FIG. 1

Schematic representation of the AAV constructs containing weak promoters

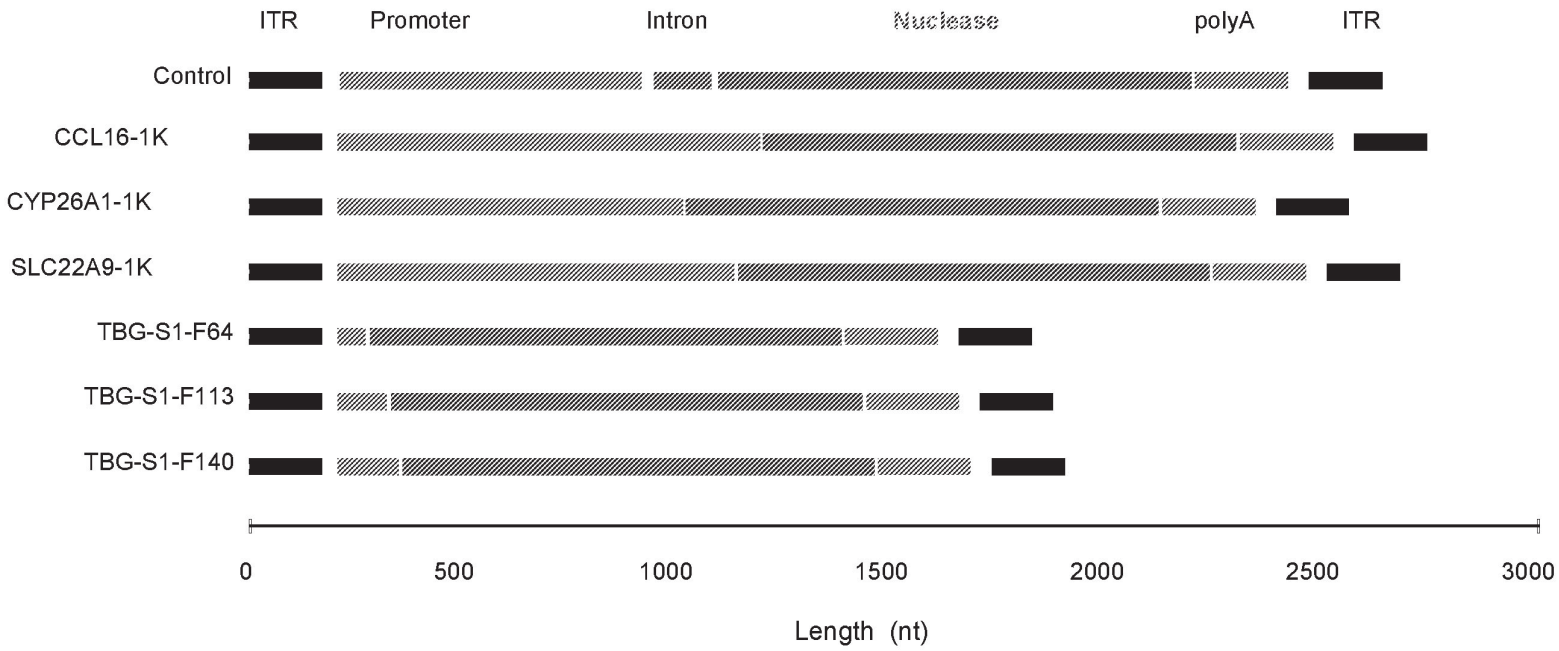

FIG. 2A

Indel% in target region

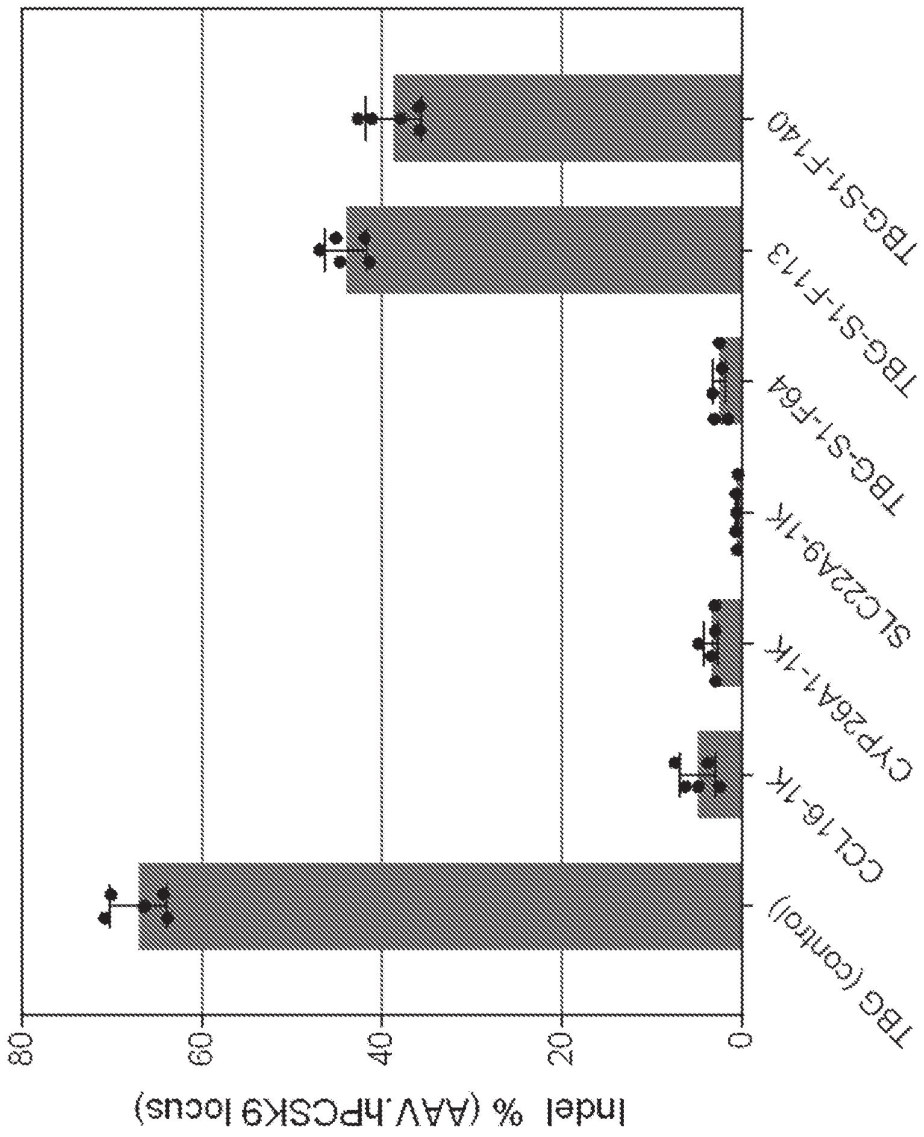

