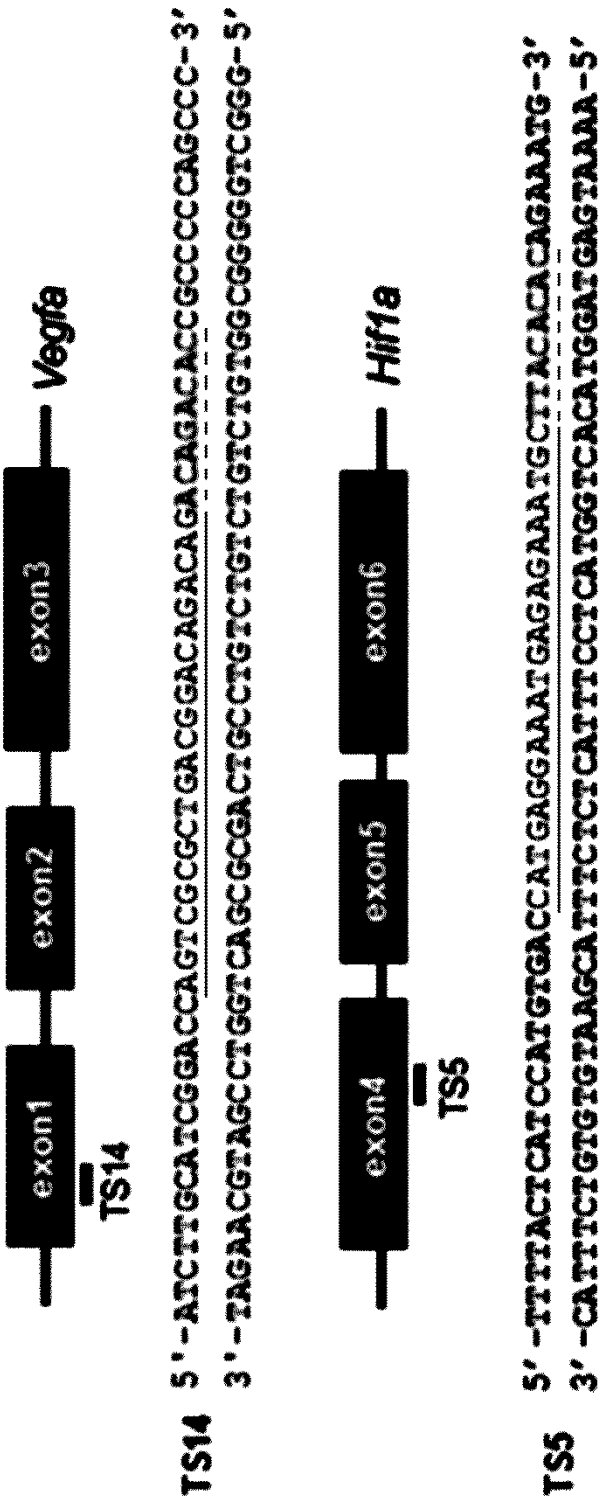

Vegfa
exon1
exon2
exon3
TS14
TS14 5'-ATCTTGCATCGGACCAGTCGCGCTGACGGACAGACAGACACCGCCCCCCAGCCC-3'
3'-TAGAACGTAGCCTGGTCAGCGCGACTGCCTGTCTGTCTGTGGCGGGGGTCGGG-5'
Hif1a
exon4
exon5
exon6
TS5
TS5 5'-TTTACTCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG-3'
3'-CATTTCTGTGTAAGCATTTCTCTCATTTCCTCATGGTCACATGGATGAGTAAAA-5'

FIG. 1a

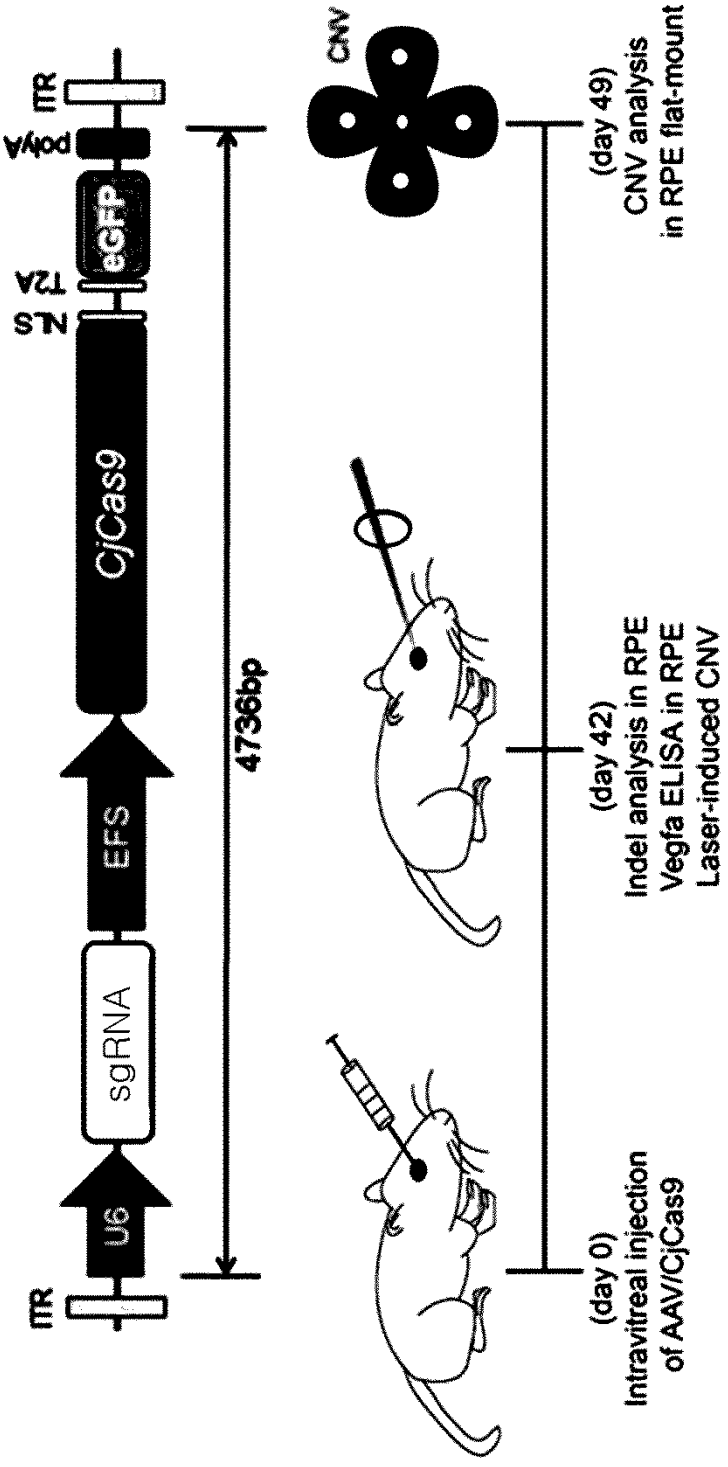

ITR
U6
sgRNA
EFS
CjCas9
NLS
T2A
eGFP
polyA
ITR
4736bp
CNV
(day 0)
Intravitreal injection
of AAV/CjCas9
(day 42)
Indel analysis in RPE
Vegfa ELISA in RPE
Laser-induced CNV
(day 49)
CNV analysis
in RPE flat-mount

