

Summary

[0005] Provided herein are genetically engineered T cells and compositions, methods, uses, kits, and articles of manufacture related to genetically engineered T cells. In some of any of the provided examples, the genetically engineered T cell comprises a modified cluster of differentiation 247 (*CD247*) locus. In some of any examples, the modified *CD247* locus comprises a transgene sequence encoding a chimeric receptor or a portion thereof. In provided examples, the transgene sequence is in-frame with an open reading frame or a partial sequence thereof of the endogenous *CD247* locus. Thus, in provided examples, the modified *CD247* locus encodes a chimeric receptor that includes sequences encoded from the transgene sequence and sequences encoded from the endogenous *CD247* locus. In particular examples, the chimeric receptor contains an intracellular region that comprises a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain, for example the entire CD3ζ signaling domain, or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences (e.g., an open reading frame) at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0006] Provided herein are genetically engineered T cells that contain a modified *CD247* locus. In some of any examples, the modified *CD247* locus comprises a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3zeta (CD3ζ) signaling domain. In some of any examples, the nucleic acid sequence comprises a transgene sequence encoding a portion of the chimeric receptor, the transgene sequence having been integrated at the endogenous *CD247* locus. In some of any examples, the integration occurs via homology directed repair (HDR). In some of any examples, all or a fragment of the CD3ζ signaling domain of the intracellular region of the chimeric receptor is encoded by an open reading frame or a partial sequence thereof of the endogenous *CD247* locus. In some of any examples, the nucleic acid sequence comprises an in-frame fusion of (i) a transgene sequence encoding a portion of the chimeric receptor and (ii) an open reading frame or a partial sequence thereof of the endogenous *CD247* locus. In particular examples, the modified *CD247* locus encodes a chimeric receptor that contains an intracellular region that comprises a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain, for example the entire CD3ζ signaling domain, or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0006a] In one aspect, the present disclosure refers to a genetically engineered T cell, comprising a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3zeta (CD3ζ) signaling domain.

[0007] Provided herein are genetically engineered T cells that contain a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3ζ signaling domain, wherein the nucleic acid sequence comprises an in-frame fusion of (i) a transgene sequence encoding a portion of the chimeric receptor and (ii) an open reading frame or a partial sequence thereof of an endogenous *CD247* locus encoding the CD3ζ signaling domain. In particular examples, the modified *CD247* locus encodes a chimeric receptor that contains an intracellular region that comprises a CD3zeta (CD3ζ) signaling domain, in which the

CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0008] In some of any examples, the transgene sequence is in-frame with one or more exons of the open reading frame or partial sequence thereof of the endogenous *CD247* locus.

[0009] In some of any examples, the transgene sequence does not comprise a sequence encoding a 3' UTR. In some of any examples, the transgene sequence does not comprise an intron.

[0010] In some of any examples, the transgene sequence encodes a fragment of the CD3ζ signaling domain. For example, in particular examples, the CD3ζ signaling domain or a fragment thereof of the chimeric receptor is encoded together by sequences of the transgene sequence and by genomic sequences (e.g., an open reading frame) at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0011] In some of any examples, the transgene sequence does not encode the CD3ζ signaling domain or a fragment thereof. For example, in particular examples the entire or full-length of the CD3ζ signaling domain or a fragment thereof of the chimeric receptor is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0012] In some of any examples, the open reading frame or a partial sequence thereof comprises at least one intron and at least one exon of the endogenous *CD247* locus. In some of any examples, the open reading frame or a partial sequence thereof encodes a 3' UTR of the endogenous *CD247* locus.

[0013] In some of any examples, the transgene sequence is downstream of exon 1 and upstream of exon 8 of the open reading frame of the endogenous *CD247* locus. In some of any examples, the transgene sequence is downstream of exon 1 and upstream of exon 3 of the open reading frame of the endogenous *CD247* locus.

[0014] In some of any examples, at least a fragment of the CD3ζ signaling domain, such as the entire CD3ζ signaling domain, of the encoded chimeric receptor is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof. In some of any examples, the CD3ζ signaling domain is encoded by a sequence of nucleotides comprising at least a portion of exon 2 and exons 3-8 of the open reading frame of the endogenous *CD247* locus. In some of any examples, the CD3ζ signaling domain is encoded by a sequence of nucleotides that does not comprise exon 1, does not comprise the full length of exon 1 and/or does not comprise the full length of exon 2 of the open reading frame of the endogenous *CD247* locus.

[0015] In some of any examples, the encoded chimeric receptor is capable of signaling via the CD3ζ signaling domain.

[0016] In some of any examples, the encoded CD3ζ signaling domain comprises the sequence selected from any one of SEQ ID NOS:13-15, or a sequence that exhibits at least 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS: 13-15, or a fragment thereof. In some examples, the encoded CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:13. In some examples, the encoded CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:14. In some examples, the encoded CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:15,

[0017] In some of any examples, the chimeric receptor is or comprises a functional non-T cell receptor (non-TCR) antigen receptor.

[0018] In some of any examples, the chimeric receptor is a chimeric antigen receptor (CAR). In some of any examples, the chimeric receptor further comprises an extracellular region and/or a transmembrane domain.

[0019] In some of any examples, the transgene sequence comprises a sequence of nucleotides encoding one or more regions of the chimeric receptor. In some of any examples, the transgene sequence comprises a sequence of nucleotides encoding one or more of an extracellular region, a transmembrane domain and/or a portion of the intracellular region. In some of any examples, the extracellular region comprises a binding domain. In some of any examples, the binding domain is an antibody or an antigen-binding fragment thereof. In some of any examples, the binding domain comprises an antibody or an antigen-binding fragment thereof.

[0020] In some of any examples, the binding domain is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition. In some of any examples, the target antigen is a tumor antigen. In some of any examples, the target antigen is selected from among $\alpha\beta6$ integrin ($\alpha\beta6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G protein-coupled receptor class C group 5 member D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family

Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0021] In some of any examples, the extracellular region comprises a spacer. In some of any examples, the spacer is operably linked between the binding domain and the transmembrane domain. In some of any examples, the spacer comprises an immunoglobulin hinge region. In some of any examples, the spacer comprises a C_H2 region and a C_H3 region.

[0022] In some of any examples, the portion of the intracellular region encoded by the transgene sequence comprises one or more costimulatory signaling domain(s). In some of any examples, the one or more costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some examples, the costimulatory signaling domain is a signaling domain of human CD28. In some examples, the costimulatory signaling domain is a signaling domain of human 4-1BB. In some examples, the costimulatory signaling domain is a signaling domain of human ICOS. In some of any examples, the one or more costimulatory signaling domain comprises an intracellular signaling domain of 4-1BB, such as human 4-1BB.

[0023] In some of any examples, the modified *CD247* locus encodes a chimeric receptor that comprises, from its N to C terminus in order: the extracellular binding domain, the spacer, the transmembrane domain and an intracellular signaling region. In particular examples, the intracellular region contains a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0024] In some of any examples, the transgene sequence comprises in order: a sequence of nucleotides encoding an extracellular binding domain; a spacer; and a transmembrane domain; a costimulatory signaling domain. In some of any examples, the modified *CD247* locus comprises in order: a sequence of nucleotides encoding an extracellular binding domain; a spacer; and a transmembrane domain; and an intracellular region containing a costimulatory signaling domain and a

CD3zeta (CD3ζ) signaling domain. In particular examples, the intracellular signaling region contains a costimulatory signaling domain and a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences (e.g., an open reading frame) at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell. In some of any examples, the transgene sequence comprises in order a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that includes a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, that is that also includes a C_H2 region and/or a C_H3 region; and a transmembrane domain, that is from human CD28; a costimulatory signaling domain, that is from human 4-1BB. In some of any examples, the modified *CD247* locus comprises in order a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that includes a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, that is that also includes a C_H2 region and/or a C_H3 region; and a transmembrane domain, that is from human CD28; and an intracellular region containing a costimulatory signaling domain that is from human 4-1BB, and the CD3ζ signaling domain. In particular examples, the intracellular region contains a costimulatory signaling domain and a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences (e.g., an open reading frame) at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell.

[0025] In some of any examples, the chimeric receptor is a CAR that is a multi-chain CAR.

