DSCAMS AND SIDEKICKS DIRECT LAMINA-SPECIFIC SYNAPTIC CONNECTIONS IN VERTEBRATE RETINA

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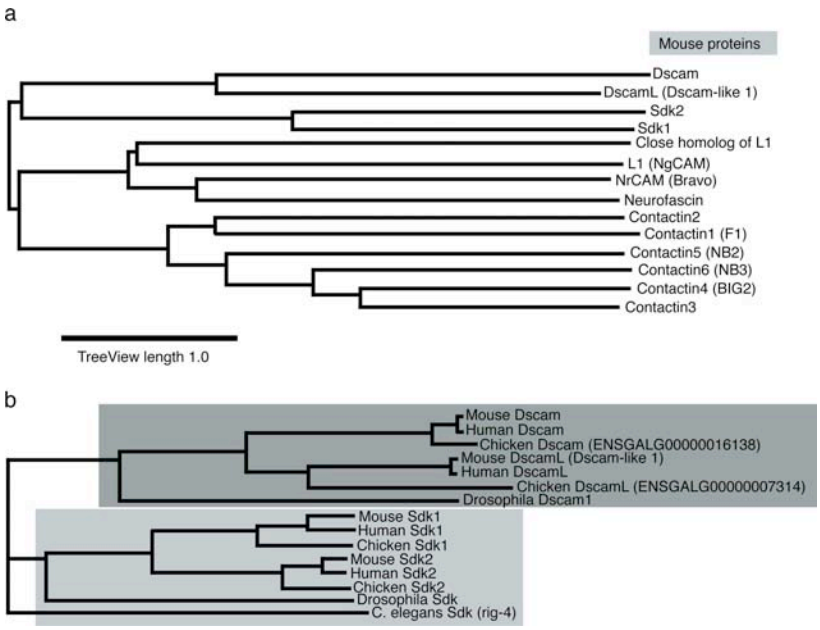
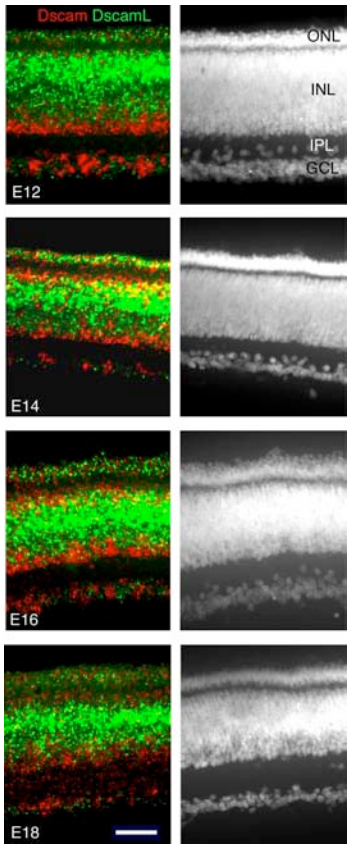
Supplementary Table: Percent of cells positive for Dscams and Sidekicks in the ganglion cell layer (GCL) of E16 retina.

Gene	% of positive cells in GCL (Range of values)
Dscam	**19.0±2.8 (16.9-22.5)**
DscamL	**20.0±7.1 (12.3-36.7)**
Sdk1	**9.5±2.4 (4.4-13.8)**
Sdk2	**14.5±2.1 (11.8-17.1)**

