

The Predicted Splicing Variant c.11+5G>A in *RPE65* Leads to a reduction in mRNA Expression in a Cell-Specific Manner

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Table S1. List of primers employed in this work.

Name	Sequence with <u>tag/tails</u> (5'-3')	Sequence without tag/tails (5'-3')	Tm(°C)	PCR product (bp)
<u>Splicing vectors</u>				
<i>RPE65_5'UTR_Fwd</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCTTC</u> CAGAGAATGGTGCCAAGGTC	CAGAGAATGGTGCCAAGGTC	65.1	3,832
<i>RPE65_intron3_Rev</i>	<u>GGGGACCACTTTGTACAAGAAAGCTGGGTG</u> TTGCCAAGGTTACCTCCTA	TTGCCAAGGTTACCTCCTA	63.7	
<i>SDMut_RPE65_c.11+5_Fwd</i>	n/a	CTGGAAGAAAATGTCTATCCAGTAAGTATCTCTGGGAGAC	73.8	3,832
<i>SDMut_RPE65_c.11+5_Rev</i>	n/a	CTGGAAGAAAATGTCTATCCAGTAAGTATCTCTGGGAGAC	73.8	
<u>Luciferase-reporter assay constructs</u>				
<i>RPE65_XS_Intron 1.1_Fwd</i>	<u>ACTGGAAGAAAATGTCTATCCAGTAACCTTGGCAACA</u>	ACTGGAAGAAAATGTCTATCCA	58.0	5,508
<i>RPE65_XS_5'UTR_Rev</i>	CCTGGGTGTTGCCAAGGTTAC <u>ATTATCATGAATCC</u>	CCTGGGTGTTGCCAAGGTTAC	60.0	
<i>RPE65_S_Intron 1_Fwd</i>	CACCTTCATGGATTCATGATAAAT <u>GTAACCTTGGCAACA</u>	CACCTTCATGGATTCATGATAAAT	58.0	5,561
<i>RPE65_S_5'UTR_Rev</i>	CCTGGGTGTTGCCAAGGTTAC <u>ATTATCATGAATCC</u>	CCTGGGTGTTGCCAAGGTTAC	60.0	
<i>RPE65_M_Intron 2.1_Fwd</i>	CGCTACCTAATTGGAATTCCTCC <u>GTAACCTTGGCAACA</u>	CGCTACCTAATTGGAATTCCTC	60.0	6,992
<i>RPE65_M_5'UTR_Rev</i>	CCTGGGTGTTGCCAAGGTTAC <u>ATTATCATGAATCC</u>	CCTGGGTGTTGCCAAGGTTAC	60.0	
<i>RPE65_L_Intron 2.2_Fwd</i>	ACTGTGAAAGCTGAGAGGCC <u>GTAACCTTGGCAACA</u>	ACTGTGAAAGCTGAGAGGCC	60.0	8,056
<i>RPE65_L_5'UTR_Rev</i>	CCTGGGTGTTGCCAAGGTTAC <u>ATTATCATGAATCC</u>	CCTGGGTGTTGCCAAGGTTAC	60.0	
<u>RT-PCR</u>				
<i>RPE65_exon1_Fwd</i>	n/a	CTTCATTCTGCAGTTGGTGCC	62.0	164
<i>RPE65_exon3_Rev</i>	n/a	CCACATCGAAGGAGACTGCC	58.0	
<i>ACTB_exon3_Fwd</i>	n/a	ACTGGGACGACATGGAGAAG	60.5	398
<i>ACTB_exon3_Rev</i>	n/a	TCTCAGCTGTGGTGGTGAAG	60.5	
<u>qPCR</u>				
<i>OCT3/4_Fwd</i>	n/a	GTTCTTCATTCACCTAAGGAAGG	58.4	101
<i>OCT3/4_Rev</i>	n/a	CAAGAGCATCATTGAACCTCAC	58.4	

