

Gene	Protein	Function in synaptic signaling	No. of alleles
<i>egl-30</i>	Gα _q	Activates PLCβ and Rho-specific GEF domain of Trio	34
<i>unc-2</i>	N/P/Q-type Calcium Channel	Mediates voltage-gated calcium entry in neurons	4
<i>unc-31</i>	CAPS	Mediates dense core vesicle exocytosis. Function overlaps with Gαs pathway	19
<i>unc-73</i> RhoGEF domain	Rho-specific GEF domain of Trio	Gαq effector that activates RhoA	8
<i>unc-108</i>	Rab2	Subject of this study	5
Other			18

The diagram illustrates the genetic map of Chromosome I, highlighting the *unc-108* gene and its surrounding region. The map is divided into three main sections:

- Top Section (Chromosome I):** A horizontal line representing the chromosome with a scale bar indicating 2 Mb. A 1090 Kb interval is marked between the *ceP62* and *ceP64* loci. A 200 Kb interval is marked between the *ceP194* and *ceP64* loci.
- Middle Section:** A detailed view of the *unc-108* gene region. The *unc-108* gene is shown as a blue box. The *ceP62* locus is marked at the left end, and the *ceP194* locus is marked at the right end. A 416 Kb interval is marked between *ceP62* and *ceP194*. A 40 Kb interval is marked between *ceP194* and *ceP195*. The *unc-108* gene is located between *ceP194* and *ceP195*. The *unc-108* gene is flanked by a splice donor (red box) and a splice acceptor (red box). The *unc-108* gene is located between the *ceP194* and *ceP195* loci. The *unc-108* gene is flanked by a splice donor (red box) and a splice acceptor (red box). The *unc-108* gene is located between the *ceP194* and *ceP195* loci.
- Bottom Section:** A detailed view of the *unc-108* gene region. The *unc-108* gene is shown as a blue box. The *ceP62* locus is marked at the left end, and the *ceP194* locus is marked at the right end. A 416 Kb interval is marked between *ceP62* and *ceP194*. A 40 Kb interval is marked between *ceP194* and *ceP195*. The *unc-108* gene is located between *ceP194* and *ceP195*. The *unc-108* gene is flanked by a splice donor (red box) and a splice acceptor (red box). The *unc-108* gene is located between the *ceP194* and *ceP195* loci.

Conservation of Rabs in the Animal Kingdom		
<i>C. elegans</i> Rab	Human Orthologue (Accession No.)	% Identity
UNC-108	Rab2 (NP_002856)	87.9
RAB-14	Rab14 (CAG33675)	82.8
RAB-1	Rab1 (NP_004152)	79.5
RAB-11.1	Rab11 (P62491)	79.2
RAB-6.2	Rab6 (AAD27707)	77.5
RAB-5	Rab5 (NP_004153)	74.2
RAB-8	Rab8 (CAA40065)	72.2
RAB-3	Rab3C (AAK08968)	69.5
RAB-10	Rab10 (CAG33584)	69.3
RAB-7	Rab7 (AAD02565)	69.2
RAB-35	Rab35 (CAG46484)	66.7
RAB-18	Rab18 (CAG38486)	63.6
AEX-6	Rab27 (NP_899059)	59.0
RAB-30	Rab30 (CAG46903)	53.4
RAB-39	Rab39B (NP_741995)	47.4
RAB-21	Rab21 (NP_055814)	47.6
RAB-28	Rab28 (CAA64364)	42.2
RAB-37	Rab37 (NP_783865)	41.2
RAB-33	Rab33b (Q9H082)	37.3
RAB-19	Rab19 (NP_001008749)	35.1

The 20 *C. elegans* Rabs with their human orthologues and Accession numbers, listed in order from highest to lowest percent identity. Percent identity is calculated from Vector NTI Align X, which uses the Clustal W algorithm. Worm proteins are from WormBase Release WS190 (<http://ws190.wormbase.org>).

Figure S1. **A genetic screen ties the most highly conserved animal Rab protein to a synaptic signaling function.** (A) Partial summary of the results of the *goa-1* ($G\alpha_o$) suppressor screen. GEF, guanine nucleotide exchange factor. (B) Scaled low to high resolution drawings show SNP markers used to map the mutations. The location of each SNP is noted in Materials and Methods. The amino acid changes caused by each mutation and the approximate location of each mutation are indicated. The wild-type amino acid at each of the missense locations is identical in humans. The location of the recently identified *unc-108(nu415)* null deletion mutation (Chun et al. 2008. *Mol. Biol. Cell.* 19:2682–2695), which is used extensively in this study, is also indicated. (C) Comparison of the 20 *C. elegans* Rab proteins with their human orthologues. Accession numbers are from the NCBI Protein database.

