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 transforming growth factor-beta receptor type-2 (*TGFBR2*) locus. In some of any examples, the modified *TGFBR2* locus comprises a transgene sequence encoding a recombinant receptor or a portion thereof.

[0006] Provided herein are genetically engineered T cells that contain a modified transforming growth factor-beta receptor type-2 (*TGFBR2*) locus, said modified *TGFBR2* locus comprising a transgene sequence encoding a recombinant receptor or a portion thereof. In some of any examples, the transgene sequence has been integrated at the endogenous *TGFBR2* locus. In some of any examples, the integration is via homology directed repair (HDR).

[0006a] In one aspect, the present disclosure refers to a genetically engineered T cell, comprising a modified transforming growth factor-beta receptor type-2 (*TGFBR2*) locus, said modified *TGFBR2* locus comprising a transgene sequence encoding a recombinant receptor or portion thereof.

[0007] In some of any examples, the modified *TGFBR2* locus does not encode a functional TGFBRII polypeptide. In some of any examples, the modified *TGFBR2* locus does not encode a TGFBRII polypeptide or the expression of TGFBRII polypeptide is eliminated. In some of any examples, the modified *TGFBR2* locus does not encode a full length TGFBRII polypeptide or encodes a partial TGFBRII polypeptide. In some of any examples, the modified *TGFBR2* locus encodes a dominant negative TGFBRII polypeptide. In some of any examples, the encoded TGFBRII polypeptide comprises an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60 In some of any examples, the encoded TGFBRII polypeptide comprises 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 an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60 or a fragment thereof. In some of any examples, the transgene sequence is in-frame with one or more exons of an open reading frame or partial sequence thereof of the endogenous *TGFBR2* locus.

[0008] In some of any examples, the transgene sequence is downstream of exon 1 and upstream of exon 6 of the open reading frame of the endogenous *TGFBR2* locus. In some of any examples, the transgene sequence is downstream of exon 4 and upstream of exon 6 of the open reading frame of the endogenous *TGFBR2* locus.

[0009] In some of any examples, the recombinant receptor is or comprises recombinant T cell receptor (TCR). In some of any examples, the recombinant receptor is a recombinant TCR and the transgene sequence encodes a TCR alpha (TCRα) chain, a TCR beta (TCRβ) chain or both. In some of any examples, the recombinant receptor is a functional non-T cell receptor (non-TCR) antigen receptor. In some of any examples, the recombinant receptor comprises a functional non-T cell receptor (non-TCR) antigen receptor. In some of any examples, the recombinant receptor is a chimeric antigen receptor (CAR). In some of any examples, the CAR comprises an extracellular region, a transmembrane domain, and an 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. In some of any examples, the binding domain is capable of binding to a target antigen that is associated with, specific to, or expressed on a cell or tissue of a disease, disorder or condition.

[0010] In some of any examples, the target antigen is a tumor antigen. In some of any examples, the target antigen is selected from among αvβ6 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-22Rα), IL-13 receptor alpha 2 (IL-13Rα2), 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.

[0011] 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. In some of any examples, the intracellular region comprises an intracellular signaling domain. In some of any examples, the intracellular signaling domain is an intracellular signaling domain of a CD3 chain, such as a CD3-zeta (CD3ζ) chain, or a signaling portion thereof. In some of any examples, the intracellular signaling domain comprises an intracellular signaling domain of a CD3 chain, such as a CD3-zeta (CD3ζ) chain, or a signaling portion thereof. In some of any examples, the intracellular region 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 of any examples, the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0012] In some of any examples, the modified *TGFBR2* locus encodes a recombinant 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.

[0013] 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; and an intracellular signaling region. In some of any examples, the modified *TGFBR2* locus comprises in order: a sequence of nucleotides encoding an extracellular binding domain; a spacer; and a transmembrane domain; a costimulatory signaling domain; and an intracellular signaling region.

[0014] 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 comprises a sequence from a human immunoglobulin hinge, that is an IgG1, IgG2 or IgG4 or a modified version thereof, that further comprises 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; and an intracellular signaling region, that is a CD3ζ chain or a portion thereof. In some of any examples, the modified *TGFBR2* locus comprises in order: a sequence of nucleotides encoding an extracellular binding domain, that is an scFv; a spacer, that comprises a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, that further comprises 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; and an intracellular signaling region, that is a CD3ζ chain or a portion thereof.

[0015] In some of any examples, the CAR is a multi-chain CAR. In some of any examples, the transgene sequence comprises a sequence of nucleotides encoding at least one further protein.

