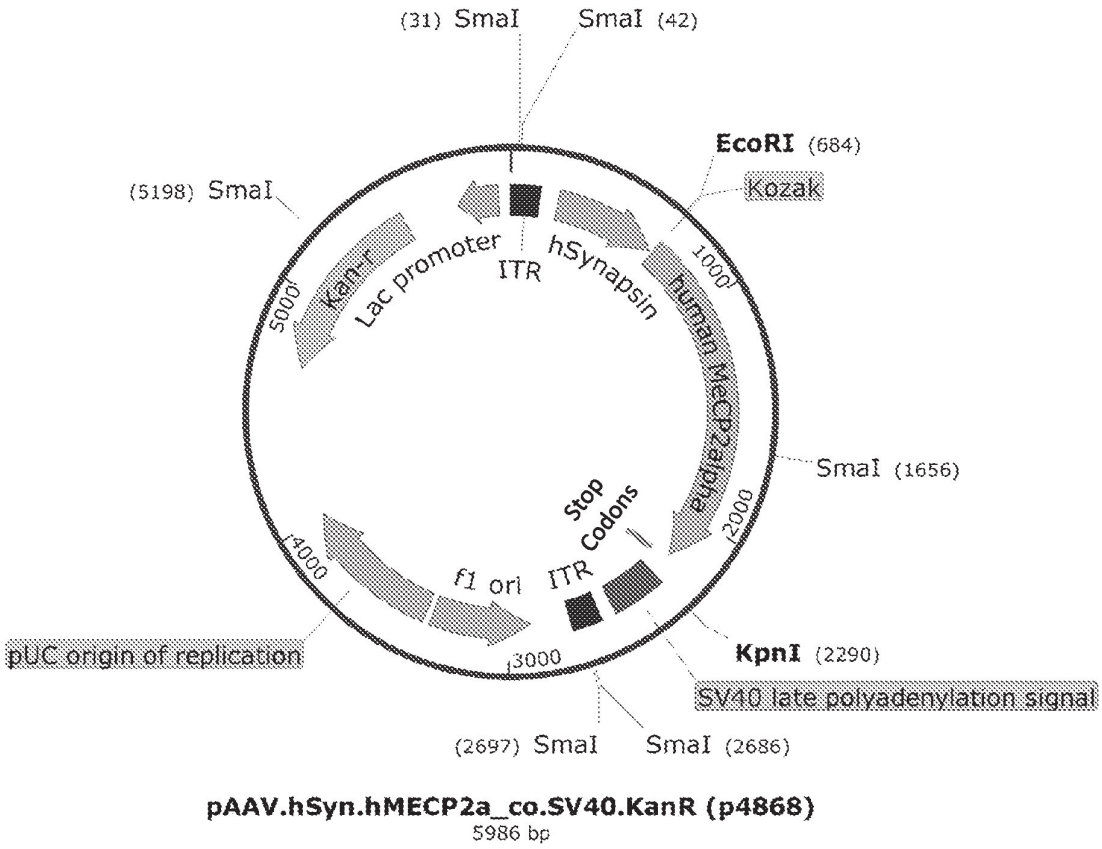

(31) SmaI
SmaI (42)
EcoRI (684)
Kozak
(5198) SmaI
Kan-r
Lac promoter
ITR
hSynapsin
1000
human MeCP2alpha
5000
SmaI (1656)
Stop Codons
2000
4000
f1 ori
ITR
pUC origin of replication
3000
KpnI (2290)
SV40 late polyadenylation signal
(2697) SmaI
SmaI (2686)
pAAV.hSyn.hMECP2a_co.SV40.KanR (p4868)
5986 bp

FIG. 1

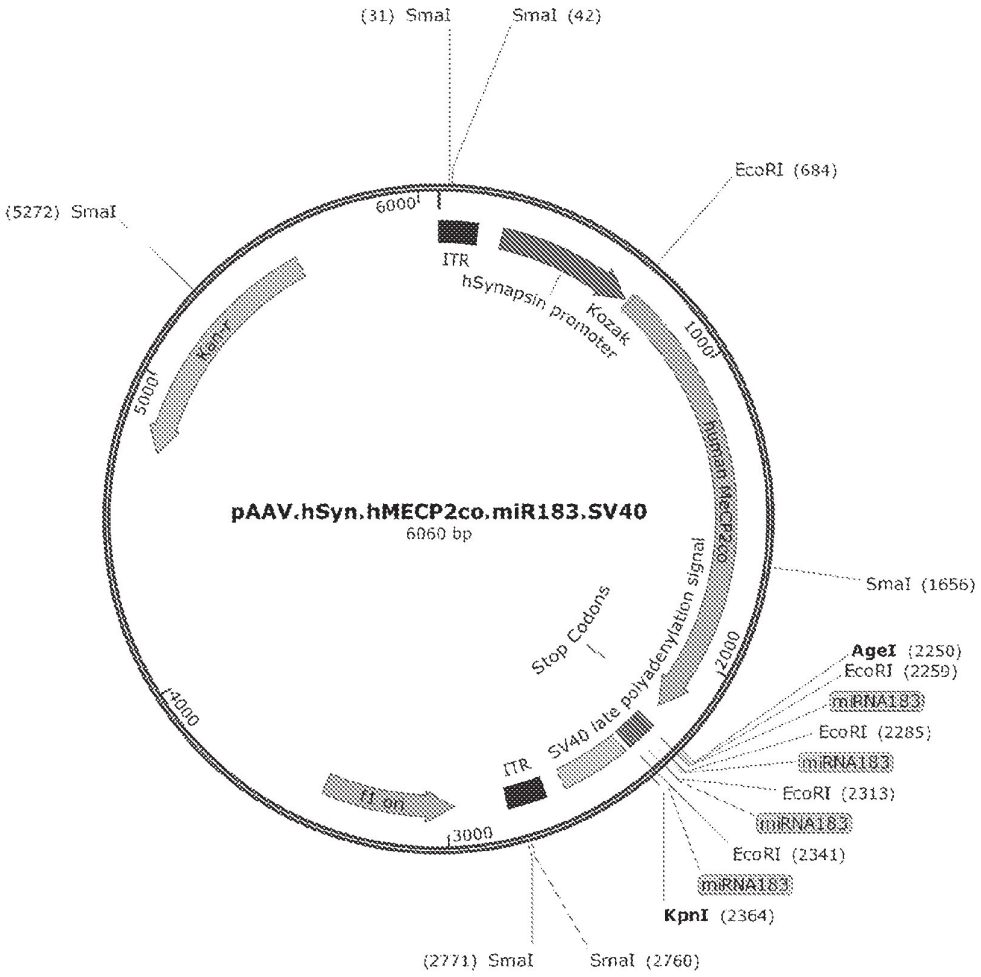

(31) SmaI
SmaI (42)
EcoRI (684)
(5272) SmaI
6000
ITR
hSynapsin promoter
Kozak
1000
Kan-r
5000
human MeCP2co
pAAV.hSyn.hMECP2co.miR183.SV40
6060 bp
SmaI (1656)
Stop Codons
SV40 late polyadenylation signal
2000
AgeI (2250)
EcoRI (2259)
miRNA183
EcoRI (2285)
miRNA183
EcoRI (2313)
miRNA183
EcoRI (2341)
miRNA183
KpnI (2364)
4000
ITR
f1 on
3000
(2771) SmaI
SmaI (2760)

