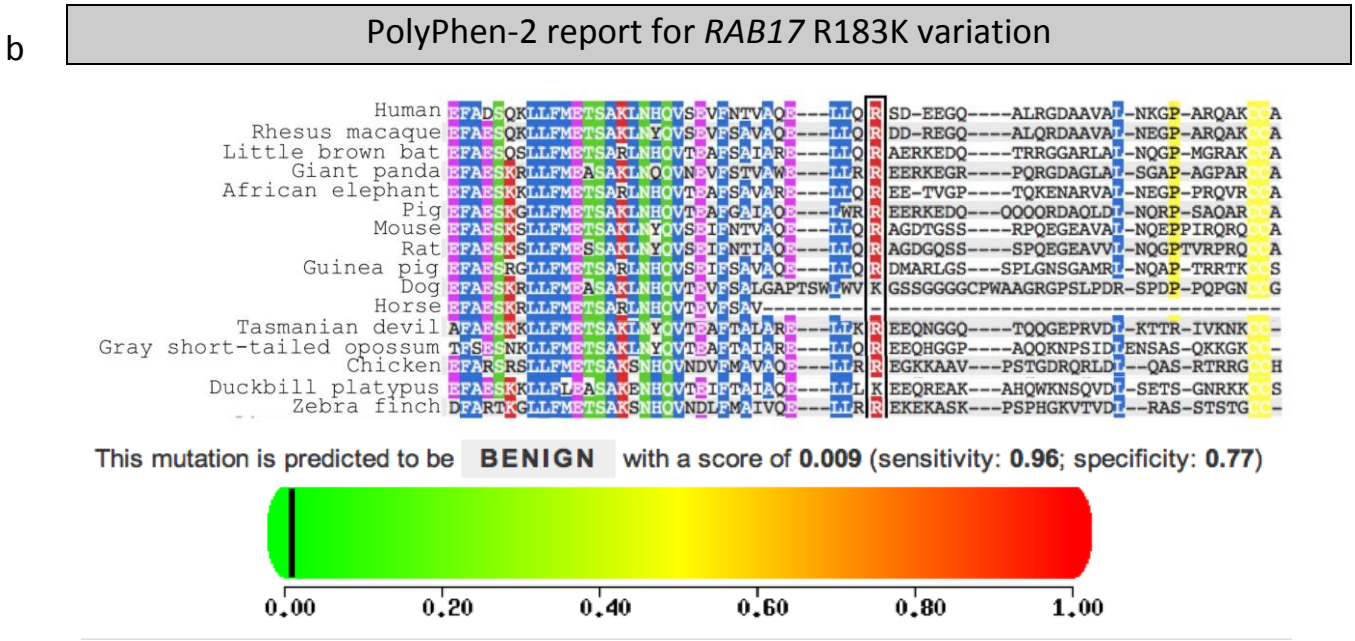
