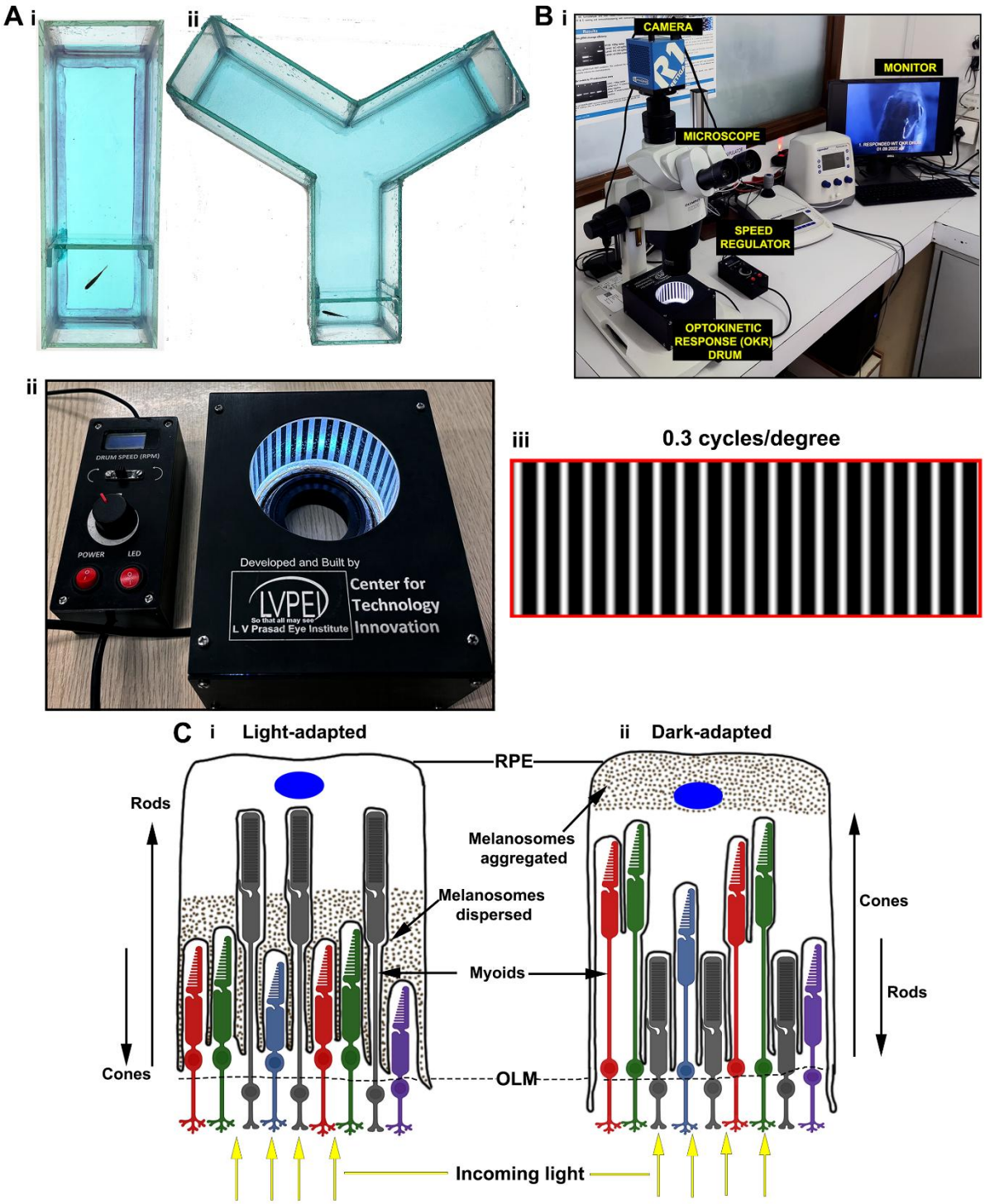
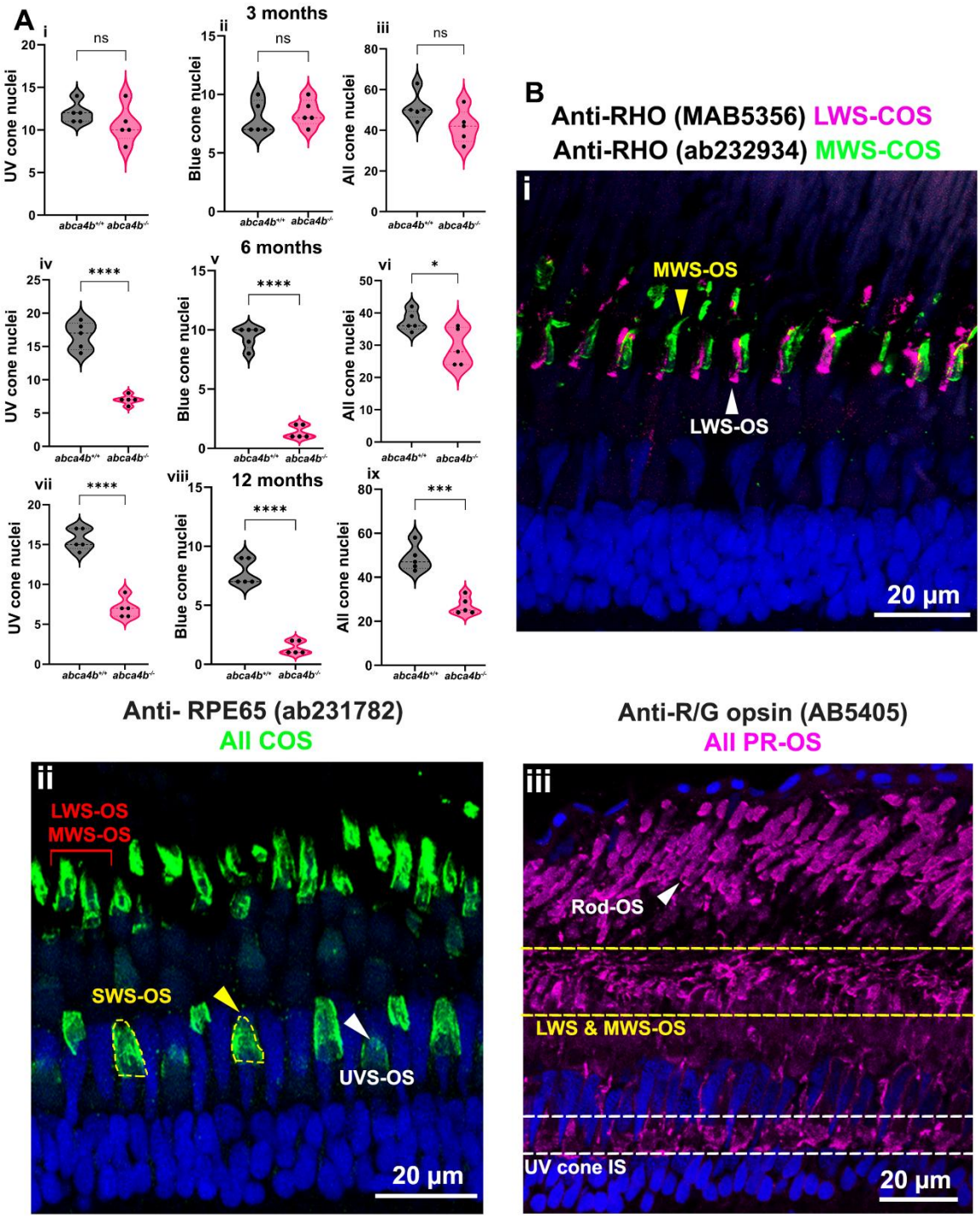
Supplementary Figures:

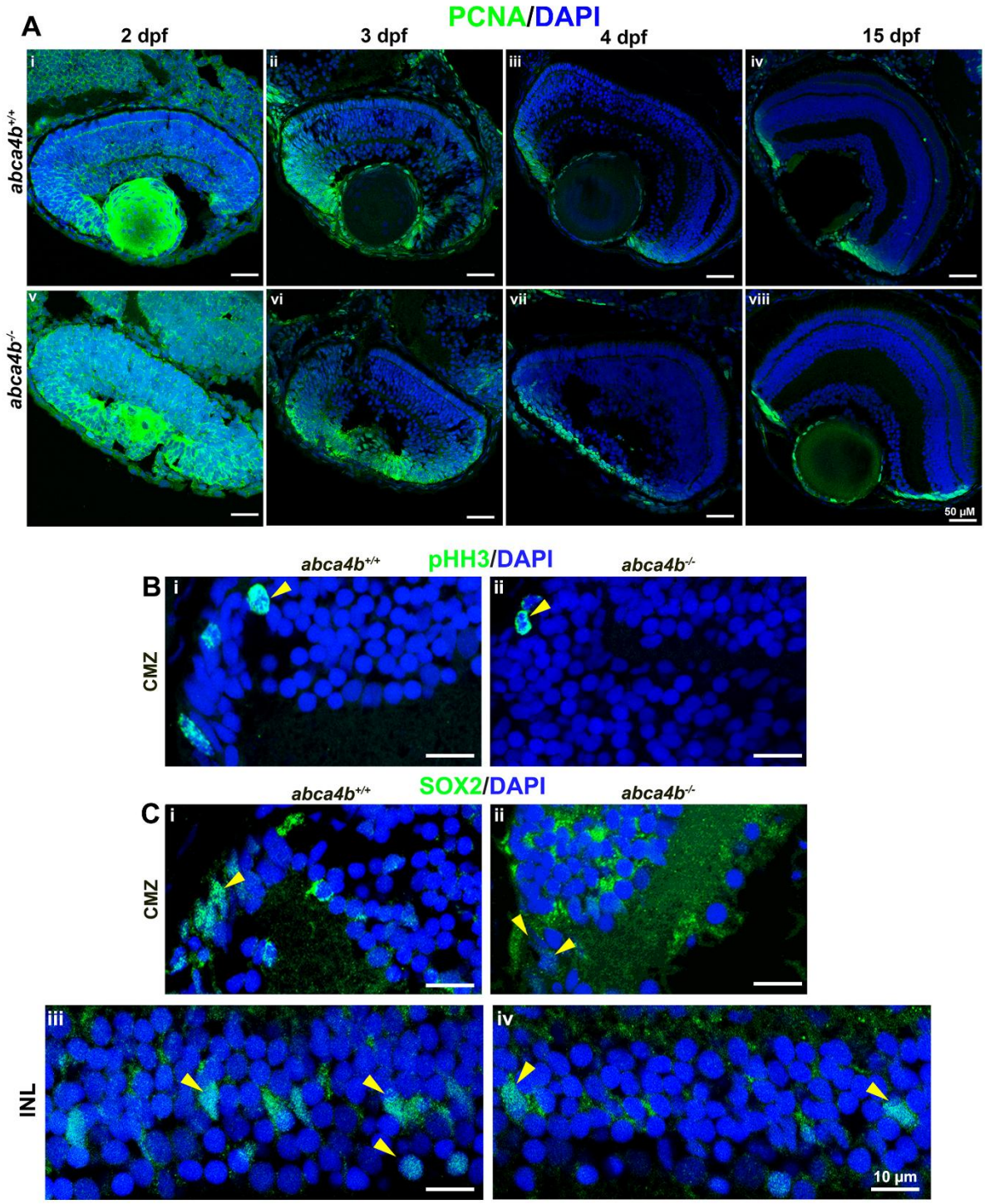


Supplementary Figure 1. A. *Representative images of rectangular (i) and Y-maze (ii) set up for feed-capture behavioural studies.* ***B.*** *OKR drum set up mounted on the stage of a stereo-zoom microscope. The eye movements of the test animals are visualized through the microscope and are video recorded using the camera mounted to the microscope (i). The OKR set up has a rotatable vertical drum lined by sine wave gratings and a central hollow space to position*

the larval or adult fishes, to visualize and record their OKR behaviour. A speed controller regulates the drum rotational speed and the direction of rotation (ii). A representative sine wave gratings of 0.3 cpd spatial frequency and 100% black and white contrast (iii). ***C.*** *Cartoon representation of the photoreceptor and RPE cell layers showing light-adapted (i) and dark-adapted (ii) retinomotor movements.*