FIG. 2A

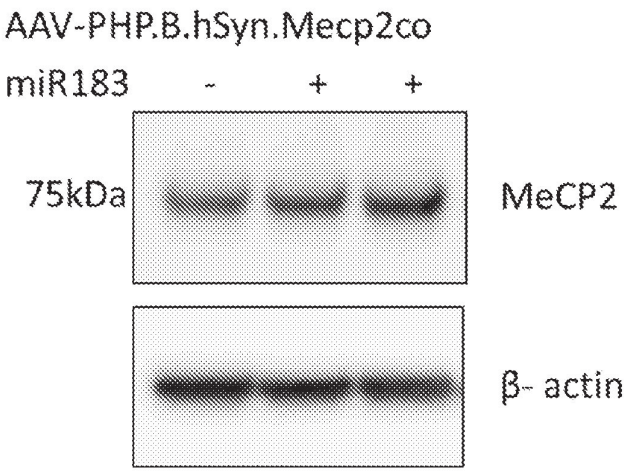


FIG. 2B

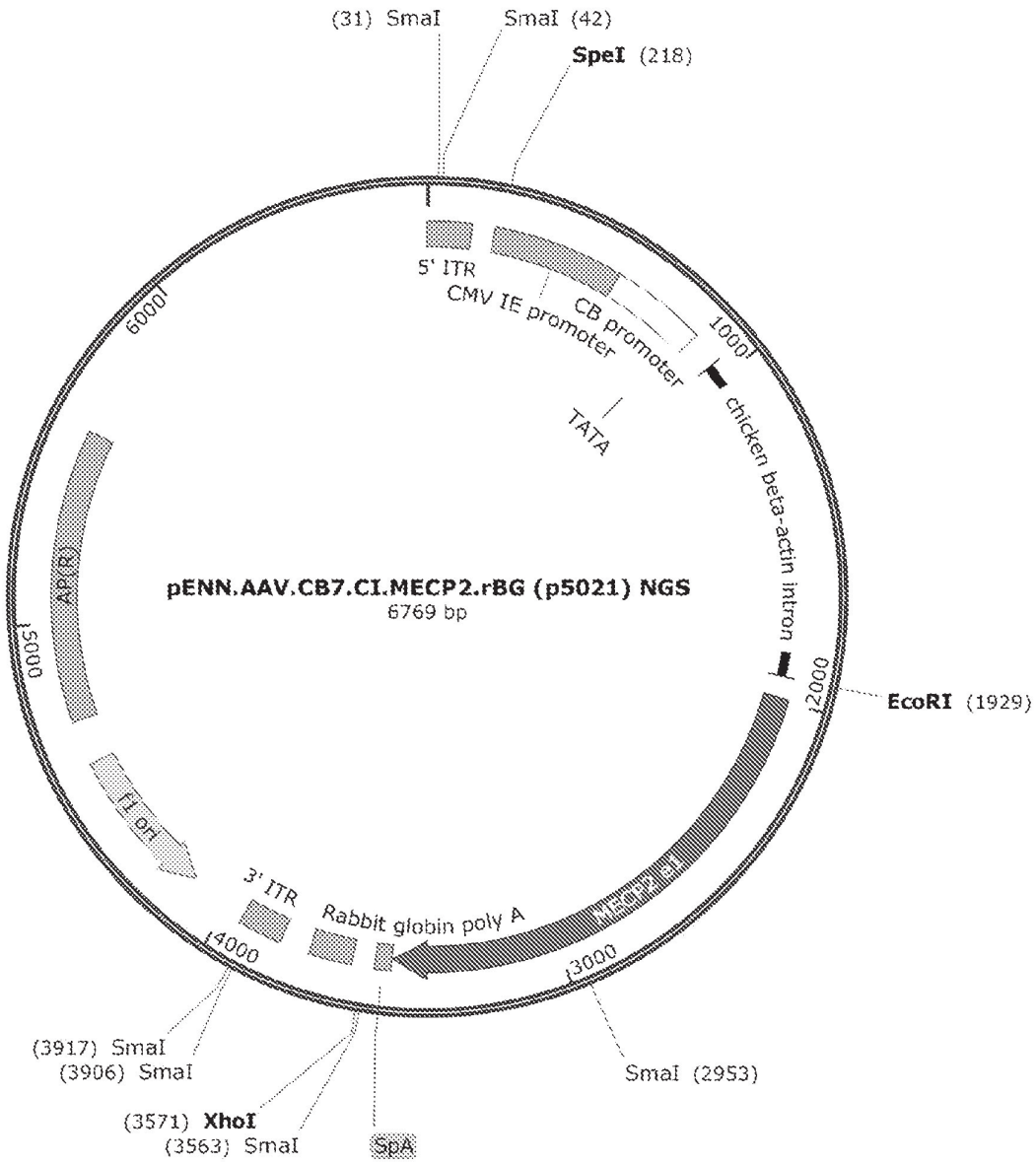

(31) SmaI
SmaI (42)
SpeI (218)
5' ITR
CMV IE promoter
CB promoter
1000
chicken beta-actin intron
TATA
pENN.AAV.CB7.CI.MECP2.rBG (p5021) NGS
6769 bp
6000
AP^R
5000
f1 ori
3' ITR
Rabbit globin poly A
4000
2000
EcoRI (1929)
3000
SmaI (2953)
(3917) SmaI
(3906) SmaI
(3571) XhoI
(3563) SmaI
SpA

FIG. 3

Mecp2-ko cortex (neg. ctrl)

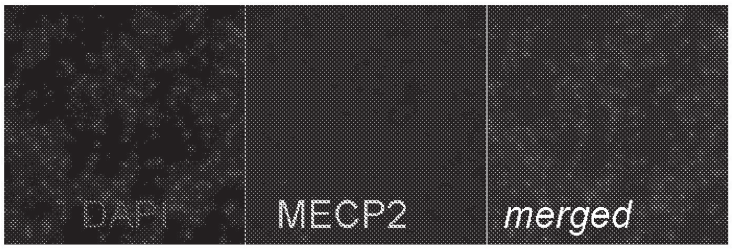


FIG. 4A FIG.4B FIG. 4C

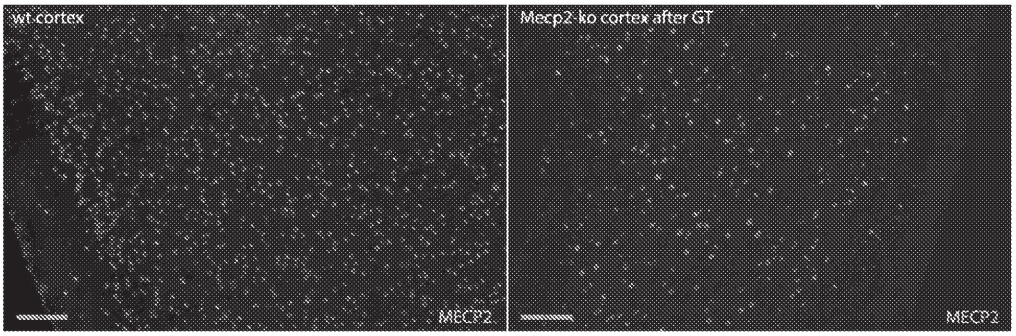


FIG. 4D FIG. 4E

FIG. 5A

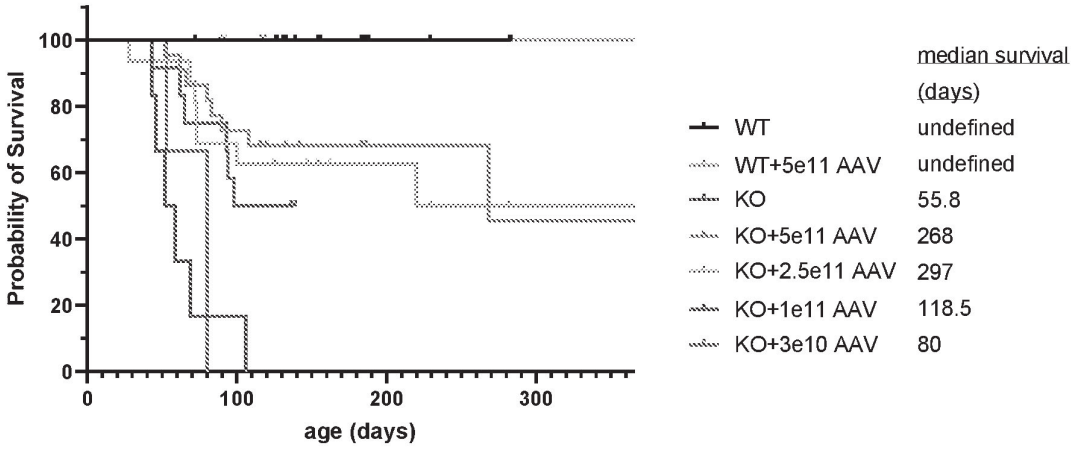

Probability of Survival
100
80
60
40
20
0
0
100
200
300
age (days)
median survival (days)
WT undefined
WT+5e11 AAV undefined
KO 55.8
KO+5e11 AAV 268
KO+2.5e11 AAV 297
KO+1e11 AAV 118.5
KO+3e10 AAV 80

FIG. 5B

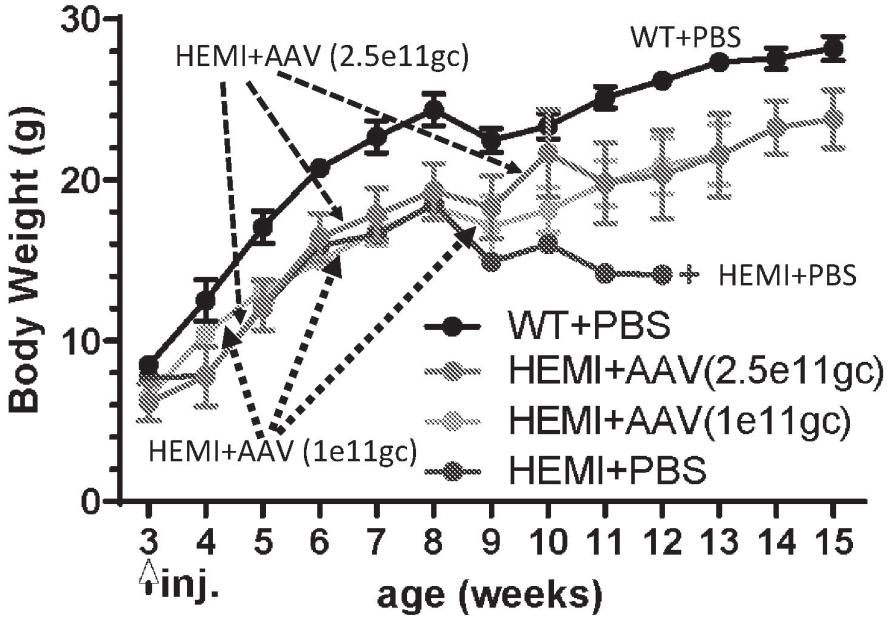

Body Weight (g)
30
20
10
0
HEMI+AAV (2.5e11gc)
WT+PBS
HEMI+PBS
HEMI+AAV (1e11gc)
WT+PBS
HEMI+AAV(2.5e11gc)
HEMI+AAV(1e11gc)
HEMI+PBS
3 4 5 6 7 8 9 10 11 12 13 14 15
inj.
age (weeks)

FIG. 5C

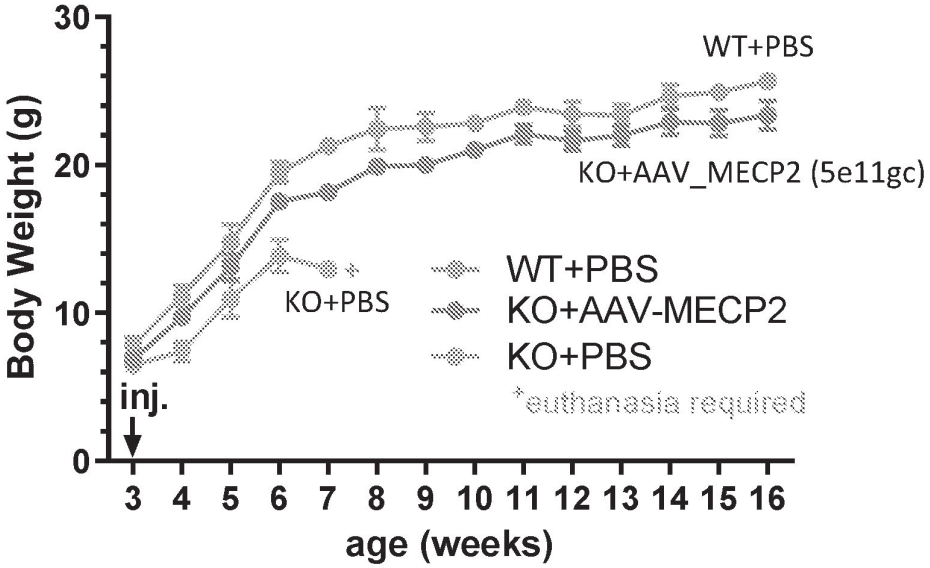

Body Weight (g)
30
20
10
0
WT+PBS
KO+AAV_MECP2 (5e11gc)
KO+PBS
WT+PBS
KO+AAV-MECP2
KO+PBS
*euthanasia required
inj.
3 4 5 6 7 8 9 10 11 12 13 14 15 16
age (weeks)