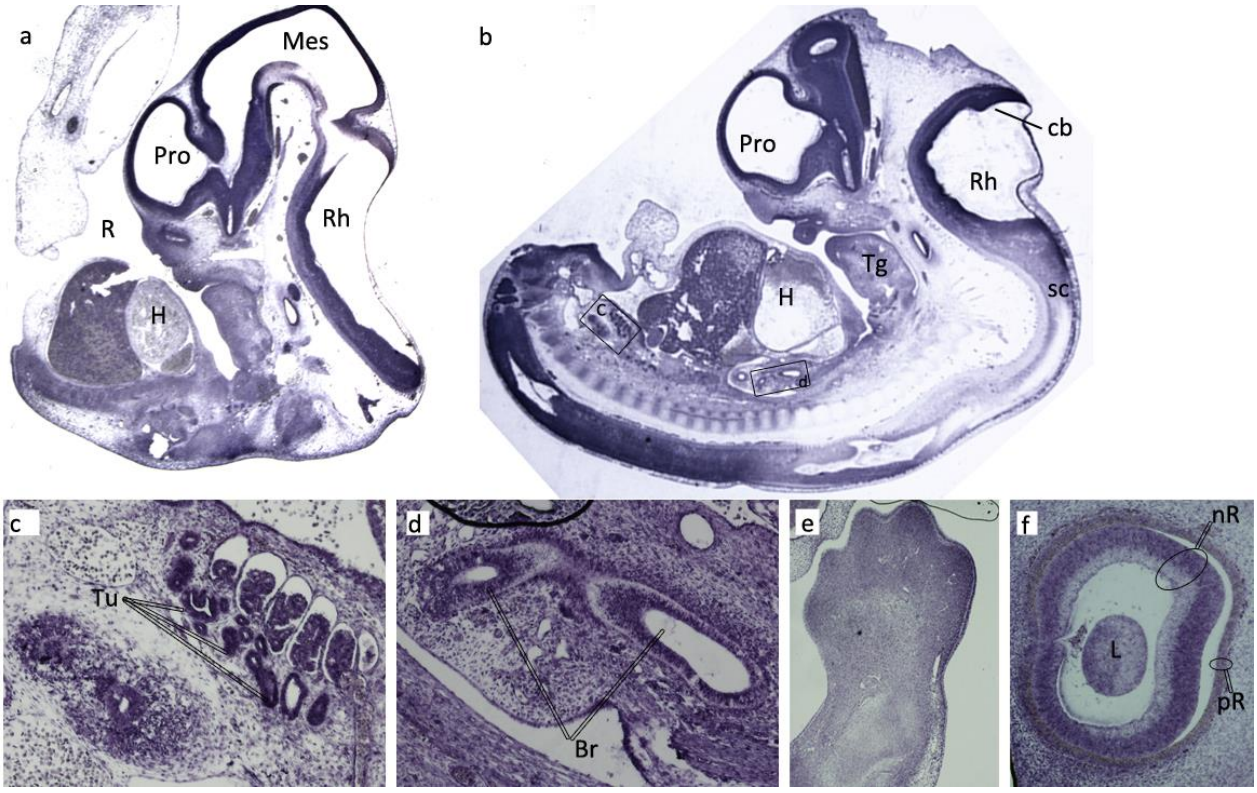
a

