Supplemental Information

Figure S1

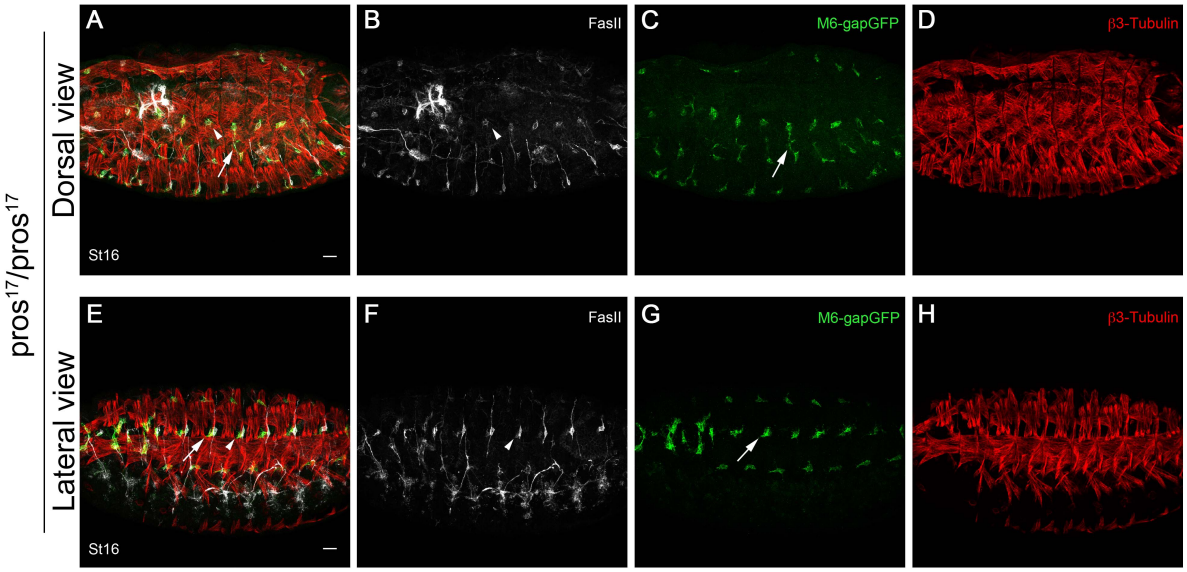


Figure S1. Motor axons are not necessary for behavior of embryonic AMPs. Dorsal view (**A**-**D**) and lateral view (**E**-**H**) of stage 16 *prospero* mutants combined with the M6-gapGFP sensor. In this context motor axons do not exit the CNS properly leaving only a minor extension toward the most ventral muscles, and the Fas2 positive extension of the LBD in the dorsal region. White arrows point toward the unaffected migration of DL-AMPs (**C**) and L-AMPs (**G**). Arrowheads highlight the Fas2 expression associated with AMP cells (**B**, **F**). Scale bars represent 20 μm.

