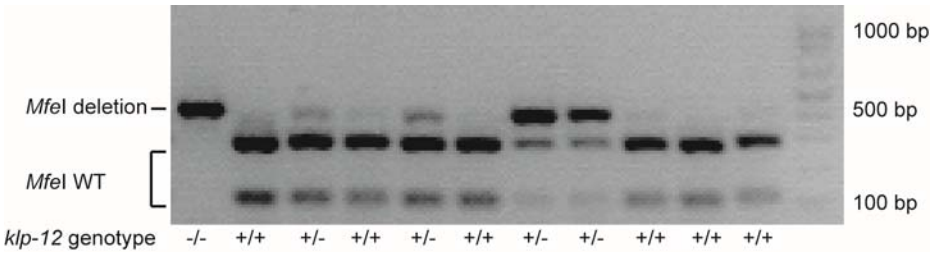


Figure S1 Representative *klp-12* deletion experiment. Injection of 50 ng/µl of CRISPR/Cas9 plasmid targeting a previously described PAM in the *klp-12* locus (FRIEDLAND *et al.* 2013) results in deletion of an adjacent *Mfe*I restriction site. WT and mutated *Mfe*I digested PCR products are indicated. Products were run on a 1.5% TAE-agarose gel and the 1KB+ (Invitrogen) size standard is provided. *klp-12* genotype inferred by *Mfe*I digestion is indicated. WT animals have complete *Mfe*I digestion of the PCR product, mutant homozygotes have no digestion of the PCR product, and heterozygotes have a mixture of digested and undigested PCR products.

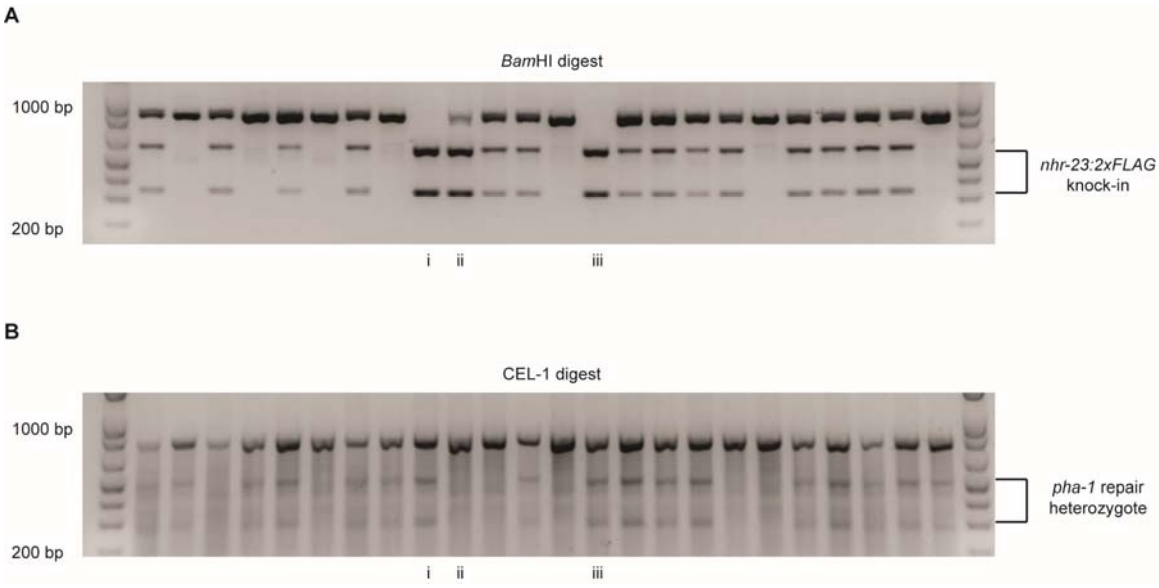


Figure S2 Representative genotyping of the progeny of an *nhr-23::2xFLAG* knock-in heterozygote. (A) *Bam*HI digestion to genotype *nhr-23::2xFLAG* knock-in. Twenty-four progeny from an *nhr-23::2xFLAG* knock-in heterozygote were plated out, allowed to lay progeny, and the parental animal was genotyped. Candidate homozygotes are indicated by i, ii, and iii. (B) CEL-1 digestion of PCR products amplifying the *pha-1(ts)* repair site. CEL-1 cuts mismatches. (i and iii) are *pha-1* repair heterozygotes, as indicated by the digestion product. (ii) is a *pha-1(ts)* repair homozygote. *pha-1* and *nhr-23::2xFLAG* genotypes were confirmed by sequencing of the PCR products. The 1KB+ (Invitrogen) size standard is provided in A and B.

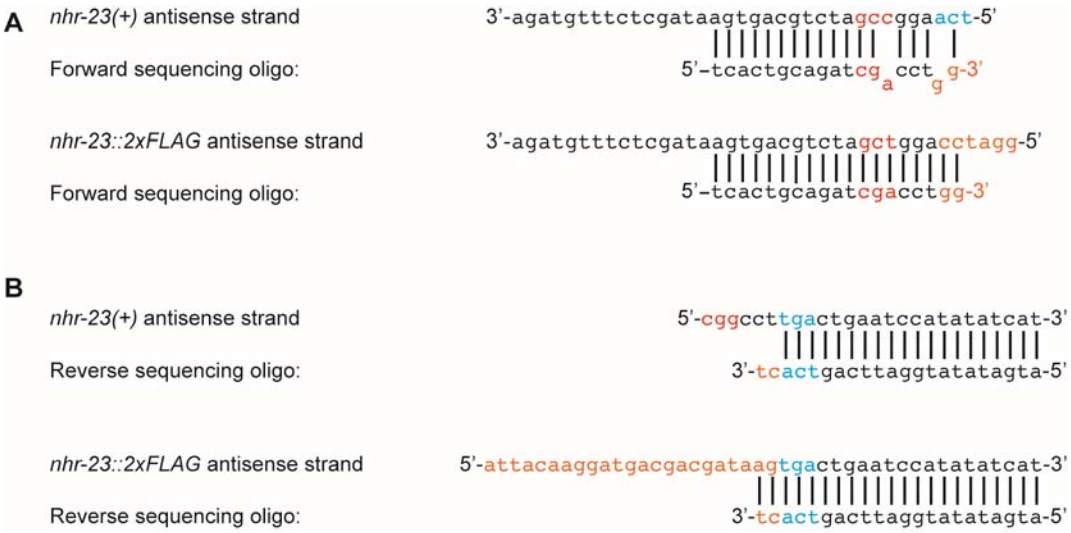
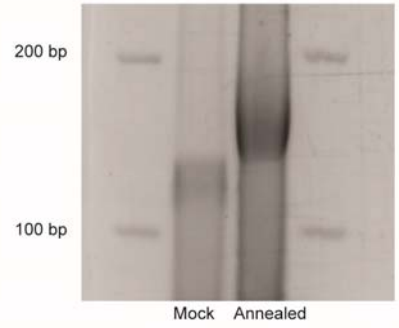


Figure S3 Oligo design to sequence FLAG knock-in heterozygotes. Design of oligos to sequence into 5' end (A) and 3' end (B) of *nhr-23* 2x and 3x FLAG knock-ins. The non-coding strand of *nhr-23 (+)* is shown paired with the sequencing oligo. The stop codon (blue text), PAM #1 (red text), and a portion of the 2xFLAG knock-in sequence (orange text) are indicated. The oligo is designed to bind the genomic sequence at the insertion site with the last two bases binding bases in the insert. In cases where sequence is too poor to confirm correct insertion of an epitope, an additional round of PCR can be performed using one of the insert-specific oligos and an external primer that binds in the genomic sequence. Purification of this product followed by sequencing using the primer that binds in the genomic sequence provides the entire epitope sequence. In events where 2 bp of knock-in sequence is not sufficient to confer specificity, increasing the knock-in specific homology will correct the problem at the expense of sequencing coverage of the knock-in.

