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Supplemental information

A developmental pathway for epithelial-to-motoneuron transformation in *C. elegans*

Alina Rashid, Maya Tevlin, Yun Lu, and Shai Shaham

SUPPLEMENTAL FIGURES

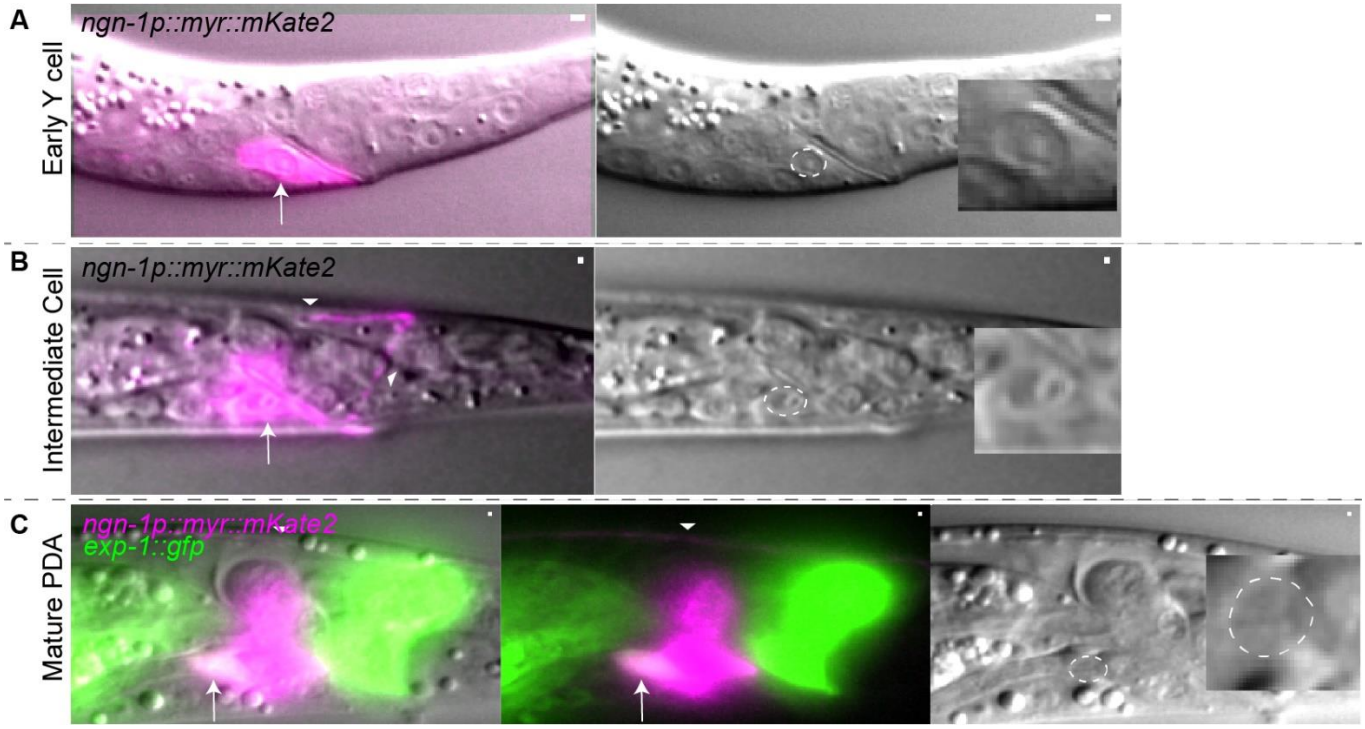

Figure S1: PDA adopts a neuron-like speckled nuclear morphology after axon outgrowth is complete. Related to Figure 1. (A) Early Y cell has a "fried egg" epithelial-like nuclear morphology. (B) Y/PDA with a process still has a "fried egg" nuclear morphology. (C) Mature PDA (with *exp-1p::gfp* expression) adopts a neuron-like speckled nuclear morphology. Arrows, cell body. Arrowheads, cellular process. Dashed circle, outline of cell body. Scale bars, 5 µm.

