File S1

Supporting Materials and Methods

Strain List

N2	Wild type	Figure 4A, B
RM2256	*pha-1(e2123)III*	Recipient for transgenic injections
RM523	*unc-17(cn355)IV*	Figure 4C, D
RM3288	*pha-1(e2123)III; mdEx833[Punc-17::dual-reporter(wt) pBX pBS]*	Figure 3; 4E, F; 5A
RM3302	*pha-1(e2123)III; mdEx847[Punc-17::dual-reporter(cn355) pBX pBS]*	Figure 4G, H
RM3376	*pha-1(e2123)III; mdEx909[dual-reporter(wt) pBX pBS]*	No promoter (Control)
RM3317	*pha-1(e2123)III; mdEx857[Punc-17::dual-reporter(R1u:scr)* pBX pBS]*	Figure 5B
RM3323	*pha-1(e2123)III; mdEx863[Punc-17::dual-reporter(R1ud:scr) pBX pBS]*	Figure 5C
RM3319	*pha-1(e2123)III; mdEx859[Punc-17::dual-reporter(R2d:scr) pBX pBS]*	Figure 5D
RM3321	*pha-1(e2123)III; mdEx861[Punc-17::dual-reporter(R2ud:scr) pBX pBS]*	Figure 5E
RM3347	*pha-1(e2123)III; mdEx885[Punc-17::dual-reporter(R1uR2d:scr) pBX pBS]*	Figure 5F

*Note: R1u:scr = R1 upstream element scrambled (see Table S1);

R1ud:scr = R1 upstream and downstream elements scrambled; etc.

Details for the *unc-17(cn355)* allele (see Figure 4)

Nature of mutation: A>G transition

Flanking Sequences: AAATTTAGAAAAAATAAAATATTCC/ **A>G** /GGGGGAGAGAGAGAGATGGGCTTCA

(in direction of transcription)

Sources and accession numbers of genomic CGL sequences

Genomic sequences for all Caenorhabditis species were downloaded from WormBase, Release WS240 (www.wormbase.org).

Downloaded from the Sanger Center (www.sanger.ac.uk/resources/downloads/helminths/globodera-pallida.html):

Globodera pallida pathogens_Gpal_scaffold_214.1

pathogens_Gpal_scaffold_214.2

pathogens_Gpal_scaffold_146

Downloaded from NCBI (www.ncbi.nlm.nih.gov):

Ciona intestinalis	NW_001955240 REGION: 1..30000
Drosophila melanogaster	NT_033777 REGION: 14525001..14560000
Meloidogyne hapla	ABLG01001183 **and** ABLG01002582
Mus musculus	AC167565
Rattus norvegicus	NW_047469 REGION: complement(7900000..8006000)
Saccoglossus kowalevskii	NW_003151267 REGION: 100001..150000
Schistosoma mansoni	NS_000200 REGION: 148400001..148540000
Takifugu rubripes *	CGL a (chr 1): NC_018890 REGION: complement(3185000..3200000)
	CGL b (chr 4): NC_018893 REGION: 10170000..10190000

* Note: The genomes of *T. rubripes* and other teleosts reflect a whole genome duplication, and therefore have 2 (somewhat diverged) copies of many genes, including the CGL. Official *T. rubripes* gene names for the CGLs are still unassigned, but we provisionally refer to the CGL on Chromosome 1 as CGL a, and the locus on Chromosome 4 as CGL b.

DNA/RNA sequence analysis

Sequence analysis utilized Vector NTI® software (Life Technologies Corporation, Carlsbad, CA) or the Lasergene® Suite (DNASTAR, Inc., Madison, WI). For several species, genomic annotation of the CGL was incomplete or incorrect; in such cases, the genomic organization of the *unc-17* and *cha-1* homologs was deduced through analysis with TBlastN, identification of splice-site consensus sequences, and direct determination of homology. RNA structures were analyzed with Mfold (Zuker 2003) or Sfold (Ding and Lawrence 2003; Ding *et al.* 2005). The criteria for R1-like elements (in addition to complementarity) were that they flank the complete VAChT coding sequence but do not include any part of the ChAT coding sequence; the criterion for R2-like elements was that they flank or overlap the splice site of the first VAChT coding exon.

Construction of dual-reporter plasmid RM#942p from 7 fragments cloned into pCRII-TOPO

Starting Plasmids:

pBS: pBlueScript (Stratagene)

TOPO: pCRII-TOPO (Invitrogen)

pAA64: mCherry plasmid with 3 artificial introns (McNally *et al.* 2006; Green *et al.* 2008).

RM#651p: Fire-type modular plasmid with an empty cloning site 1 and GFP in cloning site 2.

RM#691p: Fire-type plasmid with CFP driven by 4.4 kb of genomic sequence upstream of the *unc-17* start codon.

