Developmental stage-dependent transcriptional regulatory pathways control neuroblast lineage progression

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SUMMARY
Neuroblasts generate neurons with different functions by asymmetric cell division, cell cycle exit and differentiation. The underlying transcriptional regulatory pathways remain elusive. Here, we performed genetic screens in C. elegans and identified three evolutionarily conserved transcription factors (TFs) essential for Q neuroblast lineage progression. Through live cell imaging and genetic analysis, we showed that the storkhead TF HAM-1 regulates spindle positioning and myosin polarization during asymmetric cell division and that the PAR-1-like kinase PIG-1 is a transcriptional regulatory target of HAM-1. The TEAD TF EGL-44, in a physical association with the zinc-finger TF EGL-46, instructs cell cycle exit after the terminal division. Finally, the Sox domain TF EGL-13 is necessary and sufficient to establish the correct neuronal fate. Genetic analysis further demonstrated that HAM-1, EGL-44/EGL-46 and EGL-13 form three transcriptional regulatory pathways. We have thus identified TFs that function at distinct developmental stages to ensure appropriate neuroblast lineage progression and suggest that their vertebrate homologs might similarly regulate neural development.

KEY WORDS: C. elegans, Asymmetric cell division, Neuroblast development

INTRODUCTION
Neuroblast lineage progression is a fundamental biological process in which neural stem cells asymmetrically divide, exit the cell cycle and differentiate into distinct neurons. Defects in neuroblast lineage progression cause abnormal neural circuits and neurological disorders (Ming and Song, 2011; Zhao et al., 2008). Despite recent progress in understanding the transcriptional control of neurogenesis (Hsieh, 2012), the full inventory of transcription factors (TFs) and regulatory networks underlying neuroblast development remains largely unknown.

The C. elegans Q neuroblasts divide three times to generate three distinct neurons (oxygen sensory, mechanosensory and interneuron) and two apoptotic cells in L1 larvae (Fig. 1A) (Sulston and Horvitz, 1977). The anterior and posterior daughters of Q neuroblasts, Q.a and Q.p, employ distinct cellular mechanisms in their asymmetric divisions (Ou et al., 2010). A polarization of myosin-based contractility is the primary driver for the production of two distinct daughter cells in Q.a division (Ou et al., 2010), and the myosin polarization also occurs in Drosophila neuroblast asymmetric division (Cabant Porn et al., 2010). In Q.p division, asymmetric daughter cell sizes and fates arise from the initial displacement of the mitotic spindle towards one side of the cell, which is similar to what occurs in the first division of the C. elegans embryo (Gönczy, 2008; Ou et al., 2010). Pioneering C. elegans genetics uncovered that a POU domain TF, UNC-86, acts to modify latent reiterative cell lineages of the Q neuroblast; Q.a divides normally but Q.p repeats the division pattern of the mother neuroblast in unc-86 mutants (Chalfie et al., 1981). Additional transcriptional regulatory pathways that determine the distinct Q cell division patterns (e.g. Q.a division) have yet to be identified.

Neuroblast division needs to be precisely regulated. In Q neuroblasts, Q.a only divides once whereas Q.p divides twice (Sulston and Horvitz, 1977). The Hippo signaling pathway consists of a core kinase cascade that controls organ size and the development of human cancers. Upon activation in Drosophila, Hippo kinase phosphorylates and activates Warts kinase, which inactivates Yorkie by phosphorylation; Yorkie is a transcriptional coactivator of a TEA domain (TEAD) family TF, Scalloped, which induces gene transcription to promote cell proliferation and inhibit apoptosis (Pan, 2010; Zhao et al., 2011). In C. elegans, CST-1/2, WTS-1, YAP-1 and EGL-44 are homologs to Hippo, Warts, Yorkie and Scalloped, respectively, and they play diverse roles in life span, development and neuronal fate determination (Cai et al., 2009; Iwasa et al., 2013; Kang et al., 2009; Wu et al., 2001). However, none of them has been shown to regulate C. elegans cell cycle progression. Prior genetic studies reported that EGL-44 and EGL-46 (a homolog of human insulinoma-associated protein) control the fate of the touch cell FLP and that EGL-46 is essential for cell cycle exit in Q.ap and Q.paa cells (Wu et al., 2001), but the function of EGL-44/Scalloped in C. elegans neuroblast proliferation remains unclear.

Neuronal differentiation involves numerous cell type-specific transcriptional regulatory cascades that determine neuronal fate (Hobert, 2011). A LIM domain TF, MEC-3, positively regulates the expression of mechanosensory genes in one of three Q cell progenies (Way and Chalfie, 1988). Equally intriguing is the inhibition of the touch fate in non-mechanosensory neurons. The C. elegans C2H2-type zinc-finger TF PAG-3 is the homolog of the Drosophila Senseless proteins and represses touch neuron-specific genes in BDU interneurons (Cameron et al., 2002; Jia et al., 1996; Jia et al., 1997). However, little is known about the repression of the touch fate in Q cell progenies that do not function as the mechanosensory neuron.

This study performed large-scale forward genetic screens, through which we identified three evolutionarily conserved TFs.
We confirmed that these mutants were correctly cloned. All of the canonical alleles of these genes have the same phenotypes as our newly isolated mutant alleles (supplementary material Fig. S1B). GFP- or mCherry-tagged translational fusion constructs of these genes successfully rescued the corresponding mutant phenotypes (supplementary material Fig. S1C).

Live cell imaging
C. elegans L1 larvae were anesthetized with 0.1 mM levamisole (Sigma) in M9 buffer, mounted on 3% agarose pads, and maintained at 20°C. Our imaging system includes an Axio Observer Z1 microscope (Carl Zeiss MicroImaging) equipped with a 100×, 1.45 N.A. objective, an EM CCD camera (Andor XePlex DU-897D-C00-01B), and the 488-nm and 568-nm lines of a Saphire CW CDRH USB laser system or an argon and krypton laser attached to a spinning disk confocal scan head (Yokogawa CSU-X1 Spinning Disk Unit). Time-lapse images were acquired with exposure time of 300 milliseconds every 30 or 60 seconds by Focus Image software (developed by Mr Xiang Zhang at the Institute of Biophysics, Chinese Academy of Sciences). We used ImageJ software (NIH) to process images. In all images shown, the anterior of Q cells or C. elegans is to the left.

Quantification and statistical analysis
Quantification of spindle position and daughter cell size ratio followed our published protocol (Ou et al., 2010). Spindle positioning was quantified by the ratio of the distance of the anterior and posterior centrosomes to the anterior and posterior cell poles (Pa, Pp). Daughter cell size ratio was calculated by Q.tp/Q.p to Q.pp/Q.pa. Quantification of GFP fluorescence intensity of PIG-1::GFP in wild type (WT) and mutants followed our published methods (Ou and Vale, 2009). The fluorescence intensity of PIG-1 was calculated as fluorescence divided by area using ImageJ. Student’s t-test or χ2 analysis was used to examine statistical differences in daughter Q cell size ratio or extra neuron phenotypes in WT and mutants.

HAM-1 binding peak display
Integrated Genome Browser 6.7.3 was used to analyze HAM-1 binding peaks at the pig-1 promoter. The CHIP-Seq data were downloaded from the modENCODE consortium (www.modencode.org).

Yeast two-hybrid analysis
Sequences encoding EGL-44 and EGL-46 full-length proteins or truncations were cloned into a DNA activation domain (AD) vector (pGADT7, Clontech) and a binding domain (BD) vector (pGBK7T, Clontech) by the in-fusion cloning technique. Plasmids were verified by DNA sequencing. The resulting AD and BD constructs were transformed into the yeast strain AH109 using the Yeastmaker Yeast Transformation System 2 (Clontech). Transformants carrying both AD and BD vectors were initially selected on selective complete agar –Leu –Trp –Ade –His medium. To screen protein-protein interaction, a single clone was streaked on the interaction selection media (synthetic complete agar –Leu –Trp –Ade –His) to score growth.

GST fusion protein pull-down assay
E. coli BL21 (DE3) was used to express GST, GST-EGL-44 or His-EGL-46 with induction by 0.3 mM IPTG for 12 hours at 16°C. Bacterial culture was collected and disrupted in lysis buffer (PBS pH 7.32, with 140 mM NaCl, 2.7 mM KCl, 10 mM NaHPO4 and 1.8 mM KH2PO4) using a microfluidizer. Bacterial lysate containing His-EGL-46 was incubated with GST lysate or GST-EGL-44 for 30 minutes at 4°C, and glutathione-agarose beads 4B (GE Healthcare) were added to immobilize GST or GST-EGL-44 for another hour at 4°C. The beads were washed three times with PBS. The bound proteins were eluted with elution buffer (10 mM reduced glutathione in PBS) and the eluents were subjected to western blotting with anti-His and anti-GST antibodies (Zhonghan Golden Bridge).