<i>PAX6_Fwd</i>	n/a	GCTGCAAAGAAATAGAACATCC	58.4	111
<i>PAX6_Rv</i>	n/a	TTGGCTGCTAGTCTTTCTCG	58.4	
<i>CRX_Fwd</i>	n/a	CCCCAGTGTGGATCTGATG	59.5	116
<i>CRX_Rv</i>	n/a	CAAAACAGTGCTCCAGCTC	59.5	
<i>OPN1SW_Fwd</i>	n/a	TTCTTCTCCAAGAGTGCTTGC	59.5	97
<i>OPN1SW_Rv</i>	n/a	CCTTCCCACACACCATCTTC	60.5	
<i>BEST1_Fwd</i>	n/a	TCAGTGTGGACACCTGTATGC	61.2	84
<i>BEST1_Rv</i>	n/a	AAGCTGTACACCGCCACAG	59.5	
<i>RPE65_Fwd</i>	n/a	TTACTACGCTTGACAGAGACC	62.1	105
<i>RPE65_Rv</i>	n/a	GCCCCATTGACAGAGACATAG	61.2	
<i>MERTK_Fwd</i>	n/a	TTGCAGCATTGAGTCAAGGAAGC	65.2	106
<i>MERTK_Rv</i>	n/a	GGCTTGACGCTGCTTGATTGGTA	65.2	
<i>DCT_Fwd</i>	n/a	CTTTGCCGGCTATAATTGTG	56.4	101
<i>DCT_Rv</i>	n/a	TCAAGGAATGGATGTTCTGC	56.4	
<i>ABCA4_Fwd</i>	n/a	CATCCTGTTCCACCACCTCA	60.5	113
<i>ABCA4_Rv</i>	n/a	CTGTGTCCTCCAACATGGCT	60.5	
<i>GUSB_Fwd</i>	n/a	AGAGTGGTGCTGAGGATTGG	60.5	80
<i>GUSB_Rv</i>	n/a	CCCTCATGCTCTAGCGTGTC	62.5	

Underlined sequences corresponded to tail/tag sequences. SDMut means side-directed mutagenesis. n/a means non-applicable for that primer.

Table S2. List and combinations of antibodies used for immunocytochemistry.

Gene	Antibody Catalog #	Manufacturer	Properties	Dilution
Primary antibodies				
BEST1	NB300-164	Novus Biologicals (CO, USA)	Monoclonal, Mouse	1:200
EZRIN	E8897	Sigma-Aldrich	Monoclonal, Mouse	1:250
ZO-1	40-2300	Invitrogen	Polyclonal, Rabbit	1:500
RPE65	MAB5428	Sigma-Aldrich	Monoclonal, Mouse	1:500
Secondary antibodies				
Goat anti-rabbit 488	A11008	Molecular Probes (ORE, USA)	Polyclonal	1:500
Goat anti-mouse 568	A11031	Life Technologies	Polyclonal	1:500
Others				
DAPI	0100-20	Southern Biotechnologies (AL, USA)		1:300 (of a 1:100 dilution)
Combinations				
Antibody 1	Secondary Antibody 1	Antibody 2	Secondary antibody 2	DAPI
ZO-1	Goat anti-rabbit 488	BEST1	Goat anti-mouse 568	Yes
ZO-1	Goat anti-rabbit 488	EZRIN	Goat anti-mouse 568	Yes
ZO-1	Goat anti-rabbit 488	RPE65	Goat anti-mouse 568	Yes

Table S3. List of antibodies used for Western blot analysis study

Primary antibodies	Target protein	Produced by	Antibody Catalog #	Host	Diluent	WB dilution
	RPE65	Sigma-Aldrich	MAB5428	Mouse (Monoclonal)	WestVisionTM block diluent	1:1000
	Histone H3	Abcam	ab32356	Rabbit (Monoclonal)	WestVisionTM block diluent	1:1000
	MERTK (Y323)	Abcam	ab52968	Rabbit (Monoclonal)	WestVisionTM block diluent	1:1000
Secondary antibodies	Target host	Produced by	Antibody Catalog #	Host / Fluorophore	Diluent	WB dilution
	Rabbit	Molecular Probes	A21076	Goat / Alexa Fluor 680	WestVisionTM block diluent	1:10000
	Mouse	Li-Cor Biosciences	926-32210	Goat / IRDye800	WestVisionTM block diluent	1:20000

Table S4. Summary of the protein analysis of the ~35 kDa and 65 kDa protein band from the IP of RPE65. The last two columns represent the total intensity of the detected peptide sequence from lower intensity (in red) to higher intensity (green), yellow colours indicate intermediate intensity. In bold, RPE65 protein which have an intensity affinity with the 65-kDa protein as expected. Proteins with coverage lower than 5.0, probability or top peptide probability lower than 0.999 and/or highest intensity for both bands lower than 50,000 were discarded during the filtrating process.

