

WHAT IS CLAIMED:

1. A recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3':

- a. a first polynucleotide sequence that encodes a chimeric antigen receptor (CAR) that comprises an extracellular antigen-binding domain that specifically binds to CD19, a transmembrane domain, and a cytoplasmic domain;
- b. a second polynucleotide sequence that comprises an F2A element;
- c. a third polynucleotide sequence that encodes a fusion protein that comprises IL-15, or a functional fragment or functional variant thereof, and IL-15R α , or a functional fragment or functional variant thereof;
- d. a fourth polynucleotide sequence that comprises a T2A element; and
- e. a fifth polynucleotide sequence that encodes a marker protein.

2. The recombinant vector of claim 1, wherein said F2A element comprises (a) a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications; (b) a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141; (c) a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications; or (d) a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.

~~3. The recombinant vector of claim 1, wherein said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141.~~

~~4. The recombinant vector of claim 1, wherein said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications.~~

~~5. The recombinant vector of claim 1, wherein said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.~~

~~6.3.~~ 6.3. The recombinant vector of ~~any one of claim 1-5~~ claim 1 or 2, wherein said T2A element comprises (a) a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications; (b) a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143; (c) a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications; or (d) a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.

~~7. The recombinant vector of any one of claims 1-5, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143.~~

~~8. The recombinant vector of any one of claims 1-5, wherein said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications.~~

~~9. The recombinant vector of any one of claims 1-5, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.~~

~~10.4.~~ 10.4. The recombinant vector of any one of claims 1-~~9~~3, wherein said antigen-binding domain comprises: a heavy chain variable region (VH) comprising complementarity determining regions VH CDR1, VH CDR2, and VH CDR3; and a light chain variable region (VL) comprising complementarity determining regions VL CDR1, VL CDR2, and VL CDR3.

~~11. The recombinant vector of claim 10, wherein said antigen-binding domain comprises an scFv that comprises said VH and said VL operably linked via a first peptide linker.~~

~~12. The recombinant vector of claim 10 or 11, wherein said VH comprises the VH CDR1, VH CDR2, and VH CDR3 amino acid sequences set forth in SEQ ID NO: 2.~~

~~13. The recombinant vector of claim 10 or 11, wherein~~

- ~~a. said VH CDR1 comprises the amino acid sequence of SEQ ID NO: 6; or the amino acid sequence of SEQ ID NO: 6, comprising 1, 2, or 3 amino acid modifications;~~

- b. ~~said VH CDR2 comprises the amino acid sequence of SEQ ID NO: 7; or the amino acid sequence of SEQ ID NO: 7, comprising 1, 2, or 3 amino acid modifications; and~~
 - c. ~~said VH CDR3 comprises the amino acid sequence of SEQ ID NO: 8; or the amino acid sequence of SEQ ID NO: 8, comprising 1, 2, or 3 amino acid modifications.~~
14. ~~The recombinant vector of any one of claims 10-13, wherein said VL comprises the VL CDR1, VL CDR2, and VL CDR3 amino acid sequences set forth in SEQ ID NO: 1.~~
15. ~~The recombinant vector of any one of claims 10-13, wherein~~
- a. ~~said VL CDR1 comprises the amino acid sequence of SEQ ID NO: 3; or the amino acid sequence of SEQ ID NO: 3, comprising 1, 2, or 3 amino acid modifications;~~
 - b. ~~said VL CDR2 comprises the amino acid sequence of SEQ ID NO: 4; or the amino acid sequence of SEQ ID NO: 4, comprising 1, 2, or 3 amino acid modifications; and~~
 - c. ~~said VL CDR3 comprises the amino acid sequence of SEQ ID NO: 5; or the amino acid sequence of SEQ ID NO: 5, comprising 1, 2, or 3 amino acid modifications.~~
16. ~~The recombinant vector of any one of claims 10-15, wherein said VH comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 2.~~
17. ~~The recombinant vector of any one of claims 10-16, wherein said VH is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 20.~~
18. ~~The recombinant vector of any one of claims 10-17, wherein said VL comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 1.~~
19. ~~The recombinant vector of any one of claims 10-18, wherein said VL is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 19.~~

