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Supplemental Data

Human iPSC Modeling Reveals Mutation-Specific Responses to Gene Therapy in a Genotypically Diverse Dominant Maculopathy

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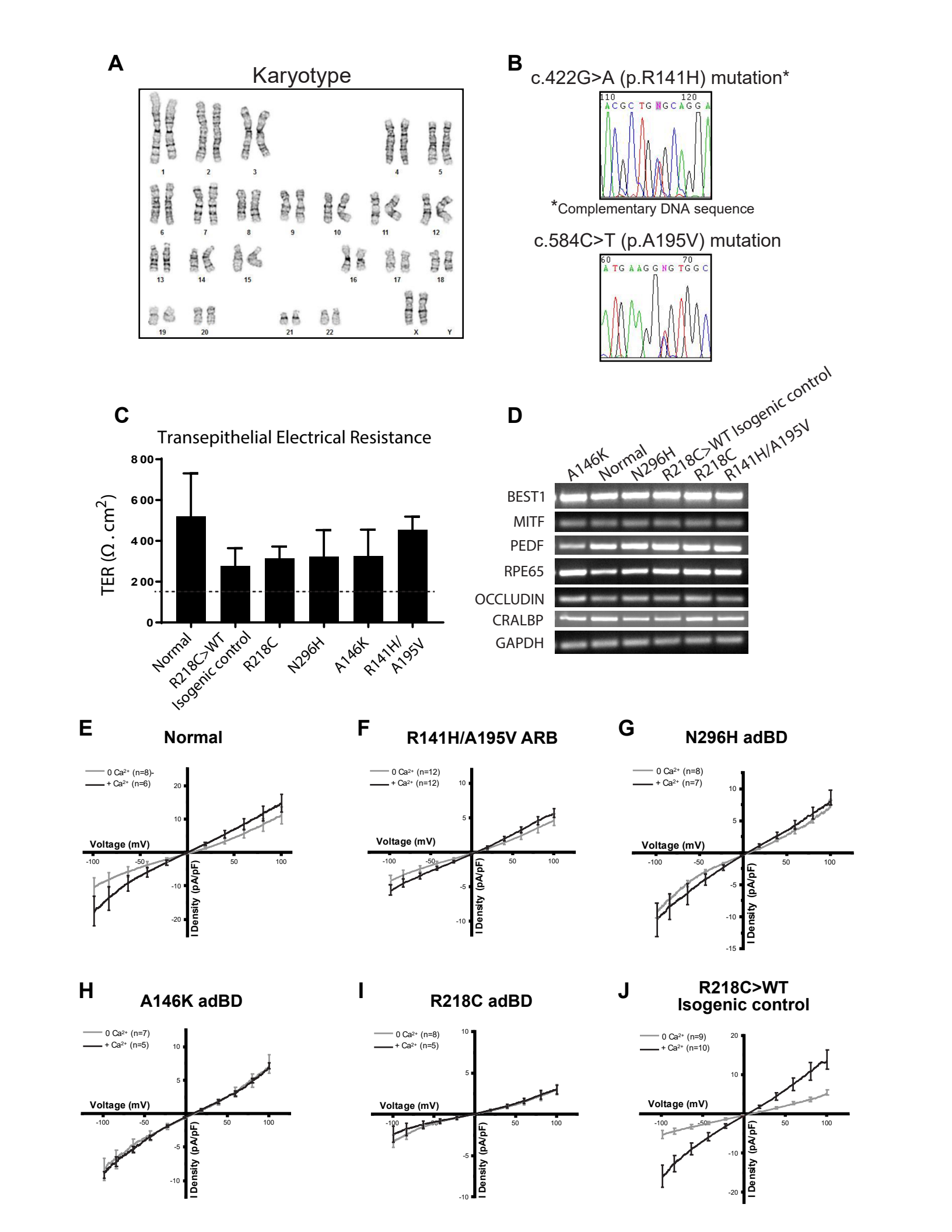
A
Karyotype
B
c.422G>A (p.R141H) mutation*
*Complementary DNA sequence
c.584C>T (p.A195V) mutation
C
Transepithelial Electrical Resistance
TER (Ω . cm2)
Normal
R218C>WT Isogenic control
R218C
N296H
A146K
R141H/ A195V
D
A146K
Normal
N296H
R218C>WT Isogenic control
R218C
R141H/A195V
BEST1
MITF
PEDF
RPE65
OCCLUDIN
CRALBP
GAPDH
E
Normal
F
R141H/A195V ARB
G
N296H adBD
H
A146K adBD
I
R218C adBD
J
R218C>WT Isogenic control
Voltage (mV)
I Density (pA/pF)

