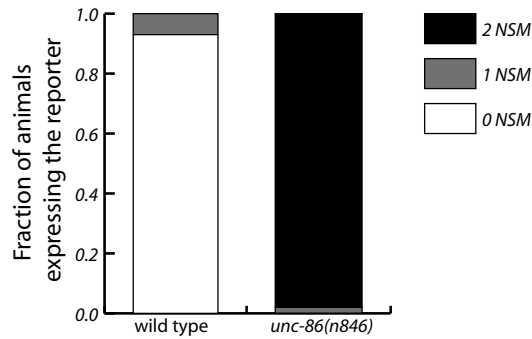
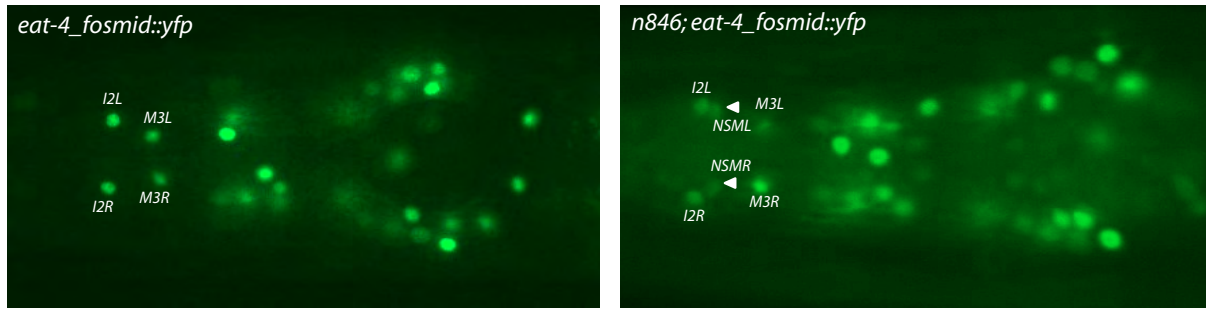
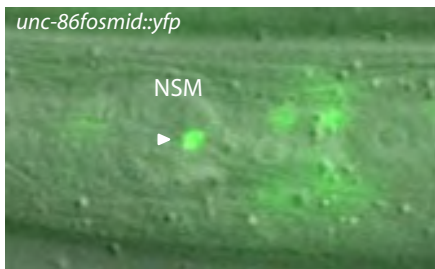


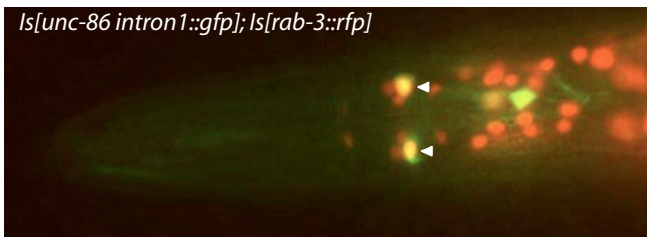
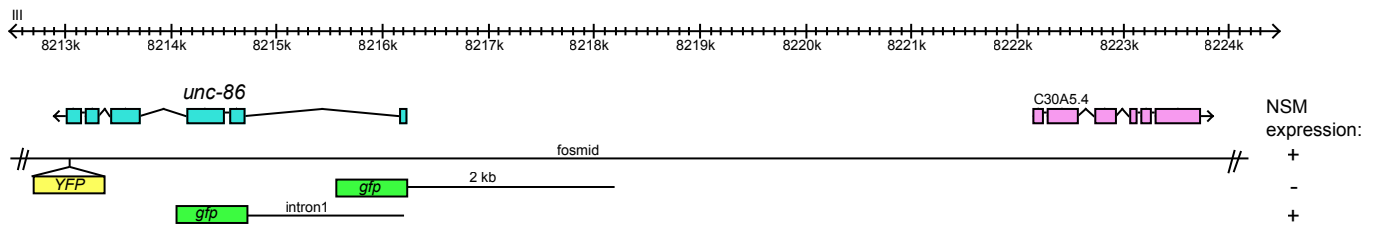
A



B



C



	% NSM on	n
<i>ls[unc-86 intron1::gfp]</i>	93%	28
<i>n846; ls[unc-86 intron1::gfp]</i>	0%	56

