Supplementary Material:

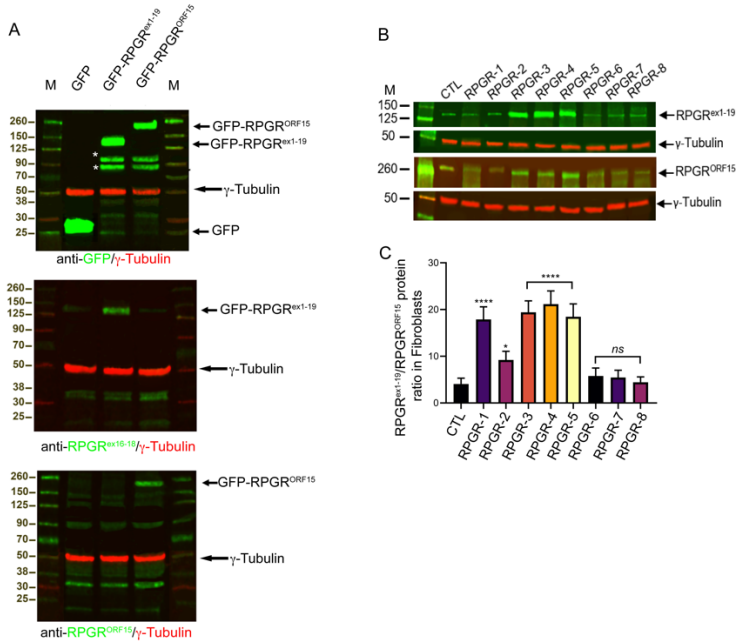
Generation and characterization of RPGR isoform-specific antibodies: The RPGR isoform-specific antibodies were generated against the protein domains present specifically in each isoform (Abclonal). For $RPGR^{ex1-19}$-specific antibody, the protein domain encoded by amino acids 923-1039 encompassing exons 16-18 of the RPGR protein was used to generate antibodies in rabbits. For $RPGR^{ORF15}$-specific antibody, we used the protein domain encoded by amino-acids 563-804 encompassing exon ORF15.

Human photoreceptor stem cell maintenance and retinal differentiation culture

The human embryonic and iPS stem cell lines (Rb2 from Wicell and iPS RPGR cell lines) were maintained in feeder free conditions with E8 (Thermo Fisher) media on geltrex coated 6 well plates. Briefly, when 80% confluent hPSCs were dissociated using Versene solution for 10 minutes. PSC small clumps were collected, washed twice with PBS and resuspended in E8 media for further maintenance culture on 6 well plates. For retinal neuroepithelial differentiation, human PSCs were maintained as described above until 90-95% confluent, then media without FGF (E6, Thermo Fisher) was added to the cultures for two days (D1 and 2 of differentiation) followed by a neural induction period (up to 7 weeks) in proneural induction media (Advanced DMEM/F12, MEM non essential amino acids, N2 Supplement, 100mM Glutamine and Pen/Strep). Lightly- pigmented islands of retinal pigmented epithelium (RPE) appeared as early as week 3 in culture. Optic vesicles were formed from within the RPE region between weeks 4 and 7. During this period neuroretinal vesicles were manually excised with 21G needles and kept individually in low binding 96 well plates in retinal differentiation media (DMEM, F12,

