

Supplementary Information

mRNA trans-splicing dual AAV vectors for (epi)genome editing and gene therapy

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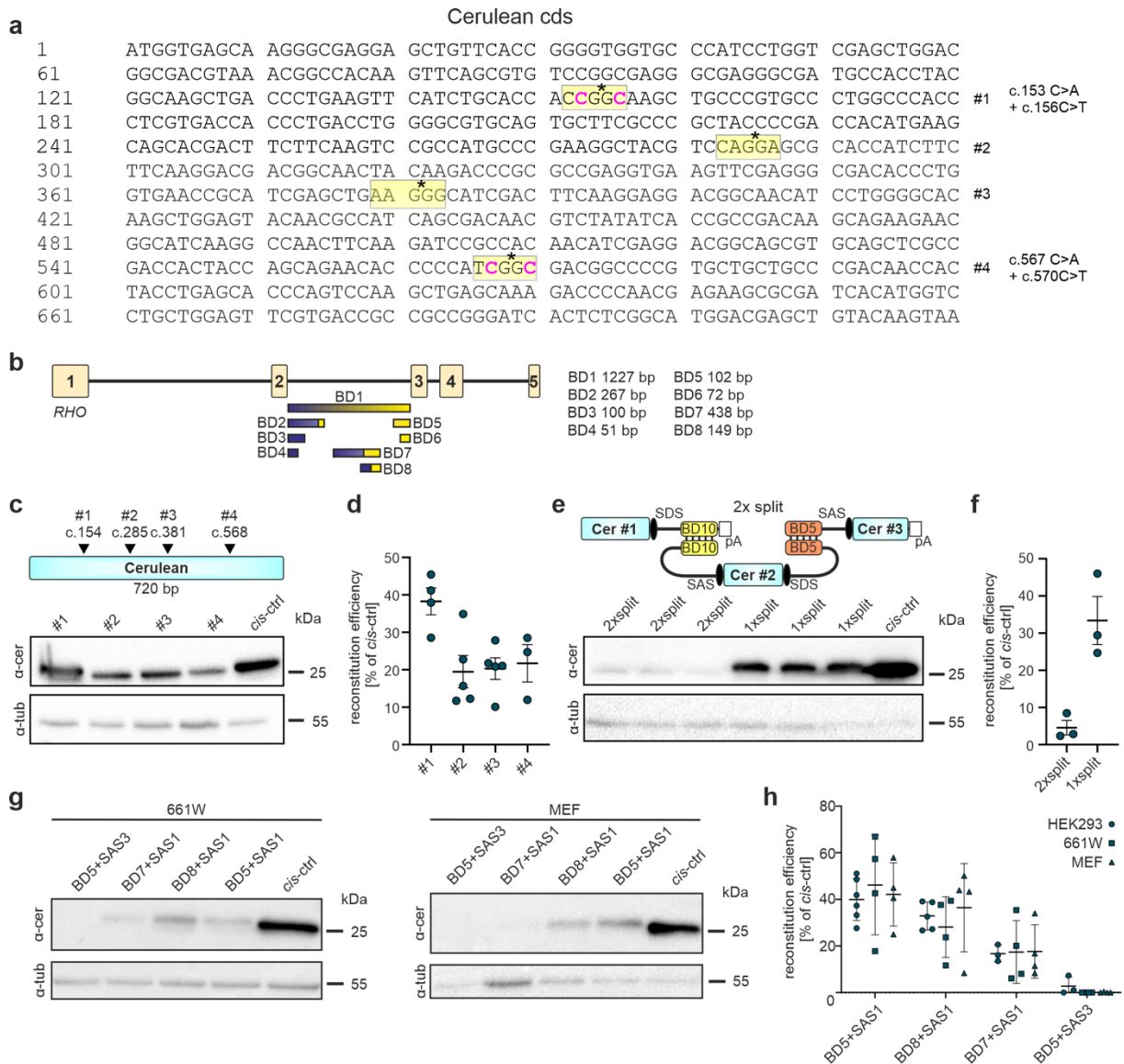


Fig. S1 *In vitro* evaluation of REVeRT. **a** Coding sequence (cds) of cerulean. The split sites (#1-4) used are highlighted in yellow. Asterisks mark the exact split position. Introduced silent mutations are highlighted in pink. **b** Scheme depicting *RHO* intron 2 used for BD1-BD8. The respective sequences originate from different regions of the intron as indicated by the color gradient. BD2, BD7 and BD8 represent fusion sequences containing the parts of the 5' end and parts of the 3' end of the intron. **c** Upper panel, Position of the split sites within the cerulean sequence. Lower panel, Western Blot of HEK293 cells transfected with cerulean vectors split at positions #1-4. **d** Ratiometric quantification of reconstitution efficiency relative to the cis-ctrl determined from western blots **c**. $n=4$ (#1), $n=5$ (#2, #3) and $n=3$ (#4) transfections. **e** Upper panel, Scheme depicting mRNA reconstitution for cerulean split into three fragments. Lower panel, Western Blot of HEK293 cells transfected with cerulean constructs split into two (1xsplit) or three fragments (2xsplit). **f** Ratiometric quantification of reconstitution efficiency relative to the cis-ctrl determined from western blots **e**. $n=3$ transfections. **g** Western blot of 661W (left) or mouse embryonic fibroblast (MEF, right) cells transfected with split cerulean constructs in presence of different BDs or SASs. **h** Ratiometric quantification of the reconstitution efficiency relative to the cis-ctrl determined from western blots **g**. The corresponding HEK293 western blots are shown in Fig 1. Scatter plots show mean $\pm$ SEM. Minimum of $n=3$ transfections. All source data are provided as a Source Data file.

