Supplementary Methods

Expression constructs

A 970 bp promoter fragment upstream of the *unc-50* start ATG was used to control expression of *gfp*, replacing the *myo-3* promoter in pPD115.62 as a HindIII/KpnI fragment creating *unc-50::gfp*. The *myo-3::unc-50* construct was obtained by subcloning *unc-50* cDNA as AgeI/EcoRI fragments into pPD115.62 to replace *gfp*. To generate the *myo-3::mRFP-unc-50* construct, mRFP coding region (Campbell et al., 2002) was PCR amplified without a stop codon and subcloned inframe as a KpnI/AgeI fragment into the *myo-3::unc-50* vector. Expression of the *unc-50* cDNA under the control of the heat shock promoter (*hsp-16.48::unc-50*) was achieved by replacing the *myo-3* promoter in *myo-3::unc-50* with a HindIII/BamHI *hsp-16.48* promoter PCR fragment. The ER marker *myo-3::GFP-cytochrome b5* (cb5) and Golgi marker *myo-3::Mans-gfp* were constructed by replacing the *glr-1* promoter from the original vector (Rolls et al., 2002), respectively.

Transgenic animals

If not otherwise stated the coinjection markers pL15EK[*lin-15*(+)]and pPD115.62(*myo-3::gfp*) were used at 40 and10 ng/μl, respectively. All *unc-50* expression constructs were injected at a concentration of 2 ng/μl. The injection mix was always adjusted to a total DNA concentration of 100 ng/μl by adding pBlueScript SKII (Stratagene). The expression constructs for in-vivo immuno-labeling of cell-surface expressed receptors are as described previously (Gottschalk et al., 2005).

Electrophysiology

Electrophysiological methods were performed as described previously (Richmond and Jorgensen, 1999). Muscle recordings were made in the whole-cell voltage-clamp configuration (holding potential -60mV) with an EPC-10 patch-clamp amplifier and digitized at 2.9 kHz. Data were acquired by Pulse software (HEKA). The bath solution contained 150mM NaCl, 5mM KCl, 5mM CaCl2, 1mM MgCl2, 10mM glucose and 15mM HEPES, pH 7.35, about 340 mOsm. The pipette solution was prepared as described previously (Richmond and Jorgensen, 1999). Subsequent analysis and graphing were performed with Pulsefit (HEKA) and Igor Pro. All statistically derived values are given as means ± s.e.m.

In vivo Immunolabelling of cell-surface exposed epitopes

α-HA (16B12) coupled to AlexaFluor593 (Molecular Probes) and α-cMYC (9E10), coupled to Cy3 (SIGMA) were used for epitope-staing. Injections and imaging were performed as previously described (Gottschalk and Schafer, 2006). For quantitative analysis, stretches of the ventral nerve cord near the vulva were photographed and analyzed using ImageJ (NIH). Line scans of fluorescence intensity were traced along the nerve cord puncta. After background correction, fluorescence values were averaged for individual line scans, then the averaged values of line scans from 10-20 different animals of identical genotype and experimental conditions were averaged.

Protein extraction and western blotting

Stage L4 worms or a mixed staged population of worms were collected and approx. 500μl worm pellet was frozen at -80°C until usage. For extract preparation worm pellets were ground under liquid nitrogen to a fine powder in a ceramic mortar and thawed on ice. While thawing 10 volumes of ice cold homogenization buffer (20 mM HEPES pH 7,4; 10 mM KCl; 1 mM EDTA; 400 μM Pefabloc (Roche) and Complete Mini Protease inhibitor cocktail, minus EDTA (Roche)) were added and the suspension was further homogenised with ten strokes using a 2 ml tight fitting glass tissue homogeniser. Afterwards an equal volume of homogenization buffer containing 0,5 M sucrose was added and the suspension was spun twice at 500g for 10 min to remove worm debris and nuclei. The resulting nuclear pellets were pooled and extracted twice with 5 ml of homogenization buffer containing 0,25 M sucrose. The post nuclear supernatants were pooled and subsequently centrifuged at 150000g for 1h. The membrane pellets were solubilized in SDS buffer and separated on a SDS polyacraylamide gel, blotted onto a nitrocellulose membrane, and subsequently probed with the appropriate primary antibody and a horseradish peroxidase coupled secondary antibody (DAKO). The signal was revealed with Lumilight reagents (Roche) and normalized against staining of the transmembrane subunit of the vacuolar ATPase VHA-5 (Liegeois et al., 2006).

