Supplementary Information for

Limited time window for retinal gene therapy in a preclinical model of ciliopathy

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Supplementary Information Text

Extended Materials and Methods

Sucrose gradient ultracentrifugation

Mouse eyes were collected and homogenized with Polytron PT 1200E in ice-cold PBS Lysis Buffer (PBS with 0.7% Triton X-100). After centrifugation at 20,000 x*g* for 15 min at 4 °C, supernatants were concentrated with Microcon Centrifugal Filter devices (20,000 MWCO; Millipore), loaded on a 4-ml 10-40% sucrose gradient in PBS with 0.04% Triton X-100, and spun at 166,400 xg_{avg} for 15 hours (Sorvall WX100 Ultra Centrifuge; Thermo Scientific). A total of 19 fractions (~210 µl each) were collected from the bottom of each centrifuge tube using a 26-G needle. Fifteen microliters of each fraction were used for SDS-PAGE and immunoblotting following standard protocols.

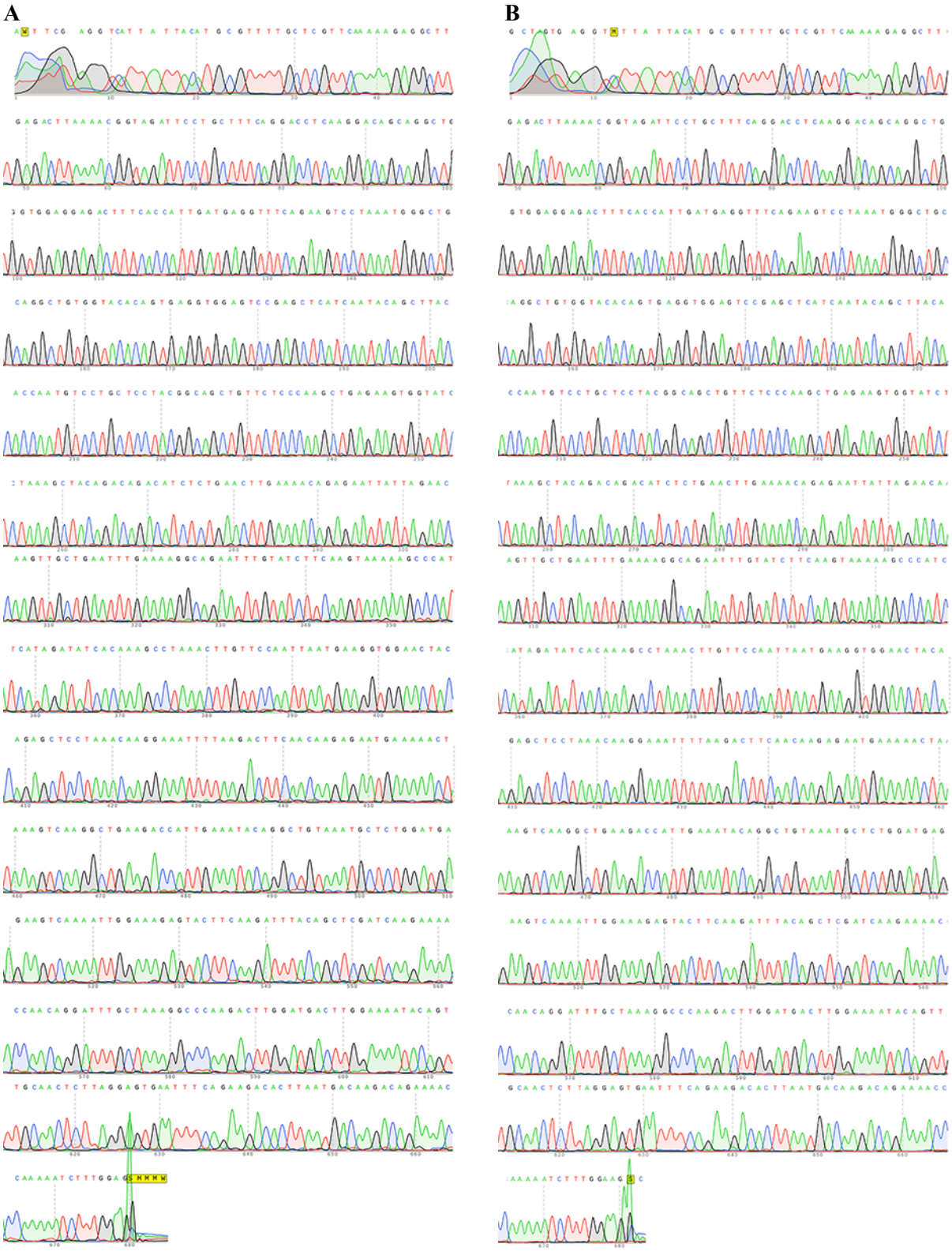

Fig. S1. Sequencing results of *Lztfl1* cDNAs (between exons 2 and 8)

A) Sequence of *Lztfl1* cDNA from normal (*Lztfl1*$^{+/gt}$) mouse eyes.