[0026] In some of any examples, the transgene sequence comprises a sequence of nucleotides encoding at least one further protein. For example, the at least one further protein may be another chain of the CAR. In some examples, the at least one further protein is a surrogate marker or truncated receptor for co-expression on a cell with the chimeric receptor. In some of any examples, the transgene sequence comprises one or more multicistronic element(s), such as separating the chimeric receptor and the one or more further proteins. In some of any examples, the multicistronic element(s) is positioned between the sequence of nucleotides encoding the portion of the chimeric receptor and the sequence of nucleotides encoding the at least one further protein. In some of any examples, the at least one further protein is a surrogate marker. In some of any examples, the surrogate marker is a truncated receptor. In some of any examples, the truncated receptor lacks an intracellular signaling domain and/or is not capable of mediating intracellular signaling when bound by its ligand. In some of any examples, the chimeric receptor is a multi-chain CAR, and a multicistronic element is positioned between a sequence of nucleotides encoding one chain of the multi-chain CAR and a sequence of nucleotides encoding another chain of the multi-chain CAR. In some of any examples, the one or more multicistronic element(s) are upstream of the sequence of nucleotides encoding the portion of the chimeric receptor. In some of any examples, the one or more multicistronic element is or comprises a

ribosome skip sequence. In some of any examples, the ribosome skip sequence is a T2A, a P2A, an E2A, or an F2A element.

[0027] In some of any examples, the modified *CD247* locus comprises the promoter and/or regulatory or control element of the endogenous *CD247* locus operably linked to control expression the nucleic acid sequence encoding the chimeric receptor. In some of any examples, the modified locus comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the nucleic acid sequence encoding the chimeric receptor. In some of any examples, the one or more heterologous regulatory or control element comprises a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, a splice acceptor sequence and/or a splice donor sequence. In some of any examples, the heterologous promoter is or comprises a human elongation factor 1 alpha (EF1 α) promoter or an MND promoter or a variant thereof.

[0028] In some of any examples, the T cell is a primary T cell derived from a subject. In some of any examples, the subject is a human. In some of any examples, the T cell is a CD8⁺ T cell or subtypes thereof. In some of any examples, the T cell is a CD4⁺ T cell or subtypes thereof. In some of any examples, the T cell is derived from a multipotent or pluripotent cell. In some of any examples, the pluripotent cell is an iPSC. In some of any examples, the T cell is derived from a multipotent or pluripotent cell, which is an iPSC.

[0029] Also provided herein are polynucleotides, such as polynucleotides that can be used for integration of a transgene sequence encoding a chimeric receptor into the *CD247* locus. In some of any examples, the polynucleotides include (a) a nucleic acid sequence encoding a chimeric receptor or a portion thereof; and (b) one or more homology arm(s) linked to the nucleic acid sequence, wherein the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a *CD247* locus or a partial sequence thereof. In some of any examples, integration of the polynucleotide into the *CD247* locus encodes a chimeric receptor that comprises an intracellular region (e.g., an intracellular region comprising a CD3 ζ signaling domain) and the nucleic acid sequence of (a) is a nucleic acid sequence encoding a portion of the chimeric receptor, in which said portion does not include the full intracellular region of the chimeric receptor. In some examples, the full intracellular region includes a CD3zeta (CD3 ζ) signaling domain. In some examples, the full intracellular region includes a costimulatory signaling domain and a CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) encodes a portion of the chimeric receptor that does not include the entire or full length sequence encoding a CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) does not contain any sequence encoding the CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) encodes an intracellular region that comprises a fragment of the CD3zeta (CD3 ζ) signaling domain. In any of such examples, the nucleic acid sequence of (a) may encode a costimulatory signaling domain of the intracellular region.

[0029a] In one aspect, the present disclosure refers to a polynucleotide, comprising: (a) a nucleic acid sequence encoding a chimeric receptor or a portion thereof; and (b) one or more homology arm(s) linked to the nucleic acid sequence, wherein the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a *CD247* locus or a partial sequence thereof.

[0030] Also provided herein are polynucleotides that contain (a) a nucleic acid sequence encoding a portion of a chimeric receptor, said chimeric receptor comprising an intracellular region (e.g., an intracellular region comprising a CD3 ζ signaling domain), wherein the portion of the chimeric receptor includes less than the full intracellular region of the chimeric receptor; and (b) one or more homology arm(s) linked to the nucleic acid sequence, wherein the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a *CD247* locus or a partial sequence thereof. In some examples, the polynucleotide can be used for integration of a transgene sequence encoding the chimeric receptor into the *CD247* locus. In some examples, the full intracellular region includes a CD3zeta (CD3 ζ) signaling domain. In some examples, the full intracellular region includes a costimulatory signaling domain and a CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) encodes a portion of the chimeric receptor that does not include the entire or full length sequence encoding a CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) does not contain any sequence encoding the CD3zeta (CD3 ζ) signaling domain. In some examples, the nucleic acid sequence of (a) encodes an intracellular region that comprises a fragment of the CD3zeta (CD3 ζ) signaling domain. In any of such examples, the nucleic acid sequence of (a) may encode a costimulatory signaling domain of the intracellular region.

[0031] In some of any examples, the full intracellular region of the chimeric receptor comprises a CD3zeta (CD3 ζ) signaling domain or a fragment thereof, wherein at least a portion of the intracellular region is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof when the chimeric receptor is expressed from a cell introduced with the polynucleotide.

[0032] In some of any examples, the nucleic acid sequence encoding the portion of the chimeric receptor and the one or more homology arm(s) together comprise at least a fragment of a sequence of nucleotides encoding the intracellular region of the chimeric receptor, wherein at least a portion of the intracellular region comprises the CD3 ζ signaling domain or a fragment thereof encoded by the open reading frame of the *CD247* locus or a partial sequence thereof when the chimeric receptor is expressed from a cell introduced with the polynucleotide.

[0033] In some of any examples, the nucleic acid sequence of (a) does not comprise a sequence encoding a 3' UTR. In some of any examples, the nucleic acid sequence of (a) does not comprise an intron.

[0034] In some of any examples, the nucleic acid sequence of (a) encodes a fragment of the CD3 ζ signaling domain. In such examples, when the chimeric receptor is expressed from a cell introduced with the polynucleotide, at least a portion of the CD3 ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3 ζ) of the engineered cell such as a T cell. For example, in particular examples, the CD3 ζ signaling domain or a fragment thereof of the chimeric receptor is encoded together by sequences of the transgene sequence

and by genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3 ζ) of the engineered cell such as a T cell.

[0035] In some of any examples, the nucleic acid sequence of (a) does not encode the CD3 ζ signaling domain or a fragment thereof. In such examples, when the chimeric receptor is expressed from a cell introduced with the polynucleotide the entire or full-length of the CD3 ζ signaling domain or a fragment thereof of the chimeric receptor is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3 ζ) of the engineered cell such as a T cell.

[0036] In some of any examples, the open reading frame or a partial sequence thereof of the endogenous *CD247* locus comprises at least one intron and at least one exon of the endogenous *CD247* locus. In some of any examples, the open reading frame or a partial sequence thereof encodes a 3' UTR of the endogenous *CD247* locus.

[0037] In some of any examples, at least a fragment of the CD3 ζ signaling domain, such as the entire CD3 ζ signaling domain, of the encoded chimeric receptor is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof, when the chimeric receptor is expressed from a cell introduced with the polynucleotide.

[0038] In some of any examples, the nucleic acid sequence of (a) is a sequence that is exogenous or heterologous to an open reading frame of the endogenous genomic *CD247* locus a T cell, such as a human T cell.

[0039] In some of any examples, the nucleic acid sequence of (a) comprises a sequence of nucleotides that is in-frame with one or more exons of the open reading frame or a partial sequence thereof of the *CD247* locus comprised in the one or more homology arm(s).

[0040] In some of any examples, the one or more region(s) of the open reading frame of the endogenous *CD247* locus or a partial sequence thereof is or comprises sequences that are upstream of exon 8 of the open reading frame of the *CD247* locus. In some of any examples, the one or more region(s) of the open reading frame is or comprises sequences that are upstream of exon 3 of the open reading frame of the *CD247* locus. In some of any examples, the one or more region(s) of the open reading frame is or comprises sequences that includes exon 3 of the open reading frame of the *CD247* locus. In some of any examples, the one or more region(s) of the open reading frame is or comprises sequences that includes at least a portion of exon 2 of the open reading frame of the *CD247* locus. In some of any examples, the one or more homology arm(s) does not comprise exon 1, does not comprise the full length of exon 1 and/or does not comprise the full length of exon 2 of the open reading frame of the endogenous *CD247* locus.

