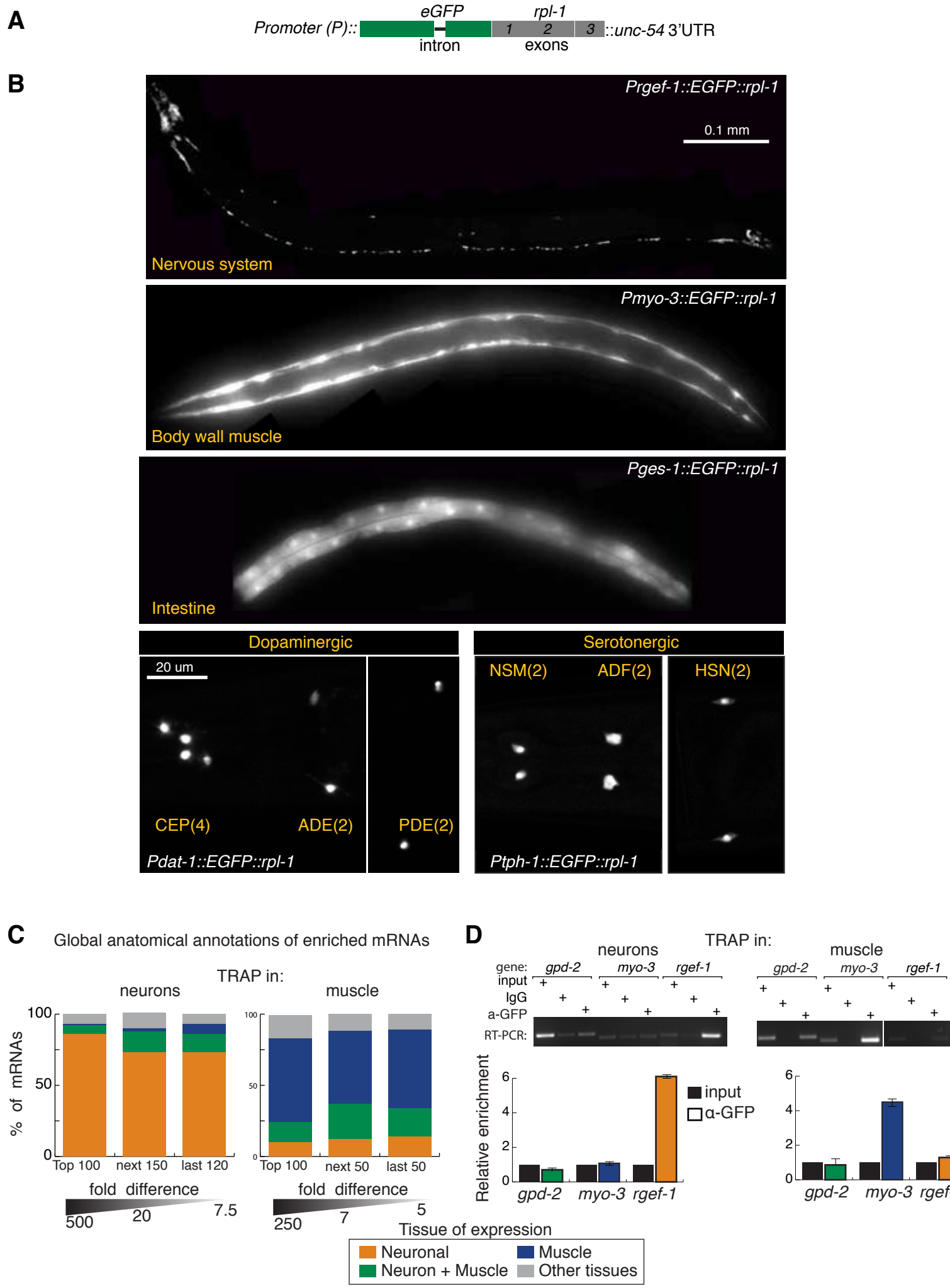
Supplemental figure 1



Supplemental Figure Legends

Figure S1. Establishment and validation of Translating Ribosome Affinity Purification (TRAP) in *C. elegans.* Related to Figure 1.

(A) Schematic of ribosomal protein RPL-1 tagged with GFP used for TRAP. Enhanced GFP (eGFP) sequence contains a synthetic intron and is fused to the *rpl-1* (Y71F9AL.13) cDNA sequence.

(B) Transgenic lines expressing the ribosomal fusions in different cell types. Promoters used for expression are the standard sequences of canonical genes used as markers: *rgef-1* is exclusively expressed in the nervous system and in most neurons (Altun-Gultekin et al., 2001). *myo-3* is expressed in the body-wall muscle (Miller et al., 1986). *ges-1* encodes for a gut-specific esterase (Kennedy et al., 1993); *dat-1* for a plasma membrane dopamine transporter (Carvelli et al., 2004), and *tph-1* for tryptophan hydroxylase (Sze et al., 2000), the rate-limiting enzyme in serotonin biosynthesis.

(C) Bar graphs show global anatomical annotations of enriched mRNAs in TRAP. The immunoprecipitated (IP) mRNAs of a given tissue correspond to genes previously reported to be expressed in these tissues (documented in Wormbase). Left: neuronal IP, right: muscle IP. For enriched TRAP mRNAs with reported anatomical expression, bins were sorted according to relative enrichment (RPKMs) in the TRAP material versus whole-animal RNA. Plot shows on x-axis bins with fold enrichment, and on y-axis the percentage of these mRNAs with any of four anatomical categories "tissue of expression". A "Neuronal+Muscle" expressed mRNA is defined as being present in neurons, muscle and possibly other additional tissues. "Other tissue" represents mRNAs not found in neurons or in muscle.

(D) TRAP enriches *bona fide* tissue-specific mRNAs. Specificity of IP was tested in a mock IP using IgG coupled to beads; TRAP IP uses anti-GFP antibodies. Same transgenic lysate was used for mock and TRAP IP. Gel shows semi-quantitative reverse-transcription PCR (RT-PCR) results amplifying cDNA from three different genes: *gpd-2* is a house keeping gene, *rgef-1* is a neuron-specific gene, and *myo-3* is a body-wall muscle –specific gene. Color scheme as in (C). Anti-GFP antibodies (TRAP), but not IgG mock IP enriches for the tissue-specific mRNA. Left: neurons, right: muscle.

