

CLAIMS

1. A polynucleotide comprising a nucleic acid sequence encoding a zinc finger nuclease (ZFN) that cleaves a *CIITA* gene, wherein the ZFN comprises a Zinc Finger DNA-binding domain that binds to a DNA sequence in the *CIITA* gene and a cleavage domain,

wherein the ZFN is capable of cleaving the *CIITA* gene between amino acids 28 and 29 corresponding to SEQ ID NO: 1 or between amino acids 461 and 462 corresponding to SEQ ID NO: 1.

2. The polynucleotide of claim 1, wherein the DNA-binding domain binds to GCCACCATGGAGTTG (SEQ ID NO: 9) and/or CTAGAAGGTGGCTACCTG (SEQ ID NO:15).

3. The polynucleotide of claim 1, wherein the DNA-binding domain binds to ATTGCT and GAACCGTCCGGG (SEQ ID NO: 38) or GATCCTGCAGGCCAT (SEQ ID NO: 29).

4. The polynucleotide of claims 1 or 2, wherein the DNA-binding domain comprises five zinc fingers, which comprises finger 1 (F1) comprising SEQ ID NO: 10 [RPYTLRL], finger 2 (F2) comprising SEQ ID NO: 11 [RSANLTR], finger 3 (F3) comprising SEQ ID NO: 12 [RSDALST], finger 4 (F4) comprising SEQ ID NO: 13 [DRSTRTK], and finger 5 (F5) comprising SEQ ID NO: 14 [DRSTRTK].

5. The polynucleotide of claims 1 or 2, wherein the DNA-binding domain comprises six zinc fingers, which comprises F1 comprising SEQ ID NO: 16 [RSDVLSA], F2 comprising SEQ ID NO: 17 [DRSNRIK], F3 comprising SEQ ID NO: 18 [DRSHLTR], F4 comprising SEQ ID NO: 19 [LKQHLTR], F5 comprising SEQ ID NO: 20 [QSGNLAR], and F6 comprising SEQ ID NO: 21 [QSTPRTT].

6. The polynucleotide of claims 1 or 3, wherein the DNA-binding domain comprises six zinc fingers, which comprises F1 comprising SEQ ID NO: 23 [RSDHLR], F2 comprising SEQ ID NO: 24 [DSSDRKK], F3 comprising SEQ ID NO: 25 [RSDTLSE], F4 comprising 26 [QSGDLTR], and F5 comprising SEQ ID NO: 27 [QSSDLR], and F6 comprising SEQ ID NO: 28 [YKWTLRN].

7. The polynucleotide of claims 1 or 3, wherein the DNA-binding domain comprises five zinc fingers, which comprises F1 comprising SEQ ID NO: 30 [SNQNLTT], F2 comprising SEQ ID NO: 31 [DRSHLAR], F3 comprising SEQ ID NO: 32 [QSGDLTR], F4 comprising SEQ ID NO: 33 [WKHDLTN], and F5 comprising SEQ ID NO: 34 [TSGNLTR].
8. The polynucleotide of any one of claims 1 or 3, wherein the DNA-binding domain comprises SEQ ID NO: 54 or SEQ ID NO: 56.
9. The polynucleotide of any one of claims 1 or 3, which encodes a zinc finger nuclease pair comprising a first zinc finger DNA-binding domain and a second zinc finger DNA-binding domain, wherein the first DNA-binding domain comprises SEQ ID NO: 54 and the second DNA-binding domain comprises SEQ ID NO: 56.
10. The polynucleotide of any one of claims 1, 2, 4, or 5, which encodes a zinc finger nuclease pair comprising a first zinc finger DNA-binding domain and a second zinc finger DNA-binding domain, wherein the first zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 5 and the second zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 6.
11. The polynucleotide of any one of claims 1, 3, 6, or 7, which encodes a zinc finger nuclease pair comprising a first zinc finger DNA-binding domain and a second zinc finger DNA-binding domain, wherein the first zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 7 and the second zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 8.
12. The polynucleotide of any one of claims 1, 3, 6, or 7, which encodes a zinc finger nuclease pair comprising a first zinc finger DNA-binding domain and a second zinc finger DNA-

binding domain, wherein the first zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 54 and the second zinc finger DNA-binding domain comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 56.

13. The polynucleotide of any one of the preceding claims, wherein the cleavage domain comprises a *FokI* cleavage domain.

14. The polynucleotide of claim 13, wherein the *FokI* cleavage domain further comprises one or more mutations at positions 418, 432, 441, 448, 476, 479, 481, 483, 486, 487, 490, 496, 499, 523, 525, 527, 537, 538 and 559 of SEQ ID NO 35.

15. The polynucleotide of claim 14, wherein the *FokI* cleavage domain comprise SEQ ID NO: 36 (*FokELD*); wherein the *FokI* cleavage domain comprises one or more mutations at positions 490, 537, and/or 538; or wherein the *FokI* cleavage domain comprises SEQ ID NO: 37 (*FokKKR*).

16. The polynucleotide of any one of claims 13 to 15, wherein the *FokI* cleavage domain forms a dimer prior to DNA cleavage or wherein the *FokI* dimer comprises a heterodimer.

17. The polynucleotide of any one of claims 1, 2, 4, and 5, wherein the ZFN comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 5 or to SEQ ID NO: 6.

18. A polynucleotide comprising a sequence at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, or least about 98%, at least about 99% or about 100% sequence identity to SEQ ID NO: 39.

19. The polynucleotide of any one of claims 1, 3, and 6, wherein the ZFN comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least

about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 7 or SEQ ID NO: 54.

20. The polynucleotide of any one of claims 1, 3, and 7, wherein the ZFN comprises the amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 8 or SEQ ID NO: 56.

21. A polynucleotide comprising a sequence at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, or least about 98%, at least about 99% sequence identity to SEQ ID NO: 40, SEQ ID NO: 53, SEQ ID NO: 55, or SEQ ID NO: 57.

22. A polypeptide encoded by the polynucleotide of any one of claims 1 to 17.

23. An isolated cell comprising the polynucleotides of claims 1 to 21, the ZFNs of claim 22, or the ZFN pairs according to claim 22.

24. The isolated cell of claim 23, wherein the isolated cell comprises a T cell, a NK cell, a tumor infiltrating lymphocyte, a stem cell, Mesenchymal stem cells (MSC), hematopoietic stem cells (HSC), fibroblasts, cardiomyocytes, pancreatic islet cells, or a blood cell.

25. The isolated cell of claim 23 or 24, which is allogeneic or autologous.

26. A method of preparing a T cell, comprising contacting an isolated T cell with the polynucleotides of claims 1 to 21, the ZFNs according to claims 22, or the ZFN pair of any one of claims 22.

27. The method of claim 26, wherein the T cell comprises a chimeric antigen receptor T cell, a T cell receptor cell, a Treg cell, a Tumor infiltrating lymphocyte, or any combination thereof.

28. A method of treating a subject in need of a cellular therapy comprising administering the isolated cell of any one of claims 23 to 25 to the subject.

29. The method of claim 28, wherein the isolated cells are allogeneic or autologous.