manageable clinical efficacy of CAR T cells is currently limited to CD19-positive B cell malignancies and to NY-ESO-1-peptide expressing synovial sarcoma patients expressing HLA-A2.

SUMMARY

[0005] There is a clear need to improve genetically engineered T cells to more broadly act against various human malignancies.

[0005a] In one aspect, provided herein is a recombinant nucleic acid comprising: (a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising (i) a TCR subunit comprising (1) at least a portion of a TCR extracellular domain, and (2) a transmembrane domain, and (ii) an antibody or fragment thereof comprising an antigen binding domain; and (b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR gamma constant domain, a TCR delta constant domain, or a TCR gamma constant domain and a TCR delta constant domain; wherein the TCR subunit and the antibody or fragment thereof are operatively linked; wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR; optionally, wherein the antibody or fragment thereof is murine, human or humanized; and optionally, wherein the antibody or fragment thereof binds to a cell surface antigen on the surface of a tumor cell.

[0005b] In another aspect, provided herein is a recombinant nucleic acid comprising: (a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising (i) a TCR subunit comprising (1) at least a portion of a TCR extracellular domain, and (2) a transmembrane domain, and (ii) an antibody or fragment thereof comprising an antigen binding domain; and (b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR delta constant domain; wherein the TCR subunit and the antibody or fragment thereof are operatively linked; wherein the TCR extracellular domain comprises an extracellular domain of a TCR gamma chain or portion thereof; wherein the recombinant nucleic acid further comprises a sequence encoding a second antigen binding domain, wherein the second antigen binding domain is operatively linked to the TCR constant domain; wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR; and wherein the TCR constant domain functionally incorporates into the TCR complex when expressed in the modified T cell.

[0005c] In another aspect, provided herein is a modified T cell comprising: (i) the recombinant nucleic acid as described herein, wherein the modified T cell comprises a functional disruption of an endogenous TCR; or (ii) the TFP and the TCR constant domain encoded by the recombinant nucleic acid as described herein, wherein the modified T cell comprises a functional disruption of an endogenous TCR.

[0005d] In another aspect, provided herein is a medicament comprising: (a) the modified T cells as described herein; and (b) a pharmaceutically acceptable carrier.

[0005e] In another aspect, provided herein is a method of producing the modified T cell as described herein, the method comprising: (i) (a) disrupting an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; thereby producing a T cell containing a functional disruption of an endogenous TCR gene; and (b) transducing the T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid as described herein; optionally, wherein disrupting comprises transducing the T cell with a nuclease protein or a nucleic acid sequence encoding a nuclease protein that targets the endogenous gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; or (ii) transducing a T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid as described herein; optionally, wherein the T cell containing a functional disruption of an endogenous TCR gene is a T cell containing a functional disruption of an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; and optionally, wherein the T cell containing a functional disruption of an endogenous TCR gene has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell; optionally, wherein the T cell is a human T cell; and optionally, wherein the sequence comprised by the recombinant nucleic acid or the vector is inserted into the endogenous TCR subunit gene at the cleavage site, and wherein the insertion of the sequence into the endogenous TCR subunit gene functionally disrupts the endogenous TCR subunit.

[0005f] In another aspect, provided herein is a use of a pharmaceutical composition in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, the pharmaceutical composition comprising: (i) the medicament as described herein, or (ii) a medicament comprising (a) a modified T cell produced according to the method as described herein, and (b) a pharmaceutically acceptable carrier, optionally, wherein the modified T cell is an allogeneic T cell; wherein a therapeutically effective amount of (i) the medicament as described herein, or (ii) the medicament comprising (a) a modified T cell produced according to the method as described herein, and (b) a pharmaceutically acceptable carrier is to be administered to the subject; and optionally, wherein: (a) the cancer is a solid cancer, a lymphoma or a leukemia; and/or (b) the cancer is selected from the group consisting of renal cell carcinoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, kidney cancer, and stomach cancer.

[0005g] In another aspect, provided herein is the recombinant nucleic acid as described herein or the modified T cell as described herein for use as a medicament or in the preparation of a medicament.

[0006] Described herein are modified T cells comprising fusion proteins of TCR subunits, including CD3 epsilon, CD3gamma, CD3 delta, TCR gamma, TCR delta, TCR alpha and TCR beta chains with binding domains specific for cell surface antigens that have the potential to overcome limitations of existing approaches. Additionally, these modified T cells may have functional disruption of an endogenous TCR (e.g., TCR alpha, beta or both). These modified T cells may have the ability to kill target cells more efficiently than CARs, but release comparable or lower levels of pro-inflammatory cytokines. These modified T cells and methods of their use may represent an advantage for these cells relative to CARs because elevated levels of these cytokines have been associated with dose-limiting toxicities for adoptive CAR T therapies.

[0007] Provided herein are modified T cells comprising T-cell receptor (TCR) fusion protein (TFP) and a TCR constant domain, methods of producing the modified T cells, and methods of use thereof for the treatment of diseases. The TCR constant domain described herein can be a TCR alpha constant domain, a TCR beta constant domain, a TCR alpha constant domain and a TCR beta constant domain, a TCR gamma constant domain, a TCR delta constant domain, or a TCR gamma constant domain and a TCR constant domain. The TFP described herein can comprise a TCR subunit and an antibody or fragment thereof. The TFP described herein can comprise a TCR subunit and a binding ligand or fragment thereof. The TCR subunit can be derived from any of the TCR chains such as TCR alpha chain, TCR beta chain, TCR gamma chain, TCR delta chain, CD3 epsilon, CD3 delta, or CD3 gamma. The antibody or fragment thereof can be murine, human, or humanized.

[0008] In an example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a transmembrane domain, and an antibody comprising an antigen binding domain; and a sequence encoding a TCR constant domain(s), wherein the TCR constant domain is a TCR gamma constant domain or a TCR delta constant domain, or a sequence encoding a TCR gamma constant domain and a TCR delta constant domain; wherein the TCR subunit and the antibody are operatively linked, and wherein

the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0009] In another example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, a transmembrane domain, and a binding ligand or a fragment thereof that is capable of binding to an antibody or fragment thereof; and a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR gamma constant domain or a TCR delta constant domain, or a sequence encoding a TCR gamma constant domain and a TCR delta constant domain; wherein the TCR subunit and the binding ligand or fragment thereof are operatively linked, and wherein the TFP functionally incorporates into TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0010] In some examples, the TCR subunit further comprises an intracellular domain of TCR alpha, TCR beta, TCR gamma, or TCR delta or an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, or CD3 delta. In some examples, the TCR constant domain is a TCR delta constant domain. In some examples, the TCR delta constant domain comprises SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243 or SEQ ID NO:265, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the intracellular domain is an intracellular domain of TCR gamma. In some examples, the sequence encoding the TCR delta constant domain further encodes a second antigen binding domain or ligand binding domain that is operatively linked to the sequence encoding the TCR delta constant domain. In some examples, the second antigen binding domain or ligand binding domain is the same or different as the antigen binding domain or ligand binding domain of the TFP. In some examples, the second antigen binding domain or ligand binding domain is operatively linked to the sequence encoding the TCR delta constant domain via a linker. In some examples, the TCR constant domain is a TCR gamma constant domain. In some examples, the TCR gamma constant domain comprises SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the intracellular domain is an intracellular domain of TCR delta. In some examples, the sequence encoding the TCR gamma constant domain further encodes a second antigen binding domain or ligand binding domain that is operatively linked to the sequence encoding the TCR gamma constant domain. In some examples, the second antigen binding domain or ligand binding domain is the same or different as the antigen

binding domain or ligand binding domain of the TFP. In some examples, the second antigen binding domain or ligand binding domain is operatively linked to the sequence encoding the TCR gamma constant domain via a linker.

