

## Methods and Supplementary Figures for:

### Wnt controls the medial-lateral subdivision of the *Drosophila* head

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## MATERIAL AND METHODS

***Drosophila* genetics.** Targeted gene expression manipulation was carried out using the GAL4/UAS system (1). *oc2-GAL4* is an ocellar complex-specific driver line (2). The *optix2/3-GAL4* driver (“optix-GAL4”) is expressed in the undifferentiated region of the eye domain within the EAD (3, 4). UAS lines used were UAS-dAxinA2-28 (5), UAS-dTCF<sup>DN</sup> (6), UAS-DEcad-5i (7), UAS-otdRNAi (8), UAS-ey (9), UAS-otd (Flybase: 0103635), UAS-GFP (10). The *wg* reporter *wg-Z* is described in (11). Larvae were raised at 29°C.

**Immunofluorescence.** Primary antibodies were: guinea pig anti-Otd (1/1000; gift of Tiffany Cook, WSU Center for Molecular Medicine and Genetics, Detroit, USA), rabbit anti-Optix (1/500; gift of Francesca Pignoni, Upstate Medical University, Syracuse, USA) and guinea pig anti Hth (1/59; GP51)(12). Rat-anti-Elav (1/1000, 7E8A10) and mouse anti-Ey (1/200) were from the DSHB, University of Iowa. Anti-mouse, anti-rabbit and anti-rat secondary antibodies, conjugated with Alexa 488, 568 or 647 were from Molecular Probes. Immunofluorescence in eye imaginal discs and embryos was carried out according to standard protocols. Image acquisition was carried out using a Leica SP2 AOBS confocal microscope. Images were processed with Photoshop CS5 (Adobe).

**X-gal histochemistry.** Discs of *wg-Z* larvae were dissected in cold PBS and fixed in 0.5% glutaraldehyde for 15 min on ice. X-gal staining was performed in 1× PBS, 0,1% Triton X-100 (PBT), 2 mM MgCl<sub>2</sub>, 5 mM K<sub>3</sub>Fe(CN)<sub>6</sub>, 5 mM K<sub>4</sub>Fe(CN)<sub>6</sub>, 1 mg/ml X-gal at 37°C. After staining, discs were washed in PBS and mounted in 80% glycerol. Adult cuticle X-gal staining was as in (13).

