FIG. 1

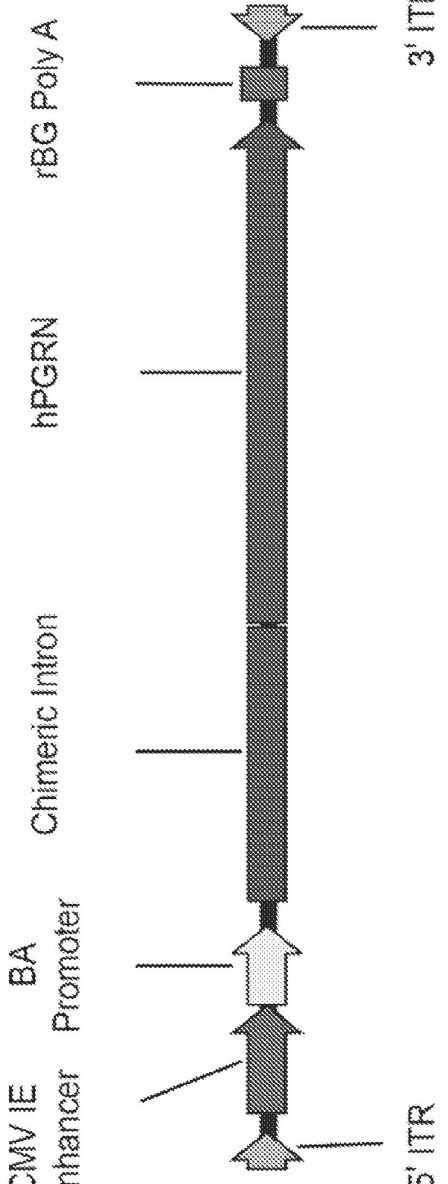

CMV IE Enhancer
BA Promoter
Chimeric Intron
hPGRN
rBG Poly A
5' ITR
3' ITR

FIG. 2

CMV IE Enhancer
BA Promoter
Chimeric Intron
hPGRN
rBG Poly A
3' ITR
f1 Ori
pUC Ori
KanR
5' ITR
pENN.AAV.CB7.CI.hPGRN.rBG.KanR (p4862)

FIG. 3A

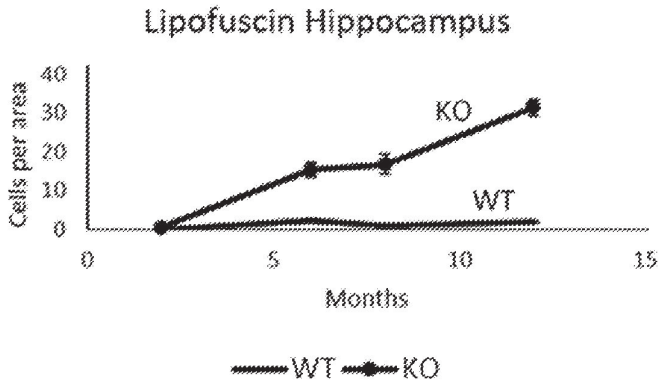


FIG. 3B

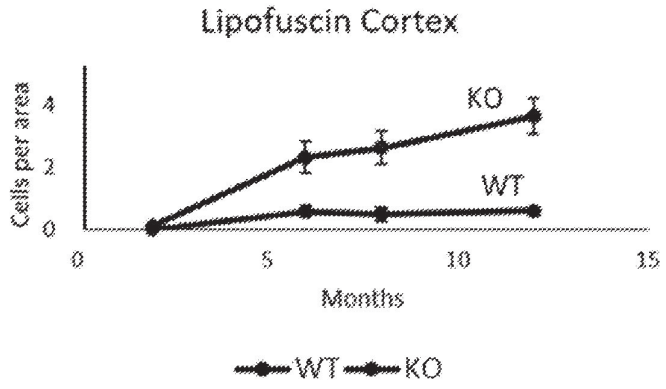


FIG. 3C

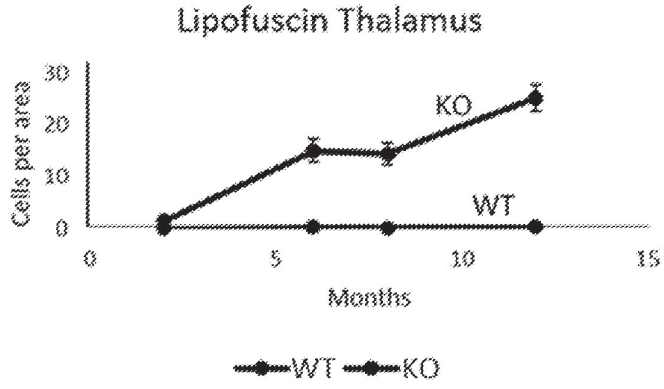


FIG. 3D

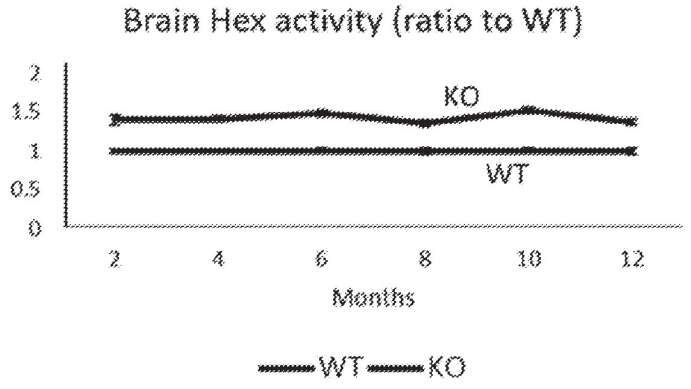


FIG. 4

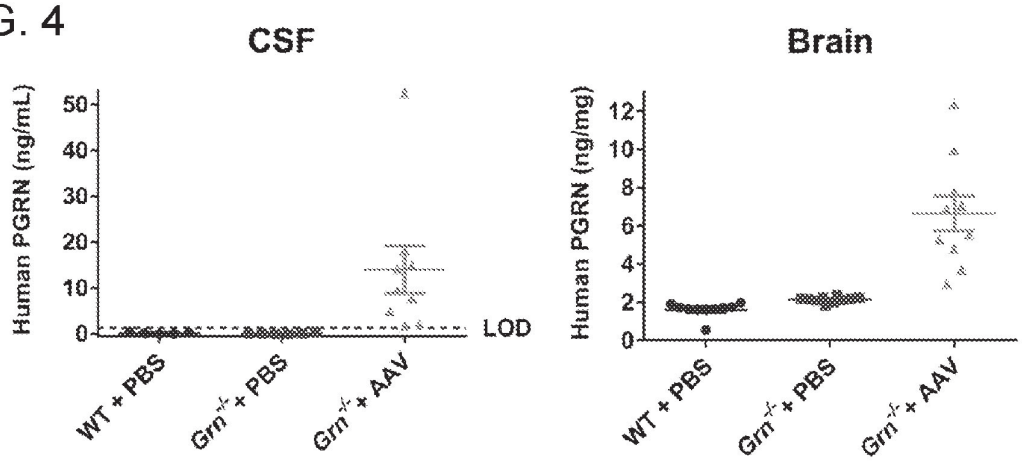


FIG. 5

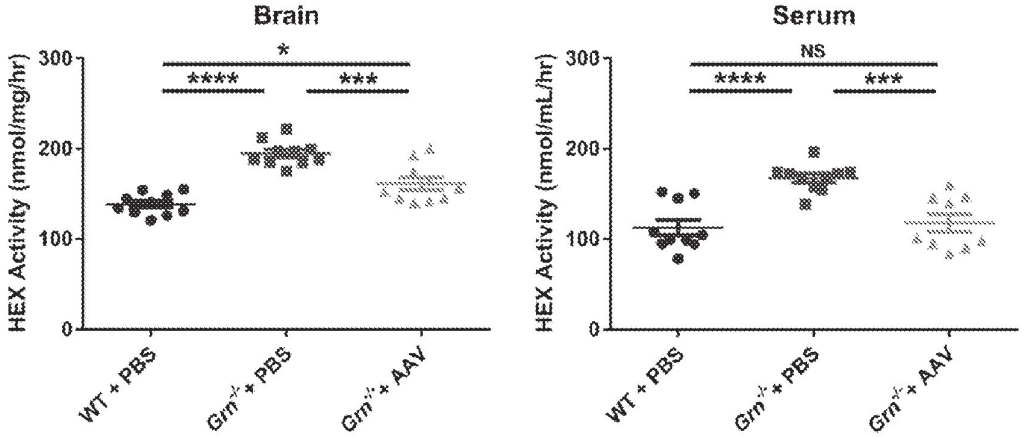


FIG. 6

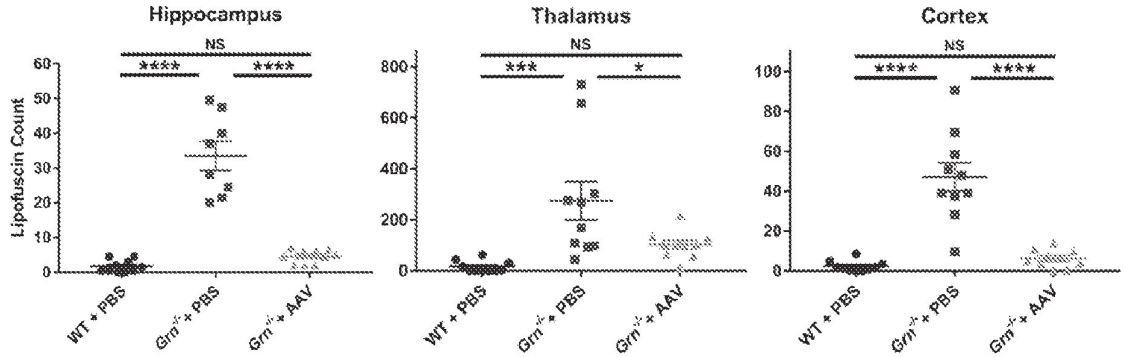


FIG. 7A

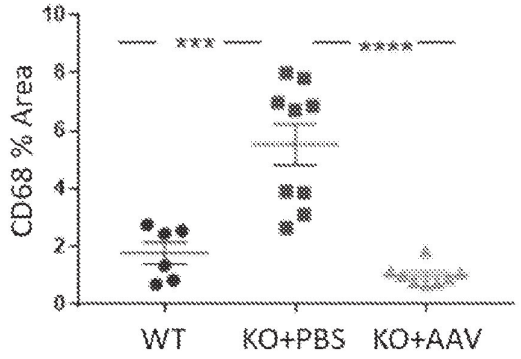


FIG. 7B

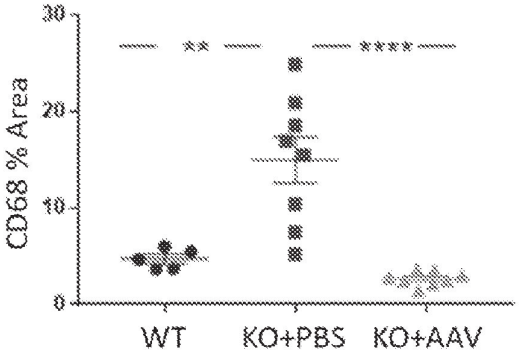


FIG. 7C

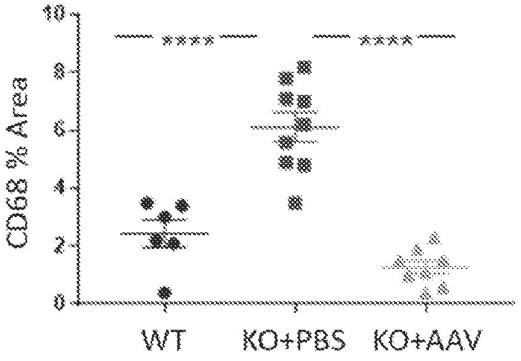


FIG. 8

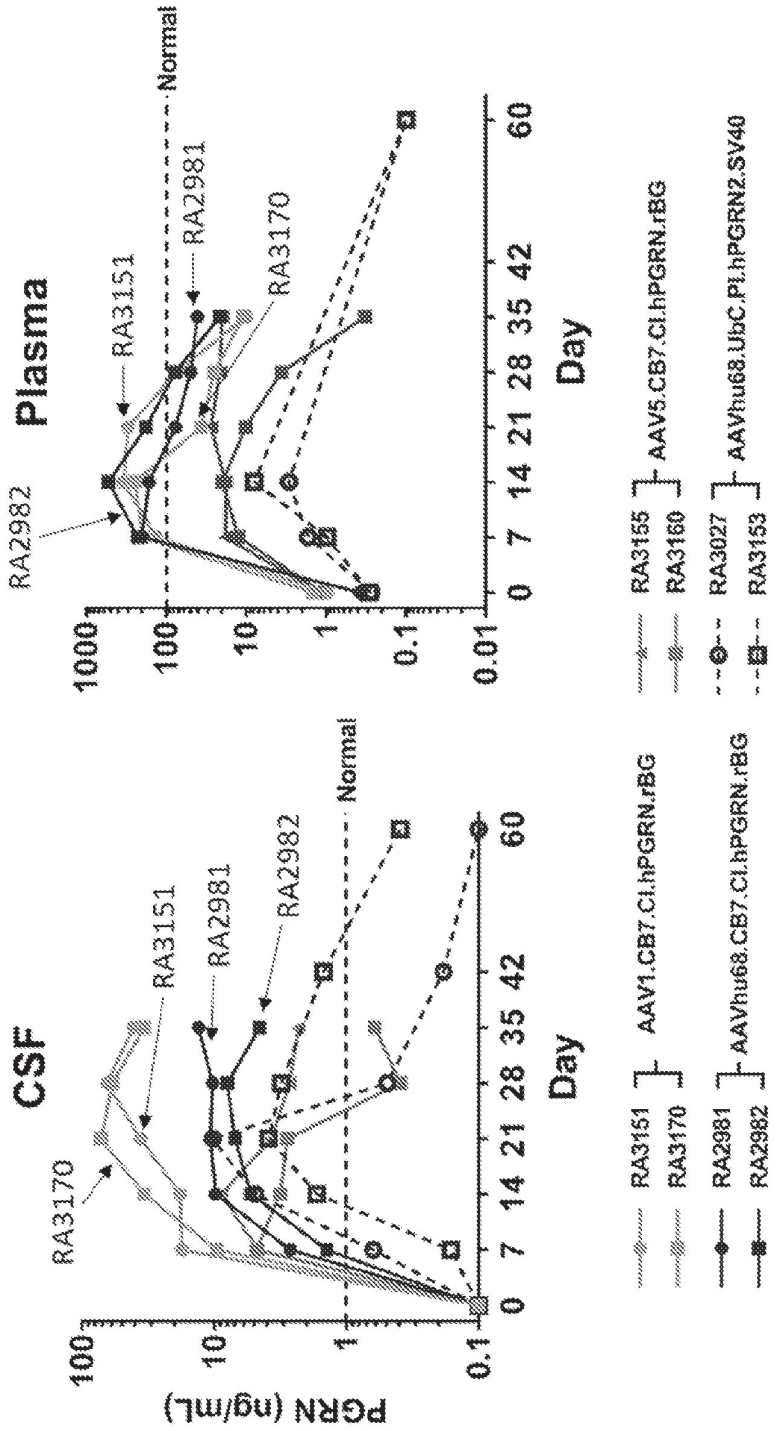

CSF
Plasma
PGRN (ng/mL)
Day
RA3170
RA3151
RA2981
RA2982
Normal
RA3155
RA3160
RA3027
RA3153
AAV1.CB7.CI.hPGRN.rBG
AAV5.CB7.CI.hPGRN.rBG
AAVhu68.CB7.CI.hPGRN.rBG
AAVhu68.UbC.PI.hPGRN2.SV40

