FIG 1

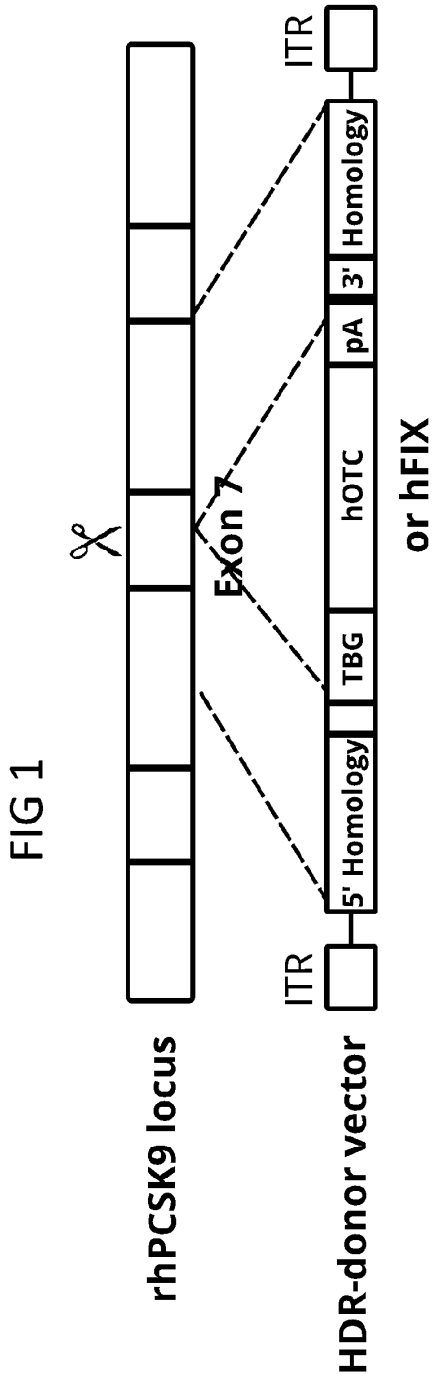

rhPCSK9 locus
Exon 7
ITR
5' Homology
TBG
hOTC
pA
3'
Homology
ITR
HDR-donor vector
or hFIX

FIG 2

Vector genome, gene expression, on/off-target editing, histology
1st liver biopsy
2nd liver biopsy
Newborn or 3-month-old
AAVhu37 injection
Day
0
7
14
28
42
56
70
84
bleeds, ~ every 2-4 weeks
(serum chem/CBC, hFIX exp in plasma, PCSK9 in serum, lipids, NAb)

FIG 3A

FIG 3B

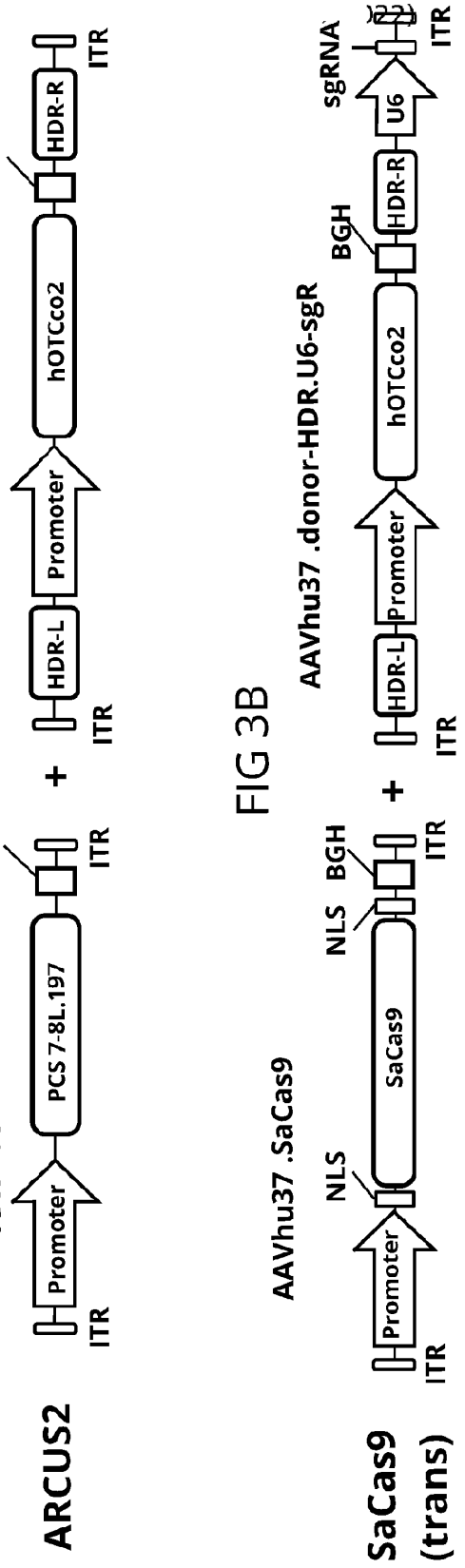


FIG 3C

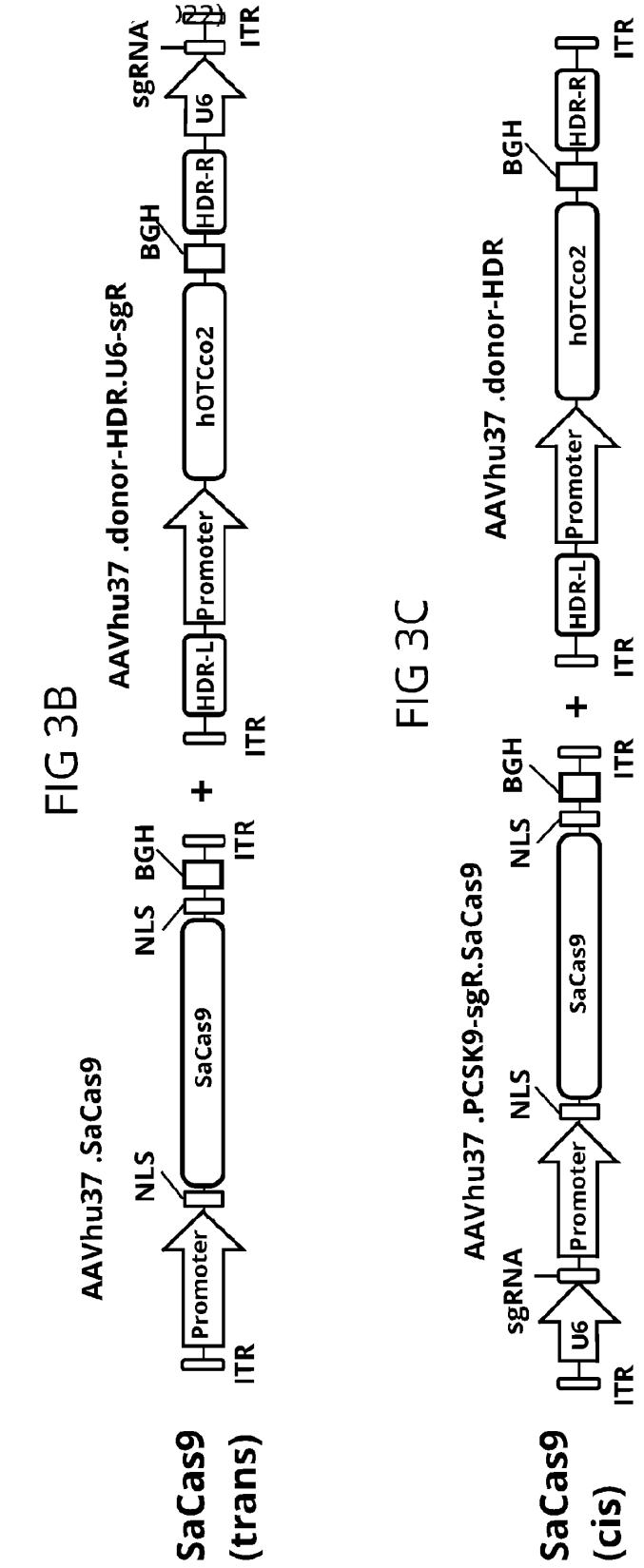


FIG 4A

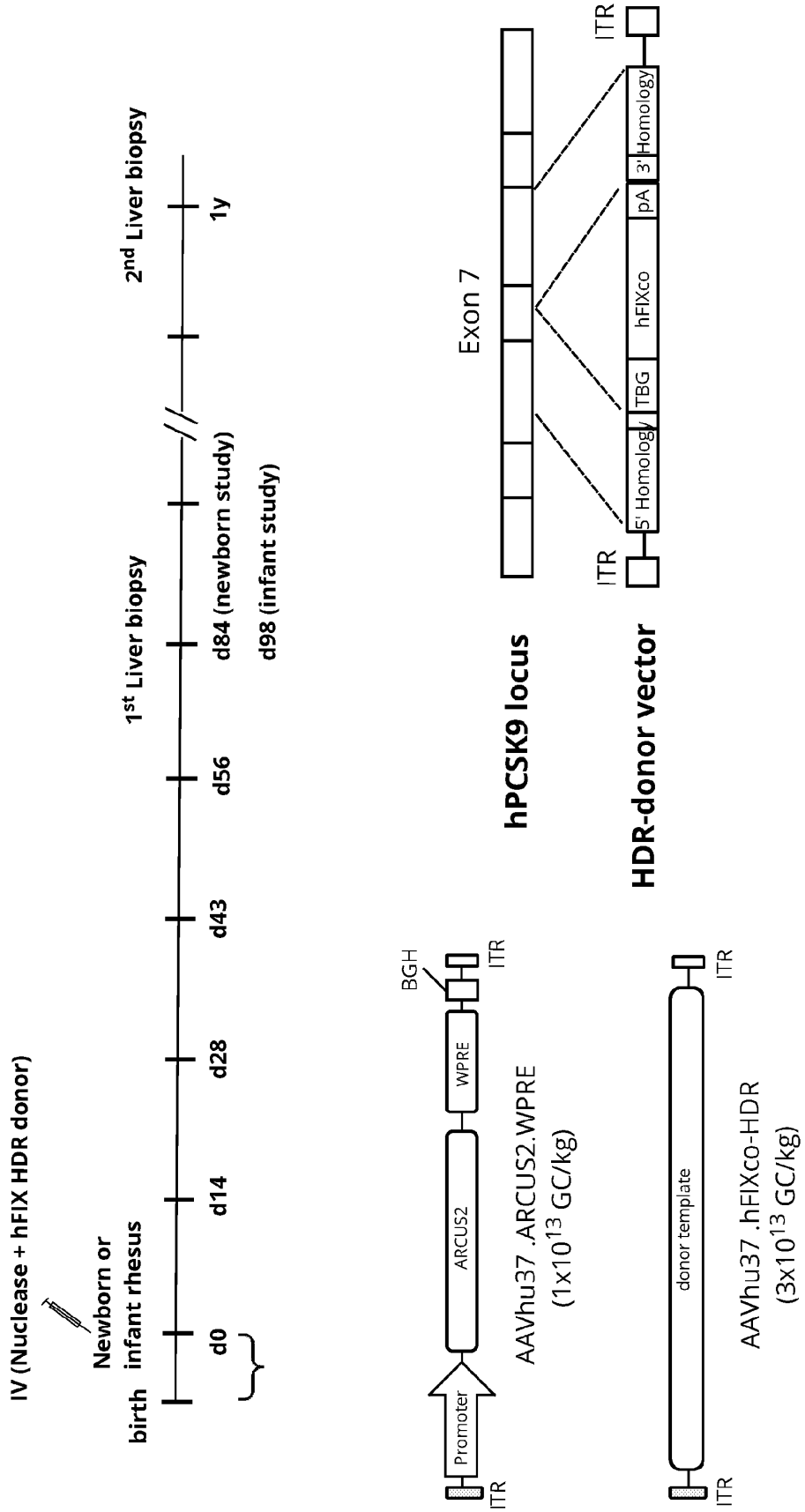

IV (Nuclease + hFIX HDR donor)
Newborn or infant rhesus
birth
d0
d14
d28
d43
d56
1st Liver biopsy
d84 (newborn study)
d98 (infant study)
2nd Liver biopsy
1y
Exon 7
hPCSK9 locus
HDR-donor vector
ITR
5' Homology
TBG
hFIXco
pA
3' Homology
ITR
BGH
ITR
WPRE
ARCUS2
Promoter
ITR
AAVhu37 .ARCUS2.WPRE
(1x10^13 GC/kg)
ITR
donor template
ITR
AAVhu37 .hFIXco-HDR
(3x10^13 GC/kg)

FIG 4B

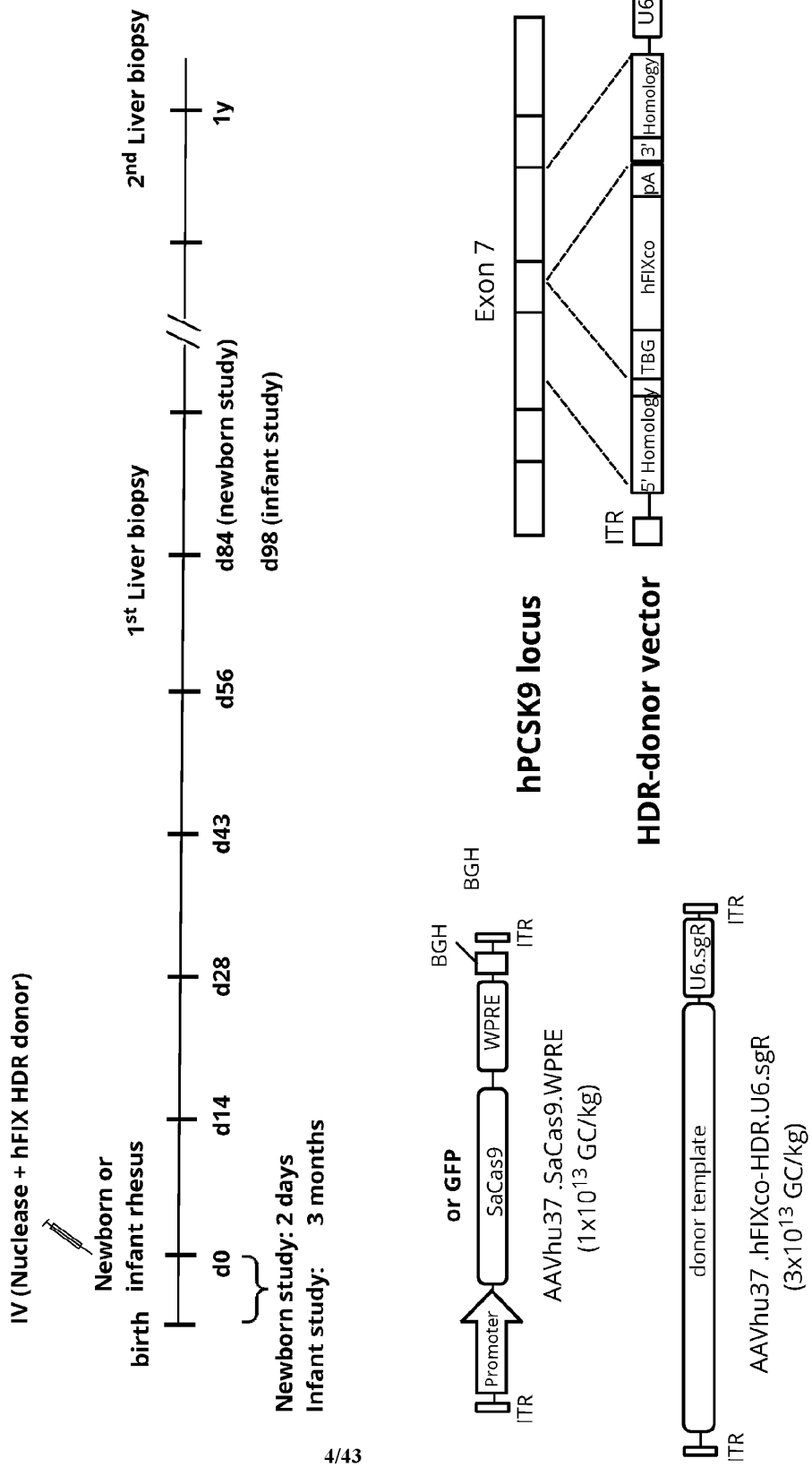

IV (Nuclease + hFIX HDR donor)
Newborn or infant rhesus
birth
d0
d14
d28
d43
d56
d84 (newborn study)
d98 (infant study)
1st Liver biopsy
2nd Liver biopsy
1y
Newborn study: 2 days
Infant study: 3 months
or GFP
SaCas9
WPRE
BGH
ITR
Promoter
AAVhu37 .SaCas9.WPRE
(1x10^13 GC/kg)
donor template
U6.sgR
AAVhu37 .hFIXco-HDR.U6.sgR
(3x10^13 GC/kg)
hPCSK9 locus
Exon 7
HDR-donor vector
5' Homology
TBG
hFIXco
pA
3'
Homology

FIG 4C

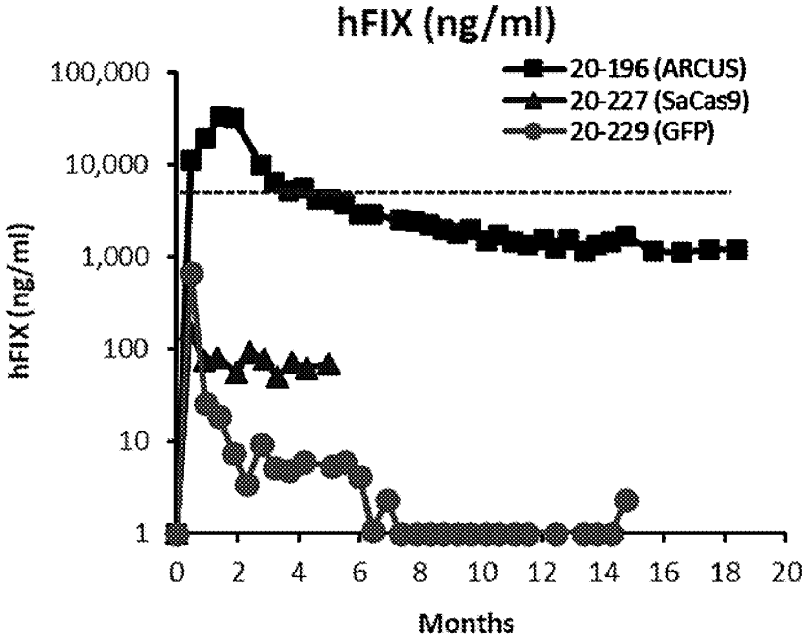

hFIX (ng/ml)
20-196 (ARCUS)
20-227 (SaCas9)
20-229 (GFP)
100,000
10,000
1,000
100
10
1
hFIX (ng/ml)
0 2 4 6 8 10 12 14 16 18 20
Months

FIG 4D

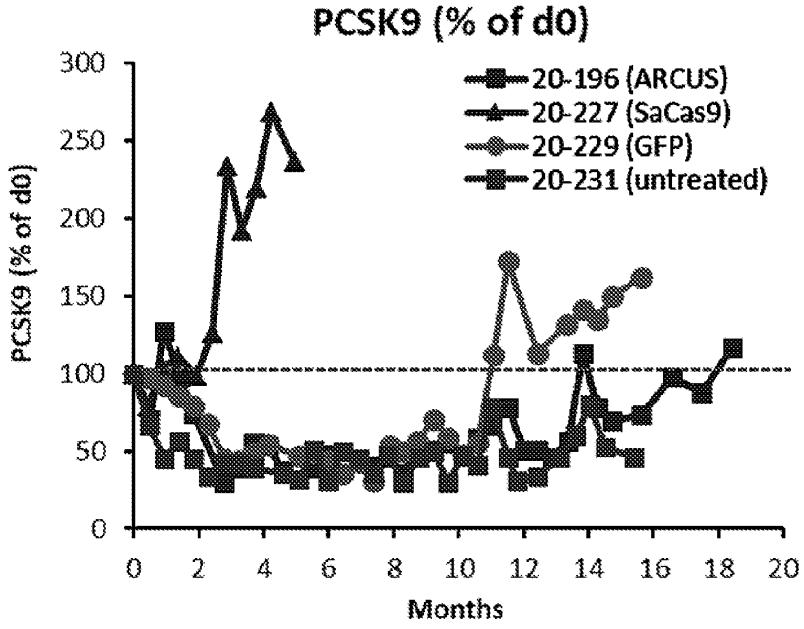

PCSK9 (% of d0)
20-196 (ARCUS)
20-227 (SaCas9)
20-229 (GFP)
20-231 (untreated)
300
250
200
150
100
50
0
PCSK9 (% of d0)
0 2 4 6 8 10 12 14 16 18 20
Months

