SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transgenes

The following transgenes were obtained from other labs:
wgIs73[ceh-14::TY1::EGFP::3xFLAG] (Sarov et al., 2012)
wgIs55[mec-3::TY1::EGFP::3xFLAG] (Sarov et al., 2012)
otIs92[flp-10::gfp] (Mehta et al., 2004)
rtEx251[nlp-15::gfp] (Nathoo et al., 2001)
gmIs21[nlp-1::gfp] (Frank et al., 2003)
zwEx107[inx-7::gfp] (Altun et al., 2009)
otEx537[ser-2b::gfp] (Tsalik et al., 2003)
kyIs174[slt-1::gfp] (Hao et al., 2001)
otIs14[zig-3::gfp] (Aurelio et al., 2003)
jsEx896[hid-1::gfp] (Mesa et al., 2011)
xdEx44[cam-1b::gfp] (Zhang et al., 2013)
jsEx740[aex-6::gfp] (Mahoney et al., 2006)
adIs1240[eat-4::gfp] (Lee et al., 1999)
uIs22[mec-3::gfp] (gift from M. Chalfie). This construct contains begins 2372 bp upstream of the *mec-3* translational start site and is fused to GFP at the end of exon 3.
uIs3[mec-7::gfp] (gift from M. Chalfie)
zdIs5[mec-4::gfp] (Clark and Chiu, 2003)
arEx1127[glt-3::mCherry] (a gift from D. Shaye)
qIs74[pop-1::gfp]
qIs95[sys-1::venus] (Phillips et al., 2007).
uIs115[mec-17::rfp] (gift from M. Chalfie)

The following transgenes were generated in this study:

Reporter for transcription factors:
otEx181[ceh-14^{prom}::gfp; rol-6]

otIs337[unc-86^{fosmid}::SL2::NLS::YFP::H2B; ttx-3::mCherry]
otEx5851 [unc-86 ^{fosmid}::NLS::mChOpti; lin-44::yfp]
otIs429[pag-3^{fosmid}::mChOpti; ttx-3::gfp]

Terminal identity markers:
otIs544[cho-1^{fosmid}::mChOpti]
otIs439[lad-2^{prom}::gfp; pha-1(+)]
otIs358[ser-2b::gfp; pha-1] (integration of *otEx537*)
otEx5480[ahr-1::TY1::EGFP::3xFLAG; ttx-3::mCherry]

Ectopic expression:
otEx5440, otEx5441, otEx5442 [unc-86::mec-3; ttx-3::gfp]
otEx5853 [hsp::unc-86; ttx-3::mCherry]
otEx5852[hsp::mec-3; ttx-3::dsRed]

ser-2 and *ceh-14 cis*-regulatory analysis:
otEx5779, otEx5780, otEx5781[ser-2$^{4.7kb\ prom}$::gfp; pha-1(+)]
otEx5782, otEx5783, otEx5784[ser-2$^{2.4kb\ prom}$::gfp; pha-1(+)]
otEx5785, otEx5786, otEx5787[ser-2$^{1.3kb\ prom}$::gfp; pha-1(+)]
otEx5788, otEx5789, otEx5790[ser-2$^{700bp\ prom}$::gfp; pha-1(+)]
otEx5791, otEx5792, otEx5793[ser-2$^{370bp\ prom}$::gfp; pha-1(+)]
otEx5795, otEx5796, otEx5797[ser-2$^{370bpmutA\ prom}$::gfp; pha-1(+)]
otEx5798, otEx5799, otEx5800[ser-2$^{370bpmutB\ prom}$::gfp; pha-1(+)]
otEx5801, otEx5802, otEx5803[ser-2$^{370bpmutA/B\ prom}$::gfp; pha-1(+)]
otEx5804, otEx5805, otEx5806[ser-2$^{370bpmutC\ prom}$::gfp; pha-1(+)]
otEx5807, otEx5808, otEx5809[ser-2$^{370bpmutD\ prom}$::gfp; pha-1(+)]
otEx5810, otEx5811, otEx5812[ceh-14$^{1kb\ prom}$::gfp; pha-1(+)]
otEx5813, otEx5814, otEx5815[ceh-14$^{480bp\ prom}$::gfp; pha-1(+)]
otEx5816, otEx5817, otEx5818[ceh-14$^{480bpmutA\ prom}$::gfp; pha-1(+)]
otEx5819, otEx5820, otEx5821[ceh-14$^{480bpmutB\ prom}$::gfp; pha-1(+)]
otEx5822, otEx5823, otEx5824[ceh-14$^{480bpmutC\ prom}$::gfp; pha-1(+)]

