Supplemental Figure 1. Phylogenetic comparison of the intron retained in JPH-1B.

A) Top: Exon-intron diagrams for *C. elegans jph-1* isoform A and B.
Bottom: We performed a BLASTn search of *C. elegans jph-1* against 26 *Caenorhabditis* genomes published on Caenorhabditis.org (Stevens et al. 2019). Aligned sequences are thick black lines and unaligned sequences are thin black lines. Darker lines indicate stronger hits. Boundaries between aligned and unaligned regions often match up with exon-intron boundaries. 10 *Caenorhabditis* sequences align with the intron retained in JPH-1B.
B) We translated the introns of these 10 species in the same reading frame as *C. elegans jph-1* and aligned the amino acid sequences using MUSCLE (Madeira et al. 2019). The sequences vary in length because most encounter stop codons, except for sister species *C. sulstoni* and *C. afra*, which have no stop codons in the intron and are in frame with the following exon. Beyond the first three amino acids there is little amino acid conservation between sequences.

Supplemental Figure 2. Expression pattern of a *jph-1* transcriptional reporter and a JPH-1B translational fusion reporter.

A) *jph-1* is expressed in neurons and most muscles. Top: Illustration of *jph-1* promoter::GFP expression construct [P*jph-1*-GFP(*juEx8013* and *juEx8014*)]. Bottom: GFP expression was seen in head ganglia neurons and pharyngeal, body wall, vulval, uterine, stomatointestinal, anal sphincter, and anal depressor muscles. The large fluorescent circle marked by an asterisk is a coelomocyte labeled by the coinjection marker [P*unc-122*-RFP], as the filter used for imaging permitted simultaneous detection of both GFP and RFP.
B) *jph-1* localization in an L1 stage animal. Top: Illustration of expression construct [P*jph-1*-GFP::JPH-1A(*juSi387*)]. Bottom: Confocal images of an L1 stage animal. A plane near the surface of the animal shows expression in the body wall muscle, while a plane taken through the middle of the animal shows expression in the pharyngeal muscle and head ganglia neurons.
C) JPH-1B has a diffuse localization. Top: Illustration of construct expressing *jph-1b* cDNA under the *jph-1* promoter [P*jph-1*-GPF::JPH-1B(*juEx8038*)]. Bottom: Confocal projection of an L4 stage

animal head shows a diffuse localization in neurons and muscles.
Scale bars 20 µm.

Supplemental Figure 3. *jph-1(0)* mutants do not alter touch neuron morphology or enhance axon fusion after injury.

A) Touch neuron morphology is normal in *jph-1(ju1683)* animals. Representative images of wild-type and *jph-1(ju1683)* day-1 adult animals expressing the touch neuron marker P*mec-7*-GFP(*muIs32*). Labels indicate ALM, PLM, AVM, and PVM neuron cell bodies. The bright spot below the *jph-1(ju1683)* ALM cell body is likely fluorescence from the ALM on the opposite side of the body. Scale bar, 100 µm.
B) Distance regrown by PLM axon 24h post-injury. Control animals expressed GFP Degron in the touch neurons. *jph-1(ju1683)* animals expressing GFP-tagged JPH-1A under the *jph-1* promoter [P*jph-1*-GFP::JPH-1A(*juSi387*)] also expressed GFP Degron in the touch neurons, predicted to degrade GFP::JPH-1 specifically in touch neurons. There was no statistically significant difference between groups. Number of animals per genotype indicated below X-axis tick marks. Data are shown as individual data points and mean±SEM. Statistics: Student's t-test. ns not significant.
C) Percentage of animals with axon-axon fusion 24h post-injury. *jph-1(ok2823)* mutants had increased axon fusion while null mutants *ju1683* and *ju1684* exhibited wild-type levels of axon fusion. Number of animals per genotype indicated below X-axis tick marks. Statistics: Fisher's exact test performed pairwise. ns not significant, **p<0.01.
D) JPH-1(*ok2823*) localizes to the nucleus. Top: Illustration of construct expressing *jph-1(ok2823)* cDNA from original start to stop codon under the *jph-1* promoter [P*jph-1*-GFP::JPH-1(*ok2823*)(*juEx8035)*]. A premature stop codon in the middle of JPH-1(*ok2823*) truncates the C-terminal two-thirds of the protein. Bottom: Confocal projection of L4-stage animal tail with arrows indicating neuronal nuclei labeled by JPH-1(ok2823). Scale bar, 20 µm.