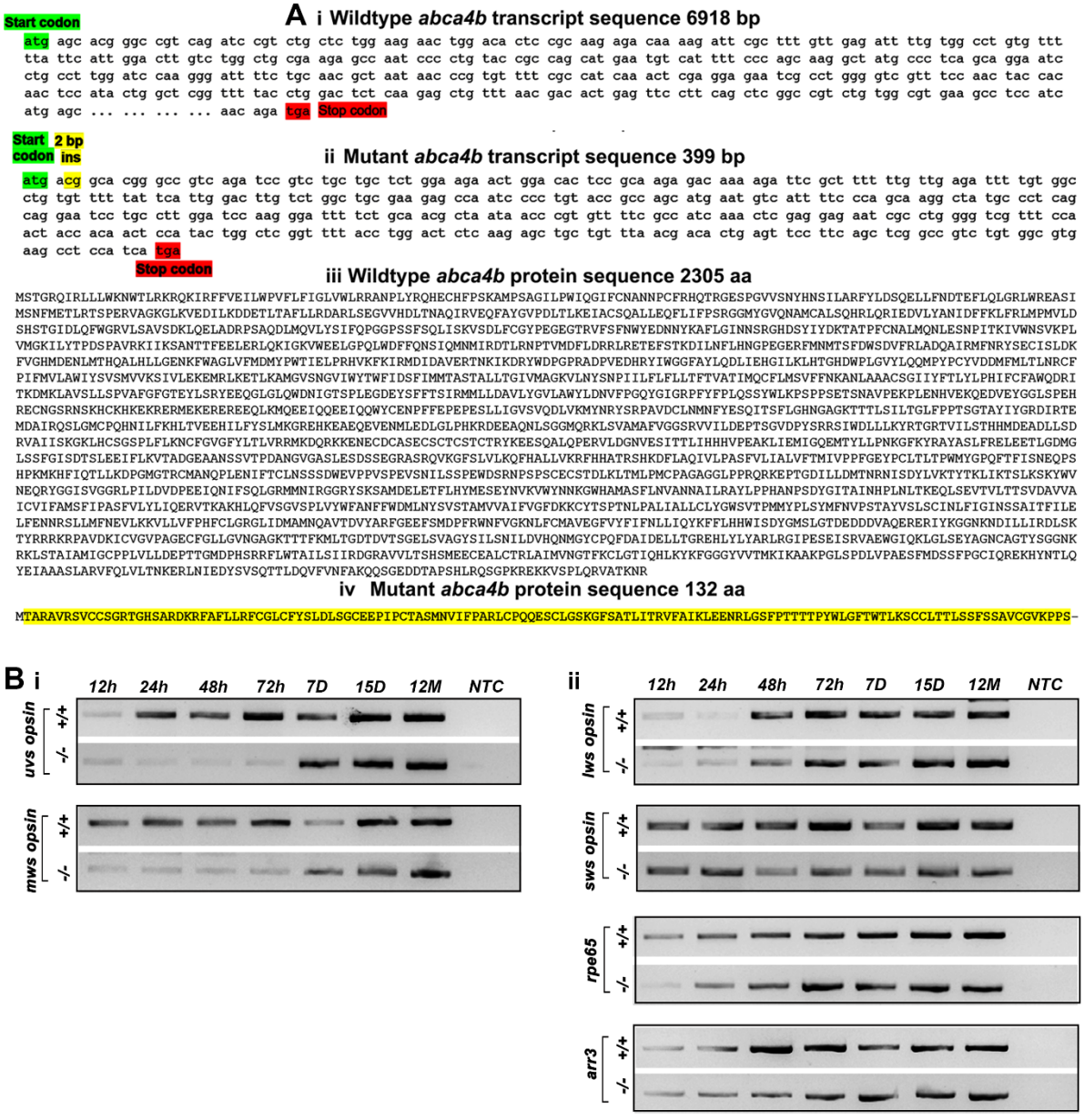


Supplementary Figure 2. A. *Violin plots of the SWS, UVS cone subtypes and all cone cell counts in wt and mutant retinas at 3M, 6M, 12M. Note that the mutants at 3M showed no significant difference when compared to the wt in all parameters tested (i-iii).* ***At*** *6M, the mutants displayed a drastic reduction in the numbers of blue and UV cone nuclei (iv-vi). At 12M, the mutants displayed a highly significant reduction in numbers of all the cone sub types (vii-ix). The data was analysed using the unpaired t-test for statistical significance. ns= p>0.05 (not significant), * p<0.05, **** p<0.0001. N=5.* ***B.*** *Zebrafish adult retinal sections immunostained with, (i) anti-RHO (MAB5356) to label the LWS-COS in magenta (white arrowheads) and anti-RHO (ab232934) to label the MWS-COS in green (yellow arrowheads); (ii) anti-RPE65 (ab231782) to label all COS in green, showing UVS-COS (white arrowheads), SWS-COS (yellow arrowheads) and LWS/MWS-COS (red square brackets); (iii) anti-R/G Opsin (AB5405) to label all POS in magenta, showing UV cone IS (white dashed line), LWS/MWS-COS (yellow dashed line) and Rod-OS (white arrowhead). Scale bar: 20µm.*



Supplementary Figure 3. A*. Confocal images of developing retinal sections of 2 dpf, 3 dpf, 4 dpf and 15 dpf larvae of wild type (i-iv) and mutant zebrafish (v-viii), immunolabelled with anti-PCNA (in green) and counterstained with DAPI to label the nuclei (in blue). Note the presence of* pcna^{+} *cells throughout the retina at 2 dpf, which gradually gets restricted to the retinal periphery at 3-4 dpf and are retained only by a minor population of CMZ cells at 15 dpf, both in wt and mutant larvae. Scale bar: 50 µm. N=5.* ***B.*** *Confocal images of retinal*

sections of 3M adult retina of wt and mutant zebrafish immunolabelled with anti-pHH3 (in green) and counterstained with DAPI (in blue) (i-ii). Note the decrease in mitotic activity at the CMZ of 3M abca4b$^{-/-}$ mutants. ***C.*** *Confocal images of retinal sections of 3M adult retina of wt and mutant zebrafish immunolabelled with anti-SOX2 (in green) and counterstained with DAPI (in blue) (i-iv). Note the reduction in SOX2 positive cells at the CMZ (i-ii) and in the INL (iii-iv) of mutant retinas (yellow arrowheads), as compared to wt retinas. Scale bar: 10 µm, N=3.*



Supplementary Figure 4. A. *Coding sequences of the abca4b wt transcript (cDNA length: 6918 bases) (i) and null mutant transcript displaying an insertion of 2 bases 'CG' (highlighted*

in yellow), resulting in codon frameshift and premature termination (cDNA length: 399 bases) (ii). Amino acid sequences of the abca4b full length wt protein (2305 aa) (iii) and the truncated mutant protein (132 aa), with totally altered peptide sequences (highlighted in yellow) (iv). ***B.*** *Agarose gel images of RT-PCR products of various retinal transcripts expressed at 12, 24, 48, 72 hpf, 7D, 15D and 12M of wt and mutant retinas. Note the delayed onset of expression of uvs and mws opsin in mutants, which got peaked at 7 dpf and are comparable to wt expression at later time points (i). The expression patterns of other mature photoreceptor-specific transcripts such as, lws opsin, sws opsin, arr3 and mature RPE-specific transcripts such as, rpe65 are comparable between wt and mutant retinas at all time-points tested (ii).*

Supplementary methodology:

Zebrafish breeding

Adult zebrafish were fed with freshly hatched artemia larvae twice daily (INVE aquaculture, inc. Nellore, India) and solid dry feed (PL 500) once daily. Young larvae beyond 3-5-days post fertilization (dpf) were fed with fine powder feed (PL 150) three times daily and a combination of PL 150 and live artemia larvae from 5-10 dpf, followed by only live artemia until 30 dpf. For mating, well fed males and females (1:1/1:2) were moved to mating cages the previous evening and kept isolated by a plastic divider. The divider was removed early in the morning when the light is turned on, to enable spawning and external fertilization. The fertilized eggs were then collected and transferred to a petri dish with fish water (0.3 g/L of sea salt in RO water) containing 0.1% (w/v) methylene blue and were incubated at 28°C for 72 h until the larvae hatched.