RESULTS
Identification of three evolutionarily conserved transcription factors essential for Q neuroblast lineage progression
To identify factors that control Q neuroblast lineage progression, we carried out GFP- and mCherry-based genetic screens. We used
a mechanosensory neuron-specific reporter construct (Pmec-4::gfp) and an oxygen sensory neuron-specific reporter construct (Pgcy-32::mCherry) to isolate mutations that alter the numbers of both neuronal types (Fig. 1B). The mec-4 promoter drives GFP expression in six mechanosensory neurons, two of which, A VM and PVM (A/PVM), are derived from Q.p asymmetric divisions. The gcy-32 promoter drives mCherry expression in four oxygen sensory neurons, two of which, AQR and PQR (A/PQR), are produced by the Q.a lineage. Genetic screens isolated mutations with extra neurons from Q cell lineages. Pmec-4::gfp marks AVM and PVM (green) and Pgcy-32::mCherry labels AQR and PQR (red). See text for further details of the screen. (C) Percentages of extra AVM/PVM (green) or AQR/PQR (red) in mutants. Mutations altered ham-1, egl-44, egl-46 and egl-13 (see Materials and methods). n=127-194. (D) Gene structures. The genomic region corresponding to the largest unspliced RNA of each isolated gene is indicated; boxes represent exons, solid lines represent introns. Coding regions of conserved domains Storkhead, TEA/ATTS, zinc-finger and Sox are in orange, blue, purple and green, respectively. Nucleotide substitution or deletion mutations are indicated. Asterisks indicate mutation loci.

We identified 13 mutants that did not map to the known loci. Using genetic techniques, we showed that these mutants caused molecular lesions in three evolutionarily conserved TFs (Fig. 1C,D; supplementary material Fig. S1, Fig. S2A; see Materials and methods; BLAST E-values range from 6.5e−21 to 2.7e−71): HAM-1 is a homolog of human storkhead box 1 (STOX1) (Frank et al., 2005); EGL-44 is a homolog of human transcriptional enhancer factor TEAD5 (TEAD3) (Wu et al., 2001); and EGL-13 is a homolog of the human TF SOX5 (Hanna-Rose and Han, 1999). The functions
of these TFs in neuroblast lineage progression are not well understood. Our study addresses the interaction between EGL-44 and EGL-46, and we isolated six alleles of egl-46 (Fig. 1C,D).

We next used live cell imaging analysis to determine which cellular processes were defective in these mutants. Among existing mutants, we focused on ham-1(cas46), egl-44(cas6), egl-46(cas36) and egl-13(cas11) because these alleles include a deletion (cas46), the most severe extra neuron phenotype (cas6), or the earliest stop codon (cas36 and cas11).

**HAM-1 regulates spindle positioning and myosin polarization**

HAM-1 was previously shown to control *C. elegans* embryonic but not larval neuroblast asymmetric cell divisions (Frank et al., 2005; Guenther and Garriga, 1996). Although human STOX1/HAM-1 is abundantly expressed in the brain (van Dijk et al., 2010), its neuronal function is largely unknown. We confirmed that Q.p development is independent of ham-1 as no extra A/PQR were generated in ham-1 mutants (Fig. 1C). However, ham-1 mutants generated extra A/PQR neurons (Fig. 1C), indicating that HAM-1 specifically regulates Q.a development.

We studied Q cell asymmetric divisions in ham-1 and other TF mutants by visualizing the dynamics of the GFP-tagged centrosome, mCherry-labeled chromosome and plasma membrane. We found that only Q.a asymmetric division was defective in ham-1 mutants (Fig. 2A,E). In WT animals, Q.a positioned its spindle in the cell center, and an anterior accumulation of myosin during cytokinesis was the likely cause of the generation of a small Q_aa and a large Q_ap (Fig. 2A,C) (Ou et al., 2010). In ham-1 mutants, 62% of QR.a (n=13) properly positioned their spindles, but myosin was evenly distributed in the contractile ring, producing two equal daughter cells; for the remaining 38% of QR.a, the spindle was shifted towards the posterior and myosin symmetrically distributed, producing a large QR.aa and a small QR.ap (Fig. 2A-D; supplementary material Fig. S2B,C).

HAM-1 contains a predicted winged helix DNA-binding motif (Fig. 1D), suggesting that it might function as a TF inside the nucleus. However, prior immunofluorescence studies reported that HAM-1 is asymmetrically localized on the cortex in neuroblasts (Frank et al., 2005; Guenther and Garriga, 1996). We examined the expression and localization of HAM-1 in Q cells using a GFP reporter. The modENCODE consortium constructed an integrant line expressing a GFP-tagged HAM-1 protein under the control of the ham-1 promoter (Pham-1). The Pham-1::ham-1::gfp transgene was functional as it reduced the extra A/PQR phenotype from 31% (n=196) to 9% (n=203) in ham-1 mutants (supplementary material Fig. S1C). By quantifying daughter cell size asymmetry, we found that the transgene partially rescued the asymmetric cell division defects in ham-1 mutants (P<0.01, Student’s t-test; the distribution of the raw data is shown in supplementary material Fig. S1D), which correlated with the partial rescue of the extra neuron phenotype. Using this reporter, HAM-1::GFP fluorescence was visible in both Q.a and Q.p as well as in their neighboring cells (supplementary material Movie 1). Time-lapse imaging analysis showed that HAM-1::GFP was restricted to interphase nuclei as with other TFs (e.g. EGL-44, EGL-46 and EGL-13, supplementary material Fig. S3B) and that HAM-1::GFP was evenly distributed in the cytoplasm of dividing Q.a (n=14) and Q.p (n=10) cells (Fig. 3A; supplementary material Movie 1). The dynamic distribution of HAM-1 suggested that it might function as a TF in Q cells.
HAM-1 promotes pig-1 expression for Q cell asymmetric division

To understand how HAM-1 regulates Q.a asymmetric division, we searched for its transcriptional regulatory target. The modENCODE consortium used chromatin immunoprecipitation coupled with high-throughput DNA sequencing (CHIP-seq) to search for the genomewide binding sites of HAM-1 in *C. elegans* L1 larvae. From the original datasets, we uncovered that HAM-1 is bound to the promoter region of the *pig-1* gene (−266 to −42 bp relative to the ATG; Fig. 3B). PIG-1 is a member of the conserved PAR-1/Kin1/SAD-1 family of serine/threonine kinases that regulate polarity and asymmetric cell division, and the inhibition of *pig-1* phenocopied the *ham-1* extra neuron phenotype in the Q.a cell (Cordes et al., 2006). Our previous imaging analysis showed that myosin polarization during Q.a cytokinesis was disrupted in *pig-1* mutants (Ou et al., 2010). Furthermore, the prior genetic analysis suggested that *pig-1* acted downstream of *ham-1* (Cordes et al., 2006). Thus, *pig-1* is a likely functional target of HAM-1.

We first examined whether HAM-1 promoted *pig-1* expression in Q cells. We constructed a strain expressing P*pig-1::pig-1::gfp* and showed that the transgene was functional because it reduced extra A/PQR neurons in *pig-1*(gm344) mutants from 19% (n=154) to 2% (n=174). After crossing the transgene into *ham-1* mutants, we compared the expression of P*pig-1::pig-1::gfp* in WT and *ham-1* mutants. The P*pig-1::gfp* fluorescence was visible in all the WT Q.a cells (n=18), but was significantly reduced in *ham-1* mutants (Fig. 4A,B). PIG-1::GFP fluorescence did not change in neighboring seam cells of *ham-1* mutants (Fig. 4A,B, asterisks), suggesting that we could use PIG-1::GFP in seam cells as a fiduciary marker to further quantify PIG-1::GFP changes in Q cells. We measured the fluorescence intensity ratio between Q.a and seam cells within the same animal. PIG-1::GFP in Q.a was 3.3-fold brighter than that of the seam cell (n=18) in WT animals, but this intensity ratio was reduced to 1.4 in *ham-1* mutants (n=24; P<0.005, Student’s t-test) (Fig. 4C). Thus, HAM-1 positively regulates *pig-1* expression in Q.a.

To study the significance of the HAM-1 binding site in the *pig-1* promoter, we deleted it from a functional P*pig-1::pig-1::gfp* plasmid. This completely abolished *pig-1::gfp* expression in the Q.a cell (100%, four independent transgenic lines, 10-15 worms were examined from each line; Fig. 4D, right). As a negative control, the deletion of another region in the pig-1 promoter (−633 to −407 from 266 to −407 bp relative to the ATG) phenocopied the *ham-1* extra neuron phenotype in the Q.a cell (100%, four independent transgenic lines, 10-15 worms were examined from each line; Fig. 4D, left). Still images show PIG-1::GFP fluorescence in WT (left) and *ham-1* mutant (right). PIG-1::GFP was expressed by the *pig-1* promoter (green, top). Q cell plasma membrane and chromosomes were marked with mCherry (red, middle). In the merged images (bottom) arrows point to the Q.a cell. Asterisks (A,D) indicate seam cells. (B) Quantification of PIG-1::GFP fluorescence intensities in the Q.a cell or seam cell of WT or *ham-1* mutants. (C) Quantification of the PIG-1::GFP fluorescence intensity ratio between the Q.a cell and seam cell in WT and *ham-1* mutants. (B,C) n=18-24; error bars indicate s.d. Student’s t-tests were used. (D) PIG-1::GFP expression under the control of the *pig-1* promoter (left) or with the deletion of a negative control fragment (middle) or the HAM-1 binding site (right). PIG-1::GFP (green, top) Q cell (mCherry, red, middle) and merge (bottom), in which arrows point to Q,a and Q.p. (E) The extra A/PQR neuron phenotype in *ham-1* mutants and in *ham-1* animals that expressed *pig-1::gfp* via HAM-1-independent promoters. χ2 analysis was used; N.S., not significant. n=52-154. AP indicates that anterior is to left. Scale bars: 5 μm.
the ATG) did not change PIG-1::GFP fluorescence in three independent transgenic lines (Fig. 4D, middle). Thus, the HAM-1 binding site in the pig-1 promoter is essential for pig-1 expression.