	Number of variations in whole exome	
Total variants called	73,103	
Exonic + Splice-sites (SS) variants	18,040	
Non-synonymous + SS + frameshifts	9,738	
Unknown variants (dbSNP132/1K genome/in-house database filtering)	338	
Homozygous variations	25	
Mapping analysis	2	*RAB17*, p.R183K *PDE6D* c.140-1G>A

b

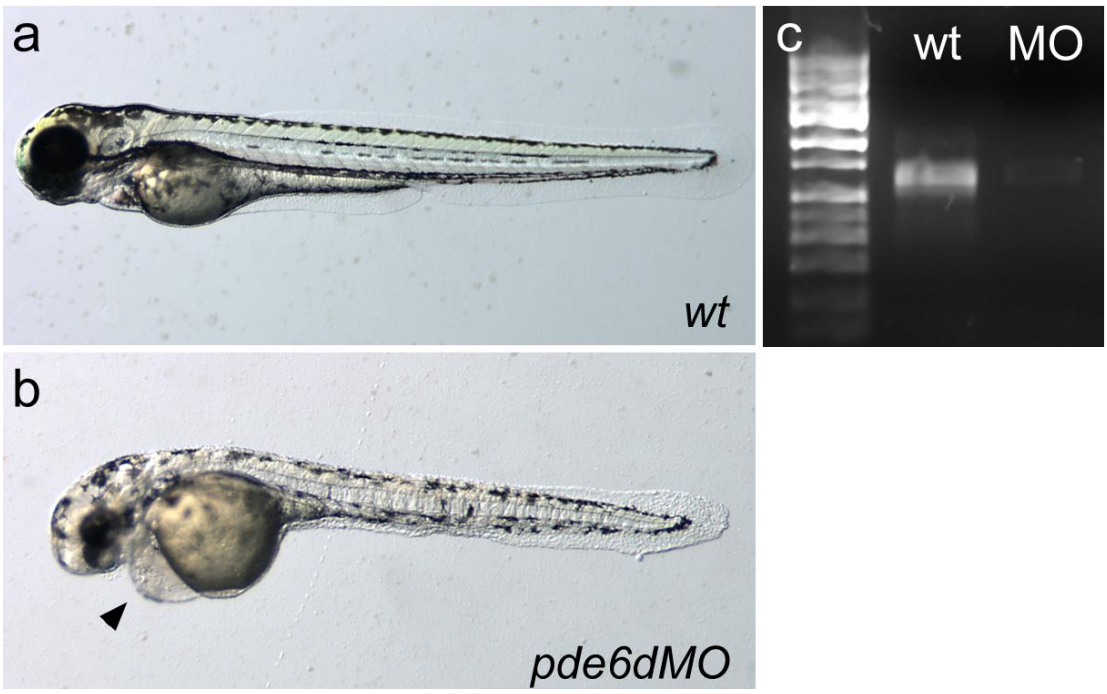


Supp. Figure S1. Exome sequencing. Exome capture with the SureSelect Human All Exon kit (Agilent) was followed by single-end sequencing on an Illumina Genome Analyzer. **(a)** Filtering of the data identified a unique truncating mutation within the targeted homozygous region. **(b)** Polyphen 2 analysis of the RAB17 R183K predicts it is benign. Arg at position 183 is not conserved and is replaced by a lysin in Dog (Canis familiaris) and Duckbill platypus (Ornithorhynchus anatinus).



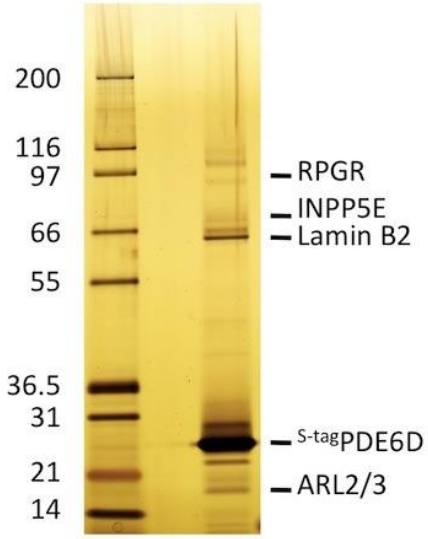
Supp. Figure S2. PDE6D expression pattern during human development. Immunohistochemistry was performed on normal human embryos obtained after elective pregnancy termination in agreement with French legislation (law no. 2004-800), National Ethics Committee recommendations (report no. 1 of May 22, 1984) and the Necker Hospital ethics committee. Embryonic stages were established according to Carnegie staging (CS) classification. After citrate buffer antigen retrieval treatment, adjacent sections were incubated overnight with or without rabbit polyclonal anti-PDE6D antibody (Sigma, HPA037434) and subsequently with goat polyclonal anti-rabbit IgG coupled to alkaline phosphatase (AbCam, ab6722).

PDE6D is ubiquitously expressed within human embryonic tissues with a more intense staining in the entire central nervous system (prosencephalon (Pr), mesencephalon (Mes) and rhombencephalon (Rh) **(a,b)**, in renal tubules **(c)** and in epithelial cells of the bronchus **(d)**. PDE6D is also localized in the limb bud mesenchyme **(e)** and in the neural layer of the optic cup (future neural retina) **(f)**. Br, bronchus; Cb, cerebellum; H, heart; L, lens; nR, neural retina; pR, pigment layer of retina; R, Rathke's pouch; sc, spinal cord; tg, tongue; Tu, renal tubules.



