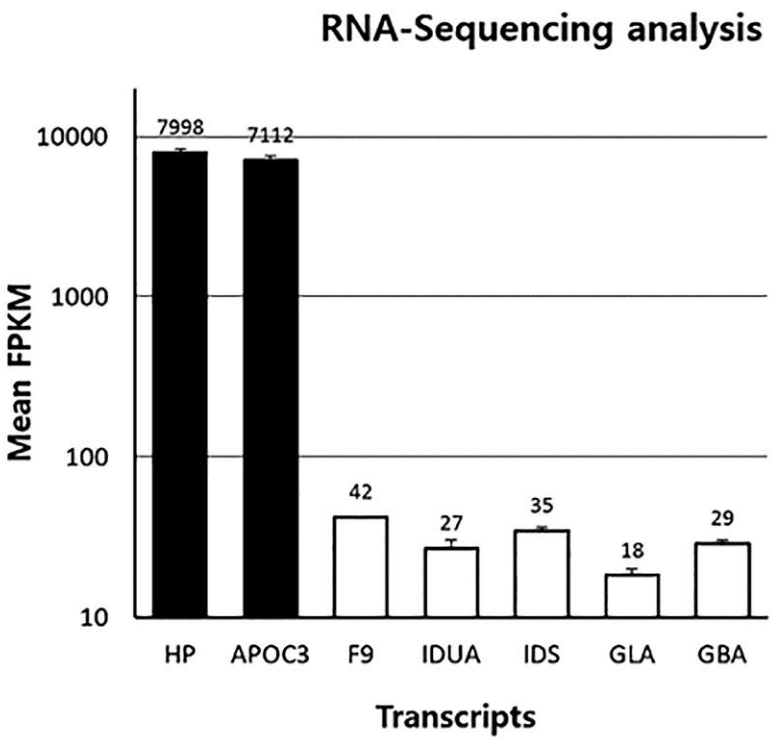
FIG. 1



F9: Gene for Hemophilia B
IDUA: Gene for MPSI(Hurler disease)
IDS: Gene for MPSII(Hunter disease)
GLA: Gene for Fabry disease
GBA: Gene for Gaucher disease