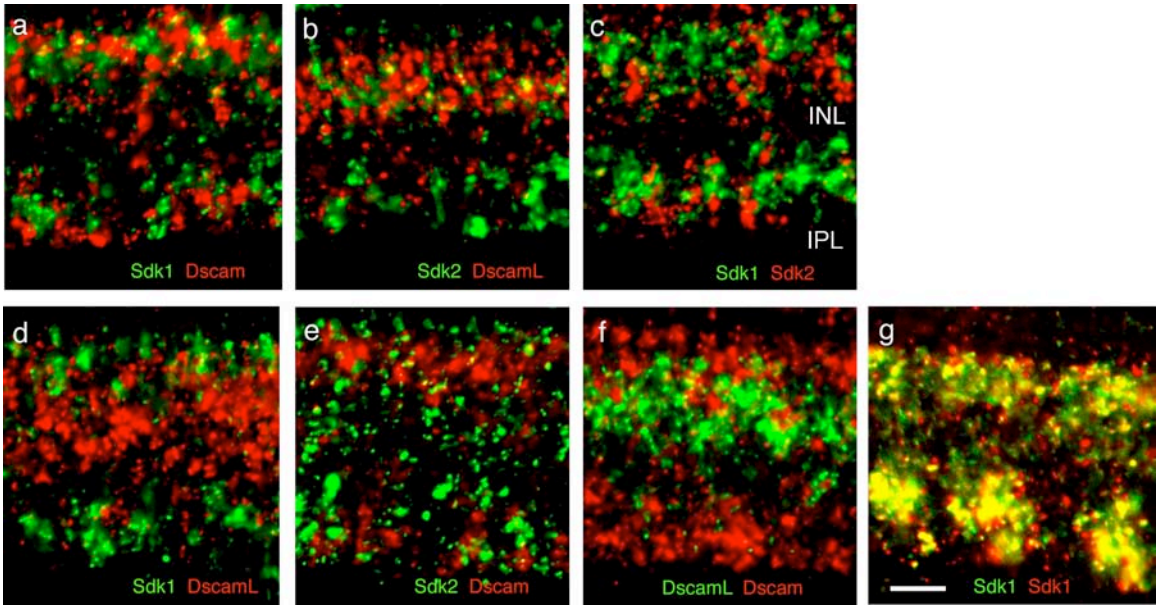
Total number of cells in sections of GCL was determined by DAPI staining; Dscam- and Sidekick-positive cells determined by in situ hybridization (see Fig. 1c). Values are mean percent from 10 fields from 2 or more retinae; range and standard deviation are also shown. In chick the GCL contains about 90% RGCs and 10% displaced amacrine cells[31]. Double-staining with the pan-RGC marker, thy-1 (ref. 32) showed that the Dscam- and Sidekick-positive cells in the GCL are RGCs (data not shown).



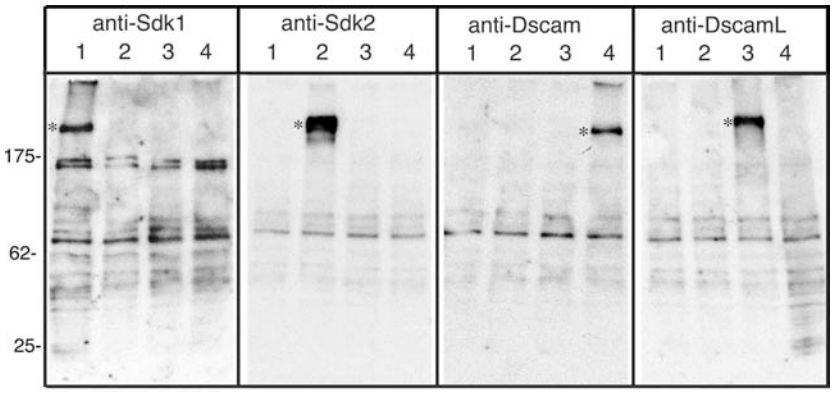
Supplementary Figure 1.
Phylogenetic tree of Dscam and Sidekick proteins showing their relationship in the IgSF.
a. Mouse proteins, for which best annotated sequences of IgSF genes are available. Dscams and Sidekicks are more closely related to each other than to their next closest relatives, which are those of the L1 (L1, NrCAM, Neurofascin) and Contactin subfamilies. Highest homology between Dscams and Sidekicks is observed in the middle of their ectodomain (e.g., residues 450-950 sdk2 vs residues 650-1200 of dscamL). **b.** Independent alignment of Dscam and Sidekicks from multiple species. Alignments are based on the entire protein coding sequence except for chicken Dscam-1, for which genomic annotation is incomplete. ENSEMBL accession numbers are indicated in the Figure. **a** was generated by Treefam (http://www.treefam.org/) and **b** was generated using ClustalW with PAM matrix (http://www.ebi.ac.uk/clustalw/).



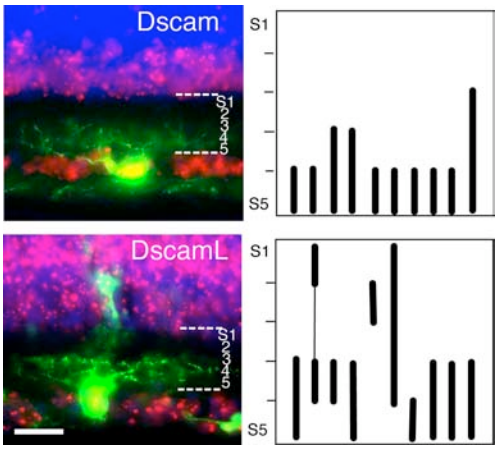
Supplementary Figure 2.
Double label in situ hybridization to sections of E12, E14, E16 and E18 retina with probes for Dscam and DscamL, and counterstained for DAPI (blue). Dscam and DscamL are expressed by complementary subsets of cells throughout this period. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Yellow signal reflects overlap of multiple cells in these relatively thick sections. Bar is 50μm.



