PNAS
www.pnas.org

Supplementary Information for

Tmem138 is localized to the connecting cilium essential for rhodopsin localization and outer segment biogenesis

Dianlei Guo[1]*, Jiali Ru[1]*, Lijing Xie[1], Mingjuan Wu[1], Yingchun Su[1], Shiyong Zhu[1], Shujuan Xu[1], Bin Zou[1], Yanhong Wei[2], Xialin Liu[1], Yizhi Liu[1$], Chunqiao Liu[1,3$]

Chunqiao Liu

Email: liuchunq3@mail.sysu.edu.cn;

This PDF file includes:

Supplementary Information Text

SUPPLEMENTARY MATERIALS AND METHODS

Animal husbandry

All procedures involving the use of mice were approved by Zhongshan Ophthalmic Center Animal Care and Use Committee (ACUC). Transgenic mice were generated using Cyagen service (www.cyagen.com).

Generation of Tmem138 mutant alleles

The 'knockout-first' strategy was used to create the *Tmem138* gene-trap allele (*Tmem138*$^{a/a}$) by homologous recombination in ES cells (Fig. 1A) (1, 2). Briefly, a gene-trap construct was engineered with an Frt site and *En2* splicing acceptor (SA) preceding an *eGfp* reporter, followed by a polyadenylation signal, *loxP*, *PGK-neo*, *Frt*, and *loxP*. This gene-trap cassette was placed between *Tmem138* exon 1 and 2. A third *loxP* was placed right after exon 3, which will be used for the generation of a conditional knockout allele. Southern analysis was performed as described previously (2) to identify the F1 *Tmem138*$^{a/a}$ allele. A 5'-probe outside of the left arm was used to identify 8.3kb- and 12.4kb- ApaLI fragments from wild type and *Tmem138*$^{a/a}$ alleles, respectively (Fig.1B).

For the generation of *Tmem138*$^{b/b}$ straight knockout allele, *Sox2-Cre* transgenic females (equivalent to the germline *Cre* transgenic lines, JAX laboratory) were used to cross with the *Tmem138*$^{a/a}$ to delete exon 2 and 3, and leaving the *eGfp* reporter driven by the endogenous *Tmem138* promoter. For the generation of the *Tmem138*$^{c/c}$ conditional allele, the *CAG-Flp* transgenic line (Model Animal Research Center of Nanjing University, China) was used to delete the gene-trap cassette, leaving two loxP sites flanking the exon 2 and 3 (Fig. 1A). Further crossing *Rho-Cre* transgenic line (Cyagen, China) with the *Tmem138*$^{c/c}$ allele generated rod-specific *Tmem138* mutants.

Genotyping and detection of *Tmem138* allelic transcripts and proteins

All primers used in this study were listed in Suppl. Table 1. Primers for genotyping and detection of *Tmem138* allelic transcripts were illustrated in Suppl. Fig. 1 & 7. Briefly, primer pairs of p1-p2 and p3-p4 were used for genotyping of wild type and *Tmem138* $^{b/+}$ straight knockout allele (Tm138 b), respectively. Primer pair p13-p14 was for genotyping of both wild type and *Tmem138* $^{c/+}$ conditional knockout allele, and primer pair p15-p16 was for genotyping *Rho-Cre* transgenic allele. PCR programs for p1-p2, p3-p4, p13-p14 and p15-p16 are all the same as follows 94℃ 5min; 94℃ 10s, 61℃ 30s, 72℃ 30s, 35 cycle; 72℃ 10min.

For RT-PCR, retinas from wild type and *Tmem138* mutant mice at postnatal 12 days were dissected in PBS on ice. Fresh retinas were subjected to RNA extraction using RNAsimple Total RNA Kit (DP419, TIANGEN, China) per the manufacturer's instructions. Total RNAs were reverse transcribed into cDNAs (A5001, Promega, China), and PCR reactions were performed to identify transcripts from different *Tmem138* alleles. P5-p6, p7-p8, p9-p10, and p11-p12 were used for genotyping exon (E) 1, *Gfp*, E2, and E5, respectively. PCR programs for p5-p6, p7-p8, p9-p10 and p11-p12 are all the same as follows 94℃ 5min; 94℃ 10s, 60℃ 30s, 72℃ 30s, 35 cycles; 72℃ 10min.

Southern blotting was performed as described previously (2). Briefly, α^{32}P-labeled probes outside the left (5'-probe) and the right (3'-probe) homologous arms were hybridized to nylon membranes carrying ApaLI- and KpnI- digested wild type and *Tmem138* $^{a/a}$ genomic DNA, respectively. The 5' -probe detected 8.3kb wild type and 12.4kb mutant fragments, and the 3' -probe detected 10.2kb wild type and 13.2kb mutant fragments. The Southern probe primer sequences are provided in Suppl. Table 1.

For Western blotting, protein samples were blotted onto polyvinylidene difluoride (PVDF) membranes by a wet transblot system (Mini-Protein Tetra; Biorad) using the standard protocol recommended by the manufacturer. Antibody against Tmem138 (HPA042373; Sigma, Suppl. Table 2) was used to probe the blot using a standard protocol. Chemiluminescent images were taken by FluorChem R (Proteinsimple).

Retinal cell dissociation, histology, immunohistochemistry, and TUNEL labeling

Retinas were dissected in fresh cold (4℃) PBS, transferred to a 1.5ml Eppendorf tube, and dissociated with 300µl digestion solution (including papain; 2mg/ml; Collagenase I: 1mg/ml; DNase I: 0.05mg/ml in DMEM basal medium) at 37℃ for 5min. Mechanical trituration was performed by pipetting 30 times with a P1000 pipette. Cells were centrifuged at 400 ×g for 5 min at 4 °C. The dissociated cells were gently resuspended in 200 µl of 2%PFA, spread on slides, and quickly dried for 10min. Slides were washed with PBS three times and subjected to immunostaining as described previously (3).

For plastic sectioning, tissues (eyeball, brain, cerebellum, kidney, testis) were dissected and fixed with Carnoy's fixative (60% alcohol, 30% chloroform,10% Acetic acid) overnight at room temperature, dehydrated with a graded series of ethanol, and embedded using Technovit kit according to the manufacturer's instruction (14653, Technovit, Germany). 3 µm plastic sections were cut with a microtome (RM 223, Leica, Germany) and stained with hematoxylin and eosin according to the manufacturer's instruction (UI0402, UBIO, China).

Immunohistochemistry and TUNEL (Roche, Cat. 11684795910) detection of apoptosis were performed as described previously by Guo et. al (3). Antibodies were listed in Suppl. Table 2 with vendor catalog numbers and experimental conditions.

Optical coherence tomography (OCT) and Electroretinography (ERG)

For full-field ERG testing, mice were dark-adapted overnight and anesthetized by an intraperitoneal injection of 4-5 µl/g 10% chloral hydrate. Pupils were dilated using Compound Tropicamide Eye Drops (SINQI, China). ERG responses were recorded using CELERIS system (D430, Diagnosys LLC, USA) with a stimulating electrode in contact with the cornea through Hypromellose eye drops (ZOC, China). Scotopic and photopic ERGs were recorded using strobe flash stimuli from 5-averaged responses of 0.003

~10cd.s/m^2 and 0.3~30 cd-s/m^2, respectively. Light adaptation with background intensity at 30 cd/m^2 was performed for 5 minutes between scotopic and photopic recordings. The values of a- and b-waves were exported to GraphPad Prism 5 for statistical analysis. Student *t-tests* were performed to determine statistical significance. A total of 6 animals were used for each genotype.

For OCT, anesthetization and pupil dilation were performed as described for ERG recording. OCT images were acquired using a Spectralis HRA + OCT system (Heidelberg Engineering, Heidelberg, Germany). Mice were placed on a stage for alignment and corneas were kept moist with drops of 0.9% sterile saline. Images were taken centered on the optic nerve head, and horizontal or radial scans were obtained from each eye. The thickness of the outer retina (RPE + OS + ONL +OPL) and the inner retina (INL + IPL +GCL) were analyzed using Heidelberg Spectralis system software, and values of a- and b-waves were exported to GraphPad Prism 5 for statistical analysis. Student *t-test* was performed to obtain the statistic powers. A total of 6~10 animals were used for each genotype.

Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and immuno-electron microscopy (Immuno-EM)

For both TEM and SEM, P0~P14 mice were euthanized by decapitation or cervical dislocation as described previously (4). Eyes were enucleated and immersed in 2%PFA for 10 min, and the retinas were dissected out, cut into small pieces (about 2mm x 1mm), and fixed further in 2% PFA/2.5%/glutaraldehyde in PBS at 4℃ overnight. Adult mice were deeply anesthetized with 10% chloral hydrate (5ul/g), and cardiac perfusion was performed first with 50 ml 0.9% saline followed by 50 ml of fixative (2% PFA, 2.5% glutaraldehyde in PBS) for about 10 min. The enucleated eyes were further fixed in the same fixative for 24 h and cut on a vibratome (VT1000S, Leica, Germany) at 200μm thickness.

