Table S1: Fly strains
Wild type and mutant stocks
w^{1118}
Canton S
Apterous-lacZ
$numb^{15}$, P{neoFRT}40A/Cyo
P{ry[+t7.2]=neoFRT}82B e^{s}, $spdo^{G104}$/TM3 Sb
$nkx6^{D25}$, P{FRT(w[hs])}2A/TM6b Tb
$hb9^{ij154}$, P{FRT(w[hs])}2A/TM6b Tb
tup^{isl-1}, P{ry[+t7.2]=neoFRT}40A/CyO
dac^{E462}P{neoFRT}40A/CyO
Fly lines used for the Gal4/UAS experiments
UAS-hb9
UAS-nkx6
UAS-unc4
Gene Switch-Elav-Gal4
Fly lines used for MARCM analysis
P{GawB}elav[C155] ; P{FRT(w[hs])}G13, P{tubP-GAL80}LL2
P{GawB}elav[C155], P{FRT(w[hs])}G13 P{UAS-mCD8::GFP.L}LL5
P{hsFLP}1; P{FRT(w[hs])}G13 P{tubP-GAL80}LL2/CyO, P{FRT(w[hs])}G13 P{tubP-GAL80}LL2
P{tubP-GAL80}LL10 P{neoFRT}40A/CyO
y[1] w^{1118}; P{tubP-GAL80}LL9 P{FRT(w[hs])}2A/TM3, Sb

Table S2: Antibodies used in gene expression studies		
Antibody Specificity	Species	Reference
Hb9	Rabbit, guinea pig	Broihier and Skeath, 2002
Lim3	Guinea pig	Broihier et al., 2004
Islet	Rat	Broihier et al., 2004
Nkx6	Rat	Broihier et al., 2004
Dbx	Guinea pig	Lacin et al., 2009
Nmr1	Rabbit	Leal et al., 2009
Msh	Rabbit	McDonald et al., 1998
Bar-H1/2	Rabbit	Higashijima et al., 1992
Unc4	Rabbit	this paper
Dichaete	Rabbit	Nambu and Nambu, 1996
Vestigial	Rabbit	Guss et al., 2008
NOS	Guinea pig	Lacin et al., submitted
GFP	Rabbit	Torrey Pines and Roche
β-galactosidase	Mouse and Rabbit	Invitrogen
Invected/engrailed 4D9	Mouse	Patel et al., 1989
Cut 2B10	Mouse	Blochinger et al., 1993
FasII 1D4	Mouse	van Vactor et al., 1993
Chinmo	Rabbit	Zhu et al., 2006
Broad-Complex	Mouse	Emery, 1994

Table S3: Genes assayed for expression in post-embryonic neurons			
Transcription factors expressed in discrete clusters of neurons in post-embryonic lineages			
Vestigial	Msh	Cut	Dichaete
Unc4	Dbx	Nmr1	BarH1/2
Engrailed	Apterous	Hb9	Lim3
Islet	Nkx6	Dachshund	
Transcription factors expressed in subsets of neurons in most or all post-embryonic lineages			
Zfh-1	Lim1	Castor	Hunchback
Kruppel	Pdm2		
Transcription factors not expressed in clusters of neurons in post-embryonic lineages			
Pdm3	Dimmed	FoxD	Pointed
Eve (may label one lineage)	Eagle (expressed in progenitors)	Eyes Absent	

Table S4: Transcription factor expression in post-embryonic lineages		
Lineage	**A hemi-lineage**	**B hemi-lineage**
Lineage 0	En (n>10*)	PCD*
Lineage 1	Msh (n>10)	Nmr1 (n=4)
Lineage 2	?	PCD
Lineage 3	Nkx6 (n>10); Nmr1 (n=3)	Dbx (n>10); Dacs (n=4)
Lineage 4	PCD	Hb9 (n>10); Nkx6 (n=4); Ap-lacZ (n=0)**
Lineage 5	PCD	Vg (n=2); Cut (n=7)
Lineage 6	?	En (n=5); Vg (n=3); Cut (n=4)
Lineage 7	PCD	Unc4 (n>10)
Lineage 8	?	Lim3 (n=5)
Lineage 9	Msh	Lim3 (n=7); Islet (n=0)**
Lineage 10	PCD	Hb9 (n>10); Nkx6 (n=3); Lim3 (n=5)
Lineage 11	Nkx6 (n=4); Unc4 (n=6)	?
Lineage 12	Unc4 (n>10)	Nkx6 (n>10); Nmr1 (n=4)
Lineage 13	Dbx (n>10)	Dichaete (n=7); Vg (n=1)
Lineage 14	Msh (n>10)	PCD; Lim3 (n=4); Islet (n=0)**
Lineage 15	PCD	Lim3 (n>10); Nkx6 (n>10); Islet (n=0)**
Lineage 16	PCD	Hb9 (n=>10); Lim3 (n=7))
Lineage 17	Unc4 (n=4); Islet (n=2)	PCD
Lineage18	PCD	Unc4 (n=6)
Lineage 19	Dbx (n=6)	Unc4 (n=8)
Lineage 20	BarH (n=7)#	PCD*/BarH
Lineage 21	Msh (n>10)	PCD*
Lineage 22	BarH(n=7)#	PCD*/BarH
Lineage 23	PCD	Unc4 (n=5)
Lineage 24	PCD	Nkx6/? (n=2 of 4)

PCD: Indicates that most cells in hemilineage die

*: denotes number of wild-type clones assayed for this lineage and transcription factor.

**: Islet expression in lineages 9, 14, and 15 based on co-expression with Lim3; Ap-lacZ expression in lineage 4 based on co-expression with Hb9

#: 7 $BarH^{+}$ clones were generated for lineages 20 and 22.

Figure S1

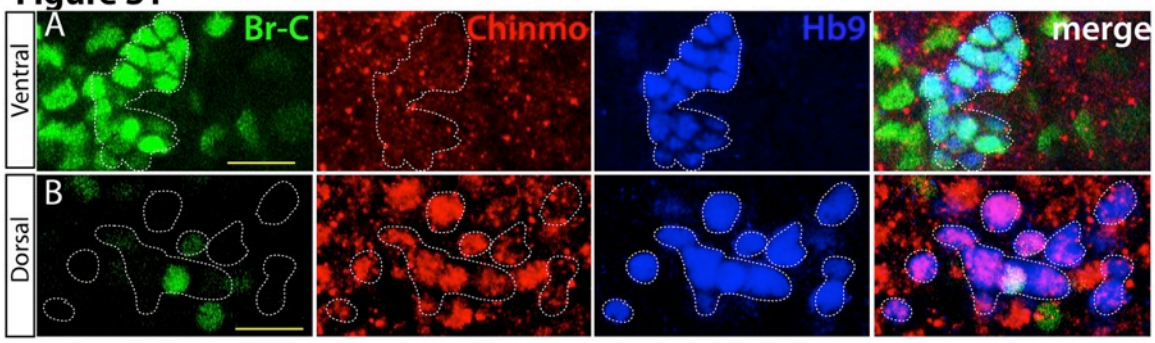

Figure S1) Hb9, Chinmo, and Broad expression in a thoracic neuromere of a late third instar larva.
(A, B) Ventral (A) and dorsal (B) views of a portion of a thoracic hemineuromere labeled for Broad (green), Chinmo (red), and Hb9 (blue). (A) Most small, ventral $Hb9^{+}$ neurons express Broad. (B) Most large, dorsal $Hb9^{+}$ neurons express Chinmo; a few express Broad. Anterior - up; scale bar: 10um.