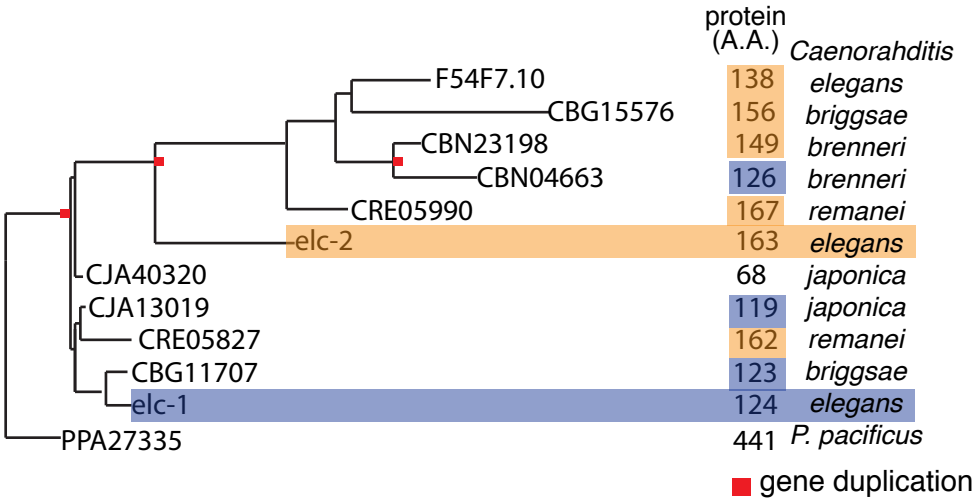
Supplemental figure 2

A

		Tissues				Subtype	
		Whole animal	Intestine	Muscle	Neurons	Dopamine	Serotonin
Elongins	*elc-2*	0.6	0.2	0.1	24	1	100
	elc-1	15	6	45	54	7	13
	F54F7.10	0.3	0.3	0.0	8	0.1	0.0
	Elongin A	29	33	16	72	26	18
	elb-1	52	56	79	154	37	85
Cullins	*cul-2*	16	19	10	34	26	23
	cul-5	10	8	3	20	8	11
	pVHL/vhl-1	11	20	7	28	7	13

RPKM values

B



C

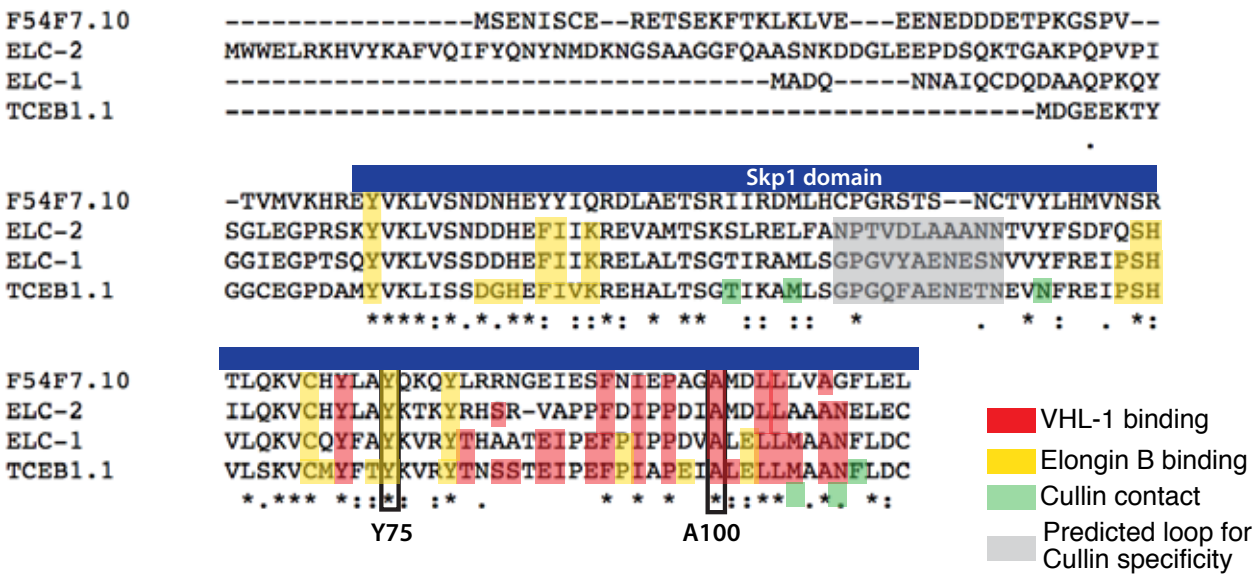


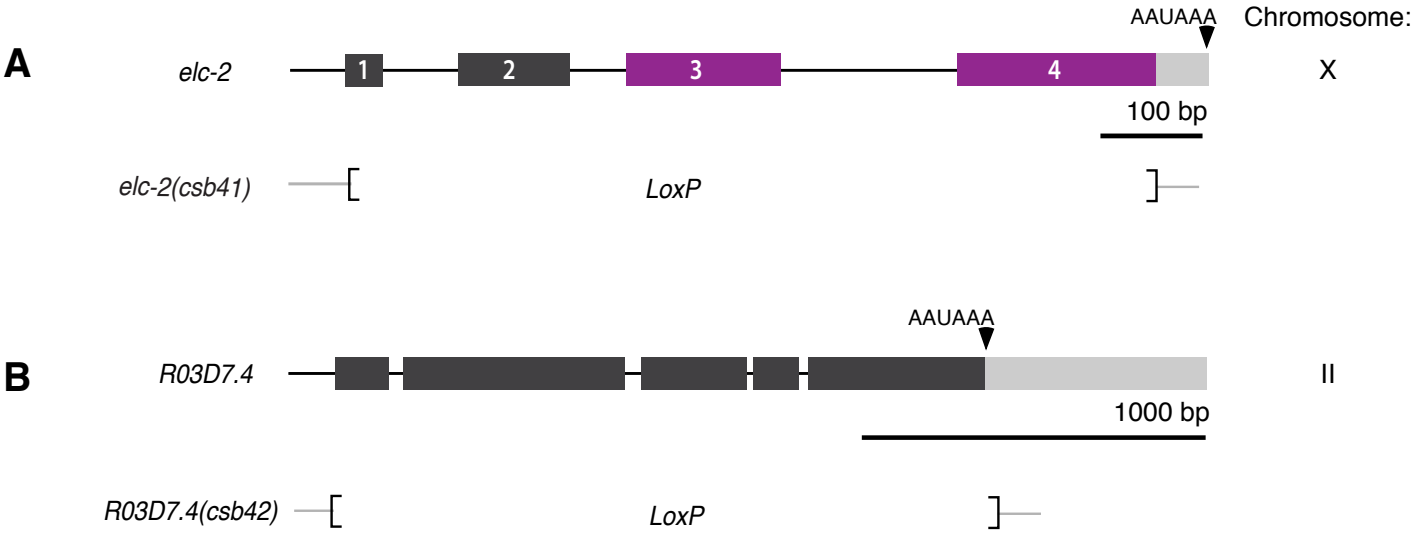
Figure S2. Elongin C orthologs in *C. elegans.* Related to Figure 2.