FIG. 6A

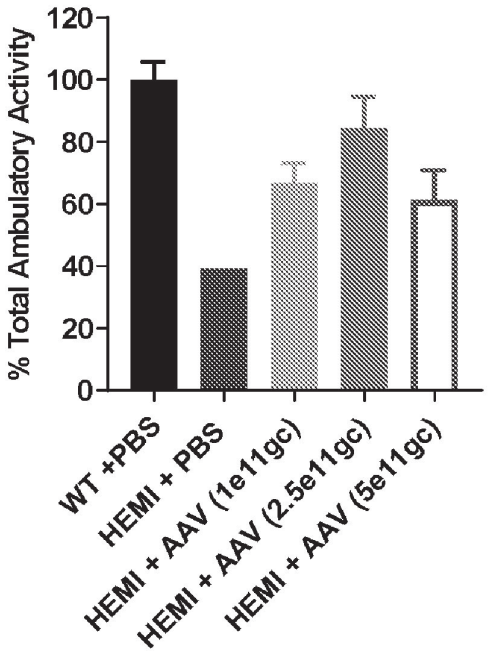

% Total Ambulatory Activity
120
100
80
60
40
20
0
WT +PBS
HEMI + PBS
HEMI + AAV (1e11gc)
HEMI + AAV (2.5e11gc)
HEMI + AAV (5e11gc)

FIG. 6B

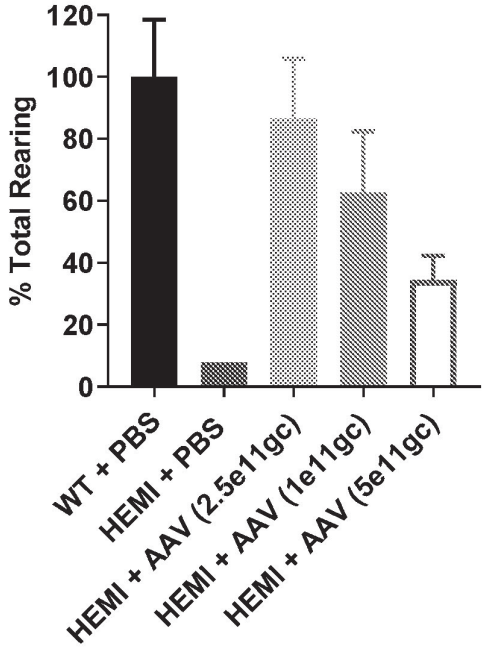

% Total Rearing
120
100
80
60
40
20
0
WT + PBS
HEMI + PBS
HEMI + AAV (2.5e11gc)
HEMI + AAV (1e11gc)
HEMI + AAV (5e11gc)

FIG. 6C

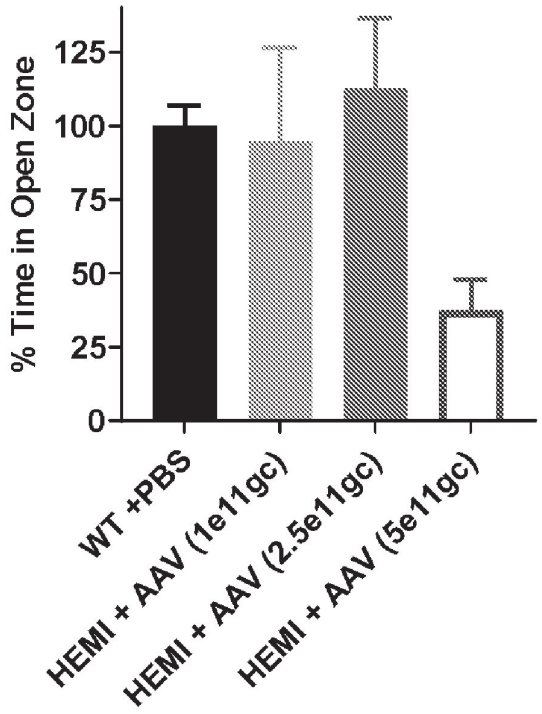
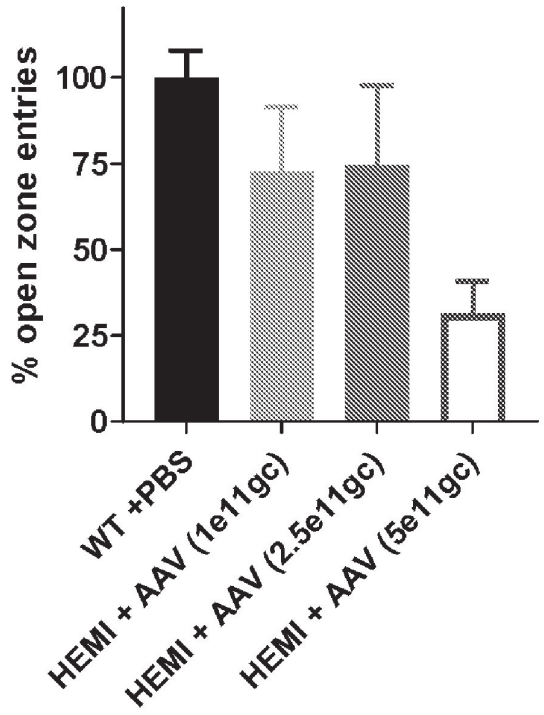

% Time in Open Zone
125
100
75
50
25
0
WT +PBS
HEMI + AAV (1e11gc)
HEMI + AAV (2.5e11gc)
HEMI + AAV (5e11gc)

FIG. 6D


% open zone entries
100
75
50
25
0
WT +PBS
HEMI + AAV (1e11gc)
HEMI + AAV (2.5e11gc)
HEMI + AAV (5e11gc)

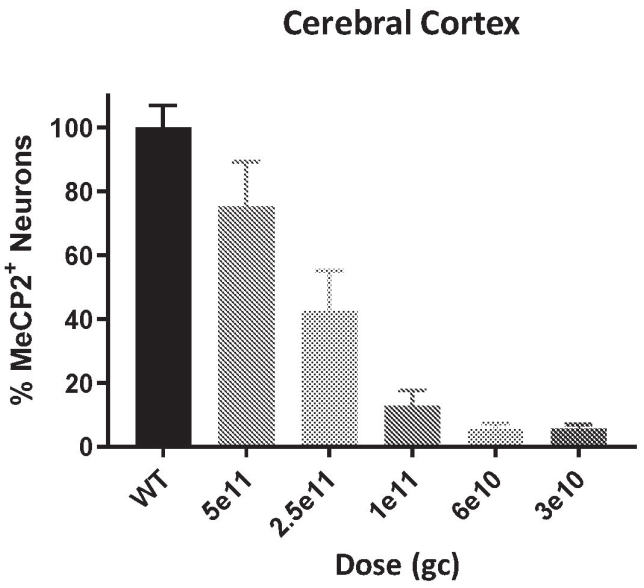

Cerebral Cortex
% MeCP2+ Neurons
100
80
60
40
20
0
WT
5e11
2.5e11
1e11
6e10
3e10
Dose (gc)

FIG. 6E

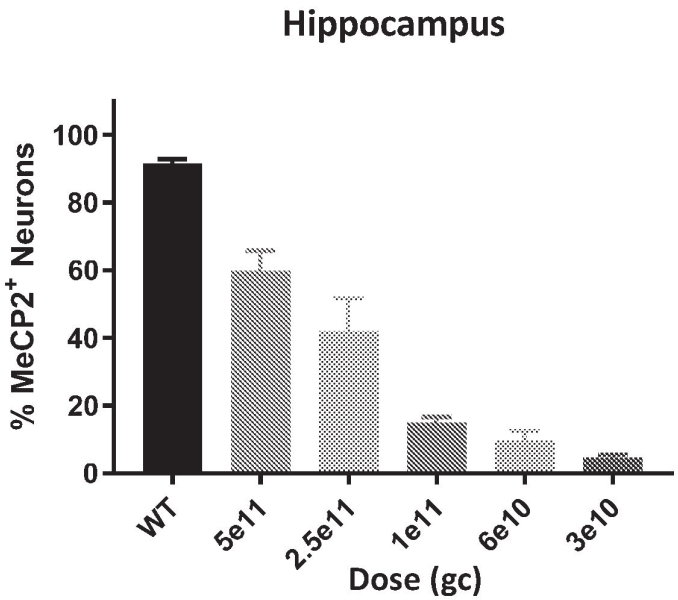

Hippocampus
% MeCP2+ Neurons
100
80
60
40
20
0
WT
5e11
2.5e11
1e11
6e10
3e10
Dose (gc)

FIG. 6F

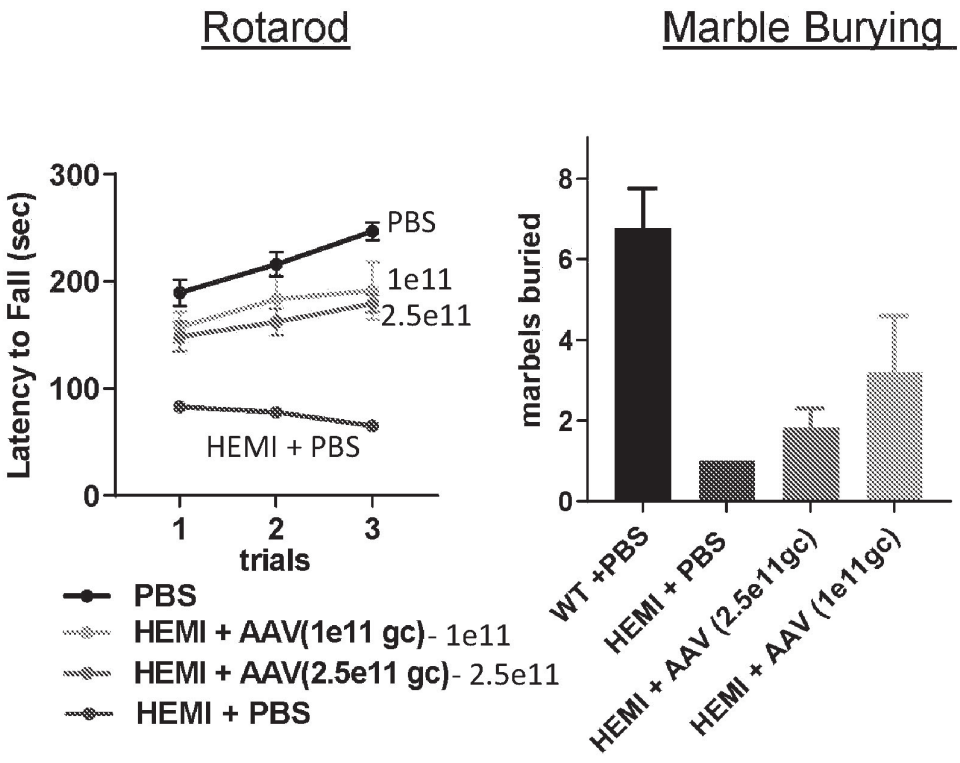
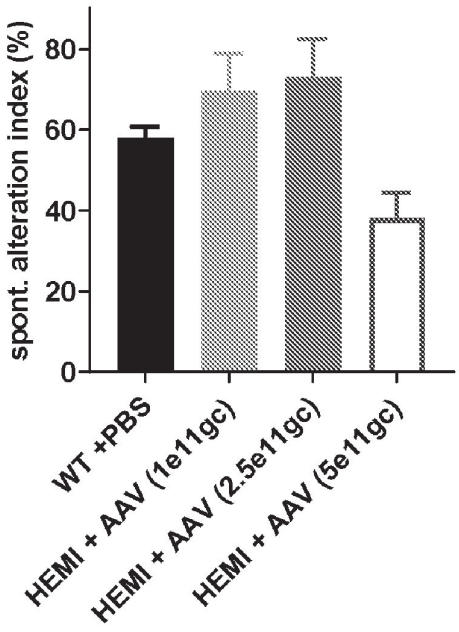

