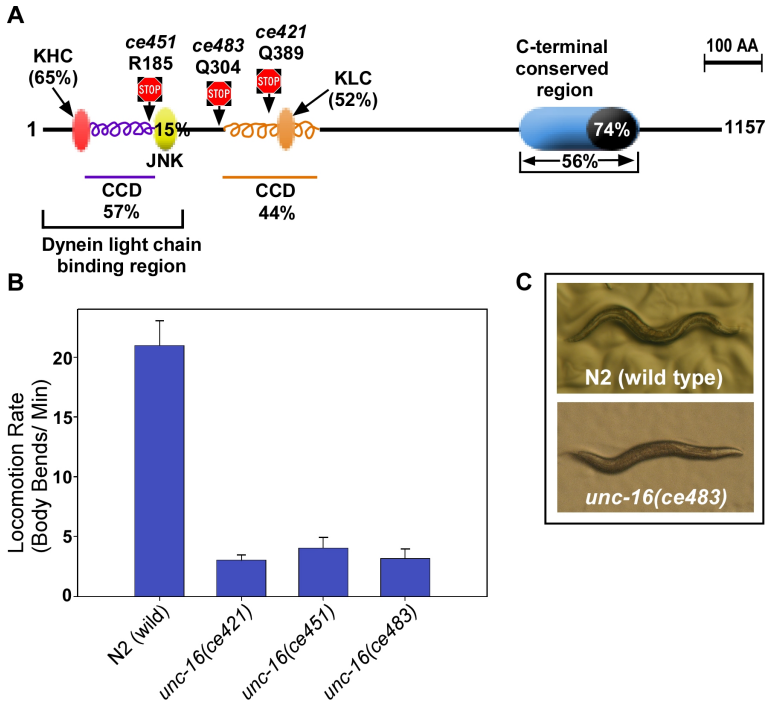

Figure S1 Three *goa-1(sa734)* suppressor mutations disrupt UNC-16, a conserved protein required for normal locomotion rates.

(A) Scale drawing of UNC-16 showing the location of nonsense mutations isolated in this study, conserved regions, and regions known to interact with other proteins from previous studies of UNC-16 and its JIP3 orthologs. Percentages refer to percent identities when comparing the indicated regions of UNC-16 (ZK1098.10b.1 from www.wormbase.org; freeze WS200; (HARRIS *et al.* 2009) to its human JIP3 ortholog (NP_003962). References for interaction sites are as follows: KHC (Kinesin Heavy Chain) (SUN *et al.* 2011); JNK (c-Jun N-terminal kinase) (KELKAR *et al.* 2000), KLC (Kinesin Light Chain) (KELKAR *et al.* 2005; NGUYEN *et al.* 2005; SAKAMOTO *et al.* 2005; VERHEY *et al.* 2001). The dynein light intermediate chain has been shown to interact with amino acids 1-240 of *C. elegans* UNC-16 (ARIMOTO *et al.* 2011). To compute percent identities we used Clustal W (THOMPSON *et al.* 1994) run through Vector NTI Advance 11.0 (InVitrogen). To predict coiled-coil domains (labeled CCD in figure) we used the COILS server on www.ch.embnet.org (LUPAS *et al.* 1991). The 100 AA scale bar indicates 100 amino acids.

(B) *unc-16* mutations confer sluggish locomotion. Graph shows spontaneous locomotion rates of N2 (wild type) compared to the indicated genotypes. Error bars are standard errors of the means (SEMs) of 10 animals each. The mean locomotion rates of the three *unc-16* mutants are not significantly different.

(C) Representative adult animals are shown for each genotype. Body length of wild type is ~1 mm, and both images have similar magnification.

