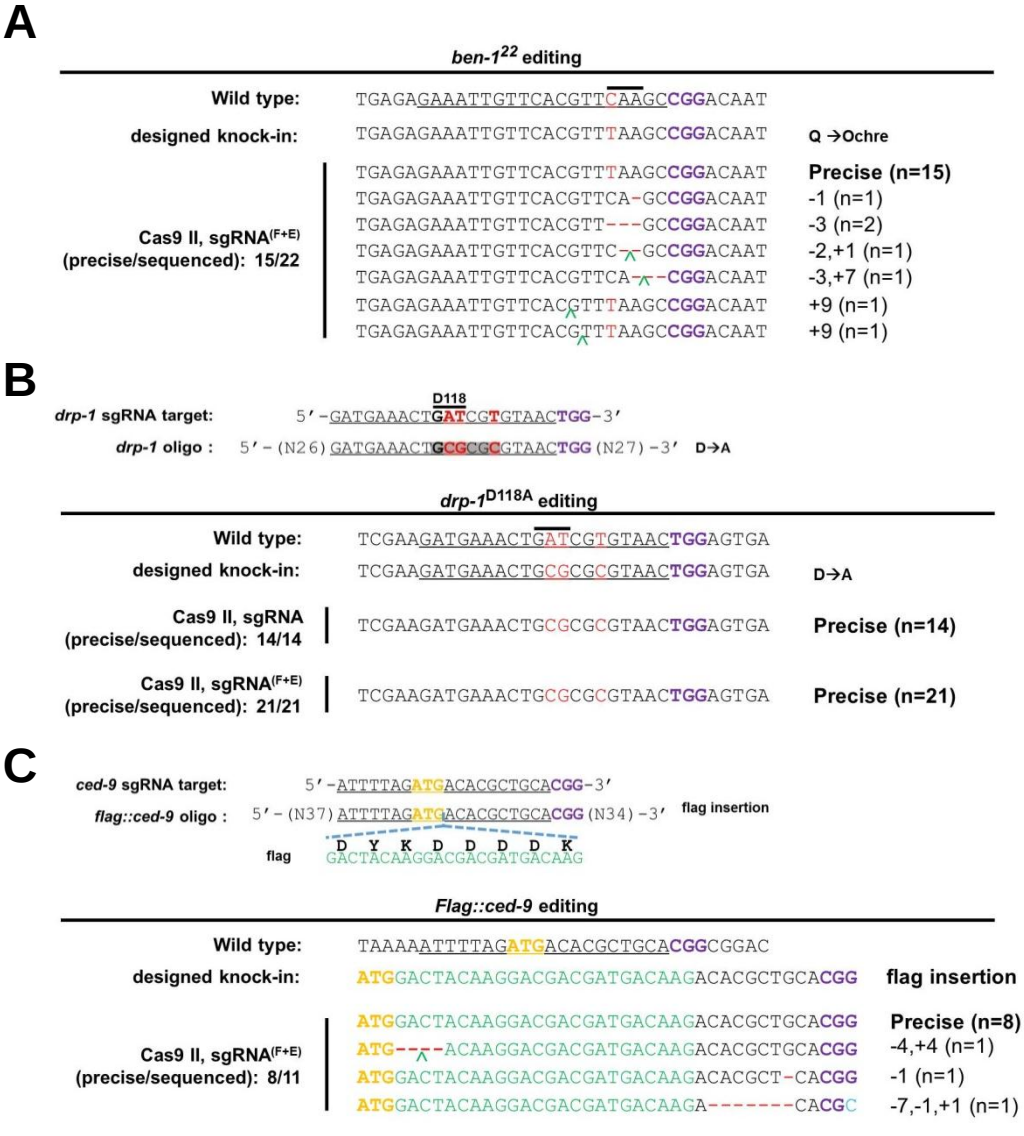
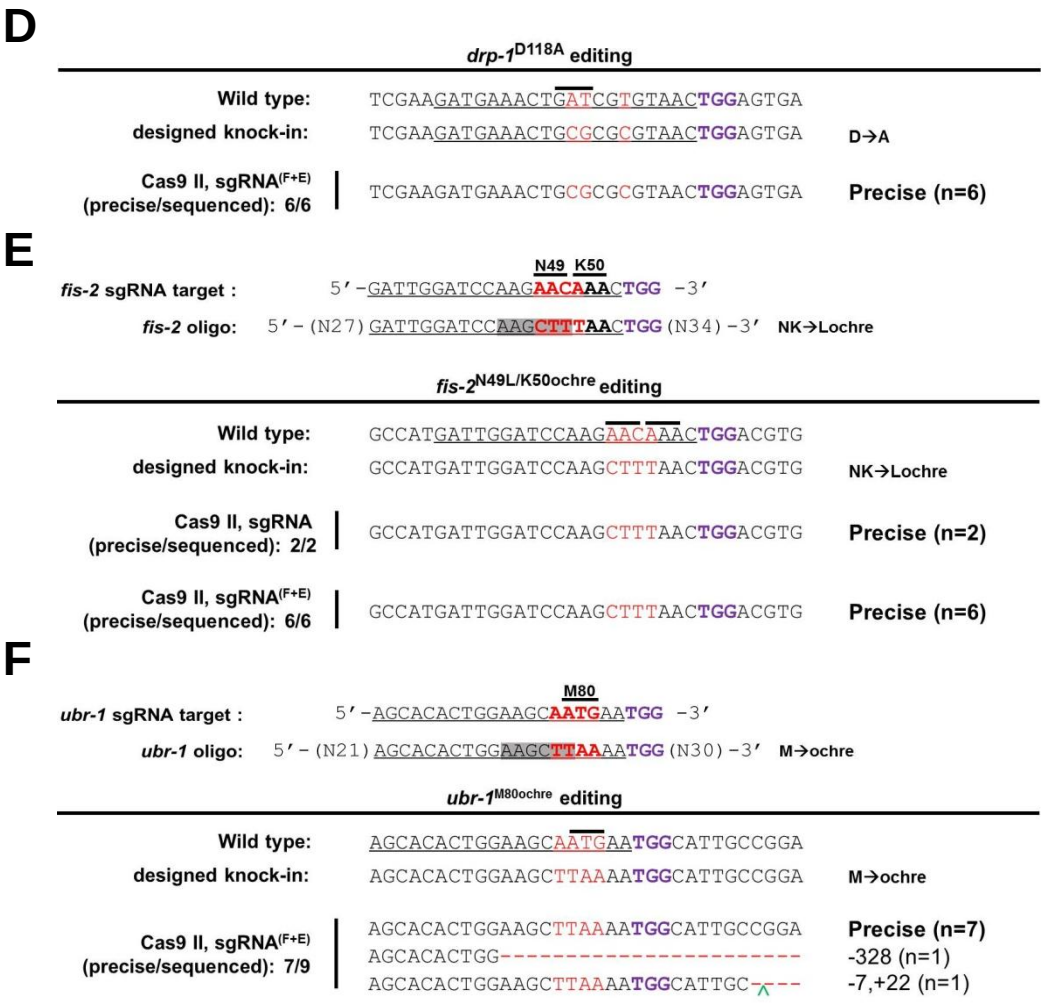