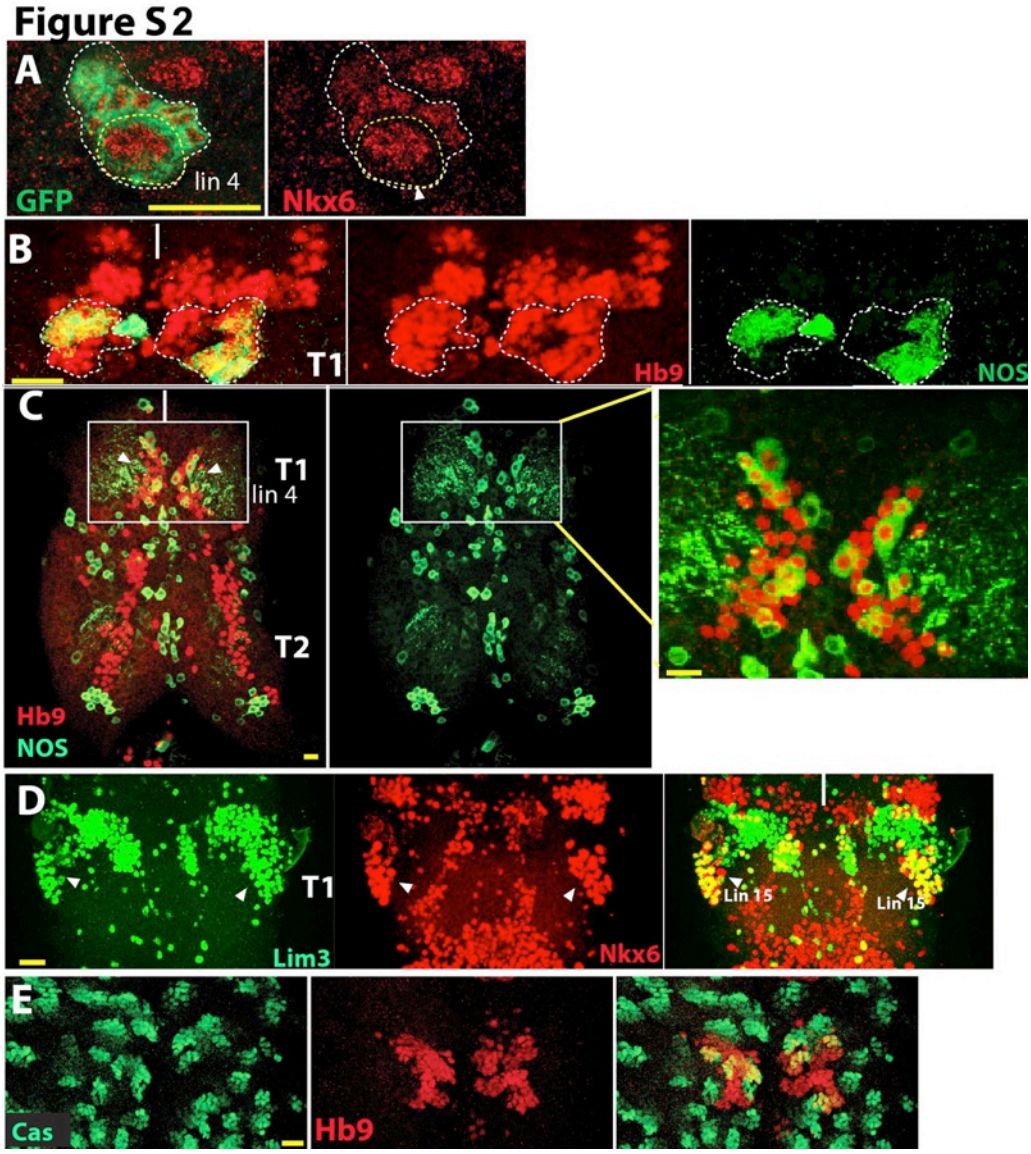

Figure S2) Hb9, Nkx6, Lim3, and Cas expression in thoracic post-embryonic lineages.
(A) Wild-type lineage 4 MARCM clone in late-third instar larval CNS labeled for Nkx6 expression (red); arrowhead points to the NB, identified based on its large size and ventral position within clone. (B) Hb9 (red) and NOS (green) co-expression in lineage 4 neurons (dashed lines) of a late-third instar larva. (C) NOS expression (green) is maintained in $Hb9^{+}$ neurons (red; arrowheads) in the first thoracic segment of the adult nerve cord. (D) In the adult CNS, Lim3 and Nkx6 co-expression in lineage 15 neurons (arrowheads), all of which are motor neurons. For simplicity, only the first thoracic segment is shown. (E) In late-third instar larvae, Cas (green) and Hb9 (red) expression in post-embryonic neuronal lineages. In B-D T1, T2, and T3 refer to identity of thoracic segment. Anterior is up; white line marks ventral midline. Scale bar – 10um.