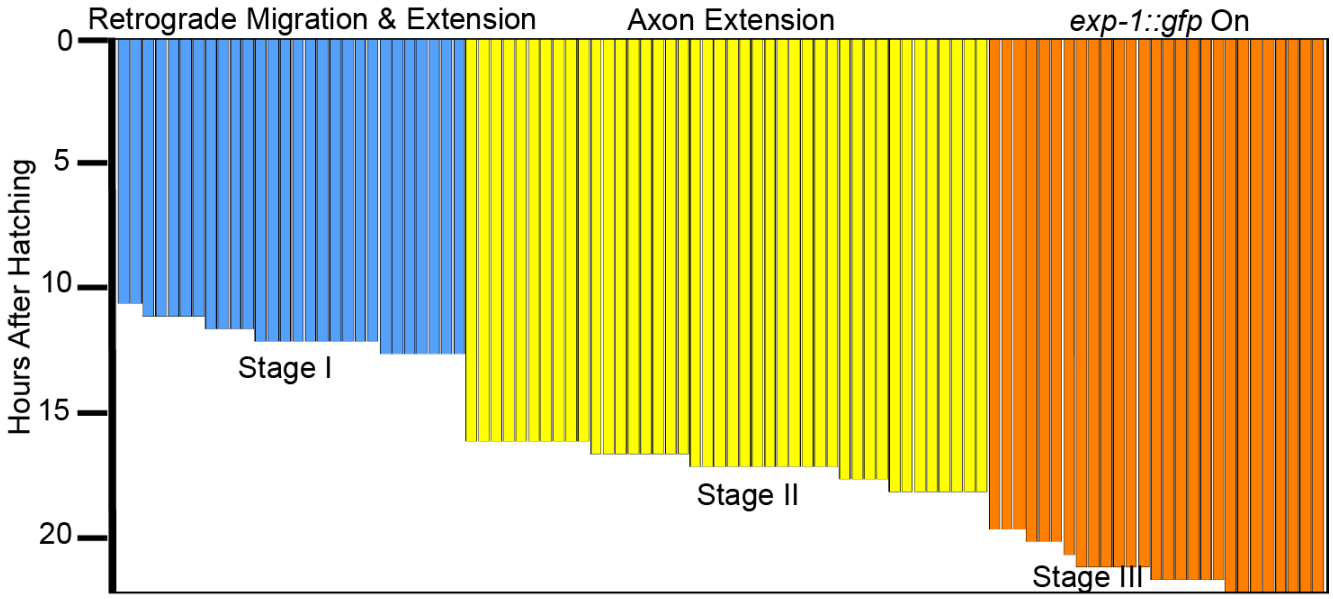

Figure S2: Starvation does not affect the timing or nature of Y-to-PDA transformation events. Related to Figure 1. Each horizontal bar represents Y-to-PDA stage of a single animal. Stage I: n = 28, mean = 11.77 ± 0.63 hrs, Stage II: n = 42, mean = 16.9 ± .71 hrs, Stage III: n = 27, mean = 21.11 ± 0.86 hrs.