Bibliography

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Supplementary Figures

Suppl. Figure 1: The clustering of the ACR-16 type nAChR is not affected in *unc-50* mutants. In *unc-49* mutants, in which the GABA-receptor is eliminated, the evoked NMJ response predominantly resulting from ACR-16 AChR activation is unaffected in *unc-50* mutant. This indicates that ACR-16 nAChR clustering at the NMJ is unaffected by loss of UNC-50. Selective block of the ACR-16 AChR by dihydro-β-erythroidine (DHβE) in *unc-49* mutants isolates the evoked post-synaptic current due to Lev-AChR activation, which is eliminated in *unc-50* mutants.

Suppl. Figure 2: Molecular identification of the *unc-50* gene. *unc-50* mutants were initially isolated based on impaired locomotion (Brenner, 1974) and shown to be to resistant to paralysis and subsequent death caused by 1 mM levamisole (Lewis et al., 1980). *unc-50* was mapped to a narrow interval very close to *unc-69* and *ced-9* on chromosome III. The cosmid T07A5 that covers this region was able to rescue the *unc-50* associated phenotypes. To further refine the minimal rescuing sequence of T07A5 we generated subfragments of T07A5 by restriction digest and subcloning. These subfragments were injected into *unc-50* animals and their rescuing activity was assayed. A minimal HindIII/PstI subfragment of T07A5 was sufficient for rescue of the *unc-50* mutant phenotypes (indicated in grey). This fragment contained an operon of two predicted genes T07A5.5 and T07A5.2. Using RT-PCR analysis we showed that gene T07A5.5 is spliced to the SL1 splice leader and encodes a peptide of 35 amino acids with a predicted signal sequence for secretion. A sequence comparison revealed that T07A5.5 represents the *C. elegans* ortholog of Ost4p from the yeast *S. cerevisiae*, a subunit of the oligosaccharyltransferase complex of the ER lumen, which catalyzes aspargine-linked glycosylation of newly synthesized proteins. The second gene T07A5.2 is SL2 spliced as typical for the down stream gene in an operon (data not shown; Blumenthal, 1995). Sequencing of the *unc-50* mutant strains revealed that all mutations are found within the coding region of gene T07A5.2 (Fig. 5A; Suppl. Table 1). In order to further demonstrate the identity of *unc-50* we subcloned the T07A5.2 cDNA under the muscle specific myo-3 promoter and assayed levamisole sensitivity of the transgenic animals. As shown in Fig 6. The *myo-3*::T07A5.2 construct was sufficient not only to confer levamisole sensitivity but to also revert the uncoordinated (Unc) phenotype of *unc-50* mutants. Therefore, we conclude that T07A5.2 is the gene *unc-50*. The gene structure as shown in Fig. 5B was confirmed by sequencing the ESTs yk668g11 and yk125e8 (kindly provided by Y. Kohara) and by 3' and 5' rapid amplification of cDNA ends (RACE) RT-PCR according to the manufactures protocol (Clontech).

Suppl. Figure 3: Phylogenetic tree of the UNC-50 like proteins is shown. The sequences used are *C. elegans* NP499279, *C. briggsae* CAE56800, human CAG38495, mouse AAH19484, rat AAH61976, chicken XP416887, Zebrafish AAH47831, *Drosophila melanogaster* AAF54267, *Anapheles gambiae* EAA08030, rice XP449984, *Arabidopsis thaliana* AAK96450, *Cryptococcus neoformans* EAL18336, *Yarrowia lipolytica* XP504552, *Saccharomyces cerevisiae* CAA82102, *Kluyveromyces lactis* XP456033, *Eremothecium gossypii* NP985620, *Debaryomyces hansenii*

