

FIG 1A

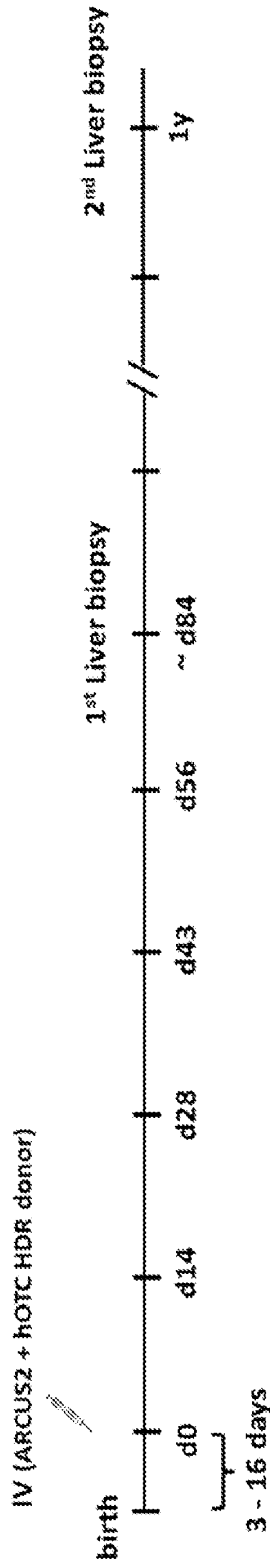
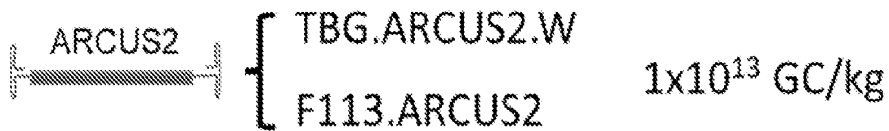
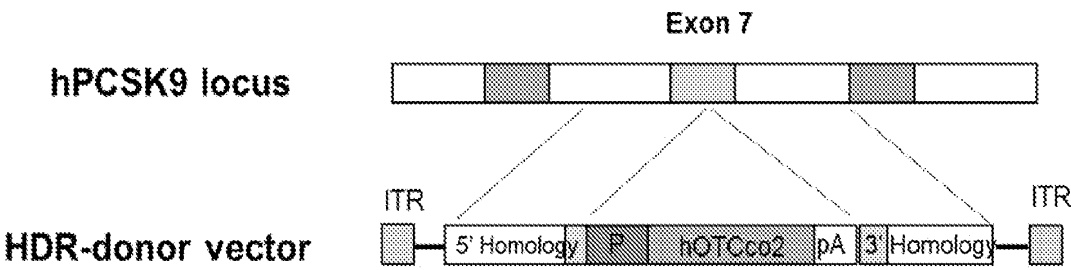


FIG 1B

Group	AAV Nuclease Targeting the PCSK9 Gene	Dose (GC/kg)	AAV Containing the hOTC Donor Transgene	Dose (GC/kg)	N
1	N/A	N/A	AAVrh79.rhHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
2	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0 x 10 ¹³	AAVrh79.rhHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
3	AAVrh79.TBG-S1-F113.M2PCSK9.bGH	1.0 x 10 ¹³	AAVrh79.rhHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
4	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0 x 10 ¹³	AAVrh79.shHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
5	AAVrh79.TBG-S1-F113.M2PCSK9.bGH	1.0 x 10 ¹³	AAVrh79.shHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
6	AAVrh79.TBG.PI.M2PCSK9.WPRE.bGH	1.0 x 10 ¹³	AAVrh79.rhHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2
7	AAVrh79.TBG-S1-F113.M2PCSK9.bGH	1.0 x 10 ¹³	AAVrh79.rhHDR.TBG.hOTCco.bGH	3.0 x 10 ¹³	2

Abbreviations: AAV, adeno-associated virus; GC, genome copies; hOTC, human ornithine transcarbamylase; IV, intravenous; N/A, not applicable; N, number of animals; PCSK9, proprotein convertase subtilisin/kexin type 9 (gene).

FIG 2



1x10¹³ GC/kg

3x10¹³ GC/kg

Total N = 14

Serotypes: AAVhu37 (n=4); AAVrh79 (n=8+2 ctl)

FIG 3

HDR Arms	Promoter (nuclease vector)	Serotype	Group	Animal ID	Sex	Age at dosing (day)	Anti- AAV BAB	Biopsy (day)	Transduction %		Amplicon-seq Indel%	ITR-seq # of OT sites
									OTC ISH	OTC IF		
Long	-	AAVrh79	G1	21-180	M	13	50	85	0.1	0.6	0.09	3
				21-204	F	1	50	84	1	0.7	0.06	2
Short	F113	AAVrh79	G5	21-146	F	7	50	84	2.3	1.3	1.5	2
				21-113 ^a	F	15	1600	86	0	0	0.1	N.T.
	TBG	AAVrh79	G4	21-111 ^a	F	16	400	85	0	0	0.1	N.T.
				21-122 ^a	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	4
				21-090	M	6	25	86	0.9	1.1	0.7	3
		AAVrh79	G7	21-178	M	10	N.T. ^b	85	1.9	2.2	0.9	2
				21-188	M	3	25	93	1.6	2.6	0.7	6
	TBG	AAVhu37	G2	21-053	F	9	100	86	4.5	4	4.5	5
				21-071	M	9	50	84	3.7	2.9	3.9	5
		AAVrh79	G6	21-157	F	6	25	84	16.9	18.6	12.8	27
				21-175	F	11	<25	84	10.3	11.9	13.5	40

^aNewborns with Day 0 samples tested highly positive in the anti-AAV binding antibody (BAB) assay. Animals were euthanized on Day 126–128.

^bDay 0 sample of 21-178 was collected post vector dosing which would interfere with the BAB assay.

Abbreviations: AAV, adeno-associated virus; BAB, binding antibody; F, female; HDR, homology dependent recombination; IF, immunofluorescence; ISH, *in situ* hybridization; ITR, inverted terminal repeat; M, male; N.T., not tested; OT, off-target; OTC, ornithine transcarbamylase.