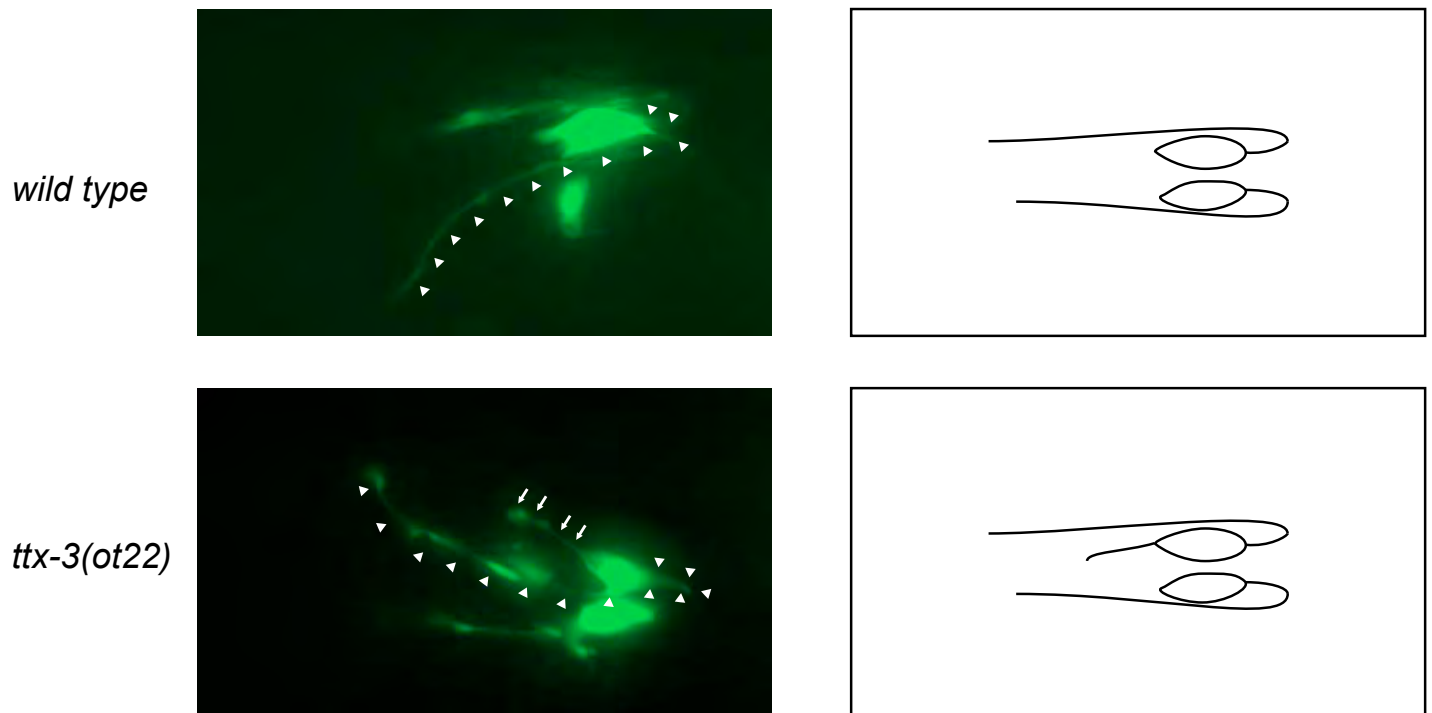
Suppl. Figure 1: Analysis of the NSM neurons.

A: A fosmid reporter of the *eat-4* locus was kindly provided by E. Serrano and will be published elsewhere.

B: A *unc-86* fosmid reporter construct is expressed in the NSM neurons of adult animals.

C: A *cis*-regulatory element from the *unc-86* locus drives expression in NSM and this expression depends on *unc-86*.