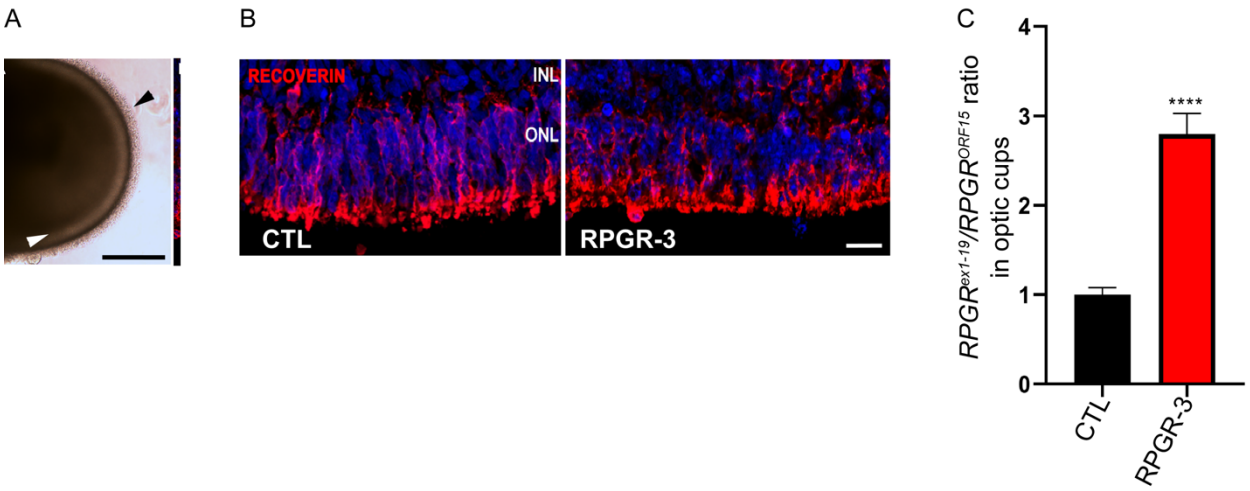
Pen/Strep and B27 without retinoic acid). At 6 wks of differentiation retinal differentiation medium was supplemented with 10% FBS, 100uM taurine (Sigma, T4871) and 2mM glutamax and at 10 wks 1 uM retinoic acid (RA) was added (RDM+ Factors media). At 10 wks of culture vesicles were transferred to low binding 24 well plates (5 vesicles/well). At 12 wks of differentiation, media was changed again to ALT media (Advanced DMEM/F12, B27 without retinoic acid, N2 Supplement, 4 mM glutamax, 7.5mM glucose, 100uM taurine, 0.5 μM RA and Pen/Strep). Maintenance cultures of hPSCs were feed daily and differentiation cultures were feed every 2-3 days. At 24 weeks 10 retinal organoids were collected per sample and snap frozen or placed in Trizol and stored at -20^{o}C.

Supplementary Figure 1:



A. Cell extracts from HEK293 cells transiently transfected with plasmids encoding GFP, GFP-RPGR^{ORF15} or GFP-RPGR^{ex1-19} were analyzed by SDS-PAGE and immunoblotting using indicated antibodies. Green channel indicates the bands obtained using the GFP or RPGR antibodies. Immunoblotting using anti- γ-tubulin (red) was used as loading control. M: apparent molecular weight marker (kDa). **B**. Protein extracts (100 μg) of the indicated fibroblasts were analyzed by SDS-PAGE and immunoblotting using RPGR^{ex1-19} or RPGR^{ORF15}-specific (green) or γ-tubulin (red; loading control) antibodies. **C**. The RPGR^{ex1-19}/RPGR^{ORF15} protein ratio was calculated relative to the γ-tubulin levels, which were uniform among all samples. Data are mean ± SD from three independent experiments (with n>100/experiment). **D**. *RPGR^{ex1-19}/RPGR^{ORF15}* mRNA expression was calculated in control (CTL) and RPGR-3 optic cups. Statistically significant differences are indicated (****: p<0.0001; *p<0.01).

