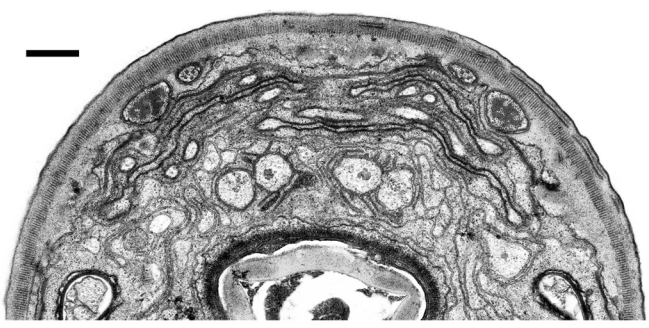
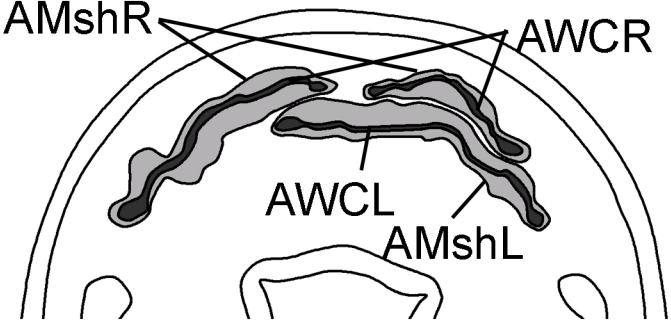
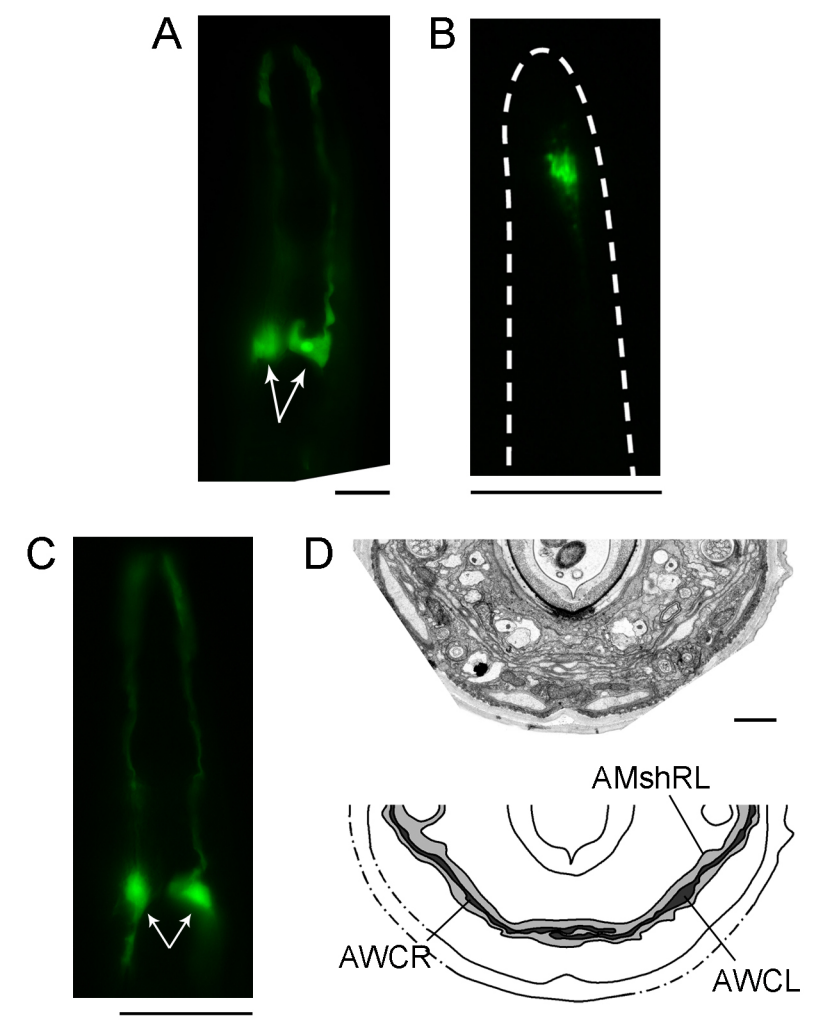
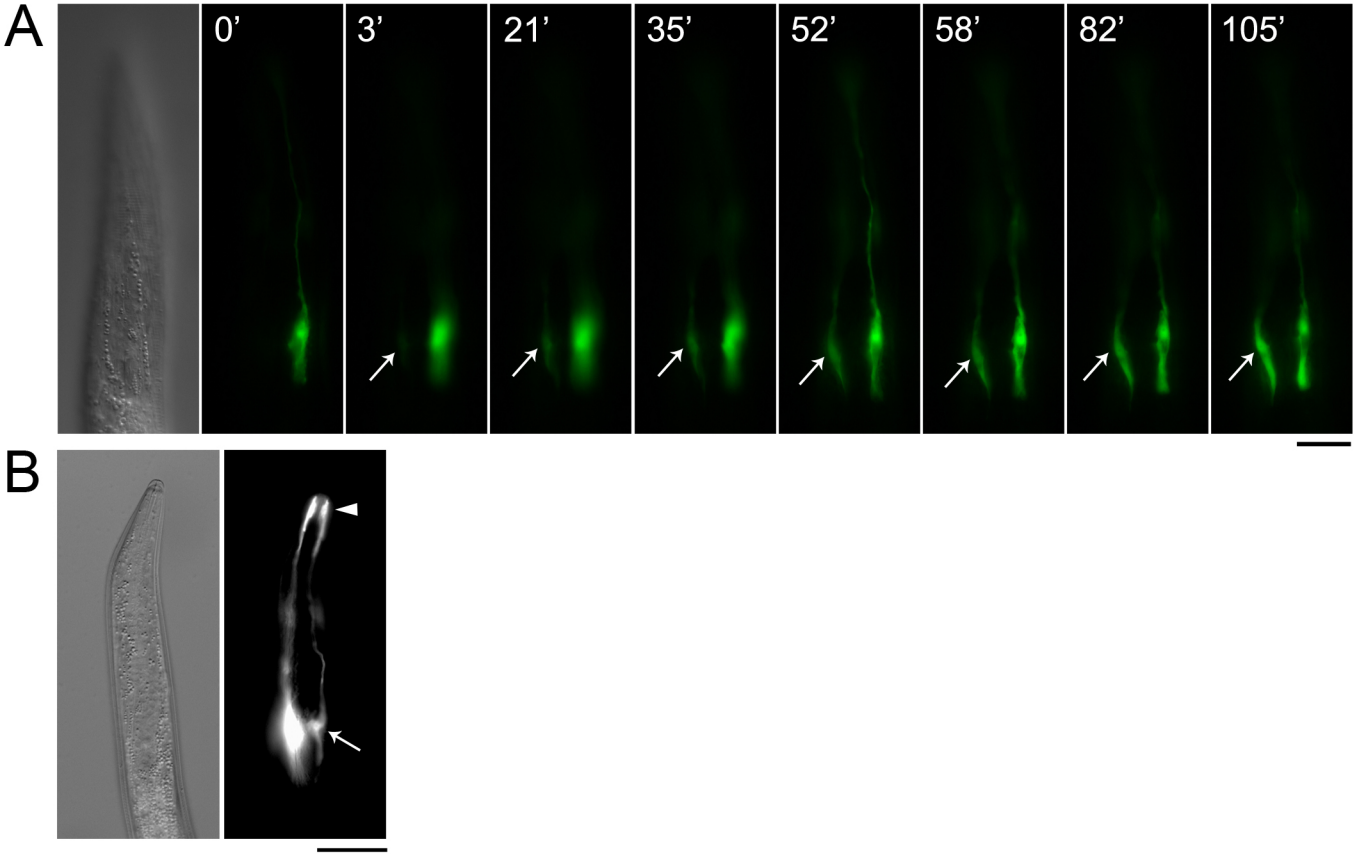
daf-7(e1372) dauer