Isolation of genomic DNA from zebrafish tailfin clips

About 2-3 mm of wildtype zebrafish tailfin clips were lysed using 100 µL of lysis buffer (50 mM Tris, 0.1% SDS, 5 mM EDTA, pH 8), followed by addition of Proteinase K (1 µg/µL) in lysis buffer for overnight digestion at 56°C. The DNA was precipitated using equal volumes of ice-cold isopropanol and $1/10^{th}$ volume of 5M NaCl. The contents of the tubes were mixed by inversion and incubated at 4°C for 10 min and centrifuged at 500 *g* for 10 min. The DNA pellet was washed with 70% ethanol, air dried, dissolved in TE buffer, pH 8 containing 0.4 mg/mL of RNase and incubated overnight at room temperature. The quality of the purified genomic DNA was checked on 1% agarose gel and further quantified using NanoDrop™ (Thermo Fisher Scientific).

T7 endonuclease (T7E) assay

The DNA substrate or the targeted genomic region was PCR amplified and purified. The amplicon (a mix of normal and edited DNA) was denatured and reannealed to enable heteroduplex formation and creation of mismatch loops at edit sites, which are then recognized and cleaved by the T7 endonuclease to yield smaller DNA fragments. For this, a 20 μL reaction was set up with 200 ng of PCR product in 1X NEB 2.1 buffer, denatured at 95°C for 5 min and cooled gradually to room temperature to facilitate heteroduplex formation. To this reaction, 0.5 μL of T7 endonuclease I (10 U/μL) was added and incubated at 37°C for 1 hour. The cleaved DNA products were analysed on 2.5% agarose gels, to evaluate target-specific edits and edit efficiencies.

Genetic screening to identify mosaic founder fishes (F_0)

The RNP injected embryos were allowed to develop for a month to the juvenile stage. The test animals are anesthetized using 0.02% tricaine and 2 mm tail-fin clips were taken for genomic DNA isolation. The gRNA2 target spanning region was PCR amplified using specific primer sets and the amplicons were analysed by Sanger sequencing, to screen and identify F_0 founders carrying target site specific edits. The spectrum of in-del mutations generated in F_0 founders was further identified by cloning the target region amplicon into pMOS-Blue vector and multiple clones were sequence confirmed using M13 forward and T7 sequencing primers.

Confirmation of germline edits and the generation of *abca4b*$^{-/-}$ homozygotes

The adult F_0 mosaic founder fish carrying a frameshift in-del was backcrossed with the wildtype animals *(abca4b*$^{+/+}$*)* to obtain F_1 progenies, which were individually genotyped by tailfin clip genomic DNA screens to confirm the inheritance of mutant alleles and to identify the heterozygous mutants. The mutations that got germline transmitted was confirmed by cloning and sequencing of the target region PCR amplicons. The F_1 generation heterozygotes *(abca4b*$^{+/-}$*)* with identical in-del mutations were then inter-bred to generate F_2 generation progenies, which are further genotyped individually to identify the 25% homozygous null recessive mutants *(abca4b*$^{-/-}$*)*. The adult null mutants were fertile and are interbred for further colony expansion.

OCT embedding of zebrafish tissues

The wt and mutant zebrafish heads were collected, washed with 1X PBS and fixed in 4% paraformaldehyde with overnight incubation at 4°C, to enable better penetrance of the fixative and preservation of cells. Well-fixed tissues were washed thrice with 1X PBS and dehydrated using 30% sucrose solution in 1X PBS at 4°C overnight, until the tissues settled to the bottom. This prevents ice crystal formation during OCT embedding. The tissues were then transferred to cryomolds, properly oriented and excess sucrose solution was blotted out. The optimal cutting

temperature (OCT) medium was then added to fully submerge the tissue. The moulds were then placed on a cryotome stand to allow the freezing and solidification of the OCT medium. Once solidified, the moulds were labelled and stored at -80°C. Thin cryosections were taken on charged glass slides using a Cryostat (CM1860 UV, Leica, Germany) and the slides were preserved at -80°C until further use.

Paraffin embedding of zebrafish tissues

Whole larval and adult zebrafish heads were fixed in 4% paraformaldehyde for 48 h, prior to processing for paraffin embedding. The fixed tissue was decalcified using 8% EDTA for 48-72 h. The tissue was then placed inside a biopsy cassette and loaded into an automated bench top tissue processor (TP1020, Leica, Germany) for sequential dehydration and clearing. The tissue was sequentially immersed in 60%, 70%, 80%, 90% isopropanol for 1 h each, 100% isopropanol (2 changes, 1 h each), Xylene (2 changes, 1 h each) and transferred into melted paraffin wax at an optimum temperature of 58-60°C (3 changes, 1 h each). The tissue sample was finally embedded in paraffin, allowed to solidify and then sectioned using a microtome (HistoCore MULTICUT, Leica, Germany) to obtain 4-5 μm thick tissue slices. The sections were collected on silane coated glass slides, and dried in oven set at 37°C. The slides were further processed for staining with hematoxylin and eosin (H&E) for histological analysis.

Deparaffinization of paraffin tissue sections

Before proceeding for H&E staining of tissue sections, deparaffinization was done to remove paraffin wax from the tissue samples. For this, the slides with tissue sections are heated at 70°C on a heating plate for 15-20 min to melt the paraffin. The tissue sections were then given three changes of xylene washes (I, II, III) for 3 min each, to ensure the complete removal of paraffin. The tissue was then re-hydrated by immersing them in three different percentages of isopropanol (100%, 90%, and 80%) for 3 min each. The sample slides were washed in distilled water and further processed for hematoxylin and eosin staining.