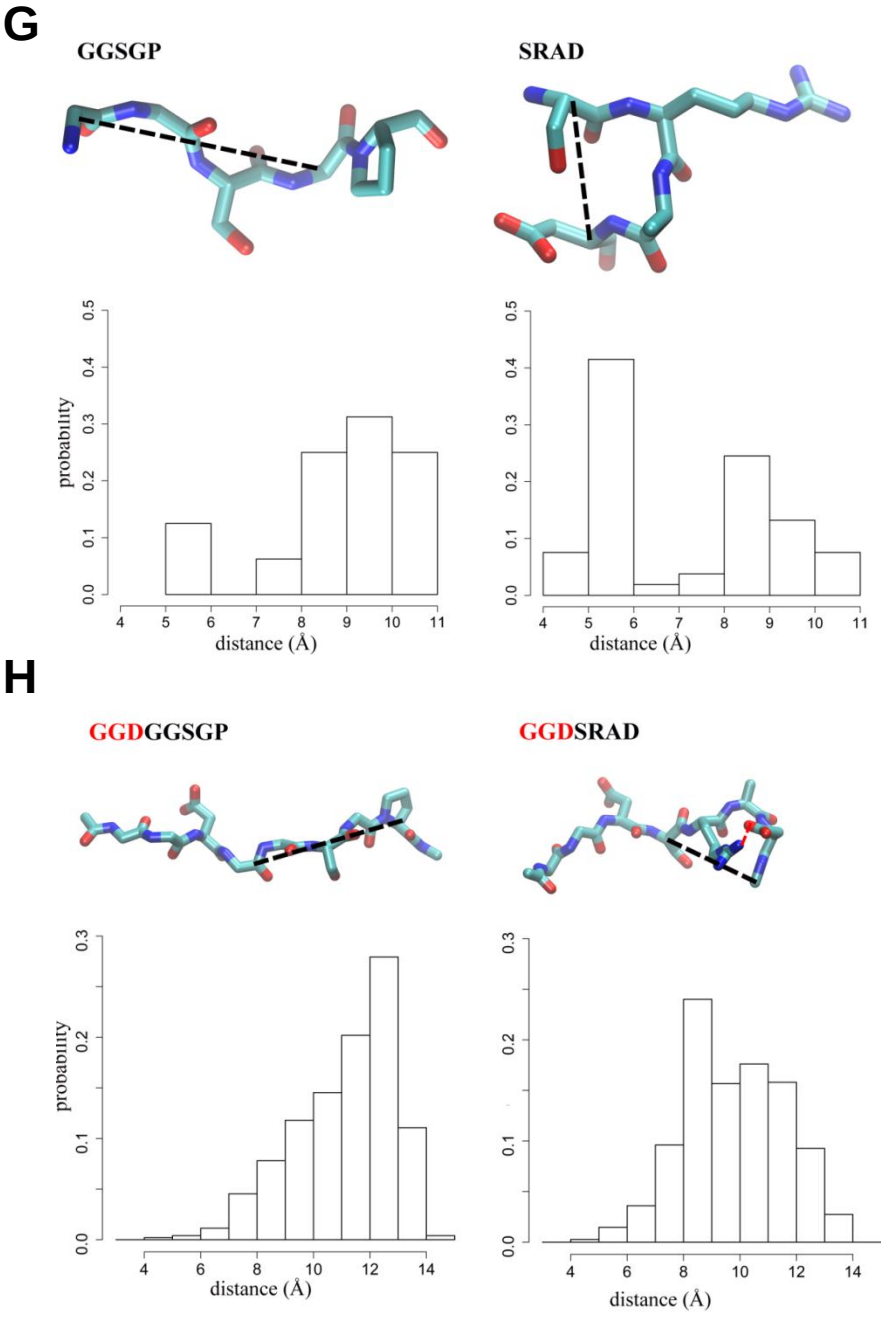