B) Sequence of *Lztfl1* cDNA from rescued *Lztfl1*$^{gt/gt}$ mouse eyes.

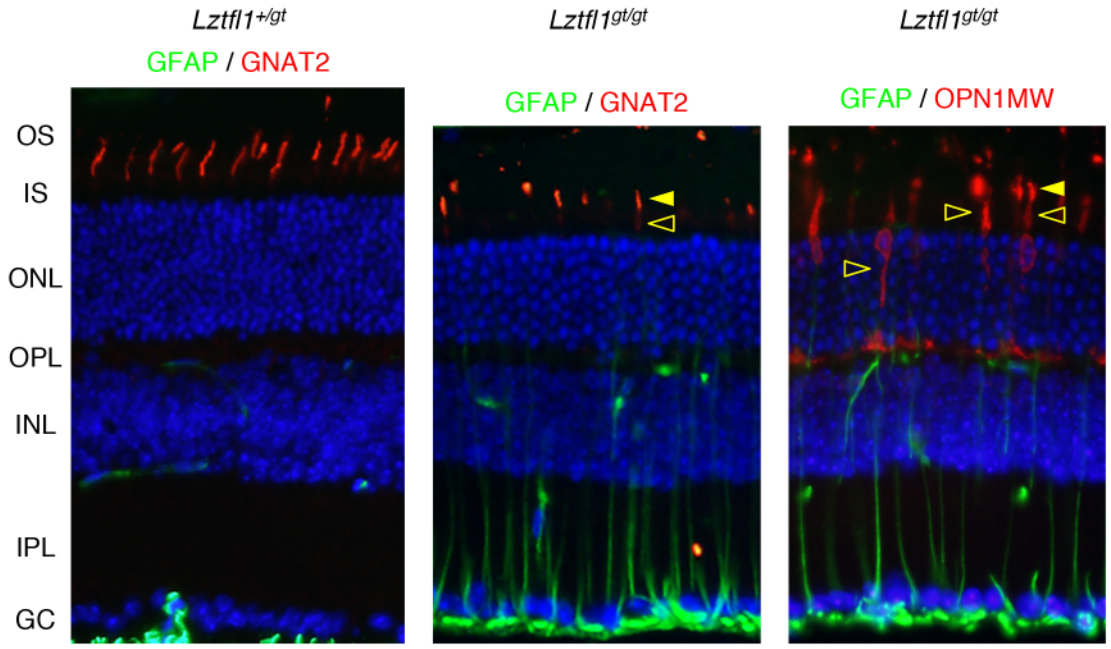

Fig. S2. Shortening of the cone OS in *Lztfl1* mutant retinas.

Retinal sections from *Lztfl1*$^{+/gt}$ and *Lztfl1*$^{gt/gt}$ mice at P45 were decorated with GFAP (green), GNAT2 (red), and OPN1MW (red) antibodies. Cone OSs in *Lztfl1* mutant retinas were marked by yellow arrowheads. Mislocalization of OPN1MW to the IS was marked by open arrowheads.