otEx5825, otEx5826, otEx5827[ceh-14$^{480bpmutA/B/C\ prom}$::gfp; pha-1(+)]
otEx5828, otEx5829, otEx5830[ceh-14$^{480bpmutD\ prom}$::gfp; pha-1(+)]
otEx5831, otEx5832, otEx5833[ceh-14$^{480bpmutE\ prom}$::gfp; pha-1(+)]
otEx5834, otEx5835, otEx5836[ceh-14$^{480bpmutF\ prom}$::gfp; pha-1(+)]
otEx5837, otEx5838, [ceh-14$^{480bpmutD/E/F\ prom}$::gfp; pha-1(+)]
otEx5839, otEx5840, otEx5841[ceh-14$^{480bpmutA/D/E/F\ prom}$::gfp; pha-1(+)]
otEx5842, otEx5843, otEx5844[ceh-14$^{480bpmutB/D/E/F\ prom}$::gfp; pha-1(+)]
otEx5845, otEx5846, otEx5847[ceh-14$^{329bp\ prom}$::gfp; pha-1(+)]
otEx5848, otEx5849, otEx5850[ceh-14$^{170bp\ prom}$::gfp; pha-1(+)]

EMSA probe sequences

EMSA probe sequences are as follows (underlining indicates the sequence added for complementarity to the short labeled oligonucleotide):

ceh-14 promoter probe:
5'-
AATTGTTTTCATTTAAAATGAGCAACTGTAATTTTCTATTCATTAAAGTATTTTTTTTA
CCATTTAAAAGGAACCCATTCATGAAAAGTTGGTCAAGGTCGTTTCC-3'

tph-1 promoter probe:
5'-
CCCAACACCACATTATTCATGTATTTCCTCCAAACCACTGAACCATCTCATTCTCAA
ACCAGTTTCTATCCGTTTGTTTGCATTCAATTAAATTTTTGGTCAAGGTCGTTTCC-3'

unlabeled *mec-3* promoter probe:
5'-
ACATTTGAAAAAACAACAAATTCATTCGAAATGCATTGCCCATAATGAATCGACCGA
AAAACACAAGTGACCGTCAGGAGATCGATAGAG-3'

ceh-14 mutated promoter probe:
5'-
AATTGTTTTCATTTAAAATGAGCAACTGCCATTTTCTATTCCCTAAAGTATTTTTTTTA
CCATTTAAAAGGAACCCATTCCCGAAAAGTT-3'

unlabeled competitor *ceh-14* promoter probes:
Site D: 5' - TTCTATTCATTAAAGTATT - 3'
Site D (mut): 5' - TTCTATTCCCCCAAGTATT - 3'
Site E: 5' - CCCTCTCTTAATTGCTTTT - 3'
Site E(mut): 5' - CCCTCTCTCCCCTGCTTTT - 3'
Site F: 5' - TGACATCAATTAAGTTGAA - 3'
Site F (mut): 5' - TGACATCACCCCAGTTGAA - 3'

Single Molecule FISH

Oligonucleotides for smFISH consisted of 48 20-nucleotide probes against *pag-3* mRNA coupled to CAL Fluor 610 Red Dye (Biosearch Technologies). smFISH was performed as described at biosearchtech.com/stellarisprotocols. Briefly, L4 stage animals were fixed in 3.7% formaldehyde and hybridized to oligonucleotides at a 1:2000 concentration. *pag-3* mRNAs were quantified by taking stacks of images 0.3 um apart and counting individual fluorescent dots.

SUPPLEMENTAL REFERENCES

Altun, Z.F., Chen, B., Wang, Z.W., and Hall, D.H. (2009). High resolution map of Caenorhabditis elegans gap junction proteins. Dev Dyn *238*, 1936-1950.

Aurelio, O., Boulin, T., and Hobert, O. (2003). Identification of spatial and temporal cues that regulate postembryonic expression of axon maintenance factors in the C. elegans ventral nerve cord. Development *130*, 599-610.

