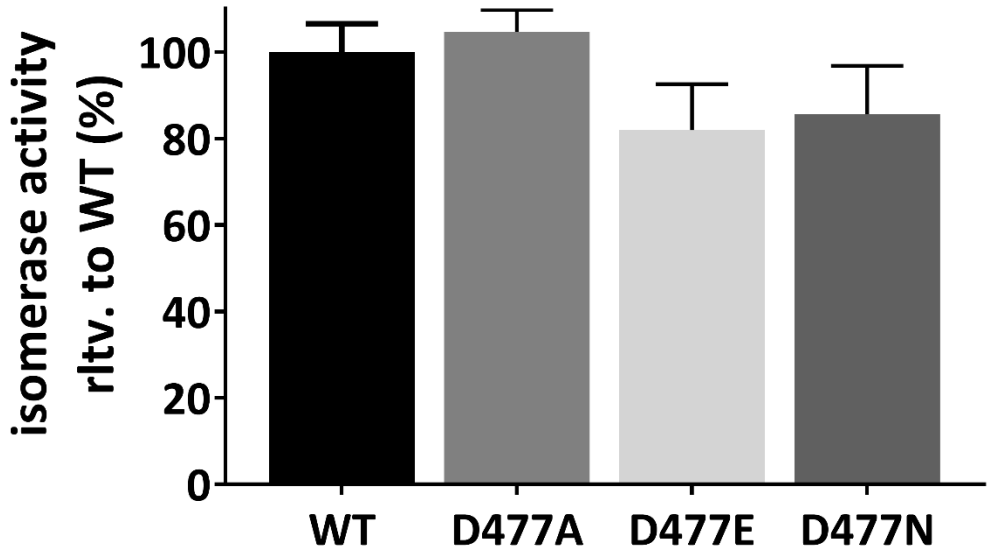
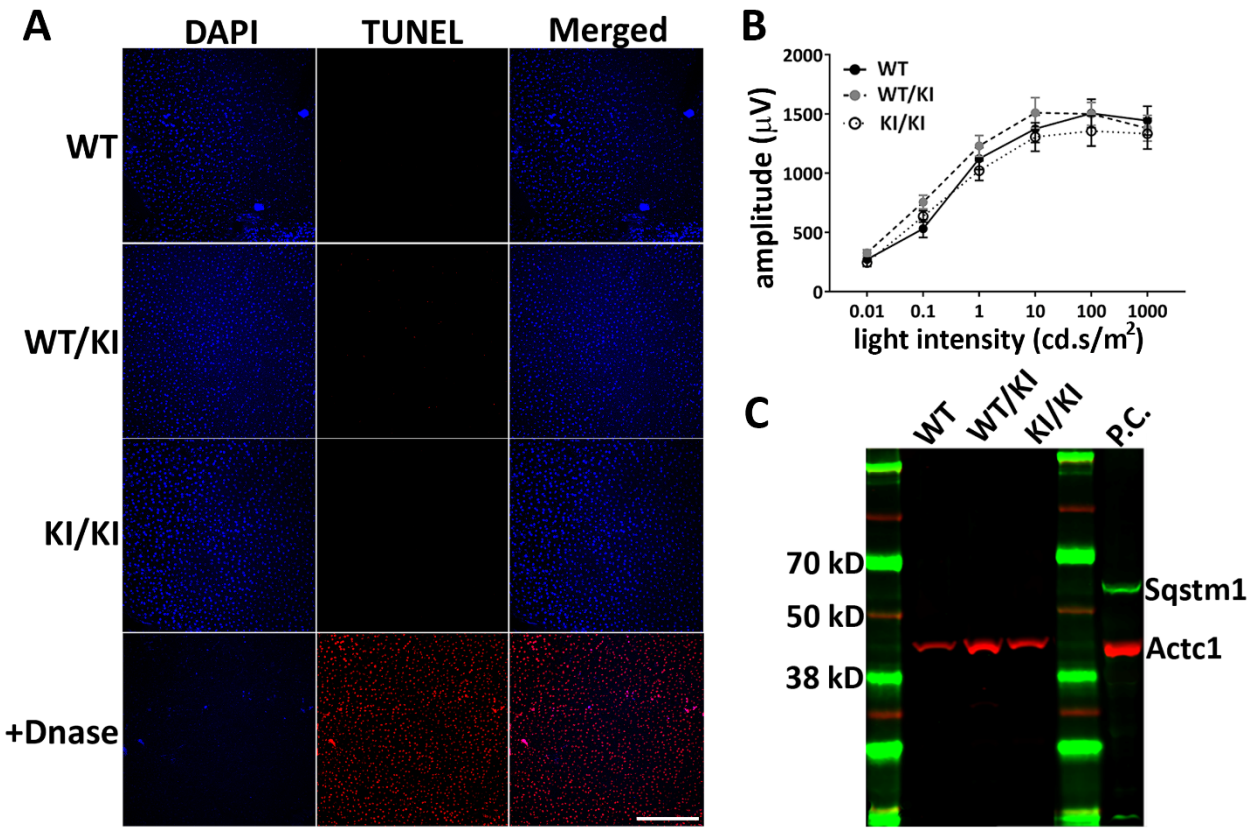
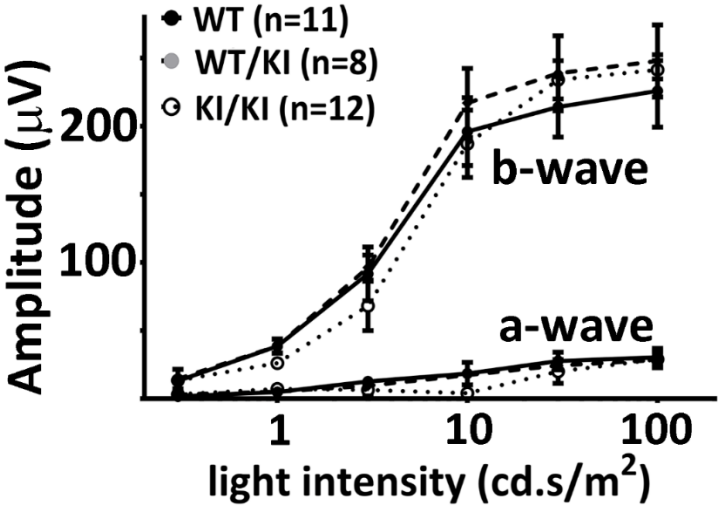


