Supplemental Information

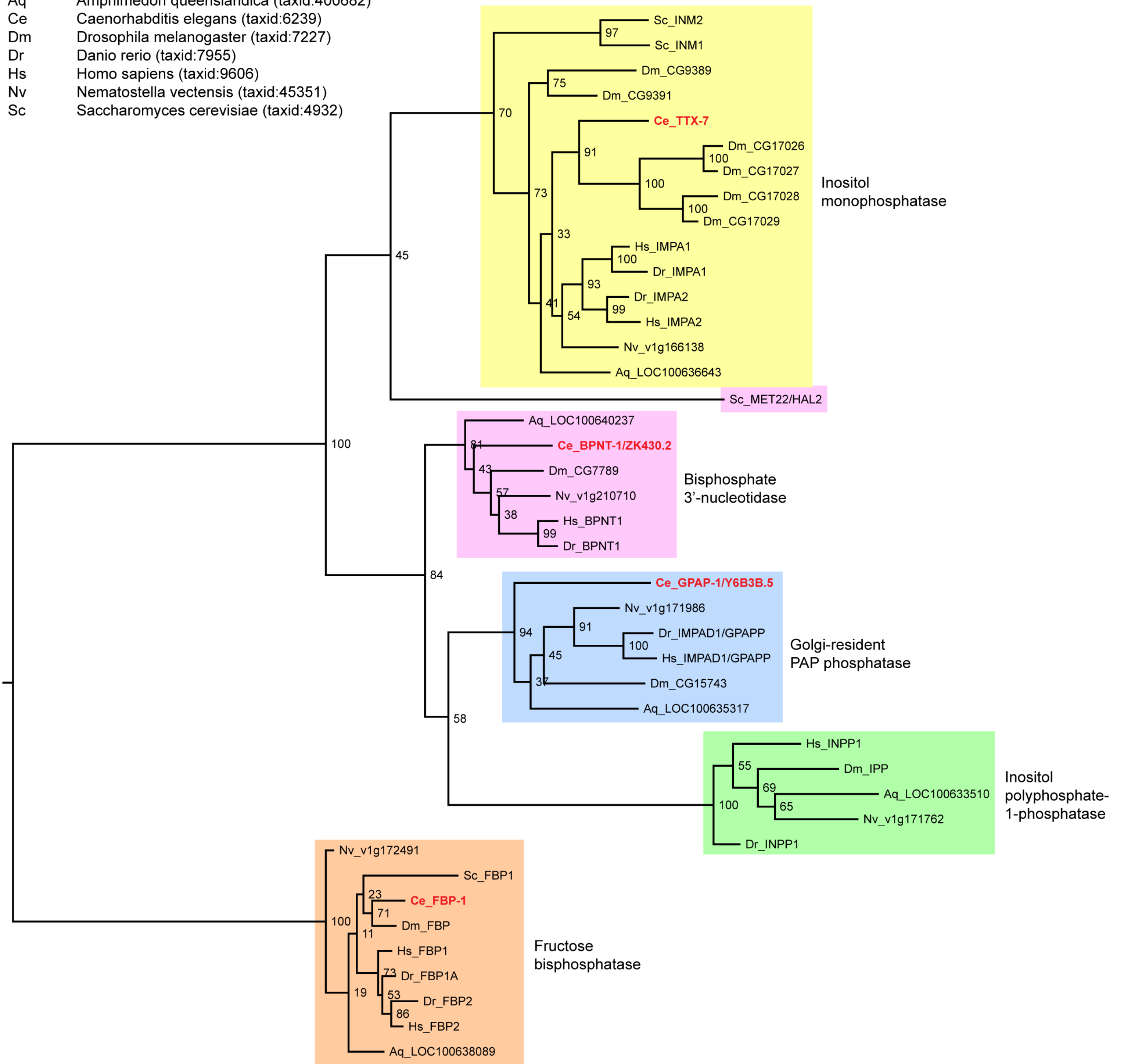
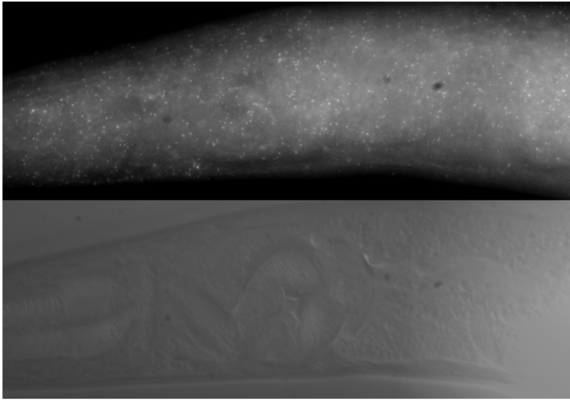
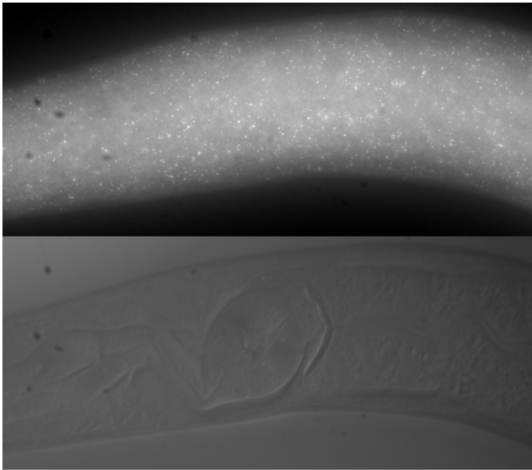


