (2003). Gene Transfer and Expression in Mammalian Cells. Amsterdam, NL: Elsevier Sciences B.V., the disclosures of which are incorporated herein by reference).

[0623] Any methods known to those skilled in the art for the insertion of nucleic acid fragments into a vector can be used to construct expression vectors comprising a polynucleotide disclosed herein. These methods can include in vitro recombinant DNA and synthetic techniques and in vivo (genetic) recombination. The polynucleotide disclosed herein can be operably linked to nucleic acid element sequences in the expression vector(s) to ensure the expression of the polypeptide of the disclosure. Such nucleic acid element sequences may include, but are not limited to, leader or signal sequences, promoters (*e.g.,* naturally associated or heterologous promoters), ribosomal binding sites, enhancer or activator elements, translational start and termination sequences, and transcription start and termination sequences, and are chosen to be compatible with the host cell chosen to express the polypeptide of the disclosure. Constitutive or inducible promoters as known in the art are also contemplated. The promoters may be either naturally occurring promoters, hybrid promoters that combine elements of more than one promoter, or synthetic promoters. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome such as in a gene locus. In some embodiment, the expression vector includes a selectable marker gene to allow the selection of transformed host cells. Some embodiments, include an expression vector comprising a nucleotide sequence encoding a variant polypeptide operably linked to at least one regulatory control sequence. Regulatory control sequences for use herein include promoters, enhancers, and other expression control elements. In some embodiments, an expression vector is designed for the choice of the host cell to be transformed, the particular variant

polypeptide desired to be expressed, the vector's copy number, the ability to control that copy number, and/or the expression of any other protein encoded by the vector, such as antibiotic markers.

[0624] Examples of suitable mammalian promoters include, for example, MND promoter, EF1a promoter, sEF1 promoter, gamma retroviral LTR promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, or a CD3z promoter. In some embodiments, the promoter is a minimal TATA promoter, a pGK, actin promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter,CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NF-κB promoter, AP-1 promoter, TNF-alpha promoter, and CD130 promoter, NR4A1 promoter, NR4A2, or NR4A3 promoter. Exemplary constitutive promoters include, *e.g.*, simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV), human Ubiquitin C promoter (UBC), human elongation factor la promoter (EFla), mouse phosphoglycerate kinase 1 promoter (PGK), and chicken P-Actin promoter coupled with CMV early enhancer (CAG). In some embodiments, the constitutive promoter is a synthetic

or modified promoter. In some embodiments, the promoter is or comprises an MND promoter, a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer (see Challita et al. (1995) J. Virol. 69(2):748-755). In some embodiments, the promoter is a tissue-specific promoter. In another embodiment, the promoter is a viral promoter. In another embodiment, the promoter is a non-viral promoter. In some embodiments, exemplary promoters can include, but are not limited to, human elongation factor 1 alpha (EFla) promoter or a modified form thereof or the MND promoter.

[0625] Examples of suitable mammalian promoters include, for example: TRAC promoter, TRBC1 promoter, TRBC2 promoter, CD3alpha promoter, CD3beta promoter, CD3epsilon promoter, CD3delta promoter, CD3zeta promoter, CARD11 promoter, PIK3R3 promoter, B2M promoter, CIITA promoter, PD-1 promoter, CXCR4 promoter, an albumin promoter, an F3 promoter (also known as CD142), a MICA promoter, a MICB promoter, a LRP1 promoter (also known as CD91), a HMGB1 promoter, an ABO promoter, a RHD promoter, a FUT1 promoter, a KDM5D promoter (also known as HY), a CCR5 promoter, a PDGFRa promoter, a OLIG2 promoter, and/or a GFAP promoter, elongation factor 1 alpha (EFla) promoter, CAG promoter, ubiquitin/S27a promoter of the hamster (WO 97/15664), Simian vacuolating virus 40 (SV40) early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, the long terminal repeat region of Rous Sarcoma Virus (RSV), mouse mammary tumor virus promoter (MMTV), Moloney murine leukemia virus Long Terminal repeat region, and the early promoter of human Cytomegalovirus (CMV). Examples of other heterologous mammalian promoters are the actin, immunoglobulin or heat shock promoter(s). In additional embodiments, promoters for use in mammalian host cells can be obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 Jul. 1989), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40). In further embodiments, heterologous mammalian promoters are used. Examples include the actin promoter, an immunoglobulin promoter, and heat-shock promoters. The early and late promoters of SV40 are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature 273: 113-120 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a Hindlll restriction enzyme fragment (Greenaway et al., Gene 18: 355-360 (1982)). The foregoing references are incorporated by reference in their entirety.

[0626] In some embodiments, the vector is a pseudotyped, self-inactivating lentiviral vector that carries the polypeptide of the disclosure. In some embodiments, the vector is a selfinactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the polynucleotide encoding the polypeptide of the disclosure.

[0627] In some embodiments, the viral vector is an AAV vector that carries the polypeptide of the disclosure. In some of any embodiments, the AAV vector is an AAV6 vector that carries the polynucleotide encoding the polypeptide of the disclosure.

[0628] The vector can include, but is not limited to, viral vectors, plasmid DNA, and closed-end DNA. Viral vectors can include, but are not limited to, adenoviral vectors, lentiviral vectors, retroviral vectors, adeno-associated viral vectors, vaccinia viruses, poxviruses, and herpes simplex viruses, or any of the viruses described elsewhere herein. Commonly, expression vectors contain selection markers such as ampicillin-resistance, hygromycin-resi stance, tetracycline resistance, kanamycin resistance, or neomycin resistance to permit detection of those cells transformed with the desired DNA sequences. Suitable vectors, promoter, and enhancer elements are known in the art; many are commercially available for generating subject recombinant constructs. In some embodiments, the vector is a polycistronic vector. In some embodiments, the vector is a bicistronic vector or a tricistronic vector. Bicistronic or multi ci str onic expression vectors may include (1) multiple promoters fused to each of the open reading frames; (2) insertion of splicing signals between genes; (3) fusion of genes whose expressions are driven by a single promoter; and (4) insertion of proteolytic cleavage sites between genes (self-cleavage peptide) or insertion of internal ribosomal entry sites (IRESs) between genes.

[0629] A polycistronic vector is used to co-express multiple genes in the same cell. Two strategies are most commonly used to construct a multi cistronic vector. First, an Internal Ribosome Entry Site (IRES) element is typically used for bi-cistronic vectors. The IRES element, acting as another ribosome recruitment site, allows initiation of translation from an internal region of the mRNA. Thus, two proteins are translated from one mRNA. IRES elements are quite large (usually 500-600 bp) (Pelletier et al., 1988; Jang et al., 1988). In some embodiments, the polypeptide of the disclosure disclosed herein have a smaller size compared to the endogenous or patient-derived counterpart polypeptides, and thus could be expressed using IRES element in a multi cistronic vectors having limited packaging capacity.

[0630] In some embodiments, the polycistronic construct comprises two expression cassettes, i.e., is bicistronic. In some embodiments, the polycistronic construct comprises three expression cassettes, i.e., is tricistronic. In some embodiments, the polycistronic construct comprises four expression cassettes, i.e., is quadcistronic. In some embodiments, the polycistronic construct comprises more than four expression cassettes. In any of these embodiments, each of the expression cassettes comprises a nucleotide sequence encoding a protein of interest (*e.g.,* an polypeptide of the disclosure). In certain embodiments, the two or more genes being expressed are under the control of a single promoter and are separated from one another by one or more cleavage sites to achieve co-expression of the proteins of interest from one transcript. In other embodiments, the two or more genes may be under the control of separate promoters. As the name suggests, a polycistronic construct allows simultaneous expression of two or more separate proteins from one mRNA transcript in a host cell. Cleavage sites can be used in the design of a polycistronic construct to achieve such co-expression of multiple genes.

[0631] In some embodiments, the one or more cleavage sites comprise one or more selfcleaving sites. The second strategy relies on “self-cleaving” 2A peptides. These peptides, first discovered in picomaviruses, are short (about 20 amino acids) and produce equimolar levels of multiple genes from the same mRNA. The term "self-cleaving" is not entirely accurate, as these peptides are thought to function by making the ribosome skip the synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream (Kim et al., 2011). The "cleavage" occurs between the glycine and proline residues found on the C-terminus. Thus, the upstream cistron will have a few additional residues added to the end, while the downstream cistron will start with the proline. There are four 2A peptides commonly employed in molecular biology, T2A, P2A, E2A, and F2A, the sequences of which are summarized in Table **12**. A glycine-serine-glycine (GSG) linker is optionally added to the N-terminal of a 2A peptide to increase cleavage efficiency. The use of “()” around a sequence in the present disclosure means that the enclosed sequence is optional.

Table 12. Sequences of 2A Peptides

Component	Sequence	SEQ ID NO:
T2A	(GSG)EGRGSLLTCGDVEENPGP	376
P2A	(GSG) ATNFSLLKQAGDVEENPGP	377

E2A	(GSG) QCTNYALLKLAGDVESNPGP	378
F2A	(GSG)VKQTLNFDLLKLAGDVESNPGP	379

[0632] In some embodiments, the one or more cleavage sites additionally comprise one or more protease sites. The one or more protease sites can either precede or follow the self-cleavage sites (*e.g.*, 2 A sites) in the 5' to 3' order. The protease site may be cleaved by a protease after translation of the full transcript or after translation of each expression cassette such that the first expression product is released prior to translation of the next expression cassette. In these embodiments, having a protease site in addition to the 2A site, especially preceding the 2A site in the 5' to 3' order, may reduce the number of extra amino acid residues attached to the expressed proteins of interest. In some embodiments, the protease site comprises a furin site, also known as a Paired basic Amino acid Cleaving Enzyme (PACE) site. There are at least three furin cleavage sequences, FC1, FC2, and FC3, the amino acid sequences of which are summarized in **Table 13**. Similar to the 2A sites, one or more optional glycine-serine-glycine (GSG) sequences can be included for cleavage efficiency.

Table 13. Sequences of Furin Sites

Component	Sequence	SEQ ID NO:
FC1	RRRR (GSG)	380
FC2	RKRR (GSG)	381
FC3	RKRR (GSG) TPDPW (GSG)	382

[0633] In some embodiments, the one or more cleavage sites comprise one or more self-cleaving sites, one or more protease sites, and/or any combination thereof. For example, the cleavage site can include a 2A site alone. For another example, the cleavage site can include a FC2 or FC3 site, followed by a 2A site. In these embodiments, the one or more self-cleaving sites may be the same or different. Similarly, the one or more protease sites may be the same or different.

[0634] In some embodiments, the polycistronic construct may be in the form of a vector. Any type of vector suitable for introduction of nucleotide sequences into a host cell can be used, including, for example, plasmids, adenoviral vectors, adenoviral-associated vectors,

retroviral vectors, lentiviral vectors, phages, and homology-directed repair (HDR)-based donor vectors.

[0635] In some embodiments, the vector herein is a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule, including into the cell or into the genome of a cell. The transferred nucleic acid is generally linked to, *e.g.,* inserted into, the vector nucleic acid molecule. A vector may include sequences that direct autonomous replication in a cell or may include sequences sufficient to allow integration into host cell DNA. In some embodiments, the vector herein is a plasmid. In some embodiments, the vector herein is a DNA vector. In some embodiments, the vector herein is a closed linear DNA vector. In some embodiments, the vector herein is a phagemid vector. In some embodiments, the vector herein is a synthetic DNA vector. In some embodiments, the vector herein is a single stranded DNA vector or a double stranded DNA vector. In some embodiments, the vector herein is a synthetic RNA vector. Useful vectors include, for example, plasmids (*e.g.,* DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Useful viral vectors include, *e.g.,* replication defective retroviruses and lentiviruses. Non-viral vectors may require a delivery vehicle to facilitate entry of the nucleic acid molecule into a cell.

[0636] A viral vector can comprise a nucleic acid molecule that includes virus-derived nucleic acid elements that typically facilitate transfer of the nucleic acid molecule or integration into the genome of a cell or to a viral particle that mediates nucleic acid transfer. Viral particles will typically include various viral components and sometimes also host cell components in addition to nucleic acid(s). A viral vector can comprise, *e.g.,* a virus or viral particle capable of transferring a nucleic acid into a cell, or to the transferred nucleic acid (*e.g.,* as naked DNA). Viral vectors and transfer plasmids can comprise structural and/or functional genetic elements that are primarily derived from a virus. A retroviral vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, that are primarily derived from a retrovirus.

[0637] In some embodiments, the vector is a viral vector, such as a retroviral vector. In some embodiments, the polynucleotide encoding the polypeptide of the disclosure are introduced into the cell via retroviral or lentiviral vectors, or via transposons (see, *e.g.,* Baum et al. (2006) Molecular Therapy: The Journal of the American Society of Gene Therapy. 13:1050-1063; Frecha et al. (2010) Molecular Therapy 18:1748-1757; and Hackett et al. (2010) Molecular Therapy 18:674-683).

[0638] In some vectors described herein, at least part of one or more protein coding regions that contribute to or are essential for replication may be absent compared to the corresponding wild-type virus. This makes the viral vector replication-defective. In some embodiments, the vector is capable of transducing a target non-dividing host cell and/or integrating its genome into a host genome.

[0639] In some embodiments, the vector is a retroviral vector. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector is a VSV-G pseudotyped lentivirus. In some embodiments, the vector is an AAV vector. In some embodiments, the vector is an AAV6 vector. In some embodiments, the vector is an AAV9 vector. In some embodiments, the vector is a split-intein dual AAV vector. In some embodiments, the vector is a redirected lentiviral vector. In some embodiments, the vector is a fusosome. In some embodiments, the vector is a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein. In some embodiments, the vector is an enveloped delivery vehicle. In some embodiments, the vector is a self-replicating RNA virus. In some embodiments, the vector is an mRNA-packaging virus-like particle. In some embodiments, the vector is RNP-packaging virus-like particle. In some embodiments, the vectors used in the context of this disclosure are the vectors described in WO2023193015A1; WO2022164935A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." *Cell* (2022); Hamilton, Jennifer R., et al. "In vivo human T cell engineering with enveloped delivery vehicles." *Nature Biotechnology* (2024): 1-9; Lundstrom, Kenneth. "Self-replicating RNA viruses for RNA therapeutics." *Molecules* 23.12 (2018): 3310.

[0640] In some embodiments, the retroviral nucleic acid comprises one or more of (*e.g.,* all of): a 5' promoter (*e.g.,* to control expression of the entire packaged RNA), a 5' LTR (*e.g.,* that includes R (polyadenylation tail signal) and/or U5 which includes a primer activation signal), a primer binding site, a psi packaging signal, a RRE element for nuclear export, a promoter directly upstream of the transgene to control transgene expression, a transgene (or other exogenous agent element), a polypurine tract, and a 3' LTR (*e.g.,* that includes a mutated U3, a R, and U5). In some embodiments, the retroviral nucleic acid further comprises one or more of a cPPT, a WPRE, and/or an insulator element (*e.g.,* as described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," Biomedicines, 4(1):4 (2016)).

[0641] A retrovirus typically replicates by reverse transcription of its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA

into a host genome. The structure of a wild-type retrovirus genome often comprises a 5' long terminal repeat (LTR) and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a host cell genome and gag, pol and env genes encoding the packaging components which promote the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell. In the provirus, the viral genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are involved in proviral integration and transcription. LTRs also serve as enhancerpromoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

[0642] The LTRs themselves are typically similar (*e.g.,* identical) sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

[0643] For the viral genome, the site of transcription initiation is typically at the boundary between U3 and R in one LTR and the site of poly (A) addition (termination) is at the boundary between R and U5 in the other LTR. U3 contains most of the transcriptional control elements of the provirus, which include the promoter and multiple enhancer sequences responsive to cellular and in some cases, viral transcriptional activator proteins. Some retroviruses comprise any one or more of the following genes that code for proteins that are involved in the regulation of gene expression: tot, rev, tax and rex.

[0644] With regard to the structural genes gag, pol and env themselves, gag encodes the internal structural protein of the virus. Gag protein is proteolytically processed into the mature proteins MA (matrix), CA (capsid) and NC (nucleocapsid). The pol gene encodes the reverse transcriptase (RT), which contains DNA polymerase, associated RNase H and integrase (IN), which mediate replication of the genome. The env gene encodes the surface (SU) glycoprotein and the transmembrane (TM) protein of the virion, which form a complex that interacts with cellular receptor proteins. This interaction promotes infection, *e.g.,* by fusion of the viral membrane with the cell membrane.

[0645] In a replication-defective retroviral vector genome gag, pol and env may be absent or not functional. The R regions at both ends of the RNA are typically repeated sequences. U5 and U3 represent unique sequences at the 5' and 3' ends of the RNA genome respectively. Retroviruses may also contain additional genes which code for proteins other than gag, pol and env. Examples of additional genes include (in HIV), one or more of vif, vpr, vpx, vpu, tat, rev and nef. EIAV has (amongst others) the additional gene S2.

[0646] Illustrative retroviruses suitable for use in particular embodiments, include, but are not limited to: Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus(MoMSV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), feline leukemia virus (FLV), spumavirus, Friend murine leukemia virus, Murine Stem Cell Virus (MSCV) and Rous Sarcoma Virus (RSV) and human immunodeficiency virus (HIV).

[0647] In some embodiments the retrovirus is a Gammretrovirus. In some embodiments the retrovirus is an Epsilonretrovirus. In some embodiments the retrovirus is an Alpharetrovirus. In some embodiments the retrovirus is a Betaretrovirus. In some embodiments the retrovirus is a Deltaretrovirus. In some embodiments the retrovirus is a Spumaretrovirus. In some embodiments the retrovirus is an endogenous retrovirus. In some embodiments the retrovirus is a lentivirus.

[0648] In some embodiments, a retroviral or lentivirus vector further comprises one or more insulator elements, *e.g.,* an insulator element described in Browning et al., "Insulators to Improve the Safety of Retroviral Vectors for HIV Gene Therapy," Biomedicines, 4(1):4 (2016). In various embodiments, the vectors comprise a promoter operably linked to a polynucleotide encoding an exogenous agent. The vectors may have one or more LTRs, wherein either LTR comprises one or more modifications, such as one or more nucleotide substitutions, additions, or deletions. The vectors may further comprise one of more accessory elements to increase transduction efficiency (*e.g.,* a cPPT/FLAP), viral packaging (*e.g.,* a Psi (Y) packaging signal, RRE), and/or other elements that increase exogenous gene expression (*e.g.,* poly (A) sequences), and may optionally comprise a WPRE or HPRE. In some embodiments, a lentiviral nucleic acid comprises one or more of, *e.g.,* all of, *e.g.,* from 5' to 3', a promoter (*e.g.,* CMV), an R sequence (*e.g.,* comprising TAR), a U5 sequence (*e.g.,* for integration), a PBS sequence (*e.g.,* for reverse transcription), a DIS sequence (*e.g.,* for genome dimerization), a psi packaging signal, a partial gag sequence, an RRE sequence (*e.g.,* for nuclear export), a cPPT sequence (*e.g.,* for nuclear import), a promoter to drive

expression of the exogenous agent, a gene encoding the exogenous agent, a WPRE sequence (*e.g.,* for efficient transgene expression), a PPT sequence (*e.g.,* for reverse transcription), an R sequence (*e.g.,* for polyadenylation and termination), and a U5 signal (*e.g.,* for integration).

[0649] Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2); visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). In some embodiments, HIV based vector backbones (i.e., HIV cis-acting sequence elements) are used. A lentivirus vector can comprise a viral vector or plasmid containing structural and functional genetic elements, or portions thereof, including LTRs that are primarily derived from a lentivirus.

[0650] In embodiments, a lentivirus vector (*e.g.,* lentiviral expression vector) may comprise a lentiviral transfer plasmid (*e.g.,* as naked DNA) or an infectious lentiviral particle. With respect to elements such as cloning sites, promoters, regulatory elements, heterologous nucleic acids, etc., it is to be understood that the sequences of these elements can be present in RNA form in lentiviral particles and can be present in DNA form in DNA plasmids.

[0651] In embodiments, a lentivirus vector is a vector with sufficient retroviral genetic information to allow packaging of an RNA genome, in the presence of packaging components, into a viral particle capable of infecting a target cell. Infection of the target cell can comprise reverse transcription and integration into the target cell genome. The recombinant lentivirus vector (RLV) typically carries non-viral coding sequences which are to be delivered by the vector to the target cell. In embodiments, an RLV is incapable of independent replication to produce infectious retroviral particles within the target cell. Usually, the RLV lacks a functional gag-pol and/or env gene and/or other genes involved in replication. The vector may be configured as a split-intron vector, *e.g.,* as described in PCT patent application WO 99/15683, which is herein incorporated by reference in its entirety.

[0652] In some embodiments, the lentivirus vector comprises a minimal viral genome, *e.g.,* the viral vector has been manipulated so as to remove the non-essential elements and to retain the essential elements in order to provide the required functionality to infect, transduce and deliver a nucleotide sequence of interest to a target host cell, *e.g.,* as described in WO 98/17815, which is herein incorporated by reference in its entirety.

[0653] A minimal lentiviral genome may comprise, *e.g.,* (5')R-U5-one or more first nucleotide sequences-U3-R(3'). However, the plasmid vector used to produce the lentiviral genome within a source cell can also include transcriptional regulatory control sequences operably linked to the lentiviral genome to direct transcription of the genome in a source cell. These regulatory sequences may comprise the natural sequences associated with the transcribed retroviral sequence, *e.g.,* the 5' U3 region, or they may comprise a heterologous promoter such as another viral promoter, for example the CMV promoter. Some lentiviral genomes comprise additional sequences to promote efficient virus production. For example, in the case of HIV, rev and RRE sequences may be included.

[0654] In some embodiments, the vector can be a vector of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), or the pEX series (Clontech, Palo Alto, Calif.). In some cases, bacteriophage vectors, such as ZG I O. XGT11, XZapII (Stratagene), XEMBL4, and ZNM I 149, also can be used. In some embodiments, plant expression vectors can be used and include pBIOl, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). In some embodiments, animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech).

[0655] In some embodiments, the vector (*e.g.,* a viral vector) is a double stranded DNA vector. In some embodiments, the DNA vector is a closed-end vector. In some embodiments, the closed-end vector is Doggybone™ DNA (dbDNA). Closed-end DNA vectors, including dbDNA, are known in the art and have been described in *e.g.,* Karda et al., Gene Ther (2019) 26:86-92.

[0656] In some cases, a vector may be used that does not require that the cells, *e.g.,* T cells, are activated. In some such instances, the cells may be selected and/or transduced prior to activation. Thus, the cells may be engineered prior to, or subsequent to culturing of the cells, and in some cases at the same time as or during at least a portion of the culturing.

[0657] In some embodiments, the cells are activated prior to introduction of the polynucleotide or vector. In some embodiments, the T cells are incubated with *e.g.,* anti-CD3/anti-CD28 antibodies. In some embodiments, the T cells are incubated with apololipoprotein E (ApoE) prior to, during, and/or subsequent to introduction of the polynucleotide or vector. For example, in some instances, the T cells are incubated with ApoE (e.g. ApoE4) prior to incubation with LNPs containing the polynucleotide or a composition thereof. In some embodiments, the T cells are incubated with about 1 pg/mL

ApoE4 prior to incubation with LNPs containing the polynucleotide or a composition thereof.

IX. DELIVERY OF NUCLEIC ACIDS

[0658] Introduction of the nucleic acid molecules or amino acid molecules of the disclosure into cells can be achieved by methods known to those skilled in the art such as, for example, viral infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, nucleofection, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro-injection, nanoparticle-mediated nucleic acid delivery, naked nucleic acid delivery, as nucleic acid complexed with materials such as a liposome, nanoparticle or poloxamer, or can be delivered by viruses (*e.g.,* adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)), and the like. Introduction of the nucleic acid molecules or amino acid molecules of the disclosure into cells can be ex vivo or in vivo.

[0659] In some embodiments, introducing the polynucleotides encoding the engineered polypeptides described herein into cells can be achieved by any suitable technique. Suitable techniques include calcium phosphate, lipid-mediated transfection, electroporation, nucleofection, fusogens, transposons (*e.g.,* a Sleeping Beauty system), and transduction or infection using a viral vector, as discussed herein. In some embodiments, the polynucleotides are introduced into a cell via viral transduction (*e.g.,* AAV transduction, lentiviral transduction) or otherwise delivered on a viral vector (*e.g.,* fusogen-mediated delivery). In some embodiments, the polynucleotides are introduced into a cell via a fusogen-mediated delivery or a transposase system selected from the group consisting of conditional or inducible transposases, conditional or inducible PiggyBac transposons, conditional or inducible Sleeping Beauty (SB11) transposons, conditional or inducible Mosl transposons, and conditional or inducible Tol2 transposons.

[0660] The process of introducing the nucleic acid molecule into the cell can be achieved by any suitable technique. Suitable techniques include calcium phosphate or lipid-mediated transfection, electroporation, and transduction or infection using a viral vector. In some embodiments, the nucleic acid molecule comprises DNA. In some embodiments, the nucleic acid molecule comprises a modified DNA. In some embodiments, the nucleic acid molecule

comprises RNA. In some embodiments, the nucleic acid molecule comprises mRNA. In some embodiments, the nucleic acid molecule comprises a modified mRNA.

[0661] In some embodiments, the nucleic acid molecules can be delivered by conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (2012, supra) and other standard molecular biology laboratory manuals, such as, calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction, nucleoporation, hydrodynamic shock, and infection.

[0662] In some embodiments, one or more polynucleotide(s) are introduced into a cell using electroporation (see, *e.g.,* Chicaybam et al, (2013) PLoS ONE 8(3): e60298 and Van Tedeloo et al. (2000) Gene Therapy 7(16): 1431-1437). For examples, in some embodiments, one or more RNA molecules is introduced into a T cell using electroporation. Methods and systems for electroporation are known in the art, including e.g. Nucleofection® Technology (Lonza). In some embodiments, recombinant nucleic acids are transferred into T cells via transposition (see, *e.g.,* Manuri et al. (2010) Hum Gene Ther 21(4): 427-437; Sharma et al. (2013) Molec Ther Nucl Acids 2, e74; and Huang et al. (2009) Methods Mol Biol 506: 115-126). Other methods of introducing and expressing genetic material, *e.g.,* polynucleotides and/or vectors, into immune cells include calcium phosphate transfection (*e.g.,* as described in Current Protocols in Molecular Biology, John Wiley & Sons, New York. N.Y.), protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., Mol. Cell Biol., 7: 2031-2034 (1987) and other approaches described in, *e.g.,* International Pat. App. Pub. No. WO 2014055668, and U.S. Patent No. 7,446,190.

[0663] In some embodiments, the nucleic acid molecules can be delivered by viral or non-viral delivery vehicles known in the art. For example, the nucleic acid molecule can be stably integrated in the host genome, or can be episomally replicating, or present in the recombinant host cell as a mini-circle expression vector for transient expression. Accordingly, in some embodiments, the nucleic acid molecule is maintained and replicated in the recombinant host cell as an episomal unit. In some embodiments, the nucleic acid molecule is stably integrated into the genome of the recombinant cell. Stable integration can be achieved using classical random genomic recombination techniques or with more precise

techniques such as guide RNA-directed CRISPR/Cas9 genome editing, or DNA-guided endonuclease genome editing with NgAgo (Natronobacterium gregoryi Argonaute), or TALENs genome editing (transcription activator-like effector nucleases). In some embodiments, the nucleic acid molecule is present in the recombinant host cell as a mini-circle expression vector for transient expression.

[0664] In some embodiments, the nucleic acid molecules can be encapsulated in a viral capsid or a lipid nanoparticle, or can be delivered by viral or non-viral delivery means and methods known in the art, such as electroporation. For example, introduction of nucleic acids into cells may be achieved by viral transduction. In a non-limiting example, adeno-associated virus (AAV) is engineered to deliver nucleic acids to target cells via viral transduction. Several AAV serotypes have been described, and all of the known serotypes can infect cells from multiple diverse tissue types. AAV is capable of transducing a wide range of species and tissues *in vivo* with no evidence of toxicity, and it generates relatively mild innate and adaptive immune responses.

[0665] In some embodiments, the polypeptide of the disclosure is delivered using viral transduction, for example, with a vector. In some embodiments, the vector is a pseudotyped, self- inactivating lentiviral vector that carries the exogenous polynucleotide. In some embodiments, the vector is a self-inactivating lentiviral vector pseudotyped with a vesicular stomatitis VSV-G envelope, and which carries the exogenous polynucleotide.

[0666] In some embodiments, a lentiviral vector can be used for delivery. Lentiviral-derived vector systems are useful for nucleic acid delivery and gene therapy via viral transduction. Lentiviral vectors offer several attractive properties as gene-delivery vehicles, including: (i) sustained gene delivery through stable vector integration into host genome; (ii) the capability of infecting both dividing and non-dividing cells; (iii) broad tissue tropisms, including important gene- and cell-therapy-target cell types; (iv) no expression of viral proteins after vector transduction; (v) the ability to deliver complex genetic elements, such as polycistronic or intron-containing sequences; (vi) a potentially safer integration site profile; and (vii) a relatively easy system for vector manipulation and production.

[0667] Methods of lentiviral transduction are known. Exemplary methods are described in, *e.g.,* Wang et al. (2012) J. Immunother. 35(9): 689-701; Cooper et al. (2003) Blood. 101:1637-1644; Verhoeyen et al. (2009) Methods Mol Biol. 506: 97-114; and Cavalieri et al. (2003) Blood. 102(2): 497-505. In some embodiments, the polynucleotide encoding the recombinant receptor and/or one or more additional polypeptide(s), is introduced into a

population containing cultured cells, such as by retroviral transduction, transfection, or transformation.

[0668] In some embodiments, a recombinant adeno-associated virus (AAV) vector can be used for delivery. Techniques to produce rAAV particles, in which an AAV genome to be packaged that includes the polynucleotide to be delivered, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (*e.g.,* not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes can be from any AAV serotype for which recombinant virus can be derived, and can be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, international patent application publication number WO 01/83692.

[0669] In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a retrovirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a VSV-G pseudotyped lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV6. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an AAV9. I In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a split-intein dual AAV. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a redirected lentivirus. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a fusosome. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a enveloped delivery vehicle. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with a self-replicating RNA virus. In some embodiments, the nucleic

acid encoding the engineered protein of the present disclosure is delivered with an mRNA-packaging virus-like particle. In some embodiments, the nucleic acid encoding the engineered protein of the present disclosure is delivered with an RNP-packaging virus-like particle. In some embodiments, the delivery used in the context of this disclosure are described in WO2023193015A1; WO2022164935A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." *Cell* (2022); Hamilton, Jennifer R., et al. "In vivo human T cell engineering with enveloped delivery vehicles." *Nature Biotechnology* (2024): 1-9; Lundstrom, Kenneth. "Self-replicating RNA viruses for RNA therapeutics." *Molecules* 23.12 (2018): 3310.

[0670] In some embodiments, the nucleic acid is or comprises mRNA encoding the engineered protein of the disclosure. The mRNA may be delivered to the cell using any appropriate technique, such as electroporation or using a lipid nanoparticle (LNP). In some embodiments, the nucleic acid is delivered with a lipid nanoparticle (LNP). In some embodiments, the nucleic acid is delivered with a selective organ targeting (SORT) LNP). In some embodiments, the nucleic acid is delivered with an antibody targeted LNP. In some embodiments, the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid). In some embodiments, the nucleic acid is delivered with a polymer nanoparticle. In some embodiments, the nucleic acid is delivered with a protein nanoparticle. In some embodiments, the delivery used in the context of this disclosure are described in WO2024006960A1; WO2019152557A1; Raguram, Aditya, Samagya Banskota, and David R. Liu. "Therapeutic in vivo delivery of gene editing agents." *Cell* (2022); Rurik, Joel G., et al. "CAR T cells produced in vivo to treat cardiac injury." *Science* 375.6576 (2022): 91-96.

[0671] In some embodiments, the polynucleotides disclosed herein can be delivered or introduced using one or more agent(s), capable of inducing a genetic disruption (*e.g.,* CRISPR/Cas and/or gRNA components), to a cell, using any of a number of known delivery methods or vehicles for introduction or transfer to cells, for example, using viral delivery vectors, or any of the known methods or vehicles for delivering CRISPR/Cas molecules and gRNAs. Exemplary methods are described in, *e.g.,* Wang et al. (2012) J. Immunother. 35(9): 689-701; Cooper et al. (2003) Blood. 101:1637-1644; Verhoeyen et al. (2009) Methods Mol Biol. 506: 97-114; and Cavalieri et al. (2003) Blood. 102(2): 497- 505. In some embodiments, nucleic acid sequences encoding one or more components of one or more

agent(s) capable of inducing a genetic disruption is introduced into the cells, *e.g.,* by any methods for introducing nucleic acids into a cell described herein or known. In some embodiments, a vector encoding components of one or more agent(s) capable of inducing a genetic disruption such as a CRISPR guide RNA and/or a Cas enzyme can be delivered into the cell.

[0672] In some embodiments, the one or more agent(s) capable of inducing a genetic disruption (*e.g.,* one or more agent(s) that is a CRISPR/Cas/gRNA), is introduced into the cell as a ribonucleoprotein (RNP) complex. RNP complexes include a sequence of ribonucleotides, such as an RNA or a gRNA molecule, and a protein, such as a Cas9 protein or variant thereof. For example, the Cas9 protein is delivered as RNP complex that comprises a Cas9 protein and a gRNA molecule targeting the target sequence, *e.g.,* using electroporation or other physical delivery method. In some embodiments, the RNP is delivered into the cell via electroporation or other physical means, *e.g.,* particle gun, Calcium Phosphate transfection, cell compression or squeezing. In some embodiments, the RNP can cross the plasma membrane of a cell without the need for additional delivery agents (*e.g.,* small molecule agents, lipids, etc.). In some embodiments, delivery of the one or more agent(s) capable of inducing genetic disruption, *e.g.,* CRISPR/Cas9, as an RNP offers an advantage that the targeted disruption occurs transiently, *e.g.,* in cells to which the RNP is introduced, without propagation of the agent to cell progenies. For example, delivery by RNP minimizes the agent from being inherited to its progenies, thereby reducing the chance of off-target genetic disruption in the progenies. In such cases, the genetic disruption and the integration of transgene can be inherited by the progeny cells, but without the agent itself, which may further introduce off-target genetic disruptions, being passed on to the progeny cells.

[0673] Agent(s) and components capable of inducing a genetic disruption (*e.g.,* a CRISPR/Cas molecule and gRNA molecule), can be introduced into target cells in a variety of forms using a variety of delivery methods and formulations, as set forth in **Tables 14 and 15,** or methods described in, *e.g.,* WO 2015/161276; US 2015/0056705, US 2016/0272999, US 2017/0211075; or US 2017/0016027. As described further herein, the delivery methods and formulations can be used to deliver template polynucleotides and/or other agents to the cell (such as those required for engineering the cells) in prior or subsequent steps of the methods described herein. When a CRISPR/Cas nuclease or gRNA component is encoded as DNA for delivery, the DNA may typically but not necessarily include a control region, *e.g.,*

comprising a promoter, to effect expression. Exemplary promoters for CRISPR/Cas nuclease sequences include, *e.g.,* CMV, EFla, EFS, MSCV, PGK, or CAG promoters. Useful promoters for gRNAs include, *e.g.,* Hl, EF-la, tRNA or U6 promoters. Promoters with similar or dissimilar strengths can be selected to tune the expression of components. Sequences encoding a CRISPR/Cas nuclease molecule may comprise a nuclear localization signal (NLS), *e.g.,* an SV40 NLS. In some embodiments a promoter for a CRISPR/Cas nuclease molecule or a gRNA molecule may be, independently, inducible, tissue specific, or cell specific. In some embodiments, an agent capable of inducing a genetic disruption is introduced RNP complexes.

Table 14. Exemplary Delivery Methods

Elements		**Comments**
Cas9 Molecule(s)	**gNRA Molecule(s)**	
DNA	DNA	In this embodiment, a Cas9 molecule and a gRNA are transcribed from DNA. In this embodiment, they are encoded on separate molecules.
DNA		In this embodiment, a Cas9 molecule and a gRNA are transcribed from DNA, here from a single molecule
DNA	RNA	In this embodiment, a Cas9 molecule is transcribed from DNA, and a gRNA is provided an in vitro transcribed or synthesized RNA.
mRNA	RNA	In this embodiment, a Cas9 molecule is translated from in vitro transcribed mRNA, and a gRNA is provided an in vitro transcribed or synthesized RNA.
mRNA	DNA	In this embodiment, a Cas9 molecule is translated from in vitro transcribed mRNA, and a gRNA is transcribed from DNA
Protein	DNA	In this embodiment, a Cas9 molecule is provided as a protein, and a gRNA is transcribed from DNA.
Protein	RNA	In this embodiment, a Cas9 molecule is provided as a protein, and a gRNA is provided as transcribed or synthesized RNA.

Table 15. Comparison of Exemplary Delivery Methods

Delivery Vector/Mode		**Delivery into Non-Dividing Cells**	**Duration of Expression**	**Genome Integration**	**Type of Molecule Delivered**
Physical (*e.g.*, electroporation, particle gun, Calcium Phosphate transfection, cell compression or squeezing)		YES	Transient	NO	Nucleic Acids and Proteins
Viral	**Retrovirus**	YES	Stable	YES/NO with modifications	RNA
	Lentivirus	YES	Stable	NO	RNA
	Adenovirus	YES	Transient	NO	DNA
	AAV	YES	Stable	NO	DNA
	Vaccinia Virus	YES	Very Transient	NO	DNA
	Herpes Simplex Virus	YES	Stable	NO	DNA
Non-Viral	**Cationic Liposomes**	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
	Polymeric Nanoparticles	YES	Transient	Depends on what is delivered	Nucleic Acids and Proteins
Biological Non-Viral Delivery Vehicles	**Attenuated Bacteria**	YES	Transient	NO	Nucleic Acids
	Engineered Bacteriophages	YES	Transient	NO	Nucleic Acids
	Mammalian Virus-Like Particles	YES	Transient	NO	Nucleic Acids
	Biological liposomes: Erythrocyte Ghosts and Exosomes	YES	Transient	NO	Nucleic Acids

[0674] In some embodiments, the polynucleotide encoding the engineered proteins of the disclosure can be delivered into cells by known methods or as described herein. For example, by vectors (*e.g.*, viral or non-viral vectors), non-vector based methods (*e.g.*, using naked DNA or DNA complexes), or a combination thereof. In some embodiments, the

polynucleotide containing the agent(s) and/or components thereof is delivered by a vector (*e.g.,* viral vector/virus or plasmid). The vector may be any described herein.

[0675] In some embodiments, the polynucleotide encoding the engineered proteins of the disclosure is delivered by a non-vector based method (*e.g.,* using naked DNA or DNA complexes). For example, the DNA or RNA or proteins or combination thereof, *e.g.,* ribonucleoprotein (RNP) complexes, can be delivered, *e.g.,* by organically modified silica or silicate (Ormosil), electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Lett 12: 6322-27, Kollmannsperger et al (2016) Nat Comm 7, 10372), gene gun, sonoporation, magnetofection, lipid-mediated transfection, dendrimers, inorganic nanoparticles, calcium phosphates, or a combination thereof.

[0676] In some embodiments, delivery via electroporation comprises mixing the cells with the polynucleotide encoding the engineered proteins of the disclosure or RNP complex in a cartridge, chamber or cuvette and applying one or more electrical impulses of defined duration and amplitude. In some embodiments, delivery via electroporation is performed using a system in which cells are mixed with the DNA in a vessel connected to a device (*e.g.,* a pump) which feeds the mixture into a cartridge, chamber or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel.

[0677] In some embodiments, the delivery vehicle is a non-viral vector. In some embodiments, the non-viral vector is an inorganic nanoparticle. Exemplary inorganic nanoparticles include, *e.g.,* magnetic nanoparticles (*e.g.,* FesMnCh) and silica. The outer surface of the nanoparticle can be conjugated with a positively charged polymer (*e.g.,* polyethylenimine, polylysine, polyserine) which allows for attachment (*e.g.,* conjugation or entrapment) of payload. In some embodiments, the non-viral vector is an organic nanoparticle. Exemplary organic nanoparticles include, *e.g.,* SNALP liposomes that contain cationic lipids together with neutral helper lipids which are coated with polyethylene glycol (PEG), and protamine-nucleic acid complexes coated with lipid. Exemplary lipids and polymers for gene transfer include those described in, for example, WO 2019/195492 and WO 2020/223535.

[0678] In some embodiments, the vehicle has targeting modifications to increase target cell update of nanoparticles and liposomes, *e.g.,* cell specific antigens, monoclonal antibodies, single chain antibodies, aptamers, polymers, sugars, and cell penetrating peptides. In some embodiments, the vehicle uses fusogenic and endosome-destabilizing

peptides/polymers. In some embodiments, the vehicle undergoes acid-triggered conformational changes (*e.g.,* to accelerate endosomal escape of the cargo). In some embodiments, a stimulus-cleavable polymer is used, *e.g.,* for release in a cellular compartment. For example, disulfide-based cationic polymers that are cleaved in the reducing cellular environment can be used.

[0679] In some embodiments, the delivery vehicle is a biological non-viral delivery vehicle. In some embodiments, the vehicle is an attenuated bacterium (*e.g.,* naturally or artificially engineered to be invasive but attenuated to prevent pathogenesis and expressing the transgene (*e.g.,* Listeria monocytogenes, certain Salmonella strains, Bifidobacterium longum, and modified Escherichia coll), bacteria having nutritional and tissue- specific tropism to target specific cells, bacteria having modified surface proteins to alter target cell specificity). In some embodiments, the vehicle is a genetically modified bacteriophage (*e.g.,* engineered phages having large packaging capacity, less immunogenicity, containing mammalian plasmid maintenance sequences and having incorporated targeting ligands). In some embodiments, the vehicle is a mammalian virus-like particle. For example, modified viral particles can be generated (*e.g.,* by purification of the "empty" particles followed by ex vivo assembly of the virus with the desired cargo). The vehicle can also be engineered to incorporate targeting ligands to alter target tissue specificity. In some embodiments, the vehicle is a biological liposome. For example, the biological liposome is a phospholipid-based particle derived from human cells (*e.g.,* erythrocyte ghosts, which are red blood cells broken down into spherical structures derived from the subject (*e.g.,* tissue targeting can be achieved by attachment of various tissue or cell-specific ligands), or secretory exosomes - subject-derived membrane-bound nanovescicles (30 -100 nm) of endocytic origin (*e.g.,* can be produced from various cell types and can therefore be taken up by cells without the need for targeting ligands).

[0680] In some embodiments, an RNA polynucleotide encoding the engineered proteins of the disclosure can be delivered into cells, *e.g.,* target cells described herein, by known methods or as described herein. For example, RNA can be delivered, *e.g.,* by microinjection, electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Let 12: 6322-27), lipid-mediated transfection, peptide-mediated delivery, *e.g.,* cell-penetrating peptides, or a combination thereof.

[0681] In some embodiments, delivery via electroporation comprises mixing the cells with the RNA in a cartridge, chamber or cuvette and applying one or more electrical impulses of

defined duration and amplitude. In some embodiments, delivery via electroporation is performed using a system in which cells are mixed with the RNA encoding CRISPR/Cas nuclease molecules and/or gRNA molecules in a vessel connected to a device (*e.g.,* a pump) which feeds the mixture into a cartridge, chamber or cuvette wherein one or more electrical impulses of defined duration and amplitude are applied, after which the cells are delivered to a second vessel.

[0682] In some embodiments, engineered polypeptide molecules can be delivered into cells by known methods or as described herein. For example, protein molecules can be delivered, *e.g.,* by microinjection, electroporation, transient cell compression or squeezing (such as described in Lee, et al. (2012) Nano Let 12: 6322-27), lipid-mediated transfection, peptide-mediated delivery, or a combination thereof. Delivery can be accompanied by DNA encoding a polypeptide (*e.g.,* an engineered polypeptide, gRNA, CAR, TCR, etc).

[0683] In some embodiments, the exogenous Cas protein can be introduced into the cell in polypeptide form. In certain embodiments, the Cas proteins can be conjugated to or fused to a cell-penetrating polypeptide or cell-penetrating peptide. As used herein, "cell-penetrating polypeptide" and "cell-penetrating peptide" refers to a polypeptide or peptide, respectively, which facilitates the uptake of molecule into a cell. The cell-penetrating polypeptides can contain a detectable label.

[0684] In many embodiments, Cas proteins can be conjugated to or fused to a charged protein (*e.g.,* that carries a positive, negative or overall neutral electric charge). Such linkage may be covalent. In some embodiments, the Cas protein can be fused to a superpositively charged GFP to significantly increase the ability of the Cas protein to penetrate a cell (Cronican et al. ACS Chem Biol. 2010; 5(8):747-52). In certain embodiments, the Cas protein can be fused to a protein transduction domain (PTD) to facilitate its entry into a cell. Exemplary PTDs include Tat, oligoarginine, and penetratin. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a cell-penetrating peptide. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a PTD. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a tat domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to an oligoarginine domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a penetratin domain. In some embodiments, the Cas9 protein comprises a Cas9 polypeptide fused to a superpositively charged GFP. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a cell-penetrating peptide. In some embodiments, the Casl2a

protein comprises a Casl2a polypeptide fused to a PTD. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a tat domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to an oligoarginine domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a penetratin domain. In some embodiments, the Casl2a protein comprises a Casl2a polypeptide fused to a superpositively charged GFP.

[0685] In some embodiments, the Cas protein can be introduced into a cell containing the target polynucleotide sequence in the form of a nucleic acid encoding the Cas protein. The process of introducing the nucleic acids into cells can be achieved by any suitable technique. Suitable techniques include those described herein.

X. CELLS

[0686] In another aspect, the present disclosure provides an engineered cell comprising (i) a recombinant polypeptide comprising or consisting of the epitope or mimotope of the disclosure; and/or (ii) a polynucleotide encoding the polypeptide. In some embodiments, the polynucleotide is comprised in a vector. In some embodiments, the peptide epitope or mimotope is expressed on the surface of the engineered cell. In some embodiments, the engineered cell is transfected or transduced by a vector of the disclosure. In some embodiments, the engineered cell is contacted by a particle of the disclosure.

[0687] In some embodiments, the recombinant polypeptide expressed by the engineered cell is a recombinant receptor (*e.g.,* CAR or TCR) that comprises the epitope or mimotope of the disclosure. In some embodiments, the engineered cell expresses a recombinant receptor (*e.g.,* CAR or TCR) and a different recombinant polypeptide comprising the epitope or mimotope of the disclosure — thus, in some embodiments, the epitope or mimotope is not part of the engineered receptor (*e.g.,* CAR or TCR).

[0688] In some embodiments, the engineered cell comprises a recombinant receptor (*e.g.,* CAR or TCR) capable of binding to the same antigen (*e.g.,* an autoimmune associated antigen or cancer associated antigen of the disclosure) as that of the monoclonal antibody that can bind to the epitope or mimotope of the disclosure. For example, in some embodiments, the engineered cell comprises (i) a recombinant receptor (*e.g.,* CAR or TCR) capable of binding to DLL3 antigen, and (ii) an epitope or mimotope that is capable of being bound by an anti-DLL3 monoclonal antibody (and the epitope or mimotope may be comprised within the engineered receptor or in a separate recombinant polypeptide).

[0689] In some embodiments, the engineered cell further comprises a second recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein, or a functional fragment thereof, of the disclosure. In some embodiments, the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

[0690] In some embodiments, the engineered cell comprises one or more additional peptide epitope(s) or mimotope(s) present on the cell surface, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope or different from the peptide epitope or mimotope. In some embodimetns, the one or more additional peptide epitope(s) or mimotope(s) are different from the modified peptide epitope. In some embodiments, the recombinant polypeptide comprises 1, 2, 3, or 4 additional peptide epitopes or mimotopes.