FIG. 1b

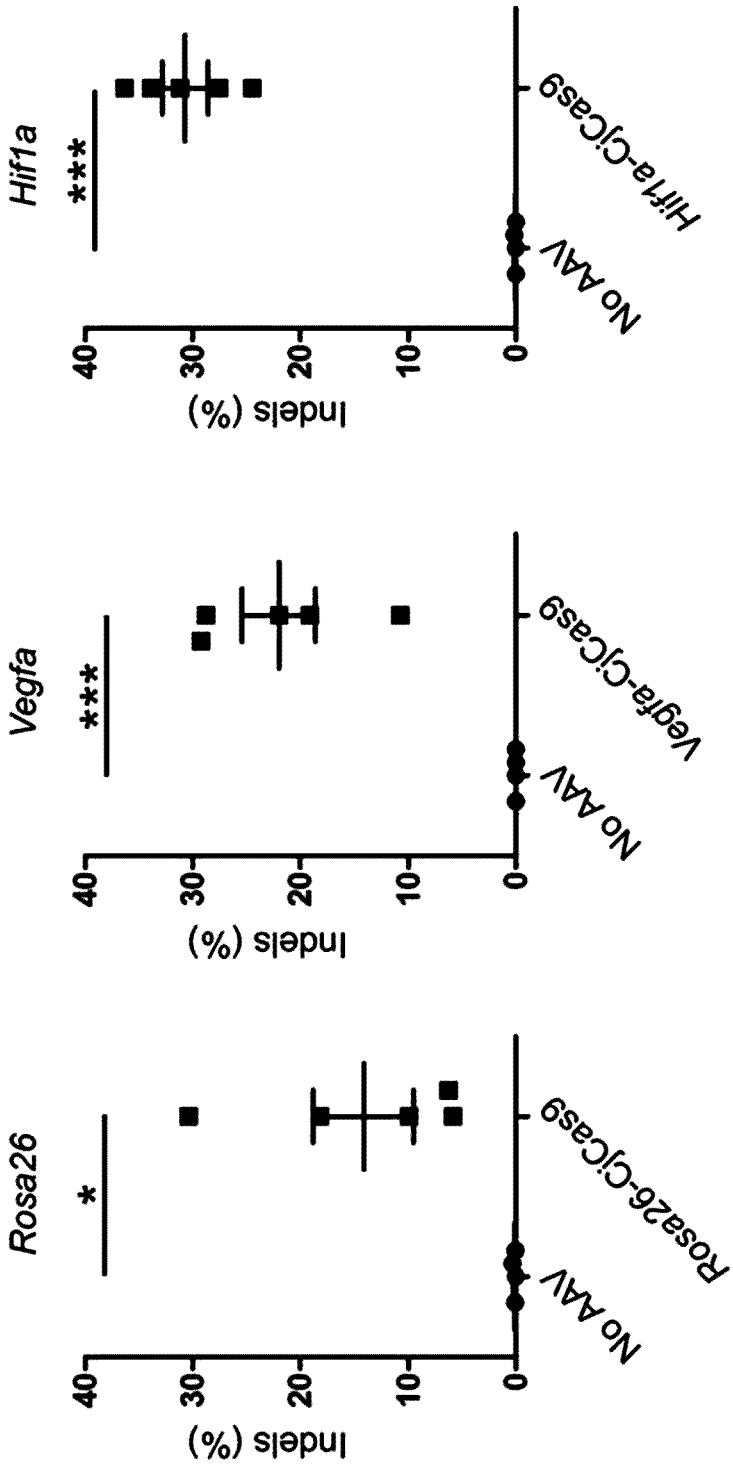

Hif1a
***
Hif1a-CjCas9
No AAV
40
30
20
10
0
Indels (%)
Vegfa
***
Vegfa-CjCas9
No AAV
40
30
20
10
0
Indels (%)
Rosa26
*
Rosa26-CjCas9
No AAV
40
30
20
10
0
Indels (%)

FIG. 1c

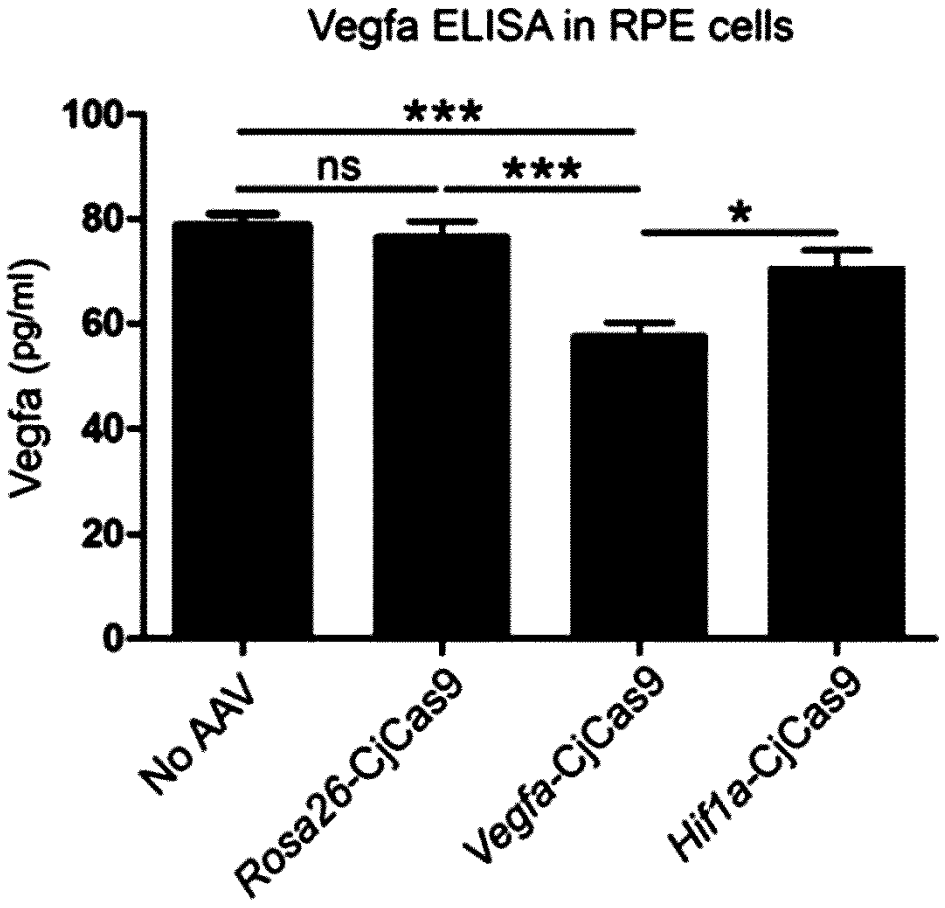

Vegfa ELISA in RPE cells
Vegfa (pg/ml)
100
80
60
40
20
0
***
ns
***
*
No AAV
Rosa26-CjCas9
Vegfa-CjCas9
Hif1a-CjCas9

