

**Supplementary Table I. Mutation effect of select lines (*ey<sup>sny10</sup>*, *ey<sup>sny13</sup>*, *ey<sup>sny14</sup>*, and *ey<sup>sny593</sup>*)**

DNA sequence (from nucleotide#121 of the initiation codon)

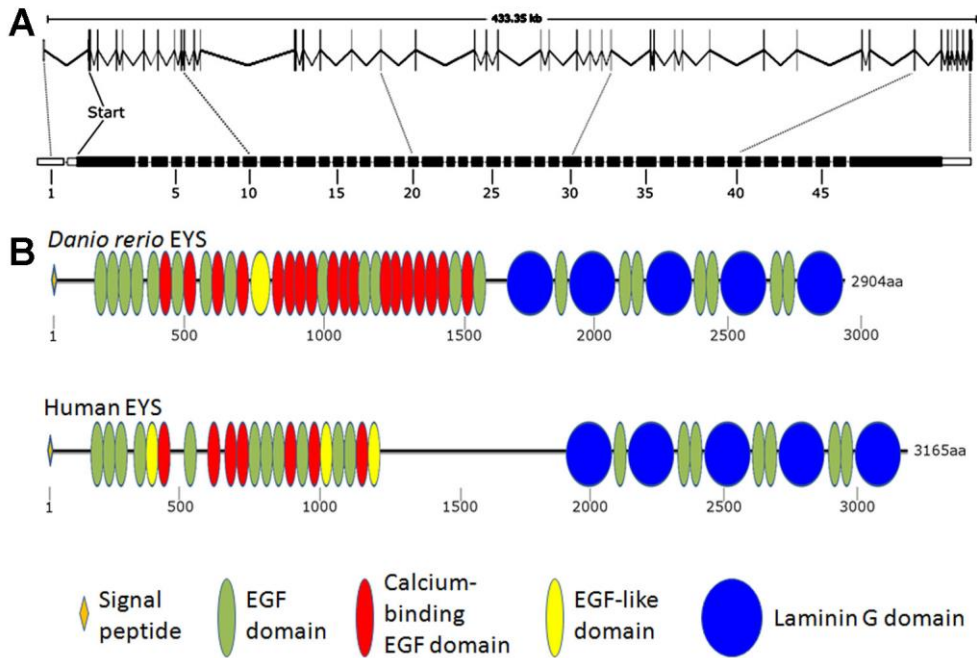
Wildtype: AACATCAGCGTGAGGTGGACCCTCATGGAGAACACCTGCAGCAGTTTGACCCAGTGCTGGAG  
*ey<sup>sny10</sup>*: AACATCAGCGTGAGGTGGA-----GAACACCTGCAGCAGTTTGACCCAGTGCTGGAG  
*ey<sup>sny13</sup>*: AACATCAGCGTGAGG-----AGAACACCTGCAGCAGTTTGACCCAGTGCTGGAG  
*ey<sup>sny14</sup>*: AACATCAGCGTGAGGTGGACCCT-----GCAGCAGTTTGACCCAGTGCTGGAG  
*ey<sup>sny593</sup>*: AACATCAGCGTGAGGTGGACCCTCATG-Δ593-TCTTTGTCCTGCTCCATTACAGgtaagg

Mutation effects on EYS protein (underlined residues indicate the result of the frameshift mutations)

*ey<sup>sny10</sup>*: MRNPKLAIIVFLLSCVIYGPVYSQVTCRRATSREWHTQPKNISVRWRTPAAV- T47Rfs5X  
*ey<sup>sny13</sup>*: MRNPKLAIIVFLLSCVIYGPVYSQVTCRRATSREWHTQPKNISVRRTPAAV- W46Rfs6X  
*ey<sup>sny14</sup>*: MRNPKLAIIVFLLSCVIYGPVYSQVTCRRATSREWHTQPKNISVRWTLQQFDPVLEQLCRNKWTL  
LDNWAISLSSTLPFGTSTWRPLVCFRRNA- M49Qfs46X  
*ey<sup>sny593</sup>*: MRNPKLAIIVFLLSCVIYGPVYSQVTCRRATSREWHTQPKNISVRWTLMSLSCSIHRCQLL-  
E50Sfs11X

Supplementary Table II. Sequences of primers

Primers	Sequence
Red opsin-forward	AACCGACAGTTCCGCGT
Red opsin-reverse	CCCCATCACTCCAAGGGAAAT
Green opsin-forward	ACTTGTGGGCTACATGGCTC
Green opsin-reverse	CGGAAGATATCTGGACCAGCC
Blue opsin-forward	CATTGCCGGCTGCATACTTC
Blue opsin-reverse	GCTTGAGCTTTGGCTGCTAAT
UV opsin-forward	CCCAAATGGGCGTTCTACCT
UV opsin-reverse	CGTCACAAGACCTGCAATCG
GNAT1-forward	ACGTCCTGCGTTCAAGAGTG
GNAT1-reverse	ATGTGGTGGCGAAGTAACGG
Actb1-forward	AGATCAAGATCATTGCTCCCC
Actb1-reverse	AGATCAAGATCATTGCTCCCC
EYS RT-PCR-forward	GTCTCGTGAATGGCACACT
EYS RT-PCR-reverse	CGTCGGCAGAAACAAACAAGA