Figure S1. Characterization of iPSC-RPE. (A) Karyotype analysis for ARB iPSCs. **(B)** DNA sequencing confirming p.Arg141His and p.Ala195Val encoding mutations in ARB iPSCs. **(C)** Net transepithelial electrical resistance (TER) ($\Omega \cdot cm^2$) for iPSC-RPE from all six lines. The dashed line demarcates the minimum expected TER (150 $\Omega \cdot cm^2$). Replicates: n=12 for each line (4 transwells from 3 replicates each), error bars represent mean ± SD. **(D)** Gene expression analysis (RT-PCR) of selected RPE-specific markers in all six lines. **(E-J)** Chloride current traces, measured in the presence (*black*) or absence (*gray*) of calcium over a voltage ramp (-100 to +100 mV), that were used to generate CaCC current density plots in Figure 1E. 4.5 μM calcium was used for +calcium conditions. The number (n) of individual cells patch clamped in the presence or absence of calcium in order to calculate CaCC current densities is shown in the top left corner of each graph. Data were obtained from at least two replicates. Error bars represent mean ± SEM.

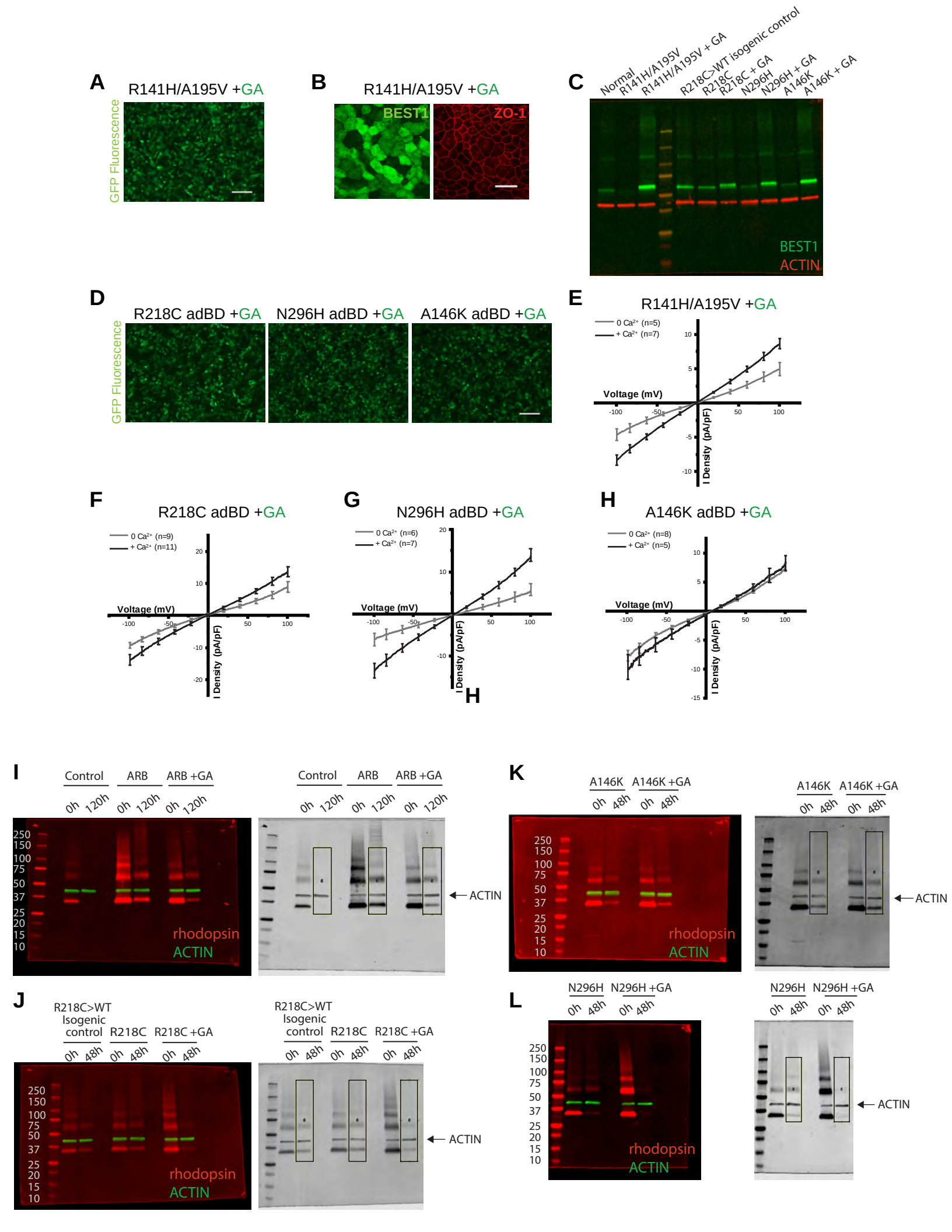