FIG. 1d

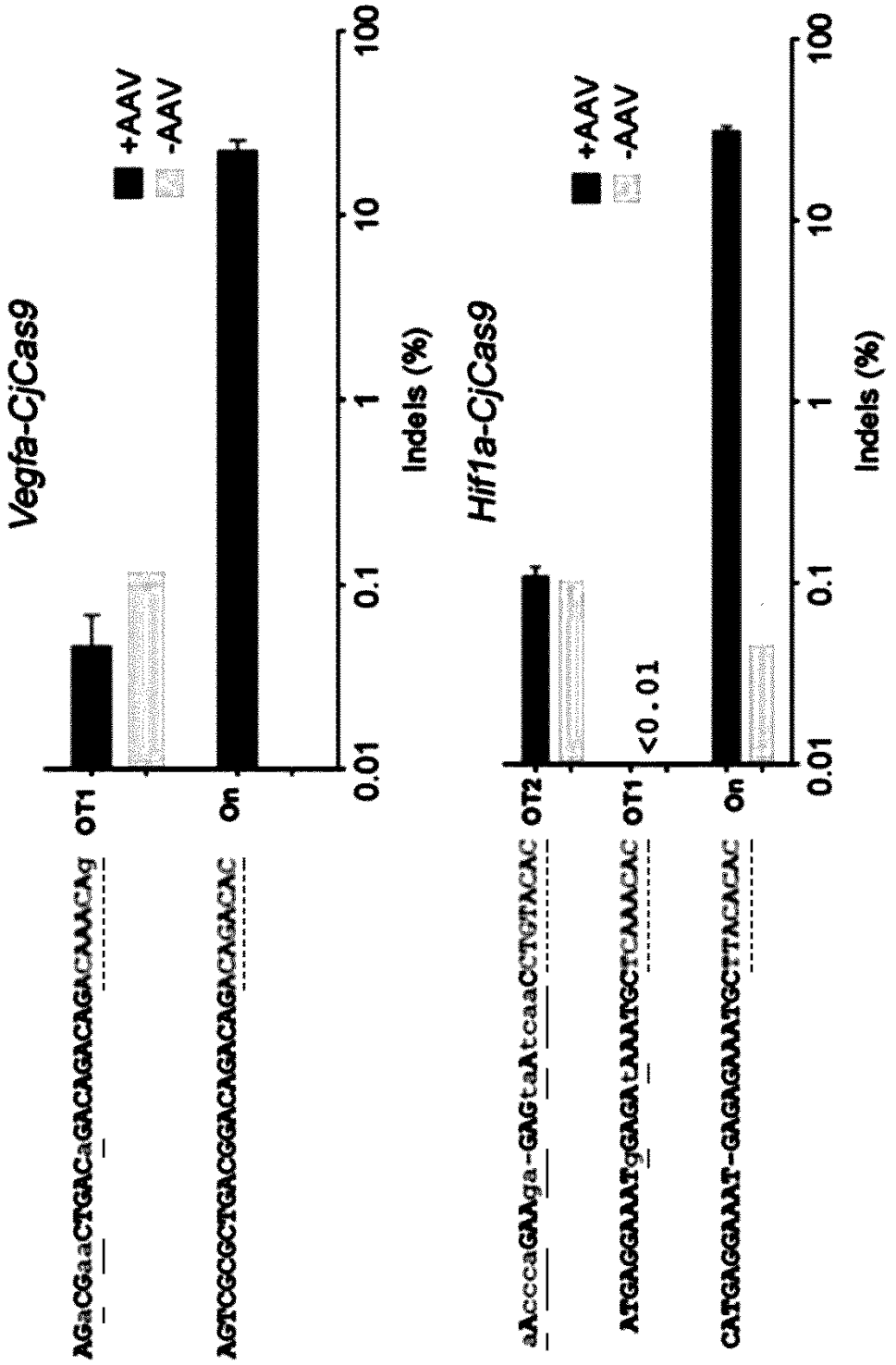

Vegfa-CjCas9
+AAV
-AAV
Indels (%)
0.01
0.1
1
10
100
OT1
On
AGaCGaaCTGACaGACAGACAGACAAACAg
AGTCGCGCTGACGGACAGACAGACAC
Hif1a-CjCas9
+AAV
-AAV
Indels (%)
0.01
0.1
1
10
100
OT2
OT1
On
<0.01
aAcccaGAAga-GAGtaAtcaaCCTGTACAC
ATGAGGAAATgGAGAtAAATGCTCAAACAC
CATGAGGAAAT-GAGAGAAATGCTTACACAC

FIG. 1e

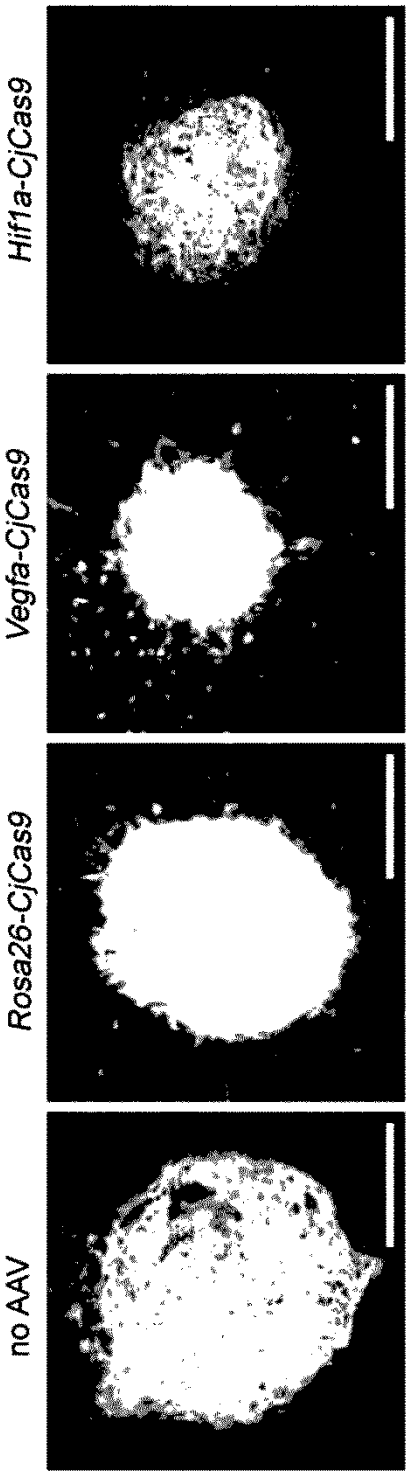


FIG. 1f

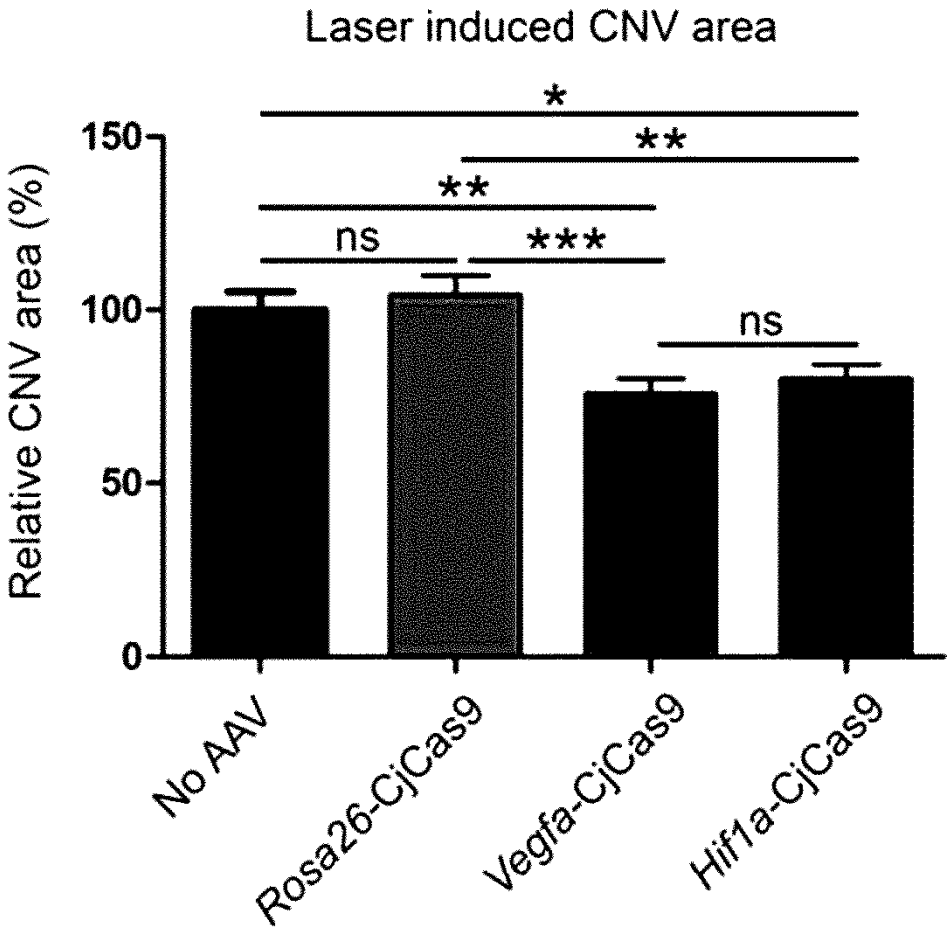

Laser induced CNV area
Relative CNV area (%)
150
100
50
0
*
**
**
ns
***
ns
No AAV
Rosa26-CjCas9
Vegfa-CjCas9
Hif1a-CjCas9