FIG. 9

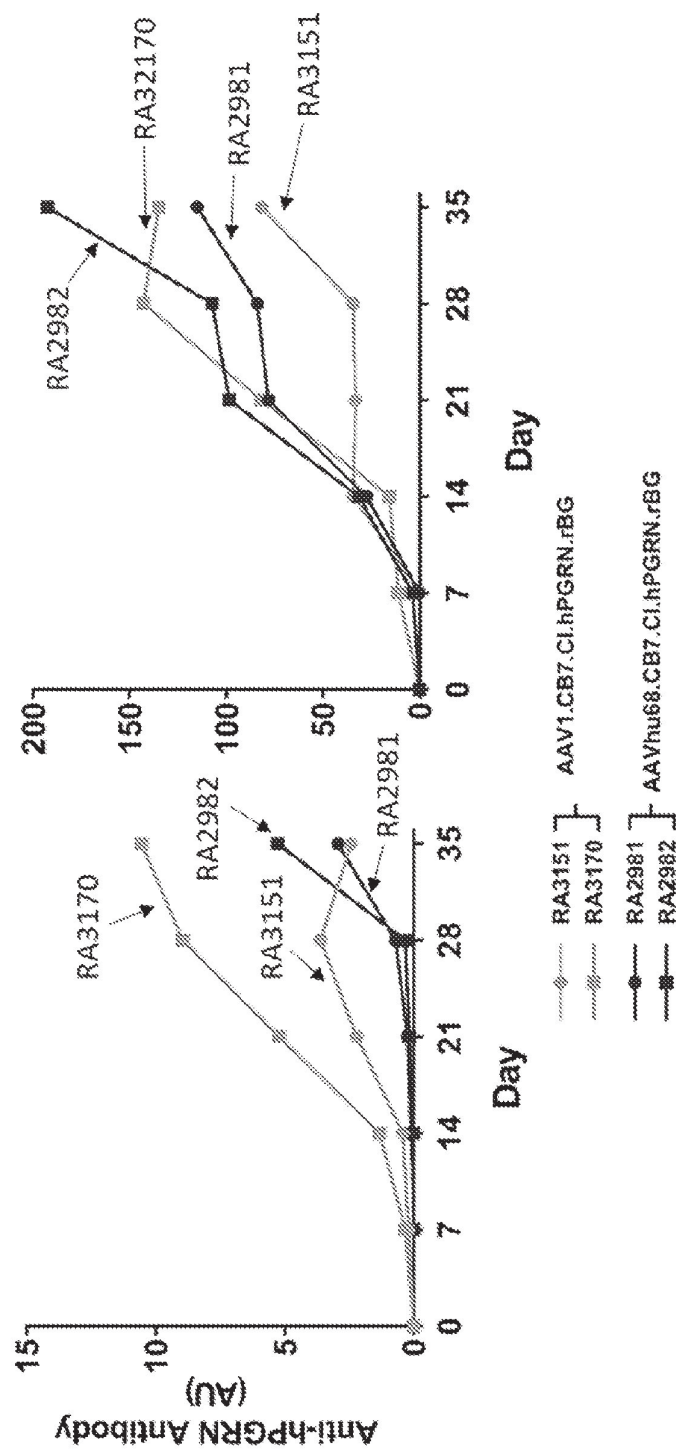

CSF
Serum
Anti-hPGRN Antibody (AU)
Day
Day
RA3170
RA2982
RA3151
RA2981
RA2982
RA32170
RA2981
RA3151
0
5
10
15
0
50
100
150
200
0
7
14
21
28
35
RA3151
RA3170
RA2981
RA2982
AAV1.CB7.CI.hPGRN.rBG
AAVhu68.CB7.CI.hPGRN.rBG

FIG. 10

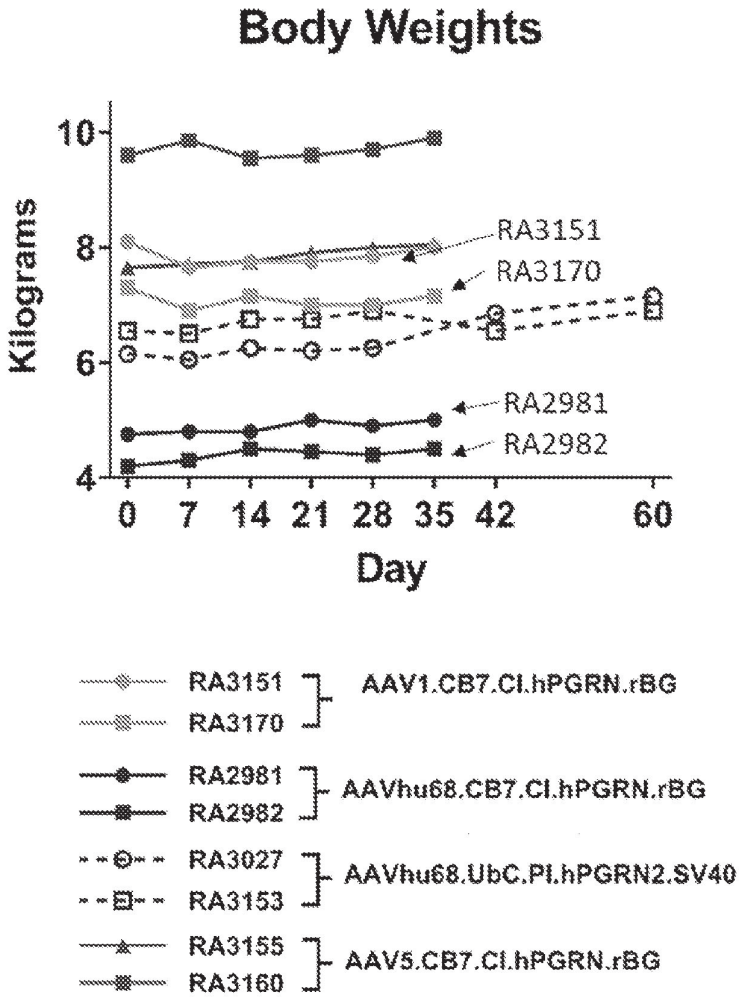

Body Weights
Kilograms
10
8
6
4
0
7
14
21
28
35
42
60
Day
RA3151
RA3170
RA2981
RA2982
RA3151
RA3170
AAV1.CB7.CI.hPGRN.rBG
RA2981
RA2982
AAVhu68.CB7.CI.hPGRN.rBG
RA3027
RA3153
AAVhu68.UbC.PI.hPGRN2.SV40
RA3155
RA3160
AAV5.CB7.CI.hPGRN.rBG

FIG. 11

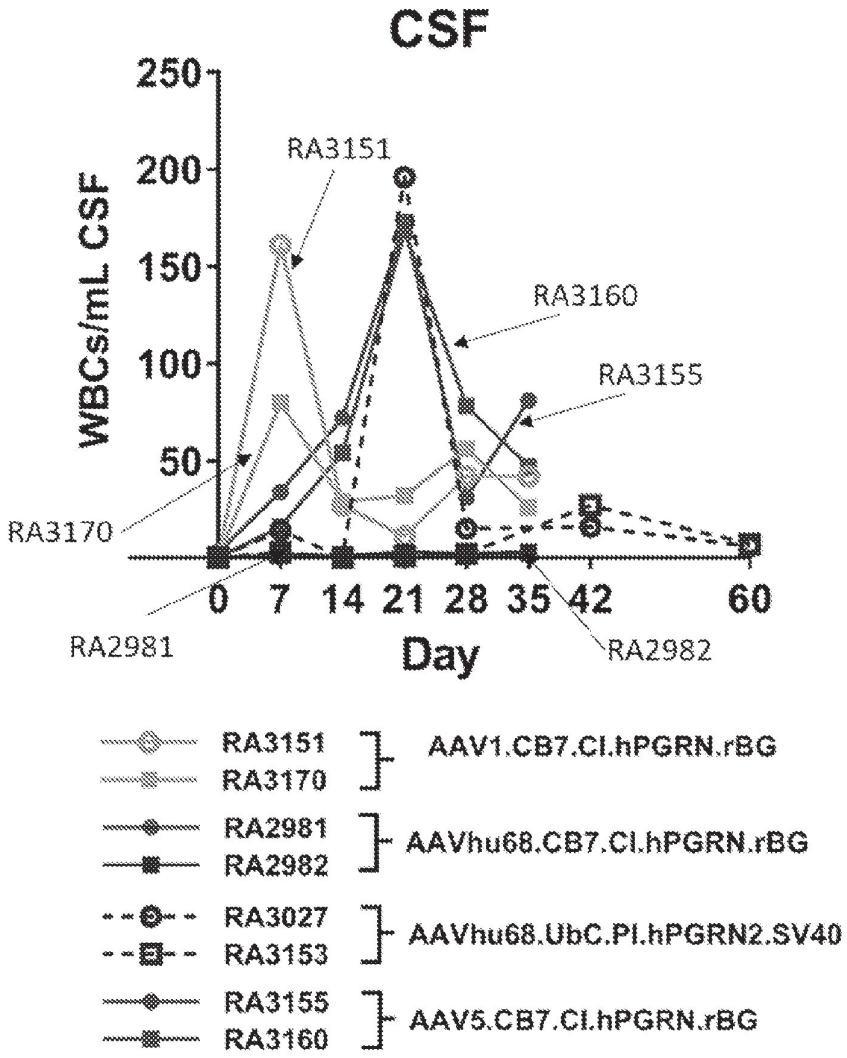

CSF
WBCs/mL CSF
250
200
150
100
50
0
7
14
21
28
35
42
60
Day
RA3151
RA3160
RA3155
RA3170
RA2981
RA2982
RA3151
RA3170
AAV1.CB7.CI.hPGRN.rBG
RA2981
RA2982
AAVhu68.CB7.CI.hPGRN.rBG
RA3027
RA3153
AAVhu68.UbC.PI.hPGRN2.SV40
RA3155
RA3160
AAV5.CB7.CI.hPGRN.rBG

FIG. 12

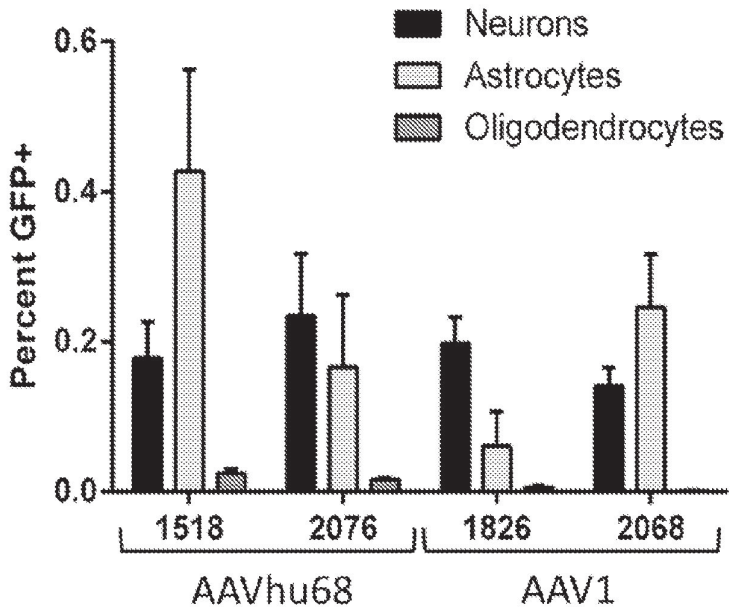

Neurons
Astrocytes
Oligodendrocytes
Percent GFP+
0.6
0.4
0.2
0.0
1518
2076
1826
2068
AAVhu68
AAV1