[0011] In some examples, the recombinant nucleic acid comprises a sequence encoding a TCR gamma constant domain and a TCR delta constant domain. In some examples, the TCR gamma constant domain comprises SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the sequence encoding the TCR gamma constant domain further encodes a TCR gamma variable domain, thereby encoding a full TCR gamma domain. In some examples, the full TCR gamma domain is gamma 9 or gamma 4. In some examples, the full TCR gamma domain comprises SEQ ID NO:255, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR delta constant domain comprises SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243 or SEQ ID NO:265, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the sequence encoding the TCR delta constant domain further encodes a TCR delta variable domain, thereby encoding a full TCR delta domain. In some examples, the full TCR delta domain is delta 2 or delta 1. In some examples, the full TCR delta constant domain comprises SEQ ID NO:256, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the intracellular signaling domain is CD3 epsilon, CD3 gamma, or CD3 delta. In some examples, the intracellular signaling domain is CD3 epsilon. In some examples, the recombinant nucleic acid further comprises at least one leader sequence and at least one linker. In some examples, the recombinant nucleic acid further comprises a portion of a TCR alpha constant domain, a portion of a TCR beta domain, or both. In some examples, the sequence comprises, from 5' to 3', a first leader sequence, an antigen binding domain sequence, a linker, a TRDC gene sequence, a cleavable linker sequence, a second leader sequence, and a TRGC gene sequence. In some examples, the sequence comprises, from 5'-3', a first leader sequence, a TRDC gene sequence, a cleavable linker sequence, a second leader sequence, an antigen binding domain sequence, a linker sequence, and a TRGC gene sequence. In some examples, the sequence comprises, from 5'-3', a first leader sequence, an antigen binding domain sequence, a first linker sequence, a TRDC gene sequence, a cleavable linker, a second leader sequence, a second antigen binding domain sequence, a second linker sequence, and a TRGC gene sequence. In some examples, the sequence comprises, from 5'-3', a first leader sequence, a TRDC gene

sequence, a first cleavable linker sequence, a second leader sequence, a TRGC gene sequence, a second cleavable linker sequence, a third leader sequence, an antigen binding domain sequence, a linker sequence, and a CD3 epsilon gene sequence. In some examples, the sequence comprises, from 5'-3', a first leader sequence, a first antigen binding domain sequence, a first linker sequence, a TRDC gene sequence or fragment thereof, a TRAC gene sequence or fragment thereof, a cleavable linker sequence, a second leader sequence, a second antigen binding domain sequence, a second linker sequence, a TRGC gene sequence or fragment thereof, and a TRBC gene sequence or fragment thereof.

[0012] In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:1. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:2. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:3. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:4. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:5. In some examples, the sequence encodes a sequence of SEQ ID NO:242. In some examples, the sequence encodes a sequence of SEQ ID NO:244. In some examples, the sequence encodes a sequence of SEQ ID NO:245. In some examples, the sequence encodes a sequence of SEQ ID NO:246. In some examples, the sequence encodes a sequence of SEQ ID NO:248. In some examples, the sequence encodes a sequence of SEQ ID NO:250. In some examples, the sequence encodes a sequence of SEQ ID NO:252. In some examples, the sequence encodes a sequence of SEQ ID NO:257. In some examples, the sequence encodes a sequence of SEQ ID NO:263. In some examples, the sequence encodes a sequence of SEQ ID NO:264.

[0013] In some examples, the binding ligand is capable of binding an Fc domain of the antibody. In some examples, the binding ligand is capable of selectively binding an IgG1 antibody. In some examples, the binding ligand is capable of specifically binding an IgG4 antibody. In some examples, the antibody or fragment thereof binds to a cell surface antigen. In some examples, the antibody or fragment thereof is murine, human or humanized. In some examples, the antibody or fragment thereof binds to a cell surface antigen on the surface of a tumor cell. In some examples, the binding ligand comprises a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the binding ligand does not comprise an antibody or fragment thereof. In some examples, the binding ligand comprises a CD16 polypeptide or fragment thereof. In some examples, the binding ligand comprises a CD16-binding polypeptide. In some examples, the binding ligand is human or humanized. In some examples, the recombinant nucleic acid further comprises a nucleic acid sequence encoding an antibody or

fragment thereof capable of being bound by the binding ligand. In some examples, the antibody or fragment thereof is capable of being secreted from a cell.

[0014] In another example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a TCR extracellular domain, and a transmembrane domain, and an antigen binding domain comprising a ligand or a fragment thereof that binds to a receptor or polypeptide expressed on a surface of a cell; and a sequence encoding a TCR constant domain(s), wherein the TCR constant domain is a TCR gamma constant domain or a TCR delta constant domain; or a sequence encoding a TCR gamma constant domain and a TCR delta constant domain; wherein the TCR subunit and the antigen binding domain are operatively linked, and wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0015] In some examples, the TCR subunit further comprises an intracellular domain of TCR alpha, TCR beta, TCR gamma, or TCR delta or an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, or CD3 delta. In some examples, the recombinant nucleic acid further comprises at least a partial sequence encoding a TCR alpha constant domain, a TCR beta constant domain, or a partial sequence of both a TCR alpha constant domain and a TCR beta constant domain. In some examples, the antigen binding domain comprises a ligand. In some examples, the ligand binds to the receptor of a cell. In some examples, the ligand binds to the polypeptide expressed on a surface of a cell. In some examples, the receptor or polypeptide expressed on a surface of a cell comprises a stress response receptor or polypeptide. In some examples, the receptor or polypeptide expressed on a surface of a cell is an MHC class I-related glycoprotein. In some examples, the MHC class I-related glycoprotein is selected from the group consisting of MICA, MICB, RAET1E, RAET1G, ULBP1, ULBP2, ULBP3, ULBP4 and combinations thereof. In some examples, the antigen binding domain comprises a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the antigen binding domain comprises a monomer or a dimer of the ligand or fragment thereof. In some examples, the ligand or fragment thereof is a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the ligand or fragment thereof is a monomer or a dimer. In some examples, the antigen binding domain does not comprise an antibody or fragment thereof. In some examples, the antigen binding domain does not comprise a variable region. In some examples, the antigen binding domain does not comprise a CDR. In some examples, the

ligand or fragment thereof is a Natural Killer Group 2D (NKG2D) ligand or a fragment thereof. In some examples, the TCR constant domain incorporates into a functional TCR complex when expressed in a T cell. In some examples, the TCR constant domain incorporates into a same functional TCR complex as the functional TCR complex that incorporates the TFP when expressed in a T cell. In some examples, the sequence encoding the TFP and the sequence encoding the TCR constant domain(s) are contained within a same nucleic acid molecule. In some examples, the encoded TFP and the encoded TCR constant domains are operatively linked by a first linker sequence. In some examples, the first linker comprises a protease cleavage site. In some examples, the protease cleavage site is a 2A, e.g., a T2A or a P2A cleavage site. In some examples, the sequence encoding the TFP and the sequence encoding the TCR constant domain(s) are contained within different nucleic acid molecules. In some examples, the TCR subunit and the antibody domain, the antigen binding domain or the binding ligand or fragment thereof of the TFP are operatively linked by a second linker sequence. In some examples, the second linker sequence comprises (G4S)n, wherein n=1 to 4. In some examples, the transmembrane domain is a TCR transmembrane domain from CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha, TCR beta, TCR delta, or TCR gamma. In some examples, the intracellular domain is derived from only CD3 epsilon, only CD3 gamma, only CD3 delta, only TCR alpha, TCR beta, TCR gamma, or TCR delta. In some examples, the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, wherein at least two of (i), (ii), and (iii) are from the same TCR subunit. In some examples, the TCR extracellular domain comprises an extracellular domain or portion thereof of a protein selected from the group consisting of a TCR gamma chain, a TCR delta chain, a CD3 epsilon TCR subunit, a CD3 gamma TCR subunit, a CD3 delta TCR subunit, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain of a TCR gamma chain or a TCR delta chain. In some examples, the TCR extracellular domain comprises the extracellular portion of a constant domain of a TCR gamma chain or a TCR delta chain, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain is or comprises a delta constant domain, or a fragment thereof. In some examples, the delta constant domain has the sequence of SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243 or SEQ ID NO:265,

functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR subunit comprising (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain is or comprises a gamma constant domain. In some examples, the gamma constant domain has the sequence of SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the extracellular domain of the TFP does not comprise the variable domain of a gamma chain or a delta chain. In some examples, the TCR subunit comprises a TCR intracellular domain comprising a stimulatory domain of a protein selected from an intracellular signaling domain of CD3 epsilon, CD3 gamma or CD3 delta, or an amino acid sequence having at least one modification thereto. In some examples, the TCR subunit of the TFP comprises the extracellular, transmembrane and intracellular domain of CD3 epsilon. In some examples, the TCR subunit of CD3 epsilon comprises the sequence of SEQ ID NO:258, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications.

