FIG. 1A

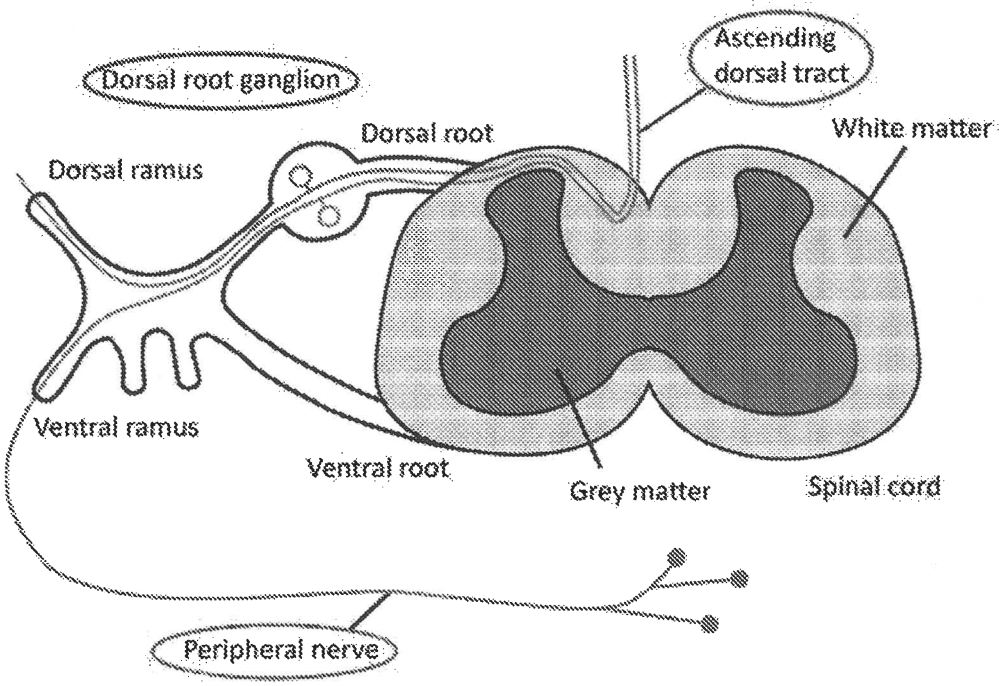

Ascending dorsal tract
Dorsal root ganglion
Dorsal root
White matter
Dorsal ramus
Ventral ramus
Ventral root
Grey matter
Spinal cord
Peripheral nerve

FIG. 1B

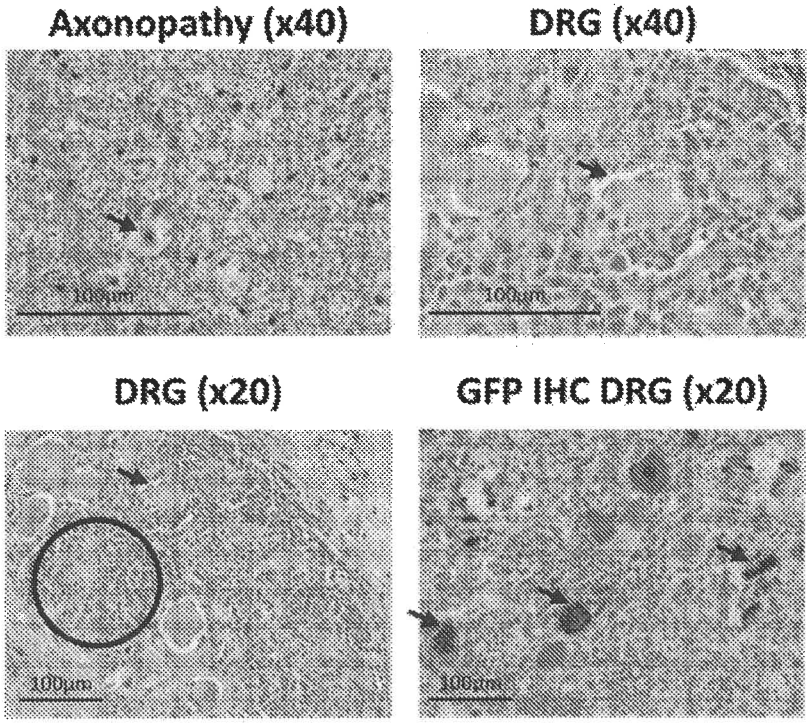

Axonopathy (x40)
DRG (x40)
DRG (x20)
GFP IHC DRG (x20)
100μm
100μm
100μm
100μm

FIG. 1C

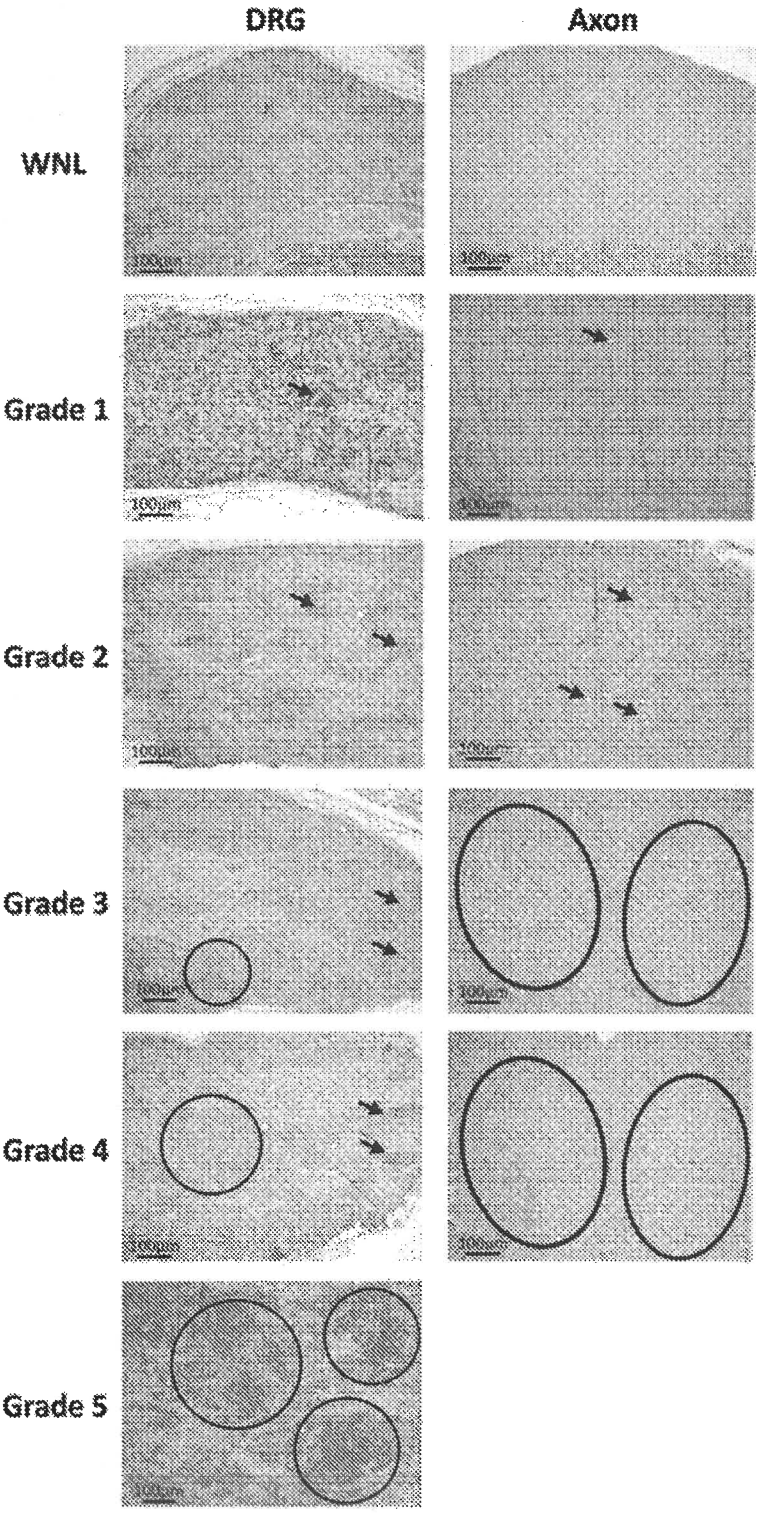


FIG. 2A

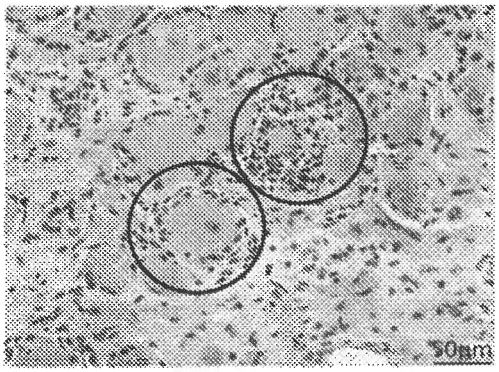


FIG. 2B

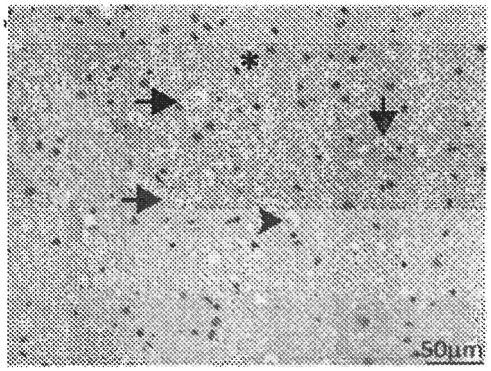


FIG. 2C

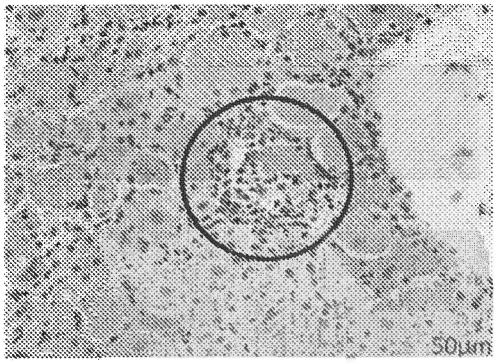


FIG. 2D

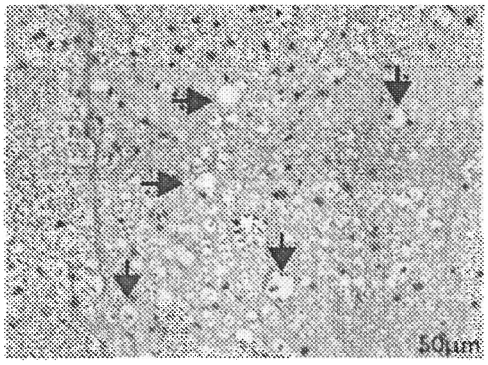


FIG. 2E

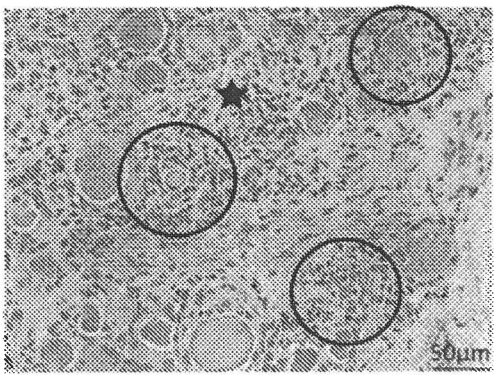


FIG. 2F

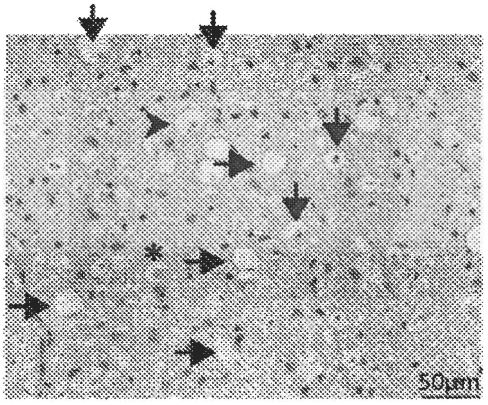


FIG. 3A

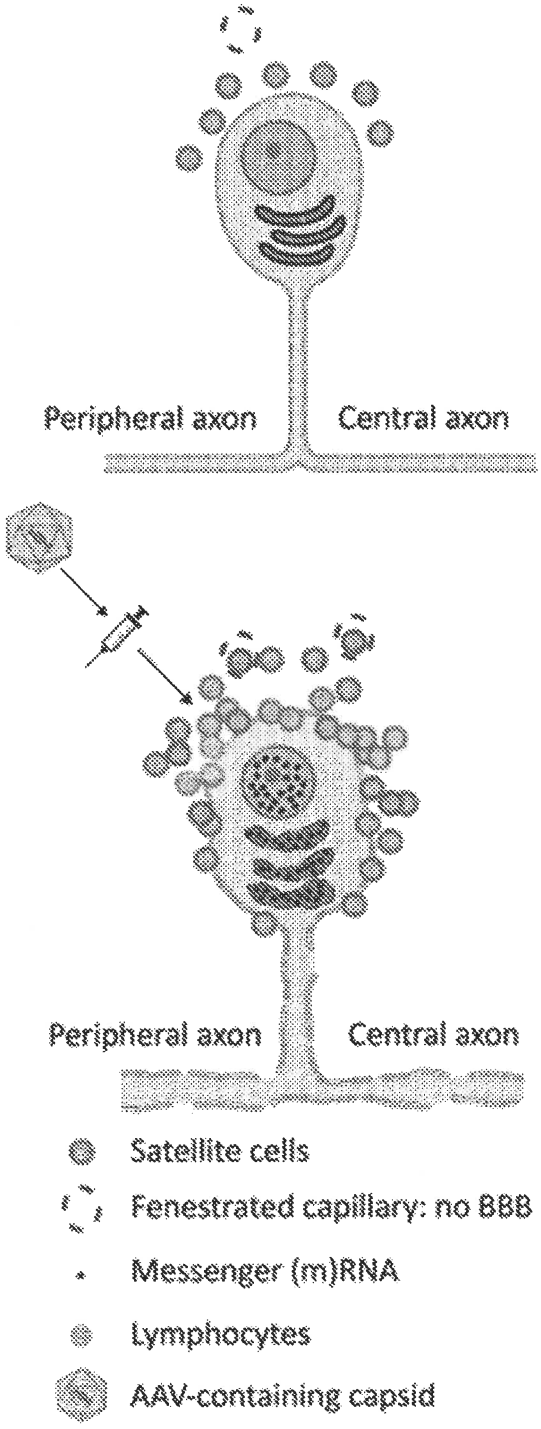

Peripheral axon
Central axon
Peripheral axon
Central axon
Satellite cells
Fenestrated capillary: no BBB
Messenger (m)RNA
Lymphocytes
AAV-containing capsid

FIG. 3B

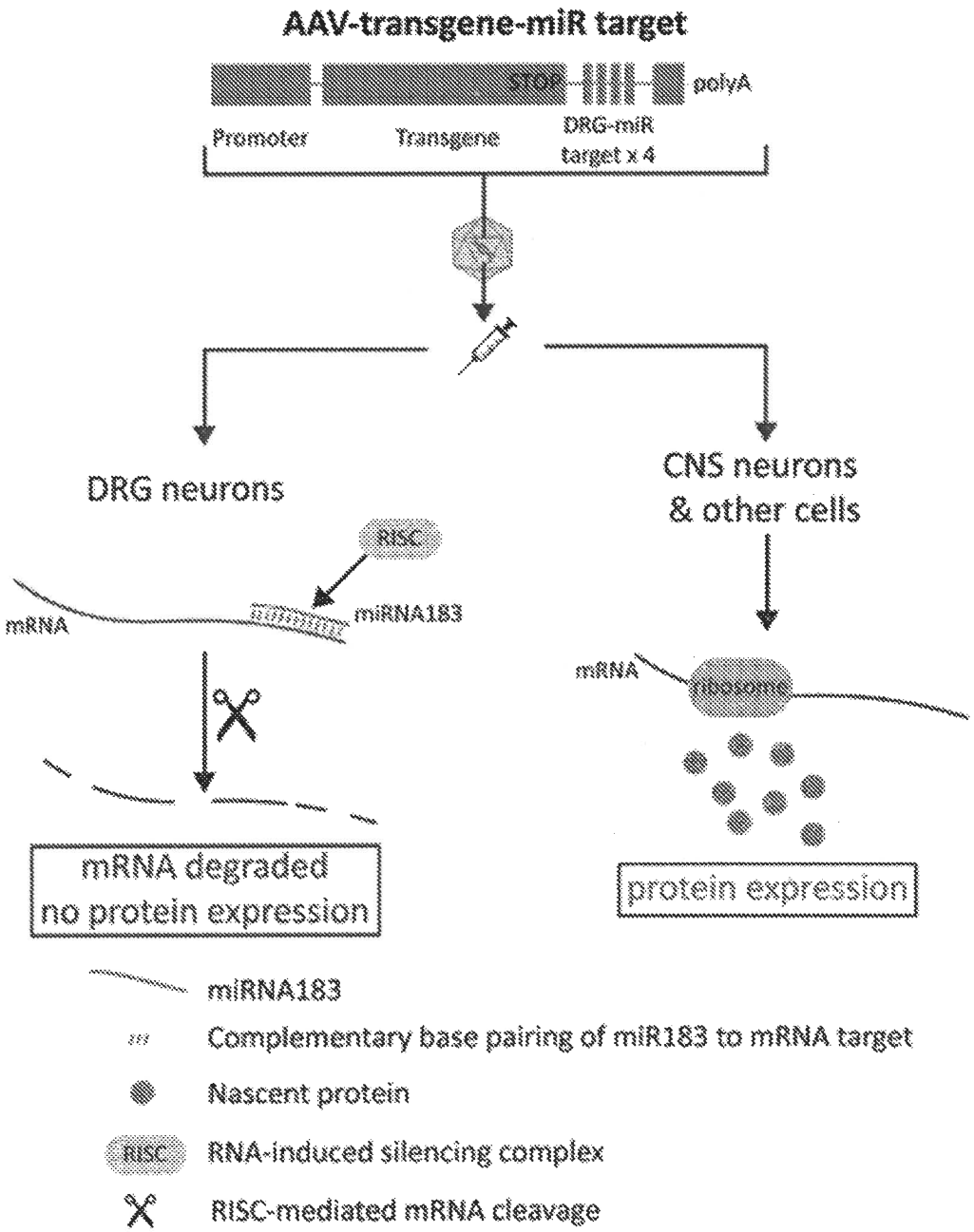

AAV-transgene-miR target
STOP
polyA
Promoter
Transgene
DRG-miR target x 4
DRG neurons
CNS neurons & other cells
RISC
mRNA
miRNA183
mRNA
ribosome
mRNA degraded no protein expression
protein expression
miRNA183
Complementary base pairing of miR183 to mRNA target
Nascent protein
RISC
RNA-induced silencing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

FIG. 4A

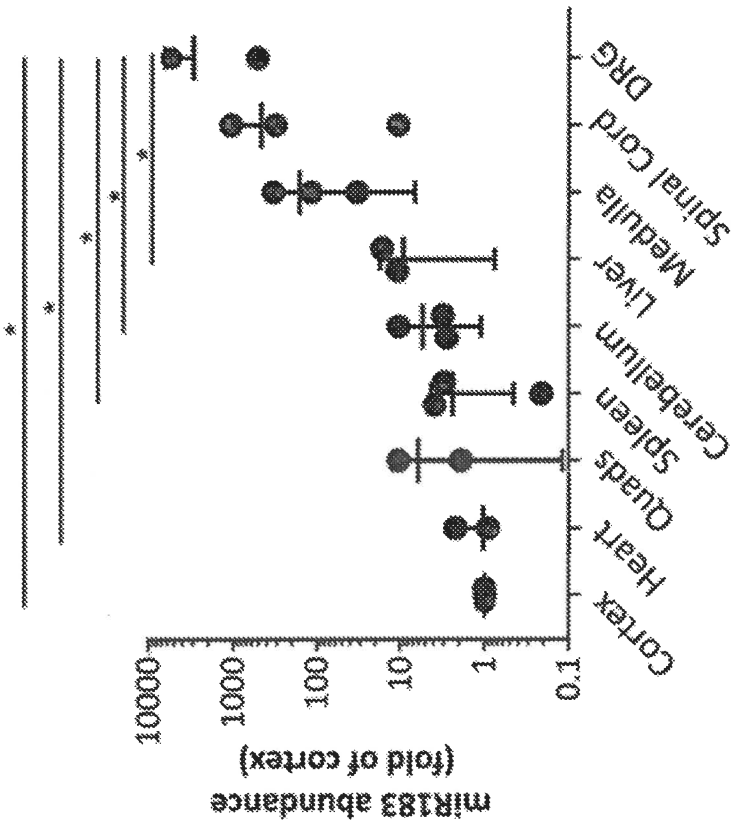

miR183 abundance
(fold of cortex)
10000
1000
100
10
1
0.1
Cortex
Heart
Quads
Spleen
Cerebellum
Liver
Medulla
Spinal Cord
DRG
*
*
*
*
*