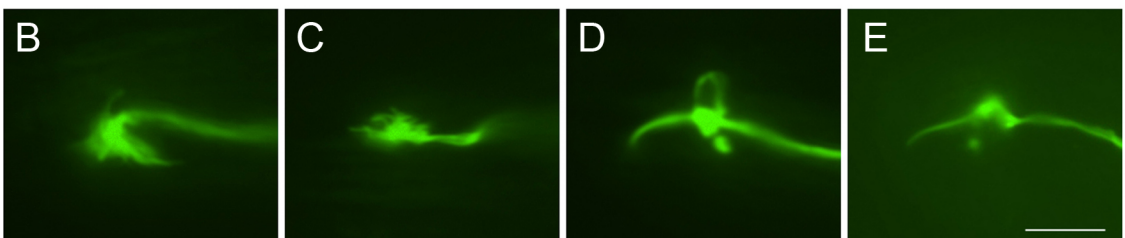


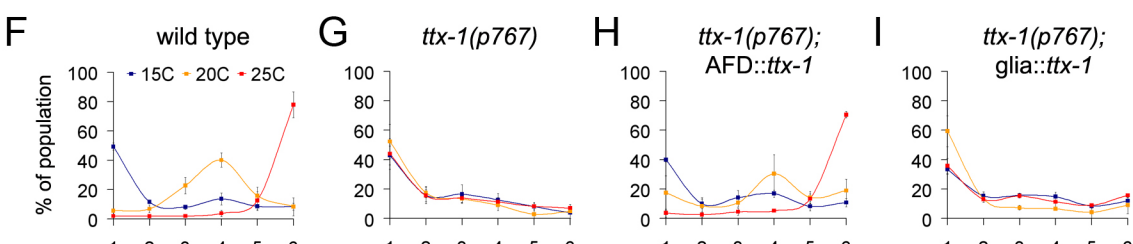

A
B
C
D
AMshRL
AWCR
AWCL


A
0’
3’
21’
35’
52’
58’
82’
105’
B

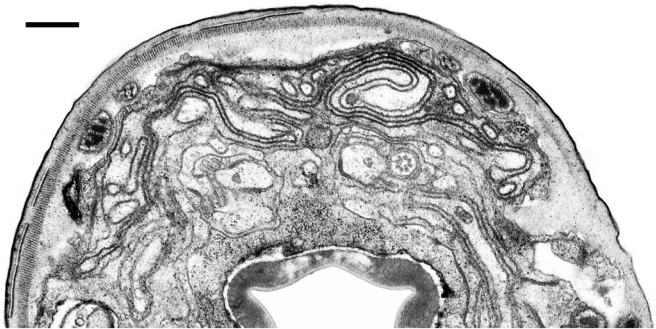
A

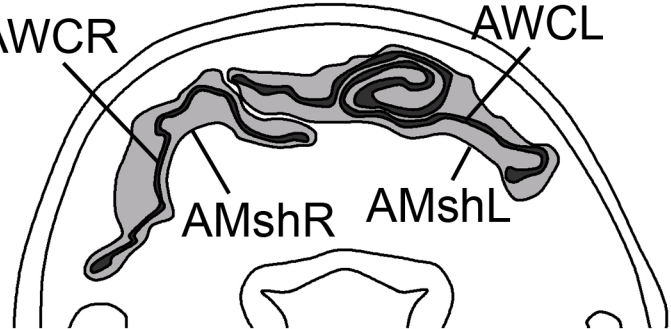
Genotype	AFD morphology at 25°C		
	absence of cilium	elongated single cilium	n
wild type	30	0	30
ttx-1(oy26)	5	24	29
ttx-1(oy26); AFD::*ttx-1*	14	1	15
ttx-1(oy26); AMsh::*ttx-1*	1	14	15
ttx-1(oy26); AM+PHsh::*ttx-1*	1	17	18