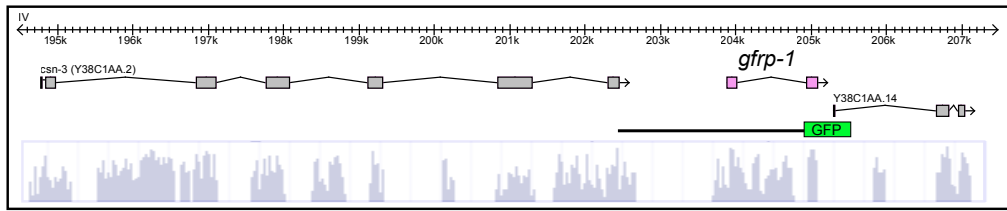
AIA neuron morphology



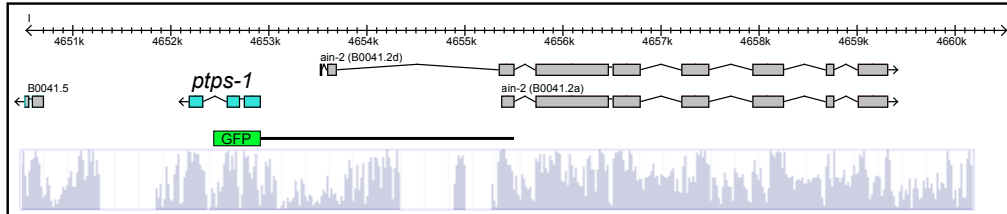
Suppl. Figure 2: AIA morphology in wildtype and *ttx-3(ot22)* mutant animals.

AIA morphology was visualized with *ins-1::gfp (otIs326)*. Left panels show AIA morphology schematically. White triangles in the *gfp* images indicate one of the main axons (normal axon). White arrows indicate the ectopic branching from the cell body in *ttx-3* mutants. Note also the blebbing of the main axon.

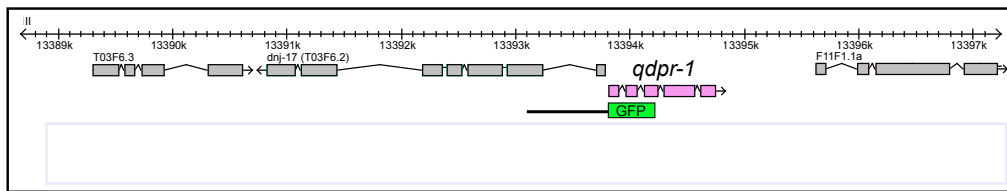
Expression pattern:



not NSM,
not HSN
a few very dim cells in the head



NSM
HSN
VC4 and VC5
no ADF
more VNC neurons
tail neuron



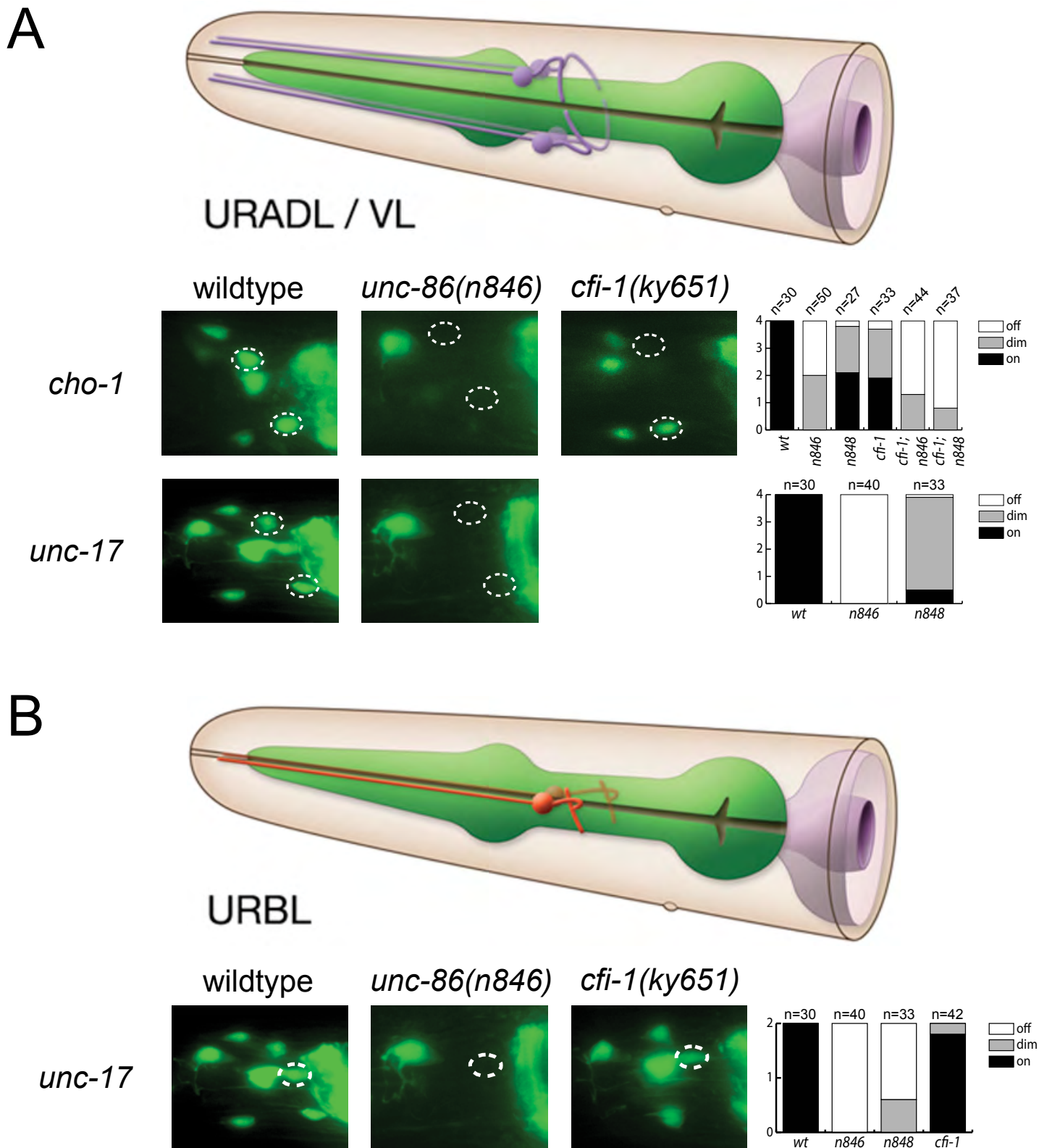
not NSM,
not HSN
several dim cells in the head (ADF?)
tail and head hypodermal cells