[0041] In some of any examples, when expressed by a cell introduced with the polynucleotide, the encoded chimeric receptor is capable of signaling via the CD3 ζ signaling domain. In some of any examples, the CD3 ζ signaling domain of the full intracellular region encoded by the chimeric receptor comprises the sequence selected from any one of SEQ ID NOS:13-15, or a sequence that

exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:13-15, or a fragment thereof. In some examples, the CD3 ζ signaling domain has the sequence set forth in SEQ ID NO: 13. In some examples, the CD3 ζ signaling domain has the sequence set forth in SEQ ID NO: 14. In some examples, the CD3 ζ signaling domain has the sequence set forth in SEQ ID NO: 15.

[0042] In some of any examples, the one or more homology arm comprises a 5' homology arm and a 3' homology arm. In some of any examples, the polynucleotide comprises the structure [5' homology arm]-[nucleic acid sequence of (a)]-[3' homology arm].

[0043] In some of any examples, the 5' homology arm and the 3' homology arm independently are from at or about 50 to at or about 2000 nucleotides, from at or about 100 to at or about 1000 nucleotides, from at or about 100 to at or about 750 nucleotides, from at or about 100 to at or about 600 nucleotides, from at or about 100 to at or about 400 nucleotides, from at or about 100 to at or about 300 nucleotides, from at or about 100 to at or about 200 nucleotides, from at or about 200 to at or about 1000 nucleotides, from at or about 200 to at or about 750 nucleotides, from at or about 200 to at or about 600 nucleotides, from at or about 200 to at or about 400 nucleotides, from at or about 200 to at or about 300 nucleotides, from at or about 300 to at or about 1000 nucleotides, from at or about 300 to at or about 750 nucleotides, from at or about 300 to at or about 600 nucleotides, from at or about 300 to at or about 400 nucleotides, from at or about 400 to at or about 1000 nucleotides, from at or about 400 to at or about 750 nucleotides, from at or about 400 to at or about 600 nucleotides, from at or about 600 to at or about 1000 nucleotides, from at or about 600 to at or about 750 nucleotides or from at or about 750 to at or about 1000 nucleotides in length. In some of any examples, the 5' homology arm and the 3' homology arm independently are at or about 200, 300, 400, 500, 600, 700 or 800 nucleotides in length, or any value between any of the foregoing. In some of any examples, the 5' homology arm and the 3' homology arm independently are greater than at or about 300 nucleotides in length. In some of any examples, the 5' homology arm and the 3' homology arm independently are at or about 400, 500 or 600 nucleotides in length, or any value between any of the foregoing.

[0044] In some of any examples, the 5' homology arm comprises the sequence set forth in SEQ ID NO:80, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:80 or a partial sequence thereof. In some examples, the 5' homology arm comprises the sequence set forth in SEQ ID NO:80. In some examples, the 5' homology arm consists or consists essentially of the sequence set forth in SEQ ID NO: 80. In some of any examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:81, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:81 or a partial sequence thereof. In some examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:81. In some examples, the 3' homology arm consists or consists essentially of the sequence set forth in SEQ ID

NO: 81.

[0045] In some of any examples, the chimeric receptor is or comprises a functional non-T cell receptor (non-TCR) antigen receptor.

[0046] In some of any examples, the chimeric receptor is a chimeric antigen receptor (CAR).

[0047] In some of any examples, the nucleic acid sequence of (a) comprises a sequence of nucleotides encoding an extracellular region a sequence of nucleotides encoding a transmembrane domain and/or a portion of the intracellular region. In some of any examples, the nucleic acid sequence of (a) comprises a sequence of nucleotides encoding an extracellular region, a sequence of nucleotides encoding a transmembrane domain and a sequence of nucleotides encoding a portion of the intracellular region. In some of any examples, the extracellular region comprises a binding domain. In some of any examples, the binding domain is or comprises an antibody or an antigen-binding fragment thereof.

[0048] In some of any examples, the binding domain is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition. In some of any examples, the target antigen is a tumor antigen. In some of any examples, the target antigen is selected from among $\alpha\beta6$ integrin (avb6 integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G protein-coupled receptor class C group 5 member D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane

antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0049] In some of any examples, the extracellular region comprises a spacer. In some of any examples, the spacer is operably linked between the binding domain and the transmembrane domain. In some of any examples, the spacer comprises an immunoglobulin hinge region. In some of any examples, the spacer comprises a C_H2 region and a C_H3 region.

[0050] In some of any examples, the portion of the intracellular region encoded by the nucleic acid of a) comprises one or more costimulatory signaling domain(s). In some of any examples, the one or more costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof. In some examples, the costimulatory signaling domain is a signaling domain of human CD28. In some examples, the costimulatory signaling domain is a signaling domain of human 4-1BB. In some examples, the costimulatory signaling domain is a signaling domain of human ICOS. In some of any examples, the one or more costimulatory signaling domain comprises an intracellular signaling domain of 4-1BB, such as human 4-1BB.

[0051] In some of any examples, the encoded chimeric receptor comprises, from its N to C terminus in order: the extracellular binding domain, the spacer, the transmembrane domain and an intracellular signaling region, when the chimeric receptor is expressed from a cell introduced with the polynucleotide. In particular examples, when expressed from a cell such as a T cell, the intracellular region of the encoded chimeric receptor contains a CD3zeta (CD3ζ) signaling domain, in which the entire CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ).

[0052] In some of any examples, the sequence of (a) comprises in order: a sequence of nucleotides encoding an extracellular binding domain; a spacer; and a transmembrane domain; and a costimulatory signaling domain. In some of any examples, the sequence of (a) comprises in order: a sequence of nucleotides encoding an extracellular binding domain; a spacer; a transmembrane domain; and an intracellular signaling region containing a a costimulatory signaling domain and a fragment of the CD3ζ signaling domain. In particular examples, when expressed from a cell such as a T cell, the polynucleotide encodes a chimeric receptor with an intracellular signaling region that contains a costimulatory signaling domain and a CD3zeta (CD3ζ) signaling domain, in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the

endogenous *CD247* locus (the genomic locus encoding CD3 ζ) of the engineered cell such as a T cell.

[0053] In some of any examples, the nucleic acid sequence of (a) comprises in order a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that includes a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, and that also includes a C_H2 region and/or a C_H3 region; and a transmembrane domain, that is from human CD28; and a costimulatory signaling domain, that is from human 4-1BB. In some of any examples, the sequence of (a) comprises in order: a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that includes a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, and that also includes a C_H2 region and/or a C_H3 region; a transmembrane domain that is from human CD28; and an intracellular region that contains a costimulatory signaling domain that is from human 4-1BB, and a fragment of the CD3 ζ signaling domain. In particular examples, when expressed from a cell such as a T cell, the polynucleotide encodes a chimeric receptor with an intracellular signaling region that contains a human 4-1BB costimulatory signaling domain and a CD3zeta (CD3 ζ) signaling domain, in which the CD3 ζ signaling domain or at least a portion of the CD3 ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3 ζ) of the engineered cell such as a T cell. In some of any examples, the modified *CD247* locus, following introduction of the polynucleotide into a T cell, comprises in order a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that includes a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, and that also includes a C_H2 region and/or a C_H3 region; and a transmembrane domain, that is from human CD28; a costimulatory signaling domain, that is from human 4-1BB.

[0054] In some of any examples, the CAR is a multi-chain CAR. In some of any examples, the nucleic acid sequence of (a) comprises a sequence of nucleotides encoding at least one further protein.

[0055] In some of any examples, the nucleic acid sequence of (a) comprises one or more multicistronic element(s). In some of any examples, the multicistronic element(s) is positioned between the sequence of nucleotides encoding the portion of the chimeric receptor and the sequence of nucleotides encoding the at least one further protein. In some of any examples, the at least one further protein is a surrogate marker. In some of any examples, the surrogate marker is a truncated receptor. In some of any examples, the truncated receptor lacks an intracellular signaling domain and/or is not capable of mediating intracellular signaling when bound by its ligand. In some of any examples, the chimeric receptor is a multi-chain CAR, and a multicistronic element is positioned between a sequence of nucleotides encoding one chain of the multi-chain CAR and a sequence of nucleotides encoding another chain of the multi-chain CAR. In some of any examples, the one or more multicistronic element(s) are upstream of the sequence of nucleotides encoding the portion of the chimeric receptor. In some of any

examples, the one or more multicistronic element is or comprises a ribosome skip sequence. In some of any examples, the ribosome skip sequence is a T2A, a P2A, an E2A, or an F2A element.

