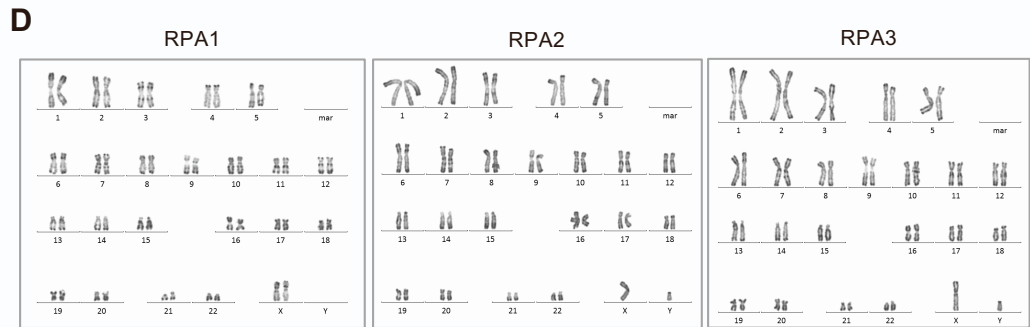
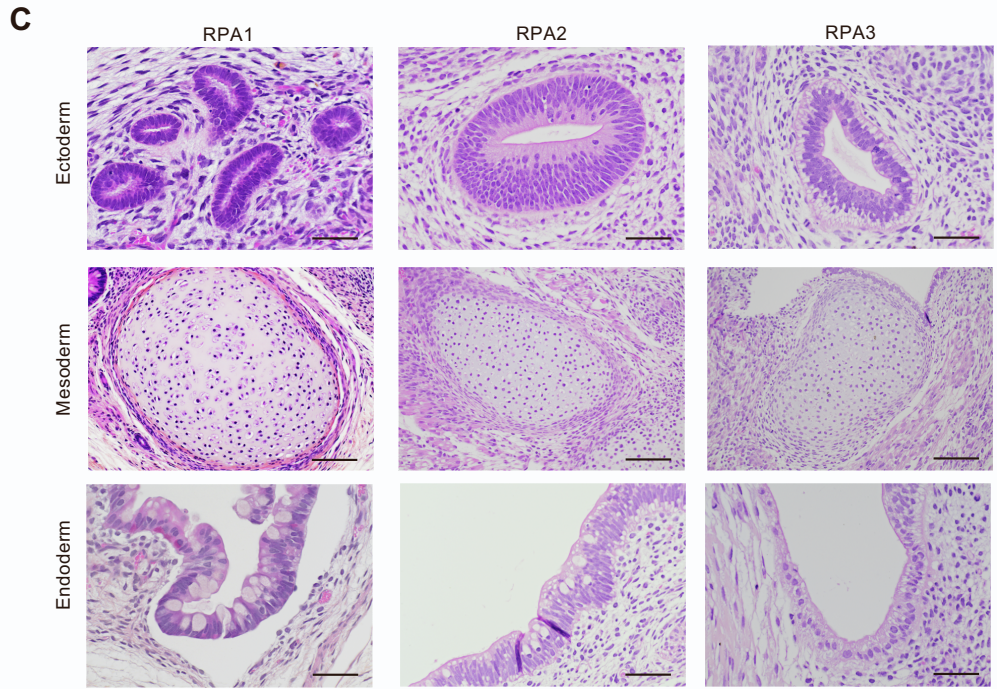
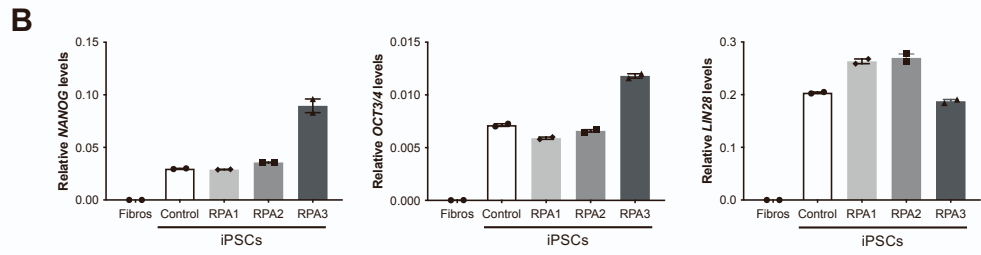
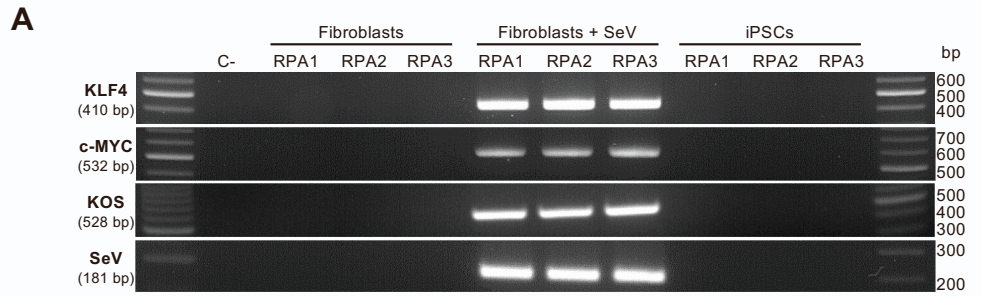