FIG. 13

		Region						
Cell marker	Animal ID	1	2	3	4	5		Average
NeuN	1518	0.13	N/A	0.205	0.34	0.167	0.049	0.178
	2076	0.106	0.227	0.258	0.528	0.053	N/A	0.234
	1826	0.1	0.318	0.195	0.202	0.171	N/A	0.197
	2068	0.218	N/A	0.153	0.141	0.128	0.065	0.141
GFAP	1518	0.152	N/A	0.202	0.914	0.39	0.476	0.427
	2076	0.011	0.064	0.235	0.511	0.011	N/A	0.166
	1826	0.019	0.013	0.021	0.244	0.007	N/A	0.061
	2068	0.066	N/A	0.245	0.13	0.459	0.33	0.246
Olig2	1518	0.02	N/A	0.03	0.04	0.009	0.023	0.024
	2076	0.02	0.023	0.011	0.013	0.013	N/A	0.016
	1826	0	0.001	0.008	0.016	N/A	0.001	0.005
	2068	0.002	0	N/A	0.001	0.002	0.002	0.001

FIG. 14

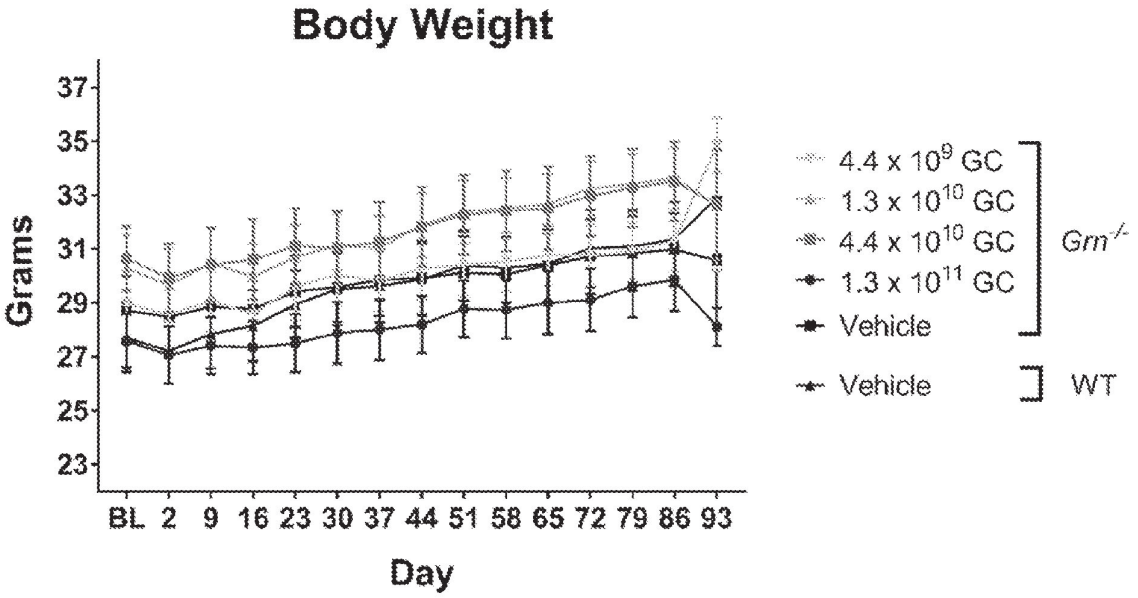

Body Weight
Grams
37
35
33
31
29
27
25
23
BL 2 9 16 23 30 37 44 51 58 65 72 79 86 93
Day
4.4 x 10^9 GC
1.3 x 10^10 GC
4.4 x 10^10 GC
1.3 x 10^11 GC
Vehicle
Gm-/-
Vehicle
WT

FIG. 15

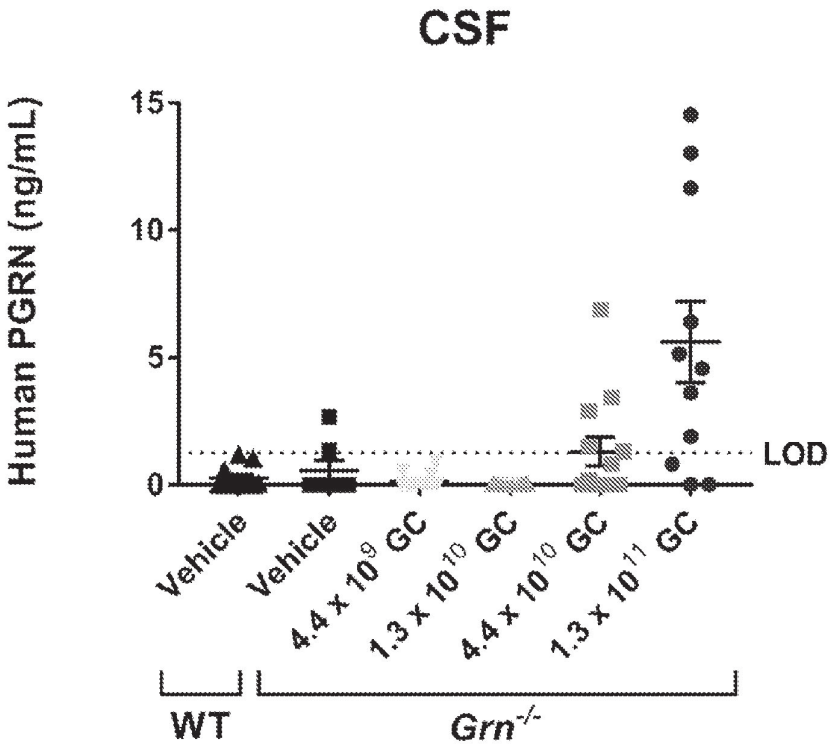
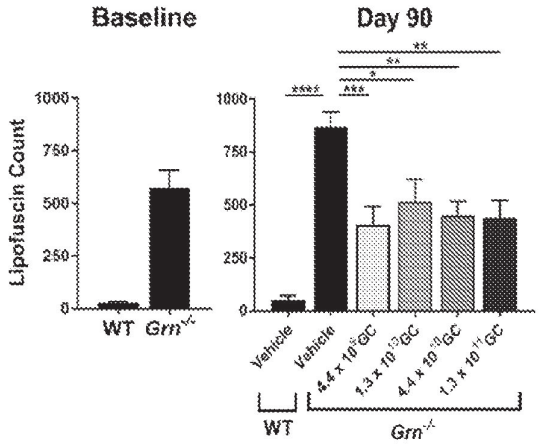

CSF
Human PGRN (ng/mL)
15
10
5
0
LOD
Vehicle
Vehicle
4.4 x 10^9 GC
1.3 x 10^10 GC
4.4 x 10^10 GC
1.3 x 10^11 GC
WT
Grn-/-

FIG. 16A



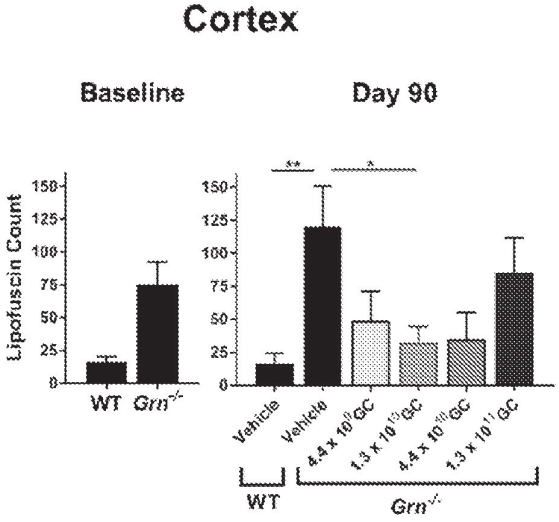


FIG. 16B

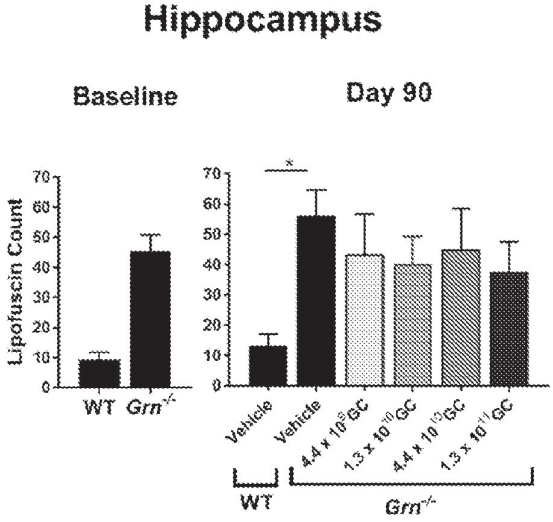
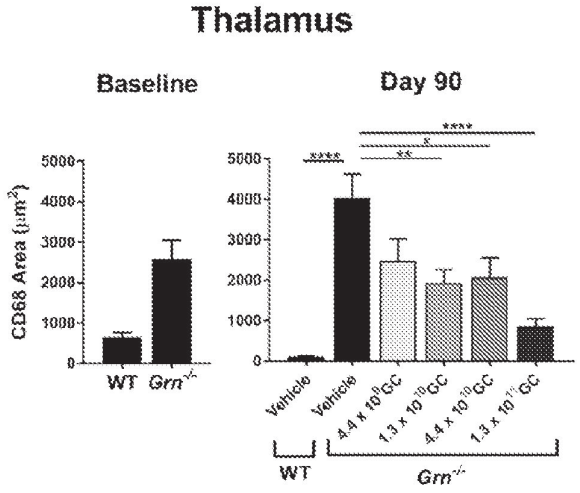


FIG. 16C

FIG. 17A



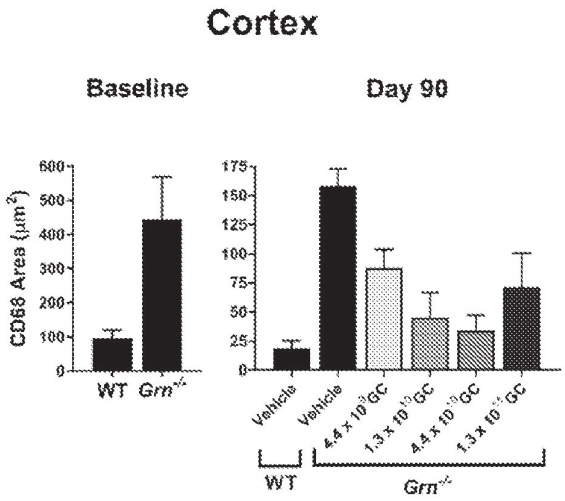


FIG. 17B

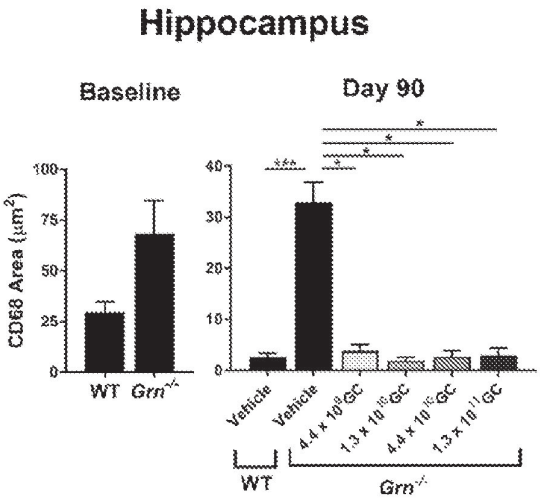


FIG. 17C

FIG. 18

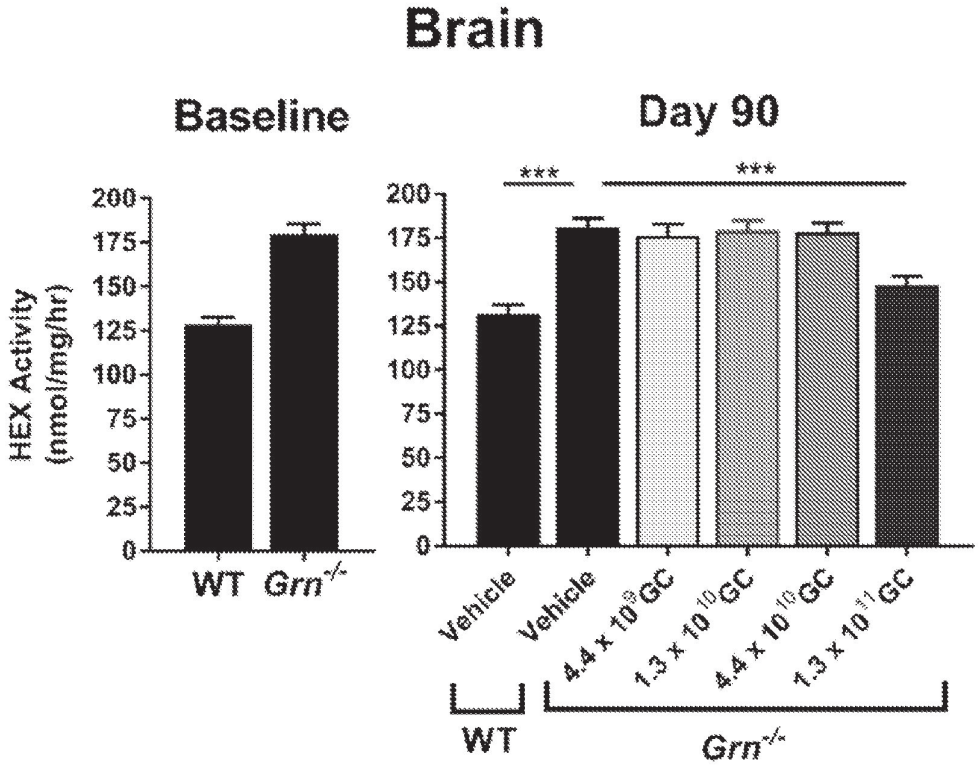

Brain
Baseline
Day 90
HEX Activity (nmol/mg/hr)
200
175
150
125
100
75
50
25
0
WT
Grn-/-
***
***
Vehicle
Vehicle
4.4 x 10^9 GC
1.3 x 10^10 GC
4.4 x 10^10 GC
1.3 x 10^11 GC
WT
Grn-/-