[0056] In some of any examples, the modified *CD247* locus, following introduction of the polynucleotide into a T cell, comprises the promoter and/or regulatory or control element of the endogenous *CD247* locus operably linked to control expression the nucleic acid sequence encoding the chimeric receptor. In some of any examples, the modified locus comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the nucleic acid sequence encoding the chimeric receptor. In some of any examples, the one or more heterologous regulatory or control element comprises a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, a splice acceptor sequence and/or a splice donor sequence. In some of any examples, the heterologous promoter is or comprises a human elongation factor 1 alpha (EF1 α) promoter or an MND promoter or a variant thereof.

[0057] In some of any examples, the nucleic acid sequence of (a) comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the nucleic acid sequence encoding the chimeric receptor. In some of any examples, the one or more heterologous regulatory or control element comprises a promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, a splice acceptor sequence and/or a splice donor sequence. In some of any examples, the heterologous promoter is or comprises a human elongation factor 1 alpha (EF1 α) promoter or an MND promoter or a variant thereof.

[0058] In some of any examples, the polynucleotide is comprised in a viral vector. In some of any examples, the viral vector is an AAV vector. In some of any examples, the AAV vector is selected from among AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 or AAV8 vector. In some of any examples, the AAV vector is an AAV2 or AAV6 vector. In some of any examples, the viral vector is a retroviral vector. In some of any examples, the viral vector a lentiviral vector.

[0058a] In one aspect, the present disclosure refers to a viral vector comprising the polynucleotide disclosed herein, optionally wherein the viral vector is an AAV vector, optionally wherein the AAV vector is an AAV2 or AAV6 vector; or wherein the viral vector is a retroviral vector, optionally a lentiviral vector.

[0059] In some of any examples, the polynucleotide is a linear polynucleotide. In some of any examples, a double-stranded polynucleotide or a single-stranded polynucleotide.

[0060] In some of any examples, the polynucleotide is at least at or about 2500, 2750, 3000, 3250, 3500, 3750, 4000, 4250, 4500, 4760, 5000, 5250, 5500, 5750, 6000, 7000, 7500, 8000, 9000 or 10000 nucleotides in length, or any value between any of the foregoing. In some of any examples, the polynucleotide is between at or about 2500 and at or about 5000 nucleotides, at or about 3500 and at or about 4500 nucleotides, or at or about 3750 nucleotides and at or about 4250 nucleotides in length.

[0061] Also provided herein are methods of producing a genetically engineered T cell, the method involving introducing the polynucleotides of any of the examples provided herein into a T cell comprising a genetic disruption at a *CD247* locus.

[0062] Also provided herein are methods of producing a genetically engineered T cell, the method involving: (a) introducing, into a T cell, one or more agent(s) capable of inducing a genetic disruption at

a target site within an endogenous *CD247* locus of the T cell; and (b) introducing any of the polynucleotides described herein into a T cell comprising a genetic disruption at a *CD247* locus, wherein the method produces a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding the chimeric receptor comprising an intracellular region comprising a CD3 ζ (CD3 ζ) signaling domain.

[0063] In some of any examples, the polynucleotide comprises a nucleic acid sequence encoding a chimeric receptor or a portion thereof, and the nucleic acid sequence encoding a chimeric receptor or a portion thereof is integrated within the endogenous *CD247* locus via homology directed repair (HDR).

[0064] Also provided herein are methods of producing a genetically engineered T cell, the method involving introducing, into a T cell, a polynucleotide comprising a nucleic acid sequence encoding a chimeric receptor or a portion thereof, said T cell having a genetic disruption within a *CD247* locus of the T cell, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is integrated within the endogenous *CD247* locus via homology directed repair (HDR).

[0064a] In one aspect, the present disclosure refers to a method of producing a genetically engineered T cell, the method comprising introducing the polynucleotide disclosed herein, or the viral vector disclosed herein, into a T cell under conditions to induce homology directed repair (HDR).

[0064b] In one aspect, the present disclosure refers to a method of producing a genetically engineered T cell, the method comprising introducing, into a T cell, a polynucleotide comprising a nucleic acid sequence encoding a chimeric receptor or a portion thereof, said T cell having a genetic disruption within a *CD247* locus of the T cell, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is integrated within the endogenous *CD247* locus via homology directed repair (HDR).

[0065] In some of any examples, the genetic disruption is carried out by introducing, into a T cell, one or more agent(s) capable of inducing a genetic disruption at a target site within an endogenous *CD247* locus of the T cell.

[0065a] In one aspect, the present disclosure refers to a method of producing a genetically engineered T cell, the method comprising: (a) introducing, into a T cell, one or more agent(s) capable of inducing a genetic disruption at a target site within an endogenous *CD247* locus of the T cell; and (b) introducing the polynucleotide disclosed herein, or the viral vector disclosed herein, into the T cell, wherein steps (a) and (b) are simultaneous or sequential, in any order.

[0066] In some of any examples, the method produces a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3 ζ (CD3 ζ) signaling domain.

[0067] In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof encodes a portion of the chimeric receptor. In some of any examples, the polynucleotide further comprises one or more homology arm(s) linked to the nucleic acid sequence, wherein the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a *CD247* locus.

[0068] In some of any examples, the full intracellular region of the chimeric receptor comprises a CD3zeta (CD3 ζ) signaling domain or a fragment thereof, wherein at least a portion of the intracellular region is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof in a cell generated by the method.

[0069] In some of any examples, the nucleic acid sequence encoding the portion of the chimeric receptor and the one or more homology arm(s) together comprise at least a fragment of a sequence of nucleotides encoding the intracellular region of the chimeric receptor, wherein at least a portion of the intracellular region comprises the CD3 ζ signaling domain or a fragment thereof encoded by the open reading frame of the *CD247* locus or a partial sequence thereof in a cell generated by the method.

[0070] In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof does not comprise a sequence encoding a 3' UTR. In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof encodes a fragment of the CD3 ζ

signaling domain, in a cell generated by the method. In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof does not encode the CD3 ζ signaling domain or a fragment thereof, in a cell generated by the method. In some of any examples, at least a fragment of the CD3 ζ signaling domain, such as the entire CD3 ζ signaling domain, of the encoded chimeric receptor is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof, in a cell generated by the method.

[0071] In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof is a sequence that is exogenous or heterologous to an open reading frame of the endogenous genomic *CD247* locus a T cell, such as a human T cell.

[0072] In some of any examples, the nucleic acid sequence encoding a chimeric receptor or a portion thereof comprises a sequence of nucleotides that is in-frame with one or more exons of the open reading frame or a partial sequence thereof of the *CD247* locus comprised in the one or more homology arm(s).

[0073] In some of any examples, when expressed by a cell introduced with the polynucleotide, the chimeric receptor is capable of signaling via the CD3 ζ signaling domain. In some of any examples, the CD3 ζ signaling domain of the full intracellular region comprises the sequence selected from any one of SEQ ID NOS:13-15, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:13-15, or a fragment thereof. In some examples, the CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:13. In some examples, the CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:14. In some examples, the CD3 ζ signaling domain comprises the sequence set forth in SEQ ID NO:15.

[0074] In some of any examples, the one or more homology arm comprises a 5' homology arm and a 3' homology arm. In some of any examples, the polynucleotide comprises the structure [5' homology arm]-[nucleic acid sequence encoding a chimeric receptor or a portion thereof]-[3' homology arm].

[0075] In some of any examples, the 5' homology arm and the 3' homology arm independently are from at or about 50 to at or about 2000 nucleotides, from at or about 100 to at or about 1000 nucleotides, from at or about 100 to at or about 750 nucleotides, from at or about 100 to at or about 600 nucleotides, from at or about 100 to at or about 400 nucleotides, from at or about 100 to at or about 300 nucleotides, from at or about 100 to at or about 200 nucleotides, from at or about 200 to at or about 1000 nucleotides, from at or about 200 to at or about 750 nucleotides, from at or about 200 to at or about 600 nucleotides, from at or about 200 to at or about 400 nucleotides, from at or about 200 to at or about 300 nucleotides, from at or about 300 to at or about 1000 nucleotides, from at or about 300 to at or about 750 nucleotides, from at or about 300 to at or about 600 nucleotides, from at or about 300 to at or about 400 nucleotides, from at or about 400 to at or about 1000 nucleotides, from at or about 400 to at or about 750

nucleotides, from at or about 400 to at or about 600 nucleotides, from at or about 600 to at or about 1000 nucleotides, from at or about 600 to at or about 750 nucleotides or from at or about 750 to at or about 1000 nucleotides in length. In some of any examples, the 5' homology arm and the 3' homology arm independently are at or about 200, 300, 400, 500, 600, 700 or 800 nucleotides in length, or any value between any of the foregoing. In some of any examples, the 5' homology arm and the 3' homology arm independently are greater than at or about 300 nucleotides in length. In some of any examples, the 5' homology arm and the 3' homology arm independently are at or about 400, 500 or 600 nucleotides in length, or any value between any of the foregoing.

