

CLAIMS

What is claimed is:

1. A chimeric antigen receptor comprising a polypeptide, wherein the polypeptide comprises one or more of:
 - a) an LILRB1 hinge domain or functional fragment or variant thereof;
 - b) an LILRB1 transmembrane domain or a functional variant thereof; or
 - c) an LILRB1 intracellular domain or an intracellular domain comprising at least one immunoreceptor tyrosine-based inhibitory motif (ITIM), wherein the ITIM is selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
2. The receptor of claim 1, wherein the polypeptide comprises an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYA EV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
3. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs NLYAAV (SEQ ID NO: 8) and VTYA EV (SEQ ID NO: 9).
4. The receptor of claim 3, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 12.
5. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs VTYA EV (SEQ ID NO: 9) and VTYAQL (SEQ ID NO: 10).
6. The receptor of claim 5, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 13.
7. The receptor of claim 2, wherein the intracellular domain comprises both ITIMs VTYAQL (SEQ ID NO: 10) and SIYATL (SEQ ID NO: 11).
8. The receptor of claim 5, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 14.
9. The receptor of claim 1, wherein the polypeptide comprises an intracellular domain comprising at least three immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8),

- VTYAEV (SEQ ID NO: 9) , VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
10. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), and VTYAQL (SEQ ID NO: 10).
 11. The receptor of claim 10, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 15.
 12. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
 13. The receptor of claim 12, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 16.
 14. The receptor of claim 2, wherein the intracellular domain comprises the ITIMs NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
 15. The receptor of claim 14, wherein the intracellular domain comprises a sequence at least 95% identical to SEQ ID NO: 17.
 16. The receptor of claim 15, wherein the intracellular domain comprises a sequence at least 95% identical to the LILRB1 intracellular domain (SEQ ID NO: 7).
 17. The receptor of claim 1, wherein the intracellular domain comprises a sequence of SEQ ID NOS: 12-17.
 18. The receptor of any one of claim 1-17, wherein the polypeptide comprises the LILRB1 transmembrane domain or a functional variant thereof.
 19. The receptor of claim 18, wherein the LILRB1 transmembrane domain or a functional variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 5.
 20. The receptor of claim 19, wherein the LILRB1 transmembrane domain comprises SEQ ID NO: 5.
 21. The receptor of any one of claim 1-20, wherein the polypeptide comprises the LILRB1 hinge domain or functional fragment or variant thereof.
 22. The receptor of claim 21, wherein the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93.

23. The receptor of claim 21, wherein the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84 or SEQ ID NO: 93.
24. The receptor of claim 21, wherein the LILRB1 hinge domain or functional fragment or variant thereof comprises a sequence at least 95% identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.
25. The receptor of claim 21, wherein the LILRB1 hinge domain comprises a sequence identical to SEQ ID NO: 4, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.
26. The receptor of any one of claims 1-25, wherein the polypeptide comprises:
- a) an LILRB1 hinge domain or functional fragment or variant thereof, and
 - b) the LILRB1 transmembrane domain or a functional variant thereof.
27. The receptor of claim 26, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 20.
28. The receptor of any one of claims 1-27, wherein the polypeptide comprises:
- a) the LILRB1 transmembrane domain or a functional variant thereof, and
 - b) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYAEEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).
29. The receptor of claim 28, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 21.
30. The receptor of claim 28, wherein the polypeptide comprises a sequence of SEQ ID NO: 21.
31. The receptor of claim 1, wherein the polypeptide comprises:
- a) an LILRB1 hinge domain or functional fragment or variant thereof;
 - b) an LILRB1 transmembrane domain or a functional variant thereof; and
 - c) an LILRB1 intracellular domain and/or an intracellular domain comprising at least two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), wherein each

ITIM is independently selected from NLYAAV (SEQ ID NO: 8), VTYAEV (SEQ ID NO: 9), VTYAQL (SEQ ID NO: 10), and SIYATL (SEQ ID NO: 11).

32. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 95% identical to SEQ ID NO: 2 or SEQ ID NO: 3.
33. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 20.
34. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 21.
35. The receptor of claim 1, wherein the polypeptide comprises a sequence at least 99% identical to SEQ ID NO: 2 or SEQ ID NO: 3.
36. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 20.
37. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 21.
38. The receptor of claim 1, wherein the polypeptide comprises a sequence identical to SEQ ID NO: 2 or SEQ ID NO: 3.
39. The receptor of claim 1, wherein the polypeptide comprises antigen-binding domain.
40. The receptor of claim 39, wherein the antigen-binding domain is an antigen-binding domain other than the LILRB1 extracellular ligand binding protein.
41. The receptor of claim 39, wherein the polypeptide comprises two or more antigen-binding domains
42. The receptor of any one of claims 1-41, wherein the receptor is an inhibitory receptor.
43. The receptor of claim 39, wherein the antigen-binding domain comprises a single chain variable fragment (scFv).
44. The receptor of claim 1, wherein the receptor comprises a second polypeptide.
45. The receptor of claim 44, wherein the first polypeptide comprises a first chain of an antibody and the second polypeptide comprise a second chain of said antibody.
46. The receptor of claim 44, wherein the receptor comprises a Fab fragment of an antibody.
47. The receptor of claim 44, wherein:
 - a) the first polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody, and

