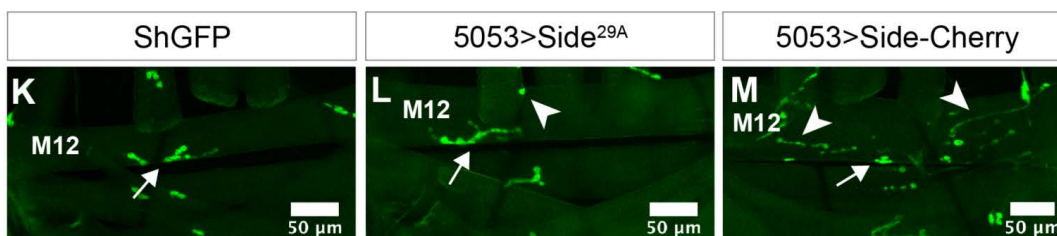
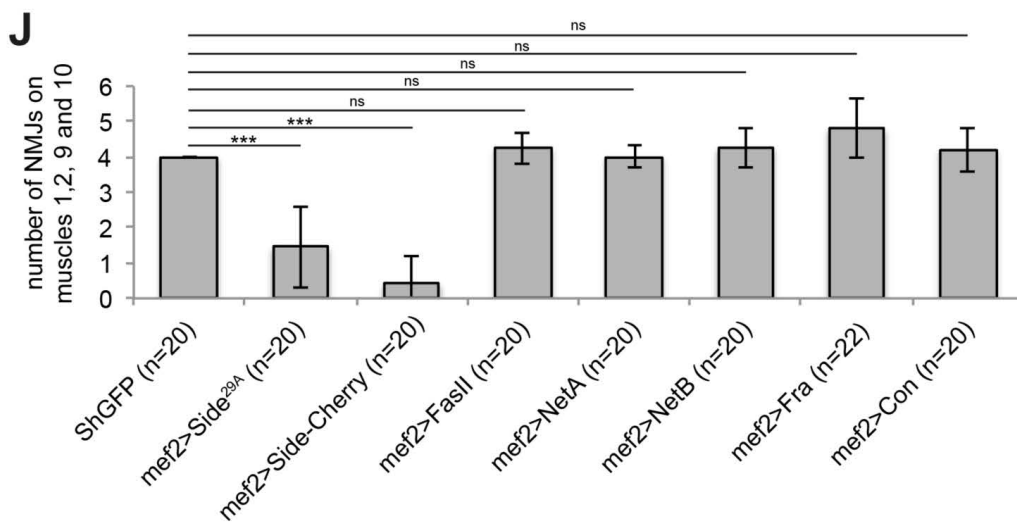
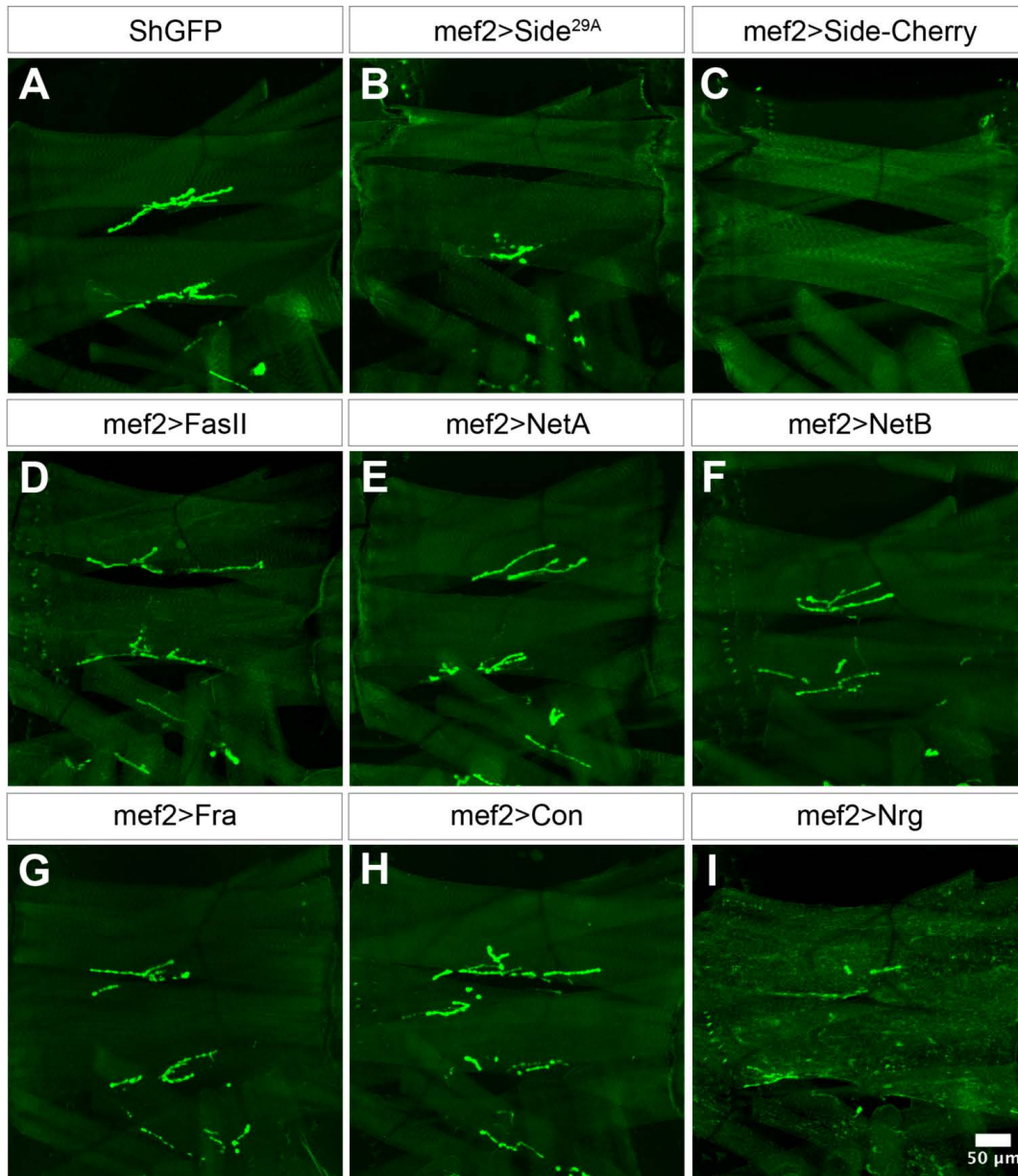