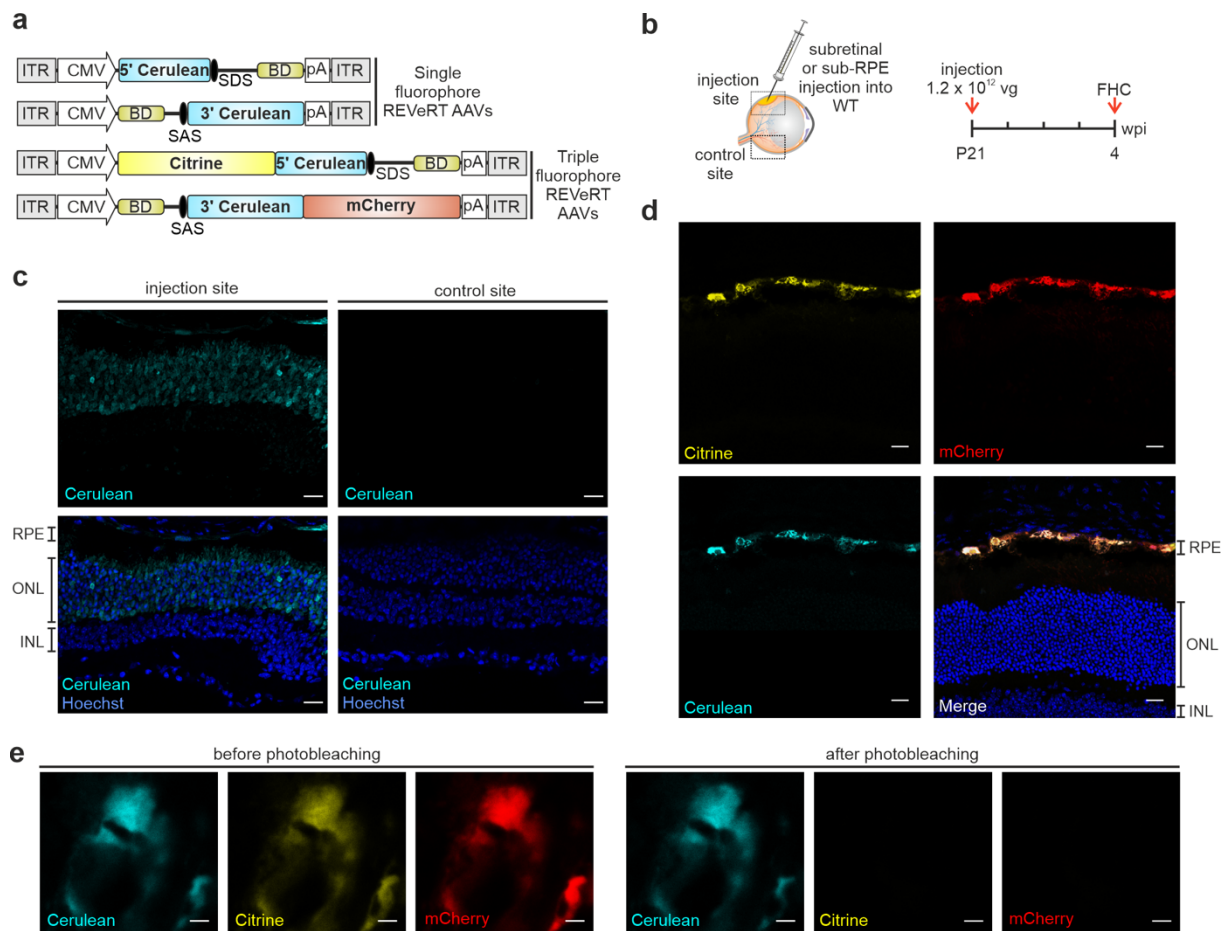


Fig. S2 Reconstitution of fluorescent reporter genes via dual REVeRT AAVs *in vivo*. **a** Design of dual REVeRT AAVs used for experiments shown in **c** (single fluorophore), **d** and **e** (triple fluorophore). ITR, inverted terminal repeats. **b** Experimental setup. FHC, fluorescence histochemistry. wpi, weeks post injection. **c** Representative FHC images of retinas obtained from mice injected with single fluorophore REVeRT AAVs. Left panel, cerulean expression along the injection site; Right panel, sections from the non-injected control site within the same retina. RPE, retinal pigment epithelium; ONL, outer nuclear layer; INL, inner nuclear layer. Scale bar, 20 μ m. **d**, **e** FHC of a retina injected with triple fluorophore REVeRT AAVs (citrine, split cerulean and mCherry) system. Scale bar, 30 μ m. **e** High magnification images of RPE cell expressing all three fluorophores before and after selective photobleaching of the mCherry and citrine signal. Photobleaching confirmed the presence of reconstituted cerulean. Scale bar, 2 μ m. All results in **c-e** are derived from non-amplified (i.e., antibody-free) fluorophore signals.

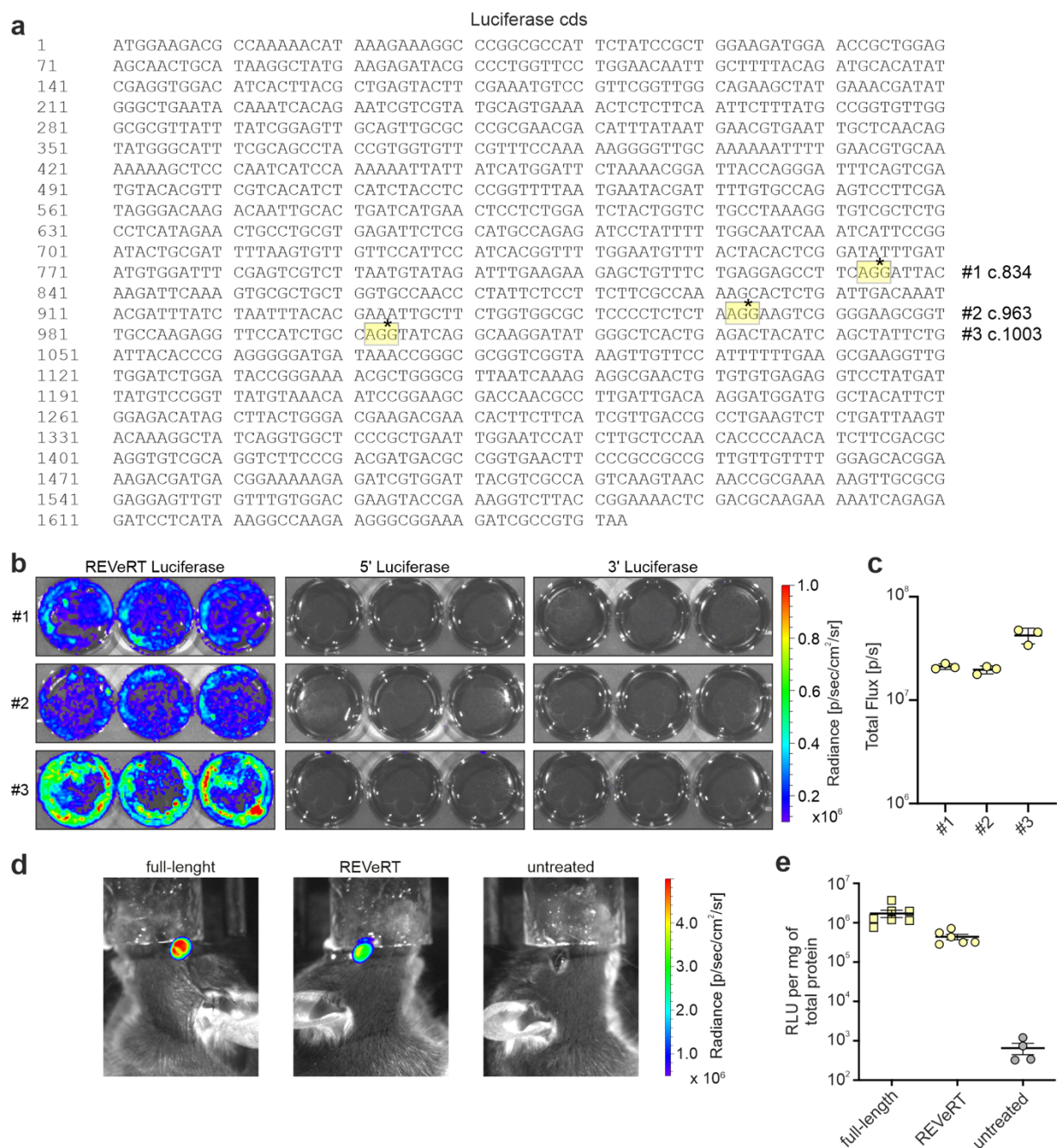


Fig. S3 Split luciferase reporter assay. **a** Luciferase coding sequence (cds). The split sites (#1-#3) used in this study are highlighted in yellow. Asterisks mark the exact split position. **b** Luminescence images of HEK293T cells transfected with luciferase constructs split at different positions as indicated. The cells were either transfected with dual vectors (REVeRT Luciferase) or with a single vector (5' Luciferase, 3' Luciferase) as negative control. **c** Quantification of the results shown in **b**. Scatter plot shows mean $\pm$ SEM. $n=3$ transfections. **d** Luminescence images of mice subretinally injected with split (REVeRT) or full-length luciferase constructs at three weeks post injection. **e** Relative quantification of luciferase expression in injected retinas at three weeks post injection. RLU, relative light units. Plots show mean $\pm$ SEM. $n=7$ retinas (full-length), $n=6$ retinas (REVeRT), $n=4$ retinas (untreated). All source data are provided as a Source Data file.

