Supplemental Figures

Supplementary Fig. 1 Identification of *Nrl* and *cpfl1* wild-type and mutant allele. PCR analysis using primers listed in Supplementary table 2. Genomic DNA was prepared using an ear punch obtained from indicated mice. Genotyping results were further confirmed by sequencing.

Supplementary Fig. 2 Normalized intensity-luminance plot. Data from a-wave (***A***) and b-wave (***B***) responses shown in Fig. 2.

Supplementary Fig. 3 Western blots demonstrating the specificity of antibodies used in this study. ***A.*** Equal amounts of cellular extracts (150 μg protein) from transiently transfected HEK293 cells with indicated plasmids were separated by PAGE gels followed by immunoblotting with subunit specific PDE6 antibodies. ***B.*** Rod photoreceptor specific proteins, GC-F and Gαt1 are absent in retinal extracts from *Nrl* $^{-/-}$ and *Nrl* $^{-/-}$ *cpfl1* mice. Retinal extracts from *cpfl1*/+ mice serve as positive control demonstrating expression of GC-F and Gαt1 in the rod-dominated retina.

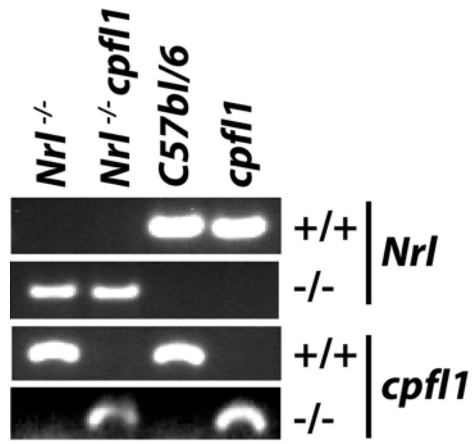
Supplementary Fig. 4 Alignment of amino acid residues from PDE6 catalytic subunits showing unique peptides identified by mass-spectrometry. PDE6 present in *Nrl*$^{-/-}$ *cpfl1* retinal extracts, immunoprecipitated by ROS-I monoclonal antibody, were separated on 4-20% gradient PAGE gel. Coomassie-stained proteins in the range of PDE6 molecular weight were cut out and analyzed by MALDI-LC MS/MS (Applied Biomics). Trypsin digestion followed by MS/MS found 27 peptides identifying Rod PDE6α and β at 100% confidence. No peptides corresponding to cone PDE6α' were found in this analysis.

Supplementary Fig. 5 Expression of rod PDE6, GC-E, and absence of M-opsin in adult *Nrl* $^{-/-}$ *cpfl1* mice. Frozen retinal sections from P30 animals were used for immunolocalization. Rod PDE6 proteins were identified using MOE, an antibody that recognizes rod PDE6αβγ and rod PDE6β subunit specific antibody. PNA, a cone marker, is stained in red. ToPRO3 staining in blue marks the nuclei. PDEαβγ staining (***AB,*** *upper panel*), PDEβ (***AB,*** *middle panel*), GC-E (***AB,*** *bottom panel*), S-opsin (***CD***, *upper panel*) and M-opsin (***CD***, *lower panel*) staining are shown in green.

