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Supplemental information

Efficient correction of *ABCA4* variants by CRISPR-Cas9 in hiPSCs derived from Stargardt disease patients

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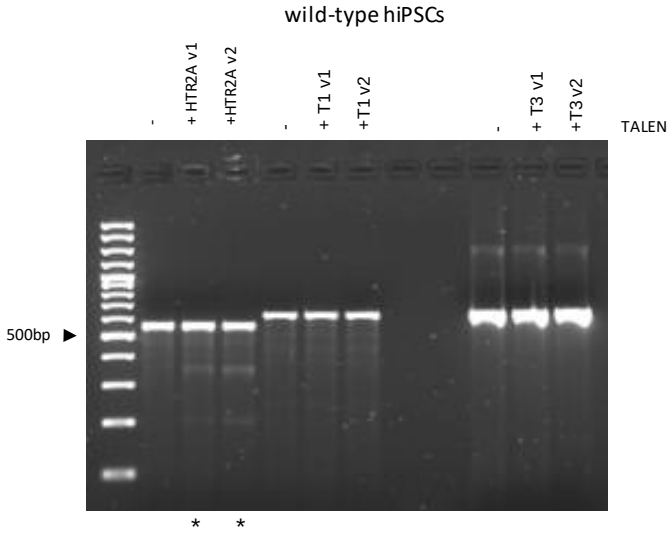
Figure S1

A



B

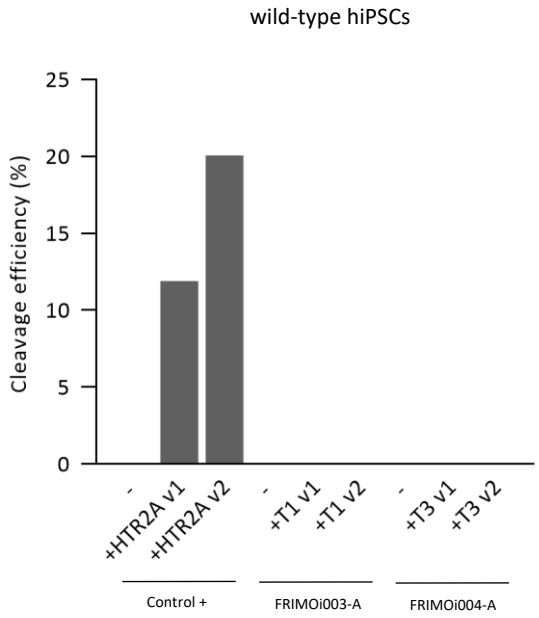
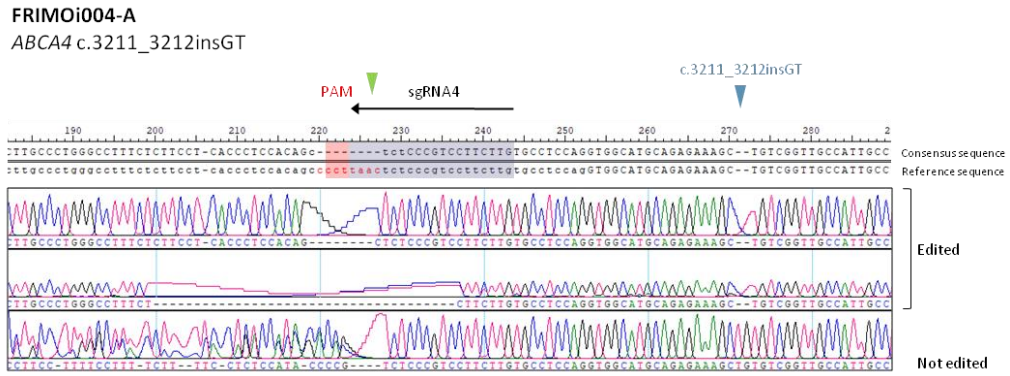


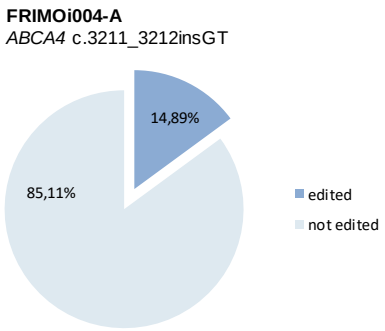
Figure S1. No DNA cleavage achieved by TALEN technology to target *ABCA4* pathogenic variants. (A) PCR products after genomic cleavage detection assay resolved on a 2% agarose gel. Asterisks show gel lanes with expected fragments resulting from T7E1 cutting. Untreated genomic DNA from same wild-type hiPSCs used for transfections was used as a negative control, showing the uncleaved parental band. Two different electroporation conditions were used for each TALEN pair (referred as v1 and v2). **(B)** Band intensity quantification of gel in (A).

