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SUPPLEMENTARY MATERIAL

corresponding to:

Eye-specific expression of an ancestral jellyfish *PaxB* gene interferes with *Pax6* function despite its conserved Pax6/Pax2 characteristics

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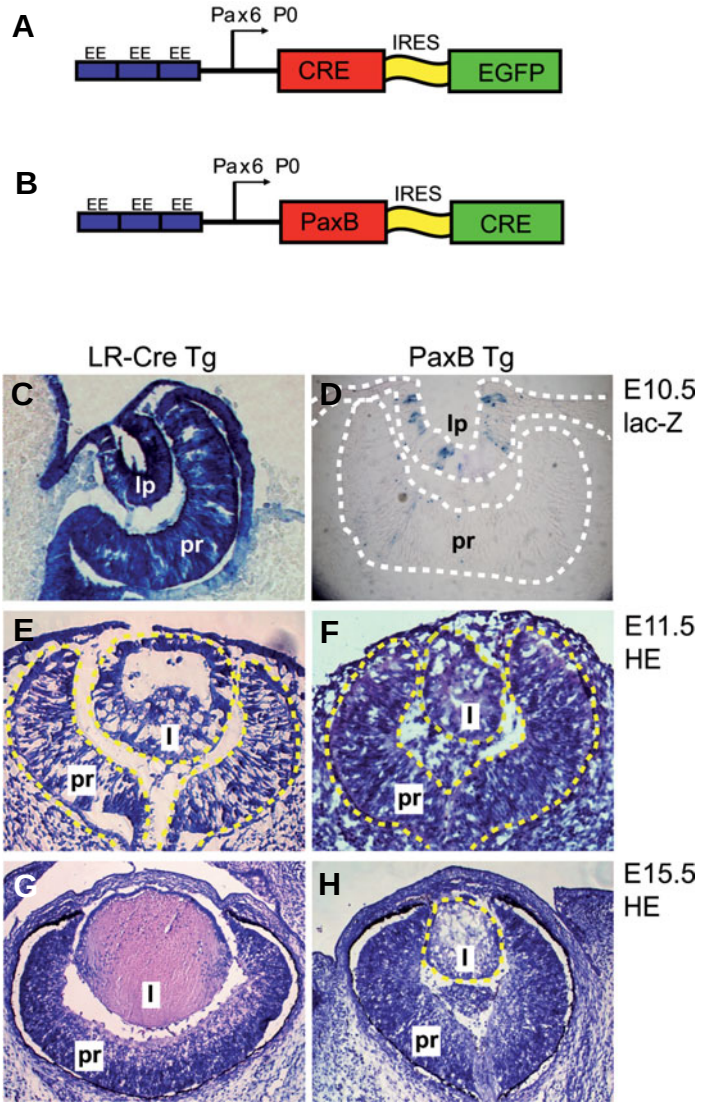
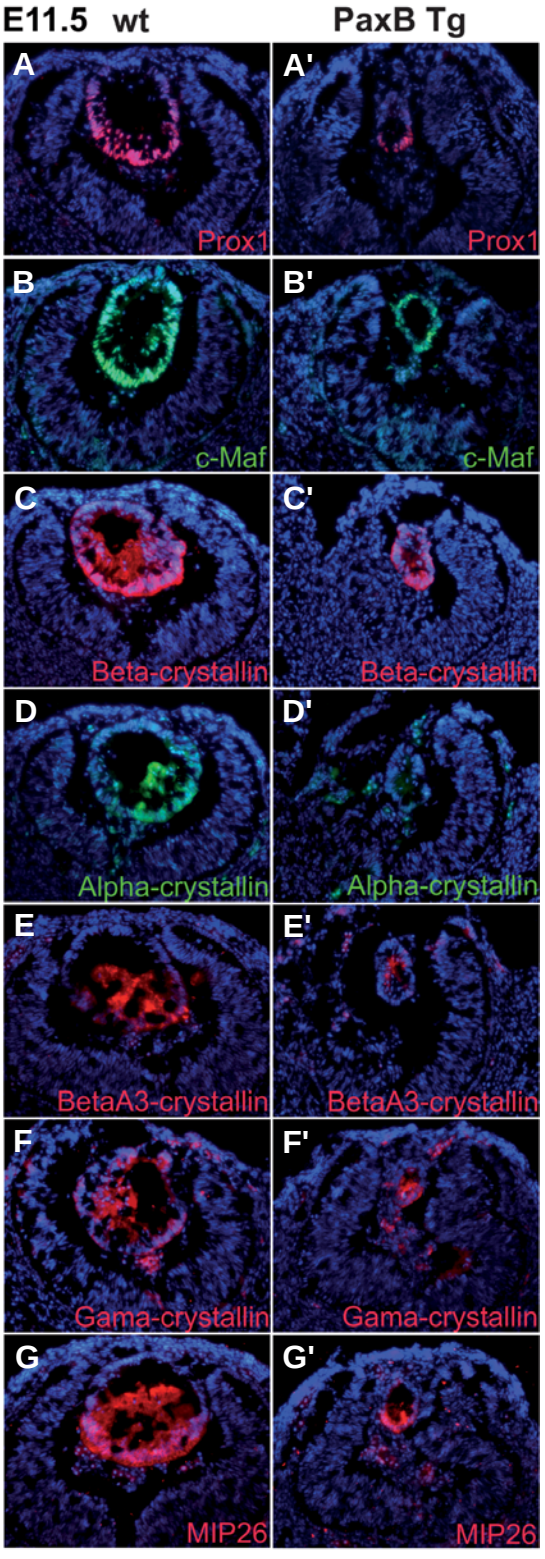
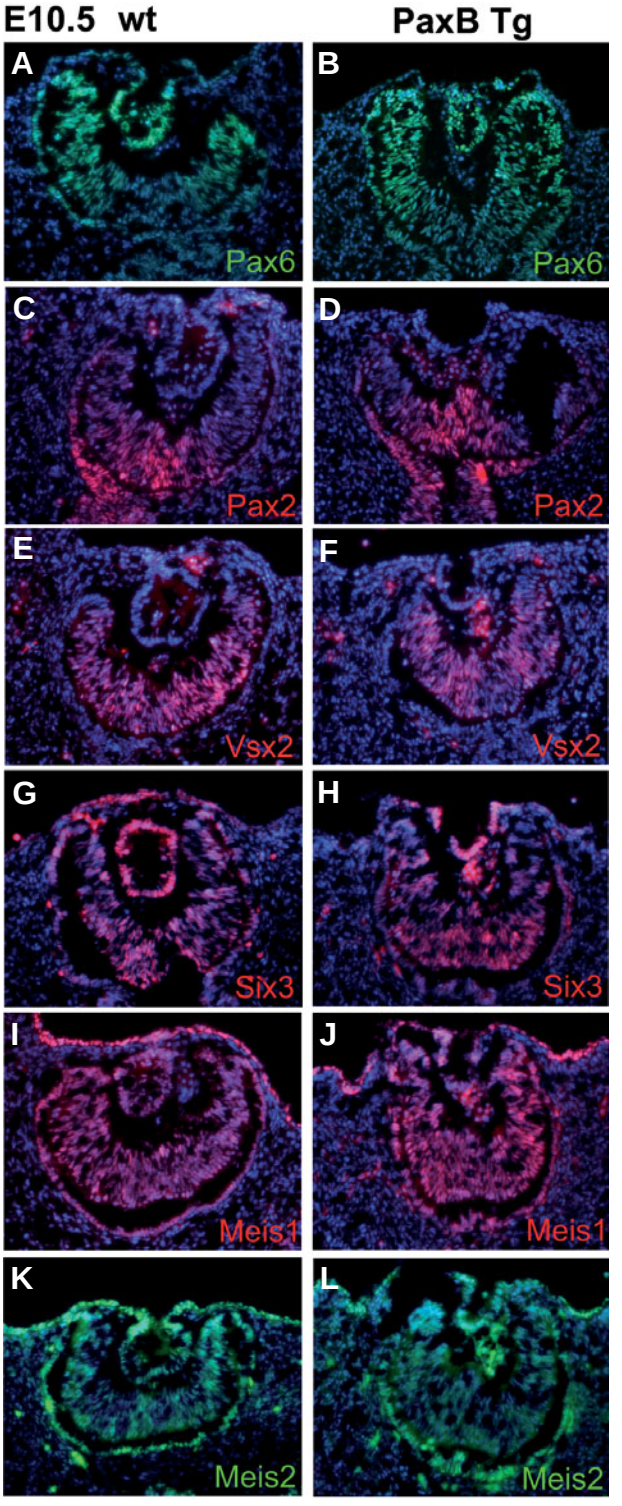