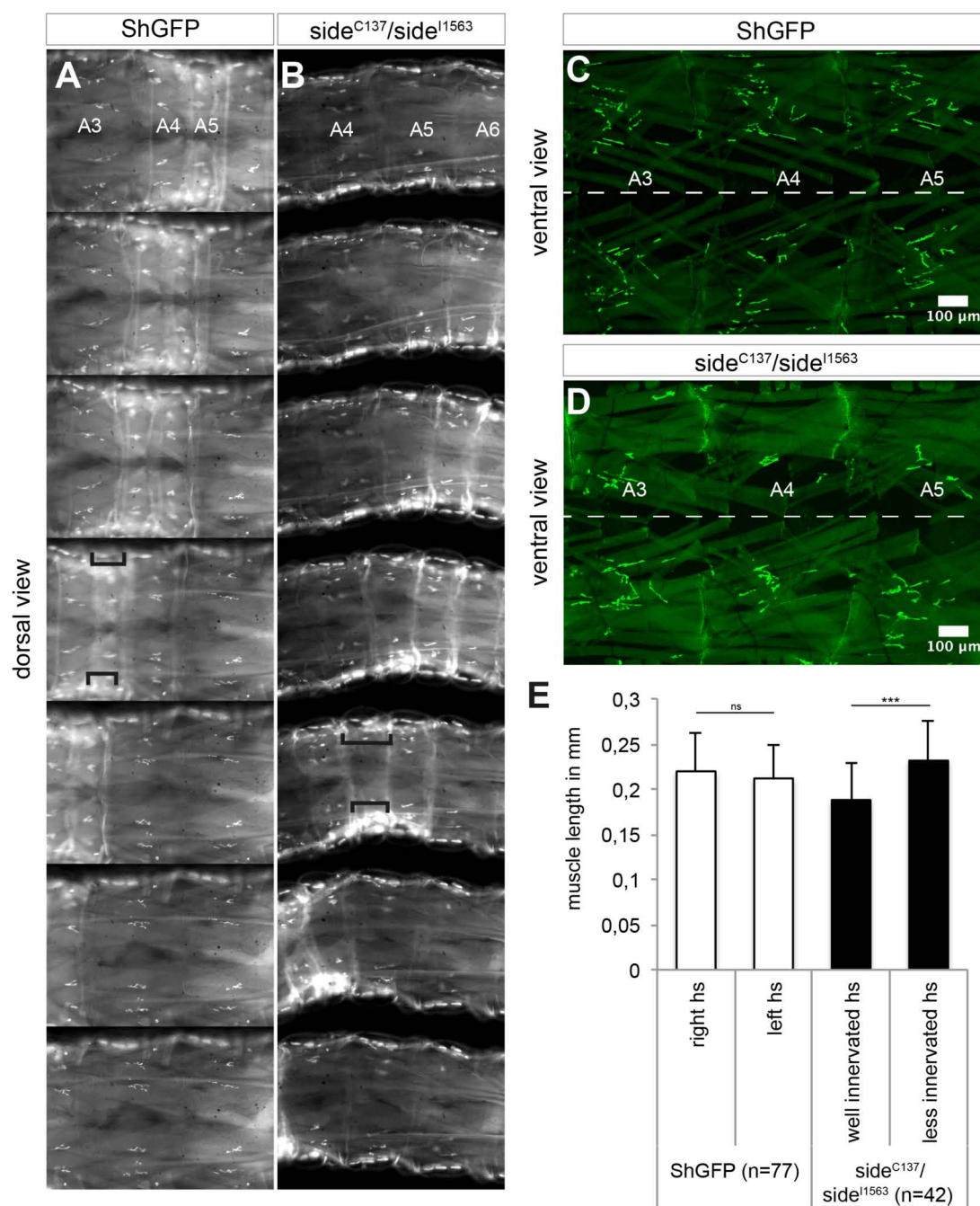
### Supplementary Fig. S1 Variations in *side* mutant wiring phenotypes.