FIG 4A

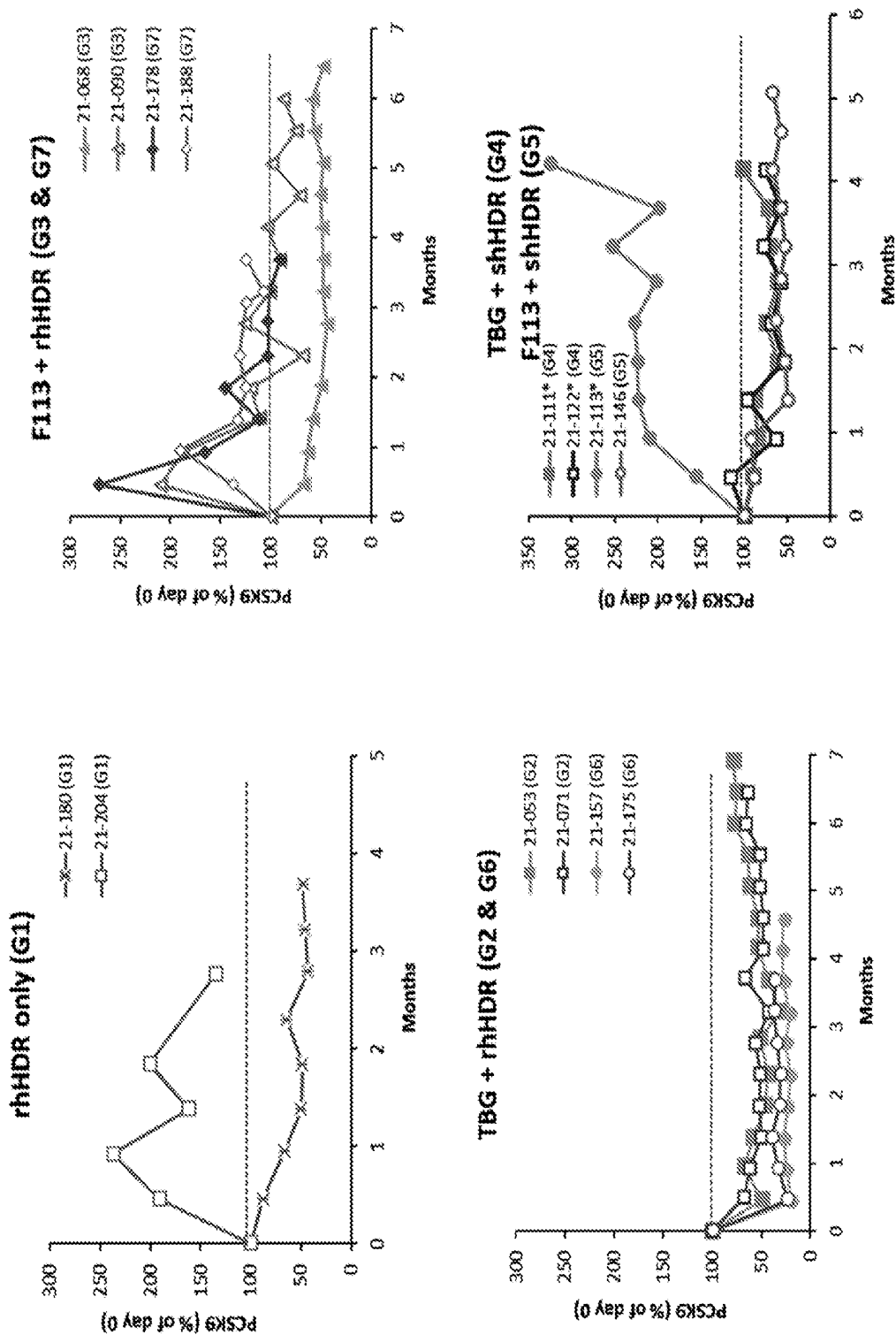


FIG 4B

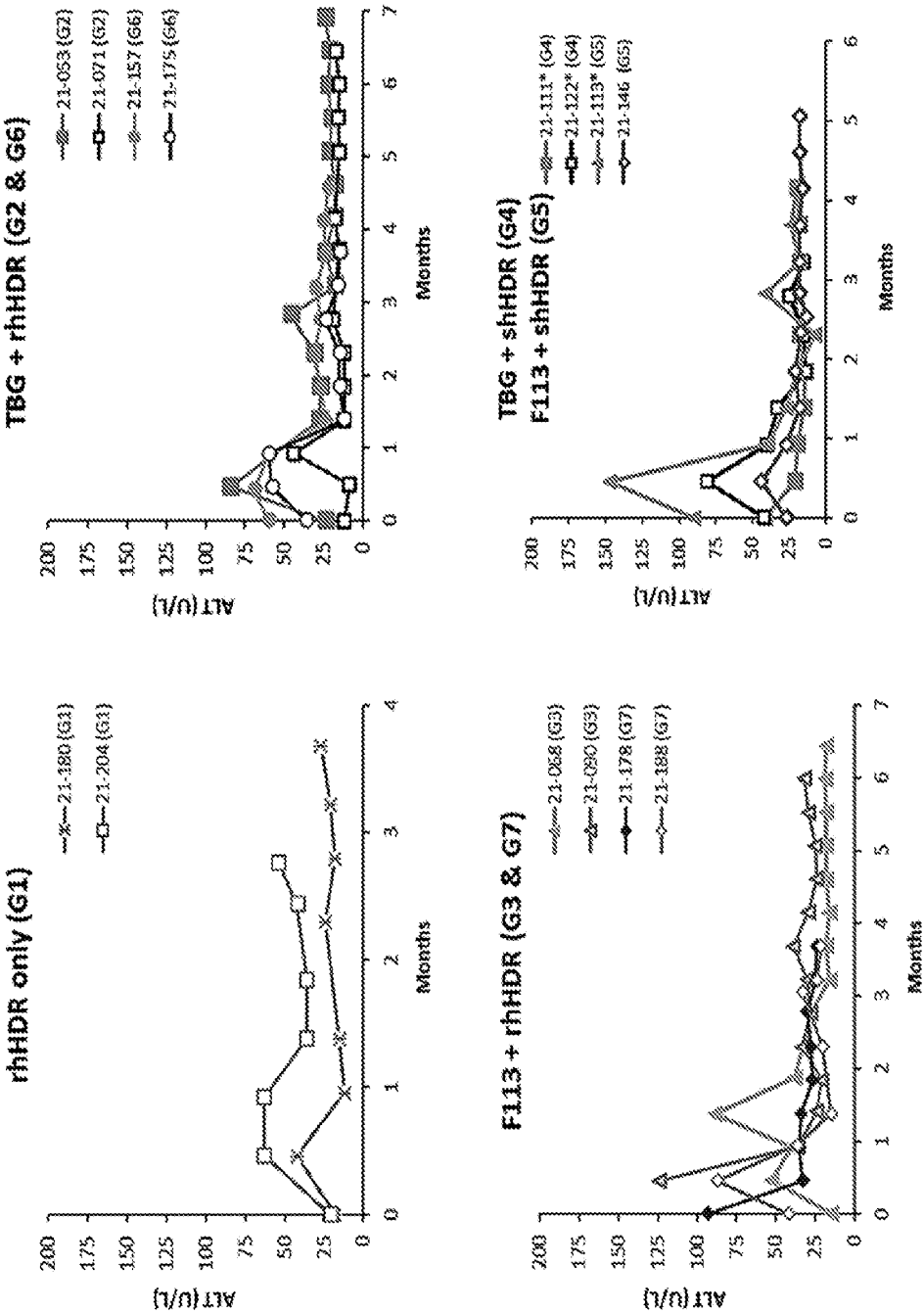


FIG 4C

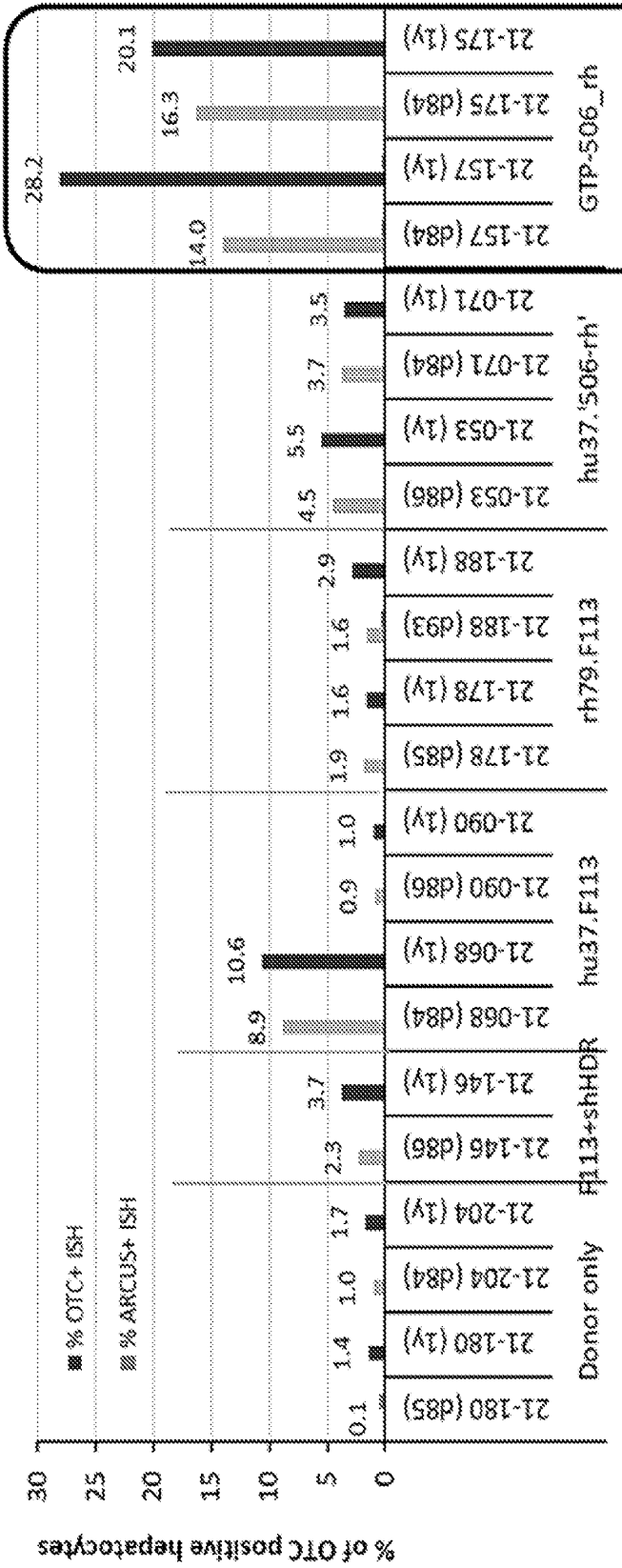


FIG 4D

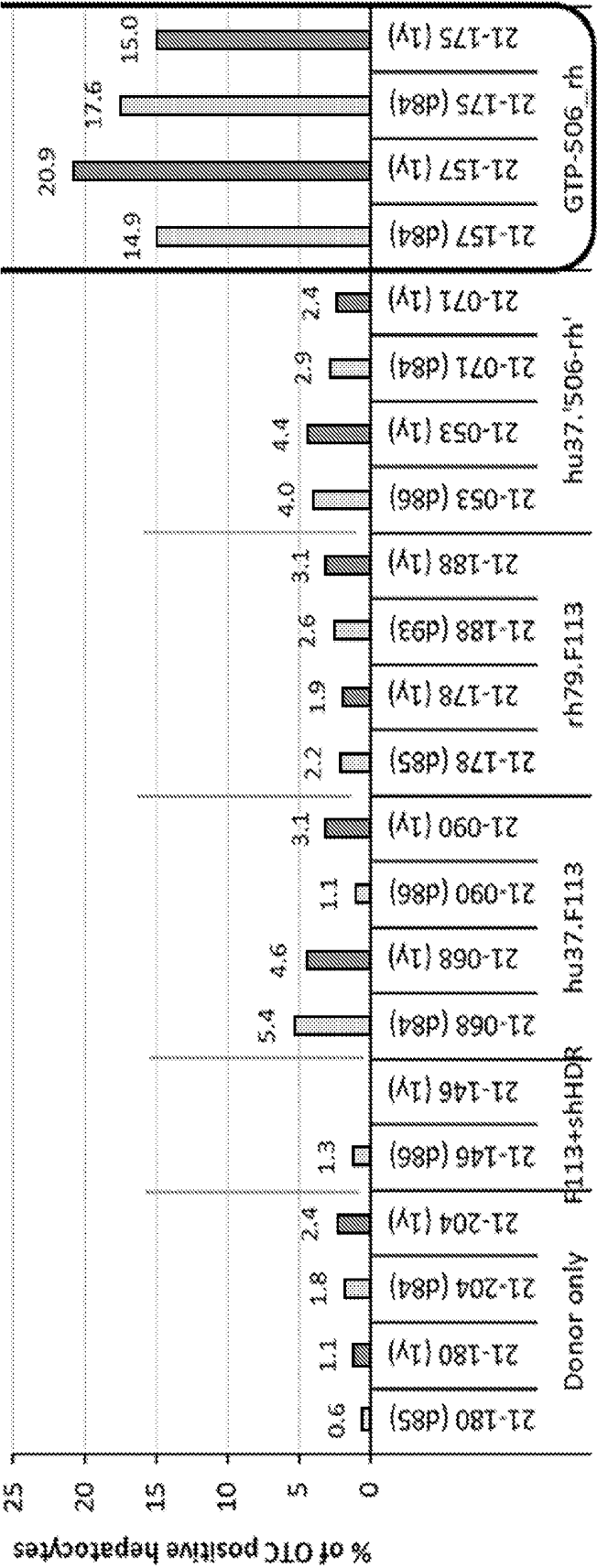