A



B

nhr-23::2xFLAG oligo	Viable injected P0	Rescued F1	P0 with rescued F1	PCR hits	Precise Knockins	% Knockins/ rescued F1	% Knockins/ P0
sense 200mer	102	27	n/a	10	7	25.9	6.9
sense 200mer mock annealed	20	5	2	3	1	20.0	5.0
sense+antisense 200mer mock annealed	32	9	2	1	1	11.1	3.1
sense+antisense 200mer annealed	24	4	4	0	0	0.0	0.0

Figure S4 dsDNA is not a more effective template than ssDNA for introduction of a *2xFLAG* epitope at the 3' end of *nhr-23*. (A) 50 ng/µl of sense of and antisense *nhr-23::2xFLAG* oligos in annealing buffer (TE buffer with 50 mM NaCl) were either annealed by heating to 95ºC for two minutes and then slowly cooling to 25ºC over 30 minutes in a thermocycler, or mock annealed (kept at 25ºC). Annealing was confirmed by resolving the annealed and mock annealed oligos on a 4% TAE-agarose gel and staining with GelRed. The 1KB+ (Invitrogen) size standard is provided. (B) Table comparing the knock-in efficiencies of sense oligos, and either mock annealed or annealed sense+antisense *nhr-23::2xFLAG* 200mers. The sense 200mer data is pooled from all experiments using *pha-1(ts)* sense oligos and *nhr-23::2xFLAG* sense 200mers (Figures 1 and 3). A control where the sense oligo was injected in annealing buffer was performed to ensure that the buffer did not affect knock-in efficiency.

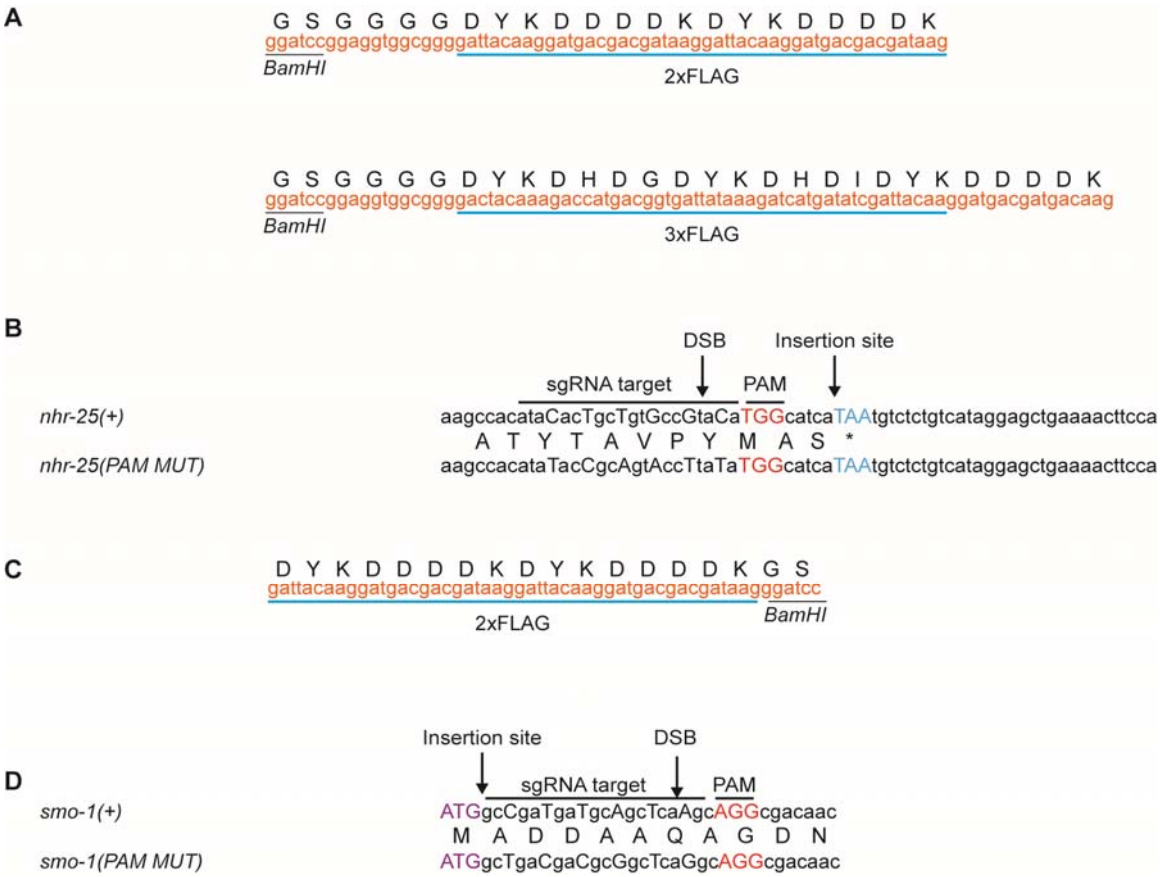
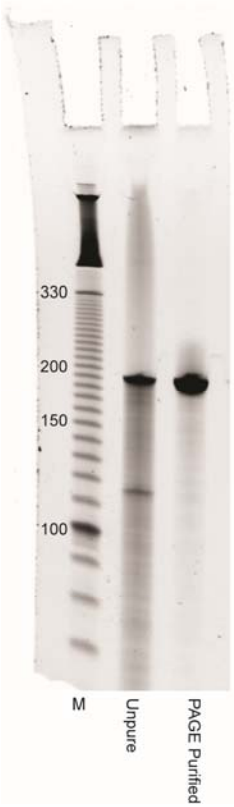


