FIG. 1

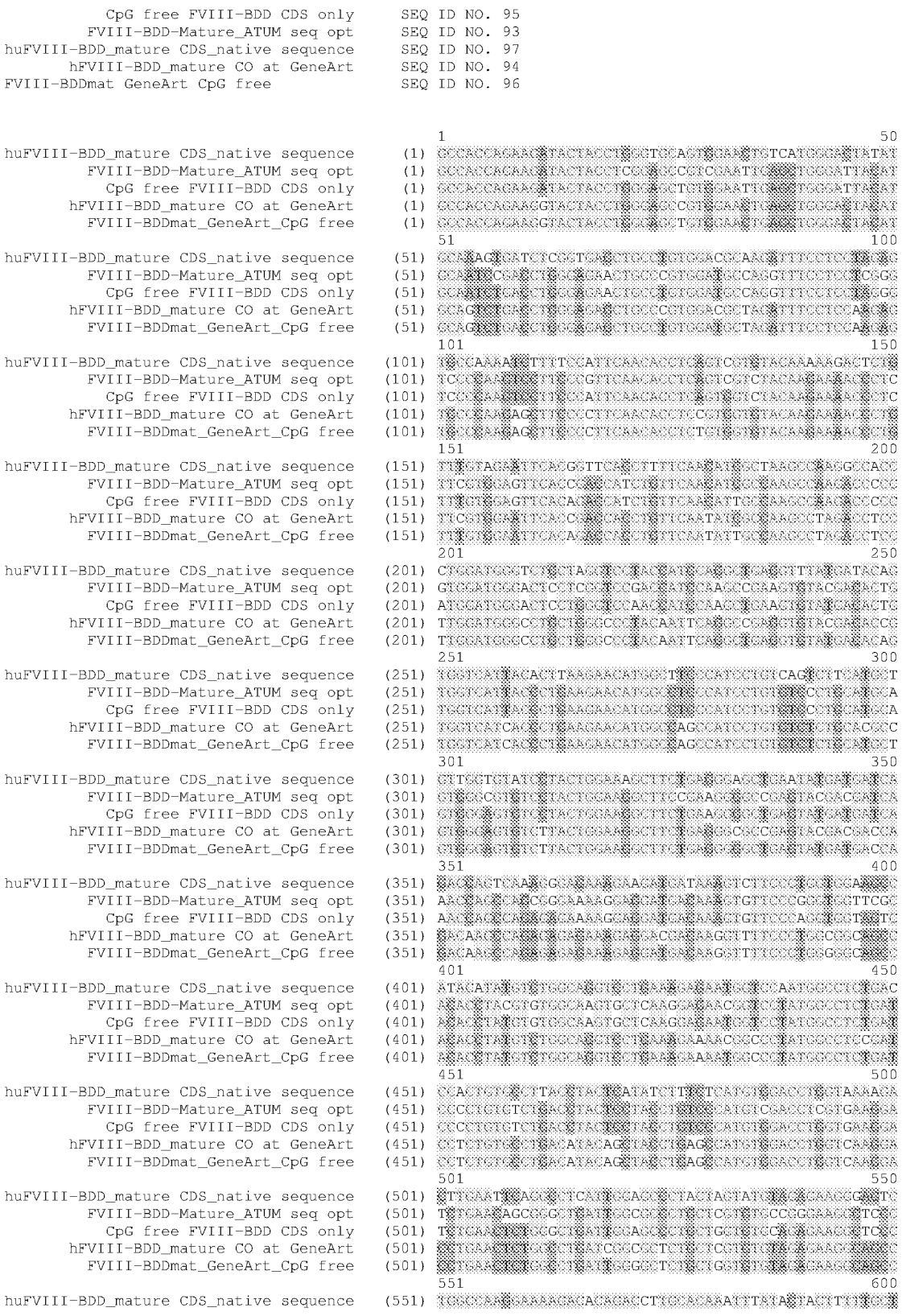

CpG free FVIII-BDD CDS only SEQ ID NO. 95
FVIII-BDD-Mature_ATUM seq opt SEQ ID NO. 93
huFVIII-BDD_mature CDS_native sequence SEQ ID NO. 97
hFVIII-BDD_mature CO at GeneArt SEQ ID NO. 94
FVIII-BDDmat GeneArt CpG free SEQ ID NO. 96
1 50
huFVIII-BDD_mature CDS_native sequence (1)
FVIII-BDD-Mature_ATUM seq opt (1)
CpG free FVIII-BDD CDS only (1)
hFVIII-BDD_mature CO at GeneArt (1)
FVIII-BDDmat_GeneArt_CpG free (1)
51 100
huFVIII-BDD_mature CDS_native sequence (51)
FVIII-BDD-Mature_ATUM seq opt (51)
CpG free FVIII-BDD CDS only (51)
hFVIII-BDD_mature CO at GeneArt (51)
FVIII-BDDmat_GeneArt_CpG free (51)
101 150
huFVIII-BDD_mature CDS_native sequence (101)
FVIII-BDD-Mature_ATUM seq opt (101)
CpG free FVIII-BDD CDS only (101)
hFVIII-BDD_mature CO at GeneArt (101)
FVIII-BDDmat_GeneArt_CpG free (101)
151 200
huFVIII-BDD_mature CDS_native sequence (151)
FVIII-BDD-Mature_ATUM seq opt (151)
CpG free FVIII-BDD CDS only (151)
hFVIII-BDD_mature CO at GeneArt (151)
FVIII-BDDmat_GeneArt_CpG free (151)
201 250
huFVIII-BDD_mature CDS_native sequence (201)
FVIII-BDD-Mature_ATUM seq opt (201)
CpG free FVIII-BDD CDS only (201)
hFVIII-BDD_mature CO at GeneArt (201)
FVIII-BDDmat_GeneArt_CpG free (201)
251 300
huFVIII-BDD_mature CDS_native sequence (251)
FVIII-BDD-Mature_ATUM seq opt (251)
CpG free FVIII-BDD CDS only (251)
hFVIII-BDD_mature CO at GeneArt (251)
FVIII-BDDmat_GeneArt_CpG free (251)
301 350
huFVIII-BDD_mature CDS_native sequence (301)
FVIII-BDD-Mature_ATUM seq opt (301)
CpG free FVIII-BDD CDS only (301)
hFVIII-BDD_mature CO at GeneArt (301)
FVIII-BDDmat_GeneArt_CpG free (301)
351 400
huFVIII-BDD_mature CDS_native sequence (351)
FVIII-BDD-Mature_ATUM seq opt (351)
CpG free FVIII-BDD CDS only (351)
hFVIII-BDD_mature CO at GeneArt (351)
FVIII-BDDmat_GeneArt_CpG free (351)
401 450
huFVIII-BDD_mature CDS_native sequence (401)
FVIII-BDD-Mature_ATUM seq opt (401)
CpG free FVIII-BDD CDS only (401)
hFVIII-BDD_mature CO at GeneArt (401)
FVIII-BDDmat_GeneArt_CpG free (401)
451 500
huFVIII-BDD_mature CDS_native sequence (451)
FVIII-BDD-Mature_ATUM seq opt (451)
CpG free FVIII-BDD CDS only (451)
hFVIII-BDD_mature CO at GeneArt (451)
FVIII-BDDmat_GeneArt_CpG free (451)
501 550
huFVIII-BDD_mature CDS_native sequence (501)
FVIII-BDD-Mature_ATUM seq opt (501)
CpG free FVIII-BDD CDS only (501)
hFVIII-BDD_mature CO at GeneArt (501)
FVIII-BDDmat_GeneArt_CpG free (501)
551 600
huFVIII-BDD_mature CDS_native sequence (551)

FIG. 1 (CONTINUED)

