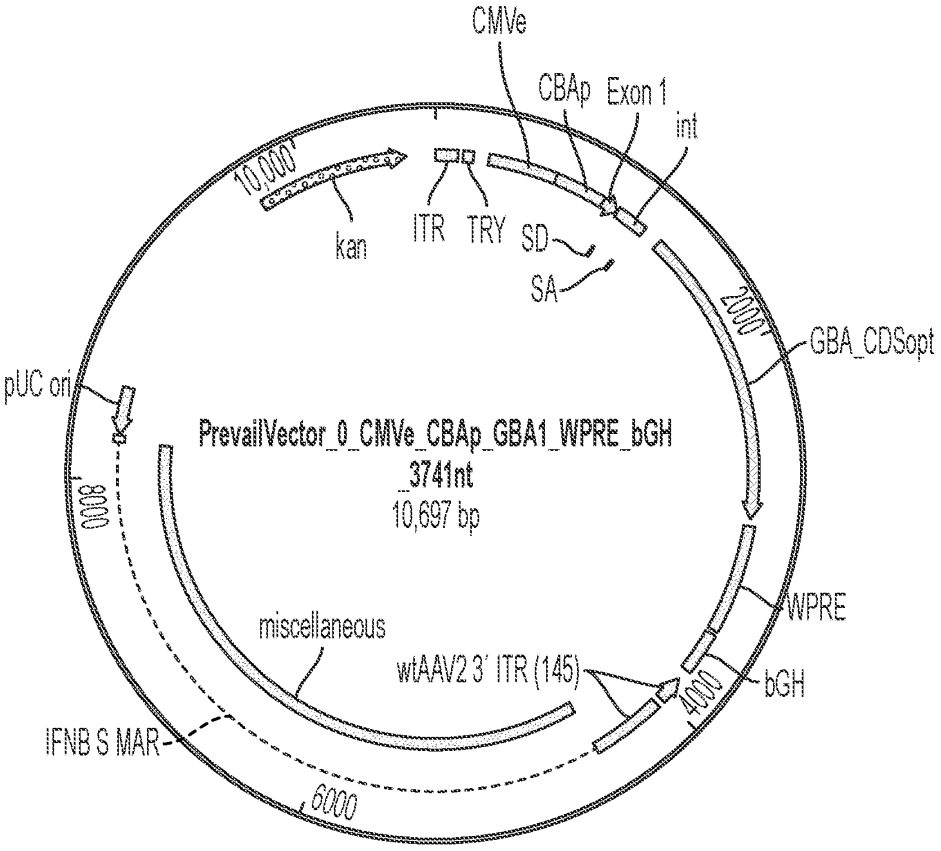

CMVe
CBAp
Exon 1
int
10,000
kan
ITR
TRY
SD
SA
2000
GBA_CDSopt
pUC ori
PrevailVector_0_CMVe_CBAp_GBA1_WPRE_bGH
_3741nt
10,697 bp
8000
miscellaneous
WPRE
wtAAV2 3' ITR (145)
4000
bGH
IFNB S MAR
6000

FIG. 1

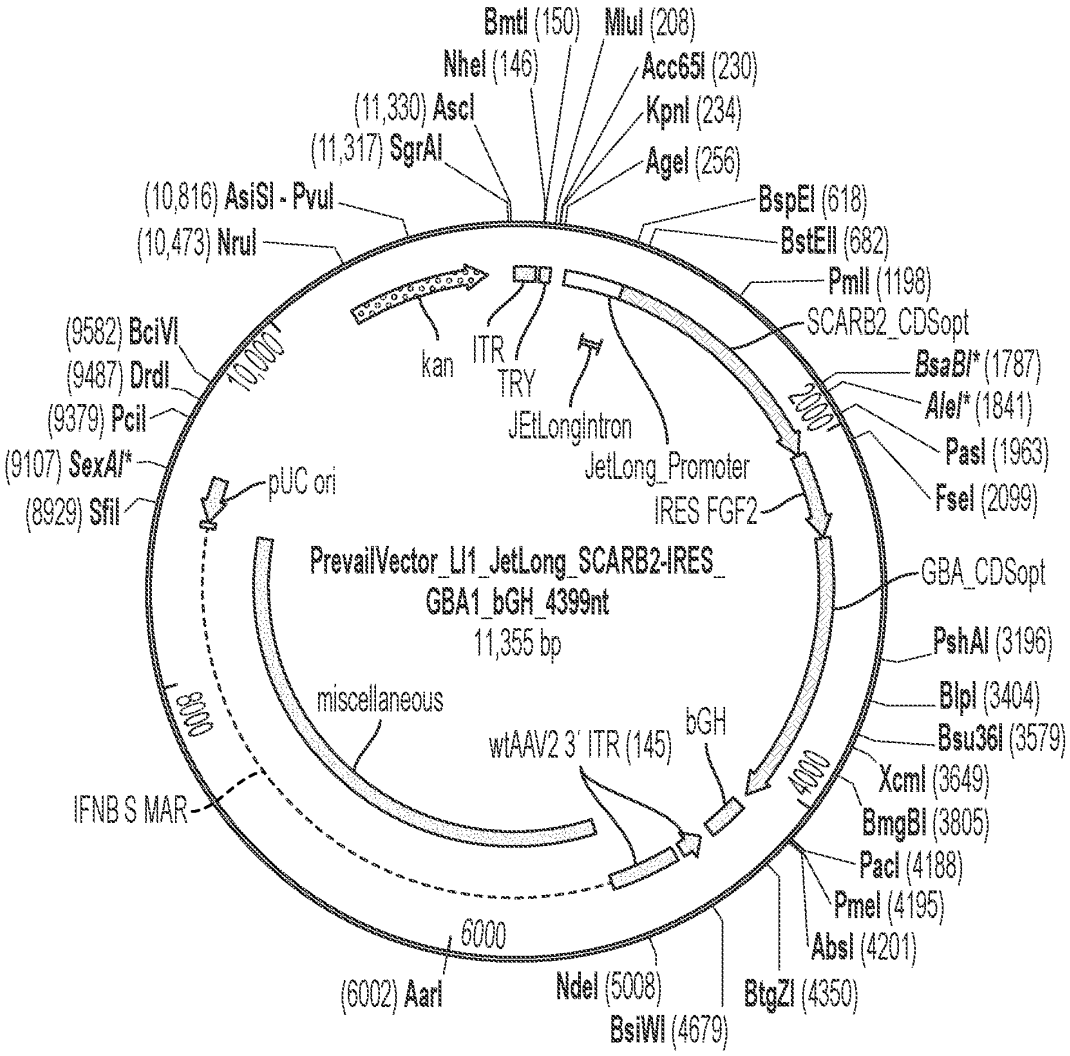
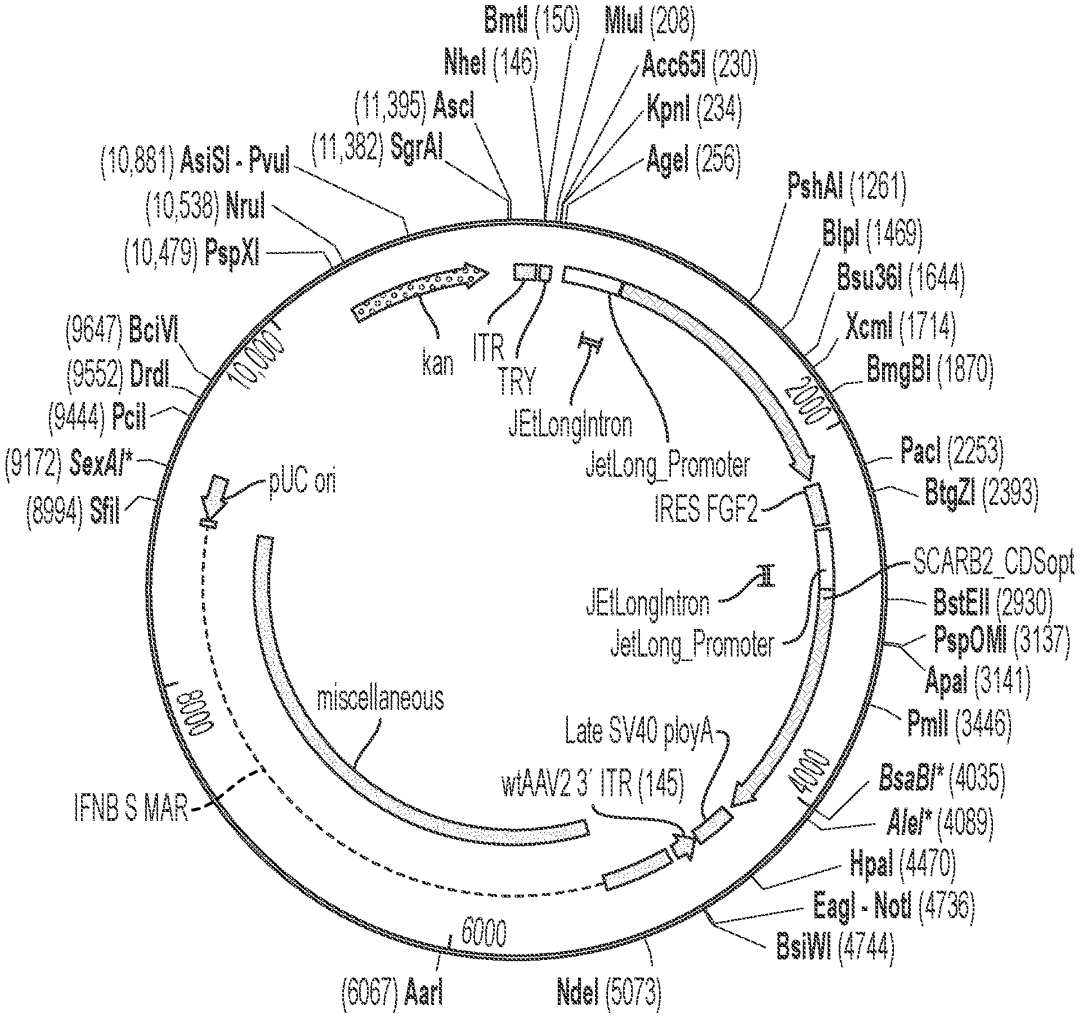

BmtI (150)
MluI (208)
NheI (146)
Acc65I (230)
(11,330) AscI
KpnI (234)
(11,317) SgrAI
AgeI (256)
(10,816) AsiSI - PvuI
BspEI (618)
(10,473) NruI
BstEII (682)
PmlI (1198)
SCARB2_CDSopt
(9582) BciVI
(9487) DrdI
(9379) PciI
(9107) SexAI*
(8929) SfiI
kan
ITR
TRY
JEtLongIntron
JetLong_Promoter
IRES FGF2
BsaBI* (1787)
AleI* (1841)
PasI (1963)
FseI (2099)
pUC ori
PrevailVector_LI1_JetLong_SCARB2-IRES_
GBA1_bGH_4399nt
11,355 bp
GBA_CDSopt
PshAI (3196)
BlpI (3404)
Bsu36I (3579)
miscellaneous
wtAAV2 3' ITR (145)
bGH
XcmI (3649)
BmgBI (3805)
IFNB S MAR
PacI (4188)
PmeI (4195)
AbsI (4201)
(6002) AarI
NdeI (5008)
BtgZI (4350)
BsiWI (4679)
10,000
2000
8000
4000
6000

FIG. 2



PrevailVector_FP1_JetLong_GBA1_bGH_JetLong_SCARB2_SV40L_4464nt
11,420 bp

FIG. 3

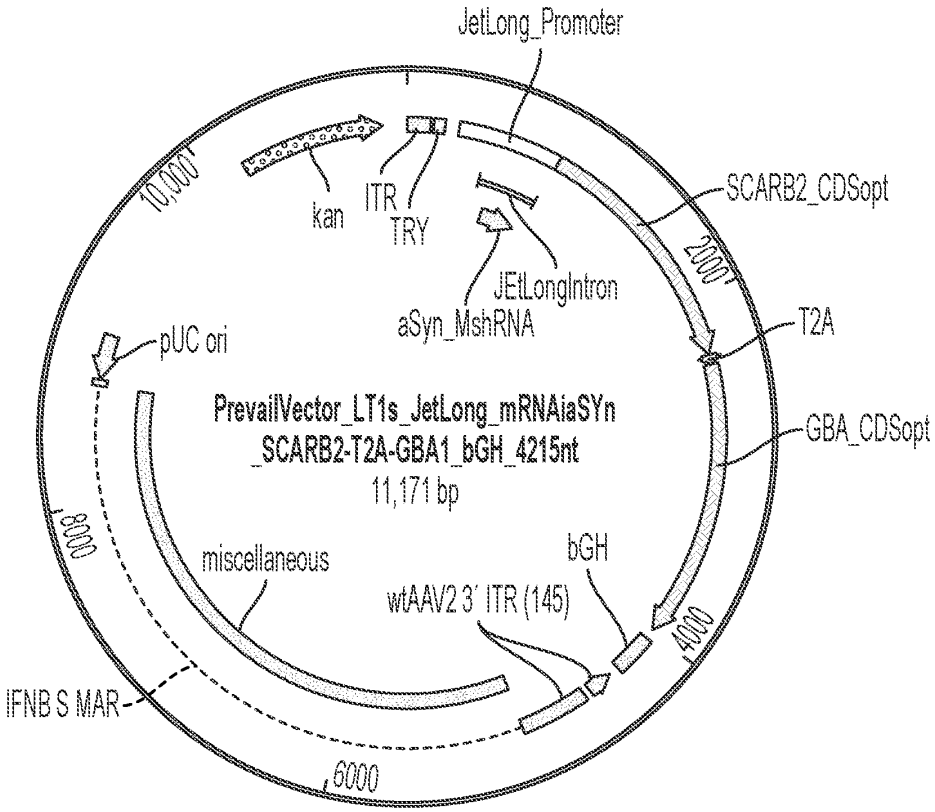

JetLong_Promoter
SCARB2_CDSopt
T2A
GBA_CDSopt
bGH
wtAAV2 3' ITR (145)
miscellaneous
IFNB S MAR
pUC ori
aSyn_MshRNA
JEtLongIntron
TRY
ITR
kan
PrevailVector_LT1s_JetLong_mRNAiaSYn
_SCARB2-T2A-GBA1_bGH_4215nt
11,171 bp
2000
4000
6000
8000
10,000

FIG. 4

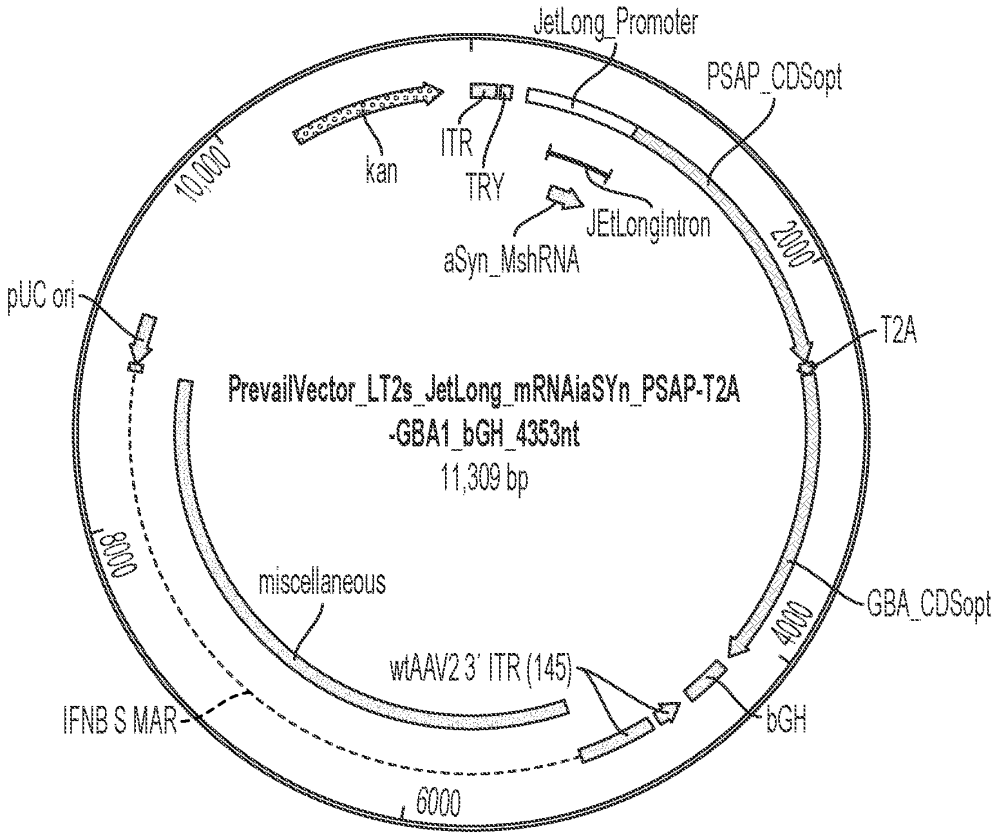

JetLong_Promoter
PSAP_CDSopt
kan
ITR
TRY
JEtLongintron
aSyn_MshRNA
10,000
2000
pUC ori
T2A
PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-T2A
-GBA1_bGH_4353nt
11,309 bp
8000
miscellaneous
GBA_CDSopt
4000
wtAAV2 3' ITR (145)
IFNB S MAR
bGH
6000

FIG. 5

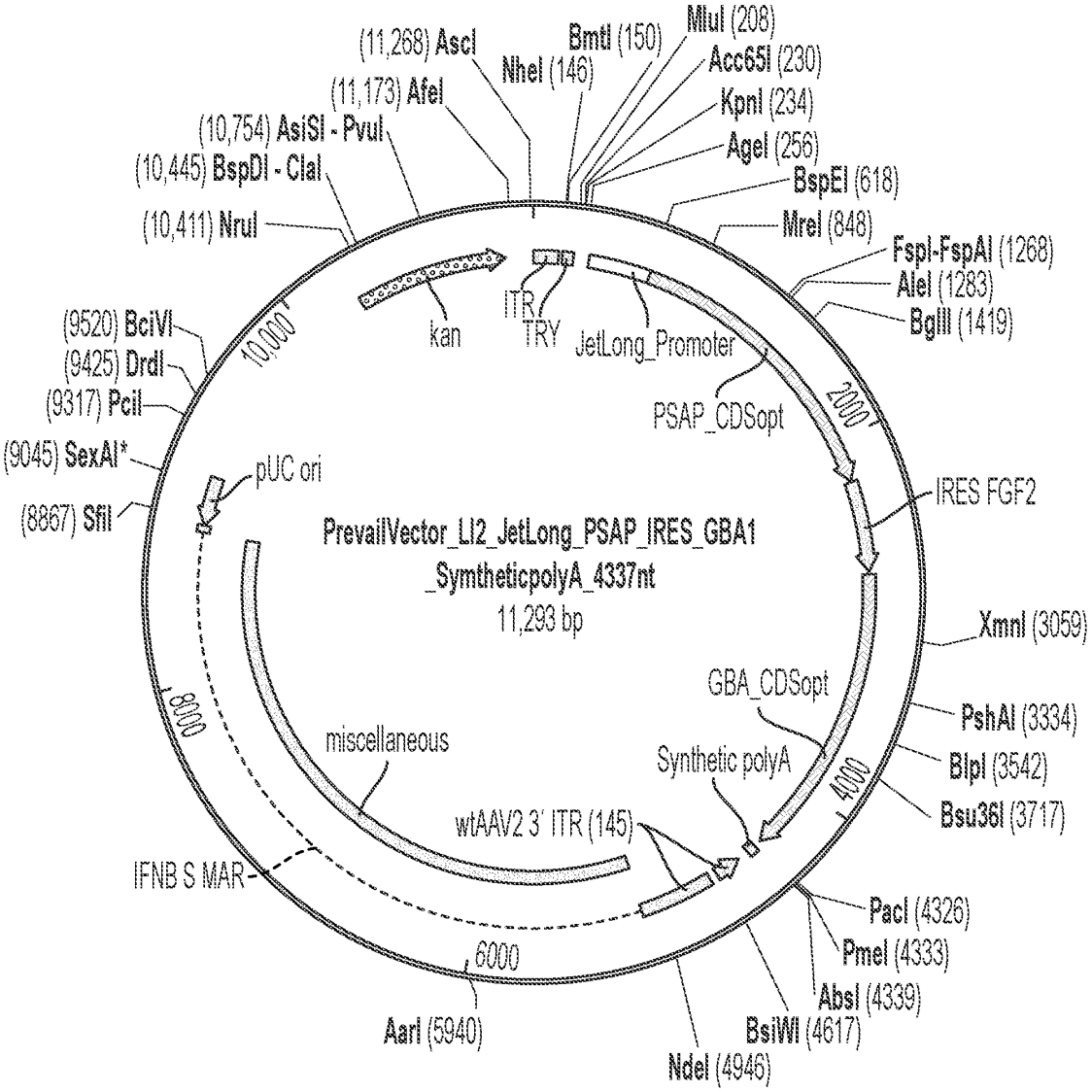

(11,268) AscI
BmtI (150)
MluI (208)
NheI (146)
Acc65I (230)
(11,173) AfeI
KpnI (234)
(10,754) AsiSI - PvuI
AgeI (256)
(10,445) BspDI - ClaI
BspEI (618)
(10,411) NruI
MreI (848)
FspI-FspAI (1268)
AleI (1283)
BglII (1419)
(9520) BciVI
(9425) DrdI
(9317) PciI
(9045) SexAI*
(8867) SfiI
kan
ITR
TRY
JetLong_Promoter
PSAP_CDSopt
IRES FGF2
pUC ori
PrevailVector_LI2_JetLong_PSAP_IRES_GBA1
_SyntheticpolyA_4337nt
11,293 bp
XmnI (3059)
GBA_CDSopt
PshAI (3334)
BlpI (3542)
miscellaneous
Synthetic polyA
Bsu36I (3717)
wtAAV2 3' ITR (145)
IFNB S MAR
PacI (4326)
PmeI (4333)
AbsI (4339)
AarI (5940)
BsiWI (4617)
NdeI (4946)
2000
4000
6000
8000
10,000

FIG. 6

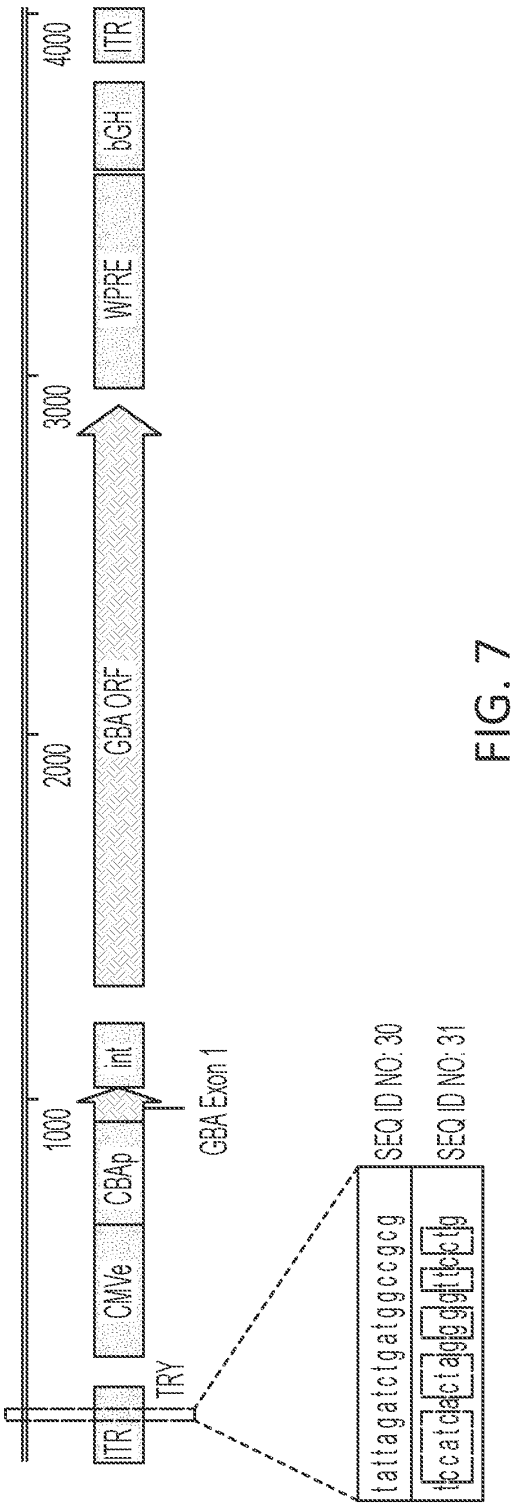

1000
2000
3000
4000
ITR
CMVe
CBAp
int
TRY
GBA ORF
WPRE
bGH
ITR
GBA Exon 1
SEQ ID NO: 30
SEQ ID NO: 31

FIG. 7

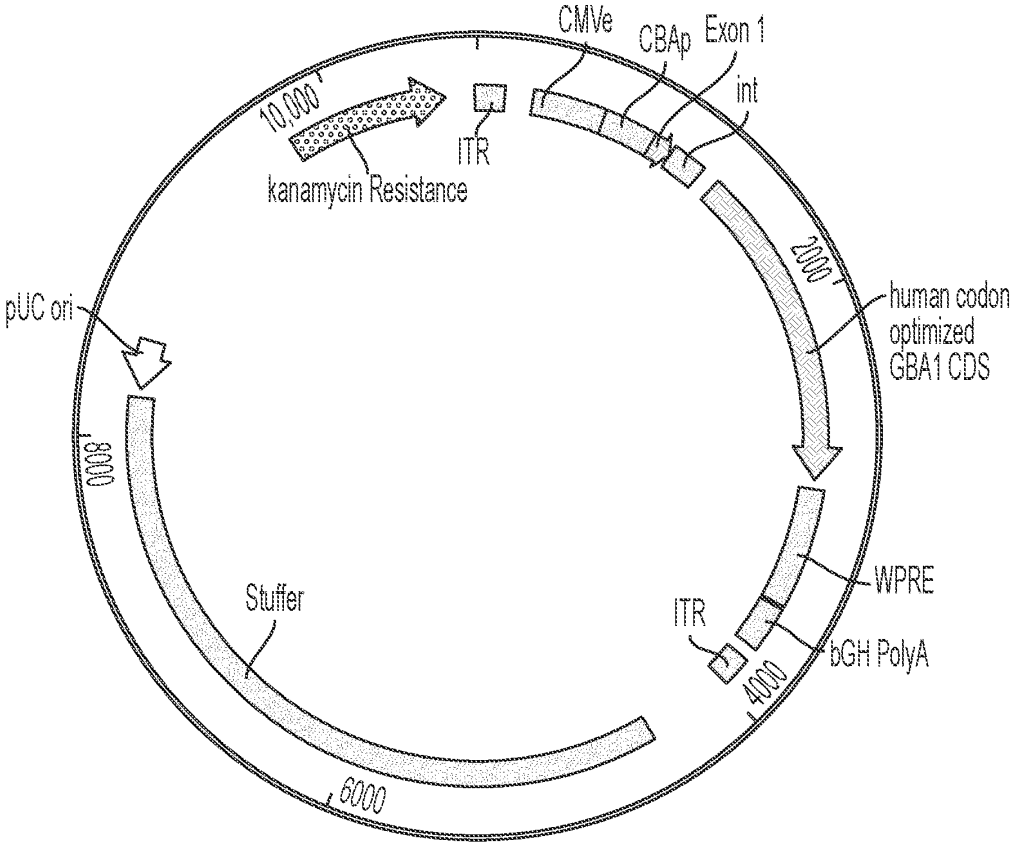

CMVe
CBAp
Exon 1
int
10,000
ITR
kanamycin Resistance
2000
human codon optimized GBA1 CDS
pUC ori
8000
Stuffer
ITR
WPRE
bGH PolyA
4000
6000