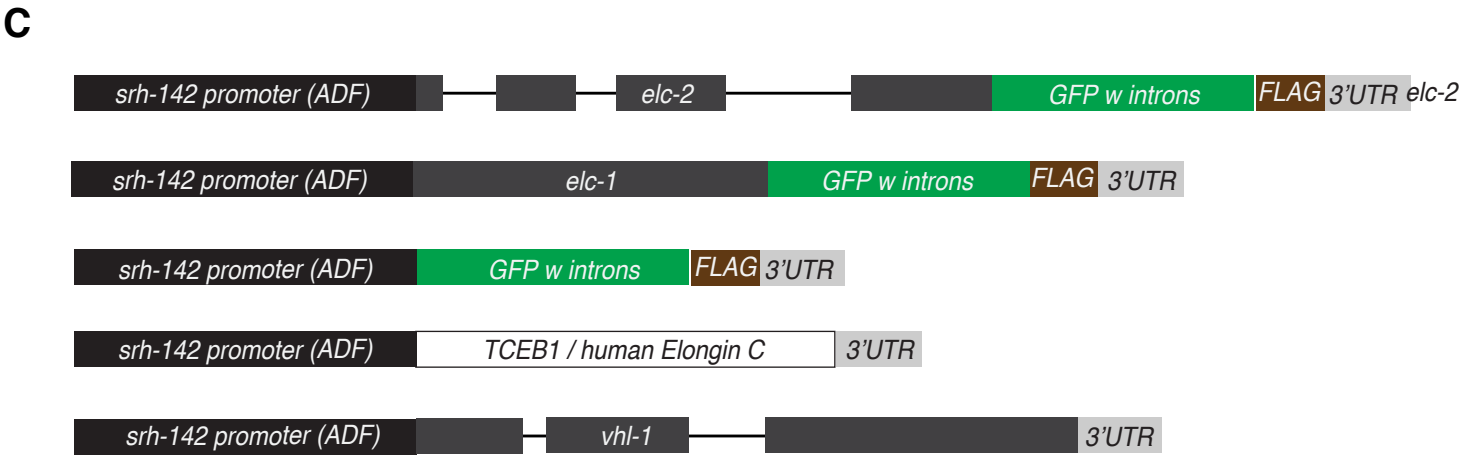
(A) RPKM values of heat map in Figure 2A depicting relative abundance of components of the Elongin complex and ECS components across different tissues, dopaminergic neurons, and serotonergic neurons as detected by TRAP.

(B) Evolutionary dendrogram of Elongin C in *Caenorhabditis* model species. *C. elegans* ELC-1 and ELC-2 are in two distinct branches, and most of the species shown have two distinct Elongin orthologs of a bigger (orange) and smaller (blue) size. Red square is duplication node. Tree was generated with Ensembl Gene/Protein Tree (Vilella et al., 2009).

(C) Protein alignment of three *C. elegans* and human (TCEB1) Elongin C orthologs.
Skp1 domain is indicated. Asterisks indicate fully conserved residues. Colons indicates strong similarity. Periods indicate weak similarity. Amino-acid identity of Skp1 domain across orthologs, relative to TCEB1: ELC-1 78%, ELC-2 47%, F54F7.10 37%. Alignments done with CLUSTAL O(1.2.3). Predicted Cullin contacts, and three-dimensional loop required for Cullin specificity are shadowed in green and gray, respectively (Yan et al., 2004). Residues that make contacts with VHL are shadowed in red, those contacting Elongin B in yellow (Stebbins et al., 1999). Note that key amino acids mediating pVHL interaction are conserved across the *C. elegans* orthologs. Squares indicate mutation hotspots in clear cell carcinoma patients: Y75S, Y75C, and A100P; these mutations affect pVHL and Cul2 binding (Sato et al., 2013).

Supplemental figure 3





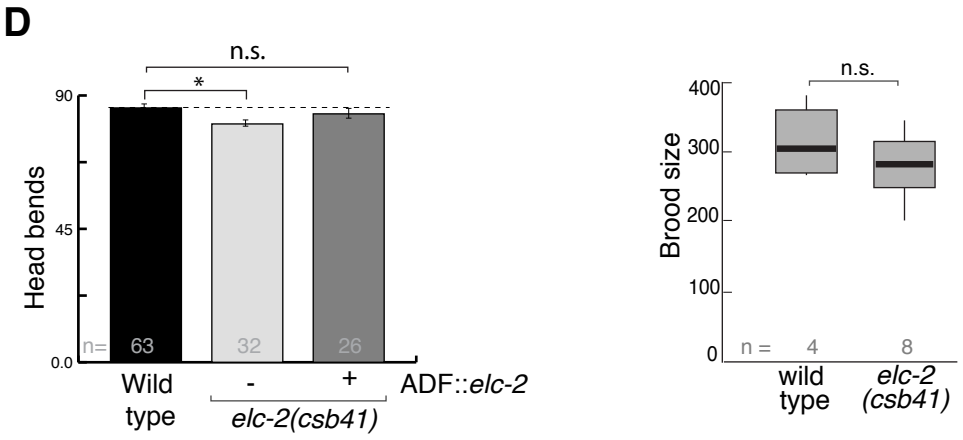


Figure S3. Genetic modifications using CRISPR, and single-copy transgenic integration. Related to Figure 3-5.

(A) Genetic disruptions using CRISPR. Top: The *elc-2* coding sequence is replaced with a LoxP-flanked selection cassette containing a GFP sequence under a pharynx promoter and a G418 resistance gene. Cre injection excises the promoter and GFP sequence leaves a single LoxP site behind in the genome (Norris et al., 2015).

(B) Same strategy as above for Elongin A.

(C) Single-copy genome integrations using modified Mos transposon system (miniMos). *shr-142* promoter (black) drives expression in ADF. *elc-2* sequence was amplified from *elc-2* fosmid and contains *C. elegans* optimized GFP and FLAG sequence. Human Elongin C TCEB1 cDNA sequence contains a *C. elegans* synthetic intron. All constructs contain *elc-2* 3'UTR.

(D) General fitness comparisons of *elc-2* mutants. Left: Number of head beads for 5 minutes on buffer drop. Single copy expression of *elc-2* in ADF. Right: Total number of brood size counted as adult.

