

WHAT IS CLAIMED IS:

1. A chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain, a transmembrane domain, and an intracellular signaling domain comprising at least one co-stimulatory signaling domain that comprises a CD28 polypeptide
5 comprising a mutated YMNM motif.
2. The CAR of claim 1, wherein the CD28 polypeptide has reduced recruitment of a p85 subunit of a phosphoinositide 3-kinase (PI3K) as compared to a CD28 molecule comprising a native YMNM motif.
3. The CAR of claim 1 or 2, wherein a p85 subunit of a PI3K does not bind to the
10 mutated YMNM motif.
4. The CAR of claim 3, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YxNx (SEQ ID NO: 21), wherein x is not a methionine (M).
5. The CAR of claim 3 or 4, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22), YSNV (SEQ ID NO: 23),
15 YKNL (SEQ ID NO: 24), YENQ (SEQ ID NO: 25), YKNI (SEQ ID NO: 26), YINQ (SEQ ID NO: 27), YHNK (SEQ ID NO: 28), YVNQ (SEQ ID NO: 29), YLNP (SEQ ID NO: 30), YLNT (SEQ ID NO: 31), YDND (SEQ ID NO: 66), YENI (SEQ ID NO: 67), YENL (SEQ ID NO: 68), YKNQ (SEQ ID NO: 72), YKNV (SEQ ID NO: 73), or YANG (SEQ ID NO: 87).
- 20 6. The CAR of any one of claims 3-5, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23), YENV (SEQ ID NO: 22), or YKNI (SEQ ID NO: 26).
7. The CAR of any one of claims 3-6, wherein the mutated YMNM motif consists of the amino acid sequence set forth in YSNV (SEQ ID NO: 23).
- 25 8. The CAR of any one of claims 3-7, wherein the mutated YMNM motif binds to growth factor receptor bound receptor 2 (Grb2) and/or Grb2-related adaptor downstream of Shc (GADS).
9. The CAR of claim 1 or 2, wherein the mutated YMNM motif does not bind to Grb2 and/or GADS.

10. The CAR of claim 9, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMxM (SEQ ID NO: 20), wherein x is not an aspartic acid (N).
11. The CAR of claim 9 or 10, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32), YMPM (SEQ ID NO: 79), YMRM (SEQ ID NO: 37), or YMSM (SEQ ID NO: 80).
12. The CAR of any one of claims 9-11, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32).
13. The CAR of claim 9, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YbxM (SEQ ID NO: 33), wherein x is not an aspartic acid (N), and b is not a methionine (M).
14. The CAR of claim 13, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YTHM (SEQ ID NO: 34), YVLM (SEQ ID NO: 35), YIAM (SEQ ID NO: 36), YVEM (SEQ ID NO: 83), YVKM (SEQ ID NO: 85), or YVPM (SEQ ID NO: 86).
15. The CAR of claim 9, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMxb (SEQ ID NO: 65), wherein x is not an aspartic acid (N), and b is not a methionine (M).
16. The CAR of claim 15, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMAP (SEQ ID NO: 77).
17. The CAR of any one of claims 9-16, wherein a p85 subunit of a PI3K signaling binds to the mutated YMNm motif.
18. The CAR of claim 1 or 2, wherein the mutated YMNm motif does not bind to Grb2 and/or GADS or a p85 subunit of a PI3K.
19. The CAR of claim 18, wherein the mutated YMNm motif consists of the amino acid sequence set forth in Ybxb (SEQ ID NO: 43), wherein x is not an aspartic acid (N), and b is not a methionine (M).
20. The CAR of claim 19, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44), YAAA (SEQ ID NO: 45), YFFF (SEQ ID NO: 46), YETV (SEQ ID NO: 69), YQQQ (SEQ ID NO: 70), YHAE (SEQ ID

NO: 71), YLDL (SEQ ID NO: 74), YLIP (SEQ ID NO: 75), YLRV (SEQ ID NO: 76), YTAV (SEQ ID NO: 82), or YVHV (SEQ ID NO: 84).

21. The CAR of any one of claims 18-20, wherein the mutated YNMN motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 44).

5 22. The CAR of claim 1 or 2, wherein the mutated YNMN motif is capable of modulating PI3K signaling by limiting the number of methionine residues that can bind to a p85 subunit of PI3K.

23. The CAR of claim 22, wherein the mutated YNMN motif consists of the amino acid sequence set forth in YMN_x (SEQ ID NO: 38) or Y_xNM (SEQ ID NO: 39), wherein
10 _x is not a methionine (M).

24. The CAR of claim 22 or 23, wherein the mutated YNMN motif consists of the amino acid sequence set forth in YMN_V (SEQ ID NO: 40), YENM (SEQ ID NO: 41), and YMN_Q (SEQ ID NO: 42), YMN_L (SEQ ID NO: 78), or YSNM (SEQ ID NO: 81).