FIG 4E

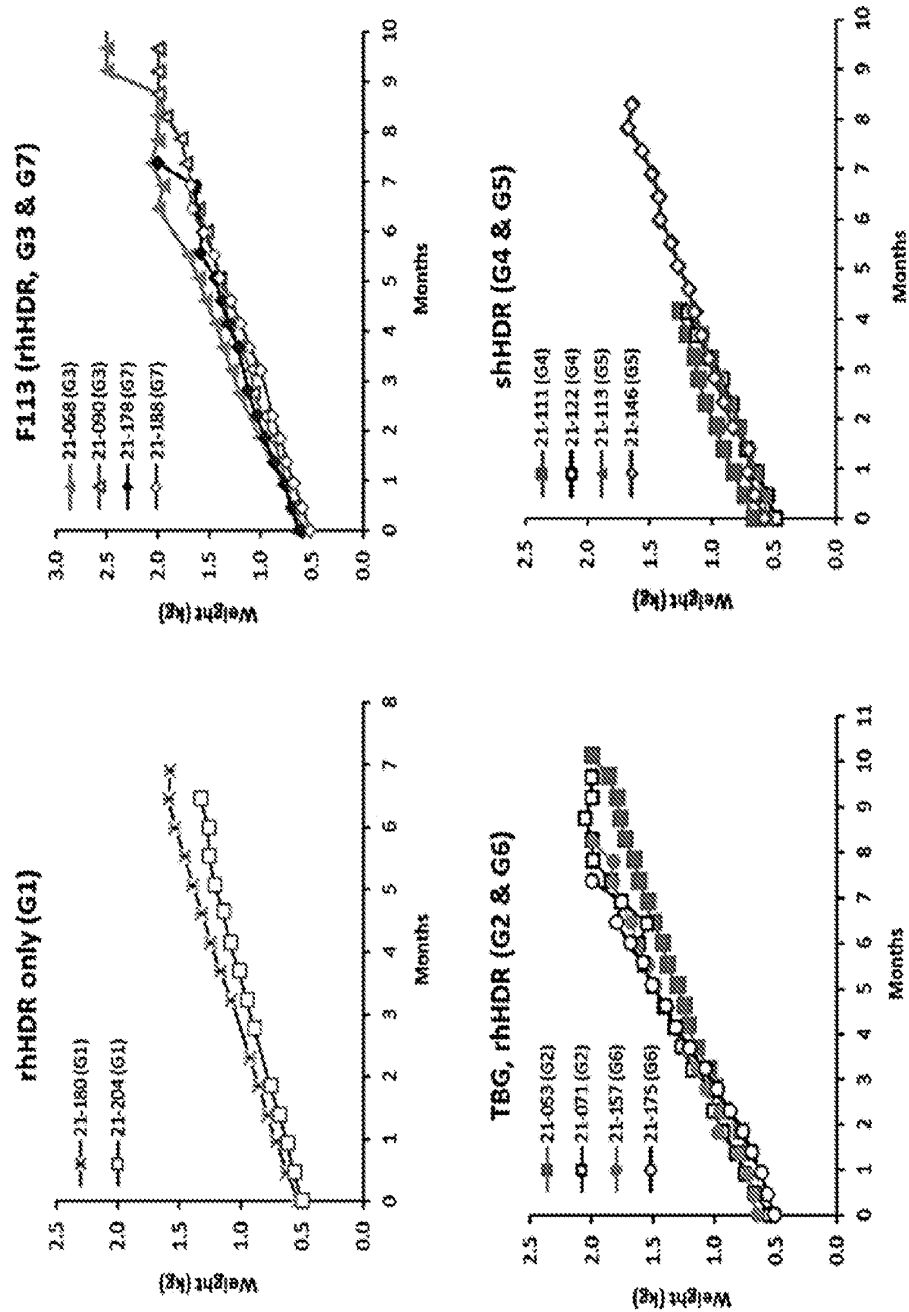


FIG. 4F

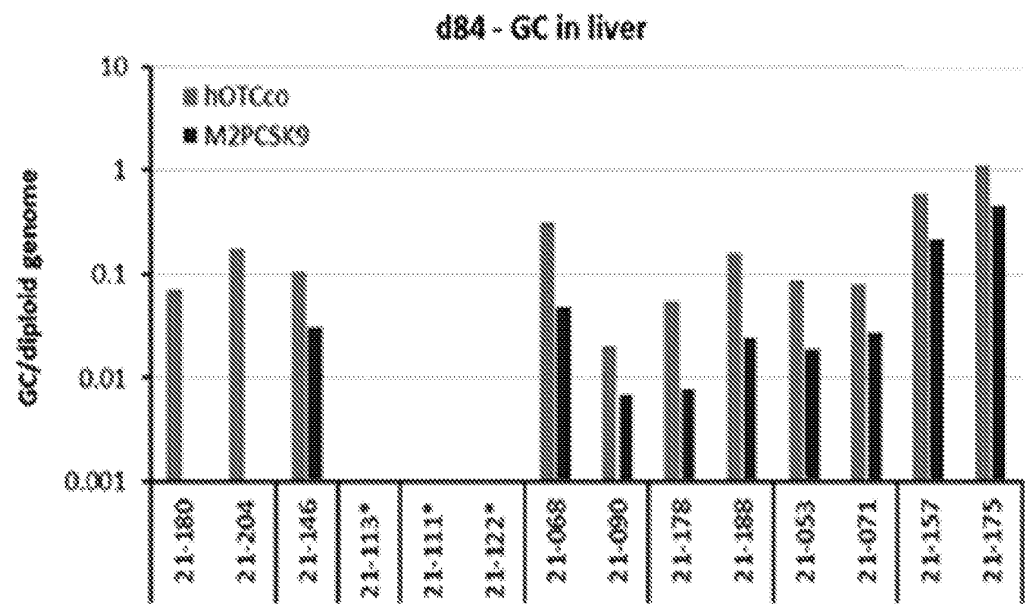


FIG. 4G

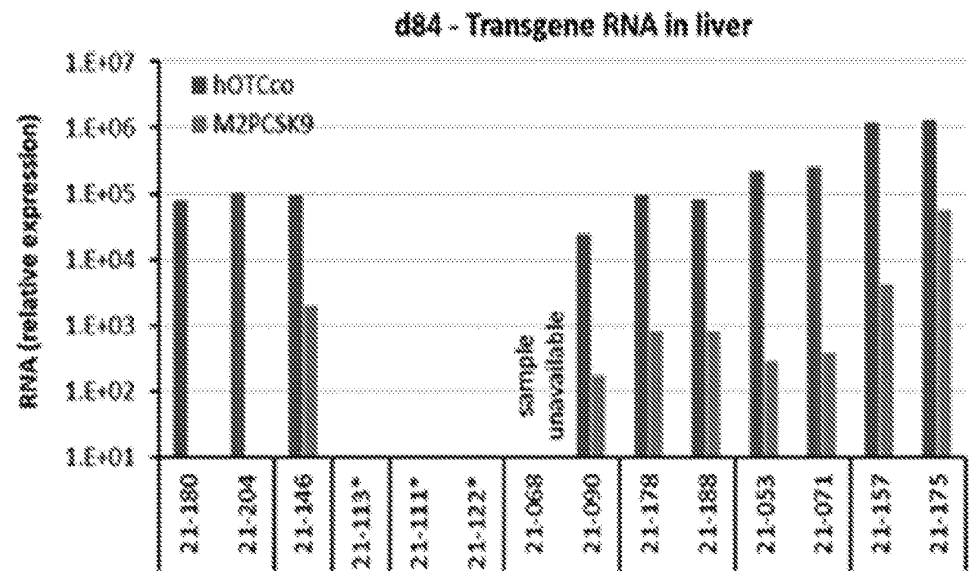


FIG. 4H

On-target Indel by amplicon-seq

