Functional Disruption of a Disease Modifier Gene Using Antisense Oligomers: A Potential Molecular Therapy for *PRPF31*-associated Retinitis Pigmentosa 11

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Introduction

Pre-messenger RNA (Pre-mRNA) processing factor 31 (PRPF31) is essential for the pre-mRNA splicing process, a critical step in eukaryotic gene expression. Heterozygous mutations in *PRPF31* can cause retinitis pigmentosa 11 (RP11). Functional defects in PRPF31 lead to degeneration of photoreceptor and retinal pigment epithelial cells, which subsequently causes loss of peripheral vision and potential for complete blindness.

RP11 features incomplete penetrance within affected families, due to variable expression levels of functional PRPF31 from the remaining healthy allele. CCR4-NOT transcription complex subunit 3 (CNOT3) is a negative transcriptional regulator of *PRPF31*, and is found at higher levels in RP11 patients, compared to asymptomatic family members carrying the same *PRPF31* mutations.

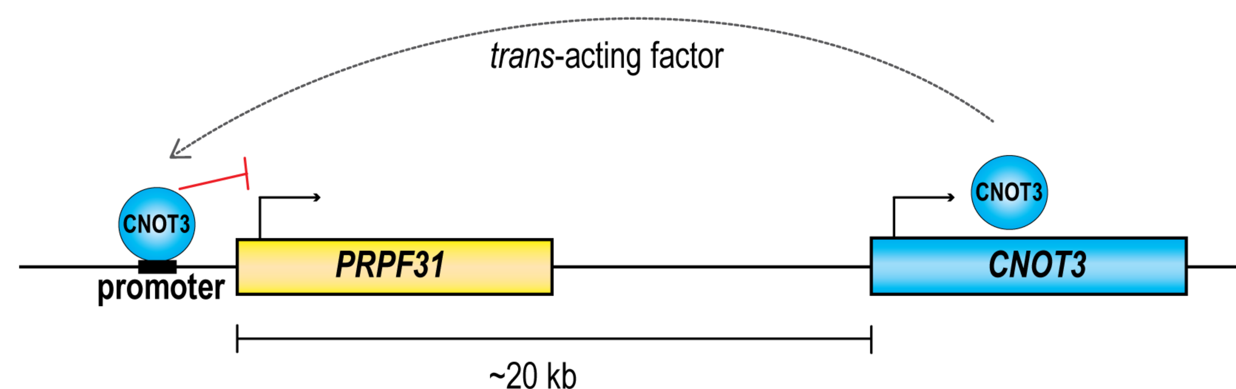

Figure 1. CNOT3 is a transcriptional regulator of PRPF31. *CNOT3* is located approximately 20 kb downstream of the *PRPF31* and acts as a *trans-acting* factor to inhibit *PRPF31* transcription.

Study Design

ASOs are designed to target selected CNOT3 exon(s) for exclusion during pre-mRNA processing in order to (i) induce a translational frameshift and downregulate CNOT3 expression and (ii) remove in-frame exon(s) encoding essential functional domains resulting in production of truncated CNOT3 isoforms. PRPF31 expression is determined as a consequence of CNOT3 modulation at mRNA and protein levels in RP11 cells. Primary cilia that are crucial for complete retinal pigment epithelium maturation will be assessed to evaluate an improved PRPF31 function.