[0691] In some embodiments, activating the engineered cell results in the secretion of IL-2 by the cell at a level that is at least 10%, at least 20%, at least 50%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, least 30-fold, or at least 100-fold higher than the IL-2 secretion level of a control cell lacking the recombinant polypeptide.

[0692] In some embodiments, expression of the recombinant polypeptide by the cell is under the control of an endogenous promoter. Non-limiting examples of endogenous promoters include a TCRa promoter, a TCRb promoter, a CD3d promoter, a CD3g promoter, a CD3e promoter, a CD3z promoter, a CARD9 promoter, a CARD10 promoter, a CARD11 promoter, a CARD14 promoter, or a PIK3R3 promoter. In some embodiments, expression of the recombinant polypeptide by the cell is under the control of an exogenous promoter. Non-limiting examples of endogenous promoters include a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter,

GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein- I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of oloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

[0693] The present disclosure additionally provides engineered cells wherein the nucleic acid construct is inserted in a T-cell receptor (TCR) locus, a CD3 locus, a B2 microglobulin (B2M) locus, a class II transactivator (CIITA) locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, a PIK3R3 locus, or a safe harbor locus. In some embodiments, the TCR locus is or comprises a TRAC locus, a TRBC1 locus, a TRBC2 locus, or a TRAJ locus. In some embodiments, the TRAJ locus is or comprises a TRAJ intron splice acceptor locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRAC locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRBC1 locus. In some embodiments,

the cell does not express a functional gene product of an endogenous TRBC2 locus. In some embodiments, the cell does not express a functional gene product of an endogenous TRAJ locus. In some embodiments, the CD3 locus is or comprises a CD3d locus, a CD3g locus, a CD3e locus, or a CD3z locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3d locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3g locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3e locus. In some embodiments, the cell does not express a functional gene product of an endogenous CD3z locus. In some embodiments, the safe harbor locus is or comprises an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus. In some embodiments, the nucleic acid construct is inserted in an exon, an intron, between an intron and an exon, or in a regulatory region.

[0694] In some embodiments, the disclosure provides a population of cells comprising one or more engineered cells. In some embodiments, the disclosure provides a population of cells comprising at least 10^2, at least 10^3, at least 10^4, at least 10^5, at least 10^6, at least 10^7, at least 10^8, at least 10^9, at least 10^10, at least 10^11, or at least 10^12, engineered cells of the disclosure.

[0695] In some embodiments, the cell further comprises **(i)** a chimeric antigen receptor (CAR) capable of binding a target antigen; and/or **(ii)** a recombinant T cell receptor (TCR) capable of binding a target antigen. In some embodiments, the engineered cell comprises a nucleic acid construct expressing the CAR or recombinant TCR. In some embodiments, the nucleic acid construct is an RNA construct. In some embodiments, this CAR or TCR is a second type of immune receptor in the cell (*e.g.,* a cell that expresses the recombinant CAR/TCR comprising the deletion tag and this second CAR/TCR). In some embodiments, the target antigen for this CAR/TCR is CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1,

DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, ggcc, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combinations thereof. In some embodiments, the target antigen for this CAR/TCR is B7H3, BCMA, CD19, CD20, CD22, CD70, CD79a, CD79b, CLDN6, CLDN18.2, DLL3, GCC, GD2, GD3, GPC3, GPRC5D, KLK3, LY6G6D, p53R175H, PRAME, ROPN1, STEAP1, or STEAP2. In some embodiments, the engineered cell has cell surface expression of the CAR/TCR.

In some embodiments, the cell further comprises a chimeric auto antigen receptor (CAAR) capable of binding a target antigen. In some embodiments, the antigen is pancreatic P-cell antigen, synovial joint antigen, myelin basic protein, proteolipid protein, myelin oligodendritic glycoprotein, MuSK, keratinocyte adhesion protein desmoglein 3 (Dsg3), Ro-RNP complex, La antigen, myeloperoxidase, proteinase 3, cardiolipin, citrullinated proteins, carbamylated proteins, or a3 chain of basement membrane collagen. In some embodiments, the engineered cell has cell surface expression of the CAAR.

In some embodiments, the cell further comprises a B-cell autoantibody receptor (BAR) capable of binding a target antigen. In some embodiments, the engineered cell has cell surface expression of the BAR.

In some embodiments, the engineered cell further comprises a non-coding RNA. In some embodiments, the non-coding RNA comprises a shRNA or a microRNA.

In some embodiments, the cell further comprises an additional potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, a tolerogenic factor, a safety switch, or any combination thereof. In some embodiments, the additional potency enhancement polypeptide is or comprises a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (*e.g.,* TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, or CD8alpha/beta.

[0696] In some embodiments, the safety switch is or comprises a herpesvirus thymidine kinase (HSV-tk) gene, an Escherichia coli cytosine deaminase (EC-CD) gene, or an inducible Caspase9 (iCasp9) protein.

[0697] In some embodiments, the engineered cell is a prokaryotic cell or a eukaryotic cell. In some embodiments, the cell is *in vivo*. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is *in vitro*. In some embodiments, the engineered cell is a eukaryotic cell. In some embodiments, the engineered cell is an animal cell. In some embodiments, the animal cell is a mammalian cell. In some embodiments, the animal cell is a human cell. In some embodiments, the cell is a non-human primate cell. In some embodiments, the cell is a canine cell. In some embodiments, the cell is a non-natural cell or has been genetically engineered (i.e., an engineered cell). In some embodiments, the polynucleotide is an exogenous nucleic acid. In some embodiments, the mammalian cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell.

[0698] In some embodiments, the cell is an immune cell selected from the group of consisting of a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector

T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T (NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, and a progenitor cell of the lymphoid lineage. In some embodiments, the cell is a T cell, a macrophage, a monocyte, or a natural killer (NK) cell. In some embodiments, the cell is a T cell. In some embodiments, the T cell is a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, or an engineered T cell comprising a transcriptional receptor.

[0699] Sources for the T cells include, but are not limited to, peripheral blood, bone marrow, or other sources of hematopoietic cells. T cells can be isolated by methods well known in the art, including commercially available isolation methods (see, for example, Rowland-Jones et al., Lymphocytes: A Practical Approach, Oxford University Press, New York (1999), Su et al., Methods Mol. Biol. 806:287-299 (2012); Bluestone et al., Sci. Transl. Med. 7(315) (doi: 10.1126/scitranslmed.aad4134)(2015); Miyara et al., Nat. Rev. Rheumatol. 10:543-551 (2014); Liu et al., J. Exp. Med. 203:1701-1711 (2006); Seddiki et al., J. Exp. Med. 203:1693-1700 (2006); Ukena et al., Exp. Hematol. 39:1152-1160 (2011); Chen et al., J. Immunol. 183:4094-4102 (2009); Putnam et al., Diabetes 58:652-662 (2009); Putnam et al., Am. Tranplant. 13:3010-3020 (2013); Lee et al., Cancer Res. 71:2871-2881 (2011); MacDonald et al., J Clin. Invest. 126:1413-1424 (2016)).

[0700] In some embodiments, the engineered cell is an autologous cell or an allogeneic cell. In some embodiments, the engineered cell is a primary cell. In some embodiments, the engineered cell is derived from a stem cell. In some embodiments, the engineered cell is genetically modified. In some embodiments, the engineered cell has reduced or eliminated cell surface expression of an endogenous T cell receptor. In some embodiments, the engineered cell is more prone to display central memory phenotype than a control cell lacking the recombinant polypeptide. In some embodiments, the engineered cell further comprises one or more modifications that inactivate or disrupt one or more alleles of TGFbeta, Regnase-1, FAS, PTPN2, NR4A3, CD52, PD-1, B2M, CIITA, SOCS1, CBLB, DGKalpha, and/or DGKζ.

[0701] In some embodiments, the cell further comprises a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, an RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing

endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof. In some embodiments, the cell further comprises Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional fragment thereof. In some embodiments, the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

[0702] Various known techniques can be employed to isolate or enrich for desired immune cells such as T cells. If a particular type of T cell is to be isolated, various cell surface markers or combinations of markers, including but not limited to, CD3, CD4, CD8, CD34 (for hematopoietic stem and progenitor cells) and the like, can be used to separate the cells, as is well known in the art (see Kearse, T Cell Protocols: Development and Activation, Humana Press, Totowa N.J. (2000); De Libero, T Cell Protocols, Vol. 514 of Methods in Molecular Biology, Humana Press, Totowa N.J. (2009)) Negative selection methods can be used to remove cells that are not the desired immune cells. Additionally, positive selection methods can be used to isolate or enrich for desired T cells. In some instances, a combination of both positive and negative selection methods can be used.

[0703] In some embodiments, the engineered cells provided by the disclosure are modified such that they are able to survive and proliferate when administered to a patient. In some embodiments, the engineered cells provided by the disclosure are modified such that they have increased signaling through certain pathways. These effects on survival, proliferation, and/or signaling may be related to the CARD-containing polypeptide or functional fragment thereof that is comprised by the engineered cell. The engineered cell may demonstrate one or more of the following characteristics: improved binding to the antigen expressed on the

surface of a target cell, reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell, reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell, reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, and/or increase intratumoral presence in vivo. In some embodiments, the engineered cell may demonstrate comparable levels of binding to the antigen expressed on the surface of a target cell; comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell. In some embodiments, the engineered cell may demonstrate increased signaling through has increased or decreased signaling through the CARD11-BCL10-MALT1 complex, NF-κB, AP-1, NFAT, JAK/STAT, and/or MEK/ERK pathways. In some embodiments, these characteristics are measured with respect to (*e.g.,* compared to) a control cell expressing a recombinant polypeptide that does not comprise the peptide epitope or mimotope.

[0704] In some embodiments, the provided modified cells are modified such that they are able to evade immune recognition and responses when administered to a patient (*e.g.,* recipient subject). The cells can evade killing by immune cells in vitro and in vivo. In some embodiments, the cells evade killing by macrophages and NK cells. In some embodiments, the cells are ignored by immune cells or a subject's immune system. In other words, the cells administered in accordance with the methods described herein are not detectable by immune cells of the immune system. In some embodiments, the cells are cloaked and therefore avoid immune rejection.

[0705] Methods of determining whether a modified cell provided herein evades immune recognition include, but are not limited to, IFN-y Elispot assays, microglia killing assays, cell engraftment animal models, cytokine release assays, ELISAs, killing assays using bioluminescence imaging or chromium release assay or Xcelligence analysis, mixed-lymphocyte reactions, immunofluorescence analysis, etc. In some embodiments, the immunogenicity of the cells is evaluated in a complement-dependent cytotoxicity (CDC) assay. CDC can be assayed in vitro by incubating cells with IgG or IgM antibodies targeting an HLA-independent antigen expressed on the cell surface in the presence of serum containing complement and analyzing cell killing. In some embodiments, CDC can be

assayed by incubating cells with ABO blood type incompatible serum, wherein the cells comprise A antigens or B antigens, and the serum comprises antibodies against the A antigens and/or B antigens of the cells.

[0706] The cell may be a vertebrate cell, for example, a mammalian cell, such as a human cell, a canine cell, or a mouse cell. The cell may also be a vertebrate stem cell, for example, a mammalian stem cell, such as a human stem cell (or a cell derived from such a stem cell), a canine stem cell, or a mouse stem cell (or a cell derived from such a stem cell). In embodiments, the cell or stem cell or a cell derived from such a stem cell is amenable to modification.

[0707] In some embodiments, the cells that are engineered or modified as provided herein are cells from a subject that is known or suspected of having a particular disease or condition to be treated.

[0708] In some embodiments, the cells that are engineered or modified as provided herein are cells from a healthy subject, such as a subject that is not known or suspected of having a particular disease or condition to be treated. For instance, if T cells are isolated or obtained from a donor subject, such as for treating a cancer, the donor subject is a healthy subject not known or suspected of suffering from cancer or another disease or condition.

[0709] The polynucleotide encoding the polypeptide of the disclosure can be introduced into a target cell, such as, for example, a human T lymphocyte, to produce a recombinant or engineered cell containing the nucleic acid molecule.

[0710] Among the cells expressing the polypeptide of the disclosure are engineered cells. The genetic engineering generally involves introduction of a nucleic acid encoding the recombinant or engineered component into a composition containing the cells, such as by retroviral transduction, transfection, or transformation.

[0711] In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0712] The cells generally are eukaryotic cells, such as mammalian cells, and typically are human cells. In some embodiments, the cells are derived from the blood, bone marrow,

lymph, or lymphoid organs, are cells of the immune system, such as cells of the innate or adaptive immunity, *e.g.,* myeloid or lymphoid cells, including lymphocytes, typically T cells and/or NK cells. Other exemplary cells include stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells typically are primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the cells include one or more subsets of T cells or other cell types, such as whole T cell populations, CD4+ cells, CD8+ cells, and subpopulations thereof, such as those defined by function, activation state, maturity, potential for differentiation, expansion, recirculation, localization, and/or persistence capacities, antigen-binding, type of antigen receptor, presence in a particular organ or compartment, marker or cytokine secretion profile, and/or degree of differentiation. With reference to the subject to be treated, the cells may be allogeneic and/or autologous. Among the methods include off-the-shelf methods. In some aspects, such as for off- the-shelf technologies, the cells are pluripotent and/or multipotent, such as stem cells, such as induced pluripotent stem cells (iPSCs). In some embodiments, the methods include isolating cells from the subject, preparing, processing, culturing, and/or engineering them, and re introducing them into the same subject, before or after cryopreservation.

[0713] In other embodiments, the methods include isolating cells from a donor, preparing, processing, culturing, and/or engineering them, and introducing them into a subject who is not the donor, before or after cryopreservation.

[0714] Among the sub-types and subpopulations of T cells and/or of CD4+ and/or of CD8+ T cells are naive T (TN) cells, effector T cells (TEFF), memory T cells and sub-types thereof, such as stem cell memory T (TSCM), central memory T (TCM), effector memory T (TEM), or terminally differentiated effector memory T cells, tumor-infiltrating lymphocytes (TIL), immature T cells, mature T cells, helper T cells, cytotoxic T cells, mucosa-associated invariant T (MAIT) cells, naturally occurring and adaptive regulatory T (Treg) cells, helper T cells, such as TH1 cells, TH2 cells, TH3 cells, TH17 cells, TH9 cells, TH22 cells, follicular helper T cells, alpha/beta T cells, and delta/gamma T cells.

[0715] In some embodiments, the cells are natural killer (NK) cells. In some embodiments, the cells are monocytes or granulocytes, *e.g.,* myeloid cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, and/or basophils.

[0716] Among the sub-types and subpopulations of NK cells are CD56+ bright and CD56+ dim NK cells. CD56+ bright NK cells, known for their high expression of CD56,

play a pivotal role in immune regulation and cytokine production, particularly in response to interleukin-12 (IL-12), IL-15, and IL-18. These cells are potent producers of cytokines like interferon-gamma (IFN-γ) but have limited cytotoxic capabilities. In contrast, CD56+ dim NK cells, which express CD56 at lower levels, are highly cytotoxic and specialize in directly killing virus-infected cells and tumor cells. Without wishing to be bound by theory, CD56+ dim NK cells achieve this through the release of granules containing perforin and granzymes. Additionally, there are other NK subsets based on the expression of receptors like NKG2A, NKG2C, and KIRs (Killer-cell Immunoglobulin-like Receptors), which modulate NK cell activity and specificity. These NK subsets demonstrate variations in response to viral infections, tumor surveillance, and interaction with other immune cells.

[0717] In some embodiments, the cells include one or more nucleic acids introduced via genetic engineering, and thereby express recombinant or genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, i.e., normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature, including one comprising chimeric combinations of nucleic acids encoding various domains from multiple different cell types.

[0718] In some embodiments, the cells are isolated from a biological sample from a patient or donor. In some embodiments, the sample include tissue, fluid, and other samples taken directly from the subject or donor, as well as samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (*e.g.,* transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including processed samples derived therefrom.

[0719] In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid

tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, *e.g.,* adoptive cell therapy, samples from autologous and allogeneic sources.

[0720] In some embodiments, the cells are derived from cell lines, *e.g.,* T cell lines. The cells in some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, non-human primate, dog, and pig.

b. Primary Cells

[0721] In some embodiments, the cell is a primary cell. Primary cells are isolated directly from human or animal tissue using enzymatic or mechanical methods. Once isolated, they are placed in an artificial environment in plastic or glass containers supported with specialized medium containing essential nutrients and growth factors to support proliferation. Primary cells could be of two types: adherent or suspension. Adherent cells require attachment for growth and are said to be anchorage-dependent cells. Adherent cells are usually derived from tissues of organs. Suspension cells do not require attachment for growth and are said to be anchorage independent cells. Most suspension cells are isolated from the blood system, but some tissue- derived cells can also be used in suspension, such as hepatocytes or intestinal cells. Although primary cells usually have a limited lifespan, they offer a number of advantages compared to cell lines. Primary cell culture enables researchers to study donors and not just cells. Several factors such as age, medical history, race, and sex can be considered when building an experimental model. With a growing trend towards personalized medicine, such donor variability and tissue complexity can be achieved with use of primary cells, but are difficult to replicate with cell lines that are more systematic and uniform in nature and do not capture the true diversity of a living tissue.

[0722] In some embodiments, the starting material is a primary cell collected from a subject (*e.g.,* a patient). In some embodiments, the starting material is a primary blood cell collected from a subject (*e.g.,* a patient), *e.g.,* via a leukopak. For example, in some embodiments, the starting material are unmodified T cells obtained from a subject (*e.g.,* a patient). In some embodiments, the starting material is a primary cell collected from a donor. In some embodiments, the starting material is a primary blood cell collected from a donor, *e.g.,* via a leukopak. For example, in some embodiments, the starting material are unmodified T cells obtained from a donor. In some embodiments, the starting material is otherwise modified or engineered to have altered expression of one or more genes.

[0723] In some embodiment the cells that are engineered as provided herein comprise cells derived from primary cells obtained or isolated from one or more individual subjects or donors. In some embodiments, the cells are derived from a pool of isolated primary cells obtained from a subject (*e.g.,* the patient administered the cells). In some embodiments, the cells are derived from a pool of isolated primary cells obtained from one or more (*e.g.,* two or more, three or more, four or more, five or more, ten or more, twenty or more, fifty or more, or one hundred or more) different donor subjects. In some embodiments, the primary cells isolated or obtained from the plurality of different donor subjects (*e.g.,* two or more, three or more, four or more, five or more, ten or more, twenty or more, fifty or more, or one hundred or more) are pooled together in a batch and are engineered in accord with the provided methods.

c. Pluripotent Stem Cells and Stem Cell Derived Cells

[0724] In some embodiments, the cell is a stem cell. In some embodiments, the cell is a pluripotent stem cell. In some embodiments, the cell is derived from a pluripotent stem cell. Pluripotent stem cells are cells that have the capacity to self-renew by dividing and to develop into the three primary germ cell layers of the early embryo and therefore into all cells of the adult body, but not extra-embryonic tissues such as the placenta. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are pluripotent stem cells. In some embodiments, the cell is an ESC. ESCs are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. In some embodiments, the cell is an iPSC. iPSCs are derived from adult somatic cells that have been genetically reprogrammed back into a pluripotent state that enables the development of an unlimited source of any type of cell needed for therapeutic purposes.

[0725] "Pluripotent stem cells" as used herein have the potential to differentiate into any of the three germ layers: endoderm (*e.g.,* the stomach lining, gastrointestinal tract, lungs, etc.), mesoderm (*e.g.,* muscle, bone, blood, urogenital tissue, etc.) or ectoderm (*e.g.,* epidermal tissues and nervous system tissues). The term "pluripotent stem cells," as used herein, also encompasses ESCs and iPSCs (or iPS cells). In some embodiments, a pluripotent stem cell is produced or generated from a cell that is not a pluripotent cell. In other words, pluripotent stem cells can be direct or indirect progeny of a non-pluripotent cell. Examples of parent cells include somatic cells that have been reprogrammed to induce a pluripotent, undifferentiated phenotype by various means. Such "iPS" or "iPSC" cells can be created by inducing the expression of certain regulatory genes or by the exogenous application of

certain proteins. Methods for the induction of iPS cells are known in the art and are further described below. (See, *e.g.,* Zhou et al., Stem Cells 27 (11): 2667-74 (2009); Huangfu et al., Nature Biotechnol. 26 (7): 795 (2008); Woltjen et al., Nature 458 (7239): 766-770 (2009); and Zhou et al., Cell Stem Cell 8:381-384 (2009); each of which is incorporated by reference herein in their entirety.) As used herein, "hiPSCs" are human induced pluripotent stem cells. In some embodiments, the cell is a mesenchymal stem cell (MSCs).

[0726] In some embodiments, the cell is a differentiated cell. In some embodiments, the cell is differentiated from a pluripotent stem cell. Differentiated cells are cells that have undergone differentiation. They are mature cells that perform a specialized function. Some examples of differentiated cells are T cells, NK cells, etc. Generally, these cells have a unique morphology, metabolic activity, membrane potential, and responsiveness to signals facilitating their function in a body tissue or organ.

[0727] In some embodiments, the engineered cells described are derived from an ESC or iPSC or a progeny thereof. As used herein, the term "derived from an ESC or iPSC or a progeny thereof" encompasses the initial ESC or iPSC that is generated and any subsequent progeny thereof. As used herein, the term "progeny" encompasses, *e.g.,* a first-generation progeny, i.e., the progeny is directly derived from, obtained from, obtainable from or derivable from the initial ESC or iPSC by, *e.g.,* traditional propagation methods. The term "progeny" also encompasses further generations such as second, third, fourth, fifth, sixth, seventh, or more generations, i.e., generations of cells which are derived from, obtained from, obtainable from or derivable from the former generation by, *e.g.,* traditional propagation methods. The term "progeny" also encompasses modified cells that result from the modification or alteration of the initial ESC or iPSC or a progeny thereof.

[0728] In embodiments, the cell or stem cell, or a cell derived from such a stem cell, has or is believed to have therapeutic value, such that the cell or stem cell or a cell derived or differentiated from such stem cell may be used to treat a disease, disorder, defect or injury in a subject in need of treatment for same.

[0729] In some embodiments, the cell is a stem cell or progenitor cell (*e.g.,* iPSC, ESC, hematopoietic stem cell, mesenchymal stem cell, endothelial stem cell, epithelial stem cell, adipose stem or progenitor cells, germline stem cells, lung stem or progenitor cells, mammary stem cells, olfactory adult stem cells, hair follicle stem cells, multipotent stem cells, amniotic stem cells, cord blood stem cells, or neural stem or progenitor cells). In some embodiments, the stem cells are adult stem cells (*e.g.,* somatic stem cells or tissue specific

stem cells). In some embodiments, the stem or progenitor cell is capable of being differentiated (*e.g.,* the stem cell is totipotent, pluripotent, or multipotent). In some embodiments, the cell is isolated from embryonic or neonatal tissue. In some embodiments, the cell is a fibroblast, monocytic precursor, B cell, exocrine cell, pancreatic progenitor, endocrine progenitor, hepatoblast, myoblast, preadipocyte, progenitor cell, hepatocyte, chondrocyte, smooth muscle cell, K562 human erythroid leukemia cell line, bone cell, synovial cell, tendon cell, ligament cell, meniscus cell, adipose cell, dendritic cells, or natural killer cell. In some embodiments, the cell is manipulated (*e.g.,* converted or differentiated) into a muscle cell, erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell, endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or brown adipocyte. In some embodiments, the cell is a muscle cell (*e.g.,* skeletal, smooth, or cardiac muscle cell), erythroid-megakaryocytic cell, eosinophil, iPS cell, macrophage, T cell, islet beta-cell, neuron, cardiomyocyte, blood cell (*e.g.,* red blood cell, white blood cell, or platelet), endocrine progenitor, exocrine progenitor, ductal cell, acinar cell, alpha cell, beta cell, delta cell, PP cell, hepatocyte, cholangiocyte, or white or brown adipocyte. In some embodiments, the cell is a hormone-secreting cell (*e.g.,* a cell that secretes insulin, oxytocin, endorphin, vasopressin, serotonin, somatostatin, gastrin, secretin, glucagon, thyroid hormone, bombesin, cholecystokinin, testosterone, estrogen, or progesterone, renin, ghrelin, amylin, or pancreatic polypeptide), an epidermal keratinocyte, an epithelial cell (*e.g.,* an exocrine secretory epithelial cell, a thyroid epithelial cell, a keratinizing epithelial cell, a gall bladder epithelial cell, or a surface epithelial cell of the cornea, tongue, oral cavity, esophagus, anal canal, distal urethra, or vagina), a kidney cell, a germ cell, a skeletal joint synovium cell, a periosteum cell, a bone cell (*e.g.,* osteoclast or osteoblast), a perichondrium cell (*e.g.,* a chondroblast or chondrocyte), a cartilage cell (*e.g.,* chondrocyte), a fibroblast, an endothelial cell, a pericardium cell, a meningeal cell, a keratinocyte precursor cell, a keratinocyte stem cell, a pericyte, a glial cell, an ependymal cell, a cell isolated from an amniotic or placental membrane, or a serosal cell (*e.g.,* a serosal cell lining body cavities).

[0730] In some embodiments, the cell is a T cell or NK cell that has been differentiated from an engineered ESC or iPSC. In some embodiments, the cell is an engineered cell that has been modified from a primary cell. In some embodiments, the cell comprises increased expression of the polypeptide of the disclosure .

[0731] As will be appreciated by those in the art, there are a variety of different methods for the generation of ESCs and iPCSs. The original induction of iPSCs was done from mouse embryonic or adult fibroblasts using the viral introduction of four transcription factors, Oct3/4, Sox2, c-Myc and Klf4; see Takahashi and Yamanaka Cell 126:663-676 (2006), hereby incorporated by reference in its entirety and specifically for the techniques outlined therein. Since then, a number of methods have been developed; see Seki et al, World J. Stem Cells 7(1): 116-125 (2015) for a review, and Lakshmipathy and Vermuri, editors, Methods in Molecular Biology: Pluripotent Stem Cells, Methods and Protocols, Springer 2013, both of which are hereby expressly incorporated by reference in their entirety, and in particular for the methods for generating hiPSCs (see for example Chapter 3 of the latter reference).

[0732] Generally, iPSCs are generated by the transient expression of one or more reprogramming factors in the host cell, usually introduced using episomal vectors. Under these conditions, small amounts of the cells are induced to become iPSCs (in general, the efficiency of this step is low, as no selection markers are used). Once the cells are "reprogrammed", and become pluripotent, they lose the episomal vector(s) and produce the factors using the endogeneous genes.

[0733] Any of the pluripotent stem cells described herein can be differentiated into any cells of an organism and tissue. In an aspect, provided herein are engineered cells that are differentiated into different cell types from ESCs or iPSCs for subsequent transplantation into recipient subjects. Differentiation can be assayed as is known in the art, generally by evaluating the presence of cell-specific markers. As will be appreciated by those in the art, the differentiated engineered pluripotent cell derivatives can be transplanted using techniques known in the art that depends on both the cell type and the ultimate use of these cells. In some embodiments, the iPSCs are differentiated into cell types selected from T cells, NK cells.

d. T Cells

[0734] In some embodiments, the cell is a T cell. In some embodiments, the T cell comprises a polynucleotide encoding the polypeptide of the disclosure. T cells of the disclosure or precursors thereof can be immune cells of the lymphoid lineage. Relevant information concerning T cells as referred to in the context of the present disclosure is known in the art, including certain information regarding desired features of T cells when used for cell therapy. It will be understood that embodiments concerning T cells described

herein may be readily and appropriately combined. It will be understood that embodiments concerning T cells described herein may be readily and appropriately combined with other embodiments described herein (for example, but not limited to, the polypeptide of the disclosure).

[0735] The T cells described herein may be used to treat or prevent a disease in a subject.

[0736] A T cell is a type of lymphocyte. T cells are one of the white blood cells of the immune system and play a central role in the adaptive immune response. T cells can be derived from T cells in a patient's own blood (autologous) or derived from the T cells of another healthy donor (allogeneic). 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, antibody-conjugated bead-based methods such as MACS™ separation (Miltenyi).

[0737] In some embodiments, the T cells can be CD4+ or CD8+ and can include, but are not limited to, regulatory T cells, cytotoxic T cells, T helper cells (CD4+), and memory T cells, including central memory T cells (TCM), stem memory T cells (TSCM), stem-cell-like memory T cells (or stem-like memory T cells), and effector memory T cells such as, for example, TEM cells and TEMRA (CD45RA+) cells, effector T cells, Th1 cells, Th2 cells, Th9 cells, Th17 cells, Th22 cells, Tfh (follicular helper) cells, T regulatory cells, natural killer T cells, mucosal associated invariant T cells (MAIT), and γδ T cells. Major T cell subtypes include TSCM (stem cell memory), TCM (central memory), TTM (Transitional Memory), TEM (Effector memory), TTE (Terminal Effector) and TN (naive). In some embodiments, the T cells express CCR7, CD27, CD28, and CD45RA. In some embodiments, the central T cells express CCR7, CD27, CD28, and CD45RO. In other embodiments, the effector memory T cells express PD-1, CD27, CD28, and CD45RO. In other embodiments, the effector memory RA T cells express PD-1, CD57, and CD45RA.

[0738] In some embodiments, the T cells of the disclosure can be hematopoietic stem and/or progenitor cells of the lymphoid lineage that can differentiate into T cells. Hematopoietic stem and/or progenitor cells can be derived from bone marrow, umbilical cord blood, and adult peripheral blood.

[0739] In some embodiments, the T cells as provided herein are primary T cells. In some embodiments, the primary T cells are isolated or obtained from and individual subject or one or more donors, such as one or more healthy donors (*e.g.,* a subject that is not known or suspected of, *e.g.,* not exhibiting clinical signs of, a disease or infection). In some instances, the T cells are populations of primary T cells. In some instances, the T cells are subpopulations or sub-types or subsets of primary T cells. Subpopulations and sub-types and subsets of primary T cells are herein described in further detail below. As will be appreciated by those in the art, methods of isolating or obtaining T lymphocytes from an individual can be achieved using known techniques. Provided herein are engineered primary T lymphocytes that contain modifications (*e.g.,* genetic modifications) described herein for subsequent transplantation or engraftment into subjects (*e.g.,* recipients).

[0740] In some embodiments, primary T cells are obtained (*e.g.,* harvested, extracted, removed, or taken) from a subject or an individual. In some embodiments, primary T cells are produced from a pool of T cells such that the T cells are from one or more subjects (*e.g.,* one or more human including one or more healthy humans). In some embodiments, the pool of primary T cells is from 1-100, 1-50, 1-20, 1-10, 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, or 100 or more subjects. In some embodiments, the donor subject is different from the patient (*e.g.,* the recipient that is administered the therapeutic cells). In some embodiments, the pool of T cells does not include cells from the patient. In some embodiments, one or more of the donor subjects from which the pool of T cells is obtained are different from the patient.

[0741] In some embodiments, primary T cells are primary T cells of a T cell subset or a sub-type or subpopulation. Such T cell subsets may be found in US2018/0319862A1 and/or WO20 16/090190A1, the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, the T cell subset, such as a CD62L+ T cell subset, that is increased in subjects upon administration of the genetically engineered cells are or include or share phenotypic characteristics with memory T cells or particular subsets thereof, such as long-lived memory T cells. In some embodiments, such memory T cells are central memory T cells (Tcm) or T memory stem cells (Tsc) cells. In some embodiments, the memory T cells are Tsc cells. Tsc cells may be described as having one or more phenotypic differences or functional features compared to other memory T cell subsets or compared to naive T cells, such as being less differentiated or more naive (see *e.g.,* Ahlers and Belyakov (2010) Blood, 115: 1678); Cieri et al (2015) Blood, 125:2865; Flynn et al. (2014) Clinical & Translational

Immunology, 3, e20; Gattinoni et al. (2012) Nat. Med., 17: 1290-1297; Gattinoni et al. (2012) Nat .Reviews, 12:671; Li et al. (2013) PLOS ONE, 8:e67401; and published PCT Appl. No. W02014/039044). In some cases, Tsc cells are thought to be the only memory T cells able to generate effector T cells and all three subsets of memory T cells (Tsc, Tcm, and Tern). In some aspects, Tsc cells have the highest survival and proliferation response to antigenic or homeostatic stimuli of all the memory T cell subsets, and the least attrition absent cognate antigen. In some embodiments, the less - differentiated Tsc cells may exhibit greater expansion, long-term viability, and target cell destruction following adoptive transfer than other memory T cells, and thus may be able to mediate more effective treatment with fewer transferred cells than would be possible for either Tcm or Tern cells.

[0742] In some embodiments, the cells as provided herein are T lymphocytes differentiated from engineered pluripotent cells that contain modifications (*e.g.,* genetic modifications) described herein and that are differentiated into T lymphocyte. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. In some embodiments, the cells differentiated into a T lymphocyte may be used for subsequent transplantation or engraftment into subjects (*e.g.,* recipients).

[0743] Methods for generating T cells from pluripotent stem cells (*e.g.,* iPSC) are described, for example, in Iriguchi et al., Nature Communications 12, 430 (2021); Themeli et al. 16(4):357-366 (2015); Themeli et al., Nature Biotechnology 31 :928-933 (2013). In some embodiments, the cell is an iPSC-derived T cell that is engineered to contain modifications (*e.g.,* genetic modifications) described herein. In some embodiments, the cell is a primary T cell that is engineered to contain modifications (*e.g.,* genetic modifications) described herein. In some embodiments, the cell comprises overexpression or increased expression of one or more complement inhibitor. In some embodiments, the T cell can be engineered with a chimeric antigen receptor (CAR), including any as described herein. In some embodiments, the engineered T cell can be used to treat a variety of indications with allogenic cell therapy, including any as described herein. In some embodiments, the engineered T cell can be used to treat cancer.

[0744] Cells disclosed herein may be T cells, for example a population of T cells. It will be understood that any reference to “a cell” e.g. “a T cell” below also applies to “a population of cells” e.g. “a population of T cells” as described in the present application.

e. NK Cells

[0745] In some embodiments, the cell is a natural killer (NK) cell. In some embodiments, the NK cell comprises a polynucleotide encoding the polypeptide of the disclosure. Relevant information concerning NK cells as referred to in the context of the present disclosure is known in the art, including certain information regarding desired features of NK cells when used for cell therapy and for example may be found from WO2017214569A1, WO 2011/068896A1, the contents of which are herein incorporated by reference. It will be understood that embodiments concerning NK cells described herein may be readily and appropriately combined with other embodiments described herein (for example, but not limited to, the polypeptide of the disclosure).

[0746] The NK cells described herein may be used to treat or prevent a disease in a subject.

[0747] NK cells, which arise through the lymphoid lineage and are part of the innate immune system, may be used in anti-cancer therapy as they have been found to detect and kill certain types of tumor cells. Natural killer (NK) cells are cytotoxic lymphocytes capable of human immune surveillance. WO2017214569A1 (the contents of which are incorporated herein by reference in their entirety) describes genome-edited primary NK cells, methods of making those cells, and methods of administering those cells.

[0748] In some embodiments, the cells that are engineered or modified as provided herein are Natural Killer (NK) cells. In some embodiments, the NK cells are isolated or obtained from an individual subject or one or more donors, such as one or more healthy donor (*e.g.,* a donor that is not known or suspected of, *e.g.,* not exhibiting clinical signs of, a disease or infection). As will be appreciated by those in the art, methods of isolating or obtaining NK cells from an individual can be achieved using known techniques. Provided herein are engineered primary NK cells that contain modifications (*e.g.,* genetic modifications) described herein for subsequent transplantation or engraftment into subjects (*e.g.,* recipients. For instance, the engineered NK cells are administered to a subject (*e.g.,* recipient, such as a patient), by infusion of the engineered NK cells into the subject.

[0749] In some embodiments, the NK cells are primary NK cells. In some embodiments, NK cells, including primary NK cells isolated from an individual subject or one or more donors (*e.g.,* healthy donors) or NK cells differentiated from iPSCs derived from one or more donors (*e.g.,* healthy donors) express CD56 (*e.g.,* CDSb^111 or CD56bright) and lack CD3 (*e.g.,* CD3neg). In some embodiments, NK cells as described herein may also express

the low-affinity Fey receptor CD16, which, without wishing to be bound by theory, mediate ADCC. In some embodiments, the NK cells also express one or more natural killer cell receptors NKG2A and NKG2D or one or more natural cytotoxicity receptors NKp46, NKp44, NKp30. For example, for the case of primary NK cells, in specific cases, the primary cells may be isolated from a starting source of NK cells, such as a sample containing peripheral blood mononuclear cells (PBMCs), by depletion of cells positive for CD3, CD14, and/or CD19. For instance, the cells may be subject to depletion using immunomagnetic beads having attached thereto antibodies to CD3, CD 14, and/or CD19, respectively), thereby producing an enriched population of NK cells. In other cases, primary NK cells may be isolated from a starting source that is a mixed population (*e.g.,* PBMCs) by selecting cells for the presence of one or more markers on the NK cells, such as CD56, CD16, NKp46, and/or NKG2D.

[0750] In some embodiments, the NK cells can be immature NK cells and can be CD56+ and CD16-. In some embodiments, the NK cells can be mature NK cells and can be CD56- and CD16+, or CD561o and CD16+ (as described in WO 2011/068896A1 (the contents of which are incorporated herein by reference in their entirety)).

[0751] A primary NK cell may express CD16 and/or CD56. In some embodiments, an NK cell does not express CD3. In some embodiments, an "NK cell" is preferably defined as a cell that is CD56+ and CD3-. In some embodiments, an "NK cell" is defined as a cell that is CD16+ and CD3-. NK cells are lymphocytes of the innate immune system that kill virally infected or transformed cells. Like T cells, NK cells are cytotoxic lymphocytes. Unlike T cells, NK cells do not require antigen recognition, and require integration of signals from many activating and inhibitory receptors to perform their function. Despite their similarities to T cells, NK cells behave differently under stimulation conditions and do not tolerate electroporation in the same way as T cells (as described in WO2017214569A1 (the contents of which are incorporated herein by reference in their entirety)).

[0752] A primary NK cell may be isolated from, for example, peripheral blood, umbilical cord cells, ascites, and/or a solid tumor (as described in WO2017214569A1, the contents of which are incorporated herein by reference in their entirety). In some embodiments, a "primary NK cell" is an NK cell that is freshly isolated. In some embodiments, a "primary NK cell" is an NK cell that has undergone up to 5 replications or divisions after being isolated, up to 10 replications or divisions after being isolated, up to 15 replications or divisions after being isolated, up to 20 replications or divisions after being isolated, up to 25

replications or divisions after being isolated, up to 30 replications or divisions after being isolated, up to 35 replications or divisions after being isolated, or up to 40 replications or divisions after being isolated. In some embodiments, the primary NK cell is a non-clonal cell. In some embodiments, primary NK cell is a proliferating cell. In some embodiments, primary NK cell is an expanded cell.

[0753] Some desired features of NK cells when used for cell therapy are described herein. NK cells may be distinguished using a number of assays, for example as set out in WO 2011/068896A1 (the contents of which is hereby incorporated by reference in its entirety). For example, IL12-p70 secretion may be measured: IL12p70 is secreted by mature DCs in order to elicit a Thl-directed response from CD4+ T cells. Human BM-derived DCs can produce >500pg/ml of IL12p70 yet blast-derived DCs did not produce any detectable IL12p70 upon maturation. Mixed lymphocyte reaction (MLR) assay: The MLR assay determines the ability of DCs to stimulate proliferation of allogenic T cells. Cord blood mononuclear cells (CBMCs, which include T cells) were used as responders, fluorescently labeled, and their proliferation was measured after 4-5 days coculture with immature or mature blast- derived DCs. Preliminary results show that the responder cells proliferate in response to mature (m)DCs. Hemangioblast-derived natural killer cells: Cell surface markers CD45, CD7, CD94, CD56, CD16, and NKG2D were evaluated in blast-derived and human bone marrow-derived NK cells.

[0754] Methods of generating natural killer (NK) cells are known in the art, for example methods described in WO 2011/068896A1 (the contents of which are incorporated by reference herein in their entirety) comprising: providing hemangioblasts; culturing the hemangioblasts on methylcellulose and a first cytokine mixture comprising IL2, IL3, IL6, IL7, IL15, SCF and FL; harvesting the cultured cells; and culturing the harvested cells in liquid media comprising human serum, and a second cytokine mixture comprising IL7, IL15, SCF and FL to generate NK cells.

[0755] In some embodiments, the cells as provided herein are NK cells differentiated from engineered pluripotent cells that contain modifications (*e.g.,* genetic modifications) described herein and that are differentiated into NK cells. As will be appreciated by those in the art, the methods for differentiation depend on the desired cell type using known techniques. In some embodiments, the cells differentiated into NK cells may be used for subsequent administration to a subject (*e.g.,* recipient, such as a patient), such as by infusion of the differentiated NK cells into the subject. Methods for generating NK cells from

pluripotent stem cells (*e.g.,* iPSC) are described, for example, in U.S. Patent No. 10626373; Shankar et al. Stem Cell Res Ther. 2020; 11 : 234; Euchner et al. Frontiers in Immunology, 2021; 12, Article 640672. doi=10.3389/fimmu.2021.640672;. Methods of differentiating NK cells are known in the art, for example as described in WO 2011/068896A1 (the contents of which are hereby incorporated by reference in their entirety). An exemplary method as described in WO 2011/068896A1. In one embodiment, the initial differentiation procedure for both cell types may be the same and may involve a 4 day culture of hESCs in Stemline II (Sigma) plus cytokines in order to generate embryoid bodies (EBs). The cytokines, VEGF and BMP4 may be used throughout the EB culture while bFGF may be added after the first 2 days. After 4 days total, the resulting EBs may be disaggregated with 0.05% trypsin and then the trypsin may be inactivated with serum- containing media. Individual cells may be subsequently filtered through a 40 pM cell strainer, counted, and seeded into H4436 or H4536 methylcellulose (Stem Cell Technologies) containing additional cytokines, such as TPO (50pg/ml), VEGF(50pg/ml), FL (50pg/ml) and bFGF (20-50pg/ml). For NK differentiation, the cytokines IL2 (l-10pg/ml), IL7 (l-20pg/ml), and/or IL15 (l-10pg/ml) may also be added at this stage. Methylcellulose cultures may be plated at a concentration of 50,000 to 150,000 cells per ml for the production and expansion of a hemangioblastic population. Blast-like cells may be harvested from methylcellulose between day 6 and 10 and further differentiated by one of the following procedures.

[0756] Cells disclosed herein may be natural killer (NK) cells, for example a population of NK cells. It will be understood that any reference to "a cell" e.g. "a NK cell" below also applies to "a population of cells" e.g. "a population of NK cells" as described in the present application.

XI. METHODS OF GENOME MODIFICATION

[0757] In some embodiments, a recombinant nucleic acid of the disclosure may be inserted into the genome of a cell. The insertion may be non-targeted (i.e., insertion into a random genomic locus of the cell) or targeted (i.e., insertion into a specific genomic locus of the cell), using any of the non-targeted or site-directed (targeted) insertion methods described herein.

f. Non-Targeted Insertion

[0758] In some embodiments, a recombinant nucleic acid of the disclosure can be inserted into a random genomic locus of a host cell. As known to a person skilled in the art, viral vectors, including, for example, retroviral vectors, lentiviral vectors, adenoviral vectors, and adeno-associated viral vectors, are commonly used to deliver genetic material into host cells and randomly insert the foreign or exogenous nucleic acid into the genome of the cell to facilitate stable expression and replication of the foreign or exogenous nucleic acid.

g. Homology-Directed Repair (HDR)

[0759] In some embodiments, homology-directed repair (HDR) can be utilized for targeted integration or insertion of one or more recombinant nucleic acids of the disclosure, *e.g.,* transgene sequences, at one or more target site(s) in the genome. In some embodiments, the nuclease-induced HDR can be used to alter a target sequence, integrate a transgene at a particular target location, and/or to edit or repair a mutation in a particular target gene.

[0760] Alteration of nucleic acid sequences at the target site can occur by HDR with an exogenously provided polynucleotide (also referred to as donor polynucleotide or template sequence). For example, the template polynucleotide provides for alteration of the target sequence, such as insertion of the transgene contained within the template polynucleotide. In some embodiments, a plasmid or a vector can be used as a template for homologous recombination. In some embodiments, a linear DNA fragment can be used as a template for homologous recombination. In some embodiments, a single stranded template polynucleotide can be used as a template for alteration of the target sequence by alternate methods of homology directed repair (*e.g.,* single strand annealing) between the target sequence and the template polynucleotide. Template polynucleotide-effected alteration of a target sequence depends on cleavage by a nuclease, *e.g.,* a targeted nuclease such as CRISPR/Cas9. Cleavage by the nuclease can comprise a double strand break or two single strand breaks.

[0761] In some embodiments, a template polynucleotide comprises the following components: [5’ homology arm] -[transgene] -[3’ homology arm]. The homology arms provide for recombination into the chromosome, thus insertion of the transgene into the DNA at or near the cleavage site, *e.g.,* target site(s). In some embodiments, the homology arms flank the most distal target site(s).

[0762] In some embodiments, "recombination" refers to a process of exchange of genetic information between two polynucleotides. In some embodiments, "homologous recombination (HR)" refers to the specialized form of such exchange that takes place, for example, during repair of double-strand breaks in cells via homology-directed repair mechanisms. This process requires nucleotide sequence homology, uses a template polynucleotide to template repair of a target DNA (i.e., the one that experienced the double-strand break, *e.g.,* target site in the endogenous gene), and is variously known as "non-crossover gene conversion" or "short tract gene conversion," because it leads to the transfer of genetic information from the template polynucleotide to the target. In some embodiments, such transfer can involve mismatch correction of heteroduplex DNA that forms between the broken target and the template polynucleotide, and/or "synthesis-dependent strand annealing," in which the template polynucleotide is used to resynthesize genetic information that will become part of the target, and/or related processes. Such specialized HR often results in an alteration of the sequence of the target molecule such that part or all of the sequence of the template polynucleotide is incorporated into the target polynucleotide. As described herein, the genetic disruption of the target site or target position can be created by any mechanisms, such as ZFNs, TALENs, CRISPR/Cas9 system, or TtAgo nucleases.

[0763] In some embodiments, double strand cleavage is affected by a nuclease, *e.g.,* a Cas9 molecule having cleavage activity associated with an HNH-like domain and cleavage activity associated with a RuvC-like domain, *e.g.,* an N-terminal RuvC-like domain, *e.g.,* a wild type Cas9.

[0764] In some embodiments, DNA repair mechanisms can be induced by a nuclease after (1) a single double-strand break, (2) two single strand breaks, (3) two double stranded breaks with a break occurring on each side of the target site, (4) one double stranded break and two single strand breaks with the double strand break and two single strand breaks occurring on each side of the target site (5) four single stranded breaks with a pair of single stranded breaks occurring on each side of the target site, or (6) one single stranded break. In some embodiments, a single-stranded template polynucleotide is used and the target site can be altered by alternative HDR.

[0765] Template polynucleotide-effected alteration of a target site depends on cleavage by a nuclease molecule. Cleavage by the nuclease can comprise a nick, a double strand break, or two single strand breaks, *e.g.,* one on each strand of the DNA at the target site. After

introduction of the breaks on the target site, resection occurs at the break ends resulting in single stranded overhanging DNA regions.