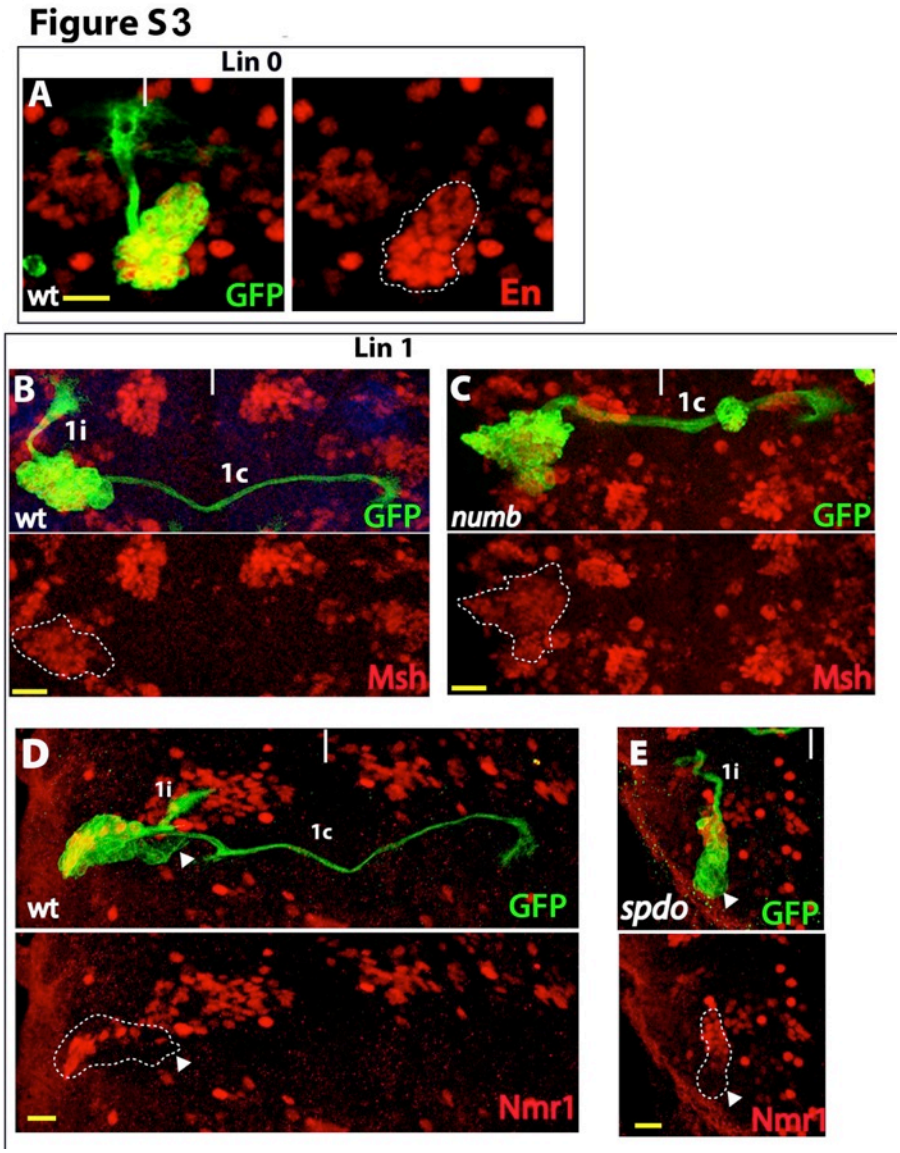

Figure S3) MARCM analysis of lineages 0 and 1 in late-third instar larvae.
(A) Wild-type lineage 0 clone labeled for Engrailed. (B, D) Wild type lineage 1 MARCM clones labeled for Msh (B) and Nmr1 (D) extend neurite bundles contralaterally (1c) and ipsilaterally (1i). (C) *numb* mutant lineage 1 MARCM clone labeled for Msh contains mostly Msh^{+} neurons and extends a neurite bundle contralaterally (1c). (E) *spdo* mutant lineage 1 MARCM clone labeled for Nmr1 contains mostly $Nmr1^{+}$ neurons and extends a neurite bundle ipsilaterally (1i). Arrowheads in D, E point to the NB, identified based on size and ventral position within clone, which do not express Nmr1. Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar – 10um.

Figure S4

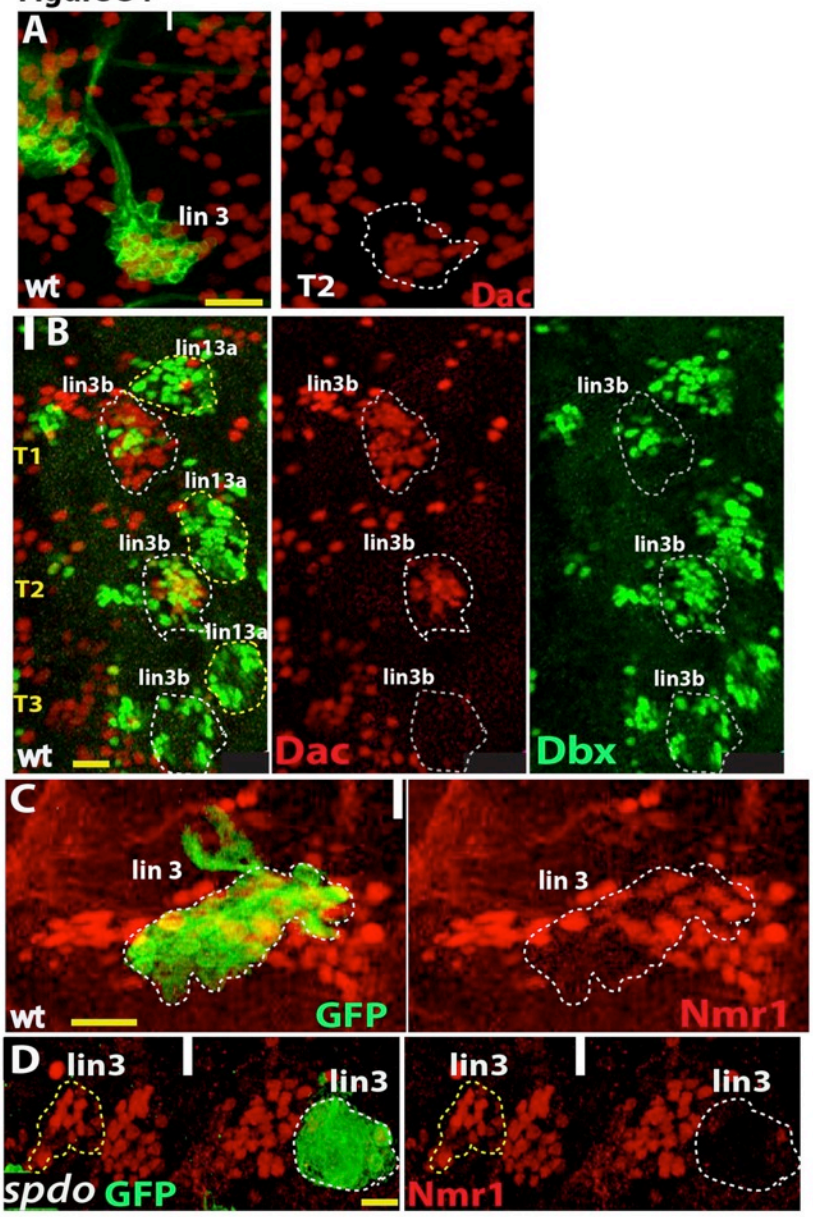

Figure S4) Dachsund, Dbx, Nkx6, and Nmr1 expression in lineage 3 in thoracic segments of late-third instar larvae.
(A) Wild-type lineage 3 MARCM clone labeled for Dachshund (Dac, red). (B) Dac and Dbx expression in lineage 3b neurons. Dashed lines outline lineage 3b and lineage 13a neurons. These neurons were mapped to the indicated lineages based on analysis of MARCM clones generated in other animals (e.g. see panel A). In A and B, T1, T2, and T3 refer to first, second, and third thoracic segments, the borders of which are identified by the Dbx^{+} lineage 13a, which only occurs in T1, T2, and T3. (C) Wild-type lineage 3 MARCM clones labeled for Nmr1 (red). (D) *spdo* mutant lineage 3 MARCM clone (green) lacks Nmr1 (red) expression; Nmr1 expression in lineage 3 neurons is shown in contralateral hemisegment.Anterior is up; white bar indicates midline. Scale bar – 10um.

