Dynamic Decapentaplegic signaling regulates patterning and adhesion in the *Drosophila* pupal retina

DEV002972 Supplementary Material

Files in this Data Supplement:

- **Supplemental Table S1** - **Adobe PDF**
- **Supplemental Figure S1** - **Fig. S1. pMad staining in the *Drosophila* pupal retina is specific.** (**A-C**) Magnified view of a single ommatidium from a 20 hours APF retina stained with anti-pMad (A, red; C) and anti-Armadillo (B, green; C). Asterisks indicate the position of the primary pigment cells. (**D-I**) Clones of Mad^{12} were dissected at either 24 (D-G) or 31 hours APF (H,I) and stained with anti-pMad (E,G,I). Clonal tissue is marked by the absence of GFP (green; D,F,H) or outlined by dashed lines (E,G,I). p-Mad from the nuclei of cone cells (E), IPCs (G) and sensory bristles (I) was abolished in Mad^{12} clones, demonstrating the specificity of the antibody. D-G correspond to the same clone looking at the plane of either the cone cells (D,E) or IPC nuclei (F,G). Note that the nuclei of the IPCs and sensory bristles (G and I, respectively) are in the same focal plane but the p-Mad staining in the bristles becomes more evident after 24 hours APF (G). Time on the left refers to hours APF.
- **Supplemental Figure S2** - **Fig. S2. Dpp signaling does not affect Rst, Rho1 or Tubulin levels or localization in the *Drosophila* pupal retina.** Clones of tkv^{4} were dissected at 25 hours APF and stained with anti-Rst (red; **A**,**B**), anti-Rho1 (red; **C**,**D**) or anti-Tubulin (red; **E-H**). Clonal tissue is marked by the absence of GFP (green; B,D,F,H) or outlined by dashed lines (A,C,E) or arrows (G). Arrows in A,B point to areas of especially severe IPC patterning defects.
- **Supplemental Figure S3** - **Fig. S3. Rst regulates Tkv post-transcriptionally.** (**A-F**) *tkv* expression in retinas was assessed by placing a *tkv-lacZ* enhancer-trap line in either a control (A-C) or rst^{CT} background (D-F). *tkv* transcription was visualized by anti-β-galactosidase antibody staining (B,E, red; C,F); membranes were stained with anti-Armadillo (A,D, green; C, F). Full genotypes: (A-C) *+/+; tkv-lacZ/+*; (D-F) rst^{CT}*/Y; tkv-lacZ/+*. (**G**) Transcript levels of *tkv* (top panel) were determined by semi-quantitative RT-PCR from control (lanes 1, 2 and 3) and rst^{CT} retinas (lanes 4, 5 and 6). Different concentrations of template demonstrated that the reactions fell within the linear range of template versus PCR product. *rp49* transcripts were used as a loading control (bottom panel) and RNA from *GMR>tkv* retinas (lanes 7, 8 and 9) was used as a positive control. Genotypes

were as indicated. Time refers to hours APF. The asterisk in G indicates a non-specific product.

- **Movie 1** - **Movie 1. Visualizing pupal retinal morphogenesis.** Control movie (*GMR>α-Catenin-GFP*) ranging from 25 to 30 hours APF (see Materials and methods for details). Green pseudo-coloring highlights examples of neighboring IPCs and their cell shape and behavior as morphogenesis progressed in the pupal retina. Importantly, neighboring IPCs from control retinas were never observed to lose their contacts, a phenotype we commonly observed in *tkv* mutant retinas (see Movie 2). Brown pseudo-coloring highlights the normal complement of IPCs around a sensory bristle. Note that unlike in *tkv* mutant retinas (see Movie 2), only three IPCs contact a sensory bristle. This pupal retina at the end of the movie was similar to a wild-type eye from a similar developmental stage (see Fig. 1D). Furthermore, the resulting adult was viable and had eyes of wild-type appearance (not shown), suggesting that neither the control genotype nor the visualization process significantly affected pupal retinal morphogenesis or animal viability.

Movie 2 - **Movie 2. Abnormal morphogenesis in *tkv-IR* pupal retinas.** Movie from 25 to 30 hours APF to assess a *GMR>tkv-IR* experimental animal; the full genotype was *GMR>α-Catenin-GFP; UAS-tkv-IR(4X)/tkv*^{8}. See Materials and methods for experimental details. Pseudo-colored in green are examples of IPCs that detach from each other temporarily, leaving primary pigment cells from adjacent ommatidia in direct contact. Blue pseudo-coloring indicates examples of IPCs subject to abnormal cell shape changes. Brown pseudo-coloring highlights examples of an abnormal arrangement of IPCs around a sensory bristle. Arrows point to cell junctions that temporarily disappear. Note that the IPC patterning defects were comparable to the ones observed in retinas with reduction in different components of the Dpp signaling pathway (see Fig. 2, Fig. 3E-J), suggesting that imaging or co-expression of *α-Catenin-GFP* did not significantly modify the *GMR>tkv-IR* phenotype.