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.