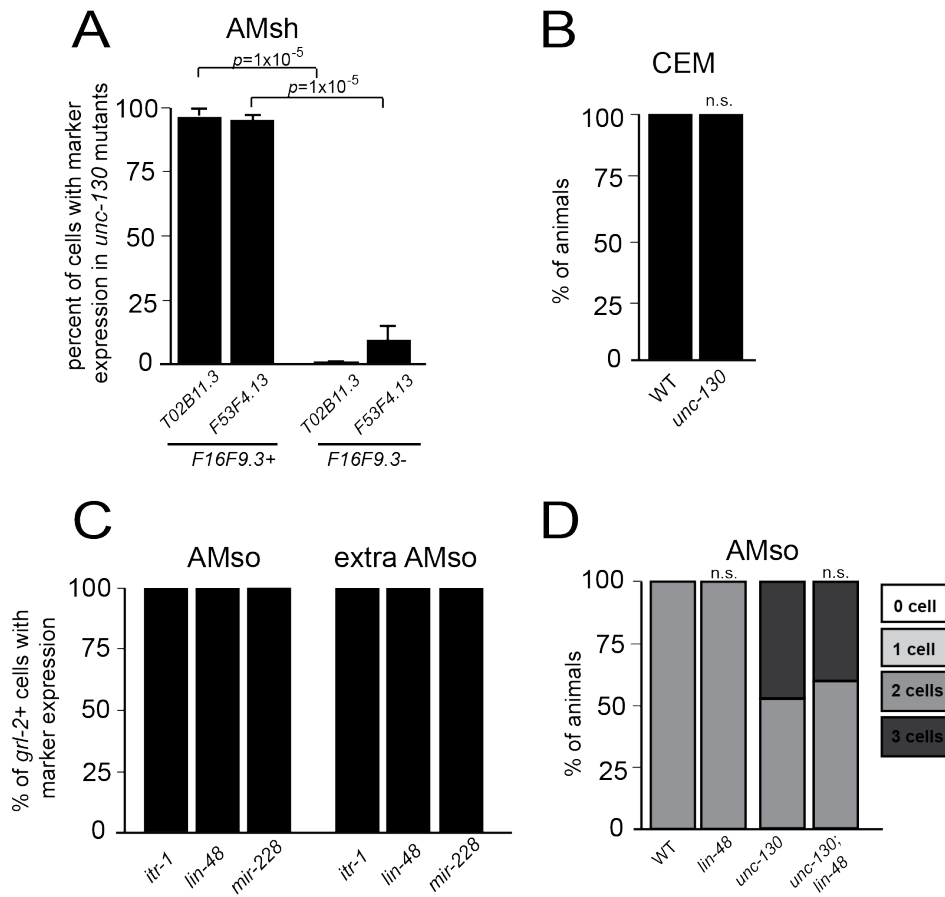**Fig. S1. Related to Fig. 1**

(A) Two example ILsoD (ABpxaapaa) lineages from wild-type and *unc-130* mutant embryos. Light blue arrowheads denote division of wild-type ILsoD progenitors and dark blue arrowheads denote divisions of *unc-130* mutant ILsoD progenitors that produce ILsoD and hyp3 cells. (B) Cell cycle lengths of ILsoL, ILsoR, ILsoVL, ILsoVR, ILsoDL, and ILsoDR progenitor cells in wild-type and *unc-130* mutant embryos. n = 6 for wild type, n = 8 for *unc-130* mutants. Center lines represent population median. Asterisks denote *p*-value < 0.05 as calculated by Welch's t-test.
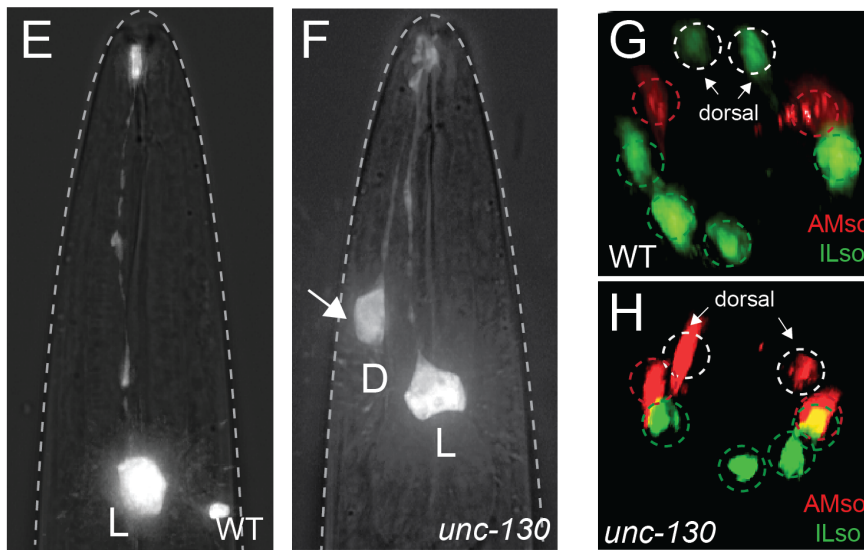
**Fig. S2. Related to Fig. 2**

(A) Percentage of *F16F9.3+* and *F16F9.3-* cells co-expressing AMsh markers,

*T02B11.3*pro:GFP and *F53F4.13*pro:GFP, in *unc-130* mutant animals. n ≥ 20 cells per marker.

