Supplementary table 1. List of primers used for testing gene editing in potential off-target regions.

Name	Primers (5'→3')	Region
Cerkl 53 DR274F	taggCCTTATATGAGGGGGTGGTC	5'
Cerkl 53 pX330F	caccCCTTATATGAGGGGGTGGTC	5'
Cerkl 53 R	aaacGACCACCCCCTCATATAAGG	5'
Cerkl 54 DR274F	taggCCTGACCACCCCCTCATATA	5'
Cerkl 54 pX330F	caccCCTGACCACCCCCTCATATA	5'
Cerkl 54 R	aaacTATATGAGGGGGTGGTCAGG	5'
Cerkl 33 DR274F	taggATCTTCTGCTTGGGCTTAGG	3'
Cerkl 33 pX330F	caccATCTTCTGCTTGGGCTTAGG	3'
Cerkl 33R	aaacCCTAAGCCCAAGCAGAAGAT	3'
Cerkl 34 DR274F	taggACAAACCTGAGCATTTATGC	3'
Cerkl 34 pX330F	caccACAAACCTGAGCATTTATGC	3'
Cerkl 34R	aaacGCATAAATGCTCAGGTTTGT	3'

Supplementary Table 2. List of reported *CERKL* mutations in human patients, classifed by their molecular effect, indicating the nucleotide and amino acid change, if reported in homo- or hetero-zygosis, and the associated clinical retinal phenotype (references at the end).

CERKL non-synonymous mutations (non-sense and missense)				
Mutation	**Localization**	**Aminoacid change**	**Zygosis**	**Retinal dystrophy**
GAG-TAG	c.193G>T	p.E65*	HM [1] HT [2]	RCD, MD
CGT-TGT	c.316C>T	p.R106C	HT [1]	arRP
CGT-AGT	c.316C>A	p.R106S	HM [3]	arRP
GGT-GAT	c.356G>A	p.G119D	HT [4]	CRD
CTC-CGC	c.365T>G	p.L122R	HM [5]	RP
TGC-TGG	c.375C>G	p.C125W	HM [6]	CRD
CTA-CCA	c.398T>C	p.L133P	HT [7]	RP
TGG-GGG	c.451T>G	p.W151G	HT [8]	RD
CCG-CTG	c.497C>T	p.P166L	HT [9]	MD
AAA-TAA	c.598A>T	p.K200*	HT [10]	RP
CAG-TAG	c.664C>T	p.Q222*	HT [11]	arRP
GAT-GTT	c.674A>T	p.D225V	HT [12]	RP
GGA-AGA	c.772G>A	p.G258R	HT [13]	CRD
CTG-CCG	c.812T>C	p.L271P	HM [14]	RP
CGA-TGA	c.847C>T	p.R283*	HM [15] HT [2]	RP, CRD
ATA-ACA	c.890T>C	p.I297T	HM [16]	RP, RCD
TGC-TGA	c.999C>A	p.C333*	HM [17]	CRD
CGA-TGA	c.1090C>T	p.R364*	HM [18]	CD, CRD
TGT-TGA	c.1164T>A	p.C388*	HT [19]	RD
CAG-TAG	c.1270C>T	p.Q424*	HM [20]	RP
CGA-TGA	c.1381C>T	p.R461*	HT [21]	RP
AGC-TGC	c.1651A>T	p.S551C	HT [22]	MD, CD, CRD

CERKL splicing mutations			
Mutation	**Localization**	**Zygosis**	**Retinal dystrophy**
IVS1 ds G-A +1	c.238+1G>A	HM [23]	RD
IVS2 ds T-G +2	c.481+2T>G	HM [20]	CRD
IVS9 as T-A -3	c.1212-3T>A	HT [24]	RP
IVS11 as C-G -3	c.1347-3C>G	HT [25]	RD
IVS1 ds T-C +2	c.238+2T>C	HT [26]	RP, CRD

Small CERKL deletions				
Mutation	**Localization**	**Aminoacid change**	**Zygosis**	**Retinal dystrophy**
CACTT^139 GATCTtATTAATTTAA	c.420delT	p.(Ile141 Leufs*3)	HT [10]	RP
ACTGT^149 GACATatGGTTTAGACA	c.450_451 delAT	p.(Ile150 Metfs*3)	HM [27]	CRD
GTA^204ACAA_EI_ GTAagTAATTTTCAG	c.613+4_613+5 delAG		HT [25]	RP
TA^204ACAA_EI_ GTAAgtaaTTTTCAGAAT	c.613+5_613+8 delGTAA		HM [28]	RP

