Supplementary material

The major ciliary isoforms of RPGR build different interaction complexes with INPP5E and RPRGIP1L

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		PAM Score	Gene Loo	cus
A T G T A T C A	A C A <mark>G</mark> A C <mark>T</mark> A G A	GAGTGG 100.0	RPGR ChrX:-3	8288222
 A -	• • • <mark>c</mark> • • • • • •	•••GA•1.7	- Chr10:+	107931947
• A • • C • • •	· <mark>A</mark> - -	• • • A • • 1.7	- Chr4:+6	5425111
• • • <mark>6 T</mark> • • •	· • • <mark>A</mark> • • • • • •	• • • G A • 1.5	- Chr14:+	72332247 potential o
• • • <mark>• 6</mark> • • <mark>1</mark>	• • <mark>•</mark> • • • • • •	• • • A • • 1.5	- Chr3:+1	.04917303 target site
• • • <mark>6 C</mark> • • •	· · · · <mark>G</mark> · · · · ·	• • • A A • 1.4	RBM14 Chr11:+	66644278
•• <mark>T</mark> G•• <mark>G</mark> (<mark>,</mark>	• • • G • • 1.0	- Chr4:+9	400518
Locus	Forward Primer 5'-3'	Reverse Primer 5'-3'	Comment	Size (bp)
Locus Chr10:+107931947	Forward Primer 5'-3' ttctggtgtgtgtgtggca	Reverse Primer 5'-3'	Comment 65°C annealing	Size (bp) 357
Locus Chr10:+107931947 Chr4:+65425111	Forward Primer 5'-3' ttctggtgtgtgtgtgtgggca tcacgcatccctatagccaac	Reverse Primer 5'-3' tgtgaggcagggagtcatta gcatagcagtgagaagtggg	Comment 65°C annealing 65°C annealing	Size (bp) 357 393
Locus Chr10:+107931947 Chr4:+65425111 Chr14:+72332247	Forward Primer 5'-3' ttctggtgtgtgtgtggca tcacgcatccctatagccaac gctagacactgttgagccca	Reverse Primer 5'-3' tgtgaggcagggagtcatta gcatagcagtgagaagtggg tccatctcactgctttccct	Comment 65°C annealing 65°C annealing 65°C annealing	Size (bp) 357 393 364
Locus Chr10:+107931947 Chr4:+65425111 Chr14:+72332247 Chr3:+104917303	Forward Primer 5'-3' ttctggtgtgtgtgtgtggca tcacgcatccctatagccaac gctagacactgttgagccca ccaacaacagagccataaccc	Reverse Primer 5'-3' tgtgaggcagggagtcatta gcatagcagtgagaagtggg tccatctcactgctttccct agtgagagaagaagaatggcct	Comment 65°C annealing 65°C annealing 65°C annealing 60°C annealing	Size (bp) 357 393 364 340
Locus Chr10:+107931947 Chr4:+65425111 Chr14:+72332247 Chr3:+104917303 Chr11:+66644278	Forward Primer 5'-3' ttctggtgtgtgtgtggca tcacgcatccctatagccaac gctagacactgttgagccca cccaacaacagagccataaccc gcgcggaattctctgtacga	Reverse Primer 5'-3' tgtgaggcagggagtcatta gcatagcagtgagaagtggg tccatctcactgctttccct agtgagagaagaagaatggcct acccaagattcccagcaca	Comment 65°C annealing 65°C annealing 65°C annealing 60°C annealing 66°C annealing	Size (bp) 357 393 364 340 366
Locus Chr10:+107931947 Chr4:+65425111 Chr14:+72332247 Chr3:+104917303 Chr11:+66644278 Chr4:+9400518	Forward Primer 5'-3' ttctggtgtgtgtgtggca tcacgcatccctatagccaac gctagacactgttgagccca ccaacaacagagccataaccc gcgcggaattctctgtacga ccagagcatctgaaaccttgtaag	Reverse Primer 5'-3' tgtgaggcagggagtcatta gcatagcagtgagaagtgggg tccatctcactgctttccct agtgagagaagaaggaatggcct acccaagattcccagcaca ctaggaatattaccaggaagggg	Comment 65°C annealing 65°C annealing 65°C annealing 60°C annealing 66°C annealing	Size (bp) 357 393 364 340 366 434