[0076] In some of any examples, the 5' homology arm comprises the sequence set forth in SEQ ID NO:80, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:80 or a partial sequence thereof. In some examples, the 5' homology arm comprises the sequence set forth in SEQ ID NO:80. In some examples, the 5' homology arm consists or consists essentially of the sequence set forth in SEQ ID NO:80. In some of any examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:81, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:81 or a partial sequence thereof. In some examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:81. In some examples, the 3' homology arm consists or consists essentially of the sequence set forth in SEQ ID NO:81.

[0077] In some of any examples, the one or more agent(s) capable of inducing a genetic disruption comprises a DNA binding protein or DNA-binding nucleic acid that specifically binds to or hybridizes to the target site, a fusion protein comprising a DNA-targeting protein and a nuclease, or an RNA-guided nuclease. In some of any examples, the one or more agent(s) comprises a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), or and a CRISPR-Cas9 combination that specifically binds to, recognizes, or hybridizes to the target site.

[0078] In some of any examples, the each of the one or more agent(s) comprises a guide RNA (gRNA) having a targeting domain that is complementary to the at least one target site. In some of any examples, the one or more agent(s) is introduced as a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas9 protein.

[0079] In some of any examples, the RNP is introduced via electroporation, particle gun, calcium phosphate transfection, cell compression or squeezing. In some of any examples, the RNP is introduced via electroporation.

[0080] In some of any examples, the concentration of the RNP is at or about 1, 2, 2.5, 5, 10, 20, 25, 30, 40 or 50 μ M, or a range defined by any two of the foregoing values. In some of any examples, the concentration of the RNP is at or about 25 μ M.

[0081] In some of any examples, the molar ratio of the gRNA and the Cas9 molecule in the

RNP is at or about 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 or 1:5, or a range defined by any two of the foregoing values. In some of any examples, the molar ratio of the gRNA and the Cas9 molecule in the RNP is at or about 2.6:1.

[0082] In some of any examples, the gRNA has a targeting domain sequence selected from CACCUUCACUCUCAGGAACA (SEQ ID NO:87); GAAUGACACCAUAGAUGAAG (SEQ ID NO:88); UGAAGAGGAUCCAUCAGC (SEQ ID NO:89); and UCCAGCAGGUAGCAGAGUUU (SEQ ID NO:90). In some of any examples, the gRNA has a targeting domain sequence of CACCUUCACUCUCAGGAACA (SEQ ID NO:87). In some of any examples, the gRNA has a targeting domain sequence of UGAAGAGGAUCCAUCAGC (SEQ ID NO:89)

[0083] In some of any examples, the T cell is a primary T cell derived from a subject. In some of any examples, the subject is a human. In some of any examples, the T cell is a CD8⁺ T cell or subtypes thereof. In some of any examples, the T cell is a CD4⁺ T cell or subtypes thereof. In some of any examples, the T cell is derived from a multipotent or pluripotent cell. In some of any examples, the multipotent or pluripotent cell is an iPSC. In some of any examples, the T cell is derived from a multipotent or pluripotent cell, which is an iPSC.

[0084] In some of any examples, the polynucleotide is comprised in a viral vector. In some of any examples, the viral vector is an AAV vector. In some of any examples, the AAV vector is selected from among AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 or AAV8 vector. In some of any examples, the AAV vector is an AAV2 or AAV6 vector. In some of any examples, the viral vector is a retroviral vector. In some of any examples, a lentiviral vector.

[0085] In some of any examples, the polynucleotide is a linear polynucleotide. In some of any examples, the linear polynucleotide is a double-stranded polynucleotide or a single-stranded polynucleotide.

[0086] In some of any examples, the one or more agent(s) and the polynucleotide are introduced simultaneously or sequentially, in any order. In some of any examples, the polynucleotide is introduced after the introduction of the one or more agent(s).

[0087] In some of any examples, the polynucleotide is introduced immediately after, or within about 30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 6 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours or 4 hours after the introduction of the agent.

[0088] In some of any examples, prior to the introducing of the one or more agent, the method comprises incubating the cells, *in vitro* with a stimulatory agent(s) under conditions to stimulate or activate the one or more immune cells. In some of any examples, the stimulatory agent(s) comprises anti-CD3 and/or anti-CD28 antibodies, such as anti-CD3/anti-CD28 beads. In some of any examples, the bead to cell ratio is or is about 1:1.

[0089] In some of any examples, the methods also include removing the stimulatory agent(s)

from the one or more immune cells prior to the introducing with the one or more agents.

[0090] In some of any examples, the method also includes incubating the cells prior to, during or subsequent to the introducing of the one or more agents and/or the introducing of the polynucleotide with one or more recombinant cytokines. In some of any examples, the one or more recombinant cytokines are selected from the group consisting of IL-2, IL-7, and IL-15. In some of any examples, the one or more recombinant cytokine is added at a concentration selected from a concentration of IL-2 from at or about 10 U/mL to at or about 200 U/mL, such as at or about 50 IU/mL to at or about 100 U/mL; IL-7 at a concentration of 0.5 ng/mL to 50 ng/mL, such as at or about 5 ng/mL to at or about 10 ng/mL and/or IL-15 at a concentration of 0.1 ng/mL to 20 ng/mL, such as at or about 0.5 ng/mL to at or about 5 ng/mL.

[0091] In some of any examples, the incubation is carried out subsequent to the introducing of the one or more agents and the introducing of the polynucleotide for up to or approximately 24 hours, 36 hours, 48 hours, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 days, such as up to or about 7 days.

[0092] In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells, such as T cells, in a plurality of engineered cells generated by the method comprise a genetic disruption of at least one target site within a *CD247* locus. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells, such as T cells, generated by the method express the chimeric receptor or antigen-binding fragment thereof. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells generated by the method express the chimeric receptor or antigen-binding fragment thereof.

[0093] In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells, such as T cells, generated by the method comprise a genetic disruption of at least one target site within a *CD247* locus. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells, such as T cells, generated by the method express the chimeric receptor. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells, such as T cells, generated by the method express the chimeric receptor, in which the chimeric receptor contains an intracellular region containing a CD3zeta (CD3ζ) signaling domain and in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell. In some examples, a least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus. In some examples, the entire or full CD3ζ signaling domain of the

intracellular region of the chimeric receptor is encoded by the genomic sequences at the endogenous *CD247* locus.

[0094] Also provided are engineered T cells or a plurality of engineered T cells generated using any of the methods described herein.

[0094a] In one aspect, the present disclosure refers to a genetically engineered T cell or a plurality of genetically engineered T cells generated using the method disclosed herein.

[0095] Also provided are compositions that include any of the engineered T cells described herein.

[0095a] In one aspect, the present disclosure refers to a composition, comprising the genetically engineered T cell disclosed herein.

[0096] Also provided are compositions that include a plurality of T cells that include any of the engineered T cells described herein. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the T cells in the composition comprise a genetic disruption of at least one target site within a *CD247* locus. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the T cells in the composition express the chimeric receptor. In some of any examples, at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the T cells in the composition express the chimeric receptor, in which the chimeric receptor contains an intracellular region containing a CD3zeta (CD3ζ) signaling domain and in which the CD3ζ signaling domain or at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus (the genomic locus encoding CD3ζ) of the engineered cell such as a T cell. In some examples, at least a portion of the CD3ζ signaling domain is encoded by the genomic sequences at the endogenous *CD247* locus. In some examples, the entire or full CD3ζ signaling domain of the intracellular region of the chimeric receptor is encoded by the genomic sequences at the endogenous *CD247* locus.

[0097] In some of any examples, the composition comprises CD4⁺ and/or CD8⁺ T cells. In some of any examples, the composition comprises CD4⁺ and CD8⁺ T cells and the ratio of CD4⁺ to CD8⁺ T cells is from or from about 1:3 to 3:1, such as 1:1.