FIG 4E

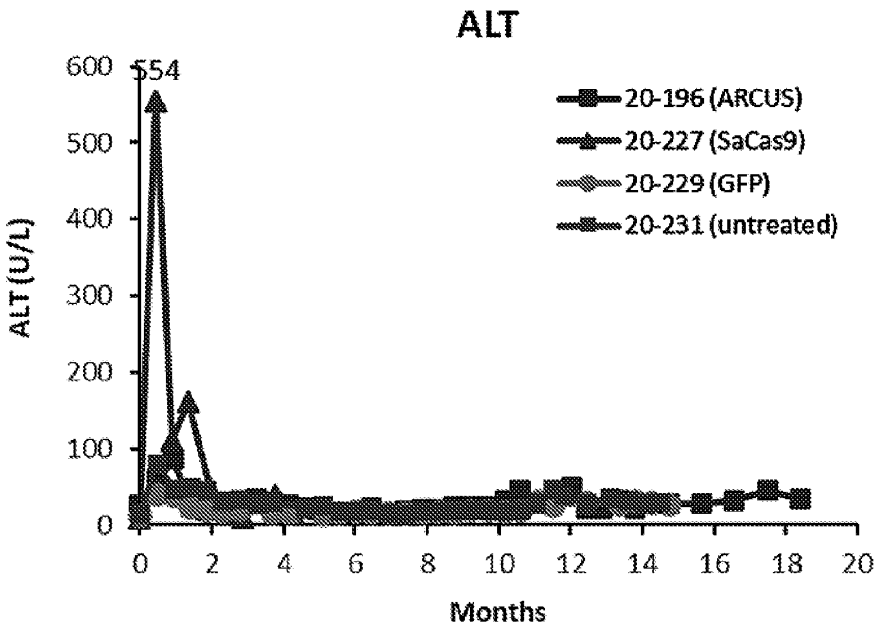

ALT
554
600
500
400
300
200
100
0
ALT (U/L)
0
2
4
6
8
10
12
14
16
18
20
Months
20-196 (ARCUS)
20-227 (SaCas9)
20-229 (GFP)
20-231 (untreated)

FIG 4F

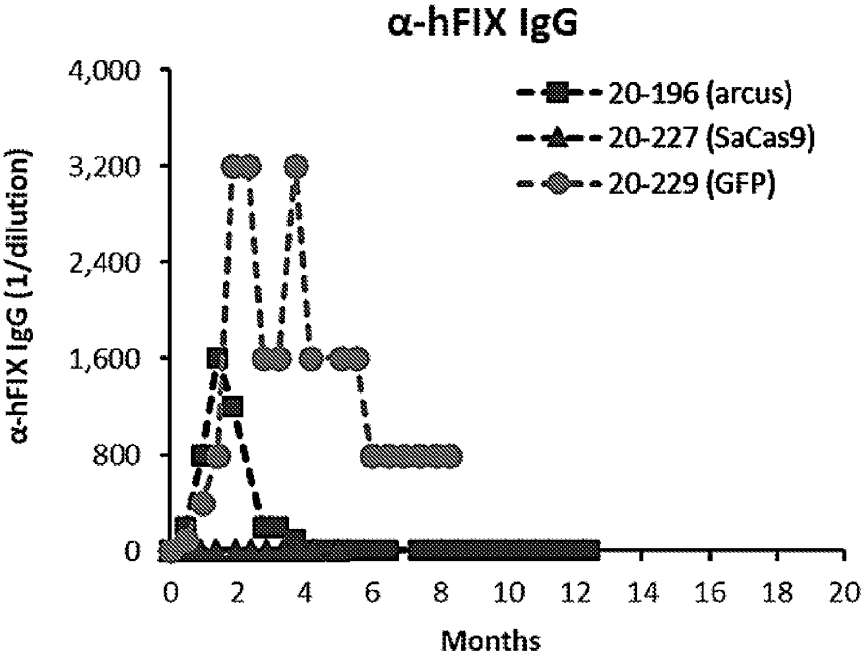

α-hFIX IgG
4,000
3,200
2,400
1,600
800
0
α-hFIX IgG (1/dilution)
0
2
4
6
8
10
12
14
16
18
20
Months
20-196 (arcus)
20-227 (SaCas9)
20-229 (GFP)

FIG 4G

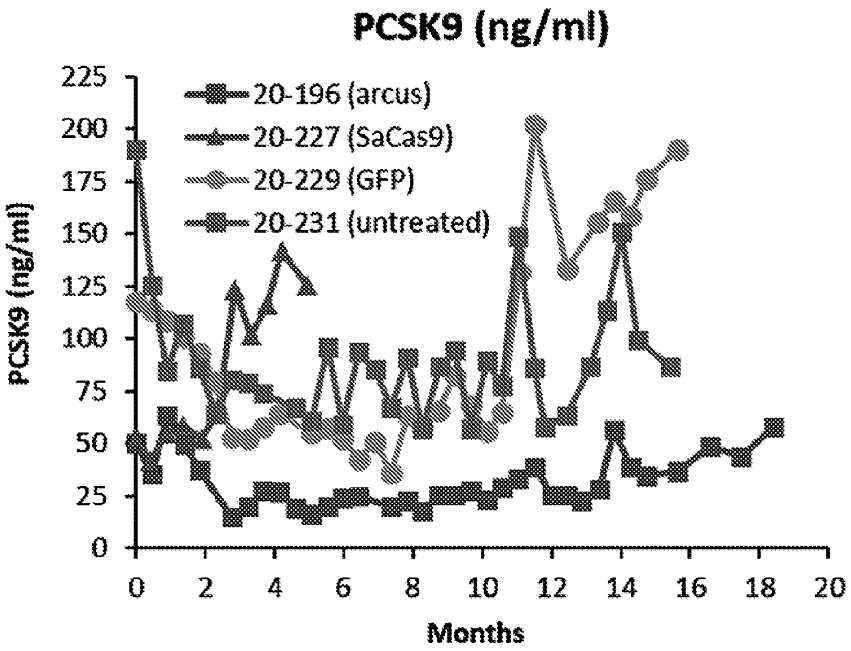

PCSK9 (ng/ml)
PCSK9 (ng/ml)
225
200
175
150
125
100
75
50
25
0
0 2 4 6 8 10 12 14 16 18 20
Months
20-196 (arcus)
20-227 (SaCas9)
20-229 (GFP)
20-231 (untreated)

FIG 4H

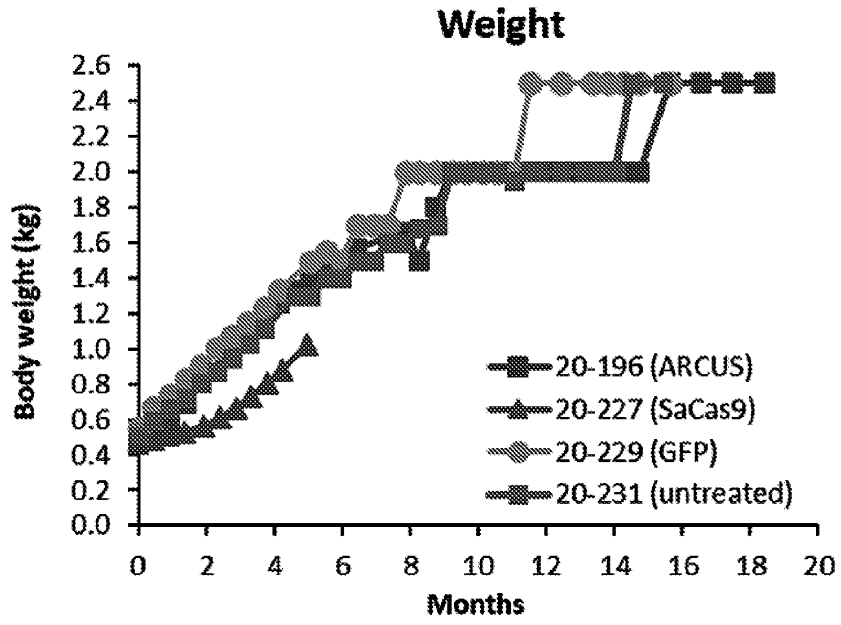

Weight
Body weight (kg)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 2 4 6 8 10 12 14 16 18 20
Months
20-196 (ARCUS)
20-227 (SaCas9)
20-229 (GFP)
20-231 (untreated)

FIG 5A

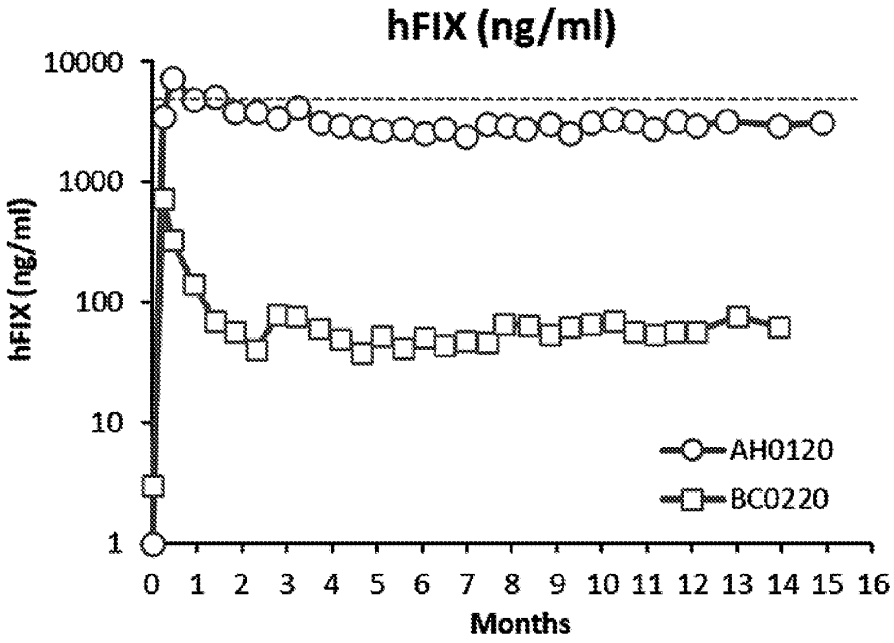

hFIX (ng/ml)
10000
1000
100
10
1
hFIX (ng/ml)
AH0120
BC0220
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months

FIG 5B

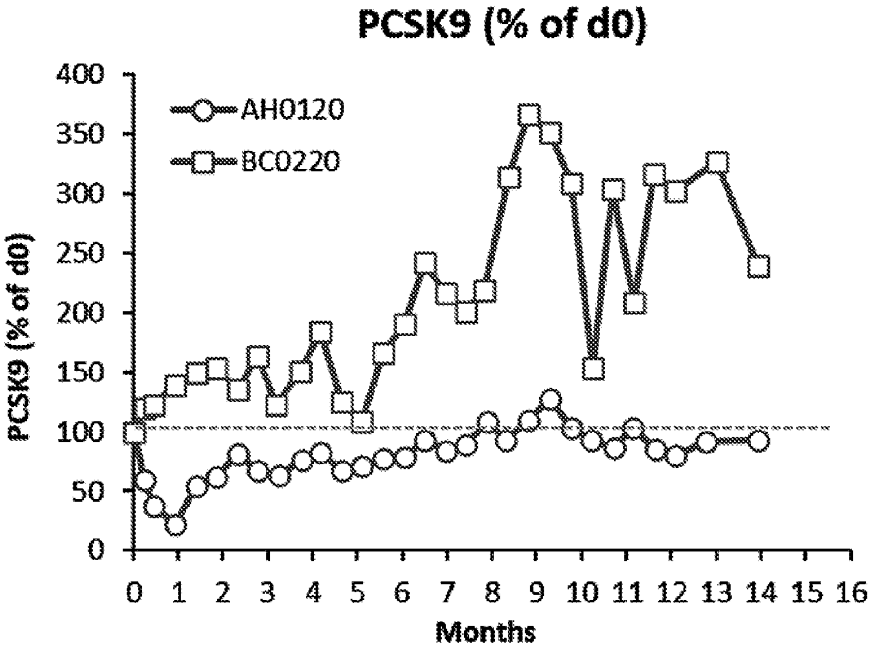

PCSK9 (% of d0)
400
350
300
250
200
150
100
50
0
PCSK9 (% of d0)
AH0120
BC0220
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months

FIG 5C

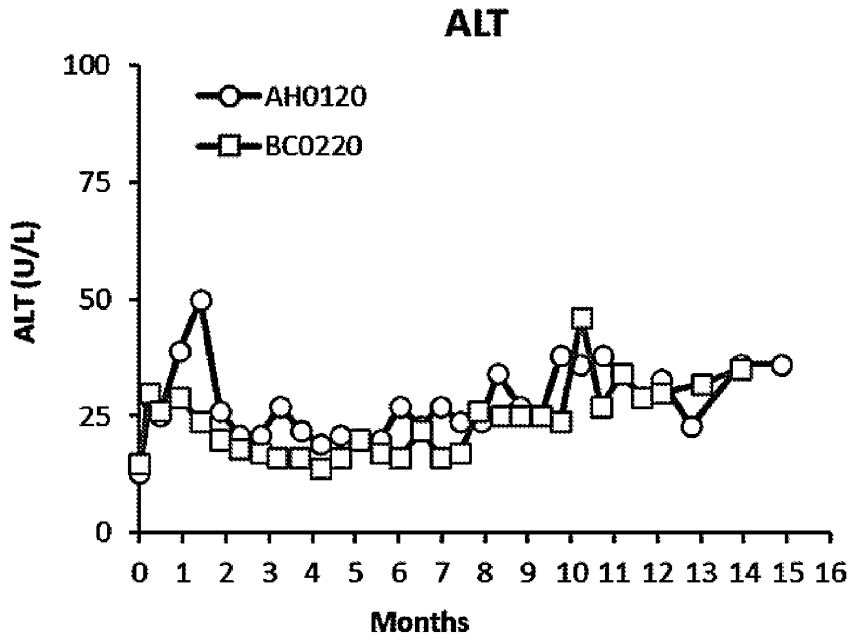

ALT
AH0120
BC0220
ALT (U/L)
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months

FIG 5D

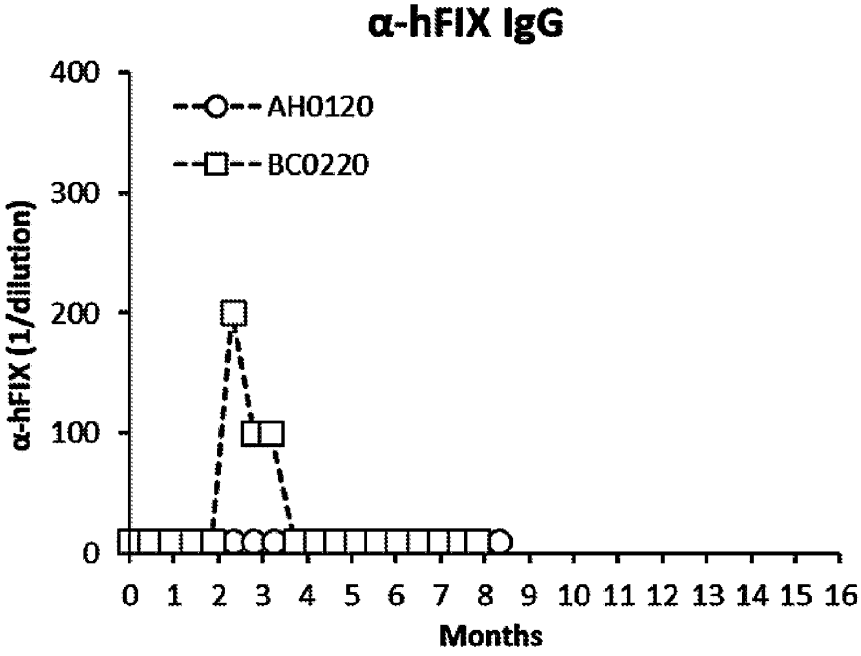

α-hFIX IgG
AH0120
BC0220
α-hFIX (1/dilution)
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months

FIG 5E

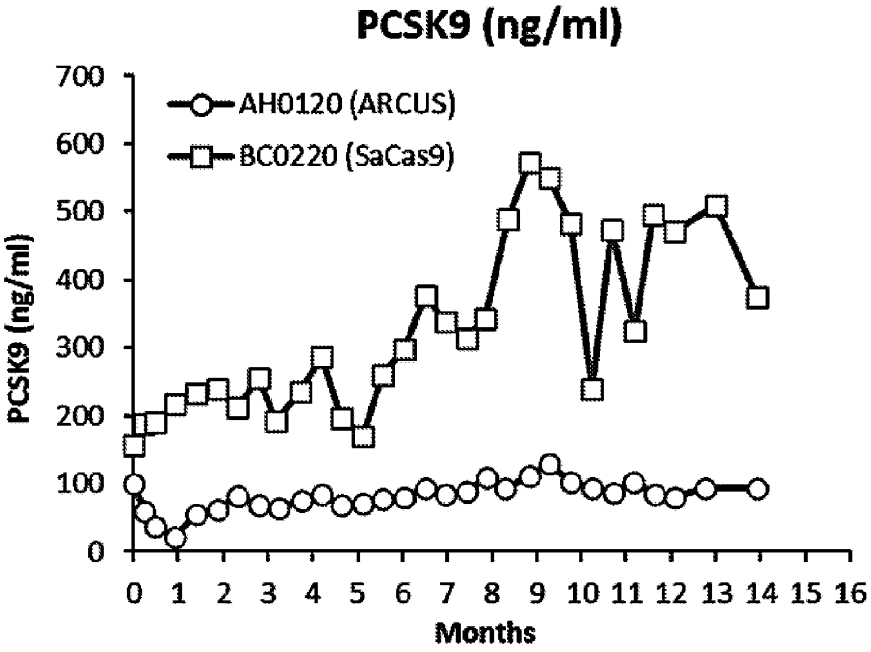

PCSK9 (ng/ml)
AH0120 (ARCUS)
BC0220 (SaCas9)
PCSK9 (ng/ml)
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months

FIG 5F

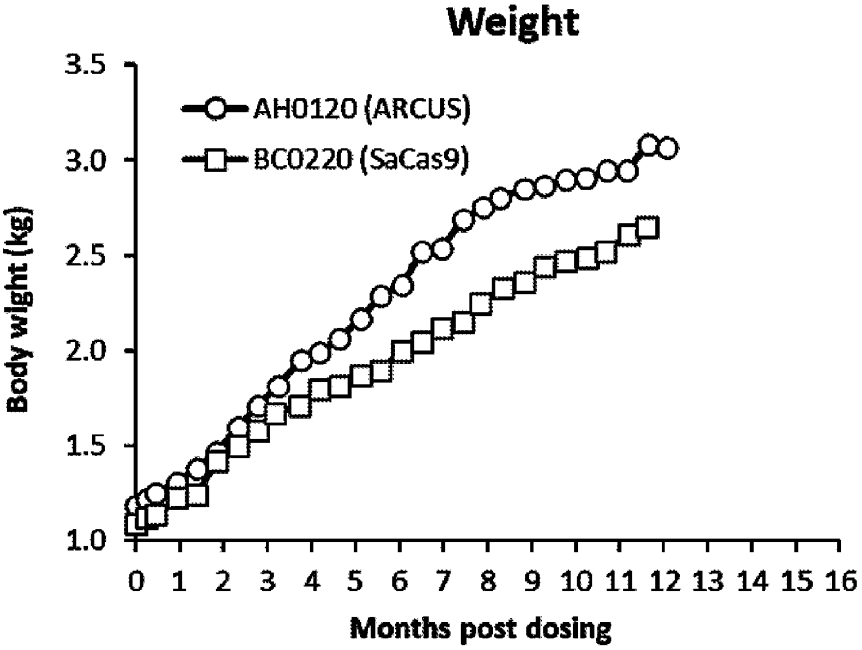

Weight
AH0120 (ARCUS)
BC0220 (SaCas9)
Body wight (kg)
3.5
3.0
2.5
2.0
1.5
1.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Months post dosing