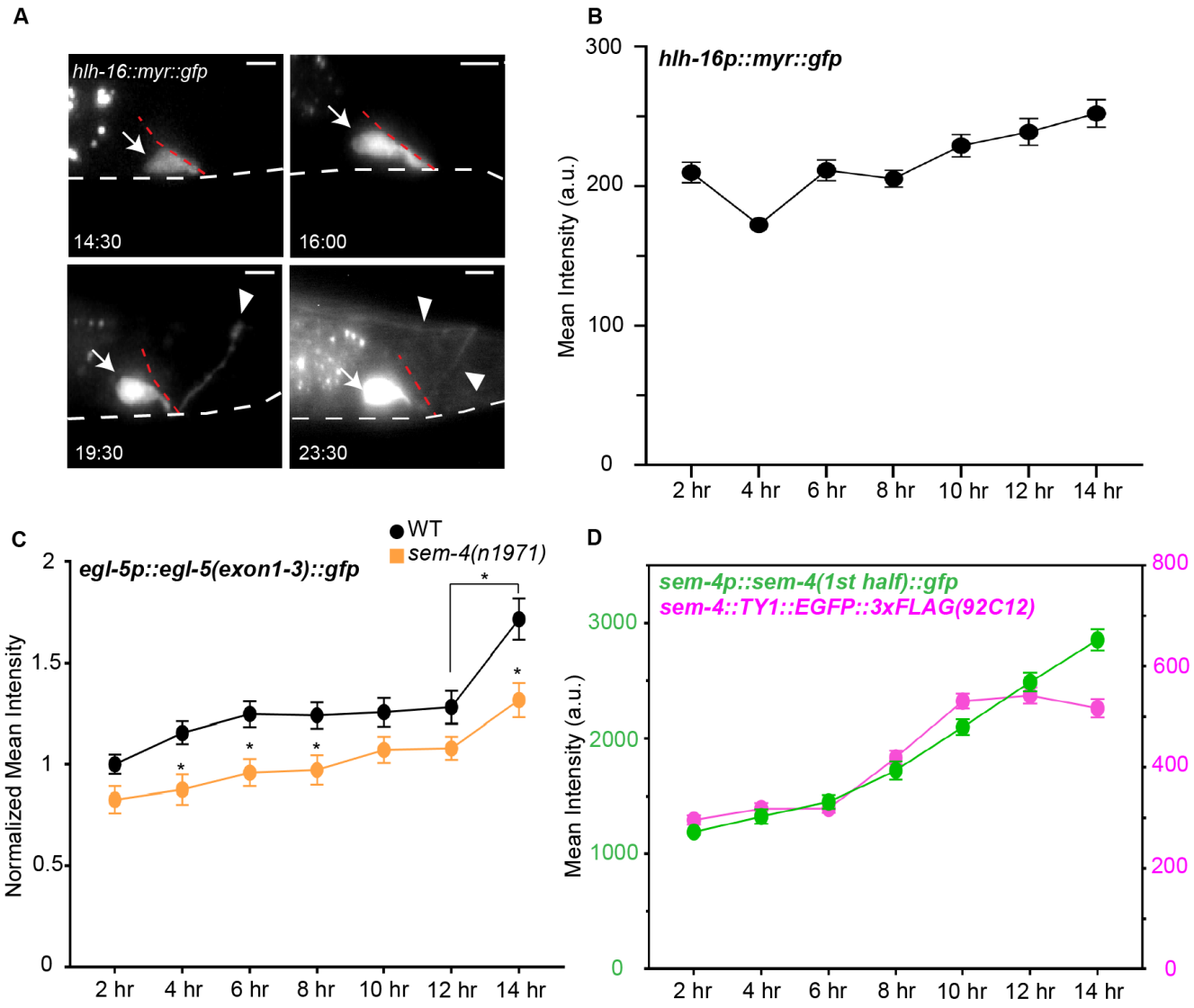

Figure S3: *hlh-16, egl-5,* and *sem-4* reporter expression in early stages of Y-to-PDA transformation. Related to Figure 3. (A) Following Y-to-PDA transformation using *hlh-16p::myr::gfp::hlh-16 3'utr*. (Top left) Y cell before transformation begins. (Top right) Retrograde cell-body migration and formation of thick process that remains attached to rectal epithelium. (Bottom left) Axon extension. (Bottom right) PDA process extension is complete. Note that *hlh-16* expression becomes fainter at this time point. Arrow, cell body, arrowhead, axon. White dashed line, ventral surface of animal. Dashed red line, rectal slit. Scale bars, 5 µm. Time stamps indicate hour after L1 arrest exit. (B) GFP fluorescence intensity in wild-type animals harboring an *nsIs943* [*hlh-16p*::*myr*::*gfp*] reporter transgene. Mean grey value intensity at the center slice of an 80 pixel x 80 pixel region containing the Y cell. Error bars, SEM. n > 50 animals for each time point. (C) GFP fluorescence intensity in animals expressing a *bxIs7* [*egl-5p*::*egl-5(exon1-3)*::*gfp*] reporter in the indicated genotypes. Mean grey value intensity in the center slice in an ROI drawn around the cell and normalized to the average values for the corresponding wild type at the 2 hr time point. n > 23 animals for each genotype per time point. t-tests were performed at each time point. Error Bars, SEM. Significance at $p < 0.01$. (D) GFP fluorescence intensity in wild-type animals expressing *kuIs34* [*sem-4p*::*sem-4(5' half)*::*gfp*] and *wgIs57* [*sem-4*::TY1::EGFP::3xFLAG(92C12)] reporters. Mean grey value intensity in the center slice in and ROI drawn around the cell body. Error bars, SEM. n > 24 animals for each genotype per time point.