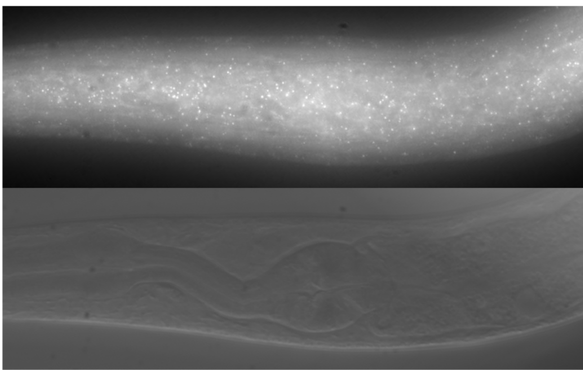
Figure S1. Phylogenetic analysis of the conserved family of lithium-sensitive phosphatases, related to Figure 1. Maximum-likelihood phylogenetic analysis of conserved regions of the inositol monophosphatase, bisphosphate 3'-nucleotidase (BPNT-1), Golgi-resident PAP phosphatase, inositol polyphosphate-1-phosphatase, and fructose bisphosphatase proteins. Colored boxes denote functional homologs. Nodes are labeled with bootstrap values (out of 100 replicates). Branch lengths are proportional to number of amino acid changes. *C. elegans* genes are indicated in red.

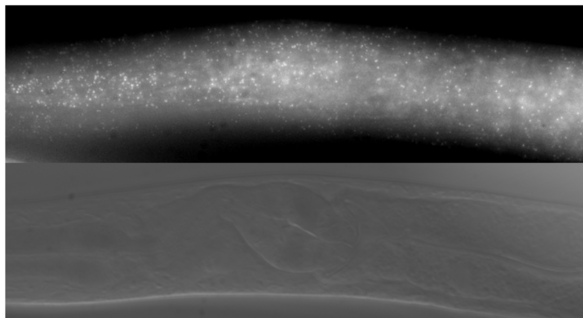
wild type

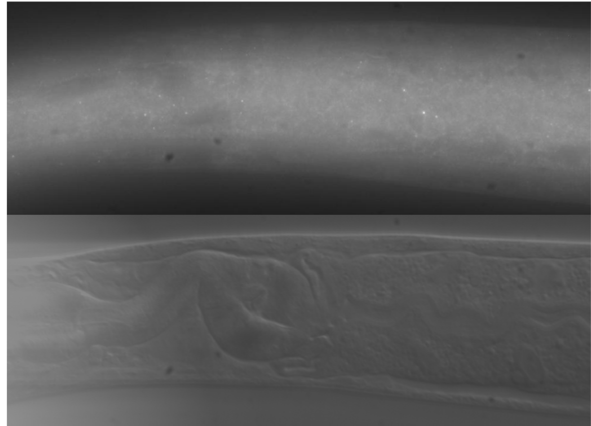
bpnt-1(qd303)