Haematoxylin and Eosin (H&E) staining of tissue sections

Following deparaffinization, the slides are incubated in hematoxylin solution for 3-5 min. After which the slides were rinsed/washed under running tap water for 5-10 min. To eliminate any non-specific binding, the slides are immersed in 1% acid alcohol for 30 sec, followed by thorough washes under running tap water. The slides were then counterstained with Eosin Y for 2 min and subsequently dehydrated using graded ethanol solutions i.e. 95% and 100%. The sections were cleared using two changes of xylene 5 min each, air-dried and mounted with DPX

(Dibutylphthalate Polystyrene Xylene) mounting medium. The stained sections were imaged using an upright bright field microscope (Axio Scope.A1, Carl Zeiss, Germany).

Antigen retrieval and immunohistochemical analysis of tissue sections

Antigen retrieval was done for paraffin embedded retinal sections. Tissue sections of 4-5 μm thickness on silane-coated microscopic slides were deparaffinized and antigen retrieval was performed by immersing the tissue slides in Coplin jars containing sodium citrate buffer (pH 6.0) with 0.05% Tween 20 and heated at 95-100°C for 20 min in microwave oven. The tissues were then permeabilized using 0.5% Triton X-100 for 20 min, followed by blocking with 10% FBS for 1 h. The phenotype of the zebrafish retina was characterised by labelling them using major retinal antigen specific primary antibodies and are visualized using fluorescent dye conjugated, species-specific secondary antibodies (Supp. Table no.4).

For the immunolabelling of the retinal tissues with FITC-conjugated PNA lectin, the samples were deparaffinized. PNA is a plant lectin that binds specifically to the galactose moieties of glycolipids found in photoreceptor cell membranes. Unlike the protein epitopes, the glycolipids modifications remain unaltered during formaldehyde fixation and cross-linking. Therefore, the antigen retrieval step required for the unmasking of protein epitopes can be skipped for PNA staining. The lectin-stained slides were counter stained with DAPI, mounted on glass slides using the Vectashield mountant. The samples were imaged using the fluorescence and confocal laser scanning microscope (Axio Observer 7 and LSM 880 with Airyscan, Carl Zeiss, Germany) and all images were acquired and analysed using the Zen Black and Zen Blue software respectively. All figure composites were prepared using the Adobe Photoshop CS6 image analysis software.

Total RNA isolation and semi-quantitative RT-PCR assay

The zebrafish embryo, larval and adult retinal tissues were minced and lysed in 1 mL of TRIzol™ reagent (Thermo Fisher Scientific) and processed for total RNA isolation as per the manufacturer recommended protocol. The final RNA pellet was washed with 70% ethanol, air-dried on ice, dissolved in nuclease-free DEPC-treated water and quantified using Nanodrop. For semi-quantitative RT-PCR analysis, the total RNA was reverse transcribed into cDNA using the Superscript III Reverse Transcriptase (Thermo Fisher Scientific), as per the manufacturer's instructions. The resulting cDNA was used as templates for semi-quantitative RT-PCR analysis, to assess the expression levels of mRNA targets across different samples analysed. Gene-specific primers used for semi-quantitative RT-PCR analysis are listed in Table no. 5

Guide RNA target loci:

gRNAs targeting exon 2 of zebrafish *abca4b* gene

GACATGATGTTTTCCCAGGTTTGTTGTTGATTGGTGGCCCCTGCGTGGGGTTAATCTGGAGCC
ATGAGCACGGGCCGTCAGATCCGTCTGCTGCTCTGGAAGAACTGGACACTCCGCAAGAGAC
AAAAGGTACAATTAACCACTCGCTGGAACACAAAAGCACTCATCATCCTGGCCTGGAGTCTCCAAAG
TCTTTGGAAAGCTTATATTTAGAGCGCTCTGTTTATATCACGTAATACAAAC**CTCAGAAATGTGGTT
CCAAGTG**

Off target prediction analysis done by CHOPCHOP web based tool.

Table 1. Predicted off targets for gRNA1 (GTTGATTGGTGGCCCCTGCGTGG)

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr2:15209638	0	GTTGATTGGTGGCCCCTGCGTGG
chr3:30843165	3	GTTGATTGaTGGCCtgTGCGTGG
chr3_KZ115070v1_alt:433094	3	GTTGATTGaTGGCCtgTGCGTGG

Table 2. Predicted off targets for gRNA2 (CCATGAGCACGGGCCGTCAGATC)

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr2:15209674	0	CCATGAGCACGGGCCGTCAGATC
chr15:34213914	3	CCGTGAGCgCGcGCCGTCcGATC

Table 3. Predicted off targets for gRNA3 (GTCTGCTGCTCTGGAAGAACTGG)

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr2:15209698	0	GTCTGCTGCTCTGGAAGAACTGG
chr19:26621084	3	CCTGaaCTTtCAGAGCAGCAGAC
chr2:32211831	3	GTCTGCTGCTCTGGAAttAtGGG

Off-targets		
Location	**Number of mismatches**	**Sequence (including mismatches)**
chr2:38046259	3	CCTGTTCTTCCAGctCAGCAGgC
chr23:44652569	3	CCAGaTCaTCCAGAGCAtCAGAC
chr24:28561956	3	GgCTGCTcCTCTGGAAaAACTGG
chr2_KZ115043v1_alt:89125	3	CCTGTTCTTCCAGctCAGCAGgC
chr6:1447800	3	aTCTcCaGCTCTGGAAGAACTGG

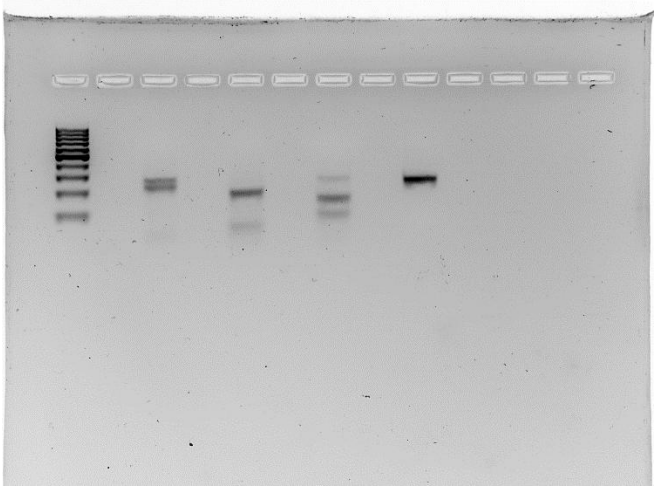
Table 4. List of antibodies used

Antibodies list		
Antibody	**Dilution**	**Catalog number**
Arrestin 3 (Mouse Monoclonal)	1:300	Abcam ab174435
BODIPY™ 665/676 (Lipid Peroxidation Sensor)	1:1000	Invitrogen™ B3932
PCNA (Rabbit Polyclonal)	1:300	GeneTex GTX124496
PNA Alexa Fluor™ 488 Conjugate	1:300	Thermo Fisher Scientific L21409
Red/Green Opsin (Rabbit Polyclonal)	1:300	Merck Millipore AB5405
Rhodopsin (Mouse Monoclonal-1D4 clone)	1:300	Merck Millipore MAB5356
Rhodopsin (Rabbit polyclonal)	1:300	Abcam ab232934
RPE65 antibody [EPR22579-44] (Rabbit monoclonal)	1:300	Abcam ab231782
pHH3 [HL1752] (Rabbit monoclonal)	1:500	Thermo Fisher Scientific MA5-46900
SOX2 (Rabbit polyclonal)	1:500	Abcam ab97959