To validate the role of HAM-1 in the transcriptional regulation of pig-1 further, we examined whether the expression of pig-1 by HAM-1-independent promoters, such as Pegl-17, Pgcv-32 or Pmec-7, could rescue the defects in Q.a asymmetric division in ham-1 mutants. We introduced a transgene that expresses egl-17::pig-1::gfp mutants. By quantifying A/PQR neuron numbers, we found that the Pegl-17::pig-1::gfp transgene partially reduced the occurrence of extra A/PQR from 31% to 17% in ham-1 mutants (P<0.01 by χ² analysis; Fig. 4E), suggesting that bypassing HAM-1 regulation of the pig-1 promoter partially rescued asymmetric division defects in ham-1 mutants. The rescue depended on the developmental stage. We expressed pig-1::gfp under the Pgcv-32 or Pmec-7 promoter, neither of which is active until Q cell differentiation, and did not observe any rescue of the extra A/PQR phenotype (Fig. 4E).

Taken together, HAM-1 positively regulates pig-1 expression during Q.a asymmetric division, possibly through the pig-1 promoter region.

EGL-44 and EGL-46 bind to each other and control cell cycle exit

Prior studies reported that EGL-44 and EGL-46 repress touch cell fate in FLP cells and that EGL-46 regulates cell cycle exit in Q cell lineages (Desai and Horvitz, 1989; Wu et al., 2001). However, whether EGL-46 is involved in cell cycle regulation has not been determined. We uncovered multiple alleles of egl-44 and egl-46 with the ectopic gain of A/PQR neurons (Fig. 1C,D). Time-lapse recording showed that EGL-44 controls cell cycle exit in Q.ap and Q.paa cells (Fig. 5A; supplementary material Movies 2, 3). Using QR.ap as an example, QR.ap differentiated into AQR in WT animals (Fig. 5A); however, QR.ap had one extra round of division at 158±19 minutes after birth in egl-44 mutants (n=9) or 158±34 minutes after birth in egl-46 animals (n=6) (Fig. 5A). Both QR.ap daughter cells differentiated into neurons expressing oxygen sensory neuron-specific genes, resulting in extra A/PQR in egl-44 or egl-46 mutants (Fig. 1C, Fig. 5A; supplementary material Movies 2, 3).

We next examined the expression pattern of egl-44 and egl-46. egl-44 was expressed throughout the Q cell lineage, whereas egl-46 was only expressed in a subset of this lineage. Initially, egl-46 was not expressed in the Q neuroblast, and in the Q.a lineage it started to be expressed in the Q.a cell, whereas in the Q.p lineage its expression was restricted to Q.paa and was absent from Q.pap (supplementary material Fig. S3A) (Wu et al., 2001). Cell cycle exit in egl-46 mutants is only defective in Q.ap and Q.paa cells, indicating that EGL-46 is specifically expressed in cells where it is essential.

Since egl-44 and egl-46 mutants have the same phenotype in terms of cell cycle regulation and FLP cell differentiation, we examined whether they physically bind to each other. Yeast two-hybrid assays detected the interaction between full-length EGL-44 and EGL-46 (Fig. 5B,C). We narrowed down the minimal regions for their association; the EGL-44 N-terminal fragment, including the TEAD domain, was sufficient to bind to the EGL-46 C-terminal fragment, including the zinc-finger domain (Fig. 5B,C). We used

Fig. 5. EGL-44 binds to EGL-46 and both control Q cell cycle exit. (A) QR.ap differentiated in WT (upper) or underwent one extra round of division in egl-44 (middle) or egl-46 (lower) mutants. Arrows indicate the QR.ap dendrite in WT (140, 175 minutes) or an extra round of division of QR.ap in egl-44 and egl-46 (150 minutes). Time is in minutes. Scale bar: 5 μm. (B) Overview of EGL-44 and EGL-46 interaction. Dashed lines indicate interaction domains. (C) Interaction between EGL-44 and EGL-46 in the yeast two-hybrid system. Yeast transformants expressing both Gal4 DNA-binding domain (BD)-EGL-44 full-length (1-286) or N(1-108) or C(109-286) terminal truncation fusions and the Gal4 transcription activation domain (AD)-EGL-46 full-length (1-486) or N(1-170) or C(171-486) terminal truncation fusions were streaked on synthetic complete medium lacking Trp and Leu (SC-2, left) or lacking Trp, Leu, His and Ade (SC-4, right). Growth on SC-4 indicated interaction between two tested fusion proteins. (D) Interaction between EGL-44 and EGL-46 in a GST fusion protein pull-down assay. GST-EGL-44, His-EGL-46 and the control GST were used in the binding reactions. Shown are detections of GST-EGL-44 (top), GST (middle) and His-EGL-46 (bottom) in western blots. (E) Q cell-specific expression of the human EGL-44 homolog TEF5 (TEAD3) under the control of the egl-17 promoter rescued the extra AQR/PQR phenotype of the egl-44 mutant. *P<0.005, Student’s t-test; n=22-35. (F) Yeast two-hybrid assay of C. elegans EGL-46 and human TEF5.
protein pull-down assays with glutathione S-transferase (GST) fusion proteins to show that His-tagged EGL-46 bound to GST-tagged EGL-44 but not to the GST protein alone (Fig. 5D). Thus, EGL-44 and EGL-46 might function in a transcriptional regulatory protein complex to promote cell cycle exit.

**EGL-13 determines neuronal fate**

EGL-13 belongs to an SRY box (Sox)-containing gene family (Fig. 1D). EGL-13 was initially identified from defects in the connection between the uterus and the vulva in *C. elegans* (Cinar et al., 2003; Hanna-Rose and Han, 1999). SOX5 and SOX6, which are vertebrate homologs of EGL-13, are involved in chondrogenesis and in the cell cycle progression of neural progenitors in the chick spinal cord; however, little is known about their function in neural signaling molecules specifically for oxygen sensation. A/PQR neurons are ciliated, and the ciliogenesis depends on the action of intraflagellar transport proteins (e.g. OSM-6). We compared the expression of A/PQR-specific genes in WT and *egl-13* mutants. All the WT PQR neurons expressed these markers, but PQR neurons in *egl-13* mutants expressed them in 6-73% of *mec-4; gcy-32; gcy-36; tax-4; and glb-5* mutants (Fig. 6C; supplementary material Fig. S4A). In A/PQR, *gcy-32; gcy-36; tax-4; and glb-5* encode membrane receptors or signaling molecules specifically for oxygen sensation. A/PQR neurons are ciliated, and the ciliogenesis depends on the action of intraflagellar transport proteins (e.g. OSM-6). We compared the expression of A/PQR-specific genes in WT and *egl-13* mutants. All the WT PQR neurons expressed these markers, but PQR neurons expressing them in 6-73% of *mec-4; gcy-32; gcy-36; tax-4; and glb-5* mutants (Fig. 6C; supplementary material Fig. S4A). EGL-13 thus determines the neuronal fate in Q cell lineages.

MEC-3 is a key TF for *mec* gene expression, and the ectopic expression of *mec* genes in Q cell progenies do not express *mec* genes in *egl-13; mec-3* double mutants (supplementary material Fig. S4C, right). We also

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**Fig. 6. EGL-13 determines neuronal fate.**

(A) Mechanosensory neurons were marked by *Pmec-4::gfp* in WT and *egl-13* mutants. Q cells were labeled by mCherry fused with a myristoylation signal and histone. (B) Oxygen sensory neurons were marked by *Pgcy-32::mCherry* (top) or *Pgcy-36::gfp* (bottom) in WT and *egl-13* mutants. Q cells were marked by cytosolic GFP (top) or were labeled as in A (bottom). (C) Quantification of *QLap* expression in A/PQR, showing that not every *mec* mutant (green). *n*=12-33. The *unc-25* gene was used as a control to show that not every neuronal fate was changed in *egl-13* mutants. (D) Schematics showing that ectopic expression of *egl-13* in AVM and PVM changed their neuronal fates. In WT animals, the Qap cell specifically expressed *egl-13* (yellow circle) and differentiated into oxygen sensory neurons (red). The Qap cell lost the properties of an oxygen sensory neuron but acquired the fate of a mechanosensory neuron (green) in *egl-13* mutants. The ectopic expression of *egl-13* caused the abnormal gain of the oxygen sensory property and the loss of the mechanosensory property in A/PVM. (E) Percentage of extra A/PQR neurons that ectopically expressed *Pgcy-32::mCherry* in *egl-13* related genetic backgrounds. *n*=66-102. (F) Quantification of *Pmec-4::gfp* expression in AVM (left) and PVM (right) in different *egl-13*-related genetic backgrounds. No (blue), one (red) or two (green) neurons expressed *Pmec-4::gfp*. *n*=45-98.
found that egl-13 single mutants and egl-13; mec-3 double mutants did not differ in the loss of A/PQR (supplementary material Fig. S4C), indicating that the loss of A/PQR in egl-13 worms does not require mec-3. EGL-13 might repress the touch fate by inhibiting mec-3 and support A/PQR fate independently of mec-3.