[0766] In canonical HDR, a double-stranded template polynucleotide is introduced, comprising homologous sequence to the target site that will either be directly incorporated into the target site or used as a template to insert the transgene or correct the sequence of the target site. After resection at the break, repair can progress by different pathways, *e.g.,* by the double Holliday junction model (or double strand break repair, DSBR, pathway) or the synthesisdependent strand annealing (SDSA) pathway.

[0767] In some embodiments, other DNA repair pathways such as single strand annealing (SSA), single- stranded break repair (SSBR), mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), intrastrand cross-link (ICL), translesion synthesis (TLS), error-free postreplication repair (PRR) can be employed by the cell to repair a doublestranded or single-stranded break created by the nucleases.

h. Site-Directed Insertion

[0768] In some embodiments, the methods for genetically modifying cells to knock out, knock down, or otherwise modify one or more genes comprise using a site-directed nuclease, including, for example, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), meganucleases, transposases, and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas systems, as well as nickase systems, base editing systems, prime editing systems, and any other gene editing systems known in the art.

i. ZFN

[0769] ZFNs are fusion proteins comprising an array of site-specific DNA binding domains adapted from zinc finger-containing transcription factors attached to the endonuclease domain of the bacterial FokI restriction enzyme. A ZFN may have one or more (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) DNA binding domains or zinc finger domains. See, *e.g.,* Carroll et al., Genetics Society of America (2011) 188:773-782; Kim et al., Proc. Natl. Acad. Sci. USA (1996) 93: 1156-1160. The individual DNA binding domains are typically referred to as "fingers" or "zinc fingers." Each zinc finger domain is a small protein structural motif stabilized by one or more zinc ions and usually recognizes a 3- to 4-bp DNA sequence. Tandem domains can thus potentially bind to an extended nucleotide sequence

that is unique within a cell's genome. Each finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A DNA binding domain binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid, zinc-chelating, DNA-binding subdomain. Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues coordinated with zinc along with the two cysteine residues of a single beta turn (see, *e.g.,* Berg & Shi, Science 271 : 1081-1085 (1996)).

[0770] Various zinc fingers of known specificity can be combined to produce multifinger polypeptides which recognize about 6, 9, 12, 15, or 18-bp sequences. Various selection and modular assembly techniques are available to generate zinc fingers (and combinations thereof) recognizing specific sequences, including phage display, yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells. Zinc fingers can be engineered to bind a predetermined nucleic acid sequence. Criteria to engineer a zinc finger to bind to a predetermined nucleic acid sequence are known in the art. See, *e.g.,* Sera et al., Biochemistry (2002) 41 :7074-7081; Liu et al., Bioinformatics (2008) 24: 1850-1857.

[0771] ZFNs containing FokI nuclease domains or other dimeric nuclease domains function as a dimer. Thus, a pair of ZFNs are required to target non-palindromic DNA sites. The two individual ZFNs must bind opposite strands of the DNA with their nucleases properly spaced apart. See Bitinaite < / al., Proc. Natl. Acad. Sci. USA (1998) 95: 10570-10575. To cleave a specific site in the genome, a pair of ZFNs are designed to recognize two sequences flanking the site, one on the forward strand and the other on the reverse strand. Upon binding of the ZFNs on either side of the site, the nuclease domains dimerize and cleave the DNA at the site, generating a DSB with 5' overhangs. HDR can then be utilized to introduce a specific mutation, with the help of a repair template containing the desired mutation flanked by homology arms. The repair template is usually an exogenous double-stranded DNA vector introduced to the cell. See Miller et al., Nat. Biotechnol. (2011) 29: 143-148; Hockemeyer et al., Nat. Biotechnol. (2011) 29:731-734.

ii. TALENS

[0772] TALENs are another example of an artificial nuclease which can be used to edit a target gene. A "TALE-nuclease" (TALEN) is a fusion protein consisting of a nucleic acidbinding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic

domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, for instance I-TevI, ColE7, NucA and Fok-I. In numerous embodiments, the TALE domain can be fused to a meganuclease, for instance LCrel and I-Onul or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE- Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927.

[0773] Transcription Activator like Effector (TALE) are proteins from the bacterial species Xanthomonas and comprise a plurality of repeated sequences. Each repeat is 33 to 35 amino acids in length, with two adjacent amino acids (termed the repeat-variable di-residue, or RVD) in position 12 and 13 that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALEN kits are sold commercially.

[0774] TALENs are produced artificially by fusing one or more TALE DNA binding domains (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) to a nuclease domain, for example, a FokI endonuclease domain. See Zhang, Nature Biotech. (2011) 29: 149-153. Several mutations to FokI have been made for its use in TALENs; these, for example, improve cleavage specificity or activity. See Cermak et al., Nucl. Acids Res. (2011) 39:e82; Miller et al., Nature Biotech. (2011) 29: 143-148; Hockemeyer et al., Nature Biotech. (2011) 29:731-734; Wood et al., Science (2011) 333 :307; Doyon et al., Nature Methods (2010) 8:74-79; Szczepek et al., Nature Biotech (2007) 25:786-793; Guo et al., J. Mol. Biol. (2010) 200:96. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding

domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI nuclease domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al., Nature Biotech. (2011) 29: 143-148.

[0775] By combining engineered TALE repeats with a nuclease domain, a site-specific nuclease can be produced specific to any desired DNA sequence. Similar to ZFNs, TALENs can be introduced into a cell to generate DSBs at a desired target site in the genome, and so can be used to knock out genes or knock in mutations in similar, HDR-mediated pathways. See Boch, Nature Biotech. (2011) 29: 135-136; Boch et al., Science (2009) 326: 1509-1512; Moscou et al., Science (2009) 326:3501.

iii. Meganucleases

[0776] Meganucleases are sequence-specific enzymes in the endonuclease family which are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs). (Chevalier, B. S. and B. L. Stoddard, Nucleic Acids Res., 2001, 29, 3757-3774). They can cleave unique sites in living cells, thereby enhancing gene targeting by 1000-fold or more in the vicinity of the cleavage site (Puchta et al., Nucleic Acids Res., 1993, 21, 5034-5040; Rouet et al., Mol. Cell. Biol., 1994, 14, 8096-8106; Choulika et al., Mol. Cell. Biol., 1995, 15, 1968-1973; Puchta et al., Proc. Natl. Acad. Sci. USA, 1996, 93, 5055-5060; Sargent et al., Mol. Cell. Biol., 1997, 17, 267-77; Donoho et al., Mol. Cell. Biol, 1998, 18, 4070-4078; Elliott et al., Mol. Cell. Biol., 1998, 18, 93-101; Cohen-Tannoudji et al., Mol. Cell. Biol., 1998, 18, 1444- 1448).

[0777] Meganucleases are grouped into families based on their structural motifs which affect nuclease activity and/or DNA recognition. The most widespread and best known meganucleases are the proteins in the LAGLID ADG family, which owe their name to a conserved amino acid sequence. See Chevalier et aL, Nucleic Acids Res. (2001) 29(18): 3757- 3774. On the other hand, the GIY-YIG family members have a GIY-YIG module, which is 70- 100 residues long and includes four or five conserved sequence motifs with four invariant residues, two of which are required for activity. See Van Roey et al., Nature Struct. Biol. (2002) 9:806-811. The His-Cys family meganucleases are characterized by a highly conserved series of histidines and cysteines over a region encompassing several hundred amino acid residues. See Chevalier et al., Nucleic Acids Res. (2001) 29(18):3757-

3774. Members of the NHN family are defined by motifs containing two pairs of conserved histidines surrounded by asparagine residues. See Chevalier et al, Nucleic Acids Res. (2001) 29(18):3757-3774.

[0778] Because the chance of identifying a natural meganuclease for a particular target DNA sequence is low due to the high specificity requirement, various methods including mutagenesis and high throughput screening methods have been used to create meganuclease variants that recognize unique sequences. Strategies for engineering a meganuclease with altered DNA-binding specificity, *e.g.,* to bind to a predetermined nucleic acid sequence are known in the art. See, *e.g.,* Chevalier et al., Mol. Cell. (2002) 10:895-905; Epinat et aL, Nucleic Acids Res (2003) 31 :2952-2962; Silva et al., J Mol. Biol. (2006) 361 :744-754; Seligman et al., Nucleic Acids Res (2002) 30:3870-3879; Sussman et aL, J Mol Biol (2004) 342:31-41; Doyon et aL, J Am Chem Soc (2006) 128:2477-2484; Chen et al, Protein Eng Des Sei (2009) 22:249-256; ArnovAd et aL, JMol Biol. (2006) 355:443-458; Smith et aL, Nucleic Acids Res. (2006) 363(2):283-294.

[0779] Like ZFNs and TALENs, Meganucleases can create DSBs in the genomic DNA, which can create a frame-shift mutation if improperly repaired, *e.g.,* via NHEJ, leading to a decrease in the expression of a target gene in a cell. Alternatively, foreign DNA can be introduced into the cell along with the meganuclease. Depending on the sequences of the foreign DNA and chromosomal sequence, this process can be used to modify the target gene. See Silva et al., Current Gene Therapy (2011) 11 : 11-27.

iv. Transposases

[0780] Transposases are enzymes that bind to the end of a transposon and catalyze its movement to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. By linking transposases to other systems such as the CRISPER/Cas system, new gene editing tools can be developed to enable site specific insertions or manipulations of the genomic DNA. There are two known DNA integration methods using transposons which use a catalytically inactive Cas effector protein and Tn7-like transposons. The transposase-dependent DNA integration does not provoke DSBs in the genome, which may guarantee safer and more specific DNA integration.

v. CRISPR/Cas

[0781] The CRISPR system was originally discovered in prokaryotic organisms (*e.g.*, bacteria and archaea) as a system involved in defense against invading phages and plasmids that provides a form of acquired immunity. Now it has been adapted and used as a popular gene editing tool in research and clinical applications.

[0782] CRISPR/Cas systems generally comprise at least two components: one or more guide RNAs (gRNAs) and a Cas protein. The Cas protein is a nuclease that introduces a DSB into the target site. CRISPR-Cas systems fall into two major classes: class 1 systems use a complex of multiple Cas proteins to degrade nucleic acids; class 2 systems use a single large Cas protein for the same purpose. Class 1 is divided into types I, III, and IV; class 2 is divided into types II, V, and VI. Different Cas proteins adapted for gene editing applications include, but are not limited to, Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, and LEG14. The most widely used Cas9 is described herein as illustrative. These Cas proteins may be originated from different source species. For example, Cas9 can be derived from S. pyogenes or S. aureus.

[0783] In the original microbial genome, the type II CRISPR system incorporates sequences from invading DNA between CRISPR repeat sequences encoded as arrays within the host genome. Transcripts from the CRISPR repeat arrays are processed into CRISPR RNAs (crRNAs) each harboring a variable sequence transcribed from the invading DNA, known as the "protospacer" sequence, as well as part of the CRISPR repeat. Each crRNA hybridizes with a second transactivating CRISPR RNA (tracrRNA), and these two RNAs form a complex with the Cas9 nuclease. The protospacer-encoded portion of the crRNA directs the Cas9 complex to cleave complementary target DNA sequences, provided that they are adjacent to short sequences known as "protospacer adjacent motifs" (PAMs).

[0784] Since its discovery, the CRISPR system has been adapted for inducing sequence specific DSBs and targeted genome editing in a wide range of cells and organisms spanning from bacteria to eukaryotic cells including human cells. In its use in gene editing applications, artificially designed, synthetic gRNAs have replaced the original crRNA:tracrRNA complex. For example, the gRNAs can be single guide RNAs (sgRNAs) composed of a crRNA, a tetraloop, and a tracrRNA. The crRNA usually comprises a complementary region (also called a spacer, usually about 20 nucleotides in length) that is user-designed to recognize a target DNA of interest. The tracrRNA sequence comprises a scaffold region for Cas nuclease binding. The crRNA sequence and the tracrRNA sequence are linked by the tetraloop and each have a short repeat sequence for hybridization with each other, thus generating a chimeric sgRNA. One can change the genomic target of the Cas nuclease by simply changing the spacer or complementary region sequence present in the gRNA. The complementary region will direct the Cas nuclease to the target DNA site through standard RNA-DNA complementary base pairing rules.

[0785] In order for the Cas nuclease to function, there must be a PAM immediately downstream of the target sequence in the genomic DNA. Recognition of the PAM by the Cas protein is thought to destabilize the adjacent genomic sequence, allowing interrogation of the sequence by the gRNA and resulting in gRNA-DNA pairing when a matching sequence is present. The specific sequence of PAM varies depending on the species of the Cas gene. For example, the most commonly used Cas9 nuclease derived from S. pyogenes recognizes a PAM sequence of 5'-NGG-3' or, at less efficient rates, 5 '-NAG-3', where "N" can be any nucleotide. Other Cas nuclease variants with alternative PAMs have also been characterized and successfully used for genome editing, which are summarized in **Table 16** below.

Table 16. Exemplary Cas nuclease variants and their PAM sequences

CRISPR Nuclease	Source Organism	PAM Sequence (5'to 3')	SEQ ID NO
SpCas9	Streptococcus pyogenes	NGG OR NAG	N/A
SaCas9	Staphylococcus aureus	NGRRT OR NGRRN	N/A
NmeCas9	Neisseria meningitidis	NNNNGATT	N/A
CjCas9	Campylobacter jejuni	NNNNRYAC	N/A

StCas9	Streptococcus thermophilus	NNAGAAW	N/A
TdCas9	Treponema denticola	NAAAC	N/A
LbCas12a (Cpf1)	Lachnospiraceae bacterium	TTTV	N/A
AsCas12a (Cpf1)	Acidaminococcus sp.	TTTV	N/A
AacCas12b	Alicyclobacillus acidiphilus	TTN	N/A
BhCas12b v4	Bacillus Hisashi	AAATN, TTTN, OR GTTN	N/A
NmeCas9	Neisseria meningitidis	NNNNGATT	N/A

R = A or G; Y = C or T; W = A or T; V = A or C or G; N = any base

[0786] In some embodiments, Cas nucleases may comprise one or more mutations to alter their activity, specificity, recognition, and/or other characteristics. For example, the Cas nuclease may have one or more mutations that alter its fidelity to mitigate off- target effects (*e.g.,* eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9). For another example, the Cas nuclease may have one or more mutations that alter its PAM specificity.

[0787] Nuclease domains of the Cas, in particular the Cas9, nuclease can be mutated independently to generate enzymes referred to as DNA "nickases". Nickases are capable of introducing a single-strand cut with the same specificity as a regular CRISPR/Cas nuclease system, including for example CRISPR/Cas9. Nickases can be employed to generate doublestrand breaks which can find use in gene editing systems (Mali et al., Nat Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957-963 (2013); Mali et al, Science, 339(6121):823-826 (2013)). In some instances, when two Cas nickases are used, long overhangs are produced on each of the cleaved ends instead of blunt ends which allows for additional control over precise gene integration and insertion (Mali et al., Nat Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957-963 (2013); Mali et al., Science, 339(6121):823-826 (2013)). As both nicking Cas enzymes must effectively nick their target DNA, paired nickases can have lower off-target effects compared to the double-strand-cleaving Cas-based systems (Ran et al., Cell, 155(2):479- 480(2013); Mali et al, Nat

Biotech, 31(9):833-838 (2013); Mali et al. Nature Methods, 10:957- 963 (2013); Mali et al., Science, 339(6121):823-826 (2013)).

[0788] The molecular machinery of such Cas proteins that allows the CRISPR/Cas system to alter target polynucleotide sequences in cells include RNA binding proteins, endo- and exo-nucleases, helicases, and polymerases. In some embodiments, the CRISPR/Cas system includes a Cas protein and at least one to two ribonucleic acids that are capable of directing the Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. As used herein, "protein" and "polypeptide" are used interchangeably to refer to a series of amino acid residues joined by peptide bonds (i.e., a polymer of amino acids) and include modified amino acids (*e.g.,* phosphorylated, glycated, glycosylated, etc.) and amino acid analogs. Exemplary polypeptides or proteins include gene products, naturally occurring proteins, homologs, paralogs, fragments and other equivalents, variants, and analogs of the above.

[0789] In some embodiments, a Cas protein comprises one or more amino acid substitutions or modifications. In some embodiments, the one or more amino acid substitutions comprises a conservative amino acid substitution. In some instances, substitutions and/or modifications can prevent or reduce proteolytic degradation and/or extend the half-life of the polypeptide in a cell. In some embodiments, the Cas protein can comprise a peptide bond replacement (*e.g.,* urea, thiourea, carbamate, sulfonyl urea, etc.). In some embodiments, the Cas protein can comprise a naturally occurring amino acid. In some embodiments, the Cas protein can comprise an alternative amino acid (*e.g.,* D-amino acids, beta-amino acids, homocysteine, phosphoserine, etc.). In some embodiments, a Cas protein can comprise a modification to include a moiety (*e.g.,* PEGylation, glycosylation, lipidation, acetylation, end-capping, etc.).

[0790] In some embodiments, a Cas protein comprises a core Cas protein, isoform thereof, or any Cas-like protein with similar function or activity of any Cas protein or isoform thereof. In some embodiments, a Cas protein comprises a core Cas protein. Exemplary Cas core proteins include, but are not limited to Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8 and Cas9. In some embodiments, a Cas protein comprises type V Cas protein. In some embodiments, a Cas protein comprises a Cas protein of an E. coli subtype (also known as CASS2). Exemplary Cas proteins of the E. Coli subtype include, but are not limited to Csel, Cse2, Cse3, Cse4, and Cas5e. In some embodiments, a Cas protein comprises a Cas protein of the Ypest subtype (also known as CASS3). Exemplary Cas proteins of the Ypest subtype

include, but are not limited to Csyl, Csy2, Csy3, and Csy4. In some embodiments, a Cas protein comprises a Cas protein of the Nmeni subtype (also known as CASS4). Exemplary Cas proteins of the Nmeni subtype include, but are not limited to Csnl and Csn2. In some embodiments, a Cas protein comprises a Cas protein of the Dvulg subtype (also known as CASS1). Exemplary Cas proteins of the Dvulg subtype include Csdl, Csd2, and Cas5d. In some embodiments, a Cas protein comprises a Cas protein of the Tneap subtype (also known as CASS7). Exemplary Cas proteins of the Tneap subtype include, but are not limited to, Cstl, Cst2, Cas5t. In some embodiments, a Cas protein comprises a Cas protein of the Hmari subtype. Exemplary Cas proteins of the Hmari subtype include, but are not limited to Cshl, Csh2, and Cas5h. In some embodiments, a Cas protein comprises a Cas protein of the Apem subtype (also known as CASS5). Exemplary Cas proteins of the Apem subtype include, but are not limited to Csal, Csa2, Csa3, Csa4, Csa5, and Cas5a. In some embodiments, a Cas protein comprises a Cas protein of the Mtube subtype (also known as CASS6). Exemplary Cas proteins of the Mtube subtype include, but are not limited to Csml, Csm2, Csm3, Csm4, and Csm5. In some embodiments, a Cas protein comprises a RAMP module Cas protein. Exemplary RAMP module Cas proteins include, but are not limited to, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, and Cmr6. See, *e.g.,* Klompe et al., Nature 571, 219-225 (2019); Strecker et al., Science 365, 48-53 (2019). Examples of Cas proteins include, but are not limited to: Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and/or GSU0054. In some embodiments, a Cas protein comprises Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and/or GSU0054. Examples of Cas proteins include, but are not limited to: Cas9, Csn2, and/or Cas4. In some embodiments, a Cas protein comprises Cas9, Csn2, and/or Cas4. In some embodiments, Examples of Cas proteins include, but are not limited to: CaslO, Csm2, Cmr5, CaslO, Csxl 1, and/or CsxlO. In some embodiments, a Cas protein comprises a CaslO, Csm2, Cmr5, CaslO, Csxl 1, and/or CsxlO. In some embodiments, examples of Cas proteins include, but are not limited to: Csfl. In some embodiments, a Cas protein comprises Csfl. In some embodiments, examples of Cas proteins include, but are not limited to: Casl2a, Casl2b, Casl2c, C2c4, C2c8, C2c5, C2cl0, and C2c9; as well as CasX (Casl2e) and CasY (Casl2d). Also see, *e.g.,* Koonin et al., Curr Opin Microbiol. 2017; 37:67-78: "Diversity, classification and evolution of CRISPR- Cas systems." In some embodiments, a Cas protein comprises Casl2a, Casl2b, Casl2c, Casl2d, Casl2e, Casl2d, and/or Casl2e. In some embodiments, a Cas protein comprises Casl3, Casl3a, C2c2, Casl3b, Casl3c, and/or Casl3d. In some embodiments, the CRISPR/Cas

system comprises a Cas effector protein selected from the group consisting of: a) Cas3, Cas8a, Cas5, Cas8b, Cas8c, CaslOd, Csel, Cse2, Csyl, Csy2, Csy3, and GSU0054; b) Cas9, Csn2, and Cas4; c) CaslO, Csm2, Cmr5, CaslO, Csxl l, and CsxlO; d) Csfl; e) Casl2a, Casl2b, Casl2c, C2c4, C2c8, C2c5, C2cl0, C2c9, CasX (Casl2e), and CasY (Casl2d); and f) Casl3, Casl3a, C2c2, Casl3b, Casl3c, and Casl3d.

[0791] In some embodiments, a Cas protein comprises any one of the Cas proteins described herein or a functional portion thereof. As used herein, "functional portion" when used in reference to a Cas protein refers to a portion of a peptide which retains its ability to complex with at least one ribonucleic acid (*e.g.,* guide RNA (gRNA)) and cleave a target polynucleotide sequence. In some embodiments, the functional portion comprises a combination of operably linked Cas9 protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional portion comprises a combination of operably linked Casl2a (also known as Cpfl) protein functional domains selected from the group consisting of a DNA binding domain, at least one RNA binding domain, a helicase domain, and an endonuclease domain. In some embodiments, the functional domains form a complex. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of a RuvC-like domain. In some embodiments, a functional portion of the Cas9 protein comprises a functional portion of the HNH nuclease domain. In some embodiments, a functional portion of the Casl2a protein comprises a functional portion of a RuvC-like domain.

[0792] In some embodiments, the Cas protein is complexed with one to two ribonucleic acids. In some embodiments, the Cas protein is complexed with two ribonucleic acids. In some embodiments, the Cas protein is complexed with one ribonucleic acid. In some embodiments, the Cas protein is encoded by a modified nucleic acid, as described herein (*e.g.,* a synthetic, modified mRNA).

[0793] The methods of the present disclosure contemplate the use of any ribonucleic acid that is capable of directing a Cas protein to and hybridizing to a target motif of a target polynucleotide sequence. In some embodiments, at least one of the ribonucleic acids comprises tracrRNA. In some embodiments, at least one of the ribonucleic acids comprises CRISPR RNA (crRNA). In some embodiments, a single ribonucleic acid comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, at least one of the ribonucleic acids

comprises a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. In some embodiments, both of the one to two ribonucleic acids comprise a guide RNA that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell. The ribonucleic acids of the present disclosure can be selected to hybridize to a variety of different target motifs, depending on the particular CRISPR/Cas system employed, and the sequence of the target polynucleotide, as will be appreciated by those skilled in the art. The one to two ribonucleic acids can also be selected to minimize hybridization with nucleic acid sequences other than the target polynucleotide sequence. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least two mismatches when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids hybridize to a target motif that contains at least one mismatch when compared with all other genomic nucleotide sequences in the cell. In some embodiments, the one to two ribonucleic acids are designed to hybridize to a target motif immediately adjacent to a deoxyribonucleic acid motif recognized by the Cas protein. In some embodiments, each of the one to two ribonucleic acids are designed to hybridize to target motifs immediately adjacent to deoxyribonucleic acid motifs recognized by the Cas protein which flank a mutant allele located between the target motifs.

[0794] In some embodiments, each of the one to two ribonucleic acids comprises guide RNAs that directs the Cas protein to and hybridizes to a target motif of the target polynucleotide sequence in a cell.

[0795] In some embodiments, one or two ribonucleic acids (*e.g.,* guide RNAs) are complementary to and/or hybridize to sequences on the same strand of a target polynucleotide sequence. In some embodiments, one or two ribonucleic acids (*e.g.,* guide RNAs) are complementary to and/or hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.,* guide RNAs) are not complementary to and/or do not hybridize to sequences on the opposite strands of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (*e.g.,* guide RNAs) are complementary to and/or hybridize to overlapping target motifs of a target polynucleotide sequence. In some embodiments, the one or two ribonucleic acids (e.g, guide RNAs) are complementary to and/or hybridize to offset target motifs of a target polynucleotide sequence.

[0796] In some embodiments, CRISPR systems of the present disclosure comprise TnpB polypeptides. In some embodiments, TnpB polypeptides may comprise a Ruv-C-like domain. The RuvC domain may be a split RuvC domain comprising RuvC-I, RuvC-II, and RuvC-III subdomains. In some embodiments, a TnpB may further comprise one or more of a HTH domain, a bridge helix domain and a zinc finger domain. TnpB polypeptides do not comprise an HNH domain. In some embodiments, a TnpB protein comprises, starting at the N-terminus: a HTH domain, a RuvC-I subdomain, a bridge helix domain, a RuvC-II sub-domain, a zinger finger domain, and a RuvC-III sub-domain. In some embodiments, a RuvC-III sub-domain forms the C- terminus of a TnpB polypeptide. In some embodiments, a TnpB polypeptide is from Epsilonproteobacteria bacterium, Actinoplanes lobatus strain DSM 43150, Actinomadura celluolosilytica strain DSM 45823, Actinomadura namibiensis strain DSM 44197, Alicyclobacillus macrosprangiidus strain DSM 17980, Lipingzhangella hal ophila strain DSM 102030, or Ktedonobacter recemifer. In some embodiments, a TnpB polypeptide is from Ktedonobacter racemifer, or comprises a conserved RNA region with similarity to the 5' ITR of K. racemifer TnpB loci. In some embodiments, a TnpB may comprise a Fanzor protein, a TnpB homolog found in eukaryotic genomes. In some embodiments, a CRISPR system comprising a TnpB polypeptide binds a target adjacent motif (TAM) sequence 5' of a target polynucleotide. In some embodiments, a TAM is a transposon-associated motif. In some embodiments, a TAM sequence comprises TCA. In some embodiments, a TAM sequence comprises TTCAN. In some embodiments, a TAM sequence comprises TTGAT. In some embodiments, a TAM sequence comprises ATAAA.

[0797] In certain embodiments, the first and/or the second transgene may function as a DNA repair template to be integrated into the target site through HDR in associated with a gene editing system (*e.g.,* the CRISPR/Cas system) as described. Generally, the transgene to be inserted would comprise at least the expression cassette encoding the protein of interest (*e.g.,* the tolerogenic factor or CAR) and would optionally also include one or more regulatory elements (*e.g.,* promoters, insulators, enhancers). In certain of these embodiments, the transgene to be inserted would be flanked by homologous sequence immediately upstream and downstream of the target, i.e., left homology arm (LHA) and right homology arm (RHA), specifically designed for the target genomic locus to serve as template for HDR. The length of each homology arm is generally dependent on the size of the insert being introduced, with larger insertions requiring longer homology arms.

[0798] In some embodiments, target-primed reverse transcription (TPRT) or prime editing may be used to engineer exogenous genes into specific loci. In some embodiments, prime editing mediates targeted insertions, deletions, all 12 possible base-to-base conversions, and combinations thereof in human cells without requiring DSBs or donor DNA templates.

[0799] Prime editing is a genome editing method that directly writes new genetic information into a specified DNA site using a nucleic acid programmable DNA binding protein ("napDNAbp") working in association with a polymerase (i.e., in the form of a fusion protein or otherwise provided in trans with the napDNAbp), wherein the prime editing system is programmed with a prime editing (PE) guide RNA ("PEgRNA") that both specifies the target site and templates the synthesis of the desired edit in the form of a replacement DNA strand by way of an extension (either DNA or RNA) engineered onto a guide RNA (*e.g.,* at the 5' or 3' end, or at an internal portion of a guide RNA). The replacement strand containing the desired edit (*e.g.,* a single nucleobase substitution) shares the same sequence as the endogenous strand of the target site to be edited (with the exception that it includes the desired edit). Through DNA repair and/or replication machinery, the endogenous strand of the target site is replaced by the newly synthesized replacement strand containing the desired edit. In some cases, prime editing may be thought of as a "search-and- replace" genome editing technology since the prime editors search and locate the desired target site to be edited, and encode a replacement strand containing a desired edit which is installed in place of the corresponding target site endogenous DNA strand at the same time. For example, prime editing can be adapted for conducting precision CRISPR/Cas-based genome editing in order to bypass double stranded breaks. In some embodiments, a homologous protein is or encodes for a Cas protein-reverse transcriptase fusions or related systems to target a specific DNA sequence with a guide RNA, generate a single strand nick at the target site, and use the nicked DNA as a primer for reverse transcription of an engineered reverse transcriptase template that is integrated with the guide RNA. In some embodiments, a prime editor protein is paired with two prime editing guide RNAs (pegRNAs) that template the synthesis of complementary DNA flaps on opposing strands of genomic DNA, resulting in the replacement of endogenous DNA sequence between the PE-induced nick sites with pegRNA-encoded sequences.

[0800] In some embodiments, a gene editing technology is associated with a prime editor that is a reverse transcriptase, or any DNA polymerase known in the art. Thus, in one aspect, a prime editor may comprise Cas9 (or an equivalent napDNAbp) which is programmed to

target a DNA sequence by associating it with a specialized guide RNA (i.e., PEgRNA) containing a spacer sequence that anneals to a complementary protospacer in the target DNA. Such methods include any disclosed in Anzalone et al., (doi.org/10.1038/s41586-019-1711-4), or in PCT publication Nos. WO2020191248, WO2021226558, or W02022067130, which are hereby incorporated in their entirety.

[0801] In some embodiments, the base editing technology may be used to introduce singlenucleotide variants (SNVs) into DNA or RNA in living cells. Base editing is a CRISPR-Cas9-based genome editing technology that allows the introduction of point mutations in RNAs or DNAs without generating DSBs. Base editors (BEs) are typically fusions of a Cas ("CRISPR- associated") domain and a nucleobase modification domain (*e.g.,* a natural or evolved deaminase, such as a cytidine deaminase that include AP0BEC1 ("apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1"), CD A ("cytidine deaminase"), and AID ("activation-induced cytidine deaminase")) domains. In some embodiments, base editors may also include proteins or domains that alter cellular DNA repair processes to increase the efficiency and/or stability of the resulting single-nucleotide change. Two major classes of base editors have been developed: cytidine base editors (CBEs) (*e.g.,* BE4) that allow C:G to T:A conversions and adenine base editors (ABEs) (*e.g.,* ABE7.10) that allow A:T to G:C conversions. Base editors are composed by a catalytically dead Cas9 (dCas9) or a nickase Cas9 (nCas9) fused to a deaminase and guided by a sgRNA to the locus of interest. The d/nCas9 recognizes a specific PAM sequence and the DNA unwinds thanks to the complementarity between the sgRNA and the DNA sequence usually located upstream of the PAM (also called protospacer). Then, the opposite DNA strand is accessible to the deaminase that converts the bases located in a specific DNA stretch of the protospacer. Compared to HDR- based strategies, base editing is a promising tool to precisely correct genetic mutations as it avoids gene disruption by NHEJ associated with failed HDR-mediated gene correction. Rat deaminase AP0BEC1 (rAPOBECl) fused to deactivated Cas9 (dCas9) has been used to successfully convert cytidines to thymidines upstream of the PAM of the sgRNA. In some embodiments, this first BE system was optimized by changing the dCas9 to a "nickase" Cas9 D10A, which nicks the strand opposite the deaminated cytidine. Without being bound by theory, this is expected to initiate long- patch base excision repair (BER), where the deaminated strand is preferentially used to template the repair to produce a U:A base pair, which is then converted to T:A during DNA replication.

[0802] In some embodiments, a base editor is a nucleobase editor containing a first DNA binding protein domain that is catalytically inactive, a domain having base editing activity, and a second DNA binding protein domain having nickase activity, where the DNA binding protein domains are expressed on a single fusion protein or are expressed separately (*e.g.,* on separate expression vectors). In some embodiments, a base editor is a fusion protein comprising a domain having base editing activity (*e.g.,* cytidine deaminase or adenosine deaminase), and two nucleic acid programmable DNA binding protein domains (napDNAbp), a first comprising nickase activity and a second napDNAbp that is catalytically inactive, wherein at least the two napDNAbp are joined by a linker. In some embodiments, a base editor is a fusion protein that comprises a DNA domain of a CRISPR-Cas (*e.g.,* Cas9) having nickase activity (nCas; nCas9), a catalytically inactive domain of a CRISPR-Cas protein (*e.g.,* Cas9) having nucleic acid programmable DNA binding activity (dCas; *e.g.,* dCas9), and a deaminase domain, wherein the dCas is joined to the nCas by a linker, and the dCas is immediately adjacent to the deaminase domain. In some embodiments, a base editor is an adenine-to-thymine or "ATBE" (or thymine-to-adenine or "TABE") transversion base editor. Exemplary base editor and base editor systems include any as described in patent publication Nos. US20220127622, US20210079366, US20200248169, US20210093667, US20210071163, W02020181202, WO2021158921, WO2019126709, W02020181178, W02020181195, WO2020214842, W02020181193, which are hereby incorporated in their entirety.

[0803] In some embodiments, a gene editing technology is Programmable Addition via Site-specific Targeting Elements (PASTE). In some aspects, PASTE is platform in which genomic insertion is directed via a CRISPR-Cas9 nickase fused to both a reverse transcriptase and serine integrase. As described in Ioannidi et al. (doi.org/10.1101/2021.11.01.466786), PASTE does not generate double stranded breaks, but allows for integration of sequences as large as ~36 kb. In some embodiments, a serine integrase can be any known in the art. In some embodiments, a serine integrase has sufficient orthogonality such that PASTE can be used for multiplexed gene integration, simultaneously integrating at least two different genes at at least two genomic loci. In some embodiments, PASTE has editing efficiencies comparable to or better than those of homology directed repair or non-homologous end joining based integration, with activity in non-dividing cells and fewer detectable off-target events.

i. Genomic Loci for Insertion

[0804] The specific site for insertion within a gene locus may be located within any suitable region of the gene, including but not limited to a gene coding region (also known as a coding sequence or "CDS"), an exon, an intron, a sequence spanning a portion of an exon and a portion of an adjacent intron, or a regulatory region (*e.g.,* promoter, enhancer). In some embodiments, the insertion occurs in one allele of the specific genomic locus. In some embodiments, the insertion occurs in both alleles of the specific genomic locus. In either of these embodiments, the orientation of the transgene inserted into the target genomic locus can be either the same or the reverse of the direction of the endogenous gene in that locus.

[0805] In some embodiments, polynucleotides of the present disclosure are inserted into a T cell receptor alpha chain (TRAC) locus. The TRAC gene encodes the TCR alpha chain constant region. The human TRAC gene resides on chromosome 14 at 22,547,506-22,552,156, forward strand. The TRAC genomic sequence is set forth in Ensembl ID ENSG00000277734.

[0806] In some embodiments, polynucleotides of the present disclosure are inserted into a T cell receptor beta chain (TRBC) locus. The TRBC genes encode the TCR beta chain constant region. TRBC1 and TRBC2 are analogs of the same gene, and T cells mutually exclusively express either TRBC1 or TRBC2. The human TRBC1 gene resides on chromosome 7 at 142,791,694-142,793,368, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000211751. The human TRBC2 gene resides on chromosome 7 at 142,801,041-142,802,748, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000211772.

[0807] In some embodiments, polynucleotides of the present disclosure are inserted into a CD247 (also known as the CD3 zeta chain) locus. The CD247 gene encodes the zeta chain of the T-cell receptor. The CD247 gene resides on chromosome 1: 167,425,027-167,518,640, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000198821.

[0808] In some embodiments, polynucleotides of the present disclosure are inserted into a beta-2-microglobulin (B2M) locus. The B2M gene encodes the B2M component of class I major histocompatibility complex (MHC I). The B2M gene resides on chromosome 15: 45,003,675-45,011,075, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000166710.

[0809] In some embodiments, polynucleotides of the present disclosure are inserted into a class II, major histocompatibility complex, transactivator (CIITA) locus. The CIITA gene encodes the CIITA protein that is a positive regulator of class II major histocompatibility complex (MHC II) gene transcription. The CIITA gene resides on chromosome 16: 10,866,222-10,943,021, forward strand, and its genomic sequence is set forth in Ensembl ID ENSG00000179583.

[0810] In some embodiments, polynucleotides of the present disclosure are inserted into a caspase recruitment domain family member 11 (CARD11) locus. In some embodiments, polynucleotides encoding the PIK3R3 protein, or a portion thereof, of the present disclosure are inserted into a CARD11 locus. The CARD11 gene encodes the CARD11 protein which plays a role in various immune responses including, amoung others, lymphocyte activation, proliferation, and differentiation, and plays a role as a positive regulator of NF-kB activation. The CARD11 gene resides on chromosome 7: 2,906,142-3,044,228, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000198286.

[0811] In some embodiments, polynucleotides of the present disclosure are inserted into a phosphoinositide-3-kinase regulatory subunit 3 (PIK3R3) locus. In some embodiments, polynucleotides encoding the CARD11 protein, or a portion thereof, of the present disclosure are inserted into a PIK3R3 locus. The PIK3R3 gene encodes the PIK3R3 protein which plays a role in inhibiting cell senescence and enhancing cell proliferation. The PIK3R3 gene resides on chromosome 1: 46,040,140-46,133,036, reverse strand, and its genomic sequence is set forth in Ensembl ID ENSG00000117461.

[0812] In some embodiments, polynucleotides of the present disclosure are inserted into a safe harbor locus. Non-limiting examples of safe harbor loci include, but are not limited to, an AAVS1 (also known as PPP1R12C; Ensembl ID: ENSG00000125503), ABO (Ensembl ID: ENSG00000175164), CCR5 (Ensembl ID: ENSG00000160791), CLYBL (Ensembl ID: ENSG00000125246), CXCR4 (Ensembl ID: ENSG00000121966), F3 (also known as CD142; Ensembl ID: ENSG00000117525), FUT1 (Ensembl ID: ENSG00000174951), HMGB1 (Ensembl ID: ENSG00000189403), KDM5D (Ensembl ID: ENSG00000012817), LRP1 (also known as CD91; Ensembl ID: ENSG00000123384), MICA (Ensembl ID: ENSG00000204520), MICB (Ensembl ID: ENSG00000206449), RHD (Ensembl ID: ENSG00000187010), human ROSA26 (GRCh37/hg19, chr3: 9,415,082–9,414,043), and SHS 231 (GRCh37/hg19, chr4: 58,976,613–58,976,632).

i. gRNAs for Site-Directed Insertion

[0813] In some embodiments, provided are gRNAs for use in site-directed insertion of a polynucleotides according to various embodiments provided herein, especially in association with the CRISPR/Cas system. The gRNAs comprise a crRNA sequence, which in turn comprises a complementary region (also called a spacer) that recognizes and binds a complementary target DNA of interest. The length of the spacer or complementary region is generally between 15 and 30 nucleotides, usually about 20 nucleotides in length, although will vary based on the requirements of the specific CRISPR/Cas system. In certain embodiments, the spacer or complementary region is fully complementary to the target DNA sequence. In other embodiments, the spacer is partially complementary to the target DNA sequence, for example at least 80%, 85%, 90%, 95%, 98%, or 99% complementary.

[0814] In certain embodiments, the gRNAs provided herein further comprise a tracrRNA sequence, which comprises a scaffold region for binding to a nuclease. The length and/or sequence of the tracrRNA may vary depending on the specific nuclease being used for editing. In certain embodiments, nuclease binding by the gRNA does not require a tracrRNA sequence. In those embodiments where the gRNA comprises a tracrRNA, the crRNA sequence may further comprise a repeat region for hybridization with complementary sequences of the tracrRNA.

[0815] In some embodiments, the gRNAs provided herein comprise two or more gRNA molecules, for example, a crRNA and a tracrRNA, as two separate molecules. In other embodiments, the gRNAs are single guide RNAs (sgRNAs), including sgRNAs comprising a crRNA and a tracrRNA on a single RNA molecule. In certain of these embodiments, the crRNA and tracrRNA are linked by an intervening tetraloop.

[0816] In some embodiments, one gRNA can be used in association with a site-directed nuclease for targeted editing of a gene locus of interest. In other embodiments, two or more gRNAs targeting the same gene locus of interest can be used in association with a site-directed nuclease.

[0817] In some embodiments, exemplary gRNAs (*e.g.,* sgRNAs) for use with various common Cas nucleases that require both a crRNA and tracrRNA, including Cas9 are provided in **Table 17** below. See, *e.g.,* Jinek et al., Science (2012) 337 (6096):816-821; Dang et al., Genome Biology (2015) 16:280; Ran et al., Nature (2015) 520: 186-191; Strecker et al., Nature Comm. (2019) 10:212. For each exemplary gRNA, sequences for

different portions of the gRNA, including the complementary region or spacer, crRNA repeat region, tetraloop, and tracrRNA, are shown.

Table 17. Exemplary gRNA Structure and Sequences for CRISPR/Cas

SEQ ID NO:	Sequence (5' to 3')	Description
N/A	nnnnnnnnnnnnnnnnnnnn	Exemplary spCas9 1 complementary region (spacer)
365	guuuuagagcua	Exemplary spCas9 1 crRNA repeat region
N/A	gaaa	Exemplary spCas9 1 crRNA tetraloop
367	uagcaaguuaaaauaaggcuaguccguuaucaacuu gaaaaaguggcaccgagucggugcuuuuuu	Exemplary spCas9 1 crRNA tracrRNA
N/A	nnnnnnnnnnnnnnnnnnnn	Exemplary spCas9 2 complementary region (spacer)
369	guuusagagcuaugcug	Exemplary spCas9 2 crRNA repeat region
N/A	gaaa	Exemplary spCas9 2 crRNA tetraloop
371	cagcauagcaaguusaaauaaggcuaguccguuauc aacuugaaaaaguggcaccgagucggugcuuuuuu	Exemplary spCas9 2 crRNA tracrRNA
N/A	nnnnnnnnnnnnnnnnnnnn	Exemplary saCas9 complementary region (spacer)
373	guuuuaguacucug	Exemplary saCas9 crRNA repeat region
N/A	gaaa	Exemplary saCas9 tetraloop
375	cagaaucuacuaaaacaaggcaaaaugccguguuua ucucgucaacuuguuggcgagauuuuuu	Exemplary saCas9 tracrRNA

s = c or g; n = any base

[0818] In some embodiments, the gRNA comprises a complementary region specific to a target gene locus of interest, for example, a TRAC locus, a TRBC1 locus, a TRBC2 locus, a TRAJ locus, a CD3zeta locus, a B2M locus, a CIITA locus, a CARD11 locus, a PIK3R3 locus, or a safe harbor locus selected from the group consisting of an AAVS1 locus, an ABO locus, a CCR5 locus, a CLYBL locus, a CXCR4 locus, a F3 locus, a FUT1 locus, a HMGB1 locus, a KDM5D locus, a LRP1 locus, a MICA locus, a MICB locus, a RHD locus, a ROSA26, locus and a SHS231 locus. The complementary region may bind a sequence in any region of the target gene locus, including for example, a CDS, an exon, an intron, a

sequence spanning a portion of an exon and a portion of an adjacent intron, or a regulatory region (*e.g.,* promoter, enhancer). Where the target sequence is a CDS, exon, intron, or sequence spanning portions of an exon and intron, the CDS, exon, intron, or exon/intron boundary may be defined according to any splice variant of the target gene. In some embodiments, the genomic locus targeted by the gRNA is located within 4000 bp, within 3500 bp, within 3000 bp, within 2500 bp, within 2000 bp, within 1500 bp, within 1000 bp, or within 500 bp of any of the loci or regions thereof as described. Further provided herein are compositions comprising one or more gRNAs provided herein and a Cas protein or a nucleotide sequence encoding a Cas protein. In certain of these embodiments, the one or more gRNAs and a nucleotide sequence encoding a Cas protein are comprised within a vector, for example, a viral vector.

[0819] In some embodiments, provided are methods of identifying new loci and/or gRNA sequences for use in the site-directed genomic insertion approaches as described. For example, for CRISPR/Cas systems, when an existing gRNA for a particular locus (*e.g.,* within an endogenous TCR gene locus) is known, an "inch worming" approach can be used to identify additional loci for targeted insertion of transgenes by scanning the flanking regions on either side of the locus for PAM sequences, which usually occurs about every 100 base pairs (bp) across the genome. The PAM sequence will depend on the particular Cas nuclease used because different nucleases usually have different corresponding PAM sequences. The flanking regions on either side of the locus can be between about 500 to 4000 bp long, for example, about 500 bp, about 1000 bp, about 1500 bp, about 2000 bp, about 2500 bp, about 3000 bp, about 3500 bp, or about 4000 bp long. When a PAM sequence is identified within the search range, a new guide can be designed according to the sequence of that locus for use in site-directed insertion of transgenes. Although the CRISPR/Cas system is described as illustrative, any gene editing approaches as described can be used in this method of identifying new loci, including those using ZFNs, TALENs, meganucleases, and transposases.

[0820] In some embodiments, the activity, stability, and/or other characteristics of gRNAs can be altered through the incorporation of chemical and/or sequential modifications. As one example, transiently expressed or delivered nucleic acids can be prone to degradation by, *e.g.,* cellular nucleases. Accordingly, the gRNAs described herein can contain one or more modified nucleosides or nucleotides which introduce stability toward nucleases. While not being bound by a particular theory, it is believed that certain modified gRNAs described

herein can exhibit a reduced innate immune response when introduced into a population of cells, particularly the cells of the present technology. As used herein, the term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, generally of viral or bacterial origin, which involves the induction of cytokine expression and release, *e.g.,* the interferons, and cell death. Other common chemical modifications of gRNAs to improve stabilities, increase nuclease resistance, and/or reduce immune response include 2'-O-methyl modification, 2'-fluoro modification, 2'-O-methyl phosphorothioate linkage modification, and 2'- O-methyl 3' thioPACE modification.

[0821] One common 3' end modification is the addition of a poly(A) tract comprising one or more (and typically 5-200) adenine (A) residues. The poly(A) tract can be contained in the nucleic acid sequence encoding the gRNA or can be added to the gRNA during chemical synthesis or following in vitro transcription using a polyadenosine polymerase (*e.g.,* E. coll poly(A) polymerase). In vivo, poly(A) tracts can be added to sequences transcribed from DNA vectors through the use of polyadenylation signals. Examples of such signals are provided in Maeder. Other suitable gRNA modifications include, without limitations, those described in U.S. Patent Application No. US 2017/0073674 Al and International Publication No. WO 2017/165862 Al, the entire contents of each of which are incorporated by reference herein.

[0822] In some embodiments, a tool for designing a gRNA as disclosed herein comprises: Benchling, Broad Institute GPP, CasOFFinder, CHOPCHOP, CRISPick, CRISPOR, Deskgen, E- CRISP, Geneious, Guides, Horizon Discovery, IDT, Off-Spotter, Synthego, or TrueDesign (ThermoFisher). One of ordinary skill in the art would understand that a tool that predicts both activity and specificity (*e.g.,* to limit off-target modification) would be useful for designing a gRNA in certain instances as disclosed herein.

XII. OTHER FEATURES

j. Expression of Polynucleotides

[0823] In some embodiments, the polynucleotides of the present disclosure are expressed under the control of an endogenous promoter. In some embodiments, the endogenous promoter, is a TRAC promoter, TRBC1 promoter, TRBC2 promoter, CD3alpha promoter, CD3beta promoter, CD3epsilon promoter, CD3delta promoter, CD3zeta promoter, CARD11 promoter, PIK3R3 promoter, B2M promoter, CIITA promoter, PD-1 promoter, CXCR4 promoter, an albumin promoter, an F3 promoter (also known as CD142), a MICA promoter,

a MICB promoter, a LRP1 promoter (also known as CD91), a HMGB1 promoter, an ABO promoter, a RHD promoter, a FUT1 promoter, a KDM5D promoter (also known as HY), a CCR5 promoter, a PDGFRa promoter, a OLIG2 promoter, and/or a GFAP promoter.

