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Figures and figure supplements

Dysregulated Dscam levels act through Abelson tyrosine kinase to enlarge presynaptic arbors

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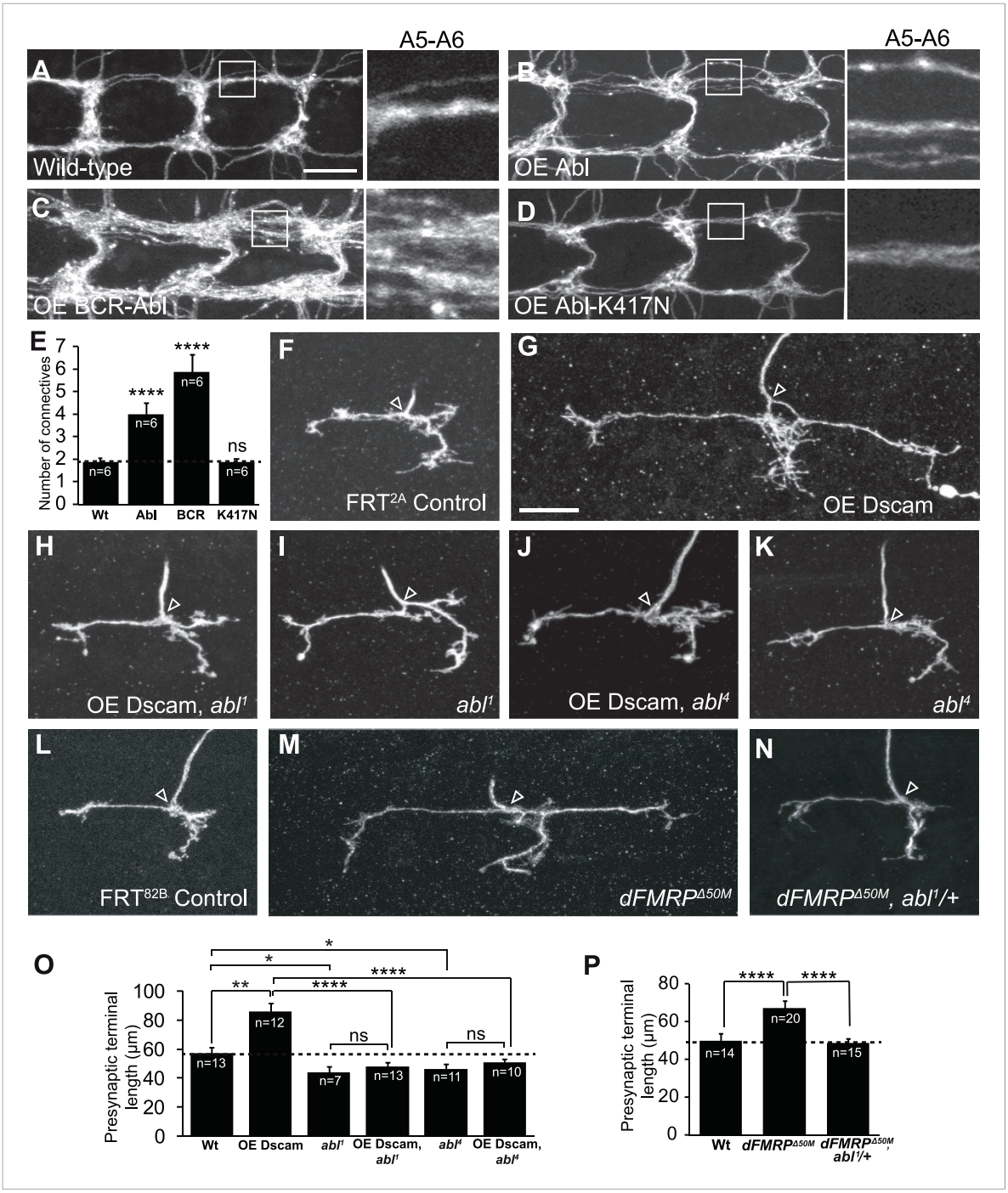

Figure 1. Dscam requires Abl to promote presynaptic terminal growth. (**A**–**E**) Abl is sufficient to cause presynaptic terminal overgrowth in C4da neurons. Transgenes were expressed with a C4da neuron-specific Gal4 driver, *ppk*-Gal4, and presynaptic terminals were visualized with a membrane monomeric RFP (mCD8-mRFP) transgene. Overexpression of Abl (**B**) leads to a modest increase in presynaptic terminal growth as compared to control (**A**). Overexpression of the constitutively active BCR-Abl (**C**) leads to robustly increased presynaptic terminal growth, while overexpression of kinase-dead Abl-K417N (**D**) is indistinguishable from control. Quantification of the number of axon connectives is shown in (**E**). Scale bar is 10 µm. (**F**–**K**) Abl is required in C4da neurons for Dscam to instruct presynaptic terminal growth. The arrowhead in each panel points to the location where an axon elaborates the presynaptic terminal arbor. The MARCM technique was used to generate and visualize single mutant C4da neurons. While overexpression of Dscam::GFP (**G**) in single C4da presynaptic terminals leads to increased length when compared to control (**F**), overexpression of Dscam in *abl*^{1} mutant neurons (**H**) leads to presynaptic terminal lengths that are indistinguishable from *abl*^{1} mutant neurons (**I**). Similarly, overexpression of Dscam in *abl*^{4} mutant neurons (**J**) does not significantly change presynaptic terminal length when compared to *abl*^{4} mutant neurons (**K**). (**L**–**N**) Abl is required to instruct presynaptic terminal growth in *dFMRP* mutants. (**M**) Loss of *dFMRP* leads to increased presynaptic terminal growth, which has previously been shown to require

Figure 1. continued on next page

Figure 1. Continued

Dscam. Loss of one copy of *abl* in *dFMRP*$^{\Delta 50M}$ mutant neurons (**N**) leads to presynaptic terminal lengths that are indistinguishable from control (**L**). Scale bar is 10 μm. (**O** and **P**) Quantification of the presynaptic terminal length in C4da neurons of indicated genotypes. Sample number is shown in white within each bar.