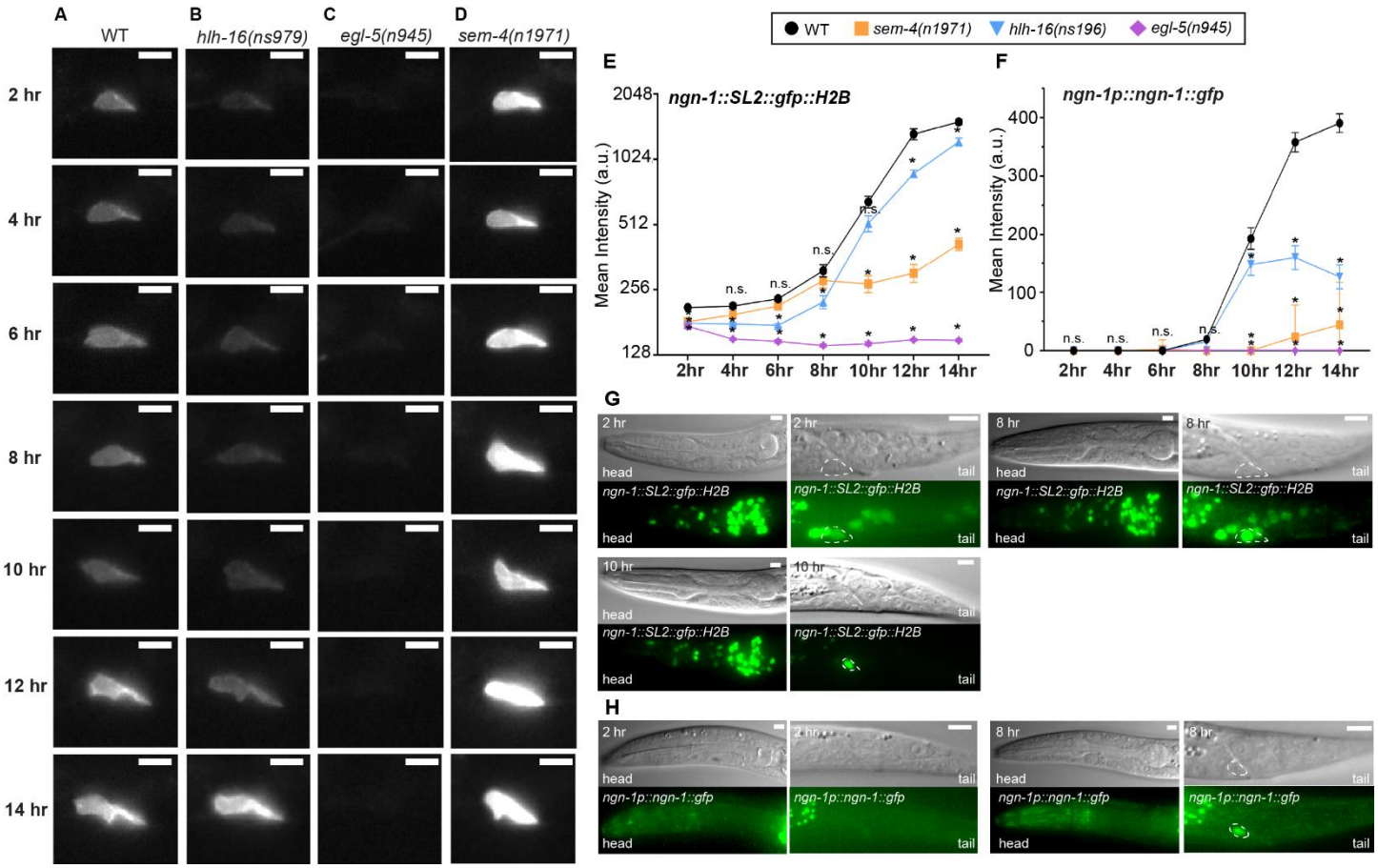

Figure S4: Following Y-to-PDA transformation using *ngn-1* reporters. Related to Figure 3. Time stamps indicate hours after L1 arrest exit. Scale bars, 5 µm. (A-D) Representative images of Y cell in indicated mutant backgrounds used for quantification of Figure 3A. (E,F) GFP fluorescence intensity in animals harboring either *ngn-1(syb5813)* endogenous transcriptional GFP fusion (E) or *ngn-1(syb5802)* endogenous translational GFP fusion (F) in indicated mutant backgrounds. *ngn-1(syb5802)* expression is not detectable before the 8-hour time point. Mean grey value intensity at the center slice of an 25 pixel x 25 pixel circle containing the Y cell. Animals scored as lacking fluorescence were assigned a value of 0.00 a.u. Two-way ANOVA with Dunnett's post-hoc test. n > 28 animals per time point for each genotype. Significance at $p < 0.01$. Error bars, SEM. Statistics in Table S2. (E) Y-axis is plotted on a log2 scale starting at 128. (G,H) Images of the *ngn-1(syb5813)* and *ngn-1(syb5802)* transcriptional and translational GFP fusions in the head and tail of the animal at different time points. Dashed white circle, Y cell. (G) The transcriptional *ngn-1* fusion initially shows broad expression in the head and tail at 2 hrs after L1 arrest exit. Over time, expression in the tail becomes more specific to the Y cell. (H) The translational NGN-1 fusion initially shows no expression in the head or tail at 2 hrs after L1 arrest exit. Expression in the Y cell in the tail is initiated between 8-10 hrs after L1 arrest exit.