**EGL-13 is sufficient for neuronal fate determination**

We studied the expression pattern of egl-13 in Q cell lineages. Using a transcriptional fusion reporter with gfp, we found that egl-13 started to be expressed after Q.a divisions and that GFP fluorescence reached the maximum level in Q.ap upon differentiation (supplementary material Fig. S4D, Movie 4). By contrast, the GFP signal was barely detected in the Q.p lineage, and the mCherry fluorescence in Q cells did not change during Q cell development (supplementary material Fig. S4D, Movie 4). egl-13 was continuously expressed in A/PQR during larval development (supplementary material Fig. S3C, e.g. L3 larval stage), indicating that EGL-13 can be involved in neuronal fate initiation and maintenance.

To address whether EGL-13 is sufficient for neuronal fate determination, we ectopically expressed it in A/PVM, where EGL-13 is normally absent. Two promoters were used for EGL-13::gfp expression; Pegl-17 was active throughout the entire Q cell lineage, whereas Pmec-7 was only switched on in A/PVM upon differentiation (Fig. 6D). Both transgenes could induce ectopic A/PQR neurons (Fig. 6E). For instance, extra AQR neurons were produced in 40% of transgenic animals expressing Pegl-7::egl-13::gfp. Since the Pmec-7 promoter was stronger than Pegl-17 during differentiation, Pegl-7::egl-13::gfp induced a higher percentage of extra A/PQR neurons than Pegl-17::egl-13::gfp (Fig. 6E). In addition, both transgenes inhibited the touch fate in A/PVM. In WT animals, 100% of A/PVM expressed the Pegl-4::gfp reporter. The Pegl-7::egl-13::gfp transgene significantly reduced the number of GFP-positive A/PVM, and 71% of AVM and 94% of PVM lost their GFP fluorescence from Pegl-4::gfp (Fig. 6F). The Pegl-17::egl-13::gfp transgene also inhibited the PVM fate (Fig. 6F).

Collectively, these data demonstrated that egl-13 is necessary and sufficient to determine the neuronal fate in the Q cell lineage.

**The additive action of ham-1, egl-44/egl-46 and egl-13 in Q cell development**

We performed double-mutant analysis to investigate interactions between the TFs. Single-mutant analysis showed that ham-1 controlled Q.a asymmetric division, whereas egl-44/egl-46 regulated Q.ap cell cycle exit (supplementary material Fig. S5A). We found that 7.8% of ham-1::egl-44 (n=51) and 20.5% of ham-1::egl-46 (n=34) double mutants generated four PQR-like neurons (supplementary material Fig. SSB), indicating that the disruption of asymmetric cell division in ham-1 and of cell cycle exit in egl-44/46 could be additive. EGL-13 determined the neuronal property of Q.ap (supplementary material Fig. S5A). We found that 12% of ham-1::egl-13 double mutants (n=80) produced three PVM-like neurons, two of which could arise from additive defects in Q.a asymmetric division of ham-1 and of daughter cell differentiation in egl-13 (supplementary material Fig. SSB). Similarly, 4.2% of egl-44; egl-13 double mutants (n=120) generated three PVM-like neurons, probably resulting from the failures of QL.ap cell cycle exit in egl-44 and of QL.ap daughter cell differentiation in egl-13 (supplementary material Fig. SSB). The double-mutant analysis suggested that three transcriptional regulatory pathways additively control Q neuroblast development.

**Evolutionarily conserved functions of EGL-44 and EGL-13**

To explore the conserved function of the TFs, we expressed human TEF5/EGL-44 and SOX5/EGL-13 in *C. elegans* egl-44 and egl-13 mutants, respectively. The extra A/PQR phenotype was reduced by TEF5 from 92% to 30% in egl-13 worms (Fig. 5E) and the extra A/PVM phenotype was also reduced by SOX5 in egl-13 mutants (Fig. 6F). The ectopic expression of SOX5 in mechanosensory neurons inhibited the touch fate of A/PVM (Fig. 6F). Furthermore, the yeast two-hybrid assay detected the direct interaction of human TEF5 and *C. elegans* EGL-46 (Fig. 5F). These results suggest that human TEF5 and SOX5 could be functional homologs of *C. elegans* EGL-44 and EGL-13.

**DISCUSSION**

This study identified three transcriptional regulatory pathways that sequentially control asymmetric cell divisions, cell cycle exit and differentiation in *C. elegans* Q neuroblast development (supplementary material Fig. S5A). We suggest that homologs of these TFs might have conserved functions in vertebrate neural development.

Our work first demonstrated that HAM-1 specifically controls Q.a but not Q.p division, providing molecular insight into their distinct cell division patterns. We propose that HAM-1 and PIG-1 regulate myosin polarization and the asymmetric segregation of cell fate determinants during the Q.a cell division. In ham-1 or pig-1 mutants, the Q.a cell divided symmetrically and the equal segregation of neural fate determinants transformed the normally apoptotic daughter cell into its sister cell. Alternatively, HAM-1 and PIG-1 might independently control asymmetric cell divisions and the asymmetric segregation of fate determinants. Consistent with this model, Q.a daughter cell size asymmetry was 100% disrupted in *ham-1* or pig-1 mutants, but extra A/PQR neurons were generated in only 31% of *ham-1* or 19% of *pig-1* mutants, indicating that daughter cell sizes do not fully specify neuronal fates. To generate extra neurons, the HAM-1/PIG-1 pathway must cooperate with others pathways (e.g. an inhibition of apoptosis) to allow daughter cell survival and differentiation.

PIG-1 might act differently in Q.a and Q.p development. Although more ectopic neurons are generated from the Q.p lineage (42% extra A/PVM) than those from the Q.a lineage (19% extra A/PQR) in pig-1 mutants (Cordes et al., 2006), Q.a and Q.p asymmetric cell divisions are 100% converted to symmetric division in pig-1 mutants (Ou et al., 2010). These observations are consistent with the notion that PIG-1 may play independent roles in asymmetric cell division and in the asymmetric segregation of fate determinants (Cordes et al., 2006). For neuronal fate determination, PIG-1 might be more essential in the Q.a lineage than in the Q.p lineage, as pig-1 mutants produce more ectopic A/PVM than ectopic A/PQR. For asymmetric cell division, PIG-1 might be more crucial for Q.a asymmetric division than Q.p division, as the PIG-1 protein level is reduced in both Q.a and Q.p of *ham-1* mutants but only Q.a asymmetric division is altered.

HAM-1 might have different functions in embryonic and postembryonic neuroblast lineages. Immunofluorescence studies reported that HAM-1 is asymmetrically localized on the cortex in embryonic neuroblasts and that it is not expressed in larvae (Frank et al., 2005; Guenther and Garriga, 1996). The absence of HAM-1 signal by immunostaining in larvae might be due to inefficient antibody penetration owing to the thick cuticle of *C. elegans*. Using a functional *Pham-1::ham-1::gfp* strain, we did not detect any asymmetric localization of HAM-1 throughout the Q.a and Q.p cell
cycle (Fig. 3A; supplementary material Movie 1). Furthermore, our CHIP-seq data analysis and functional studies demonstrated that HAM-1 could function as a TF to promote pig-1 expression in the Q.a cell.

HAM-1 might transcribe other genes for Q.a divisions. In pig-1 mutants, all the Q.a division was converted into symmetric division. In ham-1 mutants, 68% of Q.a divided symmetrically, while the other 32% made a large Q.a and a small Q.ap by completely reversing the polarity (Fig. 2A,D), which might be caused by the loss of other HAM-1 targets than pig-1. Consistently, the introduction of pig-1 under the control of the egl-17 promoter into Q cells only partially reduced the extra A/PQR of ham-1 mutants (Fig. 4E). It might be that multiple TFs regulate pig-1 expression, as although deletion of the HAM-1 binding site in the pig-1 promoter completely abolished pig-1 expression throughout the entire animal, pig-1 expression was only reduced in the Q cells of ham-1 mutants (Fig. 4A-D). pig-1 expression was reduced in Q.p of ham-1 mutants but Q.p divisions were normal (Fig. 4A, Fig. 2B,D), suggesting that Q.p division might require less PIG-1 than Q.a division.

