# Supporting Online Material for

### Glia Are Essential for Sensory Organ Function in C. elegans

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Materials and Methods Figs. S1 to S7 Tables S1 and S2 References

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### **Supporting Online Material**

#### Materials and Methods

Strains

*C. elegans* strains were cultured at 20°C as described (1), unless otherwise indicated. Wild-type animals were Bristol strain N2. Other alleles used in this work were: LGV: ttx-1(p767), osm-6(p811), fig-1(tm2079); LGX: che-2(e1033). The following transgenic arrays were used:

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"no glia 1" nsIs109 [F16F9.3 pro::DT-A(G53E) + unc-122 pro::GFP],
"no glia 2" nsIs113 [F16F9.3 pro::DT-A(G53E) + unc-122 pro::GFP] X,
cbIs1 [vap-1 pro::nlsGFP + lin-15(+)] I,
kyIs90 [odr-3 pro::odr-3(1^{st} 35 aa's)::GFP + lin-15(+)] III,
ntIs1 [gcv-5 pro::GFP + lin-15(+)] V,
ovIs17 [gcv-8 pro::GFP + lin-15(+)] V,
oyIs45 [odr-1 pro::RFP] V,
kvIs156 [str-1 pro::odr-10::GFP] X,
oyIs51 [T08G3.3 pro::RFP],
kyEx728 [sra-6 pro::G-CaMP],
kvEx1440 [sra-6 pro::chop2::Cherry + elt-2 pro::GFP],
kvEx1449 [sra-6 pro::chop2::Cherry + elt-2 pro::GFP],
myEx10 [che-11 pro::che-11::GFP, rol-6(su1006)],
nsEx1705 [dyf-11 pro::dyf-11::GFP, rol-6(su1006)],
nsEx856 [F16F9.3 pro::GFP, rol-6(su1006)],
nsEx864 [F11C7.2 pro::GFP, rol-6(su1006)],
nsEx1066 [F53F4.13 pro::GFP, rol-6(su1006)],
nsEx1086 [T02B11.3 pro::GFP, rol-6(su1006)],
nsEx1758 [fig-1 pro::GFP, rol-6(su1006)],
nsEx2192 [C38G2, rol-6(su1006)],
nsEx2209 [fig-1 pro::fig-1 (long), exp-1 pro::GFP],
nsIs184 [fig-1 pro::fig-1 (short), exp-1 pro::GFP],
nsEx2155 [T02B11.3 pro::fig-1 (short), exp-1 pro::GFP].
nsEx2150 [sra-6 pro::fig-1 (short), exp-1 pro::GFP],
nsEx2212 [sra-6 pro::odr-10::GFP, rol-6(su1006), pSL1180],
nsEx2215 [odr-1 pro::odr-10::GFP, rol-6(su1006), pSL1180],
nsEx2218 [odr-10 pro::odr-10::GFP, rol-6(su1006), pSL1180],
nsEx2221 [odr-1 pro::tax-4::GFP, rol-6(su1006), pSL1180],
nsEx2224 [odr-1 pro::tax-4::GFP, rol-6(su1006), pSL1180].
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 $P_{exp-I}$ GFP was kindly provided by Eric Jorgensen (2), pSL1180 is an empty cloning vector used to increase the DNA concentration of injection mixtures. Germline transformations were performed as described (3). Stable transgenes were obtained via psoralen integration (4).

#### Ablations

Laser ablations were performed as described (*5*) in L1 larvae of a strain expressing GFP in amphid sheath glia (*cbIs1*). Ablation success was determined by lack of GFP expression and also confirmed in eight animals by EM reconstruction. All cilia morphologies were determined in laser ablated animals as well as transgenic lines lacking glia. An attenuated form of diphtheria toxin A was expressed specifically within amphid and phasmid sheath cells using the F16F9.3 promoter region to kill these cells genetically. Transgenic animals carrying pTB29 [F16F9.3 pro::DT-A(G53E)], injected at 2 ng/μL, and pEP51 [*unc-122* pro::GFP], a gift of Elliot Perens, were obtained by germline injection followed by psoralen integration. In the two lines characterized, "no glia 1" (*nsIs109*) and "no glia 2" (*nsIs113*), the amphid sheath glia appear to die in late embryos or in early L1 larvae. Laser-ablated animals were tested in NaCl chemotaxis and osmosensation assays and they performed similarly to genetically-ablated animals, indicating that the two ablations are essentially equivalent.

### Behavioral analysis

NaCl chemotaxis and odortaxis assays were performed as previously described (6, 7). Attractants were assayed on circular plates; repellents and diacetyl in the experiment shown in Fig. 1G were assayed on square plates. All data shown is from 12 assays. The ring assay was used to test osmosensation (8). Briefly, a 1-cm ring of 4 M fructose containing the dye Congo Red was made on an NGM plate. Animals were placed inside the ring and followed over the next 10 min to determine the response to the osmotic barrier. Animals avoiding the ring more than six times were classified as normal; those exiting the ring in less than six attempts were deemed defective in osmosensation.

Thermotaxis assays were performed on a 18°-26°C linear temperature gradient (9). Animals were allowed to lay for 8-24 hours and removed from plates. The staged progeny were tested on the first day of adulthood. Briefly, animals were washed twice with S-Basal and spotted onto a 10-cm plate containing 12 mL of NGM agar. The plate was placed onto the temperature gradient with the addition of 1 mL glycerol to its bottom to improve thermal conductivity. The plate was covered with a flat piece of glass. The assay was stopped after 45 min by inverting the plate over chloroform thus killing the animals. The plates have an imprinted 6x6 square pattern which formed the basis of the 6 temperature bins. The data shown is the average of four assays.

### Dye filling

Stock solutions (5 mg/mL) of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) in *N*,*N*-dimethylformamide were stored at -20°C. To assay dye uptake, animals were soaked in 5 µg/mL DiI diluted in M9 for 20-60 min.

Microscopy and Imaging

GFP expression patterns were analyzed in stable transgenic lines by conventional fluorescence microscopy using an Axioplan II microscope equipped with an AxioCam camera. Alternatively, imaging was performed on a Zeiss Axiovert 200M microscope equipped with an UltraView spinning disk confocal head using a 100x/1.45 NA objective.

Calcium imaging was performed using a microfluidic device as described (10). Images were captured at 10 frames/sec and were analyzed using MetaMorph and Matlab (10).

### Electron Microscopy

Animals were fixed, stained, embedded in resin, and serially sectioned using standard methods (11). Imaging was performed with a transmission electron microscope equipped with a digital camera.

### Channelrhodopsin2

An overnight culture (5mL) of  $E.\ coli$  (strain OP50) was pelleted and concentrated to 50  $\mu$ L. To this, 1  $\mu$ L of 50 mM retinal (a gift of Navin Pokala and Cori Bargmann) was added. After vortexing, the mixture was spotted in NGM plates. Animals expressing ChR2-mCherry were transferred and cultivated on these plates for at least 2 hours. Animals were assayed on a dissecting microscope by exposing them for about 1 second to excitation light using a GFP Plant fluorescence filter 470/40nm. Animals initiating backward movement within 2 seconds were scored responsive. The animals were not responding to UV light per se as omission of retinal, a channelrhodopsin2 obligate cofactor, resulted in unresponsive animals.

#### Cell Culture

Embryonic cells were obtained using methods previously described (12, 13). Briefly, embryos were isolated from gravid adults following lysis in a hypochlorite solution. Eggs released by this treatment were pelleted by centrifugation and washed with sterile egg buffer containing 118 mM NaCl, 48 mM KCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, and 25 mM HEPES (pH 7.3). Eggshells were removed by incubation in 5 ml chitinase (0.5 U/ml in egg buffer) for 45 minutes. Embryos were pelleted by brief centrifugation, and the egg buffer was removed and replaced with 2 ml L-15 medium supplemented with 10% FBS, 50 U/ml penicillin and 50  $\mu$ g/ml streptomycin. The osmolarity of the culture medium was adjusted to 340 mOsm with sucrose.

The embryos were dissociated by passage through a  $5\mu m$  syringe filter. Cells were plated on poly-L-lysine (0.01%) coated cell culture dishes at a density of  $10^7$  cells/ml and maintained in L-15 media at  $22^{\circ}C$  in a humidified chamber. The cells were cultured for 24 hr to allow differentiation of GFP-labeled amphid sheath cells. The *vap-1::GFP* transgene used also drives faint expression in the AFD neuron.

### FACS analysis

Sorting experiments were performed on a FACSVantage SE/DiVa, equipped with a 488 nm laser. The machine was flushed with egg buffer prior to sorting to enhance cell

viability. Autofluorescence levels were established by flow cytometry of cells isolated from the non-GFP-expressing wild-type strain (N2). The sorting gate for size and granularity was empirically adjusted to exclude cell clumps and debris and to achieve ~95% enrichment for GFP-labeled cells, which represented 0.1% of the total cell count. The cells were collected directly into RNA extraction buffer. As a reference, equal amounts of non-GFP cells were collected by FACS for RNA isolation.

