FIG. 1A

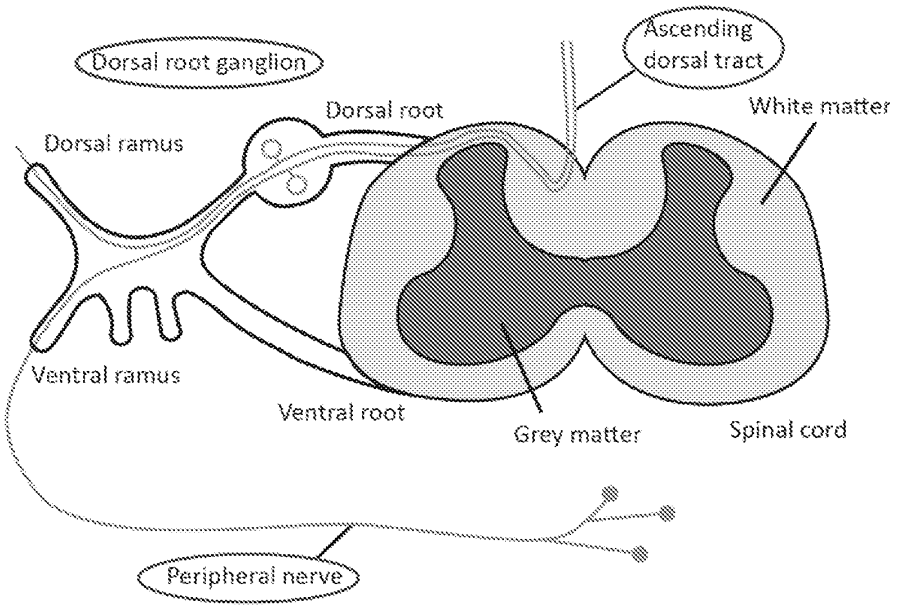

Dorsal root ganglion
Ascending dorsal tract
Dorsal root
White matter
Dorsal ramus
Ventral ramus
Ventral root
Grey matter
Spinal cord
Peripheral nerve

FIG. 1B

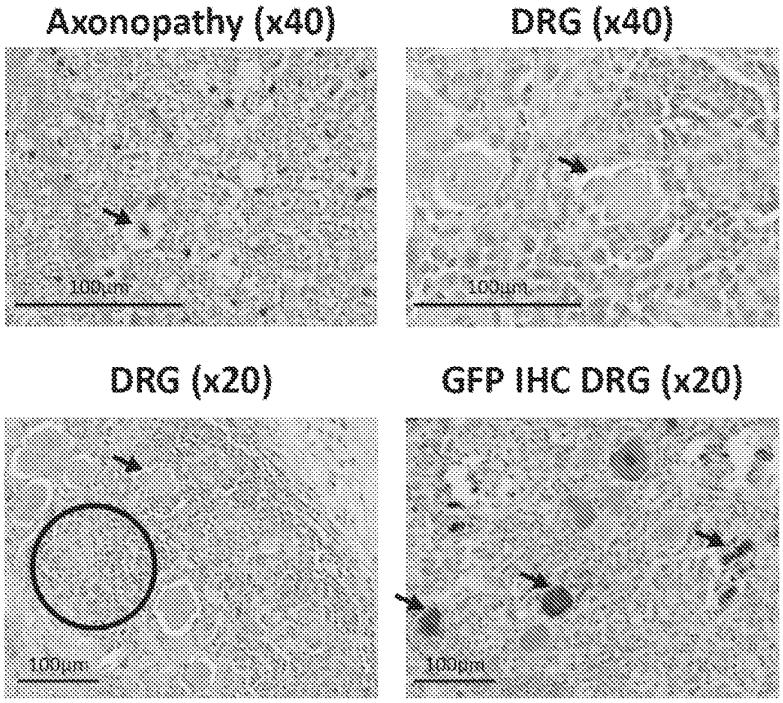

Axonopathy (x40)
DRG (x40)
DRG (x20)
GFP IHC DRG (x20)
100μm
100μm
100μm
100μm

FIG. 1C

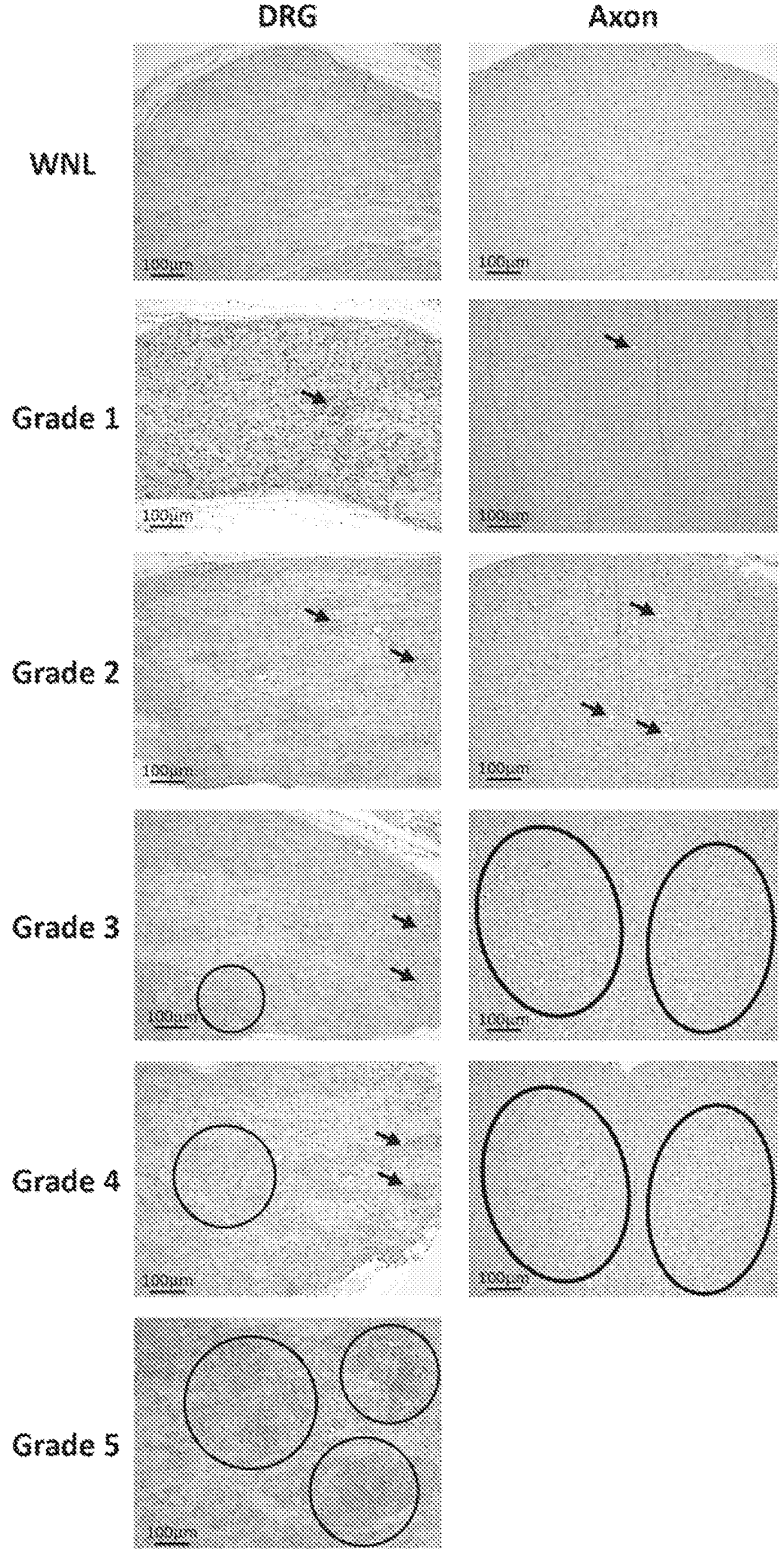


FIG. 2

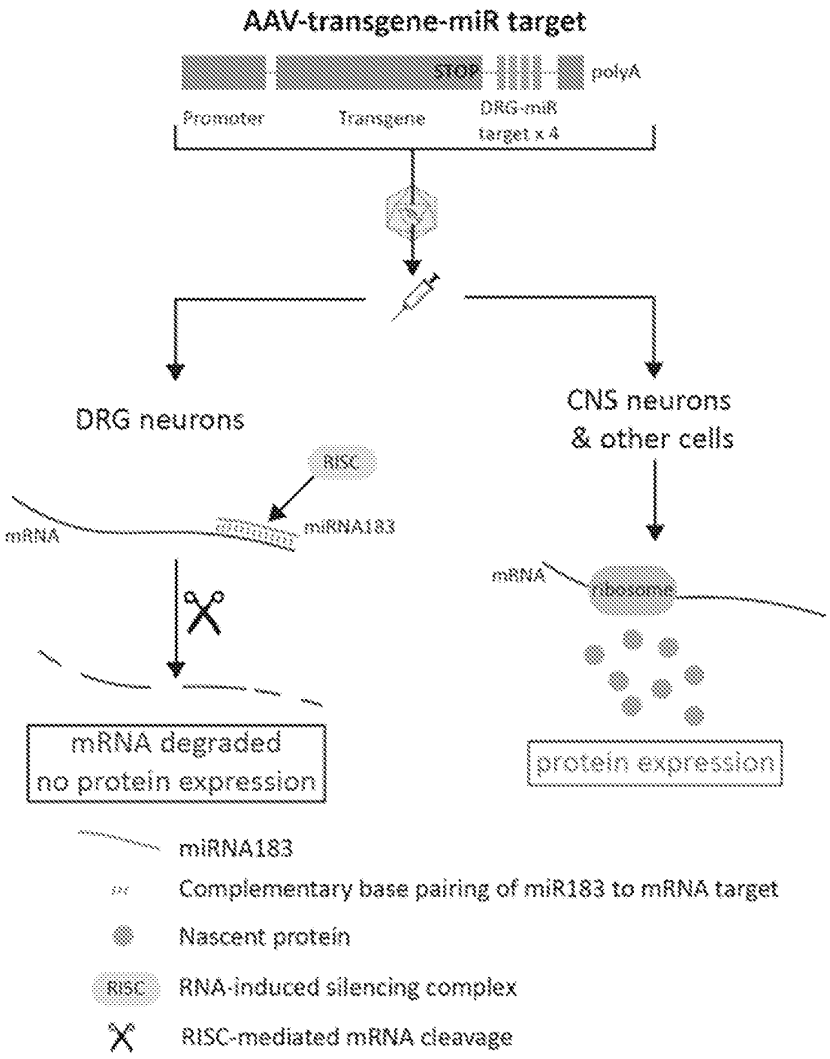

AAV-transgene-miR target
STOP
polyA
Promoter
Transgene
DRG-miR target x 4
DRG neurons
CNS neurons & other cells
RISC
mRNA
miRNA183
mRNA
ribosome
mRNA degraded no protein expression
protein expression
miRNA183
Complementary base pairing of miR183 to mRNA target
Nascent protein
RISC
RNA-induced silencing complex
RISC-mediated mRNA cleavage

FIG. 3A

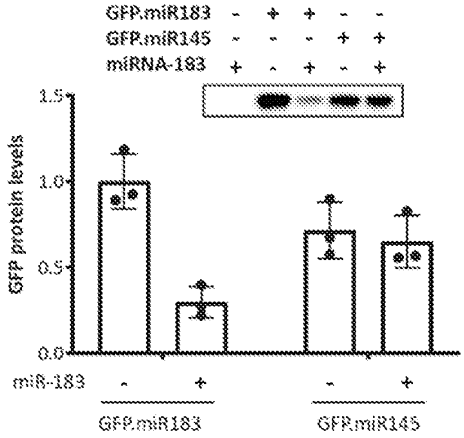



FIG. 3B

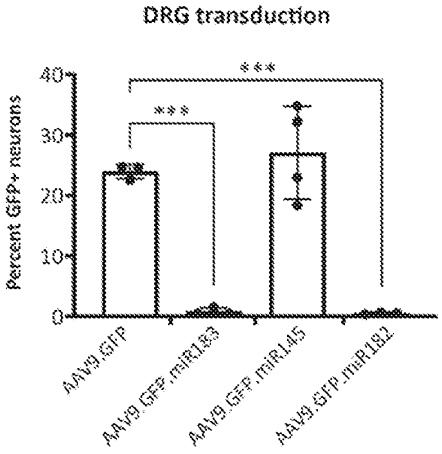



FIG. 3C

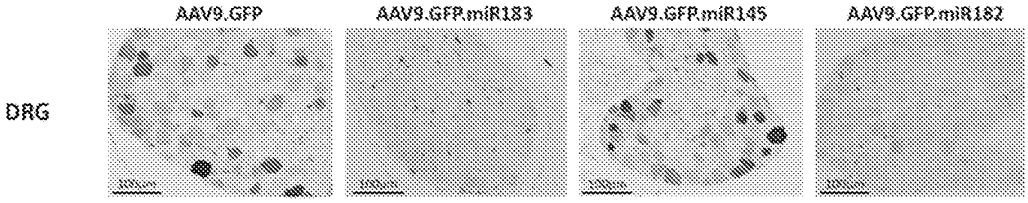



FIG. 3D

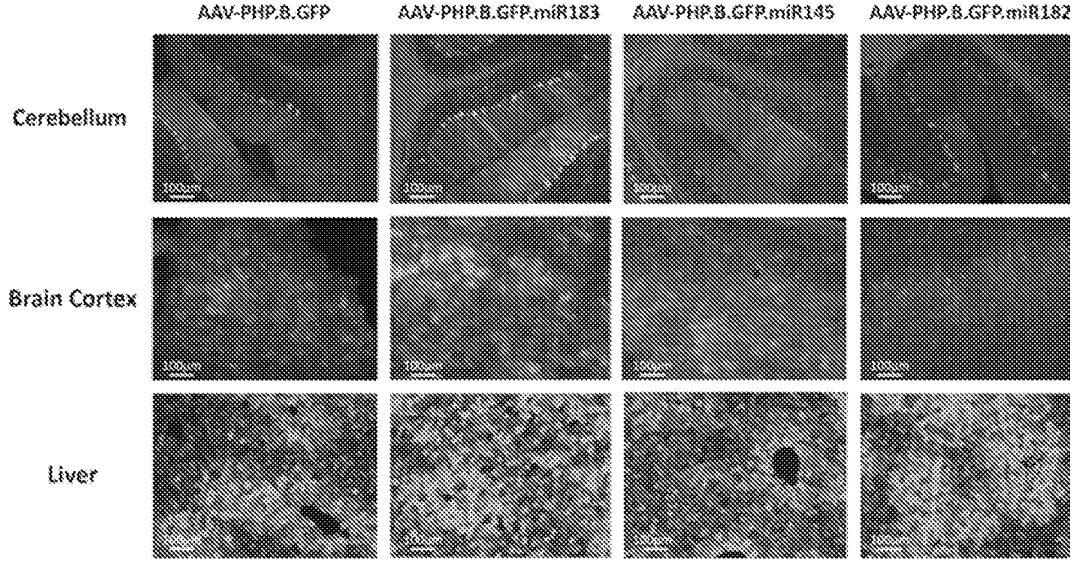


FIG. 4A

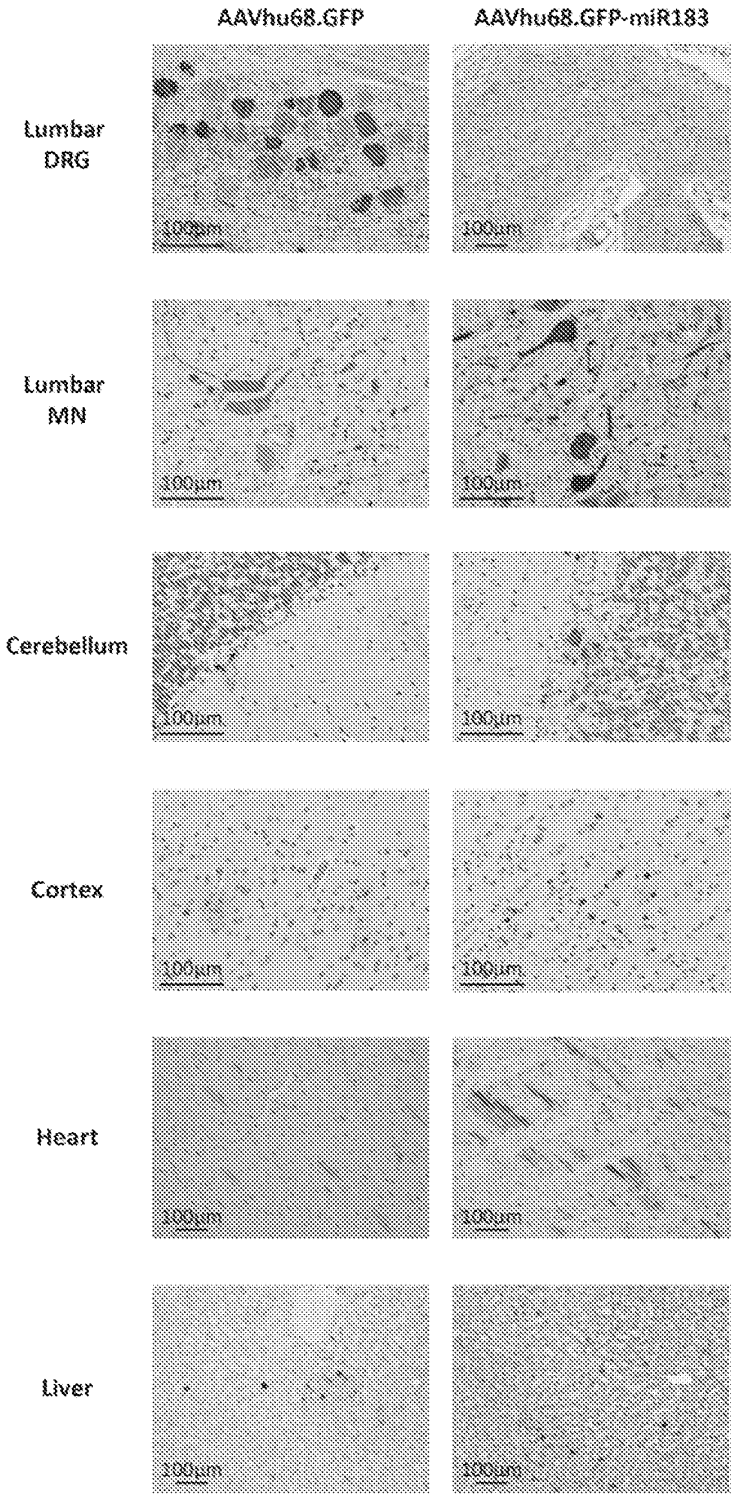

AAVhu68.GFP
AAVhu68.GFP-miR183
Lumbar DRG
Lumbar MN
Cerebellum
Cortex
Heart
Liver
100μm