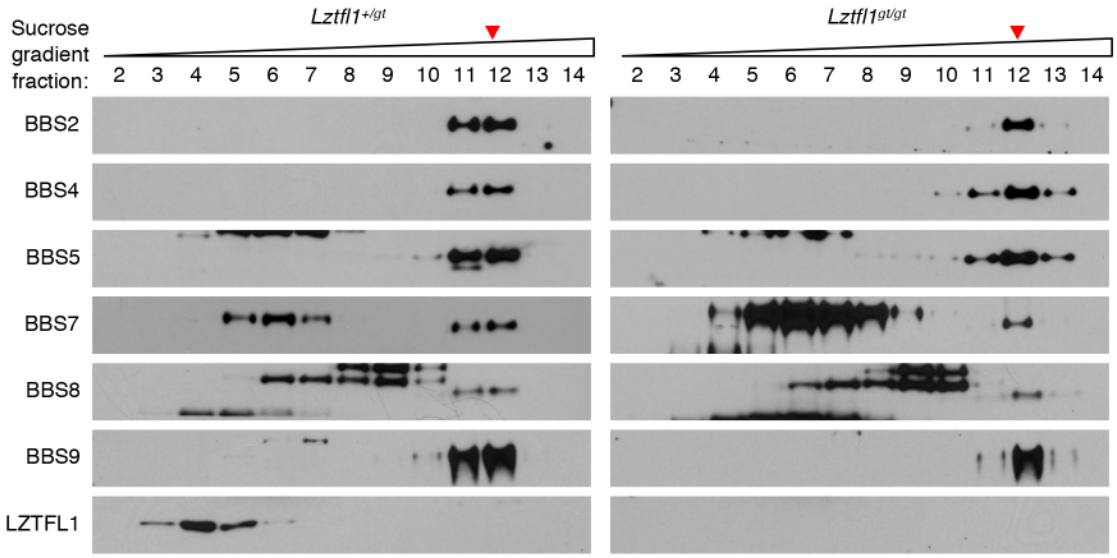

Fig. S3. BBSome assembly is not altered in *Lztfl1*$^{gt/gt}$ mutant eyes.

Protein extracts from normal (*Lztfl1*$^{+/gt}$) and *Lztfl1*$^{gt/gt}$ mutant eyes were loaded on 10-40% sucrose gradients and proteins were separated by sucrose gradient ultracentrifugation. Sedimentation of individual BBSome components was examined by SDS-PAGE and immunoblotting. Red arrowheads mark the peak at which the BBSome components were co-fractionated.