[0016] In some of any examples, the transgene sequence comprises one or more multicistronic element(s). In some of any examples, the one or more multicistronic element is positioned between

the sequence of nucleotides encoding the CAR 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 is not capable of mediating intracellular signaling when bound by its ligand. In some of any examples, the truncated receptor lacks an intracellular signaling domain or is not capable of mediating intracellular signaling when bound by its ligand.

[0017] In some of any examples, the recombinant receptor is a recombinant TCR, and a multicistronic element is positioned between a sequence of nucleotides encoding the TCRα and a sequence of nucleotides encoding the TCRβ.

[0018] In some of any examples, the recombinant 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.

[0019] In some of any examples, the one or more multicistronic element(s) are upstream of the sequence of nucleotides encoding the recombinant receptor.

[0020] 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.

[0021] In some of any examples, the modified *TGFBR2* locus comprises the promoter and regulatory or control element of the endogenous *TGFBR2* locus operably linked to control expression the nucleic acid sequence encoding the recombinant receptor. In some of any examples, the modified *TGFBR2* locus comprises the promoter or regulatory or control element of the endogenous *TGFBR2* locus operably linked to control expression the nucleic acid sequence encoding the recombinant 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 recombinant receptor. In some of any examples, the one or more heterologous regulatory or control element comprises a heterologous promoter, an enhancer, an intron, a polyadenylation signal, a Kozak consensus sequence, a splice acceptor sequence or a splice donor sequence. In some of any examples, the heterologous promoter is or comprises a human elongation factor 1 alpha (EF1α) promoter or an MND promoter or a variant thereof.

[0022] 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 T cell is derived from a multipotent or pluripotent cell, which is an iPSC.

[0023] Provided herein are polynucleotides, comprising a nucleic acid sequence encoding a

recombinant receptor or a portion thereof; and one or more homology arm(s) linked to the nucleic acid sequence. In some of any examples, the one or more homology arm(s) comprise a sequence homologous to one or more region(s) of an open reading frame of a transforming growth factor-beta receptor type-2 (*TGFBR2*) locus. In some of any examples, the recombinant receptor or a portion thereof is encoded by a modified *TGFBR2* locus comprising the nucleic acid sequence encoding the recombinant receptor or a portion thereof when the recombinant receptor is expressed from a cell introduced with the polynucleotide. In some of any examples, the nucleic acid sequence is a sequence that is exogenous or heterologous to an open reading frame of the endogenous genomic *TGFBR2* locus a T cell. In some of any examples, the nucleic acid sequence is a sequence that is exogenous or heterologous to an open reading frame of the endogenous genomic *TGFBR2* locus a T cell, which is a human T cell.

[0023a] In one aspect, the present disclosure refers to a polynucleotide, comprising: (a) a nucleic acid sequence encoding a recombinant receptor or 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 transforming growth factor-beta receptor type-2 (*TGFBR2*) locus.

[0024] In some of any examples, the one or more homology arm(s) comprise at least one intron or at least one exon of the open reading frame of the *TGFBR2* locus. In some of any examples, the modified *TGFBR2* locus does not encode a functional TGFBRII polypeptide, in a cell introduced with the polynucleotide. In some of any examples, the modified *TGFBR2* locus does not encode a TGFBRII polypeptide or the expression of TGFBRII polypeptide is eliminated, in a cell introduced with the polynucleotide.

[0025] In some of any examples, the modified *TGFBR2* locus does not encode a full length TGFBRII polypeptide or encodes a partial TGFBRII polypeptide, in a cell introduced with the polynucleotide. In some of any examples, the modified *TGFBR2* locus encodes a dominant negative TGFBRII polypeptide, in a cell introduced with the polynucleotide. In some of any examples, the encoded TGFBRII polypeptide in a cell introduced with the polynucleotide comprises an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60 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 an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60 or a fragment thereof. In some of any examples, the nucleic acid sequence is in-frame with one or more exons of the open reading frame of the *TGFBR2* locus comprised in the one or more homology arm(s).

[0026] In some of any examples, the one or more region(s) of the open reading frame is or comprises sequences that are downstream of exon 1 of the open reading frame of the endogenous *TGFBR2* 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 4 or downstream of exon 4 of the open reading frame of the *TGFBR2* locus.

[0027] 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]. 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.

[0028] In some of any examples, the 5' homology arm comprises the sequence set forth in SEQ ID NOS: 69-71 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 NOS: 69-71 or a partial sequence thereof. In some of any examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:72, 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:72 or a partial sequence thereof.