FIG. 4B

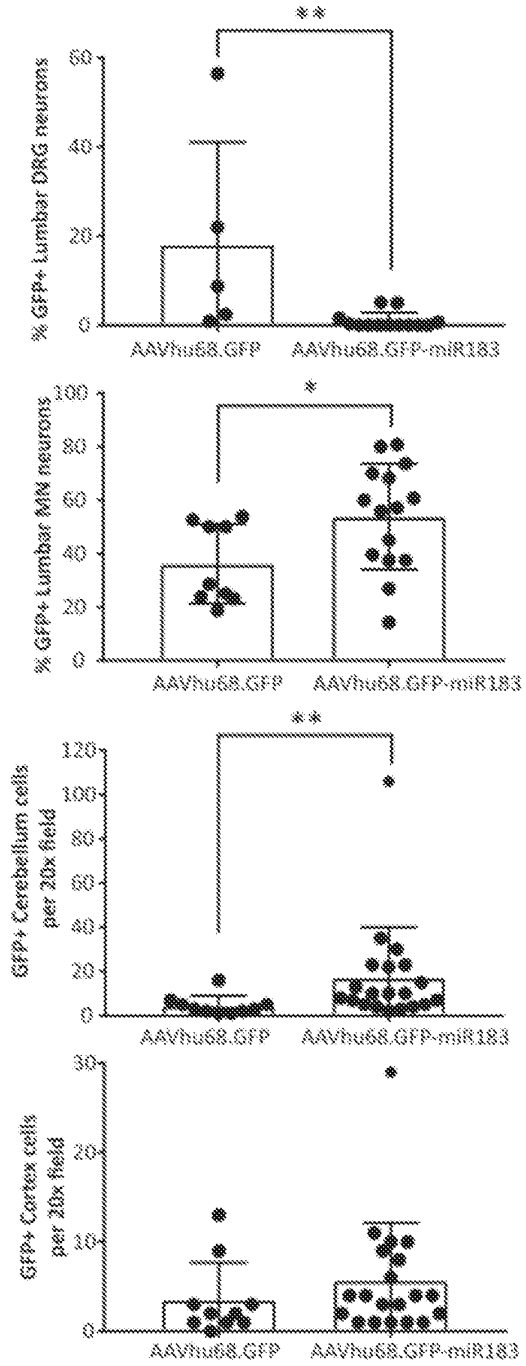

**
% GFP+ Lumbar DRG neurons
60
40
20
0
AAVhu68.GFP
AAVhu68.GFP-miR183
*
% GFP+ Lumbar MN neurons
100
80
60
40
20
0
AAVhu68.GFP
AAVhu68.GFP-miR183
**
GFP+ Cerebellum cells per 20x field
120
100
80
60
40
20
0
AAVhu68.GFP
AAVhu68.GFP-miR183
GFP+ Cortex cells per 20x field
30
20
10
0
AAVhu68.GFP
AAVhu68.GFP-miR183

FIG. 4C

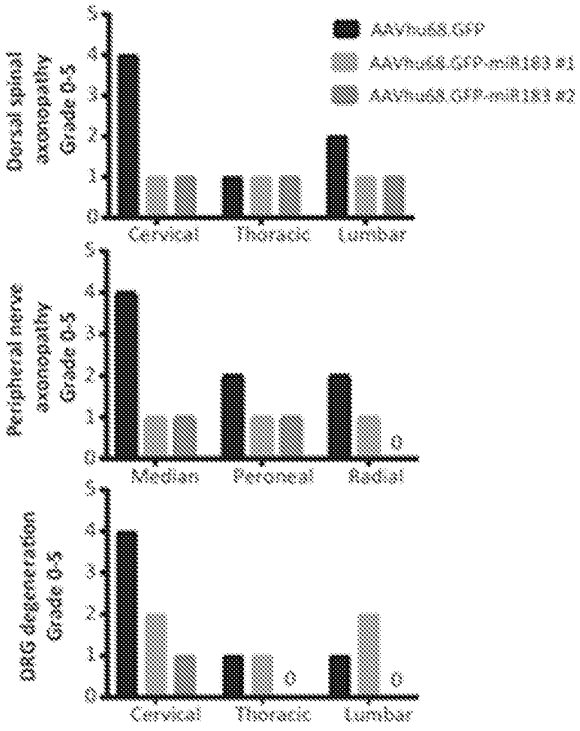

AAVhu68.GFP
AAVhu68.GFP-miR183 #1
AAVhu68.GFP-miR183 #2
Dorsal spinal axonopathy Grade 0-5
Cervical
Thoracic
Lumbar
Peripheral nerve axonopathy Grade 0-5
Median
Peroneal
Radial
DRG degeneration Grade 0-5
Cervical
Thoracic
Lumbar

FIG. 5

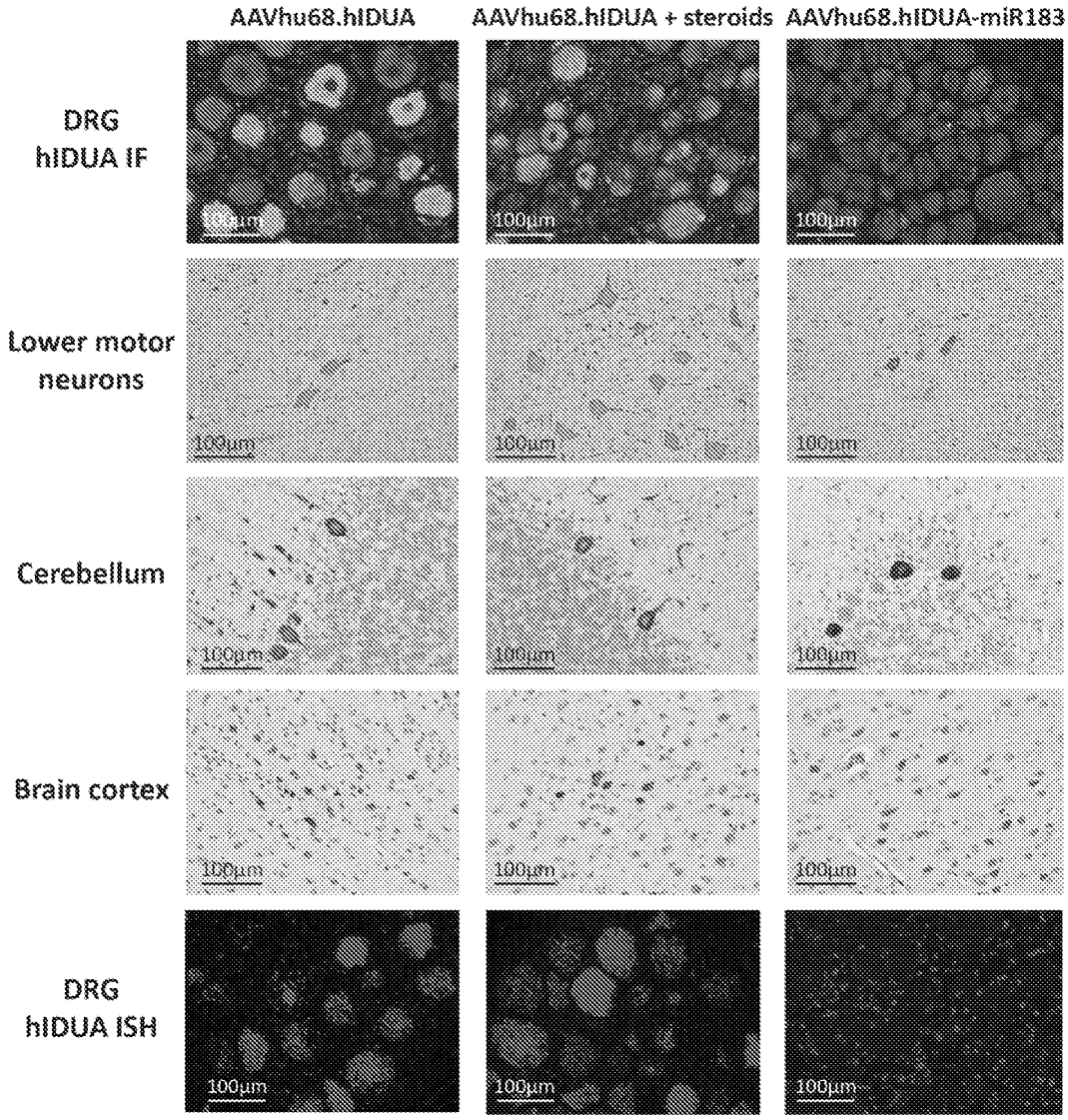

AAVhu68.hIDUA
AAVhu68.hIDUA + steroids
AAVhu68.hIDUA-miR183
DRG hIDUA IF
Lower motor neurons
Cerebellum
Brain cortex
DRG hIDUA ISH
100µm

FIG. 6A

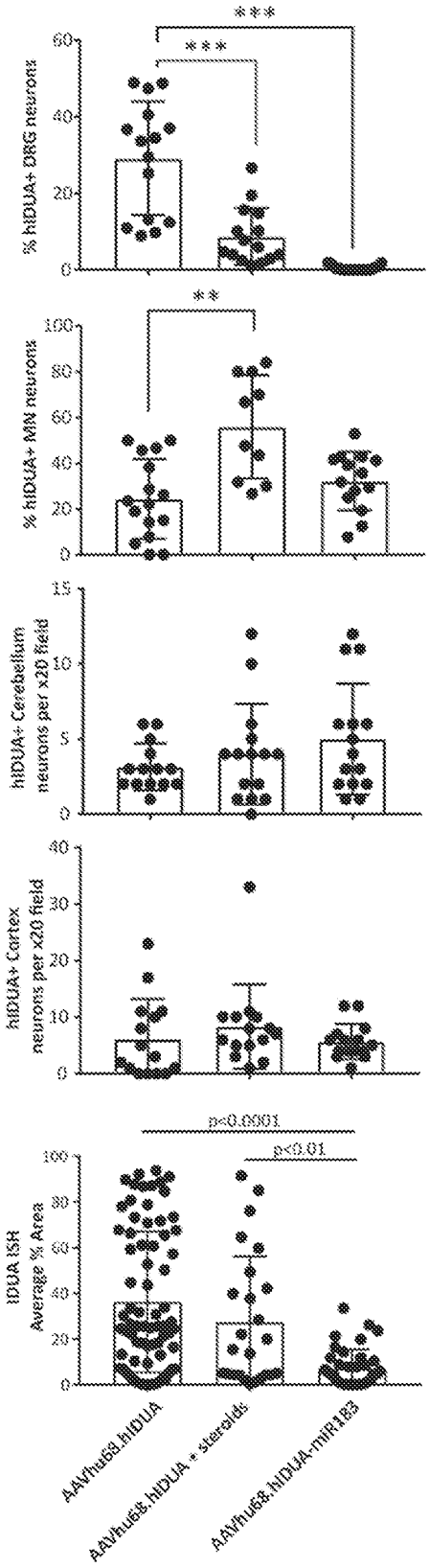
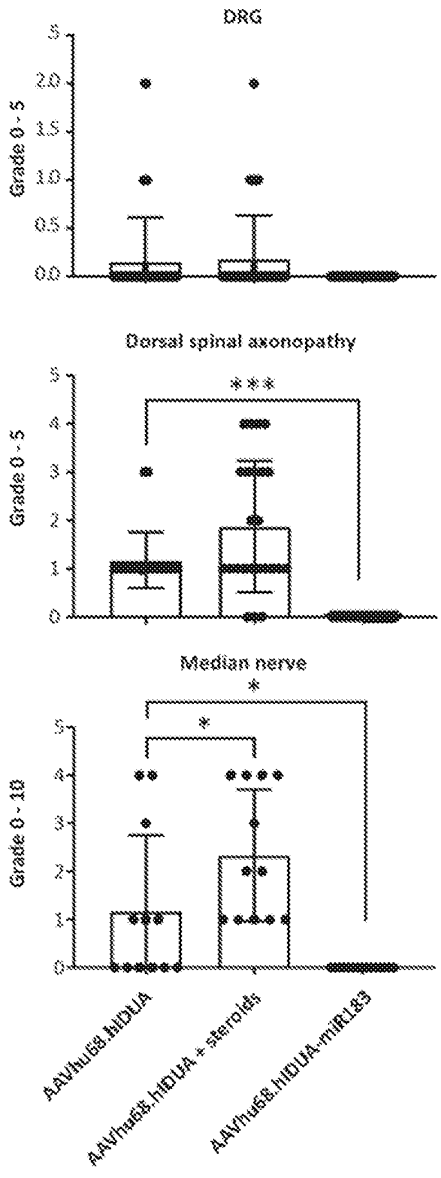



FIG. 6B



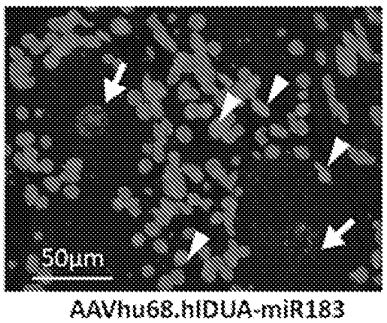




FIG. 6C

FIG. 7A

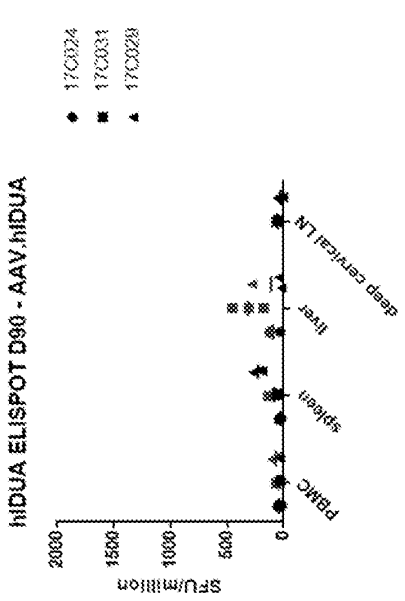



FIG. 7B

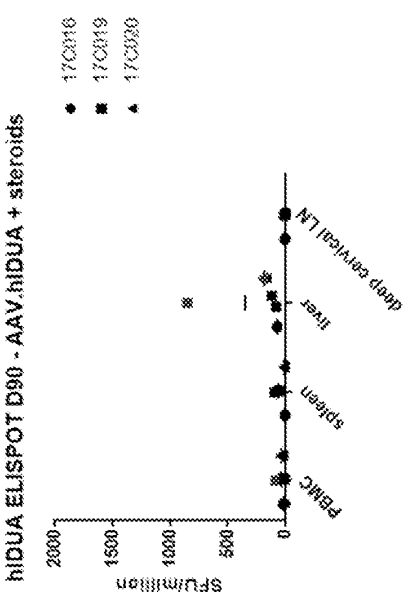



FIG. 7C

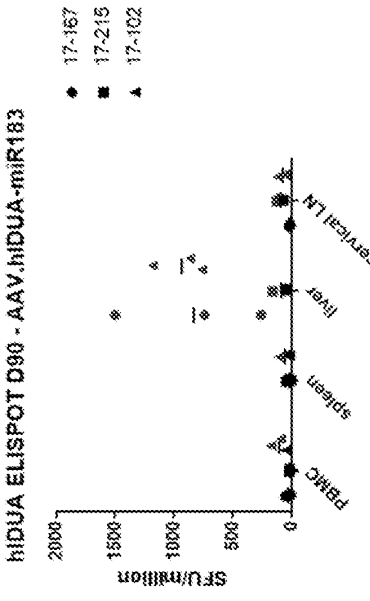



FIG. 7D

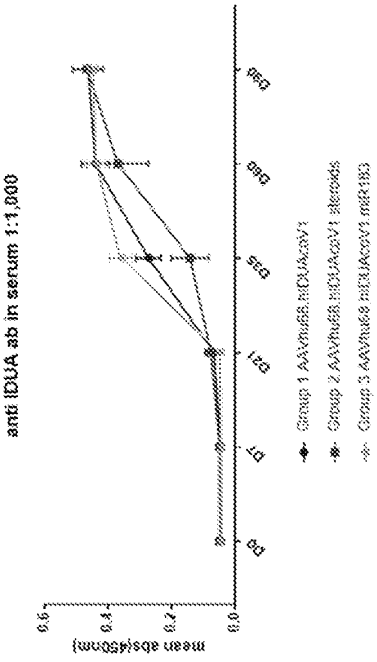


FIG. 8

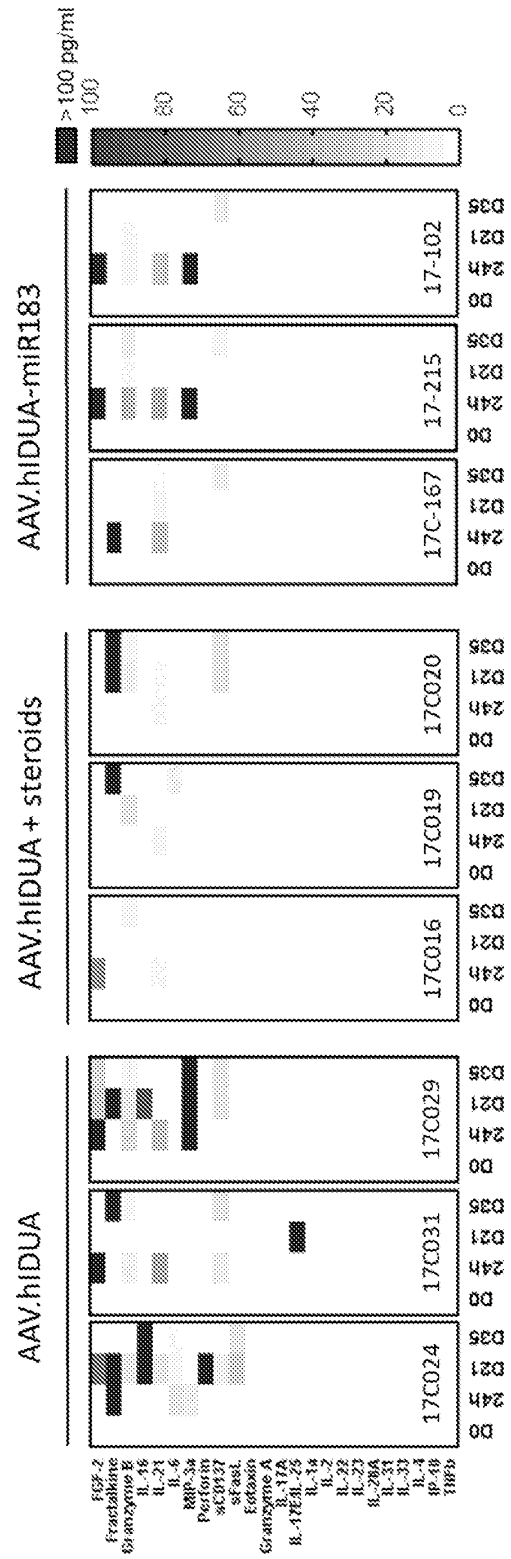

>100 pg/ml
100
80
60
40
20
0
AAV.hIDUA
AAV.hIDUA + steroids
AAV.hIDUA-miR183
17C024
17C031
17C029
17C016
17C019
17C020
17C-167
17-215
17-102
D0
24h
D21
D35
FGF-2
Fractalkine
Granzyme B
IL-15
IL-21
IL-6
MIP-3a
Perforin
sCD137
sFas L
Eotaxin
Granzyme A
IL-17A
IL-17E/IL-25
IL-1a
IL-2
IL-23
IL-28A
IL-31
IL-3
IL-4
IP-10
TNFb