[0016] In some examples, the TFP, the TCR gamma constant domain, the TCR delta constant domain, and any combination thereof is capable of functionally interacting with an endogenous TCR complex and/or at least one endogenous TCR polypeptide. In some examples, the TCR constant domain is a TCR gamma constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR delta, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof; the TCR constant domain is a TCR delta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR gamma, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof; or the TCR constant domain is a TCR gamma constant domain and a TCR delta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof. In some examples, the at least one but not more than 20 modifications thereto comprise a modification of an amino acid that mediates cell signaling or a modification of an amino acid that is phosphorylated in response to a ligand binding to the TFP. In some examples, the antibody is an antibody fragment. In some examples, the antibody fragment is a scFv, a single domain antibody domain, a VH domain or a VL domain. In some examples, an antigen binding domain is selected from a group consisting of an anti-CD19 binding domain, an anti-B-cell maturation antigen (BCMA) binding domain, an anti-mesothelin (MSLN) binding domain, an anti-CD20 binding domain, an anti-CD70 binding domain, an anti-79b binding domain, an anti-

HER2 binding domain, an anti-PMSA binding domain, an anti-MUC16 binding domain, an anti-CD22 binding domain, an anti-PD-L1 binding domain, an anti BAFF or BAFF receptor binding domain, an anti-Nectin-4 binding domain, an anti-TROP-2 binding domain, an anti-GPC3 binding domain, and an anti-ROR-1 binding domain. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID NO:60, a CDR2 of SEQ ID NO:61, and a CDR3 of SEQ ID NO:62. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID NO:63, a CDR2 of SEQ ID NO:64, and a CDR3 of SEQ ID NO:65. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID NO:66, a CDR2 of SEQ ID NO:67, and a CDR3 of SEQ ID NO:68. In some examples, the anti-MSLN binding domain comprises a sequence with at least about 80% sequence identity to a sequence of SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:71. In some examples, the anti-CD19 binding domain comprises a CDR1 of SEQ ID NO:73, a CDR2 of SEQ ID NO:75, and a CDR3 of SEQ ID NO:77. In some examples, the anti-CD19 binding domain comprises a CDR1 of SEQ ID NO:79, a CDR2 of SEQ ID NO:81, and a CDR3 of SEQ ID NO:83. In some examples, the anti-CD19 binding domain comprises a sequence with at least about 80% sequence identity to a sequence of SEQ ID NO:85 and/or SEQ ID NO:87. In some examples, the recombinant nucleic acid further comprises a sequence encoding a TCR alpha transmembrane domain. In some examples, the recombinant nucleic acid further comprises a sequence encoding a TCR beta transmembrane domain. In some examples, the recombinant nucleic acid further comprises a sequence encoding a TCR alpha transmembrane domain and a sequence encoding a TCR beta transmembrane domain.

[0017] In another example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a murine TCR alpha or murine TCR beta extracellular domain, and a murine TCR alpha or murine TCR beta transmembrane domain, and an antibody or fragment thereof comprising an antigen binding domain; and a sequence encoding a TCR constant domain(s), wherein the TCR constant domain is a murine TCR alpha constant domain or a murine TCR beta constant domain; or a sequence encoding a murine TCR alpha constant domain and a murine TCR beta constant domain; wherein the TCR subunit and the antibody are operatively linked, and wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0018] In another example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a murine TCR alpha or murine TCR beta extracellular domain,

and a murine TCR alpha or murine TCR beta transmembrane domain, and a binding ligand or a fragment thereof that is capable of binding to an antibody or fragment thereof; and a sequence encoding a TCR constant domain(s), wherein the TCR constant domain is a murine TCR alpha constant domain or a murine TCR beta constant domain; or a sequence encoding a murine TCR alpha constant domain and a murine TCR beta constant domain; wherein the TCR subunit and the binding ligand or fragment thereof are operatively linked, and wherein the TFP functionally incorporates into TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0019] In some examples, the TCR subunit comprises an intracellular domain of murine TCR alpha or murine TCR beta. In some examples, the TCR constant domain is a TCR alpha constant domain. In some examples, the TCR alpha constant domain comprises SEQ ID NO:17, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:146, or SEQ ID NO:207, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR alpha constant domain comprises a murine TCR alpha constant domain. In some examples, the murine TCR alpha constant domain comprises amino acids 2-137 of the murine TCR alpha constant domain. In some examples, the murine TCR alpha constant domain comprises amino acids 2-137 of SEQ ID NO:146. In some examples, the murine TCR alpha constant domain comprises a sequence of SEQ ID NO:207. In some examples, the murine TCR alpha constant domain comprises amino acids 82-137 of SEQ ID NO:146. In some examples, the murine TCR alpha constant domain comprises a sequence of SEQ ID NO:17. In some examples, the intracellular domain is an intracellular domain of TCR beta. In some examples, the sequence encoding the TCR alpha constant domain further encodes a second antigen binding domain or ligand binding domain that is operatively linked to the sequence encoding the TCR alpha constant domain. In some examples, the second antigen binding domain or ligand binding domain is the same or different as the antigen binding domain or ligand binding domain of the TFP. In some example, the second antigen binding domain or ligand binding domain is operatively linked to the sequence encoding the TCR alpha constant domain via a linker. In some examples, the TCR constant domain is a TCR beta constant domain. In some examples, the TCR beta constant domain comprises SEQ ID NO:18, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:152, or SEQ ID NO:209, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, TCR beta constant domain comprises a murine TCR beta constant domain. In some examples, murine TCR beta constant domain comprises amino acids 2-173 of the murine TCR beta constant domain. In some

examples, murine TCR beta constant domain comprises amino acids 2-173 of SEQ ID NO:152. In some examples, murine TCR beta constant domain comprises SEQ ID NO:209. In some examples, the TCR beta constant domain comprises amino acids 123-173 of SEQ ID NO:152. In some examples, the TCR beta constant domain comprises SEQ ID NO:18. In some examples, the intracellular domain is an intracellular domain of TCR alpha. In some examples, the sequence encoding the TCR beta constant domain further encodes a second antigen binding domain or ligand binding domain that is operatively linked to the sequence encoding the TCR beta constant domain. In some examples, the second antigen binding domain or ligand binding domain is the same or different as the antigen binding domain or ligand binding domain of the TFP. In some example, the second antigen binding domain or ligand binding domain is operatively linked to the sequence encoding the TCR beta constant domain via a linker. In some examples, the recombinant nucleic acid comprise sequence encoding a TCR alpha constant domain and a TCR beta constant domain. In some examples, the TCR alpha constant domain comprises SEQ ID NO:17, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:146, or SEQ ID NO:207, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR beta constant domain comprises SEQ ID NO:18, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:152, or SEQ ID NO:209, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the intracellular signaling domain is CD3 epsilon, CD3 gamma, or CD3 delta. In some examples, the intracellular signaling domain is CD3 epsilon.