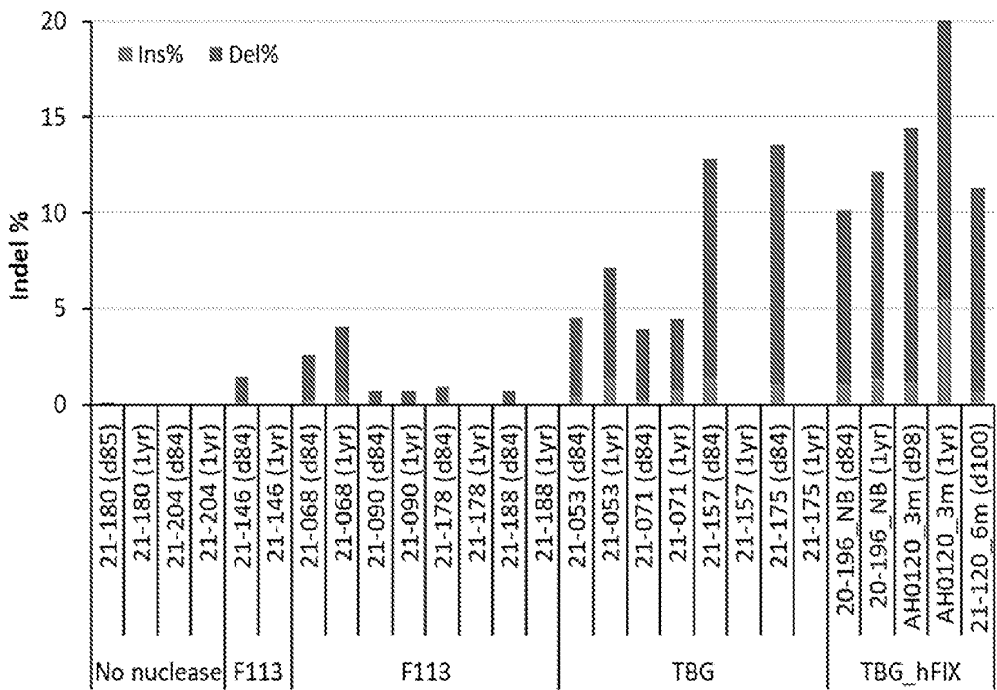


FIG. 4I

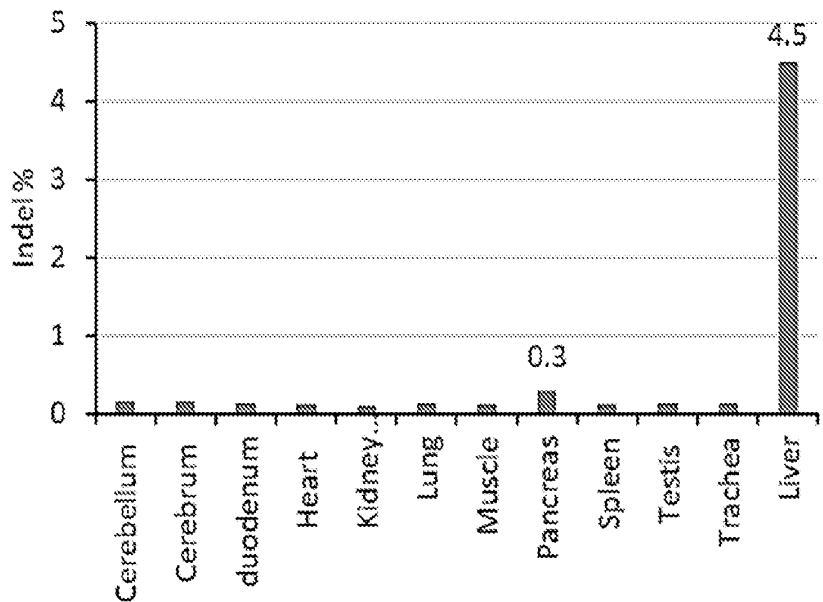


FIG 4J

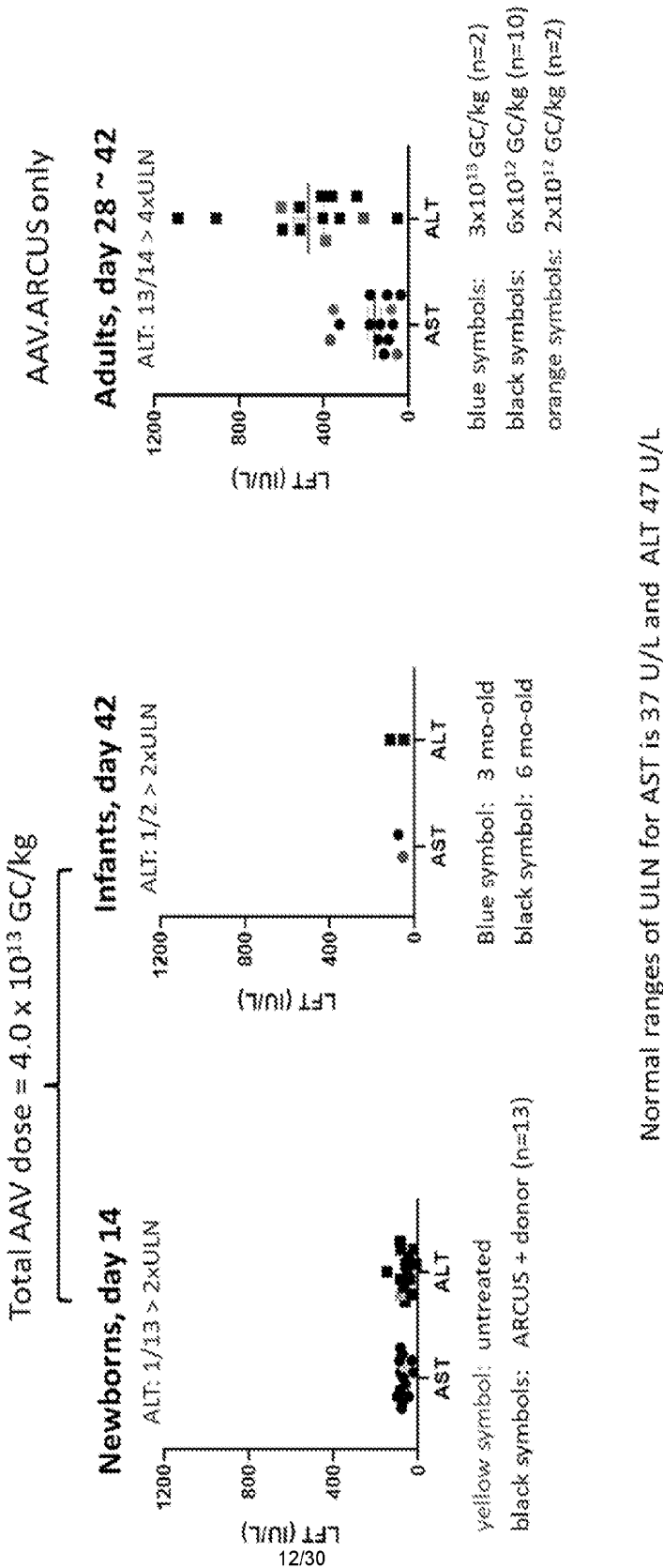


FIG 4K

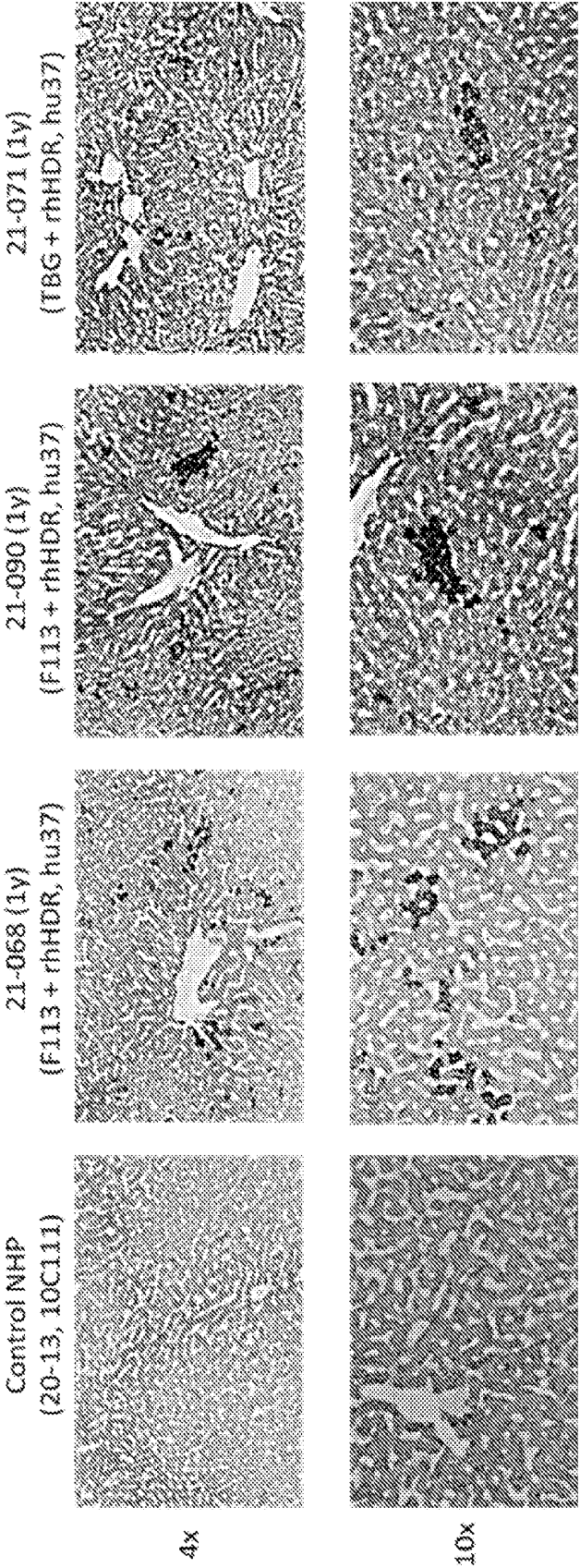