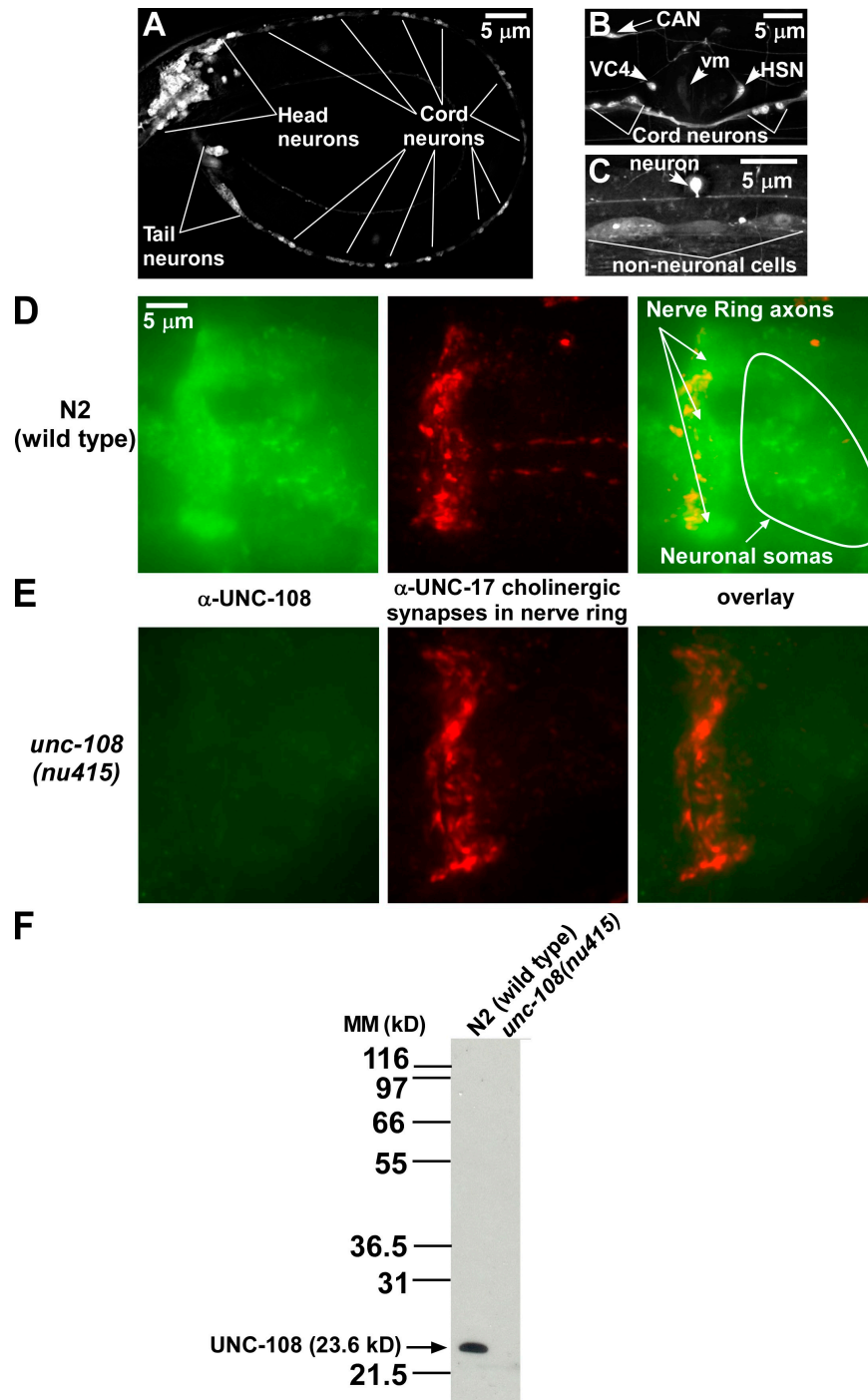
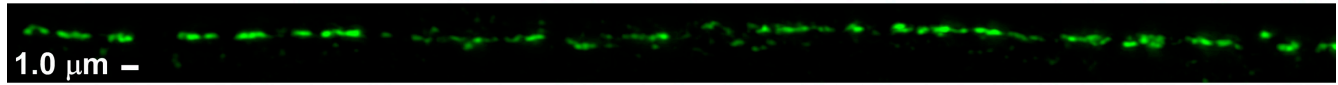