[0020] In some examples, the sequence comprises, from 5' to 3', a first leader sequence, an antigen binding domain sequence, a linker, a TRAC gene sequence, a cleavable linker sequence, a second leader sequence, and a TRBC gene sequence. In some examples, the sequence comprises, from 5' to 3', a first leader sequence, an antigen binding domain sequence, a linker, a TRAC gene sequence, a cleavable linker sequence, a second leader sequence, and a TRBC gene sequence. In some examples, the sequence comprises, from 5' to 3', a first leader sequence, a TRAC gene sequence, a cleavable linker sequence, a second leader sequence, an antigen binding domain sequence, a linker, and a TRBC gene sequence. In some examples, the sequence comprises, from 5' to 3', a first leader sequence, an antigen binding domain sequence, a linker, a TRAC gene sequence, a cleavable linker sequence, a second leader sequence, an antigen binding domain sequence, a linker, and a TRBC gene sequence. In some examples, the sequence comprises, from 5'-3', a first leader sequence, a TRAC gene sequence, a first cleavable linker sequence, a second leader sequence, a TRBC gene sequence, a second cleavable linker sequence,

a third leader sequence, an antigen binding domain sequence, a linker sequence, and a CD3 epsilon gene sequence. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:10. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:204. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:206. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:210. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:211. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:217. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:218. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:219. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:220. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:259. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:261. In some examples, the sequence encodes the polypeptide as set forth in SEQ ID NO:262. In some examples, the recombinant nucleic acid further comprises at least one leader sequence and at least one linker. In some examples, the binding ligand is capable of binding an Fc domain of the antibody. In some examples, the binding ligand is capable of selectively binding an IgG1 antibody. In some examples, the binding ligand is capable of specifically binding an IgG4 antibody. In some examples, the antibody or fragment thereof binds to a cell surface antigen. In some examples, the antibody or fragment thereof is murine, human or humanized. In some examples, the antibody or fragment thereof binds to a cell surface antigen on the surface of a tumor cell. In some examples, the binding ligand comprises a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the binding ligand does not comprise an antibody or fragment thereof. In some examples, the binding ligand comprises a CD16 polypeptide or fragment thereof. In some examples, the binding ligand comprises a CD16-binding polypeptide. In some examples, the binding ligand is human or humanized. In some examples, the recombinant nucleic acid further comprises a nucleic acid sequence encoding an antibody or fragment thereof capable of being bound by the binding ligand. In some examples, the antibody or fragment thereof is capable of being secreted from a cell.

[0021] In another example, the present disclosure provides a recombinant nucleic acid comprising a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising a TCR subunit comprising at least a portion of a murine TCR alpha or murine TCR beta extracellular domain, and a murine TCR alpha or murine TCR beta transmembrane domain, and an antigen binding domain comprising a ligand or a fragment thereof that binds to a receptor or polypeptide

expressed on a surface of a cell; and a sequence encoding a TCR constant domain(s), wherein the TCR constant domain is a murine TCR alpha constant domain or a murine TCR beta constant domain; or a sequence encoding a murine TCR alpha constant domain and a murine TCR beta constant domain; wherein the TCR subunit and the antigen binding domain are operatively linked, and wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR.

[0022] In some examples, the TCR subunit comprises an intracellular domain of murine TCR alpha or murine TCR beta. In some examples, the extracellular domain comprises the extracellular portion of a TCR alpha constant domain or TCR beta constant domain, or a fragment thereof. In some examples, the recombinant nucleic acid further comprises at least a partial sequence encoding a TCR gamma constant domain, a TCR delta constant domain, or a partial sequence of both a TCR gamma constant domain and a TCR delta constant domain. In some examples, the antigen binding domain comprises a ligand. In some examples, the ligand binds to the receptor of a cell. In some examples, the ligand binds to the polypeptide expressed on a surface of a cell. In some examples, the receptor or polypeptide expressed on a surface of a cell comprises a stress response receptor or polypeptide. In some examples, the receptor or polypeptide expressed on a surface of a cell is an MHC class I-related glycoprotein. In some examples, the MHC class I-related glycoprotein is selected from the group consisting of MICA, MICB, RAET1E, RAET1G, ULBP1, ULBP2, ULBP3, ULBP4 and combinations thereof. In some examples, the antigen binding domain comprises a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the antigen binding domain comprises a monomer or a dimer of the ligand or fragment thereof. In some examples, the ligand or fragment thereof is a monomer, a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octomer, a nonamer, or a decamer. In some examples, the ligand or fragment thereof is a monomer or a dimer. In some examples, the antigen binding domain does not comprise an antibody or fragment thereof. In some examples, the antigen binding domain does not comprise a variable region. In some examples, the antigen binding domain does not comprise a CDR. In some examples, the ligand or fragment thereof is a Natural Killer Group 2D (NKG2D) ligand or a fragment thereof. In some examples, the TCR constant domain incorporates into a functional TCR complex when expressed in a T cell. In some examples, the TCR constant domain incorporates into a same functional TCR complex as the functional TCR complex that incorporates the TFP when expressed in a T cell. In some examples, the sequence encoding the TFP and the sequence encoding the TCR constant domain(s) are contained within a same

nucleic acid molecule. In some examples, the TFP and the TCR constant domains are operatively linked by a first linker sequence. In some examples, the first linker comprises a protease cleavage site. In some examples, the protease cleavage site is a 2A, e.g., a T2A or a P2A cleavage site. In some examples, the sequence encoding the TFP and the sequence encoding the TCR constant domain(s) are contained within different nucleic acid molecules. In some examples, the TCR subunit and the antibody domain, the antigen binding domain or the binding ligand or fragment thereof of the TFP are operatively linked by a second linker sequence. In some examples, the second linker sequence comprises (G4S)n, wherein n=1 to 4. In some examples, the transmembrane domain is a TCR transmembrane domain from TCR alpha or TCR beta, e.g., murine TCR alpha or TCR beta. In some examples, the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, of TCR alpha or TCR beta. In some examples, the TCR extracellular domain comprises the extracellular portion of a constant domain TCR alpha chain or a TCR beta chain, e.g., a murine TCR alpha chain or a TCR beta chain, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR subunit comprises a transmembrane domain comprising a transmembrane domain of a TCR alpha chain or a TCR beta chain, e.g., a murine TCR alpha chain or a TCR beta chain, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TCR subunit comprises a TCR intracellular domain of a TCR alpha chain or a TCR beta chain, e.g., a murine TCR alpha chain or a TCR beta chain, or an amino acid sequence having at least one modification thereto. In some examples, the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain is or comprises an alpha constant domain. In some examples, the alpha constant domain has the sequence of SEQ ID NO:17, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:146, or SEQ ID NO:207 functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the encoded TCR comprising (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain is or comprises a beta constant domain. In some examples, the beta constant domain has the sequence of SEQ ID NO:18, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:152, or SEQ ID NO:209, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the extracellular domain of the TCR subunit does not comprise the variable domain of an alpha chain or a beta chain. In some examples, the TCR subunit comprises a

TCR intracellular domain comprising a stimulatory domain of a protein selected from an intracellular signaling domain of CD3 epsilon, CD3 gamma or CD3 delta, or an amino acid sequence having at least one modification thereto. In some examples, the TCR subunit of the TFP comprises the extracellular, transmembrane and intracellular domain of CD3 epsilon. In some examples, the TCR subunit of CD3 epsilon comprises the sequence of SEQ ID NO:258, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications. In some examples, the TFP, the TCR alpha constant domain, the TCR beta domain, and any combination thereof is capable of functionally interacting with an endogenous TCR complex and/or at least one endogenous TCR polypeptide.

[0023] In some examples, the TCR constant domain is a TCR alpha constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof;

[0024] the TCR constant domain is a TCR beta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof; or the TCR constant domain is a TCR alpha constant domain and a TCR beta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof.

[0025] In some examples, the at least one but not more than 20 modifications thereto comprise a modification of an amino acid that mediates cell signaling or a modification of an amino acid that is phosphorylated in response to a ligand binding to the TFP. In some examples, the antibody is an antibody fragment. In some examples, the antibody fragment is a scFv, a single domain antibody domain, a VH domain or a VL domain. In some examples, an antigen binding domain is selected from a group consisting of an anti-CD19 binding domain, an anti-B-cell maturation antigen (BCMA) binding domain, an anti-mesothelin (MSLN) binding domain, an anti-CD20 binding domain, an anti-CD70 binding domain, an anti-79b binding domain, an anti-HER2 binding domain, an anti-PMSA binding domain, an anti-MUC16 binding domain, an anti-CD22 binding domain, an anti-PD-L1 binding domain, an anti BAFF or BAFF receptor binding domain, an anti-Nectin-4 binding domain, an anti-TROP-2 binding domain, an anti-GPC3 binding domain, and an anti-ROR-1 binding domain. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID NO:60, a CDR2 of SEQ ID NO:61, and a CDR3 of SEQ ID NO:62. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID NO:63, a CDR2 of SEQ ID NO:64, and a CDR3 of SEQ ID NO:65. In some examples, the anti-MSLN binding domain comprises a CDR1 of SEQ ID

NO:66, a CDR2 of SEQ ID NO:67, and a CDR3 of SEQ ID NO:68. In some examples, the anti-MSLN binding domain comprises a sequence with at least about 80% sequence identity to a sequence of SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:71. In some examples, the anti-CD19 binding domain comprises a CDR1 of SEQ ID NO:73, a CDR2 of SEQ ID NO:75, and a CDR3 of SEQ ID NO:77. In some examples, the anti-CD19 binding domain comprises a CDR1 of SEQ ID NO:79, a CDR2 of SEQ ID NO:81, and a CDR3 of SEQ ID NO:83. In some examples, the anti-CD19 binding domain comprises a sequence with at least about 80% sequence identity to a sequence of SEQ ID NO:85 and/or SEQ ID NO:87.