### Isolation and amplification of RNA

For each experiment, mRNA was isolated from 90,000 - 120,000 cells using the Absolutely RNA microprep kit. 90-150 ng of obtained total RNA was subjected to linear amplification, fragmentation and biotin labeling using the Ovation TM Biotin RNA Amplification and Labeling System as recommended by the manufacturer. The quality and size distribution of obtained cDNA were assessed by gel electrophoresis.

### Array hybridization and data analysis

mRNA isolated and amplified in three independent experiments was hybridized to GeneChip  $\it C. elegans$  Genome Arrays containing 22,500 predicted transcripts. 5 µg of fragmented cDNA were used for each hybridization. The GeneSpring software was used to carry out data analysis. For the probe intensity values generated by the Affymetrix scanner, Robust Multichip Average algorithm was used for normalization and statistical processing. Data were then filtered to remove genes with low expression values (<10). t-test was performed to sequentially filter out genes with unreliable signal level between replicates (p < 0.05). Finally, genes were filtered for fold change.

### RNAi

RNAi was performed as described using published clones (14). To screen the candidate genes, 4 L4 larvae were placed on seeded RNAi plates and their progeny screened after 4 days by dye filling.

### Plasmid Constructions

Initial attempts to clone PCR-amplified *fig-1* cDNA into standard vectors failed to yield *E. coli* transformants with the expected inserts, apparently because the cDNA is toxic to *E. coli*. The toxicity problem was resolved by creating expression vector pMT1, which has minimal transcriptional activity. Specifically, the *SphI-ApaI* fragment of pPD95-75 (*15*) was replaced with pBS-KS Multiple Cloning Site Region without flanking T7 and T3 promoters. To facilitate cDNA expression, the *unc-54* 3'UTR was amplified by PCR from pPD95-75 and the resulting amplicon was ligated to the above plasmid digested with *SalI* and *ApaI*.

FIG-1 isoforms: Two predicted isoforms of F53B7.5 (*fig-1*) are annotated (wormbase.org, release 190). While we could isolate cDNA for the short isoform, we could not amplify cDNA of the long isoform. PCR-amplification from several cDNA libraries resulted in isolation of clones that contained the last 4 exons spliced to each other, but these were not properly spliced to the rest of the gene and should not result in

successful translation. Conservation of these last 4 exons in other species leads us to believe that they are likely to be part of the gene locus. It is possible that the long form is only spliced under certain conditions which were not represented in the animals used to make the cDNA libraries.

For pMT2, fig-1 cDNA (short) was amplified by PCR from cDNA and digested with NotI and XhoI. The resulting amplicon was ligated to pMT1 digested with NotI and SalI.

For  $P_{fig-I}$ GFP (pMT3), we PCR-amplified a 5.2 kb genomic DNA fragment containing sequences upstream of the fig-I ATG. The resulting amplicon was ligated to pPD95-75 digested with SphI and KpnI. This construct also gave faint expression in two pairs of ventral cord projecting neurons. This neuronal expression is not seen when using a 2.2 kb promoter region.

For P<sub>F16F9.3</sub>GFP (pMT4), we PCR-amplified a 2 kb genomic DNA fragment containing sequences upstream of the predicted ATG. The resulting amplicon was ligated to pPD95-75 digested with *Hind*III and *Kpn*I.

For P<sub>F53F4.13</sub>GFP (pMT5), we PCR-amplified a 650 bp genomic DNA fragment containing sequences upstream of the predicted ATG. The resulting amplicon was ligated to pPD95-75 digested with *Hind*III and *Kpn*I.

For  $P_{T02B11.3}$ GFP (pMT6), we PCR-amplified a 2.5 kb genomic DNA fragment containing sequences upstream of the predicted ATG. The resulting amplicon was ligated to pPD95-75 digested with *Sph*I and *Kpn*I.

For P<sub>F11C7.2</sub>GFP (pMT7), we PCR-amplified a 350 bp genomic DNA fragment containing sequences upstream of the predicted ATG. The resulting amplicon was ligated to pPD95-75 digested with *Hind*III and *Kpn*I.

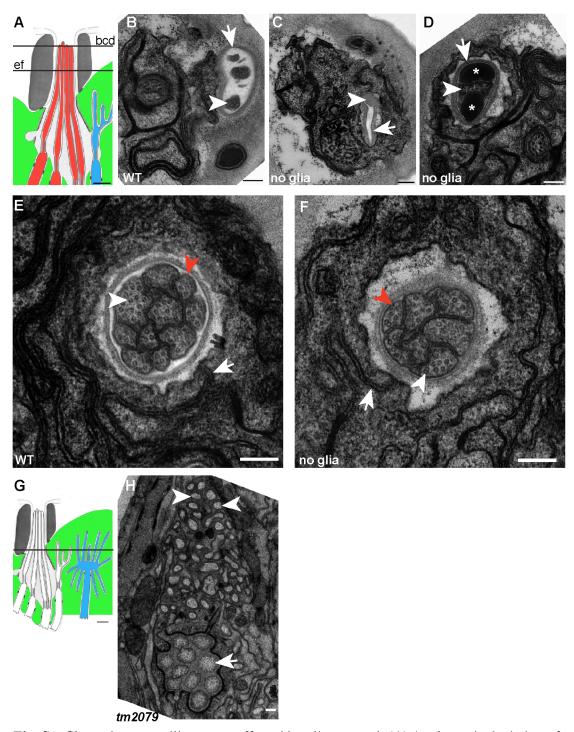
For  $P_{fig-1}fig-1$  (pMT8), we PCR-amplified a 2.2kb genomic DNA fragment containing sequences upstream of fig-1 ATG. The resulting amplicon was ligated to pMT2 digested with SacI and NotI.

For  $P_{sra-6}fig-1$  (pMT10), we PCR-amplified a 2.4kb genomic DNA fragment containing sequences upstream of sra-6 ATG. The resulting amplicon was ligated to pMT2 digested with SacI and NotI.

For P<sub>T02B11.3</sub>fig-1 (pMT11), we PCR-amplified a 2.5kb genomic DNA fragment containing sequences upstream of the predicted ATG. The resulting amplicon was ligated to pMT2 digested with *Sac*I and *Not*I.

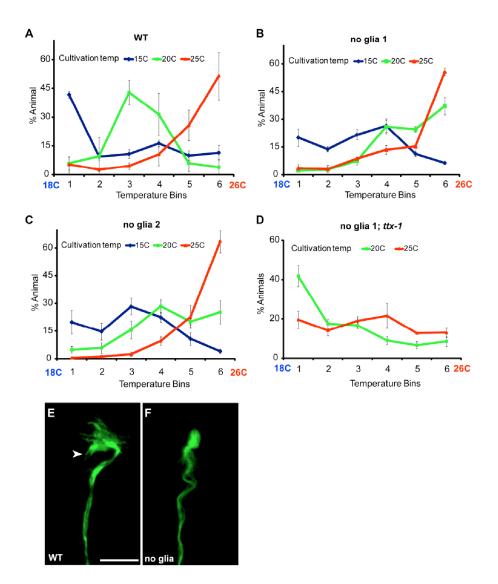
For  $P_{fig-1}fig-1$  long (pMT15), we PCR-amplified the genomic region containing the last 5 exons of fig-1 (long isoform). This was ligated into pMT8 taking advantage of an endogenous BspEI site in the last exon of the short isoform.

For  $P_{F16F9.3}DT$ -A(G53E) (pTB29), we performed site-directed mutagenesis on pJF142 [ $P_{unc-122}DT$ -A(K52E)], a gift of Hanna Fares (16), to obtain the diphtheria toxin A G53E mutant. Additionally, we found that DT-A also contained the D79G mutation. The 2056 bp region upstream of the F16F9.3 start site (-2057 to -1, relative to the ATG), was PCR-amplified and ligated as a PstI/BamIII fragment into the vector generated by site-directed mutagenesis.

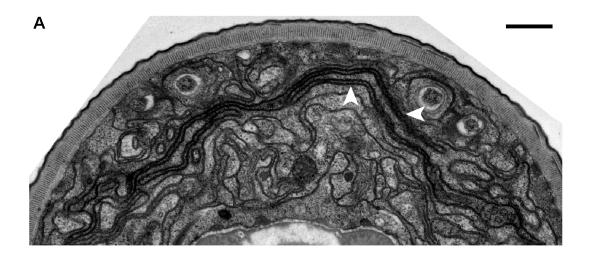


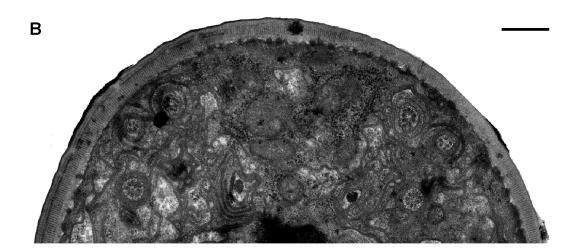
**Fig. S1.** Channel neuron cilia are not affected by glia removal. **(A)** A schematic depiction of the amphid opening indicating the level of the cross sections in **B** to **F**. Adapted from *(18)*.