FIG. 1g

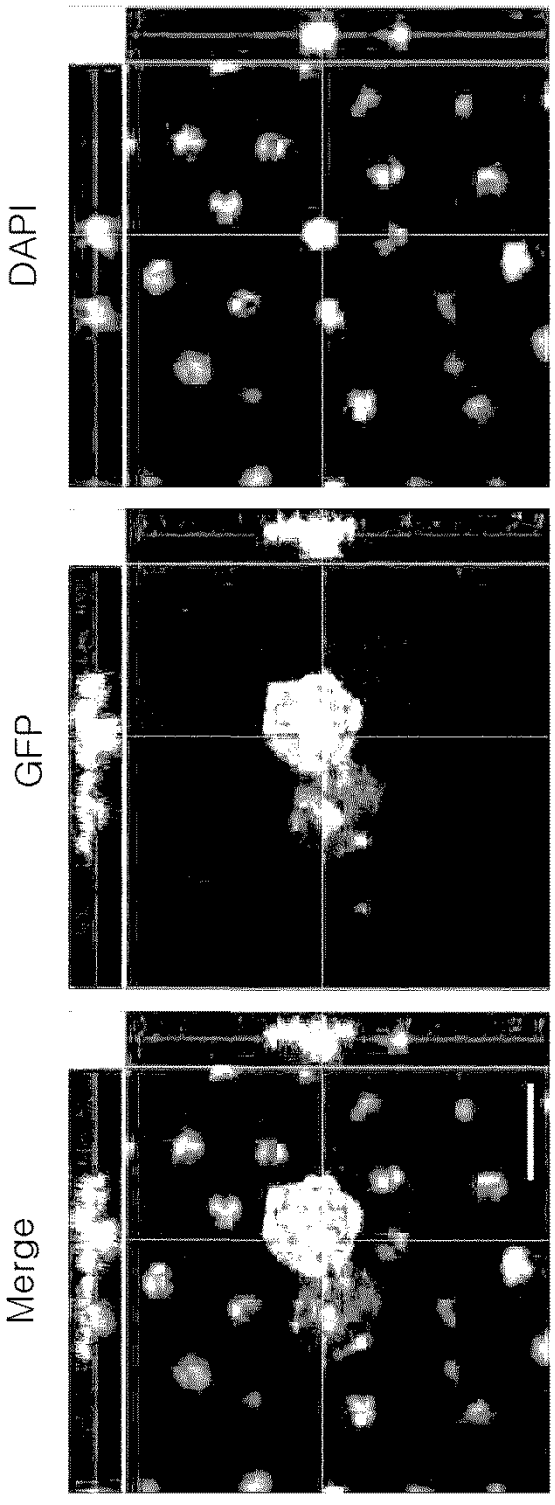


FIG. 2

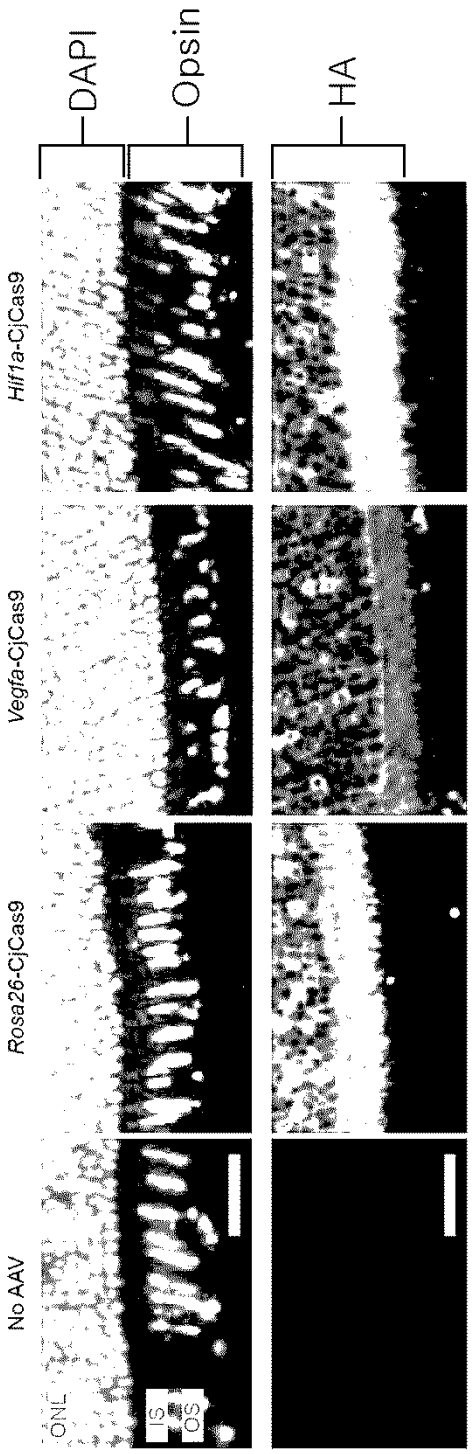

DAPI
Opsin
HA
Hif1a-CjCas9
Vegfa-CjCas9
Rosa26-CjCas9
No AAV
ONL
IS
OS

FIG. 3a

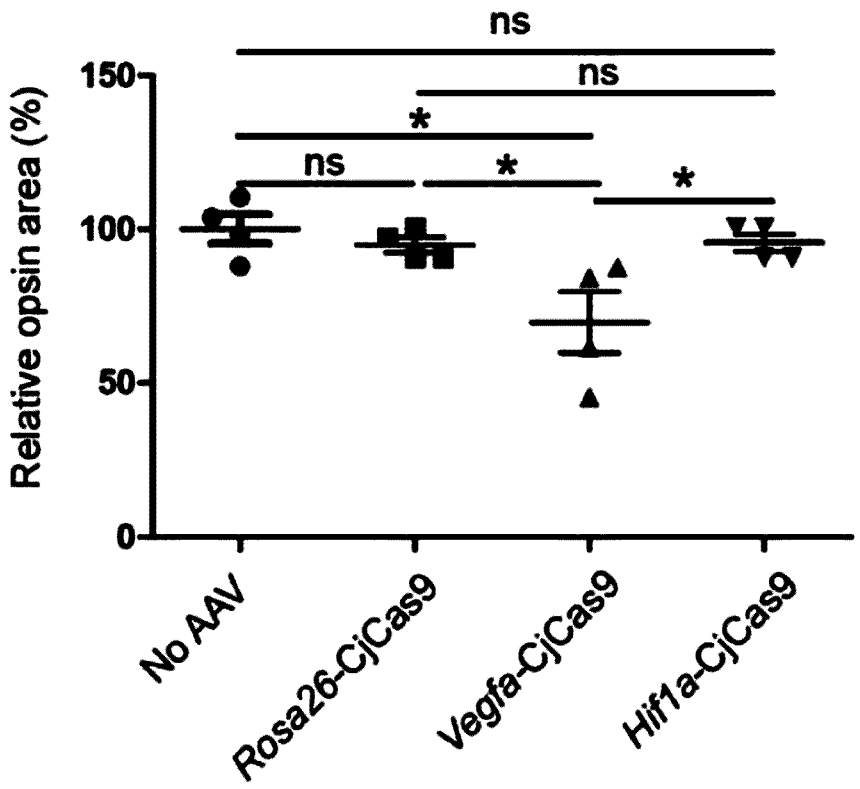

ns
ns
*
ns
*
*
150
100
50
0
Relative opsin area (%)
No AAV
Rosa26-CjCas9
Vegfa-CjCas9
Hif1a-CjCas9

