Supplemental Materials and Methods

Quantitative fluorescence imaging of live animals and image analysis

Growth of Strains: For strains with wild type growth, we plated 12 L2-stage larvae on each of 5 locomotion plates and grew them 1 d at 14° + 5 d at 20° (for growth and imaging at 20°) to produce next generation young adult progeny. Growth times and plating numbers were modified after growth tests for slow growing or lower fertility strains, such as strains containing *unc-104(ce782)* or *nud-2(ok949)*. ~55 - 65 young adults were selected and transferred to an unseeded plate immediately prior to mounting as described below.

Agarose pad slide production: We cleaned the glass slides before applying the agarose pad by placing sets of slides in slide staining dishes and shaking them for 30 min in dilute dishwasher soap followed by 1 min rinses in tap water and house distilled water, and 5 min rinses in 70% EtOH + 1% HCl and house distilled water followed by drying in a 60° oven. Cleaned slides were stored between layers of Kimwipes in a plastic container. We produced ~18-19 mm diameter 2% agarose pads using the method of (Sulston and Hodgkin 1988), with the agarose made in M9 buffer. We produced agarose pad slides in batches and stored them with the pads covered with a 24 x 30 mm coverslip to keep the surface smooth for up to 3-4 weeks in humidified containers at 4°.

Mounting animals on agarose pad slides: We transferred the pre-picked animals into a 30 μl drop of 30 mg/ ml BDM (2, 3-Butanedione monoxime; Sigma B0753) in M9 buffer in one pick-full and incubated them for 10 min on a water-moistened 1.5 cm square pad of folded Kimwipe tissue under a Petri plate lid. After the incubation, we removed ~27 μl of the solution using a P20 microinjection tip (Eppendorf 5242 956.003), leaving the worms behind in the remaining anesthetic and inverted the coverslip onto a ~18-19 mm diameter 2% agarose pad that had been dried without its protective coverslip for the final 4 min of the incubation. After straightening the coverslip slightly and nudging it ~1 mm to possibly help with dorsal/ ventral orientation, we sealed two diagonal corners with a dab of clear nail polish and imaged animals over the next 35 – 55 min.

Image Acquisition: We acquired images using a Nikon Eclipse Ti-E inverted microscope equipped with a Nikon CFI Apo TIRF 100X/ 1.49 N.A. objective, a Nikon motorized high resolution z-drive, and a motorized filter turret containing GFP, YFP, and Texas Red filter cubes (Semrock). Our illumination source was a SOLA Light Engine LED source (Lumencor). We acquired images with an ORCA Flash 4.0 16-bit camera (Hamamatsu, Bridgewater, NJ) controlled by Metamorph v. 7.7. We controlled exposure times by using Metamorph to turn the LEDs on and off rather than using a shutter. We only collected images from animals with their ventral or dorsal surfaces facing the objective. Z-series interval sizes (0.312 μm) and plane numbers (16) were the same for all strains and transgenes. Exposure times were as follows: *ceIs56* strains: 30 mSec, Texas Red filter; *ceIs259* strains: 10 mS, Texas Red filter; *ceIs267* strains: 85 mS, GFP filter. Because of the large field of view of the camera, we only collected the center quadrant of the camera's chip. The LEDs on the SOLA Light Engine were turned off via software control between successive planes. Before imaging each strain, we measured the light power of the peak emission wavelength at the objective using an XR2100 power meter (Lumen Dynamics) and an XP750 objective plane light sensor (Lumen Dynamics) with the stage position set at a standard distance (z-position) from the objective. We then adjusted the percent power of the SOLA Light Engine to produce the targeted mW power for the experiment. The percent power required to reach the target value varied by <=1% over the entire course of the experiment.

Processing Images: We used AutoDeblur Gold CWF (Media Cybernetics) to deconvolve the image stacks using the Adaptive PSF blind method and 10 iterations at the low noise setting. After deconvolving, we used Metamorph to make maximum intensity projections of each image stack.