Sheath glia, green; socket glia forming the pore, dark grey; channel neurons, red; sheath embedded neurons, blue. In the cartoons, anterior is up, scale bar, 1  $\mu$ m. (B) The amphid opening of a wild-type animal. The beginning of a cilium (arrowhead) is seen in the cuticle-bound channel (arrow). (C) A glia-ablated animal in which the amphid channel appears open. (D) Another glia-ablated animal in which the beginning of a cilium is seen (arrowhead), An abnormal EM-dense matrix (asterisk) is seen within the channel. This matrix does not cause the dye-filling defects observed in glia-ablated animals as in some animals the socket channel is unobstructed (see C) but 100% of the animals are dyefilling defective. Furthermore, the channel opening is completely normal in fig-1(tm2079) mutants, which also exhibit dye filling defects. This matrix is also not the cause of the behavioral defects observed, as glia-ablated animals are defective in avoidance of volatile compounds such as 1-octanol which do not require an open channel for entry. Specifically, we tested daf-6(e1377) animals, in which the channel cilia cannot access the environment and are embedded within the sheath (17), and found them to avoid 1-octanol (chemotaxis index -0.43) much better than glia-ablated animals (average chemotaxis index -0.10). (E) Wild-type channel cilia (red arrowhead) displaying the proper microtubule (arrowhead) arrangement. Note that the amphid socket glia is not affected by the ablation. The characteristic junction that the socket forms unto itself is indicated by the arrow. (F) Glia-ablated animals also have normal channel cilia (red arrowhead) with normal microtubules (arrowhead). (G) Amphid cartoon with the AFD neuron shown in blue, note the villi-like projections at the level of the cross section. (H) In fig-1(tm2079) animals, the AFD villi appear normal (arrowhead) as do the channel cilia (arrow). In EM images, dorsal is up; scale bar, 200 nm.

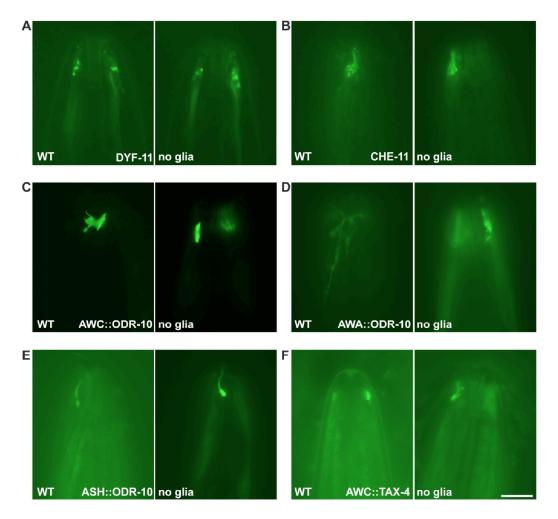


**Fig. S2.** AFD functional and morphological defects in glia ablated animals. (**A**) Thermotaxis profile of wild-type animals cultivated at three different temperatures. (**B** and **C**) Glia ablated animals fail to migrate to their cultivation temperature, especially if grown at 20°C, and thermotax to warmer temperatures. (**D**) Glia-ablated ttx-1(p767) animals migrate to cold temperatures, the reported behavior of ttx-1(p767) animals. (**E** and **F**) Glia-ablated animals lack the AFD villi seen in wild-type animals (arrowhead). Scale bar, 5  $\mu$ m.

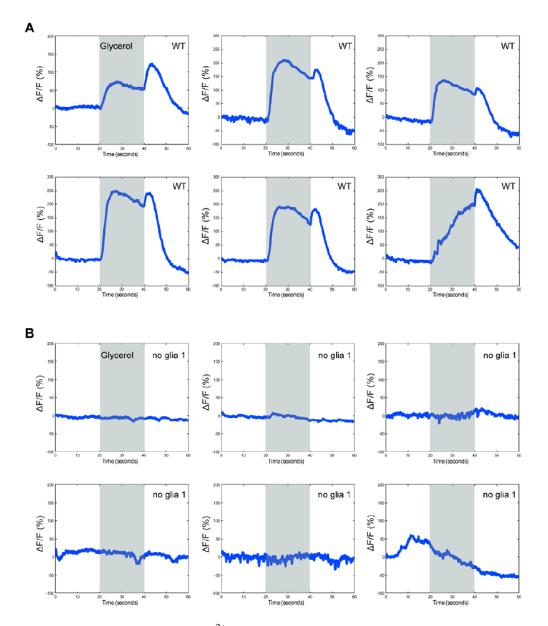




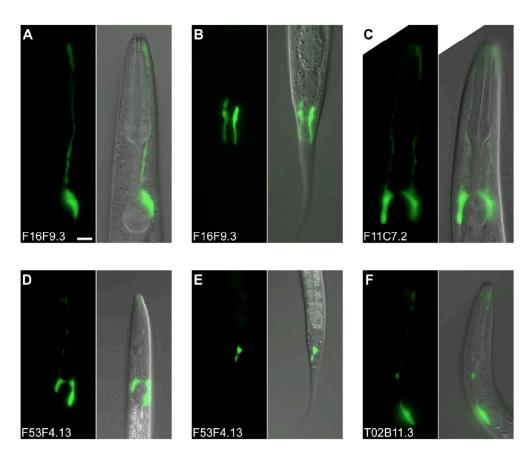
**Fig. S3.** AWC fails to extend wing-like cilia during dauer in absence of glia. **(A)** EM image of a wild-type dauer animal in cross section. Two overlapping AWC wings can be seen, arrowheads. **(B)** A glia-ablated animal (*nsIs109*) at the same cross section level has no AWC cilia extensions. Dorsal is up. Scale bar, 500 nm.



**Fig. S4.** Cilia components localize properly in absence of glia. (**A**) DYF-11, an IFT-B particle component (*19*) and (**B**) CHE-11, an IFT-A particle component (*20*), localize normally in amphid cilia of glia-ablated animals. ODR-10, an odorant receptor (*21*), localizes normally when expressed in the AWC (**C**), AWA (**D**), or ASH (**E**) cilia of glia-ablated animals. (**F**) TAX-4, a cyclic nucleotide gated channel subunit (*22*), localizes normally in glia-ablated animals. Anterior is up, scale bar 10 μm.



**Fig. S5.** Glia are required for Ca<sup>2+</sup> changes in ASH. (**A**) Six traces of G-CaMP fluorescence in the ASH neuron of different animals exposed to 1M glycerol. Stimulus is presented during the shaded region. (**B**) In absence of glia, no G-CaMP changes are observed as a result of stimulus application.



**Fig. S6.** Expression pattern of sheath-enriched genes. (**A** and **B**) F16F9.3, the most enriched gene in the microarray, is expressed only in amphid (**A**) and phasmid (**B**) sheath glia. (**C** to **F**) F11C7.2, F53F4.13 and T02B11.3 promoter fusion constructs are also expressed in amphid and phasmid sheath glia. Anterior is up, scale bar, 10 μm.

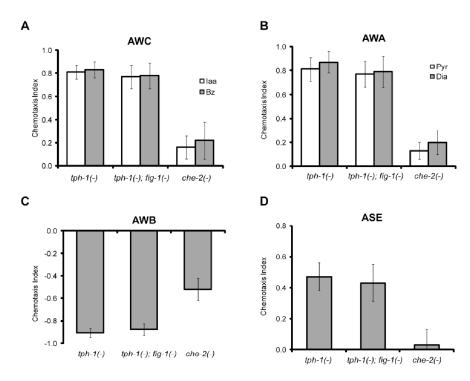


Fig. S7. fig-1 mutants are normal for some odortaxis and chemotaxis behaviors. AWC (A), AWA (B), AWB (C), and ASE (D) function is not affected in fig-1(tm2079) animals. fig-1(tm2079) animals are sluggish. We found that this defect could be suppressed by tph-1(mg280), a gene required for serotonin synthesis. As chemotaxis results are difficult to interpret if the animals have locomotory defects, we performed these assays in the tph-1(mg280) background. che-2, che-2(e1033) chemosensory mutants; error bars, standard deviation of 8 or more assays

**Table S1.** Amphid sheath glia enriched mRNAs. Genes enriched more than 4-fold are shown. The transgene used to label sheath glia, vap-1::GFP, expresses faintly in AFD also. The Affymetrix microarray probe used is included (Affy probe). TM, predicted transmembrane or secreted protein.