FIG. 3b

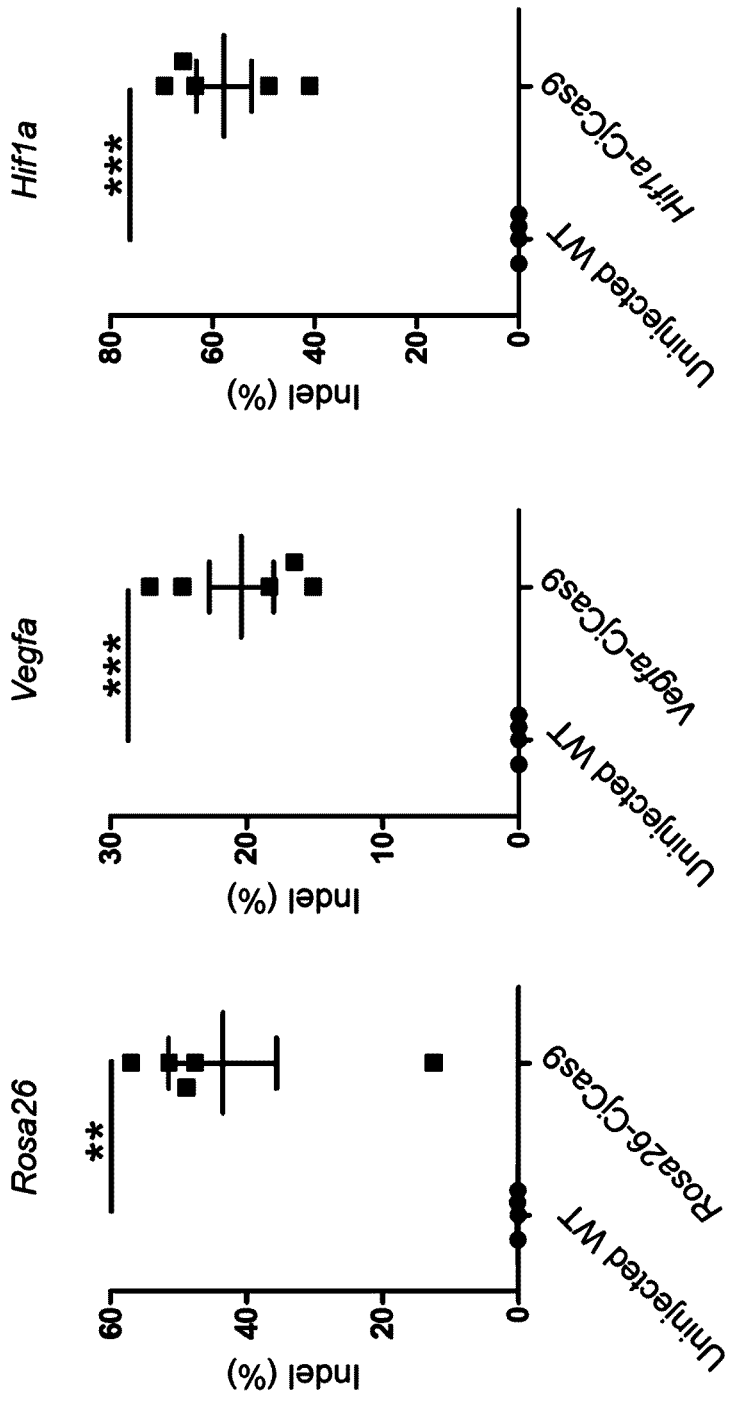

Hif1a
***
Indel (%)
80
60
40
20
0
Uninjected WT
Hif1a-CjCas9
Vegfa
***
Indel (%)
30
20
10
0
Uninjected WT
Vegfa-CjCas9
Rosa26
**
Indel (%)
60
40
20
0
Uninjected WT
Rosa26-CjCas9

FIG. 4a

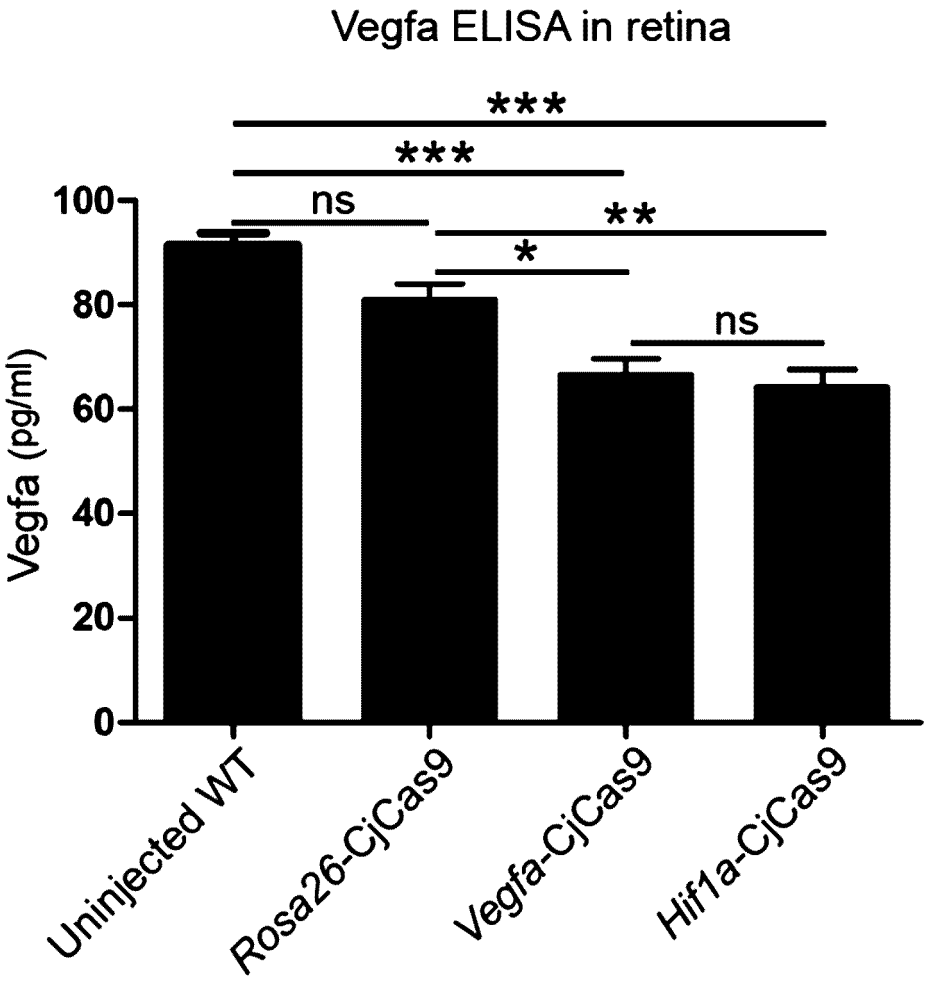

Vegfa ELISA in retina
***
***
ns
**
*
ns
100
80
60
40
20
0
Vegfa (pg/ml)
Uninjected WT
Rosa26-CjCas9
Vegfa-CjCas9
Hif1a-CjCas9

FIG. 4b

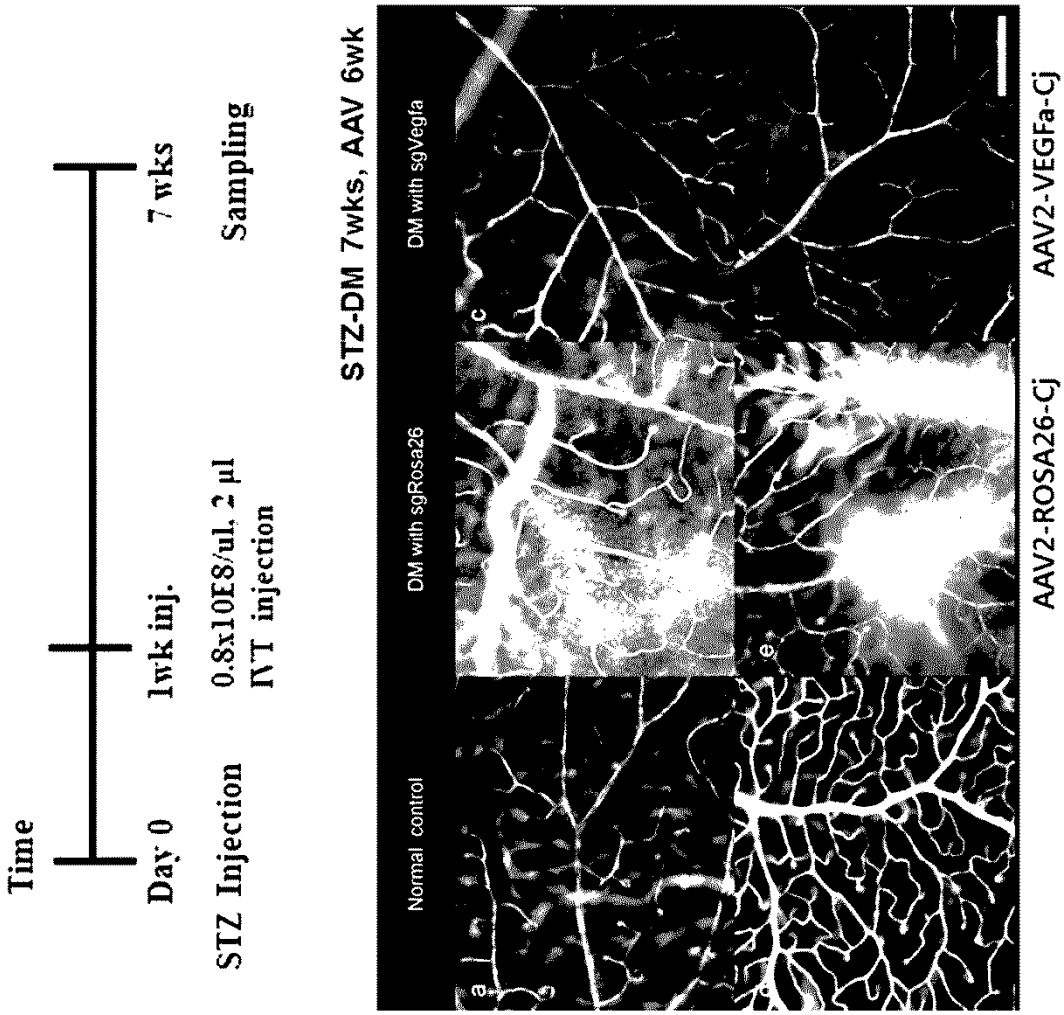

(a)
Time
Day 0
1wk inj.
7 wks
STZ Injection
0.8x10E8/ul, 2 μl
IVT injection
Sampling
(b)
STZ-DM 7wks, AAV 6wk
Normal control
DM with sgRosa26
DM with sgVegfa
AAV2-ROSA26-Cj
AAV2-VEGFa-Cj