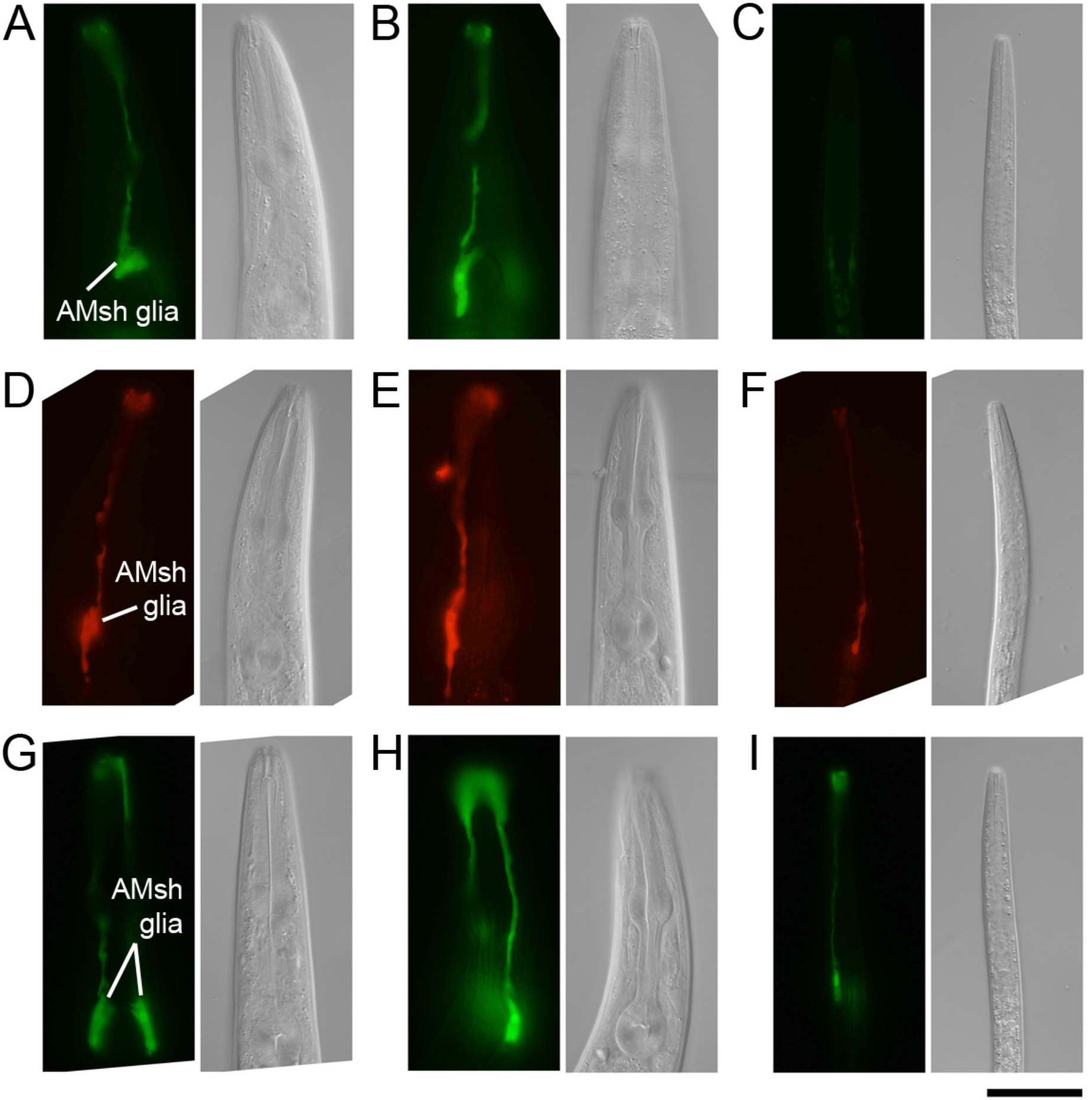




ttx-1(p767); daf-7(e1372) dauer






A
AMsh glia
B
C
D
AMsh
glia
E
F
G
AMsh
glia
H
I

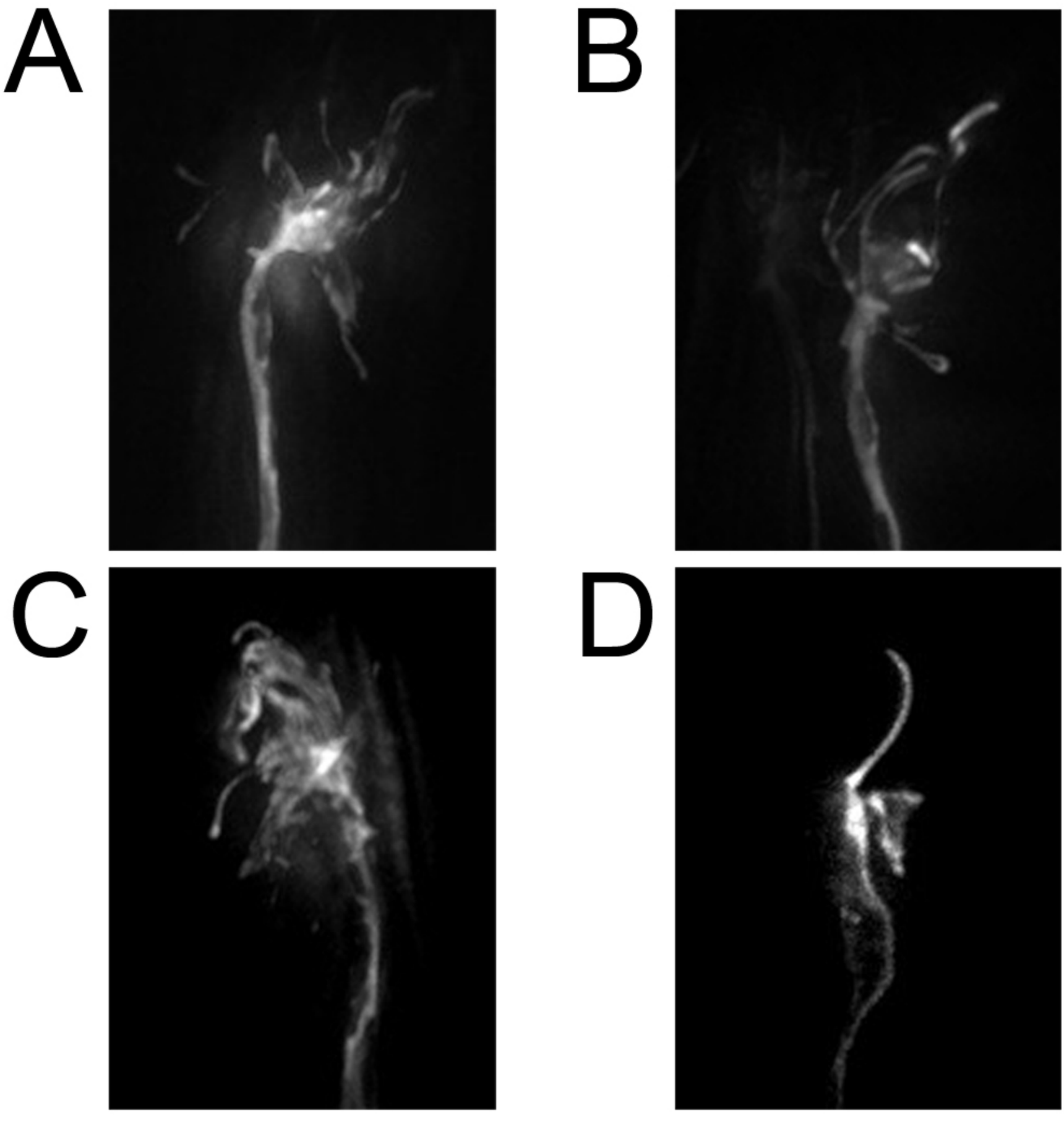

A
B
C
D

Table S1. **A summary of *ver-1* promoter deletion studies**

	sheath glia GFP expression at:				regulated by
Fragment coordinates[a]	15°C	25°C	dauer 15°C	dauer 25°C	***ttx-1*?**[b]
-2110 to +263 (in-frame)	–	++	++	++	yes
-2110 to +262 (-1 frame)[c]	–	++	nd	nd	nd
-2110 to +261 (-2 frame)[c]	–	++	nd	nd	nd
-2110 to -1	–	–	nd	nd	nd
+1 to +263	–	++	++	++	yes
+57 to +263	–	+	+	++	yes
+112 to +263	–	–	–	+	yes
+130 to +263	–	–	–	+	yes
+170 to +263	–	–	–	–	nd
+201 to +263	–	–	–	–	nd
+1 to +243	–	+	+	++	nd
+1 to +220	–	+	–	+	yes
+1 to +201	–	–	–	–	nd
+1 to +263 ATTA→GGGG[d]	–	–	–	–	nd

[a]The indicated fragments were fused to *gfp*, introduced into animals, and assayed for GFP expression. "–" indicates no expression, "+" weak expression, and "++" moderate to high expression, while "nd" indicates not determined. All constructs were injected at 60 ng/µl with 60 ng/µl pRF4. Coordinates refer to positions relative to the WormBase predicted ATG start codon of *ver-1*.

[b]To test if a *ver-1* reporter was regulated by *ttx-1*, a single array was crossed to *ttx-1(p767)* and scored for reduced GFP intensity.

[c]Frame-shift reporters likely give GFP expression using the *gfp* start site rather than the *ver-1* start, and demonstrate that regulation of GFP expression by temperature and dauer is transcriptional rather than translational.