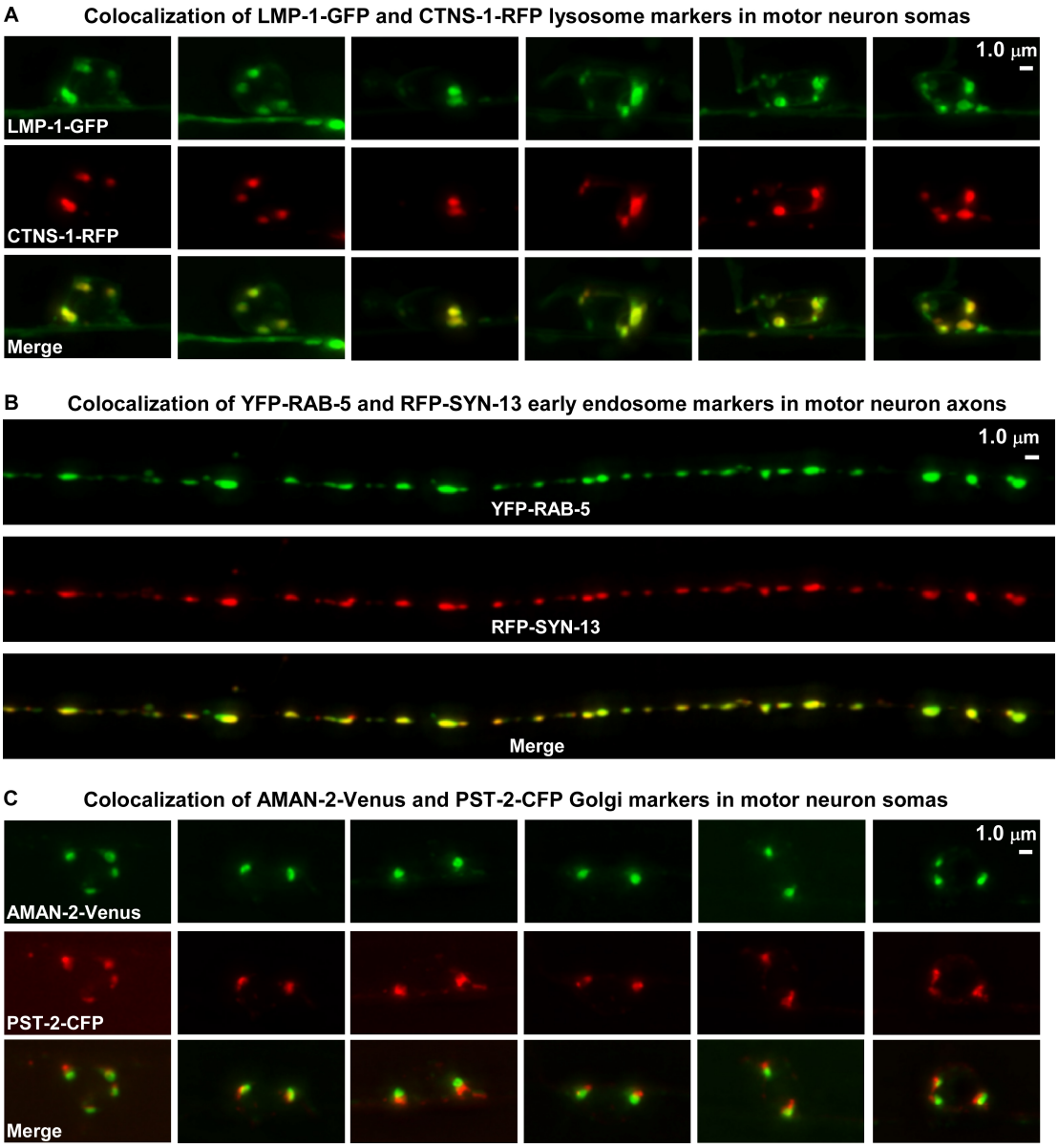

Figure S2 Demonstration of organelle marker specificity by co-localization of two organelle-specific markers.

(A) The lysosomal markers LMP-1-GFP and CTNS-1-RFP (mCherry) co-localize in motor neuron somas. Shown are six representative images collected from 6 different animals carrying both of the integrated transgenes *ceIs181* [unc-129::CTNS-1A-mCherry] and *ceIs192* [unc-129::LMP-1-GFP]. LMP-1 is the *C. elegans* ortholog of human Lysosome-Associated Membrane Protein/ CD68 (KOSTICH *et al.* 2000). CTNS-1 is the *C. elegans* ortholog of Cystinosin, which is a lysosome-specific Cysteine transporter (transports Cysteine from the lysosome lumen out to the cytosol to recycle it after lysosomal protein degradation) (KALATZIS *et al.* 2001). It has previously been shown to mark the lysosomes that are recruited to degrade cell corpses in *C. elegans* (KALATZIS *et al.* 2001; MANGAHAS *et al.* 2008).

(B) The early endosomal markers YFP-RAB-5 and RFP-SYN-13 co-localize in dorsal motor neuron axons. Shown is a representative image from a strain carrying the *ceEx346* [unc-129::RFP-SYN-13, unc-129::YFP-RAB-5] transgene.

(C) The Golgi markers AMAN-2-Venus and PST-2-CFP co-localize in motor neuron somas. Shown are six representative images collected from 6 different animals carrying both of the integrated transgenes *ceIs195* [unc-129::AMAN-2-Venus] and *ceIs185* [unc-129::PST-2A-CFP]. AMAN-2 is the *C. elegans* ortholog of a-Mannosidase II, which is known to localize to the Golgi in *C. elegans* and other animals (ORCI *et al.* 2000; ROLLS *et al.* 2002; SUMAKOVIC *et al.* 2009; VELASCO *et al.* 1993), PST-2 is a *C. elegans* ortholog of a Golgi-resident 3'-phosphoadenosine 5'-phosphosulfate (PAPS) transporter that is required for the production of extracellular sulfated molecules (DEJIMA *et al.* 2010). The two markers exhibit overlapping, but

slightly offset, patterns, suggesting that they localize to different compartments within the Golgi stacks. Unlike vertebrate cells that have a single juxtanuclear Golgi stack, most invertebrates neurons, including *C. elegans*, have 2-4 small Golgi "mini-stacks" (CHEN *et al.* 2006).

