Supplementary Information

The Retinol Binding Protein Receptor 2 (Rbpr2) is required for Photoreceptor Outer Segment Morphogenesis and Visual Function in Zebrafish

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Supplementary Information

Supplementary Figure S1: Rbpr2 mRNA expression patterns in staged zebrafish larvae

(**A**) Rbpr2 expression patterns were determined in staged zebrafish larvae by semi-quantitative RT-PCR. Total RNA from zebrafish larvae (1 - 5.5 dpf.) were subjected to semi-quantitative RT-PCR using zebrafish *rbpr2, stra6* and *gapdh* gene specific primers. Amplified PCR products were electrophoresed on 2 % agarose gels, alongside a low-molecular weight DNA ladder. Zebrafish *rbpR2* mRNA was observed as being expressed from early to late larvae stages. A portion of Zebrafish *stra6* was amplified and used as positive control. A portion of zebrafish *gapdh* was also amplified at same developmental time points and this served as the endogenous loading control.

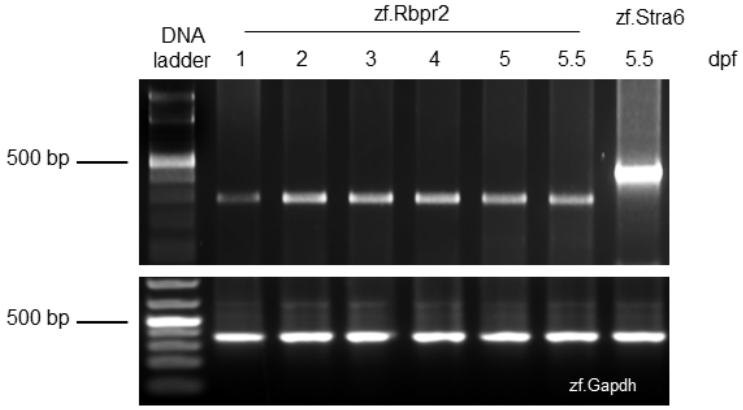
Supplementary Figure S2: Eye sections of zebrafish at 5 dpf from rescue experiments

Histological analysis of *rbpr2*^{musc97} mutant larvae eyes after rescue experiments at 5 dpf showed normal retinal lamination and eye patterning which were comparable to WT animals.

Supplementary Figure S3: ZIRC *rbpr2* mutants (*rbpr2*^{sa10706}) show eye phenotypes consistent with retinol deficiency

A *rbpr2* mutant zebrafish line (G>A mutation; *rbpr2*^{sa10706}) from the Zebrafish International Resource Center (ZIRC) which affects the essential splice site of exon

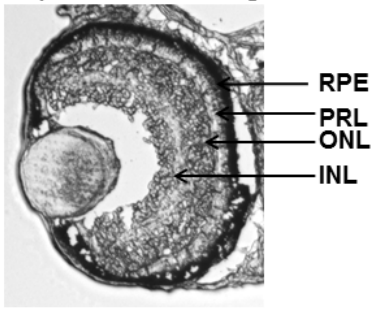
5/intron 6, was obtained and analyzed by light microscopy, histology and immunohistochemistry for cones at 5.5 dpf. The resulting eye phenotype observed was similar to the TALEN generated *rbpr2* mutant phenotype (*rbpr2*^{musc97}) described. *Rbpr2*^{sa10706} mutants showed gross defects, which included: *hydrocephaly; **smaller eyes, ***pericardial edema and ****slight tail curvature.



