The American Journal of Human Genetics, Volume *108*

Supplemental information

Impaired cholesterol efflux in retinal pigment epithelium of individuals with juvenile macular degeneration

Yi-Ting Tsai, Yao Li, Joseph Ryu, Pei-Yin Su, Chia-Hua Cheng, Wen-Hsuan Wu, Yong-Shi Li, Peter M.J. Quinn, Kam W. Leong, and Stephen H. Tsang

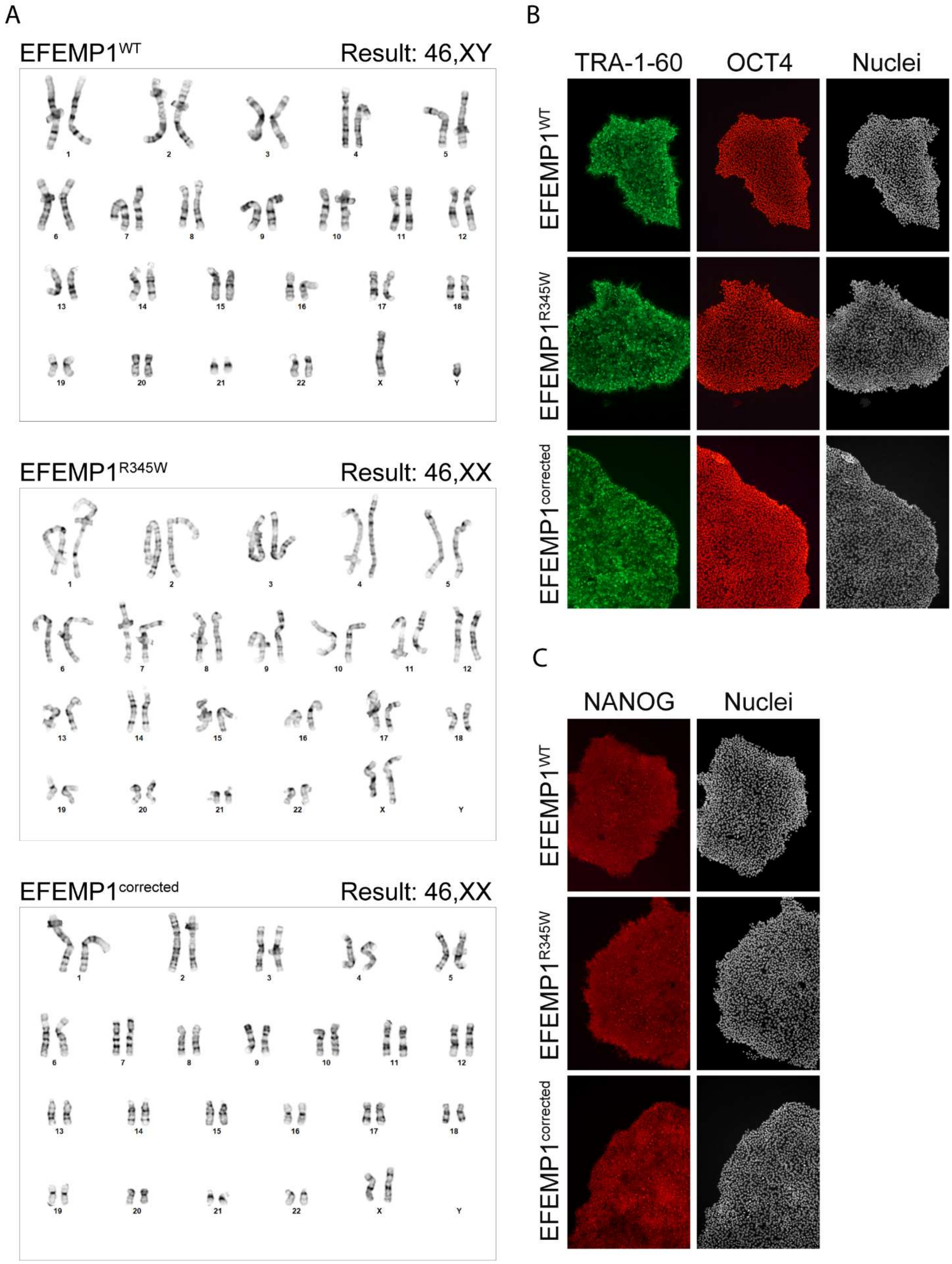


Figure S1. Characterization of iPSC cells. (A) Karyotyping result of the EFEMP1^{WT}, EFEMP1^{R345W} and EFEMP1^{corrected} iPSCs. **(B)** Immunostaining of stem cells markers TRA-1-60 and OCT4. **(C)** Immunostaining of stem cells marker NANOG.