Quantifying Images: We used Metamorph 7.7 for all analysis and quantification. To quantify fluorescence intensities per micron, we used the Trace Region tool to trace the region and used the Multiline tool to obtain the length of the traced region. We then copied and moved the region to a similar "on animal" background region for use in background subtraction. For dorsal axons, we traced the entire axon length across the image. To trace the dendrite regions around DA6 and DB6, we used the multi-line tool to trace 20 microns along the dendrite starting at one of the cell soma boundaries that faces the other cell soma. If the region between and including the two somas was >20 μm, we deleted these lines and started each of the two dendrite regions at the outer edge of each soma, proceeding outward to each edge of the image. If the region between and including the two somas was <=20 μm, we made a 2nd line that follows the dendrite in the opposite direction starting at the inner boundary of the other cell soma and proceeding in the opposite direction. If we came to the other cell soma before reaching 20 μm, we continued measuring across the soma to the dendrite on the other side of the soma until we reached 20 μm. We then started our dendrite traces at the end of each of these two lines, proceeding outward to each edge of the image using the Trace Region tool and combining the data from the two dendrite regions. Axonal and dendritic data were logged to a spreadsheet, which subtracted the background and computed the total fluorescence per micron of length. To quantify DA6 and DB6 cell soma intensity per square micron, we traced each soma separately and added them together, again using the traces to make "on animal" background regions for subtraction. To quantify puncta per micron, we set a minimum pixel intensity threshold after viewing a series of images collected from *unc-16* mutant dorsal axons. We then used the Threshold plug-in of Metamorph to highlight all pixels in the region that exceeded the threshold and counted the pixel clusters that exceeded this value, irrespective of the number of pixels in the cluster. We used the same threshold value in all strains throughout the experiment.

Producing Representative Images: After quantifying an image set we produced representative images for display by saving 8-bit versions of an image that was close to mean +/- standard error for the set. All representative images were scaled identically.

Time lapse video microscopy of organelle active transport in live animals

Growth of Strains: Animals were grown to the young adult stage as described above for quantitative fluorescence imaging, except we used as many as 30-40 locomotion plates to provide sufficient numbers of animals for the many time lapse mountings.

Agarose pad slide production: To avoid variability in agarose pad dryness, which affects animal compression and can affect active transport activity, we standardized a protocol for agarose pad slide production for time lapse imaging. After melting 0.2 g of agarose in

10 mls of M9 in a 50 ml glass bottle, we poured 0.5 ml of the molten agarose into several 1.5 ml microcentrifuge tubes in a 100° Isotemp block (Fisher). We inserted a blunt P200 pipet tip into each of 4 microcentrifuge tubes filled with distilled water and heated them to 100° in the Isotemp block. We preheated 8 clean slides (see above protocol for cleaning slides) along the front and back plastic surfaces of the Isotemp block outside of the metal tube holding blocks. We then arranged 4 pairs of spacer slides (slides with 1 layer of lab labeling tape + 1 layer of ¾ inch Scotch tape to raise them off the bench), tape side up, in front of the Isotemp block. To make the agarose pads, we placed one of the warm bottom slides between the first pair of spacer slides. We then moved one of the warm top slides to a Sardstedt Styrofoam block that is in front of the first pair of spacer slides such that the slide is in perpendicular orientation relative to the bottom slide. We removed the first hot blunt P200 tip from its hot water tube in the Isotemp block, shook it to get rid of excess water, and attached it to a P100 set on 50 μl. We then pipetted 50 μl from the open tube of molten agarose, lifting the tube out of the block briefly during pipetting. We began pipetting immediately when the tip touched the agarose, and then set the tube back in the block and pipetted the agarose onto the bottom slide between the spacers. After hovering the top slide over the agarose drop for ~1 sec, we then applied the top slide perpendicular to the bottom slide. The amount of pressure applied over the spacer slides depends on the how the drop spreads/ forms. The goal is for an average size pad to be 18-19 mm in diameter. After the 4 slides had been made, we pre-warmed another set of 8 slides and 4 blunt P200 tips for the next set. We then removed the top slide from the first slide pair and immediately applied a 24 X 30 mm coverslip to the agarose pad such that the long dimensions of the coverslip and slide are perpendicular to each other and the coverslip hangs off the edge of the slide, and then transferred this to a humidified plastic container. We repeated this with the remaining 3 slides, and then repeated this for another set of 4 slides if needed. Slides were stored at 4° until they were used the next day.