			Gene	LOCUS	
C A G T A C A	T T T <mark>G G</mark> T T <mark>A G</mark>	T T <mark>A G</mark> G G G ^{100.0}	RPGR	ChrX:-38284000) -On-target s
A - C		• • • C T A • 2.0	-	Chr8:-12645163	8
A • • • <mark>G</mark> • •	A C	••••AA• 0.9	-	Chr6:+80072108	в
тт А	• <mark>c</mark> • • • • • • •	••••T•• 0.9	-	Chr15:-6918014	18
• • • • • <mark>G</mark> •	A A • • • • • • • •	• • • • A • 0.9		ChrX:-12647599	potential o
т - <mark>А</mark> <mark>Т</mark> -	A	••••A•• 0.9	-	ChrX:-14301061	l6 target site
a • • <mark>a</mark> • a •	• • <mark>6</mark> • • • • • •	• • • • C A • 0.9	-	Chr4:+48616944	4
<mark>G • • <mark>C</mark> • • •</mark>	• <mark>A</mark> • • <mark>A</mark> • • • •	• • • • A • • 0.5	SERPINB8	Chr18:-6398798	19
-			PTPN18	Chr2:-13037402	
••••	· <mark>·</mark> · · · · · <mark>·</mark> ·	•••• • A • 0.3		cm215057402	."
	Forward Primer	Reverse Primer	Comm	ent Size (
Locus	Forward Primer 5'-3'	Reverse Primer 5'-3'	Comm	ent Size (I	bp)
Locus Chr8:-126451638	Forward Primer 5'-3'	Reverse Primer 5'-3' acagacagctaggcctctct	Comm	eent Size (I	t (dd (dd
Locus Chr8:-126451638 Chr6:+80072108	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgttctatgctggtt	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt	Comm 66°C ann 65°C ann	ealing 304	bp)
Locus Chr8:-126451638 Chr6:+80072108 Chr15:-69180148	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgtctatgctggtt ggaggctgaggcacaagaattgc	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt gaggtacttacagagcctagagc	Comm 66°C ann 65°C ann 68°C ann	ealing 304 ealing 381 ealing 449	bp) 1 1
Locus Chr8:-126451638 Chr6:+80072108 Chr15:-69180148 ChrX:-12647599	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgtctatgctggtt ggaggctgaggcacaagaattgc acaccagccagagactagaga	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt gaggtacttacagagcctagagc gagaagccaggagaatgcca	Comm 66°C ann 65°C ann 68°C ann 68°C ann	ealing 304 ealing 381 ealing 449 ealing 534	bp) 1 1 1 1 1
Locus Chr8:-126451638 Chr6:+80072108 Chr15:-69180148 ChrX:-12647599 ChrX:-143010616	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgtctatgctggtt ggagggctgaggcacaagaattgc acaccagccagagactagaga agcaacagcaagaagagggtc	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt gaggtacttacaggagcatggcca gagaagccaggagaatgcca gagtcatcaaggcaagctgg	Comm 66°C anno 65°C anno 68°C anno 67°C anno 65°C anno	ealing 304 ealing 381 ealing 449 ealing 534 ealing 363	bp) 4 1 2 3
Locus Chr8:-126451638 Chr6:+80072108 Chr15:-69180148 ChrX:-12647599 ChrX:-143010616 Chr4:+48616944	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgtctatgctggtt ggaggctgaggcacaagaattgc acaccagccagagactagaga agcaacagcaagagagggtc tcctctgtagactgtgtgcct	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt gaggtacttacagagcctagagc gagaagccaggagaatgcca gagtcatcaaggcaagctgg aatacagtggccttgatagctaa	Comm 66°C anno 65°C anno 68°C anno 67°C anno 65°C anno 65°C anno	ealing 304 ealing 381 ealing 449 ealing 534 ealing 363 ealing 475	bp) 1 1 2 3 5
Locus Chr8:-126451638 Chr6:+80072108 Chr15:-69180148 ChrX:-12647599 ChrX:-143010616 Chr4:+48616944 Chr4:+63987989	Forward Primer 5'-3' aagcaggaacagggttgaca tgctgctgtctatgctggtt ggagggtgaggcacaagaattgc acaccagccagagactagaga agcaacagcaagaagagggtc tcctctgtagactgtgtgcct tccataagcctgagatacaagtt	Reverse Primer 5'-3' acagacagctaggcctctct tgggtaagcttgtgattcagt gaggtacttacagagcctagagc gagaagccaggagaatgcca gagtcatcaaggcaagctgg aatacagtggccttgatagctaa tgctgtgtgttggaattgtgg	Comm 66°C anno 65°C anno 68°C anno 65°C anno 65°C anno 59°C anno	ealing 304 ealing 381 ealing 449 ealing 534 ealing 363 ealing 475 ealing 334	bp) 1 1 1 2 3 5 1