FIG. 4B

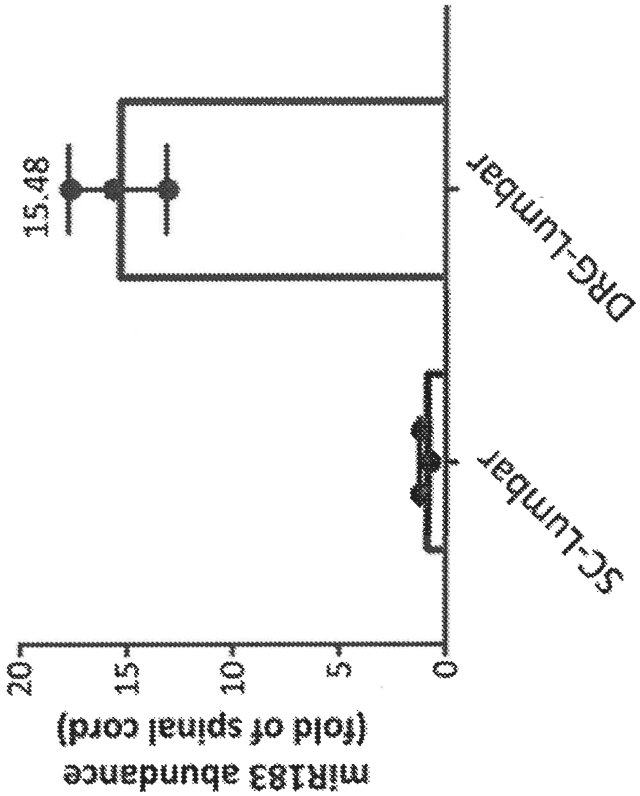

miR183 abundance
(fold of spinal cord)
20
15
10
5
0
15.48
SC-Lumbar
DRG-Lumbar

FIG. 5A

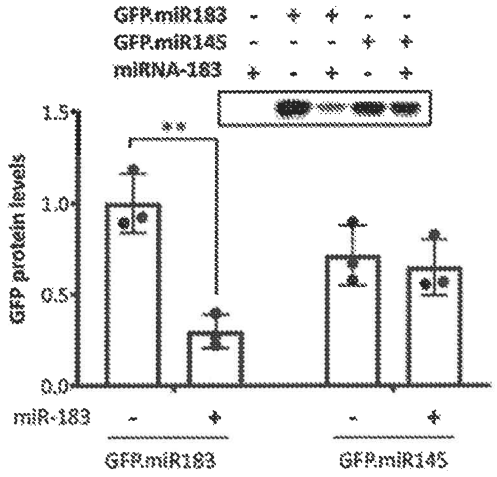


FIG. 5B

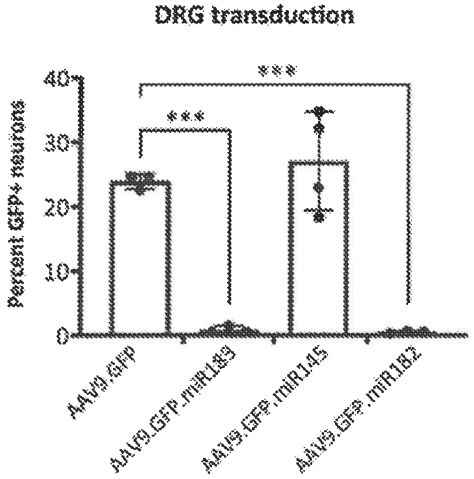


FIG. 5C

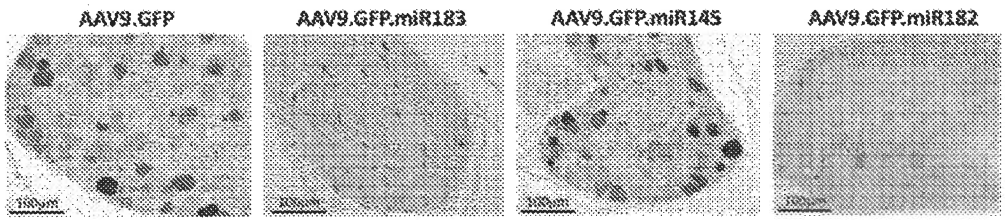


FIG. 5D

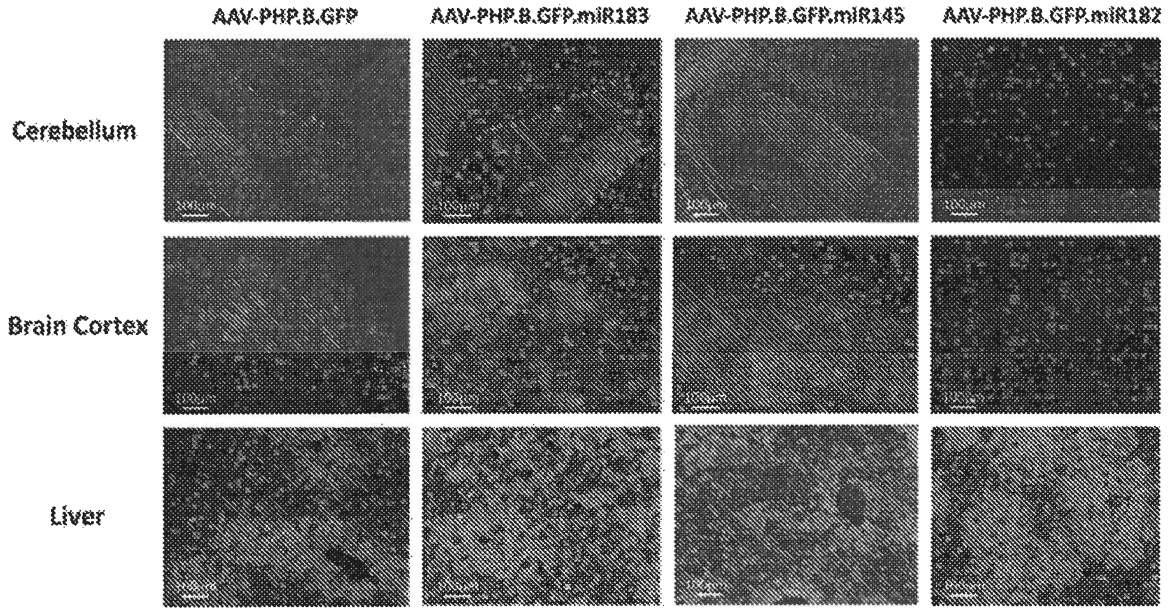


FIG. 6A

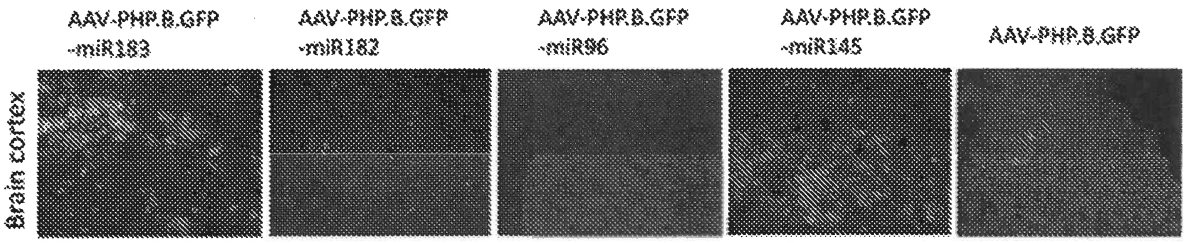

AAV-PHP.B.GFP -miR183
AAV-PHP.B.GFP -miR182
AAV-PHP.B.GFP -miR96
AAV-PHP.B.GFP -miR145
AAV-PHP.B.GFP
Brain cortex

FIG. 6B

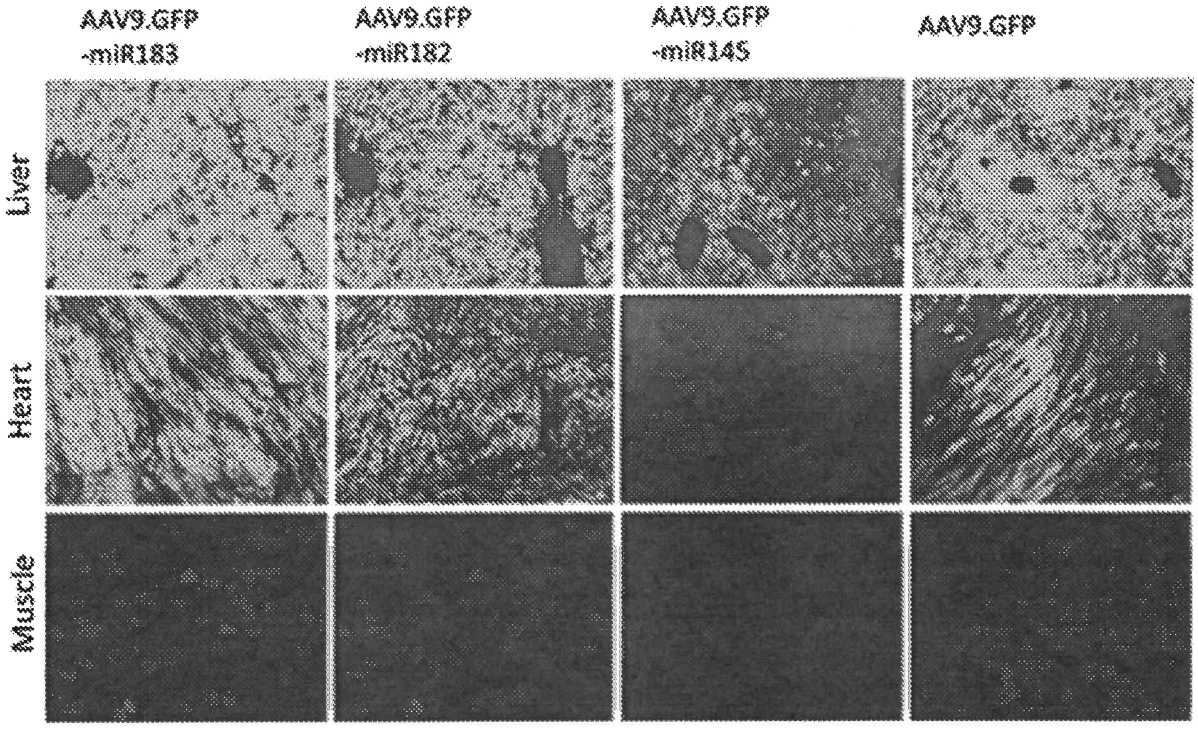

AAV9.GFP -miR183
AAV9.GFP -miR182
AAV9.GFP -miR145
AAV9.GFP
Liver
Heart
Muscle

FIG. 6C

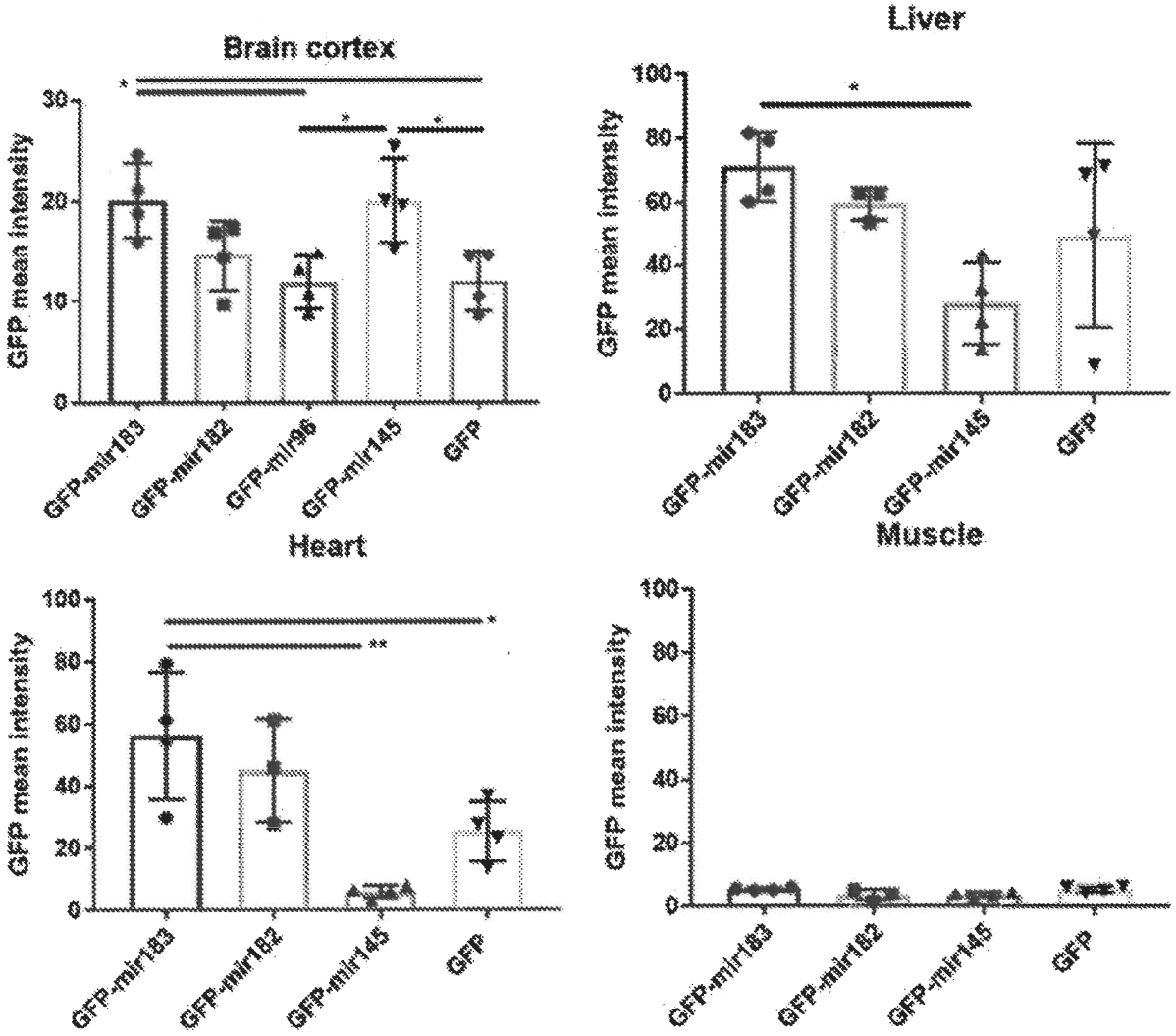

Brain cortex
GFP mean intensity
30
20
10
0
*
*
*
GFP-mir183
GFP-mir182
GFP-mir96
GFP-mir145
GFP
Liver
GFP mean intensity
100
80
60
40
20
0
*
GFP-mir183
GFP-mir182
GFP-mir145
GFP
Heart
GFP mean intensity
100
80
60
40
20
0
**
*
GFP-mir183
GFP-mir182
GFP-mir145
GFP
Muscle
GFP mean intensity
100
80
60
40
20
0
GFP-mir183
GFP-mir182
GFP-mir145
GFP

FIG. 7A

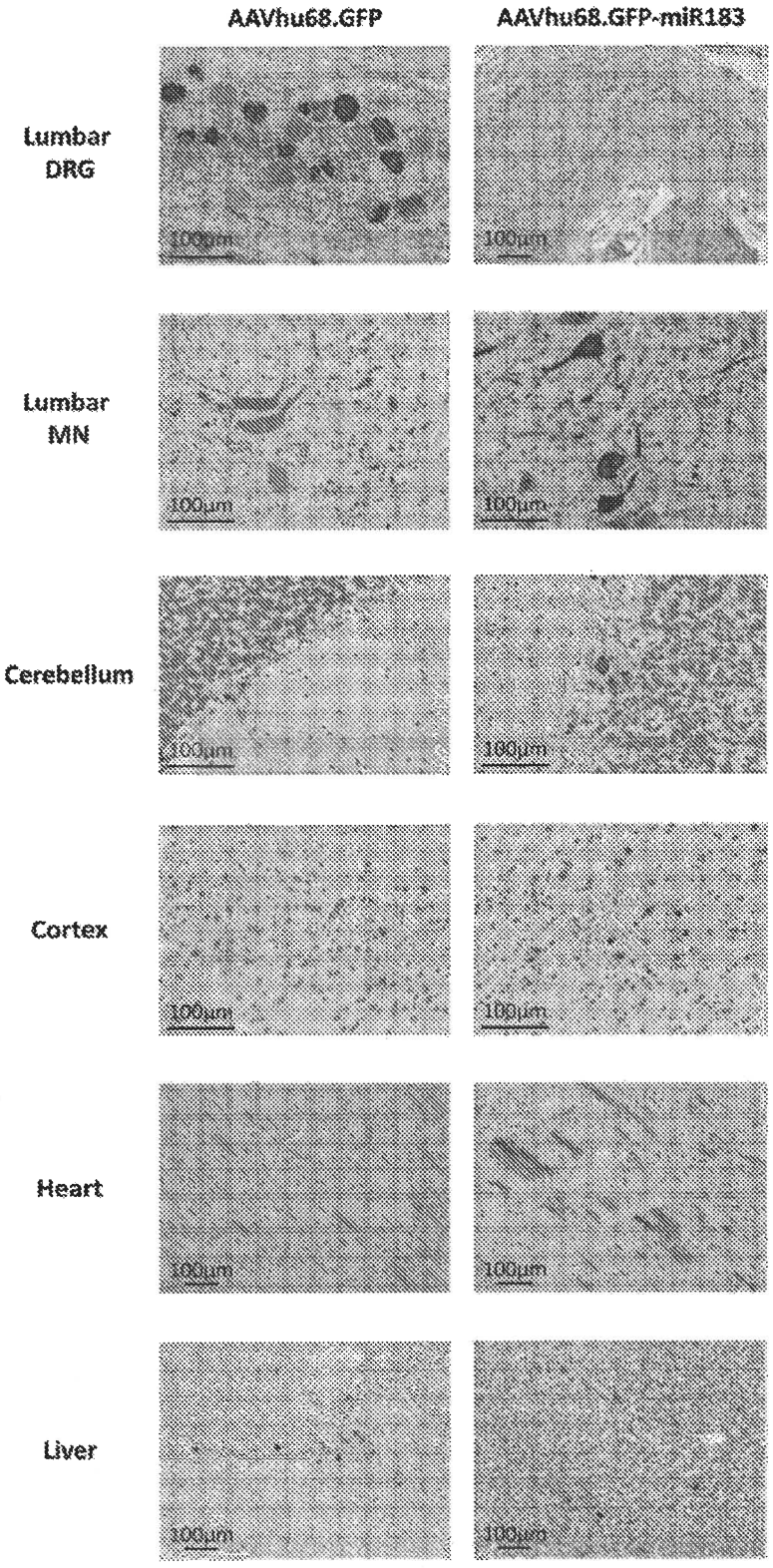

AAVhu68.GFP
AAVhu68.GFP-miR183
Lumbar DRG
Lumbar MN
Cerebellum
Cortex
Heart
Liver
100μm

FIG. 7B

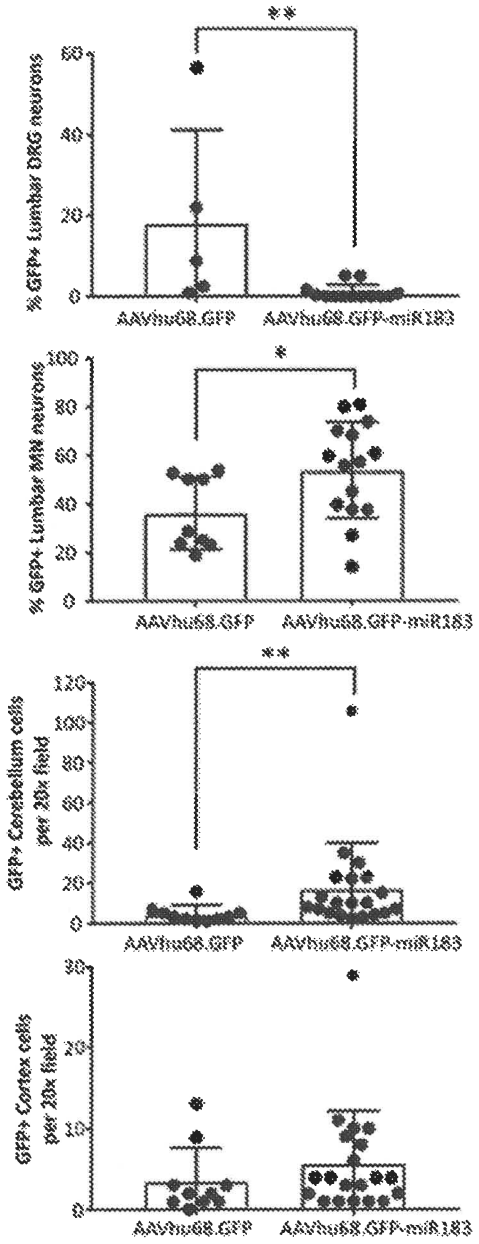

**
% GFP+ Lumbar DRG neurons
AAVhu68.GFP
AAVhu68.GFP-miR183
*
% GFP+ Lumbar MN neurons
AAVhu68.GFP
AAVhu68.GFP-miR183
**
GFP+ Cerebellum cells per 20x field
AAVhu68.GFP
AAVhu68.GFP-miR183
GFP+ Cortex cells per 20x field
AAVhu68.GFP
AAVhu68.GFP-miR183

FIG. 7C

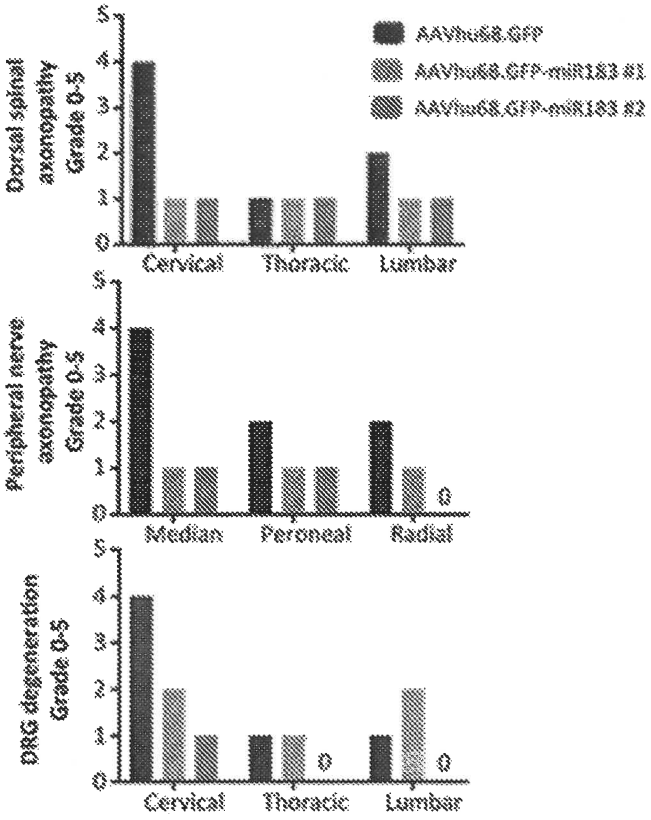

AAVhu68.GFP
AAVhu68.GFP-miR183 #1
AAVhu68.GFP-miR183 #2
Dorsal spinal axonopathy Grade 0-5
Cervical
Thoracic
Lumbar
Peripheral nerve axonopathy Grade 0-5
Median
Peroneal
Radial
DRG degeneration Grade 0-5
Cervical
Thoracic
Lumbar