FIG. 19

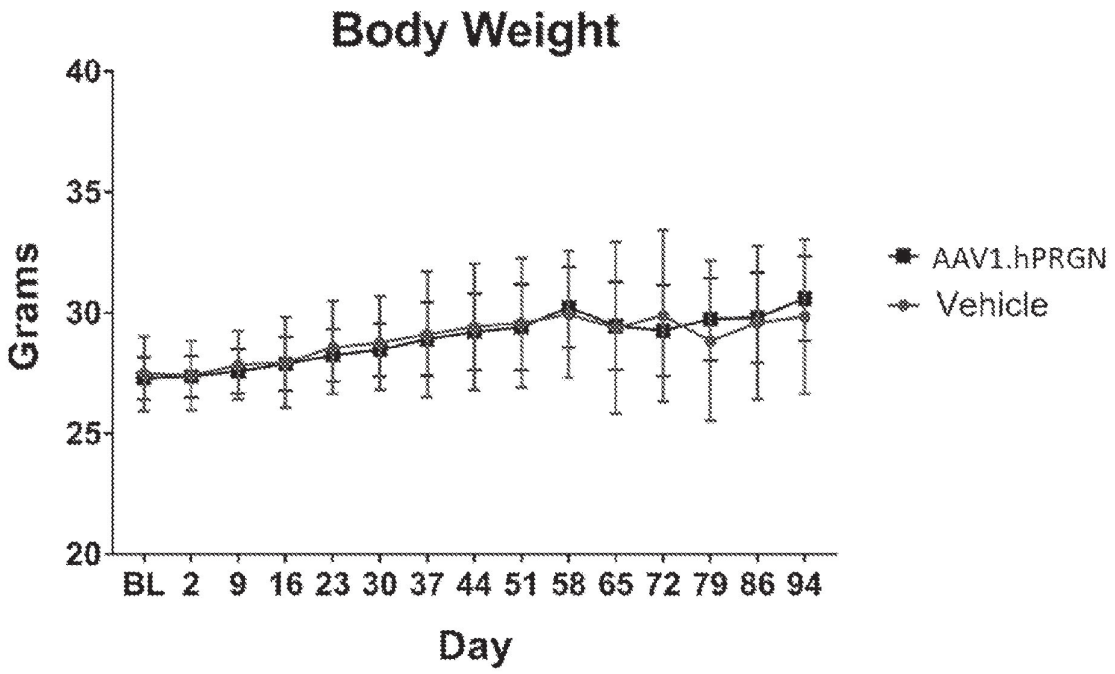

Body Weight
Grams
40
35
30
25
20
BL 2 9 16 23 30 37 44 51 58 65 72 79 86 94
Day
AAV1.hPRGN
Vehicle

FIG. 20

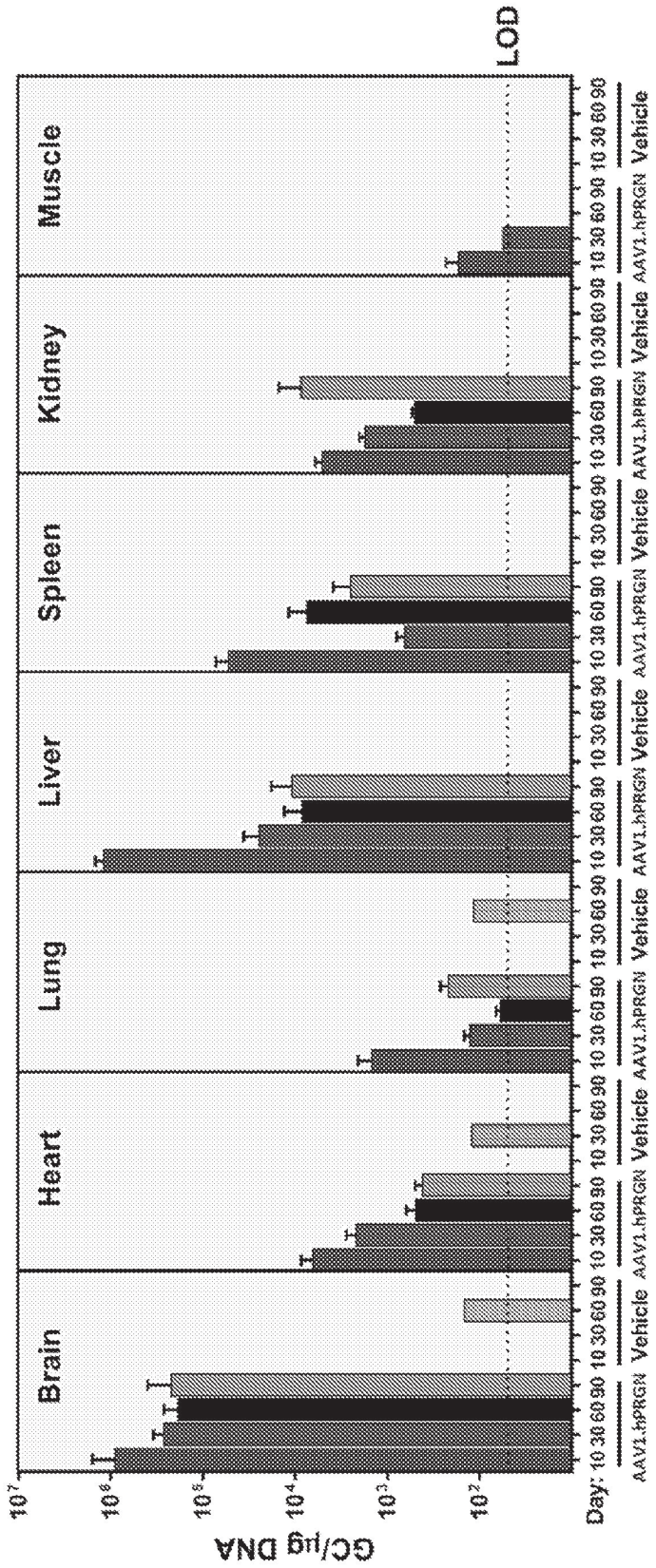

Biodistribution
Brain
Heart
Lung
Liver
Spleen
Kidney
Muscle
LOD
GC/μg DNA
10^7
10^6
10^5
10^4
10^3
10^2
Day: 10 30 60 90 10 30 60 90 10 30 60 90 10 30 60 90 10 30 60 90 10 30 60 90 10 30 60 90
AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle AAV1.hPRGN Vehicle

FIG. 21

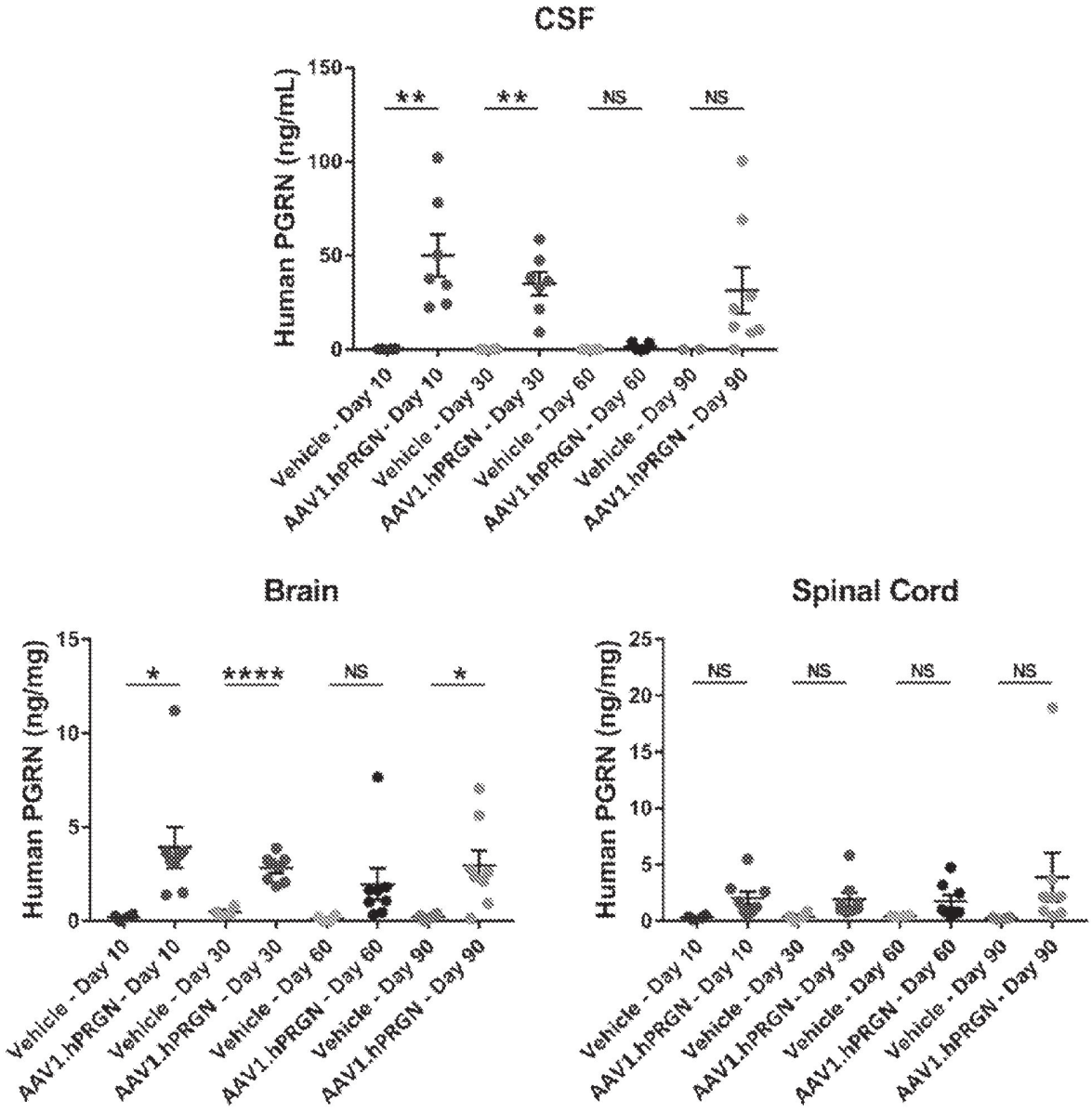

CSF
Human PGRN (ng/mL)
150
100
50
0
**
**
NS
NS
Vehicle - Day 10
AAV1.hPRGN - Day 10
Vehicle - Day 30
AAV1.hPRGN - Day 30
Vehicle - Day 60
AAV1.hPRGN - Day 60
Vehicle - Day 90
AAV1.hPRGN - Day 90
Brain
Human PGRN (ng/mg)
15
10
5
0
*
****
NS
*
Spinal Cord
Human PGRN (ng/mg)
25
20
15
10
5
0
NS
NS
NS
NS

FIG. 22

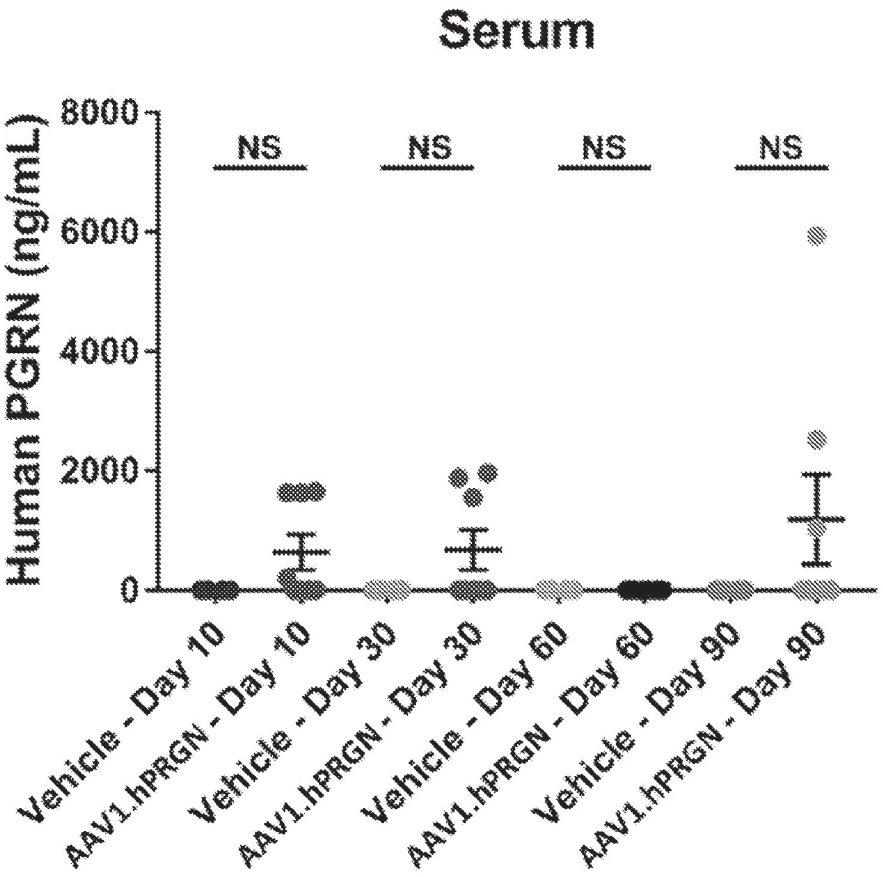

Serum
Human PGRN (ng/mL)
8000
6000
4000
2000
0
NS
NS
NS
NS
Vehicle - Day 10
AAV1.hPRGN - Day 10
Vehicle - Day 30
AAV1.hPRGN - Day 30
Vehicle - Day 60
AAV1.hPRGN - Day 60
Vehicle - Day 90
AAV1.hPRGN - Day 90