For TEM, retina pieces or sections were then rinsed with 50 mM cacodylate buffer (PH 7.4) and incubated in 1% reduced OsO4 in cacodylate buffer on ice

for 90 min. Sections were dehydrated through a gradient series of ethanol, infiltrated with propylene oxide, and embedded in EMBED812 resins (14120, EMS, USA) at 60℃ for 36-48 hours. Ultrathin sections at 60-80 nm were cut on an ultramicrotome (Leica EM UC7, Leica, Germany) and collected onto copper grids (FCF200-Cu-50, Electron Microscopy Sciences, USA). Sections were poststained with 1% uranyl acetate for 30min and Sato's lead for 3min. Electron microscopy images were acquired on HT-7700 (Hitachi, Japan).

For SEM, the cornea, sclera, and choroid were quickly removed from the freshly dissected eyeballs. Retinas with lens and RPE were then fixed in the same fixative as for TEM. Vibratome sections were prepared at 200 μm thickness after removal of the lens, dehydrated with ethanol and acetone. The samples were then immersed in isoamyl acetate, dried, sputter-coated, and imaged using scanning electron microscopy (S-3400N, Hitachi, Japan).

For immuno-EM, P5, P8, and P14 retinas were cut into small pieces about 2mm x 1mm after 2%PFA fixation. Retina pieces were fixed in 2% PFA/0.1% glutaraldehyde in PBS at 4℃ overnight. The retina pieces were rinsed with PBS, dehydrated with ethanol, and embedded in capsules filled with LR white resins (14381-UC, EMS, USA). The resins were let to polymerize at 60℃ for 36-48 hours in an oven. 60-80nm ultrathin sections were cut and collected onto nickel grids (FCF200-Ni-50, EMS, USA). Sections were treated with 50 mM Tris-glycine buffer (pH7.4) for 10 min, rinsed with PBS, and blocked with 10% BSA in PBS at room temperature (RT) for 30 min. After incubating overnight at 4℃ with 1D4 antibody (anti-rhodopsin C terminus), grids were washed with 1% BSA in PBS for 5 times, 5min each, and probed with IgG-conjugated 10nm colloidal gold particles (25825, EMS, USA) for 2h at RT. Grids were rinsed and counterstained with 1% uranyl acetate for 5-10min and Sato's lead for 30s-1min.

Detection of Protein interaction

Plasmid constructs: pLVX-*3F-TST-Tmem138*: 3xFlag and Twin-Strep-tagged (TST) tags at the N-terminus; pRK5 *HA-rhodopsin*: HA tag at the N-terminus; pRK5-*Ahi1-HA/Flag* and pRK5-*Tmem231-HA/Flag*: HA or Flag tag at the C-

terminus; pRK5-*Tmem67-Flag,* pRK5-*Arl13b-Flag,* and pRK5-*Rab8a-Flag*: Flag tag at the C-terminus. Construct diagrams are listed in Suppl. Fig. 11A.

HEK293T cells were co-transfected with equimolar amounts of the tagged plasmids (total 10ug/100mm^2 dish, ~7x10^6 cells) and were harvested after 48 h in culture. Briefly, cells were incubated on ice for 15min in 1ml of TBS lysis buffer containing 30mM Tris-HCl (PH 7.4), 150mM NaCl, 0.5%NP-40 (I8896, Sigma, USA), and a protease inhibitor cocktail (P8340, Sigma, USA). The lysed cells were centrifuged for 15 min (10,000xg at 4° C). For 3F-TST-tagged proteins, the supernatant was incubated with 100μl Strep-TactinXT resins (2-4010-010, IBA, Germany). For HA- or Flag-tagged proteins, the supernatant was incubated with 100μl Anti-HA Affinity gel (D111140, Sangon Biotech, China) or Anti-Flag M2 Affinity Gel (A2220, Sigma, USA). Incubation lasted overnight at 4℃ with rotation. The mixtures were then loaded into empty chromatography columns, and resins or agarose beads were packed by gravitation. Columns were washed 3 times withTBS buffer to clear off residual unbound proteins and eluted with 3 times 100 μ l biotin for TST column, or HA, or Flag peptides for Sepharose columns. The eluant from each fraction was examined by Western blotting using anti-Flag or anti-HA antibody. The eluant having the maximal amount of bait proteins was used for the detection of protein interaction. Schematic illustration of affinity purification is shown in in Suppl. Fig. 11B.

For immune-pull-down experiments, 14 retinas from 3-week-old mice were dissected and snap-frozen in liquid nitrogen. Retinas were then transferred into TBS lysis buffer (30mM Tris-HCl (PH 7.4), 150mM NaCl, 0.5%NP-40) supplemented with protease inhibitor cocktail (P8340, Sigma, USA) and gently vortexed for 10 seconds. The OS and CC components were separated into supernatant from the retinal tissue chunks by spinning down at 2000G for 10S (5). Let the supernatant stay in the lysis buffer for another 15 min on ice, and centrifuged at 15000 x g at 4°C to remove the tissue debris.

For protein pull-down assays, 100 µl/sample Pierce protein A/G magnetic beads (88803, Thermo, USA) were blocked with 10% BSA at 4℃ overnight on a

rotary plate. The above-prepared OS and CC protein extracts were incubated with Tmem138 antibody at 4°C overnight with rotating. The sample/antibody mixtures were transferred to a 1.5mL microcentrifuge tube containing the pre-blocked magnetic beads and incubated at room temperature for 1 hour with rotating. The magnetic beads were then collected with a magnetic stand, and proteins were eluted with 100μl of glycine (50 mM, pH2.0) and analyzed by SDS-PAGE electrophoresis and Western blotting.

Quantification and statistics

For quantification of the photoreceptor ciliary structures, TEM images from P3 and P5 retinas were acquired as previously described. Cilia between RPE and the ONL at each stage of photoreceptor ciliogenesis were counted from 3 retinal sections/retina, each longitudinally spanning over a 100μm length in mid-peripheral retinal areas. The numbers of cilia from 3 sections (3x100 μm=300 μm total span) for each stage were used for plotting the graphs. One retina for each genotype was counted.

For measuring ciliary density, Ac-tub stained flat-mount retina was divided into three areas: central, mid-peripheral, and peripheral. Images were taken with a 40x/1.40 oil objective at 1024 x 1024-pixel resolution. The cilia density was determined by counting cilia numbers in the above three areas using ImageJ and normalized to the counting area (per 100 μm^2).

For measuring lengths of ciliary domains stained with immunofluorescent markers, images were taken with a 100x/1.40 oil objective and 3.0x optical zoom factor at 1024 x 1024 resolution, using Zeiss LSM880 equipped with Airyscan. A total of 100~150 cilia of each retina section/animal (total 3 animals) were used. The lengths of stained ciliary domains were determined by NIH ImageJ software.

In quantifying rhodopsin gold particles in the CC, full-length cilia of both controls and mutants were counted at P5 since OS does not form at this age. For P8 controls, gold particles along the CC (between OS and IS) were counted. Because OS does not develop in the mutants and similar Ac-tub domain length existed between the controls and the mutants, we determined using the average

length of the control CC to count that of the mutants. For each genotype, a total of 8 sections were counted.

For quantifying the outer segment rhodopsin and peripherin contents, outer segment extracts were prepared as previously described in "**Detection of Protein interaction"** section. Western blots were probed with anti-Rho and Rds antibodies. Signal intensity was detected by NIH imageJ. Three measurements for each of three blots from 3 animals, respectively, were averaged for statistical analysis.

All statistical analyses were performed with the GraphPad Prism 7.0 software and presented as mean ± SD. Statistical powers were determined by the Student's t-test. '*', $p < 0.05$; '**', $p < 0.01$.