FIG. 8A

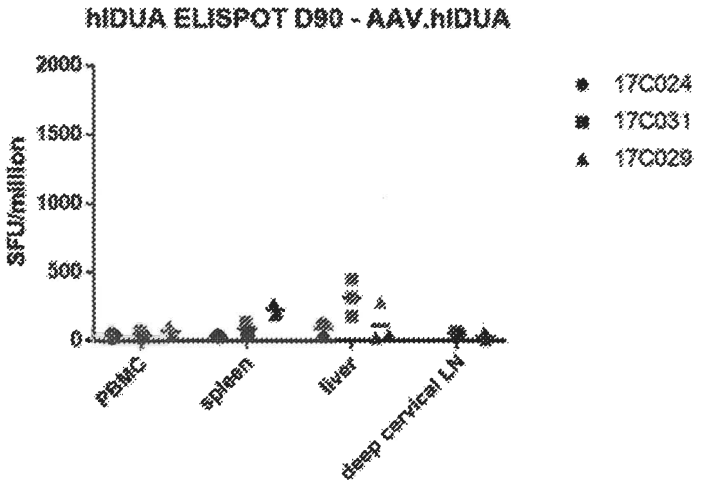


FIG. 8B

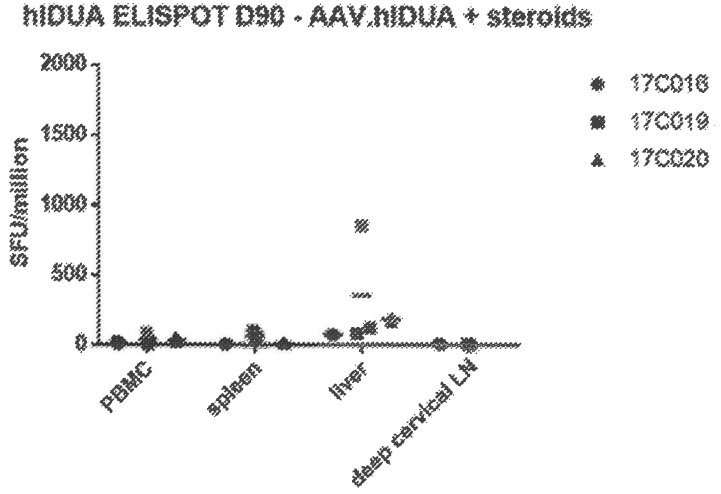


FIG. 8C

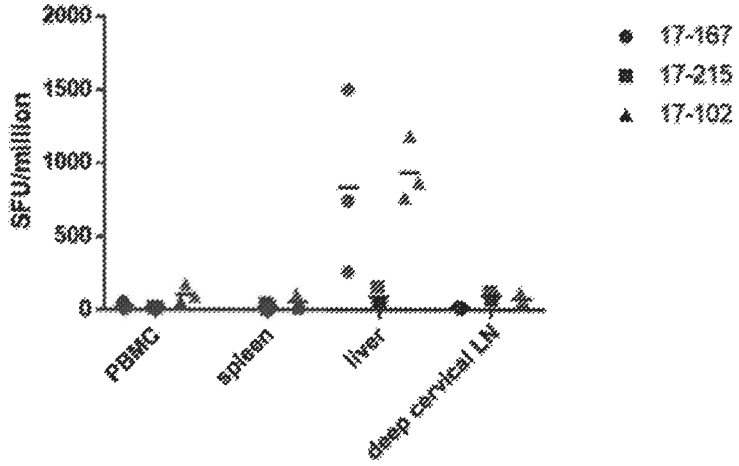


FIG. 8D

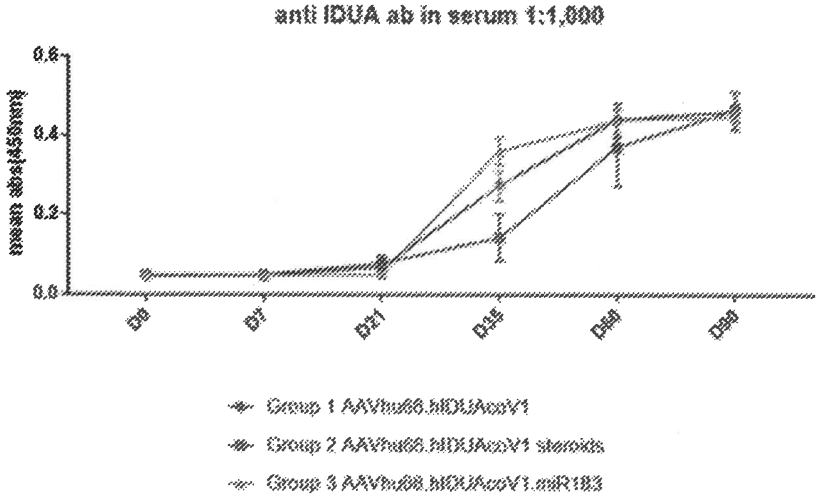


FIG. 9

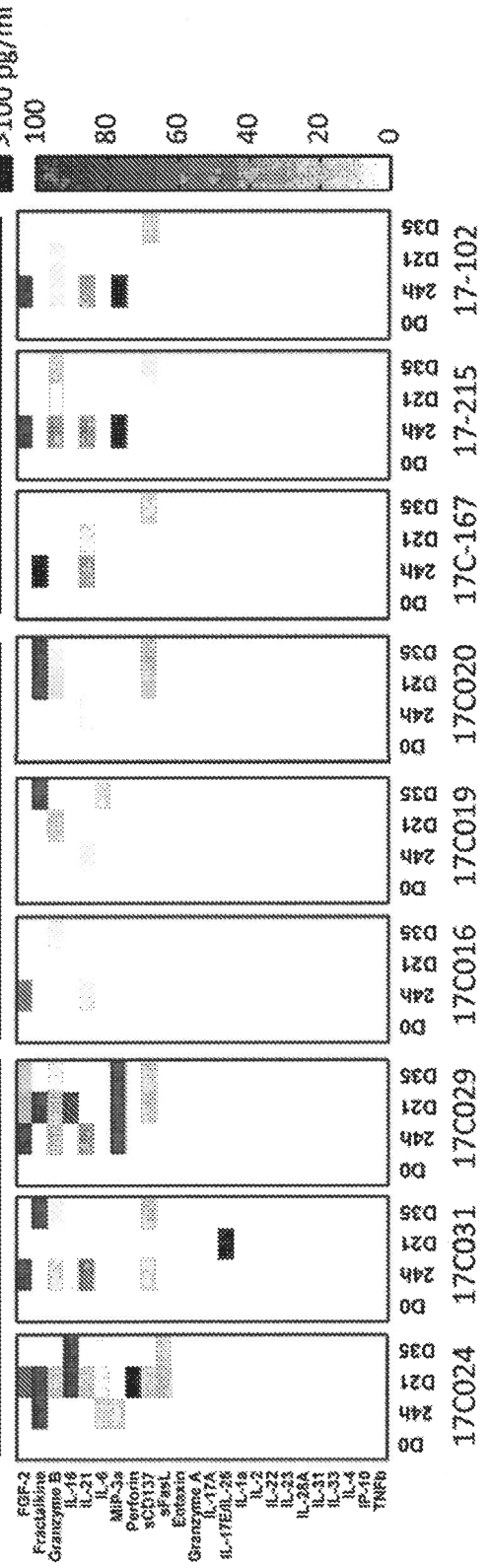

>100 pg/ml
100
80
60
40
20
0
AAV.IDUA
AAV.IDUA + Steroids
AAV.IDUA-miR183
FGF-2
Fractalkine
Granzyme B
IL-1α
IL-21
IL-4
MIP-3a
Perforin
sCD137
sFasL
Eotaxin
Granzyme A
IL-17A
IL-17E/IL-25
IL-1β
IL-12
IL-22
IL-23
IL-28A
IL-31
IL-33
IL-4
IP-10
TNFα
D0
24h
D21
D35
17C024
17C031
17C029
17C016
17C019
17C020
17C-167
17-215
17-102

FIG. 10

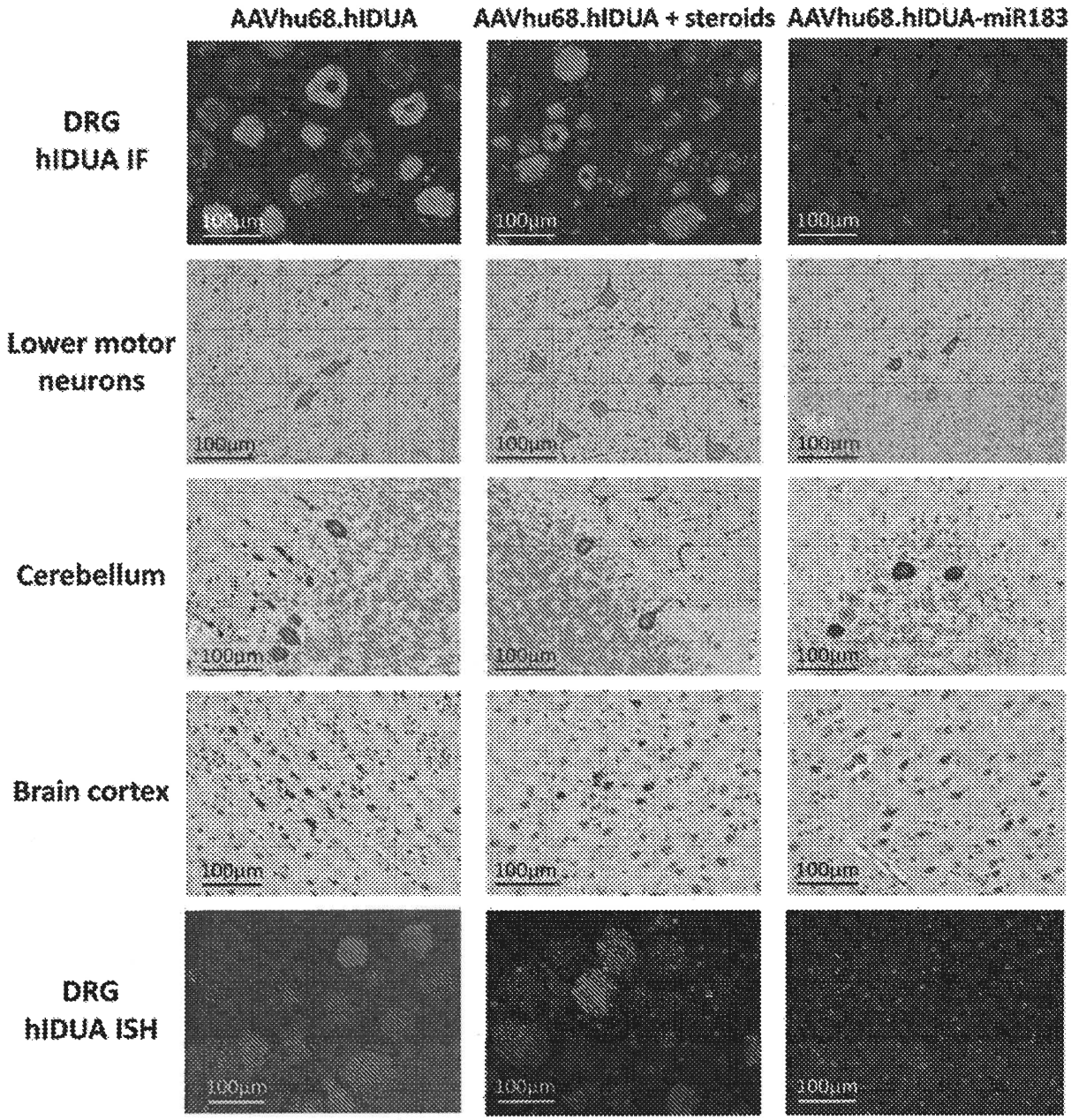

AAVhu68.hIDUA
AAVhu68.hIDUA + steroids
AAVhu68.hIDUA-miR183
DRG hIDUA IF
Lower motor neurons
Cerebellum
Brain cortex
DRG hIDUA ISH
100µm

FIG. 11A

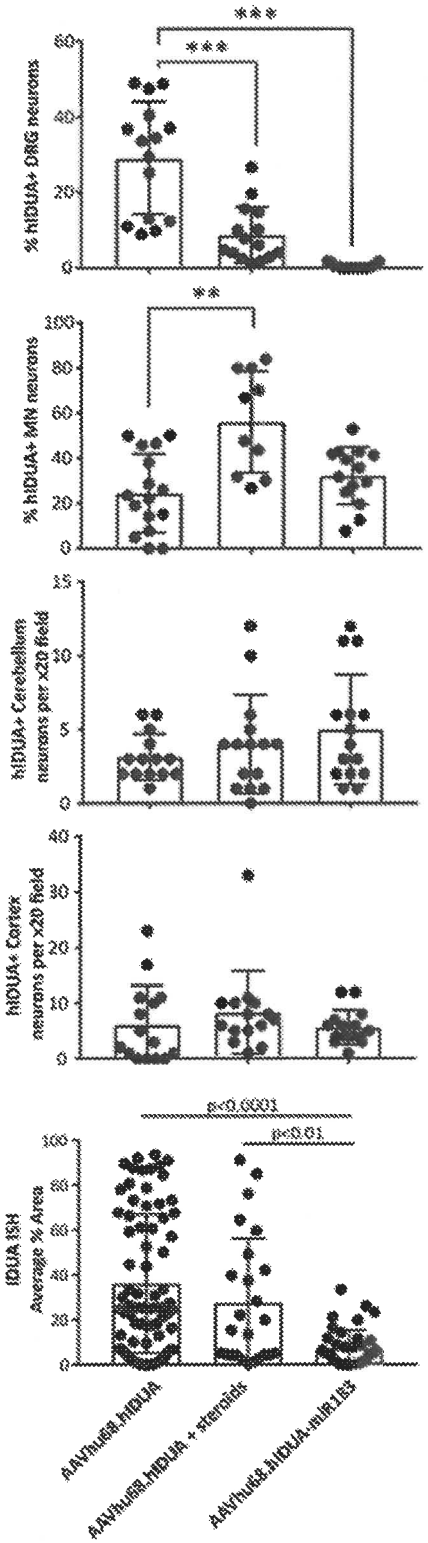
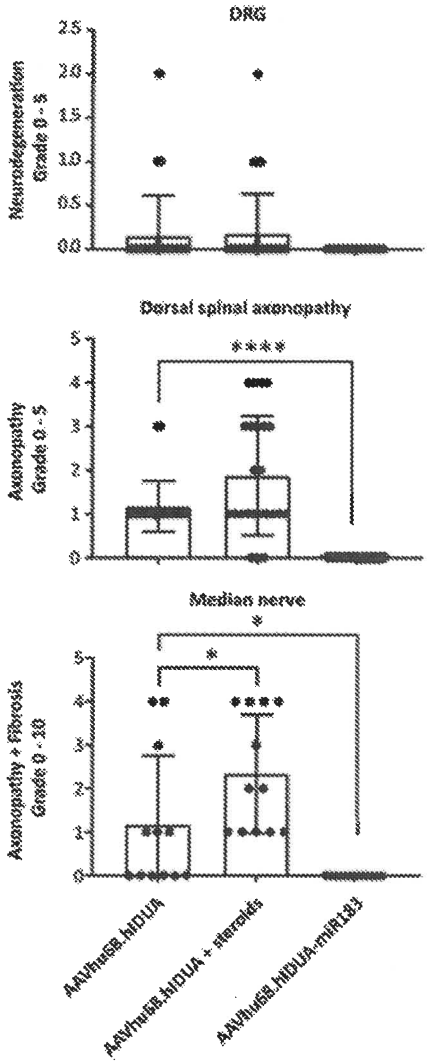
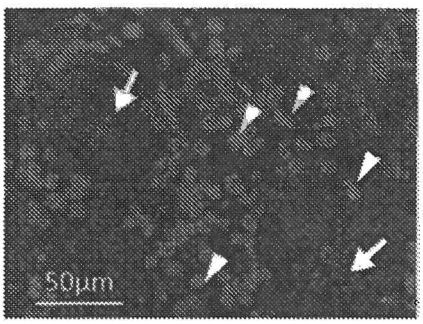


FIG. 11B





AAVhu68.hIDUA-miR183

FIG. 11C

FIG. 12

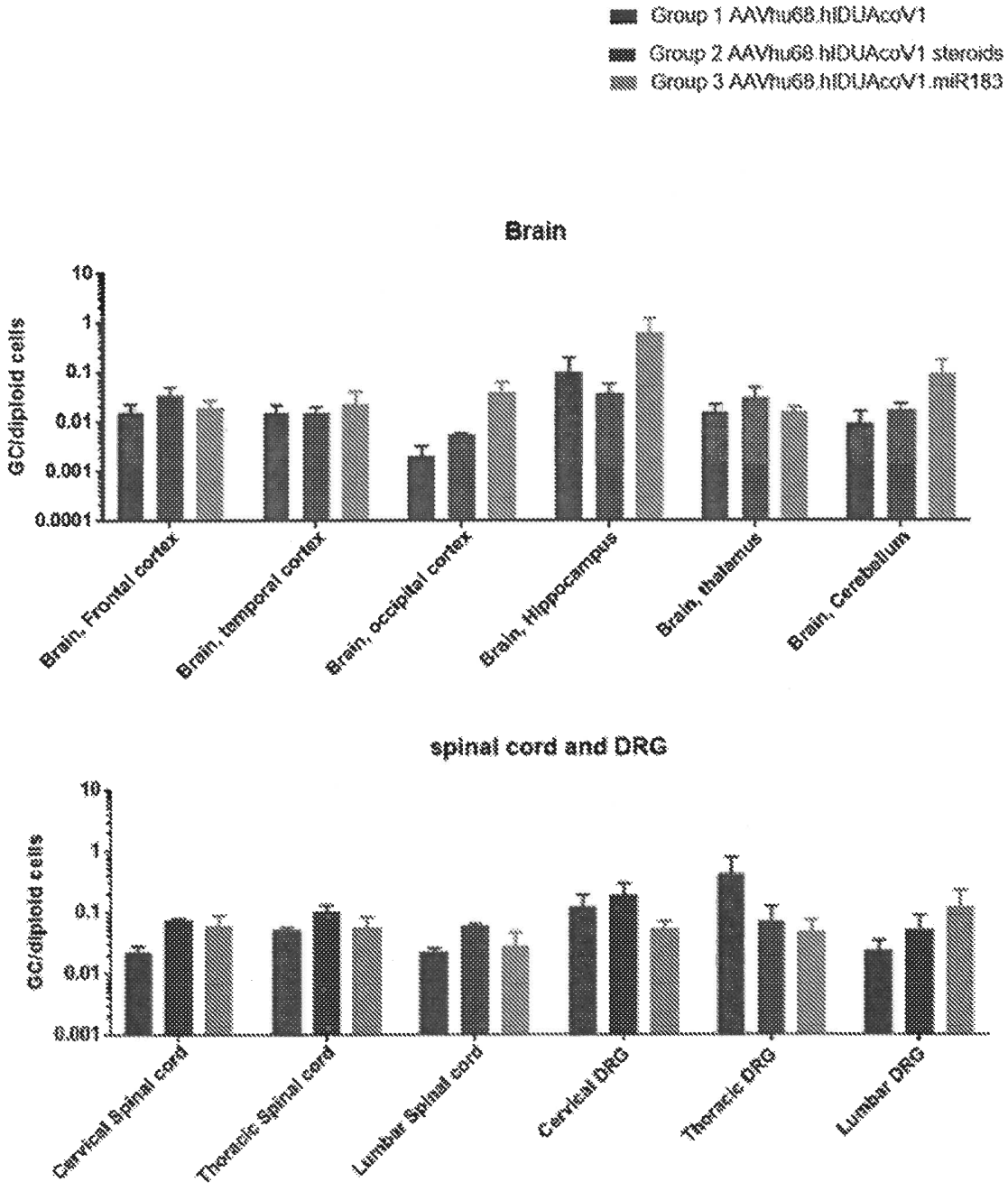

Group 1 AAVhu68.hIDUAcoV1
Group 2 AAVhu68.hIDUAcoV1 steroids
Group 3 AAVhu68.hIDUAcoV1.miR183
Brain
GC/diploid cells
10
1
0.1
0.01
0.001
0.0001
Brain, Frontal cortex
Brain, temporal cortex
Brain, occipital cortex
Brain, Hippocampus
Brain, thalamus
Brain, Cerebellum
spinal cord and DRG
GC/diploid cells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

FIG. 13A

AAV.hu68.eGFP

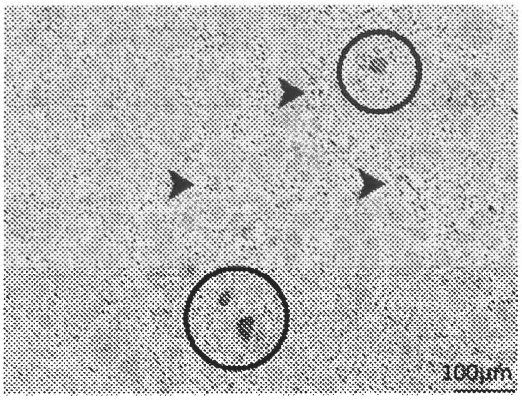


FIG. 13B

AAV.hu68.hIDUA

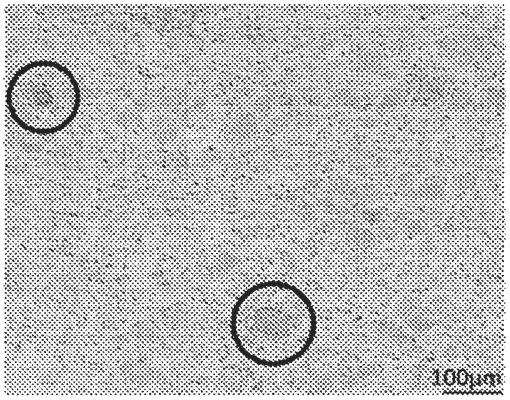


FIG. 13C

AAVhu68.eGFP.miR183

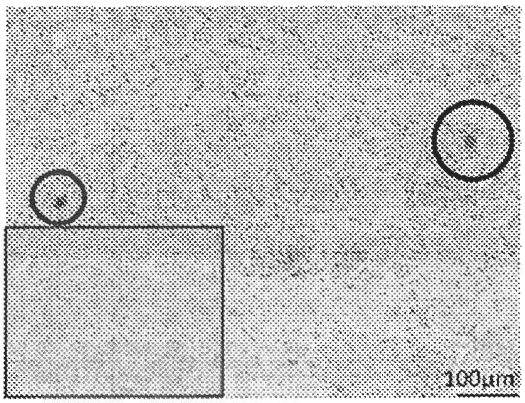


FIG. 13D

AAVhu68.hIDUA.miR183

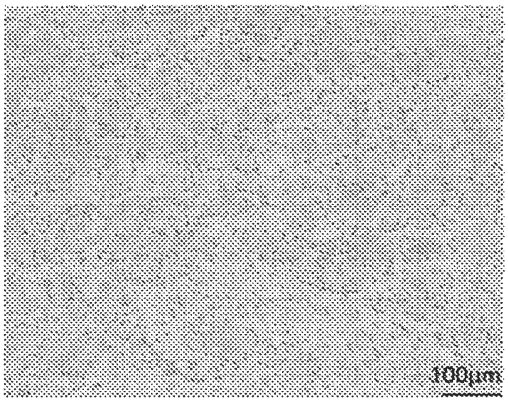


FIG. 13E

Non-AAV-injected control

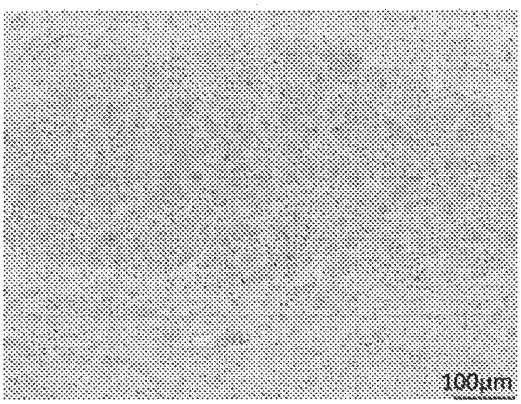


FIG. 13F

Spleen (from AAVhu68-injected NHP)