FIG. 2

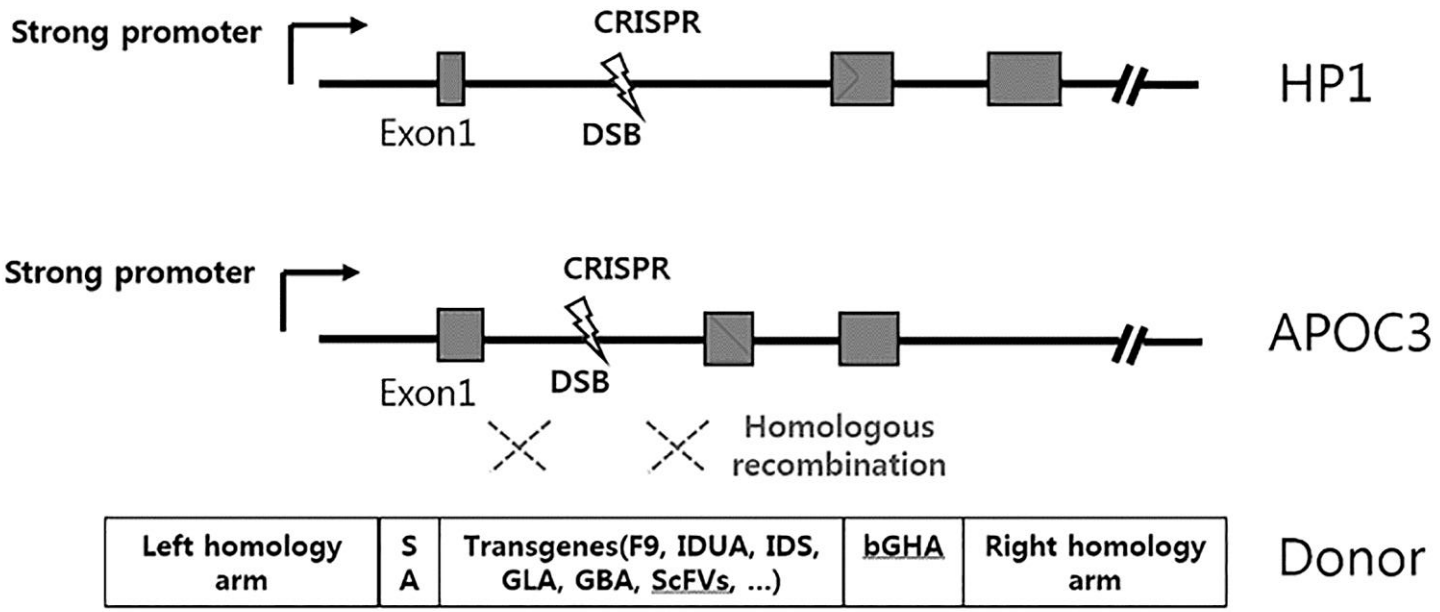

Strong promoter
CRISPR
Exon1
DSB
HP1
Strong promoter
CRISPR
Exon1
DSB
APOC3
Homologous recombination
Left homology arm
S A
Transgenes(F9, IDUA, IDS, GLA, GBA, ScFVs, ...)
bGHA
Right homology arm
Donor