A
R141H/A195V +GA
GFP Fluorescence
B
R141H/A195V +GA
BEST1
ZO-1
C
Normal
R141H/A195V
R141H/A195V + GA
R218C>WT isogenic control
R218C
R218C + GA
N296H
N296H + GA
A146K
A146K + GA
BEST1
ACTIN
D
R218C adBD +GA
N296H adBD +GA
A146K adBD +GA
GFP Fluorescence
E
R141H/A195V +GA
0 Ca2+ (n=5)
+ Ca2+ (n=7)
Voltage (mV)
I Density (pA/pF)
F
R218C adBD +GA
0 Ca2+ (n=9)
+ Ca2+ (n=11)
Voltage (mV)
I Density (pA/pF)
G
N296H adBD +GA
0 Ca2+ (n=6)
+ Ca2+ (n=7)
Voltage (mV)
I Density (pA/pF)
H
A146K adBD +GA
0 Ca2+ (n=8)
+ Ca2+ (n=5)
Voltage (mV)
I Density (pA/pF)
I
Control
ARB
ARB +GA
0h 120h 0h 120h 0h 120h
250 150 100 75 50 37 25 20 15 10
rhodopsin
ACTIN
Control
ARB
ARB +GA
0h 120h 0h 120h 0h 120h
ACTIN
K
A146K
A146K +GA
0h 48h 0h 48h
250 150 100 75 50 37 25 20 15 10
rhodopsin
ACTIN
A146K
A146K +GA
0h 48h 0h 48h
ACTIN
J
R218C>WT
Isogenic
control
R218C
R218C +GA
0h 48h 0h 48h 0h 48h
250 150 100 75 50 37 25 20 15 10
rhodopsin
ACTIN
R218C>WT
Isogenic
control
R218C
R218C +GA
0h 48h 0h 48h 0h 48h
ACTIN
L
N296H
N296H +GA
0h 48h 0h 48h
250 150 100 75 50 37 25 20 15 10
rhodopsin
ACTIN
N296H
N296H +GA
0h 48h 0h 48h
ACTIN