FIG 5G

Animal ID	Nuclease	Age at dosing (day)	Biopsy or Necropsy Time points (day)	Peak hFIX (ng/ml) (time point)	Recent hFIX (ng/ml) (time point)	Anti-hFIX IgG	hFIX transduction %	WPRE transduction %	indel %	# of loci by ITR-seq
20-196	ARCUS	2	84	32,446 (d43)	1,169 (d560)	Transient (d14 -d98)	12.3	0.20	10.3	12, 29
			366				11.8	0.11	12.1	3, 28
AH0120	ARCUS	89	98	5,185 (d42)	3,161 (d448)	< detection limit	20.4	0.33	14.4	7, 18
			364				20.5	0.05	22.2	29, 19
21-120	ARCUS	191	100	7,327 (d28)	753 (d84)	< detection limit	To be done			
20-227	SaCas9	2	151	160 (d13)	68 (d151)	< detection limit	0.8	0.02	0.11	4
BC0220	SaCas9	89	96	733 (d7)	62 (d421)	Transient (d70 -d96)	1.6	0.02	0.14	4, 0
			364				0.07	0.00	0.10	1
20-229	EGFP	2	84	672 (d14)	1 (d448)	Persistent (d28 - present)	0.1	0.00	0.10	0
20-231	Untreated	3*	84	NA	NA	NA	0.01	0.00	0.04	0

FIG 5H

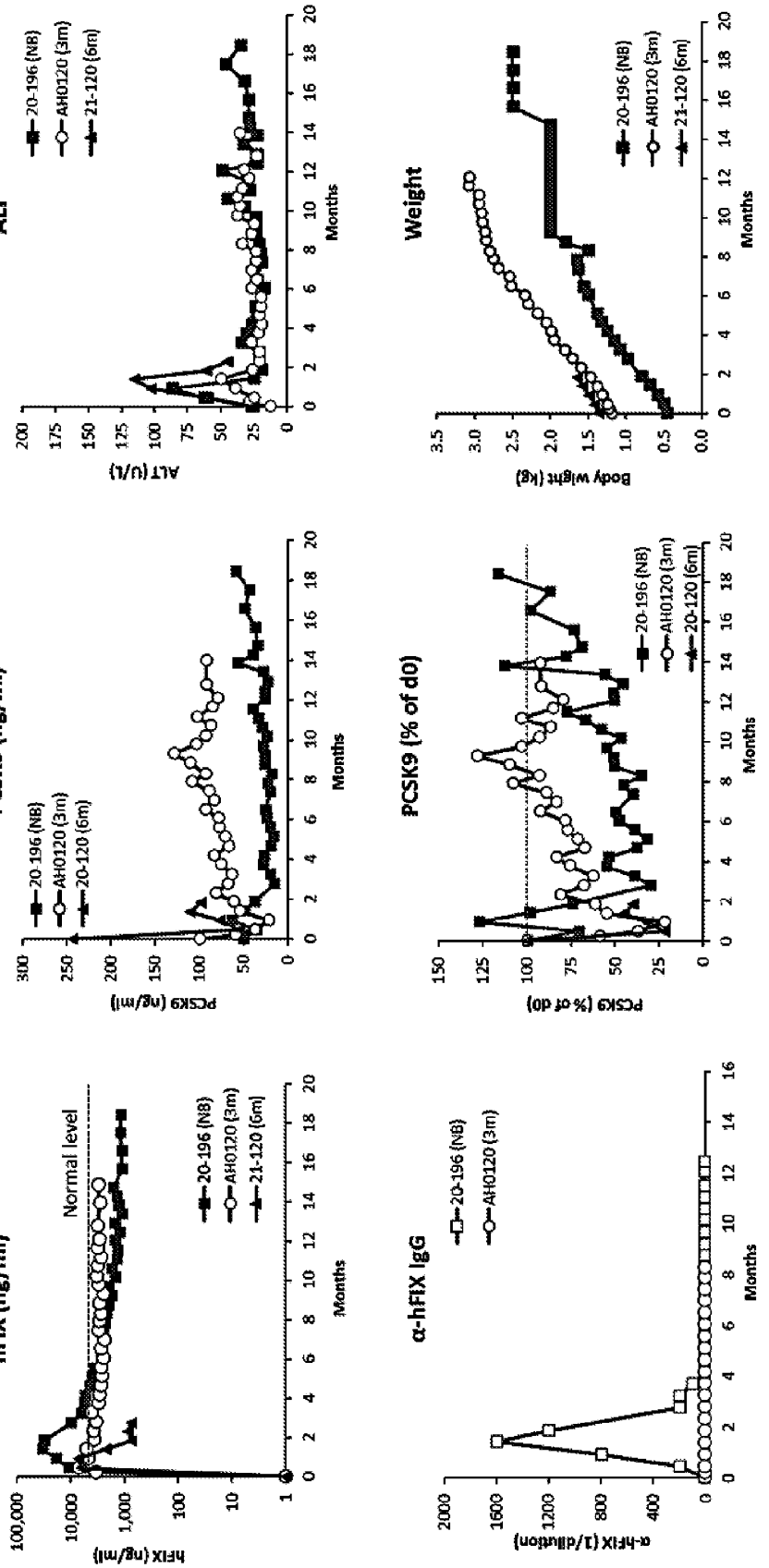

hFIX (ng/ml)
Normal level
20-196 (NB)
AH0120 (3m)
21-120 (6m)
Months
PCSK9 (ng/ml)
PCSK9 (% of d0)
α-hFIX IgG
α-hFIX (1/dilution)
ALT
ALT (U/L)
Weight
Body weight (kg)

FIG 6A

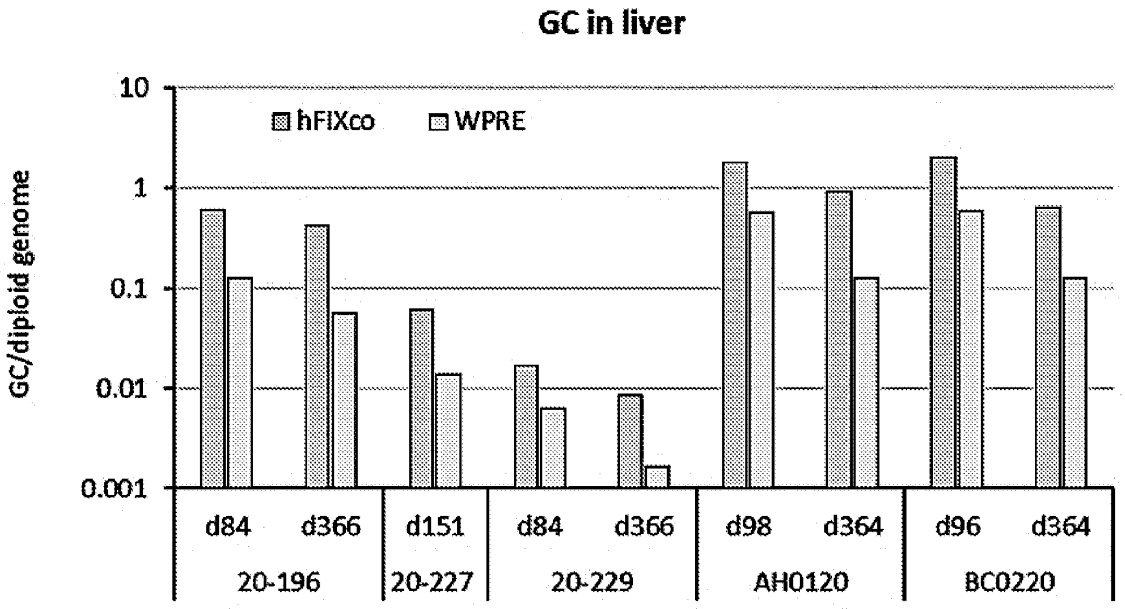

GC in liver
GC/diploid genome
10
1
0.1
0.01
0.001
hFIXco
WPRE
d84
d366
d151
d84
d366
d98
d364
d96
d364
20-196
20-227
20-229
AH0120
BC0220

FIG 6B

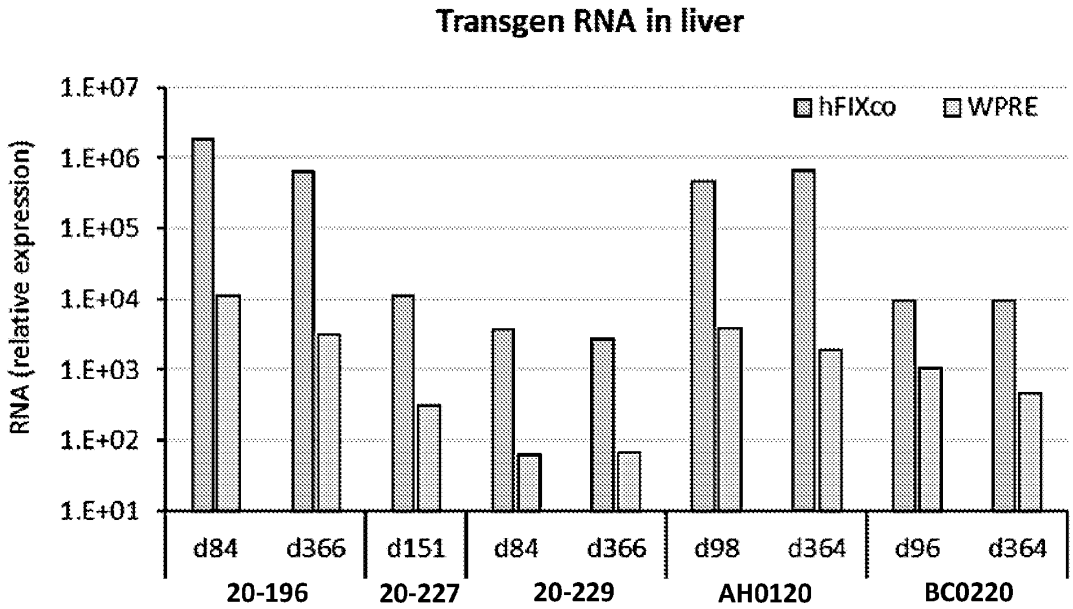

Transgen RNA in liver
RNA (relative expression)
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
hFIXco
WPRE
d84
d366
d151
d84
d366
d98
d364
d96
d364
20-196
20-227
20-229
AH0120
BC0220

FIG 6C

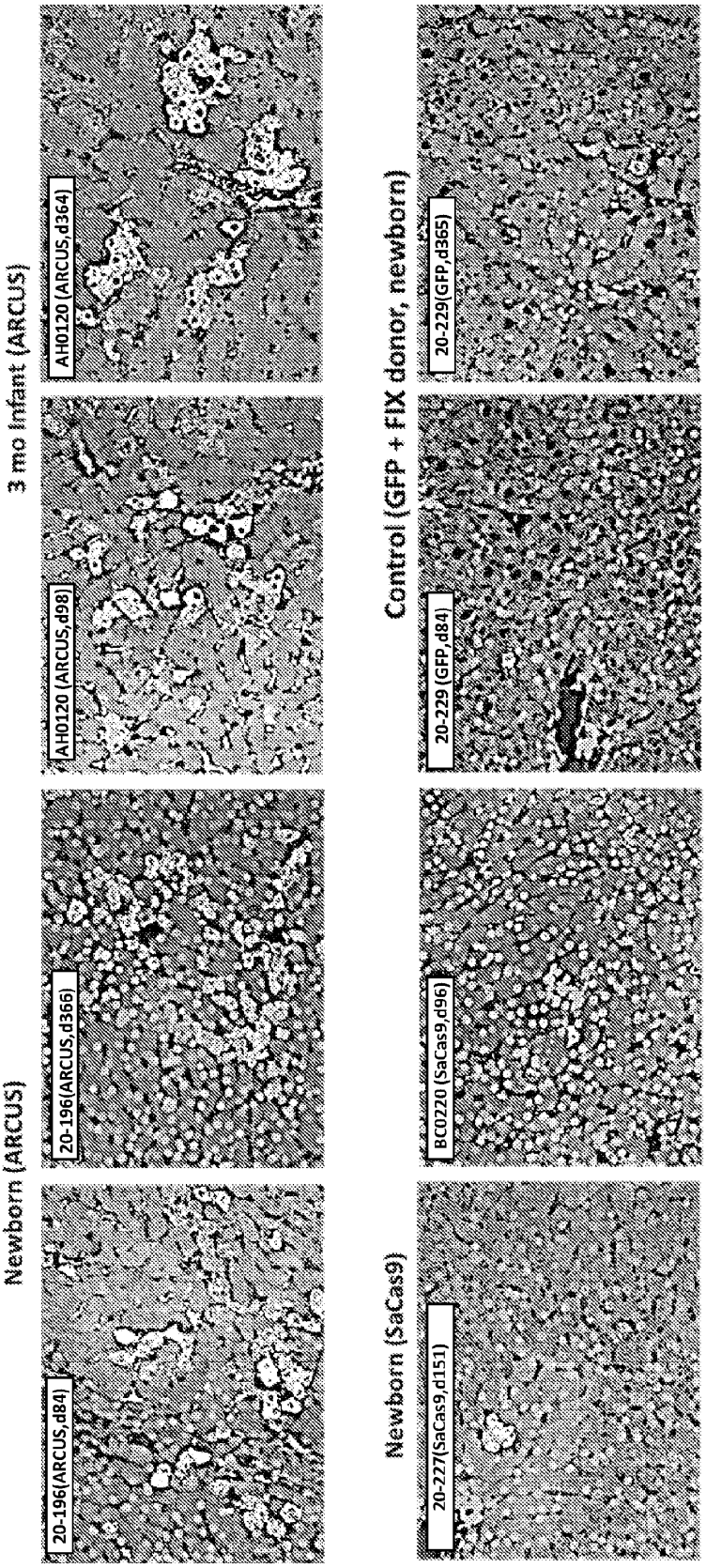

Stable Transduction in Liver
Dual ISH (green=hFIXco, red=ARCUS or GFP)
Newborn (ARCUS)
3 mo Infant (ARCUS)
Control (GFP + FIX donor, newborn)
Newborn (SaCas9)
20-196(ARCUS,d84)
20-196(ARCUS,d366)
AH0120 (ARCUS,d98)
AH0120 (ARCUS,d364)
20-227(SaCas9,d151)
BC0220 (SaCas9,d96)
20-229 (GFP,d84)
20-229(GFP,d365)

FIG 6D

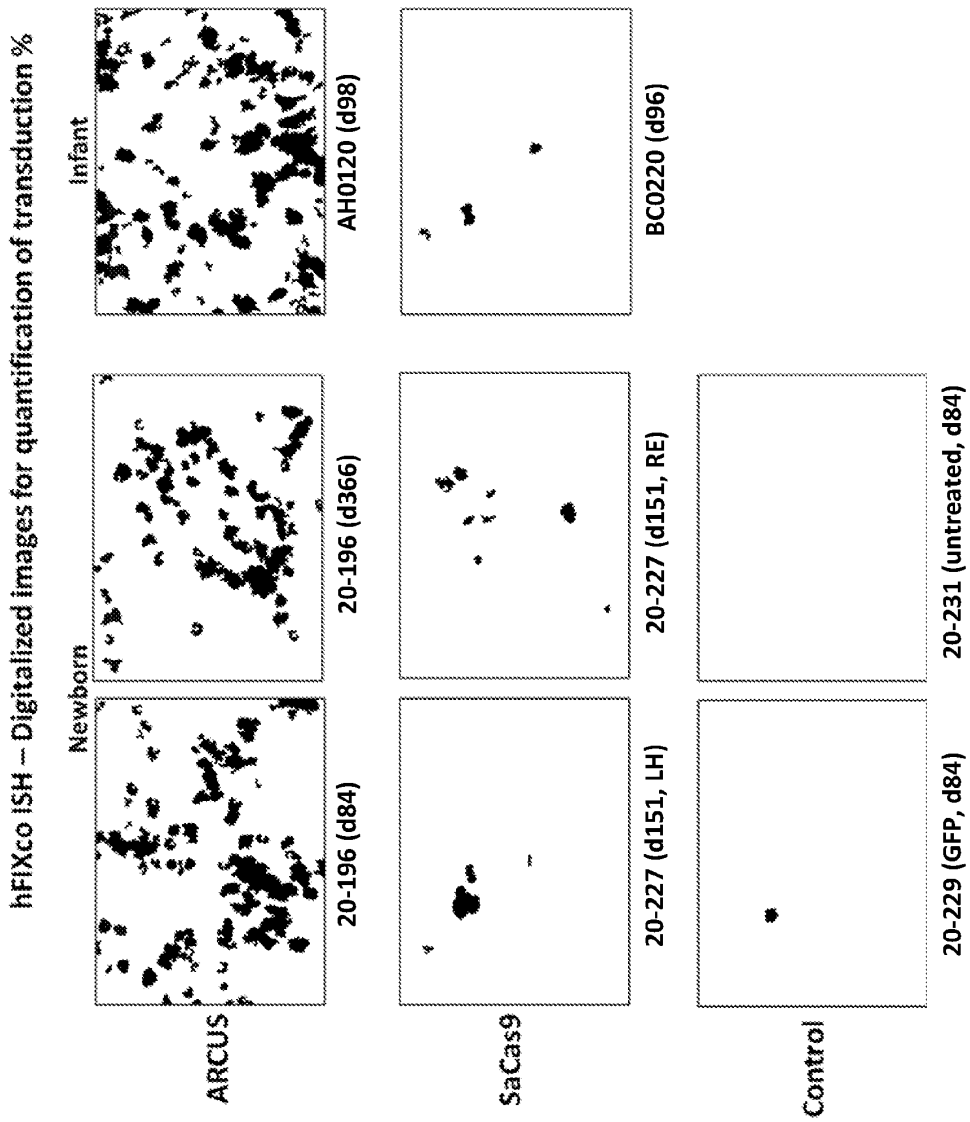
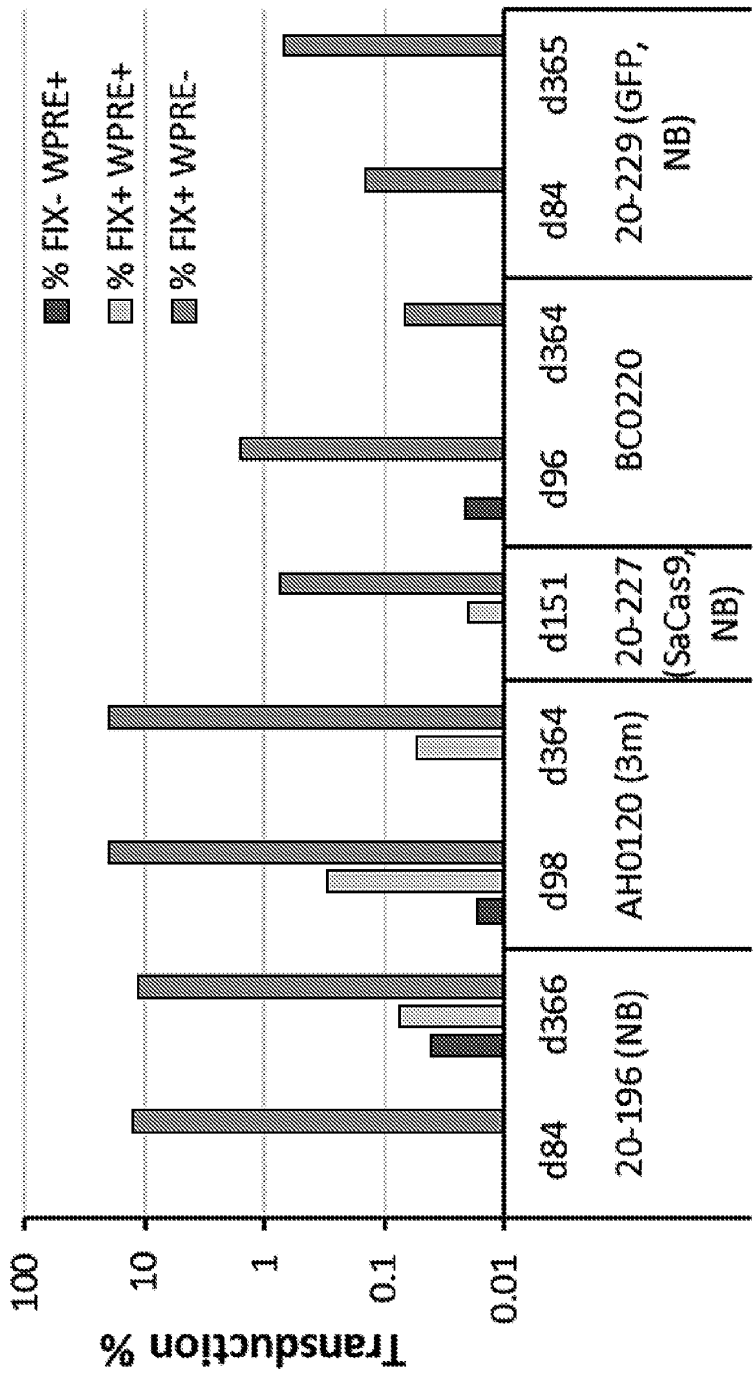

hFIXco ISH – Digitalized images for quantification of transduction %
Newborn
Infant
ARCUS
SaCas9
Control
20-196 (d84)
20-196 (d366)
AH0120 (d98)
20-227 (d151, LH)
20-227 (d151, RE)
BC0220 (d96)
20-229 (GFP, d84)
20-231 (untreated, d84)