Figure S5

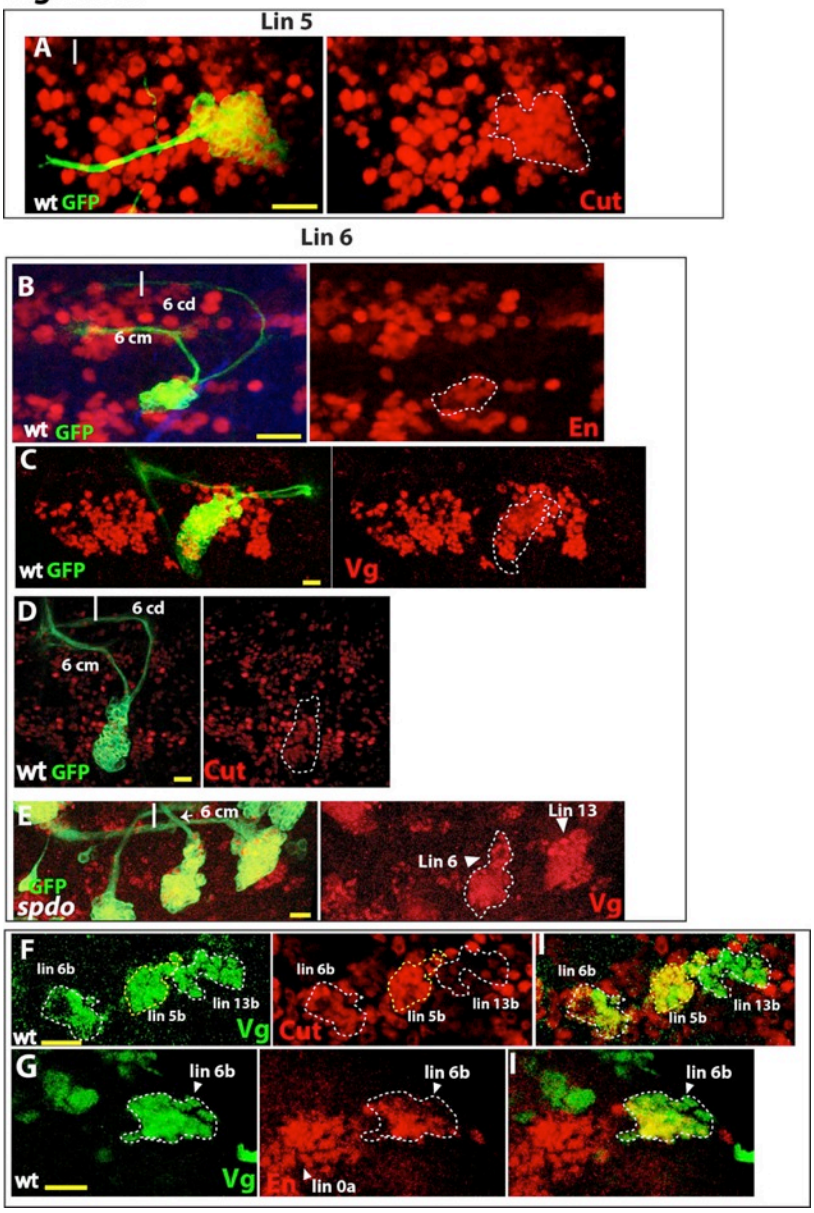

Figure S5) MARCM analysis of lineages 5 and 6 in late-third instar larvae.
(A) Wild-type lineage 5 MARCM clone labeled for Cut. (B-D) Wild-type lineage 6 MARCM clones labeled for Engrailed (En, B), Vestigial (Vg, C), and Cut (D) extend two neurite bundles contralaterally (6cm, 6cd). (E) In *spdo* mutant lineage 6 MARCM clones, most neurons express Vestigial; Vestigial expression in lineage 13 is also indicated. (F) Co-expression of Vg (green) and Cut (red) in lineage 5b and 6b neruons; Vestigial expression in lineage 13b neurons is also shown. (G) Co-expression of Vg (green) and Engrailed (red) in many lineage 6b neurons; Engrailed expression is also shown in lineage 0a neurons. Anterior is up; white bar indicates midline. Scale bar – 10um.

Figure S6

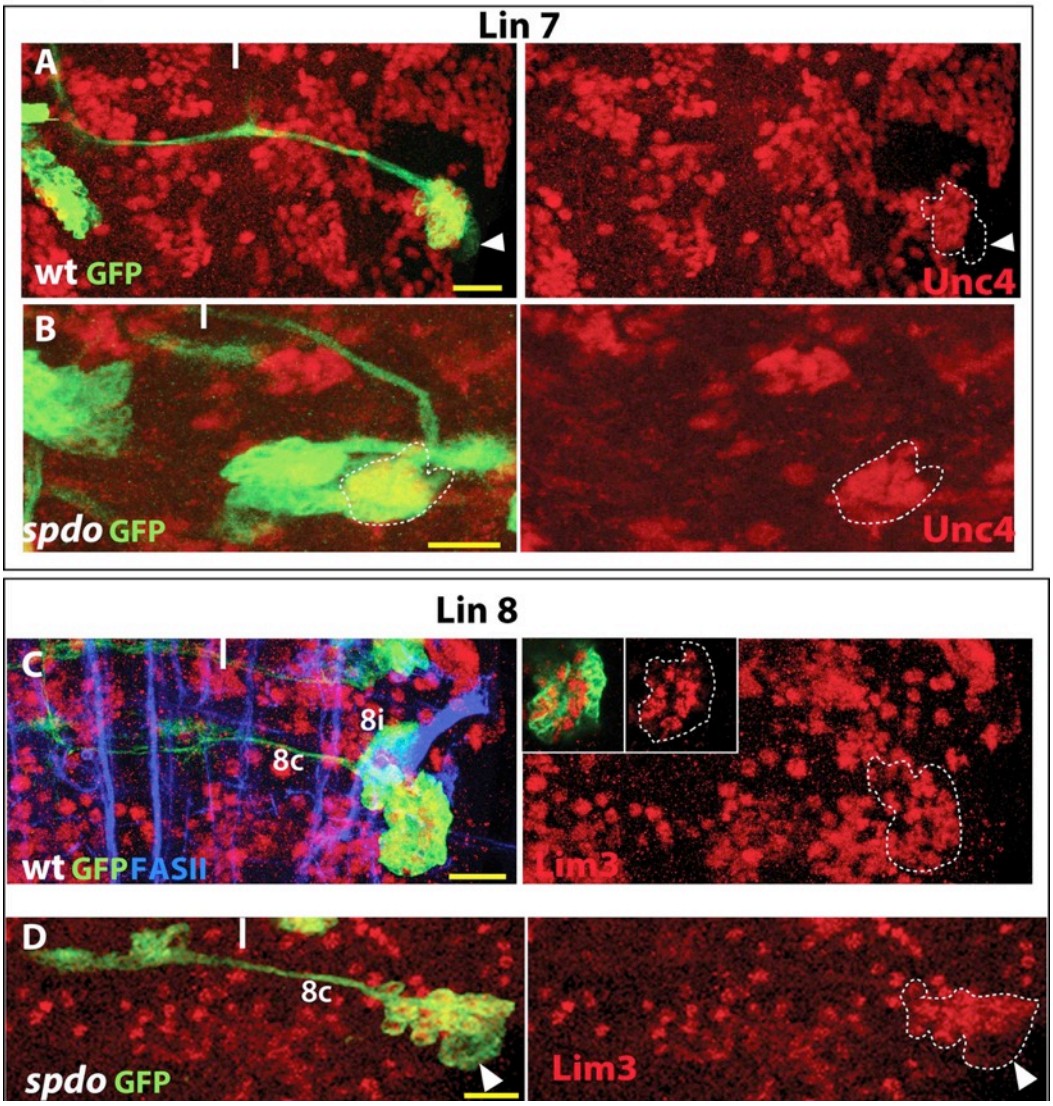

Figure S6) MARCM analysis of lineages 7 and 8 in late-third instar larvae.
(A) Wild-type lineage 7 MARCM clone labeled for Unc4 (red). Arrowhead points to NB, identified based on its large size and ventral position in clone, and adjacent GMCs that do not express Unc4. (B) *spdo* mutant lineage 7 MARCM clone labeled for Unc4 (red): the number of Unc4-expressing neurons increases. Note that two adjacent MARCM clones obscure boundary of lineage 7 clone. (C) Wild-type lineage 8 MARCM clone labeled for Lim3 (red) and Fas II (blue) extend neurite bundles ipsilaterally (8i) and contralaterally (8c). Inset shows a single Z-section of the clone revealing Lim3 expression in a subset of neurons in clone. (D) *spdo* mutant lineage 8 MARCM clone, most neurons express Lim3 and project axons contralaterally (8c). Arrowhead points to NB, identified based on size and ventral position, which lacks Lim3 expression. Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar – 10um.