FIG. 8

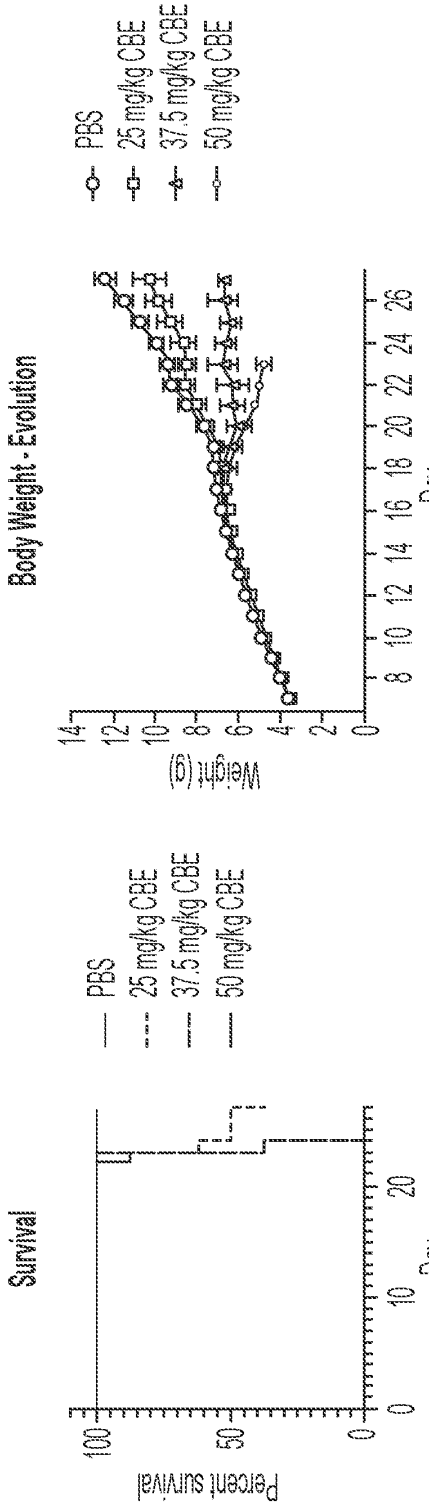


FIG. 9A

FIG. 9B

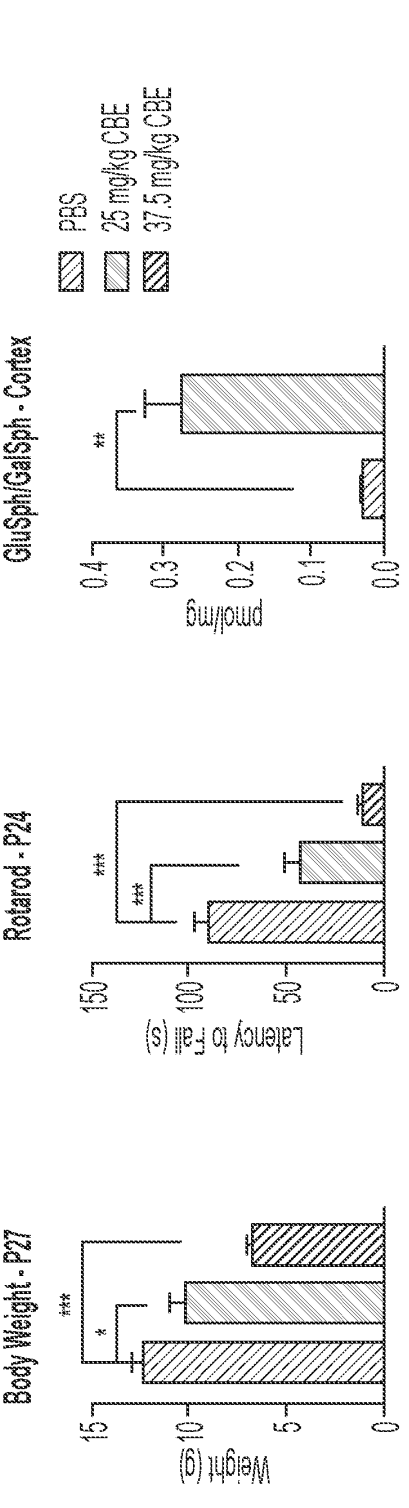


FIG. 9C

FIG. 9D

FIG. 9E

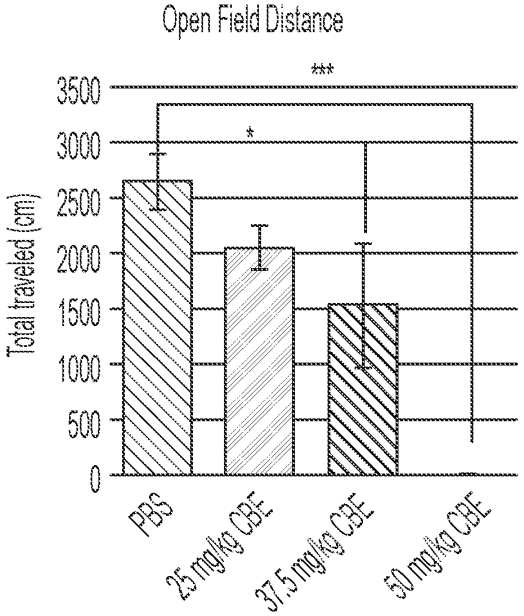

Open Field Distance
***
*
3500
3000
2500
2000
1500
1000
500
0
Total traveled (cm)
PBS
25 mg/kg CBE
37.5 mg/kg CBE
50 mg/kg CBE

FIG. 9F

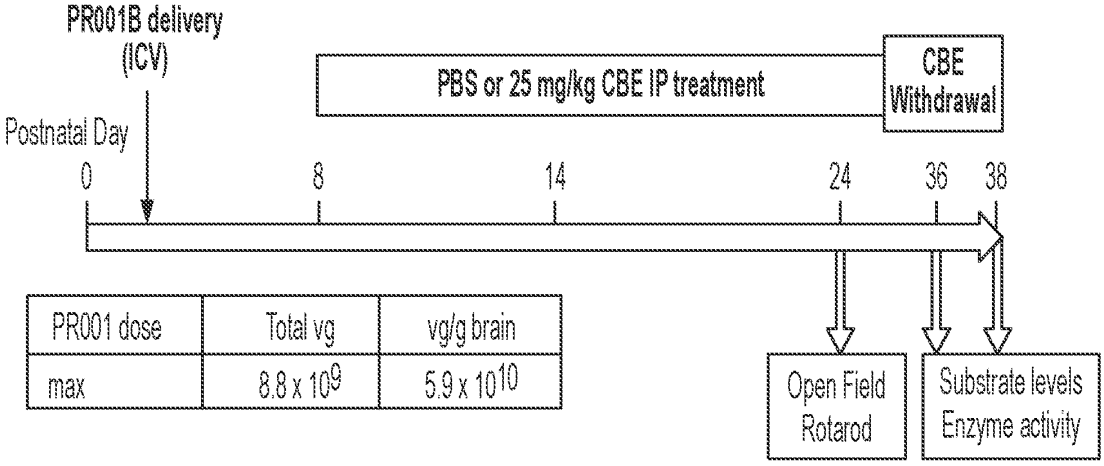

PR001B delivery
(ICV)
Postnatal Day
0
8
14
24
36
38
PBS or 25 mg/kg CBE IP treatment
CBE
Withdrawal
PR001 dose
Total vg
vg/g brain
max
8.8 x 10^9
5.9 x 10^10
Open Field
Rotarod
Substrate levels
Enzyme activity

FIG. 10

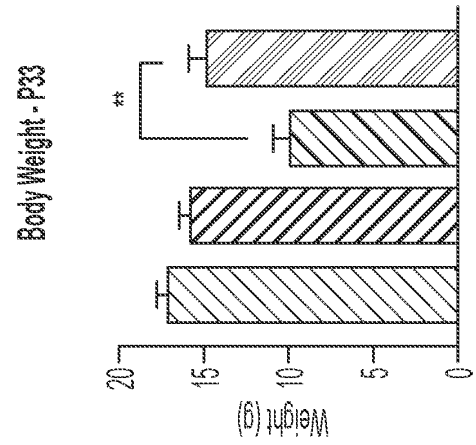


FIG. 11B

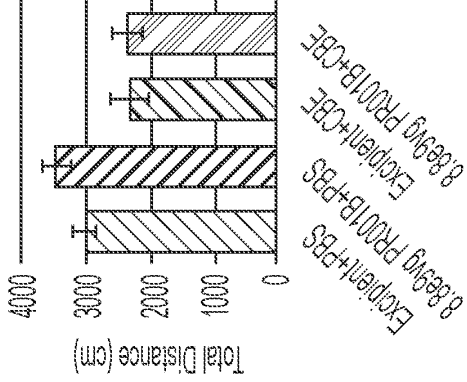


FIG. 11D

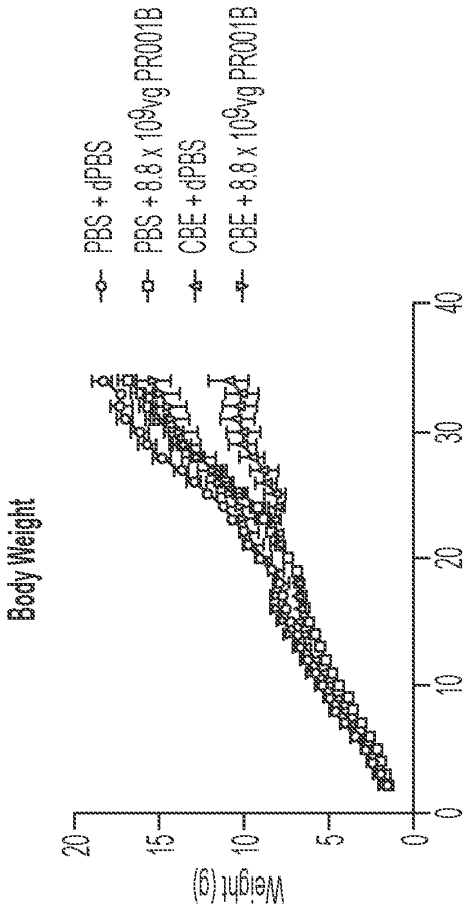


FIG. 11A

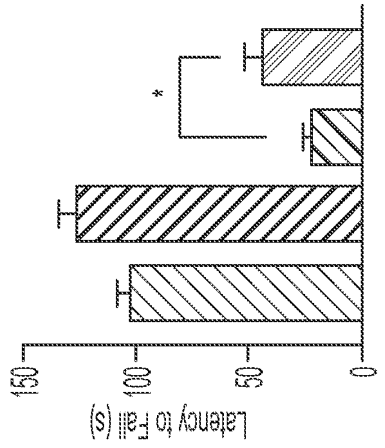


FIG. 11C

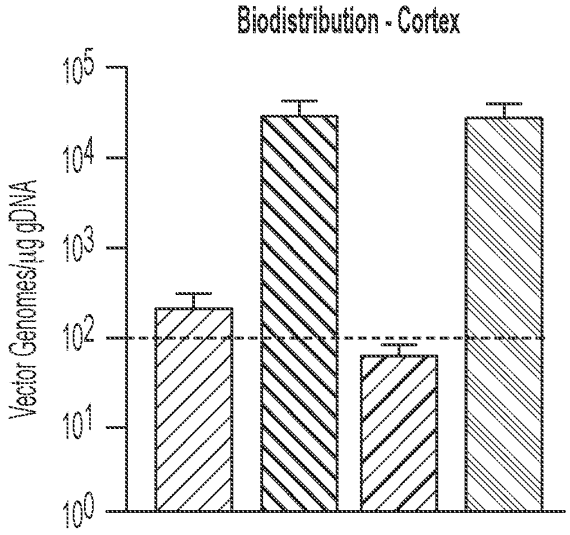


FIG. 12A

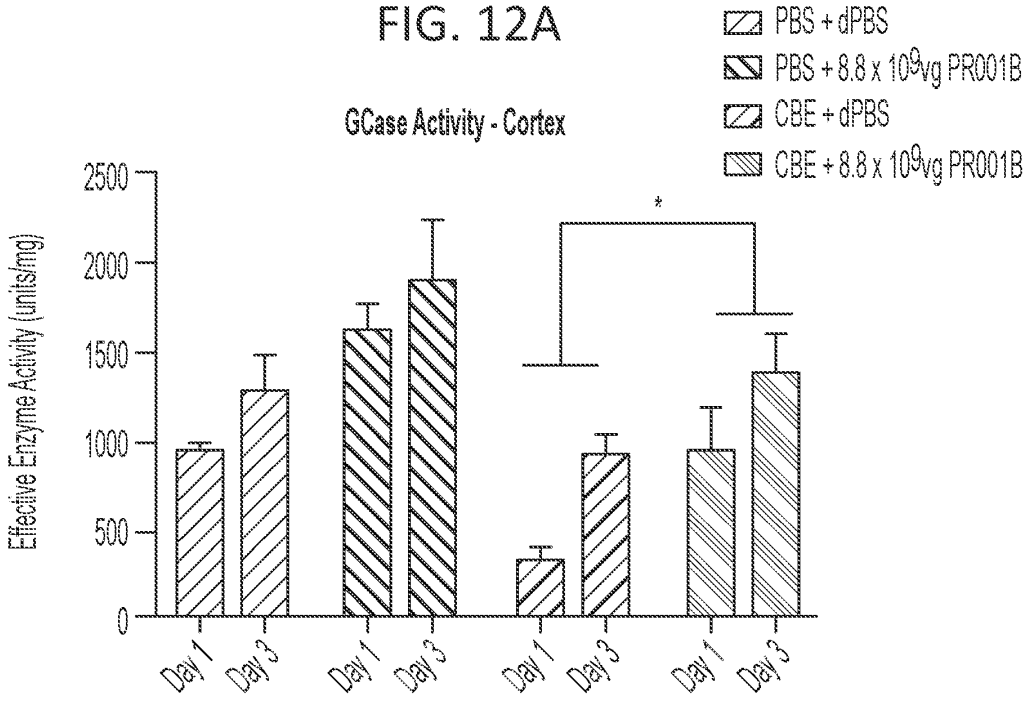


FIG. 12B

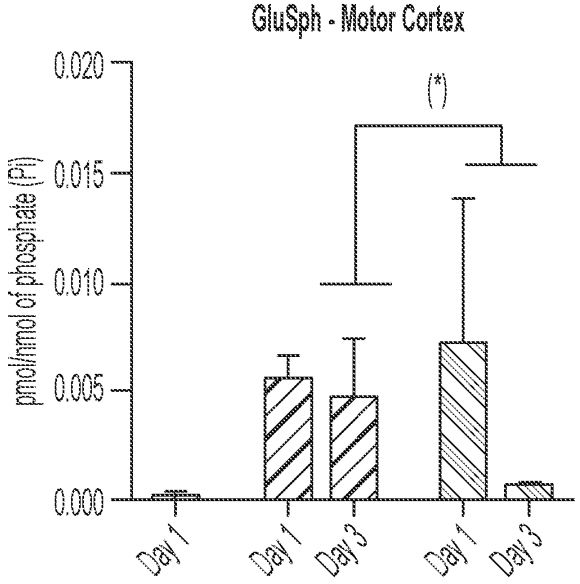


FIG. 12C

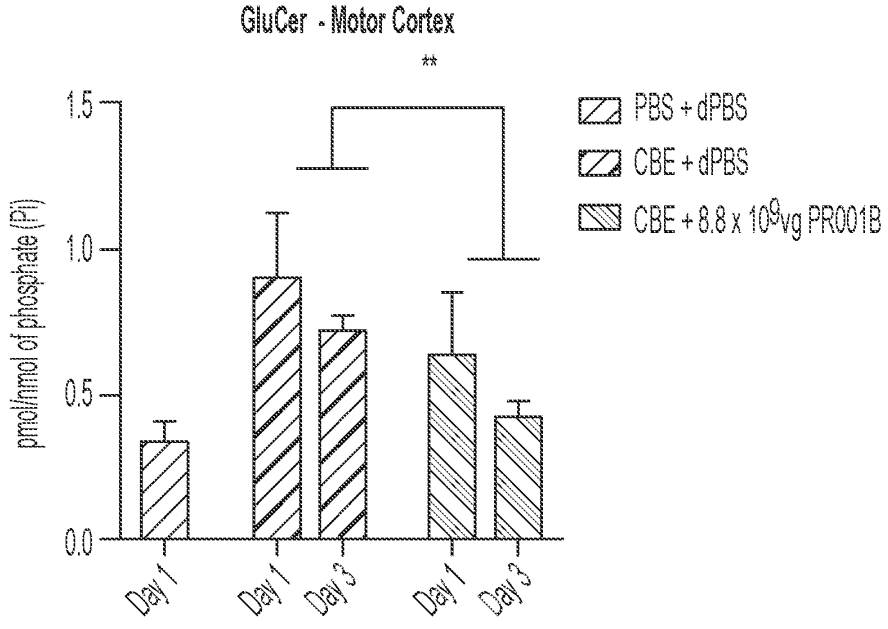


FIG. 12D

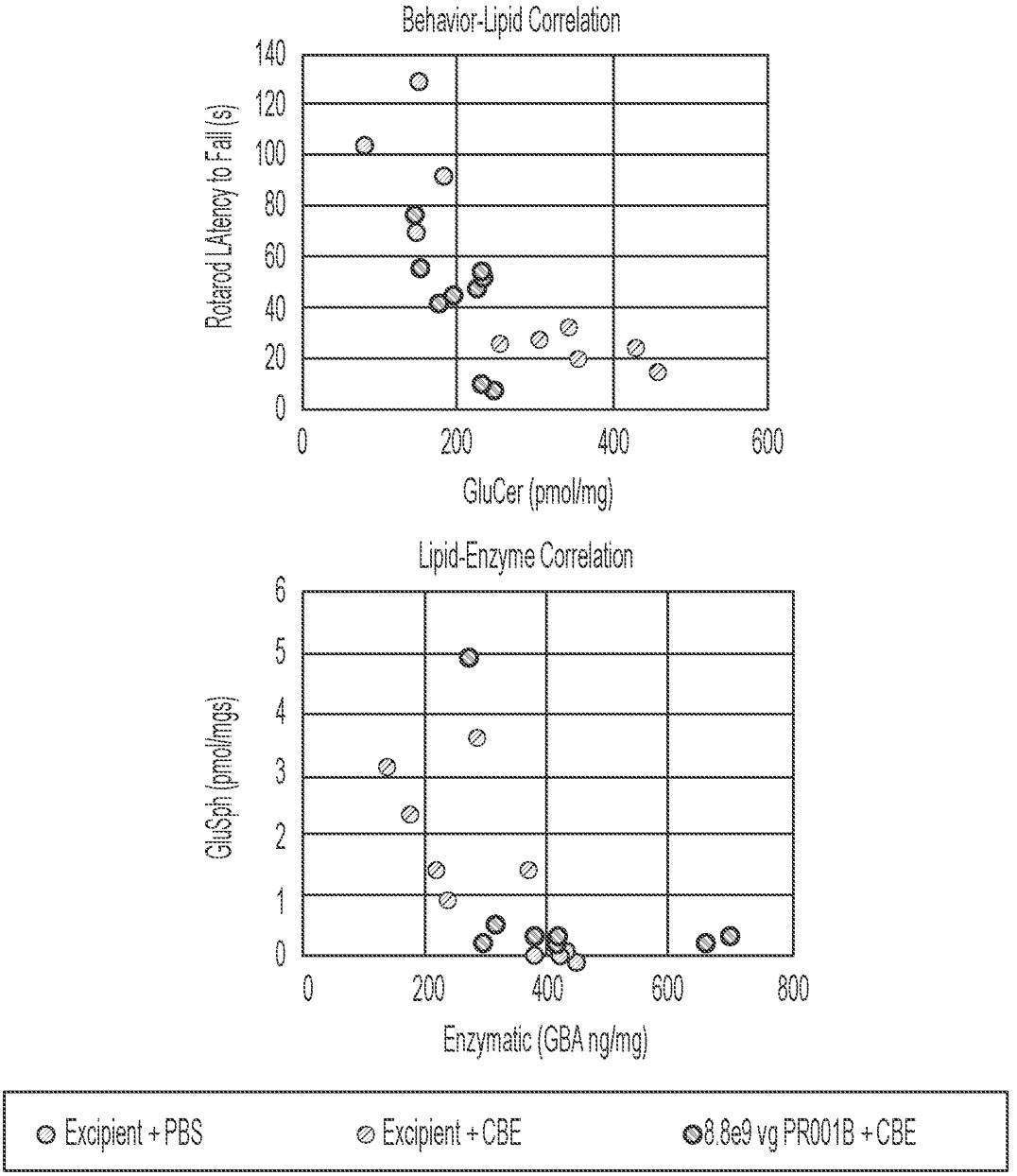

Behavior-Lipid Correlation
Rotarod LAtency to Fall (s)
140
120
100
80
60
40
20
0
0
200
400
600
GluCer (pmol/mg)
Lipid-Enzyme Correlation
GluSph (pmol/mgs)
6
5
4
3
2
1
0
0
200
400
600
800
Enzymatic (GBA ng/mg)
Excipient + PBS
Excipient + CBE
8.8e9 vg PR001B + CBE

FIG. 13

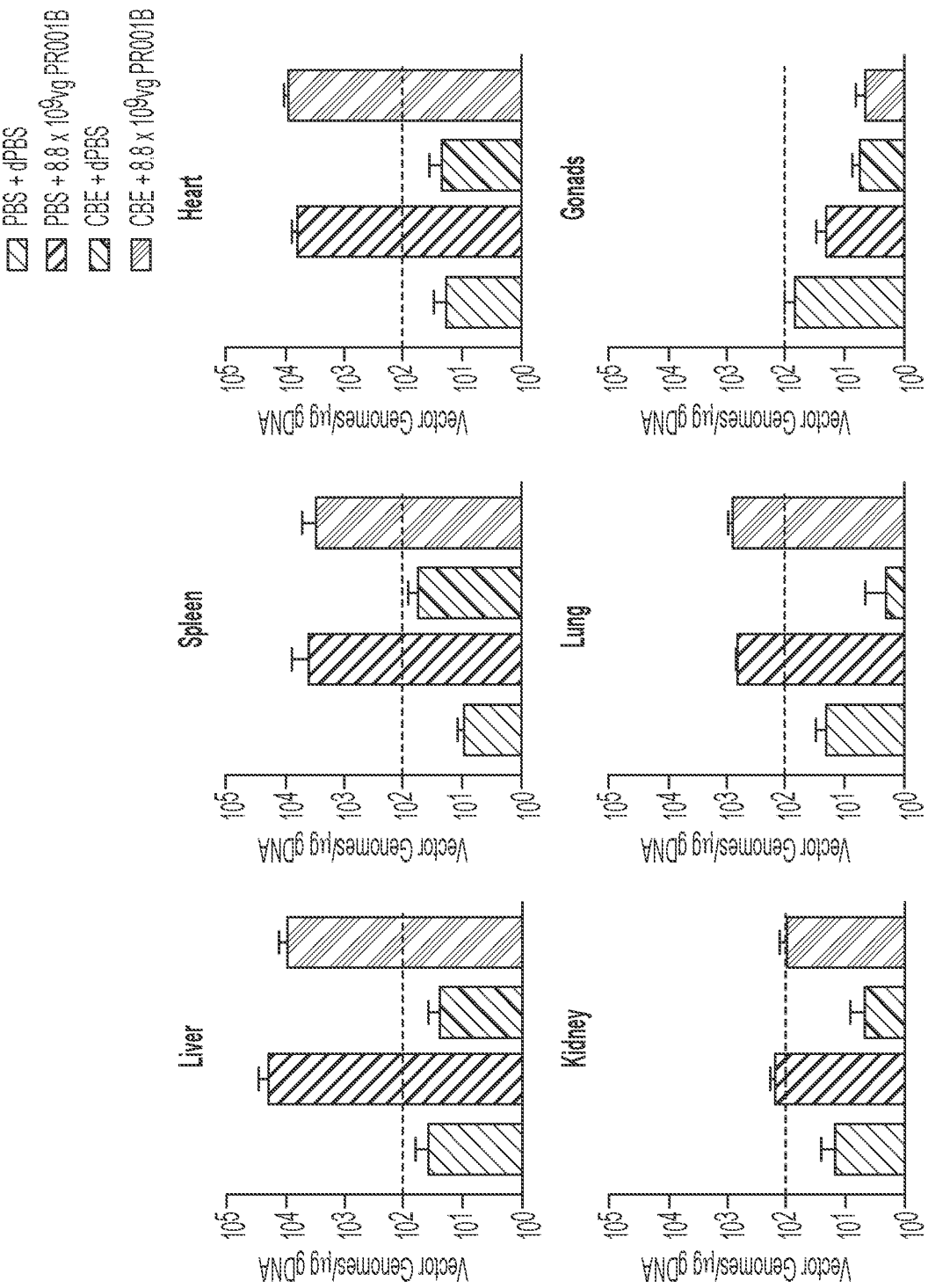

PBS + dPBS
PBS + 8.8 x 10^9vg PR001B
CBE + dPBS
CBE + 8.8 x 10^9vg PR001B
Heart
Gonads
Spleen
Lung
Liver
Kidney
Vector Genomes/µg gDNA
10^5
10^4
10^3
10^2
10^1
10^0