FIG 6E


% of Transduction by dual ISH
% FIX- WPRE+
% FIX+ WPRE+
% FIX+ WPRE-
Transduction %
100
10
1
0.1
0.01
d84
d366
20-196 (NB)
d98
d364
AH0120 (3m)
d151
20-227 (SaCas9, NB)
d96
d364
BC0220
d84
d365
20-229 (GFP, NB)

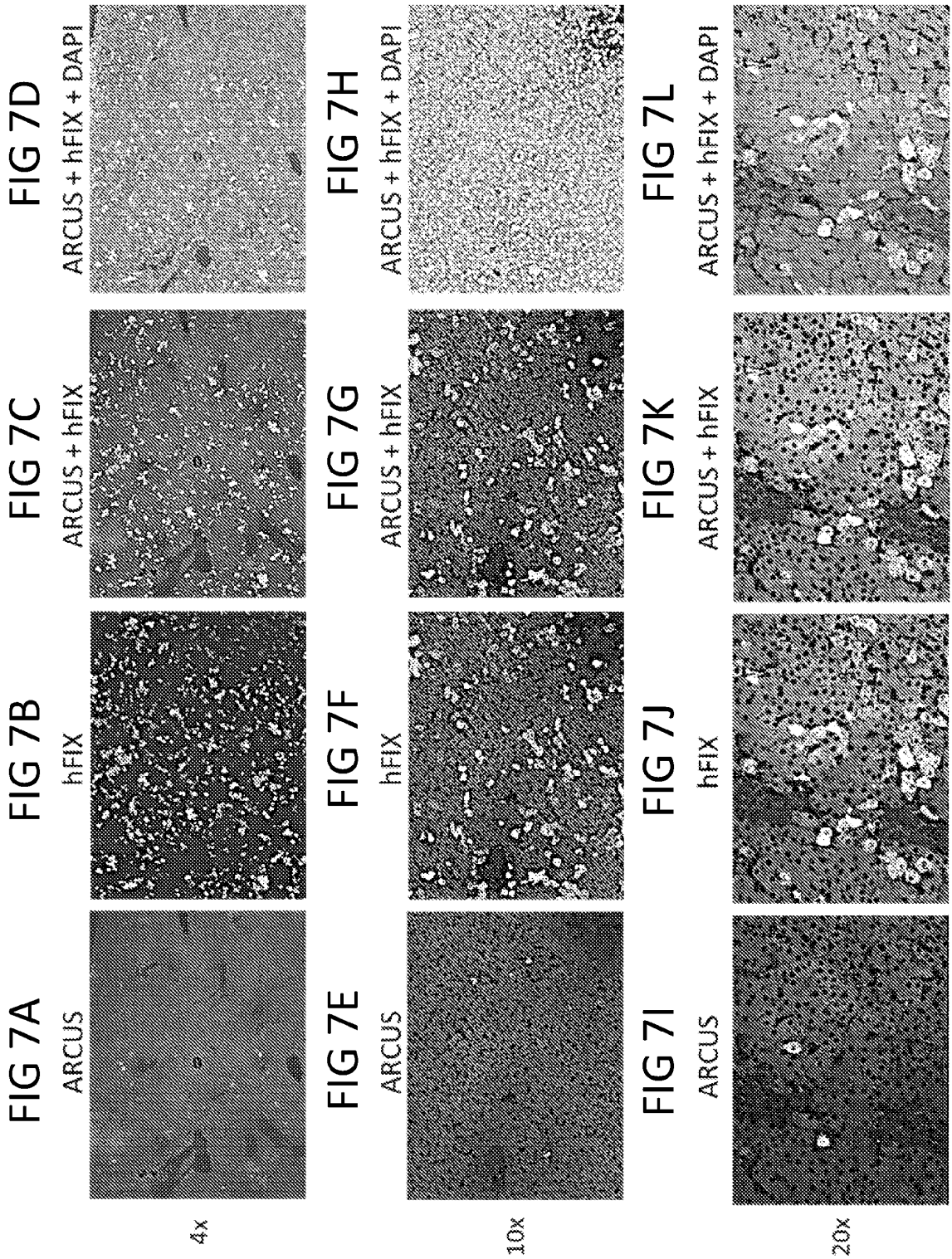


FIG 7A
ARCUS
FIG 7B
hFIX
FIG 7C
ARCUS + hFIX
FIG 7D
ARCUS + hFIX + DAPI
FIG 7E
ARCUS
FIG 7F
hFIX
FIG 7G
ARCUS + hFIX
FIG 7H
ARCUS + hFIX + DAPI
FIG 7I
ARCUS
FIG 7J
hFIX
FIG 7K
ARCUS + hFIX
FIG 7L
ARCUS + hFIX + DAPI
4x
10x
20x

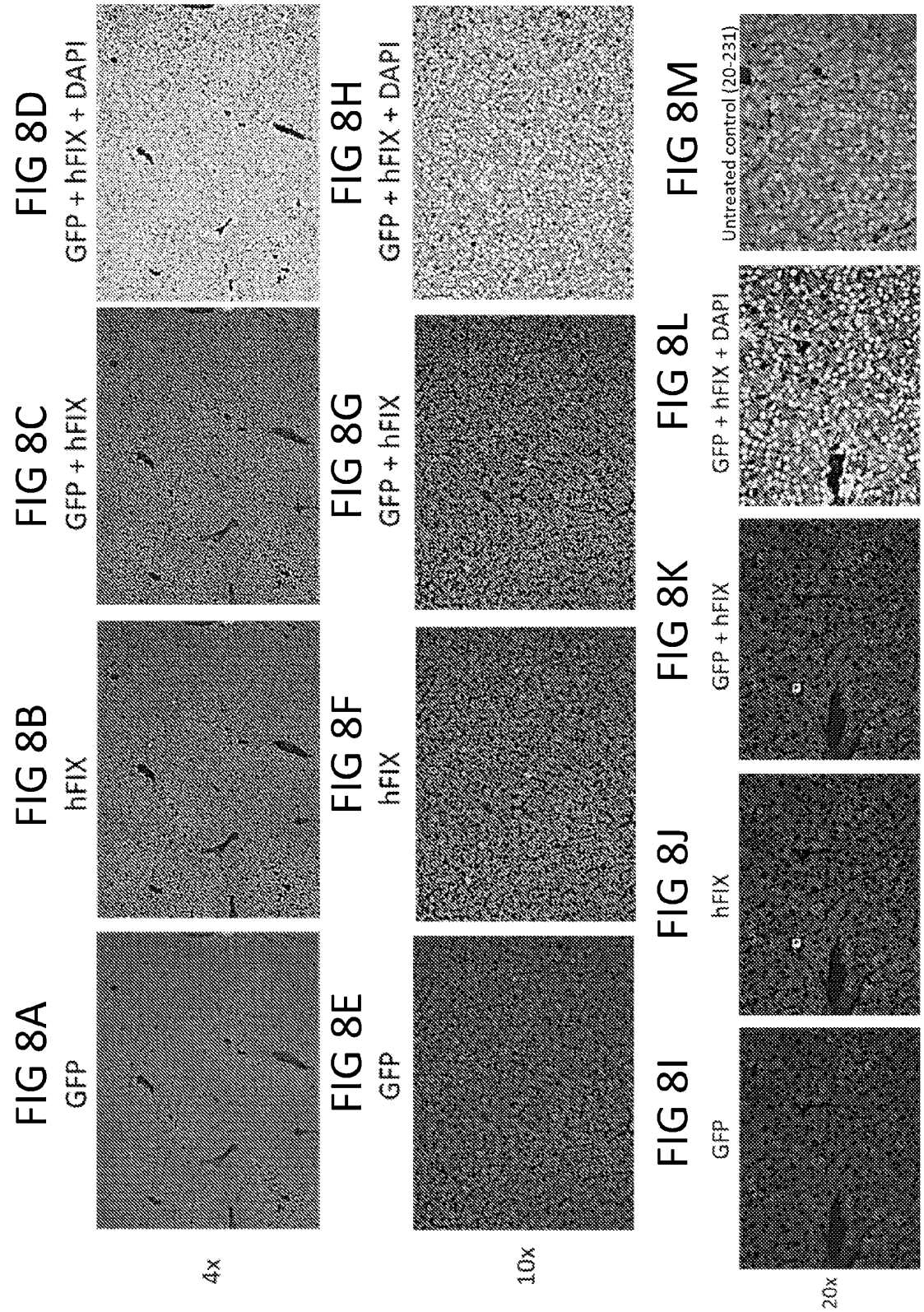


FIG 8A
GFP
FIG 8B
hFIX
FIG 8C
GFP + hFIX
FIG 8D
GFP + hFIX + DAPI
FIG 8E
GFP
FIG 8F
hFIX
FIG 8G
GFP + hFIX
FIG 8H
GFP + hFIX + DAPI
FIG 8I
GFP
FIG 8J
hFIX
FIG 8K
GFP + hFIX
FIG 8L
GFP + hFIX + DAPI
FIG 8M
Untreated control (20-231)
4x
10x
20x

FIG 9

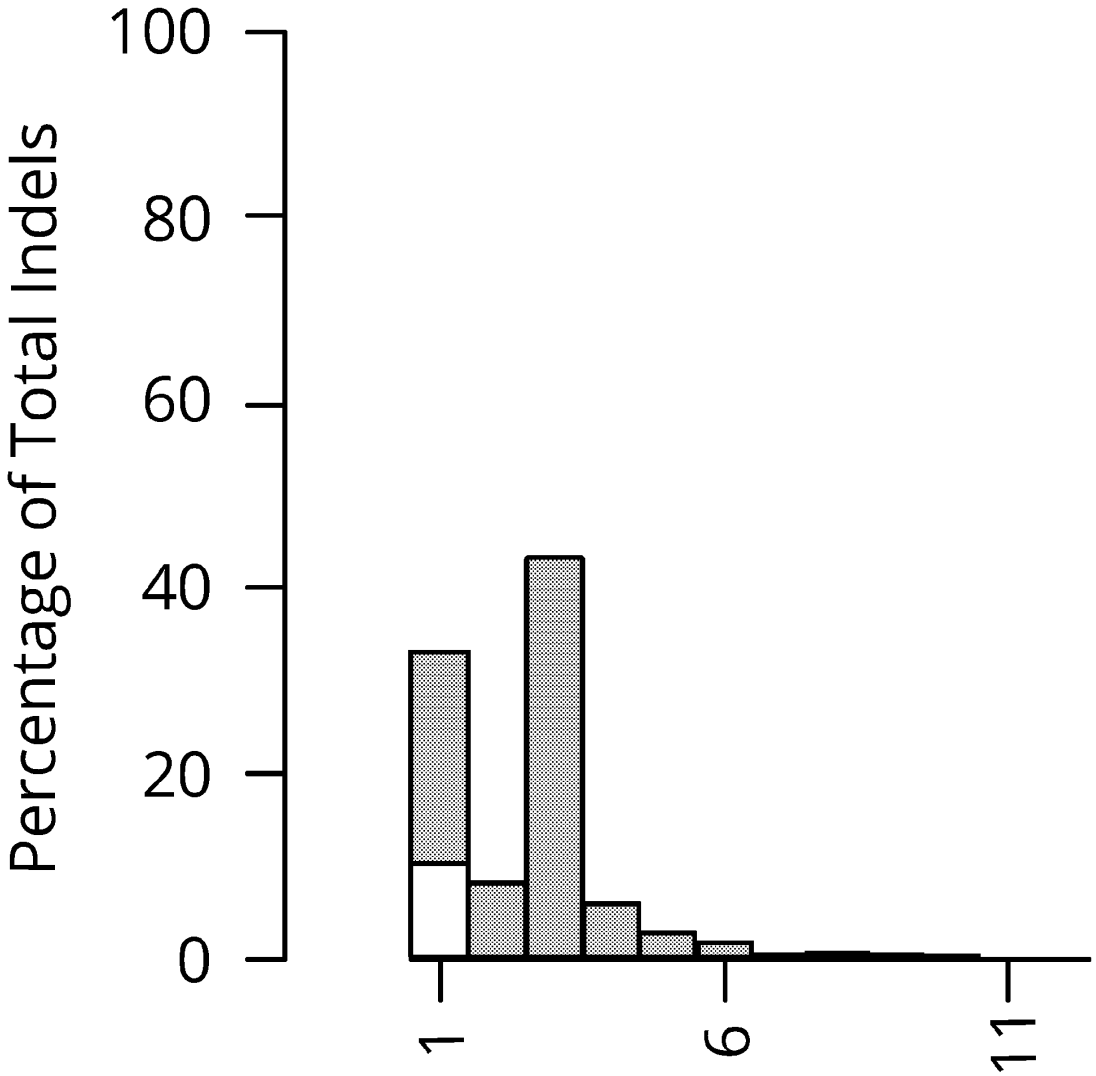


FIG 10A

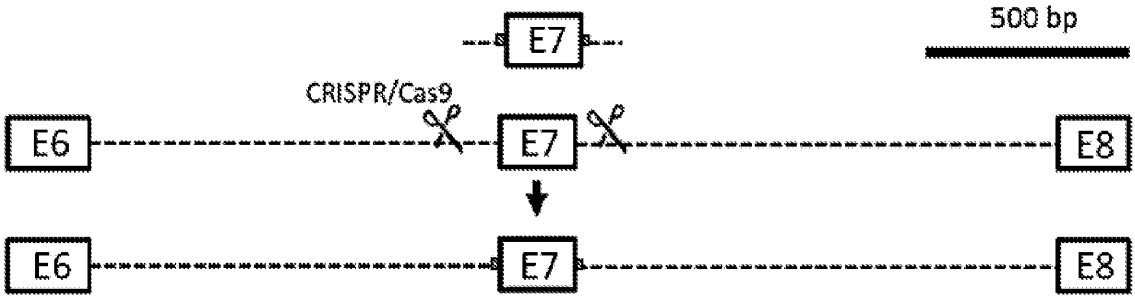


FIG 10B

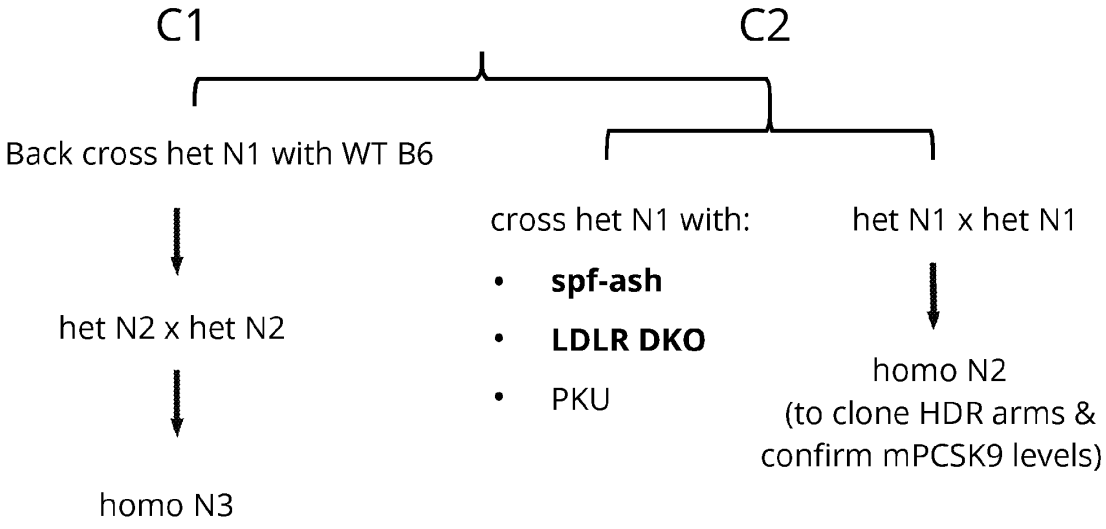


FIG 11A

HDR ARMS	Promoter (nuclease vector)	Serotype	Group	Animal ID	Sex	Age at dosing (day)	Anti-AAV Bab titer (on day 0)	Biopsy (day)	Transduction		Amplicon-seq	ITR-Seq
									OTC ISH	OTC IF	Indel %	# of OT sites[c]
Long	-	AAVrh79	G1	21-180	M	13	50	85	0.1	0.6	0.09	0
				21-204	F	1	50	84	1.0	0.7	0.06	0; 0
Short	F113	AAVrh79	G5	21-146	F	7	50	84	2.3	1.3	1.5	0; 0
				21-133[a]	F	15	1600	86	0	0	0.1	N.T.
	TBG	AAVrh79	G4	21-131[a]	F	16	400	85	0	0	0.1	N.T.
				21-122[b]	F	11	1600	85	0	0	0.1	N.T.
Long	F113	AAVhu37	G3	21-068	M	7	50	84	8.9	5.4	2.6	2; 2
				21-090	M	6	25	86	0.9	1.1	0.7	0; 0
		AAVrh79	G7	21-178	M	10	N.T.[a]	85	1.9	2.2	0.9	0; 0
				21-188	M	3	25	93	1.6	2.6	0.7	0; 1
	TBG	AAVhu37	G2	21-053	F	9	100	86	4.5	4.0	4.5	4; 3
				21-071	M	9	50	84	3.7	2.9	3.9	2; 0; 2
		AAVrh79	G6	21-157	F	6	25	84	16.9	18.6	12.8	27; 18
				21-175	F	11	<25	84	10.3	11.9	13.5	40; 27

