CLAIMS

1. An engineered Cas9 protein comprising an HNH domain, a RuvC domain, and two or more modifications in a groove between the HNH and RuvC domains, in which each of the modifications comprises an amino acid substitution of a positively charged residue of wild-type Cas9 with an uncharged residue, in which the modifications are amino acid substitutions at positions selected from the group consisting of 780, 810, 848, 855, 976, 982, 1003, and 1060, with reference to amino acid position numbering of *S. pyogenes* Cas9 (SpCas9).

2. An engineered Cas9 protein according to Claim 1, in which the uncharged residue is alanine.

3. An engineered Cas9 protein according to Claim 1, in which the modifications alter binding kinetics at DNA:RNA:protein interface.

4. An engineered Cas9 protein according to Claim 1, in which the modifications comprise amino acid substitutions, with reference to numbering of SpCas9: 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 R780A and R1060A, or K810A and R1060A, or K848A and R1060A, or K848A and E1108A, or K810A and K1003A, or R780A and R1060A, or K810A and R1060A, or K848A and R1060A.

5. An engineered Cas9 protein according to Claim 1, in which modifications comprise amino acid substitutions, with reference to numbering of SpCas9: 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.

6. An engineered Cas9 protein according to Claim 1, in which the modifications comprise, with reference to the amino acid numbering of SpCas9: K848A, K1003A, and R1060A.

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

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

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

10. An engineered Cas9 protein according to Claim 1, in which the protein comprises three or more modifications.

11. A CRISPR-Cas complex, comprising an engineered Cas9 protein according to Claim 1 and a guide polynucleotide.

12. A CRISPR-Cas complex according to Claim 11, in which the guide polynucleotide comprises a guide sequence, a tracr mate sequence, and a tracr sequence.

13. A CRISPR-Cas complex according to Claim 12, in which the guide polynucleotide is a chimeric guide polynucleotide in which the tracr mate sequence is linked to the tracr sequence.

14. A method of modulating gene expression or modifying a target sequence in a cell or cell line, comprising introducing a CRISPR-Cas complex according to Claim 11 into the cell or cell line, whereby the gene expression is modulated or the target sequence is modified resulting in a modified cell or cell line.

15. A method according to Claim 14, further comprising culturing the modified cell or cell to produce progeny.

16. A method according to Claim 14, in which the cell or cell line is a eukaryotic cell or cell line.

17. A method according to Claim 14, in which the CRISPR-Cas complex is introduced into the cell or cell line *ex vivo*.

18. A method of producing a plant having a modified trait of interest encoded by a gene of interest, comprising delivery to a plant cell of a CRISPR-Cas complex according to Claim 11, whereby the gene of interest is either modified or introduced into the cell and a modified plant cell is obtained, and regenerating the plant from the modified plant cell.

19. A method according to Claim 18, further comprising obtaining seed from the plant.

20. A method of modifying a non-human organism by manipulation of one or more target sequences, comprising delivering to the organism a CRISPR-Cas complex according to Claim 11, thereby obtaining a modified non-human organism.

21. A method according to Claim 20, in which the non-human organism is a plant or algae.

22. A method according to Claim 20, in which the non-human organism is an animal.