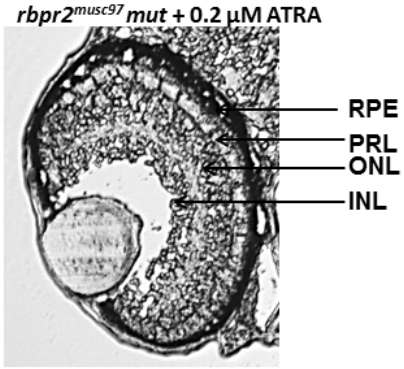
Semi-quantitative PCR at 25 cycles

Supplementary Figure 1

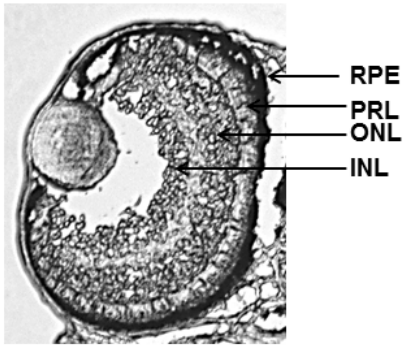
rbpr2^{musc97} *mut* + 150 ng mRNA



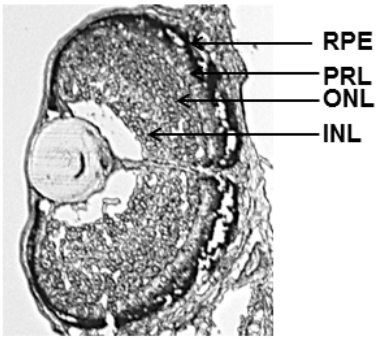
rbpr2^{musc97} *mut* + 0.2 µM ATRA



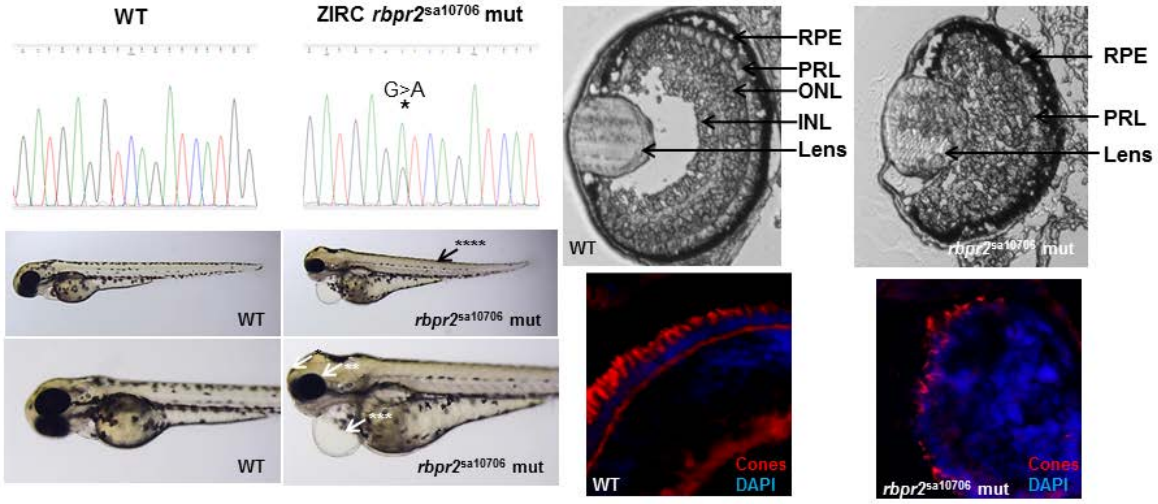
rbpr2^{musc97} *mut* + 250 ng mRNA



rbpr2^{musc97} *mut* + 0.5 µM ATRA

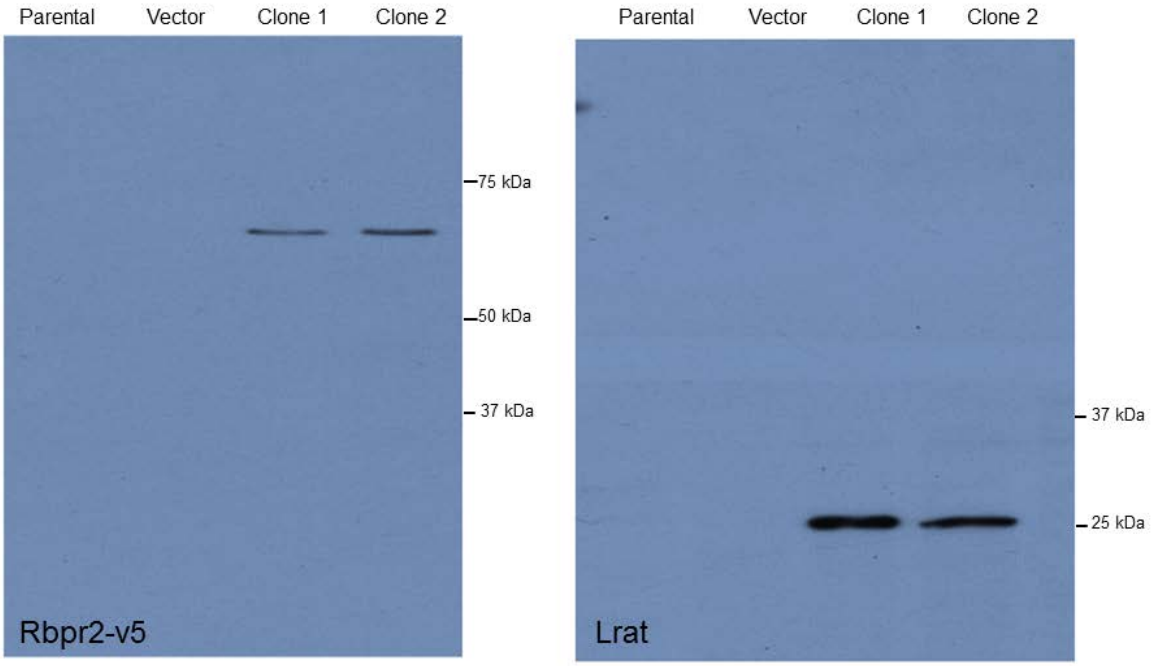


Supplementary Figure 2

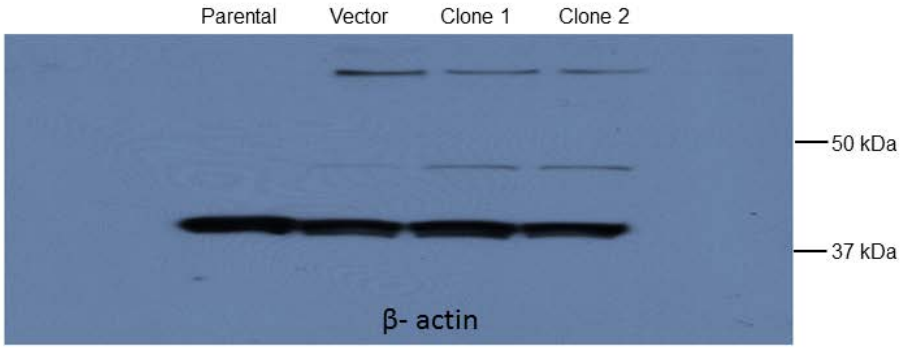


Supplementary Figure 3

Full image of unedited gel showing expression of V5-tagged Rbpr2- (V5 antibody) and LRAT in stable cell lines: Supporting Figure 1A



Full image of unedited gel showing expression of β-actin in stable cell lines: Supporting Figure 1A



Full image of SDS-PAGE gel and Commassie Blue staining showing expression of purified RBP4 protein: Supporting Figure 1D