

What Is Claimed Is:

1. A population of genetically engineered T cells, comprising:
 - (i) a disrupted gene involved in cell self-renewal;
 - (ii) a disrupted gene involved in apoptosis;
 - 5 (iii) a disrupted gene involved in regulation of T cell exhaustion; or
 - (iv) a combination of any one of (i)-(iii);wherein the population of genetically engineered T cells, as compared to the non-engineered T counterparts, have one or more of the following features: (a) enhanced expansion capacity in culture, (b) enhanced proliferation capacity, (c) a reduced apoptosis
10 level, and (d) an enhanced frequency of activation.
2. The population of genetically engineered T cells of claim 1, wherein the T cells comprise a combination of (i)-(iii).
- 15 3. The population of genetically engineered T cells of claim 1 or claim 2, wherein (i) comprises *TET2*, and optionally wherein the genetically engineered T cells do not comprise (ii), (iii) or both.
4. The population of genetically engineered T cells of any one of claims 1-3,
20 wherein the disrupted *TET2* is genetically edited in an exon selected from the group consisting of exon 1, exon 3, exon 4, exon 5, and exon 6, or a combination thereof, optionally wherein the mutated *TET2* is genetically edited in exon 3, exon 4, exon 5, or exon 6.
5. The population of genetically engineered T cells of any one of claims 1-4,
25 wherein the disrupted *TET2* gene is genetically edited by CRISPR/Cas-mediated gene editing.
6. The population of genetically engineered T cells of claim 5, wherein the disrupted *TET2* gene is genetically edited by CRISPR/Cas-mediated gene editing with a
30 guide RNA (gRNA) comprising a nucleotide sequence of SEQ ID NO: 14, 18, 22, 26, 112, 116, or 120.
7. The population of genetically engineered T cells of any one of claims 1-6; wherein (ii) comprises *FAS*.

8. The population of genetically engineered T cells of any one of claims 1-7, wherein (iii) comprises *CD70*.
9. The population of genetically engineered T cells of claim 7 or claim 8,
5 wherein the disrupted *FAS* and/or *CD70* gene is genetically edited by CRISPR/Cas-mediated gene editing.
10. The population of genetically engineered T cells of claim 9, wherein the disrupted *FAS* gene is genetically edited by CRISPR/Cas-mediated gene editing with a guide
10 RNA (gRNA) comprising the nucleotide sequence of SEQ ID NO: 69, 73, 77, 81, or 85, and/or wherein the disrupted *CD70* gene is genetically edited by CRISPR/Cas-mediated gene editing with a gRNA comprising the nucleotide sequence of SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.
- 15 11. The population of genetically engineered T cells of any one of claims 1-10, wherein the T cells further comprise a disrupted beta-2-microglobulin (*β2M*) gene.
12. The population of genetically engineered T cells of any one of claims 1-11, wherein the T cells further comprise a disrupted T cell receptor alpha chain constant region
20 (*TRAC*) gene.
13. The population of genetically engineered T cells of any one of claims 1-12, wherein the T cells are further engineered to express a chimeric antigen receptor (CAR).
- 25 14. The population of genetically engineered T cells of claim 13, wherein the CAR targets a tumor antigen.
15. The population of genetically engineered T cells of claim 14, wherein the tumor antigen is CD19, B cell maturation antigen (BCMA), or CD70.
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16. The population of genetically engineered T cells of any one of claims 13-15, wherein the T cells comprise a nucleic acid encoding the CAR, and wherein the nucleic acid is inserted in the genome of the T cells.

17. The population of genetically engineered T cells of claim 16, wherein the disrupted *TRAC* gene has an insertion of the nucleotide acid encoding the chimeric antigen receptor.

5 18. The population of genetically engineered T cells of any one of claims 1-17, wherein the T cells are derived from primary T cells of one or more human donors.

19. The population of genetically engineered T cells of any one of claims 1-18, wherein the T cells show cytokine-dependent growth.

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20. A method for preparing the population of genetically engineered T cells of any one of claims 1-10, the method comprising:

- (a) providing a plurality of cells, which are T cells or precursor cells thereof;
- (b) genetically editing one or more of the (i)-(iii) genes; and
- 15 (c) producing the population of genetically engineered T cells.

21. The method of claim 20, wherein step (b) is performed by delivering to the plurality of cells a RNA-guided nuclease, and one or more gRNAs targeting one or more of the (i)-(iii) genes.

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22. The method of claim 21, wherein (i) comprises *TET2* and optionally wherein the gRNA targeting *TET2* is specific to an exon of the *TET2* gene selected from the group consisting of exon 1, exon 3, exon 4, exon 5, and exon 6.

25 23. The method of claim 22, wherein the guide RNA (gRNA) targeting *TET2* comprises a nucleotide sequence of SEQ ID NO: 14, 18, 22, 26, 112, 116, or 120.

24. The method of any one of claims 20-23, wherein (ii) comprises *FAS*.

30 25. The method of any one of claims 20-24, wherein (iii) comprises *CD70*.

26. The method of claim 24 or claim 25, wherein the gRNA targeting *FAS* comprises the nucleotide sequence of SEQ ID NO: 69, 73, 77, 81, or 85, and/or wherein the

gRNA targeting *CD70* comprises the nucleotide sequence of SEQ ID NO: 34, 38, 42, 46, 50, 54, or 58.