Figure S2. Gene augmentation (GA) restores CaCC function in ARB iPSC-RPE, and Arg218Cys and Asn296His adBD iPSC-RPE, but not in Ala146Lys adBD iPSC-RPE. (A) GFP fluorescence in Arg141His/Ala195Val ARB iPSC-RPE transduced with lentivirus expressing *BEST1*. Scale bar = 100 µm. **(B)** ICC analysis of BEST1 and ZO-1 in Arg141His/Ala195Val iPSC-RPE transduced with lentivirus expressing *BEST1*. Increased BEST1 levels are observed in Arg141His/Ala195Val iPSC-RPE cells following gene augmentation. Scale bar = 50 µm (applies to both images). **(C)** Representative western blot showing levels of BEST1 in iPSC-RPE. Protein samples from the rhodopsin degradation assays were used to assess BEST1 levels. **(D)** GFP fluorescence in adBD iPSC-RPE transduced with lentivirus expressing *BEST1*. Scale bar = 100 µm (applies to all three images). **(E)** Chloride current traces of Arg141His/Ala195Val iPSC-RPE after gene augmentation measured in the presence (*black*) or absence (*gray*) of calcium. Error bars represent mean ± SEM. **(F-H)** Chloride current traces for adBD iPSC-RPE after gene augmentation, measured in the presence (*black*) or absence (*gray*) of calcium over a voltage ramp (-100 to +100 mV), that were used to obtain CaCC current density. 4.5 µM calcium was used for +calcium conditions. Cells with green fluorescence were used for all patch clamp measurements after gene augmentation. The number (n) of individual cells patch clamped in the presence or absence of calcium (in order to calculate CaCC current densities) is shown in the top left corner of each graph. Data were obtained from at least two replicates. Error bars represent mean ± SEM. **(I-L)** Western blots used for the rhodopsin degradation assay (left), and corresponding grayscale images (right) of western blots used to quantify levels of rhodopsin shown in Figures 2 and 3 (boxes represent areas used for quantification). For each lane, the boxed area was selected to include bands corresponding to fully denatured rhodopsin and its aggregated forms.

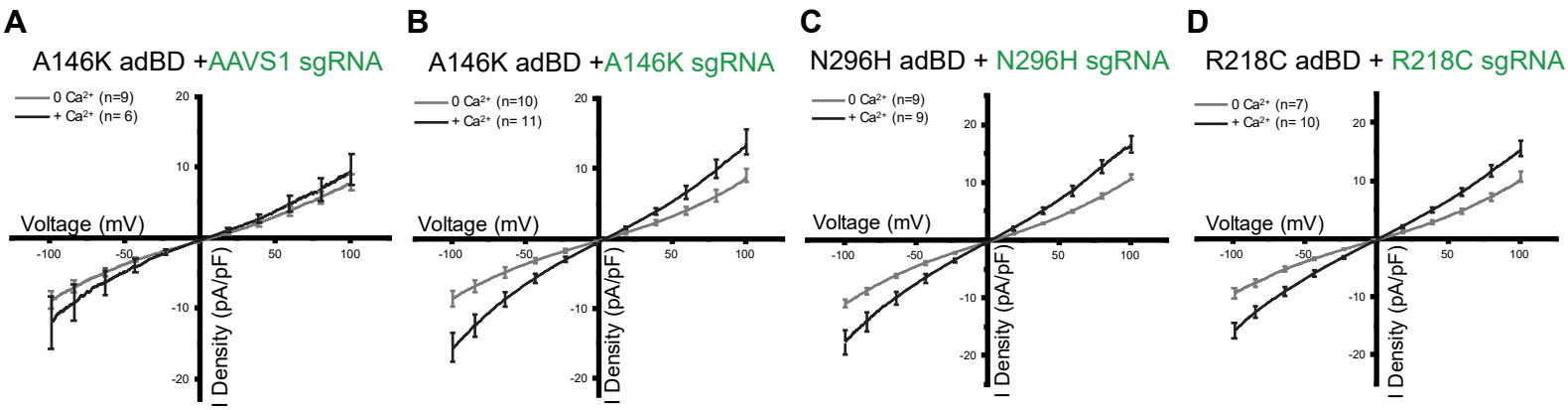
A
A146K adBD +AAVS1 sgRNA
0 Ca2+ (n=9)
+ Ca2+ (n= 6)
Voltage (mV)
I Density (pA/pF)
B
A146K adBD +A146K sgRNA
0 Ca2+ (n=10)
+ Ca2+ (n= 11)
Voltage (mV)
I Density (pA/pF)
C
N296H adBD + N296H sgRNA
0 Ca2+ (n=9)
+ Ca2+ (n= 9)
Voltage (mV)
I Density (pA/pF)
D
R218C adBD + R218C sgRNA
0 Ca2+ (n=7)
+ Ca2+ (n= 10)
Voltage (mV)
I Density (pA/pF)