FIG. 3

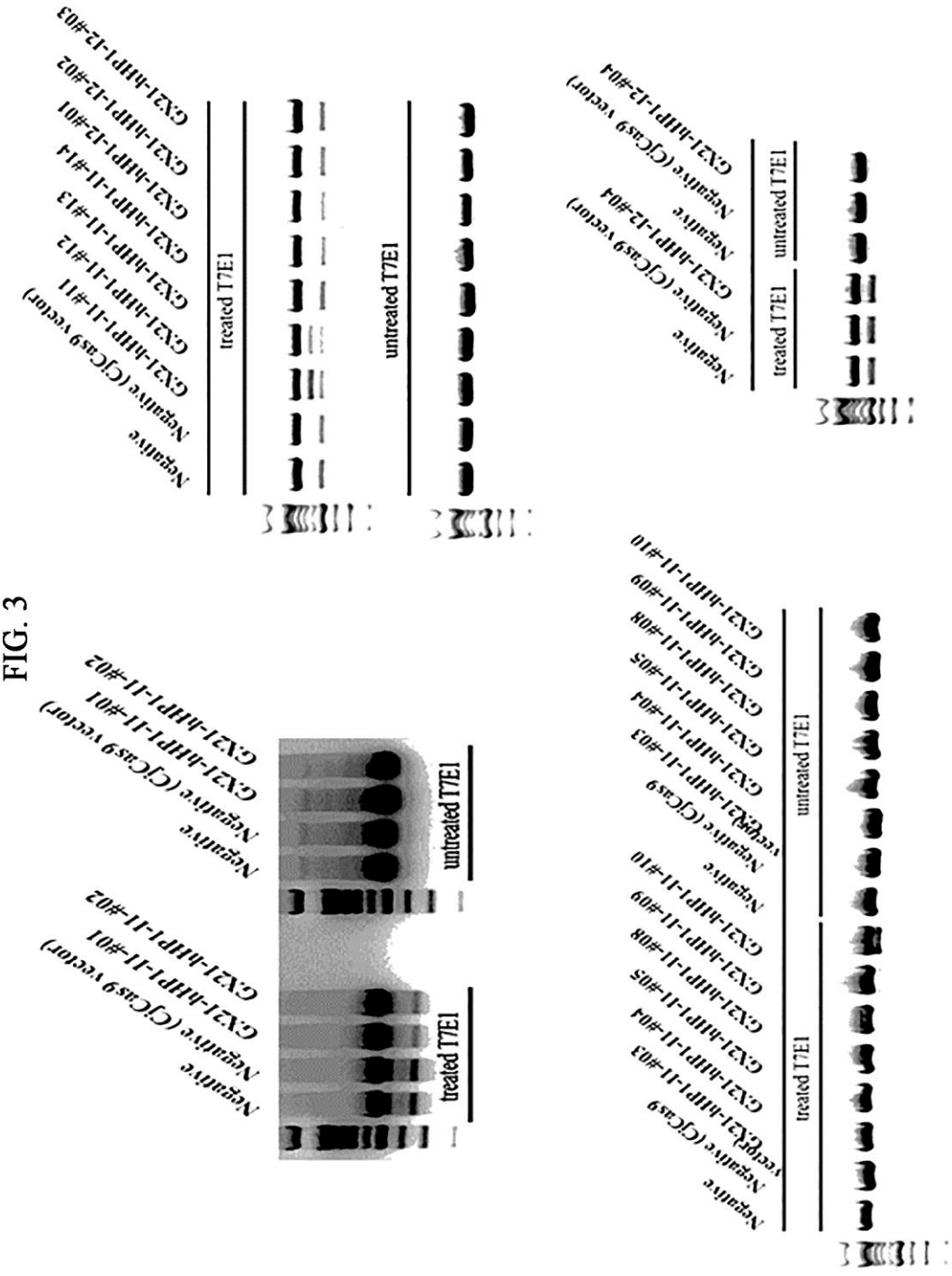
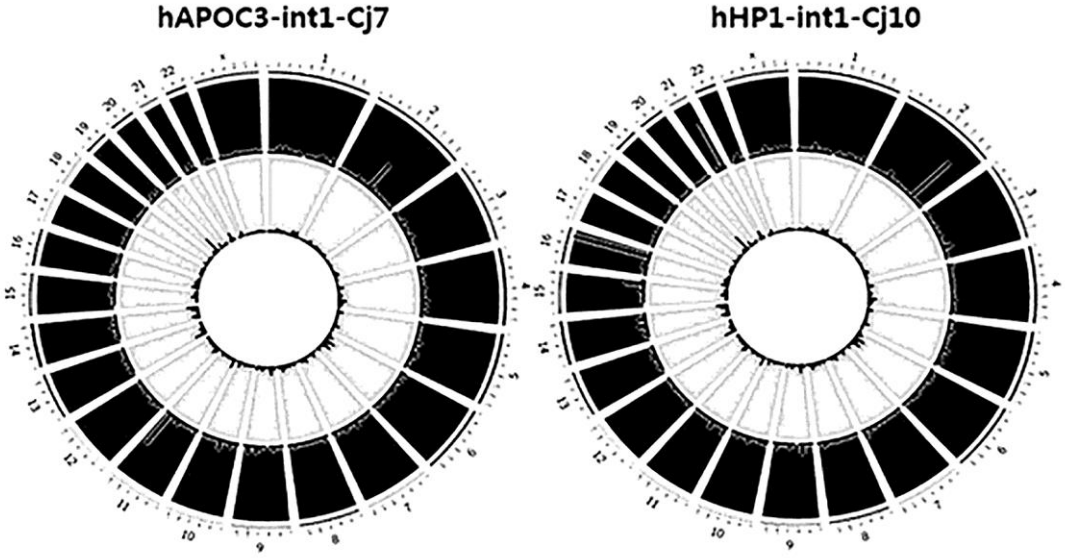

