Supporting Information

Supplemental Figure Legends

Figure S1. *The effects of LRAT on the in vitro activity assay of human RPE65 and 13cIMH.* Plasmid transfection of human RPE65 and 13cIMH into 293A-LRAT [1] and 293A cells, Western blot analysis and *in vitro* enzyme activity assays using atRE-incorporated liposomes were performed as described in Materials and Methods. Following the normalization by the RPE65 or 13cIMH expression levels (**a**), the human RPE65 in 293A cells (no-LRAT) generated both of the 11-*cis* and 13-*cis* retinol peaks (**b**). In contrast, activity of human RPE65 in 293A-LRAT cells exhibited a higher 11cROL peak but no 13cROL peak (**c**). On the other hand 13cIMH in 293A cells showed a significantly higher peak (**d**) of 13cROL than that of 293A-LRAT cells, but no 11cROL (**e**). It is likely that the generated 13cROL is used as the LRAT substrate and is esterified back to retinyl ester. As 11cROL was not an efficient substrate [2] and CRALBP can not stabilize 13cROL, LRAT selectively esterifies less 11cROL. Peak 1; atRE, 2; 11cROL, 3; 13cROL, 4; atROL.

Figure S2. *Immunohistochemistry of the cross section at the optic tectum of zebrafish brain.* **(1)** The diagram shows zebrafish brain (modified from Castro, A. et al. [3]) and a line indicates the cutting line. Tel; Telencephalon, OT; Optic Tectum CB; Cerebellum. **(2)** A phase contrast image of a cross section of zebrafish brain. **(3, 4)** The brain section was incubated without the monoclonal antibody for 13cIMH (Negative control, **3**; FITC channel, **4**; DAPI). **(5-7)** The brain section was incubated with the monoclonal antibody for 13cIMH. Green fluorescence signals, indicated by red arrows, for 13cIMH (**5**; 13cIMH, **6**; DAPI, **7**; merged) were detected in the preventricular grey zone (PGZ) of optic tectum (OT). Scale bar = 200 μm.

Figure S3. *Specificity of the anti-RPE65 antibodies to recombinant zebrafish 13cIMH.* **(a)** The expression plasmids were transfected into 293A cells and cultured for 48 hours. The cells were harvested, equal amounts (20 μg) of total cellular protein were resolved by SDS-PAGE, and the protein expression was confirmed by Western blot analysis with a polyclonal anti-human RPE65 [4], mouse monoclonal anti-His-tag, and goat polyclonal anti-β-actin antibodies at same time. **Pc**, positive control (bovine RPE microsomal protein); **Nc** negative control (cells expressing RFP); **Hum**, human RPE65; **zRPE65**, zebrafish RPE65 without 6xHis-tag; **13cIMH**, zebrafish 13cIMH without 6xHis-tag; **zRPE65-His**, zebrafish RPE65 with 6xHis-tag; **13cIMH-His**, zebrafish 13cIMH with 6xHis-tag. Subcellular fractionation was performed as explained in Materials and Methods. The polyclonal antibody recognized zebrafish 13cIMH but not zebrafish RPE65. **(b)** The cells expressing 13cIMH were fractionated. The same amounts of protein from each fraction (5 μg) were blotted with a monoclonal anti-human RPE65 antibody (Millipore, Billerica, MA) and a rabbit polyclonal antibody to calnexin (ER membrane marker, Abcam, Cambridge, MA). The monoclonal antibody recognized zebrafish 13cIMH but not zebrafish RPE65 (zRPE65). **T**; total cell lysates, **C**; cytosolic, **M**; membrane, **N**; nuclear fractions and **S**; detergent-insoluble fraction including cytoskeleton and inclusion body.

References

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3. Castro A, Becerra M, Manso MJ & Anadon R (2006) Calretinin immunoreactivity in the brain of the zebrafish, Danio rerio: distribution and comparison with some neuropeptides and neurotransmitter-synthesizing enzymes. II. Midbrain, hindbrain, and rostral spinal cord. *J Comp Neurol* **494**, 792-814.
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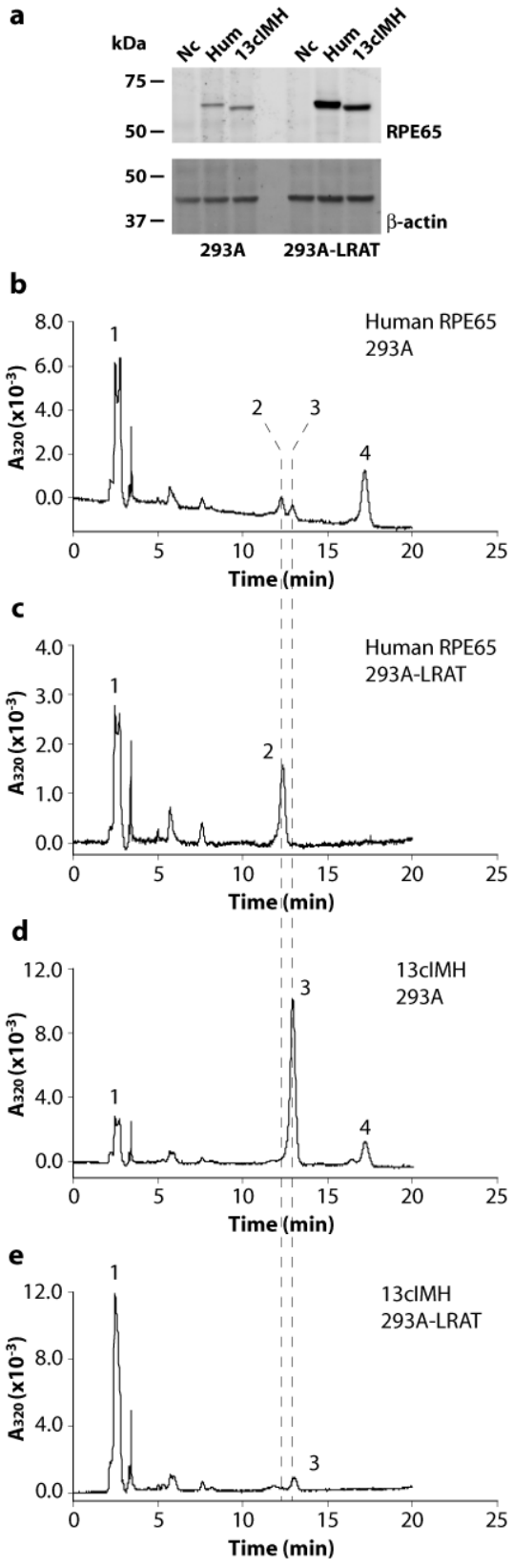

