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#### **Supplemental information**

#### Characterization and AAV-mediated CRB gene

# augmentation in human-derived CRB1<sup>KO</sup>

## and *CRB1<sup>KO</sup>CRB2<sup>+/-</sup>* retinal organoids

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Previously published	Description	Gender
LUMC04iCTRL10	$\underline{\text{ISO-4.10}} = \text{Isogenic control}$	Male
No	<u><i>CRB1</i><sup>KO</sup> CL19</u> = homozygous c.500del ; p.(Ser44Serfs*)	Male
No	<u><i>CRB1</i><sup>KO</sup>CL26</u> = homozygous c.500del ; p.(Ser44Serfs*)	Male
No	<u>CRB1<sup>KO</sup>CL72</u> = homozygous c.500del ; p.(Ser44Serfs*)	Male
No	$\underline{CRB1^{KO}CRB2^{+/-}CL4} =$	Male
	CRB1: homozygous c.500del ; p.(Ser44Serfs*),	
	CRB2: heterozygous c.576_598del; p.(Cys193Argfs*)	
No	$\underline{CRB1^{KO}CRB2^{+/-}CL9} =$	Male
	CRB1: homozygous c.500del ; p.(Ser44Serfs*),	
	CRB2: heterozygous c.576_598del; p.(Cys193Argfs*)	
No	$\underline{CRB1^{KO}CRB2^{+/-}CL17} =$	Male
	CRB1: homozygous c.498_507delinsTGCC ; p.(Ser44Lysfs*),	
	CRB2: heterozygous c.583_584del; p.(His195Trpfs*)	

## **Table S1**. hiPSC line information, related to Figure S1.

### **Table S2**. PCR primer sequences for confirmation mutations.

Target		PCR primers
CRB1	FW	GACAATGATTGTTCTTGTTCAGACACAGCC
	REV	CATCCACTTCCAAGTCGCAGTGTC
		Sanger sequencing primers
CRB1	FW	GGACAAAGACTGTGACAACATGAAAGACC
	REV	GGACACAGAAGCAGGAGTAACCATC
Target		NGS primers (5' to 3') (Illumina adapter overhang in blue)
CRB2	FW	GATGTGTATAAGAGACAGGTGTCCATCCTGCACCCTGTG
	REV	CGTGTGCTCTTCCGATCTTCGCTCACCCGTTGACCAGGT

<b>Fable S3</b> . Barcode PCR	primers used in	the NGS ampli	icon sequence analysis.
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Barcode	Primer Sequence (5' to 3')	Barcode	Read in
name		in primer	Miseq
719	CAAGCAGAAGACGGCATACGAGAT   TACTACGC	TACTACGC	TACTACGC
	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		
720	CAAGCAGAAGACGGCATACGAGAT   AGGCTCCG	AGGCTCCG	AGGCTCCG
	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		
721	CAAGCAGAAGACGGCATACGAGAT   GCAGCGTA	GCAGCGTA	GCAGCGTA
	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		
722	CAAGCAGAAGACGGCATACGAGAT   GAGCGCTA	GAGCGCTA	GAGCGCTA
	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT		
508	AATGATACGGCGACCACCGAGATCTACAC   GTACTGAC	GTACTGAC	GTACTGAC
	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG		