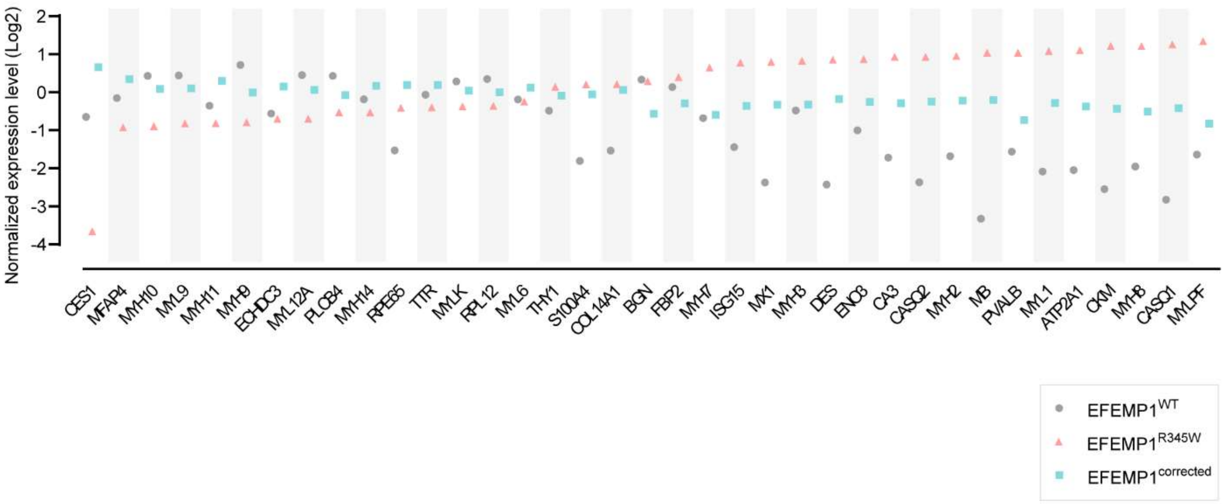


Figure S2. Proteomic analysis of iRPE cells. Expression level of the 37 DEPs shown in Fig. 2(A) determined by mass spectrometry. The order of the proteins is ranked by the mean expression level of the diseased group. EFEMP1^{WT}(n=6), EFEMP1^{R345W} (n=6), and EFEMP1^{corrected} clones (n=6).

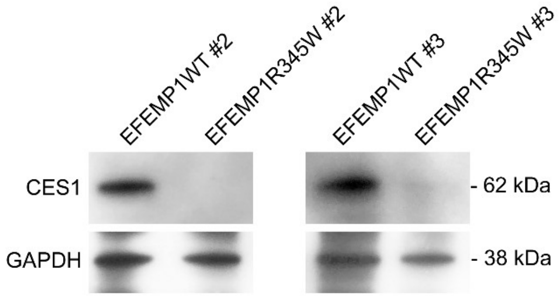


Figure S3. Expression of CES1 in two healthy donor and two DHRD patient iRPE cells. The cell lysates from iRPE derived from healthy donor #2, #3 and DHRD patient #2, #3 were determined by western blot.