Mutation	Localization	Aminoacid change	Zygosis	Retinal dystrophy
TTTTCTAG_IE_ TGtT^254GTCTGTGTT	c.759delT	p.(Val254 Serfs*12)	HT [29]	RD
AATGCT^278 GGGAtGGAAACAGAC	c.836delT	p.(Met279 Argfs*7)	HT [30]	RP
GAATC^285 CTGACtCCTGTCAGAG	c.858delT	p.(Pro287 Leufs*10)	HT [10]	RP
ATTGCAC^322 ATTatAATGG_EI_GTAAG	c.968_969delTA	p.(Ile323 Asnfs*46)	HM [31]	
GTTCTCA^34 8GCCatGTTTGGCTTT	c.1045_1046 delAT	p.(Met349 Valfs*20)	HM [2]	RD
AAA^383CTTAA_EI_ GTaagtCTTTTTCTTA	c.1151+3_1151+6 delAAGT		HM [25]	RP
GCAGAA^387 GACTgtGAAATATCAT	c.1164_1165 delTG	p.(Cys388*)	HT [32]	
CTGTT^493 GAGGAaGTAAAAGTTC	c.1482delA	p.(Val495*)	HM [33]	RD

Small *CERKL* insertions

Mutation	Localization	Aminoacid change	Zygosis	Retinal dystrophy
GGGCATC^52 TTCtGAGATCGGGA	c.156_157insT	p.(Glu53*)	HM [30]	RP
GCGAG^66CGAGC gagcACTGCGGTGG	c.197_200dup GAGC	p.(Leu68 Serfs*15)	HT [24]	MD, CD, CRD
TGAGACT^490 TACttacACTGTTGAGG	c.1467_1470 dupTTAC	p.(Thr491 Leufs*4)	HM [34]	RP
ATCAGT^547 CTTTctttATGGAGGAAG	c.1639_1642 dupCTTT	p.(Tyr548 Serfs*19)	HT [7]	RP

Large *CERKL* deletions

Mutation	Localization	Zygosis	Retinal dystrophy
gDNA	Exon 1-2	HT [35]	RD
gDNA	Exon 2	HT [24]	RP
gDNA	Exon 2	HT [25]	MD, CD, CRD
gDNA	Exon 1	HT [26]	RP, CRD
gDNA	Exon 2	HT [26]	RP, CRD

ar: Autosomic recessive. **HM**: Homozygosis. **HT**: Heterozygosis. **RD**: Retinal Dystrophy. **RP**: Retinitis Pigmentosa. **CD**: Cone Dystrophy. **CRD**: Cone-Rod Dystrophy. **MD**: Macular degeneration.

(Mutations listed on *Human Gene Mutation Database* (HGMD) http://www.hgmd.cf.ac.uk/ac/index.php).

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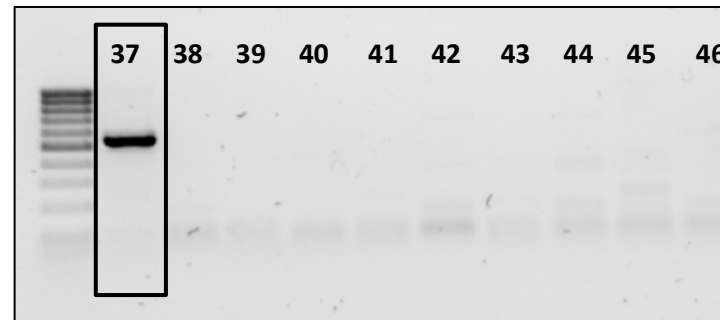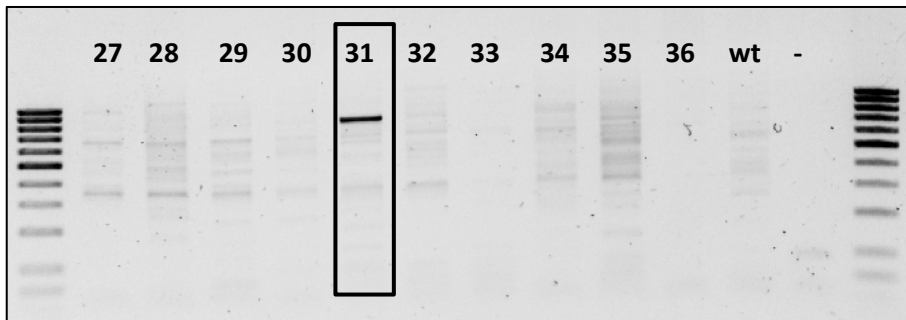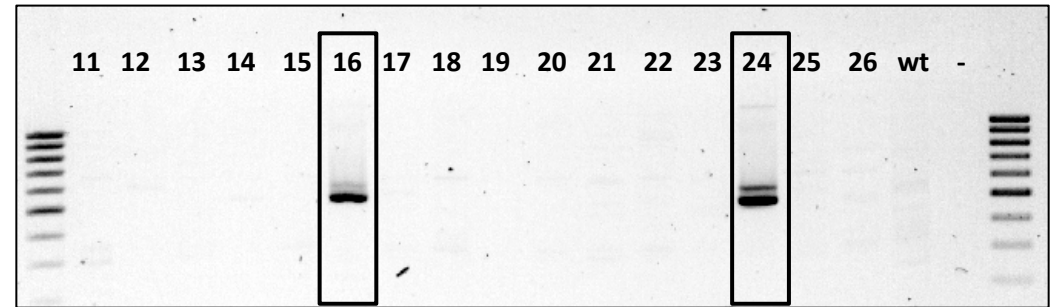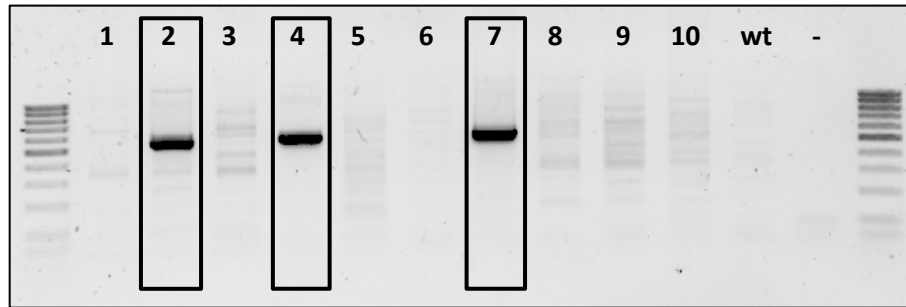
Figure S1. CRISPR/Cas9 guide position. To perform *Cerkl* gene edition we used four guides, two guides at 5' **(A)**, and two guides at 3' **(B)**.