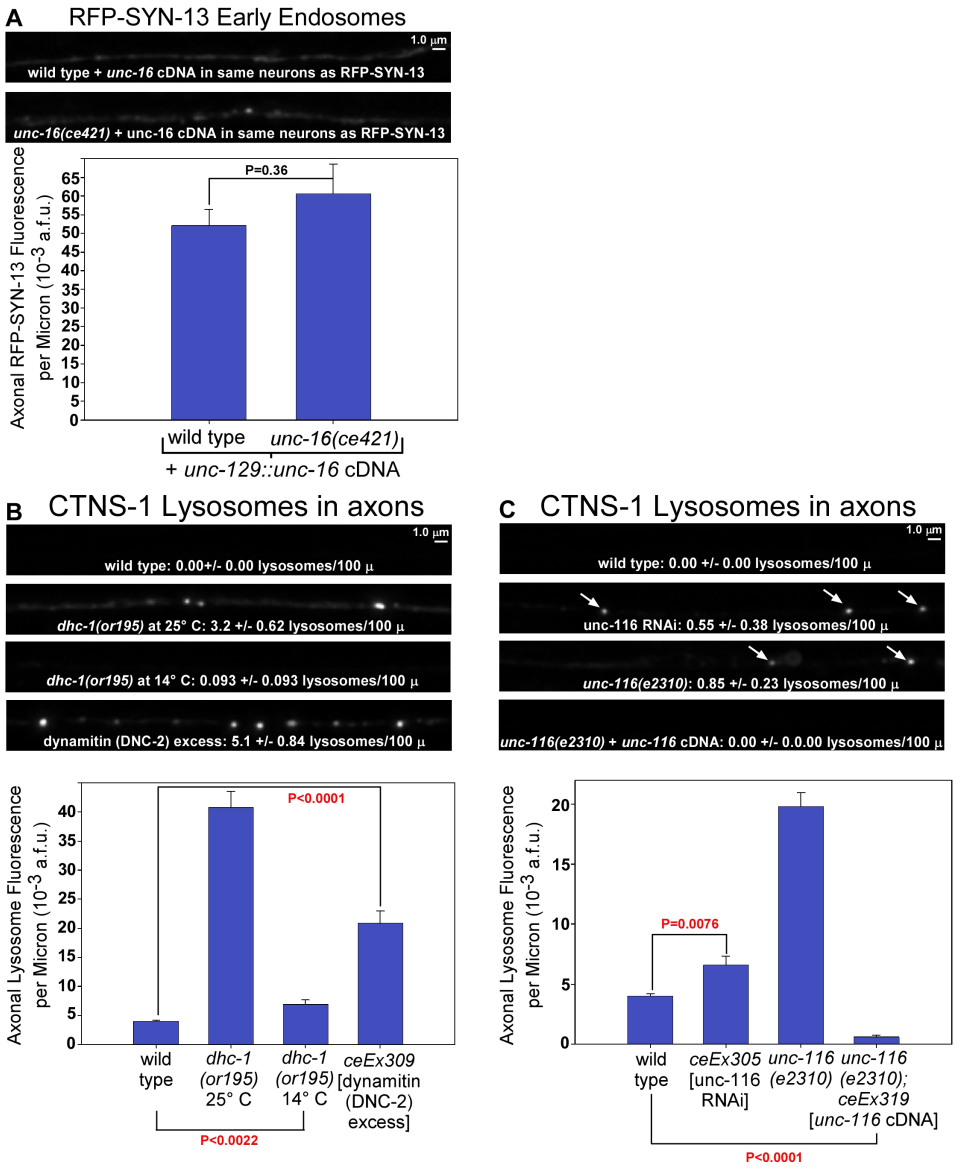

Figure S3 Impairing the function of UNC-16, DHC-1, or UNC-116 causes cell autonomous axonal organelle accumulation.

(A) Cell autonomous rescue of axonal early endosome accumulation in an *unc-16* null mutant. Representative images and quantification of RFP-SYN-13 early endosome fluorescence in a defined dorsal axon region of the indicated genotypes. Strains carry the genomically integrated transgene *ceIs77*. Identically-scaled representative images precede a graph showing the means and standard errors of the integrated fluorescence per micron of nerve cord length from images acquired from 14 animals each.

(B) – (C) Each set of panels consists of a group of identically-scaled representative images followed by a graph showing the mean integrated fluorescence per micron of nerve cord length from images acquired from 9 (*unc-116(e2310); ceEx319*) or 14 (all others) animals each. Error bars are standard errors of the mean. All strains carry the genomically integrated transgene *ceIs56* (to visualize lysosomes) in addition to the other indicated mutations or transgenes. Arrows in some panels indicate lysosomal puncta (also visible but not indicated in other images). The text data on each image states the mean number of lysosomes/ 100 mm +/- standard errors of the mean, counted by defining a threshold level (see Methods).

(B) Reducing the function of DHC-1 causes cell autonomous axonal organelle accumulation. The *ceEx309* transgene overexpresses DNC-2 (dynamitin), which transgenically reduces the function of native DNC-1 (see main text for references).

(C) Reducing the function of UNC-116 causes cell autonomous axonal organelle accumulation. The *ceEx305* transgene expresses sense and antisense *unc-116* RNAs from the same promoter used for the lysosomal marker. The *ceEx319* transgene expresses the wild type *unc-116* cDNA from the same promoter used for the lysosomal marker.

Files S1-S5

Supporting Movies

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.147348/-/DC1.

File S1. Lysosomes in wild type commissures tend to move back into the cell soma.

This movie contains four clips, each showing a different wild type cell soma with its axon initial segment. These are green due to expression of soluble GFP. Lysosomes tagged with CTNS-1-mCherry are red. We collected red images at 2 second intervals and green images every 10th frame. Playback is sped up 20-fold. The animal's genotype is *ceIs134* [unc-17b::CTNS-1A-mCherry, -GFP].

File S2. Lysosomes in *unc-16* mutant commissures tend to escape the axon initial segment and move deeper into the commissure.

This movie contains five clips, each showing a different *unc-16* mutant cell soma with its axon initial segment. These are green due to expression of soluble GFP. Lysosomes tagged with CTNS-1-mCherry are red. A 6th clip is a repeat of the 5th clip shown in monochrome to better view the escape of the lysosome from the cell soma. We collected red images at 2 second intervals and green images every 10th frame. Playback is sped up 20-fold. The animal's genotype is *unc-16(ce483); ceIs134* [unc-17b::CTNS-1A-mCherry, -GFP].