Mounting animals on slides: We first equilibrated the agarose pad slides at the intended temperature in their humidified container and prepared fresh 6 mM Levamisole in M9 from a powder stock of Levamisole (Acros Organics; AC187870100; <6 months old). To mount animals on a pad, we pre-picked 30-40 young adults to an unseeded plate, applied 30 μl of 6 mM levamisole to a 24 X 30 mm coverslip, picked the 30 young adults to the droplet in one pick-full, and incubated them for 6:10 min on a moist Kimwipe square under a Petri plate lid. Immediately after picking the worms to the drop, we removed the coverslip from one of the agarose pad slides and added 10 μl of M9 + 6 mM Levamisole to the coverslip, and then re-applied the coverslip to the pad, leaving it on the pad until ~40 sec remained on the count-down timer. When removing the coverslip we also removed as much of the 10 μl as possible along with the coverslip by tilting the coverslip up as soon as it slides off the pad and dragging the liquid away from the pad. We then blotted around the edge of the pad with a Kimwipe to remove excess liquid. When the 6:10 timer finished, we put the coverslip back on the Petri plate lid (wiping the moisture off of the part that contacted the wet Kimwipe square first) and removed 23.5 - 25.5 μl of liquid (average 24.5, but adjusted as needed depending on pad size and wetness after removing the M9) by pipetting under the stereomicroscope. We used a gel loading tip inserted onto a P20 set on the desired volume to remove the liquid in one attempt. The tip of the gel loading tip should be bent sharply, so that the opening points down at the liquid. When applying the new coverslip with worms face down onto the pad, we used a pair of jeweler's forceps to gently lower it onto the pad. On properly mounted coverslips, a small amount of liquid should wick across in all directions and slightly overflow the pad.

Image Acquisition: We acquired images using the same microscope, camera, and computer system described above for static imaging. We adjusted the light power of a SOLA LED light engine to 18% (a good level for reducing bleaching when using the Texas Red filter during the time lapse without compromising signal). We mounted the slide on the microscope and scanned the pad left to right, top to bottom using transmitted light and DIC optics to find the first animal oriented with its ventral cord facing the objective. Using the YFP filter, we then located the DB7 motor neuron and its dendrite and positioned the stage to allow viewing of the soma and as much of the dendrite as possible. At 5:30 min after applying the coverslip, we started the time lapse and continued collecting for 10 min. Each time lapse consisted of 306 frames collected at 2 sec intervals.

Processing Time Lapse Images and Converting them to Kymographs: We used the Review Multidimensional Data Metamorph plug-in to convert the time lapse images into a multi-image TIFF file. We then used the Multi-line tool to trace along the center of the DB7 neuron dendrite, starting at the cell soma boundary and proceeding anterogradely. During tracing we moved back and forth between tracing and the slider such that we could visualize the precise path as CTNS-1-RFP lysosomal puncta moved through the dendrite (i.e. following the puncta by clicking as they move along the dendrite). After ending the trace, we used the Kymograph plug-in to set the line width at a value that included all of the puncta throughout the movie. If animal movement shifted the dendrite's position during the movie, we adjusted the line width to the minimum width that allowed all puncta along the commissure to be included in the boundaries (up to ~50 pixels maximum). If necessary, we additionally shifted the position of the line slightly to compensate for movement. In some cases where animal movement prevented us from make a kymograph that included all frames in the movie, we made kymographs that only included the segments during which puncta moved and used those for analysis, noting that no puncta movements occurred in the frames not included if that was the case. Frames were excluded from the total frame count (used in some of the time calculations) if puncta movements occurred that could not be quantified via kymographs. We then created, reviewed, and saved the Kymograph, used the Save Regions plug-in to save the line traces associated with the file, and noted the optimal line width for each trace.