Projections of confocal images acquired through the translucent cuticle of undissected *side* mutant larvae expressing ShGFP (green) in muscles and dsRed in motor axons (magenta). Muscles M1/9, M22 and M13 are labelled for orientation. Entire hemisegments of three different larvae are depicted. Arrows highlight innervation or non-innervation of M1/9 and M2/10 (A, D, G), M21 (B, E, H) and M13 (C, F, I). (A-C) Entire hemisegment showing predominant lack of ventral muscles (C) but lateral muscles M5, M21 and M24 are also not innervated (B). (D-F) Continuous hemisegment showing an existing NMJ on M13 but at an aberrant position (F). Miswiring is also evident at the level of lateral muscles (E). (G-I) Hemisegment showing absence of dorsal NMJs but innervation defects are also obvious in lateral (H) and ventral (I) regions. Genotypes: (A-I) w; OK371-Gal4/+; ShGFP *side*<sup>C137</sup> /ShGFP *side*<sup>I1563</sup> UAS-dsRed. Scale bars 50 $\mu$ m.



**Supplementary Fig. S2 Overexpression of Side but not of other guidance molecules in muscles cause non-innervation of dorsal muscles.**

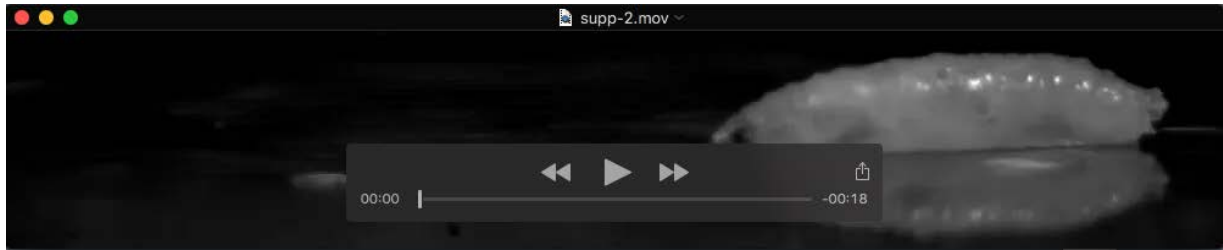
(A-I) Confocal images (projections) showing dorsal-most NMJs in intact third instar larvae overexpressing various attractants in muscles using Mef2-Gal4. FasII, FasciclinII; NetA, NetrinA; NetB, NetrinB; Fra, Frazzled; Con, Connectin; Nrg, Neuroglian<sup>180</sup>. (J) Number of NMJs on dorsal-most muscles M1/9 and 2/10 in the indicated genotypes. Only Side prevents innervation of dorsal-most muscles. Asterisks indicate  $p < 0.001$  (\*\*\*) determined by Mann-Whitney U test; ns, not significant; error bars show s.d.; n = number of hemisegments. (K-M) Muscle 12 normally carries a single NMJ apparatus at a ventral-anterior position (arrows). Overexpression of Side-Cherry, and to a lesser extent untagged Side, only in muscle 12 during embryonic development using 5053-Gal4 induces the formation of additional NMJs (arrowheads). Genotypes (K) *w*; ShGFP, (L) *w*; UAS-Side<sup>29A</sup> ShGFP/5053-Gal4, (M) *w*; UAS-Side-Cherry/+; 5053-Gal4/ShGFP. Scale bars 50 $\mu$ m.