Figure S5 FLAG tag sequences and schematic of insertion sites in *nhr-25* and *smo-1*. (A) DNA and amino acid sequence of 2x and 3x FLAG epitopes used in *nhr-23* and *nhr-25* editing experiments. A GSGGGG amino acid linker sequence precedes the epitope; a *Bam*HI site is encoded in this linker sequence for genotyping by restriction digestion. (B) Sequence of the *nhr-25* genomic locus targeted. The PAM (red text), sgRNA target sequence, stop codon (blue text), and position of the DSB are indicated. The amino acid sequence of the targeted locus is provided. The bases mutated in the oligo template to inactivate the PAM (*nhr-25(PAM MUT)* are in uppercase font in the sgRNA target sequence, with the corresponding WT bases in *nhr-25(+).* (C) DNA and amino acid sequence of the 2x FLAG epitope used in *smo-1* editing experiments. A glycine-serine dipeptide linker encoding a *Bam*HI site for diagnostic restriction digestion follows the epitope. (D) Sequence of the *smo-1* genomic locus targeted. The PAM (red text), sgRNA target sequence, start codon (purple text), and position of the DSB are indicated. The amino acid sequence of the targeted locus is provided. The bases mutated in the oligo template to inactivate the PAM (*smo-1(PAM MUT)* are in uppercase font in the sgRNA target sequence, with the corresponding WT bases in *smo-1(+).*

A



B

P0 strain	Oligo	Succesfully injected P0	F1 screened	PCR hits	Precise Knockins	% Knockins per F1	% Knockins per P0
pha-1(ts); control(RNAi)	Unpure	8	3	0	0	0.0	0.0
pha-1(ts); cku-80(RNAi)	Unpure	25	11	1	1	9.1	4.0
pha-1(ts); control(RNAi)	PAGE pure	11	3	1	1	33.3	9.1
pha-1(ts); cku-80(RNAi)	PAGE pure	7	2	1	1	50.0	14.3

C

Oligo	Succesfully injected P0	F1 screened	PCR hits	Precise Knockins	% Knockins per F1	% Knockins per P0
Unpure	80	26	4	2	7.7	2.5
PAGE pure	18	5	2	2	40.0	11.1

Figure S6 PAGE purification of oligos results in increased knock-in efficiency. (A) Comparison of PAGE purified and unpurified *nhr-25::3xFLAG* oligos. 200 ng of oligos were resolved on a denaturing 8% TBE-Urea polyacrylamide gel and stained with SYBR Gold. A 100 bp ladder (Invitrogen; sizes in bp) is provided as a standard. (B) Comparison of knock-in efficiency of unpurified and PAGE purified oligos in animals grown on HB101 and then transferred to either control RNAi or *cku-80* RNAi. (C) Comparison of all experiments using unpurified vs PAGE purified *nhr-25::3xFLAG* oligos.

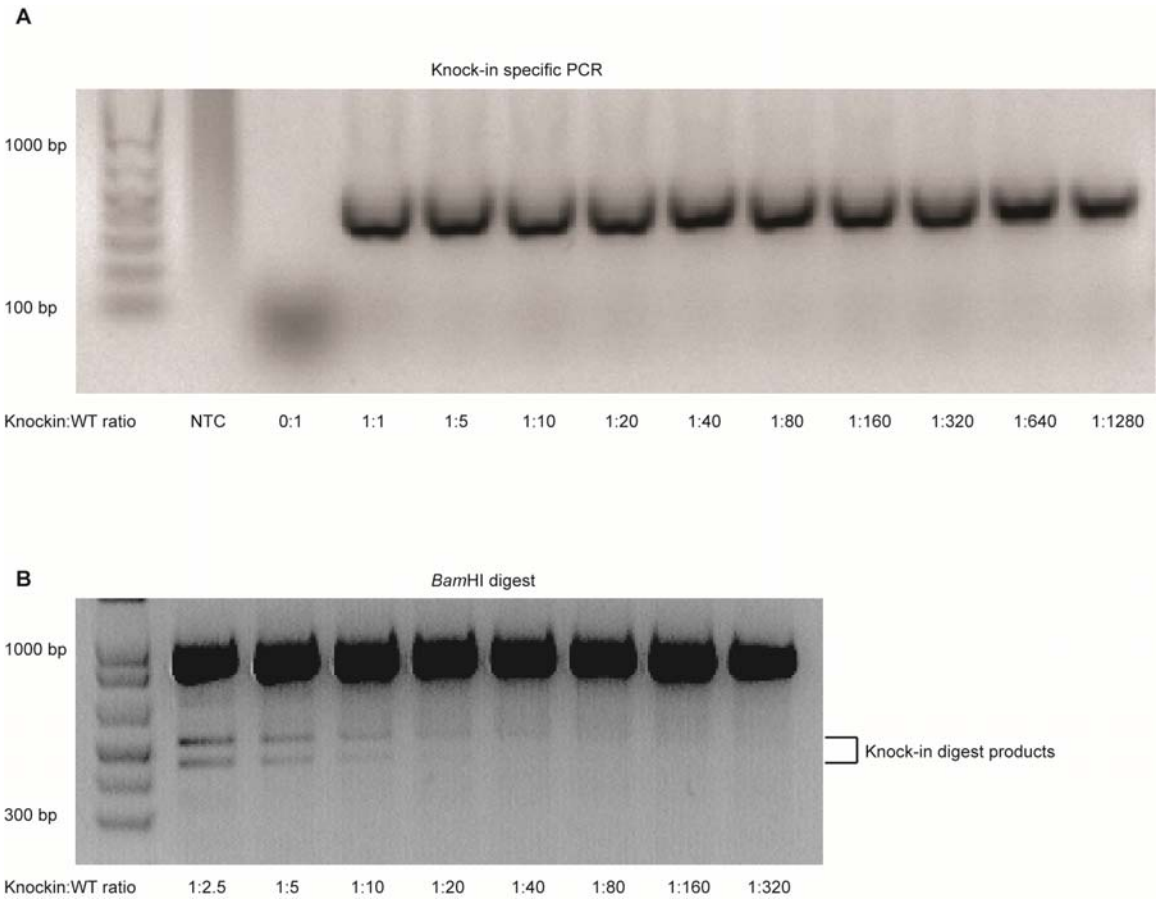


Figure S7 Detection of knock-ins by knock-in specific PCR and diagnostic restriction digestion. (A) For *nhr::23::2xFLAG* direct screening, a knock-in specific PCR approach was developed to minimize the number of PCRs required to identify knock-ins. Using the *nhr-25::2xFLAG* strain generated by direct selection (Table S8) as a control, oligos were designed to bind within the inserted sequence (oligo #1715) and outside of the insertion area. *nhr-25::2xFLAG* knock-in lysate was diluted as indicated with WT lysate and used as template in a knock-in specific genotyping PCR. No product was detected in the absence of *nhr-25::2xFLAG* lysate and knock-in product could be detected across the dilution range, to 1:1280. (NTC; no template control). (B) Identification of *nhr-25:2xFLAG* knock-in by diagnostic restriction digest. *nhr-25::2xFLAG* lysate was diluted and used in genotyping PCR as in (A). The product was then digested by *Bam*HI to detect knock-ins. Knock-in product could be detected up to a 1:20 dilution. The 1KB+ (Invitrogen) size standard is provided in A and B.