FIG. 14

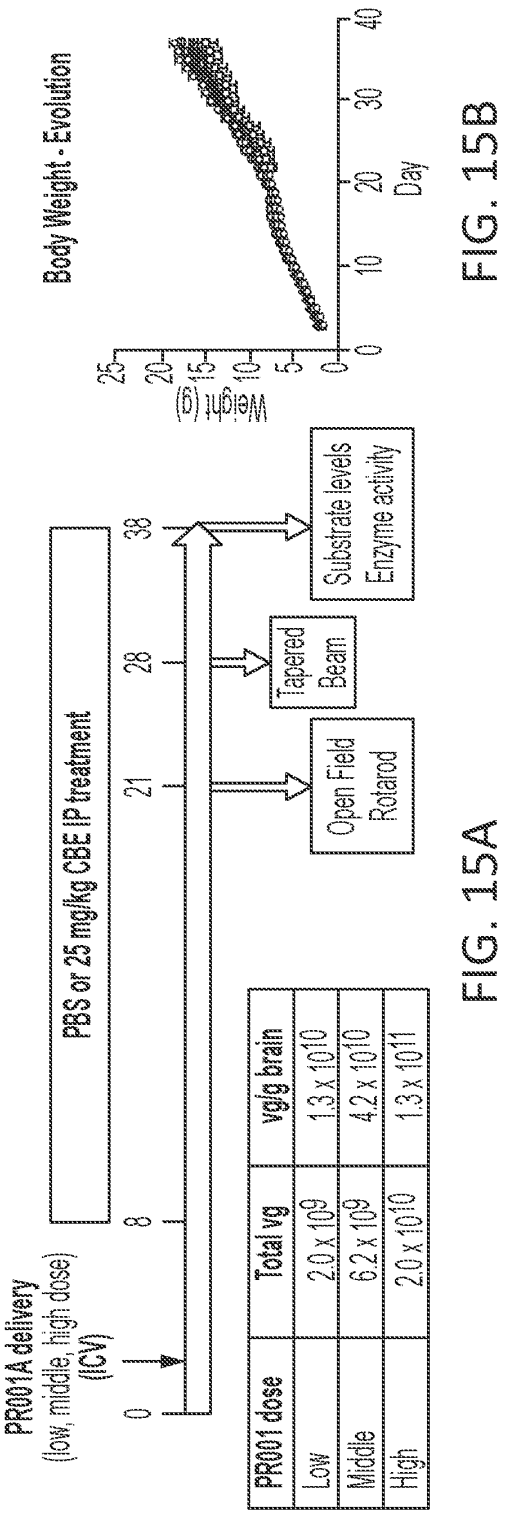


FIG. 15A

FIG. 15B

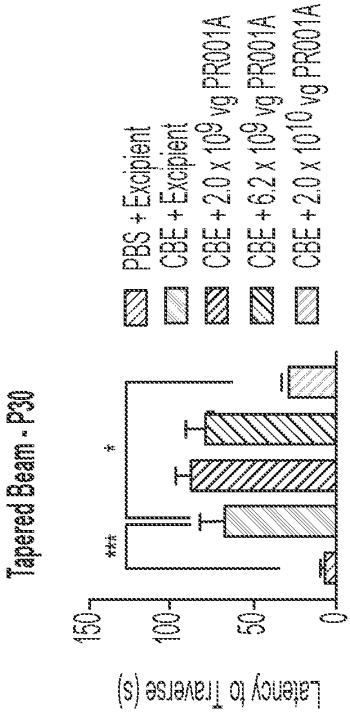


FIG. 15E

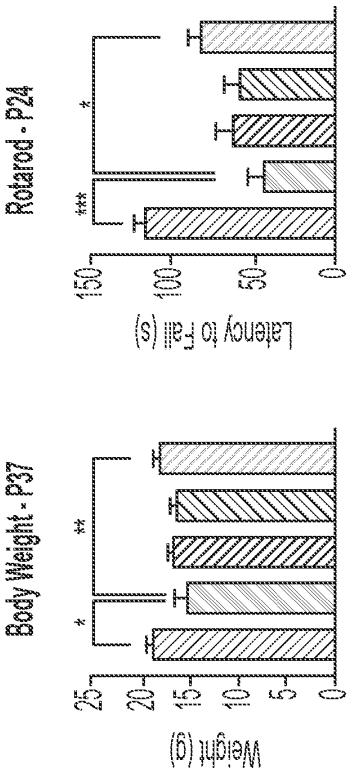


FIG. 15D

FIG. 15C

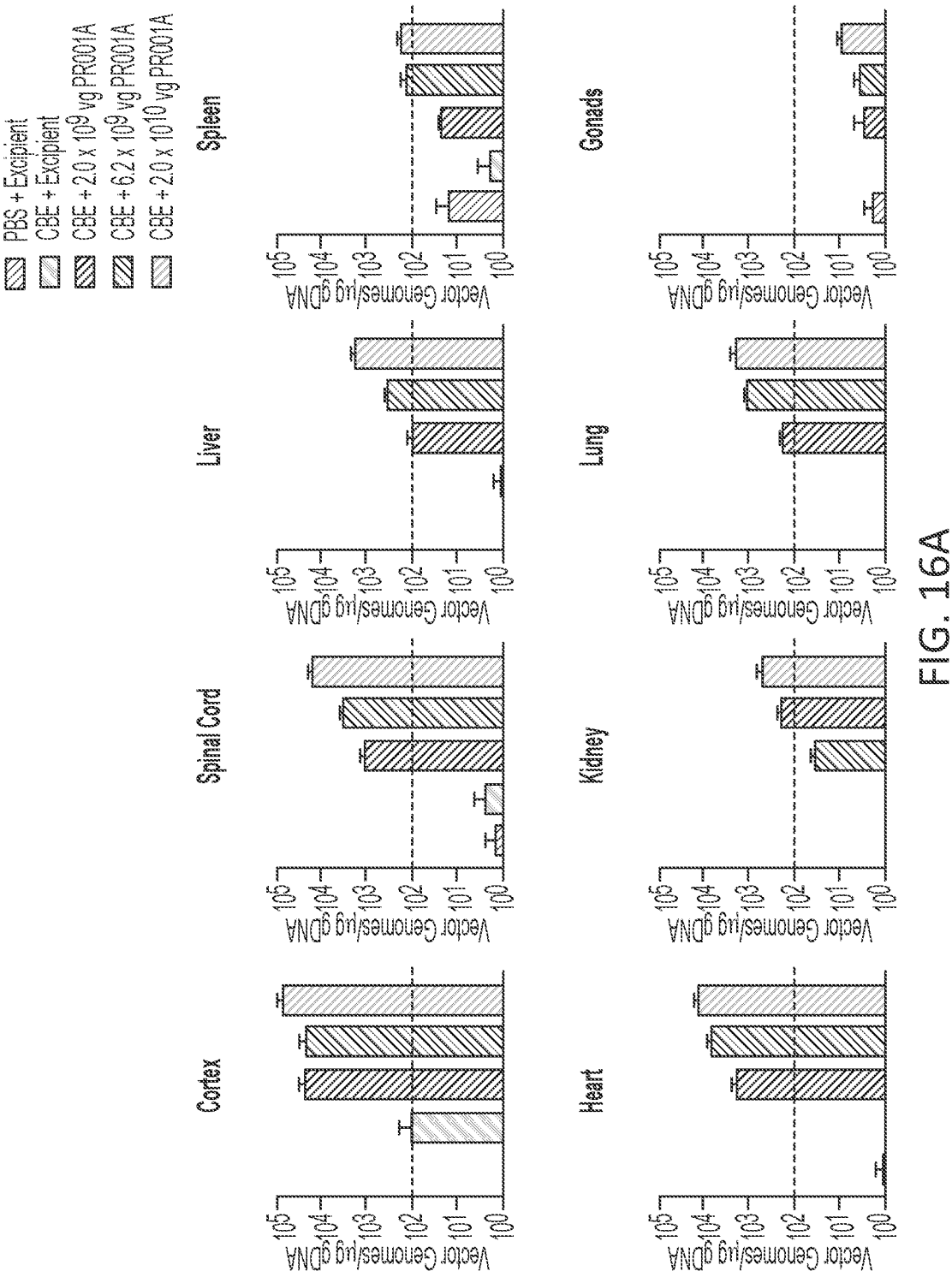

PBS + Excipient
CBE + Excipient
CBE + 2.0 x 10^9 vg PR001A
CBE + 6.2 x 10^9 vg PR001A
CBE + 2.0 x 10^10 vg PR001A
Cortex
Spinal Cord
Liver
Spleen
Heart
Kidney
Lung
Gonads
Vector Genomes/µg gDNA
10^5
10^4
10^3
10^2
10^1
10^0

FIG. 16A

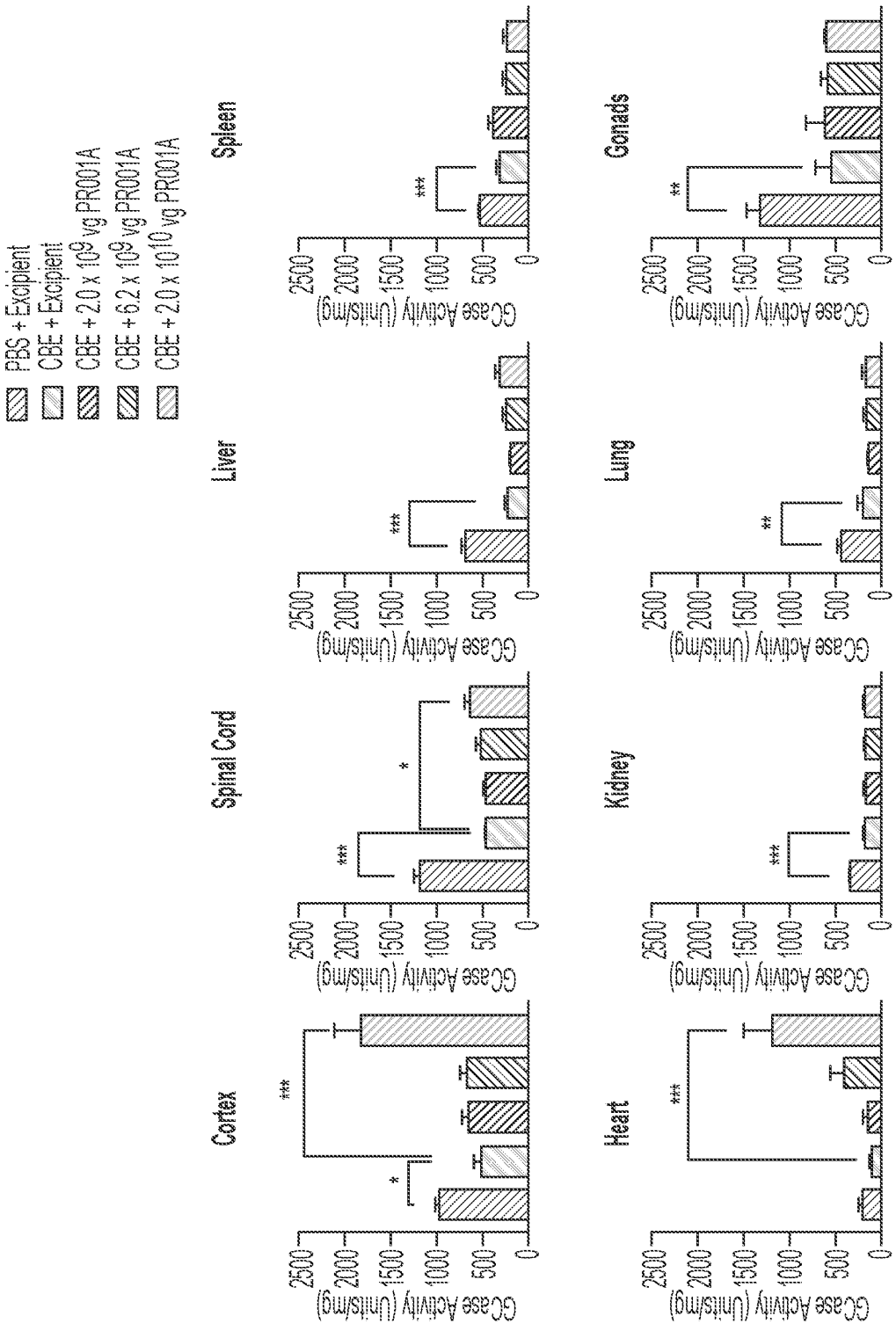

PBS + Excipient
CBE + Excipient
CBE + 2.0 x 10^9 vg PR001A
CBE + 6.2 x 10^9 vg PR001A
CBE + 2.0 x 10^10 vg PR001A
Cortex
Spinal Cord
Liver
Spleen
Heart
Kidney
Lung
Gonads
GCase Activity (Units/mg)
0
500
1000
1500
2000
2500
*
**
***

FIG. 16B

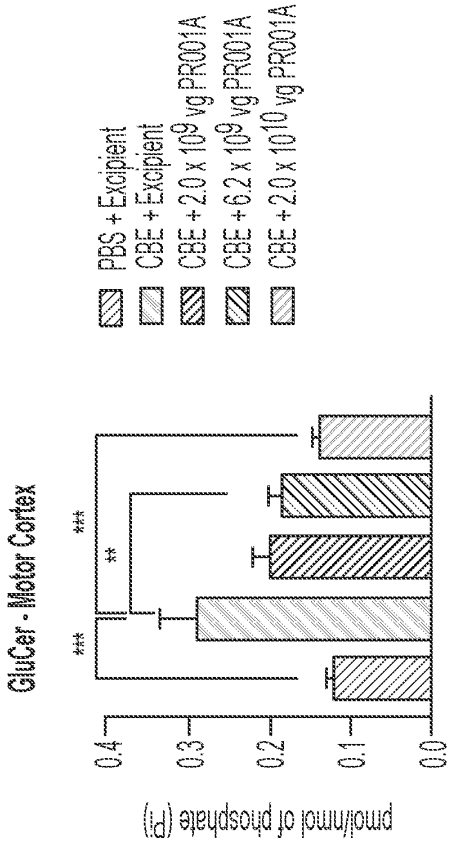


FIG. 16D

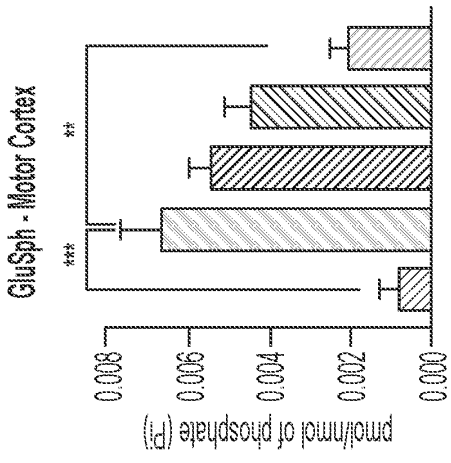


FIG. 16C

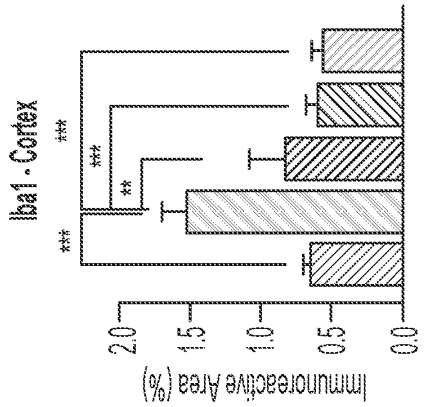


FIG. 16F

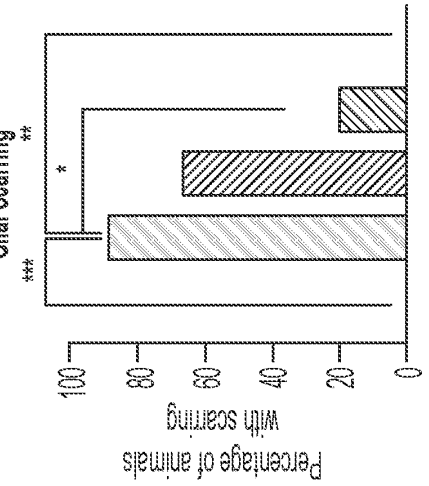


FIG. 16E

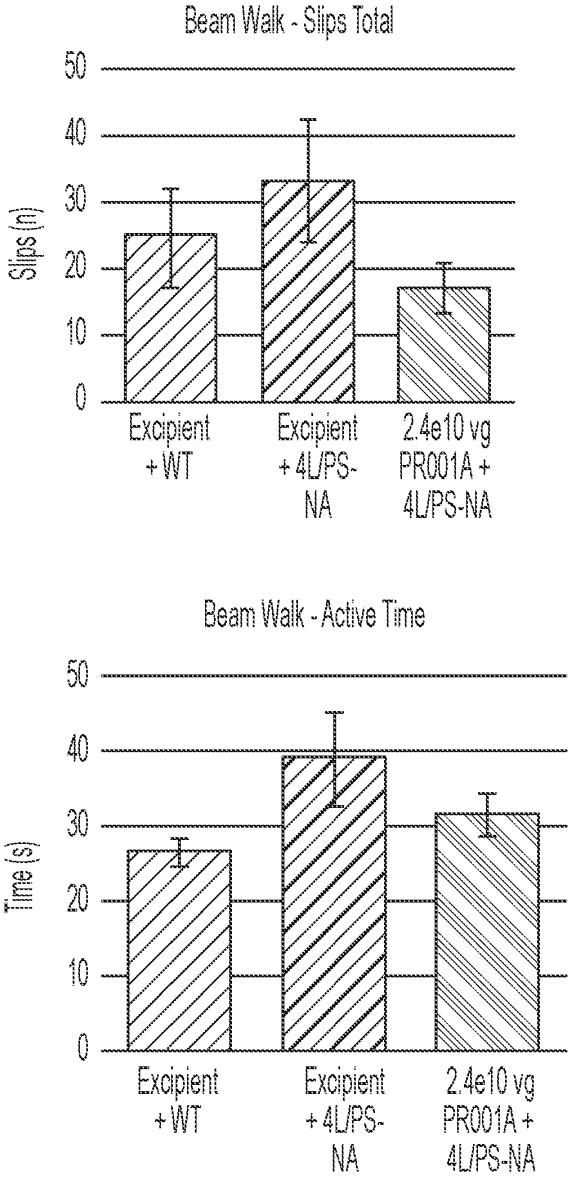

Beam Walk - Slips Total
Slips (n)
50
40
30
20
10
0
Excipient + WT
Excipient + 4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA
Beam Walk - Active Time
Time (s)
50
40
30
20
10
0
Excipient + WT
Excipient + 4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA

FIG. 17

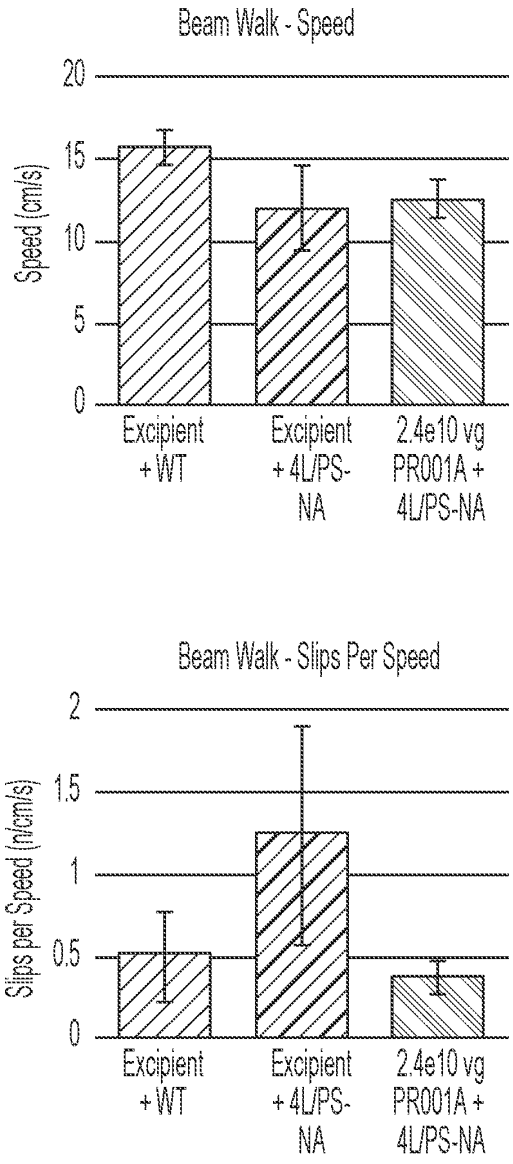

Beam Walk - Speed
Speed (cm/s)
20
15
10
5
0
Excipient + WT
Excipient + 4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA
Beam Walk - Slips Per Speed
Slips per Speed (n/cm/s)
2
1.5
1
0.5
0
Excipient + WT
Excipient + 4L/PS-NA
2.4e10 vg PR001A + 4L/PS-NA

FIG. 17 cont.

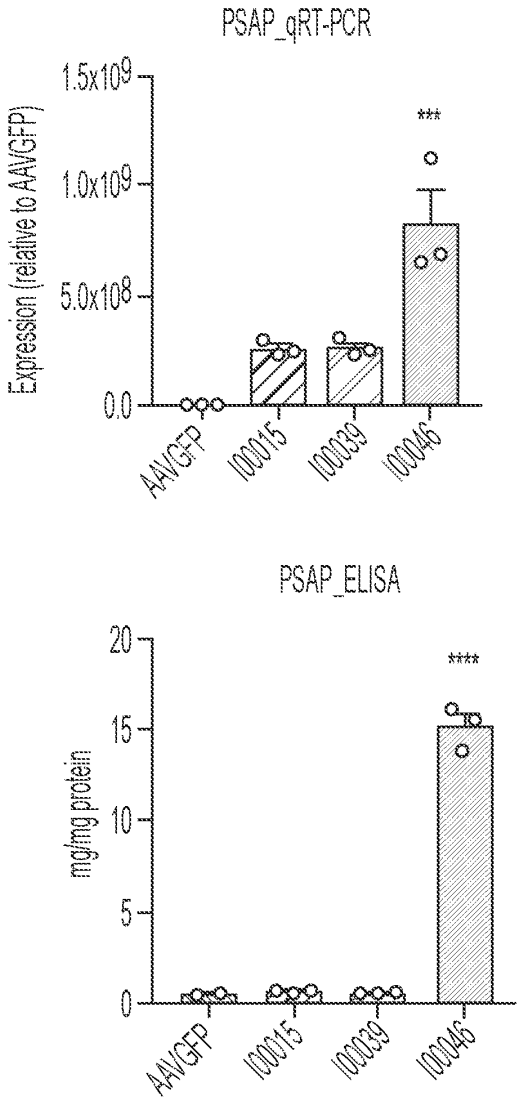

PSAP_qRT-PCR
Expression (relative to AAVGFP)
1.5x10^9
1.0x10^9
5.0x10^8
0.0
***
AAVGFP
I00015
I00039
I00046
PSAP_ELISA
mg/mg protein
20
15
10
5
0
****
AAVGFP
I00015
I00039
I00046

FIG. 18

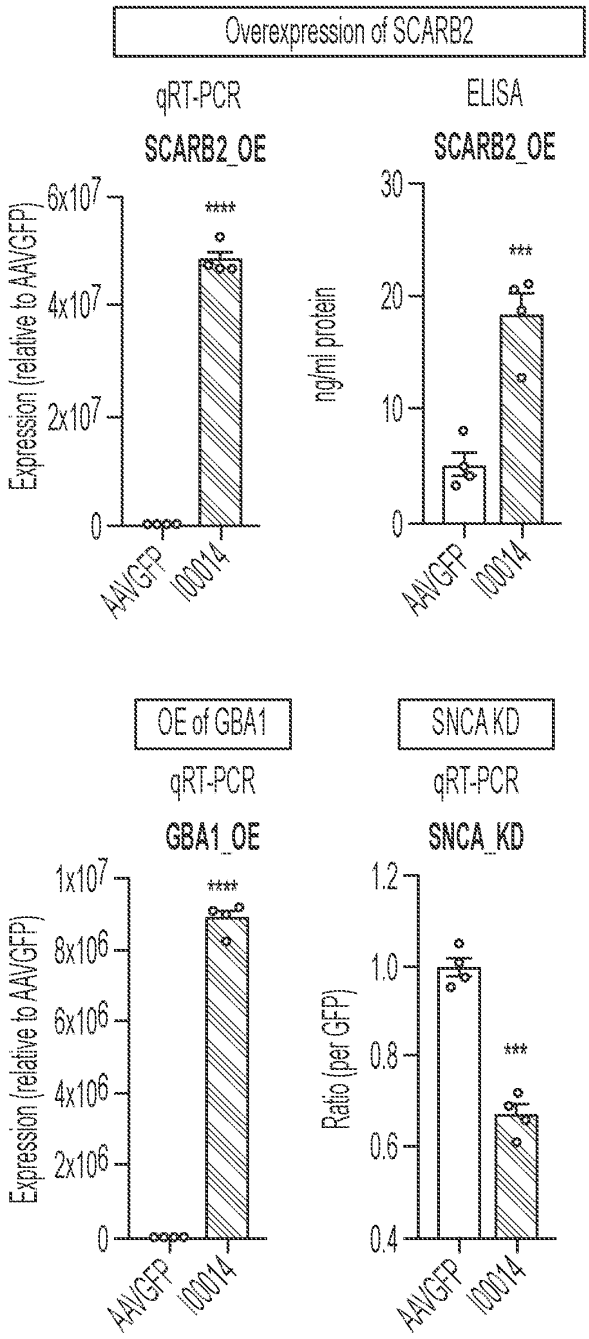

Overexpression of SCARB2
qRT-PCR
SCARB2_OE
Expression (relative to AAVGFP)
6x10^7
4x10^7
2x10^7
0
****
AAVGFP
I00014
ELISA
SCARB2_OE
ng/ml protein
30
20
10
0
***
AAVGFP
I00014
OE of GBA1
qRT-PCR
GBA1_OE
Expression (relative to AAVGFP)
1x10^7
8x10^6
6x10^6
4x10^6
2x10^6
0
****
AAVGFP
I00014
SNCA KD
qRT-PCR
SNCA_KD
Ratio (per GFP)
1.2
1.0
0.8
0.6
0.4
***
AAVGFP
I00014

FIG. 18 cont.