Figure S3. Gene editing (GE) restores CaCC activity in iPSC-RPE from all tested adBD lines. **(A-D)** Chloride current traces, measured in the presence (*black*) or absence (*gray*) of calcium over a voltage ramp (-100 to +100 mV), that were used to calculate CaCC current density plots after gene editing of adBD iPSC-RPE. iPSC-RPE was edited using lentiviral genome editors encoding sgRNAs that target **(A)** the *AAVS1* site in Ala146Lys adBD iPSC-RPE, or **(B-D)** *BEST1* mutation sites in **(B)** Ala146Lys adBD iPSC-RPE, **(C)** Asn296His adBD iPSC-RPE, or **(D)** Arg218Cys adBD iPSC-RPE. Cells with GFP fluorescence were used for whole cell patch clamp measurements and 4.5 μM calcium was used for +calcium conditions. The number (n) of individual cells patch clamped with or without calcium is shown at the top left corner of each graph. Data were obtained from two replicates. Error bars represent mean ± SEM.

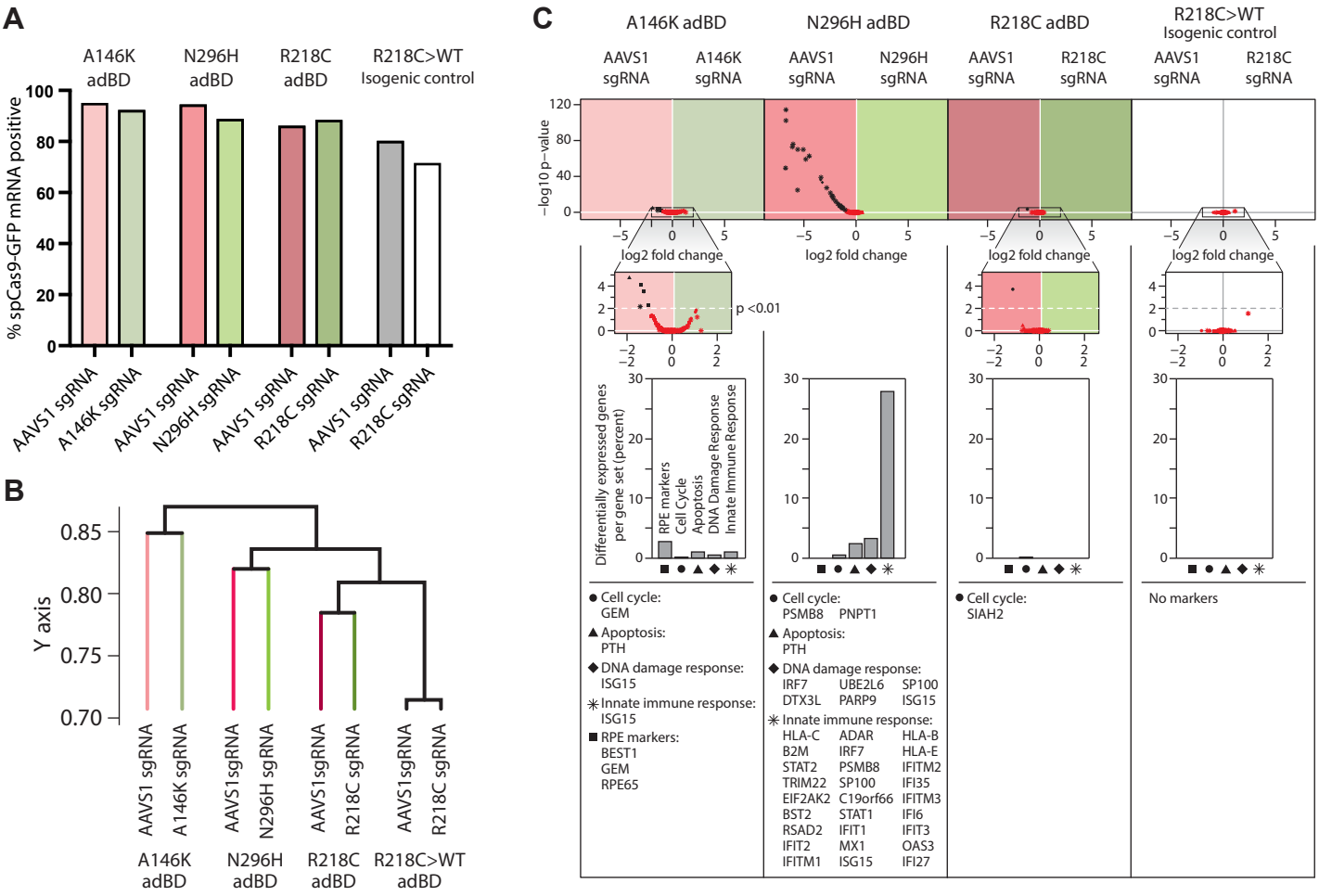
A
A146K adBD
N296H adBD
R218C adBD
R218C>WT Isogenic control
% spCas9-GFP mRNA positive
AAVS1 sgRNA
A146K sgRNA
N296H sgRNA
R218C sgRNA
B
Y axis
0.85
0.80
0.75
0.70
C
AAVS1 sgRNA
−log10 p−value
log2 fold change
p <0.01
Differentially expressed genes per gene set (percent)
RPE markers
Cell Cycle
Apoptosis
DNA Damage Response
Innate Immune Response
Cell cycle: GEM
Apoptosis: PTH
DNA damage response: ISG15
Innate immune response: ISG15
RPE markers: BEST1 GEM RPE65
Cell cycle: PSMB8 PNPT1
Apoptosis: PTH
DNA damage response: IRF7 UBE2L6 SP100 DTX3L PARP9 ISG15
Innate immune response: HLA-C ADAR HLA-B B2M IRF7 HLA-E STAT2 PSMB8 IFITM2 TRIM22 SP100 IFI35 EIF2AK2 C19orf66 IFITM3 BST2 STAT1 IFI6 RSAD2 IFIT1 IFIT3 IFIT2 MX1 OAS3 IFITM1 ISG15 IFI27