~~20. The recombinant vector of any one of claims 11-19, wherein said first peptide linker comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 17, or the amino acid sequence of SEQ ID NO: 9 or 17, comprising 1, 2, or 3 amino acid modifications.~~

~~21. The recombinant vector of any one of claims 11-20, wherein said first peptide linker is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 27 or SEQ ID NO: 35.~~

~~22. The recombinant vector of claim 21, wherein said first peptide linker is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 27.~~

23.5. The recombinant vector of any one of claims 1-~~224~~, wherein said CAR further comprises a hinge region positioned between said antigen-binding domain and said transmembrane domain of said CAR.

~~24. The recombinant vector of claim 23, wherein said hinge region comprises the amino acid sequence of SEQ ID NO: 37, 38, or 39, or the amino acid sequence of SEQ ID NO: 37, 38, or 39, comprising 1, 2, or 3 amino acid modifications.~~

~~25. The recombinant vector of claim 23, wherein said hinge region is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 40, 41, or 42.~~

26.6. The recombinant vector of any one of claims 1-~~25~~, wherein said transmembrane domain of said CAR comprises the amino acid sequence of SEQ ID NO: 43, 44, or 45, or the amino acid sequence of SEQ ID NO: 43, 44, or 45, comprising 1, 2, or 3 amino acid modifications.

~~27. The recombinant vector of any one of claims 1-25, wherein said transmembrane domain of said CAR is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 49, 50, 51, or 52.~~

~~28. The recombinant vector of any one of claims 23-27, wherein said hinge region and said transmembrane domain together comprise the amino acid sequence of SEQ ID NO: 46, 47, or 48, or the amino acid sequence of SEQ ID NO: 46, 47, or 48, comprising 1, 2, or 3 amino acid modifications.~~

~~29. The recombinant vector of any one of claims 23-27, wherein said hinge region and said transmembrane domain together are encoded by a polynucleotide sequence at least 75%, 80%,~~

~~85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 53, 54, 55, or 56.~~

~~30.7.~~ The recombinant vector of any one of claims 1-~~296~~, wherein said cytoplasmic domain comprises a primary signaling domain of human CD3 ζ , or a functional fragment or functional variant thereof.

~~31. The recombinant vector of claim 30, wherein said cytoplasmic domain comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 60.~~

~~32. The recombinant vector of claim 30, wherein said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 67 or 68.~~

~~33.8.~~ The recombinant vector of any one of claims 1-~~327~~, wherein said cytoplasmic domain comprises a co-stimulatory domain, or functional fragment or variant thereof, of a protein selected from the group consisting of CD28, 4-1BB, OX40, CD2, CD7, CD27, CD30, CD40, CDS, ICAM-1, LFA-1, B7-H3, and ICOS.

~~34. The recombinant vector of claim 33, wherein said protein is CD28 or 4-1BB.~~

~~35. The recombinant vector of claim 33 or 34, wherein said protein is CD28.~~

~~36. The recombinant vector of any one of claims 33-35, wherein said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 57 or 58, or the amino acid sequence of SEQ ID NO: 57 or 58, comprising 1, 2, or 3 amino acid modifications.~~

~~37. The recombinant vector of any one of claims 33-35, wherein said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 64 or 65.~~

~~38. The recombinant vector of claim 33 or 34, wherein said protein is 4-1BB.~~

~~39. The recombinant vector of any one of claims 33, 34, or 38, wherein said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 59, or the amino acid sequence of SEQ ID NO: 59, comprising 1, 2, or 3 amino acid modifications.~~

~~40. The recombinant vector of any one of claims 33, 34, 38, or 39, wherein said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 66.~~

~~41. The recombinant vector of any one of claims 1-40, wherein said cytoplasmic domain comprises the amino acid sequence of SEQ ID NO: 61, 62, or 63, or the amino acid sequence of SEQ ID NO: 61, 62, or 63, comprising 1, 2, or 3 amino acid modifications.~~

~~42. The recombinant vector of any one of claims 1-41, wherein said cytoplasmic domain is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 69, 70, or 71.~~

~~43.9.~~ The recombinant vector of any one of claims 1-~~428~~, wherein said CAR (a) comprises an amino acid sequence at least at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 72, 74, 76, 77, 78, 79, 80, or 81; or (b) is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 82, 83, 86, 87, 90, 91, 92, 93, 94, or 95.