Clark, S.G., and Chiu, C. (2003). C. elegans ZAG-1, a Zn-finger-homeodomain protein, regulates axonal development and neuronal differentiation. Development *130*, 3781-3794.

Frank, C.A., Baum, P.D., and Garriga, G. (2003). HLH-14 is a C. elegans achaete-scute protein that promotes neurogenesis through asymmetric cell division. Development *130*, 6507-6518.

Hao, J.C., Yu, T.W., Fujisawa, K., Culotti, J.G., Gengyo-Ando, K., Mitani, S., Moulder, G., Barstead, R., Tessier-Lavigne, M., and Bargmann, C.I. (2001). C. elegans Slit Acts in Midline, Dorsal-Ventral, and Anterior-Posterior Guidance via the SAX-3/Robo Receptor. Neuron *32*, 25-38.

Lee, R.Y., Sawin, E.R., Chalfie, M., Horvitz, H.R., and Avery, L. (1999). EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate cotransporter, is necessary for glutamatergic neurotransmission in caenorhabditis elegans. J Neurosci *19*, 159-167.

Mahoney, T.R., Liu, Q., Itoh, T., Luo, S., Hadwiger, G., Vincent, R., Wang, Z.W., Fukuda, M., and Nonet, M.L. (2006). Regulation of synaptic transmission by RAB-3 and RAB-27 in Caenorhabditis elegans. Mol Biol Cell *17*, 2617-2625.

Mehta, N., Loria, P.M., and Hobert, O. (2004). A genetic screen for neurite outgrowth mutants in Caenorhabditis elegans reveals a new function for the F-box ubiquitin ligase component LIN-23. Genetics *166*, 1253-1267.

Mesa, R., Luo, S., Hoover, C.M., Miller, K., Minniti, A., Inestrosa, N., and Nonet, M.L. (2011). HID-1, a new component of the peptidergic signaling pathway. Genetics *187*, 467-483.

Nathoo, A.N., Moeller, R.A., Westlund, B.A., and Hart, A.C. (2001). Identification of neuropeptide-like protein gene families in Caenorhabditiselegans and other species. Proc Natl Acad Sci U S A *98*, 14000-14005.

Phillips, B.T., Kidd, A.R., 3rd, King, R., Hardin, J., and Kimble, J. (2007). Reciprocal asymmetry of SYS-1/beta-catenin and POP-1/TCF controls asymmetric divisions in Caenorhabditis elegans. Proc Natl Acad Sci U S A *104*, 3231-3236.

Sarov, M., Murray, J.I., Schanze, K., Pozniakovski, A., Niu, W., Angermann, K., Hasse, S., Rupprecht, M., Vinis, E., Tinney, M.*, et al.* (2012). A genome-scale resource for in vivo tag-based protein function exploration in C. elegans. Cell *150*, 855-866.

Tsalik, E.L., Niacaris, T., Wenick, A.S., Pau, K., Avery, L., and Hobert, O. (2003). LIM homeobox gene-dependent expression of biogenic amine receptors in restricted regions of the C. elegans nervous system. Dev Biol *263*, 81-102.

Zhang, J., Li, X., Jevince, A.R., Guan, L., Wang, J., Hall, D.H., Huang, X., and Ding, M. (2013). Neuronal target identification requires AHA-1-mediated fine-tuning of Wnt signaling in C. elegans. PLoS Genet *9*, e1003618.

SUPPLEMENTAL FIGURE LEGENDS

Fig.S1 – Related to Fig.1

A: BDU-mediated mechanosensory responses are mediated by BDU expressed neuropeptides. Gravid adults were touched with a platinum pick in the anterior half of the midbody. The number of head swings of backwards movement before animals stopped, reversed direction, or did an omega turn was scored. *n* is given at the bottom of each bar. NS, not significant **p<.001 ***p<.0001 (t-test with Bonferroni correction). Error bars: s.e.m.

A1: Mechanosensory response of wildtype (WT), neuropeptide mutants, and neuropeptide processing mutants.

A2: Rescue of the *nlp-1(ok1469)* phenotype by expressing *nlp-1* under control of two different BDU promoters.