Figure S2

A



B



C

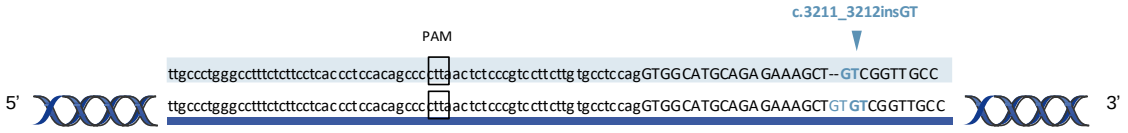
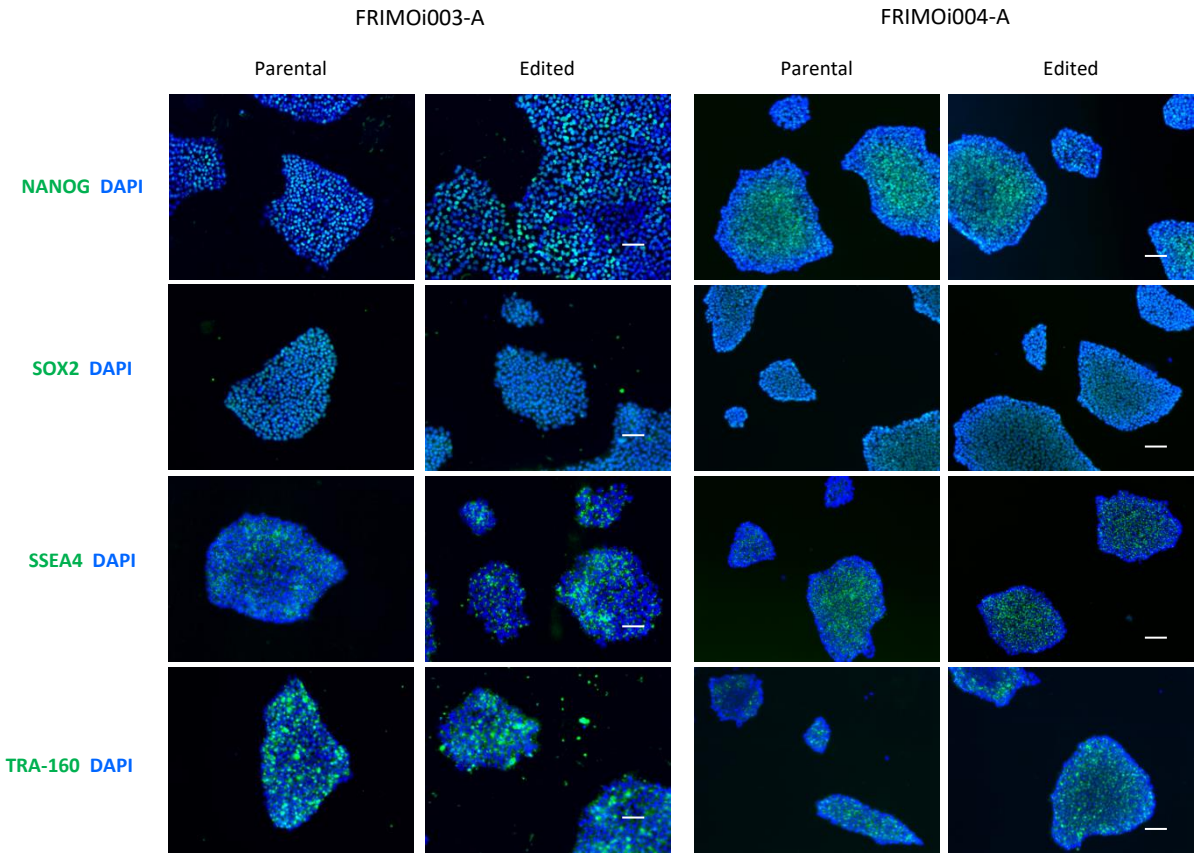