Negative
Negative (CjCas9 vector)
GA21-hHP1-11-#01
GA21-hHP1-11-#02
treated T7E1
untreated T7E1
Negative
Negative (CjCas9 vector)
GA21-hHP1-11-#11
GA21-hHP1-11-#12
GA21-hHP1-11-#13
GA21-hHP1-11-#14
GA21-hHP1-12-#01
GA21-hHP1-12-#02
GA21-hHP1-12-#03
treated T7E1
untreated T7E1
Negative
Negative (CjCas9 vector)
GA21-hHP1-12-#04
Negative
Negative (CjCas9 vector)
GA21-hHP1-12-#04
treated T7E1
untreated T7E1
Negative
Negative (CjCas9 vector)
GA21-hHP1-11-#03
GA21-hHP1-11-#04
GA21-hHP1-11-#05
GA21-hHP1-11-#08
GA21-hHP1-11-#09
GA21-hHP1-11-#10
treated T7E1
untreated T7E1
Negative
Negative (CjCas9 vector)
GA21-hHP1-11-#03
GA21-hHP1-11-#04
GA21-hHP1-11-#05
GA21-hHP1-11-#08
GA21-hHP1-11-#09
GA21-hHP1-11-#10

FIG. 4



hAPOC3-Int1-Cj7 (Cut off = 0.1)			
Chro #	Location	DNA cleavage score	Target sites
chr11	116700996	4.9	GAGGGCCAGAAATCACCCAAAGACACAC
chr2	129161569	3.9	GAGGGCAGGAAATCACCCAAGGTCACAC
chr12	41199184	2.3	GCCTAATAGAAATCACCCAAATTGACAC
chr16	17906433	1.9	AATTTAAAGAAATCACCCAAGAGCACAC
chr22	39135227	1.0	GATCAGCAGAAATCACCCAACCTAGTAC
chr8	19489673	0.7	AAGAAAGAGAAATCACCCAATTTCACAT
chr4	90615709	0.1	AGGGTTTAGAAATCACCCAAATCCACAT

hHP1-Int1-Cj10 (Cut off = 0.1)			
Chro #	Location	DNA cleavage score	Target sites
chr16	72089721	10.1	CATTCAGGAAAGTACATTGGCAATAATAC
chr2	189715402	6.6	ATTTGCGGAAAG-ACATTGGCTTTAGTAC
chr21	41767757	6.6	TTGGGGAGAAAG-ACATTGGCATTCACAC
chr15	90493185	2.4	TTTGGGGGAAAGTACA-TGGCATAAACAC
chr1	65942213	0.6	AGGCAGGAAAAATACATTGGCAAAAACAT
chr12	5061011	0.4	CACTCCAGAAAGTAC-TTGGCTGCCACAC
chr12	89500941	0.2	TGCTATTGTAAATACATTGGCCTGTACAC
chr19	54254073	0.2	TGTTGAAGAATGTACATTGTCTCACACAC
chr19	54193739	0.2	TGTTGAAGAATGTACATTGTCGCACACAC
chr3	177600564	0.2	CTAGATTAAATGTACATTGGCCAAAACAT
chrX	47761599	0.1	AGAGAAAGAGAGTACATTGGCGAAGATAC

