

Supplementary Information, Table S1A List of oligonucleotide repair templates

used in this study.

Oligo name	Sequence 5'-3'
<i>ben-1</i> ²² oligo	TCATATAACTCAAAAAGAACCTGGAAAAATGAGAGAAATT GTTCACGTTAACGCCGACAATGTGGAATCAAATCGGAGCCA AGTTCTGGGAAGTGAT
<i>ben-1</i> ¹⁵³ oligo (± 50 nt)	ACTTATAAGGGAGAAAGTGAATTGCAGTTGGAAAGAATCAAT GTCTACTAGAACATGAGGCTAATGGTGAGAAATTAGCTTTTA TTCGATTTCAGATT
<i>ben-1</i> ¹³⁴⁰ oligo	TTCAGCCTCTTCGAACACTACGTCGAGCACATTATCGACAAGTT CGGCTCATTGGTAGTGACCCCTGGCCCAGTTGTTCCGGCT CCGGATTGTCCGA
<i>ben-1</i> ¹⁴⁹⁹ oligo	AATTAATCCAACCTTGGCGACGGAACAAACCGAGAAAGAACT CATAAATTCAATCTGGATACTCTCACGGATTTGAAATGAGA AGAGTTCCCATTCCAG
<i>drp-1</i> oligo	TCGATGCCGTGCGAAAAGAAATCGAACAGATGAAACTGCGCG TAACTGGAGTGAATAAGGAATCTCTCTTCC
<i>flag::ced-9</i> oligo	TCTAGAATGTATATTGATTATGAAAACGAATAAAAATTAA GATGGACTACAAGGACGACGATGACAAGACACGCTGCACGGC GGACAACCTGCTGACGAATCCGGCGTATCGGCG
<i>fis-2</i> oligo	GTCGACTTTGCCTTGACATGCCATGATTGGATCCAAGCTTT AACTGGACGTGAAGGAGGGAATCGTGTCTTGAGAGTGA
<i>ubr-1</i> oligo	CTAGACGTTCGAACACAAAGAGCACACTGGAAGCTAAAAT GGCATTGCCGGAGTTGATCCTGCAAGACGGGG
<i>ben-1</i> ¹⁵³ oligo (± 25 nt)	AGTTGGAAAGAACATCAATGTCTACTAGAACATGAGGCTAATGGTGA GAAATTAA
<i>ben-1</i> ¹⁵³ oligo (± 20 nt)	GAAAGAACATGTCTACTAGAACATGAGGCTAATGGTGAGAA
<i>ben-1</i> ¹⁵³ oligo (± 15 nt)	AATCAATGTCTACTAGAACATGAGGCTAATGGT
<i>ben-1</i> ¹⁵³ oligo (± 10 nt)	ATGTCTACTAGAACATGAGGCTA
<i>ben-1</i> ¹³⁶ oligo	ACTTATAAGGGAGAAAGTGAATTGCAGTTGGAAATGAATCAATG TCTACTATAATGAGGCTAATGGTGAGAAATTAGCTTTTATT CGATTTCAGATT
<i>ben-1</i> ¹²⁷ oligo	AACTTATAAGGGAGAAAGTGAATTGTAGTTGGAAAGAACATCAAT GTCTACTATAATGAGGCTAATGGTGAGAAATTAGCTTTTATT TCGATTTCAGATT
<i>ben-1</i> ¹¹⁵ oligo	CCAGCCTGATGGAACCTATAAGGGATAAAGTGATTGCAGTTG GAAAGAACATGTCTACTATAATGAGGCTAATGGTGAGAAATT TAGCTTTTATTGATTTTCAGATT

Supplementary Information, Table S1B List of primers used in this study.

Usage	Primer	Sequence (5'-3')
Genotyping	<i>ben-1</i> F1	CCCTGGCTAGTTCAAACGAAGAG
	<i>ben-1</i> R1	CCCATAGGTTCCCGTATGTC
	<i>ben-1</i> F2	TGCGCATTGCGCTCTATAGCAACC
	<i>ben-1</i> R2	CGTCCACGGAACATTGCAGCTACAG
	<i>drp-1</i> F	TCCACAGATTGTAGTCGTCG
	<i>drp-1</i> R	CTTCAGTGCAGGAAGACAG
	<i>ced-9</i> F	TTTGCCTTCGATTCACCG
	<i>ced-9</i> R	TGTGCATTCCAACCGTCCG
	<i>fis-2</i> F	ACTATTCTCGAGGAACGGAC
	<i>fis-2</i> R	ACCCGTTCACTTTGTTCCG
Allele-specific primers	<i>ubr-1</i> F	TACGCCAGCTGCTGCTCAAG
	<i>ubr-1</i> R	CCGTCAAATATTGCTGTGCC
	<i>ced-9</i> flag F	TTGTTTGAAACGCACCGCC
	<i>ced-9</i> flag R	CATCGTCGTCCTTGTAGTCC
	<i>ced-9</i> N2 F	CGAATAAAAATTTAGATGACACGC
	<i>ced-9</i> N2 R	GTGTCCTCCAGTTGTC

To confirm that precise knock-ins occurred at the targeted sites in edited animals, genomic DNA spanning each sgRNA target site was PCR amplified using “genotyping” primers and subjected to sequencing analysis. The *ben-1* F1/R1 primer pair was used to amplify the genomic DNA spanning the *ben-1*²² and *ben-1*¹⁵³ positions. The *ben-1* F2/R2 primer pair was used to amplify the genomic DNA spanning *ben-1*¹³⁴⁰, *ben-1*¹⁴⁹⁹ sgRNA target sites. *drp-1* F/R, *ced-9* F/R, *fis-2* F/R and *ubr-1* F/R primer pairs were used to amplify the genomic DNA spanning the *drp-1*, *ced-9*, *fis-2* and *ubr-1* sgRNA target sites, respectively. For *drp-1*, *fis-2* and *ubr-1* editing experiments, the same PCR products were digested with restriction enzymes to identify the knock-in animals. In the *flag::ced-9* editing experiments, we used allele-specific primers to identify *flag::ced-9* knock-in animals (*ced-9* flag F/R) from benomy^L F1 animals and homozygous F2 animals with knock-ins (*ced-9* flag F/R and *ced-9* N2 F/R).