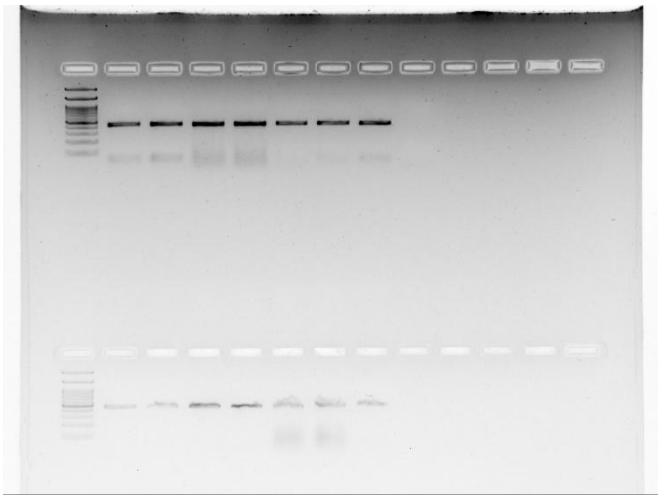
Table 5. List of primers used

S. no.	Name of the primer	Primer Sequence (5' to 3')	Product size (bp)
1.	ZF-OPN-SW-UV-F	GATCATTGGTACCGCCTGTGCT	261 bp
	ZF-OPN-SW-UV-R	CTCTCTGCCTTCTGGGTGGACT	
2.	ZF-OPN-SW-BL-F	GGACCTGACTGGTATACGACTAAC	265 bp
	ZF-OPN-SW-BL-R	CCACCCAGATTGCAAAGATAGC	
3.	ZF-OPN-LW1-2-F	ACTGTGGTCGTTGACTGTCATCTC	360 bp
	ZF-OPN-LW1-2-R	TGTGTGGACTCAGAATCCTTCTGC	
4.	ZF-OPN-MW1-4-F	GTGGTTTGCAAGCCAATGGG	332 bp
	ZF-OPN-MW1-4-R	CTCTCAGCCTTCTGAGTGGA	
5.	ZF-VSX2-RT-F	GGATGTGTACGCCAGAGAAATGCT	527 bp
	ZF-VSX2-RT-R	AGTCCAGCGAACAGATAGACGCAA	
6.	ZF-CRX-RT-F	AATCAACCTTCCCGAGTCCAGAGT	561 bp
	ZF-CRX-RT-R	AGACTGCCTTGACTACAAGGACCA	
8.	ZF-PA6AX-RT-F	GAGACCCAAATGAGGCTTCAGCTT	614 bp
	ZF-PA6AX-RT-R	ACTTGAACGGGTACAGACACTCCA	
9.	ZF-ABCA4B-RT-F	TGTACCTTTATGCTCGCCTTCGTG	544 bp
	ZF-ABCA4B-RT-R	GAGGCAGCTGCGATTTCATACTGT	
10.	ZF-RPE65-A-RT-F	GGGTTCATCGTGTTTGACCTGTGT	575 bp
	ZF-RPE65-A-RT-R	TGCTAAAGATCTGTCGGAGATCGC	
11.	ZF-ARR3A-RT-F	AGAAGGATACCTGCCGACTTGTCA	566 bp
	ZF-ARR3A-RT-R	AGTGATGTGACAGTAGAGCTGCCT	
12.	5'UTR-L2	GACATGATGTTTTCCCAGGTTT	266 bp
	E1-R2	CTCAGAAATGTGGTTCCAAGTG	

Supplementary information: Original gel pictures

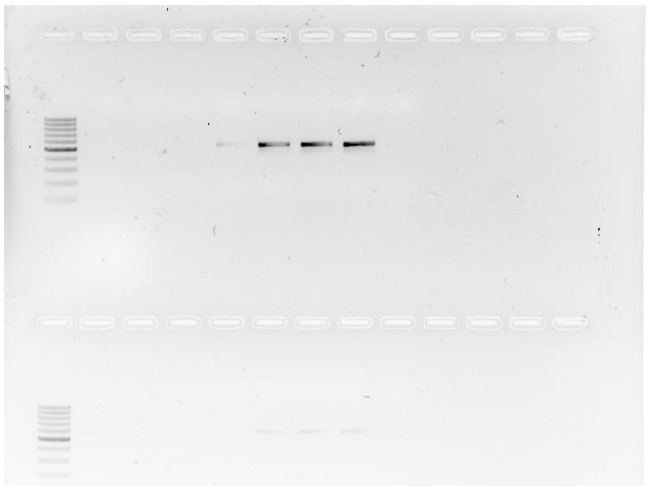


Original gel image of *in vitro* cleavage assay: The gel images displays 100 bp ladder, cleaved products by g1, g2 and g3 and the negative control

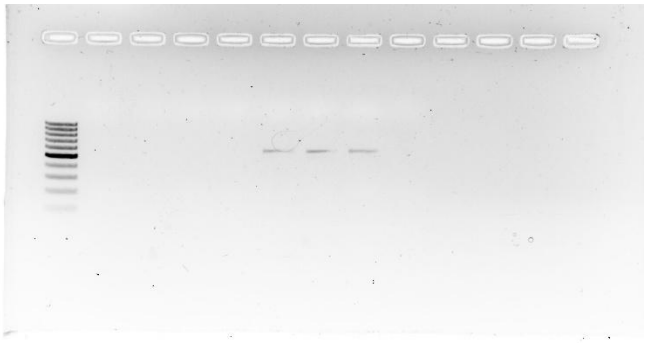


Original gel image of wildtype gapdh standardisation 12hpf (hour post fertilization), 24hpf, 48hpf, 72hpf, 7dpf (days post fertilization), 15dpf and 12M (top lane).