Supp. Figure S3. Zebrafish *pde6d* morpholino knockdown. **(a)** Control zebrafish larva at 3 dpf. **(b)** *pde6d* morphant at 3 dpf after injection of an exon 2 splice donor-blocking morpholino oligonucleotide (MO), (Gene-Tools, LLC) with sequence 5'-TAATGCTGAGAACCACAAACCTTCA-3', or a translation blocking MO 5'-TTCGTCCGAAGACATCTTTTTCCTT-3'. *pde6d*-deficient embryos exhibit defects in eye development and pericardial edema (arrowhead). A control random sequence morpholino did not cause a phenotype at similar injection concentration. **(c)** RTPCR analysis of control and morphant mRNA revealed near complete absence of *pde6d* mRNA in exon 2 splice donor morphants (MO) compared with controls (wt).

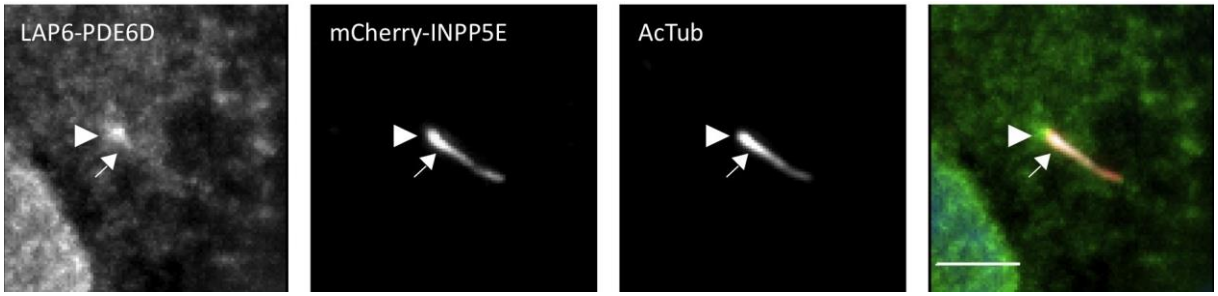
A



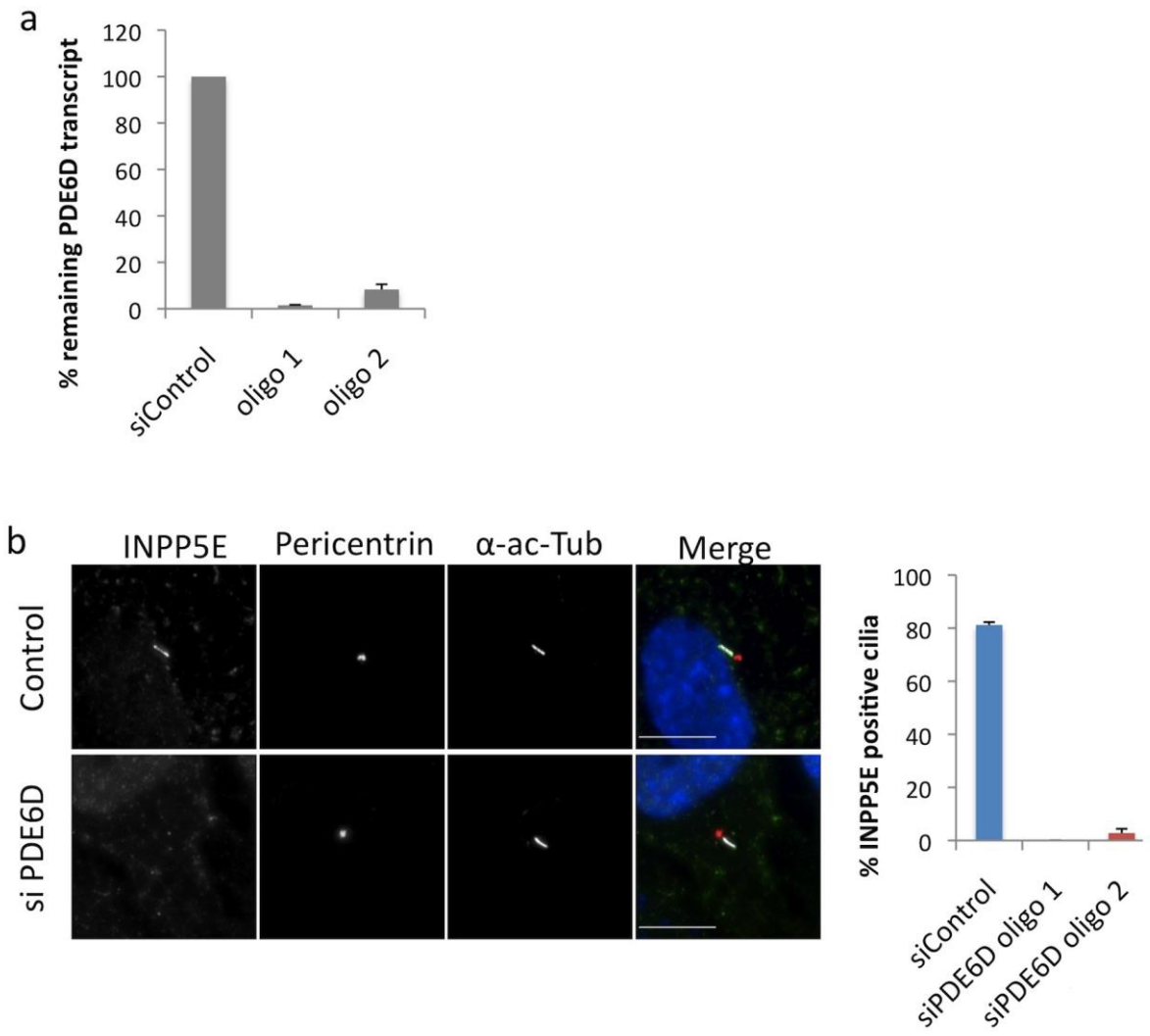
B

Gene	Peptides	CaaX box
PDE6D (bait)	3307	no
Lamin B2	1166	yes
RPGR	501	yes*
Rab28	424	yes
INPP5E	318	yes
Pre-Lamin A/C	246	yes
ARL3	188	no
ARL2	76	no

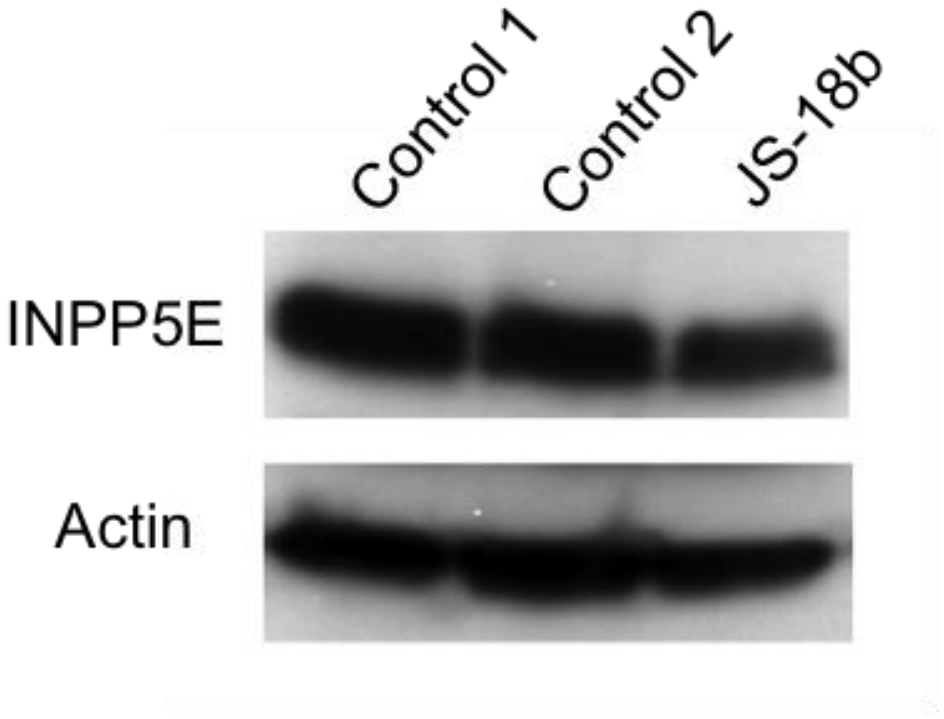
Supp. Figure S4. Isolation of PDE6D interacting proteins. **(A)** Silver stained gel showing the eluate from tandem affinity purification of LAP-tagged PDE6D. **(B)** Table of proteins with or without a CaaX-box identified by mass spectrometry as PDE6D interactors. Asterisk denotes a CaaX-box predicted to be geranylgeranylated.