FIG. 9

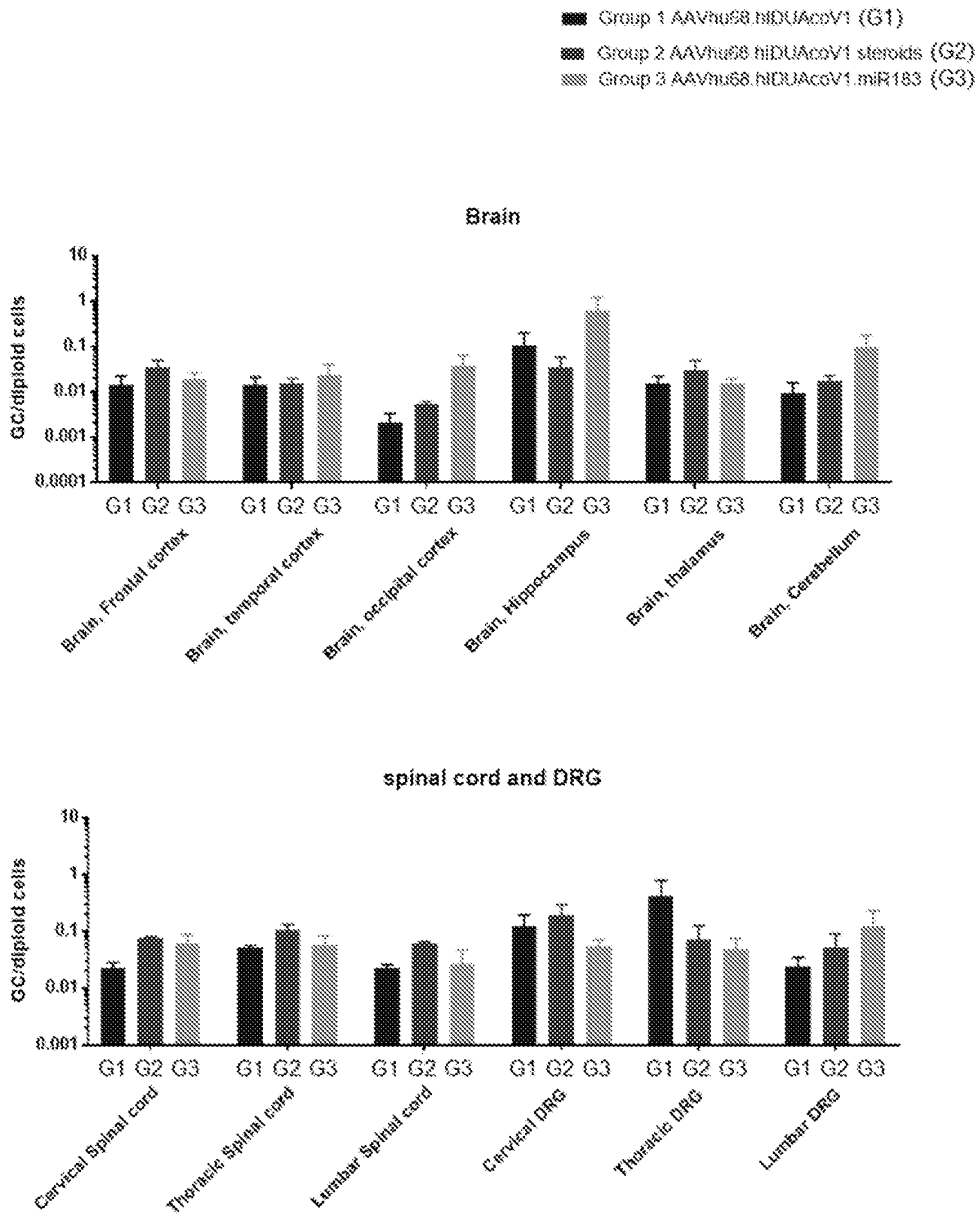

Group 1 AAVhu68.hIDUAcoV1 (G1)
Group 2 AAVhu68.hIDUAcoV1 steroids (G2)
Group 3 AAVhu68.hIDUAcoV1.miR183 (G3)
Brain
GC/diploid cells
10
1
0.1
0.01
0.001
0.0001
G1 G2 G3
Brain, Frontal cortex
Brain, temporal cortex
Brain, occipital cortex
Brain, Hippocampus
Brain, thalamus
Brain, Cerebellum
spinal cord and DRG
GC/diploid cells
10
1
0.1
0.01
0.001
G1 G2 G3
Cervical Spinal cord
Thoracic Spinal cord
Lumbar Spinal cord
Cervical DRG
Thoracic DRG
Lumbar DRG

FIG. 10A

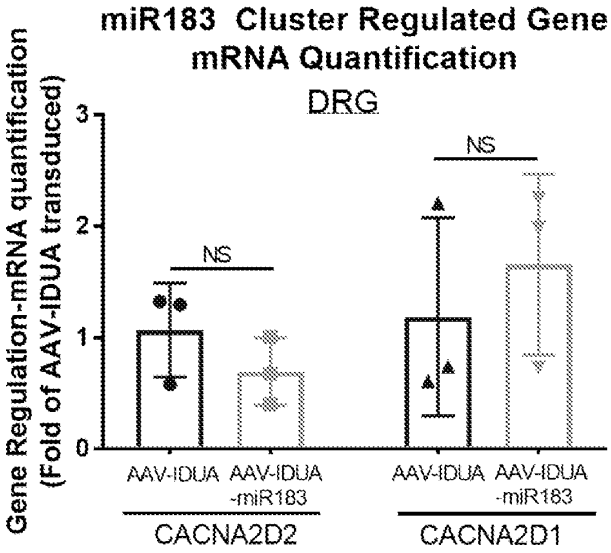



FIG. 10B

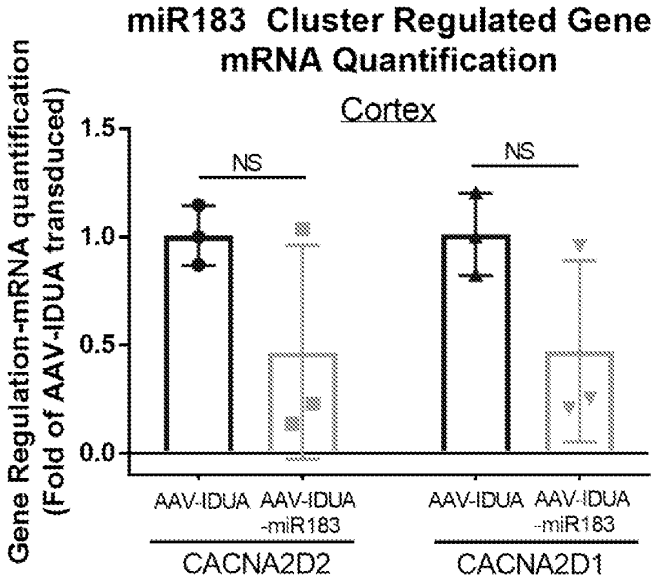


FIG. 11

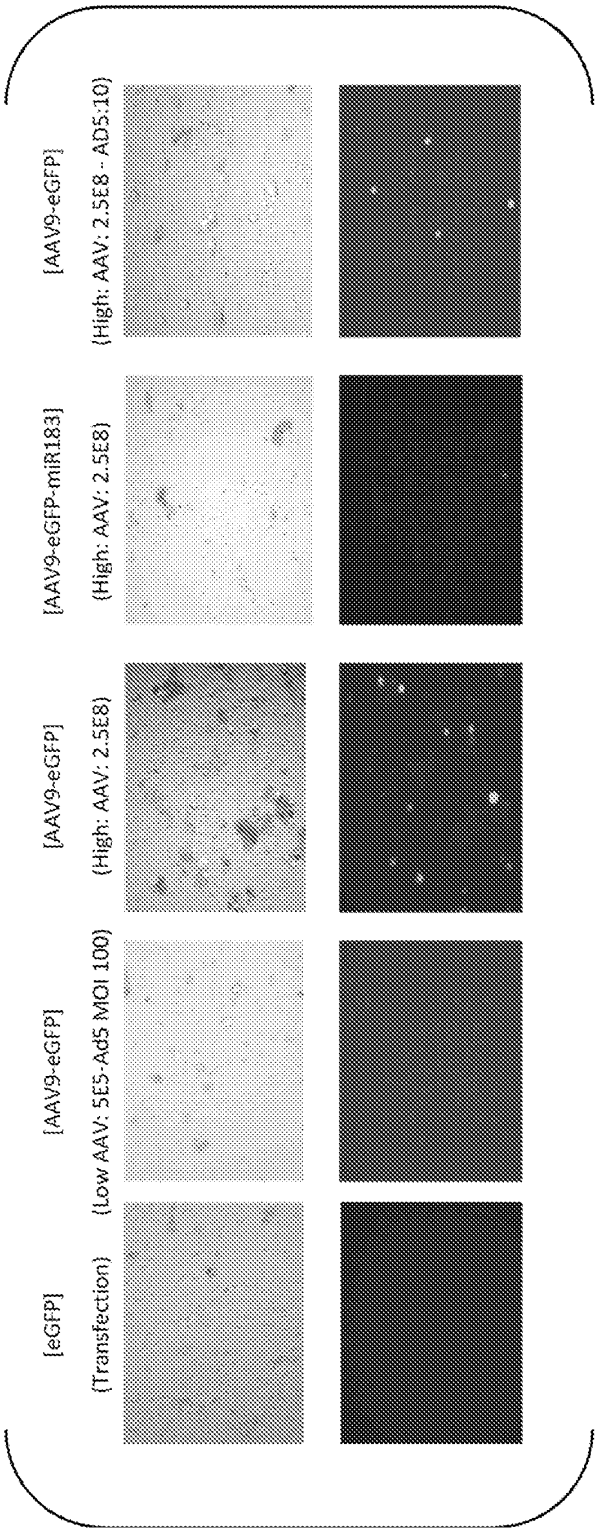

[eGFP]
(Transfection)
[AAV9-eGFP]
(Low AAV: 5E5-Ad5 MOI 100)
[AAV9-eGFP]
(High: AAV: 2.5E8)
[AAV9-eGFP-miR183]
(High: AAV: 2.5E8)
[AAV9-eGFP]
(High: AAV: 2.5E8 - AD5:10)

FIG. 12

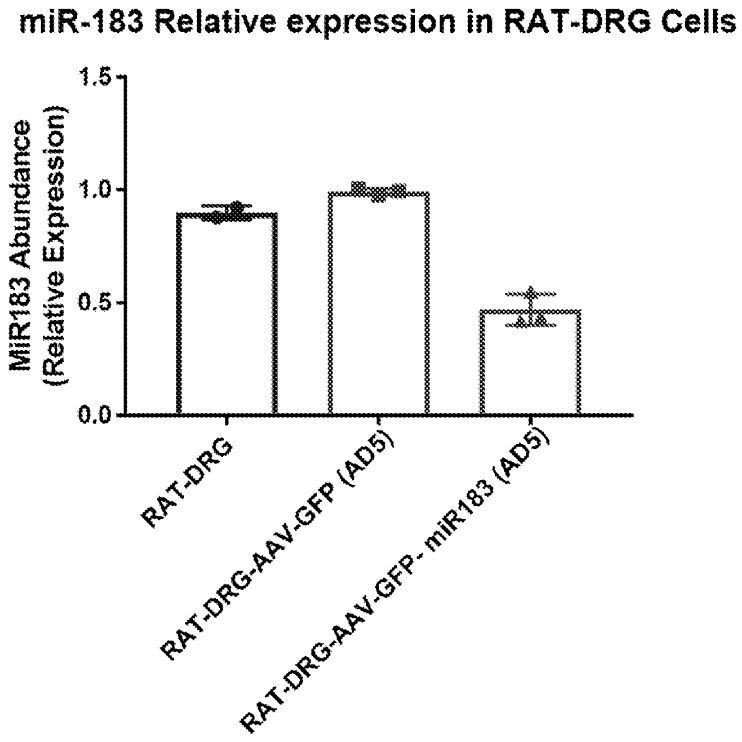

miR-183 Relative expression in RAT-DRG Cells
MiR183 Abundance (Relative Expression)
1.5
1.0
0.5
0.0
RAT-DRG
RAT-DRG-AAV-GFP (AD5)
RAT-DRG-AAV-GFP- miR183 (AD5)