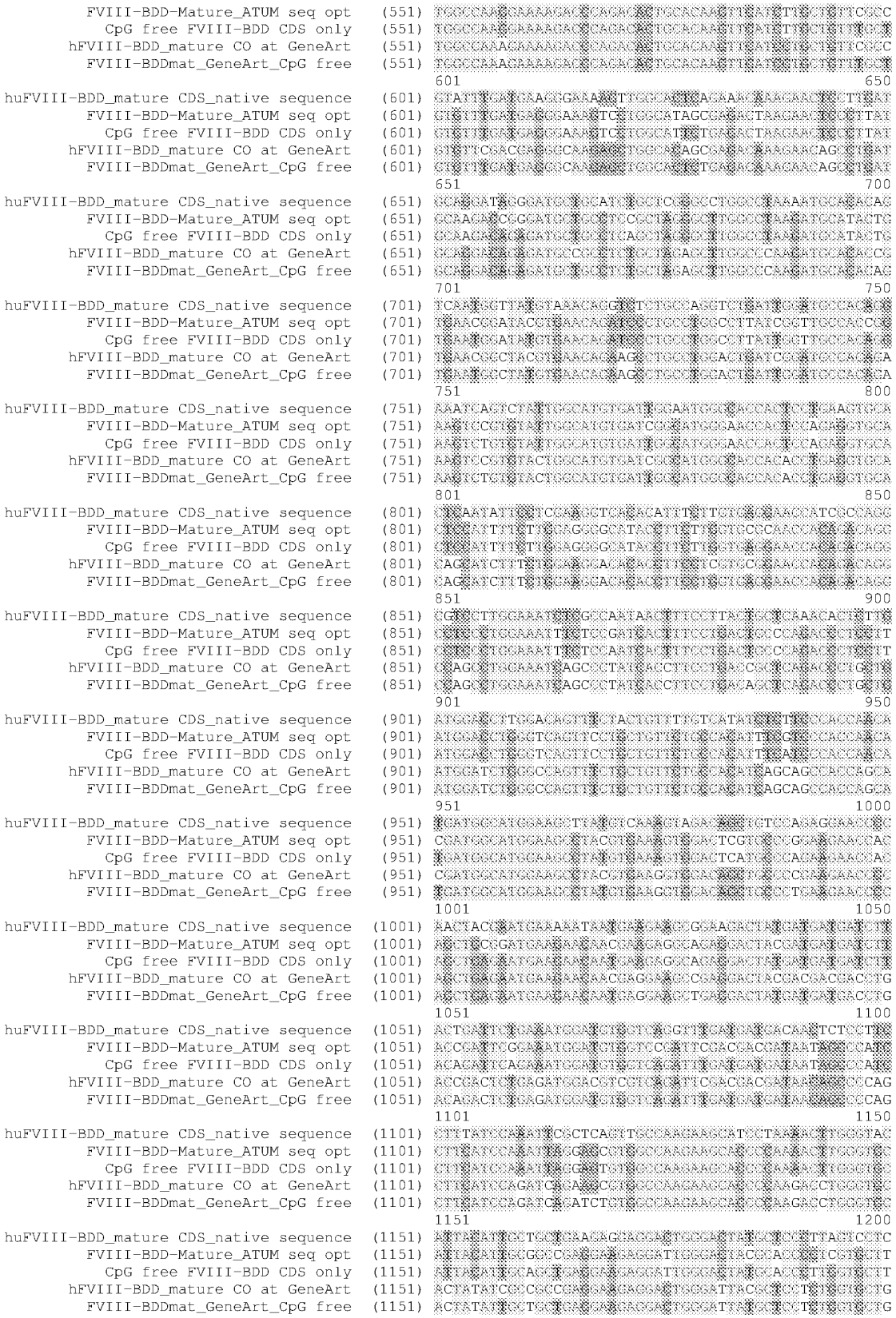


FIG. 1 (CONTINUED)

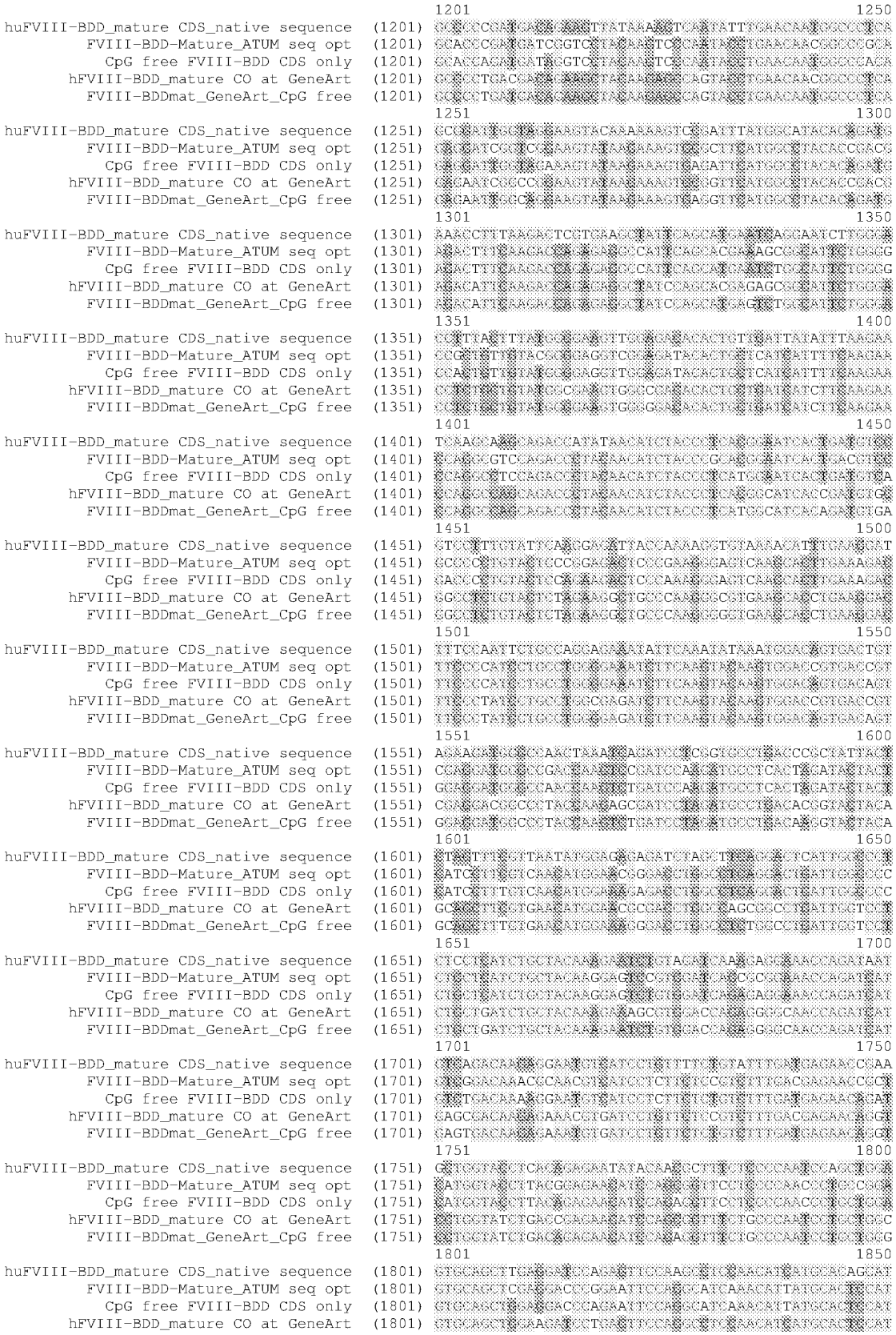


FIG. 1 (CONTINUED)

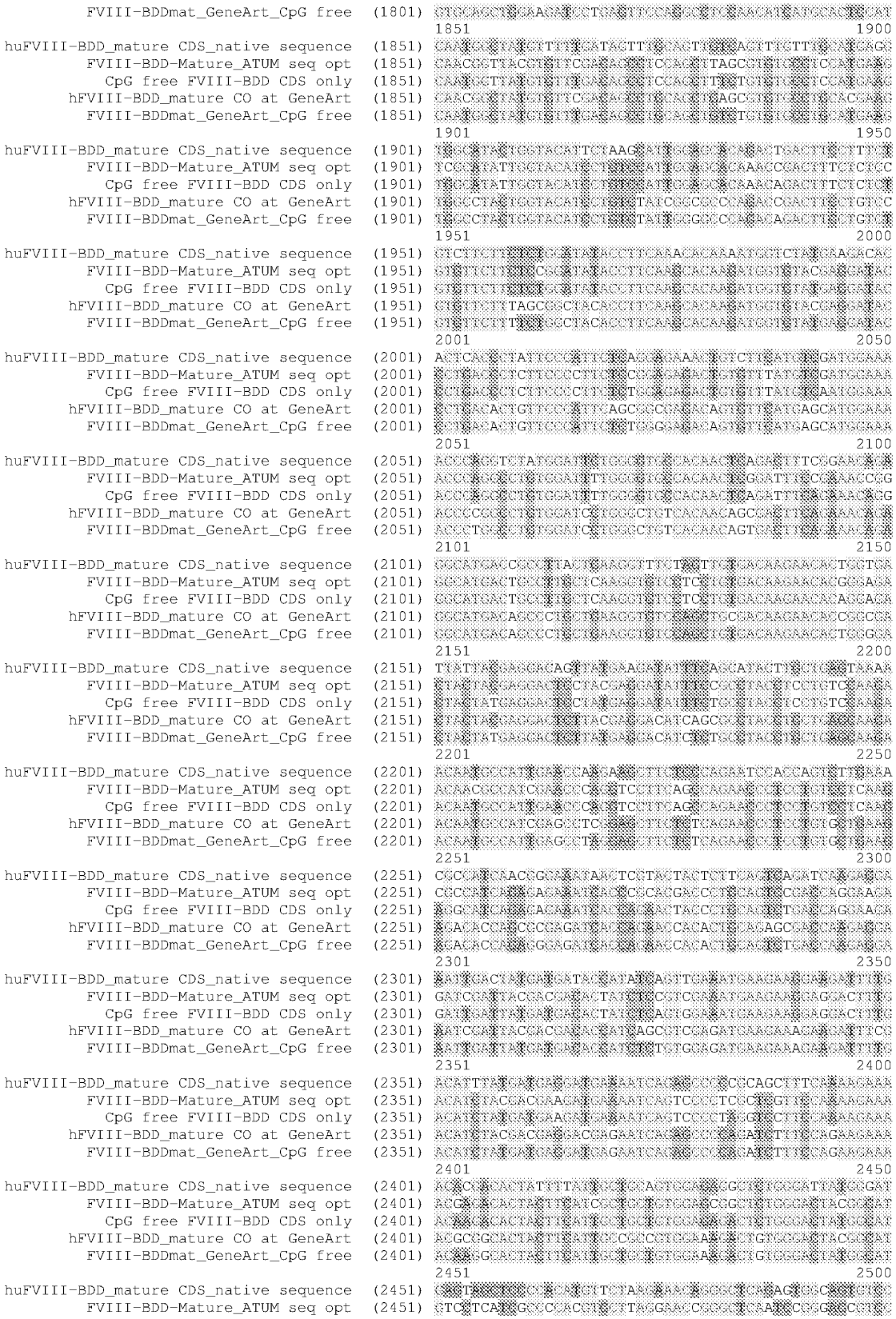


FIG. 1 (CONTINUED)