Rotarod
Marble Burying
Latency to Fall (sec)
300
200
100
0
1
2
3
trials
PBS
1e11
2.5e11
HEMI + PBS
PBS
HEMI + AAV(1e11 gc)- 1e11
HEMI + AAV(2.5e11 gc)- 2.5e11
HEMI + PBS
marbels buried
8
6
4
2
0
WT +PBS
HEMI + PBS
HEMI + AAV (2.5e11gc)
HEMI + AAV (1e11gc)

FIG. 7A

FIG. 7B

FIG. 7C


Y-Maze
spont. alteration index (%)
0
20
40
60
80
WT +PBS
HEMI + AAV (1e11gc)
HEMI + AAV (2.5e11gc)
HEMI + AAV (5e11gc)

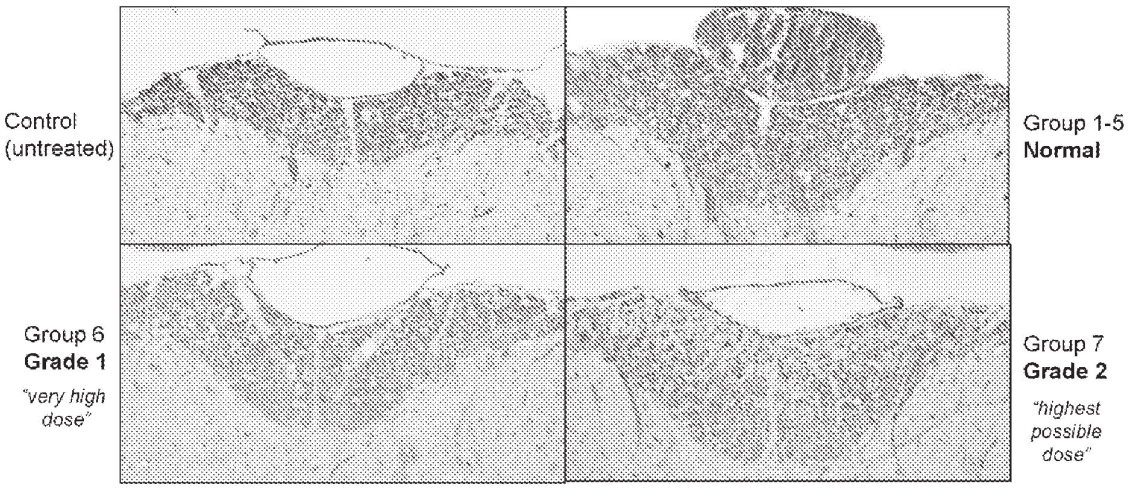

Control
(untreated)
Group 1-5
Normal
Group 6
Grade 1
"very high dose"
Group 7
Grade 2
"highest possible dose"

FIG. 8A

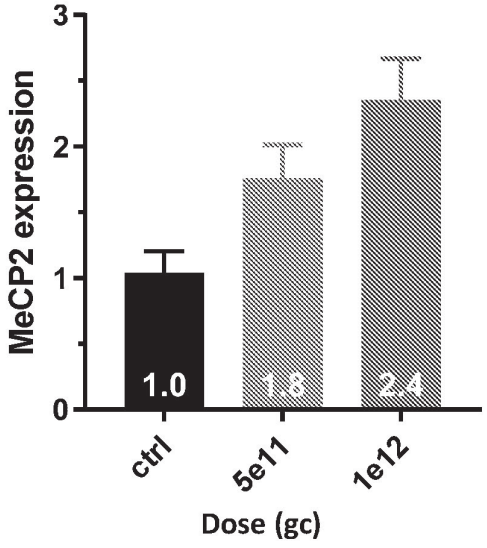

MeCP2 expression
3
2
1
0
1.0
1.8
2.4
ctrl
5e11
1e12
Dose (gc)

FIG. 8B

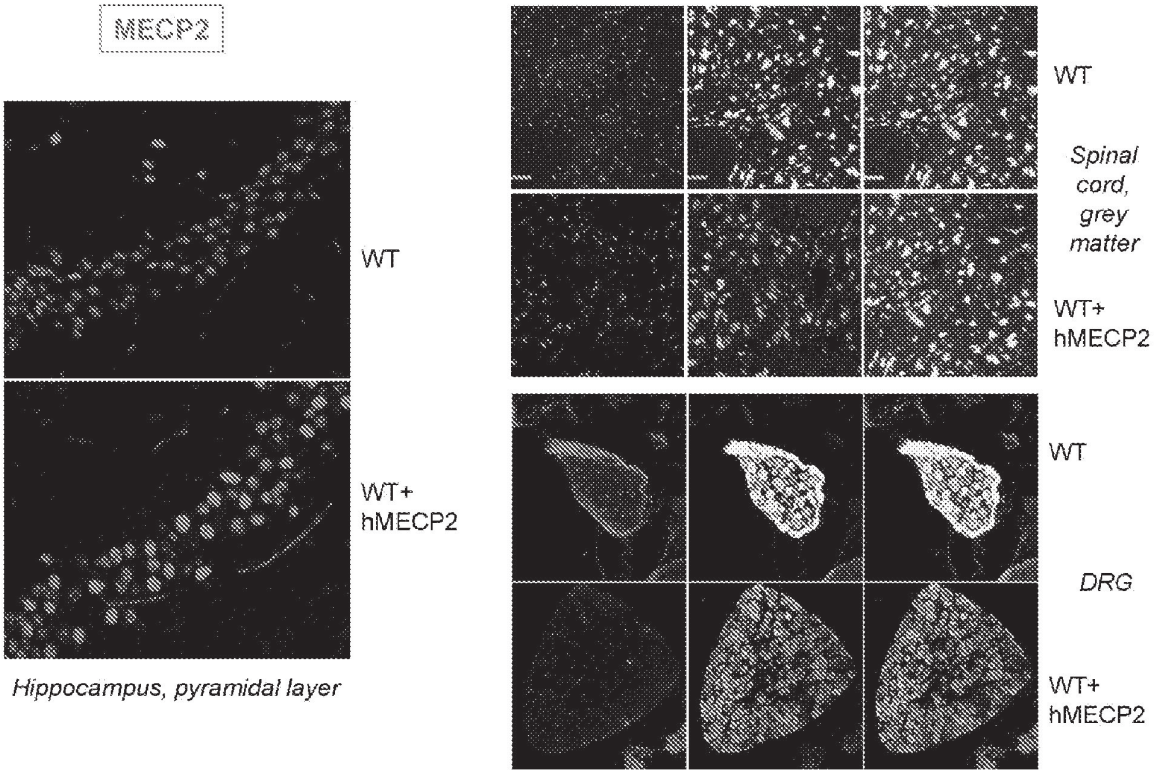

MECP2
WT
WT+
hMECP2
Hippocampus, pyramidal layer
WT
Spinal
cord,
grey
matter
WT+
hMECP2
WT
DRG
WT+
hMECP2