A) Whole region

PCR <u>whole deletion</u> (Primers: Cerkl UF – Cerkl DR)

(guides underlined) (small insertions) (small deletions)

```
wt 5'        tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcatataaggacttcacaaa
wt 3'        GGCGTTCTGCCTCCTAAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTGAGCATTTATGCTGGCATTGCAAACATTATTTTGT

mouse 02     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcat...                          ...TGCAAACATTATTTTGT

mouse 04     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcattcat...                       ...TGCTGGCATTGCAAACATTATTTTGT

mouse 07     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcacaatgctcaggtttgtatttata...       ...ATGCTGGCATTGCAAACATTATTTTGT

mouse 16     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcagggac...                       ...TGCTGGCATTGCAAACATTATTTTGT

mouse 24     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctc...                            ...AAACATTATTTTGT

mouse 37     tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctc...                            ...ATTATTTTGT
```

B) 5' or upstream region

PCR <u>5' or upstream</u> (Primers: Cerkl UF2 – Cerkl UR)
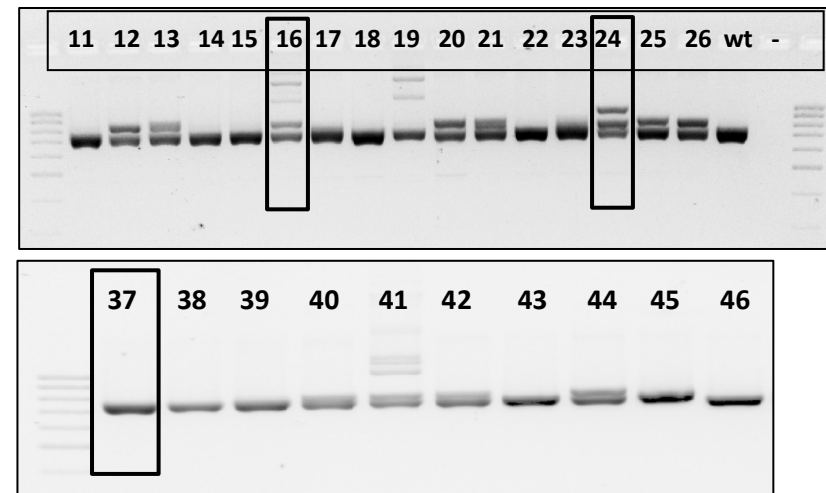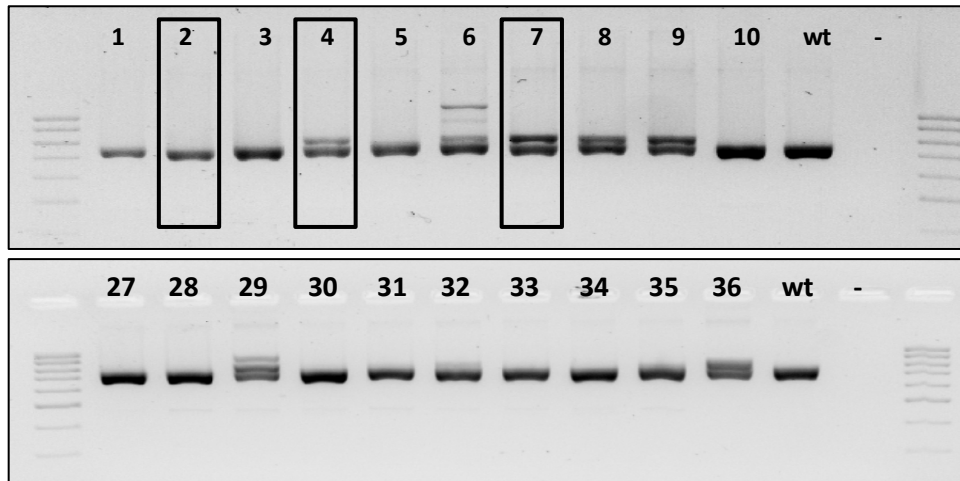

```
wt 5'              tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcatataaggacttcacaaa

mouse 02  (11/11)  tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctca-------------caaa

mouse 04a (10/12)  tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcataataaggacttcacaaa

mouse 04b (2/12)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctca-------------aaa

mouse 07  (6/12)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctca------------caaa

mouse 16a (5/10)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgacc---ccctcatataaggacttcacaaa

mouse 16b (4/10)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctc----------ttcacaaa

mouse 16c (1/10)   tagtacagaaaccccagtttttaaatcacccttcaatgcc---cgaccccctcatataaggacttcacaaa

mouse 24a (3/14)   tagtacagaaaccccagtttttaaatcacccttcaatgcagagaggtggtgcatatgtatttatacaatgtgctaactttctggcttttaatcttctgcttgggcattgaaggacttcacaaa

mouse 24b (9/14)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccac---------taaggacttcacaaa

mouse 24c (2/14)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcataataaggacttcacaaa

mouse 37  (1/12)   tagtacagaaaccccagtttttaaatcacccttcaatgcctgaccacccccctcattataaggacttcacaaa
```
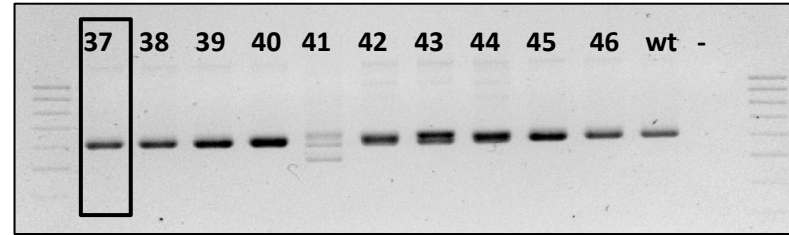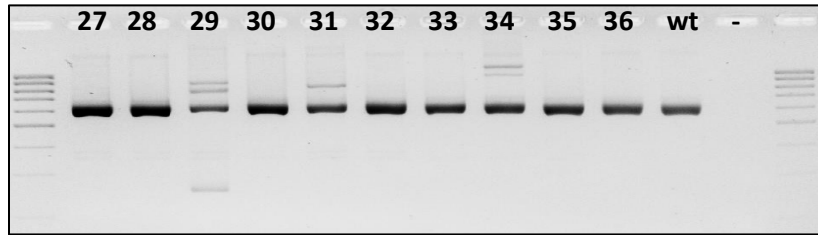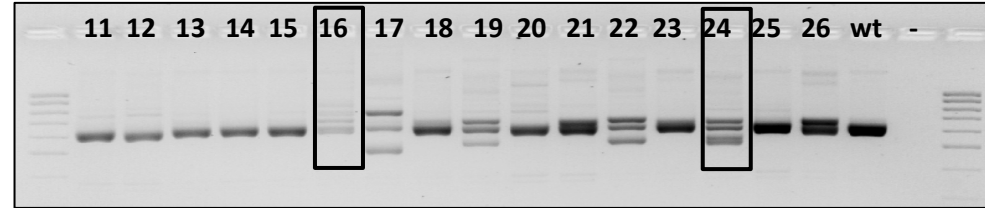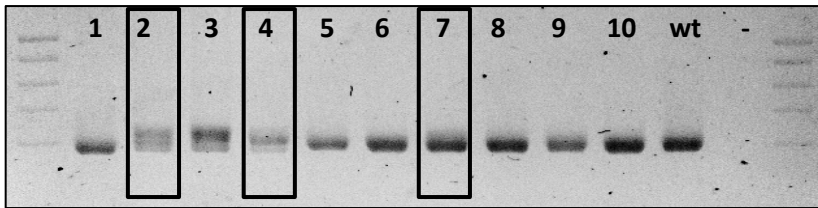
C) 3' or downstream region (mice 7 & 37 wild type)