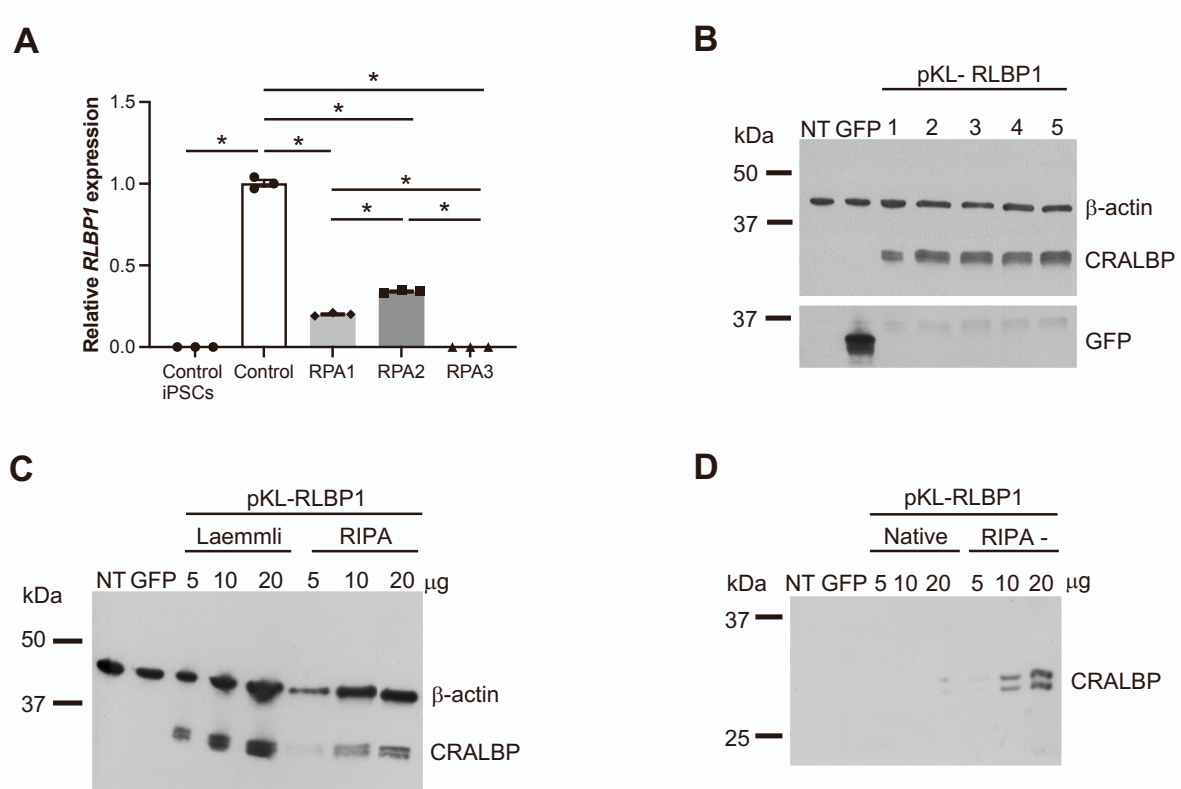
## **Supplemental Information**

### **Dual CRALBP isoforms unveiled: iPSC-derived retinal modeling and AAV2/5-RLBP1 gene transfer raise considerations for effective therapy**

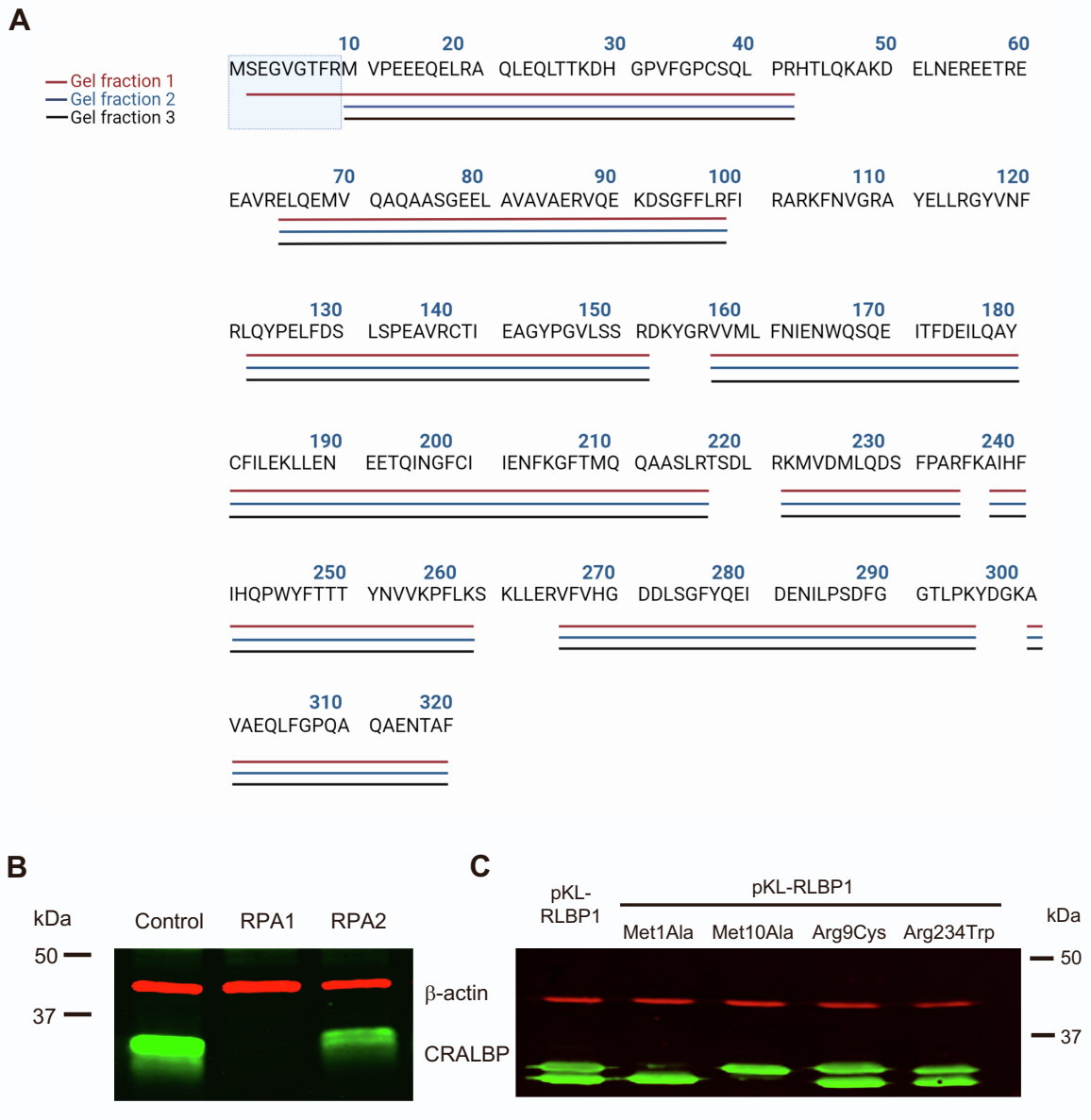
**Krishna Damodar, Gregor Dubois, Laurent Guillou, Daria Mamaeva, Marie Pequignot, Nejla Erkilic, Carla Sanjurjo-Soriano, Hassan Boukhaddaoui, Florence Bernex, Béatrice Bocquet, Jérôme Vialaret, Yvan Arsenijevic, T. Michael Redmond, Christopher Hirtz, Isabelle Meunier, Philippe Brabet, and Vasiliki Kalatzis**



