YMTHE, Volume *31*

Supplemental Information

In vivo CRISPR gene editing

in patients with herpetic stromal keratitis

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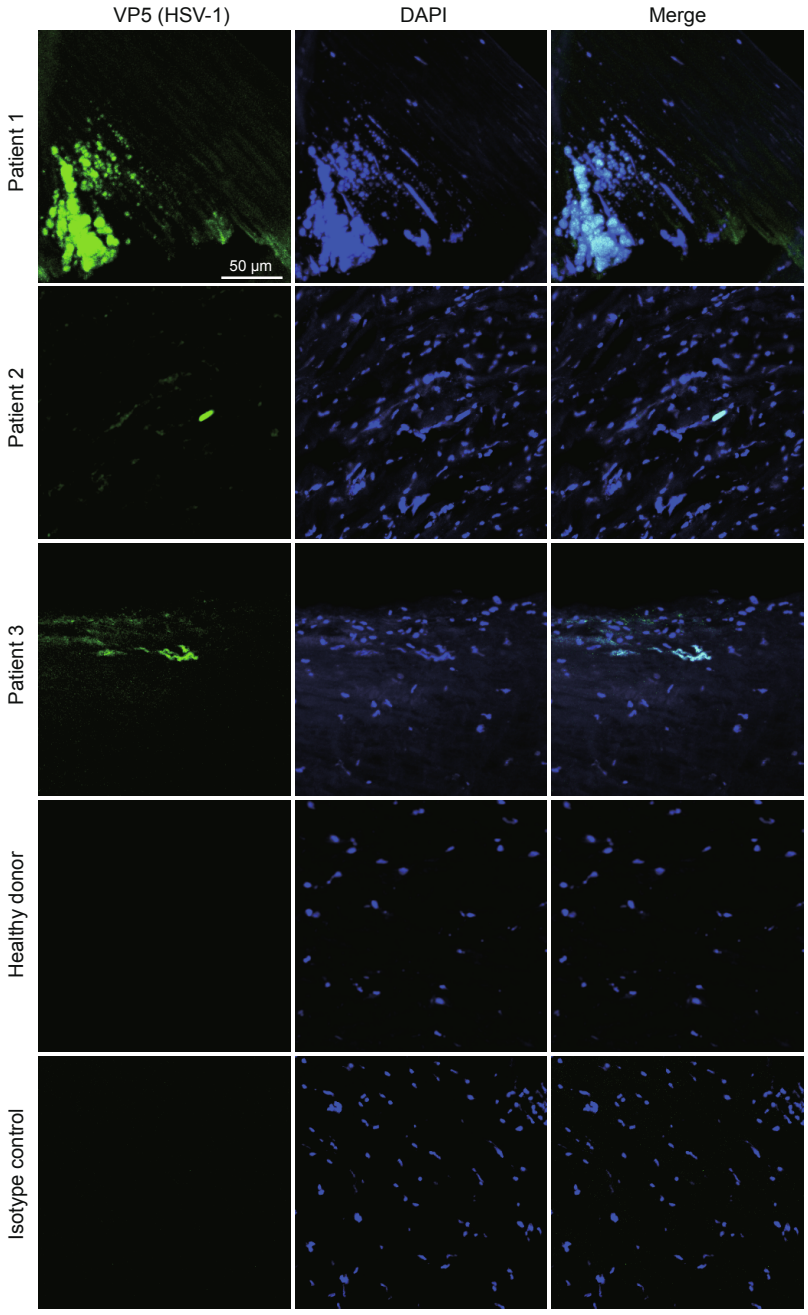


Fig. S1. Fluorescence microscopy analysis of HSV-1 in the removed corneal buttons. HSV-1 capsid protein VP5 was presented in green while DAPI was in blue. Their perfect overlap after merging suggests the presence of HSV-1 in the patient corneal tissue before CRISPR treatment.

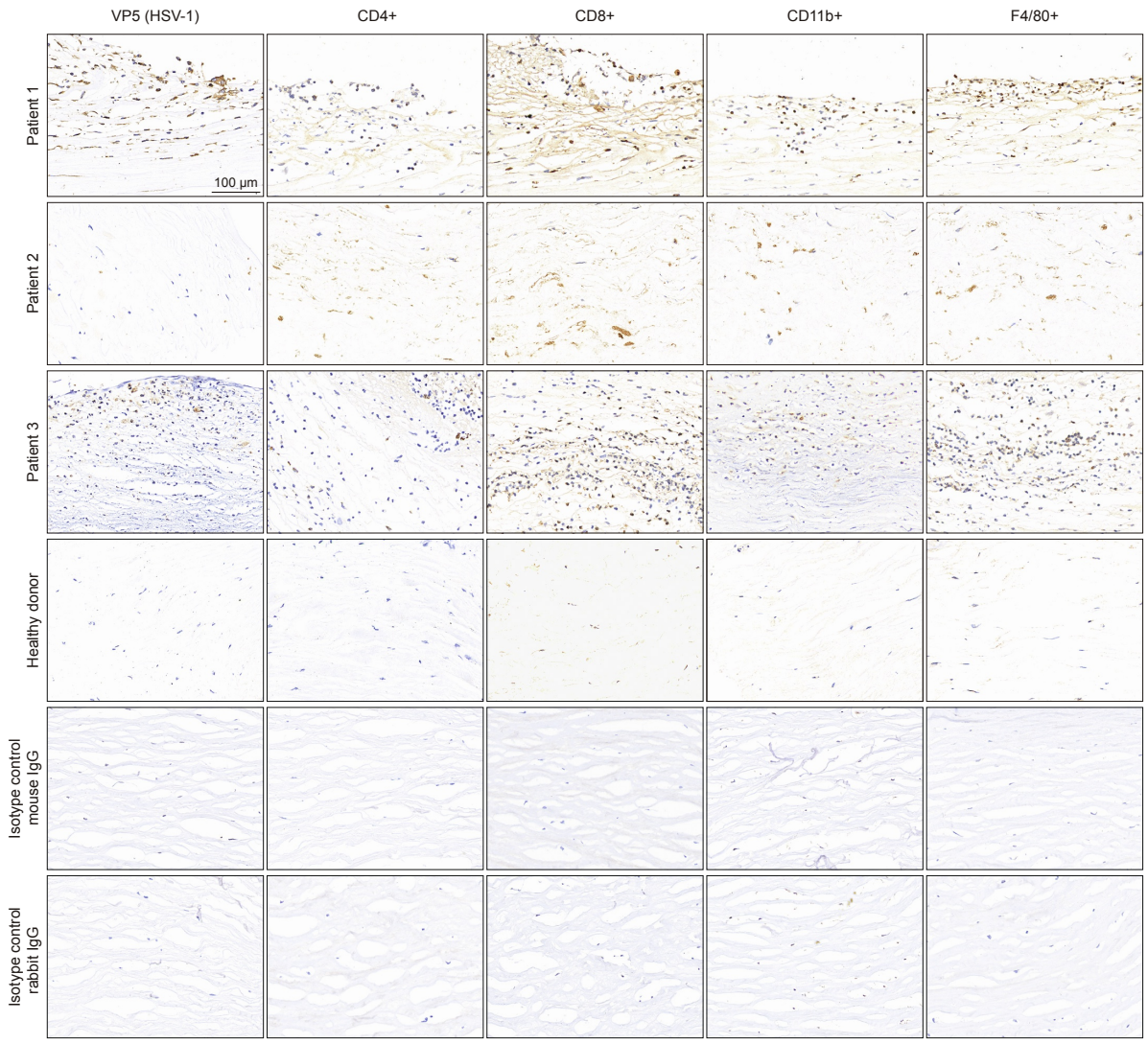
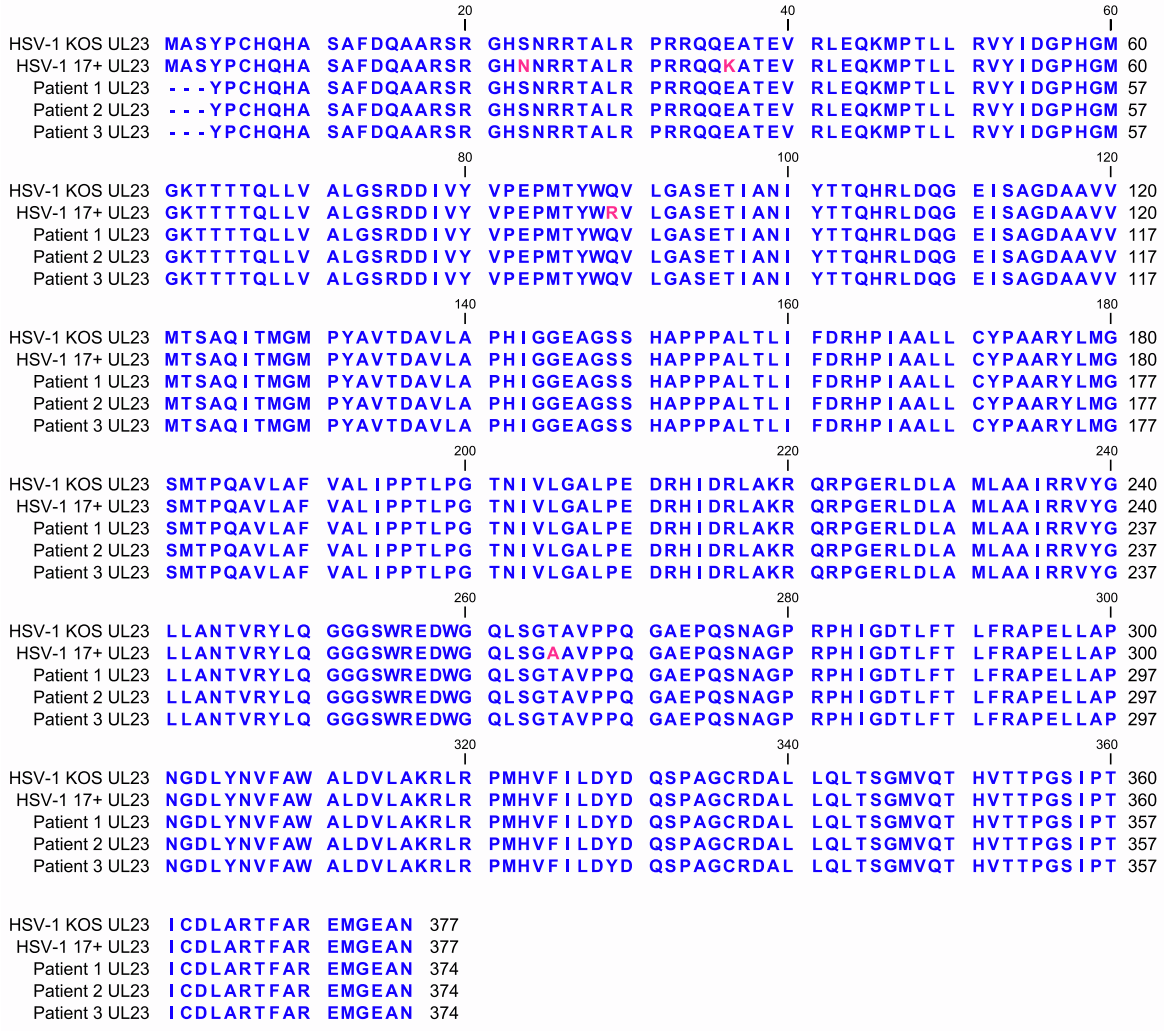


Fig. S2. Immunohistochemistry analysis of immune cells in the corneal button. The removed corneal buttons from the patients were stained for T cells (CD4+ and CD8+), myeloid-derived cells (CD11b+) and macrophages (F4/80+). Immunohistochemistry confirmed excess infiltration of inflammatory cells compared to the healthy cornea.

A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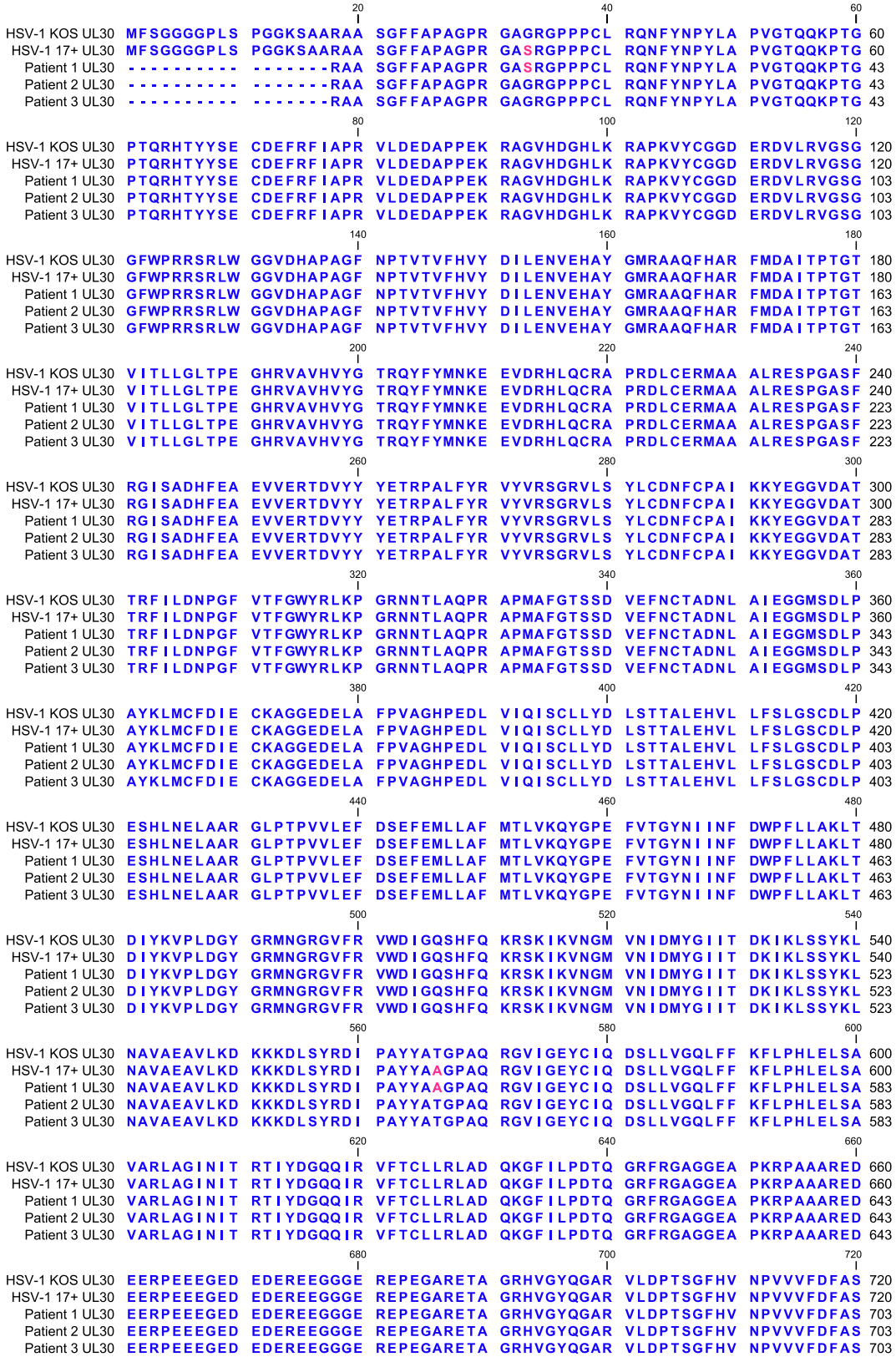
B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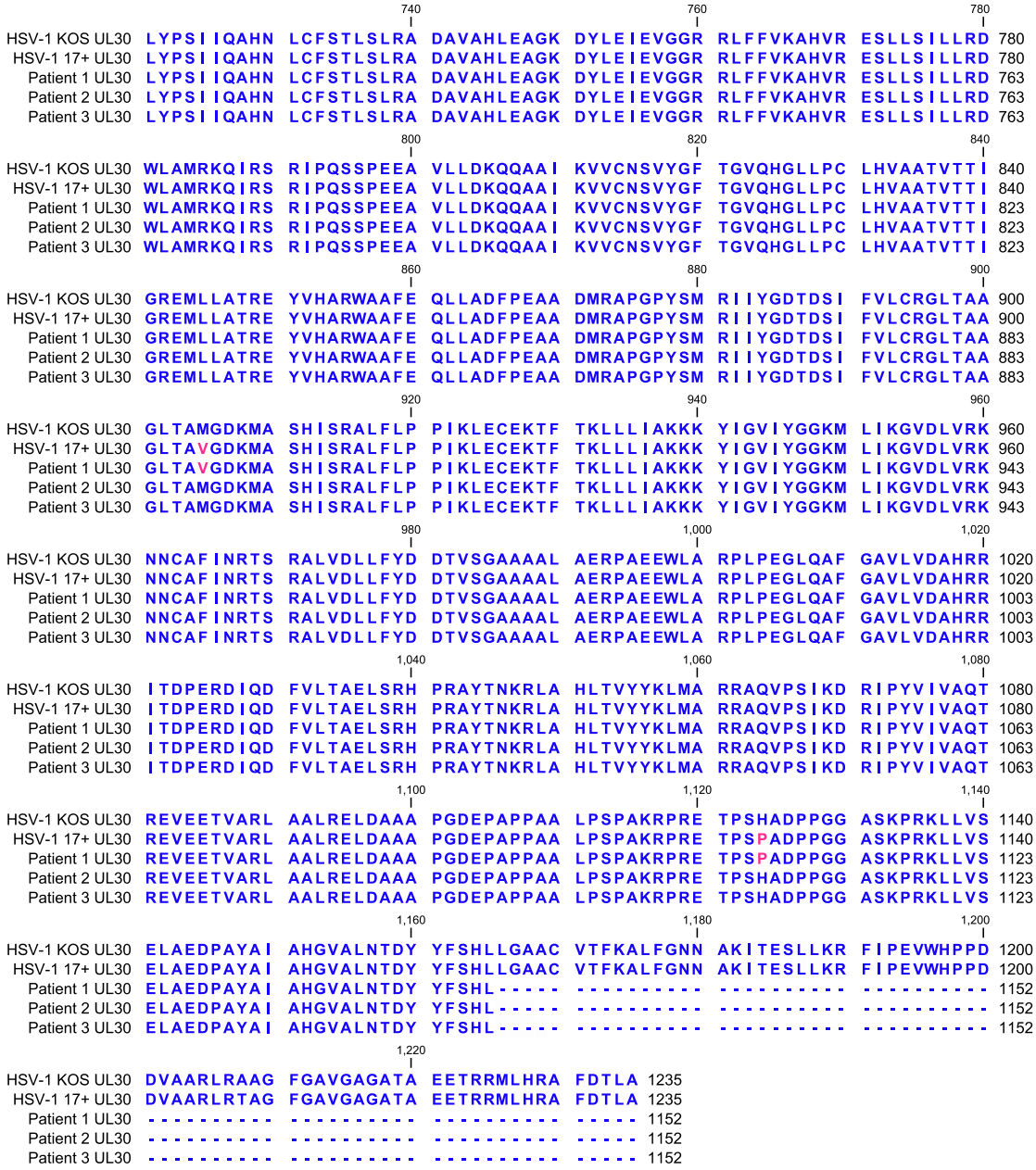

Fig. S3. Amino acids alignment for TK and DNA pol. HSV-1 strains were isolated from the three participants and sequenced for genes encoding thymidine kinase (TK, UL23) and DNA polymerase (DNA pol, UL30). Letter in red color indicated changed amino acids in TK (**A**) and DNA pol (**B**). HSV-1 KOS and 17syn+ strains were used as references. Only amino-acid changes different from both KOS and 17syn+ simultaneously are considered as ACV resistant mutations.