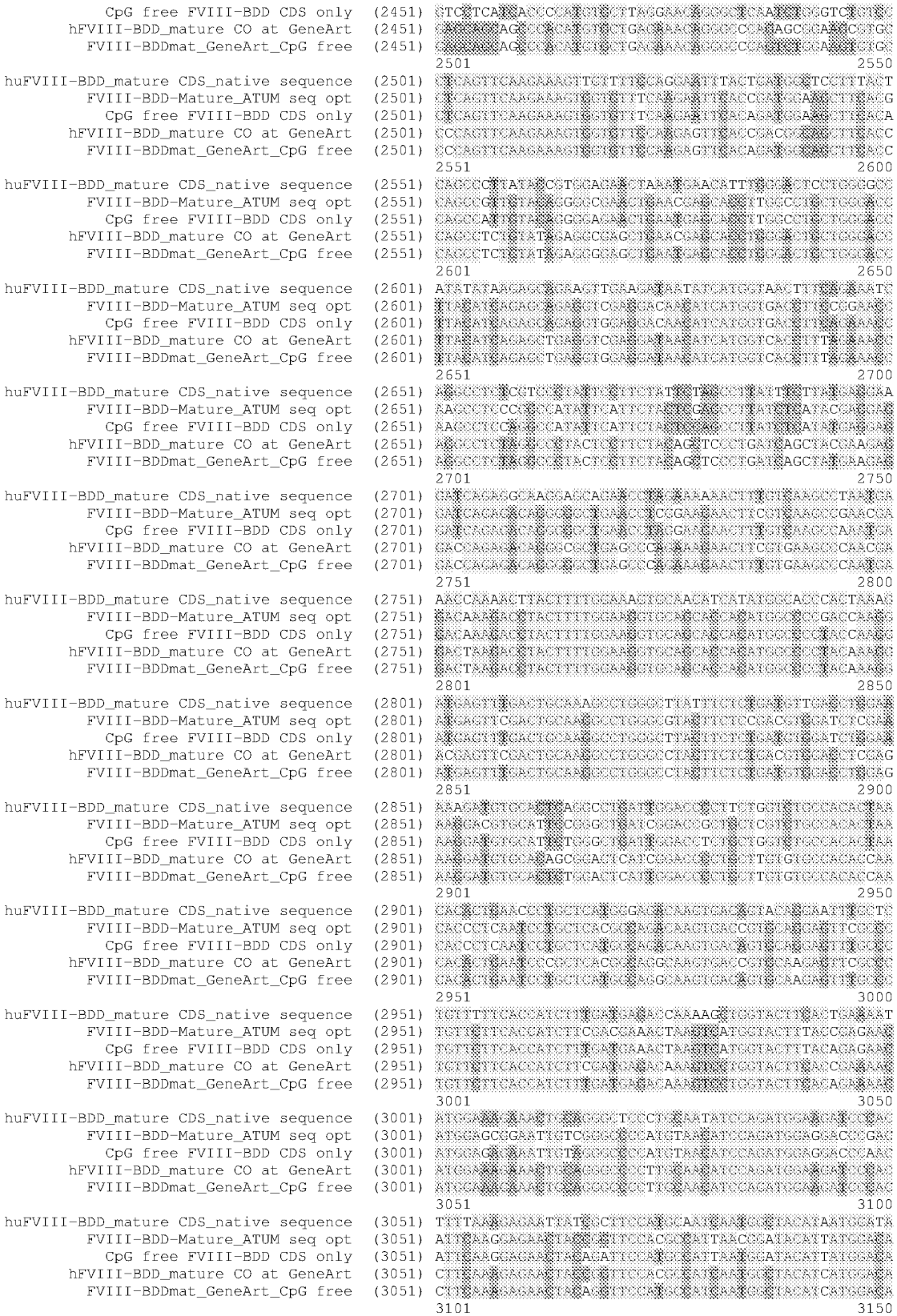


FIG. 1 (CONTINUED)

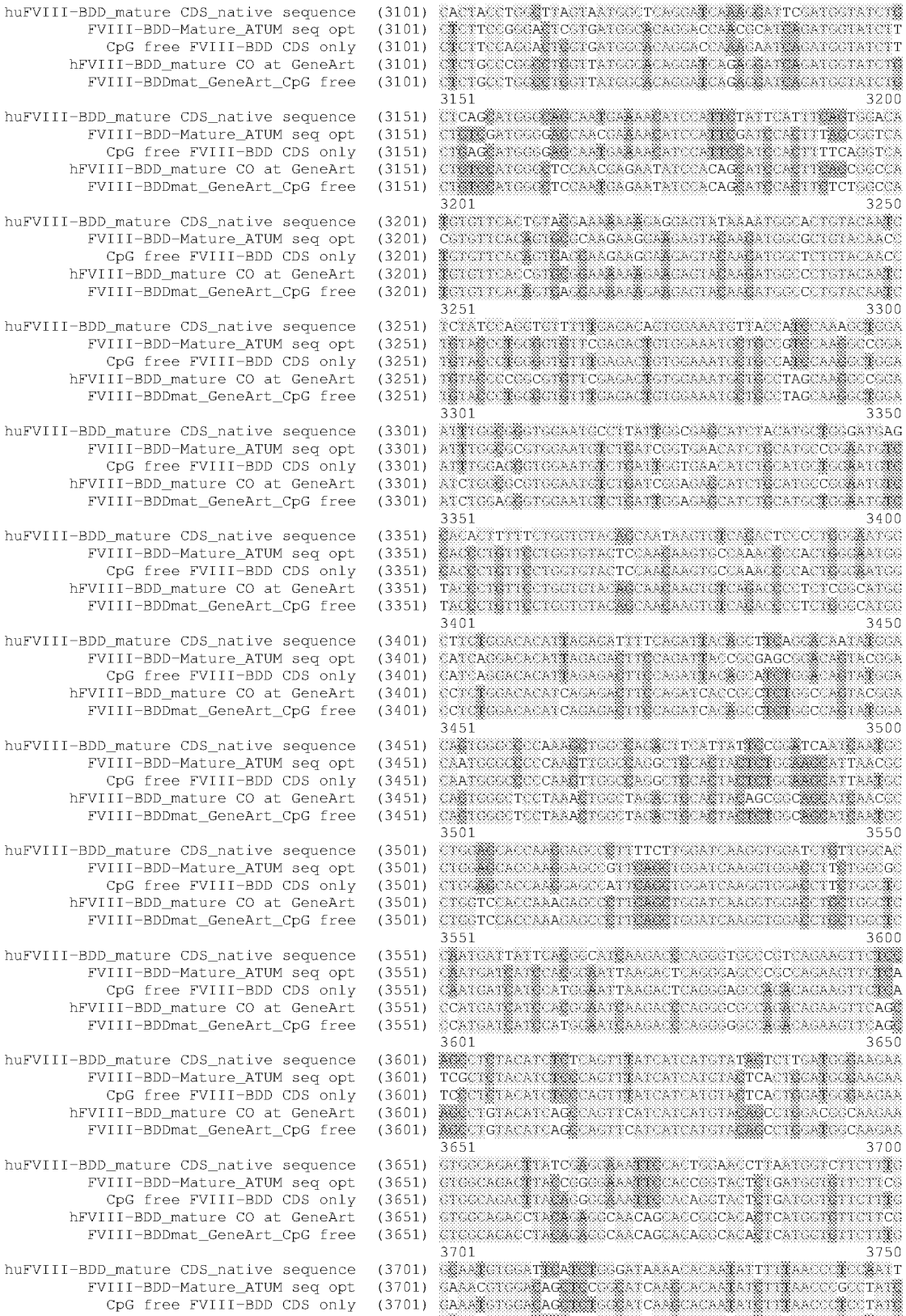


FIG. 1 (CONTINUED)

