Supplemental Information

Dscam Expression Levels Determine Presynaptic Arbor Sizes in *Drosophila* Sensory Neurons

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Inventory of Supplemental Information

Supplemental Data

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related to Figure 1 and Figure 3.

Figure S2.
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Supplemental Figure Legends

Supplemental References

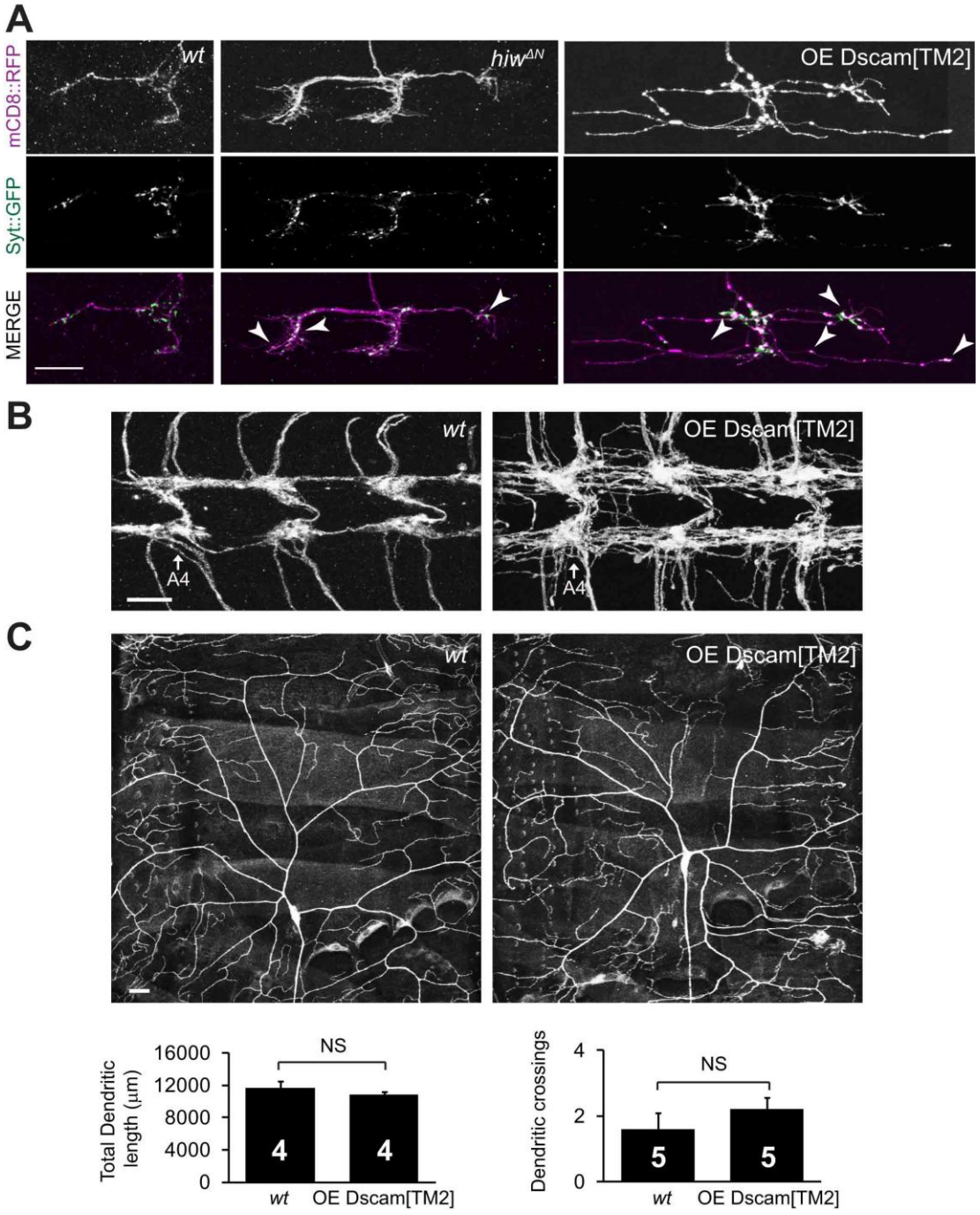

Figure S1. Hiw and Dscam regulate presynaptic arbor growth in C4da neurons. (Related to Figure 1 and Figure 3)

