CLAIMS

What is claimed is:

1. A zinc finger protein comprising 3 to 6 zinc finger DNA-binding domains, wherein each zinc finger DNA-binding domain comprises two beta sheets, an alpha helix, a recognition helix region that binds to a nucleotide sequence, and further wherein one or more of the zinc finger DNA-binding domains comprise mutations in amino acid residues (-5), (-9) and/or (-14), numbered relative to the start of the alpha helix region.

2. The zinc finger protein of claim 1, wherein the amino acids at (-5), (-9) and/or (-14) are mutated to an alanine (A), a leucine (L), a serine (S), an aspartic acid (N), glutamine (E), tyrosine (Y) and/or glutamine (Q) reside.

3. The zinc finger protein of claim 1 or claim 2, wherein the Arg (R) at position -5 is changed to a Tyr (Y), Asp (N), Glu (E), Leu (L), Gln (Q) or Ala (A) residue; the Arg (R) at position (-9) is replaced with Ser (S), Asp (N), or Glu (E); and/or the Arg (R) at position (-14) is replaced with Ser (S) or Gln (Q) residue.

4. A zinc finger nuclease comprising the zinc finger protein of any of claims 1 to 3 and a cleavage domain.

5. The zinc finger nuclease according to claim 4, wherein the cleavage domain comprises an engineered *Fok*I cleavage domain.

6. The zinc finger nuclease according to claim 4 or claim 5, wherein the engineered cleavage domain comprises one or more mutations in the dimerization domain.

7. The zinc finger nuclease of claim 5 or 6, wherein the engineered cleavage domain comprises one or more substitution mutations as follows:

(i) the wild-type Gln (Q) residue at position 481 is replaced with an Ala (A), Cys (C), Asp (D), Glu (E) or Ser (S) residue (Q481A, Q481C, Q481D, Q481E or Q481S);

(ii) the wild-type Ser (S) residue at position 418 is replaced with a Glu (E) or Asp (D) residue (S418E or S418D);

(iii) the wild-type Ile (I) residue at position 479 is replaced with a Gln (Q) or Thr (T) residue (I479Q or I479T);

(iv) the wild-type Pro (P) residue at position 478 is replaced an Asp (D) residue (P478D);

(v) the wild-type Lys (K) residue at position 525 is replaced with an Ala (A), Cys (C), Glu (E), Ile (I), Ser (S), Thr (T) or Val (V) residue (K525A, K525C, K525E, K252I, K525S, K5252T or K525V);

(vi) the wild-type Arg (R) residue as position 416 is replaced with an Asp (D), Glu (E), His (H) or Asn (N) residue (R416D, R416E, R416H, or R416N);

(vii) the wild-type Gly (G) residue at position 480 is replaced with an Asp (D) residue (G480D);

(viii) the wild-type Ser (S) residue a position 472 is replaced with an Asp (D) residue (S472D);

(ix) the wild-type Asn (N) residue at position 476 is replaced with a Glu (E) or Gly (G) residue (N476E or N476G);

(x) the wild-type Asn (N) residue at position 527 is replaced with an Asp (D) residue (N527D);

(xi) the wild-type Gln (Q) residue at position 531 is replaced with an Arg (R) or Thr (T) residue (Q531R or Q531T);

(xii) the wild-type Arg (R) residue at position 422 is replaced with a His (H) residue (R422H);

(xiii) the wild-type Ser (S) residue at position 446 is replaced with an Asp (D) residue (S446D);

(xiv) the wild-type residue at position 448 is replaced with an Ala (A) residue (K448A);

(xv) the wild-type His (H) residue at position 523 is replaced with a Glu (E) residue (H523E);

(xvi) the wild-type Leu (L) residue at position 424 is replaced with a Phe (F) residue (L424F); and

(xvi) the wild-type Asn (N) residue at position 542 is replaced with an Asp (D) residue (N542D), wherein the amino acid residues are numbered relative to full length *Fok*I wild-type cleavage domain as shown in SEQ ID NO:1, beginning at amino acid residue 384 of the full length FokI protein.

8. The zinc finger nuclease of any of claims 5 to 7, wherein the wild type Gln (Q) residue at position 481 is replaced with an Ala (A) residue in the engineered FokI cleavage domain.

9. The zinc finger nuclease of any of claims 5 to 8, wherein the engineered FokI cleavage domain comprises substitution mutations as follows: the R416D, R416E, R416H, or R416N mutation and the R422H mutation; the R416D, R416E, R416H, or R416N mutation and the K448A mutation; the K448A and I479Q mutations; the K448A and Q481A mutations; and/or K448A mutation and the K525A, K525C, K525E, K252I, K525S, K5252T or K525V mutation

10. The zinc finger nuclease of any of claims 4 to 9, wherein the cleavage domain is *FokI* cleavage domain further comprising an amino acid mutation at one or more of positions 432, 441, 483, 486, 487, 490, 496, 499, 527, 537, 538 and 559.

11. A zinc finger nuclease comprising a first zinc finger nuclease according to any of claims 4 to 10 and a second zinc finger nuclease.

12. One or more polynucleotides encoding the zinc finger protein of any of claims 1 to 3 or the zinc finger nuclease according to any of claims 4 to 11.

