FIG. 1

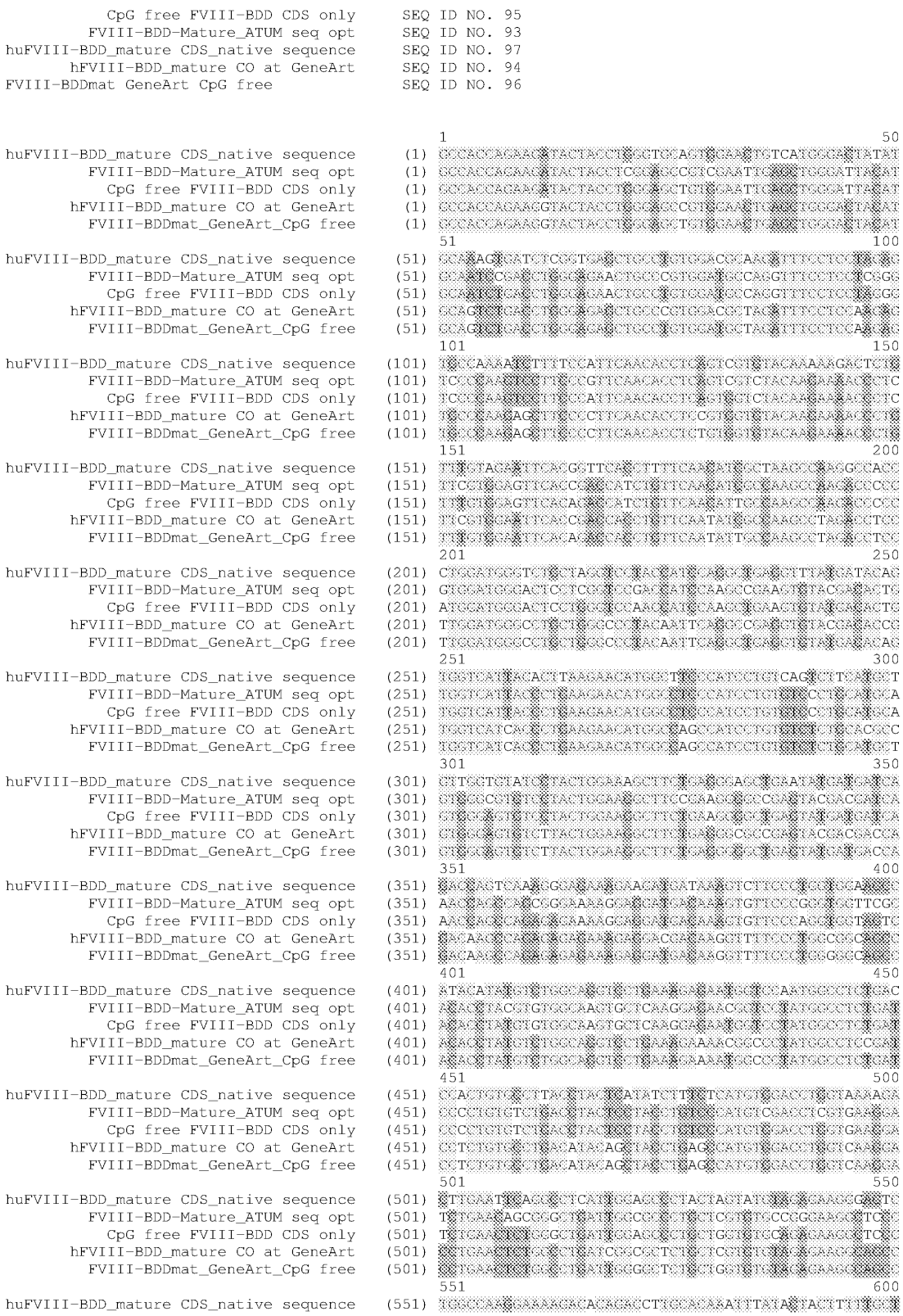

CpG free FVIII-BDD CDS only SEQ ID NO. 95
FVIII-BDD-Mature_ATUM seq opt SEQ ID NO. 93
huFVIII-BDD_mature CDS_native sequence SEQ ID NO. 97
hFVIII-BDD_mature CO at GeneArt SEQ ID NO. 94
FVIII-BDDmat GeneArt CpG free SEQ ID NO. 96
1 50
huFVIII-BDD_mature CDS_native sequence (1) GCCACCAGAAGTACTACCTGGGTGCAGTGGAACTGTCATGGGACTATAT
FVIII-BDD-Mature_ATUM seq opt (1) GCCACCAGAAGTACTACCTCGGCGCCGTCGAATTGAGCTGGGATTACAT
CpG free FVIII-BDD CDS only (1) GCCACCAGAAGTACTACCTGGGCGCTGTGGAATTGAGCTGGGATTACAT
hFVIII-BDD_mature CO at GeneArt (1) GCCACCAGAAGGTACTACCTGGGCGCCGTGGAACTGAGCTGGGACTACAT
FVIII-BDDmat_GeneArt_CpG free (1) GCCACCAGAAGGTACTACCTGGGCGCTGTGGAACTGAGCTGGGACTACAT
51 100
huFVIII-BDD_mature CDS_native sequence (51) GCAAAGTGATCTCCGTGAGCTGCCTGTGGACGCAAGATTTCCTCTGGGC
FVIII-BDD-Mature_ATUM seq opt (51) GCAAATCCGACTGGCGAACTGCCCGTGGATGCCAGGTTTCCTCCTCGGG
CpG free FVIII-BDD CDS only (51) GCAAATCTGACTGGCGAACTGCCTGTGGATGCCAGGTTTCCTCCTGGGC
hFVIII-BDD_mature CO at GeneArt (51) GCAGTCTGACTGGCGAGCTGCCCCCTGGACGCTAGATTTCCTCCAGGC
FVIII-BDDmat_GeneArt_CpG free (51) GCAGTCTGACTGGCGAGCTGCCTGTGGACGCTAGATTTCCTCCAGGC
101 150
huFVIII-BDD_mature CDS_native sequence (101) TGCCAAAATCTTTTCCATTCAACACCTCGTCTGTGTACAAAAAGACTGTG
FVIII-BDD-Mature_ATUM seq opt (101) TCCCGAACTCGTTCCGTTCAACACCTCGTCTGTCTACAAGAAGACGCTC
CpG free FVIII-BDD CDS only (101) TCCCGAACTCTTTCCATTCAACACCTCGTGTCTCTACAAGAAGACGCTC
hFVIII-BDD_mature CO at GeneArt (101) TGCCGAAGAGTTTCCCTTCAACACCTCCGTGTGTACAAGAAGACGCTG
FVIII-BDDmat_GeneArt_CpG free (101) TGCCGAAGAGTTTCCCTTCAACACCTCTGTGTGTACAAGAAGACGCTG
151 200
huFVIII-BDD_mature CDS_native sequence (151) TTTGTAGATTCACGGTTCAGCTTTTCAAGATGGCTAAGCCAAGGCCACC
FVIII-BDD-Mature_ATUM seq opt (151) TTCCTGCAGTTCACCGGCCATCTGTTCAACATCGCGAAGCCAAGCCCCC
CpG free FVIII-BDD CDS only (151) TTTCTGCAGTTCACACGCCATCTGTTCAACATTGCGAAGCCAAGCCCCC
hFVIII-BDD_mature CO at GeneArt (151) TTCGTGCAATTCACCGACCAGCTGTTCAATATCGCGAAGCCTAGACCTCC
FVIII-BDDmat_GeneArt_CpG free (151) TTTGTGCAGTTCACACGCCAGCTGTTCAATATTGCGAAGCCTAGACCTCC
201 250
huFVIII-BDD_mature CDS_native sequence (201) CTGCATGGGTCTGCTAGGTCCTACGATCCAGGCTGAGCTTTATGATACAG
FVIII-BDD-Mature_ATUM seq opt (201) GTGCATGGCACTCCTCGTCCGACGATCCAAGCCCAAGTGTACGACACTG
CpG free FVIII-BDD CDS only (201) ATGCATGGCACTCCTGGGTCCAACGATCCAAGCTGAAGTGTATGACACTG
hFVIII-BDD_mature CO at GeneArt (201) TTGCATGGCCTTCTGGCCCTACAATTCAGCCCGAGCTGTACGACACCG
FVIII-BDDmat_GeneArt_CpG free (201) TTGCATGGCCTTGCTGGCCCTACAATTCAGCCTCAGCTGTATGACACAG
251 300
huFVIII-BDD_mature CDS_native sequence (251) TGGTCATTACACTTAAGAACATGGCTTCCCATCCTGTCAGCTTTCATGCT
FVIII-BDD-Mature_ATUM seq opt (251) TGGTCATTACGCTGAAGAACATGGCCTTCCATCCTCTGTCCCTGCATGCA
CpG free FVIII-BDD CDS only (251) TGGTCATTACGCTGAAGAACATGGCCTTCCATCCTGTGTCCCTGCATGCA
hFVIII-BDD_mature CO at GeneArt (251) TGGTCATCACGCTGAAGAACATGGCAGCCATCCTGTGTCTCTGCACGCC
FVIII-BDDmat_GeneArt_CpG free (251) TGGTCATCACGCTGAAGAACATGGCAGCCATCCTGTGTCTCTGCATGCT
301 350
huFVIII-BDD_mature CDS_native sequence (301) GTTGGTGTATCGTACTGGAAAGCTTCGAGCGAGCTGAATATGATGATCA
FVIII-BDD-Mature_ATUM seq opt (301) GTGGGCGTGTCGTACTGGAAGGCTTCCGAAGCGGCCGAGTACGACGATCA
CpG free FVIII-BDD CDS only (301) GTGGGCGTGTCGTACTGGAAGGCTTCTGAAGCGGCTGAGTATGATGATCA
hFVIII-BDD_mature CO at GeneArt (301) GTGGGCGTGTCTTACTGGAAGGCTTCGAGCGGCTGAGTATGATGACCA
FVIII-BDDmat_GeneArt_CpG free (301) GTGGGCGTGTCTTACTGGAAGGCTTCGAGCGGCTGAGTATGATGACCA
351 400
huFVIII-BDD_mature CDS_native sequence (351) GACAAGTCAAAGGGAAAGAAGAATGATAAGTCTTCCCATGCTGGAACCC
FVIII-BDD-Mature_ATUM seq opt (351) AACGACCAGCGGAAAAGGAGCATCAGAAAGTGTTCCCGCCTGGTTCGC
CpG free FVIII-BDD CDS only (351) AACGACCAGCGACAAAAGGAGCATCAGAAAGTGTTCCCAGGTGGTTCC
hFVIII-BDD_mature CO at GeneArt (351) GACAACCACCGCCGCAGAAGCAGCACGACGAAGTTTTCCCTGGCGGCACCC
FVIII-BDDmat_GeneArt_CpG free (351) GACAACCACCGTCGCAGAAGCAGCACGACGAAGTTTTCCCTGGGGGCACCC
401 450
huFVIII-BDD_mature CDS_native sequence (401) ATACATTGTGTGGCAGTGCTGAAGCAGAAGCACCCAATGGCCTCTGAC
FVIII-BDD-Mature_ATUM seq opt (401) AGACGTACGTGTGGCAAGTGCTCAAGGAGAACCGCCCTATGGCCTCTGAT
CpG free FVIII-BDD CDS only (401) AGACGTATGTGTGGCAAGTGCTCAAGGCAGAACGCCCTATGGCCTCTGAT
hFVIII-BDD_mature CO at GeneArt (401) AGACGTACTGTGGCAGCTGCTGAAGCAAAAGCCGCCCTATGGCCTCCGAT
FVIII-BDDmat_GeneArt_CpG free (401) AGACGTATCTGTGGCAGCTGCTGAAGCAAAAGCCGCCCTATGGCCTCTGAT
451 500
huFVIII-BDD_mature CDS_native sequence (451) CCACTGTGGCTTACGTACTCATATCTTTCTCATGTGGACCTGCTAAAAGA
FVIII-BDD-Mature_ATUM seq opt (451) CCCCTGTGTCTGACGTACTCGTACCTGTCCCATGTCGACCTCGTGAAGGA
CpG free FVIII-BDD CDS only (451) CCCCTGTGTCTGACGTACTCCTACCTGTCCCATGTGGACCTGGTGAAGGA
hFVIII-BDD_mature CO at GeneArt (451) CCTCTGTGCCTGACATACAGCTACCTGAGCCATGTGGACCTGGTCAAGGA
FVIII-BDDmat_GeneArt_CpG free (451) CCTCTGTGCCTGACATACAGGTACCTGAGCCATGTGGACCTGGTCAAGGA
501 550
huFVIII-BDD_mature CDS_native sequence (501) GTTCAATTCAGCGCTCATTCGAGCGCTACTATATATTGGCGAAGCGGCTC
FVIII-BDD-Mature_ATUM seq opt (501) TTTCAAGAGCGGCTGATTCGCCGCCTGTCTCGTGTGCCGGAAGCGTCCC
CpG free FVIII-BDD CDS only (501) TGTCAACTCTGGCGCTGATTCGAGCGCTGCTGGTGTGCCGCGAAGCGTCCC
hFVIII-BDD_mature CO at GeneArt (501) GCTCAACTCTGGCCTGATCCGCCGCTCTCTCGTGTCTCCGCAAGCGACCC
FVIII-BDDmat_GeneArt_CpG free (501) GCTGAACTCTGGCCTGATTGGCGCCTCTGCTGGTGTCTGCCAAGCGACCC
551 600
huFVIII-BDD_mature CDS_native sequence (551) TGGCCAAGAAAAGACACACGACCTTGCACAAATTTATACTACTTTTGCGT