Supplemental figure 4

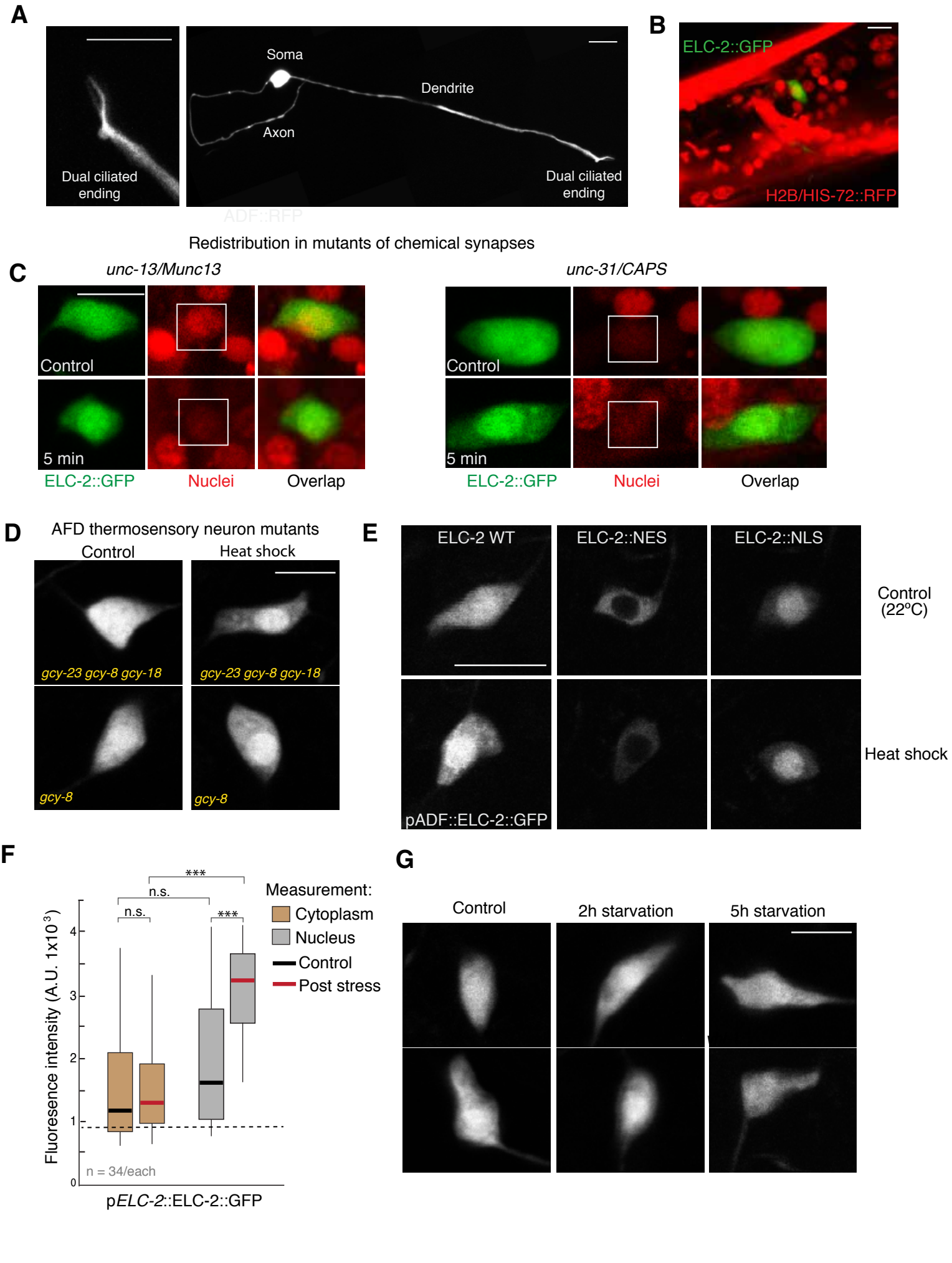


Figure S4. ELC-2::GFP protein distribution in different paradigms. Related to Figure 4.

(A) Visualization of ADF gross morphology using a cytoplasmically localized RFP gene expressed under the control of the *srh-142* promoter region. Representative images from *elc-2* mutant animals.

(**B**) Confocal microscopy image shows strain with H2B/HIS-72 tagged with RFP to overlap ELC-2 GFP expression from fosmid (green) with nuclei (red). Scale bar: 5 μm.

(C) Imaging same as in (Figure 4B), but in *unc-13* or *unc-31* mutants of chemical synapses prior to heat stress, and 5 minutes after heat stress.

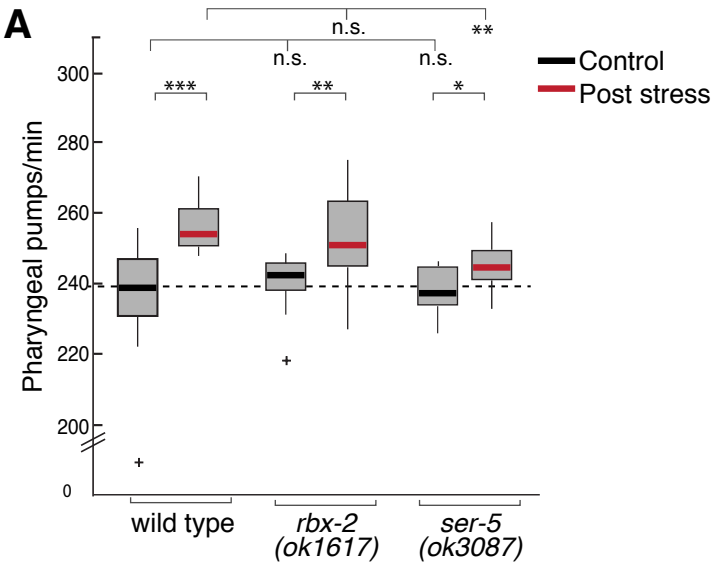
(D) ELC-2 protein redistribution in two mutants that are defective in thermosensation of the major thermosensory neurons AFD.