FIG. 23A

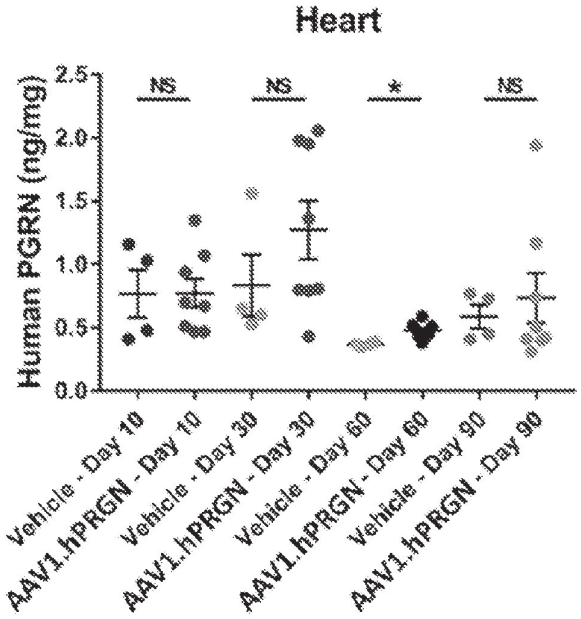


FIG. 23B

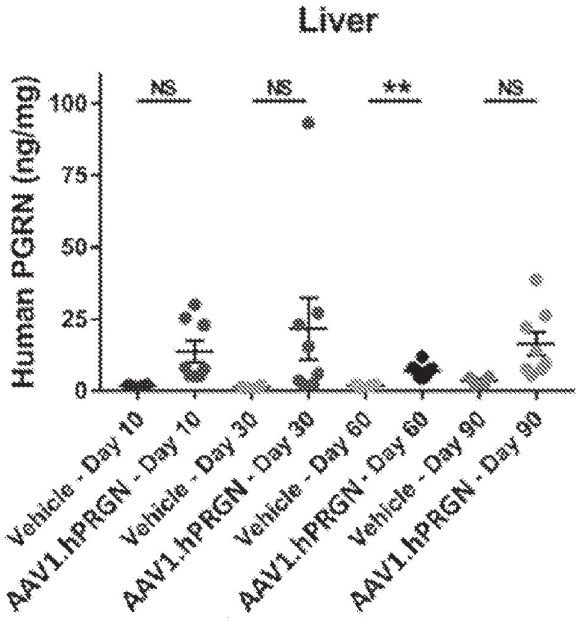


FIG. 23C

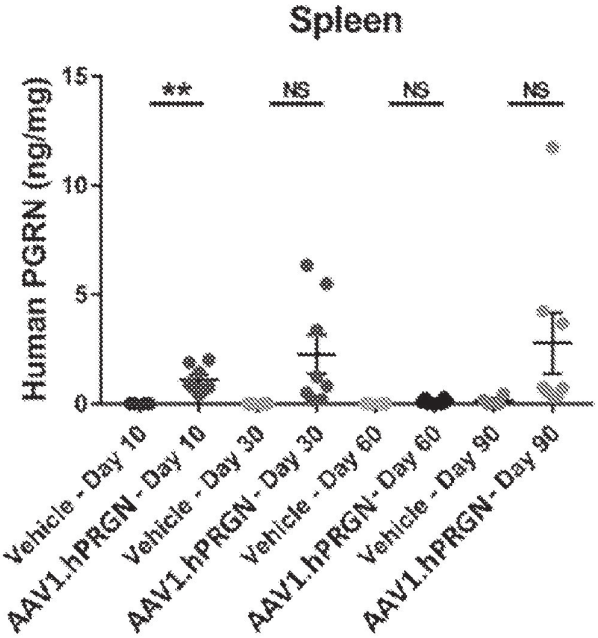


FIG. 23D

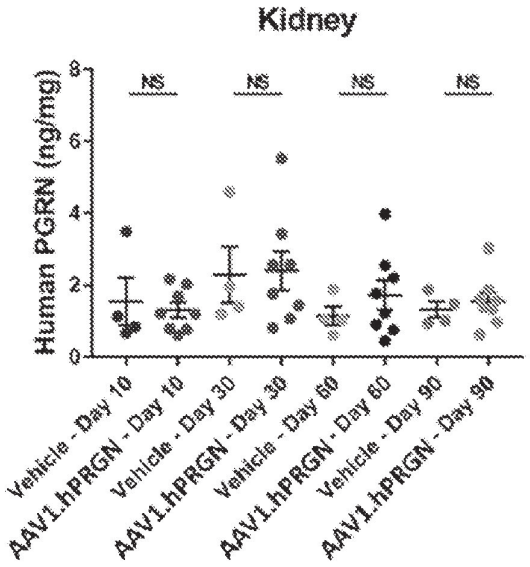


FIG. 23E

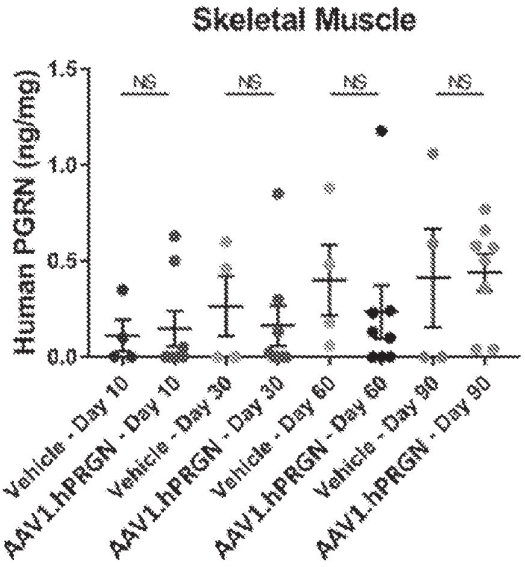


FIG. 23F

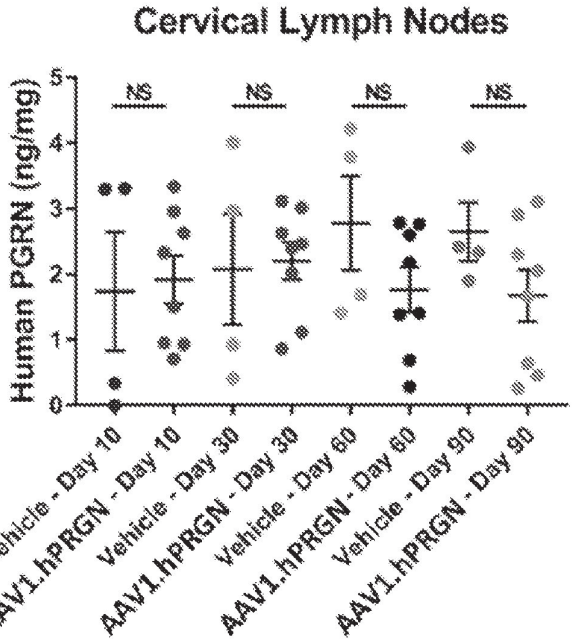
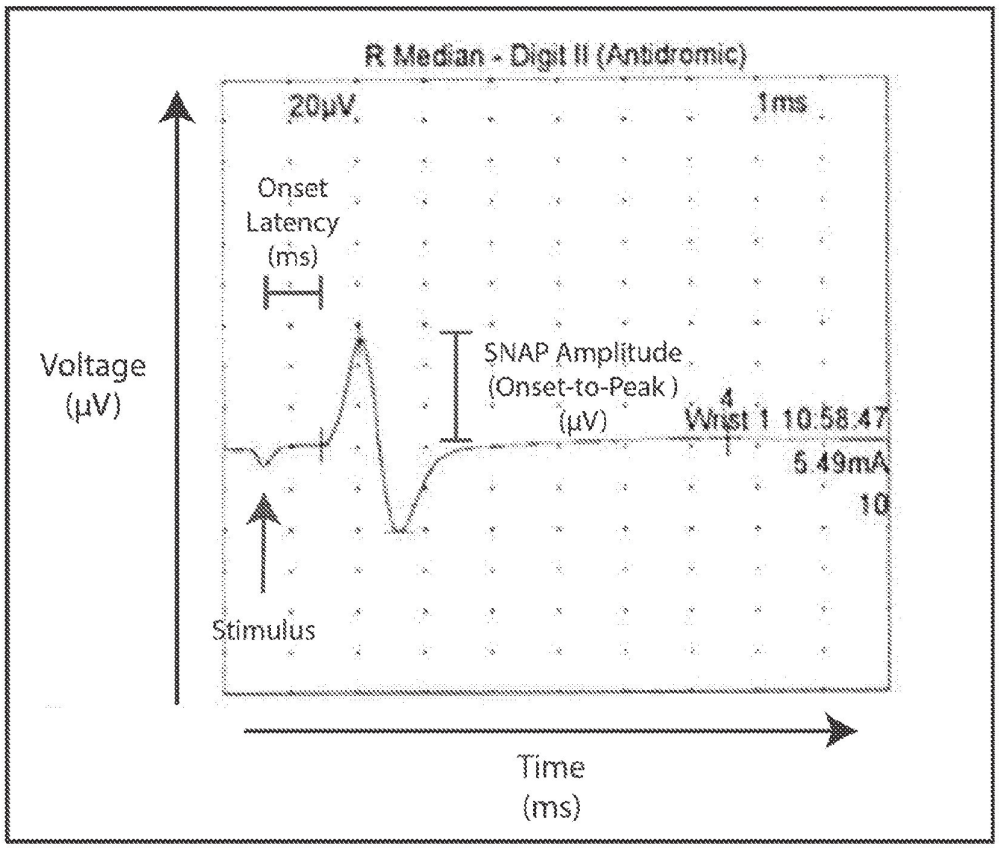


FIG. 24


R Median - Digit II (Antidromic)
20µV
1ms
Onset
Latency
(ms)
SNAP Amplitude
(Onset-to-Peak)
(µV)
Wrist 1 10:58:47
5.49mA
10
Voltage
(µV)
Stimulus
Time
(ms)

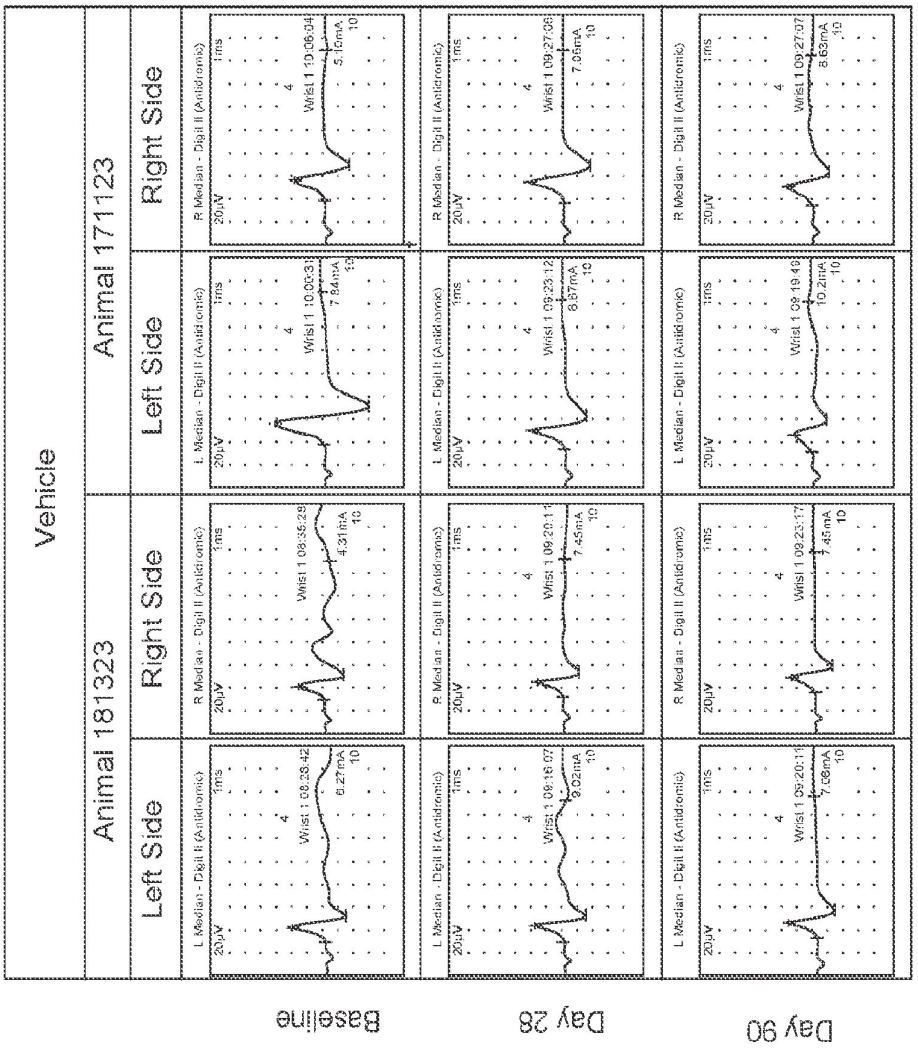

Vehicle
Animal 181323
Animal 171123
Left Side
Right Side
Left Side
Right Side
Baseline
Day 28
Day 90