FIG. 13A

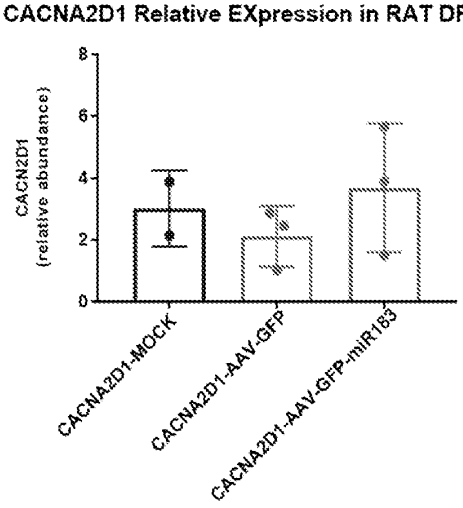



FIG. 13B

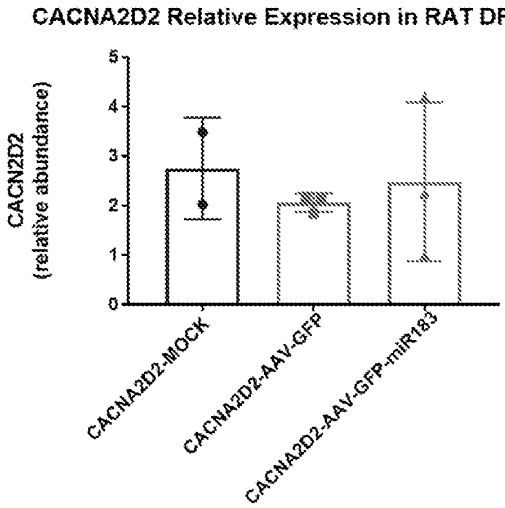



FIG. 13C

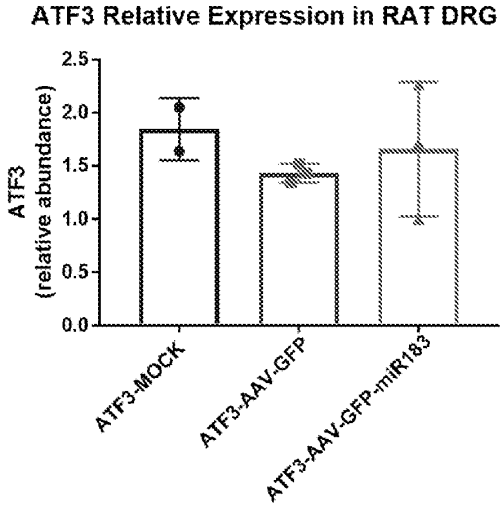


FIG. 14

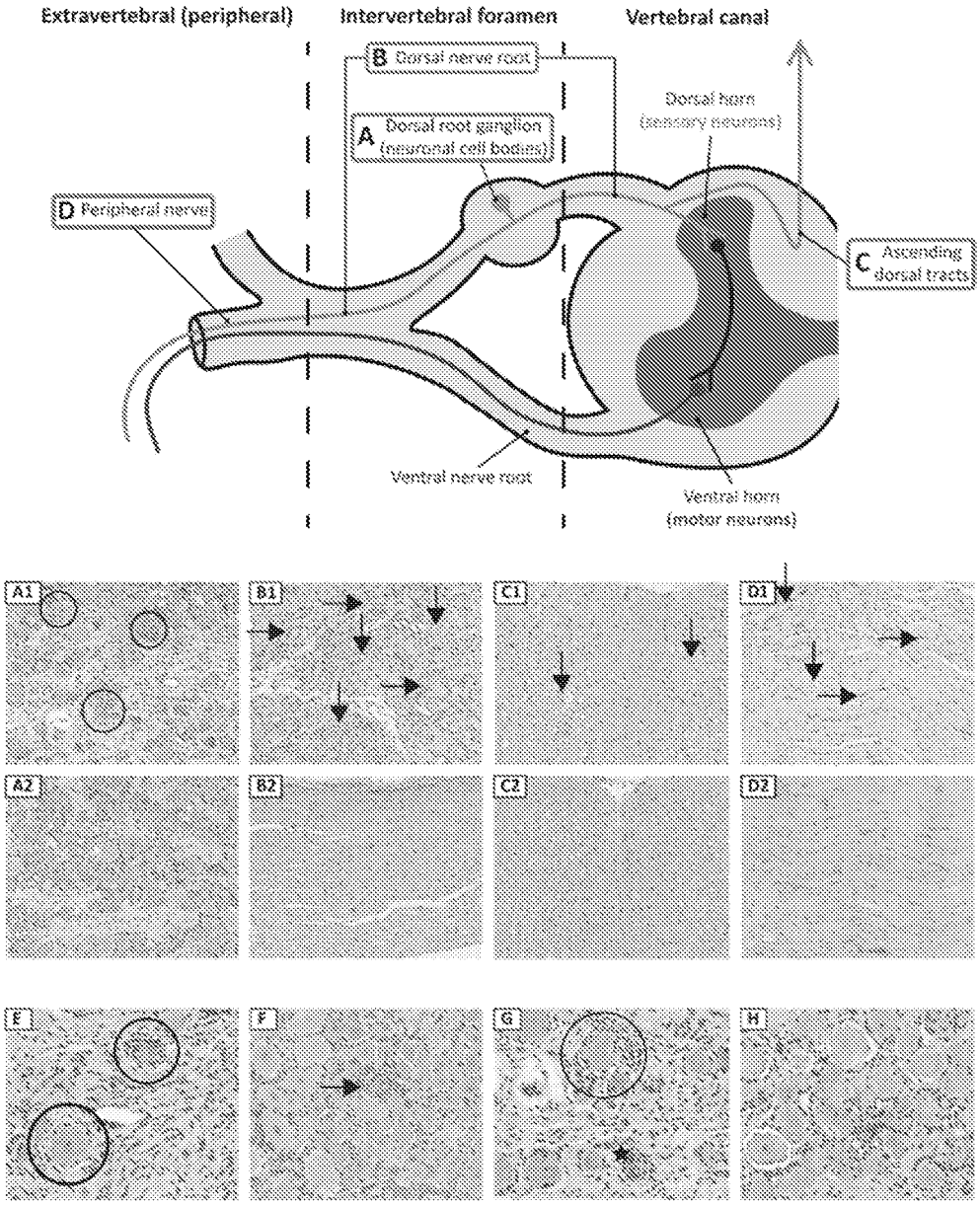

Extravertebral (peripheral)
Intervertebral foramen
Vertebral canal
B Dorsal nerve root
A Dorsal root ganglion (neuronal cell bodies)
Dorsal horn (sensory neurons)
D Peripheral nerve
C Ascending dorsal tracts
Ventral nerve root
Ventral horn (motor neurons)
A1
B1
C1
D1
A2
B2
C2
D2
E
F
G
H

FIG. 15A

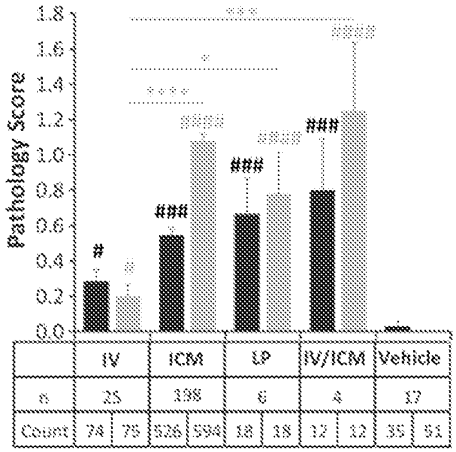



FIG. 15B

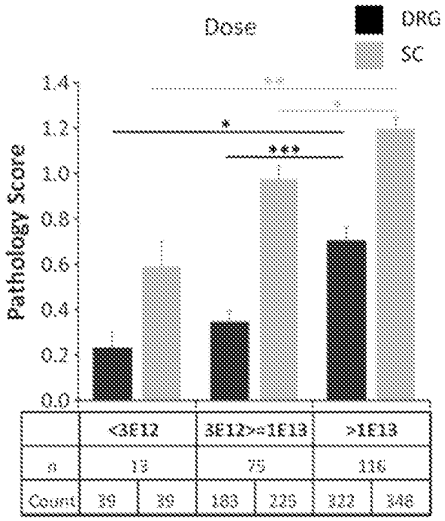



FIG. 15C

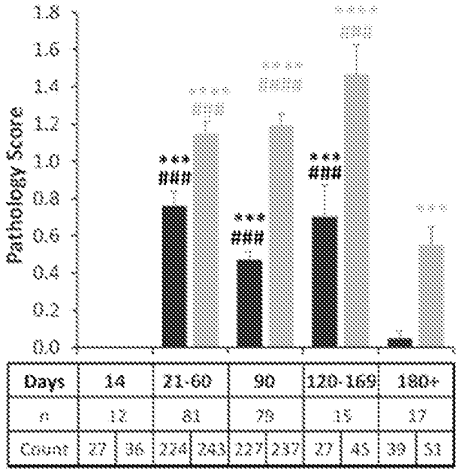



FIG. 15D

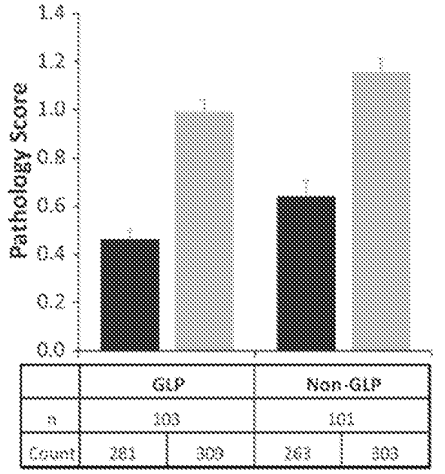


FIG. 16A

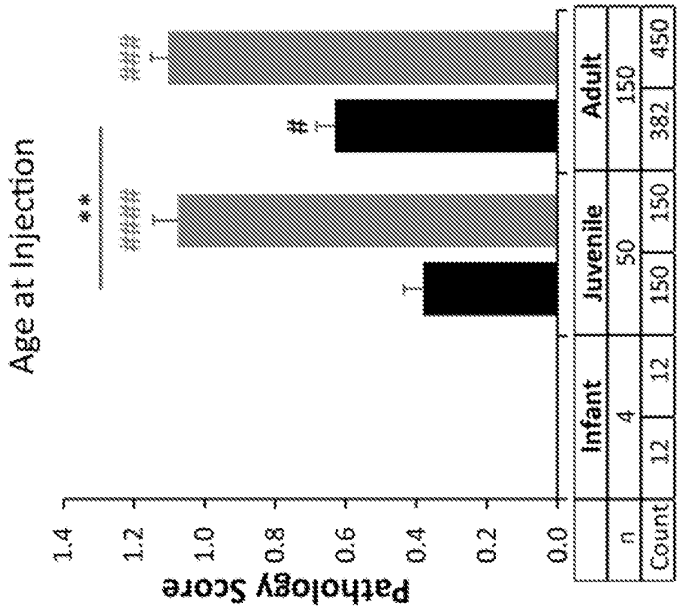



FIG. 16B

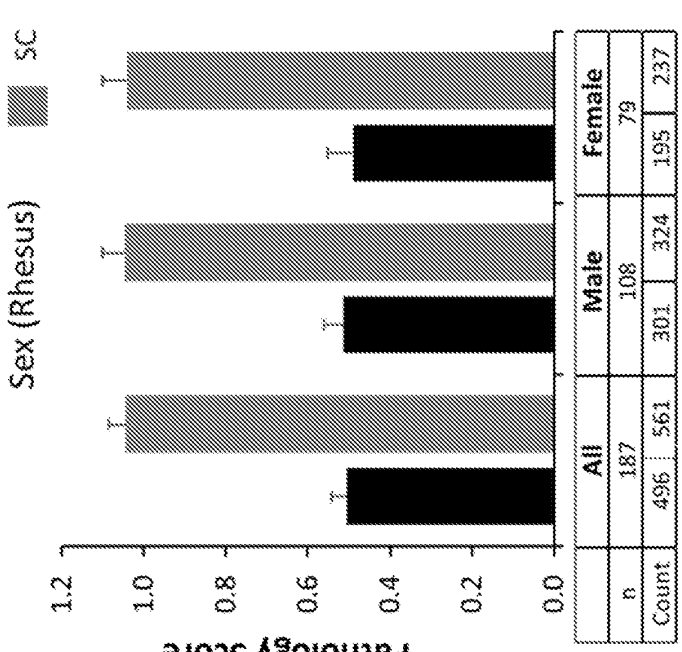
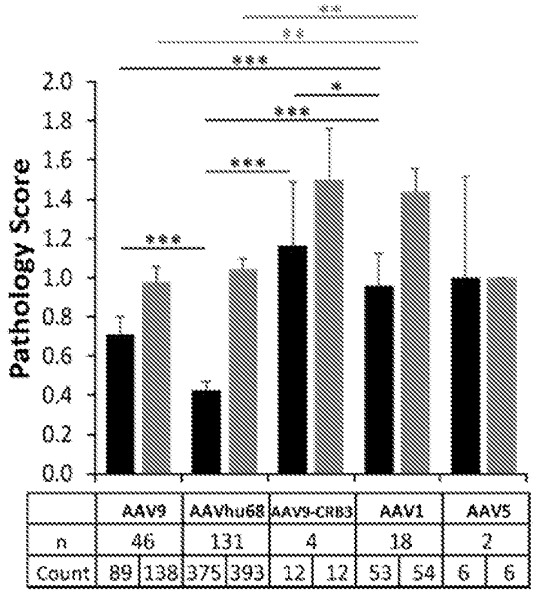
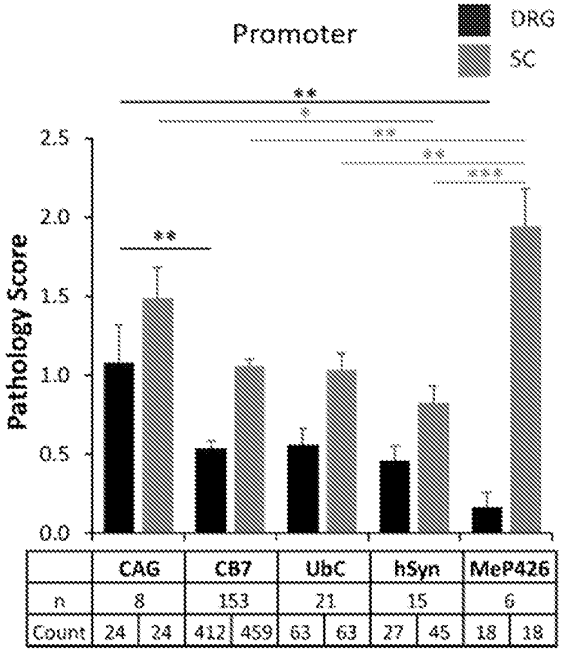


FIG. 17A



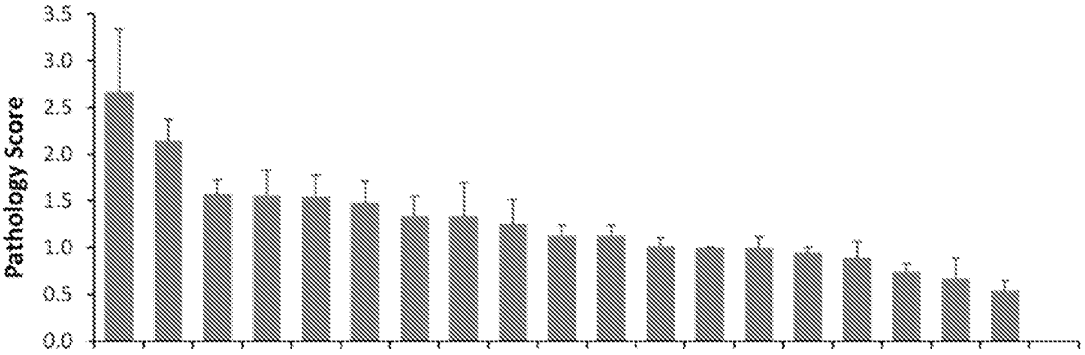

| | AAV9 | | AAVhu68 | | AAV9-CRB3 | | AAV1 | | AAV5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| n | 46 | | 131 | | 4 | | 18 | | 2 | |
| Count | 89 | 138 | 375 | 393 | 12 | 12 | 53 | 54 | 6 | 6 |

FIG. 17B




| | CAG | | CB7 | | UbC | | hSyn | | MeP426 | |
|---|---|---|---|---|---|---|---|---|---|---|
| n | 8 | | 153 | | 21 | | 15 | | 6 | |
| Count | 24 | 24 | 412 | 459 | 63 | 63 | 27 | 45 | 18 | 18 |

SC Pathology by Transgene




| TG | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | 2 | 7 | 13 | 6 | 6 | 3 | 2 | 2 | 8 | 23 | 18 | 32 | 2 | 11 | 3 | 9 | 38 | 4 | 12 | 3 |
| Count | 6 | 21 | 39 | 18 | 18 | 9 | 6 | 6 | 24 | 69 | 54 | 96 | 6 | 33 | 9 | 27 | 114 | 12 | 36 | 9 |

FIG. 17C

FIG. 17D

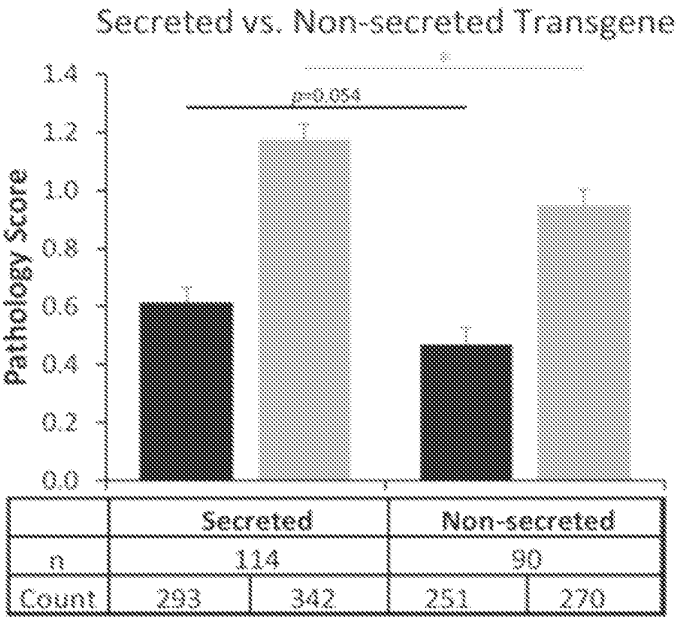

Secreted vs. Non-secreted Transgene
p=0.054
Pathology Score
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Secreted
Non-secreted
n
114
90
Count
293
342
251
270