[0029] In some of any examples, the encoded recombinant receptor is or comprises recombinant T cell receptor (TCR). In some of any examples, the encoded recombinant receptor is a recombinant TCR and the nucleic acid sequence in (a) encodes a TCR alpha (TCRα) chain, a TCR beta (TCRβ) chain or both.

[0030] In some of any examples, the encoded recombinant receptor is a functional non-T cell receptor (non-TCR) antigen receptor. In some of any examples, the encoded recombinant receptor comprises a functional non-T cell receptor (non-TCR) antigen receptor. In some of any examples, the encoded recombinant receptor is a chimeric antigen receptor (CAR).

[0031] In some of any examples, the CAR comprises an extracellular region, a transmembrane domain, and an 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. In some of any examples, the binding domain is capable of binding to a target antigen that is associated with, specific to, or expressed on a cell or tissue of a disease, disorder or condition.

[0032] In some of any examples, the target antigen is a tumor antigen. In some of any examples, the target antigen is selected from among αvβ6 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-22Rα), IL-13 receptor alpha 2 (IL-13Rα2), 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.

[0033] In some of any examples, the extracellular region comprises a spacer. In some of any examples, the extracellular region comprises a spacer which 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. In some of any examples, the intracellular region comprises an intracellular signaling domain. In some of any examples, the intracellular signaling domain is an intracellular signaling domain of a CD3 chain. In some of any examples, the intracellular signaling domain is an intracellular signaling domain of a CD3 chain, which is a CD3-zeta (CD3ζ) chain, or a signaling portion thereof. In some of any examples, the intracellular signaling domain comprises an intracellular signaling domain of a CD3 chain. In some of any examples, the intracellular signaling domain comprises an intracellular signaling domain of a CD3 chain, which is a CD3-zeta (CD3ζ) chain, or a signaling portion thereof. In some of any examples, the intracellular region 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 of any examples, the costimulatory signaling region comprises an intracellular signaling domain of 4-1BB.

[0034] In some of any examples, the modified *TGFBR2* locus encodes a recombinant 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 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; and an intracellular signaling region.

[0035] 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 comprises a sequence from a human immunoglobulin hinge, that is from IgG1, IgG2 or IgG4 or a modified version thereof, that further comprises 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; and an intracellular signaling region, that is a CD3ζ chain or a portion thereof.

[0036] In some of any examples, the CAR is a multi-chain CAR. In some of any examples, the nucleic acid sequence comprises a sequence of nucleotides encoding at least one further protein.

[0037] In some of any examples, the nucleic acid sequence comprises one or more multicistronic element(s). In some of any examples, the one or more multicistronic element is positioned between the sequence of nucleotides encoding the CAR and the sequence of nucleotides encoding the at least one further protein.

[0038] In some of any examples, the at least one further protein is a surrogate marker. In some of any examples, the at least one further protein is a surrogate marker which is a truncated receptor. In some of any examples, the at least one further protein is a surrogate marker which is a truncated receptor which lacks an intracellular signaling domain and is not capable of mediating intracellular signaling when bound by its ligand. In some of any examples, the at least one further protein is a

surrogate marker which is a truncated receptor which lacks an intracellular signaling domain or is not capable of mediating intracellular signaling when bound by its ligand.

[0039] In some of any examples, the recombinant receptor is a recombinant TCR, and a multicistronic element is positioned between a sequence of nucleotides encoding the TCRα and a sequence of nucleotides encoding the TCRβ.

[0040] In some of any examples, the recombinant 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.

[0041] In some of any examples, the one or more multicistronic element(s) are upstream of the sequence of nucleotides encoding the recombinant 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 one or more multicistronic element is or comprises a ribosome skip sequence which is a T2A, a P2A, an E2A, or an F2A element.

[0042] In some of any examples, the nucleic acid sequence comprises one or more heterologous or regulatory control element(s) operably linked to control expression of the recombinant receptor when expressed from a cell introduced with the polynucleotide. In some of any examples, the one or more heterologous regulatory or control element comprises a heterologous 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 (EF1α) promoter or an MND promoter or a variant thereof.

[0043] 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 is a retroviral vector which is a lentiviral vector.

[0044] In some of any examples, the polynucleotide is a linear polynucleotide. In some of any examples, the polynucleotide is a linear polynucleotide, which is a double-stranded polynucleotide or a single-stranded polynucleotide. 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.

[0045] Provided herein are methods of producing a genetically engineered T cell, the method involving introducing any of the provided polynucleotides into a T cell comprising a genetic disruption at

a *TGFBR2* locus.

[0045a] 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 into a T cell under conditions to induce homology directed repair (HDR), comprising a genetic disruption at a *TGFBR2* locus.