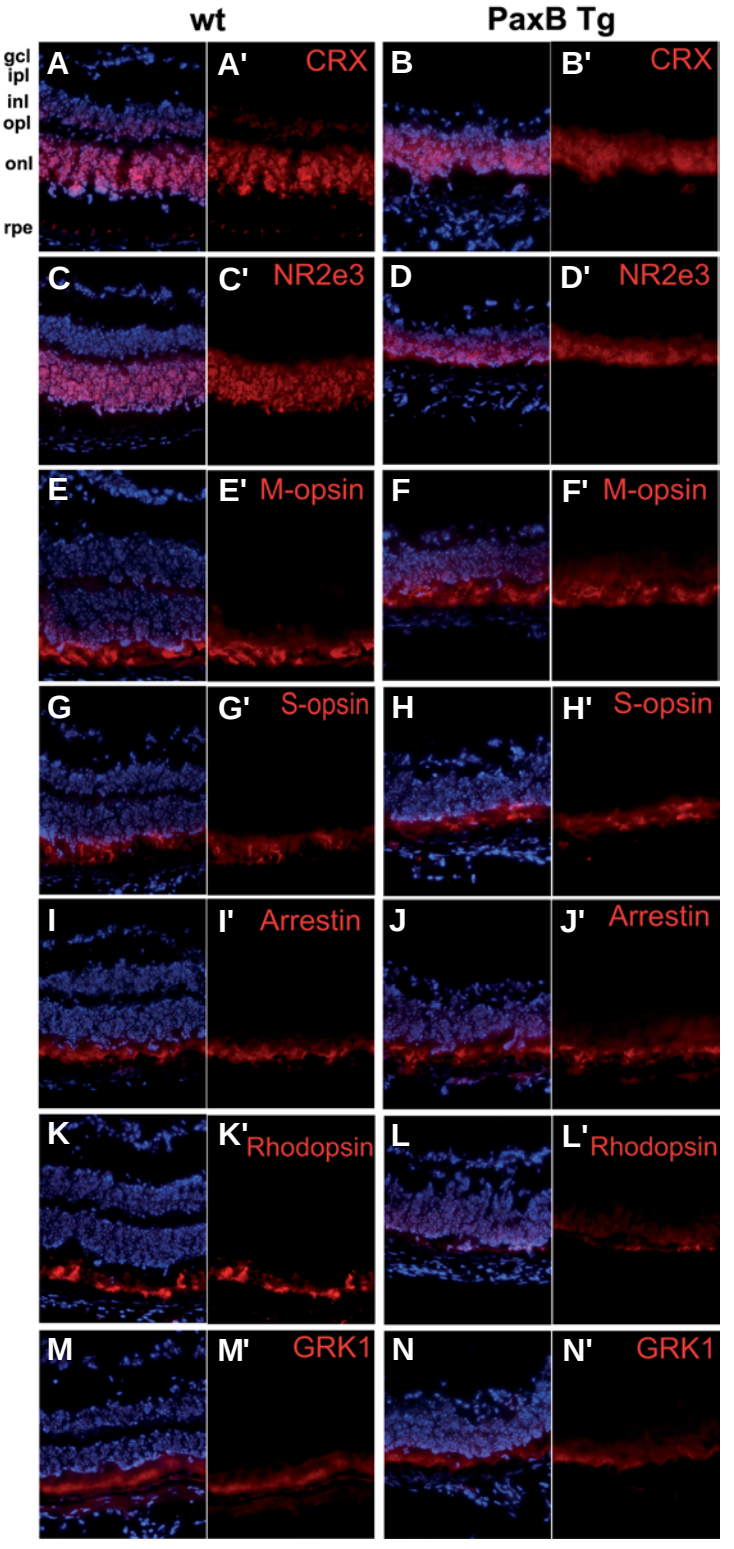
Supplementary Fig. 1. Normal gross morphology of the control LR-Cre transgenic eyes. *Schematic diagram of LR-Cre* **(A)** *and PaxB* **(B)** *transgenic constructs. Three copies of the lens-specific ectoderm enhancer (EE) derived from the* Pax6 *gene were cloned upstream of the Pax6 P0 minimal promoter to drive the expression of Cre and EGFP* **(A; LR-Cre Tg)** *or the expression of PaxB and Cre cDNAs* **(B; PaxB Tg)** *in bicistronic constructs via internal ribosomal entry site (IRES). Cre recombinase activity in LR-Cre* **(C)** *and* PaxB *transgenic* **(D)** *mice (detected using ROSA26R reporter mice) at E10.5. In comparison to* PaxB *transgenic mice the Cre recombinase activity in LR-Cre transgenic mice is much stronger in all developing eye tissues. Note the intensive staining in the presumptive retina of LR-Cre transgenic mice. Cryosections at the indicated embryonic stages from the LR-Cre* **(E,G)** *and* PaxB *transgenic* **(F,H)** *mice stained with hematoxylin and eosin. The morfology of E11.5 and E15.5 LR-Cre transgenic eyes appears normal as compared to* PaxB *transgenic mice despite stronger expression of Cre. Abbreviations used in this are as follows: l, lens; lp, lens pit; pr, presumptive retina.*