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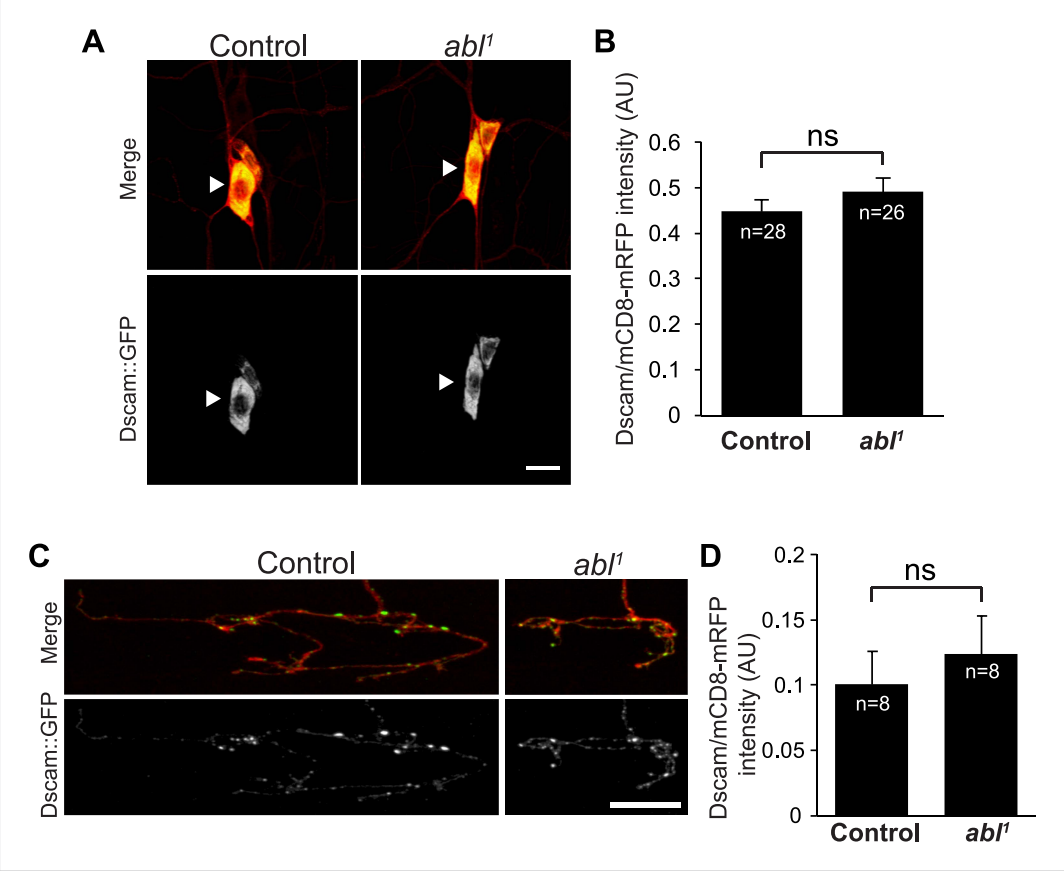

Figure 1—figure supplement 1. Loss of *abl* does not affect Dscam::GFP expression level. (**A** and **B**) Loss of *abl* does not affect Dscam::GFP expression in C4da cell bodies. (**A**) Example images of C4da neuron cell bodies (white arrowheads) in control (left) or *abl*^{1} homozygous mutant (right) animals. Upper images show merged signals of mCD8::mRFP and Dscam::GFP, while lower images show Dscam::GFP alone. Scale bar is 10 μm. (**B**) Quantification of the relative intensity of Dscam::GFP fluorescence normalized to mCD8::mRFP. Sample number is shown inside each bar. (**C** and **D**) Loss of *abl* does not affect Dscam::GFP expression in C4da presynaptic terminals. The MARCM technique was used to generate and visualize single mutant C4da neurons. (**C**) Example images of C4da presynaptic terminals in control (left) and *abl*^{1} mutant clones. Upper images show merged signals of mCD8::mRFP and Dscam::GFP, lower images show Dscam::GFP alone. Scale bar is 10 μm. (**D**) Quantification of the relative intensity of Dscam::GFP normalized to mCD8::mRFP. Sample number is shown inside each bar.

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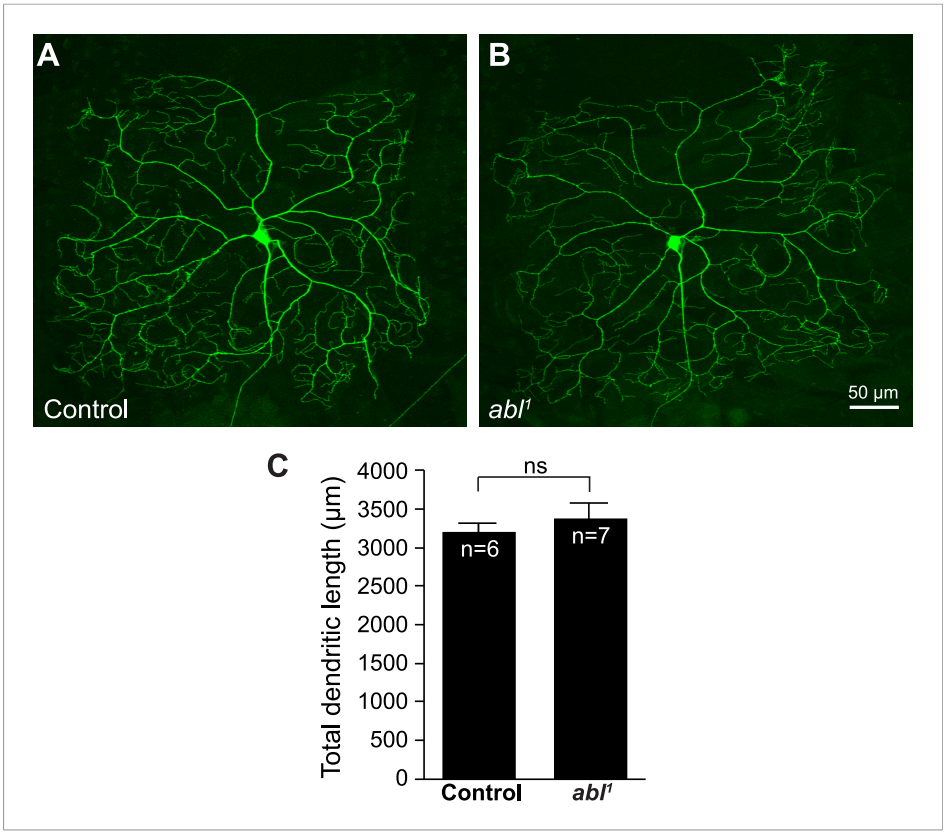

Figure 1—figure supplement 2. Loss of *abl* does not affect C4da dendritic length or morphology. Representative images of control (**A**) and *abl*^{1} mutant C4da neuron clones (**B**). The average total dendritic length is not significantly different between these two conditions (**C**). Scale bar is 50 µm.