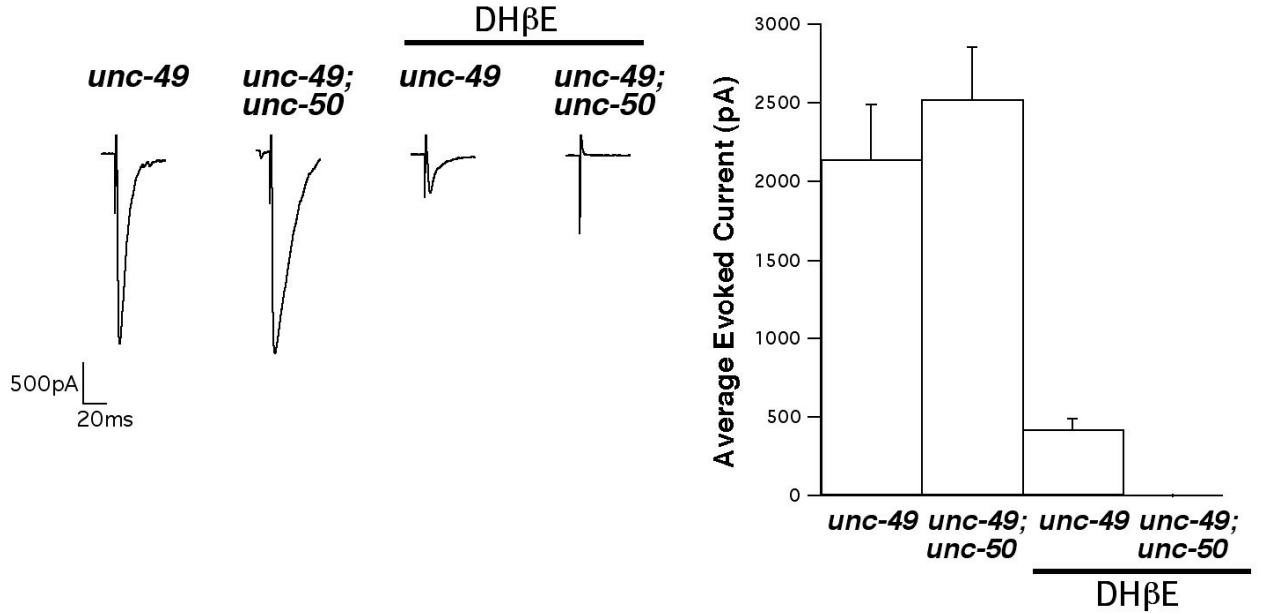
CAG85007, *Candida glabrata* XP448907, *Schizosaccharomyces pombe* CAB08773, *Aspergillus nidulans* XP661043. The protein sequences for *Ciona intestinalis* and *Zeay mays* were extracted from EST data and for *C. remanei* assembled from whole genome shot gun sequence assemblies, respectively.

Suppl. Figure 4: TMHMM2 likelihood prediction for the occurrence of transmembrane domains with the UNC-50 protein.

