Figures and figure supplements

Replacing the PDZ-interacting C-termini of DSCAM and DSCAML1 with epitope tags causes different phenotypic severity in different cell populations

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Figure 1. The C-terminus of DSCAM is not required for protein stability or localization. (A) In the DscamAC allele, the sequence encoding the final ten amino acids was replaced with a Myc tag by homologous recombination. (B) Western blots of protein immunoprecipitated from HEK293T cells co-

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transfected with MAGI-3 and V5-tagged DSCAM intracellular domain (ICD) or V5-tagged DSCAM-ΔC ICD (ΔC) demonstrates that the ΔC mutation disrupts the PDZ-binding of DSCAM’s C-terminus. (C) Western blots of DSCAM protein immunoprecipitated from neonatal brains showed no change in the size or amount of DSCAM in Dscam^AC/AC mutants. The antibody specificity is confirmed by the lack of signal from Dscam^−/− brains. (D–F) Immunofluorescent labeling for DSCAM in vertically sectioned retinas from 3-week old mice demonstrated that the protein is found in a normal, punctate localization in the synaptic plexiform layers in Dscam^+/+ (D) and Dscam^{AC/AC} (E) mice, consistent with earlier reports for wild type DSCAM (de Andrade et al., 2014). No staining above background was found in Dscam^−/− retinas (F), demonstrating the specificity of the DSCAM antibody. (G–I) Hematoxylin and eosin staining of adult retinas shows that, compared to controls (G), Dscam^−/− retinas (I) are severely expanded and disorganized. Dscam^{AC/AC} retinas (H) have modest expansion, but not the extensive disorganization found in the null mutant. Scale bar is 100 μm. See also Figure 1—figure supplement 1 and Figure 1—figure supplement 2.

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**Figure 1—figure supplement 1.** DSCAM’s C-terminus interacts with PDZ domains. (A, B) Both MAGI-2 and MAGI-3 were found to interact with DSCAM by yeast two-hybrid. The C-terminal 20 amino acids of DSCAM (PDZ) were used as bait and the Gal4 binding domain alone (GBD) was used as a negative control. Successful interaction is revealed by the expression of β-galactosidase on LacZ (A, note colony color) and expression of HIS3 promoting survival on plates without histidine (B, -His). When transfected into HEK293T cells, both DSCAM (C) and DSCAM-ΔC (D) protein localized to the cell surface as revealed by live cell staining with an antibody raised against the entire extracellular domain.

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DSCAM protein localization is grossly unchanged in Dscam<sup>DC/DC</sup> retinas. (A–F) Confocal images of P9 retinas immunolabeled for DSCAM and melanopsin show normal colocalization between the proteins in control (A–C) Dscam<sup>++</sup> animals (D–F). The colocalization is quantified in G. N = 4 retinas per genotype. *p<0.05. (H–L) Confocal images of P7 retinas immunolabeled for DSCAM collected under standardized microscope settings from Dscam<sup>++</sup> (H), Dscam<sup>−/−</sup> (I), Dscam<sup>DC/DC</sup> (J), and Dscam<sup>−/−</sup> (K) retinas show the relative staining intensities in each mouse. (L) Intensities were measured along a 10 μm line adjacent and perpendicular to the INL, a region that includes S1. Fluorescence intensity in Dscam<sup>DC/DC</sup> retinas was not reduced. Box plots represent the median, first and third quartile, range, and outliers. N = 6 retinas each for Dscam<sup>++</sup>, Dscam<sup>−/−</sup>, and Dscam<sup>DC/DC</sup> genotypes and 2 retinas for Dscam<sup>−/−</sup>. *p<0.05.