Quantifying Movements from Kymographs: After opening the multi-plane tiff file, using the Load Regions plug-in to re-load the line traces, and setting the line width at the above determined optimum, we re-created the kymograph (previously saved kymographs can't be used to log data). We then used the Line tool to trace each anterograde movement and logged the Distance and Time data for each movement before repeating for the retrograde movements. A "movement" was defined as being >= 1.25 microns. There is no velocity minimum if the movement is >= 2.0 microns. However, if a movement is < 2.0 microns, it must have a velocity of >= 0.1 microns/ sec. A movement continues until it pauses for >= 30 sec or until it reverses direction, or until it reaches the end of the time course, or until it merges with another punctum and the other punctum does not move (if it merges with another punctum and the combined puncta continue moving, then the movement is considered to continue). Movements that changed their velocity without pausing or changing direction were treated as single point-to-point straight line movements, with one end of the line at the beginning of the movement and the other end at the end of the movement (thus creating an average distance and time from point A to point B). Lysosomal puncta were often elongated, in which case we made point-to-point measurements from the center of the tubule. Sometimes elongated puncta will either stretch, contract, or break apart without actually moving from point to point. These events were obvious from watching the movie and were not treated as point-to-point movements. To quantify puncta entering or exiting the cell soma, we clicked through each time lapse movie to score exit and entry events. If a punctum near the boundary of the soma was elongated, we did not score it as an exit or entry event unless or until the entire length of the punctum had left or entered the cell soma.

Quantifying Percent of Time in Paused State: Pauses were analyzed on all movies having at least 280 frames for movement analysis (out of a maximum of 306 frames). These 280-306 – frame sets were observed for movements/ pauses both frame-by-frame and using kymographs. Because of the occasional movement of puncta out of the field of view, each punctum's time spent paused was compared only to its own total time visible and quantified as a proportion of time visible spent paused (i.e. 0.00 indicating that the punctum never paused and 1.00 indicating that the punctum was paused for the entire time it was visible).

Time lapse movies: We annotated multi-plane TIFF files using Metamorph Display > Graphics > Text or Arrow. We converted selected multi-plane TIFF files to movies using Metamorph Stack > Make Movie > AVI, specifying 3/30ths for each frame (which is 20X faster than real time). Movies were saved as full frames, uncompressed and converted to Quicktime using Wondershare Video Converter Platinum.

C. elegans non- wild type Strains

Strain name	Genotype (origin and/ or first use cited if not produced in this study)
NG4251	*cdk-5(gm336)* [6X outcrossed] (Juo *et al.* 2007)
KG4755	*cdk-5(gm336); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4751	*cdk-5(gm336); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4485	cdk-5(gm336); *ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4543	*cdk-5(gm336); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4626	*cdk-5(gm336); sad-1(ce749) syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4578	*cdk-5(gm336); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4665	*cdk-5(tm613)* [2X outcrossed]
KG2998	*ceIs134 [unc-17::CTNS-1-RFP, -GFP]* (Edwards *et al.* 2013)
KG4671	*ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4710	*ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG2430	*ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]* (Edwards *et al.* 2009)
KG4575	*nud-2(ok949); cdk-5(gm336); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4505	*nud-2(ok949); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4574	*nud-2(ok949); syd-2(ce759) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4400	*sad-1(ce749)* [2X outcrossed]
KG4483	*sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4596	*sad-1(ce749) syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4757	*sad-1(ce749); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4693	*sad-1(ce749); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4541	*sad-1(ce753)* [2X outcrossed]
KG4401	*syd-2(ce759)* [2X outcrossed]
KG4599	*syd-2(ok217)* [3X outcrossed]
KG4542	*syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4756	*syd-2(ok217); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4712	*syd-2(ok217); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG2338	*unc-16(ce483)* [5X outcrossed]
KG4481	*unc-16(ce483) cdk-5(gm336)*
KG4758	*unc-16(ce483) cdk-5(gm336); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4691	*unc-16(ce483) cdk-5(gm336); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4497	*unc-16(ce483)* cdk-5(gm336); *ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4579	*unc-16(ce483) cdk-5(gm336); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]; ceEx450 [unc-129::CDK-5 cDNA]*
KG4522	*unc-16(ce483) cdk-5(gm336); pct-1(tm2175) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4544	*unc-16(ce483) cdk-5(gm336); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4627	*unc-16(ce483) cdk-5(gm336); sad-1(ce749) syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4597	*unc-16(ce483) cdk-5(gm336); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4654	*unc-16(ce483) cdk-5(tm613)*
KG4608	*unc-16(ce483) strd-1(ok2283); ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG3035	*unc-16(ce483); ceIs134 [unc-17::CTNS-1-RFP, -GFP]*
KG4192	*unc-16(ce483); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4625	*unc-16(ce483); nab-1(ok943); ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4638	*unc-16(ce483); nab-1(ok943); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4639	*unc-16(ce483); nab-1(ok943); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4588	*unc-16(ce483); nud-2(ok949); cdk-5(gm336); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4573	*unc-16(ce483); nud-2(ok949); ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4587	*unc-16(ce483); nud-2(ok949); syd-2(ce759) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4521	*unc-16(ce483); pct-1(tm2175); ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4694	*unc-16(ce483); pptr-2(ok1467); ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4482	*unc-16(ce483); sad-1(ce749)*
KG4498	*unc-16(ce483); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4841	*unc-16(ce483); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]; ceEx472 [unc-129::sad-1 cDNA]*
KG4538	*unc-16(ce483); sad-1(ce749) syd-2(ce759) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*

