CLAIMS

What is claimed is:

1. A polypeptide having at least 50% sequence identity to *Streptococcus pyogenes* Cas9 having the amino acid sequence set forth in SEQ ID NO:1 over its entire length, wherein said polypeptide comprises at least one combination of deletions selected from the group consisting of ΔHNH ΔRuvCIII-b (Δ775-909 Δ1002-1074), ΔREC3 ΔHNH ΔRuvCIII-b (Δ498-712 Δ775-909 Δ1002-1074), ΔREC2 ΔREC3 ΔHNH ΔRuvCIII-b (Δ180-308 Δ498-712 Δ775-909 Δ1002-1074), and ΔREC2 ΔHNH ΔRuvCIII-b (Δ180-308 Δ775-909 Δ1002-1074), wherein the polypeptide comprises a PI domain (1099-1368), wherein the position numbering is in accordance with SEQ ID NO:1; and wherein the polypeptide has CRISPR-Cas DNA-binding activity.

2. The polypeptide of claim 1, wherein the polypeptide further comprises at least one missense mutation selected from the group consisting of G12R, T13K, T13R, N14K, N497K, T657K, T657R, N767K, T770K, T770R, Q920K, Q920R, S1109R, D1135K, D1135R, S1338R and combinations thereof.

3. The polypeptide of claim 1, wherein the polypeptide comprises at least one combination of modifications selected from the group consisting of ΔREC2 ΔHNH ΔRuvCIII-b T13K, ΔREC2 ΔHNH ΔRuvCIII-b T657K, ΔREC2 ΔHNH ΔRuvCIII-b T657R, ΔREC2 ΔHNH ΔRuvCIII-b 770K, ΔREC2 ΔHNH ΔRuvCIII-b Q920K, ΔREC2 ΔHNH ΔRuvCIII-b S1109R, ΔREC2 ΔHNH ΔRuvCIII-b D1135K, and combinations thereof.

4. The polypeptide of claim 1, wherein the polypeptide has the amino acid sequence set forth in any one of SEQ ID NOs: 16, 23, 24, 25, 26, 29, and 30.

5. A nucleic acid molecule comprising or consisting of a nucleic acid encoding a polypeptide of claim 1.

6. The nucleic acid molecule of claim 5, wherein the nucleic acid molecule is a recombinant expression vector.

7. The nucleic acid molecule of claim 6, wherein the recombinant expression vector further comprises at least one regulatory element for controlling expression of the polypeptide.

8. A composition comprising the polypeptide of claim 1 and a guide RNA.

9. An in vitro method of site-specific engineering of a target DNA, the method comprising: contacting the target DNA with a CRISPR-Cas system comprising the polypeptide of claim 1, wherein the CRISPR-Cas DNA-binding activity of the polypeptide recruits an effector domain to the target DNA.

10. The method of claim 9, wherein the target DNA is extrachromosomal or is part of a chromosome in vitro, or in a cell.

11. A CRISPR-Cas system comprising the polypeptide of claim 1, for use in site-specific engineering of a target DNA, wherein the CRISPR-Cas DNA-binding activity of the polypeptide recruits an effector domain to the target DNA, and wherein the target DNA is extrachromosomal or part of a chromosome *in vivo* or in a cell.

12. Use of a CRISPR-Cas system comprising the polypeptide of claim 1 in the manufacture of a medicament for site-specific engineering of a target DNA, wherein the CRISPR-Cas DNA-binding activity of the polypeptide recruits an effector domain to the target DNA, wherein the target DNA is extrachromosomal or part of a chromosome *in vivo* or in a cell.