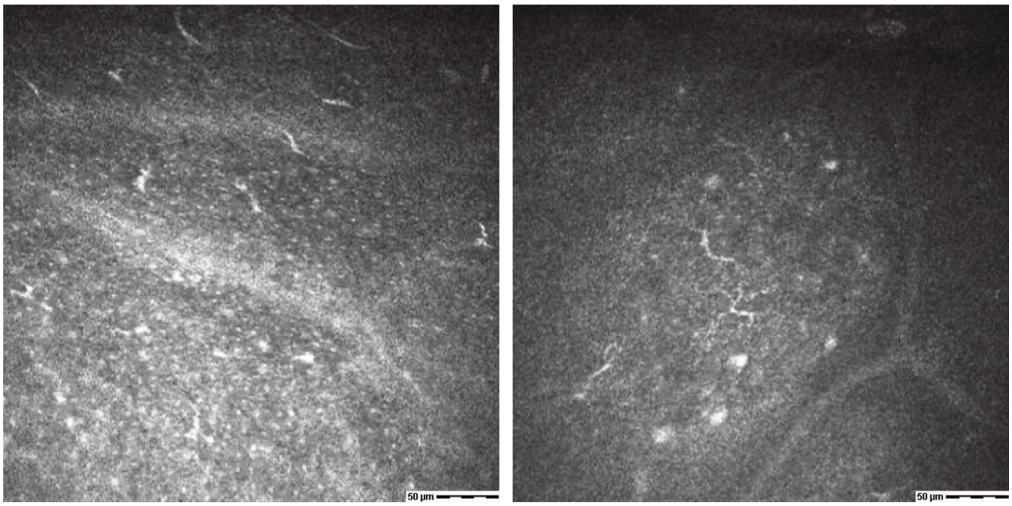

Fig. S4. Postoperative IVCM examination of patient 1. IVCM at 12-month post-injection showed very few corneal subbasal nerves in the transplanted corneal graft of patient 1.

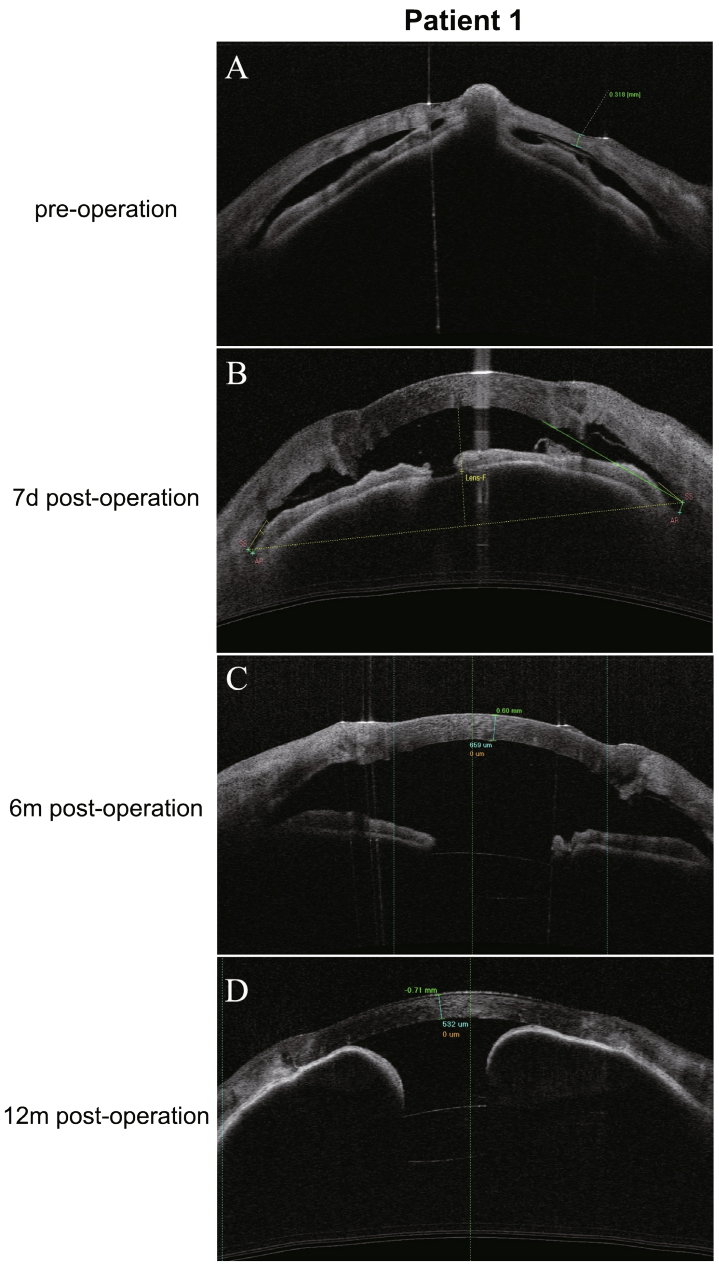

Fig. S5. Time-domain OCT of the anterior segment of patient 1. This image shows the time-domain OCT of the anterior segment of patient 1 at different time points (**A-D**). Before the injection, there was iris incarceration in the thinning area of the cornea. OCT showed a slightly shallow anterior chamber after 7 days post-injection and an iris adhesion at the 12-month visit, yet the intraocular pressure of patient 1 was within the normal range. Prophylactic laser therapy was conducted to prevent glaucoma.

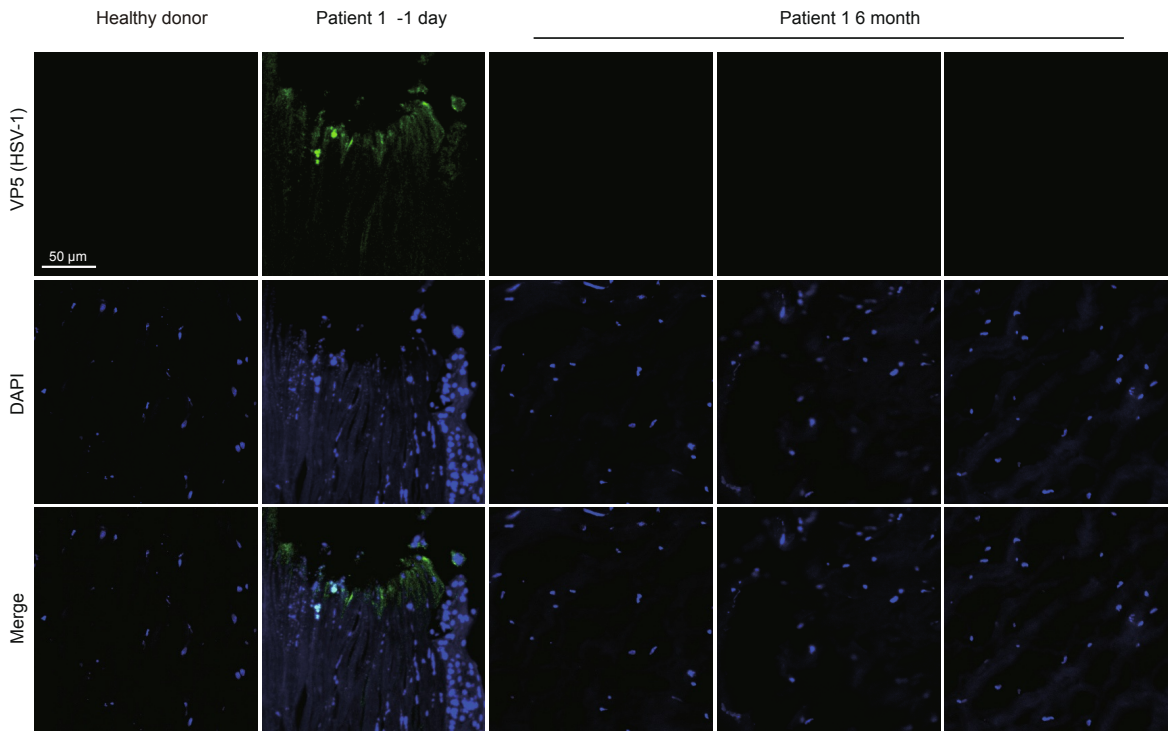

Fig. S6. Fluorescence microscopy analysis of HSV-1 in the removed corneal button. After the second corneal transplantation on the right eye of patent 1 at 6 months after PK, the removed corneal button showed no signs of HSV-1 capsid protein VP5 (green) by the fluorescence microscopy. Scale bars, 50 μm.

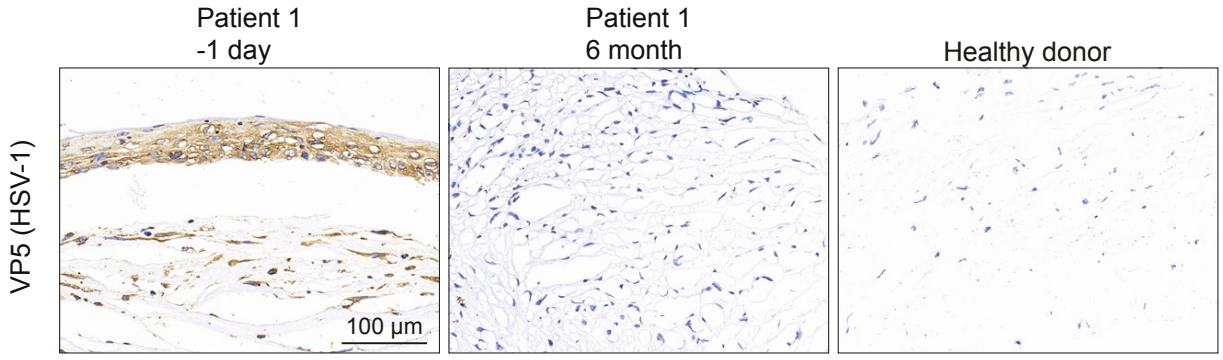

Fig. S7. Immunohistochemistry analysis of HSV-1 in the removed corneal button from patient 1. After the second corneal transplantation on his right eye 6 months after PK, the removed corneal button showed no signs of HSV-1 capsid protein VP5 (brown) by immunohistochemistry analysis.

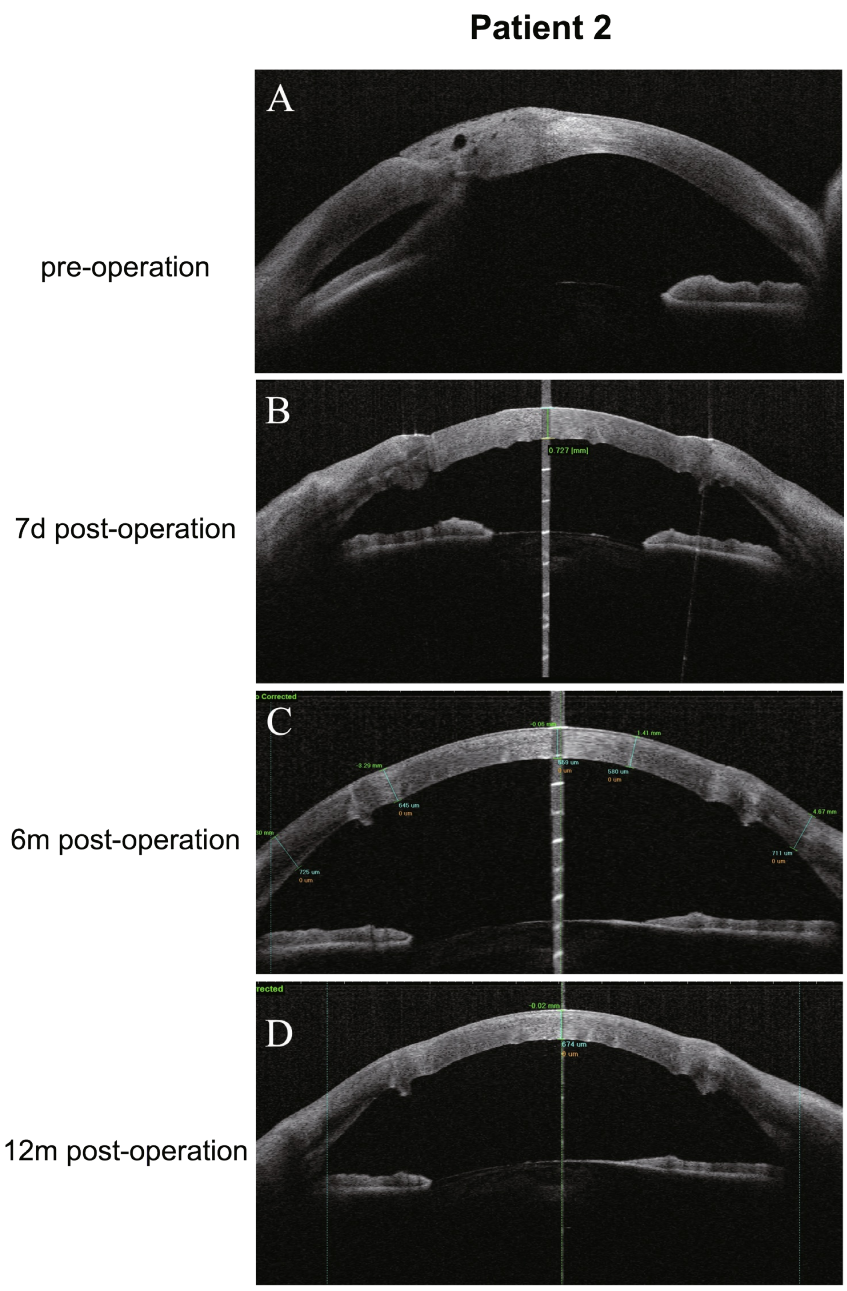

Fig. S8. Time-domain OCT of the anterior segment of patient 2. The pre-injection OCT of the anterior segment showed a possible corneal perforation site in patient 2 (**A**). Post-injection OCT images (**B-D**) indicate a deepened anterior chamber, which was maintained for over 12 months after the corneal transplantation.

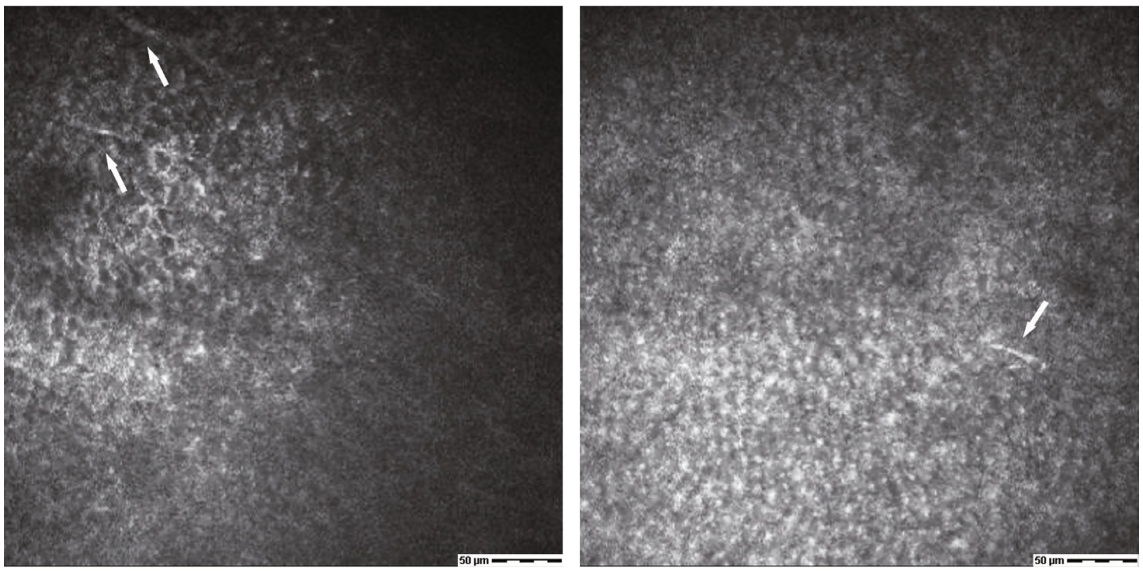

Fig. S9. Postoperative IVCM examination of patient 2. IVCM showed slight corneal nerve regeneration (white arrow) in the transplanted corneal graft at the 12 month-follow-up of patient 2.

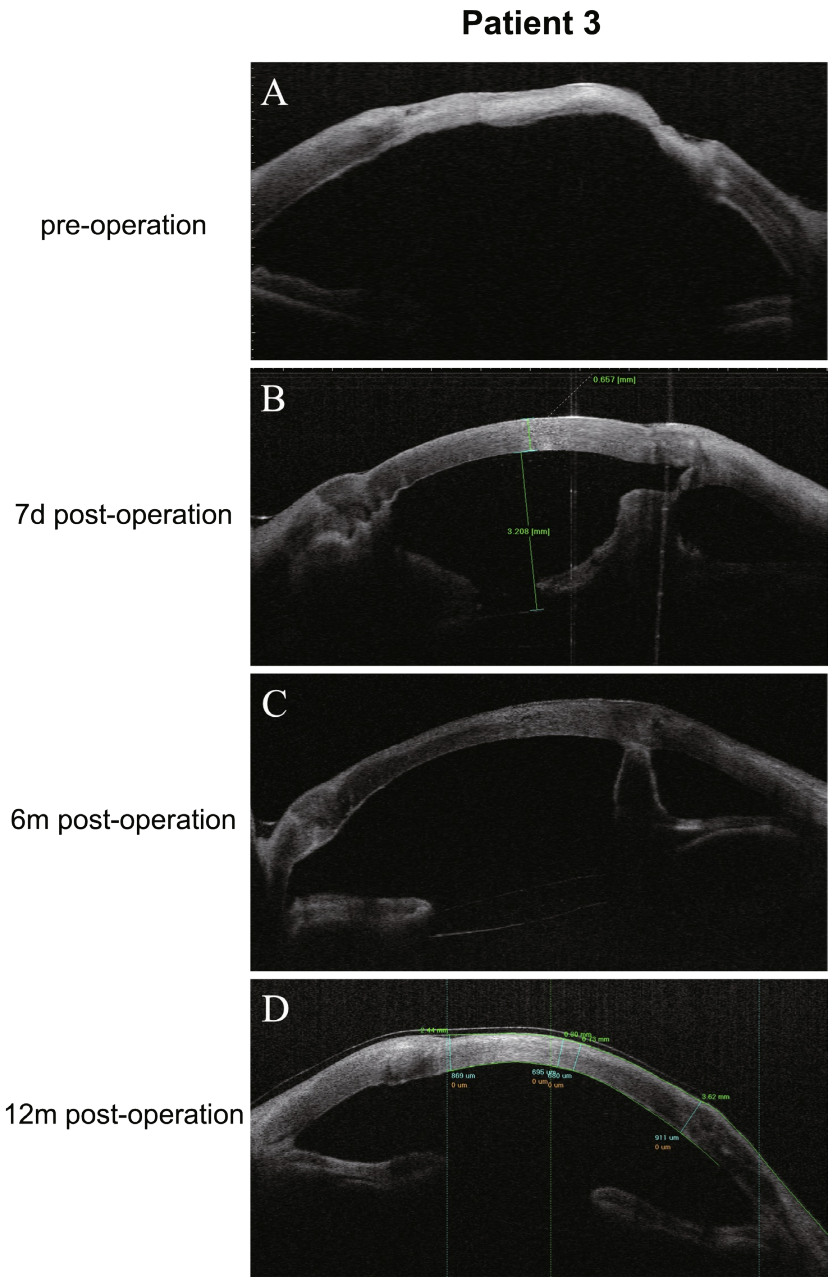

Fig. S10. Time-domain OCT of the anterior segment of patient 3. The pre-injection OCT of the anterior segment showed a corneal perforation channel in patient 3 (**A**). Post-injection OCT images (**B-D**) indicate an in-position intraocular lens and a slightly adhered iris to the posterior cornea after PK.

