

signaling domain from 4-1BB/CD137 and five additional amino acids. In one aspect, the present disclosure provides an immune cell comprising a chimeric antigen receptor (CAR), wherein the (CAR) comprises: (a) an extracellular domain comprising an antigen-binding domain; (b) a transmembrane domain; and (c) an intracellular domain comprising a costimulatory endodomain, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids.

In some embodimentexamples, the chimeric antigen receptor is a single polypeptide. In some embodimentexamples, the chimeric antigen receptor is comprised of two polypeptides.

In some embodimentexamples, the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids, wherein the five additional amino acids are encoded by SEQ ID NO: 1. In some embodimentexamples, the costimulatory endodomain comprises SEQ ID NO: 2.

In some embodimentexamples, the antigen-binding domain is humanized. In some embodimentexamples, the antigen-binding domain is human. In some embodimentexamples, the antigen-binding domain is an scFv. In some embodimentexamples, the antigen-binding domain specifically binds an antigen associated with a disease. In some embodimentexamples, the antigen-binding domain specifically binds a tumor antigen. In some embodimentexamples, the antigen-binding domain specifically binds to an antigen selected from the group consisting of: glypican-3 (GPC3), malignancy variant receptor (MVR), and CD19.

In some embodimentexamples, the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8 alpha, CD8 beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6. In some embodimentexamples, the transmembrane domain is a transmembrane domain from

CD8 α . In some [embodimentexamples](#), the intracellular domain further comprises an intracellular domain from CD3 ζ .

In some [embodimentexamples](#), the chimeric antigen receptor further comprises a signal peptide or leader sequence. In some [embodimentexamples](#), the chimeric antigen receptor further comprises a hinge region. In some [embodimentexamples](#), the hinge region is a CD8 α hinge. In some [embodimentexamples](#), the chimeric antigen receptor further comprises an additional antigen-binding domain. In some [embodimentexamples](#), the additional antigen-binding domain is an scFv.

In some [embodimentexamples](#), the immune cell is a human immune cell. In some [embodimentexamples](#), the human immune cell is an autologous human immune cell. In some [embodimentexamples](#), the human immune cell is an allogenic human immune cell. In some [embodimentexamples](#), the immune cell is a T cell. In some [embodimentexamples](#), the immune cell is an NK cell.

Provided herein are nucleic acids encoding a chimeric antigen receptor (CAR), wherein the chimeric antigen receptor comprises: (a) an extracellular domain comprising an antigen-binding domain; (b) a transmembrane domain; and (c) an intracellular domain comprising a costimulatory endodomain, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids. In another aspect, the present disclosure provides a nucleic acid encoding a chimeric antigen receptor (CAR), wherein the chimeric antigen receptor comprises: an extracellular domain comprising an antigen-binding domain; a transmembrane domain; and (c) an intracellular domain comprising a costimulatory endodomain, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids.

In some [embodimentexamples](#), the chimeric antigen receptor is a single polypeptide. In some [embodimentexamples](#), the chimeric antigen receptor is comprised of two polypeptides.

In some [embodimentexamples](#), the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids, wherein the five additional amino acids are encoded by a nucleotide sequence of SEQ ID NO: 1. In some [embodimentexamples](#), the costimulatory endodomain comprises SEQ ID NO: 2.

In some [embodimentexamples](#), the antigen-binding domain is humanized. In some [embodimentexamples](#), the antigen-binding domain is human. In some [embodimentexamples](#), the antigen-binding domain is an scFv. In some [embodimentexamples](#), the antigen-binding domain specifically binds an antigen associated with a disease. In some [embodimentexamples](#), the antigen-binding domain specifically binds a tumor antigen. In some [embodimentexamples](#), the antigen-binding domain specifically binds to an antigen selected from the group consisting of: glycan-3 (GPC3), malignancy variant receptor (MVR), and CD19.

In some [embodimentexamples](#), the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell

receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8 alpha, CD8 beta, CD96 (Tactile),
 5 CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9
 10 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6. In some [embodiment examples](#), the transmembrane domain is a transmembrane domain from CD8 alpha.

In some [embodiment examples](#), the intracellular domain further comprises an intracellular domain from CD3 ζ . In some [embodiment examples](#), the chimeric antigen receptor further comprises a signal peptide or leader sequence. In some [embodiment examples](#), the
 20 chimeric antigen receptor further comprises a hinge region. In some [embodiment examples](#), the hinge region is a CD8 α hinge.

