Neuron, Volume *73*

Supplemental Information

Integrins Regulate Repulsion-Mediated Dendritic Patterning of *Drosophila* Sensory Neurons by Restricting Dendrites in a 2D Space

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INVENTORY OF SUPPLEMENTAL ITEMS

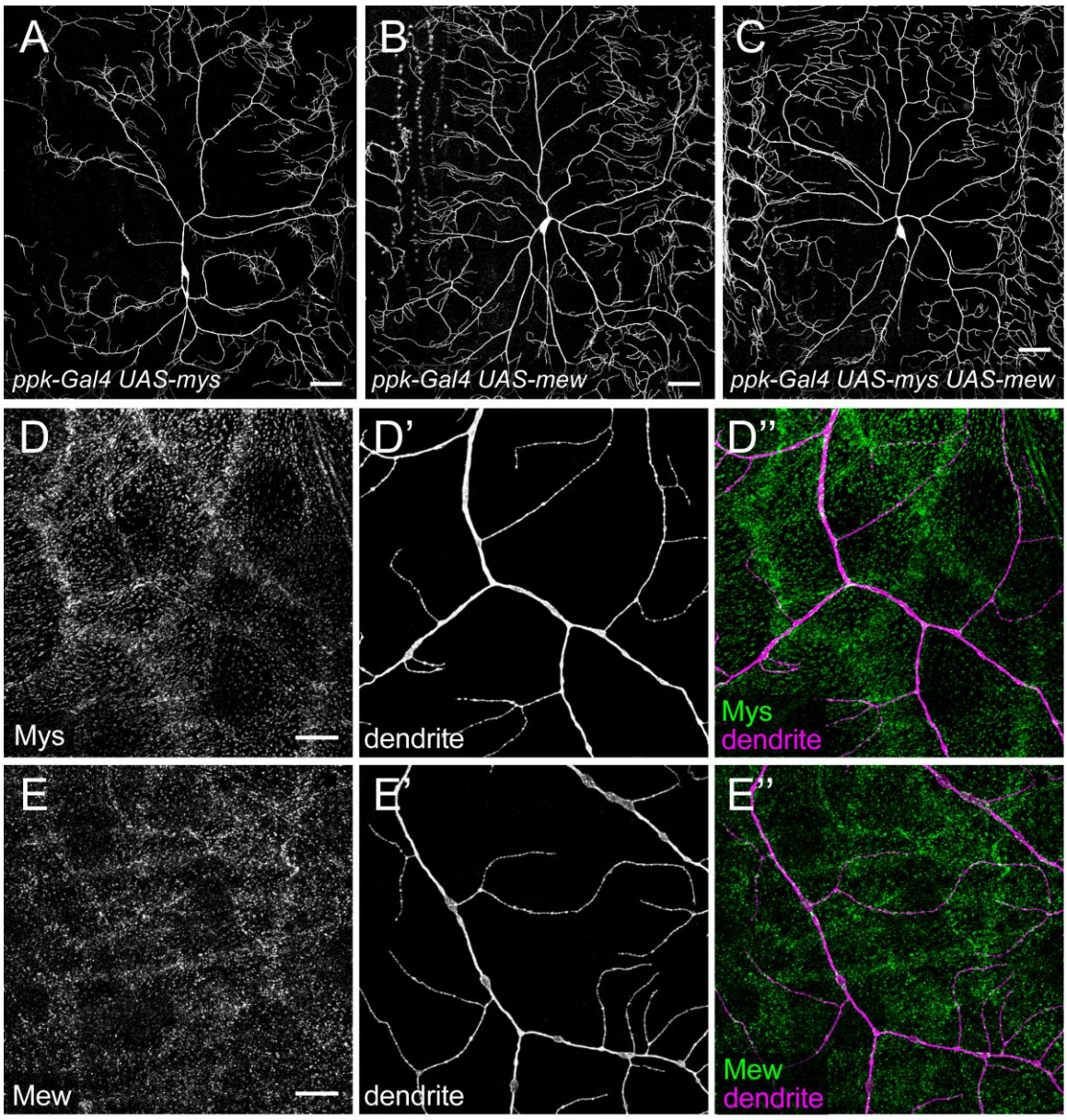

Figure S1. Integrin-overexpression phenotypes in ddaC and endogenous integrin expression, related to Figure 4.