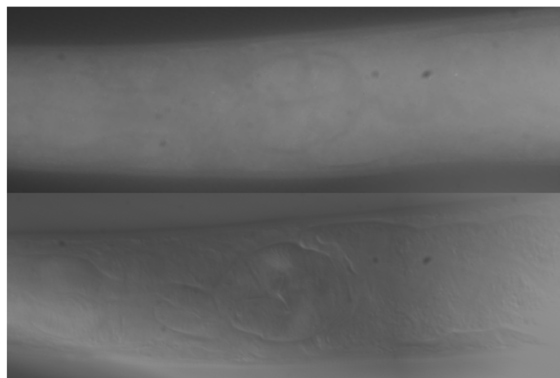


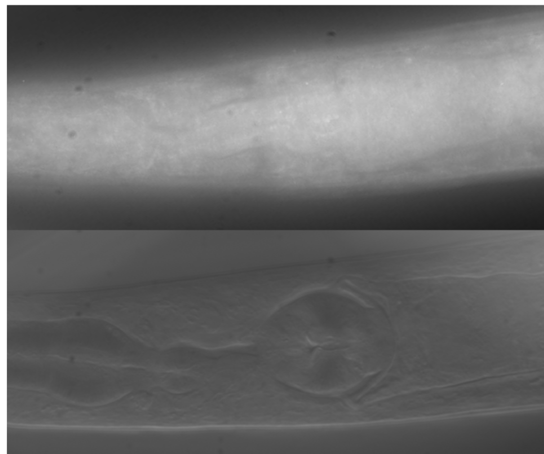












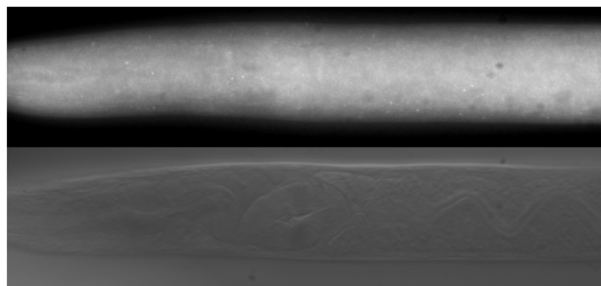


Figure S2. The *bpnt-1* expression pattern is broad and not limited to the ASJ neurons, related to Figure 1. Single molecule fluorescent *in situ* hybridization of *bpnt-1* mRNA in wild type and *bpnt-1(qd303)* mutant animals. Images presented are maximum intensity z-projections of 30 stacked exposures of *bpnt-1* mRNA (above) and trans-illumination (below). Animals presented are representative samples from a mixed-stage population. All images were taken at 100x magnification.