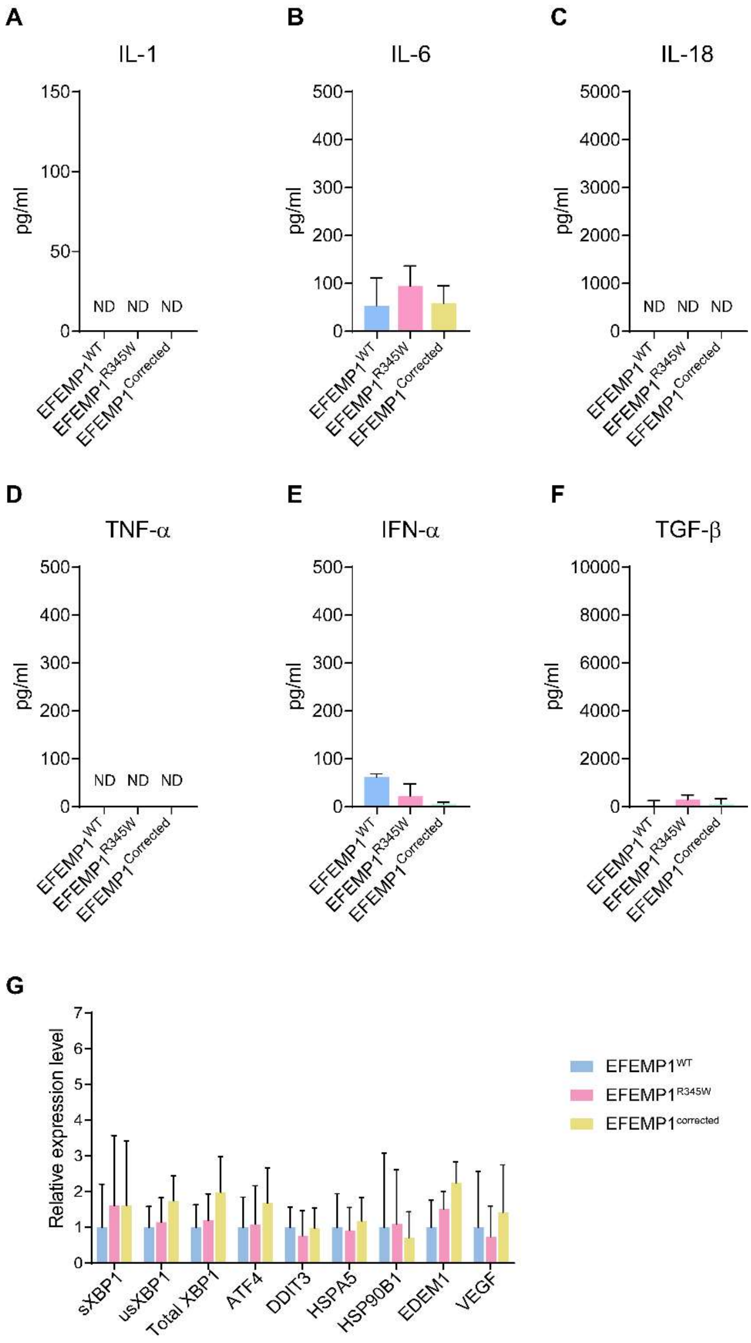


Figure S4. Absence of cytokine changes and UPR in DHRD patient-derived iRPE cells. (A) IL-1, **(B)** IL-6, **(C)** IL-18, **(D)** TNF-alpha, **(E)** IFN-alpha and **(F)** TNF-beta cytokine level in the supernatant of EFEMP1^{WT} (n=12), EFEMP1^{R345W} (n=12), and EFEMP1^{corrected} (n=16) iRPE cell cultures. One-way ANOVA analysis showed no significant differences between the groups. Note: The scale range of each graph was set according to the range of the standard provided in the respective ELISA kits by the manufacturer. **(G)** Real-time PCR analysis of UPR-related gene expression level in EFEMP1^{WT} (n=5), EFEMP1^{R345W} (n=6), and EFEMP1^{Corrected} (n=8) iRPE cells. Geometric mean and standard deviation were used to determine the p-values. Data represented as geometric mean ± geometric SD. One-way ANOVA analysis showed no significant differences between the groups.