A3: To ensure that *ceh-14(ch3)* and *nlp-1(ok1469)* mutants are not generally defective in backwards response, animals were exposed to the noxious chemical copper chloride. No difference was seen between wild type and *ceh-14* or *nlp-1* animals.

B: Crossregulation of transcription factors in ALM and BDU.

B1: Expression of fosmid-based reporters of transcription factors in embryo and adult. *unc-86^{fosmid}::yfp* (*otIs337*) is expressed in ALM and BDU. *ceh-14^{fosmid}::gfp* (*wgIs73*) is expressed in BDU. *mec-3* (*wgIs55*) is expressed exclusively expressed in ALM. *pag-3 ^{fosmid}::rfp* (*otIs429*) is expressed in ALM and BDU. Arrows in embryo images indicate ALM, arrowheads indicate BDU.

B2: Expression levels of *pag-3* in BDU and ALM are similar, as determined by mRNA counting with smFISH. smFISH was performed on L4 stage animals and expression of *pag-3* was compared between ALM and BDU neurons. Each data point represents the number of mRNAs seen in either ALM or BDU of a single animal. See Supplementary Experimental procedures for more details on smFISH.

B3: Cross-regulation of BDU transcription factors. While expression of *unc-86* is unaffected by either *pag-3(ls20)* or *ceh-14(ch3)*, expression of *pag-3* is off in BDU in *unc-86(n846)* and expression of *ceh-14* is off in both *unc-86(n846)* and *pag-3(ls20)*. Animals were scored at the L4 stage. $n \geq 50$.

B4: Summary of transcription factor interactions. Regulation of *ceh-14* by both *pag-3* and *unc-86* ("feedforward loop") is inferred from the analysis of the *cis*-regulatory architecture of the *ceh-14* locus described in Fig.3B. *mec-3* regulation by *unc-86* was previously shown (Xue et al. 1992).

Fig.S2 – Related to Fig.2

A: UNC-86 binds to a probe derived from the *ceh-14* promoter. This binding is competed away with addition of cold oligos corresponding to putative UNC-86 binding sites (see main text), but not by mutated forms of those binding sites. EMSA was performed with 100 nM UNC-86. See Supplementary Experimental Procedures for probe sequences.

B: Specificity of UNC-86 binding to *ceh-14* promoter. UNC-86 binding is competed away with the addition of cold oligos identical to the probe but not by a mutated form of the oligo missing the UNC-86 binding site. See Supplementary Experimental Procedures for probe sequences.

Fig.S3 – Related to Fig.5

A: The *unc-86p::mec-3* transgene rescues the mutant phenotypes of *mec-3.* *mec-17::rfp*, an ALM reporter, was examined for expression in ALM in wild type, *mec-3(e1338),* and the *mec-3* mutant rescued by *mec-3* driven by the *unc-86* 5.2kb promoter