Cell cycle: SIAH2
No markers

Figure S4. Single cell transcriptome analysis in gene-edited adBD iPSC-RPE. (A) Percent of analyzed cells per sample for which *spCas9-T2A-GFP* transcripts were captured using scRNA-seq. **(B)** Dendrogram depicting relative similarity between samples. Non-negative matrix factorization comparison across samples indicates that greater transcriptional variability exists between iPSC-RPE lines than in the same iPSC-RPE line treated with lentiviral genome editors (*AAVS1* lentiviral genome editor versus *BEST1* mutant allele-targeted lentiviral genome editor). The dendrogram shows the similarity of the transcriptomes from each sample, derived from the average Jaccard coefficient between gene clusters from one sample and those from another sample. The y-axis denotes 1-average Jaccard coefficient and indicates the distance between different samples (tree tips) as well as between groups of samples (internal nodes). **(C)** Differential gene expression in 5 curated gene sets associated with cell cycle regulation (*circles*), apoptosis (*triangles*), DNA damage response (*diamonds*), innate immune response (*asterisks*), or RPE-identity (*squares*) in control (*AAVS1*) lentiviral genome editor versus mutant allele-targeted lentiviral genome editor treated samples. For one sample pair (Asn296His iPSC-RPE), genes associated with a potential adverse treatment effect were upregulated in control lentiviral genome editor-treated sample compared to the mutant allele-targeted lentiviral genome editor sgRNA-treated sample. $p < 0.01$ was the threshold for determining significant changes in gene expression.