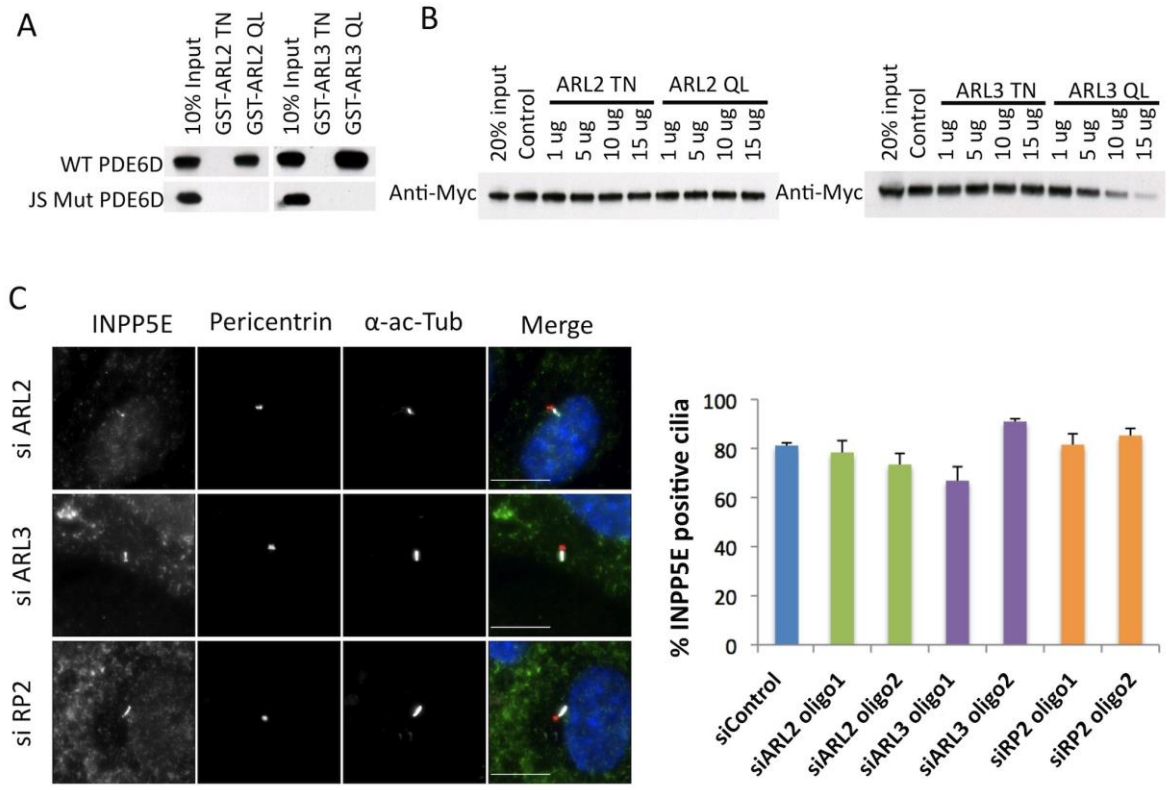
Supp. Figure S5. Partial co-localization of PDE6D and INPP5E. GFP-tagged PDE6D localizes to the transition zone and proximal end of the cilium while INPP5E localizes uniformly along the length of the axoneme.