**C**

ATGAGAAACCCAAAGCTAGCTATCATTTGCTCTTCCTGCTCAGCTGTGTAATATATGGCCCTGTGTACAGCCAGGTAA  
 CCTGCCGCAGAGCCACGCTCTCGTGAATGGCACACTCAGCCAAGAACATCAGCGTGAGGTTGACCCATGAGGA  
 ACACCTGCAGCAGTTTGACCCAGT GCTGGAGCAGCTTGCAGAAACAAATGGACACTTCTGGACAACTGGGCCAT  
 ATCACTTCTCAACTTTGCCCTTTGGAACTTCAACTGGAGACCTCTTGTTTGTTCGCGACGGAACGCTTGAAC  
 AACACGGTGTCACATACTAA TCAAGGTTTTCTAAAGAGGAGTTTGATAAGTGC GCGATTTT AGAGCCCCGAAAGGAGC  
 AGTTGGTATTTGCCAGCAGTATAATGGTACTTTGCAAGTGGAAATCAAAGTGGCTGATGTCTGGTGTGAATTACTT  
 TACGATCAATAAGAGGGAGTAGTCATCTGTGCAGGTTTGGCCTACGCA TTGCA GTTCTGGTCAAGCCTCAGCTC  
 TGCCAGAGCTCACCTCTCCTCGGC TGTGTT CGGGAAAGGAGAAATGCAGAACTACGCTTAAAGATGATTCAATT  
 CCTGTGGGTGCACAAACTTTT CAGGACGTACTGCAGAAACGTTGACGGATGCTATGAGCAGCCATGCTTAAA  
 TGGAGGCACCTGCTGAGTGAGGGAAGTGCCTACACAGATCTTCACCGTACACATGCTTTGTCTCTCTCCATTC  
 ACAG

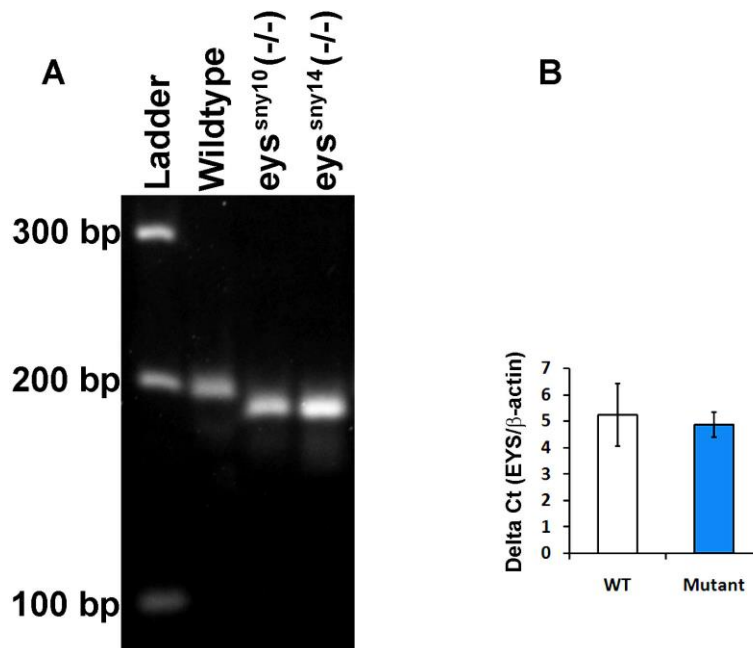
**D**

zEYS Antigen VDLHLSYNCLCLPGWEGEYCQRETNECLSNPCKNNATCTDLLNAYRCVCPQGWTLGDCDE  
 -----CECTSGWTGQCSE  
 \* \* . \* \* \* \* : \* \*

zEYS Antigen DVKECSSSPCLNGAHCVE SDTPGEFSC TCPPFFTGPLCEQPYDPCQLQRNPCLHNSTCRA  
 E INECDSDPCMNGGLCHESTI PGQFVCLCPPLYTGQFCHQRYNLCLLHNPCR-----  
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Supplementary Fig.1. Zebrafish EYS protein is predicted to be similar to human EYS protein

