Supporting Information

Chang et al. 10.1073/pnas.0907720106



Fig. S1. Nearly complete loss of cones in 5-month-old *cpfl1* mice. Retinal sections of a 5-month-old cpfl1 mutant were immunostained with an antibody against the M opsin (*A*) or stained with HRP-conjugated peanut agglutinin that specifically binds to cone sheaths (*B*), respectively. In comparison with an age-matched C57BL/6J control (*C* and *D*) there were only very few cones left in the *cpfl1* retina.



Fig. S2. Linkage mapping and positional cloning of *cpfl1*. (*A*) Recombination data localizing *cpfl1* to a 0.5 cM interval on mouse Chromosome 19. The 125 progeny from a backcross and 377 progeny from F2 intercross between C57BL/6J-*cpfl1/cpfl1* and CAST/Ei were phenotyped and genotyped. The columns of squares represent haplotypes (filled boxes, *cpf11/cpfl1* allele; open boxes, CAST/Ei allele). (*B*) Physical map of the critical *cpfl1* region derived from UCSC Genome Browser v123. The map shows informative markers and the candidate genes, including *Pde6c*. In addition, the markers flanking the *cpfl1* locus as well as *Pde6c* co-localize on the YAC clone 398-A-5.



Fig. S3. Comparative Southern Blot analysis of parts of *Pde6c* in cpfl1 and C57BL/6J wild-type mice. (A) Hybridization of a murine *Pde6c* cDNA fragment covering exons 3–7 against *Xbal-*, *Ncol-*, or *Bam*HI-digested *cpfl1* and C57BL/6J (WT) mouse DNA revealed increased lengths of fragments covering intron 4 in *cpfl1*. λ H and λ HE denote size standards (λ -DNA digested with *Hind*III and *Hind*III+*Eco*RI, respectively); corresponding fragment sizes are given at the margins. (*B*) Schematic map of parts of the *Pde6c* gene in C57BL/6J (WT) and the *cpfl1* mutant indicating the origin of fragments (as predicted for C57BL/6J) detected in *A*. Note that the 1,522-bp insertion in the *cpfl1* allele introduced an additional *Ncol* site.

1	TATTTTCATATCTTATGTTGGCTTCCTTCCTTTATGCAGCCATTTAAGATTCTCATGAAATTCATTC
81	TGTCTTCTTTTATTTATTTAAACATTTTTATCTTTACTCCTGGAAATTTCTT
161	TTTTTTTTTTTTGCCAAGATAATCTCATTTTATTGCATTTCGATAGATTCTTCTCTGTAAATTGTTGGTAATGTCATCCTT
241	TAATGACTAGAGAGGTACGAGGATTCTAATTTTATTTGAGATTTTTATTCATTTAAGGGGAAGAGTTCTCTCTACTACTT
321	TTTAGAAATAGCAAAACTATGACTTATTTAAATAATTGTAAACTGCTCATTTCTTTTTCCATTAGCAGAATATTGCTGC
401	CTAACAAAGGGTATGATCTTAGGGGAAAAAAAAAAAAAGGCAAACACTAATGAGTAGCAGCAGGAGCAACAAGCAGCACTGGG
481	TATTTTTTATCCCTTGTCTTTCTTATCATATCAACAGATTTCATTTTCCACACGAATTTTTTCCTCATAAAAATATTT
561	TCAAATTTTCTCCTGTACACACTGAGTCTAGTATATGACCTCAATCATTTTGAGACTCAAGGGCTCAAAGTAGTTAAAGT
641	TTATATATTAGGTAGAAAAAATACTGCTGAATTTTCTATCATAAATTTGGACTCACTATGCGGCAACAAATAATTGAAGA
721	ATCATTACTTTTGCATGGGTCCTTTAAAAGTTGGATCTCAACTGTATAATTTTATATGATTATATTACCTACC
801	${\tt TTTCAGATTACTACTTCTTTTAAATATCTATCTATCTATC$
881	TGTATATATCTGAGTGCATATATATATATATATATATATA
961	AGGAGCCCATGGAAGTCAGAAGAGGATAATGGAACCCTTCAAACTGGAATGACAGAATCCTCAGTATGGGTGTCAAGCAT
1041	gagtetgtgtacteteeaatteagaaagtteeatgag <mark>gtaatgteaetttgttttgt</mark>
1121	TTCTTTTGTAATGGTGAGACTCTAACAAAAAGTCTCATACAAGTGAAGCCTCACTGTCTTTGACAGCTAATAAACTGAAA
1201	CCTATATTTGAGATAATTATTTAACATCTTTATCTTGGTTATAAGTCAACCTAATGTAATC
1281	TTTTTTTTTTTTTTTTTTTTTCCATTTTTTTTTTAGGTATTTAGCTCATTTACATTTCCAATGCTATACCAAAAGTCCCCCT
1361	TACCCACCCCCCCCCCCCCCCCCCCCCCCCCCTTGGCCCTGGCGTTCCCCTGTACCGGGGCACACAAAGT
1441	CTGCGTGTCCAATGGGCCTCTCTTTCCAGTGATGGCCGACTAGGCCATCTTTTGATACATATGCAGCTAGAGTCAAGAGC
1521	TCAGGGGTACTGGTTAGTTCATAATGTTGTTCCACCTATAGGGTTGAAGATCCCTTTAGCTCCTTGGGTACTTTCTCTAG
1601	CTCCTCCATTGGGAGCCCTGTGATCCATCCATTAGCTG <mark>CTCCTGGAAATTTCTT</mark> GTCTGGATTTTCTACTAAGTTACTGT
1681	CATTGGGGGGCATTTGCTGTGAGATTTGTTAGATTTTAGAGGGGACATGTGGTCTTGGGCTTTCATTTTGTGTTTTGTATT