Results

1. CNOT3 and PRPF31 expression in RP11, non-penetrant carrier and wildtype iPSC-derived retinal pigment epithelium (RPE)

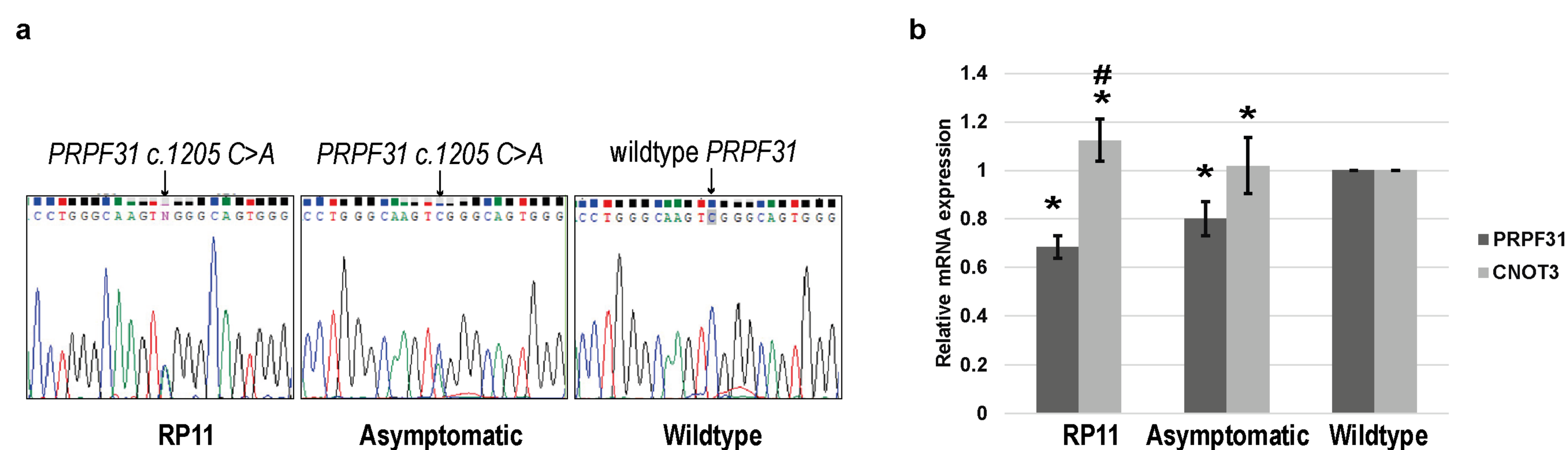

Figure 2. *CNOT3* inveresely correlated to *PRPF31* expression. **(a)** Sanger sequencing analysis of PRPF31 mutation in an RP11 patient and non-penetrant individual within the same pedigree and compared to wildtype sequence from an unaffected subject. **(b)** RT-qPCR analysis of *PRPF31* and *CNOT3* mRNA expression normalised with TATA-binding protein (TBP) expression in iPSCs-derived retinal pigment epithelium from RP11, asymptomatic and wild type (WT) individuals. Bar chart represent mean±standard error of the mean (SEM) from three independent RT-qPCR. Expression of *CNOT3* and *PRPF31* transcripts in wild type was set to 1. Student's *t* test. **p*<0.05 compared with wildtype. #*p*<0.05 compared with asymptomatic subject.