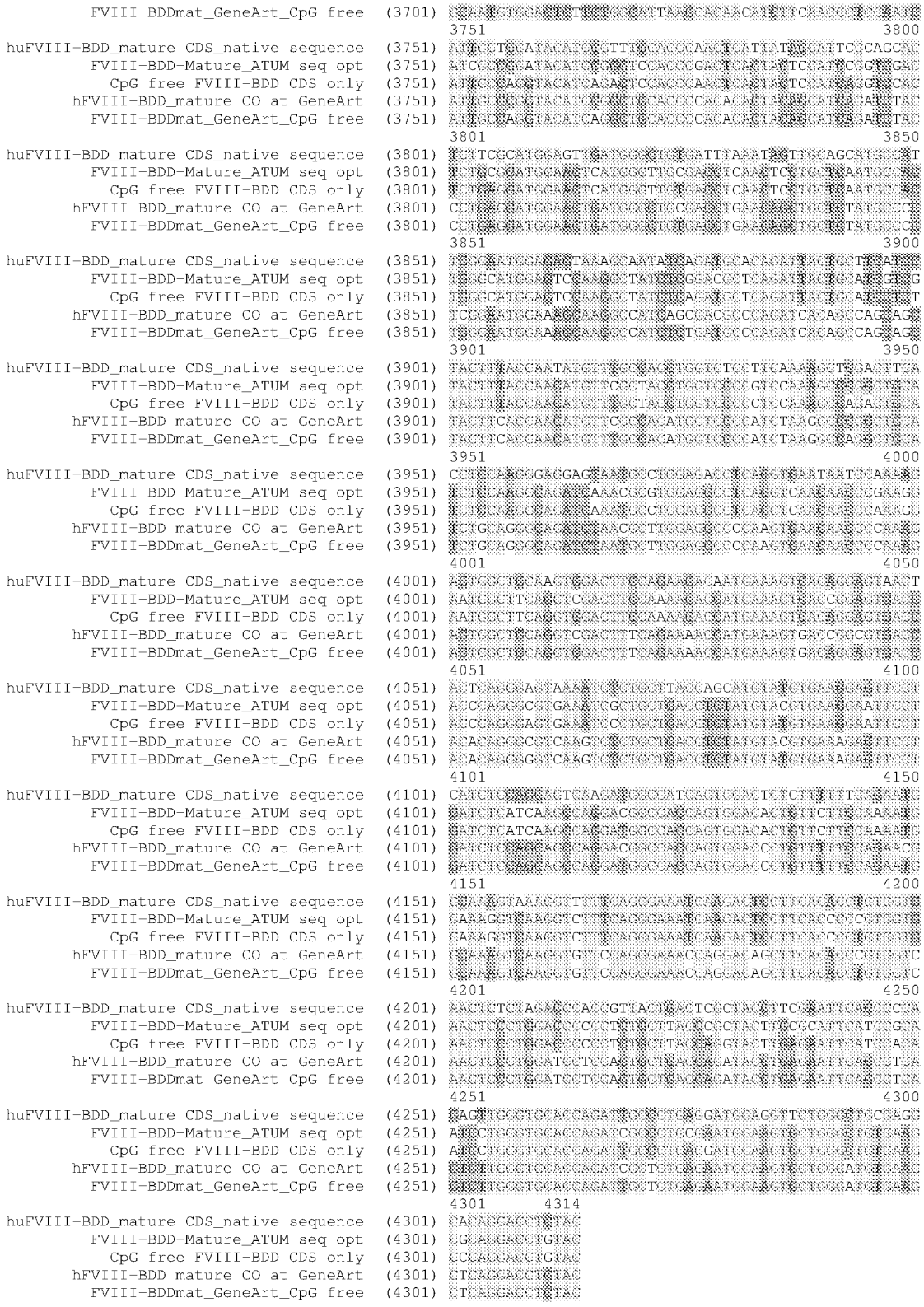


FIG. 2

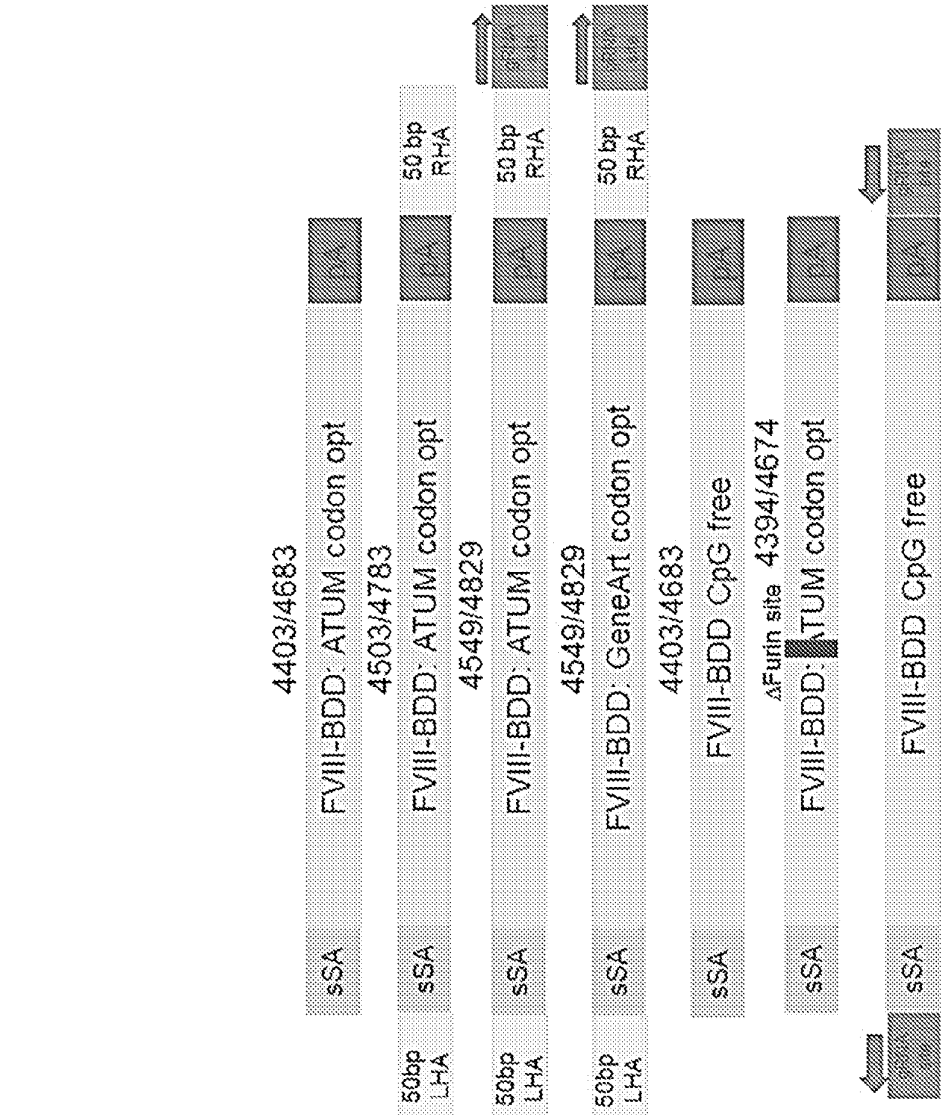
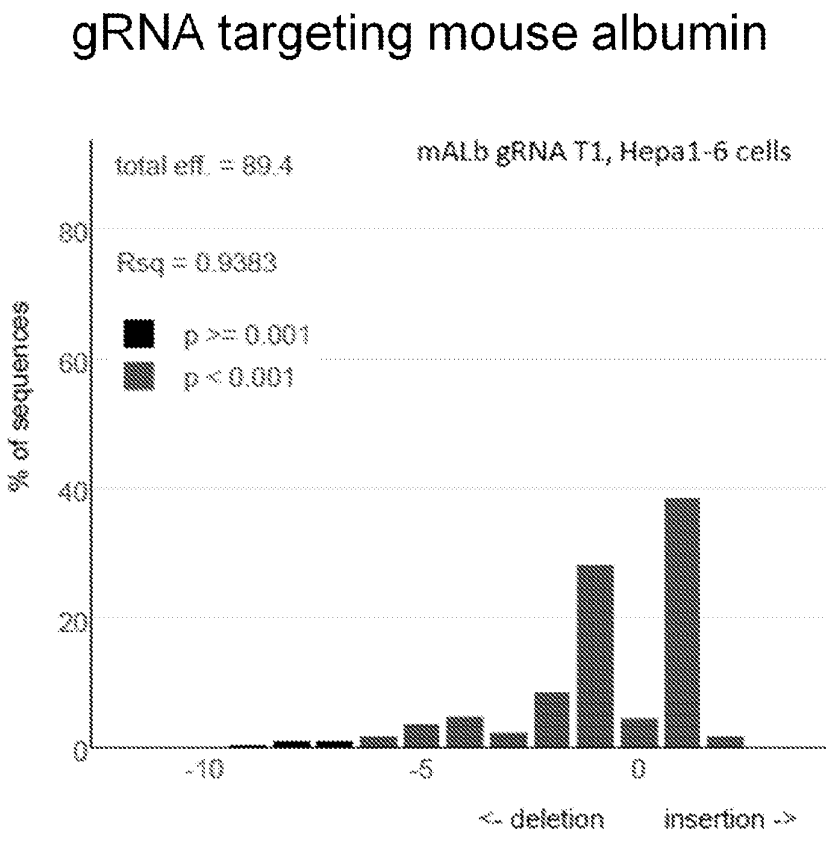

"MAB8A"
4403/4683
sSA
FVIII-BDD: ATUM codon opt
"MAB8B"
4503/4783
50bp LHA
sSA
FVIII-BDD: ATUM codon opt
50 bp RHA
"MAB8C"
4549/4829
50bp LHA
sSA
FVIII-BDD: ATUM codon opt
50 bp RHA
"MAB8D" (aka CGA)
4549/4829
50bp LHA
sSA
FVIII-BDD: GeneArt codon opt
50 bp RHA
"MAB8E"
4403/4683
sSA
FVIII-BDD CpG free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

