

A

*ben-1*²² sgRNA target: GAAATTGTTACGTTCAAGCCGG
*ben-1*²² repair oligo: (N36) GAAATTGTTACGTTAAGCCGG (N42) Q →Ochre
*ben-1*¹³⁴⁰ sgRNA target: CCGAAGGAGCCGAACTTGTCGAT
*ben-1*¹³⁴⁰ repair oligo: (N45) CCGAATGAGCCGAACTTGTCGAT (N33) G →Opal
*ben-1*¹⁵³ sgRNA target: AATCAATGCTACTTATAATGAGG
*ben-1*¹⁵³ repair oligo: (N35) AATCAATGCTACTAGAATGAGG (N43) Y →Amber
*ben-1*¹⁴⁹⁹ sgRNA target: CCAGATAGAATTATGAGTTCTTT
*ben-1*¹⁴⁹⁹ repair oligo: (N44) CCAGATTGAATTATGAGTTCTTT (N34) R →Opal

B

	Cas9	Benomyl ^R /F1	Knock-ins/Sequenced	Precise knock-ins/Sequenced	Normalized precise knock-ins/F1
<i>ben-1</i> 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)
<i>ben-1</i> 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)
<i>ben-1</i> ¹³⁴⁰	I	0% (0/894)	NA	NA	0% (0/894)
	II	3% (24/802)	88% (21/24)	71% (17/24)	2% (17/802)
<i>ben-1</i> 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 oligonucleotide templates used to edit the *ben-1*¹³⁴⁰ position and the *ben-1*¹⁴⁹⁹ position are reverse complement to the sequences of the oligonucleotides 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.

C**ben-1¹⁵³ editing**

Wild type:	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	
designed knock-in:	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Y→Amber
Cas9 I (precise/sequenced): 11/54	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Precise (n=11)
	GAAAGAATCAATACTACTAGAATGAGGCTAAT	-1,+1 (n=1)
	GAAAGAATCAATGTCTACTA-AATGAGGCTAAT	-1 (n=1)
	GAAAGAATCAATGTCTACTATA-TGAGGCTAAT	-1 (n=11)
	GAAAGAATCAATGTCTACTA--ATGAGGCTAAT	-2 (n=1)
	GAAAGAATCAATGTCTA----ATGAGGCTAAT	-5 (n=1)
	GAAAGAATCAATGTCTACT----GAGGCTAAT	-5 (n=3)
	GAAAGAATCAATGTCTACTAT----GGCTAAT	-5 (n=1)
	GAAAGAATCAATGTCTACTATA-----T	-10 (n=1)
	GAAAGAATCAATGTCTACTATA-----	-11 (n=1)
	GAAAGAATCAATGTCTA-----AT	-14 (n=1)
	GAAAGAATCAATGTCTACTATA-----	-16 (n=1)
	GAAAGAATCAATGT-----	-22 (n=1)
	GAA-----GGCTAAT	-23 (n=1)
	GA-----GGCTAAT	-24 (n=1)
	-----TGAGGCTAAT	-45(n=1)
	GTGATTT-----//-----ATTAGC	-48 (n=1)
	GAAAGAATCAATGTCTACTA-----	-59 (n=1)
	-----GGCTAAT	-276 (n=1)
	GAAAGAATCAATGTCTACT- [^] -TGAGGCTAAT	-4,+3 (n=1)
	GAAAGA-----TAAT	-23,+17 (n=1)
	GAAAG-----GGCTAAT	-21,+1 (n=1)
	GAAA-----	-29,+3 (n=1)
	-----GAGGCTAAT	-50,+13 (n=1)
	GAAAGAATCAATGTCTACTAGA [^] -----	-29,+47 (n=1)
	-----AATGAGGCTAAT	-22,+43 (n=1)
	GAAAGAATCAATGTCTACTA-----AT	-11,+56 (n=1)
GAAAGAATCAATGTCTACT- [^] -----GAGGCTAAT	-5,+178 (n=1)	
GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	+14 (n=1)	
GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	+13 (n=1)	
GAAAGAATCAATGTCTACTATAATGAGGCTAAT	+24 (n=1)	
GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	+16 (n=1)	
Cas9 II (precise/sequenced): 45/50	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Precise (n=45)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	-1,+1 (n=1)
	AAAAGAATCAATGTCTACTAGAATGAGGCTAAT	-1,+1 (n=1)
	GAAAGAATCAATGTCTACTATA-TGAGGCTAAT	-1 (n=1)
	GAAAGAATCAAT-TCTACTAGAATGAGGCTAAT	-1 (n=1)
GAAAGAATCAATGTCTACTATAATGAGGCTAAT	+1 (n=1)	