[0824] In some embodiments, the polynucleotides of the present disclosure are expressed under the control of a contitutive promoter. Examples of suitable promoters include, for example, MND promoter, EF1a promoter, sEF1 promoter, gamma retroviral LTR promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, or a CD3z promoter. In some embodiments, the promoter is a minimal TATA promoter, a pGK, actin promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter,CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NF-κB promoter, AP-1 promoter, TNF-alpha promoter, and CD130 promoter, NR4A1 promoter, NR4A2, or NR4A3 promoter. Exemplary constitutive promoters include, *e.g.,* simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV), human Ubiquitin C promoter (UBC), human elongation factor la promoter (EFla), mouse phosphoglycerate kinase 1 promoter (PGK), and chicken P-Actin

promoter coupled with CMV early enhancer (CAG). In some embodiments, the constitutive promoter is a synthetic or modified promoter. In some embodiments, the promoter is or comprises an MND promoter, a synthetic promoter that contains the U3 region of a modified MoMuLV LTR with myeloproliferative sarcoma virus enhancer (see Challita et al. (1995) J. Virol. 69(2):748-755). In some embodiments, the promoter is a tissue-specific promoter. In another embodiment, the promoter is a viral promoter. In another embodiment, the promoter is a non-viral promoter. In some embodiments, exemplary promoters can include, but are not limited to, human elongation factor 1 alpha (EFla) promoter or a modified form thereof or the MND promoter.

i. Cell Therapy Potency Enhancements

[0825] In some embodiments, the disclosure provides polynucleotides, polypeptides, and methods for improving adoptive immune cell (*e.g.,* T cell) therapies. In some embodiments, the polynucleotides and polypeptides of the disclosure improves the therapeutic efficacy of the immune cells (*e.g.,* T cells) by altering cell signaling, decreasing cell exhaustion, and/or by enhancing *in vivo* persistence, fitness, control and/or safety of engineered cells.

[0826] Adoptive cell therapies comprising a chimeric antigen receptor (CAR) or recombinant T cell receptor (TCR)) have shown therapeutic success in hematological malignancies. However, effectively targeting solid tumors has been limited, in part due to challenges related to the immune suppressive tumor microenvironment, T cell exhaustion, poor T cell activity, and/or poor *in vivo* persistence, fitness, control and/or safety of engineered T cells. T cell-based therapies, such as adoptive T cell therapies (including those involving the administration of engineered cells expressing recombinant, engineered or chimeric receptors specific for a disease or disorder of interest, such as a recombinant T cell receptor (TCR) or other recombinant, engineered or chimeric receptors, such as a chimeric antigen receptor (CAR)) can be effective in the treatment of cancer and other diseases and disorders. In certain contexts, other approaches for generating engineered cells for adoptive cell therapy may not always be entirely satisfactory. In some aspects, efficacy or potency of the engineered cells can depend on various factors, including T cell exhaustion, immunosuppressive tumor microenvironment (TME), poor cell infiltration into the target, *e.g.,* tumor, and a lack of endogenous anti-tumor immune response.

[0827] In some contexts, optimal activity or outcome can depend on the ability of the administered cells to express the recombinant receptor on the surface, recognize and bind to

a target, to traffic, localize to and successfully enter appropriate sites within the subject, tumors, and environments thereof. In some contexts, optimal activity or outcome can depend on the ability of the administered cells to uniformly and/or continuously express the recombinant receptor, become activated, expand, to exert various effector functions, including cytotoxic killing and secretion of various factors such as cytokines, to persist, including long-term, to differentiate, transition or engage in reprogramming into certain phenotypic states (such as long-lived memory, less-differentiated, and effector states), to avoid or reduce immunosuppressive conditions in the local microenvironment of a disease, to provide effective and robust recall responses following clearance and re-exposure to target ligand or antigen, and avoid or reduce exhaustion, anergy, peripheral tolerance, terminal differentiation, and/or differentiation into a suppressive state.

[0828] In some embodiments, the polypeptides of the disclosure, or the polynucleotides of the disclosure can alter the therapeutic efficacy of engineered T cells. Alteration of therapeutic efficacy can include, without limitation, a decrease in T cell exhaustion, increased proliferative capacity, enhanced anti-tumor effect, enhanced replicative lifespan, decreased replicative senescence, enhanced ability to kill, enhanced fitness of engineered T cells and/or other functions or activities of T cells.

[0829] In some embodiments, the polypeptides of the disclosure, or the polynucleotides of the disclosure can improve the control or safety of engineered T cells. The improvement of control or safety can include, without limitation, the ability to deplete the engineered T cells when an adverse effect occurs.

1. Potency Enhancements

[0830] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter the *in vivo* persistence of T cells in tumors or the other functions or activities of T cells described herein.

[0831] In some embodiments of the recombinant nucleic constructs and/or recombinant nucleic acids of the disclosure, the polypeptides can include more than one mutation. For example, one or two or three or four or five or more mutations. In some embodiments, the mutations are a combination of different types of mutations. The different types of mutations can be point mutations, gene fusions, substitutions, gain-of-function mutations, stop-gain mutations, insertion mutations, deletion mutations, duplication mutations or translocations.

The mutation can be a T cell lymphoma mutation or a mutation in a clonally expanded population of T cells. In some embodiments, the mutation is a point mutation or substitution.

[0832] In some embodiments, the mutation is a gene fusion. In some embodiments, the gene fusion comprises a polypeptide comprising a caspase-associated recruitment domain (CARD). In some embodiments, the gene fusion comprises a polypeptide comprising a CARD containing protein or a functional fragment thereof. A functional fragment of a CARD containing protein can be, for example, a fragment that provides NF-κB transcriptional activity, AP-1 transcriptional activity, and/or MALT1 proteolytic activity activity at a level at least 70%, 75%, 80%, 85%, 90%, or 95% of that of a full-length protein, as determined by the in vitro CAR Jurkat assay. In some embodiments, the gene fusion comprises a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a phosphorylated tyrosine (pTyr). In some embodiments, the gene fusion comprises a CARD containing protein and a domain capable of binding to (i) a substrate localized to the intracellular side of the plasma membrane of a cell and/or (ii) a target polypeptide comprising a pTyr.

[0833] In some embodiments, the domain is capable of binding to a substrate indirectly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. Indirect localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with another polypeptide or lipid that is directly localized to the intracellular side of the plasma membrane. In some embodiments, the domain is capable of binding to a substrate directly localized to the intracellular side of the plasma membrane. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, binding of the substrate to the intracellular side of the plasma membrane itself. Direct localization can refer to localization of the substrate to the intracellular side of the plasma membrane through, for example, interaction of the substrate with the intracellular side of the plasma membrane itself.

[0834] The polypeptides of the disclsoure, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, can alter T cell signaling in various ways. In some embodiments, the altering of the T cell signaling can be by enhancing, promoting, improving, reducing, regulating, or modulating the signaling pathway within a T

cell. In some embodiments, the altering of the T cell signaling can be by activating, increasing, suppressing, inhibiting, or other means of changing the signaling.

[0835] The polypeptides of the disclsoure, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, can alter T cell signaling through one or more T cell signaling pathways. In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter T cell signaling by increasing one or more pathways and/or reducing one or more pathways, or by a combination of enhancing and/or reducing various pathways. In some embodiments, the mutation can alter cytokine production. The cytokine can be, without limitation, IL-2, IL-4, IL-5, TNF alpha, IFN- gamma, IL-13 and/or other cytokines. In some embodiments, the cytokine is IL-2. In some embodiments, the cytokine is IL-2, and the production of IL-2 is increased.

[0836] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter T cell signaling through one or more T cell signaling pathways. Signaling pathways can be, without limitation, the NFAT pathway, NF-κB pathway, AP-1 pathway, JAK/STAT pathway, RAS/MEK/ERK, and/or phospholipase gamma signaling, or described elsewhere herein.

[0837] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter CARD11-BCL10-MALT1 complex signaling, co-stimulatory molecule signaling, cytokine production and/or transcription factor activity in T cells.

[0838] The disclosure also relates to polypeptides having one or more mutations that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increase intratumoral presence of therapeutic T cells. The disclosure further relates to recombinant nucleic acids encoding polypeptides having one or more mutations, that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increse intratumoral presence of therapeutic T cells.

[0839] T cell fitness can refer to the ability of a T cell to generate an immune response. The ability of a T cell to perform T cell functions such as signaling, cytokine production, survival, and persistence in a tumor, may contribute to the fitness of a T cell. T cell exhaustion may contribute to reducing its fitness.

[0840] In some embodiments, polypeptides of the disclosure, or the recombinant nucleicacid constructs and/or recombinant nucleic acids of the disclosure can alter *in vivo* persistence in tumors of therapeutic T cells including the mutation. The *in vivo* persistence of therapeutic T cells can refer to the length of time that the therapeutic T cells exist within the tumor of the host after infusion. In some embodiments, *in vivo* persistence of therapeutic T cells is enhanced. Enhanced *in vivo* persistence of therapeutic T cells can include at least a positive log2 fold change in therapeutic T cells as compared to the input total.

[0841] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter the *in vivo* persistence of T cells in tumors or the other functions or activities of T cells described herein, through one or more of the following genes: BCL6, BCOR, BRAF, CARD11, CARMIL2, CCND3, CD28, CD3E, CSNK1A1, CSNK2B, ECSIT, EIFS1, FYN, GATA, GNAQ, IRF4, ITGB2, JAK1, JAK3, JUNB, KCNQ1, LATS1, MSC, MYCN, NFKB1, NFKB2, NRAS, PDCD1, PLCG1, PRKCB1 , RARA, RASGRP1, RHOA, SMARCB1, STAT3, STAT5, TBL1XR1, TNFRSF1B, TP53 and VAV1.

[0842] In some embodiments, the genes include caspase recruitment domain family member 11 (CARD11). In some embodiments, the genes include capping protein regulator and myosin 1 linker 2 (CARMIL2), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), B-cell lymphoma 6 (BCL6), B-cell lymphoma 10 (BCL10), and MYCN. In some embodiments, the gene can be a STAT3, STAT5B, JAK1, JAK2, or JAK3 gene. In some embodiments, the gene can be BRAF, or RASGRP1. In some embodiments, the gene can be phospholipase C gamma 1 (PLCG1) gene. In some embodiments, the gene can be NFKB1, NFKB2, or JUNB gene. In some embodiments, the gene can be TNFRSF1B.

[0843] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes, a polypeptide comprising a CARD11 polypeptide having a substitution at amino acid 357, 361, 615, 634, and/or 655. In some embodiments, the substitution at amino acid 357 is D357N. In some embodiments, the substitution at amino acid 361 is Y361C. In some embodiments, the substitution at amino acid 615 is S615F. In some embodiments, the substitution at amino acid 634 is E634K. In some embodiments, the substitution at amino acid 655 is S655C.

[0844] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes, a polypeptide comprising a BCL3 polypeptide having a substitution at amino acid 647. In some embodiments, the substitution at amino acid 647 is S647R.

[0845] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes, a polypeptide comprising a CARMIL2 polypeptide having a substitution at amino acid 575. In some embodiments, the substitution at amino acid 575 is Q575E.

[0846] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a MYCN polypeptide having a substitution at amino acid 44. In some embodiments, the substitution at amino acid 44 is P44L.

[0847] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a JAK1 polypeptide having a substitution at amino acid 1097. In some embodiments, the substitution at amino acid 1097 is an G1097A substitution.

[0848] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a JAK3 polypeptide having a substitution at amino acid 573. In some embodiments, the substitution at amino acid 573 is an A573V substitution.

[0849] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a STAT3 polypeptide having a substitution at amino acid 618. In some embodiments, the substitution at amino acid 618 is an G618R substitution. In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a STAT3 polypeptide having a substitution at amino acid 647. In some embodiments, the substitution at amino acid 647 is an N647I substitution. In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a STAT3 polypeptide having a substitution at amino acid 661. In some embodiments, the substitution at amino acid 661 is an D661I substitution.

[0850] In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a STAT5 polypeptide having a substitution at amino acid 628. In some embodiments, the substitution at amino acid 628 is an T628S substitution. In some embodiments, the polypeptide of the disclosure has, or the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure encodes a polypeptide comprising a STAT5 polypeptide having a substitution at amino acid 665. In some embodiments, the substitution at amino acid 665 is an Y665F substitution.

[0851] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a TNFRSF1B polypeptide having a substitution at amino acid 256 and/or 377. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a TNFRSF1B polypeptide having a substitution at amino acid 256. In some embodiments, the substitution at amino acid 256 is an G256C substitution. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a TNFRSF1B polypeptide having a substitution at amino acid 337. In some embodiments, the substitution at amino acid 337 is an T337I substitution.

[0852] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a CD28 polypeptide having a substitution at amino acid 51, 77, and/or 195. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a CD28 polypeptide having a substitution at amino acid 51. In some embodiments, the substitution at amino acid 51 is an F51V substitution. In some embodiments, the substitution at amino acid 51 is an F51I substitution. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a CD28 polypeptide having a substitution at amino acid 77. In some embodiments, the substitution at amino acid 77 is an Q77P substitution. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a CD28 polypeptide having a substitution at amino acid 195. In some embodiments, the substitution at amino acid 195 is an T195P substitution.

[0853] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising an ICOS-CD28 polypeptide.

[0854] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a CD28-CTLA4 polypeptide.

[0855] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a BRAF polypeptide having a substitution at amino acid 469 and/or 594. In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a BRAF polypeptide having a substitution at amino acid 469. In some embodiments, the substitution at amino acid 469 is an G469R substitution. In some embodiments, the substitution at amino acid 469 is an G469A substitution. In some embodiments, the substitution at amino acid 594 is an D594N substitution.

[0856] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a RASGRP1 polypeptide having a substitution at amino acid 261. In some embodiments, the substitution at amino acid 261 is an M261I substitution.

[0857] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a PLCG1 polypeptide having a substitution at amino acid 47, 48, 520, 1163, 1165 or any combination thereof. In some embodiments, the substitution at amino acid 47 is an E47K substitution. In some embodiments, the substitution at amino acid 48 is an R48W substitution. In some embodiments, the substitution at amino acid 520 is an S520F substitution. In some embodiments, the substitution at amino acid 1163 is an E1163K substitution. In some embodiments, the substitution at amino acid 1165 is an D1165H substitution.

[0858] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a NFKB1 polypeptide having a substitution at amino acid 67. In some embodiments, the substitution at amino acid 67 is an H67Y substitution.

[0859] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a NFKB2 polypeptide having a substitution at amino acid 656. In some embodiments, the substitution at amino acid 656 is an K656X substitution.

[0860] In some embodiments of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure, the nucleic acid encodes a polypeptide comprising a JUNB polypeptide having a substitution at amino acid 282. In some embodiments, the substitution at amino acid 282 is an A282V substitution.

1. Additional Potency Enhancements and/or Cell Armours

[0861] In some embodiments, the cells disclosed herein further comprise an additional potency enhancement or cell armours. In some embodiments, the cells disclosed herein comprise polypeptides comprising additional potency enhancement or cell armour. In some embodiments, the cells disclosed herein comprise polynucleotides encoding the polypeptides comprising additional potency enhancement or cell armours. In some embodiments, the additional potency enhancement or cell armours that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increase intratumoral presence of therapeutic T cells. The disclosure further relates to recombinant nucleic acids encoding polypeptides having one or more mutations, that can reduce T cell exhaustion, increase proliferation, alter effector function, resist T cell dysfunction, increase T cell fitness, enhance *in vivo* persistence, and/or increse intratumoral presence of therapeutic T cells. Such disclosure is applicable to the methods, uses and cells described herein. In some embodiments, the cells are T cells (*e.g.*, CAR-T cells) or NK cells.

[0862] Described here are engineered cells that comprise one or more modifications. In some embodiments, the engineered cells comprise a modification to increase expression of one or more additional potency enhancement or cell armours. In some embodiments, the additional potency enhancement or cell armours is one or more of a dominant-negative transforming growth factor-β receptor type II (dnTGFβRII), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha,IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, or CD8alpha/beta.

[0863] Described here are engineered cells that comprise one or more modifications. In some embodiments, the engineered cells comprise a modification to inactivate or disrupt one or more alleles of TGFbeta receptor II (TGFBRII), Regnase-1, FAS, PTPN2, NR4A3, CD52, PD-1, B2M, CIITA, SOCS1, CBLB, DGKalpha, and DGKζ.

ii. Tolerogenic Factors

[0864] In some embodiments, the cells are further modified to enable immune evasion. When transplanted in vivo without immunosuppression, immune evasive cells have reduced expression of one or more MHC class I molecules and/or MHC class II molecules and thus there may be no evidence of a systemic immune response against the transplanted cells from the recipient's immune system, such as no T cell activation, antibody production, or NK cell activity. Disclosure relating to edited cells is provided herein. Such disclosure is applicable to the methods, uses and cells described herein. In some embodiments, the cells are T cells (*e.g.,* CAR-T cells).

[0865] One modification that may be desirable for the cell therapy product is that the engineered cells and populations thereof exhibit reduced expression of one or more MHC class I molecules and/or MHC class II molecules. A further modification considered desirable for the cell therapy product is that the engineered cells and populations thereof exhibit increased expression of one or more tolerogenic factor.

[0866] Provided herein are methods and compositions for alleviating and/or evading the effects of immune system reactions to allogenic transplants. To overcome the problem of immune rejection of cell-derived and/or tissue transplants, disclosed herein is an engineered immune- evasive cell, or population or pharmaceutical composition thereof, that represents a viable source for any transplantable cell type. The engineered cells disclosed herein provide for reduced recognition the recipient subject's immune system, regardless of the subject's genetic make-up, or any existing response within the subject to one or more previous allogeneic transplants, previous autologous chimeric antigen receptor (CAR) T rejection, and/or other autologous or allogenic therapies wherein a transgene is expressed. The engineered cells may include, but are not limited to, T cells, NK cells, and blood cells.

[0867] Described here are engineered cells that comprise one or more modifications. In some embodiments, the provided engineered cells also contain a modification of one or more target polynucleotide sequences that regulates the expression of one or more MHC

class I molecules, one or more MHC class II molecules, or one or more MHC class I molecules and one or more MHC class II molecules.

[0868] In some embodiments, the provided engineered cells also include a modification to increase expression of one or more tolerogenic factor. In some embodiments, the tolerogenic factor is one or more of A20/TNFAIP3, B2M-HLA-E, CD16, CD16 Fc receptor, CD24, CD27, CD35, CD39, CD46, CD47, CD52, CD55, CD59, CD64, CD200, CCL21, CCL22, CTLA4-Ig, Cl inhibitor, CR1, DUX4, FASL, HLA-C, HLA-E, HLA-E heavy chain, HLA-F, HLA-G, H2-M3, IDO1, IL-10, IL15-RF, IL-35, IL-39, MANF, Mfge8, PD-L1, Serpinb9, or any combination thereof.

iii. Safety Features

1. Safety Switch

[001] In some embodiments, the cells comprise a "suicide gene" or "suicide switch". A suicide gene or suicide switch can be incorporated to function as a "safety switch" that can cause the death of the engineered cell (*e.g.,* primary engineered cell or cell differentiated from an engineered pluripotent stem cell), such as after the engineered cell is administered to a subject and if they cells should grow and divide in an undesired manner. The "suicide gene" ablation approach includes a suicide gene in a gene transfer vector encoding a protein that results in cell killing only when activated by a specific compound. A suicide gene may encode an enzyme that selectively converts a nontoxic compound into highly toxic metabolites. The result is specifically eliminating cells expressing the enzyme. In some embodiments, the suicide gene is the herpesvirus thymidine kinase (HSV-tk) gene, and the trigger is ganciclovir. In other embodiments, the suicide gene is the Escherichia coli cytosine deaminase (EC-CD) gene, and the trigger is 5 -fluorocytosine (5-FC) (Barese et al, Mol. Therap. 20(10): 1932-1943 (2012), Xu et al, Cell Res. 8:73-8 (1998), both incorporated herein by reference in their entirety).

[002] In other embodiments, the suicide gene is an inducible Caspase protein. An inducible Caspase protein comprises at least a portion of a Caspase protein capable of inducing apoptosis. In some embodiments, the inducible Caspase protein is iCasp9. It comprises the sequence of the human FK506-binding protein, FKBP12, with an F36V mutation, connected through a series of amino acids to the gene encoding human caspase 9. FKBP12-F36V binds with high affinity to a small-molecule dimerizing agent, API 903. Thus, the suicide function of iCasp9 is triggered by the administration of a chemical inducer of dimerization (CID). I n

some embodiments, the CID is the small molecule drug API 903. Dimerization causes the rapid induction of apoptosis. (See WO2011146862; Stasi et al, N. Engl. J. Med 365; 18 (2011); Tey et al, Biol. Blood Marrow Transplant. 13:913-924 (2007), each of which are incorporated by reference herein in their entirety.)

[003] Inclusion of a safety switch or suicide gene allows for controlled killing of the cells in the event of cytotoxicity or other negative consequences to the recipient, thus increasing the safety of cell-based therapies, including those using the engineered poliypeptides disclosed herein.

[004] In some embodiments, a safety switch can be incorporated into, such as introduced into, the engineered cells provided herein to provide the ability to induce death or apoptosis of engineered cells containing the safety switch, for example if the cells grow and divide in an undesired manner or cause excessive toxicity to the host. Thus, the use of safety switches enables one to conditionally eliminate aberrant cells in vivo and may improve the use of certain cell therapies in the clinic. Safety switches and their uses thereof are described in, for example, Duzgune§, Origins of Suicide Gene Therapy (2019); Duzgune§ (eds), Suicide Gene Therapy. Methods in Molecular Biology, vol. 1895 (Humana Press, New York, NY) (for HSV-tk, cytosine deaminase, nitroreductase, purine nucleoside phosphorylase, and horseradish peroxidase); Zhou and Brenner, Exp Hematol 44(11): 1013-1019 (2016) (for iCaspase9); Wang et al., Blood 18(5): 1255-1263 (2001) (for huEGFR); U.S. Patent Application Publication No. 20180002397 (for HERl); and Philip et al., Bloodl24(8): 1277-1287 (2014) (for RQR8).

[005] In some embodiments, the safety switch can cause cell death in a controlled manner, for example, in the presence of a drug or prodrug or upon activation by a selective exogenous compound. In some embodiments, the safety switch is selected from the group consisting of herpes simplex virus thymidine kinase (HSV-tk), cytosine deaminase (CyD), nitroreductase (NTR), purine nucleoside phosphorylase (PNP), horseradish peroxidase, inducible caspase 9 (iCasp9), and rapamycin-activated caspase 9 (rapaCasp9).

[006] In some embodiments, the safety switch may be a transgene encoding a product with cell killing capabilities when activated by a drug or prodrug, for example, by turning a nontoxic prodrug to a toxic metabolite inside the cell. In these embodiments, cell killing is activated by contacting an engineered cell with the drug or prodrug. In some cases, the safety switch is HSV- tk, which converts ganciclovir (GCV) to GCV-triphosphate, thereby interfering with DNA synthesis and killing dividing cells. In some cases, the safety switch is

CyD or a variant thereof, which converts the antifungal drug 5 -fluorocytosine (5-FC) to cytotoxic 5 -fluorouracil (5-FU) by catalyzing the hydrolytic deamination of cytosine into uracil. 5-FU is further converted to potent anti-metabolites (5- FdUMP, 5-FdUTP, 5-FUTP) by cellular enzymes. These compounds inhibit thymidylate synthase and the production of RNA and DNA, resulting in cell death. In some cases, the safety switch is NTR or a variant thereof, which can act on the prodrug CB 1954 via reduction of the nitro groups to reactive N-hydroxylamine intermediates that are toxic in proliferating and nonproliferating cells. In some cases, the safety switch is PNP or a variant thereof, which can turn prodrug 6-methylpurine deoxyriboside or fludarabine into toxic metabolites to both proliferating and nonproliferating cells. In some cases, the safety switch is horseradish peroxidase or a variant thereof, which can catalyze indole-3 -acetic acid (IAA) to a potent cytotoxin and thus achieve cell killing.

[007] In some embodiments, the safety switch may be an iCasp9. Caspase 9 is a component of the intrinsic mitochondrial apoptotic pathway which, under physiological conditions, is activated by the release of cytochrome C from damaged mitochondria. Activated caspase 9 then activates caspase 3, which triggers terminal effector molecules leading to apoptosis. The iCasp9 may be generated by fusing a truncated caspase 9 (without its physiological dimerization domain or caspase activation domain) to a FK506 binding protein (FKBP), FKBP12- F36V, via a peptide linker. The iCasp9 has low dimer-independent basal activity and can be stably expressed in host cells (*e.g.,* human T cells) without impairing their phenotype, function, or antigen binding. However, in the presence of chemical inducer of dimerization (CID), such as rimiducid (API 903), AP20187, and rapamycin, iCasp9 can undergo inducible dimerization and activate the downstream caspase molecules, resulting in apoptosis of cells expressing the iCasp9. See, *e.g.,* PCT Application Publication No. WO2011/146862; Stasi et al., N. Engl. J. Med. 365; 18 (2011); Tey et al., Biol. Blood Marrow Transplant 13:913-924 (2007). For example, the rapamycininducible caspase 9 variant is called rapaCasp9. See Stavrou et al., Mai. Ther. 26(5): 1266- 1276 (2018). Thus, iCasp9 can be used as a safety switch to achieve controlled killing of the host cells.

2. Graft Versus Host Disease (GVHD)

[0869] In some embodiments, the cells expressing the engineered polypeptide of the disclosure do not comprise a functional endogenous TCR. T cell therapies have shown

clinical efficacy in bone marrow and organ transplantation, cancer immunotherapy, viral infections, and autoimmune diseases. Unfortunately, T cells may also engage in deleterious side effects. "On-target but off-tumor" adverse events have been reported in cancer immunotherapy clinical trials using both engineered T cell receptor (TCR) T cells and engineered chimeric antigen receptor (CAR) T cells. These include B cell aplasia in chronic lymphocytic leukemia patients treated with T cells expressing a CD19 CAR, fatal acute respiratory distress syndrome after anti-ERBB2 CAR T cell infusion thought to result from cross- reactivity on lung epithelium, and TCR-induced fatalities from cardiac myonecrosis or neurological toxicity incurred in patients treated with TCRs recognizing cancer-testis antigens. Similarly, the curative gains of donor lymphocyte infusion (DLI) in allogeneic bone marrow transplantation are hampered by the induction of both acute and chronic graft-versus-host disease (GVHD) and bone marrow aplasia.

[0870] In some embodiments, cells of the present disclosure comprise shRNA targeting TRAC, TRBC1, TRBC2, and/or CD3, thereby generating a cell that does not comprise a functional endogenous TCR. In some embodiments, cells of the present disclosure have been modified, using methods described herein, to eliminate expression of TRAC, TRBC1, TRBC2, and/or CD3, thereby generating a cell that does not comprise a functional endogenous TCR.

3. Depletion Tag

[0871] In some embodiments, the safety switch may be a membrane-expressed protein which allows for cell depletion after administration of a specific antibody to that protein. Safety switches of this category may include, for example, one or more transgene encoding CCR4, CD16, CD19, CD20, CD30, EGFR, GD2, HER1, HER2, MUC1, PSMA, or RQR8 for surface expression thereof. These proteins may have surface epitopes that can be targeted by specific antibodies. In some embodiments, the safety switch comprises CCR4, which can be recognized by an anti-CCR4 antibody. Non-limiting examples of suitable anti-CCR4 antibodies include mogamulizumab and biosimilars thereof. In some embodiments, the safety switch comprises CD16 or CD30, which can be recognized by an anti-CD16 or anti-CD30 antibody. Non-limiting examples of such antiCD16 or anti-CD30 antibody include AFM13 and biosimilars thereof. In some embodiments, the safety switch comprises CD19, which can be recognized by an anti-CD19 antibody. Non-limiting examples of such anti-CD19 antibody include MOR208 and biosimilars thereof. In some embodiments, the safety

switch comprises CD20, which can be recognized by an anti-CD20 antibody. Non-limiting examples of such anti-CD20 antibody include binutuzumab, ublituximab, ocaratuzumab, rituximab, rituximab-Rllb, and biosimilars thereof. Cells that express the safety switch are thus CD20-positive and can be targeted for killing through administration of an anti- CD20 antibody as described. In some embodiments, the safety switch comprises EGFR, which can be recognized by an anti-EGFR antibody. Non-limiting examples of such anti-EGFR antibody include tomuzotuximab, RO5083945 (GA201), cetuximab, and biosimilars thereof. In some embodiments, the safety switch comprises GD2, which can be recognized by an anti-GD2 antibody. Non-limiting examples of such anti-GD2 antibody include Hul4.18K322A, Hul4.18- IL2, Hu3F8, dinituximab, c.60C3-Rllc, and biosimilars thereof. In some embodiments, the safety switch may be an exogenously administered agent that recognizes one or more tolerogenic factor on the surface of the engineered cell. In some embodiments, the exogenously administered agent is an antibody directed against or capable of binding a tolerogenic agent (*e.g.,* an anti-CD47 antibody). By recognizing and blocking a tolerogenic factor on the engineered cell, an exogenously administered antibody may block the immune inhibitory functions of the tolerogenic factor thereby re-sensitizing the immune system to the engineered cells. For instance, for an engineered cell that overexpresses CD47 an exogenously administered anti-CD47 antibody may be administered to the subject, resulting in masking of CD47 on the engineered cell and triggering of an immune response to the engineered cell.

k. Reduced Expression of Target genes

[0872] In some embodiments, knocking down (*e.g.,* decreasing, eliminating, or inhibiting) gene expression can be achieved by RNA silencing or RNA interference (RNAi). Useful RNAi methods include those that utilize synthetic RNAi molecules, short interfering RNAs (siRNAs), PlWI-interacting NRAs (piRNAs), short hairpin RNAs (shRNAs), microRNAs (miRNAs), and other transient knock down methods recognized by those skilled in the art. Reagents for RNAi including sequence specific shRNAs, siRNA, miRNAs and the like are commercially available.

XIII. COMPOSITIONS

[0873] In another aspect, the present disclosure provides a composition comprising the recombinant polypeptide of the disclosure. In another aspect, the present disclosure provides a composition comprising the engineered cells comprising the recombinant nucleic acid encoding the recombinant polypeptide of the disclosure, and/or a vector comprising the recombinant nucleic acid.

[0874] The disclosure additionally provides compositions comprising or consisting of a population of engineered cells, within which some portion of the engineered cells express a recombinant polypeptide of the disclosure. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a gene product of an endogenous TRAC locus. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express a recombinant polypeptide disclosed herein; at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the CAR or recombinant TCR; and/or at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a gene product of an endogenous TRAC locus.

[0875] In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide of the disclosure. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the receptor (*e.g.,* the CAR, T-body, HIT receptor, or recombinant TCR). In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus.

[0876] In some embodiments, the composition comprises CD4+ T cells and CD8+ T cells. In some embodiments of a composition comprising CD4+ T cells and CD8+ T cells, the

percentage of CD4+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the percentage of CD8+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition. In some embodiments, the ratio of CD4+ T cells to CD8+ T cells is from at or about 1:3 to at or about 3:1, optionally at or about 1:1.

[0877] As used herein, the term "composition" includes, but is not limited to, a pharmaceutical composition. A "pharmaceutical composition" refers to an active pharmaceutical agent formulated in pharmaceutically acceptable or physiologically acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy. It will also be understood that, if desired, the compositions of the invention may be administered in combination with other agents, such as, *e.g.,* cytokines, growth factors, hormones, small molecules, chemotherapeutics, pro-drugs, drugs, antibodies, or other various pharmaceutically active agents. There is virtually no limit to other components that may also be included in the compositions, provided that the additional agents do not adversely affect the ability of the composition to deliver the intended therapy. The phrase "pharmaceutically acceptable" is used herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0878] The compositions may also comprise a pharmaceutically acceptable carrier, diluent, or excipient. As used herein "pharmaceutically acceptable carrier, diluent, or excipient" includes, without limitation, any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose, and sucrose; starches, such as com starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; tragacanth; malt; gelatin; talc; cocoa butter; waxes; animal and vegetable fats; paraffins; silicones; bentonites; silicic acid; zinc oxide; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil,

olive oil, corn oil, and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol, and polyethylene glycol; esters, such as ethyl oleate, and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and any other compatible substances employed in pharmaceutical formulations.

[0879] The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline; Ringers solution; isotonic sodium chloride; fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium; polyethylene glycols; glycerin; propylene glycol or other solvents; antibacterial agents, such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents, such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates, or phosphates; and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

[0880] The composition may be suitably developed for intravenous, intratumoral, oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration.

[0881] In some embodiments, the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure and recombinant cells of the disclosure can be incorporated into compositions, including pharmaceutical compositions. Such compositions generally include the recombinant nucleic acids, recombinant polypeptides, and/or recombinant cells of the disclosure, and a pharmaceutically acceptable excipient, *e.g.,* carrier.

[0882] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™. (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, the composition should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a

solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.,* sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be generally to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[0883] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0884] Systemic administration of the subject recombinant nucleic acid constructs and/or recombinant nucleic acids and recombinant cells of the disclosure can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0885] In some embodiments, the recombinant nucleic acid constructs and/or recombinant nucleic acids and recombinant cells of the disclosure can also be administered by transfection or infection using methods known in the art, including but not limited to the methods described in McCaffrey et al. (Nature 418:6893, 2002), Xia et al. (Nature

Biotechnol. 20: 1006-1010, 2002), or Putnam (Am. J. Health Syst. Pharm. 53: 151-160, 1996, erratum at Am. J. Health Syst. Pharm. 53:325, 1996).

[0886] In some embodiments, the recombinant nucleic acid constructs and/or recombinant nucleic acids and recombinant cells of the disclosure are prepared with carriers that will protect the recombinant polypeptides against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811. As described in greater detail below, the polypeptides of the present disclosure may also be modified to achieve extended duration of action such as by PEGylation, acylation, Fc fusions, linkage to molecules such as albumin, etc. In some embodiments, the recombinant polypeptides can be further modified to prolong their half-life in vivo and/or ex vivo. Non-limiting examples of known strategies and methodologies suitable for modifying the recombinant polypeptides of the disclosure include (1) chemical modification of a recombinant polypeptide described herein with highly soluble macromolecules such as polyethylene glycol ("PEG") which prevents the recombinant polypeptides from contacting with proteases; and (2) covalently linking or conjugating a recombinant polypeptide described herein with a stable protein such as, for example, albumin. Accordingly, in some embodiments, the polypeptides of the disclosure can be fused to a stable protein, such as, albumin. For example, human albumin is known as one of the most effective proteins for enhancing the stability of polypeptides fused thereto and there are many such fusion proteins reported.

[0887] In some embodiments, the pharmaceutical compositions of the disclosure include one or more PEGylation reagents. In some embodiments, the PEGylation reagent is selected from methoxy polyethylene glycol-succinimidyl propionate (mPEG-SPA), mPEG-succinimidyl butyrate (mPEG-SBA), mPEG-succinimidyl succinate (mPEG-SS), mPEG-succinimidyl carbonate (mPEG-SC), mPEG-Succinimidyl Glutarate (mPEG-SG), mPEG-N-hydroxyl-succinimide (mPEG-NHS), mPEG-tresylate and mPEG-aldehyde. In some

embodiments, the PEGylation reagent is polyethylene glycol. In some embodiments, the PEGylation reagent is polyethylene glycol with an average molecular weight of 20 kD covalently bound to the N-terminal methionine residue of the recombinant polypeptides of the disclosure., or about 80 kD covalently bound to the N-terminal methionine residue of the polypeptides of the disclosure. In some embodiments, the PEGylation reagent is polyethylene glycol with an average molecular weight of about 40 kD covalently bound to the N-terminal methionine residue of the polypeptides of the disclosure.

[0888] Accordingly, in some embodiments, recombinant nucleic acid constructs and/or recombinant nucleic acids and recombinant cells of the disclosure are chemically modified with one or more polyethylene glycol moieties, *e.g.,* PEGylated; or with similar modifications, e.g. PASylated. In some embodiments, the PEG molecule or PAS molecule is conjugated to one or more amino acid side chains of the disclosed recombinant polypeptide. In some embodiments, the PEGylated or PASylated polypeptide contains a PEG or PAS moiety on only one amino acid. In other embodiments, the PEGylated or PASylated polypeptide contains a PEG or PAS moiety on two or more amino acids, *e.g.,* attached to two or more, five or more, ten or more, fifteen or more, or twenty or more different amino acid residues. In some embodiments, the PEG or PAS chain is 2000, greater than 2000, 5000, greater than 5,000, 10,000, greater than 10,000, greater than 10,000, 20,000, greater than 20,000, and 30,000 Da. The PASylated polypeptide may be coupled directly to PEG or PAS (*e.g.,* without a linking group) through an amino group, a sulfhydryl group, a hydroxyl group, or a carboxyl group. In some embodiments, the recombinant polypeptide of the disclosure is covalently bound to a polyethylene glycol with an average molecular weight of 20,000 Daltons. In some embodiments, the recombinant polypeptide of the disclosure is covalently bound to a polyethylene glycol with an average molecular weight ranging from about 1 kD to about 200 kD such as, *e.g.,* about 10 kD to about 150 kD, about 50 kD to about 100 kD, about 5 kD to about 100 kD, about 20 kD to about 80 kD, about 30 kD to about 70 kD, about 40 kD to about 60 kD, about 50 kD to about 100 kD, about 100 kD to about 200 kD, or about 1 150 kD to about 200 kD. In some embodiments, the recombinant polypeptide of the disclosure is covalently bound to a polyethylene glycol with an average molecular weight of about 5 kD, about 10 kD, about 20 kD, about 30 kD, about 40 kD, about 50 kD, about 60 kD, about 70 kD, or about 80 kD. In some embodiments, the recombinant polypeptide of the disclosure is covalently bound to a polyethylene glycol with an average molecular weight of about 40 kD.

XIV. KITS, COMPONENTS, AND ARTICLES OF MANUFACTURE

[0889] Systems or kits of the present disclosure include one or more of any of the recombinant nucleic acids, recombinant polypeptides, engineered cells, or pharmaceutical compositions disclosed herein as well as syringes (including pre-filled syringes) and/or catheters (including pre-filled syringes) used to administer any of the recombinant nucleic acids, recombinant polypeptides, engineered cells, or pharmaceutical compositions to a subject. The kits also include written instructions for using of any of the recombinant nucleic acids, recombinant polypeptides, engineered cells, or pharmaceutical compositions disclosed herein as well as syringes and/or catheters for use with their administration.

[0890] Any of the above-described systems and kits can further include one or more additional reagents, where such additional reagents can be selected from: dilution buffers; reconstitution solutions, wash buffers, control reagents, control expression vectors, negative control polypeptides, positive control polypeptides, reagents for *in vitro* production of the chimeric receptor polypeptides.

[0891] In some embodiments, the components of a system or kit can be in separate containers. In some other embodiments, the components of a system or kit can be combined in a single container.

[0892] In some embodiments, a system or kit can further include instructions for using the components of the kit to practice the methods. The instructions for practicing the methods are generally recorded on a suitable recording medium. For example, the instructions can be printed on a substrate, such as paper or plastic, etc. The instructions can be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or sub-packaging), etc. The instructions can be present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, flash drive, etc. In some instances, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (*e.g.,* via the internet), can be provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions can be recorded on a suitable substrate.

XV. METHODS OF THE DISCLOSURE

[0893] The disclosure also relates to methods including the recombinant polypeptides, recombinant nucleic acids, vectors, particles, or the cells of the disclosure.

Methods for Preparing Immune Cells (e.g., T cells) for Use in Cell Therapy

[0894] The disclosure also provides methods for preparing an immune cell (*e.g.,* T cell) for use in cell therapy. The immune cell (*e.g.,* T cell) can further comprise a CAR, a TCR, and/or a transcriptional receptor. The immune cell can be an NK cell or a tumor infiltrating lymphocyte from a patient with cancer. Preparing the cell can include introducing (*e.g.,* by transducing) into a cell any one or more of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure. Preparing the cell can include introducing (*e.g.,* by transducing) into a cell any one or more vectors comprising any one or more of the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure. Preparing the cell can include contacting the cell with any one or more particles comprising any one or more of the recombinant nucleic acids of the disclosure. Preparing the cell can include introducing to the cell any one or more of the recombinant nucleic acids of the disclosure. Preparing the cell can include expressing in the cell any one or more of the polypeptides of the disclosure. The cell can be genetically modified for expression of the polypeptide. The cell can further comprise expression of an engineered receptor that binds to a target in a tumor cell.

[0895] In some methods of preparing an engineered cell (*e.g.,* an immune cell), the method comprises expressing in the cell: (a) the recombinant polypeptide of the disclosure; or (b) at least the following two polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope. In some embodiments, the method comprises enriching the engineered cells expressing the recombinant polypeptide using a binding agent that binds to the peptide epitope or mimotope; optionally, wherein the binding agent comprises a monoclonal antibody. In some embodiments, at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

[0896] In some embodiments, the cell is in vivo. In some embodiments, the cell is prepared in vivo. In some embodiments, the cell is prepared in the body of a subject in need of treatment.

[0897] In some embodiments, the recombinant polypeptide is expressed in the cell, and/or the nucleic acid construct is introduced into the cell, by contacting the cell with a vector or a particle. In some embodiments, the vector is a plasmid, a synthetic DNA vector, a single stranded DNA vector,a double stranded DNA vector, a linear DNA vector, a closed linear DNA vector, a synthetic RNA vector, a phagemid vector, an RNA vector, an mRNA vector, or a viral vector. In some embodiments, the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector. In some embodiments, the retrovirus is a lentivirus, optionally a VSV-G pseudotyped lentivirus. In some embodiments, the AAV vector is an AAV6 vector or an AAV9 vector. In some embodiments, the vector is a fusosome, a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein, or an enveloped delivery vehicle. In some embodiments, the vector is a self-replicating RNA virus, an mRNA-packaging virus-like particle, or a ribonucleoprotein (RNP)-packaging virus-like particle.

[0898] In some embodiments, the particle is a lipid nanoparticle (LNP), a selective organ targeting (SORT) LNP, or an antibody targeted LNP. In some embodiments, the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid). In some embodiments, the particle is a polymer nanoparticle or a protein nanoparticle.

[0899] In some embodiments, the cell is ex vivo. In some embodiments, the cell is prepared ex vivo. In some embodiments, the cell is prepared outside of the body of a subject in need of treatment. In some embodiments, the nucleic acid construct is introduced into the cell by electroporation or transfection. In some embodiments, the transfection is lipofection. In some embodiments, the nucleic acid construct is a plasmid, a synthetic DNA vector, a single stranded DNA vector, a double stranded DNA vector, a linear DNA vector, a closed linear DNA vector, a synthetic RNA vector, a phagemid vector, an RNA vector, or an mRNA vector. In some embodiments, the nucleic acid construct is introduced via a viral vector. In some embodiments, the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector. In some embodiments, the retrovirus is a lentivirus,

optionally a VSV-G pseudotyped lentivirus. In some embodiments, the AAV vector is an AAV6 vector or an AAV9 vector.

[0900] In some embodiments, the method comprises contacting the cell with an AAV vector comprising a recombinant nucleic acid encoding: (a) arecombinant polypeptide of the disclosure or (b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide.

[0901] In some embodiments, the method comprises contacting the cell with a particle comprising a recombinant nucleic acid encoding: (a) a recombinant polypeptide of the disclosure, or (b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide; optionally, wherein the particle is a lipid nanoparticle (LNP).

[0902] In some embodiments, the method comprises genetically modifying the cell for expression of the recombinant polypeptide. In some embodiments, the method comprises introducing into the cell one or more nucleic acids encoding a sequence specific nuclease or a nucleic acid programmable DNA binding protein. In some embodiments, the method additionally comprises introducing into the cell one or more guide RNAs.

[0903] In some embodiments, the sequence-specific nuclease is an RNA guided nuclease. In some embodiments, the equence-specific nuclease or nucleic acid programmable DNA binding domain is a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

[0904] In some embodiments, the Cas nuclease is Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2,

Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional portion thereof . In some embodiments, the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

[0905] In some embodiments, the method comprises introducing into the cell a ribonucleoprotein (RNP) complex comprising the sequence-specific nuclease or nucleic acid programmable DNA binding. In some embodiments, introducing the nucleic acid construct comprises contacting the cell with a particle or a vector disclosed herein. In some embodiments, the nucleic acid construct is introduced via electroporation. In some embodiments, the nucleic acid construct is introduced via AAV. In some embodiments, the nucleic acid construct is introduced via lentivirus, optionally a VSV-G pseudotyped lentivirus. In some embodiments, the nucleic acid construct is introduced via LNP.

[0906] In some embodiments, the nucleic acid construct is inserted into an endogenous locus of the cell. In some embodiments, the endogenous locus is or comprises a TRAC gene locus, a TRBC1 gene locus, a TRBC2 gene locus, a TRAJ locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, or a PIK3R3 locus. the TRAJ locus is or comprises a TRAJ intron splice acceptor locus. In some embodiments, the endogenous locus is or comprises a TRAC gene locus. In some embodiments, the endogenous locus is or comprises a TRAJ locus. In some embodiments, the TRAJ locus is or comprises a TRAJ intron splice acceptor locus. In some embodiments, the endogenous locus is or comprises a safe harbor locus. In some embodiments, the afe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus. In some embodiments, the first insertion site is an exon. In some embodiments, the first insertion site is an intron. In some embodiments, the first insertion site is between an intron and an exon. In some embodiments, the first insertion site is in a regulatory region.

[0907] In some embodiments, the first insertion site is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is ngg, nag, ngrrt, ngrm, nnnngatt, nnnnryac, nnagaaw, naaaac, tttv, ttn, attn, tttn, gttn, or yttn and wherein: (i) r = a or g, (ii) y = c or t, (iii) w = a or t, (iv) v = a or c or g, and (v) n = a, c, t, or g.

[0908] In some embodiments, the cell produced by the method comprises a modified TRAC gene locus. In some embodiments, the cell has reduced or eliminated surface expression of an endogenous TCR.

[0909] In some embodiments, the method further comprises introducing a gene editing machinery into the cell. In some embodiments, the step of introducing comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery. In some embodiments, the gene editing machinery is CRISPR/Cas9. In some embodiments, the gene editing machinery is introduced via electroporation. In some embodiments, the nucleic acid construct is introduced via AAV. In some embodiments, the nucleic acid construct is introduced via LNP. In some embodiments, the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the engineered receptor (*e.g.,* CAR); and (iii) the modified TRAC gene locus. In some embodiments, method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.,* CAR); and (iii) the modified TRAJ locus. In some embodiments, the cell is for use in a cell therapy.

[0910] In some embodiments, the cells can be transduced with a recombinant nucleic acid construct that can alter T cell signaling via the NFAT pathway, NF-κB pathway, AP-1 pathway, JAK/STAT pathway, RAS/MEK/ERK, phospholipase gamma signaling or other T cell signaling pathways.