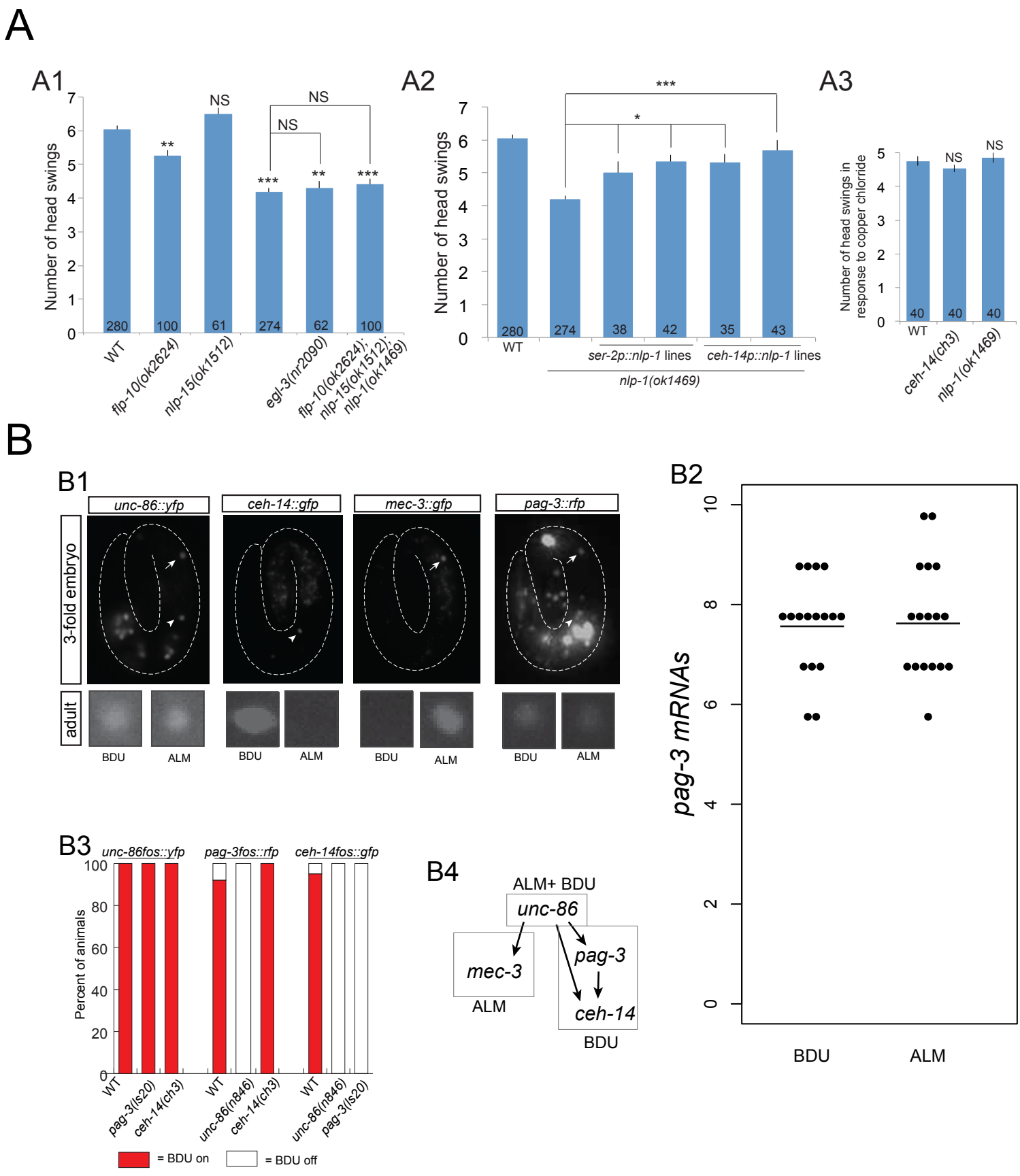
B: The *unc-86p::pag-3* transgenes rescues the mutant phenotypes of *pag-3*. *flp-10::gfp*, a BDU reporter, was examined for expression in BDU in wild type, *pag-3(ls20)*, and the *pag-3* mutant rescued by *pag-3* driven by the *unc-86* 5.2kb promoter.

Fig.S4 – Related to Fig.6

A: Competition for UNC-86 binding is dependent on the presence of an unlabeled competitor probe. EMSA was performed with or without the presence of an unlabeled *mec-3* promoter probe using 100 nM UNC-86 and 200 nM MEC-3.