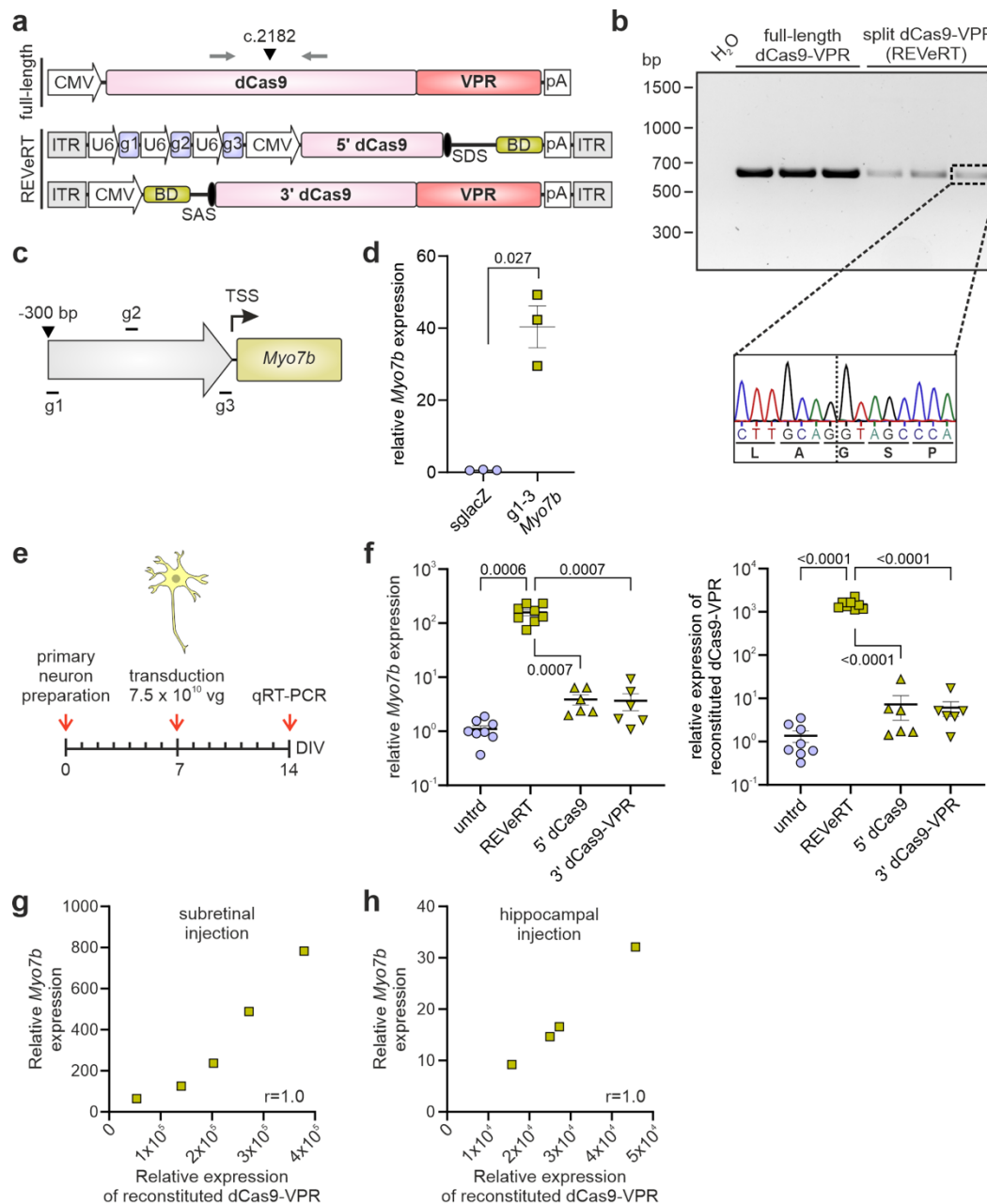


Fig. S4 *Myo7b* transactivation using split dCas9-VPR reconstituted via REVeRT. **a** Split (REVeRT) and full-length dCas9-VPR constructs used in **b-h**. Split site (arrowhead) and junction-spanning primers (arrows) are indicated. **b** RT-PCR using indicated primer pair. Sequencing of the splicing junction shown below. The RT-PCR was repeated twice. **c** Binding positions of three sgRNAs targeting the region upstream of the transcription start site (TSS) of *Myo7b*. **d** qRT-PCR from 661W cells co-transfected with split dCas9-VPR and either *lacZ*- or *Myo7b*-targeting (g1-g3) sgRNAs. *lacZ*-transfected cells served as reference. Mann-Whitney test was used for statistical analysis. $n=3$ independent transfections. **e** Time scale for experiments in mouse primary neurons transduced with dual REVeRT AAV8Y733F vectors. DIV, days *in vitro*. **f** Relative expression of *Myo7b* and reconstituted dCas9-VPR in transduced mouse primary neurons ($n=8$ cultures). Welch ANOVA with Dunnett T3 was used for statistical analysis. Untreated cells (untrd) served as reference ($n=8$ cultures). Neurons transduced with single vectors (5' dCas9, 3' dCas9-VPR) served as controls ($n=6$ cultures). **g**, **h** Dose-dependency between dCas9-VPR reconstitution and transcriptional activation of *Myo7b* upon subretinal (**g**) or hippocampal injection (**h**). Spearman correlation coefficient shown in the corner. Scatter plots show mean $\pm$ SEM. All source data are provided as a Source Data file.

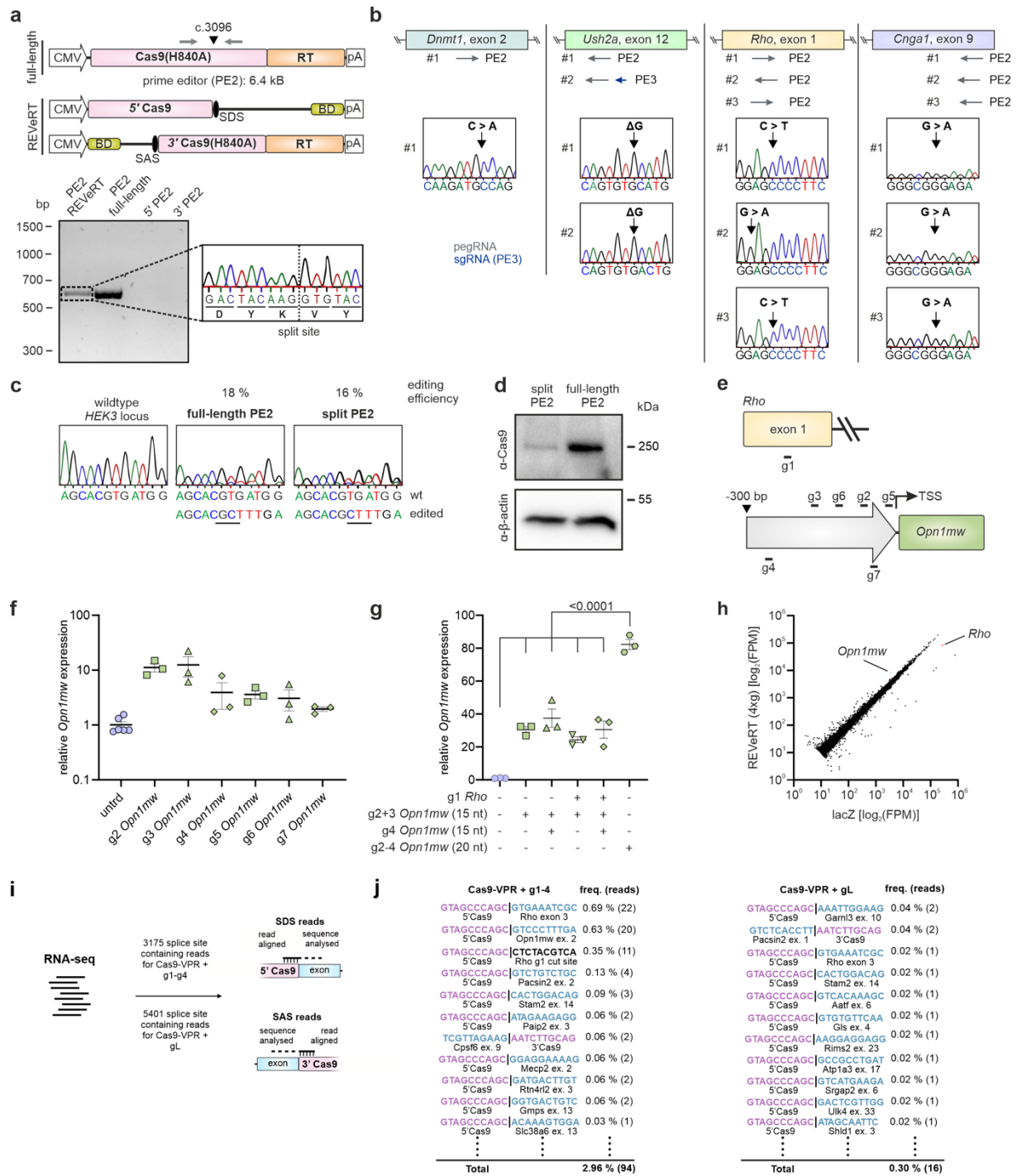


Fig. S5 *In vitro* prime editing and CONNACT strategy via REVERT. **a** Upper panel, split and full-length prime editor 2 (PE2) constructs used in **b** and **c**. Split site (arrowhead) and junction-spanning primers (arrows) are indicated. Lower panel, RT-PCR of reconstituted PE2 using junction-spanning primers and sequencing result of the splicing junction. Single vectors (5' PE2 and 3' PE2) served as controls. The RT-PCR has been repeated three times. **b** Sequencing results of the targeted genomic loci after co-transfection of 661W and MEF cells with PE2 or PE3 (PE2 + additional sgRNA introducing a nick) and corresponding pegRNAs. Target exons and binding position of the pegRNAs are depicted above. **c** Sequencing of the *HEK3* locus after co-transfection of HEK293T cells with split or full-length PE2 and pegRNA. **d** Western blot of 661W cells transfected with split or full-length PE. The western blot has been repeated once. **e** Binding positions of the sgRNAs targeting *Rho* exon 1 (g1) or the region upstream of the TSS of *Opn1mw* (g2-g7). **f** qRT-PCR from MEF cells co-transfected with