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 ->

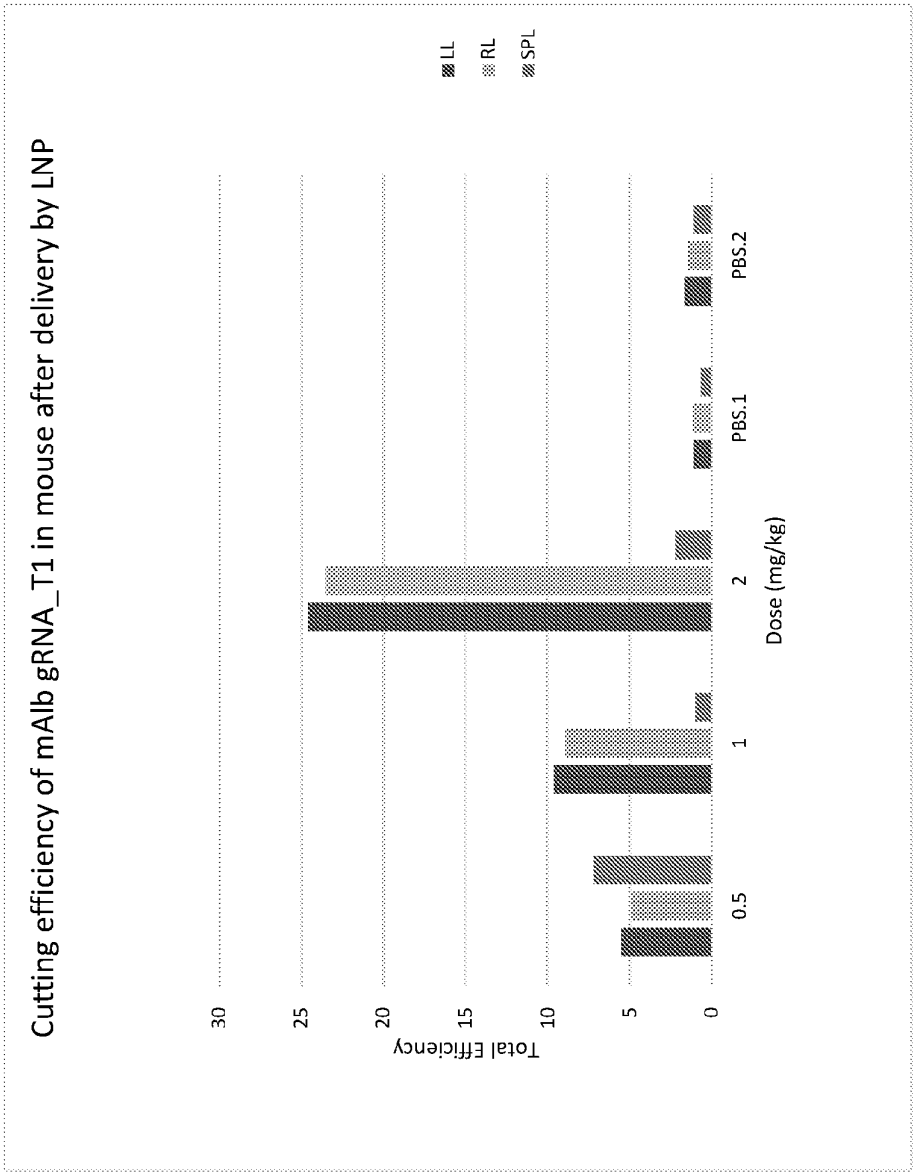

Cutting efficiency of mAlb gRNA_T1 in mouse after delivery by LNP
LL
RL
SPL
Total Efficiency
30
25
20
15
10
5
0
0.5
1
2
PBS.1
PBS.2
Dose (mg/kg)

FIG. 4

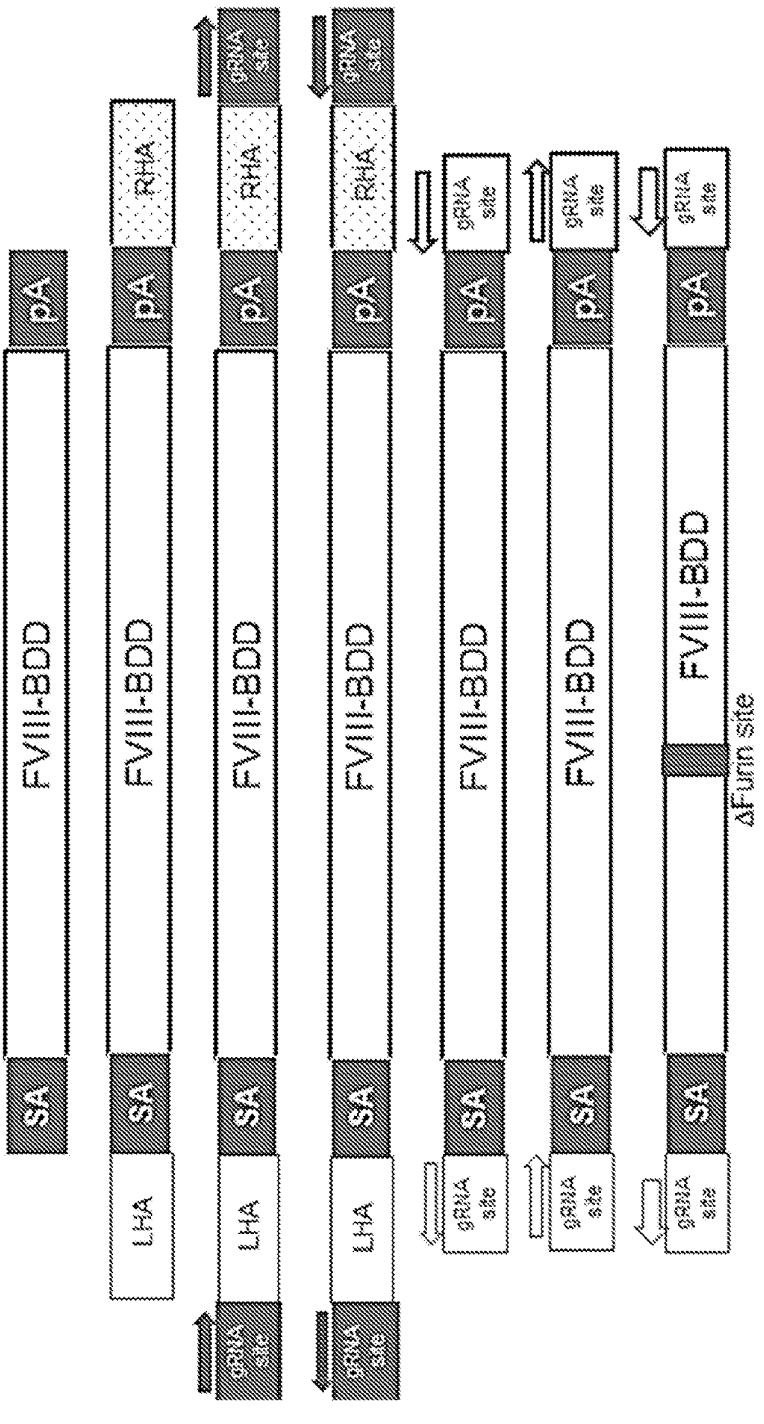


FIG. 5

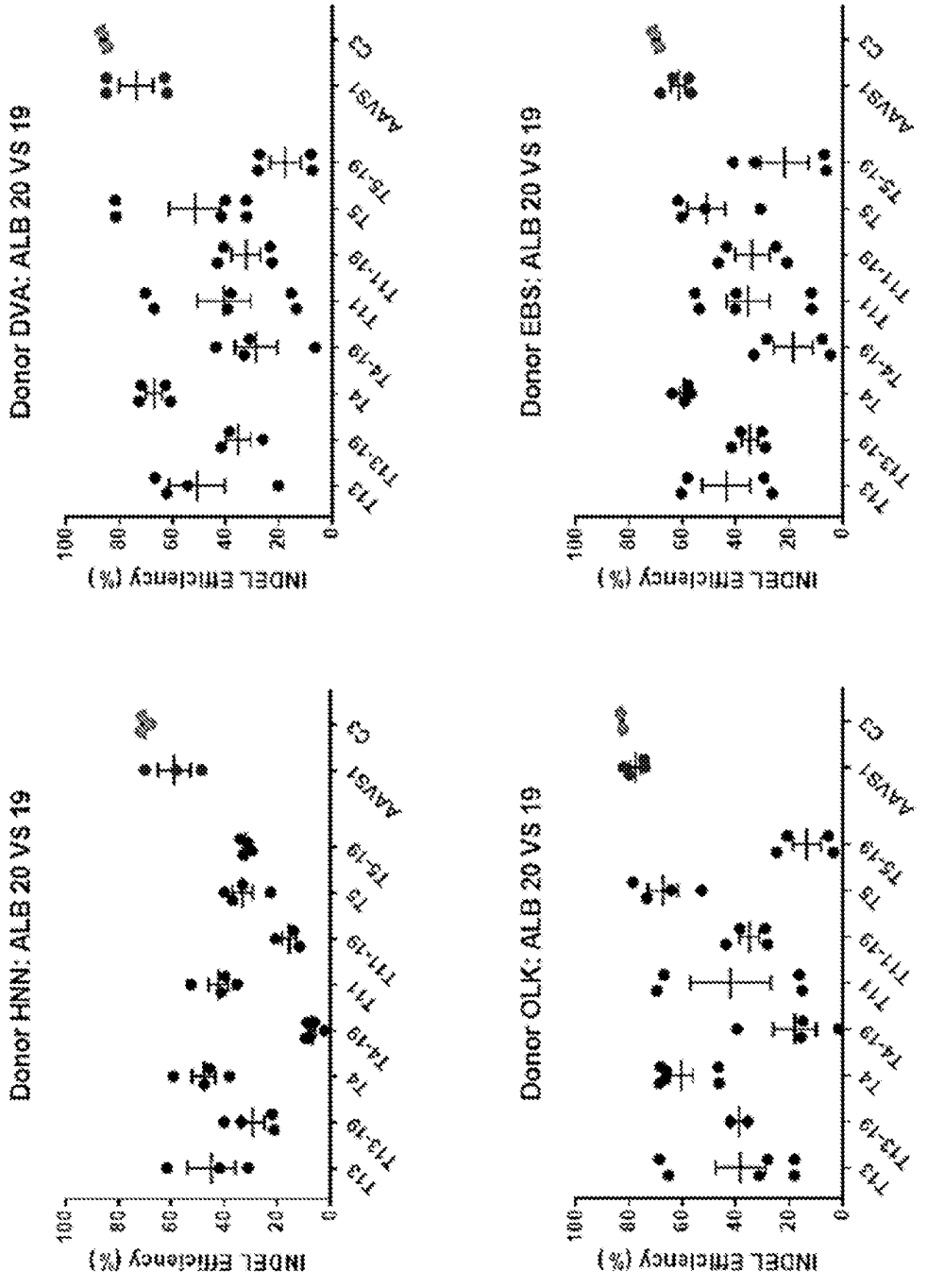

Donor DVA: ALB 20 VS 19
Donor EBS: ALB 20 VS 19
Donor HNN: ALB 20 VS 19
Donor OLK: ALB 20 VS 19
INDEL Efficiency (%)
100
80
60
40
20
0
T13
T13-19
T4
T4-19
T11
T11-19
T5
T5-19
AAVS1
C3