Original gel image of *abca4b* mutant gapdh standardisation 12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



Original gel image of *abca4b* transcript expression in wildtype zebrafish 12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).



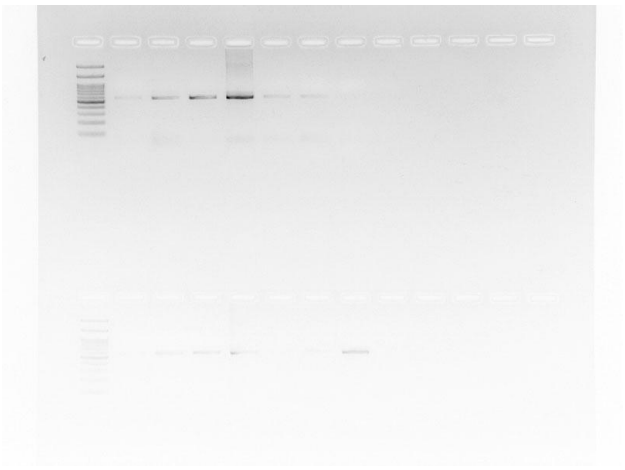
Original gel image of *abca4b* transcript expression in *abca4b* mutants 12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.

Original gel image of *pax6* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).

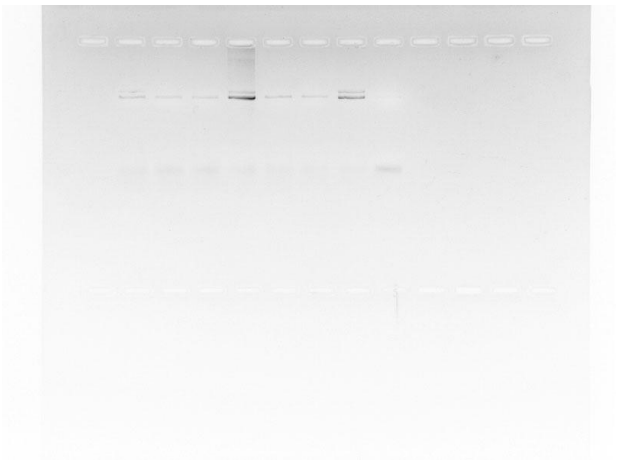
Original gel image of *pax6* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.

Original gel image of *chx10* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).

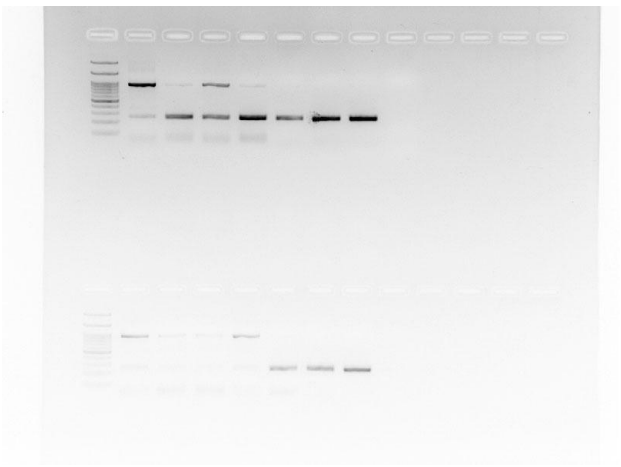
Original gel image of *chx10* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



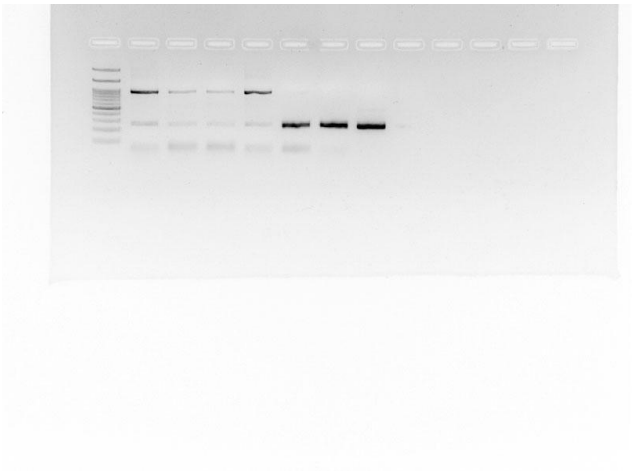
Original gel image of *crx* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).



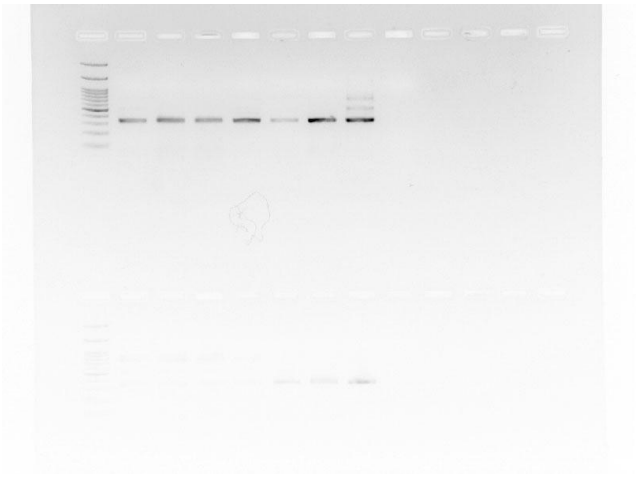
Original gel image of *crx* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



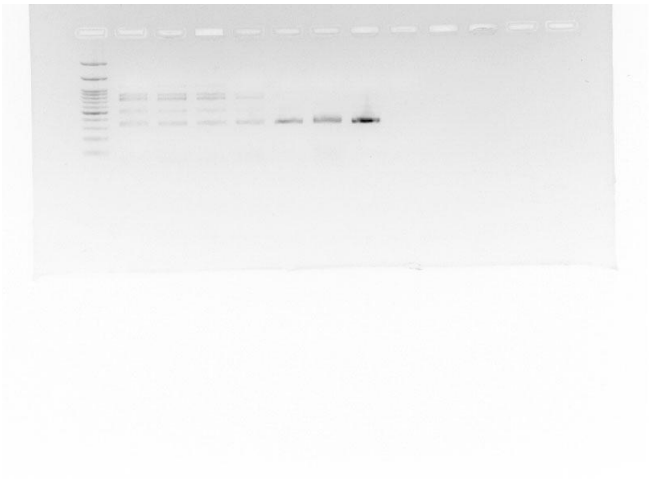
Original gel image of *uvsopsin* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).