File S3. Rapid bi-directional processive transport of lysosomes in *unc-16* null mutant commissures.

This movie shows the central region of a DA-type cholinergic motor neuron commissure in a young adult animal. The cell soma and main axon of the neuron are not visible. Lysosomes are tagged with CTNS-1-GFP. To simultaneously image this large region of the commissure across a large focal depth we collected a 16-plane z-stack at each time point (50 mSec exposure times) and produced maximum intensity projections. The movie contains 10 frames collected at 60 sec intervals for a total of 10 min in real time but has been sped up 60-fold. The animal's genotype is *unc-16(ce483); ceIs83* [unc-17::CTNS-1A-GFP].

File S4. Lysosomes in the synaptic region of *unc-16* null mutants are much less active than those in the commissures.

This movie shows the intersection of a commissure of a DB-type cholinergic motor neuron with its main axon tract in the dorsal cord of a young adult animal. The cell soma of the neuron is on the other side of the animal (not visible). Lysosomes are tagged with CTNS-1-GFP. To simultaneously image this large region of the commissure and dorsal cord across a large focal depth we collected a 16-plane z-stack at each time point (50 mSec exposure times) and produced maximum intensity projections. The movie contains 15 frames collected at 60 sec intervals for a total of 15 min in real time but has been sped up 60-fold. Note the many inactive puncta in the main axon tracts of the dorsal cord compared with the bi-directionally active lysosomal puncta in the commissure. The animal's genotype is *unc-16(ce483); ceIs83* [unc-17::CTNS-1A-GFP].

File S5. Active transport of Lysosomes in *unc-16* null mutant commissures leads to their deposition in the main axon.

This movie contains two clips. Each shows the intersection of cholinergic motor neuron commissures with their main axon tract in the dorsal cord. The cell somas are on the other side of the animal (not visible). Lysosomes are tagged with CTNS-1-GFP. The first clip shows a CTNS-1-GFP punctum leaving the commissure and entering the main axon in the dorsal cord where it joins other lysosomes. The movie contains 190 frames (25 mSec exposure times) collected at 2 sec intervals for a total of 6 min 20 sec in real time but has been sped up 20-fold. The animal's genotype is *unc-16(ce483); ceIs113* [unc-129::CTNS-1A-GFP]. The second clip shows the intersection of two commissures with the dorsal cord. The lower commissure contains an elongated lysosome. The upper commissure shows a spherical lysosome that appears to move in and out of the main axon. Three bright lysosomes that have been previously deposited in the axon appear inactive. The movie contains 80 frames (50 mSec exposure time) collected at 2 sec intervals for a total of 2 min 40 sec in real time but has been sped up 10-fold. The animal's genotype is *unc-16(ce483); ceIs56* [unc-129::CTNS-1A-mCherry].

File S6

Strains, Plasmids, Transgenes, and Supporting References

C. elegans strains (strain names and complete genotypes; alpha-numerically listed by genotype), DNAs (plasmids and PCR products along with basic construction details, listed alpha-numerically by plasmid name), and transgenes.

C. elegans Strains

Strain name	Genotype
KG1640	*ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus] (EDWARDS *et al.* 2009)
KG2664	*ceIs56; ceEx305* [unc-129::unc-116 sas RNAi]
KG2475	*ceIs56; ceEx309* [unc-129::dnc-2 gene]
KG1643	ceIs59 [unc-129::YFP-rab-5]
KG1834	*ceIs70* [unc-129::aman-2-Venus, RFP-syn-13]
KG2052	ceIs77 [unc-129::unc-16, RFP-syn-13]
KG2126	*ceIs79* [unc-129::GFP-pisy-1]
KG2178	*ceIs82* [unc-129::GFP-tram-1]
KG2443	*ceIs83* [unc-17::CTNS-1-GFP, RFP-RAB-3]
KG2882	*ceIs123* [unc-129::GFP]
KG2998	*ceIs134* [unc-17b::CTNS-1-mCherry, -GFP]
KG4125	*ceIs195* [unc-129::AMAN-2-Venus]
EU828	*dhc-1(or195)* (HAMILL *et al.* 2002; KOUSHIKA *et al.* 2004) (*Caenorhabditis* Genetics Center)
KG1829	*dhc-1(or195); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG2463	*dhc-1(or195); unc-16(ce421); ceIs56* [unc-129::CTNS-1-RFP, unc-129::NLP-21-Venus]
JT9887	*goa-1(sa734)* (ROBATZEK and THOMAS 2000)
KG2164	*jnk-1(gk7); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG1746	*unc-16(ce421)*
KG1788	*unc-16(ce421); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG1821	*unc-16(ce421); ceIs59* [unc-129::YFP-rab-5]
KG1836	*unc-16(ce421); ceIs70* [unc-129::aman-2-Venus, RFP-syn-13]
KG2053	*unc-16(ce421); ceIs77* [unc-129::unc-16, RFP-syn-13]
KG2127	*unc-16(ce421); ceIs79* [unc-129::GFP-pisy-1]
KG2179	*unc-16(ce421); ceIs82* [unc-129::GFP-tram-1]
KG2165	*unc-16(ce421); jnk-1(gk7); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG1758	*unc-16(ce451)*
KG1943	*unc-16(ce451); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG2338	*unc-16(ce483)*
KG2692	*unc-16(ce483); ceEx346* [unc-129::RFP-SYN-13, unc-129::YFP-RAB-5]
KG2101	*unc-16(ce483); ceIs56* [unc-129::CTNS-1-RFP, unc-129::NLP-21-Venus]
KG2444	*unc-16(ce483); ceIs83* [unc-17::CTNS-1-GFP, RFP-RAB-3]
KG4109	*unc-16(ce483); ceIs123* [unc-129::GFP]
KG3035	*unc-16(ce483); ceIs134* [unc-17b::CTNS-1-mCherry, -GFP]
KG4179	*unc-16(ce483)/+; ceIs181* [unc-129::CTNS-1A-mCherry]/+*; ceIs192* [unc-129::LMP-1-GFP]/+
KG4183	*unc-16(ce483)/+; ceIs185* [unc-129::PST-2A-CFP]/+*; ceIs195* [unc-129::AMAN-2-Venus]/+
KG4152	*unc-16(ce483); ceIs195* [unc-129::AMAN-2-Venus]
FF41	*unc-116(e2310)* (PATEL *et al.* 1993) (also known as *e2281*)
KG1942	*unc-116(e2310); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG1944	*unc-116(e2310) unc-16(ce421); ceIs56* [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]
KG2486	*unc-116(e2310); ceIs56; ceEx319* [unc-129::unc-116 cDNA]

