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Supplemental Information

Prevention of Photoreceptor Cell Loss in a *Cln6*^{nclf} Mouse Model of Batten Disease Requires *CLN6* Gene Transfer to Bipolar Cells

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Supporting information – Results

Table S1: Summary of all subretinal AAV2/8 injections performed in *Cln6*^{nclf} mice. Mean ± SD of the scotopic a-wave amplitudes (at 10 cd.s/m^2, 2 months post-treatment), the main functional readout for the therapeutic effect, did not show a significant increase in rod photoreceptor function in treated *Cln6*^{nclf} eyes. Mean scotopic a-wave amplitudes of the contralateral untreated *Cln6*^{nclf} eyes and wild type mice are provided for reference. N = number of independent animal cohorts, *n* = number of eyes in independent animals.

Treatment age	Promoter and transgene	Titre	Scotopic a-wave amplitudes (mean ±SD)	*n*	N
P9-P10	CMV.hCLN6	1.5x10^{10} vg/eye	-140.53 ±33.34	8	2
P9-P10	CMV.hCLN6	1.5x10^{9} vg/eye	-178.41 ±40.72	7	2
P10	CMV.hCLN6	1.5x10^{8} vg/eye	-159.81 ±22.38	6	1
P5	CMV.hCLN6	1x10^{9} vg/eye	-125.91 ±23.29	5	2
P5	CMV.hCLN6	1x10^{8} vg/eye	-152.06 ±35.75	5	2
P9	CMV.mCln6	1.5x10^{9} vg/eye	-103.68 ±25.85	5	1
P9	CMV.mCln6	1.5x10^{8} vg/eye	-152.30 ±14.86	3	1
P10	MOPS.hCLN6	1.5x10^{11} vg/eye	-100.96 ±11.56	3	1
P9	MOPS.hCLN6	1.5x10^{10} vg/eye	-105.62 ±21.54	6	1
P9-P10	MOPS.hCLN6	1.5x10^{9} vg/eye	-147.27 ±71.89	5	2
P9	null control	1.5x10^{10} vg/eye	-157.27 ±19.29	4	1
Untreated *Cln6*^{nclf}	-	-	-145.06 ±30.99	47	9
WT C57BL/6J	-	-	-338.44 ±24.19	7	1

Table S2: Summary of mice used for the ERG recordings following the treatment with 7m8.CMV.hCLN6 (as shown in figure 4). N = number of independent animal cohorts, *n* = number of eyes in independent animals.

Treatment age	**Promoter and transgene**	**Titre**	***n* at 1-6 months**				**N**	***n***	**N**
			1M	2M	4M	6M	1M-6M	9M	9M
P5-P6	CMV.hCLN6	$1x10^{10}$ vg/eye	12	12	12	12	4	4	2
P5-P6	CMV.hCLN6	$1x10^{19}$ vg/eye	12	13	13	11	4	5	2
P5-P6	null control	$1x10^{10}$ vg/eye	3	6	6	5	2	-	-
Untreated *Cln6*^{nclf}	-	-	10	10	11	11	4	5	2
WT C57BL/6J	-	-	7	5	6	5	2	7	1

Table S3: Summary of mice used for the ERG recordings following the treatment with 7m8.Grm6.hCLN6 and 7m8.PCP2.hCLN6 (as shown in figure 5). N = number of independent animal cohorts, *n* = number of eyes in independent animals.

Treatment age	**Promoter and transgene**	**Titre**	***n* at 1-6 months**				**N**	***n***	**N**
			1M	2M	4M	6M	1M-6M	9M	9M
P5-P6	PCP2.hCLN6	$1x10^{10}$ vg/eye	11	11	10	10	4	-	-
P5-P6	Grm6.hCLN6	$1x10^{10}$ vg/eye	14	14	14	14	5	7	3
Untreated *Cln6*^{nclf}	-	-	7	7	9	9	3	5	2
WT C57BL/6J	-	-	7	5	6	5	2	7	1