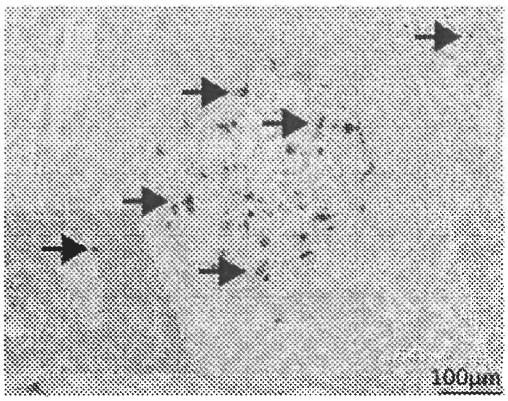


FIG. 14A

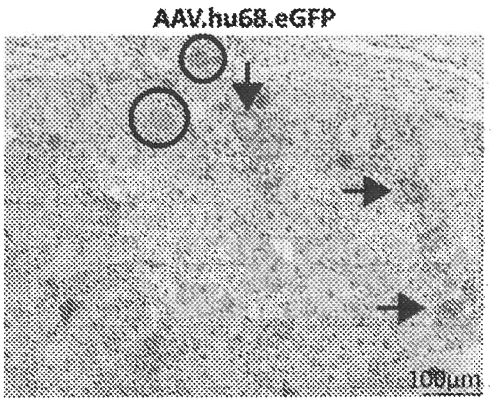


FIG. 14B

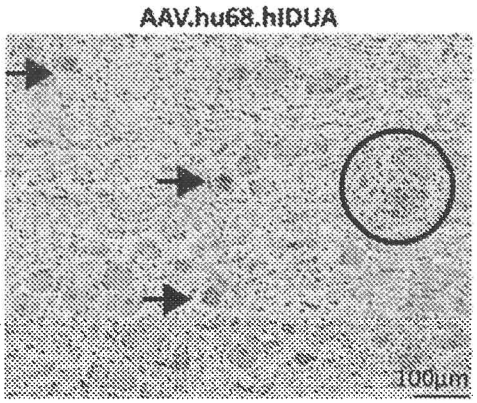


FIG. 14C

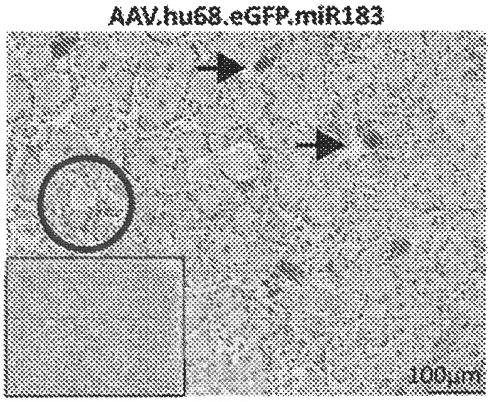


FIG. 14D

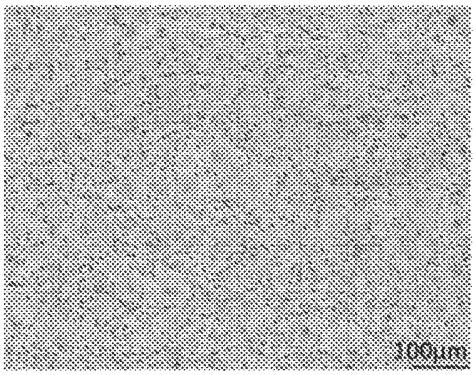


FIG. 14E

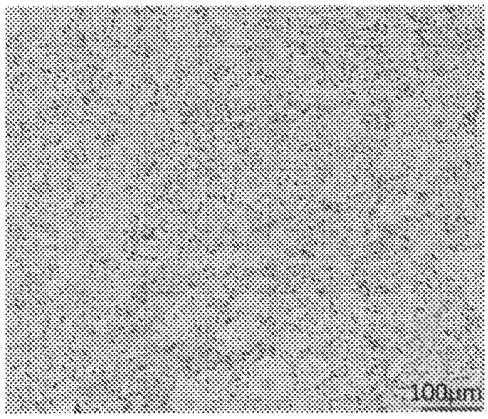


FIG. 15A

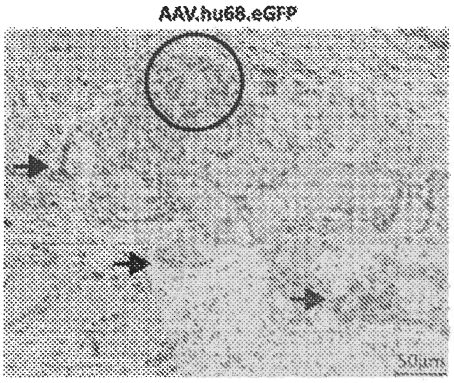


FIG. 15B

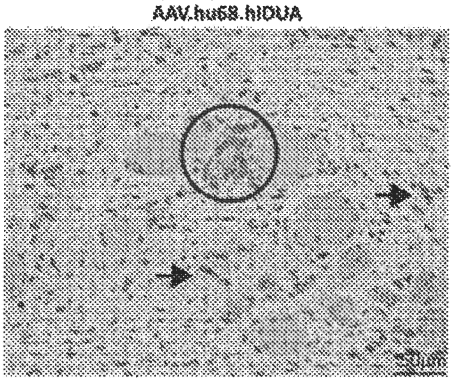


FIG. 15C

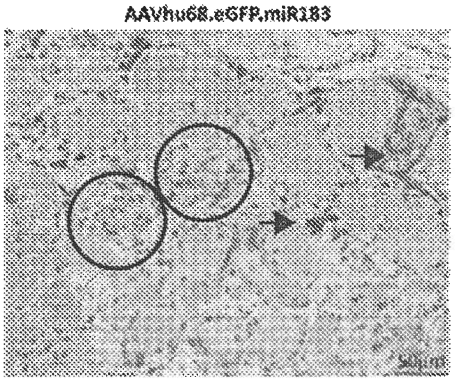


FIG. 15D

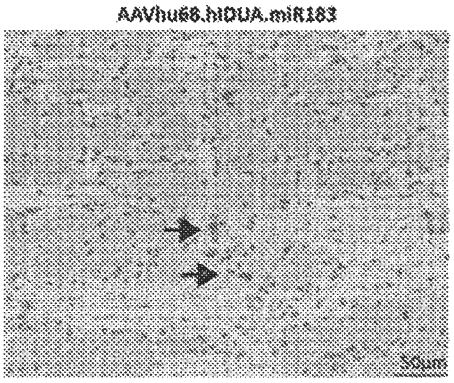


FIG. 15E

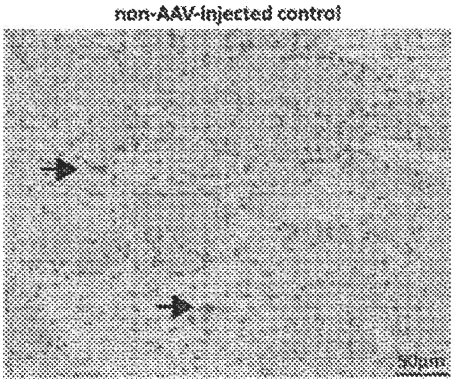


FIG. 16A

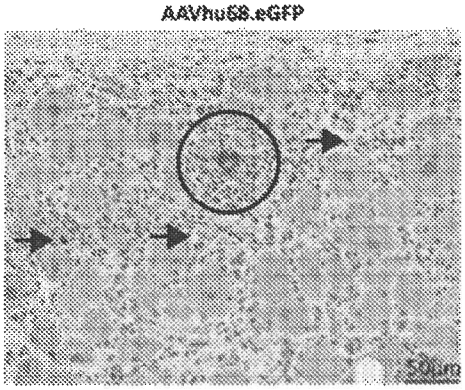


FIG. 16B

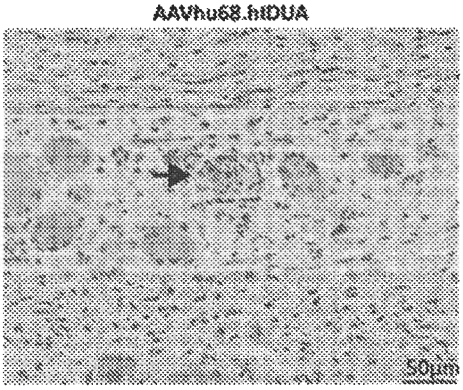


FIG. 16C

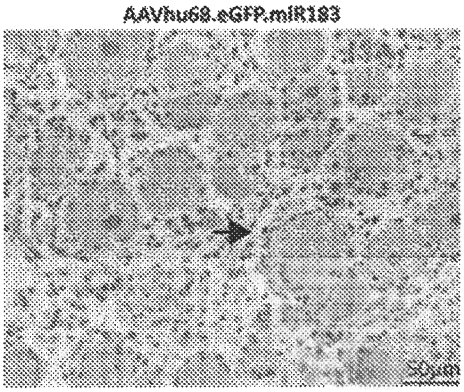


FIG. 16D

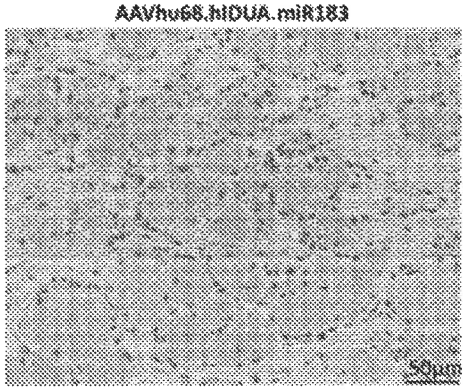


FIG. 16E

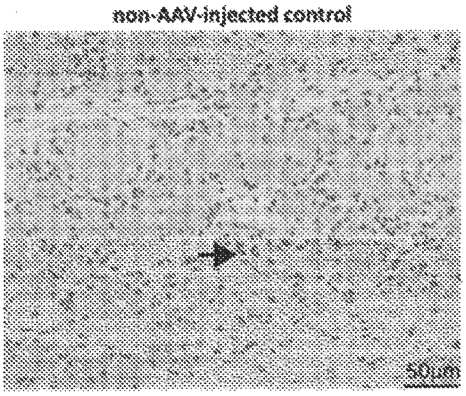


FIG. 16F

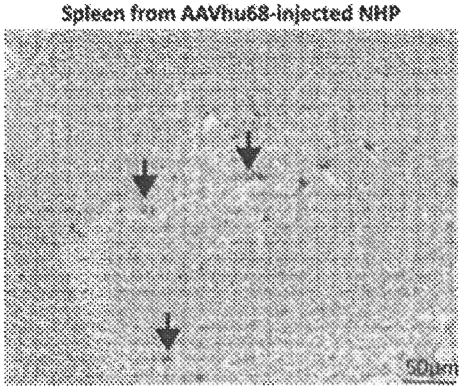


FIG. 17A

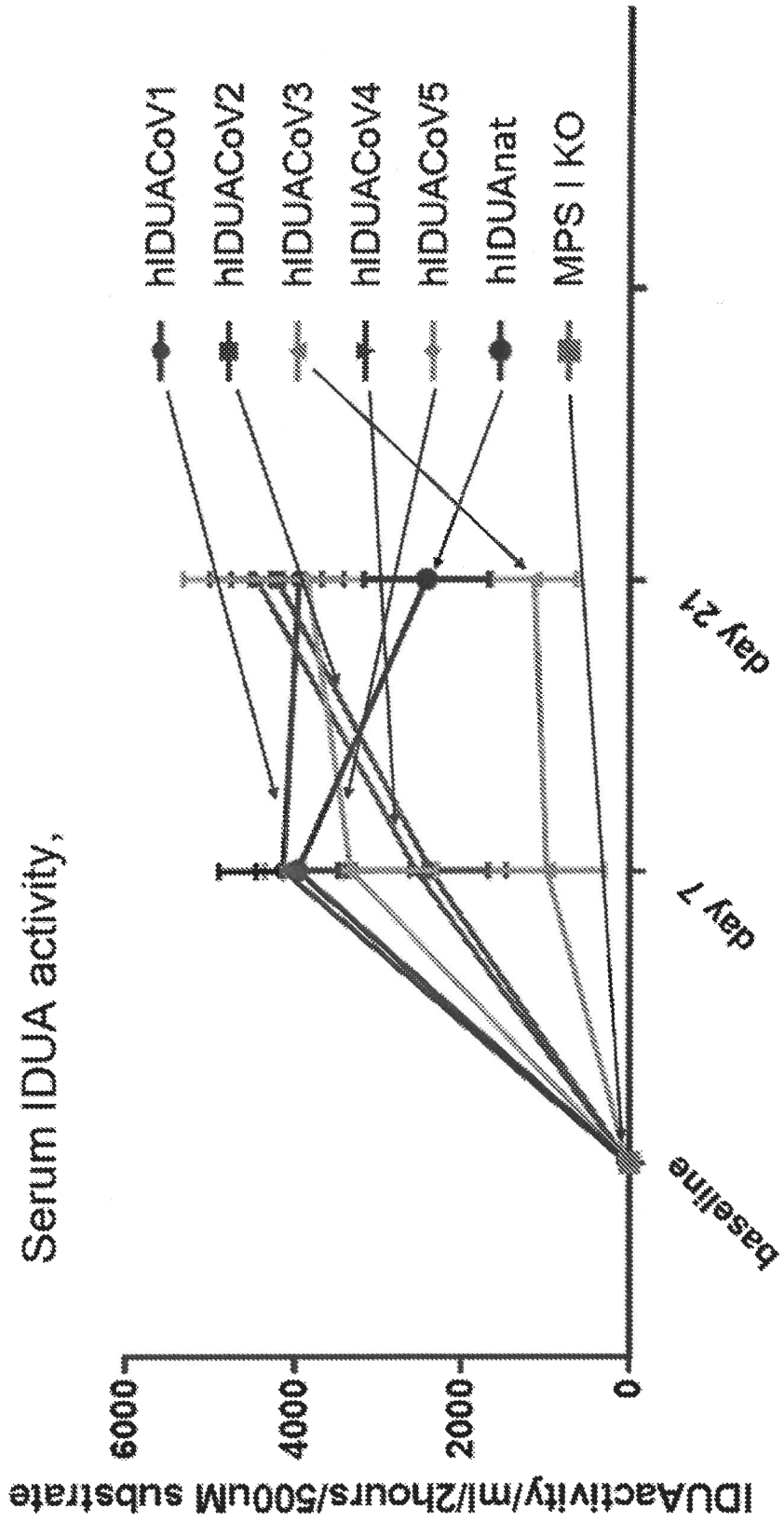

Serum IDUA activity,
IDUAactivity/ml/2hours/500uM substrate
6000
4000
2000
0
baseline
day 7
day 21
hIDUACoV1
hIDUACoV2
hIDUACoV3
hIDUACoV4
hIDUACoV5
hIDUAmat
MPS I KO

FIG. 17B

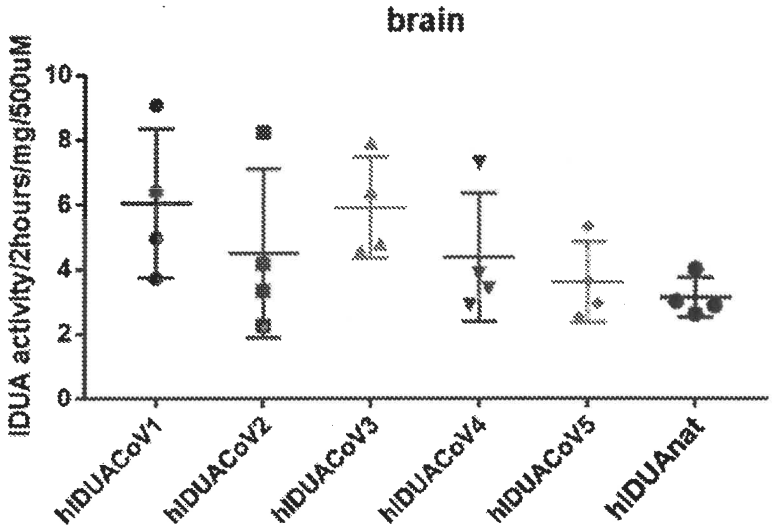


FIG. 17C

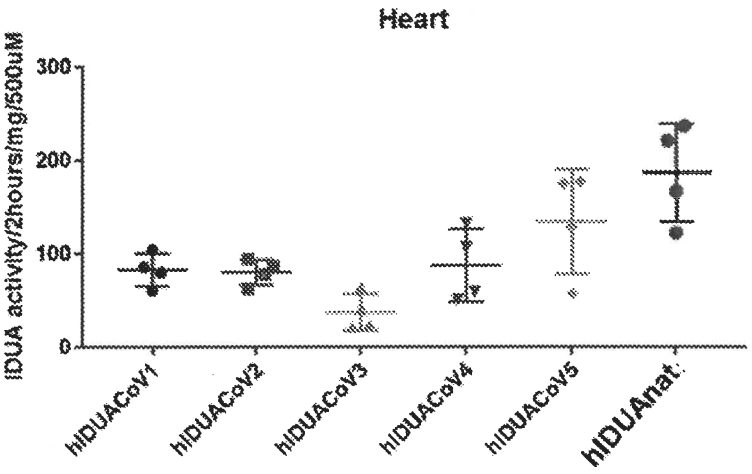


FIG. 17D

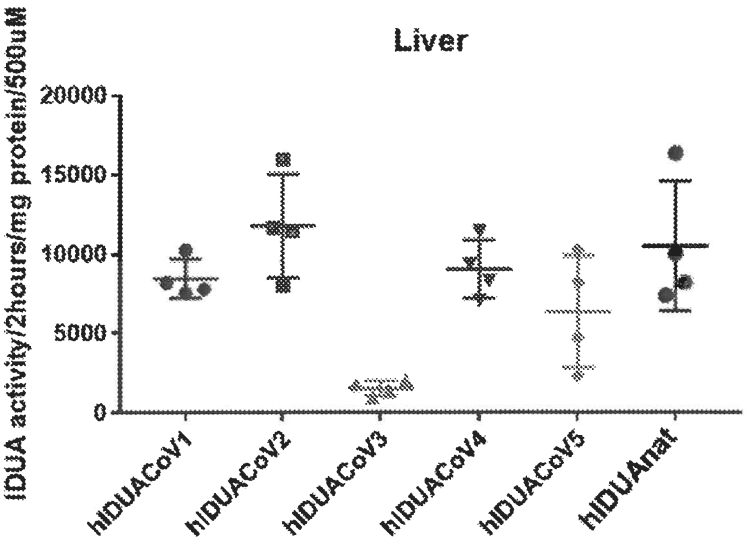


FIG. 18A

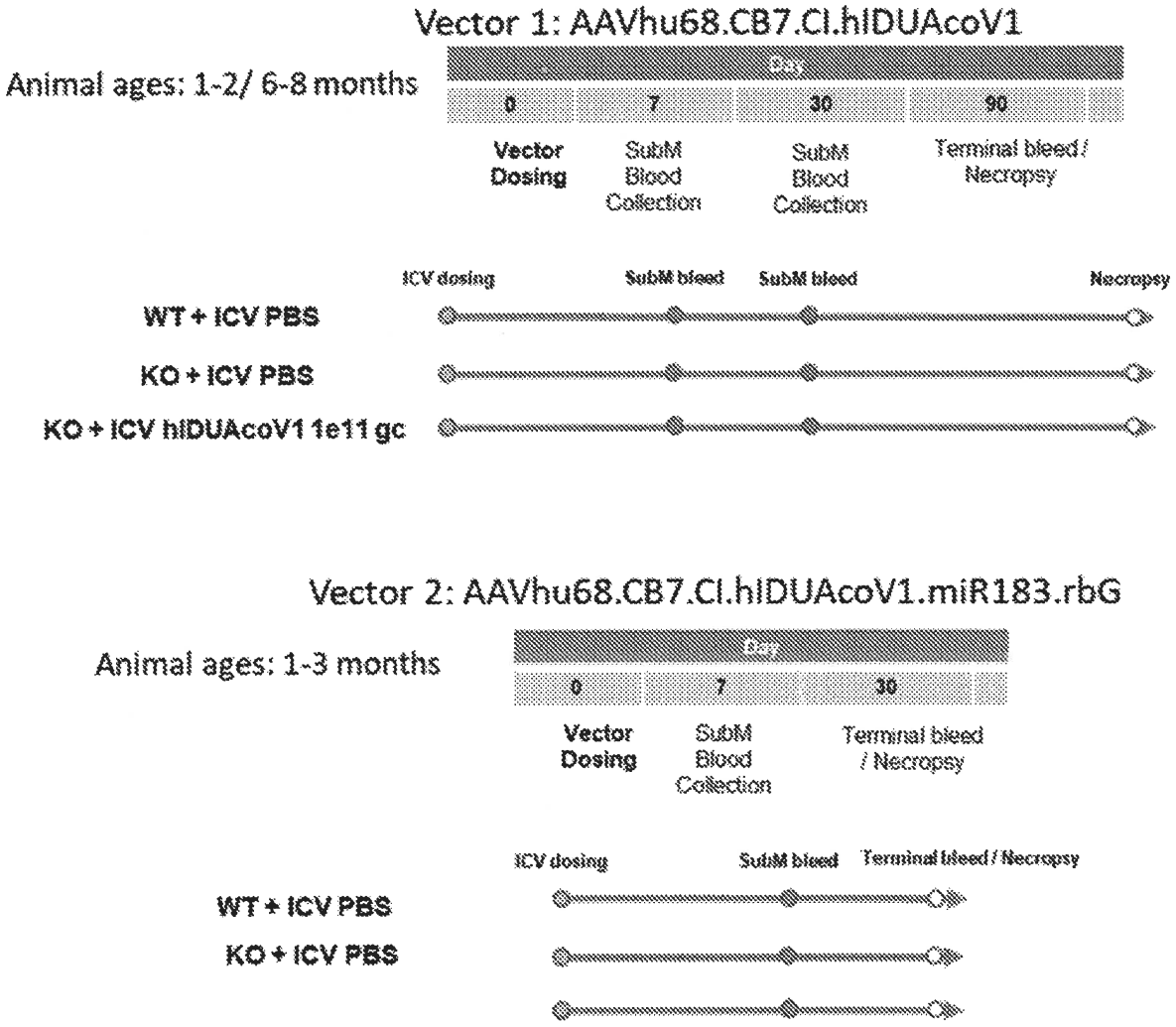

Vector 1: AAVhu68.CB7.CI.hIDUAcoV1
Animal ages: 1-2/ 6-8 months
Day
0
7
30
90
Vector Dosing
SubM Blood Collection
SubM Blood Collection
Terminal bleed / Necropsy
ICV dosing
SubM bleed
SubM bleed
Necropsy
WT + ICV PBS
KO + ICV PBS
KO + ICV hIDUAcoV1 1e11 gc
Vector 2: AAVhu68.CB7.CI.hIDUAcoV1.miR183.rbG
Animal ages: 1-3 months
Day
0
7
30
Vector Dosing
SubM Blood Collection
Terminal bleed / Necropsy
ICV dosing
SubM bleed
Terminal bleed / Necropsy
WT + ICV PBS
KO + ICV PBS