(A) Presynaptic arbors of single ddaC neurons labeled with the presynaptic marker synaptotagmin::GFP (Syt::GFP). The flip-out technique, which employs an excisable GAL80 (Gordon and Scott, 2009), was used to express the membrane marker mCD8::mRFP (magenta) and the presynaptic marker Syt::GFP (green) in *wild-type* (*wt*), *hiw* mutant (*hiw*$^{\Delta N}$), and Dscam[TM2]-overexpressing C4da neurons (OE Dscam[TM2]). Arrowheads point to ectopic synaptic regions formed in overgrown axon terminals. Scale bar: 10 μm. (B-C) Overexpression

of Dscam[TM2] does not change dendritic growth in C4da neurons. A *Dscam[TM2]* transgene was expressed using the *ppk-Gal4* driver. Axonal (B) and dendritic arbors (C) were visualized with mCD8::GFP. Quantification of total dendritic length and dendritic crossings are shown in the bottom panels. Scale bar: 10 μm. Sample numbers are shown in the bars.

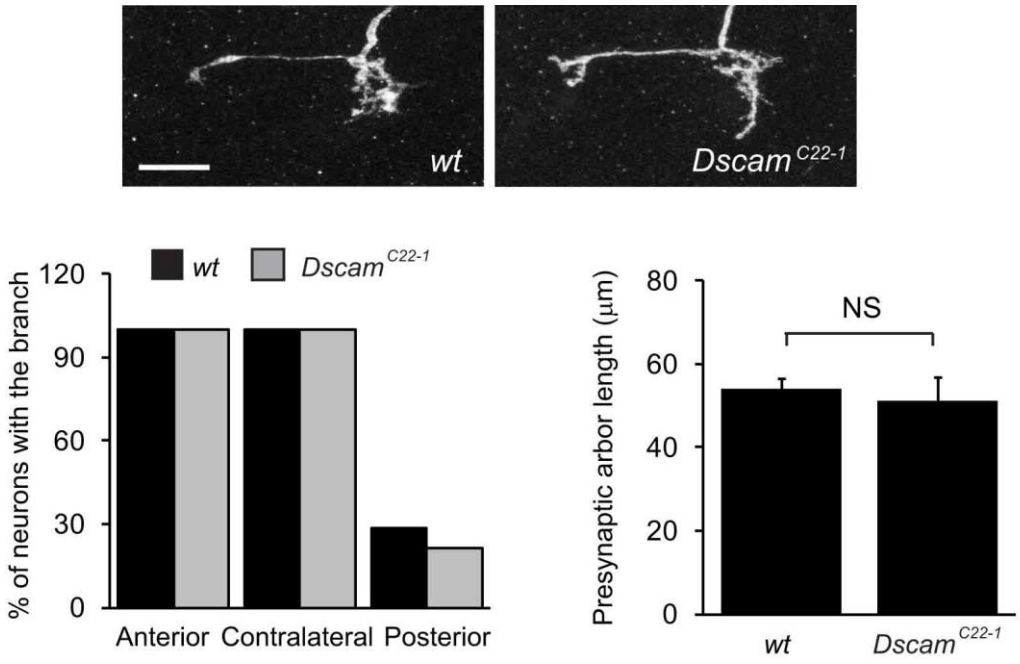

Figure S2. Reducing the diversity of Dscam ectodomain does not affect either the targeting or the growth of C4da presynaptic arbors. (Related to Figure 2)

Representative images and quantification of single C4da neuron presynaptic arbors that are *wild-type* (*wt*), or homozygous for a *Dscam* allele with reduced diversity (*Dscam*$^{C22\text{-}1}$). *Dscam*$^{C22\text{-}1}$ contains a partial deletion of the exon 4 cluster, resulting in 75% reduction of alternative exon 4 (Wang et al., 2004). Scale bar: 10 μm. *wt*, n = 14; *Dscam*$^{C22\text{-}1}$, n = 14.