Figure S2

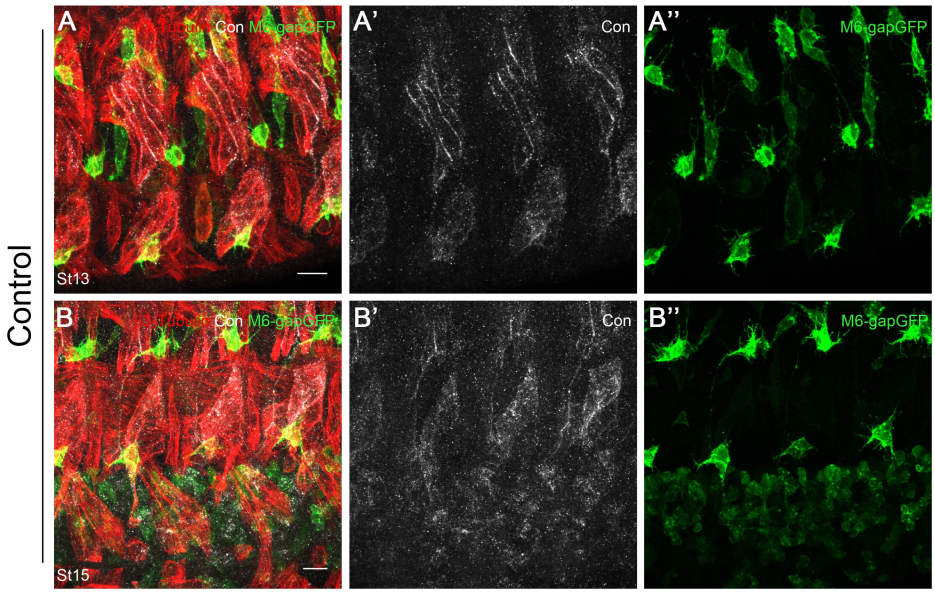


Figure S2. L-AMPs do not express the cell adhesion molecule Connectin. Ventrolateral view of stage 13 (**A**-**D**) and stage 16 (**E**-**H**) M6-gapGFP embryos stained with anti-Connectin. Connectin expression is observed in lateral and ventral acute muscles, and in some cells in the CNS. AMPs are Connectin-negative. Scale bars represent 10 μm.

Figure S3

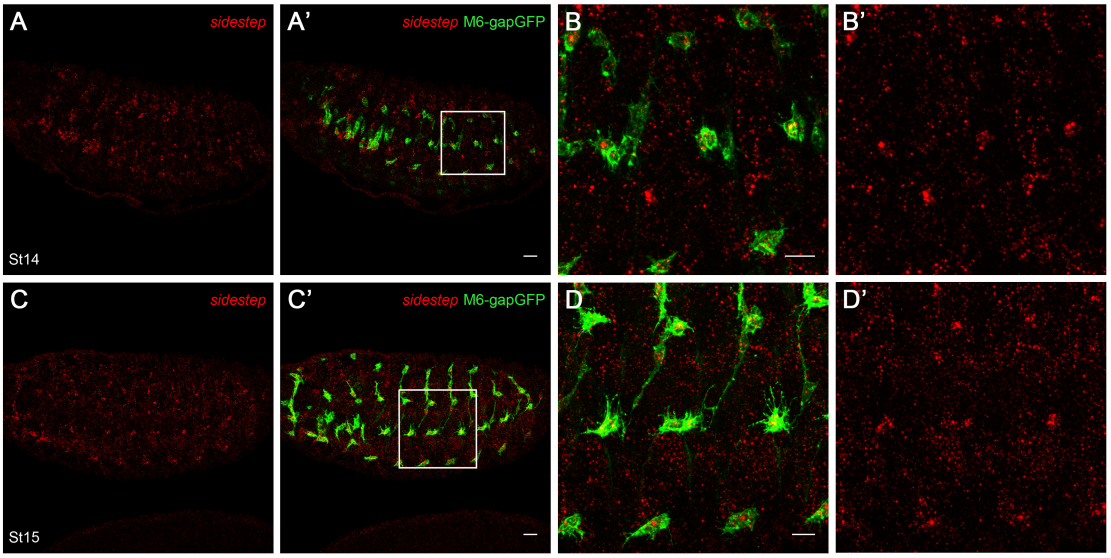


Figure S3. Embryonic *sidestep* expression in AMPs. (**A**-**D'**) Fluorescent *in situ* hybridization (red) of *sidestep* in a stage early 14 (**A**-**B'**) and 15 (**C**-**D'**) M6-gapGFP embryos. As shown in the zoomed views (**B**,**B'**,**D**,**D'**) *sidestep* transcripts are particularly enriched in DL-AMPs, L-AMPs and V-AMPs. Notice that in stage 14 embryos *side* expression is also detected in a dorsally located cluster of cells, presumably sensory neurons. Scale bars represent 20 μm and 10 μm for the zoomed view.