Plasmids and PCR Products

DNA Name	Common Name	Description or reference
KG#59	rab-3:: expression vector	(SCHADE *et al.* 2005)
KG#65	unc-17b:: expression vector	(CHARLIE *et al.* 2006)
KG#67	ttx-3::GFP	Gift of Oliver Hobert, Columbia University
KG#79	unc-17b::SNB-1-GFP	Used Pfu Ultra polymerase and primers engineered with restriction sites to amplify the 2286 bp snb-1-GFP region from pSB120.65 and cloned into Nhe I/ Apa I cut KG#65
KG#94	unc-17:: expression vector	(Edwards et al., 2008)
KG#100	unc-17b::SNB-1-GFP	Used Age I/ Apa I to cut out the ~1800 bp GFP/ 3' control region from KG#79, leaving the 3600 bp vector fragment containing the unc-17b promoter. To this vector fragment, we ligated the 1800 bp Age I/ Apa I fragment cut from pPD94.81, which contains the S65C GFP variant.
KG#130	unc-17b::SNT-1	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the full length snt-1 cDNA minus the stop codon (~1.4 Kb) from *C. elegans* mRNA. Used Accuprime Pfx and primers engineered with restriction sites to amplify and clone into Nhe I/ Kpn I cut KG#65 (4.2 Kb).
KG#146	unc-17b:: -GFP	(EDWARDS *et al.* 2009)
KG#148	unc-17b::SNT-1-CFP	Used Kpn I/ Spe I to cut out the ~1000 bp 3' control region from KG#130, leaving the 4600 bp vector fragment containing the unc-17beta promoter hooked to the 1.4 kb snt-1 stop codon-less cDNA. To this vector fragment, we will ligate the 1800 bp Kpn I/ Spe I fragment cut from pPD136.61, which contains CFP.
KG#150	unc-25::GFP	(EDWARDS *et al.* 2009)
KG#230	unc-129:: expression vector	(EDWARDS *et al.* 2009)
KG#238	unc-17b::___-RFP	(EDWARDS *et al.* 2009)
KG#240	unc-129::___-RFP expression vector	(EDWARDS *et al.* 2009)
KG#244	unc-129:: expression vector with Not I site	(EDWARDS *et al.* 2009)
KG#253	unc-129::___-CFP	Used Age I/ Apa I to cut out the ~1000 bp unc-54 3' control region from KG#230, leaving the 5.4 kb vector fragment containing the unc-129 promoter. To this vector fragment, we ligated the 1800 bp Age I/ Apa I fragment (containing CFP + the unc-54 3' control region) cut from pPD136.61.
KG#255	ttx-3::RFP	(EDWARDS *et al.* 2009)
KG#344	acr-2:: expression vector	Used Herculase II and primers engineered with restriction sites to amplify the 3.2 Kb acr-2 promoter from KP#704 and cloned it into Pst I/ Bam HI cut pPD96.52 (6.1 Kb).
KG#353	acr-2::___-GFP	Used Age I/ Spe I to cut out the 1000 bp unc-54 3' UTR from KG#344 leaving the 6.1 Kb vector fragment containing the acr-2 promoter. To this fragment, we ligated the like-digested 1800 bp GFP + unc-54 3' UTR cut from pPD94.81.
KG#354	unc-25::CTNS-1A-GFP	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the full length ctns-1a cDNA (1.2 Kb) lacking a stop codon from *C. elegans* mRNA for in-frame fusion to GFP. Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify and clone the cDNA into Nhe I/ Age I cut KG#150 (5.8 Kb).
KG#355	acr-2::CTNS-1a-GFP	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the full length ctns-1a cDNA (1.2 Kb) lacking a stop codon from *C. elegans* mRNA for in-frame fusion to GFP. We then used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Age I cut KG#353 (7.9 Kb).
KG#357	unc-129::YFP-RAB-5	Used Herculase II and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1430 bp YFP-rab-5 insert from pCZ677 unc-25::YFP-rab-5 and clone it into Not I cut KG#244 unc-129:: (6.4 Kb). Analyzed 6 clones to identify those with the correct insert size and use PCR with vector and GFP primers to identify clones with correctly oriented insert.
KG#358	unc-129::CFP-RAB-7	Used Herculase II and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1430 bp CFP-rab-7 insert from pCZGY168 unc-25::CFP-rab-7 and cloned it into Not I cut KG#244 unc-129:: (6.4 Kb). Analyzed 8 clones to identify those with the correct insert size and used PCR