Supp. Figure S1. Effect of RPE65 missense mutation at position 477 on RPE65 activity. Mutation of D477 residue into alanine (D477A), glutamic acid (D477E), and asparagine (D477N) did not interfere with 11-*cis* retinol synthesis when compared to the wild type construct (WT). Isomerase activities of the mutants are quantitated relative to that of WT, and expressed as the mean and SD of two determinations.

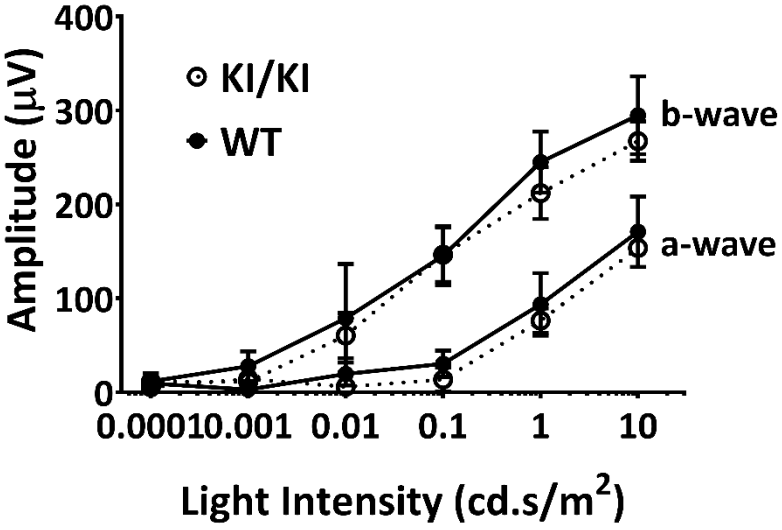
Supp. Figure S2. Sequence alignment of RPE65 gene and protein in region of c.1430A. (A) DNA sequence alignment between mouse and human RPE65 surrounding c.1430A. Asterisk marks c.1430A. (B) Protein sequence alignment among different species. Notice the conservation of Asp477 among the species while amino acid at 475 can be either His or Gln, or Ser in zebrafish. Asterisk marks residue D477.



