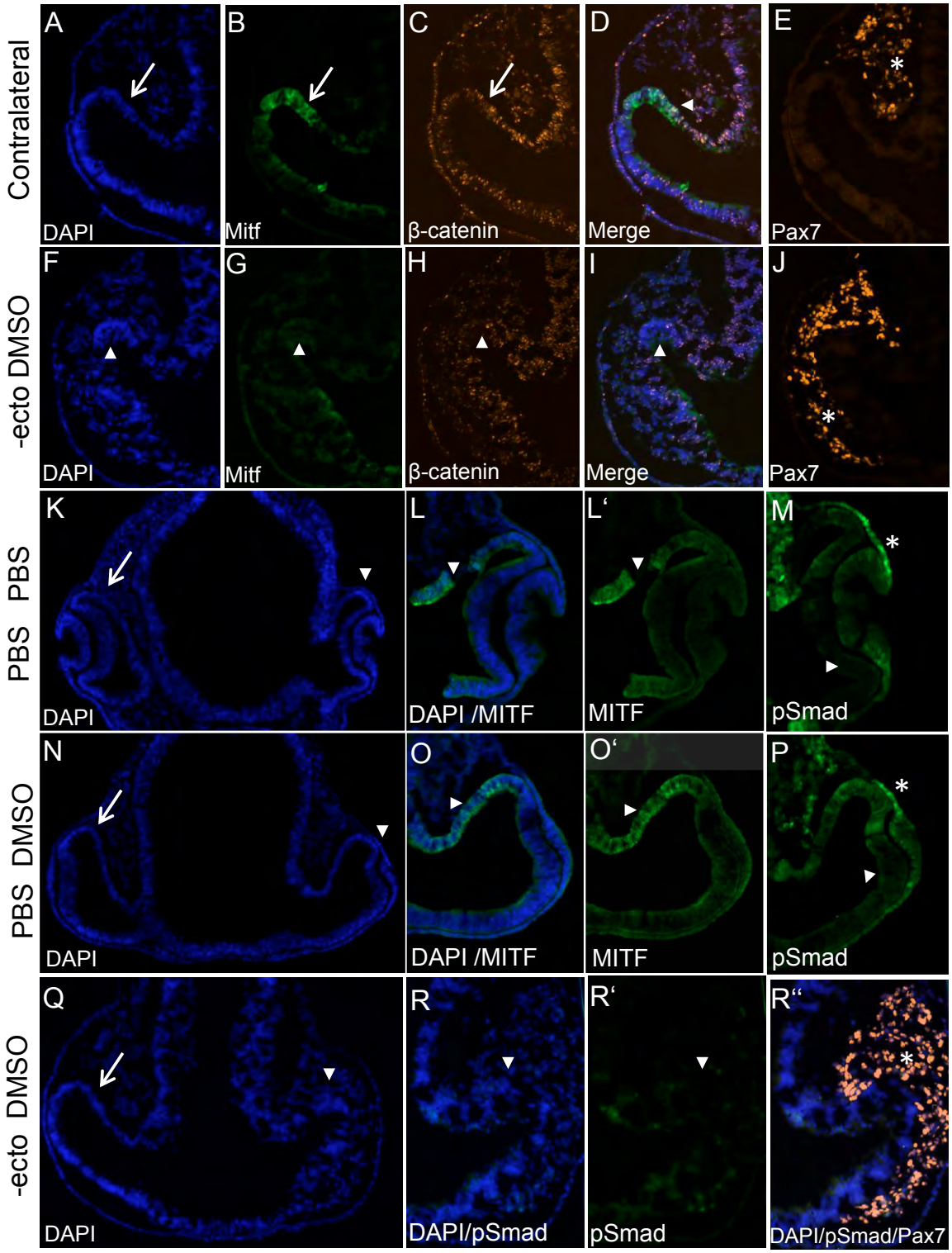

Fig. S1. Expression of proteins following implantation of PBS- and/or DMSO-soaked beads in the presence or absence of the surface ectoderm. (A-E) Contralateral ov (arrows) at stage 12/13, showing MITF (B), β-catenin (C) and Pax7 (E) expression following removal of the surface ectoderm and implantation of a DMSO-soaked bead. The overlay shown in (D) shows that at this stage nuclear staining of β-catenin starts to co-localise with a few MITF-positive cells (arrowhead). (E) In a parallel section Pax7 marks the neural crest-derived mesenchyme dorsally to the ov (asterisk). (F-J) Expression of MITF (G), β-catenin (H), and overlay (I) following surface ectoderm removal and implantation of a DMSO-soaked bead. MITF expression is lost (arrowhead in G), although in this case Pax-7 positive mesenchymal cells are surrounding the entire ov (asterisk in J). (K-P) Effects on protein expression at early optic cup stages, following implantation of two beads soaked in PBS (K-M), or one in PBS and one in DMSO (N-P). MITF expression is detected in the dorsal RPE (arrowheads in L, L′; O, O′) and pSmad1 labelling is not detected ventrally (arrowheads in M and P). Note that pSmad labelling is detected in the presumptive RPE, dorsal NR and dorsal surface ectoderm (asterisks). (Q-R”) Effect on protein expression following surface ectoderm removal and implantation of two beads, one soaked in PBS and one soaked in DMSO. In the absence of the surface ectoderm, pSmad is not detected in the ov (arrowheads in R-R”), although Pax-7 positive mesenchymal cells (asterisk) are surrounding the optic vesicle.