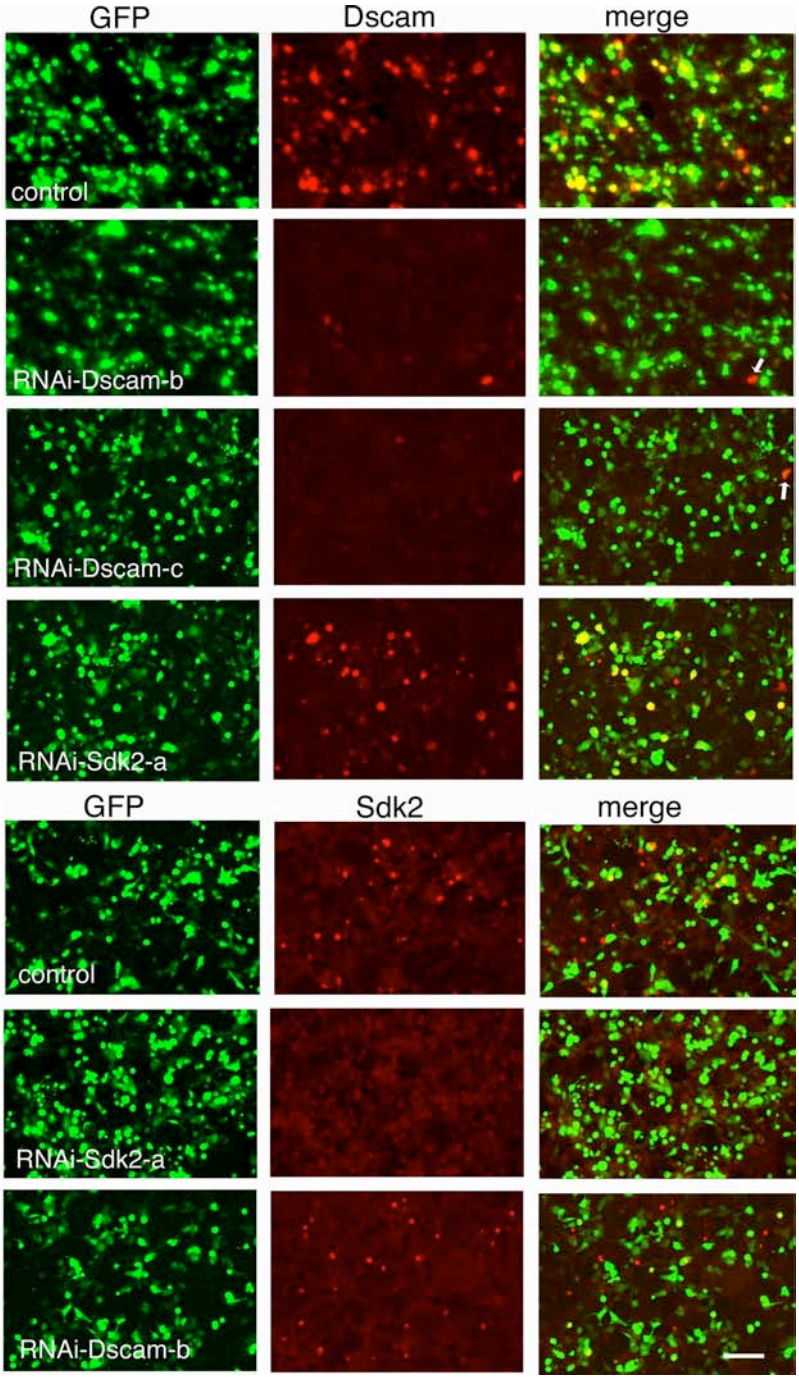
Supplementary Figure 3.
Double label in situ hybridization to sections of E15 retina showing that Dscam, DscamL, Sidekick-1 and Sidekick-2 are expressed by mutually exclusive subsets of cells in the inner nuclear layer (INL). **g** shows a section hybridized with two probes to Sidekick-1, to illustrate pattern expected for co-expression. Bar is 20μm.



