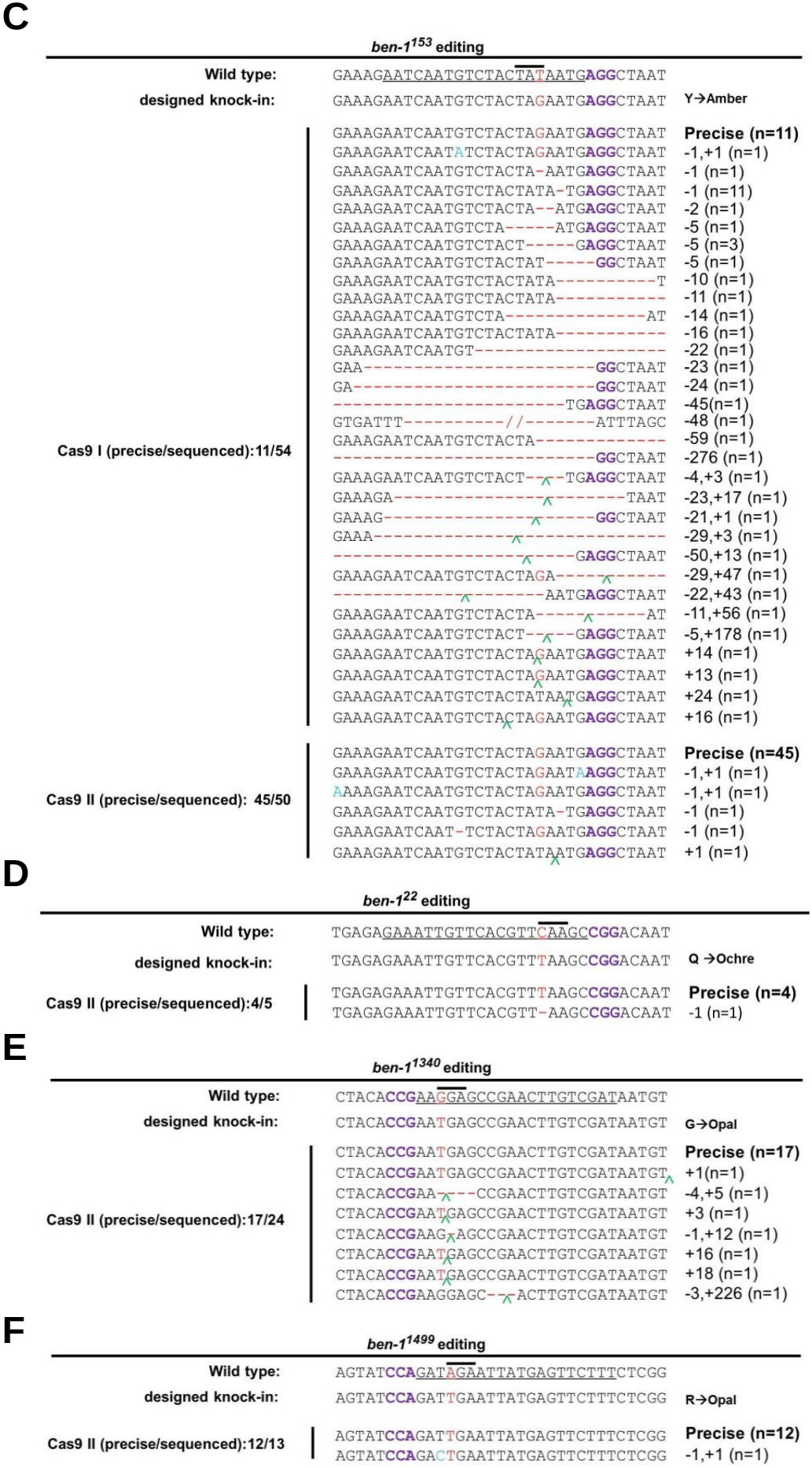
A

B

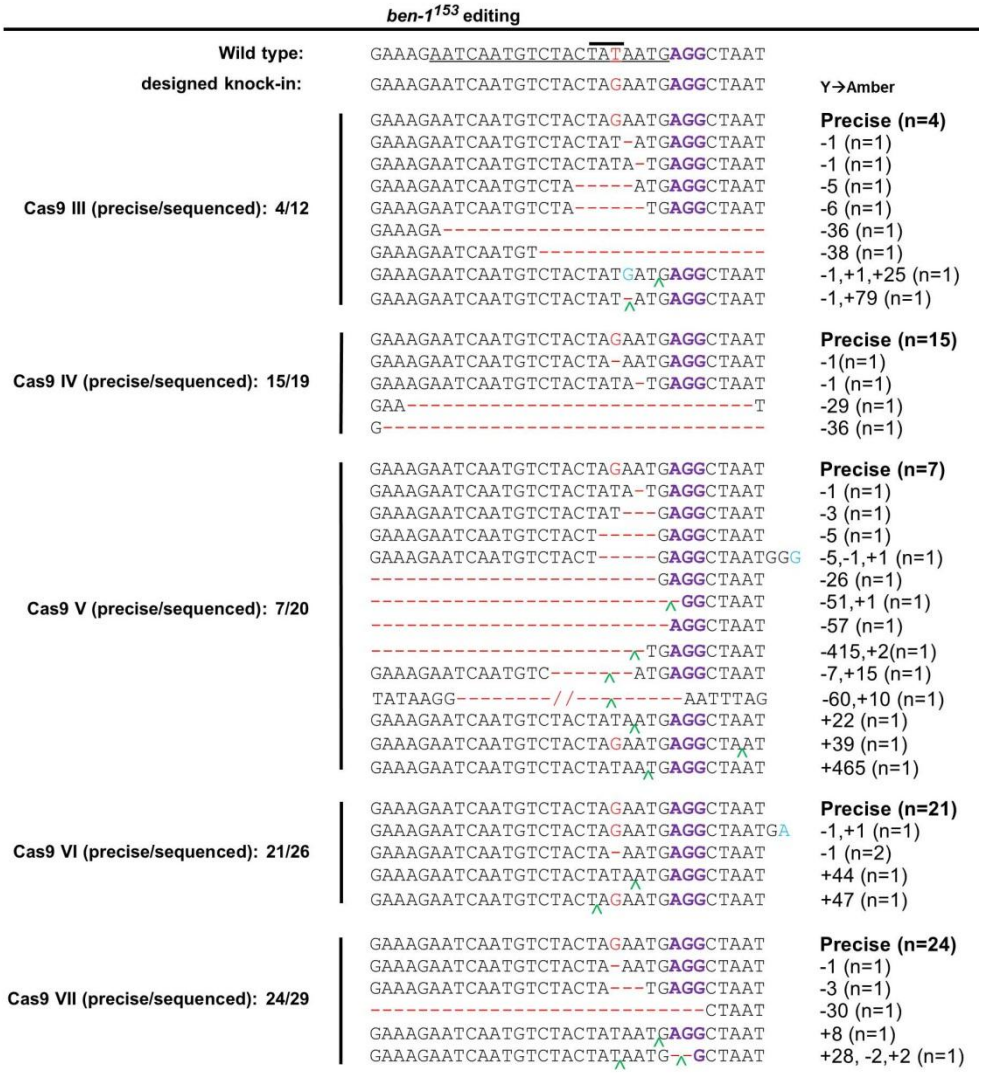
	Cas9	Benomyl^R/F1	Knock-ins/Sequenced	Precise knock-ins/Sequenced	Normalized precise knock-ins/F1
ben-1^{22}	I	0% (0/865)	NA	NA	0% (0/865)
	II	0.8% (5/625)	80% (4/5)	80% (4/5)	0.6% (4/625)
ben-1^{153}	I	15% (128/848)	30% (16/54)	20% (11/54)	3% (26/848)
	II	66% (498/759)	96% (48/50)	90% (45/50)	59% (448/759)
ben-1^{1340}	I	0% (0/894)	NA	NA	0% (0/894)
	II	3% (24/802)	88% (21/24)	71% (17/24)	2% (17/802)
ben-1^{1499}	I	0% (0/855)	NA	NA	0% (855)
	II	25% (211/843)	100% (13/13)	92% (12/13)	23% (195/843)

Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(A)** Sequences of different *ben-1* sgRNA target sites and corresponding oligonucleotide repair templates used in the editing experiments. The protospacer adjacent motif (PAM) sequences are highlighted with purple and the sequences targeted by sgRNAs are underlined. The nucleotide altered in each targeted site is indicated in red. The codons of the modified amino acids are marked by a dash on the top. The oligonucloeotide templates used to edit the *ben-1*^{1340} position and the *ben-1*^{1499} position are reverse complement to the sequences of the oligonucloeotides shown. The numbers in parentheses indicate the lengths of homology arms not shown in the repair templates. **(B)** Comparison of the editing efficiencies by Cas9 I and Cas9 II at the *ben-1*^{22}, *ben-1*^{153}, *ben-1*^{1340} and *ben-1*^{1499} positions, respectively. NA, not applicable. The number of benomyl-resistant F1 animals identified from the number of total F1 animals screened (benomyl^R/F1), the number of knock-ins from the number of benomyl^R F1 animals sequenced (Knock-ins/Sequenced), and the number of precise knock-ins from the number of benomyl^R F1 animals sequenced (Precise knock-ins/Sequenced) are shown, respectively. The normalized percentage of precise knock-ins identified from the total number of F1 animals screened (Normalized precise knock-ins/F1) is also shown. For each experiment, 5-7 wild-type N2 animals were injected.



Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(C-F)** Sequences of wild type animals and Cas9-edited (Cas9 I or Cas9 II) animals around the *ben-1*^{153} **(C)**, *ben-1*^{22} **(D)**, *ben-1*^{1340} **(E)**, and *ben-1*^{1499} **(F)** positions are shown. The protospacer adjacent motif (PAM) sequences are highlighted in purple and the sequences targeted by sgRNAs are underlined. The nucleotide altered at each target site is indicated in red. The codon for the modified amino acid is marked by a dash on the top. The red dashes (-) depict the deleted nucleotides. The green insertion marker (∧) indicates the position of the inserted nucleotides or sequences. The double slashes (//) indicate that there is a long DNA sequence not shown. 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.

G



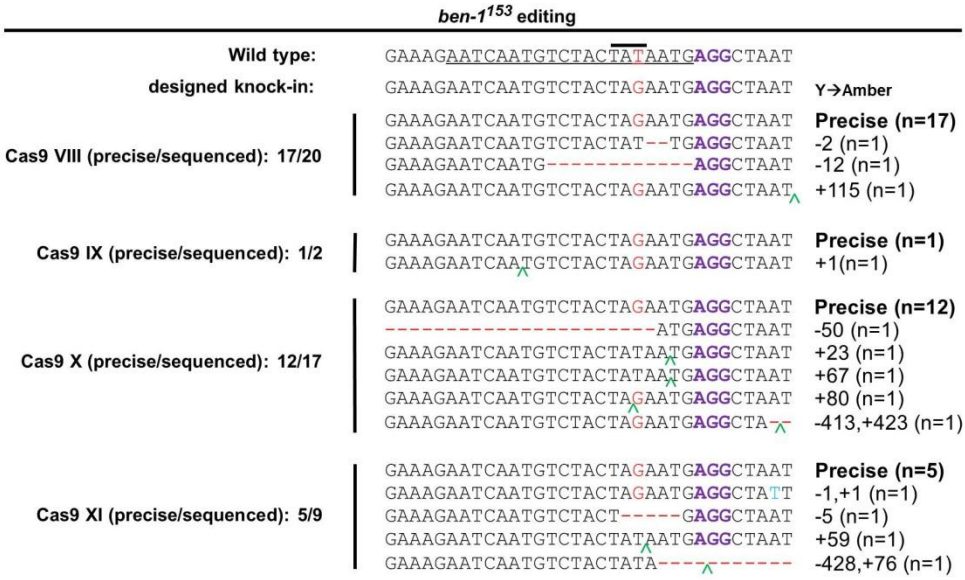
Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(G)** Sequences of wild type animals and different Cas9-edited animals around the *ben-1*[153] position. Sequences of wild type animals and different Cas9-edited (Cas9 III, Cas9 IV, Cas9 V, Cas9 VI and Cas9 VII) animals around the *ben-1*[153] position are shown. Sequences of Cas9 I- and Cas9 II-edited animals around the *ben-1*[153] position are presented in Supplementary information, Figure S1C. The protospacer adjacent motif (PAM) sequences are highlighted in purple and the sequences targeted by sgRNAs are underlined. The nucleotide altered at each target site is indicated in red. The codon for the modified amino acid is marked by a dash on the top. The red dashes (-) depict the deleted nucleotides. The green insertion marker (∧) indicates the position of the inserted nucleotides or sequence. The double slashes (//) indicate that there is a long DNA sequence not shown. 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.

H

I

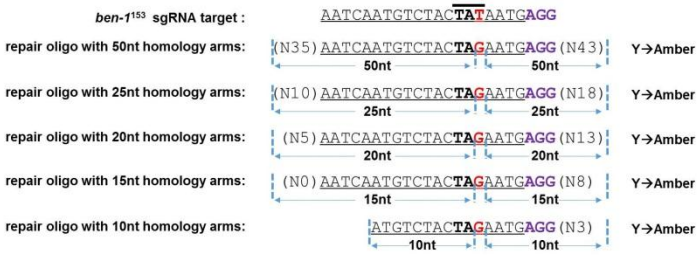
	Cas9	Benomyl^{R}/F1	Knock-ins/Sequenced	Precise knock-ins/Sequenced	Normalized precise knock-ins/F1
	II	66% (498/759)	96% (48/50)	90% (45/50)	59% (448/759)
	VIII	4% (43/1156)	90% (18/20)	85% (17/20)	3% (37/1156)
***ben-1*^{153}**	IX	0.3% (3/1092)	100% (2/2)	50% (1/2)	0.1% (1/1092)
	X	12% (133/1156)	82% (14/17)	71% (12/17)	8% (94/1156)
	XI	6% (72/1116)	67% (6/9)	56% (5/9)	4% (40/1116)