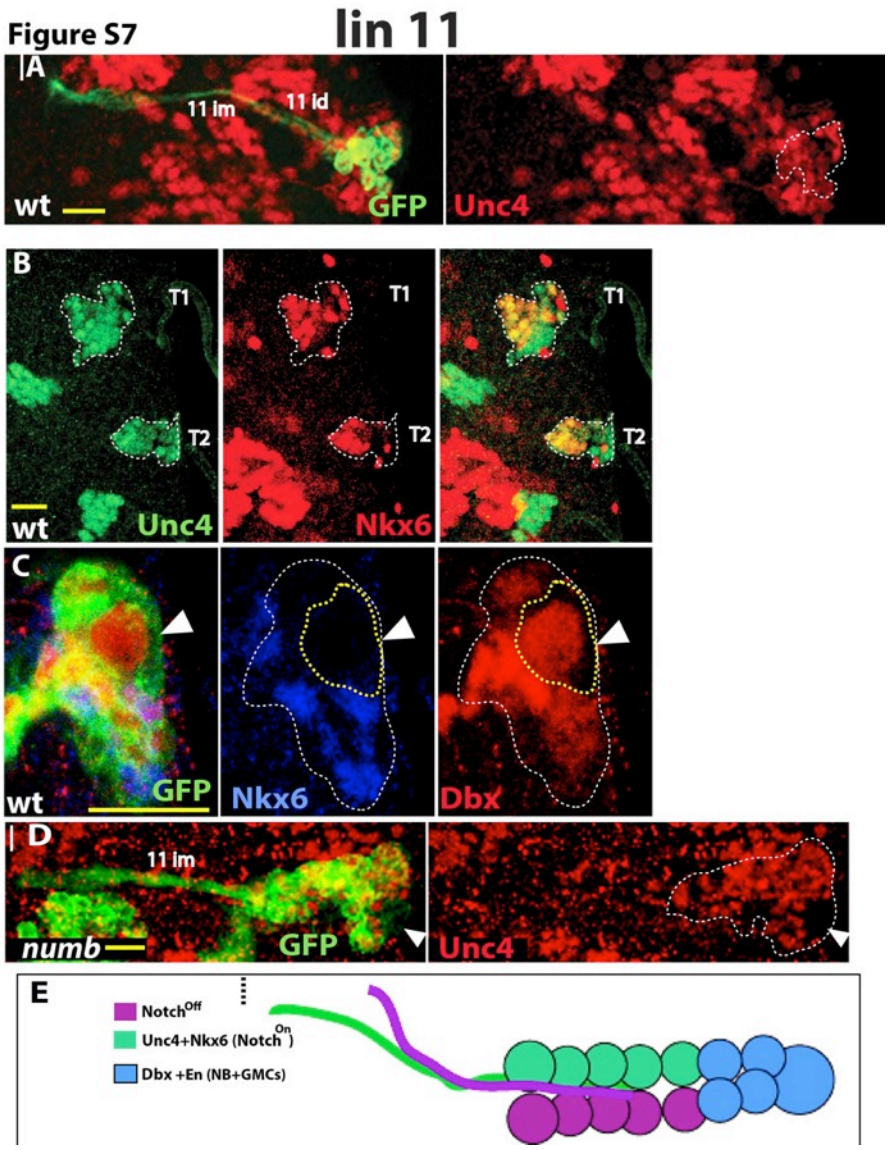

Figure S7) MARCM analysis of lineage 11 in late-third instar larvae.
(A) Wild-type lineage 11 MARCM clone labeled for Unc4 (red) extends a neurite bundle that forks as it approaches the midline (11im, 11id). (B) Unc4 (green) and Nkx6 (red) co-expression in lineage 11 neurons (arrowheads; dashed lines). Predicted boundaries of lineage 11 neurons based on analysis of Unc4 and Nkx6 expression in lineage 11 MARCM clones generated in other animals. T1 and T2 refer to first and second thoracic segments. (C) Dbx (red) and Nkx6 (blue) expression in a wild-type lineage 11 MARCM clone. Dbx expressed in the NB (arrowhead), identified based on its size, and adjacent GMCs. (D) *numb* mutant lineage 11 MARCM clone labeled for Unc4 (red): the clone is larger than in wild-type and contains mostly $Unc4^{+}$ neurons. Arrowhead points to NB, identified by its size and ventral position in clone, which does not express Unc4.(E) Schematic of gene expression in lineage 11.Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar – 10um.