~~44. The recombinant vector of any one of claims 1-43, wherein said CAR is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 82, 83, 86, 87, 90, 91, 92, 93, 94, or 95.~~

~~45.10.~~ The recombinant vector of any one of claims 1-~~449~~, wherein said IL-15, or said functional fragment or functional variant thereof, is operably linked to said IL-15R α , or said functional fragment or functional variant thereof, via a second peptide linker.

~~46.11.~~ The recombinant vector of any one of claims 1-~~4510~~, wherein said fusion protein (a) comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 119, 121, or 180; or (b) is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 126, 127, 130, 131, or 181.

~~47. The recombinant vector of any one of claims 1-46, wherein said fusion protein is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 126, 127, 130, 131, or 181.~~

~~48.12.~~ The recombinant vector of any one of claims 1-~~4711~~, wherein said marker protein comprises: domain III of HER1, or a functional fragment or functional variant thereof; an N-terminal portion of domain IV of HER1; and a transmembrane domain of CD28, or a functional fragment or functional variant thereof.

~~49. The recombinant vector of claim 48, wherein said domain III of HER1, or a functional fragment or functional variant thereof, comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 98.~~

50. —The recombinant vector of claim 48, wherein said domain III of HER1, or a functional fragment or functional variant thereof, is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 110 or 164.

51. —The recombinant vector of any one of claims 48-50, wherein said N-terminal portion of domain IV of HER1 comprises amino acids 1-40, 1-39, 1-38, 1-37, 1-36, 1-35, 1-34, 1-33, 1-32, 1-31, 1-30, 1-29, 1-28, 1-27, 1-26, 1-25, 1-24, 1-23, 1-22, 1-21, 1-20, 1-19, 1-18, 1-17, 1-16, 1-15, 1-14, 1-13, 1-12, 1-11, or 1-10 of SEQ ID NO: 99.

52. —The recombinant vector of any one of claims 48-51, wherein said N-terminal portion of domain IV of HER1 comprises amino acids 1-21 of SEQ ID NO: 99.

53. —The recombinant vector of any one of claims 48-52, wherein said N-terminal portion of domain IV of HER1 comprises the amino acid sequence of SEQ ID NO: 100, or the amino acid sequence of SEQ ID NO: 100, comprising 1, 2, or 3 amino acid modifications.

54. —The recombinant vector of any one of claims 48-53, wherein said N-terminal portion of domain IV of HER1 is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 112.

55. —The recombinant vector of any one of claims 48-54, wherein said transmembrane region of CD28 comprises the amino acid sequence of SEQ ID NO: 101, or the amino acid sequence of SEQ ID NO: 101, comprising 1, 2, or 3 amino acid modifications.

56. —The recombinant vector of any one of claims 48-55, wherein said transmembrane region of CD28 is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 113.

57.13. The recombinant vector of any one of claims 1-~~56~~12, wherein said marker protein (a) comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 96, 97, 166, or 167; or (b) is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 107, 108, 109, 162, 173, or 174.

58. —The recombinant vector of any one of claims 1-57, wherein said marker protein is encoded by a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 107, 108, 109, 162, 173, or 174.

~~59.14.~~ The recombinant vector of any one of claims 1-~~58~~13, wherein said regulatory element comprises a promoter.

~~60.~~—The recombinant vector of claim ~~59~~, wherein said promoter is a human elongation factor 1-alpha (hEF-1 α) hybrid promoter.