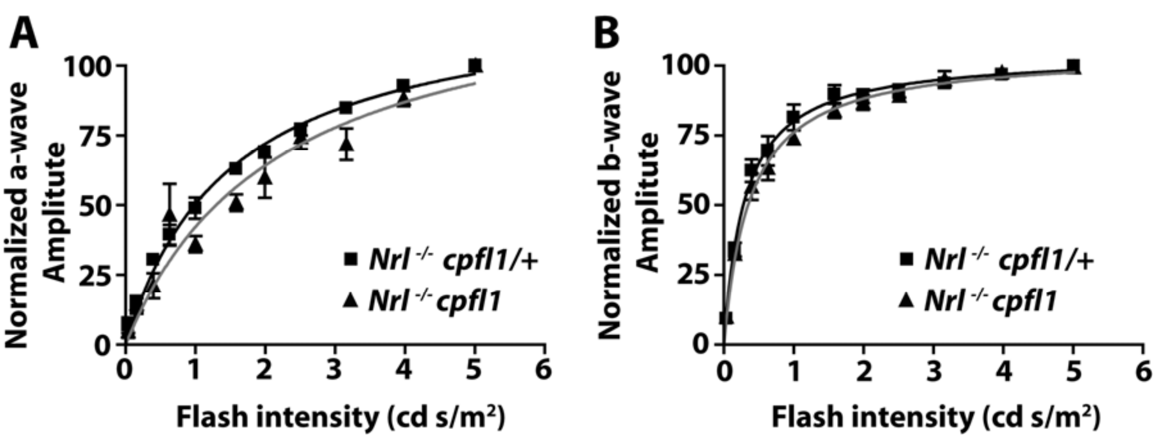
Supplementary Fig. 6 M-opsin mis-localization in the absence of cone PDE6 is not due to cell death. Propidium iodide (PI) staining (red) in retinal sections from *cpfl1* mice at P14 showed few apoptotic cells (brighter red, indicated with an arrow). Note that M-opsin staining (green) does not overlap with PI staining.

Supplementary Fig. 7 Mislocalization of M-opsin (green, panel B). In contrast, S-opsin (green, panel A) is localized to outer segments. Residual PDE6α subunit is transported to outer segments (green, panel C). As expected, PDE6β subunit is undetectable (panel D). Retinal sections are from *Nrl* $^{-/-}$ *cpfl1 rd* mutant mice at P12. Red staining by PNA marks cone photoreceptor cells.