FIG. 2C

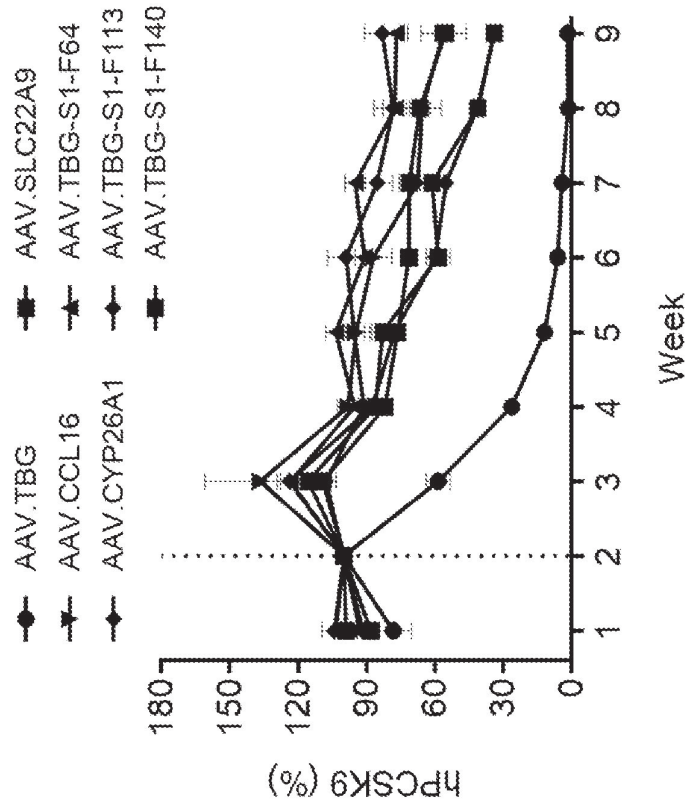
AAV.TBG
AAV.CCL16
AAV.CYP26A1
AAV.SLC22A9
AAV.TBG-S1-F64
AAV.TBG-S1-F113
AAV.TBG-S1-F140
hPCSK9 (%)
180
150
120
90
60
30
0
1
2
3
4
5
6
7
8
9
Week