FIG. 5

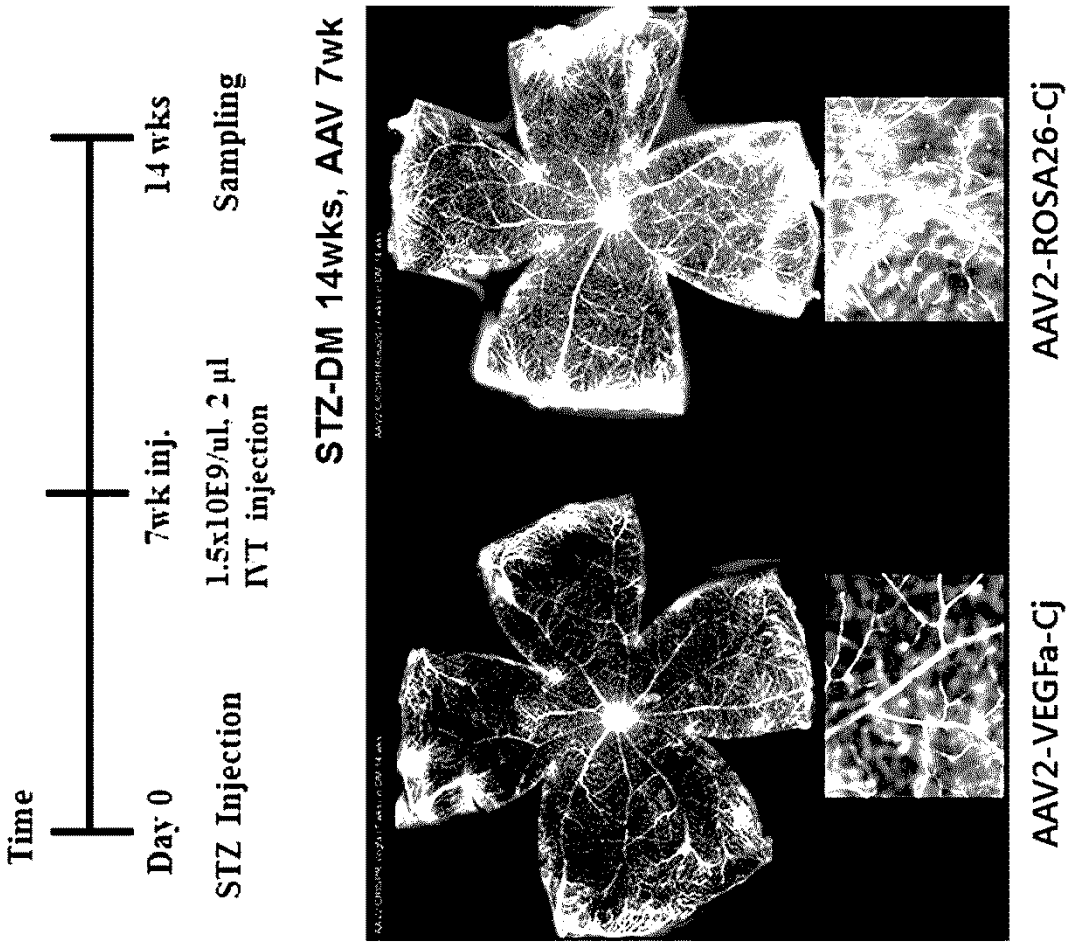

(a)
Time
Day 0
14 wks
STZ Injection
7wk inj.
1.5x10E9/ul, 2 µl
IVT injection
Sampling
(b)
STZ-DM 14wks, AAV 7wk
AAV2-VEGFa-Cj
AAV2-ROSA26-Cj

FIG. 6a

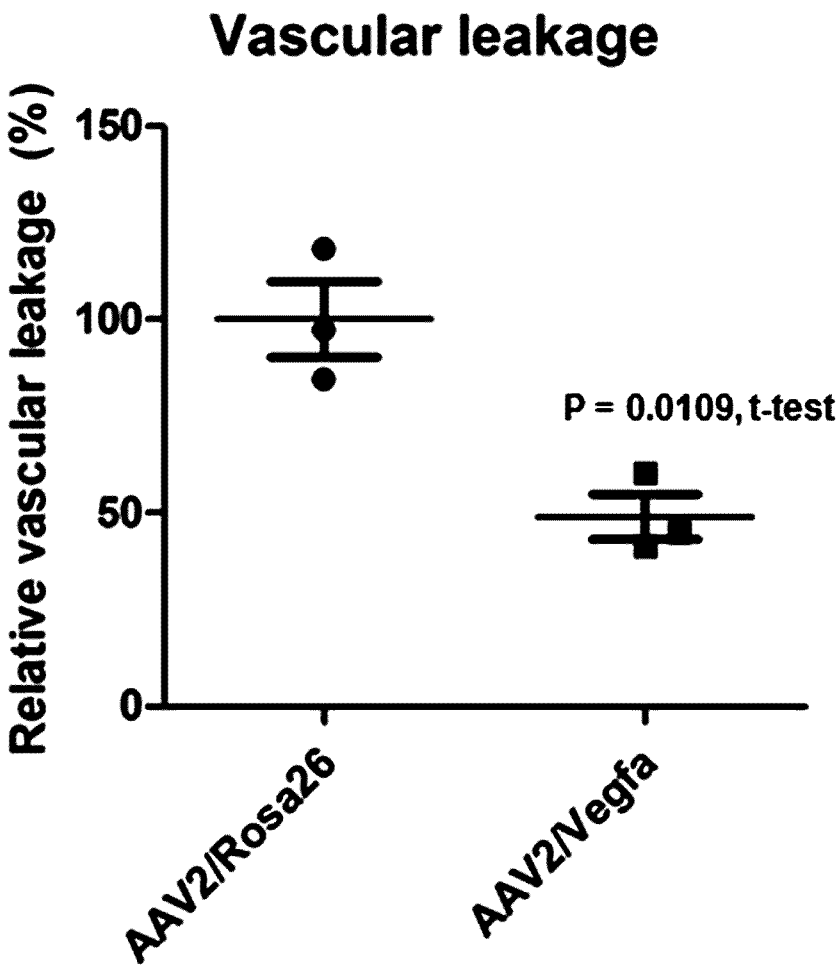
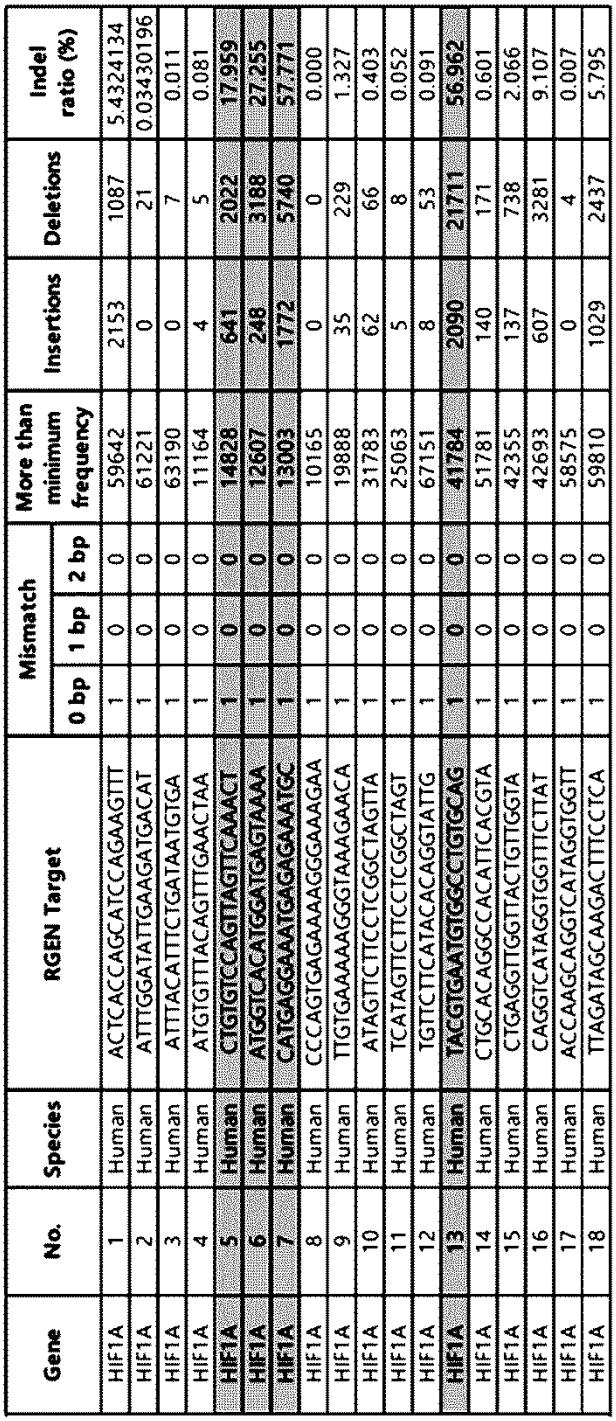