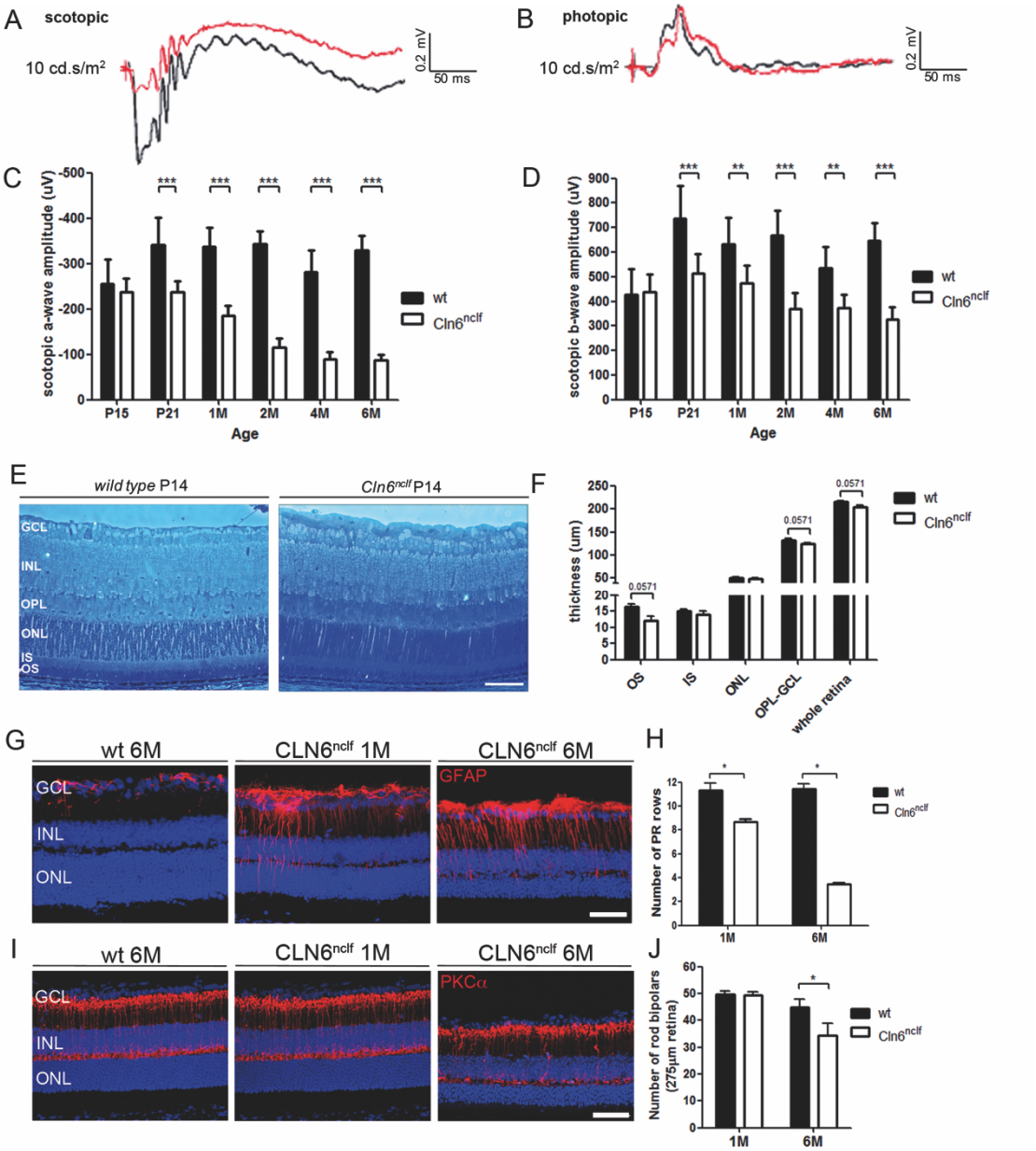

Figure S1: Characterisation of the ocular phenotype in *Cln6*^{nclf} mice. Representative (A) scotopic and (B) photopic ERG traces at 10 cd.s/m from wild type (black) and *Cln6*^{nclf} (red) mice at 2 months. (C) Scotopic a-wave and (D) b-wave amplitudes from right eyes at 10 cd.s/m^2 over time (means ± SD). *Cln6*^{nclf}: n = 6-10 eyes, wild type: n = 5-7 eyes per time point. (E) Representative images of semithin retinal sections revealed no obvious morphological abnormalities, (F) no significant differences were detected in the thickness of the retina at P14 in mutant and wild type mice. A non-parametric Mann-Whitney U test was performed. *Cln6*^{nclf} n = 3 eyes, wild type n = 4 eyes. (G) Mueller glia cell activation indicated by increased GFAP staining (red) and loss of photoreceptors labelled with DAPI nuclear staining (blue) were present from 1 month in mutant retinas. (H) At 1 month and 6 months the number of photoreceptor rows was significantly decreased in mutant mice (means ± SD), n = 5 eyes per group. (I) Immunostaining for PKCα (red) highlighted a mild loss of rod bipolar cells at 6 months. (J) The number of PKCα positive cells was reduced in *Cln6*^{nclf} mice at 6 months but not at 1 month (means ± SD). *Cln6*^{nclf}: n = 5 eyes, wild type: n = 4 eyes. Two-way ANOVA with Bonferroni post-test were performed to determine significance between wild type and *Cln6*^{nclf} mice. $p < 0.01$ = **, $p < 0.001$ = ***. P = postnatal day, M = month, GCL = ganglion cell layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, OS = outer segments, IS = inner segments. Scale bars 50 μm.