FIG. 2B

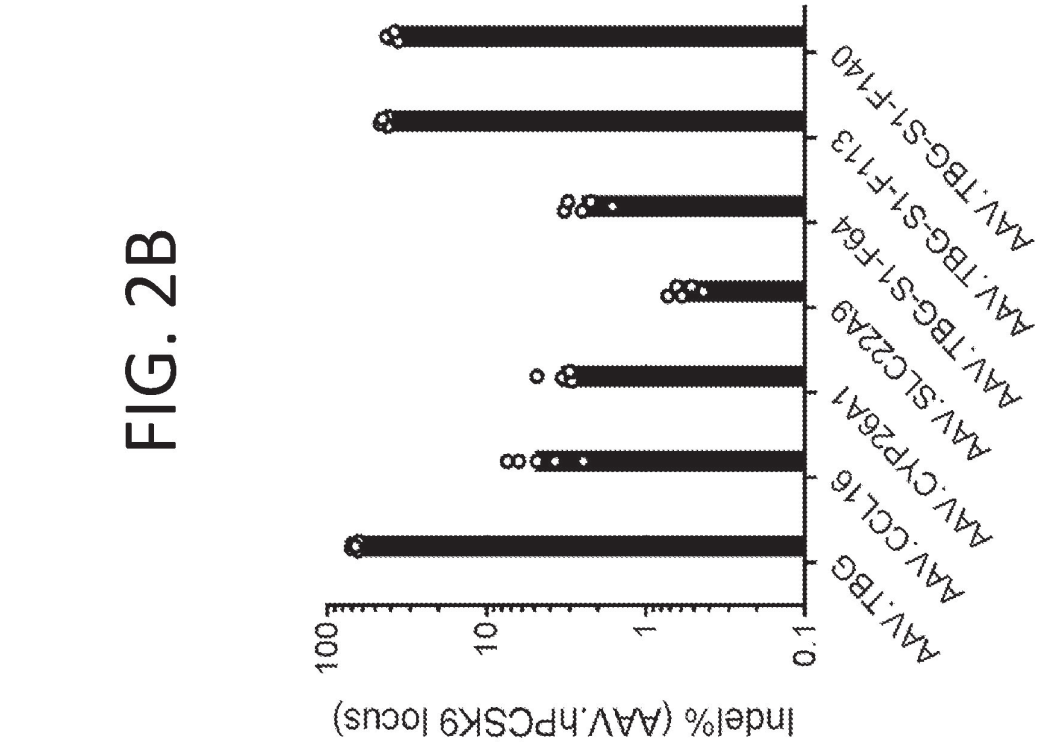
Indel% (AAV.hPCSK9 locus)
100
10
1
0.1
AAV.TBG
AAV.CCL16
AAV.CYP26A1
AAV.SLC22A9
AAV.TBG-S1-F64
AAV.TBG-S1-F113
AAV.TBG-S1-F140