**Supplementary Fig. S3 Maintenance of left-right symmetric muscle contraction and onset of contraction in *side* mutant larvae.**

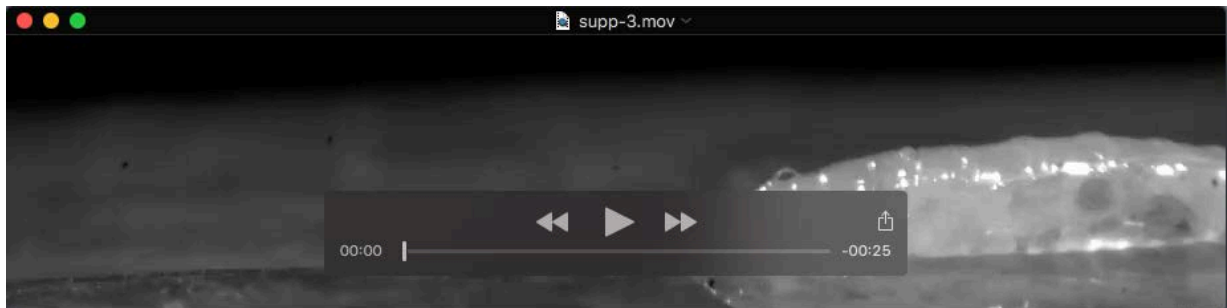
(A, B) Representative still images of ShGFP control and *side* mutant third instar larvae crawling under UV-illumination (dorsal views). Contraction waves travel from posterior (A6) to anterior (A3) and are recognizable as slightly brighter belt-like areas. Measurements were taken on the right and left sides of contracted segments, exemplified by black brackets. (C, D) Confocal images of ventral views of ShGFP

and *side* mutant larvae after the crawl. Body bending in *side* mutants was only observed when 3-4 consecutive hemisegments on one side of the body were lacking NMJs (upper 3 hemisegments in (D)). (E) Evaluation of length measurements shows that dorsal regions of left and right hemisegments in control larvae contract symmetrically during crawling. In *side* mutants, asymmetric innervation in 3-4 hemisegments in a row, induced bending towards well innervated sides due to stronger muscle contraction. Asterisks indicate  $p < 0.001$  (\*\*\*) calculated by students t-test; ns, not significant; error bars show s.d.; n = number of cycles.



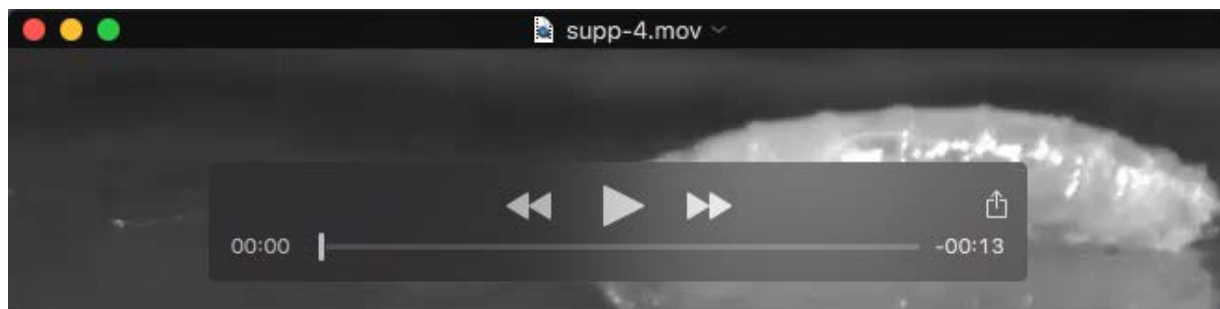
### Movie S1

ShGFP control larva crawling on a 2% agarose substrate and recorded at 125 fps using a high-speed video camera. Although limbless, the segments at the very posterior end stamp on the substrate.



### Movie S2

Transheterozygous *side* mutant larva (*side*<sup>C137</sup>/*side*<sup>I1563</sup>) crawling on a 2% agarose substrate. Loss of substrate contact causes visible "arching" phenotypes.



### Movie S3

Third instar larva overexpressing untagged Side in muscles under control of Mef2-Gal4 shows excessive head lifting and body wall "arching" phenotypes.



### Movie S4

Larvae overexpressing Side-Cherry in muscles crawl slower and show extreme head and tail lifting phenotypes as well as body wall "arching".



### Movie S5

Dorsal view of a ShGFP control larva crawling on a 2% agarose substrate (125 fps).



### Movie S6

Dorsal view of a larvae overexpressing Side-Cherry in muscles showing extreme shifts of the dorso-ventral axis to the left during the contraction wave.