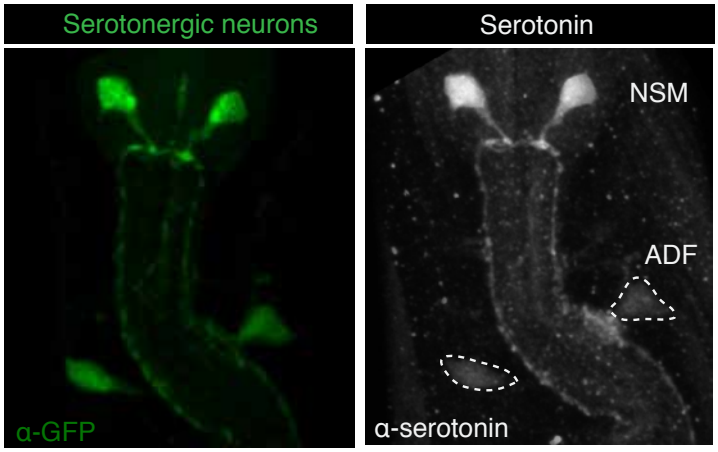
(E) Expression of ELC-2::GFP using an ADF specific promoter. NES: Nuclear export signal: LPPLERLTL from HIV Rev protein, or NLS: nuclear localization sequence from EGL-13 MSRRRKANPTKLSENAKKLAKEVEN, was placed between ELC-2 and GFP.

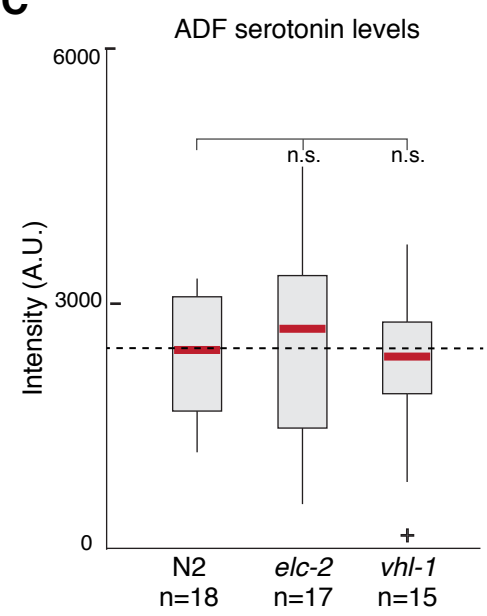
(A) Measurement of confocal Z-stack GFP intensity of the nucleus and cytoplasm before and after heat stress. Done as in Figure 4D, except ELC-2 is expressed from a fosmid construct containing its endogenous promoter p*ELC-2*. Student's *t* test: *** $p<0.0005$, ** $p<0.005$, *$p<0.05$, n.s. not significant.

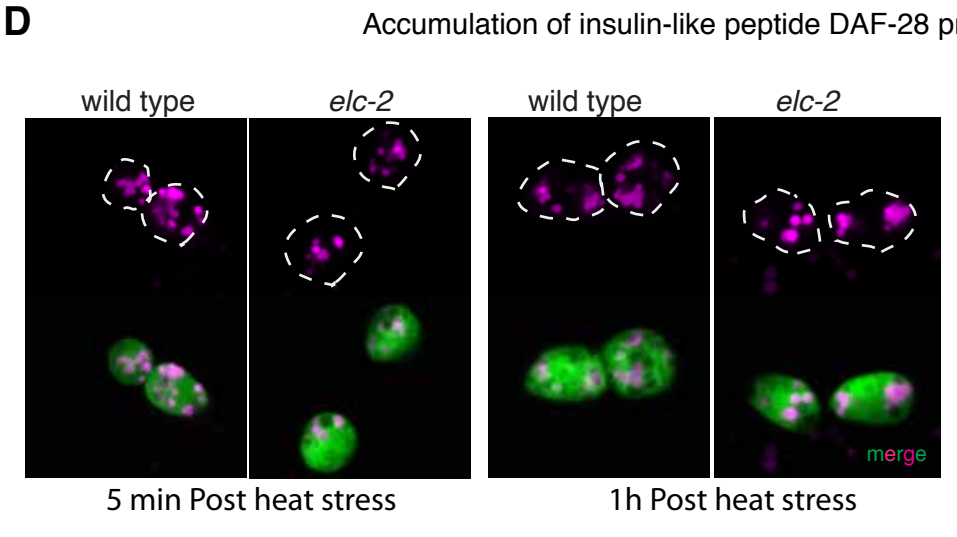
(G) Confocal microscopy images of ELC-2::GFP showing two ADF neuronal soma of control (well-fed adults), or 2-hour or 5-hour starved animals (washed 2x with M9 and put on NGM plates without food).

Supplemental figure 5









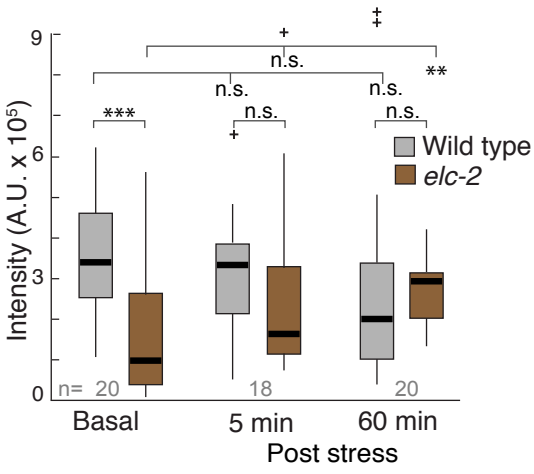


Figure S5. Anti-serotonin immunostaining and quantification. Related to Figure 6.

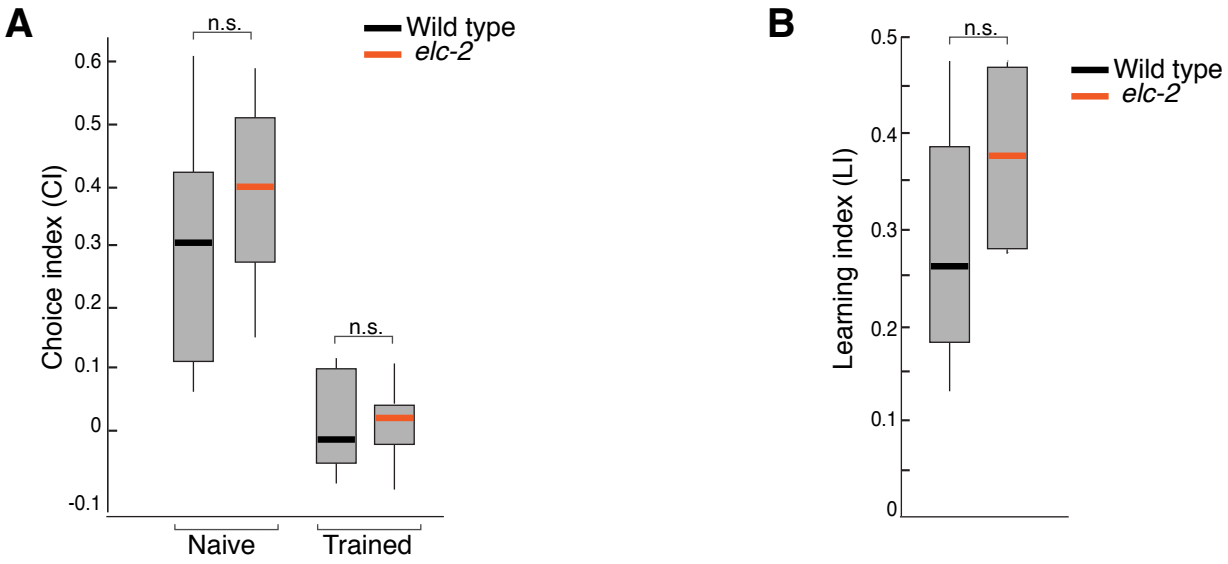
(A) Pharyngeal pumping rates before (control) and after one-hour recovery from heat shock (post stress). Genotype is indicated on x-axis. 1-way ANOVA with Dunnet's correction is used for comparing across genotypes; Student's *t* test is used for comparison within genotype. *** $p<0.0005$, ** $p<0.005$, *$p<0.05$, n.s. not significant.