FIG. 3
Number of off-target loci

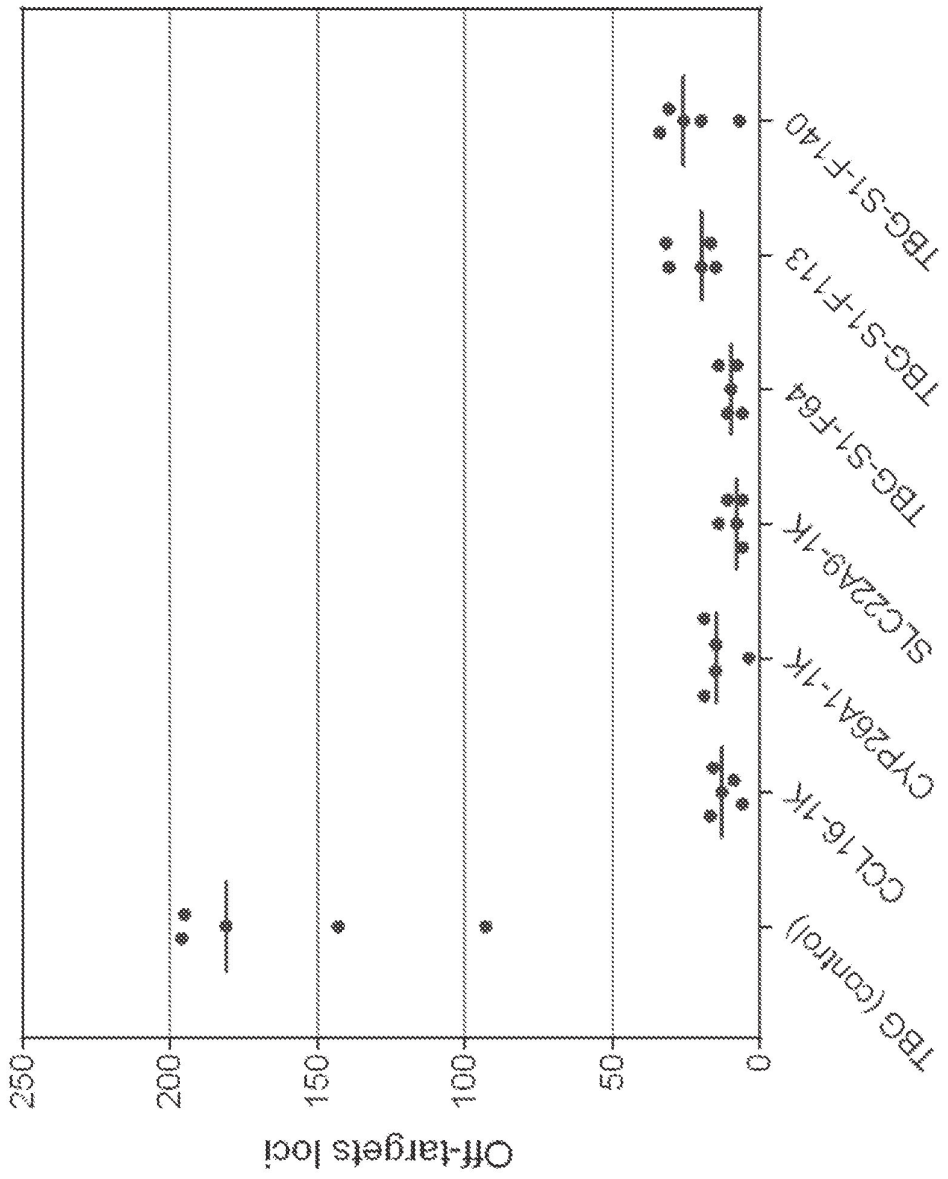

FIG. 4

Indels in a subset of identified off-targets

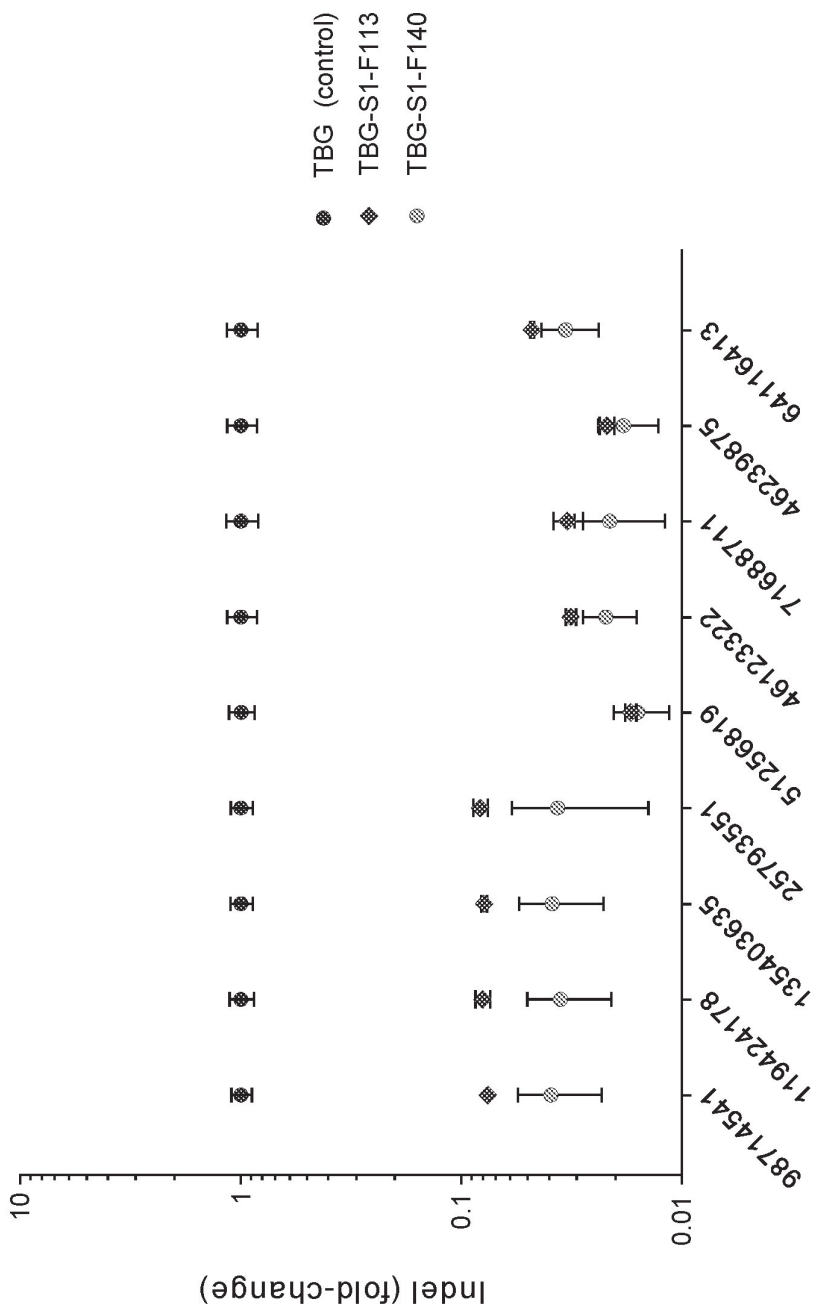

FIG. 5

Test of AAV constructs in vivo (AAV.hPCSK9)