File S1
Supplemental Methods

PCR-based knock-in screening

For direct screening of *nhr-25::2xFLAG* knock-ins, WT animals or *lig-4(ok716)* mutants were injected with 100 ng/µl of pJW1185 (*nhr-25* targeting CRISPR/Cas9), 10 ng/µl of a *myo-2::tdTomato* co-injection marker, and 100 ng/µl of a *nhr-25::2xFLAG* 135 mer (oligo #1580, Table S2). P0 animals were plated in single wells of 12-well plates containing NGM-lite agar seeded with OP50 *E. coli*. Following incubation at 25ºC for three days, wells were scored for the presence of marker positive F1 progeny. From these wells, the marker positive F1 were picked off and discarded and 1 ml of M9+gelatin was added. This step was performed because previous reports suggested that edits occurred in marker negative F1 (ZHAO *et al.* 2014). Marker negative F1s from these wells were pipetted into 30 µl of M9+gelatin in a 96-well plate; four worms were pipetted into each well. A multichannel pipette was used to add 30 µl of 2xOP50 food to each well. This food was made by inoculating a one liter culture of LB+streptomycin (50 µg/ml) with a single colony of OP50, shaking for 16 hours at 225 rpm at 37ºC, pelleting the culture by spinning at 4000 rpm, and resuspending in 10 ml of M9. For the 2xOP50 food, 5 ml of this concentrated OP50 was added to 45 ml of M9+gelatin containing 10 µg/ml cholesterol. Plates were parafilmed and incubated at 25ºC for 3-4 days. Lysates were made by using a multichannel pipette to transfer 10 µl of worm culture to 10 µl of single-worm lysis buffer containing proteinase K in a 96-well plate. The lysates were then processed, and genotyping PCRs and BamHI digests performed as described in the "Genotyping PCRs and restriction digestion" section in the main-text Methods. For wells with a hit, in order to identify homozygotes, 24 F2 progeny were transferred to single wells of a 96-well plate and incubated and screened as above.

For pooled screening of *nhr-23::2xFLAG* knock-ins, WT animals or *lig-4(ok716)* mutants were injected with 50 ng/µl of pJW1254 (*nhr-23* PAM #1 targeting CRISPR/Cas9), 5 ng/µl of a *myo-2::tdTomato* co-injection marker, and 50 ng/µl of a sense *nhr-23::2xFLAG* 199mer (oligo #1719, Table S2). P0 animals were plated and marker positive wells were identified as above. All progeny from marker positive wells were washed out with 1 ml of M9+gelatin and diluted to ~10 worms/30µl and this volume (30 µl) was plated in 96 wells using a repeat pipetter. The number of worms/well was averaged in two rows of the plate to confirm the estimated concentration of 10 worms/well. Food was added, the plates were incubated, and lysates made as described above. Knock-in specific PCRs were performed using an oligo that internally bound the *2xFLAG* sequence (#1715) and an oligo that bound external to the knock-in sequence. Four rows were pooled for each PCR reaction with 0.5 µl of lysate from each row used in a 20 µl PCR. For wells with a hit, in order to identify homozygotes, 48-96 F2 progeny were transferred to single wells of a 96-well plate and incubated and screened as above.

Generation of lysates for immunoblotting

For the immunoblot in Figure 2, animals of the indicated genotype were synchronized by alkaline bleaching followed by plating overnight in the absence of food. Approximately 3000 arrested L1s were plated on 10 cm NGM-lite plates seeded with OP50 and incubated at 25ºC for 48 hours, at which point the animals were gravid adults. Animals were washed off of the plates with M9+gelatin, pelleted and transferred to a 1.5 ml tube and washed four times with 1 ml

of M9. The M9 was aspirated, leaving 150 µl and the pellet flash-frozen in liquid nitrogen and stored at -80ºC. The pellet was resuspended by adding 150 µl 2xRIPA buffer (100 mM Tris-HCl, 900 mM NaCl, 2% NP-40, 1% Sodium deoxycholate and 0.2% SDS, pH 7.4) supplemented with Protease Inhibitor Cocktail Set III, EDTA-free (Calbiochem, #539134-1SET), 1mM PMSF, 10 µM MG-132 proteasome inhibitor (Caymon, #10012628), and 1 mM DTT. Worms were lysed by three cycles of sonication on ice (10 sec, 20% amplitude). Debris was pelleted by centrifugation at 14,000 rpm for 10 minutes at 4ºC and protein concentration was determined by a 660nm Protein Assay (Pierce). Four micrograms of total protein was resolved by SDS-PAGE using a Mini-PROTEAN TGX Stain-Free 4-15% gradient gel (Bio-Rad, #456-8086).

For the immunoblot in Figure 5, ten gravid adults were placed on 10 cm plates and incubated for four days at 25ºC. Lysates were made by washing crowded, mixed stage animals off of these 10 cm plates in M9+gelatin, pelleting at 700xg for two minutes, and transferring to a 1.5 ml tube. The worms were washed four additional times with 1 ml of M9+gelatin. The M9+gelatin was aspirated to just above the worm pellet and the pellet was rapidly freeze-thawed three times (cycling between liquid nitrogen and a 42ºC water bath) before 4x Laemmli buffer was added to a final concentration of 1x. The samples boiled for 10 minutes, then the lysate was frozen for 15 minutes on dry ice and then boiled again for 10 minutes. Debris was pelleted by centrifugation at 14,000 rpm in a microcentrifuge for 5 minutes. Ten microlitres of lysate was resolved by SDS-PAGE on a Mini-PROTEAN TGX 4-15% gradient gel (Bio-Rad, catalog #456-1086) at run at 250 V.

PEG/DMSO DH5a competent cells

A single DH5 alpha colony from a freshly struck plate was used to inoculate a 5 ml LB culture and and incubated overnight at 37ºC shaking at 225 rpm. This culture was used to inoculate 500 ml of LB which was shaken at 37ºC until an OD600 of 0.5-0.6 was reached. Cells were pelleted by centrifugation for 5 min at 2000 rpm, 4ºC. Cells were gently resuspended in 25 ml of ice cold TSB buffer (LB pH 6.1, 10% PEG-3350, 5% DMSO, 10 mM $MgCl_2$, 10 mM $MGSO_4$), incubated on ice for 10 minutes, aliquoted, flash frozen in liquid nitrogen, and stored at -80ºC. To transform the cells, an aliquot was thawed on ice and the DNA to be transformed was mixed with 5xKCM (500 mM KCl, 150 mM $CaCl_2$, 250 mM $MgCl_2$) and dH20 to a final volume of 100 µl at 1xKCM final concentration. An equal amount of cells was added, mixed by gentle inversion, and incubated on ice for 20 minutes. The mixture was then incubated at room temperature for 10 minutes before 1 ml of SOC or LB was added and the transformation was shaken for 1 hr at 37ºC before plating on LB containing appropriate antibiotics.