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Figure 2. DSCAM-mediated self-avoidance requires C-terminal interactions in only some amacrine cell types. (A–C) Dopaminergic amacrine cells (stained for tyrosine hydroxylase, TH) are non-randomly spaced in wild type retinas (A), but lose mosaic spacing and form neurite fascicles in two-week old DscamAC/AC (B) and Dscam−/− (C) retinas. (D) In both mutants, there was a significant increase in cell density. Spacing was quantified by DRP analysis; relative cell densities normalized to the overall density at increasing distances from reference cells are plotted in (E). By Voronoi (F) and nearest neighbor (G) analyses spacing in DscamAC/AC retinas was not significantly different than in Dscam−/− animals. (H–J) Conversely, bNOS-positive amacrine cells were not visibly different between controls (H) and DscamAC/AC retinas (I) despite clear fascication and loss of mosaic spacing in Dscam−/− mice (J). DscamAC/AC values were intermediate between control and DscamAC/AC in cell density (K), DRP (L), Voronoi (M), and nearest neighbor (N) analyses, but differences from control were not statistically significant. Means ± s.e.m. are represented in D–E, K–L. Box plots represent the median, first and third quartile, range, and outliers. N = 4–8 retinas per genotype. *p<0.05; **p<0.01; ***p<0.001; n.s. is not significant by Tukey post-hoc test between indicated genotypes or compared to controls. Scale bar is 100 μm. Representative Voronoi domains are in Figure 2—figure supplement 1.

Figure 2 continued on next page
Figure 2—figure supplement 1. Examples of Voronoi tessellation domains in Dscam mutants. Representative Voronoi tessellation domains of TH-positive DA cells (A–C) and bNOS-positive amacrine cells (D–F) show the differential effect of the C-terminal deletion.

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Figure 3. RGCs also display differential dependence on DSCAM C-terminal interactions for self-avoidance. (A–C) Melanopsin-positive intrinsically photoresponsive retinal ganglion cells are found in a mosaic pattern in wild type retinas (A), but at two weeks of age, ipRGC cell bodies in DscamACAC (B) retinas are pulled into clusters similar to those in Dscam−/− (C) retinas. (D) Overall ipRGC density was not significantly increased in DscamACAC retinas. (E) By DRP, cell body clustering was intermediate between Dscam+/+ and Dscam−/− retinas. Voronoi (F) and nearest neighbor (G) analyses also revealed a clear intermediate defect. Values from DscamACAC retinas were significantly different from both control and Dscam−/− mutants. (H–J) Cdh3-GFP RGCs are mosaically spaced in control retinas (H), and this spacing is not perturbed in the DscamACAC retinas (I), but these cells form clusters in Dscam−/− animals (J), indicating interactions mediated by DSCAM’s C-terminus are dispensable to prevent these cells from clustering. (K) Cdh3-GFP RGC overall cell density was significantly increased in Dscam−/− retinas, but not in DscamACAC mutants. (L) A clear exclusion zone is detectable by DRP analysis in Dscam+/+ and DscamACAC retinas. This exclusion zone is lost in Dscam−/− animals, where cell density is increased adjacent to any given cell, Figure 3 continued on next page
Figure 3 continued

indicative of clustering. Similarly, Voronoi (M) and nearest neighbor (N) analyses describe a clear spacing defect in Dscam<sup>−/−</sup> but not in Dscam<sup>AC/AC</sup> retinas. Means ± s.e.m. are represented in D–E, K–L. Box plots represent the median, first and third quartile, range, and outliers. N = 6 retinas per genotype. *p<0.05; **p<0.01; ***p<0.001; n.s. is not significant by Tukey post-hoc test between indicated genotypes or compared to controls. Scale bar is 250 μm. Representative Voronoi domains are in Figure 3—figure supplement 1.

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Figure 3—figure supplement 1. Examples of Voronoi tessellation domains in Dscam mutants. Representative Voronoi tessellation domains of melanopsin-positive ipRGCs (A–C) and Cdh3-GFP RGCs (D–F) show the differential effect of the C-terminal deletion.

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Figure 4. DSCAML1-mediated self-avoidance requires C-terminal interactions in only some cell types. (A) Dscam1\textsuperscript{ΔC/ΔC} mutant mice were generated by replacing the sequence encoding the final ten amino acids with an HA tag by homologous recombination. See also Figure 4—figure supplement 1.