dCas9-VPR and g2 – g7. Untreated cells (untrd) served as reference. n=3 independent transfections. **g** qRT-PCR from MEF cells co-transfected with dCas9-VPR and different combinations of sgRNAs as indicated. Spacer lengths of *Opn1mw*-targeting sgRNAs are shown in brackets. Untreated cells served as reference. One-way ANOVA with Dunnett's T3 test was used to compare expression levels using a 15 nt vs. 20 nt spacer. Scatter plots show mean $\pm$ SEM. n=3 independent transfections. **h** RNA-Seq analysis of retinas originating from three different animals (dark pink data points in Fig. 4d) injected with REVeRT(4xg) or *lacZ* vectors. *Opn1mw* and *Rho* transcripts are highlighted. FPM, fragments per million. **i** Schematic depiction of RNA-Seq data analysis to identify REVeRT splicing events with endogenous transcripts. **j** Identity of transcripts containing a splicing event with an endogenous mRNA. Splicing events have been analyzed for retinas injected with Cas9-VPR and targeting sgRNAs (g1-g4) and injected with a non-targeting sgRNA (gL). The full list of identified sequences can be found in Table S4. All source data are provided as a Source Data file.

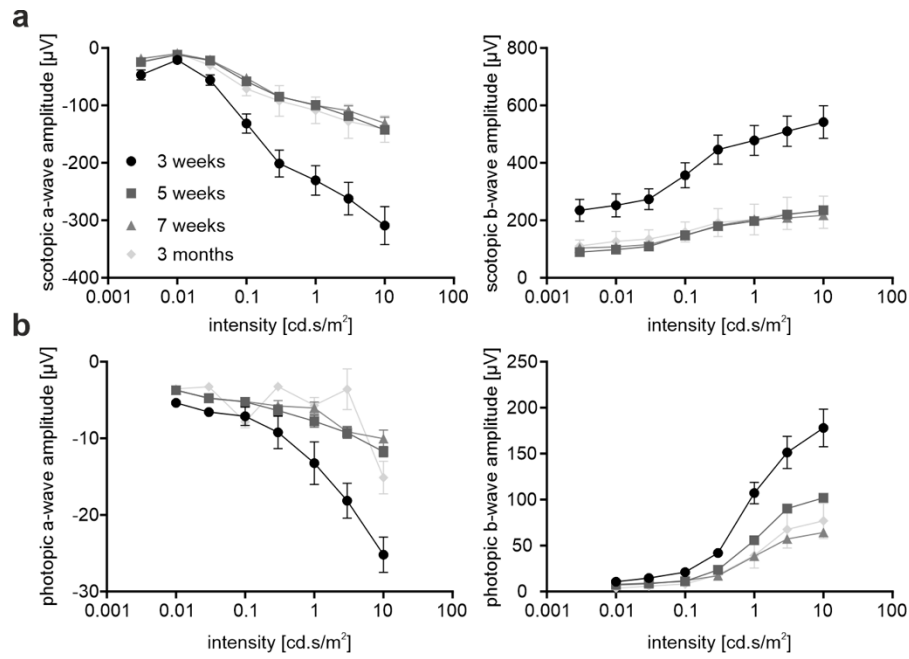


Fig. S6 Natural history in *Abca4*-deficient mice. **a, b** Scotopic (**a**) and photopic (**b**) ERG measurements for untreated *Abca4*^{-/-}/*Rdh8*^{-/-} mice between 3 weeks and 3 months of age as indicated. Plots show mean ± SEM. n=4 mice. Both eyes were measured. All source data are provided as a Source Data file.

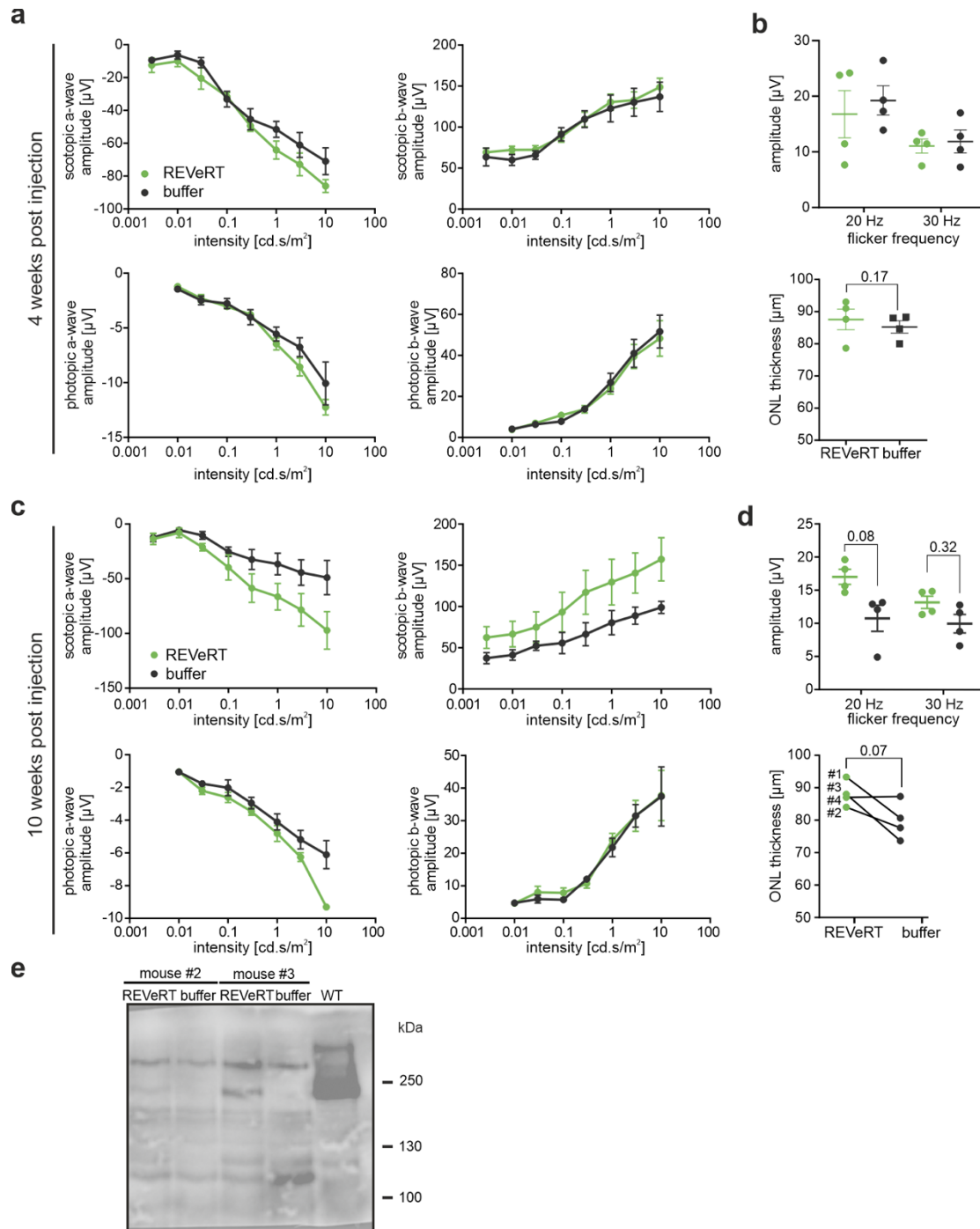


Fig. S7. Functional analysis of REVeRT- and buffer-injected eyes in *Abca4*^{-/-} *Rdh8*^{-/-} mice. **a** ERG measurements of injected *Abca4*^{-/-}/*Rdh8*^{-/-} mice at 4 wpi under scotopic (upper panel) and photopic (lower panel) conditions. **b** Upper panel, Amplitudes from flicker stimuli at 20 Hz and 30 Hz under photopic conditions at 4 wpi. Lower panel, OCT measurements of injected *Abca4*^{-/-}/*Rdh8*^{-/-} mice at 4 wpi. **c** ERG measurements under scotopic (upper panel) or photopic (lower panel) conditions at 10 wpi. **d** Upper panel, Results from flicker at 20 Hz and 30 Hz under photopic conditions at 10 wpi. Lower panel, Pairwise comparison of OCT measurements for the treated and control eye of the single animals at 10 wpi. Two-way ANOVA with Šidák's multiple comparisons test was used for statistical analysis. Plots show mean $\pm$ SEM. Wpi, weeks post injection. n=4 injected mice for **a** – **d**. **e** Uncropped western blot shown in Fig. 5e. All source data are provided as a Source Data file.