[0046] Provided herein are methods of producing a genetically engineered T cell, the method involving introducing, into a T cell, one or more agent(s) capable of inducing a genetic disruption at a target site within an endogenous *TGFBR2* locus of the T cell; and introducing the polynucleotide into a T cell comprising a genetic disruption at a *TGFBR2* locus, wherein the method produces a modified *TGFBR2* locus, said modified *TGFBR2* locus comprising a nucleic acid sequence encoding the recombinant receptor or a portion thereof. In some of any examples, the nucleic acid sequence encoding a recombinant receptor or a portion thereof is integrated within the endogenous *TGFBR2* locus via homology directed repair (HDR).

[0046a] 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 *TGFBR2* locus of the T cell; and (b) introducing the polynucleotide disclosed herein into the T cell, wherein steps (a) and (b) are simultaneous or sequential, in any order.

[0047] 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 recombinant receptor or a portion thereof, said T cell having a genetic disruption within a *TGFBR2* locus of the T cell, wherein the nucleic acid sequence encoding the recombinant receptor or a portion thereof is integrated within the endogenous *TGFBR2* locus via homology directed repair (HDR). 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 *TGFBR2* locus of the T cell. In some of any examples, the method produces a modified *TGFBR2* locus, said modified *TGFBR2* locus comprising a nucleic acid sequence encoding a recombinant receptor or a portion thereof. 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 transforming growth factor-beta receptor type-2 (*TGFBR2*) locus.

[0047a] 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 recombinant receptor or portion thereof, said T cell having a genetic disruption within a *TGFBR2* locus of the T cell, wherein the nucleic acid sequence encoding the recombinant receptor or portion thereof is integrated within an endogenous *TGFBR2* locus via homology directed repair (HDR).

[0048] In some of any examples, the modified *TGFBR2* locus does not encode a functional TGFBRII polypeptide, in a cell generated by the method. In some of any examples, the modified *TGFBR2* locus does not encode a TGFBRII polypeptide or the expression of TGFBRII polypeptide is eliminated, in a cell generated by the method. In some of any examples, the modified *TGFBR2* locus does not encode a full length TGFBRII polypeptide or encodes a partial TGFBRII polypeptide, in a cell generated by the method. In some of any examples, the modified *TGFBR2* locus encodes a dominant negative TGFBRII polypeptide, in a cell generated by the method.

[0049] 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]-[the nucleic acid sequence encoding a recombinant receptor or a portion thereof]-[3' homology arm]. 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.

[0050] In some of any examples, the 5' homology arm comprises the sequence set forth in SEQ ID NOS: 69-71 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 NOS: 69-71 or a partial sequence thereof. In some of any examples, the 3' homology arm comprises the sequence set forth in SEQ ID NO:72, 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:72 or a partial sequence thereof.

[0051] In some of any examples, the encoded recombinant receptor is a recombinant T cell receptor (TCR). In some of any examples, the encoded recombinant receptor comprises a recombinant T cell receptor (TCR). In some of any examples, the encoded recombinant receptor is a chimeric antigen receptor (CAR).

[0052] 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. 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. In some of any examples, the RNP is introduced via electroporation, particle gun, calcium phosphate transfection, cell compression or squeezing, such as via electroporation. In some of any examples, the

concentration of the RNP is from at or about 1 μM to at or about 5 μM. In some of any examples, wherein the concentration of the RNP is at or about 2 μM. In some of any examples, the gRNA has a targeting domain sequence of GUGGAUGACCUGGCUAACAG (SEQ ID NO:73).

[0053] 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 T cell is derived from a multipotent or pluripotent cell, which is an iPSC.

[0054] 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 is a retroviral vector, which is a lentiviral vector.

[0055] In some of any examples, the polynucleotide is a linear polynucleotide. In some of any examples, the polynucleotide is a linear polynucleotide which is a double-stranded polynucleotide or a single-stranded polynucleotide. 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). 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.

[0056] 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 and anti-CD3 and anti-CD28 antibodies. In some of any examples, the stimulatory agent(s) comprises and anti-CD3 or anti-CD28 antibodies. In some of any examples, the stimulatory agent(s) comprises and anti-CD3 and anti-CD28 antibodies, which are anti-CD3/anti-CD28 beads. In some of any examples, the stimulatory agent(s) comprises and anti-CD3 or anti-CD28 antibodies, which are anti-CD3/anti-CD28 beads. In some of any examples, the stimulatory agent(s) comprises and anti-CD3 and anti-CD28 antibodies, which are anti-CD3/anti-CD28 beads, where the bead to cell ratio is or is about 1:1. In some of any examples, the stimulatory agent(s) comprises and anti-CD3 or anti-CD28 antibodies, which are anti-CD3/anti-CD28 beads, where the bead to cell ratio is or is about 1:1.