FIG. 1 (CONTINUED)

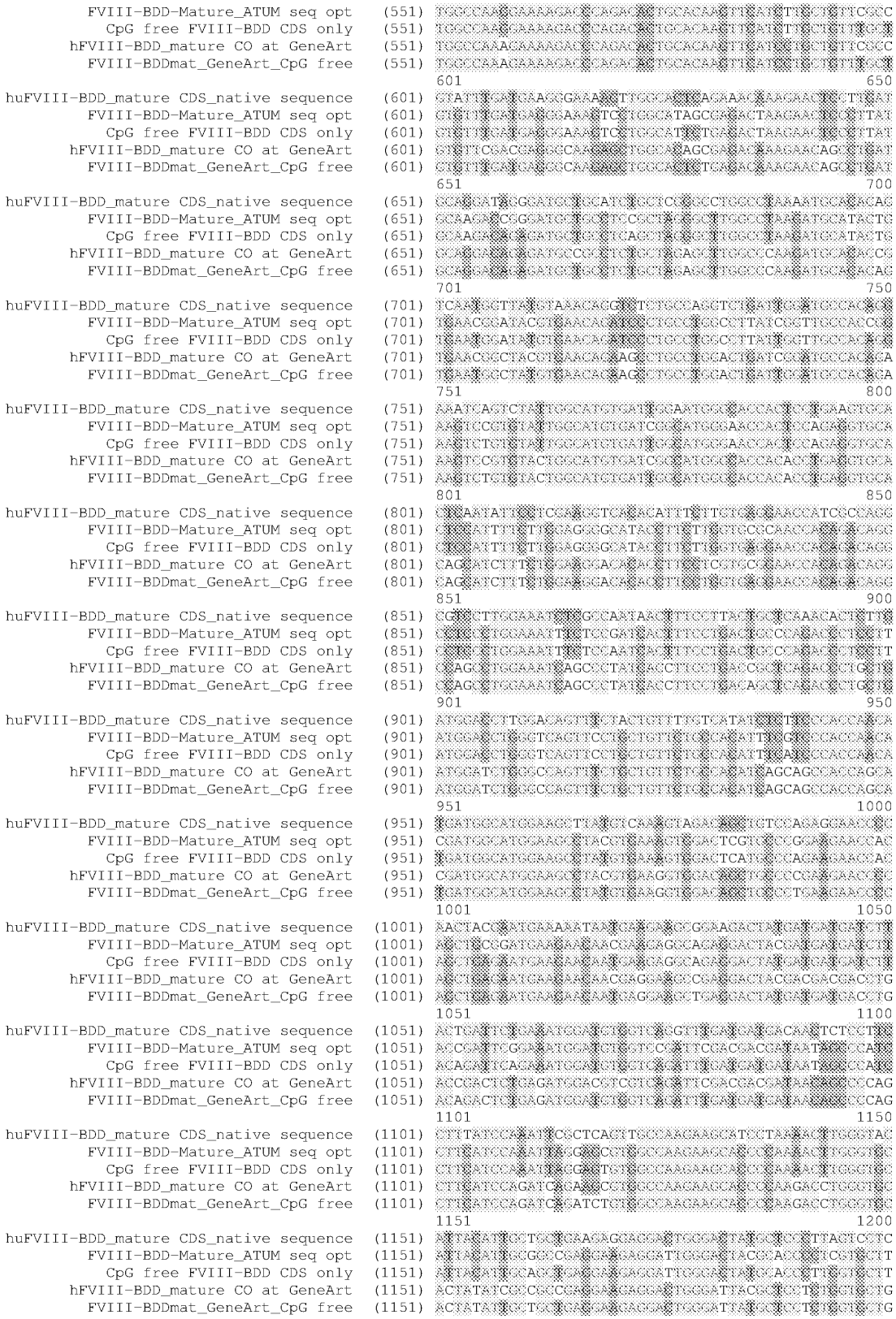


FIG. 1 (CONTINUED)

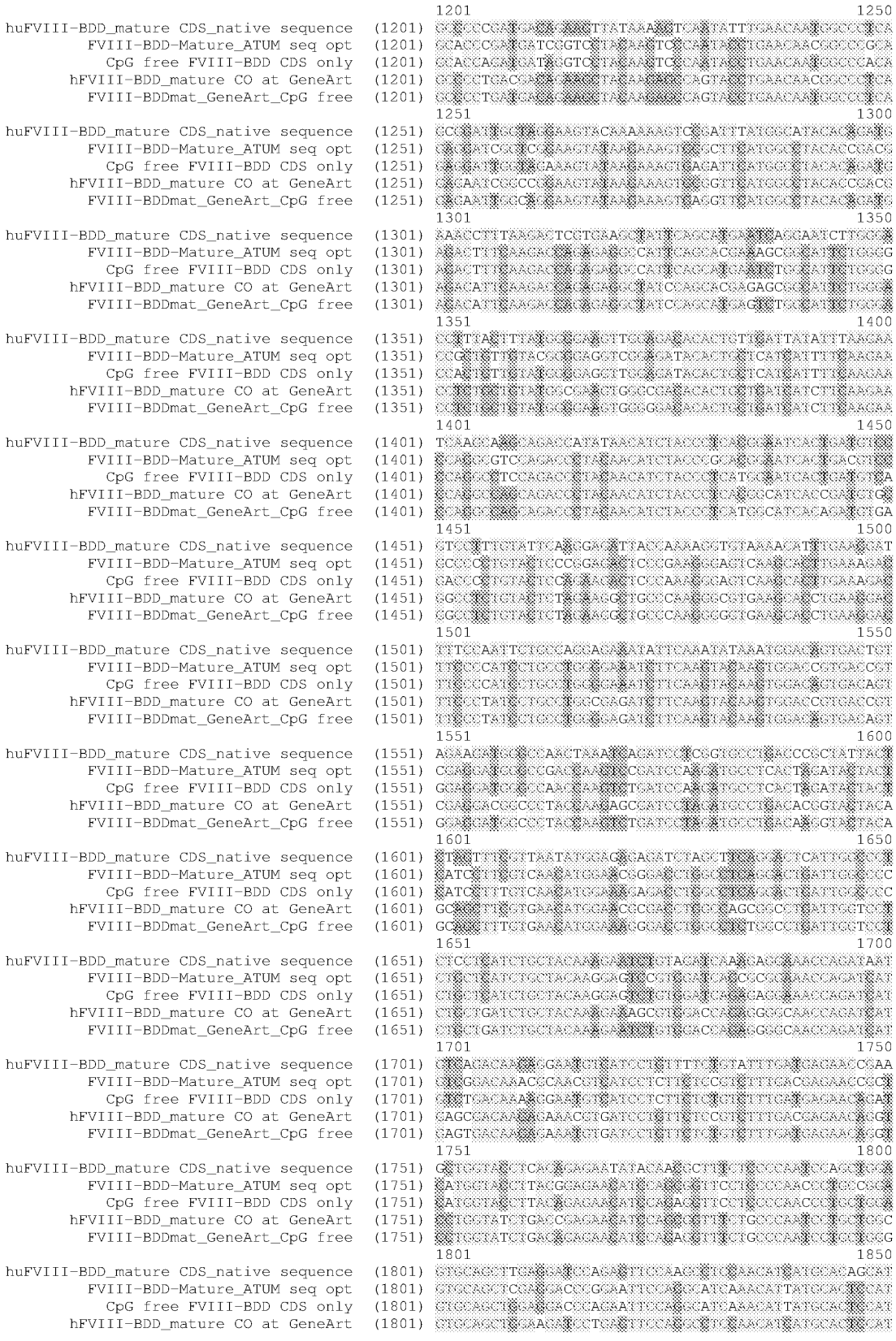


FIG. 1 (CONTINUED)