This study links C. elegans Hippo components to cell cycle regulation. EGL-44, human TEF5 and Drosophila Scalloped are homologous TFs, but our work showed that EGL-44 promotes cell cycle exit (Fig. 5A), which is opposite to the function of TEF5 and Scalloped in promoting cell proliferation. Thus, Hippo components might have modulated functions across species. The physical association of EGL-44 and EGL-46 implied that the vertebrate EGL-46 homolog INSM1 might be a previously unknown component in the Hippo signaling pathway. INSM1 plays a pan-neurogenic role in promoting basal progenitor formation in the radial thickness of the cortical plate, whereas its ectopic expression allows neuroepithelial cells to undergo self-amplification. INSM1 might have modulated functions across species. The physical association of EGL-44 and EGL-46 implied that the vertebrate EGL-46 homolog INSM1 might be a previously unknown component in the Hippo signaling pathway. INSM1 plays a pan-neurogenic role in promoting basal progenitor formation in the neocortex (Farkas et al., 2008), and inhibition of INSM1 reduces the radial thickness of the cortical plate, whereas its ectopic expression allows neuroepithelial cells to undergo self-amplification (Farkas et al., 2008), indicating its potential function in cell cycle regulation. The Drosophila homolog of EGL-46, Nerfin-1, is expressed in neuroblasts and regulates early axon guidance in the central nervous system (Kuzin et al., 2005; Stivers et al., 2000), but its function in neuroblast divisions has yet to be determined.

Extra A/PQR neurons were generated in egl-44/eegl-46 and unc-86 mutants. In egl-44/eegl-46 mutants, an extra round of Q.ap cell division gave rise to extra A/PQR neurons. In unc-86 mutants, extra A/PQR neurons were produced by the reiteration of the Q neuroblast lineage in the Q.p cell (Chalfie et al., 1981). We did not find any changes in egl-44/eegl-46 expression in unc-86 mutants (supplementary material Fig. S3D). EGL-44/EGL-46 determined the touch fate of FLP neurons (Wu et al., 2001) but not in Q cell lineages (Fig. 5A; supplementary material Fig. S5A), and we found that egl-13 was expressed in two AQR neurons of egl-44 mutants (supplementary material Fig. S3E).

Our study has uncovered a novel function of EGL-13/SOX5 family TFs in neuronal fate determination. Vertebrate EGL-13/SOX5 regulates the differentiation of prechondrocytes into chondroblasts (Smits et al., 2004). In the nervous system, SOX5 controls the timing of cell cycle exit by opposing the activity of Wnt/β-catenin in the chicken spinal cord (Martinez-Morales et al., 2010). Mammalian SOX5 regulates the pace of differentiation of corticalfugal neurons by fine-tuning the identity of the various closely related subtypes (Lai et al., 2008). This work has shown that EGL-13 represses the touch fate in non-mechanosensory neurons of the Q cell lineage (Fig. 6). The fate of touch neurons can be inhibited by different transcriptional regulatory mechanisms in different lineages. In C. elegans pag-3 mutants, the mechanosensory genes of ALM touch neurons are ectopically expressed in lineally related BDU interneurons (Cameron et al., 2002; Jia et al., 1996; Jia et al., 1997). Taken together, our study demonstrates that three transcriptional regulatory pathways function at distinct development stages to ensure neuroblast lineage progression in C. elegans. The identification of additional transcriptional targets will further advance our understanding of neuroblast development.

Acknowledgements
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Competing interests statement
The authors declare no competing financial interests.

Author contributions

Supplementary material
Supplementary material available online at http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.098723/-/DC1

References


Fig. S1. *ham-1, egl-44, egl-46 and egl-13 cloning.* (A) Snip-SNP markers (top) and number of recombinants (bottom) of each gene are shown. (B) Extra neuron phenotypes in the canonical alleles. (C) Rescue of extra neuron phenotypes using GFP- or mCherry-tagged HAM-1, EGL-44, EGL-46 and EGL-13. (D) Rescue of daughter cell size asymmetry in *ham-1* mutants by *Pham-1::ham-1::gfp*. In WT animals, Q.aa is half the size of Q.ap. In *ham-1* mutants, Q.aa was 1.1-fold larger than Q.ap, and *Pham-1::ham-1::gfp* reduced the Q.aa/Q.ap ratio to 0.9.
A

Homologues of HAM-1, EGL-44, EGL-46 and EGL-13

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B

Still images show the distribution of GFP-tagged non-muscle Myosin II (NMY-2) during QR.a cytokinesis in WT (asymmetry, B) and ham-1 mutants (symmetry, two examples in C). Plasma membrane and chromosomes (red) were labeled by mCherry fused with a myristoylation signal and histone (HIS-24). Time in seconds is on the top left. Scale bars: 5 μm.

C

Fig. S2. ham-1, egl-44, egl-46 and egl-13 homologs and myosin II asymmetry in the QR.a cell of WT and ham-1 mutants. (A) ham-1, egl-44, egl-46 and egl-13 gene homologs across species. Protein names, protein ID and BLAST E-value are shown. (B,C) Still images show the distribution of GFP-tagged non-muscle Myosin II (NMY-2) during QR.a cytokinesis in WT (asymmetry, B) and ham-1 mutants (symmetry, two examples in C). Plasma membrane and chromosomes (red) were labeled by mCherry fused with a myristoylation signal and histone (HIS-24). Time in seconds is on the top left. Scale bars: 5 μm.
Fig. S3. Expression and localization of EGL-44, EGL-46 and EGL-13 in WT and mutants. (A) Still images of Pegl-44::gfp and Pegl-46::gfp expression patterns at different developmental stages of the Q neuroblast lineage. (B) Still images show the nuclear localization of EGL-44, EGL-46 and EGL-13 at the L1 larval stage. Either GFP (EGL-46 and EGL-13) or mCherry (EGL-44) was fused with the coding sequence of these TFs. Q cells were labeled with GFP (left) or mCherry (middle and right). Merged images are in the bottom row. (C) Expression of egl-13 in AQR (top) and PQR (bottom) in the L3 larval stage. A/PQR was marked with mCherry. (D) Expression of egl-44 (left) and egl-46 (right) in WT (top) and unc-86 mutants (bottom). (E) Expression of egl-13 in AQR of WT (top) and egl-44 mutants (bottom). Scale bars: 5 μm.
**Figure S4.** The neuronal fate in WT and egl-13 mutants. (A) The fate of mechanosensory neurons in WT and egl-13 mutants. Q cell plasma membrane and chromosomes (red) were labeled by mCherry. (B) The fate of oxygen sensory neurons in WT and egl-13 mutants. Cells were marked by cytosolic GFP or labeled as in A with mCherry. The unc-25 gene (bottom right) was used as a control to show that not every neuronal fate was changed in egl-13 mutants. (C) Percentage of AVM, PVM, AQR, PQR or ectopic A/PVM in WT (blue bars), mec-3 (red), egl-13 (green) and mec-3; egl-13 double mutants (purple). $n=62-103$. (D) Still images (left) show egl-13::gfp; membrane chromosome (Q cell). (Middle) Quantification from the frames on the left and Movie 4. (Right) The fluorescence intensity ratio of Pegl-13::gfp markers in Q.a and Q.p cells at birth and at the end of their development ($n=11$ each measurement). mCherry fluorescence in Q cells was used as an internal control. Scale bars: 5 μm.
**Fig. S5. Sequential actions of TFs in Q cell lineage progression.** (A) Summary of Q.a cell phenotype in *ham-1, pig-1, egl-44, egl-46* and *egl-13*. Single-headed arrow shows that HAM-1 promotes *pig-1* expression. Double-headed arrow shows that EGL-44 and EGL-46 bind to each other. (B) Double-mutant phenotypes (left) and summary (right). In *ham-1; egl-44* or *ham-1; egl-46* double mutants, four cells (1-4) express *Pgcy-32::mCherry*. In *ham-1; egl-13* or *egl-44; egl-13* double mutants, two extra cells (1 and 2) express *Pmec-4::gfp*.

**Movie 1. HAM-1::GFP dynamics in Q.a and Q.p asymmetric cell division.** Transgenic *C. elegans* strain (GOU418) expressing GFP-tagged HAM-1 protein (green) and mCherry labeling of Q cell membrane and histone in Q cells (red). Frames were taken every 30 seconds for 40 minutes. The display rate is seven frames per second.
Movie 2. An extra round of division of QR.ap in the egl-44 mutant. *C. elegans* strain (GOU247) expressing mCherry labels the Q cell membrane and histone in Q cells (red) in the egl-44 mutant. Frames were taken every minute for 247 minutes. The display rate is seven frames per second.

Movie 3. An extra round of division of QR.ap in the egl-46 mutant. *C. elegans* strain (GOU463) expressing mCherry labels the Q cell membrane and histone in Q cells (red) in the egl-46 mutant. Frames were taken every 30 seconds for 250 minutes. The display rate is seven frames per second.

