CLAIMS

What is claimed is:

1. A zinc finger protein comprising at least three zinc finger DNA-binding domains, wherein each zinc finger DNA-binding domain comprises two beta sheets, an alpha helix region, 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 at any one of positions (-5), (-9) and/or (-14), numbered relative to the start of the alpha helix region, and wherein the amino acid residues at positions (-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) residue.

2. The zinc finger protein of claim 1, 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.

3. A zinc finger nuclease comprising the zinc finger protein of any one of claim 1 or claim 2 and a cleavage domain.

4. The zinc finger nuclease according to claim 3, wherein the cleavage domain comprises an engineered *Fok*I cleavage domain.

5. The zinc finger nuclease according to claim 3 or claim 4, wherein the engineered cleavage half-domain comprises one or more mutations in residues 416, 421, 422, 424, 472, 478, 480, 525 or 542 or in which the wild-type residue at position 418 is replaced with a Glu (E) or Asp (D) residue, the wild-type residue at position 446 is replaced with an Asp (D) residue, the wild-type residue at position 448 is replaced with an Ala (A) residue (K448A), the wild-type residue at position 479 is replaced with a Gln (Q) or Thr (T) residue (I479Q or I479T), the wild type residue at position 481 is replaced with an Ala (A), Cys (C), Asp (D), Ser (S), or Glu (E) residue (Q481A, Q481C, Q481D, Q481S, Q481E), or the wild-type residue at position 476 is

replaced with a Glu (E) residue or a Glu (G) residue (N476E, N476G), wherein the amino acid residues are numbered relative to full length FokI wild-type cleavage domain as shown in SEQ ID NO:1.

6. The zinc finger nuclease of claim 5, comprising mutations as follows: mutations at residues 416 and 422; a mutation at position 416 and the K448A mutation; the K448A and I479Q mutations; the K448A and Q481A mutations; and/or the K448A mutation and a mutation at position 525.

7. The zinc finger nuclease of claim 6, wherein:

the wild-residue at position 416 is replaced with a Glu (E), Asp (D), His (H) or Asn (N) residue (R416E, R416D, R416H, R416N);

the wild-type residue at position 421 is replaced with a Ser (S) residue (D421S);

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

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

the wild-type residue at position 472 is replaced with an Asp (D) residue (S472D);

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

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

the wild-type 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, K525I, K525S, K525T, K525V); and/or

the wild-type residue at position 542 is replaced with an Asp (D) residue (N542D).

8. The zinc finger nuclease of any one of claims 3 to 7, wherein the cleavage domain is *FokI* cleavage half-domain further comprising an additional amino acid mutation at one or more of positions 432, 441, 483, 486, 487, 490, 496, 499, 527, 537, 538 and 559.

9. A zinc finger nuclease comprising a first and a second dimerizing zinc finger nuclease according to any one of claims 3 to 6 and a second zinc finger nuclease.

10. One or more polynucleotides encoding the zinc finger protein of any one of claim 1 or claim 2 or the zinc finger nuclease according to any one of claims 3 to 9.

11. An isolated cell comprising the one or more polynucleotides of claim 10.

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

expressing the zinc finger nuclease according to any one of claims 3 to 9 or the one or more polynucleotides according to claim 10 in a cell *in vitro*,

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

13. The *in vitro* method according to claim 12, wherein the cell is contacted *in vitro* with a donor polynucleotide; wherein cleavage of the cellular chromatin facilitates homologous recombination between the donor polypeptide and the cellular chromatin.

14. An isolated cell or cell line comprising the zinc finger protein of any of claim 1 or claim 2, the zinc finger nuclease of any one of claims 3 to 9 and/or the one or more polynucleotides of claim 10.

15. A composition comprising first and second polynucleotides encoding the first and second zinc finger nucleases of claim 9 wherein the first polynucleotide encodes the first zinc finger nuclease and the second polynucleotide encodes the second zinc finger nuclease 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.