Figure S2. ***unc-108* (Rab2) is strongly expressed in the nervous system.** (A–C) Images of a GFP reporter transgene driven by the *unc-108* promoter used in the rescue experiment in Fig. 1 B. The genotype is *unc-108*(*ce365*); *cels44* [*unc-108::GFP*]. (A) A first larval stage animal showing strong expression of the GFP reporter throughout the nervous system. (B) The vulval region of an adult animal showing strong expression in the neurons of this region (CAN, VC4, and HSN [hermaphrodite-specific neuron]) and weaker expression in one of the vulval muscles (vm). (C) Weak expression in nonneuronal cells in the body of an adult animal compared with strong expression of one of the body neurons. (D and E) The nerve ring region of wild type (D) or a mutant lacking UNC-108 (Rab2; E) coimmunostained with an affinity-purified antibody raised against recombinant UNC-108 (green) and a monoclonal antibody recognizing cholinergic synaptic vesicles (UNC-17; red). These representative images, scaled identically, illustrate the specificity of the UNC-108 antibody. The nerve ring contains only axons, so the images demonstrate that native Rab2 is present in axons. The nerve ring contains many noncholinergic axons as well; thus, because UNC-108 is not cholinergic specific and does not concentrate at synapses, it is more broadly distributed in the nerve ring than UNC-17. Images are maximum projections of 50-plane image stack z series, with each plane separated by 0.5 μ m. UNC-108 images were not deconvolved because of low signal to noise in the *unc-108* mutant. (F) Western blot of total *C. elegans* lysates (2,625 L1 larvae per lane) probed with the UNC-108 antibody. The *unc-108*(*nu415*) lysate lacks the prominent band at 23.6 kD, which is the predicted molecular mass (MM) of UNC-108.

A N2 (wild type): 0.38 +/- 0.014 synapses per micrometer (n = 9 animals)



unc-108(ce363): 0.38 +/- 0.0095 synapses per micrometer (n = 9 animals)

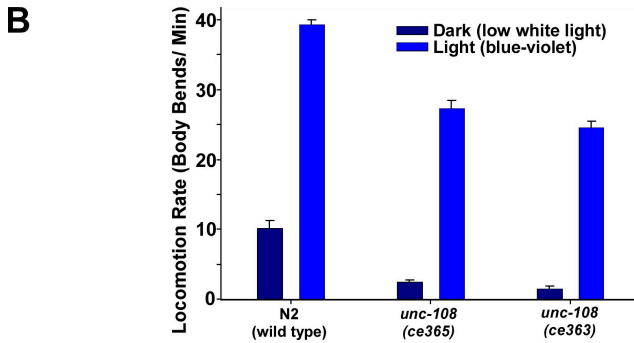
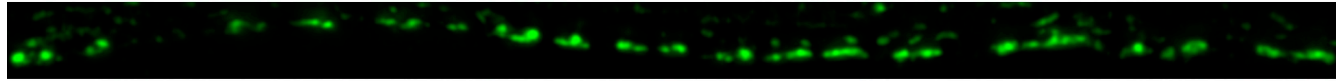


Figure S3. ***unc-108* mutant phenotypes are not associated with permanent developmental defects or permanently damaged synapses.** (A) Cholinergic synaptic density is unaltered in an *unc-108* mutant. Representative images and quantitative data derived from immunostaining the indicated genotypes with an antibody against the UNC-17 cholinergic synaptic vesicle marker. A region of the ventral nerve cord in the mid body region was used for imaging and quantification. Minor regions of UNC-17 immunoreactivity were not included in the analysis. The person quantifying the images was blind to the genotype. Data above each image represent means and SEMs. (B) The *C. elegans* ultraviolet light response restores normal levels of locomotion to *C. elegans unc-108* loss-of-function mutants. Even though *unc-108* mutants are normally sluggish and only weakly responsive to harsh physical stimuli, the *C. elegans* ultraviolet light response restores the locomotion rate in *unc-108* mutants to levels greater than that of wild type under ambient light conditions, showing that synapses must be developed properly and not damaged by the absence of UNC-108. Data are the means and SEM from populations of 10 animals per genotype during 6-min locomotion assays under optimal power blue-violet light (1,460 $\mu\text{W}/\text{mm}^2$) produced through a CFP filter.