PCR 3' or downstream (Primers: CerkI DF – CerkI DR)

```
wt 3'          GGCGTTCTGCCTCCTAAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTGAGCATTTATGCTGGCATTGCAAACATTATTTTGT

mouse 02a (7/11)   GGCGTTCTGCCTCCTAAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTGAGCATTTATTGCTGGCATTGCAAACATTATTTTGT

mouse 02b (4/11)   GGCGTTCTGCCTCCTAAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAAT------------------GCTGGCATTGCAAACATTATTTTGT

mouse 04 (6/9)     GGCGTTCTGCCTCC-AAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTGA-----------GCATTGCAAACATTATTTTGT

mouse 16a (2/9)    GCGTTCTGCCTC---------AAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTG-----------GCATTGCAAACATTATTTTGT

mouse 24a (3/8)    GGCGTTCTGC-------------------------------------------------------------------------------------------------------TGGCATTGCAAACATTATTTTGT

mouse 24b (3/8)    GGCGTTCTGC-----------------------------------------------------------------------------------------------------------AAACATTATTTTGT

mouse 24c (2/8)    GGCGTTCTGCCT-----GCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAACCTG---------CTGGCATTGCAAACATTATTTTGT
```

```
wt (16b reference)
GAAAACCTGGGGCTATCTGGTTAAAGTTTCAAAGCTATAAAGATGTCAGAATGATGATGAAACTTAATTCTAATAGGGGCCACCATGAAGCATCATGCTTTTTTTTGTTTTTTTTTTTTTTTTTTTTTTTTTTTGTTATATTTAGTTCAATTTTCCCTTTGTG
GAGACTTATATCATTGAAGAAGTAAAAATTCACCCAAAGAGTAATAGCAATGGATACAGCCCACAGGAGGAGGGTGGAAACCATACAACTGCTTCAGAACACGGGTTTCCGTGGAATGTAGATGGTGACTTAATGGAAGCTGCATCAGAGGTTCACATTA
GGTAAGCCCAACTATTTTATGACTTTCTGTGCAGAATACTAGATGCAACACTATTAATATAATAAGGGCGTTCTGCCTCCTAAGCCCAAGCAGAAGATTAAAAGCCAGAAAGTTAGCACATTGTATAAATACATATGCACCACCTCTCAATAAATACAAA
CCTGAGCATTTATGCTGGCATTGCAAACATTATTTTGTATAAATGATACAGACATGTTGACAATTTACATTGTTTGTCTTTGAGGATATTCTTAAACCTTATAAGGTTAATGTAGTGCCATACTCTCTAGCCCTGCTCCC

mouse 16b (7/9)
GAAAACCTGGGGCTATCTGGTTAAAGTTTCAAAGCTATAAAGATGTCAGAATGATGATGAAACTTAATTCTAATAGGGGCCACCATGAAGCATC---CTTTTGTGAA
GCTGGCATTGCAAACATTATTTTGTATAAATGATACAGACATGTTGACAATTTACATTGTTTGTCTTTGAGGATATTCTTAAACCTTATAAGGTTAATGTAGTGCCATACTCTCTAGCCCTGCTCCCA
```