(A-C) Representative dendritic patterns of ddaC neurons overexpressing *UAS-mys* (A), *UAS-mew* (B), or both *UAS-mys* and *UAS-mew* (C) driven by *ppk-Gal4*. Scale bars, 50 μm. (D-E") Immunostaining of endogenous Mys (D-D") and Mew (E-E") in the epidermis of *ppk-Gal4 UAS-CD4-tdTom* animals. Scale bars, 10 μm.

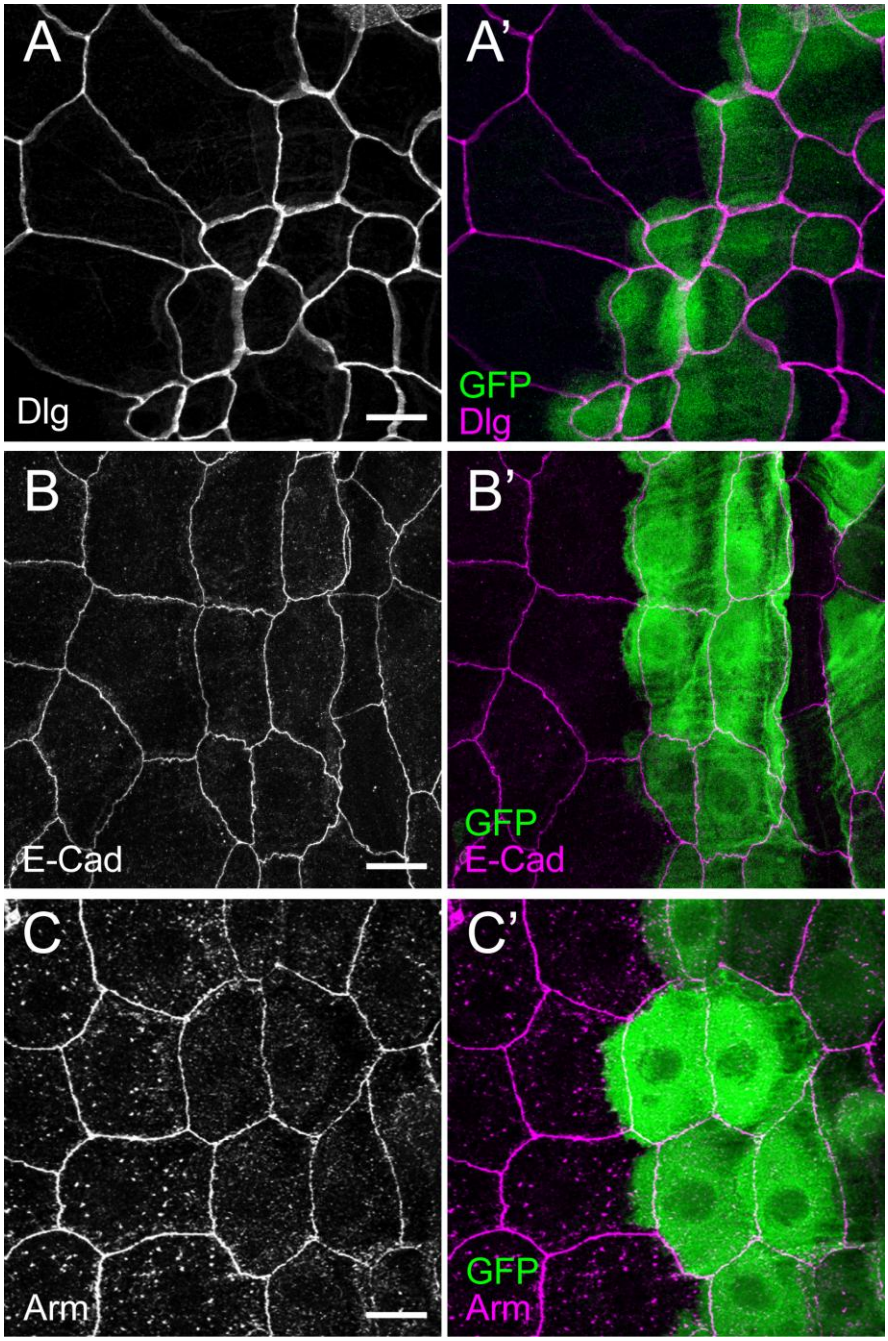

Figure S2. Expression of epithelial polarity markers in *wb* RNAi animals, related to Figure 5.

Expression of a septate junction marker, Discs large (Dlg) (A-A'), and adherens junction markers E-cadherin (E-cad) (B-B') and Armadillo (Arm) (C-C') in animals expressing *UAS-dcr-2*, *UAS-wb-RNAi*, and *UAS-EGFP* driven by *hh-Gal4*. The *UAS-wb-RNAi* expressing cells are labeled by GFP. The localization of Dlg, E-cad, and Arm at cell junctions appears normal. Scale bars, 20 μm.