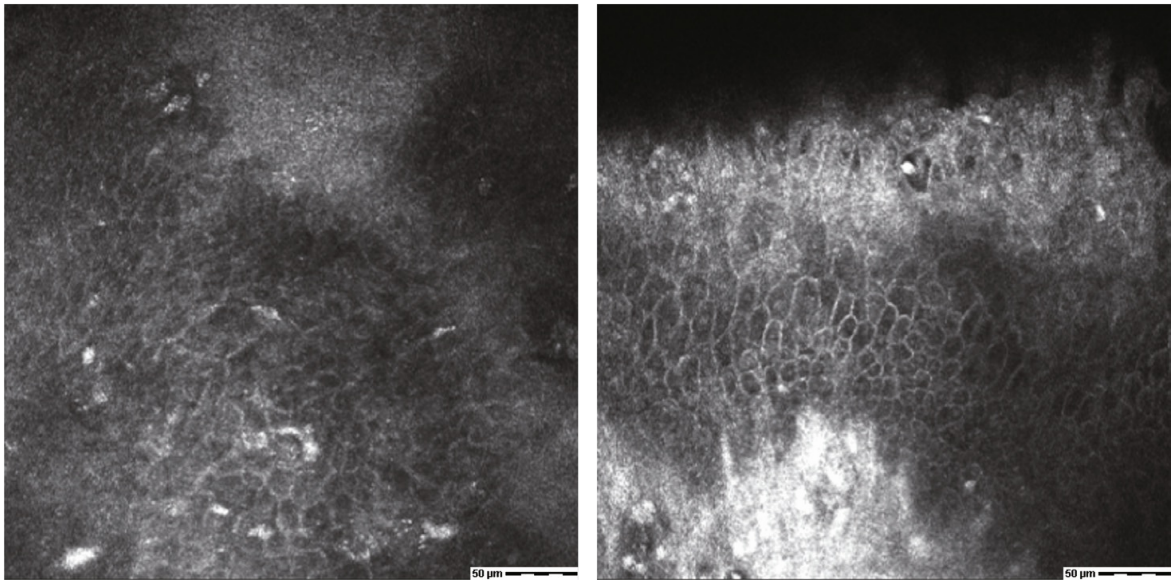

Fig.S11. Postoperative IVCM examination of patient 3. IVCM showed no signs of corneal nerve regeneration at the 12-month follow-up of patient 3. His corneal epithelium density decreased and showed irregular morphologic changes, which indicated the possibility of corneal neurological dystrophy related to HSK.

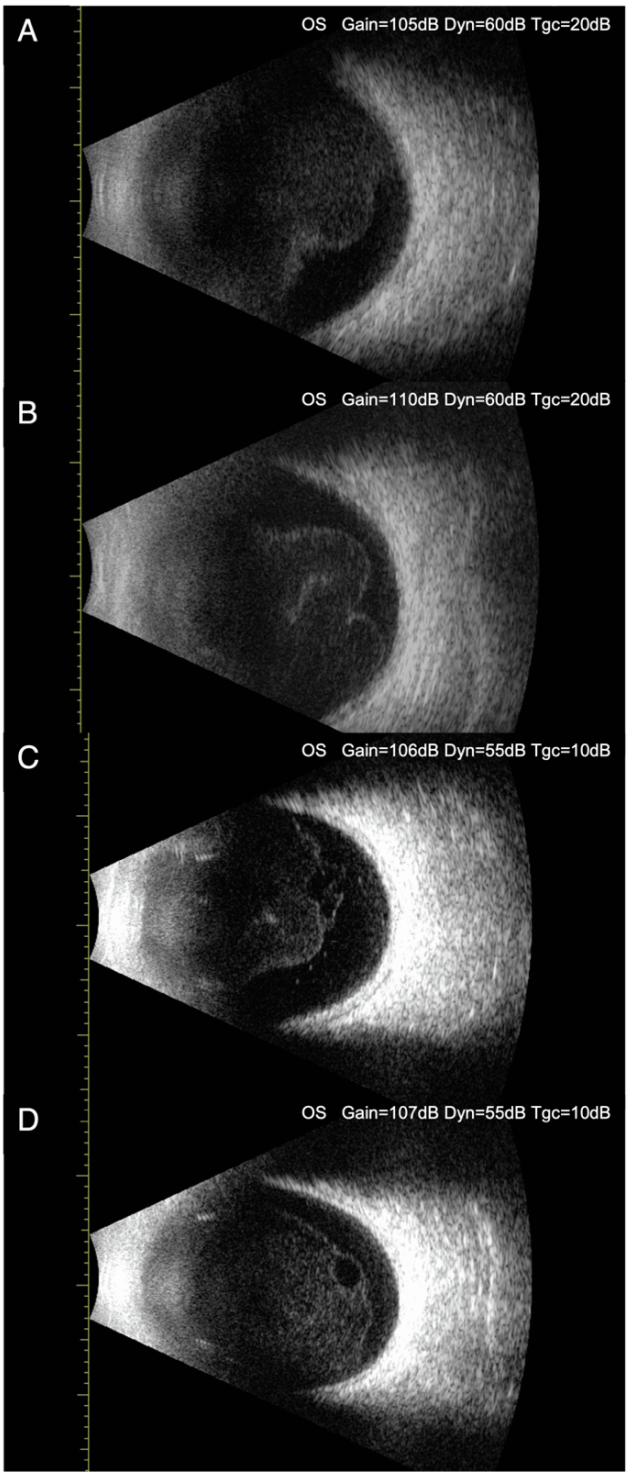

Fig. S12. Postoperative B-scan ultrasonography of patient 3. B-scan ultrasonography of the patient 3 on 7 days (**A**), 1 month (**B**), 3 months (**C**) and 6 months (**D**) post-injection. Continuous ribbon-like echo was found in the posterior vitreous body, indicating vitreous opacity with posterior detachment.

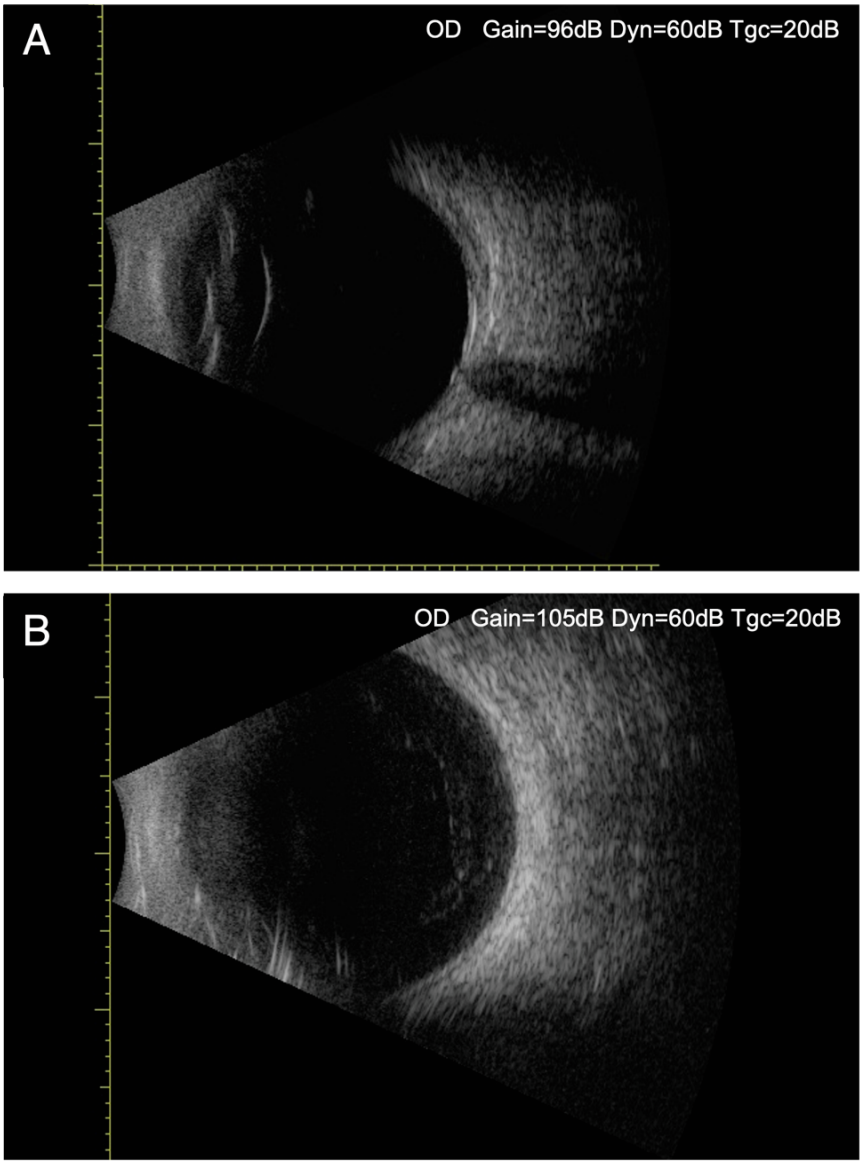

Fig. S13. Postoperative B-scan ultrasonography of patient 2. B-scan ultrasonography results of patient 2 on 6 months (**A**) and 9 months (**B**) post-injection showed no remarkable changes in the vitreous body.

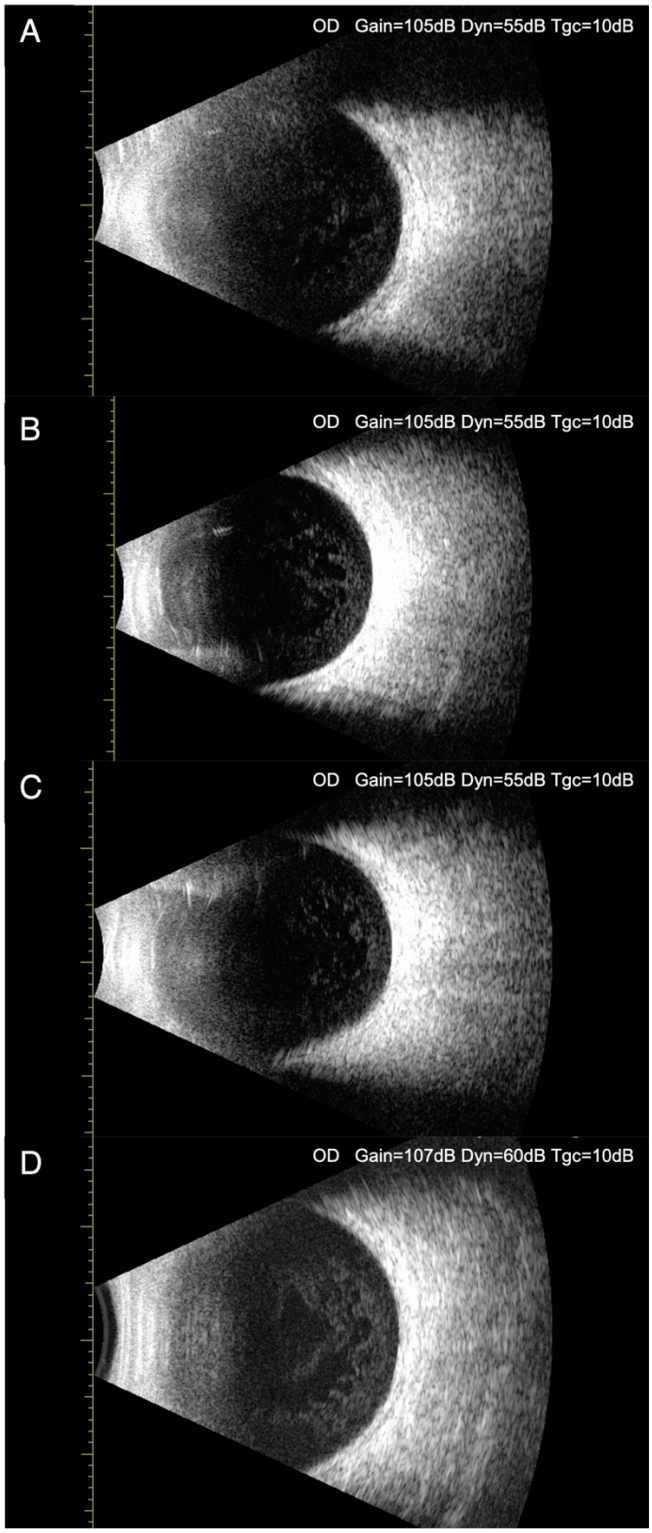

Fig. S14. Postoperative B-scan ultrasonography of patient 1. B-scan ultrasonography of patient 1 on 7 days (**A**), 1 month (**B**), 6 months (**C**) and 12 months (**D**) post-injection revealed no remarkable changes in the vitreous body.

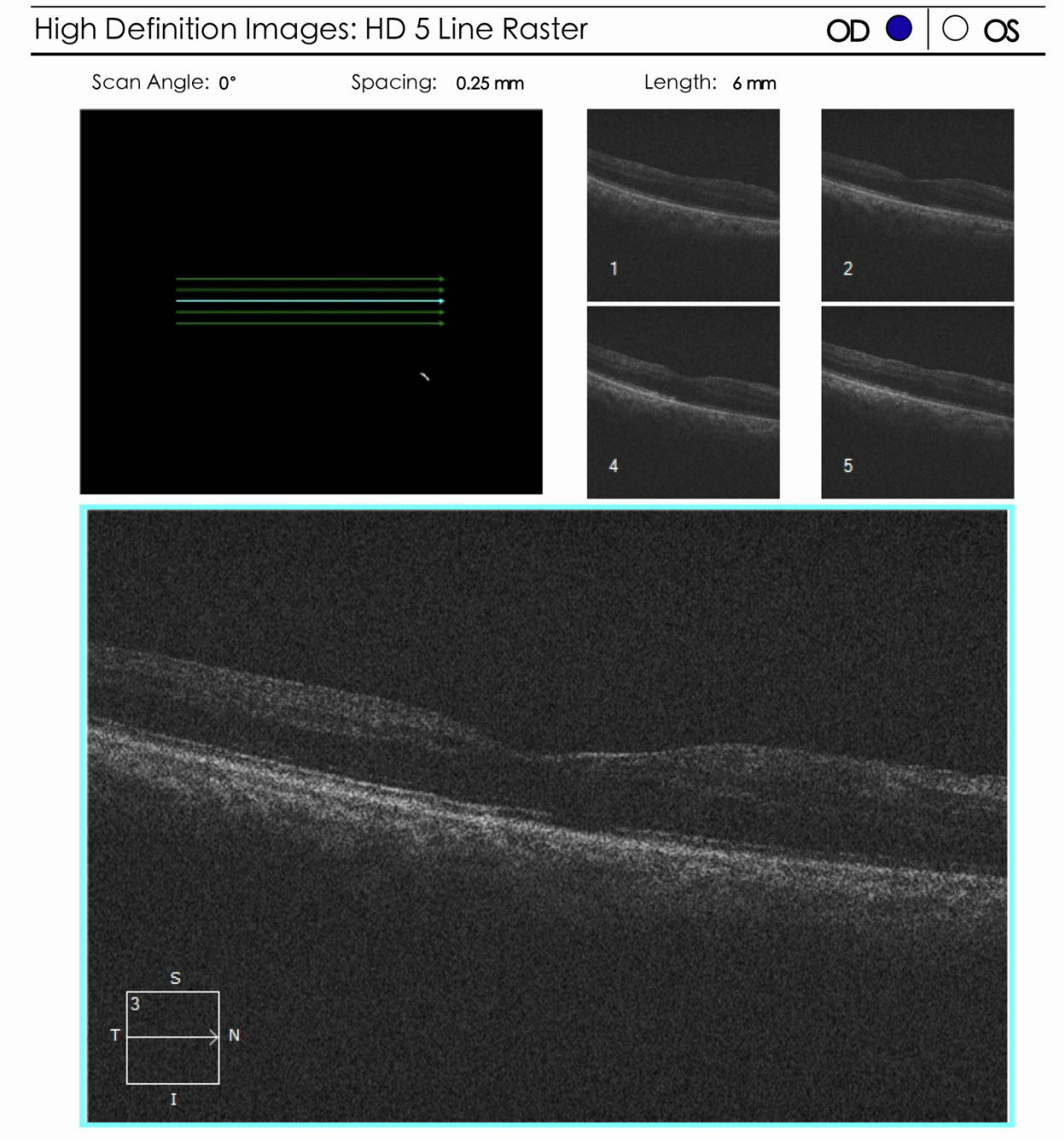

Fig. S15. Spectral-domain OCT of the retina of patient 2. The spectral-domain OCT of patient 2's retina was performed at 6 months post-injection using Cirrus OCT 5000 (Zeiss). The structure of his fundus showed no obvious exception after the administration of HELP.

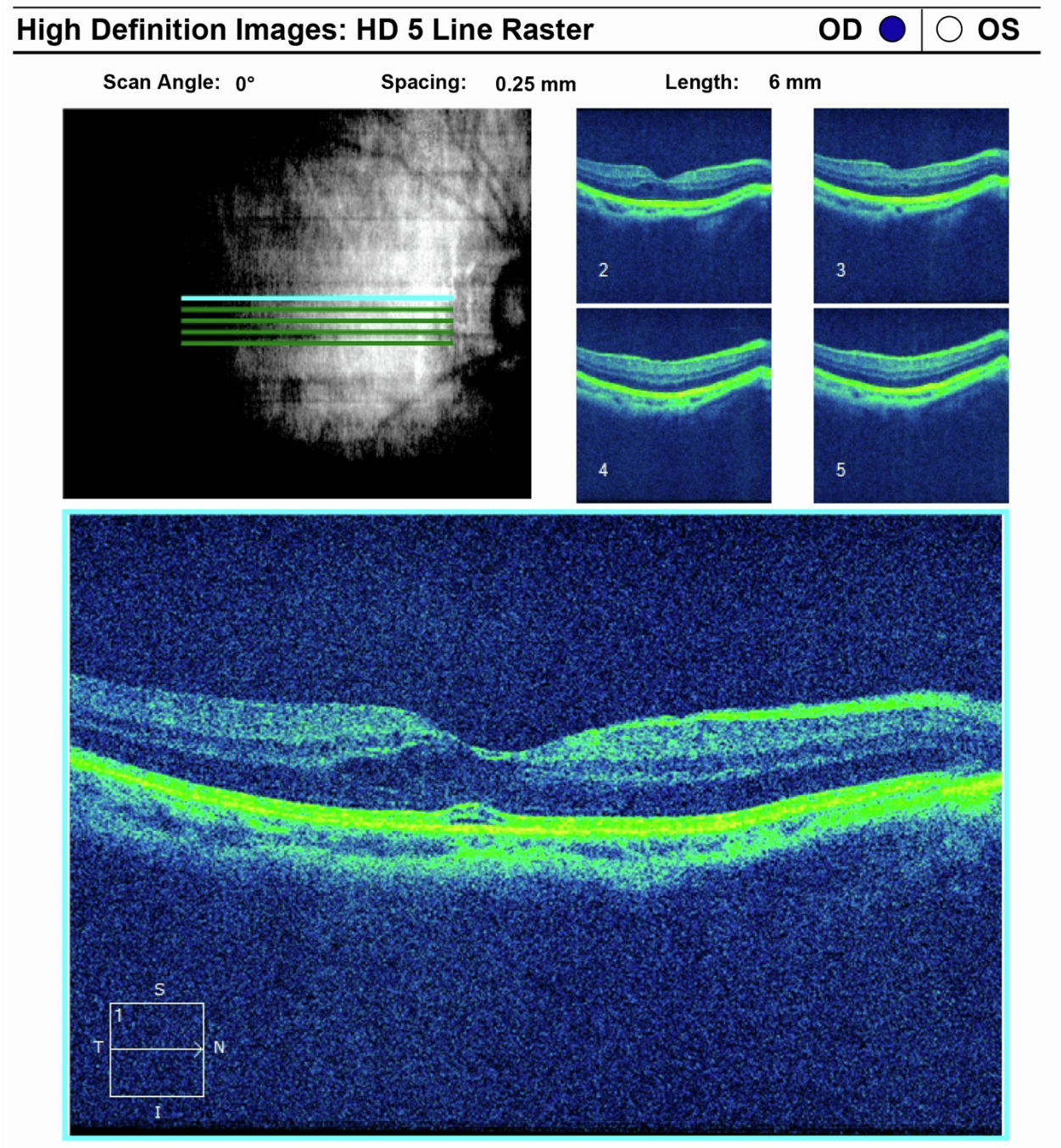

Fig. S16. Spectral-domain OCT of the retina of patient 1. The optical coherence tomography revealed mild center-involved intraretinal fluid and subfoveal fluid in the right eye of patient 1 at six months after the treatment. The ellipsoid zone was intact. We continue to follow up on this situation without any intervention.