Gene	**Forward Primer**	**Reverse Primer**
BEST1	ATTTATAGGCTGGCCCTCACGGAA	TGTTCTGCCGGAGTCATAAAGCCT
MITF	TTCACGAGCGTCCTGTATGCAGAT	TTGCAAAGCAGGATCCATCAAGCC
PEDF	AATCCATCATTCACCGGGCTCTCT	TGCACCCAGTTGTTGATCTCTTGC
RPE65	GCCCTCCTGCACAAGTTTGACTTT	AGTTGGTCTCTGTGCAAGCGTAGT
OCCLUDIN	TCATTGCCGCGTTGGTGATCTTTG	ATGATGCCCAGGATAGCACTCACT
CRALBP	TTCCGCATGGTACCTGAAGAGGAA	ACTGCAGCCGGAAATTCACATAGC
GAPDH	CAACGGATTTGGTCGTATTGG	GCAACAATATCCACTTTACCACAGTTAA

Table S1: RPE-specific RT-PCR primers used.

GE Vector Name	sgRNA Name	Vector Backbone	Backbone Source
VMD2.*AAVS1*	*AAVS1*	*VMD2-spCas9-T2A-GFP*	Alfred Lewin (University of Florida)
VMD2.R218C	R218C	*VMD2-spCas9-T2A-GFP*	Alfred Lewin (University of Florida)
VMD2.N296H	N296H	*VMD2-spCas9-T2A-GFP*	Alfred Lewin (University of Florida)
VMD2.A146K	A146K	*VMD2-spCas9-T2A-GFP*	Alfred Lewin (University of Florida)

Table S2. List of gene editing vectors used.

Primer Name	Primer sequence
LCv2-GFP.Gib.F	GATTACAAAGACGATGACGATAAGGGATCCGGTGAGGGCAGAGGAAGTC
LCv2-GFP.Gib.	ACAGTCGAGGCTGATCAGCGGGTTTAAACCTACTACTGCTAGAGATTTTCCACAC
LCv2-GFP.seq.L	ACCGGCCTGTACGAGACACG
LCv2-GFP.seq.R	GAAAGGACAGTGGGAGTGGCACC
VMD2.LCv2.GFP.Gib.F	GTGGCACCGAGTCGGTGCTTTTTTGAATTCCAATTCTGTCATTTTACTAGGGTGATGAAATTC
VMD2.LCv2.GFP.Gib.R	TGTACTTCTTGTCCATGGTGGCAGCGCTCTATCGGCCGCGGGTACA
VMD2.LCv2.GFP.seq.L	GAATGAATACCGGGCTGCAGTCAAC
VMD2.LCv2.GFP.seq.R	GTCGGTGATCACGGCCCAG

Table S3. List of primers for lentiviral plasmid generation.

sgRNA Name	Sequence 5' - 3'	PAM	Chr	Position	Strand	Off-Target Score	On-Target Score
A146K	CTTTGGTGCTGACGCTGCGC	AGG	11	61955893	-1	81.2	51.6
R218C	GTGTCCACACTGAGTACACA	AGG	11	61957403	-1	56.3	67.2
N296H	CATCATCCTCTCCAAAGGGG	TGG	11	61959521	-1	54.0	64.6
AAVS1	GGGGCCACTAGGGACAGGAT	TGG	19	55115755	+1	55.8	54.5

Table S4. List of sgRNAs. Off-target [47] and on-target [48] scores are also presented. Scores range from 0-100 with higher scores being better for both scoring systems. Highest ranked off-target cut sites for each sgRNA are available in Supplemental Data File C.