[0057] In some of any examples, the method comprises removing the stimulatory agent(s) from the one or more immune cells prior to the introducing with the one or more agents.

[0058] In some of any examples, the method further comprises incubating the cells prior to, during or subsequent to the introducing of the one or more agents and/or the introducing of the template polynucleotide with one or more recombinant cytokines. In some of any examples, the method further comprises incubating the cells prior to, during or subsequent to the introducing of the one or more agents and/or the introducing of the template polynucleotide with one or more recombinant cytokines, where 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. 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, which is 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. 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, which is 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, which is 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. 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 template 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. 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 template 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, which can be up to or about 7 days.

[0059] 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 comprise a genetic disruption of at least one target site within a *TGFBR2* 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 generated by the method express the recombinant receptor or antigen-binding fragment thereof.

[0060] Provided herein are engineered T cells or a plurality of engineered T cells generated using any of the methods described herein.

[0060a] 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.

[0061] Provided herein are compositions comprising the engineered T cell from any of the examples described herein.

[0061a] In one aspect, the present disclosure refers to a composition, comprising the genetically engineered T cell disclosed herein; or a plurality of the genetically engineered T cell disclosed herein.

[0062] Provided herein are compositions comprising a plurality of the engineered T cell from any of the examples described herein. 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. 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, which can be 1:1. In some of any examples, cells expressing the recombinant 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.

[0063] Provided herein are methods of treatment comprising administering the engineered cell, plurality of engineered cells or composition of any of the examples described herein to a subject having a disease or disorder.

[0063a] 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.

[0064] Provided herein are uses of the engineered cell, plurality of engineered cells or composition of any of the examples described herein for the treatment of a disease or disorder.

[0065] Provided herein are uses of the engineered cell, plurality of engineered cells or composition of any of the examples described herein in the manufacture of a medicament for treating a disease or disorder.

[0066] Provided herein are uses of the engineered cell, plurality of engineered cells or composition of any of the examples described herein is for use in the treatment of a disease or disorder.

[0066a] 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, optionally 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, 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).

[0067] In some of any examples of the method, use or the engineered cell, plurality of engineered cells or composition for use of any of the examples described herein, the disease or disorder is a cancer or a tumor.

[0068] In some of any examples, the cancer or the tumor is a hematologic malignancy, such as 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).

[0069] 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).

[0070] Provided herein are kits that include one or more agent(s) capable of inducing a genetic disruption at a target site within a *TGFBR2* locus; and the polynucleotide of any of the examples provided herein.

[0070a] 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 *TGFBR2* locus; and the polynucleotide disclosed herein.

[0071] Provided herein are kits that include one or more agent(s) capable of inducing a genetic

disruption at a target site within a *TGFBR2* locus; and a polynucleotide comprising a nucleic acid sequence encoding recombinant receptor or a portion thereof, wherein the transgene encoding the recombinant receptor or a fragment, such as an antigen-binding fragment, a domain and/or a 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.

[0071a] 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 *TGFBR2* locus; and a polynucleotide comprising a nucleic acid sequence encoding a recombinant receptor or portion thereof, wherein the nucleic acid sequence encoding the recombinant receptor or 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

[0072] **FIGS. 1A-1D** show the anti-tumor activity of the adoptively transferred anti-ROR1 CAR+ T cells, as determined by the change in tumor volume in a tumor-bearing mouse xenograft model NOD.Cg.Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ (NSG) injected subcutaneously with H1975 non-small cell lung cancer cells. **FIGS. 1A** and **1C** (group mean; Donor 1 and 2, respectively) and **FIGS. 1B** and **1D** (individual mice; Donor 1 and 2, respectively) show the change in tumor volume for mice administered engineered primary human T cell compositions generated from one of two independent donors (Donor 1, Donor 2), as follows: (1) engineered T cells expressing the anti-ROR1 CAR R12 by lentiviral delivery (LV only), (2) engineered T cells expressing the anti-ROR1 CAR R12 by lentiviral delivery and *TGFBR2* knockout (LV+KO), or (3) engineered T cells expressing the anti-ROR1 CAR R12 and DN-TGFBRII by lentiviral delivery (LV+DN), administered at a dose of 1 x 10^6 cells (low dose; top panels) or 3 x 10^6 cells (high dose; bottom panels); and 3 x 10^6 mock treated cells (mock KO) or were untreated (tumor only) as controls.