FIG. 18B

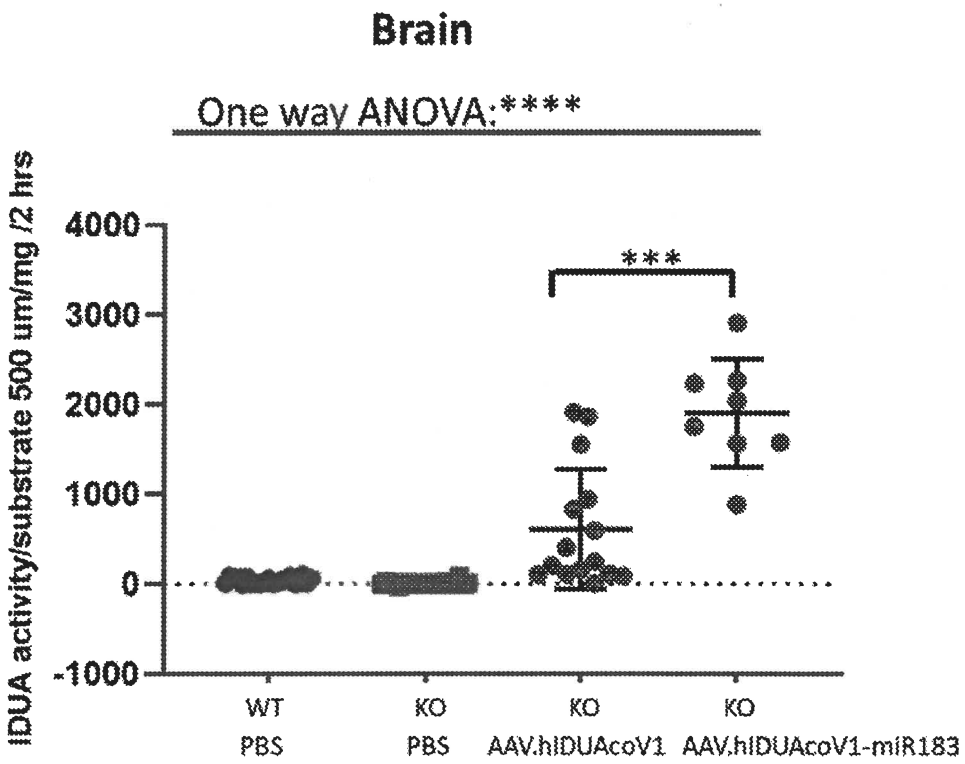


FIG. 18C

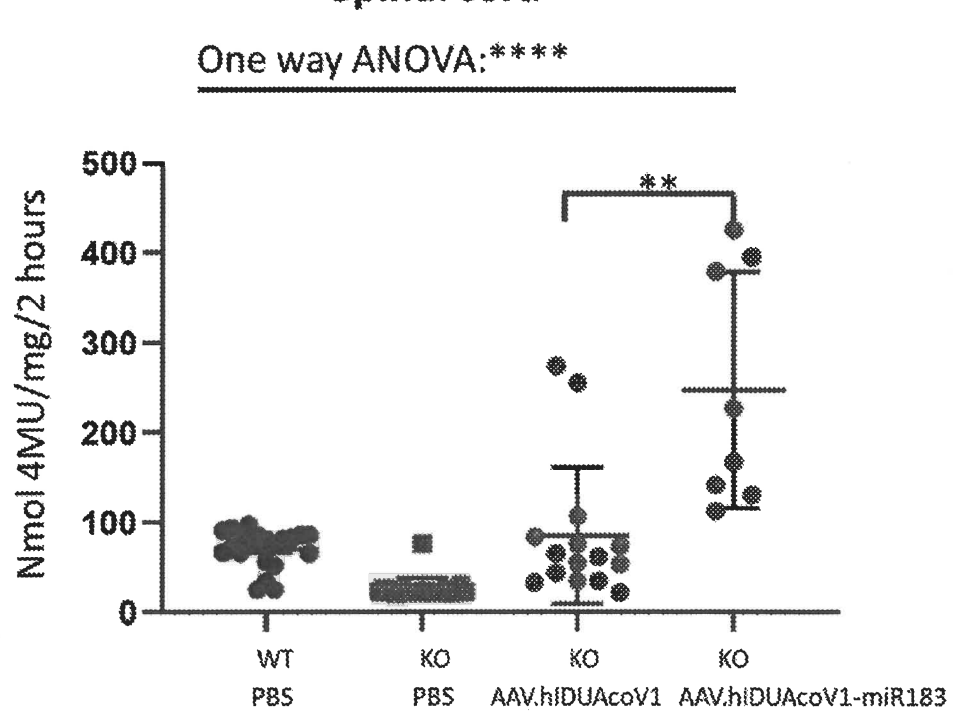


FIG. 18D

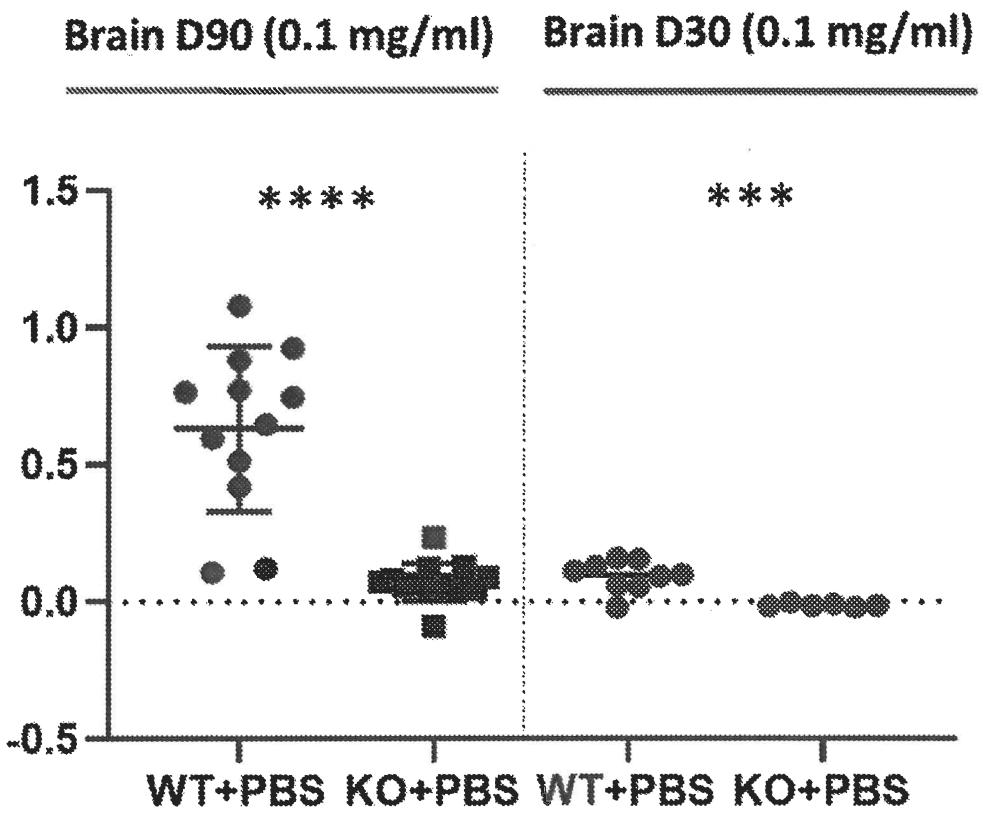

Brain D90 (0.1 mg/ml)
Brain D30 (0.1 mg/ml)
****
***
1.5
1.0
0.5
0.0
-0.5
WT+PBS
KO+PBS
WT+PBS
KO+PBS

FIG. 18E

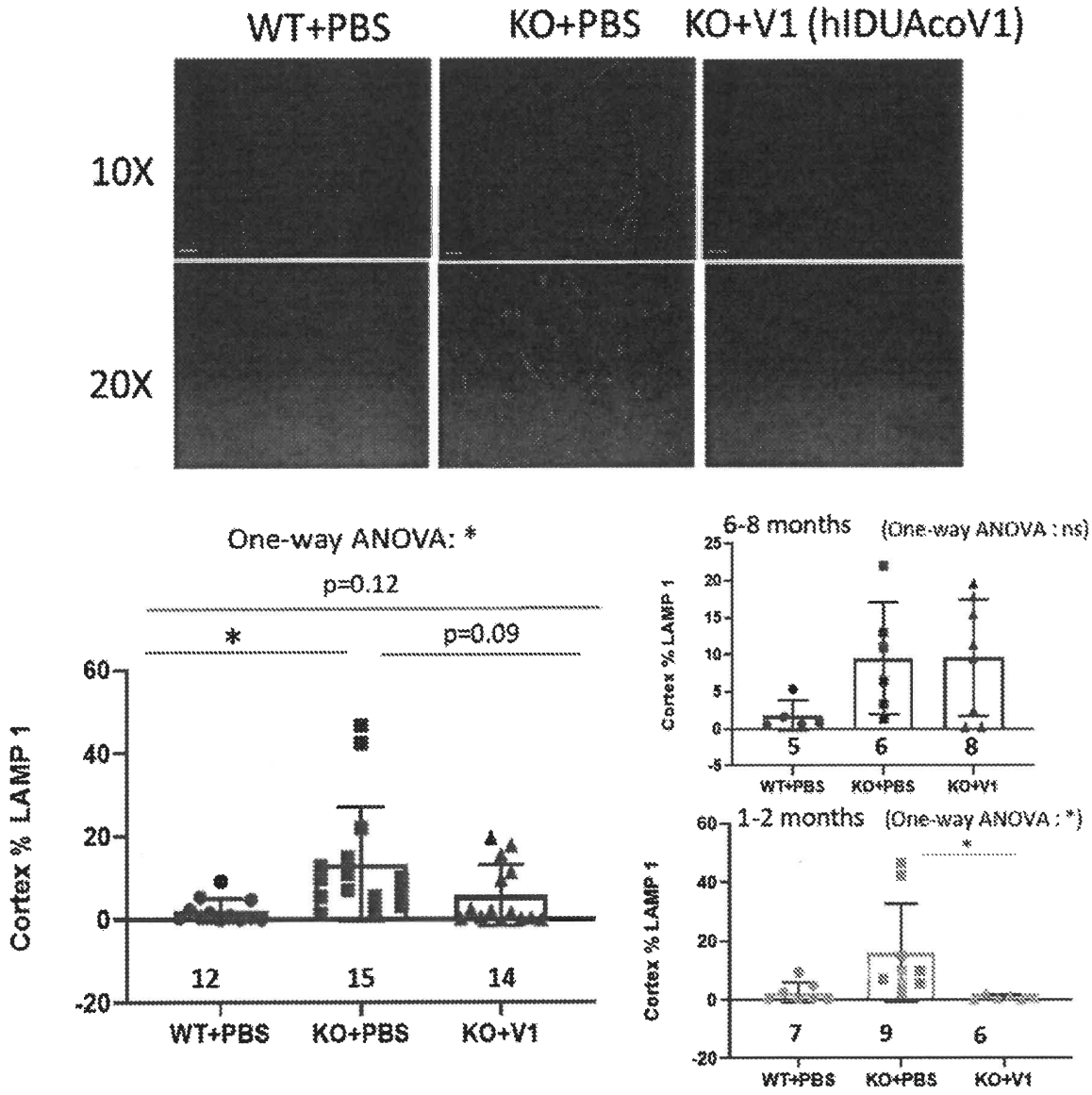
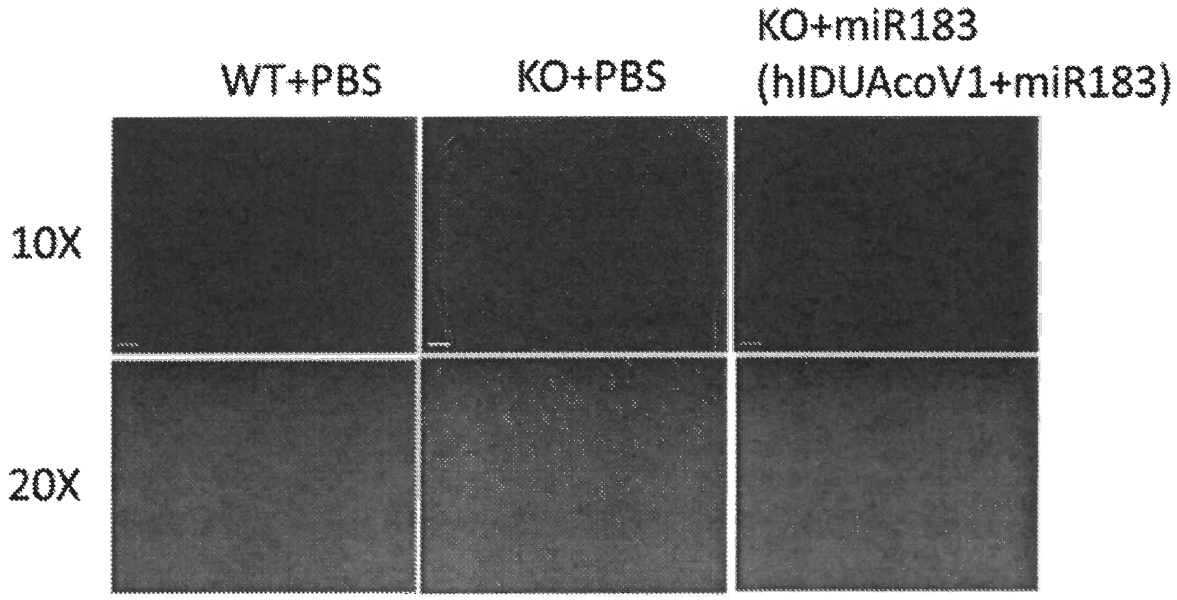

WT+PBS
KO+PBS
KO+V1 (hIDUAcoV1)
10X
20X
One-way ANOVA: *
p=0.12
*
p=0.09
Cortex % LAMP 1
60
40
20
0
-20
12
15
14
WT+PBS
KO+PBS
KO+V1
6-8 months
(One-way ANOVA : ns)
Cortex % LAMP 1
25
20
15
10
5
0
-5
5
6
8
WT+PBS
KO+PBS
KO+V1
1-2 months
(One-way ANOVA : *)
*
Cortex % LAMP 1
60
40
20
0
-20
7
9
6
WT+PBS
KO+PBS
KO+V1

FIG. 18F


WT+PBS
KO+PBS
KO+miR183
(hIDUAcoV1+miR183)
10X
20X

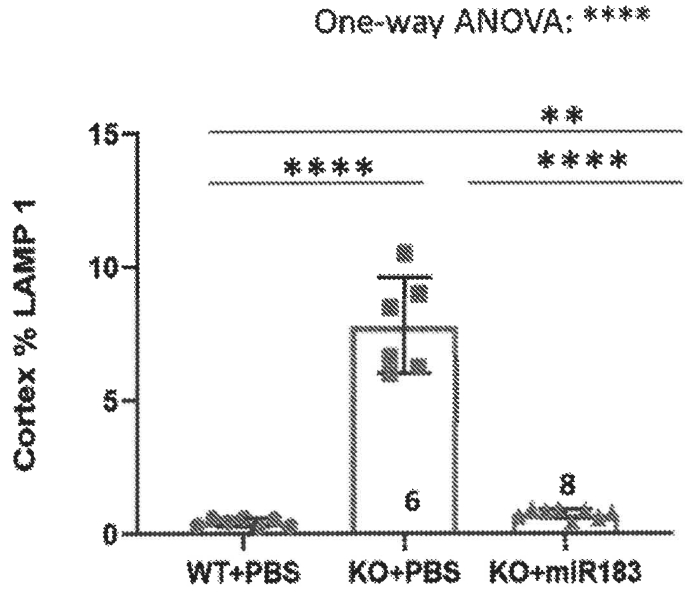

One-way ANOVA: ****
**
****
****
Cortex % LAMP 1
15
10
5
0
6
8
WT+PBS
KO+PBS
KO+miR183

FIG. 19A

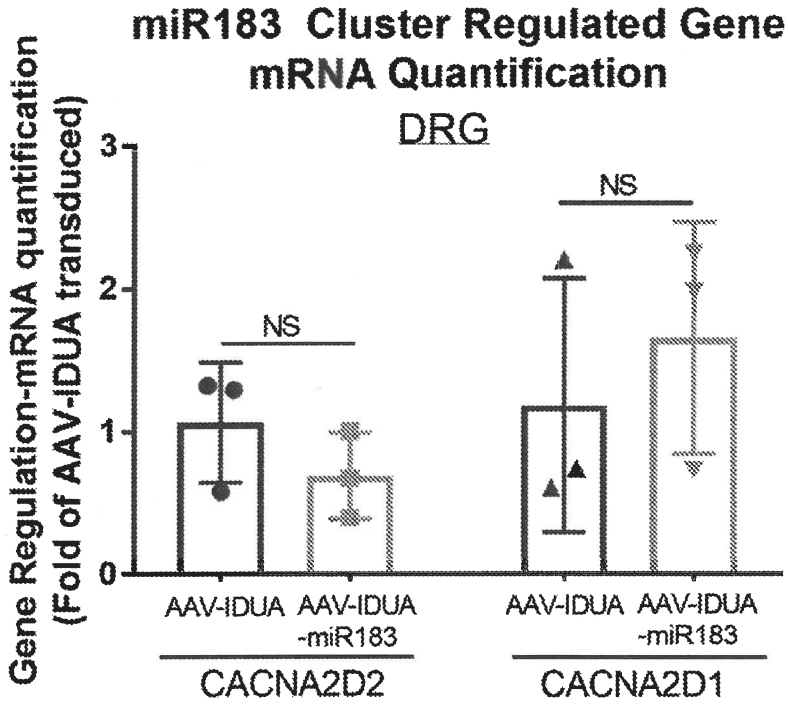


FIG. 19B

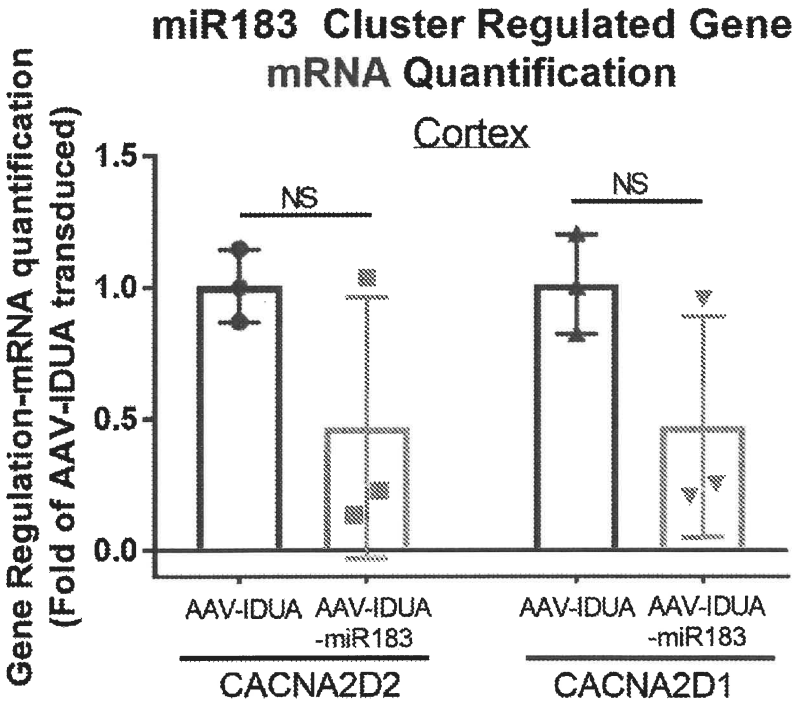


FIG. 20

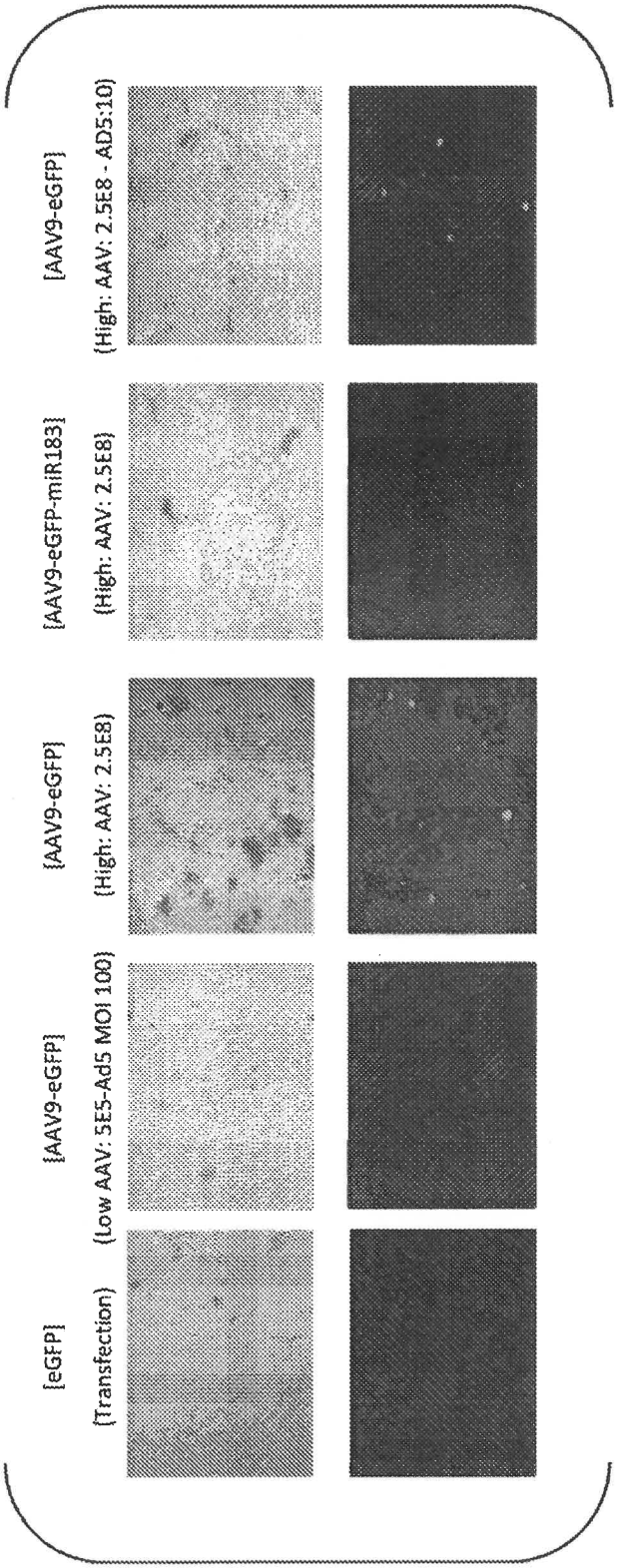

[eGFP]
(Transfection)
[AAV9-eGFP]
(Low AAV: 5E5-Ad5 MOI 100)
[AAV9-eGFP]
(High: AAV: 2.5E8)
[AAV9-eGFP-miR183]
(High: AAV: 2.5E8)
[AAV9-eGFP]
(High: AAV: 2.5E8 - AD5:10)