not NSM,
not HSN
not ADF
a couple very faint cells in head
no obvious neuronal expression
tail hypodermal cells
pharyngeal muscle expression

Suppl. Figure 3: BH4 pathway reporters.

Reporter genes and overview of expression pattern of BH4 pathway genes. Expression patterns were observed with multiple lines.



Suppl. Figure 4: *unc-86* controls the identity of the cholinergic URA and URB neurons.

A: *unc-86* and *cfi-1* affect URA identity. Lateral views (anterior to left) are shown. Bar graphs indicate average number of cells expressing *gfp* in the four URA neurons.

B: *unc-86*, but not *cfi-1* affects URB identity as assessed with two different *unc-86* allele, *n846* and *n848*. Lateral views (anterior to left) are shown. Bar graphs indicate average number of cells expressing *gfp* in the two URB neurons.

Table S1. Rescue of NSM and AIA differentiation defects of *ttx-3* mutant animals

	% animals expressing <i>mgl-1::mcherry</i> in NSM	% animals expressing <i>mgl-1::mcherry</i> in AIA	<i>n</i>
wild type	100	100	>100
<i>ttx-3(ot22)</i>	0	0	>100
<i>ttx-3(ot22); Ex[cat-1^{prom}::ttx-3cDNA] line 1</i>	92	0	36
<i>ttx-3(ot22); Ex[cat-1^{prom}::ttx-3cDNA] line 2</i>	89	0	37
<i>ttx-3(ot22); Ex[ins-1^{prom}::ttx-3cDNA] line 1</i>	0	79	38
<i>ttx-3(ot22); Ex[ins-1^{prom}::ttx-3cDNA] line 2</i>	0	58	36
<i>ttx-3(ot22); Ex[ins-1^{prom}::ttx-3cDNA] line 3</i>	0	79	38

Injection marker: *rol-6(d)*. Note that the *ins-1* promoter is still weakly expressed in *ttx-3* mutants and can hence be used to drive *ttx-3* in *ttx-3* mutants.