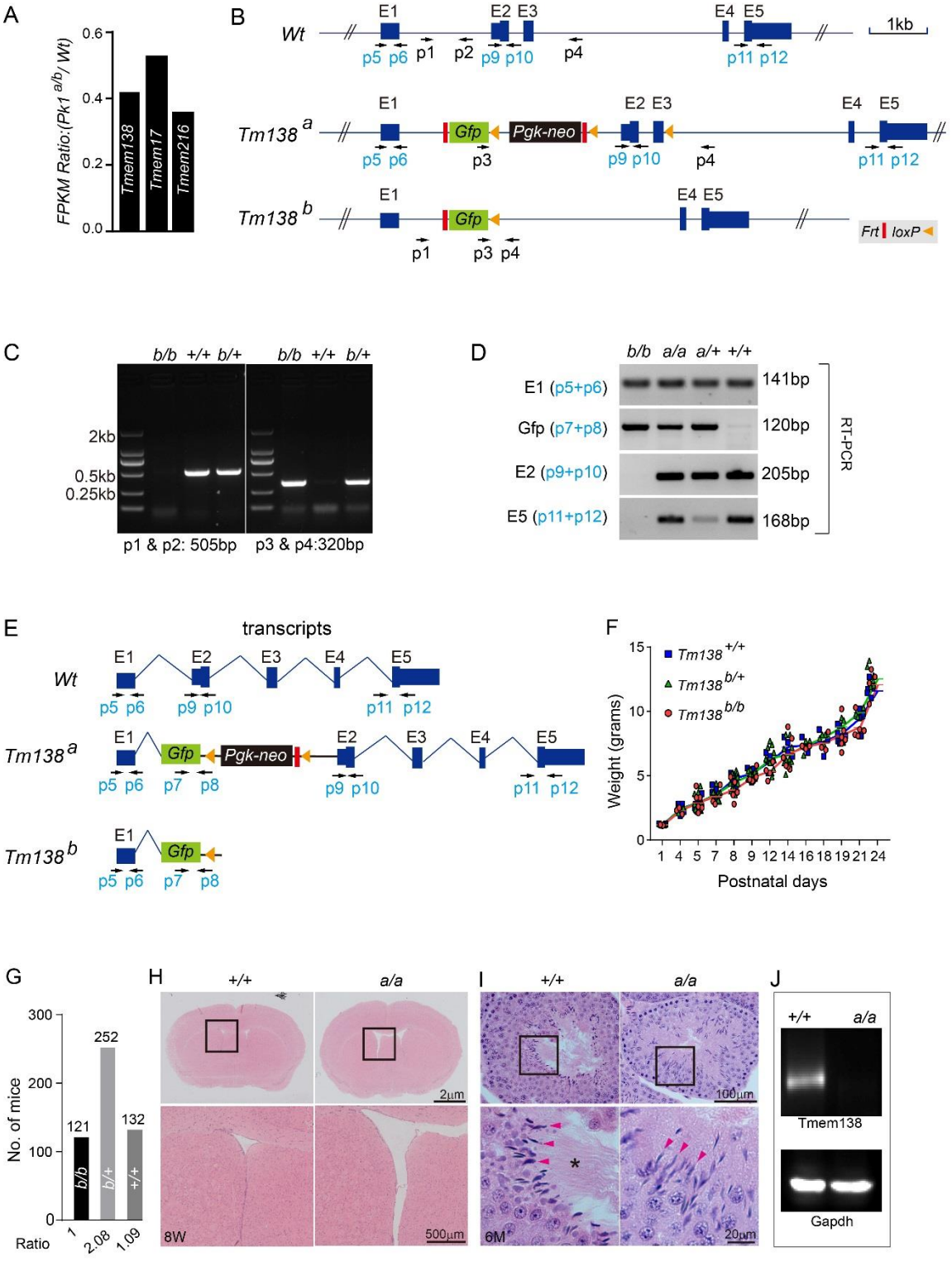
Tmem138 tertiary structure prediction and analysis of scRNA-seq data

Tmem138 3D structure was obtained from AlphaFold Protein Structure Database developed by DeepMind, in which the protein structure was predicted from the amino acid sequence (https://alphafold.ebi.ac.uk/).

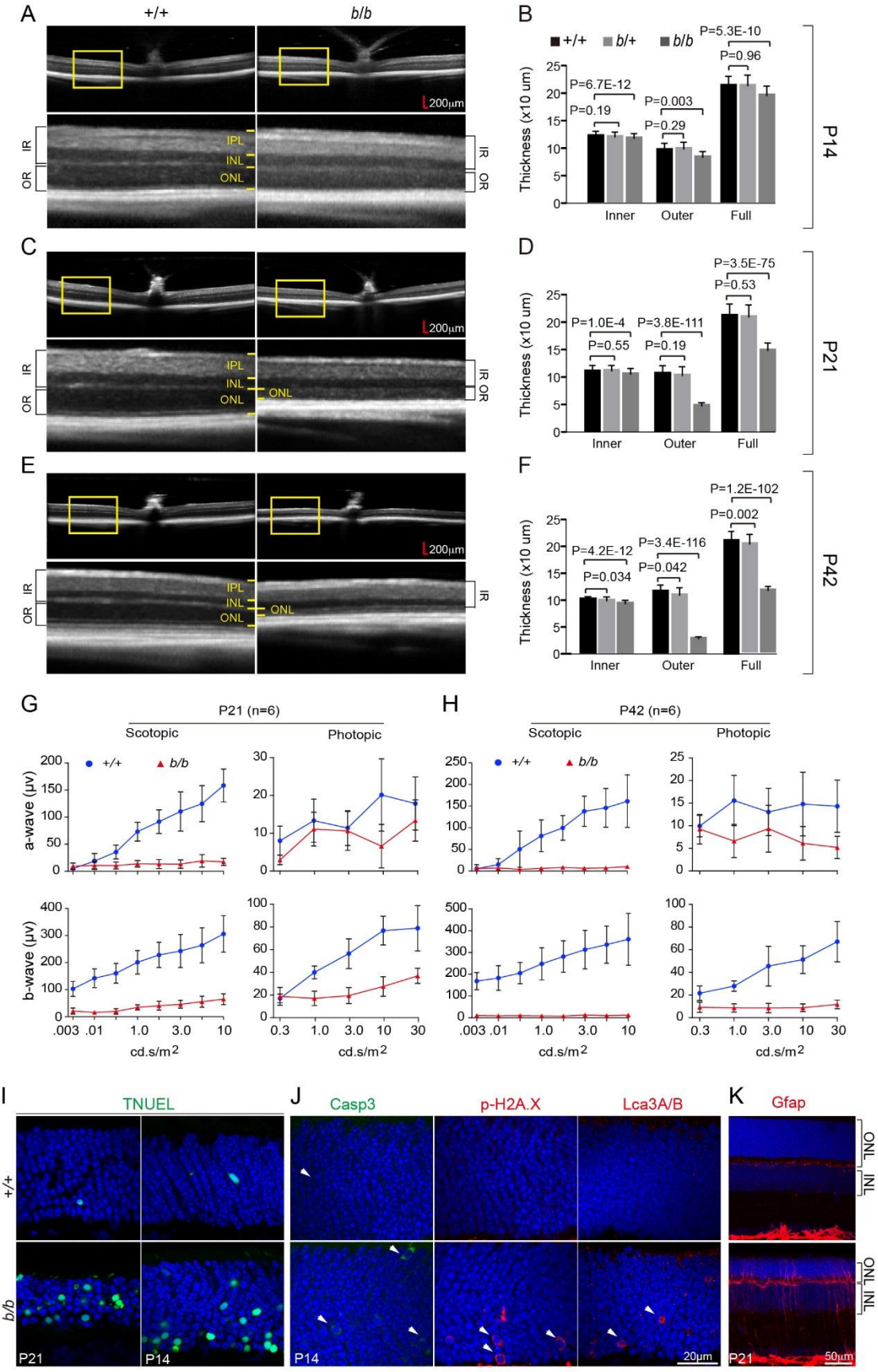
For analysis of scRNA-seq data (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147573), gene count matrix was imported to the Seurat toolkit (Version 4.0.2). Cells were excluded if more than 15% of detected genes were from mitochondrial and if less than 200 genes were transcribed. The Seurat object used Log normalization and set 2000 genes to find variable features. Dimensionality reduction was then performed using PCA and UMAP plots generated by the RunUMAP function.

Cells were clustered using the Louvain algorithm based on a shared nearest-neighbor network. The final resolution of all subsequent clustering analyses was determined by the biological questions and the need for details as explored in the subsequent annotation. In order to determine cell types, we compared clusters to those of the Immgen database using SingleR (V1.0.6) package. Differential expression of marker genes was performed using the FindAllMarkers function in Seurat with default parameters. Then, clusters were further annotated by directly comparing their transcriptional state with the known clusters using Single R