[0026] In some examples, the nucleic acid is selected from the group consisting of a DNA and an RNA. In some examples, the nucleic acid is an mRNA. In some examples, the nucleic acid is a circRNA. In some examples, the recombinant nucleic acid comprises a nucleic acid analog, wherein the nucleic acid analog is not in an encoding sequence of the recombinant nucleic acid. In some examples, the nucleic analog is selected from the group consisting of 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, T-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), T-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), 2'-O-N-methylacetamido (2'-O-NMA) modified, a locked nucleic acid (LNA), an ethylene nucleic acid (ENA), a peptide nucleic acid (PNA), a 1',5'- anhydrohexitol nucleic acid (HNA), a morpholino, a methylphosphonate nucleotide, a thiolphosphonate nucleotide, and a 2'-fluoro N3-P5'-phosphoramidite. In some examples, the recombinant nucleic acid further comprises a leader sequence. In some examples, the recombinant nucleic acid further comprises a promoter sequence. In some examples, the recombinant nucleic acid further comprises a sequence encoding a poly(A) tail. In some examples, the recombinant nucleic acid further comprises a 3'UTR sequence. In some examples, the nucleic acid is an isolated nucleic acid or a non-naturally occurring nucleic acid. In some examples, the nucleic acid is an in vitro transcribed nucleic acid. In another example, the present disclosure provides a vector comprising the recombinant nucleic acid. In some examples, the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, an adeno-associated viral vector (AAV), a Rous sarcoma viral (RSV) vector, or a retrovirus vector. In some examples, the vector is an AAV6 vector. In some examples, the method further comprises a promoter. In some examples, the vector is an in vitro transcribed vector.

[0027] In another example, the present disclosure provides a modified T cell comprising the recombinant nucleic acid, or the vector, wherein the modified T cell comprises a functional disruption of an endogenous TCR.

[0028] In another example, the present disclosure provides a modified T cell comprising the sequence encoding the TFP of the nucleic acid or a TFP encoded by the sequence of the nucleic acid encoding the TFP, wherein the modified T cell comprises a functional disruption of an endogenous TCR.

[0029] In another example, the present disclosure provides a modified allogenic T cell comprising the sequence encoding the TFP or a TFP encoded by the sequence of the nucleic acid encoding the TFP.

[0030] In some examples, the T cell further comprises a heterologous sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR gamma constant domain, a TCR delta constant domain or a TCR gamma constant domain and a TCR delta constant domain. In some examples, the T cell further comprises a heterologous sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR alpha constant domain, a TCR beta constant domain or a TCR alpha constant domain and a TCR beta constant domain. In some examples, the TCR constant domain, e.g., the TCR alpha constant domain, the TCR beta constant domain or the TCR alpha constant domain and the TCR beta constant domain, is a murine TCR constant domain, e.g., a murine TCR alpha constant domain, a murine TCR beta constant domain or a murine TCR alpha constant domain and a murine TCR beta constant domain. In some examples, the endogenous TCR that is functionally disrupted is an endogenous TCR alpha chain, an endogenous TCR beta chain, or an endogenous TCR alpha chain and an endogenous TCR beta chain. In some examples, the endogenous TCR that is functionally disrupted has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell. In some examples, the functional disruption is a disruption of a gene encoding the endogenous TCR. In some examples, the disruption of a gene encoding the endogenous TCR is a removal of a sequence of the gene encoding the endogenous TCR from the genome of a T cell. In some examples, the T cell is a human T cell selected from CD4 cells, CD8 cells, naive T-cells, memory stem T-cells, central memory T- cells, double negative T-cells, effector memory T-cells, effector T-cells, ThO cells, TcO cells, Th1 cells, Tc1 cells, Th2 cells, Tc2 cells, Th17 cells, Th22 cells, alpha/beta T cells, gamma/delta T cells, natural killer (NK) cells, natural killer T (NKT) cells, hematopoietic stem cells and pluripotent stem cells. In some examples, the T cell is a CD8+ or CD4+ T cell. In some examples, the T cell is an allogenic T cell. In some examples, the method further comprises a nucleic acid encoding an inhibitory molecule that comprises a first polypeptide comprising at least a portion of an inhibitory molecule, associated with a second polypeptide comprising a positive signal from an intracellular signaling domain. In some examples, the inhibitory molecule comprises the first

polypeptide comprising at least a portion of PD1 and the second polypeptide comprising a costimulatory domain and primary signaling domain.

[0031] In another example, the present disclosure provides a pharmaceutical composition comprising: the modified T cells; and a pharmaceutically acceptable carrier.

[0032] In another example, the present disclosure provides a method of producing the modified T cell, the method comprising disrupting an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; thereby producing a T cell containing a functional disruption of an endogenous TCR gene; and transducing the T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid or the vector.

[0033] In some examples, the method further comprises disrupting comprises transducing the T cell with a nuclease protein or a nucleic acid sequence encoding a nuclease protein that targets the endogenous gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain.

[0034] In another example, the present disclosure provides a method of producing the modified T cell, the method comprising transducing a T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid, or the vector.

[0035] In some examples, the T cell containing a functional disruption of an endogenous TCR gene is a T cell containing a functional disruption of an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain.

[0036] In some examples, the T cell is a human T cell.

[0037] In some examples, the T cell containing a functional disruption of an endogenous TCR gene has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell.

[0038] In some examples, the nuclease is a meganuclease, a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a CRISPR/Cas nuclease, or a megaTAL nuclease.

[0039] In some examples, the sequence comprised by the recombinant nucleic acid or the vector is inserted into the endogenous TCR subunit gene at the cleavage site, and wherein the insertion of the sequence into the endogenous TCR subunit gene functionally disrupts the endogenous TCR subunit.

[0040] In some examples, the nuclease is a meganuclease.

[0041] In some examples, the meganuclease comprises a first subunit and a second subunit, wherein the first subunit binds to a first recognition half-site of the recognition sequence, and wherein the second subunit binds to a second recognition half-site of the recognition sequence.

[0042] In some examples, the meganuclease is a single-chain meganuclease comprising a linker, wherein the linker covalently joins the first subunit and the second subunit.

[0043] In another example, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition.

[0044] In another example, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising (a) a modified T cell produced according to the method described herein; and (b) a pharmaceutically acceptable carrier.

[0045] In another example, the present disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising (a) a modified T cell produced according to the method described herein; and (b) a pharmaceutically acceptable carrier.

[0046] In some examples, the modified T cell is an allogeneic T cell. In some examples, less cytokines are released in the subject compared a subject administered an effective amount of an unmodified control T cell. In some examples, less cytokines are released in the subject compared a subject administered an effective amount of a modified T cell comprising the recombinant nucleic acid, or the vector. In some examples, the method comprises administering the pharmaceutical composition in combination with an agent that increases the efficacy of the pharmaceutical composition. In some examples, the method comprises administering the pharmaceutical composition in combination with an agent that ameliorates one or more side effects associated with the pharmaceutical composition. In some examples, the cancer is a solid cancer, a lymphoma or a leukemia. In some examples, the cancer is selected from the group consisting of renal cell carcinoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, kidney and stomach cancer. In some examples, less cytokines are released in the subject compared to a subject administered an effective amount of an autologous T cell expressing the TFP described herein. In some examples, the method does not induce graft versus host disease. In some examples, the subject has a reduced risk of developing graft versus host disease compared to a subject administered an effective amount of an autologous T cell expressing the TFP described herein.