FIG 5A

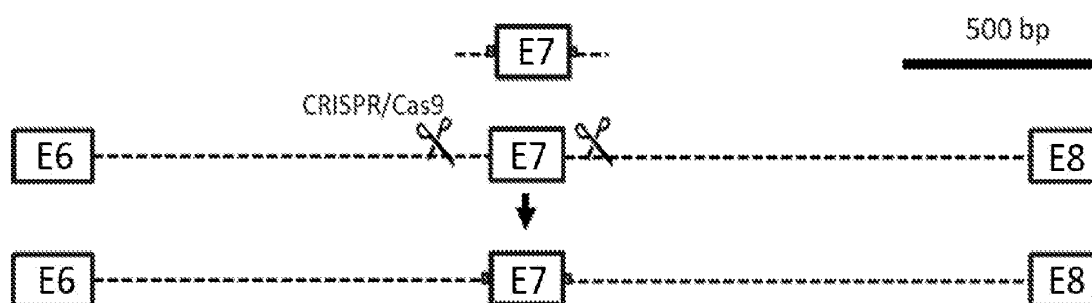


FIG 5B

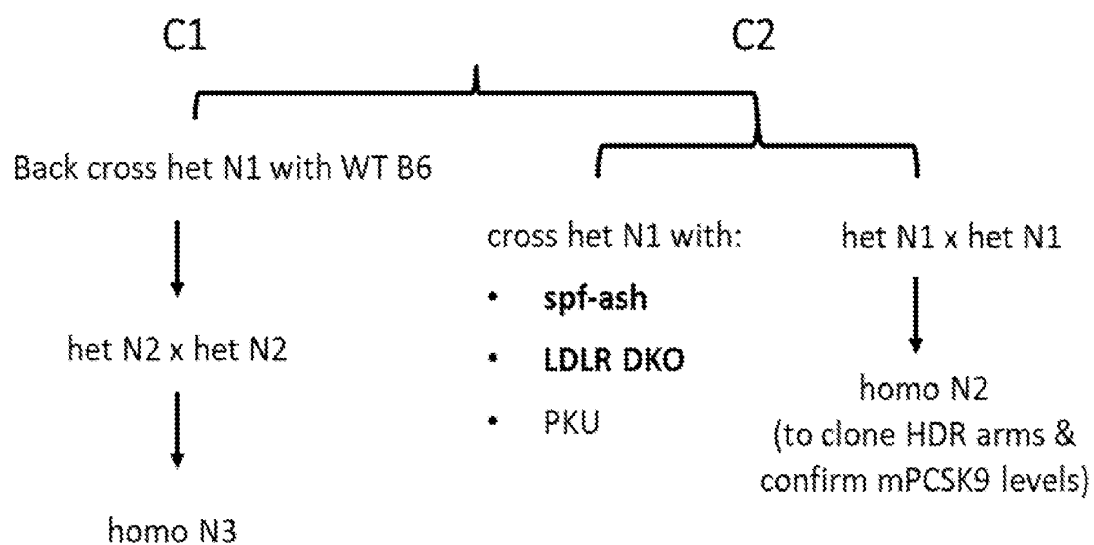


FIG 5C

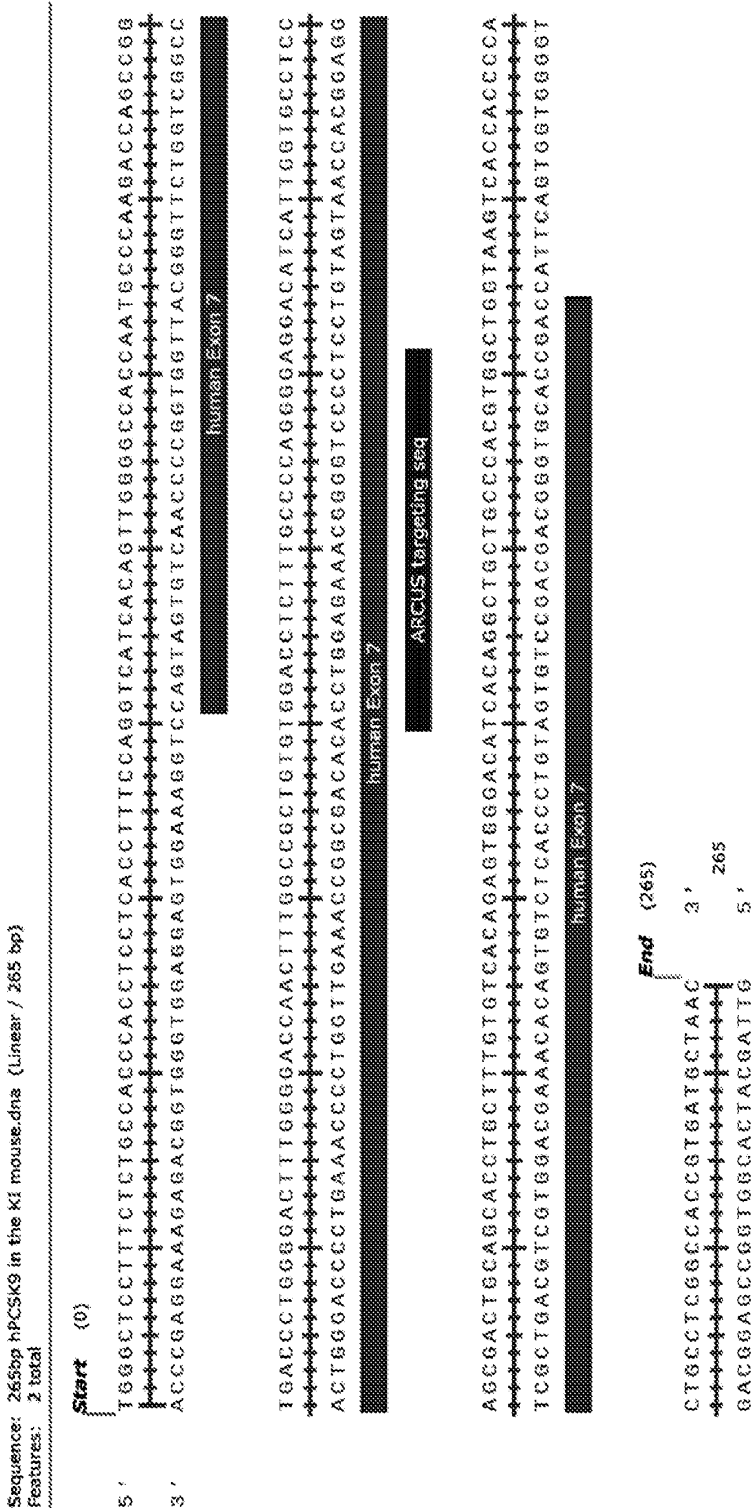


FIG 6

[illegible]