KG4268	*unc-16(ce483); sad-1(ce749); ceIs134 [unc-17::CTNS-1-RFP, -GFP]*
KG4759	*unc-16(ce483); sad-1(ce749); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4719	*unc-16(ce483); sad-1(ce749); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4656	*unc-16(ce483); sad-1(ce753)*
KG4598	*unc-16(ce483); sad-1(ce753) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4630	*unc-16(ce483); strd-1(ok2283); sad-1(ce749) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4623	*unc-16(ce483); syd-1(tm6234); ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4644	*unc-16(ce483); syd-1(tm6234); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4657	*unc-16(ce483); syd-2(ce759)*
KG4499	*unc-16(ce483); syd-2(ce759) ceIs56 [unc-129::CTNS-1-RFP, -129::nlp-21-Venus]*
KG4658	*unc-16(ce483); syd-2(ok217)*
KG4563	*unc-16(ce483); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]*
KG4843	*unc-16(ce483); syd-2(ok217) ceIs56 [unc-129::CTNS-1-RFP, unc-129::nlp-21-Venus]; ceEx474 [unc-129::SYD-2 gene]*
KG4760	*unc-16(ce483); syd-2(ok217); ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4720	*unc-16(ce483); syd-2(ok217); ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4754	*unc-16(ce483; ceIs267 [unc-129::PST-2-GFP, unc-129::RFP]*
KG4692	*unc-16(ce483; ceIs259 [unc-129::RFP-SYN-13, unc-129::Venus]*
KG4375	*unc-104(ce782); unc-16(ce483); ceIs134*
KG4405	*unc-104(e1265); unc-16(ce483); ceIs134*

Mutation Lesions and Methods Used for Genotyping in Strain Constructions

Mutation	Description of Molecular Lesion	Effect on Protein	Method(s) used for genotyping	References for mutant isolation and/ or mutation description
cdk-5(gm336)	760 bp deletion that starts in proximal promoter and deletes exons 1, 2, and most of exon 3 (>60% of coding region)	Putative null	PCR with primers inside deleted region	(Juo *et al.* 2007)
cdk-5(tm613)	428 bp deletion extending from middle of exon 3, including all of exon 4, and ending in intron 4.	Putative null	PCR with primers inside deleted region	Japanese National Bioresource Project for the Experimental Animal "Nematode *C. elegans*" and this study.
nab-1(ok943)	1032 bp deletion that deletes coding region exons common to all isoforms, including all of the region encoding the coiled-coil domain.	Putative null	PCR with primers inside deleted region	*C. elegans* Gene Knockout Consortium and (Hung *et al.* 2007)
nud-2(ok949)	1109 bp deletion and a 1 bp insertion. Starts at the predicted ATG of nud-2 and deletes the entire open reading frame except the last part of the last exon.	Putative null	PCR with primers inside deleted region	*C. elegans* Gene Knockout Consortium and (Fridolfsson *et al.* 2010)
pct-1(tm2175)	733 bp deletion with a 5 bp insertion (GAGAG). Removes the kinase domain in all 3 isoforms.	Putative null	PCR with primers inside deleted region	Japanese National Bioresource Project for the Experimental Animal "Nematode *C. elegans*" and (Ou *et al.* 2010)
sad-1(ce749)	Q57Stop (out of 835 or 914 amino acids, depending on the isoform). Mutation position is the same in both isoforms.	Putative null	Make 250 bp PCR product centered on the mutation, followed by restriction digest with Mse I (the mutation creates an Mse I site).	This study
sad-1(ce753)	R147Stop nonsense mutation	Putative null	Make 500 bp PCR product centered on the mutation, followed by restriction digest with Bst UI (site is only present in wild type).	This study
strd-1(ok2283)	1015 bp deletion that deletes from amino acid R174 onward	Putative null	PCR with primers inside deleted region	*C. elegans* Gene Knockout Consortium and