[0911] The methods of preparing an immune cell (*e.g.,* T cell) according to the disclosure can include transducing immune cells with recombinant nucleic acid constructs and/or recombinant nucleic acids capable of enhancing or promoting or improving or reducing or regulating or modulating the pathway or activating or increasing or suppressing or inhibiting or other means of changing the immune cell signaling. The methods of preparing an immune cell (*e.g.,* T cell) according to the disclosure can include transducing cells with recombinant nucleic acid constructs and/or recombinant nucleic acids that can alter CARD11-BCL10-MALT1 complex signaling, co-stimulatory molecule signaling, and cytokine production and/or transcription factor activity in the immune cell (*e.g.,* T cell) .

[0912] In an embodiment, the method of preparing an immune cell (*e.g.,* T cell) for use in a cell therapy includes transducing the immune cell with a recombinant nucleic acid construct and/or recombinant nucleic acid having a mutation capable of altering (i) immune cell signaling through NFAT, NF-κB and/or AP-1 pathways, (ii) cytokine production, and/or (iii) *in vivo* persistence of immune cells in tumors.

[0913] In some embodiments of the methods of preparing an immune cell (*e.g.,* T cell) for use in a cell therapy includes polypeptides and/or recombinant nucleic acid constructs and/or recombinant nucleic acids that can alter *in vivo* persistence in tumors of therapeutic immune cells including the mutation. In some embodiments, the recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter the therapeutic efficacy, decrease immune cell exhaustion, increase proliferative capacity, enhance anti-tumor effect, enhance the replicative lifespan, decrease replicative senescence, and enhance ability to kill, enhance the fitness of engineered immune cells and/or other functions or activities of immune cells.

[0914] In some embodiments, the polypeptides or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure can alter the *in vivo* persistence in tumors, *in vivo* accumulation in tumors, or the other functions or activities of immune cells described herein, through a mutation in one or more of the genes described herein. In some embodiments, the genes include caspase recruitment domain family member 11 (CARD11). In some embodiments, the genes include capping protein regulator and myosin 1 linker 2 (CARMIL2), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), B-cell lymphoma 6 (BCL6), B-cell lymphoma 10 (BCL10) and MYCN. The gene can be a signal transducer and activator of transcription gene (STAT) including STAT3, STAT5B. The gene can be a janus kinase gene such as JAK1, JAK2, or JAK3 gene. The gene can be v-raf murine sarcoma viral oncogene homolog B1 (BRAF). The gene can be RAS Guanyl Releasing Protein 1 (RASGRP1). The gene can be Tumor necrosis factor receptor superfamily member 1B (TNFRSF1B). The gene can be phospholipase C gamma 1 (PLCG1) gene. The gene can be nuclear factor kappa light chain enhancer of activated B cells (NF-κB1), or (NF-κB2) or JunB Proto-Oncogene, AP-1 Transcription Factor Subunit (JUNB) gene.

[0915] The disclosure also provides methods of enriching and/or isolating engineered cells disclosed herein. In some embodiments, the method comprises contacting the engineered cells with a binding agent that binds to the epitope or the mimotope. In some embodiments, the binding agent comprises an antibody. In some embodiments, the binding agent comprises a purification marker; optionally, wherein the purification marker comprises biotin, avidin, a myc tag, an influenza hemagglutinin (HA) tag, a FLAG tag, a glutathione-S transferase (GST) tag, a V5 tag, a poly-Histidine (His) tag, a Fc tag, calmodulin binding

protein (CBP), maltose-binding protein (MBP), thioredoxin, or a chitin-binding protein. In some embodiments, the binding agent is bound to a solid substrate or a purification bead.

[0916] In some embodiments, the method results in a population of purified engineered cells in which at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cell expresses the recombinant polypeptide.

[0917] The discosure also provides methods of measuring the number of the engineered cells disclosed herein. In some embodiments, the method comprises administering, or contacting the engineered cells with, a binding agent that binds to the epitope or the mimotope. In some embodiments, the binding agent comprises an antibody.

Methods of Treating a Subject

[0918] The disclosure also provides methods of treating a subject in need of cell therapy, comprising administering to the subject a cell (*e.g.,* T-cell) as described herein or a cell (*e.g.,* T cell) prepared by any of the methods described herein. The disclosure also provides methods for enhancing the *in vivo* persistence of a cell (*e.g.,* a T cell, or specifically a therapeutic T cell) in a subject in need thereof, by administering to the subject a therapeutically effective amount of the cells of the disclosure. The cells can comprise any of the polypeptides and/or recombinant nucleic acid constructs and/or recombinant nucleic acids of the disclosure.

[0919] In some embodiments, the method comprises, before the administration, generating engineered cells according to a method of preparing an engineered cell described herein. In some embodiments, the engineered cells are derived from immune cells: (i) obtained and/or isolated from the subject, (ii) obtained and/or isolated from one or more donors, and/or (iii) derived from one or more stem cells.

[0920] The disclosure also provides methods of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering a therapeutically effective number of the engineered cells disclosed herein.

[0921] In some embodiments, the method of treating an autoimmune disease in a subject in need thereof comprises administering an effective amount of a recombinant nucleic, a vector, or a particle disclosed herein, to the subject. In some embodiments, the peptide epitope or mimotope is bound by an antibody for an antigen associated with the autoimmune disease. In some embodiments, the autoimmune disease is lupus, systemic lupus

erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjogren's syndrome, Hashimoto's thyroiditis, or celiac disease.

[0922] In some embodiments, the method of treating cancer in a subject in need thereof comprises administering an effective amount of a recombinant nucleic, a vector, or a particle disclosed herein, to the subject. In some embodiments, the peptide epitope or mimotope is capable of being bound by antibody for an antigen associated with the cancer.

[0923] The cancer may be any kind of cancer known in the art. In some embodiments, the cancer is a hematological cancer. Non-limiting examples of hematological cancer include myeloid neoplasm, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blast crisis chronic myelogenous leukemia (bcCML), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, or B-cell lymphoma. In some embodiments, the cancer is a solid tumor. Non-limiting examples of a solid tumor include small cell lung cancer, colorectal cancer, testicular cancer, ovarian cancer, melanoma, lymphoma, leukemia, multiple myeloma, prostate cancer, breast cancer, non-small cell lung cancer, gastric cancer, esophageal cancer, liver cancer, kidney cancer, head & neck cancer, glioblastoma, neuroblastoma, soft tissue sarcoma, uterine cancer, brain cancer, skin cancer, renal cancer, bladder cancer, pancreatic cancer, thyroid cancer, eye cancer, gastrointestinal cancer, carcinoma, or sarcoma.

[0924] The cancer may express one or more cancer-associated antigen. Non-limiting examples of cancer-associated antigens include CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation

antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, ggcc, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combination thereof. In some embodiments, the cancer expresses B7H3, BCMA, CD19, CD20, CD22, CD70, CD79a, CD79b, CLDN6, CLDN18.2, DLL3, GCC, GD2, GD3, GPC3, GPRC5D, KLK3, LY6G6D, p53R175H, PRAME, ROPN1, STEAP1, or STEAP2. In some embodiments, the cancer expresses KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

[0925] In some embodiments, the cancer is selected from Table 2. In some embodiments, the cancer and the corresponding cancer-associated antigen are selected from Table 2.

[0926] The disclosure also provides methods of treatment of a subject in need thereof, wherein the peptide epitope or mimotope is bound byan antibody. Contacting an engineered cell comprising a peptide epitope or mimotope or a recombinant polypeptide described herein can be useful to increase the safety or efficacy of the method of treatment.

[0927] In some embodiments, the peptide epitope or mimotope is bound by an antibody for a cancer-associated antigen of T cell lymphoma. In some embodiments, the cancer-associated antigen of T cell lymphoma is CCR4, CCR7, or CD3. In some embodiments,

[0928] In some embodiments, the method further comprises administering an antibody to the subject, wherein the antibody binds the tag, the peptide epitope, or the mimotope.

[0929] In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; and (ii) CD3. In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; and (ii) CD16. In some embodiments, the antibody is a tri-specific or multi-specific antibody that is capable of specifically binding to (i) the peptide epitope or the mimotope; (ii) 41BB or albumin, and (ii) CD3.

[0930] In some embodiments, the antibody is tarlatamab.

[0931] In some embodiments, the antibody is mogamulizumab.

[0932] In some embodiments, the antibody is CAP-100.

[0933] Treatment with the engineered cells of the disclosure may be associated with certain adverse events. The disclosure also provides methods of treatment wherein the method further comprises monitoring the subject for an adverse event. Non-limiting examples of adverse events include cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), hemophagocytic lymphohistiocytosis (HLH), on-target off-tumor toxicity, tumor lysis syndrome, lymphoproliferative disorder, splenomegaly, lymphadenopathy, an abnormal ratio of engineered cells to other blood cells, acute toxicity, potency enhancement mediated cytotoxicity, potency enhancement mediated GVHD, uncontrolled expansion of engineered cells, and engineered cell mediated lymphoma.

[0934] Following treatment with the engineered cells of the disclosure, certain treatment outcomes may occur. The disclosure also provides methods of treatment wherein the method further comprises monitoring the subject for one or more treatment outcomes. Non-limiting examples of treatment outcomes include reduction or elimination of the cancer or the autoimmune disease.

[0935] Upon observing one or more adverse events, depletion of the engineered cells of the disclosure may be advantageous (*e.g.,* for safety). The disclosure also provides methods of reducing or eliminating at least one symptom of an adverse event caused by engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells. In some embodiments, the antibody is administered after the detection or occurrence of an adverse event. In some

embodiments, administration of the antibody reduces or eliminates at least one symptom of the adverse event.

[0936] In some embodiments, the subject has been administered the engineered cells disclosed herein within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody. In some embodiments, the subject has been administered a recombinant nucleic acid, a vector, or particle of the disclosure within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

[0937] In some embodiments, the antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells expressing the tag, the peptide epitope, or the mimotope in the subject. In some embodiments, the antibody is administered after the detection or occurrence of an adverse event, and administration of the antibody reduces or eliminates at least one symptom of the adverse event. In some embodiments, the method additionally comprises administration of a treatment of the one or more adverse events known to one skilled in the art. In some embodiments, the method additionally comprises administration of a corticosteroid, tocilizumab, and/or anakyrin.

[0938] Upon observing one or more treatment outcomes, depletion of the engineered cells of the disclosure may be advantageous (*e.g.,* for safety). The disclosure also provides methods wherein the antibody is administered after the detection or occurrence of a treatment outcome. In some embodiments, the treatment outcome is reduction or elimination of the cancer or the autoimmune disease.

[0939] Following administration of the antibody, reduction or elimination of at least one symptom of the adverse event may occur. The disclosure also provides methods additionally comprises monitoring the subject for reduction or elimination of at least one symptom of the adverse event. In some embodiments, the monitoring comprises clinical laboratory tests of ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count. In some embodiments, the monitoring comprises imaging, wherein the imaging is PET, CT, MRI, and/or x-ray. In some embodiments, the monitoring comprises clinical assessment of body temperature, blood pressure, blood oxygenation, heart rate, cognitive status, and/or organ function. In some

embodiments, the monitoring comprises standardized assessment used for detecting or monitoring one or more adverse events. In some embodiments, the standardized assessment comprises the Immune Effector Cell–Associated Encephalopathy (ICE) assessment, the Cornell Assessment, and/or the Pediatric Delirium (CAPD) assessment. In some embodiments, the monitoring comprises any combination thereof.

[0940] In some embodiments, the method results in depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cells in the subject. In some embodiments, the method results in depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of regulatory T cells in the subject. In some embodiments, the method results in clinical laboratory results within the normal range for ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count. In some embodiments, the method results in imaging results indicating a reduction in cancer or reduction in at least one symptoms of an adverse event. In some embodiments, the method results in reduction or absence of fever, hypotension, hypoxia, tachycardia, aphasia, cognitive impairment, coagulopathy, respiratory failure, lymphadenopathy, and/or splenomegaly. In some embodiments, the method results in an improved score on a standardized assessment used for detecting or monitoring one or more adverse events. In some embodiments, the method results in the ability to reduce or eliminate administration of corticosteroids, tocilizumab, and/or anakyrin.

[0941] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event. In some emobdiments, the method results in higher therapeutic efficacy than a control method to reduce or eliminate the at least one symptom of the adverse event, optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin. In some embodiments, the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0942] In some embodiments, the adverse event is an acute adverse event. Non-limiting examples of acute adverse events include CRS, ICANS, HLH, on-target off-tumor toxicity, and tumor lysis.

[0943] In some embodiments, the antibody is a bi-specific or multi-specific antibody that is capable of specifically binding to the peptide epitope or the mimotope. In some embodiments, the bi-specific or multi-specific antibody is capable of specifically binding to (i) the peptide epitope or the mimotope, in addition to a second epitope or antigen. In some embodiments, the bi-specific or multi-specific antibody is capable of specifically binding to (i) the peptide epitope or the mimotope and (ii) CD3. In some embodiments, the bi-specific or multi-specific antibody is capable of specifically binding to (i) the peptide epitope or the mimotope and (ii) CD16. In some embodiments, the bi-specific or multi-specific antibody is capable of specifically binding to (i) the peptide epitope or the mimotope, (ii) 41BB or albumin, and (ii) CD3. In some embodiments, the antibody is tarlatamab. In some embodiments, the antibody is mogamulizumab. In some embodiments, the antibody is CAP-100.

[0944] The disclosure also provides methods of administering ranges or amounts of the antibody. In some embodiments, a dose of between 0.003 mg/kg and 10 mg/kg, between 0.01 mg/kg and 10 mg/kg, between 0.003 mg/kg and 0.3 mg/kg, between 0.05 mg/kg and 5 mg/kg, or between 0.1 mg/kg and 1 mg/kg of the antibody is administered to the subject. In some embodiments, a dose of between about 0.003 mg/kg and about 0.01 mg/kg, between about 0.01 mg/kg and about 0.03 mg/kg, between about 0.03 mg/kg and about 0.1 mg/kg, between about 0.1 mg/kg and about 0.2 mg/kg, between about 0.2 mg/kg and about 0.5 mg/kg, between about 0.5 mg/kg and about 1 mg/kg, between about 1 mg/kg and about 2 mg/kg, between about 2 mg/kg and about 5 mg/kg, or between about 5 mg/kg and about 10 mg/kg, of the antibody is administered to the subject. In some embodiments, a dose of between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg, of the antibody is administered to the subject.

[0945] In some embodiments, the antibody is administered to the subject once. In some embodiments, the antibody is administered to the subject more than once. In some embodiments, the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks.

[0946] In some embodiments, the method comprises (a) administering the engineered cell, the recombinant nucleic acid, the vector, or the particle into a brain of the subject; and (b) administering the antibody outside of the brain of the subject. In some embodiments, the method comprises intraventricular administration of the engineered cells, the recombinant nucleic acid, the vector, or the particle.

[0947] In some embodiments, the method comprises systemic administration of the antibody. In some embodiments, the method comprises intravenous administration of the antibody. In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell that have crossed the blood brain barrier than a control method that reduces or eliminates the at least one symptom of the adverse event. In some embodiments, the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0948] In some embodiments, the antibody is administered more than once. In some embodiments, the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks. In some embodiments, the antibody is administered more than once with an interval of at least 1, 2, 3, or 4 weeks. In some embodiments, the antibody is administered two, three, or four times.

[0949] In some embodiments, the antibody and the binding region of the recombinant polypeptide can specifically bind the same antigen.

[0950] Further descriptions of the antibody dosing regimen can be found, for example, in Sato et al., N Engl J Med. 2018 Feb 8;378(6):529-538; Meyerowitz et al., Clin Infect Dis. 2023 Sep 18;77(6):851-856; Sato et al., Brain. 2023 Aug 1;146(8):3181-3191; Sato et al., J Neurol. 2024 Mar 2 (advanced publication; doi: 10.1007/s00415-024-12239-x); world wide web (www): accessdata.fda.gov/drugsatfda_docs/label/2018/761051s000lbl.pdf; world wide web (www): accessdata.fda.gov/drugsatfda_docs/label/2023/761342s000lbl.pdf; the content of each of which is herein incorporated by reference in its entirety.

[0951] In some embodiments, the antibody is mogamulizumab, and the method further comprises administering one or more of: (a) at least one retinoid; (b) interferon alpha; and/or (c) extracorporeal photopheresis (ECP). In some embodiments, the retinoid and/or interferon alpha is delivered systemically. In some embodiments, the method comprises administering mogamulizumab and extracorporeal photopheresis (ECP). In some embodiments, the

method comprises administering mogamulizumab and interferon alpha. In some embodiments, the method comprises administering mogamulizumab, interferon alpha, systemic retinoid, and extracorporeal photopheresis (ECP). Further descriptions of the use of retinoid, interferon alpha, and/or ECP can be found, for example, in Ninosu et al., Cancers (Basel). 2024 Jan; 16(1): 141; Weiner et al., Dermatologic Therapy (2023) Volume 2023, Article ID 7625926; Treitman et al., Hematological Oncology, volume 41, Issue S2 Supplement: 17th International Conference on Malignant Lymphoma, Palazzo dei Congressi, Lugano, Switzerland, 13 - 17 June 2023, Pages 772-774, the content of each of which is incorporated by reference in its entirety.

[0952] The administering step can be accomplished using any method of implantation delivery in the art. For example, the engineered cells of the disclosure can be infused directly in the individual's bloodstream or otherwise administered to the individual.

[0953] In some embodiments, the methods disclosed herein include administering which term is used interchangeably with the terms "introducing," implanting," and "transplanting," engineered cells into an individual, by a method or route that results in at least partial localization of the introduced cells at a desired site such that a desired effect(s) is/are produced. The engineered cells or their differentiated progeny can be administered by any appropriate route that results in delivery to a desired location in the individual where at least a portion of the administered cells or components of the cells remain viable. The period of viability of the cells after administration to an individual 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 lifetime of the individual, i.e., long-term engraftment.

[0954] When provided prophylactically, the engineered cells described herein can be administered to an individual in advance of any symptom of a disease or condition to be treated. Accordingly, in some embodiments the prophylactic administration of an engineered cell population prevents the occurrence of symptoms of the disease or condition.

[0955] When provided therapeutically in some embodiments, engineered cells are provided at (or after) the onset of a symptom or indication of a disease or condition, *e.g.,* upon the onset of disease or condition.

[0956] For use in the various embodiments described herein, an effective amount of engineered cells as disclosed herein, can be at least 10^2 cells, at least 5×10^2 cells, at least 10^3 cells, at least 5×10^3 cells, at least 10^4 cells, at least 5×10^4 cells, at least 10^5 cells, at least 2×10^5 cells, at least 3×10^5 cells, at least 4×10^5 cells, at least 5×10^5 cells, at least 6

$\times 10^5$ cells, at least 7×10^5 cells, at least 8×10^5 cells, at least 9×10^5 cells, at least 1×10^6 cells, at least 2×10^6 cells, at least 3×10^6 cells, at least 4×10^6 cells, at least 5×10^6 cells, at least 6×10^6 cells, at least 7×10^6 cells, at least 8×10^6 cells, at least 9×10^6 cells, or multiples thereof. The engineered cells can be derived from one or more donors or can be obtained from an autologous source. In some embodiments, the engineered cells are expanded in culture prior to administration to an individual in need thereof.

[0957] In some embodiments, the delivery of an engineered cell composition (*e.g.,* a composition including a plurality of engineered cells according to any of the cells described herein) into an individual by a method or route results in at least partial localization of the cell composition at a desired site. A composition including engineered cells can be administered by any appropriate route that results in effective treatment in the individual, *e.g.,* administration results in delivery to a desired location in the individual where at least a portion of the composition delivered, *e.g.,* at least 1×10^4 cells, is delivered to the desired site for a period of time. Modes of administration include injection, infusion, and instillation. "Injection" includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracerebrospinal, and intrasternal injection and infusion. In some embodiments, the route is intravenous. For the delivery of cells, delivery by injection or infusion is a preferred mode of administration.

[0958] In some embodiments, the engineered cells are administered systemically, *e.g.,* via infusion or injection. For example, a population of engineered cells are administered other than directly into a target site, tissue, or organ, such that it enters the individual's circulatory system and, thus, is subject to metabolism and other similar biological processes.

[0959] The efficacy of a treatment including any of the compositions provided herein for the treatment of a disease or condition can be determined by a skilled clinician. However, one skilled in the art will appreciate that a treatment is considered effective if any one or all of the signs or symptoms or markers of disease are improved or ameliorated. Efficacy can also be measured by failure of an individual to worsen as assessed by decreased 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 an individual or an animal (some non-limiting examples include a human, or a mammal) 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.

[0960] As discussed above, a therapeutically effective amount includes an amount of a therapeutic composition that is sufficient to promote a particular beneficial effect when administered to an individual, such as one who has, is suspected of having, or is at risk for a disease. In some embodiments, an effective amount 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.

[0961] In some embodiments of the disclosed methods, the individual is a mammal. In some embodiments, the mammal is a human. In some embodiments, the individual has or is suspected of having a disease associated with inhibition of cell signaling mediated by a cell surface ligand or antigen. The diseases suitable for being treated by the compositions and methods of the disclosure include, but are not limited to, cancers, autoimmune diseases, inflammatory diseases, and infectious diseases. In some embodiments, the disease is a cancer or a chronic infection.

[0962] Further descriptions of methods for CAR design, delivery and expression in T cells, and the manufacturing of clinical-grade CAR-T cell populations can be found, for example, in Lee et al., Clin Cancer Res (2012) 18(10):2780-90, hereby incorporated by reference in its entirety. For example, the engineered CARs may be introduced into T cells using retroviruses, which efficiently and stably integrate a nucleic acid sequence encoding the chimeric antigen receptor into the target cell genome.

[0963] Other methods known in the art include, but are not limited to, lentiviral transduction, transposon-based systems, direct RNA transfection, and CRISPR/Cas systems (*e.g.,* type I, type II, or type III systems using a suitable Cas protein such Cas3, Cas4, Cas5, Cas5e (or CasD), Cas6, Cas6e, Cas6f, Cas7, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9, Cas10, Cas10d, Cas12a (Cpf1), Cas13a (C2c2), Cas13b, Cas13d, CasF, CasG, CasH, Csy1, Csy2, Csy3, Cse1 (or CasA), Cse2 (or CasB), Cse3 (or CasE), CasX, CasY, Cse4 (or CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6,

Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csz1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cu1966, etc.).

[0964] In some embodiments, a recombinant adeno-associated virus (AAV) vector can be used for delivery. Techniques to produce rAAV particles, in which an AAV genome to be packaged that includes the polynucleotide to be delivered, rep and cap genes, and helper virus functions are provided to a cell are known in the art. Production of rAAV requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (*e.g.,* not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes can be from any AAV serotype for which recombinant virus can be derived, and can be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, international patent application publication number WO 01/83692.

[0965] The engineered cells (*e.g.,* CAR-T cells), once they have been expanded ex vivo in response to, for example, an autoimmune disease antigen, can be reinfused into the subject in a therapeutically effective amount.

[0966] The precise amount of engineered cells (*e.g.,* CAR T cells) to be administered can be determined by a physician with consideration of individual differences in age, weight, extent of disease and condition of the subject.

[0967] Administration of cell (*e.g.,* T cell) therapies may be defined by number of total cells per infusion or number of cells per kilogram of body weight, especially for pediatric subjects (*e.g.,* patients). As immune cells (*e.g.,* T cells) replicate and expand after transfer, the administered cell dose may not resemble the final steady-state number of cells. In some embodiments, a pharmaceutical composition including the immune cells (*e.g.,* CAR T cells) of the present disclosure may be administered at a dosage of 10^4 to 10^{10} total cells. In another embodiment, a pharmaceutical composition including the immune cells (*e.g.,* CAR T cells) of the present disclosure may be administered at a dosage of 10^3 to 10^8 cells/kg body weight, including all integer values within those ranges.

[0968] Compositions including the immune cells (*e.g.,* CAR T cells) of the present disclosure may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are known in the art (see, for example, Rosenberg et al., New Engl J Med, (1988) 319:1676). The optimal dosage and treatment

regimen for a particular subject can be determined by one skilled in the art by monitoring the subject for signs of disease and adjusting the treatment accordingly.

[0969] In some embodiments, administration of any of the compositions embodied herein, for the treatment of, for example, an autoimmune or inflammatory disease, can be combined with other cell-based therapies, for example, stem cells, antigen presenting cells, pancreatic islets etc.

[0970] The composition of the present disclosure may be prepared in a manner known in the art and in a manner suitable for parenteral administration to mammals, particularly humans, including a therapeutically effective amount of the composition alone, with one or more pharmaceutically acceptable carriers or diluents.

[0971] Compositions include those suitable for parenteral administration, including subcutaneous, intramuscular, intravenous and intradermal administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any method well known in the art of pharmacy. Such methods include preparing the carrier for association with the CAR-T cells. In general, the compositions are prepared by uniformly and intimately bringing into association any active ingredients with liquid carriers.

[0972] In some embodiments, the composition is suitable for parenteral administration. In another embodiment, the composition is suitable for intravenous administration. Compositions suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes, which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

[0973] Enhancing the *in vivo* persistence of immune cells (*e.g.,* T cells) in a subject can be enhancing the fitness or enhancing the function and/or enhancing the efficacy of the immune cells. The enhancing can be measured by determining the accumulation of the number of T cell in a tumor.

[0974] In some embodiments, *in vivo* persistence can be measured by promoting the intra-tumoral increase of effector cytokines. In some embodiments, the *in vivo* persistence can be by increasing the expression of the stemness-associated transcription factor TCF1 in TILs. In some embodiments, the *in vivo* persistence can be by increasing the expression of TNF-α, IFN-γ, and/or IL-2.

[0975] In some embodiments, enhancing the *in vivo* persistence can be measured by measuring differences in gene expression. In some embodiments, the genes include but are not limited to activation markers (IL2RA, CD69), cytotoxic and effector molecules (IFNG, TNF, IL4, IL5, IL13, GZMA, GZMB), chemokines (CCL4, CCL20, CCL8), and co-stimulatory molecules (ICOS, OX40, 4-1BB, GITR).

[0976] In some embodiments, the subject has cancer or an autoimmune disease. In some embodiments, the cancer can be a solid tumor. In some embodiments, the cancer can be a hematological cancer. In some embodiments, the cancer expresses a tumor-associated antigen, as described herein. In some embodiments, the cancer expresses DLL3, LY6G6D, Claudin 6, GCC, p53R175H, and/or PRAME.

[0977] In some embodiments, the polypeptides, recombinant nucleic acids, vectors, particles, or engineered cells of the disclosure are for treating cancer or tumor. In some embodiments, the disclosure provides methods for using the engineered cells for treating cancer or tumor. In some embodiments, the engineered cells comprise T cells. In some embodiments, the engineered cells comprise NK cells.

[0978] In some embodiments, the cancer or tumor is metastatic or has metastasized.

[0979] In some embodiments, the tumor is a solid tumor. Exemplary solid tumors include, without limitation, small cell lung cancer, colorectal cancer, testicular cancer, ovarian cancer, or melanoma, lymphoma, leukemia, multiple myeloma, prostate cancer, breast cancer, non-small cell lung cancer, gastric cancer, esophageal cancer, liver cancer, kidney cancer, head & neck cancer, glioblastoma, neuroblastoma, soft tissue sarcoma, uterine cancer, brain cancer, skin cancer, renal cancer, bladder cancer, pancreatic cancer, thyroid cancer, eye cancer, gastrointestinal cancer, carcinoma, and sarcoma.

[0980] In some embodiments, the cancer is selected from the group consisting of B cell acute lymphoblastic leukemia (B-ALL), diffuse large B-cell lymphoma, liver cancer, pancreatic cancer, breast cancer, ovarian cancer, colorectal cancer, lung cancer, non-small cell lung cancer, acute myeloid lymphoid leukemia, multiple myeloma, gastric cancer, gastric adenocarcinoma, pancreatic adenocarcinoma, glioblastoma, neuroblastoma, lung squamous cell carcinoma, hepatocellular carcinoma, and bladder cancer.

[0981] In some embodiments, the method of treatment does not comprise administration of lymphodepletive agents within 7 days prior to administration of the cell therapy. Non-limiting examples of lymphodepletive agents include cyclophosphamide, fludarabine, and/or bendamustine within 7 days prior to administration of the cell therapy. In some

embodiments, the method of treatment does not comprise administration of at least 600,000 IU/kg of IL-2 every 8 hours. In some embodiments, the method of treatment does not comprise a checkpoint therapy which blocks PD-1 or CTLA-4 signaling.

[0982] In one aspect, the present disclosure provides methods of treating cancer or an autoimmune disease in a subject in need thereof, comprising: (a) administering an effective amount of an engineered cell, a recombinant nucleic acid, a vector, or a particle disclosed herein, to the subject, wherein the engineered cell or the recombinant polypeptide comprises two or more of the tags, the peptide epitopes or the mimotopes that are capable of binding to mogamulizumab, and wherein the tags, the peptide epitopes or the mimotopes are the same or different; and (b) administering at least one dose of mogamulizumab to the subject.

[0983] In some embodiments, each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

[0984] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

[0985] In some embodiments, the engineered cell, the recombinant nucleic acid, the vector, or the particle is administered at least 1, 2, 3, 4, 5, 6, 7, 8 weeks, or at least 2, 3, 4, 5, 6, 8 or 10 months, or at least 1, 2, 3, 4, or 5 years, before the first dose of mogamulizumab is administered to the subject.

[0986] In one aspect, the present disclosure provides methods of treating cancer in a subject in need thereof, comprising: (a) intraventricularly administering an effective amount of an engineered cell, a recombinant nucleic acid, a vector, or a particle disclosed herein, to the subject; and (b) systemically administering at least one dose of mogamulizumab to the subject.

[0987] In some embodiments, each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and

about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

[0988] In some embodiments, the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cells outside the brain than a control method that reduces or eliminates the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin. In some embodiments, the method comprises administering at least two, at least three, or at least four doses of mogamulizumab to the subject.

[0989] Procedures for separation of cells include, but are not limited to, flow cytometry, affinity chromatography, density gradient centrifugation, magnetic separation with antibody-coated magnetic beads, conjugation to particles that modify cell density, cytotoxic agents joined to or used in conjunction with a monoclonal antibody (mAb) or any other appropriate technique.

[0990] In some embodiments, the isolated immune cells (*e.g.,* T cells) are genetically engineered ex vivo for recombinant expression of a transgene. In some embodiments, isolated immune cells (*e.g.,* T cells) are genetically engineered ex vivo for recombinant expression of a CAR or a transcriptional receptor as described in more details supra. In some embodiments, the cells can be genetically engineered for recombinant expression. Methods suitable for genetically engineering cells for recombinant expression are well known in the art.

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[0991] All publications and patent applications mentioned in this disclosure are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0992] No admission is made that any reference cited herein constitutes prior art. The discussion of the references states what their authors assert, and the inventors/disclosers reserve the right to challenge the accuracy and pertinence of the cited documents. It will be clearly understood that, although a number of information sources, including scientific journal articles, patent documents, and textbooks, are referred to herein; this reference does

not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0993] The discussion of the general methods given herein is intended for illustrative purposes only. Other alternative methods and alternatives will be apparent to those of skill in the art upon review of this disclosure, and are to be included within the spirit and purview of this application.

EXAMPLES

[0994] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, cell biology, biochemistry, nucleic acid chemistry, and immunology, which are well known to those skilled in the art. Such techniques are explained fully in the literature cited above.

[0995] Additional embodiments are disclosed in further detail in the following examples, which are provided by way of illustration and are not in any way intended to limit the scope of this disclosure or the claims.

Example 1: Design of chimeric antigen receptors harboring epitope tags

[0996] This Example describes the design of chimeric antigen receptors (CARs) with epitopes that are capable of being bound by monoclonal antibodies inserted into the extracellular region. Specifically, the CAR constructs designed for these experiments comprised (from N-terminus to C-terminus) a CD8 signal sequence (CD8ss) (SEQ ID NO: 414), a VL domain (SEQ ID NO: 415), a (G4S)3 linker (SEQ ID NO: 416), a VH domain (SEQ ID NO: 419), a CD8 hinge (SEQ ID NO: 420), a CD8 transmembrane (CD8 TM) (SEQ ID NO: 421), a 41BB co-stimulatory domain (SEQ ID NO: 422), and a CD3Z signaling domain (SEQ ID NO: 423). For constructs labeled as N terminal epitope tagged, the epitope tag and any glycine/serine flexible linker sequences were placed between the CD8 signal sequence and the VL domain. For constructs labeled as linker epitope tagged, the epitope tag and any glycine/serine flexible linker sequences were placed between the VL and VH domains. For constructs labeled as hinge epitope tagged, the epitope tag and any glycine/serine flexible linker sequences were placed between the VH domain and an N-terminal truncated CD8 hinge sequence consisting of the sequence PEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 487).

[0997] This Example further describes the design of CARs with native (unmodified) epitopes and modified epitopes (**FIG. 1A-1D** and **Table 18**). Without wishing to be bound by theory, it is hypothesized that the epitope tags could be modified to: **1)** allow for improved binding of CARs that comprise an epitope tag (relative to a CAR that comprises an unmodified epitope tag), **2)** reduce antigen-independent CAR signaling and/or antigen-independent cell activation (relative to a CAR that comprises an unmodified epitope tag), and/or **3)** reduce cross-linking between CARs expressed on the cell surface of the cell (relative to a CAR that comprises an unmodified epitope tag). In a non-limiting example, a CCR4 epitope (SEQ ID NO: 430) recognized by the monoclonal antibody mogamulizumab was modified to eliminate a C-terminal cysteine (SEQ ID NO: 431).

[0998] A schematic detailing the CARs used in this Example is depicted in **FIG. 1A-1D** and the corresponding sequences are presented in **Table 18**.

Table 18. CAR Constructs

Amino Acid SEQ ID NO	Nucleic Acid SEQ ID NO	Name	Design of CAR Construct
401	489	ML73-p	[CD8ss]-[VL]-[3xG4S-linker]-[VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
402	490	ML122-p	[CD8ss]-[TROP2-Tag]-[SG4S-linker]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
403	491	ML141-p	[CD8ss]-[G4S-linker]-[DLL3_EGF3-Tag]-[SG4S-linker]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
404	492	ML142-p	[CD8ss]-[G4S-linker]-[CCR4-Tag]-[SG4S-linker]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
405	493	ML158-p	[CD8ss]-[anti-DLL3-VL]-[G4S-linker]-[CCR4-Tag]-[SG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
406	494	ML159-p	[CD8ss]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[G4S]-[CCR4-Tag]-[SG4S-linker]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
407	495	ML175-p	[CD8ss]-[G4S-linker]-[CCR4-delta-Cys-Tag]-[SG4S-linker]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
408	496	ML193-p	[CD8ss]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[G4S]-[CCR4-delta-ProLysProCys-Tag]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]

409	497	ML206-p	[CD8ss]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[G4S]-[CCR4-delta-Cys-Tag]-[SG4S-linker]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
410	498	ML207-p	[CD8ss]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CCR4-Tag]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
411	499	ML234-p	[CD8ss]-[anti-DLL3-VL]-[G4S-linker]-[CCR4-delta-Cys-Tag]-[SG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
412	500	ML235-p	[CD8ss]-[CCR4-delta-Cys-Tag]-[anti-DLL3-VL]-[3xG4S-linker]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
413	501	ML236-p	[CD8ss]-[anti-DLL3-VL]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
525	537	ML367-p	[CD8ss]-[anti-CD19-LC]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-CD19-HC]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
526	538	ML413-p	[CD8ss]-[anti-CD19-LC]-[3xG4S-linker]-[anti-CD19-HC]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
527	539	ML365-p	[CD8ss]-[anti-CLDN6-VL]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-CLDN6-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
528	540	ML411-p	[CD8ss]-[anti-CLDN6-VL]-[3xG4S-linker]-[anti-CLDN6-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
529	541	ML366-p	[CD8ss]-[anti-DLL3-VL]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
530	542	ML412-p	[CD8ss]-[anti-DLL3-VL]-[3xG4S-linker] -[anti-DLL3-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
531	543	ML363-p	[CD8ss]-[anti-KLK2-VL]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-KLK2-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
532	544	ML409-p	[CD8ss]-[anti-KLK2-VL]-[3xG4S-linker] -[anti-KLK2-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
533	545	ML364-p	[CD8ss]-[anti-LY6G6D-VL]-[GGS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti- LY6G6D -VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
534	546	ML410-p	[CD8ss]-[anti-LY6G6D-VL]-[3xG4S-linker] -[anti-LY6G6D-VH]-[CD8hinge]-[CD8tm]-[41BB]-[CD3z]
535	547	ML832-p	[CD8ss]-[anti-CD19-HC]-[mmTRBC2]-[Furin]-[P2A]-[hulgKss]-[GS]-[CCR4-delta-cys-Tag]-[SGGS]-[anti-CD19-LC]-[mmTRAC+LVL]
536	548	ML840-p	[CD8ss]-[anti-CD19-HC]-[mmTRBC2]-[Furin]-[P2A]-[hulgKss]-[anti-CD19-LC]-[mmTRAC+LVL]
549	554	ML493-p	[P2A]-[anti-p53R175H-TCRb variable]-[mmTRBC2]-[P2A]-[hulgKss]-[CCR4-delta-cys-Tag]-[SGGGGS]-[anti-p53R175H-TCRa variable]-[TRAC] *note that, due to knock-in, TRAC sequence is only partial

550	555	ML162-p	[P2A]-[anti-p53R175H-TCRb variable]-[mmTRBC2]-[SGSG]-[P2A]-[anti-p53R175H-TCRa variable]-[TRAC] *note that, due to knock-in, TRAC sequence is only partial

Example 2: Testing expression and functionality of anti-DLL3 CARs harboring unmodified and modified epitope tags

[0999] This Example describes functional assessments of T cells expressing CARs comprising native epitope tags or modified epitope tags inserted at different extracellular locations.

Methods:

[1000] *Cloning:* Nucleic acid encoding the engineered CARs comprising either native or modified epitope tags were synthesized and cloned into lentiviral vectors containing an MND promoter.

[1001] *T Cell Manufacturing:* Pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells with the CAR transgene expression vector and viral packaging plasmids using Mirus TransIT-Lenti. The cells were cultured and viral supernatants were harvested and filtered through a 0.45 uM PES filter 48 hours post transfection. T cells were cultured in T cell media comprising X-VIVO15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells were activated with MACS TransAct (CD3 and CD28 activation) for 24 hours before being transduced with the lentivirus. 24 hours after transduction, cells were centrifuged and the media was removed. Then, the cells were plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells were harvested for assays.

[1002] *Flow cytometry:* Cells were harvested and resuspended in PBS + 2% FBS (Gibco) for staining. For CAR constructs carrying the CCR4 epitope recognized by mogamulizumab, cells were stained with soluble FITC tagged protein, to identify CAR expression, and a biotinylated mogamulizumab bio-similar (ThermoFisher) followed by FITC anti-biotin secondary staining, to identify epitope tag expression. For CAR constructs carrying the DLL3 epitope recognized by tarlatamab or the TROP2 epitope recognized by sacituzumab, cells were stained with soluble FITC tagged protein, to identify CAR expression. Cells were assessed for activation via staining for CD69 with a BV711 conjugated anti-CD69 antibody

(ThermoFisher). Cells were then analyzed on a flow cytometer (ThermoFisher) to assess staining.

[1003] *Cytokine analysis:* Target cells expressing DLL3 and T cells expressing the anti-DLL3 CARs described in **Example 1** were plated at a 1:1 ratio and cultured in X-VIVO15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). Following overnight culture, supernatants were obtained and evaluated for IL-2 and IFNgamma expression using the V-PLEX Human IL-2 Kit and the V-PLEX Human IFNgamma Kit per manufacturer instructions (Meso Scale Discovery). For negative control or mogamulizumab treatment conditions, antibody was added to T cells at a concentration of 1.25-10 ug/mL, depending on the experiment.

[1004] *Target cell killing:* 25,000 red fluorescent nuclear protein expressing target cells expressing DLL3 and 25,000 anti-DLL3 CAR positive T cells carrying the indicated construct were plated in each well of a 96 well flat bottom plate in X-VIVO15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). Four images per well were acquired every 2-4 hours utilizing a CellCyte Live Cell Imager (Cytena).

[1005] *Cell Lines:* Small cell lung cancer (SCLC) cell lines that are antigen negative (A549), DLL3 low (DMS-273), and DLL3 high (SHP-77) were used in the present experiments.

Results:

Tarlatamab DLL3 Epitope Tag

[1006] Primary human T cells from a healthy donor were obtained and either: 1) not transduced (UTD), 2) transduced with an anti-DLL3 CAR that does not comprise an epitope tag (CAR untagged) (ML73-p), or 3) transduced with an anti-DLL3 CAR that comprises a DLL3 epitope that can be bound by tarlatamab (CAR+Tag) (ML141-p). **FIG. 2A** demonstrates that there was no difference in the percentage of CAR positive T cells between the CAR untagged and CAR+Tag groups. **FIG. 2B** shows a decrease in CAR MFI between the two groups. **FIG. 2C** shows that when co-cultured with a cancer cell line that expresses DLL3 at a low level, the cells expressing the CAR untagged and the cells expressing CAR+Tag were able to effectively control the growth of the cancer cells. Finally, **FIG. 2D** demonstrates that the cells expressing the CAR untagged and the cells expressing CAR+Tag secreted similar amounts of IFNgamma when co-cultured with a cancer cell line expressing low levels of DLL3 but did not secrete IFNgamma when co-cultured with a DLL3 negative

cell line. Taken together, these results demonstrate that T cells expressing CAR constructs tagged with an epitope that can be bound by a monoclonal antibody (tarlatamab) are expressed at similar levels to CAR constructs without a tag (CAR untagged) and retain functionality against antigen positive cell lines.

[1007] Next, T cell depletion was assessed by culturing the T cells with tarlatamab, a monoclonal antibody that recognizes the DLL3 epitope inserted into the CAR+Tag construct. **FIG. 3A** shows that tarlatamab treatment did not change the percentage of CAR positive T cells expressing the CAR untagged construct (ML73-p). However, tarlatamab treatment eliminated nearly all CAR positive T cells that express the CAR+Tag construct (ML141-p). Further, **FIG. 3B** shows that tarlatamab treatment resulted in activation of a larger percentage of total T cells (CAR- and CAR+) in the culture containing T cells expressing the CAR+Tag construct, compared to the culture containing the CAR untagged construct. Without wishing to be bound by theory, this may indicate that tarlatamab results in fratricidal T cell activation, i.e. bystander T cells in the culture are activated against the T cells that express the CAR+Tag construct, consistent with the presumed mechanism of tarlatamab activity as a bispecific T cell engager. Cells are presumed to be activated because the DLL3 binding region of tarlatamab binds to the tag of the CAR+Tag-expressing T cells, and the anti-CD3e binding region binds to a T cell in the culture to induce T cell activation. In the T cells expressing the CAR untagged construct, the DLL3 epitope is absent and therefore the DLL3 binding region of tarlatamb is unable to bind.

Mogamulizumab CCR4 Epitope Tag

[1008] Primary human T cells from a healthy donor were obtained and either: 1) not transduced (UTD), 2) transduced with an anti-DLL3 CAR that does not comprise an epitope tag (ML73-p), 3) transduced with an anti-DLL3 CAR that comprises a CCR4 epitope that can be bound by mogamulizumab inserted at the N-terminus of the CAR construct (ML142-p; N-term tag), 4) transduced with an anti-DLL3 CAR that comprises the CCR4 epitope inserted in the linker of the CAR construct (ML158-p; Linker tag), 5) transduced with an anti-DLL3 CAR that comprises the CCR4 epitope inserted in the hinge of the CAR construct (ML159-p; Hinge tag), 6) transduced with an anti-DLL3 CAR that comprises a modified CCR4 epitope with a C-terminal cysteine deleted (SEQ ID NO: 431) inserted at the N-terminus of the CAR construct (ML175-p; N term deltaCys tag), or 7) transduced with an anti-DLL3 CAR that comprises a modified CCR4 epitope (LDESIYSNYYLYESI (SEQ ID

NO: 437)) with no linkers inserted in the hinge of the CAR construct (ML193-p; Hinge minimal tag).

[1009] **FIGS. 4A-4C** demonstrate that there were no significant differences in the percentage of CAR positive T cells between the untagged CAR (ML73-p) and tagged CAR constructs. **FIGS. 4D-4F** show that some constructs showed a decrease in CAR MFI. **FIGS. 5A-5C** show the percentage of T cells that can be stained with Mogamulizumab. **FIGS. 5D-5F** show that the placement of the CCR4 epitope tag and modification of the tag result in differences in mogamulizumab binding. Specifically, an epitope tag inserted into the linker of the CAR construct was bound by mogamulizumab at higher rate than an epitope tag inserted in N-terminus of the CAR construct (**FIG. 5D**). Further, a modified epitope tag (with a C-terminal cysteine removed) inserted in the N-terminus of the CAR construct was bound by mogamulizumab at a higher rate than an unmodified epitope tag inserted in either the N-terminus of the CAR construct or the hinge of the CAR construct (**FIG. 5E**). Finally, an unmodified epitope tag inserted in the N-terminus of the CAR construct was bound by mogamulizumab at a higher rate than an unmodified epitope tag inserted in either the hinge of the CAR construct or the minimal epitope tag inserted in the hinge of the CAR construct (**FIG. 5F**).

[1010] *In vitro* functional data (**FIGS. 6A-6F**) show that cells expressing CAR constructs containing an unmodified CCR4 epitope tag or a modified CCR4 epitope tag released both IL-2 and IFNgamma in response to antigen stimulation. Indeed, **FIGS. 6A-6C** demonstrate that while all CAR constructs with an epitope tag release IL-2 in response to stimulation with low antigen and high antigen cell lines, the placement of the epitope tag and modification of the epitope tag resulted in differences in IL-2 release. Further, **FIGS. 6D-6F** demonstrate that while all CAR constructs with an epitope tag release IFNgamma in response to stimulation with low antigen and high antigen cell lines, the placement of the epitope tag and modification of the epitope tag resulted in differences in IFNgamma release.

[1011] *In vivo* functional data (**FIGS. 7A-7D**) show that cells expressing CAR constructs containing an unmodified CCR4 epitope tag or a modified CCR4 epitope tag were able to effectively control the growth of both low antigen and high antigen cell lines. Specifically, **FIGS. 7A-7B** show that there is not a significant difference in the ability of the CAR constructs with an epitope tag to control the growth of a high antigen cancer cell line *in vivo* compared to a CAR without an epitope tag (ML73-p). Further, **FIGS. 7C-7D** show that there is not a significant difference in the ability of the CAR constructs with an epitope tag

to control the growth of a low antigen cancer cell line *in vivo* compared to a CAR without an epitope tag (ML73-p).

Sacituzumab TROP2 Epitope Tag

[1012] Primary human T cells from a healthy donor were obtained and: 1) not transduced, 2) transduced with an anti-DLL3 CAR that does not comprise an epitope tag (ML73-p), or 3) transduced with an anti-DLL3 CAR that comprises a TROP2 epitope that can be bound by Sacituzumab inserted in the N-terminus of the CAR construct (ML122-p; TROP2 tag). **FIGS. 8A-8B** demonstrate that while there is no significant difference in the percentage of CAR positive T cells between the untagged CAR (ML73-p) and tagged CAR constructs, the CAR construct with the TROP2 epitope is not bound by Sacituzumab (**FIG. 8B**).

Example 3: In vitro and in vivo assessment of CAR T depletion after treatment with mogamulizumab

[1013] This Example describes the *in vitro* and *in vivo* depletion of CAR T cells expressing the modified mogamulizumab epitope tag.