FIG 11B-1

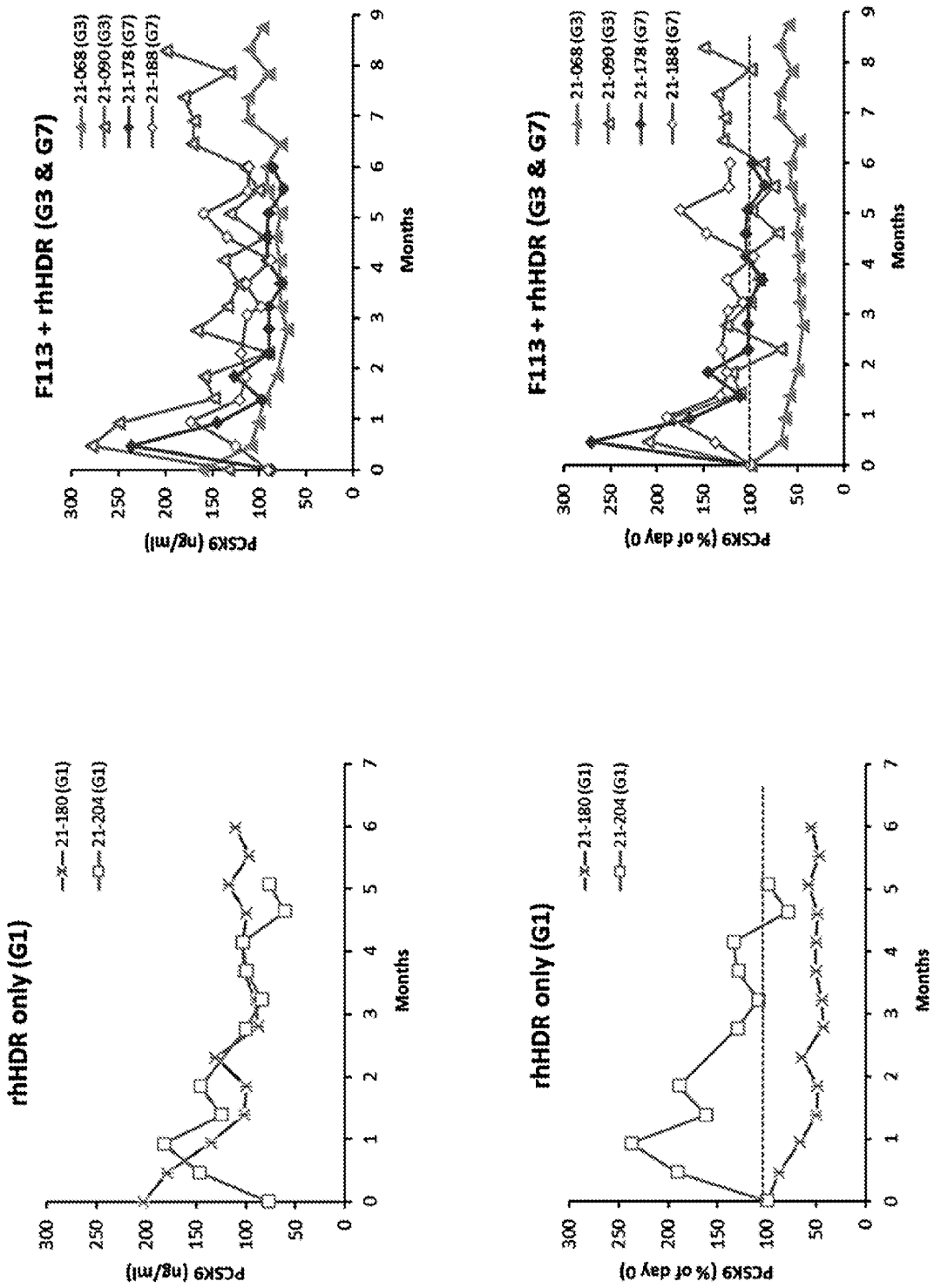

F113 + rhHDR (G3 & G7)
21-068 (G3)
21-090 (G3)
21-178 (G7)
21-188 (G7)
PCSK9 (ng/ml)
PCSK9 (% of day 0)
Months
rhHDR only (G1)
21-180 (G1)
21-204 (G1)

FIG 11B-2

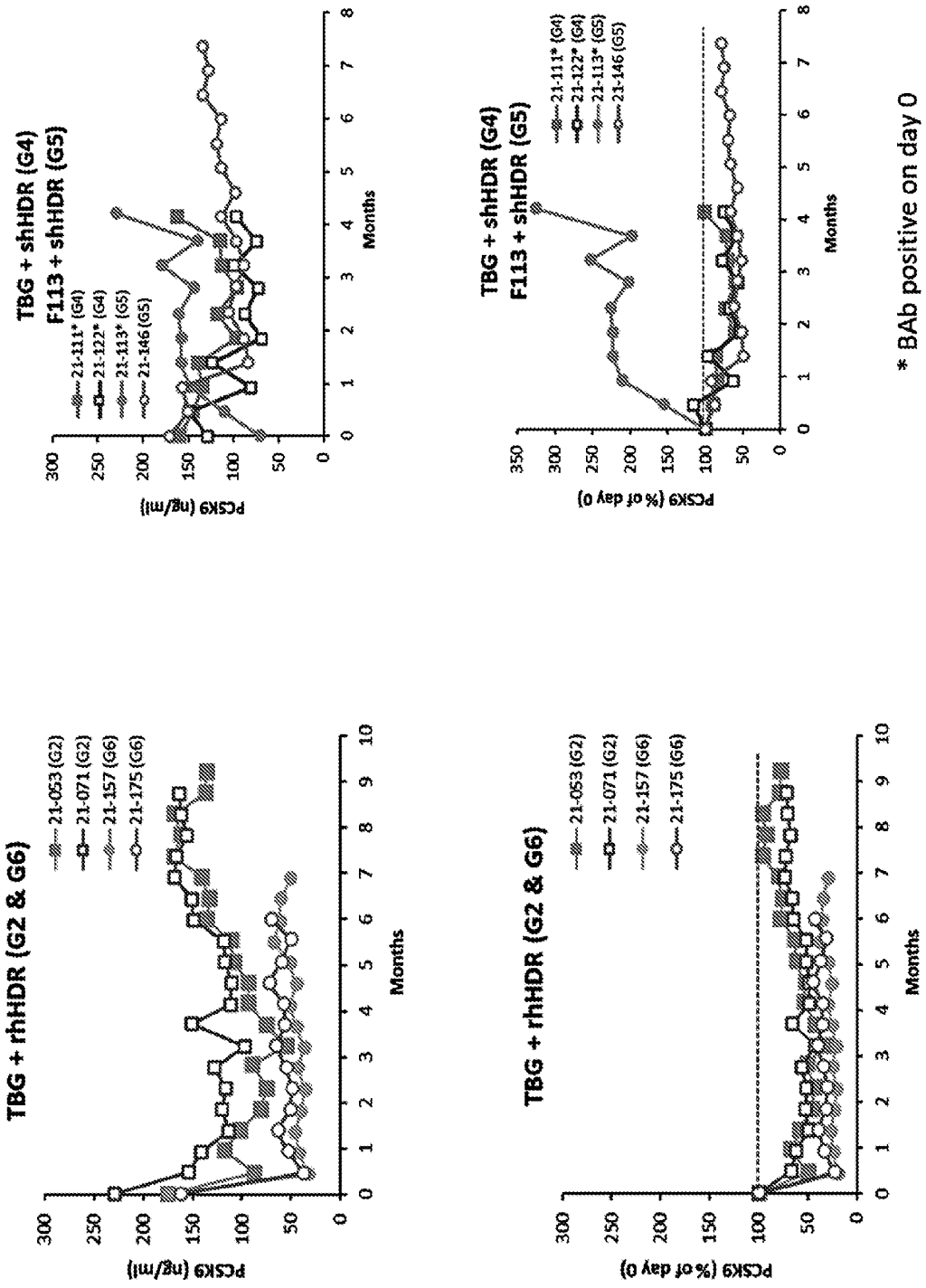

TBG + shHDR (G4)
F113 + shHDR (G5)
21-111* (G4)
21-122* (G4)
21-113* (G5)
21-146 (G5)
PCSK9 (ng/ml)
Months
TBG + shHDR (G4)
F113 + shHDR (G5)
PCSK9 (% of day 0)
Months
* BAb positive on day 0
TBG + rhHDR (G2 & G6)
21-053 (G2)
21-071 (G2)
21-157 (G6)
21-175 (G6)
PCSK9 (ng/ml)
Months
TBG + rhHDR (G2 & G6)
PCSK9 (% of day 0)
Months

FIG 11C-1

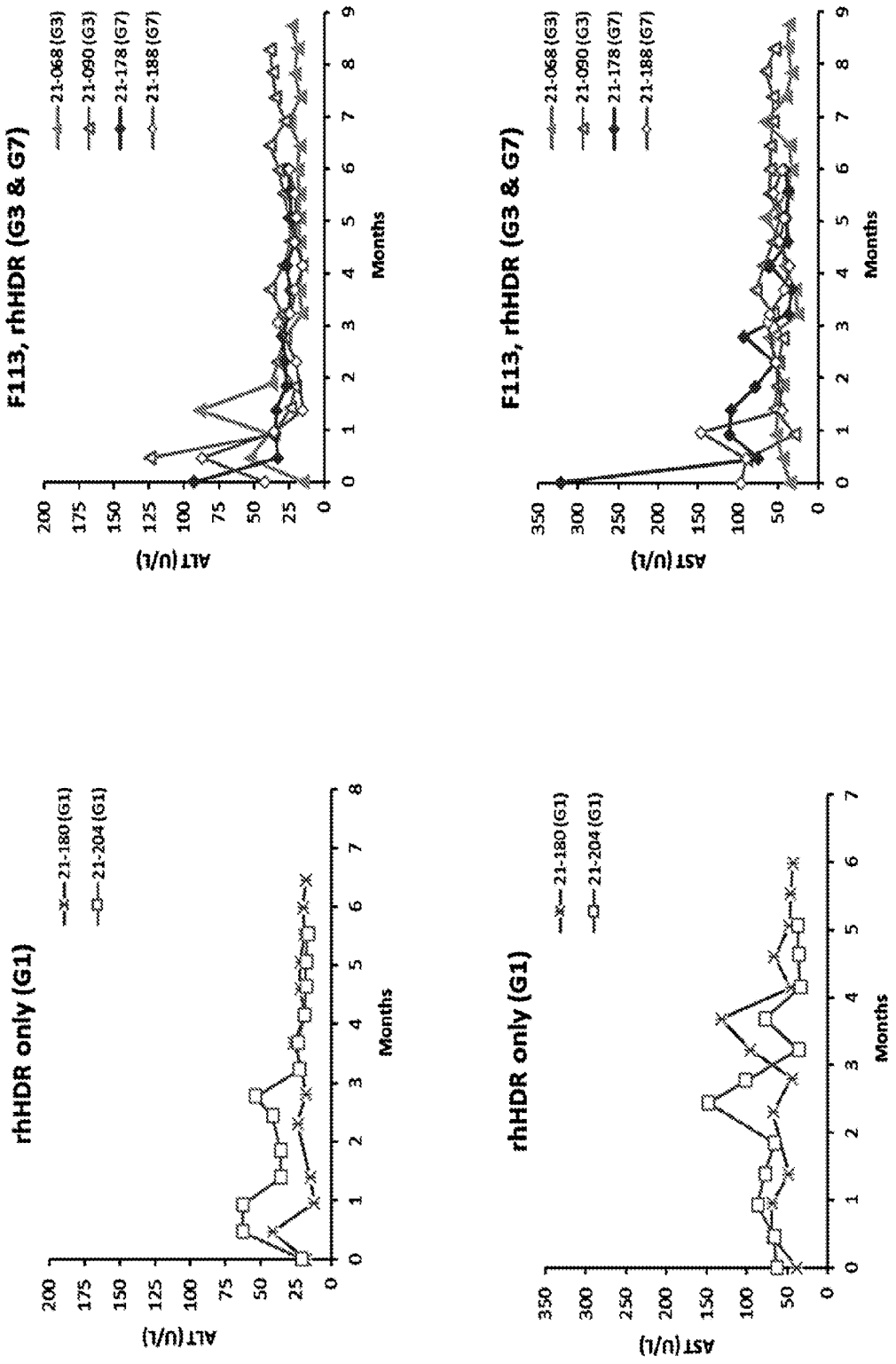
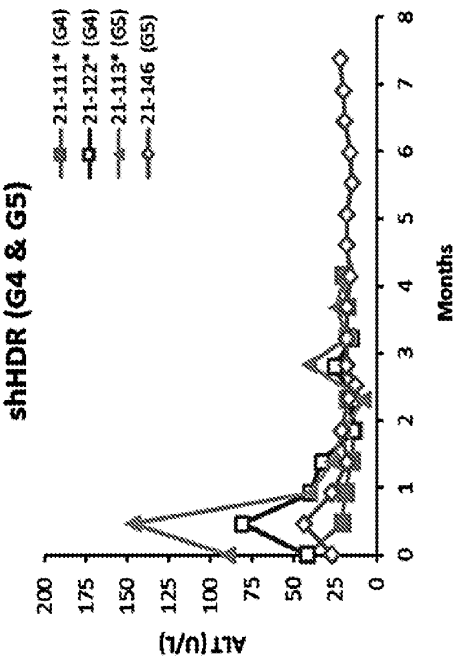
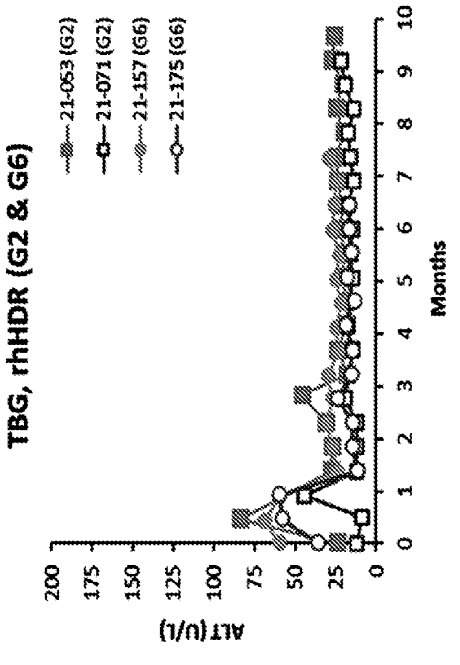
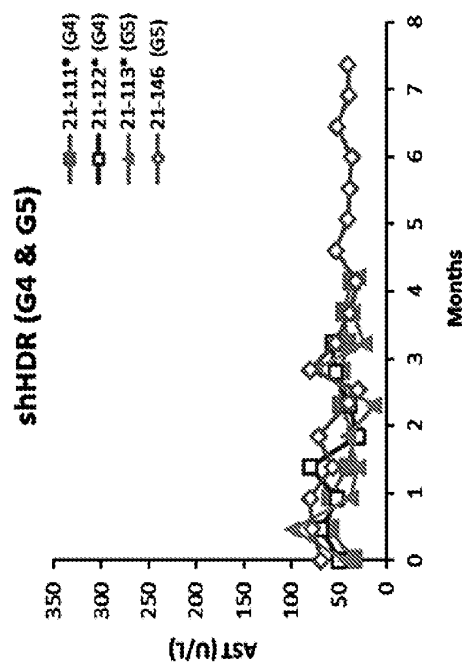

F113, rhHDR (G3 & G7)
21-068 (G3)
21-090 (G3)
21-178 (G7)
21-188 (G7)
ALT (U/L)
AST (U/L)
Months
rhHDR only (G1)
21-180 (G1)
21-204 (G1)

FIG 11C-2


shHDR (G4 & G5)
21-111* (G4)
21-122* (G4)
21-113* (G5)
21-146 (G5)
ALT (U/L)
Months


TBG, rhHDR (G2 & G6)
21-053 (G2)
21-071 (G2)
21-157 (G6)
21-175 (G6)
ALT (U/L)
Months


shHDR (G4 & G5)
21-111* (G4)
21-122* (G4)
21-113* (G5)
21-146 (G5)
AST (U/L)
Months

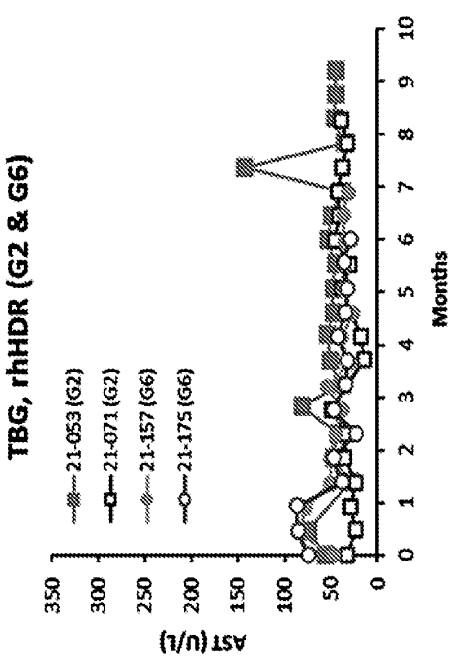

TBG, rhHDR (G2 & G6)
21-053 (G2)
21-071 (G2)
21-157 (G6)
21-175 (G6)
AST (U/L)
Months

FIG 11D

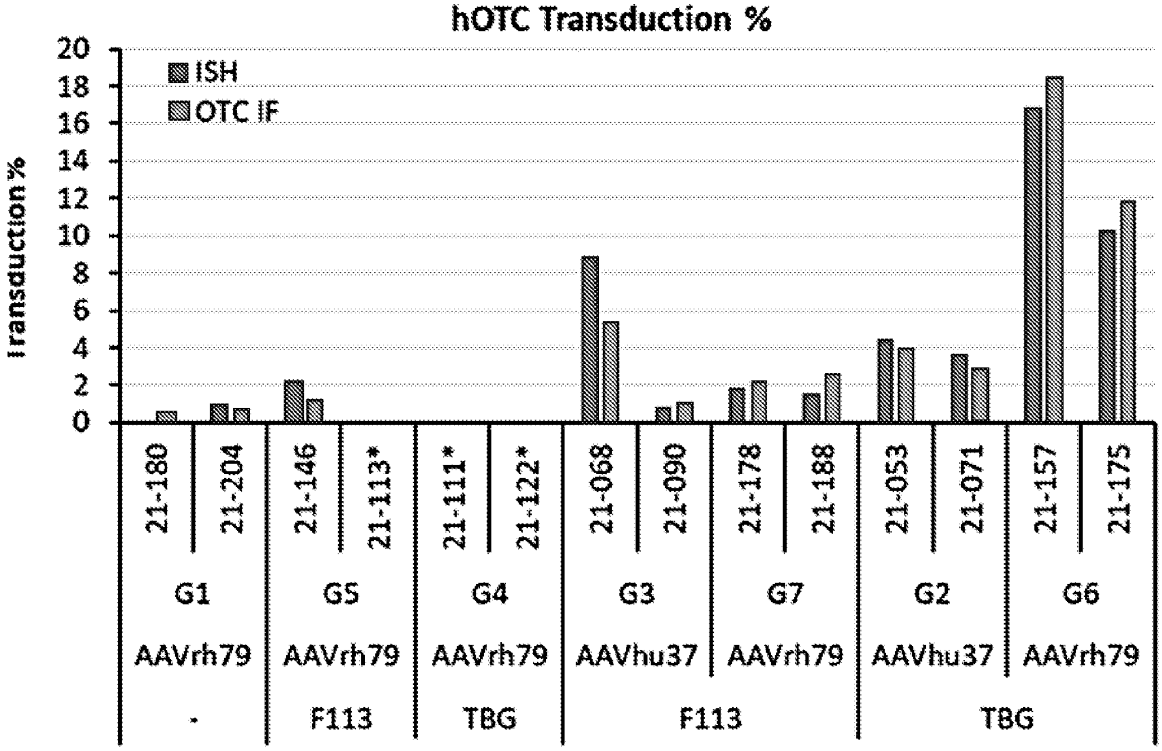
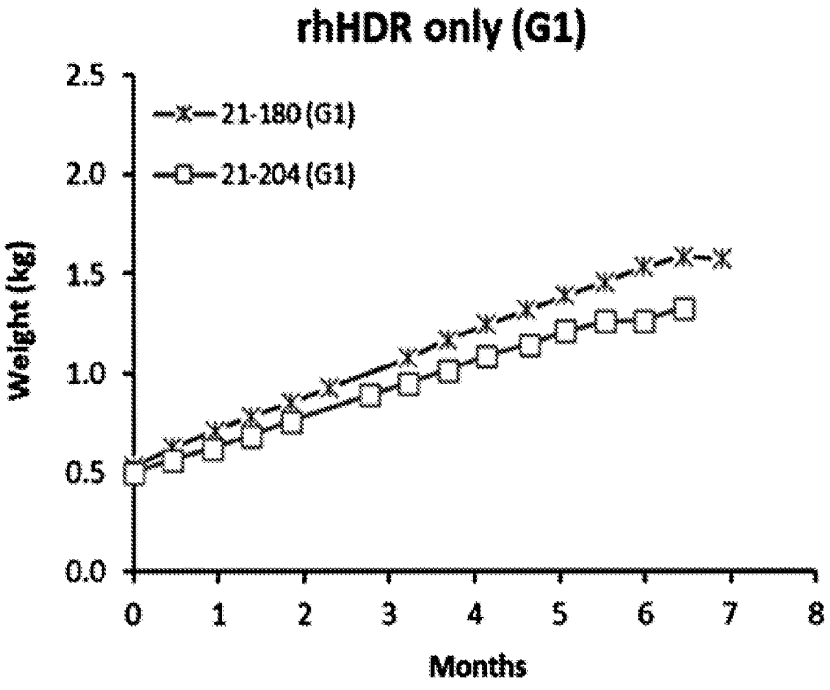

hOTC Transduction %
ISH
OTC IF
Transduction %
20
18
16
14
12
10
8
6
4
2
0
21-180
21-204
21-146
21-113*
21-111*
21-122*
21-068
21-090
21-178
21-188
21-053
21-071
21-157
21-175
G1
G5
G4
G3
G7
G2
G6
AAVrh79
AAVrh79
AAVrh79
AAVhu37
AAVrh79
AAVhu37
AAVrh79
-
F113
TBG
F113
TBG