Gene name	Locus	Affy Probe	Fold	Description	TM
F16F9.3		181258_at	416	small secreted peptide with a Calcium-binding EF-	Y
				hand domain	
F14D7.7		178799_at	329	similarit to Chlorobium tepidum Peptidyl-prolyl	Y
				cis-trans isomerase	
T02B11.4		186124_at	307	Small secreted protein with conserved cysteines	Y
T02B11.3		174051_s_at	222	Small secreted protein with conserved cysteines	Y
**		185805_at	38.0		
F53F4.13		182491_at	177	integral membrane protein	Y
F53B7.5		173307_s_at	163	thrombospondin, type I and C6 repeat containg	Y
				protein, secreted protein	
**		191533_s_at	27.6		
R05A10.3		183692_s_at	134	contains similarity to S. cerevisiae Conserved	Y
				Oligomeric Golgi complex 1	
R05A10.4		183248_at	132	contains similarity to Pfam domain PF07403	Y
				Protein of unknown function (DUF1505) contains	
				similarity to Interpro domain IPR009981 (Protein	
				of unknown function DUF1505)	
R13D7.2		176714_at	101	contains similarity to Pfam domain PF03236	
				Domain of unknown function DUF263 contains	
				similarity to Interpro domain IPR004920 (Protein	
				of unknown function DUF263)	
F15D4.7		179655_at	96.7	transposon	
F52E1.2		180478_at	91.1	contains similarity to Pfam domain PF00059 Lectin	Y
				C-type domain	
C33G8.4		183569_at	83.8	putative secreted or extracellular protein family	Y
				member precursor	
F07C6.3		177784_at		transmembrane protein	Y
R07A4.4		178781_at	68.7	contains similarity to Thrombospondin, type I,	Y
				IPR003609 (Apple-like), IPR003014 (N/apple	
				PAN), IPR008266 (Tyrosine protein kinase, active	
				site)	
C08F11.1		184129_at	68.2	putative secreted or extracellular protein family	Y
				member precursor	
R06F6.11	tag-209	187447_at	64.7	putative secreted or extracellular protein precursor	Y
Y54G2A.10		174184_at	63.2	putative secreted or extracellular protein precursor	Y
ZK822.4		178629_at	61.8	contains similarity to Pfam domain PF02520	
				Domain of unknown function DUF148 contains	
				similarity to Interpro domain IPR003677 (Protein	
				of unknown function DUF148)	
F11C7.3	vap-1	182409_s_at	61.7	vap-1 encodes a predicted secreted protein that is	Y
				similar to the venom allergen-like proteins found in	
				a number of invertebrates, including parasitic	
				nematodes	

Gene name	Locus	Affy Probe	Fold	Description	TM
"		171730_x_at	55.8	-	
"		172026_x_at	37.7		
C44H4.1		193311_at		Leucine Rich Repeat (2 copies) (6 domains)	Y
R05A10.2		182944_at	56.6	contains similarity to Saccharomyces cerevisiae Actin-binding protein that stabilizes actin filaments; Tpm1,	Y
F35B12.9		193419_at	55.7	contains similarity to Homo sapiens Titin; TR:Q8WZ42	Y
Y69H2.3		177839_s_at	53.4	trypsin inhibitor-like, cysteine-rich TIL region precursor family member, Fibrillins and related proteins containing Ca2+-binding EGF-like domains	Y
Y23H5B.3		176889_at	52.8	putative secreted or extracellular protein precursor	Y
C05B5.9		189071_at	46.1	pseudogene	
Y46B2A.2		189905_s_at	44.3	DNA helicase PIF1/RRM3	
F11C7.2		182121_at	43.6	thrombospondin, type I family member. Secreted or transmembrane	Y
F20A1.1		172426_x_at	43.3	putative secreted or extracellular protein family member precursor	Y
F59A7.2		183014_at	38.7	putative secreted or extracellular protein family member precursor	Y
K09B3.1		181495_at	37.3	metalloproteinase 1 like, Metridin-like ShK toxin;ShK domain-like	Y
Y69A2AR.22		176280_at	37.2	·	Y
T02B11.7	nas-32	188595_s_at	36.6	metalloprotease III family member	Y Y
Y38H6A.3		182558_at		contains similarity to Interpro domain IPR006150 (Cysteine-rich repeat)	Y
K06A9.1		194259_x_at	34.8	homolog of human TCOF1, which when mutated leads to Treacher-Collins syndrome (OMIM:154500).	Y
"		184777_s_at	12.0		
***		172070_x_at	7.24		
F58B4.1a	nas-31	189892_at	34.7	Meprin A metalloprotease	Y
F14D2.7		180618_at		Zinc finger, Rad18-type putative	Y
K11E4.1		179237_at	29.8	integral membrane protein, contains similarity to Pfam domain PF01764 (Lipase (class 3)	Y
F35E2.5		177877_at	28.6	contains similarity to Pfam domain PF03761  Domain of unknown function (DUF316) contains similarity to Interpro domain IPR005514 (Protein of unknown function DUF316)	Y
F40F8.4		178505_s_at	27.5	putative secreted or extracellular protein precursor with WD40 repeat	Y
C25E10.11		187011_at	26.9	putative secreted or extracellular protein	Y
C37E2.5	ceh-37	192014_s_at	26.3	ceh-37 has an OTX-like homeodomain but lacks other domains found in OTX proteins, and the CEH-37 homeodomain is predicted to resemble the Myb domain of telomere-binding proteins; CEH-37	
		173897_at	5.18	binds the telomeric sequence 'TTAGGC	
Y105C5B.5		185106_at	25.0		
1 100C0D.0		105100_at	∠೨.0		

Gene name	Locus	Affy Probe	Fold	Description	TM
F59A7.5		183007_at		putative secreted or transmembrane protein	Y
C56E10.3		185563_at	24.2	contains similarity to Drosophila melanogaster Flybase gene name is CLIP-190-PA, FLYBASE:CG50	
"		172586_at	11.0		
B0285.6		190632_at		predicted sodium-coupled carboxylate transporter related to Drosophila Indy and the mammalian NaDC1 and NaDC3 transporters	Y
C56C10.4		184220_at		contains similarity to Pfam domain PF01682 DB module contains similarity to Interpro domain IPR002602	Y
C12D8.9		179347_at	23.4		
C05D12.1		184125_at	22.3	contains similarity to Interpro domains IPR005018 (DOMON related), IPR003006 (Immunoglobulin/major histocompatibility complex, conserved site), IPR006593 (Cytochrome b561 / ferric reductase transmembrane)	Y
F13B6.3		180766_at	21.4	contains similarity to Homo sapiens Mucin-5AC precursor (Fragment)	Y
C23H3.7	tre-5	187729_s_at	20.8	trehalase (tre-5)	Y
F07F6.7		190471_at		apolipoprotein L like	Y
K02E11.3		181315_at	20.3	contains similarity to Interpro domain IPR002213 (UDP-glucuronosyl/UDP-glucosyltransferase)	Y
H22K11.1	asp-3	191956_at	20.0	asp-3 encodes an aspartyl protease homolog that is required, in parallel with ASP-4 but downstream of CLP-1 and TRA-3, for degenerative (necrotic-like) cell death in neurons induced by mutations such as mec-4(d), deg-3(d), or gsa-1(gf).	Y
K02E11.5		180905_at		UDP-glucuronosyl/UDP-glucosyltransferase	
C41C4.1		187005_at	18.3	contains similarity to Pfam domain PF04590 Protein of unknown function, DUF595 contains similarity to Interpro domain IPR007669 (Protein of unknown function DUF595)	Y
C06E2.2		182220_at	16.9	,	
F16H6.10		176708_at	16.8		
Y71G12B.17		176293_at	16.5	Phosphatidylinositol transfer protein [KOG3668]	
Y39H10A.1		176555_at	14.9	integral membrane protein	Y
T05C1.1		182235_at	14.7	putative protein, with a transmembrane domain, a coiled coil-4 domain (2E769), mRNA.	Y
K02E11.4		181328_at	14.6	contains similarity to Interpro domain IPR007110 (Immunoglobulin-like)	Y
Y73B6A.3		185418_at	14.6	putative secreted or extracellular protein family member precursor, UDP-glucuronosyl/UDP- glucosyltransferase; Histone H5	Y
F36H2.3		192520_s_at	14.5	Complement factor H precursor like	Y
T23C6.3		183310_s_at	13.5		
M01G12.14		185163_s_at	13.4	contains similarity to Rhizobium meliloti Putative transposase of insertion sequence ISRM19 protein	
Е02Н9.3		181022_s_at	13.0	contains similarity to Pfam domain PF03312 Protein of unknown function, DUF272 contains similarity to Interpro domain IPR004987	