Provided herein are vectors comprising any one of the nucleic acids described herein. In one aspect, the present disclosure provides a vector comprising the nucleic acid disclosed herein. In some [embodiment examples](#), the vector further comprises a promoter operationally linked to the nucleic acid. In some [embodiment examples](#), the promoter is a constitutive promoter. In some [embodiment examples](#), the promoter is an inducible promoter. In some [embodiment examples](#), the vector is a viral vector. In some [embodiment examples](#), the viral vector is a lentiviral vector.

Provided herein are methods of producing an engineered immune cell, the method comprising: introducing into an immune cell any one of the nucleic acids described herein or any one of the vectors described herein, thereby producing the engineered immune cell. In another aspect, the present disclosure provides a method of producing an engineered immune cell, the method comprising: introducing into an immune cell a nucleic acid disclosed herein or a vector disclosed herein, thereby producing the engineered immune cell. In some [embodiment examples](#), the method further comprises, after the introducing step, culturing the engineered immune cell.
 30 In some [embodiment examples](#), the immune cell is a T cell. In some [embodiment examples](#), the immune cell is a NK cell.

In some embodimentexamples, the method further comprises, before the introducing step, obtaining the immune cell from a subject. In some embodimentexamples, the method further comprises administering the engineered immune cell to the subject. In some embodimentexamples, the subject has been diagnosed or identified as having a cancer.

5 Provided herein are engineered immune cells produced by any one of the methods described herein. In one aspect, the present disclosure provides an engineered immune cell produced by the method disclosed herein.

10 Provided herein are pharmaceutical compositions comprising any one of the engineered immune cells described herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a pharmaceutical composition comprising the engineered immune cell disclosed herein and a pharmaceutically acceptable carrier.

15 Provided herein are methods of treating a cancer in a subject, the method comprising administering to the subject any one of the engineered immune cells described herein or any one of the pharmaceutical compositions described herein. In yet another aspect, the present disclosure provides use of the engineered immune cell disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a cancer in a subject. In some embodimentexamples, the cancer is an anti-glypican-3-associated cancer, an anti-CD19-associated cancer, or an anti-MVR-associated cancer. In some embodimentexamples, the cancer is carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphomas), blastoma, sarcoma, leukemia, squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, lung adenocarcinoma, squamous cell carcinoma of the lung, peritoneal cancer, hepatocellular carcinoma, gastric cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary carcinoma, kidney cancer, prostate cancer, vulvar cancer, thyroid cancer, liver carcinoma, other lymphoproliferative disorders, and various types of head and neck cancer. In some embodimentexamples, the subject has previously been administered one or more additional anticancer therapies selected from the group consisting of: ionizing radiation, a chemotherapeutic agent, a therapeutic antibody, and a checkpoint inhibitor. In some embodimentexamples, the subject has been identified or diagnosed as having the cancer.

30 BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows a schematic of an exemplary MVR CAR construct.

FIG. 2 shows exemplary enzyme mapping results after MVRL2H2-4-1BB cloning.

35 **FIG. 3** shows restriction enzyme digestion results of huGC33(VH-VL)-euBBz, wherein the expected size is marked on the DNA ladders and the results are shown in gel electrophoresis images.

WHAT IS CLAIMED IS:

1. An immune cell comprising a chimeric antigen receptor (CAR), wherein the (CAR) comprises:
 - (a) an extracellular domain comprising an antigen-binding domain;
 - 5 (b) a transmembrane domain; and
 - (c) an intracellular domain comprising a costimulatory endodomain, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids.
- 10 2. The immune cell of claim 1, wherein the chimeric antigen receptor is a single polypeptide.
3. The immune cell of claim 1, wherein the chimeric antigen receptor is comprised of two polypeptides.
- 15 4. The immune cell of any one of claims 1-3, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids, wherein the five additional amino acids are encoded by SEQ ID NO: 1.
- 20 5. The immune cell of claim 4, wherein the costimulatory endodomain comprises SEQ ID NO: 2.
6. The immune cell of any one of claims 1-5, wherein the antigen-binding domain is humanized.
- 25 7. The immune cell of any one of claims 1-6, wherein the antigen-binding domain is human.
8. The immune cell of any one of claims 1-7, wherein the antigen-binding domain is an scFv.