FIG 7

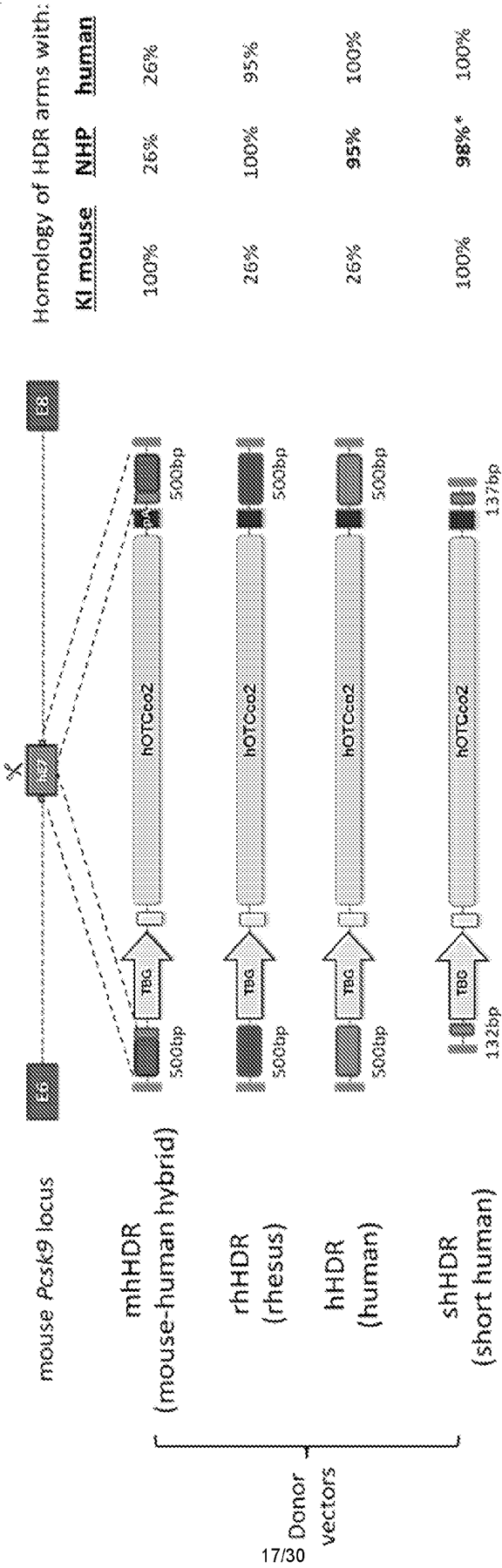


FIG 8A

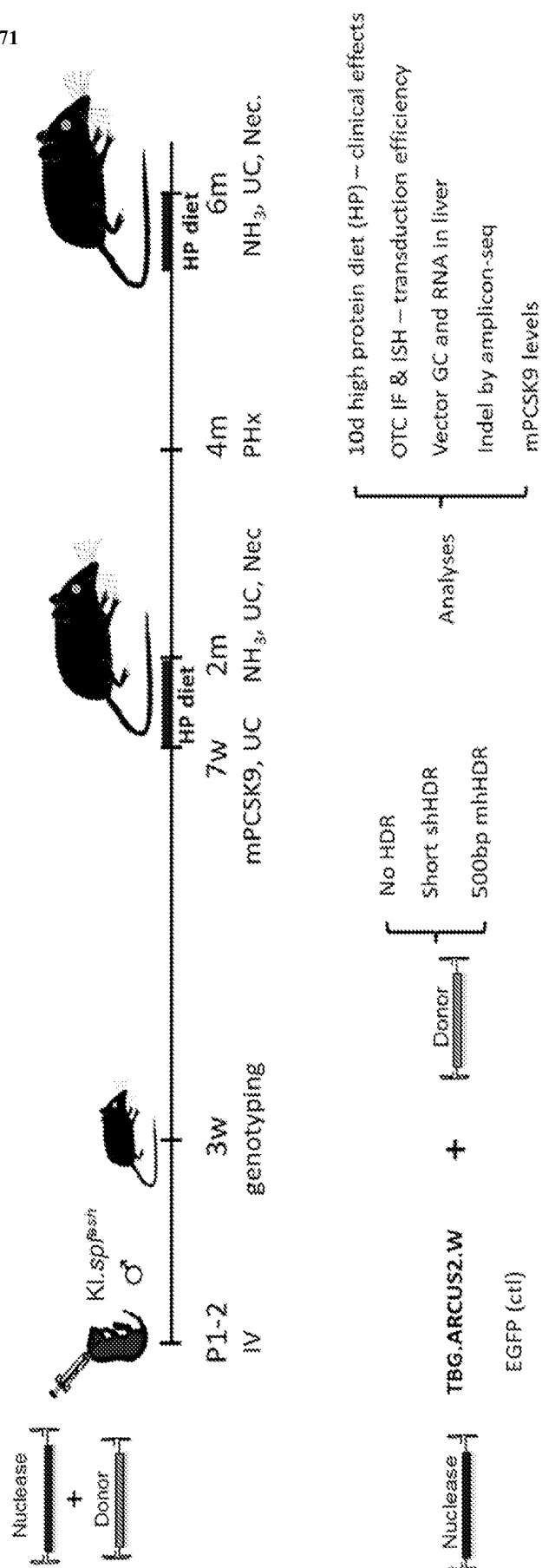


FIG 8B

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

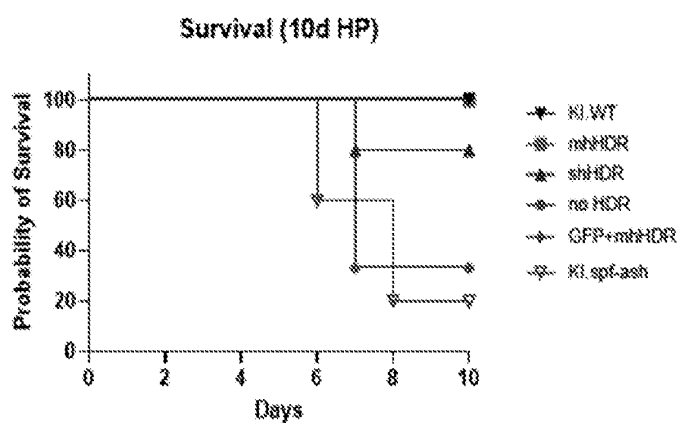


FIG 9A

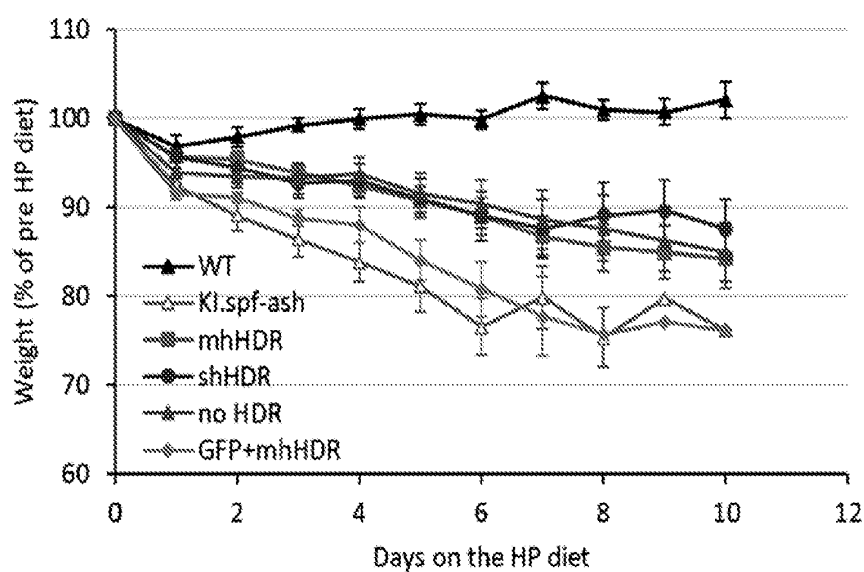


FIG 9B

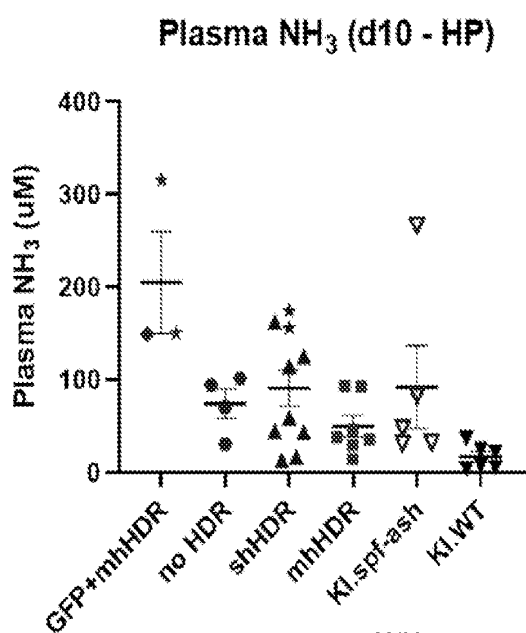


FIG 9C

FIG 9F

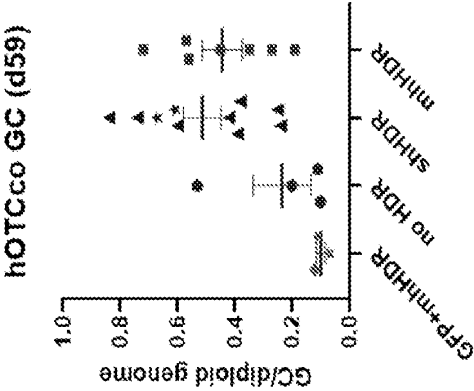


FIG 9E

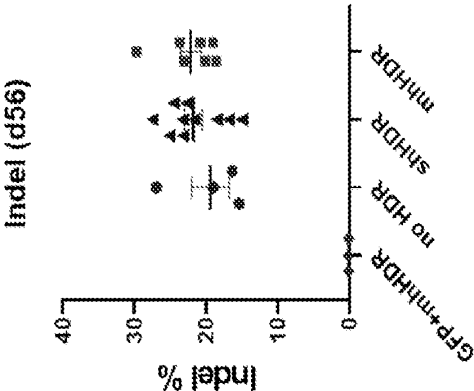


FIG 9D

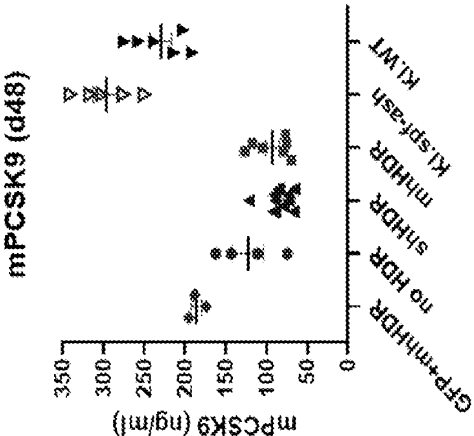


FIG 10

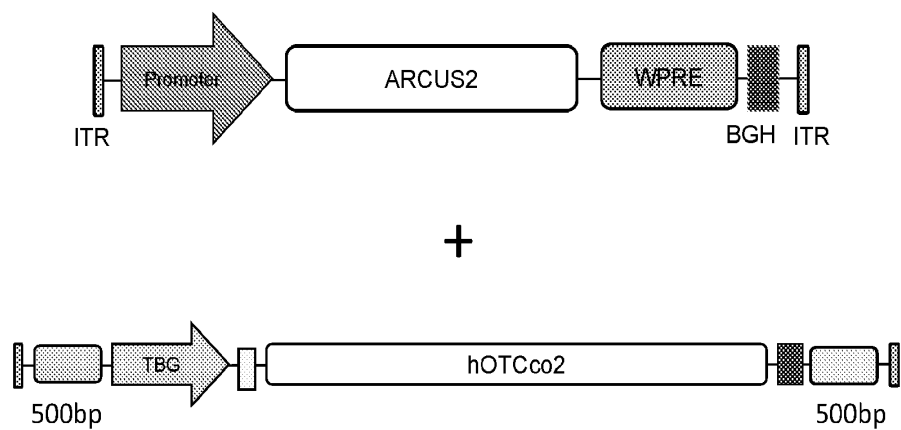


FIG 11

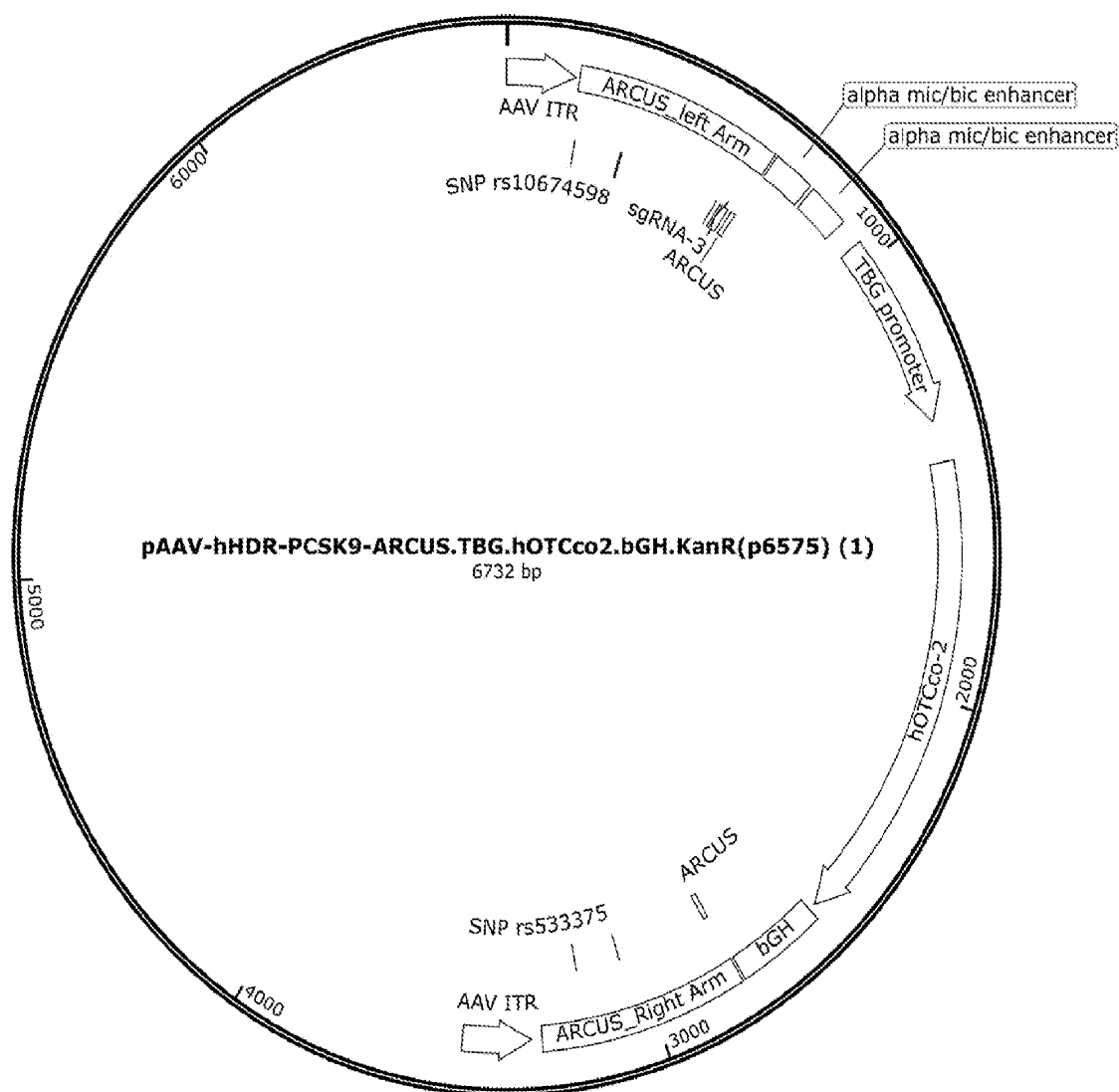
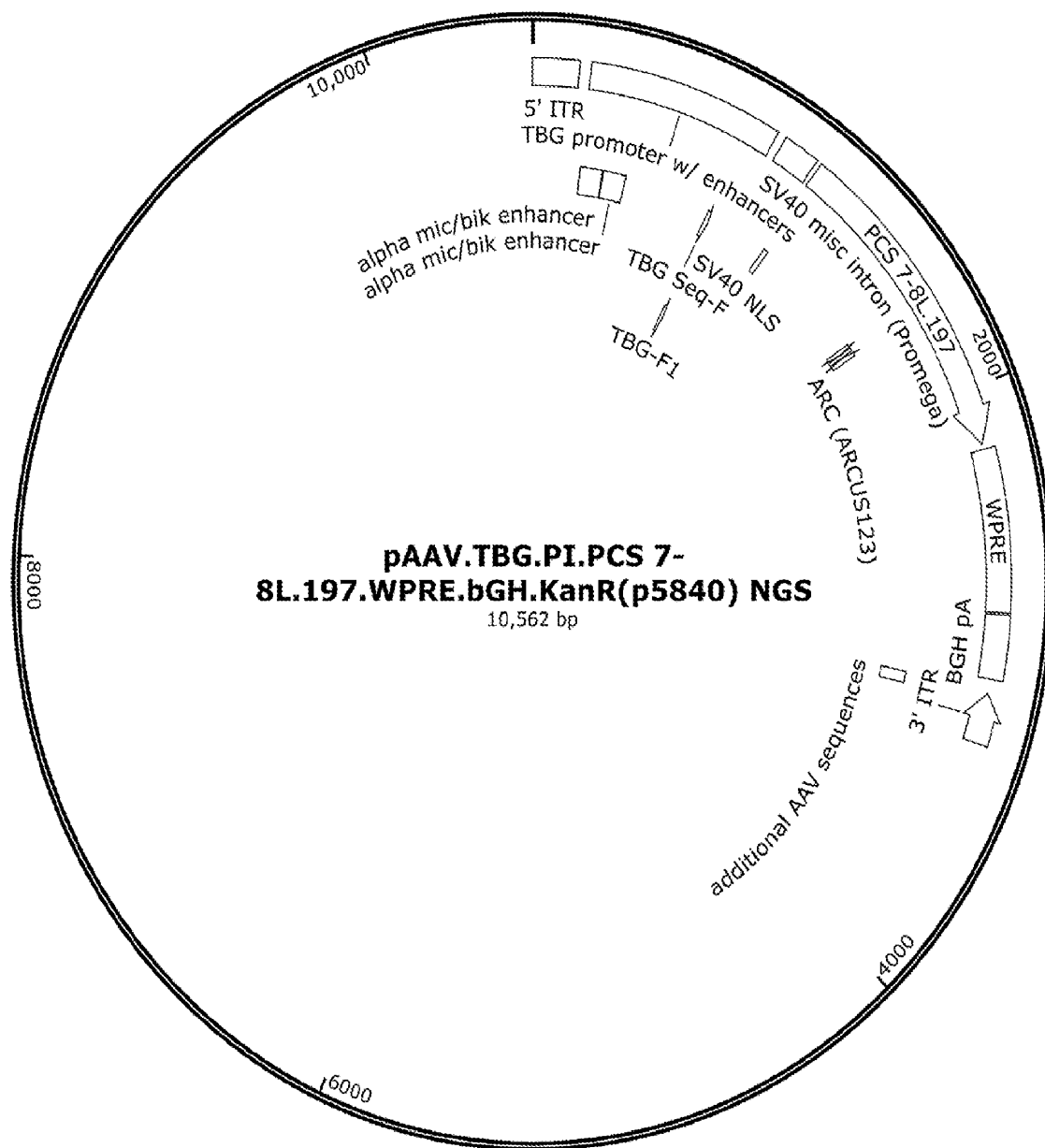


FIG 12



HDRs				
Name	Left	SEQ ID NO	Right	SEQ ID NO
short human	tgggctcctttctctgccaccacctcctcac ctttccagGTCATCACAGTTGGGGC CACCAATGCCCAAGACCAGCCG GTGACCCTGGGGACTTTGGGGAC CAACTTTGGCCGCTGTGTGGACC TCTTTGC	7	TTGCCCCAGGGGAGGACATCATTG GTGCCTCCAGCGACTGCAGCACCTG CTTTGTGTACAGAGTGGGACATCA CAGGCTGCTGCCACGTGGCTGgta agtcaccacccactgcctcgccaccgtgatg ctaac	8
long human	acctaggtcccccgccctatcaaggcttcc ctggcgccgaatttaaaggcatcaagcaa acaaagcccaacacatctctgccttgctc tcagtttccccctggcacttagaaccact tgatacaccgaatagttccggtctatctcc cccactaggatgtaaaactccacaggggcat tgggaatgctgcctggctatggtagggaca gaggggagcaccagggcggggaggggt gccagagtctgcctgggagtcagattttc cttaggaggggacatttgagtgggacccaa acaggtgtatagcagttgtccagcccagct ggcaaggcctgagtcgctctgcaaccct ctctgggctccttctctgccaccacctcct cactttccagGTCATCACAGTTGGG GCCACCAATGCCAGGACCAGC CGGTGACCCTGGGGACTTTGGGG ACCAACTTTGGCCGCTGTGTGGA CCTCTTGC	9	TTGCCCCAGGGGAGGACATCATTG