FIG. 25-1

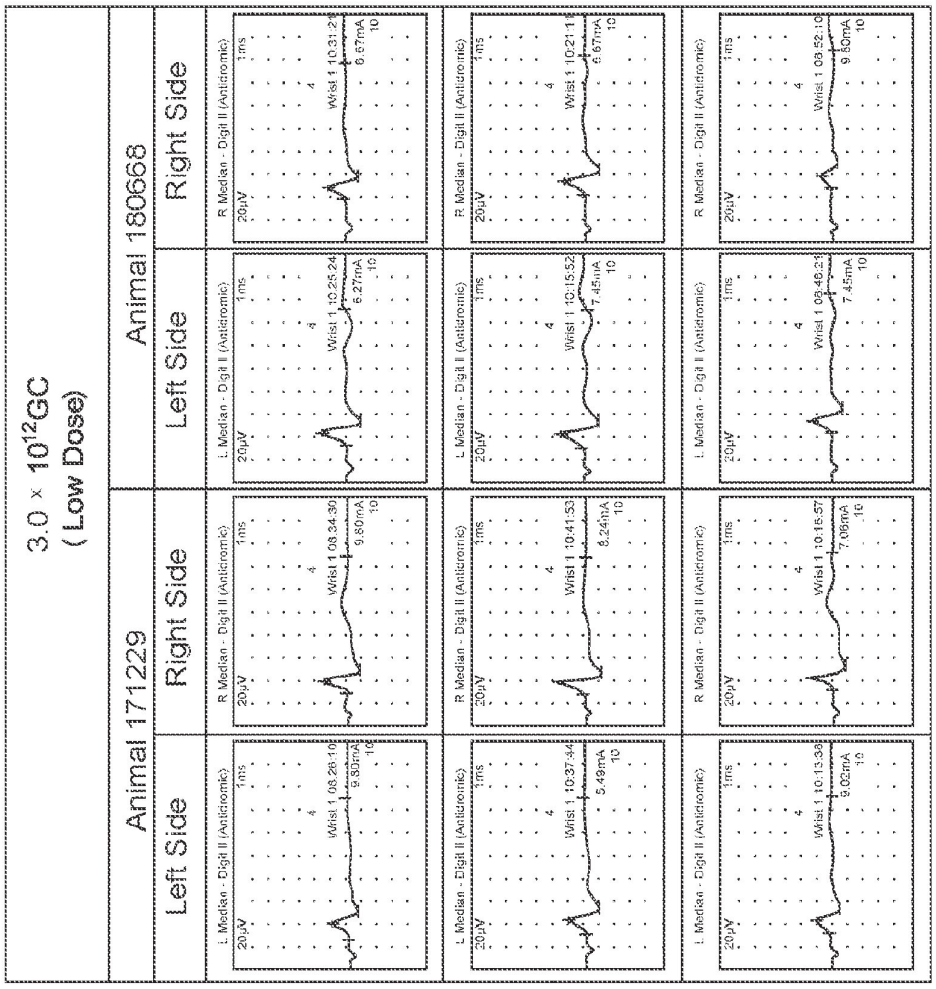

3.0 x 10^12GC
(Low Dose)
Animal 171229
Left Side
Right Side
Animal 180668
Left Side
Right Side
L Median - Digit II (Antidromic)
R Median - Digit II (Antidromic)
20µV
1ms
Wrist 1 08:28:10
9.80mA
Wrist 1 08:34:30
9.80mA
Wrist 1 10:25:24
6.27mA
Wrist 1 10:31:2
6.67mA
Wrist 1 10:37:46
5.49mA
Wrist 1 10:41:53
8.24mA
Wrist 1 10:15:52
7.45mA
Wrist 1 10:21:1
6.67mA
Wrist 1 10:13:38
9.02mA
Wrist 1 10:16:57
7.08mA
Wrist 1 08:46:21
7.45mA
Wrist 1 08:52:10
9.80mA

FIG. 25-2

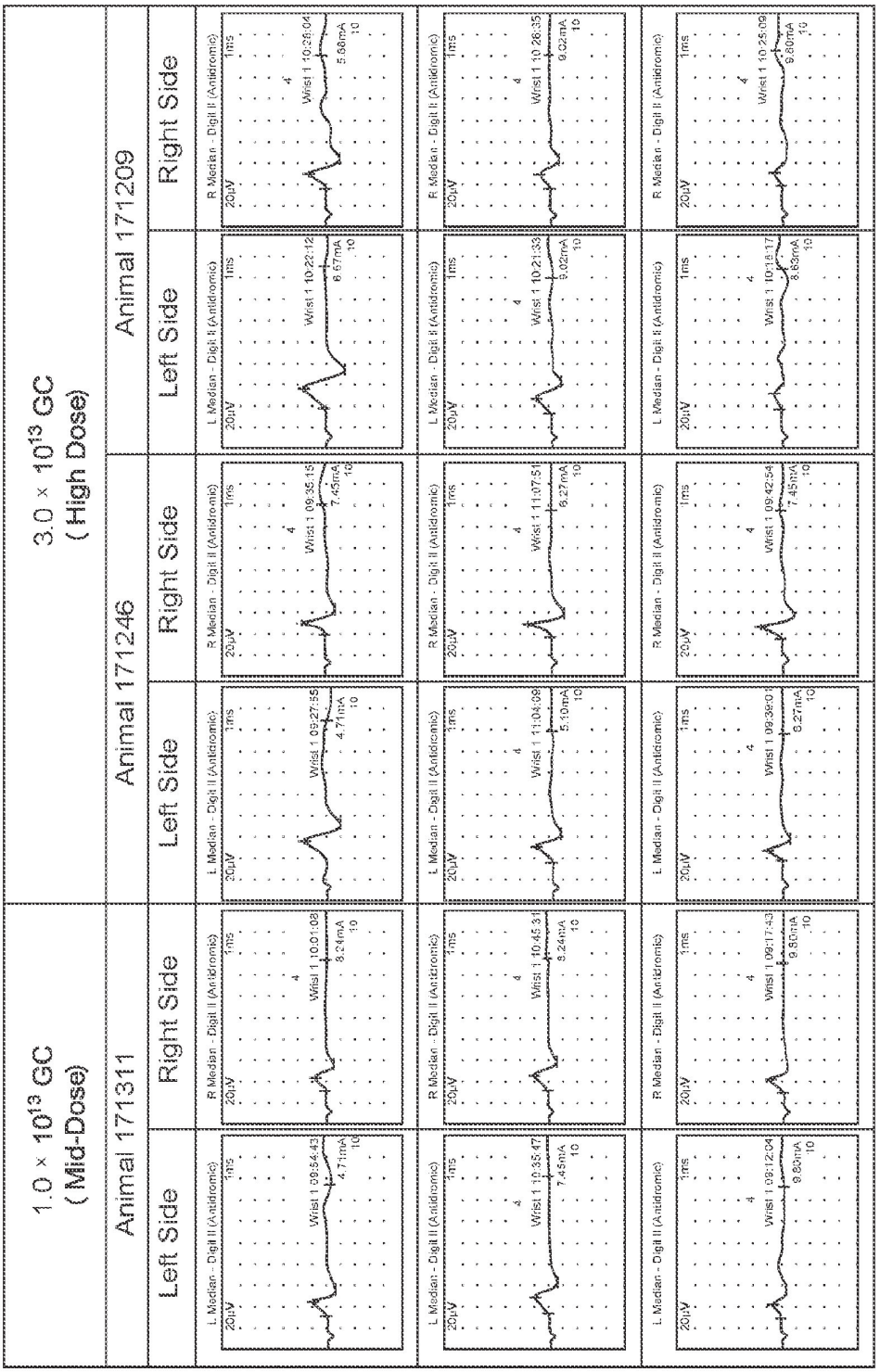
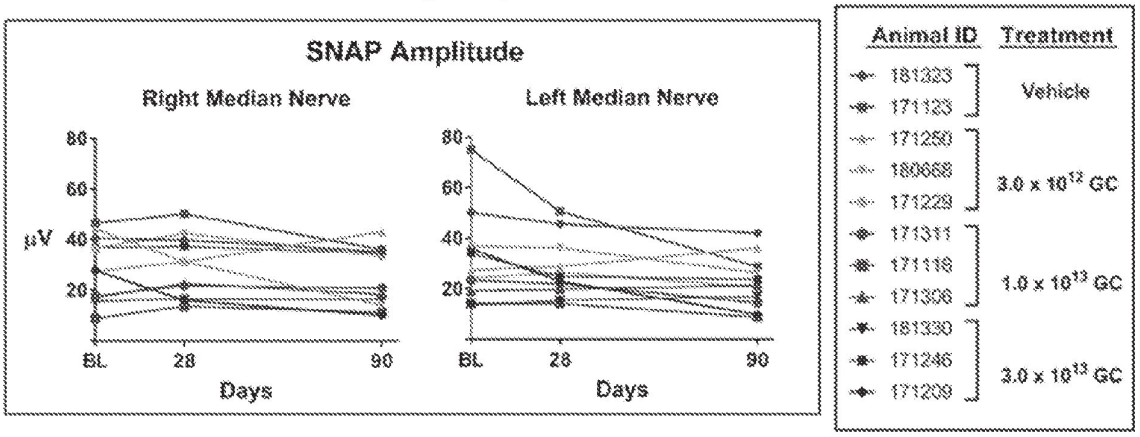

1.0 × 10^13 GC
(Mid-Dose)
3.0 × 10^13 GC
(High Dose)
Animal 171311
Animal 171246
Animal 171209
Left Side
Right Side
Left Side
Right Side
Left Side
Right Side
L Median - Digit II (Antidromic)
R Median - Digit II (Antidromic)
L Median - Digit II (Antidromic)
R Median - Digit II (Antidromic)
L Median - Digit II (Antidromic)
R Median - Digit II (Antidromic)
20µV
1ms
Wrist 1 09:54:43
4.71mA
Wrist 1 10:01:08
8.24mA
Wrist 1 09:27:55
4.71mA
Wrist 1 09:35:13
7.45mA
Wrist 1 10:22:12
6.57mA
Wrist 1 10:26:04
5.66mA
Wrist 1 10:35:47
7.45mA
Wrist 1 10:45:31
8.24mA
Wrist 1 11:04:08
5.10mA
Wrist 1 11:07:56
6.27mA
Wrist 1 10:21:39
5.02mA
Wrist 1 10:26:35
6.02mA
Wrist 1 09:12:04
9.80mA
Wrist 1 09:17:43
9.80mA
Wrist 1 09:36:07
6.27mA
Wrist 1 09:42:54
7.45mA
Wrist 1 10:18:17
6.83mA
Wrist 1 10:25:09
9.80mA

FIG. 25-3

FIG. 26A



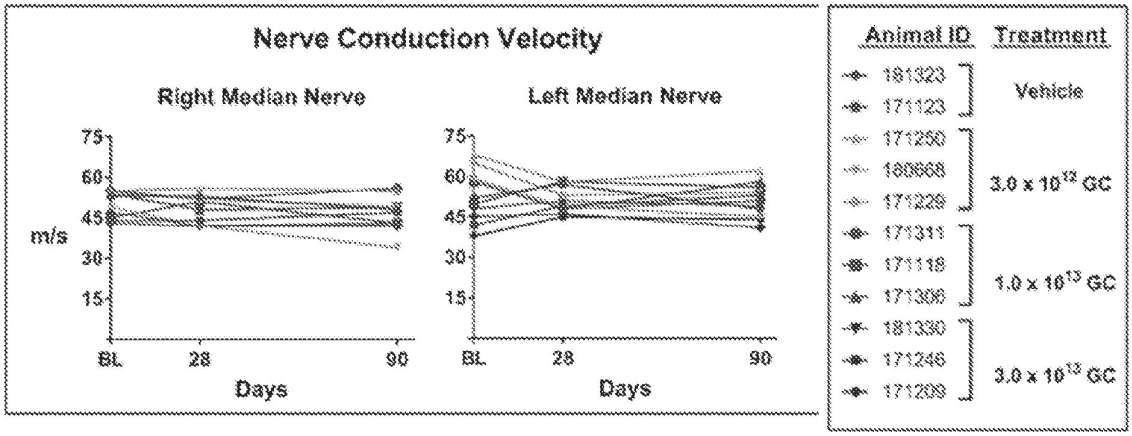


FIG. 26B

FIG. 27

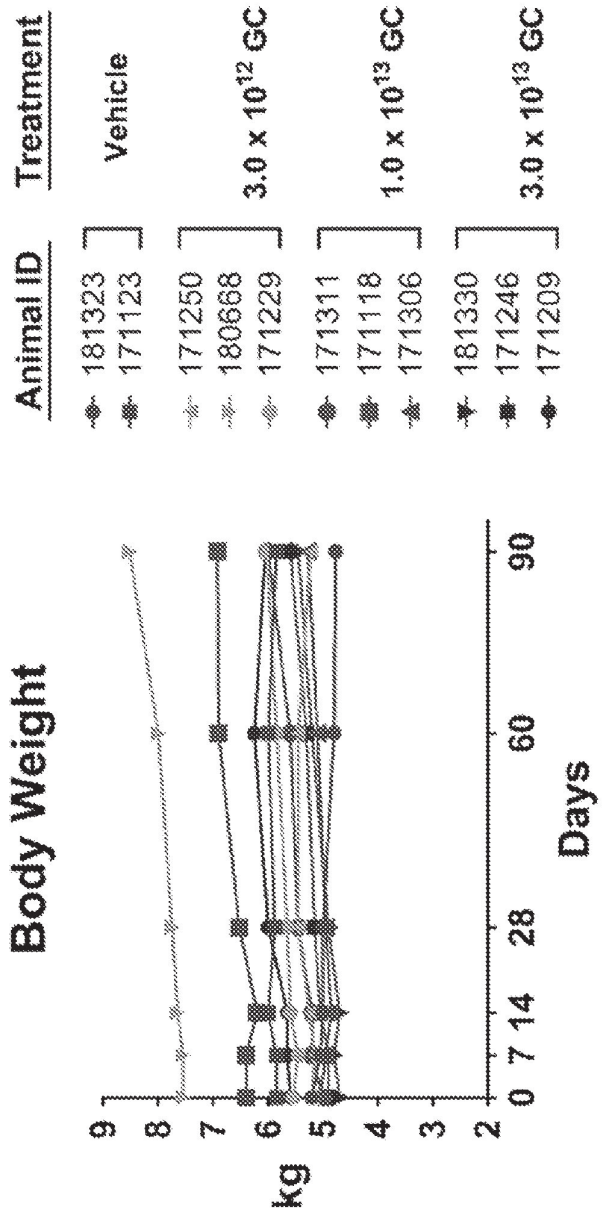

Body Weight
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC
kg
9
8
7
6
5
4
3
2
0 7 14 28 60 90
Days