A

Diagnosis:

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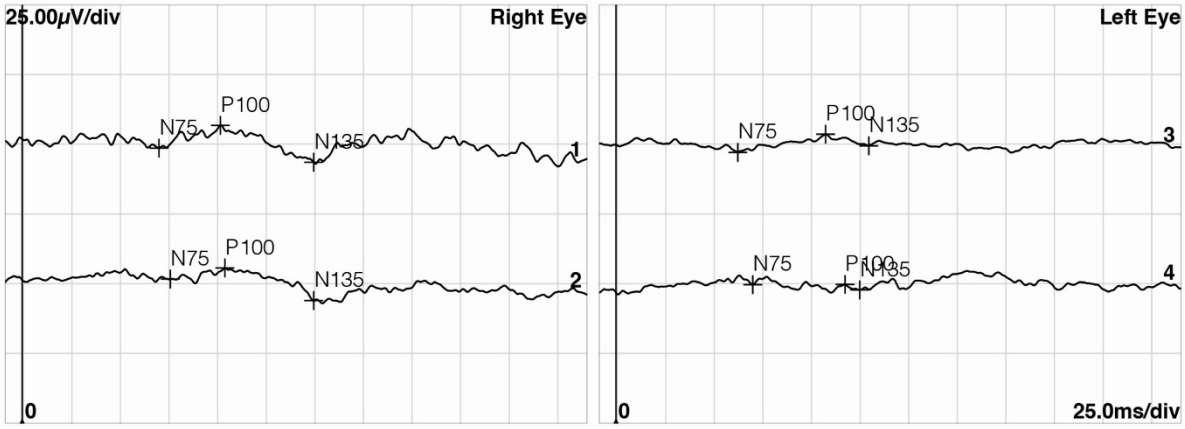

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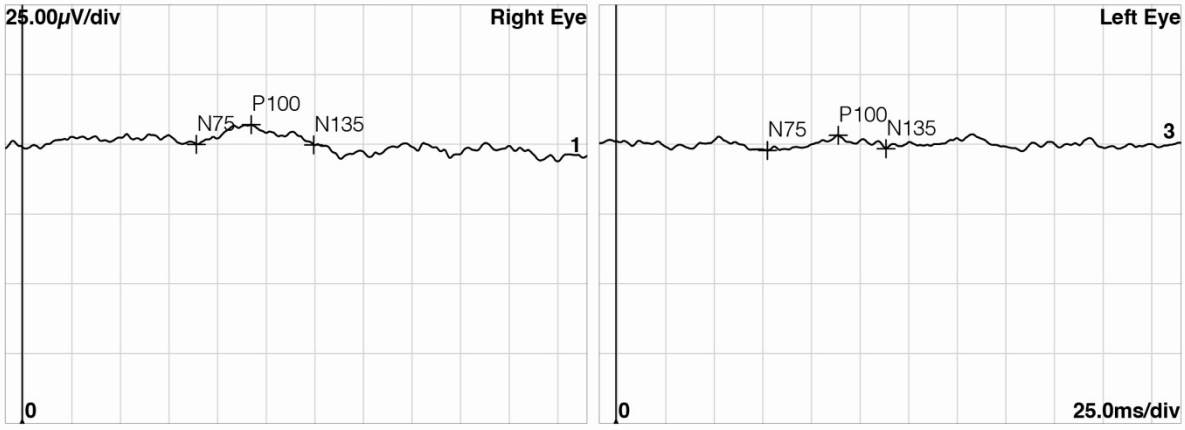
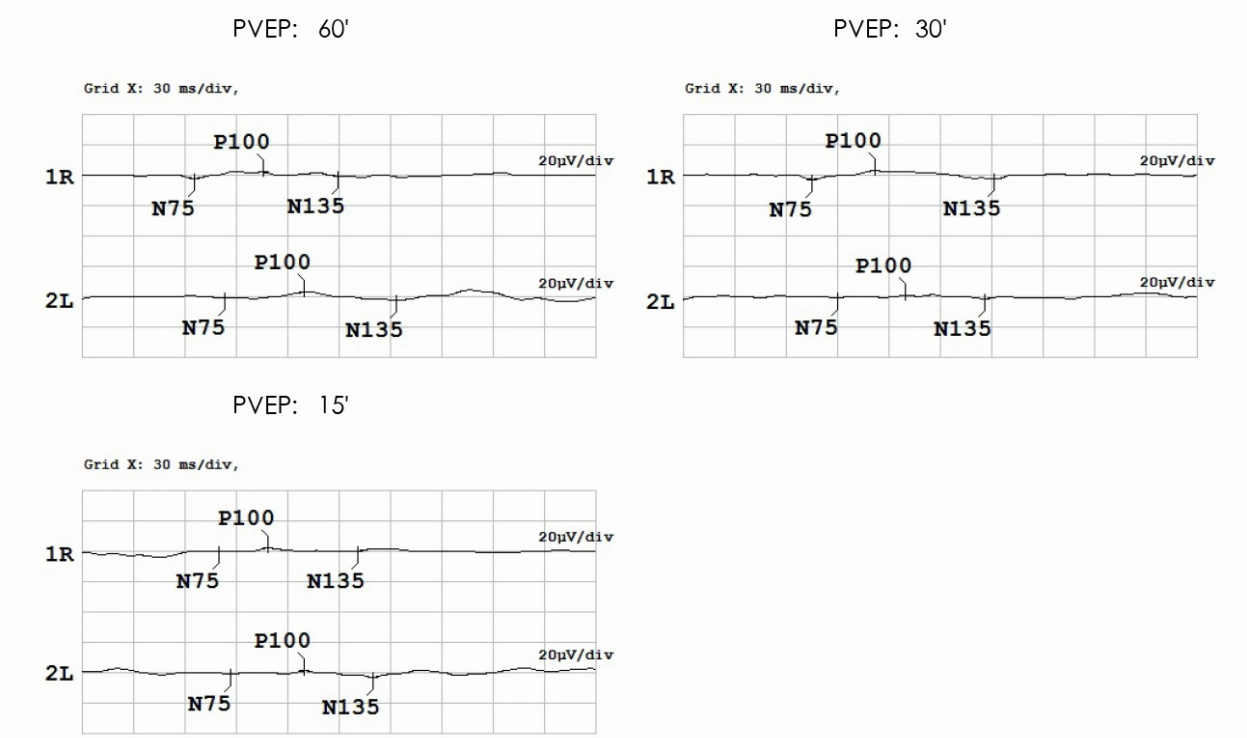
2 R1 1,0 deg	76.3	104.5	150.3	3.94µV (!)	11.7µV
3 L1 2,0 deg	62.8	108.0	130.3	6.51µV (!)	4.14µV
4 L1 1,0 deg	70.5	118.0 (!)	125.6	83.5nV (!)	2.01µV

2_Pattern-VEP 30 min&15 min (Monitor)



Normals	-	105-126	-	7.00µV-42.5µV	
Channel	**N75 [ms]**	**P100 [ms]**	**N135 [ms]**	**N75-P100**	**P100-N135**
1 R1 30 min	89.8	118.0	150.3	7.13µV	7.29µV
2 R1 15 min					
3 L1 30 min	78.1	114.5	139.1	5.40µV (!)	4.80µV
4 L1 15 min					

B



PVEP: 图形翻转.60'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	66.0	106.0	149.5	4.9	3.4
2L	83.5	130.0	183.5	4.8	5.8

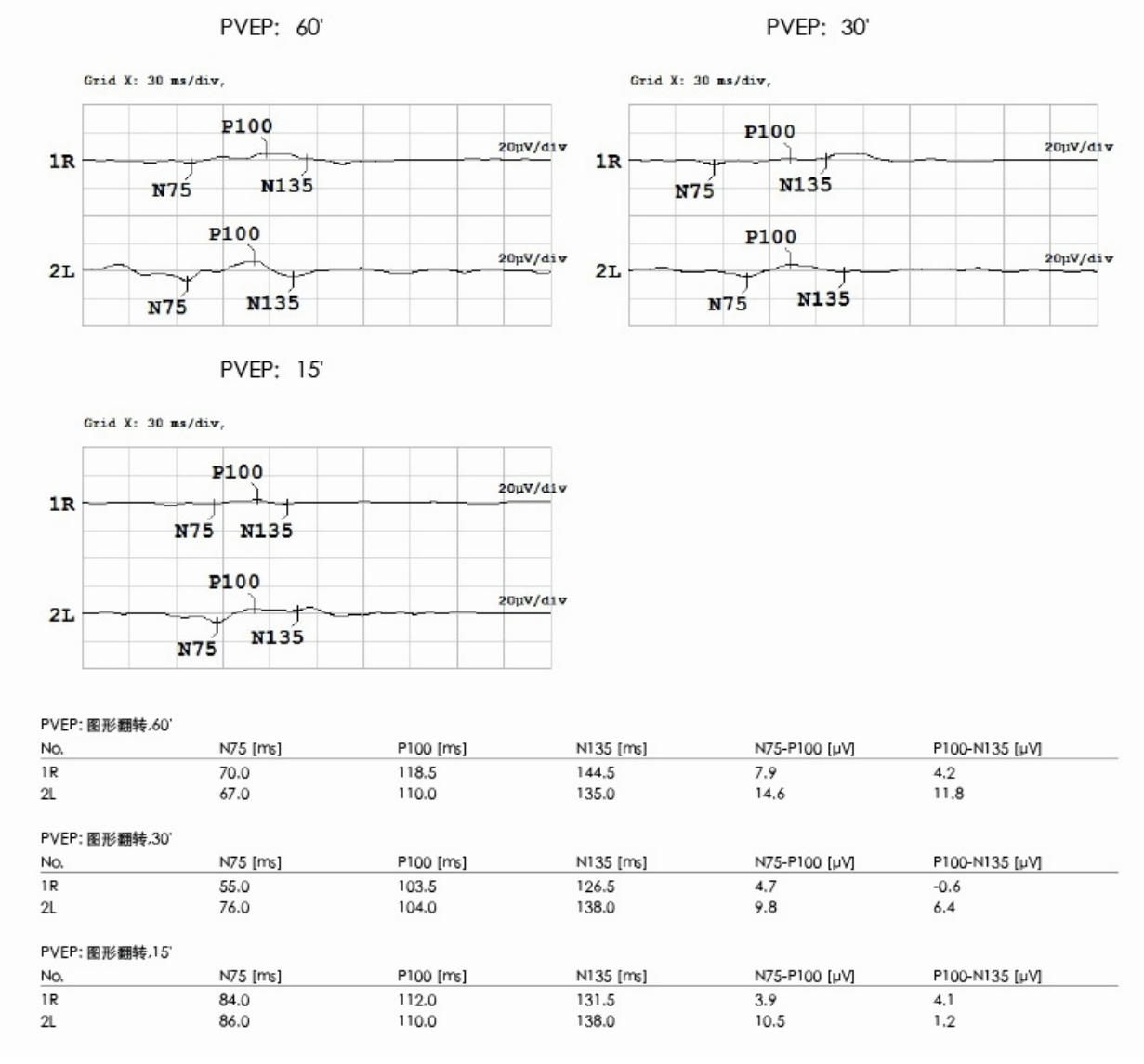
PVEP: 图形翻转.30'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	75.5	112.0	182.0	6.3	6.0
2L	90.5	130.0	176.0	2.8	3.1

PVEP: 图形翻转.15'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	80.0	109.0	161.5	3.2	1.9
2L	87.0	130.0	170.0	3.0	4.9

Fig. S17. Retina ERG of patient 3. The light-stimulated electrical activity of the retina indicates no remarkable change in terms of retinal function in patient 3 (**A**: pre-injection; **B**: six months post-injection).



PVEP: 图形翻转.60'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	70.0	118.5	144.5	7.9	4.2
2L	67.0	110.0	135.0	14.6	11.8

PVEP: 图形翻转.30'

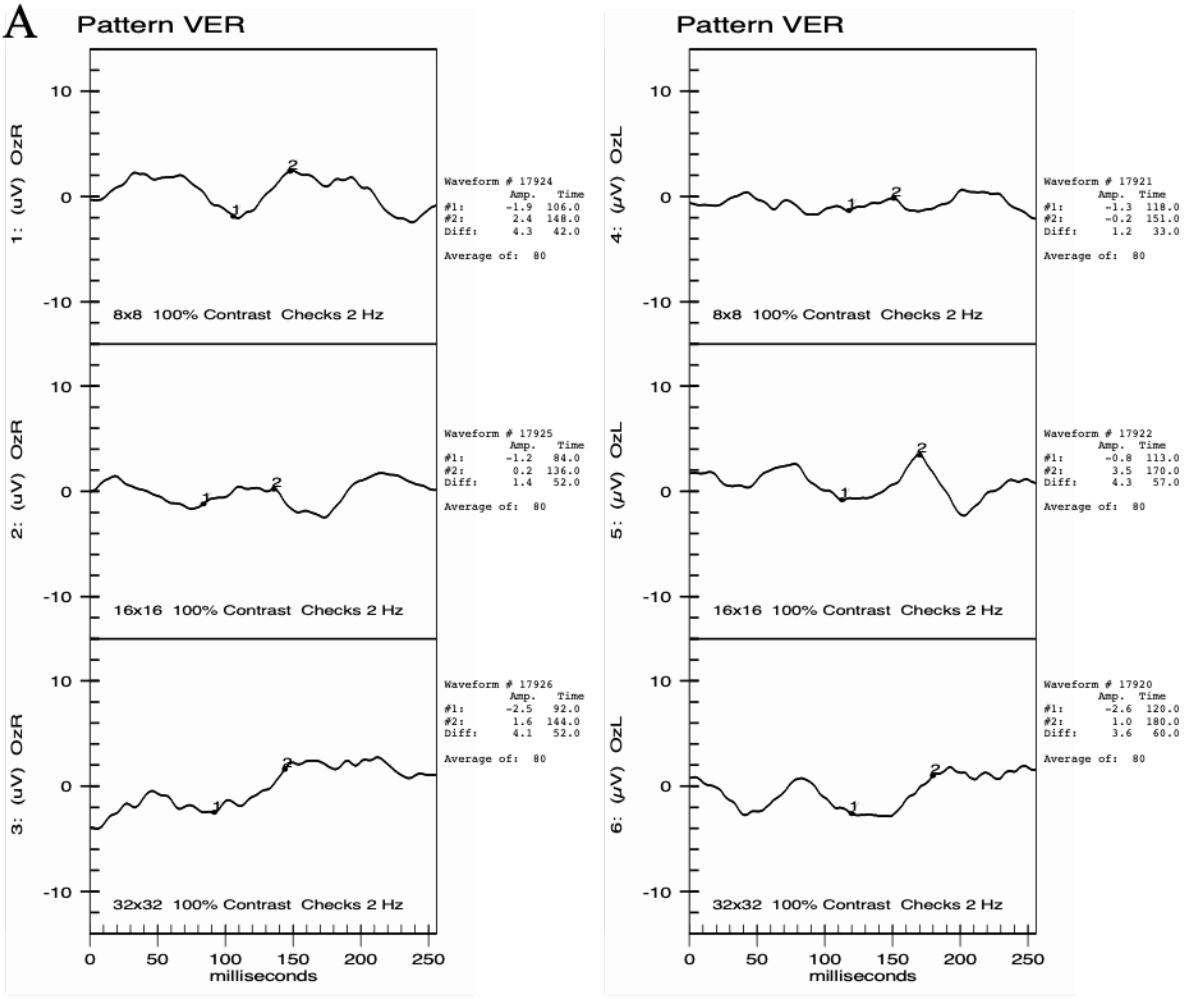
No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	55.0	103.5	126.5	4.7	-0.6
2L	76.0	104.0	138.0	9.8	6.4

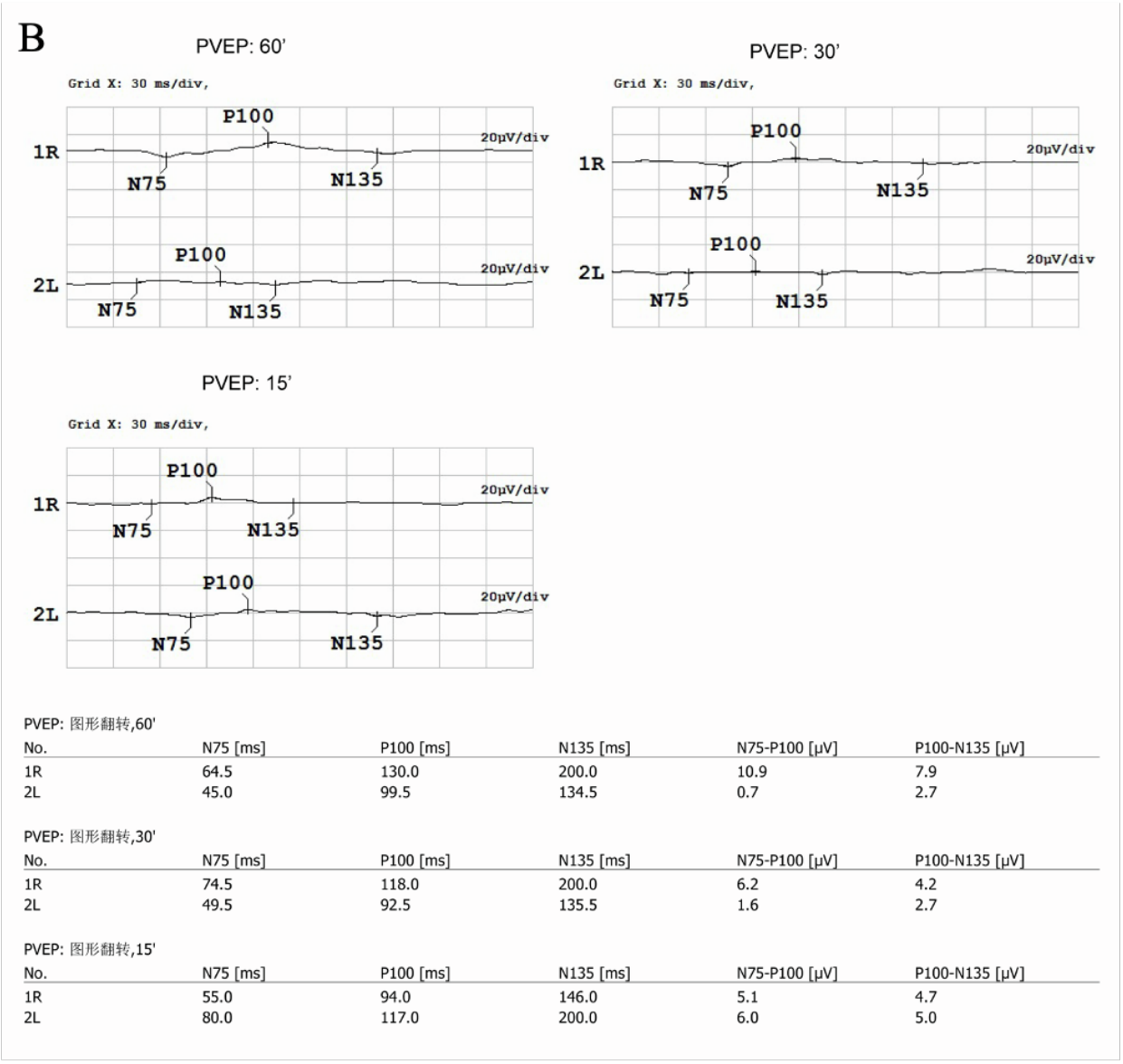
PVEP: 图形翻转.15'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	84.0	112.0	131.5	3.9	4.1
2L	86.0	110.0	138.0	10.5	1.2

Fig. S18. Retina ERG of patient 2. The light-stimulated electrical activity of retina indicates no significant change in terms of retinal function in patient 2 twelve months after the treatment.