Figure S2. Genotyping PCRs for CRISPR-edited alleles in mosaic pups, and sequences gene-edited alleles per mice. A) Specific PCRs for the whole *Cerkl* locus deletion allele with primers flanking the PAM sites for the Cas9 D10A nickase at 5' and 3' sequences, allowed to detect the pups (out of 46 born alive) carrying the full locus deletion. **B)** Specific primers for gene-editing in the upstream *Cerkl* region allowed to detect mosaic pups carrying alleles where Cas9 D10A nickase only cut at the 5' sites. **C)** Specific primers for gene-editing in the downstream *Cerkl* region allowed to detect mosaic pups carrying alleles where Cas9 D10A nickase only cut at the 3' sites. Per each specific primer pair, PCRs from animals carrying gene-edited alleles are highlighted. Bands were excised, cloned and sequenced. Very few alleles were present per animal (indicated by a and b sequences) and sequences are indicated below. Small indels at the edited sites were detected in some cases (blue nucleotides indicate small insertions and red nucleotides, small deletions). Mouse 16 b allele (gene-editing occurred only at the 3' *Cerkl* region carried a relatively larger deletion). Mouse 2 carrying the full locus deletion was chosen as a founder for our colony (highlighted in yellow).
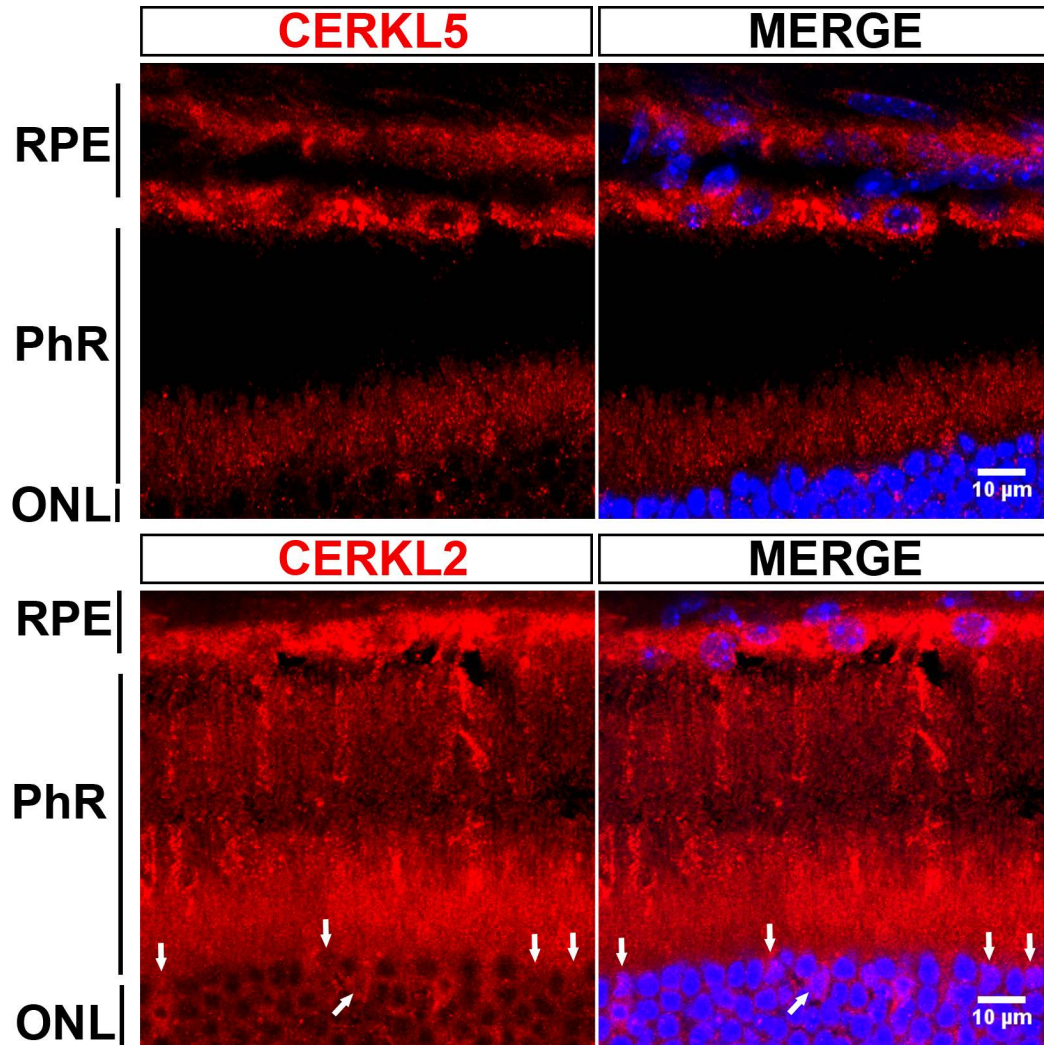
Figure S3. CERKL (in red) is highly expressed in the Retinal Pigment Epithelium (RPE) cells, as detected using two anti-CERKL antibodies recognizing different protein isoforms. Nuclei are counterstained with DAPI (blue). Note that some CERKL isoforms (detected with anti-CERKL2) are also localized in the nuclei of RPE cells and some cones (white arrows) (see main text). **PhR-** Photoreceptor layer (include the outer and inner segments of photoreceptors). **ONL-** Outer nuclear layer.