Table S1 Strains generated for this study

Strain name	Genotype
KRY41	*lig-4(ok716)* III*; nhr-25(kry1[nhr-25::2xFLAG])* X
KRY42	*nhr-25(kry1[nhr-25::2xFLAG])* X
KRY46	*nhr-23(kry4[nhr-23::2xFLAG])* I*; pha-1(kry5[Y169C*e2123])* III
KRY47	*nhr-23(kry6[nhr-23::2xFLAG])* I*; pha-1(kry7[Y169C*e2123])* III
KRY48	*nhr-23(kry8[nhr-23::2xFLAG])* I*; pha-1(kry9[Y169C*e2123])* III
KRY49	*nhr-23(kry10[nhr-23::2xFLAG])* I*; pha-1(kry11[Y169C*e2123])* III
KRY50	*nhr-23(kry12[nhr-23::2xFLAG])* I*; pha-1(kry13[Y169C*e2123])* III
KRY51	*nhr-23(kry14[nhr-23::2xFLAG])* I*; pha-1(kry15[Y169C*e2123])* III
KRY52	*nhr-23(kry16[nhr-23::2xFLAG])* I*; pha-1(kry17[Y169C*e2123])* III
KRY53	*nhr-23(kry18[nhr-23::2xFLAG])* I*; pha-1(kry19[Y169C*e2123])* III
KRY54	*nhr-23(kry20[nhr-23::2xFLAG])* I*; pha-1(kry21[Y169C*e2123])* III
KRY55	*nhr-23(kry22[nhr-23::2xFLAG])* I*; pha-1(kry23[Y169C*e2123])* III
KRY56	*nhr-23(kry24[nhr-23::2xFLAG])* I*; pha-1(kry25[Y169C*e2123])* III
KRY57	*nhr-23(kry26[nhr-23::2xFLAG])* I*; pha-1(kry27[Y169C*e2123])* III
KRY58	*nhr-23(kry28[nhr-23::2xFLAG])* I*; pha-1(kry29[Y169C*e2123])* III
KRY59	*nhr-23(kry30[nhr-23::2xFLAG])* I*; pha-1(kry31[Y169C*e2123])* III
KRY60	*nhr-23(kry32[nhr-23::2xFLAG])* I*; pha-1(kry33[Y169C*e2123])* III
KRY64	*pha-1(kry34[Y169C*e2123])* III*; nhr-25(kry35[nhr-25::2xFLAG])* X
KRY65	*pha-1(kry36[Y169C*e2123])* III*; nhr-25(kry37[nhr-25::2xFLAG])* X
KRY66	*pha-1(kry38[Y169C*e2123])* III*; nhr-25(kry39[nhr-25::2xFLAG])* X
KRY67	*pha-1(kry40[Y169C*e2123])* III*; nhr-25(kry41[nhr-25::2xFLAG])* X
KRY70	*pha-1(kry42[Y169C*e2123])* III
KRY71	*pha-1(kry43[Y169C*e2123])* III
KRY72	*nhr-23(kry44[nhr-23::3xFLAG])* I*; pha-1(kry45[Y169C*e2123])* III
KRY73	*nhr-23(kry46[nhr-23::3xFLAG])* I*; pha-1(kry47[Y169C*e2123])* III
KRY74	*pha-1(kry48[Y169C*e2123])* III*; nhr-25(kry49[nhr-25::3xFLAG])* X
KRY75	*nhr-23(kry50[nhr-23::3xFLAG])* I*; pha-1(kry51[Y169C*e2123])* III*; nhr-25(kry52[nhr-25::3xFLAG])* X
KRY76	*nhr-23(kry6[nhr-23::2xFLAG])* I
KRY77	*nhr-23(kry44[nhr-23::3xFLAG])* I
KRY78	*nhr-23(kry50[nhr-23::3xFLAG])* I
KRY79	*nhr-25(kry35[nhr-25::2xFLAG])* X
KRY80	*nhr-25(kry52[nhr-25::3xFLAG])* X
KRY81	*smo-1(kry53[2xFLAG::smo-1]) I; pha-1(kry54[Y169C*e2123]) III*
KRY82	*smo-1(kry55[2xFLAG::smo-1]) I; pha-1(kry56[Y169C*e2123]) III*

Table S2 Repair oligos used for this study

Primer	Description	Sequence
1580	PAGE purified *nhr-25::2x FLAG* (sense 135mer)	aagccacatacactgctgtgccgtacatggcatcaggatccggaggtggcggggattacaaggatg acgacgataaggattacaaggatgacgacgataagtaatgtctctgtcataggagctgaaaacttc caa
1719	*nhr-23::2xFLAG* (sense 199mer; PAM #1 mutated)	tgtctgatccaacatcatctgaaaagcttcctgccctctacaaagagctattcactgcagatcgacct ggatccggaggtggcggggattacaaggatgacgacgataaggattacaaggatgacgacgata agtgactgaatccatatatcatcaatagttttatccatgctctccttccctatccccgtccatgaat
1831	*nhr-23::2xFLAG* (sense 200mer; PAM #1 and #2 mutated)	aaaacttccgaatgtctgatccaacatcatctgaaaagcttcctgcactctacaaagagctattcact gcagatcgacctggatccggaggtggcggggattacaaggatgacgacgataaggattacaagga tgacgacgataagtgactgaatccatatatcatcaatagttttatccatgctctccttccctatccc
1832	*nhr-23::2xFLAG* (antisense 200mer; PAM #1 and #2 mutated)	gggatagggaaggagagcatggataaaactattgatgatatatggattcagtcacttatcgtcgtca tccttgtaatccttatcgtcgtcatccttgtaatccccgccacctccggatccaggtcgatctgcagtg aatagctctttgtagagtgcaggaagcttttcagatgatgttggatcagacattcggaagtttt
1899	PAGE purified *pha-1* repair (sense 80mer)	caaaatacgaatcgaagactcaaaaagagtatgctgtatgattacagatgttcatcaagttattcat aaatcattgatag
1985	PAGE purified *pha-1* repair (antisense 80mer)	ctatcaatgatttatgaataacttgatgaacatctgtaatcatacagcatactctttttgagtcttcgat tcgtattttg
1986	*pha-1* repair (sense 60mer)	aatcgaagactcaaaaagagtatgctgtatgattacagatgttcatcaagttattcataa
1987	*pha-1* repair (sense 200mer)	ggagttttgttgtacattacatttcaggttcttaaaacaaaccatgaagattatggtaatcaaaatac gaatcgaagactcaaaaagagtatgctgtatgattacagatgttcatcaagttattcataaatcatt gataggttcagattgtaagtcttgattatctatcgttttgtaaagtgactaaactttaatcatta
1989	*nhr-25::2xFLAG* (sense 175mer; PAM mutated)	caggtcccagcaatccaactgccaaccccacaagccacatataccgcagtaccttatatggcatca ggatccggaggtggcggggattacaaggatgacgacgataaggattacaaggatgacgacgata agtaatgtctctgtcataggagctgaaaacttccaatggagtcag
2014	*nhr-25::3xFLAG* (sense 193mer; PAM mutated)	actcaggtcccagcaatccaactgccaaccccacaagccacatataccgcagtaccttatatggcat caggatccggaggtggcggggactacaaagaccatgacggtgattataaagatcatgatatcgatt acaaggatgacgatgacaagtaatgtctctgtcataggagctgaaaacttccaatggagt
2015	*nhr-23::3xFLAG* (sense 193mer; PAM #1 and #2 mutated)	ttccgaatgtctgatccaacatcatctgaaaagcttcctgcactctacaaagagctattcactgcaga tcgacctggatccggaggtggcggggactacaaagaccatgacggtgattataaagatcatgatat cgattacaaggatgacgatgacaagtgactgaatccatatatcatcaatagttttatccatgctc
2085	*nhr-25::3xFLAG* (sense 193mer; PAM mutated)-PAGE purified	actcaggtcccagcaatccaactgccaaccccacaagccacatataccgcagtaccttatatggcat caggatccggaggtggcggggactacaaagaccatgacggtgattataaagatcatgatatcgatt acaaggatgacgatgacaagtaatgtctctgtcataggagctgaaaacttccaatggagt