				(Kim *et al.* 2010)
syd-1(tm6234)	1266 bp deletion starting in the intron between exons 7 and 8 and ends in the middle of exon 11 of the "b" isoform. The deletion eliminates R249 – Y471 (out of 942 amino acids total) and results in a frame shift predicted to prevent translation of the remaining protein.	Putative null	Behavioral phenotypes and PCR with primers inside deleted region	Japanese National Bioresource Project for the Experimental Animal "Nematode *C. elegans*" and this study.
syd-2(ce759)	Q387Stop (out of 1139 amino acids total)	Putative null	Behavioral phenotypes and PCR followed by sequencing	This study
syd-2(ok217)	~2 Kb deletion covering most of the N-terminal coiled coil domains. Results in a frame shift and stop codon at amino acid 200 (out of 1139 amino acids total).	Putative null	Behavioral phenotypes and PCR with primers inside deleted region	*C. elegans* Gene Knockout Consortium and (Wagner *et al.* 2009; Kittelmann *et al.* 2013)
unc-16(ce483)	Q304Stop (out of1157 amino acids of the ZK1098.10b isoform)	Putative null	Behavioral phenotypes and PCR followed by sequencing	(Edwards *et al.* 2013)
unc-104(ce782)	Missense mutation G105E in motor domain	Conditional strong reduction-of-function or null	Behavioral phenotypes and PCR followed by sequencing	(Edwards et al., co-submitted)
unc-104(e1265)	Missense mutation D1497N in the cargo binding domain	strong reduction-of-function	Behavioral phenotypes and PCR followed by sequencing	(Kumar *et al.* 2010)