J



Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(H)** A schematic diagram of Cas9 proteins with different N-terminal and C-terminal tags. The black boxes depict Cas9-coding exons and the blue lines depict the introns. The red boxes depict the GGSGP linker, the purple boxes depict SV40 NLS, the yellow boxes depict the HA tag, and the blue box depicts the PGSGG linker. **(I)** Comparison of the gene editing efficiencies at the *ben-1*^{153} position by five different Cas9 proteins shown in H. The number of benomyl-resistant (benomyl^{R}) F1 animals identified from the number of total F1 animals screened, the number of knock-ins from the number of benomyl^{R} F1 animals sequenced, and the number of precise knock-ins from the number of benomyl^{R} F1 animals sequenced are shown, respectively**.** The normalized percentage of precise knock-ins identified from the total number of F1 animals screened is also shown. For each experiment, 5-7 wild-type N2 animals were injected. **(J)** Sequences of wild type animals and different Cas9-edited (Cas9 VIII, Cas9 IX, Cas9 X and Cas9 XI) animals around the *ben-1*^{153} position are shown as described in Supplementary information, Figure S1C.

K

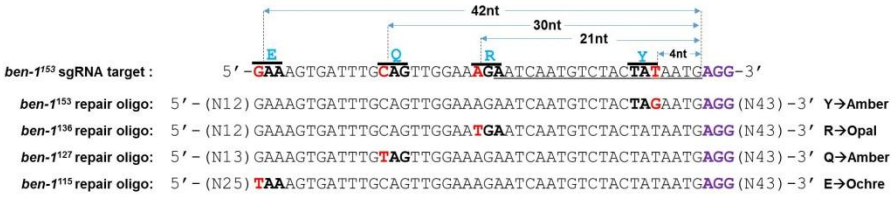


L

	Length of homology arms	10nt	15nt	20nt	25nt	50nt
ben-1^{153}	**Knock-ins/sequenced**	14% (3/21)	20% (5/25)	79% (19/24)	92% (22/24)	96% (48/50)
	Precise knock-ins/sequenced	14% (3/21)	20% (5/25)	79% (19/24)	92% (22/24)	90% (45/50)

Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(K)** Sequences of the *ben-1*^{153} sgRNA target site and five different oligonucleotide repair templates used in the editing experiments, which contain 50nt, 25nt, 20nt, 15nt and 10nt homology arms, respectively, as described in Figure S1A. The lengths of homology arms in each oligonucleotide are indicated. **(L)** Comparison of the editing efficiencies at the *ben-1*^{153} position when different lengths of oligonucleotide repair templates are used. The number of knock-ins from the number of benomyl-resistant F1 (benomyl^{R} F1) animals sequenced (Knock-ins/Sequenced) and the number of precise knock-ins from the number of benomyl^{R} F1 animals sequenced (Precise knock-ins/Sequenced) are shown. For each experiment, 5-7 wild-type N2 animals were injected.

M



N

Editing	*ben*-1^{153}	*ben*-1^{136}	*ben*-1^{127}	*ben*-1^{115}
Distance between PAM and the knock-in site	4nt	21nt	30nt	42nt
Knock-ins/Sequenced	96% (48/50)	71% (15/21)	33% (7/21)	10% (2/20)
Precise knock-ins/Sequenced	90% (45/50)	52% (11/21)	14% (3/21)	10% (2/20)

Supplementary information, Figure S1 Cas9 proteins with different C-terminal tags display dramatically different editing efficiencies. **(M)** Sequences of the *ben-1*^{153} sgRNA target site and oligonucleotide repair templates used to create stop codons at four different *ben-1* positions. The PAM sequence is highlighted with purple and the sequence targeted by the sgRNA is underlined. The nucleotide altered in each knock-in site is indicated in red. The codons for the modified amino acids are marked by a dash on the top and the amino acids altered shown in blue and listed on the top of the dash. The numbers in parentheses indicate the lengths of homology arms not shown in the repair templates. The distance between the PAM sequence and the nucleotide altered is indicated by a pair of blue arrows. **(N)** Comparison of the editing efficiencies of Cas9 II at four different *ben-1* sites, *ben-1*^{153}, *ben-1*^{136}, *ben-1*^{127} and *ben-1*^{115}, respectively. The same *ben-1*^{153} sgRNA was used in these editing experiments. The distance between the PAM sequence and the nucleotide altered, the number of knock-ins from the number of benomyl-resistant F1 (benomyl^{R} F1) animals sequenced (Knock-ins/Sequenced), and the number of precise knock-ins from the number of benomyl^{R} F1 animals sequenced (Precise knock-ins/Sequenced) are shown, respectively. For each experiment, 5-7 wild-type N2 animals were injected.