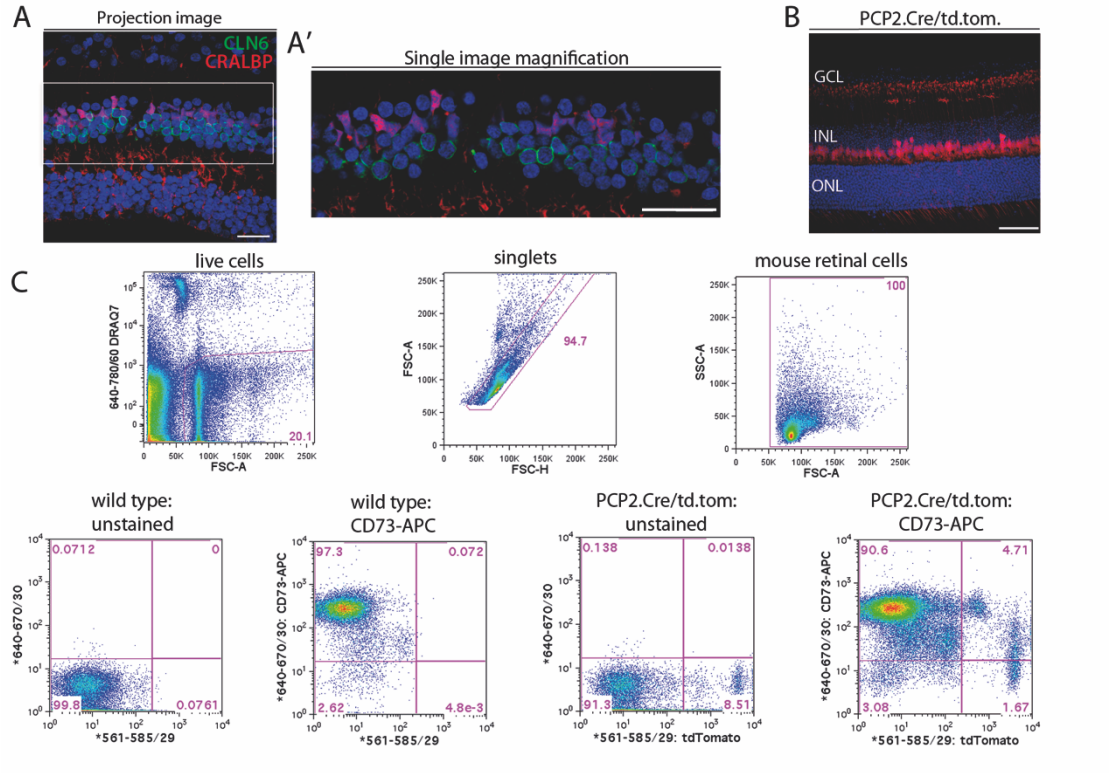

Figure S2: Assessment of CLN6 in inner retinal cells

(A-B') Immunostaining for CLN6 (green) and PKCα (red) on human retinal cross-section showing that CLN6 is expressed in rod bipolar cells. (C-C') Staining for CLN6 (green) and CRALBP (red) showing that CLN6 is not expressed in Mueller glia cells. (D) Representative retinal cross-section of a PCP2.Cre/td.tomato mouse. Following the pairing of PCP2.Cre and flox.STOP.flox.td.tomato mice, the retinas of the Cre-positive offspring were used for further analysis. Td.tomato expression was detected in bipolar cells and also in photoreceptors. (E) FACS-gating strategy to isolate CD73-positive photoreceptors and td.tomato-positive/CD73-negative bipolar cells to extract cell type specific RNA for qPCR analyses. Scale bars 50 µm.