[d]The core ATTA nucleotides of the predicted TTX-1 binding site (GGATTATC) are at position +176.

Table S2. Effects of mutations in genes controlling thermotaxis, neuronal morphology, dauer, and the heat-shock/UPR pathways on *ver-1* promoter::*gfp* expression

	***ver-1* expression 15°C**			***ver-1* expression 25°C**		
Genotype[a]	% PHsh on	% AMsh on	n	% PHsh on	% AMsh on	n
wild type	6	0	80	100	93	80
AFD, AIY or AIZ neurons (thermotaxis circuit)						
ttx-1(p767)	0	0	58	2	0	51
ttx-1(oy26)	0	0	47	0	0	44
ceh-14(ch3)	0	0	42	96	94	50
dac-1(gk211)	2	0	48	100	92	49
tax-2(p691)	0	0	41	100	88	50
tax-4(p678)	2	0	46	100	83	76
pkc-1(nj1)	5	0	40	100	88	40
pkc-1(nj3)	3	0	40	100	95	40
pkc-1(nj4)	5	0	40	100	95	40
pkc-2(ok328)	8	0	40	100	95	40
ttx-3(ks5)	5	0	39	100	80	51
ttx-3(mg158)	2	0	40	100	95	45
lin-11(n389)	5	0	41	100	98	54
lin-11(n566)	2	0	40	100	92	50
unc-86(e1416)	2	0	45	100	91	45
unc-86(n846)	2	0	43	100	94	54
***Otx/otd* transcription factors**						
ceh-37(ok642)	10	0	40	100	85	40
ceh-37(ok272)	10	0	40	100	83	40
ceh-36(ky646)	20	3	40	100	98	40
Neuronal cilia and dendritic morphology						
che-2(e1033)	0	0	55	100	86	51
che-13(e1805)	0	0	49	100	94	52
osm-6(p811)	0	0	44	100	83	48
dyf-7(ns89)	0	0	44	88	98	50
dyf-7(m537)	0	0	43	88	98	50
Dauer neuroendocrine pathways						
daf-7(e1372)	5	0	42	100[b]	100[b]	53
daf-12(m20)	0	0	40	100	90	50
daf-12(m25)	0	0	54	100	90	50
daf-2(m41)	0	0	39	100[c]	98[c]	54
daf-16(mu86)	5	0	40	100	100	40
Heat-shock and UPR pathways						
wild type	8	0	40	93[d]	43[d]	80
ire-1(zc14)	8	0	40	98[d]	45[d]	40
hsf-1(sy441)	13	0	40	100[d]	53[d]	40