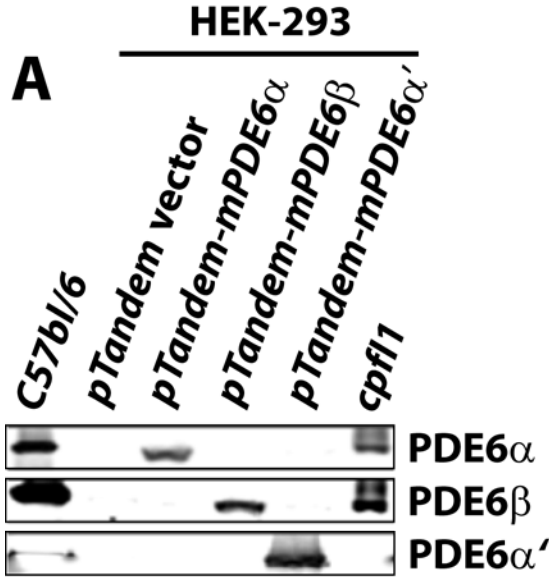
Supplementary Fig. 1

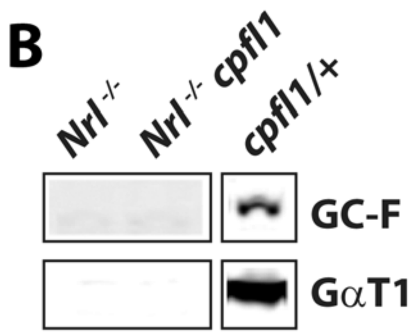


Supplementary Fig. 2



Supplementary Fig. 3





Supplementary Fig. 4

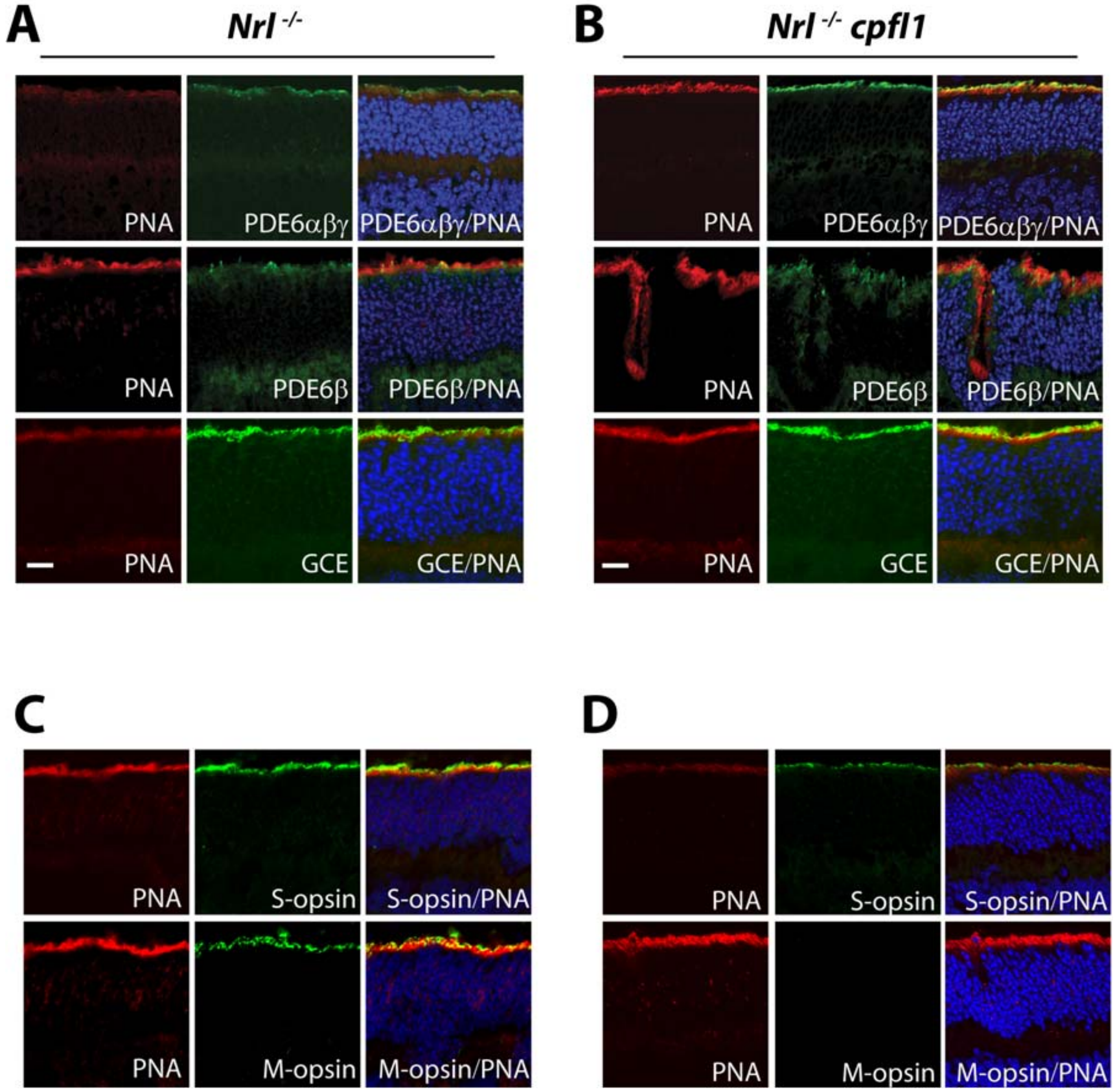
```
mPDE6α     MGEVTAEEVEKFLDSNIGFAKQYYNFHYRGKVISDLLGAKEAAVDFS-NYHDVNSVEESE 59
mPDE6β      MSLSEEQVRSFLDGNPTFAHQYFGKKLSPENVAGAC-EDGWLADCG-SLRELCQVEESA 57
mPDE6α'    MGEISQEAVERYLEKNPCFAKEYFDKKLRVEALGVIFKNSHAGVQTGLSLPEMTQVEESA 60

mPDE6α     IIFDLLRDVQENLQ-AEKCTFNVMKKLCFLLRADRMSLFMYRTRNGIAELATRLFNVHKD 118
mPDE6β     ALFELVQDMQESVN-MERVVFKILRRLCTILHADRCSLFMYRQRNGIAELATRLFSVQPD 116
mPDE6α'    VCLELLQCMQDEAGSAEQMAHRALQRLAQLLQADCCSMFSCRARNGIPEVASRLLNVTPT 120

mPDE6α     AVLEDCLVMPDSEIVFPLDMGVVGHVAHSKKIANVPNTEEDEHFCDFVDNLTEYQTKNIL 178
mPDE6β     SLLEDCLVPPDSEIVFPLDIGIVGHVAQTKKMINVQDVAECPHFSSFADELTDYVTKNIL 176
mPDE6α'    SKFEDNLVAPDREVVFPLDIGIVGWVAHVKKALNVSDVKKNSHFSDFMDKQTGYVTRNLL 180

mPDE6α     ASPIMNGKDVVAIIMAVNKIDEPHFTKRDEEILLKYLNFVNLIMKVFHLSYLHNCETRRG 238
mPDE6β     STPIMNGKDVVAVIMAVNKLDGPCFTSEDEDVFTKYLNFATLNLKIYHLSYLHNCETRRG 236
mPDE6α'    AVPIVAGKEVLAVVMAVNKISAPEFSKQDEEVFSKYLSFVAVALRLQHTSYLYSVESRRS 240

mPDE6α     QILLWSGSKVFEELTDIERQFHKALYTVRAFLNCDRYSVGLLDMTKQKEFFDVWPVLMGE 298
mPDE6β     QVLLWSANKVFEELTDIERQFHKAFYTVRAYLNCERYSVGLLDMTKEKEFFDVWPVLMGE 296
mPDE6α'    QILMWSANKVFEELTDVERQFHKALYTIRTYLNCDRYSIGLLDMTKEKEFYDEWPIKLGE 300

mPDE6α     APAYSGPRTPDGREINFYKVIDYILHGKEDIKVIPNPPADHWALVSGLPTYVAQNGLICN 358
mPDE6β     AQPYSGPRTPDGREIVFYKVIDYILHGKEDIKVIPTPPADHWALASGLPTYVAESGFICN 356
mPDE6α'    VEPYKGPKTPDGREIIFYKIIDYILHGKEEINVIPSPPADHWTLVSGLPTYVAENGFICN 360

mPDE6α     IMNAPAEDFFEFQKEPLDESGWMIKNVLSMPIVNKKEEIVGVATFYNRKDGKPFDDMDET 418
mPDE6β     IMNASADEMFNFQEGPLDDSGWVIKNVLSMPIVNKKEEIVGVATFYNRKDGKPFDDQDEV 416