Protein ID	Entry Name	Gene	Protein Length	Coverage	Organism	Protein Existence	Protein Probability	Top Peptide Probability	35 Total Intensity	65 Total Intensity
O43852	CALU_HUMAN	CALU	315	10.8	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	93,961.51	0
P00505	AATM_HUMAN	GOT2	430	24.2	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	162,246.56	24,242.09
P02545	LMNA_HUMAN	LMNA	664	23.9	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	105,971.945
P02768	ALBU_HUMAN	ALB	609	11.8	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	2,501,452	5,493,824
P04406	G3P_HUMAN	GAPDH	335	13.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	67,913.35	7,994.7446
P04843	RPN1_HUMAN	RPN1	607	11.5	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	80,513.95
P10809	CH60_HUMAN	HSPD1	573	25	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	113,916.625
P11166	GTR1_HUMAN	SLC2A1	492	12.4	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	345,835	56,481.434
P29401	TKT_HUMAN	TKT	623	27.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	203,020.9
P38646	GRP75_HUMAN	HSPA9	679	25.2	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	279,579.03
P39656	OST48_HUMAN	DDOST	456	8.6	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	57,738.64	0
Q07065	CKAP4_HUMAN	CKAP4	602	30.4	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	133,184.78
Q9NPH2	INO1_HUMAN	ISYNA1	558	7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	67,233.82
A0A075B6S2	KVD29_HUMAN	IGKV2D-29	120	16.7	Homo sapiens OX=9606	3:Protein inferred from homology	1	0.999	700,050.06	128,539.875

O75874	IDHC_HUMAN	IDH1	414	10.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	104,416.69	0
O94905	ERLN2_HUMAN	ERLIN2	339	14.2	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	174,234.78	0
P00558	PGK1_HUMAN	PGK1	417	30.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	164,836.4	0
P01859	IGHG2_HUMAN	IGHG2	326	16.3	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	67,411.27	29,636.666
P01857	IGHG1_HUMAN	IGHG1	330	13.3	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	86,852.52	79,244.375
P02790	HEMO_HUMAN	HPX	462	15.4	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	193,785.7
P04075	ALDOA_HUMAN	ALDOA	364	38.5	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	95,4295.2	0
P09972	ALDOC_HUMAN	ALDOC	364	8.2	Homo sapiens OX=9606	1:Experimental evidence at protein level	0.9995	0.999	117,448.11	0
P04264	K2C1_HUMAN	KRT1	644	55	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	2,632,790.8	2,522,892.5
P35908	K22E_HUMAN	KRT2	639	51.2	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,435,906	1,496,954
P13647	K2C5_HUMAN	KRT5	590	28.3	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	986,652.44	560,298.8
P02538	K2C6A_HUMAN	KRT6A	564	30.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,063,048	846,167.06
P04259	K2C6B_HUMAN	KRT6B	564	26.8	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,531,210.5	1,464,059.1
P11142	HSP7C_HUMAN	HSPA8	646	39.9	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	536,350.4
P0DMV8	HS71A_HUMAN	HSPA1A	641	21.8	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	406,626.2
P13645	K1C10_HUMAN	KRT10	584	48.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,595,172.6	1,333,126.6
P35527	K1C9_HUMAN	KRT9	623	43.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,872,941.2	1,926,071.8
P08727	K1C19_HUMAN	KRT19	400	61	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	984,447.1	415,205.28
P02533	K1C14_HUMAN	KRT14	472	44.5	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,072,700	816,618.3
Q04695	K1C17_HUMAN	KRT17	432	24.8	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	984,447.1	415,205.28