Movie 4. The expression of GFP under the control of the egl-13 promoter. Transgenic *C. elegans* strain (GOU774) expressing Pegl-13::gfp (green) and mCherry labeling of the Q cell membrane and histone in Q cells (red). Frames were taken every minute for 131 minutes. The display rate is seven frames per second.
**Table S1. C. elegans strains used in this study**

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<td>zdIs5[Pmec-4::gfp; lin-15(+)]; rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-5:his-24; pRF4(+)]</td>
<td>Cross zdIs5 with rdlvls1</td>
<td></td>
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<tr>
<td><strong>GOU376</strong></td>
<td>ham-1(cas46); zdIs5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>EMS mutagenesis and outcross</td>
<td></td>
</tr>
<tr>
<td><strong>GOU378</strong></td>
<td>ham-1(cas46); casls22[Pegl-17::gfp-TEV-S:cmd-1, Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-5:his-24; pRF4(+)]</td>
<td>Cross cas46 with casls22</td>
<td></td>
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<tr>
<td><strong>GOU381</strong></td>
<td>egl-13(cas11); casEx1505[Pegl-13::egl-13::gfp; Pegl-17::myri-mCherry; Pegl-17::mCherry-TEV-5:his-24; unc-76(+)]</td>
<td>Microinjection</td>
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<tr>
<td><strong>GOU388</strong></td>
<td>ham-1(cas46); wgls102[Pham-1::ham-1::TY1::EGFP::3Xflag; unc-119(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Cross cas46; wgls102; casls35</td>
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<tr>
<td><strong>GOU392</strong></td>
<td>egl-13(cas11); casEx553[Pglb-5::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-5:his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td></td>
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<tr>
<td><strong>GOU393</strong></td>
<td>egl-13(cas11); casEx551[Pgcy-36::gfp; Pegl-17::Myri-mCherry;</td>
<td>Microinjection</td>
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<tr>
<td>Gene Symbol</td>
<td>Modified Gene</td>
<td>Modification</td>
<td>Source</td>
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<tr>
<td>Pegl-17::mCherry-TEV-S::his-24; unc-76(+)</td>
<td>egl-13(cas11); casEx554[Ptax-4::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU394</td>
<td>zdIs5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]; egl-13(cas12)</td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
</tr>
<tr>
<td>GOU396</td>
<td>muls32[Pmec-7::gfp; lin-15(+)]; rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-S::his-24; pRF4]</td>
<td>cross rdlvls1 with muls32</td>
<td>This study</td>
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<tr>
<td>GOU397</td>
<td>rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; muls17[Posm-6::osm-6::gfp; unc-36(+)]</td>
<td>cross rdlvls1 with muls17</td>
<td>This study</td>
</tr>
<tr>
<td>GOU398</td>
<td>zdls5[Pmec-4::gfp; mec-3(e1338)IV; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>cross zdls5; casls35 with e1338</td>
<td>This study</td>
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<tr>
<td>GOU406</td>
<td>zdls5[Pmec-4::gfp; lin-15(+)]; mec-3(e1338)IV; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>cross e1338 with egl-13(cas11); zdls5; casls35</td>
<td>This study</td>
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<td>GOU407</td>
<td>egl-13(cas11); rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; muls32[Pmec-7::gfp; lin-15(+)]</td>
<td>cross rdlvls1 with muls32</td>
<td>This study</td>
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<td>GOU424</td>
<td>casEx1506[Pmec-3::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<td>GOU429</td>
<td>egl-13(cas11); egl-44(cas6); zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Cross egl-13(cas11) with egl-44(cas6); zdls5; casls35</td>
<td>This study</td>
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<tr>
<td>GOU430</td>
<td>egl-13(cas11); rdlvls1[Pegl-17::Myri-mCherry; Pegl-17::mig-10::yfp; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; casEx1506[Pmec-3::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
<td>Strain</td>
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<tr>
<td>GOU438</td>
<td>pegl-17::mCherry-TEV-S::his-24; unc-76(+)</td>
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<tr>
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<td>egl-13(cas11); rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; mns17[Posm-6::osm-6::gfp; unc-36(+)] cross rdvs1 with mns17 This study</td>
<td></td>
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<tr>
<td>GOU444</td>
<td>egl-13(cas11); zdls5[Pmec-4::gfp; lin-15(+)]; rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)] cross cas11 with rdvs1 This study</td>
<td></td>
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<tr>
<td>GOU454</td>
<td>egl-13(cas11); ham-1(cas46); zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)] cross cas11 with cas46 This study</td>
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<tr>
<td>GOU463</td>
<td>egl-46(cas36); rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)] cross cas36 with rdvs1 This study</td>
<td></td>
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<tr>
<td>GOU464</td>
<td>rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; juls76[Punc-25::GFP; lin-15(+)] cross rdvs1 with juls76 This study</td>
<td></td>
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<tr>
<td>GOU465</td>
<td>egl-13(cas11); rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; juls76[Punc-25GFP; lin-15(+)] cross rdvs1 with juls76 This study</td>
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<td>GOU473</td>
<td>egl-46(cas36); ham-1(cas46); casls22[pegl-17::gfp-TEV-S::cmd-1, pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)] Cross with cas36; cas46; casls22 This study</td>
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<td>GOU477</td>
<td>egl-44(cas6); ham-1(cas46); casls22[pegl-17::gfp-TEV-S::cmd-1, pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)] Cross with cas6; cas46; casls22 This study</td>
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<td>GOU489</td>
<td>zuls20[par-3::par-3::ZF1::GFP; unc-119(+)]; rdvs1[pegl-17::Myri-mCherry; pegl-17::mCherry-TEV-S::his-24; pRF4(+)] Cross zuls20 with rdvs1 This study</td>
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<tr>
<td>GOU498</td>
<td>ham-1(gm279); zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)] Cross gm279 with GOU246 This study</td>
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<tr>
<td>Line</td>
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<td>Method</td>
<td>Source</td>
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<td>GOU517</td>
<td>ham-1(cas46); zuls45[PNmy-2::nmy-2::gfp; unc-119(+)]; rdvls1[ Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]</td>
<td>Cross with cas46; zuls45; rdvls1</td>
<td>This study</td>
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<tr>
<td>GOU518</td>
<td>egl-13(cas11); casEx588[ Pmec-18::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
<td>GOU519</td>
<td>egl-13(cas11); ham-1(gm279); zdls5[ Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Cross cas11 with casls35</td>
<td>This study</td>
</tr>
<tr>
<td>GOU60</td>
<td>casls22[ Pegl-17::gfp-TEV-S::cmd-1, Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]</td>
<td>Microinjection and integration</td>
<td>This study</td>
</tr>
<tr>
<td>GOU619</td>
<td>egl-13(cas11); ayls9[Pegl-17::gfp; dpy-20(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>cross cas11 with ayls9</td>
<td>This study</td>
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<tr>
<td>GOU631</td>
<td>casEx609[Pegl-17::sox5::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; egl-13(cas11); zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU663</td>
<td>casEx609[Pegl-17::sox5::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU670</td>
<td>casEx1513[ Pegl-17::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU672</td>
<td>egl-13(cas11); casEx1513[ Pegl-17::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU673</td>
<td>casEx1515[Pmec-7::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]; zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>Code</td>
<td>Description</td>
<td>Method</td>
<td>Notes</td>
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<tr>
<td>GOU734</td>
<td><code>par-2(or640); casls22[Pegl-17::gfp-TEV-S::cmd-1; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]</code></td>
<td>Cross <code>or640</code> with <code>casls22</code></td>
<td>This study</td>
</tr>
<tr>
<td>GOU735</td>
<td><code>par-2(or640); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]</code></td>
<td>Cross with <code>or640</code>; <code>zdls5</code>; <code>casls35</code></td>
<td>This study</td>
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<tr>
<td>GOU739</td>
<td><code>ham-1(cas27); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU740</td>
<td><code>ham-1(cas137); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU764</td>