Gene name	Locus	Affy Probe		Description	TM
C40H5.2		178802_at	12.4	contains similarity to Bifidobacterium longum Possible magnesium and cobalt transport protein.;	
				TR:Q8G4E0	
F49F1.7		181132_s_at	12.3	contains similarity to Pfam domain PF01549 ShK	Y
				domain-like contains similarity to Interpro domain IPR003582 (Metridin-like ShK toxin)	
H11L12.1		172299_x_at	12.1	,	
11		172550_x_at	10.1		
C16H3.2	lec-9	192336_at	12.0	lec-9 encodes a predicted lectin that affects	
F59E11.2		185750_at	12.0	embryonic viability.	
F39E11.2		185/50_at	12.0	Short-chain dehydrogenase/reductase SDR;Glucose/ribitol dehydrogenase;Insect alcohol	
				dehydrogenase family;NAD(P)-binding;short chain	
				dehydrogenase danny, (VAD(1)-olliding, short chain dehydrogenase	
C12C8.1	hsp-70	192266_s_at	11.8	hsp-70 encodes a heat-shock protein that is a	
0.200.1	nop / o	1,2200_0_0_0	11.0	member of the hsp70 family of molecular	
				chaperones.	
F31F6.5	daf-6	187906_at	11.6	a Patched-like gene required for amphid sheath	Y
				channel formation, daf-6 interacts genetically with	
				che-14	
C27C7.1		172213_x_at	11.5	contains similarity to Homo sapiens Hypothetical	
CLIFF	1 150	100005 4	11.6	protein FLJ13213	
C17E7.6	nhr-158	190925_at		nuclear receptor NHR-51 like family member	37
F08G5.6 T19H12.3		178843_at 185822_s_at		CUB-like region; CUB-like domain putative secreted or extracellular protein family	Y
119112.3		163622_8_at	11.3	member precursor UDP-glucuronosyl/UDP-	1
				glucosyltransferase	
M03E7.2		181683_at	11.1	contains similarity to Plasmodium berghei 58 kDa	Y
		_		phosphoprotein (Heat shock-related protein)	
				(HRP).; SW:Q08168	
Y54F10BM.3		173344_at	10.8	Protein-tyrosine phosphatase, receptor/non-receptor	Y
				type;Protein-tyrosine phosphatase;Protein-tyrosine	
				phosphatase, catalytic	
C38D9.2		179425_at		Zinc finger, CCHC-type	
F59D6.7		180406_at	10.4	contains similarity to Pfam domain PF00036 EF	
				hand contains similarity to Interpro domains IPR011992 (EF-Hand type), IPR002048 (Calcium-	
				binding EF-hand), IPR008080 (Parvalbumin),	
				IPR003299 (Flagellar calcium-binding protein	
				(calflagin))	
F59D12.3		180289_at	10.4	contains similarity to Pfam domain PF04590	Y
		_		Protein of unknown function, DUF595 contains	
				similarity to Interpro domain IPR007669 (Protein	
				of unknown function DUF595)	
T13B5.9		175491_at	10.3	Peptidase M, neutral zinc metallopeptidases, zinc-	
GOSTIO 2		150005	0.0	binding site	
C25F9.2		178896_at	9.9	DNA polymerase type B, organellar and viral family member (5T899), predicted mRNA.	
				ramny member (31899), predicted mkina.	

Gene name	Locus	Affy Probe	Fold	Description	TM
F10A3.1		177627_at	9.8	claudin homolog that may be required for normal	Y
				cohesion of apical junctions in epithelia; F10A3.1	
				is worm-specific, with obvious homologs only in C.	
77.00 1.0.0	1 0	100505		elegans	**
K02A2.3	kec-3	193537_s_at	9.7	Bumetanide-sensitive Na-K-C1 cotransporter	Y
C25A1.2	fkh-10	190179_at	9.7	fkh-10 encodes one of 15 forkhead transcription	
W/01 A 11 4	1 10	100001 -4	0.6	factors	
W01A11.4	lec-10	190281_at	9.6	lec-10 encodes a galectin, a soluble galactose-	
				binding lectin; recombinant lec-10 can bind to	
W06D11.3		183074_at	0.6	sugar in an in vitro assay.	
W 00D11.3		1830/4_at	9.6	Homologous to E. coli RecQ and human BLM and	
				WRN proteins that are defective in Bloom's	
C11H1.9		177796_at	9.4	syndrome and Werner's syndrome contains similarity to Pfam domain PF01105	Y
CHIII.9		177790_at	9. <del>4</del>	emp24/gp25L/p24 family/GOLD contains	1
				similarity to Interpro domains IPR001251 (Cellular	
				retinaldehyde-binding/triple function, C-terminal),	
				IPR000348 (emp24/gp25L/p24)	
C14F11.5	hsp-43	190097_s_at	9.4	heat shock protein	
Y38H6C.7	пэр-чэ	181511 at	9.3	pseudogene	
F21H12.4	ptc-2	193368_s_at	9.3	PATCHED homolog, has sterol sensing domain	Y
1211112.4	ptc-2	175500_5_at	7.5	(SSD); PTC-2 is required for normal fat storage,	1
				normal egg osmotic integrity, locomotion, egg	
				laying, and viability in RNAi assays	
K12II4.1	ceh-26	176620_at	9.3	ceh-26 encodes a protein that contains a prospero-	
111211111	CCH 20	170020_ut	7.5	related homeodomain; expressed in nuclei of the	
				adult head, tail neurons, and one cell in the	
				postdeirids.	
K10C2.3		189595_s_at	9.3	aspartic proteinase family member	Y
C14F11.6		193712_s_at	9.1	DTDP-4-dehydrorhamnose 3,5-epimerase (RFBC	
				gene)	
T04A11.1		182091_s_at	9.1	Phenazine biosynthesis PhzC/PhzF	
				protein; Phenazine biosynthesis-like protein	
Y55B1BL.1		186903_at	9.1	integral membrane protein	Y
F53F4.6	rdy-2	178699_at	9.1	RDY-2 (rod-like lethal Dye-filling defective);	Y
				contains similarity to Interpro domain IPR017441	
				(Protein kinase ATP binding, conserved site)	
C05B5.3	pqn-8	186853_s_at	8.9	glutamine/asparagine (Q/N)-rich ('prion') domain	Y
C38D9.5		178878_at	8.9	BRCT; Ankyrin; Protein of unknown function	Y
				WSN;Domain of unknown function	
C56C10.6		189105_s_at	8.8	Casein kinase	
C33C12.3		189972_at	8.5	contains similarity to Pfam domain PF02055 O-	Y
				Glycosyl hydrolase family 30 contains similarity to	
				Interpro domains IPR017853 (Glycoside hydrolase,	
				catalytic core)	
Y46C8AL.2	clec-174	172185_x_at	8.4	C-type lectin	<u>Y</u>
R05D7.5		178659_at	8.3	putative membrane protein	Y
Y57E12B.3		176402_at	8.2	contains similarity to Pfam domains PF04083 (ab-	Y
				hydrolase associated lipase region), IPR000073	
				(Alpha/beta hydrolase fold-1), IPR000694 (Proline-	
				rich region)	

Gene name	Locus	Affy Probe	Fold	Description	TM
Y6E2A.4		179418_at	8.1	contains similarity to Pfam domains PF00104	
				(Ligand-binding domain of nuclear hormone	
				receptor), PF00105 (Zinc finger, C4 type (two	
				domains))	
Y65B4BL.2	deps-1	186952_at	8.0	unfamiliar P-granule-associated protein required for	
				germline RNAi, orthologs in other Caenorhabditis	
				species, but has no non-nematode orthologs.	
Y110A2AL.4		187531_at	8.0	putative membrane protein	Y
11		175367_s_at	6.7		
F32B5.4		183820_at	8.0	putative membrane protein	Y
F29B9.9	col-111	188461_s_at	7.9	COLlagen structural gene	Y
Y69H2.1		177981_at	7.9	contains similarity to Myxine glutinosa NADH-	Y
				ubiquinone oxidoreductase chain 4	
C05B5.8		186950_at	7.8	contains similarity to Shigella flexneri Putative	Y
				transport protein.; TR:Q83RB7	
F14D2.4	bath-29	181360_s_at	7.8	Meprin/TRAF-like MATH and BTB/POZ domain	
				containing protein family member	
"		181302_s_at	6.1		
T13C2.2		179776_at	7.6		Y
"		180200_at	5.6		
C17C3.5		183228_at	7.6	Protein of unknown function DUF38,	
				Caenorhabditis species;FTH domain	
R09H3.3		179945_at	7.5		
W09B6.5		181510_at	7.4		
ZC449.2		183234_s_at	7.4	PAN domain and Apple-like domain, secreted or	Y
				transmembrane	
"		191797_at	6.1		
C12D5.7	cyp-33A1	189430_at	7.4	cytochrome p450 2C33v4 family member (5I160)	Y
C17E7.9		175231_s_at	7.3	integral membrane protein	Y
C04F12.9	rnh-1.3	184160_at	7.3	ribonuclease H	
Y105C5A.25		187110_at	7.3	pseudogene	
F59H6.5		185671_s_at	7.3	contains similarity to Pfam domain PF05970	
				Eukaryotic protein of unknown function (DUF889)	
				contains similarity to Interpro domains IPR010285	
				(Protein of unknown function DUF889, eukaryote),	
				IPR003593 (AAA+ ATPase, core), IPR006034	
				(Asparaginase/glutaminase)	
C34D4.10		181223_at	7.2		Y
C49A9.4		182898_at	7.1	orthologous to the human gene CYSTATIN B	
				(STEFIN B) (CSTB; OMIM:601145), which when	
				mutated leads to disease.	
C10H11.10	kca-1	186482 s at	7.1		
T04G9.7		179805_s_at	7.1		Y
"		174857_at	6.0		
C28H8.11		192112_s_at	7.0	Tryptophan 2,3-dioxygenase	
Y38H6C.8		193307_at	7.0	Lectin C-type domain short and long forms	Y
T14G8.4		179625_at	7.0	contains similarity to Mus musculus Gamma-	Y
		_		tubulin complex component GCP5 homolog.;	
				TR:Q8BKN5	
F59B1.2		182919_at	7.0	·	Y
					-