FIG 11E-1


rhHDR only (G1)
21-180 (G1)
21-204 (G1)
Weight (kg)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
Months

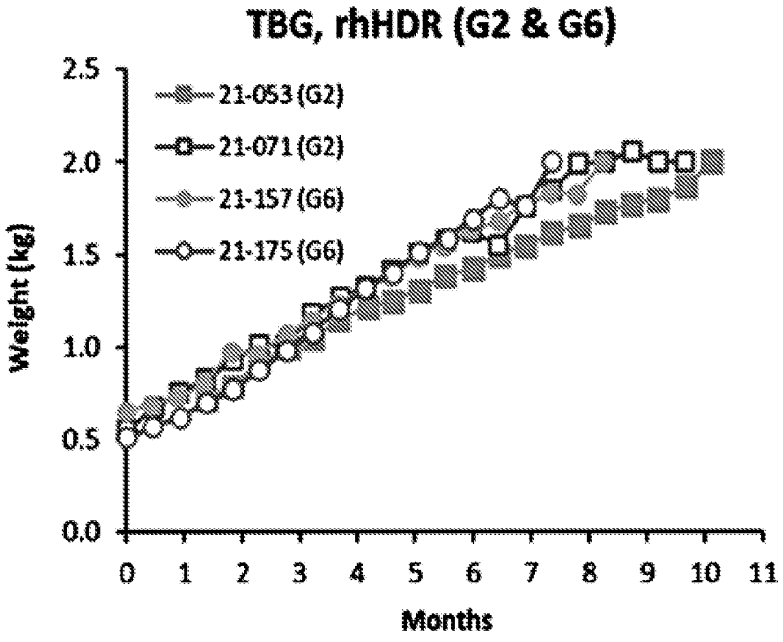
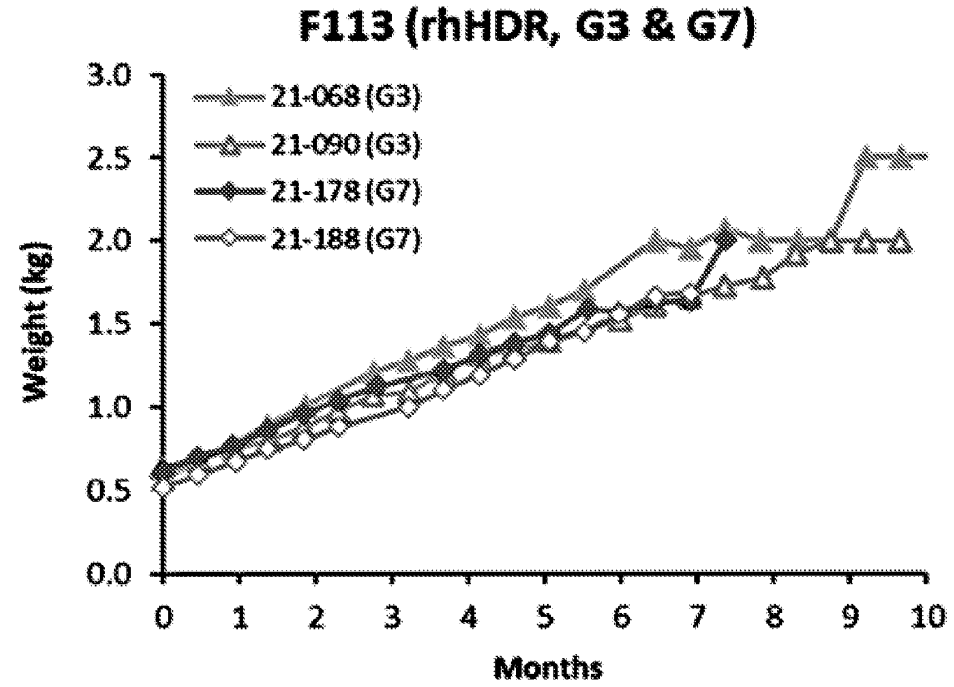

TBG, rhHDR (G2 & G6)
21-053 (G2)
21-071 (G2)
21-157 (G6)
21-175 (G6)
Weight (kg)
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
10
11
Months

FIG 11E-2


F113 (rhHDR, G3 & G7)
21-068 (G3)
21-090 (G3)
21-178 (G7)
21-188 (G7)
Weight (kg)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10
Months

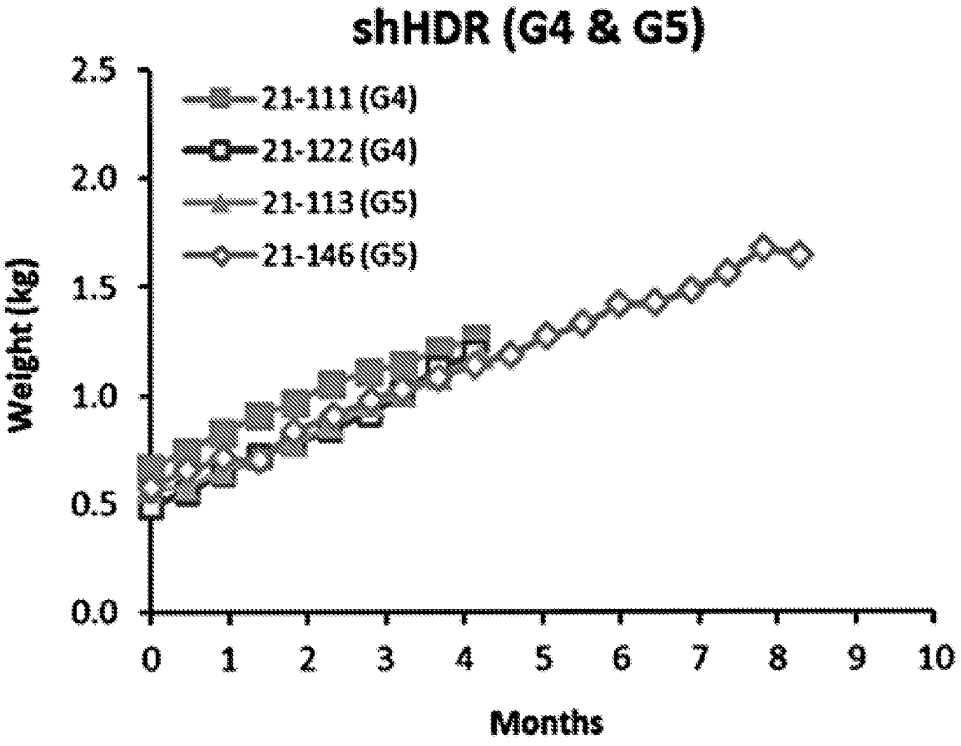

shHDR (G4 & G5)
21-111 (G4)
21-122 (G4)
21-113 (G5)
21-146 (G5)
Weight (kg)
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10
Months

FIG. 11G

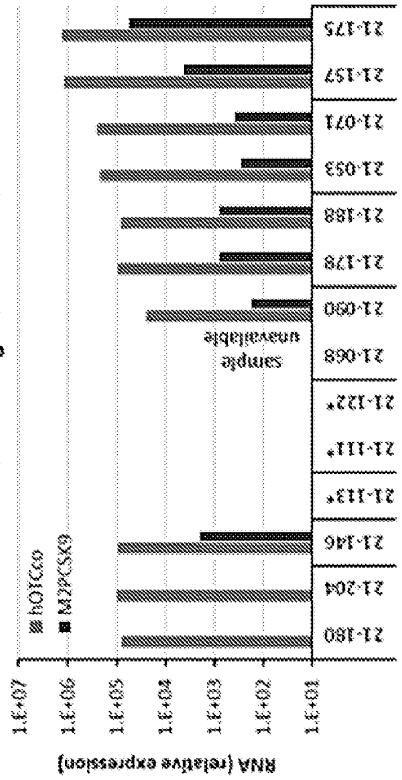


FIG. 11F

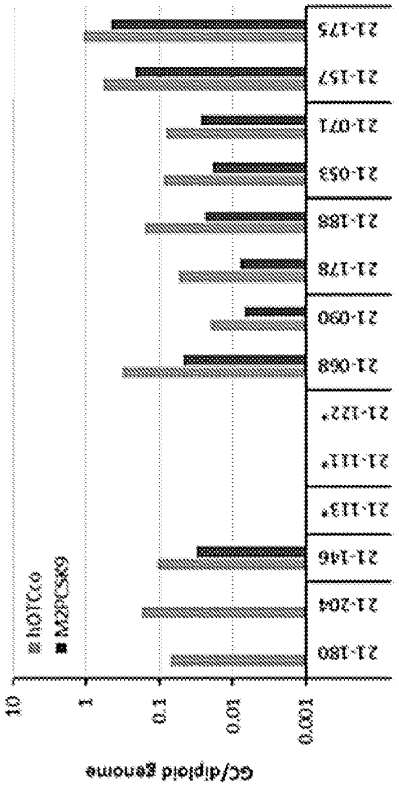


FIG. 11H

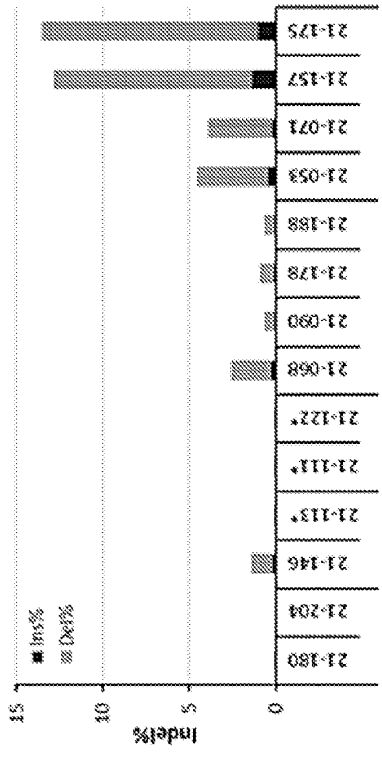


FIG 11I

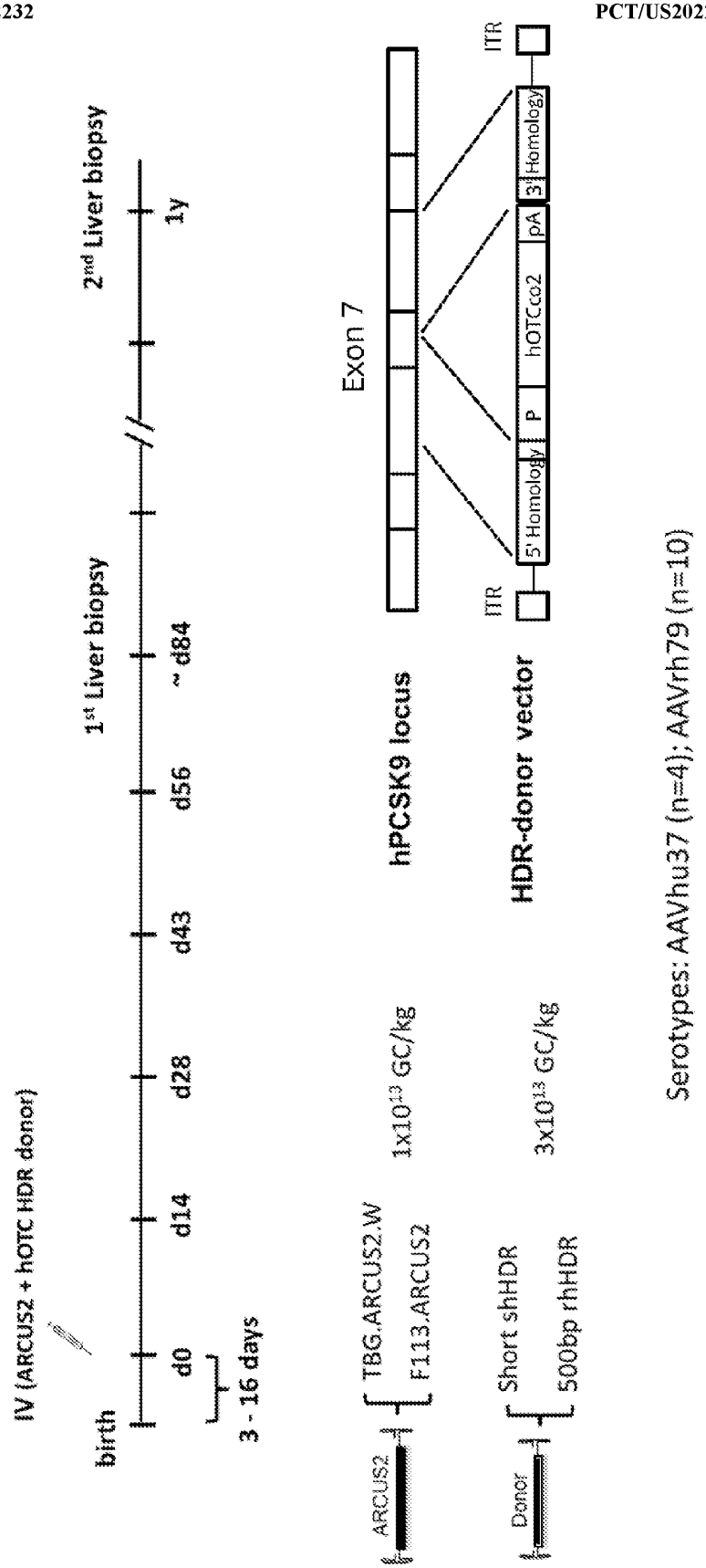

IV (ARCUS2 + hOTC HDR donor)
birth
d0
d14
d28
d43
d56
~ d84
1y
3 - 16 days
1st Liver biopsy
2nd Liver biopsy
ARCUS2
TBG.ARCUS2.W
F113.ARCUS2
1x10^13 GC/kg
Donor
Short shHDR
500bp rhHDR
3x10^13 GC/kg
hPCSK9 locus
HDR-donor vector
Exon 7
ITR
5' Homology
P
hOTCco2
pA
3'
Homology
ITR
Serotypes: AAVhu37 (n=4); AAVrh79 (n=10)

FIG 12

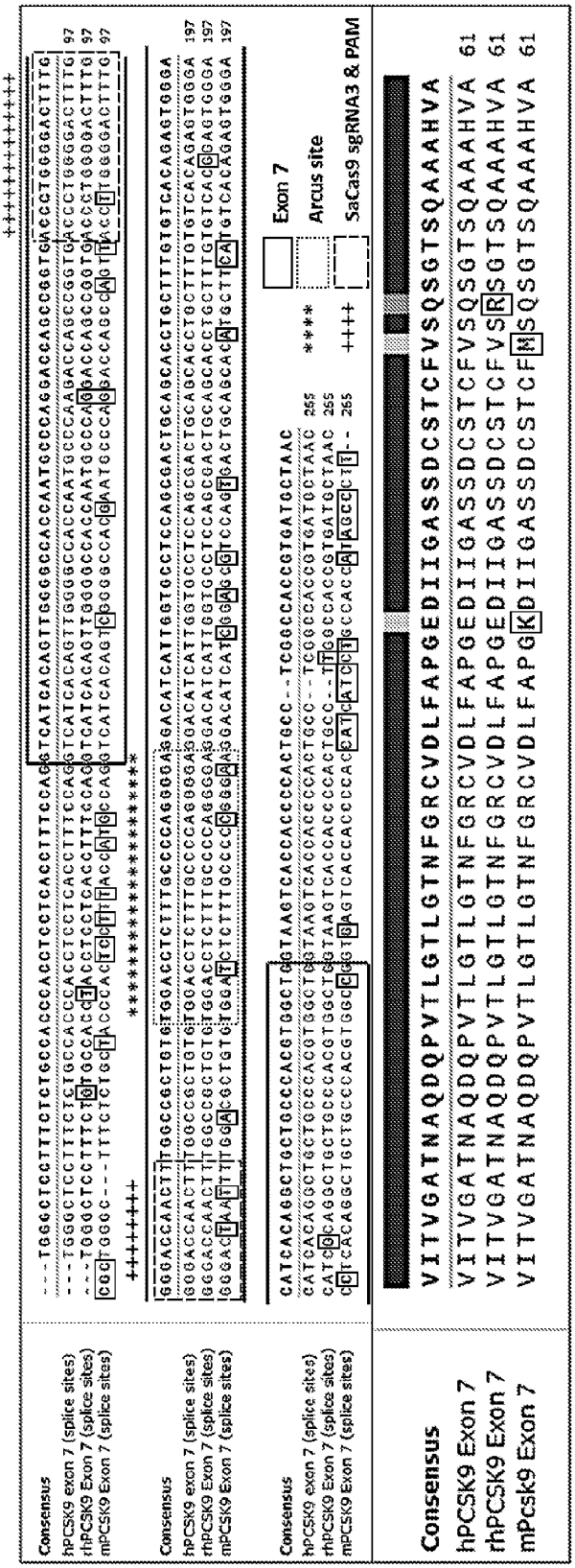

Exon 7
Arcus site
SaCas9 sgRNA3 & PAM
Consensus
hPCSK9 Exon 7
rhPCSK9 Exon 7
mPcsk9 Exon 7
VITVGATNAQDQPVTLGTLGTNFGRCVDLFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVA 61
VITVGATNAQDQPVTLGTLGTNFGRCVDLFAPGEDIIGASSDCSTCFVSRSGTSQAAAHVA 61
VITVGATNAQDQPVTLGTLGTNFGRCVDLFAPGKDIIGASSDCSTCFMSQSGTSQAAAHVA 61