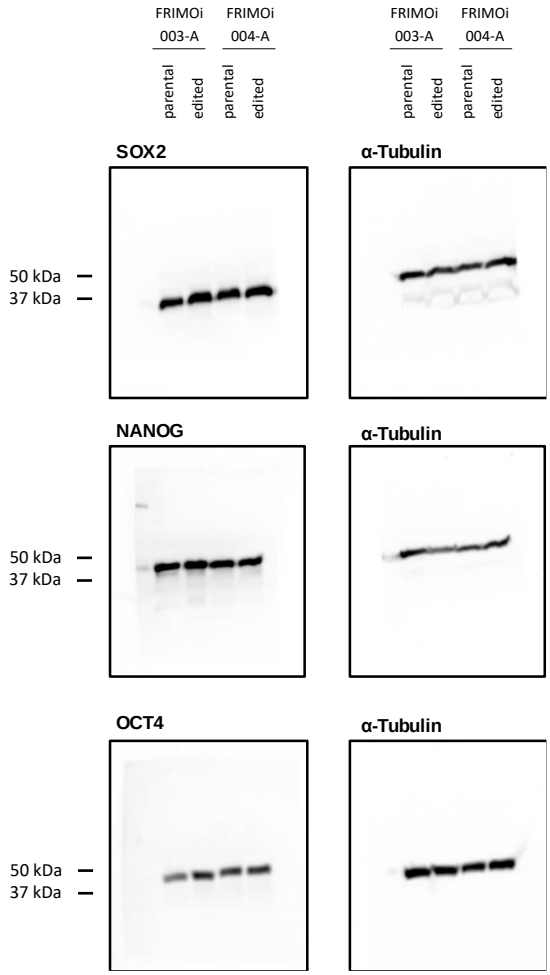
Figure S2. CRISPR/Cas9-mediated gene editing could result in genomic aberrations before experimental design optimization. **(A)** Representative captures of chromatograms showing Sanger sequencing reads (with reverse primers) of PCR products from *ABCA4* specific locus. On top are consensus and reference sequences and below examples of edited and not edited clones. Highlighted are the sgRNA and PAM motif (in red). Green arrowhead point out predicted DNA cut site. Small and large deletions appear near PAM and cut site. **(B)** Pie chart of the percentage of edited and not edited clones according to variant correction in FRIMOi004-A hiPSCs. **(C)** ssODN design for CRISPR/Cas9-mediated gene editing for FRIMOi004-A hiPSC correction before assay optimization. Note that PAM was not modified.

Figure S3

A


FRIMOi003-A
FRIMOi004-A
Parental
Edited
Parental
Edited
NANOG DAPI
SOX2 DAPI
SSEA4 DAPI
TRA-160 DAPI

B



C

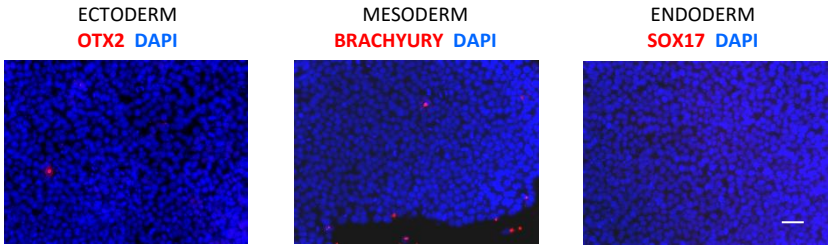


Figure S3. hiPSC clones preserve the expression of pluripotency markers after gene editing. **(A)** Immunofluorescence pictures of pluripotency markers NANOG, SOX2, SSEA4 and TRA-160 counterstained with DAPI in parental and edited clones colonies in FRIMOi003-A and FRIMOi004-A. Representative images are shown. Scale bar represents 100 µm. **(B)** Full unedited blots for Figure 4E. **(C)** As in Figure 4F, immunofluorescence pictures of lineage markers OTX2, BRACHYURY and SOX17 counterstained with DAPI in edited hiPSCs not subjected to the differentiation protocol. Representative images are shown. Scale bar represents 50 µm.

Table S1. List of primers used for genotyping each *ABCA4* locus depending on the sgRNA and TALEN.