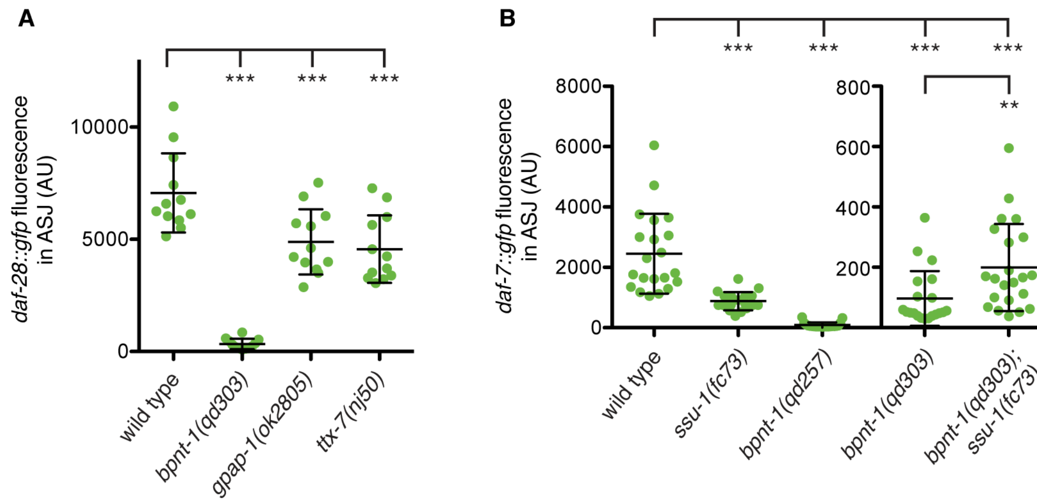


Figure S3. BPNT-1, but not GPAP-1 or TTX-7, is required for ASJ function partially due to expression of cytosolic sulfotransferase SSU-1, related to Figures 2 and 3. (A) Maximum fluorescence of *daf-28::gfp* in the ASJ neurons. All genotypes contain *mgIs40[daf-28p::nls-GFP]*. **(B)** Maximum fluorescence of *daf-7p::gfp* in the ASJ neurons after 16 h exposure to *P. aeruginosa*. All genotypes contain *ksIs2[daf-7p::gfp]*. *** $P < 0.001$, ** $P < 0.01$ as determined by one-way ANOVA followed by Dunnett's Multiple Comparison Test. Error bars indicate standard deviation.

Supplemental Experimental Procedures:

C. elegans Strains

C. elegans was maintained on *E. coli* OP50 as previously described [S1]. BPNT-1 was formerly known as *C. elegans* TAG-231/ZK430.2. GPAP-1 was formerly known as *C. elegans* Y6B3B.5. The *bpnt-1(qd257)* allele (D119N) was isolated from an EMS mutagenesis screen and cloned through SNP mapping followed by whole-genome sequencing. The *bpnt-1(qd303)* allele (insertion/deletion) was generated using CRISPR-based targeting [S2]. The *bpnt-1(gk577587)* (G287R) and *bpnt-1(gk469190)* (W294X) alleles were isolated from the *C. elegans* Million Mutation Project and also conferred *daf-7* expression defects in the ASJ neurons. For assays in which a synchronized population of animals was required, strains were egg-prepped in bleach and arrested overnight in M9 buffer at the L1 larval stage. For a complete list of strains used in this study see below.

ASJ Gene Expression and Morphology

To measure the expression of *daf-7* in the ASJ neurons on *P. aeruginosa* PA14*,* an overnight culture of PA14 was grown in 3 mL LB at 37˚C, and the following morning 7 µL of culture was seeded onto 3.5 cm SKA plates as previously described [S3]. PA14 plates were grown overnight at 37˚C and then grown for an additional two days at room temperature. Animals at the L4 larval stage were then picked onto the center of the bacterial lawn, incubated at 25˚C, and scored for GFP expression 16 h later. For quantifying the area of the ASJ cell body, animals were incubated in the lipophilic dye DiI (Molecular Probes) at 10 µg/ml in M9 buffer for 3 hours. Animals were then destained for 1 hour on a lawn of *E. coli* prior to imaging. To measure the expression of

daf-28, gpa-9, and *trx-1* in the ASJ neurons, L4 larval animals were transferred to *E. coli* plates with or without 15 mM LiCl and incubated overnight at 20˚C. Images were acquired with an Axioimager Z1 microscope using animals anaesthetized in 50 mM sodium azide. To quantify fluorescence animals were imaged at 40x magnification and the maximum intensity value within the ASJ neuron was determined using FIJI software. Statistical analysis was performed using GraphPad Prism Software.

P. aeruginosa Avoidance and Dauer Exit Assays

Plates for *P. aeruginosa* avoidance assays were prepared as above. 30 animals at the L4 larval stage were transferred to the center of PA14 lawns, incubated at 25˚C, and scored for avoidance 15-20 h later. For dauer exit assays, all strains carried a mutation in the dauer constitutive gene *daf-2*. Gravid adults were egg-laid at 25˚C on plates with *E. coli* and the experiment began two days later when all animals had entered the dauer developmental stage. Dauer animals were moved onto NGM plates seeded with *E. coli* with or without 15 mM LiCl. Animals continued to be incubated at 25˚C and scored over the next week for dauer exit.