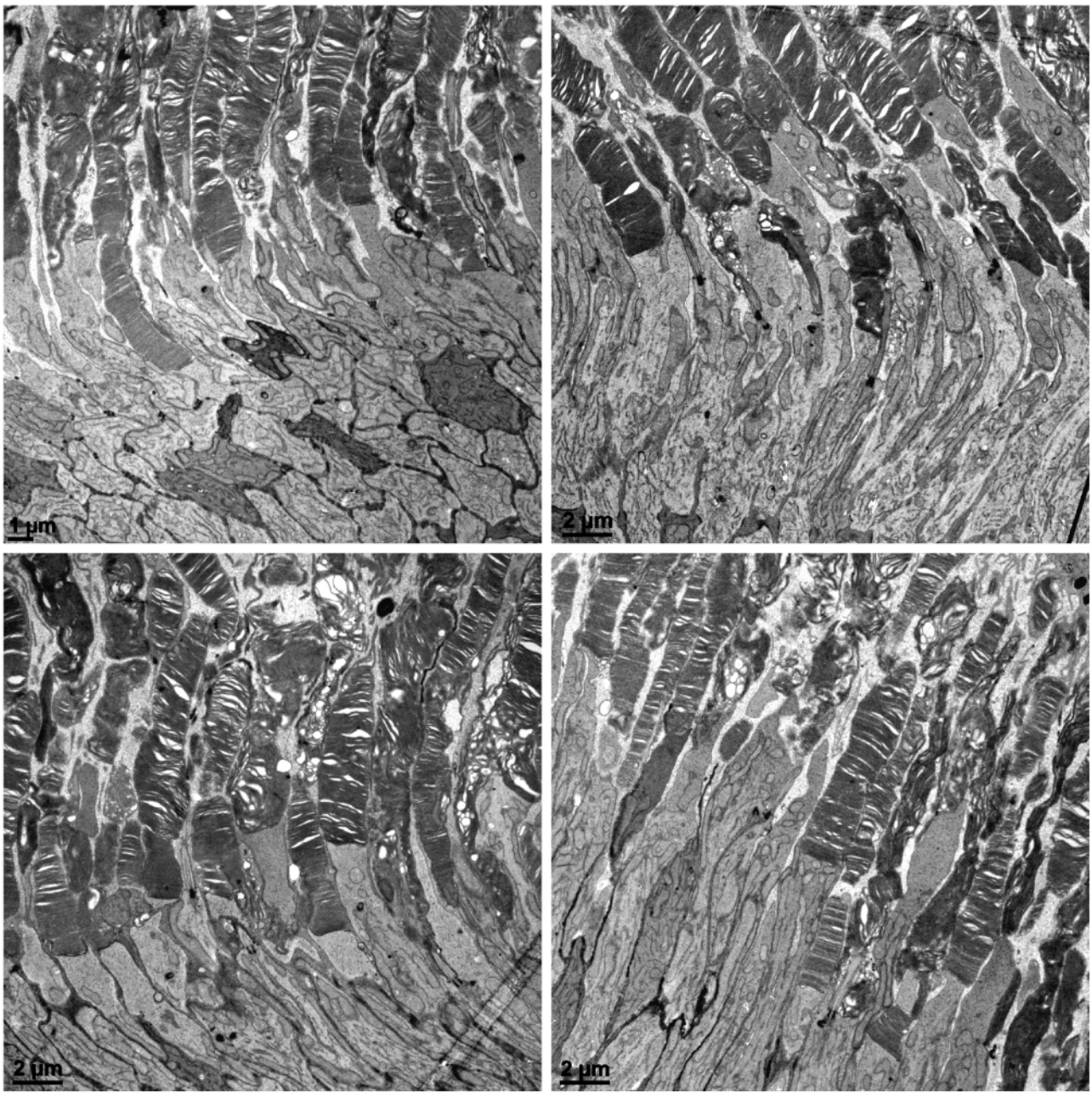

Fig. S4. TEM images of P5-rescued *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice at 3 months PTI.

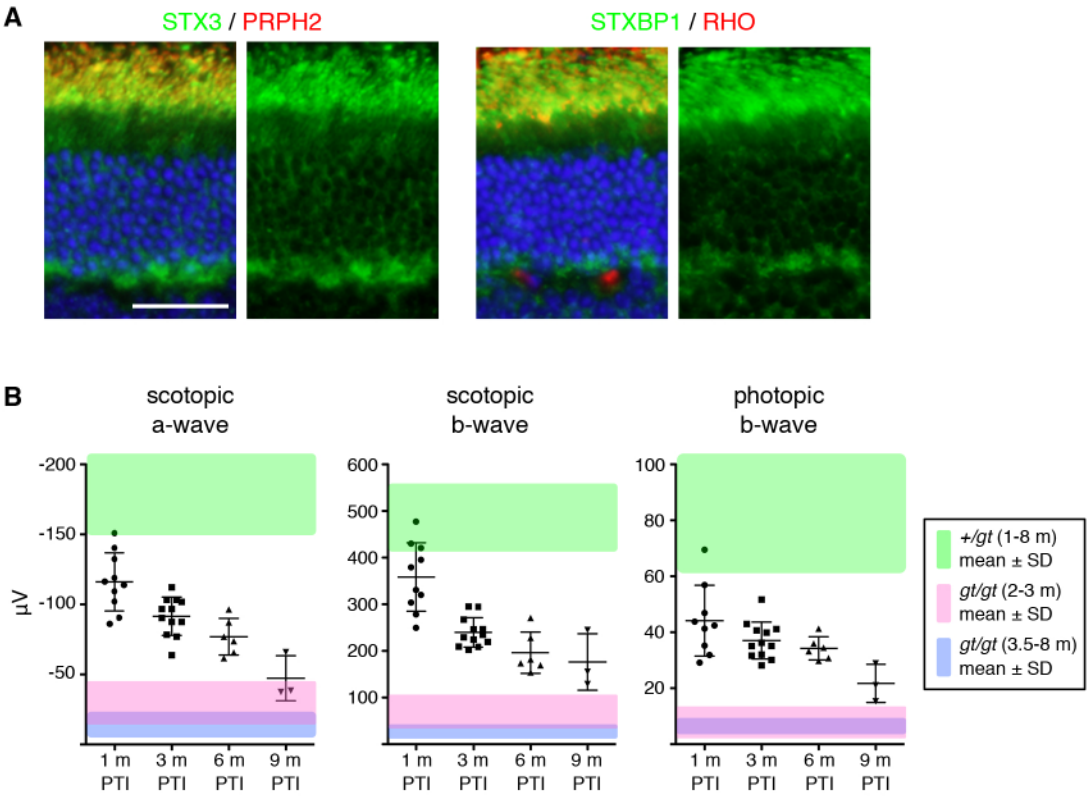

Fig. S5. Protein mislocalization and ERG in P5-rescued *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice at 9 months PTI.

A) Retinal sections from P5-rescued *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice were collected at 9 months PTI and stained with anti-STX3 (green) and anti-STXBP1 (green) antibodies. Anti-PRPH2 (red) and anti-RHO (red) antibodies were used as a marker of the OS. Merged images are shown on the left. Scale bar denotes 25 μm.

B) ERG responses of P5-rescued *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice at 9 months PTI. Data were added to the graphs presented in Figure 3 for direct comparisons with prior time points (1 m, 3 m, and 6 m PTI). Statistical analyses were not conducted because of the small number of animals examined.