Error bars – standard error of proportion. *p*-values calculated by z-score for two population

proportions. (B) Percentage of wild-type and *unc-130* mutant males expressing the CEM neuron

marker, *pkd-2*pro:GFP. The CEM neuron is the sister cell of AMso in males. (C) Percentage of

*grl-2+* cells co-expressing AMso markers, *itr-1*pro:GFP and *lin-48*pro:GFP, and glial marker

*mir-228*pro:histone-GFP in endogenous and extra cells in *unc-130* mutants. n ≥ 20 cells per

marker. (D) Percentage of animals expressing AMso-specific marker *grl-2*pro:YFP in zero, one,

two, three or more cells in wild type, *lin-48*, *unc-130*, and *lin-48*; *unc-130* double mutants. n=50

animals per genotype. *p*-values were calculated by Fisher's Exact test. Lateral views of wild-type

(E) and *unc-130* mutant (F) animals expressing AMso marker, *grl-2*pro:YFP. Arrow indicates

extra cell. In lateral views, the other endogenous AMso cell is not visible. D – dorsal, L – lateral.

En face views of wild-type (G) and *unc-130* mutant (H) animals co-expressing *grl-18*pro:GFP to

mark ILso glia and *grl-2*pro:mApple to mark AMso glia. Arrows indicate cells in the dorsal

position. There are two extra AMso glia in this particular *unc-130* mutant animal.
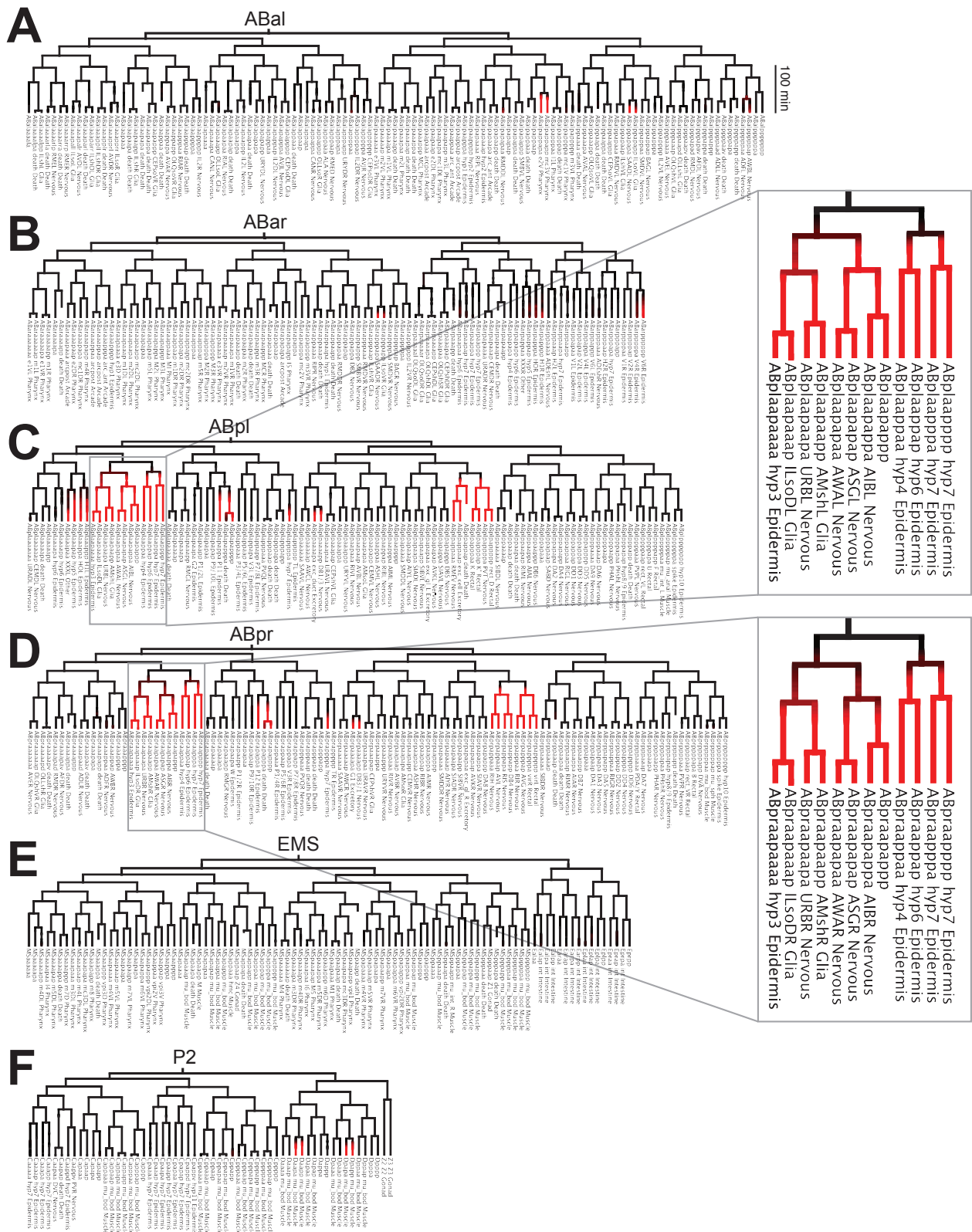
**Fig. S3. Related to Fig. 3**