FIG. 9

FIG. 10A

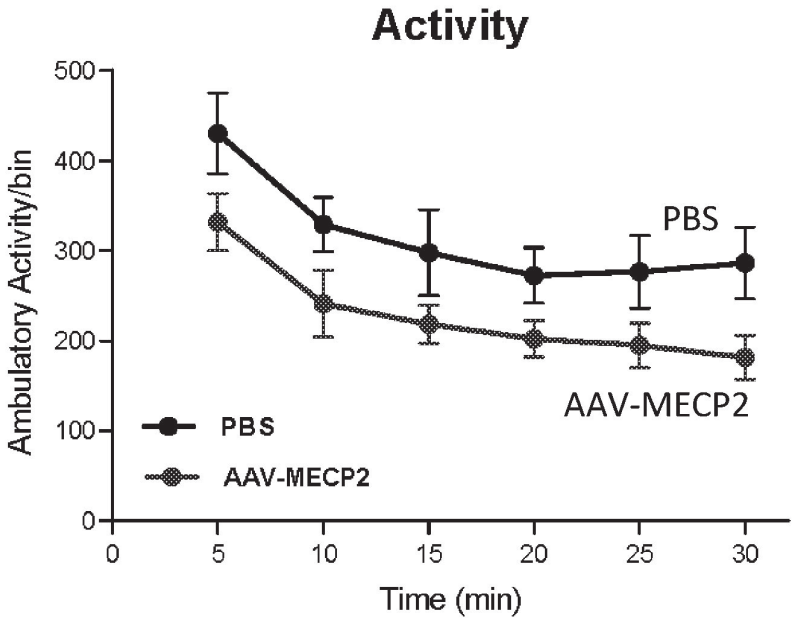


FIG. 10B

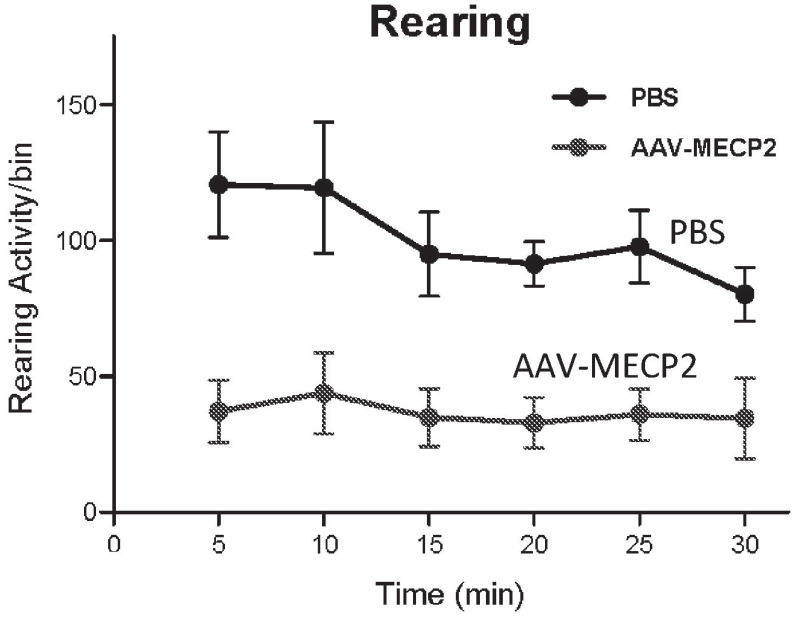


FIG. 10C

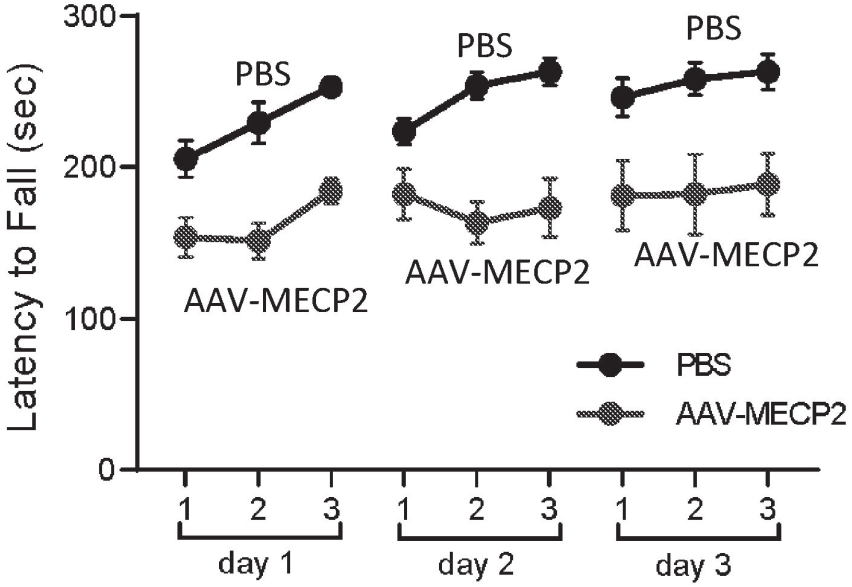


FIG. 11

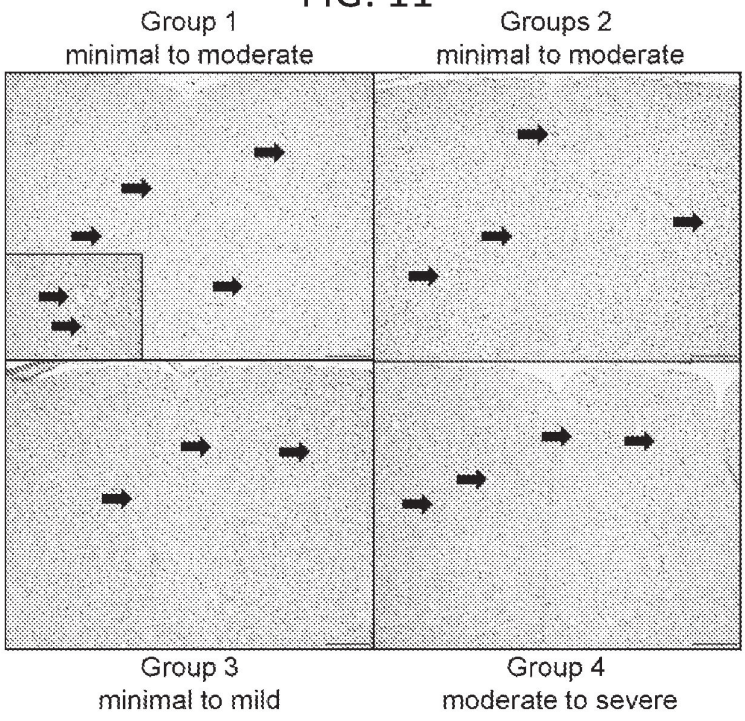
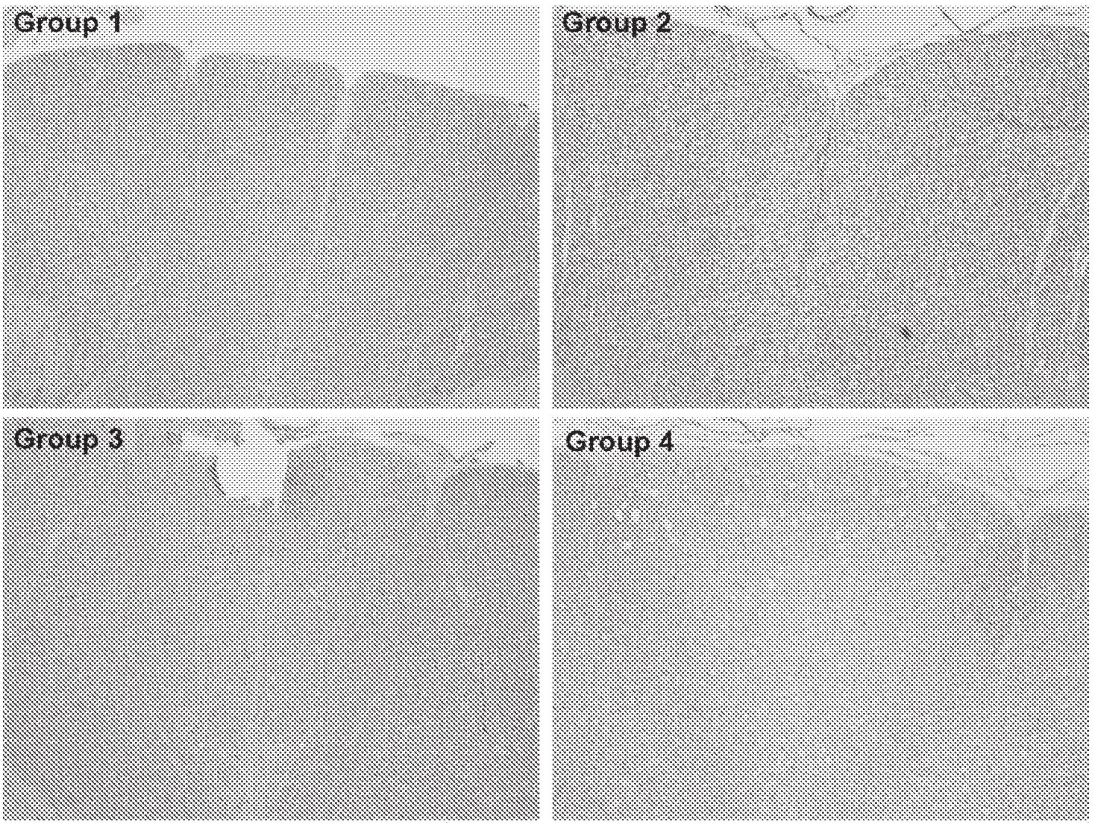


FIG. 12



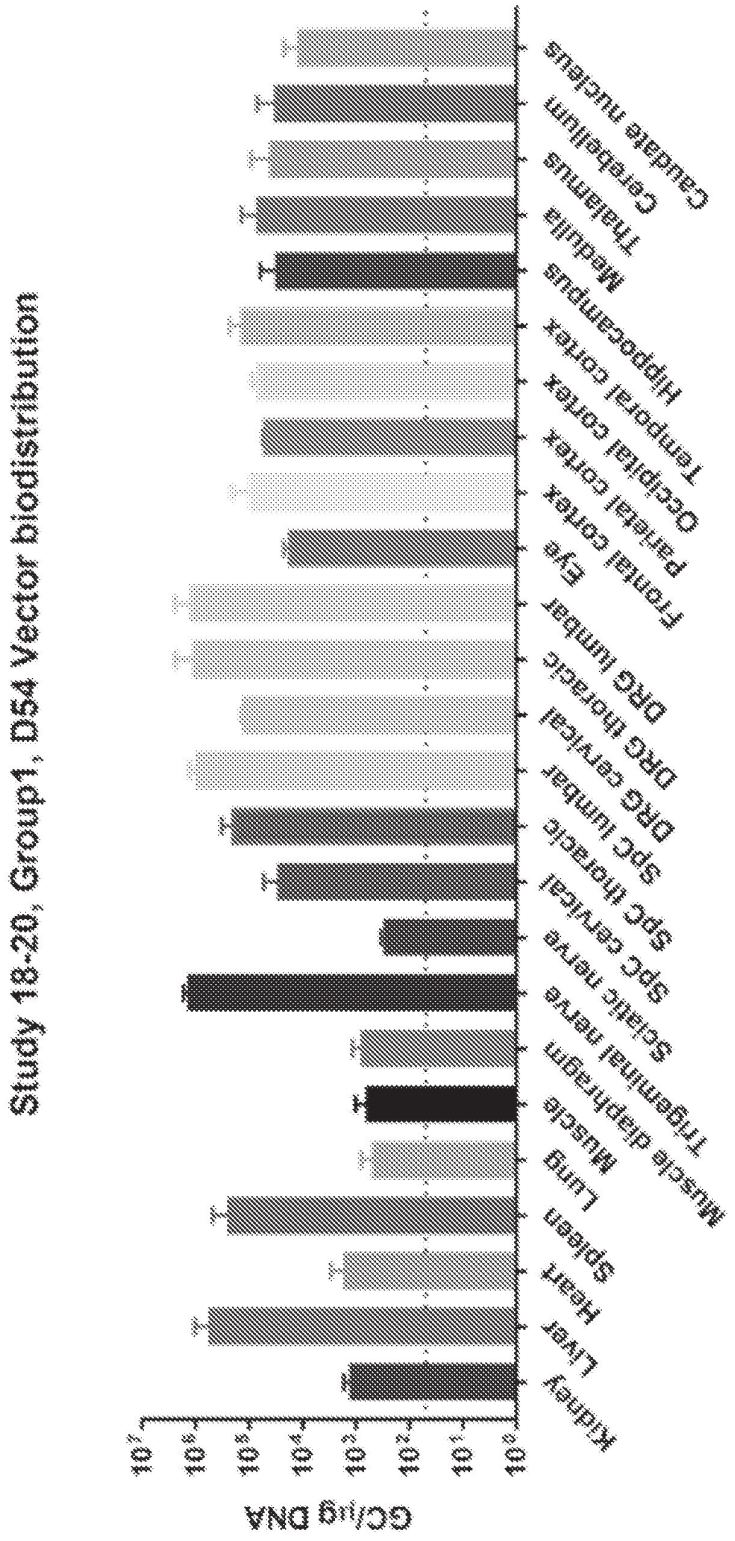

Study 18-20, Group1, D54 Vector biodistribution
GC/µg DNA
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 13A