**Figure S1: Quality controls of RPA1, RPA2 and RPA3 iPSCs.** **A)** RT-PCR analysis of the clearance of the Sendai virus (SeV) reprogramming vectors in iPSCs at P12 (RPA2) and P16 (RPA1 and RPA3) using primers specific to the transgene cassettes (KLF4, c-MYC, KOS) or viral backbone (SeV). C-, control without cDNA; Fibroblasts, negative control; SeV-transduced fibroblasts (+ SeV), positive control. **B)** qPCR analysis of the relative expression of host pluripotency genes *NANOG*, *OCT3/4* and *LIN28A* in control iPSCs (positive control) and iPSCs of RPA1, RPA2 and RPA3 patients; Fibroblasts (Fibros), negative control. **C)** Teratoma assay showing the differentiation of RPA1, RPA2 and RPA3 iPSCs into: ectoderm, as determined by the presence of neural tubes (row 1); mesoderm, as determined by the presence of cartilage (row 2); endoderm, as determined by the presence of intestinal epithelium with typical mucous cells (row 3). Scale bar = 50  $\mu\text{m}$  (ectoderm and endoderm) and 100  $\mu\text{m}$  (mesoderm). **D)** Karyotype analysis of RPA1 iPSCs with a normal 46, XX karyotype at P16, and RPA2 and RPA3 iPSCs with a normal 46, XY karyotype at P14 and P19, respectively.