A


Pattern VER
1: (uV) OzR
2: (uV) OzR
3: (uV) OzR
8x8 100% Contrast Checks 2 Hz
16x16 100% Contrast Checks 2 Hz
32x32 100% Contrast Checks 2 Hz
Waveform # 17924
Amp. Time
#1: -1.9 106.0
#2: 2.4 148.0
Diff: 4.3 42.0
Average of: 80
Waveform # 17925
Amp. Time
#1: -1.2 84.0
#2: 0.2 136.0
Diff: 1.4 52.0
Average of: 80
Waveform # 17926
Amp. Time
#1: -2.5 92.0
#2: 1.6 144.0
Diff: 4.1 52.0
Average of: 80
milliseconds
Pattern VER
4: (μV) OzL
5: (μV) OzL
6: (μV) OzL
8x8 100% Contrast Checks 2 Hz
16x16 100% Contrast Checks 2 Hz
32x32 100% Contrast Checks 2 Hz
Waveform # 17921
Amp. Time
#1: -1.3 118.0
#2: -0.2 151.0
Diff: 1.2 33.0
Average of: 80
Waveform # 17922
Amp. Time
#1: -0.8 113.0
#2: 3.5 170.0
Diff: 4.3 57.0
Average of: 80
Waveform # 17920
Amp. Time
#1: -2.6 120.0
#2: 1.0 180.0
Diff: 3.6 60.0
Average of: 80
milliseconds



PVEP: 图形翻转,60'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	64.5	130.0	200.0	10.9	7.9
2L	45.0	99.5	134.5	0.7	2.7

PVEP: 图形翻转,30'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	74.5	118.0	200.0	6.2	4.2
2L	49.5	92.5	135.5	1.6	2.7

PVEP: 图形翻转,15'

No.	N75 [ms]	P100 [ms]	N135 [ms]	N75-P100 [μV]	P100-N135 [μV]
1R	55.0	94.0	146.0	5.1	4.7
2L	80.0	117.0	200.0	6.0	5.0

Fig. S19. Retinal ERG examination of patient 1. ERG detected no obvious changes of rod or cone responses to light stimulus in patient 1. (**A**: pre-injection; **B**: twelve months post-injection)

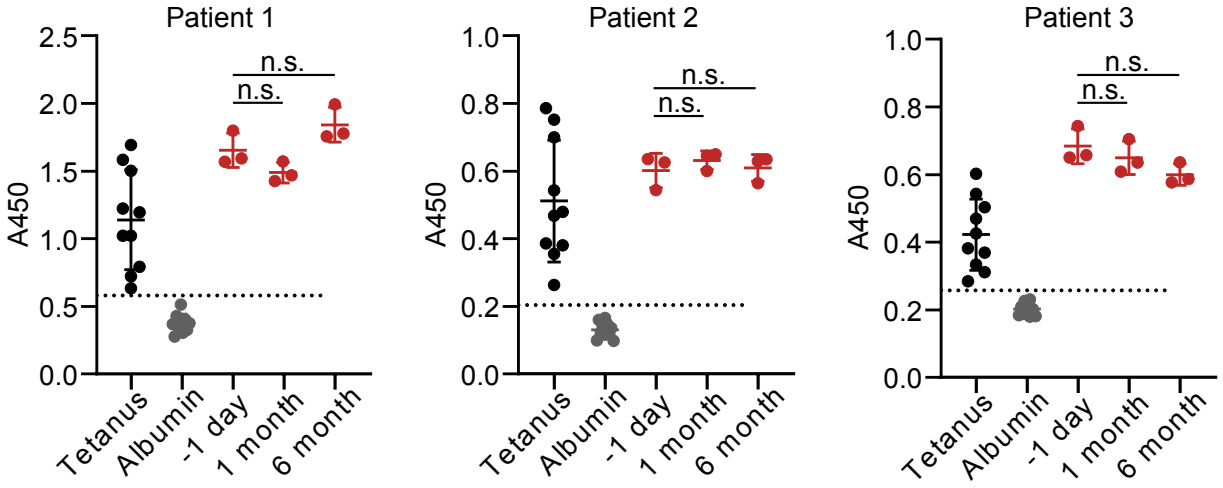


Fig. S20. ELISA results detect antibodies against SpCas9. Tetanus toxoid and human albumin in the sera from different donors served as a positive and negative control, respectively. The Cas9-specific antibodies were determined at different time points. The change in Cas9 antibody levels before and after HELP administration was insignificant. All samples above the dotted line were considered antibody-positive. The dotted line represents the mean absorbance of the negative control, human albumin, plus three s.d. from the mean. Data and error bars represent mean ± s.e.m.; n.s., non-significant; unpaired two-tailed Student's t-tests.

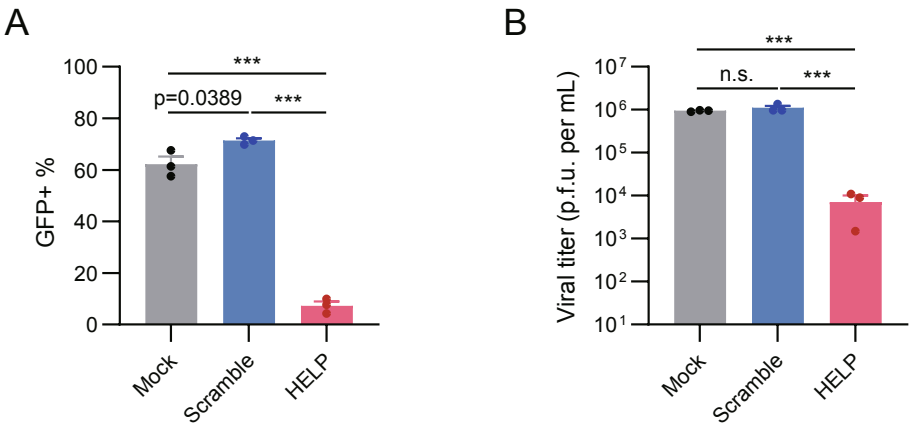


Fig. S21. In vitro antiviral activity of the clinical grade HELP. 4×10^4 293T cells were seeded in a 48-well plate and transduced with 400 ng of HELP or scramble control on the following day. The medium was refreshed 12 h post-infection (h.p.i.). 24 h after transduction, cells were infected with HSV-1-GFP at an MOI of 1. The cells and supernatants were harvested at 24 and 48 h.p.i. for flow cytometry (**A**) and plaque assay (**B**), respectively. 293T cells were transduced with HELP for 24 h and then infected with HSV-1-GFP. n=3 biologically independent samples. Data and error bars represent mean ± s.e.m.; n.s., non-significant; ***P<0.001; unpaired two-tailed Student's t-tests.

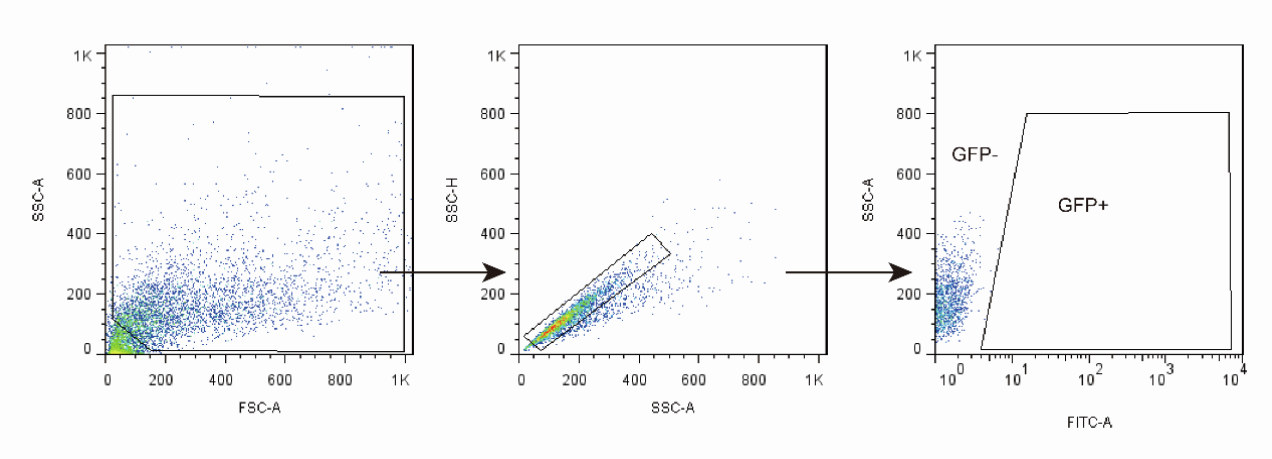


Fig. S22. Gating strategies used for cell sorting analysis. Gate strategy to sort GFP positive cells from HSV-1-GFP infected 293T cells on supplementary Fig. S21A.

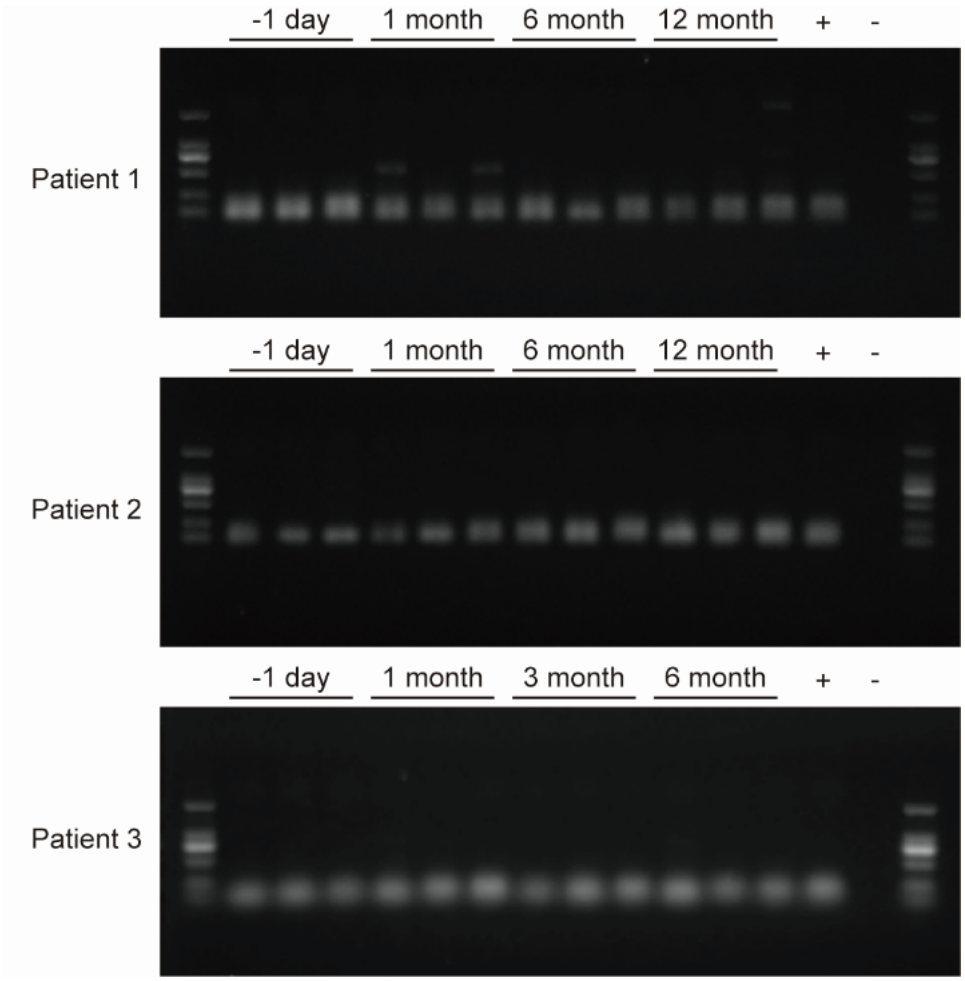


Fig. S23. Validity of patient samples. GAPDH gene in the tear swab of patients was tested by PCR to verify the validity of patient samples. Eye swab samples of 1 day pre-injection, 1 month, 6 months, 12 months post-injection of patient 1 & 2, and samples of 1 day pre-injection, 1 month, 3 months, 6 months post-injection of patient 3 were used as templates for PCR followed by nucleic acid gel electrophoresis. The results showed that GAPDH was positive in all samples, indicating that the swabs were valid.

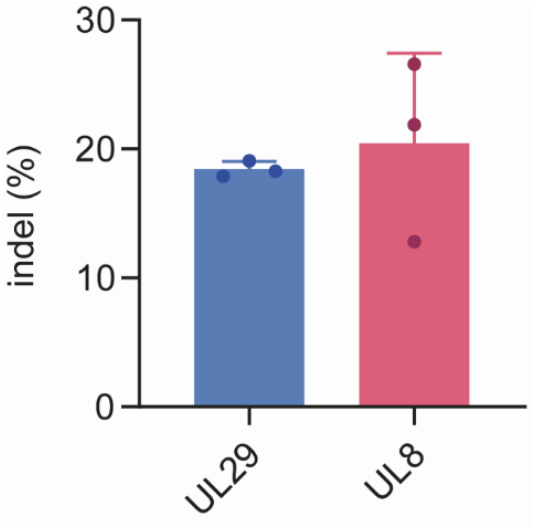
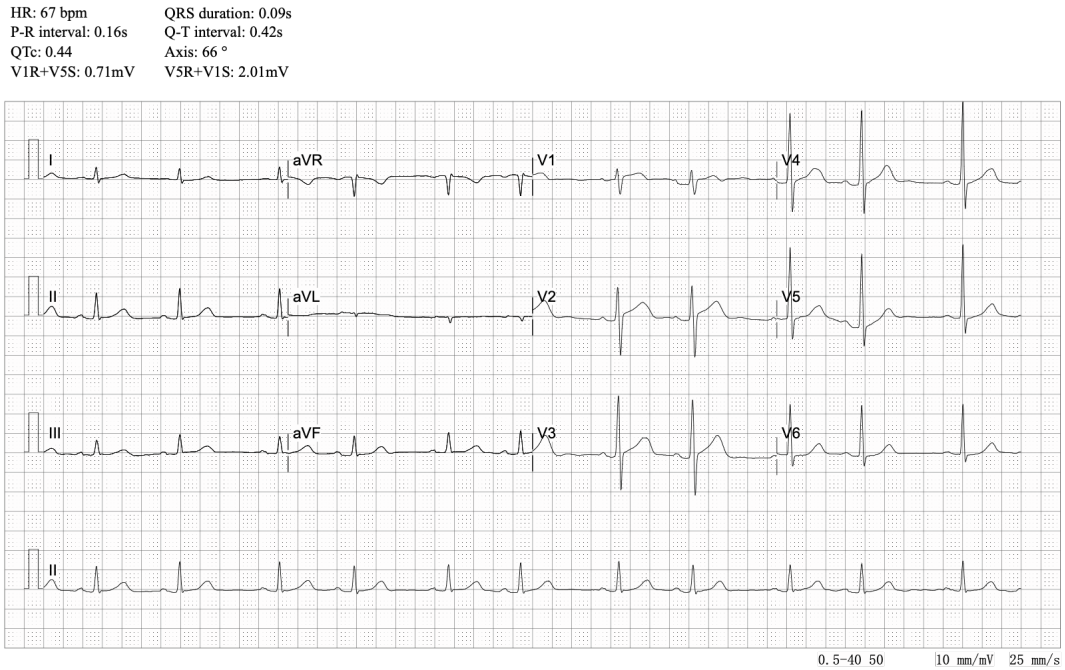


Fig. S24. Gene editing efficiency of the clinical grade HELP. TIDE analysis of indels in the HSV-1 genome. 2×10^4 293T cells were incubated with 200 ng P24 HELP, then infected with HSV-1 with MOI=1 24 h later. Virus DNA was collected at 2 days post-infection for Sanger sequencing. n=3 biologically independent samples.

A



B

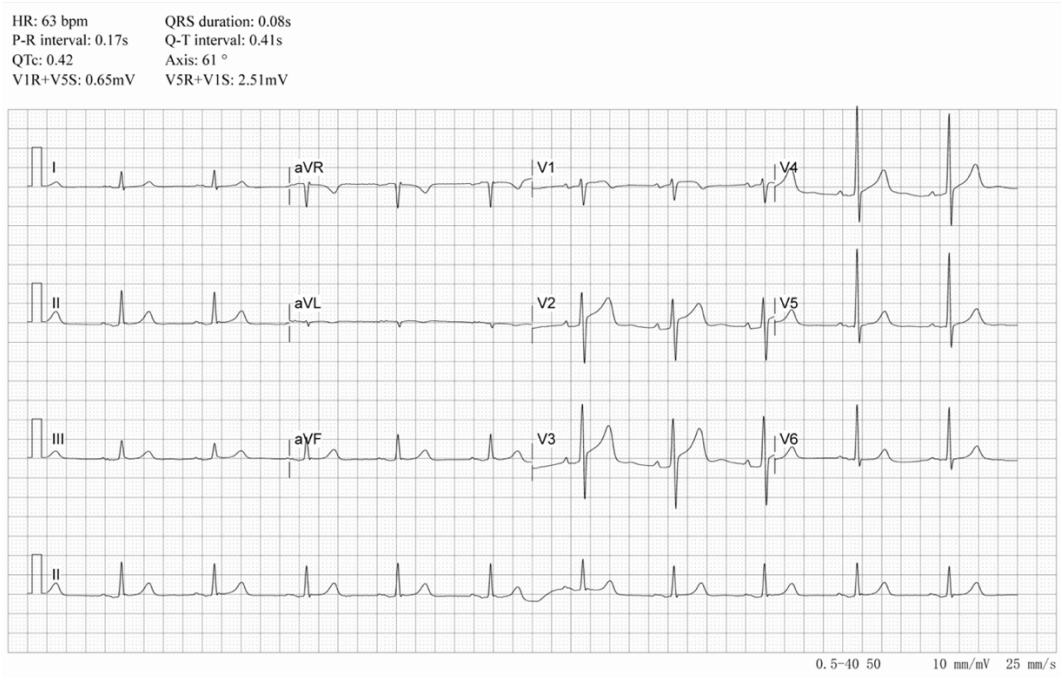
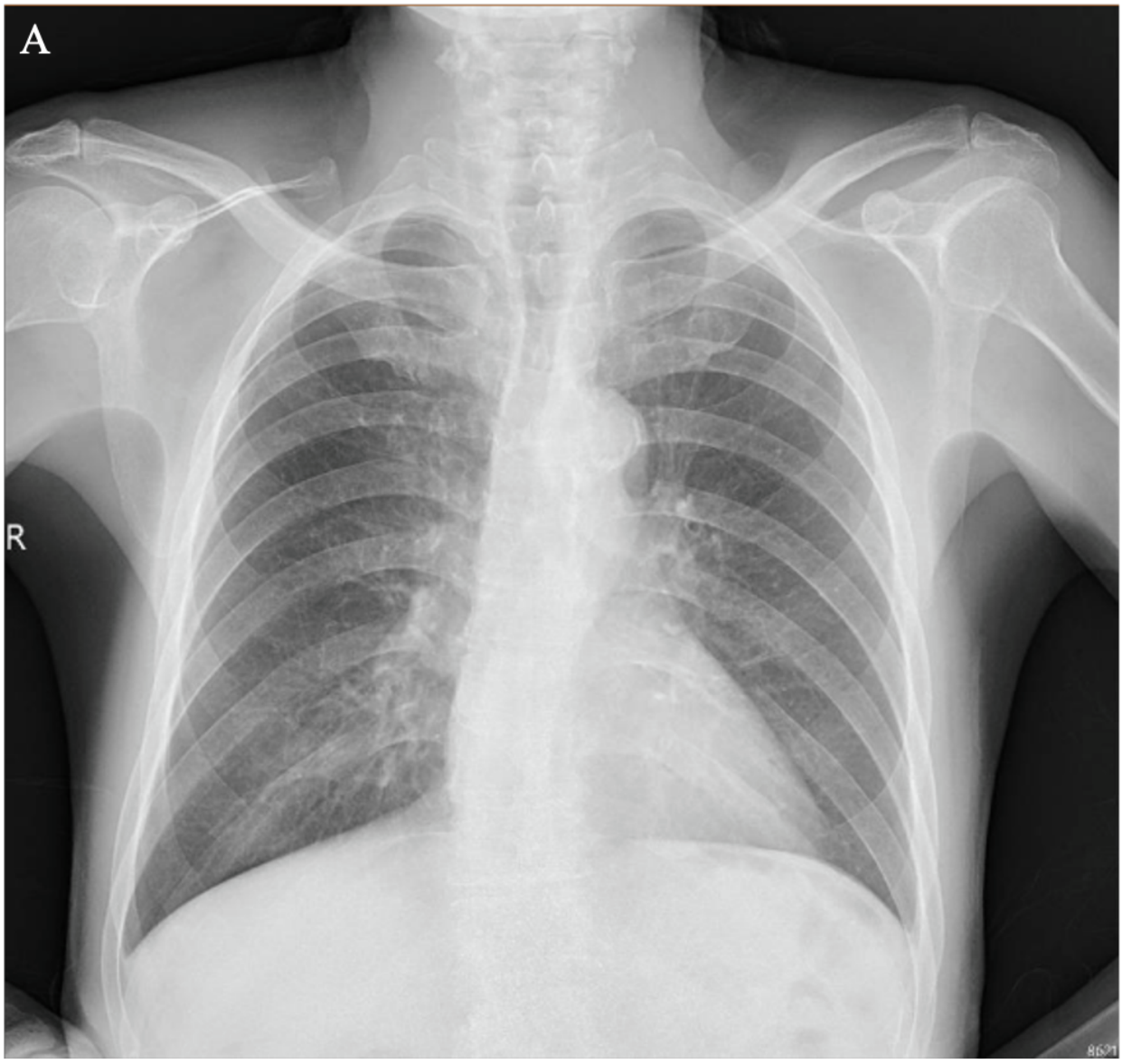