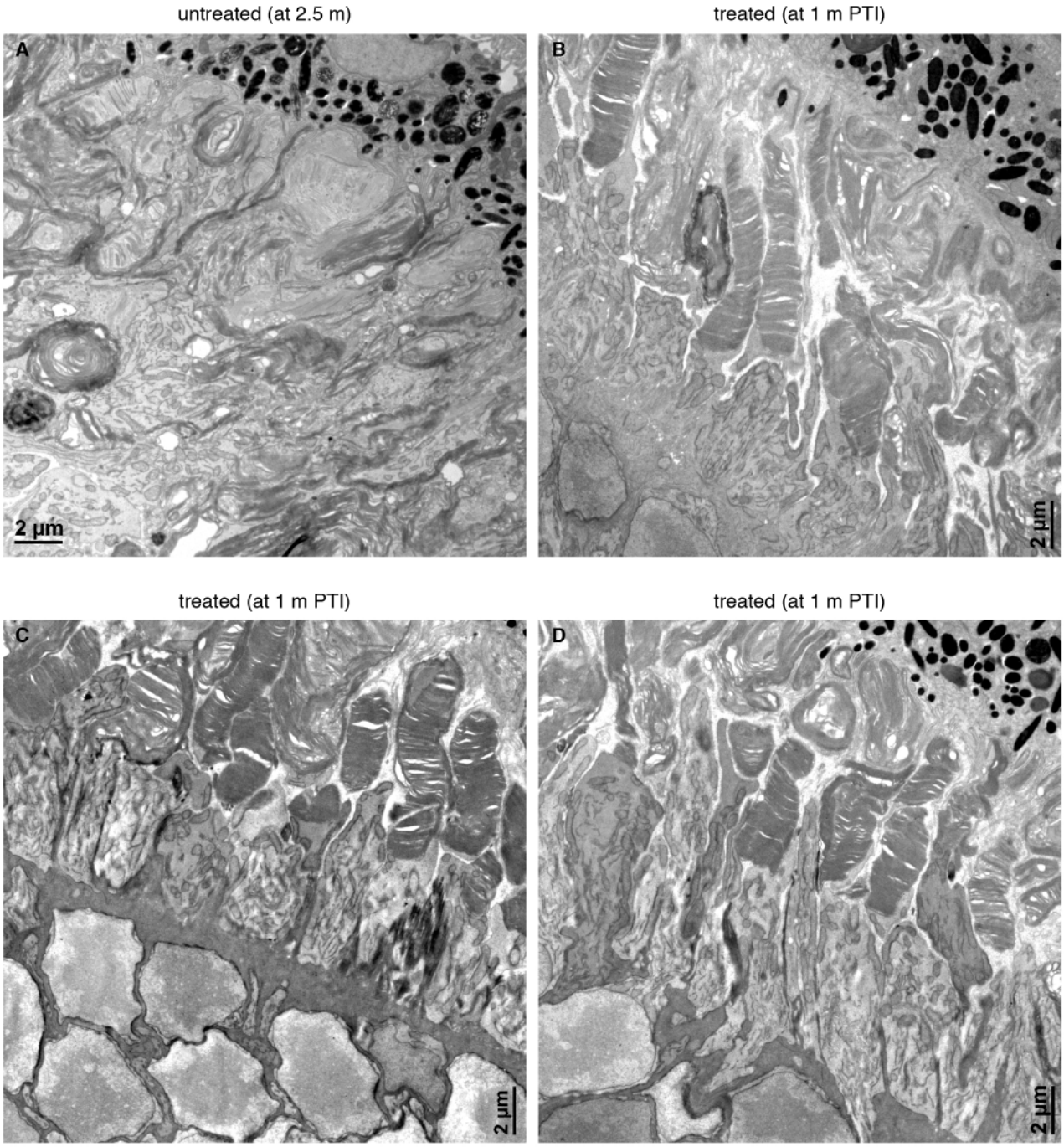

Fig. S6. TEM images of P45-rescued *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice at 1 month PTI.

Transmission electron micrographs from untreated (A; 2.5-months old) and P45-treated (B-D) *Lztfl1*$^{gt/gt}$;*FLP*$^{+}$ mice at 1 month PTI.