		with vector and GFP primers to identify clones with correctly oriented insert.
KG#359	unc-129::CFP-RAB-11	Used Herculase II and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1430 bp CFP-rab-11 insert from pCZGY171 unc-25::CFP-rab-11 and cloned it into Not I cut KG#244 unc-129:: (6.4 Kb). Analyzed 8 clones to identify those with the correct insert size and used PCR with vector and GFP primers to identify clones with correctly oriented insert.
KG#367	unc-129::___-GFP	Used Kpn I/ Spe I to cut out the ~1000 bp unc-54 3' control region from KG#230, leaving the 5.3 kb vector fragment containing the unc-129 promoter. To this vector fragment, we ligated the 1800 bp Kpn I/ Spe I fragment (containing GFP + unc-54 3' control region) cut from pPD94.81.
KG#369	unc-129::CTNS-1A-GFP	Used Nhe I/ Age I to cut out the 1.2 Kb ctns-1a cDNA from KG#354 and cloned it into the like-digested unc-129::___-GFP vector KG#367.
KG#371	unc-129::CTNS-1A-RFP cDNA	(EDWARDS *et al.* 2009)
KG#374	unc-129::Venus expression vector	(EDWARDS *et al.* 2009)
KG#411	unc-129::SYN-13-RFP	Used StrataScript Reverse Transcriptase and a primer engineered with a restriction site to make the full length syn-13 cDNA (0.75 Kb) lacking a stop codon from *C. elegans* mRNA for in-frame fusion to RFP. We used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Age I cut KG#240 (7.2 Kb).
KG#413	unc-129::SYN-13	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 0.75 Kb syn-13 cDNA (adding a stop codon at the end) from KG#411 and cloned it into Nhe I/ Age I cut KG#230 (6.4 Kb).
KG#414	unc-129::RFP-SYN-13	Used Herculase II and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 0.8 Kb RFP fragment (minus stop codon) from KG#238 unc-17 ::RFP and cloned it into Nhe I cut KG#413 unc-129::syn-13 cDNA (7.2 Kb). Used PCR and restriction digests to identify clones with the correct RFP orientation.
KG#429	unc-129::AMAN-2-Venus	Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the first 82 codons of the aman-2 gene (301 bp = all of first exon, the 55 bp first intron, and some of second exon, with reading frame adjusted for downstream Venus) from N2 genomic DNA and cloned it into Nhe I/ Age I cut KG#374 unc-129::____-Venus (7.2 Kb).
KG#432	unc-17::CTNS-1-GFP	Used Nhe I/ Apa I to cut out the ~1000 bp unc-54 3' control region from KG#94, leaving the 5.9 kb vector fragment containing the unc-17 promoter. To this fragment, we ligated the 3.05 Kb Nhe I/ Apa I fragment (containing CTNS-1-GFP + unc-54 3' control region) cut from KG#355.
KG#445	unc-129::UNC-16 cDNA	Used AffinityScript Reverse Transcriptase and a primer engineered with a restriction site to make the full length unc-16 cDNA (3471 bp) from *C. elegans* mRNA. We then used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Kpn I cut KG#230 (6.4 Kb).
KG#468	unc-129::UNC-16 cDNA	Used Use Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify and clone the 3.5 Kb unc-16 cDNA from KG#445 unc-129::unc-16 cDNA into Nhe I/ Kpn I cut KG#230 (6.4 Kb).
KG#469	rab-3::UNC-16 cDNA	Use Nhe I/ Kpn I to cut out the 3.5 Kb unc-16 cDNA from KG#468 and clone into the like-digested rab-3:: vector KG#59 (4900 bp).
KG#470	rab-3:: expression vector with Sbf I site	Synthesized 2 complementary oligos containing Nhe I and Age I sticky ends and Sbf I and Kpn I sites in between. After hybridization, we cloned it into Nhe I/ Age I cut KG#59.
KG#471	mig-13::expression vector	Used BamH I/ Apa I to cut out the ~825 bp mult-cloning site + unc-54 3' control region from KS#1 mig-13:: expression vector, leaving the 6.1 kb vector fragment containing the mig-13:: promoter. To this vector fragment, we ligated the 1000 bp BamH I/ Apa I fragment from KG#470.
KG#472	rab-3:: expression vector with Fse I site	Synthesized 2 complementary oligos containing Nhe I and Age I sticky ends and Fse I and Kpn I sites in between. After hybridization, we cloned it into Nhe I/ Age I cut KG#59 (rab-3::).
KG#476	mig-13::___-RFP expression vector	Used Kpn I/ Apa I to cut out the ~1000 bp unc-54 3' control region from KG#483, leaving the 6.0 kb vector fragment containing the mig-13 promoter. To this vector fragment, we ligate the 1800 bp Kpn I/ Apa I fragment (containing RFP + unc-54 3' control region) cut from KG#240.
KG#477	mig-13::GFP-___ expression vector	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 800 bp GFP (S65C version with 3 introns; minus stop codon) from pPD94.81 and cloned it into Asc I/ Sbf I cut KG#471 mig-13::____ (7.0 Kb).