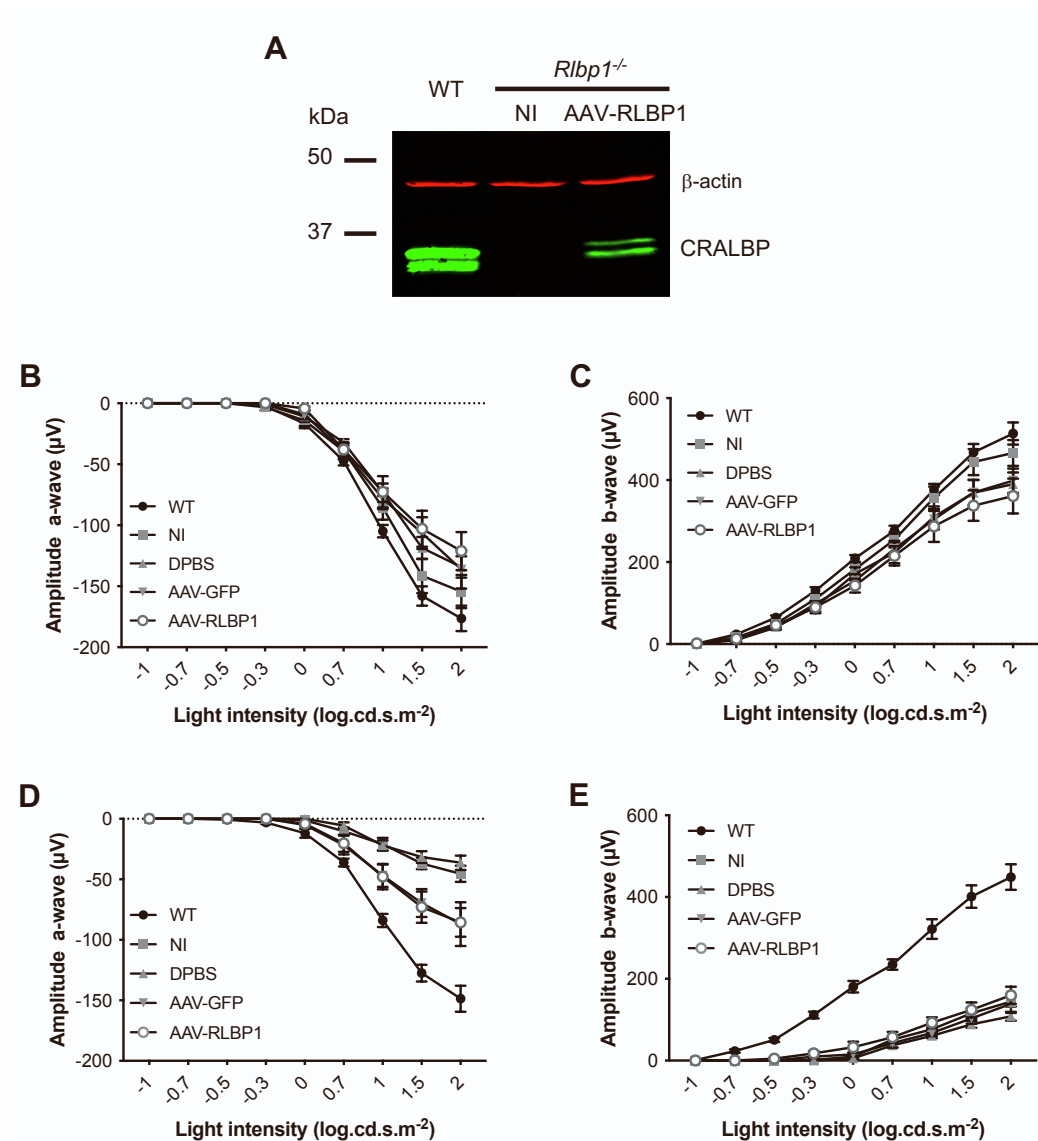


**Figure S2: *RLBP1* in iPSC-derived RPE and CRALBP expression from proviral plasmids. A)** qPCR analysis of *RLBP1* expression in control iPSCs, and in control, RPA1, RPA2 and RPA3 iPSC-derived RPE using a forward primer spanning the exon 6 to 7 junction and a reverse primer in exon 7 (situated in the deletion carried by RPA3). Data are represented as mean  $\pm$  SEM and expressed relative to control; \* $p < 0.05$ ;  $n = 3$ . **B)** Western blot analysis of CRALBP using a mouse monoclonal antibody in COS-7 cells non-transfected (NT), or transfected with a GFP-expressing plasmid or pKL-RLBP1, and scraped and collected in different combinations of protein inhibitor cocktails (PIC) and buffers: 1. Roche PIC and resuspension in Laemmli buffer (standard conditions); 2. Roche PIC and lysis in RIPA buffer; 3. Cell signalling PIC and lysis in Cell signalling lysis buffer; 4. Cell signalling PIC and lysis in RIPA buffer; 5. Roche PIC and lysis in Cell Signalling buffer;  $\beta$ -actin was used as loading control and GFP as a positive transfection control. **C)** Western blot analysis of CRALBP using a mouse monoclonal antibody in COS-7 cells NT or transfected with a GFP-expressing plasmid or pKL-RLBP1, and collected in Laemmli or RIPA buffer;  $\beta$ -actin was the loading control. **D)** Western blot analysis of CRALBP using a mouse polyclonal antibody in COS-7 cells NT or transfected with a GFP-expressing plasmid or with pKL-RLBP1, collected in Biorad Native sample buffer or lysed in RIPA (without SDS) buffer, and migrated under non-denaturing conditions;  $\beta$ -actin was the loading control.



