Supporting Information

Chanana et al. 10.1073/pnas.0901148106

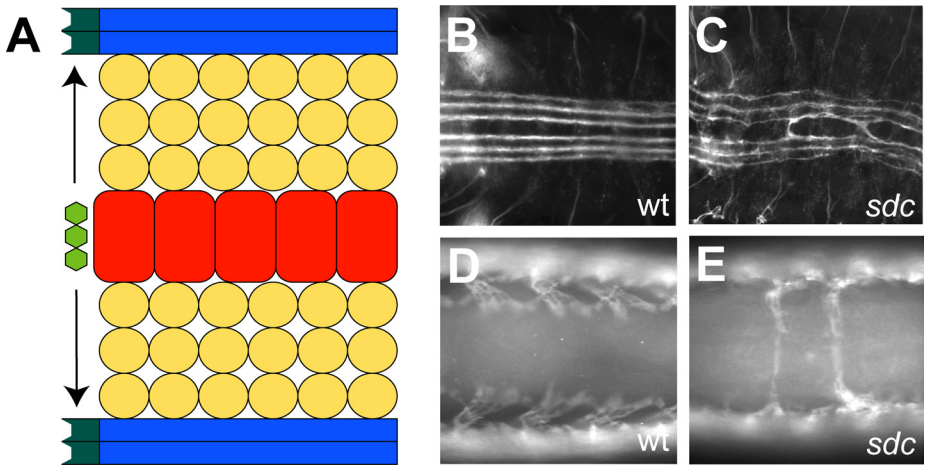

Fig. S1. The *sdc* mutants show *slit*-like axon and muscle pattern defects. (*A*) Schematic of the ventral midline. Ventral midline cells (red) secrete Slit (light green), which diffuses or is transported through the intermediate tissue (yellow) to reach the target tissue (blue), where receptor Robo (dark green) is expressed. Immunofluorescence images of stage 16 embryos stained with anti-FASII Ab (*B* and *C*) and anti-MHC Ab (*D* and *E*). (*B*) The *wt* embryos have 3 ipsilateral axon fascicles on each side of the ventral midline expressing FASII. (*C*) In *sdc* mutant embryos, the innermost axon fascicle crosses over the ventral midline in some segments. (*D*) The *wt* embryos show no muscle crossing over the ventral midline. (*E*) In an *sdc* homozygous mutant, ventral midline crossover of ventral muscles is observed. (*B–E*: ventral view; *Left*, anterior.)

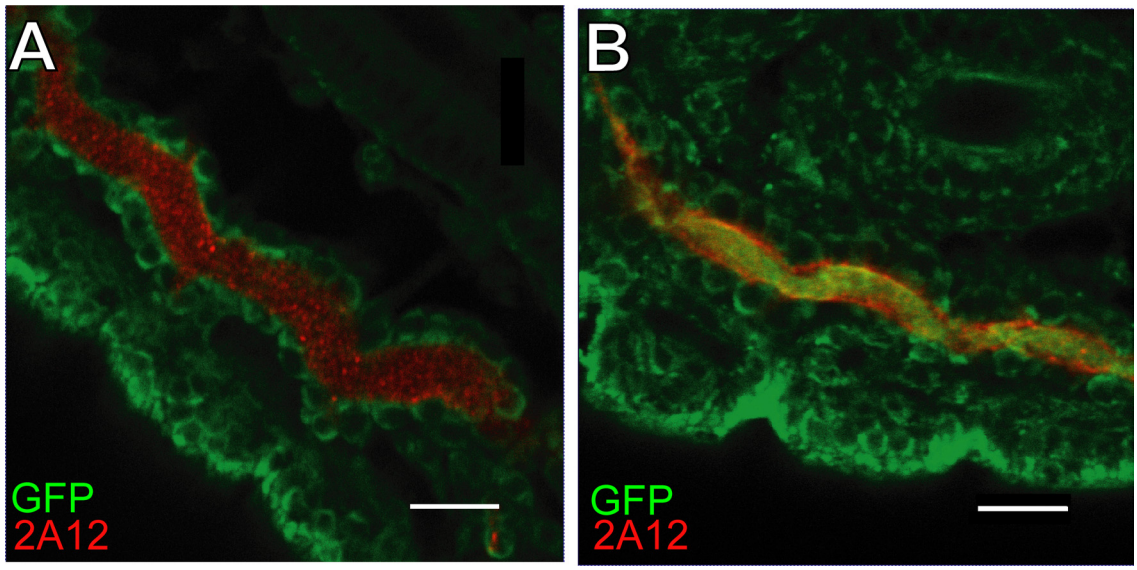

Fig. S2. Sdc-ΔTC is secreted in the tracheal lumen. Cross sections of the dorsal tracheal trunk of stage 15 embryos stained with anti-GFP Ab to detect Sdc-PA (*A*) and Sdc-ΔTC (*B*) expressed with *daG4*. The 2A12 Ab labels the tracheal lumen. Sdc-PA expressed in the tracheal system is absent from the lumen (*A*), whereas Sdc-ΔTC is secreted as seen by its colocalization with 2A12 Ab staining (*B*) (Scale bar: 10 μm.)