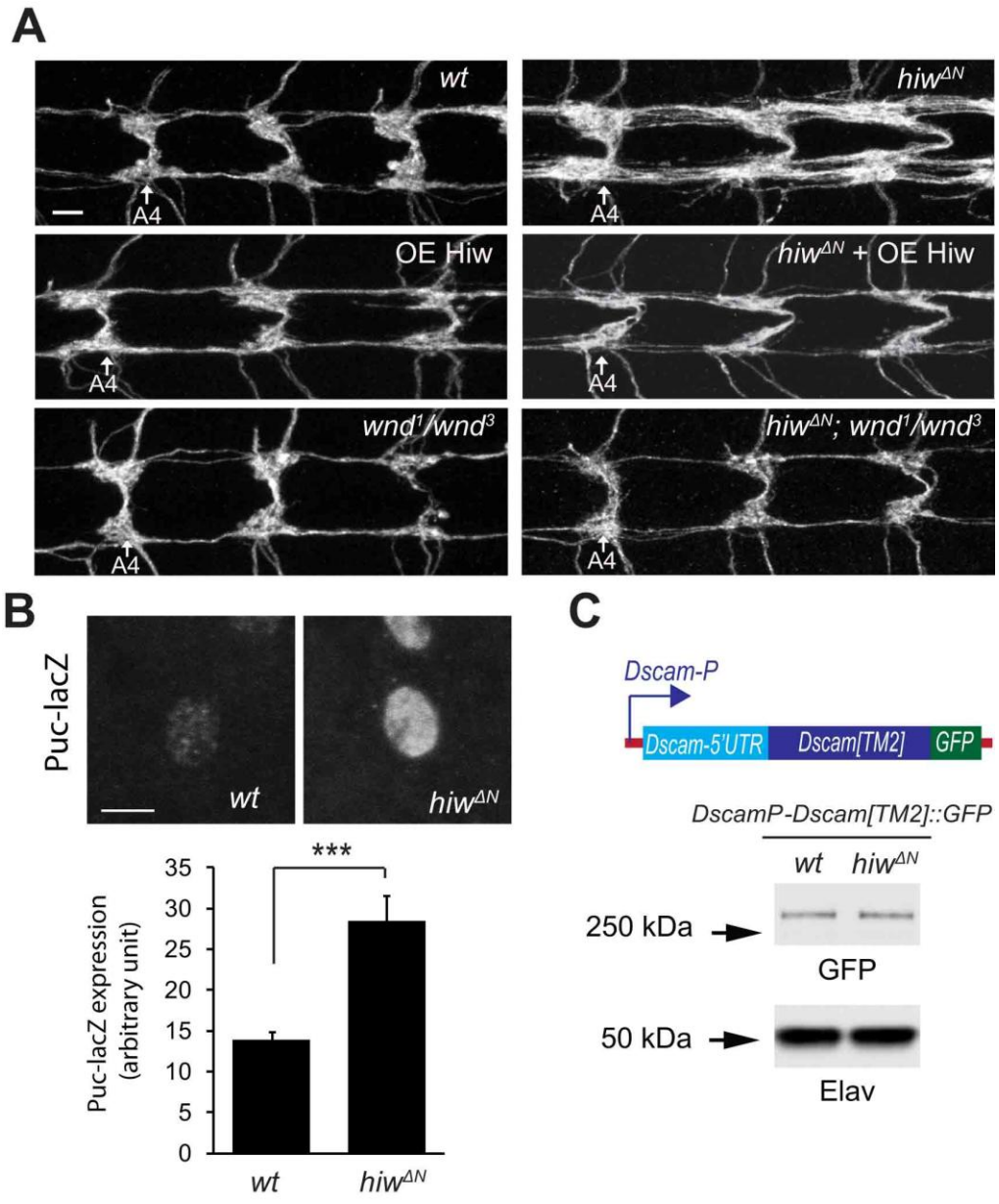

Figure S3. Hiw-Wnd pathway functions in C4da neurons and does not regulate *Dscam* promoter activity. (Related to Figure 3)

(A) Presynaptic arbors of larval C4da neurons visualized with CD4::tdTomato driven by the *ppk* enhancer. C4da neurons in *hiw*$^{\Delta N}$ larvae display extensive overgrowth of presynaptic arbors, which is completely rescued by overexpressing Hiw (*hiw*$^{\Delta N}$ + OE Hiw) in C4da neurons and by *wnd* mutations (*hiw*$^{\Delta N}$; *wnd*^{1}/*wnd*^{3}). Hiw overexpression or a *wnd* mutant background (*wnd*^{1}/*wnd*^{3}) does not cause significant changes in C4da presynaptic arbor growth. C4da presynaptic arbors in abdominal segments 4 (A4) through 6 are shown. Scale bar: 5 μm. (B) *hiw* mutations upregulate Wnd signaling in C4da neurons. The expression levels of *Puckered*-lacZ, a reporter of Wnd activity (Xiong et al., 2010), were elevated two fold in *hiw* mutant C4da nuclei (n = 11), as compared to wild-type (n = 10). (C) *Hiw* mutations do not affect *Dscam* promoter