2. Schematic strategies of ASO-mediated CNOT3 exon skipping

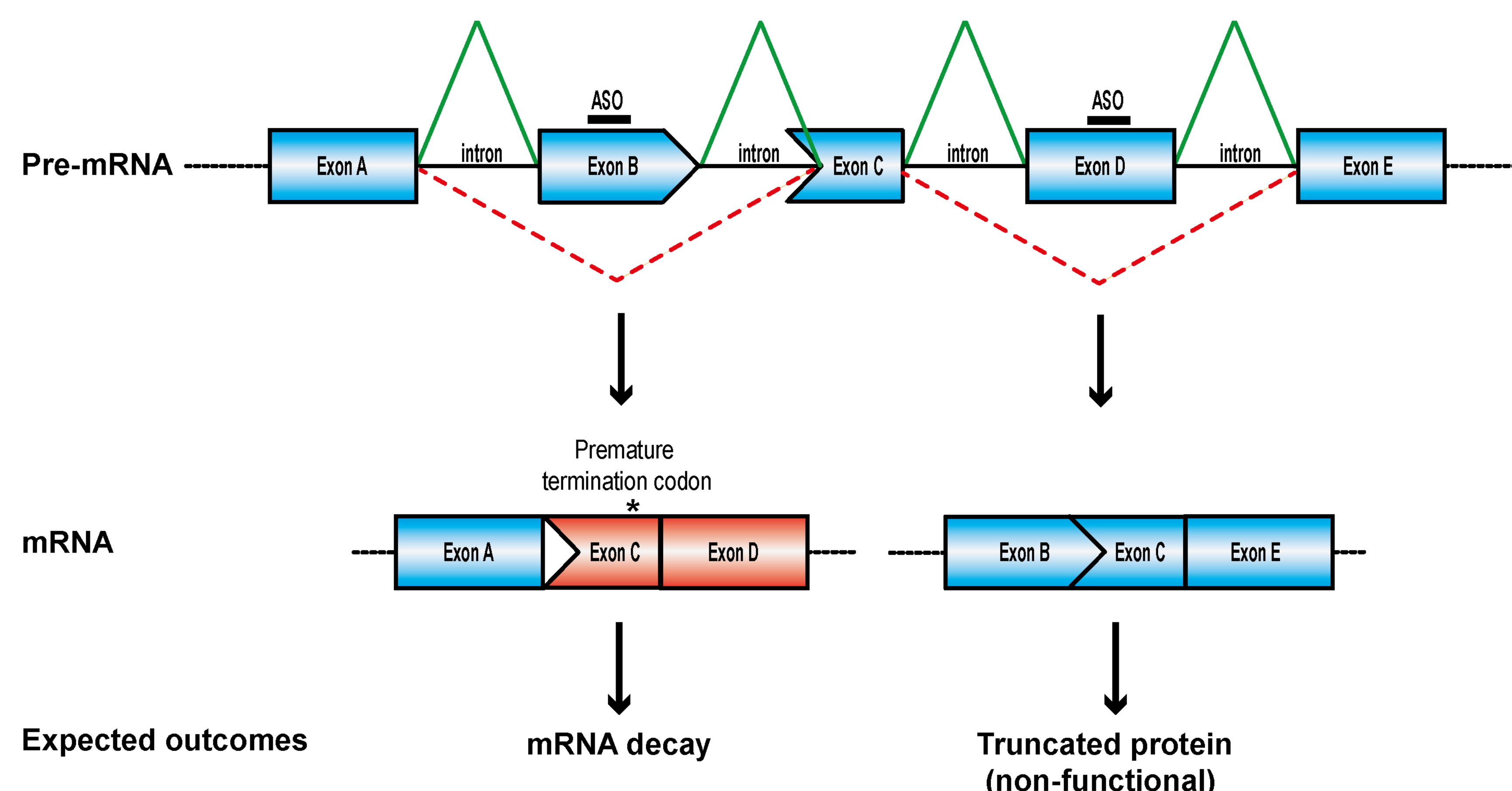

Figure 3. Antisense oligomers were designed to target removal CNOT3 exon(s) during pre-mRNA splicing to (i) exclude out-of-frame exon leading to translational frameshift that is predicted to lead to non-sense mediated mRNA decay or (ii) exclude an in-frame exon leading to production of a non/low functional truncated CNOT3 isoform.

Results (cont')