Supplementary Figure S1: Scores of potential off-target sites were calculated using the Benchling software (https://www.benchling.com/) based on the algorithms developed by Doench *et al.* and Hsu *et al.* (Doench et al., 2014; Hsu et al., 2013). The off-target score is between 0 to 100 and represents the probability of the Cas9 binding to induce double strand brakes. We considered potential off-target sites for sgRNA1 (RPGR-Intron13) and sgRNA2 (RPGR-Intron15) with up to 4 bp mismatches.

Mismatches are indicated in the coloured boxes (green, blue, red, yellow). The PAM sequence is highlighted in grey. The genomic locus of potential off-targets in the human genome is listed (Reference sequence: GRCh38 (hg38, Homo sapiens). In the table, primer combinations for all potential off-target sites are listed. Sequence alternation at the potential off-target sites were not detected.

References

- Doench, J. G., Hartenian, E., Graham, D. B., Tothova, Z., Hegde, M., Smith, I., . . . Root, D. E. (2014). Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. *Nat Biotechnol, 32*(12), 1262-1267. doi:10.1038/nbt.3026
- Hsu, P. D., Scott, D. A., Weinstein, J. A., Ran, F. A., Konermann, S., Agarwala, V., . . . Zhang, F. (2013). DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol*, *31*(9), 827-832. doi:10.1038/nbt.2647





Supplementary Figure S2: Densitometric measurement of RT-PCR product intensities of *RPGR*^{skip14/15} and *RPGR*^{ex1-19} isoforms from different tissues and HEK293T cells. For RT-PCR results, please refer to figure 1. The upper panel shows the comparison of band intensities (arbitrary units) found in RT-PCR analyses of *RPGR*^{skip14/15} and *RPGR*^{ex1-19} in different human tissues. The lower panel show the comparison between *RPGR*^{skip14/15} and *RPGR*^{ex1-19} band intensities for HEK293T cells, where we used two different cDNA concentrations per reaction (10 ng and 50 ng).



Sequencing results analyzing RPGR^{ex1-19}RT-PCR products



Supplementary Figure S3: Sanger sequencing results of RT-PCR products of the *RPGR*^{ex1-19} isoform from different human tissues and HEK293T cells. For RT-PCR results, please refer to figure 1. The sequence profiles (electropherograms) confirmed the identity of the *RPGR*^{ex1-19} splice product detected in different human tissues and HEK293T cells.



reverse sequencing primer

Sequencing results analyzing RPGR^{skip14/15} RT-PCR products



Supplementary Figure S4: Sanger sequencing results of RT-PCR products of the *RPGR*^{skip14/15} isoform from different human tissues and HEK293T cells. For RT-PCR results, please refer to Figure 1. The sequence profiles (electropherograms) confirmed the identity of the *RPGR*^{skip14/15} splice product detected in different human tissues and HEK293T cells.



D-tubulin, axoneme

RPGR

Supplementary Figure S5: Higher magnification micrographs of immunocytochemical signals detecting RPGR and the axoneme (d-tubulin) in unaltered HEK293T cells, in clone A, and in clone B. Similar results were detected between the different cell lines suggesting that the ciliary localization of RPGR was not disturbed by the CRISPR/eSpCas9-induced genomic alterations of clones A and B.