FIG. 18

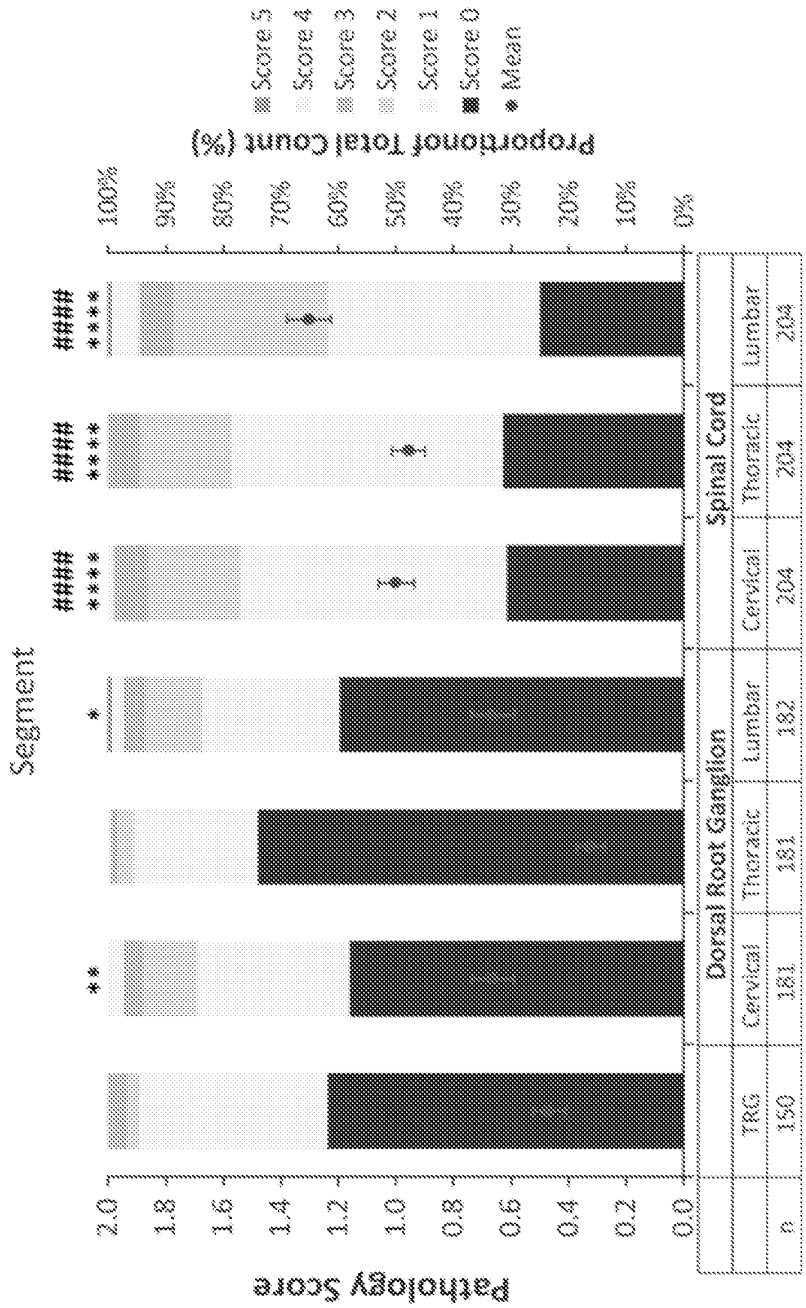
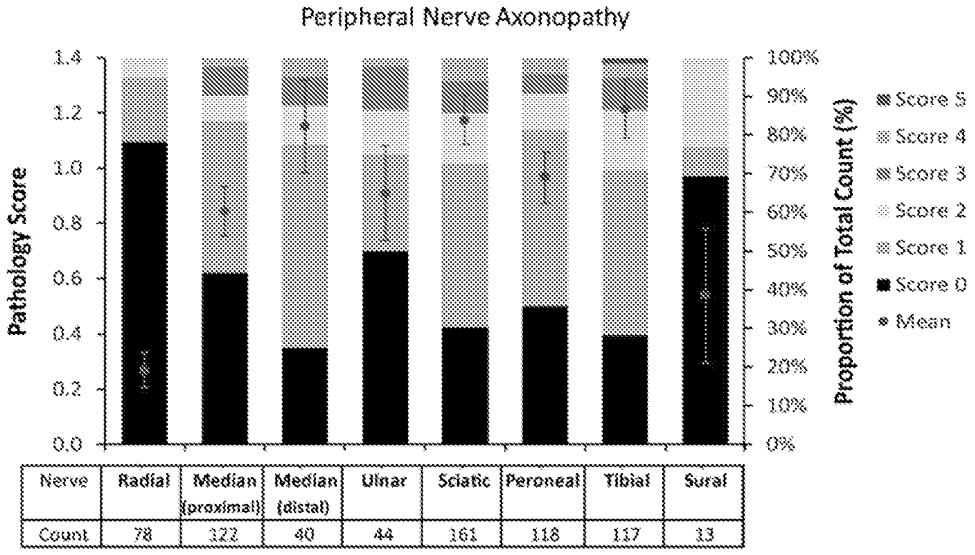

Pathology Score
Proportion of Total Count (%)
Score 5
Score 4
Score 3
Score 2
Score 1
Score 0
Mean
Segment
Dorsal Root Ganglion
Spinal Cord
TRG
Cervical
Thoracic
Lumbar
Cervical
Thoracic
Lumbar
n
150
181
181
182
204
204
204
**
*
#### ****
#### ****
#### ****

FIG. 19A



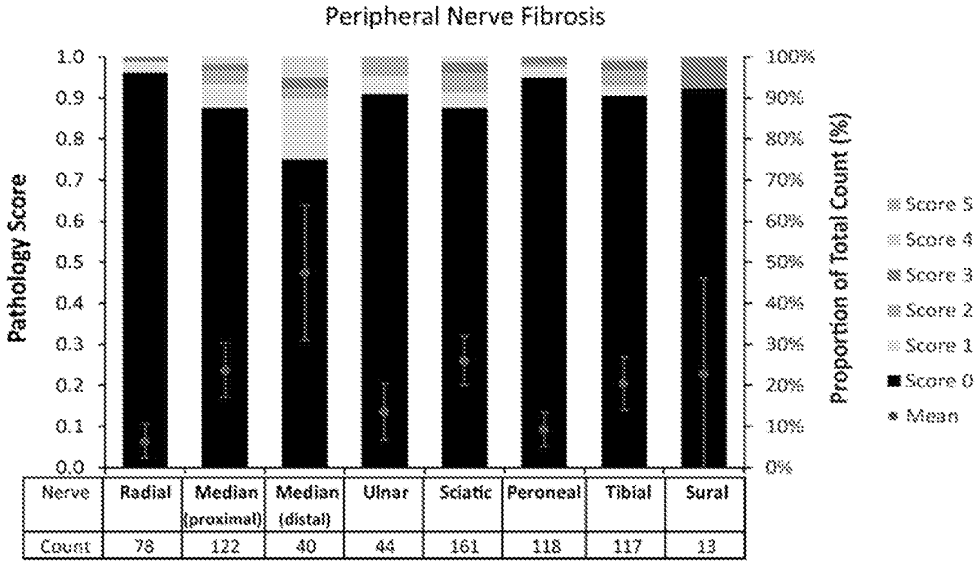




FIG. 19B

FIG. 20A

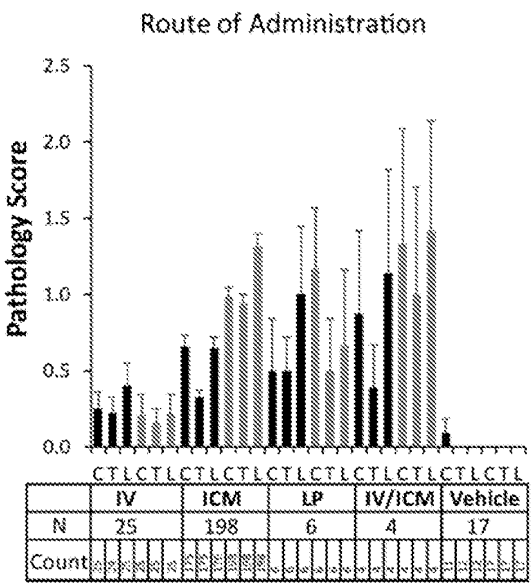



FIG. 20B

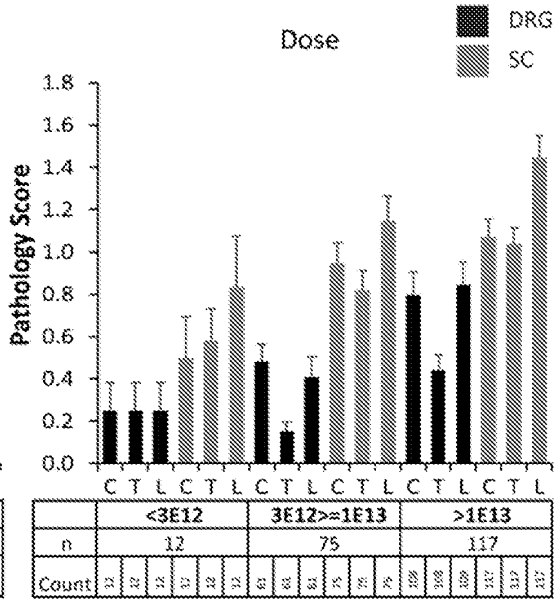



FIG. 20C

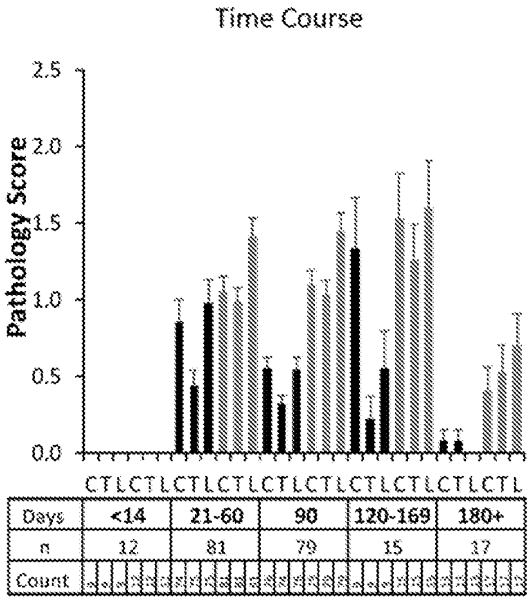



FIG. 20D

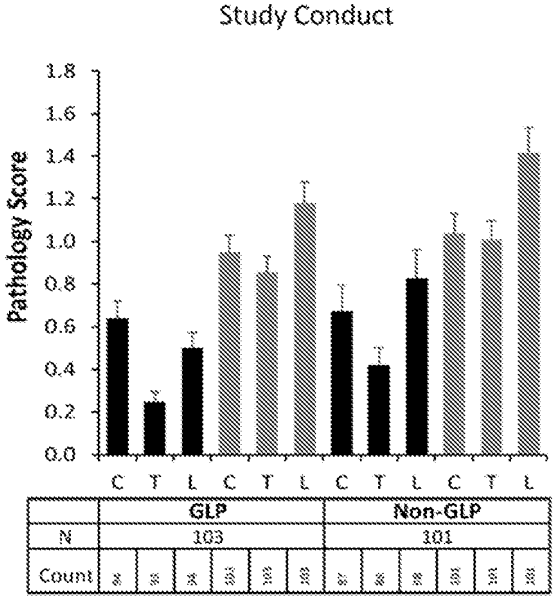


FIG. 21A

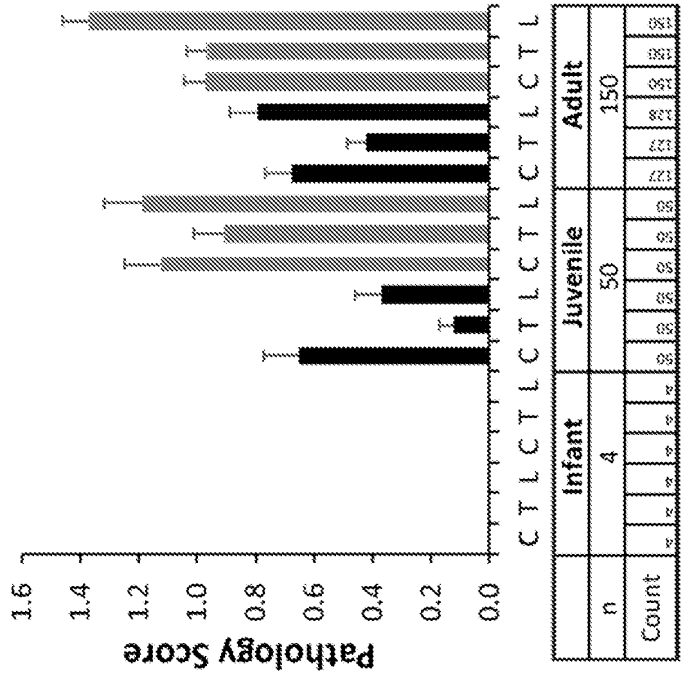



FIG. 21B

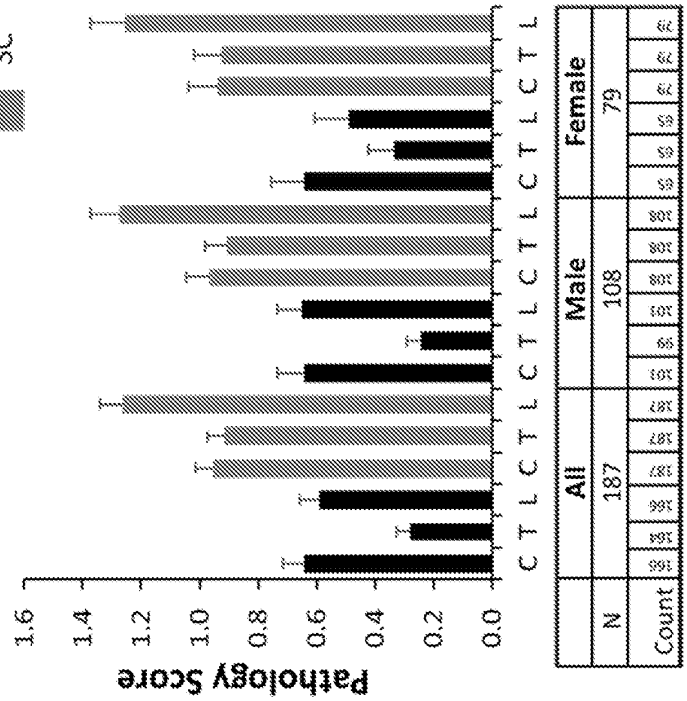


FIG. 22A

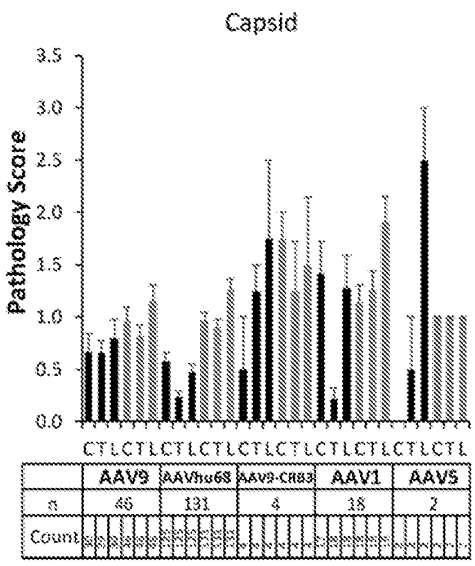
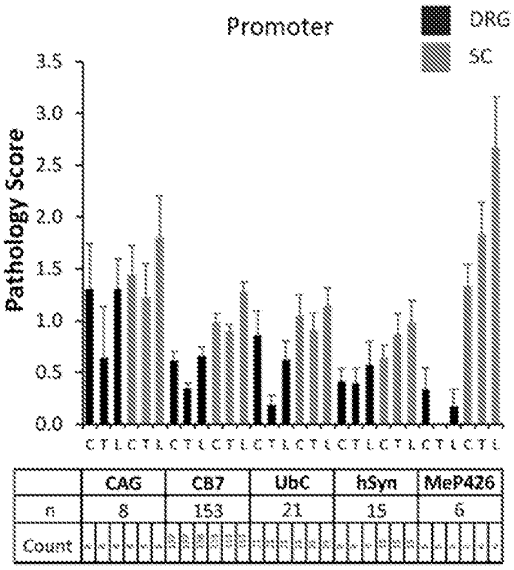



FIG. 22B



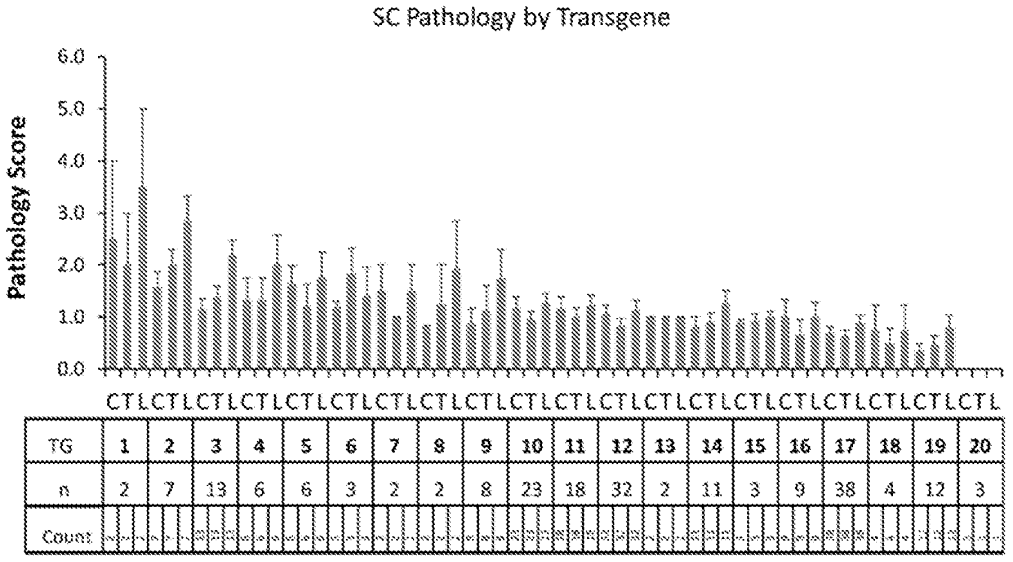




FIG. 22C

FIG. 23

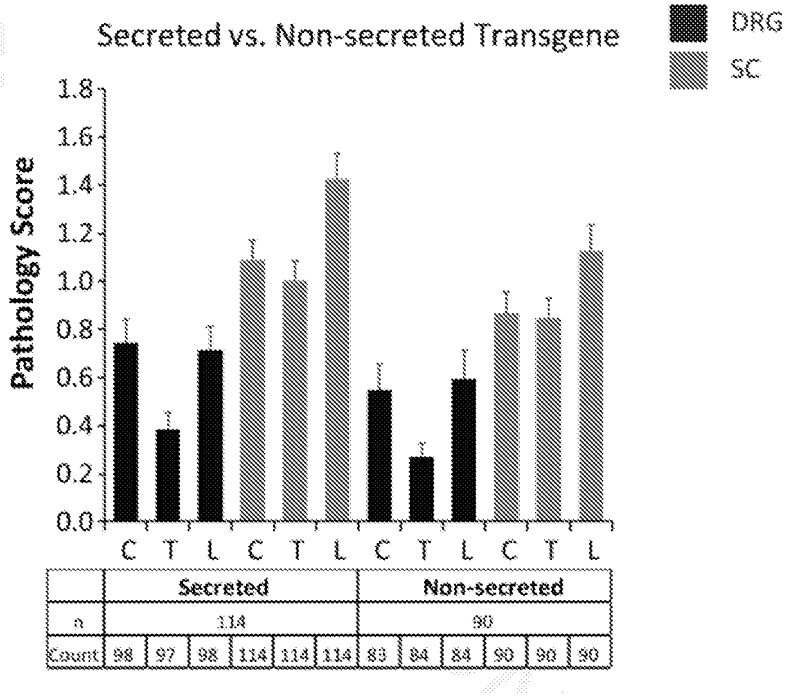

Secreted vs. Non-secreted Transgene
DRG
SC
Pathology Score
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C T L C T L C T L C T L
Secreted
Non-secreted
n
114
90
Count
98 97 98 114 114 114 83 84 84 90 90 90