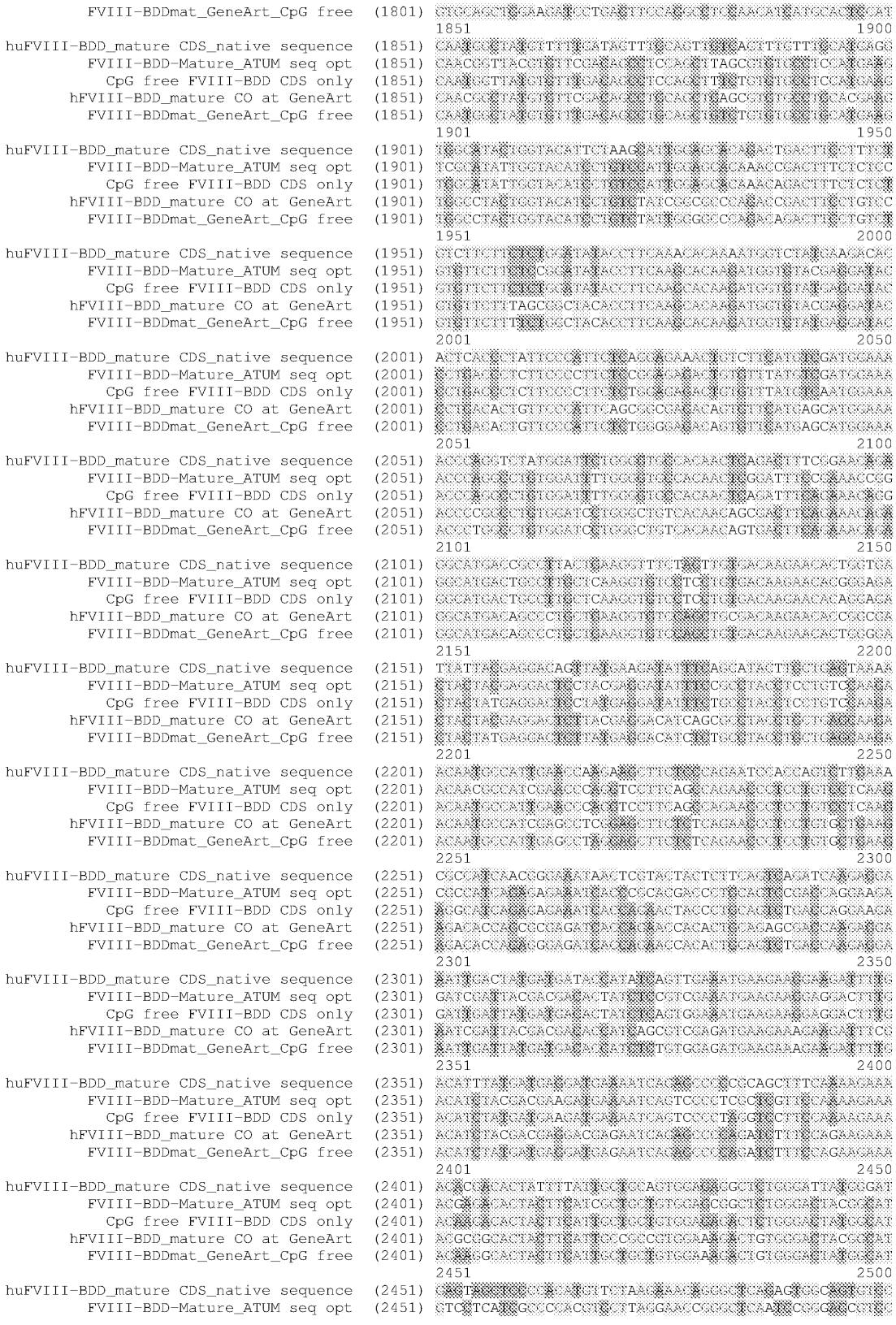


FIG. 1 (CONTINUED)

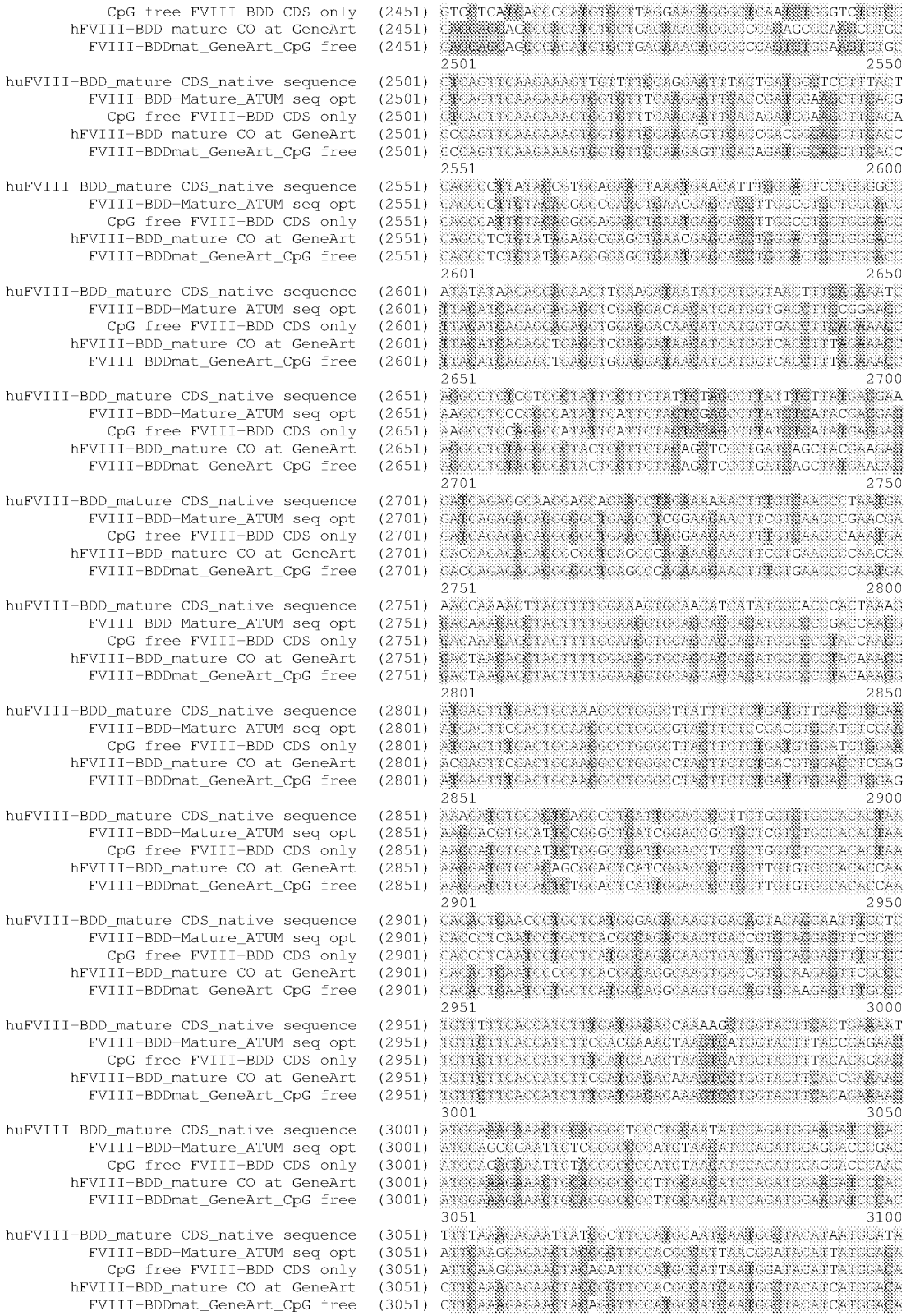


FIG. 1 (CONTINUED)