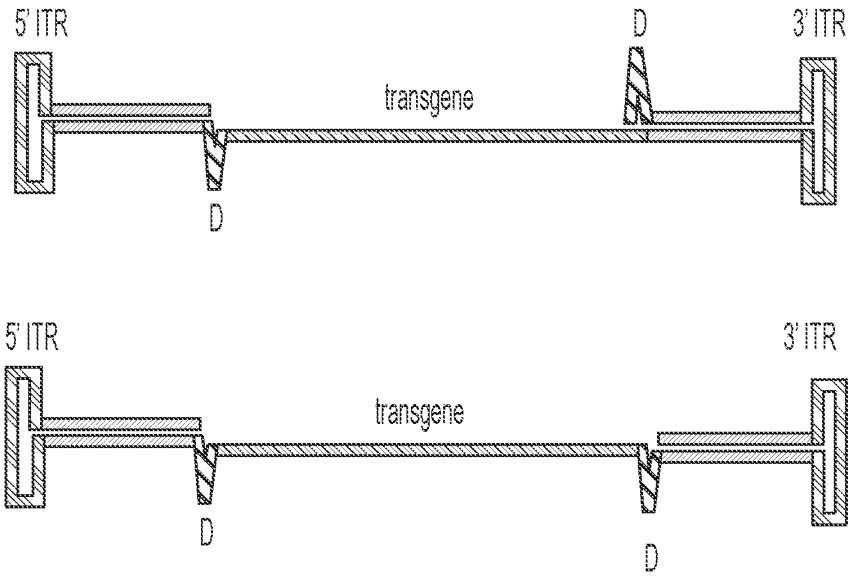

5' ITR
D
3' ITR
transgene
D
5' ITR
3' ITR
transgene
D
D

FIG. 19

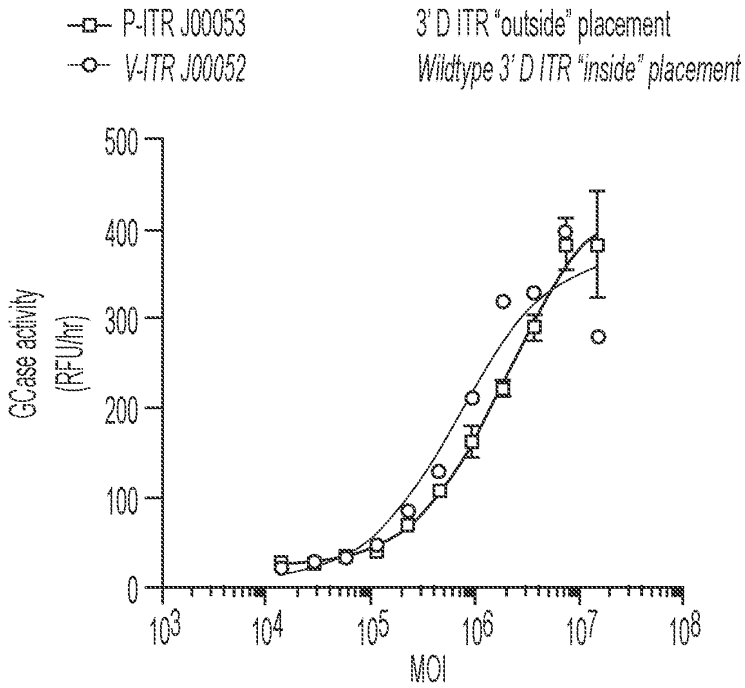

P-ITR J00053
V-ITR J00052
3' D ITR "outside" placement
Wildtype 3' D ITR "inside" placement
GCase activity (RFU/hr)
500
400
300
200
100
0
10^3
10^4
10^5
10^6
10^7
10^8
MOI

FIG. 20

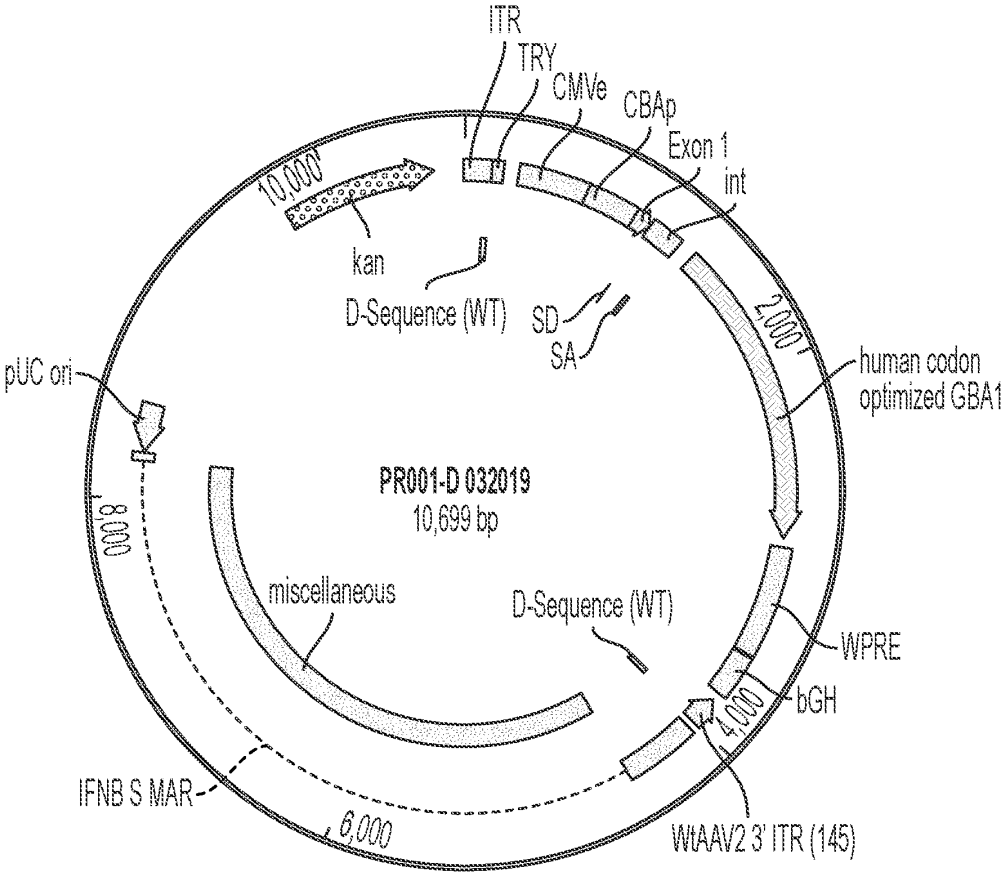

ITR
TRY
CMVe
CBAp
Exon 1
int
10,000
kan
D-Sequence (WT)
SD
SA
2,000
human codon optimized GBA1
pUC ori
PR001-D 032019
10,699 bp
8,000
miscellaneous
D-Sequence (WT)
WPRE
bGH
4,000
IFNB S MAR
6,000
WtAAV2 3' ITR (145)

FIG. 21

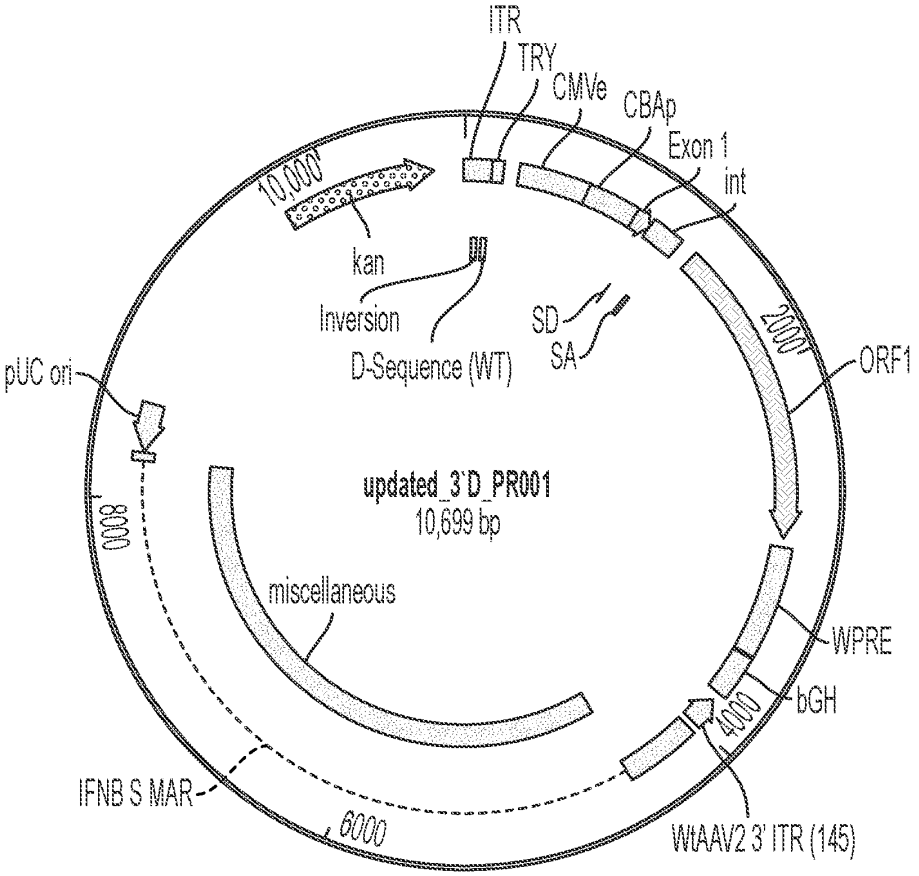

ITR
TRY
CMVe
CBAp
Exon 1
int
10,000
kan
Inversion
D-Sequence (WT)
SD
SA
2000
ORF1
pUC ori
updated_3'D_PR001
10,699 bp
8000
miscellaneous
WPRE
bGH
4000
IFNB S MAR
6000
WtAAV2 3' ITR (145)

FIG. 22

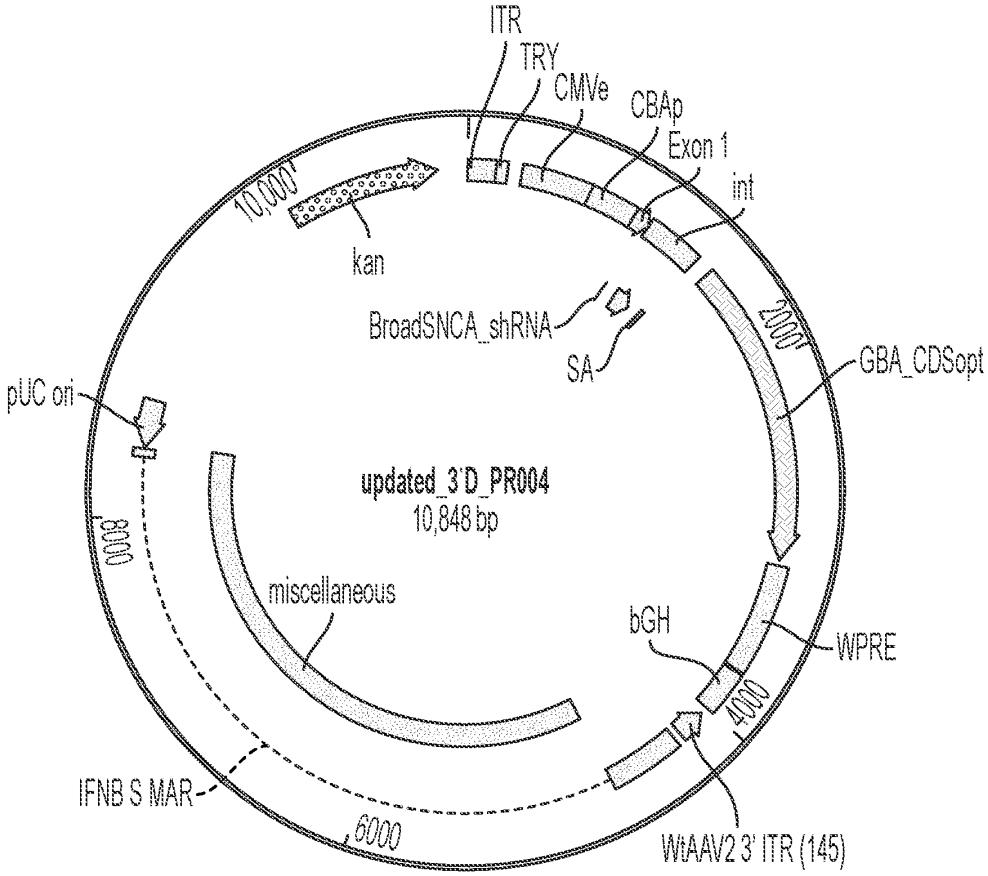
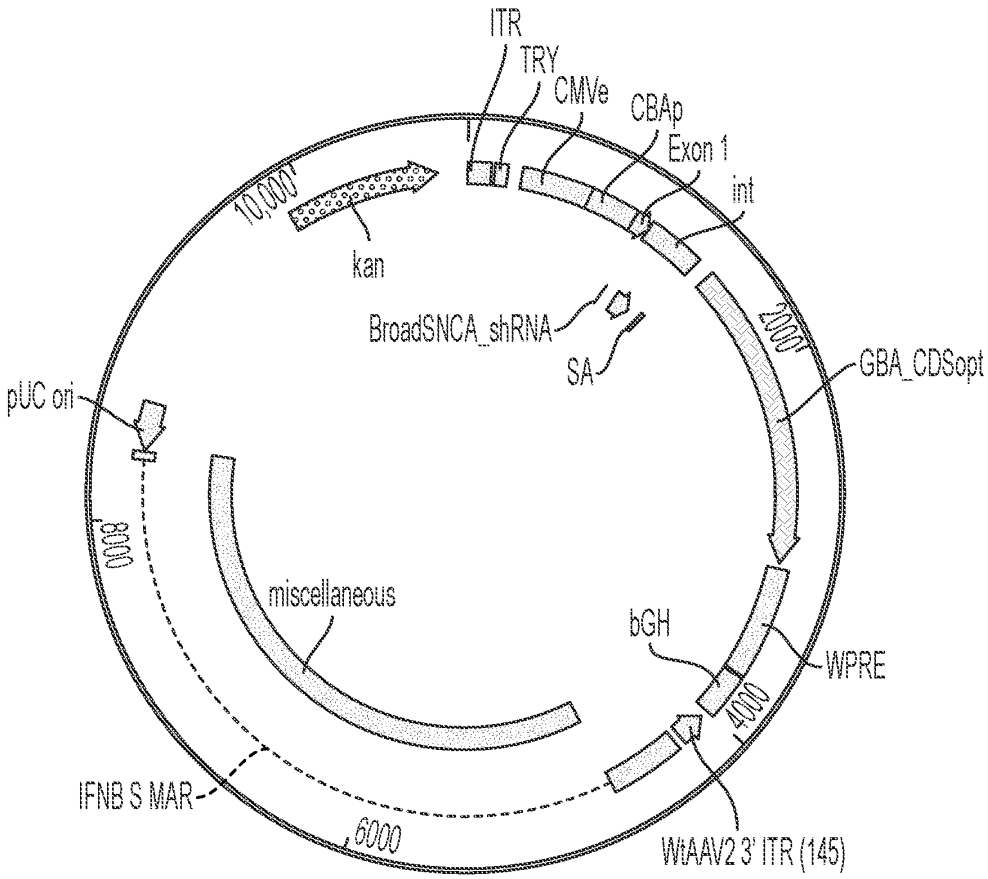

ITR
TRY
CMVe
CBAp
Exon 1
int
10,000
kan
BroadSNCA_shRNA
SA
2000
GBA_CDSopt
pUC ori
updated_3'D_PR004
10,848 bp
8000
miscellaneous
bGH
WPRE
4000
IFNB S MAR
6000
WtAAV2 3' ITR (145)

FIG. 23



updated_Intronic_SNCA_Broad_sh_GBA1_aligned
10,484 bp

FIG. 24

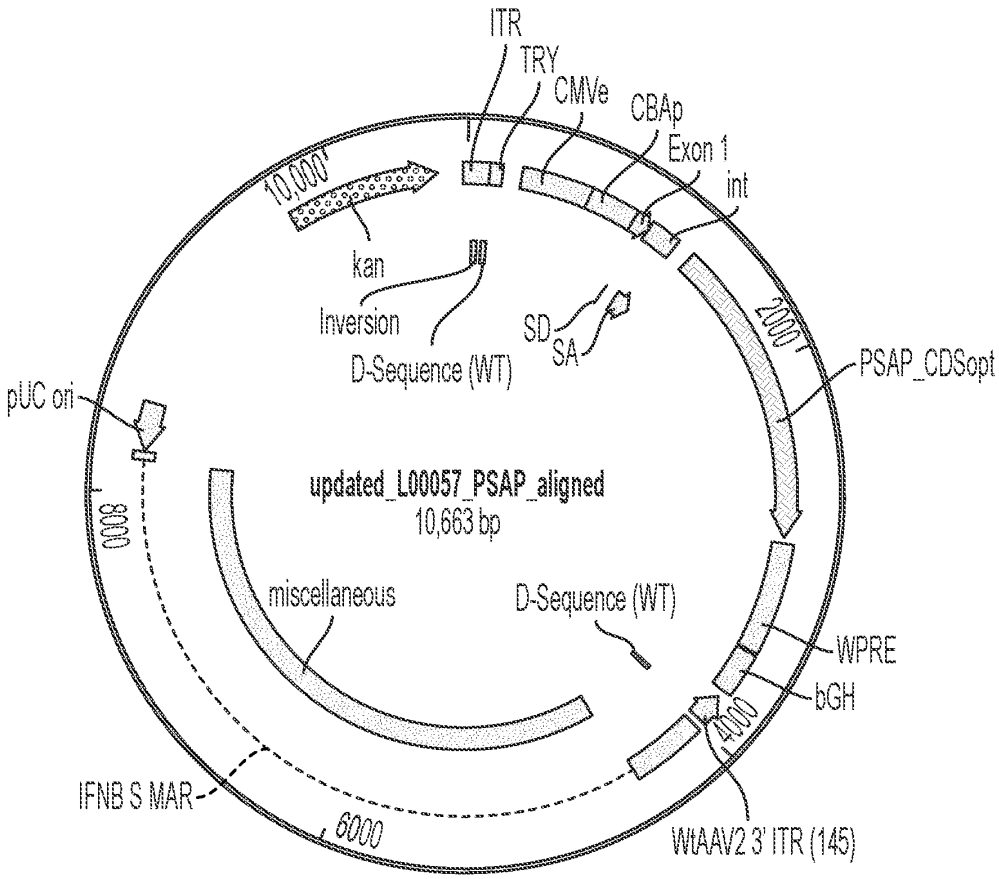

ITR
TRY
CMVe
CBAp
Exon 1
int
10,000
kan
Inversion
D-Sequence (WT)
SD
SA
2000
PSAP_CDSopt
pUC ori
updated_L00057_PSAP_aligned
10,663 bp
8000
miscellaneous
D-Sequence (WT)
WPRE
bGH
4000
IFNB S MAR
6000
WtAAV2 3' ITR (145)

FIG. 25

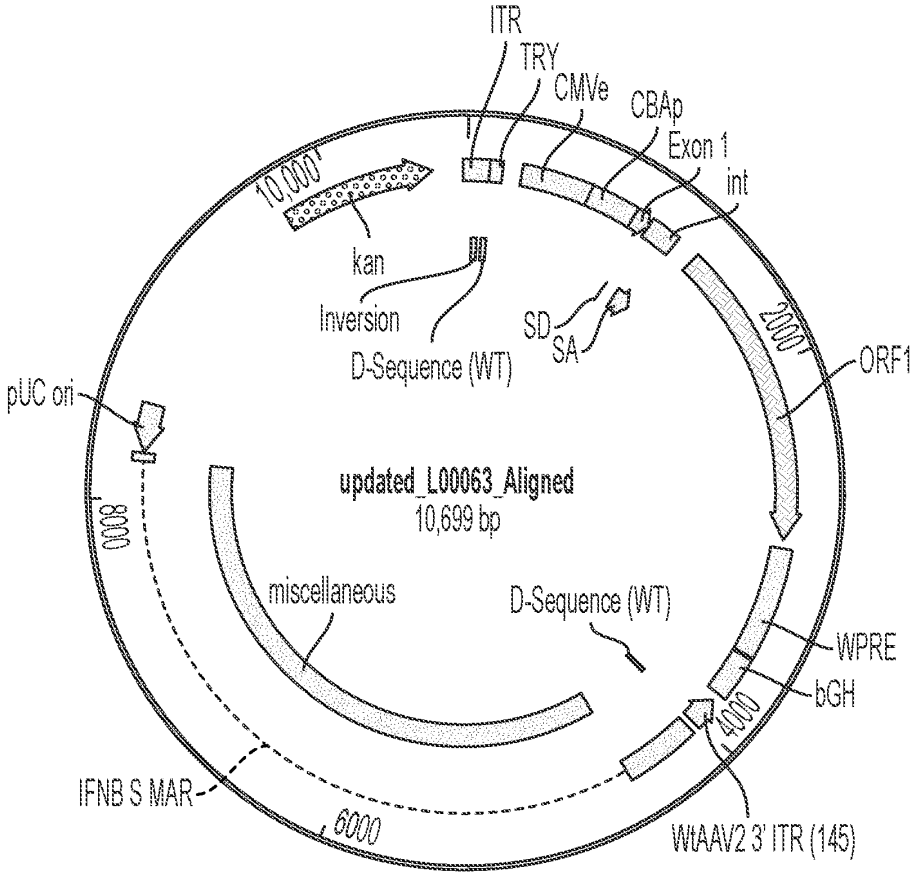
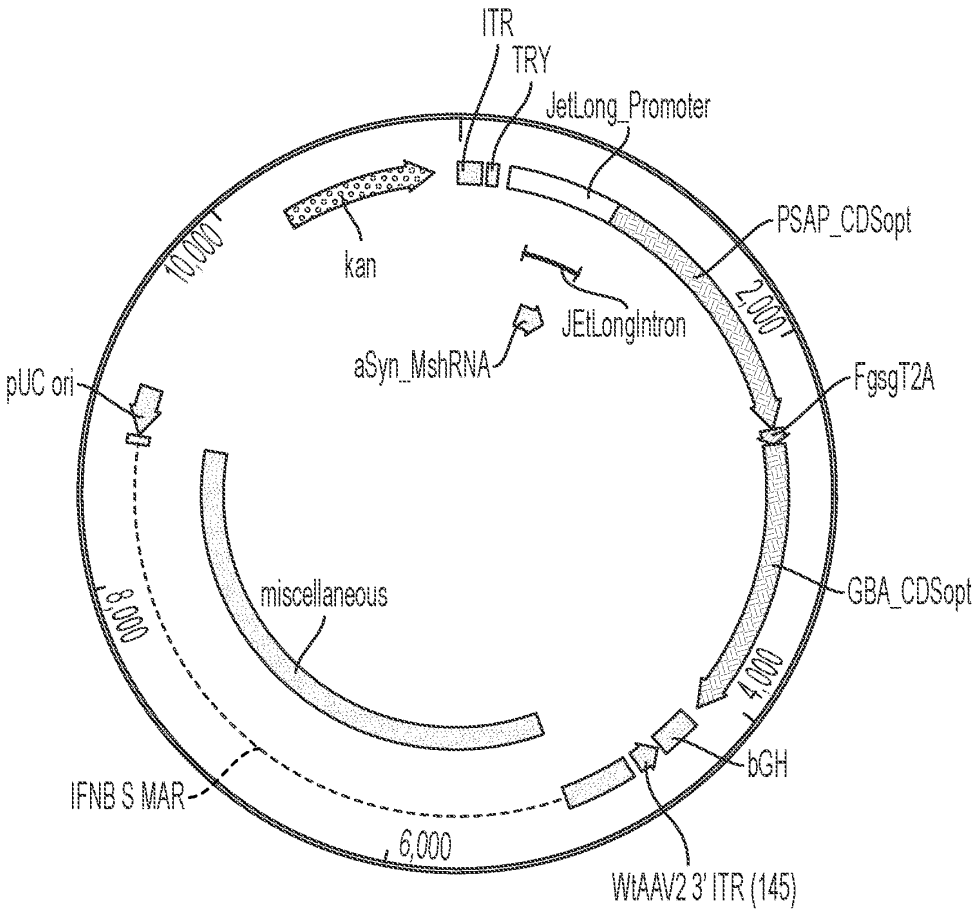

ITR
TRY
CMVe
CBAp
Exon 1
int
10,000
kan
Inversion
D-Sequence (WT)
SD
SA
2000
ORF1
pUC ori
updated_L00063_Aligned
10,699 bp
8000
miscellaneous
D-Sequence (WT)
WPRE
bGH
4000
IFNB S MAR
6000
WtAAV2 3' ITR (145)

FIG. 26



updated_PrevailVector_LT2s_JetLong_mRNAiaSYn_PSAP-FgsgT2A-GBA1_bGH_4374nt_aligned
11,292 bp

FIG. 27

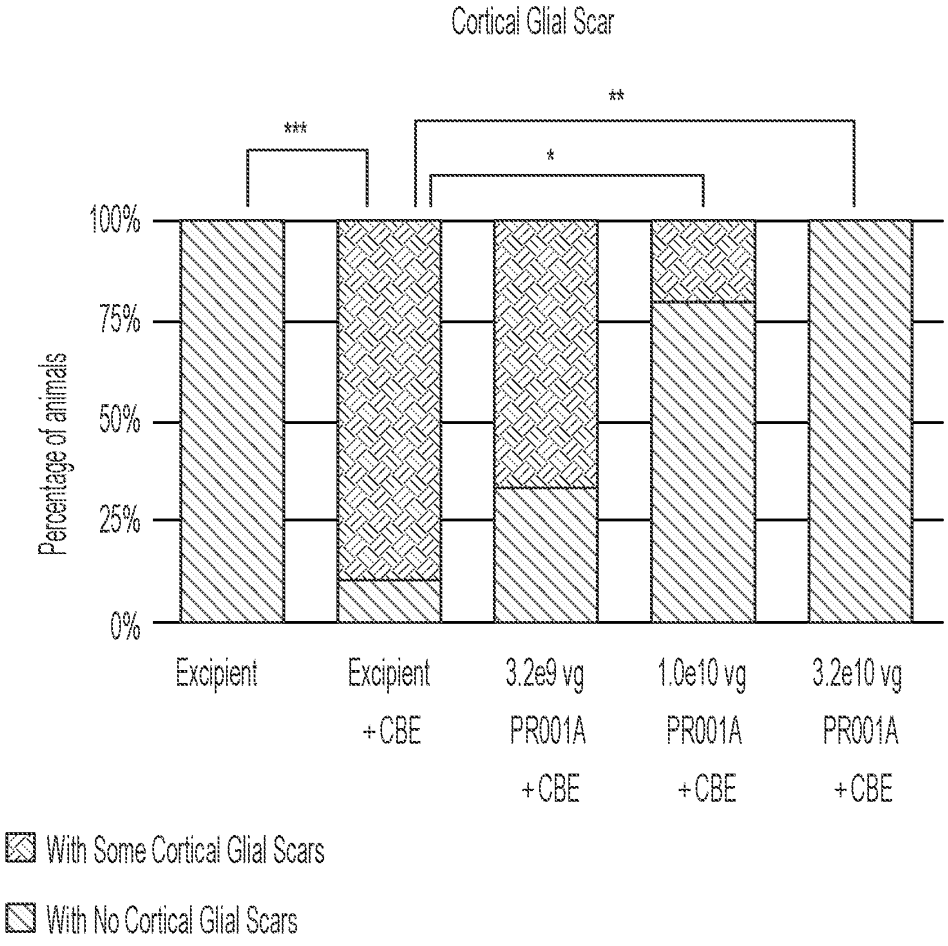

Cortical Glial Scar
***
**
*
100%
75%
50%
25%
0%
Percentage of animals
Excipient
Excipient +CBE
3.2e9 vg PR001A +CBE
1.0e10 vg PR001A +CBE
3.2e10 vg PR001A +CBE
With Some Cortical Glial Scars
With No Cortical Glial Scars

FIG. 28

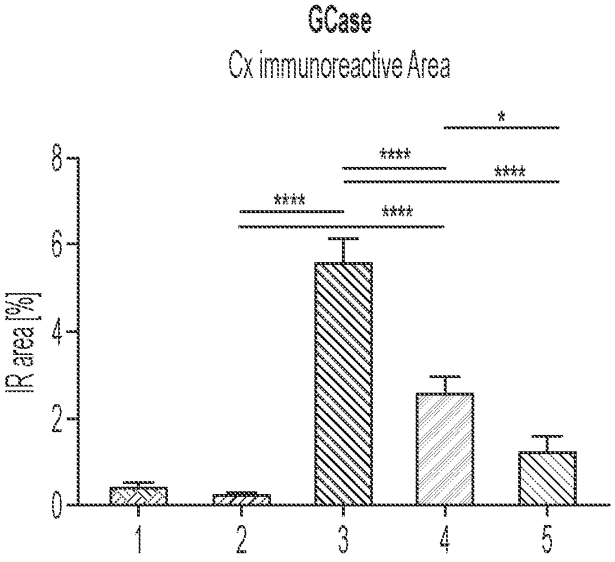


FIG. 29A

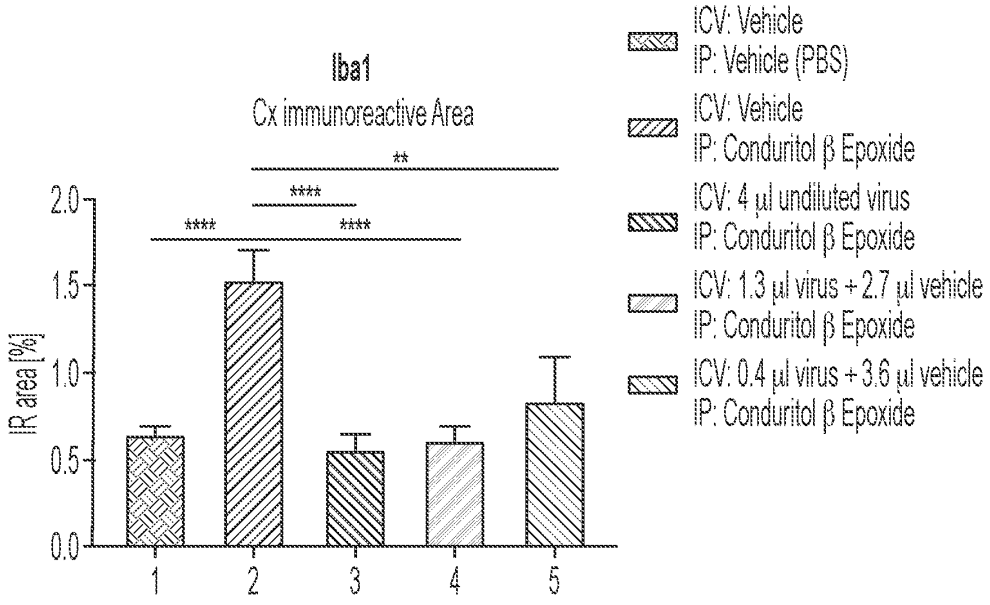


FIG. 29B

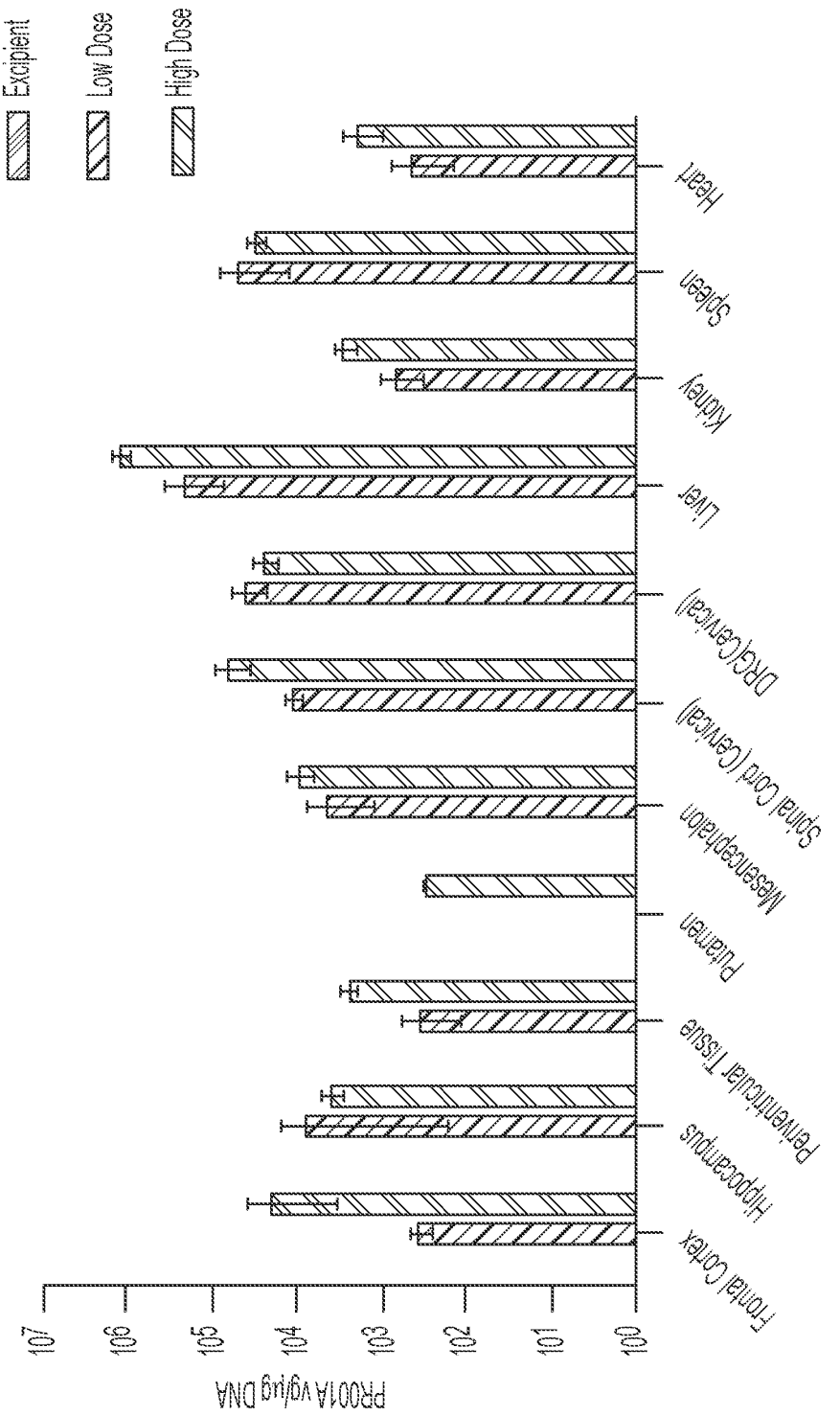

Excipient
Low Dose
High Dose
Heart
Spleen
Kidney
Liver
DRG(Cervical)
Spinal Cord (Cervical)
Mesencephalon
Pulvinar
Periventricular Tissue
Hippocampus
Frontal Cortex
107
106
105
104
103
102
101
100
PR001A vg/µg DNA