mPDE6α'    MLNAPADEYFTFQKGPVDETGWVIKNVLSLPIVNKKEDIVGVATFYNRKDGKPFDEHDEH 420

mPDE6α     LMESLTQFLGWSVLNPDTYESMNKLENRKDIFQDIVKYHVKCDNEEIQKILKTREVYGKE 478
mPDE6β     LMESLTQFLGWSVLNTDTYDKMNKLENRKDIAQDMVLYHVRCDKDEIQEILPTRDRLGKE 476
mPDE6α'    ITETLTQFLGWSLLNTDTYERVNKLESRKDIAQEMVMNLTKATPDEISSILKFKEKLNVE 480

mPDE6α     P-WECEEEELAEILQGELPDAESYEINKFHFSDLPLTELELVKCGIQMYYELRVVDKFHI 537
mPDE6β     P-ADCEEDELGKILKEELPGPTKFDIYEFHFSDLECTELELVKCGIQMYYELGVVRKFQI 535
mPDE6α'    VIEECEERQLLAILKEDLPDPRTADLYEFCFSDFPITEHELVKCGLRLFLEINVVEKFKV 540

mPDE6α     PQEALVRFMYSLSKGYRRITYHNWRHGFNVGQTMFSLLVTGKLKRYFTDLEALAMVTAAF 597
mPDE6β     PQEVLVRFLFSVSKAYRRITYHNWRHGFNVAQTMFTLLMTGKLKSYYTDLEAFAMVTAGL 595
mPDE6α'    PVEVLTRWMYTVRKGYRPVTYHNWRHGFNVGQTMFTLLMTGRLKKYYTDLEAFAMLAAAF 600

mPDE6α     CHDIDHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKTLLRDESLNIFQNLNRRQHEH 657
mPDE6β     CHDIDHRGTNNLYQMKSQNPLAKLHGSSILERHHLEFGKFLLAEESLNIYQNLNRRQHEH 655
mPDE6α'    CHDIDHRGTNNLYQMKSTSPLARLHGTSILERHHLEYSKTLLQDESLNIFQNLNKRQFET 660

mPDE6α     AIHMMDIAIIATDLALYFKKRTMFQKIVDQSKTYESTQEWTQYMMLEQTRKEIVMAMMMT 717
mPDE6β     VIHLMDIAIIATDLALYFKKRTMFQKIVDESKNYEDKKSWVEYLSLETTRKEIVMAMMMT 715
mPDE6α'    VIHLFEVAIIATDLALYFKKRTMFQKIVDTCEQMQSEEETIKYVTSDPTKKEVIMAMMMT 720

mPDE6α     ACDLSAITKPWEVQSKVALLVAAEFWEQGDLERTVLQQNPIPMMDRNKADELPKLQVGFI 777
mPDE6β     ACDLSAITKPWEVQSKVALLVAAEFWEQGDLERTVLDQQPIPMMDRNKAAELPKLQVGFI 775
mPDE6α'    ACDLSAITKPWEVQSQVALLVANEFWEQGDLERTVLQQQPIPMMDRSKKDELPKLQVGFI 780

mPDE6α     DFVCTFVYKEFSRFHEEITPMLDGITNNRKEWKALADEYEAKMKALEEEKQKQQAAKQAA 837
mPDE6β     DFVCTFVYKEFSRFHEEILPMFDRLQNNRKEWKALADEYEAKVKALEEEKKEEDRVAAK 835
mPDE6α'    DFVCTFVYKEFSRFHGEITPMLNGLQNNRVEWKSLAEEYEAKVKVTEEEAGKQEEEASDG 840

mPDE6α     SGNQPGGNPTPGGAPASKSCCIQ 860
mPDE6β     KVGTEVCNGGP--APKSSTCCIL 856
mPDE6α'    KAATDLGGSAE--DKKSKTCLML 861
```