Supplementary information, Figure S2 A structurally optimized sgRNA$^{(F+E)}$ enhances the editing efficiency of Cas9 II and structural modeling of Cas9 I and Cas9 II. **(A-C)** Sequences of wild type animals and different Cas9-edited animals (mediated by different combinations of Cas9 I or Cas9 II with regular sgRNA or sgRNA$^{(F+E)}$) around the *ben-1*^{22} **(A)**, the *drp-1*^{D118A} **(B)**, and the *ced-9* **(C)** sgRNA target sites are shown. Sequences of Cas9 II/sgRNA-edited animals around the *ben-1*^{22} position are shown in supplementary information, Figure S1D. Sequences of sgRNA target sites and oligonucleotide templates used in *drp-1*^{D118A} and *ced-9* editing experiments are shown above the corresponding sequencing results and presented as in Supplementary information, Figure S1A. The protospacer adjacent motif (PAM) sequences are highlighted in purple and the sequences targeted by sgRNAs are underlined. The nucleotides altered at each target site are indicated in red. The codon for the modified amino acid is marked by a dash on the top **(A, B).** To generate a BssH II restriction site (marked with a gray box) at the *drp-1*^{D118A} position, a silent mutation was introduced at residue Arg119 (CGT to CGC). The translation initiation codon of *ced-9* is highlighted in yellow and the Flag-coding sequence inserted after the initiation codon is highlighted in green **(C)**. The red dashes (-) depict the deleted nucleotides. The green insertion marker (^**)** indicates the position of the inserted nucleotides. The replaced nucleotides are highlighted in blue and indicated as “-1 +1”. The numbers of deleted (-n) or/and inserted (+n) nucleotides are listed to the right of each sequence. The number in the parenthesis indicates the number of independently edited animals with the edited sequence shown on the left.