(A-F) Lineage diagram of all embryonic divisions in a wild-type embryo from the lineaging

strain RW11144, which expresses UNC-130:GFP. (A) ABal, (B) ABar, (C) ABpl, (D) ABpr, (E)

MS and E, and (F) C, D, and Z lineages.  UNC-130 expression levels are indicated in red.
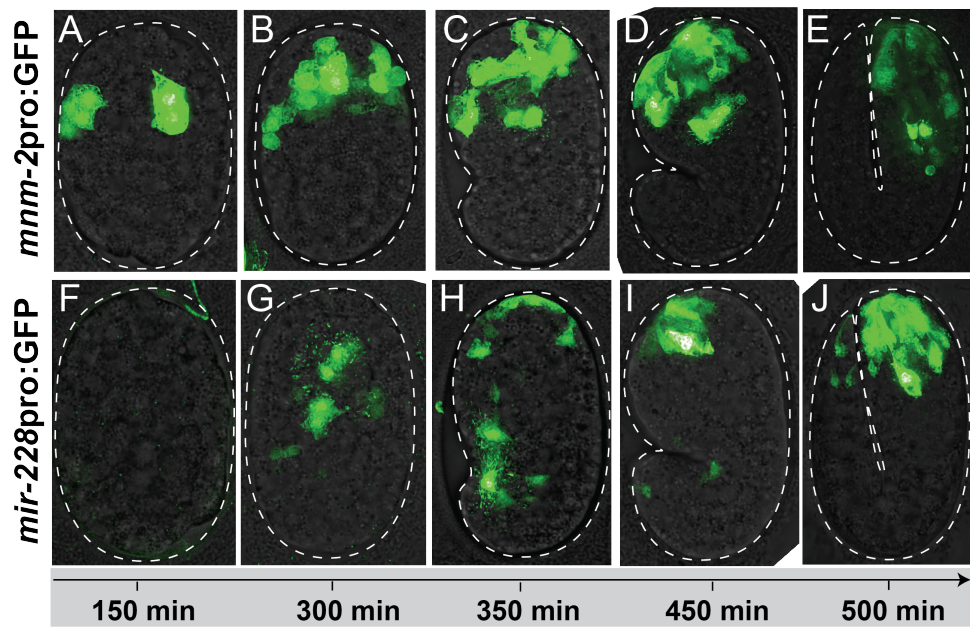
**Fig. S4. Related to Fig. 3.**