25. The CAR of any one of claims 1-24, wherein the extracellular antigen-binding
15 domain binds to an antigen.

26. The CAR of claim 25, wherein the antigen is a tumor antigen or a pathogen antigen.

27. The CAR of claim 25 or 26, wherein the antigen is a tumor antigen.

28. The CAR of claim 27, wherein the tumor antigen is selected from the group
20 consisting of CD19, mesothelin, AXL, TIM3, HVEM, MUC16, MUC1, CA1X, CEA, CD8, CD7, CD10, CD20, CD22, CD30, CLL1, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD70, CD74, CD99, CD123, CD133, CD138, EGP-2, EGP-40, EpCAM, Erb-B, FBP, Fetal acetylcholine receptor, folate receptor- α , GD2, GD3, HER-2, hTERT, IL-13R- α 2, κ -light chain, KDR, LeY, L1 cell adhesion molecule, MAGE-A1, MAGEA3,
25 CT83 (also known as KK-LC-1), p53, MART1, GP100, Proteinase3 (PR1), Tyrosinase, Survivin, hTERT, EphA2, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, WT-1, BCMA, CD44V6, NKCS1, EGF1R, EGFR-VIII, ADGRE2, CCR1, LILRB2, PRAME, HPV E6 oncoprotein, and HPV E7 oncoprotein.

30 29. The CAR of claim 28, wherein the tumor antigen is CD19.

30. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YMDM (SEQ ID NO: 32).
31. The CAR of claim 30, wherein the extracellular antigen-binding domain binds to CD19.
- 5 32. The CAR of claim 31, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 51.
33. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YKNI (SEQ ID NO: 26).
34. The CAR of claim 33, wherein the extracellular antigen-binding domain binds to
10 CD19.
35. The CAR of claim 34, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 55.
36. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YENV (SEQ ID NO: 22).
- 15 37. The CAR of claim 36, wherein the extracellular antigen-binding domain binds to CD19.
38. The CAR of claim 37, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 53.
39. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists
20 of the amino acid sequence set forth in YSNV (SEQ ID NO: 64).
40. The CAR of claim 39 wherein the extracellular antigen-binding domain binds to CD19.
41. The CAR of claim 40, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 57.
- 25 42. The CAR of any one of claims 1-29, wherein the mutated YMNm motif consists of the amino acid sequence set forth in YGGG (SEQ ID NO: 63).
43. The CAR of claim 42, wherein the extracellular antigen-binding domain binds to CD19.

44. The CAR of claim 43, wherein the CAR comprises the amino acid sequence set forth in SEQ ID NO: 61.
45. A cell comprising the CAR of any one of claims 1-44.
46. The cell of claim 45, wherein the cell is an immunoresponsive cell.
- 5 47. The cell of claim 45 or 46, wherein the cell is a cell of the lymphoid lineage or a cell of the myeloid lineage.
48. The cell of any one of claims 45-47, wherein the cell is selected from the group consisting of T cells, Natural Killer (NK) cells, stem cells from which lymphoid cells may be differentiated.
- 10 49. The cell of any one of claims 45-48, wherein the cell is a T cell.
50. The cell of claim 48 or 49, wherein the T cell is selected from the group consisting of a cytotoxic T lymphocyte (CTL), a $\gamma\delta$ T cell, a tumor-reactive lymphocyte, a tumor-infiltrating lymphocyte (TIL), a regulatory T cell, and a Natural Killer T (NKT) cell.
- 15 51. A composition comprising a cell of any one of claims 45-50.
52. The composition of claim 51, which is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.
53. The composition of claim 51 or 52, which is for treating and/or preventing a neoplasm or a tumor, and/or a pathogen infection.
- 20 54. A method of reducing tumor burden in a subject, the method comprising administering to the subject a cell of any one of claims 45-50 or a composition of any one of claims 51-53.
55. The method of claim 54, wherein the method reduces the number of tumor cells, reduces tumor size, and/or eradicates the tumor in the subject.
- 25 56. A method of treating and/or preventing a neoplasm or a tumor, the method comprising administering to the subject a cell of any one of claims 45-50 or a composition of any one of claims 51-53.
57. A method of lengthening survival of a subject having a neoplasm or a tumor, the method comprising administering to the subject a cell of any one of claims 45-50 or a
- 30 composition of any one of claims 51-53.

58. The method of any one of claims 54-57, wherein the neoplasm and/or tumor is selected from the group consisting of B cell leukemia, B cell lymphoma, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma, Burkitt lymphoma, acute myeloid leukemia (AML) and Mixed-phenotype acute leukemia (MPAL).
59. A method for producing an antigen-specific cell, the method comprising introducing into a cell a nucleic acid molecule encoding a CAR of any one of claims 1-44.
60. The method of claim 59, wherein the nucleic acid molecule is present on a vector.
61. The method of claim 60, wherein the vector is a retroviral vector.
62. A nucleic acid molecule encoding the CAR of any one of claims 1-44.
63. The nucleic acid molecule of claim 62, comprising the nucleotide sequence set forth in SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, or SEQ ID NO: 58.
64. A vector comprising the nucleic acid molecule of claim 62 or 63.
65. The vector of claim 64, wherein the vector is a γ -retroviral vector.
66. A host cell expressing the nucleic acid molecule of claim 64 or 65.
67. The host cell of claim 66, wherein the host cell is a T cell.
68. A kit comprising a CAR of any one of claims 1-44, a cell of any one of claims 35-40, a composition of any one of claims 51-53, a nucleic acid molecule of claim 62 or 63, or a vector of claim 64 or 65.
69. The kit of claim 68, wherein the kit further comprises written instructions for treating and/or preventing a neoplasm, a tumor, and/or a pathogen infection.