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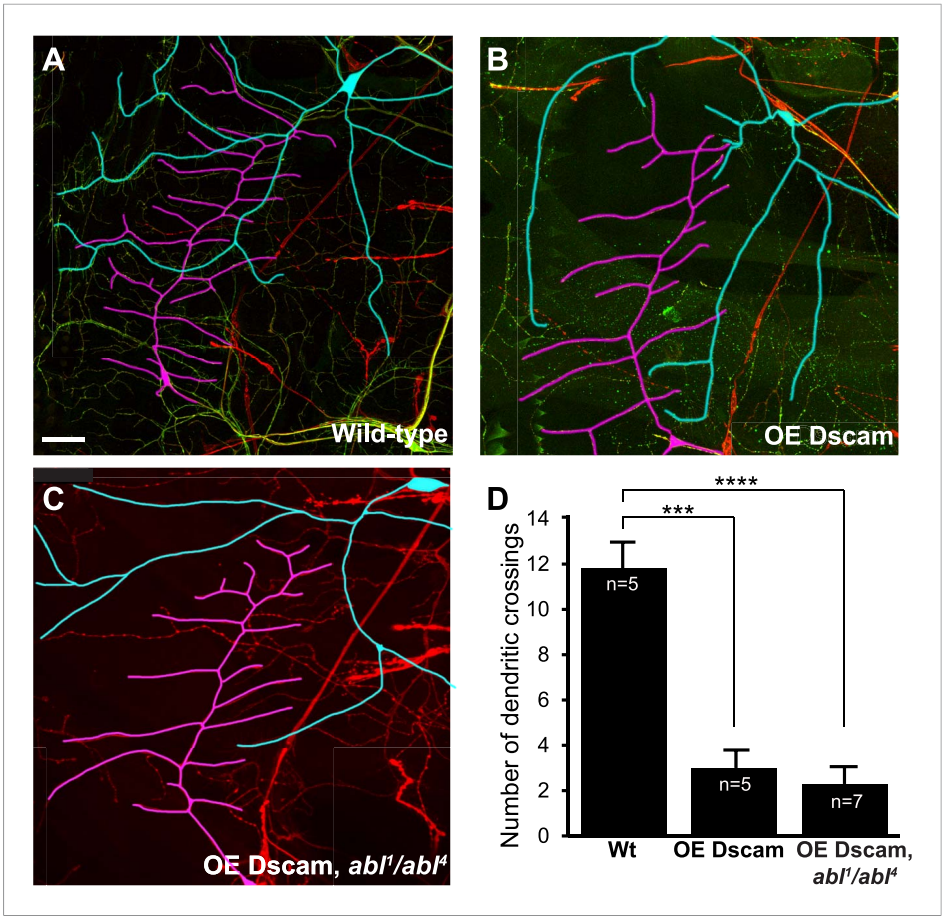

Figure 1—figure supplement 3. Single Dscam isoform-induced ectopic repulsion between class I and class III dendrites does not require *abl*. The dendritic field of the class I da neuron vpda (traced in magenta) normally overlaps extensively with that of the class III da neuron v′pda (traced in cyan) (**A**). When a transgene expressing a single Dscam isoform is overexpressed in both neurons, their dendritic fields segregate (**B**), exhibiting an ectopic repulsion. The expression of the same Dscam transgene in abl^{1} neurons also leads to ectopic repulsion (**C**). Original background images show the pan-neuronal marker labeled with anti-horseradish-peroxidase antibody (red) and Dscam::GFP transgene expression (green). (**D**) Quantification of the number of dendritic branch crossing. Sample number is shown in white inside each bar. Scale bar is 25 μm.