[a]All strains contained the *ver-1* promoter::*gfp* transgene (*nsIs22*).
Animals were scored as adults at the indicated temperature except for:
[b]Dauer-constitutive *daf-7* animals as dauer larvae at 25°C.
[c]Dauer-constitutive *daf-2* animals as dauer-recovered adults at 25°C.
[d]Due to increased lethality at high temperatures, *ire-1(zc14)* and *hsf-1(sy441)* animals were cultivated first at 15°C before shifting as L4 larvae to 25°C and scoring GFP intensity 24 h later. Wild-type control animals were shifted under the same conditions.

Table S3. Restoring *ttx-1* expression to *ttx-1(p767)* mutant adults using a heat-inducible promoter rescues *ver-1* expression

Genotype[a]	heat shock[b]	*ver-1* expression 15°C		*ver-1* expression 25°C	
		% PHsh on	% AMsh on	% PHsh on	% AMsh on
wild type	−	8	0	90	30
	+	10	0	95	40
ttx-1(p767)	−	0	0	0	0
	+	0	0	0	0
ttx-1(p767); heat shock::*ttx-1a*[c]	−	0	0	0	0
	+	13	0	55	10
ttx-1(p767); heat shock::*ttx-1b*[c]	−	0	0	0	0
	+	8	0	63	20

[a]All strains contained the *ver-1* promoter::*gfp* transgene (*nsIs22*).

[b]Animals carrying heat-shock promoter::*ttx-1* arrays were cultivated initially at 15°C. Adult animals were heat shocked at 34°C for 50 min, and then transferred either to 15°C or 25°C. *ver-1* promoter::*gfp* expression was scored 24 h later.

[c]heat-shock promoter::*ttx-1a* and heat-shock promoter::*ttx-1b* lines used were *nsEx1636* and *nsEx1680*, respectively. *ttx-1a* and *ttx-1b* are described in Table 1.

$n = 40$ for all values.

Table S4. Strains.	
Mutant alleles used:	LGI: *tax-2*(*p691*), *lin-11*(*n389, n566*), *che-13*(*e1805*), *daf-16*(*mu86*); LGII: *ire-1*(*zc14*); LGIII: *dac-1*(*gk211*), *tax-4*(*p678*), *unc-86*(*e1416, n846*), *daf-7*(*e1372*), *daf-2*(*m41*); LGIV: *osm-9*(*ky10*); LGV: *ttx-1*(*p767, oy26*), *pkc-1*(*nj1, nj3, nj4*), *osm-6*(*p811*); LGX: *ceh-14*(*ch3*), *pkc-2*(*ok328*), *ttx-3*(*ks5, mg158*), *ceh-37*(*ok642, ok272*), *ceh-36*(*ky646*), *che-2*(*e1033*), *dyf-7*(*ns89, m537*), *daf-12*(*m20, m25*), *lin-15*(*n765*).
ver-1 alleles used:	The predicted VER-1 protein is 1083 amino acids in length, and includes an amino-terminal extracellular region with immunoglobulin-like domains, flanked by a signal sequence and a single transmembrane domain. An intracellular protein kinase domain is predicted (Popovici et al., 2002). The *ver-1(tm1348)* allele has a frame-shift deletion that codes for a truncated protein (267 conserved amino acids in length) without the transmembrane and protein kinase domains, and which is followed by an additional non-homologous 21 amino acids (HSPSSETLRSETNSEKFYTFZ). A single base mutation also causes an amino acid change at position 266 (Y to C). The *ver-1(ok1738)* allele has an in-frame deletion (between residues 168 and 417) removing part of the extracellular region.
Integrated transgenes used:	LGIV: *nsIs22*[$P_{ver\text{-}1}$*gfp*; *lin-15*(+)], *nsIs53*[$P_{vap\text{-}1}$*dsRed*; *unc-119*(+)], *nsIs142*[$P_{F16F9.3}$*dsRed*; *lin-15*(+)]; LGV: *oyIs17*[$P_{gcy\text{-}8}$*gfp*; *lin-15*(+)], *oyIs45*[$P_{odr\text{-}1}$*yfp*; *lin-15*(+)]; LGX: *nsIs143*[$P_{F16F9.3}$*dsRed*; *lin-15*(+)]. The sheath glia-specific integrated *ttx-1* rescuing transgene (glia::*ttx-1*) was LGV: *nsIs219*[$P_{T02B11.3}$*ttx-1a*; $P_{unc\text{-}122}$*dsRed*; pSL1180]. The integrated AFD-specific rescuing transgene (AFD::*ttx-1*) was LGX: *nsIs99*[$P_{gcy\text{-}8}$*ttx-1a;*$P_{gcy\text{-}7}$*gfp; lin-15*(+)]. Strains carrying *nsIs99* may also contain the linked *lin-15* allele *n765*.
Extrachromosomal arrays used:	*nsEx755*[$P_{gcy\text{-}8}$*egl-1*[x2]; *rol-6(su1006)*], *nsEx1320*[$P_{ttx\text{-}1(AFD)}$*dsRed*; *rol-6(su1006)*], *nsEx1942*[$P_{ttx\text{-}1(glia)}$*gfp*; *rol-6(su1006)*], *nsEx2436*[$P_{T02B11.3}$*gfp*; *rol-6(su1006)*], *nsEx2703*[$P_{F16F9.3}$*eff-1a::gfp*; *rol-6(su1006)*], *nsEx2727*[$P_{F16F9.3}$*aff-1::gfp*; *rol-6(su1006)*], *nsEx1685/nsEx1391*[$P_{F16F9.3}$*gfp*; *rol-6(su1006)*]. *hyEx167* ($P_{aff\text{-}1}$*gfp*) and *zzEx26* ($P_{eff\text{-}1}$*gfp*) were gifts from Benjamin Podbilewicz and William Mohler. *ttx-1* rescuing arrays included: *nsEx875/nsEx897/nsEx899*[$P_{gcy\text{-}8}$*ttx-1a*; *rol-6(su1006)*], *nsEx877/nsEx893/nsEx895*[$P_{vap\text{-}1}$*ttx-1a*; *rol-6(su1006)*], *nsEx930/nsEx937/nsEx939*[$P_{F16F9.3}$*ttx-1a*; *rol-6(su1006)*], *nsEx1636*[$P_{heat\ shock}$*ttx-1a*; *rol-6(su1006)*], *nsEx1680*[$P_{heat\ shock}$*ttx-1b*; *rol-6(su1006)*]. glia::*Otx* rescuing arrays included: *nsEx1661*[$P_{F16F9.3}$*Otx1*; *rol-6(su1006)*], *nsEx1662*[$P_{F16F9.3}$*Otx2*; *rol-6(su1006)*].