P08779	K1C16_HUMAN	KRT16	473	24.3	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	1,072,700	816,618.3
P14618	KPYM_HUMAN	PKM	531	27.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	254,464.88
P16403	H12_HUMAN	H1-2	213	25.4	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	115,079.92	89,869.164
P10412	H14_HUMAN	H1-4	219	24.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	135,708.25	82,180.445
P39023	RL3_HUMAN	RPL3	403	12.9	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	109,001.164	3,278.821
P42025	ACTY_HUMAN	ACTR1B	376	9.6	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	76,152.51	0
P63261	ACTG_HUMAN	ACTG1	375	77.9	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	2.61E+07	177,051.66
P60709	ACTB_HUMAN	ACTB	375	77.9	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	2.61E+07	177,051.66
P68032	ACTC_HUMAN	ACTC1	377	46.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	2.51E+07	174,650.2
P61160	ARP2_HUMAN	ACTR2	394	21.1	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	133,726.66	0
Q15019	SEPT2_HUMAN	SEPTIN2	361	12.5	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	92,480.19	0
Q16518	RPE65_HUMAN	RPE65	533	22.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	4,680.633	904,031.3
Q53GT1	KLH22_HUMAN	KLHL22	634	23.7	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	0	196,319.53
Q9NYL9	TMOD3_HUMAN	TMOD3	352	44.6	Homo sapiens OX=9606	1:Experimental evidence at protein level	1	0.999	499,725.06	6,141.5503