Figure S4

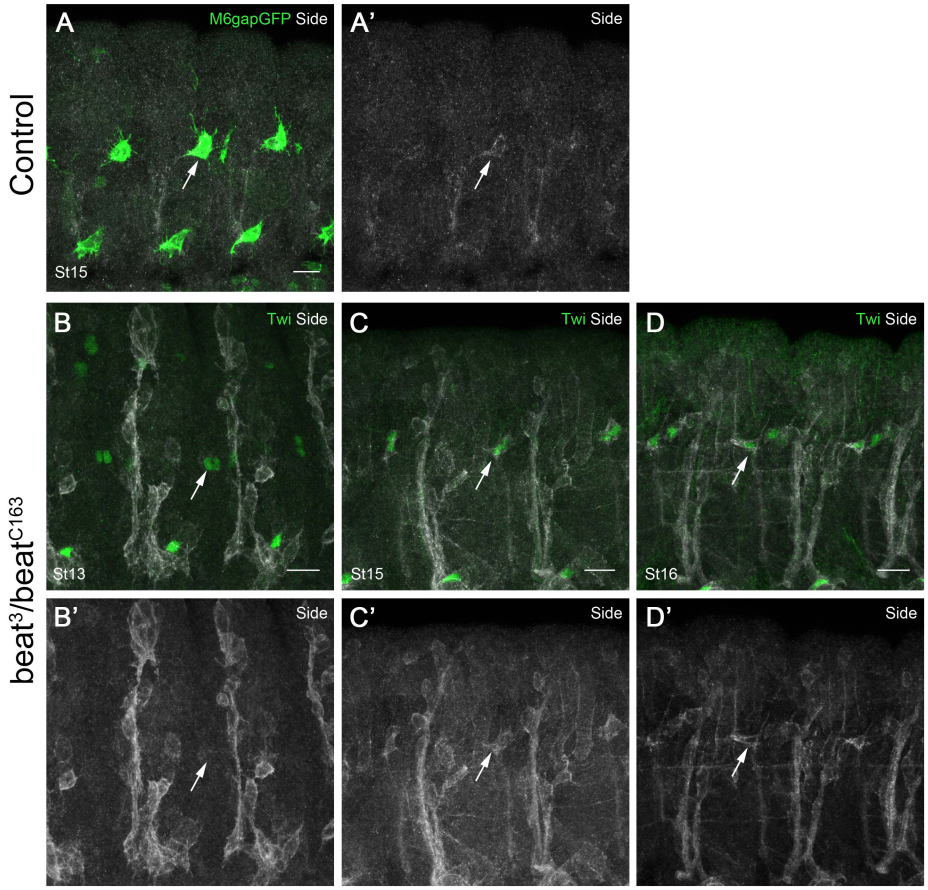


Figure S4. L-AMPs transiently express the guidance molecule Sidestep. (**A**,**A'**) Zoomed lateral view of a M6-gapGFP stage 15 embryo. Immunostaining with the anti-Sidestep antibody reveals a weak expression in L-AMPs (white arrows). (**B**-**D'**) In *beat-1a* mutant embryo Sidestep expression appears higher in Sidestep-positive cells. At stage 13 L-AMPs are Sidestep-negative (**B**,**B'**). Increased Sidestep expression in L-AMPs could be detected at stage 15 (**C**,**C'**) and persists in the anterior L-AMP in stage 16 *beat-1a* mutant embryos (**D**,**D'**). Scale bars represent 10 μm.

