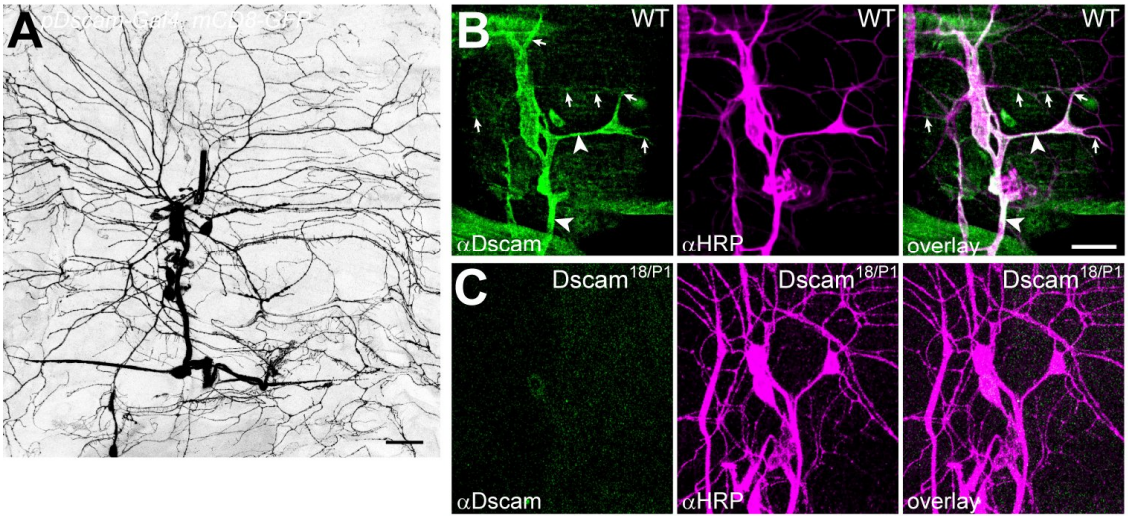
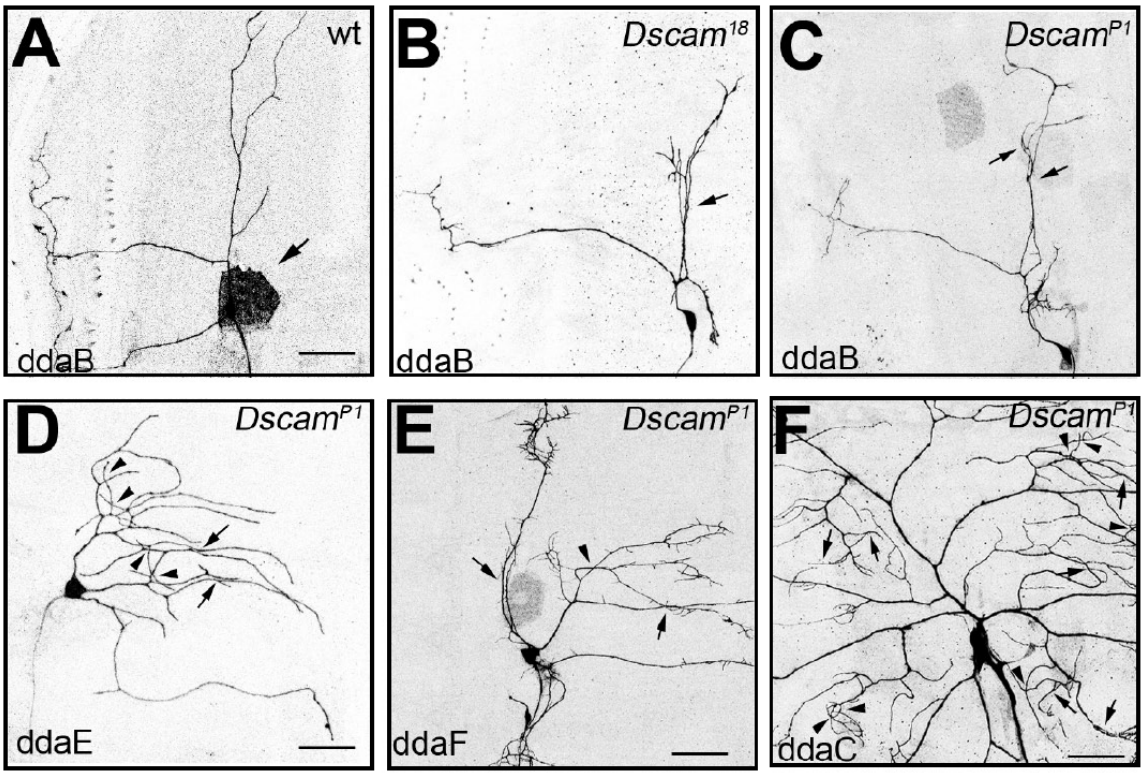
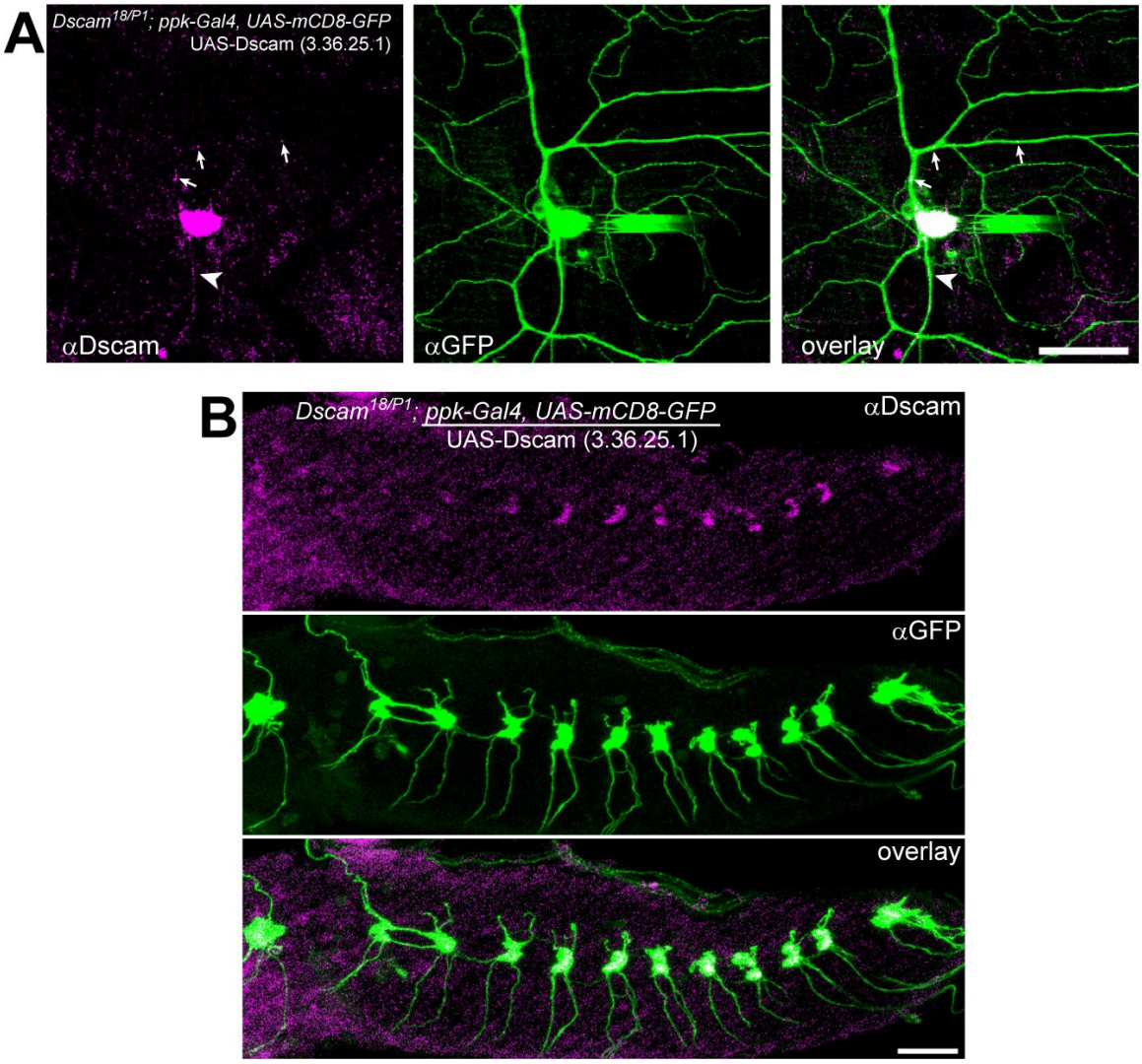


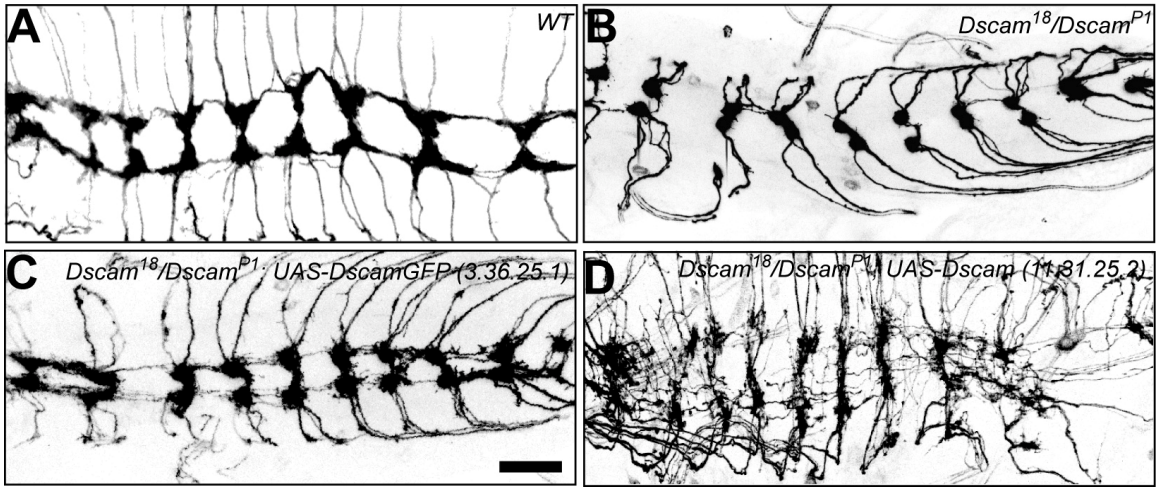
Supplemental Figure 1: Dscam is expressed in all da neurons
(A) A 4.5 kb Dcam promoter fragment (*pDscam-Gal4*) drives expression of *UAS-mCD8-GFP* in all da neurons. The dorsal da neuron cluster of a third instar larva is shown. Dorsal is up and anterior to the left. Scale bar, 30 mm. (B) Anti-Dscam and anti-HRP antibody staining of wild type early third instar larva showing the endogenous expression pattern of Dscam in all da neurons of the dorsal cluster. Dscam is detected in both axons (arrowheads) and dendrites (arrows) of da neurons (C) Anti-Dscam and anti-HRP antibody staining of $Dscam^{18}$/$Dscam^{P1}$ early third instar larva showing no detectable Dscam expression. Scale bars, 20 mm.



Supplemental Figure 2: MARCM analysis of *Dscam* mutant da neurons. (A-C) Class II ddaB da neuron MARCM clones of (A) *wild type* (arrow indicates epithelial cell clone overlapping with the da neuron cell body), (B) *Dscam*^{18} *and (C) Dscam*^{P1} third instar larva. Arrows indicate dendritic branch bundling. Scale bar equals 30 µm. (D-F) MARCM clones of *Dscam*^{P1} mutant (D) class I ddaE, (E) class III ddaF, and (F) class IV ddaC da neurons in third instar larva. Arrows indicate branch bundling, arrowheads indicate branch crosses. These experiments demonstrate that the *Dscam*^{P1} mutant allele leads to comparable phenotypes as *Dscam*^{18}. Scale bar equals 30 µm.