FIG. 30

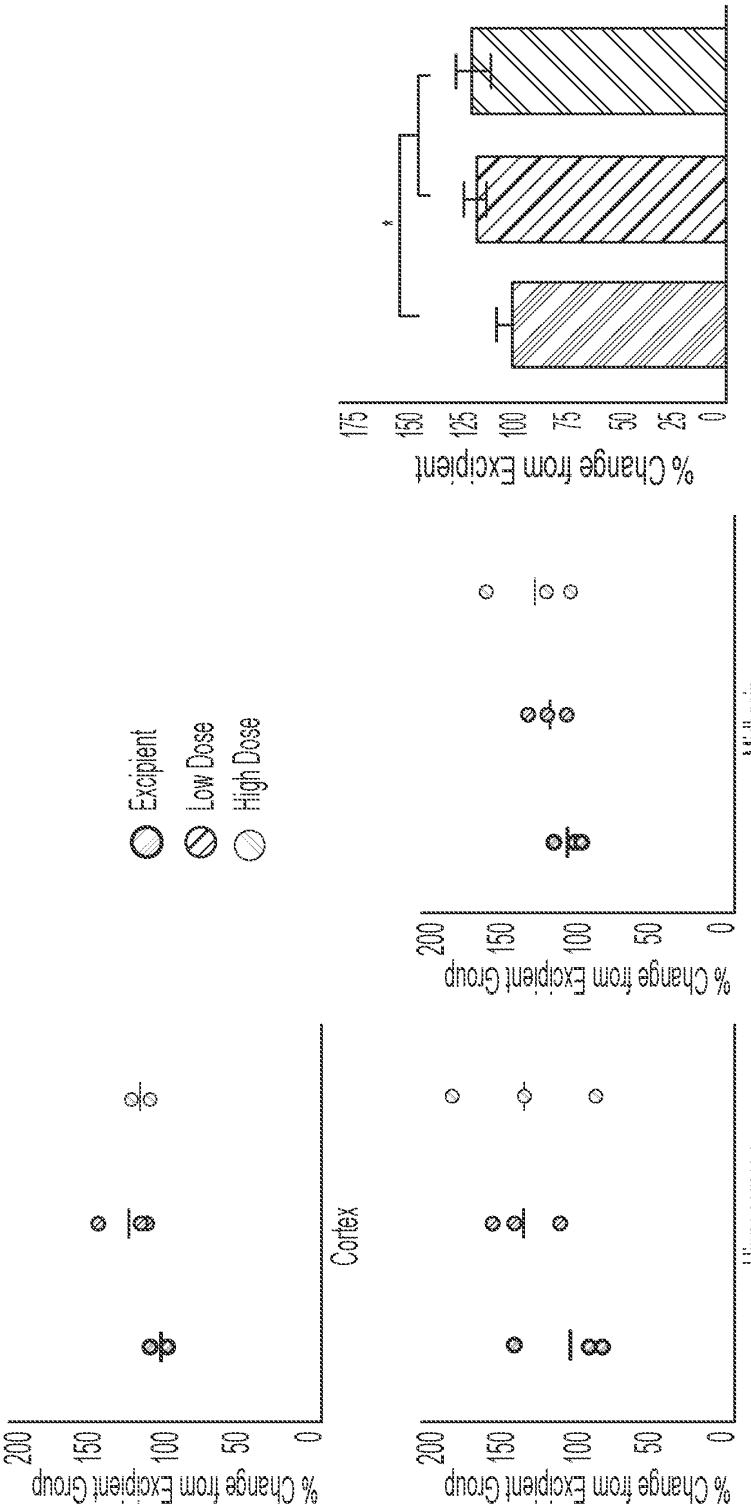


FIG. 31A

FIG. 31B

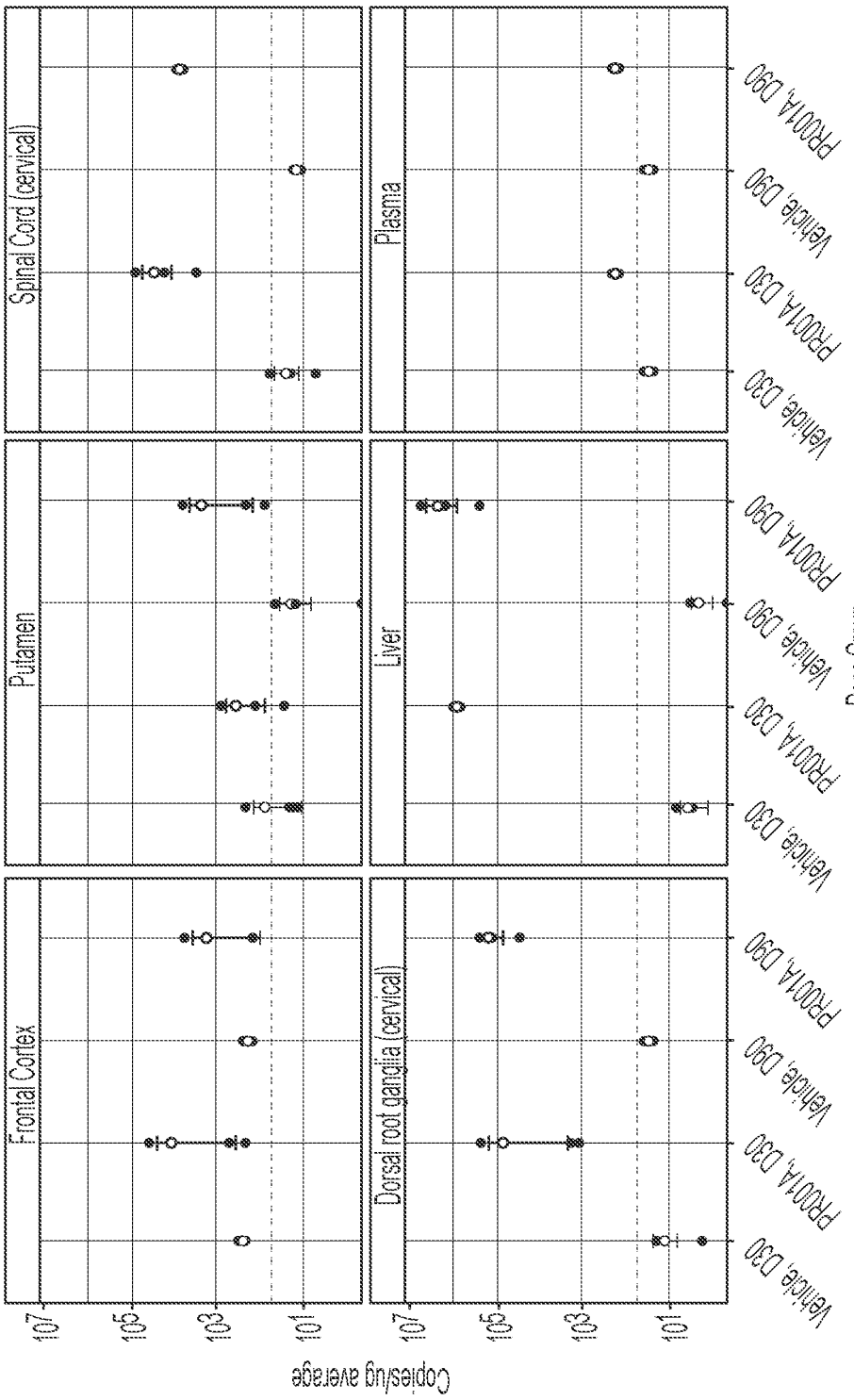

Frontal Cortex
Putamen
Spinal Cord (cervical)
Dorsal root ganglia (cervical)
Liver
Plasma
Copies/ug average
107
105
103
101
Vehicle, D30
PR001A, D30
Vehicle, D90
PR001A, D90
Dose Group

FIG. 32

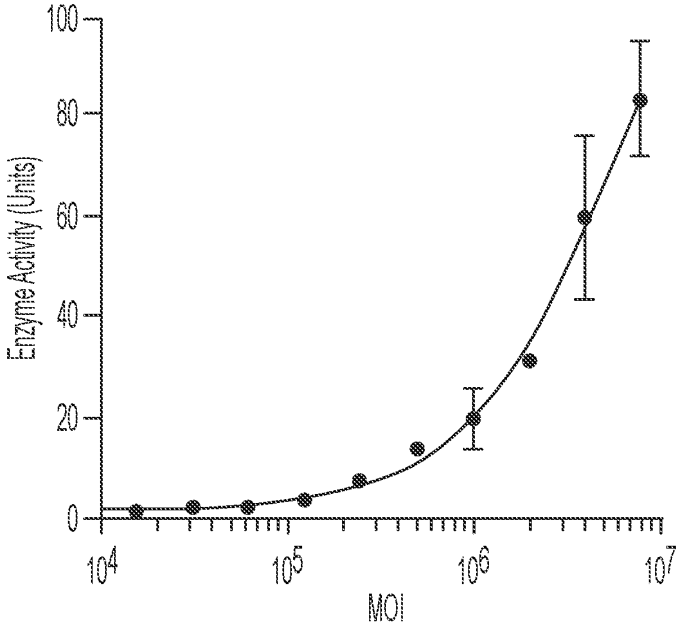


FIG. 33

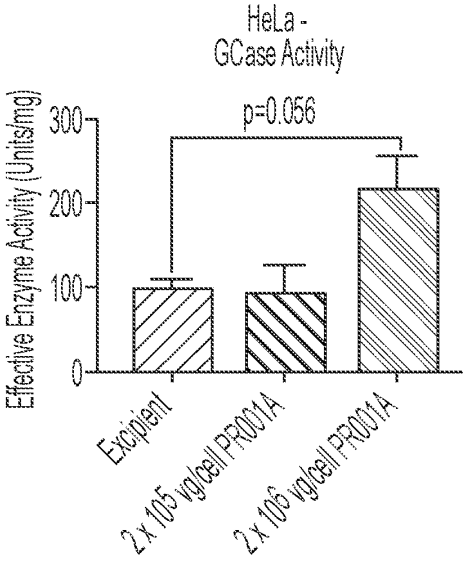


FIG. 34A

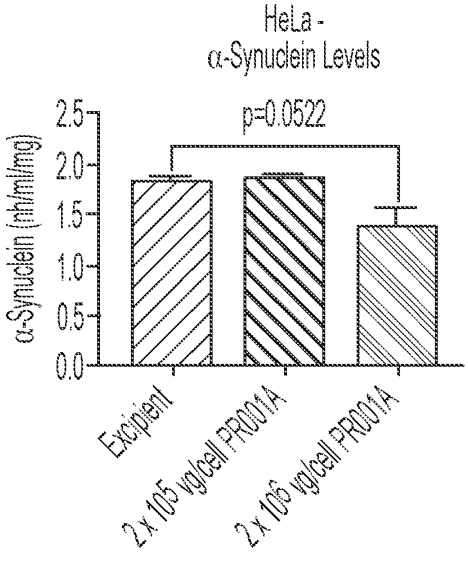


FIG. 34B

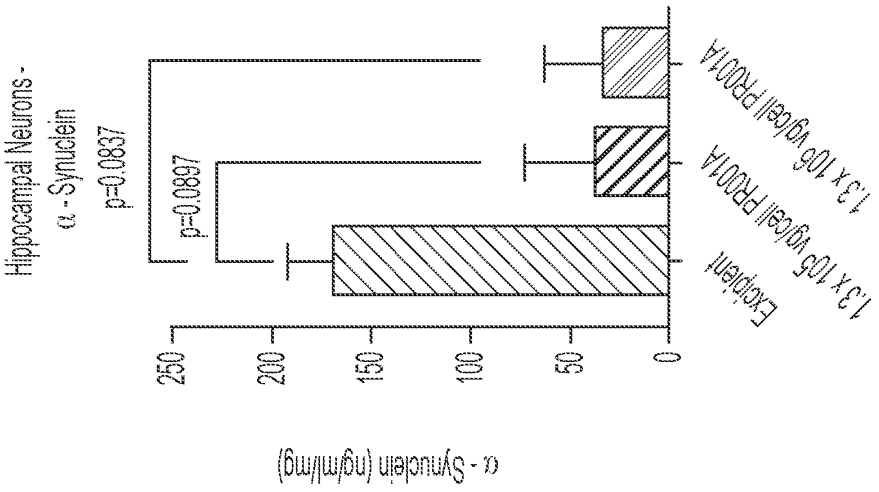


FIG. 35B

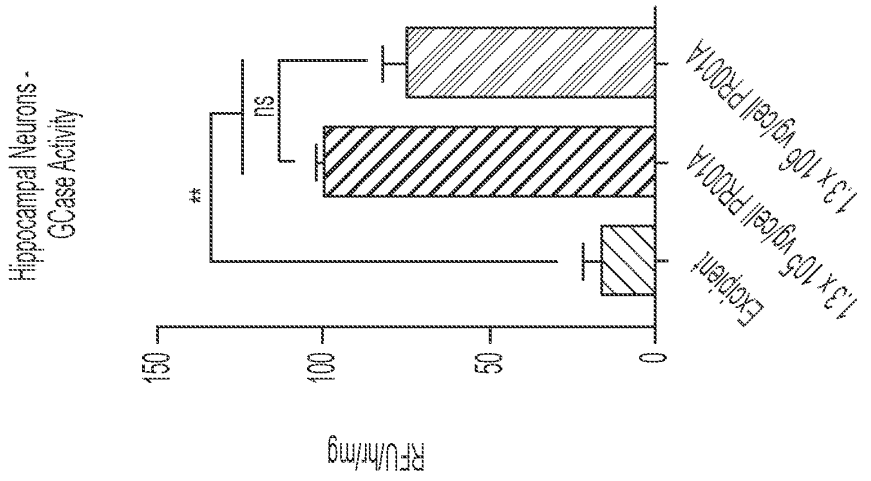


FIG. 35A

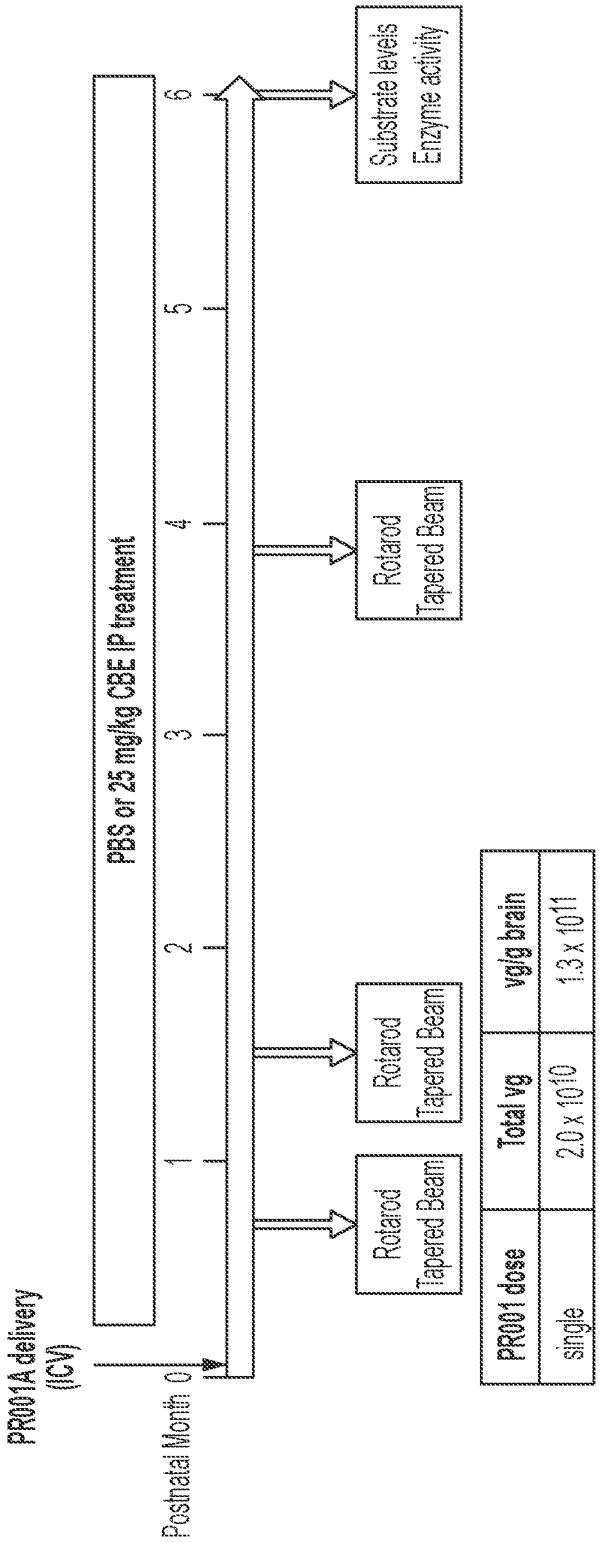

PR001A delivery
(ICV)
Postnatal Month 0
1
2
3
4
5
6
PBS or 25 mg/kg CBE IP treatment
Rotarod
Tapered Beam
Rotarod
Tapered Beam
Rotarod
Tapered Beam
Substrate levels
Enzyme activity
PR001 dose
Total vg
vg/g brain
single
2.0 x 10^10
1.3 x 10^11

FIG. 36

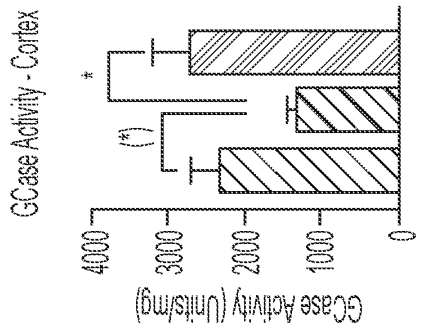


FIG. 37A

FIG. 37B

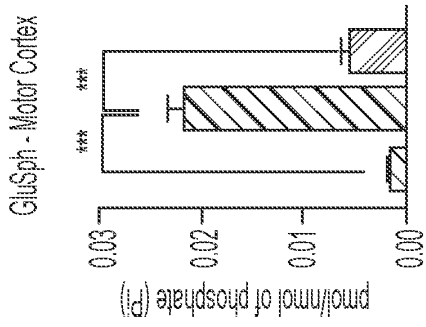


FIG. 37C

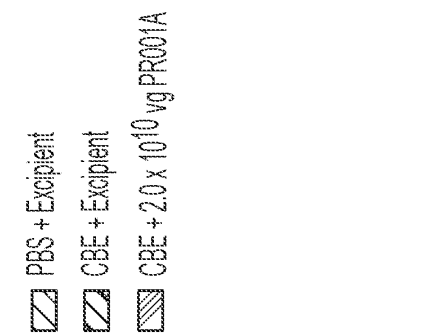


FIG. 37D

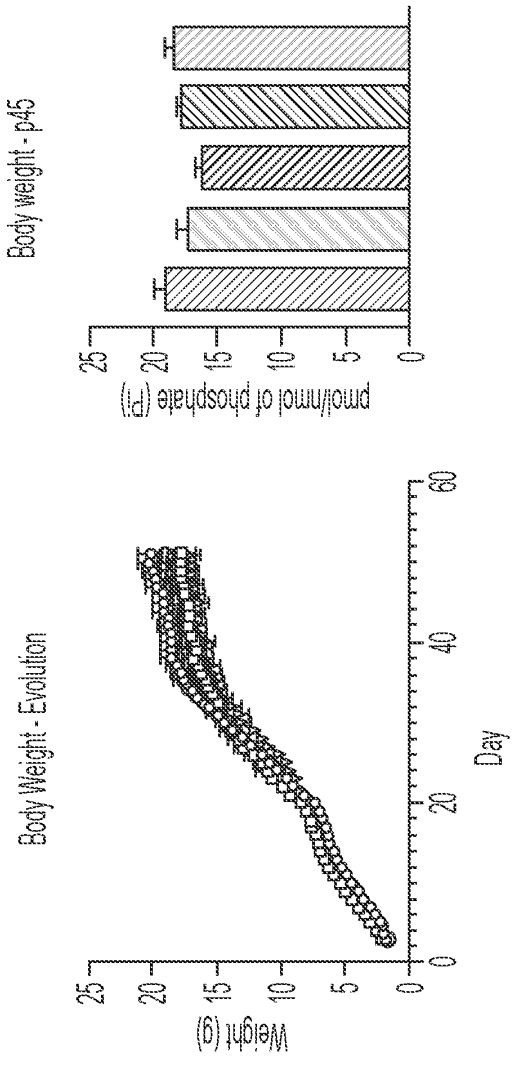


FIG. 38A

FIG. 38B

FIG. 38C

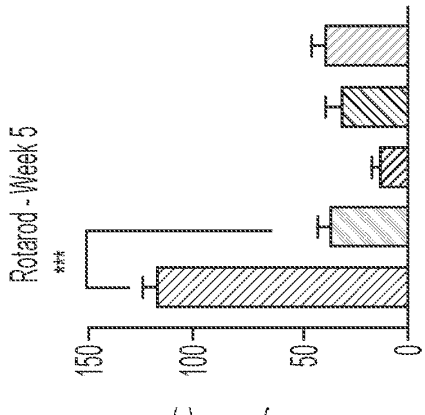


FIG. 38D

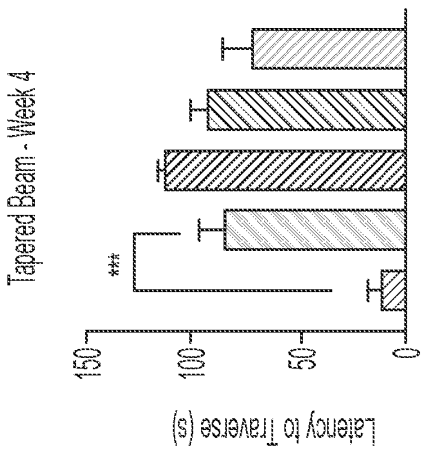


FIG. 38E

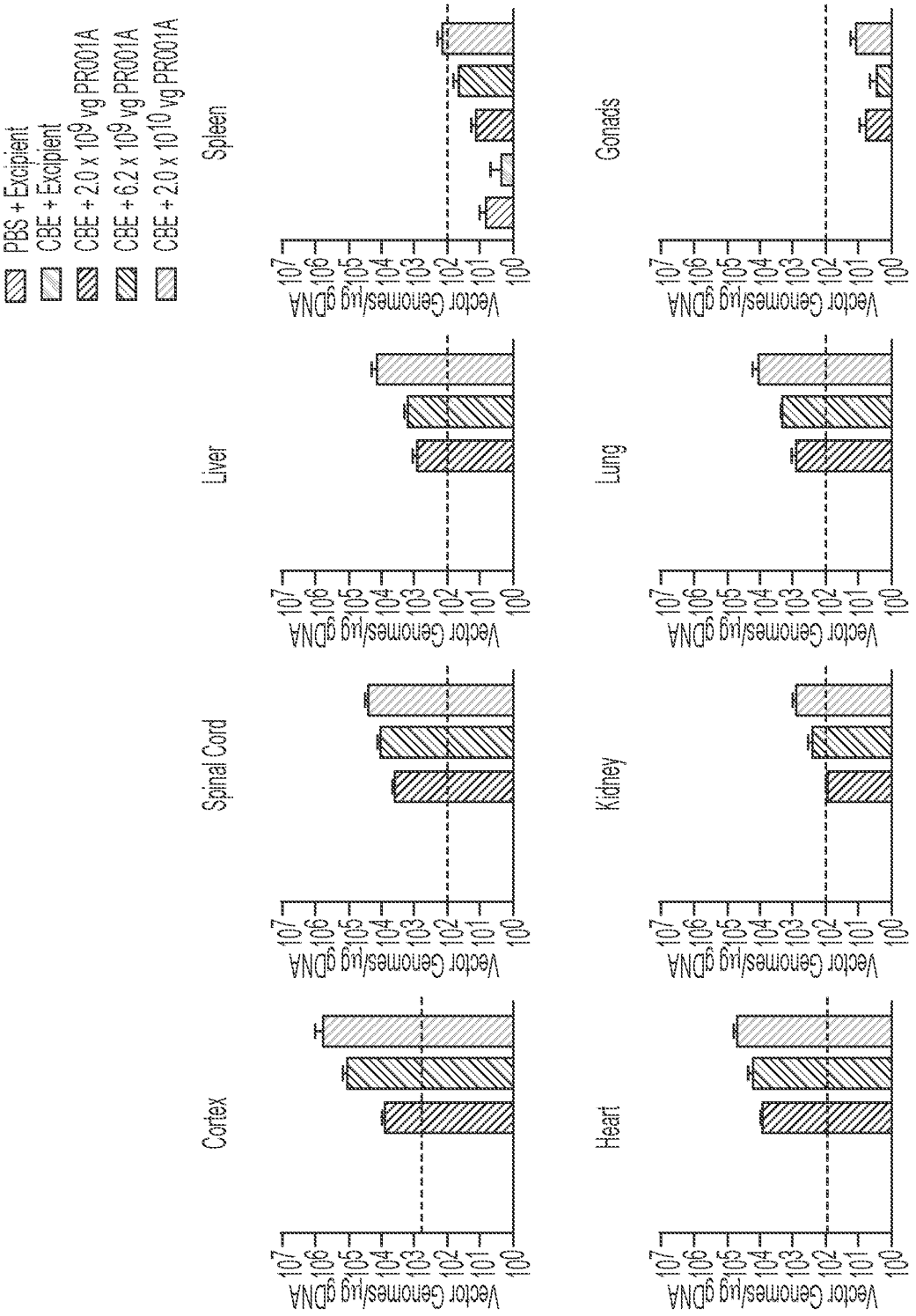

PBS + Excipient
CBE + Excipient
CBE + 2.0 x 10^9 vg PR001A
CBE + 6.2 x 10^9 vg PR001A
CBE + 2.0 x 10^10 vg PR001A
Cortex
Spinal Cord
Liver
Spleen
Heart
Kidney
Lung
Gonads
Vector Genomes/µg gDNA
100
101
102
103
104
105
106
107