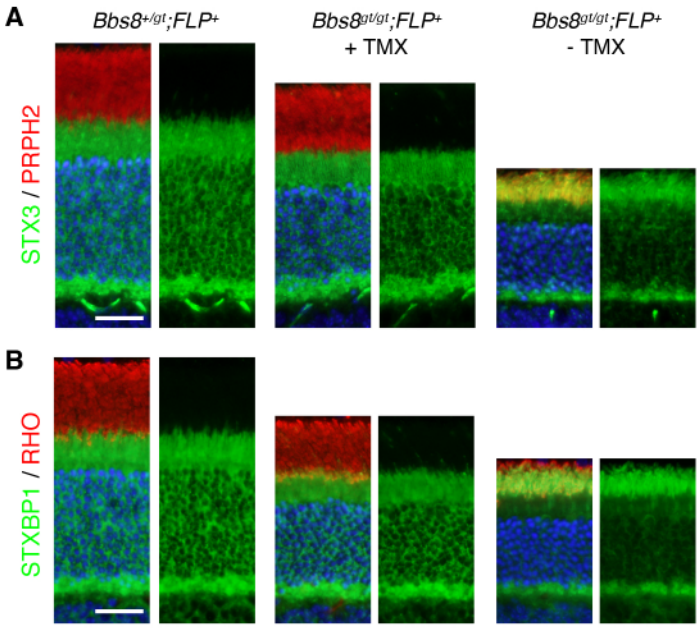

Fig. S7. Restoration of *Bbs8* expression at P25-29 completely rescues the protein mislocalization phenotype in *Bbs8*$^{gt/gt}$ mice.

Retinal sections from P25-rescued *Bbs8*$^{gt/gt}$*;FLP*$^{+}$ mice (middle) were collected at 1.5 months PTI and stained with STX3 (A; green) and STXBP1 (B; green) antibodies. Retinas from age-matched (2.5 months old) normal (*Bbs8*$^{+/gt}$*;FLP*$^{+}$; left) and untreated *Bbs8*$^{gt/gt}$*;FLP*$^{+}$ (right) mice were included as controls. PRPH2 (red) and RHO (red) antibodies were used as a marker of the OS. Scale bar denotes 25 μm.

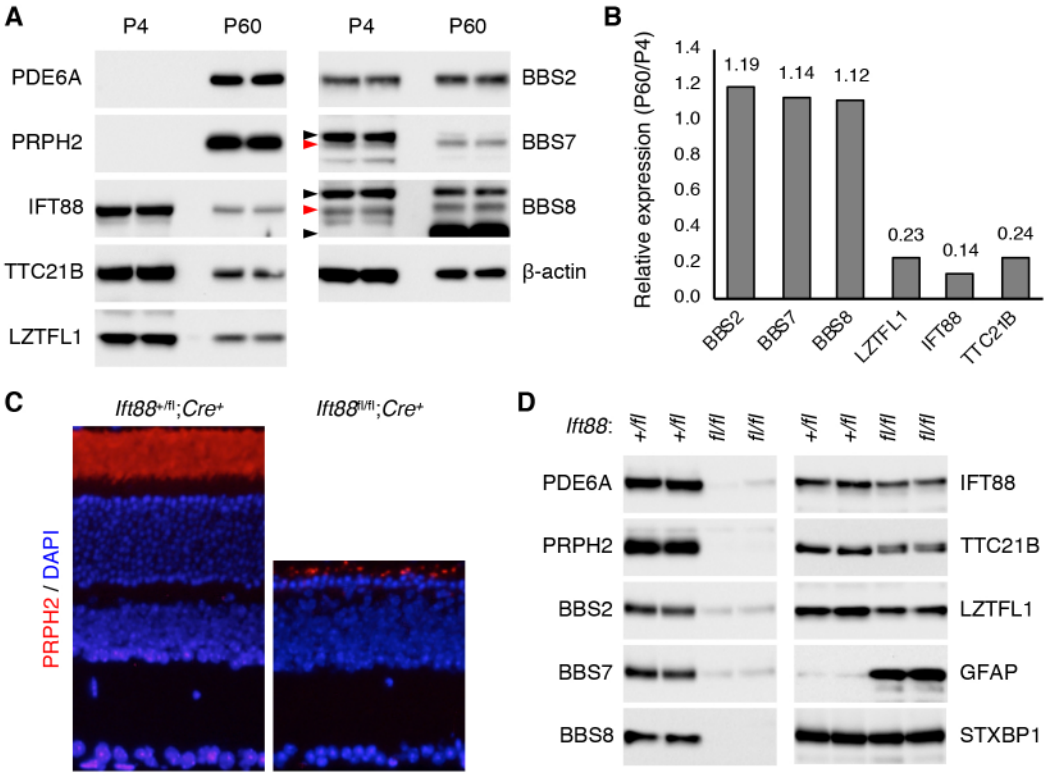

Fig. S8. **Expression of LZTFL1 is more similar to IFT proteins than BBSome components.**

A) Retinal protein extracts from 4-day and 60-day old wild-type mice were analyzed by immunoblotting. Red arrowheads mark BBS7 and BBS8 proteins, while black arrowheads indicate cross-reacting proteins.