Single Molecule Fluorescent *In Situ* Hybridization

smFISH was performed as previously described [S4]. Briefly, *C. elegans* were fixed in 4% formaldehyde for 45 min at room temperature. After washing with PBS, larvae were resuspended in 70% EtOH and incubated overnight at 4˚C. The following day fixed larvae were transferred into hybridization solution with the smFISH probe and incubated overnight at 30˚C. The *bpnt-1* probe was constructed by pooling 32 unique 20 nucleotide

DNA oligos that tile the *bpnt-1* coding region and coupling them to Cy5 dye. For a complete list of oligos see below. Images were acquired with a Nikon Eclipse Ti Inverted Microscope outfitted with a Princeton Instruments PIXIS 1024 camera. Data were analyzed using FIJI software; images presented in Figure S2 are maximum intensity z-projections of 30 stacked exposures.

Generation of Transgenic Animals

To generate the genomic *bpnt-1* rescue construct, a 3.8 kb PCR fragment containing 1.4 kb of upstream promoter was amplified from genomic DNA and cloned into the pGEM-T Easy vector. To generate the *bpnt-1(I75A)* lithium-insensitive rescue construct the above plasmid was edited using a QuikChange II XL Site-Directed Mutagenesis Kit from Agilent Technologies. To generate the ASJ-specific *bpnt-1* and *mCherry* expression constructs, the *trx-1* promoter [S5] (1.1 kb) was amplified by PCR from genomic DNA. *bpnt-1* cDNA was amplified by PCR from a cDNA library generated with an Ambion RETROscript Kit. The *unc-54* 3-prime UTR was amplified by PCR from Fire Vector pPD95.75. The *mCherry::unc-54 3'UTR* fragment was amplified from plasmid pCFJ104. DNA constructs (promoter::gene::*unc-54* 3'UTR) were synthesized using PCR fusion as previously described [S6] and cloned into the pGEM-T Easy vector. To generate the cross-species *bpnt-1* rescue constructs, *D. melanogaster* BPNT-1/CG7789 cDNA (clone LD34542) and IPP cDNA (clone RE60387) were amplified from plasmids from the Drosophila Genomic Resource Center. *H. sapiens* IPP cDNA (clone 3845956) was amplified from a plasmid from GE Healthcare Dharmacon. *S. cerevisiae* MET22/HAL2 DNA was amplified from genomic yeast DNA. *C. elegans bpnt-1* cDNA was amplified

as above. These cDNAs were then cloned into the above genomic *bpnt-1* rescue construct using Gibson Assembly. All constructs were injected into animals at a concentration of 50 ng/µL along with a plasmid carrying the co-injection marker *ofm-1p::gfp* (50 ng/µL). At least three independent transgenic lines were analyzed for each construct. For a complete list of primers used in this study see below.

Phylogenetic Analysis

NCBI protein BLAST was used to collect homologs of all lithium-sensitive phosphatases in the following species: yeast (*Saccharomyces cerevisiae)*, sponge (*Amphimedon queenslandica)*, sea anemone (*Nematostella vectensis), Caenorhabditis elegans,* fly (*Drosophila melanogaster*), zebrafish (*Danio rerio)*, and human (*Homo sapiens)*. A protein alignment of conserved residues was generated using ClustalX, and a maximum-likelihood tree with 100 bootstrapped data sets was generated using PhyML [S7]. Graphical trees were generated using FigTree.

Complete list of *C. elegans* strains used in this study

STRAIN	GENOTYPE	FIGURE
FK181	*ksIs2[daf-7p::GFP + rol6(su1006)] I*	1b-c, 3a-b, S3b
ZD886	*ksIs2; bpnt-1(qd257)*	1b-c, S3b
ZD1147	*ksIs2; bpnt-1(gk469190) 7xBackcrossed*	1b
ZD1146	*ksIs2; bpnt-1(gk577587) 6xBackcrossed*	Text
ZD1181	*ksIs2; bpnt-1(qd303)*	1b-c, 3a-b, S3b
ZD1150	*ksIs2; bpnt-1(qd257); qdEx121[bpnt-1(+) + ofm-1::gfp]*	1b
ZD1159	*ksIs2; bpnt-1(qd257); qdEx123[trx-1p::bpnt-1 cDNA + ofm-1::gfp]*	1b-c
DA650	*npr-1(215F) X*	1d
ZD1441	*bpnt-1(qd257); npr-1(215F)*	1d
ZD1442	*bpnt-1(qd303); npr-1(215F)*	1d
N2	wild type Bristol strain	1e, S2
ZD1169	*bpnt-1(qd257) II*	1e
ZD1224	*bpnt-1(qd303) II*	1e, S2

