FIG 1A



FIG 1B

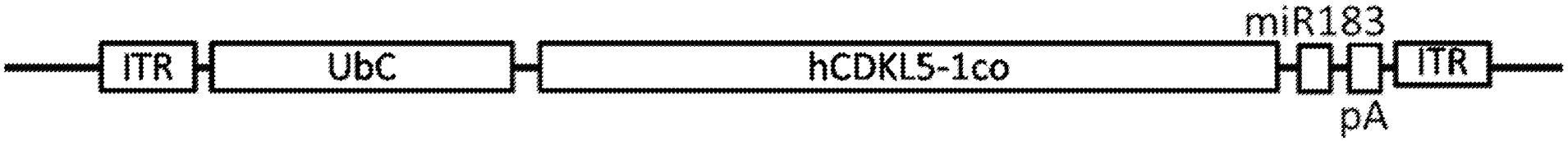


FIG 1C

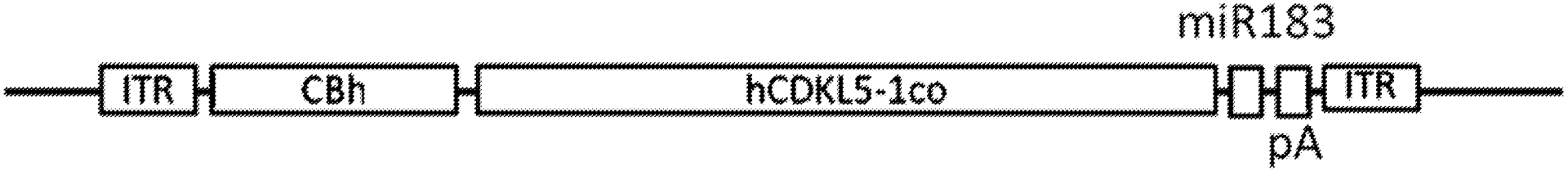


FIG 2A

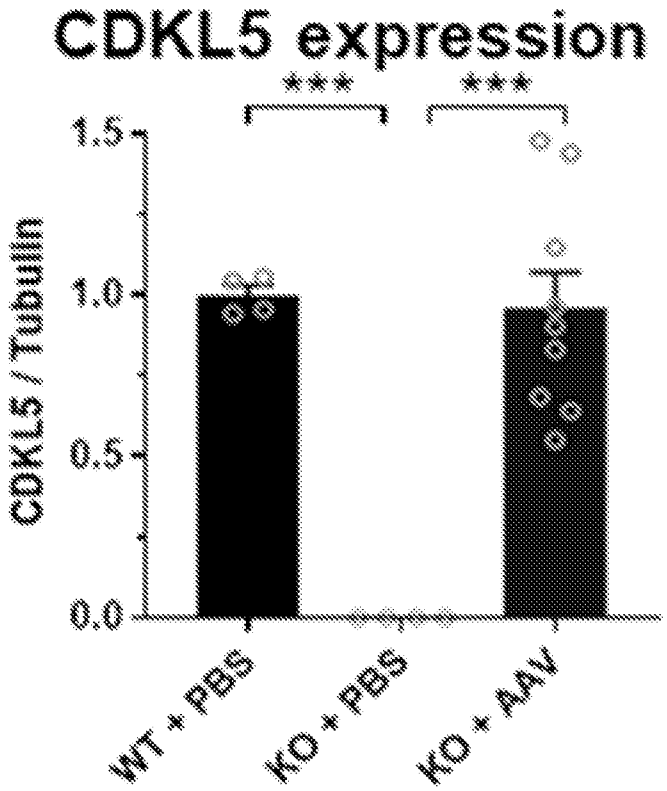

CDKL5 expression
***
***
CDKL5 / Tubulin
1.5
1.0
0.5
0.0
WT + PBS
KO + PBS
KO + AAV

FIG 2B

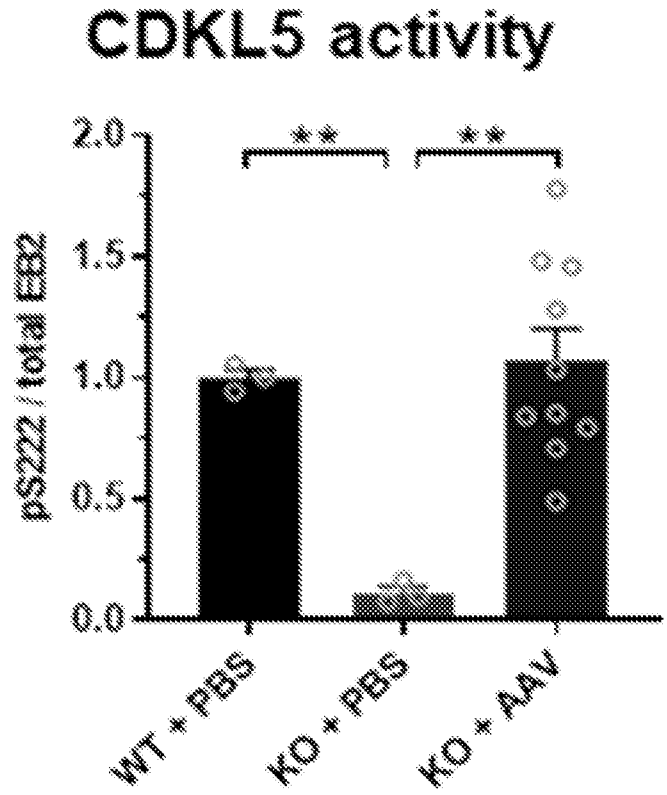

CDKL5 activity
**
**
pS222 / total EB2
2.0
1.5
1.0
0.5
0.0
WT + PBS
KO + PBS
KO + AAV

FIG 3

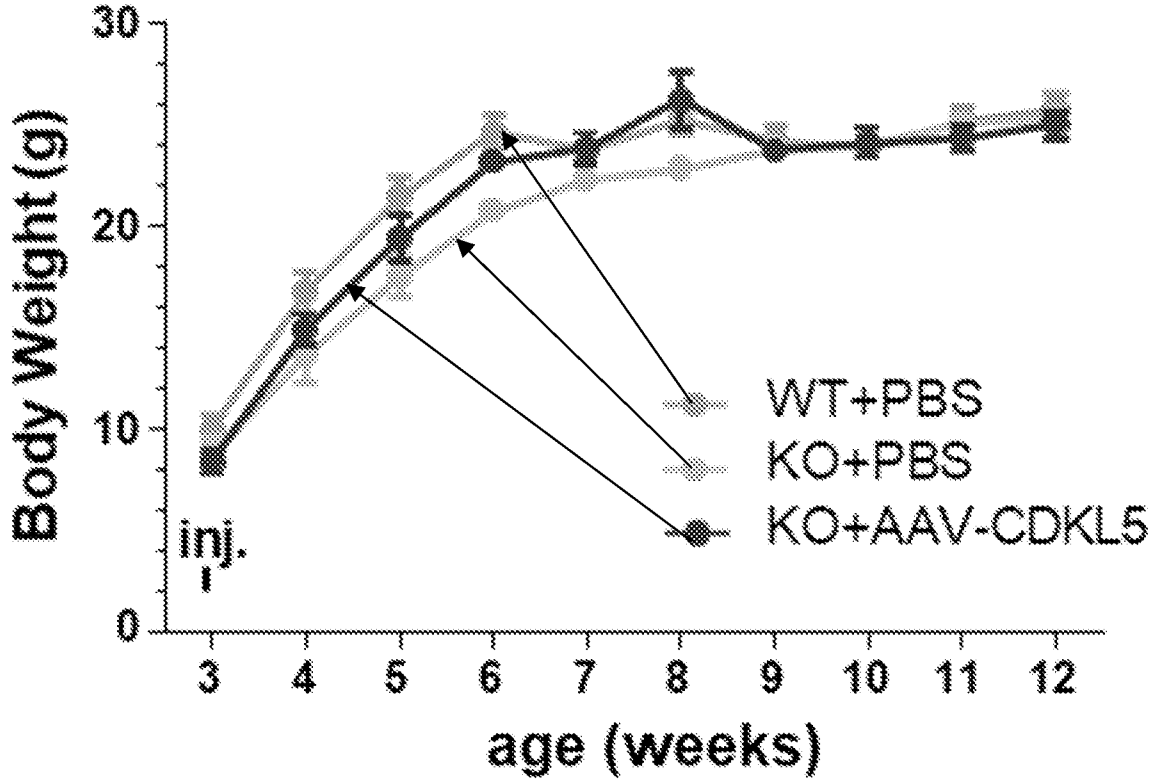

Body Weight (g)
30
20
10
0
inj.
3
4
5
6
7
8
9
10
11
12
age (weeks)
WT+PBS
KO+PBS
KO+AAV-CDKL5