B) Relative expression levels of BBS2, BBS7, BBS8, LZTFL1, IFT88, and TTC21B at P60 compared with at P4.

C) Retinal sections of 2-month old *Ift88*$^{+/fl}$*;rhodopsin-Cre*$^{+}$ and *Ift88*$^{fl/fl}$*;rhodopsin-Cre*$^{+}$ mice. More than 95% of photoreceptors are lost by this age.

D) Retinal protein extracts from 2-month old *Ift88*$^{+/fl}$*;rhodopsin-Cre*$^{+}$ and *Ift88*$^{fl/fl}$*;rhodopsin-Cre*$^{+}$ mice were analyzed by immunoblotting.

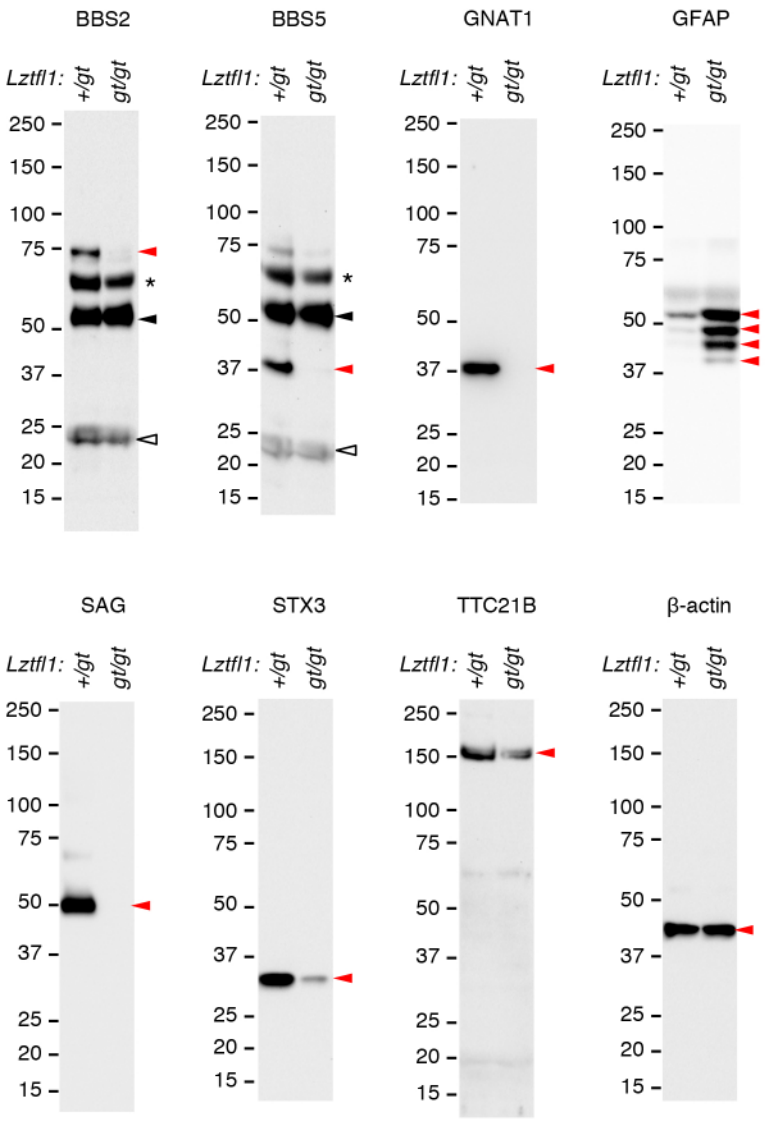

Fig S9. Specificity of the antibodies used in this study.

Whole eye protein extracts from 6-month old normal (*Lztfl1*$^{+/gt}$) and "photoreceptor-less" *Lztfl1*$^{gt/gt}$ mice were separated on an SDS-PAGE gel, and indicated antibodies were used for immunoblotting. Target protein bands were marked by red arrowheads. Black and open arrowheads indicate IgG heavy and light chains, respectively. Asterisks denote unknown, cross-reacting proteins.