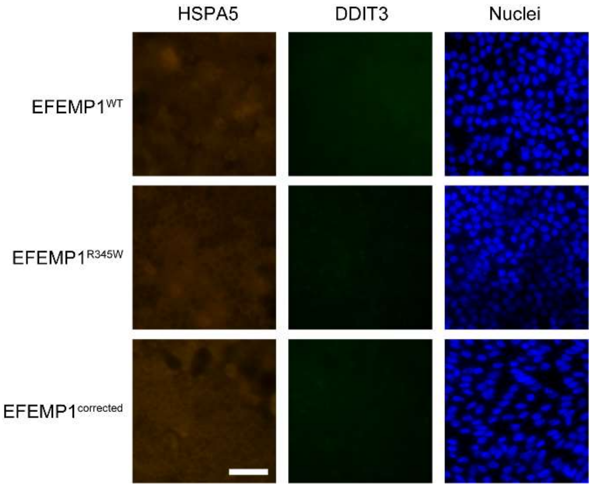


Figure S5. Immunocytochemistry of UPR markers. The staining of HSPA5 (left column) and DDIT3 (middle column) of the EFEMP1^{WT}, EFEMP1^{R345} and EFEMP1^{corrected} iRPE.

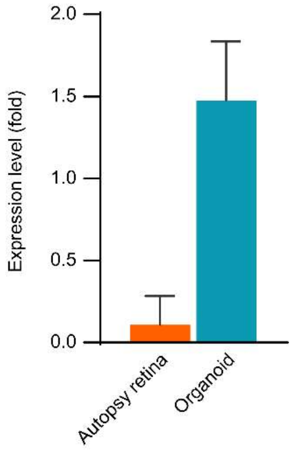


Figure S6. Relative expression of CES1 in retina. The CES1 mRNA level in of retina tissue derived from human autopsy and human iPSC-derived retinal organoid were determined by qPCR. The data is presented as the ratio of the level in retina/the level in RPE. Data represented as geometric mean ± geometric SD. (n=2)

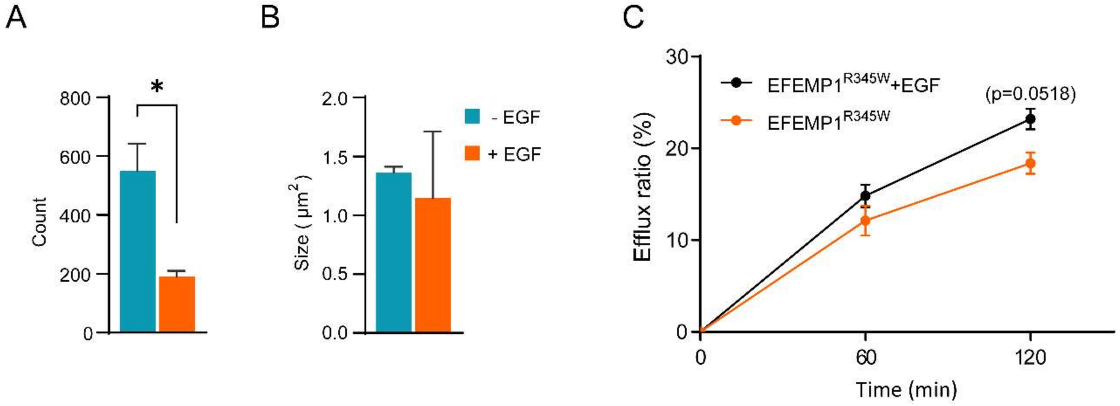


Figure S7. EGF treatment ameliorates intracellular lipid droplet accumulation and improve cholesterol efflux in EFEMP1^{R345W} iRPE. (A, B) The amount (A) and size (B) of the lipid droplet detected in EFEMP1^{R345W} iRPE with or without the treatment of 100 mM EGF for two weeks. **(C)** The cholesterol efflux of EGF-treated or untreated EFEMP1^{R345W} iRPE. Data represented as mean ± SD. (n=2)