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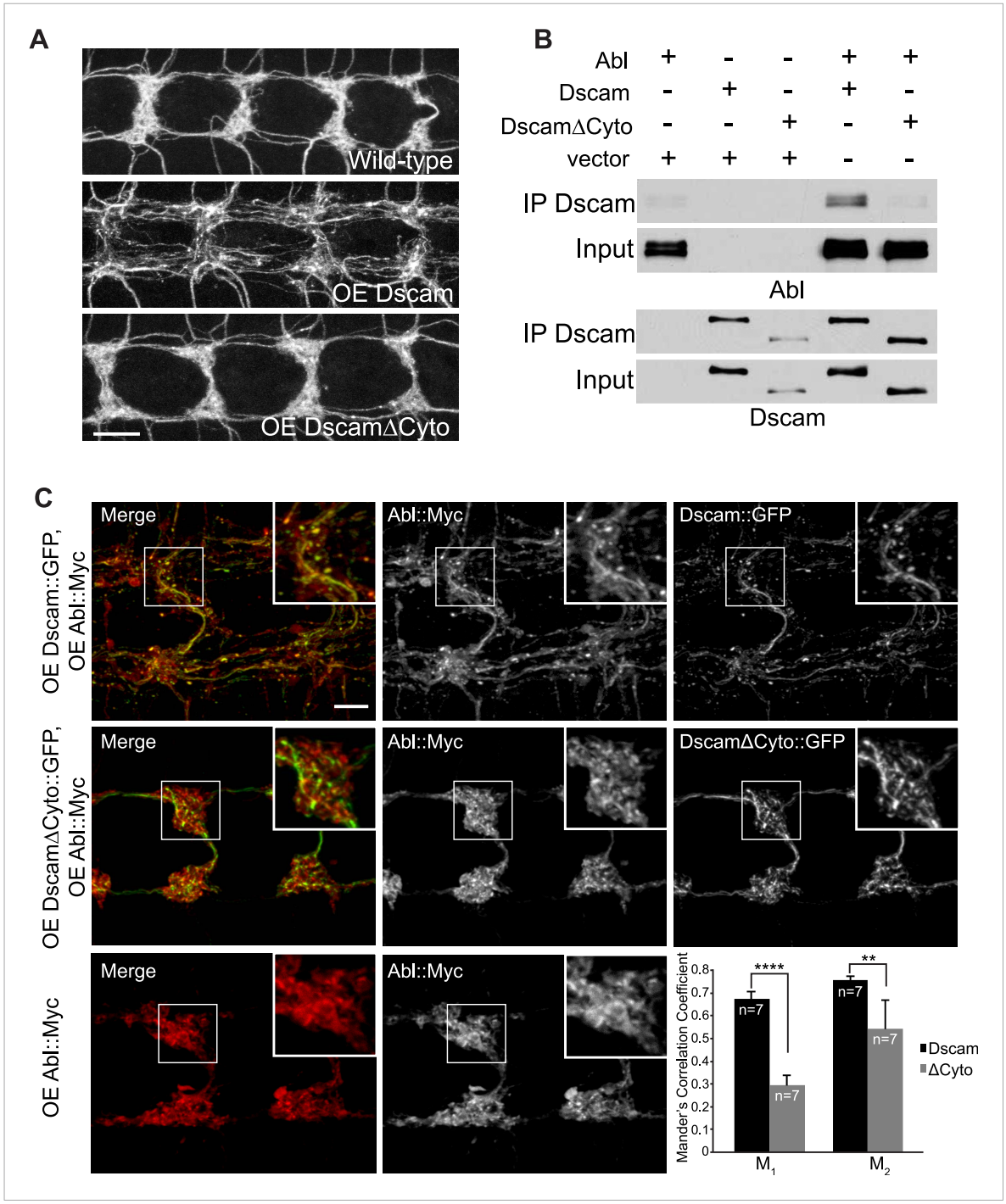

Figure 2. Dscam binds to Abl through its cytoplasmic domain. (**A**) The cytoplasmic domain of Dscam is required for instructing presynaptic terminal growth. Overexpression of full-length Dscam under the control of *ppk*-Gal4 (**A**, middle) leads to exuberant presynaptic terminal overgrowth when compared to control (**A**, top). However, overexpression of DscamΔCyto (**A**, bottom) fails to increase presynaptic terminal growth. Scale bar is 10 μm. (**B**) Dscam binds Abl via its cytoplasmic domain. S2 cells were co-transfected with Abl::Myc along with either Dscam::GFP, DscamΔCyto::GFP, or an empty vector. Dscam::GFP was immunoprecipitated with anti-GFP antibody and bound Abl::Myc was examined with anti-Myc antibody (top). Immunoprecipitated Dscam::GFP and input Dscam::GFP was examined with anti-GFP (bottom). (**C**) Abl colocalizes and redistributes with Dscam but not with DscamΔCyto in presynaptic terminals in vivo. When expressed alone, Abl::Myc shows a diffuse pattern (bottom). When expressed along with Dscam::GFP (top), Abl::Myc redistributes into punctate structures that colocalize with Dscam::GFP. When expressed along with DscamΔCyto::GFP (middle), Abl::Myc does not redistribute, displaying a similar pattern to when Abl::Myc is expressed alone (bottom). This is quantified using Manders' Correlation Coefficient. M_1 presents a measure of the fraction of Abl::Myc that overlaps with Dscam(ΔCyto)::GFP, while M_2 presents a measure of the

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fraction of Dscam(ΔCyto)::GFP that overlaps with Abl::Myc. Both M_1 and M_2 are significantly increased in Abl-Dscam coexpression when compared to Abl-DscamΔCyto coexpression. Scale bar is 5 μm.

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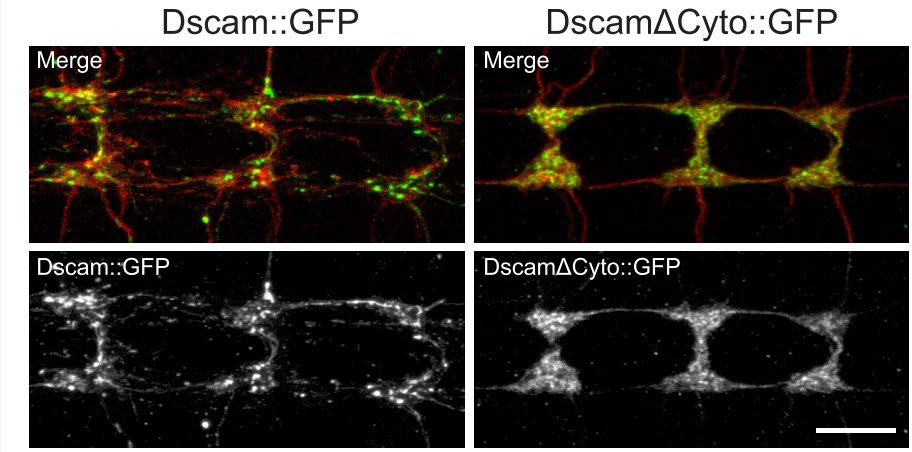

Figure 2—figure supplement 1. DscamΔCyto::GFP is trafficked to presynaptic terminals at a similar level to Dscam::GFP. Both Dscam::GFP (left) and DscamΔCyto::GFP (right) are trafficked to presynaptic terminals. In addition, presynaptic terminal overgrowth is observed 100% of the time when Dscam::GFP is overexpressed, while presynaptic terminal overgrowth is never observed when DscamΔCyto::GFP is overexpressed. Top image shows merged images mCD8::mRFP (red) and either Dscam::GFP or DscamΔCyto::GFP (green). Bottom images show Dscam::GFP or DscamΔCyto::GFP only. Scale bar is 10 μm.