[0098] In some of any examples, cells expressing the chimeric receptor make up at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more of the total cells in the composition or of the total CD4⁺ or CD8⁺ cells in the composition.

[0099] Also provided herein are methods of treatment involving administering the engineered cell, plurality of engineered cells or composition of any of the examples provided herein to a subject having a disease or disorder.

[0100] Also provided herein are uses of any of the engineered cell, plurality of engineered cells or composition described herein for the treatment of a disease or disorder. In provided examples, the chimeric receptor expressed by the engineered cell is directed to or targets an antigen associated with or expressed on a cell or tissue of the disease or condition.

[0100a] In one aspect, the present disclosure refers to the genetically engineered T cell, plurality of genetically engineered T cells, or composition disclosed herein, for use in the treatment of a disease or disorder.

[0101] Also provided herein are uses of any of the engineered cell, plurality of engineered cells or composition described herein in the manufacture of a medicament for treating a disease or disorder. In provided examples, the chimeric receptor expressed by the engineered cell is directed to or targets an antigen associated with or expressed on a cell or tissue of the disease or condition.

[0101a] In one aspect, the present disclosure refers to use of the genetically engineered T cell, plurality of genetically engineered T cells or composition disclosed herein, in the manufacture of a medicament for treating a disease or disorder.

[0102] Also provided are any of the engineered cell, plurality of engineered cells or compositions from any of the examples provided herein for use in the treatment of a disease or disorder. In provided examples, the chimeric receptor expressed by the engineered cell is directed to or targets an antigen associated with or expressed on a cell or tissue of the disease or condition.

[0103] In some of any examples, the disease or disorder is a cancer or a tumor. In some of any examples, the cancer or the tumor is a hematologic malignancy. In some of any examples, the hematological malignancy is a lymphoma, a leukemia, or a plasma cell malignancy. In some of any examples, the cancer is a lymphoma and the lymphoma is Burkitt's lymphoma, non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, Waldenstrom macroglobulinemia, follicular lymphoma, small non-cleaved cell lymphoma, mucosa-associated lymphatic tissue lymphoma (MALT), marginal zone lymphoma, splenic lymphoma, nodal monocytoid B cell lymphoma, immunoblastic lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, primary mediastinal B cell lymphoma, lymphoplasmacytic lymphoma (LPL), or mantle cell lymphoma (MCL). In some of any examples, the cancer is a leukemia and the leukemia is chronic lymphocytic leukemia (CLL), plasma cell leukemia or acute lymphocytic leukemia (ALL). In some of any examples, the cancer is a plasma cell malignancy and the plasma cell malignancy is multiple myeloma (MM).

[0104] In some of any examples, the tumor is a solid tumor. In some of any examples, the solid tumor is a non-small cell lung cancer (NSCLC) or a head and neck squamous cell carcinoma (HNSCC).

[0105] Also provided are kits. In some of any examples, the kits include one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and the polynucleotide of any of the examples provided herein.

[0105a] In one aspect, the present disclosure refers to a kit comprising: one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and the polynucleotide disclosed herein, or the viral vector disclosed herein.

[0106] Also provided are kits that include one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and a polynucleotide comprising a nucleic acid sequence encoding chimeric receptor or a portion thereof, wherein the transgene encoding the chimeric receptor or antigen-binding fragment or chain thereof is targeted for integration at or near the target site via homology directed repair (HDR); and instructions for carrying out the method of any of the examples provided herein.

[0106a] In one aspect, the present disclosure refers to a kit, comprising: one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and a polynucleotide comprising a nucleic acid sequence encoding a chimeric receptor or a portion thereof, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is targeted for integration at or near the target site via homology directed repair (HDR); and instructions for carrying out the method disclosed herein.

Brief Description of the Drawings

[0107] **FIG. 1** depicts surface expression of CD3 and TCR, as assessed by flow cytometry, in T cells that were electroporated with ribonucleoprotein (RNP) complexes containing one of four *CD247*-targeting gRNAs (gRNA 1, 2, 3, 4), for introducing a genetic disruption at the endogenous *CD247* locus by CRISPR/Cas9-mediated gene editing, or T cells subject to a mock electroporation that did not contain a gRNA (mock) as control.

Claims

1. A genetically engineered T cell, comprising a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3zeta (CD3 ζ) signaling domain.
2. The genetically engineered T cell of claim 1, wherein the modified *CD247* locus is an endogenous *CD247* locus, and wherein the nucleic acid sequence comprises a transgene sequence encoding a portion of the chimeric receptor, the transgene sequence having been integrated at the endogenous *CD247* locus of a T cell, optionally via homology directed repair (HDR).
3. The genetically engineered T cell of claim 2, wherein the entire CD3 ζ signaling domain or a fragment of the CD3 ζ signaling domain is encoded by an open reading frame or a partial sequence thereof of the endogenous *CD247* locus.
4. The genetically engineered T cell of any of claim 2 or claim 3, wherein the nucleic acid sequence encoding the chimeric receptor comprises an in-frame fusion of (i) the transgene sequence encoding a portion of the chimeric receptor and (ii) an open reading frame or a partial sequence thereof of the endogenous *CD247* locus.
5. The genetically engineered T cell of any of claims 2-4, wherein the transgene sequence is in-frame with one or more exons of the open reading frame or partial sequence thereof of the endogenous *CD247* locus.
6. The genetically engineered T cell of any of claims 2-5, wherein the transgene sequence does not comprise a sequence encoding a 3' UTR and/or does not comprise an intron.
7. The genetically engineered T cell of any of claims 2-6, wherein:
the transgene sequence encodes a fragment of the CD3 ζ signaling domain or does not encode the CD3 ζ signaling domain or a fragment thereof.
8. The genetically engineered T cell of any of claims 3-7, wherein the open reading frame or a partial sequence thereof comprises at least one intron and at least one exon of the endogenous *CD247* locus, and/or encodes a 3' UTR of the endogenous *CD247* locus.
9. The genetically engineered T cell of any of claims 2-8, wherein the transgene sequence is downstream of exon 1 and upstream of exon 8 of the open reading frame of the endogenous *CD247*

locus; optionally downstream of exon 1 and upstream of exon 3 of the open reading frame of the endogenous *CD247* locus.

10. The genetically engineered T cell of any of claims 2-9, wherein at least a fragment of the CD3 ζ signaling domain, optionally the entire CD3 ζ signaling domain, of the encoded chimeric receptor is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof, optionally wherein the CD3 ζ signaling domain is encoded by a sequence of nucleotides comprising at least a portion of exon 2 and exons 3-8 of the open reading frame of the endogenous *CD247* locus; or a sequence of nucleotides that does not comprise exon 1, does not comprise the full length of exon 1 and/or does not comprise the full length of exon 2 of the open reading frame of the endogenous *CD247* locus.

11. The genetically engineered T cell of any of claims 1-10, wherein the encoded chimeric receptor is capable of signaling via the CD3 ζ signaling domain.

12. The genetically engineered T cell of any of claims 1-11, wherein the encoded CD3 ζ signaling domain comprises the sequence selected from any one of SEQ ID NOS:13-15, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:13-15, or a fragment thereof.

13. The genetically engineered T cell of any of claims 1-12, wherein the chimeric receptor is a chimeric antigen receptor (CAR).

14. The genetically engineered T cell of any of claims 1-13, wherein the chimeric receptor comprises (i) an extracellular region comprising a binding domain, (ii) a transmembrane domain, and (iii) an intracellular region, optionally wherein the binding domain is or comprises an antibody or an antigen-binding fragment thereof.

15. The genetically engineered T cell of claim 14, wherein the binding domain is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder or condition, optionally wherein the target antigen is a tumor antigen, and/or wherein the target antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III

epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G protein-coupled receptor class C group 5 member D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV, or other pathogens.

16. The genetically engineered T cell of claim 14 or 15, wherein the extracellular region comprises a spacer, optionally wherein the spacer is operably linked between the binding domain and the transmembrane domain, and/or the spacer comprises an immunoglobulin hinge region and/or a C_H2 region and a C_H3 region.

17. The genetically engineered T cell of any of claims 1-16, wherein the intracellular region comprises one or more costimulatory signaling domain(s), optionally wherein the one or more costimulatory signaling domain comprises an intracellular signaling domain of a CD28, a 4-1BB or an ICOS or a signaling portion thereof.