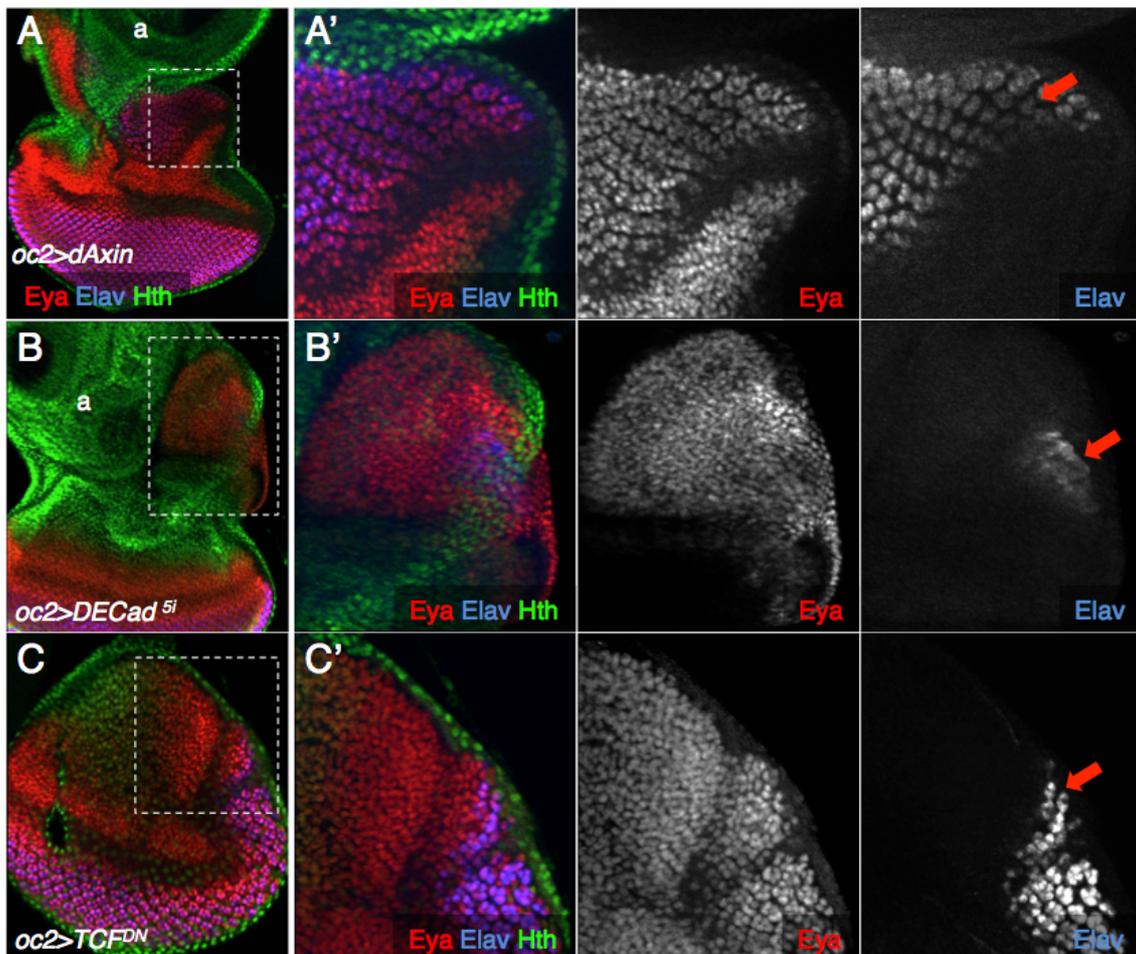
**Scanning electron microscopy (SEM).** Adult flies were transferred to 75% ethanol for 24 h at room temperature. Flies were dehydrated through an ethanol series (80%, 90%, 95% and twice 100%; 12–24 h each step). Flies were then air-dried and mounted onto SEM stubs covered with carbon tape and sputter-coated with gold (Edwards Six Sputter). Images were obtained using a JEOL 6460LV scanning electron microscope at the CITIUS facilities (Universidad de Sevilla).

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**SUPPLEMENTARY FIGURES.**

**Figure S1. Attenuation of the Wnt signaling pathway in the prospective head vertex causes the development of ectopic compound eyes.** Confocal images of imaginal discs of the indicated genotypes (A-C). The boxed regions are shown at higher magnification in (A'-C'). Discs are stained for the retinal determination gene *Eya* (red) and the photoreceptor marker *Elav* (blue). *Hth* (*homotorax*, green), which marks the prospective head capsule and antenna ("a")(12), is used as a counterstain. In (A'-C') merged, *Eya* and *Elav* channels are shown. Red arrows point to *Elav*-expressing photoreceptors within the ectopic eye tissue.



**Figure S2. Ey and Otd are coexpressed in EAD and this coexpression might specify periorbital cuticle.** (A,C,D) Dorsal views of adult heads from wildtype (A), *oc2>ey* (C) and *optix>otd* individuals (D). *oc2-GAL4* is a dorsal head specific driver, while *optix2/3-GAL4* (“*optix*”) drives expression in the undifferentiated region of the eye primordium. The periorbital cuticle (red double-headed arrow) is extended in *oc2>ey* heads. Similarly, when *otd* is expressed in the developing eye, head-like cuticle develops at the expense of the compound eye (CE). (B) Confocal image showing the boundary between the Otd- and Ey-expressing regions in the eye-antennal disc of a late L3 wild type larva. There is a region of overlap between Otd and Ey domains that corresponds to the prospective periorbital region (bracketed). Compound eye (CE) is pseudocolored in red.