D**ben-1²² editing**

Wild type:	TGAGAGAAATTGTTACGTTCAAGCCGGACAAT	
designed knock-in:	TGAGAGAAATTGTTACGTTAAGCCGGACAAT	Q →Ochre
Cas9 II (precise/sequenced): 4/5	TGAGAGAAATTGTTACGTTAAGCCGGACAAT	Precise (n=4)
	TGAGAGAAATTGTTACGTT-AAGCCGGACAAT	-1 (n=1)

E**ben-1¹³⁴⁰ editing**

Wild type:	CTACACCGAAGGAGCCGAACCTGTGCGATAATGT	
designed knock-in:	CTACACCGAATGAGCCGAACCTGTGCGATAATGT	G→Opal
Cas9 II (precise/sequenced): 17/24	CTACACCGAATGAGCCGAACCTGTGCGATAATGT	Precise (n=17)
	CTACACCGAATGAGCCGAACCTGTGCGATAATGT [^]	+1(n=1)
	CTACACCGAA [^] --CCGAACCTGTGCGATAATGT	-4,+5 (n=1)
	CTACACCGAATGAGCCGAACCTGTGCGATAATGT	+3 (n=1)
	CTACACCGAAG [^] AGCCGAACCTGTGCGATAATGT	-1,+12 (n=1)
	CTACACCGAATGAGCCGAACCTGTGCGATAATGT	+16 (n=1)
	CTACACCGAATGAGCCGAACCTGTGCGATAATGT	+18 (n=1)
	CTACACCGAAGGAGC [^] ACTTGTGCGATAATGT	-3,+226 (n=1)

F**ben-1¹⁴⁹⁹ editing**

Wild type:	AGTATCCAAGATAGAATTATGAGTTCTTTCTCGG	
designed knock-in:	AGTATCCAAGATGAATTATGAGTTCTTTCTCGG	R→Opal
Cas9 II (precise/sequenced): 12/13	AGTATCCAAGATGAATTATGAGTTCTTTCTCGG	Precise (n=12)
	AGTATCCAAGACTGAATTATGAGTTCTTTCTCGG	-1,+1 (n=1)

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-1¹⁵³* **(C)**, *ben-1²²* **(D)**, *ben-1¹³⁴⁰* **(E)**, and *ben-1¹⁴⁹⁹* **(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 (-) or/and inserted (+) 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.

	Wild type:		
	GAAGAATCAATGTCTACTAT <u>GAATGAGGCTAAT</u>		
	designed knock-in:	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT	Y→Amber
Cas9 III (precise/sequenced): 4/12	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		Precise (n=4)
	GAAGAATCAATGTCTACTAT-ATGAGGCTAAT		-1 (n=1)
	GAAGAATCAATGTCTACTATA-TGAGGCTAAT		-1 (n=1)
	GAAGAATCAATGTCTA-----ATGAGGCTAAT		-5 (n=1)
	GAAGAATCAATGTCTA-----TGAGGCTAAT		-6 (n=1)
	GAAAGA-----		-36 (n=1)
	GAAGAATCAATGT-----		-38 (n=1)
	GAAGAATCAATGTCTACTATGATGAGGCTAAT		-1,+1,+25 (n=1)
	GAAGAATCAATGTCTACTATATGAGGCTAAT		-1,+79 (n=1)
	Cas9 IV (precise/sequenced): 15/19	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT	
GAAGAATCAATGTCTACTATA-AATGAGGCTAAT			-1 (n=1)
GAAGAATCAATGTCTACTATA-TGAGGCTAAT			-1 (n=1)
GAA-----T			-29 (n=1)
G-----			-36 (n=1)
Cas9 V (precise/sequenced): 7/20	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		Precise (n=7)
	GAAGAATCAATGTCTACTATA-TGAGGCTAAT		-1 (n=1)
	GAAGAATCAATGTCTACTAT--GAGGCTAAT		-3 (n=1)
	GAAGAATCAATGTCTACT-----GAGGCTAAT		-5 (n=1)
	GAAGAATCAATGTCTACT-----GAGGCTAATGGG		-5,-1,+1 (n=1)
	-----GAGGCTAAT		-26 (n=1)
	-----GGCTAAT		-51,+1 (n=1)
	-----AGGCTAAT		-57 (n=1)
	-----TGAGGCTAAT		-415,+2(n=1)
	GAAGAATCAATGTCT-----ATGAGGCTAAT		-7,+15 (n=1)
	TATAAGG-----//-----AATTAG		-60,+10 (n=1)
	GAAGAATCAATGTCTACTATAATGAGGCTAAT		+22 (n=1)
	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		+39 (n=1)
GAAGAATCAATGTCTACTATAATGAGGCTAAT		+465 (n=1)	
Cas9 VI (precise/sequenced): 21/26	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		Precise (n=21)
	GAAGAATCAATGTCTACTAGAAATGAGGCTAATGA		-1,+1 (n=1)
	GAAGAATCAATGTCTACTA-AATGAGGCTAAT		-1 (n=2)
	GAAGAATCAATGTCTACTATAATGAGGCTAAT		+44 (n=1)
	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		+47 (n=1)
Cas9 VII (precise/sequenced): 24/29	GAAGAATCAATGTCTACTAGAAATGAGGCTAAT		Precise (n=24)
	GAAGAATCAATGTCTACTA-AATGAGGCTAAT		-1 (n=1)
	GAAGAATCAATGTCTACTA--TGAGGCTAAT		-3 (n=1)
	-----CTAAT		-30 (n=1)
	GAAGAATCAATGTCTACTATAATGAGGCTAAT		+8 (n=1)
GAAGAATCAATGTCTACTATAATGCTAAT		+28,-2,+2 (n=1)	