Table S1. List of PCR primers used

Purpose	Primer Name	Sequence	Product size
Lztfl1^{gt} genotyping	F-Lztfl1-comm	TAACATGCCACTTGGACATCATGG	wt: 409 bp gt: 543 bp
	R-Lztfl1-wt	ATTCCATGAAAGCTGGTGTTGTGA	
	R-Lztfl1-gt	CCACAACGGGTTCTTCTGTTAGTC	
FlpER genotyping	F-FLP-comm	AAAGTCGCTCTGAGTTGTTAT	wt: 603 bp FLP: 309 bp
	R-FLP-wt	GGAGCGGGAGAAATGGATATG	
	R-FLP-tg	TTATGTAACGCGGAACTCCA	
Ift88 genotyping	F-Ift88-flox	GACCACCTTTTTAGCCTCCTG	wt: 209 bp fl: 254 bp
	R-Ift88-flox	TTCTGGCTCTGAACACAATCC	
iCre75 genotyping	F-iCre75	TCAGTGCCTGGAGTTGCGCTGTGG	wt: none Tg: 650 bp
	R-iCre75	CTTAAAGGCCAGGGCCTGCTTGGC	
Lztfl1^{gt} excision	F-Lz-exc-comm	GCGCATAACGATACCACGATA	unexcised: 599 bp excised: 292 bp
	R-Lz-unexcised	AGAGACAGGTGAGAGGAGATG	
	R-Lz-excised	GGTACAGCAGTGATTTCCCTATT	
Lztfl1 exon 2 qRT-PCR	F-Lz-exon2	GGCCTAAATGAGCACCATCA	103 bp
	R-Lz-exon2	GGTCCTGAAAGCAGGAATCTAC	
Lztfl1 exon 10 qRT-PCR	F-Lz-exon10	CCCAAATACGCCTCTGTCAT	95 bp
	R-Lz-exon10	GGCTCTTCTTCCCACTCTAAAC	
Lztfl1 cDNA (exons 2-8)	F-Lz-RT2	GCAGAGTTGGGCCTAAATGA	722 bp
	R-Lz-RT8	GCTGCTAGGTTCTCTTCCAAA	
Rpl19 qRT-PCR	F-Rpl19-qRT	GCAAGCCTGTGACTGTCCATT	106 bp
	R-Rpl19-qRT	GCATTGGCAGTACCCTTCCTC	

Table S2. List of antibodies used

Antibody	Source / Reference	Cat#	Verification
Mouse anti-β-actin monoclonal (clone: AC-15)	Sigma-Aldrich	A1978	(1), this study
Mouse anti-BBS2 monoclonal (clone: A-12)	Santa Cruz	sc-365355	this study
Rabbit anti-BBS4 polyclonal	Maxence Nachury (2)	N/A	(2, 3)
Mouse anti-BBS5 polyclonal (clone: B-11)	Santa Cruz	sc-515331	this study
Rabbit anti-BBS7 polyclonal	ProteinTech Group	18961-1-AP	(4)
Rabbit anti-BBS8 polyclonal	Sigma-Aldrich	HPA003310	(4)
Rabbit anti-BBS9 polyclonal	Sigma-Aldrich	HPA021289	(3)
Mouse anti-GFAP monoclonal (clone: GA5)	EMD Millipore	MAB3402	(5), this study
Rabbit anti-GNAT1 polyclonal	ProteinTech Group	55167-1-1AP	this study
Rabbit anti-GNAT2 polyclonal	abcam	ab97501	(6)
Rabbit anti-IFT88 polyclonal	ProteinTech Group	13967-1-1AP	(7, 8)
Rabbit anti-LZTFL1 polyclonal	Seongjin Seo (3)	N/A	(3, 6)
Rabbit anti-PDE6A polyclonal	ProteinTech Group	21200-1-1AP	(6)
Rabbit anti-PRPH2 polyclonal	ProteinTech Group	18109-1-AP	(6)
Mouse anti-RHO monoclonal (clone: 1D4)	EMD Millipore	MAB5356	(9)
Mouse anti-SAG monoclonal (clone: E-3)	Santa Cruz	sc-166383	this study
Mouse anti-STX3 monoclonal (clone: 1-146)	EMD Millipore	MAB2258	this study
Rabbit anti-STXBP1 polyclonal	ProteinTech Group	11459-1-AP	(6)
Rabbit anti-TTC21B polyclonal	Sigma-Aldrich	HPA035495	this study

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