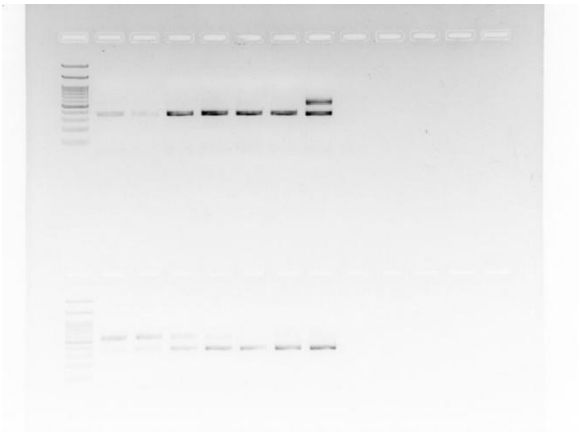
Original gel image of *uvsopsin* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



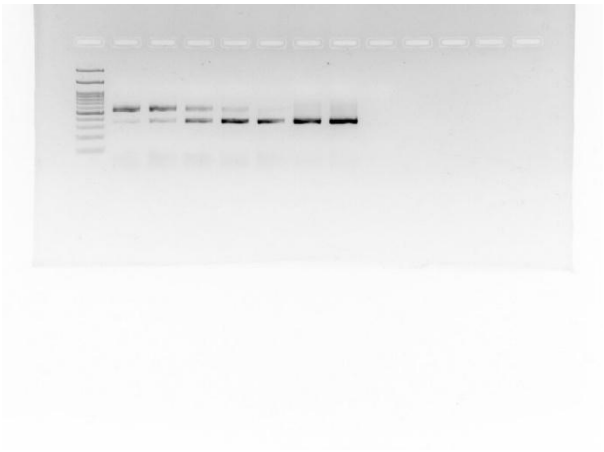
Original gel image of *mwsopsin* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



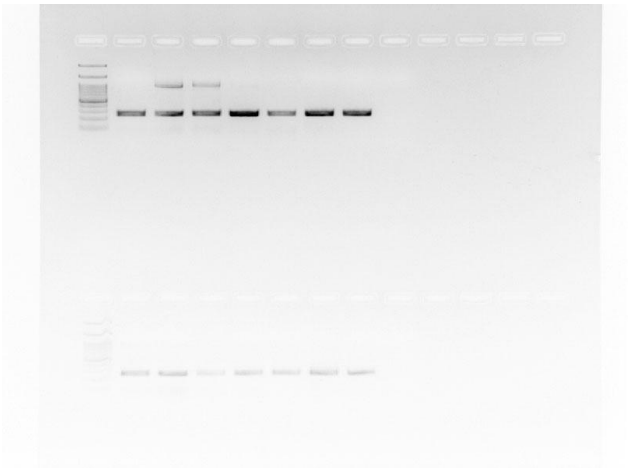
Original gel image of *mwsopsin* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



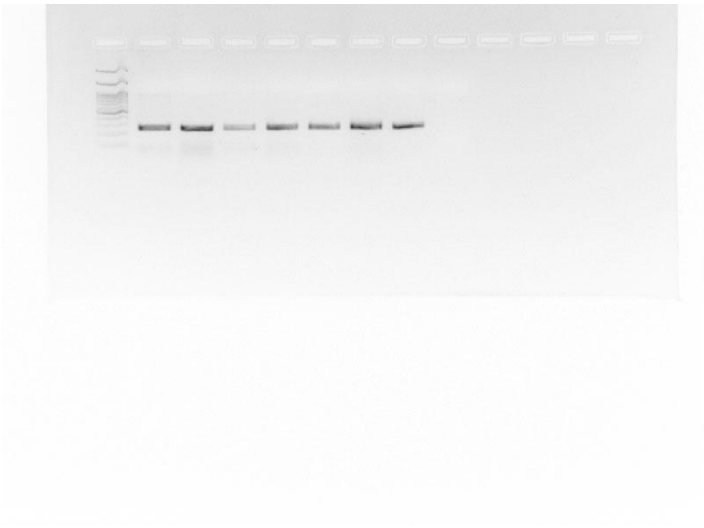
Original gel image of *lwsopsin* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).



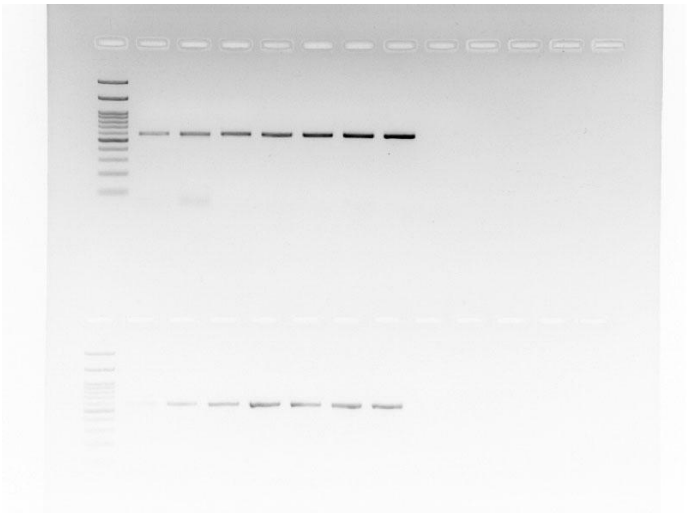
Original gel image of *lwsopsin* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).



Original gel image of *swsopsin* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).

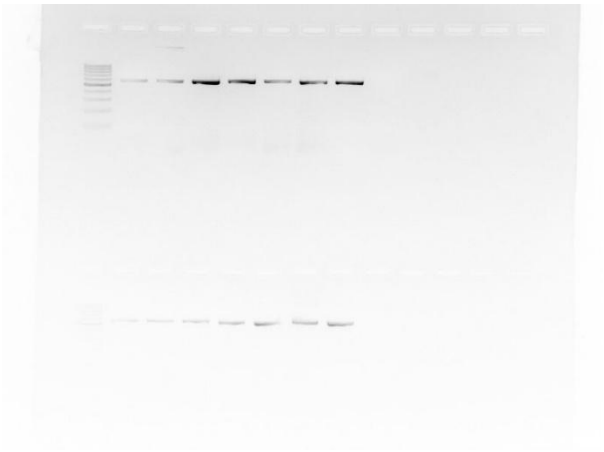


Original gel image of *swsopsin* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



Original gel image of *rpe65* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).

Original gel image of *rpe65* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.



Original gel image of *arr3* transcript expression in wildtype zebrafish-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M (top lane).

Original gel image of *arr3* transcript expression in *abca4b* mutants-12hpf, 24hpf, 48hpf, 72hpf, 7dpf, 15dpf and 12M.