FIG. 24

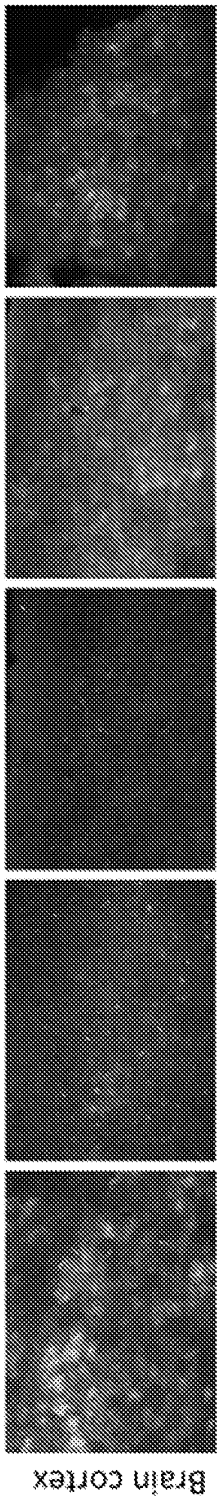

Brain cortex
AAV-PHP.B.GFP -miR183
AAV-PHP.B.GFP -miR182
AAV-PHP.B.GFP -miR96
AAV-PHP.B.GFP -miR145
AAV-PHP.B.GFP

FIG. 25A

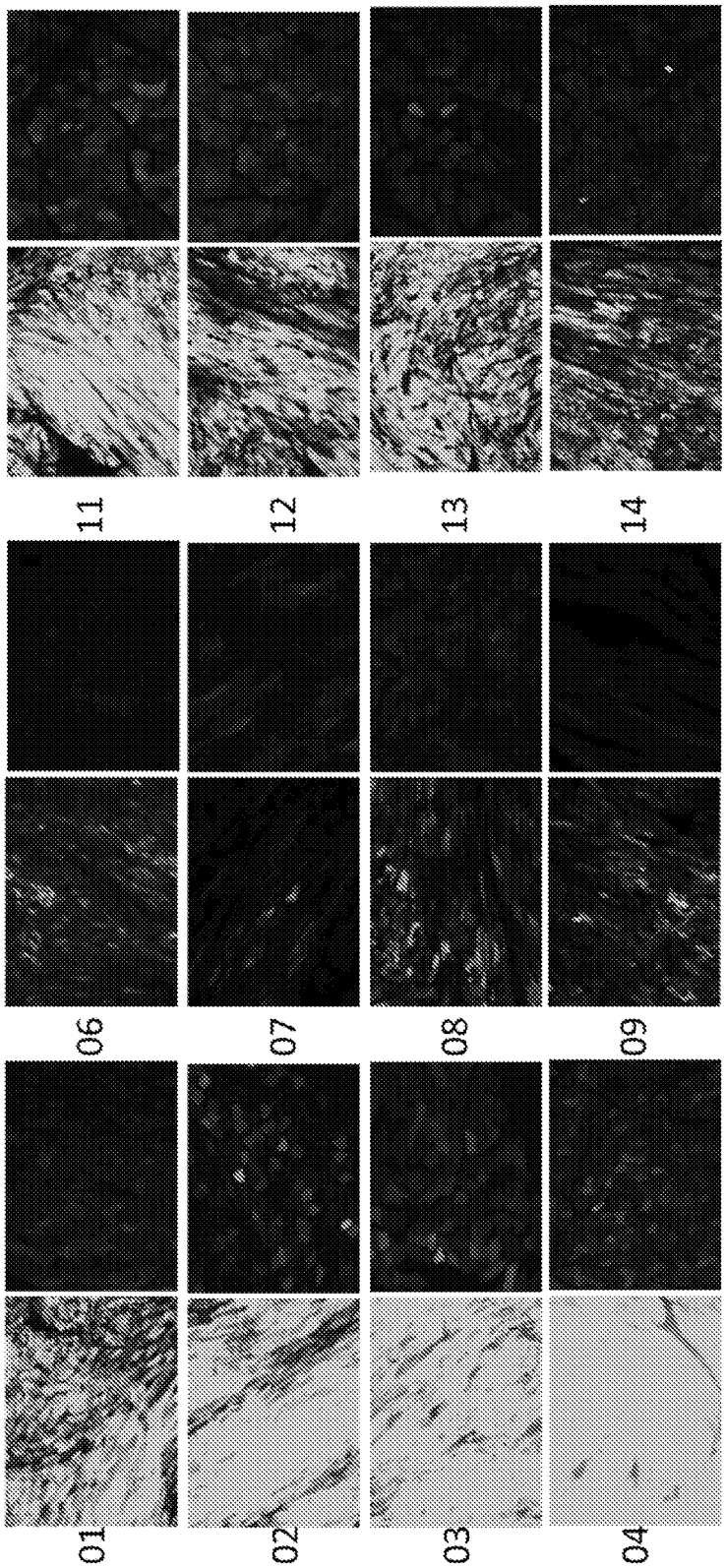

AAV9. CB7.CI.eGFP.miR183.rBG
AAV9. CB7.CI.eGFP.miR145.rBG
AAV9. CB7.CI.eGFP. rBG
Heart (3s)
Muscle (10s)
01
02
03
04
06
07
08
09
11
12
13
14

FIG. 25B

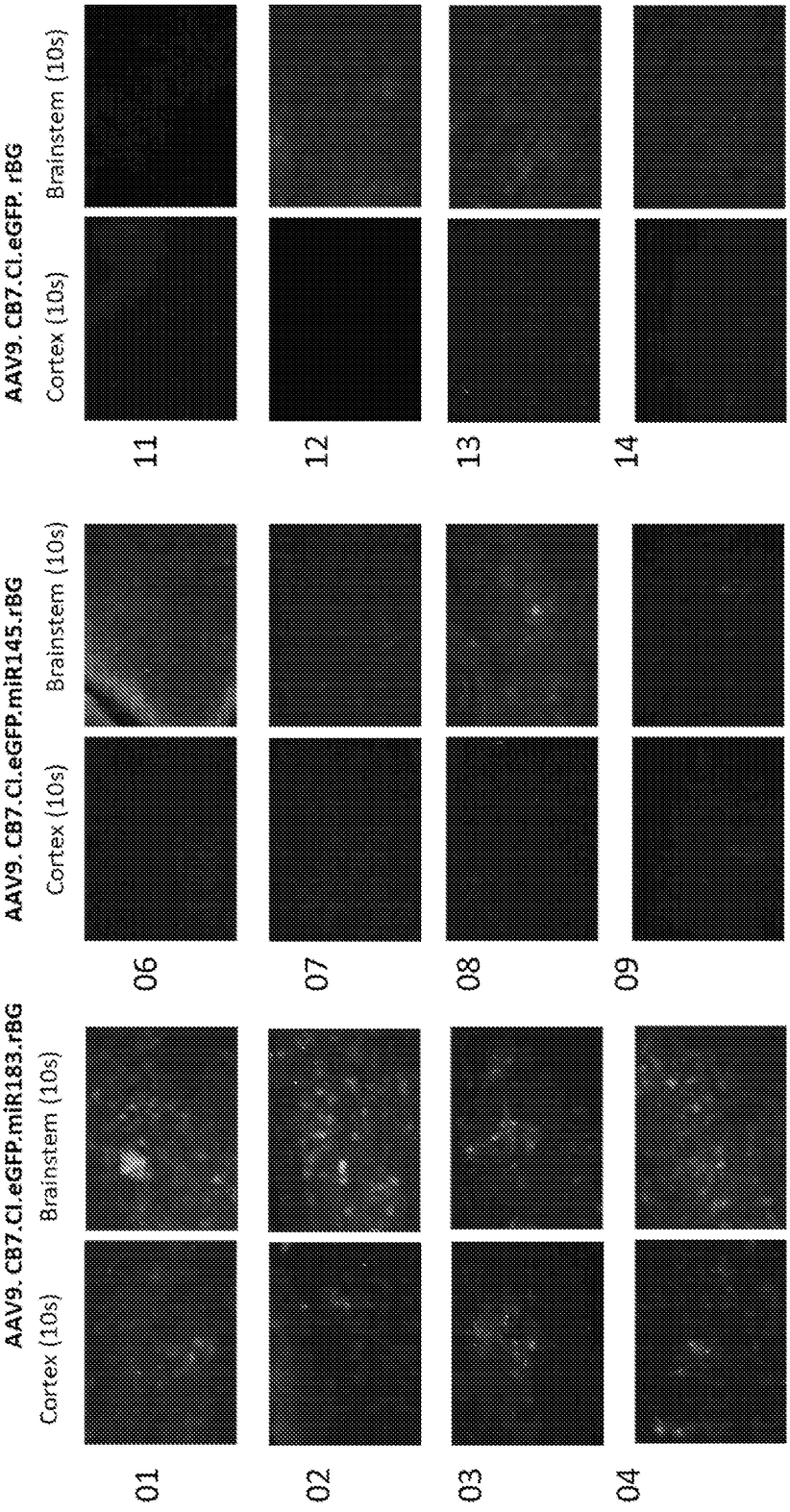

AAV9. CB7.CI.eGFP.miR183.rBG
Cortex (10s)
Brainstem (10s)
AAV9. CB7.CI.eGFP.miR145.rBG
Cortex (10s)
Brainstem (10s)
AAV9. CB7.CI.eGFP. rBG
Cortex (10s)
Brainstem (10s)
01
02
03
04
06
07
08
09
11
12
13
14

FIG. 25C

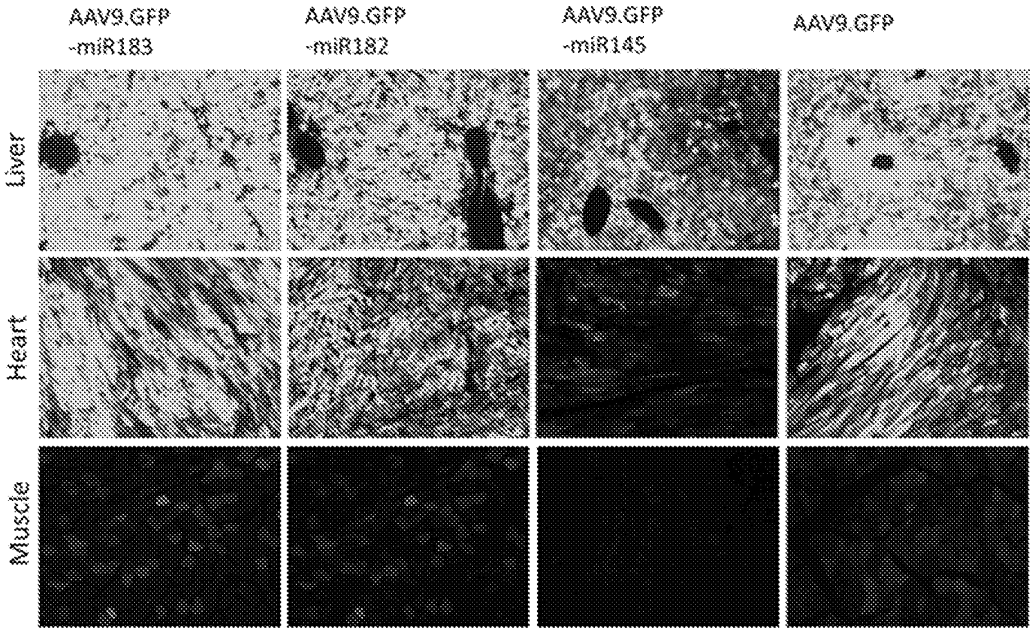



FIG. 25D

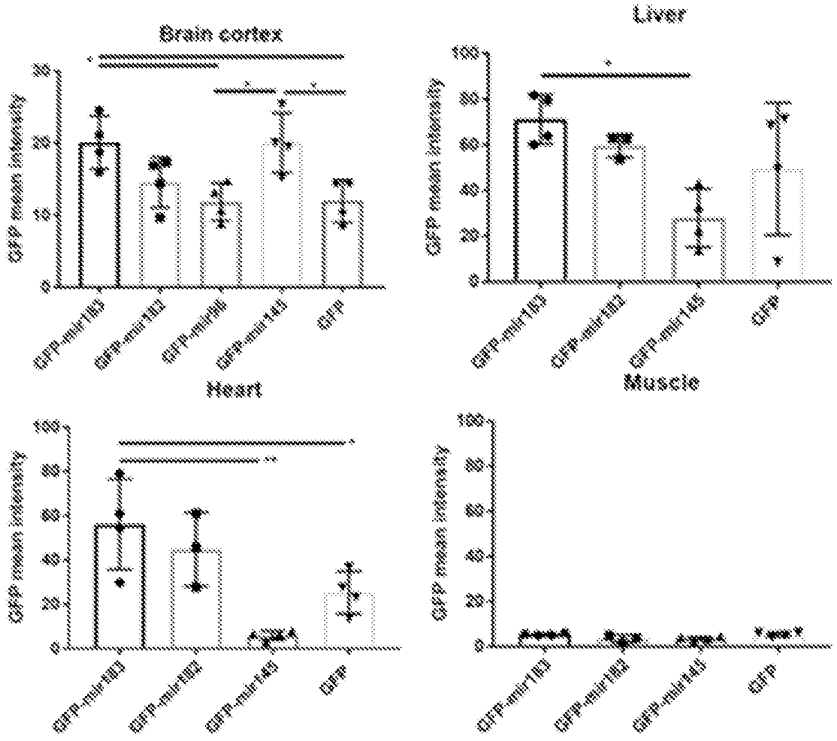


FIG. 26A

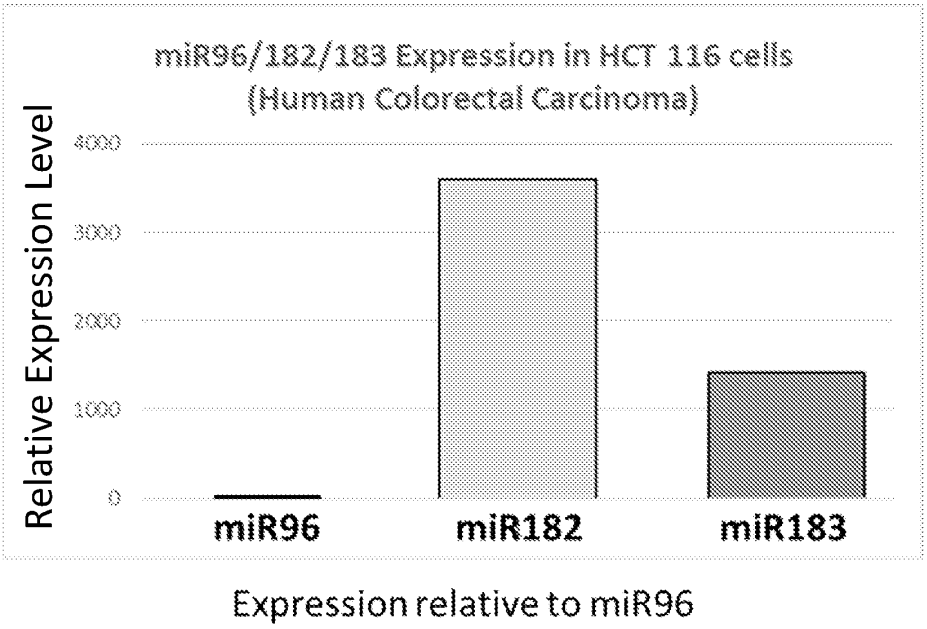



FIG. 26B

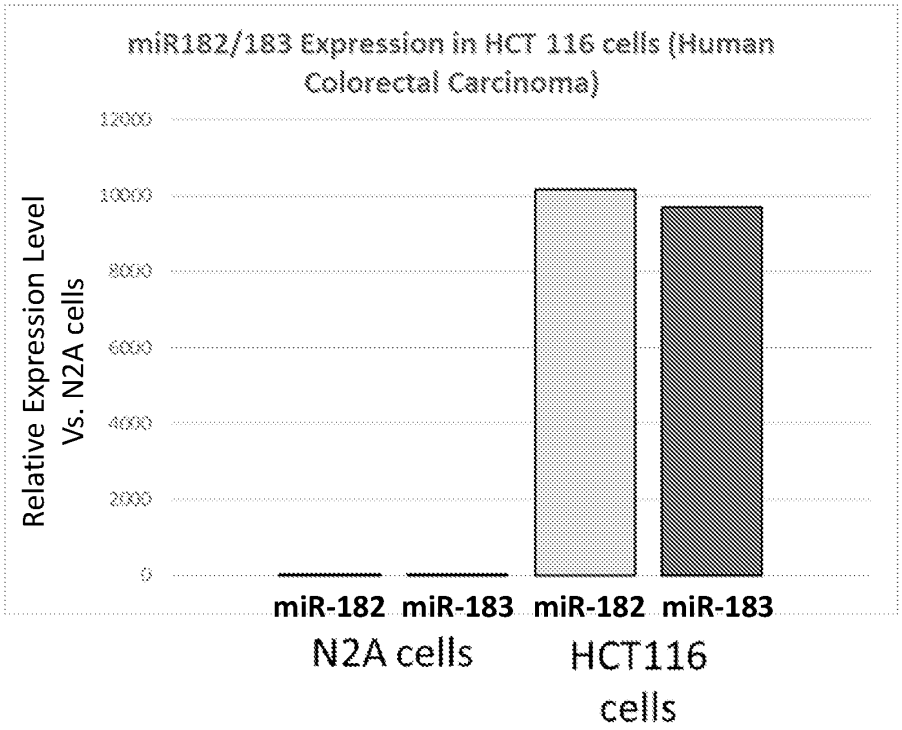


FIG. 26C

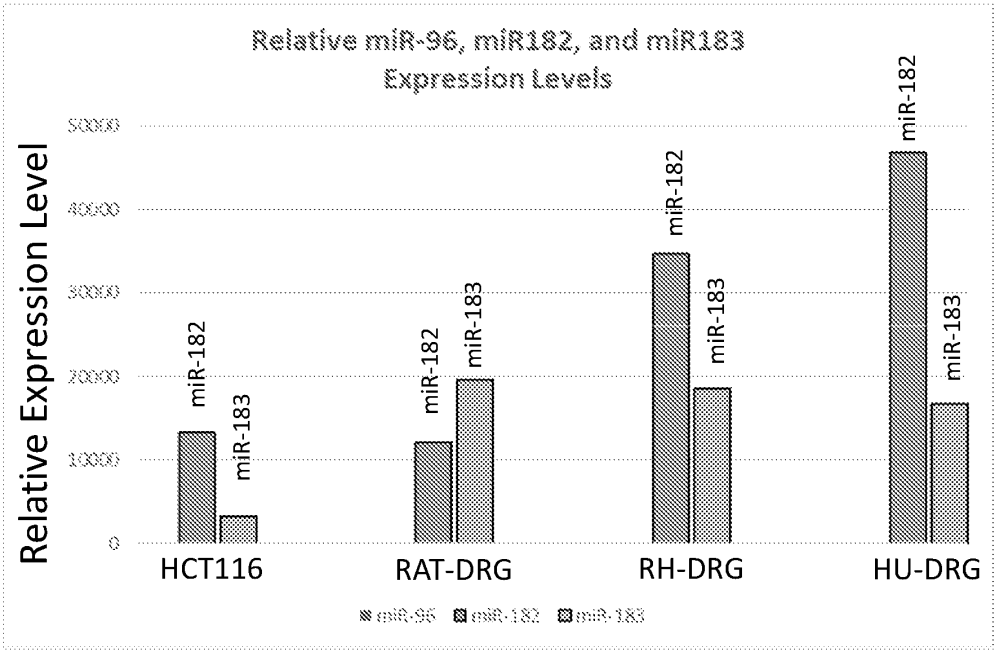

Relative miR-96, miR182, and miR183 Expression Levels
Relative Expression Level
50000
40000
30000
20000
10000
0
miR-182
miR-183
miR-182
miR-183
miR-182
miR-183
miR-182
miR-183
HCT116
RAT-DRG
RH-DRG
HU-DRG
miR-96
miR-182
miR-183