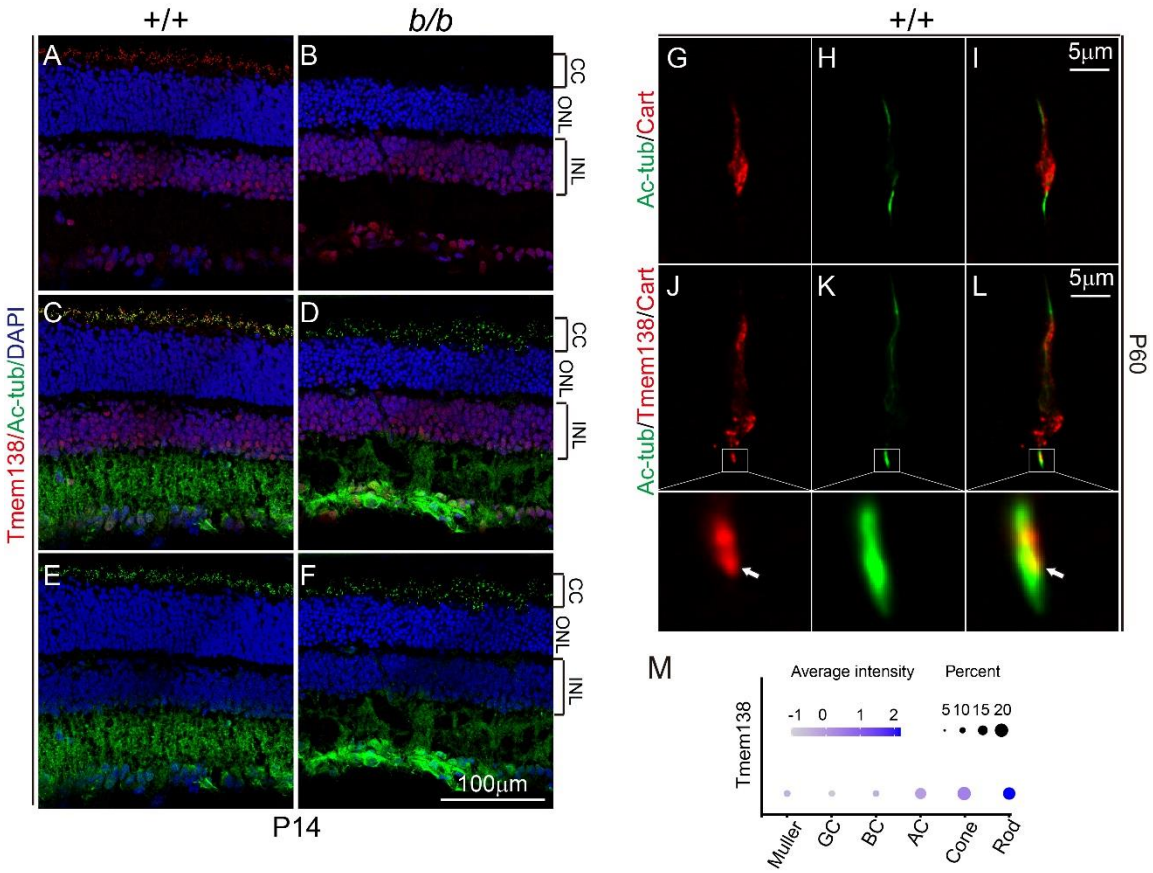
Package and top differentially expressed genes with cell type-specific expression known from the literature. Clusters highly expressed similar marker genes indicating the same cell types were merged for broad categories.


A
FPKM Ratio:(Pk1 a/b/ Wt)
0.6
0.4
0.2
0.0
Tmem138
Tmem17
Tmem216
B
Wt
Tm138 a
Tm138 b
E1
E2 E3
E4 E5
1kb
p5 p6
p1
p2
p9 p10
p4
p11 p12
Gfp
Pgk-neo
p3
Frt
loxP
C
b/b +/+ b/+
2kb
0.5kb
0.25kb
p1 & p2: 505bp
p3 & p4:320bp
D
b/b a/a a/+ +/+
E1 (p5+p6)
Gfp (p7+p8)
E2 (p9+p10)
E5 (p11+p12)
141bp
120bp
205bp
168bp
RT-PCR
E
transcripts
Wt
Tm138 a
Tm138 b
p7 p8
F
Tm138 +/+
Tm138 b/+
Tm138 b/b
Weight (grams)
Postnatal days
G
No. of mice
121
252
132
b/b
b/+
+/+
Ratio
1
2.08
1.09
H
+/+
a/a
2μm
8W
500μm
I
6M
100μm
20μm
J
Tmem138
Gapdh

S**upplemental Figure 1.** (A), Three transmembrane protein genes downregulated in *Pk1* mutant mouse (*Pk1*$^{a/b}$) retina. (B), Genomic locations of primers used to differentiate genetic alleles and to detect transcripts from each allele by RT-PCR. Primers in black (P1-P4) were used for genotyping. Primers in turquoise (P5-P12) were used for RT-PCR. (C), Genotyping *Tmem138*$^{+/+}$(*Wt*), *Tmem138*$^{b/+}$, and *Tmem138*$^{b/b}$ mice. (D), RT-PCR- detected transcripts from selected exons. (E), Possible longest transcripts for each allele inferred from the results of (C). (F), Comparable growth curves from different *Tmem138* alleles. (G), Genotype ratio of pooled postnatal mice. (H), H&E-stained coronal brain sections from wild-type and homozygous *Tmem138*$^{a/a}$ *(a/a)* mice. Boxed areas are magnified below each panel. (I), H&E-stained testis sections from 6-month-old wild type and the '*a/a'* mice, respectively. Boxed areas are magnified below each panel. Red arrows and asterisk indicate sperm heads and tails, respectively; (J) Western blotting of P14 retinal extracts from wild type and the '*a/a'* mice using an anti-Tmem138 antibody.


A
+/+
b/b
200µm
IR
OR
IPL
INL
ONL
B
+/+
b/+
b/b
Thickness (x10 um)
P=5.3E-10
P=0.96
P=6.7E-12
P=0.19
P=0.003
P=0.29
Inner
Outer
Full
P14
C
P=3.5E-75
P=0.53
P=1.0E-4
P=0.55
P=3.8E-111
P=0.19
P21
E
P=1.2E-102
P=0.002
P=4.2E-12
P=0.034
P=3.4E-116
P=0.042
P42
G
P21 (n=6)
Scotopic
Photopic
a-wave (µv)
b-wave (µv)
cd.s/m2
H
P42 (n=6)
I
TNUEL
P21
P14
J
Casp3
p-H2A.X
Lca3A/B
20µm
K
Gfap
ONL
INL
50µm

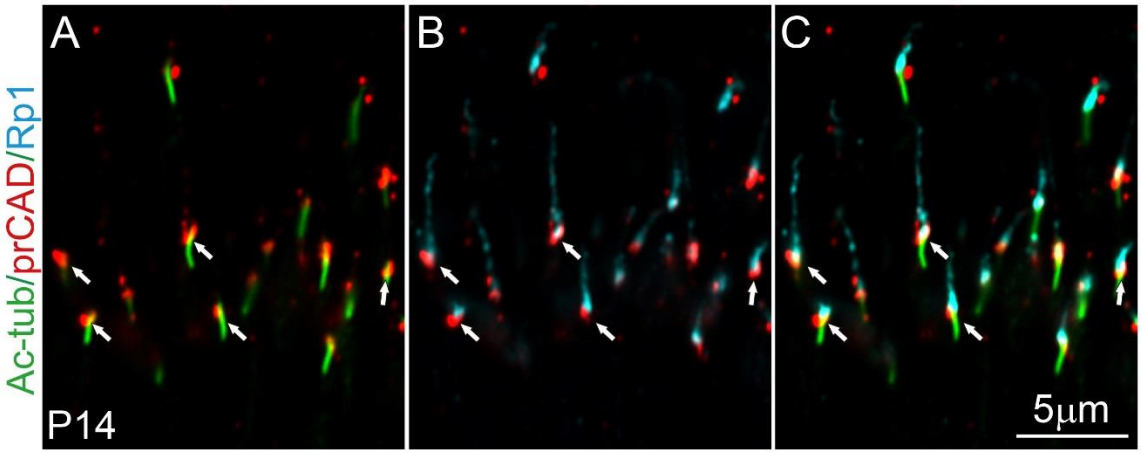
Supplemental Figure 2. (A, B), (C, D), and (E, F) are OCT images and respective quantifications at P14, P21, and P42. Boxed areas were magnified below each panel. (G, H), Quantification of scotopic and photopic a- and b-waves of ERG at P21 (G) and P42 (H). (I), Detection of cell death by TNUEL at P21 and P14. (J), Detection of cell death using anti-Caspase-3 and p-H2A. X antibodies, and detection of autophagy using an anti-Lca3A/B antibody. (K), Muller glia activation in response to retinal injury. ONL, outer nuclear layer; INL, inner nuclear layer.