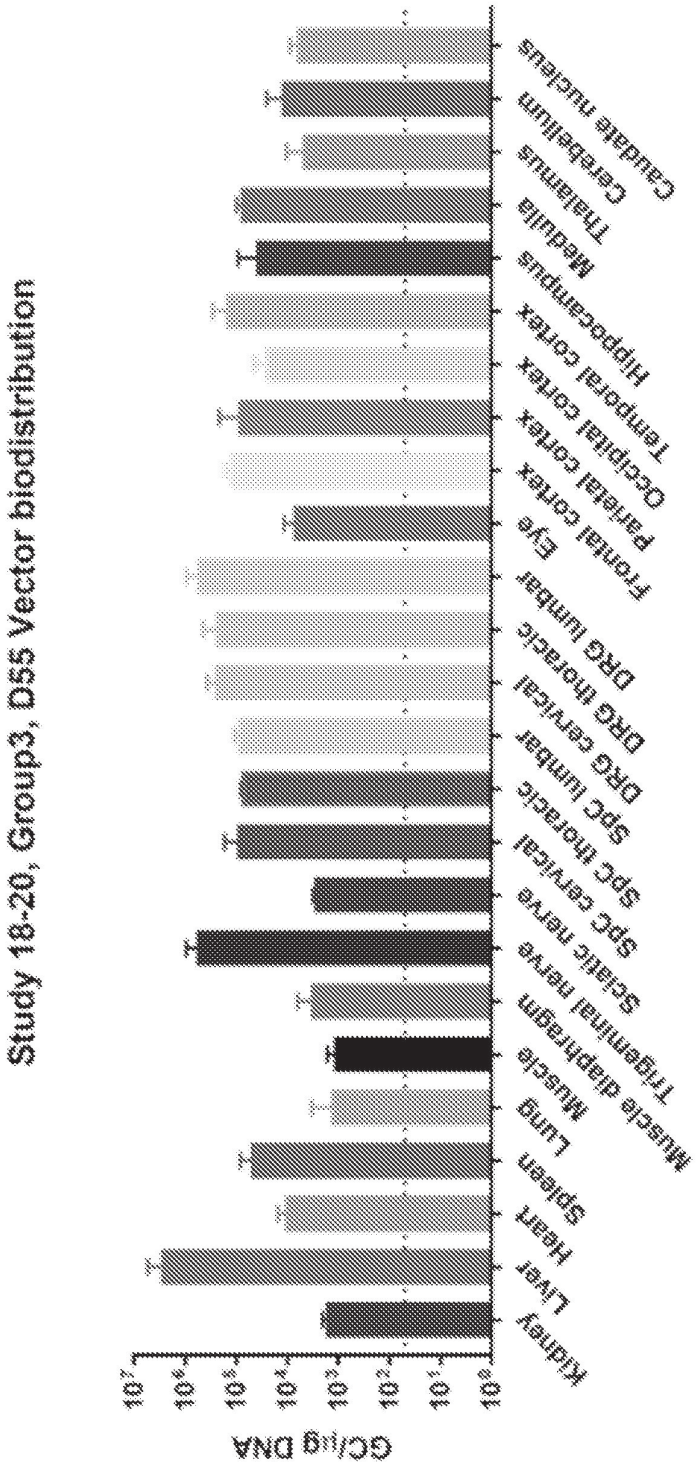

Study 18-20, Group3, D55 Vector biodistribution
GC/µg DNA
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 13B

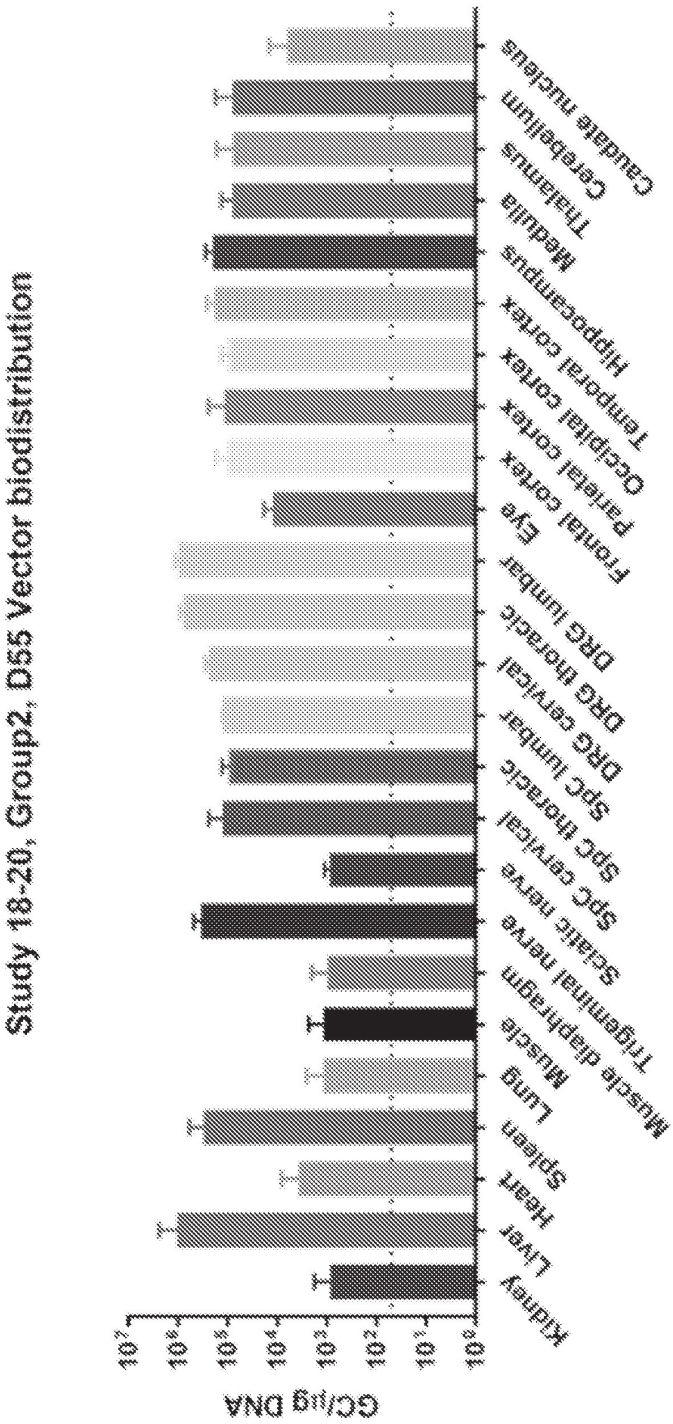

Study 18-20, Group2, D55 Vector biodistribution
GC/μg DNA
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 13C

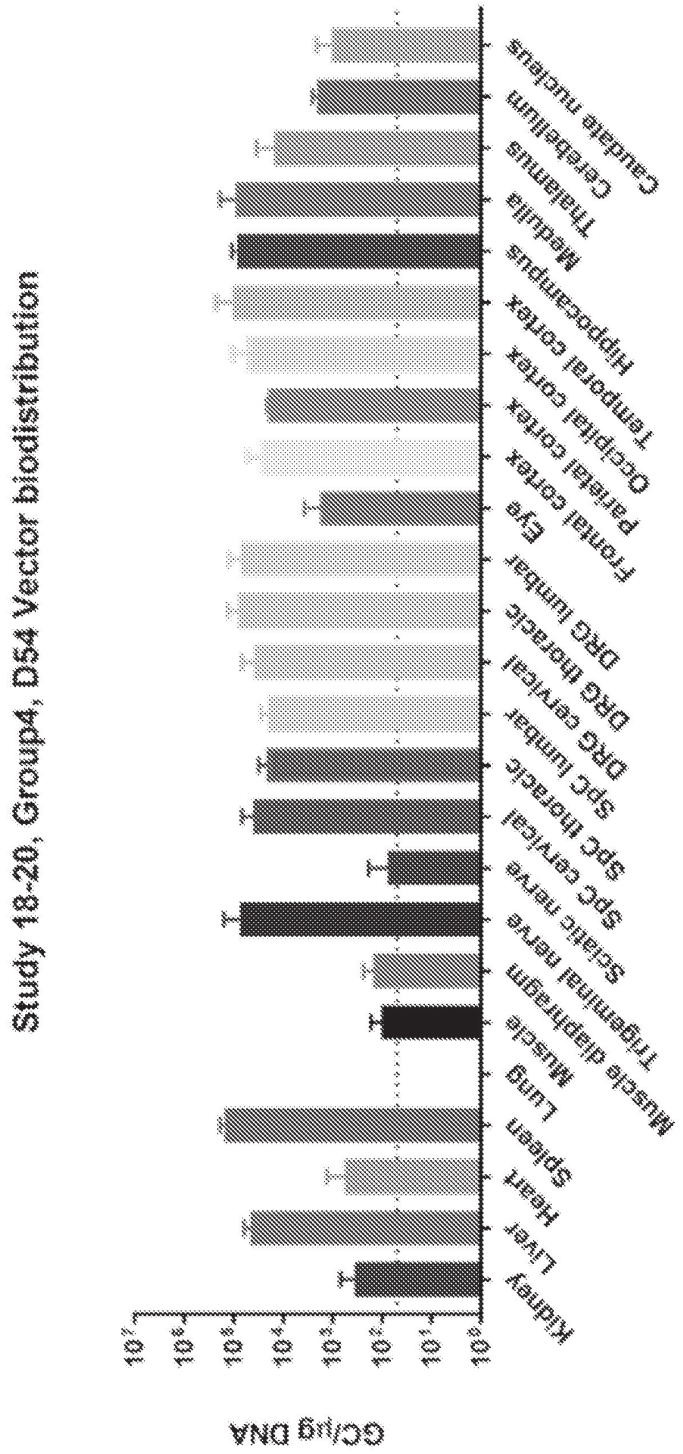

Study 18-20, Group4, D54 Vector biodistribution
GC/µg DNA
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 13D