18. The genetically engineered T cell of any of claims 2-17, wherein:
the transgene sequence comprises a sequence of nucleotides encoding, in order: (i) a binding

domain, optionally a single chain Fv fragment (scFv); (ii) a spacer, optionally comprising a sequence from a human immunoglobulin hinge, optionally from IgG1, IgG2, IgG4, or a modified version thereof, optionally further comprising a C_H2 region and/or a C_H3 region; (iii) a transmembrane domain, optionally from human CD28; and (iv) an intracellular region comprising a costimulatory signaling domain, optionally from human 4-1BB; and/or

the modified *CD247* locus comprises a sequence of nucleotides encoding, in order: (i) a binding domain, optionally scFv; (ii) a spacer, optionally comprising a sequence from a human immunoglobulin hinge, optionally from IgG1, IgG2, IgG4, or a modified version thereof, optionally further comprising a C_H2 region and/or a C_H3 region; (iii) a transmembrane domain, optionally from human CD28; and (iv) an intracellular region comprising a costimulatory signaling domain, optionally from human 4-1BB; and the CD3ζ signaling domain.

19. The genetically engineered T cell of any of claims 2-18, wherein the transgene sequence comprises a sequence of nucleotides encoding at least one further protein, optionally wherein the at least one further protein is a surrogate marker, optionally wherein the surrogate marker is a truncated receptor, optionally wherein the truncated receptor lacks an intracellular signaling domain and/or is not capable of mediating intracellular signaling when bound by its ligand.

20. The genetically engineered T cell of any of claims 2-19, wherein the transgene sequence comprises one or more multicistronic element(s), optionally wherein the one or more multicistronic element is or comprises a ribosome skip sequence, optionally wherein the ribosome skip sequence is a T2A, a P2A, an E2A, or an F2A element.

21. The genetically engineered T cell of claim 20, wherein:
the transgene sequence comprises a sequence of nucleotides encoding a portion of the chimeric receptor, and the one or more multicistronic element(s) are positioned upstream of the sequence of nucleotides encoding the portion of the chimeric receptor; and/or positioned between the sequence of nucleotides encoding the portion of the chimeric receptor and the sequence of nucleotides encoding the at least one further protein; and/or

the chimeric receptor is a CAR that is a multi-chain CAR and the one or more multicistronic element(s) are positioned between a sequence of nucleotides encoding one chain of a multi-chain CAR and a sequence of nucleotides encoding another chain of the multi-chain CAR.

22. The genetically engineered T cell of any of claims 2-21, wherein the modified *CD247* locus comprises a promoter and/or regulatory or control element of the endogenous *CD247* locus operably linked to control expression of the nucleic acid sequence encoding the chimeric receptor; or

wherein the modified *CD247* locus comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the chimeric receptor or a portion thereof.

23. The genetically engineered T cell of any of claims 1-22, wherein the T cell is a primary T cell derived from a subject, optionally wherein the subject is a human.

24. The genetically engineered T cell of any of claims 1-23, wherein the T cell is a CD8⁺ T cell or subtype thereof, or a CD4⁺ T cell or subtype thereof.

25. A polynucleotide, comprising:
(a) a nucleic acid sequence encoding a chimeric receptor or a portion thereof; and
(b) one or more homology arm(s) linked to the nucleic acid sequence, wherein the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a *CD247* locus or a partial sequence thereof.

26. The polynucleotide of claim 25, wherein the nucleic acid sequence of (a) encodes a portion of a chimeric receptor, wherein the portion of the chimeric receptor encoded by the nucleic acid sequence comprises (i) an extracellular region comprising a binding domain and (ii) a transmembrane domain, and wherein the nucleic acid sequence of (a) does not encode the entire CD3zeta (CD3ζ) signaling domain of an intracellular region.

27. The polynucleotide of claim 25 or 26, wherein the nucleic acid sequence of (a) is a sequence that is exogenous or heterologous to an open reading frame of the endogenous genomic *CD247* locus of a T cell, optionally a human T cell.

28. The polynucleotide of any of claims 25-27, wherein the open reading frame or a partial sequence thereof comprises at least one intron and at least one exon and/or a 3' UTR of the endogenous *CD247* locus of a T cell, optionally a human T cell.

29. The polynucleotide of any of claims 25-28, wherein at least a fragment of the CD3ζ signaling domain, optionally the entire CD3ζ signaling domain, is encoded by the open reading frame of the endogenous *CD247* locus or a partial sequence thereof, when a chimeric receptor is expressed from a cell introduced with the polynucleotide.

30. The polynucleotide of any of claims 25-29, wherein the nucleic acid sequence of (a) encodes a fragment of the CD3ζ signaling domain.

31. The polynucleotide of any of claims 25-29, wherein the nucleic acid sequence of (a) does not encode the CD3 ζ signaling domain or a fragment thereof.

32. The polynucleotide of any of claims 25-31, wherein the nucleic acid sequence of (a) does not comprise a sequence encoding a 3' UTR; and/or does not comprise an intron.

33. The polynucleotide of any of claims 25-32, wherein the nucleic acid sequence of (a) comprises a sequence of nucleotides that is in-frame with one or more exons of the open reading frame or a partial sequence thereof of the *CD247* locus comprised in the one or more homology arm(s), optionally wherein the one or more region(s) of the open reading frame is or comprises sequences that are upstream of exon 8 of the open reading frame of the *CD247* locus; sequences that are upstream of exon 3 of the open reading frame of the *CD247* locus, optionally sequences that include exon 3 of the open reading frame of the *CD247* locus; and/or sequences that include at least a portion of exon 2 of the open reading frame of the *CD247* locus.

34. The polynucleotide of any of claims 25-33, wherein the one or more homology arm(s) does not comprise exon 1, does not comprise the full length of exon 1, and/or does not comprise the full length of exon 2 of the open reading frame of the endogenous *CD247* locus.

35. The polynucleotide of any of claims 25-34, wherein the one or more homology arm comprises a 5' homology arm and a 3' homology arm and the polynucleotide comprises the structure [5' homology arm]-[nucleic acid sequence of (a)]-[3' homology arm], optionally wherein the 5' homology arm and the 3' homology arm independently are at or about 200, 300, 400, 500, 600, 700 or 800 nucleotides in length, or any value between any of the foregoing, or are greater than at or about 300 nucleotides in length, optionally at or about 400, 500 or 600 nucleotides in length, or any value between any of the foregoing.

36. The polynucleotide of any of claims 25-35, wherein the 5' homology arm comprises the sequence set forth in SEQ ID NO:80, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:80 or a partial sequence thereof; and/or the 3' homology arm comprises the sequence set forth in SEQ ID NO:81, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:81 or a partial sequence thereof.

37. The polynucleotide of any of claims 25-36, wherein the chimeric receptor is a chimeric antigen receptor (CAR).

38. The polynucleotide of any of claims 26-37, wherein the binding domain comprises an antibody or an antigen-binding fragment thereof.

39. The polynucleotide of any of claims 26-38, wherein the binding domain is capable of binding to a target antigen that is associated with, specific to, and/or expressed on a cell or tissue of a disease, disorder, or condition, optionally wherein the target antigen is a tumor antigen, and/or wherein the target antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G protein-coupled receptor class C group 5 member D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R $\alpha 2$), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV, or other pathogens.

40. The polynucleotide of any of claims 25-39, wherein the nucleic acid sequence of (a)

comprises a sequence of nucleotides encoding at least one further protein, optionally wherein the at least one further protein is a surrogate marker, optionally wherein the surrogate marker is a truncated receptor, optionally wherein the truncated receptor lacks an intracellular signaling domain and/or is not capable of mediating intracellular signaling when bound by its ligand.

41. The polynucleotide of any of claims 25-40, wherein the nucleic acid sequence of (a) comprises one or more multicistronic element(s), optionally wherein the one or more multicistronic element is or comprises a ribosome skip sequence, optionally wherein the ribosome skip sequence is a T2A, a P2A, an E2A, or an F2A element.

42. The polynucleotide of claim 41, wherein:
the nucleic acid of (a) comprises a sequence of nucleotides encoding a portion of the chimeric receptor, and the one or more multicistronic element(s) are positioned upstream of the sequence of nucleotides encoding the portion of the chimeric receptor; and/or positioned between the sequence of nucleotides encoding the portion of the chimeric receptor and the sequence of nucleotides encoding the at least one further protein; and/or

the chimeric receptor is a CAR that is a multi-chain CAR and the one or more multicistronic element(s) are positioned between a sequence of nucleotides encoding one chain of a multi-chain CAR and a sequence of nucleotides encoding another chain of the multi-chain CAR.