FIG 13

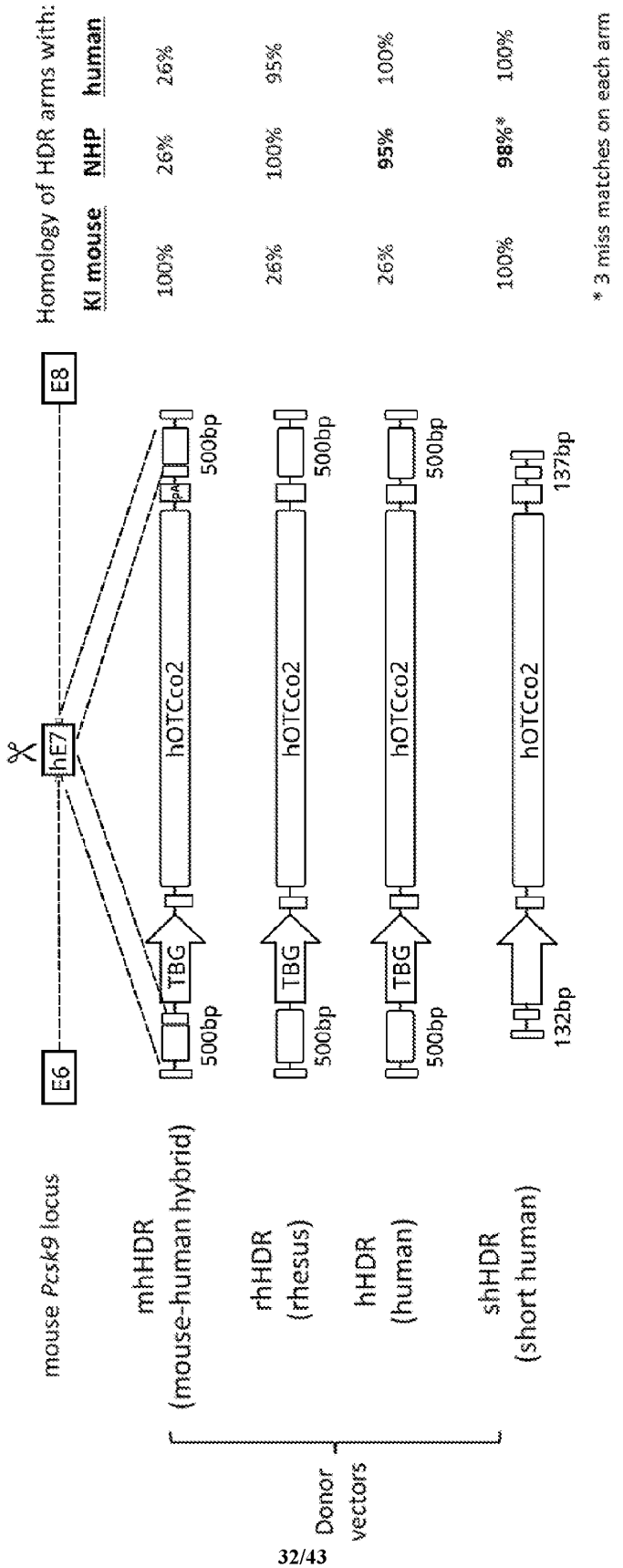

Homology of HDR arms with:
KI mouse
NHP
human
100%
26%
26%
26%
100%
95%
26%
95%
100%
100%
98%*
100%
* 3 miss matches on each arm
mouse Pcsk9 locus
E6
hE7
E8
mhHDR
(mouse-human hybrid)
rhHDR
(rhesus)
hHDR
(human)
shHDR
(short human)
Donor
vectors
TBG
hOTCco2
pA
500bp
500bp
500bp
500bp
132bp
137bp

FIG 14A

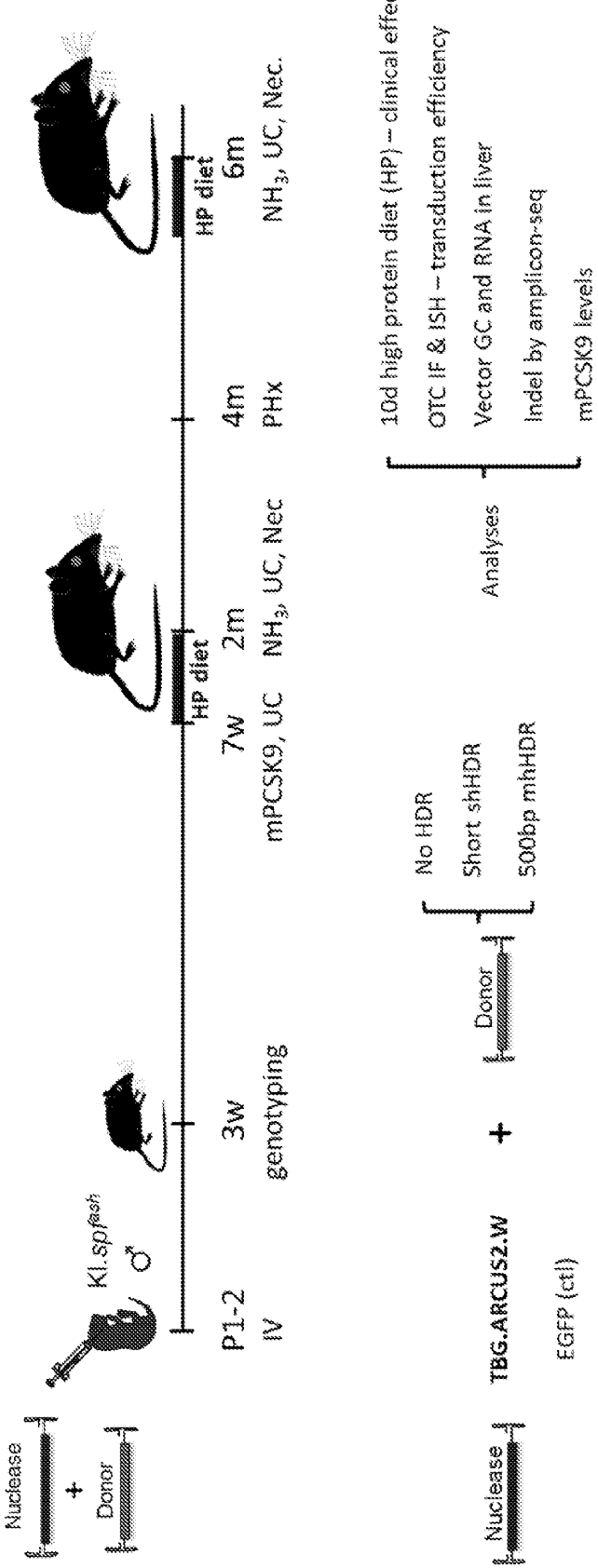

Nuclease
+
Donor
Kl.sp^fish
♂
P1-2
IV
3w
genotyping
7w
mPCSK9, UC
HP diet
2m
NH3, UC, Nec
4m
PHx
HP diet
6m
NH3, UC, Nec.
TBG.ARCUS2.W
+
EGFP (ctl)
Donor
No HDR
Short shHDR
500bp mhHDR
Nuclease
Analyses
10d high protein diet (HP) – clinical effects
OTC IF & ISH – transduction efficiency
Vector GC and RNA in liver
Indel by amplicon-seq
mPCSK9 levels

FIG 14B

Group	N	M2PCSK9 Meganuclease-Expressing AAV Vector (Targeting the *PCSK9* Gene)	Dose (GC/kg)	AAV Donor Vector	Dose (GC/kg)
1	20	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0×10^{13}	AAVrh79.mhHDR.TBG.hOTCco.bGH	3.0×10^{13}
2	20	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0×10^{13}	AAVrh79.shHDR.TBG.hOTCco.bGH	3.0×10^{13}
3	20	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0×10^{13}	AAVrh79.TBG.hOTCco.bGH	3.0×10^{13}
4	20	AAVrh79.TBG.PI.EGFP.WPRE.bGH (negative control)	1.0×10^{13}	AAVrh79.mhHDR.TBG.hOTCco.bGH	3.0×10^{13}
5	20	NA	NA	NA	NA

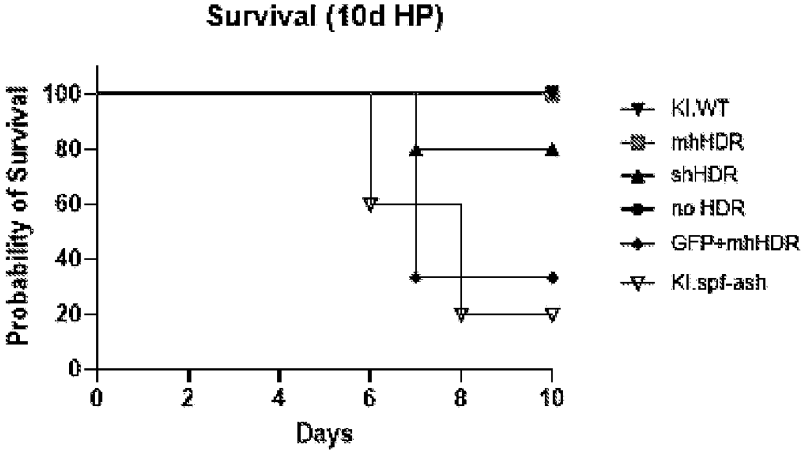


FIG 14C

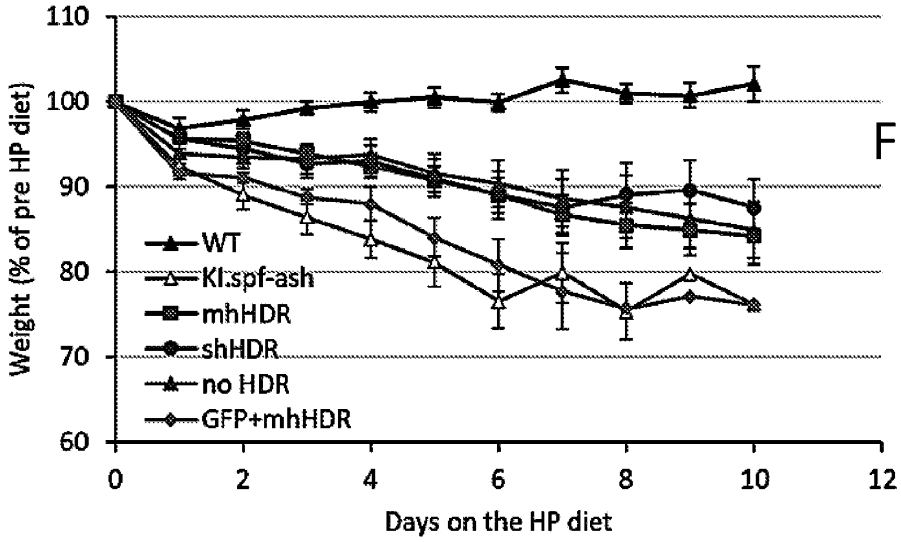


FIG 14D

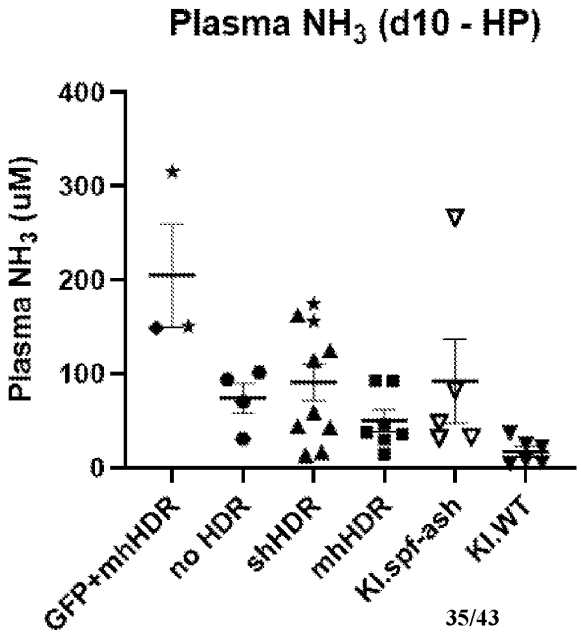


FIG 14E

FIG. 14H

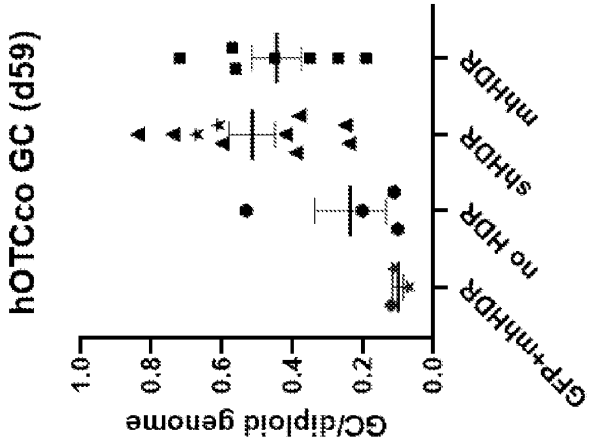


FIG 14G

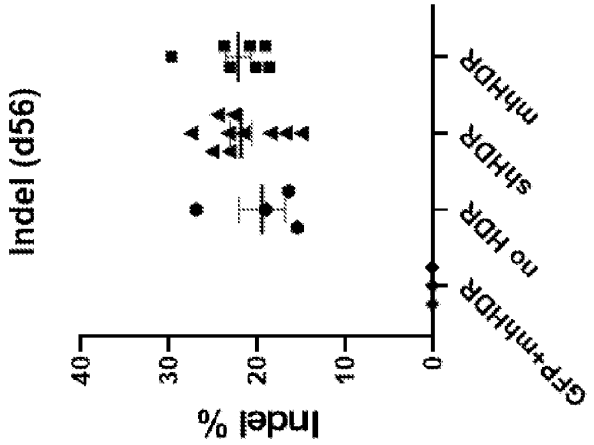


FIG 14F

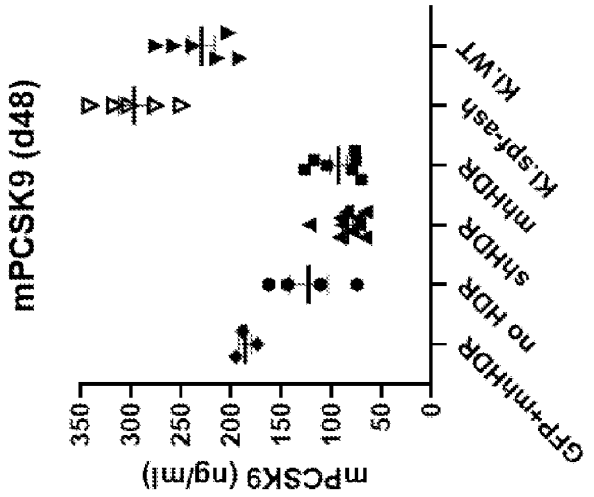


FIG 14I

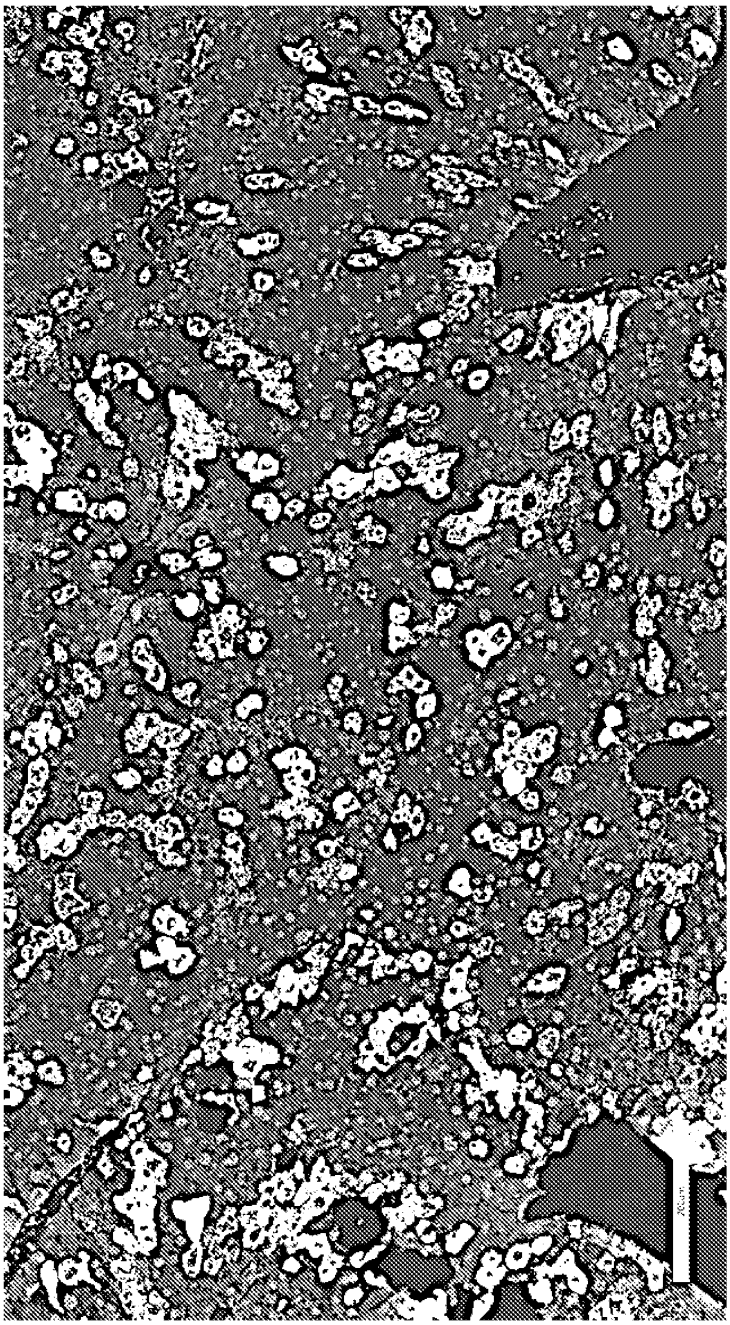


FIG 15

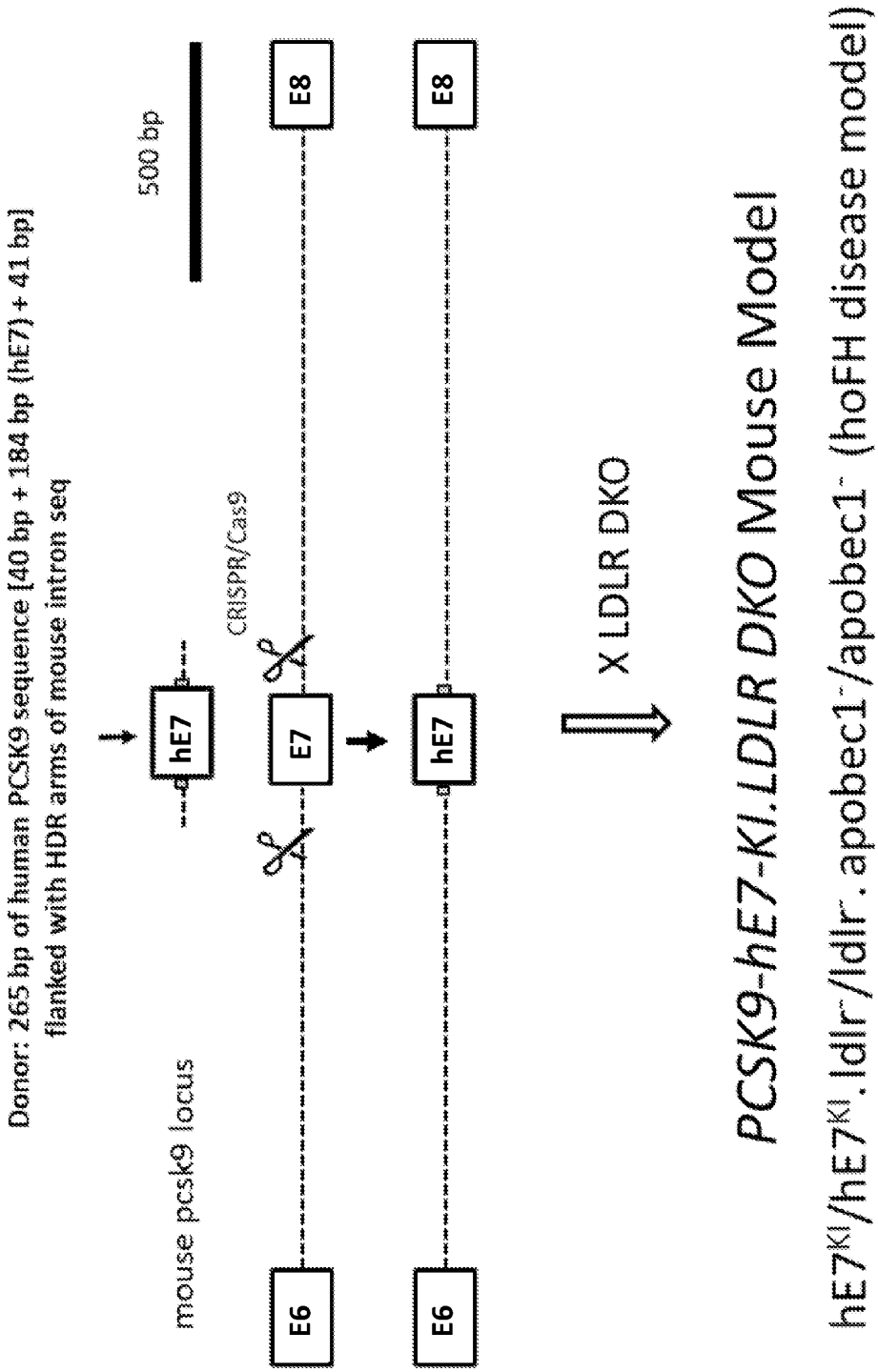

Donor: 265 bp of human PCSK9 sequence [40 bp + 184 bp (hE7) + 41 bp]
flanked with HDR arms of mouse intron seq
500 bp
mouse pcsk9 locus
hE7
CRISPR/Cas9
E7
E8
E6
hE7
E8
E6
X LDLR DKO
PCSK9-hE7-KI.LDLR DKO Mouse Model
hE7KI/hE7KI.ldlr-/ldlr-.apobec1-/apobec1- (hoFH disease model)