FIG. 21

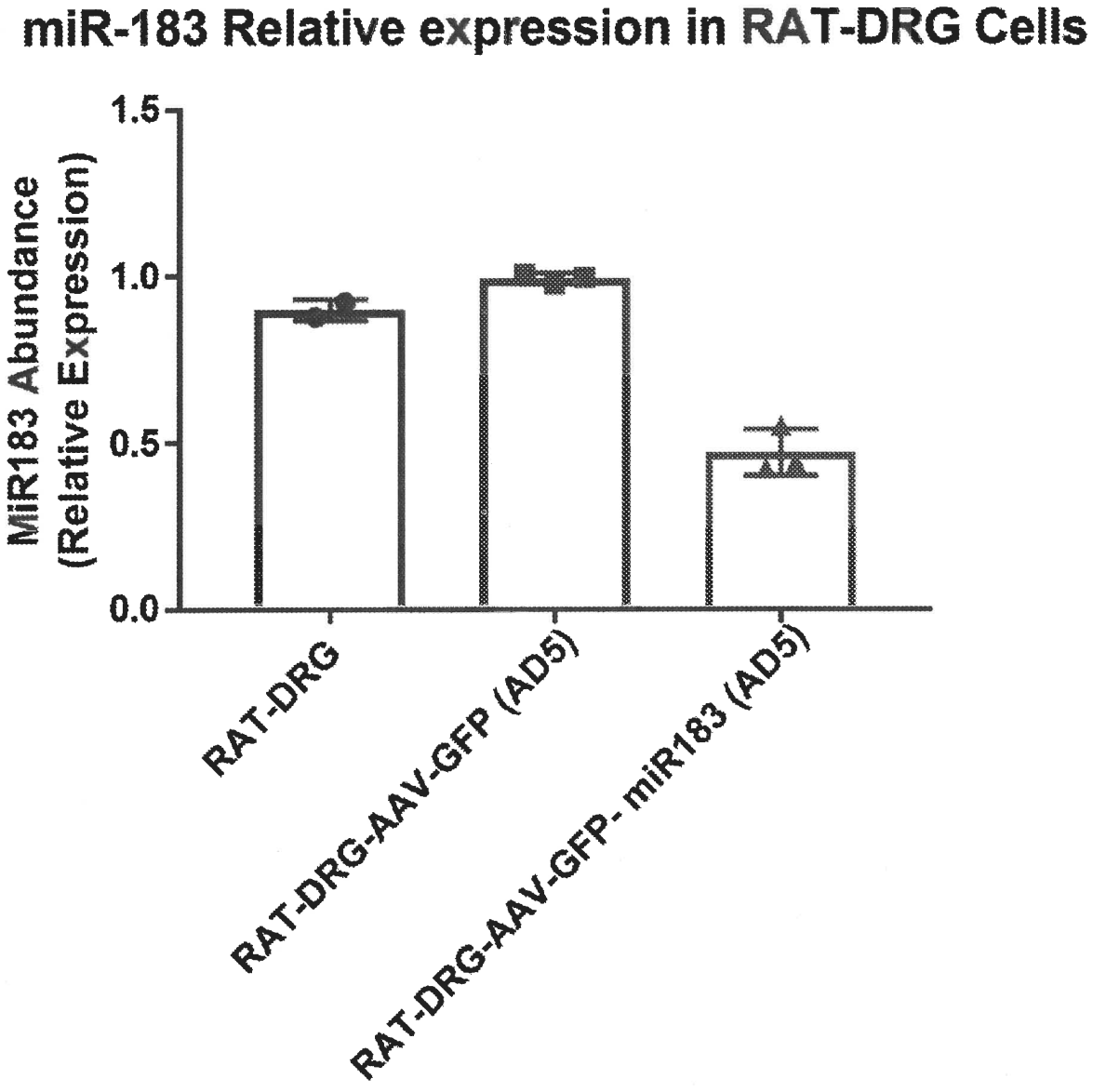

miR-183 Relative expression in RAT-DRG Cells
MiR183 Abundance
(Relative Expression)
1.5
1.0
0.5
0.0
RAT-DRG
RAT-DRG-AAV-GFP (AD5)
RAT-DRG-AAV-GFP- miR183 (AD5)

FIG. 22A

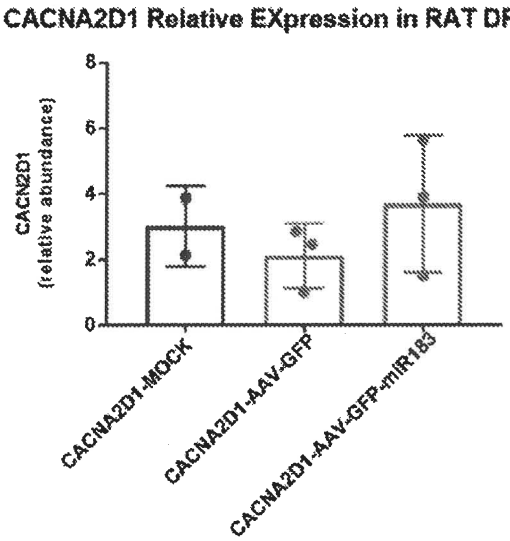


FIG. 22B

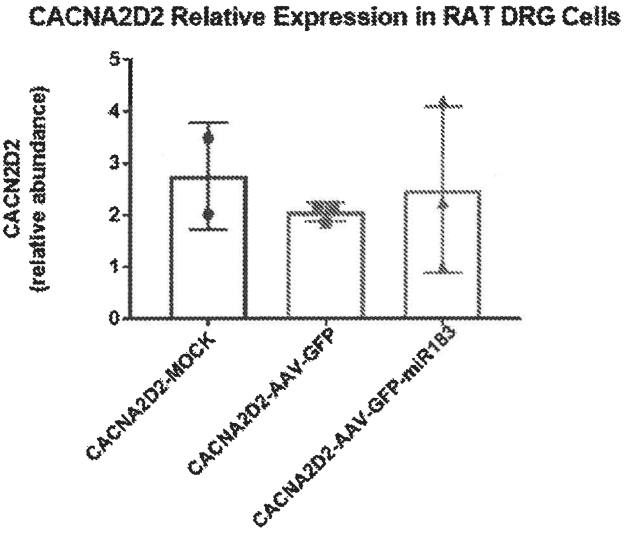


FIG. 22C

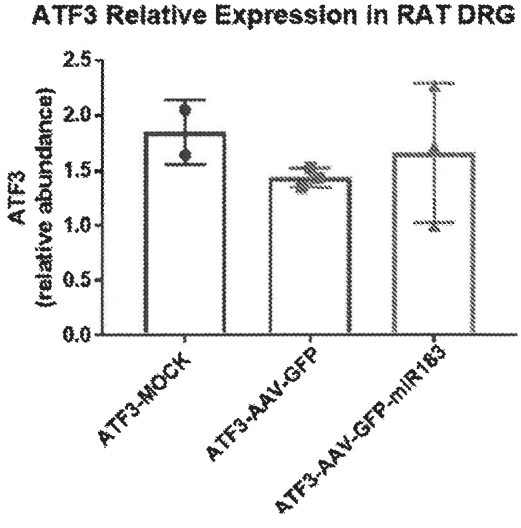


FIG. 23

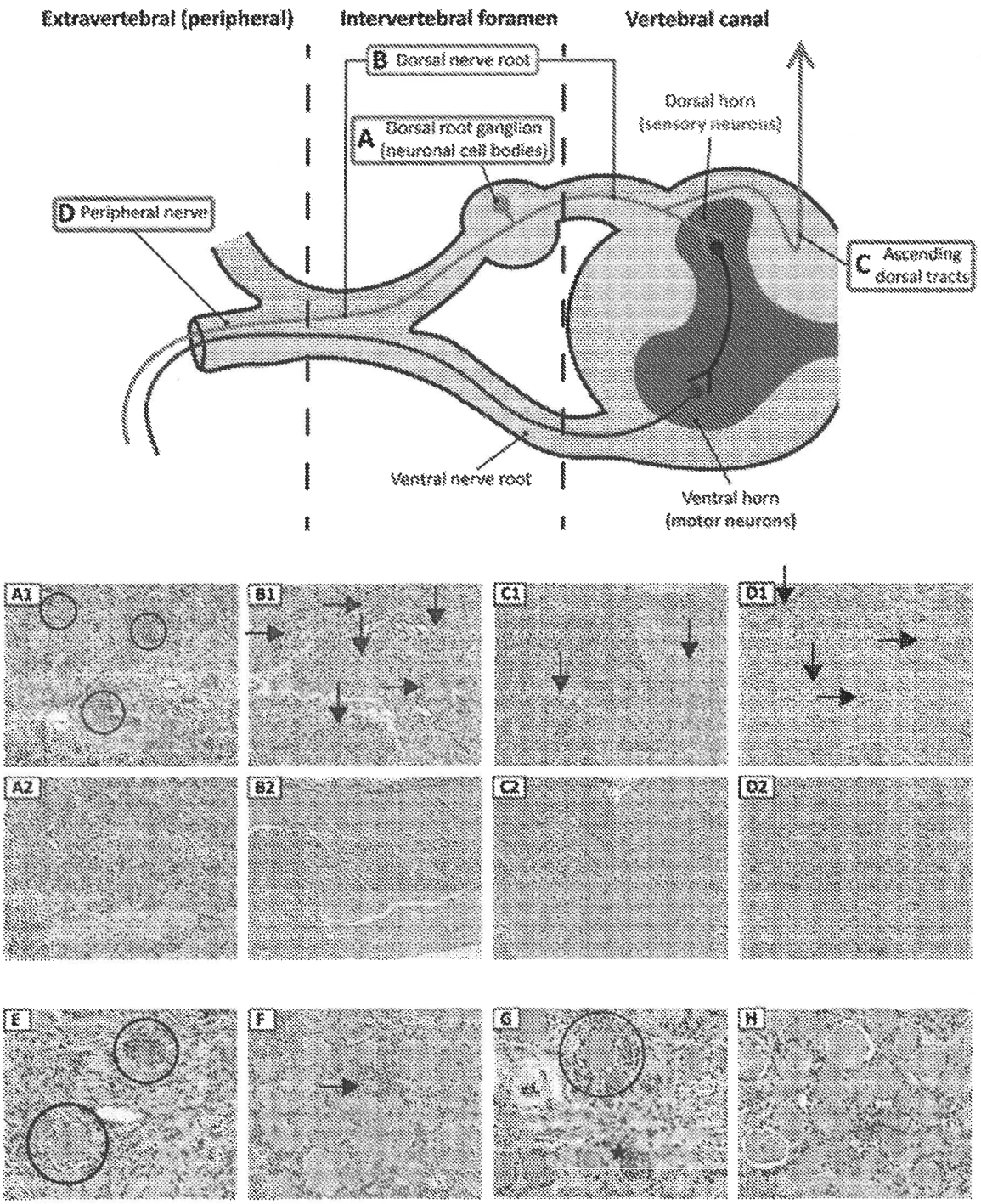

Extravertebral (peripheral)
Intervertebral foramen
Vertebral canal
B Dorsal nerve root
A Dorsal root ganglion (neuronal cell bodies)
Dorsal horn (sensory neurons)
D Peripheral nerve
C Ascending dorsal tracts
Ventral nerve root
Ventral horn (motor neurons)
A1
B1
C1
D1
A2
B2
C2
D2
E
F
G
H

FIG. 24A

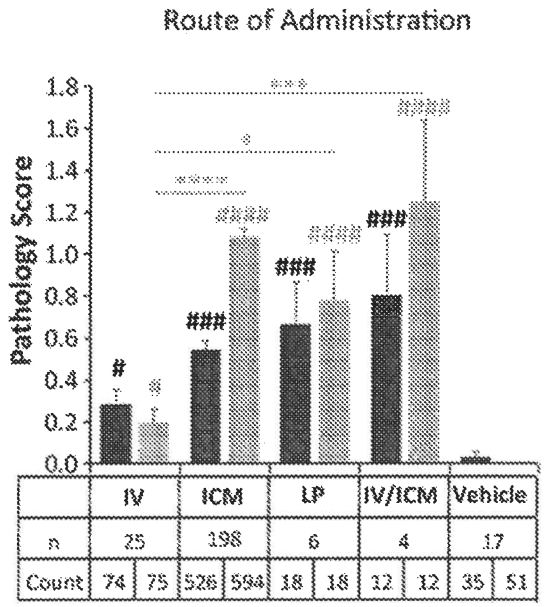
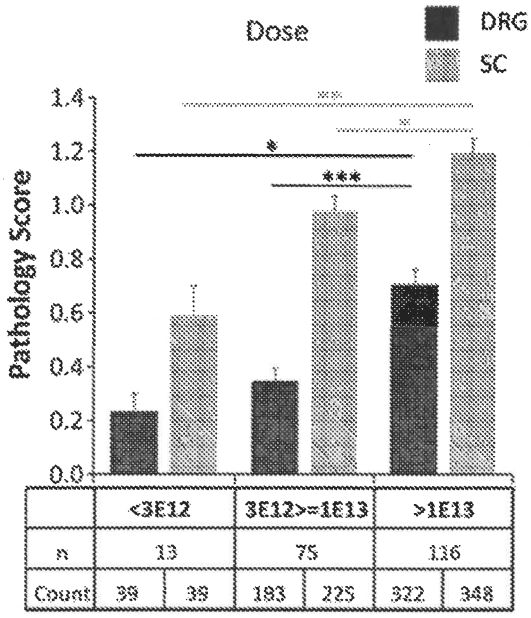


FIG. 24B



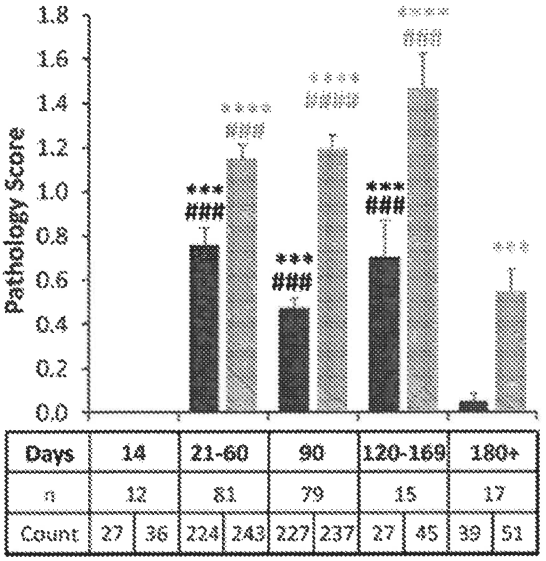


FIG. 24C

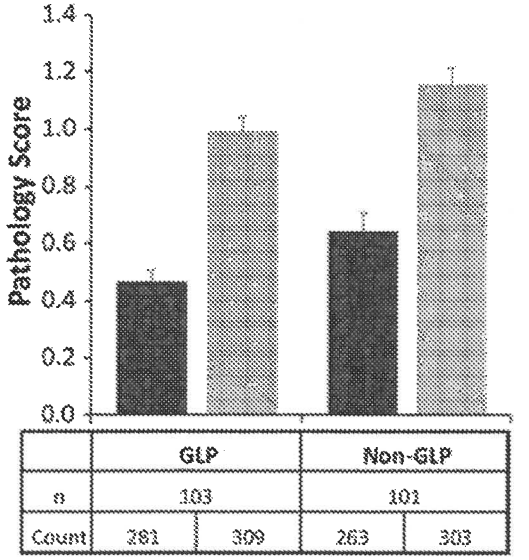


FIG. 24D

FIG. 25A

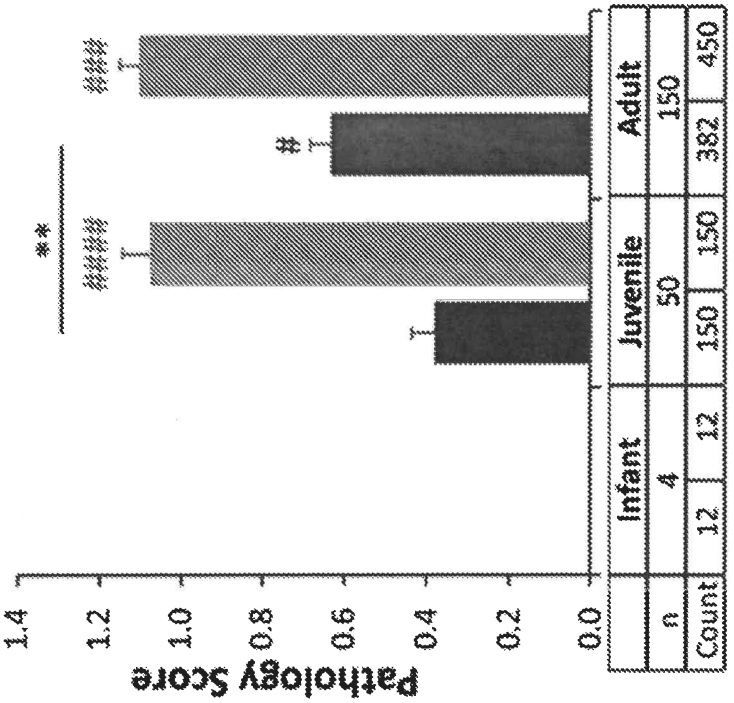


FIG. 25B

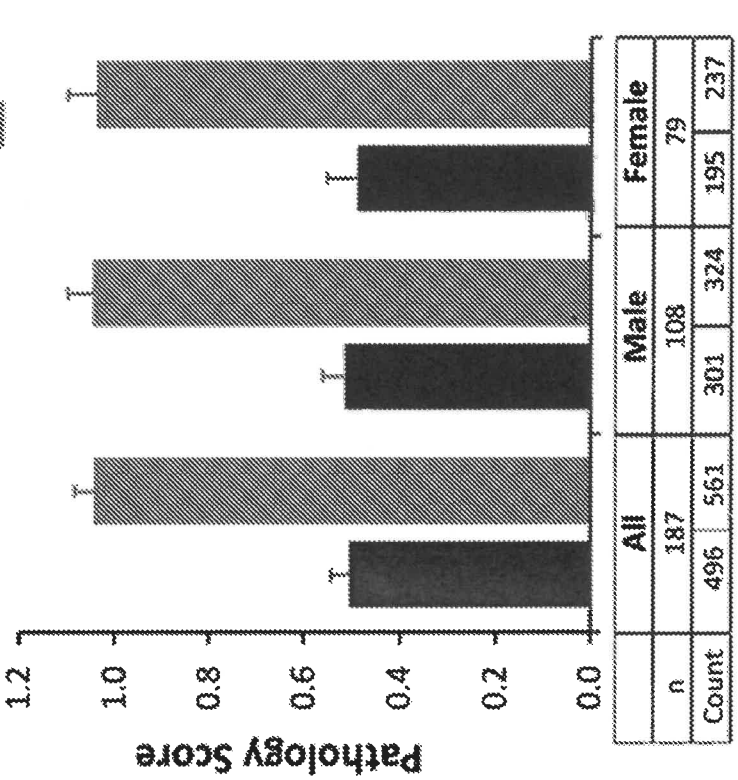
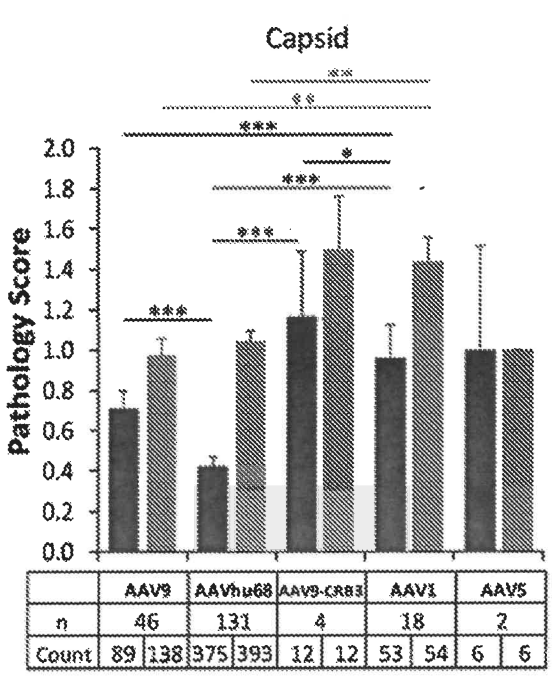
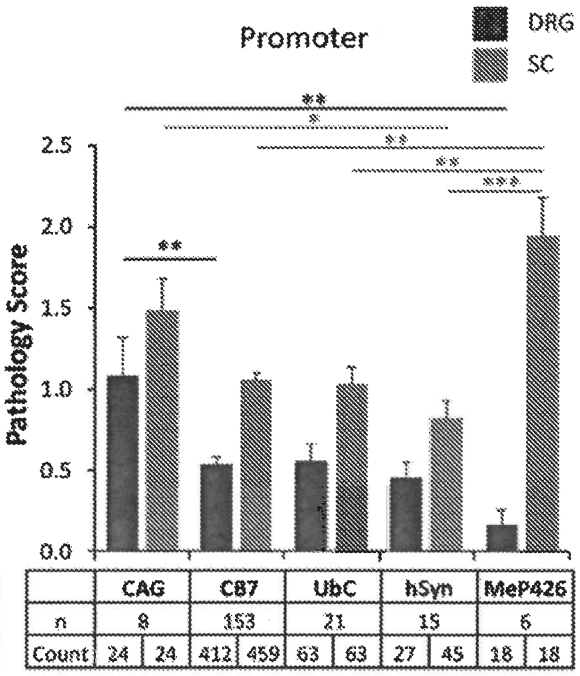


FIG. 26A



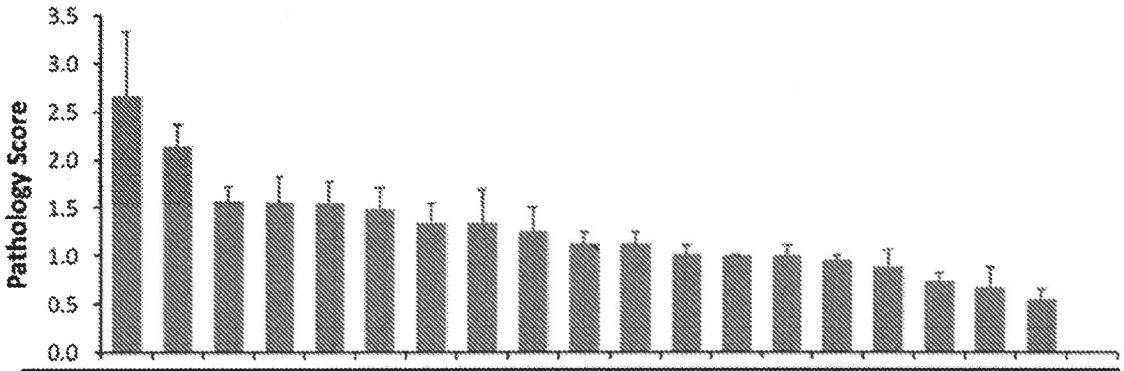
	AAV9		AAVhu68		AAV9-CR83		AAV1		AAV5	
n	46		131		4		18		2	
Count	89	138	375	393	12	12	53	54	6	6

FIG. 26B



	CAG		C87		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. 26C

FIG. 26D

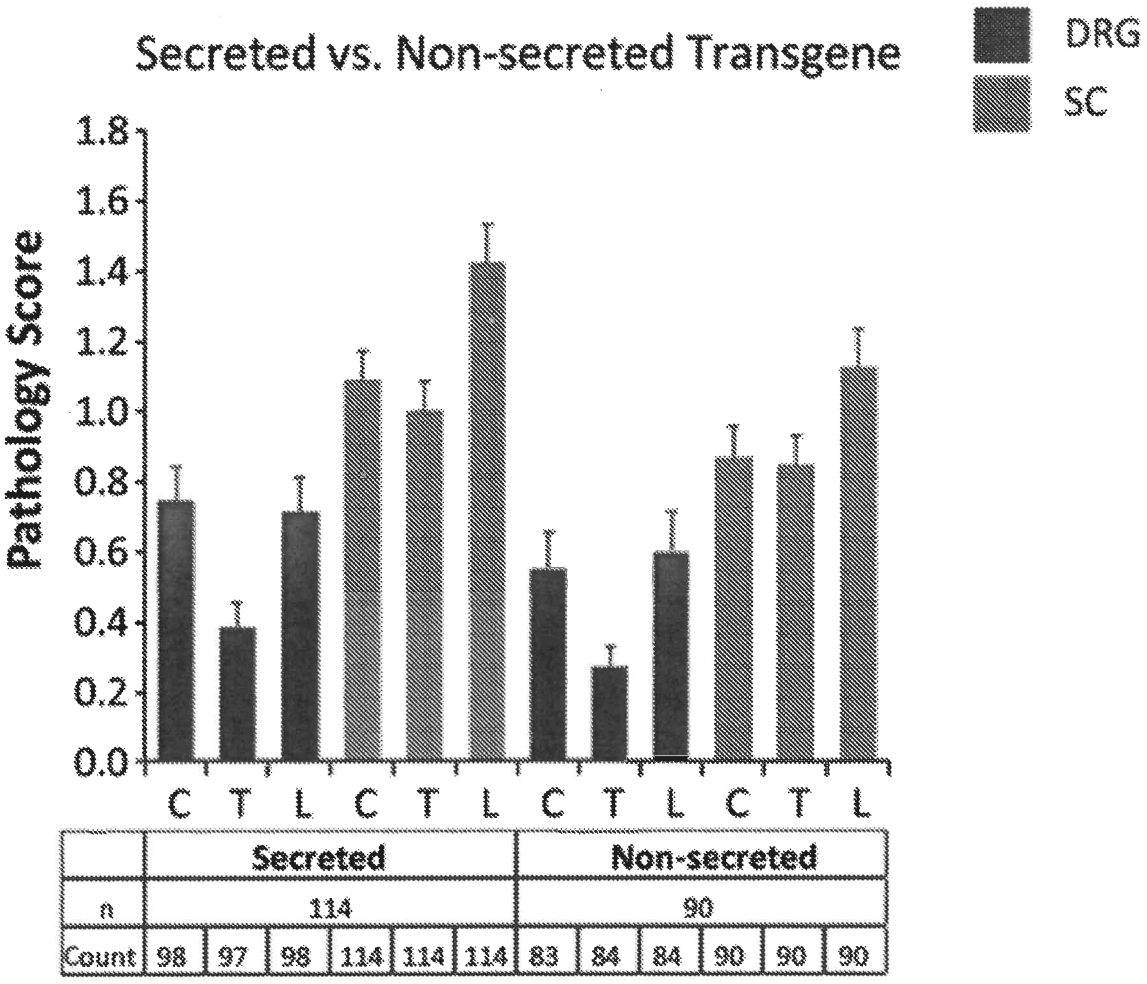

Secreted vs. Non-secreted Transgene
DRG
SC
Pathology Score
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C T L C T L C T L C T L
Secreted
Non-secreted
n
114
90
Count
98 97 98 114 114 114 83 84 84 90 90 90