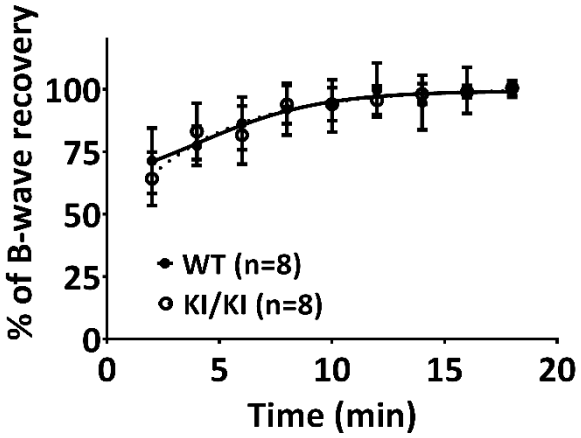
Supp. Figure S3. RPE integrity and function in the WT/KI and KI/KI mice. (A) representative images of TUNEL (red) and DAPI (purple) staining of flat-mounted RPE in WT, WT/KI, and KI/KI mice at age of 4 months. There is no significant TUNEL staining observed in either the WT/KI or KI/KI mouse RPE. WT mouse RPE treated with Dnase I (+Dnase) serves as a positive control for TUNEL staining. Scale bar=200μm. (B) c-wave ERG recordings were performed after full dark adaptation of the animals. The c-wave responses were comparable between WT, WT/KI, and of the KI/KI animals at age of 4 months. Error bars indicate the S.E. of the mean (n=10-12 for each genotype group). (c) Expression of stress-inducible protein Sqstm1 in aged mouse eye cup. Extracted proteins from the RPE/choroid/Sclera tissue were subject to western blot using polyclonal anti-Sqstm1 (rabbit), followed by a secondary anti-rabbit antibody labeled with 600nm fluorophore (green). A monoclonal anti-actin (Actc1) antibody (mouse, red) was adopted for expression normalization among different samples. There is no detectable Sqstm1 expression in WT, WT/KI, or KI/KI mouse RPE/choroid/sclera. ARPE19 cells treated with the vH^+ATPase inhibitor bafilomycin A1 (BafA1) serves as a positive control (P.C.) for the Sqstm1 expression as described in the *Materials and Methods* section.



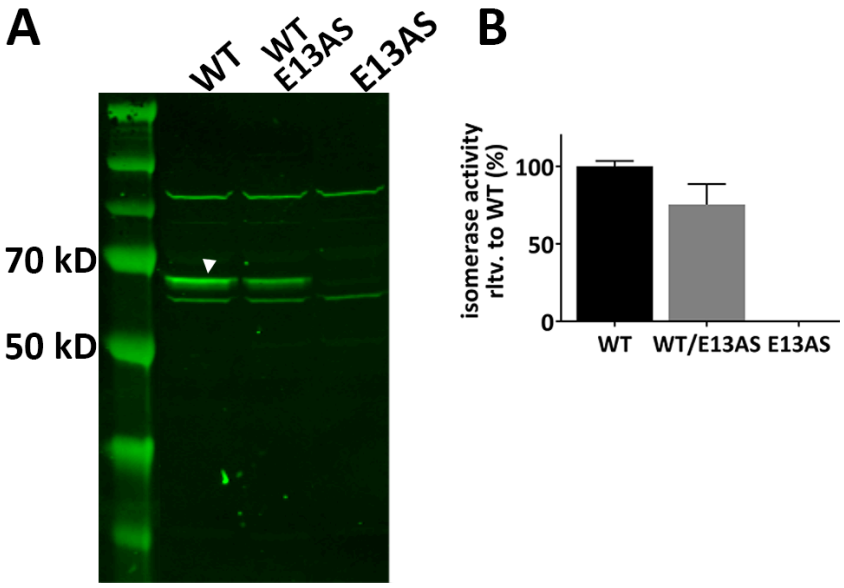
Supp. Figure S4. Cone ERG responses following short dark adaptation. Photopic ERG recordings were performed after the animals were dark adapted for only 20 minutes. Both the a-wave and b-wave response were comparable between WT, WT/KI, and KI/KI animals. Error bars represent S.D. of the mean; n=8-12 for each genotype group.



Supp. Figure S5. ERG responses after chronic light exposure. Scotopic ERG response of the KI/KI mice after chronic light exposure for 6 months. KI/KI mice were exposed to 500 lux light, on a 12h day/12 h night schedule, for 6 months, and scotopic ERG response were recorded after a full dark adaptation of ~16 hours. The ERG responses of the KI/KI mice are comparable to the WT sibling controls; n=8 for each genotype group.



Supp. Figure S6. The cone ERG recovery after light bleaching is similar in KI/KI mice raised in dim light conditions as compared to that in WT siblings. Error bars represent S.D. of the mean; n=8 for each genotype group.



Supp. Figure S7. Effect of alternative splicing on RPE65 activity and expression. Expression plasmids containing recombinant DNA comprising cDNA of the alternatively spliced RPE65 isoform (E13AS) were transfected into HEK293F cells. Mutant expression and isomerase activity were compared to cells transfected with wild type RPE65-expressing plasmid (WT), as well as cells co-transfected with WT and E13AS mutant at 1:1 ratio (WT/E13AS). (A) A putative alternatively spliced construct was not detected in the 293F cells nor was there an effect on the expression of the WT RPE65 construct when co-transfected at equimolar ratio. Arrowhead indicates the position of RPE65 expression in the cells transfected with WT; (B) There was no isomerase activity associated with the expression of the alternatively spliced isoform E13AS nor did it interfere with the activity of the WT construct when the two were co-expressed.