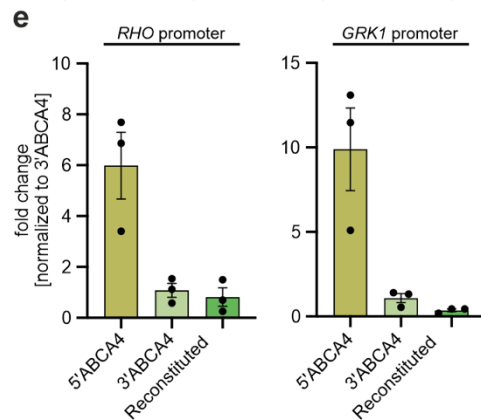
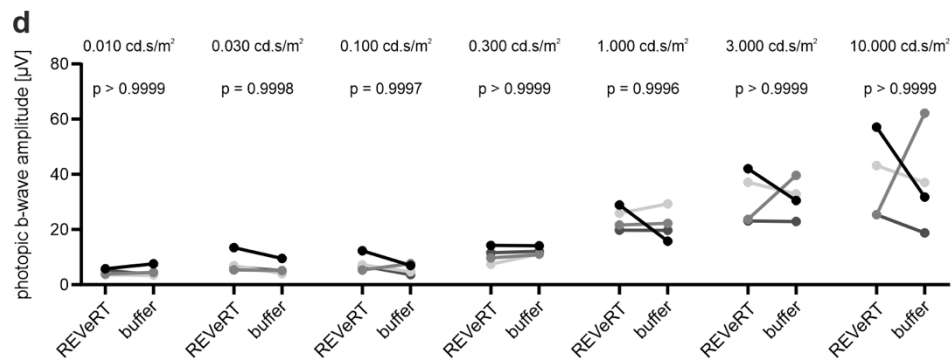
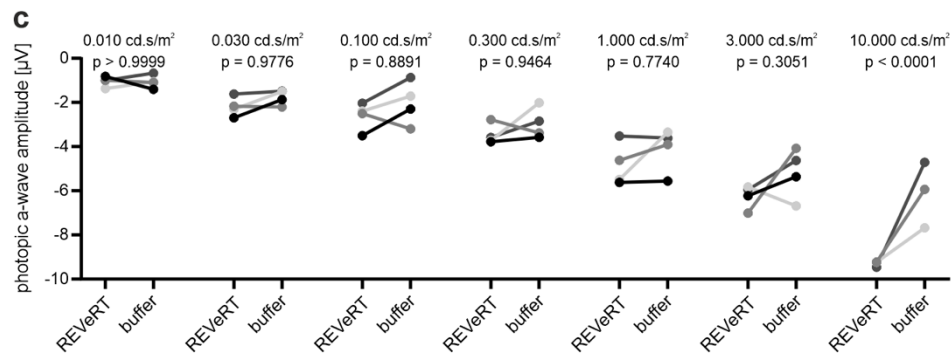
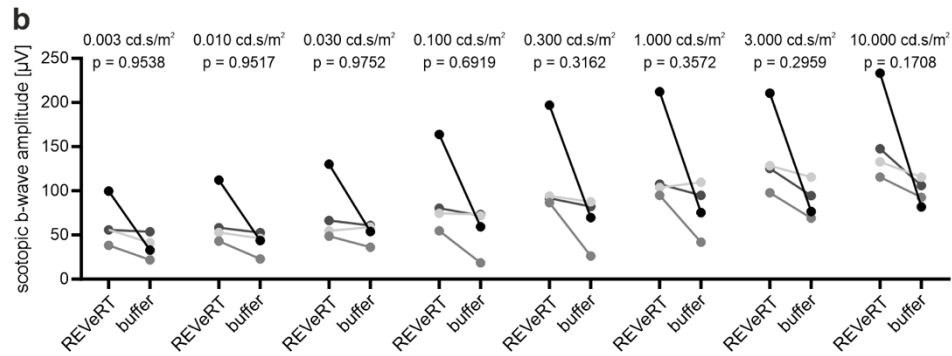
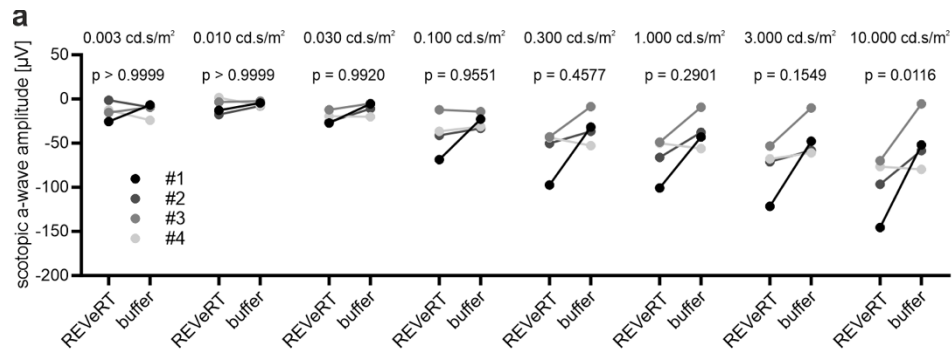


Fig. S8 Pairwise comparison of treated and buffer-injected eyes in Abca4-deficient mice.
a-d Pairwise plots for the ERG measurements shown in Fig. S7a and c under scotopic (**a, b**) or photopic (**c, d**) conditions as indicated. Light intensities and p-values (two-way ANOVA with Šídák's multiple comparison test) are shown above the corresponding measurements. n=4 injected mice. **e** Relative frequency of 5'ABCA4 mRNA, 3'ABCA4 mRNA and reconstituted mRNA in REVeRT-injected retinas of adult *Abca4*^{-/-}/*Rdh8*^{-/-} mice 4 weeks post injection. All values are relative to the frequency of 3'ABCA4 transcript. Transcript expression was driven by a RHO (left) or a GRK1 promoter (right). Error bars indicate mean ± standard error and were calculated from technical triplicate reactions from n=3 injected mice. All source data are provided as a Source Data file.

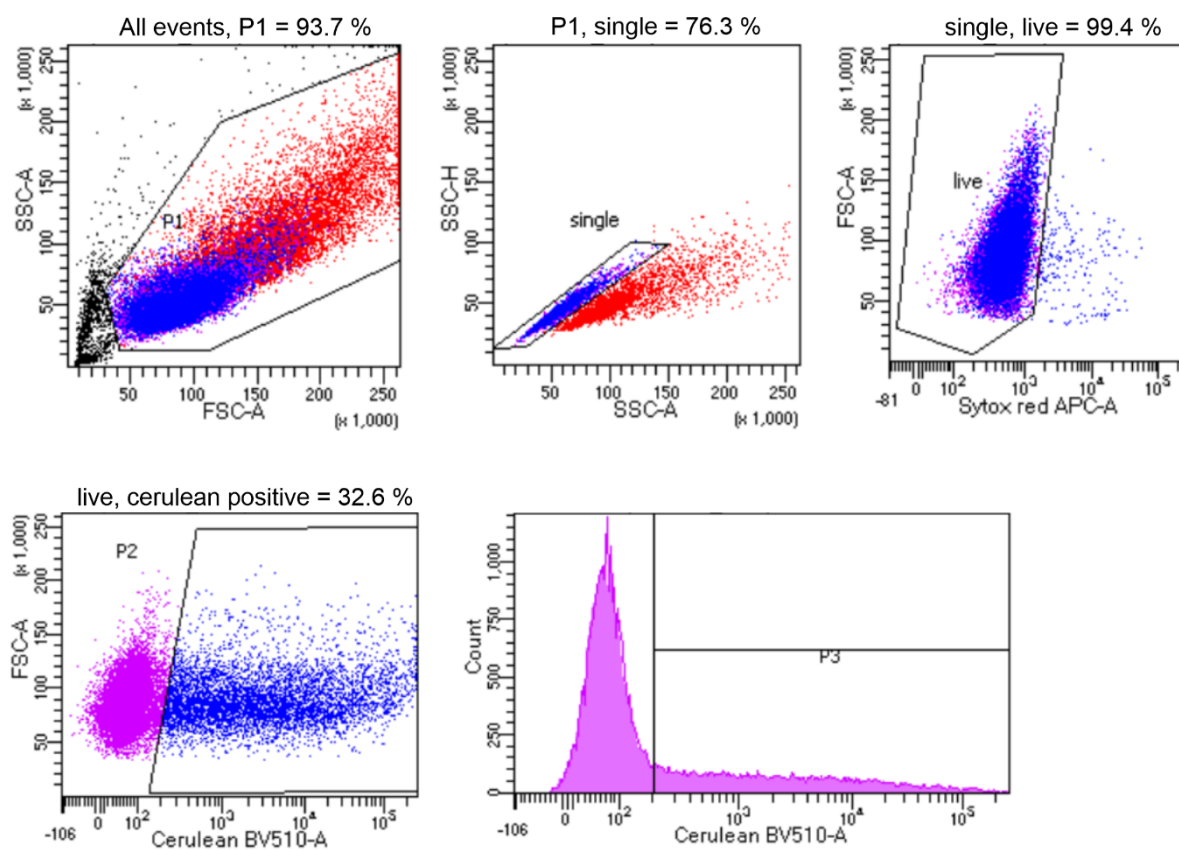


Fig. S9 Gating Strategy used for flow cytometry on HEK293 cells transfected with split cerulean constructs.