[0047] In another example, the present disclosure provides the recombinant nucleic acid, the vector, the modified T cell, or the pharmaceutical composition, for use as a medicament or in the preparation of a medicament.

INCORPORATION BY REFERENCE

[0048] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] Figure 1 is a series of graphs showing surface expression of CD3 (SK7) vs TCRαβ (IP26) in TRA-edited (left) and TRB-edited (right) cells. Wild type Jurkat cells were edited at either the TRAC or TRBC genes to disrupt TRA or TRB surface expression. Cells negative for CD3 and TCRαβ were purified using Magnetic-Activated Cell Sorting (MACS). The gates on the plots were drawn to delineate CD3 and TCRαβ negative-negative population of cells and the percentages of cells remaining in each quadrant are shown in the corners.

[0050] Figure 2 is a series of graphs showing surface expression of CD3 (SK7) vs TCRαβ (IP26) in TRA-/- (left) and TRB-/- (right) Jurkat cells. The first panel from the left is a non-transduced negative control. The second panel shows TRB-/- cells transduced with a TCRβ Full Length TFP. The third and fourth panels show TRA-/- and TRB-/- transduced with a TCRγδ TFP construct.

[0051] Figure 3 is a drawing showing various strategies to generate allogeneic TFP T cells.

[0052] Figure 4 is a schematic showing elements of the constructs used to generate allogeneic TFP T cells.

[0053] Figure 5 is a series of graphs showing surface expression of CD3 vs TCRαβ in T cells transduced with TCRγδ TFP constructs after editing the TRAC locus versus wild type cells and TRAC edited, non-transduced T cells. The graphs depict cell populations prior to MACS purification.

[0054] Figure 6 is a series of graphs showing surface expression of CD3 vs TCRαβ in T cells transduced with TCRγδ TFP constructs after editing the TRAC locus versus wild type cells and TRAC edited, non-transduced T cells. Graphs depict cell populations after MACS purification.

[0055] Figure 7 is a series of graphs showing surface expression of allogeneic TFP transduced T cells (TFP+). The left-hand panels show TFP+ percentage of T cells transduced with TCRγδ TFP

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant nucleic acid comprising:

(a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising

(i) a TCR subunit comprising

(1) at least a portion of a TCR extracellular domain, and

(2) a transmembrane domain, and

(ii) an antibody or fragment thereof comprising an antigen binding domain; and

(b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR gamma constant domain, a TCR delta constant domain, or a TCR gamma constant domain and a TCR delta constant domain;

wherein the TCR subunit and the antibody or fragment thereof are operatively linked;

wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR;

optionally, wherein the antibody or fragment thereof is murine, human or humanized; and

optionally, wherein the antibody or fragment thereof binds to a cell surface antigen on the surface of a tumor cell.

2. The recombinant nucleic acid of claim 1, wherein the TCR extracellular domain comprises an extracellular domain or portion thereof of a TCR gamma chain or functional fragment thereof, a TCR delta chain or functional fragment thereof, a CD3 epsilon subunit or functional fragment thereof, a CD3 gamma subunit or functional fragment thereof, a CD3 delta subunit or functional fragment thereof, or amino acid sequences thereof having at least one but not more than 20 modifications.

3. The recombinant nucleic acid of claim 1 or 2, wherein the TCR subunit comprises at least a portion of a TCR gamma extracellular domain or at least a portion of a TCR delta extracellular domain.

4. The recombinant nucleic acid of any one of claims 1-3, wherein the TCR constant domain is:

(i) a TCR delta constant domain, and the TCR delta constant domain comprises SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243, or SEQ ID NO:265, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications;

(ii) a TCR gamma constant domain, and the TCR gamma constant domain comprises SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications; or
(iii) a sequence encoding a TCR gamma constant domain and a TCR delta constant domain, and wherein the TCR gamma constant domain comprises SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications, and/or the TCR delta constant domain comprises SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243, or SEQ ID NO:265, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications.

5. The recombinant nucleic acid of any one of claims 1-4, wherein the sequence encoding the TCR gamma constant domain further encodes a TCR gamma variable domain, thereby encoding a full TCR gamma domain, and/or the sequence encoding the TCR delta constant domain further encodes a TCR delta variable domain, thereby encoding a full TCR delta domain;
optionally, wherein the full TCR gamma domain is gamma 9 or gamma 4, and/or the full TCR delta domain is delta 2 or delta 1, and
optionally, wherein the full TCR gamma domain comprises SEQ ID NO:255, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications, and/or the full TCR delta constant domain comprises SEQ ID NO:256, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications.

6. The recombinant nucleic acid of any one of claims 1-5, wherein the transmembrane domain of the TFP is a TCR transmembrane domain from CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha, TCR beta, TCR delta, or TCR gamma.

7. The recombinant nucleic acid of any one of claims 1-6, wherein the recombinant nucleic acid further comprises a sequence encoding a second antigen binding domain, wherein the second antigen binding domain is operatively linked to the TCR delta constant domain or TCR gamma constant domain, and wherein:
   (a) the TCR subunit comprises (i) at least a portion of a TCR gamma extracellular domain and (ii) a TCR gamma or TCR delta transmembrane domain; and the TCR constant domain is a TCR delta constant domain;
   (b) the TCR subunit comprises (i) at least a portion of a TCR delta extracellular domain and (ii) a TCR gamma or TCR delta transmembrane domain; and the TCR constant domain is a TCR gamma constant domain

wherein the second antigen binding domain is the same or different as the antigen binding domain of the TFP; and

optionally, wherein the second antigen binding domain is operatively linked to the sequence encoding the TCR constant domain via a linker

8. The recombinant nucleic acid of any one of claims 1-7, wherein the TCR constant domain is operatively linked to a TCR gamma or TCR delta transmembrane domain, and wherein:

(i) the TCR subunit comprises (i) at least a portion of a TCR gamma extracellular domain and (ii) a TCR gamma transmembrane domain; and wherein the TCR constant domain is a TCR delta constant domain and is operatively linked to a TCR delta transmembrane domain;

(ii) the TCR subunit comprises (i) at least a portion of a TCR gamma extracellular domain and (ii) a TCR delta transmembrane domain; and wherein the TCR constant domain is a TCR delta constant domain and is operatively linked to a TCR gamma transmembrane domain;

(iii) the TCR subunit comprises (i) at least a portion of a TCR delta extracellular domain and (ii) a TCR delta transmembrane domain; and wherein the TCR constant domain is a TCR gamma constant domain and is operatively linked to a TCR gamma transmembrane domain, or

(iv) the TCR subunit comprises (i) at least a portion of a TCR delta extracellular domain and (ii) a TCR gamma transmembrane domain; and wherein the TCR constant domain is a TCR gamma constant domain and is operatively linked to a TCR delta transmembrane domain.

9. The recombinant nucleic acid of any one of claims 1-8, wherein the recombinant nucleic acid further comprises:

(i) at least one leader sequence and at least one linker;

(ii) a partial sequence encoding a portion of a TCR alpha constant domain, a partial sequence encoding a portion of a TCR beta domain, or a partial sequence encoding a portion of a TCR alpha constant domain and a portion of a TCR beta constant domain; and/or

(iii) a sequence encoding a TCR alpha transmembrane domain, a sequence encoding a TCR beta transmembrane domain, or a sequence encoding a TCR alpha transmembrane domain and a sequence encoding a TCR beta transmembrane domain.

10. The recombinant nucleic acid of any one of claims 1-9, wherein the TCR constant domain incorporates into a functional TCR complex when expressed in a T cell; and

optionally, wherein the TCR constant domain incorporates into a same functional TCR complex as the functional TCR complex that incorporates the TFP when expressed in a T cell.

11. The recombinant nucleic acid of any one of claims 1-10, wherein the sequence encoding the TFP and the sequence encoding the TCR constant domain are contained within a same nucleic acid molecule or within different nucleic acid molecules.