Time course of *mnm-2*pro:GFP (A-E) and *mir-228*pro:GFP (F-J) expression in embryos.
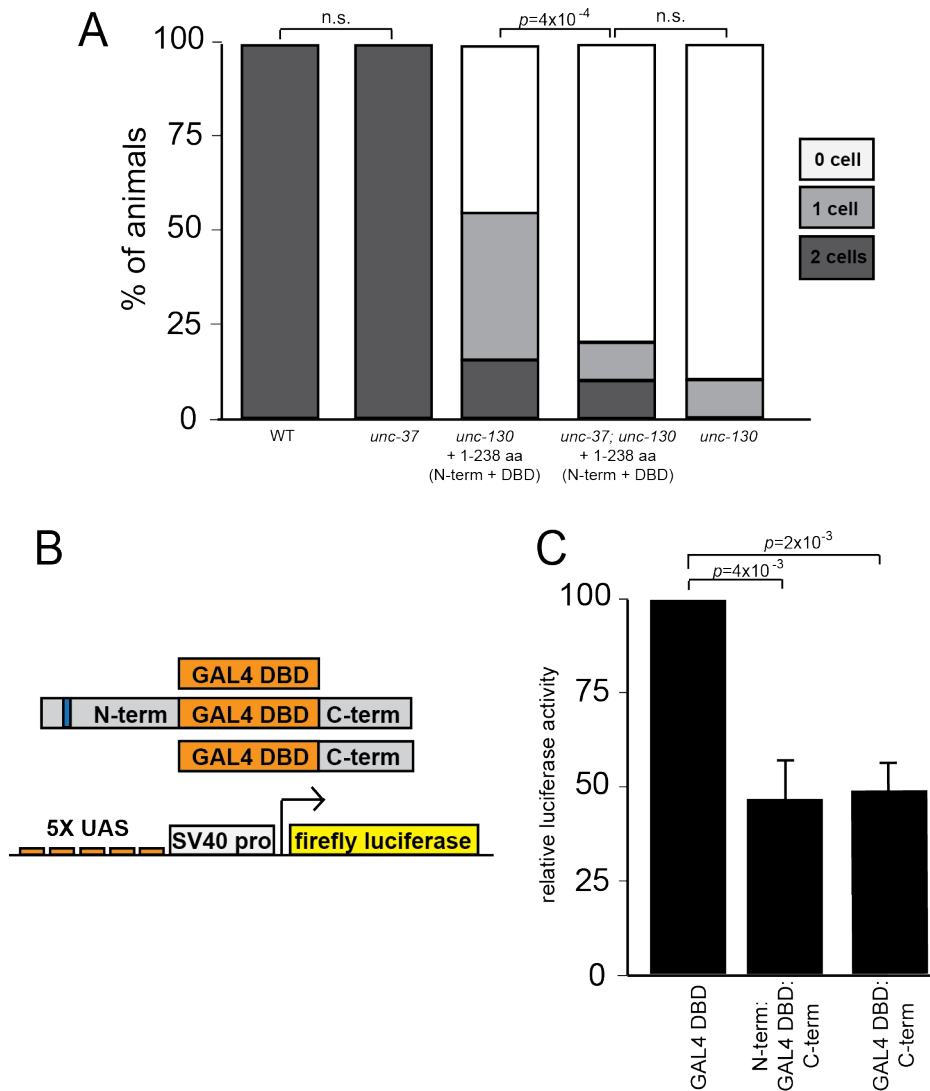
**Fig. S5. Related to Fig. 4**

(A) Percentage of animals expressing *grl-18*pro:YFP in zero, one, or two ILsoD glia in wild-type, *unc-37* mutants, and *unc-130* mutants with N-terminus rescue, *unc-130*; *unc-37* double mutants with N-terminus rescue, and *unc-130* mutants alone. n = 50 animals per condition. *p*-values were calculated by Fisher's Exact test. (B) Schematic diagram of constructs used in luciferase assays. (C) GAL4DBD alone, N-term:GAL4DBD:C-term, or GAL4DBD:C-term, along with a UAS-firefly luciferase reporter and constitutively expressed renilla luciferase were

transfected into HEK293T cells. Relative firefly luciferase activity, first normalized to renilla

luciferase bioluminescence in each sample, and then to DBD-GAL4 alone. Error bars - SD; *p*-

values calculated by Welch's t-test with Bonferroni correction for multiple t-tests.