2086	*nhr-23::2xFLAG* (sense 140mer; 35 bp homology arms)	cctgccctctacaaagagctattcactgcagatcgacctggatccggaggtggcggggattacaag gatgacgacgataaggattacaaggatgacgacgataagtgactgaatccatatatcatcaatagt tttatcca
2087	*nhr-23::2xFLAG* (sense 120mer; 25 bp homology arms)	acaaagagctattcactgcagatcgacctggatccggaggtggcggggattacaaggatgacgac gataaggattacaaggatgacgacgataagtgactgaatccatatatcatcaata
2088	*nhr-23::2xFLAG* (sense 100mer; 15 bp homology arms)	attcactgcagatcgacctggatccggaggtggcggggattacaaggatgacgacgataaggatta caaggatgacgacgataagtgactgaatccatat
2089	*nhr-23::2xFLAG* (sense 200mer; PAM #1, #2, and #3 mutated)	aaaacttccgaatgtctgatccaacatcatctgaaaagcttcctgcactctacaaagagctattcact gcagatcgacctggatccggaggtggcggggattacaaggatgacgacgataaggattacaagga tgacgacgataagtgactgaatccatatatcatcaatagttttatccatgctctccttccctatccc
2099	*lig-4* (sense 60mer; insert stop codon, delete part of exon 1)	agtagttgacgtcttcaacaagatttaaggatccgtaagacaattgggcgaactattaca
2105	*2xFLAG::smo-1* (sense 175mer; PAM mutated)	ttctcttttcaaatctaatttcgtttcagagactcccgctataaacgatggattacaaggatgacgac gataaggattacaaggatgacgacgataagggatccgctgacgacgcggcacaggcaggcgaca acgccgaatacatcaagatcaaggtcgttggacaggtaatttg

Table S3 Oligos used for this study

Primer	Description	Sequence
1335	P*U6* primer for site-directed mutagenesis	caagacatctcgcaatagg
1349	sgRNA template sequencing oligo	ctctgacacatgcagctcccgg
1432	Generation of *klp-12* CRISPR/Cas9 plasmid by Q5 mutagensis (pJW1138; pair with oligo 1335)	atccacaagttacaattggGTTTTAGAGCTAGAAATAGCAAGT
1436	*klp-12* genotyping-F	ccatcgaataatccatccacaagtt
1437	*klp-12* genotyping-R	gtttcgcttggggtgtcatgtt
1582	Generation of *nhr-25* CRISPR/Cas9 plasmid by Q5 mutagensis (pJW1185; pair with oligo 1335)	catacactgctgtgccgtacaGTTTTAGAGCTAGAAATAGCAAGT
1584	*nhr-25* C-terminal insert screening-F	agagaagagaagcatcggaag
1586	*nhr-25* C-terminal insert screening-R	tgtgagggtttgggcactagg
1586	*nhr-23* C-terminal insert screening-F	gtgtgcggtgaaaggtattctg
1587	*nhr-23* C-terminal insert screening-R	aatgaggaactctcctgcaac
1715	FLAG-specific oligo for direct screening. Pair with oligo 1585 or 1587.	gggattacaaggatgacgacg
1734	Generation of *nhr-23* CRISPR/Cas9 plasmid by Q5 mutagensis (pJW1254; pair with oligo 1335)	aagagctattcactgcagatGTTTAAGAGCTATGCTGGAAACAG
1763	Generation of *klp-12* CRISPR/Cas9 plasmid by Q5 mutagensis (pJW1236; pair with oligo 1335)	atccacaagttacaattggGTTTAAGAGCTATGCTGGAAACAG
1785	deletion of P*U6*-sgRNA template in pJW1219 to generate pJW1259-F	cgacgttgtaaaacgacggccagt
1786	deletion of P*U6*-sgRNA template in pJW1219 to generate pJW1259-R	ccgggagctgcatgtgtcagagg
1787	P*U6*-F for generating pJW1310 and cloning P*U6* for PCR-derived sgRNA templates	attgtgttcgttgagtgaccc
1788	P*U6*-R for generating pJW1310 and cloning P*U6* for PCR-derived sgRNA templates	caagacatctcgcaataggagg
1789	sgRNA-F for generating pJW1311	gtttaagagctatgctggaaac
1790	sgRNA-R for generating pJW1311 and cloning P*U6*::sgRNA templates	aaaaataggcgtatcacgagg
1793	nested P*U6*-sgRNA template-F	aacgtcgtgactgggaaaacc
1794	nested P*U6*-sgRNA template-R	ggtgtgaaataccgcacagatgc
1827	*nhr-23* PAM #3 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGgaagcttttcagatgatgtGTTTAAGAGCTATGCTGGA
1828	*nhr-23* PAM #1 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGagagctattcactgcagatGTTTAAGAGCTATGCTGGA
1829	Generation of *nhr-23* PAM#2 CRISPR/Cas9 plasmid by Q5 mutagesis (pJW1268; pair with oligo 1335)	agtgaatagctctttgtagaGTTTAAGAGCTATGCTGGAAACAG
1897	Generation of *pha-1* CRISPR/Cas9 plasmid by Q5 mutagesis (pJW1285; pair with oligo 1335)	atgaataacttgatgaacatGTTTAAGAGCTATGCTGGAAACAG