FIG. 27

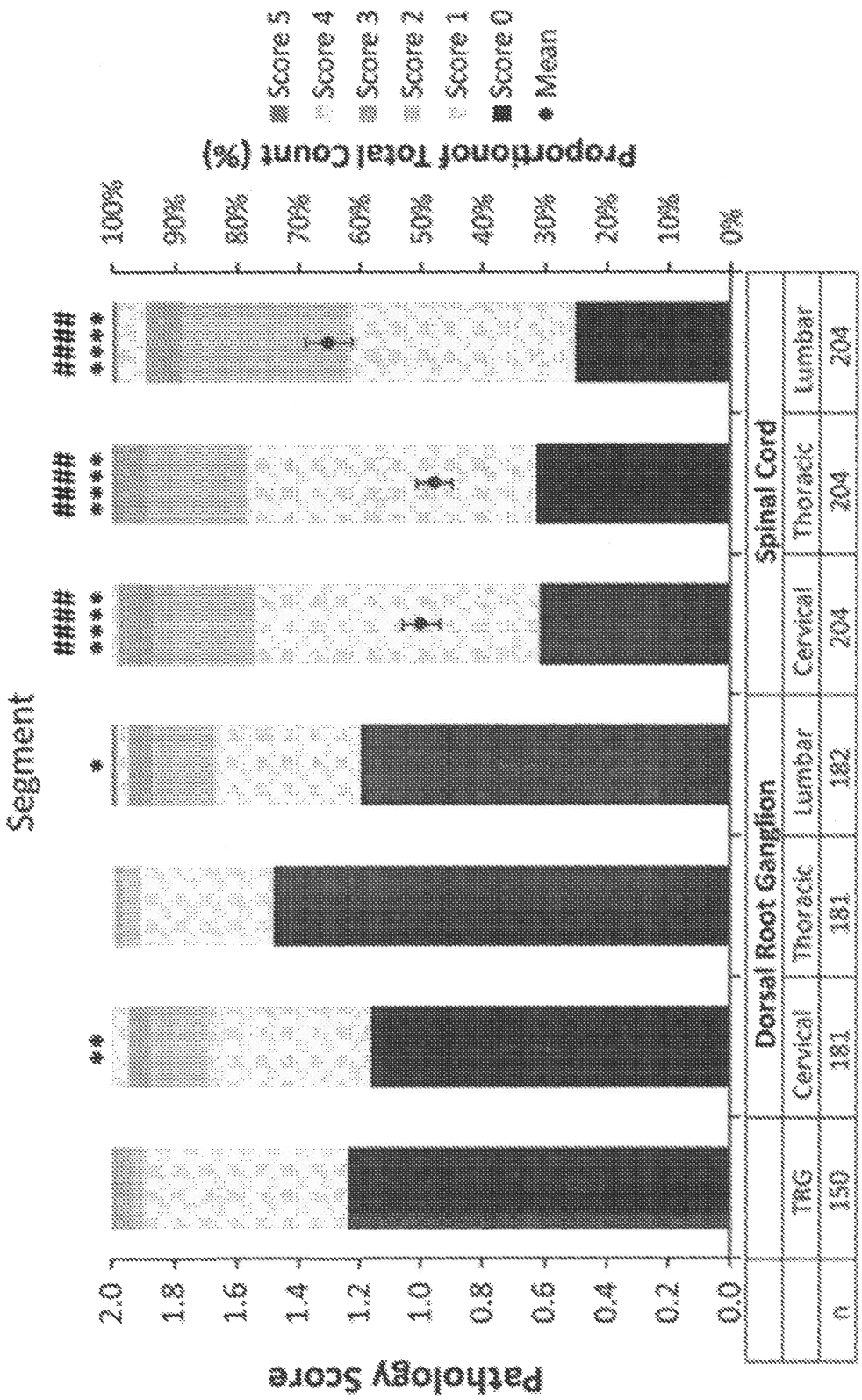
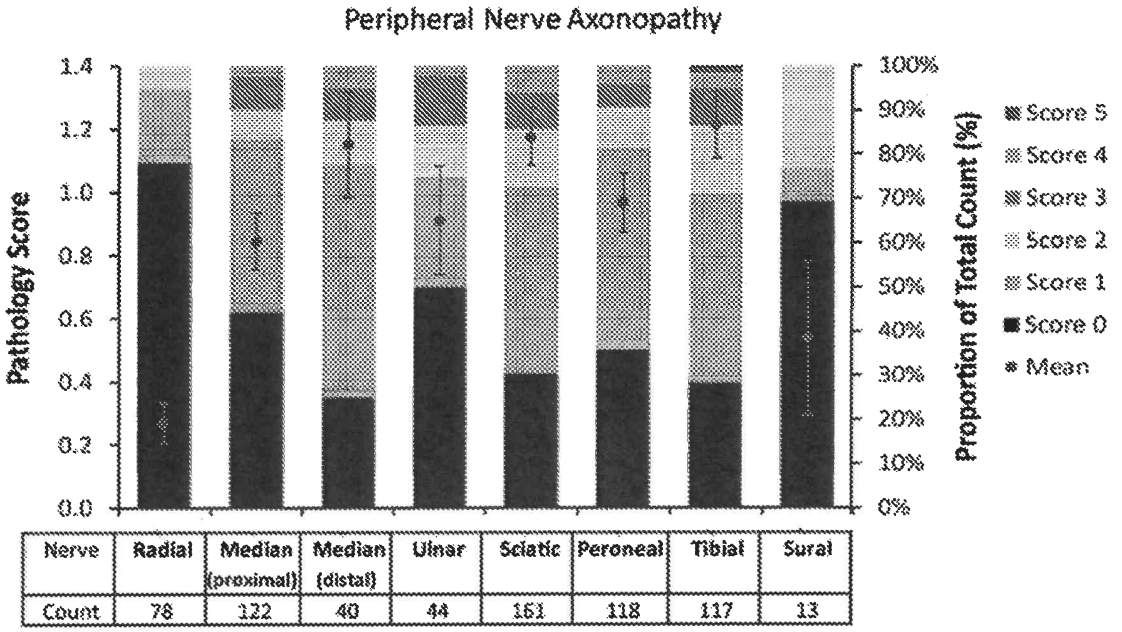

Score 5
Score 4
Score 3
Score 2
Score 1
Score 0
Mean
Proportionof Total Count (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Segment
Pathology Score
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
*
####
****
####
****
#####
****
n
150
181
181
182
204
204
204
TRG
Cervical
Thoracic
Lumbar
Cervical
Thoracic
Lumbar
Dorsal Root Ganglion
Spinal Cord

FIG. 28A



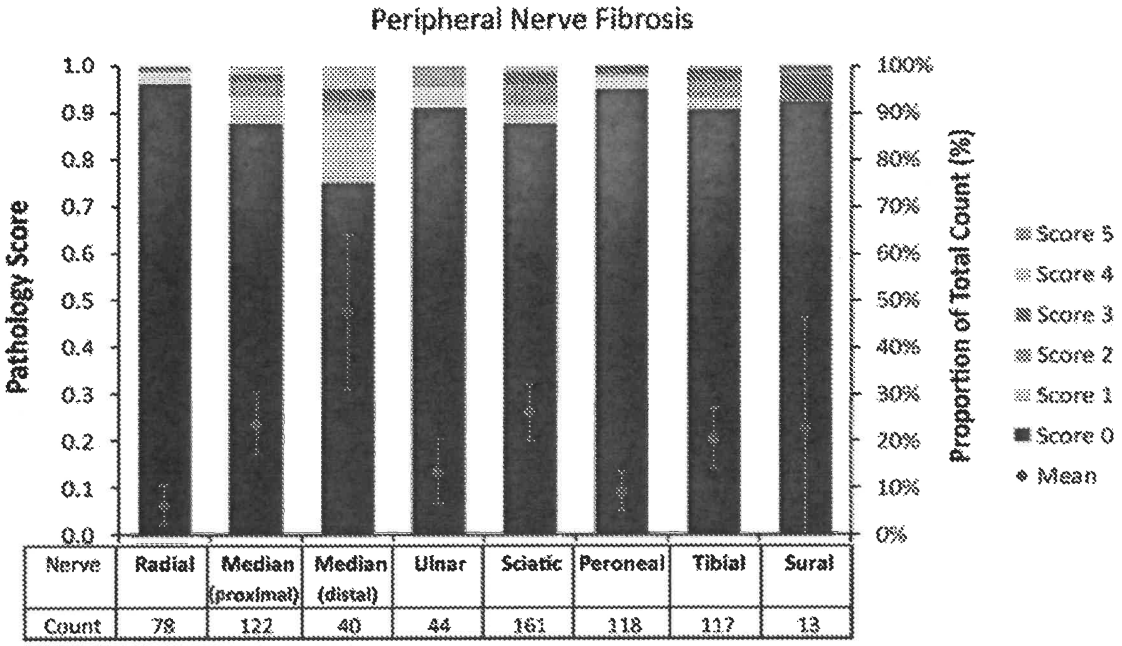


FIG. 28B

FIG. 29A

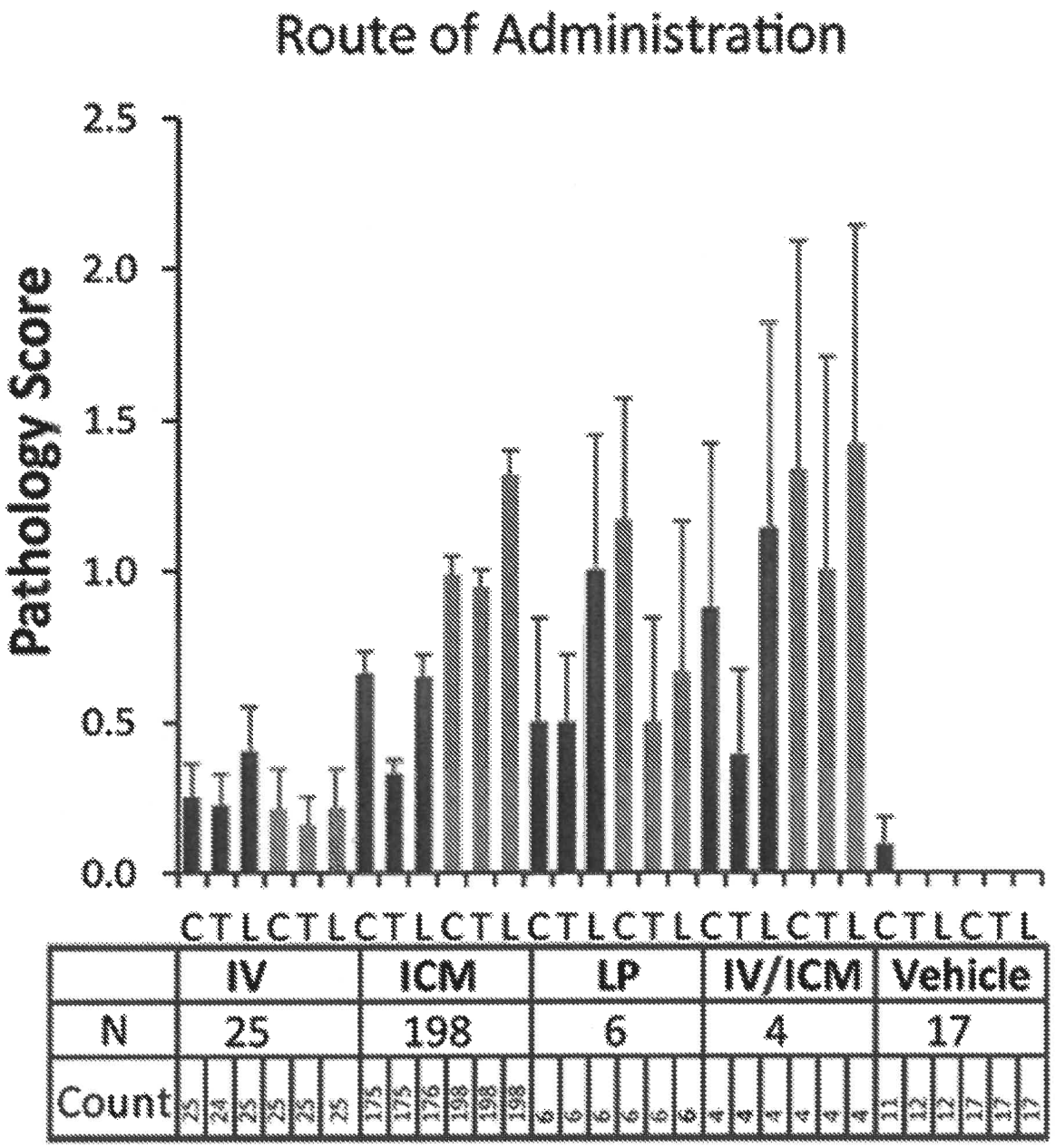
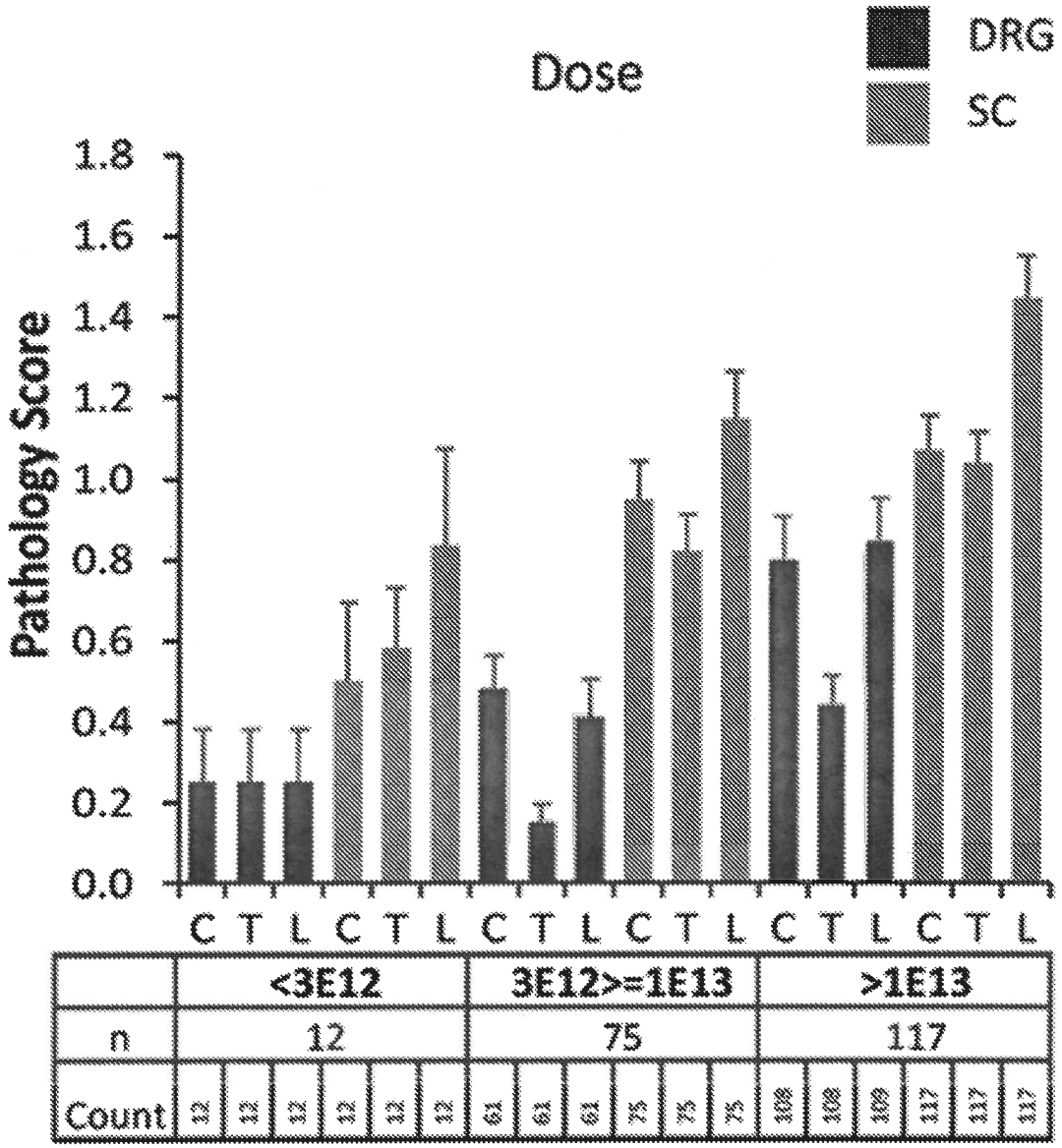

Route of Administration
Pathology Score
2.5
2.0
1.5
1.0
0.5
0.0
C T L C T L C T L C T L C T L C T L C T L C T L C T L
IV
ICM
LP
IV/ICM
Vehicle
N
25
198
6
4
17
Count

FIG. 29B


Dose
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 C T L C T L
<3E12
3E12>=1E13
>1E13
n
12
75
117
Count

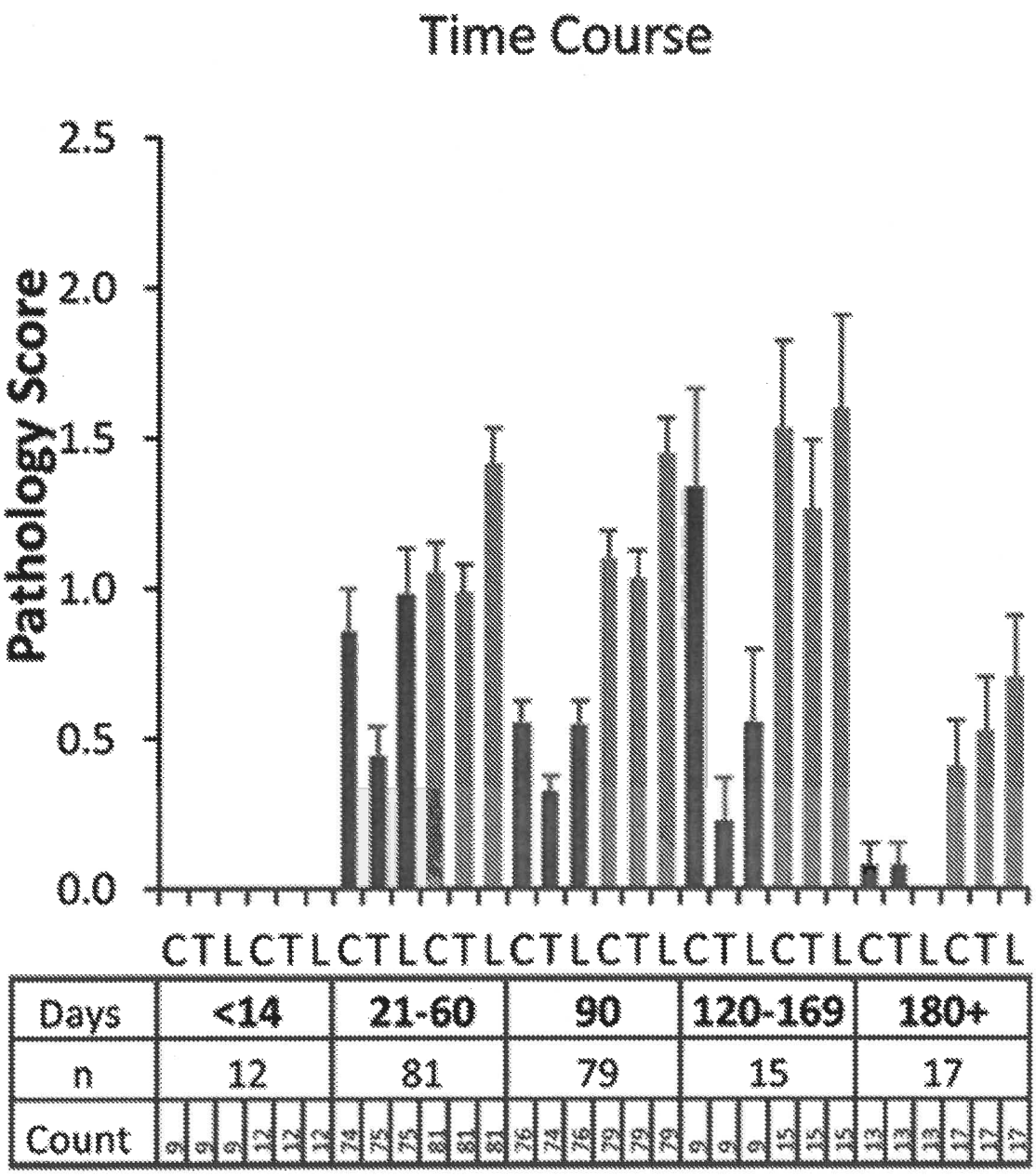

Time Course
Pathology Score
2.5
2.0
1.5
1.0
0.5
0.0
CTLCTLCTLCTLCTLCTLCTLCTLCTLCTL
Days
<14
21-60
90
120-169
180+
n
12
81
79
15
17
Count
9 9 9 12 12 12 74 75 75 81 81 81 78 73 76 79 78 78 9 9 9 15 15 15 13 13 13 17 17 17