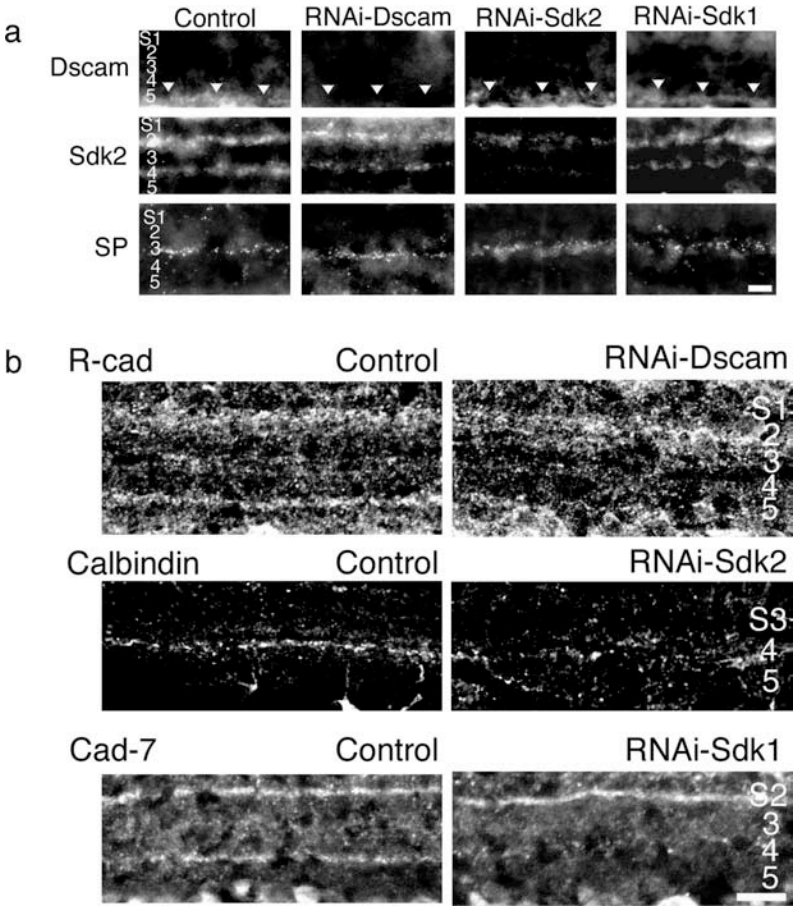
Supplementary Figure 4.
Characterization of antibodies to Dscams and Sidekicks. HEK293 cells were transfected with cDNAs encoding full-length mouse Dscam orSidekick, then lysed and immunoblotted. Each antibody recognized only the appropriate protein. Mouse cDNAs were used in this experiment because full-length chick DscamL cDNA was not available. Other experiments show that the Dscam antibodies do not recognize chicken sidekicks and that the Sidekick antibodies do not recognize chick Dscam (data not shown).



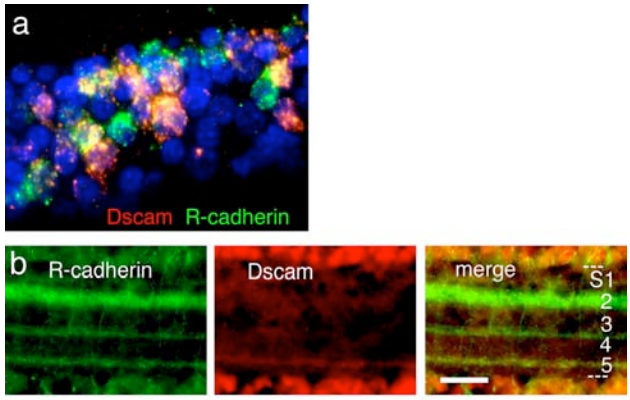
Supplementary Figure 5.
Dendritic arbors of Dscam and DscamL-positive RGCs. Cells were labeled with a piggyBac vector encoding GFP (see Fig. 4) and Dscam-positive cells were identified by in situ hybridization. Micrographs show examples and graphs summarize data from multiple cells. Dendrites of Dscam-positive RGCs arborized predominantly in S5, although processes of some cells extended into S3 and S4; dendrites of Dscam-1-positive RGCs arborized in S1,2,4 and 5, but seldom in S3. The positions of the dendrites corresponded to the sublaminae in which the Dscam and DscamL proteins are concentrated (see Fig. 1). Bar is 20μm.