FIG. 27A

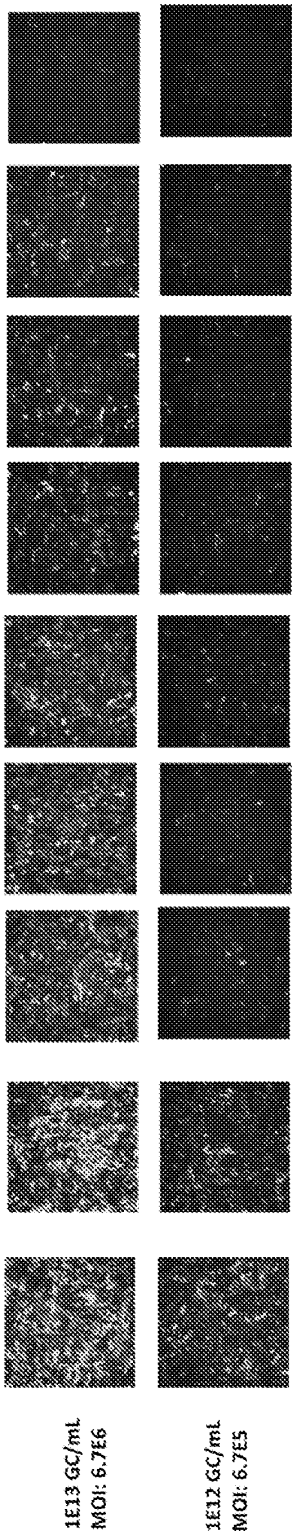



FIG. 27B

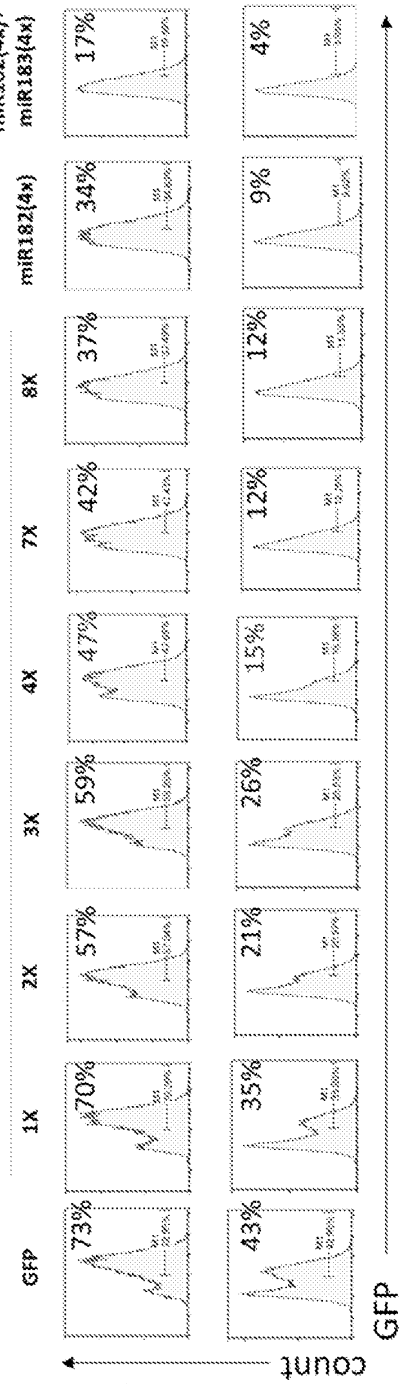


FIG. 27C

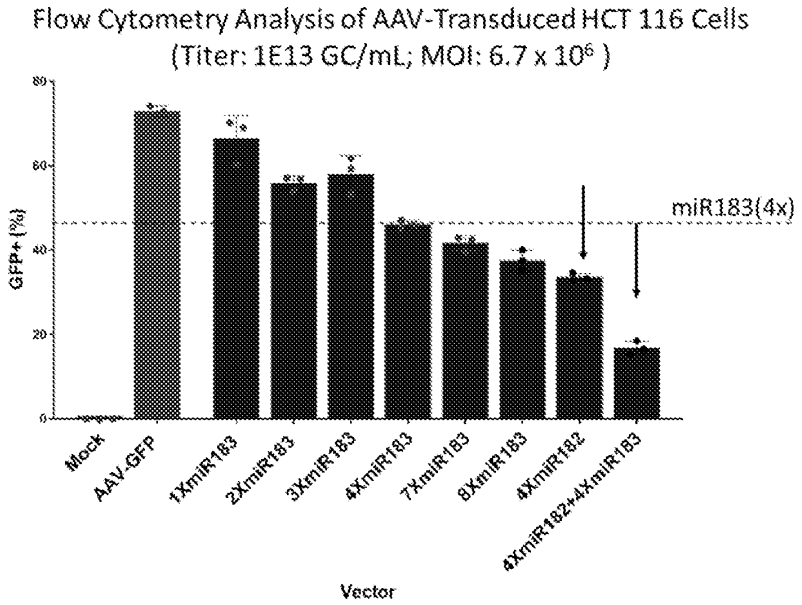



FIG. 27D

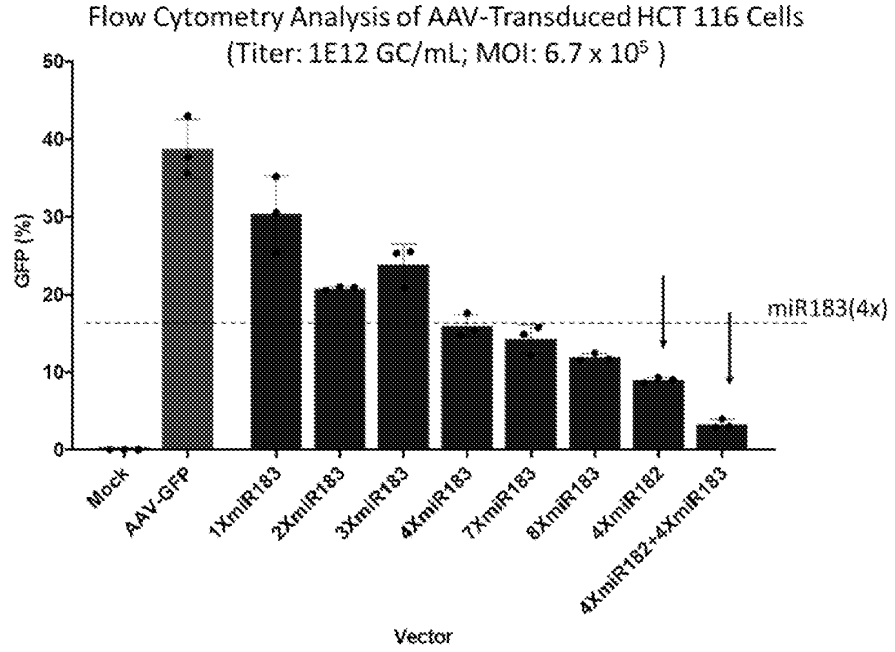


FIG. 28A

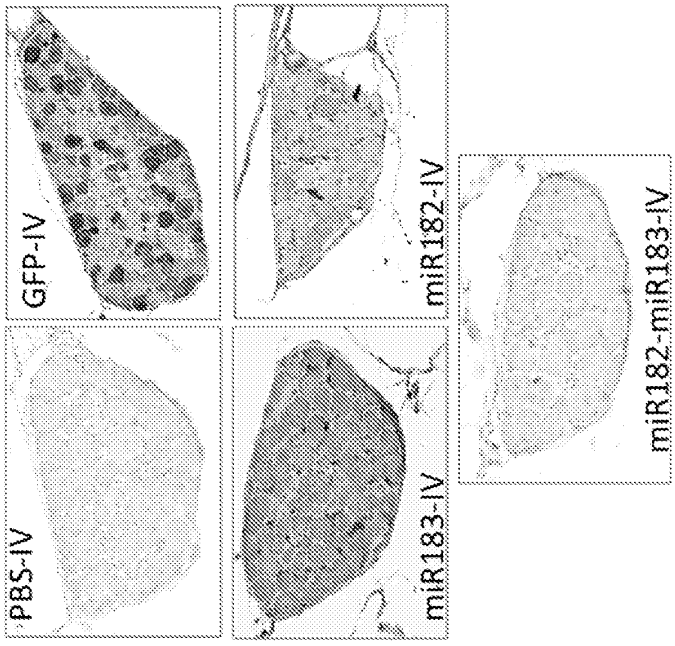



FIG. 28B

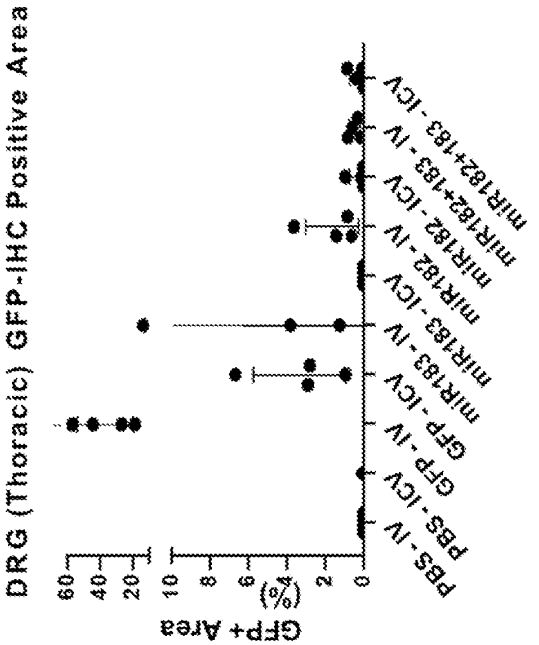
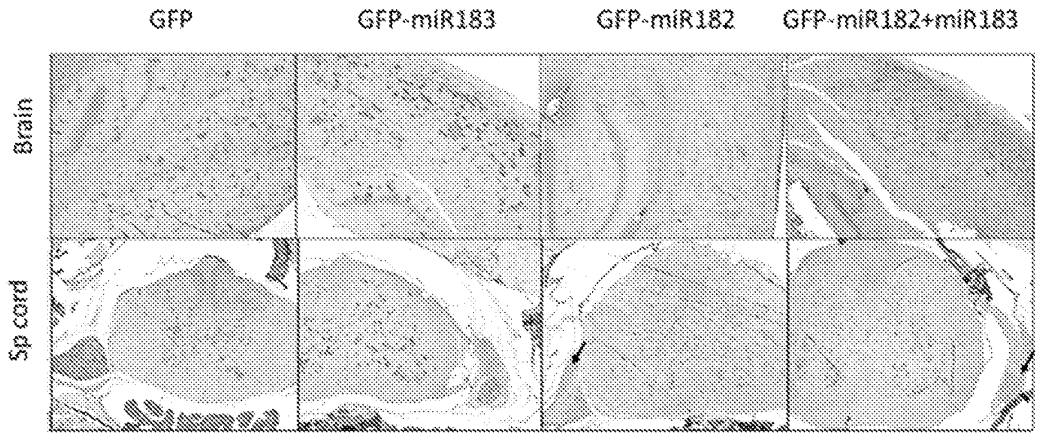


FIG. 28C



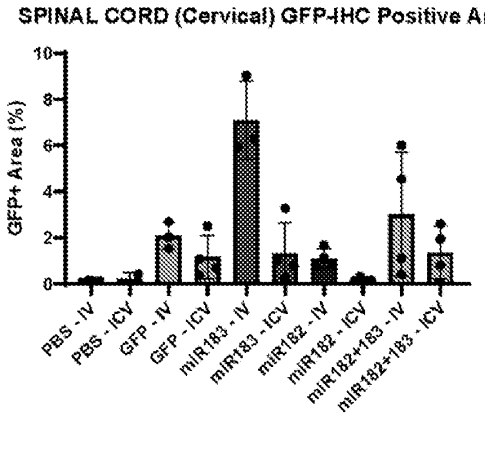




FIG. 28D

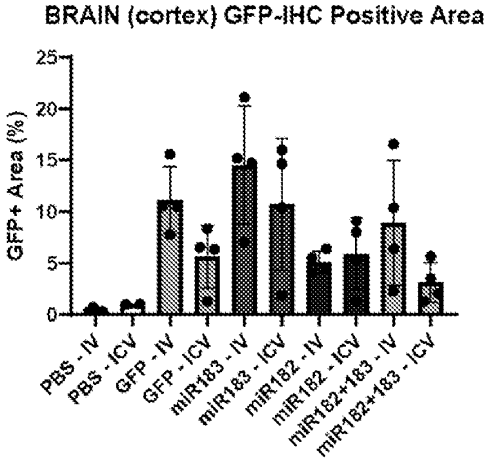



FIG. 28E

FIG. 28F

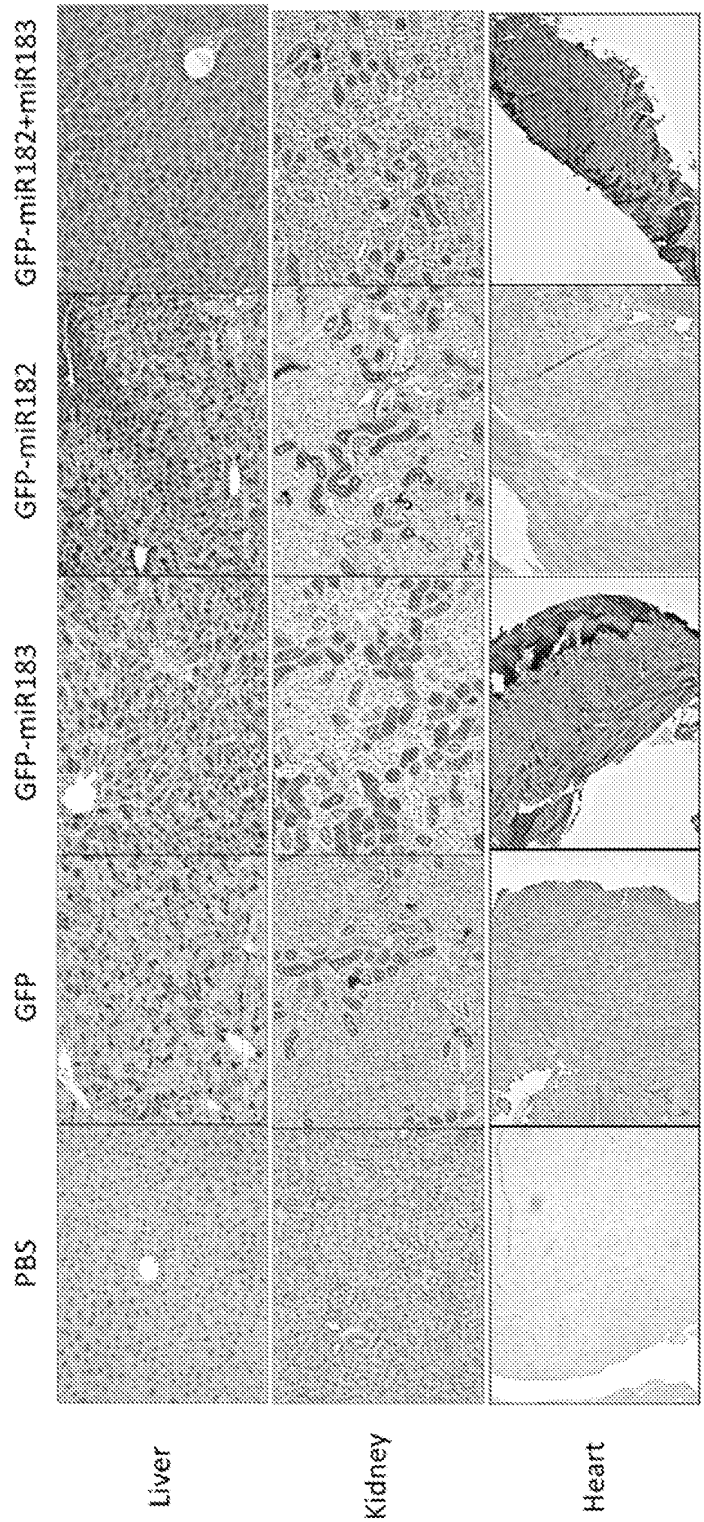

PBS
GFP
GFP-miR183
GFP-miR182
GFP-miR182+miR183
Liver
Kidney
Heart

FIG. 28G

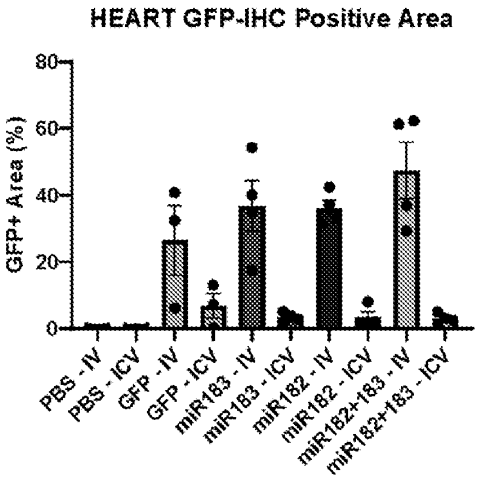
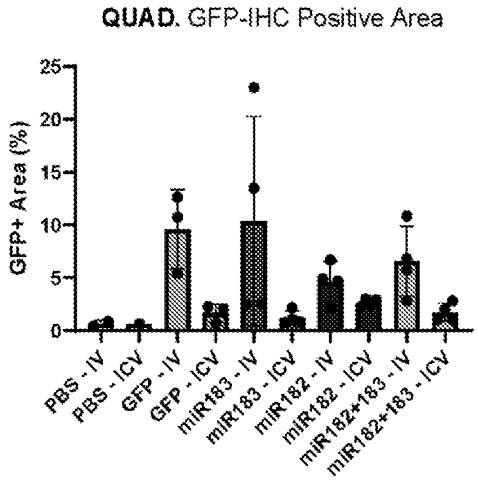