(B) Animals expressing GFP under a *tph-1* promoter were used for staining to identify serotonergic neurons**.** Pictures show anti-GFP (green) and anti-serotonin (gray) staining in the head of wild-type animal.

(C) Box plot shows serotonin levels in ADF soma at control temperature in animals of three different genotypes. Median is red bar. + denotes outliers. Student's *t* test is used for comparisons within genotype, n.s. not significant.

(D) Micrographs of Insulin-like peptide (ILP) DAF-28::mCherry (magenta) 5 min or 1 hour after heat stress. Plot shows fluorescence intensity for DAF-28::mCherry accumulation in coelomocyte scavenger cells (green). ** $p<0.005$, Student's *t* test.

Supplemental figure 6



S6. ELC-2 is not required for aversive olfactory learning on pathogenic bacteria. Related to Figure 3.

(A) The *elc-2* mutants learn to avoid pathogenic bacteria. Box plot shows choice index between *E. coli* OP50 and the pathogenic bacteria *P. aureginosa* PA14 in naive animals cultivated on OP50 and trained animals exposed to PA14 for 4-6 hours during adult stage. Choice index = (Turing rate evoked by OP50 smell – turning rate evoked by PA14 smell)/(Turing rate evoked by OP50 smell + turning rate evoked by PA14 smell), , n =32/condition, done in 4 independent assays. Student's *t* test, n.s., not significant.

(B) Leaning index (choice index in naive animals – choice index in trained animals) of wild type and the *elc-2* mutants. n = 32/condition, Student's *t* test, n.s., not significant.

Supplemental Figure References

Altun-Gultekin, Z., Andachi, Y., Tsalik, E.L., Pilgrim, D., Kohara, Y., and Hobert, O. (2001). A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in C. elegans. Development *128*, 1951-1969.

Carvelli, L., McDonald, P.W., Blakely, R.D., and DeFelice, L.J. (2004). Dopamine transporters depolarize neurons by a channel mechanism. Proc Natl Acad Sci U S A *101*, 16046-16051.

Kennedy, B.P., Aamodt, E.J., Allen, F.L., Chung, M.A., Heschl, M.F., and McGhee, J.D. (1993). The gut esterase gene (ges-1) from the nematodes Caenorhabditis elegans and Caenorhabditis briggsae. J Mol Biol *229*, 890-908.

Miller, D.M., Stockdale, F.E., and Karn, J. (1986). Immunological identification of the genes encoding the four myosin heavy chain isoforms of Caenorhabditis elegans. Proc Natl Acad Sci U S A *83*, 2305-2309.

Norris, A.D., Kim, H.M., Colaiacovo, M.P., and Calarco, J.A. (2015). Efficient Genome Editing in Caenorhabditis elegans with a Toolkit of Dual-Marker Selection Cassettes. Genetics *201*, 449-458.

Sato, Y., Yoshizato, T., Shiraishi, Y., Maekawa, S., Okuno, Y., Kamura, T., Shimamura, T., Sato-Otsubo, A., Nagae, G., Suzuki, H.*, et al.* (2013). Integrated molecular analysis of clear-cell renal cell carcinoma. Nat Genet *45*, 860-867.

Stebbins, C.E., Kaelin, W.G., Jr., and Pavletich, N.P. (1999). Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. Science *284*, 455-461.

Sze, J.Y., Victor, M., Loer, C., Shi, Y., and Ruvkun, G. (2000). Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature *403*, 560-564.
Vilella, A.J., Severin, J., Ureta-Vidal, A., Heng, L., Durbin, R., and Birney, E. (2009). EnsemblCompara GeneTrees: Complete, duplication-aware phylogenetic trees in vertebrates. Genome Res *19*, 327-335.

Yan, Q., Kamura, T., Cai, Y., Jin, J., Ivan, M., Mushegian, A., Conaway, R.C., and Conaway, J.W. (2004). Identification of Elongin C and Skp1 sequences that determine Cullin selection. J Biol Chem *279*, 43019-43026.

Materials and Methods

Maintenance of *C. elegans* and strains used in present study

Extrachromosomal arrays expressing eGFP tagged *rpl-1* were integrated using UV light, and backcrossed into N2 at least three times.

Strains:

JAC487 *elc-2(csb41[loxP])* X

JAC579 *R03D7.4* (*csb42[LoxP]*) II

JAC342 *csbEx83*[*elc-2::GFP fosmid WRM063B_B12*; *Pmyo-3::mCherry*]

ET65 *cul-2(ek1)/unc-64(e246)* III

EU640 *cul-2(or209)* III.

JAC596 *cul-5(ok1706)* V (RB1470 backcrossed 2X with N2)

JAC598 *elc-2(csb41); csbEx83*

CB5602 *vhl-1(ok161)* X

DA2100 *ser-7(tm1325)* X.

MT9772 *mod-5(n3314)* I.