Table S1. Binding domain analysis.

Binding domain	Reconstitution efficiency WB $\pm$ SEM [%]	Reconstitution efficiency FC $\pm$ SEM [%]	Length [bp]	GC content [%]
1	2.1 $\pm$ 2.1	1.7 $\pm$ 0.9	1227	55.3
2	27.0 $\pm$ 5.8	17.3 $\pm$ 1.2	267	55.1
3	5.7 $\pm$ 1.8	4.2 $\pm$ 0.4	100	57.0
4	37.3 $\pm$ 5.4	31.5 $\pm$ 1.7	51	56.9
5	39.9 $\pm$ 2.3	36.4 $\pm$ 1.4	102	62.7
6	24.2 $\pm$ 6.6	12.1 $\pm$ 0.2	72	56.9
7	16.7 $\pm$ 2.1	13.6 $\pm$ 0.5	438	56.8
8	32.9 $\pm$ 2.5	35.8 $\pm$ 1.8	105	59.0
9	29.2 $\pm$ 9.4	24.0 $\pm$ 0.7	100	47.0
10	53.5 $\pm$ 5.7	44.0 $\pm$ 1.3	100	46.0
11	30.7 $\pm$ 5.0	20.3 $\pm$ 0.7	100	48.0
12	13.2 $\pm$ 5.0	16.8 $\pm$ 0.5	100	48.0

Table S2. Trans-splicing elements used for REVeRT.

Element	Sequence
BD: BD10	CATCTGACCACCTGCGAA(Δ 5bp)TTTTTGCATC CT GCTG TTTAAT CT GCGTTGG(Δ 3bp)TTTAACCGCCTGTCTGGCTTTTTTCACTGA TGTG(Δ 1bp)ATCGCC <i>TTGATGCACT</i>
SAS: SAS1	CAACGAGTCTTTTGTCTACAG G
SDS: SDS1	AG GTAAG

BD domain contains following modifications compared to the native lacZ sequence: 11x substitution (bold), 3x deletion (Δ), 1x artificial sequence (*italic*).

Table S3. Differentially expressed genes.

Gene	ID	log2FoldChange	pvalue	padj
<i>Rad54b</i>	ENSMUSG00000078773	2.36	1.72E-16	3.79E-13
<i>Manba</i>	ENSMUSG00000028164	1.77	8.14E-38	1.08E-33
<i>Slc6a2</i>	ENSMUSG00000055368	1.65	1.88E-29	1.24E-25
<i>Gm19410</i>	ENSMUSG00000109372	1.59	4.31E-10	5.18E-07
<i>Frrs1</i>	ENSMUSG00000033386	1.46	5.76E-13	1.09E-09
<i>Podn</i>	ENSMUSG00000028600	1.39	6.49E-12	1.07E-08
<i>Opn1mw</i>	ENSMUSG00000031394	1.29	1.87E-25	8.22E-22
<i>Ifi47</i>	ENSMUSG00000078920	1.20	1.09E-06	5.61E-04
<i>Gm16062</i>	ENSMUSG00000087249	1.18	6.95E-07	4.48E-04
<i>ligp1</i>	ENSMUSG00000054072	1.13	1.91E-06	7.88E-04
<i>BC065403</i>	ENSMUSG00000097211	1.13	1.40E-04	1.01E-02
<i>Cutal</i>	ENSMUSG00000026870	1.09	6.47E-06	1.68E-03
<i>1500026H17Rik</i>	ENSMUSG00000097383	1.09	9.25E-05	7.69E-03
<i>Fam204a</i>	ENSMUSG00000057858	1.09	6.62E-17	1.75E-13
<i>Shisa2</i>	ENSMUSG00000044461	1.07	4.18E-18	1.38E-14
<i>Gucy1a2</i>	ENSMUSG00000041624	-1.07	1.00E-03	2.95E-02
<i>Malat1</i>	ENSMUSG00000092341	-1.10	1.77E-03	3.98E-02
<i>Klf12</i>	ENSMUSG00000072294	-1.17	2.02E-06	7.97E-04
<i>Kcnh5</i>	ENSMUSG00000034402	-1.18	3.85E-04	1.72E-02
<i>Dok6</i>	ENSMUSG00000073514	-1.28	5.92E-05	5.79E-03
<i>Gm11808</i>	ENSMUSG00000068240	-1.28	7.70E-04	2.51E-02
<i>Gm340</i>	ENSMUSG00000090673	-1.55	6.85E-06	1.68E-03
<i>Rho</i>	ENSMUSG00000030324	-1.69	6.85E-06	1.68E-03
<i>Cdr1</i>	ENSMUSG00000090546	-2.22	4.37E-04	1.84E-02
<i>Gm22009</i>	ENSMUSG00000089417	-2.29	1.11E-04	8.76E-03
<i>Gm26917</i>	ENSMUSG00000097971	-2.81	3.22E-04	1.58E-02
<i>Scarna2</i>	ENSMUSG00000088185	-3.37	6.60E-05	6.23E-03
<i>Hist1h4d</i>	ENSMUSG00000061482	-3.59	5.06E-04	2.00E-02
<i>Rmrp</i>	ENSMUSG00000088088	-3.68	1.87E-03	4.05E-02
<i>Gm23935</i>	ENSMUSG00000076258	-3.71	3.06E-04	1.51E-02
<i>Lars2</i>	ENSMUSG00000035202	-3.72	2.85E-04	1.45E-02
<i>Rn7sk</i>	ENSMUSG00000065037	-3.94	4.07E-04	1.76E-02
<i>Gm24187</i>	ENSMUSG00000088609	-3.96	1.35E-04	1.00E-02
<i>Gm42418</i>	ENSMUSG00000098178	-4.42	3.62E-09	3.42E-06
<i>Gm24270</i>	ENSMUSG00000076281	-4.55	4.02E-05	4.62E-03
<i>Mir6236</i>	ENSMUSG00000098973	-5.32	1.12E-05	2.13E-03
<i>Gm15564</i>	ENSMUSG00000086324	-5.37	6.06E-06	1.61E-03

Significant up- or downregulation is defined as absolute log2 fold change > 1 and adjusted p-value < 0.05. DESeq2 was used for RNA-Seq analysis. This includes using the two-sided Wald test to obtain log2 and p-values and using the Benjamini-Hochberg procedure as a correction for multiple testing to obtain adjusted p-values.

Table S4 Splicing events with endogenous transcripts in Cas9-VPR injected retinas.