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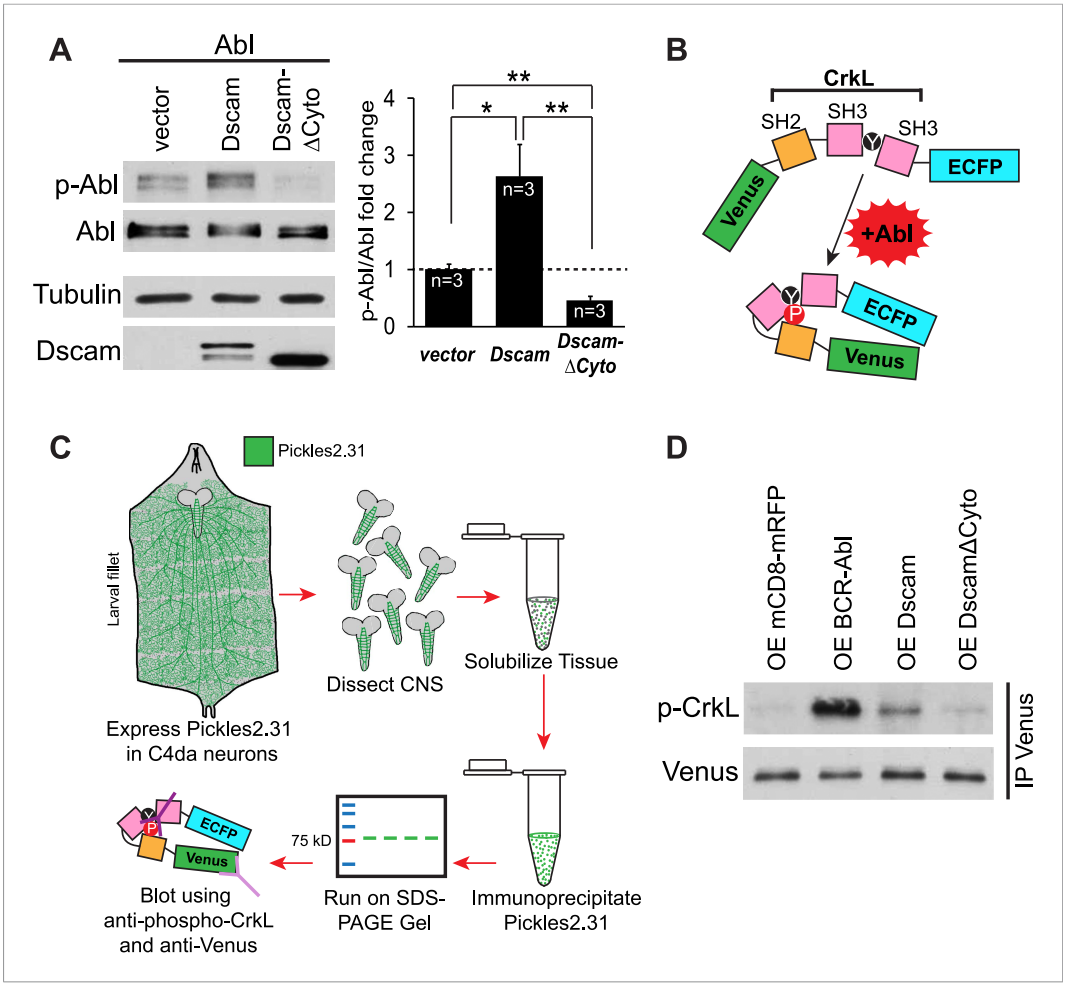

Figure 3. Dscam activates Abl kinase in culture and in vivo. (**A**) Dscam activates Abl in cultured S2 cells. Abl activation was examined in S2 cell lysates transfected with indicated constructs by using anti-phospho-Y412-Abl antibody. The intensity of phospho-Abl was quantified, normalized to total Abl::Myc, and presented as bar graph (n = 3) (**A**, right). (**B**) Schematic of Pickles2.31, an Abl activity reporter that uses phosphorylation of CrkL to report Abl kinase activity. Pickles2.31 is composed of a fragment of human CrkL that contains an Abl phosphorylation site, Y207, sandwiched between ECFP and Venus. Phosphorylation of Pickles2.31 by Abl can be detected with an anti-phospho-Y207-CrkL (p-CrkL) antibody. (**C**) Schematic of in vivo assay for detecting Abl activity in C4da presynaptic terminals. Pickles2.31 is specifically expressed in C4da neurons. As can be appreciated from the larval fillet diagram (left), the cell bodies and dendrites of C4da neurons reside in the larval body wall while their presynaptic terminals reside in the CNS. To assay Abl activity only in presynaptic terminals, larval CNS are dissected out and solubilized into lysates. Pickles2.31 in the presynaptic terminals is then immunoprecipitated with an anti-Venus antibody (left). After running on an SDS-PAGE gel, Pickles2.31 expression level can be assayed using an anti-Venus antibody, while the phosphorylation of Y207, a proxy for Abl activity level, can be ascertained by western blotting with a p-CrkL antibody. (**D**) Dscam activates Abl in presynaptic terminals in vivo. Overexpression of BCR-Abl leads to a robust increase in p-CrkL staining of Pickles2.31 when compared to the mCD8-mRFP control. Similarly, overexpression of Dscam leads to consistent, though less extreme, increase in p-CrkL when compared to control. In contrast, overexpression of DscamΔCyto is indistinguishable from the mCD8-mRFP control. This is a representative blot of three experimental repeats.