Rag1 KO mice

Week 0. IV injection of AAV.hPCSK9

Week 2. IV injection of AAV.**weak promoters**

Week 7. Necropsy

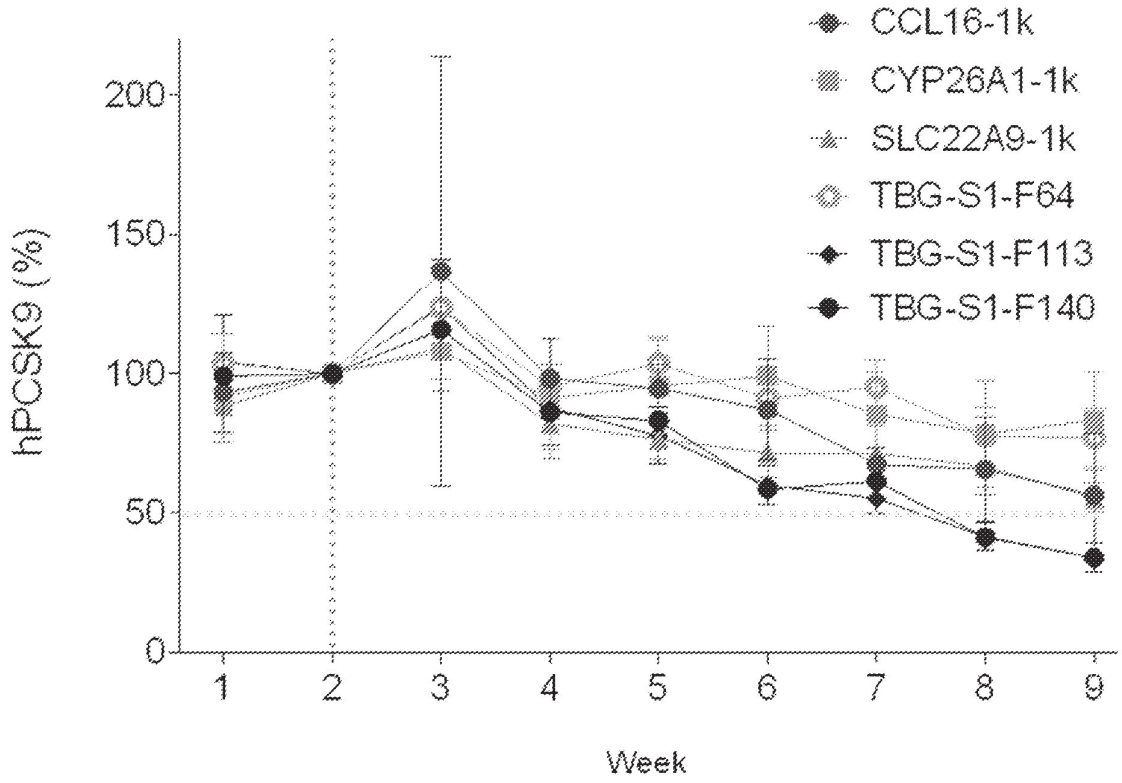

FIG. 6

Vector	On/off-target activity		PCSK9				LDL				AST		ALT	
	Indel (%)	Off-targets (ITR-Seq)	Time	Min Level (ng/mL)	Min Level (%)	Plateau (%)	Time	Min Level (ng/mL)	Min Level (%)	Plateau (%)	Time	Max Level	Time	Max Level
AAV8.TBG.ARCUS2 (no WPRE)	44%	263	d31	63.5	36%	76%	d21	22	49%	98%	d42	304	d42	1112
AAV8.TBG.ARCUS2 (no WPRE)	15%	41	d21	142.5	52%	TBD	d21	34	58%	TBD	d57	29	d57	216
AAV8.TBG.ARCUS2 (no WPRE)	33%	93	d42	89.5	31%	TBD	d21	41	46%	TBD	d57	37	d57	162
AAV8.TBG-S1-F113.ARCUS2	18%	19	d18	73.9	26%	TBD	d21	44	55%	TBD	d22	60	d22	39
AAV8.TBG-S1-F113.ARCUS2	34%	26	d28	91.2	45%	TBD	d21	24	37%	TBD	d43	54	d43	7