FIG 4

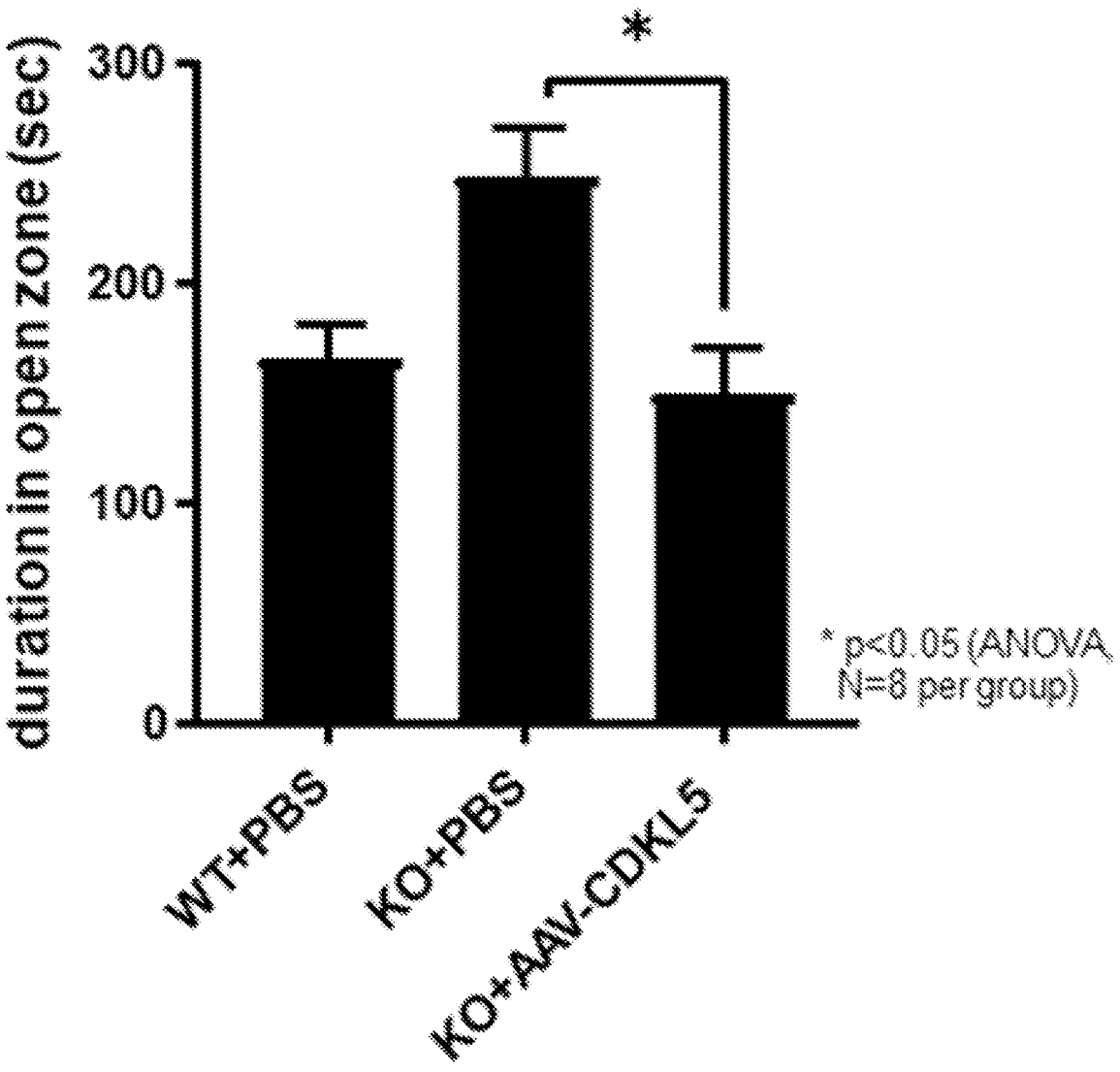

duration in open zone (sec)
300
200
100
0
*
* p<0.05 (ANOVA, N=8 per group)
WT+PBS
KO+PBS
KO+AAV-CDKL5

FIG 5A

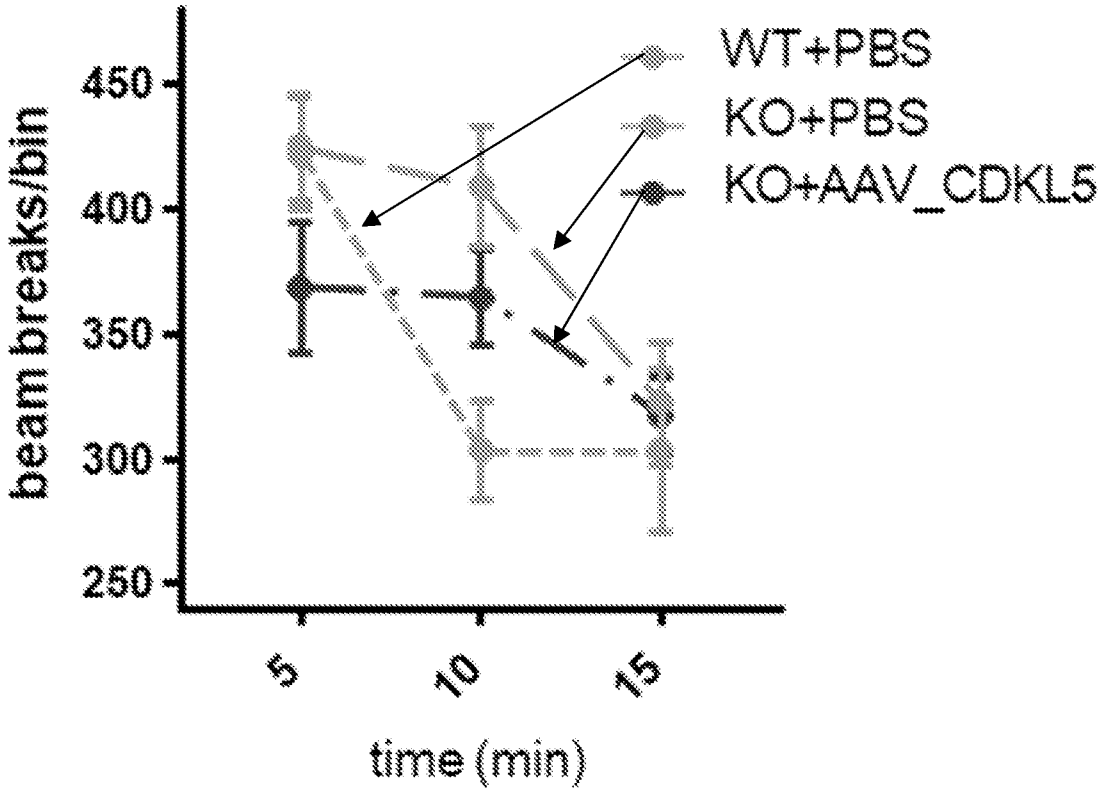

WT+PBS
KO+PBS
KO+AAV_CDKL5
beam breaks/bin
450
400
350
300
250
5
10
15
time (min)