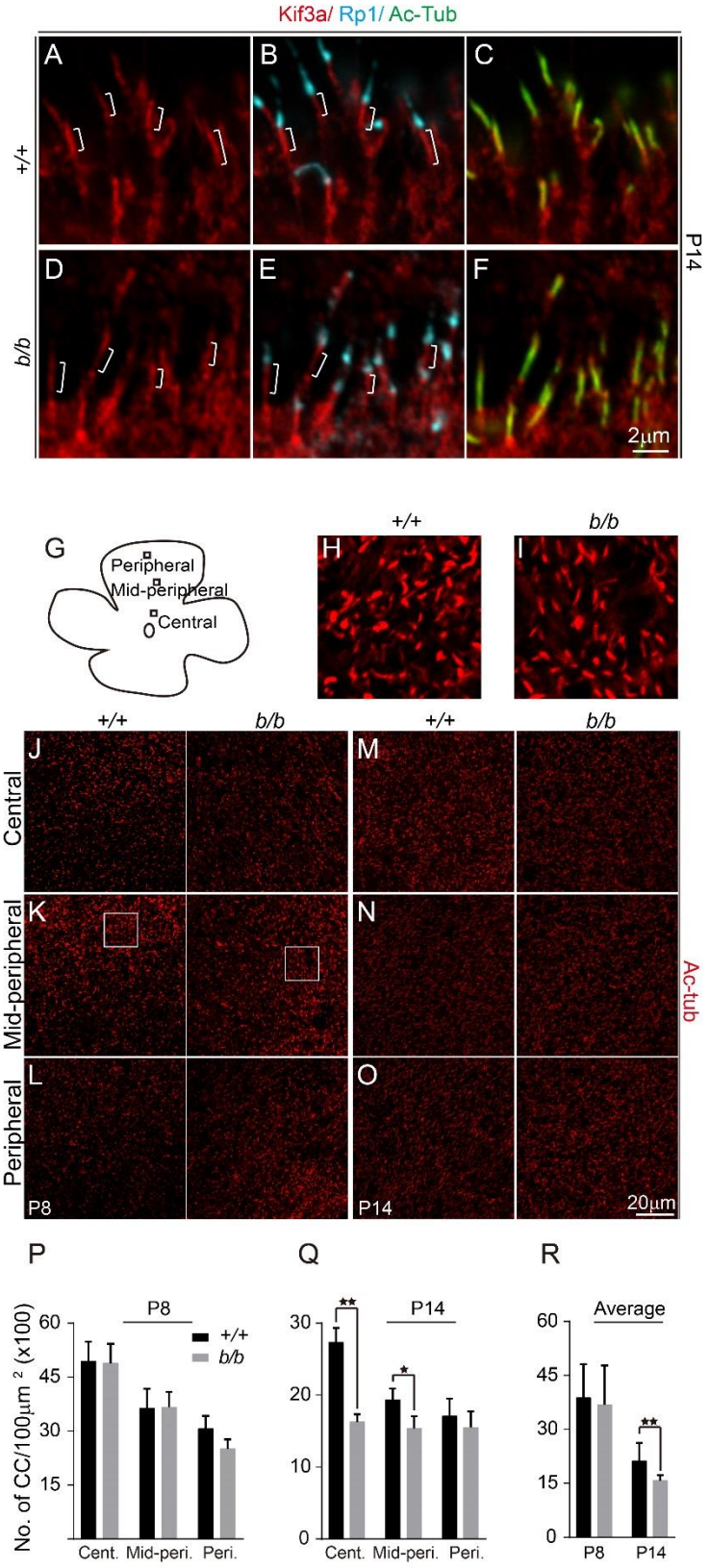
+

Supplemental Figure 3. (A-F), Immunostaining of retinal sections at P14. Ac-tub, green; Tmem138, red. Genotypes are indicated on top of the panels. (G-L), Detection of Tmem138 localization in dissociated cones: Ac-tub, green; Tmem138/cone arrestin (Cart), red. Boxed areas in J-L are magnified below each

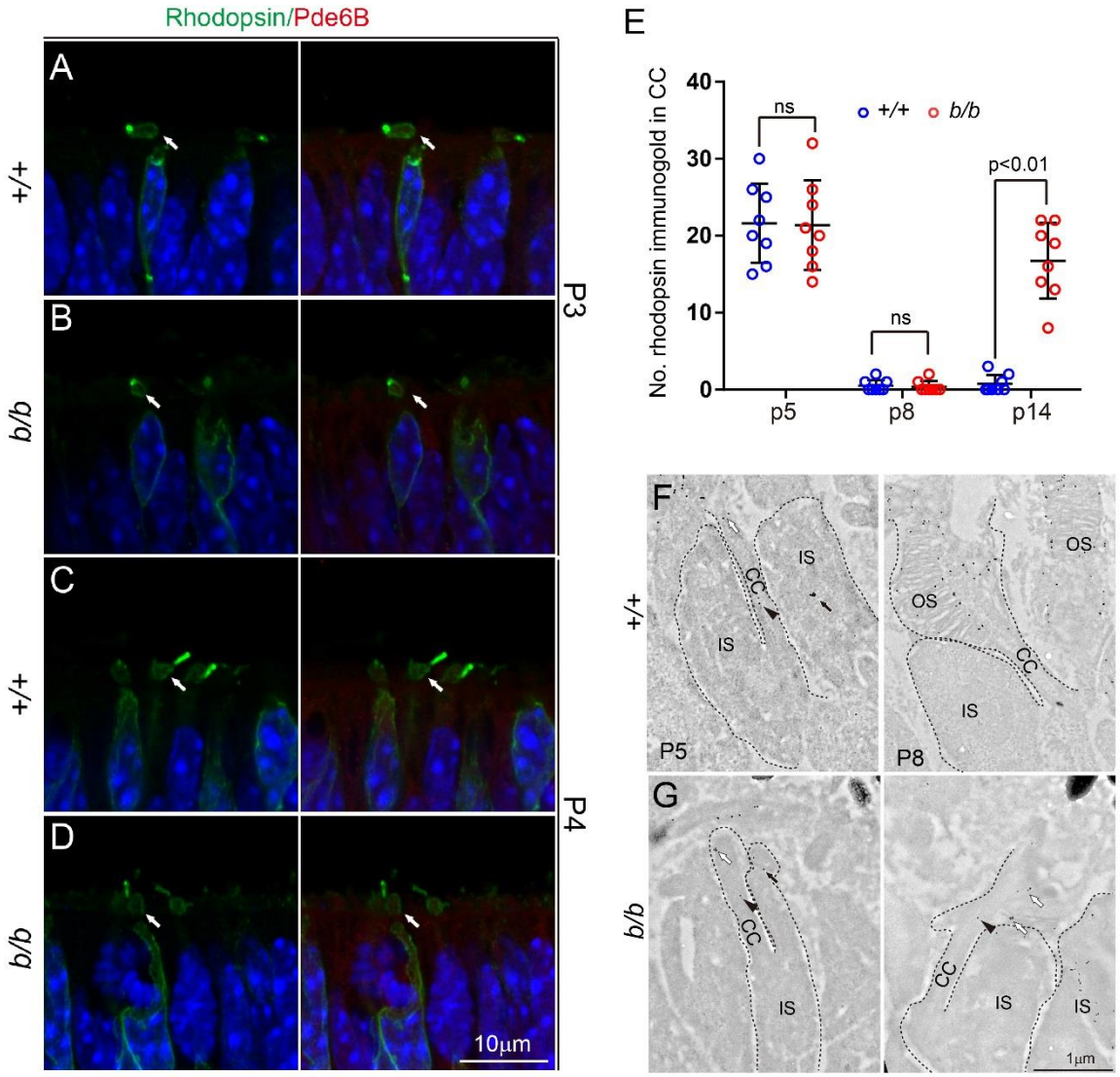
panel. Arrows point to Tmem138 staining in the CC. (M), Dot plotting single-cell expression profiles of *Tmem138* in major retinal cell types. The size of each dot represents the percentage of cells in each cell type. Grey to blue gradient indicates low to high expression. ONL, outer nuclear layer; INL, inner nuclear layer; CC, connecting cilium.



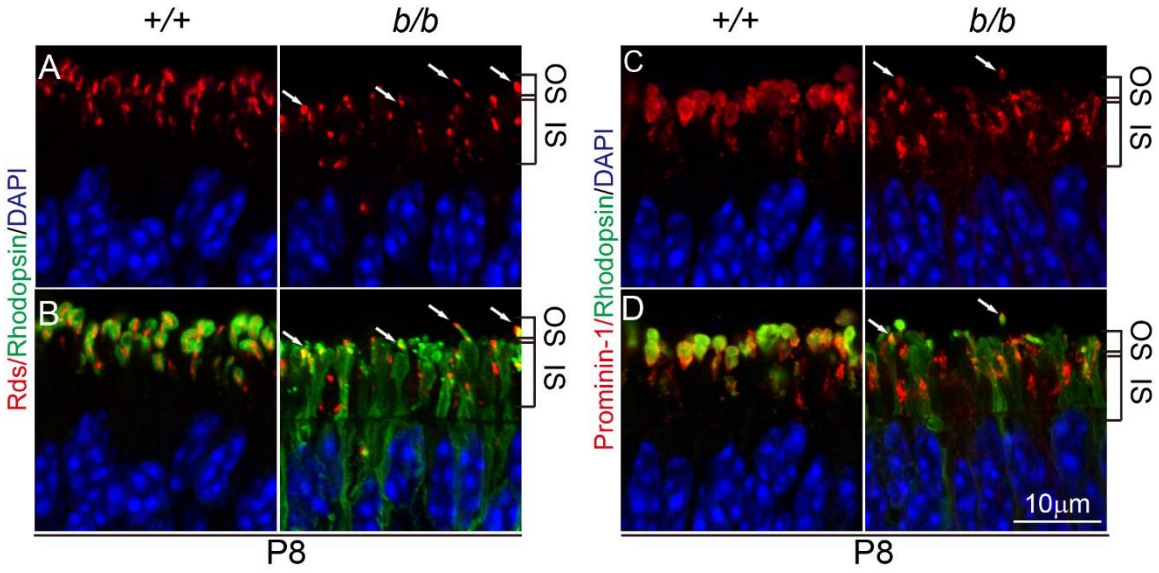
Supplemental Figure 4. Triple labeling photoreceptors with prCAD (red), Ac-tub (green), and Rp1(turquoise) at P14. Arrows point to the prCAD staining adjacent/juxtaposed to the base of the Rp1. (A), Merged channels of Ac-tub and prCAD; (B), Merged channels of prCAD and Rp1; (C), Merged all three channels.