Fig. S25. The ECG examination of patient 1. The pre- and postoperative ECG of patient 1 was unremarkable. (**A**: pre-injection; **B**: twelve months post-injection)


A
R

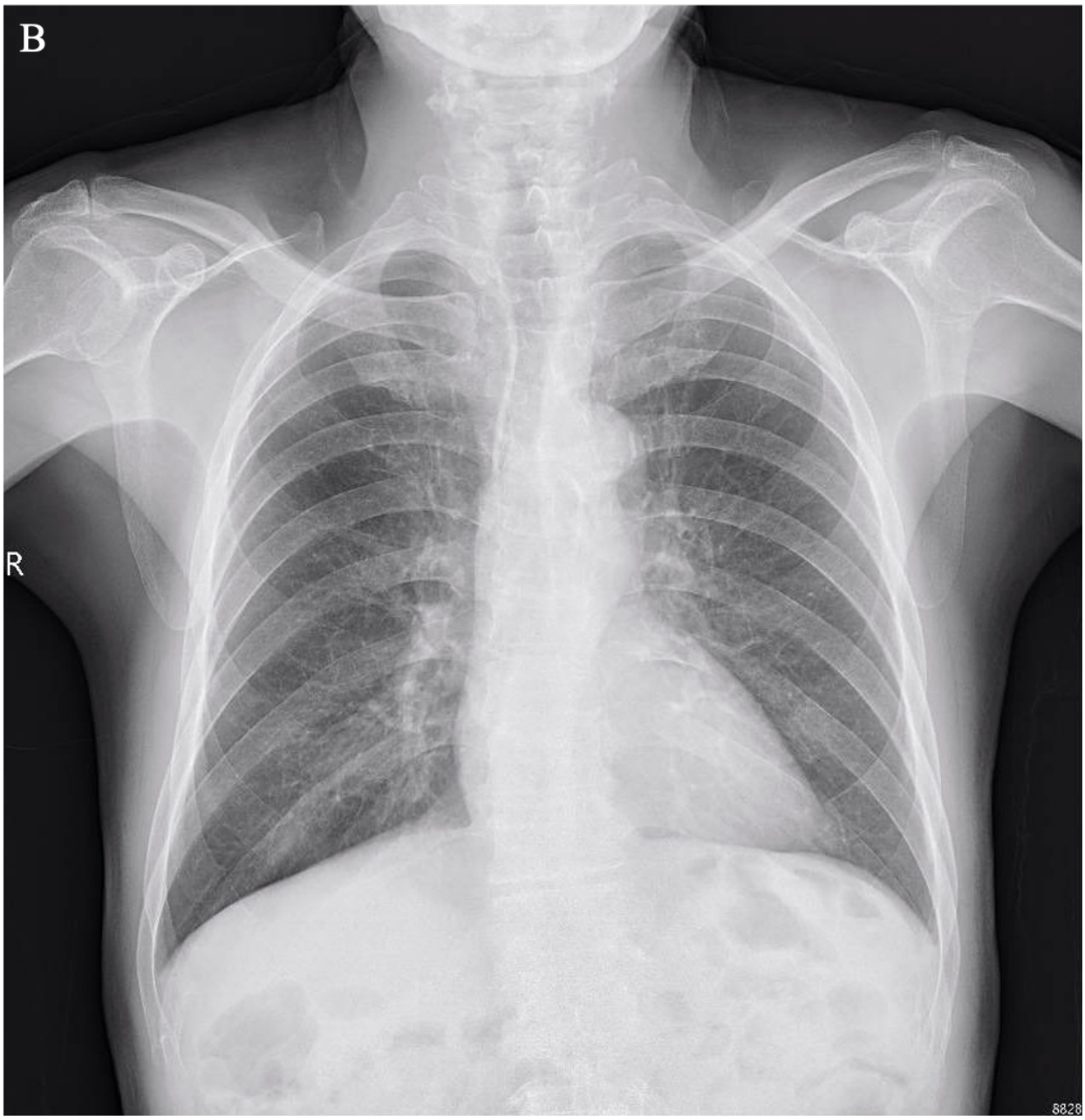


Fig. S26. Chest X-ray examination of patient 1. The Chest X-ray of patient 1 showed no remarkable changes before (**A**) and twelve months (**B**) after the injection.

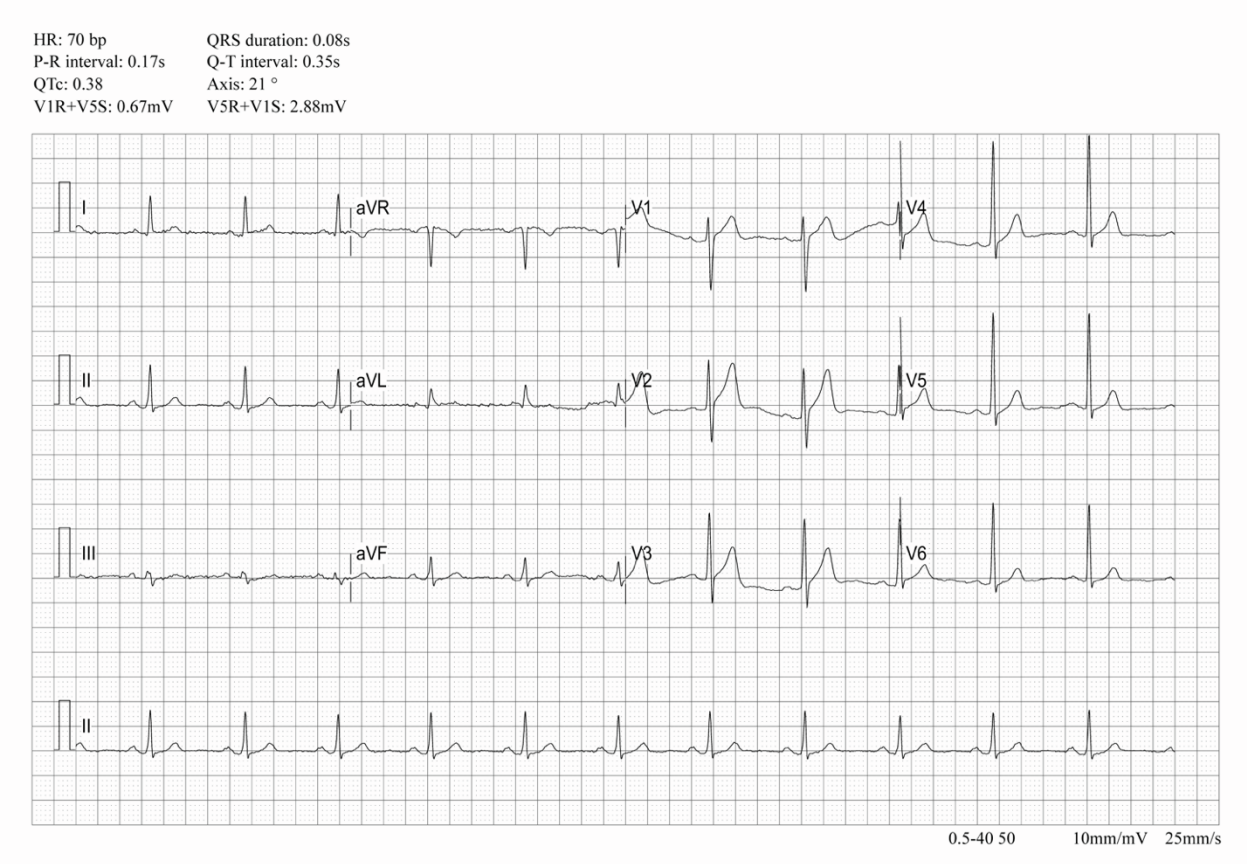


Fig. S27. The ECG of patient 2. The ECG of patient 2 showed counterclockwise transposition, indicating a possibly hypertrophic in the left ventricle. Slightly hypertrophy of the ventricle is not reckoned as a contraindication for corneal transplant surgery.

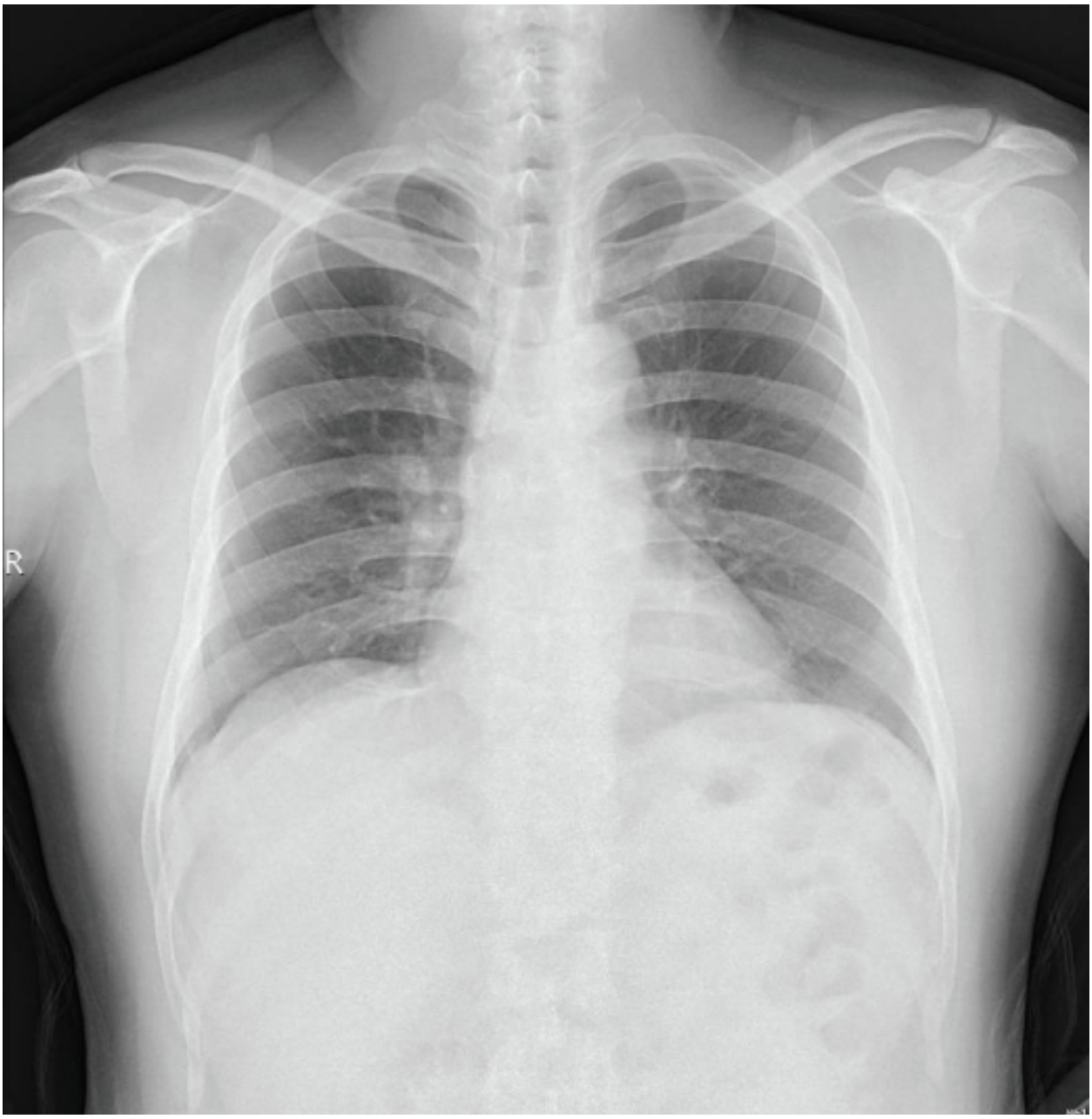


Fig. S28. Chest X-ray film of patient 2. The chest X-ray result of patient 2 was unremarkable.

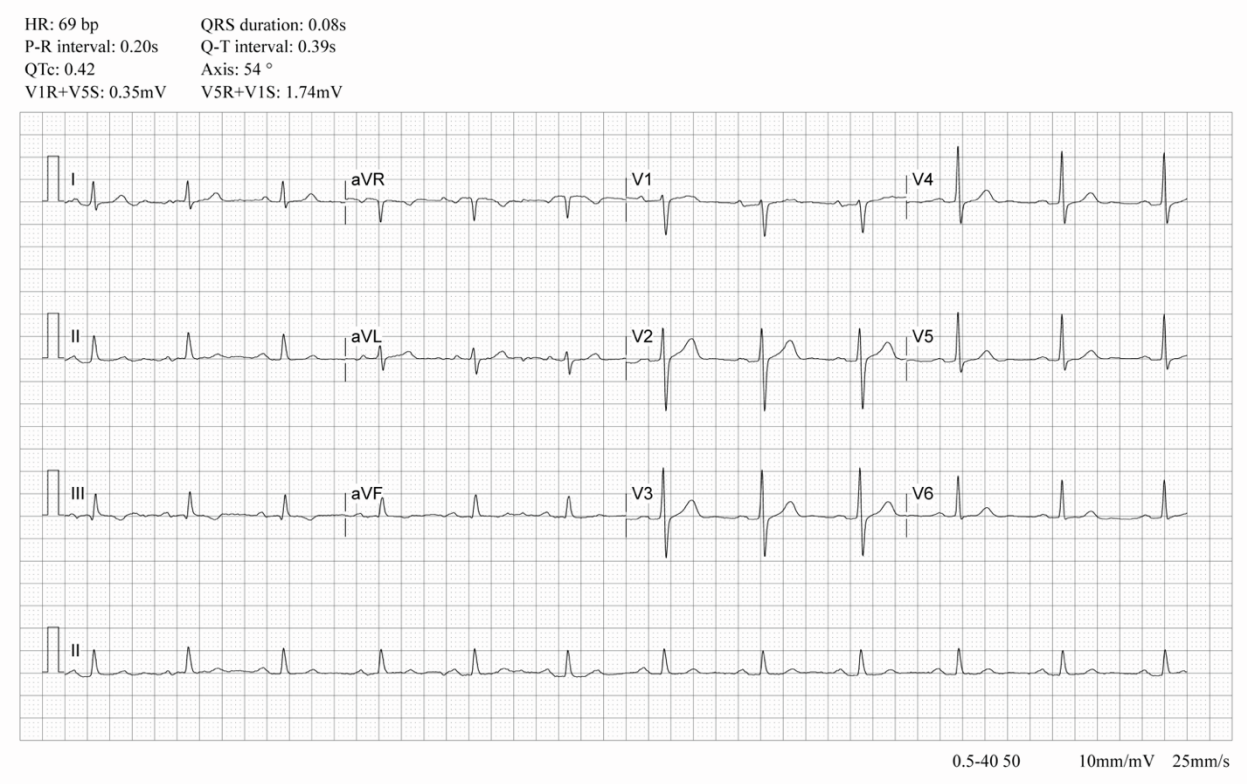


Fig. S29. The ECG examination of patient 3. The pre-operative ECG of patient 3 was unremarkable.