FIG 5B

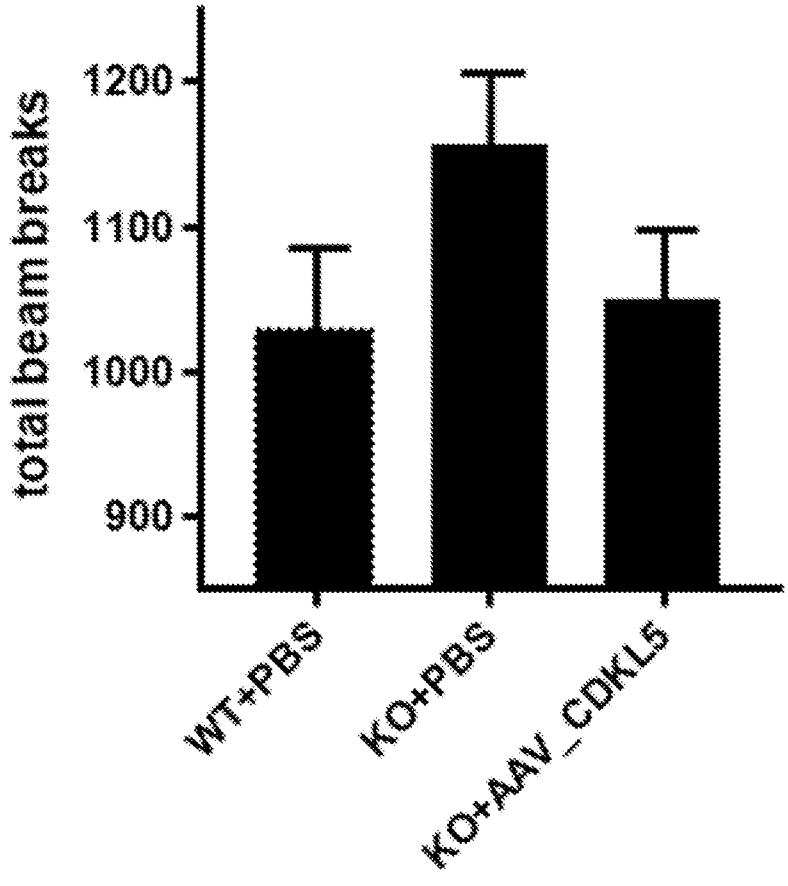

total beam breaks
1200
1100
1000
900
WT+PBS
KO+PBS
KO+AAV_CDKL5

FIG 6

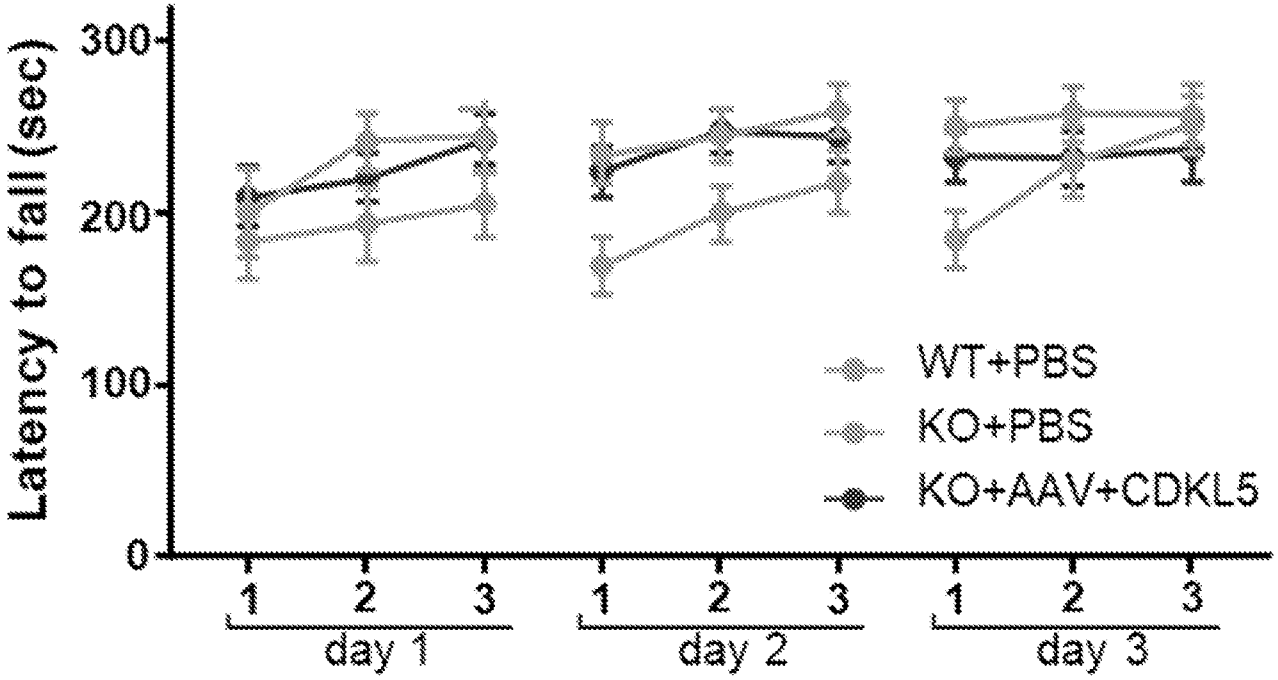

Latency to fall (sec)
300
200
100
0
WT+PBS
KO+PBS
KO+AAV+CDKL5
1
2
3
day 1
day 2
day 3

FIG 7A

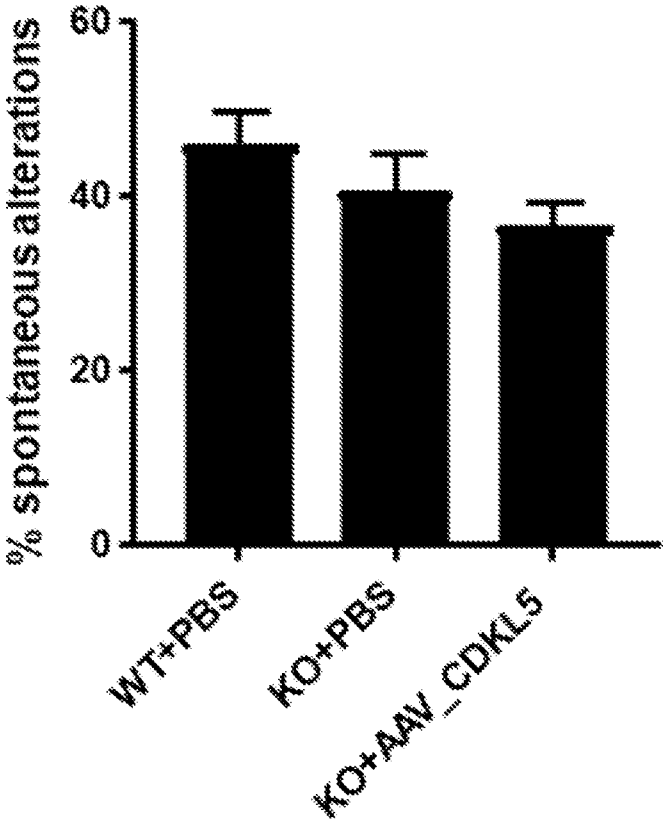

% spontaneous alterations
60
40
20
0
WT+PBS
KO+PBS
KO+AAV_CDKL5

FIG 7B

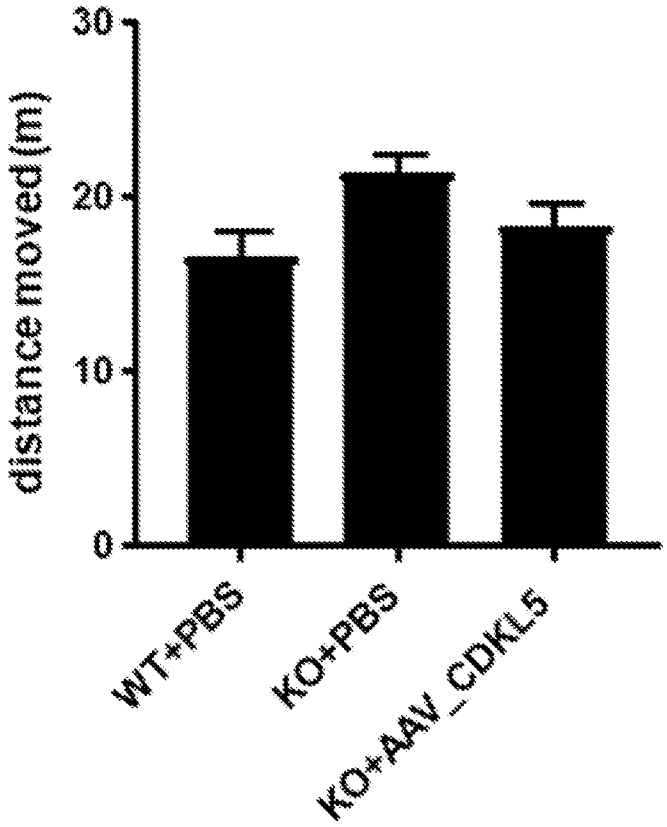

distance moved (m)
30
20
10
0
WT+PBS
KO+PBS
KO+AAV_CDKL5

FIG 8A

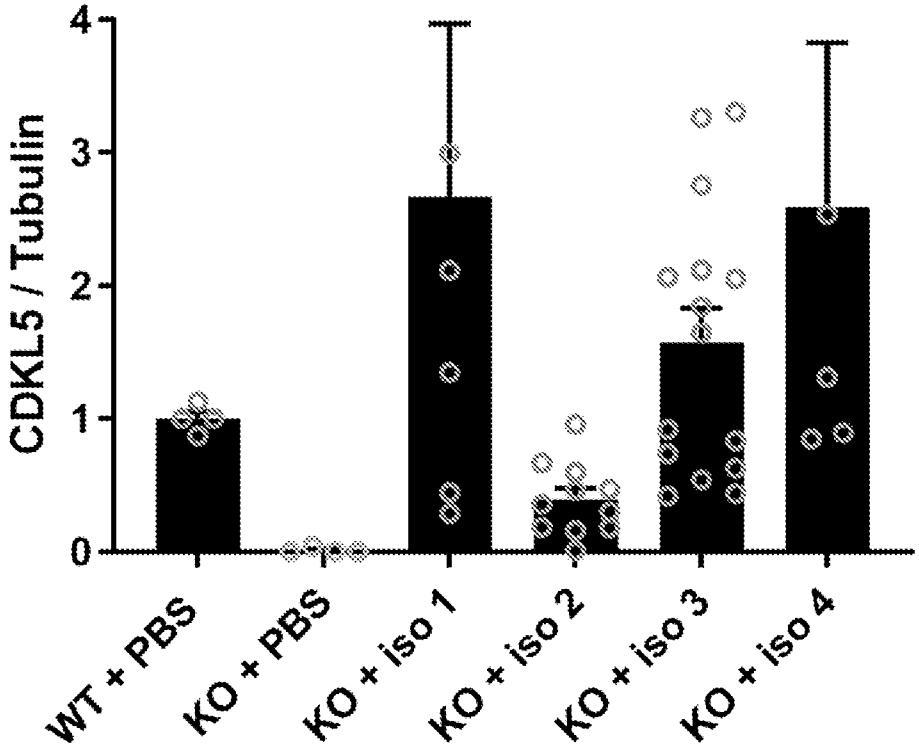

CDKL5 / Tubulin
4
3
2
1
0
WT + PBS
KO + PBS
KO + iso 1
KO + iso 2
KO + iso 3
KO + iso 4