JAC619 ADF::RFP [*oyIs51*(V)]; *CsbEx122* [*Pelc-1*::*gfp*; *Pmyo-3::mCherry*]

JAC620 ADF::RFP [*oyIs51*(V)]; *CsbEx123* [*Pvhl-1*::*gfp*; *Pmyo-3::mCherry*]

JAC625 *elc-2(csb41)*; *csbEx125* [*pADF::elc-2::gfp*; *Pmyo-3::mCherry*]

JAC626 *elc-2(csb41)*; *csbEx126* [*pADF::elc-2::NES:gfp*; *Pmyo-3::mCherry*]

JAC627 *elc-2(csb41)*; *csbEx127* [*pADF::elc-2::NLS:gfp*; *Pmyo-3::mCherry*]

Single copy insertions

JAC503 *elc-2(csb41) X; csbSi1[Pshr-142::elc-2:GFP::FLAG]*

JAC590 *vhl-1(ok161) X; csbSi2 [Psrh-142:vhl-1 genomic]*

JAC591 *elc-2(csb41) X; csbSi3[Pshr-142::GFP]*

JAC601 *elc-2(csb41) X; csbSi4[Pshr-142::human Elongin C]*

Serotonin staining:

GR1366 *mgIs42 [tph-1::GFP + rol-6(su1006)]*

GR1333 *mgIs42 [tph-1::GFP + rol-6(su1006)]*

JAC603 *elc-2(csb41) X ; mgIs42*

JAC604 *vhl-1(csb41) X; mgIs42*

Strains for TRAP:

JAC126 *csbIs4[Prgef-1::eGFP(+intron)::rpl-1(spliced)::unc-54 3′ UTR + lin-15]*

JAC127 *csbIs6[Pmyo-3::eGFP(+intron)::rpl-1(spliced)::unc-54 3′ UTR + lin-15]*

JAC379 *csbIs27[Pdat-1::eGFP(+intron)::rpl-1(spliced)::unc-54 3′ UTR + lin-15]*

JAC382 *csbIs29[Pges-1::eGFP(+intron)::rpl-1(spliced)::unc-54 3′ UTR + lin-15]*

JAC433 *csbIs32[Ptph-1::eGFP(+intron)::rpl-1(spliced)::unc-54 3′ UTR + lin-15]*

ADF neuropeptide:

KQ2523 *Ex[srh-142p::daf-28::CHERRY; unc-122p::GFP*] (kindly provided by Kaveh Ashrafi)

JAC535 *elc-2(csb41) X; Ex[srh-142p::daf-28::CHERRY; unc-122p::GFP]*

Cell specific disruption (kindly provided by Cori Bargmann):

CX13228 *tph-1(mg280) II; kySi56[moSCI (Chr IV) loxP-tph-1-genomic-loxP]*

CX1357 *tph-1(mg280) II; kySi56; kyEx4077[Psrh-142::nCre (95 ng/ul); Pmyo-3::mCherry (5ng/ul)]*

CX13572 *tph-1(mg280)* II; *kySi56; kyEx4057[Pceh-2::nCre (10 ng/ul), Pmyo-3::mCherry (5ng/ul)]*

Detailed protocol of Translating Ribosome Affinity Purification in *C. elegans*

Animal synchronization, plates and food

Animals are synchronized by bleaching gravid mothers with NaOH, bleach, water (1:1:8). We use standard NGM 15-cm plates with 25K–55K animals/plate. To prepare food, a single colony of OP50-1 (Streptomycin R) is grown during the day in 2-3 ml. 1 ml is used to inoculate 1 L LB/Strep. Culture is pelleted and concentrated 10X in liquid NGM without antibiotics. 15-cm plates are seeded with 2 ml of this concentrated food and let the food dry for two days.

Animal yield

The amount of animals (plates) needed for a TRAP immunoprecipitation (IP) depends on the number of cells expressing transgenic ribosomes. In our experience we recommend the following number of 15-cm plates. For tissues such as nervous system, intestine or muscle: 5-10 plates. For 10 cells: 10-15 plates. For 2 cells: 15-25 plates.

Worm Harvest

Chill M9/0.01%T-x100, and the 0.15 M KCl Harvest and IP Buffer. Using a long pipette, wash animals off each plate with 10 ml of chilled M9/T-X100, transfer liquid to 15-ml conical tubes standing on ice. Repeat wash with 5 ml per plate with chilled M9/T-X100. Spin tubes 670 x *g* for 1 min at 4^{o}C to collect worms at the bottom. Aspire supernatant leaving less than 1 ml per tube. Reduce the number of tubes to the half of the number of tubes you started and evenly distribute the existing pellets. Fill up tubes containing worms with M9/T-X100 (this is the 2nd wash). Aspire supernatant leaving less than 1 ml per tube. Reduce the number of tubes to two 15-ml conical tubes. Fill up tubes with 0.15 M KCl Harvest Buffer supplemented with cycloheximide (CHX) and DTT. Spin tubes 670 x *g* for 1 min at 4^{o}C. Aspire supernatant and move one worm pellet to other tube to have one final tube. Add 10 ml of 0.15 M KCl Harvest and IP Buffer supplemented with cycloheximide, DTT and protease inhibitor. Spin tubes 670 x *g* for 1 min at 4^{o}C. Remove almost all supernatant; leave a volume equal of the worm pellet (i.e. for 1 ml worm pellet, leave 1ml of buffer). Add 10 µl of RiboLock (RNase inhibitor) and flick tube. Do a quick spin for 20 s. Place tube in liquid nitrogen and store tubes at -80^{o}C. *Animals for one IP can be collected at multiple points. Keep track of the actual volume of pure worm pellet and buffer.*

Lysate preparation

Notes: designate metal spatulas and a couple of mortar and pestles to be used only for RNA work. Bake spatulas, mortar and pestle overnight at 200^{o}C. Keep on ice a 50 ml falcon tube, and 1.5 ml tubes. Use a Styrofoam container with short walls to put liquid nitrogen, place mortar and pestle on the liquid nitrogen, let it cool down for ~ 1 minute. Get tube out of freezer and shake/hit the conical tube to get the loose pellet and decant it on the mortar. First, slowly crush the frozen pellet with the pestle. Then grind for 5 min the smaller frozen chunks to make a powder. Use a spatula to transfer the powder into one 50 ml plastic tube standing on ice and let it sit for 10 min. Add a ~1 volume of chilled 0.15 M KCl Harvest

and IP Buffer supplemented with DTT/ CHX / RNAse inhibitors *(i.e. if expecting 2 ml of thawed material, then add 2 ml of buffer).* Let the sample thaw on ice. Pipette up and down, without forming bubbles, to dissolve the last pieces of ice. Transfer ~1 ml of lysate into pre-chilled 1.5 ml tubes. Spin 5 min 3,000 x g, transfer supernatant to a new tube. Spin 10 min 21,000 x *g*. Transfer supernatant to a new tube. Save a 50 µl aliquot into one tube to test quality of RNA in lysate. Freeze tubes in liquid nitrogen and store at -80^{o}C.