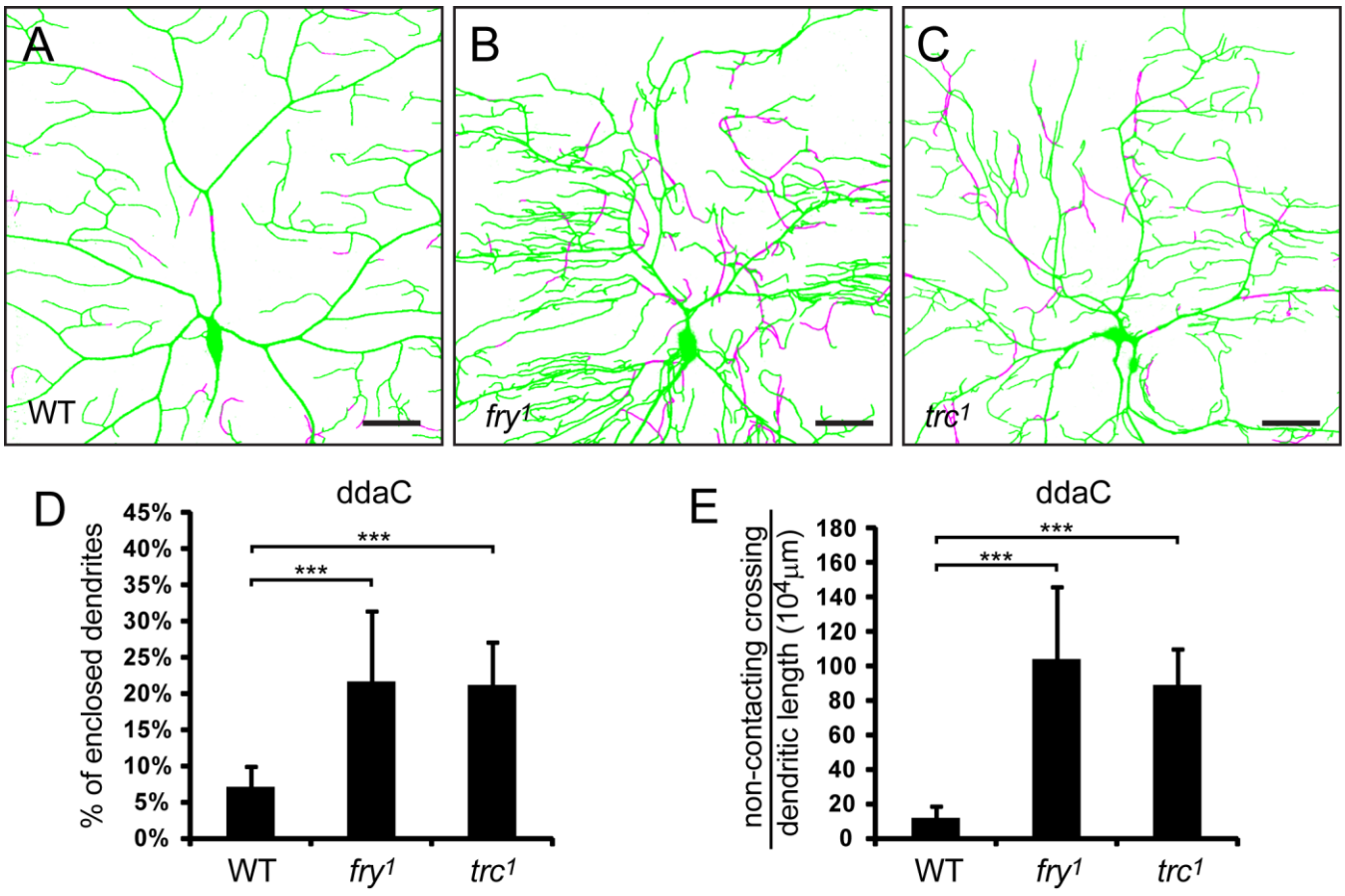

Figure S3. *fry* and *trc* act cell-autonomously to inhibit dendrite enclosure in the epidermis and non-contacting crossing, related to Figure 6

(A-C) Dendritic patterns of wildtype (A), *fry*^{1} (B), and *trc*^{1} (C) ddaC neurons generated with the MARCM system. The dendrites attached to the ECM are in green and enclosed dendrites are in magenta. Scale bars, 30 µm. (D) Quantification of enclosed dendrites in wildtype, *fry*^{1}, and *trc*^{1} mutant neurons. (E) Number of non-contacting crossings normalized to total dendritic length. The error bars represent standard deviations. ***P<0.001, one-way analysis of variance and Dunnett's test compared to the wildtype.

Movie S1. Dendrite-ECM spatial relationship in the wildtype, related to Figure 1

Animation showing cross sections of the epidermis scanning through the ddaC dendritic field at the dorsal midline of a wildtype 3rd instar larva. The dendrites are labeled by *ppk-CD4-tdTom* (magenta) and the ECM is labeled by *vkg-GFP*. The width of the dendritic field is 228 μm. The cross sections of the epidermis are oriented apical up.

Movie S2. Dendrite-ECM spatial relationship of a *mew* knock-down neuron, related to Figure 3

Animation of cross sections of the epidermis at the dorsal midline in an animal where *mew* is knocked-down from da neurons by $Gal4^{21\text{-}7}$. The labeling of dendrites and the ECM, section width and orientation are the same as in Movie S1.

Movie S3. Dendrite-epidermal cell spatial relationship in the wildtype, related to Figure 5

Animation showing cross sections of the epidermis scanning through the ddaC dendritic field at Hh-expressing epidermal cells in *hh-Gal4 UAS-EGFP UAS-dcr-2*. The dendrites are labeled by *ppk-CD4-tdTom* (magenta) and epidermal cells are labeled by EGFP expression. The width of the dendritic field is 129 μm. The sections are oriented apical up.

Movie S4. Dendrite-epidermal cell spatial relationship in *wb* RNAi, related to Figure 5

Animation showing cross sections of the epidermis scanning through the ddaC dendritic field at Hh-expressing epidermal cells in *hh-Gal4 UAS-EGFP UAS-dcr-2 UAS-wb-RNAi*. The labeling of dendrites and epidermal cells, section width and orientation are the same as in Movie S3.