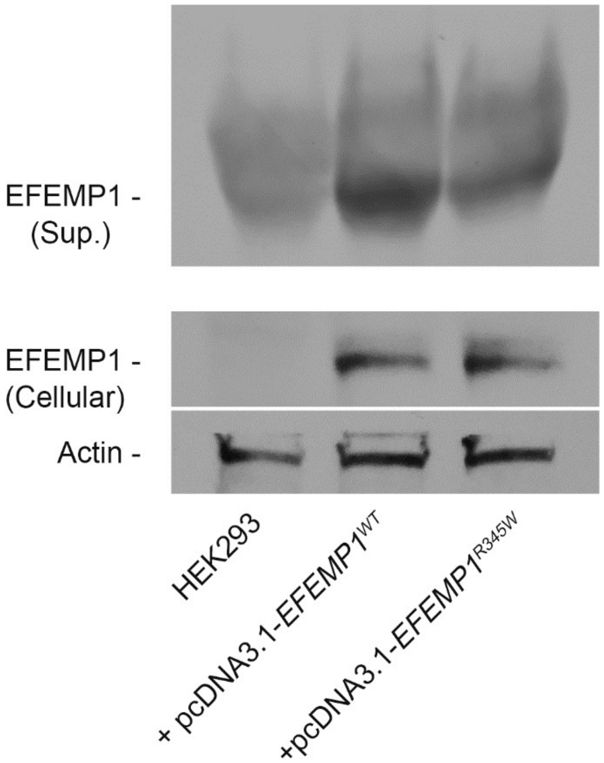
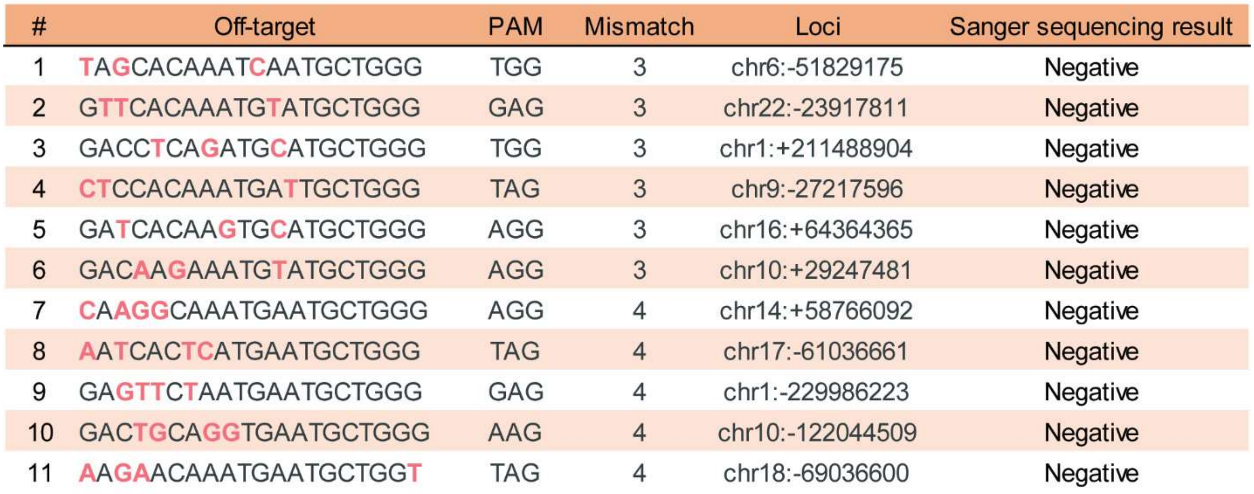
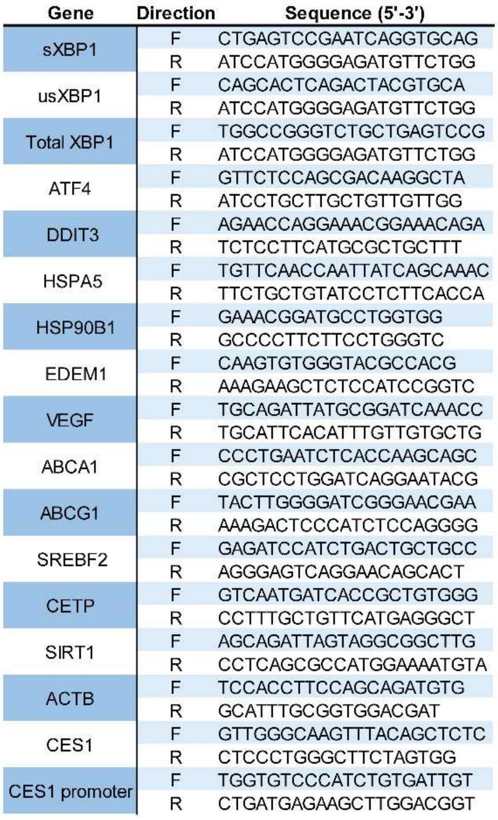