activity. Dscam[TM2]::GFP is expressed under the control of the *Dscam* promoter (*Dscam-P*) and *Dscam* 5'UTR in *wt* and $hiw^{\Delta N}$ larvae. Dscam[TM2]::GFP expression in larval brains was examined by Western blot analysis using an anti-GFP antibody and normalized to Elav (wild-type: 1.00 ± 0.08, n = 3; $hiw^{\Delta N}$: 1.21 ± 0.14, n = 3; mean ± SEM; p = 0.18, paired t-test).

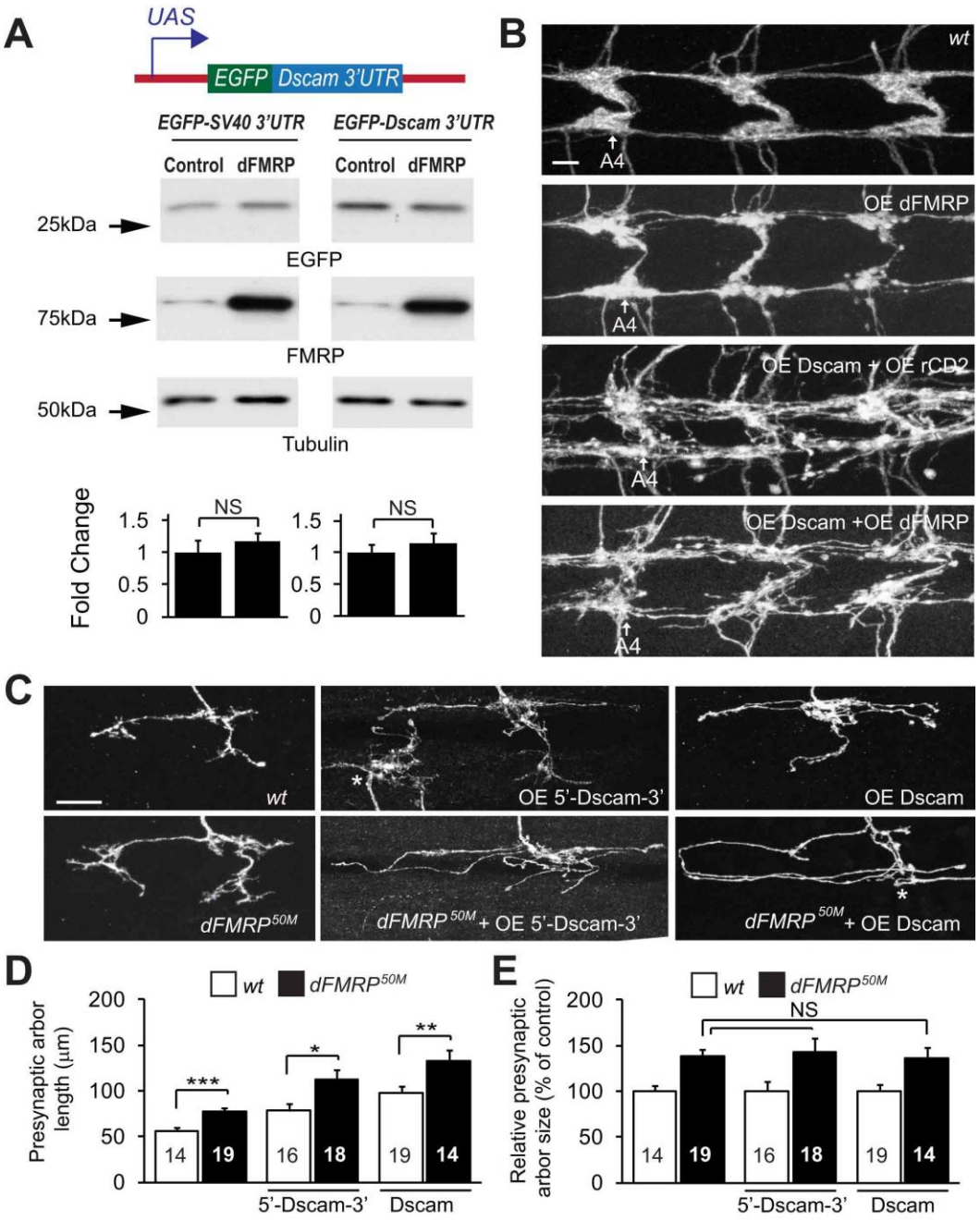

Figure S4. FMRP regulates Dscam expression through the coding region of *Dscam* mRNA. (Related to Figure 6)