FIG. 28

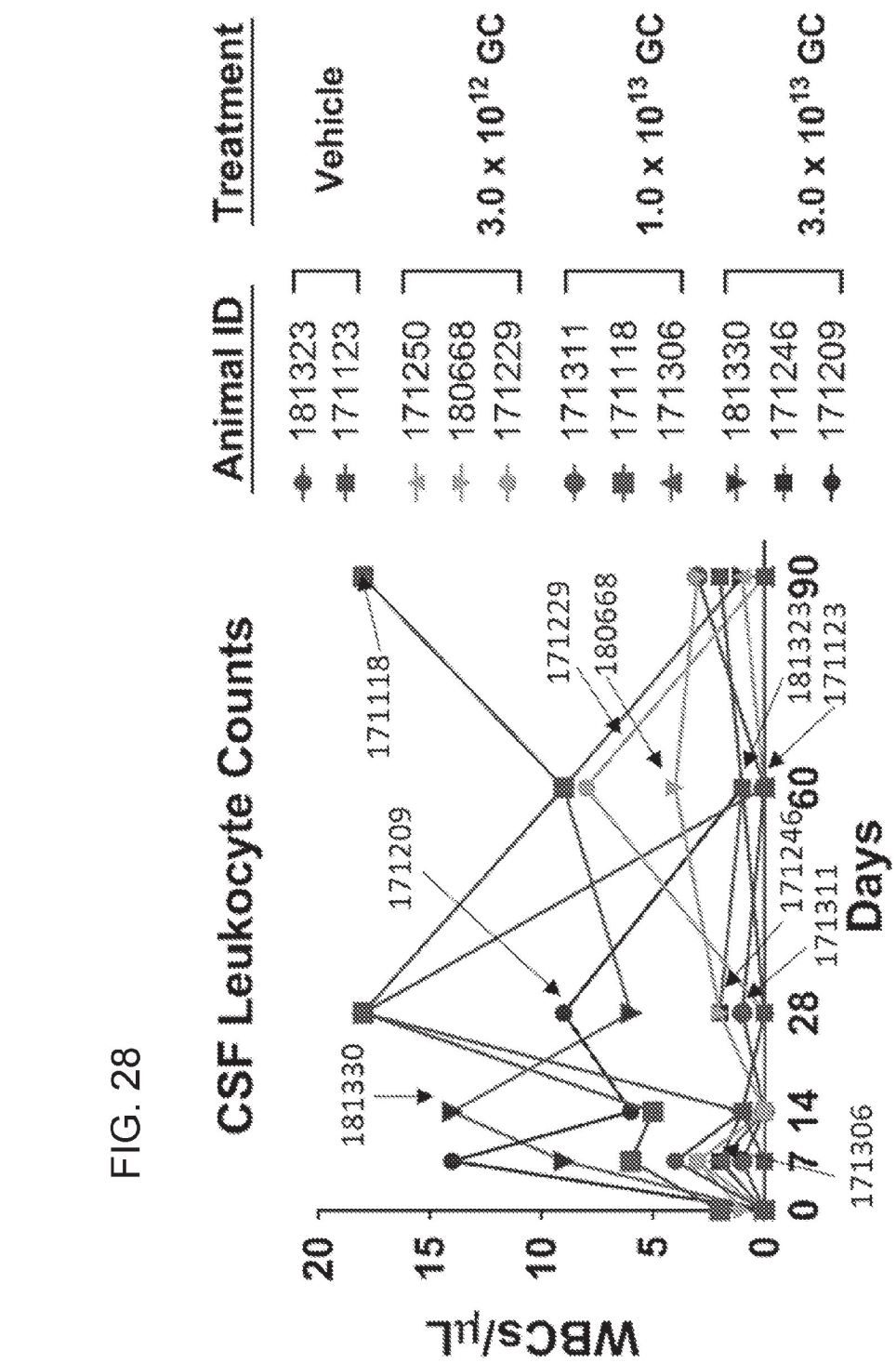

CSF Leukocyte Counts
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC
WBCs/μL
20
15
10
5
0
0
7
14
28
60
90
Days
171118
171209
171229
180668
181330
171306
171246
171311
181323
171123

FIG. 29

				IFN-γ T Cell Response											
				AAV1 (Capsid)						Human PGRN (Transgene)					
Group	Treatment	Dose (GC/Animal)	Animal ID	PBMCs			Tissue-Specific Lymphocytes			PBMCs			Tissue-Specific Lymphocytes		
				BL	Day 28	Day 90	Spleen	Liver	Bone Marrow	BL	Day 28	Day 90	Spleen	Liver	Bone Marrow
1	ITFFB	N/A	171123												
			181323												
2	AAV1.hPRGN	3.0×10^{12}	171229											X	
			171250			X						X	X	X	
			180668		X	X	X								
3	AAV1.hPRGN	1.0×10^{13}	171118					X							
			171306		X	X	X					X		X	
			171311												
4	AAV1.hPRGN	3.0×10^{13}	171209		X						X		X	X	
			171246		X	X	X	X				X		X	
			181330											X	

Abbreviations: AAV1, adeno-associated virus serotype 1; BL, baseline; GC, genome copies; ID, identification number; IFN-γ; interferon gamma; ITFFB, intrathecal final formulation buffer; N/A, not applicable; NAb, neutralizing antibody; PBMC, peripheral blood mononuclear cell; PGRN, progranulin (protein).

FIG. 30

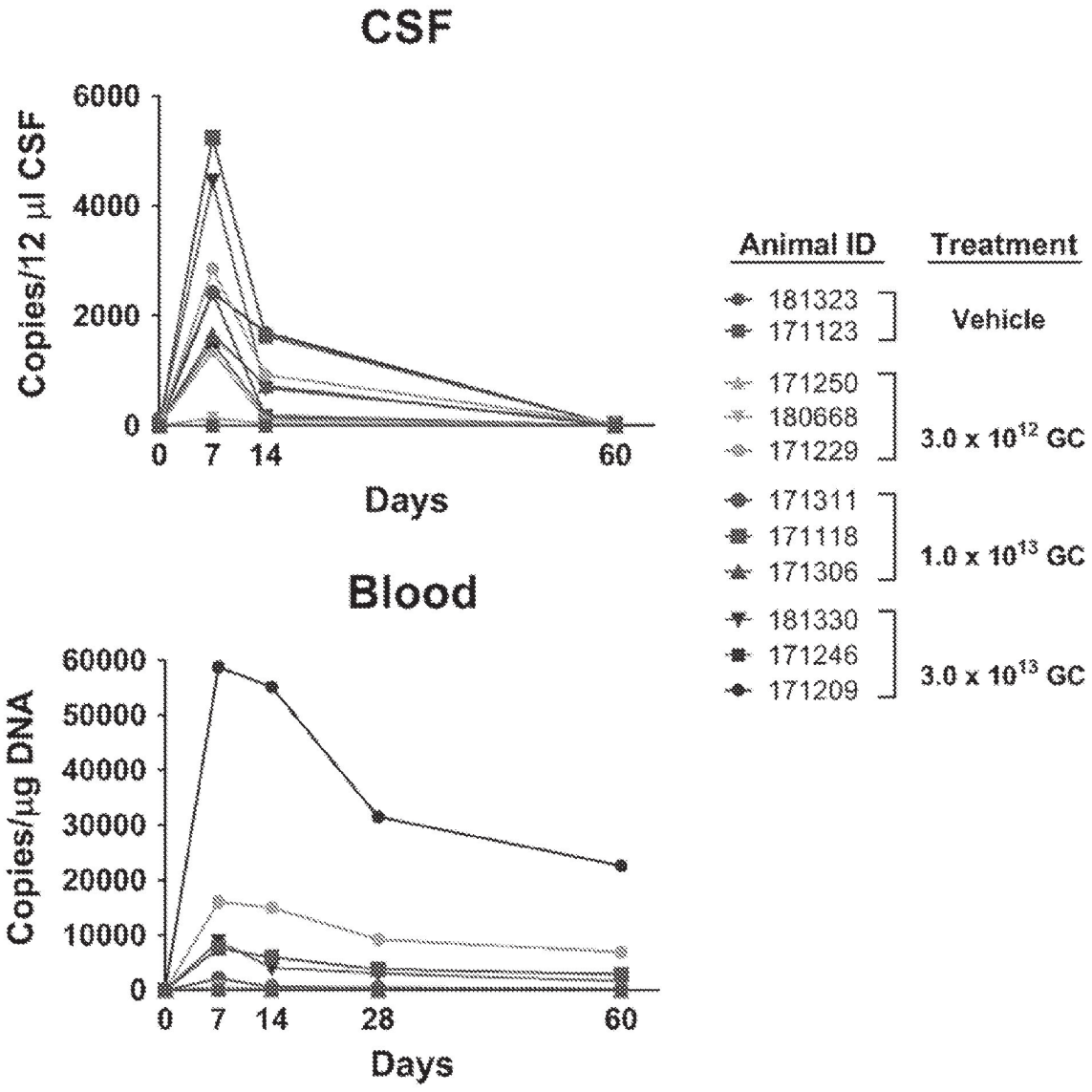

CSF
Copies/12 µl CSF
6000
4000
2000
0
0
7
14
60
Days
Blood
Copies/µg DNA
60000
50000
40000
30000
20000
10000
0
0
7
14
28
60
Days
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC

FIG. 31

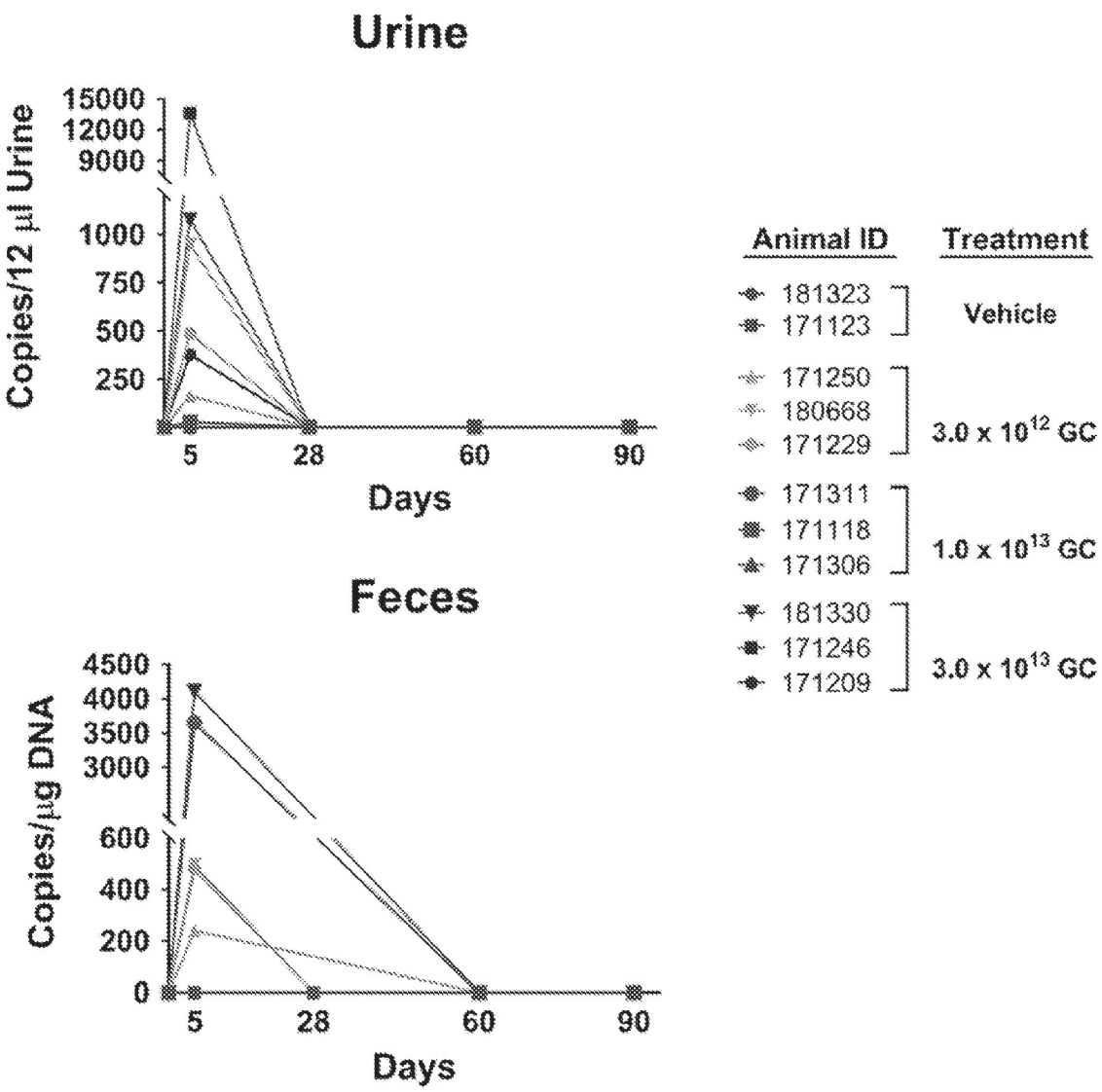

Urine
Copies/12 µl Urine
15000
12000
9000
1000
750
500
250
5
28
60
90
Days
Feces
Copies/µg DNA
4500
4000
3500
3000
600
400
200
0
5
28
60
90
Days
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC

FIG. 32

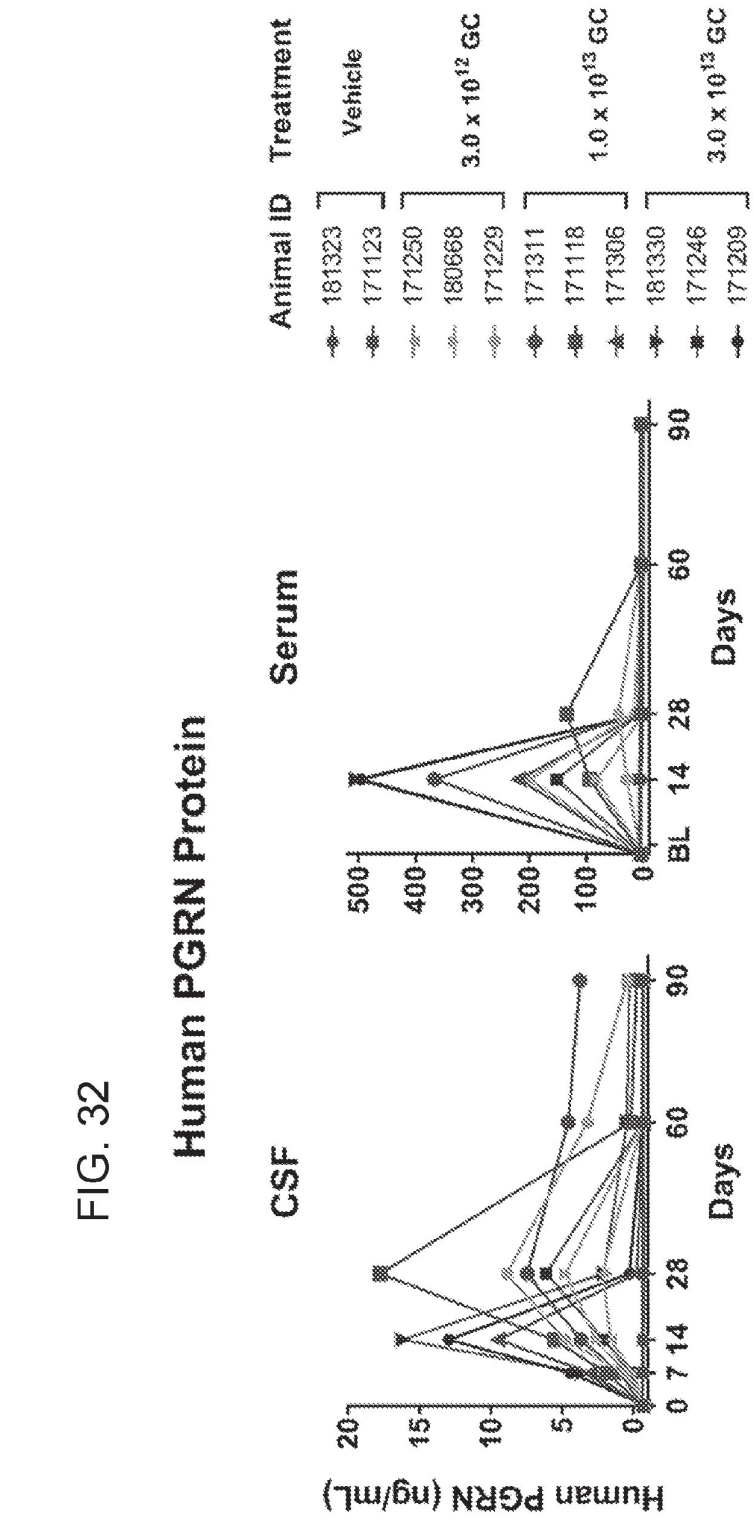

Human PGRN Protein
CSF
Serum
Human PGRN (ng/mL)
Days
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC

FIG. 33

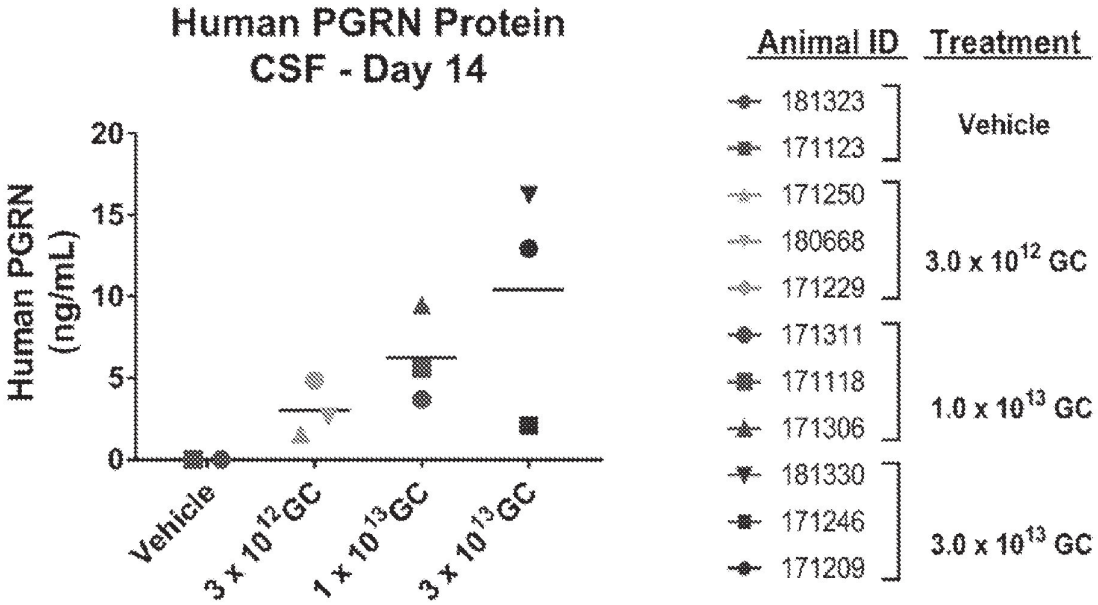

Human PGRN Protein
CSF - Day 14
Human PGRN (ng/mL)
20
15
10
5
0
Vehicle
3 x 10^12 GC
1 x 10^13 GC
3 x 10^13 GC
Animal ID
Treatment
181323
171123
Vehicle
171250
180668
171229
3.0 x 10^12 GC
171311
171118
171306
1.0 x 10^13 GC
181330
171246
171209
3.0 x 10^13 GC

FIG. 34

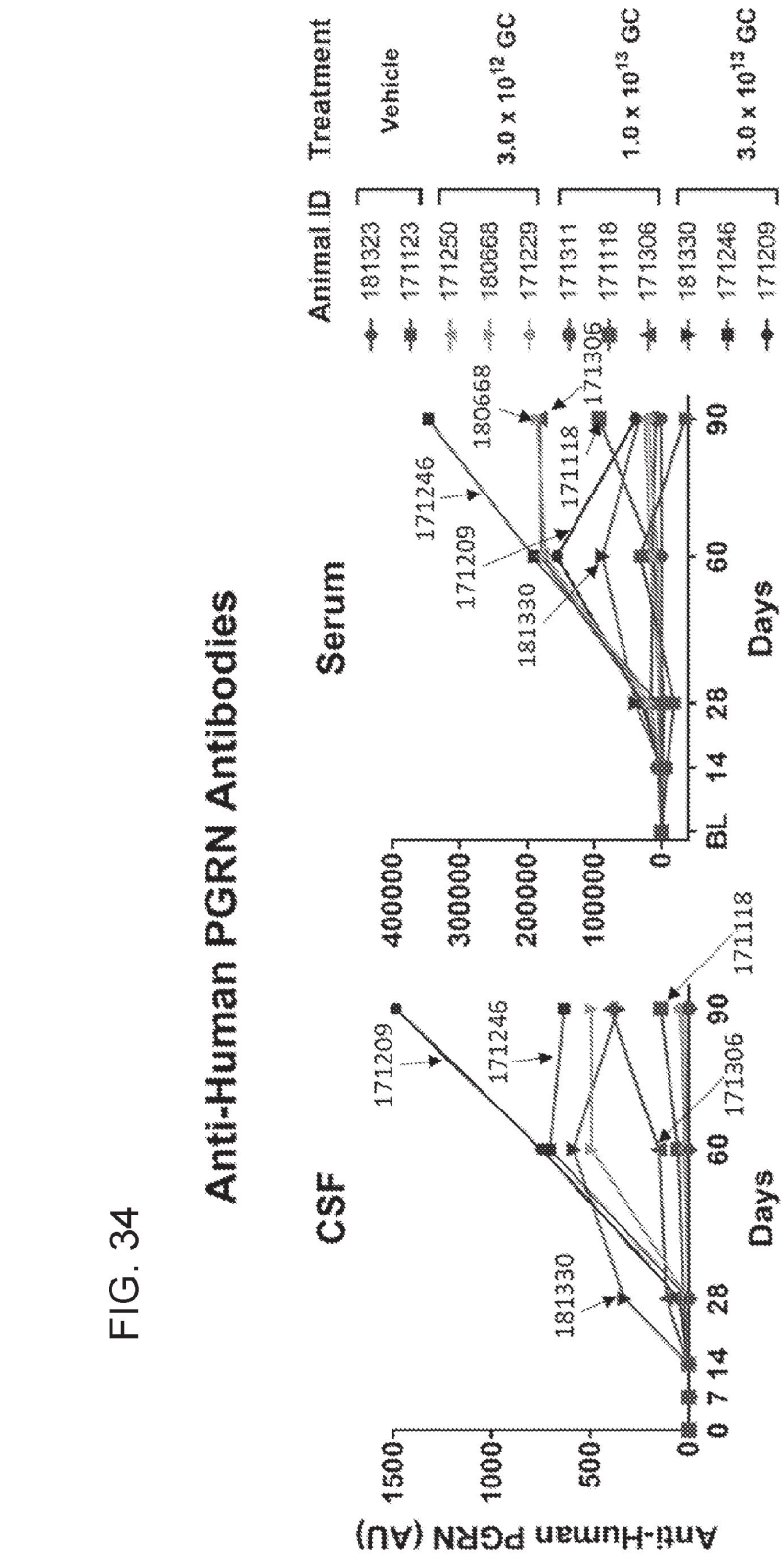
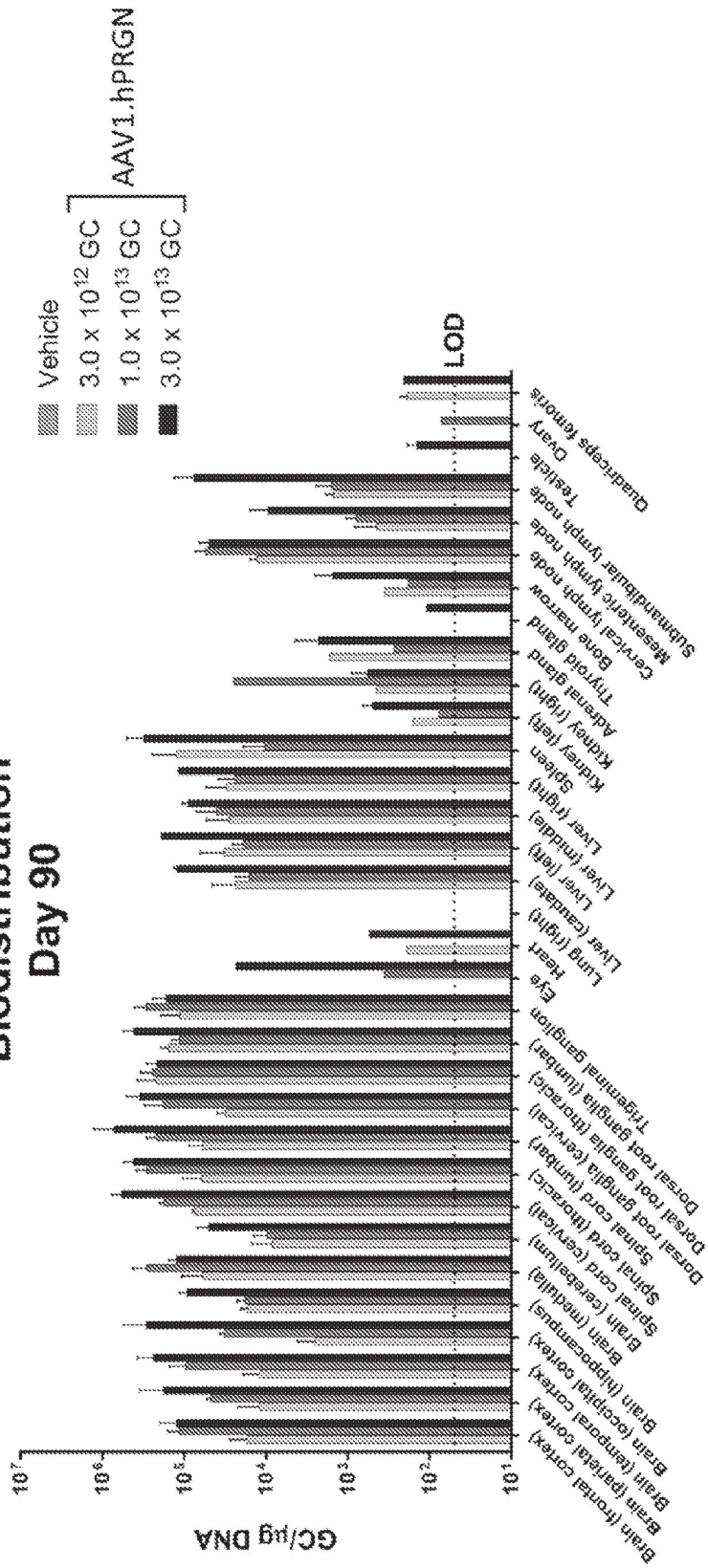

Anti-Human PGRN Antibodies
CSF
Serum
Anti-Human PGRN (AU)
Days
Animal ID
Treatment
Vehicle
3.0 x 10^12 GC
1.0 x 10^13 GC
3.0 x 10^13 GC
181323
171123
171250
180668
171229
171311
171118
171306
181330
171246
171209
BL

FIG. 35


Biodistribution
Day 90
Vehicle
3.0 x 10^12 GC
1.0 x 10^13 GC
3.0 x 10^13 GC
AAV1.hPRGN
GC/µg DNA
LOD
Brain (frontal cortex)
Brain (parietal cortex)
Brain (temporal cortex)
Brain (occipital cortex)
Brain (hippocampus)
Brain (medulla)
Brain (cerebellum)
Spinal cord (cervical)
Spinal cord (thoracic)
Spinal cord (lumbar)
Dorsal root ganglia (cervical)
Dorsal root ganglia (thoracic)
Dorsal root ganglia (lumbar)
Trigeminal ganglion
Eye
Heart
Lung (right)
Liver (caudate)
Liver (left)
Liver (middle)
Liver (right)
Spleen
Kidney (left)
Kidney (right)
Adrenal gland
Thyroid gland
Bone marrow
Cervical lymph node
Mesenteric lymph node
Submandibular lymph node
Testicle
Ovary
Quadriceps femoris