Methods:

[1014] *Cloning:* To enable visualization of mogamulizumab-induced NK-mediated antibody dependent cellular cytotoxicity (ADCC) *in vitro*, nucleic acid encoding the engineered CAR comprising the modified mogamulizumab epitope tag (ML236-p) and GFP (where noted) were synthesized and cloned into a lentiviral vector containing an MND promoter (for which nucleotide sequence is SEQ ID NO: 556, and which encodes the polypeptide of SEQ ID NO: 551). For targeted insertion into the TRAC locus (and expression controlled by the TRA promoter), nucleic acid sequences encoding the engineered CAR comprising the modified mogamulizumab epitope tag (ML236-p) and a CARD11-PIK3R3 fusion protein (SEQ ID NO: 2) was synthesized and cloned into an AAV6 viral vector for use as a homology directed repair (HDR) template for knock-in to the TRAC locus.

[1015] *T Cell Manufacturing:* T cells were cultured in T cell media comprising X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells were activated with MACS TransAct (CD3 and CD28 activation). For non-targeted insertion, T cells were transduced with the lentivirus 24 hours later. Transduced

cells were centrifuged and the media was removed after 24 hours. Then, the cells were plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells were harvested. For targeted insertion at the TRAC locus, T cells were electroporated with RNPs consisting of Cas9 protein and a single guide RNA cleaving exon 1 of the human TRAC gene 48 hours after TransAct activation. The cells were cultured in human T cell medium without serum (X-VIVO 15,10 mM HEPES, and 1x Glutamax, supplemented with 10 ng/mL IL-7 and 5 ng/mL IL-15) and transduced with an AAV6 vector providing the HDR template, rested overnight, then washed, and transferred to a G-Rex plate for expansion and harvest 2 days later.

[1016] *In vitro mogamulizumab depletion*: For NK cell-mediated ADCC assessment, CAR-positive (CAR+) T cells were co-cultured with NK cells at an NK:T ratio of 10:1, 5:1, or 2:1, in the presence of mogamulizumab or an isotype control antibody. 24 hours post in vitro ADCC co-culture CAR T cells were enumerated by GFP expression detected by flow cytometry.

[1017] *In vivo mogamulizumab depletion*: A subcutaneous inoculum of $10x10^6$ SHP77 cells were injected into 7-10 week old female NCG (Charles River) mice. At day 14 post tumor injection, mice were treated with untransduced T cells or $2x10^5$ CAR+ T cells via retro-orbital injection. The untransduced T cell dose was matched to the highest total T cells infused for the CAR+ T cell dose. Tumors were measured by caliper twice weekly and the mice were followed for survival. Mice were then treated with a single dose of 1 mg/kg mogamulizumab either 7 or 14 days after T cell infusion or multiple doses of 1 mg/kg mogamulizumab every 3 days for a total of 4 doses starting on day 5 post T cell infusion. The following tissues were collected 24 hours after the last dose of mogamulizumab and processed to single cell suspension for flow cytometry analysis: blood, spleen, and bone marrow.

[1018] *Flow cytometry*: Tissues were processed into a single cell suspension and red blood cells were lysed. Then, single cell suspensions were stained for human CD45, mouse CD45, and live/dead dye for flow cytometry analysis.

[1019] *Cell Lines*: The DLL3 positive small cell lung cancer (SCLC) cell line, SHP-77, was used.

Results:

In vitro demonstration of mogamulizumab-mediated CAR T cell depletion

As described in the methods above, the lentiviral transgene construct that codes for the CAR containing the modified mogamulizumab epitope tag co-expresses GFP to allow simple

quantification of engineered T cells by flow cytometry. **FIG. 9A** shows that 83.5% of T cells transduced with the transgene construct are GFP-positive (GFP+), and thus express the CAR with the modified mogamulizumab epitope tag, while 16.5% of the T cells were GFP-negative (GFP-), and thus do not express the modified mogamulizumab epitope tag. When the CAR T cells were co-cultured with NK cells in the presence of mogamulizumab, maximum mogamulizumab-induced T cell depletion was observed at the 10:1 NK:T co-culture condition, achieving about 70% GFP+ cell depletion relative to isotype control (**FIG. 9B**).

In vivo demonstration of mogamulizumab-mediated CAR T cell depletion

Briefly, to assess mogamulizumab-mediated depletion of CAR T cells in vivo, tumor-bearing mice were infused with T cells expressing the CAR comprising the modified mogamulizumab epitope tag, followed by treatment with mogamulizumab and subsequent assessment of T cell depletion in various tissues. T cells expressing a CAR comprising the modified mogamulizumab epitope tag were generated using primary human CD3-positive (CD3+) T cells from healthy donors via targeted AAV insertion at the TRAC locus. Mice bearing SHP77 small cell lung cancer (SCLC) tumors were infused with $2x10^5$ T cells. Administered T cells were either untransduced T cells or T cells comprising a CAR comprising the modified mogamulizumab epitope tag (ML236-p). Mice were then treated with a single intraperitoneal dose of 1 mg/kg mogamulizumab on day 7 or day 14 after T cell infusion, followed by blood collection on day 15 post T cell infusion in both cohorts. In CAR treated mice that were not treated with mogamulizumab, an average of 1,316 T cells/ul were detected in the blood (**FIG. 9C**), whereas mogamulizumab treated mice averaged 11.5 T cells/ul or 33.5 T cells/ul detected in the blood in the day 7 and day 14 treatment groups, respectively (**FIG. 9C**). In a second study, tumor-bearing mice were treated with either a single dose of mogamulizumab on day 14 after T cell infusion or multiple doses of mogamulizumab every 3 days for a total of 4 doses starting on day 5 post T cell infusion. T cells were enumerated in blood, spleen, and bone marrow 24 hours after the last dose of mogamulizumab (**FIG. 9D**). T cell counts in the blood of non-mogamulizumab treated mice averaged 2,785 T cells/ul, whereas mogamulizumab treated mice averaged 65.4 T cells/ul and 8.6 T cells/ul in the blood in the single or multi-dose treatment groups, respectively (**FIG. 9D**). The multi-dose mogamulizumab treatment achieved the most complete T cell depletion, as average T cell count in blood, bone marrow, and spleen were similar to baseline T cell count established by the UTD cohort. Taken

together, the data indicate that mogamulizumab mediates highly effective depletion of CAR T cells comprising the modified mogamulizumab epitope tag, both *in vitro* and *in vivo*.

Example 4: In vitro comparison of tonic signaling for CAR constructs comprising an unmodified mogamulizumab epitope tag and a modified mogamulizumab epitope tag

[1020] This Example describes the *in vitro* assessment of tonic activity of a CAR construct with a modified mogamulizumab epitope tag compared to a CAR construct with an unmodified mogamulizumab epitope tag.

Methods:

[1021] *Cloning:* Nucleic acid encoding a CAR alone (CAR; ML73-p), a CAR comprising the unmodified mogamulizumab epitope tag (SEQ ID NO: 430) inserted into the linker between the VH and VL binding regions of the CAR (CAR.moga; ML158-p), or a CAR comprising the modified mogamulizumab epitope tag (SEQ ID NO: 431) inserted into the linker between the VH and VL binding regions of the CAR (CAR.moga.delCys; ML236-p) were synthesized and cloned into lentiviral vectors containing a sEF1a promoter.

[1022] *T Cell Manufacturing:* Pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells with the CAR transgene expression vector and viral packaging plasmids using Mirus TransIT-2020. The cells were cultured and viral supernatants were harvested and filtered through a 0.45 uM PES filter 48 hours post transfection. T cells were cultured in T cell media comprising X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells were activated with MACS TransAct (CD3 and CD28 activation) for 24 hours before being transduced with the lentivirus. 24 hours after transduction, cells were centrifuged and the media was removed. Then, the cells were plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells were harvested for assays or cryopreserved in Cryostor media.

[1023] *Cytokine analysis:* CAR T cells were thawed and rested for 48 h before being plated and cultured alone or with 1, 10, or 20 ug/mL mogamulizumab or isotype antibody in X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). Following a 24-hour culture, supernatants were obtained and evaluated for IFNgamma expression using the V-PLEX Human IFNgamma Kit per manufacturer instructions (Meso Scale Discovery).

[1024] *Flow cytometry:* CAR T cells were thawed and rested for 48 h before being plated and cultured alone or with 1, 10, or 20 ug/mL mogamulizumab or an isotype antibody in X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). For immunophenotyping, T cells were collected after 96 hours in culture and stained with antibodies for hCD45RA and hCCR7(ThermoFisher). T cell counts were enumerated via flow cytometry after 96 hours in culture.

Results:

To assess impact of mogamulizumab epitope tag on tonic activity of the CAR, T cells comprising a CAR (CAR; ML73-p), a CAR comprising the unmodified mogamulizumab epitope tag (CAR.Moga; ML158-p), or a CAR comprising the modified mogamulizumab epitope tag (CAR.Moga.delCys; ML236-p) were generated via lentiviral transduction. Tonic activity was measured by antigen-independent IFNgamma release (**FIG. 10A**) and proliferation (**FIG. 10B**) of T cells alone in culture. Incorporation of the unmodified mogamulizumab epitope tag increased tonic activity of the CAR (CAR.Moga; ML158-p), which was exacerbated by the addition of 10 ug/mL or 20 ug/mL mogamulizumab. However, the CAR comprising the modified mogamulizumab epitope tag (CAR.Moga.delCys; ML236-p), which does not comprise the terminal cysteine, exhibited low levels of tonic signaling that were similar to CAR alone. Compared to a CAR that did not have a mogamulizumab epitope tag (CAR; ML73-p), the unmodified mogamulizumab epitope tag (CAR.Moga; ML158-p) drastically increased the ratio of effector memory T cells at the expense of naïve and Temra T cells; however, this effect was not observed in cells expressing the CAR comprising the modified mogamulizumab epitope tag (CAR.Moga.delCys; ML236-p) (**FIG. 10C**). This dataset suggests that the modified mogamulizumab epitope tag (SEQ ID NO: 431) can be inserted into the linker of a CAR while maintaining the tonic profile of the CAR.

Example 5: In vitro assessment of the compatibility of the modified mogamulizumab epitope tag in various CAR formats

[1025] This Example describes functional assessments of T cells expressing CARs that target various tumor antigens including KLK2, Ly6G6D, Claudin-6, DLL3 and CD19 as well as the incorporation of a modified mogamulizumab epitope tag into said CARs.

Methods:

[1026] *Cloning:* Nucleic acid encoding CAR alone (KLK2.CAR; ML409-p, CLDN6.CAR; ML411-p, DLL3.CAR; ML412-p, LY6G6D.CAR; ML410-p, and CD19.CAR; ML413-p) or encoding a CAR comprising the modified mogamulizumab epitope tag inserted into the linker between the VH and VL binding regions of the CAR (KLK2.moga-CAR; ML363-p, CLDN6.moga-CAR; ML365-p, DLL3.moga-CAR; ML366-p, LY6G6D.moga-CAR; ML364-p, and CD19.moga-CAR; ML367-p) were synthesized and cloned into lentiviral vectors containing an sEF1a promoter.

[1027] *T Cell Manufacturing:* Pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells with the CAR transgene expression vector and viral packaging plasmids using Mirus TransIT-2020. The cells were cultured and viral supernatants were harvested and filtered through a 0.45 uM PES filter 48 hours post transfection. T cells were cultured in T cell media comprising X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells were activated with MACS TransAct (CD3 and CD28 activation) for 24 hours before being transduced with the lentivirus. 24 hours after transduction, cells were centrifuged and the media was removed. Then, the cells were plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells were harvested for assays.

[1028] *Flow cytometry:* Cells were harvested and resuspended in PBS + 2% FBS (Gibco) for staining. For CAR constructs carrying the modified mogamulizumab epitope tag, cells were stained with soluble FITC or PE tagged protein, to identify CAR expression, and a biotinylated mogamulizumab bio-similar (ThermoFisher) followed by PE anti-biotin secondary staining, to specifically identify expression of the modified mogamulizumab epitope tag. Cells were then analyzed on a flow cytometer (ThermoFisher) to assess staining.

[1029] *Cytokine analysis:* Target cells expressing KLK2, Ly6G6D, Claudin-6, DLL3 or CD19 and T cells expressing the anti-KLK2 CAR, anti-Ly6G6D CAR, anti-Claudin-6 CAR, anti-DLL3 CAR, or anti-CD19 CAR were plated at a 1:1 ratio and cultured in X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). Following overnight culture, supernatants were obtained and evaluated for IL2 and IFNgamma expression using the V-PLEX Human IL2 Kit and the V-PLEX Human IFNgamma Kit per manufacturer instructions (Meso Scale Discovery).

[1030] *Cell Lines:* The following cell lines were used in this example: wild type K562 cell line (antigen negative), K562 cell lines engineered to overexpress CD19, K562 cell lines engineered to overexpress Claudin-6, KLK2-positive (KLK2+) LNCap cells, Ly6G6D-positive (Ly6G6D+) LS1034 cells, and DLL3-positive (DLL3+) SHP77 cells.

Results:

[1031] Primary human T cells from a healthy donor were obtained and either: 1) not transduced (UTD), 2) transduced with an anti-KLK2 CAR (ML409-p), anti-Claudin-6 CAR (ML411-p), anti-DLL3 CAR (ML412-p), anti-Ly6G6D CAR (ML410-p), or an anti-CD19 CAR (ML413-p) that does not comprise a modified mogamulizumab epitope tag (KLK2.CAR, CLDN6.CAR, DLL3.CAR, LY6G6D.CAR, and CD19.CAR), or 3) transduced with an anti-KLK2 CAR comprising a modified mogamulizumab epitope tag (KLK2.moga-CAR; ML363-p), anti-Claudin-6 CAR comprising a modified mogamulizumab epitope tag (CLDN6.moga-CAR; ML365-p), anti-DLL3 CAR comprising a modified mogamulizumab epitope tag (DLL3.moga-CAR; ML366-p), anti-Ly6G6D CAR comprising a modified mogamulizumab epitope tag (LY6G6D.moga-CAR; ML364-p), or anti-CD19 CAR comprising a modified mogamulizumab epitope tag (CD19.moga-CAR; ML367-p). **FIG. 11A** demonstrates that there was minimal to no difference in the percentage of CAR positive T cells between the CAR alone groups (KLK2.CAR, CLDN6.CAR, DLL3.CAR, LY6G6D.CAR, and CD19.CAR) and moga-CAR groups (KLK2.moga-CAR, CLDN6.moga-CAR, DLL3.moga-CAR, LY6G6D.moga-CAR, and CD19.moga-CAR). **FIG. 11B** shows minimal to no difference in CAR MFI between the CAR alone groups and moga-CAR groups. **FIG. 11C** shows mogamulizumab staining can be used to detect only CAR constructs that comprise a modified mogamulizumab epitope tag but does not detect CARs without the epitope tag. **FIG. 11D** illustrates an increase in MFI in moga-CAR groups, consistent with detection of the modified mogamulizumab epitope tag in these groups. Notably, CCR4 is expressed by CD4+ T cells which likely accounts for the basal level of CCR4 detected in CAR alone groups. **FIGS. 11E-11I** show that, when co-cultured with a cancer cell line that expresses the target antigen, the cells expressing a CAR alone and the cells expressing a CAR comprising a modified mogamulizumab epitope tag produced similar levels of IFNgamma, with the exception of the anti-Claudin-6 moga-CAR. Finally, **FIGS. 11J-11N** demonstrate that the cells expressing the CAR alone and the cells expressing moga-CAR secret comparable amounts of IL-2 when co-cultured with a cancer cell line expressing their target antigen. Taken together, these results demonstrate that T

cells expressing CAR constructs tagged with a modified mogamulizumab epitope tag are expressed at similar levels to CAR constructs without a tag and retain functionality against antigen positive cell lines.

Example 6: Moga tag on other receptor formats

[1032] This Example describes the design and functional assessment of T cells expressing signaling receptors other than CARs (*e.g.,* transgenic TCR (tTCR) and T-bodies) that comprise a modified mogamulizumab epitope tag.

Methods:

[1033] *Cloning:* Nucleic acid encoding *e.g.,*an anti P53 R175H targeted transgenic TCR (tTCR; ML493-p) comprising the modified mogamulizumab epitope tag as well as untagged receptor were synthesized and cloned into an AAV backbone for insertion into the TRAC locus. Nucleic acid encoding an anti-CD19 T-body (ML832-pXX)comprising the modified mogamulizumab epitope tag as well as untagged receptor were synthesized and cloned into a lentiviral vector comprising an MND promoter.

[1034] *T Cell Manufacturing:* For lentiviral engineering of T cells expressing the T-body, pantropic VSV-G pseudotyped lentivirus was produced via transfection of Lenti-X 293T cells with the tTCR or T-body transgene expression vector and viral packaging plasmids using Mirus TransIT-2020. The cells were cultured and viral supernatants were harvested and filtered through a 0.45 uM PES filter 48 hours post transfection. T cells were cultured in T cell media comprising X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells were activated with MACS TransAct (CD3 and CD28 activation) for 24 hours before being transduced with the lentivirus. 24 hours after transduction, cells were centrifuged and the media was removed. Then, the cells were plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells were harvested for assays. For targeted insertion at the TRAC locus, 48 hours after activation with TransAct, T cells were electroporated with RNPs consisting of Cas9 protein and a single guide RNA targeting the TRAC locus. The cells were cultured in human T cell medium without serum (X-VIVO 15,10 mM HEPES, and 1x Glutamax, supplemented with 10 ng/mL IL-7 and 5 ng/mL IL-15) and transduced with an AAV6 vector to deliver the HDR template, rested overnight, then washed and transferred to a G-Rex for expansion. 2 days later the cells were harvested.

[1035] *Flow cytometry for receptor and mogamulizumab epitope tag expression:* Cells were harvested and resuspended in PBS + 2% FBS (Gibco) for staining. To identify T cells expressing receptors comprising the modified mogamulizumab epitope tag, tTCR or T-body constructs were stained with a biotinylated mogamulizumab bio-similar (ThermoFisher) followed by PE anti-biotin secondary staining. To identify T cells expressing the P53 tTCR, both tagged and untagged, T cells were stained with TCR Vb22 (Beckman Coulter) conjugated to FITC. To identify T cells expressing the anti-CD19 T-body, both tagged and untagged, T cells were stained with anti-FMC63 (Acro Biosystems). Finally, the cells were analyzed by flow cytometry.

[1036] *In vitro mogamulizumab depletion*: For NK cell-mediated ADCC assessment, T cells expressing the anti-CD19 T-body were co-cultured with NK cells at an NK:T ratio of 10:1 or 5:1, in the presence of a mogamulizumab biosimilar (10 ug/mL) or an isotype control antibody (10 ug/mL). Cells were collected 24 hours later, stained with anti-human CD3 antibodies, and enumerated by flow cytometry.

[1037] *Cytokine analysis:* T cells expressing the anti-CD19 T-body were co-cultured with CD19-K562 target cells at a 1:1 ratio in X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), GlutaMAX (ThermoFisher), and HEPES (ThermoFisher). Following overnight culture, supernatants were collected and evaluated for IL2 and IFNgamma expression using the V-PLEX Human IL-2 kit and the V-PLEX Human IFNgamma kit per manufacturer instructions (Meso Scale Discovery).

Cell Lines: The following cell lines were used in the present experiments: wild type K562 cell line (antigen negative) and K562 lines overexpressing CD19.

Results:

To assess the impact, if any, of incorporating the modified mogamulizumab tag on expression and function of other receptor formats, the following T cells were made: 1) T cells expressing an untagged anti P53 R175H targeted transgenic TCR (tTCR), 2) T cells expressing an anti P53 R175H targeted transgenic TCR (tTCR) comprising the modified mogamulizumab epitope tag, 3) T cells expressing an untagged anti-CD19 T-body, or 4) T cells expressing an anti-CD19 T-body comprising the modified mogamulizumab epitope tag. Incorporation of the modified mogamulizumab epitope tag did not drastically impact receptor expression of either the T-body (**FIGS. 12A-C**) or the tTCR (**FIGS. 12D-F**). Further, compared to the untagged construct, incorporation of the modified mogamulizumab epitope tag did not adversely impact antigen-dependent cytokine release for T cells

expressing the T-body comprising the modified mogamulizumab epitope tag (**FIGS. 12G and 12H**). Lastly, **FIG. 12I** shows that T cells expressing a T-body comprising the modified mogamulizumab epitope tag were depleted via NK cell-mediated ADCC upon treatment with mogamulizumab, demonstrating functional depletion of T-bodies expressing the modified mogamulizumab epitope tag.

Example 7: Testing expression and functionality of anti-DLL3 CARs harboring CCR7 epitope tags

This Example describes functional assessments of T cells expressing CARs comprising native CCR7 epitope tags inserted at different extracellular locations.

Methods:

[1038] *Cloning*: Nucleic acid encoding a CAR (or TCR or T-body) comprising either a native CCR7 epitope tag (SEQ ID NO: 519) or modified CCR7 epitope tag (SEQ ID NO:520-524) are synthesized and cloned into lentiviral vectors containing an MND promoter or into AAV vectors to enable site directed insertion via HDR, for example into the TRAC locus or TRAJ intron splice acceptor locus.

[1039] *T cell manufacturing*: Pantropic VSV-G pseudotyped lentivirus is produced via transfection of Lenti-X 293T cells with the CAR (or tTCR or T-body) transgene expression vector and viral packaging plasmids using Mirus TransIT-2020. The cells are cultured and viral supernatants are harvested and filtered through a 0.45 uM PES filter 48 hours post transfection. Primary T cells are cultured in T cell media comprising X-VIVO 15 media (Lonza) with 5% Human AB serum (BioIVT), 1x GlutaMAX (ThermoFisher), 10 mM HEPES (ThermoFisher), and recombinant human IL-7 (10 ng/mL) and IL-15 (5 ng/mL). Cells are activated with MACS TransAct (CD3 and CD28 activation) for 24 hours before being transduced with the lentivirus. 24 hours after transduction, cells are centrifuged and the media is removed. Then, the cells are plated in fresh T cell media and cultured at a density of 1 million cells/mL. On day 5-7 after initial T cell activation, cells are harvested.

[1040] *Flow cytometry*: Primary T cells from healthy donors are used to make 1) Untransduced T cells, 2) CAR T cells that do not comprise a CCR7 epitope tag, 3) CAR T cells that comprise an unmodified CCR7 epitope tag at the N terminus of the CAR, 4) CAR T cells that comprise an unmodified CCR7 epitope tag in the linker of the CAR, 5) CAR T cells that comprise an unmodified CCR7 epitope tag in the hinge of the CAR, 6) CAR T cells that comprise a modified CCR7 epitope tag at the N terminus of the CAR, 7) CAR T

cells that comprise a modified CCR7 epitope tag in the linker of the CAR, and 8) CAR T cells that comprise a modified CCR7 epitope tag in the hinge of the CAR. Engineered T cells are evaluated for percent CAR positivity, CAR MFI, and expression of the CCR7 epitope tag via flow cytometry. CAR expression for all groups is validated with a soluble FITC tagged protein that is targeted by the CAR (for example, any of the proteins targeted by the CARs in Example 5 above, KLK2, CLDN6, DLL3, LY6G6D, or CD19). Expression of the CCR7 epitope tag is validated by staining with CAP-100, an anti-CCR7 monoclonal antibody, followed by a fluorophore conjugated anti-hIgG. Expression of the CAR would be absent from the untransduced T cells but would be equivalent as quantified by CAR positivity and MFI across the CAR alone T cells and each of the CAR with CCR7 epitope tag T cells. Expression of the CCR7 epitope tag would be absent from the untransduced T cells and CAR alone T cells, but would be equivalent as quantified by CAR positivity and CCR7 epitope tag staining for each of the CAR with CCR7 epitope tag T cells.

[1041] *Target cell killing*: The functional impact, if any, of integrating a CCR7 epitope tag into the CAR is evaluated in *in vitro* killing assays. Briefly, red fluorescent nuclear protein expressing tumor cells expressing a protein that is targeted by the CAR (for example, any of the proteins targeted by the CARs in Example 5 above, KLK2, CLDN6, DLL3, LY6G6D, or CD19) and CAR T cells with a CAR targeting the same protein are plated at E:T ratios ranging from 1:1 to 1:100. Longitudinal images are collected using the CellCyte Live Cell Imager (Cytena) and target killing is quantified by fluorescent intensity on the red channel. If the CCR7 epitope tag does not impact the function of the CAR, there will be no difference in the killing capabilities of the CAR T with CAR alone and CAR T with the CCR7 epitope tag even at rigorous E:T ratios as shown by complete clearance of red fluorescent nuclear protein target cells.

[1042] *Cytokine production*: The functional impact, if any, of integrating a CCR7 epitope tag into the CAR is evaluated in T cell cytokine production after target stimulation. Briefly, target cells expressing a protein that is targeted by the CAR (for example, any of the proteins targeted by the CARs in Example 5 above, KLK2, CLDN6, DLL3, LY6G6D, or CD19) and CAR T cells with a CAR targeting the same protein are plated at a 1:1 ratio. Following overnight culture, supernatants are collected and evaluated for IL-2 and IFNgamma expression using the V-PLEX Human IL-2 kit and the V-PLEX Human IFNgamma kit per manufacturer instructions (Meso Scale Discovery). Untransduced T cells would not have increased cytokine production after co-culture however both the T cells with CAR alone and

T cells with a CAR comprising a CCR7 epitope tag would have similarly increased levels of IL-2 and IFNgamma production following co-culture with target cells.

[1043] *In vivo functional data*: The functional impact, if any, of integrating a CCR7 epitope tag into the CAR is further evaluated in *in vivo* killing assays (as described in Example 3 above). Briefly, mice are inoculated with $10x10^6$ tumor cells that express the same protein that is targeted by the CAR. Then, 10-14 days post tumor injection, the mice are treated with a $2x10^5$ dose of either 1) untransduced T cells, 2) T cells that express CAR alone, or 3) T cells that express CAR with a CCR7 epitope tag. Untransduced T cells would not control the tumor cells but it is expected that the CAR alone T cells and CAR with a CCR7 epitope tag T cells would clear the tumors with similar tumor growth and rejection kinetics.

[1044] *In vitro ADCC*: *In vitro* ADCC assays are preformed to demonstrate that a CCR7 epitope tag can be used as a mechanism to sufficiently deplete cells (as described in Example 3 above). Briefly, NK cells are co-cultured with T cells engineered to express CAR alone or CAR with a CCR7 epitope tag (as described above) at NK:T cell ratios ranging from 50:1 to 1:1. Across each NK: T cell ratio varying concentrations of CAP-100, an anti-CCR7 monoclonal antibody, starting from, for example, a concentration of 100 ug/ml and titrating down log fold to, for example, a concentration of 0.1 ug/ml are used to determine the in vitro ADCC killing of T cells harboring a CCR7 epitope tag. An isotype control antibody is used as a negative control. After 24 hours the number of remaining T cells are quantified using flow cytometry and stained for CD3e and CD56 to identify the T cell population and NK cell population, respectively. T cells expressing a CAR alone (without a CCR7 epitope tag) are not anticipated to be depleted relative to treatment with an isotype control antibody, however, equivalent depletion and sensitivity to ADCC is anticipated for each of the T cells expressing a CAR with the CCR7 epitope tag as shown by depletion of the CD3e population in culture as compared to an isotype control.

[1045] *In vivo ADCC*: *In vivo* ADCC assays are preformed to demonstrate that a CCR7 epitope tag can be used as a mechanism to sufficiently deplete cells (as described in Example 3 above). Briefly, mice are inoculated with $10x10^6$ tumor cells that express the same protein that is targeted by the CAR. Then, 10-14 days post tumor injection, the mice are treated with a $1x10^6$ dose of either 1) untransduced T cells, 2) T cells that express CAR alone, or 3) T cells that express CAR with a CCR7 epitope tag. Next, $5x10^6$ NK cells are adoptively transferred into each mouse 7 days post T cell treatment and mice are treated with

either CAP-100 or a hIgG isotype control antibody. Peripheral blood is collected and processed for flow cytometry analysis to quantify CD3e-positive T cells in circulation. T cells expressing a CAR alone (without a CCR7 epitope tag) are not anticipated to be depleted, as shown by high levels of circulating CD3e-positive cells in the peripheral blood, by treatment with CAP-100 relative to treatment with an isotype control antibody. In contrast, equivalent depletion and sensitivity to ADCC, as shown by low levels of circulating CD3e-positive cells in the peripheral blood, by treatment with CAP-100 is anticipated for each of the T cells expressing a CAR with the CCR7 epitope tag relative to treatment with an isotype control antibody. Further, mice treated with T cells expressing a CAR with the CCR7 epitope tag who experience functional CAR T cell depletion after CAP-100 treatment would succumb to tumor growth while mice treated with T cells expressing a CAR without the CCR7 epitope tag who do not experience functional CAR T cell depletion after CAP-100 treatment would reject their tumors.

[1046] While particular alternatives of the present disclosure have been disclosed, it is to be understood that various modifications and combinations are possible and are contemplated within the true spirit and scope of the appended claims. There is no intention, therefore, of limitations to the exact abstract and disclosure herein presented.

EXEMPLARY EMBODIMENTS

Exemplary embodiments of the invention are provided below:

Embodiment 1. A modified peptide epitope or mimotope, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence having at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (substitutions, insertions, and/or deletions) compared to an unmodified peptide epitope or mimotope, and wherein the modified peptide epitope or mimotope is capable of being bound by a monoclonal antibody.

Embodiment 2. The modified peptide epitope or mimotope of embodiment 1, wherein the peptide epitope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 430, and wherein the peptide epitope is capable of being bound by mogamulizumab.

Embodiment 3. The modified peptide epitope or mimotope of embodiment 2, wherein the modified peptide epitope or mimotope does not comprise the cysteine that is present at the C-terminus of SEQ ID NO: 430.

Embodiment 4. The modified peptide epitope or mimotope of embodiment 2 or 3, wherein the modified peptide epitope comprises or consists of any one of SEQ ID NO: 431-438; preferably, the modified peptide epitope consists of SEQ ID NO: 431.

Embodiment 5. The modified peptide epitope or mimotope of embodiment 1, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence:
(a) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 475, and wherein the peptide epitope or mimotope is capable of being bound by rituximab;
(b) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 476, and wherein the peptide epitope or mimotope is capable of being bound by Palivizumab;
(c) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 477, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;
(d) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 478, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;
(e) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 479, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;
(f) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 480, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;
(g) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 481, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab;

(h) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 482, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab;
(i) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 483, and wherein the peptide epitope or mimotope is capable of being bound by QBend-10;
(j) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 484, and wherein the peptide epitope or mimotope is capable of being bound by Alemtuzumab;
(k) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 469, and wherein the peptide epitope or mimotope is capable of being bound by Sacituzumab;
(l) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO:470-474, and wherein the peptide epitope or mimotope is capable of being bound by an anti-GUCY2C antibody;
(m) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 439, and wherein the peptide epitope or mimotope is capable of being bound by Tarlatamab;
(n) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO: 441-468, and wherein the peptide epitope or mimotope is capable of being bound by an anti-DLL3 antibody; or
(o) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NOs: 519-524, and wherein the peptide epitope or mimotope is capable of being bound by CAP-100.

Embodiment 6. The modified peptide epitope or mimotope of any of embodiments 1-5, wherein the mutation(s) are substitutions.

Embodiment 7. The modified peptide epitope or mimotope of any of embodiments 1-6, wherein the mutation(s) are conservative substitutions.

Embodiment 8. The modified peptide epitope or mimotope of any of embodiments 1-7, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted or deleted.

Embodiment 9. The modified peptide epitope or mimotope of embodiment 8, wherein the N-terminus of the unmodified peptide epitope or mimotope contains cysteine.

Embodiment 10. The modified peptide epitope or mimotope of any of embodiments 8-9, wherein the C-terminus of the unmodified peptide epitope or mimotope contains cysteine.

Embodiment 11. The modified peptide epitope or mimotope of any of embodiments 8-10, wherein the unmodified peptide epitope or mimotope comprises at least one cysteine between the N-terminus and the C-terminus.

Embodiment 12. The modified peptide epitope or mimotope of any one of embodiments 8-11, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine, glycine, threonine, alanine, or valine; preferably, serine or valine; more preferably, serine.

Embodiment 12.1. The modified peptide epitope or mimotope of embodiment 12, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine.

Embodiment 13. The modified peptide epitope or mimotope of embodiment 12, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with valine.

Embodiment 13.1. The modified peptide epitope or mimotope of any of embodiments 1-13, wherein the modified peptide epitope or mimotope does not comprise any sulfur atoms.

Embodiment 14. The modified peptide epitope or mimotope of any of embodiments 1-13.1, wherein the modified peptide epitope or mimotope does not comprise any cysteine(s).

Embodiment 15. The modified peptide epitope or mimotope of any of embodiments 1-14, wherein the mutation(s) comprise deleting or substituting one or more amino acids that facilitate polypeptide crosslinking in the unmodified peptide epitope or mimotope.

Embodiment 16. The modified peptide epitope or mimotope of any of embodiments 1-15, wherein the mutation(s) comprise deleting or substituting one or more cysteine(s), lysine(s), aspartic acid(s), glutamic acid(s), or any combination thereof.

Embodiment 17. The modified peptide epitope or mimotope of any of embodiments 1-16, wherein the modified peptide epitope or mimotope is capable of being bound by the corresponding antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM; optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody.

Embodiment 18. The modified peptide epitope or mimotope of any of embodiments 1-17, wherein the modified peptide epitope or mimotope is capable of being bound by [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab

(talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589.

Embodiment 19. The modified peptide epitope or mimotope of any of embodiments 1-18, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4, or 5 mutations compared to the unmodified peptide epitope or mimotope.

Embodiment 20. The modified peptide epitope of any of embodiments 1-19, wherein the unmodified peptide epitope or mimotope comprises or consists of no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 consecutive amino acids in an autoimmune-associated antigen or a cancer-associated antigen.

Embodiment 21. A peptide epitope comprising or consisting of SEQ ID NO: 430, wherein the peptide epitope is capable of being bound by an anti-CCR4 antibody.

Embodiment 22. The peptide epitope of embodiment 21, wherein the anti-CCR4 antibody is mogamulizumab.

Embodiment 23. A peptide epitope comprising or consisting of any one of SEQ ID NO: 439-468, wherein the peptide epitope is capable of being bound by an anti-DLL3 antibody.

Embodiment 24. The peptide epitope of embodiment 23, wherein the anti-DLL3 antibody is tarlatamab.

Embodiment 24.1. A peptide epitope comprising or consisting of any one of SEQ ID NOs: 519-524, wherein the peptide epitope is capable of being bound by an anti-CCR7 antibody.

Embodiment 24.2. The peptide epitope of embodiment 24.1, wherein the anti-CCR7 antibody is CAP-100.

Embodiment 25. A recombinant polypeptide comprising or consisting of the peptide epitope or mimotope of any one of embodiments 1-24.2.

Embodiment 26. The recombinant polypeptide of embodiment 25, wherein the recombinant polypeptide consists of the peptide epitope or mimotope.

Embodiment 27. The recombinant polypeptide of any of embodiments 25-26, wherein the peptide epitope or mimotope is capable of being expressed on the surface of a cell.

Embodiment 28. The recombinant polypeptide of any of embodiments 25-27, wherein the peptide epitope or mimotope is located in an extracellular region of the recombinant polypeptide.

Embodiment 29. The recombinant polypeptide of any of embodiments 25-28, wherein the recombinant polypeptide is not any of CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, and CD52.

Embodiment 30. The recombinant polypeptide of any of embodiments 25-29, wherein the recombinant polypeptide is not CCR4.

Embodiment 30.1. The recombinant polypeptide of any of embodiments 25-30, wherein the recombinant polypeptide is not CCR7.

Embodiment 31. The recombinant polypeptide of any of embodiments 25-30.1, wherein the recombinant polypeptide does not comprise a cysteine at the C-terminus of the modified peptide epitope or mimotope.

Embodiment 32. The recombinant polypeptide of any of embodiments 25-31, wherein the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the modified peptide epitope or mimotope.

Embodiment 33. The recombinant polypeptide of any of embodiments 25-32, wherein the recombinant polypeptide does not comprise a cysteine at the N-terminus of the modified peptide epitope or mimotope.

Embodiment 34. The recombinant polypeptide of any of embodiments 25-33, wherein the recombinant polypeptide does not comprise a cysteine immediately before the N-terminus of the modified peptide epitope or mimotope.

Embodiment 35. The recombinant polypeptide of any of embodiments 25-34, wherein the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s), wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the modified peptide epitope or different from the modified peptide epitope.

Embodiment 36. A recombinant polypeptide comprising a tag, wherein the tag comprises or consists of a peptide epitope or a mimotope that mimics an epitope.

Embodiment 36.1. The recombinant polypeptide of embodiment 36, wherein the epitope is derived from CCR4, DLL3, or CCR7.

Embodiment 37. The recombinant polypeptide of any of embodiments 25-36.1, wherein the recombinant polypeptide comprises a binding region for an antigen.

Embodiment 38. The recombinant polypeptide of embodiment 37, wherein the antigen is expressed on the surface of a target cell.

Embodiment 39. The recombinant polypeptide of any of embodiments 25-38, wherein the recombinant polypeptide does not comprise a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

Embodiment 40. The recombinant polypeptide of any of embodiments 37-39, wherein the recombinant polypeptide comprises or consists of a T cell receptor fusion construct (TRuC) derived from a CD3 protein and comprising the binding region; optionally; the CD3 protein is CD3ε.

Embodiment 41. The recombinant polypeptide of any of embodiments 37-38, wherein the recombinant polypeptide comprises or consists of a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

Embodiment 41.1. The recombinant polypeptide of any of embodiments 37-38, wherein the recombinant polypeptide comprises or consists of a receptor comprising:

(a) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRalpha, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRbeta; or

(b) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRbeta, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRalpha,

optionally, wherein at least one of the constant region of TCRalpha or the constant region of TCRbeta are an endogenous constant region of TCRalpha or an endogenous constant region of TCRbeta,

optionally wherein the receptor is a T-body.

Embodiment 41.2. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located between the VH and the constant region of TCRalpha.

Embodiment 41.3. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located between the VL and the constant region of TCRalpha.

Embodiment 41.4. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located between the VH and the constant region of TCRbeta.

Embodiment 41.5. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located between the VL and the constant region of TCRbeta.

Embodiment 41.6. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located N-terminal to the VH.

Embodiment 41.7. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located N-terminal to the VL.

Embodiment 41.8. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located C-terminal to the constant region of TCRalpha.

Embodiment 41.9. The recombinant polypeptide of embodiment 41.1, wherein the peptide epitope or mimotope is located C-terminal to the constant region of TCRbeta.

Embodiment 42. The recombinant polypeptide of any one of embodiments 37-41.9, wherein an immune cell expressing the recombinant polypeptide exhibits one or more of the following:

(a) improved binding to the antigen expressed on the surface of the target cell;

(b) reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the immune cell; and/or

(c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell;

compared to an immune cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope.

Embodiment 43. The recombinant polypeptide of any one of embodiments 37-42, wherein an immune cell expressing the recombinant polypeptide exhibits one or more of the following:

(a) comparable levels of binding to the antigen expressed on the surface of the target cell;

(b) comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the immune cell; and/or

(c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell;

compared to an immune cell expressing a recombinant polypeptide that does not comprise a peptide epitope or mimotope.

Embodiment 44. The recombinant polypeptide of any of embodiments 37-43, comprising the peptide epitope or mimotope located N-terminal to the binding region.

Embodiment 45. The recombinant polypeptide of embodiment 44, wherein the peptide epitope or mimotope is separated from the N-terminus of the binding region by no more than 15, no more than 10, no more than 5, no more than 4, no more than 3, no more than 2, no more than 1, or zero amino acid(s).

Embodiment 46. The recombinant polypeptide of any of embodiments 37-45, comprising the peptide epitope or mimotope located C-terminal to the binding region.

Embodiment 47. The recombinant polypeptide of embodiment 46, wherein the peptide epitope or mimotope is separated from the C-terminus of the binding region by zero, no more than 5, no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, or no more than 60 amino acids.

Embodiment 48. The recombinant polypeptide of any of embodiments 37-47, wherein the peptide epitope or mimotope is separated from the binding region by no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

Embodiment 49. The recombinant polypeptide of any of embodiments 37-48, wherein the peptide epitope or mimotope is located within the binding region.

Embodiment 50. The recombinant polypeptide of any of embodiments 41-49, wherein the TCR is TCRalpha, and wherein the binding region comprises a variable region and a constant region.

Embodiment 51. The recombinant polypeptide of any of embodiments 41-49, wherein the TCR is TCRbeta, and wherein the binding region comprises a variable region and a constant region.

Embodiment 52. The recombinant polypeptide of any of embodiments 50-51, wherein the peptide epitope or mimotope is located between the variable region and the constant region.

Embodiment 53. The recombinant polypeptide of any of embodiments 50-51, wherein the peptide epitope or mimotope is located N-terminal to the variable region.

Embodiment 54. The recombinant polypeptide of any of embodiments 50-51, wherein the peptide epitope or mimotope is located C-terminal to the constant region.

Embodiment 55. The recombinant polypeptide of any of embodiments 41-49, wherein the recombinant polypeptide comprises or consists of the CAR.

Embodiment 56. The recombinant polypeptide of any of embodiments 37-49 and 55, wherein the binding region comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

Embodiment 57. The recombinant polypeptide of any of embodiments 37-49 and 55, wherein the binding region comprises a single chain variable fragment (scFv) comprising a VH and a VL.

Embodiment 58. The recombinant polypeptide of any of embodiments 37-49 and 55, wherein the binding region comprises one or more variable heavy domains of heavy chain (VHHs).

Embodiment 59. The recombinant polypeptide of any of embodiments 56-58, wherein the VH and the VL is separated by a linker, or wherein the binding region comprises at least two VHHs separated by a linker.

Embodiment 60. The recombinant polypeptide of any of embodiments 37-49 and 55, wherein the binding region comprises a first binding domain and a second binding domain, wherein the first binding domain and the second binding domain are separated by a linker.

Embodiment 61. The recombinant polypeptide of any of embodiments 37-49 and 55, wherein the recombinant polypeptide comprises a second binding region located N-terminal or C-terminal to the binding region; optionally, wherein the binding region and the second binding region are separated by a linker.

Embodiment 62. The recombinant polypeptide of any of embodiments 59-61, wherein the peptide epitope or mimotope is located within the linker.

Embodiment 63. The recombinant polypeptide of any of embodiments 59-62, wherein the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

Embodiment 64. The recombinant polypeptide of any of embodiments 37-61, wherein the peptide epitope or mimotope is located in between the binding region(s) and a transmembrane region.

Embodiment 65. The recombinant polypeptide of any of embodiments 37-61, wherein the peptide epitope or mimotope is located within a hinge region that connects the binding region(s) to a transmembrane region.

Embodiment 66. The recombinant polypeptide of embodiment 65, wherein the hinge region comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, no more than 60, no more than 65, or no more than 70 amino acids.

Embodiment 67. The recombinant polypeptide of any of embodiments 41-66, wherein the CAR or TCR comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids N-terminal to the binding region(s), excluding the signal peptide.

Embodiment 68. The recombinant polypeptide of any of embodiments 41-67, wherein the extracellular region of the CAR or TCR comprises no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, or no more than 500 amino acids.

Embodiment 69. The recombinant polypeptide of any of embodiments 41-68, wherein the CAR or TCR comprises, from N-terminus to C-terminus, the binding region(s), a hinge region, a transmembrane region.

Embodiment 70. The recombinant polypeptide of any of embodiments 37-69, wherein the antigen is an autoimmune-associated antigen or a cancer-associated antigen.

Embodiment 71. The recombinant polypeptide of any of embodiments 25-70, wherein the peptide epitope is derived from an autoimmune-associated antigen or a cancer-associated antigen or wherein the mimotope mimics an epitope of the autoimmune-associated antigen or the cancer-associated antigen.

Embodiment 72. The recombinant polypeptide of embodiment 70 or 71, wherein the autoimmune-associated antigen or the cancer-associated antigen is selected from the group

consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, and any combinations thereof.

Embodiment 73. The recombinant polypeptide of any of embodiments 70-72, wherein the cancer-associated antigen is a cancer-associated antigen for a solid tumor.

Embodiment 74. The recombinant polypeptide of embodiment 73, wherein the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin α5β1, CD51, CD27, CDH17, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, integrin α11β1, DLL3, CD2, SSTR2, TROP2, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA, LY6G6D, IL-1α, CEA, C242 antigen, CanAg (MUC1 glycoform), CTAA16.88, UCY2C, IGF2BP3, p53R175H, NKG2D ligands, PRAME, MAGE-A4, vimentin, HER2/neu, CCR4, CCR5, ROPN1, GPA33, ROR1, KK-LC-1, CA-125, folate receptor 1, folate receptor alpha, MUC1, MUC16, MSLN, CLDN6, WT1, ALPPL2, KRASG12D, KRASG12V, CD70, MSLN, CLDN18.2, HPV E6/E7, GPC3, IL-13Rα, EGFRv3, EGFR806, GD2, or CA9.

Embodiment 75. The recombinant polypeptide of embodiment 74, wherein the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin a5b1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, or Integrin α11β1.

Embodiment 75.1. The recombinant polypeptide of embodiment 74, wherein the cancer-associated antigen for the solid tumor comprises or consists of KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

Embodiment 76. The recombinant polypeptide of any of embodiments 70-75.1, wherein the cancer is a solid tumor selected from the group consisting of small cell lung cancer, prostate cancer, colorectal cancer, head and neck squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, lung cancer, pancreatic cancer, stomach cancer, cervical cancer, hepatocellular carcinoma, glioma cancer, neuroblastoma cancer, and renal cell carcinoma.

Embodiment 77. The recombinant polypeptide of any of embodiments 70-76, wherein the cancer is a hematologic cancer selected from B cell lymphoma, T cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.

Embodiment 78. The recombinant polypeptide of any of embodiments 70-77, wherein the cancer-associated antigen is or comprises an antigen selected from Table 2.

Embodiment 79. The recombinant polypeptide of any of embodiments 37-78, wherein the antigen is not CD19.

Embodiment 80. The recombinant polypeptide of any of embodiments 37-79, wherein the antigen is not CCR4.

Embodiment 80.1. The recombinant polypeptide of any of embodiments 37-79, wherein the antigen is not CCR7.

Embodiment 81. The recombinant polypeptide of any of embodiments 37-80.1, wherein the antigen is DLL3.

Embodiment 82. The recombinant polypeptide of any of embodiments 25-81, wherein the epitope is derived from a cancer-associated antigen selected from Table 2.

Embodiment 83. The recombinant polypeptide of any of embodiments 25-81, wherein the epitope is derived from DLL3.

Embodiment 84. The recombinant polypeptide of embodiment 83, wherein the peptide epitope comprises or consists of at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 consecutive amino acids in SEQ ID NO: 486, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

Embodiment 85. The recombinant polypeptide of embodiment 83, wherein the peptide epitope comprises or consists of any one of SEQ ID NO: 439-468, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

Embodiment 86. The recombinant polypeptide of embodiment 83, wherein the peptide epitope comprises or consists of SEQ ID NO: 439, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

Embodiment 87. The recombinant polypeptide of any of embodiments 37-86, wherein the peptide epitope or mimotope is capable of being bound by a monoclonal antibody that binds to the antigen for the binding region.

Embodiment 88. The recombinant polypeptide of any of embodiments 37-80, wherein the epitope is derived from a cancer-associated antigen of T cell lymphoma.

Embodiment 89. The recombinant polypeptide of any of embodiments 37-80, wherein the epitope is derived from a cancer-associated antigen of adult T-cell lymphoma (ATL) and/or cutaneous T-cell lymphoma (CTCL).

Embodiment 90. The recombinant polypeptide of any of embodiments 37-80 and 88-89, wherein the epitope is derived from a cancer-associated antigen of a Th2 cell, a cutaneous lymphocyte antigen-positive skin-homing T cell, and/or a Treg cell.