Supplementary figures

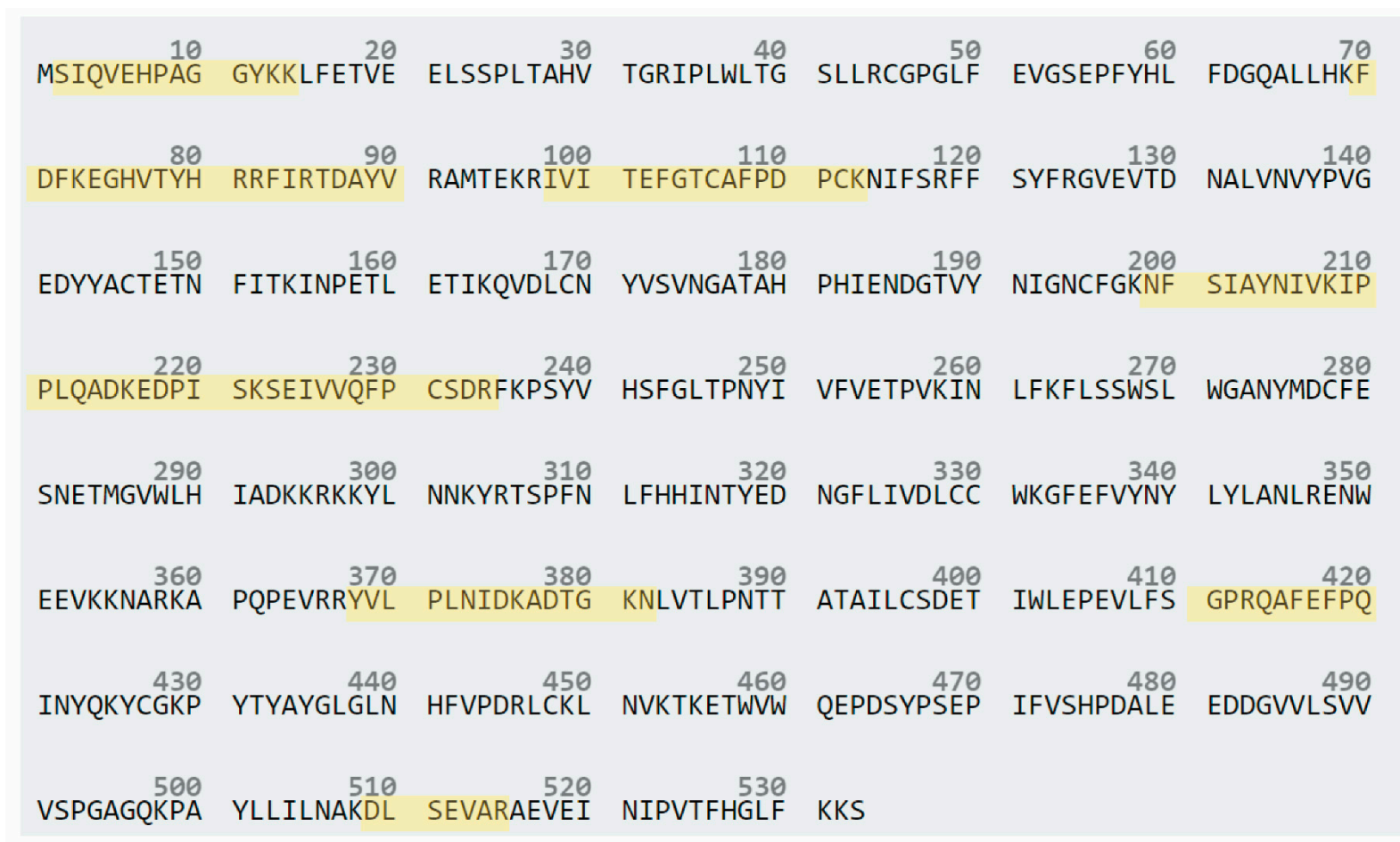


Figure S1. Peptide coverage of RPE65 in for the 65 kDa band. Peptides found by mass spectrometry after in-gel digestion of immunoprecipitating the ~35 kDa and 65 kDa bands. In yellow, coverage of peptide sequences of the 65 kDa band mapped on RPE65 protein sequence obtained from Uniprot (Q16518)

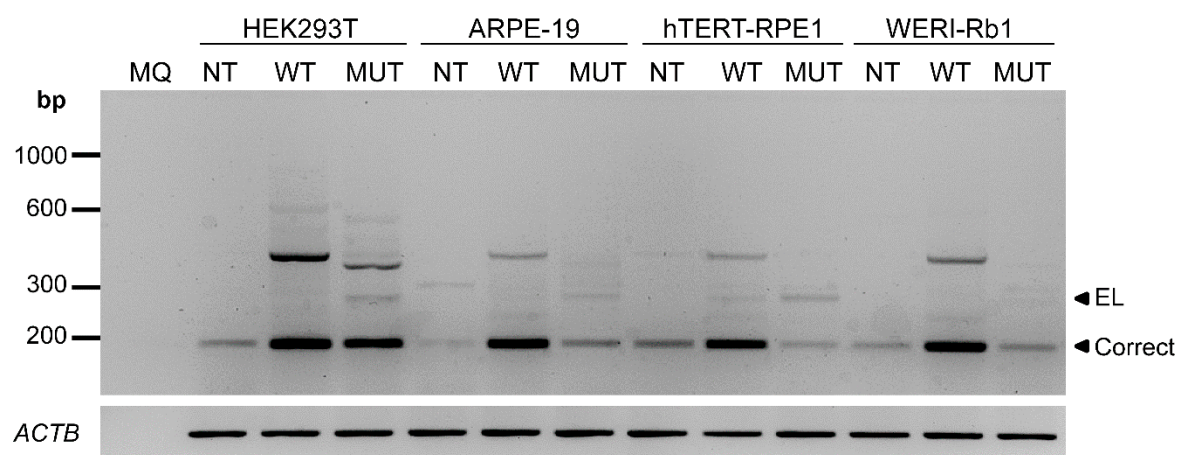


Figure S2. RT-PCR from exon 1 to 3 of the *RPE65* entry clones in different cell lines. A) Splice assays in HEK293T, ARPE-19, hTERT-RPE1 and Weri-Rb1. Representative electrophoresis gel (n=2) of the RT-PCR product after amplifying exon 1 to 3 of *RPE65* comparing wild-type (WT) and mutant (MUT) expression vectors. MQ: miliQ water; NT: non-transfected; EL: exon elongation; Correct: wild-type transcript; bp: base-pairs.