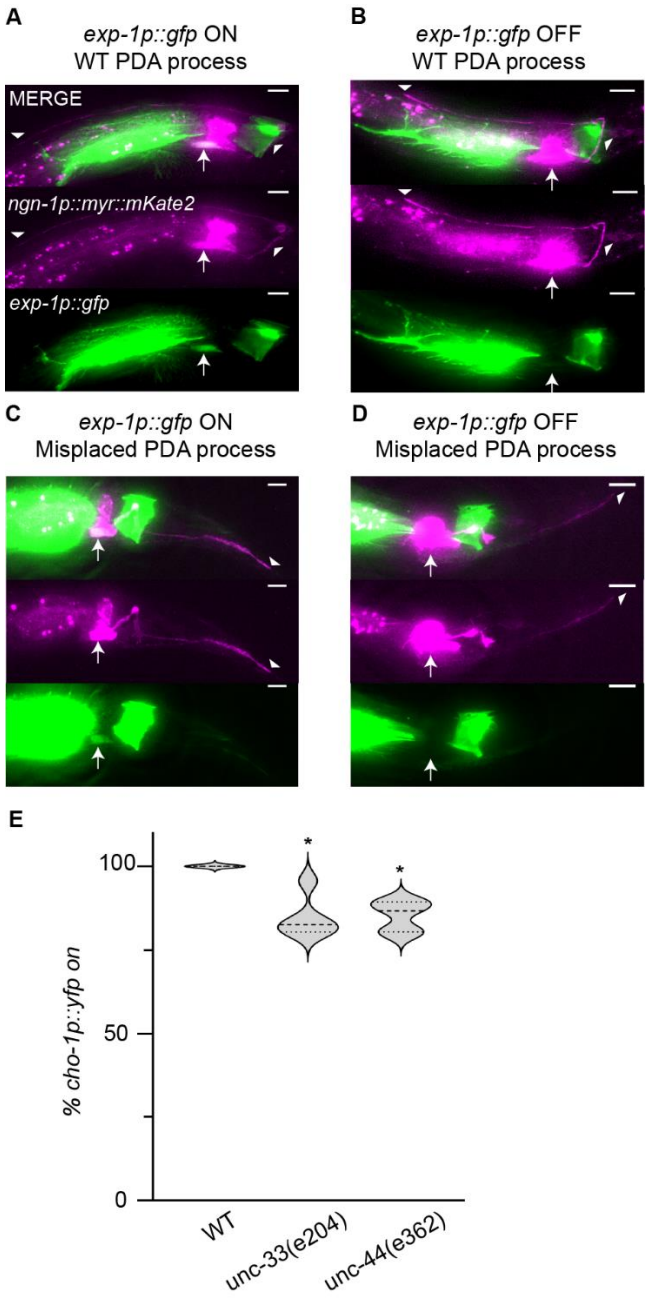

Figure S5: Defects observed in mutants of the cytoskeleton organizing genes *unc-119, unc-44,* and *unc-33*. Related to Figure 5. (A) A wild-type PDA mutant in an *unc-44(e362)* mutant. The PDA process is properly placed and *exp-1p::gfp* expression is on. (B) *unc-44(e362)* mutant; PDA process is properly placed, but *exp-1p::gfp* is not expressed. (C) *unc-44(e362)* mutant, PDA process is improperly placed, but *exp-1p::gfp* is expressed. (D) PDA process is improperly placed, and *exp-1p::gfp* is not expressed. Arrows, cell body. Arrowheads, cellular process. Scale bars, 5 µm. (E) Percent of animals expressing *cho-1p::cho-1::SL2::NLS::yfp* in the indicated genotypes in properly placed axons. Data shown as violin plots. n > 130 animals, n = 5 replicates for all genotypes. One-way ANOVA with Dunnett's post-hoc test. Significance at $p < 0.01$.

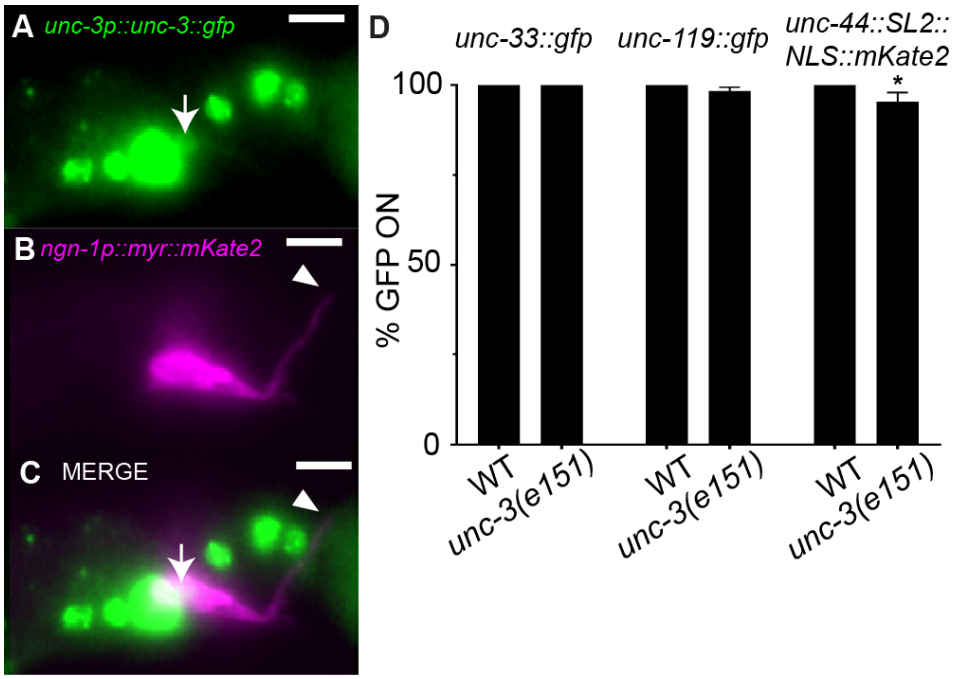