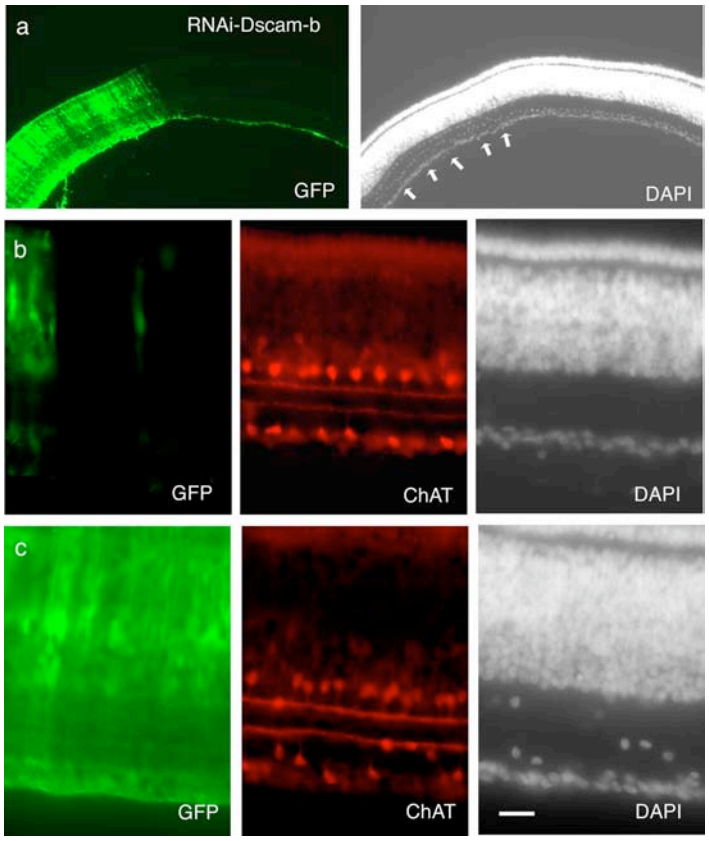
Supplementary Figure 6.
Assay of miRNA vectors directed at Dscam and Sidekick-2. HEK293 cells were co-transfected with the miRNA vector (which also encoded GFP; see Fig. 3) and a cDNA encoding chicken Dscam or Sidekick-2. Each miRNA dramatically and specifically decreased levels of corresponding IgSF protein, but Sidekick RNA had no effect on Dscam expression or visa versa. Arrows indicate cells that expressed Dscam but had not been cotransfected with the miRNA vector; IgSF expression persists in these cells. Dscam-b and Dscam-c are two different RNA sequences, both of which were effective. Similar experiments showed the efficacy and specificity of miRNA directed at Sidekick-1 (not shown). Bar, 80μm.