Variant	Sequence ID	Forward	Reverse
c.4253+4C>T	1	CTTGGGATGGCGCTAGCTCT	AAGCCCAACCCTCTCCACCA
	2	AAGCCCAACCCTCTCCACCA	ATGCTGCCTTGGGATGGCGC
	T1	CCCTGGATATATGGGCAGCA	AGTGGTCTGATGGCATGTCA
c.6089G>A p.Arg2030Gln	3	GGTAACTTCCAGCATTTTGC	TTCCTTTTCCCGTTGGCA
	T2	CCCATGCATTTCTGAAGCCA	ACTCCTATGTGGCCACAACA
c.3211_3212insGT p.Ser1071Cysfs*14	4	ACACTGGTCCTGAGTGGT	GCACCAAACCACTGCTGGGT
	5	GCCTTTCTCTTCCTCACCCT	CTCAGGAGGCTTTAGCTGGA
	6	GTCTGATCCGAGGAGGTGAG	GTCTCGAGTAAGGGTCCACC
	T3	GCCTTTCTCTTCCTCACCCT	CTCAGGAGGCTTTAGCTGGA
c.2023G>A p.Val675Ile	7	TGCTTCAGGGCTAACATGGA	TGCATTGGAGACACCCTGAT
c.6148G>C p.Val2050Leu	8	AGGCTGAAGTCCATTTCCCA	TTGGTTTAAGCCCTTGGTGC
	9	AGGCTGAAGTCCATTTCCCA	TTGGTTTAAGCCCTTGGTGC

Table S2. *In silico* analysis of potential off-targets for sgRNA2 and sgRNA6.

							Splicing alteration prediction	
sgRNA	**DNAsequence**[a]	**Chr**	**Position**	**Strand**	**Mismatches**	**Locus type**	**Donor**	**Acceptor**
sgRNA2	TTCgTCAGGTGTcaGGACTCGGG	chr1	3143299	+	3	*PRDM16* intron 1	4/4	2/4
	TTCTTCAGGTGTGtGGACaaGGG	chr1	19313503	+	3	*SLC66A1* intron 1	4/4	1/4
	cTCTTCAGGgGTGCaGACTCAGG	chr3	25324476	-	3	*RARB* intron 5	0/4	0/4
	TTCcTCAGtTGTGCGGAgTCAGG	chr9	126203473	+	3	Intergenic	-	-
	TTCTTCAGtTGTaCtGACTCTGG*	chr12	80295911	-	3	*OTOGL* intron 31	-	-
	TTCTTCAaGTcTGCaGACTCAGG	chr22	22363162	+	3	Intergenic	-	-
	TcaTTCAGGTGTGaGGACTCTGG*	chr22	46361593	+	3	*CELSR1* exon 35	-	-
sgRNA6	GCATcCAGAGAAAGCTaTGTAGG*	chr1	112903490	-	2	Intergenic	-	-
	GCcTGgAGAGAAAtCTGTGTTGG	chr1	32346517	+	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr1	69061899	-	3	Intergenic	-	-
	GCATGCAGAGgAgGCTtTGTAGG*	chr1	170663322	-	3	*PRRX1* exon 1	-	-
	GaATGCAGAGAAgGCTtTGTGGG*	chr1	183127284	+	3	*LAMC1*exon 17	-	-
	GtATGCAGAGAAAGtTGgGTGGG	chr2	27885125	-	3	*RBKS* intron 1	0/4	0/4
	GCATGCAGAGccAGCTGTGcAGG	chr2	33616987	+	3	Intergenic	-	-
	GCATGCAcAGAgAGCaGTGTGGG	chr2	129908581	+	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr2	133878772	+	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr3	30145653	-	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr3	161637539	+	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr4	99023883	+	3	*METAP1* intron 1	3/4	3/4
	tCATGCAGAGccAGCTGTGTGGG	chr5	37661519	+	3	*WDR70* intron 10	0/4	0/4
	tCATGCAGAGtcAGCTGTGTGGG	chr5	113221881	-	3	*MCC* intron 1	1/4	2/4
	GCATGCAGAGAAAGCTGgtgTGG	chr5	175897659	+	3	Intergenic	-	-
	aCATGCAGtGAAAGCTGTGgAGG*	chr6	72272584	-	3	*RIMS1* intron 22	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr6	131862447	+	3	*ENPP1* intron 9	0/4	0/4
	GCATGCtGAGAAAGCaaTGTAGG	chr6	150787396	-	3	*PLEKHG1* intron 5	2/4	4/4
	GCATGCAaAGAAAtCTGTGaTGG	chr7	14774828	-	3	*DGKB* intron 2	0/0	0/0
	tCAcGCAGAGAcAGCTGTGTAGG	chr7	20053553	-	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr7	25248655	-	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr7	44988748	+	3	Intergenic	-	-
	GCATGgAGAGActGCTGTGTGGG	chr7	69229227	+	3	Intergenic	-	-
	tCATGCAGAGccAGCTGTGTGGG	chr7	68980776	-	3	Intergenic	-	-