FIG. 29C

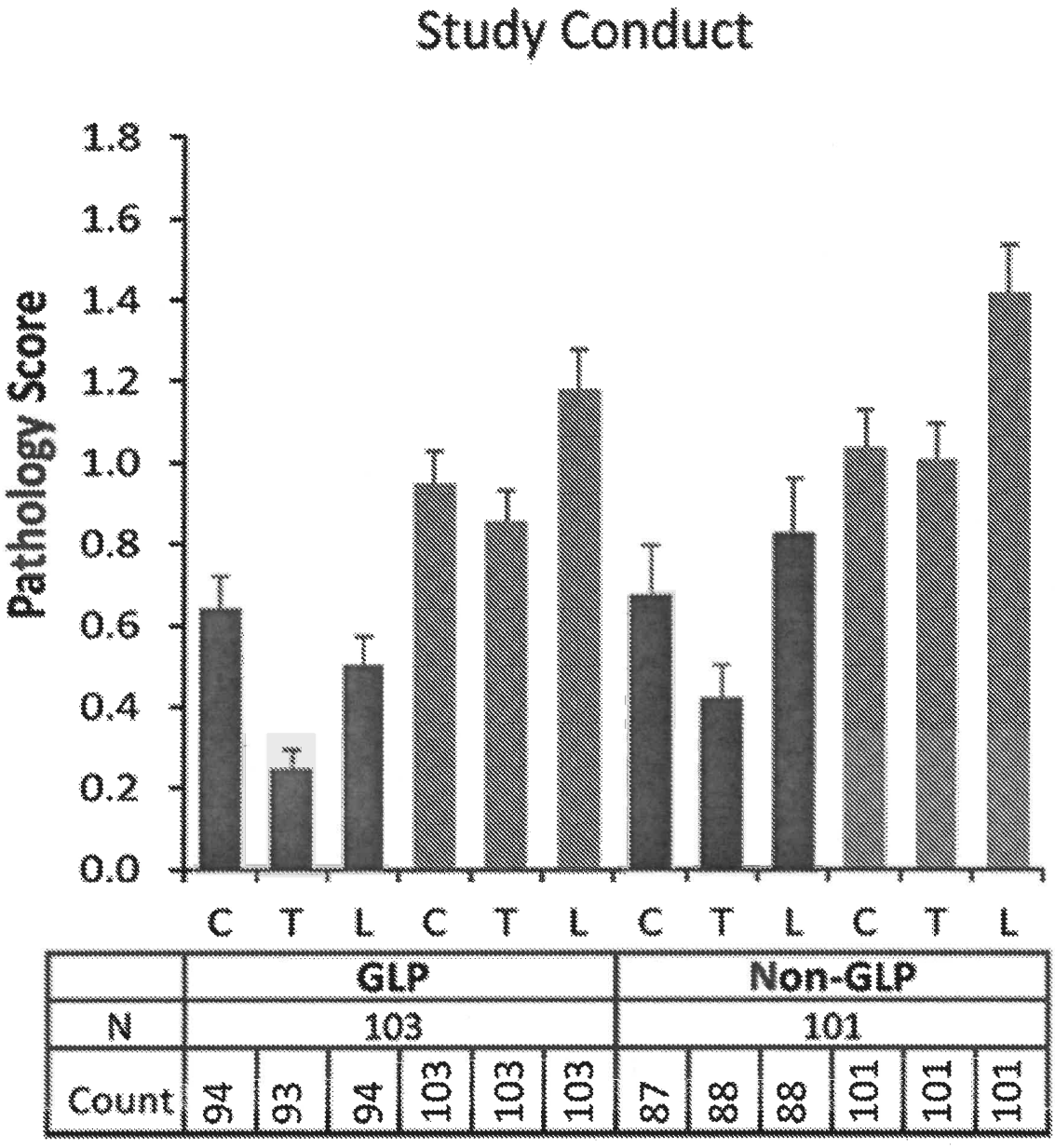

Study Conduct
Pathology Score
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C T L C T L C T L C T L
GLP
Non-GLP
N
103
101
Count
94 93 94 103 103 103 87 88 88 101 101 101

FIG. 29D

FIG. 30A

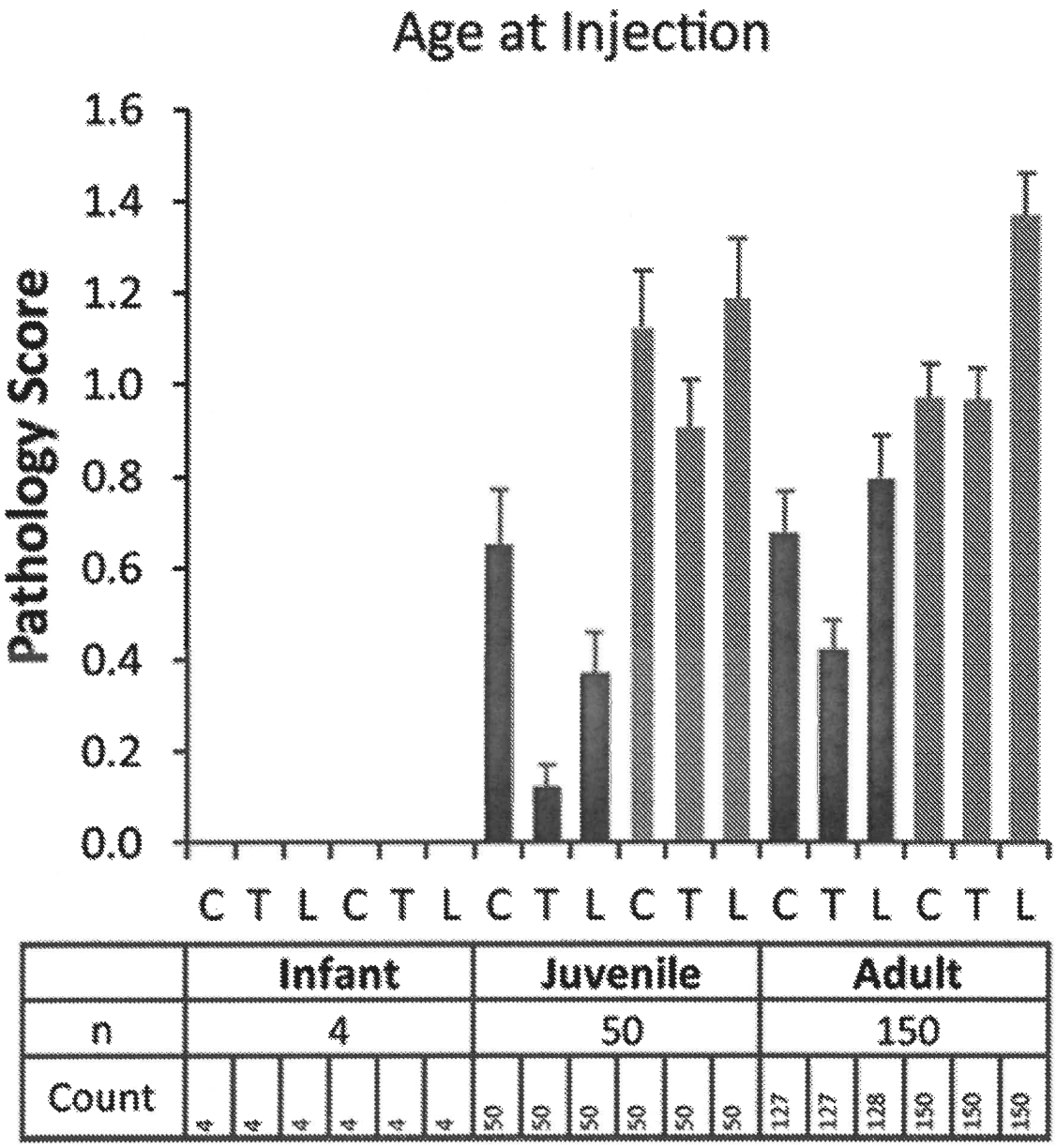

Age at Injection
Pathology Score
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C T L C T L C T L C T L C T L C T L C T L
Infant
Juvenile
Adult
n
4
50
150
Count
4 4 4 4 4 4
50 50 50 50 50 50
127 127 128 150 150 150

FIG. 30B

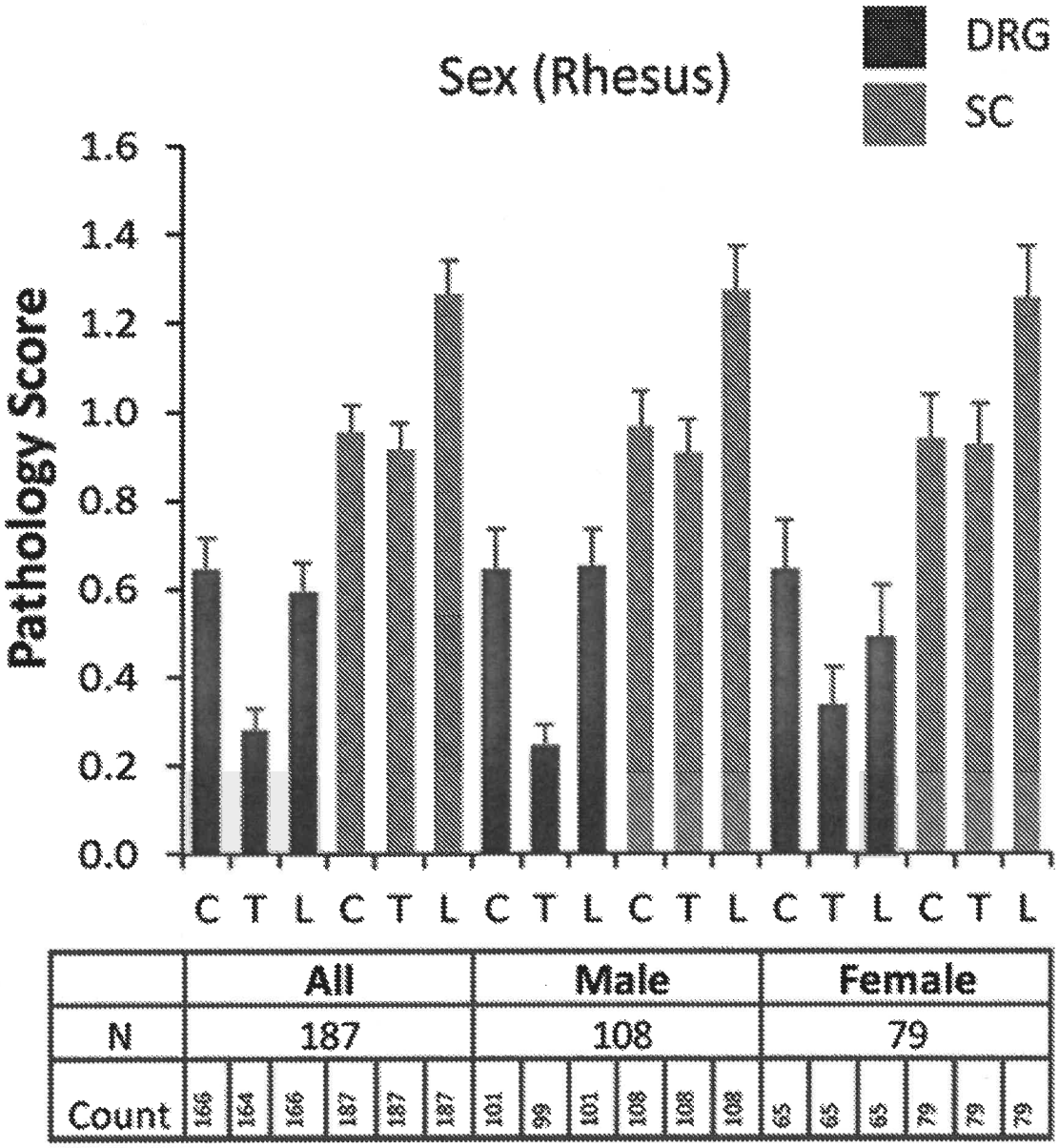

Sex (Rhesus)
DRG
SC
Pathology Score
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C T L C T L C T L C T L C T L C T L
All
Male
Female
N
187
108
79
Count
166 164 166 187 187 187 101 99 101 108 108 108 65 65 65 79 79 79

FIG. 31A

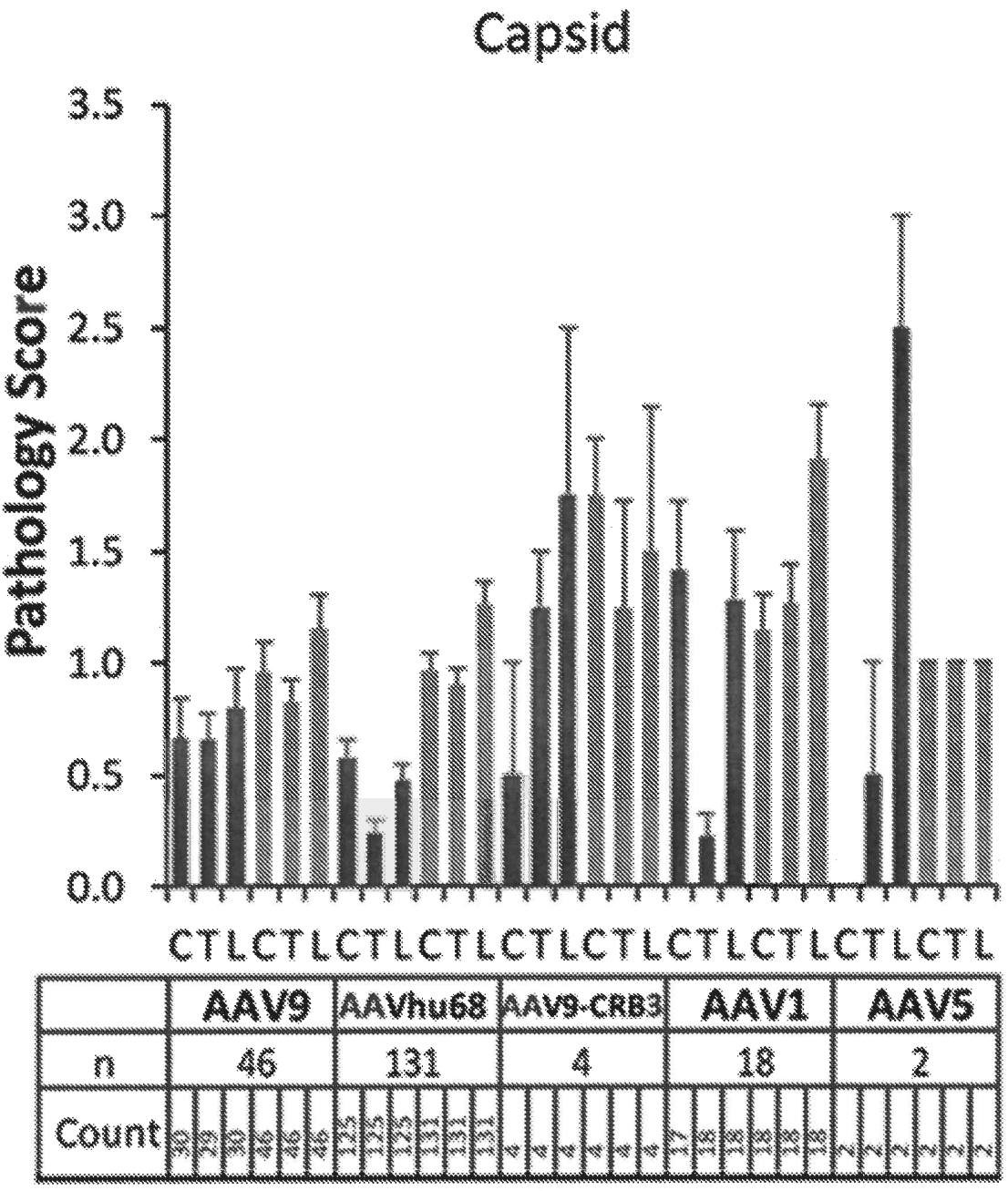
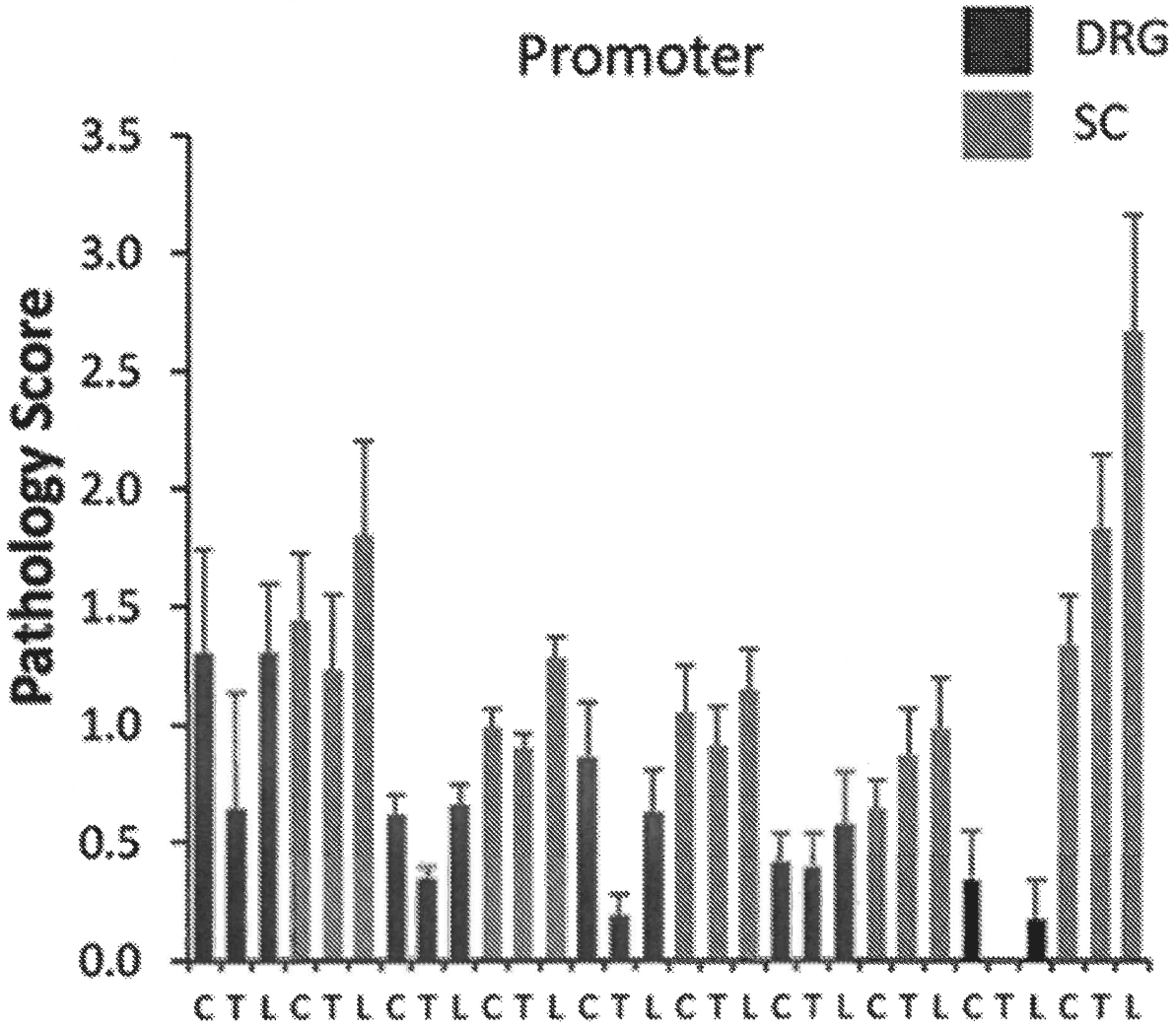

Capsid
Pathology Score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CTLCTLCTLCTLCTLCTLCTLCTLCTLCTL
AAV9
AAVhu68
AAV9-CRB3
AAV1
AAV5
n
46
131
4
18
2
Count

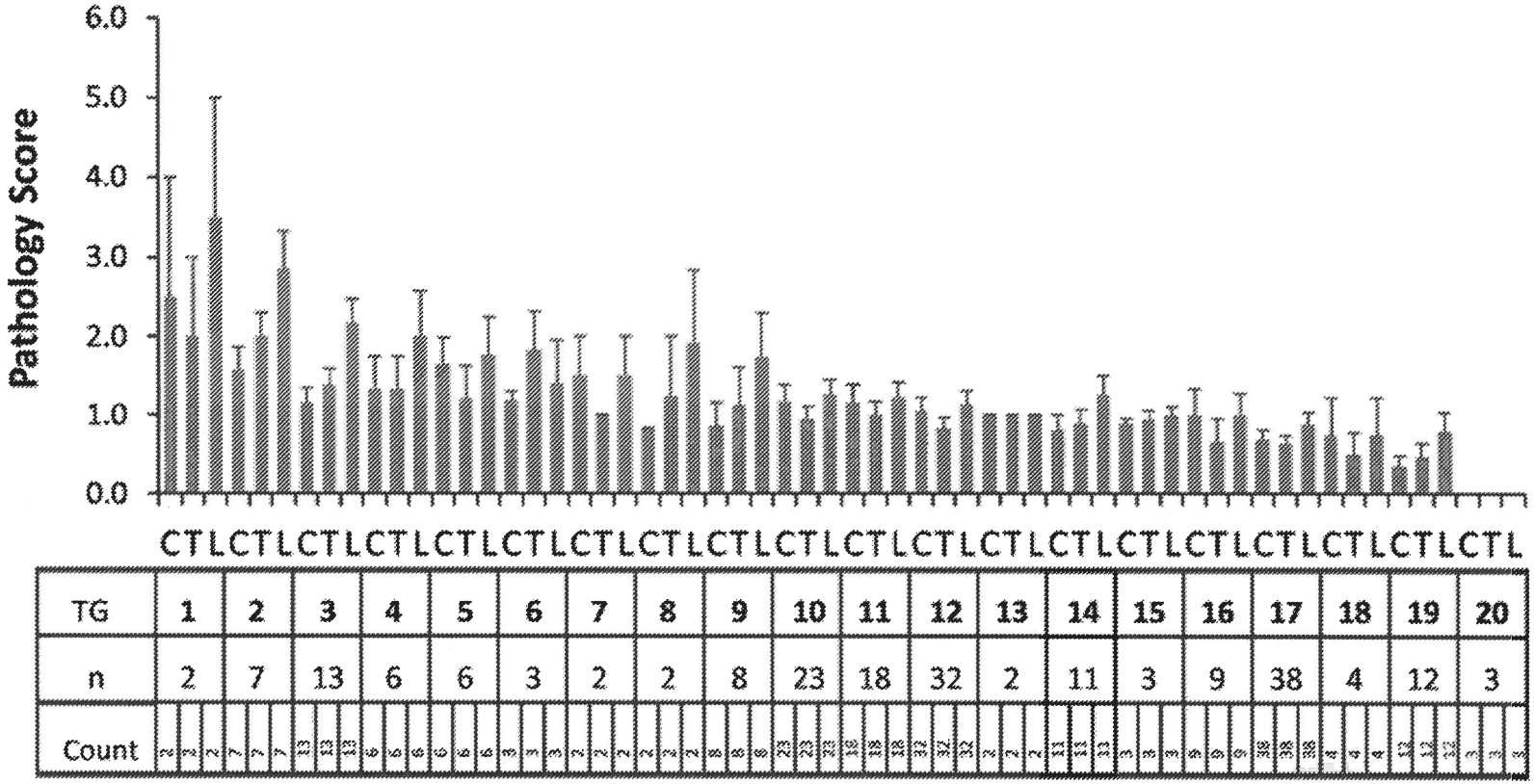
FIG. 31B



	CAG	CB7	UbC	hSyn	MeP426
n	8	153	21	15	6
Count	8 8 8 8 8 8	153 153 153 153 153 153	21 21 21 21 21 21	15 15 15 15 15 15	6 6 6 6 6 6

FIG. 31C

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	2 2 2	7 7 7	13 13 13	6 6 6	6 6 6	3 3 3	2 2 2	2 2 2	8 8 8	23 23 23	18 18 18	32 32 32	2 2 2	11 11 11	3 3 3	9 9 9	38 38 38	4 4 4	12 12 12	3 3 3