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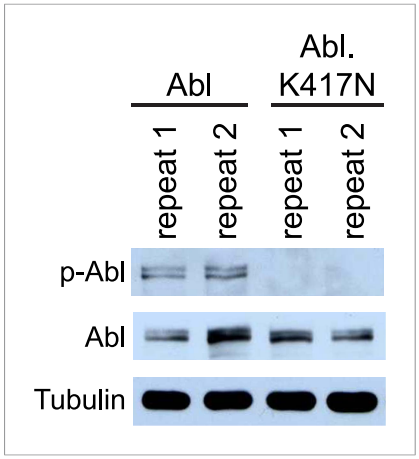

Figure 3—figure supplement 1. Phospho-Y412-Abl antibody specifically reports Abl activation. S2 cells were transfected with either Abl::Myc or Abl-K417N::Myc. Myc was blotted to report total Abl::Myc or Abl-K417N::Myc level (middle), while phosho-Y412-Abl (p-Abl) was blotted to report Abl kinase activation (top). While Abl::Myc displays a characteristic two-band pattern at the correct molecular weight when blotted for p-Abl, no signal is detected for Abl-K417N. This demonstrates that p-Abl specifically reports Abl activation.
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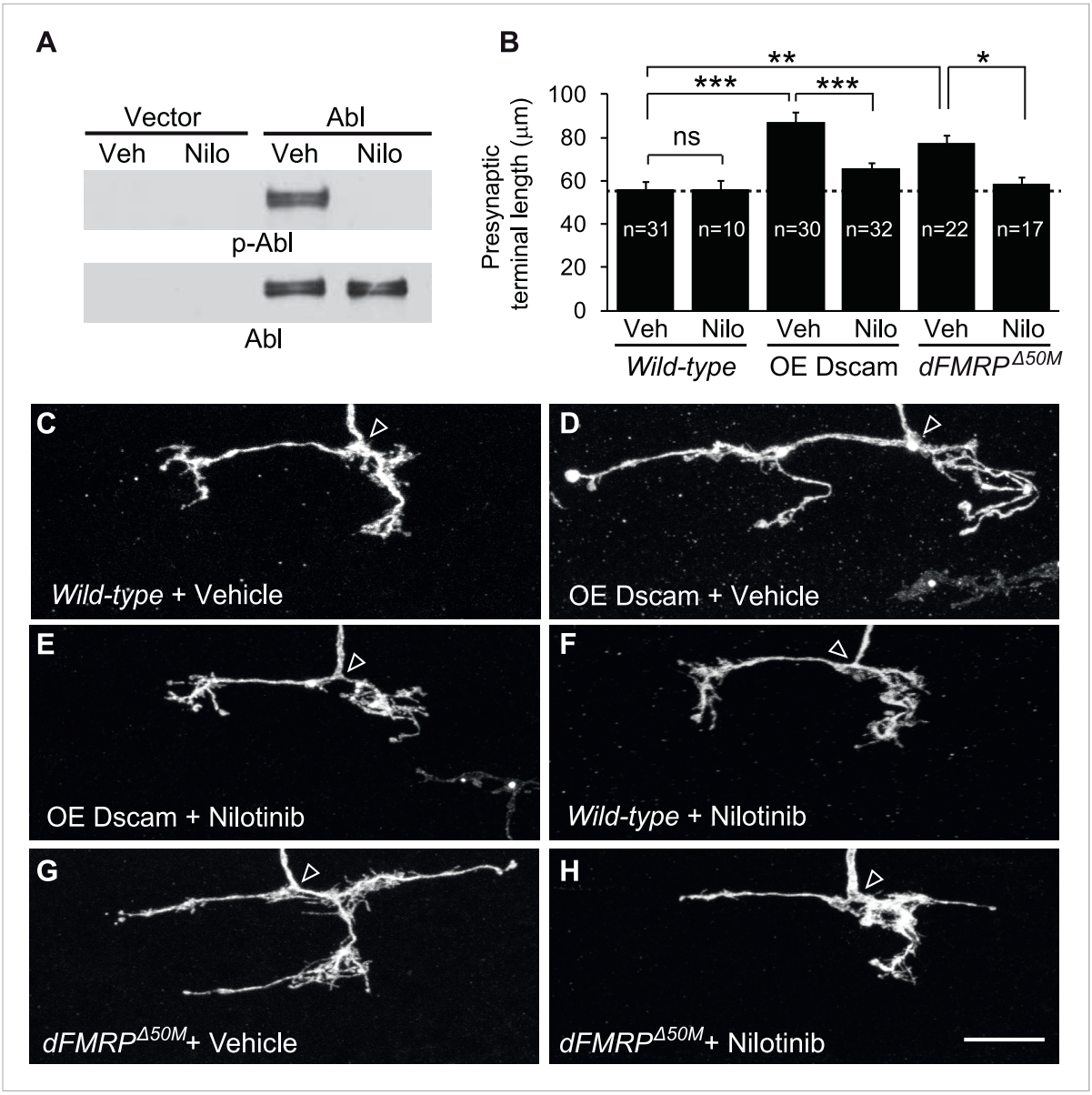

Figure 4. Pharmacological inhibition of Abl mitigates the neuronal defects caused by increased Dscam expression in vivo. (**A**) Nilotinib inhibits *Drosophila* Abl kinase. S2 cells were transfected with either Myc-vector or Abl::Myc, and then treated with either vehicle (DMSO) or 5 μM nilotinib for 6 hr. Total lysates were subjected to western blot analysis with phospho-Y412-Abl (p-Abl) (top) and Myc antibodies (bottom). (**B**) Quantification of the presynaptic terminal length of the indicated genotypes and drug treatment. Sample number is shown inside each bar. (**C**–**H**) Nilotinib treatment mitigates presynaptic arbor enlargement caused by Dscam overexpression (OE Dscam, **D** and **E**) and by *dFMRP* mutations (*dFMRP*$^{\Delta 50M}$, **G** and **H**). Nilotinib treatment alone does not affect presynaptic terminal growth (**F**). The arrowhead in each panel points to the location where an axon elaborates the presynaptic terminal arbor. The MARCM technique was used to generate and visualize single presynaptic terminals of mutant C4da neurons. *Drosophila* larvae were raised in the presence of either 380 μM nilotinib or vehicle (DMSO) for 4 days before the analysis. Scale bar is 10 μm.