KG#483	mig-13:: expression vector	Used BamH I/ Apa I to cut out the ~825 bp multi-cloning site + unc-54 3' control region from KS#1 mig-13, leaving the 6.1 kb vector fragment containing the mig-13:: promoter. To this vector fragment, we ligated the 1000 bp BamH I/ Apa I fragment from KG#472.
KG#484	unc-129::PISY-1 gene	Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1042 bp pisy-1 gene coding region from N2 genomic DNA and cloned it into Nhe I/ Age I cut KG#230 (6.4 Kb).
KG#485	unc-129::TRAM-1 gene	Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1470 bp tram-1 gene coding region from N2 genomic DNA and cloned it into Nhe I/ Age I cut KG#230 (6.4 Kb).
KG#486	unc-129::GFP-PISY-1 gene	Used Herculase II and primers engineered with restriction sites to amplify the 0.8 Kb GFP fragment (minus stop codon) from KG#146 and cloned it into Nhe I cut KG#484 (7.5 Kb).
KG#488	mig-13::CTNS-1A-RFP	Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the stop-codon-less 1215 bp ctns-1 cDNA from KG#371 and cloned it into Fse I/ Kpn I cut KG#476 (7.8 Kb).
KG#490	mig-13::GFP-RAB-5 gene	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 1.3 Kb rab-5 gene coding region from N2 genomic DNA and cloned it into Sbf I/ Kpn I cut KG#477 (7.8 Kb).
KG#491	unc-17::RFPA-RAB-3	Used Herculase II and primers engineered with restriction sites to amplify the 1.5 Kb RFP-rab-3 fragment from KS#3 mig-13::RFP-rab-3 and cloned it into Sbf I cut KG#493 unc-17:: expression vector (6.4 Kb). Used PCR (between RFP and vector) to identify a clone with the correct orientation.
KG#493	unc-17:: expression vector	Synthesized 2 complementary oligos such that hybridization produces a double stranded fragment containing Nhe I and Age I sticky ends and Sbf I and Kpn I sites in between. We then cloned this fragment into Nhe I/ Age I cut KG#94 and tested for the Sbf I site by cutting with Sbf I.
KG#494	unc-129::GFP-TRAM-1 gene	Used Herculase II and primers engineered with restriction sites to amplify the 0.8 Kb GFP fragment (minus stop codon) from KG#146 and cloned it into Nhe I cut KG#485 unc-129::tram-1 gene (7.9 Kb).
KG#546	unc-129::DNC-2 gene	Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify the 1204 bp dnc-2 gene from N2 genomic DNA and clone it into Nhe I/ Age I cut KG#230 (6.4 Kb).
KG#551	unc-129::UNC-116 cDNA	Used AffinityScript Multiple Temperature Reverse Transcriptase and a primer engineered with a restriction site to make the full length unc-116 cDNA (2448 bp) from *C. elegans* mRNA. Used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify and clone the cDNA into Nhe I/ Age I cut KG#230 (6.4 Kb).
KG#571	unc-129::LMP-1-GFP	Used AffinityScript Multiple Temperature Reverse Transcriptase and a primer engineered with a restriction site to make the full length *lmp-1* cDNA minus its stop codon (714 bp) from *C. elegans* mRNA. We then used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Age I cut KG#367 (7.2 Kb).
KG#611	unc-129::PST-2A-CFP	Used AffinityScript Multiple Temperature Reverse Transcriptase and a primer engineered with a restriction site to make the full length *pst-1* cDNA minus its stop codon (1.1 Kb; 1095 bp). Then used Herculase II polymerase and primers engineered with restriction sites and the ribosome binding site AAAA to amplify and clone the cDNA into Nhe I/ Age I cut KG#253 unc-129::___-CFP.
KG#645	unc-17b::CTNS-1A-RFP	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 0.5 Kb unc-17b promoter from KG#65. We cut this insert with Hind III/ Bam HI and cloned it into like-digested KG#371 unc-129::CTNS-1-RFP (removing the 2.4 Kb unc-129:: promoter in the process).
KG#657	bacterial tac promoter::MBP-UNC-16[1-462] cDNA	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 1.4 Kb unc-16 [AA 1-462] cDNA from KG#469 (rab-3::unc-16 cDNA) and cloned it into Bam HI/ Sal I cut pMALc2H10T (6.7 Kb).
KGsa7	unc-129::unc-116 sas genomic	Used Expand 20 kb+ to fuse the ~2.6 kb unc-129 promoter with a 2 Kb unc-116 genomic – rich region in forward and reverse orientations to allow sense and anti-sense RNAs to be formed in motor neurons for RNAi experiments. Combined the 2 sas products in equimolar concentrations.
KP#704	acr-2::GFP-SNB-1	Gift of Joshua Kaplan, Massachusetts General Hospital/ Harvard University (Jacob and Kaplan, 2003)
KP#1383	unc-129::NLP-21-Venus	Gift of Joshua Kaplan and Derek Sieburth, Massachusetts General Hospital/ Harvard University (Sieburth *et al.* 2007)
KS#1	mig-13:: expression vector	Gift of Kang Shen, Stanford University (Klassen and Shen 2007)
KS#3	mig-13::RFP-RAB-3	Gift of Kang Shen, Stanford University (Klassen and Shen 2007)
pCZ677	unc-25::YFP-RAB-5	Gift of Yish Jin, University of California San Diego (Grill *et al.* 2007)

pCZGY168	unc-25::CFP-RAB-7	Gift of Yish Jin, University of California San Diego (GRILL *et al.* 2007)
pCZGY171	unc-25::CFP-RAB-11	Gift of Yish Jin, University of California San Diego (GRILL *et al.* 2007)
pPD94.81	unc-54::GFP	Gift of Andrew Fire, Stanford University
pPD96.52	myo-3:: expression vector	Gift of Andrew Fire, Stanford University
pPD136.61	myo-3::CFP	Gift of Andrew Fire, Stanford University
pMalC2H10T	Maltose-binding protein bacterial expression vector	Gift of John Tesmer, University of Michigan
pRK793	His6-TEV [S219V] protease	Addgene
pSB120.65	snb-1::SNB-1 gene-GFP	Gift of Michael Nonet, Washington University
RM#605p	unc-17b::GFP	Gift of Jim Rand, Oklahoma Medical Research Foundation (CHARLIE *et al.* 2006)