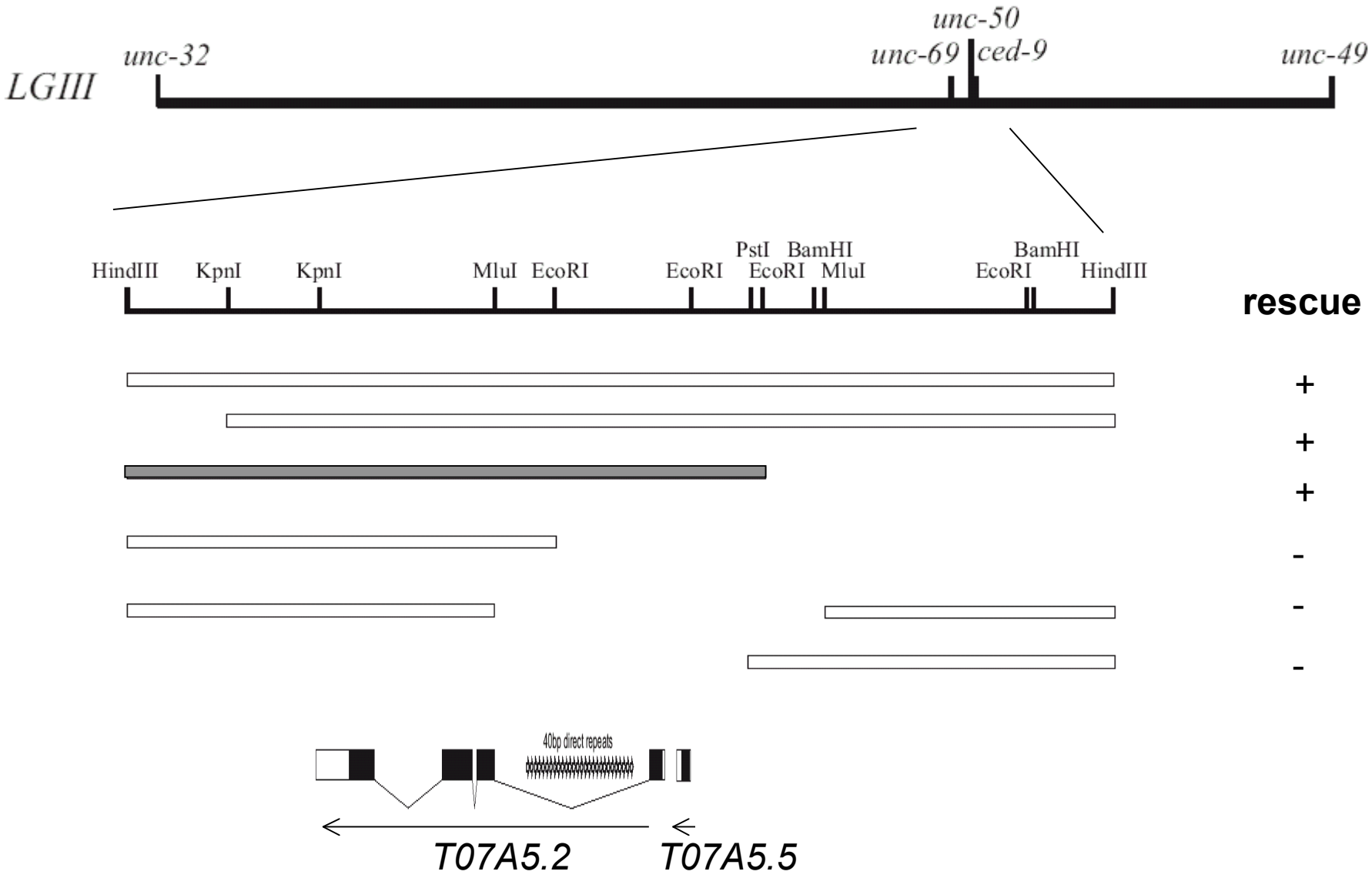
Suppl. Figure 5: UNC-50 is ubiquitously expressed. A transcriptional fusion of the *unc-50* promoter to GFP is expressed in all cell types throughout development. (**A**) *unc-50::gfp* expression in the head of an adult hermaphrodite. Expression is seen in the pharyngeal muscle, head neurons, and epidermis. (**B**) Expression is also present in the gut, ventral cord motor neurons, and epidermal seam cells. (**C**) In the mid body region expression is seen in the gut, seam cells, uterus, vulva muscles, and distal tip cell. (**D**) In the tail region *unc-50* expression can be detected in the epidermis and enteric muscles.

Suppl. Figure 6: The loss of UNC-50 does not alter ER and Golgi structures. Transgenes driving muscle-specific expression of the Golgi marker MannosidaseII-GFP (top) and the ER marker GFP-cytochrome b5 (bottom) were introduced in the *unc-50(x47)* background. In both cases, GFP distribution was similar to that observed in wild-type animals. Bar = 10 μm.