FIG. 5

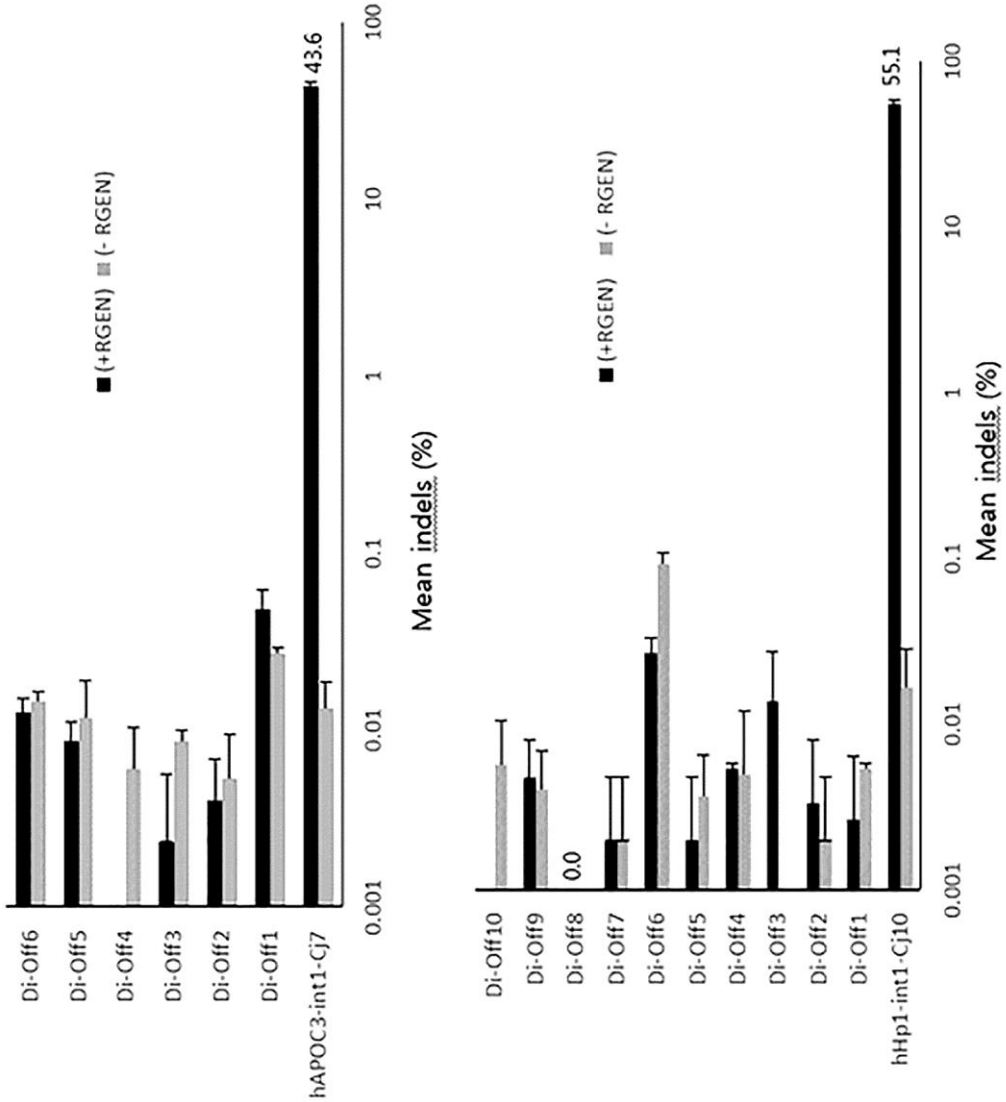

■ (+RGEN) ■ (- RGEN)
Mean indels (%)
0.001
0.01
0.1
1
10
100
Di-Off6
Di-Off5
Di-Off4
Di-Off3
Di-Off2
Di-Off1
hAPOC3-int1-Cj7
43.6
0.0
Di-Off10
Di-Off9
Di-Off8
Di-Off7
Di-Off6
Di-Off5
Di-Off4
Di-Off3
Di-Off2
Di-Off1
hHp1-int1-Cj10
55.1