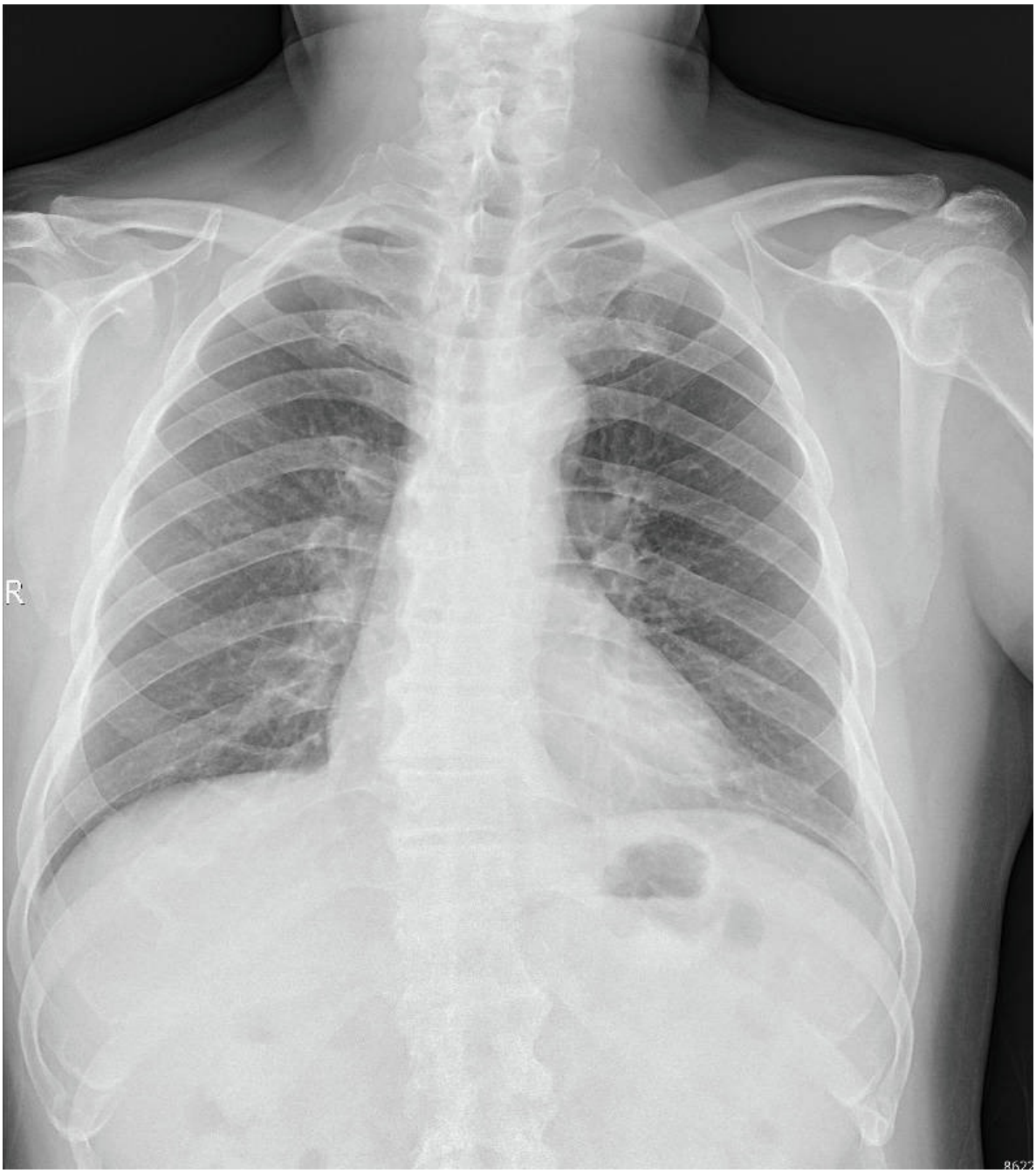


Fig. S30. Chest X-ray film of patient 3. The chest X-ray result of patient 3 was unremarkable.

Table S1. Visual acuity, intraocular pressure and virus tests.

		1 day pre-injection	7 days post-injection	1 month post-injection	2 months post-injection	3 months post-injection	6 months post-injection	12 months post-injection
Patient 1	**Best-corrected visual acuity**	Light perception	Finger count	Finger count	Finger count	Finger count	20/100	20/100
	Intraocular pressure (mmHg)	Undetectable	12.0	10.4	16	15.3	8	21.3
	HSV-1 tests (Ct value)	21.35 (Cornea) 28.37 (Aqueous) 30.55*(Tear swab)	- 24.09(Tear swab) -	28.37(Tear swab) 31.49(Tear swab) 31.08(Tear swab)	- - -	- - -	- - -	- - -
Patient 2	**Best-corrected visual acuity**	Hand motion	20/250	20/167	20/100	20/67	20/133	20/133
	Intraocular pressure (mmHg)	Undetectable	11.0	16.3	16.4	12.4	7.3	15.0
	HSV-1 tests (Ct value)	26.34 (Cornea) 36.50 (Aqueous) 35.01*(Tear swab)	- - -	- - -	- - -	- - -	- - -	- - -
Patient 3	**Best-corrected visual acuity**	Hand motion	Hand motion	Hand motion	Hand motion	Hand motion	Hand motion	Finger count
	Intraocular pressure (mmHg)	Undetectable	n.a.	15.0	24.1	18.5	18.1	19.3
	HSV-1 tests (Ct value)	34.9 (Cornea) - (Aqueous) 29.5 (Tear swab)	- - -	- - -	- - -	- - -	- - -	- - -

*Mean HSV-1 titer (Ct value) of three simultaneously sampled tear swabs; n.a., not available; -, Ct value undetectable.

Table S2. List of adverse events.

Patient	Adverse Event	Start Date	End Date	Placebo Related	Severity	Outcome	Treatment
1	corneal edema	11/2020	11/2020	possibly related	mild	resolved	concomitant medication
	conjunctival injection	11/2020	11/2020	unlikely to be related	mild	resolved	concomitant medication
	hyphema	11/2020	11/2020	unrelated	mild	resolved	none
	neurotrophic keratitis	5/2021	/	unrelated	mild	unresolved	concomitant medication
	uncontrolled corneal ulcer	9/2021	11/2021	unrelated	moderate	resolved	penetrating keratoplasty
	cataract	n.a.	11/2021	unrelated	mild	resolved	cataract surgery
	secondary glaucoma	10/2021	12/2021	unrelated	mild	resolved	concomitant medication
2	corneal edema	1/2021	1/2021	possibly related	mild	resolved	concomitant medication
	conjunctival injection	1/2021	1/2021	unlikely to be related	mild	resolved	concomitant medication
	itching	2/2021	3/2021	unrelated	mild	resolved	concomitant medication
	endophthalmitis	7/2021	7/2021	unrelated	moderate	resolved	vitrectomy and antibiotic injection
	cataract	n.a.	/	unrelated	mild	unresolved	none
3	corneal edema	5/2021	5/2021	possibly related	mild	resolved	concomitant medication
	conjunctival injection	5/2021	5/2021	unlikely to be related	mild	resolved	concomitant medication
	graft rejection	8/2021	10/2021	unrelated	mild	resolved	concomitant medication
	secondary glaucoma	7/2021	8/2021	unrelated	mild	resolved	concomitant medication
	cataract	n.a.	/	unrelated	mild	unresolved	none

Table S3. p24 antibody test.

Positive Index*	Positive control	Negative control	-1 day	1 month	3 months	6 months
Patient 1	15.3	0.49	0.45	0.45	0.45	0.45
Patient 2	13.4	0.51	0.50	0.44	0.46	0.44
Patient 3	11.62	0.49	0.46	0.49	0.5	0.47

* Calculate the mean absorbance values (OD450) for Negative Control using formula:
OD mean (negative control) = (OD (negative control 1) + OD (negative control 2)) / 2.
Calculate the cut-off value: Cut-off = OD mean (negative control) + 0.05
Calculate Positivity Index for each sample:
Positivity Index = mean OD 450 (sample) / Cut-off value.
If Positivity Index value is greater than 1.1, the result is **Positive**.
If Positivity Index value is less than 0.9, the result is **Negative**.

Table S4. Sequencing information on raw data.

Sample	Yield (Mb)	Depth (x)	Reads	Base≥Q30 (%)	Insert size (bp)
WT	192,354	64	1,282,361,462	91.60%	420.725
HELP	185,126	62	1,234,177,196	90.93%	403.405

Note:
Depth (x): The average sequencing depth (Yield bases/Human genome size).
Yield (Mb): The number of bases in raw data.
Reads: The number of paired-end reads.
Base ≥ Q30 (%): The percentage of bases with a quality score of 30 or higher. Q30 means that the sequencing error rate of a base is 0.001.

Insert size (bp): The average size of sample sequencing fragments.

Table S5. The coverage statistics of the sequence alignment.

Sample	Mapping rate	Properly mapped	Fraction of covered with ≥20x	Fraction of covered with ≥0.2 * Mean	Fraction of CDS regions covered with ≥20x
WT	92.254%	89.592%	97.168%	98.809%	98.55%
HELP	92.537%	89.771%	97.095%	98.831%	98.50%

Note:

Mapping rate (%): The percentage of total reads mapping to the reference genome.

Properly mapped: In the paired-end sequencing mode, the percentage of total reads, with both Read1 and Read2 mapping to the genome and corresponding mapping positions consistent with the fragment size distribution of the sequencing library.

Fraction of covered with ≥ 20x (%): It refers to the proportion of bases with a coverage depth not less than 20x in the genome.

Fraction of covered with ≥ 0.2 * Mean (%): It refers to the proportion of bases with a coverage depth of no less than 0.2 fold the mean depth of coverage in the genome.

Fraction of CDS regions covered with ≥ 20x (%): It refers to the proportion of bases with a coverage depth not less than 20x in the CDS regions.

Table S6. The information on potential off-target site

Pos	Strand	Sequence	Variant_Classification	Variant_type	Ref	Alt	DP	DV	AF	Distance to CDS of gene	Distance
chr8:105379575	+	GCGAGaGTAaACaacTCCCAGG	intergenic	SNP	G	T	55	3	0.0545	-10678bp to gene:DCSTAMP; +12064bp to DPYS	20

Note:
Pos: The mutation site on chromosome.
Strand: The strand where the off-target sites are located.
Sequence: Sequence of off-target sites.
Variant_Classification: The genome function regions where the mutations occurred.
Variant_type: Variant type (SNP/Indel). SNP, single nucleotide polymorphism.
Ref: The corresponding base in the reference genome (before editing) at this mutation site.
Alt: The mutated base/sequence.
DP: Sequencing depth of the locus.
DV: Sequencing depth of the mutation base.
AF: Proportion of reads carrying variant alleles.
Distance to CDS of gene: The distance between gene and the mutation.
Distance: The distance between the mutation site and the homologous region site, with negative values indicating that the mutation occurred upstream of the homologous site and positive values indicating that the mutation occurred downstream of the homologous site

Table S7. The statistics table of SV.

Group	BND	DEL	DUP	INS	INV	Total
HELP_vs _WT	7	2	0	1	0	10
WT_vs_HELP	6	1	0	0	0	7

Note:

1) Group: The first line is in the format case_vs_control, which is the experiment and control groups provided by the project. The second line is in the format control_vs_case. The WT of the project is the untreated HCSCs and HELP-treated HCSCs is the experimental group (case).
2) BND: The number of chromosomal translocation breakend variations of samples.
3) DEL: The number of large segment deletion variations of samples.
4) DUP: The number of tandem duplication variations of samples.
5) INS: The number of chromosome segment insertion variations of samples.
6) INV: The number of chromosome segment inversion variations of samples.
7) Total: Total number of structural variations detected of samples.

Table S8. SV annotation of sample group HELP_vs_WT.

Chrom	POS	FORMAT	WT	HELP	Somatic Score	SV type	Cyto Band	Gene_name	Gene count	Function_Gene	RE_gene	ExAC_delZ	ExAC_dupZ	OMIM_ID	GnomAD_pLI	ExAC_pLI	ACMG_class
chr3	60598188	PR:SR	46,0:44,0	28,2:40,3	41	DEL	p14.2	FHIT;MIR548BB	2	Intron/exonic	AAGAB(HI=3/morbid/RE=mTL_miRNA);ABHD5(morbid/RE=mTL_miRNA);.....	0.44589193	-0.556419	.	0.0057055	0.0013918	3
chr8	48662203	PR:SR	15,0:46,5	31,6	40	DEL	q11.21	.	0	intergenic	.	.	.	.	.	.	3
chr11	1628828	PR	75,0	31,6	64	BND	p15.5	KRTAP5-3	1	exonic	.	.	.	.	0.16438	0.26291	.
chr11	50325994	PR	62,0	24,0:43,8	60	BND	p11.12	.	0	intergenic	.	.	.	.	.	.	.
chr11	71238526	PR	75,0	74,8	48	BND	q13.4	KRTAP5-7	1	exonic	.	.	.	.	0.090951	0.58196	.
chr16	21031742	PR:SR	2,0:18,0	51,7	38	INS	p12.3	DNAH3	1	intron	.	-2.253479	0.84066663	.	1.54E-64	9.63E-51	.
chr16	35157286	PR	62,0	74,8	49	BND	p11.1	.	0	intergenic	.	.	.	.	.	.	.
chr4	187503324	PR	45,0	4,0:12,7	44	BND	q35.2	.	0	intergenic	.	.	.	.	.	.	.
chr4	190110388	PR	45,0	51,7	44	BND	q35.2	.	0	intergenic	.	.	.	.	.	.	.
chrY	9986510	PR	231,1	199,7	33	BND	p11.2	.	0	intergenic	.	.	.	.	.	.	.

Note:

Chrom: Name of the chromosome.

POS: The starting site of chromosome of structural variation.

FORMAT: The FORMAT column from a VCF file. PR indicates spanning paired-read support for the ref and alt alleles in the order listed. SR indicates split reads for the ref and alt alleles in the order listed

WT: The support reads of control sample which is shown in the format indicated by column “Format”

HELP: The support reads of case sample which is shown in the format indicated by column “Format”.

SomaticScore: Somatic variant quality score.

SV_type: Types of structural variation.

CytoBand: Cytogenic band annotation.

Gene_name: Gene symbol.

Gene_count: Number of overlapped genes with the copy number variation segment.

Function_Gene: The gene function regions where the variant occurred.

RE_gene: Name of the genes regulated by a regulatory element overlapped with the SV to annotate.

ExAC_delZ: Positive delZ_ExAC (Z score) from ExAC indicate gene intolerance to deletion.

ExAC_dupZ: Positive dupZ_ExAC (Z score) from ExAC indicate gene intolerance to duplication.

OMIM_ID: OMIM unique six-digit identifier.

GnomAD_pLI: Score computed by gnomAD indicating the probability that a gene is intolerant to a loss of function variation.

ExAC_pLI: Score computed by ExAC indicating the probability that a gene is intolerant to a loss of function variation. ExAC considers pLI >= 0.9 as an extremely LoF intolerant gene.

ACMG_class: SV ranking class into 1 of 5: class 1 (benign) class 2 (likely benign) class 3 (variant of uncertain significance) class 4 (likely pathogenic) class 5 (pathogenic).

Table S9. SV annotation of sample group WT_vs_HELP.

Chrom	POS	FORMAT	HELP	WT	SomaticScore	SV_type	Cyto Band	Gene_name	Gene_count	Function_Gene	RE_gene	ExAC_delZ	ExAC_dupZ	OMIM_ID	GnomAD_pLI	ExAC_pLI	ACMG_class
chr5	149029138	PR:SR	13,0:10,0	16,0:8,5	30	DEL	q32	.	0	intergenic	.	.	.	.	.	.	3
chr10	24500527	PR:SR	29,0:18,1	28,0:15,6	47	BND	p12.2	KIAA1217	1	intron	.	1.17216141	0.58181469	.	0.00023185	0.014168	.
chr10	58939351	PR	80,1	61,9	30	BND	q21.1	.	0	intergenic	.	.	.	.	.	.	.
chr15	47872183	PR:SR	47,0:63,0	48,2:54,2	34	BND	q21.1	SEMA6D	1	intron	.	0.50209124	-0.2822914	.	0.93948	0.99947	3
chr3	50478844	PR:SR	47,0:63,0	48,2:54,2	34	BND	p21.31	CACNA2D2	1	intron	.	1.68574942	1.51141627	607082	0.99997	0.99969	3
chr5	84159932	PR:SR	29,0:18,1	28,0:15,6	47	BND	q14.3	.	0	intergenic	.	.	.	.	.	.	.
chr9	123131688	PR	80,1	61,9	30	BND	q33.2	.	0	intergenic	.	.	.	.	.	.	.

Table S10. The primers for UL8 and UL29 amplification.

Target sites	gRNA Sequence (5'-3')	Primer names	Sequence (5'-3')
UL8	GGGGCAGCCATACCGCGTAA	Y1-F	gagccgtagaaatcccgcag
		Y2-R	aaacctcaccaaacagacaa
UL29	GCGAGCGTACACGTATCCC	Y3-F	gggtgtagtccgaaaagccaa
		Y4-R	cacgcccccaggtaaagtgta

Table S11. The quality report of HELP.

Source	OBiO Technology (Shanghai) Corp., Ltd.	**Specification**	0.25 ml/vial
Test Category	**Test**	**Result**	
Quantification	p24 protein content	1.19E+04 ng p24/ml	
Safety	Bacteria	Negative	
	Mycoplasma	Negative	
Conclusion	Qualified		

Table S12. Intraocular bacterial and fungal culture results of patient 2. Gram+ cocci were found in the right eye of patient 2 during the vitrectomy 6 months after PK.

Culture	Presence	Species
Bacteria	+	Gram-positive cocci
Fungus	-	-

Table S13. Blood tests of patient 1. The blood tests of patient 1 showed slight anemia, hypoproteinemia, and diabetes before the injection. We observed no abnormal changes which were related to the injection in these blood tests at 12-month follow-up.

Code	Pre-Injection	Twelve Months Post-Injection	Normal Range	Unit
White blood cell count	5.24	4.39	4.0~10.0	10^9/L
Red blood cell count	2.71 ↓	3.47 ↓	4.3~5.8	10^12/L
Hemoglobin	106 ↓	122 ↓	130~175	g/l
Packed red blood cell volume	30.7 ↓	36.9 ↓	40~50	%
Red blood cell volume distribution width-coefficient of variation	15.1 ↑	15.2 ↑	10.0~15.0	%
Red blood cell volume distribution width-standard deviation	62.3 ↑	59.8 ↑	35.0~50.0	fL
Mean corpuscular volume	113.3 ↑	106.3 ↑	80.0~100.0	fL
Mean corpuscular hemoglobin	39.1 ↑	35.2 ↑	27.0~33.0	Pg
Mean corpuscular hemoglobin concentration	345	331	320~360	g/l
Platelet count	175	213	100~400	10^9/L
Platelet distribution width	9.7	11.1	9.0~17.00	
Thrombocytocrit	0.17	0.22	0.16~0.22	%
Mean platelet volume	9.7	10.2	9.0~16.0	fL
Platelet-larger cell ratio	21.6	25.0	14.0~46.0	%
Percentage of neutrophil	61.6	56.2	50.0~70.0	%
Percentage of lymphocyte	32.1	30.8	20.0~40.0	%
Percentage of eosinophil	1.5	3.0	0.5~5.0	%
Percentage of monocyte	4.4	8.9 ↑	3.0~8.0	%
Percentage of basophil	0.4	1.1 ↑	0.0~1.0	%
Neutrophil count	3.23	2.47	2.0~7.0	10^9/L
Lymphocyte count	1.68	1.35	0.8~4.0	10^9/L
Monocyte count	0.23	0.39	0.10~0.80	10^9/L
Eosinophil count	0.08	0.13	0.00~0.50	10^9/L
Basophil count	0.02	0.05	0.00~0.10	10^9/L
Code	**Pre-Injection**	**Twelve Months Post-Injection**	**Normal Range**	**Unit**
Alanine aminotransferase	22	31	0~65	u/l
Aspartate aminotransferase	25	30	15~37	u/l
Total protein	67	71	64~82	g/l
Albumin	47	43	35~54	g/l
Globulin	20	28	20~40	g/l
Albumin/globulin	2.4	1.5	1.2~2.5	

γ-Glutamyltransferase	55		37		15~85	u/l
Prealbumin	150	↓	200		200~400	mg/l
Alkaline phosphatase	134		137	↑	50~136	u/l
Blood urea nitrogen	6.8	↑	9.2	↑	2.5~6.4	mmol/l
Creatinine	78		90		53~115	umol/l
Uric acid	0.42	↑	0.38	↑	0.202~0.417	mmol/l
Total bilirubin	10		6		0~17	umol/l
Connect bilirubin	5		2		1~5	umol/l
Total bile acid	14.5	↑	14.4	↑	0.0~10.0	umol/l
High density lipoprotein	1.73		1.86		0.910~2.060	mmol/l
Low density lipoprotein	1.33		2.21		0.00~3.36	mmol/l
Apoprotein A	1.53		1.28		1.100~1.700	g/l
Apoprotein B	0.56	↓	0.76	↓	0.800~1.550	g/l
Apoprotein E	35		38		27~45	mg/l
Lipoprotein small a	25		42		0~300	mg/l
Small dense low density lipoprotein	0.74		1.20		0.26~1.36	mmol/l
Calcium	2.21		2.21		2.04~2.74	mmol/l
Phosphorus	1.24		1.15		0.80~1.60	mmol/l
Potassium	4.6		4.8		3.5~5.4	mmol/l
Sodium	145		143		135~147	mmol/l
Chloride	109	↑	103		96~108	mmol/l
Carbon dioxide binding capacity	22		24		21~32	mmol/l
Lactate dehydrogenase	172		153		81~234	u/l
Creatine kinase	75		95		39~308	u/l
Complement 3c	0.73	↓	0.71	↓	0.9~1.8	g/l
Complement 4	0.20		0.16		0.1~0.4	g/l
Complement 1q	108.7	↓	117.7	↓	159~233	mg/l
Total complement	68.7	↑	62.7	↑	32.5~58.3	u/ml
Haptoglobin	56.50		92.00		32~205	mg/dl
Blood glucose	6.1		6.9	↑	3.9~6.1	mmol/l
Total cholesterol	3.04		4.54		2.80~5.20	mmol/l
Triacylglycerol	0.77		0.70		0.34~2.26	mmol/l

Code	**Pre-Injection**	**Twelve Months Post-Injection**	**Normal Range**	**Unit**
Prothrombin time	13.7	12.7	11.0~14.5	sec
International normalized ratio	1.03	0.98	0.80~1.20	INR
Activated partial thromboplastin time	35.9	32.6	28~45	s
Thrombin time	16.3	17.2	14.0~21.0	s

Fibrinogen	2.70		3.01	2.00~4.00	g/l
D-Dimer	0.67	↑	0.75	↑	0.00~0.50	ug/ml
Prothrombin time ratio	95		105	70~150	%

Code	**Pre-Injection**		**Twelve Months Post-Injection**		**Normal Range**	**Unit**
Glycated hemoglobin	6.2	↑	6.2	↑	4.0~6.0	%

*Red font denotes an elevated clinical index than the normal range; pink font denotes a decreased clinical index than the normal range. Similarly hereinafter.