Figure 4 continued on next page
Figure 4 continued

(B–D) Hematoxylin and eosin staining of adult retinas shows that, compared to controls (B), Dscaml1^{-/-} retinas (D) are significantly expanded and disorganized. Dscaml1^{H/C} retinas (C) have a more intermediate expansion without the extensive disorganization found in the null mutant. (E–G) All amacrine cells (Dab1-positive) are organized in a mosaic pattern in two-week old control retinas (E). This pattern is disrupted in Dscaml1^{H/C} retinas (F), but not as severely as in Dscaml1^{-/-} retinas (G), where the cells form clusters. (H) There was a significant increase in cell density in Dscaml1^{-/-} but not in Dscaml1^{H/C} animals. I) DRP analysis revealed an intermediate effect in Dscaml1^{H/C} retinas between the clear exclusion zone in control and clustering in Dscaml1^{-/-}. All amacrine spacing was slightly disrupted in Dscaml1^{H/C} retinas by Voronoi analysis (J) but not by nearest neighbor analysis (K). L–N) Conversely, compared to controls (L) the disruption of VGLUT3-positive amacrine cell spacing in Dscaml1^{H/C} (M) retinas was more similar to that in Dscaml1^{-/-} (N) retinas. (O) VGLUT3-positive amacrine cell density was significantly increased in both Dscaml1^{H/C} and Dscaml1^{-/-} animals. (P) DRP analysis reveals the loss of exclusion zone in both mutants. (Q,R) Dscaml1^{H/C} values were significantly different from controls in both Voronoi and nearest neighbor analyses. Means ± s.e.m. are represented in H–I, O–P. Box plots represent the median, first and third quartile, range, and outliers. N = 6–8 retinas per genotype. *p<0.05; ***p<0.001; n.s. is not significant by Tukey post-hoc test between indicated genotypes or compared to controls. Scale bars are 100 µm. Representative Voronoi domains are in Figure 4—figure supplement 2.

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Figure 4—figure supplement 1. DSCAML1-ΔC has a normal membrane topology, but does not interact with MAGI-3. Live staining of HEK293T cells transfected with full-length DSCAML1 with an N-terminal HA tag (A) and DSCAML1-ΔC with a C-terminal HA tag (B) show that, for both proteins as expected, the N-terminus is presented to the extracellular space and the C-terminus to the cytoplasm. (C) Western blots of protein immunoprecipitated from HEK293T cells co-transfected with MAGI-3 and V5-tagged DSCAML1 intracellular domain (ICD) or V5-tagged DSCAML1-ΔC ICD (ΔC) demonstrates that the ΔC mutation disrupts the PDZ-binding of DSCAML1’s C-terminus. DOI: 10.7554/eLife.16144.011
Figure 4—figure supplement 2. Examples of Voronoi tessellation domains in Dscaml1 mutants. Representative Voronoi tessellation domains of AII amacrine cells (A–C, Dab1) and VGLUT3-positive amacrine cells (D–F) show the differential effect of the C-terminal deletion.

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Figure 5. Increased cell density is not sufficient to explain spacing defects. (A) ipRGC cell density was similar in Dscam<sup>ΔC/ΔC</sup> and Bax<sup>-/-</sup> retinas. Despite this, clustering was more severe in Dscam<sup>ΔC/ΔC</sup> as measured by DRP (B) and Voronoi (C), but not by nearest neighbor analysis (D). (E) Likewise, DA cell density was similar in Dscam<sup>ΔC/ΔC</sup> and Bax<sup>-/-</sup> retinas. However, DA cell spacing was not significantly different between Dscam<sup>ΔC/ΔC</sup> and Bax<sup>-/-</sup> mutants (F–H). (I) VGLUT3-positive amacrine cell density was significantly higher in Bax<sup>-/-</sup> than in Dscam<sup>ΔC/ΔC</sup> retinas. Mosaic spacing was more disrupted in Dscam<sup>ΔC/ΔC</sup> as measured by Voronoi domain analysis (K) and nearest neighbor (L) but not by DRP (J). Means ± s.e.m. are represented in A–B, E–F, I–J. Box plots represent the median, first and third quartile, range, and outliers. *p<0.05; **p<0.01; ***p<0.001; n.s. is not significant by student’s t-test.
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Figure 6. Neurite fasciculation is separable from density-dependent cell body clustering. (A) DA cell neurites evenly fill their receptive fields in control mice, but form fascicles in Dscam<sup>ΔC/ΔC</sup> (B) and Dscam<sup>−/−</sup> (C) animals. (D) DA fascicles are rarely observed in Bax<sup>−/−</sup> mutants. (E) M2 ipRGC dendrites