## A

```
UNC-130  ----------MLFSMESILSSTKPKLEPPPKLEP--EVTINEQVVDLPR--SNTRLSEPST
FoxD3    MTLSGGGSASDMSGQTVLTAEDVDIDVVGEGDDGLEEKDSDAGCDSPAGPPELRLDEADE

         SASVLEHDLKFGESRKRSRSLGDEPTEDEDGVPVRKANKRNHSTSSAADSSSDDAKDDDD
         VPPAAPHHGQPQPPHQQPLTLPKEAAGAGAGPGGDVGAPEADGCKGGVGGEEGGASGGGP

         DDDSTSRKSMSGHR-KSSHAKPPYSYIALIAMSILNSPEKKLTLSEICEFIINKFEYYKE
         GAGSGSAGGLAPSKPKNSLVKPPYSYIALITMAILQSPQKKLTLSGICEFISNRFPYYRE
                            ********** * ** ** ****** ***** * **** *
         KFPAWQNSIRHNLSLNDCFVKVARGPGNPGKGNYWALDPNCEDMFDNGSFLRRRKRYKKN
         KFPAWQNSIRHNLSLNDCFVKIPREPGNPGKGNYWTLDPQSEDMFDNGSFLRRRKRFKRH
         ********************      **********  ***   *************** *
         SDTYH------EMMSHHPMPFPPFLPQGMPFP-PRMMHPMANIPMLGHPMNPRAVPNMPA
         QQEHLREQTALMMQSFGAYSLAAAAGAAGPYGRPYGLHPAAAAFAYSHPAAAAAAAAAAA

         FFIPQNID--------------------------------SQKLLSMMASRIMPMDAPVS
         LQYPYALPPVAPVLPPAVPLLPSGELGRKAAAFGSQLGPGLQLGLNSLGAAAAAAGTAGA

         SGQKRTSSSSSSSPNENGSSAVSDKLSA--------------------------------
         AGTTASLIKSEPSARPSFSINIIGGGPAAPGGSAVGAGVAGGTGGSGGGSTAQSFLRPP
         ------------------------------------------------------------
         GTVQSAALMATHQPLSLSRTTATIAPILSVPLSGQFLQPAASAAAAAAAAAQAKWPAQ
```
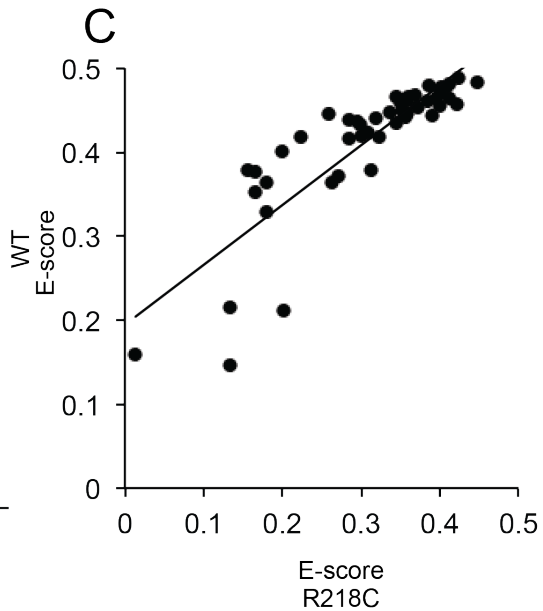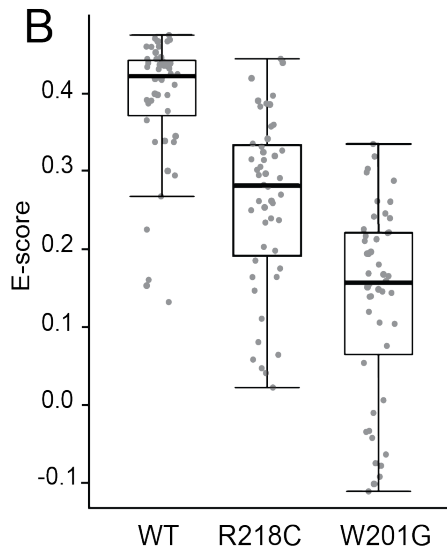
## B



## C

**Fig. S6. Related to Fig. 5**

(A) Alignment of UNC-130 (black) and FoxD3 (gray) sequences. DNA binding domain is highlighted in green. Eh1 motifs are highlighted in blue. Asterisks denote conserved amino acids in the DNA binding domain. Underlined residues are mutated in point mutants described in Fig. 5. (B) Technical replicate of PBM experiment from Fig. 5D. Scatter plot of E-scores for 8-mer DNA sequences matching [A/G][C/T]AAACA or AA[C/T]AACA from protein binding microarray (PBM) assays of wild-type, R218C, and W201G mutant proteins. Black lines represent population median, top and bottom of boxes are $25^{th}$ and $75^{th}$ percentiles, respectively, and top and bottom of whiskers are either most extreme point or 1.5x the interquartile range. *p*-values calculated by Mann-Whitney test. (C) Scatter plot of E-scores for 8-mer DNA sequences matching [A/G][C/T]AAACA or AA[C/T]AACA for wild-type versus R218C mutant proteins. Linear regression, $R^2$=0.72

**Table S1. Penetrance of phenotypes in Fig. 1**

| cell type | marker | genotype | % of animals with # of cells | | | n | *p*-value |
|---|---|---|---|---|---|---|---|
| | | | **0** | **1** | **2** | **n** | *p*-**value** |
| **ILsoD** | *grl-18* | wt | 0 | 0 | 100 | 50 | $2 \times 10^{-29}$ |
| | | *ev505* | 90 | 10 | 0 | 50 | |
| **ILsoD** | *col-53* | wt | 0 | 0 | 100 | 50 | $2 \times 10^{-29}$ |
| | | *ev505* | 88 | 12 | 0 | 50 | |
| **ILsoD** | *col-177* | wt | 0 | 6 | 94 | 50 | $2 \times 10^{-27}$ |
| | | *ev505* | 86 | 14 | 0 | 50 | |
| **ILsoL/R** | *grl-18* | wt | 0 | 0 | 100 | 50 | 1 |
| | | *ev505* | 0 | 0 | 100 | 50 | |
| **ILsoL/R** | *col-53* | wt | 0 | 6 | 94 | 50 | 0.1 |
| | | *ev505* | 0 | 0 | 100 | 50 | |
| **ILsoL/R** | *col-177* | wt | 8 | 10 | 82 | 50 | 0.4 |
| | | *ev505* | 2 | 14 | 84 | 50 | |
| **ILsoV** | *grl-18* | wt | 0 | 0 | 100 | 50 | 1 |
| | | *ev505* | 0 | 0 | 100 | 50 | |
| **ILsoV** | *col-53* | wt | 0 | 4 | 96 | 50 | 1 |
| | | *ev505* | 0 | 4 | 96 | 50 | |
| **ILsoV** | *col-177* | wt | 0 | 2 | 98 | 50 | 0.1 |
| | | *ev505* | 0 | 12 | 88 | 50 | |