FIG. 7

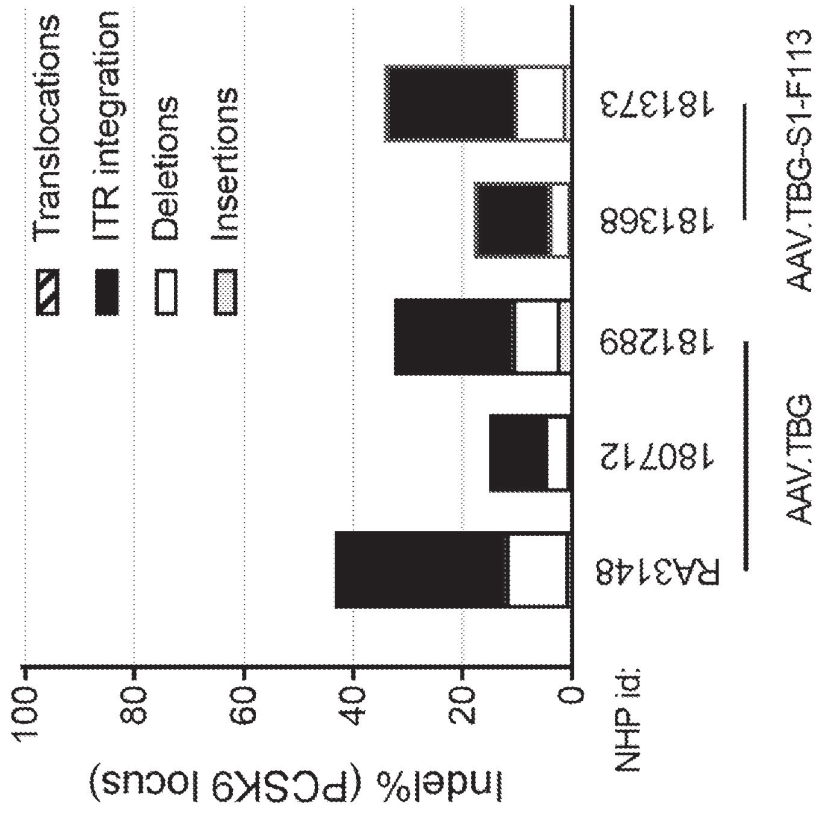
Translocations
ITR integration
Deletions
Insertions
Indel% (PCSK9 locus)
100
80
60
40
20
0
NHP id:
RA3148
180712
181289
181368
181373
AAV.TBG
AAV.TBG-S1-F113

FIG. 8

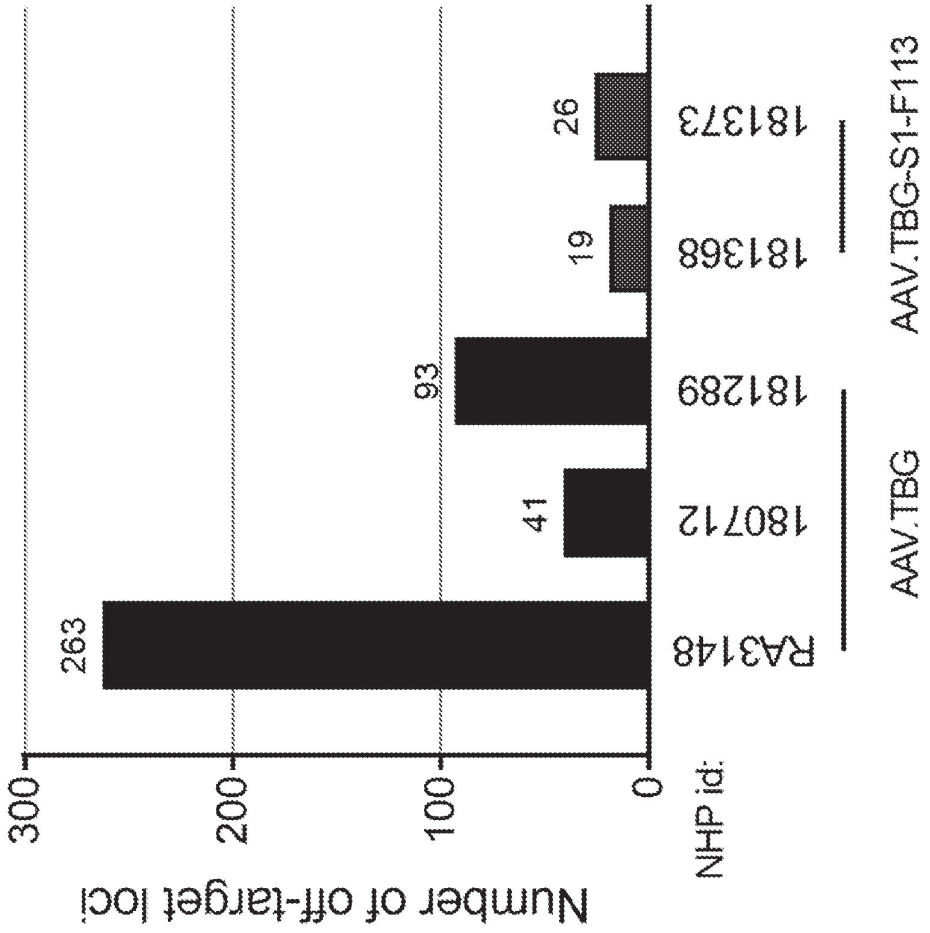
Number of off-target loci
300
200
100
0
263
41
93
19
26
NHP id:
RA3148
180712
181289
181368
181373
AAV.TBG
AAV.TBG-S1-F113