5' Gene	3' Gene	# of reads	Eye injected with
5' Cas9	Rho exon 3	22	Cas9-VPR + g1-g4
5' Cas9	Opn1mw exon 2	20	Cas9-VPR + g1-g4
5' Cas9	Rho g1 cut site	11	Cas9-VPR + g1-g4
5' Cas9	Pacsin2 exon 2	4	Cas9-VPR + g1-g4
5' Cas9	Stam2 exon 14	3	Cas9-VPR + g1-g4
5' Cas9	Rtn4rl2 exon 3	2	Cas9-VPR + g1-g4
5' Cas9	Mecp2 exon 2	2	Cas9-VPR + g1-g4
5' Cas9	Paip2 exon 3	2	Cas9-VPR + g1-g4
5' Cas9	Gmps exon 13	2	Cas9-VPR + g1-g4
Cpsf6 exon 9	3' Cas9	2	Cas9-VPR + g1-g4
5' Cas9	Slc38a6 exon 13	1	Cas9-VPR + g1-g4
5' Cas9	Slc30a9 exon 2	1	Cas9-VPR + g1-g4
5' Cas9	Lpcat1 exon 2	1	Cas9-VPR + g1-g4
5' Cas9	Sdf2 exon 4	1	Cas9-VPR + g1-g4
5' Cas9	Cfap418 exon 2	1	Cas9-VPR + g1-g4
5' Cas9	Abdhd14a exon 3	1	Cas9-VPR + g1-g4
5' Cas9	Tbc1d8 exon 2	1	Cas9-VPR + g1-g4
5' Cas9	Pard3 exon 23	1	Cas9-VPR + g1-g4
5' Cas9	Zmat1 exon 2	1	Cas9-VPR + g1-g4
5' Cas9	Opn1mw exon 1	1	Cas9-VPR + g1-g4
5' Cas9	AW046200 lncRNA	1	Cas9-VPR + g1-g4
Arid3b exon 3	3' Cas9	1	Cas9-VPR + g1-g4
5' Cas9	Garnl3 exon 10	2	Cas9-VPR + gL
Pacsin2 exon 1	3' Cas9	2	Cas9-VPR + gL
5' Cas9	Rho exon 3	1	Cas9-VPR + gL
5' Cas9	Stam2 exon 14	1	Cas9-VPR + gL
5' Cas9	Aatf exon 6	1	Cas9-VPR + gL
5' Cas9	Cox7b exon 2	1	Cas9-VPR + gL
5' Cas9	Shld1 exon 3	1	Cas9-VPR + gL
5' Cas9	Ulk4 exon 33	1	Cas9-VPR + gL
5' Cas9	Srgap2 exon 6	1	Cas9-VPR + gL
5' Cas9	Atp1a3 exon 17	1	Cas9-VPR + gL
5' Cas9	Rims2 exon 23	1	Cas9-VPR + gL
5' Cas9	Gls exon 4	1	Cas9-VPR + gL
5' Cas9	Gt(ROSA)26Sor	1	Cas9-VPR + gL

Table S5 sgRNA sequences.

sgRNA	Spacer sequence	Target and purpose	Figure
g1 Myo7b	AGACTCCAAGAACGCCAGTC	Transcriptional activation of <i>Myo7b</i>	3, S4
g2 Myo7b	GGGCACCATTAACCACTGCT	Transcriptional activation of <i>Myo7b</i>	3, S4
g3 Myo7b	GGAAGGGCTCCAAGCGGAAC	Transcriptional activation of <i>Myo7b</i>	3, S4
g1 Rho	GTACGGTGACGTAGAGCGTG	<i>Rho</i> exon 1, Knockout of <i>Rho</i>	4, S5
g2 Opn1mw	GGGGCCTTTAAGGTA	Transcriptional activation of <i>Opn1mw</i>	4, S5
g3 Opn1mw	GCCACCCCTGTGGAT	Transcriptional activation of <i>Opn1mw</i>	4, S5
g4 Opn1mw	CTTGCTTGTTTACAA	Transcriptional activation of <i>Opn1mw</i>	4, S5
g5 Opn1mw	GTCCTGTAACCCCAT	Transcriptional activation of <i>Opn1mw</i>	S5
g6 Opn1mw	GATGATCTAAGTCCT	Transcriptional activation of <i>Opn1mw</i>	S5
g7 Opn1mw	CTGCAGGATCAGCCC	Transcriptional activation of <i>Opn1mw</i>	S5
<i>Ush2a</i> exon12 #2	CAGACCTCACAAGCACTCCA	<i>Ush2a</i> exon 12, Nicking gRNA for PE3	S5

Table S6 pegRNA sequences used in Fig. S5.

pegRNA	Spacer sequence	3' extension	RT template length (nt)	PBS length (nt)
<i>HEK3_CTT</i>	GGCCCAGACTGA GCACGTGA	TCTGCCATCAAAGC GTGCTCAGTCTG	13	13
<i>Dnmt1_5d_5</i> GtoT	CGGGCTGGAGC TGTTGCGCGC	AAGATGCAAGCGC GAACAGCTCCAG	12	13
<i>Ush2a</i> exon 12 #1 + #2	AACTCTGTGATC CGCTTTCT	TGACACTGCCCAGA AAGCGGATCACAGA	15	13
<i>Rho</i> exon 1 #1	AGTACTGCGGCT GCTCGAAG	GGAGTCCCTTCGAG CAGCCGCAG	10	13
<i>Rho</i> exon 1 #2	CCAACGTCACAG GCGTGGTG	AGGGGCTTCGCAC CACGCCTGTGACG	13	13
<i>Rho</i> exon 1 #3	AGTACTGCGGCT GCTCGAAG	GTGCGGAGTCCCTT CGAGCAGCCGCA	14	12
<i>Cnga1</i> exon 9 #1	CAAGAAAGGGGA CATCGGGC	GTACATCTCTCGCC CGATGTCCCCTTT	14	13
<i>Cnga1</i> exon 9 #2	TATGCAAGAAAG GGGACATC	TACATCTCCCGTCC GATGTCCCCTTTC	17	10
<i>Cnga1</i> exon 9 #3	CAAGAAAGGGGA CATCGGGC	GTACATCTCTCGCC CGATGTCCCCTT	14	12

Table S7 Primer sequences.

Primer	Sequence (5' – 3')
Cas9 forward	AGAACGCTTGAAACTTACGCT
Cas9 reverse	TTGATGTCCAGTTCCTGATCC
Cerulean forward	ATGGTGAGCAAGGGCGAGG
Cerulean reverse	CTTGACAGCTCGTCCATGCC
Cnga1 forward	TGTGAAGCTGGTCTGTTGGT
Cnga1 reverse	CCTCCCTTTCTCTTCCAGCA
Dnmt1 forward	GTGTGGTACATGCTGCTTCCG
Dnmt reverse	CTCCCTCAAGCTCCCAGTCAAT
HEK3 forward	GCATGCATTTGTAGGCTTGA
HEK3 reverse	CTTTTCCTCTGTTGAGCTCG
HEK3 + adapter forward	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGC ATGCATTTGTAGGCTTGA
HEK3 + adapter reverse	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCT TTTCCTCTGTTGAGCTCG
ITR2 forward	GGAACCCCTAGTGATGGAGTT
ITR2 reverse	CGGCCTCAGTGAGCGA
PE2 forward	CGATGTGGACGCTATCGTGC
PE2 reverse	GCCTTGCCGATTTCTGCTC
qRT-PCR 5'ABCA4 F	ACCACCACCTTGTCCATCCT
qRT-PCR 5'ABCA4 R	ATGTGCTCAGCCACCGTGAG
qRT-PCR 3'ABCA4 F	GTCCCAGCACAGCCTATGT
qRT-PCR 3'ABCA4 R	CTGAGCAGCGTCCGGTTATT
qRT-PCR 5'+3' ABCA4 F (split-site spanning)	CTGCTCCTGAAGTATCGCTCAG
qRT-PCR 5'+3' ABCA4 R (split-site spanning)	AGCCTGTGCCAAAGCAGTTC
qRT-PCR Alas forward	TCGCCGATGCCATTCTTATC
qRT-PCR Alas reverse	GGCCCCAACTTCCATCATCT
qRT-PCR Actb forward	CACACCCGCCACCAGTTC
qRT-PCR Actb reverse	CCCACGATGGAGGGGAATAC
qRT-PCR Myo7b forward	GGGACACAAGTACAGGAAGGA
qRT-PCR Myo7b reverse	GCGTTCAAAGCCCACTAGG
qRT-PCR Cas9 reconst forward	AGCACAAGTTTCTGGCCAGGG
qRT-PCR Cas9 reconst reverse	GGTAGTTTGGTTCTCTCGGGCC
qRT-PCR Opn1mw forward	GGAGCAGGTAAGTGGCCTTATG
qRT-PCR Opn1mw reverse	GGAGGTAGCAGAGCACGATG
qRT-PCR Rho forward	GGATCATGGCGTTGGCCTGT
qRT-PCR Rho reverse	CCGCATGAACATTGCATGCCC
Rho forward	AGCCTTGGTCTCTGTCTACG
Rho reverse	TGGTGAATCCTCCGAAGACC
Ush2a forward	CCAGGGCTTAGATGCAATCG
Ush2a reverse	AAGTAGCCTGCCTTACACTG

Supplementary Note 1: Sequences of REVeRT plasmids for therapeutic purposes.