Figure S8. Overexpression of EFEMP1 in HEK293 cells. The HEK293 cells were transfected with pcDNA3.1 vector expressing either EFEMP1^{WT} or EFEMP1^{R345W} for 96 hours. The expression of EFEMP1 in cell lysate or culture supernatant was verified by western blot. HEK293 cell without plasmid transfection was used as a negative control.



#	Off-target	PAM	Mismatch	Loci	Sanger sequencing result
1	**T**A**G**CACAAAT**C**AATGCTGGG	TGG	3	chr6:-51829175	Negative
2	G**TT**CACAAATG**T**ATGCTGGG	GAG	3	chr22:-23917811	Negative
3	GACC**T**CA**G**ATG**C**ATGCTGGG	TGG	3	chr1:+211488904	Negative
4	**CT**CCACAAATGA**T**TGCTGGG	TAG	3	chr9:-27217596	Negative
5	GA**T**CACAA**G**TG**C**ATGCTGGG	AGG	3	chr16:+64364365	Negative
6	GAC**A**A**G**AAATG**T**ATGCTGGG	AGG	3	chr10:+29247481	Negative
7	**C**A**AGG**CAAATGAATGCTGGG	AGG	4	chr14:+58766092	Negative
8	**A**A**T**CAC**TC**ATGAATGCTGGG	TAG	4	chr17:-61036661	Negative
9	GA**GTT**C**T**AATGAATGCTGGG	GAG	4	chr1:-229986223	Negative
10	GAC**TG**CA**GG**TGAATGCTGGG	AAG	4	chr10:-122044509	Negative
11	**A**A**GA**ACAAATGAATGCTGG**T**	TAG	4	chr18:-69036600	Negative

Table S1. Off-targeting analysis of gene-corrected iPSC clone. The genomic DNA of EFEMP1^{corrected} iPSC clone was extracted after CRISPR engineering. The 11 loci that resemble the protospacer used for gene correction were amplified by PCR and analyzed by Sanger sequencing. The mismatched nucleotides are marked in red.