3. Screening of antisense oligomers to mediate CNOT3 exon skipping in human dermal fibroblast.

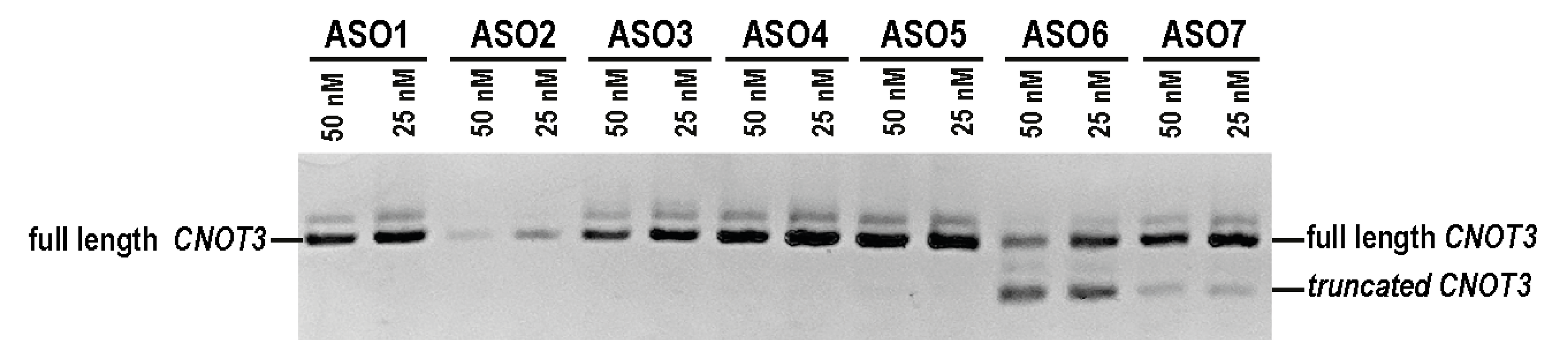

Figure 4. Human dermal fibroblasts were transfected with 2′-O-methyl-modified phosphorothioate antisense oligomers at concentrations of 50 and 25 nM and incubated for 48 h. RT-PCR was used to assess CNOT3 exon skipping.

4. Antisense oligomer-mediated CNOT3 exon skipping upregulates PRPF31 expression in RP11 iPSC-derived RPE .

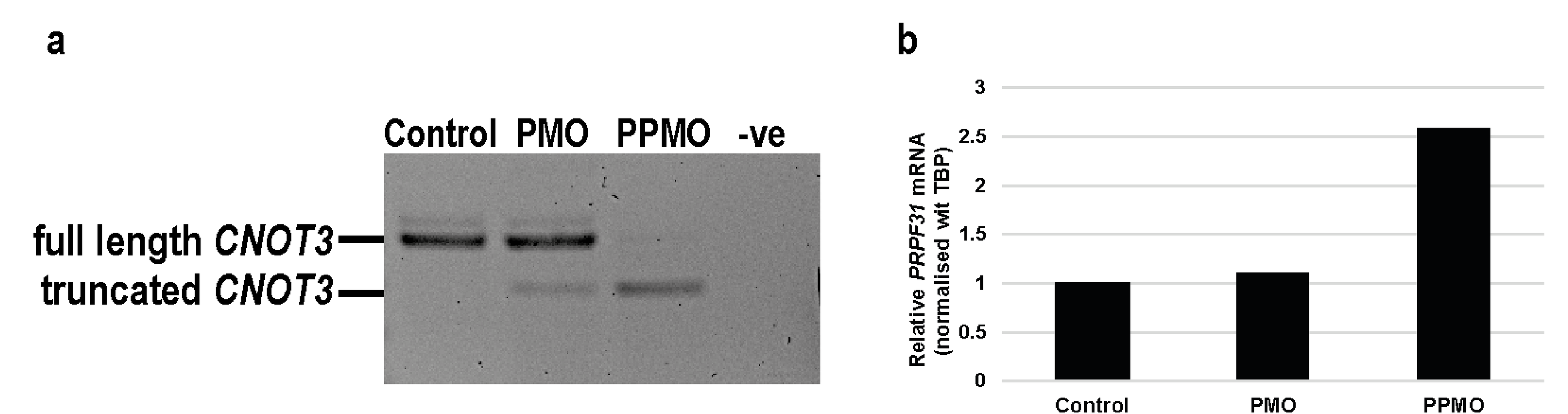

Figure 5. ASO6 was synthesized as phosphorodiamidate morpholino oligomer (PMO) and transfected into RP11 iPSC-derived RPE. **(a)** The percentage of CNOT3 exon skipping treated with PMO alone or peptide-tagged PMO (PPMO) at a concenration of 5 μM. **(b)** *PRPF31* upregulation as a consequence of CNOT3 knockdown, determined using qRT-PCR and normalised with TATA-binding protein (TBP) expression. PRPF31 expression of untreated control was set to 1.