FIG. 14

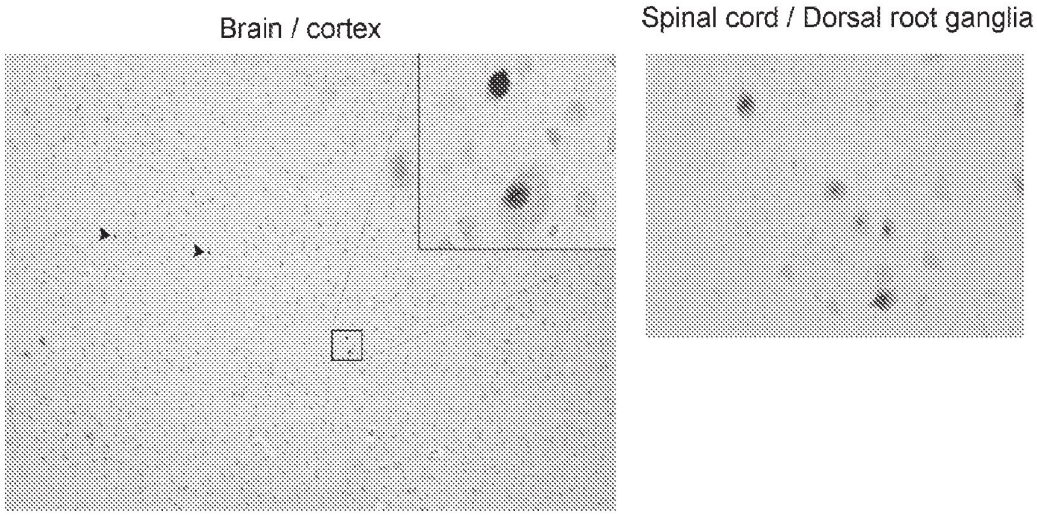
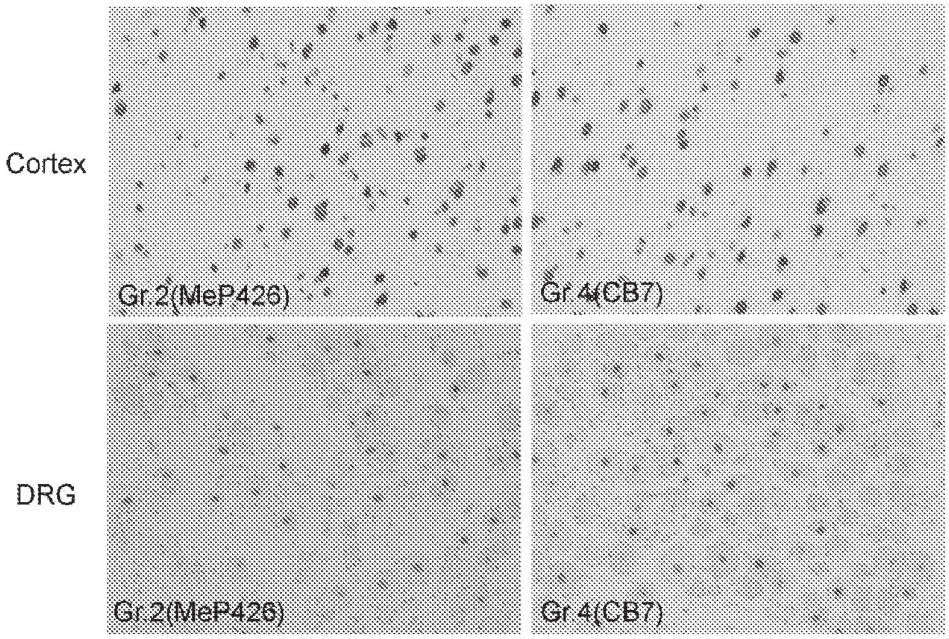

Brain / cortex
Spinal cord / Dorsal root ganglia

FIG. 15


Cortex
DRG
Gr.2(MeP426)
Gr.4(CB7)
Gr.2(MeP426)
Gr.4(CB7)

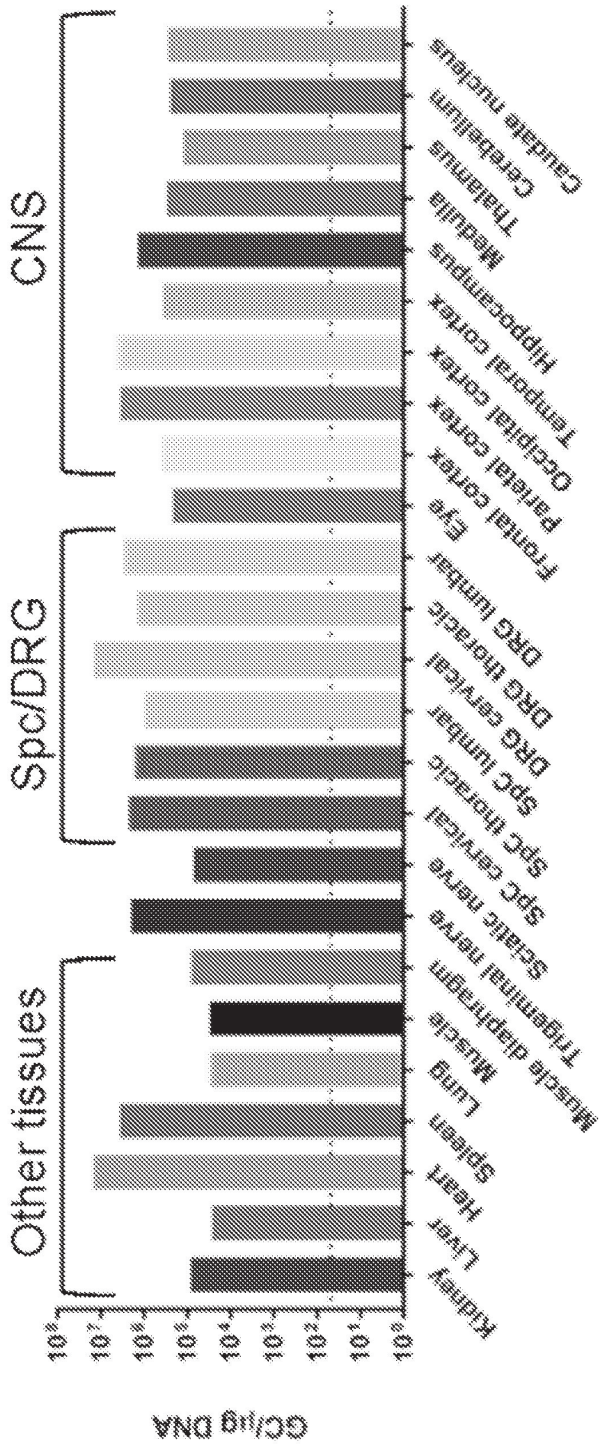

GC/µg DNA
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Other tissues
Spc/DRG
CNS
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
Spc cervical
Spc thoracic
Spc lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 16A

