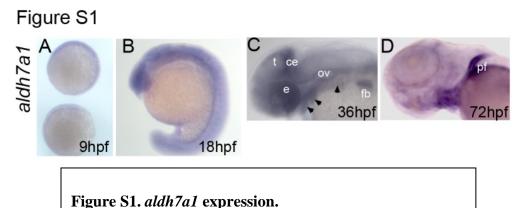
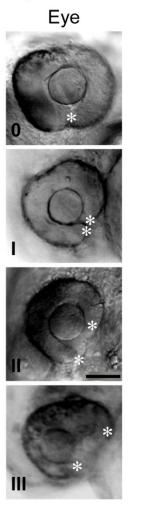
### **Supplementary Data**



Whole-mount *in situ* hybridization of shows expression of *aldh7a1* at (A) 9hpf, (B) 18hpf,(C) 36hpf, and (D) 72hpf.e,eye; t, tectum; ce,cerebellum; ov, otic vesicle; fb, fin bud; pf, pectoral fin. Arrow head indicates pharyngeal arches.

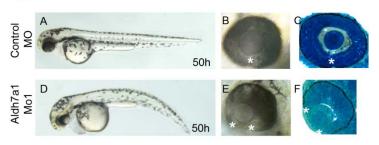


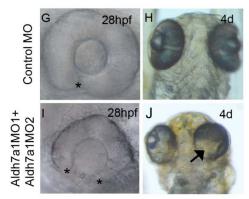


## Figure S2. Grades of severity for eye development.

Classification of phenotype severity following morpholino injections. Four grades of coloboma (0, least severe; I, II,and III, most severe) classified by distance between edges of optic fissure at ~28 hpf, marked by white asterisks. Scale bar: 65 µm

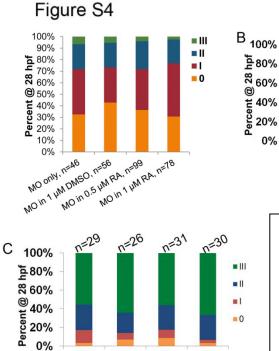
## Figure S3





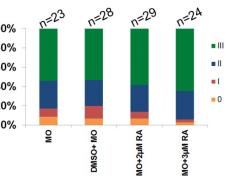
#### Figure S3. aldh7a1 loss-of-function phenotype.

(A,D) Aldh7a1MO1 injected embryos developed bent tail compare to control MO injected embryos. (A-F) Lateral view of zebrafish eye at 50 hpf. Co-injection of Aldh7a1MO1 and Aldh7a1MO2 developed coloboma(I n=36/41, J n=25/31), compared to(G,H) control MO injected embryos.(C-F) Sagittal section of eye. Asterisks indicate edges of optic fissure, arrow indicates ventral defect in the eye at 4d.



- 1

= 0



#### Figure S4. *aldh7a1* morphant phenotype is not rescued by retinoic acid.

(A-C) aldh7a1 morphant embryos were incubated in retinoic acid (RA) beginning at; (A) 20-somite stage for 1 hour, (B) 2 somite stage for 2 hours, and (C) 10-somite stage for 2 hours. The distribution of eye phenotypes (0, I, II, and III) was recorded at 28hpf. RA had no visible effect compared to DMSO control.

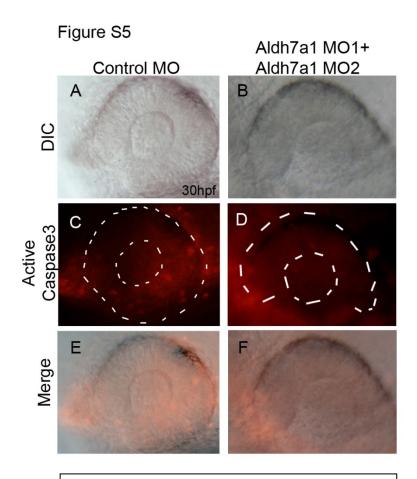
0%

MO

OM +OSMO

2µM RA+ MO

3µM RA+ MO



# Figure S5. Coloboma in *aldh7a1*morphant fish is not due to apoptosis.

Active Caspase3 staining of control (C,E) and *aldh7a1* morphants embryos (D,F). White lines in C, D demarcates the eye.

| Table S1: |                      |                     |                                     |
|-----------|----------------------|---------------------|-------------------------------------|
| Gene      | cDNA IMAGE<br>clone* | Primer <sup>†</sup> | 5' <del>→</del> 3' Sequence         |
| aldh7a1   | 2600757              | antisenseprobe_FWD  | TGGTTGTACCGTCTGCTGTC                |
|           |                      | antisenseprobe_REV  | CAAAACAAGGGTTGCAATGA                |
|           |                      | ORF_FWD             | CGGAATTCCGATGTCGACTCTGCTTATTAACCAAC |
|           |                      | ORF_REV             | CCCTCGAGGGGGGCAACATCATGATTCATTCAA   |
| nlz1      | 7405421              | ORF_FWD             | GATCCAGATGAGCGAACTGC                |
|           |                      | ORF_REV             | TAATCACTGGTACCCAAGAGCA              |
| vax2      | 9038293              | ORF_FWD             | ATGTTTGATCAAGCCACGA                 |
|           |                      | ORF_REV             | GGTAGAGGGGAGAAGATTACAGG             |
|           |                      |                     |                                     |

\*IMAGE clones obtained from OpenBiosystems (<u>www.openbiosystems.com</u>); <sup>†</sup>primers obtained from BioServe (<u>www.bioserve.com</u>).

## Table S1

List of Image clones and Oligonucleotides used in this study.