~~61.~~—The recombinant vector of claim ~~59~~ or ~~60~~, wherein said promoter comprises a polynucleotide sequence at least ~~75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the polynucleotide sequence of SEQ ID NO: 146.

~~62.~~—The recombinant vector of any one of claims ~~1-61~~, wherein said vector further comprises a polyA sequence 3' of said fifth polynucleotide sequence.

~~63.~~—The recombinant vector of claim ~~62~~, wherein said polyA sequence comprises a polynucleotide sequence at least ~~75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the polynucleotide sequence of SEQ ID NO: 148.

~~64.~~—A recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3':

- ~~a.~~—a first polynucleotide sequence that encodes a CAR that comprises an amino acid sequence at least ~~95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the amino acid sequence of SEQ ID NO: 72 or 74;
- ~~a.~~—a second polynucleotide sequence that comprises an F2A element;
- ~~b.~~—a third polynucleotide sequence that encodes a fusion protein that comprises an amino acid sequence at least ~~95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the amino acid sequence of SEQ ID NO: 119, 121, or 180;
- ~~c.~~—a fourth polynucleotide sequence that comprises a T2A element; and
- ~~d.~~—a fifth polynucleotide sequence that encodes a marker protein that comprises an amino acid sequence at least ~~95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the amino acid sequence of SEQ ID NO: 96 or 97.

~~65.~~—The recombinant vector of claim ~~64~~, wherein said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications.

~~66.~~—The recombinant vector of claim ~~64~~, wherein said F2A element comprises a polynucleotide sequence at least ~~75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%~~ identical to the polynucleotide sequence of SEQ ID NO: 141.

67. —The recombinant vector of claim 64, wherein said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications.

68. —The recombinant vector of claim 64, wherein said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.

69. —The recombinant vector of any one of claims 64-68, wherein said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications.

70. —The recombinant vector of any one of claims 64-68, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143.

71. —The recombinant vector of any one of claims 64-68, wherein said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications.

72. —The recombinant vector of any one of claims 64-68, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.

73. —A recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that comprises, from 5' to 3':

- a. —a first polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 82, 83, 86, or 87;
- a. —a second polynucleotide sequence that comprises an F2A element;
- b. —a third polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 126, 127, 130, 131, or 181;
- c. —a fourth polynucleotide sequence that comprises a T2A element; and

- d. ~~a fifth polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 107, 108, 109, or 162.~~
74. ~~The recombinant vector of claim 73, wherein said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 137, or the amino acid sequence of SEQ ID NO: 137, comprising 1, 2, or 3 amino acid modifications.~~
75. ~~The recombinant vector of claim 73, wherein said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 141.~~
76. ~~The recombinant vector of claim 73, wherein said F2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 138, or the amino acid sequence of SEQ ID NO: 138, comprising 1, 2, or 3 amino acid modifications.~~
77. ~~The recombinant vector of claim 73, wherein said F2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 142.~~
78. ~~The recombinant vector of any one of claims 73-77, wherein said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 139, or the amino acid sequence of SEQ ID NO: 139, comprising 1, 2, or 3 amino acid modifications.~~
79. ~~The recombinant vector of any one of claims 73-77, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence SEQ ID NO: 143.~~
80. ~~The recombinant vector of any one of claims 73-77, wherein said T2A element comprises a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 140 or 182, or the amino acid sequence of SEQ ID NO: 140 or 182, comprising 1, 2, or 3 amino acid modifications.~~
81. ~~The recombinant vector of any one of claims 73-77, wherein said T2A element comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 144, 145, or 165.~~
82. ~~The recombinant vector of any one of claims 1-81, further comprising a Left inverted terminal repeat (ITR) and a Right ITR, wherein said Left ITR and said Right ITR flank said polycistronic expression cassette.~~
83. ~~The recombinant vector of claim 82, which comprises, from 5' to 3':~~

- ~~a. —said Left ITR;~~
- ~~b. —said transcriptional regulatory element;~~
- ~~c. —said first polynucleotide sequence;~~
- ~~d. —said second polynucleotide sequence;~~
- ~~e. —said third polynucleotide sequence;~~
- ~~f. —said fourth polynucleotide sequence;~~
- ~~g. —said fifth polynucleotide sequence; and~~
- ~~h. —said Right ITR.~~

84.15. A recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises (a) a transcriptional regulatory element operably linked to a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 149; or (b) a transcriptional regulatory element operably linked to a polynucleotide that encodes an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.