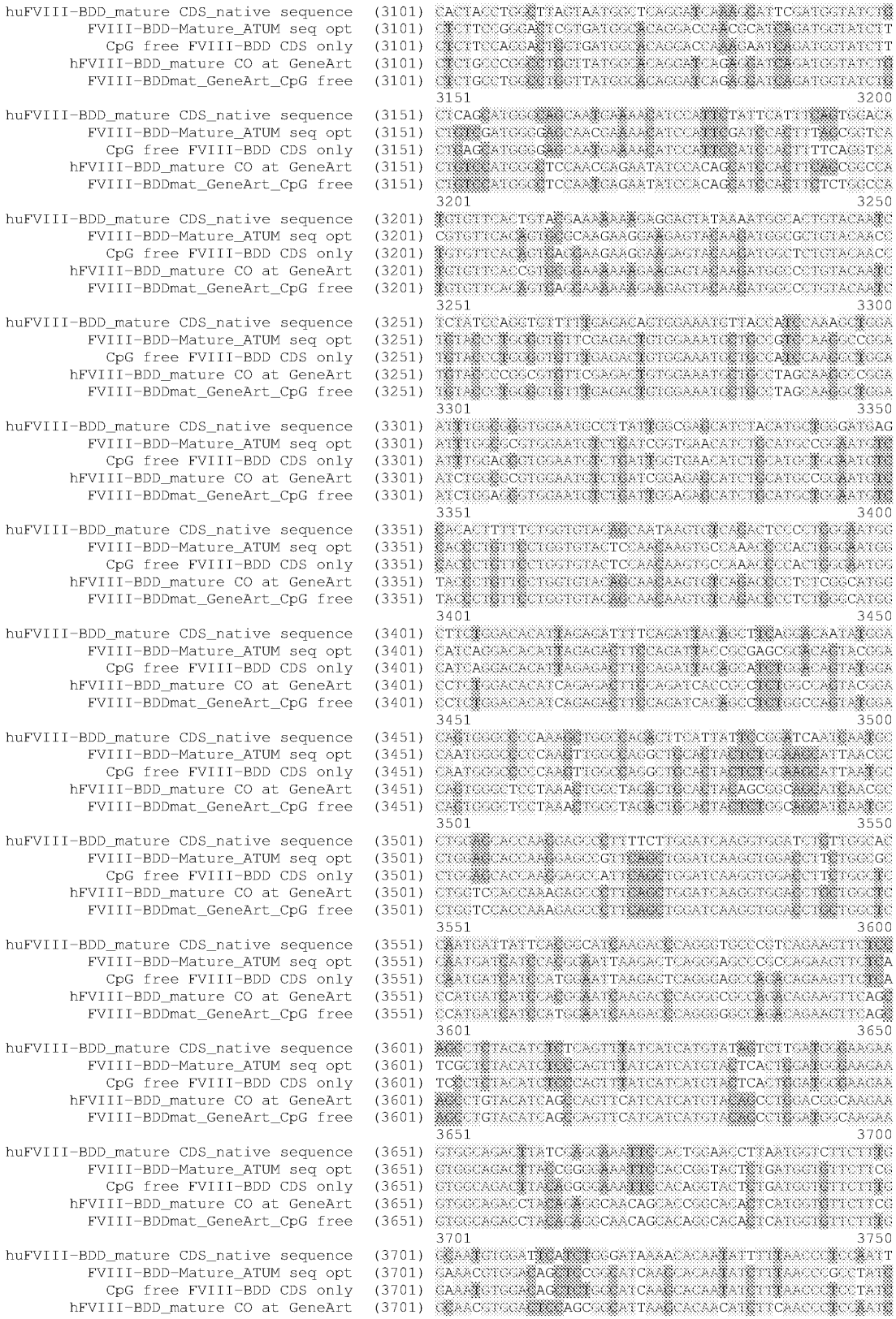


FIG. 1 (CONTINUED)

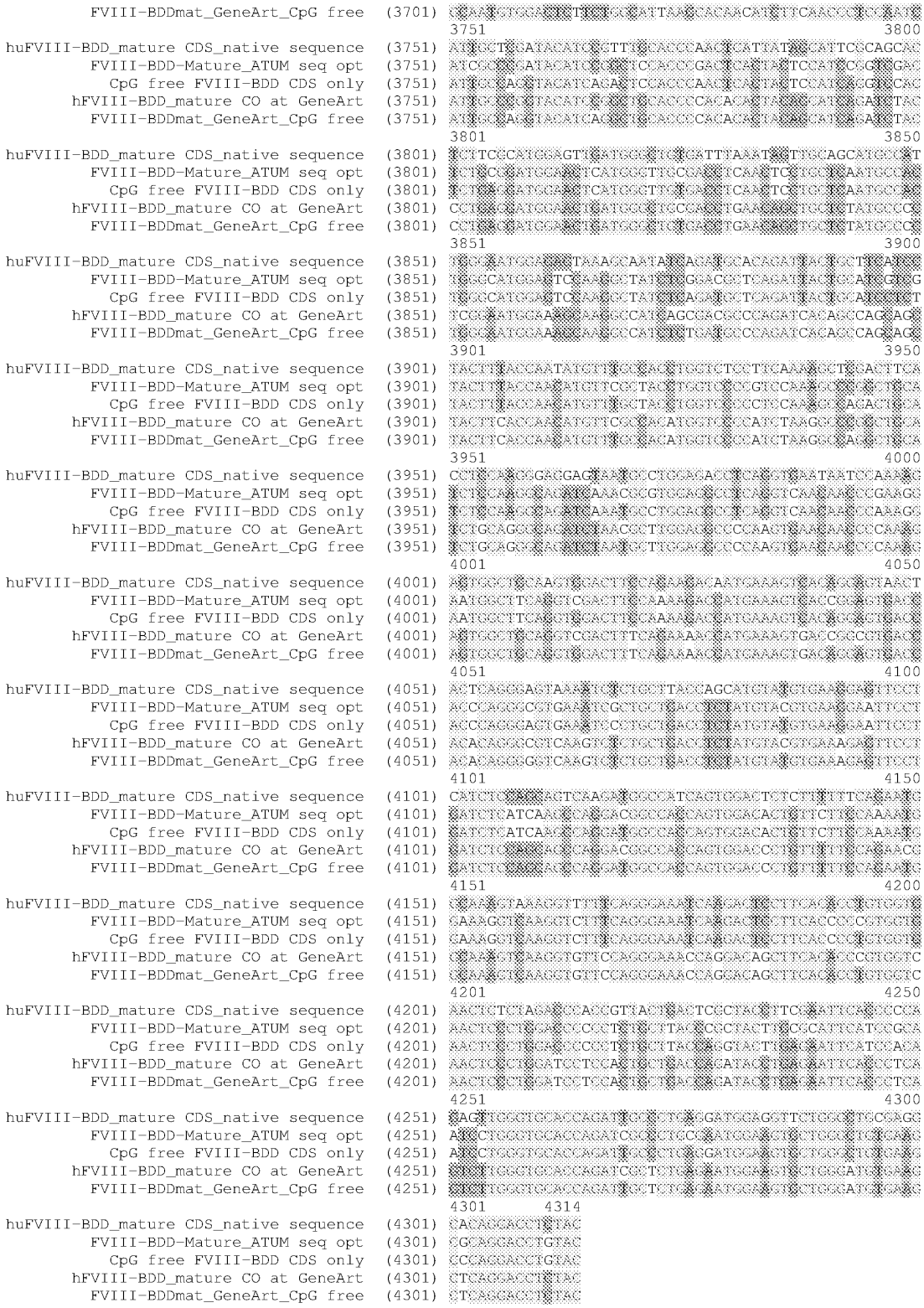


FIG. 2

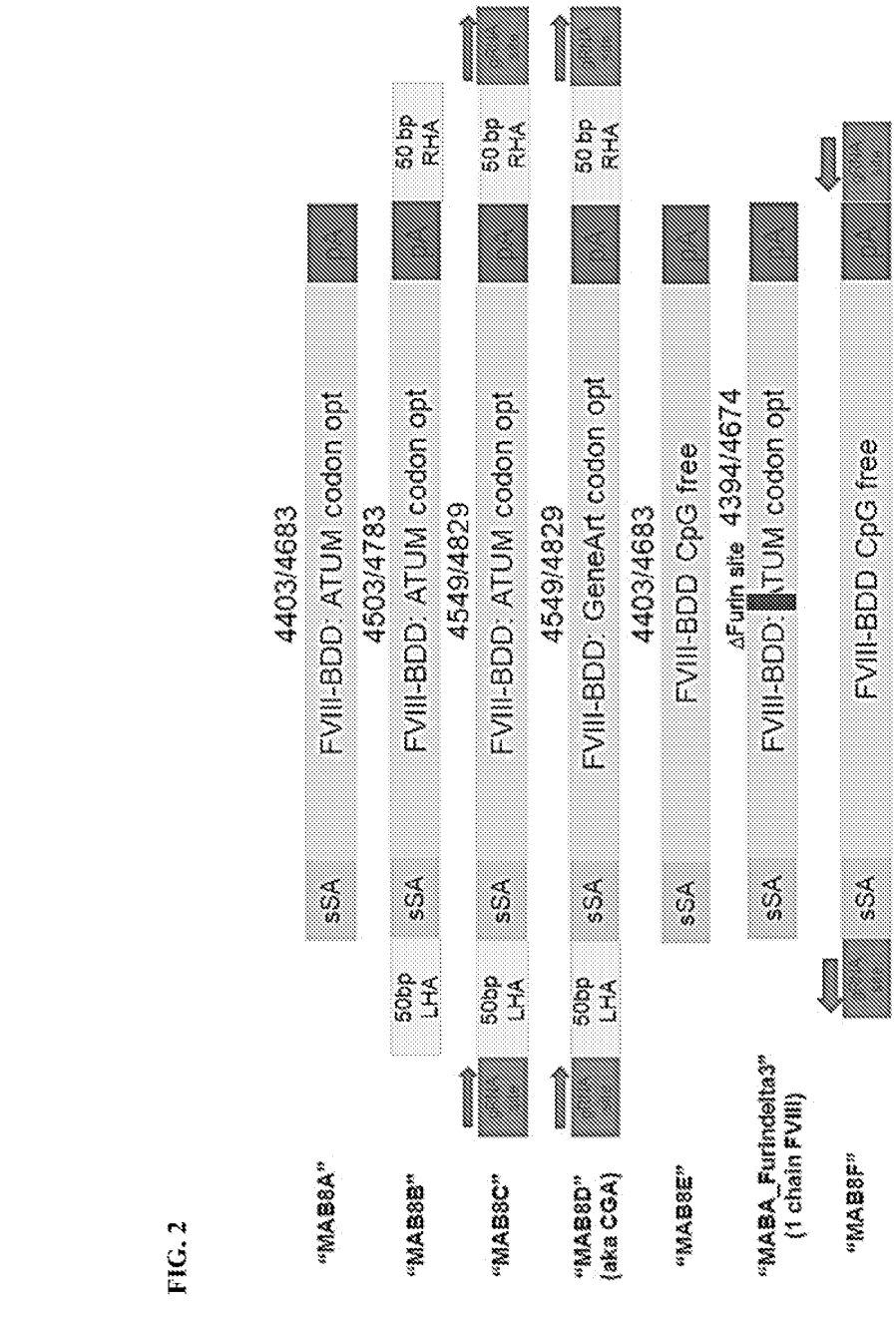
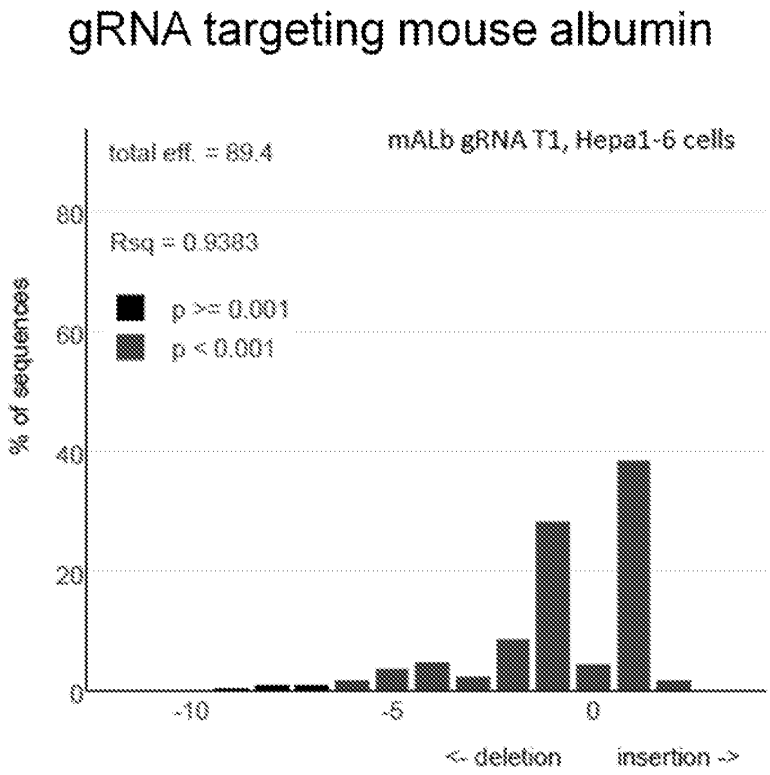