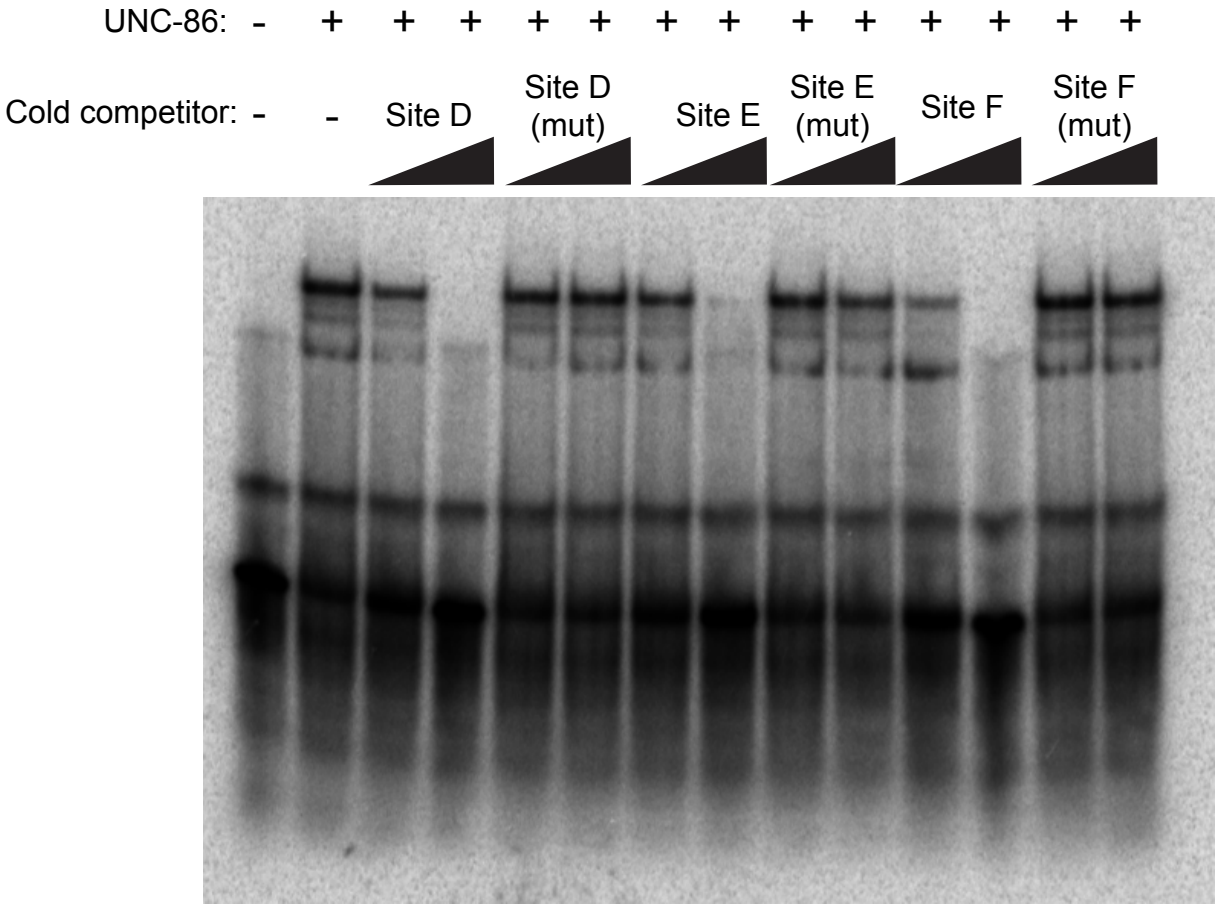
B: MEC-3, but not PAG-3, is able to compete UNC-86 away from binding to a *ceh-14* promoter. EMSA was performed with 100 nM UNC-86, 100 or 200 nM MEC-3, and 100 or 200 nM PAG-3.

C: Loss of UNC-86/MEC-3 binding abolishes the competition mechanism. UNC-86(L195F) binding to a *ceh-14* probe is unaffected by the addition of MEC-3. EMSA was performed with 100 nM UNC-86(L195F) and 100 or 200 nM MEC-3.