Table S14. Urine tests of patient 1. The pre-injection and twelve months post-injection routine urinalysis of patient 1 was unremarkable.

Code	Pre-Injection	Twelve Months Post-Injection	Normal Range	Unit
Urine glucose	-	-	-	
Ketone body	-	-	-	
Occult blood	-	-	-	
Protein	-	-	-	
Nitrite	-	-	-	
Bilirubin	-	-	-	
Specific gravity	>=1.030 ↑	1.020	1.003~1.030	
Urine PH value	6.0	6.0	5.0~6.5	
Urobilinogen	16	16	3.0~16.0	umol/l
Leukocyte	-	-	-	
White blood cell count	2	2	0~28	/ul
Red blood cell count	1	6	0~17	/ul
Squamous epithelial cells	-	2	0~28	/ul
Non-squamous epithelial cells	-	1	0~6	/ul
Trichomonas	-	-	0~1	/ul
Kidney epithelial cells	-	-	0~6	/ul
Transparent tube	-	-	0~2	/ul
Particle tube	-	-	0~1	/LPF
Cell tube	-	-	0~1	/LPF
Triphosphate crystal	-	-		
Calcium oxalate crystal	-	-		
Leucine crystal	-	-		
Cystine crystals	-	-		

Table S15. Infectious diseases tests of patient 1. The four transfusion-associated contagion tests (hepatitis B, hepatitis C, syphilis, AIDS) of patient 1 were unremarkable.

Code	Pre-Injection	Twelve Months Post-Injection
Hepatitis B virus surface antigen	Negative	Negative
Hepatitis B virus surface antibody	Negative	Negative
Hepatitis B virus e antigen	Negative	Negative
Hepatitis B virus e antibody	Negative	Negative
Hepatitis B virus core antibody	Negative	Negative
Hepatitis B virus antibody-immunoglobin M	Negative	Negative
Hepatitis B virus pre-S1 antigen	Negative	Negative
Hepatitis C virus antibody	Negative	Negative
Treponema pallidum particle agglutination test	Negative	Negative
Rapid plasma regain test	Negative	Negative
Human immunodeficiency virus antibody	Negative	Negative

Table S16. Blood tests of patient 2. The blood tests of patient 2 indicated mild hyperlipidemia, which had no obvious influence on the implementation of the surgery. We observed no notable changes related to the injection in these blood tests at 12-month follow-up.

Code	Pre-Injection	Twelve Months Post-Injection	Normal Range	Unit
White blood cell count	7.17	10.16 ↑	4.0~10.0	10^9/L
Red blood cell count	4.73	4.69	4.3~5.8	10^12/L
Hemoglobin	148	144	130~175	g/l
Packed red blood cell volume	44.0	43.1	40~50	%
Red blood cell volume distribution width-coefficient of variation	12.1	12.9	10.0~15.0	%
Red blood cell volume distribution width-standard deviation	41.6	43.1	35.0~50.0	fL
Mean corpuscular volume	93.0	91.9	80.0~100.0	fL
Mean corpuscular hemoglobin	31.3	30.7	27.0~33.0	Pg
Mean corpuscular hemoglobin concentration	336	334	320~360	g/l
Platelet count	229	236	100~400	10^9/L
Platelet distribution width	9.3	9.8	9.0~17.00	
Thrombocytocrit	0.21	0.22	0.16~0.22	%
Mean platelet volume	9.1	9.2	9.0~16.0	fL
Platelet-larger cell ratio	17.7	18.4	14.0~46.0	%
Percentage of neutrophil	64.3	77.8 ↑	50.0~70.0	%
Percentage of lymphocyte	28.7	18.3 ↓	20.0~40.0	%
Percentage of eosinophil	1.0	0.3 ↓	0.5~5.0	%
Percentage of monocyte	5.6	3.1	3.0~8.0	%
Percentage of basophil	0.4	0.5	0.0~1.0	%
Neutrophil count	4.61	7.9 ↑	2.0~7.0	10^9/L
Lymphocyte count	2.06	1.86	0.8~4.0	10^9/L
Monocyte count	0.40	0.32	0.10~0.80	10^9/L
Eosinophil count	0.07	0.03	0.00~0.50	10^9/L
Basophil count	0.03	0.05	0.00~0.10	10^9/L
Code	**Pre-Injection**	**Twelve Months Post-Injection**	**Normal Range**	**Unit**
Alanine aminotransferase	17	19	0~65	u/l
Aspartate aminotransferase	17	15	15~37	u/l
Total protein	70	68	64~82	g/l
Albumin	46	42	35~54	g/l
Globulin	24	26	20~40	g/l
Albumin/globulin	1.9	1.6	1.2~2.5	

γ-Glutamyltransferase	33		37	15~85	u/l
Prealbumin	320		350	200~400	mg/l
Alkaline phosphatase	71		80	50~136	u/l
Blood urea nitrogen	5.5		7.5 ↑	2.5~6.4	mmol/l
Creatinine	69		62	53~115	umol/l
Uric acid	0.25		0.32	0.202~0.417	mmol/l
Total bilirubin	16		10	0~17	umol/l
Connect bilirubin	5		2	1~5	umol/l
Total bile acid	2.5		11.2 ↑	0.0~10.0	umol/l
High density lipoprotein	1.24		1.61	0.910~2.060	mmol/l
Low density lipoprotein	4.36	↑	4.72 ↑	0.00~3.36	mmol/l
Apoprotein A	1.46		1.24	1.100~1.700	g/l
Apoprotein B	1.51		1.55	0.800~1.550	g/l
Apoprotein E	52	↑	46 ↑	27~45	mg/l
Lipoprotein small a	41		47	0~75	nmol/l
Small dense low density lipoprotein	3.11	↑	2.70 ↑	0.26~1.36	mmol/l
Calcium	2.34		2.25	2.04~2.74	mmol/l
Phosphorus	1.18		0.99	0.80~1.60	mmol/l
Potassium	4.2		4.0	3.5~5.4	mmol/l
Sodium	140		142	135~147	mmol/l
Chloride	104		101	96~108	mmol/l
Carbon dioxide binding capacity	23		21	21~32	mmol/l
Lactate dehydrogenase	168		182	81~234	u/l
Creatine kinase	122		128	39~308	u/l
Complement 3c	1.03		1.04	0.9~1.8	g/l
Complement 4	0.20		0.32	0.1~0.4	g/l
Complement 1q	154.5	↓	169.4	159~233	mg/l
Total complement	67.9	↑	63.5 ↑	32.5~58.3	u/ml
Haptoglobin	93.30		110.50	32~205	mg/dl
Blood glucose	5.6		6.1	3.9~6.1	mmol/l
Total cholesterol	6.12	↑	7.04 ↑	2.80~5.20	mmol/l
Triacylglycerol	2.33	↑	1.81	0.34~2.26	mmol/l

Code	**Pre-Injection**	**Twelve Months Post-Injection**	**Normal Range**	**Unit**
Prothrombin time	13.3	12.4	11.0~14.5	sec
International normalized ratio	0.99	0.95	0.80~1.20	INR
Activated partial thromboplastin time	35.3	33.0	28~45	s
Thrombin time	18.7	19.7	14.0~21.0	s

Fibrinogen	2.98	2.74	2.00~4.00	g/l
D-Dimer	0.24	0.23	0.00~0.50	ug/ml
Prothrombin time ratio	102	110	70~150	%
Code	**Pre-Injection**	**Twelve Months Post-Injection**	**Normal Range**	**Unit**
Glycated hemoglobin	5.9	5.9	4.0~6.0	%

Table S17. Routine urine test of patient 2. The routine urinalysis of patient 2 was unremarkable.

Code	Pre-Injection	Twelve Months Post-Injection	Normal Range	Unit
Urine glucose	-	-	-	
Ketone body	-	-	-	
Occult blood	-	-	-	
Protein	-	-	-	
Nitrite	-	-	-	
Bilirubin	-	-	-	
Specific gravity	>=1.030 ↑	1.025	1.003~1.030	
Urine PH value	5.5	6.0	5.0~6.5	
Urobilinogen	3.2	16	3.0~16.0	umol/l
Leukocyte	-	-	-	
White blood cell count	6	6	0~28	/ul
Red blood cell count	2	2	0~17	/ul
Squamous epithelial cells	2	1	0~28	/ul
Non-squamous epithelial cells	-	1	0~6	/ul
Trichomonas	-	-	0~1	/ul
Kidney epithelial cells	-	-	0~6	/ul
Transparent tube	-	-	0~2	/ul
Particle tube	-	-	0~1	/LPF
Cell tube	-	-	0~1	/LPF
Triphosphate crystal	-	-		
Calcium oxalate crystal	-	-		
Leucine crystal	-	-		
Cystine crystals	-	-		

Table S18. Transfusion-associated contagion tests of patient 2. The contagion tests of patient 2 showed specific antibodies to the hepatitis B virus. The other three transfusion-associated contagion tests (hepatitis C, syphilis, AIDS) were negative.

Code	Pre-Injection	Twelve Months Post-Injection
Hepatitis B virus surface antigen	Negative	Negative
Hepatitis B virus surface antibody	Weak Positive	Weak Positive
Hepatitis B virus e antigen	Negative	Negative
Hepatitis B virus e antibody	Negative	Negative
Hepatitis B virus core antibody	Positive	Positive
Hepatitis B virus antibody-immunoglobin M	Negative	Negative
Hepatitis B virus pre-S1 antigen	Negative	Negative
Hepatitis C virus antibody	Negative	Negative
Treponema pallidum particle agglutination test	Negative	Negative
Rapid plasma regain test	Negative	Negative
Human immunodeficiency virus antibody	Negative	Negative

Table S19. Blood tests of patient 3. The blood tests of patient 3 indicated mild hyperlipidemia, which had no obvious influence on the implementation of the surgery.

Code	Pre-Injection	Six Months Post-Injection	Normal Range	Unit
White blood cell count	5.45	4.83	4.0~10.0	10^9/L
Red blood cell count	5.28	4.89	4.3~5.8	10^12/L
Hemoglobin	159	152	130~175	g/l
Packed red blood cell volume	46.1	42.8	40~50	%
Red blood cell volume distribution width-coefficient of variation	11.9	12.4	10.0~15.0	%
Red blood cell volume distribution width-standard deviation	37.6	39.0	35.0~50.0	fL
Mean corpuscular volume	87.3	87.5	80.0~100.0	fL
Mean corpuscular hemoglobin	30.1	31.1	27.0~33.0	Pg
Mean corpuscular hemoglobin concentration	345	355	320~360	g/l
Platelet count	151	105	100~400	10^9/L
Platelet distribution width	10.8	12.2	9.0~17.00	
Thrombocytocrit	0.14 ↓	0.11 ↓	0.16~0.22	%
Mean platelet volume	9.5	10.7	9.0~16.0	fL
Platelet-larger cell ratio	21.5	30.8	14.0~46.0	%
Percentage of neutrophil	62.3	57.7	50.0~70.0	%
Percentage of lymphocyte	29.7	32.4	20.0~40.0	%
Percentage of eosinophil	1.7	2.3	0.5~5.0	%
Percentage of monocyte	5.9	7.3	3.0~8.0	%
Percentage of basophil	0.4	0.3	0.0~1.0	%
Neutrophil count	3.40	2.21	2.0~7.0	10^9/L
Lymphocyte count	1.62	1.24	0.8~4.0	10^9/L
Monocyte count	0.32	0.28	0.10~0.80	10^9/L
Eosinophil count	0.09	0.09	0.00~0.50	10^9/L
Basophil count	0.02	0.01	0.00~0.10	10^9/L
Code	**Pre-Injection**	**Six Months Post-Injection**	**Normal Range**	**Unit**
Alanine aminotransferase	29	53	0~65	u/l
Aspartate aminotransferase	18	26	15~37	u/l
Total protein	76	65	64~82	g/l
Albumin	49	40	35~54	g/l
Globulin	27	24	20~40	g/l
Albumin/globulin	1.8	1.66	1.2~2.5	
γ-Glutamyltransferase	37	49	15~85	u/l

Prealbumin	350		310		200~400	mg/l
Alkaline phosphatase	95		103		50~136	u/l
Blood urea nitrogen	6.6	↑	5.6		2.5~6.4	mmol/l
Creatinine	78		77		53~115	umol/l
Uric acid	0.32		0.37		0.202~0.417	mmol/l
Total bilirubin	16		16		0~17	umol/l
Connect bilirubin	7	↑	3		1~5	umol/l
Total bile acid	6		2		0.0~10.0	umol/l
High density lipoprotein	1.16		0.84	↓	0.910~2.060	mmol/l
Low density lipoprotein	2.79		2.24		0.00~3.36	mmol/l
Apoprotein A	1.29		1.00	↓	1.100~1.700	g/l
Apoprotein B	1.000		0.800		0.800~1.550	g/l
Apoprotein E	39		35		27~45	mg/l
Lipoprotein small a	38		42		0~75	nmol/l
Small dense low density lipoprotein	1.90	↑	1.87	↑	0.26~1.36	mmol/l
Calcium	2.32		2.32		2.04~2.74	mmol/l
Phosphorus	0.97		1.23		0.80~1.60	mmol/l
Potassium	4.3		4.4		3.5~5.4	mmol/l
Sodium	140		139		135~147	mmol/l
Chloride	102		105		96~108	mmol/l
Carbon dioxide binding capacity	26		23		21~32	mmol/l
Lactate dehydrogenase	149		175		81~234	u/l
Creatine kinase	75		98		39~308	u/l
Complement 3c	1.07		1.12		0.9~1.8	g/l
Complement 4	0.31		0.29		0.1~0.4	g/l
Complement 1q	195.5		210.4		159~233	mg/l
Total complement	82.5	↑	84.7	↑	32.5~58.3	u/ml
Haptoglobin	86.50		98.20		32~205	mg/dl
Blood glucose	5.7		4.7		3.9~6.1	mmol/l
Total cholesterol	4.36		3.68		2.80~5.20	mmol/l
Triacylglycerol	1.23		2.24		0.34~2.26	mmol/l

Code	**Pre-Injection**	**Six Months Post-Injection**	**Normal Range**	**Unit**
Prothrombin time	13.3	11.1	11.0~14.5	sec
International normalized ratio	1.00	0.94	0.80~1.20	INR
Activated partial thromboplastin time	37.7	32.8	28~45	s
Thrombin time	18.0	16.8	14.0~21.0	s
Fibrinogen	3.43	2.72	2.00~4.00	g/l

D-Dimer	0.28	0.31	0.00~0.50	ug/ml
Prothrombin time ratio	99	97	70~150	%

Code	**Pre-Injection**	**Six Months Post-Injection**	**Normal Range**	**Unit**
Glycated hemoglobin	5.3	4.89	4.0~6.0	%

Table S20. Routine urine test of patient 3. The routine urinalysis of patient 3 was unremarkable.

Code	Pre-Injection Results		Normal Range	Unit
Urine glucose	-		-	
Ketone body	-		-	
Occult blood	-		-	
Protein	-		-	
Nitrite	-		-	
Bilirubin	-		-	
Specific gravity	>=1.030	↑	1.003~1.030	
Urine PH value	7.0	↑	5.0~6.5	
Urobilinogen	3.2		3.0~16.0	umol/l
Leukocyte	-		-	
White blood cell count	1		0~28	/ul
Red blood cell count	1		0~17	/ul
Squamous epithelial cells	-		0~28	/ul
Non-squamous epithelial cells	-		0~6	/ul
Trichomonas	-		0~1	/ul
Kidney epithelial cells	-		0~6	/ul
Transparent tube	-		0~2	/ul
Particle tube	-		0~1	/LPF
Cell tube	-		0~1	/LPF
Triphosphate crystal	-			
Calcium oxalate crystal	-			
Leucine crystal	-			
Cystine crystals	-			

Table S21. Transfusion-associated contagion tests of patient 3. The contagion tests of patient 3 showed specific antibodies to the hepatitis B virus. The other three transfusion-associated contagion tests (hepatitis C, syphilis, AIDS) were negative.

Code	Pre-Injection Results
Hepatitis B virus surface antigen	Negative
Hepatitis B virus surface antibody	Positive
Hepatitis B virus e antigen	Negative
Hepatitis B virus e antibody	Positive
Hepatitis B virus core antibody	Positive
Hepatitis B virus antibody-immunoglobin M	Negative
Hepatitis B virus pre-S1 antigen	Negative
Hepatitis C virus antibody	Negative
Treponema pallidum particle agglutination test	Negative
Rapid plasma regain test	Negative
Human immunodeficiency virus antibody	Negative

Video S1. HELP Administration method. Each formulation, 0.2 mL in total, was drawn into a 27g ophthalmic syringe for the intrastromal injection. Penetrating keratoplasty was performed following the routine procedures. After sewing up the donor cornea, the graft bed of the recipient was injected with the HELP formulation in 6-8 locations.

Supplemental Results of Whole Genome Sequencing (WGS)

The sequencing depth of sequencing data of the two samples (HELP-treated group and WT group) was sufficient (62x and 64x, respectively) with good sequencing quality (Q30 > 90%) (Table S4). After sequence alignment, the results showed that mapping rate to human genome (hg19) was greater than 92%, and the fraction of covered with ≥20x and 0.2*Mean were greater than 97%. The fraction of CDS regions covered with ≥20x was greater than 98%. These results indicating a good quality of the sequencing data (Table S5).

The analysis of off-target sites showed that there were few potential off-target sites. In the HELP-treated cells, no potential off-target sites of UL8 sgRNA were identified, and one potential off-target site of UL29 sgRNA was identified, which was a SNP located at chr8:105,379,595 in the intergenic region and far from CDS (>10K) with low mutation frequency of 0.0545 (Table S6).

Structural variants (SVs) analysis revealed ten SVs in the HELP-treated HCSCs sample after filtering out the data of untreated HCSCs sample (WT sample), and seven SVs in the WT sample after filtering out the data of HELP-treated HCSCs sample. All the structural variants have low risk of affecting the gene function and causing pathogenicity (Table S7-9). Considering the structural variants result, it is presumed that these SVs probably occur due to the nature of the cells rather than HELP treatment.