"MAB8A"
"MAB8B"
"MAB8C"
"MAB8D" (aka CGA)
"MAB8E"
"MABA_Furindelta3" (1 chain FVIII)
"MAB8F"
sSA
50bp LHA
50 bp RHA
FVIII-BDD: ATUM codon opt
FVIII-BDD: GeneArt codon opt
FVIII-BDD CpG free
4403/4683
4503/4783
4549/4829
4394/4674
ΔFurin site

FIG. 3


gRNA targeting mouse albumin
total eff. = 89.4
mALb gRNA T1, Hepa1-6 cells
Rsq = 0.9383
p >= 0.001
p < 0.001
% of sequences
80
60
40
20
0
-10
-5
0
<- deletion
insertion ->

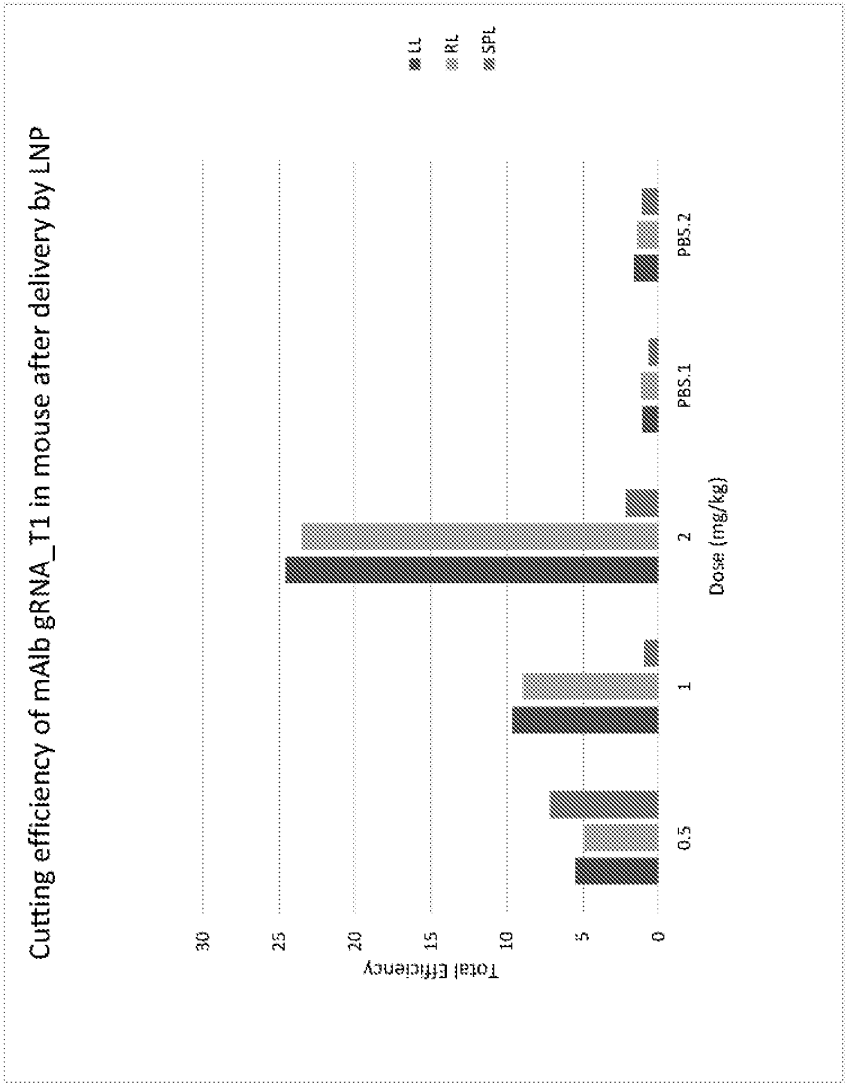

Cutting efficiency of mAlb gRNA_T1 in mouse after delivery by LNP
LL
RL
SPL
Total Efficiency
30
25
20
15
10
5
0
0.5
1
2
PBS.1
PBS.2
Dose (mg/kg)