9. The immune cell of any one of claims 1-8, wherein the antigen-binding domain specifically binds an antigen associated with a disease.
10. The immune cell of any one of claims 1-9, wherein the antigen-binding domain specifically binds a tumor antigen.
11. The immune cell of any one of claims 1-10, wherein the antigen-binding domain specifically binds to an antigen selected from the group consisting of: glypican-3 (GPC3), malignancy variant receptor (MVR), and CD19.
12. The immune cell of any one of claims 1-11, wherein the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8alpha, CD8beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class 1 molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6.
13. The immune cell of any one of claims 1-12, wherein the transmembrane domain is a transmembrane domain from CD8 α .

14. The immune cell of any one of claims 1-13, wherein the intracellular domain further comprises an intracellular domain from CD3 ζ .
- 5 15. The immune cell of any one of claims 1-14, wherein the chimeric antigen receptor further comprises a signal peptide or leader sequence.
16. The immune cell of any one of claims 1-15, wherein the chimeric antigen receptor further comprises a hinge region.
- 10 17. The immune cell of claim 16, wherein the hinge region is a CD8 α hinge.
18. The immune cell of any one of claims 1-17, wherein the chimeric antigen receptor further comprises an additional antigen-binding domain.
- 15 19. The immune cell of claim 18, wherein the additional antigen-binding domain is an scFv.
20. The immune cell of any one of claims 1-19, wherein the immune cell is a human immune cell.
- 20 21. The immune cell of claim 20, wherein the human immune cell is an autologous human immune cell.
22. The immune cell of claims 20, wherein the human immune cell is an allogenic human
- 25 immune cell.
23. The immune cell of any one of claims 1-22, wherein the immune cell is a T cell.
24. The immune cell of any one of claims 1-23, wherein the immune cell is an NK cell.
- 30

25. A nucleic acid encoding a chimeric antigen receptor (CAR), wherein the chimeric antigen receptor comprises:
- (a) an extracellular domain comprising an antigen-binding domain;
 - (b) a transmembrane domain; and
 - (c) an intracellular domain comprising a costimulatory endodomain, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids.
26. The nucleic acid of claim 25, wherein the chimeric antigen receptor is a single polypeptide.
27. The nucleic acid of claim 25, wherein the chimeric antigen receptor is comprised of two polypeptides.
28. The nucleic acid of any one of claims 25-27, wherein the costimulatory endodomain comprises an intracellular signaling domain from 4-1BB/CD137 and five additional amino acids, wherein the five additional amino acids are encoded by a nucleotide sequence of SEQ ID NO: 1.
29. The nucleic acid of claim 28, wherein the costimulatory endodomain comprises SEQ ID NO: 2.
30. The nucleic acid of any one of claims 25-29, wherein the antigen-binding domain is humanized.
31. The nucleic acid of any one of claims 25-30, wherein the antigen-binding domain is human.
32. The nucleic acid of any one of claims 25-31, wherein the antigen-binding domain is an scFv.

33. The nucleic acid of any one of claims 25-32, wherein the antigen-binding domain specifically binds an antigen associated with a disease.
34. The nucleic acid of any one of claims 25-33, wherein the antigen-binding domain specifically binds a tumor antigen.
35. The nucleic acid of any one of claims 25-34, wherein the antigen-binding domain specifically binds to an antigen selected from the group consisting of: glycan-3 (GPC3), malignancy variant receptor (MVR), and CD19.
36. The nucleic acid of any one of claims 25-35, wherein the transmembrane domain is a transmembrane domain selected from a protein selected from the group consisting of: 4-1BB/CD137, an activating NK cell receptor, an immunoglobulin protein, B7-H3, BAFFR, BLAME (SLAMF8), BTLA, CD100 (SEMA4D), CD103, CD160 (BY55), CD18, CD19, CD19a, CD2, CD247, CD27, CD276 (B7-H3), CD28, CD29, CD3 delta, CD3 epsilon, CD3 gamma, CD3 zeta, CD30, CD4, CD40, CD49a, CD49D, CD49f, CD69, CD7, CD84, CD8, CD8 alpha, CD8 beta, CD96 (Tactile), CD11a, CD11b, CD11c, CD11d, CDS, CEACAM1, CRT AM, cytokine receptor, DAP-10, DNAM1 (CD226), Fc gamma receptor, GADS, GITR, HVEM (LIGHTR), IA4, ICAM-1, Ig alpha (CD79a), IL-2R beta, IL-2R gamma, IL-7R alpha, inducible T cell costimulator (ICOS), an integrin, ITGA4, ITGA6, ITGAD, ITGAE, ITGAL, ITGAM, ITGAX, ITGB2, ITGB7, ITGB1, KIRDS2, LAT, LFA-1, a ligand that specifically binds with CD83, LIGHT, LTBR, Ly9 (CD229), lymphocyte function-associated antigen-1 (LFA-1), an MHC class I molecule, NKG2C, NKG2D, NKp30, NKp44, NKp46, NKp80 (KLRF1), OX-40, PAG/Cbp, programmed death-1 (PD-1), PSGL1, SELPLG (CD162), a Signaling Lymphocytic Activation Molecule (a SLAM protein), SLAM (SLAMF1), SLAMF4 (CD244), SLAMF6 (NTB-A), SLAMF7, SLP-76, a TNF receptor protein, TNFR2, TNFSF14, a Toll ligand receptor, TRANCE/RANKL, VLA1, and VLA-6.
37. The nucleic acid of any one of claims 25-36, wherein the transmembrane domain is a transmembrane domain from CD8 alpha.

