CLAIMS

What is claimed is:

1. A vector comprising a nucleotide sequence S1 encoding a CD8α polypeptide, a nucleotide sequence S2 encoding a CD8β polypeptide, a nucleotide sequence S3 encoding a T cell receptor (TCR)α polypeptide, and a nucleotide sequence S4 encoding a TCRβ polypeptide, wherein the nucleotide sequences are arranged in tandem in a 5' to 3' orientation selected from S1-S2-S3-S4, S1-S2-S4-S3, S2-S1-S3-S4, S2-S1-S4-S3, S3-S4-S1-S2, S3-S4-S2-S1, S4-S3-S1-S2, and S4-S3-S2-S1. .

2. The vector of claim 1, wherein the nucleotide sequences are arranged in tandem in a 5' to 3' orientation selected from S1-S2-S3-S4, S1-S2-S4-S3, S2-S1-S3-S4, and S2-S1-S4-S3.

3. The vector of claim 2, wherein the nucleotide sequences are arranged in tandem in a 5' to 3' orientation of S2-S1-S4-S3.

4, The vector of any one of claims 1-3, wherein the CD8αβ polypeptides comprise amino acid sequences at least 90% identical to SEQ ID NO: 11 and at least 90% identical to SEQ ID NO: 12.

5. The vector of any one of claims 1-4, wherein the TCRαβ polypeptides comprise amino acid sequences at least 90% identical to the amino acid sequences selected from SEQ ID NO: 13 and 14, 15 and 16, 17 and 18, 19 and 20, 21 and 22, 23 and 24, 25 and 26, 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, 47 and 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 and 60, 61 and 62, 63 and 64, 65 and 66, 67 and 68, 69 and 70, 71 and 72, 73 and 74, 75 and 76, 77 and 78, 79 and 80, 81 and 82, 83 and 84, 85 and 86, 87 and 88, or 89 and 90.

6. The vector of any one of claims 1-5, wherein the TCRαβ polypeptides comprise a amino acid sequences at least 90% identical to SEQ ID NO: 13 and at least 90% identical to SEQ ID NO:14.

7. The vector of any one of claims 1-6, further comprising a nucleotide sequence S5 encoding a 2A peptide and a nucleotide sequence S6 encoding a linker peptide, wherein S5 and S6 are positioned between S1 and S2, S1 and S3, S1 and S4, S2 and S3, S2 and S4, and/or S3 and S4.

8. The vector of claim 7, wherein the 2A peptide is selected from P2A (SEQ ID NO: 3), T2A (SEQ ID NO: 4), E2A (SEQ ID NO: 5), or F2A (SEQ ID NO: 6).

9. The vector of claim 7 or 8, wherein the linker peptide is GSG or SGSG (SEQ ID NO: 8).

10. The vector of any one of claim 1-9, further comprising a nucleotide sequence S7 encoding a furin peptide (SEQ ID NO: 2), wherein S7 is positioned between S1 and S2, S1 and S3, S1 and S4, S2 and S3, S2 and S4, and/or S3 and S4.

11. A vector comprising a first nucleotide sequence S1 encoding a protein Z1, a second nucleotide sequence S2 encoding a protein Z2, a third nucleotide sequence S3 encoding a protein Y1, and a fourth nucleotide sequence S4 encoding a protein Y2, wherein Z1 and Z2 form a first dimer and Y1 and Y2 form a second dimer, wherein the first dimer Z1Z2 is structurally different from the second dimer Y1Y2, wherein the vector is in a gene delivery system that is used for adaptive cellular therapy.

12. The vector of claim 11, wherein the S1, S2, S3, and S4 are arranged in tandem in a 5’ to 3’ orientation selected from S1-S2-S3-S4, S1-S2-S4-S3, S1-S3-S2-S4, S1-S3-S4-S2, S1-S4-S3-S2, S1-S4-S2-S3, S2-S1-S3-S4, S2-S1-S4-S3, S2-S3-S1-S4, S2-S3-S4-S1, S2-S4-S3-S1, S2-S4-S1-S3, S3-S1-S2-S4, S3-S1-S4-S2, S3-S2-S1-S4, S3-S2-S4-S1, S3-S4-S1-S2, S3-S4-S2-S1, S4-S1-S2-S3, S4-S1-S3-S2, S4-S2-S1-S3, S4-S2-S3-S1, S4-S3-S1-S2, or S4-S3-S2-S1.