Figure S5

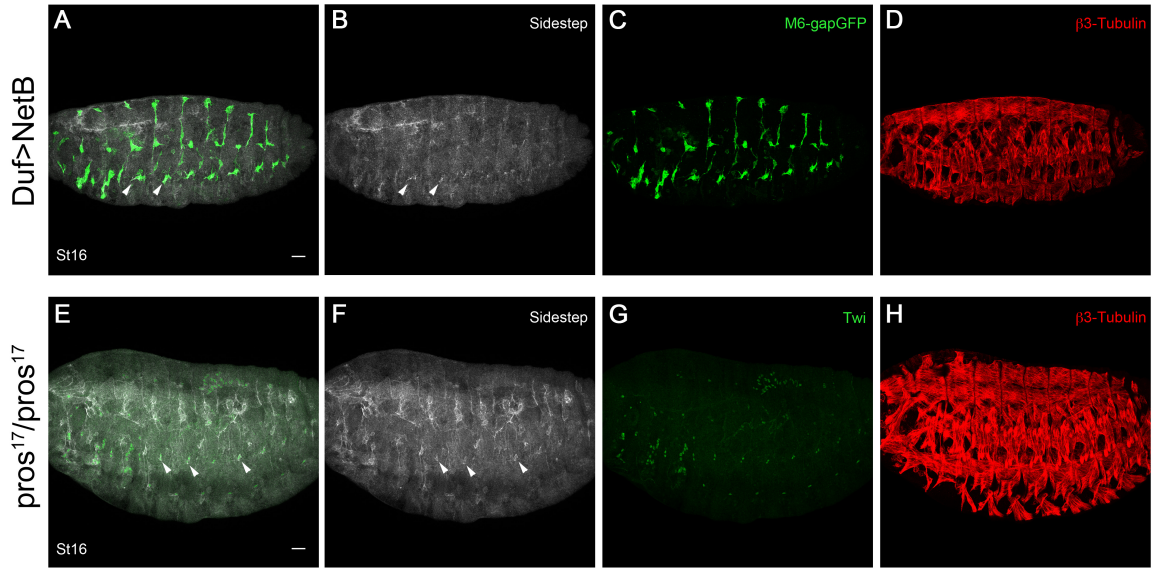


Figure S5. Sidestep expression in L-AMPs in different genetic contexts affecting the SNa. (**A**-**D**) Lateral view of stage 16 DUF>NetB embryo combined with the M6-gapGFP sensor. (**E**-**H**) Lateral view of stage 16 *prospero* mutant. In both contexts, associated with deficient formation of SNa, we observe an increase in the Sidestep staining in L-AMPs (white arrowheads). Scale bars represent 20 µm.