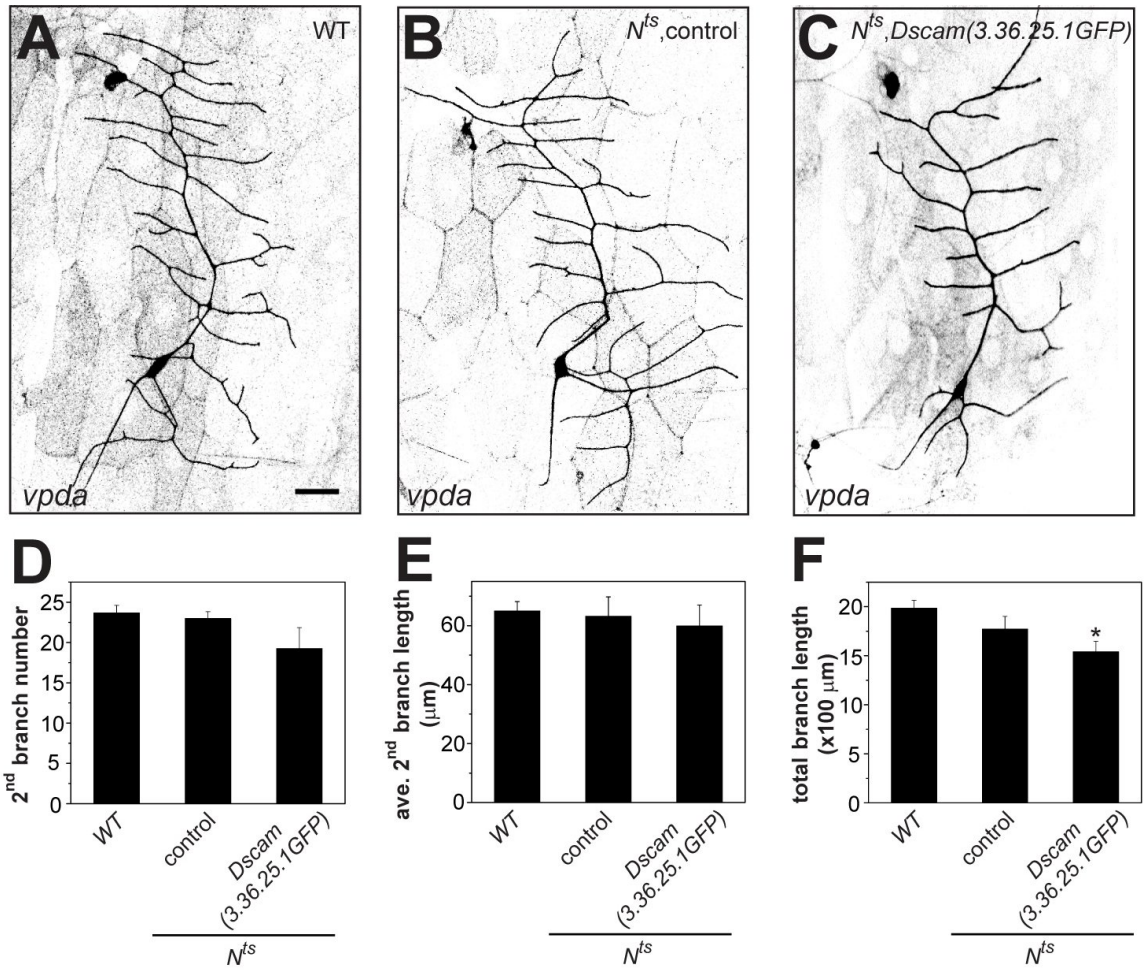


Supplemental Figure 3: Dscam TM1 isoforms localize to axons and dendrites of class IV da neurons.