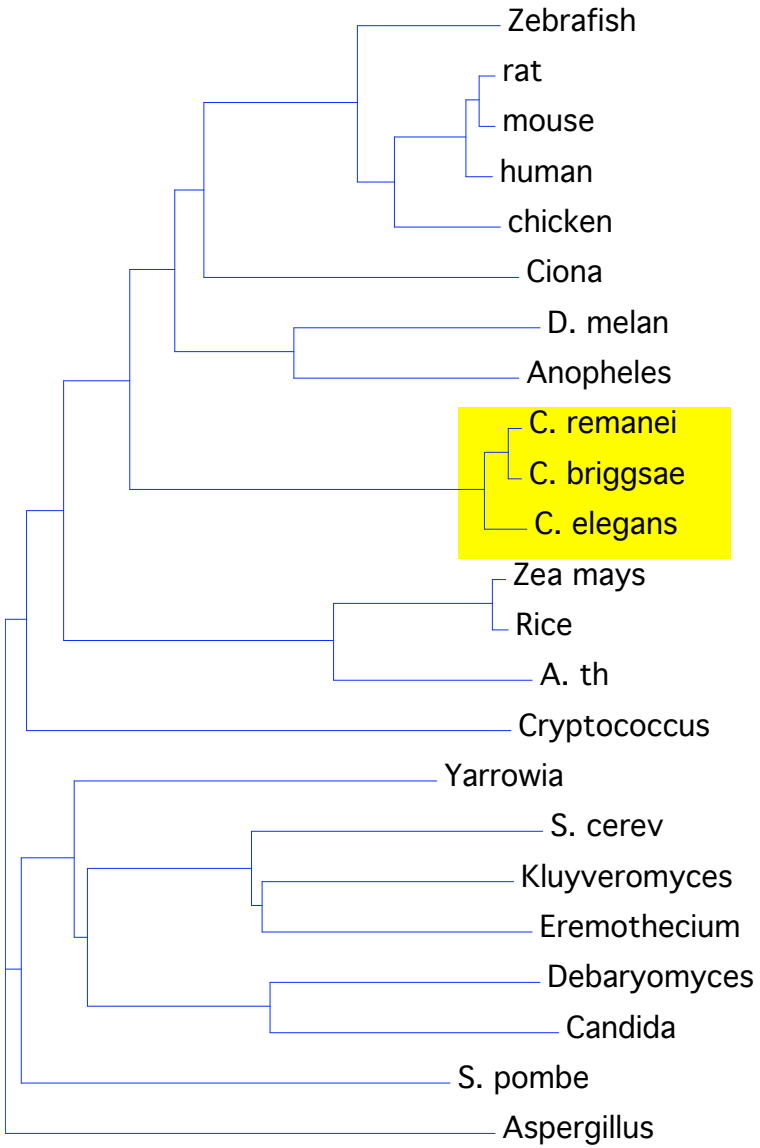
Supplementary Fig.1



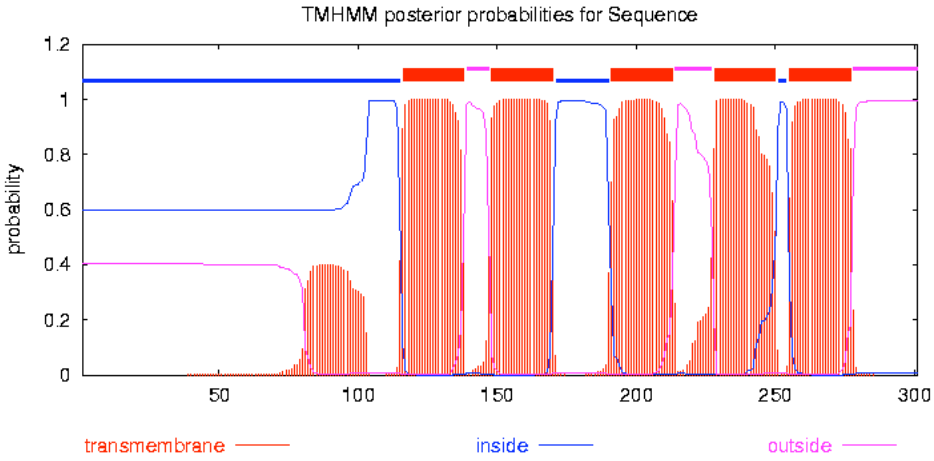
Supplementary Fig.2