Transgenic Arrays

Array name	Experimental contents	Relevant organelles labeled and references	Co-transformation marker(s)
ceEx305	KGsa7 [unc-129::UNC-116 sas RNAi fusion PCR product] (80 ng/ ml total; 40 ng/ ml each orientation)	N/A	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceEx309	KG#546 [unc-129::DNC-2 gene] (10 ng/ ml)	N/A	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceEx319	KG#551 [unc-129::UNC-116 cDNA] (25 ng/ ml)	N/A	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceEx346	KG#414 [unc-129::RFP-SYN-13] (5 ng/ ml) KG#357 [unc-129::YFP-RAB-5] (5 ng/ ml)	Early/ recycling endosomes (CHUN *et al.* 2008; PREKERIS *et al.* 1998) Early endosomes (GRILL *et al.* 2007; GROSSHANS *et al.* 2006; MAXFIELD and MCGRAW 2004)	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceIs56	KG#371 [unc-129::CTNS-1a-RFP] (5 ng/ ml)	Lysosomes (KALATZIS *et al.* 2001; MANGAHAS *et al.* 2008); this study	KG#255 [ttx-3::RFP] (15 ng/ ml) KP#1383 unc-129::NLP-21-Venus (15 ng/ ml)
ceIs59	KG#357 [unc-129::YFP-RAB-5] (5 ng/ ml)	Early endosomes (GRILL *et al.* 2007; GROSSHANS *et al.* 2006; MAXFIELD and MCGRAW 2004)	KG#255 [ttx-3::RFP] (15 ng/ ml)
ceIs70	KG#429 [unc-129::AMAN-2-Venus] (7 ng/ ml) KG#414 [unc-129::RFP-SYN-13] (5 ng/ ml)	Golgi (ORCI *et al.* 2000; ROLLS *et al.* 2002; SUMAKOVIC *et al.* 2009; VELASCO *et al.* 1993); this study Early/ recycling endosomes (CHUN *et al.* 2008; PREKERIS *et al.* 1998)	KG#255 [ttx-3::RFP] (35 ng/ ml)
ceIs77	KG#468 [unc-129::UNC-16 cDNA] (35 ng/ ml) KG#414 [unc-129::RFP-SYN-13] (5 ng/ ml)	N/A Early/ recycling endosomes (CHUN *et al.* 2008; PREKERIS *et al.* 1998)	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceIs79	KG#486 [unc-129::GFP-PISY-1] (4 ng/ ml) KG#240 [unc-129::RFP] (5 ng/ ml)	Regular/ smooth ER (ROLLS *et al.* 2002) RFP – soluble cytoplasmic protein	KG#255 [ttx-3::RFP] (25 ng/ ml)
ceIs82	KG#494 [unc-129::GFP-TRAM-1] (5 ng/ ml) KG#240 [unc-129::RFP] (5 ng/ ml)	Rough ER (ROLLS *et al.* 2002) RFP – soluble cytoplasmic protein	KG#255 [ttx-3::RFP] (25 ng/ ml)
ceIs83	KG#432 [unc-17::CTNS-1-GFP] (5 ng/ ml) KG#491 [unc-17::RFPA-RAB-3] (5 ng/ ml)	Lysosomes (KALATZIS *et al.* 2001; MANGAHAS *et al.* 2008); this study	KG#255 [ttx-3::RFP] (25 ng/ ml)
ceIs123	KG#367 [unc-129::GFP] (5 ng/ ml)	N/A	KG#255 [ttx-3::RFP] (25 ng/ ml)
ceIs134	KG#645 [unc-17b::CTNS-1A-RFP] (3.5 ng/ ml)	Lysosomes (KALATZIS *et al.* 2001; MANGAHAS *et al.* 2008); this study	RM#605p [unc-17b::GFP] (10 ng/ ml)
ceIs181	KG#371 [unc-129::CTNS-1a-RFP] (5 ng/ ml)	Lysosomes (KALATZIS *et al.* 2001; MANGAHAS *et al.* 2008); this study	KG#255 [ttx-3::RFP] (25 ng/ ml)
ceIs185	KG#611 [unc-129::PST-2A-CFP] (5 ng/ ml)	Golgi (DEJIMA *et al.* 2010); this study	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceIs192	KG#571 [unc-129::LMP-1-GFP] (3 ng/ ml)	Lyosomes (Kostich et al., 2000); this study	KG#67 [ttx-3::GFP] (25 ng/ ml)
ceIs195	KG#429 [unc-129::AMAN-2-Venus] (1 ng/ ml)	Golgi (ORCI *et al.* 2000; ROLLS *et al.* 2002; SUMAKOVIC *et al.* 2009; VELASCO *et al.* 1993); this study	KG#255 [ttx-3::RFP] (25 ng/ ml)

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