12. The recombinant nucleic acid of any one of claims 1-11, wherein:

(i) the sequence encoding the TFP and the sequence encoding the TCR constant domain are contained within a same nucleic acid molecule, and the encoded TFP and the encoded TCR constant domains are operatively linked by a first linker sequence;

optionally, wherein the first linker comprises a protease cleavage site;

optionally, wherein the protease cleavage site is a 2A; and

optionally, wherein the 2A is a T2A or a P2A cleavage site; and/or

(ii) the TCR subunit and the antibody or fragment thereof of the TFP are operatively linked by a second linker sequence; and

optionally, wherein the second linker sequence comprises $(G_4S)_n$, wherein n=1 to 4.

13. The recombinant nucleic acid of any one of claims 1-12, wherein the TCR subunit further comprises:

(i) an intracellular domain comprising a stimulatory domain from an intracellular signaling domain of CD3 epsilon, CD3 gamma, or CD3 delta or an amino acid sequence having at least one modification thereto; and/or

(ii) an intracellular domain of TCR alpha, TCR beta, TCR gamma, or TCR delta,

optionally, wherein the intracellular domain of the TCR subunit is derived from only CD3 epsilon, only CD3 gamma, only CD3 delta, only TCR alpha, only TCR beta, only TCR gamma, or only TCR delta.

14. The recombinant nucleic acid of any one of claims 6-13, wherein the TCR constant domain is operatively linked to a TCR gamma or TCR delta transmembrane domain, and wherein the TCR gamma or TCR delta transmembrane domain operatively linked to the TCR constant domain is operatively linked to an intracellular domain comprising an intracellular domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.

15. The recombinant nucleic acid of any one of claims 1-14, wherein the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain, wherein at least two of (i), (ii), and (iii) are from the same TCR subunit.

16. The recombinant nucleic acid of claim 1-16, wherein the TCR subunit comprises (i) at least a portion of a TCR extracellular domain, (ii) a TCR transmembrane domain, and (iii) a TCR intracellular domain that is or comprises a delta constant domain or fragment thereof or a gamma constant domain or fragment thereof ;
optionally, wherein the TCR extracellular domain comprises the extracellular portion of a constant domain of a TCR gamma chain or functional fragment thereof or an amino acid sequences thereof having at least one but not more than 20 modifications, the extracellular portion of a TCR delta chain or functional fragment thereof or an amino acid sequence thereof having at least one but not more than 20 modifications;
optionally, wherein the delta constant domain has the sequence of SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:243 or SEQ ID NO:265, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications, and/or the gamma constant domain has the sequence of SEQ ID NO:21 or SEQ ID NO:155, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications; and
optionally, wherein the extracellular domain of the TFP does not comprise the variable domain of a gamma chain or a delta chain.

17. The recombinant nucleic acid of claim 13, wherein the TCR subunit of the TFP comprises the extracellular, transmembrane and intracellular domain of CD3 epsilon; and
optionally, wherein the TCR subunit of CD3 epsilon comprises the sequence of SEQ ID NO:258, functional fragments thereof, or amino acid sequences thereof having at least one but not more than 20 modifications.

18. The recombinant nucleic acid of any one of claims 1-17, wherein:
(i) the TFP, the TCR gamma constant domain, the TCR delta constant domain, and any combination thereof is capable of functionally interacting with an endogenous TCR complex and/or at least one endogenous TCR polypeptide;
(ii) the TCR constant domain is a TCR gamma constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR delta, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof;
(iii) the TCR constant domain is a TCR delta constant domain and the TFP functionally integrates into a TCR complex comprising an endogenous subunit of TCR gamma, CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof; or
(iv) the TCR constant domain is a TCR gamma constant domain and a TCR delta constant domain and the TFP functionally integrates into a TCR complex comprising an

endogenous subunit of CD3 epsilon, CD3 gamma, CD3 delta, or a combination thereof.

19. The recombinant nucleic acid of any one of claims 1-18, wherein the antibody or fragment thereof is an antibody fragment, wherein the antibody fragment is a scFv, a single domain antibody domain, a V_H domain or a V_L domain.

20. The recombinant nucleic acid of any one of claims 1-19, wherein the sequence comprises, from 5' to 3':

(i) a first leader sequence, an antigen binding domain sequence, a linker, a TRDC gene sequence, a cleavable linker sequence, a second leader sequence, and a TRGC gene sequence;

(ii) a first leader sequence, a TRDC gene sequence, a cleavable linker sequence, a second leader sequence, an antigen binding domain sequence, a linker sequence, and a TRGC gene sequence;

(iii) a first leader sequence, an antigen binding domain sequence, a first linker sequence, a TRDC gene sequence, a cleavable linker, a second leader sequence, a second antigen binding domain sequence, a second linker sequence, and a TRGC gene sequence;

(iv) a first leader sequence, a TRDC gene sequence, a first cleavable linker sequence, a second leader sequence, a TRGC gene sequence, a second cleavable linker sequence, a third leader sequence, an antigen binding domain sequence, a linker sequence, and a CD3 epsilon gene sequence; or

(v) a first leader sequence, a first antigen binding domain sequence, a first linker sequence, a TRDC gene sequence or fragment thereof, a TRAC gene sequence or fragment thereof, a cleavable linker sequence, a second leader sequence, a second antigen binding domain sequence, a second linker sequence, a TRGC gene sequence or fragment thereof, and a TRBC gene sequence or fragment thereof.

21. The recombinant nucleic acid of any one of claims 1-20, wherein the sequence encodes any one selected from the group consisting of the polypeptides of SEQ ID NOs: 1-5, 242, 244-246, 248, 250, 252, 257, 263, and 264.

22. The recombinant nucleic acid of any one of claims 1-21, wherein the antigen binding domain is selected from the group consisting of an anti-CD19 binding domain, an anti-B-cell maturation antigen (BCMA) binding domain, an anti-mesothelin (MSLN) binding domain, an anti-CD20 binding domain, an anti-CD70 binding domain, an anti-79b binding domain, an anti-HER2 binding domain, an anti-PMSA binding domain, an anti-MUC16 binding domain, an anti-CD22 binding domain, an anti-PD-L1 binding domain, an anti BAFF or BAFF receptor binding domain, an anti-Nectin-4 binding domain, an

anti-TROP-2 binding domain, an anti-GPC3 binding domain, and an anti-ROR-1 binding domain; and

optionally, wherein:

(a) the anti-MSLN binding domain comprises:

(i) a CDR1 of SEQ ID NO:60, a CDR2 of SEQ ID NO:61, and a CDR3 of SEQ ID NO:62;

a CDR1 of SEQ ID NO:63, a CDR2 of SEQ ID NO:64, and a CDR3 of SEQ ID NO:65; or

a CDR1 of SEQ ID NO:66, a CDR2 of SEQ ID NO:67, and a CDR3 of SEQ ID NO:68; and/or

(ii) a sequence with at least about 80% sequence identity to the sequence of SEQ ID NO:69, SEQ ID NO:70, or SEQ ID NO:71; or

(b) the anti-CD19 binding domain comprises:

(i) a light chain CDR1 of SEQ ID NO:73, a CDR2 of SEQ ID NO:75, and a CDR3 of SEQ ID NO:77; and/or

a heavy chain CDR1 of SEQ ID NO:79, a CDR2 of SEQ ID NO:81, and a CDR3 of SEQ ID NO:83.

(ii) a light chain variable region having at least about 80% sequence identity to the sequence of SEQ ID NO:85, and/or a heavy chain variable region having at least about 80% sequence identity to the sequence of SEQ ID NO:87.