FIG. 6

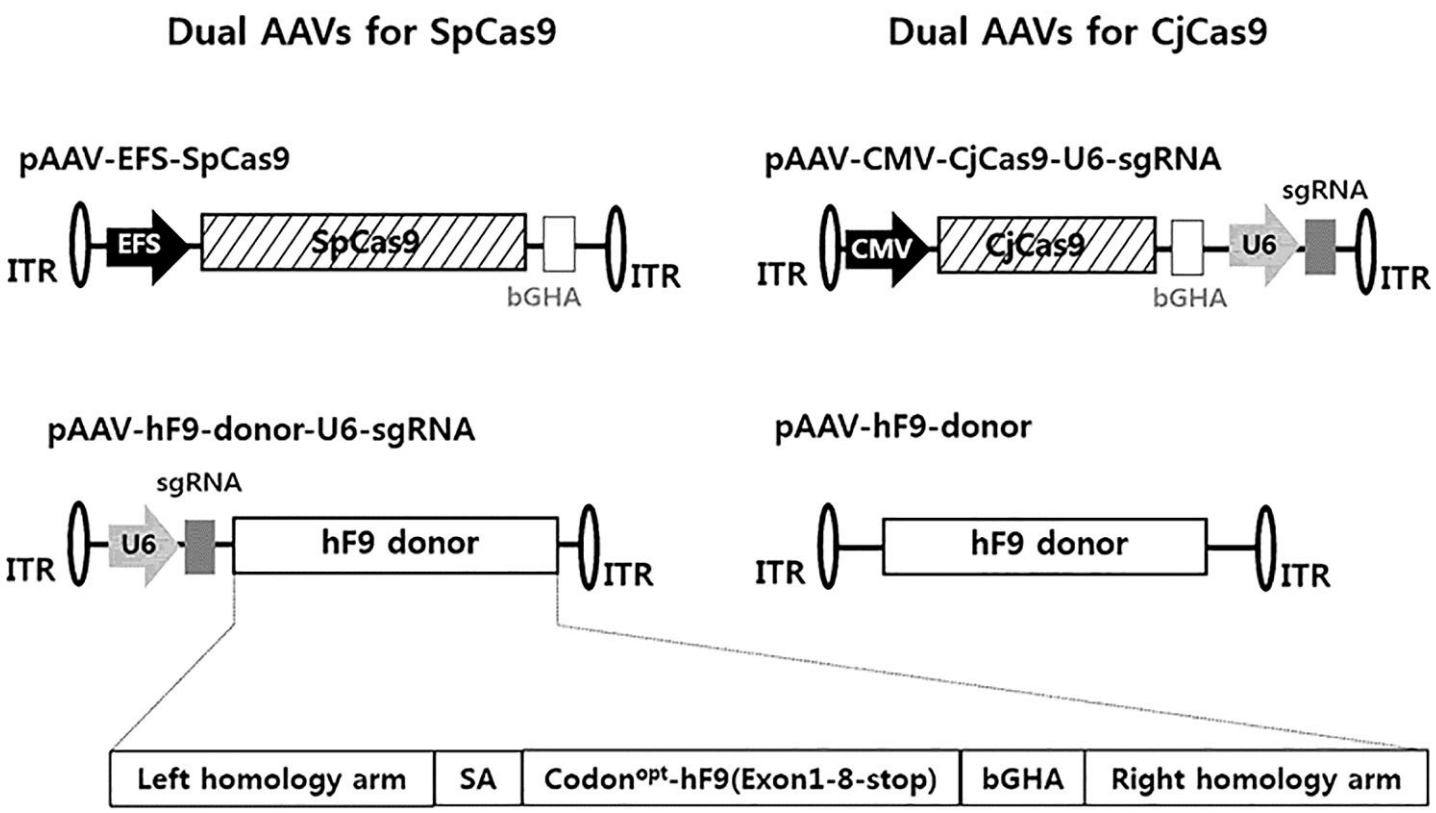

Dual AAVs for SpCas9
Dual AAVs for CjCas9
pAAV-EFS-SpCas9
ITR
EFS
SpCas9
bGHA
ITR
pAAV-CMV-CjCas9-U6-sgRNA
sgRNA
ITR
CMV
CjCas9
bGHA
U6
ITR
pAAV-hF9-donor-U6-sgRNA
sgRNA
ITR
U6
hF9 donor
ITR
pAAV-hF9-donor
ITR
hF9 donor
ITR
Left homology arm
SA
Codon^opt-hF9(Exon1-8-stop)
bGHA
Right homology arm