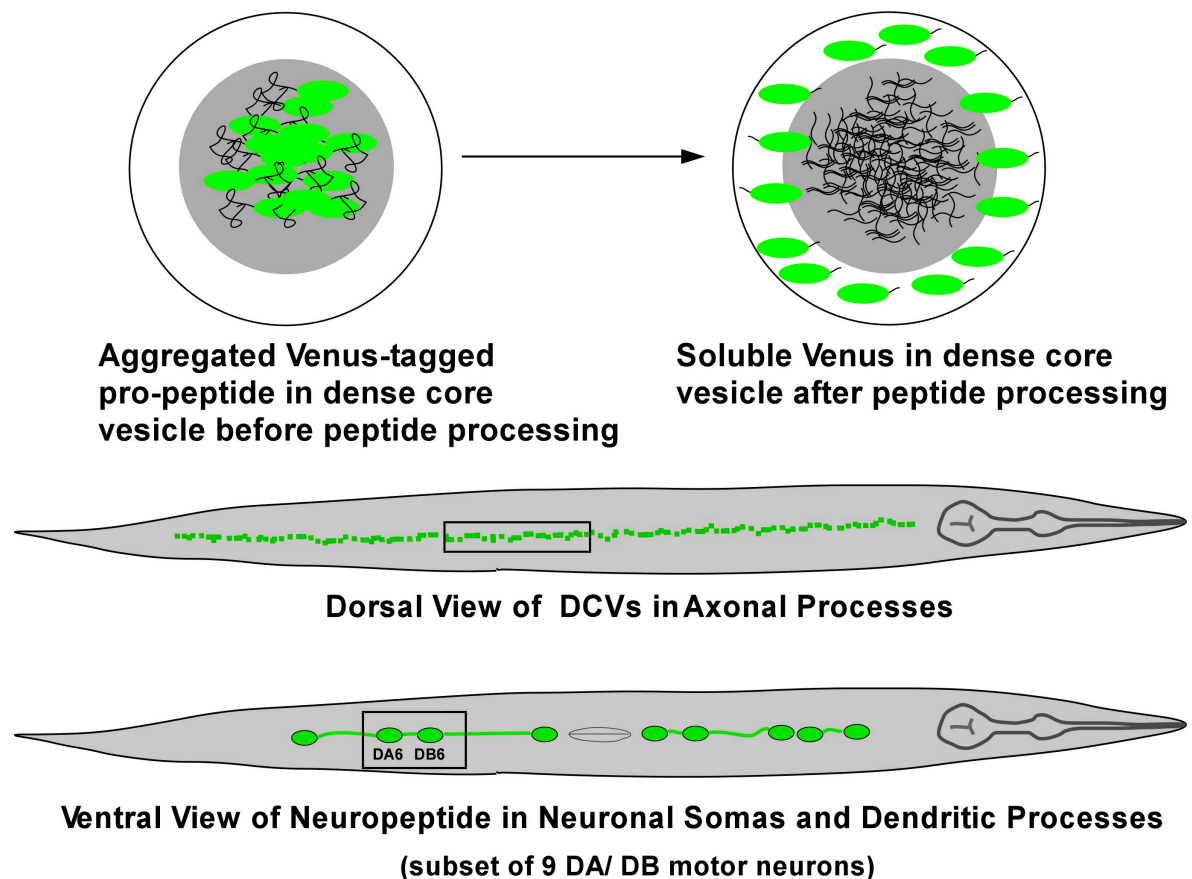
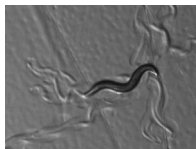


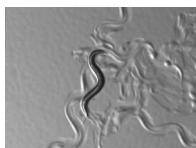
Figure S4. **Model for the physical states of Venus-tagged neuropeptides in DCVs before and after neuropeptide processing.** The top drawings show the likely status of the Venus tag before and after neuropeptide processing. The bottom drawings show dorsal and ventral views of *C. elegans* adults and the fluorescence pattern that appears when tagged neuropeptides are expressed in a subset of nine DA/DB ventral cord motor neurons. Boxed regions show the regions imaged in this study. DA6 and DB6 normally exhibit variable spacing between them, as can be seen from the images in this study.



Video 1. **Locomotion and harsh touch response of wild-type *C. elegans*.** An adult N2 (wild type) animal on a lawn of OP-50 bacteria on a culture plate is shown. The 41-s video shows spontaneous movement as well as how it responds to touch with a wire pick.



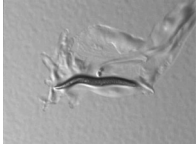
Video 2. **Locomotion and harsh touch response of the *unc-108(nu415)* mutant.** An adult *unc-108(nu415)* single mutant animal on a lawn of bacteria on a culture plate is shown. The 35-s video shows spontaneous movement as well as how it responds to touch with a wire pick.



Video 3. **Locomotion and harsh touch response of the *egl-3(nr2090)* mutant.** An adult *egl-3(nr2090)* single mutant on a lawn of bacteria on a culture plate is shown. The 37-s video shows spontaneous movement as well as how it responds to touch with a wire pick.



Video 4. **Locomotion and harsh touch response of the *unc-31(e928)* mutant.** An adult *unc-31(e928)* single mutant on a lawn of bacteria on a culture plate is shown. The 32-s video shows spontaneous movement as well as how it responds to touch with a wire pick.