1898	*pha-1* P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGatgaataacttgatgaacat GTTTAAGAGCTATGCTGG
1908	*pha-1* genotyping-F	caatttggcagccattcatgtg
1909	*pha-1* genotyping-R	tcgcgcactactgaatcagagtc
1988	*nhr-25* PAM#2 P*U6*-sgRNA template (pair with oligo 1790)	cctcctattgcgagatgtcttGatacactgctgtgccgtaca GTTTAAGAGCTATGCTGG
1995	Generation of *nhr-25* CRISPR/Cas9 plasmid by Q5 mutagensis (pJW1308; pair with oligo 1335)	atacactgctgtgccgtacaGTTTAAGAGCTATGCTG GAAACAG
2093	*nhr-23* sgRNA PAM #4 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGatgatgttggatcagacattG TTTAAGAGCTATGCTGG
2097	*lig-4* sgRNA#1 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGacgtcttcaacaagattcgg GTTTAAGAGCTATGCTGG
2098	*lig-4* sgRNA#2 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790)	cctcctattgcgagatgtcttGttgacgtcttcaacaagattG TTTAAGAGCTATGCTGG
2104	*smo-1* sgRNA#1 P*U6*-sgRNA template by PCR fusion (pair with oligo 1790); sgRNA is from (KIM *et al.* 2014)	cctcctattgcgagatgtcttGgccgatgatgcagctcaagc GTTTAAGAGCTATGCTGG
2114	*nhr-23* FLAG specific: for sequencing into 5' end of 2xFLAG and 3xFLAG tags in heterozygotes	ttcactgcagatcgacctgg
2115	*nhr-23* FLAG specific: for sequencing into 3' end of 2xFLAG and 3xFLAG tags in heterozygotes	atgatatatggattcagtcact
2117	*nhr-25* FLAG specific: for sequencing into 5' end of 2xFLAG and 3xFLAG tags in heterozygotes	gtaccttatatggcatcagg
2118	*nhr-25* FLAG specific: for sequencing into 3' end of 2xFLAG and 3xFLAG tags in heterozygotes	ctcctatgacagagacattact
2127	*smo-1* genotyping-F	cgctccccagacaatcgata
2128	*smo-1* genotyping-R	tggaaaagggatggatgggtg
2129	*lig-4* genotyping-F	ggcaagactcaagctcggat
2130	*lig-4* genotyping-R	cccatcatccattggtcccg
2135	*smo-1* FLAG specific: for sequencing into 5' end of 2xFLAG tag heterozygotes	ctcccgctataaacgatgga
2136	*smo-1* FLAG specific: for sequencing into 3' end of 2xFLAG tag heterozygotes	gtgccgcgtcgtcagcgg
	GSGGGG-2xFLAG epitope (used in *nhr-23* and *nhr-25* editing)	ggatccggaggtggcggggattacaaggatgacgacgata aggattacaaggatgacgacgataag
	GSGGGG-3xFLAG epitope (used in *nhr-23* and *nhr-25* editing)	ggatccggaggtggcggggactacaaagaccatgacggtg attataaagatcatgatatcgattacaaggatgacgatgaca ag
	2xFLAG-GS epitope (used in *smo-1* editing)	gattacaaggatgacgacgataaggattacaaggatgacga cgataagggatcc

For oligos 1828, 1898, 1988, 2093, 2097, 2098, and 2104, the underlined, lowercase sequence is the sgRNA target site. The uppercase G 1 bp 5' to the sgRNA target is the +1 base of the U6 transcript and the uppercase sequence 3' top the sgRNA target is a portion of the chimeric sgRNA.

For oligos 1432, 1582, 1734, 1763, 1829, 1897, and 1995 the lowercase sequence is the sgRNA target sequence, uppercase sequence is a portion of the chimeric sgRNA .

Table S4 gBlocks used for this study

Primer	Description	Sequence
1643	sgRNA(F+E)	tataaacacctcctattgcgagatgtcttggatggatgtgtagtcaattgtttaagagctatgctggaaacagcatagcaagtt taaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgtgaaatttctggcgtaatagcgaa gaggcccgcacc

Table S5 Brood size analysis of indicated genotypes

Strain	Genotype	Broodsize	P0 animals scored	Embryonic lethality (%)	Males (%)	Molting defects (%)
N2	WT	213±45 (n=4465)	19	0.16	0	0 (n=4458)
GE24	*pha-1(e2123)* III	128±34 (n=1520)	12	72.7	0	n/a
KRY42[b]	*nhr-25(kry1[nhr-25::2xFLAG])* X	191±42 (n=3627)	19	1.25	0	0 (n=3627)
KRY71	*pha-1(kry43[Y169C*e2123])* III	210.7±30 (n=2528)	12	1.19	0	0 (n=2528)
KRY49	*nhr-23(kry10[nhr-23::2xFLAG])* I; *pha-1(kry11[Y169C*e2123])* III	167.3±34 (n=1840)	11	1.68	0	0 (n=1840)
KRY64	*pha-1(kry34[Y169C*e2123])* III; *nhr-25(kry35[nhr-25::2xFLAG])* X	146.8±35 (n=1762)	12	2.27	0.11	0 (n=1762)
KRY72	*nhr-23(kry44[nhr-23::3xFLAG])* I; *pha-1(kry45[Y169C*e2123])* III	167.1±68 (n=1671)	10	0.78	0	0 (n=1671)
KRY74	*pha-1(kry48[Y169C*e2123])* III; *nhr-25(kry49[nhr-25::3xFLAG])* X	180.8±48 (n=1671)	12	2.00	0	0 (n=2170)
KRY75	*nhr-23(kry50[nhr-23::3xFLAG])* I;*pha-1(kry51[Y169C*e2123])* III; *nhr-25(kry52[nhr-25::3xFLAG])* X	112.7±46 (n=1352)	12	3.42	0.15	0 (n=1352)

[a]all remaining progeny arrested as larvae.

[b]from direct screening approach. *lig-4(ok716)* removed by outcrossing; strain was outcrossed 6x.

Table S6 Males recovered in injection experiments

P0 strain	Diet	Expt.	*pha-1* oligo	Repair oligo	Viable injected P0	P0 with rescued F1	♂n	♂PCR hit
pha-1(ts)	OP50	Figure 1D	80mer sense	*nhr-23::2xFLAG* sense	16	3	0	0
pha-1(ts)	OP50	Figure 1D	80mer antisense	*nhr-23::2xFLAG* sense	47	3	0	0
pha-1(ts)	OP50	Figure 1D	60mer sense	*nhr-23::2xFLAG* sense	11	3	0	0
pha-1(ts)	OP50	Figure 1D	200mer sense	*nhr-23::2xFLAG* sense	28	10	3	2
pha-1(ts)	OP50	Figure 1D	200mer sense	*nhr-23::2xFLAG* antisense	57	4	0	0
pha-1(ts)	HB101	Figure 3B	200mer sense	*nhr-23::2xFLAG* sense	27	3	0	0
pha-1(ts); control(RNAi)	OP50 then RNAi	Table 2	80mer sense	*nhr-23:::2xFLAG*	10	1	0	0
pha-1(ts); cku-80(RNAi)	OP50 then RNAi	Table 2	80mer sense	*nhr-23:::2xFLAG*	16	6	1	1
pha-1(ts); control(RNAi)	HB101 then RNAi	Table 2	200mer sense	*nhr-25:::2xFLAG*	21	4	1	0
pha-1(ts); cku-80(RNAi)	HB101 then RNAi	Table 2	200mer sense	*nhr-25:::2xFLAG*	22	12	3	0
pha-1(ts); control(RNAi)	HB101 then RNAi	Table 2	200mer sense	*nhr-23::2xFLAG* *nhr-25::3xFLAG*	34	4	0	0 0
pha-1(ts); cku-80(RNAi)	HB101 then RNAi	Table 2	200mer sense	*nhr-23::2xFLAG* *nhr-25::3xFLAG*	13	3	0	0 0
pha-1(ts); cku-80(RNAi)	HB101 then RNAi	Table 2	200mer sense	*2xFLAG::smo-1* *lig-4 stop*	15	7	0	0 0

Table S7 Conversion events associated with different DSB positions for *nhr-23::2xFLAG* knock-ins

sgRNA	DSB Distance from insert site	Sequenced animals	PAM#1 only	PAM#2 only	PAM#3 only	PAM#1+ PAM#2	PAM#2+ PAM#3	PAM#1+ PAM#2+ PAM#3	PAM#1+ FLAG	PAM#1+ PAM#2+ FLAG	PAM#1+ PAM#2+ PAM#3+ FLAG
PAM #1[a]	9 bp	22[b]	n/a	n/a	n/a	n/a	n/a	n/a	16	6	n/a
PAM #2	29 bp	12[c]	0	2	n/a	0	n/a	n/a	0	1	n/a
PAM #3	54 bp	8 [d]	0	0	0	0	1	0	0	0	1[e]

For the data presented in Figure 4, the number of animals with conversion events at the indicated PAMs, and number FLAG tag knock-in was presented. Here, a more detailed breakdown of the knock-in events is provided. There was no PAM #3 mutation present in the repair oligo used for the PAM #1 and PAM #2 sgRNA experiments.