GTGCCTCCAGCGACTGCAGCACCTG CTTTGTGTACAGAGTGGGACATCA CAGGCTGCTGCCACGTGGCTGgta agtcaccacccactgcctcgccaccgtgatg ctaacagccccttggcagtcagggtctgtgcc gggacctccagtgccaggctctgtcaggggg accagagatgaagtaggcctgatggtgccttc aaggacactcagtcctgatgggagggcaggt gcacagagggaacacgaggtcagggtctat tagaggagcccagaggaggcacctgcccag cccgagggtcagagaaggcatctggaggag ggacattgatcgggagcttgatggatgaata ggagttacctggccgataagacagcaactac caaggcttagaggtgtgagaggaggtgtctt acctcactgagtaaggactgcaggcggttac cttcgagaagagagcttag	10
long rhesus	aagcatacctgacagcggttaaccagatcc tcctggcctcgagggttccctggcagccc aatttaaaggcatcaagcaaaagccc aacacatctctgccttgcctctcagtcctcc cccgtggcacttagagccactgatacacc gagtagtttctatctcctcactagaatgt aaactccacaggggcattgggaatgctgcc tggctatggtagggacagaggggagcacc agggcggggaggggtgccagagtctgc ctggggagtcagatttcttaggagggga catttgaatgggaccaaacaggtgtatag cagttgtcagcccagctggcaaggcctca gtttgcttctgaacccctctcttgggctcctt tctgtccacctacctcctcactttccaggt catcacagttggggcccaatgccagga ccagccggtgacctggggactttggggac caacttggccgctgtgtggacctttgc	11	ttgcccaggggaggacatcattggtgcctcca gcgactgcagcacctgctttgtgtcagggagtg ggacatcgaggctgctgccacgtggctggt aagtcaccacccactgccttggccaccgtgat gtaacagccccttggcggtcagggtctgtgcc aggacctccagtgccaggctctgtcaggggg accagagatgagacaggcctgatggtgccttc atggacactcagtcctgataggagagcagtg cacagtggaacacgaggttagggctgtatta gaggagcccagaggaggcacctgcccagcc cgagggtcagagaaggcttcttggacaagg acatttgatctggagcttgatggatgaatagg agtccacctggcgataagacagcaactacca aggcttagaggtgtgacggaaggctgtcttac ctcactgtgtgagggactgcaggcggttacct tctagaagagagcttggtgtctctggt	12