23. A recombinant nucleic acid comprising:

(a) a sequence encoding a T cell receptor (TCR) fusion protein (TFP) comprising

(i) a TCR subunit comprising

(1) at least a portion of a TCR extracellular domain, and

(2) a transmembrane domain, and

(ii) an antibody or fragment thereof comprising an antigen binding domain; and

(b) a sequence encoding a TCR constant domain, wherein the TCR constant domain is a TCR delta constant domain;

wherein the TCR subunit and the antibody or fragment thereof are operatively linked;

wherein the TCR extracellular domain comprises an extracellular domain of a TCR gamma chain or portion thereof;

wherein the recombinant nucleic acid further comprises a sequence encoding a second antigen binding domain, wherein the second antigen binding domain is operatively linked to the TCR constant domain;

wherein the TFP functionally incorporates into a TCR complex when expressed in a modified T cell comprising a functional disruption of an endogenous TCR; and
wherein the TCR constant domain functionally incorporates into the TCR complex when expressed in the modified T cell.

24. The recombinant nucleic acid of any one of claims 1-23, wherein:
(i) the nucleic acid is selected from the group consisting of a DNA and an RNA; and
optionally, wherein the nucleic acid is an mRNA or a circRNA; and/or
(ii) wherein the recombinant nucleic acid comprises a nucleic acid analog, wherein the nucleic acid analog is not in an encoding sequence of the recombinant nucleic acid; and
optionally, wherein the nucleic analog is selected from the group consisting of 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl, 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), 2'-O-N-methylacetamido (2'-O-NMA) modified, a locked nucleic acid (LNA), an ethylene nucleic acid (ENA), a peptide nucleic acid (PNA), a 1',5'-anhydrohexitol nucleic acid (HNA), a morpholino, a methylphosphonate nucleotide, a thiolphosphonate nucleotide, and a 2'-fluoro N3-P5'-phosphoramidite.

25. The recombinant nucleic acid of any one of claims 1-24, further comprising a leader sequence, a promoter sequence, a sequence encoding a poly(A) tail, and/or a 3'UTR sequence.

26. The recombinant nucleic acid of any one of claims 1-25, wherein the recombinant nucleic acid is a vector;
optionally, wherein the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentivirus vector, adenoviral vector, an adeno-associated viral vector (AAV), a Rous sarcoma viral (RSV) vector, or a retrovirus vector; and
optionally, wherein the vector is an AAV6 vector.

27. A modified T cell comprising:
(i) the recombinant nucleic acid of any one of claims 1-26, wherein the modified T cell comprises a functional disruption of an endogenous TCR; or
(ii) the TFP and the TCR constant domain encoded by the recombinant nucleic acid of any one of claims 1-26,
wherein the modified T cell comprises a functional disruption of an endogenous TCR.

28. The modified T cell of claim 27, wherein:

(i) the endogenous TCR that is functionally disrupted is an endogenous TCR alpha chain, an endogenous TCR beta chain, or an endogenous TCR alpha chain and an endogenous TCR beta chain;

(ii) the endogenous TCR that is functionally disrupted has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell; and/or

(iii) the functional disruption is a disruption of a gene encoding the endogenous TCR;

optionally, wherein the disruption of a gene encoding the endogenous TCR is a removal of a sequence of the gene encoding the endogenous TCR from the genome of a T cell.

29. The modified T cell of claim 27 or 28, wherein the T cell further comprises a heterologous sequence encoding:

(i) a TCR constant domain that is a TCR gamma constant domain, a TCR delta constant domain, or a TCR gamma constant domain and a TCR delta constant domain, or

(ii) a TCR constant domain that is a TCR alpha constant domain, a TCR beta constant domain, or a TCR alpha constant domain and a TCR beta constant domain;

optionally, wherein the TCR constant domain is a murine TCR constant domain.

30. The modified T cell of any one of claims 27-29, wherein the T cell is a human T cell selected from CD4 cells, CD8 cells, naive T-cells, memory stem T-cells, central memory T- cells, double negative T-cells, effector memory T-cells, effector T-cells, ThO cells, TcO cells, Th1 cells, Tc1 cells, Th2 cells, Tc2 cells, Th17 cells, Th22 cells, alpha/beta T cells, gamma/delta T cells, natural killer (NK) cells, natural killer T (NKT) cells, hematopoietic stem cells, and pluripotent stem cells;

optionally, wherein the T cell is a CD8+ or CD4+ T cell; and

optionally, wherein the T cell is an allogenic T cell.

31. The modified T cell of any one of claims 27-30, further comprising a nucleic acid encoding an inhibitory molecule that comprises a first polypeptide comprising at least a portion of an inhibitory molecule, associated with a second polypeptide comprising a positive signal from an intracellular signaling domain; and

optionally, wherein the inhibitory molecule comprises the first polypeptide comprising at least a portion of PD1 and the second polypeptide comprising a costimulatory domain and primary signaling domain.

32. A medicament comprising:

(a) the modified T cells of any one of claims 27-31; and

(b) a pharmaceutically acceptable carrier.

33. A method of producing the modified T cell of any one of claims 27-31, the method comprising:

(i) (a) disrupting an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; thereby producing a T cell containing a functional disruption of an endogenous TCR gene; and
(b) transducing the T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid of any one of claims 1-26;
optionally, wherein disrupting comprises transducing the T cell with a nuclease protein or a nucleic acid sequence encoding a nuclease protein that targets the endogenous gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; or

(ii) transducing a T cell containing a functional disruption of an endogenous TCR gene with the recombinant nucleic acid of any one of claims 1-26;
optionally, wherein the T cell containing a functional disruption of an endogenous TCR gene is a T cell containing a functional disruption of an endogenous TCR gene encoding a TCR alpha chain, a TCR beta chain, or a TCR alpha chain and a TCR beta chain; and
optionally, wherein the T cell containing a functional disruption of an endogenous TCR gene has reduced binding to MHC-peptide complex compared to that of an unmodified control T cell;

optionally, wherein the T cell is a human T cell; and

optionally, wherein the sequence comprised by the recombinant nucleic acid or the vector is inserted into the endogenous TCR subunit gene at the cleavage site, and wherein the insertion of the sequence into the endogenous TCR subunit gene functionally disrupts the endogenous TCR subunit.

34. The method of claim 33, wherein the nuclease is a meganuclease, a zinc-finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a CRISPR/Cas nuclease, or a megaTAL nuclease; and

optionally, wherein the nuclease is a meganuclease,

optionally, wherein the meganuclease comprises a first subunit and a second subunit, wherein the first subunit binds to a first recognition half-site of the recognition sequence, and the second subunit binds to a second recognition half-site of the recognition sequence; or the meganuclease is a single-chain meganuclease comprising a linker, wherein the linker covalently joins the first subunit and the second subunit.

35. Use of a pharmaceutical composition in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, the pharmaceutical composition comprising: (i) the medicament of claim 32, or (ii) a medicament comprising (a) a

modified T cell produced according to the method of claim 33 or 34, and (b) a pharmaceutically acceptable carrier, optionally, wherein the modified T cell is an allogeneic T cell;

wherein a therapeutically effective amount of (i) the medicament of claim 32, or (ii) the medicament comprising (a) a modified T cell produced according to the method of claim 33 or 34, and (b) a pharmaceutically acceptable carrier is to be administered to the subject; and

optionally, wherein:

(a) the cancer is a solid cancer, a lymphoma or a leukemia; and/or

(b) the cancer is selected from the group consisting of renal cell carcinoma, breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer, kidney cancer, and stomach cancer.

36. The use of claim 35, wherein (i) the medicament of claim 32, or (ii) the medicament comprising (a) a modified T cell produced according to the method of claim 33 or 34, and (b) a pharmaceutically acceptable carrier is to be administered in combination with an agent that increases the efficacy of (i) the medicament of claim 32, or (ii) the medicament comprising (a) a modified T cell produced according to the method of claim 33 or 34, and (b) a pharmaceutically acceptable carrier, and/or an agent that ameliorates one or more side effects associated with (i) the medicament of claim 32, or (ii) the medicament comprising (a) a modified T cell produced according to the method of claim 33 or 34, and (b) a pharmaceutically acceptable carrier.

37. The use of claim 35 or 36, wherein the administration does not induce graft versus host disease and/or the subject has a reduced risk of developing graft versus host disease compared to a subject administered an effective amount of an autologous T cell that only expresses the TFP according to any one of claims 1-26.

38. The recombinant nucleic acid of any one of claims 1-26 or the modified T cell of any one of claims 27-31 for use as a medicament or in the preparation of a medicament.