Peptides unique to PDE6α (green)

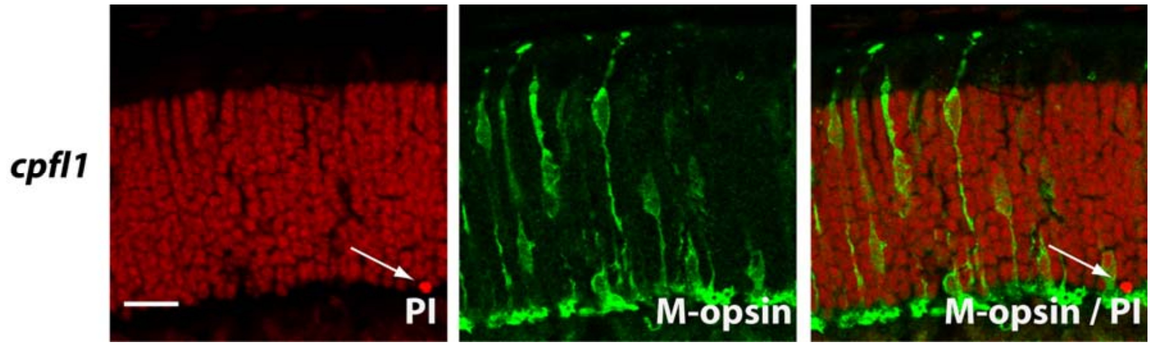
Peptides unique to PDE6β (red)

Peptides unique to rod PDE6 subunits (yellow)