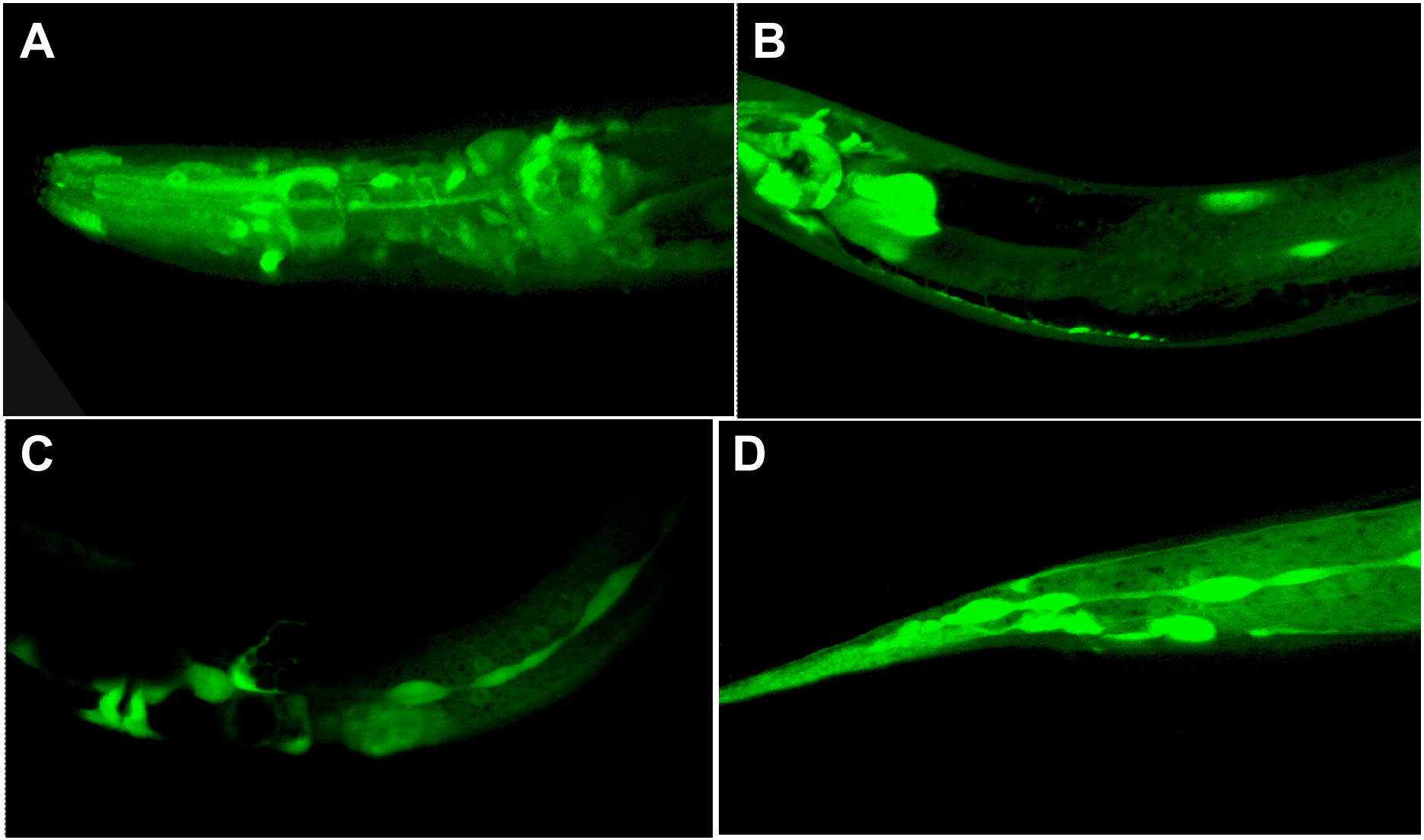
Supplementary. Fig. 3



Supplementary. Fig. 4



Supplementary. Fig. 5



Suppl. Fig. 6