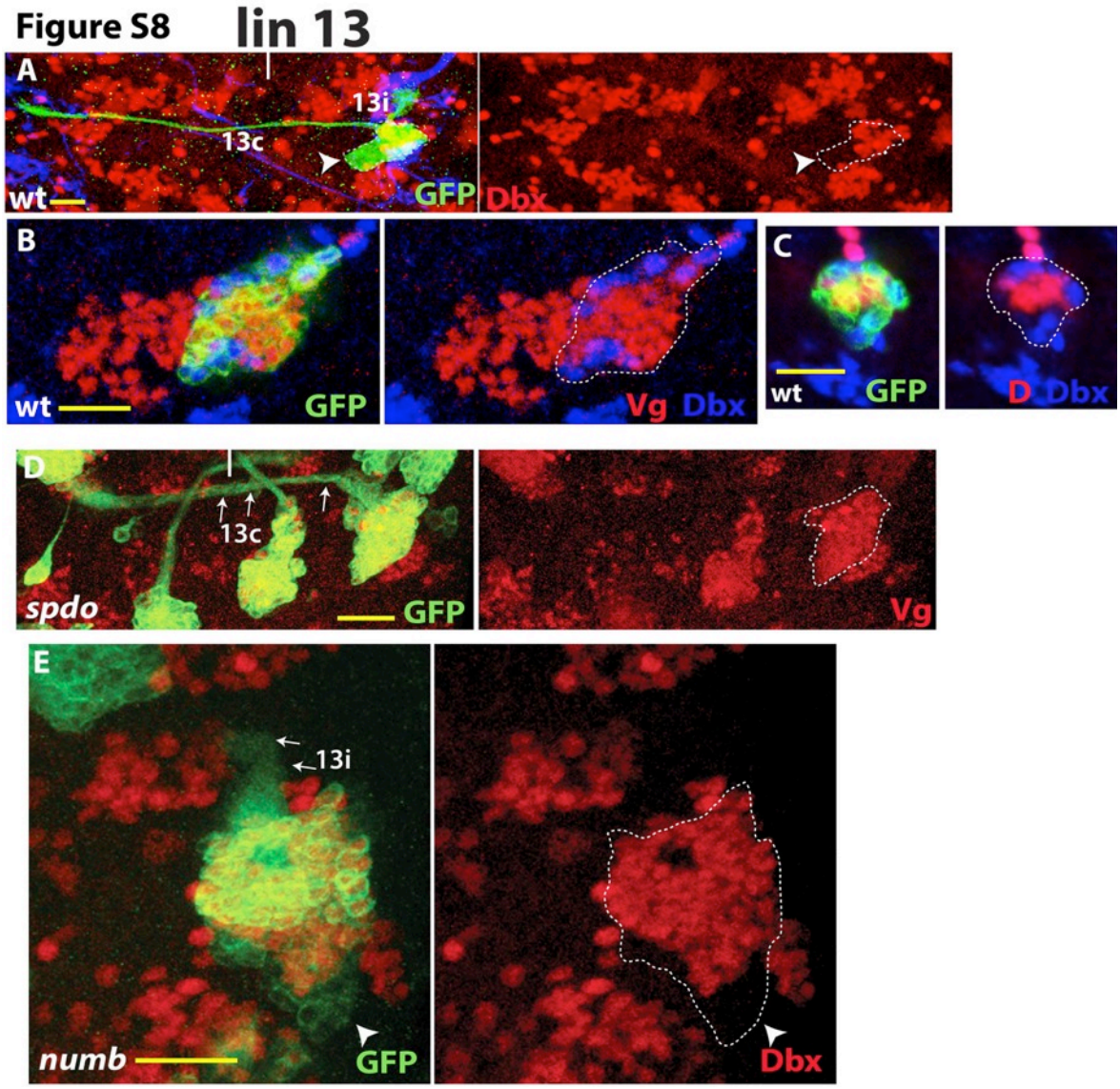

Figure S8) MARCM analysis of lineage 13 in late-third instar larvae.
(A) Wild-type lineage 13 MARCM clone labeled for Dbx (red) extends a short neurite bundle ipsilaterally (13i) and a long neurite bundle contralaterally (13c). Arrowhead points to Dbx$^-$ NB, identified based on size and position within clone.(B) Wild-type lineage 13 MARCM clone labeled for Vestigial (red) and Dbx (blue): the two proteins are expressed in mutually exclusive sets of neurons. (C) Wild-type lineage 13 MARCM clone labeled for Dichaete (red) and Dbx (blue): the two proteins are expressed in mutually exclusive sets of neurons. (D) *spdo* mutant lineage 13 MARCM clone labeled for Vestigial (red): most neurons in clone express Vg and extend the 13c neurite bundle contralaterally (arrows). (E) *numb* mutant lineage 13 MARCM clone: most neurons express Dbx and extend the short 13i neurite bundle ipsilaterally (arrows). Arrowhead points to the NB and adjacent GMCs identified based on size and ventral location in the clone, which lack Dbx expression. Anterior is up; white bar indicates midline. Scale bar: 10um.

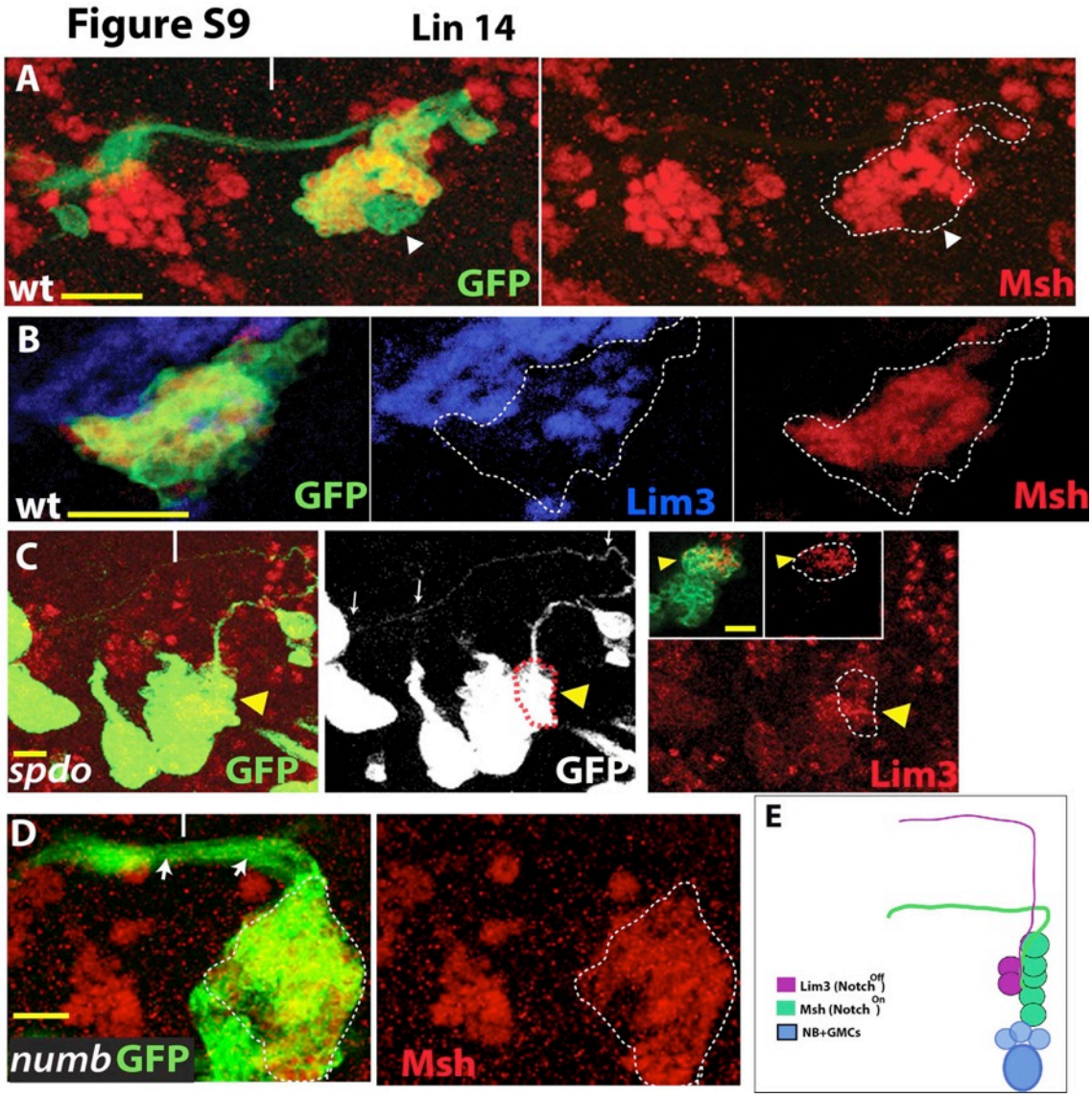

Figure S9) MARCM analysis of lineage 14 in late-third instar larvae
(A) Wild-type lineage 14 MARCM clone labeled for Msh (red) extends a thick ventral neurite bundle contralaterally and a thin dorsal neurite bundle contralaterally, the latter being too faint to be visible in the image. Arrowhead points to the Msh^{-} NB, identified by its size and position in clone, and adjacent GMCs. (B) Wild-type lineage 14 MARCM clone labeled for Lim3 (blue) and Msh (red): the two proteins are not co-expressed in neurons of this lineage. (C) *spdo* mutant lineage 14 MARCM clone (dotted lines) labeled for Lim3 expression: most neurons in the clone express Lim3 and extend a thin dorsal neurite bundle (arrows). Boundaries of lineage 14 mutant clone were determined by analysis of individual Z-sections as shown in inset. (D) *numb* mutant lineage 14 MARCM clone labeled for Msh expression: most neurons express Msh and extend a thick ventral neurite bundle (arrows). (E) Schematic of gene expression in lineage 14.
Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar – 10um.