(A) FMRP does not regulate Dscam expression through the *Dscam* 3'UTR. Western blots of lysates of cultured S2 cells expressing EGFP reporters SV40 3'UTR or *Dscam* 3'UTR along with dFMRP cDNA (dFMRP) or an empty vector (Control). Bar chart: quantification of the expression levels of EGFP reporter. EGFP expression levels were normalized to tubulin levels and presented as fold change for statistical analysis (n = 4). (B) FMRP is sufficient to suppress presynaptic arbor overgrowth caused by Dscam-overexpression. Shown are presynaptic arbors of larval C4da neurons labeled with *ppk*-CD4::tdTomato in C4da neurons that are *wild-type* (*wt*),

overexpressing dFMRP (OE dFMRP), overexpressing a *Dscam* transgene that does not contain *Dscam* 5' and 3'UTRs along with the membrane protein rat CD2 (OE Dscam + OE rCD2), and overexpressing the same Dscam transgene along with dFMRP (OE Dscam + OE dFMRP). C4da presynaptic arbors in abdominal segments 4 (A4) through 6 are shown. Scale bar: 5 μm. (C) FMRP is required to restrain presynaptic arbor overgrowth caused by Dscam-overexpression. Shown are presynaptic arbors of single ddaC MARCM clones. To avoid possible saturation of presynaptic arbor growth by Dscam-overexpression, we chose UAS-Dscam and UAS-5'-Dscam-3' transgenes that express Dscam at a lower level than those used in Figure 1B and 2D. These two transgenes are located in the same genomic locus. Scale bar: 5 μm. (D) Quantification of presynaptic arbor length. Sample numbers are shown in the bars. (E) Quantification of relative presynaptic arbor size. Relative presynaptic arbor sizes were obtained by normalizing values from *dFMRP* mutant clones to the average values of corresponding of wild-type clones (*dFMRP*^{50M} vs *wt*; *dFMRP*^{50M} + OE 5'-Dscam-3' vs OE 5'-Dscam-3'; *dFMRP*^{50M} + OE Dscam vs OE Dscam). Sample numbers are shown in the bars.

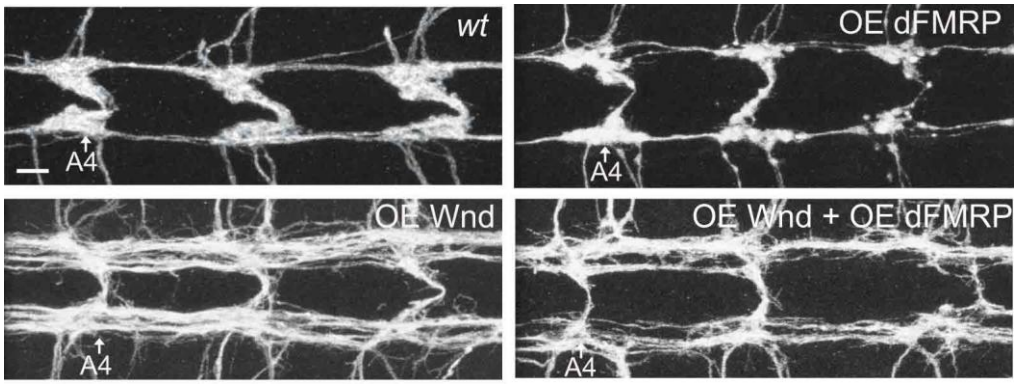

Figure S5. Wnd and FMRP converge to regulate presynaptic arbor growth. (Related to Figure 7)

Wnd was expressed using the driver *ppk-Gal4* with either the control transgene rCD2 (OE Wnd) or dFMRP (OE Wnd + OE dFMRP). Note that dFMRP suppresses extensive overgrowth of C4da presynaptic arbors caused by Wnd overexpression (OE Wnd). C4da presynaptic arbors in abdominal segments 4 (A4) through 6 are shown. Scale bar: 5 μm.

Supplemental References

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