**Table S2. Penetrance of IL neuron phenotypes.**

| cell type | marker | genotype | % of animals with # of cells | | | | n | *p*-value |
|---|---|---|---|---|---|---|---|---|
| | | | **4** | **5** | **6** | **>6** | **n** | *p*-value |
| **IL1** | *flp-3* | wt | 12 | 10 | 78 | 0 | 50 | $9 \times 10^{-4}$ |
| | | *ev505* | 16 | 4 | 58 | 22 | 50 | |
| **IL2** | *klp-6* | wt | 4 | 12 | 84 | 0 | 50 | 0.4 |
| | | *ev505* | 0 | 12 | 84 | 4 | 50 | |

**Table S3. Penetrance of phenotypes in Fig. 2.**

| cell type | marker | genotype | % of animals with # of cells | | | | | n | *p*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | | **0** | **1** | **2** | **3** | **4** | **n** | |
| **hyp3** | *ceh-10* | wt | 0 | 0 | 100 | - | - | 50 | $1 \times 10^{-10}$ |
| | | *ev505* | 0 | 30 | 70 | - | - | 50 | |
| **URB** | *nlp-6* | wt | 0 | 2 | 98 | - | - | 50 | 1 |
| | | *ev505* | 0 | 4 | 96 | - | - | 50 | |
| **AMsh** | *F16F9.3* | wt | 0 | 0 | 100 | - | - | 50 | $5 \times 10^{-7}$ |
| | | *ev505* | 6 | 32 | 62 | - | - | 53 | |
| **CEPsh** | *hlh-17* | wt | 0 | 0 | 0 | 0 | 100 | 50 | 0.2 |
| | | *ev505* | 0 | 0 | 0 | 6 | 94 | 50 | |
| **AMso** | *grl-2* | wt | 0 | 0 | 100 | 0 | 0 | 50 | $7 \times 10^{-8}$ |
| | | *ev505* | 0 | 0 | 58 | 34 | 8 | 53 | |
| **PHsh** | *F16F9.3* | wt | 0 | 12 | 88 | - | - | 50 | 1 |
| | | *ev505* | 0 | 14 | 86 | - | - | 50 | |
| **PHso** | *grl-2* | wt | 0 | 0 | 0 | 4 | 96 | 50 | 0.2 |
| | | *ev505* | 0 | 0 | 0 | 12 | 88 | 50 | |

**Table S4. Strains generated for this study**

| ID | Genotype | Figures |
|---|---|---|
| CHB3747 | *hmnEx2123* [*grl-2*pro:CFP + *grl-18*pro:YFP] | 1 |
| CHB3756 | *unc-130 (ev505); hmnEx2126* [*grl-2*pro:CFP + *grl-18*pro:YFP] | 1 |
| CHB3310 | *hmnIs47* [*grl-18*pro:mApple]*; ynIs78* [*flp-8*pro:GFP] | 1, 5 |
| CHB3311 | *unc-130 (ev505); hmnIs47* [*grl-18*pro:mApple]*; ynIs78* [*flp-8*pro:GFP] | 1 |
| CHB4124 | *hmnIs47* [*grl-18*pro:mApple]; *hmnEx2227* [*col-53*pro:GFP + pRF4] | 1 |
| CHB4143 | *unc-130 (ev505); hmnIs47* [*grl-18*pro:mApple]; *hmnEx2227* [*col-53*pro:GFP + pRF4] | 1 |
| CHB4064 | *hmnIs47* [*grl-18*pro:mApple]; *hmnEx2171* [*col-177*pro:GFP + pRF4] | 1 |
| CHB4125 | *unc-130 (ev505); hmnIs47* [*grl-18*pro:mApple]; *hmnEx2171* [*col-177*pro:GFP + pRF4] | 1 |
| CHB3933 | *ujIs113; wgIs476* | S1 |
| CHB4067 | *ev505 ujIs113; wgIs476* | S1 |
| CHB4046 | *hmnIs100* [*ceh-10*pro:GFP + pRF4] | 2 |
| CHB4047 | *unc-130 (ev505); hmnIs100* [*ceh-10*pro:GFP + pRF4] | 2 |
| CHB4066 | *hmnEx2237* [*nlp-6*pro:GFP + pRF4] | 2 |
| CHB4163 | *unc-130 (ev505); hmnEx2237* [*nlp-6*pro:GFP + pRF4] | 2 |
| CHB3562 | *unc-130 (ev505); irIs67* [*hlh-17*pro:GFP] | 2 |
| CHB1634 | *unc-130 (ev505); hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | 2 |
| CHB1549 | *hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | 2 |
| CHB3850 | *hmnEx1910* [*grl-2*pro:mCherry + *itr-1*pro:YFP + pRF4] | S2 |
| CHB3355 | *unc-130 (ev505); hmnEx1910* [*grl-2*pro:mCherry + *itr-1*pro:YFP + pRF4] | S2 |
| CHB3422 | *saIs14* [*lin-48*pro:GFP]; *hmnEx1939* [*grl-2*pro:mCherry + pRF4] | S2 |
| CHB3441 | *unc-130 (ev505); saIs14* [*lin-48*pro:GFP]; *hmnEx1951* [*grl-2*pro:mCherry; pRF4] | S2 |