38. The nucleic acid of any one of claims 25-37, wherein the intracellular domain further comprises an intracellular domain from CD3 ζ .
- 5 39. The nucleic acid of any one of claims 25-38, wherein the chimeric antigen receptor further comprises a signal peptide or leader sequence.
40. The nucleic acid of any one of claims 25-39, wherein the chimeric antigen receptor further comprises a hinge region.
- 10 41. The nucleic acid of claim 40, wherein the hinge region is a CD8 α hinge.
42. A vector comprising the nucleic acid of any one of claims 25-41.
- 15 43. The vector of claim 42 further comprising a promoter operationally linked to the nucleic acid.
44. The vector of claim 43, wherein the promoter is a constitutive promoter.
- 20 45. The vector of claim 43, wherein the promoter is an inducible promoter.
46. The vector of any one of claims 42-45, wherein the vector is a viral vector.
47. The vector of claim 46, wherein the viral vector is a lentiviral vector.
- 25 48. A method of producing an engineered immune cell, the method comprising:
introducing into an immune cell a nucleic acid of any one of claims 25-41 or a vector of
any one of claims 42-47, thereby producing the engineered immune cell.
- 30 49. The method of claim 48, further comprising, after the introducing step, culturing the
engineered immune cell.

50. The method of any one of claims 48-49, wherein the immune cell is a T cell.

51. The method of any one of claims 48-50, wherein the immune cell is a NK cell.

52. The method of any one of claims 48-51, ~~further comprising, before the introducing step, obtaining wherein~~ the immune cell is to be obtained from a subject before the introducing step.

53. The method of claim 52, wherein ~~the method further comprises administering~~ the engineered immune cell is to be administered to the subject.

54. The method of claim 52 or 53, wherein the subject has been diagnosed or identified as having a cancer.

55. An engineered immune cell produced by the method of any one of claims 48-54.

56. A pharmaceutical composition comprising the engineered immune cell of claim 55 and a pharmaceutically acceptable carrier.

57. Use of the engineered immune cell of claim 55 or the pharmaceutical composition of claim 56 in the manufacture of a medicament for ~~A method of~~ treating a cancer in a subject, ~~the method comprising administering to the subject an engineered immune cell of claim 55 or a pharmaceutical composition of claim 56.~~

58. The ~~method use~~ of claim 57, wherein the cancer is an anti-glypican-3-associated cancer, an anti-CD19-associated cancer, or an anti-MVR-associated cancer.

59. The ~~method use~~ of claim 57, wherein the cancer is carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphomas), blastoma, sarcoma, leukemia, squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, lung adenocarcinoma, squamous cell carcinoma of the lung, peritoneal cancer, hepatocellular carcinoma, gastric cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer,

breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary carcinoma, kidney cancer, prostate cancer, vulvar cancer, thyroid cancer, liver carcinoma, other lymphoproliferative disorders, and various types of head and neck cancer.

5 60. The ~~method~~-use of any of claims 57-59, wherein the subject has previously been administered one or more additional anticancer therapies selected from the group consisting of: ionizing radiation, a chemotherapeutic agent, a therapeutic antibody, and a checkpoint inhibitor.

10 61. The ~~method~~-use of any one of claims 57-60, wherein the subject has been identified or diagnosed as having the cancer.