Vascular leakage
Relative vascular leakage (%)
150
100
50
0
P = 0.0109, t-test
AAV2/Rosa26
AAV2/Vegfa

FIG. 6b

Gene	No.	Species	RGEN Target	Mismatch			More than minimum frequency	Insertions	Deletions	Indel ratio (%)
				0 bp	1 bp	2 bp				
VEGFA	1	Human	GTAGAGCAGCAAGGCAAGGCTC	1	0	0	13619	3	206	1.535
VEGFA	2	Human	CTTTCTGTCCTCAGTGGTCCCA	1	0	0	44326	128	396	1.182
VEGFA	3	Human	GAGACCCTGGTGGACATCTTCC	1	0	0	85413	884	3266	4.859
VEGFA	**4**	**Human**	**TTCCAGGAGTACCCTGATGAGA**	**1**	**0**	**0**	**60920**	**1188**	**13853**	**24.690**
VEGFA	5	Human	TTGAAGATGTACTCGATCTCAT	1	0	0	46994	68	165	0.496
VEGFA	6	Human	AGGGGCACACAGGATGGCTTGA	1	0	0	38858	10	7	0.044
VEGFA	**7**	**Human**	**AGCAGCCCCCGCATCGCATCAG**	**1**	**0**	**0**	**16605**	**684**	**6441**	**42.909**
VEGFA	8	Human	GCAGCAGCCCCCGCATCGCATC	1	0	0	36464	29	54	0.228
VEGFA	**9**	**Human**	**GTGATGTTGGACTCCTCAGTGG**	**1**	**0**	**0**	**61570**	**6313**	**38511**	**72.802**
VEGFA	**10**	**Human**	**TGGTGATGTTGGACTCCTCAGT**	**1**	**0**	**0**	**41580**	**3087**	**21321**	**58.701**
VEGFA	11	Human	CATGGTGATGTTGGACTCCTCA	1	0	0	42569	382	2057	5.730
VEGFA	12	Human	ATGCGGATCAAACCTCACCAAG	1	0	0	63996	24	357	0.595
VEGFA	13	Human	CACATAGGAGAGATGAGCTTCC	1	0	0	52511	11	77	0.168

FIG. 7



Gene	No.	Species	RGEN Target	Mismatch			More than minimum frequency	Insertions	Deletions	Indel ratio (%)
				0 bp	1 bp	2 bp				
HIF1A	1	Human	ACTCACCAGCATCCAGAAGTTT	1	0	0	59642	2153	1087	5.4324134
HIF1A	2	Human	ATTTGGATATTGAAGATGACAT	1	0	0	61221	0	21	0.03430196
HIF1A	3	Human	ATTTACATTTCTGATAATGTGA	1	0	0	63190	0	7	0.011
HIF1A	4	Human	ATGTGTTTACAGTTTGAACTAA	1	0	0	11164	4	5	0.081
HIF1A	**5**	**Human**	**CTGTGTCCAGTTAGTTCAAACT**	**1**	**0**	**0**	**14828**	**641**	**2022**	**17.959**
HIF1A	**6**	**Human**	**ATGGTCACATGGATGAGTAAAA**	**1**	**0**	**0**	**12607**	**248**	**3188**	**27.255**
HIF1A	**7**	**Human**	**CATGAGGAAATGAGAGAAATGC**	**1**	**0**	**0**	**13003**	**1772**	**5740**	**57.771**
HIF1A	8	Human	CCCAGTGAGAAAAGGGAAAGAA	1	0	0	10165	0	0	0.000
HIF1A	9	Human	TTGTGAAAAAGGGTAAAGAACA	1	0	0	19888	35	229	1.327
HIF1A	10	Human	ATAGTTCTTCCTCGGCTAGTTA	1	0	0	31783	62	66	0.403
HIF1A	11	Human	TCATAGTTCTTCCTCGGCTAGT	1	0	0	25063	5	8	0.052
HIF1A	12	Human	TGTTCTTCATACACAGGTATTG	1	0	0	67151	8	53	0.091
HIF1A	**13**	**Human**	**TACGTGAATGTGGCCTGTGCAG**	**1**	**0**	**0**	**41784**	**2090**	**21711**	**56.962**
HIF1A	14	Human	CTGCACAGGCCACATTCACGTA	1	0	0	51781	140	171	0.601
HIF1A	15	Human	CTGAGGTTGGTTACTGTTGGTA	1	0	0	42355	137	738	2.066
HIF1A	16	Human	CAGGTCATAGGTGGTTTCTTAT	1	0	0	42693	607	3281	9.107
HIF1A	17	Human	ACCAAGCAGGTCATAGGTGGTT	1	0	0	58575	0	4	0.007
HIF1A	18	Human	TTAGATAGCAAGACTTTCCTCA	1	0	0	59810	1029	2437	5.795

FIG. 8a

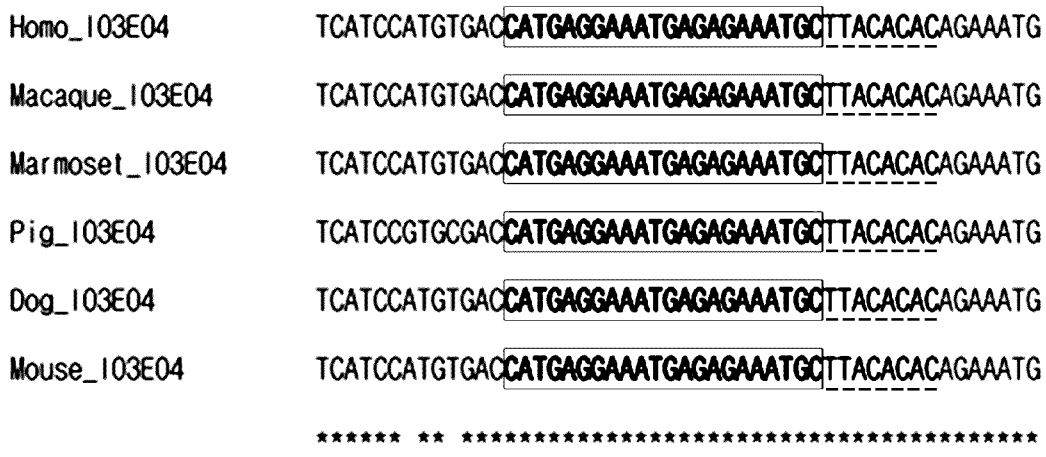
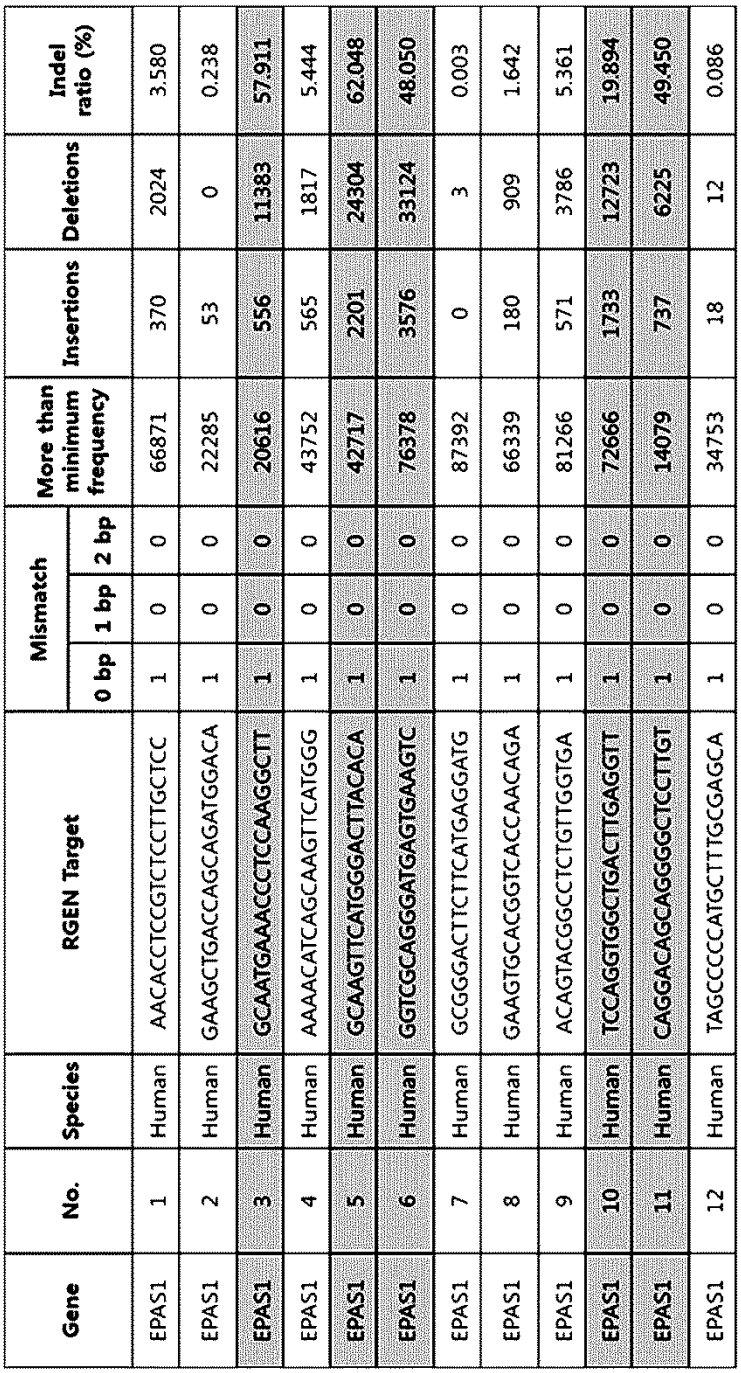