FIG. 39

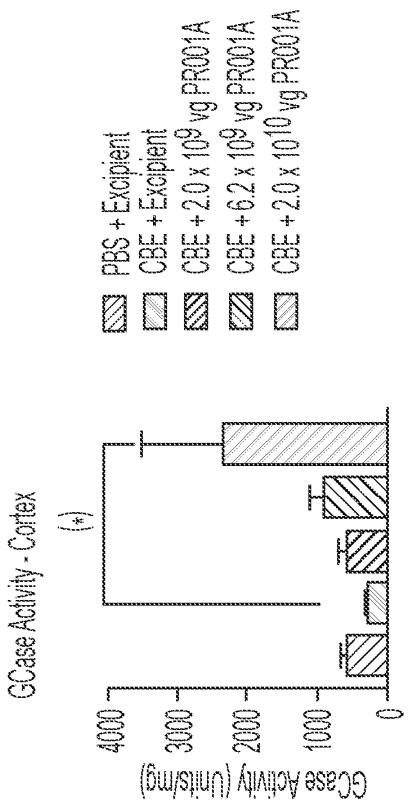


FIG. 40

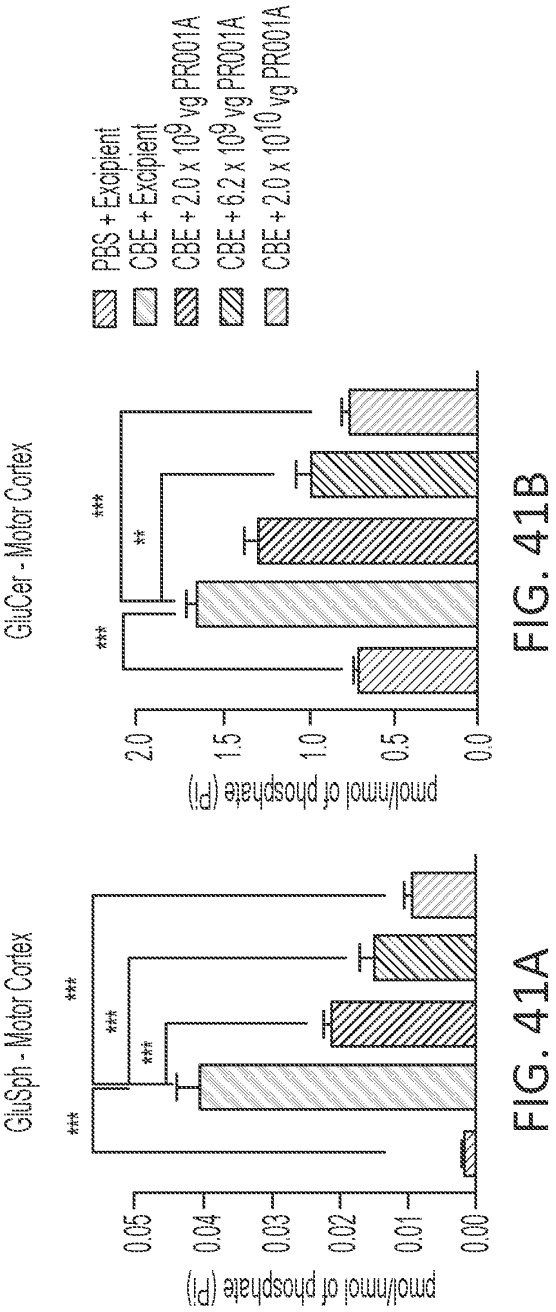


FIG. 41B

FIG. 41A

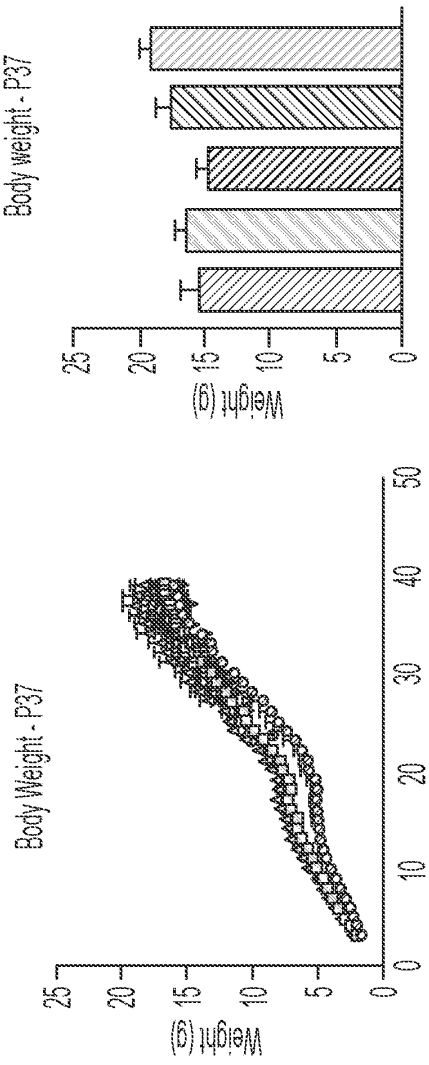


FIG. 42A

FIG. 42B

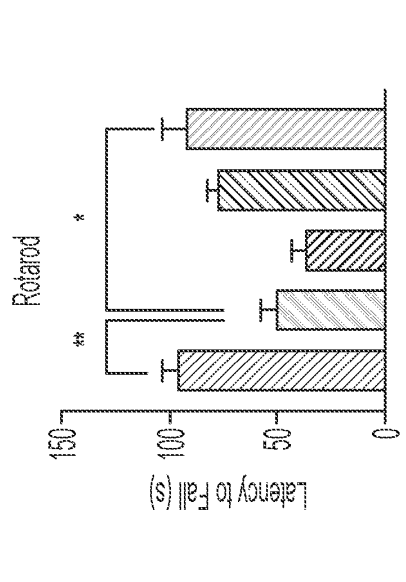


FIG. 42C

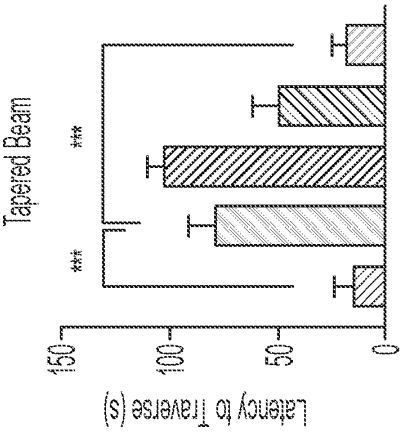


FIG. 42D

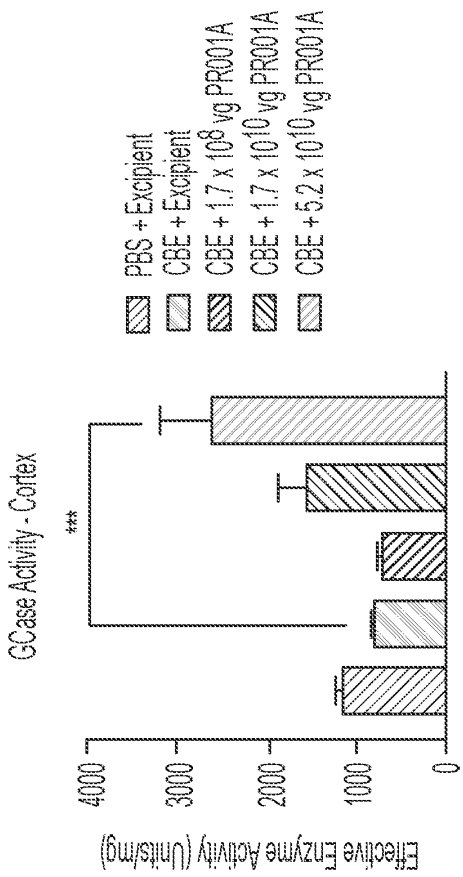


FIG. 43A

FIG. 43B

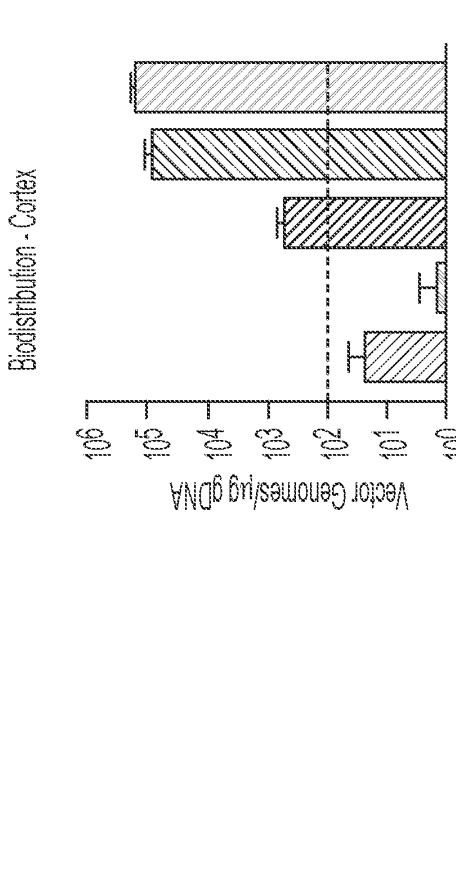


FIG. 44A

FIG. 44B

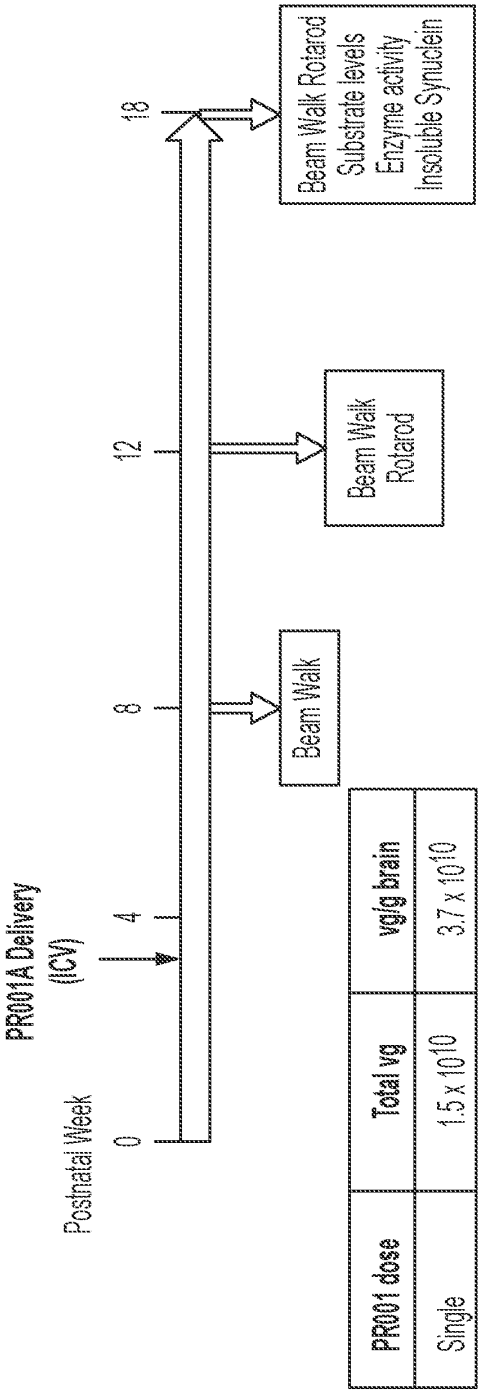

Posthatal Week
0
4
8
12
18
PR001A Delivery
(ICV)
Beam Walk
Beam Walk
Rotarod
Beam Walk Rotarod
Substrate levels
Enzyme activity
Insoluble Synuclein
PR001 dose
Total vg
vg/g brain
Single
1.5 x 10^10
3.7 x 10^10

FIG. 45

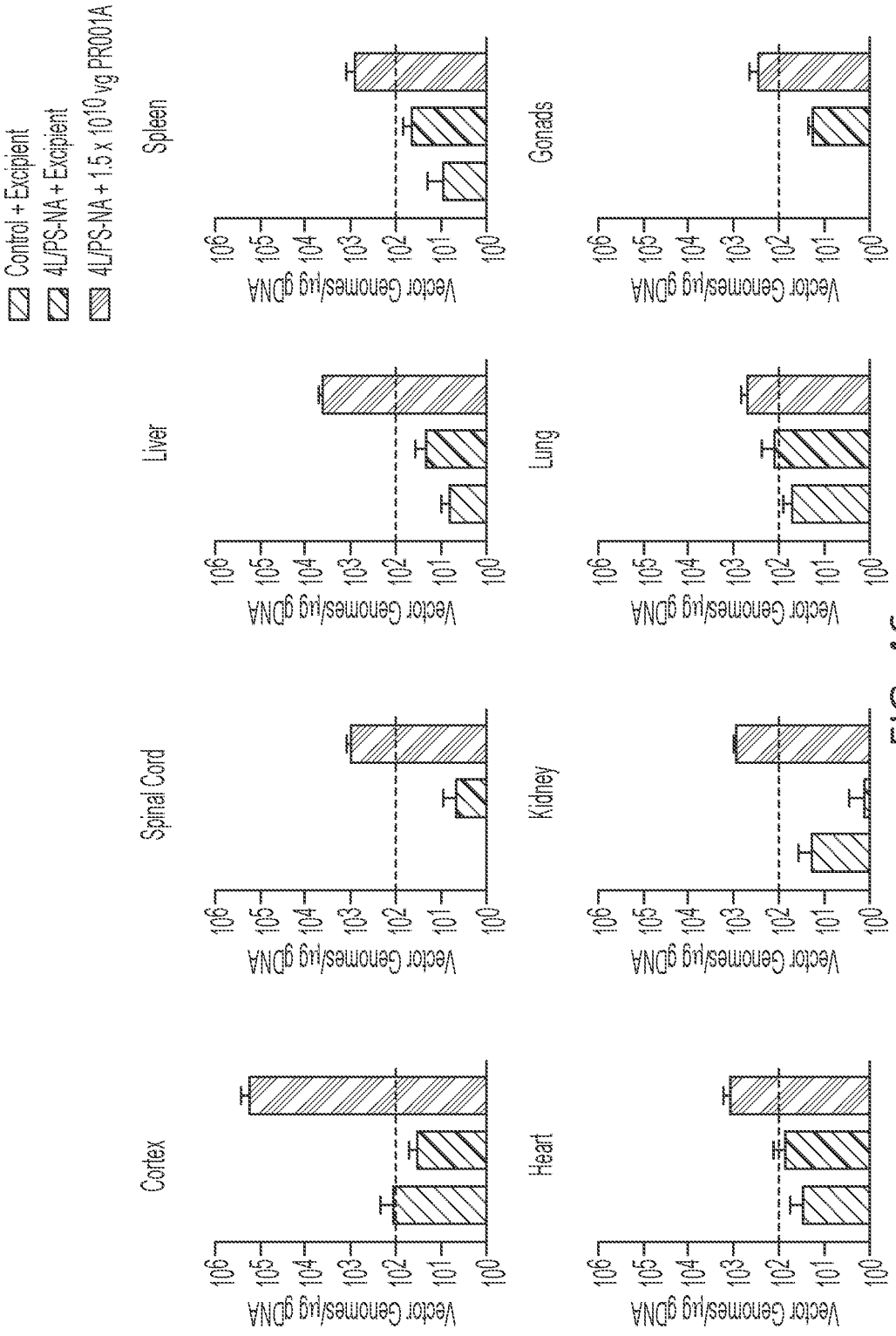

Control + Excipient
4L/PS-NA + Excipient
4L/PS-NA + 1.5 x 10^10 vg PR001A
Spleen
Liver
Spinal Cord
Cortex
Gonads
Lung
Kidney
Heart
Vector Genomes/µg gDNA
106
105
104
103
102
101
100

FIG. 46

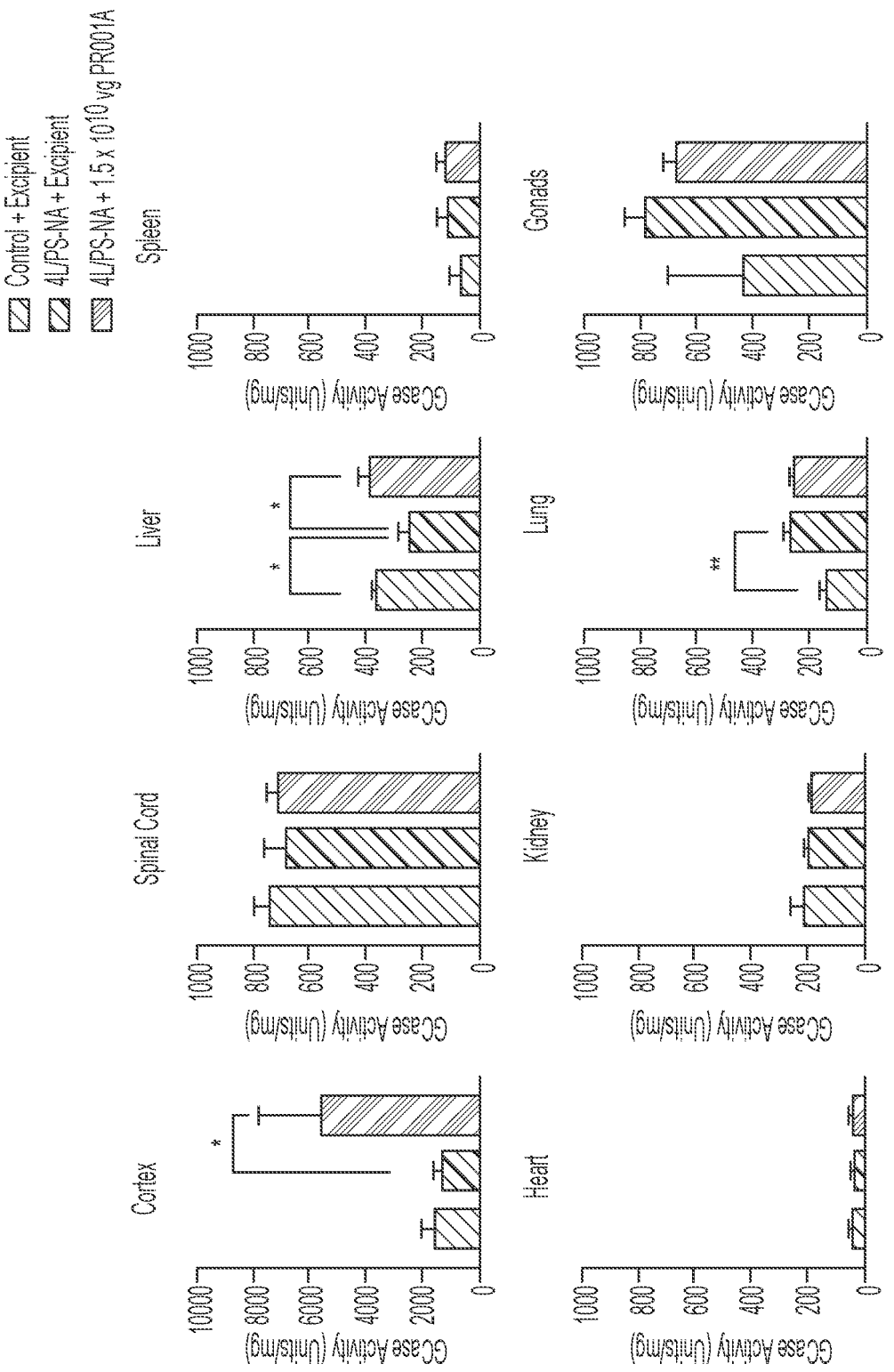

Control + Excipient
4L/PS-NA + Excipient
4L/PS-NA + 1.5 x 10^10 vg PR001A
Cortex
Spinal Cord
Liver
Spleen
Heart
Kidney
Lung
Gonads
GCase Activity (Units/mg)
0
200
400
600
800
1000
2000
4000
6000
8000
10000
*
**

FIG. 47

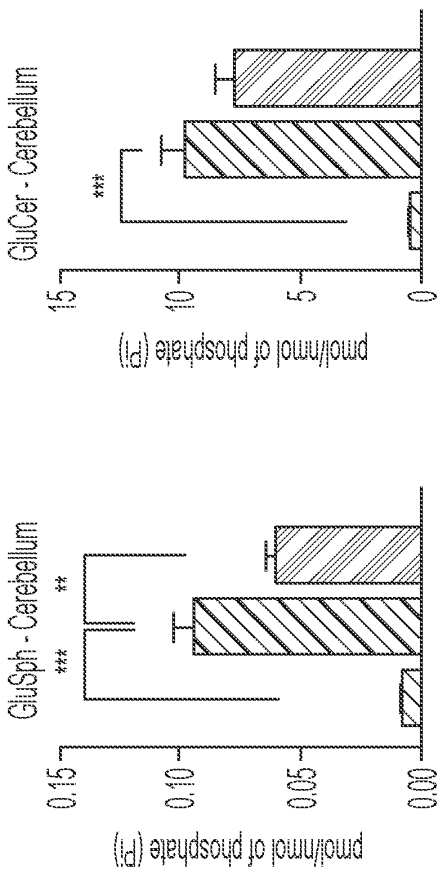


FIG. 48A

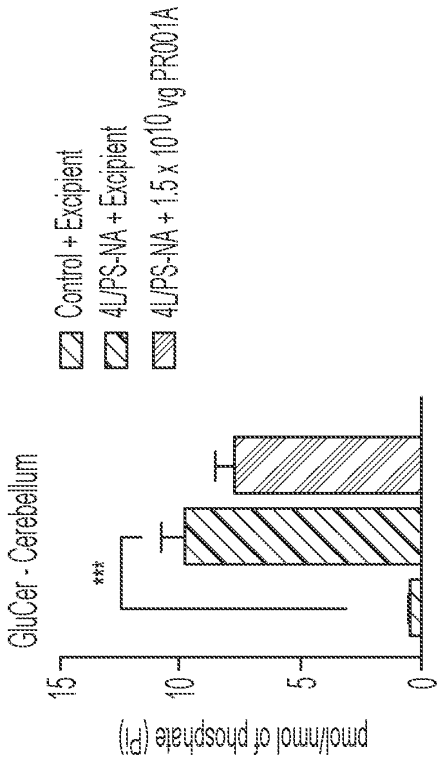


FIG. 48B

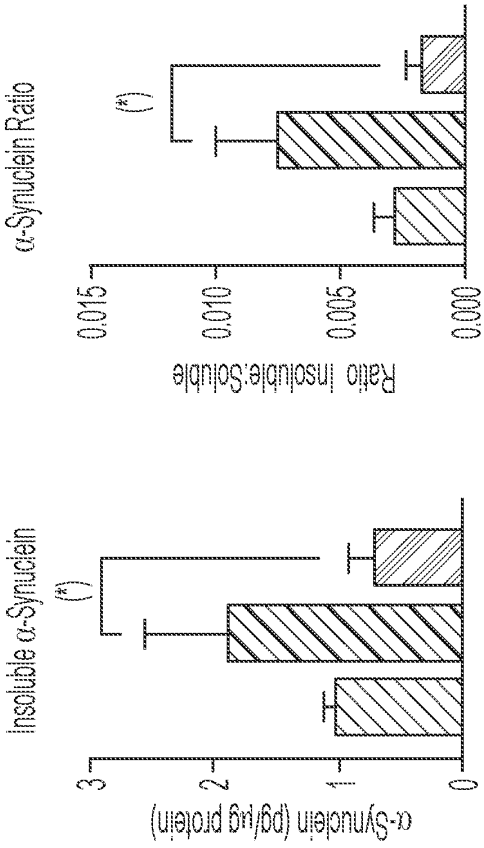
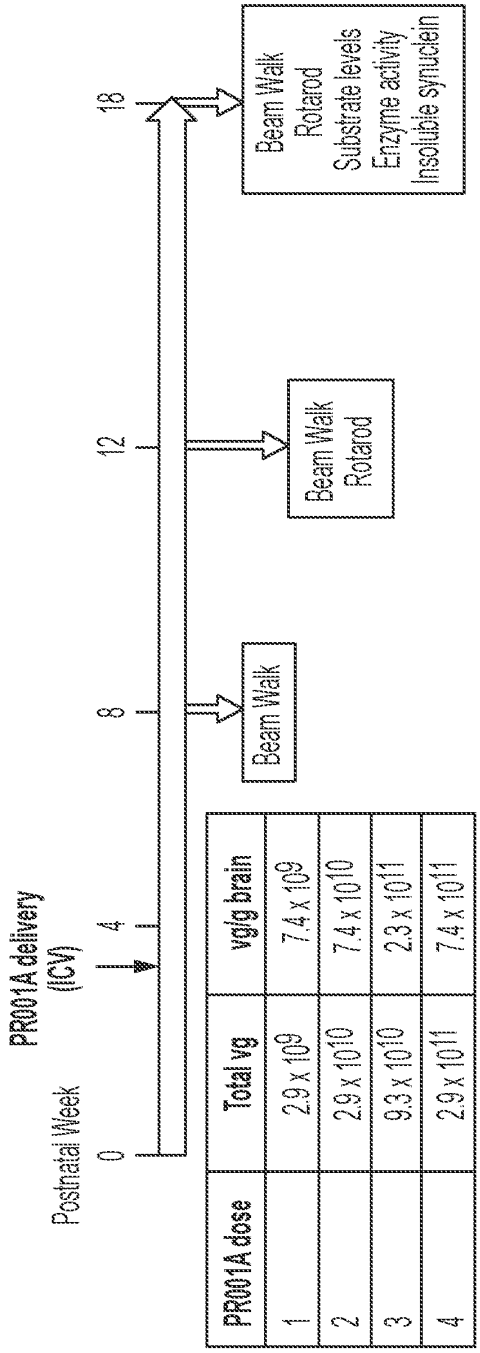


FIG. 49A

FIG. 49B



PR001A dose	Total vg	vg/g brain
1	2.9×10^9	7.4×10^9
2	2.9×10^{10}	7.4×10^{10}
3	9.3×10^{10}	2.3×10^{11}
4	2.9×10^{11}	7.4×10^{11}