FIG 8B

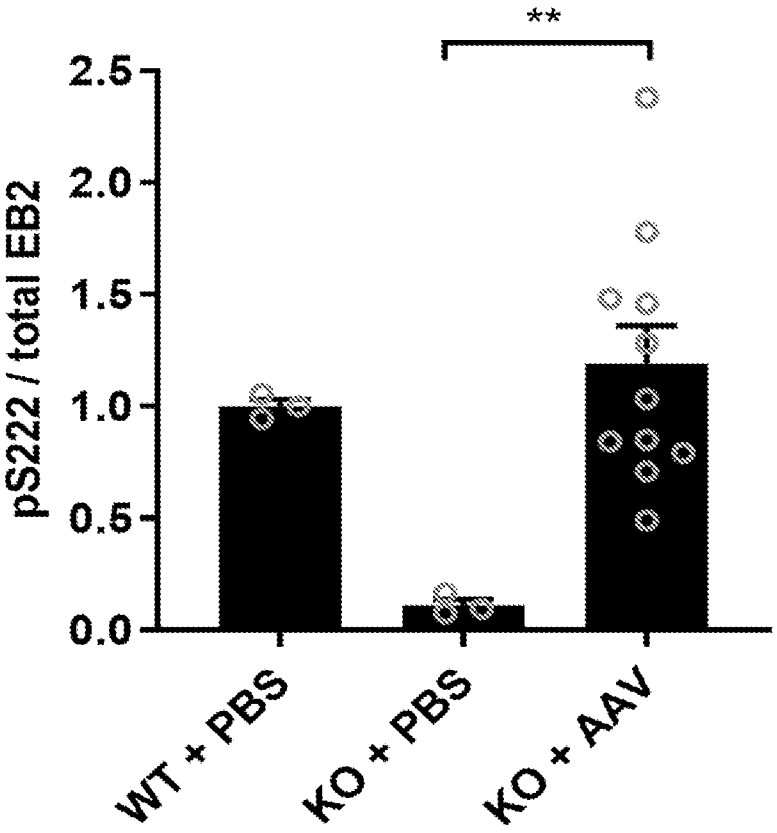
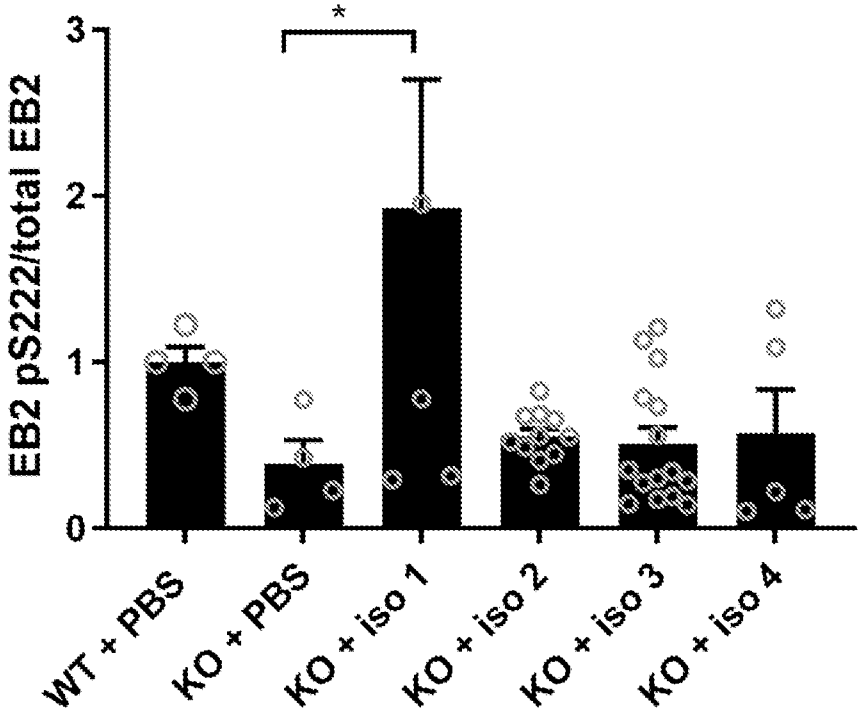

**
pS222 / total EB2
2.5
2.0
1.5
1.0
0.5
0.0
WT + PBS
KO + PBS
KO + AAV

FIG 8C



One-way ANOVA with Sidak's multiple comparison's test
*p<0.5

FIG 8D

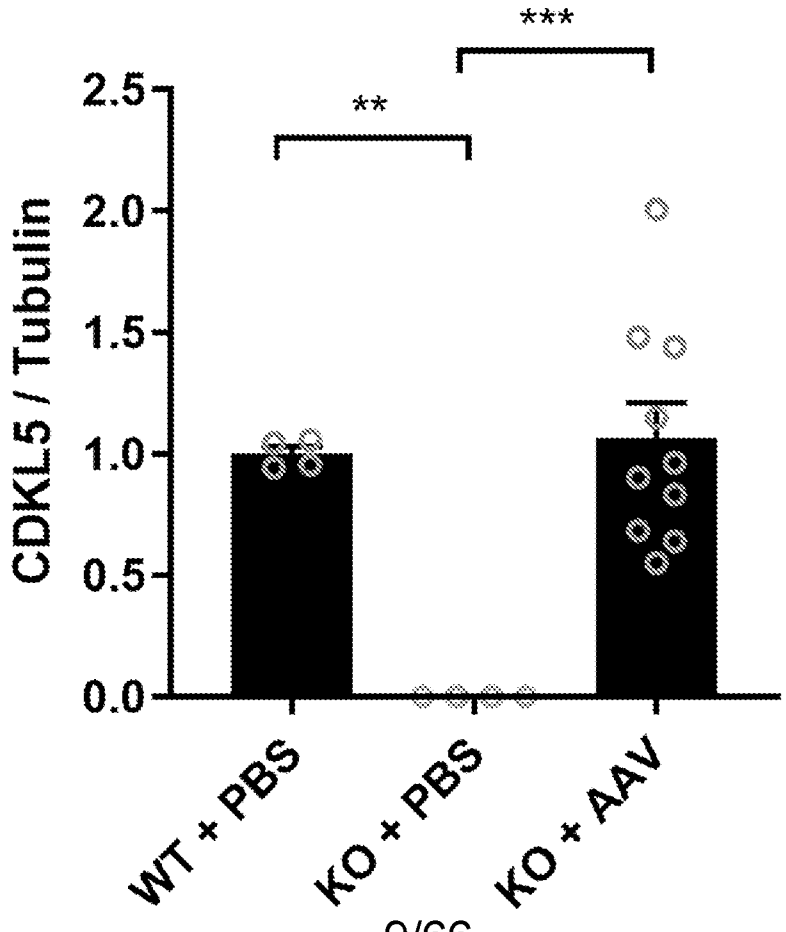


FIG 9A

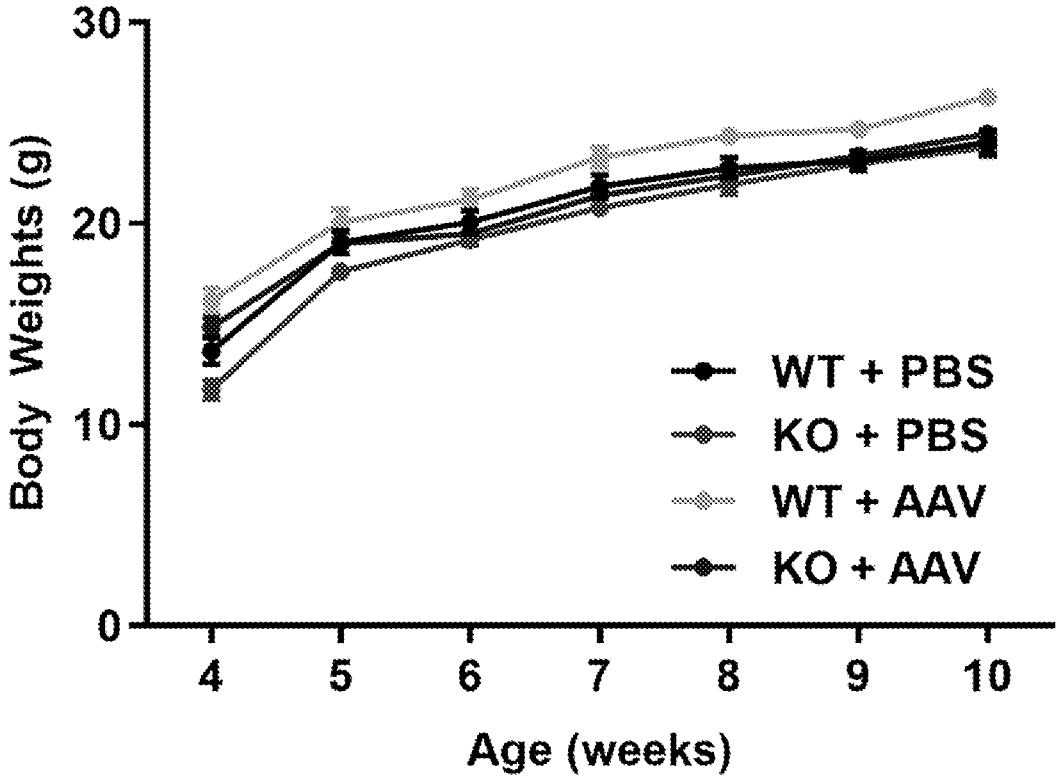

30
20
10
0
Body Weights (g)
4
5
6
7
8
9
10
Age (weeks)
WT + PBS
KO + PBS
WT + AAV
KO + AAV