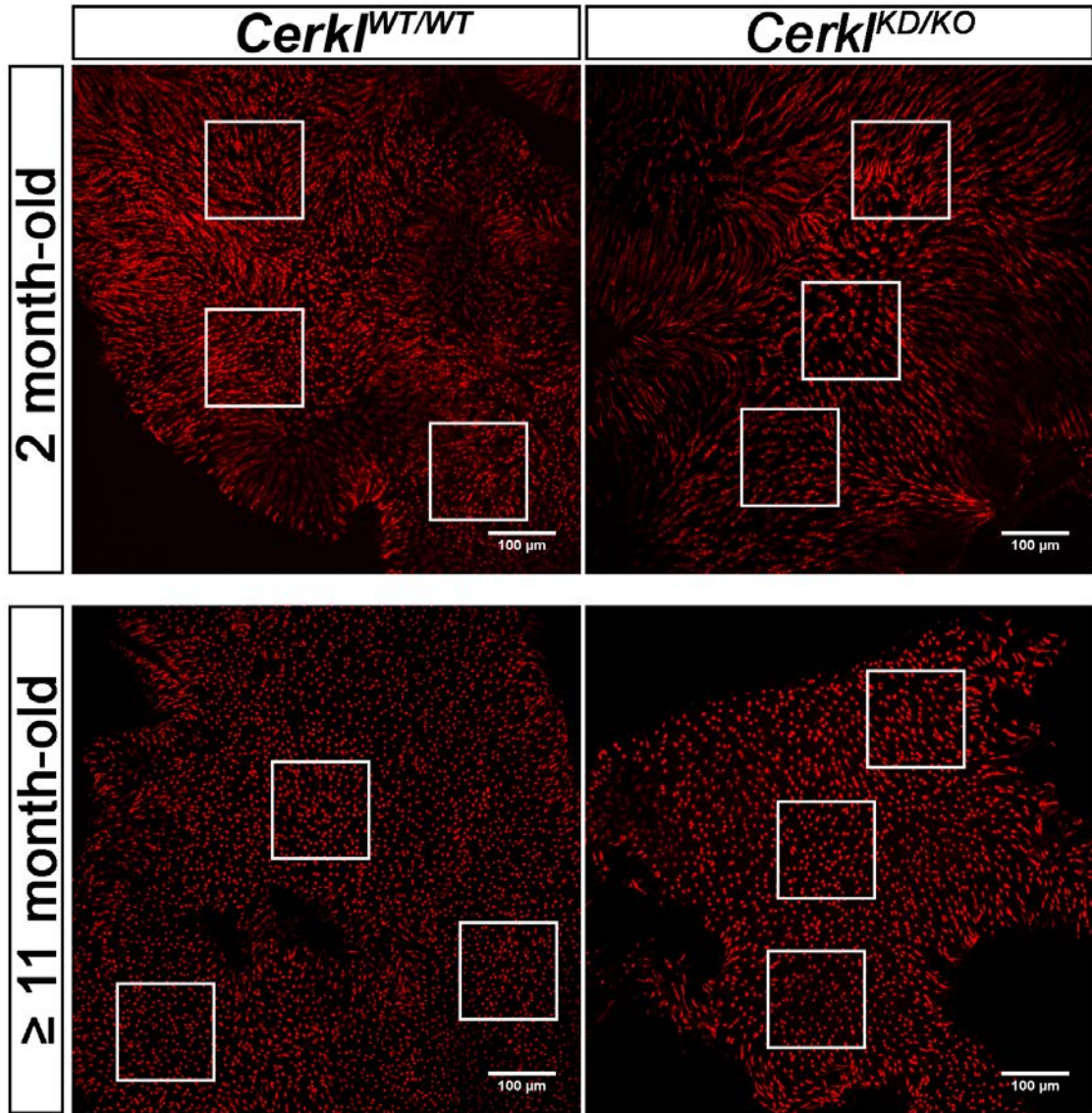
Figure S4. Representative positioning of the ROIs (regions of interest) in a retinal whole mount. The number of cones were counted on three ROIs per image and a minimum of 12 images per retina.
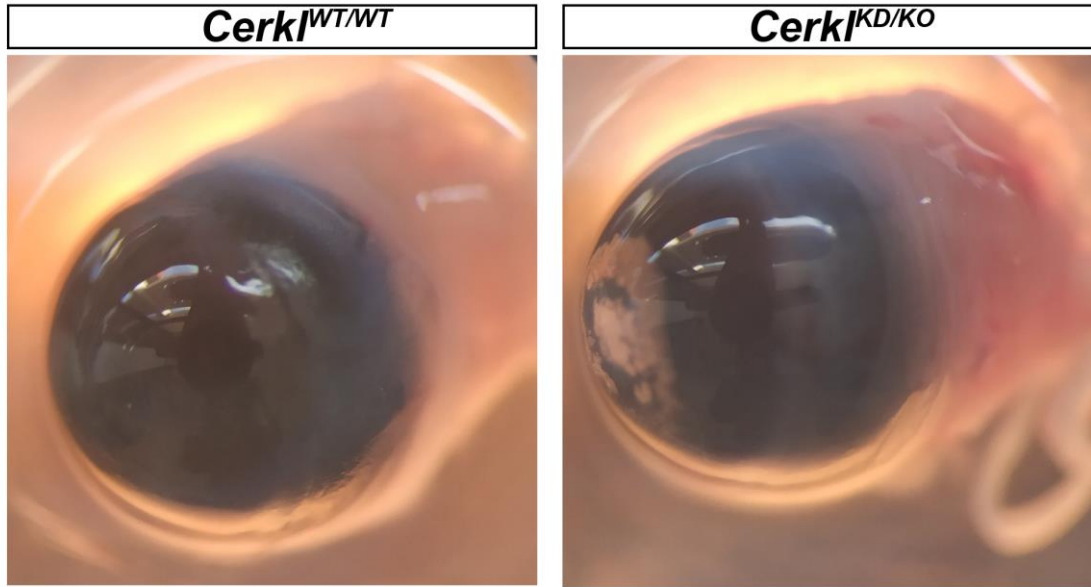
Figure S5. Corneal aggregates in aged *Cerkl*^{KD/KO} mice (≥18 months of age).
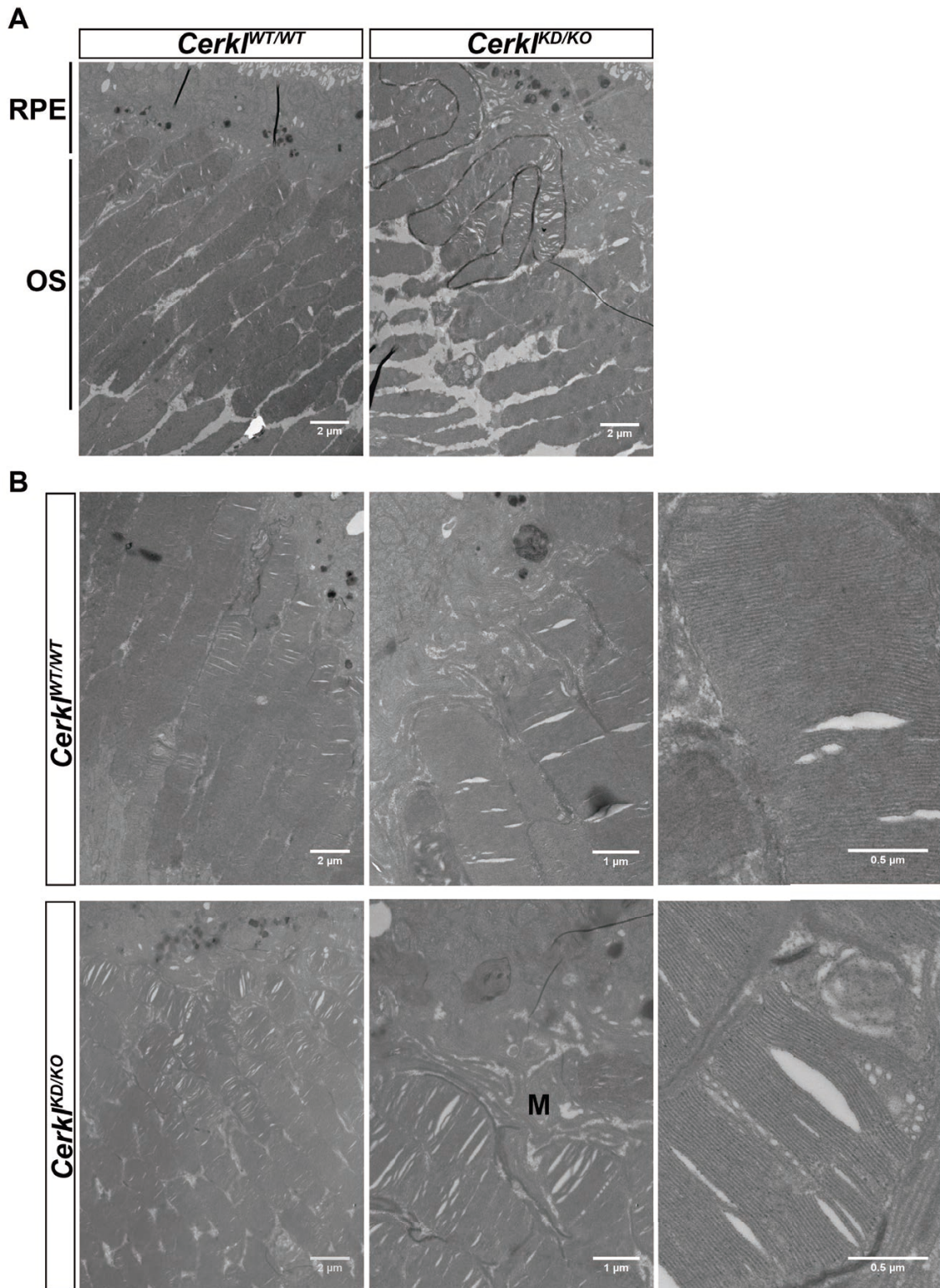
Figure S6. Transmission electron microscopy image showing the ultrastructure of retinal photoreceptors. A) *Cerkl*^{KD/KO} photoreceptors show loose stacking of the membraneous disks and disarrayed bent outer segments (outlined in black) and disorganized microvilli (M). B) Additional images from different *Cerkl*^{KD/KO} and *Cerkl*^{WT/WT} mice to show the consistency of the phenotype. Magnifications were taken at 5000x, 12000x and 40000x. Scale bars 2 µm, 1 µm and 0.5 µm respectively.