Supporting Information

Fig S1. *Specificity of gene-specific primers of zebrafish RPE65a, 13cIMH and RPE65c.* To distinguish the expression of RPE65a, 13cIMH and RPE65c in the retina separately, we designed gene specific primers (see Fig. 1 broken lines arrows and TABLE 1). The specificity of the designed primers was tested by PCR using 1 ng of each cDNA clone. PCR was performed with Taq DNA polymerase (Roche, Indianapolis, IN) at 94˚C for 5 min followed by 35 cycles of 94˚C for 30 sec, 58˚C for 30 sec, and 72˚C for 30 sec. The sizes of the PCR products were confirmed by 2.0% of agarose gel electrophoresis. Only specific primers (left side: RPE65a gene specific primer, middle: 13cIMH, right: RPE65c) and a matched template (Nc; negative control without template, RPE65a, 13cIMH and RPE65c) combination amplified the expected size of products.

Fig. S2. *Immunostaining of zebrafish retinal section using antibodies for GS and RPE65c.* The images represent RPE65c staining **(1)**, GS staining **(2)**, merged RPE65c and GS staining **(3)**, and DAPI staining **(4)**, respectively. No significant RPE65c signals were observed in the RPE layer, whereas the signals were detected in inner retina in the region between the ONL and GCL. RPE, retinal pigment epithelium; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 20 μm.

Fig S3. *Hypothesized molecular mechanisms of our in vitro assay systems and intra-retinal visual cycle.* **(A)** Our originally designed *in vitro* assay system (1), which mimics

the *in vivo* condition. The starting material, atROL, is converted to atRE by LRAT and further catalyzed to 11cROL and 13cROL by RPE65. 11cROL is stabilized by CRALBP and 13cROL can be re-esterified by LRAT. **(B)** Schematic diagrams of a recently developed method which uses an atRE incorporated in liposome as the substrate without LRAT (2). Both 11cROL and 13cROL are generated, but 11cROL will be stabilized by CRALBP.

References

1. Moiseyev, G., Chen, Y., Takahashi, Y., Wu, B. X., and Ma, J. X. (2005) RPE65 Is the Isomerohydrolase in the Retinoid Visual Cycle, *Proc. Natl. Acad. Sci. USA 102*, 12413-12418.
2. Nikolaeva, O., Takahashi, Y., Moiseyev, G., and Ma, J. X. (2009) Purified RPE65 shows isomerohydrolase activity after reassociation with a phospholipid membrane, *FEBS J. 276*, 3020-3030.

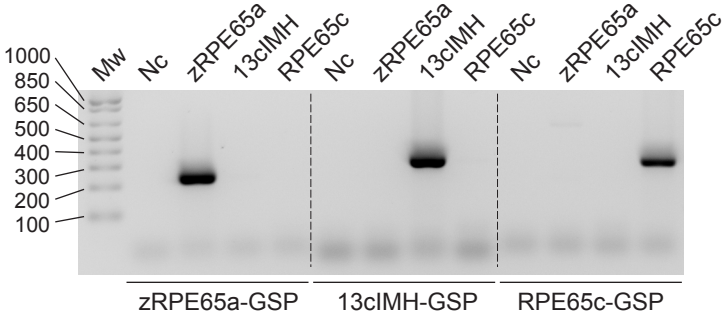
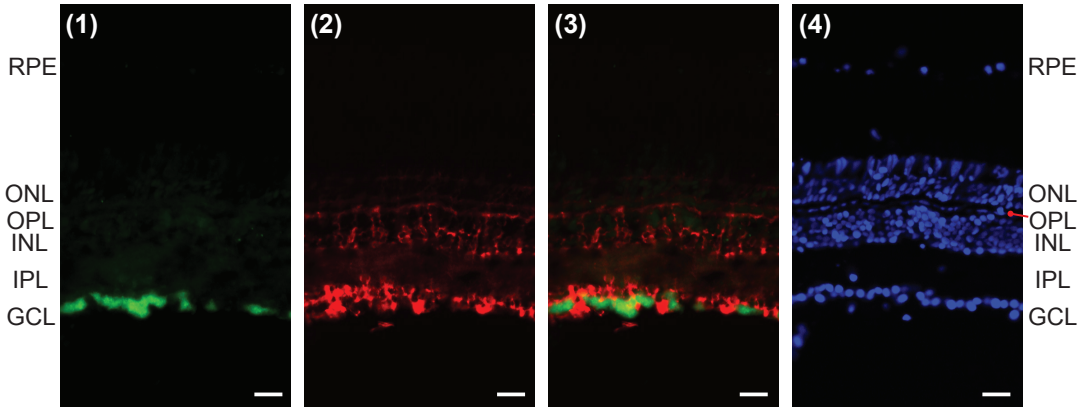


Fig. S1 Y. Takahashi et al



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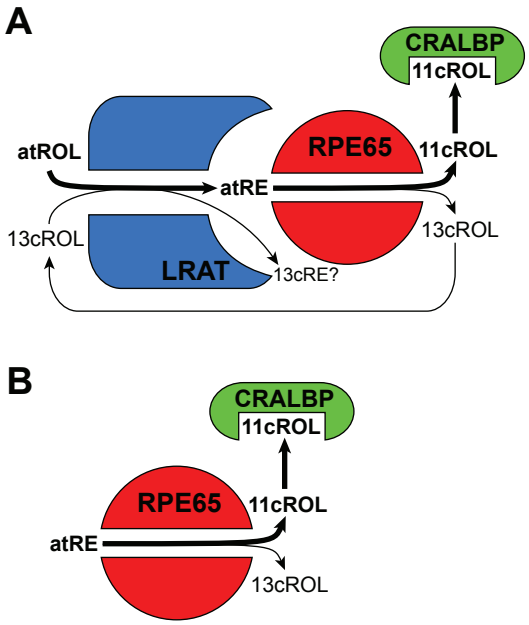

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