FIG 9B

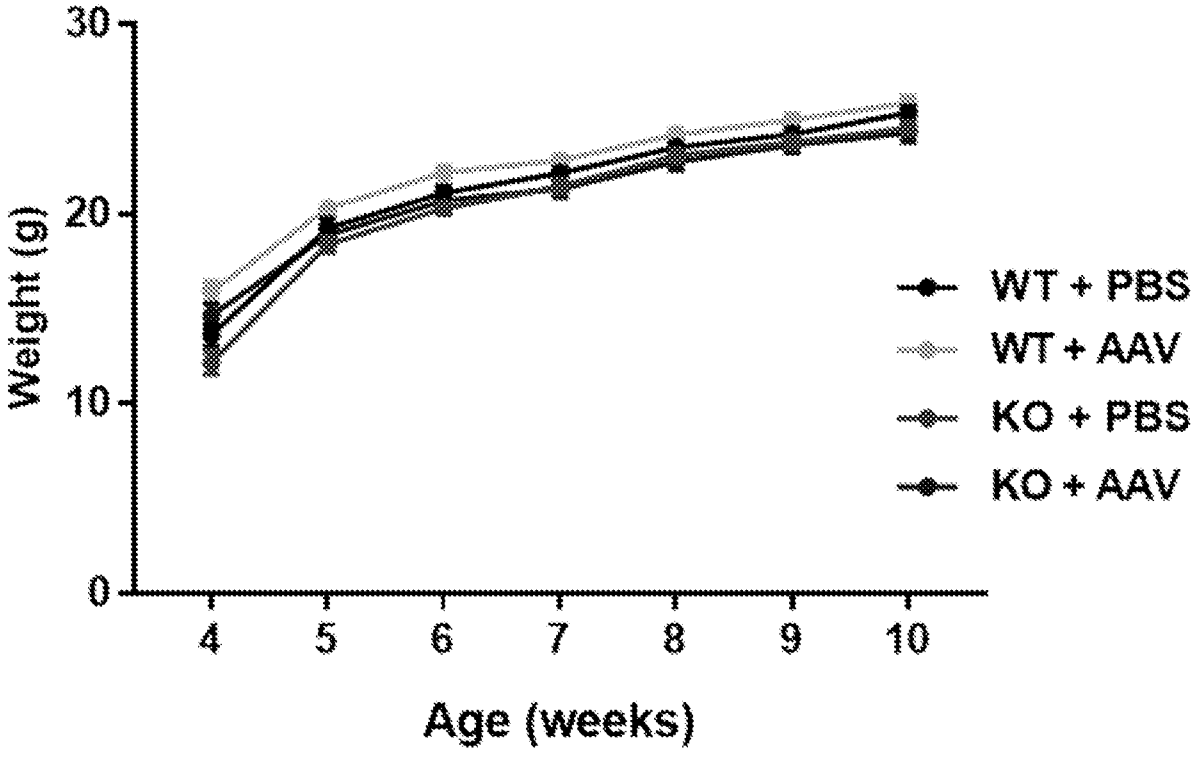

30
20
10
0
Weight (g)
4
5
6
7
8
9
10
Age (weeks)
WT + PBS
WT + AAV
KO + PBS
KO + AAV