FIG. 6

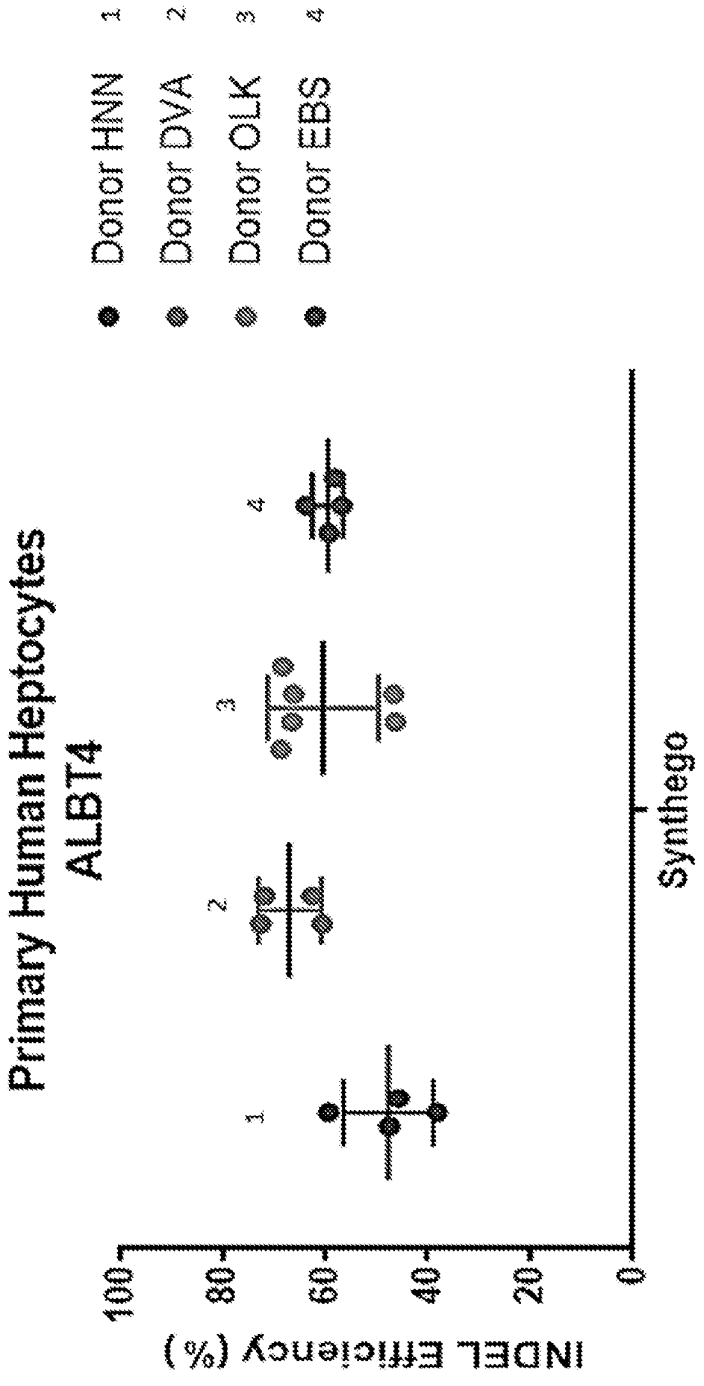

Primary Human Heptocytes
ALBT4
Donor HNN
Donor DVA
Donor OLK
Donor EBS
1
2
3
4
INDEL Efficiency (%)
100
80
60
40
20
0
Synthego

FIG. 7

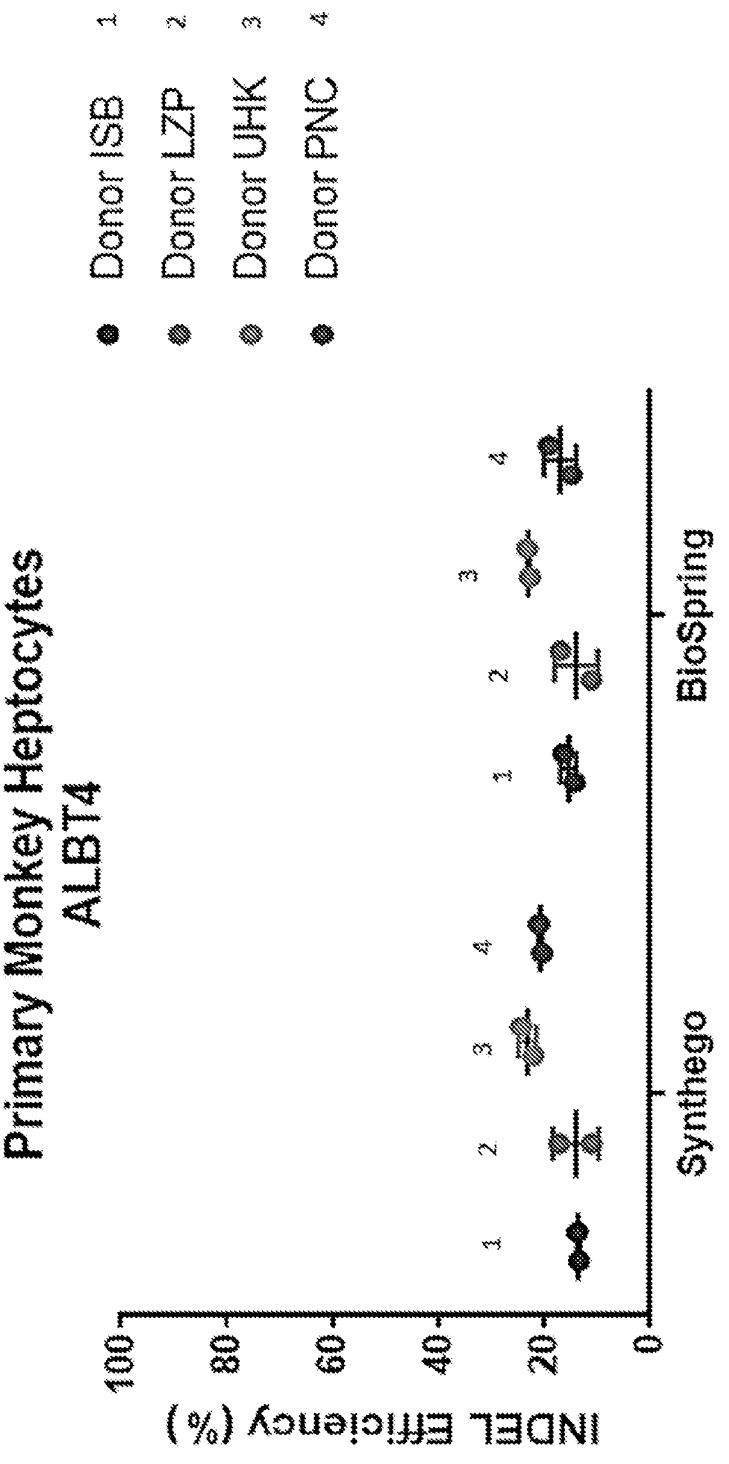
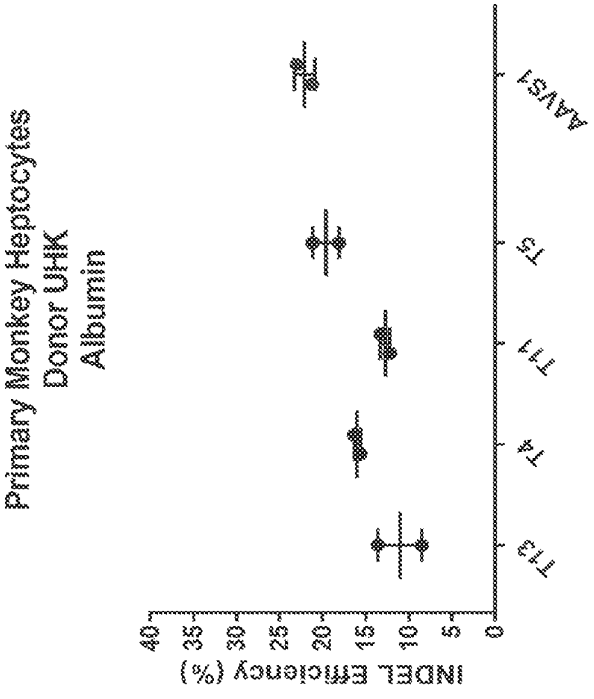


FIG. 7 (CONTINUED)

FIG. 7 (CONTINUED)