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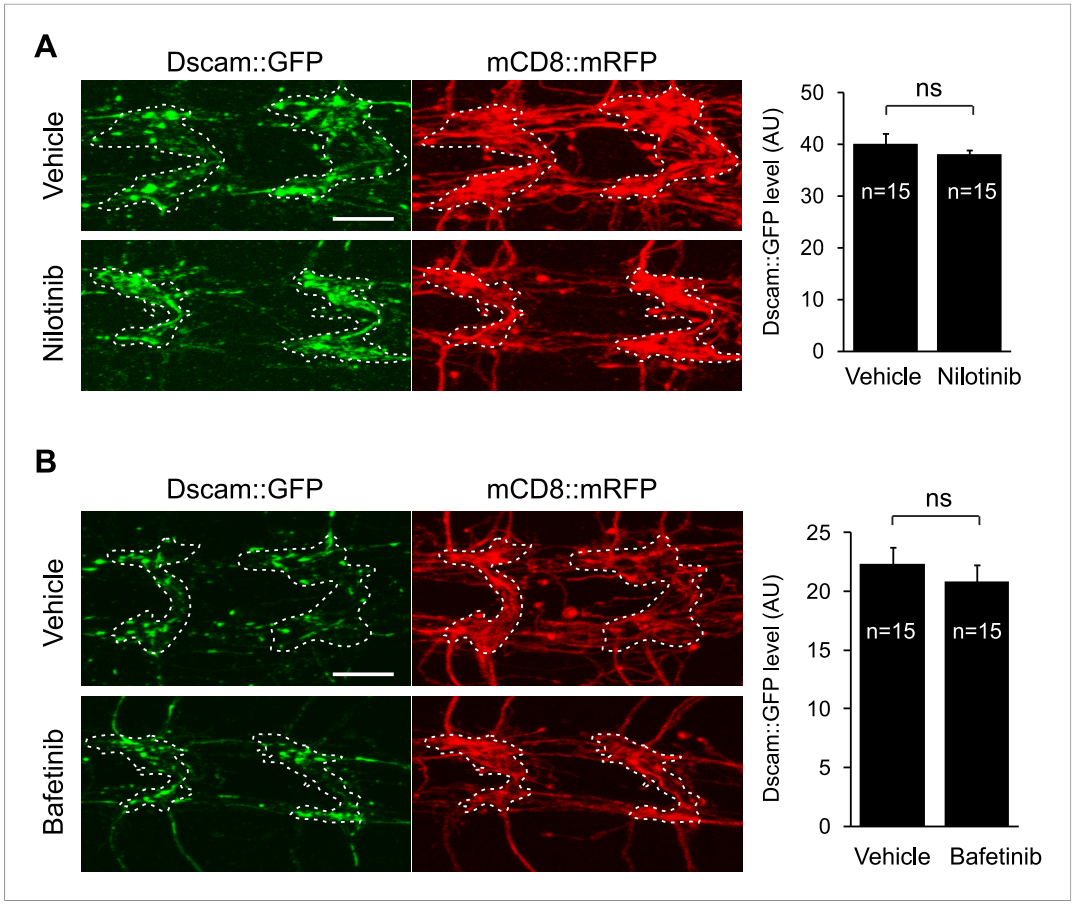

Figure 4—figure supplement 1. Nilotinib and bafatinib do not reduce Dscam transgene expression. Example images of C4da presynaptic terminals expressing Dscam::GFP in animals fed either vehicle (**A** and **B**, top), 380 μM nilotinib (**A**, bottom), or 125 μM bafetinib (**B**, bottom) throughout larval development. Images of mCD8::mRFP are shown to indicate the neuropil regions used for the quantifications (white dotted line). Scale bar is 10 μm. Quantification of the fluorescence of the Dscam::GFP transgene in neuropil region is shown on the right. Sample number is shown inside each bar.

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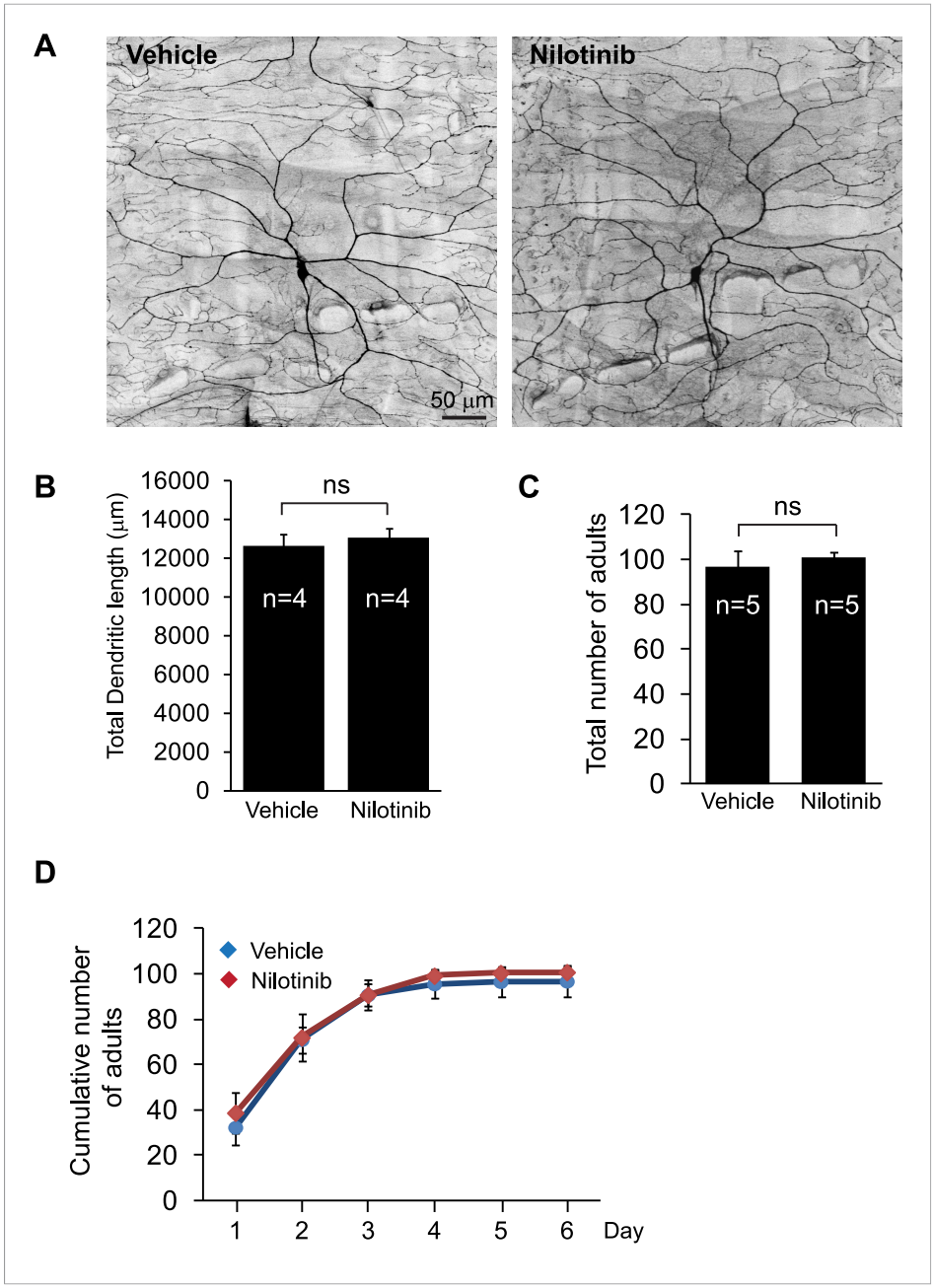