**Figure S3: Proteomic and western blot analysis of control or mutant CRALBP isoforms.**

Alignment of the CRALBP peptides identified by mass spectrometry in the different gel fractions on the full length CRALBP sequence. The light blue square indicates the differing N-terminus between the two isoforms. **B**) Western blot analysis of CRALBP in 30 µg lysate of control, RPA1 and RPA2 iPSC-derived RPE cultured in 24-well plates. **C**) Western blot analysis of the CRALBP isoforms in 15 µg lysates of HEK-293 cells transfected with the control pKL-RLBP1 plasmid and the plasmids mutated in the ATG start codons (Met1Ala, Met10Ala) or carrying the missense variants present in patients RPA1 (Arg234Trp) and RPA2 (Arg9Cys); β-actin was the loading control in B and C.



**Figure S4: CRALBP production and scotopic ERG recordings post-AAV-RLBP1 administration.** Western blot analysis of CRALBP in the eyes of wild-type (WT) and non-injected (NI) or AAV-RLBP1-injected *Rlbp1*<sup>-/-</sup> mice; β-actin represents the loading control. Baseline a-wave (**B**) and b-wave (**C**) ERG amplitudes (in μV) in dark-adapted WT and NI, DPBS-injected, AAV-GFP-injected and AAV-RLBP1-injected *Rlbp1*<sup>-/-</sup> mice following stimulation at increasing light intensities expressed in log candela second/metre<sup>2</sup>. Recordings were performed up to 8 weeks post-injection. Data are represented as mean ± SEM; *n* = 9 (WT), 10 (NI), 9 (DPBS), 7 (AAV-GFP) and 8 (AAV-RLBP1). a-wave (**D**) and b-wave (**E**) ERG amplitudes post-photobleaching of the same wild type (WT) and *Rlbp1*<sup>-/-</sup> mice recorded up to 10 weeks post-treatment. Data are represented as mean ± SEM.