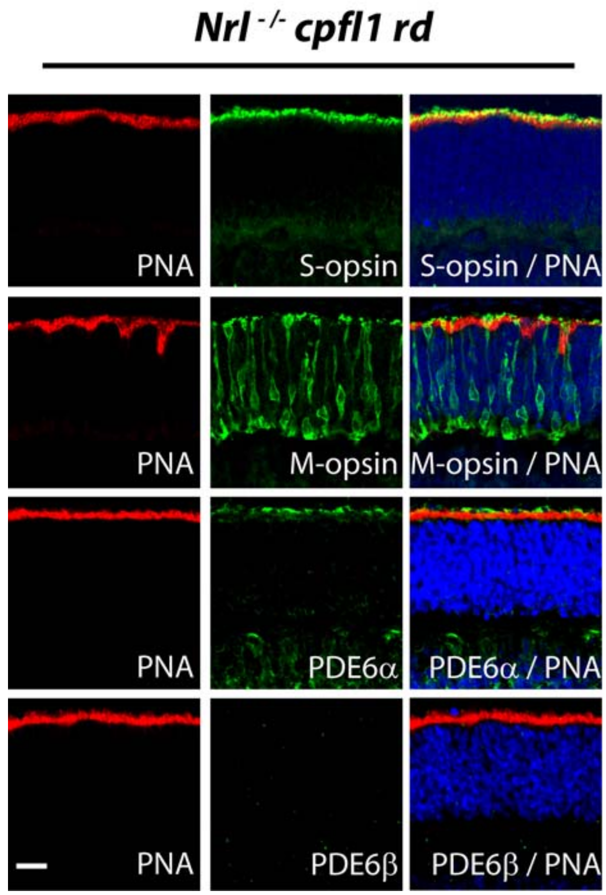
Supplementary Fig. 5



Supplementary Fig. 6



Supplementary Fig. 7



Supplementary table 1.Oligonucleotide used in this study for genotyping.

Genotype	Primer sequences	Product size (bp)
Nrl-wt	5′-GTGTTCCTTGGCTGGAAAGA- 3′ 5′- CTGTTCACTGTGGGCTTTCA-3′	300 bp
Nrl-kO	5′- TTTCTGGTTCTGACAGTGACTACG-3′ 5′- ACCAAATTAAGGGCCAGCTCATTCCT-3′	600 bp
PDE6b- wt	5′-TGACATTACTCCTTTTCCCTCAGTCTG-3′ 5′- TACCCACCCTTCCTAATTTTTCTCACGC-3′	500 bp
PDE6b- rd1	5′-TGACATTACTCCTTTTCCCTCAGTCTG-3′ 5′- GTAAACAGCAAGAGGCTTTATTGGGAAC-3′	700 bp
PDE6c- cpfl1	5′-TTCAACCATCTCTGCCCTTC-3′ 5′- AGCAGACCTCTGCGAAGAAC-3′	450 bp (wt) 750 bp (mut)

Supplementary table 2. Oligonucleotide used in this study for RT-PCR.

Gene	Primer sequences	Produdct size (bp)
Hprt	5′-CAAACTTTGCTTTCCCTGGT-3′ 5′-CAAGGGCATATCCAACAACA-3′	200 bp
Pde6a	5′-TGTGATCTCTCAGCCATCACCA-3′ 5′- CTGGTTCTTTAACTGTCCAGTGCCA-3′	516 bp
Pde6b	5′- CGATTTCACGAAGAGATCCTG-3′ 5′- CCTGTTCCTAATGGCTTATACCAA - 3′	302 bp
Pde6g	5′- CTGACAGAGTCCAGAAGCTAAGG-3′ 5′-CTAGGGACTCAGGCTCAGGTTT-3′	418 bp
Pde6c	5′- AGCGGCAGTTTGAAACGGTGA-3′ 5′- TCGCCTCGTACTCCTCCGCC-3′	500 bp
Gnat1	5′- GGGCCAGCGCTGAGGAGAAG-3′ 5′- AGCCGGCGGAGTCATTGAGC-3′	438 bp
Rho	5′- TCAAGCCTGAGGTCAACAAC- 3′ 5′- GTCTTGGAAGCGGTGGCAGAG- 3′	439 bp
Opn1sw	5′- GGTCATTGGCTTTCCTGG - 3′ 5′- TGCAGGCCCTCAGGGATG- 3′	175 bp
Opn1mw	5′- GCCCAGACGTGTTCAGCG- 3′ 5′- GACCATCACCACCACCAT- 3′	212 bp
Nrl	5′- CTATGGAAGGGCCTCTTGG- 3′ 5′- GCCACGATGCTCAGAAGTTT- 3′	540 bp