Kif3a/ Rp1/ Ac-Tub
A
B
C
D
E
F
+/+
b/b
P14
2μm
G
Peripheral
Mid-peripheral
Central
H
I
+/+
b/b
J
K
L
M
N
O
Central
Mid-peripheral
Peripheral
Ac-tub
P8
P14
20μm
P
Q
R
P8
P14
Average
+/+
b/b
No. of CC/100μm 2 (x100)
Cent. Mid-peri. Peri.
Cent. Mid-peri. Peri.
P8 P14

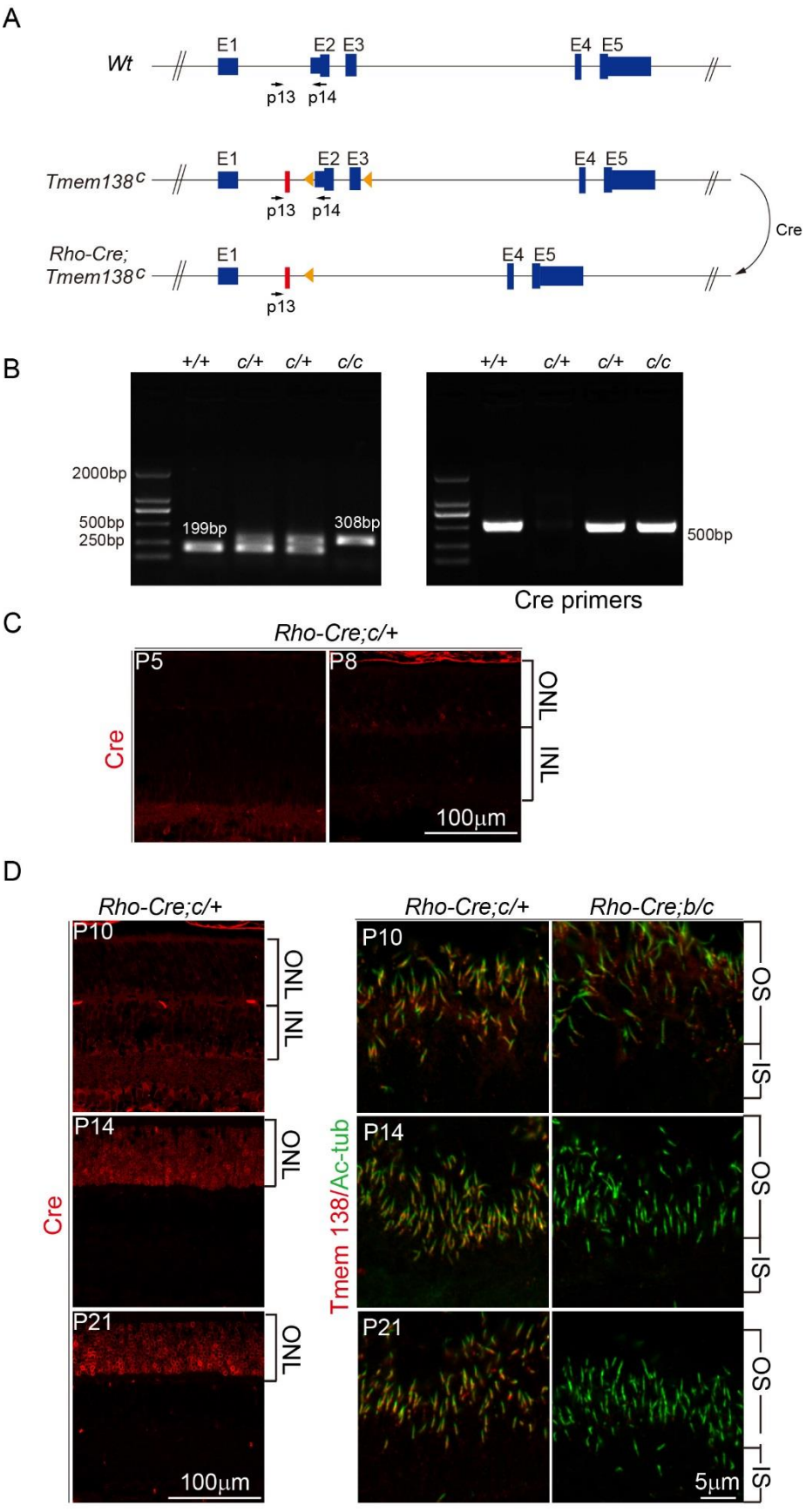
Supplemental Figure 5. (A-F), Kif3a (red), Ac-tub (green), and Rp1(turquoise) triple labeling at P14. Brackets indicate Kif3a CC staining. (G-R), Quantification of cilia number by Ac-tub-stained flat-mount retinas. (G), Schematic drawing of a retina with small squares indicating counted areas. (H, I), Magnified areas in (K) showing image examples for quantification of cilia numbers. (J-L), P8 retina with Ac-tubulin staining. (M-O), P14 retina with Ac-tubulin staining. (P, Q), Cilia numbers of P8 (J) and P14 (K) retinal areas. (R), Average numbers of cilia from different areas from P8 and P14 retinas. Student's t-test. '*', $p < 0.05$; '**', $p < 0.01$. Cent., Central; Mid-peri., Mid-peripheral; Peri., Peripheral.



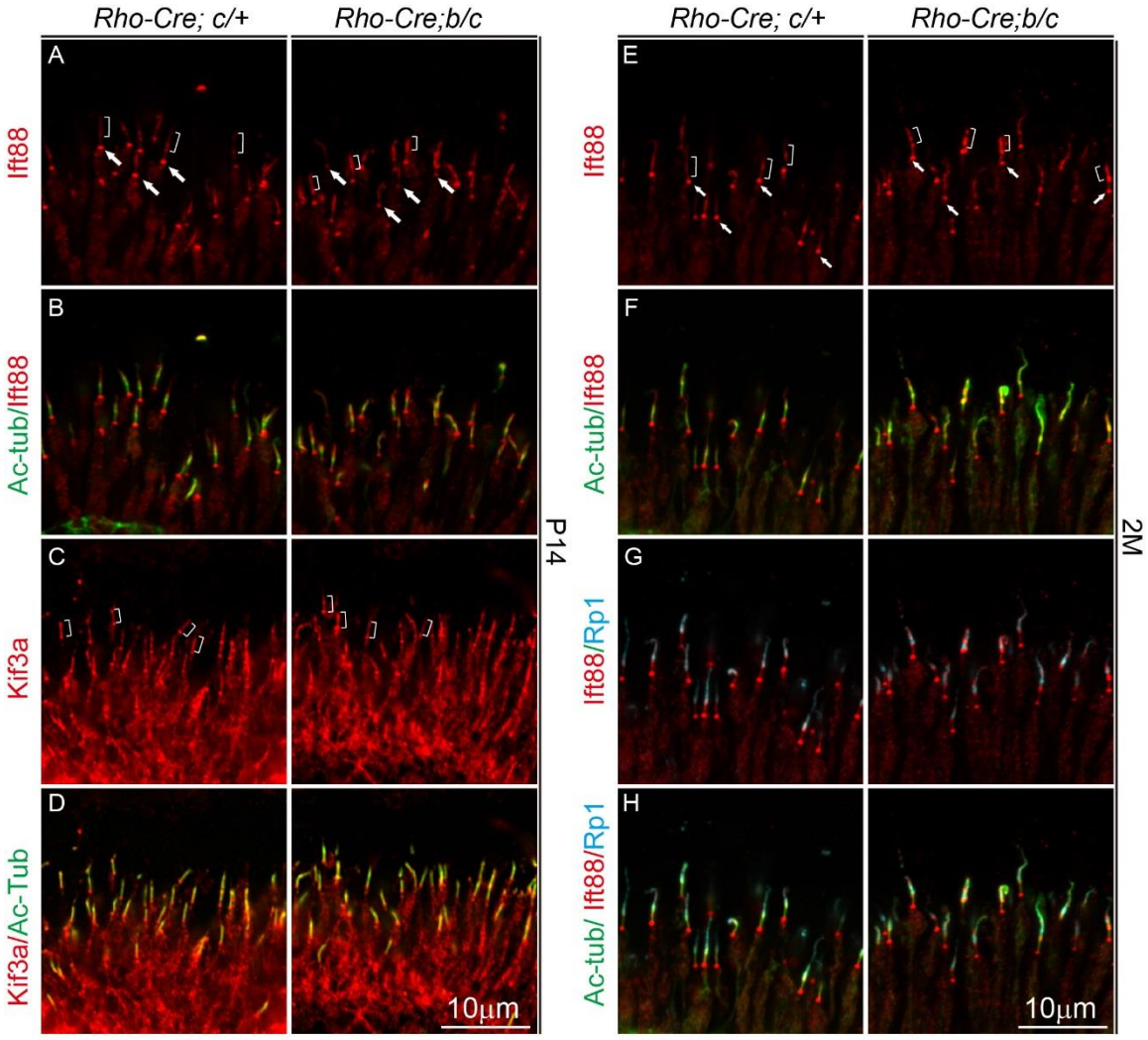
Supplemental Figure 6. rhodopsin- and Pde6B-stained P3 (A, B) and P4 (C, D) photoreceptor cells. Arrows point to the nascent inner segments. (E), Quantification of rhodopsin immunogold particles in the CC (see Materials and Methods section). A total of 8 sections for each indicated age was counted, and average CC particles/section is shown as one point of data in the graph. (F,G), Immunogold labeling of Peripherin/Rds in '+/+' and '*b/b*' mice. Black arrowheads point to the Rds labeling in the connecting cilium; Black arrows point to the Rds labeling in the IS; Open arrows point to the Rds at the ciliary tips, possibly where the nascent discs grow. OS, outer segment; IS, inner segment; CC, connecting cilium;