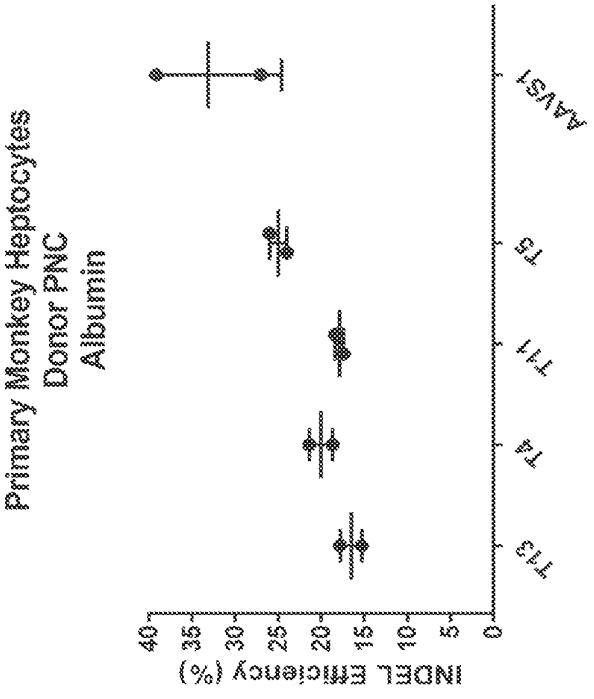


FIG. 8

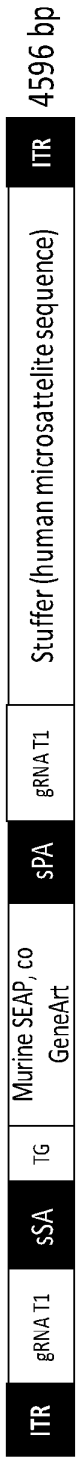
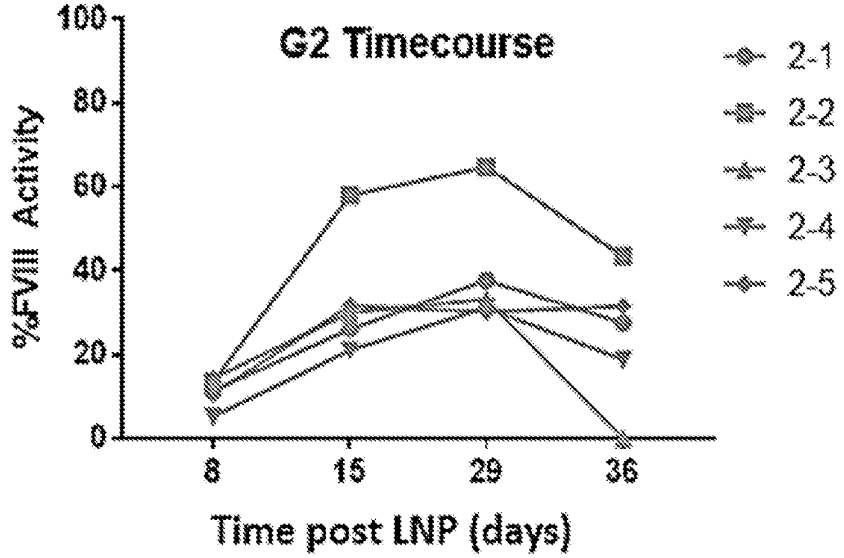
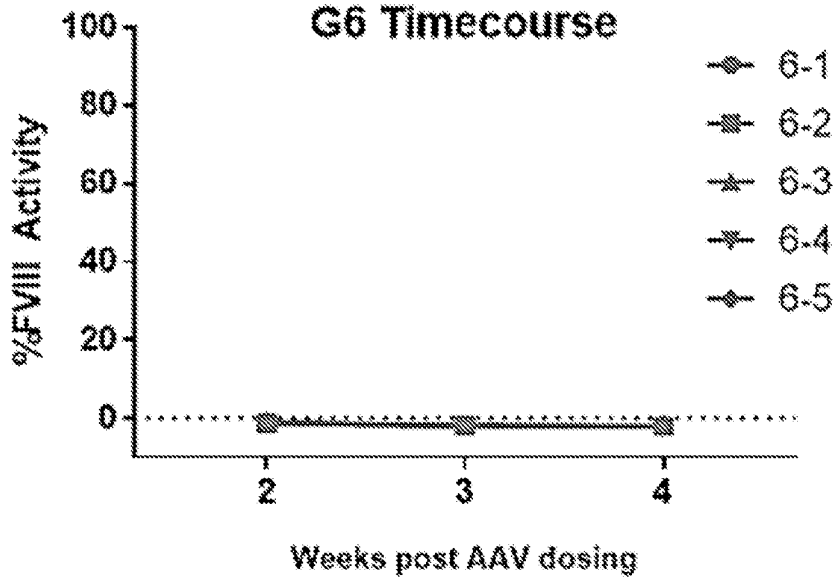

pCB0047
ITR
gRNA T1
sSA
TG
Murine SEAP, co GeneArt
sPA
gRNA T1
Stuffer (human microsattelite sequence)
ITR
4596 bp

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

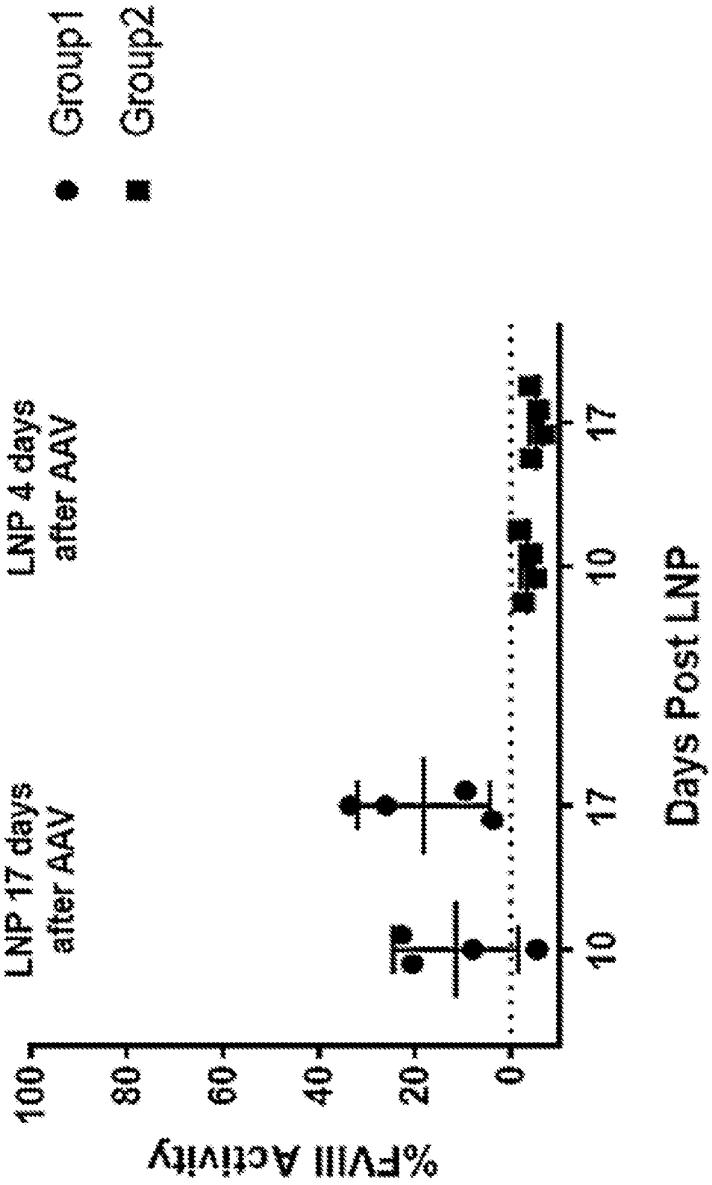

Group1
Group2
LNP 4 days after AAV
LNP 17 days after AAV
%FVIII Activity
100
80
60
40
20
0
10
17
10
17
Days Post LNP

FIG. 11

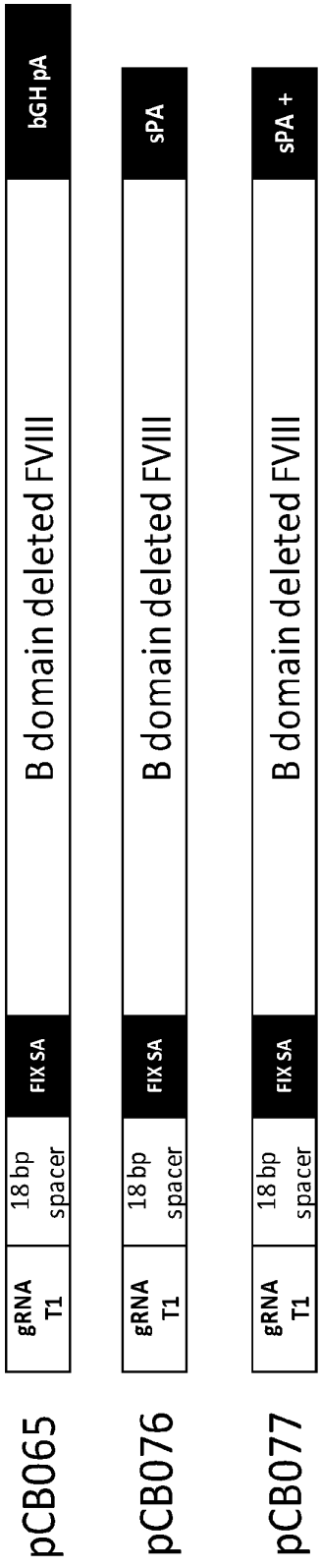
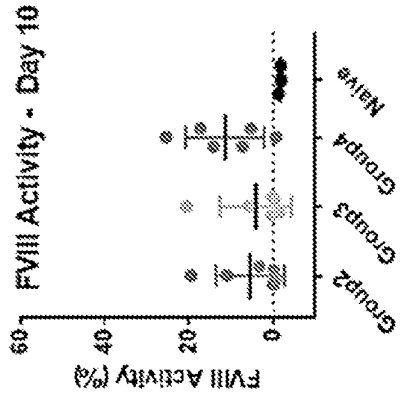

pCB065
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
bGH pA
pCB076
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
sPA
pCB077
gRNA T1
18 bp spacer
FIX SA
B domain deleted FVIII
sPA +