~~85. —A recombinant vector comprising a polycistronic expression cassette, wherein said polycistronic expression cassette comprises a transcriptional regulatory element operably linked to a polynucleotide that encodes an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.~~

86.16. The recombinant vector of any one of claims 84 or 851-15, further comprising a Left inverted terminal repeat (ITR) and a Right ITR, wherein said Left ITR and said Right ITR flank said polycistronic expression cassette.

87.17. The recombinant vector of any one of claims 82, 83, or 86claim 16, wherein said Left ITR and said Right ITR are ITRs of a DNA transposon selected from the group consisting of a Sleeping Beauty transposon, a piggyBac transposon, TcBuster transposon, and a Tol2 transposon.

~~88. —The recombinant vector of claim 87, wherein said DNA transposon is said Sleeping Beauty transposon.~~

~~89. —The recombinant vector of any one of claims 1-88, wherein said vector is a non-viral vector.~~

~~90. —The recombinant vector of claim 89, wherein said non-viral vector is a plasmid.~~

~~91. —The recombinant vector of any one of claims 1-90, wherein said vector is a viral vector.~~

92. — ~~The recombinant vector of any one of claims 1-91, wherein said vector is a polynucleotide.~~

93. — ~~A polynucleotide encoding an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 152.~~

94.18. A population of cells that comprise the recombinant vector of any one of claims 1-~~92~~17.

95. — ~~The population of cells of claim 94, wherein said vector is integrated into the genome of said population of cells.~~

96. — ~~A population of cells that comprise the polynucleotide of claim 93.~~

97. — ~~The population of cells of claim 96, wherein said polynucleotide is integrated into the genome of said population of cells.~~

98. — ~~A population of cells that comprise a polypeptide comprising an amino acid sequence encoded by the polynucleotide of claim 93.~~

99. — ~~The population of cells of any one of claims 94-98, comprising a CAR comprising the amino acid sequence of SEQ ID NO: 72, 73, 74, 75, 76, 77, 78, 79, 80, or 81; a fusion protein comprising the amino acid sequence of SEQ ID NO: 119, 120, 121, 122, 180, or 183; and a marker protein comprising the amino acid sequence of SEQ ID NO: 96, 97, 166, or 167.~~

100. — ~~The population of cells of any one of claims 94-98, comprising a CAR comprising the amino acid sequence of SEQ ID NO: 74; a fusion protein comprising the amino acid sequence of SEQ ID NO: 121; and a marker protein comprising the amino acid sequence of SEQ ID NO: 97.~~

101. — ~~The population of cells of any one of claims 94-98, comprising a CAR comprising the amino acid sequence of SEQ ID NO: 75; a fusion protein comprising the amino acid sequence of SEQ ID NO: 122; and a marker protein comprising the amino acid sequence of SEQ ID NO: 97.~~

102. — ~~The population of cells of any one of claims 94-101, wherein said cells are immune effector cells.~~

103. — ~~The population of cells of claim 102, wherein said immune effector cells are selected from the group consisting of T cells, natural killer (NK) cells, B cells, mast cells, and myeloid-derived phagocytes.~~

104. — ~~The population of cells of claim 103, wherein said immune effector cells are T cells.~~

105. — ~~The population of cells of claim 104, comprising alpha/beta T cells, gamma/delta T cells, or natural killer T (NK-T) cells.~~

106. — ~~The population of cells of claim 104 or 105, comprising CD4⁺ T cells, CD8⁺ T cells, or both CD4⁺ T cells and CD8⁺ T cells.~~

~~107.—The population of cells of any one of claims 94–106, wherein said cells are *ex vivo*.~~

~~108.—The population of cells of any one of claims 94–107, wherein said cells are human.~~

~~109.~~19. A method of producing a population of engineered cells, comprising:

- a. introducing into a population of cells the recombinant vector of ~~any one of claims 82, 83, or 86–88~~claim 16 or 17, and a DNA transposase or a polynucleotide encoding a DNA transposase; and
- b. culturing said population of cells under conditions wherein said transposase integrates the polycistronic expression cassette into the genome of said population of cells,

thereby producing the population of engineered cells.