FIG 14A

ITR-seq site		Best match to off-target consensus	SEQ ID
OT#		sequence	NO
	on-target chr1	TGGACCTCTTTGCCCCAGGGGA	18
OT1	chr10:21153025	TGGCCCTTGGTGCCACAGGGAA	19
OT2	chr16:54719552	AGGACCTTGGTGAGCCCGGGAA	20
OT3	chr16:53659419	GGGCCCTCCTTGCCCCAGGGAA	21
OT4	chr9:58529853	TGGTCTGTGGTACCCAGGGGA	22
OT5	chr19:17139526	TGGCCCTTTTACAGGAGGGCC	23
OT6	chr7:86142563	TGGCCCTCTTTCCAGAGGCCA	24
OT7	chr14:11607123	TGACCCCTGGTACCCAGGGCG	25
OT8	chr20:68895219	TGACCTCTTTCCCCAGGGTC	26
OT9	chr19:35457309	TGTCCTCTGTGCCCCAGGCCA	27
OT10	chr2:74738162	TGTCCTCTGTCCCCAGGCTA	28

FIG 14B

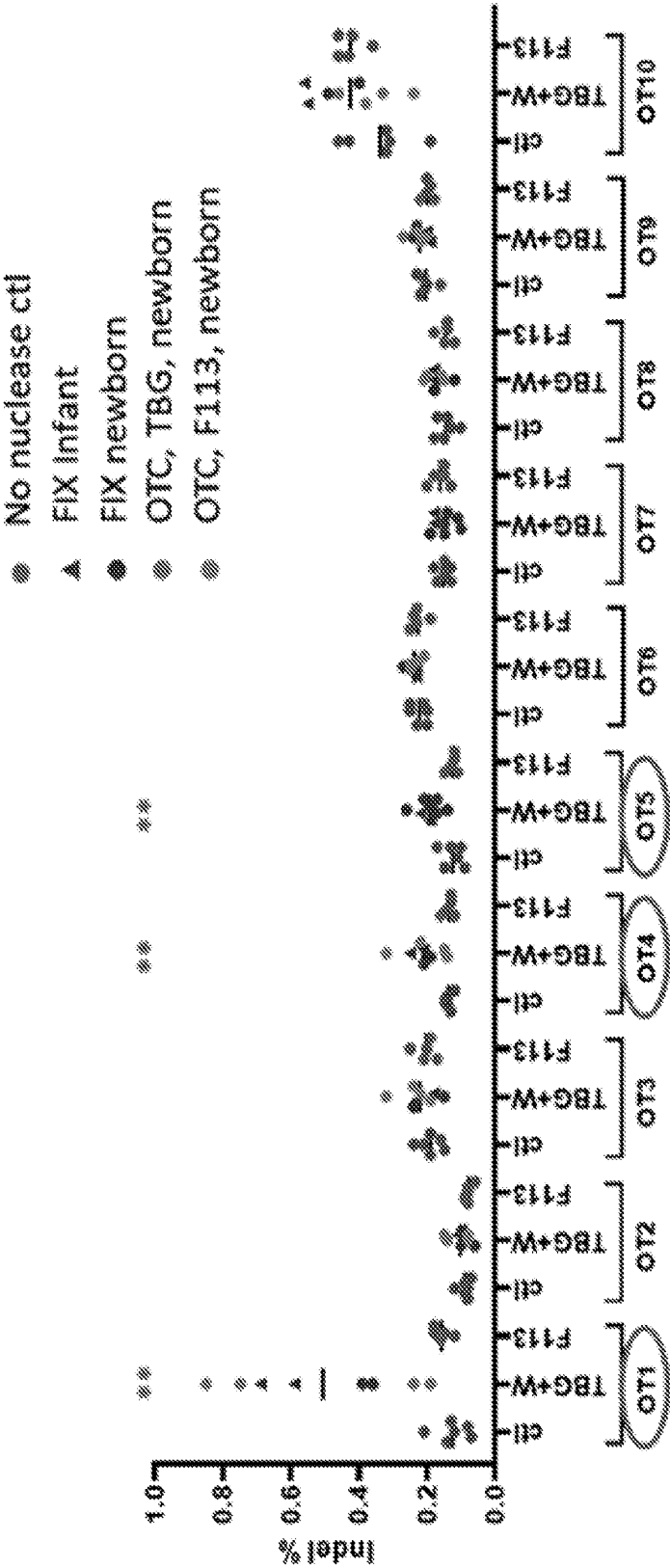


FIG 15A

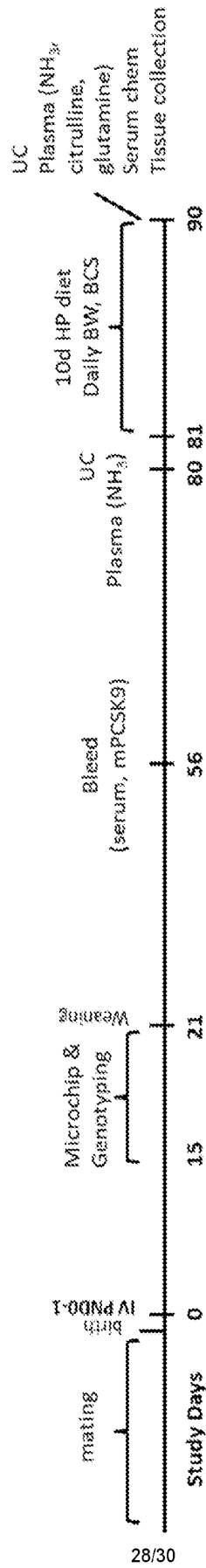


FIG 15B

Group	1	2	3	4	5	6	7	8
Number of (Male) Animals	No less than 15	No less than 15	No less than 15	No less than 15	No less than 15	No less than 15	No less than 15	No less than 15
Genotype	<i>KLspf^{ash}</i>	<i>KLspf^{azh}</i>	<i>KLspf^{azh}</i>	<i>KLspf^{azh}</i>	<i>KLspf^{as}_{ii}</i>	<i>KLWT</i>	<i>KLspf^{azh}</i>	<i>KLspf^{as}_{ii}</i>
<u>IV Dosing:</u> TA-Vector 1:	1.1×10^{12}	3.3×10^{12}	1.0×10^{13}	N/A	N/A	N/A	1.0×10^{13}	N/A
GTP-506A (GC/kg)								
<u>IV Dosing:</u> TA-Vector 2:	3.3×10^{12}	1.0×10^{13}	3.0×10^{13}	3.0×10^{13}	N/A	N/A	3.0×10^{13}	N/A
Murine donor vector (GC/kg)								
Total Dose (GC/kg)	4.4×10^{12}	1.3×10^{13}	4.0×10^{13}	3.0×10^{13}	N/A	N/A	4.0×10^{13}	N/A
High Protein Challenge	YES	YES	YES	YES	YES	YES	NO	NO
(Days 81±3 - 90±3)								

FIG 16

Study	Group	Vectors	Animal ID	DOB	Date Dosed	Day 90
						Necropsy /Biopsy
2020-007	G1	Vehicle control (d90 Nec)	22-167 (M)	7/21/2022	9/12/2022	12/12/2022
(6-9 week, GLP, OTC Tox)	G2	Vehicle control (1y Nec)	22-173 (M)	8/7/2022	9/28/2022	12/27/2022
	G3	GTP-506 (d90 Nec)	22-130 (F)	6/17/2022	8/8/2022	11/7/2022
	G3	GTP-506 (d90 Nec)	22-161 (M)	7/11/2022	8/30/22	11/28/2022
	G4	GTP-506 (1y Nec)	22-165 (M)	7/16/2022	9/12/2022	12/12/2022
	G4	GTP-506 (1y Nec)	22-176 (F)	8/12/2022	9/28/2022	12/27/2022