**SUPPLEMENTAL FIGURE LEGENDS**

**Figure S1. Structural Determination, Related to Figure 1.**

(A) Simulated annealing omit *mF*O – *DF*C electron density map for the PAM and the PAM-interacting residues is shown as a blue mesh (contoured at 4.5) (stereoview).

(B) Location of the C946A mutation. The C946A mutation is located on a surface-exposed  helix in the TOPO domain, and does not contact the bound nucleic acids.

(C) Target sequence in the human *DYRK1A* locus, used to test endogenous genome cleavage by the wild-type (WT) and C946A mutant of SaCas9. The sites cleaved by the RuvC and HNH domains are indicated by cyan and pink triangles, respectively.

(D) Comparison of the genome cleavage activities of the WT and C946A mutant of SaCas9. The activities were evaluated by the percentage of indel formation at the *DYRK1A* locus, using next-generation sequencing of targeted amplicon (n = 3 for all experiments, error bars indicate MLE) (see Experimental Procedure section for more details).

**Figure S2. Crystal Structures of SpCas9 (PDB ID 4CMP) and AnCas9 (PDB ID 4OGE) in the Apo Form, Related to Figure 1.**

The active sites of the HNH and RuvC nuclease domains are indicated by red circles. In SpCas9, the WED domain is disordered. In AnCas9, residues 99–136 and 171–224 in the REC lobe are disordered, probably due to their flexibility.

**Figure S3. Structure-Guided Sequence Alignment of SaCas9 and SpCas9, Related to Figure 1.**

The structures of SaCas9 and SpCas9 (PDB ID 4UN3) were superimposed by the secondary-structure matching (SSM) algorithm, using CueMol (http://www.cuemol.org), and then the sequence alignment was manually refined. The figure was prepared using ESPript3 (http://espript.ibcp.fr/ESPript/ESPript/). The secondary structures of SaCas9 and SpCas9 are shown above and below the sequences, respectively.

**Figure S4. Comparison of the SaCas9 and SpCas9 sgRNAs, Related to Figure 2.**

(A and B) Crystal structures (left) and nucleotide sequences (right) of the SpCas9 sgRNA (PDB ID 4OO8) (A) and the SaCas9 sgRNA (B).

(C) Effect of the truncation of stem loop 2 on the *in vitro* DNA cleavage activity. An *Eco*RI-linearized pUC119 plasmid (150 ng, 7 nM) was cleaved with the SaCas9–sgRNA complex (8, 16, 32 nM) at 37°C for 1 h, and then resolved on an ethidium bromide-stained 1% agarose gel.

(D) Electrostatic surface potential of SpCas9 (PDB ID 4OO8) (left) and SaCas9 (right).

**Figure S5. Structural Comparison of the REC Lobes, Related to Figure 4.**

(A) Structural comparison of the REC lobes (the C-terminal region) of SaCas9, SpCas9 (PDB ID 4UN3) and AnCas9 (PDB ID 4OGE). The conserved  helices are numbered.

(B) Superimposition of the REC lobes (the C-terminal region) of SaCas9, SpCas9 (PDB ID 4UN3) and AnCas9 (PDB ID 4OGE) (stereoview).

**Figure S6. Structural Comparison of the REC, WED and PI Domains, Related to Figure 4.**

(A) Structural comparison of the WED and PI domains of SaCas9, SpCas9 (PDB ID 4UN3) and AnCas9 (PDB ID 4OGE). The core -strands in the WED and PI domains are numbered. The SpCas9-specific insertion is highlight in pale blue. The PAM-interacting residues in SaCas9 and SpCas9, and the equivalent residues in AnCas9, are shown as stick models.

(B and C) Recognition of the sgRNA scaffolds in SaCas9 (B) and SpCas9 (PDB ID 4UN3) (C).

**Figure S7. RuvC and HNH Nuclease Domains, Related to Figure 6.**

(A) Target sequence in the human *DYRK1A* locus, used to test endogenous genome cleavage by the wild-type (WT) and mutants of SaCas9. The sites cleaved by the RuvC and HNH domains are indicated by cyan and pink triangles, respectively.

(B) Comparison of the genome cleavage activities of the wild-type (WT) and mutants of SaCas9. The activities were evaluated by the percentage of indel formation at the *DYRK1A* locus, using next-generation sequencing of targeted amplicon (n = 3 for all experiments, error bars indicate MLE) (see Experimental Procedure section for more details).

(C and D) Structural comparison between *T. thermophilus* RuvC in complex with a Holliday junction substrate (PDB ID 4LD0) (C) and the SaCas9 RuvC domain (D). The non-target DNA strand was modeled into the SaCas9 RuvC domain, based on the superimposition of *T. thermophilus* RuvC on SaCas9. The catalytic residues are shown as stick models. The cleavage sites of the target and non-target DNA strands are indicated by magenta circles.