Non-tagged *UAS-Dscam (3.36.25.1)* was expressed in *Dscam*$^{18/P1}$ mutant animals in class IV da neurons using *ppk-Gal4, UAS-CD8-GFP*. The distribution pattern is similar to Dscam TM1 isoforms carrying a C-terminal GFP-tag (see Figure 4). (A) Anti-Dscam and anti-GFP antibody staining of a class IV ddaC neuron is shown. The untagged TM1 isoform is mainly localized to the cell body and punctate structures in both, axons (arrowheads) and dendrites (arrows). Dorsal is up and anterior to the left. Scale bar equals 30 mm. (B) Anti-Dscam and anti-GFP antibody staining of the VNC showing the presence of Dscam (3.36.25.1) in axon terminals of class IV da neurons. Scale bars, 30 mm.

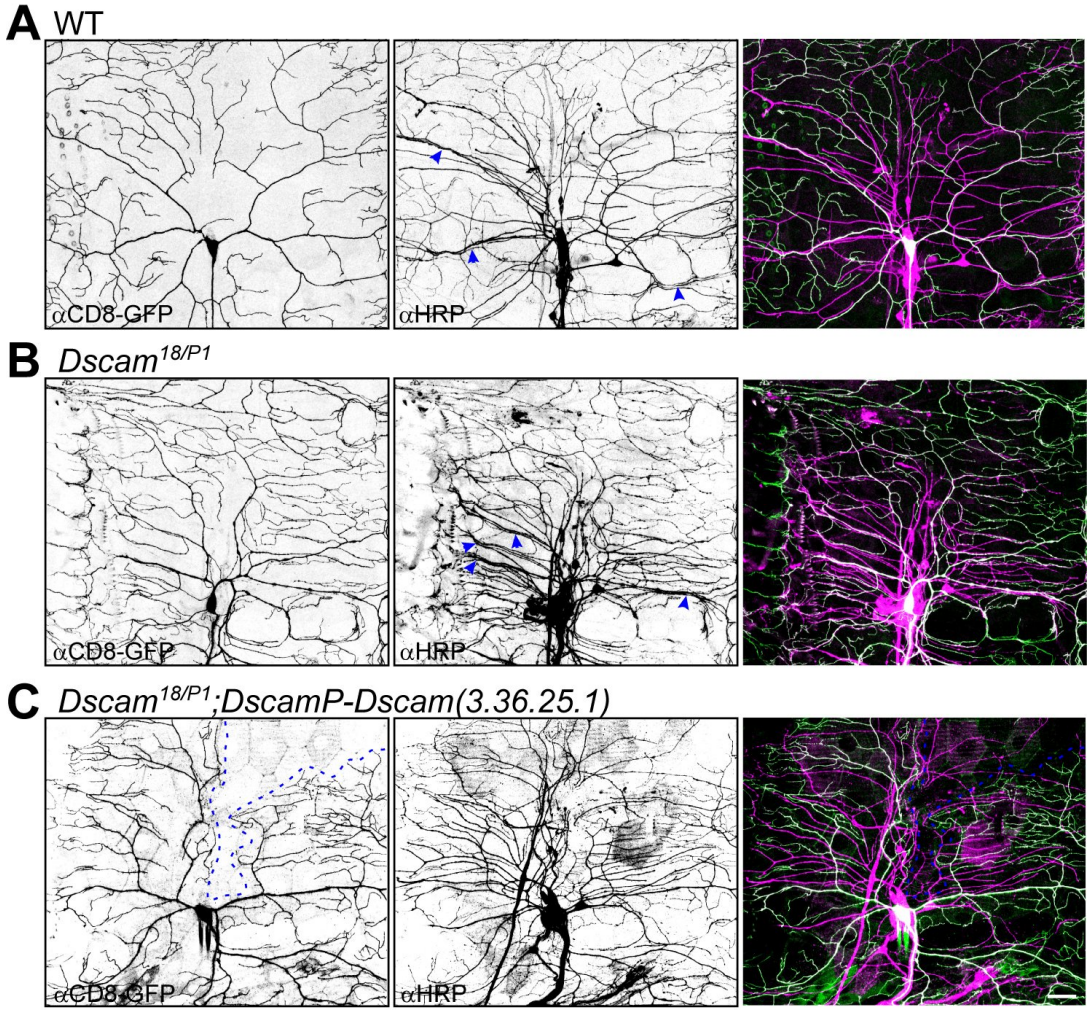


Supplemental Figure 4: Dscam is required for the proper axonal projection pattern of class IV da neurons

(A-C) Class IV da neuron axon projections in the VNC visualized in third instar larva using *ppk-Gal4, UAS-mCD8-GFP*. Wild type (A), *Dscam^{18}/Dscam^{P1}* (B), *Dscam^{18}/Dscam^{P1}; ppk-Gal4/UAS-Dscam (3.36.25.1GFP)* (C), and *Dscam^{18}/Dscam^{P1}; ppk-Gal4/UAS-Dscam (11.31.25.2)* (D). Class IV da neurons show a ladder-like pattern of commissural and longitudinal axonal projections in the wild type larval VNC (A). In *Dscam^{18}/Dscam^{P1}* mutant larvae, the longitudinal axonal projections were largely absent (B). Longitudinal defect of axonal projections were partially restored by the expression of either TM1 or TM2 single Dscam isoforms (C, D). However, the overall shape and laminar pattern of the longitudinal axon projections did not resemble wild type axon projections. These results indicate that expression of a single Dscam isoform can partially restore class IV da neurons axonal projections along the longitudinal tracks, but is not sufficient for proper lamination and targeting. Scale bar, 30 mm.



Supplemental Figure 5: Transient N^{ts} inactivation and duplication of class I vpda neurons does not affect dendrite development.