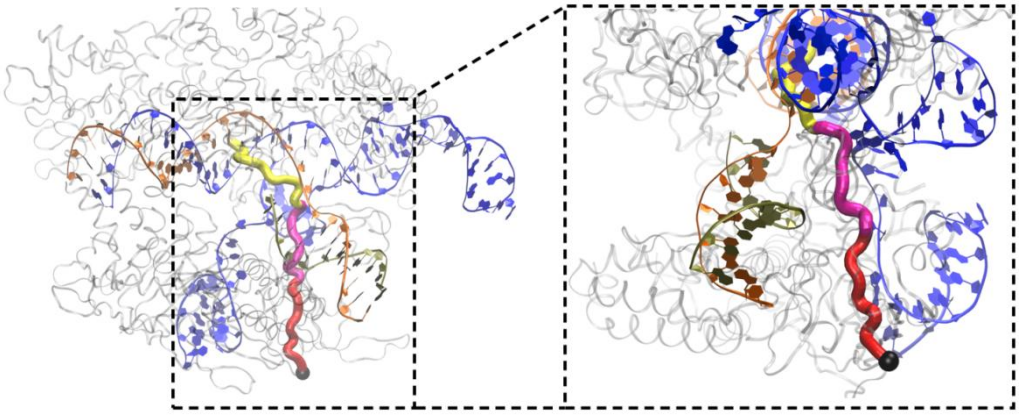


Supplementary information, Figure S2 A structurally optimized sgRNA$^{(F+E)}$ enhances the editing efficiency of Cas9 II and structural modeling of Cas9 I and Cas9 II. **(D-F)** Sequences of wild type animals and different Cas9-edited F1 homozygous animals (mediated by different combinations of Cas9 I or Cas9 II with regular sgRNA or sgRNA$^{(F+E)}$) around the *drp-1*^{D118A} **(D)**, the *fis-2*$^{N49L/K50ochre}$ **(E)**, and the *ubr-1* ^{M80ochre} **(F)** sgRNA target sites are shown. Sequences of sgRNA target sites and oligonucleotide repair templates used in the *fis-2*$^{N49L/K50ochre}$ and *ubr-1* ^{M80ochre} editing experiments are shown above the corresponding sequencing results and presented as in Supplementary information, Figure S1A. The protospacer adjacent motif (PAM) sequences are highlighted in purple and the sequences targeted by sgRNAs are underlined. The nucleotides altered at each target site are indicated in red. The codons for the modified amino acids are marked by dashes on the top. Four nucleotide changes were made in the *fis-2* gene and in the *ubr-1* gene, respectively, to generate a Hind III site (shaded in gray), resulting in a N49K missense mutation and a K50ochre nonsense mutation in the *fis-2* gene **(E)** and a silent mutation at Ala79 (GCA to GCT) and a M80ochre nonsense mutation in the *ubr-1* gene **(F)**.The red dashes (-) depict the deleted nucleotides. The green insertion marker (^) indicates the position of the inserted nucleotides or sequence. The numbers of deleted (-n) or/and inserted (+n) nucleotides are listed to the right of each sequence. The number in the parenthesis indicates the number of independently edited animals with the edited sequence shown on the left.

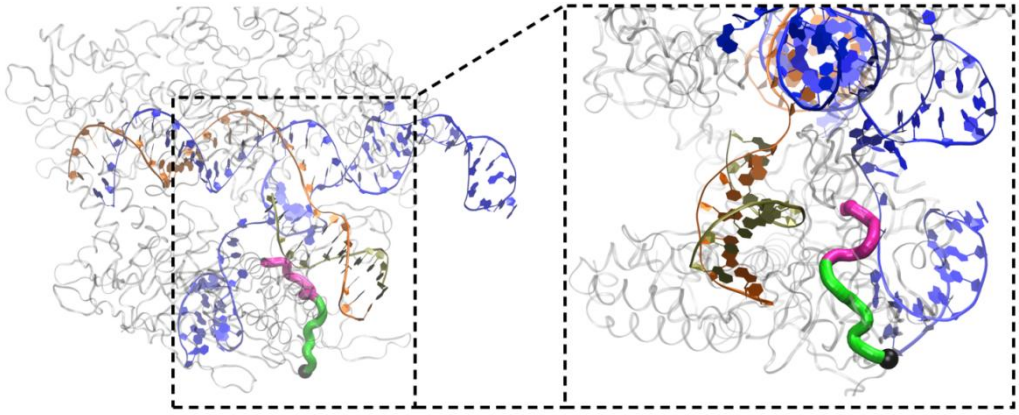


Supplementary information, Figure S2 A structurally optimized sgRNA$^{(F+E)}$ enhances the editing efficiency of Cas9 II and structural modeling of Cas9 I and Cas9 II. **(G)** The representative structures are taken from the crystal structures with PDB IDs of 2D9B and 1H6K, respectively. The EED is defined as the distance between the first and the fourth residues in the linkers, as indicated by the dashed black lines in the representative structures. **(H)** The representative structures for GGDGGSGP and GGDSRAD obtained from MD simulations on these two peptide fragments, respectively. The EED is calculated as the distance between the first and the fifth residues of GGSGP linker or between the first residue of the SRAD linker and the C terminal blocker of SRAD, as indicated by the dashed black lines in the representative structures. The dashed red line represents the electrostatic interaction between the Arg residue and the Asp residue in the SRAD linker. The probability distribution for EED is shown in histograms **(G, H)**.

