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.