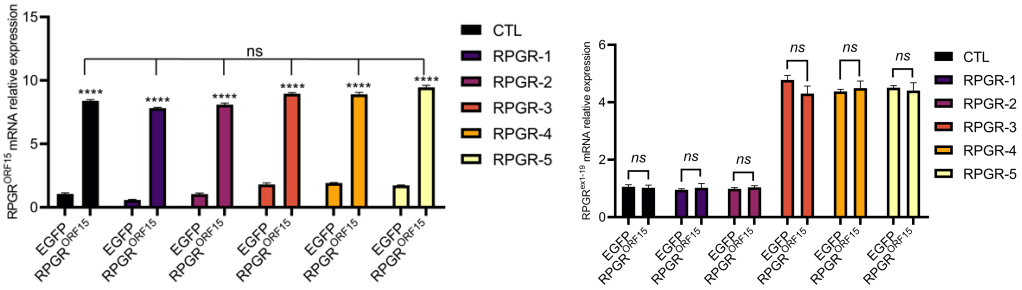
Supplementary Figure 2



A. Bright field image of a 22 weeks RPGR-3 PSC-derived retinal organoid, demonstrating retinal layers (white arrowhead) and the presence of a brush border (black arrowhead). Scale bar: 100 μm. **B.** Immunohistochemical staining of control (CTL) and RPGR-3 PSC-derived retinal organoid cryosections at 22 weeks, showing RECOVERIN+ (red) photoreceptors in the outer nuclear-like layer (ONL). DAPI in blue. Scale bar: 15 μm. **C**. Quantitative RT-PCR analysis of the RPGR isoforms extracted from the control and RPGR-3 was performed. The results show the $RPGR^{ex1\text{-}19}/RPGR^{ORF15}$ ratio in the RPGR-3 organoids relative to the control (CTL). ONL: outer nuclear layer; INL: inner nuclear layer.

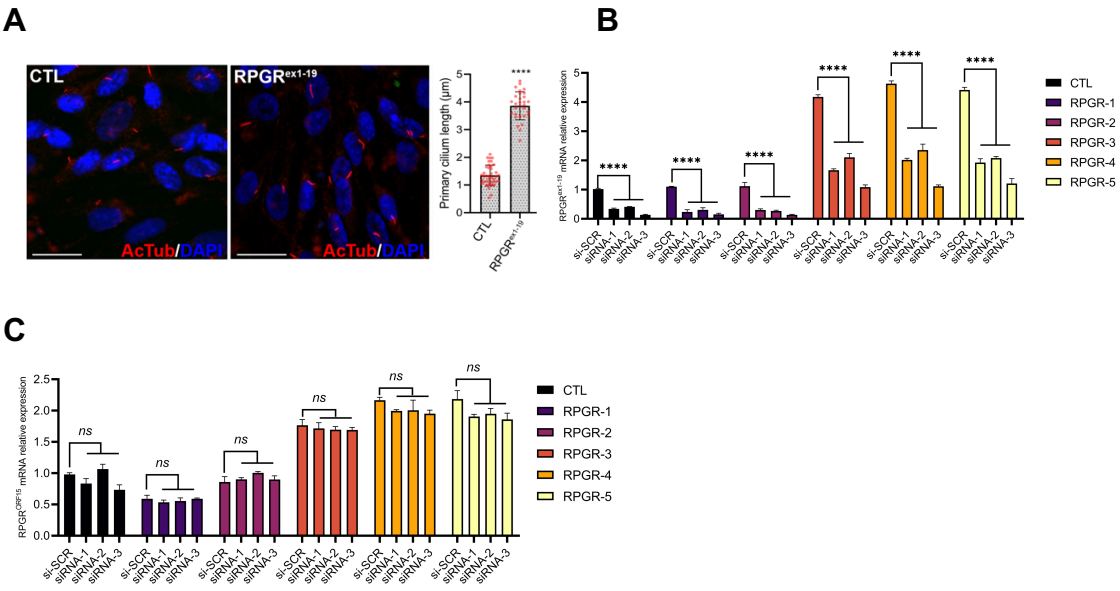
Supplementary Figure 3:

A **B**



Expression of RPGR^{ORF15} (**A**) and RPGR$^{ex1\text{-}19}$ (**B**) was analyzed by qRT-PCR after transfecting the indicated human fibroblasts with pEGFP empty vector or pEGFP-RPGR^{ORF15}. All cells overexpressed RPGR^{ORF15} to similar levels (4-5 folds higher) when compared to RPGR^{ORF15} levels in the pEGFP-expressing cells. **B**: RPGR^{ORF15}-overexpression did not affect RPGR$^{ex1\text{-}19}$ mRNA levels in fibroblasts.

Supplementary Figure 4



A: hTERT-RPE-1 cells were transiently transfected with cDNA encoding GFP alone or GFP-RPGR^{ex1-19} followed by staining with acetylated α-tubulin (AcTub; red). DAPI (blue) was used to stain the nuclei. The cilia length was quantified and represented as a bar graph. ****: p<0.0001. **B**: Three siRNAs against RPGR^{ex1-19} (siRNA-1, siRNA-2, and siRNA-3) were tested for their ability to knock down RPGR^{ex1-19} expression after transfection into the indicated human fibroblasts. Although all siRNAs exhibited significant down-regulation of the *RPGR^{ex1-19}* isoform when compared to a scrambled siRNA, siRNA-3 was the most potent. ****: p<0.0001. **C**: *RPGR^{ex1-19}* siRNA expression did not alter the expression of RPGR^{ORF15}. ns: not significant.