13. The vector of claim 11 or 12, further comprising a fifth nucleotide sequence S5 encoding a 2A peptide and a sixth nucleotide sequence S6 encoding a linker peptide, wherein S5 and S6 are positioned between S1 and S2, S1 and S3, S1 and S4, S2 and S3, S2 and S4, and/or S3 and S4.

14. The vector of claim 13, wherein the 2A peptide is selected from P2A (SEQ ID NO: 3), T2A (SEQ ID NO: 4), E2A (SEQ ID NO: 5), or F2A (SEQ ID NO: 6).

15. The vector of claim 13 or 14, wherein the linker peptide is GSG or SGSG (SEQ ID NO: 8).

16. The vector of any one of claim 11-15, further comprising a seventh nucleotide sequence S7 encoding a furin peptide (SEQ ID NO: 2), wherein S7 is positioned between S1 and S2, S1 and S3, S1 and S4, S2 and S3, S2 and S4, and/or S3 and S4.

17. The vector of any one of claims 11-16, further comprising a post-transcriptional regulatory element (PRE) sequence selected from a Woodchuck PRE (WPRE) or a hepatitis B virus (HBV) PRE (HPRE).

18. The vector of any one of claims 11-17, further comprising a promoter sequence that controls the transcription of the S2-S7, wherein the promoter sequence is selected from cytomegalovirus (CMV) promoter, phosphoglycerate kinase (PGK) promoter, myelin basic protein (MBP) promoter, glial fibrillary acidic protein (GFAP) promoter, modified MoMuLV LTR containing myeloproliferative sarcoma virus enhancer (MNDU3), Ubiqitin C promoter, EF-1 alpha promoter, or Murine Stem Cell Virus (MSCV) promoter.

19. The vector of any one of claims 11-18, wherein the first dimer Z1Z2 is selected from SEQ ID NO: 13 and 14, 15 and 16, 17 and 18, 19 and 20, 21 and 22, 23 and 24, 25 and 26, 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, 47 and 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 and 60, 61 and 62, 63 and 64, 65 and 66, 67 and 68, 69 and 70, 71 and 72, 73 and 74,

75 and 76, 77 and 78, 79 and 80, 81 and 82, 83 and 84, 85 and 86, 87 and 88, or 89 and 90.

20. The vector of any one of claims 11-19, wherein the second dimer Y1 and Y2 is SEQ ID NO: 11 and 12.

21. The vector of any one of claims 11-20, wherein the orientation is S2-S1-S4-S3.

22. The vector of claim 21, comprising the sequence selected from PTE WPRE (SEQ ID NO: 91), TPE WPRE (SEQ ID NO: 92), or PTE fn WPRE (SEQ ID NO: 93).

23. The vector of any one of claims 11-20, wherein the orientation is S4-S3-S2-S1.

24. The vector of claim 23, comprising the sequence PTE CD8 TCR WPRE (SEQ ID NO: 94).

25. The vector of any one of claims 11-24 that is a viral vector selected from adenoviruses, poxviruses, alphaviruses, arenaviruses, flaviruses, rhabdoviruses, retroviruses, lentiviruses, herpesviruses, paramyxoviruses, or picornaviruses.

26. The vector of claim 25, wherein the vector is pseudotyped with an envelope protein of a virus selected from the native feline endogenous virus (RD114), a chimeric version of RD114 (RD114TR), gibbon ape leukemia virus (GALV), a chimeric version of GALV (GALV-TR), amphotropic murine leukemia virus (MLV 4070A), baculovirus (GP64), vesicular stomatitis virus (VSV-G), fowl plague virus (FPV), Ebola virus (EboV), or baboon retroviral envelope glycoprotein (BaEV), lymphocytic choriomeningitis virus (LCMV).

27. A method of preparing T cells for immunotherapy comprising

isolating T cells from a blood sample of a human subject,

activating the isolated T cells in the presence of an aminobisphosphonate,

transducing the activated T cells with the vector of any one of claims 11-26, and

expanding the transduced T cells.