Supp. Table S1. Primers and probes used in this study.

Name	Sequence	Type	Application
gRNA_c.1430	TCA TCT TCT TCC AAG GCA CC	primer	gRNA synthesis
c.1430-169mer	AGATGAACGTCAAAACTAAAGAAATCTGGATGT GGCAAGAGCCAGATTCTTACCCATCTGAACCCA TCTTTGTTTCTCA**C**CCAG**G**TGC**CT**TGGAAGAAGA	oligo	ssODN carrying knockin mutation for homologous recombination
D477G s_F	GTT GAA TCA CTT TGTTCCTGACA	primer	genotyping via sequencing
D477G s_R410	AGT AGT GAT CTC CAA GAC CTG AC	primer	
D477G-GEN-F	CTC ATT GAT TCC ATT ACC ATC ATC TTC	primer	genotyping via ddPCR
D477G-GEN-R	CTT GGT CAT AAG CAG CTC TGT	primer	
D477G-GEN-KI-P	AGG CAC CTG GGT GAG AAA CAA AGA	probe	
D477G-GEN-REF-P	AGA ATC TGG CTC TTG CCA CAT CCA	probe	
3' RACE PCR	GGC CAC GCG TCG ACT AGT AC		3'-RACE
cDNA Cloning Primer	GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT		
E67-1262	CTC CAC TGA AAG CAG ACA AGG AAG A		
3'probe assay-F	GTT CTG AGT GTG GTG GTG AG	primer	mouse *Rpe65* taqman assay for 3' end of WT transcript
3'probe assay-P	AAA TTG CCA GGG CTG AAG TGG AGA	probe	
3'probe assay-R	CAT GGA AGG TCA CAG GGA TAT T	primer	
5'probe assay-F	GCA CAA GTT TGA CTT CAA GGA G	primer	mouse *Rpe65* taqman assay for 5' end of WT transcript
5'probe assay-P	CAC ATA CCA CAG AAG ATT CAT CCG CAC T	probe	
5'probe assay-R	CAG TCA TTG CTC GAA CAT AAG C	primer	
E13-MIS-F	ACC CAT CTG AAC CCA TCT TTG	primer	taqman assay for mouse transcript E13-MIS
E13-MIS-P	CCA CCA CAC TCA GAA CCA CAC CAT	probe	
E13-MIS-R	CCA GGA GAT ATG CAG GCT TT	primer	
E13-AS-R	CAC TCA GAA CCA CAC CAT CAT	primer	taqman assay for alternatively spliced mouse E13-AS
E13-AS-P	CTT CTT CCA AGG CAC CTT GTC AGG AAC AA	probe	
E13-AS-F	CAG AAA TTT GGA GGG AAA CCT TAT AC	primer	
E13-DEL-F	TGG AGG GAA ACC TTA TAC TTA TGC	primer	taqman assay for alternatively spliced mouse E13-DEL
E13-DEL-P	CTT TGT TCC TGA CAA GGT GTG GTT CTG AGT	probe	
E13-DEL-R	CAA GTC TTT GGC ATT CAG AAC C	primer	
human E13-MIS-F	TGG CAA GAG CCT GAT TCA TAC C	primer	taqman assay for human transcript E13-MIS
human E13-MIS-P	TTG TTT CTC ACC CAG GTG CCT TGG AA	probe	
human E13-MIS-R	CAC CAC CAC ACT CAG AAC TAC AC	primer	
human E13-AS-F	TGC GTA TGG ACT TGG CTT GAA T	primer	taqman assay for alternatively spliced human E13-AS
human E13-AS-P	CAC CAC CAC ACT CAG AAC TAC AC	probe	
human E13-AS-R	CAC TTT GTT CCA GAT AGG GTG CCT TGG A	primer	

Supp. Table S2. Comparison of gene expression profiles between WT and WT/KI eye cups with adjusted p-value (false discovery rate (FDR)<0.5)

ensembl.ID	gene symbol	Fold Change (Log_2)*	p value	FDR-adjusted p
ENSMUSG00000026616	Cr2	-2.96	5.11E-09	7.92E-05
ENSMUSG00000086503	Xist	9.50	3.69E-06	0.03
ENSMUSG00000028174	Rpe65	-0.62	5.75E-05	0.30
ENSMUSG00000028647	Mycbp	-1.06	9.40E-05	0.35
ENSMUSG00000069919	Hba-a1	-0.62	0.000112	0.35
ENSMUSG00000063415	Cyp26b1	0.66	0.000142	0.37
ENSMUSG00000007682	Dio2	1.36	0.000178	0.39
ENSMUSG00000000001	Gnai3	0.05	0.760996	1.00