Fig. S1 Y. Takahashi et al

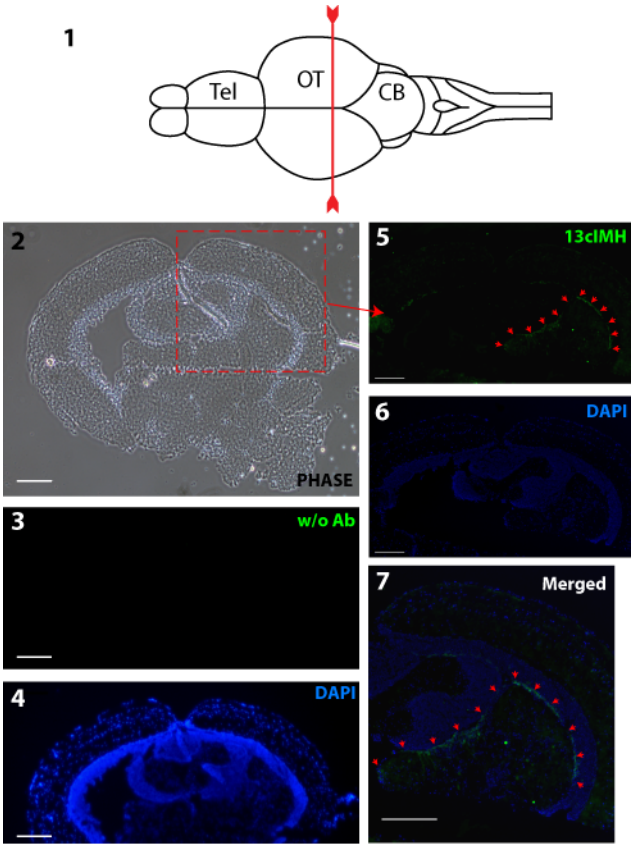

Fig. S2 Y. Takahashi et al

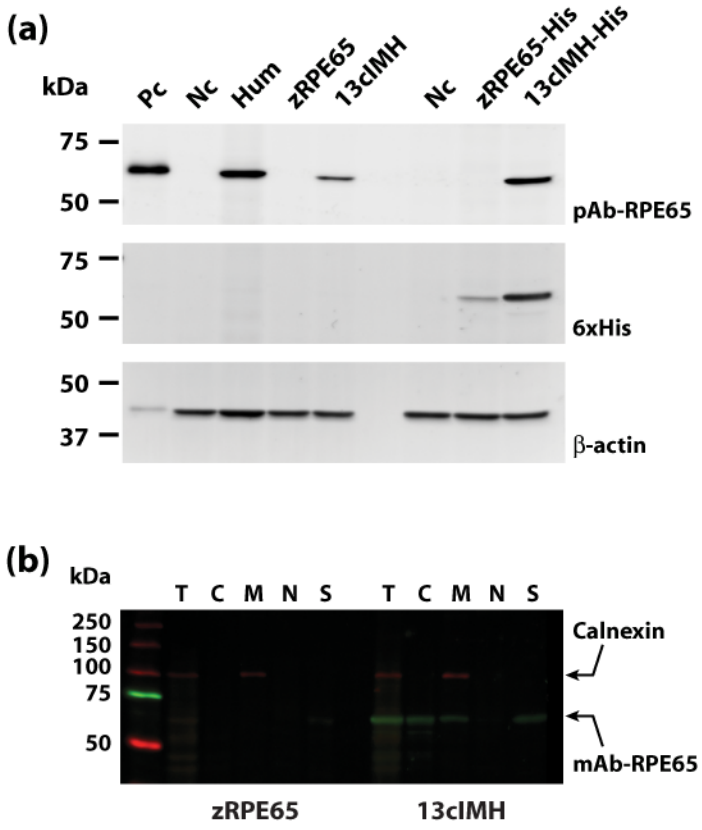

Fig. S3 Y. Takahashi et al