Video 5. **Locomotion and harsh touch response of the *unc-108(nu415); egl-3(nr2090)* double mutant.** An adult *unc-108(nu415); egl-3(nr2090)* double null mutant on a lawn of bacteria on a culture plate is shown. The 33-s video shows spontaneous movement as well as how it responds to touch with a wire pick. Note the similarity to an *unc-31* null mutant (Video 4).

Table S1. Worm strains used in this study

Strain name	Genotype
NM2777	<i>aex-6(sa24); rab-3(js49)^a</i>
KG2045	<i>aex-6(sa24); rab-3(js49); cels56 [unc-129::ctns-1-RFP, unc-129::nlp-21-Venus]</i>
KG1666	<i>ceEx231 [unc-129::GFP-unc-108, unc-129::RFP-snb-1]</i>
KG1640	<i>cels56 [unc-129::ctns-1-RFP, unc-129::nlp-21-Venus]</i>
KG1641	<i>cels57 [unc-129::2XFYVE-RFP, unc-129::nlp-21-Venus]</i>
KG1645	<i>cels61 [unc-129::flp-3-Venus]</i>
KG1646	<i>cels62 [unc-129::ANF-Venus]</i>
KG1852	<i>cels72 [unc-129::ida-1-GFP]</i>
KG774	<i>egl-3(nr2090)^{b,c}</i>
KG1855	<i>egl-3(nr2090); cels56 [unc-129::ctns-1a-RFP, unc-129::nlp-21-Venus]</i>
KP2018	<i>egl-21(n476)^d</i>
JT9887	<i>goa-1(sa734)^e</i>
KG1395	<i>nuls183 [unc-129::nlp-21-Venus]^f</i>
KG1624	<i>nuls195 [unc-129::ins-22-Venus]^f</i>
KG862	<i>unc-31(e928)^{g,h}</i>
KG1279	<i>unc-108(ce363)ⁱ</i>
KG1284	<i>unc-108(ce363) goa-1(sa734)</i>
KG1567	<i>unc-108(ce363); nuls183 [unc-129::nlp-21-Venus]</i>
KG1281	<i>unc-108(ce365)^j</i>
KG1286	<i>unc-108(ce365) goa-1(sa734)</i>
KG1452	<i>unc-108(ce365); ceEx208 [rab-3::unc-108 cDNA]</i>
KG1502	<i>unc-108(ce365); ceEx210 [myo-3::unc-108 cDNA]</i>
KG1507	<i>unc-108(ce365); cels41 [rab-3::unc-108 cDNA]</i>
KG1510	<i>unc-108(ce365); cels43 [unc-108::unc-108 cDNA]</i>
KG1511	<i>unc-108(ce365); cels44 [unc-108::GFP]</i>
KG1655	<i>unc-108(ce365); cels61 [unc-129::flp-3-Venus]</i>
KG1656	<i>unc-108(ce365); cels62 [unc-129::ANF-Venus]</i>
KG1475	<i>unc-108(ce365); nuls183 [unc-129::nlp-21-Venus]</i>
KG1625	<i>unc-108(ce365); nuls195 [unc-129::ins-22-Venus]</i>
KG1420	<i>unc-108(ce365); unc-31(e928)</i>
KG1415	<i>unc-108(ce386)</i>
KG1815	<i>unc-108(ce473)</i>
KG1831	<i>unc-108(ce493)</i>
KG1900	<i>unc-108(nu415) [gift from D. Chun and J. Kaplan]^{i,k}</i>
KG1906	<i>unc-108(nu415); egl-3(nr2090)</i>
KG1811	<i>unc-108(nu415); cels56 [unc-129::ctns-1a-RFP, unc-129::nlp-21-Venus]</i>
KG2208	<i>unc-108(nu415); cels56 [unc-129::ctns-1a-RFP, unc-129::nlp-21-Venus]; ceEx282 [unc-129::unc-108 cDNA]</i>
KG1808	<i>unc-108(nu415); egl-3(nr2090); cels56 [unc-129::ctns-1-RFP, nlp-21-Venus]</i>
KG1812	<i>unc-108(nu415); cels57 [unc-129::2XFYVE-RFP, unc-129::nlp-21-Venus]</i>
KG1853	<i>unc-108(nu415); cels72 [unc-129::ida-1-GFP]</i>
KG1907	<i>unc-108(nu415); unc-31(e928)</i>

Strain names and genotypes of the non-wild strains in this study are listed alphanumerically by genotype. References are given for strains produced in previous studies.

^aMahoney et al., 2006.

^bLiu et al., 1999.

^cKass et al., 2001.

^dJacob and Kaplan, 2003.

^eRobatzek and Thomas, 2000.

^fSieburth et al., 2007.

^gCharlie et al., 2006.

^hSpeese et al., 2007.

ⁱMangahas et al., 2008.

^jMassachusetts General Hospital, Boston, MA.