FIG. 9

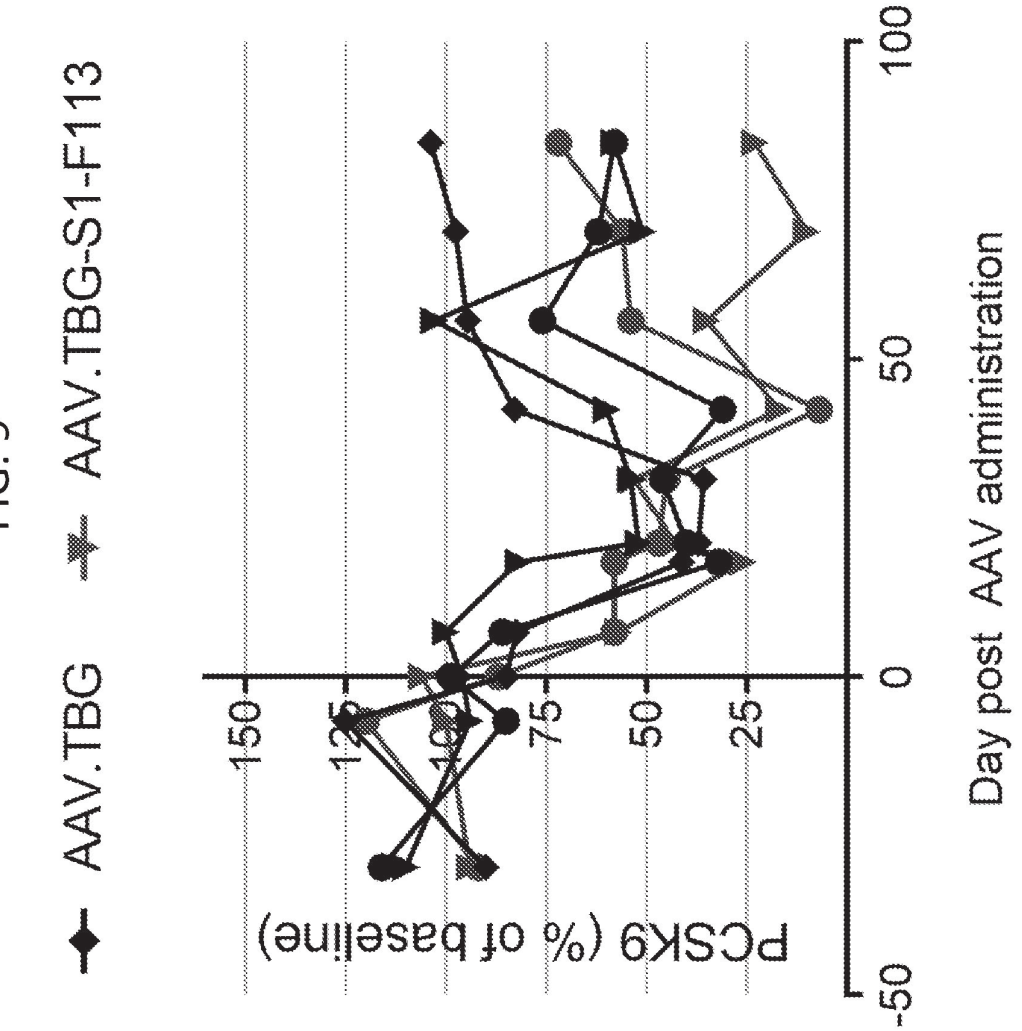
AAV.TBG
AAV.TBG-S1-F113
PCSK9 (% of baseline)
150
125
100
75
50
25
-50
0
50
100
Day post AAV administration