Primer Name	Primer sequence
MT.C.OT.5v2.HTS.F	GTTGGTTCCTGAAGATGGGCAG
MT.C.OT.5v2.HTS.R	CTGTCAAGGCCAAGTTCTGCTG
MT.C.OT.2.HTS.F	GCTAAATTCTGCTATAAAAGGAAGG
MT.C.OT.2.HTS.R	GCATTGCTTTAGAAAACTCAGAAGT
MT.C.OT.3.HTS.F	AGTGAGACCAAGTTCTGACAGCA
MT.C.OT.3.HTS.R	GGCCTCTTCATACATACACATGCAC
MT.C.OT.4.HTS.F	CCTCCACATCTGCAGAAAAGTGT
MT.C.OT.4.HTS.R	GGCAGGGTTTGGTCTCCTACTT
MT.C.OT.5.HTS.F	GGATGGCTCTGGGTGGGTTT
MT.C.OT.5.HTS.R	CTTCCAACTCTTCCTCCCACCC
MT.C.OT.6.HTS.F	TGAGGTTCAGAATAGCTCAGCA
MT.C.OT.6.HTS.R	TGTTTCTGTGAAGCAAATCAAAGCT
MT.C.OT.7.HTS.F	TGTTTCTGTGAAGCAAATCAAAGCT
MT.C.OT.7.HTS.R	TGAGGTTCAGAATAGCTCAGCA
MT.C.OT.8.HTS.F	AAAGCATGGCGGGAGTGCTAA
MT.C.OT.8.HTS.R	TGACTAAATCCCTGGCATCGCT
MT.C.OT.9.HTS.F	GCCAGTAATTTTCCAAGGCTTCT
MT.C.OT.9.HTS.R	TTCCTACTAGAACCTCCTTGAG
MT.C.OT.10.HTS.F	GTGACCTGACTTTGCTGAAAGGT
MT.C.OT.10.HTS.R	ACCTGAATTATCTCAAGCTCACT
AAVS1T2.HTS.F	ATGTGGCTCTGGTTCTGGGTAC
AAVS1T2.HTS.R	GAGACTAGGAAGGAGGAGGCCT
R218C.HTSv2.F	GTGTTCAGAACCCCATCCCC
R218C.HTSv2.R	AGCCTAGTCCTCACCTGTGT
BEST.cDNA.HTSv2.F	GGTCGAATCCGGGACCCTATC
BEST.cDNA.HTSv2.R	GCCACAGTCACCACCTGTGTAT
AAVS1T2.HTS.F	ATGTGGCTCTGGTTCTGGGTAC
AAVS1T2.HTS.R	GAGACTAGGAAGGAGGAGGCCT

Table S5. Primers for deep sequencing of DNA and cDNA.

Lentivirus	Titer (Transduction units/ml)*
VMD2-BEST1-T2A-GFP	22.00 x10^7
VMD2-spCas9-T2A-GFP R218C sgRNA (encodes sgRNA targeting the c.652C>T (p.Arg218Cys) mutant *BEST1* allele)	74.16 x10^7
VMD2-spCas9-T2A-GFP A146K sgRNA (encodes sgRNA targeting the c.436_437delinsAA (p.Ala146Lys) mutant *BEST1* allele)	74.26 x10^7
VMD2-spCas9-T2A-GFP N296H sgRNA (encodes sgRNA targeting the c.886A>C (p.Asn296His) mutant *BEST1* allele)	68.91 x10^7
VMD2-spCas9-T2A-GFP AAVS1 sgRNA (encodes sgRNA targeting *AAVS1* alleles)	74.01 x10^7

*The QuickTiter Lentivirus Titer Kit calculates Transduction units in 10-fold ranges (10^{6-7}).

Table S6. Titers for lentivirus stocks used in this study.