FIG. 29H



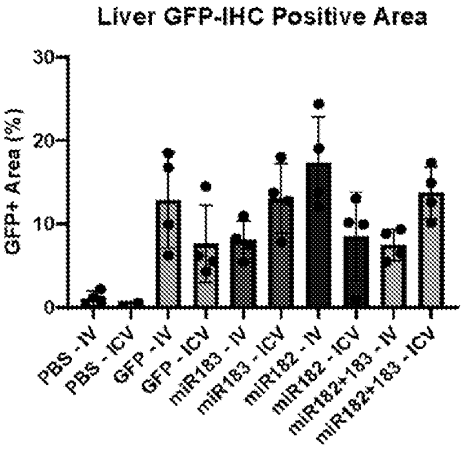




FIG. 28I

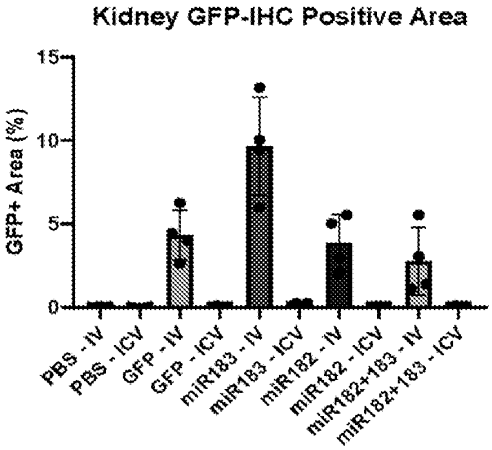



FIG. 28J

FIG. 29A

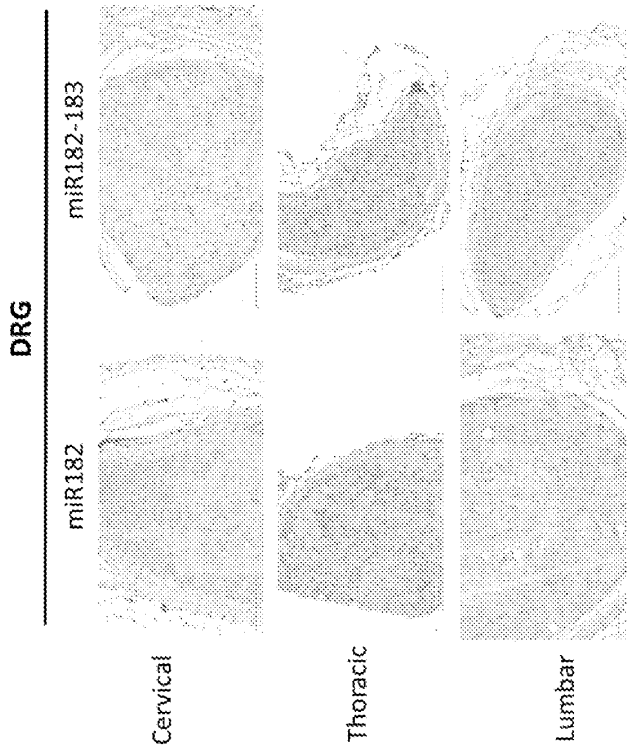



FIG. 29B

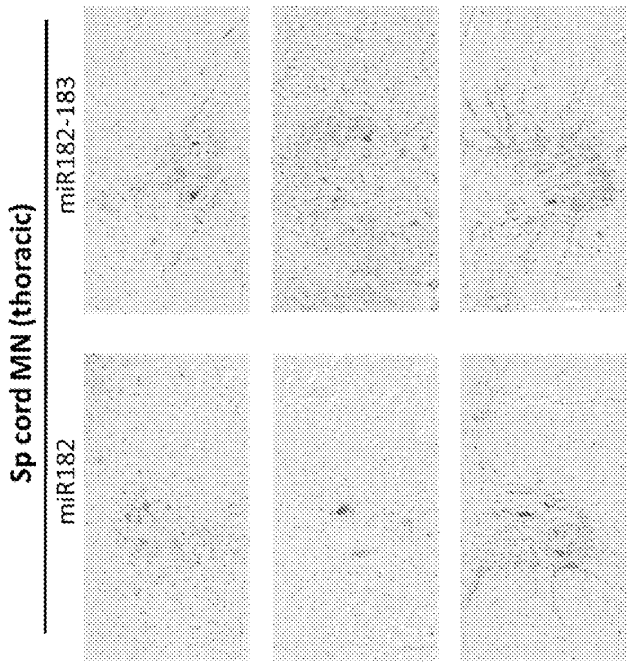
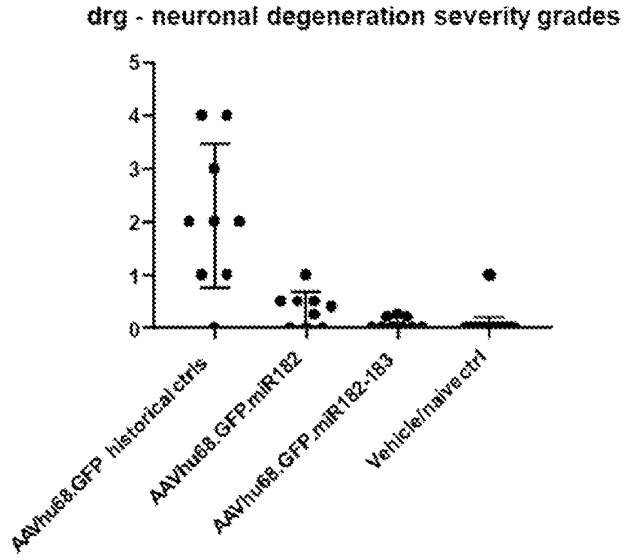


FIG. 29C



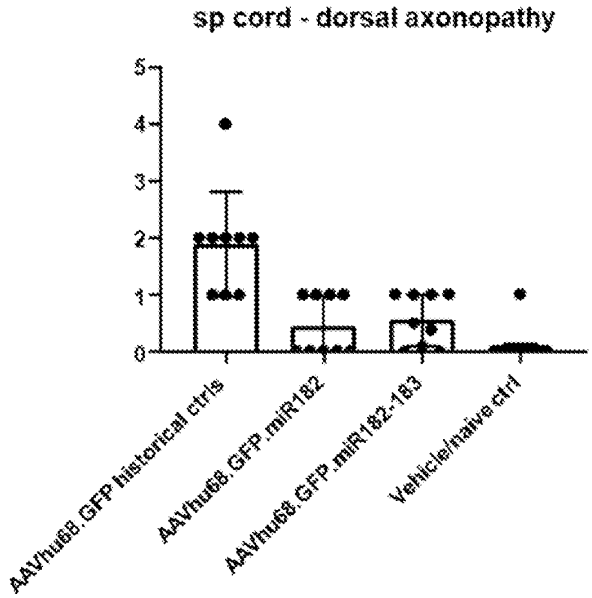




FIG. 29D

FIG. 30A

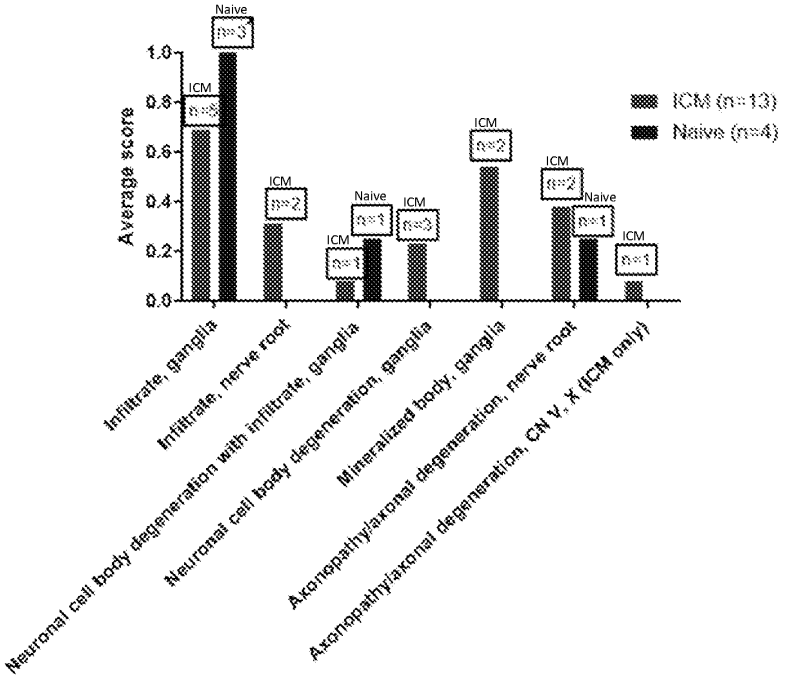



FIG. 30B

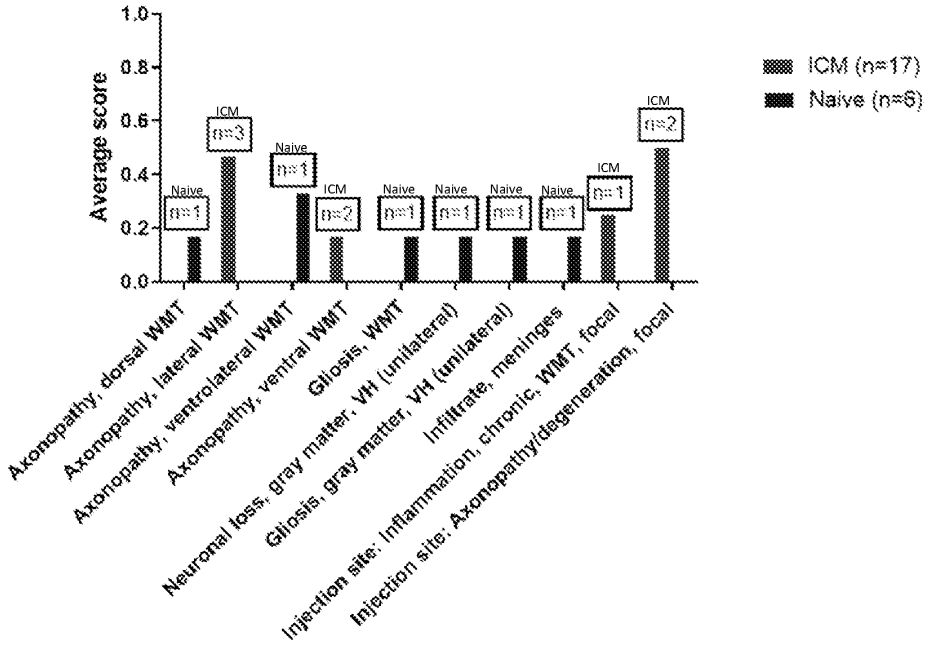


FIG. 30C

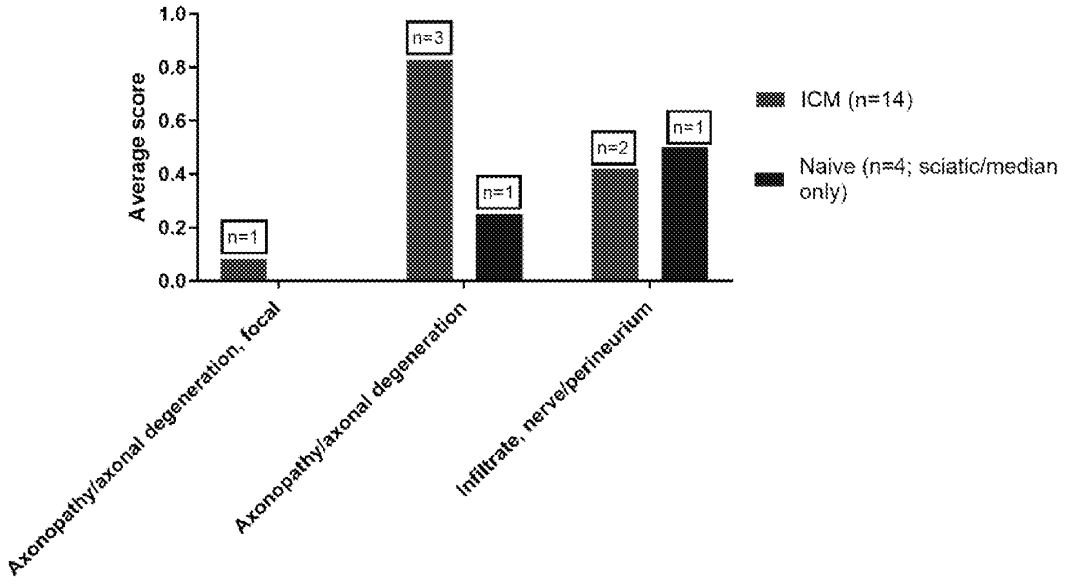
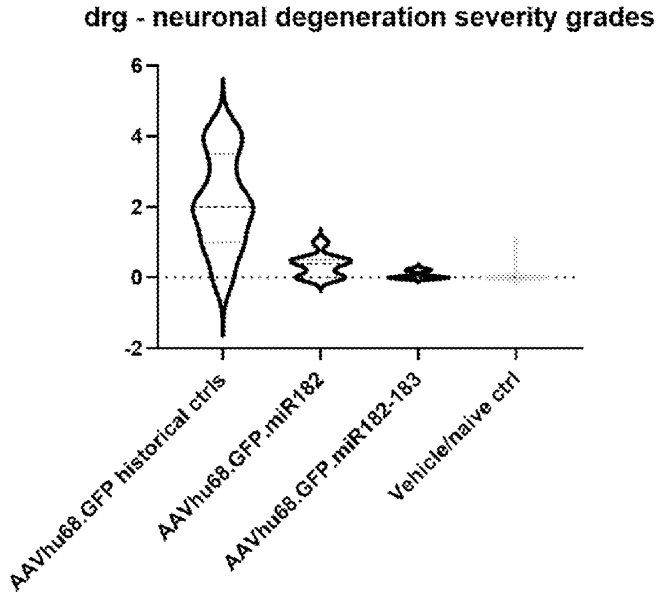

Average score
1.0
0.8
0.6
0.4
0.2
0.0
n=1
n=3
n=1
n=2
n=1
ICM (n=14)
Naive (n=4; sciatic/median only)
Axonopathy/axonal degeneration, focal
Axonopathy/axonal degeneration
Infiltrate, nerve/perineurium

FIG. 31A



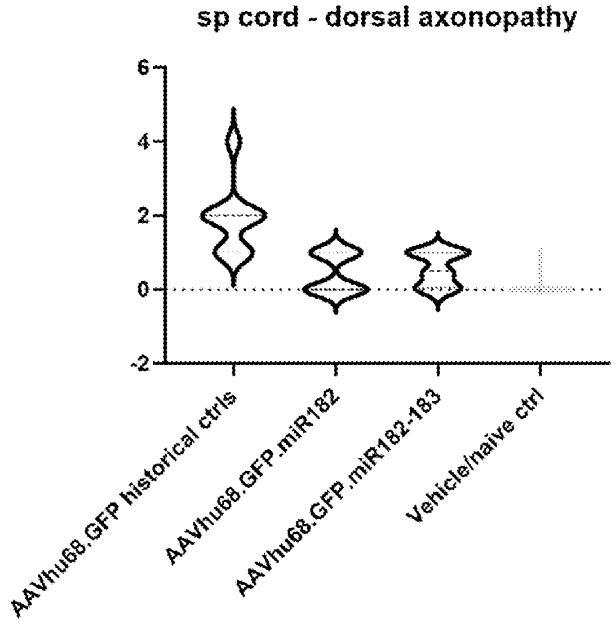




FIG. 31B