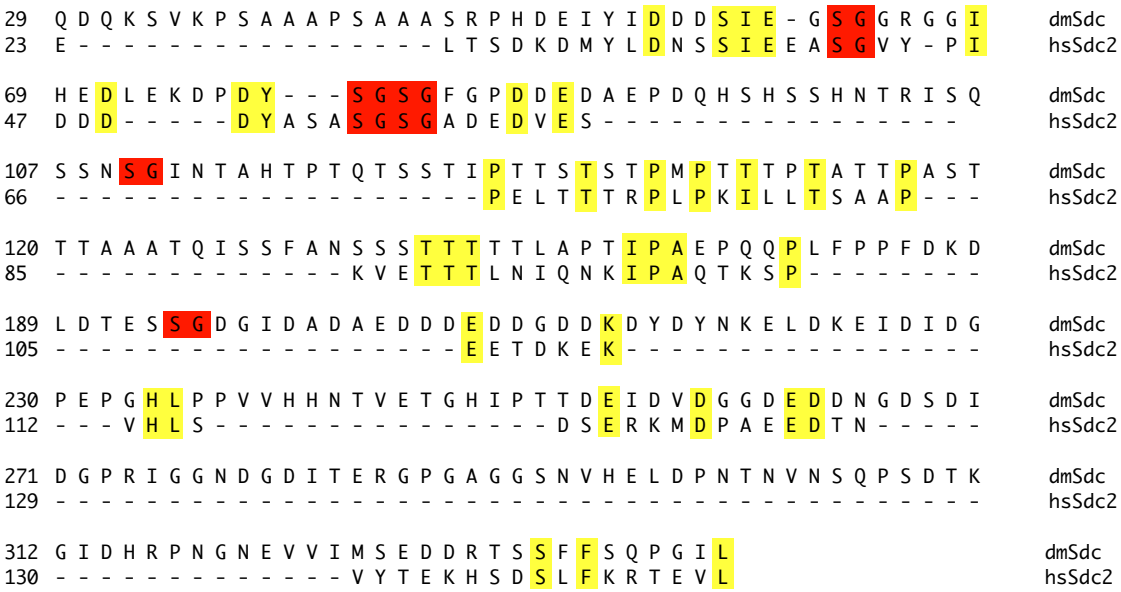

Fig. S3. Sequence comparison of the extracellular domain (without the signal peptide sequence) of *Drosophila* dmSdc and human hsSdc2. Identical amino acids are marked by yellow boxes, and the serine glycine HS attachment motifs are marked by red boxes. Alignment was performed using the ClustalW program (www.clustal.org).

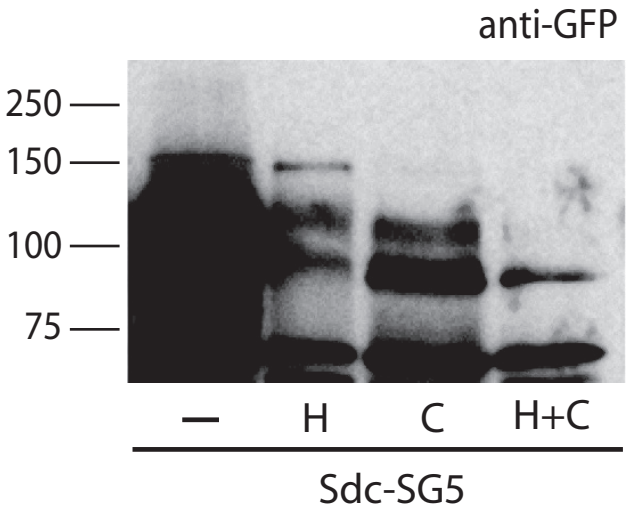

Fig. S4. Sdc-SG5 is modified by HS and CS. Extracts of *tubulinPG4*×UAS-*sdc-SG5* embryos were mock treated (—), treated with heparinase (H), treated with chondroitinase (C), or treated with both heparinase and chondroitinase (H+C). Sdc-SG5 was detected with anti-GFP Ab. Note that treatment with heparinase dramatically reduces protein stability but also reduces smearing tendency, indicating that Sdc-SG5 is still modified by HS in addition to being modified by CS , although all HS attachment sites are mutated.

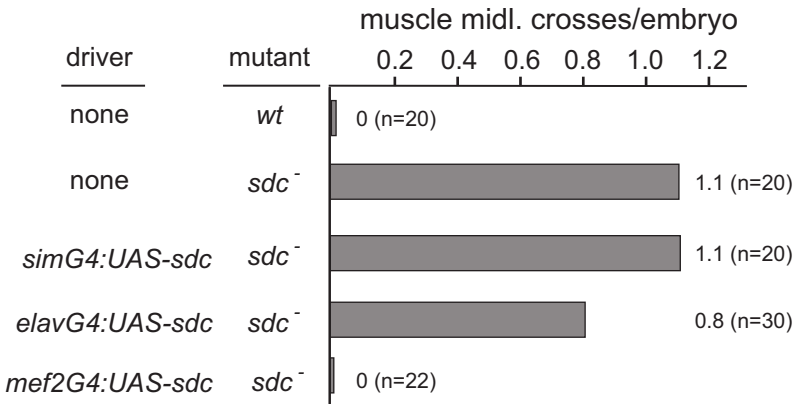

Fig. S5. Sdc is required exclusively on the target tissue. Number of ectopic ventral midline crossovers of muscles per embryo stained with α-MHC Ab in *wt*, *sdc*, and *sdc* homozygous mutants rescued with *UAS-sdc-PA* expressed in Slit-secreting midline cells (*simG4*), intermediate neuronal tissue (*elavG4*), or muscle target tissue (*mef2G4*). *n*, number of embryos analyzed.