<td><code>egl-46(cas25); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<td>GOU765</td>
<td><code>egl-46(cas36); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU766</td>
<td><code>egl-44(cas58); zdls5[Pmec-4::gfp; lin-15(+); casls35[Pgcy-32::mCherry; unc-76(+)]]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU767</td>
<td><code>egl-46(cas133); casls36[Pgcy-32::gfp; unc-76(+)]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU768</td>
<td><code>egl-44(cas140); casls36[Pgcy-32::gfp; unc-76(+)]</code></td>
<td>EMS mutagenesis and outcross</td>
<td>This study</td>
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<tr>
<td>GOU769</td>
<td><code>unc-76(e911); casEX1115[Pegl-46::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]]</code></td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
<td>GOU770</td>
<td><code>unc-76(e911); casEx1116[Pegl-44::gfp; Pegl-17::Myri-mCherry, Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]]</code></td>
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<tr>
<td>GOU771</td>
<td><code>unc-76(e911); casEX1118[Pegl-46::gfp-TEV::egl-46; Pegl-17::Myri-mCherry, Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]]</code></td>
<td>Microinjection</td>
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<tr>
<td>GOU772</td>
<td><code>unc-76(e911); casEX1122[Pegl-17::mCherry-TEV-S::egl-44 b; Pegl-17::Myri-mCherry, Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]]</code></td>
<td>Microinjection</td>
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<td>GOU773</td>
<td>egl-44(cas6); casEX1125[Pegl-17::TEAD3::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-T::his-24; pRF4(+)]</td>
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<tr>
<td>GOU774</td>
<td>unc-76(e911); casEx1501[Pegl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
<td>GOU775</td>
<td>unc-76(e911); casEx1505[Pegl-13::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU776</td>
<td>unc-76(e911); casEx1513[Pegl-17::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU777</td>
<td>unc-76(e911); casEx1515[Pmec-7::egl-13::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU778</td>
<td>unc-76(e911); casEx551[Pgcy-36::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<td>GOU779</td>
<td>unc-76(e911); casEx553[Pglb-5::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
<td>GOU780</td>
<td>unc-76(e911); casEx554[Ptax-4::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<td>GOU781</td>
<td>unc-76(e911); casEx588[Pmec-18::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<td>GOU782</td>
<td>unc-76(e911); casEx609[Pegl-17::Sox5::gfp; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU853</td>
<td>egl-44(cas6); rdlIs1[Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; pRF4(+)]; kuls29[Pegl-13::NLS::GFP; unc-119(+)]</td>
<td>cross cas6 with rdlIs1; kuls29</td>
<td>This study</td>
</tr>
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<td>GOU863</td>
<td>casEx849[pPD95.77::pig-1::pig-1a::GFP; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24]; pig-1(gm3 44); zdIs5[Pmec-4::gfp; lin-15(+)]</td>
<td>Microinjection</td>
<td>This study</td>
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<tr>
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<td>GOU868</td>
<td><code>ham-1(cas46);casEx843[Pgcy-32::pig-1a::GFP; Pegl-17::Myri-GFP; Pegl-17::GFP-TEV-S::his-24; casls35[Pgcy-32::mCherry; unc-76(+)]</code></td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU878</td>
<td><code>unc-86(e1416); casEx1115[Pegl-46::gfp; Pegl-17::Myri-mCherry, Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</code></td>
<td>cross e1416 with casEx1115</td>
<td>This study</td>
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<tr>
<td>GOU886</td>
<td><code>unc-86(e1416); casEx1116[Pegl-44::gfp; Pegl-17::Myri-mCherry, Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</code></td>
<td>cross e1416 with casEx1116</td>
<td>This study</td>
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<td>GOU923</td>
<td><code>ham-1(cas46);casEx831[mec-7::pig-1a::GFP; Pegl-17::Myri-GFP, Pegl-17::GFP-TEV-S::his-24; casls35[Pgcy-32::mCherry; unc-76(+)]</code></td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU924</td>
<td><code>ham-1(cas46);casEx840[Pegl-17::pig-1a::GFP; Pegl-17::Myri-GFP, Pegl-17::GFP-TEV-S::his-24; zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</code></td>
<td>Microinjection</td>
<td>This study</td>
</tr>
<tr>
<td>GOU937</td>
<td><code>egl-46(n1127); zdls5[Pmec-4::gfp; lin-15(+)]; casls35[Pgcy-32::mCherry; unc-76(+)]</code></td>
<td>Cross n1127 with GOU246</td>
<td>This study</td>
</tr>
<tr>
<td>GOU938</td>
<td><code>egl-46(cas36); casls223[Pegl-46::gfp-TEV::egl-46; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</code></td>
<td>Cross cas36 with casls223</td>
<td>This study</td>
</tr>
<tr>
<td>GOU939</td>
<td><code>egl-44(cas6); casls224[Pegl-17::mCherry-TEV-S::egl-44bt; Pegl-17::Myri-mCherry; Pegl-17::mCherry-TEV-S::his-24; unc-76(+)]</code></td>
<td>Cross cas6 with casls224</td>
<td>This study</td>
</tr>
<tr>
<td>PCR product</td>
<td>Primer 5'</td>
<td>Primer 3'</td>
<td>Template</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------------</td>
<td>--------------------------------</td>
<td>---------------------------</td>
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<tr>
<td>egl-13 promoter</td>
<td>TCACCTGCCCCCGACATTA</td>
<td>GGTACCAAGCTTTGGGTCTTG</td>
<td>N2 genomic DNA</td>
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<tr>
<td></td>
<td></td>
<td>TCTACGGCTCATGCTGG</td>
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<tr>
<td>gcy-36 promoter</td>
<td>CCTGCTTCGCAAAAAATCAA ACTTCACAT</td>
<td>CATGGTAACAGCTTTGGGTCT</td>
<td>N2 genomic DNA</td>
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<tr>
<td></td>
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<td>CTAAATAAAAAAAAAATTACAT GATTTC</td>
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<tr>
<td>tax-4 promoter</td>
<td>GATTTTGATATGAATCAG AAATCTTGA</td>
<td>CATGGTAACAGCTTTGGGTCT</td>
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<td>TCTTGAAAACATAATTTAATTTT GAGAA</td>
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<tr>
<td>glb-5 promoter</td>
<td>TTCTCGCCCACGATCAC ATTTATCA</td>
<td>CATGGTAACAGCTTTGGGTCT</td>
<td>N2 genomic DNA</td>
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<td></td>
<td>TCCGGTTCTTAATTCGAAATAATTT</td>
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<tr>
<td>mec-3 promoter</td>
<td>TTGATCTAAAGTTTACAT TAATCTG</td>
<td>CATGGTAACAGCTTTGGGTCT</td>
<td>N2 genomic DNA</td>
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<tr>
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<td></td>
<td>GCAATCCAACAGGAGTCTCT AGAC</td>
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<tr>
<td>mec-18 promoter</td>
<td>GCACGCGTAAAGACCC CCTGGATC</td>
<td>CATGGTAACAGCTTTGGGTCT</td>
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<td>GCTCACAACCTCTTGGAGAG CGAG</td>
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<td>gfp::unc-54 3'UTR</td>
<td>AGACCCCAAGCTTTGGTAC CATGAGTAAGGAGAAG AACTTTTCAC</td>
<td>AAGGGCCCGTACGGCCGACT AGTAGG</td>
<td>Plasmid</td>
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<tr>
<td>egl-44 promoter</td>
<td>AAACAAATACTCTTATCCGTTAGC</td>
<td>CATGGTACCAAGCTTTGGGTCT</td>
<td>Fosmid RM0612bC06</td>
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<tr>
<td></td>
<td></td>
<td>AATCTTTGGAAATAAGAACTCCG GTACG</td>
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<tr>
<td>egl-44 gene_short</td>
<td>ACATGGACAGCGGAGG GGAAGGTACTATGTCGGA AGACGTAGCAGTC</td>
<td>AATATCGCAGCTCCGCTTTC</td>
<td>Fosmid WRM0612bC06</td>
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<tr>
<td>Pegl-44::gfp</td>
<td>AAACAAATACTTTATCTTTATCCTTATCTGGTTAGCCGGAAACAGTGTTTGGTATATTGGGG</td>
<td>PCR product pegl-44 promoter and gfp::unc-54 3’ UTR</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------------------</td>
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</tr>
<tr>
<td>Pegl-17::Myri-mCherry-TEV-S::egl-44b</td>
<td>CTCCTGTTCATGGAACATCTCTTAGCAGGACATTCTATGGGAAAGTGATTGAG</td>
<td>PCR product Pegl-17::mcherry-tev-s and egl-44 gene_short</td>
<td></td>
</tr>
<tr>
<td>egl-46 promoter</td>
<td>TTCCAGATGTTTCTTCCCGGACATTATCGCATCAGTTATCC</td>
<td>N2 genomic DNA</td>
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<tr>
<td>egl-46 gene</td>
<td>ACATGGACAGCAGGAGTGGAAGGCTATATGGTGCTATGGAATGACTTTTGGGAAGACATTATCGCATCAGTTATCC</td>
<td>N2 genomic DNA</td>
<td></td>
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<tr>
<td>Pegl-46::gfp</td>
<td>TTCCAGATGTTTCTTCGGAAACAGTGTTTGGTATATTGGGG</td>
<td>PCR product egl-46 promoter and gfp::unc-54 3’ UTR</td>
<td></td>
</tr>
<tr>
<td>Pegl-46::gfp-TEVS::egl-46</td>
<td>TTCCAGATGTTTCTTCGAAGACATTATCGCATCAGTTATCC</td>
<td>PCR product egl-46 promoter, gfp-TEV-S and egl-46 gene</td>
<td></td>
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Table S3. Plasmids constructed for *C. elegans* transgenesis