Table S2. Strains and transgenes used in this study

Strain/Array	Notes
<i>ttx-3(ot22)</i>	Premature stop before the homeobox (Altun-Gultekin et al., 2001).
<i>unc-86(n846)</i>	A likely null allele resulting in protein loss (Röhrig, 2000). The molecular identity of this strong allele had not been previously described. We sequenced this allele and it to harbor a G>A splice acceptor site mutation at end of the second intron of the C30H5.7a transcript in the middle of the POU domain (aatacttcagGCGG to aatactcaaGCGG)
<i>unc-86(n848)</i>	The molecular nature of this temperature-sensitive allele is a GT to AT splice donor site mutation in intron 4 (Röhrig, 2000)
<i>cfi-1(ky651)</i>	A splice acceptor site mutation before the DNA binding domain (Shaham and Bargmann, 2002)
<i>otls224</i>	<i>Is[cat-1::gfp]</i> (Flames and Hobert, 2009)
<i>otls225</i>	<i>Is[cat-4::gfp]</i> (Flames and Hobert, 2009)
<i>otls226</i>	<i>Is[bas-1::gfp]</i> (Flames and Hobert, 2009)
<i>zdl-13</i>	<i>Is[tph-1::gfp]</i> (Clark and Chiu, 2003)
<i>wgls68</i>	<i>Is[ttx-3fosmid::EGFP-FLAG, unc-119(+)]</i> . Kindly provided by Valerie Reinke and the ModEncode consortium. Based on fosmid WRM064cD04. The tag was TY1 EGFP 3xFLAG and was added at the C-terminus.
<i>otls337</i>	<i>Is[unc-86 fosmid^{WRM0612cF07}::NLS::YFP::H2B; ttx-3::mCherry]</i> . The <i>unc-86</i> fosmid reporter was generated bacterial recombineered as previously described (Tursun et al., 2009), fusing an SL2::NLS::YFP::H2B reporter cassette at the C-terminus of <i>unc-86</i> in fosmid WRM0612cF07.
<i>vsIs33</i>	<i>Is[dop-3::dsRed]</i> . Kindly provided by Michael Koelle
<i>otls317</i>	<i>Is[mgl-1^{long prom}::mcherry, pha-1]</i> . DNA kindly provided by Kaveh Ashrafi (Greer et al., 2008)
<i>otls341</i>	<i>Is[mgl-1^{short prom}::gfp]</i> -1994 to -1374 bp upstream of ATG
<i>otls379</i>	<i>Is[cho-1^{AlA prom}::gfp; rol-6(d)]</i> -3006 to -2642 bp upstream of ATG
<i>otls326</i>	<i>Is[ins-1::gfp; rol-6(d)]</i> -289 bp upstream of ATG
<i>otEx4687</i>	<i>Ex[glr-2::gfp; rol-6(d)]</i> -1798 bp upstream of ATG
<i>otEx4886</i>	<i>Ex[ttx-3 intron7::gfp; rol-6(d)]</i> GGAAG+intron7+CGTCTACCGATGAAGATG cloned into pPD95.75
<i>otEx5056</i>	<i>Ex[flip-2::gfp; rol-6(d)]</i> -2002 bp upstream of ATG
<i>otEx4781</i>	<i>Ex[mod-5^{NSM prom}::gfp; elt-2::gfp]</i> . First intron of mod-5 cloned into pPD95.75(CACCAGCAGCTGCAAG+ intron1+ CTGAACTCTCC driving GFP)
<i>otEx5280</i>	<i>Ex[ptps-1::gfp; rol-6(d)]</i> -2600 bp upstream of ATG
<i>otEx5163</i>	<i>Ex[nlp-3::gfp; rol-6(d)]</i> . DNA kindly provided by Hart lab (Nathoo et al., 2001)
<i>otEx5364</i>	<i>Ex[mgl-3::gfp; rol-6(d)]</i> .