Embodiment 91. The recombinant polypeptide of any of embodiments 37-80 and 88-90, wherein the epitope is derived from CCR4.

Embodiment 92. The recombinant polypeptide of embodiment 91, wherein the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NO: 430-438, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

Embodiment 93. The recombinant polypeptide of embodiment 91, wherein the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 431.

Embodiment 93.1. The recombinant polypeptide of any of embodiments 37-80 and 88-90, wherein the epitope is derived from CCR7.

Embodiment 93.2. The recombinant polypeptide of embodiment 93.1, wherein the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 519-

524, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

Embodiment 93.3. The recombinant polypeptide of embodiment 93.1, wherein the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NOs: 519-524.

Embodiment 94. The recombinant polypeptide of any of embodiments 25-93.3, wherein the peptide epitope or the mimotope is about 5 to about 10, about 10 to about 20, about 15 to about 25, about 20 to about 30, about 25 to about 35, about 30 to about 40, about 35 to about 45, or about 40 to about 50 amino acids in length.

Embodiment 95. The recombinant polypeptide of any of embodiments 25-94, wherein the peptide epitope or the mimotope is no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids in length.

Embodiment 96. The recombinant polypeptide of any of embodiments 25-95, wherein the peptide epitope does not comprise any mutation compared to the native epitope derived from the antigen.

Embodiment 97. The recombinant polypeptide of any of embodiments 25-96, wherein the peptide epitope or the mimotope is capable of forming an alpha-helical conformation.

Embodiment 98. The recombinant polypeptide of any of embodiments 25-97, wherein the peptide epitope or the mimotope is capable of being bound by an antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM; optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody.

Embodiment 99. The recombinant polypeptide of embodiment 98, wherein the antibody is [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab,

Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108,

M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589 .

Embodiment 100. The recombinant polypeptide of any of embodiments 98-99, wherein the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD3.

Embodiment 101. The recombinant polypeptide of any of embodiments 98-99, wherein the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD16.

Embodiment 102. The recombinant polypeptide of any of embodiments 98-99, wherein the antibody is a tri-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; (ii) 41BB or albumin, and (ii) CD3.

Embodiment 103. The recombinant polypeptide of embodiment 98, wherein the antibody is tarlatamab.

Embodiment 104. The recombinant polypeptide of embodiment 98, wherein the antibody is mogamulizumab.

Embodiment 104.1. The recombinant polypeptide of embodiment 98, wherein the antibody is CAP-100.

Embodiment 105. The recombinant polypeptide of any of embodiments 25-104.1, wherein the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s).

Embodiment 106. The recombinant polypeptide of embodiment 105, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope in the tag.

Embodiment 107. The recombinant polypeptide of embodiment 105, wherein the one or more additional peptide epitope(s) or mimotope(s) are different from the peptide epitope or mimotope in the tag.

Embodiment 108. The recombinant polypeptide of any of embodiments 105-107, wherein the recombinant polypeptide comprises 1, 2, 3, or 4 additional peptide epitope(s) or mimotope(s).

Embodiment 109. The recombinant polypeptide of any of embodiments 25-108, wherein the recombinant polypeptide comprises an extracellular portion comprising the binding region and the peptide epitope or mimotope.

Embodiment 110. The recombinant polypeptide of any of embodiments 25-109, wherein the recombinant polypeptide comprises a transmembrane region or a glycosylphosphatidylinositol (GPI) anchor.

Embodiment 111. The recombinant polypeptide of any of embodiments 25-110, wherein the recombinant polypeptide comprises an intracellular region; optionally, the intracellular domain is located C-terminal to the transmembrane region.

Embodiment 112. The recombinant polypeptide of embodiment 111, wherein the intracellular region comprises one or more intracellular signaling domains.

Embodiment 113. The recombinant polypeptide of embodiment 112, wherein the one or more intracellular signaling domains are derived from a protein selected from the group consisting of CD3zeta, 1xxCD3zeta, CD28, 4-1BB, OX40, IL2Rb turbodomains, MYD88/CD40, MYD88/CD40 inducible costimulatory domain, CD2, B7-1/CD80; B7-2/CD86; B7-H1/PD-L1; B7-H2; B7-H3; B7-H4; B7-H6; B7-H7; BTLA/CD272; CD28; CTLA-4; Gi24/VISTA/B7-H5; ICOS/CD278; PD-1; PD-L2/B7-DC; PDCD6); 4-1BB/TNFSF9/CD137; 4-1BB Ligand/TNFSF9; BAFF/BLyS/TNFSF13B; BAFF R/TNFRSF13C; CD27/TNFRSF7; CD27 Ligand/TNFSF7; CD30/TNFRSF8; CD30 Ligand/TNFSF8; CD40/TNFRSF5; CD40/TNFSF5; CD40 Ligand/TNFSF5; DR3/TNFRSF25; GITR/TNFRSF18; GITR Ligand/TNFSF18; HVEM/TNFRSF14;

LIGHT/TNFSF14; Lymphotoxin-alpha/TNF-beta; OX40/TNFRSF4; 0X40 Ligand/TNFSF4; RELT/TNFRSF19L; TACI/TNFRSF13B; TL1A/TNFSF15; TNF-alpha; TNF RII/TNFRSF1B); 2B4/CD244/SLAMF4; BLAME/SLAMF8; CD2; CD2F-10/SLAMF9; CD48/SLAMF2; CD58/LFA-3; CD84/SLAMF5; CD229/SLAMF3; CRACC/SLAMF7; NTB- A/SLAMF6; SLAM/CD150); CD2; CD7; CD53; CD82/Kai-1; CD90/Thyl; CD96; CD160; CD200; CD300a/LMIRl; HLA Class I; HLA-DR; Ikaros; Integrin alpha 4/CD49d; Integrin alpha 4 beta 1; Integrin alpha 4 beta 7/LPAM-l; LAG-3; TCL1A; TCL1B; CRTAM; DAP12; Dectin- 1/CLEC7A; DPPIV/CD26; EphB6; TIM- 1 /KIM- 1 /HA VCR; TIM-4; TSLP; TSLP R; lymphocyte function associated antigen-1 (LFA-1); NKG2C, an immunoreceptor tyrosine based activation motif (ITAM), CD27, CD134/OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, and any combinations thereof.

Embodiment 114. The recombinant polypeptide of embodiment 113, wherein the intracellular region comprises an intracellular signaling domain derived from 4-1BB and/or an intracellular signaling domain derived from CD3zeta; optionally, the intracellular region comprises, from N-term to C-term, the intracellular signaling domain derived from 4-1BB and the intracellular signaling domain derived from CD3zeta.

Embodiment 115. The recombinant polypeptide of embodiment 114, wherein the intracellular region comprises the intracellular signaling domain derived from 4-1BB comprising a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 422.

Embodiment 116. The recombinant polypeptide of embodiment 114 or 115, wherein the intracellular region comprises the intracellular signaling domain derived from CD3zeta comprising:

(a) a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 423; or

(b) a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 488 wherein the amino acid corresponding to position 14 of SEQ ID NO: 488 is lysine.

Embodiment 117. The recombinant polypeptide of any of embodiments 64-116, wherein the transmembrane region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 421.

Embodiment 118. The recombinant polypeptide of any of embodiments 69-117, wherein the hinge region comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 420.

Embodiment 119. The recombinant polypeptide of any of embodiments 59-118, wherein the linker comprises a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to any one of SEQ ID NO: 416-418.

Embodiment 120. The recombinant polypeptide of any of embodiments 25-119, comprising a polypeptide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to any one of SEQ ID NO: 401-413.

Embodiment 121. The recombinant polypeptide of any of embodiments 25-120, wherein the recombinant polypeptide is no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, no more than 500, no more than 550, no more than 600, no more than 650, no more than 700, no more than 750, or no more than 800 amino acids in length.

Embodiment 122. A recombinant nucleic acid encoding the peptide epitope or mimotope of any one of embodiments 1-24.2, or the recombinant polypeptide of any one of embodiments 25-121.

Embodiment 123. The recombinant nucleic acid of embodiment 122, wherein the recombinant nucleic acid encodes (i) the TCRalpha comprising the peptide epitope or mimotope and a TCRbeta, or (ii) a TCRalpha and the TCRbeta comprising the peptide epitope or mimotope.

Embodiment 124. The recombinant nucleic acid of embodiment 122, wherein the recombinant nucleic acid encodes a TCRalpha, a TCRbeta, and the peptide epitope or mimotope located in between the TCRalpha and the TCRbeta.

Embodiment 125. The recombinant nucleic acid of embodiment 123 or 124, wherein the TCRalpha and the TCRbeta are encoded by the same coding region; optionally, wherein the TCRalpha and the TCRbeta are separated by a cleavage site.

Embodiment 125.1. The recombinant nucleic acid of embodiment 122, wherein the recombinant nucleic acid encodes (i) the VH and the constant region of TCRalpha of the HIT receptor, or (ii) the VL and the constant region of TCRbeta of the HIT receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope.

Embodiment 125.2. The recombinant nucleic acid of embodiment 122, wherein the recombinant nucleic acid encodes (i) the VH and the constant region of TCRbeta of the HIT receptor, or (ii) the VL and the constant region of TCRalpha of the HIT receptor, wherein (i) or (ii) comprises the peptide epitope or mimotope.

Embodiment 125.3. The recombinant nucleic acid of embodiment 125.1 or 125.2, wherein (i) and (ii) are encoded by the same coding region; optionally, wherein (i) and (ii) are separated by a cleavage site.

Embodiment 126. A recombinant nucleic acid encoding (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

Embodiment 126.1. The recombinant nucleic acid of any of embodiments 122-126, wherein the recombinant nucleic acid is less than 5.0 kb in length.

Embodiment 127. The recombinant nucleic acid of any of embodiments 122-126.1, wherein the recombinant nucleic acid is less than 4.8 kb in length.

Embodiment 127.1. The recombinant nucleic acid of any of embodiments 122-127, wherein the recombinant nucleic acid is less than 4.7 kb in length.

Embodiment 128. The recombinant nucleic acid of any of embodiments 122-127.1, wherein the recombinant nucleic acid further comprises a polynucleotide sequence encoding a second recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof.

Embodiment 129. The recombinant nucleic acid of embodiment 128, wherein the CARD containing protein comprises (i) a CARD domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 11-103 of SEQ ID NO: 1 and (ii) a Src Homology region 2 (SH2) domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 622-716 of SEQ ID NO: 1.

Embodiment 130. The recombinant nucleic acid of embodiment 128 or 129, wherein the CARD containing protein comprises a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NO: 1-4.

Embodiment 131. The recombinant nucleic acid of any one of embodiments 128-130, wherein the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

Embodiment 132. The recombinant nucleic acid of any one of embodiments 122-131, wherein the polynucleotide sequence encoding the recombinant polypeptide is operably linked to a promoter.

Embodiment 133. The recombinant nucleic acid of embodiment 132, wherein the promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter,

CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of moloney virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

Embodiment 134. The recombinant nucleic acid of embodiment 132, wherein the promoter is a constitutive promoter, optionally wherein the constitutive promoter is or comprises a CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10

promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, MND promoter or a short EF1a promoter.

Embodiment 135. The recombinant nucleic acid of any one of embodiments 122-131, wherein the polynucleotide sequence encoding the recombinant polypeptide is not operably linked to a promoter.

Embodiment 136. The recombinant nucleic acid of any one of embodiments 122-135, wherein the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more cleavable linkers, optionally wherein the one or more cleavable linkers comprise a P2A, E2A, F2A, or T2A self-cleaving peptide.

Embodiment 137. The recombinant nucleic acid of any one of embodiments 122-136, wherein the recombinant nucleic acid further comprises one or more nucleic acid element sequences, optionally wherein the one or more nucleic acid element sequences is or comprises ribosomal binding sites, enhancer elements, activator elements, translational start sequences, translational termination sequences, transcription start sequences, transcription termination sequences, polyadenylation signal sequences, a 70 bp poly(A) tract, a 100 bp poly(A) tract, a 172 bp poly(A) tract, a 200 bp poly(A) tract, a 300 bp poly(A) tract, a 325 bp poly(A) tract, replication elements, RNA processing and export elements, transposon sequences, transposase sequences, insulator sequences, internal ribosome entry sites (IRES), 5'UTRs, 3'UTRs, mRNA 3' end processing sequences, boundary elements, locus control regions (LCR), matrix attachment regions (MAR), recombination or cassette exchange sequences, linker sequences, cleavable linker sequences, secretion signals, resistance markers, anchoring peptides, localization signals, fusion tags, affinity tags, chaperonins, proteases, or any combination thereof.

Embodiment 138. The recombinant nucleic acid of any one of embodiments 122-137, wherein the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or a non-coding RNA, optionally wherein the non-coding RNA comprises a shRNA or a microRNA.

Embodiment 139. The recombinant nucleic acid of embodiment 138, wherein the one or more additional polypeptides comprise an additional potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, or any combination thereof.

Embodiment 140. The recombinant nucleic acid of embodiment 139, wherein the additional potency enhancement polypeptide is or comprises a dominant negative form of an inhibitor of a cell-mediated immune response of the immune cell (*e.g.,* TGFβR2 DNR), c-Jun, CCL19, CCL21, IL2R, IL7, IL7Ralpha, IL15, IL15RA, IL18, decoy-resistant IL18 (DR-18), MyD88/CD40, PD1-CD28 switch receptor, PD1-41BB switch receptor, CD40L-CD28 switch receptor, CTBR12 switch receptor, or CD8alpha/beta.

Embodiment 141. The recombinant nucleic acid of any one of embodiments 122-140, wherein the recombinant nucleic acid is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length.

Embodiment 141.1. The recombinant nucleic acid of embodiment 141, wherein the recombinant nucleic acid is less than about 5.0 kb in length.

Embodiment 142. The recombinant nucleic acid of embodiment 141, wherein the recombinant nucleic acid is less than about 4.7 kb in length.

Embodiment 143. A recombinant nucleic acid comprising, from 5' to 3':
(1) a promoter,
(2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,
(3) a cleavable linker encoding sequence, and
(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121.

Embodiment 144. A recombinant nucleic acid comprising, from 5' to 3':
(1) a promoter,
(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,
(3) a cleavable linker encoding sequence, and
(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

Embodiment 145. A recombinant nucleic acid comprising, from 5' to 3':
(1) a promoter,
(2) a polynucleotide sequence encoding a CARD-containing protein or a functional

fragment thereof,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

Embodiment 146. A recombinant nucleic acid comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor.

Embodiment 147. A recombinant nucleic acid comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

Embodiment 148. A recombinant nucleic acid comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof.

Embodiment 149. A recombinant nucleic acid comprising, from 5' to 3':

(1) a promoter,

(2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(5) a cleavable linker encoding sequence, and

(6) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121.

Embodiment 150. A recombinant nucleic acid comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121, and

(5) a cleavable linker encoding sequence.

Embodiment 151. A recombinant nucleic acid comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and

(5) a cleavable linker encoding sequence.

Embodiment 152. A recombinant nucleic acid comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and

(7) a cleavable linker encoding sequence.

Embodiment 153. A recombinant nucleic acid comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121, and

(7) a cleavable linker encoding sequence.

Embodiment 154. A recombinant nucleic acid comprising, from 5' to 3':

(1) a cleavable linker encoding sequence,

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

(3) a cleavable linker encoding sequence,

(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(5) a cleavable linker encoding sequence,

(6) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a

cytokine, a chemokine, or a growth factor, and
(7) a cleavable linker encoding sequence.

Embodiment 155. A recombinant nucleic acid comprising, from 5' to 3':
(1) a cleavable linker encoding sequence,
(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,
(3) a cleavable linker encoding sequence,
(4) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,
(5) a cleavable linker encoding sequence,
(6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and
(7) a cleavable linker encoding sequence.

Embodiment 156. A recombinant nucleic acid comprising, from 5' to 3':
(1) a cleavable linker encoding sequence,
(2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,
(3) a cleavable linker encoding sequence,
(4) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,
(5) a cleavable linker encoding sequence,
(6) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121, and
(7) a cleavable linker encoding sequence.

Embodiment 157. A recombinant nucleic acid comprising, from 5' to 3':
(1) a cleavable linker encoding sequence,
(2) a polynucleotide sequence encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,
(3) a cleavable linker encoding sequence,
(4) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,
(5) a cleavable linker encoding sequence,

(6) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and

(7) a cleavable linker encoding sequence.

Embodiment 158. A recombinant nucleic acid comprising:

(1) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof, and

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121,

wherein the recombinant nucleic acid is an RNA construct.

Embodiment 159. A recombinant nucleic acid comprising:

(1) a polynucleotide sequence encoding a CARD-containing protein or a functional fragment thereof,

(2) a polynucleotide sequence encoding the recombinant polypeptide of any one of embodiments 25-121, and

(3) a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor,

wherein the nucleic acid is an RNA construct.

Embodiment 160. A combination of recombinant nucleic acid constructs comprising:

(1) a first nucleic acid construct encoding a CARD-containing protein or a functional fragment thereof, and

(2) a second nucleic acid construct encoding the recombinant polypeptide of any one of embodiments 25-121,

wherein the first and the second nucleic acid constructs are both RNA constructs.

Embodiment 161. A combination of recombinant nucleic acid constructs comprising:

(1) a first nucleic acid construct encoding a CARD-containing protein or a functional fragment thereof,

(2) a second nucleic acid construct encoding the recombinant polypeptide of any one of embodiments 25-121, and

(3) a third nucleic acid construct encoding a polynucleotide encoding an additional potency enhancement polypeptide, a cytokine, a chemokine, or a growth factor, and

wherein the first, second, and third nucleic acid constructs are each RNA constructs.

Embodiment 162. The recombinant nucleic acid of any of embodiments 143-161, wherein the CARD-containing protein comprises (i) a CARD domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 11-103 of SEQ ID NO: 1 and (ii) a Src Homology region 2 (SH2) domain comprising a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acids 622-716 of SEQ ID NO: 1.

Embodiment 163. The recombinant nucleic acid of any of embodiments 143-162, wherein the CARD containing protein comprises a sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to any one of SEQ ID NO: 1-4.

Embodiment 164. The recombinant nucleic acid of any one of embodiments 143-163, wherein the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

Embodiment 165. A vector comprising the recombinant nucleic acid of any one of embodiments 122-164.

Embodiment 166. The vector of embodiment 165, wherein the vector is a plasmid.

Embodiment 167. The vector of embodiment 165, wherein the vector is a synthetic DNA vector.

Embodiment 167.1. The vector of embodiment 167, wherein the vector is a single stranded DNA vector or a double stranded DNA vector.

Embodiment 168. The vector of embodiment 165, wherein the vector is a linear DNA vector.

Embodiment 169. The vector of embodiment 165, wherein the vector is a closed linear DNA vector.

Embodiment 169.1. The vector of embodiment 165, wherein the vector is a synthetic RNA vector.

Embodiment 170. The vector of embodiment 165, wherein the vector is a phagemid vector.

Embodiment 171. The vector of embodiment 165, wherein the vector is a viral vector.

Embodiment 172. The vector of embodiment 171, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector.

Embodiment 173. The vector of embodiment 172, wherein the retrovirus is a lentivirus.

Embodiment 174. The vector of embodiment 173, wherein the lentivirus is a VSV-G pseudotyped lentivirus.

Embodiment 175. The vector of embodiment 172, wherein the AAV vector is AAV6 vector.

Embodiment 176. The vector of embodiment 172, wherein the AAV vector is AAV9 vector.

Embodiment 177. The vector of embodiment 172, wherein the AAV vector is a split-intein dual AAV vector.

Embodiment 178. The vector of embodiment 171, wherein the vector is a redirected lentiviral vector.

Embodiment 179. The vector of embodiment 171, wherein the vector is a fusosome.

Embodiment 180. The vector of embodiment 171, wherein the vector is a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein.

Embodiment 181. The vector of embodiment 171, wherein the vector is an enveloped delivery vehicle.

Embodiment 182. The vector of embodiment 171, wherein the vector is self-replicating RNA virus.

Embodiment 183. The vector of embodiment 165, wherein the vector is mRNA-packaging virus-like particle.

Embodiment 184. The vector of embodiment 165, wherein the vector is RNP-packaging virus-like particle.

Embodiment 185. A particle comprising the recombinant nucleic acid of any one of embodiments 122-164.

Embodiment 186. The particle of embodiment 185, wherein the particle is a lipid nanoparticle (LNP).

Embodiment 187. The particle of embodiment 185, wherein the particle is a selective organ targeting (SORT) LNP.

Embodiment 188. The particle of embodiment 185, wherein the particle is an antibody targeted LNP.

Embodiment 189. The particle of any one of embodiments 186-188, wherein the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid).

Embodiment 190. The particle of embodiment 185, wherein the particle is a polymer nanoparticle.

Embodiment 191. The particle of embodiment 185, wherein the particle is a protein nanoparticle.

Embodiment 192. An engineered cell, wherein the engineered cell (i) comprises the recombinant nucleic acid of any one of embodiments 122-164, (ii) is transfected or transduced by the vector of any one of embodiments 165-184, or (iii) is contacted by the particle of any one of embodiments 185-191.

Embodiment 193. An engineered cell expressing the peptide epitope or mimotope of any one of embodiments 1-24.2, or the recombinant polypeptide of any one of embodiments 25-121.

Embodiment 194. An engineered cell expressing (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

Embodiment 195. An engineered cell comprising the recombinant polypeptide of any one of embodiments 25-121 or the recombinant nucleic acid of any one of embodiments 122-164.

Embodiment 196. The engineered cell of any one of embodiments 192-195, wherein the engineered cell further expresses a second recombinant polypeptide comprising a caspase-associated recruitment domain (CARD) containing protein or a functional fragment thereof.

Embodiment 197. The engineered cell of embodiment 196, wherein the second recombinant polypeptide comprising the CARD containing protein or the functional fragment thereof comprises no more than 500, no more than 550, no more than 600, no more than 650, or no more than 700 amino acids.

Embodiment 198. The engineered cell of any one of embodiments 192-197, wherein the engineered cell comprises one or more additional peptide epitope(s) or mimotope(s) present on the cell surface, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope or different from the peptide epitope or mimotope.

Embodiment 199. The engineered cell of any one of embodiments 192-198, wherein activating the engineered cell results in the secretion of IL-2 by the cell at a level that is at least 10%, at least 20%, at least 50%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, least 30-fold, or at least 100-fold higher than the IL-2 secretion level of a control cell lacking the recombinant polypeptide.

Embodiment 200. The engineered cell of any one of embodiments 192-199, wherein expression of the recombinant polypeptide is under the control of an endogenous promoter.

Embodiment 201. The engineered cell of embodiment 200, wherein the endogenous promoter is a TCRa promoter, a TCRb promoter, a CD3d promoter, a CD3g promoter, a CD3e promoter, a CD3z promoter, a CARD9 promoter, a CARD10 promoter, a CARD11 promoter, a CARD14 promoter, or a PIK3R3 promoter.

Embodiment 202. The engineered cell of any one of embodiments 192-199, wherein expression of the recombinant polypeptide is under the control of an exogenous promoter.

Embodiment 203. The engineered cell of embodiment 202, wherein the exogenous promoter is or comprises a minimal TATA promoter, pGK promoter, actin promoter, CD4 promoter, CD8a promoter, CD8b promoter, TCRa promoter, TCRb promoter, CD3d promoter, CD3g promoter, CD3e promoter, CD3z promoter, CARD9 promoter, CARD10 promoter, CARD11 promoter, CARD14 promoter, PIK3R3 promoter, CD25 promoter, IL2 promoter, IL7 promoter, IL15 promoter, KLRG-1 promoter, HLA-DR promoter, CD38 promoter, CD69 promoter, Ki-67 promoter, CD11a promoter, CD58 promoter, CD99 promoter, CD62L promoter, CD103 promoter, CCR4 promoter, CCR5 promoter, CCR6 promoter, CCR9 promoter, CCR10 promoter, CXCR3 promoter, CXCR4 promoter, CLA promoter, Granzyme A promoter, Granzyme B promoter, Perforin promoter, CD57 promoter, CD161 promoter, IL-18Ra promoter, CD69 promoter, GzmB promoter, T-bet promoter, IFNgamma promoter, TIM3 promoter, IL4 promoter, GATA3 promoter, IL1 promoter, IL5 promoter, IL6 promoter, IL13 promoter, IL10 promoter, IL17A promoter, IL6 promoter, IL21 promoter, IL23R promoter, FoxP3 promoter, CTLA4 promoter, CD25 promoter, PD1 promoter, CD45RO promoter, CCR7 promoter, CD28 promoter, CD95 promoter, CD28 promoter, CD27 promoter, CD127 promoter, PD-1 promoter, CD122 promoter, CD132 promoter, c-Kit promoter, nuclear factor of activated T cells (NFAT) promoter, programmed death 1 (PD-1) promoter, T cell immunoglobulin mucin-3 (TIM-3) promoter, cytotoxic T lymphocyte antigen-4 (CTLA4) promoter, lymphocyte-activation protein 3 (LAG-3) promoter, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) promoter, B- and T-lymphocyte attenuator (BTLA) promoter, CD25 promoter, CD69 promoter, Fas ligand (FasL) promoter, TIGIT promoter, TGF-beta promoter, T-bet promoter, Eomes promoter, GATA3 promoter, CD45RA promoter, 2B4 promoter, Type I interferon (IFN) alpha, Type I IFN beta promoter, IFN gamma promoter, IRF3 promoter, IRF7 promoter, NFkB promoter, AP-1 promoter, TNF-alpha promoter, CD130 promoter, NR4A1 promoter, NR4A2, NR4A3 promoter, MND promoter, EF-1alpha promoter, short EF-1alpha promoter, CAG promoter, ubiquitin/S27a promoter, SV40 promoter, SV40 early promoter, adenovirus major late promoter, mouse metallothionein- I promoter, Moloney murine leukemia virus (MMLV) long terminal repeat (LTR) region, CMV promoter, immunoglobulin promoter, heat shock promoter, polyoma virus promoter, fowlpox virus promoter, bovine papilloma virus promoter, avian sarcoma virus promoter, retrovirus promoter, hepatitis-B virus promoter, PGK promoter, vaccinia virus 7.5K promoter, TK promoter of HSV, mouse mammary tumor virus (MMTV) promoter, LTR promoter of HIV, promoter of 332oloney

virus, Epstein Barr virus (EBV) promoter, Rous sarcoma virus (RSV) promoter, U6 promoter, or UBC promoter.

Embodiment 204. The engineered cell of any one of embodiments 192-203, wherein the nucleic acid construct is inserted in a T-cell receptor (TCR) locus, a CD3 locus, a B2 microglobulin (B2M) locus, a class II transactivator (CIITA) locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, a PIK3R3 locus, or a safe harbor locus.

Embodiment 205. The engineered cell of embodiment 204, wherein the TCR locus is or comprises a TRAC locus, a TRBC1 locus, a TRBC2 locus, or a TRAJ locus.

Embodiment 205.1 The engineered cell of embodiment 205, wherein the TRAJ locus is or comprises a TRAJ intron splice acceptor locus.

Embodiment 206. The engineered cell of embodiment 204, wherein the cell does not express a functional gene product of an endogenous TRAC locus.

Embodiment 207. The engineered cell of embodiment 204, wherein the cell does not express a functional gene product of an endogenous TRBC1 locus.

Embodiment 208. The engineered cell of embodiment 204, wherein the cell does not express a functional gene product of an endogenous TRBC2 locus.

Embodiment 208.1. The engineered cell of embodiment 204, wherein the cell does not express a functional gene product of an endogenous TRAJ locus.

Embodiment 209. The engineered cell of embodiment 204, wherein the CD3 locus is or comprises: a CD3d locus, a CD3g locus, a CD3e locus, or CD3z locus.

Embodiment 210. The engineered cell of embodiment 209, wherein the cell does not express a functional gene product of an endogenous CD3d locus.

Embodiment 211. The engineered cell of embodiment 209, wherein the cell does not express a functional gene product of an endogenous CD3g locus.

Embodiment 212. The engineered cell of embodiment 209, wherein the cell does not express a functional gene product of an endogenous CD3e locus.

Embodiment 213. The engineered cell of embodiment 209, wherein the cell does not express a functional gene product of an endogenous CD3z locus.

Embodiment 214. The engineered cell of embodiment 204, wherein the safe harbor locus is or comprises an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

Embodiment 215. The engineered cell of any one of embodiments 192-214, wherein the nucleic acid construct is inserted is an exon, an intron, between an intron and an exon, or a regulatory region.

Embodiment 216. The engineered cell of embodiment any one of embodiments 192-215, wherein the cell is selected from the group of consisting of a T cell, a CD4+ T cell, a CD8+ T cell, a regulatory T cell (Treg), a gamma delta T cell (γδT), an invariant natural killer T (iNKT) cell, a mucosal associated invariant T (MAIT) cell, a macrophage, a monocyte, a natural killer (NK) cell, a tumor infiltrating lymphocyte (TIL), a cytotoxic T cell, a T helper cell, a memory T cell, a central memory T (TCM) cell, a stem memory T (TSCM) cell, a stem-cell-like memory T cell (or stem-like memory T cells), an effector memory T (TEM) cell, a TEMRA (CD45RA+) cell, an effector T cell, a Th1 cell, a Th2 cell, a Th9 cell, a Th17 cell, a Th22 cell, a Tfh (follicular helper) cell, a natural killer T (NKT) cell, a transitional memory T (TTM) cell, a terminal effector T (TTE) cell, a naïve T (TN) cell, a hematopoietic stem cell, and a progenitor cell of the lymphoid lineage.

Embodiment 217. The engineered cell of embodiment 216, wherein the cell is selected from the group consisting of a T cell, a macrophage, a monocyte, and a natural killer (NK) cell.

Embodiment 218. The engineered cell of embodiment 217, wherein the cell is a T cell.

Embodiment 219. The engineered cell of embodiment 218, wherein the T cell is selected from the group consisting of a regulatory T cell (Treg), a gamma delta T cell, a CD8+ T cell, an invariant iNKT cell, a MAIT cell, a CAR T cell, a tumor-infiltrating lymphocyte, and an engineered T cell comprising a transcriptional receptor.

Embodiment 220. The engineered cell of any one of embodiments 192-219, wherein the cell is an autologous cell.

Embodiment 221. The engineered cell of any one of embodiments 192-219, wherein the cell is an allogeneic cell.

Embodiment 222. The engineered cell of any one of embodiments 192-221, wherein the cell is a primary cell.

Embodiment 223. The engineered cell of any one of embodiments 192-221, wherein the cell is derived from a stem cell.

Embodiment 224. The engineered cell of any one of embodiments 192-223, wherein the cell is genetically modified.

Embodiment 225. The engineered cell of any one of embodiments 192-224, wherein the cell has reduced or eliminated cell surface expression of an endogenous T cell receptor.

Embodiment 226. The engineered cell of any one of embodiments 192-225, wherein the cell is more prone to display central memory phenotype than a control cell lacking the recombinant polypeptide.

Embodiment 227. The engineered cell of any one of embodiments 192-226, wherein the cell further comprises one or more modifications that inactivate or disrupt one or more alleles of TGFbeta, Regnase-1, FAS, PTPN2, NR4A3, CD52, PD-1, B2M, CIITA, SOCS1, CBLB, DGKalpha, and/or DGKζ.

Embodiment 228. The engineered cell of any one of embodiments 192-227, wherein the cell is a mammalian cell.

Embodiment 229. The engineered cell of embodiment 228, wherein the cell is selected from the group consisting of a human cell, a mouse cell, and a canine cell.

Embodiment 230. The engineered cell of any one of embodiments 192-229, wherein the cell further comprises a sequence specific nuclease, a nucleic acid programmable DNA binding protein, an RNA guided nuclease, an RNA-guided nuclease comprising a Cas nuclease and a guide RNA (CRISPR-Cas combination), a ribonucleoprotein (RNP) complex comprising a gRNA and a Cas nuclease, a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-

deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

Embodiment 231. The engineered cell of embodiment 230, wherein the cell further comprises Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional fragment thereof.

Embodiment 232. The engineered cell of embodiment 231, wherein the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

Embodiment 232.1. The engineered cell of any one of embodiments 192-232, wherein the engineered cell exhibits one or more of the following:

(a) improved binding to the antigen expressed on the surface of a target cell;

(b) reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or

(c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell;

compared to a control cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope.

Embodiment 232.2. The engineered cell of any one of embodiments 192-232.1, wherein the engineered cell exhibits one or more of the following:

(a) comparable levels of binding to the antigen expressed on the surface of a target cell;

(b) comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or

(c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell;
compared to a control cell expressing a recombinant polypeptide that does not comprise the peptide epitope or mimotope.

Embodiment 233. The engineered cell of any one of embodiments 192-232.2, wherein the cell has reduced exhaustion, increased proliferative capacity, enhanced replicative lifespan, decreased replicative senescence, enhanced anti-tumor effect, reduced dysfunction, enhanced persistence, and/or increase intratumoral presence *in vivo*.

Embodiment 234. The engineered cell of any one of embodiments 192-233, wherein the cell has increased or decreased signaling through the CARD11-BCL10-MALT1 complex, NF-κB, AP-1, NFAT, JAK/STAT, and/or MEK/ERK pathways.

Embodiment 235. A composition comprising the vector of any one of embodiments 165-184, the particle of any one of embodiments 185-191, or the engineered cell of any one of embodiments 192-234.

Embodiment 236. The composition of embodiment 235, further comprising a pharmaceutically acceptable carrier.

Embodiment 237. The composition of embodiment 235 or 236, wherein at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

Embodiment 238. The composition of any one of embodiments 235-237, wherein at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

Embodiment 239. The composition of any one of embodiments 235-238, wherein at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAC locus.

Embodiment 240. The composition of any one of embodiments 235-239, wherein: at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide of any one of embodiments 1-86; at

least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the receptor, or recombinant TCR; and/or at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAC locus.

Embodiment 240.1. The composition of any one of embodiments 235-238, wherein at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus.

Embodiment 240.2. The composition of any one of embodiments 235-240.1, wherein: at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide of any one of embodiments 1-86; at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the receptor, or recombinant TCR; and/or at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition do not express a functional gene product of an endogenous TRAJ locus.

Embodiment 241. The composition of any one of embodiments 235-240.2, wherein the composition comprises CD4+ T cells and CD8+ T cells, and the percentage of CD4+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the percentage of CD8+ T cells in the composition is between at or about 20% and at or about 80%, or at or about 20%, 30%, 40%, 50%, 60%, 70%, or 80% of the total cells in the composition; and/or the ratio of CD4+ T cells to CD8+ T cells is from at or about 1:3 to at or about 3:1, optionally at or about 1:1.

Embodiment 242. A method of preparing an engineered cell, wherein the method comprises contacting the cell with the recombinant nucleic acid of any one of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191.

Embodiment 243. A method of preparing an engineered cell, wherein the method comprises expressing in the cell:
(a) the recombinant polypeptide of any of embodiments 25-121; or
(b) at least the following two polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising at least one tag

in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope.

Embodiment 244. The method of any one of embodiments 242-243, wherein the method comprises enriching the engineered cells expressing the recombinant polypeptide using a binding agent that binds to the peptide epitope or mimotope; optionally, wherein the binding agent comprises a monoclonal antibody.

Embodiment 245. The method of any one of embodiments 242-244, wherein at least at or about 70%, 75%, 80%, 85%, 90%, or 95% of the engineered cells, or of the total cells, in the composition express the recombinant polypeptide.

Embodiment 246. The method of any one of embodiments 242-245, wherein the cell is *in vivo*.

Embodiment 247. The method of any one of embodiments 242-246, wherein the cell is prepared *in vivo*.

Embodiment 248. The method of any one of embodiments 242-247, wherein the cell is prepared in the body of a subject in need of treatment.

Embodiment 249. The method of any one of embodiments 226-248, wherein the recombinant polypeptide is expressed in the cell, and/or the nucleic acid construct is introduced into the cell, by contacting the cell with a vector or a particle.

Embodiment 250. The method of embodiment 249, wherein the vector is a plasmid, a synthetic DNA vector, a linear DNA vector, a closed linear DNA vector, a phagemid vector, an RNA vector, an mRNA vector, or a viral vector.

Embodiment 251. The method of embodiment 250, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector.

Embodiment 252. The method of embodiment 251, wherein the retrovirus is a lentivirus, optionally a VSV-G pseudotyped lentivirus.

Embodiment 253. The method of embodiment 251, wherein the AAV vector is an AAV6 vector or an AAV9 vector.

Embodiment 254. The method of embodiment 249, wherein the vector is a fusosome, a lentiviral particle engineered with the anti-CD3 Cocal glycoprotein, or an enveloped delivery vehicle.

Embodiment 255. The method of embodiment 249, wherein the vector is a self-replicating RNA virus, an mRNA-packaging virus-like particle, or a ribonucleoprotein (RNP)-packaging virus-like particle.

Embodiment 256. The method of embodiment 249, wherein the particle is a lipid nanoparticle (LNP), a selective organ targeting (SORT) LNP, or an antibody targeted LNP.

Embodiment 257. The method of embodiment 256, wherein the LNP comprises: (i) an ionizable lipid (*e.g.,* an amino lipid), (ii) a sterol or other structural lipid, (iii) a non-cationic helper lipid or phospholipid, and (iv) a PEG-lipid (*e.g.,* a PEG-modified lipid).

Embodiment 258. The method of embodiment 249, wherein the particle is a polymer nanoparticle or a protein nanoparticle.

Embodiment 259. The method of any one of embodiments 242-245, wherein the cell is *ex vivo*.

Embodiment 260. The method of any one of embodiments 242-245, wherein the cell is prepared *ex vivo*.

Embodiment 261. The method of any one of embodiments 242-245, wherein the cell is prepared outside of the body of a subject in need of treatment.

Embodiment 262. The method of any one of embodiments 259-261, wherein the nucleic acid construct is introduced into the cell by electroporation or transfection.

Embodiment 263. The method of embodiment 262, wherein the transfection is lipofection.

Embodiment 264. The method of embodiment 262, wherein the nucleic acid construct is a plasmid, a synthetic DNA vector, a linear DNA vector, a closed linear DNA vector, a phagemid vector, an RNA vector, or an mRNA vector.

Embodiment 265. The method of any one of embodiments 259-261, wherein the nucleic acid construct is introduced via a viral vector.

Embodiment 266. The method of embodiment 265, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector.

Embodiment 267. The method of embodiment 266, wherein the retrovirus is a lentivirus, optionally a VSV-G pseudotyped lentivirus.

Embodiment 268. The method of embodiment 266, wherein the AAV vector is an AAV6 vector or an AAV9 vector.

Embodiment 269. A method of preparing an engineered cell, wherein the method comprises contacting the cell with an AAV vector comprising a recombinant nucleic acid encoding:
(a) the recombinant polypeptide of any of embodiments 25-121; or
(b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide.

Embodiment 270. A method of preparing an engineered cell, wherein the method comprises contacting the cell with a particle comprising a recombinant nucleic acid encoding:
(a) the recombinant polypeptide of any of embodiments 25-121; or
(b) at least the following three polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope; and (iii) a potency enhancement polypeptide;
optionally, wherein the particle is a lipid nanoparticle (LNP).

Embodiment 271. The method of any one of embodiments 242-270, wherein the method comprises genetically modifying the cell for expression of the recombinant polypeptide.

Embodiment 272. The method of embodiment 271, wherein the method comprises introducing into the cell one or more nucleic acids encoding a sequence specific nuclease or a nucleic acid programmable DNA binding protein.

Embodiment 273. The method of embodiment 272, wherein the method additionally comprises introducing into the cell one or more guide RNAs.

Embodiment 274. The method of embodiment 272 or 273, wherein the sequence-specific nuclease is an RNA guided nuclease.

Embodiment 275. The method of any one of embodiments 272-274, wherein the sequence-specific nuclease or nucleic acid programmable DNA binding domain is a homing endonuclease, a zinc finger nuclease (ZF) nucleic acid binding entity, a transcription activator-like effector (TALE) nucleic acid binding entity, a meganuclease, a Cas nuclease, a core Cas protein, a homing endonuclease, an endonuclease-deficient-Cas protein, an enzymatically inactive Cas protein, a CRISPR- associated transposase (CAST), a Type II or Type V Cas protein, or a functional portion thereof.

Embodiment 276. The method of embodiment 275, wherein the Cas nuclease is Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8a, Cas8b, Cas8c, Cas9, CaslO, Casl2, Casl2a (Cpfl), Casl2b (C2cl), Casl2c (C2c3), Casl 2d (CasY), Casl2e (CasX), Casl2f (C2cl0), Cas 12g, Casl2h, Casl2i, Cas 12k (C2c5), Casl3, Casl3a (C2c2), Casl3b, Casl3c, Casl3d, C2c4, C2c8, C2c9, Cmrl, Cmr2, Cmr3, Cmr4, Cmr5, Cmr6, Csdl, Csd2, Cas5d, Csel, Cse2, Cse3, Cse4, Cas5e, Csfl, Csml, Csm2, Csm3, Csm4, Csm5, Csnl, Csn2, Cstl, Cst2, Cas5t, Cshl, Csh2, Cas5h, Csal, Csa2, Csa3, Csa4, Csa5, Cas5a, CsxlO, Csxl l, Csyl, Csy2, Csy3, Csy4, Mad7, SpCas9, eSpCas9, SpCas9-HFl, HypaSpCas9, HeFSpCas9, and evoSpCas9 high-fidelity variants of SpCas9, SaCas9, NmeCas9, CjCas9, StCas9, TdCas9, LbCasl2a, AsCasl2a, AacCasl2b, BhCasl2b v4, TnpB, dCas (D10A), dCas (H840A), dCasl3a, dCasl3b, or a functional portion thereof.

Embodiment 277. The method of embodiment 274, wherein the RNA-guided nuclease is a Life Edit nuclease (LEG), optionally a LEG14 nuclease.

Embodiment 278. The method of any one of embodiments 272-277, wherein the method comprises introducing into the cell a ribonucleoprotein (RNP) complex comprising the sequence-specific nuclease or nucleic acid programmable DNA binding.

Embodiment 279. The method of embodiment 278, wherein introducing the nucleic acid construct comprises contacting the cell with the particle of any one of embodiments 185-191 or the vector of any one of embodiments 165-184.

Embodiment 280. The method of embodiment 279, wherein the recombinant nucleic acid is introduced via electroporation.

Embodiment 281. The method of embodiment 279, wherein the recombinant nucleic acid is introduced via AAV.

Embodiment 282. The method of embodiment 279, wherein the recombinant nucleic acid is introduced via lentivirus, optionally a VSV-G pseudotyped lentivirus.

Embodiment 283. The method of embodiment 279, wherein the recombinant nucleic acid is introduced via LNP.

Embodiment 284. The method of any one of embodiments 242-283, wherein the recombinant nucleic acid is inserted into an endogenous locus of the cell.

Embodiment 285. The method of embodiment 284, wherein the endogenous locus is or comprises a TRAC gene locus, a TRBC1 gene locus, a TRBC2 gene locus, a TRAJ locus, a CARD9 locus, a CARD10 locus, a CARD11 locus, a CARD14 locus, or a PIK3R3 locus.

Embodiment 285.1. The method of embodiment 285, wherein the TRAJ locus is or comprises a TRAJ intron splice acceptor locus.

Embodiment 286. The method of embodiment 285, wherein the endogenous locus is or comprises a TRAC gene locus.

Embodiment 286.1. The method of embodiment 285, wherein the endogenous locus is or comprises a TRAJ locus.

Embodiment 286.2. The method of embodiment 286.1, wherein the TRAJ locus is or comprises a TRAJ intron splice acceptor locus.

Embodiment 287. The method of embodiment 284, wherein the endogenous locus is or comprises a safe harbor locus.

Embodiment 288. The method of embodiment 287, wherein the safe harbor locus is an AAVS1, ABO, CCR5, CLYBL, CXCR4, F3, FUT1, HMGB1, KDM5D, LRP1, MICA, MICB, RHD, ROSA26, or SHS231 locus.

Embodiment 289. The method of any one of embodiments 284-288, wherein the first insertion site is an exon.

Embodiment 290. The method of any one of embodiments 284-288, wherein the first insertion site is an intron.

Embodiment 291. The method of any one of embodiments 284-288, wherein the first insertion site is between an intron and an exon.

Embodiment 292. The method of any one of embodiments 284-288, wherein the first insertion site is in a regulatory region.

Embodiment 293. The method of any one of embodiments 284-292, wherein the first insertion site is 25 nucleotides or less from a protospacer adjacent motif (PAM) sequence, wherein the PAM sequence is ngg, nag, ngrrt, ngrm, nnnngatt, nnnnryac, nnagaaw, naaaac, tttv, ttn, attn, tttn, gttn, or yttn and wherein:
(i) r = a or g,
(ii) y = c or t,
(iii) w = a or t,
(iv) v = a or c or g, and
(v) n = a, c, t, or g.

Embodiment 294. The method of any one of embodiments 284-293, wherein the cell comprises a modified TRAC gene locus.

Embodiment 295. The method of any one of embodiments 284-294, wherein the cell has reduced or eliminated cell surface expression of an endogenous TCR.

Embodiment 296. The method of any one of embodiments 242-295, wherein the method further comprises introducing a gene editing machinery into the cell.

Embodiment 297. The method of any one of embodiments 242-296, wherein the step of introducing comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery.

Embodiment 298. The method of embodiment 297, wherein the gene editing machinery is CRISPR/Cas9.

Embodiment 299. The method of embodiment 298, wherein the gene editing machinery is introduced via electroporation.

Embodiment 300. The method of embodiment 299, wherein the nucleic acid construct is introduced via AAV.

Embodiment 301. The method of embodiment 299, wherein the nucleic acid construct is introduced via LNP.

Embodiment 302. The method of any one of embodiments 242-301, wherein the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.,* CAR); and (iii) the modified TRAC gene locus.

Embodiment 302.1. The method of any one of embodiments 242-301, wherein the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.,* CAR); and (iii) the modified TRAJ locus.

Embodiment 303. The method of any one of embodiments 242-302.1, wherein the cell is for use in a cell therapy.

Embodiment 304. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells of any of embodiments 192-234 to the subject.

Embodiment 305. The method of embodiment 304, wherein the peptide epitope or mimotope is bound by an antibody for an antigen associated with the cancer or the autoimmune disease.

Embodiment 306. The method of embodiment 304 or 305, comprising, before the administration, generating engineered cells according to the method of any one of 242-303.

Embodiment 307. The method of any one of embodiments 304-306, wherein the engineered cells are derived from immune cells:

(i) obtained and/or isolated from the subject,

(ii) obtained and/or isolated from one or more donors, and/or

(iii) derived from one or more stem cells.

Embodiment 308. A method of treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, to the subject.

Embodiment 309. The method of embodiment 308, wherein the peptide epitope or mimotope is bound by an antibody for an antigen associated with the autoimmune disease.

Embodiment 310. The method of embodiment 308 or 309, wherein the autoimmune disease is lupus, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Crohn's disease, ulcerative colitis, Addison's disease, Graves' disease, Sjogren's syndrome, Hashimoto's thyroiditis, or celiac disease.

Embodiment 311. A method of treating cancer in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, to the subject.

Embodiment 312. The method of embodiment 311, wherein the peptide epitope or mimotope is capable of being bound by an antibody for an antigen associated with the cancer.

Embodiment 313. The method of any one of embodiments 304-307 and 311-312, wherein the cancer is a hematological cancer.

Embodiment 314. The method of any one of embodiments 304-307 and 311-313, wherein the cancer is myeloid neoplasm, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), blast crisis chronic myelogenous leukemia (bcCML), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, or B-cell lymphoma.