Figure S6

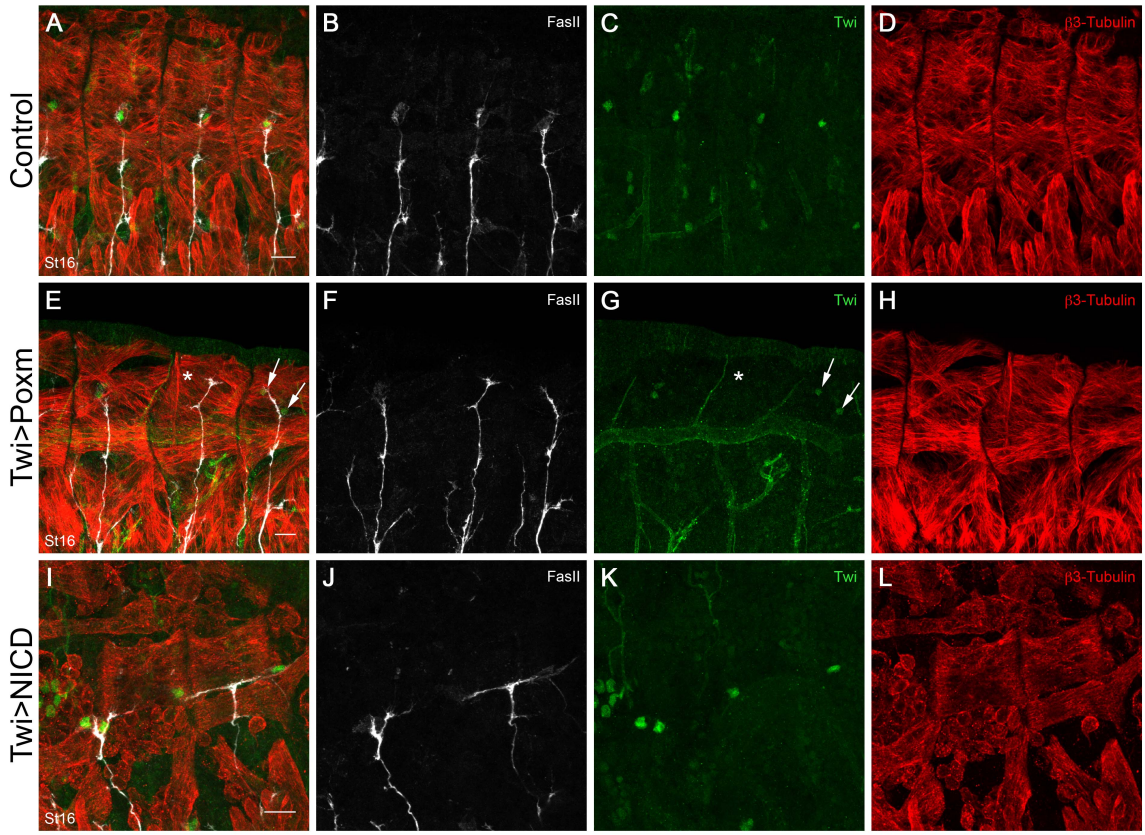


Figure S6. ISN is attracted by D-AMPs but its dorsal migration is not affected by their absence. Zoomed dorsal view of Twist-GAL4 (**A**-**D**), Twist>Pox meso (**E**-**H**) and Twist>NICD (**I**-**L**) stage 16 embryos. (**E**-**H**) The ectopic expression of Pox meso can induce either the loss of D-AMPs (white asterisk) or their duplication in a given segment (white arrows). The absence of the D-AMPs does not lead to a stall phenotype for the ISN, which can still innervate the most dorsal muscles. In the segment with duplicated D-AMPs, both retain the ability to attract the ISN. (**I**-**L**) Pan-mesodermal expression of the Notch intra-cellular domain (NICD) leads to the stochastic disruption of most of the body-wall muscles. Surviving D-AMPs are often mis-localized and are associated with altered ISN trajectory. Scale bars represent 10 μm.

Figure S7

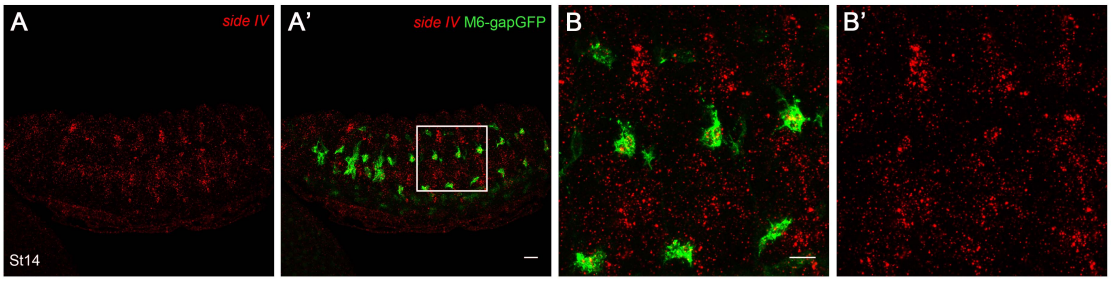
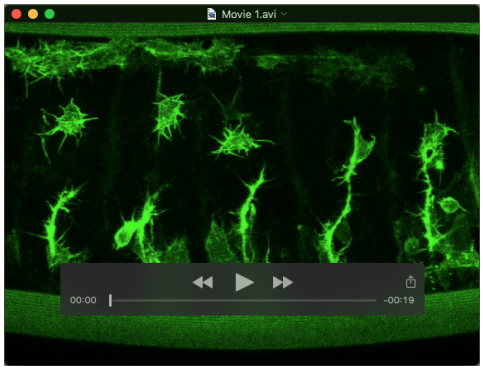


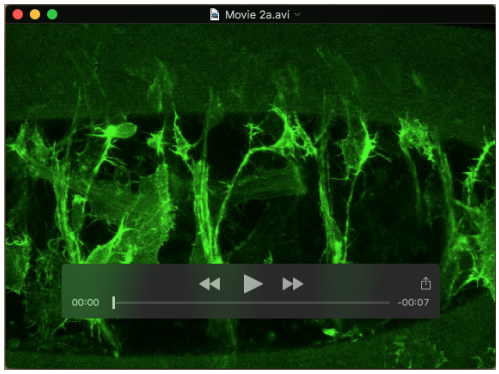
Figure S7. Embryonic *side IV* expression in L-AMPs. (**A**-**B'**) Fluorescent *in situ* hybridization (red) of *side IV* in a stage 14 M6-gapGFP embryo. The zoomed view (**B**,**B'**) highlights the enrichment of *side IV* transcripts in L-AMPs. Scale bars represent 20 μm and 10 μm for the zoomed view.