tCATGCAGAGccAGCTGTGTGGG	chr7	73391450	-	3	Intergenic	-	-
GCATGCAGAGcAgGCTGTGcAGG	chr7	128259702	+	3	Intergenic	-	-
caATGCAGAaAAAGCTGTGTTGG	chr7	140190082	+	3	Intergenic	-	-
GCATtCAGAGAAAGCTcTcTGGG	chr8	23471730	+	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr8	57401196	-	3	Intergenic	-	-
cCATGCAGAGAAAGCTtTGaAGG*	chr8	79763025	+	3	*HEY1* exon 5	-	-
atATGCAGAGAAAGCcGTGTGGG	chr8	97697883	+	3	*MTDH* intron 6	3/4	0/4
tCATGCAGAGccAGCTGTGTGGG	chr9	73872853	-	3	Intergenic	-	-
GCATGCAcAGgAAGCTGgGTGGG	chr9	126564877	-	3	Intergenic	-	-
GCATGCtGAGAgAtCTGTGTGGG	chr10	13423675	+	3	Intergenic	-	-
GCATGCAGAaAgAGCTGTcTTGG	chr11	19738132	+	3	*NAV2* intron 1	0/4	0/4
tCATGCAGAGAAgtCTGTGTGGG	chr11	83424208	-	3	Intergenic	-	-
tCATGCAGAGgcAGCTGTGTGGG	chr12	9633829	-	3	Intergenic	-	-
tCATGCAGAGtcAGCTGTGTGGG	chr12	15037314	+	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr12	39544035	+	3	Intergenic	-	-
tCATGCAGAGtcAGCTGTGTGGG	chr12	46218679	+	3	*SLC38A1* intron 5	0/4	2/4
tCATGCAGAGccAGCTGTGTGGG	chr12	101865652	+	3	Intergenic	-	-
GCAaGaAGAGAAAGCTGaGTTGG	chr13	23990943	+	3	*SPATA13* intron 2	0/4	3/4
tCATGCAGAGtcAGCTGTGTGGG	chr13	66184877	+	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr15	26091192	+	3	Intergenic	-	-
GCcTGCAGgGgAAGCTGTGTGGG	chr15	45473248	-	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr15	75167813	+	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr16	83153087	+	3	*CDH13* intron 5	0/4	0/4
GCgTtCAGAGAAgGCTGTGTAGG	chr19	39278607	-	3	Intergenic	-	-
GCATGCAGAGAAAaCTcTcTGGG	chr20	727292	+	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chr20	45444649	+	3	Intergenic	-	-
GgATGCAGtGAAAGCaGTGTGGG	chr21	43335686	+	3	Intergenic	-	-
GtATGCAGAGAAAcCTGTcTGGG	chr22	42898195	-	3	*PACSIN2* intron 2	0/4	0/4
GCAgGCAGAGAgAGCTGTGcAGG	chrX	24453027	-	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chrX	44550392	-	3	Intergenic	-	-
tCATGCAGAGccAGCTGTGTGGG	chrX	121961995	+	3	Intergenic	-	-
GCAgcCAGAGAAAGCTGTGgAGG	chrX	153652105	+	3	Intergenic	-	-
tCATGCAGAGtcAGCTGTGTGGG	chrY	11886966	-	3	Intergenic	-	-

[a]Asterisk indicates selected off-targets for Sanger sequencing

Table S3. List of primers for selected off-targets genotyping.

Off-target ID	Primer sequence		Amplicon (bp)
OT-1	Forward	ATGGTAAGTATATGAGTTGATGAG	298
	Reverse	ATGACACACTTCCACCTCCAG	
OT-2	Forward	AGTGATCAGCACAGCTGCTTC	307
	Reverse	CCTTTTCGTACTTAGGAAACGC	
OT-3	Forward	TTAGGGTCCTCATCATGCTATG	331
	Reverse	GTGAACTTCTTGAGATGAGAGG	
OT-4	Forward	ATTAGCAGCCATCTAAACCTATG	487
	Reverse	AGGAGTTTGTCTTGATCCTGAG	
OT-5	Forward	AGGAAGAAGGAGATTGTGATGG	284
	Reverse	AGTCCGACGGAGGGTGCTG	
OT-6	Forward	TATGACAGTATAGTCAGCTTATAG	418
	Reverse	TGGAACATAAGTCTTTAAGAACAG	
OT-7	Forward	TCGTTGTATCCACTGTATGTGG	419
	Reverse	GACAGCATTTTCTACATTAAGGTA	