Gene name	Locus	Affy Probe	Fold	Description	TM
F58B6.2	inft-1	183953_at	7.0	Proline-rich region; Actin-binding FH2 and DRF	
				autoregulatory;Actin-binding FH2;Formin	
				Homology 2 Domain	
Y65B4BR.8		175375_at	6.8	contains similarity to Pfam domain PF06425	
				Partner of SLD five, PSF3 contains similarity to	
				Interpro domain IPR010492 (GINS complex, Psf3	
7/20E104 12		172624	6.0	component)	37
Y38E10A.12		172624_x_at	6.8	contains similarity to Pfam domain PF07203	Y
				Protein of unknown function (DUF1412) contains	
				similarity to Interpro domain IPR009853 (Protein	
3742E9C 1	1 25	192905 -4	<i>C</i> 9	of unknown function DUF1412)	37
Y43F8C.1	nlp-25	183895_at	6.8	Neuropeptide-Like Protein (prion protein)	$\frac{Y}{Y}$
F40G12.5		180606_at	6.7	Protein of unknown function DUF263;Domain of unknown function DUF263	Y
T05F1.7		178341_at	6.7	contains similarity to Paramecium tetraurelia	
				Chromosome undetermined scaffold_33, whole	
				genome shotgun sequence.; TR:A0D056	
F20A1.10		186043_at	6.7	putative secreted protein	Y
F53A9.8		180612_s_at	6.7	contains similarity to Plasmodium chabaudi Pc-	
				fam-3 protein putative (Fragment).; TR:Q4XGP3	
Y39E4B.12	gly-5	187815_s_at	6.7	gly-5 encodes a predicted member of the UDP-	Y
				GalNAc:polypeptide N-	
				acetylgalactosaminyltransferase (ppGaNTase)	
				family.	
T27F2.4		179204_s_at	6.7	Basic-leucine zipper (bZIP) transcription factor	
Y57G7A.5		184542_at	6.7		
F20A1.9		183123_s_at	6.6	B302, (SPRY)-like; SPla/RYanodine receptor	
				SPRY;ATPase associated with various cellular	
				activities, AAA-4;SPRY domain;Divergent AAA	
T1601.5		170710		domain	
T16G1.5		179610_s_at	6.5	Protein of unknown function DUF227; Protein	
				kinase-like; Protein of unknown function DUF1679,	
				Caenorhabditis species; CHK kinase-like; Protein of unknown function (DUF1679); Domain of unknown	
				function (DUF227)	
T06C12.4	fbxa-197	182533_at	6.5	Protein of unknown function DUF38,	
100012.4	1024-177	102333_at	0.5	Caenorhabditis species; FTH domain	
C17H12.12		186931_at	6.5	BTB/POZ-like	
ZK265.2	col-63	173650_s_at	6.5	COLlagen structural gene (37.4 kD) (col-63)	V
T05E11.8	001 03	179794 at	6.5	contains similarity to Pfam domain PF02520	Y
100111.0		1/2/2/_ut	0.5	Domain of unknown function DUF148 contains	_
				similarity to Interpro domain IPR003677 (Protein	
				of unknown function DUF148)	
C04B4.2		179273 at	6.3	Calcium-binding EF-hand	
H06H21.1	srw-94	184731_at	6.3	7TM chemoreceptor, srw family	Y
B0280.12b	glr-2	189724_at	6.3	glr-2 encodes an AMPA (non-NMDA)-type	<u> Y</u>
	<i>U</i> -			ionotropic glutamate receptor subunit	-
Y37D8A.23	unc-25	194006_at	6.3	GABA neurotransmitter biosynthetic enzyme,	
		_		glutamic acid decarboxylase (GAD); unc-25	
				activity is required for GABA synthesis	
Y43F8B.9		180823_at	6.3		

Gene name	Locus	Affy Probe	Fold	Description	TM
T21G5.3	glh-1	190711_at	6.2	a putative DEAD-box RNA helicase that contains	
				four CCHC zinc fingers and is homologous to	
				Drosophila VASA, a germ-line-specific, ATP-	
EQCALO		104151 -+	( )	dependent RNA helicase	
F26A1.9 Y39A3B.3		184151_at	6.2	integral membrane protein	v
C05D2.8		184691_at	6.2	integral membrane protein	$\frac{Y}{Y}$
B0034.4		187761_s_at 187363_at	6.1		<u>Y</u>
Y8G1A.2	inx-13	193448 s at	6.0	inx-13 encodes an innexin, an integral	$\frac{1}{Y}$
1 601A.2	шх-13	193 <del>44</del> 6_8_at	0.0	transmembrane channel protein that is a structural	1
				component of invertebrate gap junctions	
F10C1.2	ifb-1	192149_s_at	6.0	ifb-1 (also known as vab-21) encodes two isoforms	
11001.2	110 1	1,211, _5_a	0.0	of an essential intermediate filament protein that is	
				coexpressed with the essential IF proteins IFA-1,	
				IFA-2, and IFA-3, along with IFA-4	
**		175020_at	5.2		
F14B4.1		174848_s_at	6.0	LDL receptor-related protein	Y
H02I12.1		180976_s_at	5.9	protein with 12 chitin-binding peritrophin-A	Y
				domains; H02I12.1(RNAi) animals have an	
				osmotically-sensitive embryonic lethal phenotype,	
				perhaps because of defects in chitin and eggshell	
				synthesis	
F53B2.3	eak-4	178868_at	5.9	eak-4 encodes a novel protein that contains an N-	Y
				myristoylation signal; eak-4 acts in parallel to akt-1	
				to regulate insulin-like signaling and dauer	
C14F5.2	rio 2	192500_at	5.8	formation zig-3 encodes a predicted secreted protein that is a	Y
C14F3.2	zig-3	192300_at	3.0	member of the immunoglobulin superfamily of	1
				proteins;	
Y97E10B.6	srx-11	187148_at	5.8	pseudogene	
F56C11.2	ptr-11	171745_x_at	5.7	ptr-11 encodes a nematode-specific member of the	Y
100011.2	L	1.1.1012		sterol sensing domain (SSD) proteins, distantly	-
				paralogous to Drosophila PATCHED (PTC) and	
				human PTCH (OMIM:601309)	
M01G12.9		179942_at	5.7		
F33D4.2	itr-1	193676_s_at	5.7	inositol (1,4,5) trisphosphate receptor that affects	Y
				the defecation cycle and pharyngeal pumping, and	
				also affects ovulation in a pathway downstream of	
				LET-23; interacts with UNC-54 in vivo	
H38K22.5	gly-6	175655_s_at	5.7	Glycosyl transferases	Y
R09H3.2	2.56	172250_x_at	5.7	probe is in intergenic region	
F31C3.10	rrn-3.56	173281_s_at	5.6	ribosomal RNA	37
F47C12.8		186276_at	5.6	contains similarity to Pfam domain PF05912 C.	Y
				elegans protein of unknown function (DUF870) contains similarity to Interpro domain IPR008588	
				(Protein of unknown function DUF870)	
		171767_x_at	4.8	(110tom of unknown function DO1010)	
F20B6.8	hpk-1	180322_at	5.6	a predicted dual-specificity protein kinase with	
12020.0	mpic 1	100522_41	٥.٠	distant homology to the vertebrate protein kinase	
				DYRK1A and the Drosophila homolog mini-brain	