- b) the second polypeptide comprises an antigen-binding fragment of the light chain of the antibody.
48. The receptor of claim 44, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of the light chain of the antibody, and
 - b) the second polypeptide comprises an antigen-binding fragment of the heavy chain of the antibody.
49. The receptor of claim 44, wherein the first polypeptide comprises a first chain of a T-cell receptor (TCR) and the second polypeptide comprises a second chain of said TCR.
50. The receptor of claim 44, wherein the receptor comprises an extracellular fragment of a T cell receptor (TCR).
51. The receptor of claim 50, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of an alpha chain of the TCR, and
 - b) the second polypeptide comprises an antigen-binding fragment of the beta chain of the TCR.
52. The receptor of claim 50, wherein:
- a) the first polypeptide comprises an antigen-binding fragment of the beta chain of the TCR, and
 - b) the second polypeptide comprises an antigen-binding fragment of the alpha chain of the TCR.
53. The receptor of claim 1, wherein the receptor comprises a single-chain TCR.
54. The receptor of claim 43, wherein the scFv comprises the complementarity determined regions (CDRs) of any one of SEQ ID NOS: 22-33.
55. The receptor of claim 43, wherein the scFv comprises a sequence at least 95% identical to any one of SEQ ID NOS: 35-46 or 125.
56. The receptor of claim 43, wherein the scFv comprises a sequence identical to any one of SEQ ID NOS: 35-46 or 125.
57. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises the heavy chain CDRs of any one of SEQ ID NOS: 25-27 or 31-33, and wherein the light

- chain of the antibody comprises the light chain CDRs of any one of SEQ ID NOS: 22-24 or 28-30.
58. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises a sequence at least 95% identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence at least 95% identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125.
59. The receptor of claim 47 or claim 48, wherein the heavy chain of the antibody comprises a sequence identical to the heavy chain portion of any one of SEQ ID NOS: 35-46 or 125, and wherein the light chain of the antibody comprises a sequence identical to the light chain portion of any one of SEQ ID NOS: 35-46 or 125.
60. The receptor of claim 1, wherein the receptor comprises an amino acid sequence at least 95% identical to any one of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122.
61. The receptor of claim 1, wherein the receptor comprises an amino acid sequence of SEQ ID NOS: 47-71, 77-79, 89-92, 120 or 122.
62. A polynucleotide comprising a nucleic acid sequence encoding the receptor of any one of claim 1-61.
63. A vector comprising the polynucleotide of claim 62.
64. The vector of claim 63, further comprising a sequence encoding a promoter operably linked to the polynucleotide.
65. An immune cell comprising the receptor of any one of claims 1-61, the polynucleotide of claim 62, or the vector of claim 63 or 64.
66. The immune cell of claim 65, wherein immune cell activation is reduced when the cell is contacted with the antigen or a cell expressing the antigen on its surface.
67. The immune cell of claim 65 or claim 66, wherein immune cell activation comprises expression of a gene operatively linked to an NFAT promoter.
68. The immune cell of any one of claims 65-67, wherein the immune cell is a T cell.
69. The immune cell of any one of claims 65-68, further comprising an activator receptor.
70. The immune cell of claim 69, wherein the activator receptor is a chimeric antigen receptor or a T cell receptor.
71. A method of making an immune cell, comprising introducing the polynucleotide of claim 62, or the vector of claim 63 or 64 into the immune cell.

72. The method of claim 69, wherein the immune cell expresses the receptor.
73. The method of claim 69 or claim 70, wherein the cell is an immune cell.
74. The method of claim 71, wherein the immune cell is a T cell.
75. The method of any one of claims 69-72, wherein immune cell activation is reduced when the cell is contacted with an antigen specific to the chimeric antigen receptor, or a cell expressing the antigen on its surface.
76. The method of claim 73, wherein immune cell activation comprises expression of a gene operatively linked to an NFAT promoter.
77. A method of treating a subject with a disease or a disorder, comprising administering to the subject a plurality of the immune cells of any one of claims 65-70.
78. The method of claim 77, wherein the disease or disorder is cancer.
79. A kit, comprising the receptor of any one of claims 1-61, the polynucleotide of claim 62, the vector of claim 63 or 64, or the immune cell of any one of claims 65-68.
80. An immune cell comprising a chimeric antigen receptor comprising a polypeptide, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 21.
81. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 3.
82. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 2.
83. The immune cell of claim 81, wherein the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 22-27, respectively.
84. The immune cell of claim 81, wherein the chimeric antigen receptor comprises an antigen-binding domain comprising CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3 sequences according to SEQ ID NO: 28-33, respectively.
85. The immune cell of claim 80, wherein the polypeptide sequence shares at least 95% identity or 100% identity to SEQ ID NO: 122.
86. The immune cell of any one of claims 80-83, wherein the immune cell is a T cell.
87. The immune cell of claim 84, wherein the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target expressed on tumor cells.

88. The immune cell of claim 87, wherein the T cell comprises a chimeric antigen receptor or T cell receptor that specifically binds to a target selected from etiolate receptor, $\alpha\text{v}\beta\beta$ integrin, BCMA, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD37, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, CEA, DLL4, EGP-2, EGP-40, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EPCAM, EphA2, EpCAM, FAP, FBP, fetal acetylcholine receptor, Fzd7, GD2, GD3, Glypican-3 (GPC3), h5T4, IL-11R, IL13R-a2, KDR, κ light chain, λ light chain, LeY, L1 CAM, MAGE-A1, mesothelin, MHC presented peptides, MUC1, MUC16, NCAM, NKG2D ligands, Notch1, Notch2/3, NY-ESO-1, PRAME, PSCA, PSMA, Survivin, TAG-72, TEMs, TERT, VEGFR2, and ROR1.
89. A method of treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of claim 87.
90. A method of treating and/or preventing cancer in a subject in need thereof, comprising administering to the subject the immune cells of claim 88.