**Table S1. PCR, sequencing, genotyping and mutagenesis primers**

Target	Experiment	Sequence (5' to 3')
<i>MluI</i> - <i>RLBP1</i> <i>RLBP1</i> - <i>XhoI</i>	Vector production	F: <b>ACG CGT</b> ATG TCA GAA GGG GTG GGC AC R: <b>CTC GAG</b> TCA GAA GGC TGT GTT CTC AG
pKL backbone <i>RLBP1</i> exon 5 <i>RLBP1</i> exon 7 <i>RLBP1</i> exon 5 <i>RLBP1</i> exon 6 <i>RLBP1</i> exon 7-8	Sanger sequencing cDNA	F: AAT CTG TGC GGA GCC GAA AT F: CGC GCA CGG AAG TTC AAC GT F: CAA GGG CTT TAC CAT GCA GC R: GAA GCC GCT GTC CTT CTC TT R: AGG CTG TCA AAG AGC TCA GG R: TCC TGG AGC ATG TCC ACC AT
<i>Rlbp1</i>	Genotyping	F: TTA GAC TCA CAG GGG CCA ACA R1: ATG ATC CTT GGT TGT GAG CTG CTC R2: TAA AGC GCA TGC TCC AGA CT
c.1ATG>GCG p.Met1Ala	Site directed mutagenesis	F: TTC GAG CGA ACG CGT <b>GCG</b> TCA GAA GGG GTG GG R: CCC ACC CCT TCT GAC GCA CGC GTT CGC TCG AA
c.28ATG>GCG p.Met10Ala	Overlapping PCR	F1: TGA TTA ATT CGA GCG AAC GCG <b>TAT GTC</b> AGA AGG GGT GGG CAC GTT CCG
		R2: CAG TTG GGC ACG GAG CTC CTG TTC CTC TTC AGG TAC <b>CGC</b> GCG GAA CGT GCC CAC C
		F3: GCT CCG TGC CCA ACT GGA GCA GCT CAC AAC CAA GGA CCA TGG ACC TGT CTT TGG CCC
		R4: CTT GGC CTT CTG CAA GGT GTG GCG GGG CAG CTG GCT GCA CGG GCC AAA GAC AGG TCC
		F5: ACC TTG CAG AAG GCC AAG GAT GAG CTG AAC GAG AGA GAG GAG ACC CGG GAG GAG GC
		R6: TGC GCC TGC ACC ATC TCC TGC AGC TCT CGC ACT GCC TCC TCC CGG G
	Flanking PCR	F: TGA TTA ATT CGA GCG AAC GCG R: TGC GCC TGC ACC ATC
c.25C>T p.Arg9Cys	Site directed mutagenesis	F: GGG TGG GCA CGT TCT GCA TGG TAC CTG AAR' R: 5'TTC AGG TAC CAT GCA GAA CGT GCC CAC CC3
c.700C>T p.Arg234Trp	Site directed mutagenesis	F: GAT TCC TTC CCA GCC TGG TTC AAA GCC ATCC R: GGA TGG CTT TGA ACC AGG CTG GGA AGG AAT C
c.25C>T in exon 4 (p.Arg9Cys; RPA2)	Sanger sequencing gDNA	F: GAC CCC ACA AAA GGA GGA GG R: GCT GGA CCC TTT TCA CAG GA
c.333T>G in exon 5 (p.Tyr111X; RPA1 and RPA2)	Sanger sequencing gDNA	F: CCT CAC CCG CAC CTA AGT TT R: GGG GGT CTG GAG GGG AAA TT
c.700C>T in exon 8 (p.Arg234Trp; RPA1)	Sanger sequencing gDNA	F: TTG CTG GCC TGG AAA TAG GA R: GGT GCC CTA AGG ATG AGG GT
Exons 7-9del (RPA3)	Long-range PCR (gDNA)	F: TGT GAA GCT GAG CAC GTC AGA T R: TTG GGA GAA CTT TGG CAT G

F – forward, R – reverse

**Table S2. RT-PCR and qPCR primer sequences**

<b>Target</b>	<b>Experiment</b>	<b>Forward primer</b>	<b>Reverse primer</b>	<b>Efficacy</b>
<i>RLBP1</i>	qPCR exons 6-7	GAA ATC ACC TTT GAT GAG AT	TCT TCC TGA GAT CTG AAG TC	2.18
<i>RLBP1</i>	qPCR exon 6	ACC CTG AGC TCT TTG ACA GC	TGA AGA GCA TGA CCA CTC GG	2.02
SeV	RT-PCR	GGA TCA CTA GGT GAT ATC GAG C	ACC AGA CAA GAG TTT AAG AGA TAT GTA TC	N/A
<i>KOS</i>	RT-PCR	ATG CAC CGC TAC GAC GTG AGC GC	ACCTTGACAATCCTGATGTGG	N/A
<i>KLF4</i>	RT-PCR	TTC CTG CAT GCC AGA GGA GCC C	AAT GTA TCG AAG GTGCTC AA	N/A
<i>c-MYC</i>	RT-PCR	TAA CTG ACT AGC AGG CTT GTC G	TCC ACA TAC AGT CCT GGA TGA TGA TG	N/A
<i>NANOG</i>	qPCR	CAA AGG CAA ACA ACC CAC TT	TCT GCT GGA GGC TGA GGT AT	2.06
<i>OCT3/4</i>	qPCR	GTA CTC CTC GGT CCC TTT CC	CAA AAA CCC TGG CAC AAA CT	1.94
<i>LIN28A</i>	qPCR	GGG GAA TCA CCC TAC AAC CT	CTT GGC TCC ATG AAT CTG GT	2.16
<i>GAPDH</i>	qPCR	AAC CAT GAG AAG TAT GAC AAC	CTT CCA CGA TAC CAA AGT T	2.02
<i>L27</i>	qPCR	ACG CAA AGC CGT CAT CGT GAA G	CTT GGC GAT CTT CTT CTT GCC	2.09