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Primer 5’</th>
<th>Primer 3’</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pOG233-Pegl-17::TEAD3::gfp</td>
<td>AGCTCACATTTCCGGGC&lt;br&gt;ACCTGAA;&lt;br&gt;CCCGAATGTGAGCT&lt;br&gt;ATGGCGTCCAACAGCT&lt;br&gt;GGAAC</td>
<td>ATGAGTAAGGAG&lt;br&gt;AAGAACKTTTCAC;&lt;br&gt;TTCTCCTTTACTCAT&lt;br&gt;GTCTTTGACGAGCT&lt;br&gt;TGTAAGAC</td>
<td>TEAD3 coding sequence was amplified from human cDNA and inserted into Pegl-17::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG234-Pegl-17::Sox5::gfp</td>
<td>CCCGAATGTGAGCT&lt;br&gt;ATGCTTACTGACCCTGA&lt;br&gt;TTTA</td>
<td>TTCTCCTTTACTCAT&lt;br&gt;GTGGGCCATGCT&lt;br&gt;CAATATG</td>
<td>Sox5 coding sequence was amplified from cDNA and inserted into Pegl-17::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG238-pGEX-6p-1-egl-44</td>
<td>GGATCCCAGGGGCC&lt;br&gt;TGGAA;&lt;br&gt;GGGCCCCTGGGATCCA&lt;br&gt;TGAACTCAATGTTTTGT&lt;br&gt;TCA</td>
<td>GAATTCGCGTTG&lt;br&gt;ACTCGAG;&lt;br&gt;TCGACCCGGGATT&lt;br&gt;CGGCGTCC&lt;br&gt;CGGCTCC</td>
<td>egl-44 coding sequence was amplified from cDNA and inserted into pGEX-6p-1 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG241-pGADT7-egl-44</td>
<td>AGCGTAATCTGGTACG&lt;br&gt;TGTA;&lt;br&gt;GTACCAGATTTAC&lt;br&gt;TGAACCTCAATGTTTTGT&lt;br&gt;TCA</td>
<td>GCAGATGAATCGTA&lt;br&gt;GATACTGA;&lt;br&gt;CTACGATTCATCTGC&lt;br&gt;TCATTCATCAGAAT&lt;br&gt;CAGGCTCC</td>
<td>egl-44 coding sequence was amplified from cDNA and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>p OG242-pGBK T7-egl-44</td>
<td>CAGGTCCTCCTGGG&lt;br&gt;ATCAG;&lt;br&gt;TCAGAGGGACCTGA&lt;br&gt;TGAACTCAATGTTTTGT&lt;br&gt;TCA</td>
<td>GCGGCGCCATAACT&lt;br&gt;AGCATGA;&lt;br&gt;TAGTATGCGGCCC&lt;br&gt;CTATTCATCAGAAT&lt;br&gt;CAGGCTCC</td>
<td>egl-44 coding sequence was amplified from cDNA and inserted into pGBK T7 plasmid via In-Fusion Advantage PCR cloning kit</td>
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<tr>
<td>pOG243-</td>
<td>AGCGTAATCTGGTACG</td>
<td>GCAGATGAATCGTA</td>
<td>egl-46 coding sequence was</td>
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<tr>
<td>Plasmid</td>
<td>DNA Sequence</td>
<td>Protein Sequence</td>
<td>Cloning Method</td>
</tr>
<tr>
<td>---------</td>
<td>--------------</td>
<td>-----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>pGADT7-egl-46</td>
<td>TCGTA; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GATACTGA; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>Amplified from cDNA and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG244-pGBKKT7-egl-46</td>
<td>CAGGTCTCCTCTGAGATCAG; TCGTA; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GCGGCGCATAACTAGCAATGAATCACTCTGA; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>egl-46 coding sequence was amplified from cDNA and inserted into pGBKKT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG251-pGADT7-egl-44(1-170aa)</td>
<td>AGCGTAATCTGGTACGTCGTA; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GCAGATGAATTCGTCGTAATCAG; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>egl-44 (1-170aa) coding sequence was amplified from pOG238 and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG252-pGADT7-egl-44(171-486aa)</td>
<td>AGCGTAATCTGGTACGTCGTA; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GCAGATGAATTCGTCGTAATCAG; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>egl-44 (171-486aa) coding sequence was amplified from pOG238 and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG253-pGBKKT7-egl-46(1-108aa)</td>
<td>CAGGTCTCCTCTGAGATCAG; TCGTA; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GCGGCGCATAACTAGCAATGAATCACTCTGA; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>egl-46 (1-108aa) coding sequence was amplified from human cDNA and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG254-pGBKKT7-egl-46(109-286aa)</td>
<td>CAGGTCTCCTCTGAGATCAG; GTACCAGATTACGCTATGGTGCCCTATGAATGACTTT</td>
<td>GCGGCGCATAACTAGCAATGAATCACTCTGA; CTACGATTCACTCTGCTTACATTGGTAATACTCT</td>
<td>egl-46 (109-286aa) coding sequence was amplified from pOG242 and inserted into pGBKKT7 plasmid via</td>
</tr>
<tr>
<td>Insertion Site</td>
<td>Forward Primer</td>
<td>Reverse Primer</td>
<td>Cloning Kit</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>pOG255-pGADT7-TEAD3</td>
<td>TGAACCTCAATGTTTTGTTCA</td>
<td>CTAACCAGACAAGTGCGGAAAGGGA</td>
<td>In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>AGCGTAATCTGGTCGTA;</td>
<td>GCAGATGAATCGTA;</td>
<td>TEAD3 coding sequence was amplified from human cDNA and inserted into pGADT7 plasmid via In-Fusion Advantage PCR cloning kit</td>
<td></td>
</tr>
<tr>
<td>GTACCAGATTAGCTATGGCGTCCAAACAGCTGGAAC</td>
<td>CTAGGATTTCATCTGCGTCtgACAGCTGGACTGAC</td>
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<tr>
<td>pOG279-pET-28a-eegl-46</td>
<td>TCGAGTGCGGCCGCAAGCTTG;</td>
<td>GCACCACCACCACCACCACTGA;</td>
<td>egl-46 coding sequence was amplified from cDNA and inserted into pET-28a(+) plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>TCGGCGGCCACTCGAATTGGTGCTCAT</td>
<td>TGGGTTGGTGGTGCTCATTGGGCCATTTGGAAATTAACCTTGA</td>
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<tr>
<td>pOG821-pPD95.77-pig-1a::gfp</td>
<td>GTACCGGTAGAAAAAAATGACCAATGATGAGTGCTCC</td>
<td>TTCTCCTTTACTCATTTGCGATTTGCGCATTTAATTTGAAAG</td>
<td>pig-1a cDNA was amplified from cDNA and inserted into pPD95.77 via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG836-pPD95.77-egl-17::pig-1a::gfp</td>
<td>GTACCGGTAGAAAAAGAGATGGATGTTTACTGCTACCACTG</td>
<td>TTCATACCTTCGCGCATACCCAATGATTGCGCCATTGCACTGAA</td>
<td>Pegl-17 was amplified from N2 genomic DNA and inserted into pOG821-pPD95.77-pig-1a::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG837-pPD95.77-Pmec-7::pig-1a::gfp</td>
<td>GTACCGGTAGAAAAAGCACATGTATGAGACCTTACACGT</td>
<td>TTCATACCTTCGCGCATACCCAATGATTGCGCCATTGCACTGAA</td>
<td>Pmec-7 was amplified from N2 genomic DNA and inserted into pOG821-pPD95.77-pig-1a::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
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<td>pOG838-pPD95.77-Pgcy-32::pig-1a::gfp</td>
<td>GTACCGGTAGAAAAACACATTATACGACTG</td>
<td>TTCATACCTTCGCGCATACCCAATGATTGCGCCATTGCACTGAA</td>
<td>Pgcy-32 was amplified from N2 genomic DNA and</td>
</tr>
<tr>
<td>p</td>
<td>AGGC</td>
<td>TGATCTTCGC</td>
<td>inserted into pOG821-pPD95.77-pig-1a::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
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<tr>
<td>pOG839-pPD95.77-Ppig-1::pig-1a::gfp</td>
<td>GTACCGGTAGAAAAAAGCAGCTGTGTCACCGATT</td>
<td>TTCATACTTGCTCATGCTG</td>
<td>pOG821-pPD95.77-pig-1a::gfp plasmid via In-Fusion Advantage PCR cloning kit</td>
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<tr>
<td>pOG851-pPD95.77-Ppig-1(deham-1 binding)::pig-1a::gfp</td>
<td>TGGAAAAATCTGGAAAGTTCATGAAACTTCGTTTT</td>
<td>TTTCCAGATTTTTCTGTAAA</td>
<td>pOG821-pPD95.77-pig-1a::gfp was amplified using primers listed to delete the ham-1 binding site via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG859-pPD95.77-Ppig-1(de_control)::pig-1a::gfp</td>
<td>TGGCCTAGTTCCGCAACTC</td>
<td>TGCCGAACCTAGGCTCTTTCCATCGCTGC</td>
<td>pOG821-pPD95.77-pig-1a::gfp was amplified using primers listed to delete the control site via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pOG1105-Pegl-13::egl-13::gfp</td>
<td>AGTGACCTGTTCGTTTCACCGCGACATTA; AGACCAAGCTTTGTTACCGTTACATCCGAGGAAGAAGACTTTTACC</td>
<td>GGTACCAAGCTTGCTTTGCCTTTCAGCGTGGTGTAGGAG; AGGTCACTAATACCAGAGGGCGCGAACGCC</td>
<td>Pegl-13::egl-13 and gfp::unc-54 3'UTR were amplified from N2 genomic DNA and plasmid respectively and inserted into pDONAR via In-Fusion Advantage PCR cloning kit</td>
</tr>
<tr>
<td>pWL30-Pmec-7::egl-13::gfp</td>
<td>AGTGACCTGTTCGTTGCAACATGTAGCTGAAGAGAGAAGAATTTCACAC</td>
<td>TCGTCTACGGCTCATGTTGGTCCTGAAATTTGGACCCGAC</td>
<td>mec-7 promoter was amplified from N2 genomic DNA and inserted into</td>
</tr>
</tbody>
</table>
| pWL32-Pegl-17::egl-13::gfp | AGTGACCTGTTCTGTTCTCCGGTCTATGGAACACTCATC | TCGTCTACGGCTCATAGCTCACATTTCGGGCACCTGAA | pOG1105 via In-Fusion Advantage PCR cloning kit
---|---|---|

*egl-17* promoter was amplified from N2 genomic DNA and inserted into pOG1105 via In-Fusion Advantage PCR cloning kit.