Table S1. KEY RESOURCES TABLE

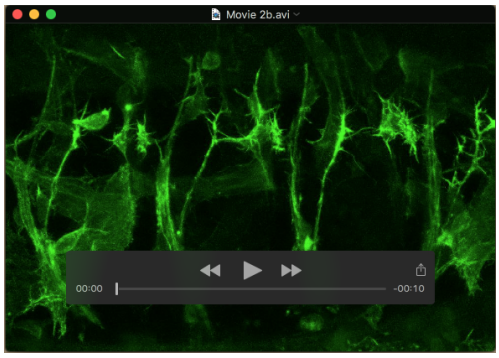
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Goat polyclonal anti-GFP	Abcam	RRID: ab5450
Mouse monoclonal anti-Sidestep	Developmental Studies Hybridoma Bank	9B8; RRID: AB_528468
Mouse monoclonal anti-Fasciclin 2	Developmental Studies Hybridoma Bank	1D4; RRID: AB_528235
Rabbit polyclonal anti-β3Tubulin	Laboratory of Renate Renkawitz-Pohl, Germany	N/A
Mouse monoclonal anti-Connectin	Developmental Studies Hybridoma Bank	C1.427; RRID: AB_10660830
Phalloidin-TRITC	Sigma-Aldrich	RRID: P1951
Guinea-pig polyclonal anti-Twist	Laboratory of Robert Zinzen, Germany	N/A
Experimental Models: Organisms/Strains		
D. melanogaster: M6-gapGFP	Laboratory of Krzysztof Jagla, France	N/A
D. melanogaster: *prosp*^{17}	Bloomington Drosophila Stock Center	RRID: BDSC_5458
D. melanogaster: *beat*^{C163}	Bloomington Drosophila Stock Center	RRID: BDSC_4742
D. melanogaster: *beat*^{3}	Bloomington Drosophila Stock Center	RRID: BDSC_4748
D. melanogaster: M6-GAL4	Laboratory of Krzysztof Jagla, France	N/A
D. melanogaster: DUF-GAL4	Laboratory of M. Ruiz Gómez, Spain	N/A
D. melanogaster: SBM-GAL4	Laboratory of A. Michelson	N/A
D. melanogaster: Twist-GAL4	Laboratory of Manfred Frasch, Germany	N/A
D. melanogaster: UAS-Numb	FlyORF	RRID: F003181
D. melanogaster: UAS-NetB	Laboratory of Thomas Kidd, USA	N/A
D. melanogaster: UAS-Reaper	Bloomington Drosophila Stock Center	RRID: BDSC_5824
D. melanogaster: UAS-LifeActGFP	Bloomington Drosophila Stock Center	RRID: BDSC_58718
D. melanogaster: UAS-Poxm	Laboratory of Markus Noll, Switzerland	N/A
Software and Algorithms		
Leica Application Suite X	Leica Application Suite X	RRID: SCR_013673
ImageJ	ImageJ	RRID: SCR_003070



Movie 1. *In vivo* imaging of M6-GAL4>UAS-LifeactinGFP stage 15 embryo. A dorso-lateral view of 4 abdominal hemisegments showing interactions of growing ISNs with D-AMPs. In reference to Fig. 1E-H.



Movie 2. *In vivo* imaging of M6-GAL4>UAS-LifeactinGFP stage 15 embryo. A ventro-lateral view of 3 abdominal hemisegments showing lateral branching of growing SNas and interactions with L-AMPs. In reference to Fig. 1I-J.



Movie 3. *In vivo* imaging of M6-GAL4>UAS-LifeactinGFP stage 15 embryo. A ventro-lateral view of 3 abdominal hemisegments showing lateral branching of growing SNas and interactions with L-AMPs. In reference to Fig. 1K-L.