FIG. 12

Group		Sample	Average FVIII value	Average TI value	Ratio
Group 2	BGH	2-1	0.000	0.108	0.00
		2-2	11.034	0.190	58.11
		2-3	0.000	0.090	0.00
		2-4	19.468	0.339	57.43
		2-5	0.000	0.060	0.00
		2-6	3.396	0.295	11.48
Group 3	sPA	3-1	5.710	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	1.42
		3-6	20.680	0.392	52.82
Group 4	sPA+Spacer	4-1	5.225	0.158	33.22
		4-2	7.238	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



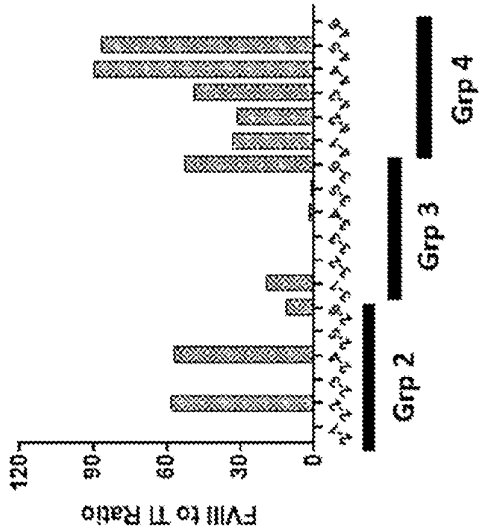


FIG. 14


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

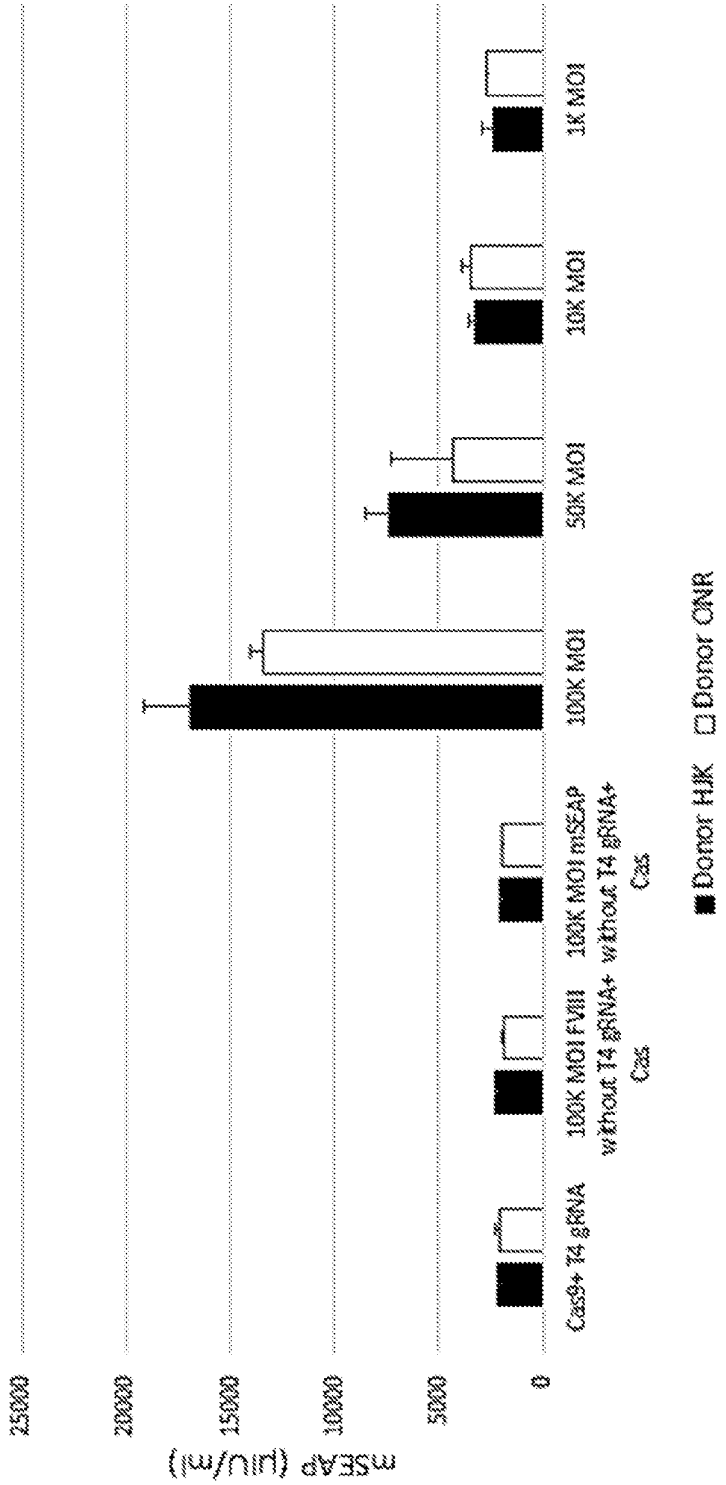
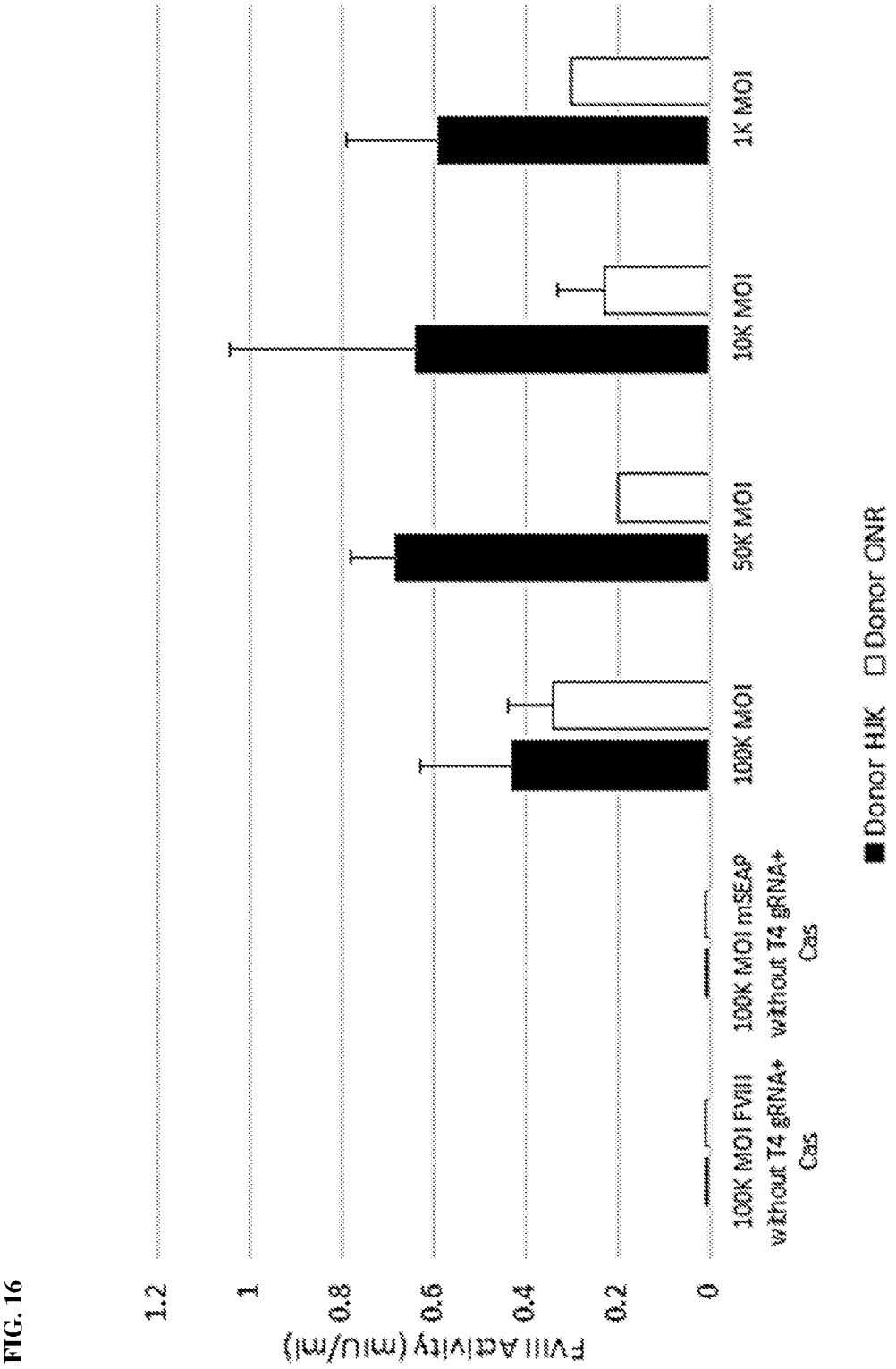

mSEAP (μIU/ml)
25000
20000
15000
10000
5000
0
Cas9+ T4 gRNA
100K MOI FVIII without T4 gRNA+ Cas
100K MOI mSEAP without T4 gRNA+ Cas
100K MOI
50K MOI
10K MOI
1K MOI
Donor HJK
Donor CNR

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 CNR