I



J



Supplementary information, Figure S2 A structurally optimized sgRNA$^{(F+E)}$ enhances the editing efficiency of Cas9 II and structural modeling of Cas9 I and Cas9 II. **(I, J)** The modeled structures of Cas9 II **(I)** and Cas9 I **(J)** (both from residue 3 to 1366 in the Cas9 portion) in complex with a sgRNA and a partial duplex DNA are shown using the New Ribbons representation in a silver and transparent mode. The structures of GGSGP (**I**) or SRAD (**J**), NLS and HA are shown using Ribbons representation in the color of red (GGDGGSGP), green (GGDSRAD), magenta (NLS) and yellow (HA), respectively. The sgRNA, target DNA and non-target DNA strands are shown using the New Ribbons representation and colored in blue, orange and tan, respectively. The Cα atoms of Gly1366 are presented as black spheres. The right panels in I and J are enlarged images of the boxed areas, which are also rotated 90 degree to show more clearly the interaction between the C-terminal tag of Cas9 and nucleic acids.

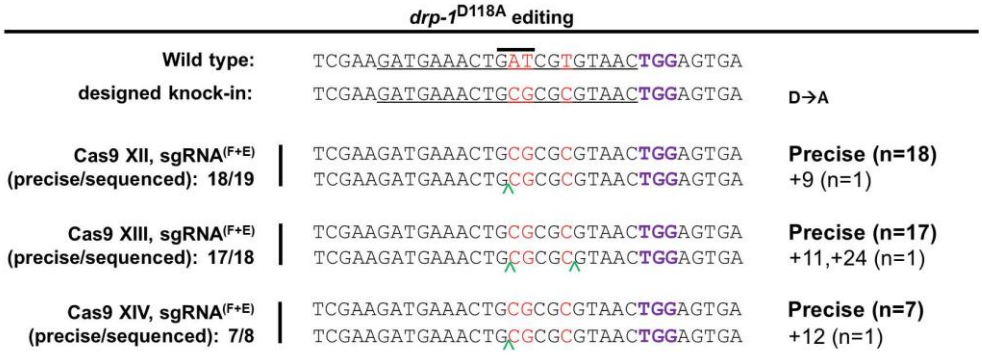
K

L

***drp-1*^{D118A}**

Cas9	Cas9 II	Cas9 XII	Cas9 XIII	Cas9 XIV
Knock-ins/Benomyl^{R} F1	50% (96/192)	32% (61/192)	13% (25/192)	4% (8/192)
Precise knock-ins/Sequenced	100% (21/21)	95% (18/19)	94% (17/18)	88% (7/8)
Normalized precise knock-ins/Benomyl^{R} F1	50% (96/192)	30% (58/192)	12% (24/192)	4% (7/192)
Homozygous knock-ins/Benomyl^{R} F1	3% (6/192)	1.6% (3/192)	1% (2/192)	0% (0/192)
Precise homozygous knock-ins/Benomyl^{R} F1	3% (6/192)	1.6% (3/192)	1% (2/192)	0% (0/192)

M



Supplementary information, Figure S2 A structurally optimized sgRNA$^{(F+E)}$ enhances the editing efficiency of Cas9 II and structural modeling of Cas9 I and Cas9 II. **(K)** A schematic diagram of Cas9 proteins with wild type and mutated HA tags. The black boxes depict the Cas9-coding exons and the blue lines depict the introns. The red boxes depict the GGSGP linker, the purple boxes depict SV40 NLS, and the yellow boxes depict wild type or modified HA tags, with the substituted amino acids highlighted in blue. **(L)** Comparison of gene editing efficiencies at the *drp-1*^{D118A} position by four different Cas9 proteins shown in K. A co-CRISPR method was used to enrich F1 edited animals at the target site. The *ben-1*^{153} sgRNA was used as a co-CRISPR driver. The number of knock-ins, homozygous knock-ins and precise homozygous knock-ins from the number of benomyl-resistant (benomyl^{R}) F1 animals isolated and the number of precise knock-ins from the number of knock-in animals sequenced are shown, respectively. The normalized percentage of precise knock-ins identified from the benomyl^{R} F1 animals screened is also shown. **(M)** Sequences of wild type animals and different Cas9-edited (Cas9 XII, Cas9 XIII and Cas9 XIV) animals around the *drp-1*^{D118A} position are shown as described in supplementary information, Figure S2B.