Lysate validation

Use the 50µl aliquot to extract RNA with Tri-Reagent or TRIZOL. Proceed to DNAse treatment (see below). Dissolve RNA pellet in 100 µl of water. Measure RNA concentration using Qubit (ThermoFisher Q33216). To test the quality of the extracted RNA, a sample can be analyzed using TapeStation (Agilent G2965AA), or running semi-quantitative RT-PCRs (Qiagen 210210) using the following primers:

House keeping gene
gpd-2
Forward 5'GTCTACAACTCAAGAGACCCAGC-3'
Reverse 5'-GTTGTCATTGATGACCTTGGCAAG-3'

Nervous system
rgef-1
Forward 5'-AAGATCGTCTCGGCCTCG-3'
Reverse 5'-TCAGGCGGTACGGTATGAC-3'

Body-wall muscle
myo-3
Forward 5'-GAGAAGAACTGGAGAAAGGCTGAAAG-3'
Reverse 5'-CAGCAATATCGGCTCTCTCTTCAG-3'

Intestine
elt-2
Forward 5'-AGACTCTTCCACACCATCAACGTCAC-3'
Reverse 5'-CATCATCCTGAACTGGCATCACATG-3'

Dopaminergic
cat-2
Forward 5'-CTCCAGAGCACAAGGACTTTGATC-3'
Reverse 5'-CATTGGACAATCTGCTGAACG-3'

Serotonergic
tph-1
Forward 5'-CGTTGTTTCAGATGGCATCCG-3'
Reverse 5'-CCATCTTGACTGTCACGAGTCTC-3'

Bead preparation and IP

Determine the volume of beads you'll need based on the following recommendations as follows:

For broad tissue IPs: 65 uL beads per 500 ug RNA in lysate. For a 2-10 cell IP: 35 uL beads per 500 ug

RNA in lysate, using a total of 3 mg worth of RNA in lysate and 210 uL worth of beads (i.e 6 tubes each containing 500 ug of RNA in lysate and 35 uL of beads).

The following is a sample of amounts of reagents for preparing beads and antibody binding. Adjust proportions accordingly for specific experiments:

Preparing beads - DynabeadsMyOneStreptavidinT1 = 150 uL, Biotinylated Protein-L 1 mg/mL = 60 uL, 1x PBS for 1 mL = 940 uL.

Antibody binding – Anti-GFP antibody 19C8 = 50 uL (25 ug), Anti-GFP antibody 19F7 = 14 uL (25 ug), 0.15M KCl buffer = 936 mL.

The volume of beads refers to direct volume out of bottle Dynabeads® MyOneTM Streptavidin T1 (LifeSciences Techonolgies 65601). Resuspend the Dynabeads® MyOneTM Streptavidin T1 thoroughly in the original bottle by hand mixing. Transfer the beads to be used to a tube and collect on the magnet. Wait 1 minute for the beads to collect on the magnet (on the DynaMag-2). Discard liquid. Wash beads once with 1 ml 1x PBS. Collect the beads on the magnet and re-suspend in the appropriate volume of 1x PBS. Incubate the beads with biotinylated Protein L in 1x PBS (aim for 1 ml total volume for 35 minutes at room temperature using gentle end-over-end rotation. In the meantime thaw the antibody stocks and spin tubes at maximum speed (>13,000 x *g*) in microcentrifuge for 10 minutes, 4°C, and transfer supernatants (antibody) to new tubes.

Collect the Protein L-coated beads on the magnet. Wash the coated beads 5 times with 1ml 1x PBS containing 3% BSA IgG-Protease-free (weight/volume). Proceed to antibody binding in 0.15 M KCl Harvest and IP Buffer 1 ml total volume for 1 hour using gentle end-over-end rotation, at room temperature. While antibody is binding, thaw the lysate stocks on ice for 50 min, then spin tubes at maximum speed (>13,000 x **g**) in microcentrifuge for 5 minutes, 4°C, and transfer supernatants (lysate) to new pre- chilled tubes. Freeze lysate remnants in liquid nitrogen. After antibody binding, wash beads 3 times with 0.15 M KCl Harvest and IP Buffer supplemented with DTT and CHX, 1 ml each. Use supplemented buffer to resuspend the beads, 200 µl per IP tube to be used. For example, for four final tubes, resuspend beads in 800 µl of IP buffer and then dispense 200 µl per tube. For the IP binding, add lysate and the necessary buffer to get each tube to 1.2 ml final volume, along with 5 uL of an RNase inhibitor such as Ribolock. Incubate for overnight at 4^{o}C using gentle end-over-end rotation.

Washing beads after IP

Prechill 0.35M KCl Wash buffer. Assemble magnetic stand on ice. Place each tube on a magnetic stand and incubate for 2 min to collect beads. Remove supernatant and save 1 ml of it if you want to examine your unbound fraction. Remove tubes from magnet and wash beads with 0.35 M KCl wash buffer. For the example, for 6 IP tubes, gather the beads of three tubes into one; ending with 2 tubes. Use 1 ml per ~100 µl of initial bead volume. Pipette up and down or flick the tube. Spin briefly and return tube to magnet for at least 1 min. While still on the magnet remove and discard supernatant. Repeat wash three times for a total of four washes. Remove supernatant, and proceed to DNAse treatment. Dissolve beads in 90 µl of water, add 10 µl of 10x DNAse buffer and 5 µl of DNAseI (Ambion AM2222). Incubate at 37^{o}C for 30 min. Proceed to RNA extraction using Trizol or Tri-Reagent. Measure RNA with Qubit. Validate IP enrichment with semi-quantitative RT-PCR.

Reagents

We are using all reagents as reported by (Heiman et al., 2014)

10x PBS Ambion # AM9625*
Rnase Zap Wipes Ambion # AM9786*
1M MgCl2 (100ml) Ambion # AM9530G*
2M KCl (100ml) Ambion # AM9640G*
Nuclease-free water (5x100ml) Ambion # AM9939*
Streptavidin MyOne T1 Dynabeads Invitrogen # 65601
HEPES (100ml) Afymetrix # 16924*
Cycloheximide Sigma # C7698-1g
DTT Sigma # D9779 Reconstitute DTT to 1M in water, filter sterilize, and store at –20 °C in single-use aliquots
10% NP-40, 10X5ml AG Scientific # P1505
IgG and Protease-free BSA JacksonImmuno #001-000-162
Roche Mini Protease Inhibitor Tablets (-EDTA) Roche #11836170001
QIAGEN OneStep RT-PCR Kit #210210