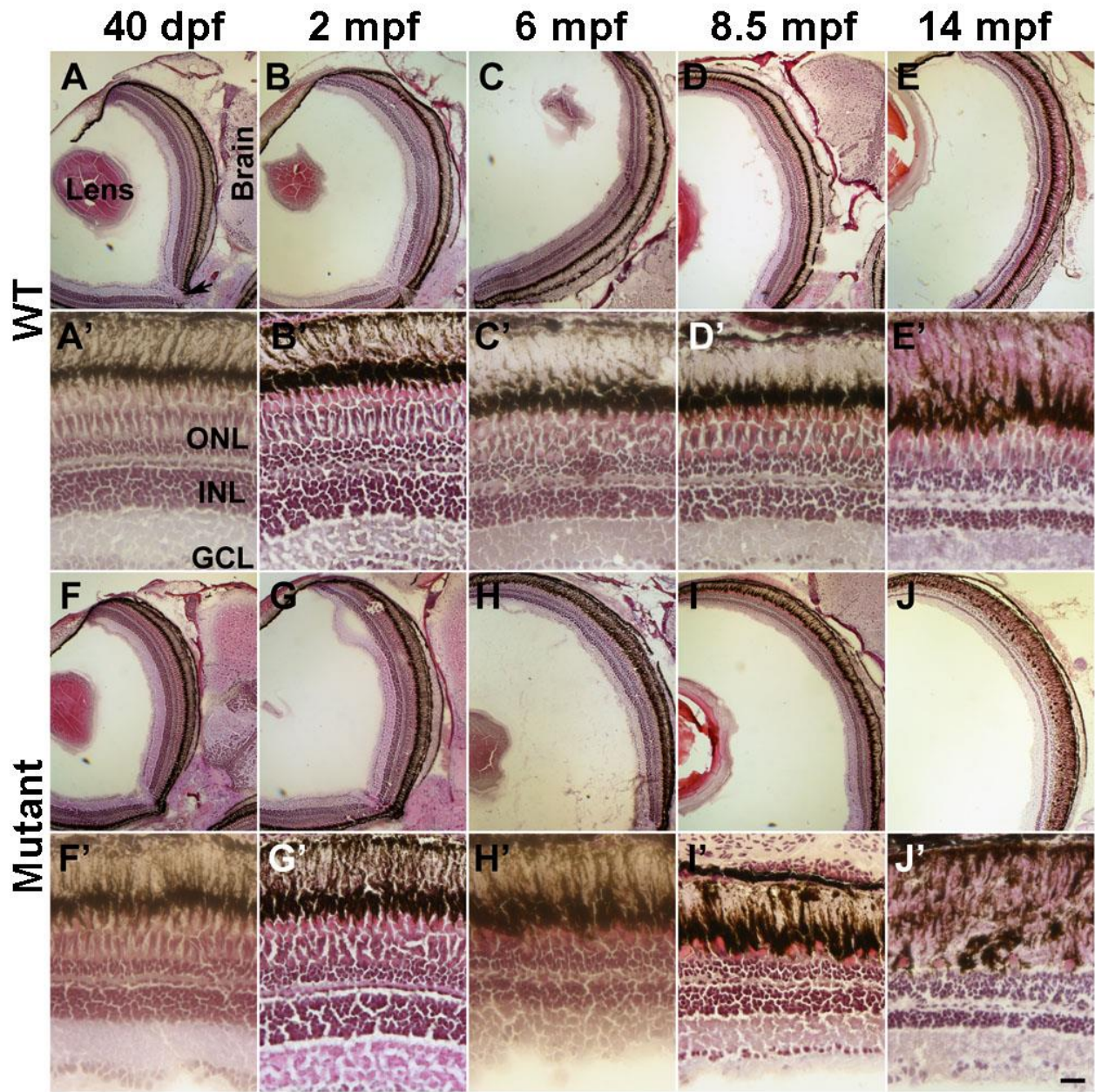
- (A) Zebrafish *eyes* genomic structure on chromosome#13.
- (B) Similar to the human EYS protein, the predicted zebrafish EYS protein has multiple EGF and laminin G domains.
- (C) Coding sequence within exon 2. Initiation codon is in bold. gRNA targets are underlined.
- (D) Alignment of the amino acid sequence of the antigen used to generate EYS antibody with zebrafish EYS amino acid residues 1081-1200. This antibody is suitable for immunofluorescence staining. However, Western blot did not produce any specific signals.