Figure S10

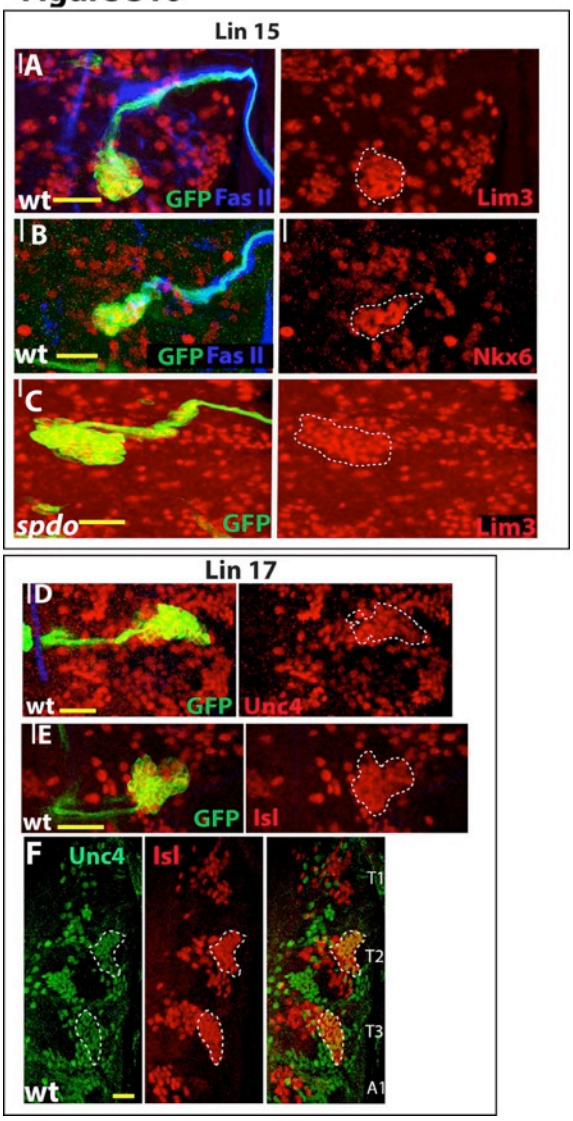

Figure S10) MARCM analysis of lineages 15 and 17 in late-third instar larvae
(A, B) Wild-type lineage 15 MARCM clone labeled for Lim3 (red in A), Nkx6 (red in B), and Fas II (blue in A, B). (C) *spdo* mutant lineage 15 MARCM clone labeled for Lim3 (red): the clone is larger and most neurons express Lim3. (D, E) Wild-type lineage 17 MARCM clone labeled for Unc4 (D) and Isl (E) extends an ipsilateral neurite bundle towards the midline. (F) Co-expression of Unc4 (green) and Isl (red) in lineage 17 occurs in the second and third, but not first, thoracic segments. Anterior is up; white bar indicates midline. Scale bar: 10um.

Figure S11

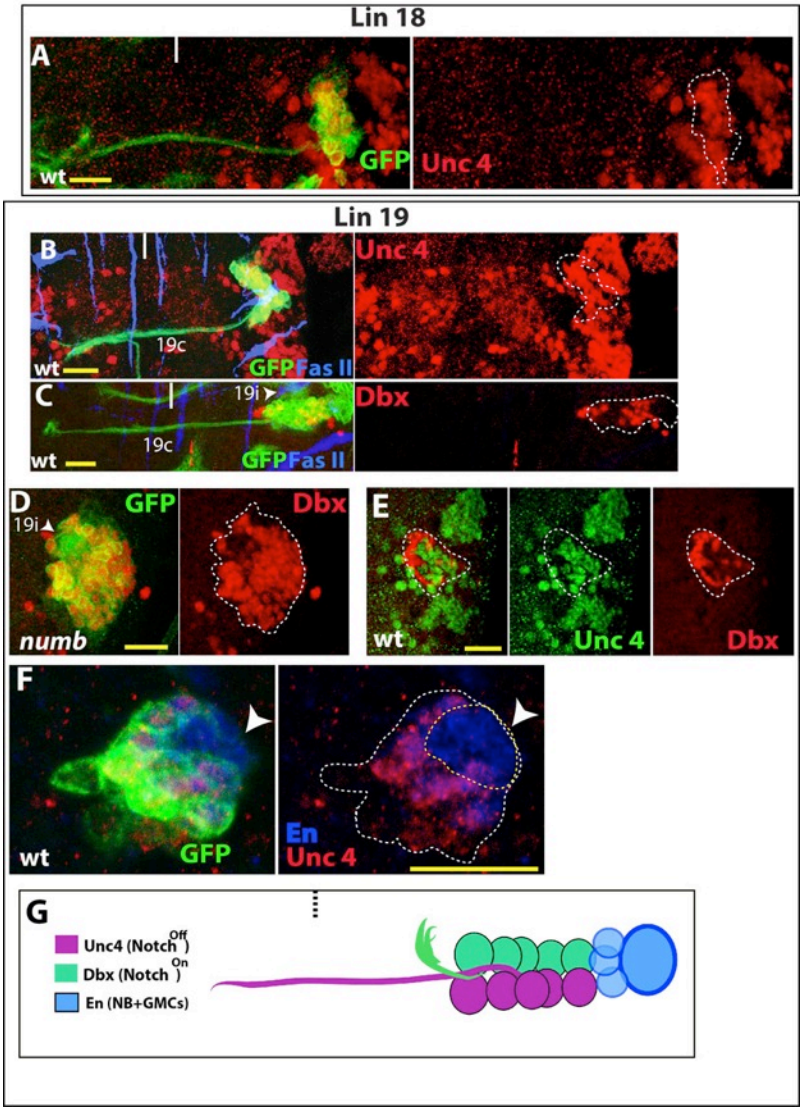

Figure S11) MARCM analysis of lineages 18 and 19 in late-third instar larvae
(A) Wild-type lineage 18 MARCM clone labeled for Unc4 (red) extends a contralateral axon projection. (B, C) Wild-type lineage 19 MARCM clone labeled for Unc4 (red, B) and Dbx (red, C) extends a short ipsilateral neurite bundle (19i, C) and a long contralateral neurite bundle (19c). (D) *numb* mutant lineage 19 MARCM clone labeled for Dbx: most neurons express Dbx. (E) Dbx (red) and Unc4 (green) expression in lineage 19: the two proteins are not co-expressed in neurons. (F) Wild-type lineage 19 MARCM clone labeled for Engrailed (blue) and Unc4 (red): arrowhead points to Engrailed^{+} NB, identified based on its size and location, and adjacent GMCs. (G) Schematic of gene expression in lineage 19. Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar: 10um.