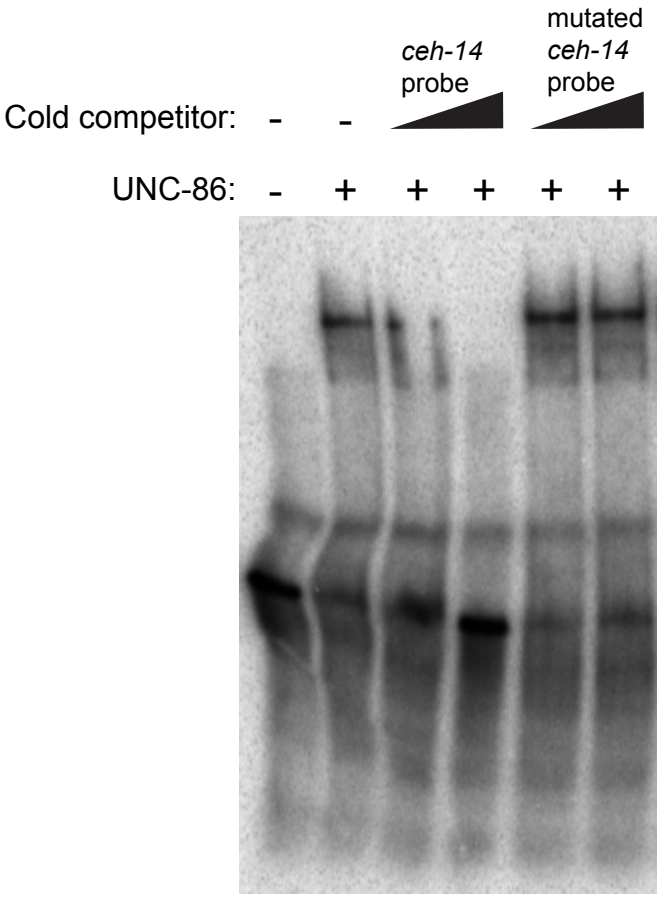


Suppl. Figure S1

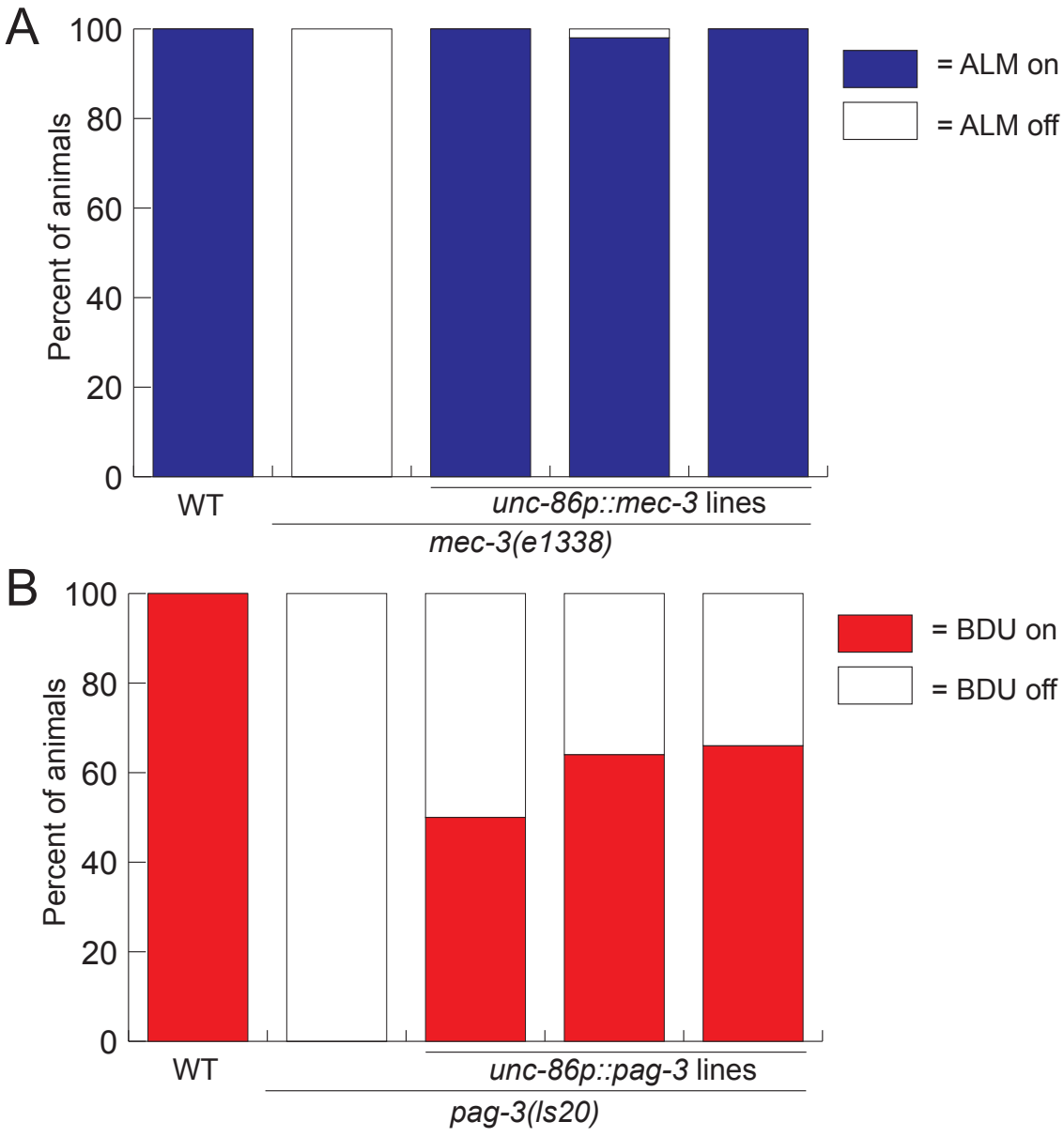