Supplementary Table 1

Western Blot				
Name	Brand	Cat number	Dilution	
RPGR ex16-18	Abclonal	Supplementary Fig 1A	1:1000	Rabbit I^{ry} Ab
$RPGR^{ORF15}$	Abclonal	Supplementary Fig 1A	1:1000	Rabbit I^{ry} Ab
IRDye 680LT anti mouse	Licor	926-68020	1:5000	Goat II^{ry} Ab
IRDye 800CW anti rabbit	Licor	926-32211	1:5000	Goat II^{ry} Ab
Anti-acetylated α-tubulin	Sigma Aldrich	T6793	1:500	Mouse I^{ry} Ab
Anti-γ-tubulin (GT4511)	Invitrogen	MA5-31482	1:500	Mouse I^{ry} Ab
Anti-polyglutamylated tubulin (GT335)	Adipogen	AG-20B-0020B-C100	1:200	Mouse I^{ry} Ab
Anti-Rhodopsin clone RET-P1	Merk Millipore	MAB5316	1:200	Mouse I^{ry} Ab
Anti-CEP290	Bethyl Laboratories	A301-659A	1:200	Rabbit I^{ry} Ab
Anti-Arl13b	Proteintech	17711-1-AP	1:500	Rabbit I^{ry} Ab
Anti-GFP	Abcam	ab13970	1:500	Chicken I^{ry} Ab
Anti-Mouse IgG (H+L), 488nm	Invitrogen	A32723	1:1000	Goat II^{ry} Ab
Anti-Mouse IgG (H+L), 546nm	Invitrogen	A11030	1:1000	Goat II^{ry} Ab
Anti-Rabbit IgG (H+L), 488nm	Invitrogen	A11008	1:1000	Goat II^{ry} Ab
Anti-Rabbit IgG (H+L), 546nm	Invitrogen	A11010	1:1000	Goat II^{ry} Ab
Anti-Chicken IgG (H+L), 488nm	Invitrogen	A11039	1:1000	Goat II^{ry} Ab

Supplementary Table 2:

RT-qPCR primers	
Name	**Sequence**
$RPGR^{ex1-19}$ E17-18 Fwd	GAACGGGCCATTTGTGAGTA
$RPGR^{ex1-19}$ E19 Rev	GGTTCTGGTCGGCATCTTTAT
$RPGR^{ORF15}$ E15 Fwd	GGAAGGAGCAGAGGATTCAAA
$RPGR^{ORF15}$ ORF15 Rev	CCTCATCTTGCCAGTGTTCT
β-actin Fwd	GACCTCTATGCCAACACAGT
β-actin Rev	AGTACTTGCGCTCAGGAGGA
RPLP0 Fwd	GCATCAGTACCCCATTCTATCAT
RPLP0 Rev	AGGTGTAATCCGTCTCCACAGA
DsiRNAs for knockdown	
Name	**Duplex sequence**
dsi-NC-SCR Fwd	CUUCCUCUCUUUCUCUCCCUUGUGA
dsi-NC-SCR Rev	AGGAAGGAGAGAAAGAGAGGGAACACU
dsi-$RPGR^{ex1-19}$.1 Fwd	CACCAAGCAAAGACAUGAAAAAAAC
dsi-$RPGR^{ex1-19}$.1 Rev	UUGUGGUUCGUUUCUGUACUUUUUUUG
dsi-$RPGR^{ex1-19}$.2 Fwd	GGAGCAGAAAGAACCAAUGAUGATA
dsi-$RPGR^{ex1-19}$.2 Rev	UUCCUCGUCUUUCUUGGUUACUACUAU
dsi-$RPGR^{ex1-19}$.3 Fwd	AUCAAAAGAUUGUCAAGAAUAACAA
dsi-$RPGR^{ex1-19}$.3 Rev	UUUAGUUUUCUAACAGUUCUUAUUGUU