[0073] **FIGS. 2A** and **2B** (Donor 1 and 2, respectively) show the tumor-free survival curve of NSG mice bearing H1975 tumors receiving an adoptive transfer of the engineered cells as described in Example 1.B.

[0074] **FIGS. 3A** (group) and **3B** (individual) show the change in tumor volume for the first 14 days after administration of 1 x 10^6 engineered T cells to NSG mice bearing H1975 tumors, prior to collection of the tumor, spleen and blood samples, as follows: (1) engineered T cells expressing the anti-ROR1 CAR R12 by lentiviral delivery (LV), (2) engineered T cells expressing the anti-ROR1 CAR R12 by lentiviral delivery and *TGFBR2* knockout (LV+KO), or (3) engineered T cells expressing the anti-ROR1 CAR R12 and DN-TGFBRII by lentiviral delivery (LV+DN) at a dose of 1 x 10^6 cells, with engineered cells in all groups subject to electroporation.

[0075] **FIGS. 4A-4B** show the frequency of CAR-expressing CD4+ (upper panels) and CD8+ (lower panels) T cells in the blood (**FIG. 4A**) or spleen (**FIG. 4B**) of mice administered cells engineered by various delivery methods as described in Example 2.B. **FIGS. 4C-4D** show the frequency of CAR-expressing CD4+ (upper panel) and CD8+ (lower panel) T cells in the tumor (**FIG. 4C**) and the frequency of CD103+ CAR-expressing CD4+ (upper panel) and CD8+ (lower panel) T cells in the tumor (**FIG. 4D**).

[0076] **FIGS. 5A-5B** show the changes in caspase 3/7 activity (**FIG. 5A**; total green object

Claims

1. A genetically engineered T cell, comprising a modified transforming growth factor-beta receptor type-2 (*TGFBR2*) locus, said modified *TGFBR2* locus comprising a transgene sequence encoding a recombinant receptor or portion thereof.

2. The genetically engineered T cell of claim 1, wherein the transgene sequence has been integrated at an endogenous *TGFBR2* locus of a T cell, optionally via homology directed repair (HDR).

3. The genetically engineered T cell of claim 1 or 2, wherein the modified *TGFBR2* locus:

does not encode a functional TGFBRII polypeptide;

does not encode a TGFBRII polypeptide or the expression of TGFBRII polypeptide is eliminated;

does not encode a full length TGFBRII polypeptide; and/or

encodes a dominant negative TGFBRII polypeptide, optionally wherein the dominant negative TGFBRII polypeptide comprises an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60, 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 an amino acid sequence corresponding to residues 22-191 of SEQ ID NO:59 or residues 22-216 of SEQ ID NO:60, or a fragment thereof.

4. The genetically engineered T cell of any of claims 1-3, wherein the transgene sequence is in-frame with one or more exons of an open reading frame or partial sequence thereof, of the endogenous *TGFBR2*.

5. The genetically engineered T cell of any of claims 1-4, wherein the transgene sequence is downstream of exon 1 and/or downstream of exon 4 and upstream of exon 6, of the open reading frame of the endogenous *TGFBR2* locus.

6. The genetically engineered T cell of any of claims 1-5, wherein the recombinant receptor is or comprises a recombinant T cell receptor (TCR), and the transgene sequence encodes a TCR alpha (TCRα) chain, a TCR beta (TCRβ) chain, or both.

7. The genetically engineered T cell of any of claims 1-6, wherein the recombinant receptor is a chimeric antigen receptor (CAR), wherein the CAR comprises an extracellular region comprising a binding domain, a transmembrane domain, and an intracellular region.

8. The genetically engineered T cell of claim 8, wherein the binding domain is or comprises an antibody or an antigen-binding fragment thereof.

9. The genetically engineered T cell of claim 7 or 8, wherein the binding domain is capable of binding to a target antigen that is associated with, specific to, or expressed on a cell or tissue of a disease, disorder or condition, optionally wherein the target antigen is a tumor antigen.

10. The genetically engineered T cell of claim 9, wherein the target antigen is selected from among αvβ6 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-22Rα), IL-13 receptor alpha 2 (IL-13Rα2), 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.

11. The genetically engineered T cell of any of claims 7-10, 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.

12. The genetically engineered T cell of any of claims 7-11, wherein the intracellular region comprises an intracellular signaling domain, optionally wherein the intracellular signaling domain is or comprises an intracellular signaling domain of a CD3 chain, optionally a CD3-zeta (CD3ζ) chain, or a signaling portion thereof.

13. The genetically engineered T cell of any of claims 7-12, wherein the intracellular region comprises one or more costimulatory signaling domain(s), 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.