FIG. 4

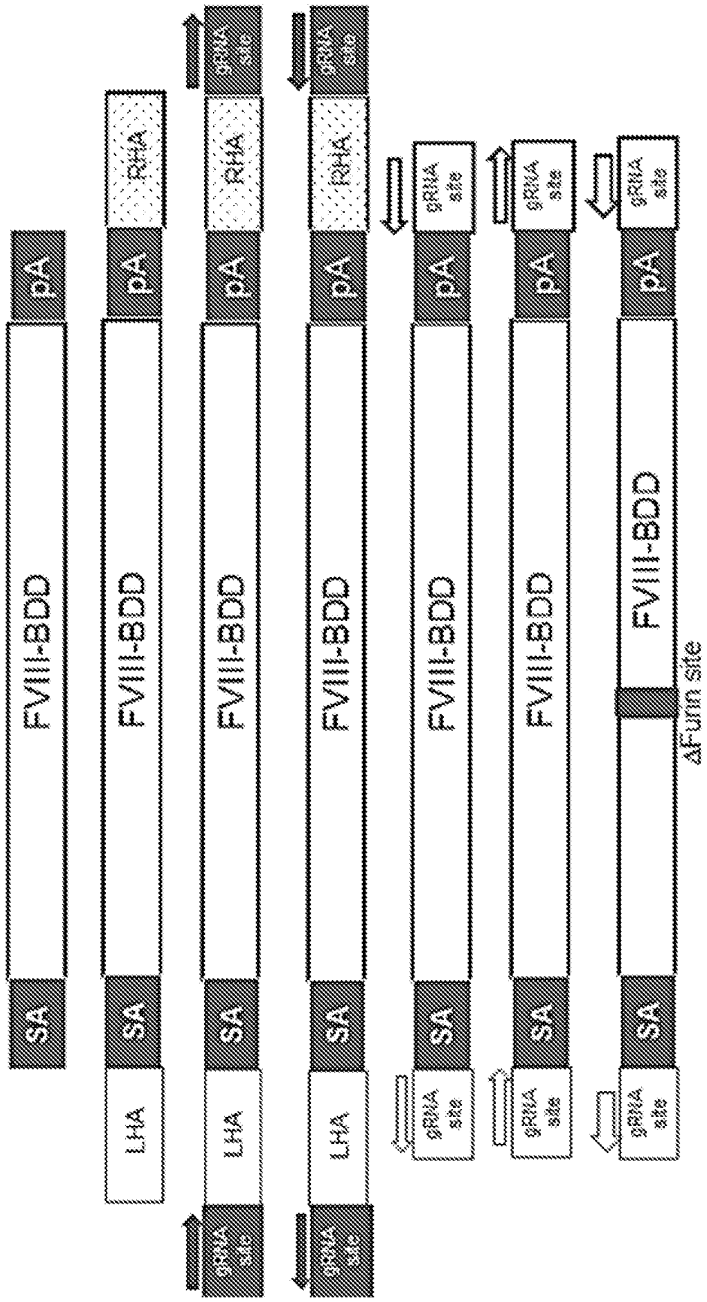


FIG. 5

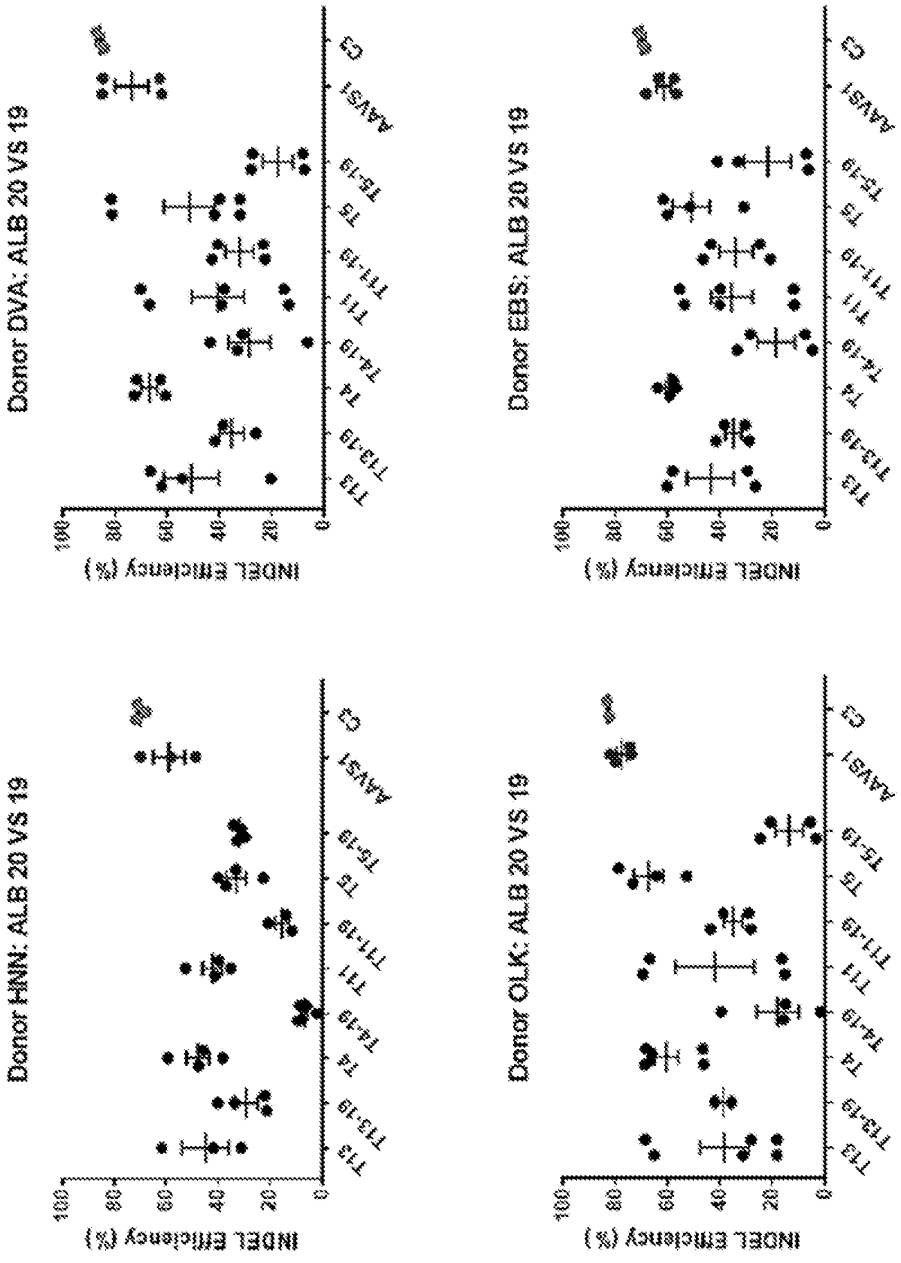

Donor DVA: ALB 20 VS 19
Donor EBS: ALB 20 VS 19
Donor HNN: ALB 20 VS 19
Donor OLK: ALB 20 VS 19
INDEL Efficiency (%)
0
20
40
60
80
100
T13
T13-19
T4
T4-19
T11
T11-19
T5
T5-19
AAVS1
C3

FIG. 6

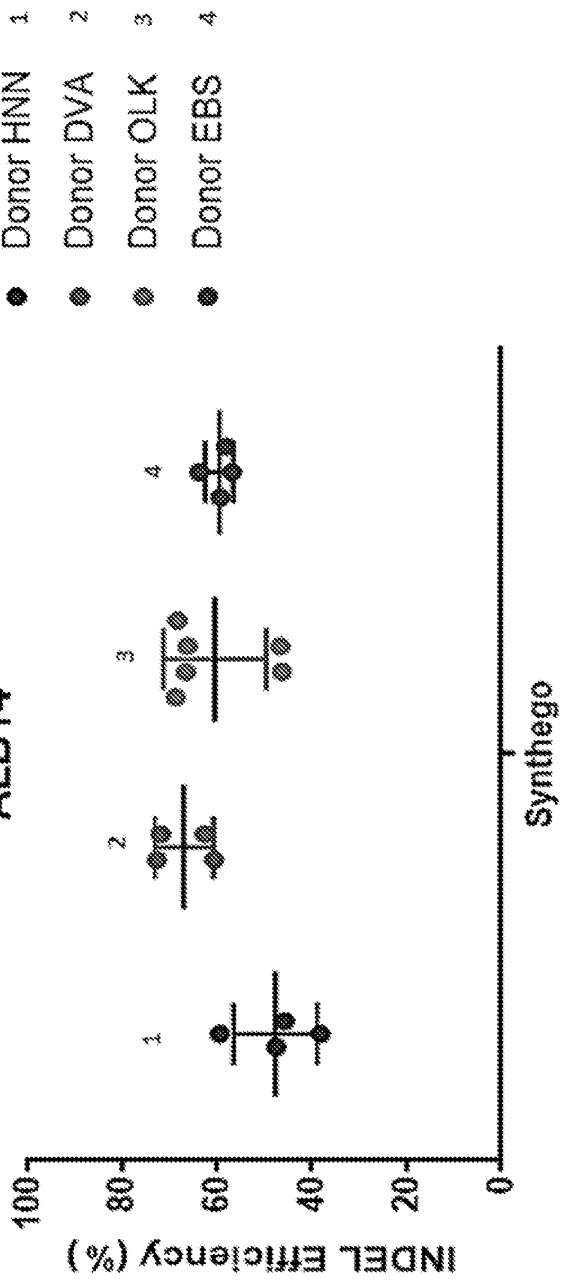

Primary Human Heptocytes
ALBT4
Donor HNN 1
Donor DVA 2
Donor OLK 3
Donor EBS 4
INDEL Efficiency (%)
100
80
60
40
20
0
1
2
3
4
Synthego

FIG. 7

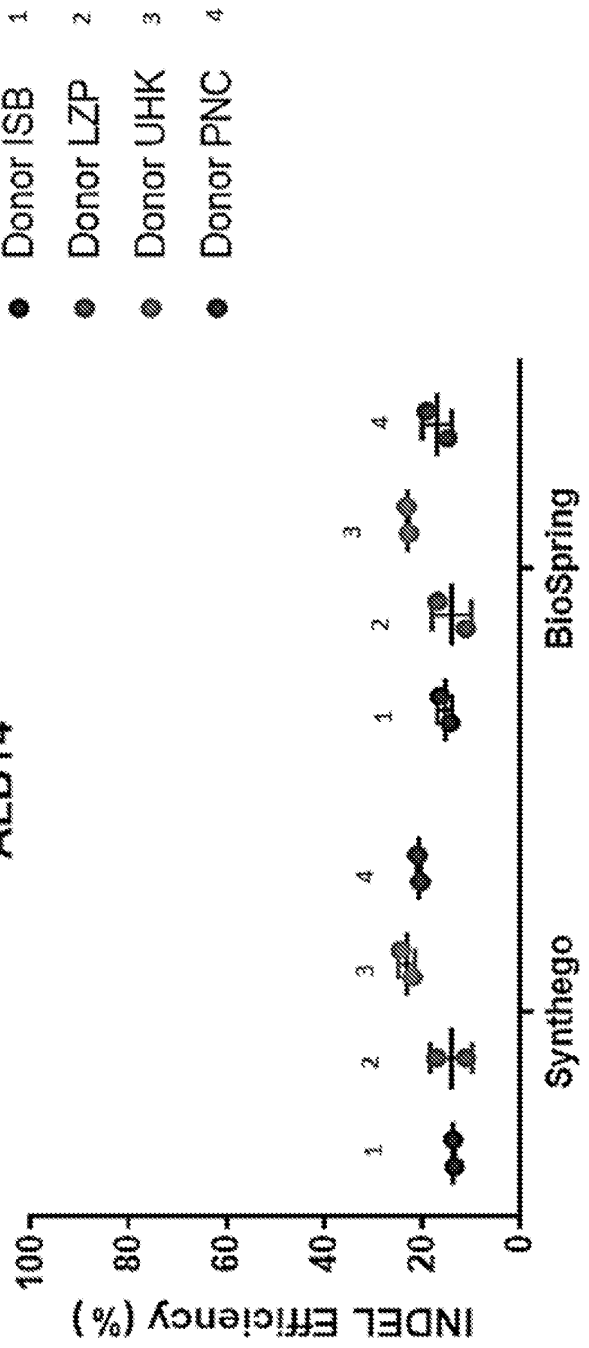
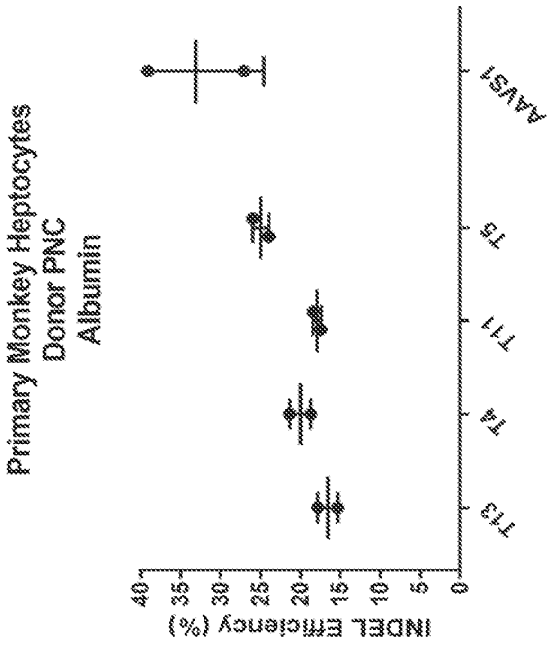


FIG. 7 (CONTINUED)



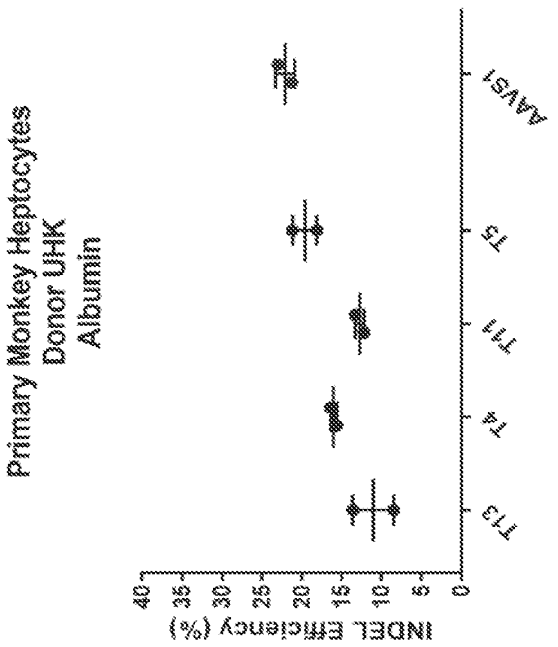


FIG. 7 (CONTINUED)