27. The method of any one of claims 20-26, wherein the cells of step (a) are
5 primary T cells of one or more human donors.

28. The method of any one of claims 20-27, wherein in step (b), gRNAs targeting a combination of (i)-(iii) are delivered to the cells.

10 29. The method of any one of claims 20-28, wherein in step (b), the RNA-guided nuclease is a Cas9 nuclease.

30. The method of claim 29, wherein the Cas9 nuclease is a *S. pyogenes* Cas9 nuclease.
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31. The method of any one of claims 20-30, wherein the RNA-guided nuclease and the one or more gRNAs are complexed in a ribonucleoprotein particle (RNP).

32. The method of any one of claims 20-31, wherein step (b) is performed by a
20 single electroporation event.

33. The method of any one of claims 20-32, wherein step (b) is performed by two sequential electroporation events.

25 34. A method for preparing genetically engineered T cells expressing a chimeric antigen receptor (CAR), the method comprising:

- (a) providing a plurality of T cells from a cell bank comprising genetically engineered T cells set forth in any one of claims 1-10;
- (b) delivering to the plurality of the T cells a nucleic acid encoding a CAR; and
- 30 (c) producing genetically engineered T cells expressing the CAR.

35. The method of claim 34, wherein the genetically engineered T cells in the cell bank further comprises a disrupted *β2M* gene.

36. The method of claim 34, wherein the method further comprises genetically editing a $\beta 2M$ gene.

37. The method of claim 36, wherein the genetically editing step comprises
5 delivering to the plurality of the T cells a gRNA targeting a $\beta 2M$ gene.

38. The method of any one of claims 34-37, wherein the genetically engineered T cells in the cell bank further comprises a disrupted *TRAC* gene.

10 39. The method of any one of claims 34-38, wherein the method further comprises genetically editing a *TRAC* gene.

40. The method of any one of claims 34-39, wherein the genetically engineered T cells in the cell bank are derived from primary T cells of one or more human donor.

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41. The method of any one of claims 34-40, wherein a RNA-guided nuclease is delivered to the T cells.

42. The method of claim 41, wherein the RNA-guided nuclease is a Cas9
20 nuclease.

43. The method of claim 42, wherein the Cas9 nuclease is a *S. pyogenes* Cas9 nuclease.

25 44. The method of any one of claims 41-43, wherein the RNA-guided nuclease and the gRNA(s) are complexed in a ribonucleoprotein particle (RNP).

45. A genetically engineered T cell population, which is prepared by a method of any one of claims 20-44.

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46. A method for eliminating undesired cells in a subject, the method comprising administering to a subject in need thereof T cells expressing a chimeric antigen receptor

targeting to the undesired cells, wherein the T cells are set forth in any one of claims 1-19 and 45.

47. The method of claim 46, wherein the undesired cells are cancer cells.

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48. A guide RNA (gRNA) targeting a *TET2* gene, comprising a nucleotide sequence specific to exon 3 or exon 5 of the *TET2* gene.

49. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence specific to exon 5.

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50. The gRNA of claim 49, wherein the nucleotide sequence targets the site of GGGATGTCCTATTGCTAAGT (SEQ ID NO: 125) or AGGGATGTCCTATTGCTAAG (SEQ ID NO: 126) in exon 5 of the *TET2* gene.

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51. The gRNA of claim 50, wherein the nucleotide sequence comprises SEQ ID NO: 18 or 22.

52. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence targeting exon 3 of the *TET2* gene.

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53. The gRNA of claim 52, wherein the nucleotide sequence target GATTCCGCTTGGTGAAAACG (SEQ ID NO: 129), CAGGACTCACACGACTATTC (SEQ ID NO: 131), or TTCCGCTTGGTGAAAACGAG (SEQ ID NO: 133) in exon 3 of the *TET2* gene.

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54. The gRNA of claim 53, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO:112, 116, or 120.

55. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence targeting exon 4 of the *TET2* gene.

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56. The gRNA of claim 55, wherein the nucleotide sequence targets CATTAGGACCTGCTCCTAGA (SEQ ID NO: 124) in exon 4 of the *TET2* gene.

57. The gRNA of claim 56, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 14.

58. The gRNA of claim 48, wherein the gRNA comprises a nucleotide sequence
5 targeting exon 6 of the *TET2* gene.

59. The gRNA of claim 58, wherein the nucleotide sequence targets
ACGGCACGCTCACCAATCGC (SEQ ID NO: 127) in exon 6 of the *TET2* gene.

10 60. The gRNA of claim 59, wherein the gRNA comprises the nucleotide sequence of SEQ ID NO: 26.

61. The gRNA of any one of claims 48-60, wherein the gRNA further comprises a
scaffold sequence.

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62. The gRNA of any one of claims 48-61, wherein the gRNA comprises one or
more modified nucleotides.

63. The gRNA of claim 62, wherein the gRNA comprises one or more 2'-O-
20 methyl phosphorothioate residues at the 5' and/or 3' terminus of the gRNA.

64. The gRNA of any one of claims 61-63, which comprises the nucleotide
sequence of SEQ ID NO: 12, 13, 16, 17, 20, 21, 24, 25, 114, 115, 118, 119, 122, or 123.

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