A



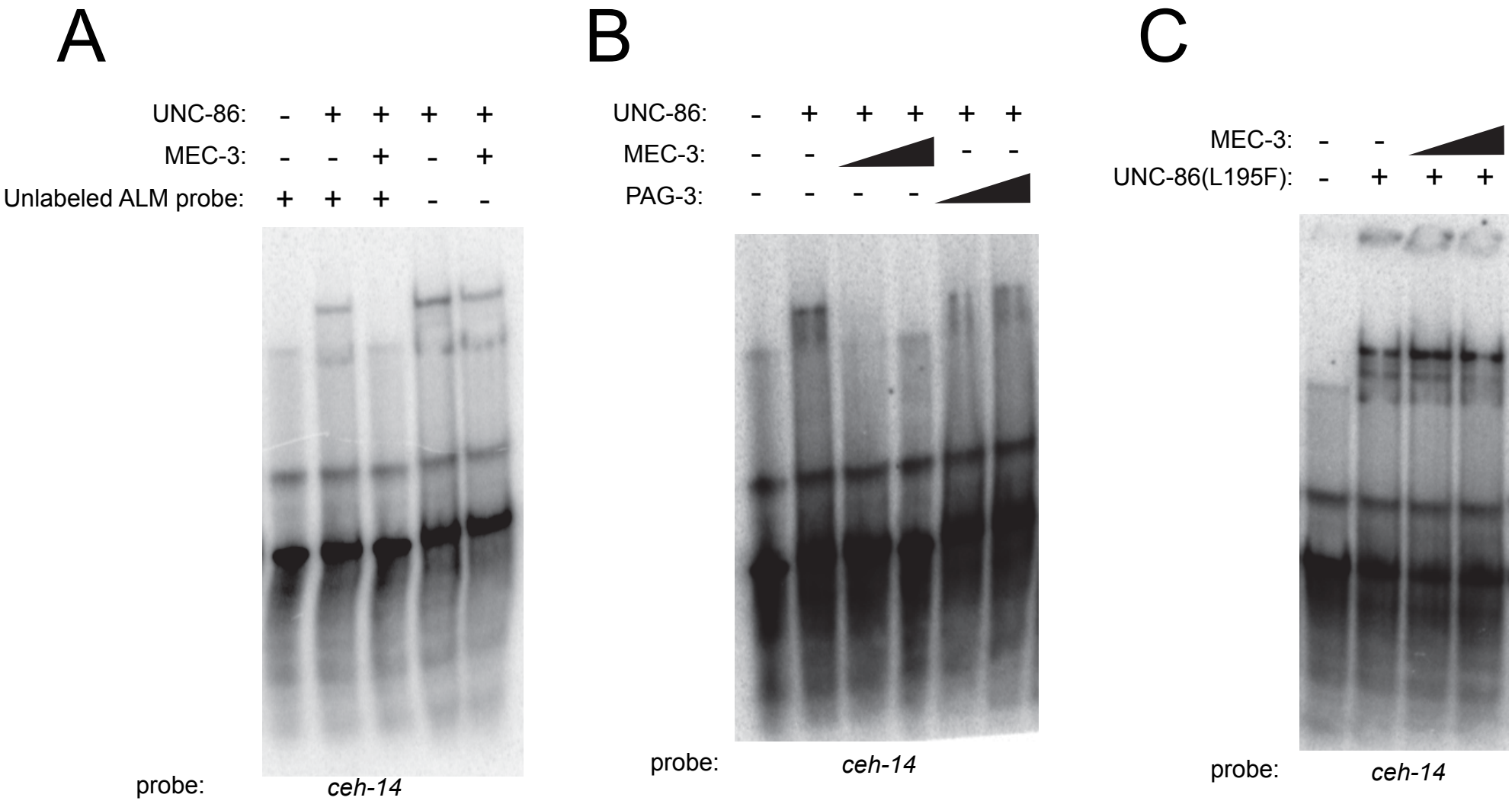
B



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4