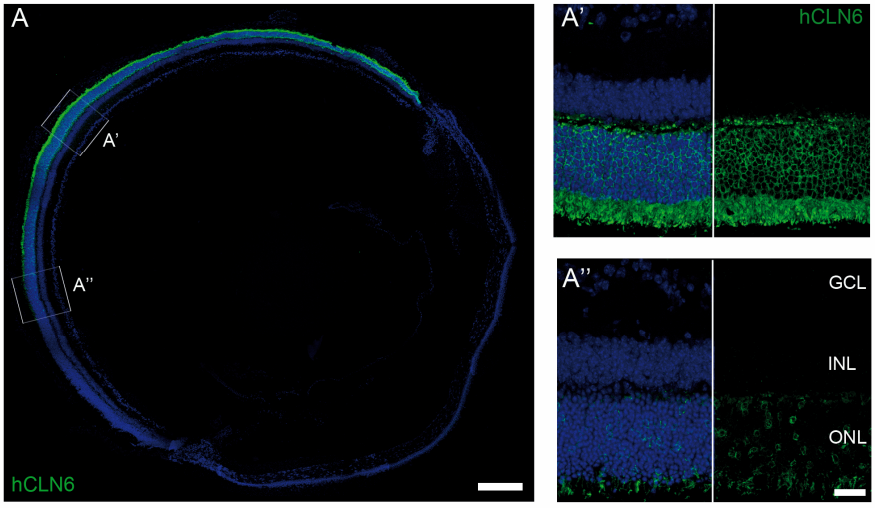

Figure S3: Subretinal administration of AAV2/8 in wild type retina

(A, A', A'') Representative images of an adult wild type retina 4 weeks after the subretinal delivery of AAV2/8.CMV.hCLN6. Immunostaining reveals a widespread expression of *CLN6* in photoreceptors following subretinal administration.

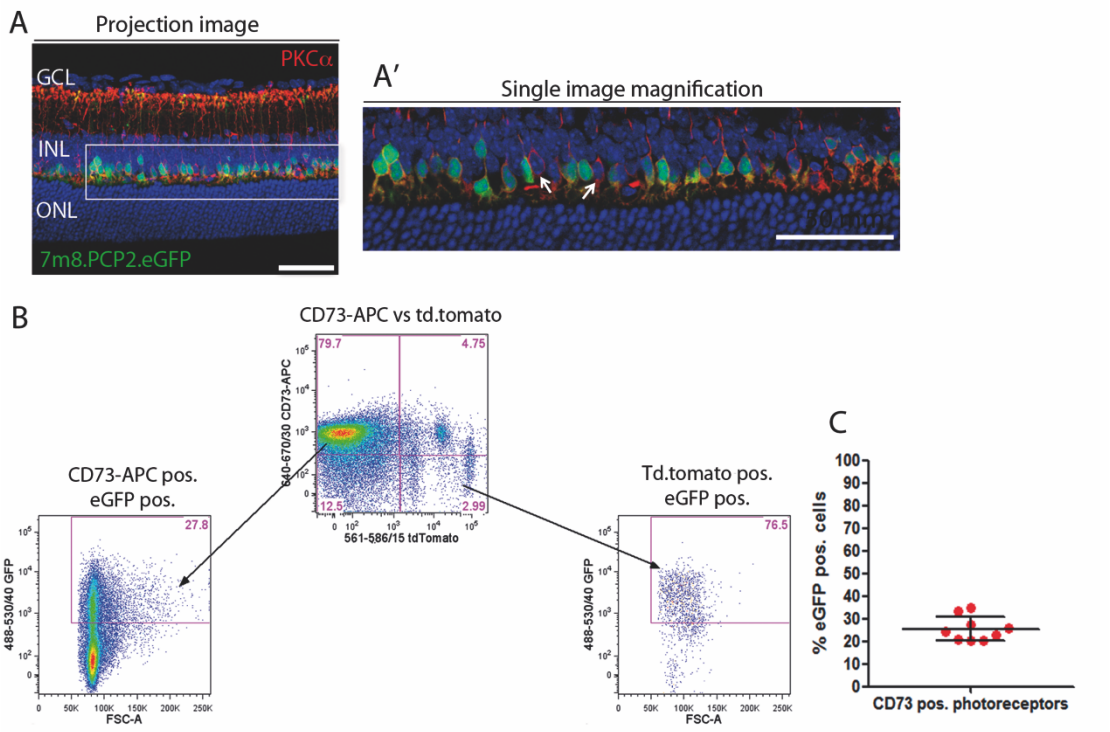

Figure S4: Transduction of retinal cell types after intravitreal injection of AAV serotype 7m8

(A, A') Representative images of wild type retinas 3 weeks after intravitreal administration of 7m8.PCP2.eGFP at P5. The majority of PKCα-positive cells expressed eGFP (arrows indicate rod bipolar cells not positive for eGFP). (B) Flow cytometry strategy to analyze the transduction efficiency of photoreceptors and bipolar cells in P5-P6 PCP2.Cre/td.tomato mice following the intravitreal delivery of 7m8.CMV.eGFP. Photoreceptor cells were labelled using the cell surface marker CD73 and bipolar cells were labelled with td.tomato in PCP2.Cre/td.tomato retinas. (C) Quantification of the photoreceptor transduction efficiency following 7m8 administration revealed that approximately 25 % of the CD73-positive cells were also positive for eGFP (mean ±SD), $n = 9$ eyes. Scale bars 50 µm.