DR1572	*daf-2(e1368) III*	1f-g, 2f
ZD1396	*bpnt-1(qd257); daf-2(e1368)*	1f-g
ZD1425	*bpnt-1(qd303); daf-2(e1368)*	1f
ZD1519	*bpnt-1(qd257); daf-2(e1368); qdEx123[trx-1p::bpnt-1 cDNA + ofm-1::gfp]*	1g
GR1455	*mgIs40[daf-28p::nls-GFP] IV*	2a, 2d-e, 2g, 3c-d, S3a
ZD1572	*bpnt-1(qd303); mgIs40*	2a, 3c-d, S3a
NL1606	*dpy-20(e1282); pkIs586[gpa-9::GFP; dpy-20]*	2b
ZD1500	*bpnt-1(qd303); pkIs586[gpa-9::GFP; dpy-20]*	2b
ZD1446	*qdEx133[trx-1p::mCherry::unc-54 3'UTR + ofm-1::gfp]*	2c
ZD1578	*bpnt-1(qd303); qdEx133[trx-1p::mCherry::unc-54 3'UTR + ofm-1::gfp]*	2c
ZD1601	*bpnt-1(qd303); mgIs40; qdEx135[bpnt-1(I75A) + ofm-1::gfp]*	2e, 2g
ZD1755	*bpnt-1(qd303); daf-2(e1368); qdEx135[bpnt-1(I75A) + ofm-1::gfp]*	2f
ZD1528	*ksIs2; bpnt-1(qd303); qdEx127[bpnt-1p::S.c. MET22 cDNA::bpnt-1 3'UTR + ofm-1::gfp]*	3a-b
ZD1529	*ksIs2; bpnt-1(qd303); qdEx128[bpnt-1p::D.m. bpnt-1 cDNA::bpnt-1 3'UTR + ofm-1::gfp]*	3a-b
ZD1530	*ksIs2; bpnt-1(qd303); qdEx129[bpnt-1p::D.m. IPP cDNA::bpnt-1 3'UTR + ofm-1::gfp]*	3a-b
ZD1533	*ksIs2; bpnt-1(qd303); qdEx131[bpnt-1p::C.e. bpnt-1 cDNA::bpnt-1 3'UTR + ofm-1::gfp]*	3a-b
ZD1527	*ksIs2; bpnt-1(qd303); qdEx126[bpnt-1p::H.s. IPP cDNA::bpnt-1 3'UTR + ofm-1::gfp]*	3b
ZD1687	*mgIs40; ssu-1(fc73)*	3c-d
ZD1648	*bpnt-1(qd303); mgIs40; ssu-1(fc73)*	3c-d
ZD1701	*gpap-1(ok2805); mgIs40*	S3a
ZD1705	*ttx-7(nj50); mgIs40*	S3a
ZD1539	*ksIs2; ssu-1(fc73)*	S3b
ZD1540	*ksIs2; bpnt-1(qd303); ssu-1(fc73)*	S3b
ZD1622	*gpap-1(ok2805) ksIs2; bpnt-1(qd303)/mIn1[mIs14 dpy-10(e128)]*	Text

Complete list of DNA oligos used in this study

All sequences are listed in the 5' to 3' direction.