Clone A: RM#691p was digested with *Xba*I and recircularized to remove the 3.2 kb *unc-17* promoter, the common exon, and the first 339 bp of the *unc-17* 1st intron.

Clone D: mCherry fragment (minus the initiation Met) amplified from pAA64 with p2288 and p2206. **Note**: A lower-case "p" followed by a number refers to a primer listed below.

Clone E: *unc-17* 3'-UTR amplified from N2 genomic DNA with p13 and p2301.

Clone F: *cha-1* 3'-UTR amplified from N2 genomic DNA with p2290 and p45.

Clone H: GFP fragment from RM#651p amplified with p2291 and p2292.

Clone I: *unc-17* 5'-UTR amplified from N2 genomic DNA with p62 and p2293.

Clone J: *unc-17* 3'-UTR amplified from N2 genomic DNA with p2294 and p49.

Cloning Steps:

Clone D was transferred from TOPO to pBS with *Xho*I+*Spe*I → Clone D'

Clone F/*Nhe*I+*Spe*I was subcloned into D'/*Spe*I → Clone D'F

Clone E/*Sac*I(blunt) was subcloned into D'F/*Sna*BI → Clone ED'F

Clone ED'F was subcloned into Clone A with *Avr*II+*Spe*I → Clone B

Clone H was transferred from TOPO to pBS with *Apa*I+*Sac*I → Clone H'

Clone I was subcloned into Clone H' with *Bam*HI → Clone IH'(B)

Clone IH'(B) was digested with *Xba*I and recircularized → Clone IH'

Clone J was subcloned into Clone IH' with *Sac*I → Clone IH'J

Clone IH'J was subcloned into Clone B with *Avr*II → RM#942p.

Primers cited:

p13	CCTTCTCTGTTACCTACAA
p45	GTCTGGTGTTTCTGGGATGA
p49	CATTTGGTGGAAGTTCGTCAAC
p62	TTCCGCATCTCTTGTTCAA
p2206	GAGCTCTTAGGATCCACTAGTCTTATAC (final amino acids of mCherry with *Sac*I site at 5'-end)

p2288 TACGTATCAAAGGGTGAAGAAGATAACA (adds *Sna*BI site; introduces a silent mutation in Val2 of mCherry)

p2290 GCTAGCGGATCCTGAATTTTATTATTATTTTTTGAG (adds the last four amino acids of mCherry with two mutations to alter the *Spe*I site to *Nhe*I - amino acid sequence from Thr-Ser to Ala-Ser - uses the *cha-1* stop codon TGA)

p2291 GGATCCAAAGGAGAAGAACTTTTCACTG (introduces a *Bam*HI site that changes GFP N-terminal amino acid sequence from Met-Ser-Lys to Gly-Ser-Lys)

p2292 GAGCTCATCCATGCCATGTGTAATC (makes two silent mutations; inserts *Sac*I site in Glu-Leu near end of GFP)

p2293 GGATCCCATCTCTCTCTCTCC (creates *Bam*HI site - changes GFP amino acid sequence from Met-Ser to Met-Gly)

p2294 GAGCTCTACAAATAGTCGTAGATTTGGATCTCTG (5'-end corresponds to the last four amino acids of GFP with two silent mutations to generate *Sac*I site)

p2301 GAGCTCATCTGGAACAAAATTTACTTCT (*Sac*I site added at 5'-end)

LITERATURE CITED

Ding, Y., C. Y. Chan and C. E. Lawrence, 2005 RNA secondary structure prediction by centroids in a Boltzmann weighted ensemble. RNA 11**:** 1157-1166.

Ding, Y., and C. E. Lawrence, 2003 A statistical sampling algorithm for RNA secondary structure prediction. Nucleic Acids Res. 31**:** 7280-7301.

Félix, M. A., C. Braendle, and A. D. Cutter, 2014 A streamlined system for species diagnosis in Caenorhabditis (Nematoda: Rhabditidae) with name designations for 15 distinct biological species. PLoS One 9: e94723.

Green, R. A., A. Audhya, A. Pozniakovsky, A. Dammermann, H. Pemble *et al.*, 2008 Expression and Imaging of Fluorescent Proteins in the *C. elegans* Gonad and Early Embryo, pp. 179-218 in *Methods in Cell Biology: Fluorescent Proteins*, edited by F. S. Kevin. Academic Press.

Huang, R. E., X. Ren, Y. Qiu and Z. Zhao, 2014 Description of *Caenorhabditis sinica* sp. n. (Nematoda: Rhabditidae), a Nematode Species Used in Comparative Biology for *C. elegans*. PLoS One 9: e110957.

McNally, K., A. Audhya, K. Oegema and F. J. McNally, 2006 Katanin controls mitotic and meiotic spindle length. J. Cell Biol. 175**:** 881-891.

Zuker, M., 2003 Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31**:** 3406-3415.