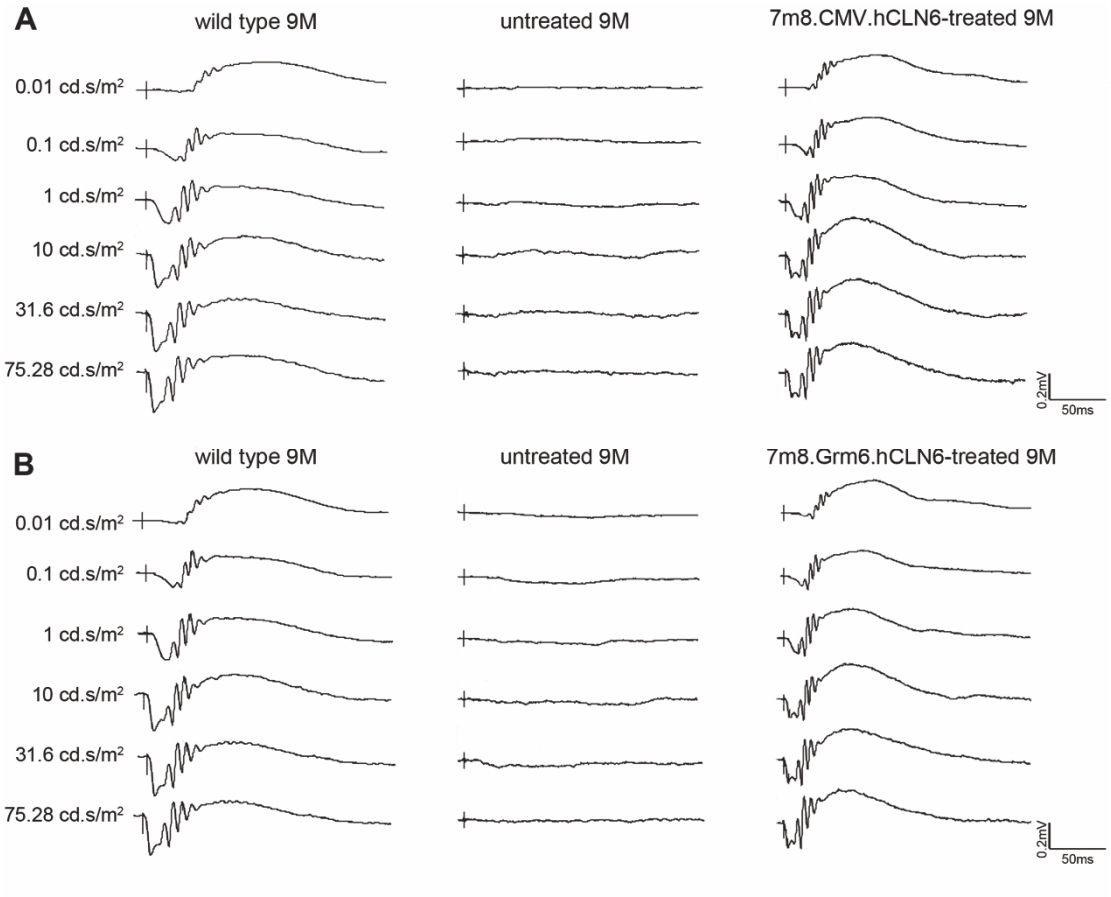

Figure S5: ERG raw traces

(A) Representative scotopic ERG raw traces from a wild type mouse at 9 months of age compared to an age-matched *Cln6*^{nclf} mouse that received intravitreal injections of 7m8.CMV.hCLN6 at a dose of 1x10^{9} vg in one eye. The contralateral eye was left uninjected. (B) Representative scotopic ERG raw traces from a wild type mouse at 9 months of age compared to an age-matched *Cln6*^{nclf} mouse that received intravitreal injections of 7m8.Grm6.hCLN6 at a dose of 1x10^{10} vg in one eye. The contralateral eye was left uninjected.