(A-C) Non-duplicated class I vpda neuronsvisualized in third instar larva using *Gal42-21*, *UAS-mCD8-GFP*. *Wild type* (A), N^{ts}, control (B) , N^{ts}, *UAS-Dscam(3.36.25.1GFP)* (C)*. Animals shown in (B) and (C) had duplicated vpda neurons in other segments.* (D-F) Quantitative analysis of the three genotpyes for secondary branch number (D), average secondary branch length (E), and total branch length (F). Note that transient *Notch* inactivation to duplicate vpda class I neurons does not affect branch number and length, while Dscam overexpression caused a slight reduction in secondary branch number (n.s.) and overall branch length compared to the control (*, $p<0.05$, n=5 for each group). Scale bar, 30 μm.



Supplemental Figure 6: Avoidance of dendrites expressing only a single Dscam isoform.

(A-C) Comparison of wild type (A), Dscam18/DscamP1 mutant (B), and *Dscam*^{18}*/Dscam*^{P1}*; DscamP-Dscam(3.36.25.1)* (C) third instar larvae expressing *UAS-mCD8-GFP* under the control of *ppk-Gal4* and immunostained with anti-CD8/GFP and anti-HRP antibodies to visualize class IV da neurons and all PNS neurons, respectively. The dorsal da neuron cluster is shown. Note that the class IV ddaC neuron evenly covers its entire field in wild type. Additionally, a subset of primary dendrites from different da neurons fasciculate while using the same growth path (A, arrows). *Dscam* mutant ddaC neurons showed self-avoidance defects as described before. Additionally, the entire da neuron cluster exhibits strongly fasciculated dendrites (B, arrows). In contrast, expression of a single isoform under the control of the Dscam promoter in the *Dscam* mutant background resulted in uneven and incomplete field coverage of the ddaC dendrites (C, indicated by dashed line). Although self-avoidance defects are reduced in all da neurons, expression of the common Dscam isoform leads to recognition and separation of the majority of dendritic processes. Thereby, in severe cases, ddaC dendrites are unable to target the dorsal field due to repulsion by other da neuron dendrites. Scale bar, 30 mm.