FIG. 16

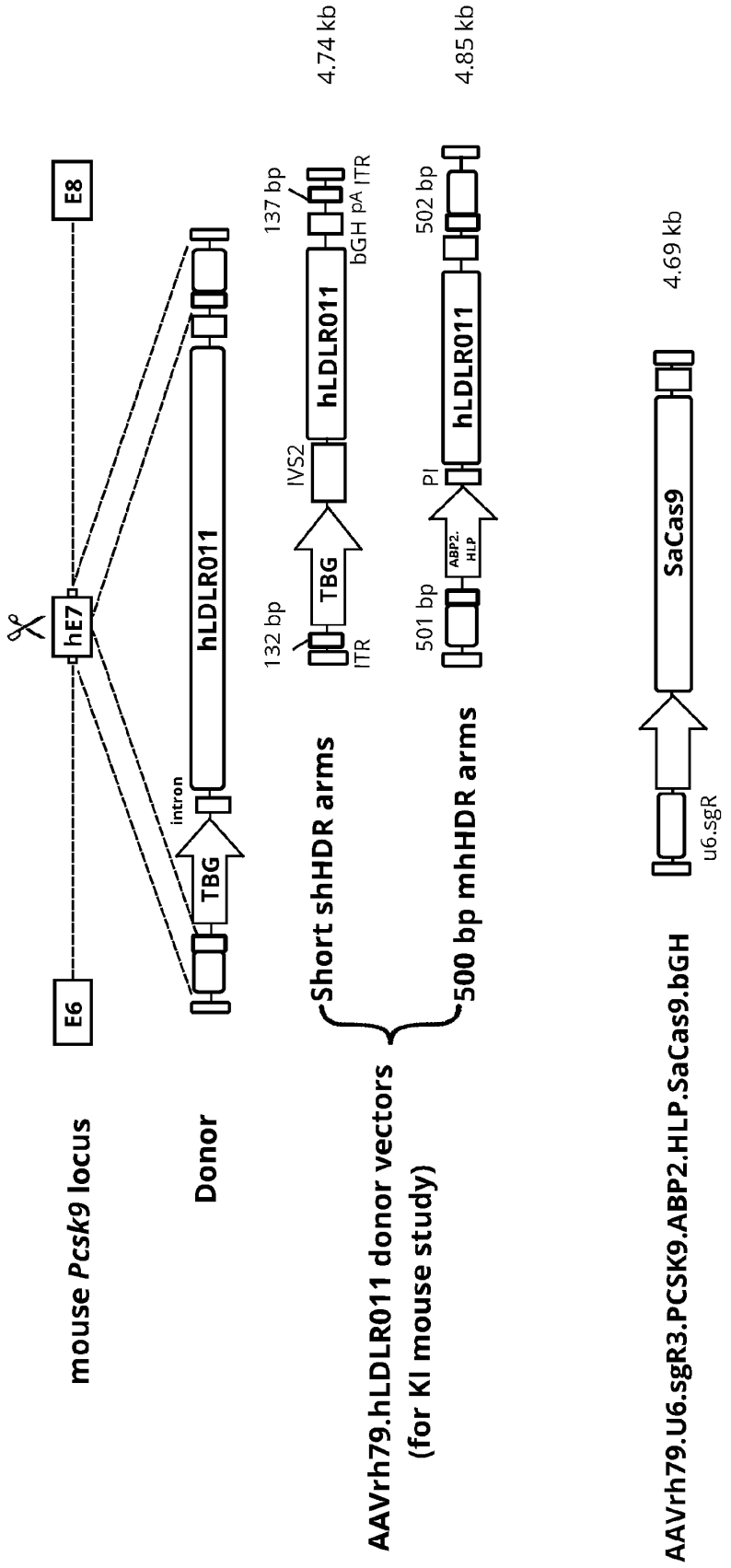
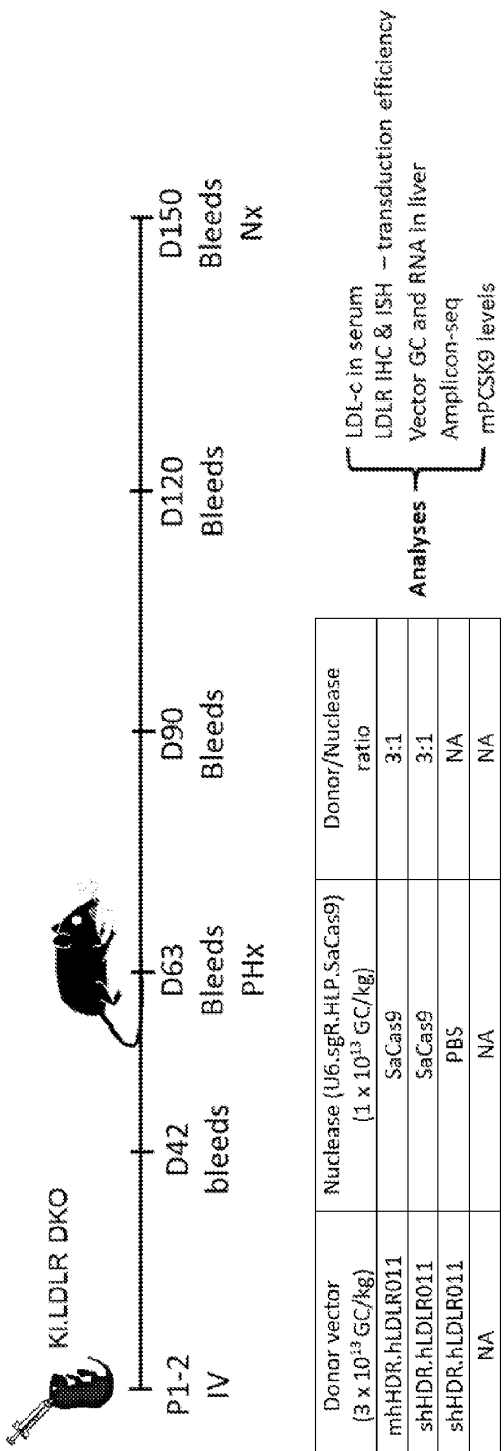

mouse Pcsk9 locus
E6
hE7
E8
Donor
TBG
intron
hLDLR011
AAVrh79.hLDLR011 donor vectors
(for KI mouse study)
Short shHDR arms
132 bp
ITR
TBG
IVS2
hLDLR011
137 bp
bGH pA
ITR
4.74 kb
500 bp mhHDR arms
501 bp
ABP2.
HLP
PI
hLDLR011
502 bp
4.85 kb
AAVrh79.U6.sgR3.PCSK9.ABP2.HLP.SaCas9.bGH
u6.sgR
SaCas9
4.69 kb

FIG 17



Group	Donor vector (3×10^{13} GC/kg)	Nuclease (U6.sgR.H1P.SaCas9) (1×10^{13} GC/kg)	Donor/Nuclease ratio
1	mhHDR.hLDLR011	SaCas9	3:1
2	shHDR.hLDLR011	SaCas9	3:1
3	shHDR.hLDLR011	PBS	NA
4	NA	NA	NA

FIG. 18A

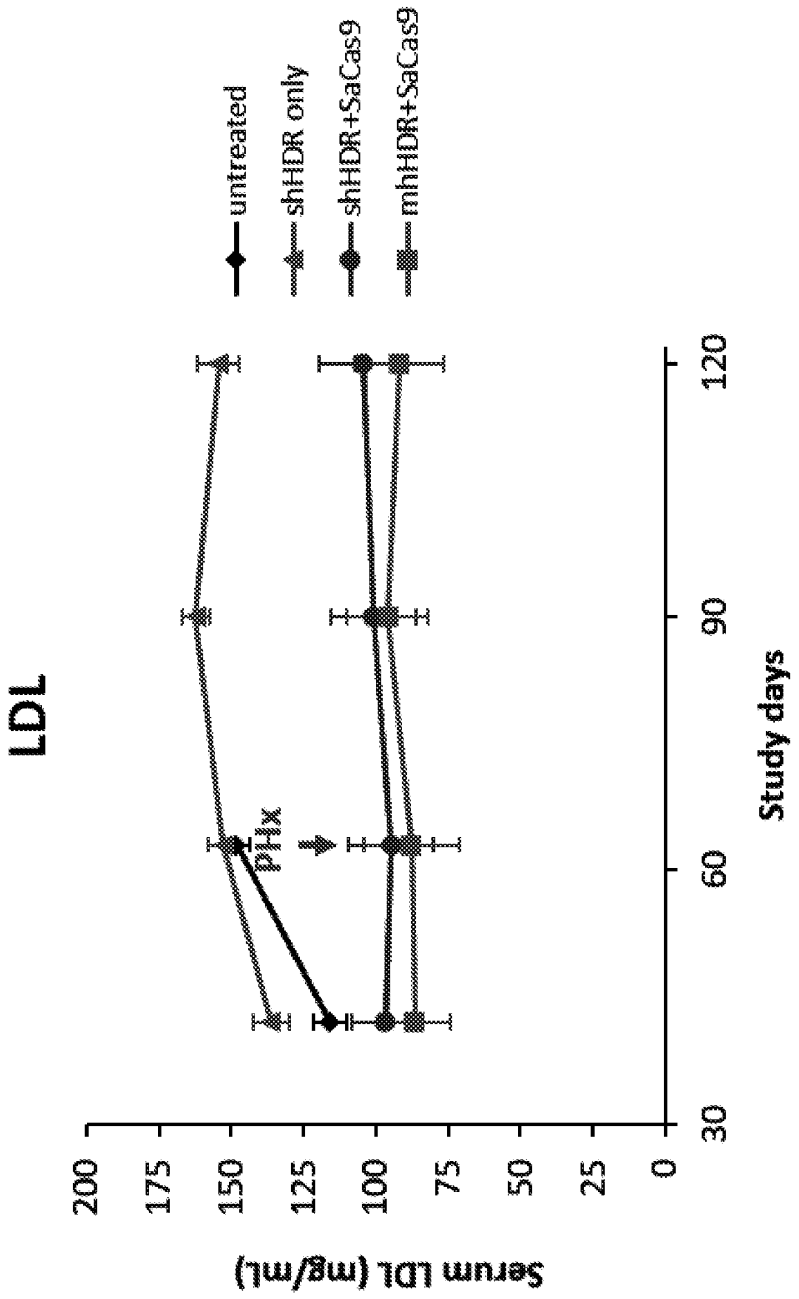

LDL
untreated
shHDR only
shHDR+SaCas9
mhHDR+SaCas9
pHx
Serum LDL (mg/mL)
200
175
150
125
100
75
50
25
0
30
60
90
120
Study days

FIG. 18D

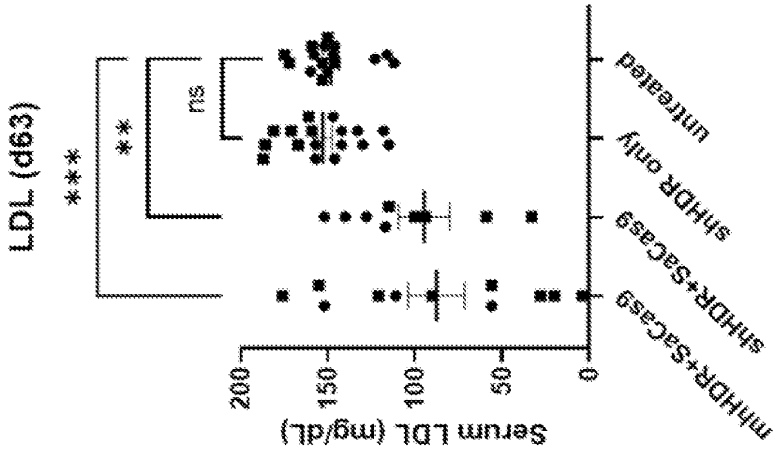


FIG. 18C

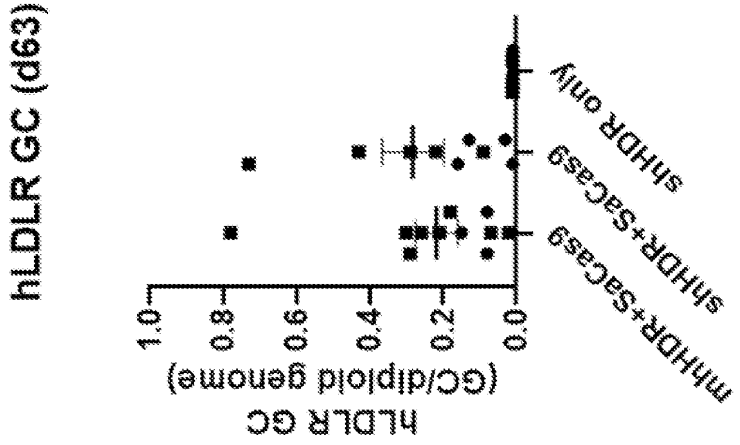


FIG. 18B

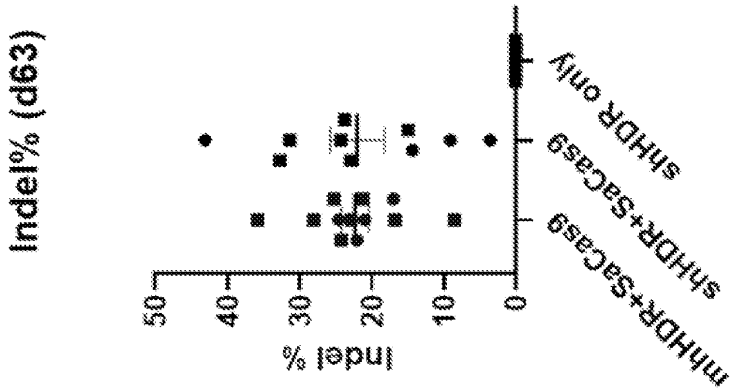
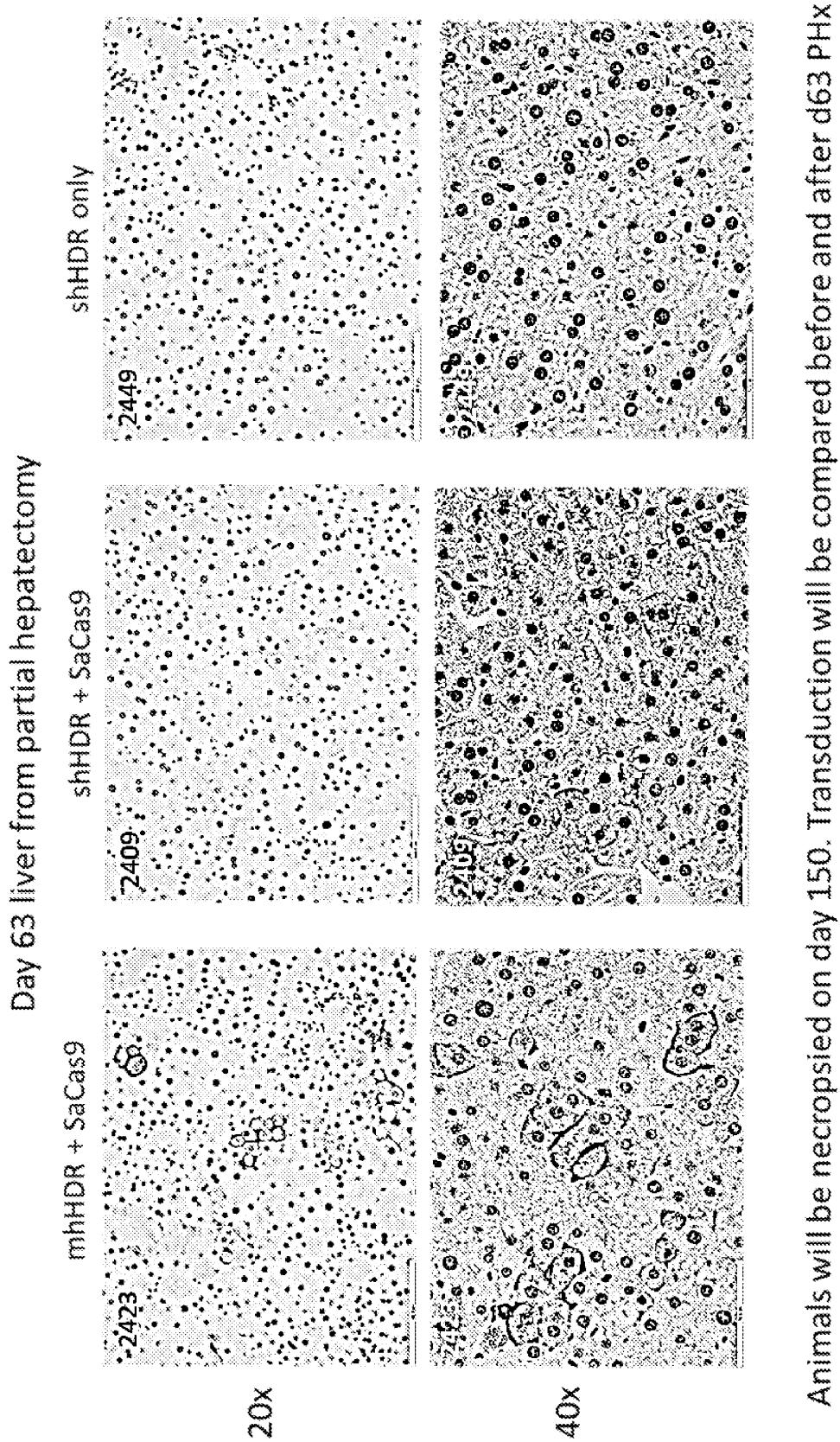


FIG. 19


Day 63 liver from partial hepatectomy
mhHDR + SaCas9
shHDR + SaCas9
shHDR only
2423
2409
2449
20x
40x
Animals will be necropsied on day 150. Transduction will be compared before and after d63 PHx