Supplementary Fig. 2. Lack of wildtype zebrafish EYS mRNA in the retina of homozygous EYS-mutant animals

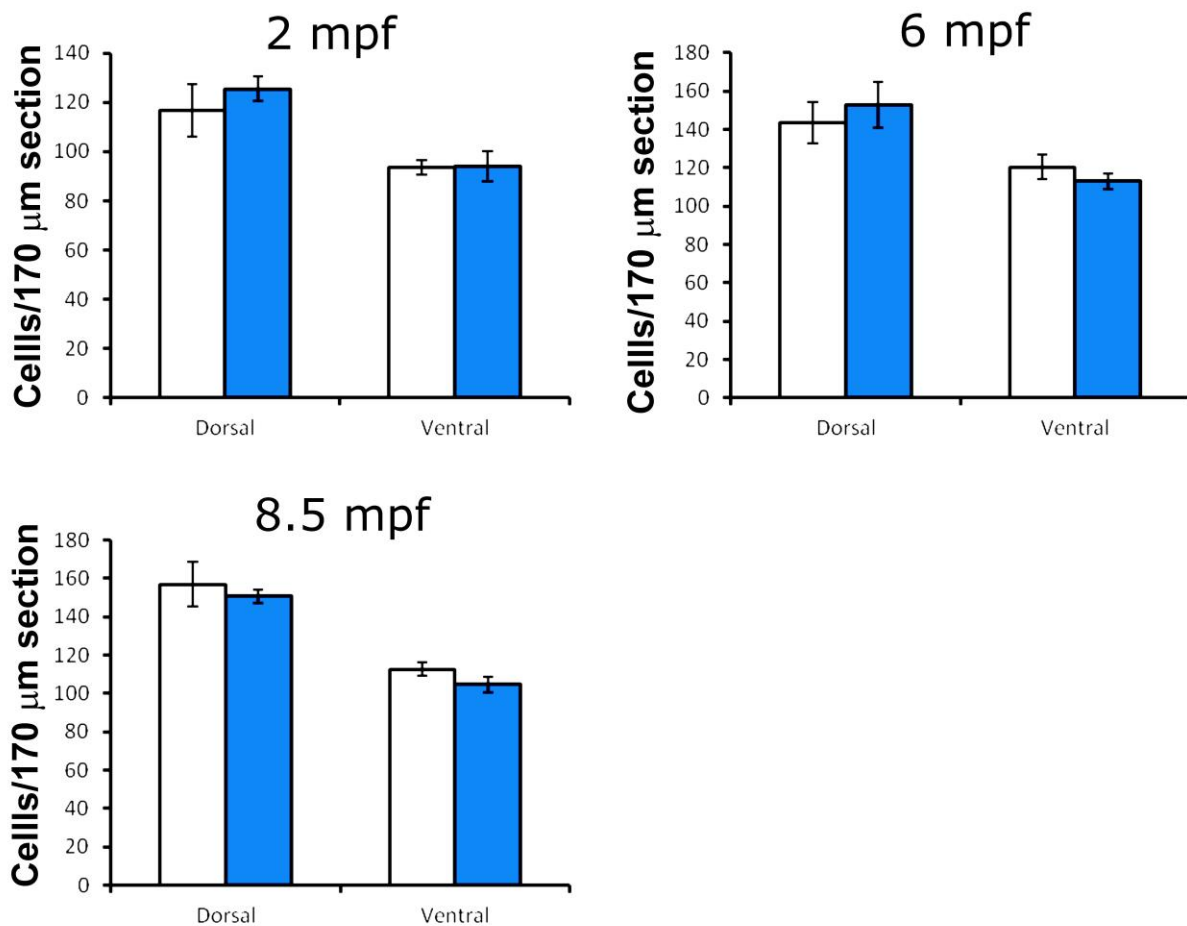
RT-PCR was carried out to evaluate the expression of EYS mRNA isolated from the retinas of wildtype and *eys<sup>sny10</sup>* and *eys<sup>sny14</sup>* homozygous mutant zebrafish. (A) The retinas of homozygous mutant zebrafish expressed only mutant EYS mRNA. The expected wildtype band was 200 bp. The sizes of mutant bands were 190 and 186 for *eys<sup>sny10</sup>* and *eys<sup>sny14</sup>*, respectively. (B) EYS real-time RT-PCR in 3 wildtype and 4 mutant animals using  $\beta$ -actin as an internal control (mean  $\pm$  SEM). There was no significant difference in EYS mRNA levels between the wildtype and the mutant retinas.





Supplementary Fig. 3. Degeneration of cone photoreceptors was revealed by H&E staining.

Eye sections from 40 dpf and 2, 6, 8.5, and 14 mpf wildtype and EYS-deficient zebrafish were stained with H&E staining. (A-E) Low magnification images of wildtype animals. (A'-E') High magnification images of wildtype animals. (F-J) Low magnification images of EYS-deficient animals. (F'-J') High magnification of EYS-deficient zebrafish. Loss of cones is apparent in EYS-deficient zebrafish at 6, 8.5, and 14 mpf.



Supplementary Fig. 4. There is no significant loss of rod photoreceptors in zebrafish at 2 to 8.5 mpf.

Rod cells in the dorsal and ventral retina near the optic nerve heads were counted. There was no significant difference between the wildtype and EYS-deficient zebrafish at 2, 6, and 8.5 mpf.