N/A – non-applicable



**Table S3: Primary antibodies**

Primary antibodies	Host	Clonality	Dilution	Company	Cat #
anti-ARL13B	Rabbit	Polyclonal	1/3000	Proteintech	17711-1-AP
anti- $\beta$ -actin, clone AC-74	Mouse	Monoclonal	1/10000	Sigma-Aldrich	A5316
anti-BEST1	Mouse	Monoclonal	1/500	Abcam	Ab2182
anti-CRALBP, clone B2	Mouse	Monoclonal	1/1000	Abcam	ab15051
anti-CRALBP	Rabbit	Monoclonal	1/1000	Abcam	ab183728
anti-CRALBP	Mouse	Polyclonal	1/1000	AgroBio	Directed against recombinant human CRALBP
anti-GFP	Rabbit	Polyclonal	1/2000	Invitrogen	A6455
anti-LRAT	Rabbit	Polyclonal	1/250	Abcam	ab166784
anti-MERTK, clone Y323	Rabbit	Monoclonal	1/250	Abcam	ab52968
anti-Perilipin-2	Guinea pig	Polyclonal	1/2000	Progen	GP47
anti-RPE65	Mouse	Monoclonal	1/1000	Abcam	ab13826
anti-ZO-1	Rabbit	Polyclonal	1/100	ThermoFisher Scientific	40-2200

**Table S4: Secondary antibodies**

Secondary antibodies	Host	Dilution	Company	Cat #
anti-Mouse IgG-HRP	Sheep	1/10000 (WB)	Jackson ImmunoResearch	515-035-003
anti-Guinea Pig-HRP	Goat	1/10000 (WB)	Jackson ImmunoResearch	106-035-003
anti-Mouse IgG IRDye 680RD	Donkey	1/20000 (WB)	LI-COR Biosciences	926-68072
anti-Rabbit IgG IRDye 800CW	Donkey	1/20000 (WB)	LI-COR Biosciences	926-32213
anti-Mouse IgG Alexa Fluor 488	Donkey	1/500	ThermoFisher Scientific	A-21202
anti-Mouse IgG Alexa Fluor 594	Donkey	1/500	ThermoFisher Scientific	A-21203
anti-Rabbit IgG Alexa Fluor 488	Donkey	1/500	ThermoFisher Scientific	A-21206
anti-Rabbit IgG Alexa Fluor 594	Donkey	1/500	ThermoFisher Scientific	A-21207
anti-Mouse IgG Alexa Fluor 546	Goat	1/500	ThermoFisher Scientific	A10036
anti-Mouse IgG Alexa Fluor 594 AffiniPure	Donkey	1/500	Jackson ImmunoResearch	715-585-150
anti-Rabbit IgG Alexa Fluor 594 AffiniPure	Donkey	1/500	Jackson ImmunoResearch	711-585-152
anti-Mouse IgG Alexa Fluor 488 AffiniPure	Donkey	1/500	Jackson ImmunoResearch	715-546-151
anti-Rabbit IgG Alexa Fluor 488 AffiniPure	Donkey	1/500	Jackson ImmunoResearch	711-545-152