Figure 6 continued on next page
imaged in the ON region of the IPL are evenly distributed in wild type mice and largely remain so in Dscam<sup>DC/DC</sup> (F) and Bax<sup>−/−</sup> (H) mutants, while severe fasciculation is evident in Dscam<sup>−/−</sup> retinas (G). I) In the OFF strata of the IPL, M1 ipRGC dendrites are diffusely organized. There is modest fasciculation in Dscam<sup>DC/DC</sup> (J) and Bax<sup>−/−</sup> (L) mice, while fasciculation in Dscam<sup>−/−</sup> retinas (K) is much more severe. (M–O) Elo ranking of fasciculation severity between genotypes demonstrates that DA neurites (M) are clearly fasciculated in Dscam<sup>DC/DC</sup> and Dscam<sup>−/−</sup> retinas, but not in Bax<sup>−/−</sup>, which had loss of mosaic cell body spacing. Conversely, ipRGCs in Dscam<sup>DC/DC</sup> did not have significantly more fasciculation than Bax<sup>−/−</sup> either in the ON (N) or OFF (O) layers, despite having a more severe cell body clustering. Box plots represent the median, first and third quartile, range, and outliers. *p<0.05; **p<0.01; ***p<0.001; n.s. is not significant by Wilcoxon rank sum test. Scale bar is 250 μm. See also Figure 6—figure supplement 1.
Figure 6—figure supplement 1. Fasciculation of Cdh3-GFP RGCs and bNOS-positive amacrine cells. Fasciculation of Cdh3-GFP RGC dendrites (A–C) and bNOS-positive neurites (D–G) were compared between genotypes at two weeks of age. (H) Elo ranking of fasciculation severity demonstrates that Cdh3-GFP RGC dendrites are clearly fasciculated in Dscam−/− retinas, but not in DscamΔC/ΔC or control retinas. (I) Similarly, bNOS-positive neurites were fasciculated in Dscam−/− retinas, but not in DscamΔC/ΔC or controls, and there was only mild fasciculation in Bax−/− animals. Box plots represent the median, first and third quartile, range, and outliers. *p<0.05; **p<0.01; n.s. is not significant by Wilcoxon rank sum test. Scale bar is 250 μm.

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Figure 7. Laminar specificity in ΔC mutants. Neurite stratification in the IPL was analyzed in immunolabeled cryosections. (A, B) bNOS-positive amacrine cells stratified normally in DscamAC/AC retinas, as did ipRGCs and DA cells (C, D), which co-stratify in the OFF region adjacent to the INL. (E) Imaged en face, DA neurites co-fasciculated with ipRGC dendrites in DscamAC/AC mutants (arrowheads), as we have previously found in Dscam−/− retinas (Fuerst et al., 2009). In Dscaml1AC/AC mutants, AII amacrine cells (F, G) and rod bipolar cells (H, I) terminate their processes normally. (J–M) TEM analysis revealed that Dscam1ΔC/ΔC retinas contained structurally normal dyad synapses between rod bipolar cells and AII/A17 amacrine cells with distinct ribbons (arrows). (K) Dscam1−/− RBC dyad synapses are characterized by excess in synaptic vesicle number and indistinct ribbons. Four retinas analyzed by TEM per genotype, > 10 synapses inspected per retina. (N, O) VGLUT3-positive amacrine cells misprojected beyond the ON ChAT layer in Dscam1ΔC/ΔC mutants. (P, Q) These ectopic neurites became associated with AII amacrine terminals adjacent to the retinal ganglion cell layer (arrowheads). This association was observable at 3 weeks of age (P) and persisted through adulthood (Q, 18 months of age). (R) These misprojections were quantified by imaging whole-mount retinas stained for VGLUT3 and Dab1 en face and calculating the percent of area occupied by VGLUT3 in projections through Dab1-positive AII amacrine terminals. Means ± s.e.m. at three threshold levels are represented in R. n = 6–8 retinas per genotype. Scale bar is 20 μm in A–O, 110 μm in E, 10 μm in P, Q, and 500 nm in J–M.

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