13. An isolated cell comprising the zinc finger protein of any of claims 1 to 3, the zinc finger nuclease according to any of claims 4 to 11, and/or the one or more polynucleotides of claim 12.

14. The zinc finger protein of any of claims 1 to 3, the zinc finger nuclease according to any of claims 4 to 11, the one or more polynucleotides of claim 12 and/or the isolated cell of claim 13 for use in the treatment or prevention of disease.

15. An *in vitro* method for cleaving genomic cellular chromatin in a region of interest, the method comprising:

expressing the zinc finger nuclease according claim 11 or the one or more polynucleotides according to claim 12 in a cell,

wherein the zinc finger nuclease is expressed in the cell and site-specifically cleaves a nucleotide sequence in the region of interest of the genomic cellular chromatin.

16. The *in vitro* method of claim 15 further comprising contacting the cell with a donor polynucleotide; wherein cleavage of the cellular chromatin facilitates homologous recombination between the donor polypeptide and the cellular chromatin.

17. An *in vitro* method for increasing engineered nuclease cleavage specificity, the method comprising contacting a cell with an engineered nuclease complex comprising a nuclease comprising a pair of zinc finger proteins according to any one of claims 1-3 or a pair of zinc finger nucleases according to any of claims 4 to 11, where the ratio of the pair is other than one to one, optionally wherein:

(i) the ratio is 1:2, 1:3, 1:4, 1:5, 1:6, 1:8, 1:9, 1:10 or 1:20, or any value therebetween.

18. A composition comprising the one or more polynucleotides of claim 12, wherein the one or more polynucleotides encode dimerizing first and second zinc finger nucleases and the ratio of the first and second zinc finger nucleases is not one to one, optionally a ratio of 1:2, 1:3, 1:4, 1:5, 1:6, 1:8, 1:9, 1:10, 1:20 or 1:30 ratio.

19. A zinc finger nuclease that cleaves a T cell receptor constant region gene (TRAC) gene, the zinc finger nuclease comprising

a first zinc finger nuclease comprising a first engineered *FokI* cleavage domain and a first zinc finger DNA-binding domain that binds to a target site in the TRAC gene, wherein the first engineered *FokI* cleavage domain comprises a polypeptide in which the wild-type Gln (Q) residue at position 486 is replaced with a Glu (E) residue, the wild-type Ile (I) residue at position 499 is replaced with a Leu (L) residue and the wild-type Asn (N) residue at position 496 is replaced with an Asp (D) residues (ELD) and

a second zinc finger nuclease comprising a second engineered *Fok*I cleavage domain and a second zinc finger DNA-binding domain, wherein the second engineered *Fok*I cleavage domain comprises a polypeptide in which the wild-type Glu (E) residue at position 490 is replaced with a Lys (K) residue, the wild-type Ile (I) residue at position 538 is replaced with a Lys (K) residue, and the wild-type His (H) residue at position 537 is replaced with an Arg (R) residue (KKR), an further wherein

(i) at least one of the first or second engineered *FokI* cleavage domains further comprises a mutation in the *Fok*I cleavage domain selected from the group consisting of: K393S, K394S, R398S, K400S, K402S, R416S, R422S, K427S, K434S, R439S, K441S, R447S, K448S, K469S, R487S, R495S, K497S, K506S, K516S, K525S, K529S, R534S, K559S, R569S, and R570S, numbered relative to wild-type FokI; and/or

(ii) at least one of the first and second zinc finger DNA-binding domains comprises one or more mutations in amino acid residues (-5), (-9) and/or (-14), numbered relative to the start of the alpha helix region.

20. The zinc finger nuclease of claim 19, wherein the mutation in the ELD and/or KKR *Fok*I cleavage domain is a R416S, R422S, R447S, K448S or K525S mutation.

21. The zinc finger nuclease of claim 19 or claim 20, wherein the amino acid at residue at position -5 is mutated in one, two or three fingers.

22. One or more polynucleotides encoding the zinc finger nuclease according to any of claims 19 to 21.

23. An isolated cell comprising the one or more polynucleotides of claim 22.

24. A composition comprising isolated cells comprising at least one site-specific genomic modification in a TRAC gene made by a zinc finger nuclease according to any of claims 19 to 21.

25. An *in vitro* method for cleaving a TCR alpha (TRAC) gene in a mammalian cell, the method comprising:

expressing the zinc finger nuclease according to any of claims 19 to 21 or the one or more polynucleotides according to claim 22 in a cell,

wherein the zinc finger nuclease is expressed in the cell and site-specifically cleaves to a nucleotide sequence in the TRAC gene.

26. The method of claim 25, further comprising contacting the cell with a donor polynucleotide; wherein cleavage of the cellular chromatin facilitates homologous recombination between the donor polypeptide and the cellular chromatin.

27. A composition comprising the one or more polynucleotides of claim 22, wherein the ratio of the first and second zinc finger nucleases is not one to one, optionally a ratio of 1:2, 1:3, 1:4, 1:5, 1:6, 1:8, 1:9, 1:10, 1:20 or 1:30 ratio.

28. The zinc finger nuclease of any of claims 19 to 21, the one or more polynucleotides of claim 23, the isolated cell of claim 24 and/or the composition of claim 25 for use in the treatment or prevention of disease.