^kChun et al., 2008.

Table S2. **Plasmids used in this study**

Plasmid name	Common name	Description or reference
KG#59	rab-3:: expression vector	Schade et al., 2005
KG#65	unc-17β:: expression vector	Charlie et al., 2006
KG#67	ttx-3::GFP	Gift from O. Hobert ^a
KG#68	rab-3::GFP	Williams et al., 2007
KG#135	unc-25:: expression vector	Used HindIII-MscI to cut out the ~2,400-bp <i>myo-3</i> promoter from pPD96.52, leaving the 3,700-bp vector fragment. To this vector fragment, we ligated the 1,322-bp HindIII-MscI <i>unc-25</i> promoter fragment cut from pSC325.
KG#146	unc-17β::GFP	Used AgeI-SpeI to cut out the ~1,000-bp AgeI-SpeI region from KG#65, leaving the 3,200-bp vector fragment containing the <i>unc-17β</i> promoter. To this vector fragment, we ligated the 1,800-bp AgeI-SpeI fragment cut from pPD94.81, which introduced the GFP (S65C) variant + <i>unc-54</i> 3' control region.
KG#150	unc-25::GFP	Used AgeI-SpeI to cut out the ~1,000-bp AgeI-SpeI region from KG#135, leaving the 4,000-bp vector fragment containing the <i>unc-25</i> promoter. To this vector fragment, we ligated the 1,800-bp AgeI-SpeI fragment cut from pPD94.81, which includes GFP (S65C) + the <i>unc-54</i> 3' control region.
KG#180 BL21(DE3)	GST-unc-47 [1–104]	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make <i>unc-47</i> (1–312 bp) cDNA. Used Accuprime Pfx and primers engineered with restriction sites to amplify and clone the cDNA fragment into BamHI-XhoI-cut pGex-KG (5.0 kb).
KG#208	rab-3:: expression vector with NotI site	Synthesized two complementary oligonucleotides containing XhoI and BglII sticky ends and a NotI site in between. Cloned into XhoI-BglII-cut KG#59.
KG#230	unc-129:: expression vector	Used Pfu Ultra polymerase and primers engineered with restriction sites to amplify the 2.6-kb <i>unc-129</i> promoter from N2 genomic DNA and clone into PstI-BamHI-cut pPD96.52. Promoter includes the region from the native Smal site (2.6 kb upstream of the start codon) down to the seventh nucleotide before the A of the ATG and is a subregion of the native <i>unc-129</i> promoter (Colavita et al., 1998) that drives expression in nine DA and DB ventral cord motor neurons.
KG#238	unc-17β::RFP	Used Pfu Ultra polymerase and primers engineered with restriction sites (and the 44-bp intron 3 from T07H6.4 on the 5' primer) to amplify the 0.7-kb mCherry coding region from mCherry plasmid and clone into AgeI-EcoRI-cut KG#146.
KG#240	unc-129::RFP expression vector	Used AgeI-ApaI to cut out the ~1,000 bp <i>unc-54</i> 3' control region from KG#230, leaving the 5.3-kb vector fragment containing the <i>unc-129</i> promoter. To this vector fragment, we ligated the 1,800-bp AgeI-ApaI fragment (containing RFP + <i>unc-54</i> 3' control region) cut from KG#238.
KG#244	unc-129:: expression vector with NotI site	Used PstI-BamHI to cut out the ~1,200-bp <i>rab-3</i> promoter from KG#208, leaving the 3,700-bp vector fragment. To this vector fragment, we ligated the 2,600-bp PstI-BamHI <i>unc-129</i> promoter fragment cut from KG#230.
KG#255	ttx-3::RFP	Used AgeI-ApaI to cut out the ~1,000-bp <i>unc-54</i> 3' control region from KG#67, leaving the vector fragment (unknown size) containing the <i>ttx-3</i> promoter. To this vector fragment, we ligated the 1,800-bp AgeI-ApaI fragment (containing RFP + <i>unc-54</i> 3' control region) cut from KG#238.
KG#303 BL21(DE3)RIL	GST-unc-108 [1–212]	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the <i>unc-108</i> cDNA 1–212 region followed by an artificial stop codon. Used Accuprime Pfx and primers engineered with restriction sites to amplify the cDNA and cloned it into XbaI-HindIII-cut pGex-KG (4.9 kb). Transformed into XL1-blue electrocompetent cells. Retransformed the DNA from this clone into the BL21(DE3)RIL bacterial expression host.
KG#317	rab-3::unc-108 cDNA	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the full-length <i>unc-108</i> cDNA (646 bp) from <i>C. elegans</i> mRNA. Then used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into NheI-KpnI-cut KG#59 (4.9 kb).
KG#318	rab-3::unc-108(Q65L) cDNA	Started with KG#317 (rab-3::unc-108 cDNA) and used Pfu Ultra and QuikChange site-directed mutagenesis to introduce the Q65L mutation. The mutation changes codon 65 of <i>unc-108</i> from CAA (Gln) to CTT (Leu; QESFRS to LESFRS). This mutation is equivalent to Ras Q61L and was shown to be a gain-of-function mutation for vertebrate Rab2 (Tisdale, 1999).
KG#323	myo-3::unc-108 cDNA	Used NheI-KpnI to cut out the 650-bp <i>unc-108</i> cDNA from KG#317 and cloned it into the like-digested <i>myo-3</i> :: expression vector pPD96-52 (6,100 bp).
KG#334	unc-108:: expression vector	Used Herculase II and primers engineered with restriction sites to amplify the 2,960-bp <i>unc-108</i> promoter from N2 genomic DNA and cloned it into PstI-SmaI-cut pPD96.52. The cloned segment includes the region from 6,668 to 9,627 bp on cosmid F53F10. This begins at the end of a noncoding RNA 3 kb upstream of the <i>unc-108</i> start codon and ends at the <i>unc-108</i> start codon after a 4-bp 5' UTR.
KG#335	unc-108::unc-108 cDNA	Used NheI-KpnI to cut out the 650-bp <i>unc-108</i> cDNA from KG#323 and clone into the like-digested <i>unc-108</i> :: promoter vector KG#334 (6,700 bp).