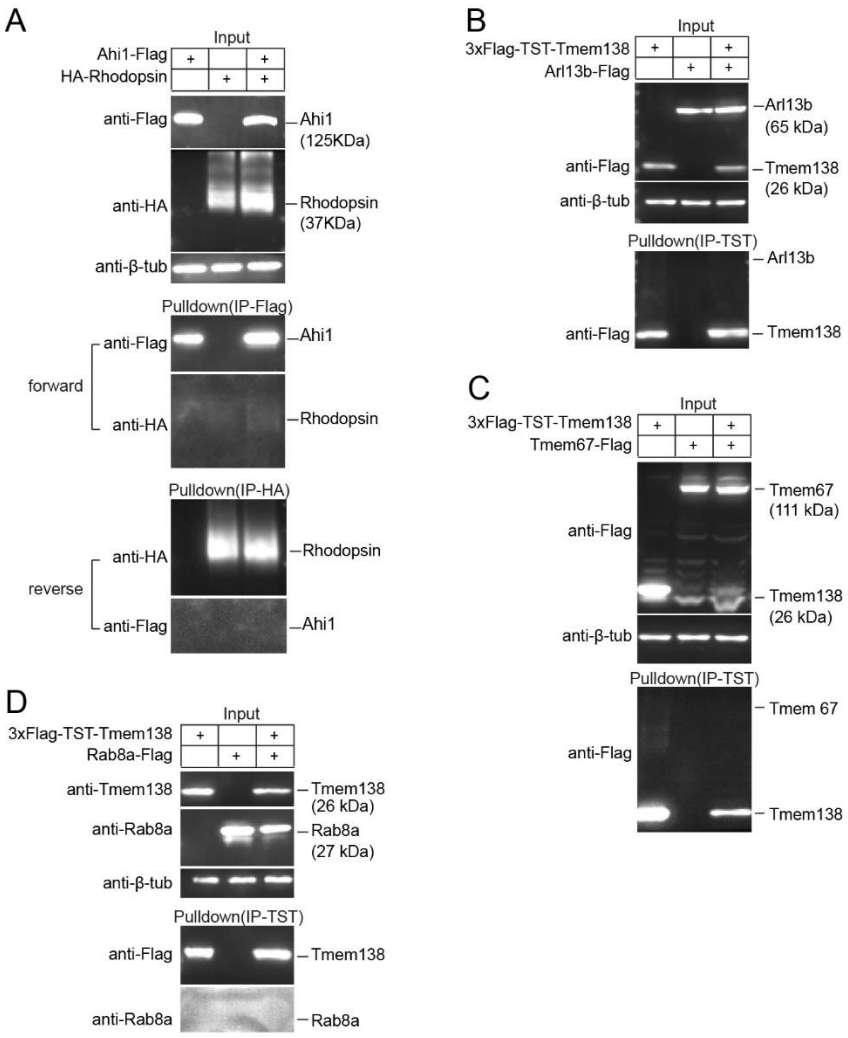
Supplemental Figure 7. (A, B), rhodopsin (green) and Rds (red) stained P8 photoreceptor cells. (C, D), rhodopsin (green) and Prominin-1 (red) stained P8 photoreceptor cells. Arrows point to the mutant ciliary tips. OS, outer segment; IS, inner segment.


A
Wt
E1
E2 E3
E4 E5
p13 p14
Tmem138c
Rho-Cre;
Tmem138c
Cre
B
+/+ c/+ c/+ c/c
2000bp
500bp
250bp
199bp
308bp
500bp
Cre primers
C
Rho-Cre;c/+
P5
P8
Cre
ONL
INL
100μm
D
Rho-Cre;c/+
Rho-Cre;b/c
P10
P14
P21
ONL
INL
OS
IS
Cre
Tmem 138/Ac-tub
100μm
5μm

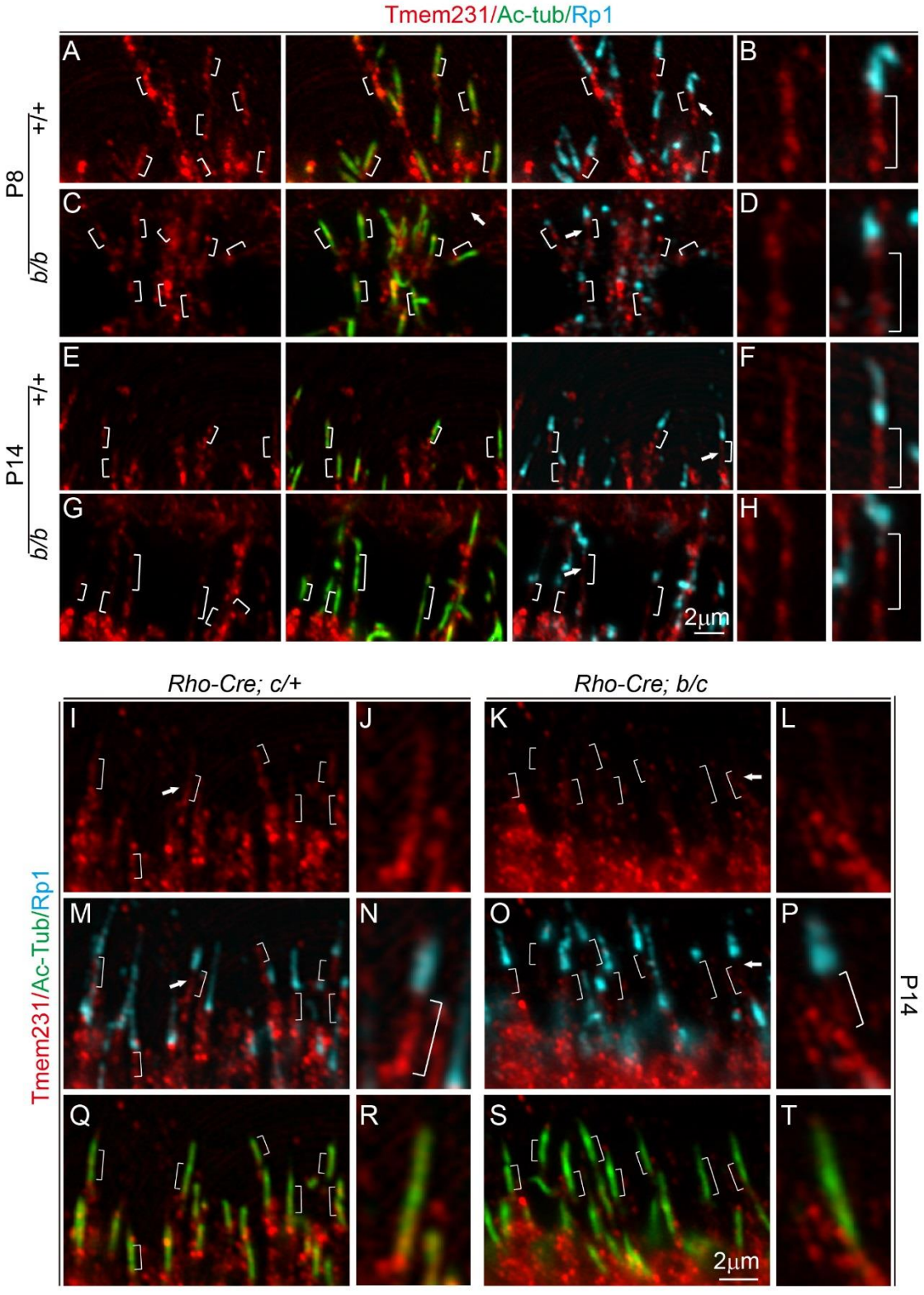
Supplemental Figure 8. *Rhodopsin-Cre* (*Rho-Cre*) was used to conditionally ablate *Tmem138* from the *Tmem138*^{c} allele*,* which was bred onto *Tmem138* null allele (*Tmem138*$^{b/+}$**)** to create compound mutants *Rho-Cre; Tmem138*$^{b/c}$ mice. (A) Primer locations used for genotyping *Tmem138*$^{c/+}$ allele. (B), Left panel: genotyping *Tmem138*$^{c/+}$ mice; right panel: genotyping *Rho-Cre* mice*;* (C), Cre protein was not detected in P5, and sparsely detected in P8 *Rho-Cre; Tmem138*$^{c/+}$ mice. (D), Cre was mildly expressed at P10, consistent with attenuated Tmem138 expression. At P14 and P21, a strong expression of Cre was detected in the ONL, and Tmem138 expression was almost gone. OS, outer segment; IS, inner segment; ONL, outer nuclear layer; INL, inner nuclear layer.