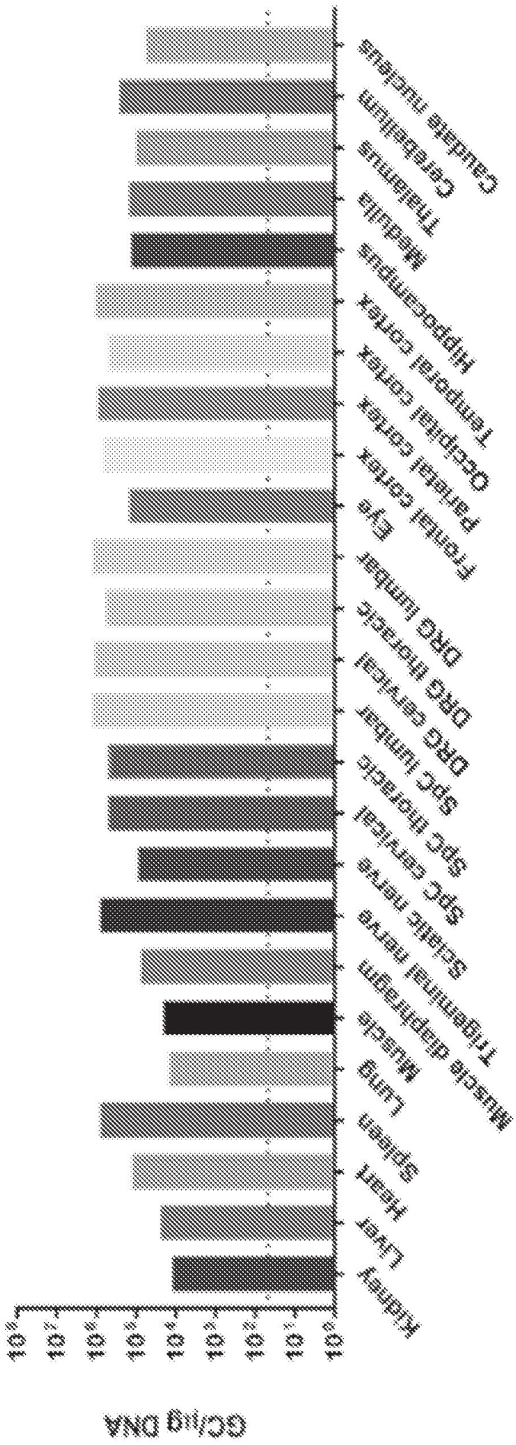

GC/µg DNA
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 16B

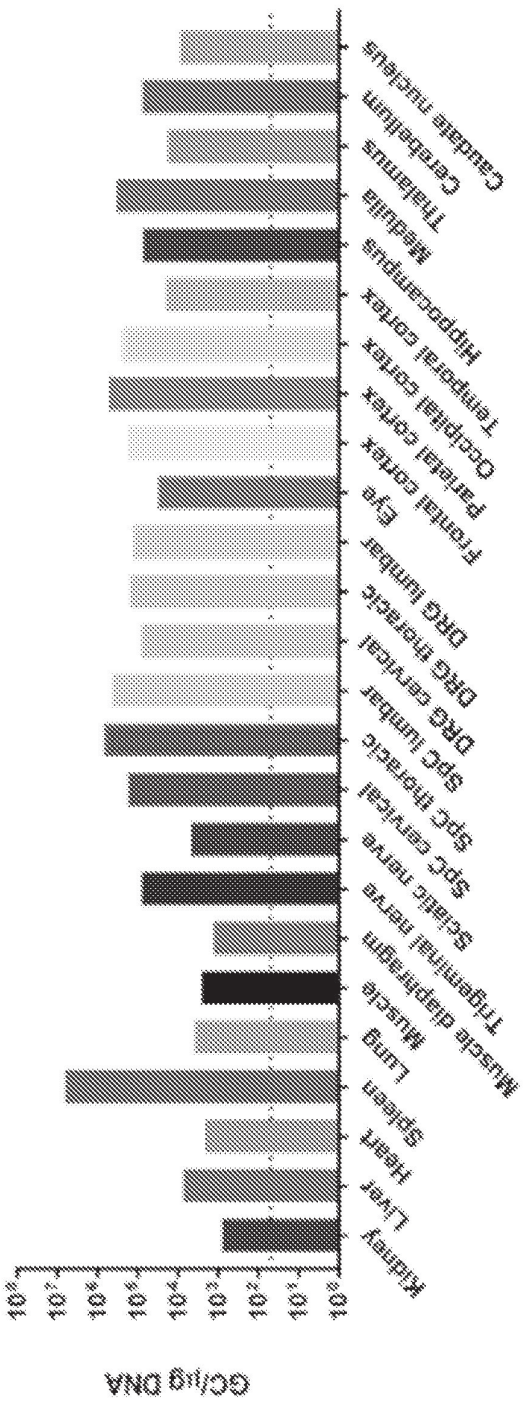
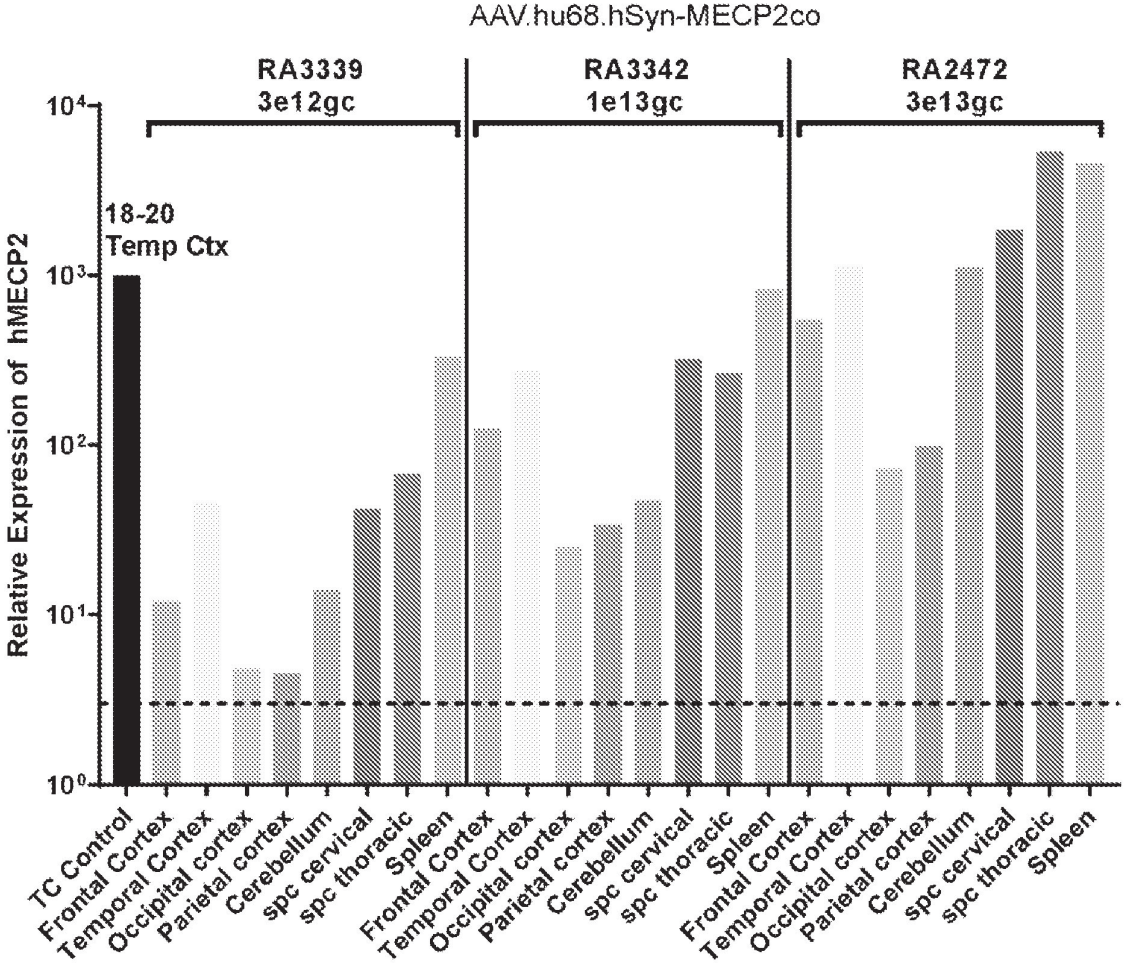

GC/µg DNA
10⁰
10¹
10²
10³
10⁴
10⁵
10⁶
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG. 16C

FIG. 17


AAV.hu68.hSyn-MECP2co
RA3339
3e12gc
RA3342
1e13gc
RA2472
3e13gc
Relative Expression of hMECP2
10^4
10^3
10^2
10^1
10^0
18-20
Temp Ctx
TC Control
Frontal Cortex
Temporal Cortex
Occipital cortex
Parietal cortex
Cerebellum
spc cervical
spc thoracic
Spleen
Frontal Cortex
Temporal Cortex
Occipital cortex
Parietal cortex
Cerebellum
spc cervical
spc thoracic
Spleen
Frontal Cortex
Temporal Cortex
Occipital cortex
Parietal cortex
Cerebellum
spc cervical
spc thoracic
Spleen

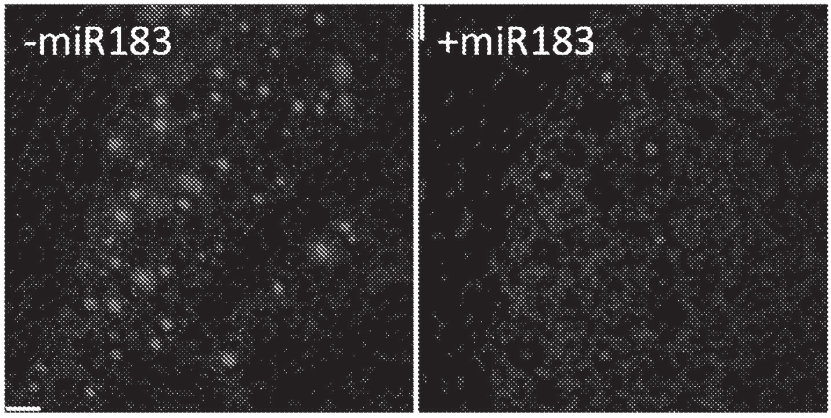


FIG. 18A

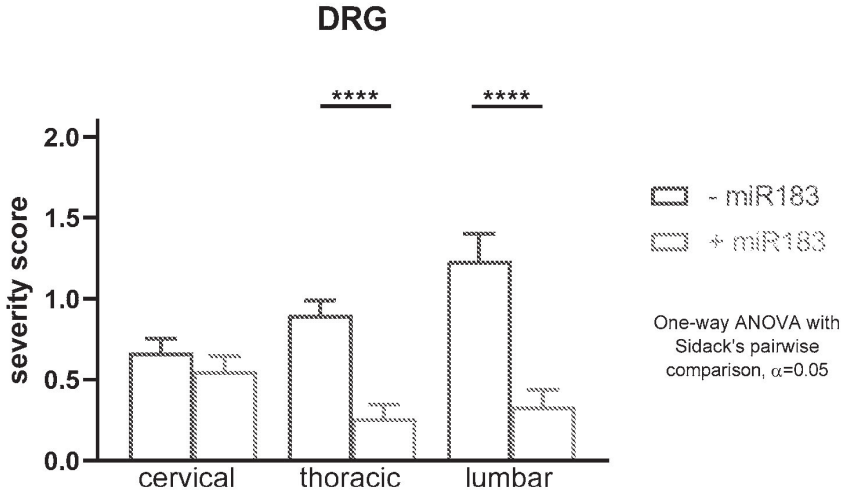


FIG. 18B

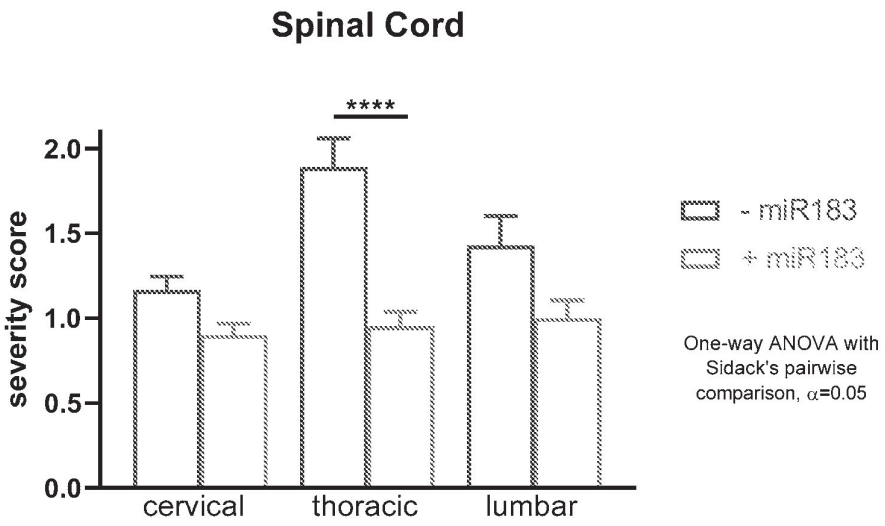

Spinal Cord
****
severity score
2.0
1.5
1.0
0.5
0.0
cervical
thoracic
lumbar
- miR183
+ miR183
One-way ANOVA with Sidack's pairwise comparison, α=0.05

FIG. 18C