FIG 9C

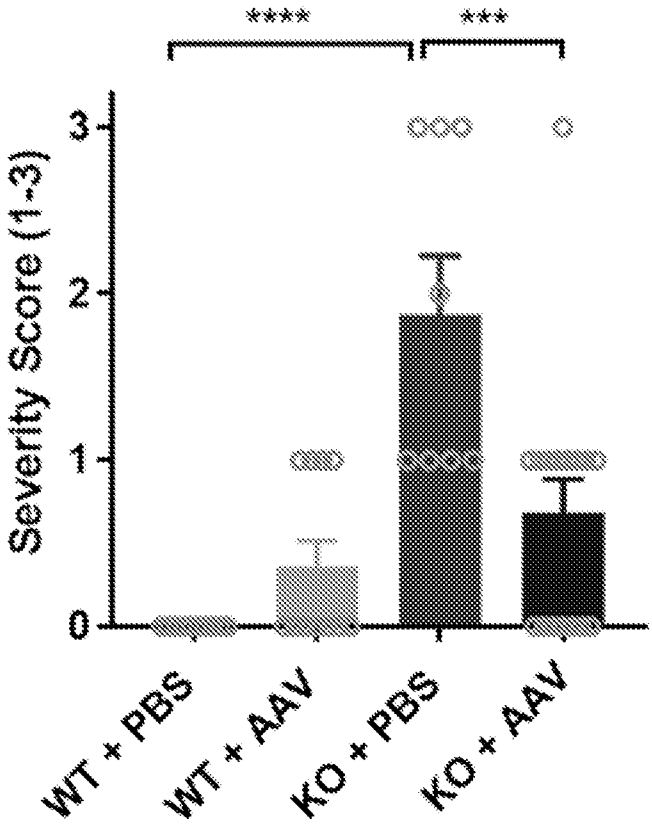

****
***
Severity Score (1-3)
0
1
2
3
WT + PBS
WT + AAV
KO + PBS
KO + AAV

FIG 9D

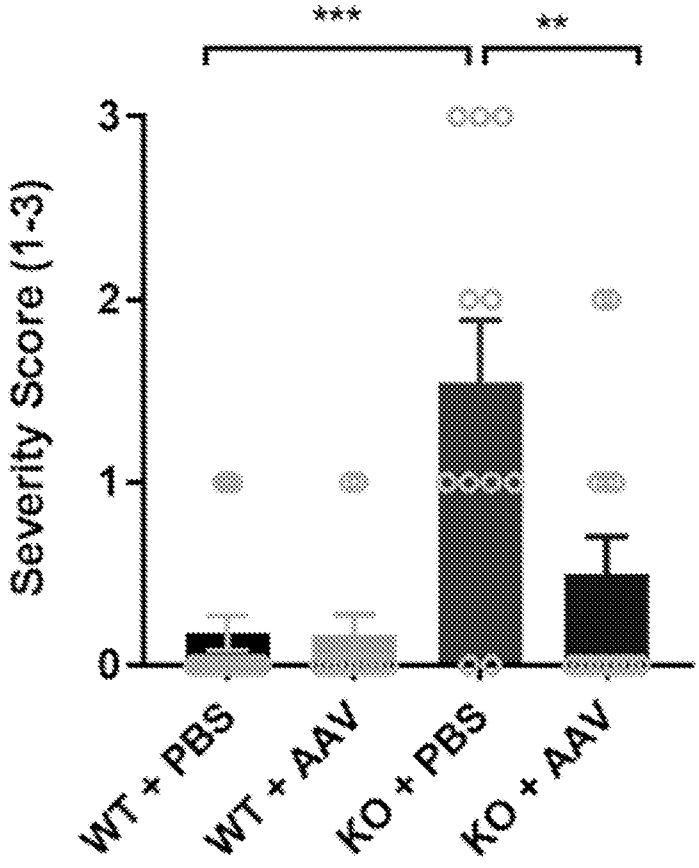

***
**
Severity Score (1-3)
0
1
2
3
WT + PBS
WT + AAV
KO + PBS
KO + AAV

FIG 9E

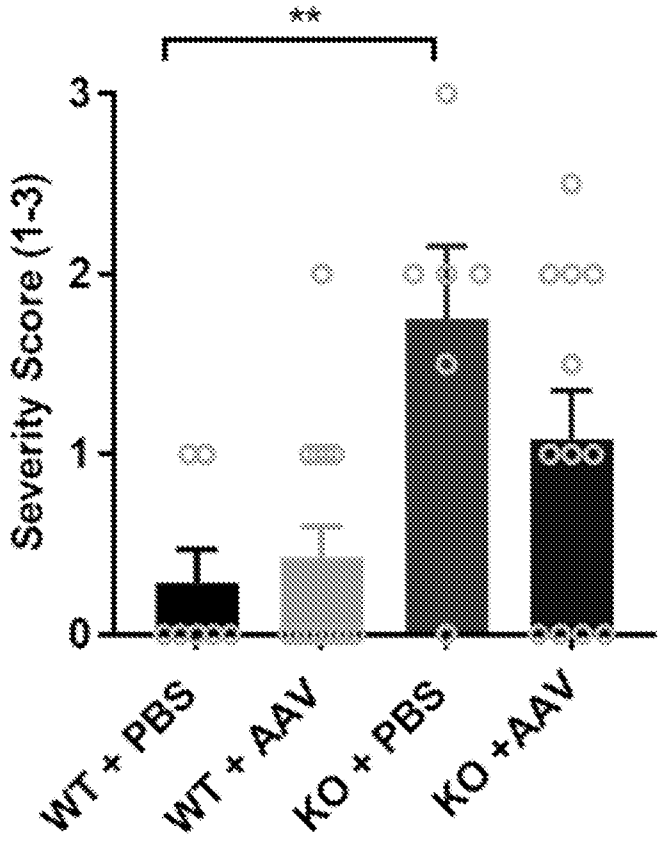

**
Severity Score (1-3)
0
1
2
3
WT + PBS
WT + AAV
KO + PBS