| CHB3221 | *hmnEx1715* [*F53F4.13*pro:GFP + pRF4]; *hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
|---|---|---|
| CHB2966 | *hmnEx1683* [*T02B11.3*pro:GFP + pRF4]; *hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
| CHB3045 | *unc-130 (ev505); hmnEx1715* [*F53F4.13*pro:GFP + pRF4]; *hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
| CHB3030 | *unc-130 (ev505); hmnEx1683* [*T02B11.3*pro:GFP + pRF4]; *hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
| CHB2223 | *unc-130 (ev505);  myIs4* [*pkd-2*pro:GFP] *him-5* (*e1490*) | S2 |
| CHB4308 | *unc-130 (ev505); hmnEx2293* [*mir-228*pro:histone-GFP]; *hmnEx1949* [*grl-2*pro:mApple + pRF4] | S2 |
| CHB4309 | *unc-130 (ev505); lin-48 (sa469);  hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
| CHB4302 | *lin-48 (sa469);  hmnIs13* [*F16F9.3*pro:mCherry + *grl-2*pro:YFP + *gcy-8*pro:CFP] | S2 |
| CHB2958 | *hmnEx1676* [*grl-2*pro:mApple + *grl-18*pro:GFP] | S2 |
| CHB2961 | *unc-130 (ev505);  hmnEx1679* [*grl-2*pro:mApple + *grl-18*pro:GFP] | S2 |
| CHB3775 | *hmnIs82* [*grl-18*pro:GFP] | 3 |
| CHB4160 | *unc-130 (ev505); hmnIs82* [*grl-18*pro:GFP] | 3 |
| CHB3313 | *unc-130 (ev505); hmnEx1903* [*unc-130*pro4:unc-130 + *grl-18*pro:GFP + *flp-8*pro:mCherry + pRF4] | 3, 4, 5 |
| CHB4174 | *unc-130 (ev505);  hmnIs82* [*grl-18*pro:GFP]; *hmnEx2273* [*mnm-2*pro:unc-130 + pRF4] | 3 |
| CHB4157 | *unc-130 (ev505); hmnIs82; hmnEx2282* [*mir-228*pro:unc-130 + *grl-18*pro:YFP + pRF4] | 3 |
| CHB4158 | *unc-130 (ev505); hmnEx2283* [*hsp16-2*pro:unc-130 +  *hsp16-41*pro:unc-130 + *grl-18*p:YFP + *unc-122*pro:RFP) | 3 |
| CHB1996 | *hmnEx1138* [*mnm-2*pro:GFP + *egl-38*pro:nls-mCherry + pRF4] | S4 |
| CHB3447 | *unc-130 (ev505); hmnEx1964* [*unc-130*pro4:DBD + *grl-18*pro:GFP + *flp-8*:mCherry + pRF4] | 4 |
| CHB3428 | *unc-130 (ev505); hmnEx1945* [*unc-130*pro4:DBD-VP64 + *grl-18*pro:GFP + *flp-8*:mCherry + pRF4] | 4 |
| CHB3381 | *unc-130 (ev505); hmnEx1922* [*unc-130*pro4:N-TERM + *grl-18*pro:GFP + *flp-8*:mCherry + pRF4] | 4 |