Figure 4—figure supplement 2. Nilotinib treatment does not cause defects in dendritic development or adult viability. (**A** and **B**) Nilotinib does not affect dendritic development. After egg collection, the animals were raised on food containing either vehicle (DMSO) or 380 μM nilotinib for 4 days. C4da dendrites were visualized by expressing mCD8::GFP with *ppk*-Gal4 (**A**). Total dendritic length was measured, quantified, and presented in the bar graph (**B**). Sample number is shown inside each bar. Scale bar is 50 μm. (**C** and **D**) Nilotinib does not affect the development of the flies. After egg collection, the animals were raised on food containing either vehicle (DMSO) or 380 μM nilotinib. Eclosed adults were counted on a daily basis. Total number and cumulative number of adults are shown in (**C**) and (**D**) respectively.

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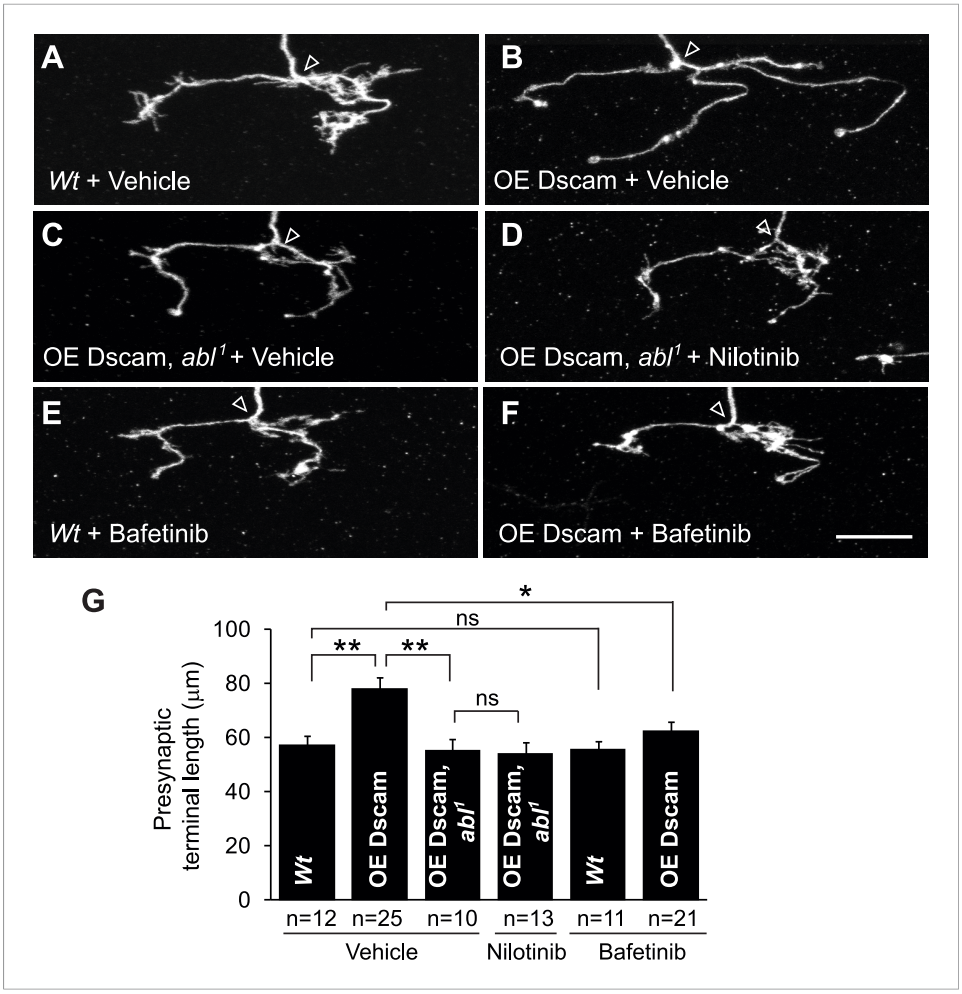

Figure 4—figure supplement 3. Nilotinib and bafetinib act through Abl inhibition to mitigate Dscam-induced presynaptic arbor enlargement in vivo. The MARCM technique was used to generate and visualize single presynaptic terminals of mutant C4da neurons. *Drosophila* larvae were raised in the presence of 380 µM nilotinib, 125 µM bafetinib, or vehicle (DMSO) for 4 days before the analysis. Scale bar is 10 µm. (**A**–**D**) Nilotinib acts through Abl inhibition to mitigate presynaptic arbor enlargement in Dscam overexpressing neurons. *Wt* (*wild-type*, FRT^{2A}), OE Dscam (overexpression of Dscam), OE Dscam, abl^1 (overexpression of Dscam in abl^1 homozygous mutations). Note that nilotinib does not further decrease the size of presynaptic arbors in abl^1 neurons overexpressing Dscam(**C** and **D**). (**E** and **F**) Bafetinib mitigates presynaptic arbor enlargement in Dscam overexpressing neurons. (**G**) Quantification of the presynaptic terminal length of the indicated genotype and drug treatment. Sample number is shown below the x-axis.

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