Α.		37700	TTCCTCTC	CRB	RB1 B.					CRB2						
	ISO-4.10	M	MMW	WW	MM	<u>M</u> N	W	IS	50-4.10	WMM	MM	WWW	www	MM	MMM	M
		ATTGG	TTC-TGTG	GCAAGA	ACTCCTGO	C A	ACAT CR	BT~CRE	32" GL4 AG	TGCCAG	AGCCAGCO	GTGCGCI	ACATGGG	GGCACG	TGCCACGAC	CTG
CKD I	CE 19, CE20, C	L'Z MAN	AAAAAAA	MMM	MAM	N N	MCR	B1KOCRE	2+/- CL9						23bp deletion	
CRB1	<i>℃CRB2</i> <sup>+,-</sup> CL4, 0	CL9 111	Chuu	M.M.	IN. MI	. (			AC	TGCCAG	AGCCAGCO	GTGCGC	ACATGGG	GGCACG	TGCCACGAC	CTG
CRE	81 <sup>KO</sup> CRB2 <sup>+/-</sup> CL1	7 M	r	M		MM		B1 <sup>KO</sup> CRE	82** CL17 AG	TGCCAG	AGCCAGCO	GTGCGCI	ACATGGG	 GGCACG	deletion	CTG
C.	0.00						000460	01.00				G	opak	0.01.70		
10	CRB1	CL19			~	0 R	JRB1	CL26			1.14		CRB1	CL/2		
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D.	CRB1 <sup>KO</sup> C	CRB2*/- CL	1				CRB1 <sup>K</sup>	°CRB2+	CL9			CRE		B2*/- C	17	
	* A	3		8.3	and a	0	-	P	43	-	1.	31	1	52 0	14	at
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	Chromosome	1p 1q	2q 3p 4	q 5q 6	iq 7p 8	Rq 9q 11	p 12p 13	q 14q 15q	16q 17p	17q 18q	19p 20q	22q Xp				
	ISO-4.10															
	CRB1 <sup>100</sup> CL19															
	CR81 <sup>50</sup> CL26															
	CRB1 <sup>KD</sup> CRB2 <sup>+/-</sup> CL4															
	CRB1***CRB2*** CL9															
	CRB1 <sup>K0</sup> CRB2 <sup>1/-</sup> CL17															
		p-value o	0.0	1 0.05	1		0.05 0.0	D1 0								
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**Figure S1: Confirmation of mutations and condition of** *CRB1*<sup>KO</sup> **and** *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> **hiPSC.** Related to all figures. (A) Confirmation of the homozygous *CRB1* mutation in *CRB1*<sup>KO</sup> and *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> hiPSC using Sanger sequencing, with *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> CL17 containing a distinct mutation. (B) Confirmation of the heterozygous *CRB2* mutation in *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> hiPSC using Sanger sequencing (upper trace) and an insertion/deletion (INDEL) detection software (ICE) (lower sequences). (C, D) Karyotyping results of (C) *CRB1*<sup>KO</sup> and (D) *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> hiPSC. (E) Meta-analysis of 90% most recurrent abnormalities in hiPSCs showing a gain in copy number variation (CNV) in chromosome 20q for hiPSC used in this study.



Figure S2: CRB1 is absent in DD180 and DD210 *CRB1*<sup>KO</sup> and *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> retinal organoids. Related to Figure 2. Representative immunohistochemical images of three *CRB1*<sup>KO</sup> and three *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> retinal organoids compared to the isogenic control at DD180 and DD210. (A) DD180 organoids expressing PALS1 (green) in all retinal organoids at the OLM and CRB1<sup>EX</sup> (red), and CRB1<sup>INT</sup> (cyan) was only detected in the isogenic control. (B) DD210 retinal organoids stained with PALS1 (green) and CRB1 (red), similar as observed in DD180. (C) MUPP1 (green) and CRB2 (red) is expressed at the OLM in control, *CRB1*<sup>KO</sup>, and *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> retinal organoids at DD210. Note: INL = Inner Nuclear Layer, ONL = Outer Nuclear Layer, OLM = Outer Limiting Membrane. Scalebar = 50µm.