Table S5. Primers used for plasmid construction.	
Primer name	**Primer sequence (restriction sites are underlined)**
aff-1 XmaI 5’	5’ cac aca ccc ggg atg cga ctg tgg caa tgg 3’
aff-1::gfp KpnI 3’	5’ cac ggt acc cag taa tca gat gaa ttc ttc ttt ttc ttc 3’
eff-1a XmaI 5’	5’ cac aca ccc ggg atg gaa ccg ccg ttt gag 3’
eff-1a::gfp 3’	5’ cac ggt acc caa atg tac tgg cta ctg cta tag tg 3’
egl1-1	5’ cac aca gga tcc atg tcc aac gtt ttt gac gtt c 3’
egl1-2	5’ cac aca cca tgg tta aaa agc gaa aaa gtc cag aag ac 3’
F16F9.3-1	5’ cac aca ctg cag gct tat gaa atg cgg aac ttg g 3’
F16F9.3-2	5’ cac aca gga tcc ttt tgt ttc tta ctg tct tgg gta 3’
F16F9.3(AgeI) 3’	5’ cac aca acc ggt ttt tgt ttc tta ctg tct tgg gta 3’
F16F9.3(NheI) 5’	5’ cac aca gct agc gct tat gaa atg cgg aac ttg g 3’
gcy8-3	5’ cac aca ctg cag aac tac ctt cct ccg cgt cca cc 3’
gcy8-4	5’cac aca gga tcc cat ttt gat gtg gaa aag gta g 3’
mmOtx1_1	5’ cac aca ccc ggg atg atg tct tac ctc aaa caa ccc 3’
mmOtx1_2	5’ cac aca gct agc tca caa gac ctg gaa ccg 3’
mmOtx2_1	5’ cac aca ccc ggg atg atg tct tat cta aag caa ccg c 3’
mmOtx2_2	5’ cac aca gct agc tca caa aac ctg gaa ttt cca t 3’
myo2-2	5’ cac aca tct aga cac ttt tca gct acc tag aat tac 3’
myo2-3	5’ cac aca ggt acc gag ggt taa aat gaa aag tgg tgg 3’
T02B11.3 BamHI 3’	5’ cac gga tcc gaa aat tta gaa tcg aga aaa atg a 3’
T02B11.3 PstI 5’	5’ cac act gca gac caa aac tat tga ttt tgt ccc ta 3’
ttx1cDNA-3	5’ cac aca cca tgg tca gct cat ttg aaa ttt cca ag 3’
ttx1cDNA-4	5’ aca gga tcc atg tcc ttg acg tct tcc tca gct cca tcc 3’
ttx1rfp-19	5’ cac aca gga tcc tat agt acc ata gga ctt tcc 3’
ttx1rfp-20	5’ cac aca ccc ggg aaa tgc tag aac att cta gag t 3’
ttx1rfp-21	5’ cac aca ccc ggg aaa act cta gaa tgt tct agc 3’
ttx1rfp-22	5’ cac aca gtc gac tgg aca cat aaa tat tgt cg 3’
ttx1rfp-23	5’ cac aca gtc gac gac aat att tat gtg tcc agc 3’
ttx1rfp-24	5’ cac aca ctc gag aac tga gtt atg gca ctt c 3’
ttx1rfp-25	5’ cac aca ctc gag aag tgc cat aac tca gtt c 3’
ttx1rfp-30	5’ cac aca ggt acc gc tga gga aga cgt caa gga 3’
ttx1rfp-35	5’ cac aca gcg gcc gcc acg gaa agt cct atg gta c 3’
ttx1rfp-38	5’ cac aca ctc gag ct agc aag tgg aga tga ccc tct tgg 3’
ttx1rfp-39	5’ cac aca gaa ttc aga atc ctt gta tac att tgg 3’
ttx1rfp-40	5’ cac aca gaa ttc cca aat gta tac aag gat tct g 3’
ttx1rfp-41	5’ cac aca gga tcc gta acc tca atc cca tgg ctg 3’
ttx1rfp-42	5’ cac aca gga tcc agc cat ggg att gag gtt ac 3’
ttx1rfp-47	5’ cac aca ctg cag cag ttt gaa tag ttc cgc gca gg 3’
ttx1rfp-48	5’ cac aca ctg cag cct gcg cgg aac tat tca aac tg 3’
ttx-1 HD BamHI 5'	5’ cac aca gga tcc ggt tcc cgt cga atg ttg 3’
ttx-1 HD XhoI 3'	5’ cac aca ctc gag ggt ggc cgg gga tga c 3’
ver-30	5’ cac aca ctg cag caa agg aat gat att tct cct gtt ac 3’
ver-31	5’ cac aca ctg cag aac tac caa taa ttt cat tgc g 3’
ver1_del-1	5’ cac aca ctg cag ttg cgc ccc aaa tct gac tg 3’
ver1_fus-6	5’ cac aca gca tgc caa tgc act gga ata ctc cag 3’