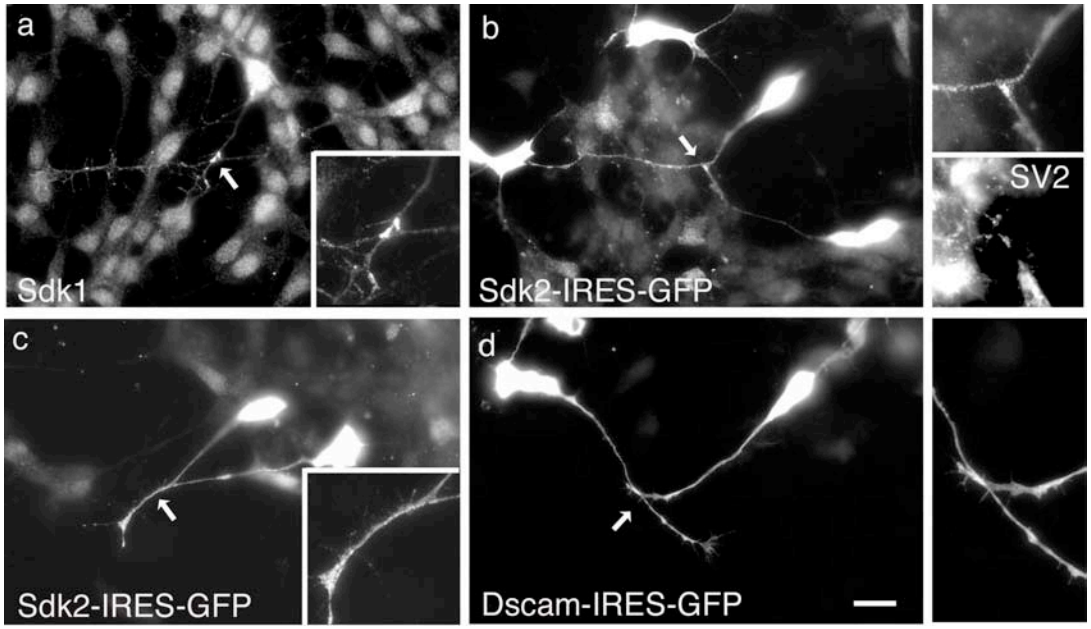
Supplementary Figure 7.
a. Specificity of miRNA vectors in vivo. Sections from areas infected by virus carrying control, Dscam, Sidekick-1 or Sidekick-2 RNA were immunostained as indicated. **b.** Confocal images of uninfected (control) and infected regions of retinae depleted of Dscam, Sidekick-1 or Sidekick-2. R-cadherin and cadherin-7 were present not only in Dscam-positive processes in S5 and Sidekick-1-positive processes in S4, but also in Dscam- and Sidekick-1-negative processes in S2; only the former were affected by the interfering RNAs Defects of the sort shown here were observed in all of 5 retinae depleted of Dscam, all 3 retinae depleted of Sidekick-1 and all 4 retinae depleted of Sidekick-2. For 1 retina of each type, we scored multiple fields in infected and uninfected region. For the Dscam retina, we observed defects in Rcadherin-positive fibers in 8/20 (40%) of fields in the infected areas and 0/20 from the uninfected area. For the Sidekick-1 retina, we observed defects in cadherin-7-positive fibers in 5/20 (25%) of fields in the infected areas and 0/20 from the uninfected area. For the Sidekick-2 retina, we observed defects in calbindin-positive fibers in 14/20 (70%) of fields in the infected areas and 2/20 (10%) from the uninfected area. In a retina infected with a control RNAi vector, we observed defects in 1/40 (2.5%) of fields stained for R-cadherin, 0/20 stained for cadherin-7 and 1/20 (5%) stained for calbindin. Bar is 5 μm in **a** and 20μm in **b**.



Supplementary Figure 8.
Dscam-positive RGCs are R-cadherin positive. **a.** Double label in situ hybridization shows that most of the Dscam-positive cells in the ganglion cell layer also express R-cadherin. **b.** Immunostaining shows that Dscam-positive processes in S5 of the IPL are also R-cadherin-positive. The R-cadherin-positive bands in S2 and S3/4 are Dscam-negative; they arise at least in part from processes of bipolar and amacrine cells. Similar experiments showed coexpression of cadherin-7 with Sidekick-1 RGCs that arborize in S4 but not S2 and calbindin with Sidekick-2 RGCs that arborize in S4 (not shown). Bar is 20μm.



Supplementary Figure 9.
Depletion of Dscam leads to mislocalization of somata of choline acetyltransferase- (ChAT-) positive amacrine cells. **a.** Section from an E17 retina following retroviral introduction of GFP and Dscam RNA at E2. Large patches of retinal cells are infected, as shown by GFP expression (green). **b.** In an uninfected (GFP-negative) area of the retina, bands of ChAT-positive processes are present in S2 and S4, and ChAT-positive somata flank the IPL on both sides. **c.** In heavily infected (GFP-positive) area, location of ChAT-positive processes is undisturbed, but some ChAT-positive somata are found in the IPL. Mislocation of somata was observed only in the most heavily infected areas, presumably reflecting most complete depletion of Dscam. In less heavily infected areas, R-cadherin-positive processes were disturbed but ChAT-positive somata were not, as shown in Fig. 3. Similar results were observed with each of two RNAs directed against Dscam (see Methods). Bar is 80μm in **a**, and 20μm in **b, c**.



Supplementary Figure 10.
Interactions of neurites that express Sidekick or Dscam. SHSY5Y neuroblastoma cells were transfected with plasmids that overexpress Sdk1 (a), Sdk2-IRES-GFP (b, c), or Dscam-IRES-GFP (d). Three days later, after cells had extended neuritis, cultures were stained with antibodies to Sdk1 (a) or GFP and SV2 (bd). Regions indicated by arrows are shown at higher power in insets. Neurites from transfected cells contacted each other, sometimes accumulated SV2 at such points of contact (b), and sometimes fasciculated with each other (see also Fig. 4g,h). Bar is 20μm in **a-d** and 10μm in insets.