Supplemental Figure 4. JPH-1 and ESYT-2 reside near cholinergic presynaptic sites.

A) *jph-1* is expressed in cholinergic neurons. Single plane confocal image of ventral nerve cord of L4 animal expressing mCherry in cholinergic neurons [P*unc-17*-mCherry(*nuIs321*)] and JPH-1A under the *jph-1* promoter [P*jph-1*-GFP::JPH-1A(*juEx7999*)]. Red arrows indicate cholinergic neuron cell bodies. Green arrowheads indicate JPH-1A puncta in cholinergic neurons.
B-C) JPH-1A mostly surrounds cholinergic synapses. Single plane confocal images of day 1 adult animals with endogenous *unc-17* tagged with mKate2 [*unc-17(ot907)*] and expressing JPH-1A under the pan-neuronal *rab-3* promoter [P*rab-3*-GFP::JPH-1A(*juEx8026*)]. Panel B shows the dorsal nerve cord and Panel C shows the dorsal sublateral cord.
D) ESYT-2 puncta flank cholinergic synapses. Single plane confocal image of day 1 adult animal dorsal nerve cord with endogenous *unc-17* tagged with mKate2 [*unc-17(ot907)*] and expressing ESYT-2 under the pan-neuronal *rab-3* promoter [*Prab-3*-GFP::ESYT-2(*juEx8113*)].
Apparent difference of UNC-17::mKate2 images in B and D reflects single-focal plane. All scale bars, 5 µm.

Supplemental Figure 5. Pharmacological responses of second alleles of *jph-1* and *esyt-2,* and transgenes.

A) *jph-1* null mutants *ju1683* and *ju1684* are both aldicarb resistant. Statistical significance shown between *jph-1(ju1684)* and wild type.
B) *esyt-2(ju1409)* animals are resistant to aldicarb, which was rescued by expression of *esyt-2* genomic DNA (*juEx7581*). Statistical significance shown between *esyt-2(ju1409)* and *esyt-2;Ex[esyt-2 gDNA].*
C) *jph-1(ju1683);esyt-2(ju1409)* double mutants exhibit a wild-type response to aldicarb. Statistical significance shown between *jph-1(ju1683)* and *jph-1(ju1683);esyt-2(ju1409)*.

Note: the choice of 0.5 mM (A, C) vs 1 mM aldicarb (B) was merely due to drug plate availability. Same responses were observed in both concentrations.
13-15 animals tested per genotype per trial, n=3 trials. Data represent mean±SEM of individual

data points. Statistics: One-way ANOVA with Tukey's post-test. ns not significant, *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 6. JPH-1A localization is unaltered in *esyt-2(0)*

Shown are single-plane confocal images of GFP::JPH-1A expressed under the *jph-1* promoter [P*jph-1*-GFP::JPH-1A(*juSi387*)] in wild-type and *esyt-2(ju1409)* backgrounds.

A) In the body wall muscle, JPH-1A localizes to rows of puncta in wild type and *esyt-2(ju1409)* animals.

B) In neurons of the head ganglia, JPH-1A localizes to reticulate structures surrounding the nucleus and forms puncta in the cell periphery of wild type and *esyt-2(ju1409)* animals. Arrows mark some of the GFP::JPH-1A puncta.

Scale bar, 5 μm**.**