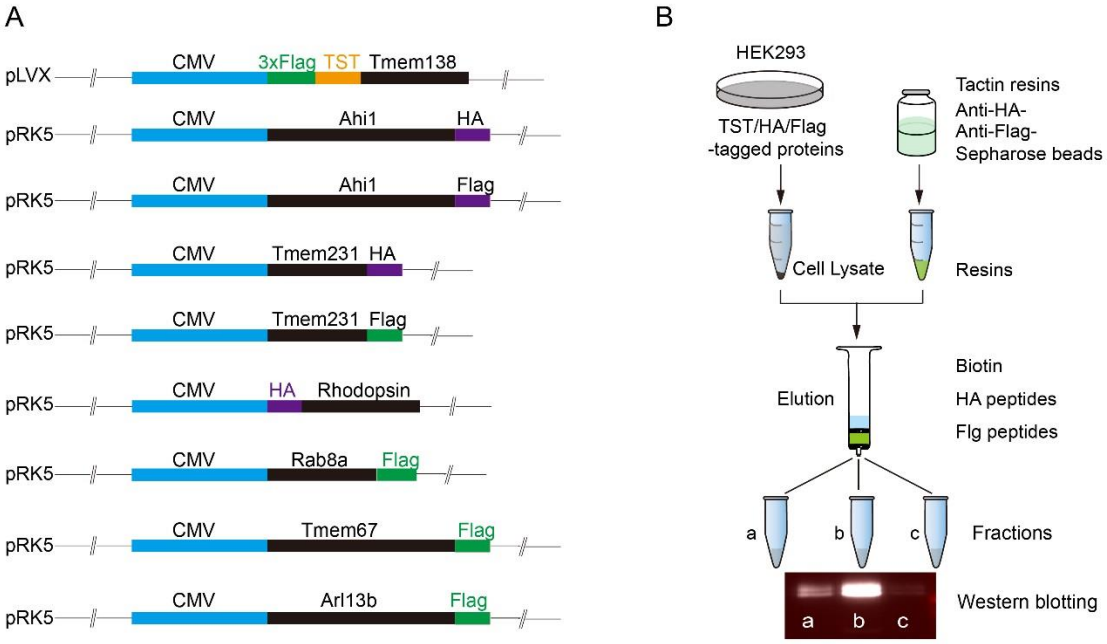
Supplemental Figure 9. Genotypes were labeled on top of the figure. (A, B) Ift88 and Ac-tub labeling photoreceptor cilia at P14. (C, D) Kif3a and Ac-tub labeling at P14. (E-H)) Ift88, Ac-tub, and Rp1 triple labeling photoreceptor cilia at P14. Arrows, the base of the cilium; Brackets, proximal axoneme.



Supplemental Figure 10. (A) Ahi1 does not interact with rhodopsin by protein pull-down assay in HEK293 cells in both forward and reverse directions. (B-D), Tmem138 does not interact with Arl13b, Tmem67, or Rab8a.


Tmem231/Ac-tub/Rp1
P8
+/+
b/b
P14
+/+
b/b
A
B
C
D
E
F
G
H
2μm
Rho-Cre; c/+
Rho-Cre; b/c
Tmem231/Ac-Tub/Rp1
P14
I
J
K
L
M
N
O
P
Q
R
S
T
2μm

Supplemental Figure 11. Triple labeling of Tmem231, Ac-tubulin, and Rp1. (A-H), Samples from germline knockouts and controls with indicated genotypes. (A-D) P8 retinal sections. (E-H) P14 retinal sections. (I-T), P14 retinas from conditional mutants and controls with indicated genotypes. Brackets indicate the CC domain of photoreceptors. Arrows point to the magnified cilia shown on the right.



Supplemental Figure 12. (A) DNA constructs used for detection of protein interaction in HEK293 cells. (B), Schematic illustration of major steps of affinity column pulldown experiment.

Table S1. Primers used for PCR and RT-PCR.

name	sequence	use
p1	5‘-GTTAGAGTTGTGTATTTGTCAGGTG-3’	genotype +/+ , b/+ (505bp)
p2	5‘-GTAAGAAAGGGAGGAAGGG-3’	
p3	5‘-CTGCTGTCCATTCCTTATTCC-3’	genotype b/+, b/b (320bp)
p4	5‘-CCAGTGCCAGCTGTAGCTAGAAGAAC-	
p5	5‘-CTCAGCGAGGCAAGATAGAA-3’	Exon1 transcripts (141bp)
p6	5‘-TCGGTTGTCATGGGAACAC-3’	
p7	5‘-CTACCAGCAGAACACCCCC-3’	Gfp transcripts (120bp)
p8	5‘-AGCAGGACCATGTGATCGC-3’	
p9	5‘-GCATTAGAGCCAAGGAAAC-3’	Exon2 transcripts (205bp)
p10	5‘-ACAGGTCATAGGACAGCAAC-3’	
p11	5‘-AGGTTAGCCTGTAGAGAGAGAC-3’	Exon5 transcripts (168bp)
p12	5‘-CAAAGTAGGAAGAGAAGGAAGT-3’	
p13	5'-TCCTGTAGAACAGGCTCTCAGGGT-3’	genotype c/+, c/c
p14	5'- CCACTGCACCATAACTTGTCATTCAT-3	
p15	5'-TCAGTGCCTGGAGTTGCGCTGTGG-3'	genotype rho-cre
p16	5'-CTTAAAGGCCAGGGCCTGCTTGGC-3'	
p17	5’-ACTGGCTACTTGAGAGGCTGAATCG-3’	Souther probe: before left arm
p18	5’-AACGCTGTCAGGTGACTGGAAGAGT-3	
p19	5’-CCTTATGGTCCCCAATCTGCTTGAG-3’	Souther probe: after right arm
p20	5’-GGAGTTGAATGTCAGGAAACAGGGC-3	

<insert page break

here>

Table S2. Antibodies for immunohistochemistry

Name	Species	Source	Cat.	Dilution	Using
Rho ID4	mouse	From Tiansen		1:1000	Hard fixed vibratome section (2% PFA and 2% glutaraldehyde fixed O/N at 4℃) & Hard fixed
PDE6B	rabbit	Invitrogen	AB-2161443	1:500	
GNAT1	mouse	santa cruz	SC-136143	1:200	Hard fixed frozen section (4% PFA fixed O/N at 4℃)
Recoverin	rabbit	millipore	AB5585	1:500	
cone arrestin	rabbit	millipore	MAB15282	1:500	
RDS	chick	From Tiansen		1:1000	
Cre	mouse	millipore	MAB3120	1:500	
Ac-tub	mouse	SIGMA	T6793	1:500	Light fixed vibratome section (2% PFA, fixed 5-10min at RT) &Light fixed frozen section (1% PFA, fixed 5-10min at RT)
AHI1	rabbit	proteintech	22045-1-AP	1:500	
Cep164	rabbit	proteintech	22227-1-AP	1:500	
IFT88	rabbit	proteintech	13967-1-ap	1:500	
RP1	chick	From Tiansen		1:1000	
Tmem138	rabbit	sigma	HPA042373	1:200	Light fixed frozen section (1% PFA, fixed 5-10min at RT)
Tmem231	rabbit	sigma	HPA042081	1:200	
Rootletin	Ms	santa cruz	sc-374056	1:500	
gamma tubulin	rabbit	abcam	ab11317	1:500	
KIF3A	rabbit	abcam	ab11259	1:500	
anti-FLAG	mouse	sigma	F1804	1:2000	Western blot
HA-tag(C29F4)	rabbit	Cell Signaling	5017	1:2000	

<insert page bre

ak here>

SI References

1. G. Testa *et al.*, A reliable lacZ expression reporter cassette for multipurpose, knockout-first alleles. *Genesis* **38**, 151-158 (2004).
2. C. Liu *et al.*, Prickle1 is expressed in distinct cell populations of the central nervous system and contributes to neuronal morphogenesis. *Human molecular genetics* **22**, 2234-2246 (2013).
3. D. Guo *et al.*, A Spatiotemporal Requirement for Prickle 1-Mediated PCP Signaling in Eyelid Morphogenesis and Homeostasis. *Investigative ophthalmology & visual science* **59**, 952-966 (2018).
4. D. Guo *et al.*, Ocular surface pathogenesis associated with precocious eyelid opening and necrotic autologous tissue in mouse with disruption of Prickle 1 gene. *Experimental eye research* **180**, 208-225 (2019).
5. M. Kakakhel *et al.*, Syntaxin 3 is essential for photoreceptor outer segment protein trafficking and survival. *Proceedings of the National Academy of Sciences of the United States of America* **117**, 20615-20624 (2020).