Movie S5. Dendrite-ECM spatial relationship in fry^{1}/fry^{6}, related to Figure 6

Animation of cross sections of the epidermis at the dorsal midline in an fry^{1}/fry^{6} mutant animal. The labeling of dendrites and the ECM, section width and orientation are the same as in Movie S1.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transmission Electron Microscopy

The immunoEM procedures for animals expressing *ppk-CD4-tdTom* were modified from a protocol described previously (Lin et al., 1994). 3rd instar larvae were dissected in cold 0.1M Sorensen's phosphate buffer (SPB) and fixed in periodate-lysine-paraformaldehyde (PLP) fixative 30 min and then in 0.01% glutaraldehyde in SPB 10

min at RT. The fixed fillets were treated with 1mM glycine 10 min in SPB to quench free aldehyde groups. After washing with SPB containing 0.05% Saponin, the samples were stained sequentially with rabbit anti-DsRed, goat anti-rabbit Biotin-SP (1:400, Jackson ImmunoResearch), and Vectastain ABC kit (Vector Laboratories). To visualize HRP, samples were incubated in SPB containing 0.5mg/ml DAB and 0.003% H_2O_2 for 20 min at RT. A post-fixation in 2% glutaraldehyde in SPB for 1 hr is necessary to stabilizing the DAB product and the tissue structures for subsequent procedures. For staining of animals expressing HRP-DsRed-GPI, dissected larvae were fixed in 2% formaldehyde and 2% glutaradehyde in SPB for 1hr at RT. After washing, the samples were directly incubated in SPB/DAB/H_2O_2 to visualize HRP.

Both types of samples were then processed the same way for embedding and sectioning. The tissues were first post-fixed with 1% OsO_4 in SPB for 1hr, and then stained en bloc with 0.5% uranyl acetate for 30 min. After dehydration with ethanol series, the fillets were embedded in Eponate 12 resin (Ted Pella, Inc). Serial sections of 70nm thickness were cut and collected with standard procedures. The sections were stained with Sato's lead solution (Sato, 1968) for 10 min before imaging with a TECNAI 12 transmission electron microscope (Philips/FEI).

Image Analysis

All image stacks were first converted into the Imaris 3.0 format and then deconvoluted using Autoquant (MediaCybernetics) with adaptive PSF for 10 iterations. 3D reconstruction and rendering were then performed with Imaris (Bitplane). Dendrites contacting ECM were identified by colocalization of *ppk-CD4-tdTom* and *vkg-GFP* signals with the ImarisColoc module. The dendrites were traced with the FilamentTracer module. To determine if two crossing dendrites are contacting, image stacks were analyzed in slice view. As the diameter of high order branches (around 200nm) is smaller than the Z resolution (larger than 500nm), contacting dendrites should appear on the same focal plane. Therefore, two dendrites were deemed non-contacting if the brightest signals were located on two separate focal planes. The image stacks were projected in Imaris to generate 2D images, which were then inverted and/or converted to green or magenta colours in Photoshop (Adobe Systems Inc).

Immunohistochemistry

Antibodies used are mouse anti-Dlg (1:100, 4F3, DSHB), rat anti-E-cad (1:100, DSHB), mouse anti-GFP (1:200, JL-8, Clontech), rabbit anti-GFP conjugated to AF488 (1:500, Invitrogen). Secondary antibodies conjugated to DyLight dyes (Jackson ImmunoResearch) were used at 1:400 dilution.

MARCM Analysis

For MARCM analyses of *fry* and *trc*, *vkg-GFP*; *fry*^{1} *FRT80B/Tm6B* or *vkg-GFP*; *trc*^{1} *FRT80B/Tm6B* female flies were crossed with *hs-Flp/Y*; *Gal4*$^{21\text{-}7}$ *UAS-CD4-tdTom*; *tub-Gal80 FRT*^{80B}*/Tm6B* to generate marked neurons mutant for *fry* or *trc*, respectively.

SUPPLEMENTAL REFERENCES

Lin, D.M., Fetter, R.D., Kopczynski, C., Grenningloh, G., and Goodman, C.S. (1994). Genetic analysis of Fasciclin II in Drosophila: defasciculation, refasciculation, and altered fasciculation. Neuron *13*, 1055-1069.
Sato, T. (1968). A modified method for lead staining of thin sections. J Electron Microsc (Tokyo) *17*, 158-159.