KO +AAV

FIG 9F

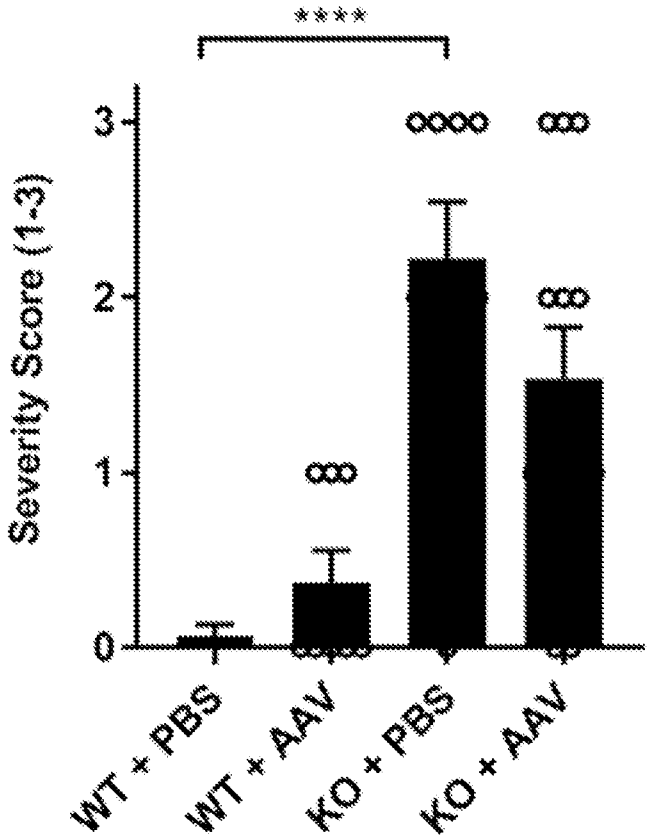

****
Severity Score (1-3)
0
1
2
3
WT + PBS
WT + AAV
KO + PBS
KO + AAV

FIG 10A

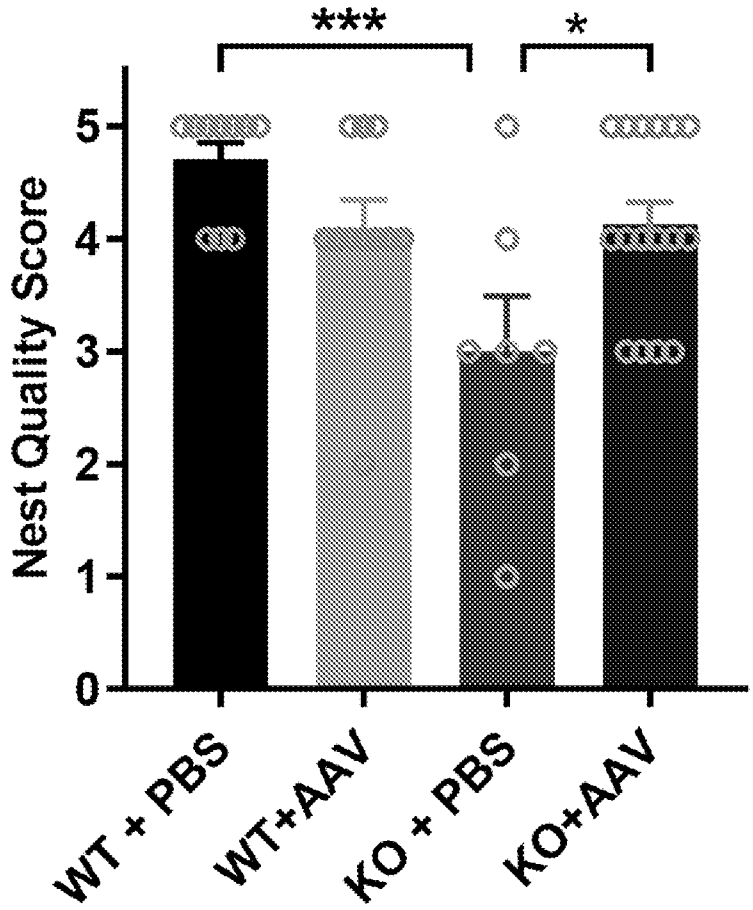


FIG 10B

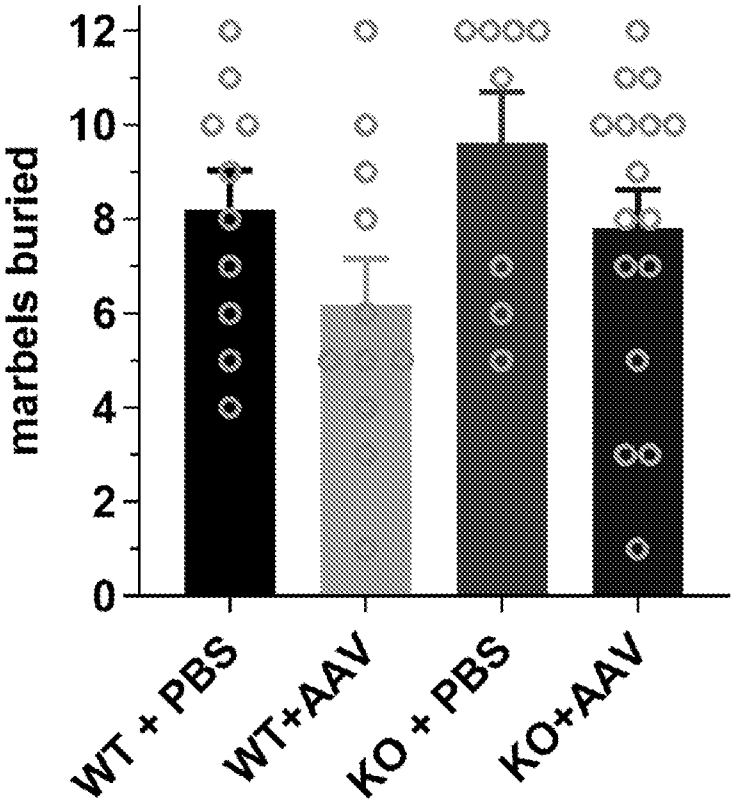


FIG 10C

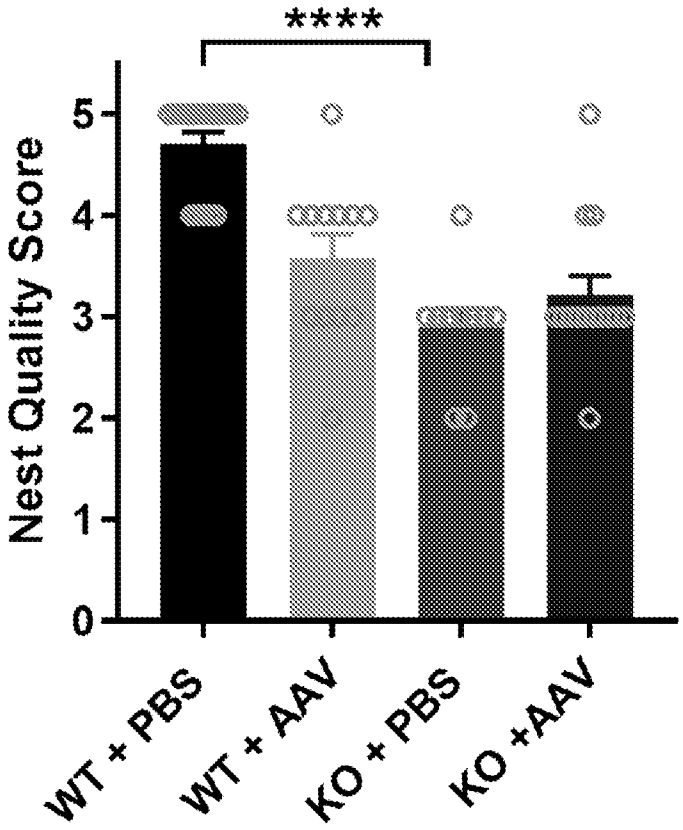


FIG 10D

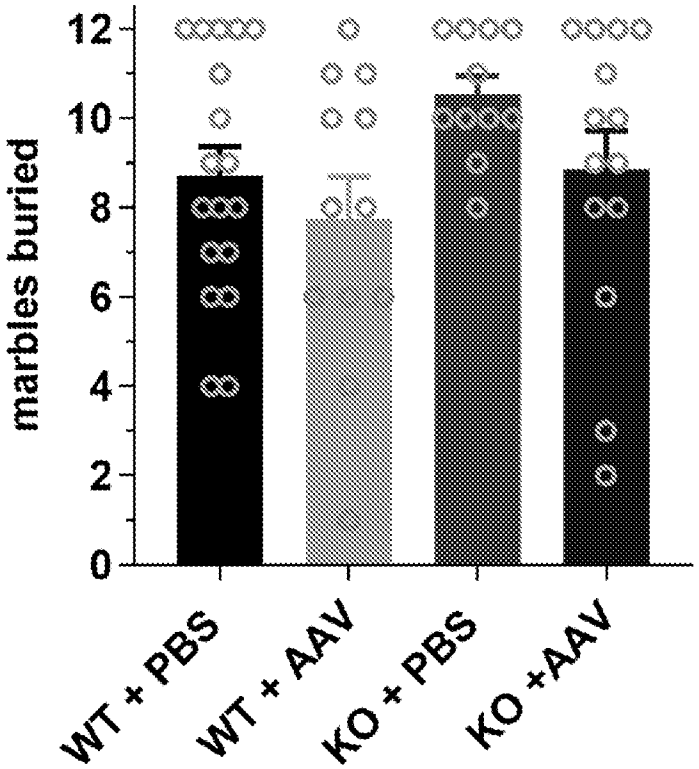


FIG 10E