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 (Λ) 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.

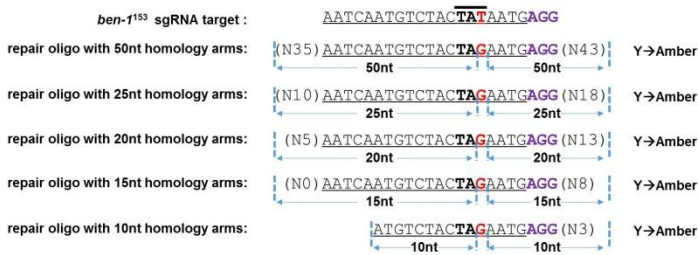
H**I**

	Cas9	Benomyl ^R /F1	Knock-ins/Sequenced	Precise knock-ins/Sequenced	Normalized precise knock-ins/F1
<i>ben-1</i> ¹⁵³	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)
	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*ben-1*¹⁵³ editing

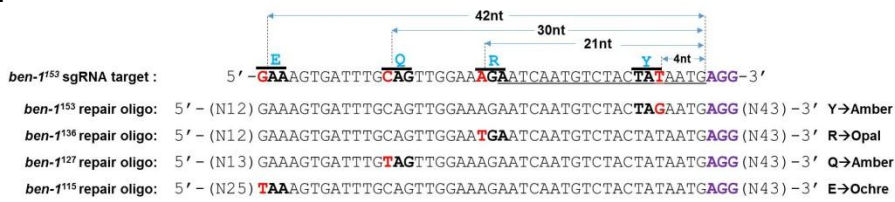
	Wild type:	designed knock-in:	
Cas9 VIII (precise/sequenced): 17/20	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Y→Amber
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAT--TGAGGCTAAT	Precise (n=17)
	GAAAGAATCAATG-----AGGCTAAT	GAAAGAATCAATG-----AGGCTAAT	-2 (n=1)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	-12 (n=1)
Cas9 IX (precise/sequenced): 1/2	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Precise (n=1)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	+1(n=1)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Precise (n=12)
	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	-50 (n=1)
Cas9 X (precise/sequenced): 12/17	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	+23 (n=1)
	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	+67 (n=1)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	+80 (n=1)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	-413,+423 (n=1)
Cas9 XI (precise/sequenced): 5/9	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	Precise (n=5)
	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	GAAAGAATCAATGTCTACTAGAATGAGGCTAAT	-1,+1 (n=1)
	GAAAGAATCAATGTCTACT-----GAGGCTAAT	GAAAGAATCAATGTCTACT-----GAGGCTAAT	-5 (n=1)
	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	GAAAGAATCAATGTCTACTATAATGAGGCTAAT	+59 (n=1)
	GAAAGAATCAATGTCTACTATA-----	GAAAGAATCAATGTCTACTATA-----	-428,+76 (n=1)

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
<i>ben-1¹⁵³</i>	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-1¹⁵³* 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-1¹⁵³* 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	<i>ben-1¹⁵³</i>	<i>ben-1¹³⁶</i>	<i>ben-1¹²⁷</i>	<i>ben-1¹¹⁵</i>
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-1¹⁵³* 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-1¹⁵³*, *ben-1¹³⁶*, *ben-1¹²⁷* and *ben-1¹¹⁵*, respectively. The same *ben-1¹⁵³* 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.