14. The genetically engineered T cell of any of claims 1-13, wherein

the transgene sequence comprises a sequence of nucleotides encoding, in order: a binding domain, optionally a single chain Fv fragment (scFv); a spacer, optionally comprising a sequence from a human immunoglobulin hinge, optionally from IgG1, IgG2 or IgG4 or a modified version thereof, optionally further comprising a C_H2 region and/or a C_H3 region; and a transmembrane domain, optionally from human CD28; a costimulatory signaling domain, optionally from human 4-1BB; and an intracellular signaling region, optionally a CD3ζ chain or portion thereof; and/or

the modified *TGFBR2* locus comprises a sequence of nucleotides encoding, in order: a binding domain, optionally an scFv; a spacer, optionally comprising a sequence from a human immunoglobulin hinge, optionally from IgG1, IgG2 or IgG4 or a modified version thereof, optionally further comprising a C_H2 region and/or a C_H3 region; and a transmembrane domain, optionally from human CD28; a costimulatory signaling domain, optionally from human 4-1BB; and an intracellular signaling region, optionally a CD3ζ chain or portion thereof.

15. The genetically engineered T cell of any of claims 1-14, 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.

16. The genetically engineered T cell of any of claims 1-15, 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.

17. The genetically engineered T cell of claim 16, wherein:

the transgene sequence comprises a sequence of nucleotides encoding the recombinant receptor or portion thereof, and the one or more multicistronic element(s) are positioned upstream of the sequence of nucleotides encoding the recombinant receptor or portion thereof; and/or positioned between the sequence of nucleotides encoding the recombinant receptor or portion thereof and the sequence of nucleotides encoding the at least one further protein; and/or

the recombinant receptor is a TCR, and the one or more multicistronic element(s) are positioned between a sequence of nucleotides encoding the TCRα and a sequence of nucleotides encoding the TCRβ; and/or

the recombinant receptor is a CAR that a multi-chain CAR, and the one or more multicistronic element(s) are 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.

18. The genetically engineered T cell of any of claims 1-17, wherein the modified *TGFBR2* locus comprises the promoter and/or regulatory or control element of the endogenous *TGFBR2* locus operably linked to control expression the transgene sequence encoding the recombinant receptor or portion thereof; or the modified *TGFBR2* locus comprises one or more heterologous regulatory or control element(s) operably linked to control expression of the recombinant receptor or portion thereof.

19. The genetically engineered T cell of any of claims 1-18, wherein the T cell is a primary T cell derived from a subject, optionally wherein the subject is a human.

20. The genetically engineered T cell of any of claims 1-19, wherein the T cell is a CD8+ T cell or subtype thereof or a CD4+ T cell or subtype thereof.

21. A polynucleotide, comprising:

(a) a nucleic acid sequence encoding a recombinant receptor or 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 transforming growth factor-beta receptor type-2 (*TGFBR2*) locus.

22. The polynucleotide of claim 21, wherein the nucleic acid sequence of (a) is a sequence that is exogenous or heterologous to the open reading frame of the endogenous *TGFBR2* locus of a T cell, optionally a human T cell.

23. The polynucleotide of claim 21 or 22, wherein the one or more homology arm(s) comprise at least one intron or at least one exon of the open reading frame of the *TGFBR2* locus of a T cell, optionally a human T cell.

24. The polynucleotide of any of claims 21-23, wherein the nucleic acid sequence of (a) is in-frame with one or more exons of the open reading frame of the *TGFBR2* locus comprised in the one or more homology arm(s).

25. The polynucleotide of any of claims 21-24, wherein the one or more region(s) of the open reading frame is or comprises sequences that are downstream of exon 1 of the open reading frame of the *TGFBR2* locus optionally wherein the one or more region(s) of the open reading frame is or comprises sequences that includes at least a portion of exon 4 or downstream of exon 4 of the open reading frame of the *TGFBR2* locus.

26. The polynucleotide of any of claims 21-25, 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.

27. The polynucleotide of claim 26, wherein the 5' homology arm comprises the sequence set forth in SEQ ID NOS: 69-71 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 NOS: 69-71 or a partial sequence thereof, and/or the 3' homology arm comprises the sequence set forth in SEQ ID NO:72, 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:72 or a partial sequence thereof.

28. The polynucleotide of claim 21, wherein:

the nucleic acid sequence of (a) comprises a sequence of nucleotides encoding the recombinant receptor or portion thereof, and the one or more multicistronic element(s) are positioned upstream of the

sequence of nucleotides encoding the recombinant receptor or portion thereof; and/or positioned between the sequence of nucleotides encoding the recombinant receptor or portion thereof and the sequence of nucleotides encoding the at least one further protein; and/or

the recombinant receptor is a TCR, and the one or more multicistronic element(s) are positioned between a sequence of nucleotides encoding the TCRα and a sequence of nucleotides encoding the TCRβ; and/or

the recombinant receptor is a CAR that a multi-chain CAR, and the one or more multicistronic element(s) are 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.