Embodiment 315. The method of any one of embodiments 304-307 and 311-312, wherein the cancer is a solid tumor.

Embodiment 316. The method of any one of embodiments 304-307, 311-312, and 315, wherein the cancer is small cell lung cancer, colorectal cancer, testicular cancer, ovarian cancer, melanoma, lymphoma, leukemia, multiple myeloma, prostate cancer, breast cancer, non-small cell lung cancer, gastric cancer, esophageal cancer, liver cancer, kidney cancer, head & neck cancer, glioblastoma, neuroblastoma, soft tissue sarcoma, uterine cancer, brain cancer, skin cancer, renal cancer, bladder cancer, pancreatic cancer, thyroid cancer, eye cancer, gastrointestinal cancer, carcinoma, or sarcoma.

Embodiment 317. The method of any one of embodiments 304-307 and 311-316, wherein the cancer expresses CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, ggcc, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, GPA33, CXORF61, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related

antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, or any combination thereof.

Embodiment 318. The method of embodiment 317, wherein the cancer expresses B7H3, BCMA, CD19, CD20, CD22, CD70, CD79a, CD79b, CDH17, CLDN6, CLDN18.2, DLL3, GCC, GD2, GD3, GPC3, GPRC5D, KLK3, LY6G6D, p53R175H, PRAME, ROPN1, STEAP1, or STEAP2.

Embodiment 318.1. The method of embodiment 317, wherein the cancer expresses KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

Embodiment 319. The method of any one of embodiments 304-307 and 311-318.1, wherein the cancer is selected from Table 2.

Embodiment 320. The method of any one of embodiments 304-307 and 311-319, wherein the cancer and the corresponding cancer-associated antigen are selected from Table 2.

Embodiment 321. The method of any one of embodiments 304-320, wherein the peptide epitope or mimotope is capable of being bound by an antibody for a cancer-associated antigen of T cell lymphoma.

Embodiment 322. The method of any one of embodiments 304-321, wherein the cancer-associated antigen of T cell lymphoma is CCR4, CCR7, or CD3.

Embodiment 323. The method of any of embodiments 304-322, wherein the method further comprises administering an antibody to the subject, wherein the antibody binds the tag, the peptide epitope, or the mimotope.

Embodiment 324. The method of any of embodiments 304-323, wherein the method further comprises monitoring the subject for an adverse event.

Embodiment 325. The method of any of embodiments 304-324, wherein the method further comprises monitoring the subject for treatment outcomes.

Embodiment 326. A method of depleting engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

Embodiment 327. A method of reducing or eliminating at least one symptom of an adverse event caused by engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

Embodiment 328. The method of any of embodiments 323-327, wherein the subject has been administered the engineered cells within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

Embodiment 328.1. The method of any of embodiments 323-327, wherein the subject has been administered the engineered cells at least 1, 2, 3, 4, 5, or 6 days, or at least 1, 2, 3, or 4 weeks, or at least 1, 2, 3, 6, or 12 months, or at least 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

Embodiment 329. The method of any of embodiments 323-327, wherein the subject has been administered the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, within 1, 2, 3, 4, 5, or 6 days, or within 1, 2, 3, or 4 weeks, or within 1, 2, 3, 6, or 12 months, or within 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

Embodiment 329.1. The method of any of embodiments 323-327, wherein the subject has been administered the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, at least 1, 2, 3, 4, 5, or 6 days, or at least 1, 2, 3, or 4 weeks, or at least 1, 2, 3, 6, or 12 months, or at least 1, 2, 3, 4, or 5 years, prior to administration of the antibody.

Embodiment 330. The method of any of embodiments 323-329.1, wherein administration of the antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells expressing the tag, the peptide epitope, or the mimotope in the subject.

Embodiment 331. The method of any of embodiments 323-330, wherein the antibody is administered after the detection or occurrence of an adverse event, and wherein administration of the antibody reduces or eliminates at least one symptom of the adverse event.

Embodiment 332. The method of any of embodiments 323-331, wherein the method additionally comprises administration of corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 333. The method of any one of embodiments 324-332, wherein the adverse event is cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), hemophagocytic lymphohistiocytosis (HLH), on-target off-tumor toxicity, tumor lysis syndrome, lymphoproliferative disorder, splenomegaly, lymphadenopathy, an abnormal ratio of engineered cells to other blood cells, acute toxicity, potency enhancement mediated cytotoxicity, potency enhancement mediated GVHD, uncontrolled expansion of engineered cells, or engineered cell mediated lymphoma.

Embodiment 334. The method of any of embodiments 323-333, wherein the antibody is administered after the detection or occurrence of a treatment outcome, optionally wherein the treatment outcome is reduction or elimination of the cancer or the autoimmune disease.

Embodiment 335. The method of any of embodiments 323-334, wherein the method additionally comprises monitoring the subject for reduction or elimination of at least one symptom of the adverse event, optionally wherein the monitoring comprises one or more of:

Clinical laboratory tests of ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count;

Imaging, wherein the imaging is PET, CT, MRI, and/or x-ray;

Clinical assessment of body temperature, blood pressure, blood oxygenation, heart rate, cognitive status, and/or organ function; and

Standardized assessment used for detecting or monitoring one or more adverse events, optionally wherein the standardized assessment comprises the Immune Effector Cell–Associated Encephalopathy (ICE) assessment, the Cornell Assessment, and/or the Pediatric Delirium (CAPD) assessment.

Embodiment 336. The method of any of embodiments 323-335, wherein the method results in at least one of:

Depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cells in the subject;

Depletion of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of regulatory T cells in the subject;

Clinical laboratory results within the normal range for ferritin, C-reactive protein (CRP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), fibrinogen, lactose dehydrogenase (LDH), absolute or relative white blood cell count, and/or absolute or relative T cell count;

Imaging results indicating a reduction in cancer or reduction in at least one symptoms of an adverse event;

Reduction or absence of fever, hypotension, hypoxia, tachycardia, aphasia, cognitive impairment, coagulopathy, respiratory failure, lymphadenopathy, and/or splenomegaly;

Improved score on a standardized assessment used for detecting or monitoring one or more adverse events; or

Ability to reduce or eliminate administration of corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 337. The method of any of embodiments 323-336, wherein the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 338. The method of any of embodiments 323-337, wherein the method results in higher therapeutic efficacy than a control method to reduce or eliminate the at least one symptom of the adverse event, optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 339. The method of any of embodiments 324-338, wherein the adverse event is an acute adverse event.

Embodiment 340. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is a bi-specific or multi-specific antibody that is capable of binding to the peptide epitope or the mimotope.

Embodiment 341. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD3.

Embodiment 342. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is a bi-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; and (ii) CD16.

Embodiment 343. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is a tri-specific or multi-specific antibody that is capable of binding to (i) the peptide epitope or the mimotope; (ii) 41BB or albumin, and (ii) CD3.

Embodiment 344. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is tarlatamab.

Embodiment 345. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is mogamulizumab.

Embodiment 345.1. The method of any of embodiments 305-307, 309-310 and 312-339, wherein the antibody is CAP-100.

Embodiment 346. The method of any of embodiments 305-307, 309-310 and 312-345.1, wherein a dose of between about 0.003 mg/kg and about 10 mg/kg, between 0.01 mg/kg and 10 mg/kg, between 0.003 mg/kg and 0.3 mg/kg, between 0.05 mg/kg and 5 mg/kg, or between 0.1 mg/kg and 1 mg/kg of the antibody is administered to the subject.

Embodiment 346.1. The method of embodiment 346, wherein a dose of between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg, of the antibody is administered to the subject.

Embodiment 347. The method of any of embodiments 305-307, 309-310 and 312-346.1, wherein the antibody is administered to the subject once, or wherein the antibody is administered more than once; optionally, wherein the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks; optionally, wherein the antibody is administered more than once with an interval of at least 1, 2, 3, or 4 weeks.

Embodiment 347.1. The method of any of embodiments 305-307, 309-310 and 312-347, wherein the method comprises (a) administering the engineered cell, the recombinant nucleic acid, the vector, or the particle into a brain of the subject; and (b) administering the antibody outside of the brain of the subject.

Embodiment 347.2. The method of embodiment 347.1, wherein the method comprises intraventricular administration of the engineered cells, the recombinant nucleic acid, the vector, or the particle.

Embodiment 347.3. The method of embodiment 347.1 or 347.2, wherein the method comprises systemic administration of the antibody; optionally, the method comprises intravenous administration of the antibody.

Embodiment 347.4. The method of any one of embodiments 347.1-347.3, wherein the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell that have crossed the blood brain barrier than a control method that reduces or eliminates the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 347.5. The method of any one of embodiments 347.1-347.4, wherein the antibody is administered more than once; optionally, wherein the antibody is administered more than once with an interval of about 1, 2, 3, or 4 weeks; optionally, wherein the antibody is administered more than once with an interval of at least 1, 2, 3, or 4 weeks.

Embodiment 347.6. The method of embodiment 347.5, wherein the antibody is administered two, three, or four times.

Embodiment 348. The method of any of embodiments 309-310 and 312-347.6, wherein both the antibody and the binding region of the recombinant polypeptide can bind the same antigen.

Embodiment 349. The method of any of embodiments 309-310 and 312-348, wherein the antibody is mogamulizumab, and wherein the method further comprises administering one or more of:

(a) at least one retinoid;

(b) interferon alpha; and/or

(c) extracorporeal photopheresis (ECP);

optionally, wherein the retinoid and/or interferon alpha is delivered systemically.

Embodiment 350. A method of isolating or enriching the engineered cells of any of embodiments 192-234, comprising contacting the engineered cells with a binding agent that binds to the epitope or the mimotope.

Embodiment 351. The method of embodiment 350, wherein the binding agent comprises an antibody.

Embodiment 352. The method of embodiment 350 or 351, wherein the binding agent comprises a purification marker; optionally, wherein the purification marker comprises biotin, avidin, a myc tag, an influenza hemagglutinin (HA) tag, a FLAG tag, a glutathione-S transferase (GST) tag, a V5 tag, a poly-Histidine (His) tag, a Fc tag, calmodulin binding protein (CBP), maltose-binding protein (MBP), thioredoxin, or a chitin-binding protein.

Embodiment 353. The method of any one of embodiments 350-352, wherein the binding agent is bound to a solid substrate or a purification bead.

Embodiment 354. The method of any one of embodiments 350-353, wherein the method results in a population of purified engineered cells in which at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% of the engineered cell expresses the recombinant polypeptide.

Embodiment 355. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering a therapeutically effective amount of engineered cells isolated or enriched by the method of any of embodiments 350-354.

Embodiment 356. A method of *ex vivo* or *in vivo* measuring the number of the engineered cells of any of embodiments 192-234, comprising administering, or contacting the engineered cells with, a binding agent that binds to the epitope or the mimotope; optionally, wherein the binding agent comprises an antibody.

Embodiment 357. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising:

(a) administering an effective amount of the engineered cell of any of embodiments 192-234, the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, to the subject, wherein the engineered cell or the recombinant polypeptide comprises two or more of the tags, the peptide epitopes or the mimotopes that are capable of being bound by mogamulizumab, and wherein the tags, the peptide epitopes or the mimotopes are the same or different; and

(b) administering at least one dose of mogamulizumab to the subject.

Embodiment 358. The method of embodiment 357, wherein each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

Embodiment 359. The method of embodiment 357 or 358, wherein the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cell than a control method to reduce or eliminate the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 360. The method of any of embodiments 357-359, wherein the engineered cell, the recombinant nucleic acid, the vector, or the particle is administered at least 1, 2, 3, 4, 5, 6, 7, 8 weeks, or at least 2, 3, 4, 5, 6, 8 or 10 months, or at least 1, 2, 3, 4, or 5 years, before the first dose of mogamulizumab is administered to the subject.

Embodiment 361. A method of treating cancer in a subject in need thereof, comprising:

(a) intraventricularly administering an effective amount of the engineered cell of any of embodiments 192-234, the recombinant nucleic acid of any of embodiments 122-164, the vector of any of embodiments 165-184, or the particle of any of embodiments 185-191, to the subject; and

(b) systemically administering at least one dose of mogamulizumab to the subject.

Embodiment 362. The method of embodiment 361, wherein each of the dose(s) of mogamulizumab is between about 0.003 mg/kg and about 0.999 mg/kg, about 0.003 mg/kg and about 0.01 mg/kg, about 0.01 mg/kg and about 0.03 mg/kg, about 0.03 mg/kg and about 0.1 mg/kg, about 0.1 mg/kg and about 0.3 mg/kg, about 0.3 mg/kg and about 0.6 mg/kg, or about 0.6 mg/kg and about 0.999 mg/kg.

Embodiment 363. The method of embodiment 361 or 362, wherein the method results in at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% higher depletion of the engineered cells outside the brain than a control method that reduces or eliminates the at least one symptom of the adverse event; optionally wherein the control method comprises administering corticosteroids, tocilizumab, and/or anakyrin.

Embodiment 364. The method of any one of embodiments 361-363, wherein the method comprises administering at least two, at least three, or at least four doses of mogamulizumab to the subject.

FURTHER NUMBERED EMBODIMENTS

Further numbered embodiments of the invention are provided below:

Embodiment A1. A recombinant polypeptide comprising (i) an extracellular region comprising a binding region for an antigen; and (ii) a transmembrane region,
wherein the extracellular region comprises a peptide epitope or mimotope capable of being bound by an anti-CCR4 antibody,
wherein the recombinant polypeptide is not CCR4.

Embodiment A2. The recombinant polypeptide of embodiment A1, wherein the recombinant polypeptide is a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

Embodiment A3. The recombinant polypeptide of embodiment A2, wherein the anti-CCR4 antibody is mogamulizumab, wherein the peptide epitope consists of SEQ ID NO: 431, and wherein the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the peptide epitope or mimotope.

Embodiment A4. The recombinant polypeptide of embodiment A3, wherein the binding region comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein the peptide epitope or mimotope is located between the VH and the VL.

Embodiment A5. An engineered immune cell expressing the recombinant polypeptide of any one of embodiments 1-4.

Embodiment A6. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells of embodiment A5 to the subject.

Embodiment A7. The method of embodiment A6, wherein the method further comprises administering the anti-CCR4 antibody to the subject, wherein the anti-CCR4 antibody is mogamulizumab.

Embodiment A8. The method of embodiment A7, wherein the anti-CCR4 antibody is administered after detection or occurrence of an adverse event caused by the engineered cells in the subject, and wherein administration of the anti-CCR4 antibody reduces or eliminates at least one symptom of the adverse event.

Embodiment A9. The method of embodiment A8, wherein administration of the anti-CCR4 antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells in the subject.

CLAIMS

1. A recombinant polypeptide comprising **(i)** an extracellular region comprising a binding region for an antigen; and **(ii)** a transmembrane region,

wherein the extracellular region comprises a peptide epitope capable of being bound by an anti-CCR4 antibody,

wherein the recombinant polypeptide is not CCR4.

2. The recombinant polypeptide of claim 1, wherein the recombinant polypeptide is a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

3. The recombinant polypeptide of claim 2, wherein the anti-CCR4 antibody is mogamulizumab, wherein the peptide epitope consists of SEQ ID NO: 431, and wherein the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the peptide epitope.

4. The recombinant polypeptide of claim 3, wherein the binding region comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein the peptide epitope or mimotope is located between the VH and the VL.

5. An engineered immune cell expressing the recombinant polypeptide of any one of claims 1-4.

6. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells of claim 5 to the subject.

7. The method of claim 6, wherein the method further comprises administering the anti-CCR4 antibody to the subject, wherein the anti-CCR4 antibody is mogamulizumab.

8. The method of claim 7, wherein the anti-CCR4 antibody is administered after detection or occurrence of an adverse event caused by the engineered cells in the subject,

and wherein administration of the anti-CCR4 antibody reduces or eliminates at least one symptom of the adverse event.

9. The method of claim 8, wherein administration of the anti-CCR4 antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells in the subject.

10. A modified peptide epitope or mimotope, wherein the peptide epitope comprises or consists of an amino acid sequence having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 430, and wherein the peptide epitope is capable of being bound by mogamulizumab.

11. The modified peptide epitope or mimotope of claim 10, wherein the modified peptide epitope or mimotope does not comprise the cysteine that is present at the C-terminus of SEQ ID NO: 430.

12. The modified peptide epitope or mimotope of claim 10 or 11, wherein the modified peptide epitope comprises or consists of any one of SEQ ID NO: 431-438; preferably, the modified peptide epitope consists of SEQ ID NO: 431.

13. The modified peptide epitope or mimotope of claim 10, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence:
(a) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 475, and wherein the peptide epitope or mimotope is capable of being bound by rituximab;
(b) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 476, and wherein the peptide epitope or mimotope is capable of being bound by Palivizumab;
(c) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 477, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;

(d) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 478, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;

(e) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 479, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;

(f) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 480, and wherein the peptide epitope or mimotope is capable of being bound by Cetuximab;

(g) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 481, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab;

(h) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 482, and wherein the peptide epitope or mimotope is capable of being bound by Nivolumab;

(i) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 483, and wherein the peptide epitope or mimotope is capable of being bound by QBend-10;

(j) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 484, and wherein the peptide epitope or mimotope is capable of being bound by Alemtuzumab;

(k) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 469, and wherein the peptide epitope or mimotope is capable of being bound by Sacituzumab;

(l) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO:470-474, and wherein the peptide epitope or mimotope is capable of being bound by an anti-GUCY2C antibody;

(m) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to SEQ ID NO: 439, and wherein the peptide epitope or mimotope is capable of being bound by Tarlatamab;

(n) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NO: 441-468, and wherein the peptide epitope or mimotope is capable of being bound by an anti-DLL3 antibody; or

(o) having 1, 2, 3, 4 or 5 mutations (substitutions, insertions, and/or deletions) compared to any one of SEQ ID NOs: 519-524, and wherein the peptide epitope or mimotope is capable of being bound by CAP-100.

14. The modified peptide epitope or mimotope of any one of claims 10-13, wherein the mutation(s) are substitutions.

15. The modified peptide epitope or mimotope of any one of claims 10-14, wherein the mutation(s) are conservative substitutions.

16. The modified peptide epitope or mimotope of any one of claims 10-15, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted or deleted.

17. The modified peptide epitope or mimotope of claim 16, wherein the N-terminus of the unmodified peptide epitope or mimotope contains cysteine.

18. The modified peptide epitope or mimotope of claim 16 or 17, wherein the C-terminus of the unmodified peptide epitope or mimotope contains cysteine.

19. The modified peptide epitope or mimotope of any one of claims 16-18, wherein the unmodified peptide epitope or mimotope comprises at least one cysteine between the N-terminus and the C-terminus.

20. The modified peptide epitope or mimotope of any one of claims 16-19, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine, glycine, threonine, alanine, or valine; preferably, serine or valine; more preferably, serine.

21. The modified peptide epitope or mimotope of claim 20, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with serine.

22. The modified peptide epitope or mimotope of claim 20, wherein the cysteine(s) in the unmodified peptide epitope or mimotope are substituted with valine.

23. The modified peptide epitope or mimotope of any one of claims 10-22, wherein the modified peptide epitope or mimotope does not comprise any sulfur atoms.

24. The modified peptide epitope or mimotope of any one of claims 10-23, wherein the modified peptide epitope or mimotope does not comprise any cysteine(s).

25. The modified peptide epitope or mimotope of any one of claims 10-24, wherein the mutation(s) comprise deleting or substituting one or more amino acids that facilitate polypeptide crosslinking in the unmodified peptide epitope or mimotope.

26. The modified peptide epitope or mimotope of any one of claims 10-25, wherein the mutation(s) comprise deleting or substituting one or more cysteine(s), lysine(s), aspartic acid(s), glutamic acid(s), or any combination thereof.

27. The modified peptide epitope or mimotope of any one of claims 10-26, wherein the modified peptide epitope or mimotope is capable of being bound by the corresponding antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM; optionally wherein the Kd is measured by surface plasmon resonance (SPR) using a sensor chip that contains the immobilized antibody.

28. The modified peptide epitope or mimotope of any one of claims 10-27, wherein the modified peptide epitope or mimotope is capable of being bound by [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk),

Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF, IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589.

29. The modified peptide epitope or mimotope of any one of claims 10-28, wherein the peptide epitope or mimotope comprises or consists of an amino acid sequence having 1, 2, 3, 4, or 5 mutations compared to the unmodified peptide epitope or mimotope.

30. The modified peptide epitope of any one of claims 10-29, wherein the unmodified peptide epitope or mimotope comprises or consists of no more than 10, no more than 15, no

more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 consecutive amino acids in an autoimmune-associated antigen or a cancer-associated antigen.

31. A peptide epitope comprising or consisting of SEQ ID NO: 430, wherein the peptide epitope is capable of being bound by an anti-CCR4 antibody.

32. The peptide epitope of claim 31, wherein the anti-CCR4 antibody is mogamulizumab.

33. A peptide epitope comprising or consisting of any one of SEQ ID NO: 439-468, wherein the peptide epitope is capable of being bound by an anti-DLL3 antibody.

34. The peptide epitope of claim 33, wherein the anti-DLL3 antibody is tarlatamab.

35. A peptide epitope comprising or consisting of any one of SEQ ID NOs: 519-524, wherein the peptide epitope is capable of being bound by an anti-CCR7 antibody.

36. The peptide epitope of claim 35, wherein the anti-CCR7 antibody is CAP-100.

37. A recombinant polypeptide comprising or consisting of the peptide epitope or mimotope of any one of claims 10-36.

38. The recombinant polypeptide of claim 37, wherein the recombinant polypeptide consists of the peptide epitope or mimotope.

39. The recombinant polypeptide of claim 37 or 38, wherein the peptide epitope or mimotope is capable of being expressed on the surface of a cell.

40. The recombinant polypeptide of any one of claims 37-39, wherein the peptide epitope or mimotope is located in an extracellular region of the recombinant polypeptide.

41. The recombinant polypeptide of any one of claims 37-40, wherein the recombinant polypeptide is not any of CCR4, CCR7, DLL3, TROP2, GUCY2C, CD20, RSV protein F, EGFR, PD-1, CD34, and CD52.

42. The recombinant polypeptide of any one of claims 37-40, wherein the recombinant polypeptide is not CCR4.

43. The recombinant polypeptide of any one of claims 37-40, wherein the recombinant polypeptide is not CCR7.

44. The recombinant polypeptide of any one of claims 37-43, wherein the recombinant polypeptide does not comprise a cysteine at the C-terminus of the modified peptide epitope or mimotope.

45. The recombinant polypeptide of any one of claims 37-44, wherein the recombinant polypeptide does not comprise a cysteine immediately after the C-terminus of the modified peptide epitope or mimotope.

46. The recombinant polypeptide of any one of claims 37-45, wherein the recombinant polypeptide does not comprise a cysteine at the N-terminus of the modified peptide epitope or mimotope.

47. The recombinant polypeptide of any one of claims 37-46, wherein the recombinant polypeptide does not comprise a cysteine immediately before the N-terminus of the modified peptide epitope or mimotope.

48. The recombinant polypeptide of any one of claims 37-47, wherein the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s), wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the modified peptide epitope or different from the modified peptide epitope.

49. A recombinant polypeptide comprising a tag, wherein the tag comprises or consists of a peptide epitope or a mimotope that mimics an epitope.

50. The recombinant polypeptide of claim 49, wherein the epitope is derived from CCR4, DLL3, or CCR7.

51. The recombinant polypeptide of any of claims 37-50, wherein the recombinant polypeptide comprises a binding region for an antigen.

52. The recombinant polypeptide of claim 51, wherein the antigen is expressed on the surface of a target cell.

53. The recombinant polypeptide of claim 51 or 52 wherein the recombinant polypeptide comprises or consists of a chimeric antigen receptor (CAR) or a recombinant T cell receptor (TCR).

54. The recombinant polypeptide of claim 51 or 52, wherein the recombinant polypeptide comprises or consists of a receptor comprising:

(a) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRalpha, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRbeta; or

(b) a heavy chain variable region (VH) located before the N-terminus of a constant region of TCRbeta, and a light chain variable region (VL) located before the N-terminus of a constant region of TCRalpha,

optionally, wherein at least one of the constant region of TCRalpha or the constant region of TCRbeta are an endogenous constant region of TCRalpha or an endogenous constant region of TCRbeta,

optionally wherein the receptor is a HLA-independent T cell (HIT) receptor.

55. The recombinant polypeptide of any one of claims 51-54, wherein an immune cell expressing the recombinant polypeptide exhibits one or more of the following:

(a) improved binding to the antigen expressed on the surface of the target cell;

(b) reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the immune cell; and/or

(c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell;
compared to an immune cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope.

56. The recombinant polypeptide of any one of claims 51-55, wherein an immune cell expressing the recombinant polypeptide exhibits one or more of the following:

(a) comparable levels of binding to the antigen expressed on the surface of the target cell;

(b) comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the immune cell; and/or

(c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the immune cell;
compared to an immune cell expressing a recombinant polypeptide that does not comprise a peptide epitope or mimotope.

57. The recombinant polypeptide of any one of claims 51-56, comprising the peptide epitope or mimotope located N-terminal to the binding region.

58. The recombinant polypeptide of claim 57, wherein the peptide epitope or mimotope is separated from the N-terminus of the binding region by no more than 15, no more than 10, no more than 5, no more than 4, no more than 3, no more than 2, no more than 1, or zero amino acid(s).

59. The recombinant polypeptide of any one of claims 51-58, comprising the peptide epitope or mimotope located C-terminal to the binding region.

60. The recombinant polypeptide of claim 59, wherein the peptide epitope or mimotope is separated from the C-terminus of the binding region by zero, no more than 5, no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, or no more than 60 amino acids.

61. The recombinant polypeptide of any one of claims 51-60, wherein the peptide epitope or mimotope is separated from the binding region by no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

62. The recombinant polypeptide of any one of claims 51-61, wherein the peptide epitope or mimotope is located within the binding region.

63. The recombinant polypeptide of any one of claims 53-62, wherein the recombinant polypeptide comprises or consists of the CAR.

64. The recombinant polypeptide of any one of claims 51-63, wherein the binding region comprises a heavy chain variable domain (VH) and a light chain variable domain (VL).

65. The recombinant polypeptide of any one of claims 51-63, wherein the binding region comprises a single chain variable fragment (scFv) comprising a VH and a VL.

66. The recombinant polypeptide of any one of claims 51-63 , wherein the binding region comprises one or more variable heavy domains of heavy chain (VHHs).

67. The recombinant polypeptide of any one of claims 64-66, wherein the VH and the VL is separated by a linker, or wherein the binding region comprises at least two VHHs separated by a linker.

68. The recombinant polypeptide of any one of claims 51-63, wherein the binding region comprises a first binding domain and a second binding domain, wherein the first binding domain and the second binding domain are separated by a linker.

69. The recombinant polypeptide of any one of claims 51-63, wherein the recombinant polypeptide comprises a second binding region located N-terminal or C-terminal to the binding region; optionally, wherein the binding region and the second binding region are separated by a linker.

70. The recombinant polypeptide of any one of claims 67-69, wherein the peptide epitope or mimotope is located within the linker.

71. The recombinant polypeptide of any one of claims 67-70, wherein the linker comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids.

72. The recombinant polypeptide of any one of claims 51-69, wherein the peptide epitope or mimotope is located in between the binding region(s) and a transmembrane region.

73. The recombinant polypeptide of any one of claims 51-69, wherein the peptide epitope or mimotope is located within a hinge region that connects the binding region(s) to a transmembrane region.

74. The recombinant polypeptide of claim 73, wherein the hinge region comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, no more than 50, no more than 55, no more than 60, no more than 65, or no more than 70 amino acids.

75. The recombinant polypeptide of any one of claims 53-74, wherein the CAR or TCR comprises no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids N-terminal to the binding region(s), excluding the signal peptide.

76. The recombinant polypeptide of any one of claims 53-75, wherein the extracellular region of the CAR or TCR comprises no more than 200, no more than 250, no more than 300, no more than 350, no more than 400, no more than 450, or no more than 500 amino acids.

77. The recombinant polypeptide of any one of claims 53-76, wherein the CAR or TCR comprises, from N-terminus to C-terminus, the binding region(s), a hinge region, a transmembrane region.

78. The recombinant polypeptide of any one of claims 51-77, wherein the antigen is an autoimmune-associated antigen or a cancer-associated antigen.

79. The recombinant polypeptide of any one of claims 37-78, wherein the peptide epitope is derived from an autoimmune-associated antigen or a cancer-associated antigen or wherein the mimotope mimics an epitope of the autoimmune-associated antigen or the cancer-associated antigen.

80. The recombinant polypeptide of claim 78 or 79, wherein the autoimmune-associated antigen or the cancer-associated antigen is selected from the group consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD3ε, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD27, CD28, CD30, CD33, CD34, CD38, CD40, CD44v6, CD45, CD46, CD47 CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD97, CD123, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD171, CD178, CD179, CD179a, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD213A2, CD246, CD252, CD253, CD261, CD262, CD272, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), CDH17, CEA, CLECL1, CLL-1, CLDN6, CLDN18.2, CS1, DLL3, LY6G6D, GCC, p53R175H, PRAME, EGFR, EGFRvIII, FGFR2, AFP, CA125, MUC-1, MAGE, ALPI, alkaline phosphatase placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (eGFP), KLK2, KLK3, Mesothelin, IL13Ra2, signal regulatory protein α (SIRPα), TCRalpha, TCRbeta, TSHR, GD2, GD3, Tn Ag, cMET, Axl, ROR1, ROR2, GPC1, GPC2, GPC3, FLT3, TAG72, CEA, EPCAM, KIT (CD117), IL-13Ra2, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, PDGFRβ, SSEA-4, folate receptor alpha, ERBB2 (Her2/neu), MUC1, MUC16, NCAM, prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, STEAP1, STEAP2, fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, folate receptor beta, TEM1/CD248, TEM7R, ROPN1, GPRC5D, GPA33, CXORF61, ALK, Polysialic acid,

PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-Al, legumain, HPV E6,E7, MAGE-A4, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, p53R175H, KRAS, mutant KRAS, KRAS G12D, prostein, surviving, telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, androgen receptor, cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2,LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, FCRL5, IGLL1, PSMA, TROP2, citrullinated vimentin, the extracellular portion of the APRIL protein, and any combinations thereof.

81. The recombinant polypeptide of any one of claims 78-80, wherein the cancer-associated antigen is a cancer-associated antigen for a solid tumor.

82. The recombinant polypeptide of claim 81, wherein the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin α5β1, CD51, CD27, CDH17, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, integrin α11β1, DLL3, CD2, SSTR2, TROP2, KLK2, KLK3, STEAP2, STEAP1, PSMA, PSCA, LY6G6D, IL-1α, CEA, C242 antigen, CanAg (MUC1 glycoform), CTAA16.88, UCY2C, IGF2BP3, p53R175H, NKG2D ligands, PRAME, MAGE-A4, vimentin, HER2/neu, CCR4, CCR5, ROPN1, GPA33, ROR1, KK-LC-1, CA-125, folate receptor 1, folate receptor alpha, MUC1, MUC16, MSLN, CLDN6, WT1, ALPPL2, KRASG12D, KRASG12V, CD70, MSLN, CLDN18.2, HPV E6/E7, GPC3, IL-13Rα, EGFRv3, EGFR806, GD2, or CA9.

83. The recombinant polypeptide of claim 82, wherein the cancer-associated antigen for the solid tumor comprises or consists of KIR2D, NRP1, CD221, VEGFR, HGF, integrin a5b1, CD51, CD27, B7H3, NYESO1, EGFR, cMET, BRD4, EpCAM, SSX, Fibronectin EDB, or Integrin α11β1.

84. The recombinant polypeptide of claim 82, wherein the cancer-associated antigen for the solid tumor comprises or consists of KLK2, Ly6G6D, CLDN6, DLL3 or CD19.

85. The recombinant polypeptide of any one of claims 78-84, wherein the cancer is a solid tumor selected from the group consisting of small cell lung cancer, prostate cancer, colorectal cancer, head and neck squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, lung cancer, pancreatic cancer, stomach cancer, cervical cancer, hepatocellular carcinoma, glioma cancer, neuroblastoma cancer, and renal cell carcinoma.

86. The recombinant polypeptide of any one of claims 78-85, wherein the cancer is a hematologic cancer selected from B cell lymphoma, T cell lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia.

87. The recombinant polypeptide of any one of claims 78-86, wherein the cancer-associated antigen is or comprises an antigen selected from Table 2.

88. The recombinant polypeptide of any one of claims 51-87, wherein the antigen is DLL3.

89. The recombinant polypeptide of any one of claims 37-88, wherein the epitope is derived from a cancer-associated antigen selected from Table 2.

90. The recombinant polypeptide of any one of claims 37-89, wherein the epitope is derived from DLL3.

91. The recombinant polypeptide of claim 90, wherein the peptide epitope comprises or consists of at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 consecutive amino acids in SEQ ID NO: 486, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

92. The recombinant polypeptide of claim 90, wherein the peptide epitope comprises or consists of any one of SEQ ID NO: 439-468, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

93. The recombinant polypeptide of claim 90, wherein the peptide epitope comprises or consists of SEQ ID NO: 439, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

94. The recombinant polypeptide of any one of claims 51-93, wherein the peptide epitope or mimotope is capable of being bound by a monoclonal antibody that binds to the antigen for the binding region.

95. The recombinant polypeptide of any one of claims 37-87, wherein the epitope is derived from a cancer-associated antigen of T cell lymphoma.

96. The recombinant polypeptide of any one of claims 37-87, wherein the epitope is derived from a cancer-associated antigen of adult T-cell lymphoma (ATL) and/or cutaneous T-cell lymphoma (CTCL).

97. The recombinant polypeptide of any one of claims 37-87, 95, and 96, wherein the epitope is derived from a cancer-associated antigen of a Th2 cell, a cutaneous lymphocyte antigen-positive skin-homing T cell, and/or a Treg cell.

98. The recombinant polypeptide of any one of claims 37-87 and 95-97, wherein the epitope is derived from CCR4.

99. The recombinant polypeptide of claim 98, wherein the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NO: 430-438, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

100. The recombinant polypeptide of claim 98, wherein the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NO: 431.

101. The recombinant polypeptide of any one of claims 37-87 and 95-97, wherein the epitope is derived from CCR7.

102. The recombinant polypeptide of claim 101, wherein the peptide epitope comprises or consists of an amino acid sequence of any one of SEQ ID NOs: 519-524, or an amino acid sequence having at most 1, at most 2, at most 3, at most 4, or at most 5 amino acid mutations thereto.

103. The recombinant polypeptide of claim 101, wherein the peptide epitope comprises or consists of an amino acid sequence of SEQ ID NOs: 519-524.

104. The recombinant polypeptide of any one of claims 37-103, wherein the peptide epitope or the mimotope is about 5 to about 10, about 10 to about 20, about 15 to about 25, about 20 to about 30, about 25 to about 35, about 30 to about 40, about 35 to about 45, or about 40 to about 50 amino acids in length.

105. The recombinant polypeptide of any one of claims 37-104, wherein the peptide epitope or the mimotope is no more than 10, no more than 15, no more than 20, no more than 25, no more than 30, no more than 35, no more than 40, no more than 45, or no more than 50 amino acids in length.

106. The recombinant polypeptide of any one of claims 37-105, wherein the peptide epitope does not comprise any mutation compared to the native epitope derived from the antigen.

107. The recombinant polypeptide of any one of claims 37-106, wherein the peptide epitope or the mimotope is capable of forming an alpha-helical conformation.

108. The recombinant polypeptide of any one of claims 37-107, wherein the peptide epitope or the mimotope is capable of being bound by an antibody with a dissociation constant (Kd) of less than 1 μM, less than 100 nM, less than 10 nM, or less than 1 nM, optionally wherein the Kd is measured by surface plasmon resonance (SPR) method using a sensor chip that contains the immobilized antibody.

109. The recombinant polypeptide of claim 108, wherein the antibody is [fam-]trastuzumab deruxtecan, (fam-trastuzumab deruxtecan-nxki), Adebrelimab, Ado-

trastuzumab emtansine, Alemtuzumab, Amivantamab, Atezolizumab, Avelumab, Belantamab mafodotin (belantamab mafodotin-blmf), Bevacizumab, Blinatumomab, Brentuximab vedotin, Cadonilimab, Camrelizumab, CAP-100, Catumaxomab, Cemiplimab (cemiplimab-rwlc), Cetuximab, Cetuximab saratolacan, Cosibelimab, Daratumumab, Dinutuximab, Disitamab vedotin, Dostarlimab, Durvalumab, Edrecolomab, Elotuzumab, Elranatamab, Emapalumab (emapalumab-lzsg), Enfortumab vedotin (enfortumab vedotin-ejfv), Enlonstobart, Envafolimab, Epcoritamab, Gemtuzumab ozogamicin, Glofitamab, Ibritumomab tiuxetan, Inotuzumab ozogamicin, Ipilimumab, Isatuximab (isatuximab-irfc), Loncastuximab tesirine, Margetuximab-cmkb, Mirvetuximab soravtansine, Mogamulizumab (mogamulizumab-kpkc), Mosunetuzumab, Moxetumomab pasudotox (moxetumomab pasudotox-tdfk), Naxitamab-gqgk, Necitumumab, Nimotuzumab, Nivolumab, Cipterbin, Obinutuzumab, Ofatumumab, Olaratumab, Panitumumab, Pembrolizumab, Penpulimab, Pertuzumab, Polatuzumab vedotin (polatuzumab vedotin-piiq), Prolgolimab, Pucotenlimab, Racotumomab, Ramucirumab, Relatlimab, Retifanlimab, Ripertamab, Rituximab, Sacituzumab govitecan (sacituzumab govitecan-hziy), Serplulimab, Sintilimab, Socazolimab, Sugemalimab, Tafasitamab (tafasitamab-cxix), Tagitanlimab, Talquetamab (talquetamab-tgvs), Tebentafusp, Teclistamab, Tislelizumab, Tisotumab vedotin, tisotumab vedotin-tftv, Toripalimab, Tositumomab-I131, Trastuzumab, Trastuzumab duocarmazine, Tremelimumab, Zimberelimab, Zolbetuximab, Zuberitamab, Odronextamab, Ivonescimab, Benmelstobart, Trastuzumab botidotin, Iparomlimab, tuvonralimab, Tarlatamab, Patritumab deruxtecan, Sacituzumab tirumotecan, Zanidatamab, Linvoseltamab, Datopotamab deruxtecan, Anbenitamab, Anvatabart opadotin, Apamistamab-Iodine (131I), Bemarituzumab, Cetrelimab, Cobolimab, Domvanalimab, Emactuzumab, Erfonrilimab, Favezelimab, Felzartamab, Fianlimab, Finotonlimab, ABBV-383, Geptanolimab, Gotistobart, Ivuxolimab, Izalontamab, Lemzoparlimab, Luveltamab tazevibulin, Magrolimab, Mecbotamab vedotin, Monalizumab, Nofazinlimab, Nurulimab, Ociperlimab, Oleclumab, Onfekafusp alfa, Oregovomab, Ozekibart, Pivekimab sunirine, Quavonlimab, Retlirafusp alfa, Rosopatamab (177Lu-DOTA), Rulonilimab, Sasanlimab, Telisotuzumab vedotin, Tiragolumab, IKS014, LCB14-0110, FS-1502, Trastuzumab mafodotin, Trastuzumab rezetecan, Vibostolimab, Vobramitamab duocarmazine, Zilovertamab vedotin, Suvemcitug, APX003, BD0801, TK001, sevacizumab, CTX-009, ES104, TR009, NOV1501, ABL001, DP303c, IAH0968, Kintuximab, gentuximab, cintuximab, MIL62, MRG002, MRG003, PM8002, MEDI5752, MCLA-128, AGEN1181, L19-IL2, L19-TNF,

IMC-F106C, JMT101, BL-B01D1, BAT1308, AZD2936, JS004, TAB004, FG-M108, M108, 9MW2821, BMS-986349, CC-93269, EM801, ABBV-181, ABBV-151, ARGX-115, DS-7300a, IBI343, SHR-A1921, or ASKB589 .

110. The recombinant polypeptide of claim 108, wherein the antibody is tarlatamab.

111. The recombinant polypeptide of claim 108, wherein the antibody is mogamulizumab.

112. The recombinant polypeptide of claim 108, wherein the antibody is CAP-100.

113. The recombinant polypeptide of any one of claims 37-112, wherein the recombinant polypeptide comprises one or more additional peptide epitope(s) or mimotope(s).

114. The recombinant polypeptide of claim 113, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope in the tag.

115. The recombinant polypeptide of claim 113, wherein the one or more additional peptide epitope(s) or mimotope(s) are different from the peptide epitope or mimotope in the tag.

116. The recombinant polypeptide of any one of claims 113-115, wherein the recombinant polypeptide comprises 1, 2, 3, or 4 additional peptide epitope(s) or mimotope(s).

117. The recombinant polypeptide of any one of claims 37-116, wherein the recombinant polypeptide comprises an extracellular portion comprising the binding region and the peptide epitope or mimotope.

118. The recombinant polypeptide of any one of claims 37-117, wherein the recombinant polypeptide comprises a transmembrane region or a glycosylphosphatidylinositol (GPI) anchor.

119. The recombinant polypeptide of any one of claims 37-118, wherein the recombinant polypeptide comprises an intracellular region; optionally, the intracellular domain is located C-terminal to the transmembrane region.

120. The recombinant polypeptide of claim 119, wherein the intracellular region comprises one or more intracellular signaling domains.

121. A recombinant nucleic acid encoding the peptide epitope or mimotope of any one of claims 10-36, or the recombinant polypeptide of any one of claims 37-120.

122. A recombinant nucleic acid encoding (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

123. The recombinant nucleic acid of claim 121 or 122, wherein the recombinant nucleic acid further comprises a polynucleotide sequence encoding one or more additional polypeptides and/or a non-coding RNA, optionally wherein the non-coding RNA comprises a shRNA or a microRNA.

124. The recombinant nucleic acid of claim 123, wherein the one or more additional polypeptides comprise an additional potency enhancement polypeptide, a cytokine, a chemokine, a growth factor, or any combination thereof.

125. The recombinant nucleic acid of any one of claims 121-124, wherein the recombinant nucleic acid is less than 10 kb, less than 9 kb, less than 8 kb, less than 7 kb, less than 6 kb, less than 5 kb, less than 4 kb, or less than 3 kb in length.

126. The recombinant nucleic acid of claim 125, wherein the recombinant nucleic acid is less than about 5.0 kb in length.

127. The recombinant nucleic acid of claim 126, wherein the recombinant nucleic acid is less than about 4.7 kb in length.

128. A vector comprising the recombinant nucleic acid of any one of claims 121-127.

129. The vector of claim 128, wherein the vector is a viral vector.

130. The vector of claim 129, wherein the viral vector is selected from a retrovirus vector, an adenovirus vector, and an adeno-associated virus (AAV) vector; optionally, wherein the viral vector is the AAV vector.

131. An engineered cell, wherein the engineered cell (i) comprises the recombinant nucleic acid of any one of claims 121-127, or (ii) is transfected or transduced by the vector of any one of claims 128-130.

132. An engineered cell expressing the peptide epitope or mimotope of any one of claims 10-36, or the recombinant polypeptide of any one of claims 37-120.

133. An engineered cell expressing (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising a tag in its extracellular region, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

134. An engineered cell comprising the recombinant polypeptide of any one of claims 37-120 or the recombinant nucleic acid of any one of claims 121-127.

135. The engineered cell of any one of claims 131-134, wherein the engineered cell comprises one or more additional peptide epitope(s) or mimotope(s) present on the cell surface, wherein the one or more additional peptide epitope(s) or mimotope(s) are the same as the peptide epitope or mimotope or different from the peptide epitope or mimotope.

136. The engineered cell of any one of claims 131-135, wherein the engineered cell exhibits one or more of the following:

(a) improved binding to the antigen expressed on the surface of a target cell;

(b) reduced recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or

(c) reduced cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell;

compared to a control cell expressing a recombinant polypeptide comprising the unmodified peptide epitope or mimotope.

137. The engineered cell of any one of claims 131-136, wherein the engineered cell exhibits one or more of the following:

(a) comparable levels of binding to the antigen expressed on the surface of a target cell;

(b) comparable levels of recombinant polypeptide-induced antigen-independent signaling and/or activation of the engineered cell; and/or

(c) comparable levels of cross-linking between the recombinant polypeptides expressed on the cell surface of the engineered cell;

compared to a control cell expressing a recombinant polypeptide that does not comprise the peptide epitope or mimotope.

138. A composition comprising the recombinant nucleic acid of any one of claims 121-127, the vector of any one of claims 128-130, or the engineered cell of any one of claims 131-137.

139. A method of preparing an engineered cell, wherein the method comprises contacting the cell with the recombinant nucleic acid of any one of claims 121-127, or the vector of any one of claims 128-130.

140. A method of preparing an engineered cell, wherein the method comprises expressing in the cell:

(a) the recombinant polypeptide of any one of claims 37-120; or

(b) at least the following two polypeptides: (i) a recombinant receptor that comprises a binding region for an antigen, and (ii) a recombinant polypeptide comprising at least one tag in its extracellular region, wherein the at least one tag comprises a peptide epitope or a mimotope that mimics an epitope.

141. The method of claim 139 or 140, wherein the step of introducing comprises homology-directed repair (HDR)-mediated insertion using the gene editing machinery.

142. The method of claim 139, wherein the recombinant nucleic acid or the vector is introduced via AAV.

143. The method of any one of claims 139-142, wherein the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.*, CAR); and (iii) the modified TRAC gene locus.

144. The method of any one of claims 139-142, wherein the method thereby produces an engineered cell comprising: (i) the recombinant polypeptide; (ii) the immune receptor (*e.g.*, CAR); and (iii) the modified TRAJ locus.

145. The method of any one of claims 139-144, wherein the cell is for use in a cell therapy.

146. A method of treating cancer or an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the engineered cells of any one of claims 131-137 to the subject.

147. A method of treating an autoimmune disease in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of any one of claims 121-127, or the vector of any one of claims 128-130, to the subject.

148. A method of treating cancer in a subject in need thereof, comprising administering an effective amount of the recombinant nucleic acid of any one of claims 121-127, or the vector of any one of claims 128-130, to the subject.

149. The method of any one of claims 146-148, wherein the method further comprises administering an antibody to the subject, wherein the antibody binds the tag, the peptide epitope, or the mimotope.

150. The method of any one of claims 146-149, wherein the method further comprises monitoring the subject for an adverse event.

151. The method of any one of claims 146-150, wherein the method further comprises monitoring the subject for treatment outcomes.

152. A method of depleting engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the

engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

153. A method of reducing or eliminating at least one symptom of an adverse event caused by engineered cells in a subject in need thereof, comprising administering to the subject an antibody that binds to a tag expressed on the surface of the engineered cells, wherein the tag comprises a peptide epitope or a mimotope that mimics an epitope.

154. The method of any one of claims 149-153, wherein administration of the antibody depletes at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 99.5%, or at least 99.9% of the engineered cells expressing the tag, the peptide epitope, or the mimotope in the subject.

155. The method of any one of claims 149-154, wherein the antibody is administered after the detection or occurrence of an adverse event, and wherein administration of the antibody reduces or eliminates at least one symptom of the adverse event.

156. The method of any one of claims 149-155, wherein the antibody is tarlatamab.

157. The method of any one of claims 149-155, wherein the antibody is mogamulizumab.

158. The method of any one of claims 149-155, wherein the antibody is CAP-100.

159. A method of isolating or enriching the engineered cells of any one of claims 131-137, comprising contacting the engineered cells with a binding agent that binds to the epitope or the mimotope.