FIG. 10

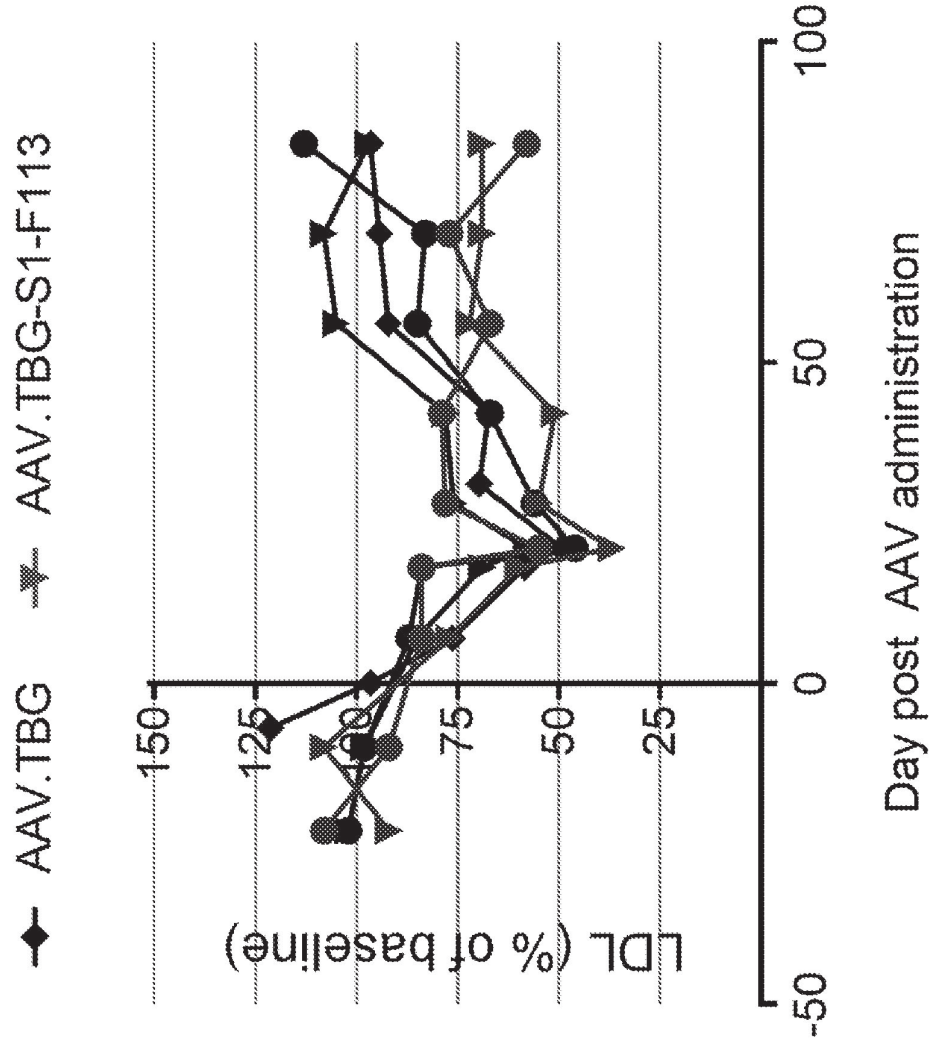
AAV.TBG
AAV.TBG-S1-F113
LDL (% of baseline)
150
125
100
75
50
25
-50
0
50
100
Day post AAV administration

FIG. 11

NHP Pharm/Tox Study Design

Group	NHP (M/F)	Dose (GC/kg)	Biopsy #1 day 18	Necropsy #1 day 28	Necropsy #2 day 120	Biopsy #2 day 180	Biopsy #3 day 364
1							
1-A	3	$1.2x10^{12}$	n=3	n=3	-	-	-
1-B	3	$1.2x10^{12}$	n=3	-	n=3	-	-
1-C	3	$1.2x10^{12}$	n=3	-	-	n=3	n=3
2							
2-A	3	$6.0x10^{12}$	n=3	n=3	-	-	-
2-B	3	$6.0x10^{12}$	n=3	-	n=3	-	-
2-C	3	$6.0x10^{12}$	n=3	-	-	n=3	n=3
3							
3-A	3	$3.0x10^{13}$	n=3	n=3	-	-	-
3-B	3	$3.0x10^{13}$	n=3	-	n=3	-	-
3-C	3	$3.0x10^{13}$	n=3	-	-	n=3	n=3

*need to include vehicle groups, n=2 per group

FIG. 12


SEQ ID NO: 25
SEQ ID NO: 6
SEQ ID NO: 7
SEQ ID NO: 8
TBG-S1 actcaaagttcaaaccttatcatttttgctttgttcctcttggccttggttttgtacat 60
F64 -- 0
F113 -- 0
F140 ----------------------------------cctcttggccttggttttgtacat 24
TBG-S1 cagctttgaaaataccatcccagggttaatgctggggttaatttataactaagagtgctc 12
F64 --gagtgctc 8
F113 ---ctttgaaaataccatcccagggttaatgctggggttaatttataactaagagtgctc 57
F140 cagctttgaaaataccatcccagggttaatgctggggttaatttataactaagagtgctc 84
****
TBG-S1 tagttttgcaatacaggacatgctataaaaatggaaagatgttgctttctgagaga 176
F64 tagttttgcaatacaggacatgctataaaaatggaaagatgttgctttctgagaga 64
F113 tagttttgcaatacaggacatgctataaaaatggaaagatgttgctttctgagaga 113
F140 tagttttgcaatacaggacatgctataaaaatggaaagatgttgctttctgagaga 140
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