Supp. Figure S6. PDE6D expression is required for INPP5E localization to the primary cilium. **(a)** Efficiency of PDE6D siRNAs tested by quantitative RT-PCR performed on RNA extracted from RPE cells 48 hours after transfection with either control or two different siRNA oligos targeting PDE6D (Dharmacon, J-004310-09 and J-004310-11). **(b)** Immunocytochemistry using antibodies raised against INPP5E, pericentrin and acetylated alpha tubulin (α-ac-Tub) in RPE1 cells transfected with either a control siRNA or a siRNA targeting PDE6D and showing that PDE6D extinction leads to to a near total loss of INPP5E localization to the primary cilium as quantified in the graph. Scale bars are 10 um. Graph is average of three independent experiments plus standard deviation.



Supp. Figure S7. Western blot of INPP5E on proteins extracted from control and patient fibroblasts. INPP5E protein is expressed in both patient and control fibroblasts. Antibodies used: INPP5E (Proteintech, 17797-1-AP) and beta-actin (Santa Cruz Biotechnology; sc-81178).



Supp. Figure S8. INPP5E primary cilia localization is independent from ARL2, ARL3 and RP2. **(A)** GST pulldowns were performed using GST-ARL2/3 TN (GDP mimicking ARL2/3 mutant protein) or GST-ARL2/3 QL (GTP mimicking ARL2/3 mutant protein) and in vitro translated myc-tagged WT or Δexon3-PDE6D. WT PDE6D binds to ARL2/3 in a GTP dependent manner while Δexon3-PDE6D is unable to bind either ARL2 or ARL3. **(B)** RPE cells were transfected with LAP6-PDE6D and myc-INPP5E, then lysed and incubated with increasing amount of either GDP (TN)- or GTP (QL)-mimicking mutant GST-ARL2 or GST-ARL3 as indicated. Immunoprecipitation of PDE6D with anti-GFP antibodies and detection of co-immunoprecipitated INPP5E by anti-Myc immunoblotting reveal specific ARL3 QL-dose dependent interaction between INPP5E and PDE6D. These results indicate that GTP-ARL3 but not GTP-ARL2-binding efficiently releases INPP5E from PDE6D. **(C)** Immunocytochemistry using antibodies raised against INPP5E, pericentrin and acetylated alpha tubulin (α-ac-Tub) in RPE1 cells transfected with either control siRNA or siRNA directed against *ARL2*, *ARL3*, and *RP2* as previously described (Wright K.J. et al. 2011) and showing that INPP5E ciliary localization is not perturbed by *ARL2*, *ARL3* or *RP2* extinction. Scale bars are 10 µm. Graph is average of three independent experiments plus standard deviation.

Supp. Table S1. Primers used for *PDE6D* genomic sequencing, RT-PCR and mutagenesis

PDE6D genomic sequencing primers	
PDE6D-1F	GAAGGAGAAGGGATCAGAAGC
PDE6D-1R	CGGCCAGTCTCCTCAGTG
PDE6D-2F	CAAGAGTTGGTGGGAGTAAACA
PDE6D-2R	GCTCACCCGCCTATTAGGTA
PDE6D-3F	CTCTGCTTTCAGCCACACAA
PDE6D-3R	GCACTGCTTTAGTTCACTGGGTA
PDE6D-4F	TGCTGATAGCTTCCTTTGGTG
PDE6D-4R	GGTCAGGAACCTGGAAACCT
PDE6D-5F	GGAATCCAGATGGAAACCAC
PDE6D-5R	TCTGGGGTTGGGAGTTTAGTC
PDE6D RT-PCR primers	
PDE6D-RT-1F	CATGTCAGCCAAGGACGAG
PDE6D-RT-5R	GAAAAGTCTCACTCTGGATGTGC
PDE6D-RT-2F	GACCTGTCTGTCCCTGGTGT
PDE6D-RT-4R	AAGCCAAACTCGAAGAACCA
Mutagenesis primers	
PDE6D-del140-265-s	ctggtgtggagcatgaGgaatggttcttcgagtttg
PDE6D-del140-265-as:	caaactcgaagaaccattcCtcatgctccacaccag