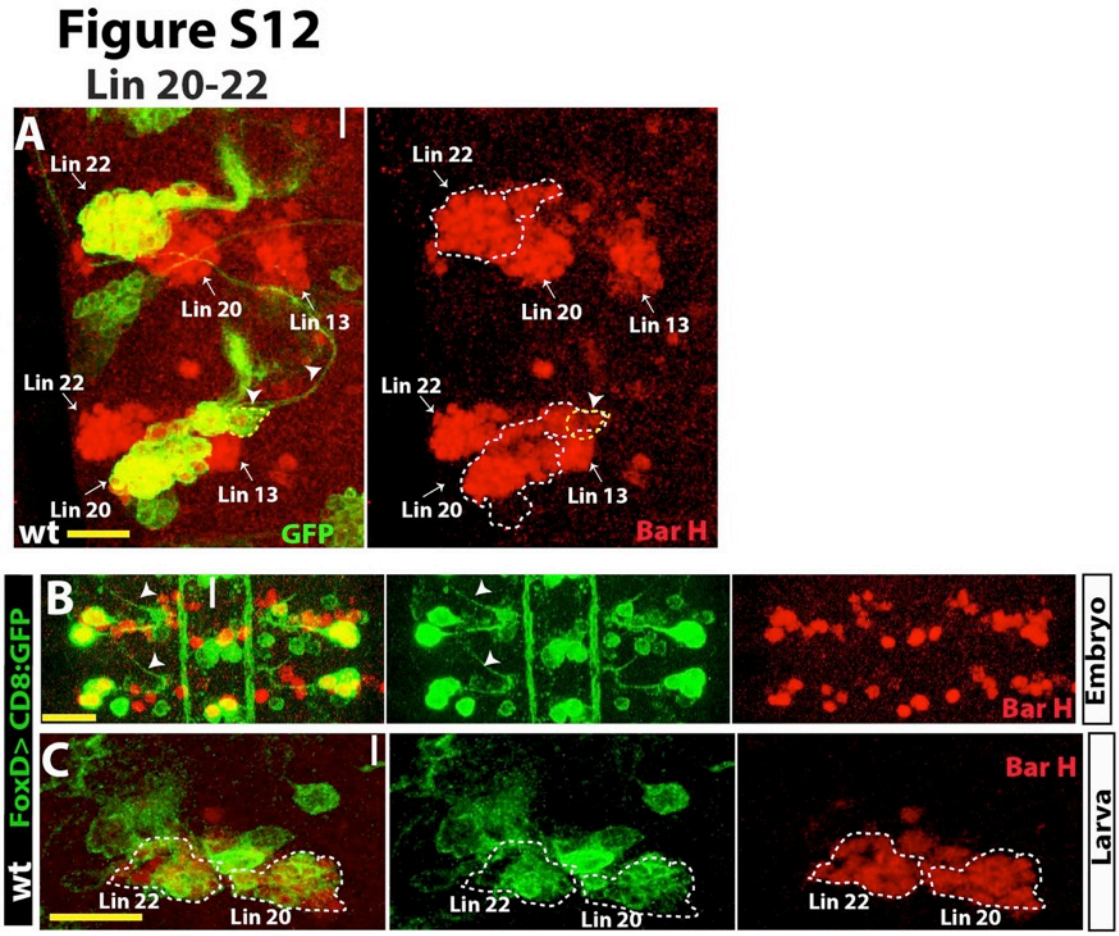

Figure S12) MARCM analysis of lineages 20 and 22
(A) Wild-type MARCM clones of lineages 20 and 22 in a late-third instar larva labeled for BarH1/2 (red). These lineages are identical in morphology; thus, the labeling is arbitrary. BarH1/2 is expressed in all neurons of lineage 20 and 22, including motor neurons. Arrowheads point to motor neuron and motor axon. (B, C) BarH1/2 (red) and GFP reporter gene expression driven by a FoxD enhancer (green) shown in the CNS of a late stage embryo (B) and late-third instar larva (C). (B) BarH1/2 is expressed in motor neurons that derive from NB 5-4 in embryo (arrowheads indicate motor neuron axons). (C) The FoxD enhancer drives reporter expression in BarH1/2-expressing neurons of post-embryonic lineages 20 and 22. Anterior is up; white bar indicates midline. Scale bar: 10um.

Figure S13

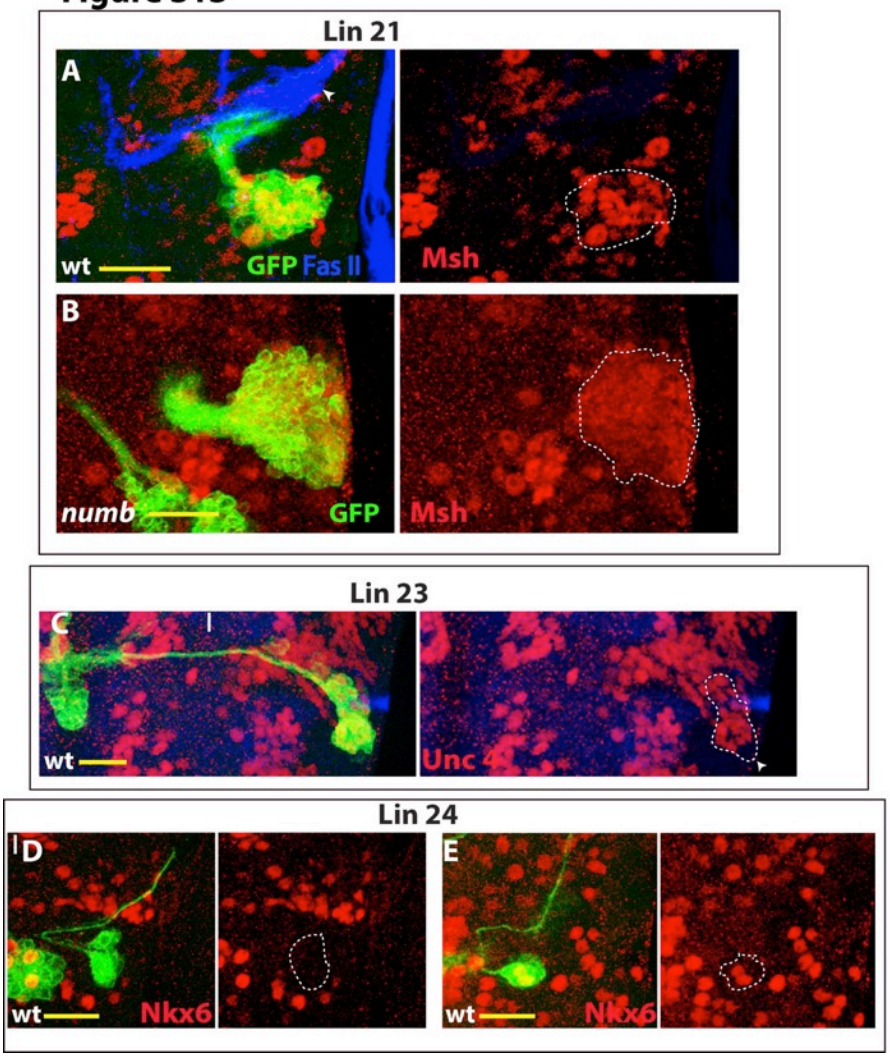

Figure S13) MARCM Analysis of lineages 21, 23 and 24 in late-third instar larvae
(A) Wild-type MARCM clone of lineage 21 labeled for Msh (red) and FasII (blue) extends an ipsilateral neurite , which terminates posterior to the lateral cylinder (arrowhead). (B) *numb* mutant lineage 21 MARCM clones labeled for Msh expression: the clones are bigger than wild-type; most neurons in them express Msh. (C) Wild-type MARCM clone of lineage 23 labeled for Unc4 (red) extends a contralateral neurite bundle. (D, E) Wild-type MARCM clones of lineage 24 labeled for Nkx6 (red): some clones lack $Nkx6^{+}$ neurons (D), and other clones contain $Nkx6^{+}$ neurons (F).
Anterior is up; white bar indicates midline; dashed lines outline clone boundaries. Scale bar:10um.

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