Figure S3: Additional phenotype quantifications of DD180 and DD210 *CRB1*<sup>KO</sup> and *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> retinal organoids. (A, B, C) Quantification of the (A) ONL, (B) retinal, and (C) INL thickness of DD180 retinal organoids. (D, E, F) Quantification of the (D) ONL, (E) retinal, and (F) INL thickness of DD210 retinal organoids. Each datapoint in the graph represents an individual organoid, of which an average has been taken of 3-6 representative images. The standard error of mean (SEM) is derived from these averages. Number of individual organoids used for the quantification per condition at DD180: 4.10 *n* =14, CRB1<sup>KO</sup> CL19 *n* = 7, CL26 *n* =5, CL72 *n* = 9, *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> CL4 *n* = 3, CL9 *n* = 8, CL17 *n* = 8; and DD210: 4.10 *n* = 12, CRB1<sup>KO</sup> CL19 *n* = 9, CL26 *n* =10, CL72 *n* = 11, *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> CL4 *n* = 8, CL17 *n* = 8 from at least two independent differentiation batches and *CRB1*<sup>KO</sup>*CRB2*<sup>+/-</sup> CL9 *n* = 5 from one differentiation batch. Statical analysis: generalized linear mixed models with p<0.05 (\*), p<0.01 (\*\*), and p<0.001 (\*\*\*).



Figure S4: AAV transduction study of control retinal organoids transduced at DD200 with AAV2.CMV.GFP or AAV5.CMV.GFP (A, B) Representative immunohistochemical images of control retinal organoids transduced with (A)  $1 \times 10^{10}$  gc, or (B)  $10 \times 10^{10}$  gc AAV2.CMV.GFP or AAV5.CMV.GFP. (C,D, E) Quantification of the number of GFP positive cells in the (D) ONL, (E) INL, and (F) GFP positive and co-localized with SOX9 in the INL (marking Müller glial cells). Statistical analysis was performed within the same AAV capsid (dose-dependent) or within the same AAV titer (comparing AAV2 with AAV5 transduction efficiency). (F, G) Immunohistochemical images of co-localization of AAV.GFP with Müller glial cells marker SOX9 (F) and photoreceptor cell marker Rhodopsin (RHO) (G). Arrows indicate colocalization of SOX9 with GFP (F), and the asterisk indicates the RPE (G). Each datapoint in the graph represents individual organoids, of which an average has been taken of 3-6 representative images. The standard error of mean (SEM) is derived from these averages. Number of individual organoids used for quantification per condition for AAV2.CMV.*GFP*:  $1 \times 10^{10}$  gc n = 6,  $10 \times 10^{10}$  gc n = 6; and for AAV5.CMV.*GFP*:  $1 \times 10^{10}$  gc n = 66,  $10 \times 10^{10}$  gc n = 6 from one differentiation batch. Note: INL = Inner Nuclear Layer, ONL = Outer Nuclear Layer. Scalebar =  $50\mu m$ , statical analysis: generalized linear mixed models with p<0.05 (\*), p<0.01 (\*\*), and p<0.001 (\*\*\*).



**Figure S5:** AAV-mediated gene therapy on *CRB1*<sup>KO</sup> organoids. (A, B) representative immunohistochemical image of (A) CRB1 in untreated and AAV.h*CRB1* and (B) CRB2 in untreated and AAV.h*CRB2* treated *CRB1*<sup>KO</sup> retinal organoids. (C,D, E, F, G, H) Quantification of the (C, D) ONL thickness, (E, F) the retinal thickness, and (G, H) INL thickness per *CRB1*<sup>KO</sup> clone (C, E, G) or all *CRB1*<sup>KO</sup> clones combined (D, F, H). Each datapoint in the graph represents individual organoids, of which an average has been taken of at least three representative images. The standard error of mean (SEM) is derived from these averages. Number of individual organoids used for quantification per condition for untreated: 4.10 n = 10, *CRB1*<sup>KO</sup> CL19 n = 7, CL26 n = 7, CL72 n = 5; AAV.h*CRB1* treated: 4.10 n = 5, *CRB1*<sup>KO</sup> CL19 n = 6, CL26 n = 8, CL72 n = 4 from two independent differentiation batches; and AAV.h*CRB2* treated: 4.10 n = 5, *CRB1*<sup>KO</sup> CL19 n = 4, CL26 n = 6, CL72 n = 3 from one differentiation batch. Note: ONL = Outer Nuclear Layer, OLM = Outer Limiting Membrane. Scalebar = 50µm, statistical analysis: generalized linear mixed models with p < 0.05 (\*), p < 0.01 (\*\*), and p < 0.001 (\*\*\*).