Primers used to generate *bpnt-1* rescue constructs	
acaaggccgaggagtctttg	*bpnt-1* genomic rescue forward
ggactcgacgaggaggaatc	*bpnt-1* genomic rescue reverse
tttttacctcttcgccggcaatattaatattcttgaaatgcttctgcagaga	*bpnt-1* QuickChange I75A forward
tctctgcagaagcatttcaagaatattaatattgccggcgaagaggtaaaaa	*bpnt-1* QuickChange I75A reverse
Primers used to generate ASJ-specific *bpnt-1* and mCherry constructs	
TGAGTTGGGCACTTCGTAGA	*trx-1* promoter forward
TGAGAAAACTCGCTTTGTTGAACATGATCAATTGCTCAAAGTCAC	*trx-1* promoter reverse + *bpnt-1* tag
CTTCTTCACCCTTTGAGACCATGATCAATTGCTCAAAGTCAC	*trx-1* promoter reverse + *mCherry* tag
ATGTTCAACAAAGCGAGTTTTCTCA	*bpnt-1* cDNA forward
TAGGGATGTTGAAGAGTAATTGGACTCACTTTTTCGATGAAATCTCCGG	*bpnt-1* cDNA reverse + *unc-54* 3'UTR tag
ATGGTCTCAAAGGGTGAAGAAG	*mCherry* forward
GTCCAATTACTCTTCAACATCCCTA	*unc-54* 3'UTR forward
CAGTTATGTTTGGTATATTGGGAATG	*unc-54* 3'UTR reverse
Primers used to generate cross-species *bpnt-1* rescue constructs	
tctgaagcattccaaatttaattttttcc	Gibson backbone forward
ttcaactggtgccgaaatcattc	Gibson backbone reverse
tcggcaccagttgaaATGTTCAACAAAGCGAGTTTTCTCA	*Ce_BPNT1* cDNA Forward + Gibson tag
ttggaatgcttcagaTCACTTTTTCGATGAAATCTCCGG	*Ce_BPNT1* cDNA Reverse + Gibson tag
tcggcaccagttgaaATGGCTGCAACTGCTCCG	*Dm_BPNT1* cDNA Forward + Gibson tag
ttggaatgcttcagaTCACTTGGCTCCCACTGC	*Dm_BPNT1* cDNA Reverse + Gibson tag
tcggcaccagttgaaATGGCATTGGAAAGAGAATTATTGGTTG	*Sc_Met22* cDNA Forward + Gibson tag
ttggaatgcttcagaTTAGGCGTTTCTTGACTGAATGAC	*Sc_Met22* cDNA Reverse + Gibson tag
tcggcaccagttgaaATGAGCGGCGTGGAGGAG	*Dm_IPP* cDNA Forward + Gibson tag
ttggaatgcttcagaTCATTGCTCGGCCAATTTGG	*Dm_IPP* cDNA Reverse + Gibson tag
tcggcaccagttgaaatgtcagatatcctccgggagc	*Hs_IPP* cDNA Forward + Gibson tag
ttggaatgcttcagactaggtatgcgtctctgcagg	*Hs_IPP* cDNA Reverse + Gibson tag
Oligos used to generate *bpnt-1* smFISH probe	
gtctcgtgagaaaactcgct	
cctctgaaactcggacagac	
atttttaataagacccccgg	
tgattttgagatctccgccg	

gatccgtgctccgatttatc	
cttccgtttgtggatcgtag	
gaactatgcagtattgagcc	
cactgaatcccatttcgatt	
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agggcgttggagacagattc	
cgccaaatttcgagttttca	
caccgactttttcaacgcta	
aaagacatacgctgcacacc	
catttcttacaaccagcact	
agtcaacacggcttcaactg	
aatgtccgtaagagttcctc	
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tactcctcccgtattattaa	
cttatgcttgacccatgaag	
ttcttgtggaatcgtatcga	
cgatgaaatctccggcaaca	

Supplemental References:

[S1] Brenner S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71–94.

[S2] Friedland AE, Tzur YB, Esvelt KM, Colaiácovo MP, Church GM, and Calarco JA. (2013). Heritable genome editing in C. elegans via a CRISPR-Cas9 system. Nat Meth *10*, 741–743.

[S3] Tan MW, Mahajan-Miklos S, and Ausubel FM. (1999). Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. Proc Natl Acad Sci USA *96*, 715–720.

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[S5] Fierro González JC, Cornils A, Alcedo J, Miranda-Vizuete A, and Swoboda P. (2011). The Thioredoxin TRX-1 Modulates the Function of the Insulin-Like Neuropeptide DAF-28 during Dauer Formation in Caenorhabditis elegans. PLoS ONE *6*, e16561.

[S6] Hobert O. (2002). PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic C. elegans. BioTechniques *32*, 728–730.

[S7] Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, and Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol *59*, 307–321.