Gene name	Locus	Affy Probe	Fold	Description	TM
ZK1037.5	nhr-247	194098_at	5.6	Ligand-binding domain of nuclear hormone	
				receptors, Zinc finger, C4 type (two domains)	
F57A8.1		188628_at	5.5	ETS domain	
Y92C3B.1	kbp-4	177029_at	5.5	Tropomyosin	
Y67A10A.9		185683_at	5.5	claudin homolog, protein with at least 3 transmembrane domains	Y
"		173027_s_at	4.3		
F59H5.3	bath-12	183672_at	5.5	pseduogene (Meprin/TRAF-like MATH and BTB/POZ domain containing protein family member)	
T08G5.3		179131_at	5.5	contains similarity to Homo sapiens hypothetical protein	Y
R166.5	mnk-1	190356_at	5.5	serine/threonine kinase	
F21H7.2		173177_s_at	5.4	contains similarity to Homo sapiens Neurofilament heavy polypeptide	
"		178141_s_at	4.0	neary polypopulae	
ZK669.5		189359_s_at	5.4	ras oncogene family	-
C39B5.2		182485_at	5.4	Cyclin-like F-box;F-box domain	
Y38E10A.15		172635_x_at	5.4	Protein of unknown function DUF1412; Protein of unknown function (DUF1412)	Y
C05D10.4		176149_at	5.4	sterile alpha motif SAM	
"		187862_at	5.2	<b>A</b>	
F35E12.6		180342_at	5.3	CUB; CUB-like region; CUB-like domain	Y
Y46H3A.2	hsp-16.41	188444_at	5.3	hsp-16.41 encodes a 16-kD heat shock protein (HSP) that is a member of the hsp16/hsp20/alphaB-crystallin (HSP16) family of heat shock proteins	
ZK20.2	kin-6	175005_at	5.3	kin-6 encodes a predicted tyrosine protein kinase.	Y
ZK488.2	nhr-90	189961_at	5.3	nuclear Hormone Receptor	
Y59E1B.1		185607_at	5.3	putative nuclear protein (XC497)	
C14C10.7	ttr-43	191018_at	5.3	ttr-43 - (TransThyretin-Related family domain) contains similarity to Interpro domain IPR001534 (Transthyretin-like)	Y
F53G12.3		184226_at	5.3	partial homolog of dual oxidase ('Ce-Duox2'), with an N-terminal peroxidase domain, two central calmodulin-binding EF hands, and a C-terminal superoxide-generating NADPH-oxidase domain	Y
C01B10.3		185295_s_at	5.3	a paralog of IPP-5, and thus may functionally overlap with ipp-5 in vivo.	
W03A5.4		183687_at	5.2	contains similarity to Homo sapiens Uncharacterized protein DLGAP1	
F49C5.8		172759_x_at	5.2	transposon	
Y75B12B.3		185413_at	5.2	contains similarity to Saccharomyces cerevisiae Mlp proteins restrict telomere length by influencing the Rif1-Tel1 pathway of telomerase regulation; also involved in the translocation of macromolecules between the nucleoplasm and the NPC	
F17C8.5	twk-6	187930_at	5.2	twk-6 encodes one of 44 C. elegans TWK (two-P domain K+) potassium channel subunits that contain two pore-forming domains and four transmembrane domains	Y

Gene name	Locus	Affy Probe	Fold	Description	TM
R02C2.4	nhr-204	191007_at	5.2	nuclear hormone receptor	Y
W04G5.2	rab-11.2	189771_at	5.2	RAB family member (rab-11.2)	Y
K08E5.3	mua-3	189800_s_at	5.1	Caenorhabditis elegans essential gene mua-3, transmembrane cell adhesion receptor precursor; matrix receptor, muscle attachment protein, MUscle Attachment abnormal	Y
R07B1.10	lec-8	175710_s_at	5.1	galectin (20.4 kD) (lec-8)	
F40F9.3		193047_at	5.1	contains similarity to Interpro domain IPR001478 (PDZ/DHR/GLGF)	
T21E8.3	pgp-8	189037_at	5.1	pgp-8 encodes an ATP-binding protein that is a member of the P-glycoprotein subclass of the ATP- binding cassette (ABC) transporter superfamily; transmembrane	Y
ZK355.5		183073_at	5.0	EGF receptor, L domain; Receptor L domain	Y
F25H5.8		178186_at	5.0	Protein of unknown function UPF0057;Uncharacterized protein family UPF0057	
Y71H2AM.22	twk-45	176220_at	5.0	twk-45 - (TWiK family of potassium channels)	Y
K04H4.2		174044_at	5.0	secreted, with an N-terminal chitin-binding peritrophin-A domain followed by up to 15 cysteine-rich domains; the general organization of K04H4.2 protein resembles that of T10E10.4	Y
C36C9.3	fbxa-170	180118_at	5.0	a protein containing an F-box and an FTH/DUF38 motif, which may also mediate protein-protein interaction.	
R10H10.4		183646_at	5.0	contains similarity to Arabidopsis thaliana T15B16.1 protein.; TR:Q9ZSH8	Y
Y57G11C.31		187086_at	5.0	Protein of unknown function DUF271; Protein of unknown function (DUF271)	Y
T24B8.6	hlh-3	192523_at	4.9	hlh-3 encodes a basic helix-loop-helix transcription factor homologous to Drosophila Achaete-scute	
Y46H3A.1	srt-42	184414_at	4.9	7TM chemoreceptor, srt family	Y
W02D7.3		180864_at	4.9	-	Y
R03H10.6		186074_at	4.8	Nucleic acid binding, OB-fold, tRNA/helicase-type; Nucleic acid-binding, OB-fold; Nucleic acid-binding, OB-fold-like; OB-fold nucleic acid binding domain	
T10D4.10	sri-43	191471_at	4.8	7TM chemoreceptor, sri family	Y
ZK470.1		183484_at	4.8	contains similarity to Interpro domain IPR007248 (Mpv17/PMP22)	Y
K05B2.3	ifa-4	193214_s_at	4.8	nonessential intermediate filament protein that is coexpressed with the essential IF protein IFB-1	
T28F4.6		178783_at	4.8	contains similarity to Interpro domain IPR016024 (Armadillo-type fold)	
T25F10.3		186385_at	4.8	EGF-like, type 3;Delta/Serrate/lag-2 (DSL) protein;EGF-like, laminin;EGF;EGF-like region, conserved site	Y
ZK770.3	inx-12	180366_s_at	4.8	inx-12 (innexin=invertebrate connexin analogue) encodes a protein of the innexin family; innexins are the only known gap junction proteins in invertebrates	Y

Gene name	Locus	Affy Probe	Fold	Description	TM
Y66D12A.17	such-1	174877_at	4.8	such-1 encodes a component of the anaphase	
				promoting complex/cyclosome (APC/C)	
W03D8.6	itx-1	188000_at	4.8	itx-1 encodes one of two Caspr orthologues found	Y
				in the C. elegans genome; Caspr proteins belong to	
				the Neurexin superfamily which mediate cell-cell	
				contacts and in the formation of specialized	
				membrane-domains in polarized epithelial and	
				nerve cells	
F55D1.2		183246_at	4.7		
B0281.4		181853_at	4.7	Potassium channel, voltage dependent, Kv,	Y
				tetramerisation;BTB/POZ fold;K+ channel	
		100000		tetramerisation domain	
W10D5.3	gei-17	188222_s_at	4.7	gei-17 encodes a protein containing a MIZ domain	
				(Msx-interacting-zinc finger) that affects	
				embryonic viability, vulval development, and body	
				morphology; interacts with GEX-3 in yeast two-	
E20E9 1		170/40+	17	hybrid assays.	
F30F8.1		178648_s_at	4.7	contains similarity to Mus musculus Nuclear factor of activated T cells 5 (T cell transcription	
				factorsNFAT5) (NF-AT5); SW:NFT5_MOUSE	
Y37E11AR.5	ugt-45	175194_at	4.7	UDP-glucoronosyl/UDP-glucosyl transferase	Y
13/ETIAN.3	ugi-43	1/319 <del>4</del> _at	4./	family member	1
F55A12.7	apm-1	193878_s_at	4.7	an ortholog of the mu1-II subunit of adaptor protein	
133A12.7	apiii-1	1936/6_8_at	4./	complex 1 (AP-1).	
C01B7.4	tag-117	189538_s_at	4.7	guanylate kinase	
T23B7.1	nspd-4	172486_x_at	4.6	Nematode Specific Peptide family, group D	-
F20A1.6	пърч-т	181821_at	4.6	remaiode specific replace fairing, group b	Y
F46F3.1	ceh-27	192317_at	4.6	ceh-27 encodes a homeodomain protein of the NK-	
1 401 3.1	CCII-27	192317_dt	7.0	2 class that contains Drosophila scarecrow and	
				human NKX-2 (OMIM:606727)	
C03G6.17		185999_at	4.6	Acyl-CoA N-acyltransferase	
C24H12.5		187836_s_at	4.6	Immunoglobulin/major histocompatibility complex,	
				conserved site	
D1086.3		177920_at	4.6	Protein of unknown function DUF19;	Y
				Uncharacterised conserved protein UPF0376	
T19D7.5		180442_at	4.6	integral membrane protein	Y
Y42G9A.2		184890_at	4.5	-	Y
R08E5.2		189653_s_at	4.5	cysteine synthase	
F08G2.6	ins-37	188047_at	4.5	ins-37 encodes an insulin-like peptide.	Y
Y37H2A.6	fbxa-211	182392_at	4.5	a protein containing an F-box and an FTH/DUF38	
F26D11.5	clec-216	185946_at	4.5	motif C-type lectin; C-type lectin fold	Y
C03E10.5	clec-213	182545_at	4.5	C-type lectin, C-type lectin fold C-type lectin, Collagen triple helix repeat (20	<u>Y</u>
COSETO.S	0100-223	1625 <del>4</del> 5_at	т.Э	copies), Proline-rich region	1
F32H2.5	fasn-1	174796 at	4.5	fatty acid synthase, orthologous to human FASN	
1 32112.3	10311-1	174750_at	ч.5	(OMIM:600212)	
Y105C5B.6	srv-15	184967_at	4.5	srv-15, serpentine Receptor, class V	Y
C28C12.4		185803_at	4.5	contains similarity to Pfam domain PF02520	Y
		_		Domain of unknown function DUF148 contains	
				similarity to Interpro domain IPR003677 (Protein	
				of unknown function DUF148)	