[a]Pooled from all 200mer *nhr-23-2xFLAG* injections (Figures 1 and 3, Table 2). As these animals were selected for based on a potential FLAG insertion, there were no "PAM only" gene conversion events that would be identified in this dataset.
[b]Includes 14 precise insertions and 8 partial insertions
[c]Of the 12 sequenced animals, nine had no knocked-in sequence
[d] Of the eight sequenced animals, six had no knocked-in sequence
[e]1 bp deletion in inserted 2xFLAG epitope

Table S8 *nhr-23::2xFLAG* and *nhr-25::2xFLAG* knock-in identification by direct screening

P0 strain	Repair oligo	Oligo polarity	Succesfully injected P0	F1 screened	PCR hits	Precise Knockins	% Knockins per F1	% Knockins per P0
WT	*nhr-25::2xFLAG*	sense	12	380	0	0	0.00	0
lig-4(ok716)	*nhr-25::2xFLAG*	sense	10	768	1	1	0.13	10
WT	*nhr-23::2xFLAG*	sense	2	200	2	0	0	0
lig-4(ok716)	*nhr-23::2xFLAG*	sense	4	800	8	0	0	0

For the *nhr-25* experiments, animals were injected with 100 ng/µl of CRISPR/Cas9 plasmid targeting the same PAM used for the experiments described in Figure 5, 10 ng/µl of a *myo-2::tdTomato* co-injection marker, and 100 ng/µl of a 135mer *nhr-25::2xFLAG* repair oligo with 35 bp homology arms (oligo #1580), which was the synthesis size limit at the time. Injected P0 animals were singly plated, and plates lacking co-injection marker positive F1 progeny were discarded. As Zhao *et al*. (2014) had reported that only non-transgenic F1s contained knock-ins, marker-negative F1 were transferred into 96-well plates (four worms/well), allowed to self-fertilize, and potential knock-ins were identified by PCR and diagnostic *Bam*HI digestion, as in the *pha-1(ts)* co-selection experiments. Oligo polarity is with respect to the coding strand.

For *nhr-23* experiments, animals were injected with 50 ng/µl of a CRISPR/Cas9 plasmid targeting *nhr-23* PAM #1 (Figure 1), 10 ng/µl of a *myo-2::tdTomato* co-injection marker, and 100 ng/µl of a 199mer *2xFLAG* repair oligo with the PAM mutated (oligos #1719). Wells containing marker-positive F1 progeny were identified, all animals from these wells were pooled, and 10 worms were plated per well of a 96-well plate. Following self-fertilization, a portion of the well was taken for genotyping and four rows were pooled for knock-in specific PCR using an oligo internal to the insert and an oligo external to the insert.

Table S9 Summary of strands to which sgRNAs bind, sgRNA activity, repair oligo strand homology, and repair oligo efficiency

Gene	sgRNA sequence[a]	Strand to which sgRNA binds[b]	sgRNA activity	Repair oligo	Repair oligo strand[c]	Repair oligo efficiency
pha-1(e2123)	atgaataacttgatga acat(cgg)	coding	Y	60, 80 and 200 mer *pha-1(ts)* repair (oligos 1899, 1986, 1987)	coding	High
				80mer *pha-1(ts)* repair (oligo 1985)	template	Weak
nhr-23 PAM #1	agagctattcactgcagat(cgg)	template	Y	200mer *nhr-23::2xFLAG* (oligo 1831, 2 PAMs mut)	coding	High
				200mer *nhr-23::2xFLAG* (oligo 1832, 2 PAMs mut)	template	Weak
				193mer *nhr-23::3xFLAG* (oligos 2015 and 2085, 2 PAMs mut)	coding	Moderate
nhr-23 PAM #2	agtgaatagctctttgtaga(ggg)	coding	Y	200mer *nhr-23::2xFLAG* (oligo 1831, 2 PAMs mut)	template	Moderate
nhr-23 PAM #3	ggaagcttttcagatgatgt(tgg)	coding	Y (moderate)	200mer *nhr-23::2xFLAG* (oligo 2089, 3 PAMs mut)	template	Moderate
nhr-23 PAM #4	atgatgttggatcagacatt(cgg)	coding	N	200mer *nhr-23::2xFLAG* (oligo 2089, 3 PAMs mut)	template	n/a (sgRNA fail)
nhr-25	atacactgctgtgccgtaca(tgg)	template	Y	175mer *nhr-25::2xFLAG* (oligo 1989; PAM mut)	template	Moderate
				193mer *nhr-25::3xFLAG* (oligo 2014; PAM mut)-unpurified	template	Weak
				193mer *nhr-25::3xFLAG* (oligo 2085; PAM mut)-PAGE purified	template	Weak
smo-1	gccgatgatgcagctcaagc(agg)	template	Y	175mer *2xFLAG::smo-1* (oligo 2105; PAM mutated)	template	High
lig-4	acgtcttcaacaagattcgg(cgg)	template	N	60mer *lig-4* stop/exon deletion (oligo 2099)	template	n/a (sgRNA fail)
	ttgacgtcttcaacaagatt(cgg)	template	N			

[a]sgRNA target sequence. The PAM sequence is provided in brackets.
[b]strand to which sgRNA binds. ie. for a target sequence with an "NGG" PAM in the coding sequence, the sgRNA would bind to the template strand
[c]strand from which oligo homology is derived

Supplemental references

FRIEDLAND A. E., TZUR Y. B., ESVELT K. M., COLAIÁCOVO M. P., CHURCH G. M., CALARCO J. A., 2013 Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. Nat Meth **10**: 741–743.

KIM H., ISHIDATE T., GHANTA K. S., SETH M., CONTE D., SHIRAYAMA M., MELLO C. C., 2014 A Co-CRISPR Strategy for Efficient Genome Editing in *Caenorhabditis elegans*. Genetics **197**: 1069–1080.

ZHAO P., ZHANG Z., KE H., YUE Y., XUE D., 2014 Oligonucleotide-based targeted gene editing in *C. elegans* via the CRISPR/Cas9 system. Cell Research **24**: 247–250.