Color Legend:

PNAS PNAS

ATCTGTG -	genuine	Pde6c se	equence
-----------	---------	----------	---------

- TTTTTTT T-Stretch
- GAGGTAC diaphanous locus
- CAAATTC cryptic exon, cDNA insertion
- GGGCCTC LINE1 element
- TGGAAAT duplication insertion site

Fig. S4. DNA sequence of the 1,552-bp insertion into intron 4 of the *Pde6c* gene of the *cpfl1* mutant.



Fig. S5. Heterologous splicing assays of putative splicing mutations. Sequence traces of PCR amplified cDNA or cDNA clones prepared from RNA of COS7 cells either transfected with mutant or wild-type *PDE6C* minigene constructs, and overview of the deduced splicing defects and their consequences. (*A*) Expression of exon 1–3 minigene constructs in COS7 cells revealed the activation of a cryptic splice site in constructs bearing the c.481–12T>A mutation. The misspliced cDNA carries a frame-shift followed by a premature termination codon. (*B*) Exon 11–14 constructs bearing the c.1483–2A>G mutation gave rise to transcripts that completely lack exon 12 and thus carry an in-frame deletion of 49 amino acid residues. (C) Gel electrophoretic separation of RT-PCR products obtained with exon 19–21 minigene constructs cloned from a control subject (1), the non-mutant (2), and the c.2368G>A mutant allele (3) of subject CHRO102/II:1. LS denote a DNA size standard (pcDNA3.1/zeo digested with *TaqI*). The agarose gel (left) shows a skipping of exon 21 in a fraction of mutant transcripts that was verified by sequencing of cDNA clones (right: bottom). Correctly spliced transcripts of the mutant construct showed the presence of the c.2368G>A substitution (right: top). Note that in this assay the minigene construct was cloned into the pSPL3 vector that includes flanking exons of the TAT gene which participates in the processing of the introduced minigene fragment.



Fig. S6. Fundus alterations in a patient with PDE6C-associated achromatopsia. Fundus photograph of the right eye of patient CHRO 9/II:3 showing the posterior pole with the macula with evidence of a weak and incomplete foveal reflex around the fovea, pigment mottling of the fovea and atrophy of the retinal pigment epithelium around the optic disc.

DN AS