28. The method of claim 27, wherein the T cells are isolated from a leukapheresis human sample.

29. The method of claim 27 or 28, wherein the aminobisphosphonate is selected from pamidronic acid, alendronic acid, zoledronic acid, risedronic acid, ibandronic acid, incadronic acid, a salt of any of the foregoing and/or a hydrate thereof.

30. The method of any one of claims 27-29, wherein the activating is further in the presence of human recombinant interleukin 2 (IL-2) and human recombinant interleukin 15 (IL-15).

31. The method of any one of claims 27-30, wherein the expanding is in the presence of IL-2 and IL-15.

32. The method of any one of claims 27-31, wherein the T cells are γδ T cells.

33. The method of any one of claims 27-32, wherein the first dimer Z1Z2 and the second dimer Y1Y2 are co-expressed on the surface of the expanded T cells.

34. A population of expanded T cells prepared by the method of any one of claims 27-32.

35. A composition comprising the population of expanded T cells of claim 34 for use in the treatment of cancer,

wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide is selected from SEQ ID NO: 98-255,

wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile

duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.

36. The composition for use of claim 35, wherein the composition further comprises an adjuvant.

37. The composition for use of claim 36, wherein the adjuvant is selected from anti-CD40 antibody, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, atezolizumab, interferon-alpha, interferon-beta, CpG oligonucleotides and derivatives, poly-(I:C) and derivatives, RNA, sildenafil, particulate formulations with poly(lactide co-glycolide) (PLG), virosomes, interleukin (IL)-1, IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-21, and IL-23.

38. A composition comprising the population of expanded T cells of claim 34 for use in eliciting an immune response,

wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide is selected from SEQ ID NO: 98-255,

wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.

39. The composition for use of claim 38, wherein the composition further comprises an adjuvant.

40. The composition for use of claim 39, wherein the adjuvant is selected from anti-CD40 antibody, imiquimod, resiquimod, GM-CSF, cyclophosphamide, sunitinib, bevacizumab, atezolizumab, interferon-alpha, interferon-beta, CpG oligonucleotides and derivatives, poly-

(I:C) and derivatives, RNA, sildenafil, particulate formulations with poly(lactide co-glycolide) (PLG), virosomes, interleukin (IL)-1, IL-2, IL-4, IL-7, IL-12, IL-13, IL-15, IL-21, and IL-23.

41. The composition for use of any one of claims 38-40, wherein the immune response comprises a cytotoxic T cell response.

42. A method of preparing T cells for immunotherapy, comprising
activating the T cells in the presence of a statin,
transducing the activated T cells with the vector of any one of claims 11-26,
wherein the vector is pseudotyped with an envelope protein of VSV-G, and
expanding the transduced T cells.

43. The method of claim 42, wherein the T cells comprise αβ T cells, γδ T cells, and/or natural killer T cells.

44. The method of claim 42 or 43, wherein the statin is selected from atorvastatin, cerivastatin, dalvastatin, fluindostatin, fluvastatin, mevastatin, pravastatin, simvastatin, velostatin, and rosuvastatin.

45. A method of preparing T cells for immunotherapy comprising
activating the T cells,
transducing the activated T cells with the vector of any one of claims 11-26, and
expanding the transduced T cells.

46. The method of claim 45, wherein the T cells comprise αβ T cells, γδ T cells, and/or natural killer T cells.

47. The method of claim 45 or 46, wherein the T cells comprise αβ T cells.

48. The method of claim 47, wherein the activating is in the presence of an anti-CD3 antibody and an anti-CD28 antibody.

49. The method of claims 47 or 48, wherein the expanding is in the presence of IL-7 and IL-15.

50. A population of expanded T cells prepared by the method of any one of claims 42-49.

51. A composition comprising the population of expanded T cells of claim 50 for use in the treatment of cancer,

wherein the T cells kill cancer cells that present a peptide in a complex with an MHC molecule on the surface, wherein the peptide is selected from SEQ ID NO: 98-255,

wherein the cancer is selected from the group consisting of non-small cell lung cancer, small cell lung cancer, melanoma, liver cancer, breast cancer, uterine cancer, Merkel cell carcinoma, pancreatic cancer, gallbladder cancer, bile duct cancer, colorectal cancer, urinary bladder cancer, kidney cancer, leukemia, ovarian cancer, esophageal cancer, brain cancer, gastric cancer, and prostate cancer.