FIG. 50

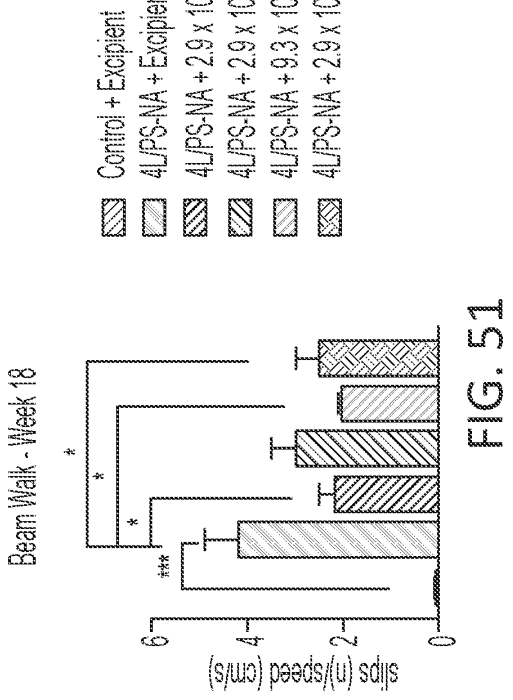


FIG. 51

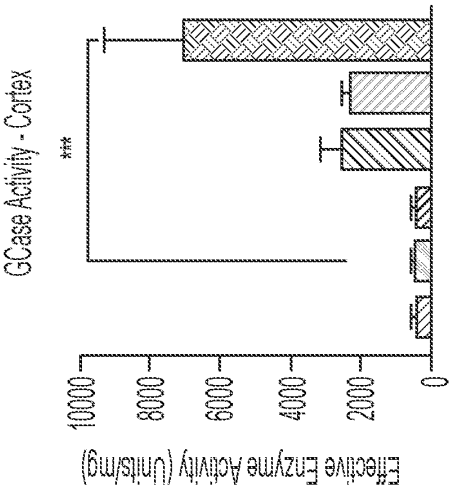


FIG. 52A

FIG. 52B

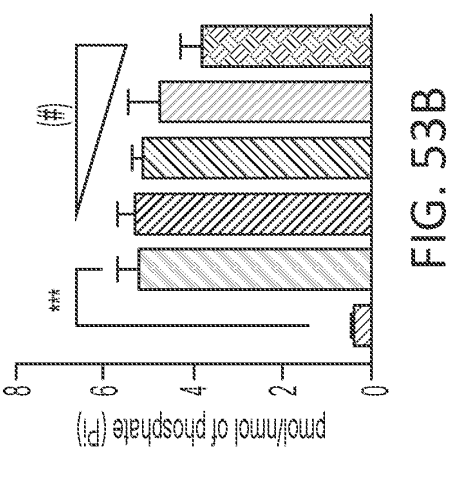


FIG. 53A

FIG. 53B

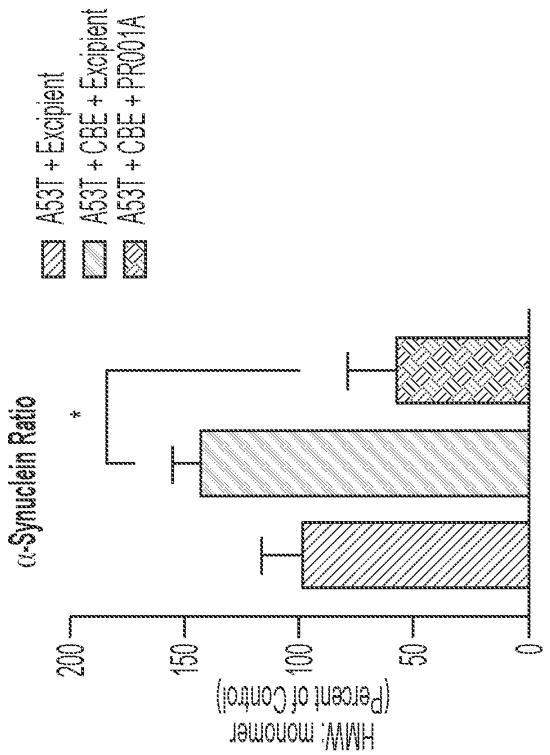


FIG. 54B

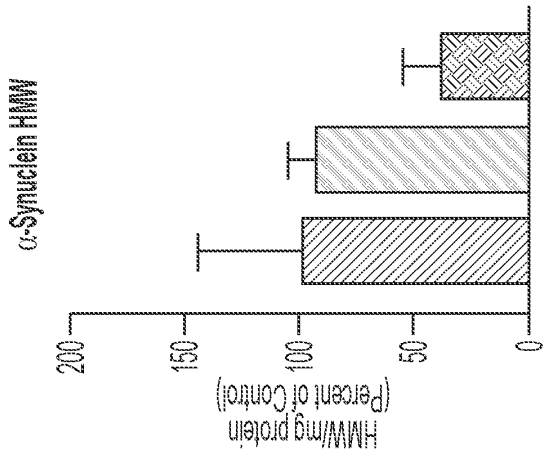


FIG. 54A

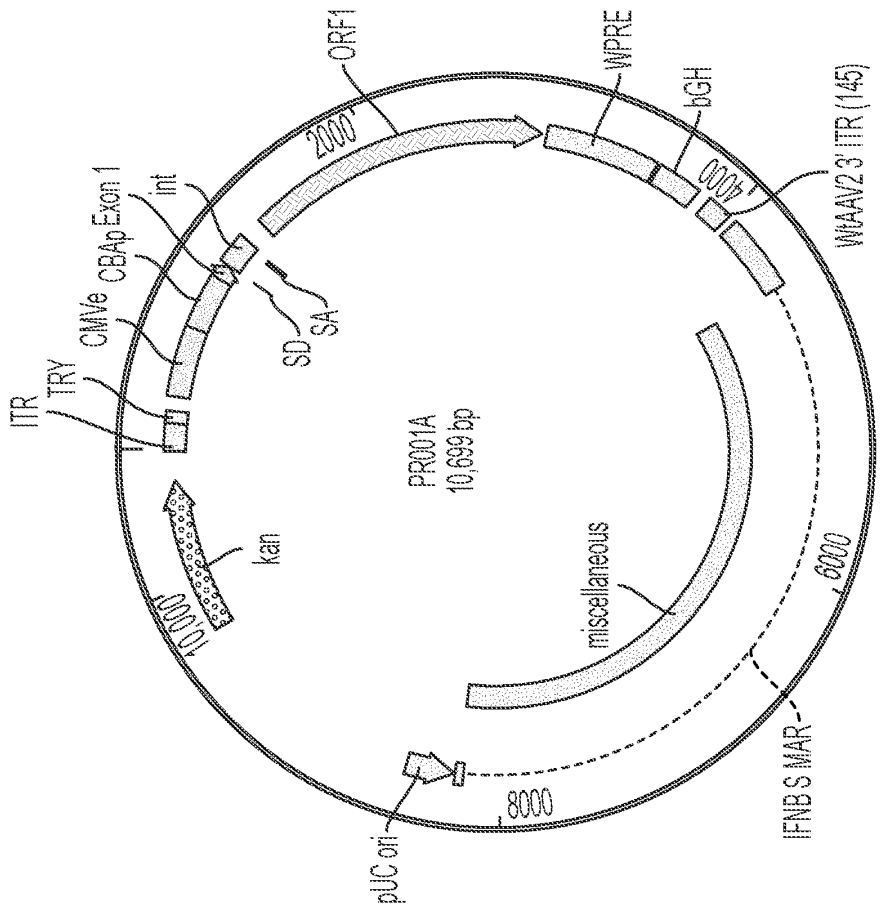

ORF1
2000
WPRE
bGH
4000
WtAAV2 3' ITR (145)
Int
CBAp Exon 1
CMVe
TRY
ITR
SD
SA
PR001A
10,699 bp
kan
10000
miscellaneous
6000
IFNB S MAR
8000
pUC ori

FIG. 55

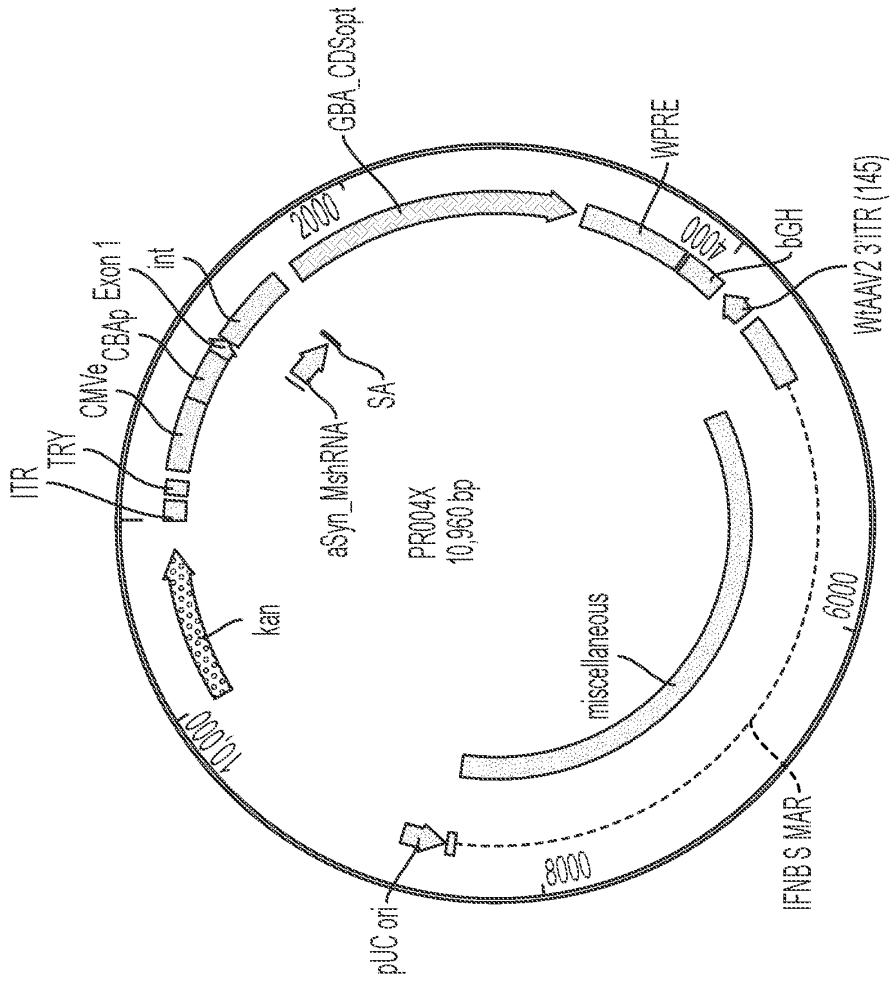

GBA_CDSopt
WPRE
bGH
WtAAV2 3'ITR (145)
IFNB S MAR
pUC ori
kan
ITR
TRY
CMVe
CBAp
Exon 1
int
SA
aSyn_MshRNA
PR004X
10,960 bp
miscellaneous
2000
4000
6000
8000
10000

FIG. 56

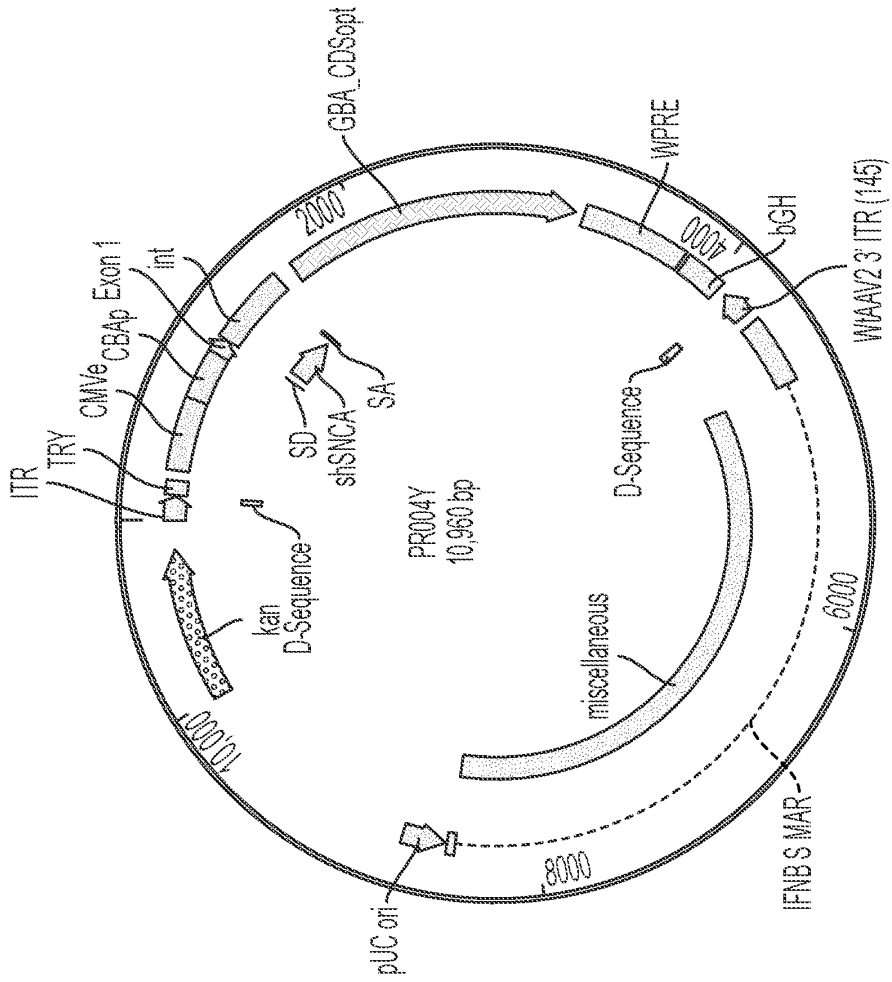

GBA_CDSopt
WPRE
bGH
WtAAV2 3' ITR (145)
Exon 1
int
CBAp
CMVe
TRY
ITR
SD
shSNCA
SA
D-Sequence
PR004Y
10,960 bp
D-Sequence
kan
D-Sequence
miscellaneous
pUC ori
IFNB S MAR
2000
4000
6000
8000
10000

FIG. 57

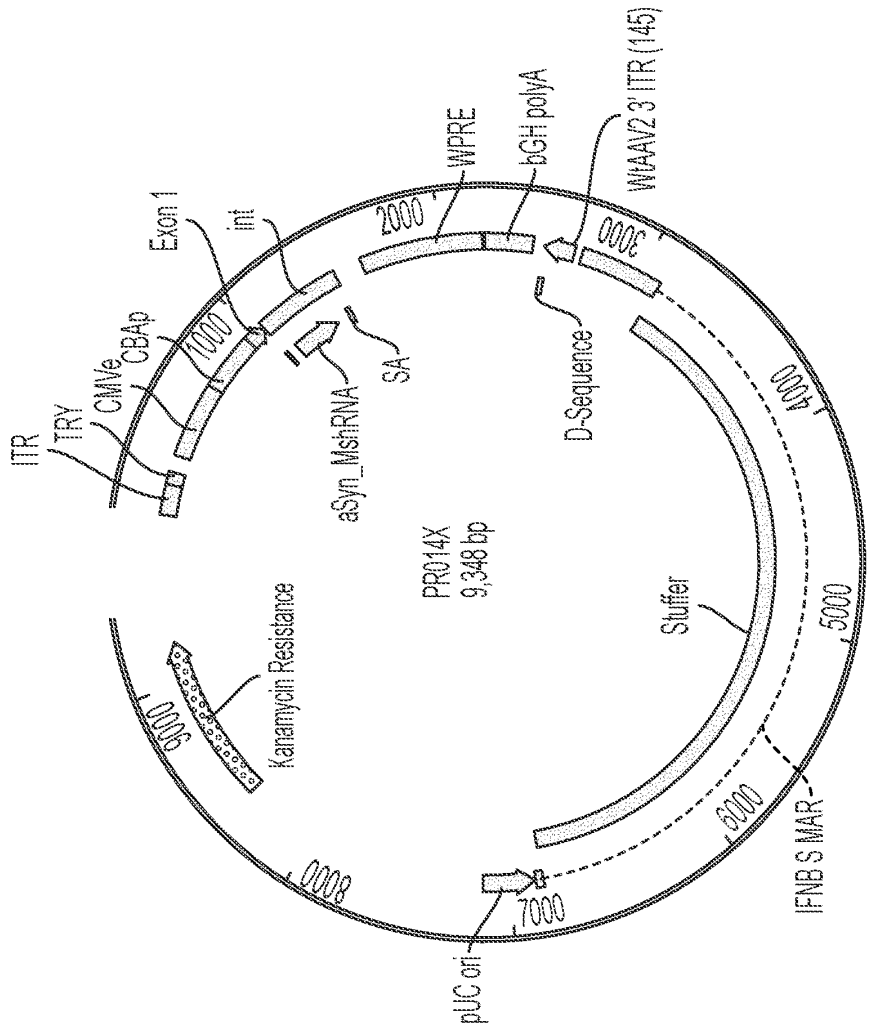

ITR
TRY
CMVe
CBAp
Exon 1
int
1000
aSyn_MshRNA
SA
2000
WPRE
bGH polyA
WtAAV2 3' ITR (145)
3000
D-Sequence
4000
PR014X
9,348 bp
Stuffer
5000
IFNB S MAR
6000
7000
pUC ori
8000
9000
Kanamycin Resistance

FIG. 58

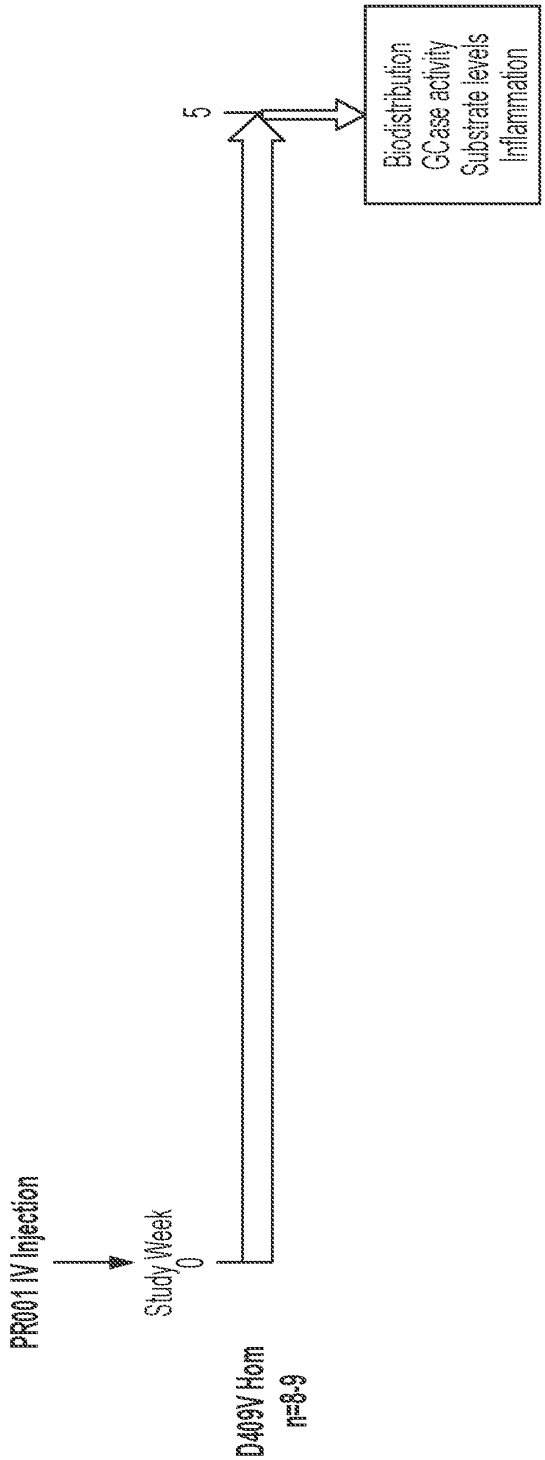

PR001 IV Injection
Study Week
0
5
D409V Hom
n=8-9
Biodistribution
GCase activity
Substrate levels
Inflammation

FIG. 59

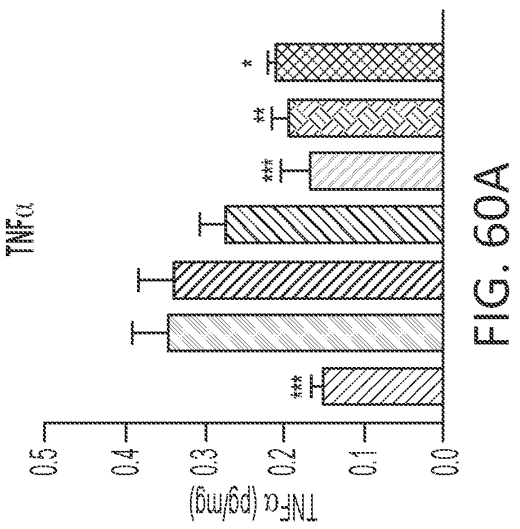


FIG. 60A

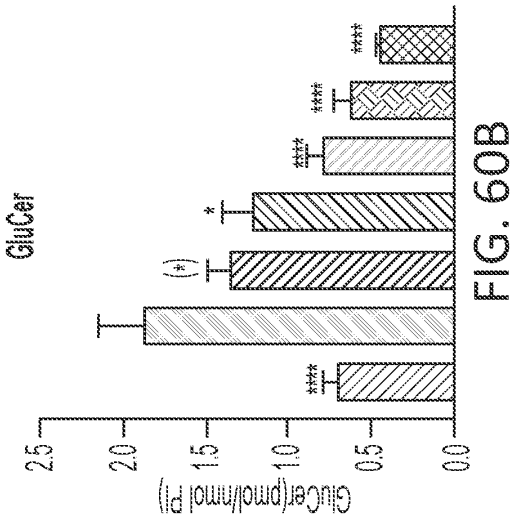
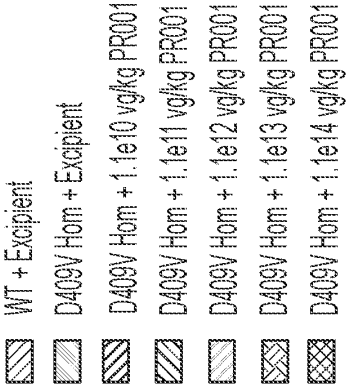


FIG. 60B



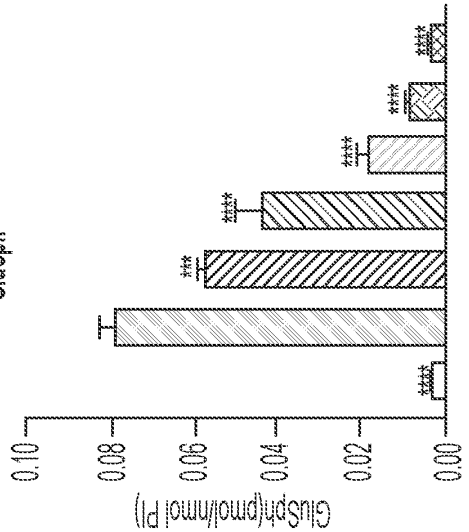


FIG. 60C

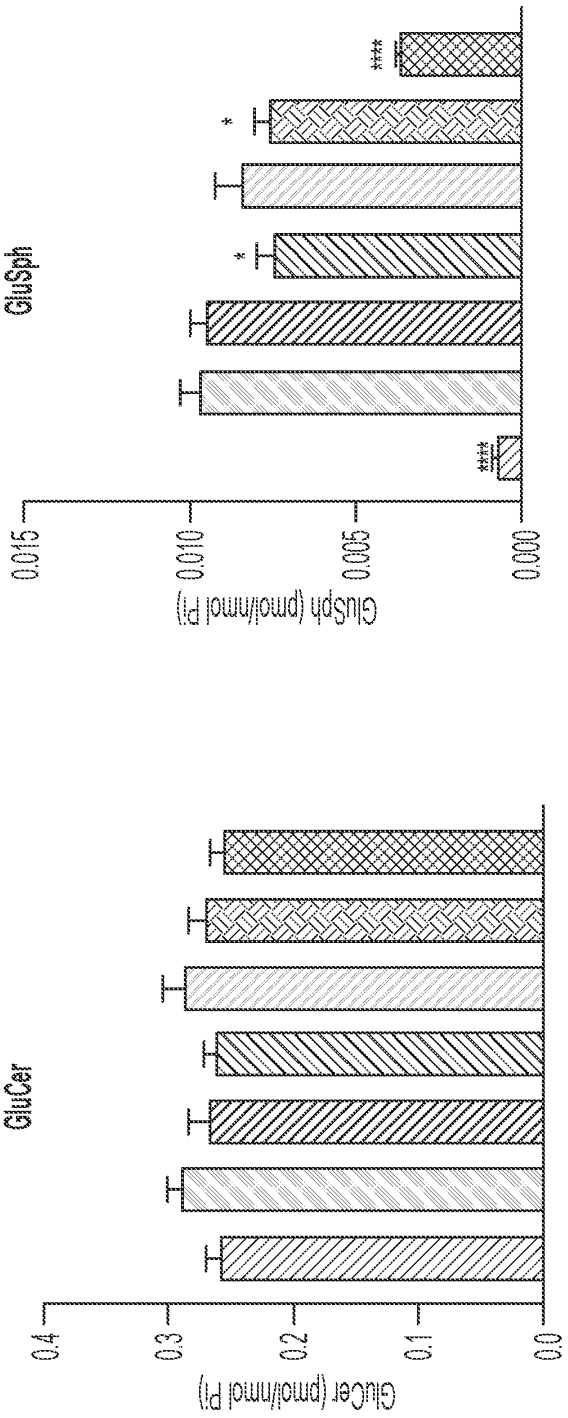


FIG. 61A

FIG. 61B

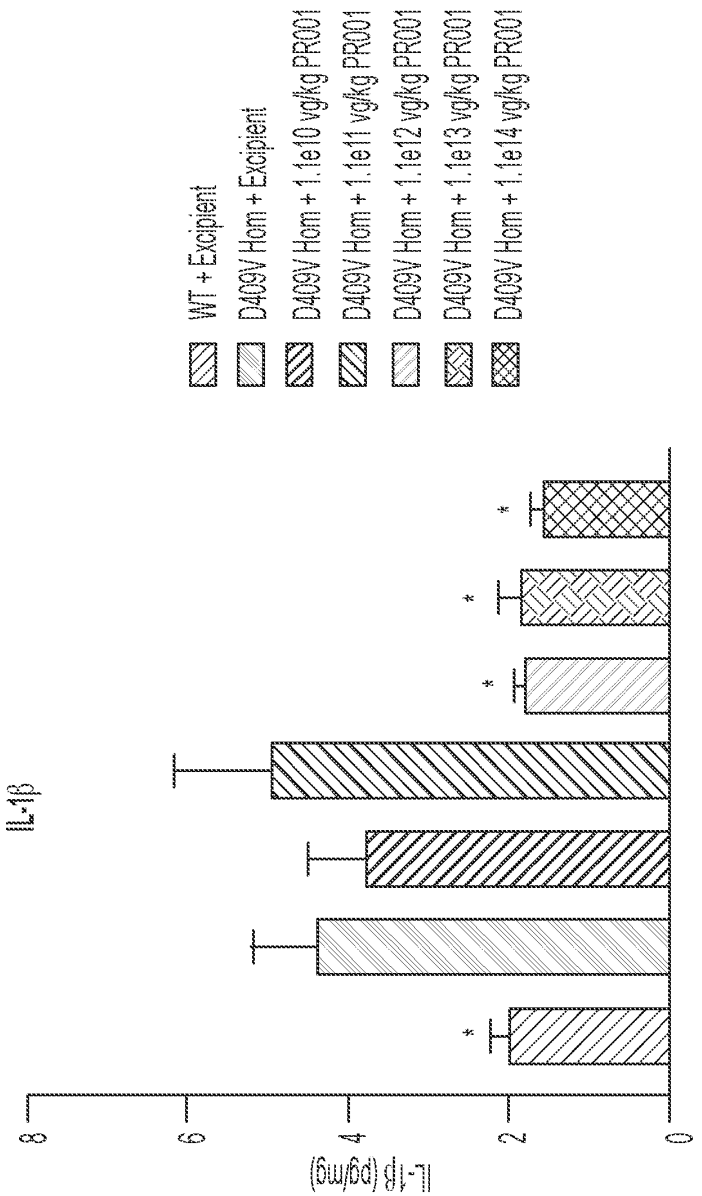

IL-1β
IL-1β (pg/mg)
0
2
4
6
8
*
*
*
*
WT + Excipient
D409V Hom + Excipient
D409V Hom + 1.1e10 vg/kg PR001
D409V Hom + 1.1e11 vg/kg PR001
D409V Hom + 1.1e12 vg/kg PR001
D409V Hom + 1.1e13 vg/kg PR001
D409V Hom + 1.1e14 vg/kg PR001