Table S2. **Plasmids used in this study** (Continued)

Plasmid name	Common name	Description or reference
KG#336	unc-108::GFP-unc-108 cDNA	Used Herculase II and primers engineered with an NheI restriction site to amplify the 0.8-kb GFP fragment (minus stop codon) from KG#146 unc-17β::GFP and cloned it into NheI-cut KG#335 unc-108::unc-108 cDNA (7.3 kb).
KG#337	unc-108::GFP	Used KpnI–ApaI to cut out the 1,000-bp unc-54 3' UTR from KG#334, leaving the 5.7-kb vector fragment containing the <i>unc-108</i> promoter. To this fragment, we ligated the like-digested 1,800-bp GFP + unc-54 3' UTR cut from pPD94.81 (4,700 bp).
KG#354	unc-25::ctns-1a–GFP	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to synthesize the full length <i>ctns-1a</i> cDNA (1.2 kb) from <i>C. elegans</i> mRNA. The cDNA lacked the stop codon for in-frame fusion to RFP. Used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into NheI–AgeI-cut KG#150 (5.8 kb).
KG#367	unc-129::GFP expression vector	Used KpnI–SpeI to cut out the ~1,000-bp unc-54 3' control region from KG#230, leaving the 5.3-kb vector fragment containing the <i>unc-129</i> promoter. To this vector fragment, we ligated the 1,800-bp KpnI–SpeI fragment (containing GFP + unc-54 3' control region) cut from pPD94.81.
KG#371	unc-129::ctns-1a–RFP cDNA	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 1.2-kb <i>ctns-1a</i> cDNA minus its stop codon and with reading frame adjusted for downstream RFP from KG#354 and cloned it into NheI–AgeI-cut KG#240 (7.2 kb).
KG#372	unc-129::2XFYVE-RFP	Used Herculase II and primers engineered with restriction sites to amplify the 2.6-kb <i>unc-129</i> promoter from KG#230 and cloned it into SphI–SalI-cut pH1 (SphI–SalI removes the <i>ced-1</i> promoter in pH1).
KG#374	unc-129::Venus expression vector	Used Herculase II and primers engineered with restriction sites to amplify the 1.7-kb Venus + unc-54 3' UTR region from KP#1383 and swap it out for the AgeI–SpeI-cut KG#230 unc-129:: expression vector (6.4 kb).
KG#375	unc-129::flp-3–Venus	Used Herculase II and primers engineered with restriction sites to amplify the 0.7-kb <i>flp-3</i> gene–coding region (minus its stop codon) from N2 genomic DNA and cloned it into NheI–AgeI-cut KG#374 such that the flp-3 reading frame was in frame with Venus.
KG#376	unc-129::ANF-Venus	Used Herculase II and primers engineered with restriction sites to amplify the 0.46-kb rat Pre-Pro-ANF cDNA (minus its stop codon) from EG3680 oxls206 (Speese et al., 2007) lysed worms and cloned it into NheI–AgeI-cut KG#374 unc-129::Venus such that the ANF reading frame was in frame with Venus.
KG#379	unc-129::GFP-unc-108 cDNA	Used Herculase II and primers engineered with restriction sites to amplify the 1.5-kb GFP–unc-108 cDNA from KG#336 unc-108::GFP–unc-108 cDNA and cloned it into NotI-cut KG#244 unc-129:: expression vector.
KG#434	unc-129::ida-1–GFP	Used Herculase II and primers engineered with restriction sites to amplify the 2.3-kb <i>ida-1</i> cDNA minus stop codon, with the reading frame adjusted for downstream GFP, from KG#141 (<i>unc-17β::ida-1</i> cloning intermediate produced by RT-PCR and sequence verified). Insert was cut with SpeI–AgeI and cloned into NheI–AgeI-cut KG#367.
KG#496	unc-129::unc-108 cDNA	Used NheI–KpnI to cut out the 650-bp unc-108 cDNA from KG#317 and cloned it into the like-digested KG#230.
KP#1283	unc-129::RFP–snb-1	Gift from J. Kaplan (Sieburth et al., 2005) ^b
KP#1383	unc-129::nlp-21–Venus	Gift from J. Kaplan (Sieburth et al., 2007) ^b
pGex-KG	Bacterial GST expression vector	Guan and Dixon, 1991
pIH1	<i>ced-1::2XFYVE-mRFP1</i>	Gift from Z. Zhou (Mangahas et al., 2008) ^c
pPD94.81	unc-54::GFP	Gift from A. Fire ^d
pPD96.52	<i>myo-3::</i> expression vector	Gift from A. Fire ^d
pPD118.20	<i>myo-3::</i> GFP	Gift from A. Fire ^d
pPD118.33	<i>myo-2::</i> GFP	Gift from A. Fire ^d
pSC325	unc-25:: promoter in pPD49.26	Gift from Y. Jin ^e