Figure S6: Determining the temporal relationship between *unc-3* and *unc-119, unc-44,* and *unc-33.* Related to Figure 5. (A-C) *unc-3p::unc-3::gfp* expression is initiated earlier than *unc-119*, *unc-44*, or *unc-33* judged by the length of the PDA axon. Compare to Figure 5F,J. Scale bars, 5 µm. Arrow, PDA nucleus. Arrowhead, axon tip. (D) Percent of animals expressing indicated reporters in indicated genotypes. Error Bars, SEM. n > 44 animals; n > 3 replicates for all genotypes. Two-way ANOVA with Sidak's post-hoc test. Significance at $p < 0.01$.

Table S1: Results of a forward genetics screen for loss of *exp-1p::gfp* expression. Related to Figure 2.

Gene Name	Allele	Number of Isolated Alleles	Molecular Lesions
hlh-16	*ns196*	1	R50C, See Figure 3
mab-9	*ns187* *ns206*	2	G282D
ngn-1	*ns185* *ns194*	2	Q17X, See Figure 3 Q29X
sem-4	*ns188* *ns189* *ns192*	3	S520G S520G G295X
unc-119	*ns200*	1	G79X, See Figure 3
Unidentified	*ns184* *ns186* *ns193* *ns195* *ns197* *ns199* *ns201*	7	