| CHB3402 | *unc-130 (ev505); hmnEx1927* [*unc-130*pro4: del eh1 N-TERM + *grl-18*pro:GFP + *flp-8*:mCherry + pRF4] | 4 |
|---------|---------|---|
| CHB3427 | *unc-130 (ev505); hmnEx1944* [*unc-130*pro4:C-TERM + *grl-18*pro:GFP + *flp-8*:mCherry + pRF4] | 4 |
| CHB4144 | *unc-130 (ev505); hmnEx2275* [*unc-130*pro4:DBD-Engrailed + *grl-18*pro:GFP + pRF4] | 4 |
| CHB4139 | *unc-130 (ev505); hmnEx2274* [*unc-130*pro4: del eh1 unc-130 + *grl-18*pro:YFP + *F16F9.3*pro:CFP + pRF4) | 4 |
| CHB4311 | *unc-130 (ev505); unc-37(e262) hmnEx2345* [*unc-130*pro4:N-TERM + *grl-18*pro:GFP + *unc-122*pro:RFP] | S5 |
| CHB4310 | *unc-37 (e262);  hmnIs82* [*grl-18*pro:GFP] | S5 |
| CHB3381 | *unc-130 (hd12); hmnIs47* [*grl-18*pro:mApple]*; ynIs48* [*flp-8*pro:GFP] | 5 |
| CHB3382 | *unc-130 (ns313); hmnIs47* [*grl-18*pro:mApple]*; ynIs48* [*flp-8*pro:GFP] | 5 |
| CHB3384 | *unc-130 (oy10); hmnIs47* [*grl-18*pro:mApple]*; ynIs48* [*flp-8*pro:GFP] | 5 |
| CHB3382 | *unc-130 (ev659); hmnIs47* [*grl-18*pro:mApple]*; ynIs48* [*flp-8*pro:GFP] | 5 |
| CHB3378 | *unc-130 (op459); hmnIs47* [*grl-18*pro:mApple]*; ynIs48* [*flp-8*pro:GFP] | 5 |
| CHB3411 | *unc-130 (ev505); hmnEx1936* [*unc-130*pro4:FOXD3, *grl-18*pro:GFP, *flp-8*:mCherry, pRF4] | 5 |

**Table S5. Strains generated in previous studies**

| ID | Genotype | Fig. | Source/Reference |
|---|---|---|---|
| OP476 | *wgIs476* [unc-86::TY1::EGFP::3xFLAG] | S1 | (Sarov et al., 2006) |
| VPR839 | *irIs67* [*hlh-17*pro:GFP]; *unc-119* | 2 | (Stout & Parpura, 2011) |
| CM117 | *saIs14* [*lin-48*pro:GFP] | S2 | (Johnson et al., 2001) |
| OP77 | *wgIs77* [unc-130::TY1::EGFP::3xFLAG] | 3 | (Sarov et al., 2006) |
| RW11144 | *itIs37* [*pie-1*pro:mCherry], *stIs10116* [*his-72*pro:his-24:mCherry]; *wgIs76* [unc-130:TY1:EGFP:3xFLAG] | 3, S3 | (Murray et al., 2012) |
| OS4260 | *nsIs198* [*mir-228*pro:GFP] | S4 | (Pierce et al., 2008) |
| PT621 | *myIs4* [*pkd-2*pro:GFP] *him-5* (*e1490*) | S2 | (Barr & Sternberg, 1999) |

**Table S6. Plasmids generated for this study**

| ID | Name |
|---|---|
| pKM47 | *grl-18*pro:YFP |
| pKM15 | *grl-2*pro:YFP |
| pKM117 | *grl-2*pro:CFP |
| pIL36 | *grl-2*pro:mCherry |
| pIL41 | *grl-2*pro:mApple |
| pKM16 | *F16F9.3*pro:CFP |
| pKM55 | pDest15 UNC-130 DBD |
| pKM69 | pDest15 R218C UNC-130 DBD |
| pKM71 | pDest15 W201G UNC-130 DBD |
| pKM59 | *unc-130*pro4:UNC-130 |
| pKM77 | *unc-130*pro4:N-term:UNC-130 DBD |
| pKM79 | *unc-130*pro4:UNC-130 DBD:C-term |
| pKM88 | *unc-130*pro4:del eh1 N-term:UNC-130 DBD |
| pKM78 | *unc-130*pro4:UNC-130 DBD |
| pKM83 | *unc-130*pro4:UNC-130 DBD VP64 |
| pKM80 | *unc-130*pro4: del eh1 UNC-130 |
| pKM115 | 5XUAS SV40pro:firefly luciferase |
| pKM114 | CAGpro: renilla luciferase |

| pKM111 | CAGpro:GAL4DBD:UNC-130 C-term |
|--------|-------------------------------|
| pKM113 | CAGpro:GAL4DBD |
| pKM108 | CAGpro:UNC-130 N-term:GAL4DBD: UNC-130 C-term |
| pKM72 | *unc-130*pro4:FOXD3 |
| pKM119 | *mir-228*pro:unc-130 |
| pKM118 | *mnm-2*pro:unc-130 |
| pKM67 | *hsp16-2*pro:unc-130 |
| pKM68 | *hsp16-41*pro:unc-130 |
| pKM126 | *nlp-6*pro:GFP |
| pKM123 | *ceh-10*pro:GFP |

**Table S7. Primers of general interest**

| Name | Sequence |
|------|----------|
| unc-130pro4_fwd | gtactCCTGCAGGctttcaattgaaaattccgaga |
| unc-130pro4_rev | gatcGGCGCGCCtgttACCGGTgtctacctagt |