Gene	Direction	Sequence (5'-3')
sXBP1	F	CTGAGTCCGAATCAGGTGCAG
	R	ATCCATGGGGAGATGTTCTGG
usXBP1	F	CAGCACTCAGACTACGTGCA
	R	ATCCATGGGGAGATGTTCTGG
Total XBP1	F	TGGCCGGGTCTGCTGAGTCCG
	R	ATCCATGGGGAGATGTTCTGG
ATF4	F	GTTCTCCAGCGACAAGGCTA
	R	ATCCTGCTTGCTGTTGTTGG
DDIT3	F	AGAACCAGGAAACGGAAACAGA
	R	TCTCCTTCATGCGCTGCTTT
HSPA5	F	TGTTCAACCAATTATCAGCAAAC
	R	TTCTGCTGTATCCTCTTCACCA
HSP90B1	F	GAAACGGATGCCTGGTGG
	R	GCCCCTTCTTCCTGGGTC
EDEM1	F	CAAGTGTGGGTACGCCACG
	R	AAAGAAGCTCTCCATCCGGTC
VEGF	F	TGCAGATTATGCGGATCAAACC
	R	TGCATTCACATTTGTTGTGCTG
ABCA1	F	CCCTGAATCTCACCAAGCAGC
	R	CGCTCCTGGATCAGGAATACG
ABCG1	F	TACTTGGGGATCGGGAACGAA
	R	AAAGACTCCCATCTCCAGGGG
SREBF2	F	GAGATCCATCTGACTGCTGCC
	R	AGGGAGTCAGGAACAGCACT
CETP	F	GTCAATGATCACCGCTGTGGG
	R	CCTTTGCTGTTCATGAGGGCT
SIRT1	F	AGCAGATTAGTAGGCGGCTTG
	R	CCTCAGCGCCATGGAAAATGTA
ACTB	F	TCCACCTTCCAGCAGATGTG
	R	GCATTTGCGGTGGACGAT
CES1	F	GTTGGGCAAGTTTACAGCTCTC
	R	CTCCCTGGGCTTCTAGTGG
CES1 promoter	F	TGGTGTCCCATCTGTGATTGT
	R	CTGATGAGAAGCTTGGACGGT

Table S2. Primers used for real-time qPCR.

	EFEMP1$^{\mathrm{WT}}$	EFEMP1$^{\mathrm{R345W}}$	EFEMP1$^{\mathrm{corrected}}$
CES1	0.637167	0.079250	1.580444
MYLPF	0.320667	2.538000	0.562722
PVALB	0.338333	2.058667	0.603778
MYH8	0.258000	2.316917	0.700889
CASQ1	0.141000	2.387500	0.747556
CKM	0.171167	2.314834	0.739778
ATP2A1	0.241750	2.153625	0.773250
MYL1	0.235500	2.121667	0.820000
MFAP4	0.900000	0.526583	1.266278
MYH7	0.623167	1.568750	0.662778
MB	0.099500	2.057500	0.869556
CA3	0.303000	1.901917	0.817667
CASQ2	0.193667	1.908417	0.842000
MYH2	0.311833	1.931834	0.858944
MYH3	0.717667	1.774250	0.801222
ISG15	0.367500	1.714250	0.777833
ENO3	0.499833	1.827833	0.837333
MX1	0.192667	1.735334	0.798167
MYH11	0.780667	0.566500	1.231334
DES	0.185667	1.810750	0.883166
MYH10	1.348500	0.537417	1.059666
MYL9	1.356000	0.564667	1.074500
ECHDC3	0.677500	0.615250	1.112333
BGN	1.260000	1.220900	0.675422
MYH9	1.645833	0.578417	0.997111
MYL12A	1.364500	0.615750	1.041222
MYH14	0.877833	0.692667	1.125500
FBP2	1.097167	1.318334	0.814833
RPE65	0.345833	0.751667	1.140167
TTR	0.956000	0.759834	1.140222
PLCB4	1.345200	0.692100	0.949122
MYLK	1.217000	0.772667	1.028778
MYL6	0.876833	0.844417	1.084833
RPL12	1.273667	0.778500	0.998944
S100A4	0.286000	1.154583	0.962945
THY1	0.715167	1.103167	0.937778
COL14A1	0.344167	1.158334	1.041389

Table S3. Normalized MS/MS result of the 37 DEs shown in Figure S2.