Sequences provided:

1. pAAV-3xsgMyo7b-CMV-5'dCas9-SDS-BD10-SV40pA (7484 bp)
2. pAAV-CMV-BD10-SAS620-3'dCas9-VPR-synpA (7699 bp)
3. pAAV-sglacZ-RHO-5'Cas9-SDS-BD10-SV40pA (6351 bp)
4. pAAV-RHO-SAS-BD10-3'Cas9-VPR-synpA (7285 bp)
5. pAAV-sgRho-2xsgOpn1mw-RHO-5'Cas9-SDS-BD10-SV40pA (7056 bp)
6. pAAV-sgRho-3xsgOpn1mw-RHO-5'Cas9-SDS-BD10-SV40pA (7413 bp)
7. pAAV-sgRho-2xsgOpn1mw-RHO-Cas9N-RmalntN-SV40pA (7154 bp)
8. pAAV-RHO-RmalntC-Cas9C-VPR-synpA (7343 bp)
9. pGL2-RHO-5'ABCA4-SDS-BD10-SV40pA (9742 bp)
10. pGL2-RHO-BD10-SAS-3'ABCA4-SV40pA (9858 bp)
11. pGL2-GRK1-5'ABCA4-BD10-SDS-SV40pA (9929 bp)
12. pGL2-GRK1-BD10-SAS-3'ABCA4-SV40pA (10045 bp)

Color code:

Gene of interest coding sequence

Intronic part of SAS

Intronic part of SDS

SV40pA

synpA

BD10

N- or C-intein

RHO promoter

CMV enhancer + promoter

hU6 promoter

GRK1 promoter

1. pAAV-3xsgMyo7b-CMV-5'dCas9-SDS-BD10-SV40pA (7484 bp)

CAGGAAACAGCTATGACCATGATTACGCCAGATTTAATTAAGGCTGCGCGCTCGCTCGCTCACTG
AGGCCGCCCCGGGCAAAGCCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG
CGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCTTGATGTTAATGATTAACC
CGCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGATCGGAATTCGCCCTTAAGGGCGCGC
CGTTTAAACGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAG
TAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGT
AACTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGAGACTCC
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AAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTCGGTGCTTTTTTTGTAAACGA
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AACGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGC
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ATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTAC
GGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTAT
GTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAAC
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GGCACCAAAATCAACGGGACTTTCCAATGTCTGTAACAACCTCCGCCCCATTGACGCAAATGGGC
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GACTAACTTTGATAAAAATCTGCCTAACGAAAAGGTGCTTCCTAAACACTCTCTGCTGTACGAGTA
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2. pAAV-CMV-BD10-SAS-3'dCas9-VPR-synpA (7699 bp)

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CGCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGATCGGAATTCGCCCTTAAGGGCGCGC
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CCAGGTGCGGCCGC

CATCTGACCACCTGCGAATTTTTGCATCCTGCTGTTAATCTGCGTTGGTT
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GAAGCTCTGCTGCAGCTGCAGTTCGACGACGAGGATCTGGGAGCCCTGCTGGGAAACAGCACC
GATCCTGCCGTGTTACCGACCTGGCCAGCGTGGACAACAGCGAGTTCCAGCAGCTGCTGAACC
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TTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCA
CGAGTGGGTACATCGAACTGGATCTCAATAGTGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGA
ACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGC
CGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGTTGAGTACTCACCAG
TCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATG
AGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTT
TTTGACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC
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AACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAG
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CGCCTACATACTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGT
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GGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTG
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GGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTC
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3. pAAV-sglacZ-RHO-5'Cas9-SDS-BD10-SV40pA (6351 bp)

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AATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTA
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ACAGCGATGTCGACAACTCTTTATCCAACCTGGTTCAGACTTACAATCAGCTTTTGAAGAGAACC
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GACGAGCGTGACACCACGATGCCTGTAGTAATGGTAACAACGTTGCGCAAACCTATTAACCTGGCGA
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CGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCC
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4. pAAV-RHO-BD10-SAS-3'Cas9-VPR-synpA (7285 bp)

CAGGAAACAGCTATGACCATGATTACGCCAGATTTAATTAAGGCTGCGCGCTCGCTCGCTCACTG
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CGAGCGCGCAGAGAGGGAGTGCCAACTCCATCACTAGGGGTTCTTGATGTTAATGATTAACC
CGCCATGCTACTTATCTACGTAGCCATGCTCTAGGAAGATCGGAATTCGCCCTTAAGGGCGCGC
CGTTTAAATAGCTAGCCCTCTCCTCCCTGACCTCAGGCTTCCTCCTAGTGTCACCTTGGCCCCCTC
TTAGAAGCCAATTAGGCCCTCAGTTTCTGCAGCGGGGATTAATATGATTATGAACACCCCCAATCT
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TCAGAACCCAGAGTCATCGGTACCACCGGTCGACTAGAGGATCCAGGTGCGGCCGC **CATCTGAC**
CACCTGCGAATTTTTGCATCCTGCTGTTAATCTGCGTTGGTTTAACCGCCTGCTGGCTTTTTT
CACTGATGTGATCGCCTTGATGCACTGCGGCCGC**CAACGAGTCTTTGTACCTACAG**GTAGCCC
AGCTATCAAAAAGGGAATACTGCAGACCGTTAAGGTCGTGGATGAACCTCGTCAAAGTAATGGGAA
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5. pAAV-sgRho-2xsgOpn1mw-RHO-5'Cas9-SDS-BD10-SV40pA (7056 bp)

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6. pAAV-sgRho-3xsgOpn1mw-RHO-5'Cas9-SDS-BD10-SV40pA (7413 bp)

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7. pAAV-sgRho-2xsgOpn1mw-RHO-Cas9N-RmaIntN-SV40pA (7154 bp)

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AATGGCTACGCCGGATACATTGACGGCGGAGCAAGCCAGGAGGAATTTTACAAATTTATTAAGCC
CATCTTGAAAAAATGGACGGCACCAGGAGCTGCTGGTAAAGCTTAACAGAGAAGATCTGTTG
CGCAAACAGCGCACTTTGACAATGGAAGCATCCCCACCAGATTACCTGGGCGAACTGCACG
CTATCCTCAGGCGGCAAGAGGATTTCTACCCCTTTTTGAAAGATAACAGGGAAAAGATTGAGAAA
ATCCTCACATTTTCGGATACCCTACTATGTAGGCCCCCTCGCCCCGGGAAATTCAGATTTCGCGT
GATGACTCGCAAATCAGAAGAGACCATCACTCCCTGGAACCTTCGAGGAAGTCGTGGATAAGGGG
GCCTCTGCCAGTCCTTCATCGAAAGGATGACTAACTTTGATAAAAAATCTGCCTAACGAAAAGGT
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CGTCACAGAAGGGATGAGAAAGCCAGCATTCTGTCTGGAGAGCAGAAGAAAGCTATCGTGGAC
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GAATGTTTCGACTCTGTTGAAATCAGCGGAGTGGAGGATCGCTTCAACGCATCCCTGGGAACGTA
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TTGAGGACATTGTCCTCACCTTACGTTGTTTGAAGATAGGGAGATGATTGAAGAACGCTTGA
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GGGGCGGCTGTCAAGAAAATGATCAATGGGATCCGAGACAAGCAGAGTGGAAGACAATCCTG

GATTTTCTTAAGTCCGATGGATTTGCCAACCAGGAAGTTCATGCAGTTGATCCATGATGACTCTCTC
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AATCCCTACTGCCTCGTATCTCGAGCTCGATGAGTTTGGACAAACCACAAGTAGAATGCAGTGA
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TCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCT
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CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCCTTTC
TCGCCACGTTCCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTT
AGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATC
GCCCGGATAGACGGTTTTTCGCCCTTTGACGCTGGAGTTCACGTTCTCAATAGTGGACTCTTGT
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CGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGT
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GGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAAC
AATTCACA

8. pAAV-RHO-RmaIntC-Cas9C-VPR-synpA (7343 bp)

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9. pGL2-RHO-5'ABCA4-SDS-BD10-SV40pA (9742 bp)

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GCCAACTTCGACTGGAGGGACATATTTAACATCACTGATCGCACCCCTCCGCTGGTCAATCAATA
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10. pGL2-RHO-BD10-SAS-3'ABCA4-SV40pA (9858 bp)

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TTATGAACACCCCCAATCTCCAGATGCTGATTACGCCAGGAGCTTAGGAGGGGGAGGTCACTTT
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11. pGL2-GRK1-5'ABCA4-BD10-SDS-SV40pA (9929 bp)

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