Supplementary Fig. 2. Lens specific transcription factors and crystallins are reduced in *PaxB* transgenic mice. *Cryosections of wild-type* **(A,B,C,D, E,F,G)** *and* PaxB *transgenic* **(A',B',C',D', E',F',G')** *embryos at E11.5 stained for Prox1* **(A,A')**, *cMaf* **(B,B')**, *β-crystallin* **(C,C')**, *α-crystallin* **(D,D')**, *βA3-crystallin* **(E,E')**, *γ-crystallin* **(F,F')** *and MIP26* **(G,G')**, *and counterstained with DAPI (blue). Lower immunoreactivity of all above mentioned markers and smaller lens was observed in the* PaxB *transgenic mice.*



Supplementary Fig. 3. Transcription factors important for the eye development were expressed normally in *PaxB* transgenic mice. *Cryosections of wild-type* **(A,C,E,G,I,K)** *and PaxB transgenic* **(B,D,F,H,J,L)** *embryos at E10.5 were stained for Pax6 (Covance,* **A,B***), Pax2* **(C,D)***, Vsx2 (Chx10)* **(E,F)***, Six3* **(G,H)***, Meis1* **(I,J)** *and Meis2* **(K,L)***, counterstained with DAPI (blue). Pax6, Pax2 and Vsx2 (Chx10) staining appeared normal in the* PaxB *transgenic mice. No difference was observed in the expression Six3, Meis1 and Meis2, transcription factors acting upstream of Pax6.*



Supplementary Fig. 4. The photoreceptor cells were not affected in the *PaxB* transgenic retina. *Immunostaining of the* PaxB *transgenic* **(right panels)** *and wild-type* **(left panels)** *adult retinal sections. Applied antibodies were as follows: CRX (cone-rod homeobox containing gene,* **A-B'***), NR2e3 (protoreceptor specific nuclear receptor,* **C-D'***), M-opsin (M-cone photoreceptors,* **E-F'***), S-opsin (S-cone photoreceptors,* **G-H'***), arrestin (rod-photoreceptors,* **I-J'***), rhodopsin (rod-photoreceptors,* **K-L'***), GRK1 (rhodopsin kinase,* **M-N'***). The nuclei were counterstained with DAPI. No difference was apparent in the photoreceptor cells using a variety of photoreceptor markers between wild-type and* PaxB *transgenic retinas. Abbreviations used in this figure are as follows: gcl, ganglion cell layer; inl, inner nuclear layer; ipl, inner plexiform layer; onl, outer nuclear layer; opl, outer plexiform layer; rpe, retinal pigment epithelium.*