Plasmids

KG#65	unc-17β:: expression vector	(Charlie *et al.* 2006)
KG#67	ttx-3::GFP	Gift of Oliver Hobert, Columbia University
KG#230	unc-129:: expression vector	(Edwards *et al.* 2009)
KG#240	unc-129::___-mCherry expression vector	(Edwards *et al.* 2009)
KG#255	ttx-3::RFP	(Edwards *et al.* 2009)
KG#367	unc-129:___-GFP	(Edwards *et al.* 2013)
KG#374	unc-129::___-Venus expression vector	(Edwards *et al.* 2009)
KG#414	unc-129::RFP-SYN-13	(Edwards *et al.* 2013)
KG#428	unc-129::SYD-2-GFP	Used Pfu Ultra polymerase and primers engineered with restriction sites to amplify the 5.4 Kb syd-2 gene coding region (minus its stop codon and with reading frame adjusted for fusing to GFP) from purified N2 genomic DNA and clone into Nhe I/ Kpn I cut KG#367 (unc-129::___-GFP).
KG#611	unc-129::PST-2A-CFP	(Edwards *et al.* 2013)
KG#695	unc-129::SAD-1A-GFP	Used AffinityScript Multiple Temperature Reverse Transcriptase and a primer engineered with a restriction site to make the sad-1 ("a" isoform) cDNA (2.7 Kb). Then used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Kpn I cut KG#367 (unc-129::___-GFP expression vector; 7.2 Kb).
KG#696	unc-17β::sad-1a cDNA	Used Herculase II polymerase and primers engineered with restriction sites to amplify the 2.7 Kb sad-1 ("a" isoform) cDNA from KG#695 and cloned it into Nhe I/ Kpn I cut KG#65 (unc-17β ::___ expression vector; 4.2 Kb).
KG#717	unc-129::SAD-1 cDNA	Used Nhe I/ Kpn I to cut out the 2.7 Kb sad-1a cDNA from KG#696 (unc-17 ::sad-1a cDNA) and cloned it into the like-digested unc-129:: vector KG#230 (6400 bp). Transform into XL1-Blue electrocompetent cells.
KG#718	unc-129::CDK-5 cDNA	Used AffinityScript Multiple Temperature Reverse Transcriptase and a primer engineered with a restriction site to make the *cdk-5* cDNA. Used Herculase II polymerase and primers engineered with restriction sites to amplify and clone the cDNA into Nhe I/ Kpn I cut KG#230.
KG#739	unc-17::__-GFP	Used Age I/ Apa I to cut out the ~1000 bp unc-54 3' control region from KG#65, leaving the 3.2 kb vector fragment containing the *unc-17β*:: promoter To this vector fragment, we ligated the 1800 bp Age I/ Apa I fragment (containing GFP + unc-54 3' control region) cut from pPD94.81.
KG#740	unc-17::PST-2A-GFP	Used Nhe I/ Age I to cut out the 1.1 Kb *pst-2a* cDNA from KG#611 (*unc-129*::PST-2A-CFP) and cloned it into like-digested KG#739 (unc-17 ::__-GFP vector).
KG#751	unc-129::PST-2A-GFP	Used Nhe I/ Age I to cut out the 1.1 Kb *pst-2a* cDNA from KG#740 (*unc-17*::PST-2A-GFP) and cloned it into the like-digested unc-129::__-GFP vector KG#367.
KG#804	unc-129::SYD-2 gene	Used the Q5 Site-Directed Mutagenesis Kit (New England Biolabs) to insert a stop codon after the SYD-2 gene in KG#428 (unc-129::SYD-2-GFP).
pPD94.81	unc-54::GFP	Gift of Andrew Fire, Stanford University
pPD118.33	myo-2::GFP	Gift of Andrew Fire, Stanford University

Transgenic Arrays and Genomically Integrated Transgenes

Array name	Insertion location	Experimental contents and injection concentrations	Co-transformation markers and injection concentrations	References for transgene or integrated insertion (if not made in this study)

ceEx450	extrachromosomal array	KG#718 [unc-129::CDK-5 cDNA] (30 ng/ μl)	KG#67 [ttx-3::GFP] (25 ng/ μl)	This study
ceEx472	extrachromosomal array	KG#717 [unc-129::SAD-1a cDNA] (2 ng/ μl)	pPD118.33 [myo-2::GFP] (0.75 ng/ μl)	This study
ceEx474	extrachromosomal array	KG#804 [unc-129::SYD-2 gene] (1.5 ng/ μl)	pPD118.33 [myo-2::GFP] (0.75 ng/ μl)	This study
ceIs56	X: ~9.0	KG#371 [unc-129::CTNS-1a-RFP] (5 ng/ μl)	KG#255 [ttx-3::RFP] (15 ng/ μl) KP#1383 unc-129::NLP-21-Venus (15 ng/ μl)	(Edwards *et al.* 2009; Edwards *et al.* 2013)
ceIs134	V: ~ -2.0	KG#645 [unc-17β::CTNS-1A-RFP] (3.5 ng/ μl)	RM#605p [unc-17β::GFP] (10 ng/ μl)	(Edwards *et al.* 2013)
ceIs259	IV: ~3.4	KG#414 [unc-129::RFP-SYN-13] (1.0 ng/ μl) KG#374 [unc-129::mCherry] (1.0 ng/ μl)	KG#255 (ttx-3::RFP) (15 ng/ μl)	This study
ceIs267	V: -1.9 or 11.2	KG#751 [unc-129::PST-2-GFP] (0.35 ng/ μl) KG#240 [unc-129::mCherry] (1.0 ng/ μl)	KG#255 (ttx-3::RFP) (15 ng/ μl)	This study

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