	DNA kindly provided by Ashrafi lab (Greer et al., 2008)
<i>otEx5163</i>	<i>[nlp-13::gfp; rol-6(d)]</i> -1967 bp upstream of ATG
<i>otEx5055</i>	<i>[scd-2::gfp; rol-6(d)]</i> -2045 bp upstream of ATG
<i>otEx5363</i>	<i>[flr-2::gfp; rol-6(d)]</i> DNA kindly provided by Takeshi Ishihara
<i>otEx4917</i>	<i>Ex[unc-86 intron1::gfp; rol-6(d)]</i> GACGACAACCGCTTCAAAAATGCAACCT+intron1+TTCAACAAC AGTTTATTTGGATCATTTCGATGACCC cloned into pPD95.75
<i>otEx4969,</i> <i>otEx4970</i>	2 independent lines of <i>Ex[cat-1^{prom14}::ttx-3; rol-6(d)]</i>
<i>otEx5073,</i> <i>otEx5074,</i> <i>otEx5075</i>	3 independent lines of <i>Ex[ins-1^{457bp_prom}::ttx-3; rol-6(d)]</i> (-457 bp upstream of ATG)
<i>Ex[gcy-28.d::gfp]</i>	the complete genotype of this array is <i>Ex[gcy-28.dp::gcy-28.d::GFP, AIA-specific ins-1p::SNB-1::mRFP, rol-6(+)]</i> (transgene kindly provided by Takeshi Ishihara)
<i>Ex[scd-2::gfp]</i>	<i>Ex[scd-2::gfp]</i> : the complete genotype of this array is <i>Ex[scd-2p::scd-2::GFP, AIA-specific ins-1p::mRFP, lin-44::gfp]</i> (transgene kindly provided by Takeshi Ishihara)
<i>olaEx1446</i>	<i>Ex [mod-5p::egfp (2ng/ul)/unc-122p::gfp (20ng/ul)]</i>
<i>olaEx1485</i>	<i>Ex[flp-4p::egfp (30ng/ul)/unc-122p::DSRED (20ng/ul)]</i>
<i>nuls9</i>	<i>Is[unc-5::gfp]</i> (transgene kindly provided by Josh Kaplan)
<i>otEx2310</i>	<i>Ex[gcy-19::gfp; unc-122::gfp]</i> (Ortiz et al., 2006)
<i>lqls3</i>	<i>Is[osm-6::gfp]</i> (transgene kindly provided by Erik Lundquist)
<i>qls56</i>	<i>Is[lag-2::gfp]</i> (transgene kindly provided by Judith Kimble)
<i>vsIs48</i>	<i>Is[unc-17::gfp]</i> (transgene kindly provided by Michael Koelle)
<i>otIs323</i>	<i>Is[cho-1_fosmid::gfp; elt-2::dsRed]</i> (transgene kindly provided by Paschalis Kratsios)

Table S3. Probe sequences for gel shift analysis

Probe	Sequence
cho-1 wt:	5'tacacacacatcgaaatatgaatcttctcttaaaaagaaggttgccaattagttcccctattcaG CTTTCGTTTCGTCGCCT
cho-1 TAAT del	5'tacacacacatcgaaatatgaatcttctcttaaaaagaaggttgccagttcccctattcaGCT TTCGTTTCGTCGCCT
mgl-1 wt	5'gtttcatactcatagtgctcattagaatagcacggatcgtgttcgcctctcgccttgtaaccgaa tctgccGCTTTCGTTTCGTCGCCT
mgl-1 TAAT del	5'gtttcatactcatagtgctcgaatagcacggatcgtgttcgcctctcgccttgtaaccgaaatctg ccGCTTTCGTTTCGTCGCCT
bas-1 wt	5'cccaacaccacattattcatgtatttctccaaccactgaaccatctcattctcaaaccagtttct atccgtttgttgcatcattcaattaaatGCTTTCGTTTCGTCGCCT
bas-1 HD mut	5'cccaacaccacgttattcatgtatttctccaaccactgaaccatctcattctcaaaccagtttct atccgtttgttgcatcagttgaatGCTTTCGTTTCGTCGCCT
bas-1 POU mut	5'cccaacaccacattattcccgtatttctccaaccactgaaccatctcattctcaaaccagtttct atccgtttgttgccctcaattaaatGCTTTCGTTTCGTCGCCT
tph-1 wt	5'tctttgttgCGcataataaaacaatcaatcaacacagcaaagaccctctcaacctcattcatg atcttGCTTTCGTTTCGTCGCCT
tph-1 HD mut	5'tctttgttgCGcatagtaaacaatcaatcaacacagcaaagaccctctcaacctcattcatg atcttGCTTTCGTTTCGTCGCCT
tph-1 POU mut	5'tctttgttgTgTataCCaCaacaaGcGatcaacacagcaaagaccctctcaacctcatt cCCgatttcttGCTTTCGTTTCGTCGCCT