29. The polynucleotide of any of claims 21-28, 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.

30. A method of producing a genetically engineered T cell, the method comprising introducing the polynucleotide of any of claims 21-29 into a T cell under conditions to induce homology directed repair (HDR), comprising a genetic disruption at a *TGFBR2* locus.

31. 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 *TGFBR2* locus of the T cell; and

(b) introducing the polynucleotide of any of claims 21-29 into the T cell, wherein steps (a) and (b) are simultaneous or sequential, in any order.

32. The method of claim 30 or 31, wherein the nucleic acid sequence encoding a recombinant receptor or portion thereof is integrated within the endogenous *TGFBR2* locus via homology directed repair (HDR).

33. 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 recombinant receptor or portion thereof, said T cell having a genetic disruption within a *TGFBR2* locus of the T cell, wherein the nucleic acid sequence encoding the recombinant receptor or portion thereof is integrated within an endogenous *TGFBR2* locus via homology directed repair (HDR).

34. The method of any of claims 30-33, wherein the method produces a modified *TGFBR2* locus in the T cell, said modified *TGFBR2* locus comprising a nucleic acid sequence encoding a recombinant receptor or portion thereof.

35. The method of any of claims 30-34, wherein, in a cell generated by the method, the modified *TGFBR2* locus:

does not encode a functional TGFBRII polypeptide, in a cell generated by the method;

does not encode a TGFBRII polypeptide or the expression of TGFBRII polypeptide is eliminated; and/or

does not encode a full length TGFBRII polypeptide or encodes a dominant negative TGFBRII polypeptide.

36. The method of claim 35, 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]-[the nucleic acid sequence encoding a recombinant receptor or portion thereof]-[3' homology arm].

37. The method of any of claims 30-36, wherein the encoded recombinant receptor is or comprises recombinant T cell receptor (TCR).

38. The method of any of claims 30-37, wherein the encoded recombinant receptor is a chimeric antigen receptor (CAR).

39. The method of any of claim 31 and 34-38, 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), or and a CRISPR-Cas9 combination that specifically binds to, recognizes, or hybridizes to the target site, optionally wherein 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.

40. The method of claim 39, 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.

41. The method of claim 40, wherein the gRNA has a targeting domain sequence of GUGGAUGACCUGGCUAACAG (SEQ ID NO:73).

42. The method of any of claims 30-41, wherein the T cell is a primary T cell derived from a subject, optionally wherein the subject is a human.

43. The method of any of claims 30-42, wherein the T cell is a CD8+ T cell or subtype thereof, or a CD4+ T cell or subtype thereof.

44. The method of any of claims 30-44, 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.

45. The method of any of claims 30-44, wherein the polynucleotide is a linear polynucleotide, optionally a double-stranded polynucleotide or a single-stranded polynucleotide.

46. The method of any of claims 31 and 34-45, 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, 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.

47. The method of any of claims 30-46, 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 *TGFBR2* 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 recombinant receptor.

48. A genetically engineered T cell or a plurality of genetically engineered T cells generated using the method of any of claims 30-47.

49. A composition, comprising the genetically engineered T cell any of claims 1-20 and claim 48; or a plurality of the genetically engineered T cell of any of claims 1-20 and claim 48.

50. The composition of claim 49, 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.

51. The composition of any of claims 49-50, wherein cells expressing the recombinant receptor make up at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, of the total cells in the composition or of the total CD4+ T cells or CD8+ T cells in the composition.

52. Use of the genetically engineered T cell, plurality of genetically engineered T cells or composition of any of claims 1-20 and 48-51 in the manufacture of a medicament for treating a disease or disorder.

53. The genetically engineered T cell, plurality of genetically engineered T cells or composition of any of claims 1-20 and 48-51 for use in the treatment of a disease or disorder

optionally 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

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).

54. A kit comprising:

one or more agent(s) capable of inducing a genetic disruption at a target site within a *TGFBR2* locus; and

the polynucleotide of any of claims 21-29.

55. A kit, comprising:

one or more agent(s) capable of inducing a genetic disruption at a target site within a *TGFBR2* locus; and

a polynucleotide comprising a nucleic acid sequence encoding a recombinant receptor or portion thereof, wherein the nucleic acid sequence encoding the recombinant receptor or 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 30-47.