5. Upregulation of PRPF31 rescues primary cilia length and number in patient iPSC-derived RPE.

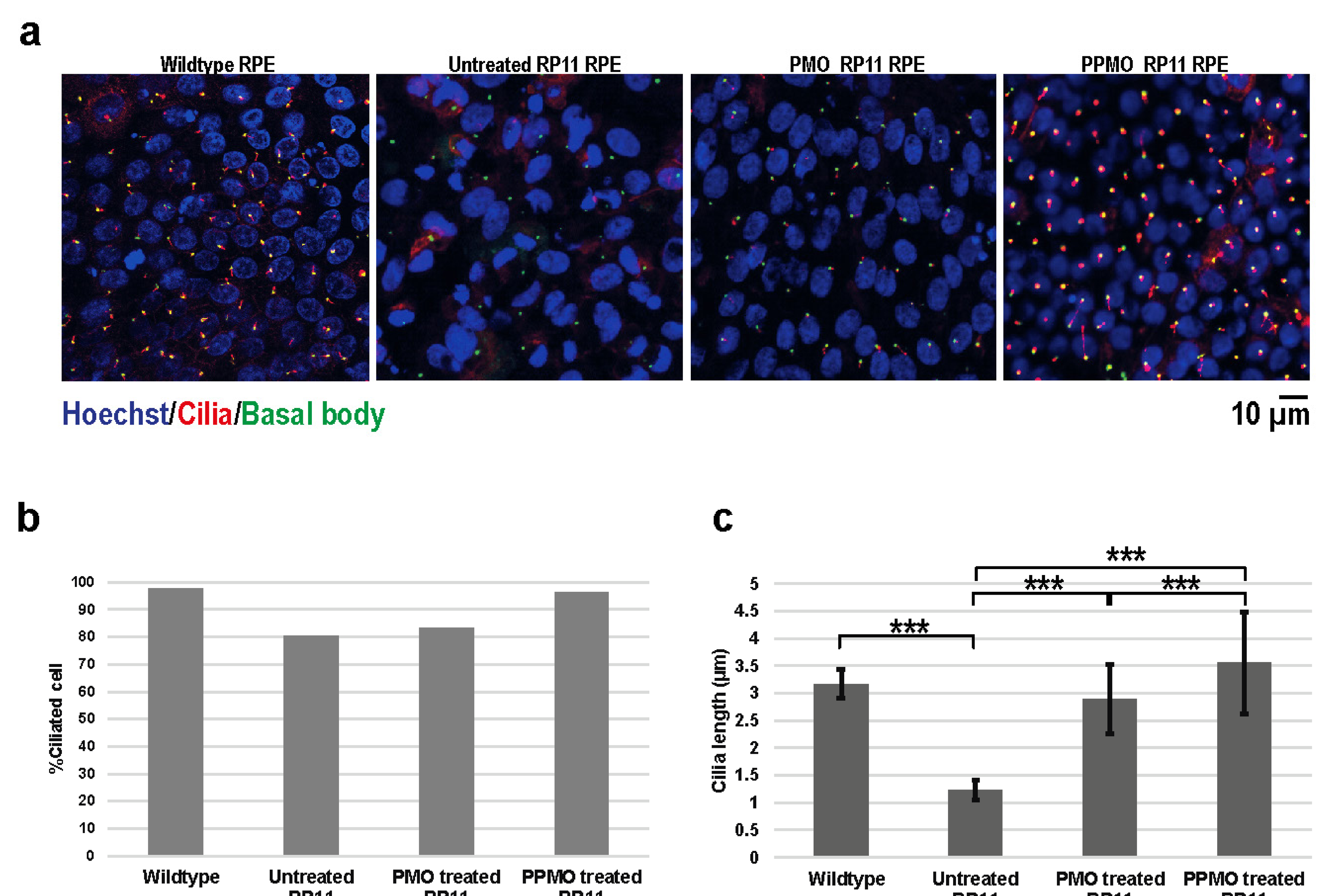

Figure 6. (a) Immunostaining of cilia (red) and basal body (green) in wildtype and RP11 RPE with or without antisense oligomer treatment. **(b)** The percentage of RPE cell expressing cilia, counted from >1,000 cells. **(c)** Cilia length, measurement using NIS-Elements Imaging software. Bar chart represents mean±SEM from ~ 300 ciliated cells. Scale bar = 10 μm. Student's *t* test. ****p*<0.001.

Conclusions

This study shows antisense oligomers can lower CNOT3 function and increase PRPF31 transcription to levels expected to provide therapeutic benefit. Transcriptome analysis will be used to evaluate pre-mRNA splicing after increased PRPF31 expression.

Acknowledgements