~~110.—The method of claim 109, wherein said Left ITR and said Right ITR are ITRs of a DNA transposon selected from the group consisting of a Sleeping Beauty transposon, a piggyBac transposon, a TcBuster transposon, and a Tol2 transposon.~~

~~111.—The method of claim 109 or 110, wherein said DNA transposon is said Sleeping Beauty transposon.~~

~~112.—The method of any one of claims 109–111, wherein said transposase is a Sleeping Beauty transposase.~~

~~113.—The method of claim 112, wherein said Sleeping Beauty transposase is selected from the group consisting of SB11, SB100X, hSB110, and hSB81.~~

~~114.—The method of claim 112 or 113, wherein said Sleeping Beauty transposase is SB11.~~

~~115.—The method of claim 114, wherein said SB11 comprises an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 160.~~

~~116.—The method of claim 114 or 115, wherein said SB11 is encoded by a polynucleotide sequence at least at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 161.~~

~~117.—The method of any one of claims 109–116, wherein said polynucleotide encoding said DNA transposase is a DNA vector or an RNA vector.~~

~~118.—The method of any one of claims 109–117, wherein said Left ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 155 or 156; and said Right ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the polynucleotide sequence of SEQ ID NO: 157, 159, or 184.~~

~~119.—The method of any one of claims 109–118, wherein said recombinant vector, and said DNA transposase or polynucleotide encoding said DNA transposase, are introduced to said population of cells using electro-transfer, calcium phosphate precipitation, lipofection, particle bombardment, microinjection, mechanical deformation by passage through a microfluidic device, or a colloidal dispersion system.~~

~~120.—The method of claim 119, wherein said recombinant vector, and said DNA transposase or polynucleotide encoding said DNA transposase, are introduced to said population of cells using electro-transfer.~~

~~121.—The method of any one of claims 109–120, wherein said method is completed in less than two days.~~

~~122.—The method of any one of claims 109–120, wherein said method is completed in 1–2 days.~~

~~123.—The method of any one of claims 109–120, wherein said method is completed in more than two days.~~

~~124.—The method of any one of claims 109–123, wherein said population of cells is cryopreserved and thawed before introduction of said recombinant vector and said DNA transposase or polynucleotide encoding said DNA transposase.~~

~~125.—The method of any one of claims 109–124, wherein said population of cells is rested before introduction of said recombinant vector and said DNA transposase or polynucleotide encoding said DNA transposase.~~

~~126.—The method of any one of claims 109–125, wherein said population of cells comprises human *ex vivo* cells.~~

~~127.—The method of any one of claims 109–126, wherein said population of cells is not activated *ex vivo*.~~

~~128.—The method of any one of claims 109–127, wherein said population of cells comprises T cells.~~

129.20. Use of (a) the population of cells of claim 18, or (b) the population of engineered cells of claim 19 in the manufacture of a medicament for~~A method of treating cancer or autoimmune disease or disorder~~ in a subject in need thereof ~~comprising administering to the subject a therapeutically effective amount of the population of cells of any one of claims 94–108, thereby treating the cancer.~~

~~130.—A method of treating cancer in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the population of engineered cells produced by the method of any one of claims 109–128, thereby treating the cancer.~~

~~131.—A method of treating an autoimmune disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the population of cells of any one of claims 94–108, thereby treating the autoimmune disease or disorder.~~

~~132.—A method of treating an autoimmune disease or disorder in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the population of engineered cells produced by the method of any one of claims 109–128, thereby treating the autoimmune disease or disorder.~~