Gene name	Locus	Affy Probe	Fold	Description	TM
C34G6.2	tyr-4	184158_at	4.4	Tyrosinase; Metridin-like ShK toxin	Y
C16B8.3		182754_at	4.4	Proline-rich region; Annexin, type VII	
T17H7.4	gei-16	174770_at	4.4	similar to the B20 antigen of the parasitic nematode	
				Onchocerca volvulus; GEI-16 is required for	
				ventral enclosure and elongation, larval	
				development, and normal rates of postembryonic	
				growth	
T10E9.3		183913_s_at	4.4	contains similarity to Pfam domain PF03351 DOMON	Y
Y38F2AL.1	nsy-4	177162_at	4.4	claudin homolog that may be required for normal	Y
				cohesion of apical junctions in epithelia;	
				Y38F2AL.1 is worm-specific, with obvious	
				homologs only in C. elegans	
C27B7.5		190420_at	4.4	Zinc finger, CCHC class	
F36H5.10		174172_at	4.4	transmembrane protein	Y
W05E10.3	ceh-32	193576_at	4.4	Six/sine oculis-type homeodomain protein most	
				closely related to the Six3/6 subfamily that contains	
				Drosophila OPTIX and human SIX3	
F56H6.2		177630 at	4.4	(holoprosencephaly 2)	Y
F56H6.2		1//630_at	4.4	Protein of unknown function DUF268, Caenorhabditis species	Y
C01B7.5		180705_at	1.1	PDZ domain (Also known as DHR or GLGF)	
R03G5.2	sek-1	192392_at	4.4 4.4	SEK-1 has MAPKK activity and belongs to the	
K0303.2	SCK-1	192392_at	7.7	MAPKK family; SEK-1 can activate both JNK-1	
				and PMK-1 in the yeast Hog pathway.	
K01C8.5	gei-14	188065_s_at	4.4	gei-14 encodes a novel protein that interacts with	
10100.5	501 11	100005_5_4		GEX-3 in yeast two-hybrid assays.	
C06A8.3		191204_s_at	4.4	Protein of unknown function DUF148;Domain of	Y
				unknown function DUF148	
H08J19.1		182541_at	4.3	contains similarity to Homo sapiens formin-like 3	
				isoform 1	
T01D1.1		181722_s_at	4.3	conserved protein, contains double-stranded beta-	
				helix domain, similar to IPR004313 (Acireductone	
				dioxygenase, ARD), IPR014710 (RmlC-like jelly	
				roll fold	
F38A5.3	lec-11	175705_s_at	4.3	Galectin, galactose-binding lectin; Concanavalin A-	Y
				like lectin/glucanase	
B0353.1		187251_at	4.3	contains similarity to Synechocystis sp	
				Serine/threonine-protein kinase C	
Y50D4B.2		176337_at	4.3	contains similarity to Homo sapiens Probable	
F21 1 0 2		100050	4.0	saccharopine dehydrogenase	
F21A9.2		189958_at	4.3	Zinc finger, C2H2-like	
F52H3.5	10. 0	179402_at	4.3	TPR repeat containing protein	
C46H11.4	lfe-2	176895_s_at	4.3	inositol (1,4,5) triphosphate-3-kinase (IP3K); Ife-2	
				activity is required negative regulation of the LET- 23 signaling pathway	
V22D7AD 2		176240 at	12	25 signating paurway	
Y22D7AR.3 C47B2.1	fbxa-140	176340_at 178679_at	4.3	protein containing an F-box and an FTH/DUF38	Y
C4/D2.1	10Ad-140	1/00/9_at	4.3	motif	1
				mour	

Gene name	Locus	Affy Probe	Fold	Description	TM
Y34D9A.10	vps-4	172130_at	4.3	AAA ATPase, core; MIT; Vps4 oligomerization, C-	
				terminal; MIT (microtubule interacting and	
				transport) domain	
F39B2.10	dnj-12	175394_at	4.2	contains a DnaJ domain, a prokaryotic heat shock	
				protein	
Y46H3A.3	hsp-16.2	188282_at	4.2	heat shock protein (hsp-16.2)	
F26A1.3		189149_at	4.2	Protein kinase	
C27D6.4		188956_at	4.2	bZIP-1, Basic leucine zipper; cAMP response	
				element binding (CREB) protein; Transcription	
				factor Jun; Eukaryotic transcription factor, Skn-1-	
76220.2		101017	1.0	like, DNA-binding	
ZC239.2		181917_at	4.2	Polymerase delta-interacting protein PDIP1 and	
DOED1 1		101001	4.0	related proteins, contain BTB/POZ domain	
R07B1.4	gst-36	191221_at	4.2	glutathione S-transferase	
Y55B1AL.1		187111_at	4.2		
C33C12.4		185158_s_at	4.2	putative membrane protein	Y
F38A3.2	ram-2	194252_x_at	4.2	ram-2 encodes a cuticle collagen that interacts with	Y
				unc-6 to affect ray cell migration, and interacts with	
C04E12.11		100025	1.0	unc-5 and unc-6 to affect embryonic viability	
C04F12.11	20	188825_s_at	4.2	transposon	
Y105E8A.16	rps-20	171977_x_at	4.1	rps-20 encodes a small ribosomal subunit S20	
E00E10.11	<i>ш</i> 1	174072 -4	4.1	protein.	
F09E10.11	tts-1	174073_at	4.1	non-coding RNA transcript of unknown function	
F19B10.10		186728_s_at	4.1	Predicted E3 ubiquitin ligase	
C17G1.5	2	179127_s_at 190313 at	4.1	town in 7 TM Dameir control (CDCD)	Y
C02D4.2	ser-2	190313_at 172363_x_at	4.1	tyramine 7-TM Domain receptor (GPCR) contains similarity to Interpro domains IPR002952	<u> </u>
F53A9.6		1/2303_x_at	4.1	(Eggshell protein), IPR002395 (HMW kininogen)	
C30G12.6		187628_s_at	4.1	Armadillo-type fold	
F31A3.3		180056_at	4.1	Armaumo-type rord	
K09F6.6		181370_s_at	4.1	Small secreted protein with conserved cysteines	
F54E7.6		182110 at	4.0	Sman secreted protein with conserved cystemes	Y
H25P19.1		175038_at	4.0		
Y39B6A.47	nhr-145	183498_s_at	4.0	nuclear hormone receptor	
C33A12.15	ttr-9	191296_at	4.0	Transthyretin-like family	
C48B4.2	rom-2	188662_at	4.0	contains similarity to Pfam domains PF01694	Y
C46D4.2	10111-2	166002_at	٦.0	(Rhomboid family), PF00036 (EF hand)	1
Y57G11C.24	eps-8	193793_s_at	4.0	eps-8 is predicted to encode five protein isoforms	
13/0110.24	срз-о	175175_s_at	т.0	with similarity to mouse epidermal growth factor	
				receptor kinase substrate	
R07B7.10		191927_at	4.0	Mitochondrial substrate carrier; Mitochondrial	Y
KU/D/.10		1)1)2/_at	7.0	carrier protein; Adenine nucleotide translocator 1	1
F58G1.4	dct-18	177978_at	4.0	Endoplasmic reticulum, targeting sequence	Y
ZK675.1	ptc-1	192536_at	4.0	an ortholog of Drosophila PATCHED (PTC) and	Y
21X0/J.1	ptc-1	172330_at	7.0	human PTCH, transmembrane protein	1
				numan F1CH, transmemorane protein	

**Table S2.** *fig-1* activity is required continuously for dye filling. Animals were cultivated on RNAi plates starting from different larval stages and were assayed for dye filling as young adults. In the case of L4 animals, this was 24 h of exposure. n = 50 for each.

### Normal Dye Filling (%)

Larval stage	Amphid	Phasmid
L1 (empty vector)	100	100
L1	80	35
L2	85	43
L4	100	72

### Supporting references and notes

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