CLAIMS

1. An engineered Cas9 protein comprising at least one modification compared to a wild-type Cas9 protein; in which the modification comprises an amino acid substitution at position 13, 63, 415, 610, 775, 779, 780, 810, 832, 848, 855, 861, 862, 866, 961, 968, 974, 976, 1000, 1003, 1014, 1047, 1060, 1107, 1108, 1109, 1114, 1129, 1240, 1289, 1296, 1297, 1300, 1311, or 1325 with reference to amino acid position numbering of *Streptococcus pyogenes* Cas9 (SpCas9).

2. An engineered Cas9 protein according to Claim 1, in which the modification comprises a substitution of Lys, His, Arg, Glu, Asp, Ser, Gly, or Thr.

3. An engineered Cas9 protein according to Claim 1, in which the modification comprises a substitution with Gly, Ala, Ile, Glu, or Asp.

4. An engineered Cas9 protein according to Claim 1, in which the modification comprises an amino acid substitution in a binding groove between RuvC and HNH domains.

5. An engineered Cas9 protein according to Claim 1, in which the modification comprises a mutation in a RuvCI, RuvCIII, RuvCIII or HNH domain.

6. An engineered Cas9 protein according to Claim 1, in which the modification at position 63, 415, 775, 779, 780, 810, 832, 848, 855, 861, 862, 866, 961, 968, 974, 976, 1000, 1003, 1014, 1047, 1060, 1107, 1108, 1109, 1114, 1129, 1240, 1289, 1296, 1297, 1300, 1311, or 1325 comprises an alanine substitution.

7. An engineered Cas9 protein according to Claim 1, in which the modification at position 13 comprises an isoleucine substitution, the modification at position 610 comprises a glycine substitution, the modification at position 799 comprises a leucine substitution, and/or in which the modification at position 1129 comprises a glutamic acid substitution.

8. An engineered Cas9 protein according to Claim 1, in which the modification comprises K775A, E779L, Q807A, R780A, K810A, R832A, K848A, K855A, K862A, K961A, K968A, K974A, R976A, H983A, K1000A, K1014A, K1047A, R1060A, K1003A, S1109A, H1240A, K1289A, K1296A, H1297A, K1300A, H1311A, or K1325A.

9. An engineered Cas9 protein according to Claim 1, in which the modification comprises R783A and A1322T, or R780A and K810A, or R780A and K855A, or R780A and R976A, or

K848A and R976A, or K855A and R976A, or R780A and K848A, or K810A and K848A, or K848A and K855A, or K810A and K855A, or H982A and R1060A, or H982A and R1003A, or K1003A and R1060A, or R780A and H982A, or K810A and H982A, or K848A and H982A, or K855A and H982A, or R780A and K1003A, or K810A and R1003A, or K848A and K1003A, or K848A and K1007A, or R780A and R1060A, or K810A and R1060A, or K848A and R1060A, or R780A and R1114A, or K848A and R1114A, or R63A and K855A, or R63A and H982A, or H415A and R780A, or H415A and K848A, or K848A and E1108A, or K810A and K1003A, or R780A and R1060A, or K810A and R1060A, or K848A and R1060A.

10. An engineered Cas9 protein according to Claim 1, in which the modification comprises H982A, K1003A, and K1129E; or R780A, K1003A, and R1060A; or K810A, K1003A, and R1060A; or K848A, K1003A, and R1060A; or K855A, K1003A, and R1060A; or H982A, K1003A, and R1060A; or R63A, K848A, and R1060A; or T13I, R63A, and K810A; or G12D, R63A, and R1060A.

11. An engineered Cas9 protein according to Claim 1, in which the modification comprises R63A, E610G, K855A, and R1060A; or R63A, K855A, R1060A, and E610G.

12. An engineered Cas9 protein according to Claim 1, in which the engineered Cas9 is a mutant of a Cas9 protein from a genus comprising *Streptococcus*, *Campylobacter*, *Nitratifractor*, *Staphylococcus*, *Parvibaculum*, *Roseburia*, *Neisseria*, *Gluconacetobacter*, *Azospirillum*, *Sphaerochaeta*, *Lactobacillus*, *Eubacterium* or *Corynebacter*.

13. An engineered Cas9 protein according to Claim 1, in which the engineered Cas9 protein comprises one or more nuclear localization signal (NLS) domains.

14. An engineered Cas9 protein according to Claim 1, in which the engineered Cas9 protein comprises at least two or more NLSs.

15. An engineered Cas9 protein according to Claim 1, in which the engineered Cas9 protein comprises one or more heterologous functional domains.

16. An engineered Cas9 protein according to Claim 15, in which the one or more heterologous functional domains comprises one or more transcriptional activation domains.

17. An engineered Cas9 protein according to Claim 16, in which the transcriptional activation domain comprises VP64.

18. An engineered Cas9 protein according to Claim 15, in which the one or more heterologous functional domains comprises one or more transcriptional repression domains.

19. An engineered Cas9 protein according to Claim 18, in which the transcriptional repression domain comprises a KRAB domain or a SD domain.

20. An engineered Cas9 protein according to Claim 15, in which the one or more heterologous functional domains comprises one or more nuclease domains.

21. An engineered Cas9 protein according to Claim 20, in which a nuclease domain comprises Fok1.

22. An engineered Cas9 protein according to Claim 15, in which the one or more heterologous functional domains have one or more of the following activities: methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, single-strand RNA cleavage activity, double-strand RNA cleavage activity, single-strand DNA cleavage activity, double-strand DNA cleavage activity and nucleic acid binding activity.

23. A nucleic acid molecule comprising a nucleotide sequence encoding an engineered Cas9 protein according to Claim 1, in which the nucleotide sequence is codon optimized for expression in a eukaryote.

24. A composition comprising an engineered Cas9 protein according to Claim 1 and one or more guide polynucleotides comprising a guide sequence, a tracr mate sequence, and a tracr sequence.

25. A CRISPR-Cas9 system comprising an engineered Cas9 protein according to Claim 1 in complex with a guide polynucleotide comprising a guide sequence, a tracr mate sequence, and a tracr sequence.

26. A vector system comprising one or more vectors, in which the one or more vectors comprises:

(a) a first regulatory element operably linked to a nucleotide sequence encoding an engineered Cas9 protein according to Claim 1; and

(b) a second regulatory element operably linked to one or more nucleotide sequences encoding one or more nucleic acid molecules comprising a guide polynucleotide comprising a guide sequence, a tracr sequence, and a tracr mate sequence, in which components (a) and (b) are located on same or different vectors.

27. An isolated eukaryotic cell comprising a vector system according to Claim 26.

28. An *in vitro* or *ex vivo* method of modifying a target locus of interest, the method comprising delivering to the locus an engineered Cas9 protein according to Claim 1, and one or more guide polynucleotides, in which the engineered Cas9 protein forms a complex with the one or more guide polynucleotides and upon binding of the complex to a target locus of interest, the Cas9 protein induces a modification of the target locus of interest.

29. An isolated host cell or cell line comprising an engineered Cas9 protein according to Claim 1.