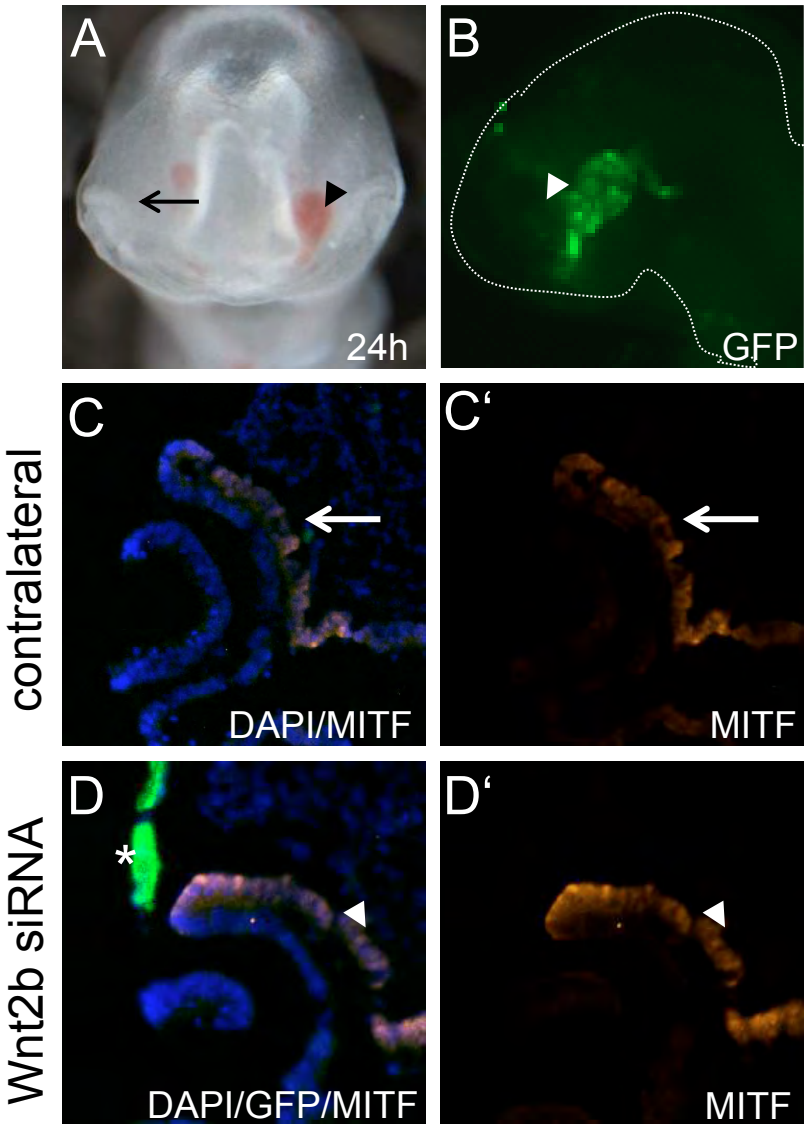

Fig. S2. Electroporation of Wnt2b siRNA does not affect MITF expression. (A) Frontal view of an embryo one day following electroporation of Wnt2b siRNA at ov stages. On the treated (arrowhead) and untreated (arrow) side an eye cup developed. (B) The electroporated ov of this embryo is GFP-positive (arrowhead). (C, C′) MITF expression in the RPE of the untreated side (arrow). (D, D′) MITF expression (arrowheads) appears to be unaffected following electroporation of Wnt2b siRNA at ov stages. The asterisk marks the GFP-positive surface ectoderm.

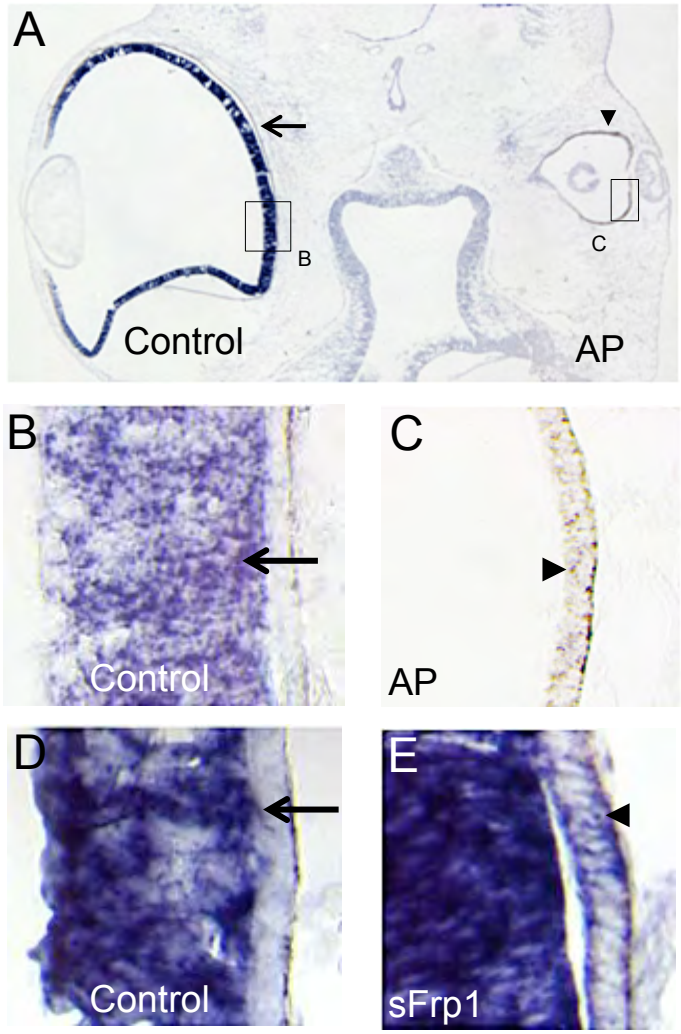

Fig. S3. Effects on *Vsx2* expression following activation and inhibition of Wnt signalling at ov stages. (A) Expression of *Vsx2*, 3 days following GSK-3β inhibition at ov stages. On the Alsterpaullone (AP)-treated side, a small microphthalmic and pigmented eye vesicle (arrowhead) develops with decreased *Vsx2* expression, when compared to the untreated side (arrow). (B) Higher magnification of the untreated eye showing *Vsx2* expression in the NR (arrow). (C) Following GSK-3β inhibition, *Vsx2* expression is dramatically downregulated and the distal ov is pigmented and has got RPE-like morphology (arrowhead). (D) *Vsx2* expression of a stage 25 chick eye. *Vsx2* expression is restricted to the NR and expression is not detected in the RPE (arrow). (E) Following implantation of beads soaked with the Wnt inhibitor sFrp1, faint *Vsx2* expression is detected in the RPE (arrowhead).