ver1_fus-7	5’ cac aca gga tcc ggg aag agg cgt gat ggt gtg 3’
ver1(+176) ATTA mut 5'	5’ gtt ttg gaa atg tac aac ggg ggg tcg gat tca ggt aag taa c 3’
ver1(+176) ATTA mut 3'	5’ gtt act tac ctg aat ccg acc ccc cgt tgt aca ttt cca aaa c 3’
ver1(3'del+243)BamHI 3'	5’ cac agg atc ctt ttg att ttt ctg aaa gtt ttc atg 3’
ver1(3'del+220)BamHI 3'	5’ cac agg atc cat gtt aaa ccg ctt caa agt agt tac 3’
ver1(3'del+201)BamHI 3'	5’ cac gga tcc gta gtt act tac ctg aat ccg ata atc c 3’
ver1 63bp	5’ cac aca ctg cag ctt tga agc ggt tta aca tga aaa c 3’
ver1 94bp	5’ cac aca ctg cag caa cgg att atc gga ttc agg 3’
ver1cDNA-1	5’ cac aca ctg cag atg act cat cga acc ccc aat 3’
VERframe1	5’ cac aca gga tcc aac tct aca aac ttt cca att ttt tga 3’
VERframe2	5’ cac aca gga tcc act cta caa act ttc caa ttt ttt gat 3’
VERframe3	5’ cac aca gga tcc ctc tac aaa ctt tcc aat ttt ttg att 3’

Table S6. Plasmids.	
Plasmid name	**Plasmid construction**
ver-1::gfp reporter constructs	The initial $P_{ver\text{-}1}$*gfp* construct was a gift from R. Roubin and C. Popovici. It includes promoter sequence from -2110 to +263 relative to the *ver-1* ATG start site. Regions -2110 to -1 (plasmid pCP.17), +1 to +263 (pCP.36), +57 to +263 (pCP.98), +112 to +263 (pCP.99), +130 to +263 (pCP.43), +170 to +263 (pCP.109), +201 to +263 (pCP.110), +1 to +243 (pCP.132), +1 to +220 (pCP.133), and +1 to +201 (pCP.134) were PCR amplified from N2 genomic DNA using primers ver1_fus-6 and ver1_fus-7, ver1cDNA-1 and VERframe1, ver-30 and VERframe1, ver-31 and VERframe1, ver1_del-1 and VERframe1, ver1 94bp and VERframe1, ver1 63bp and VERframe1, ver1_fus6 and ver1(3'del+243)BamHI 3', ver1_fus6 and ver1(3'del+220)BamHI 3', and ver1_fus6 and ver1(3'del+201)BamHI 3', respectively. The -2110 to -1 amplicon was inserted as an SphI-BamHI fragment into vector pPD95.69, while all other fragments were inserted as PstI-BamHI fragments into pPD95.75. The frame-shift *ver-1* reporters -2110 to +262 (-1 frame) (pCP.33) and -2110 to +262 (-2 frame) (pCP.34) were PCR amplified with primers ver1_fus-6 and VERframe2, and ver1_fus-6 and VERframe3, respectively, and inserted into pPD95.75 at SphI-BamHI sites. The ATTA TTX-1 binding site at position +176 to +179 was mutated to GGGG by point mutagenesis of plasmid pCP.36 using primers ver1(+176) ATTA mut 5' and ver1(+176) ATTA mut 3' to create pCP.131.
ttx-1 reporter expressed in AFD (7.5 kb upstream): $P_{ttx\text{-}1\text{pro1}}$*dsRed* (pCP.26)	Four separate PCR fragments covering -7482 to +23 relative to the ATG start site of the *ttx-1* gene were amplified from N2 genomic DNA using primers ttx1rfp-19 and ttx1rfp-20, ttx1rfp-21 and ttx1rfp-22, ttx1rfp-23 and ttx1rfp-24, and ttx1rfp-25 and ttx1rfp-30. These were cloned as BamHI-XmaI, XmaI-SalI, SalI-XhoI and XhoI-KpnI fragments into pBluescript KS(+), then as a single NotI-KpnI fragment into the pEP.9 *dsRed* vector (Elliot Perens).
ttx-1 reporter expressed in glia (3.5 kb further upstream): $P_{ttx\text{-}1\text{pro2}}$*gfp* (pMH.28)	Four separate PCR fragments covering -10965 to -7451 relative to the ATG start site of the *ttx-1* gene were amplified from N2 genomic DNA using primers ttx1rfp-38 and ttx1rfp-39, ttx1rfp-40 and ttx1rfp-48, ttx1rfp-47 and ttx1rfp-41, and ttx1rfp-42 and ttx1rfp-35. These were cloned as XhoI-EcoRI, EcoRI-PstI, PstI-BamHI, and BamHI-NotI fragments into pBluescript KS(+), then as a single NheI-NotI fragment into the pEP.9 *dsRed* vector (Elliot Perens). To facilitate expression, the *myo-2* 160 bp minimal promoter was amplified with primers myo2-2 and myo2-3 from genomic DNA and inserted as an XbaI-KpnI fragment (Okkema et al., 1993). *dsRed* was then replaced with the *gfp* coding sequence from vector pPD95.75 as a KpnI-ApaI fragment. When injected,