FIG. 7

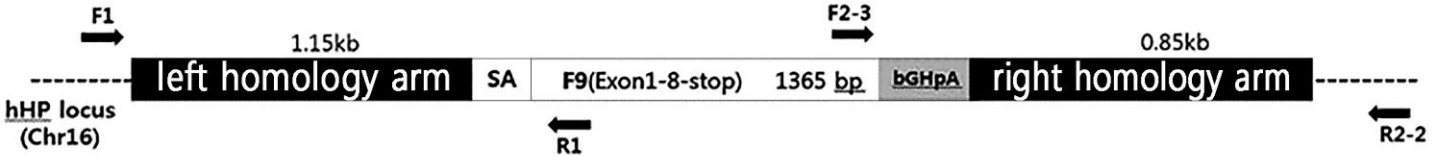

F1
1.15kb
F2-3
0.85kb
left homology arm
SA
F9(Exon1-8-stop)
1365 bp
bGHpA
right homology arm
hHP locus
(Chr16)
R1
R2-2

FIG. 8

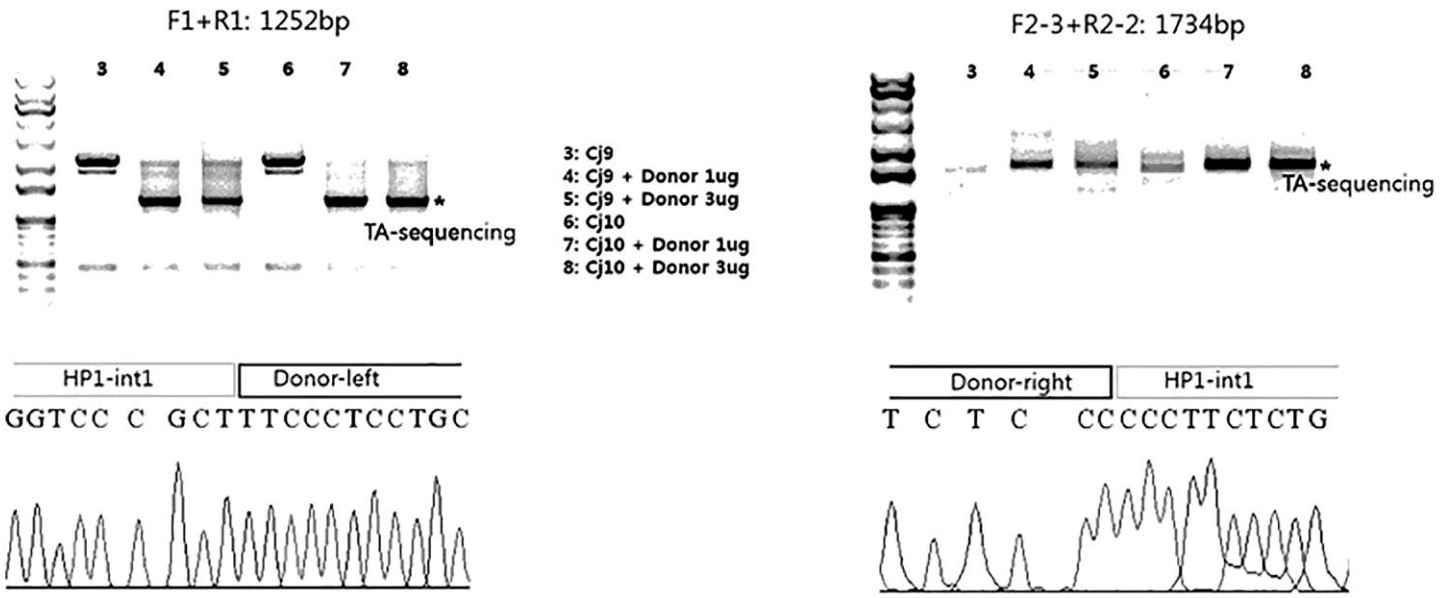
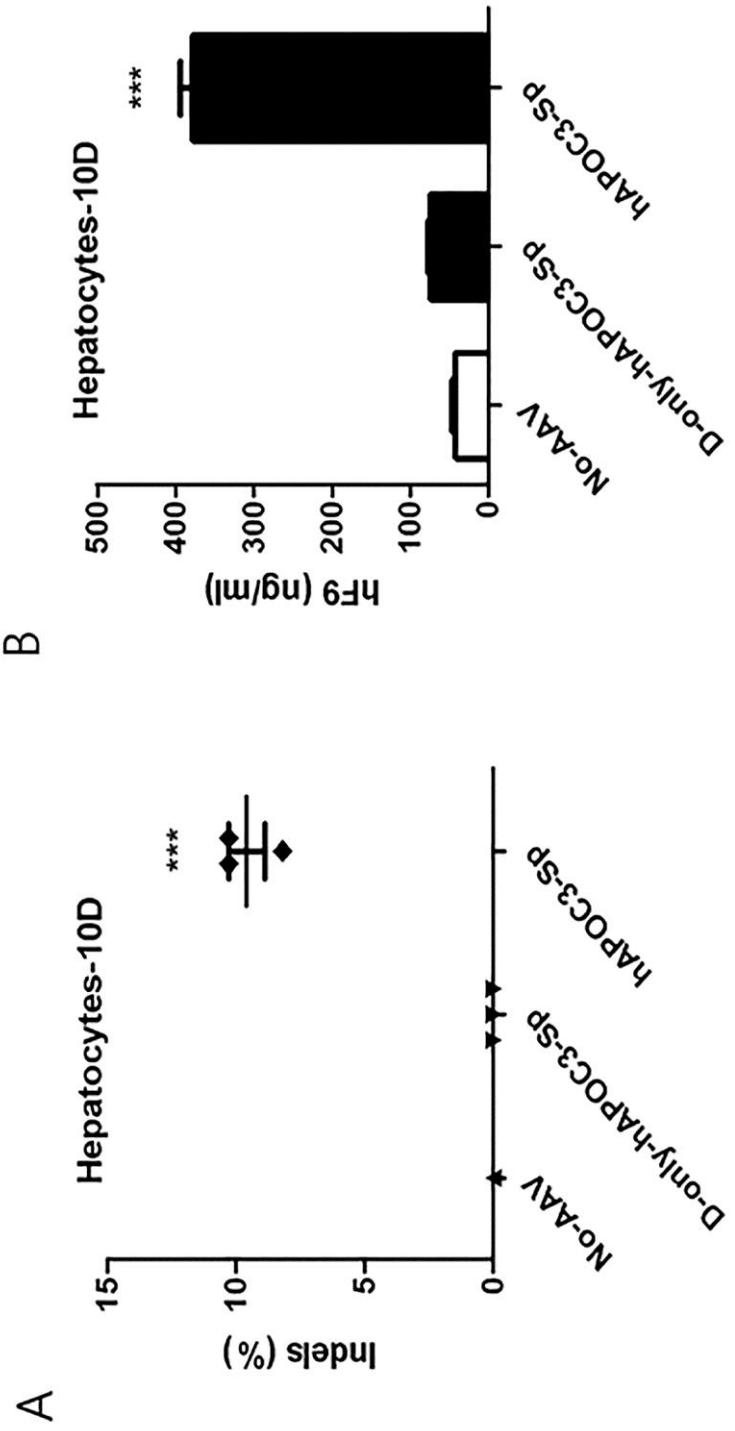

F1+R1: 1252bp
3 4 5 6 7 8
TA-sequencing
3: Cj9
4: Cj9 + Donor 1ug
5: Cj9 + Donor 3ug
6: Cj10
7: Cj10 + Donor 1ug
8: Cj10 + Donor 3ug
HP1-int1
Donor-left
GGTCC C GCTTTCCCTCCTGC
F2-3+R2-2: 1734bp
3 4 5 6 7 8
TA-sequencing
Donor-right
HP1-int1
T C T C CCCCCTTCTCTG

FIG. 9


A
Hepatocytes-10D
Indels (%)
15
10
5
0
***
No-AAV
D-only-hAPOC3-SP
hAPOC3-SP
B
Hepatocytes-10D
hF9 (ng/ml)
500
400
300
200
100
0
***
No-AAV
D-only-hAPOC3-SP
hAPOC3-SP