43. The polynucleotide of any of claims 25-42, wherein the nucleic acid sequence of (a) comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the chimeric receptor or a portion thereof.

44. The polynucleotide of any of claims 25-44, wherein the polynucleotide is a linear polynucleotide, optionally a double-stranded polynucleotide or a single-stranded polynucleotide, optionally wherein the polynucleotide is between at or about 2500 and at or about 5000 nucleotides, at or about 3500 and at or about 4500 nucleotides, or at or about 3750 nucleotides and at or about 4250 nucleotides in length.

45. A viral vector comprising the polynucleotide of any of claims 25-44, optionally wherein the viral vector is an AAV vector, optionally wherein the AAV vector is an AAV2 or AAV6 vector; or wherein the viral vector is a retroviral vector, optionally a lentiviral vector.

46. A method of producing a genetically engineered T cell, the method comprising introducing the polynucleotide of any of claims 25-44, or the viral vector of claim 45, into a T cell under

conditions to induce homology directed repair (HDR).

47. The method of claim 46, wherein the T cell comprises a genetic disruption at a *CD247* locus.

48. A method of producing a genetically engineered T cell, the method comprising:
(a) introducing, into a T cell, one or more agent(s) capable of inducing a genetic disruption at a target site within an endogenous *CD247* locus of the T cell; and
(b) introducing the polynucleotide of any of claims 25-44, or the viral vector of claim 45, into the T cell,
wherein steps (a) and (b) are simultaneous or sequential, in any order.

49. The method of claim 48, wherein the polynucleotide is introduced after the introduction of the one or more agent(s).

50. The method of any of claims 46-49, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is integrated within the endogenous *CD247* locus via homology directed repair (HDR).

51. A method of producing a genetically engineered T cell, the method comprising introducing, into a T cell, a polynucleotide comprising a nucleic acid sequence encoding a chimeric receptor or a portion thereof, said T cell having a genetic disruption within a *CD247* locus of the T cell, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is integrated within the endogenous *CD247* locus via homology directed repair (HDR).

52. The method of any of claims 46-51, wherein the method produces a modified *CD247* locus, said modified *CD247* locus comprising a nucleic acid sequence encoding a chimeric receptor comprising an intracellular region comprising a CD3 ζ signaling domain, wherein at least a fragment of the CD3 ζ signaling domain is encoded by an open reading frame of the endogenous *CD247* locus.

53. The method of any of claims 51-52, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof does not comprise a sequence encoding a 3' UTR and/or does not comprise an intron.

54. The method of any of claims 52-53, wherein, when expressed by a cell introduced with the polynucleotide, the chimeric receptor is capable of signaling via the CD3 ζ signaling domain.

55. The method of any of claims 52-54, wherein the encoded CD3 ζ signaling domain comprises the sequence selected from any one of SEQ ID NOS:13-15, or a sequence that exhibits at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:13-15, or a fragment thereof.

56. The method of any of claims 53-55, wherein the one or more homology arm comprises a 5' homology arm and a 3' homology arm, and the polynucleotide comprises the structure [5' homology arm]-[nucleic acid sequence encoding a chimeric receptor or a portion thereof]-[3' homology arm].

57. The method of any of claims 48 and 51-56, wherein the one or more agent(s) capable of inducing a genetic disruption comprises a DNA binding protein or DNA-binding nucleic acid that specifically binds to or hybridizes to the target site, a fusion protein comprising a DNA-targeting protein and a nuclease, or an RNA-guided nuclease, optionally wherein the one or more agent(s) comprises a zinc finger nuclease (ZFN), a TAL-effector nuclease (TALEN), and/or a CRISPR-Cas9 combination that specifically binds to, recognizes, or hybridizes to the target site, optionally wherein each of the one or more agent(s) comprises a guide RNA (gRNA) having a targeting domain that is complementary to the at least one target site.

58. The method of claim 57, wherein the one or more agent(s) is introduced as a ribonucleoprotein (RNP) complex comprising the gRNA and a Cas9 protein, optionally wherein the RNP is introduced via electroporation, particle gun, calcium phosphate transfection, cell compression or squeezing.

59. The method of claim 57 or 58, wherein the gRNA has a targeting domain sequence selected from CACCUUCACUCUCAGGAACA (SEQ ID NO:87); GAAUGACACCAUAGAUGAAG (SEQ ID NO:88); UGAAGAGGAUCCAUCAGC (SEQ ID NO:89); and UCCAGCAGGUAGCAGAGUUU (SEQ ID NO:90).

60. The method of any of claims 57-59, wherein the gRNA has a targeting domain sequence of CACCUUCACUCUCAGGAACA (SEQ ID NO:87).

61. The method of any of claims 57-59, wherein the gRNA has a targeting domain sequence of UGAAGAGGAUCCAUCAGC (SEQ ID NO:89).

62. The method of any of claims 46-61, wherein the T cell is a primary T cell derived from a subject, optionally wherein the subject is a human.

63. The method of any of claims 46-62, wherein the T cell is a CD8⁺ T cell or subtype thereof, or a CD4⁺ T cell or subtype thereof.

64. The method of any of claims 46-63, wherein the polynucleotide is comprised in a viral vector, optionally wherein the viral vector is an AAV vector, optionally wherein the AAV vector is an AAV2 or AAV6 vector; or wherein the viral vector is a retroviral vector, optionally a lentiviral vector.

65. The method of any of claims 46-63, wherein the polynucleotide is a linear polynucleotide, optionally a double-stranded polynucleotide or a single-stranded polynucleotide.

66. The method of any of claims 48 and 51-65, wherein the polynucleotide is introduced after the introduction of the one or more agent(s), optionally wherein the polynucleotide is introduced immediately after, or within about 30 seconds, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours or 4 hours after the introduction of the agent.

67. The method of any of claims 48 and 51-66, wherein prior to the introducing of the one or more agent, the method comprises incubating the cells, in vitro with one or more stimulatory agent(s) under conditions to stimulate or activate the one or more immune cells, optionally wherein the one or more stimulatory agent(s) comprises anti-CD3 and/or anti-CD28 antibodies, optionally anti-CD3/anti-CD28 beads, optionally wherein the bead to cell ratio is or is about 1:1.

68. The method of any of claims 46-67, wherein at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells generated by the method comprise a genetic disruption of at least one target site within a *CD247* locus; and/or at least or greater than 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 90% of the cells in a plurality of engineered cells generated by the method express the chimeric receptor.

69. A genetically engineered T cell or a plurality of genetically engineered T cells generated using the method of any of claims 46-68.

70. A composition, comprising the genetically engineered T cell any of claims 1-24 and 69; or a plurality of the genetically engineered T cell any of claims 1-24 and 69.

71. The composition of claim 70, wherein the composition comprises CD4⁺ T cells and/or CD8⁺ T cells, optionally wherein the composition comprises CD4⁺ T cells and CD8⁺ T cells and the

ratio of CD4⁺ to CD8⁺ T cells is from or from about 1:3 to 3:1, optionally 1:1.

72. The composition of claim 70 or 71, wherein cells expressing the chimeric receptor make up at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, of the total cells in the composition or of the total CD4⁺ T cells or CD8⁺ T cells in the composition.

73. The genetically engineered T cell, plurality of genetically engineered T cells, or composition of any of claims 1-24 and 70-72, for use in the treatment of a disease or disorder.

74. Use of the genetically engineered T cell, plurality of genetically engineered T cells or composition of any of claims 1-24 and 70-72, in the manufacture of a medicament for treating a disease or disorder.

75. The genetically engineered T cell, plurality of genetically engineered T cells or composition for use of claim 73 or the use of claim 74, wherein the disease or disorder is a cancer or a tumor, optionally wherein the cancer or the tumor is a hematologic malignancy, optionally a lymphoma, a leukemia, or a plasma cell malignancy, or optionally wherein the cancer or the tumor is a solid tumor, optionally wherein the solid tumor is a non-small cell lung cancer (NSCLC) or a head and neck squamous cell carcinoma (HNSCC).

76. A kit comprising:
one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and
the polynucleotide of any of claims 25-44, or the viral vector of claim 45.

77. A kit, comprising:
one or more agent(s) capable of inducing a genetic disruption at a target site within a *CD247* locus; and
a polynucleotide comprising a nucleic acid sequence encoding a chimeric receptor or a portion thereof, wherein the nucleic acid sequence encoding the chimeric receptor or a portion thereof is targeted for integration at or near the target site via homology directed repair (HDR); and
instructions for carrying out the method of any of claims 46-68.