FIG. 62

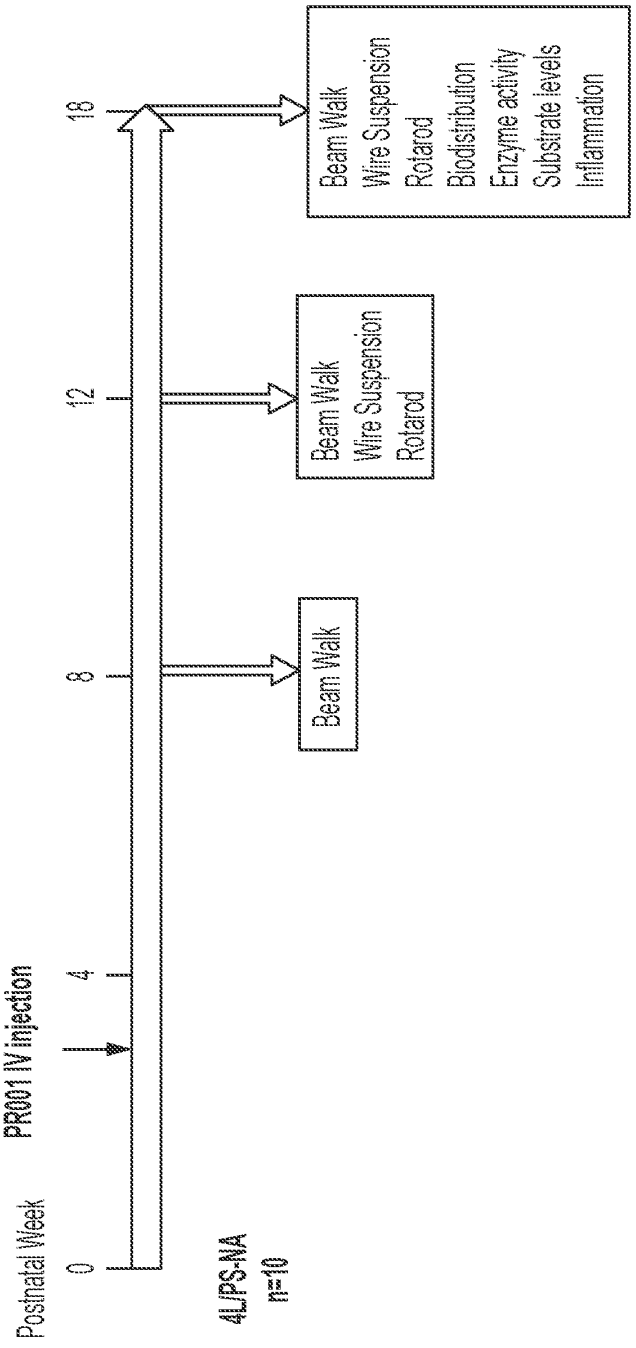

Postnatal Week
0
4
8
12
18
PR001 IV injection
4L/PS-NA
n=10
Beam Walk
Beam Walk
Wire Suspension
Rotarod
Beam Walk
Wire Suspension
Rotarod
Biodistribution
Enzyme activity
Substrate levels
Inflammation

FIG. 63

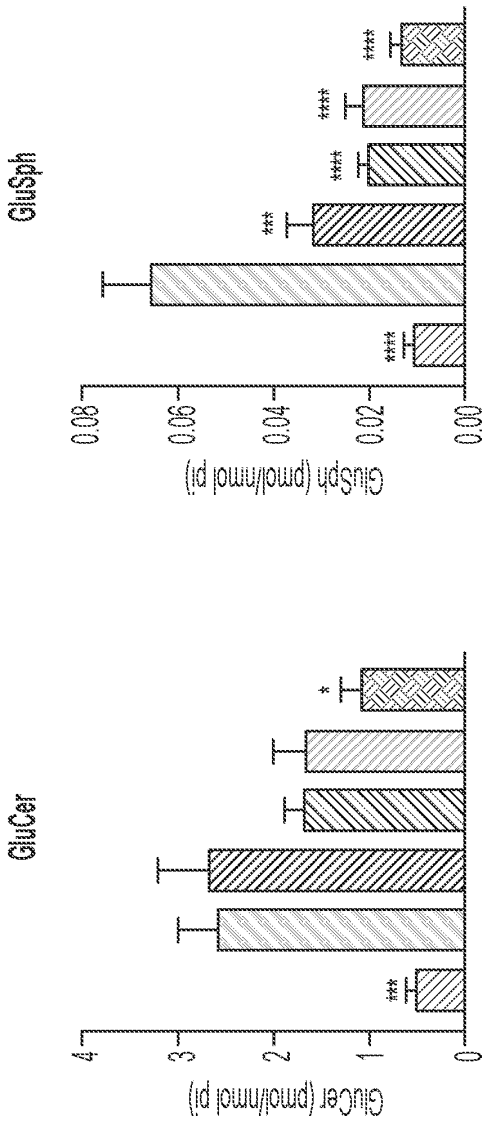


FIG. 64A

FIG. 64B

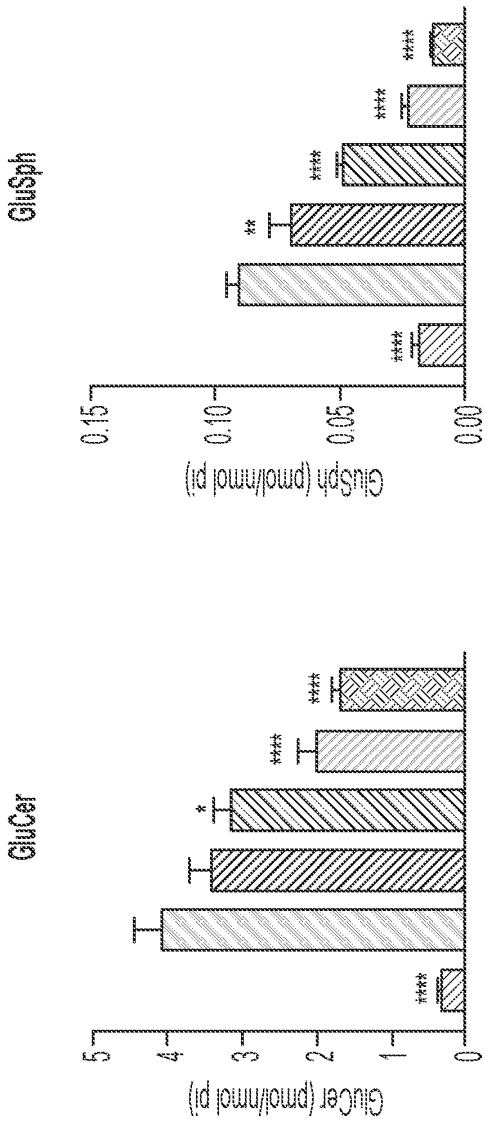


FIG. 65A

FIG. 65B

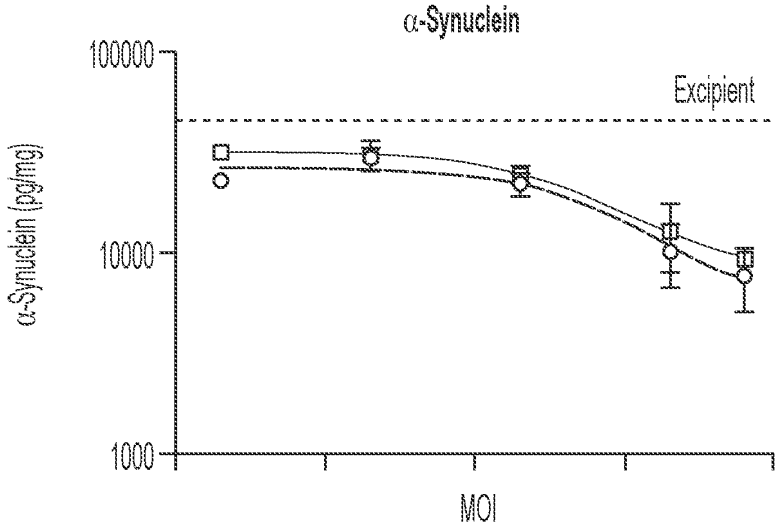


FIG. 66A

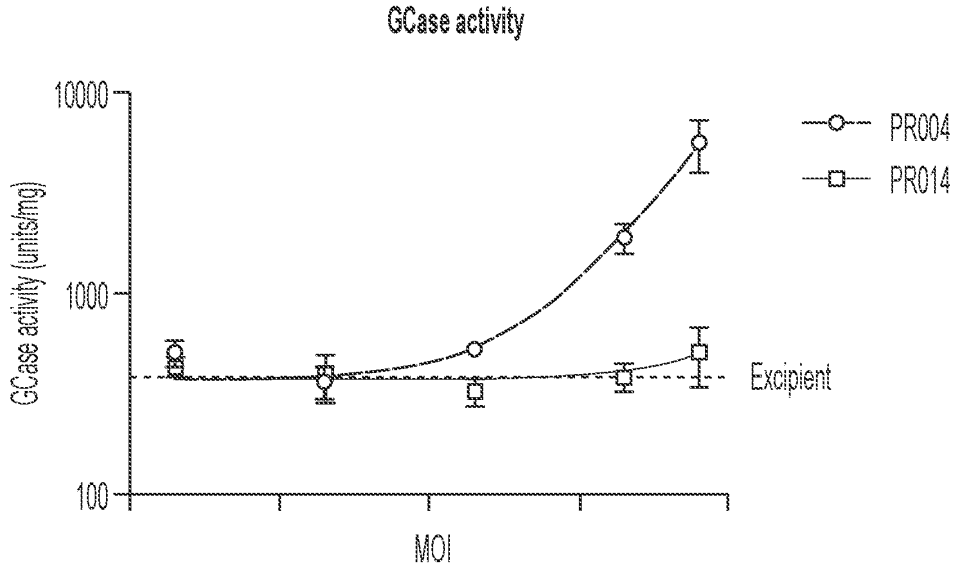


FIG. 66B

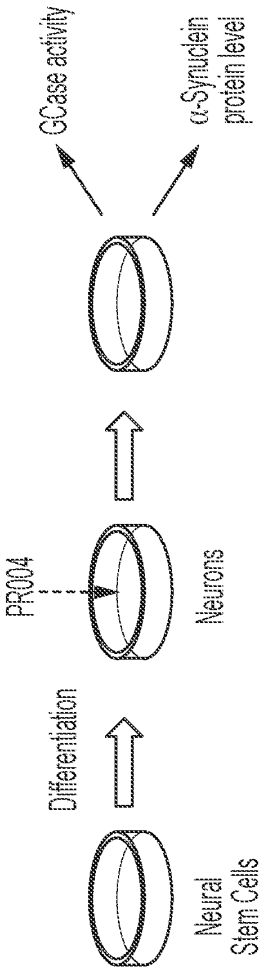
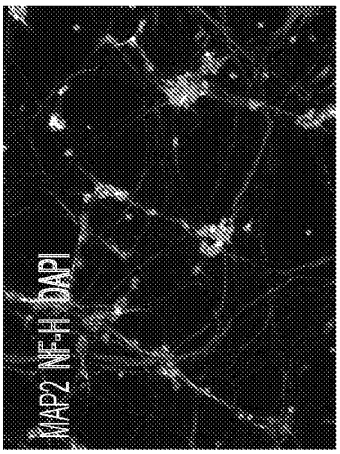


FIG. 67A



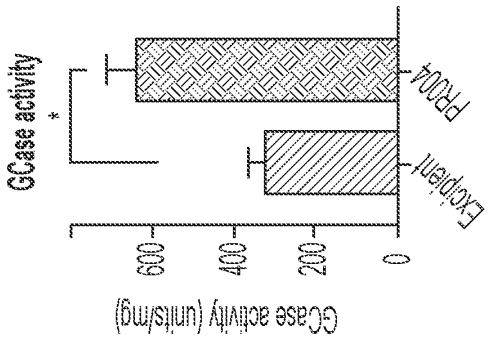


FIG. 67B

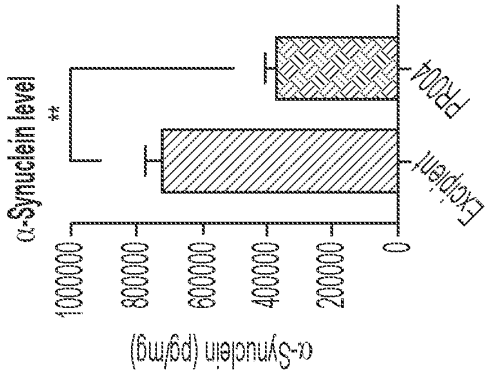


FIG. 67C

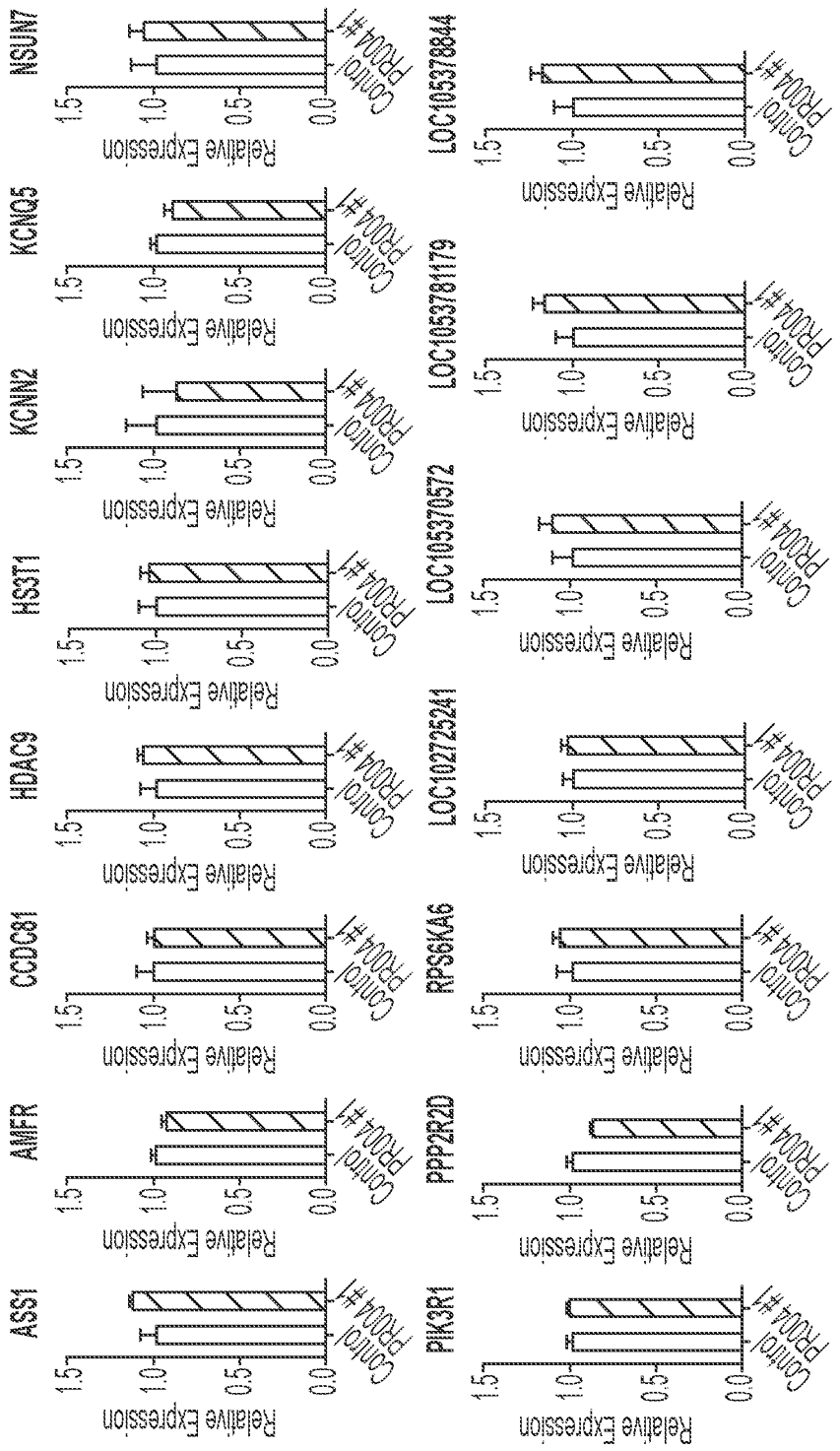

ASS1
AMFR
CCDC81
HDAC9
HS3T1
KCNN2
KCNQ5
NSUN7
PIK3R1
PPP2R2D
RPS6KA6
LOC102725241
LOC105370572
LOC105378179
LOC105378844
Relative Expression
1.5
1.0
0.5
0.0
Control
PR004 #1

FIG. 68A

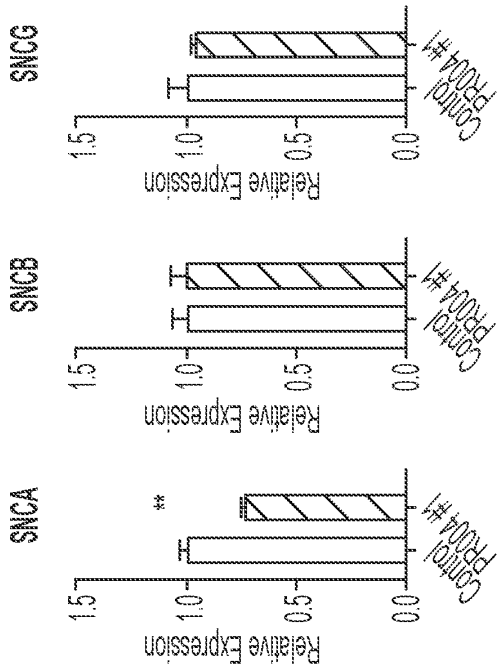

SNCA
SNCB
SNCG
Relative Expression
1.5
1.0
0.5
0.0
**
Control
PD04 #1

FIG. 68B

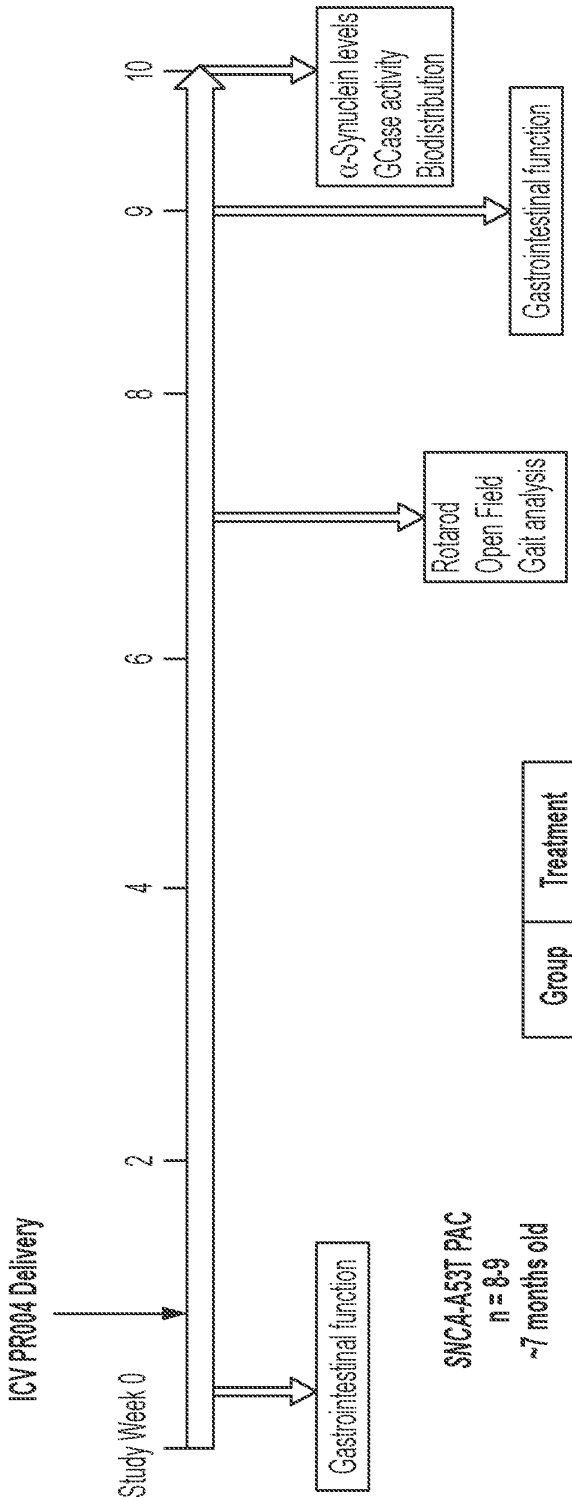

ICV PR004 Delivery
Study Week 0
2
4
6
8
9
10
Gastrointestinal function
α-Synuclein levels
GCase activity
Biodistribution
Gastrointestinal function
Rotarod
Open Field
Gait analysis
SNCA-A53T PAC
n = 8-9
~7 months old
Group
Treatment
1
Excipient
2
PR004 Dose 1
3
PR004 Dose 2

FIG. 69

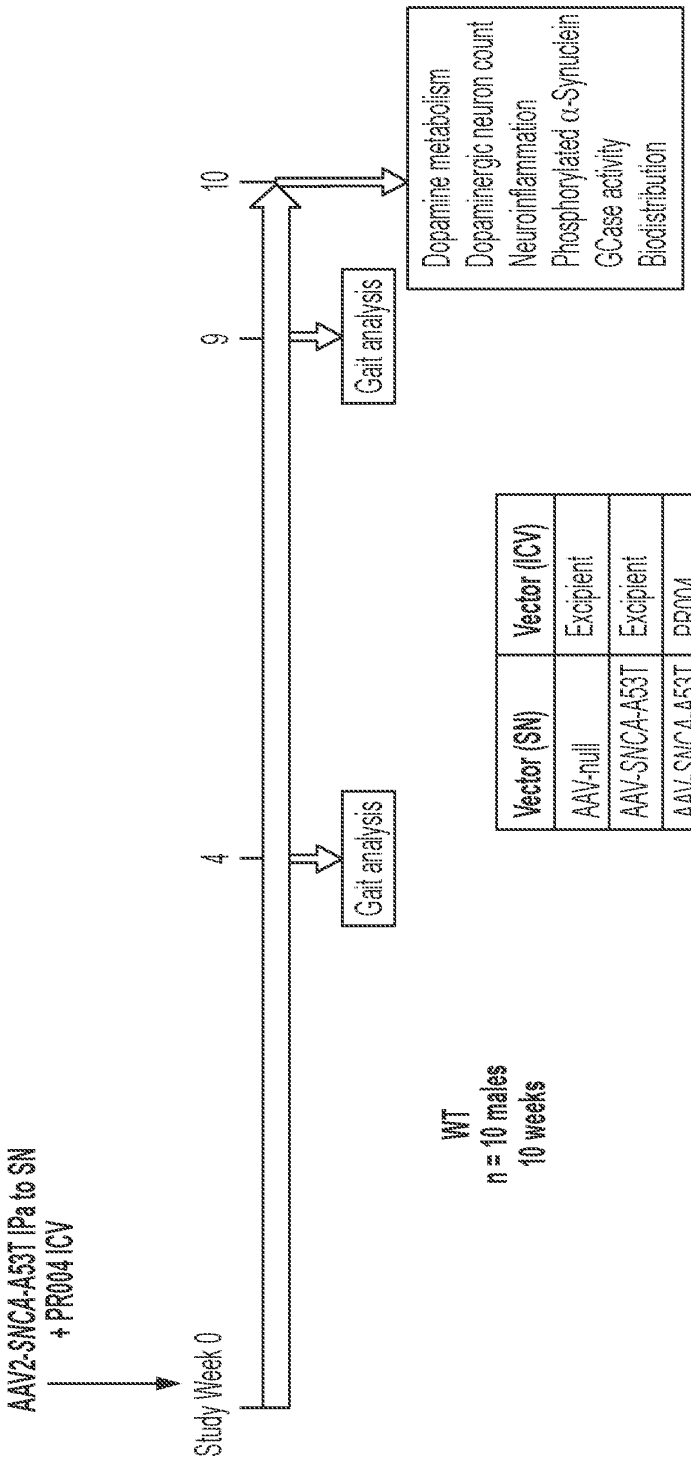

AAV2-SNCA-A53T IPa to SN
+ PR004 ICV
Study Week 0
4
9
10
Gait analysis
Gait analysis
Dopamine metabolism
Dopaminergic neuron count
Neuroinflammation
Phosphorylated α-Synuclein
GCase activity
Biodistribution
WT
n = 10 males
10 weeks
Vector (SN)
Vector (ICV)
AAV-null
Excipient
AAV-SNCA-A53T
Excipient
AAV-SNCA-A53T
PR004

FIG. 70

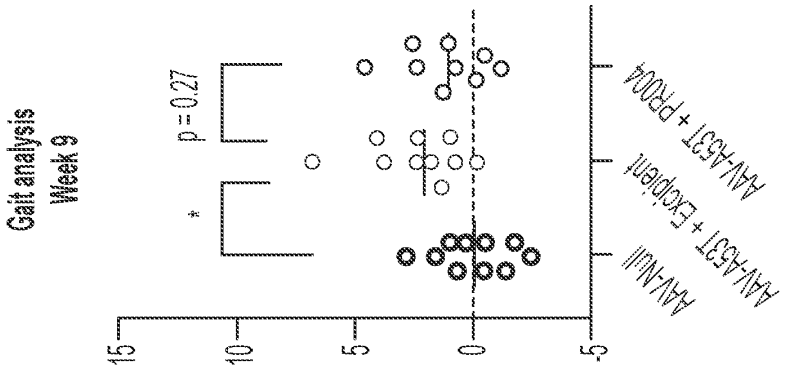


FIG. 71B

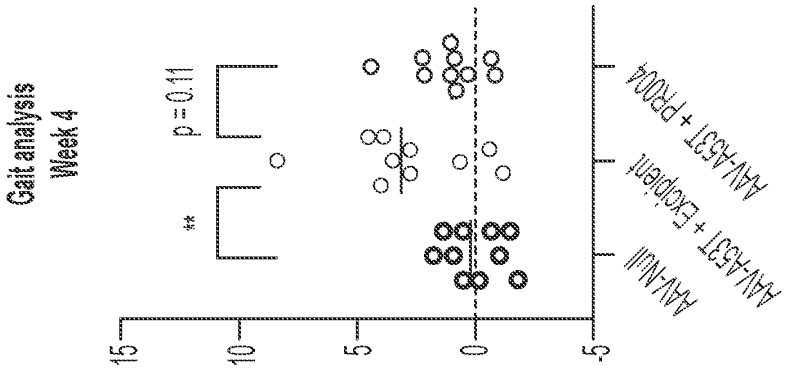


FIG. 71A