Homo_103E04 TCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
Macaque_103E04 TCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
Marmoset_103E04 TCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
Pig_103E04 TCATCCGTGCGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
Dog_103E04 TCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
Mouse_103E04 TCATCCATGTGACCATGAGGAAATGAGAGAAATGCTTACACACAGAAATG
****** ** **

FIG. 8b

Gene	No.	Species	RGEN Target	Mismatch			More than minimum frequency	Insertions	Deletions	Indel ratio (%)
				0 bp	1 bp	2 bp				
ANGPT2	1	Human	TCAGGTCCAGCATGGGTCCTGC	1	0	0	40481	0	20	0.0494
ANGPT2	2	Human	CGGCGCGTCCCTCTGCACAGCA	1	0	0	25826	1018	3294	16.696
ANGPT2	3	Human	GCTGTGCAGAGGGACGCGCCGC	1	0	0	33526	3233	13686	50.465
ANGPT2	4	Human	ATCGTATTCGAGCGGCGCGTCC	1	0	0	48216	30	56	0.178
ANGPT2	5	Human	GATGTTCTCCAGCACTTGCAGC	1	0	0	38296	0	0	0.000
ANGPT2	6	Human	AGTGCTGGAGAACATCATGGAA	1	0	0	47581	854	12527	28.123
ANGPT2	7	Human	ACAACATGAAGAAAGAAATGGT	1	0	0	24395	1370	10712	49.527
ANGPT2	8	Human	AAATGGTAGAGATACAGCAGAA	1	0	0	24648	296	984	5.193
ANGPT2	9	Human	TTCTATCATCACAGCCGTCTGG	1	0	0	36702	17	15	0.087
ANGPT2	10	Human	AAGTTCAAGTCTCGTGGTCTGA	1	0	0	35269	95	374	1.330
ANGPT2	11	Human	ACGAGACTTGAACTTCAGCTCT	1	0	0	28139	7	95	0.362
ANGPT2	12	Human	AAGAAGGTGCTAGCTATGGAAG	1	0	0	17482	210	4907	29.270
ANGPT2	13	Human	GATGATGTGCTTGTCTTCCATA	1	0	0	7694	0	3	0.039

FIG. 9



Gene	No.	Species	RGEN Target	Mismatch			More than minimum frequency	Insertions	Deletions	Indel ratio (%)
				0 bp	1 bp	2 bp				
EPAS1	1	Human	AACACCTCCGTCTCCTTGCTCC	1	0	0	66871	370	2024	3.580
EPAS1	2	Human	GAAGCTGACCAGCAGATGGACA	1	0	0	22285	53	0	0.238
EPAS1	3	Human	GCAATGAAACCCTCCAAGGCTT	1	0	0	20616	556	11383	57.911
EPAS1	4	Human	AAAACATCAGCAAGTTCATGGG	1	0	0	43752	565	1817	5.444
EPAS1	5	Human	GCAAGTTCATGGGACTTACACA	1	0	0	42717	2201	24304	62.048
EPAS1	6	Human	GGTCGCAGGGATGAGTGAAGTC	1	0	0	76378	3576	33124	48.050
EPAS1	7	Human	GCGGGACTTCTTCATGAGGATG	1	0	0	87392	0	3	0.003
EPAS1	8	Human	GAAGTGCACGGTCACCAACAGA	1	0	0	66339	180	909	1.642
EPAS1	9	Human	ACAGTACGGCCTCTGTTGGTGA	1	0	0	81266	571	3786	5.361
EPAS1	10	Human	TCCAGGTGGCTGACTTGAGGTT	1	0	0	72666	1733	12723	19.894
EPAS1	11	Human	CAGGACAGCAGGGGCTCCTTGT	1	0	0	14079	737	6225	49.450
EPAS1	12	Human	TAGCCCCCATGCTTTGCGAGCA	1	0	0	34753	18	12	0.086

FIG. 10

Gene	No.	Species	RGEN Target	Mismatch			Indel ratio (%)
				0 bp	1 bp	2 bp	
ANGPTL4	1	Human	GCATCAGGGCTGCCCCGGCCGT	1	0	0	0.152
ANGPTL4	2	Human	CACGGGTCCGCCCTGAGCGCTC	1	0	0	0.014
ANGPTL4	3	Human	GGACGCAAAGCGCGGCGACTTG	1	0	0	0.006
ANGPTL4	4	Human	TCCTGGGACGAGATGAATGTCC	1	0	0	0.137
ANGPTL4	5	Human	CTGCAGCTCGGCCAGGGGCTGC	1	0	0	0.000
ANGPTL4	6	Human	CCAGGGGCTGCGCGAACACGCG	1	0	0	0.000
ANGPTL4	7	Human	CCCTCGGTTCCCTGACAGGCGG	1	0	0	1.383
ANGPTL4	8	Human	ACCCTGAGGTCCTTCACAGCCT	1	0	0	9.746
ANGPTL4	9	Human	TTCCACAAGGTGGCCCAGCAGC	1	0	0	0.298
ANGPTL4	10	Human	CAGCAGCAGCGGCACCTGGAGA	1	0	0	4.803
ANGPTL4	11	Human	TCCTAGTTTGGCCTCCTGGACC	1	0	0	5.192
ANGPTL4	12	Human	GACCCGGCTCACAATGTCAGCC	1	0	0	0.000
ANGPTL4	13	Human	GCTGTTGCGGTCCCCCGTGATG	1	0	0	0.041
ANGPTL4	14	Human	GGCGTTGCCATCCCAGTCCCGC	1	0	0	0.000
ANGPTL4	15	Human	AACGCCGAGTTGCTGCAGTTCT	1	0	0	0.000
ANGPTL4	16	Human	ATAGGCCGTGTCCTCGCCACCC	1	0	0	0.000
ANGPTL4	17	Human	GTTCTCCGTGCACCTGGGTGGC	1	0	0	6.136
ANGPTL4	18	Human	ACACGGCCTATAGCCTGCAGCT	1	0	0	0.000
ANGPTL4	19	Human	CCACCGTCCCACCCAGCGGCCT	1	0	0	0.150
ANGPTL4	20	Human	GTGATCCTGGTCCCAAGTGGAG	1	0	0	13.605
ANGPTL4	21	Human	GACCCCGGCAGGAGGCTGGTGG	1	0	0	2.379
ANGPTL4	22	Human	TGCAGCCATTCCAACCTCAACG	1	0	0	0.245
ANGPTL4	23	Human	TGCCGCTGCTGTGGGATGGAGC	1	0	0	13.909

FIG. 11