	all lines gave GFP expression in the amphid and phasmid socket cells. Some of these lines also had sheath cell expression. Occasionally weak fluorescence was also observed in other cell types.
AFD::*ttx-1a* and AFD::*ttx-1b* (P_{gcy-8}*ttx-1a*, pCP.6, and P_{gcy-8}*ttx-1b*, pCP.5); AMsh::*ttx-1a* and AMsh::*ttx-1b* (P_{vap-1}*ttx-1a*, pCP.2, and P_{vap-1}*ttx-1b*, pCP.1); AM+PHsh::*ttx-1a* and AM+PHsh::*ttx-1b* ($P_{F16F9.3}$*ttx-1a*, pCP.19, and $P_{F16F9.3}$*ttx-1b*, pCP.20); AFD::*egl-1*[x2] (P_{gcy-8}*egl-1*[x2], pCP.9)	2 kb of *gcy-8* and F16F9.3 promoters were PCR amplified from N2 genomic DNA using primers gcy8-3 and gcy8-4, and F16F9.3-1 and F16F9.3-2, respectively. Both promoters were ligated into vector pPD49.78 at PstI-BamHI sites, replacing the *hsp-16.2* promoter, to generate cell-specific expression vectors. The *vap-1* expression vector (pTB vap1) was made by Taulant Bacaj in the same way, using ~5 kb of upstream *vap-1* sequences. cDNA template was generated by washing animals from mixed-stage plates at 15°C and 25°C, and extracting total RNA using TRIzol Reagent (Invitrogen). Poly(A) RNA was further purified using the Poly(A) Purist kit (Ambion). cDNA was generated using SuperScript II Reverse Transcriptase (Invitrogen). *ttx-1a* and *ttx-1b* cDNA isoforms were PCR amplified using primers ttx1cDNA-4 and ttx1cDNA-3, and ligated into expression vectors at sites BamHI-NcoI. The *egl-1* cDNA was amplified using primers egl1-1 and egl1-2, and ligated into the *gcy-8* expression vector at BamHI-NcoI. A double insertion of the *egl-1* cDNA occurred, generating two consecutive *egl-1* cDNA's both running 5' to 3' following the promoter. The sequences of all cDNAs generated by PCR were confirmed by sequencing. (WormBase release: WS206.)
glia::*ttx-1* ($P_{T02B11.3}$*ttx-1a*, pCP.161)	2.5 kb of T02B11.3 promoter was PCR amplified from N2 genomic DNA using primers T02B11.3 PstI 5' and T02B11.3 BamHI 3', and ligated into vector pPD49.78 at PstI-BamHI, replacing the heat-shock promoter, to create plasmid pCP.158. The T02B11.3 promoter gives specific expression in AMsh and PHsh glia only (Bacaj et al., 2008). The *ttx-1a* cDNA from pCP.2 above was inserted into pCP.158 as a BamHI-SpeI fragment to make pCP.161.
heat shock::*ttx-1a* (pCP.94) and heat shock::*ttx-1b* (pCP.95)	The *ttx-1a* and *ttx-1b* cDNAs generated above were inserted into vector pPD49.78 at BamHI-NcoI.
Mammalian cDNA expression vectors, glia::*Otx1* ($P_{F16F9.3}$*Otx1*, pCP.100) and glia::*Otx2* ($P_{F16F9.3}$*Otx2*, pCP.101)	Mouse *Otx1* and *Otx2* cDNAs were PCR amplified from Marathon-Ready mouse cDNA (Clontech) using primers mmOtx1_1 and mmOtx1_2, and mmOtx2_1 and mmOtx2_2, respectively. The amplicons were inserted into the F16F9.3 expression vector generated above at XmaI-NheI sites.
$P_{F16F9.3}$*gfp* (pCP.41)	To create pCP.41, the F16F9.3 promoter was PCR amplified from

and $P_{F16F9.3}$*dsRed* (pCP.81)	N2 genomic DNA using primers F16F9.3-1 and F16F9.3-2, and inserted into vector pPD95.75 at PstI-BamHI. For pCP.81, the promoter was amplified using primers F16F9.3(NheI) 5' and F16F9.3(AgeI) 3', and inserted into vector pEP9 (Elliot Perens) at NheI/AgeI.
$P_{F16F9.3}$*eff-1a::gfp* (pCP.179) and $P_{F16F9.3}$*aff-1::gfp* (pCP.186)	*eff-1a* and *aff-1* cDNA's were PCR amplified from cDNA template using primers eff-1a XmaI 5' and eff-1a::gfp KpnI 3', and aff-1 XmaI 5' and aff-1::gfp KpnI 3', respectively, and inserted into pCP.41 at XmaI-KpnI sites.
GST::TTX-1(HD) (pCP.142)	The homeodomain of *ttx-1* was PCR amplified from *ttx-1* cDNA with primers ttx-1 HD BamHI 5' and ttx-1 HD XhoI 3', and inserted into GST fusion vector pGEX-5X-1 at BamHI-XhoI to create plasmid pCP.142. This construct removes the first 154 and last 95 amino acids of the TTX-1a protein.