FIG. 8

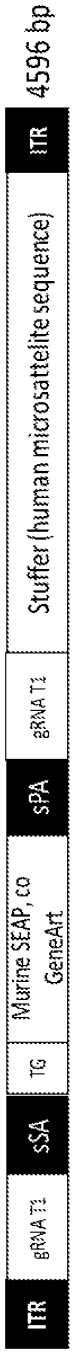
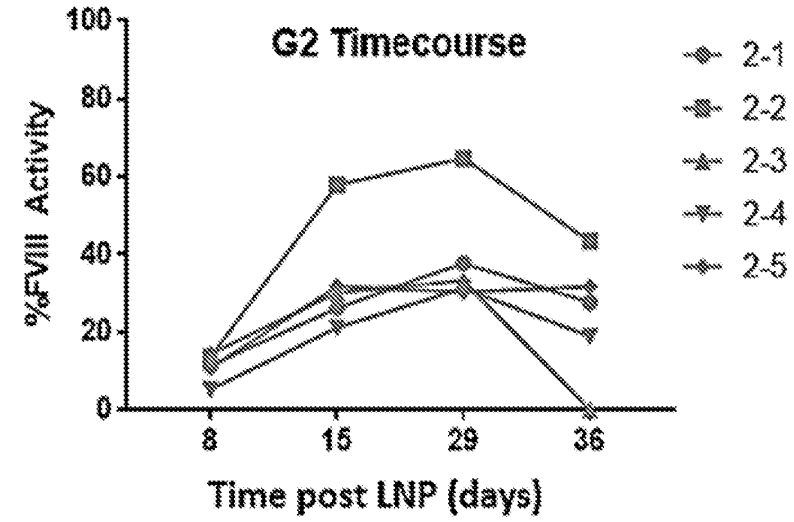
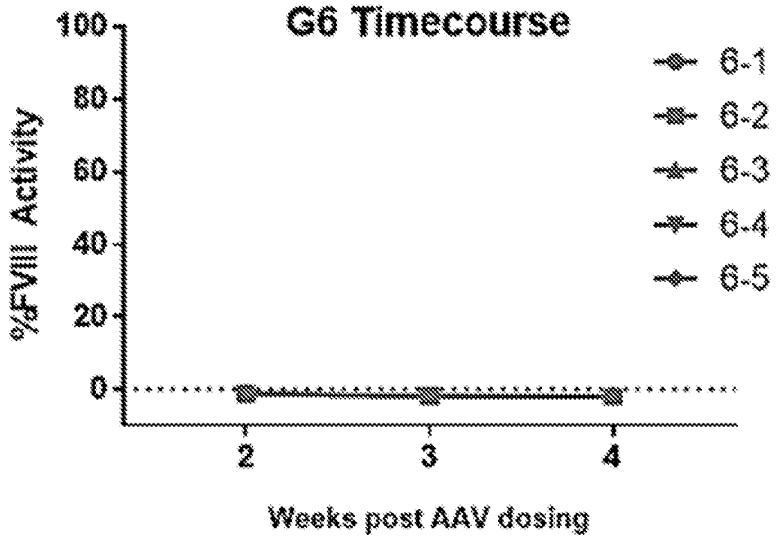

pCB0047
ITR
gRNA T1
sSA
TG
Murine SEAP, co GeneArt
sPA
gRNA T1
Stuffer (human microsatteiite sequence)
ITR
4596 bp

FIG. 9

pCB056
ITR
gRNA T1
mAlb SA
B domain deleted FVIII
UTR
sPA
gRNA T1
ITR

FIG. 10


G2 Timecourse
%FVIII Activity
100
80
60
40
20
0
8
15
29
36
Time post LNP (days)
2-1
2-2
2-3
2-4
2-5


G6 Timecourse
%FVIII Activity
100
80
60
40
20
0
2
3
4
Weeks post AAV dosing
6-1
6-2
6-3
6-4
6-5

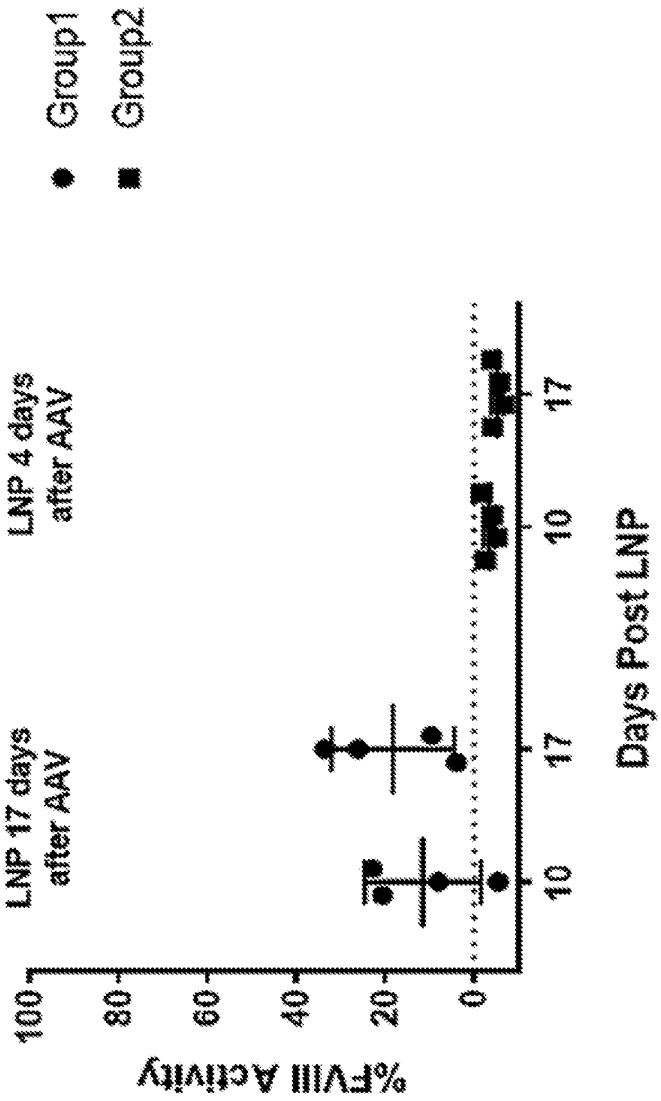

Group1
Group2
LNP 17 days after AAV
LNP 4 days after AAV
%FVIII Activity
100
80
60
40
20
0
10
17
10
17
Days Post LNP

FIG. 11

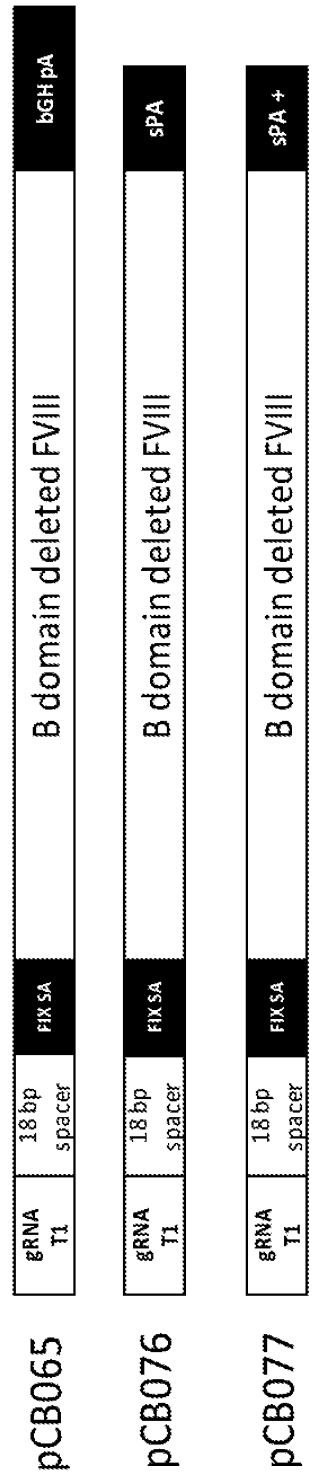

pCB065
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
bGH pA
pCB076
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
sPA
pCB077
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
sPA +

FIG. 12

Group		Sample	Average FVIII value	Average TI value	Ratio
Group 2	BDH	2-1	0.000	0.108	0.00
		2-2	11.034	0.190	58.11
		2-3	0.000	0.094	0.00
		2-4	19.468	0.339	57.40
		2-5	0.000	0.060	0.00
		2-6	3.390	0.295	11.48
Group 3	SPA	3-1	5.716	0.291	19.65
		3-2	0.000	0.043	0.00
		3-3	0.000	0.134	0.00
		3-4	0.175	0.097	1.81
		3-5	0.175	0.123	1.43
		3-6	20.680	0.392	52.82
Group 4	SPA+Spacer	4-1	5.225	0.158	33.23
		4-2	7.338	0.230	31.48
		4-3	25.425	0.521	48.82
		4-4	17.307	0.192	89.98
		4-5	14.038	0.162	86.57
		4-6	0.000	0.125	0.00

FIG. 13

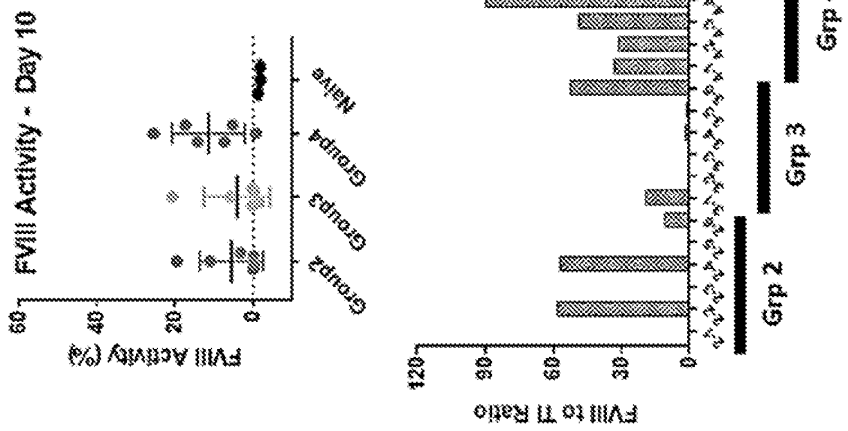


FIG. 14

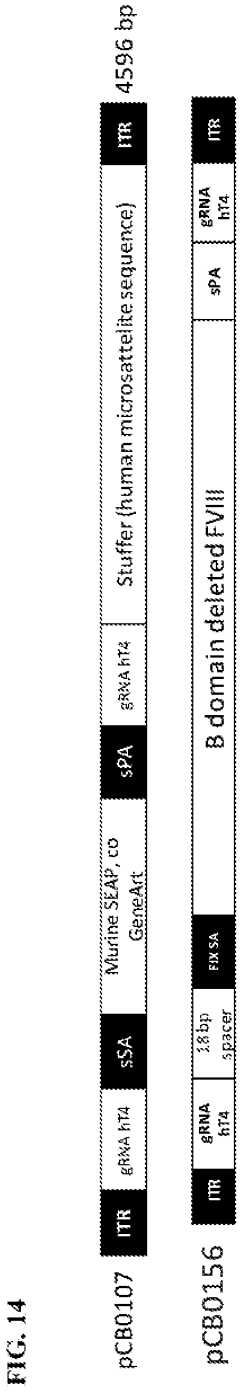

pCB0107
ITR
gRNA hT4
sSA
Murine SEAP, co GeneArt
sPA
gRNA hT4
Stuffer (human microsattelite sequence)
ITR
4596 bp
pCB0156
ITR
gRNA hT4
18 bp spacer
FIX SA
B domain deleted FVIII
sPA
gRNA hT4
ITR