UTR, untranslated region. Accuprime Pfx was purchased from Invitrogen; Pfu Ultra polymerase, StrataScript Reverse Transcriptase, Herculase II polymerase, and QuikChange were purchased from Agilent Technologies.

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^bMassachusetts General Hospital, Boston, MA.

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Table S3. Transgenic arrays used in this study

Array name	Experimental contents	Marker
ceEx208	30 ng/μl KG#317 [rab-3::unc-108 cDNA]	15 ng/μl KG#67 [ttx-3::GFP] + 15 ng/μl KG#68 [rab-3::GFP]
ceEx210	10 ng/μl KG#323 [myo-3::unc-108 cDNA]	12 ng/μl pPD118.20 [myo-3::GFP]
ceEx231	5 ng/μl KG#379 [unc-129::GFP-unc-108] + 5 ng/μl KP#1283 [unc-129::RFP-snb-1]	15 ng/μl KG#255 [ttx-3::RFP]
ceEx282	30 ng/μl KG#496 [unc-129::unc-108 cDNA]	25 ng/μl KG#67 [ttx-3::GFP]
cels41	30 ng/μl KG#317 [rab-3::unc-108 cDNA]	15 ng/μl KG#67 [ttx-3::GFP] + 15 ng/μl KG#68 [rab-3::GFP]
cels43	4 ng/μl KG#335 [unc-108::unc-108 cDNA]	15 ng/μl KG#67 [ttx-3::GFP] + 15 ng/μl KG#68 [rab-3::GFP]
cels44	12 ng/μl KG#337 [unc-108::GFP]	None
cels54	5 ng/μl KG#358 [unc-129::CFP-rab-7]	15 ng/μl KG#255 [ttx-3::RFP] + 15 ng/μl KP#1383 [unc-129::nlp-21-Venus]
cels55	5 ng/μl KG#359 [unc-129::CFP-rab-11]	15 ng/μl KG#255 [ttx-3::RFP] + 15 ng/μl KP#1383 [unc-129::nlp-21-Venus]
cels56	5 ng/μl KG#371 [unc-129::ctns-1α-RFP]	15 ng/μl KG#255 [ttx-3::RFP] + 15 ng/μl KP#1383 [unc-129::nlp-21-Venus]
cels57	5 ng/μl KG#372 [unc-129::2XFYVE-RFP]	15 ng/μl KG#255 [ttx-3::RFP] + 15 ng/μl KP#1383 [unc-129::nlp-21-Venus]
cels59	5 ng/μl KG#357 [unc-129::YFP-rab-5]	15 ng/μl KG#255 [ttx-3::RFP]
cels61	15 ng/μl KG#375 [unc-129::flp-3-Venus]	15 ng/μl KG#255 [ttx-3::RFP] + 5 ng/μl KP#1283 [unc-129::RFP-snb-1]
cels62	15 ng/μl KG#376 [unc-129::ANF-Venus]	15 ng/μl KG#255 [ttx-3::RFP] + 5 ng/μl KP#1283 [unc-129::RFP-snb-1]
cels72	25 ng/μl KG#434 [unc-129::ida-1-GFP]	35 ng/μl KG#255 [ttx-3::RFP]

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