FIG. 15

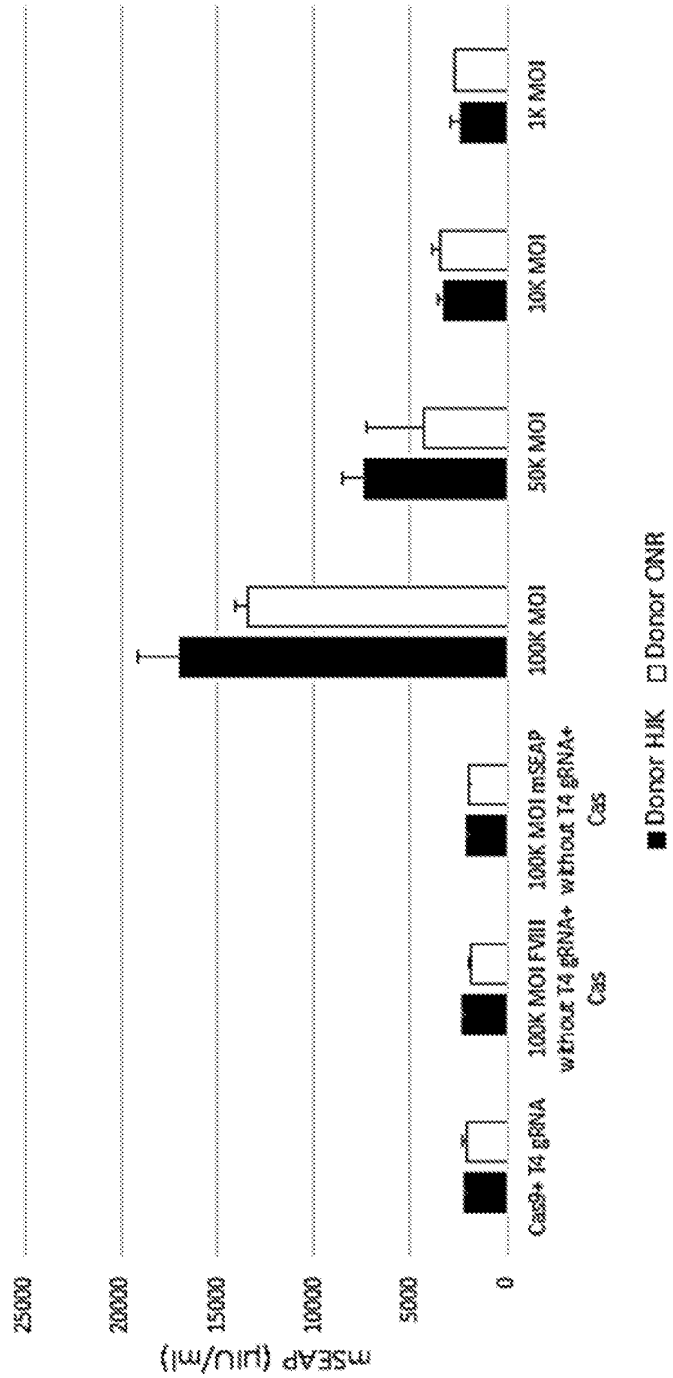
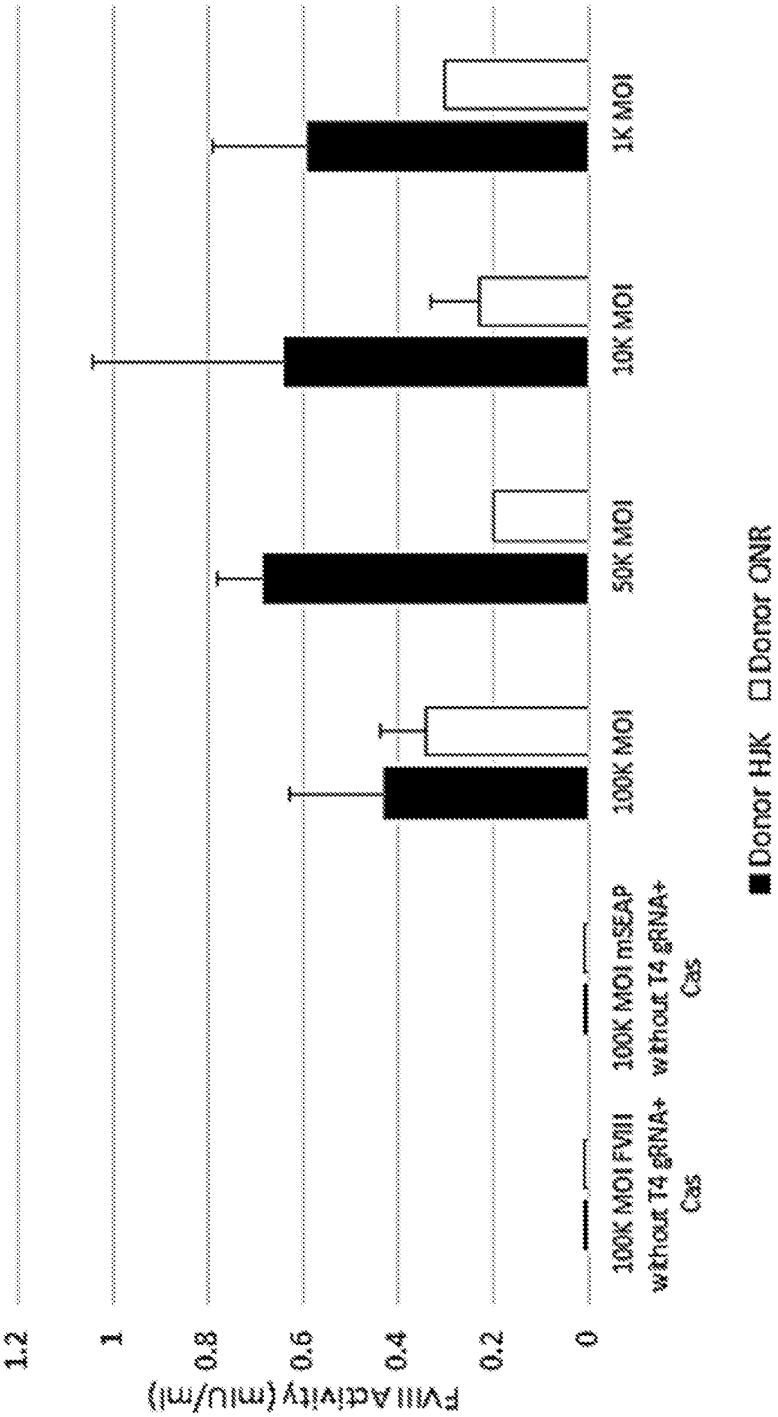

mSEAP (μIU/ml)
25000
20000
15000
10000
5000
0
Cas9+ T4 gRNA
100K MOI FVIII without T4 gRNA+ Cas
100K MOI mSEAP without T4 gRNA+ Cas
100K MOI
50K MOI
10K MOI
1K MOI
Donor HJK
Donor CNR

FIG. 16


FVIII Activity (mIU/ml)
1.2
1
0.8
0.6
0.4
0.2
0
100K MOI FVIII without T4 gRNA+ Cas
100K MOI mSEAP without T4 gRNA+ Cas
100K MOI
50K MOI
10K MOI
1K MOI
Donor HJK
Donor ONR

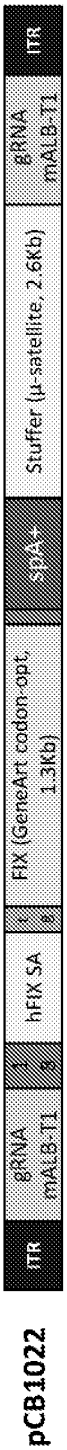


FIG. 17A

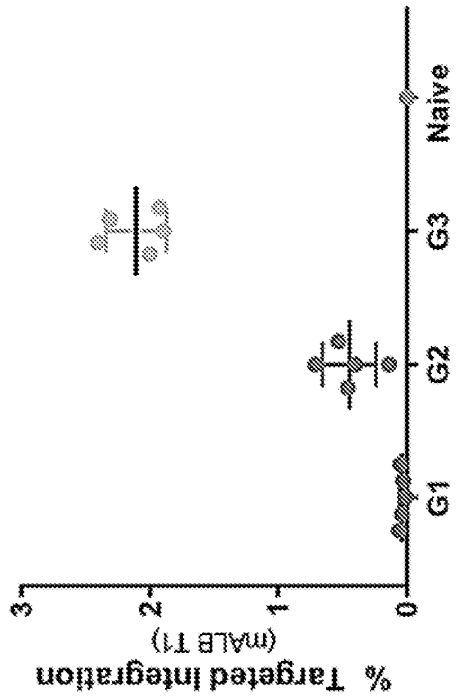


FIG. 17B



FIG. 18A

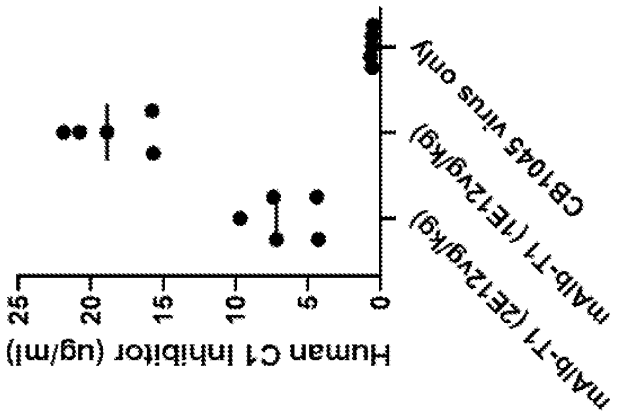


FIG. 18B