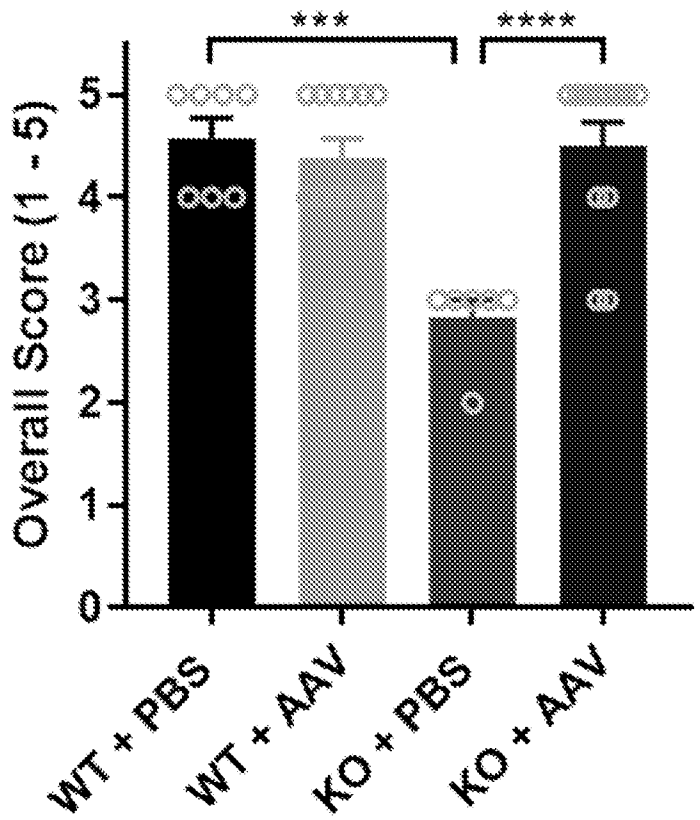


FIG 10F

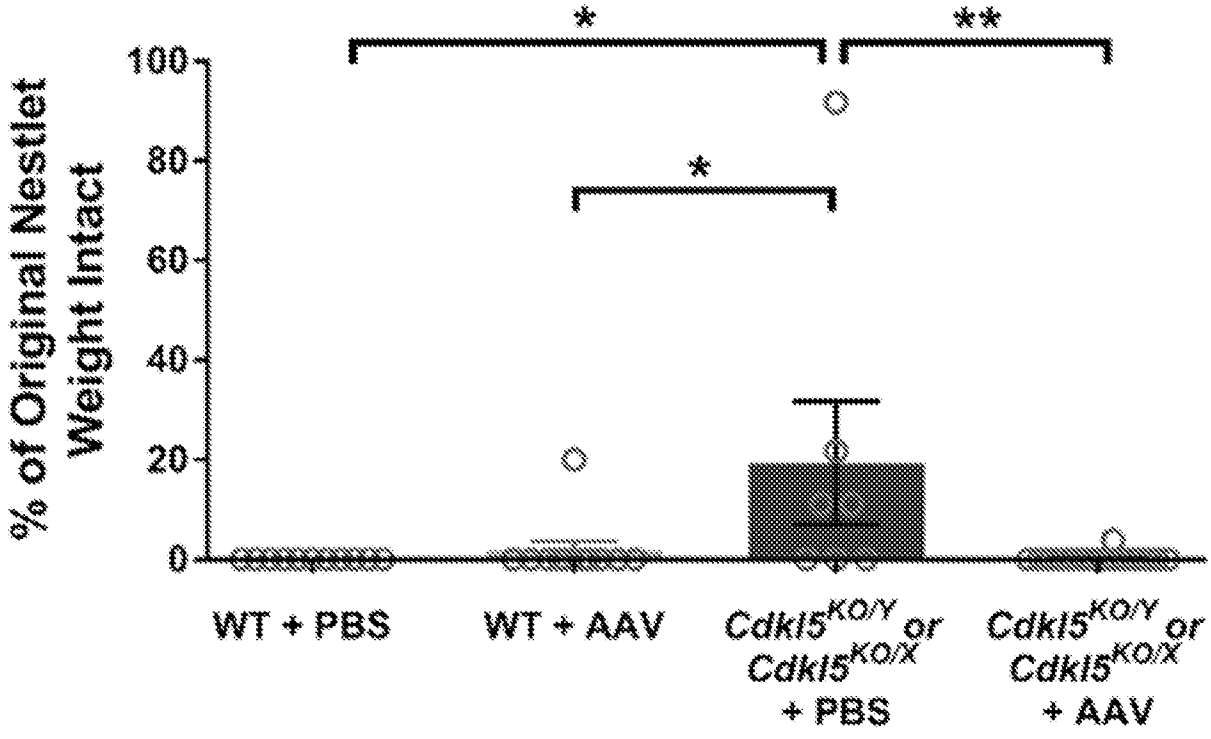


FIG 11A

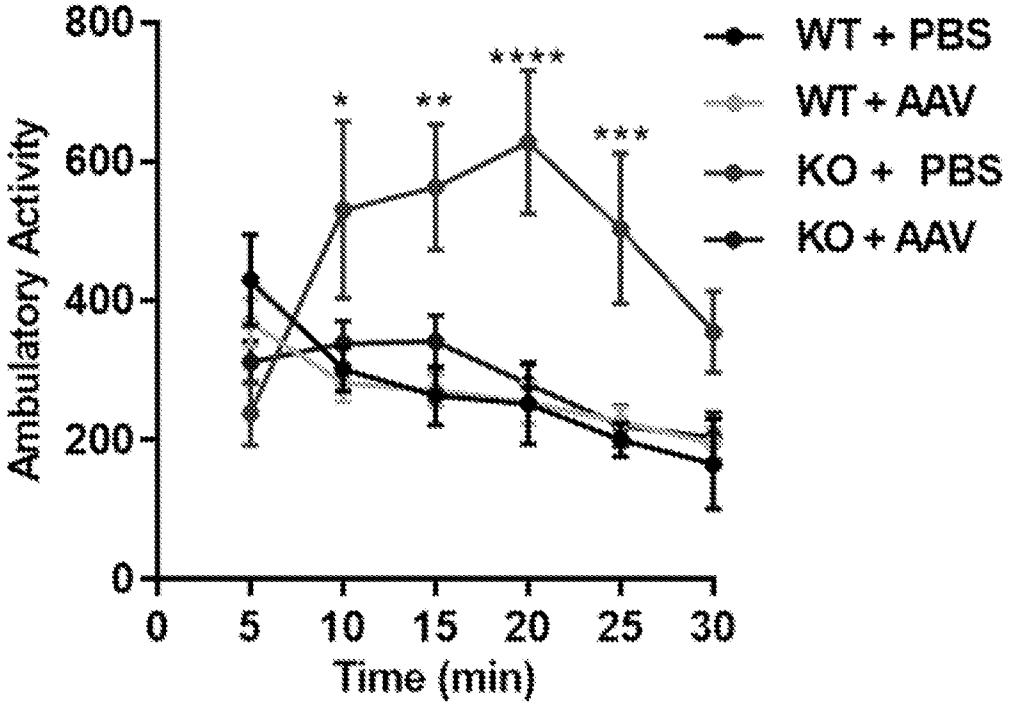


FIG 11B

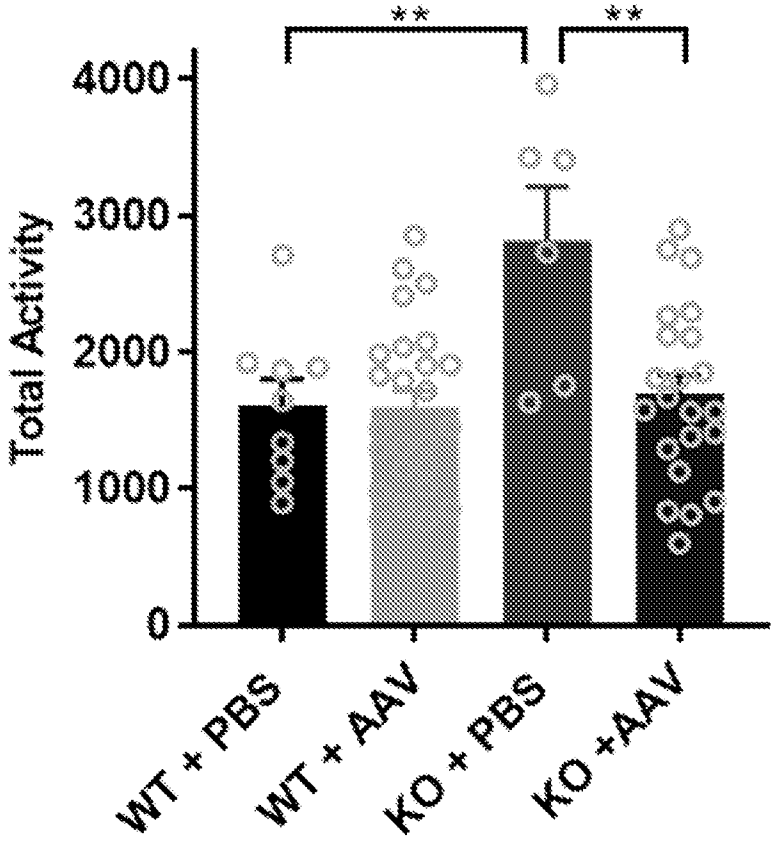


FIG 11C

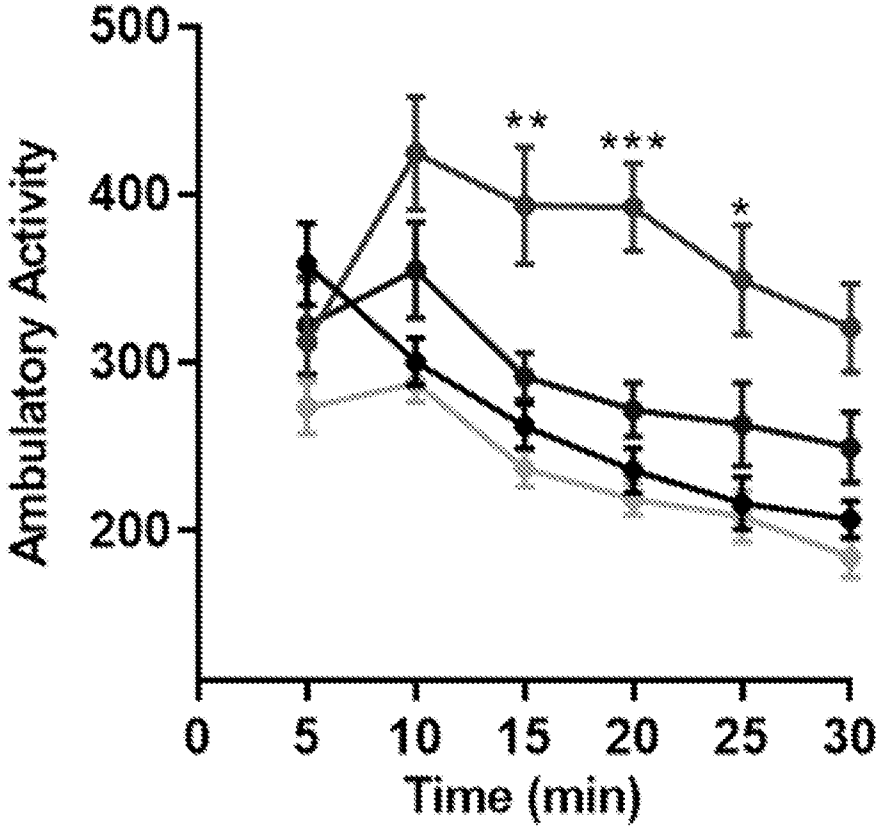


FIG 11D

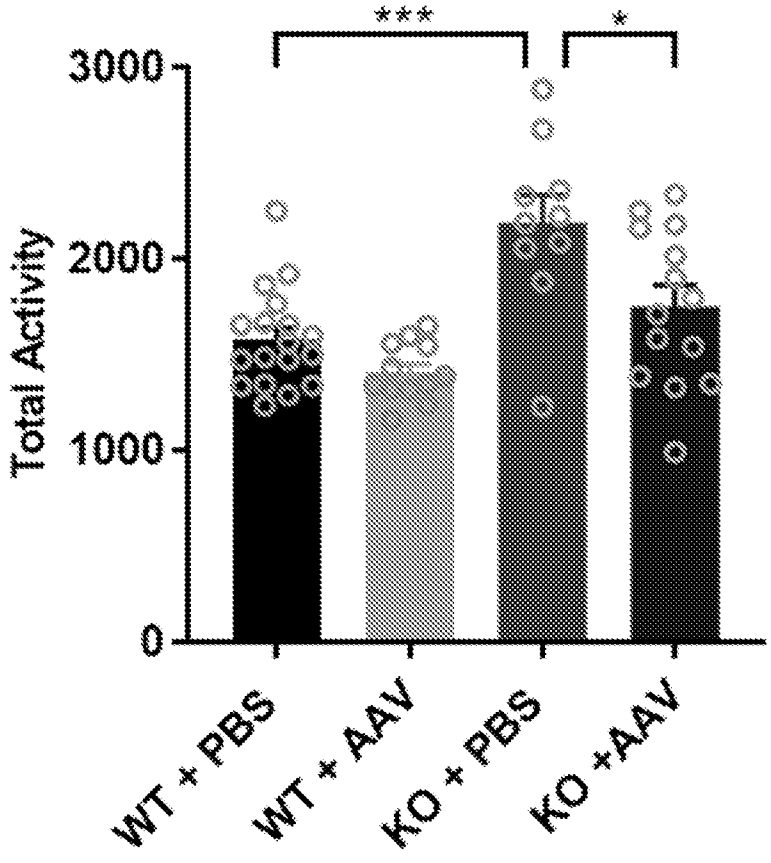


FIG 11E

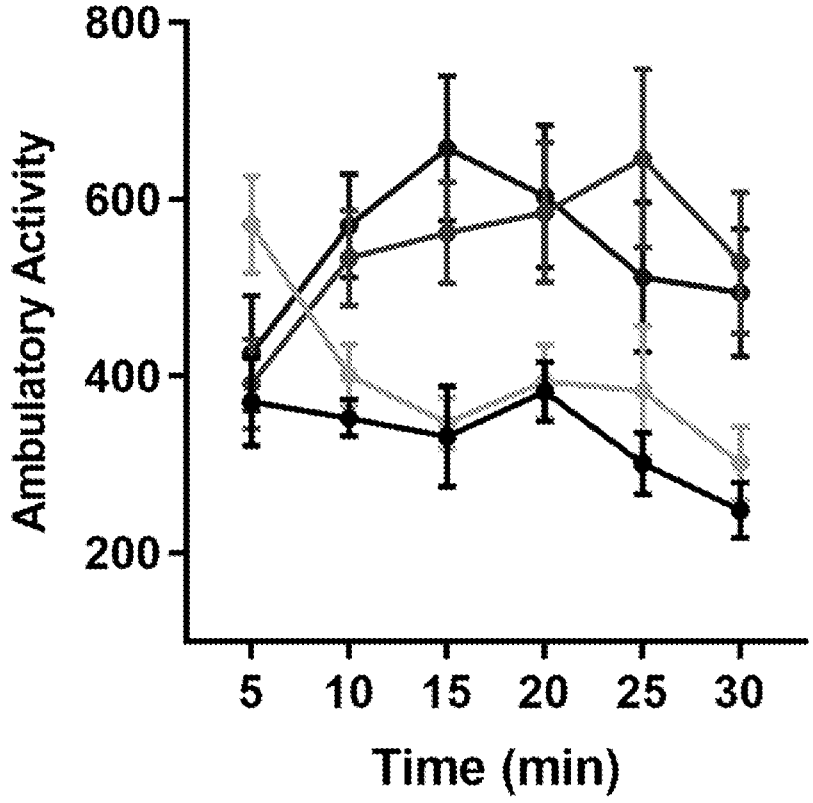


FIG 11F

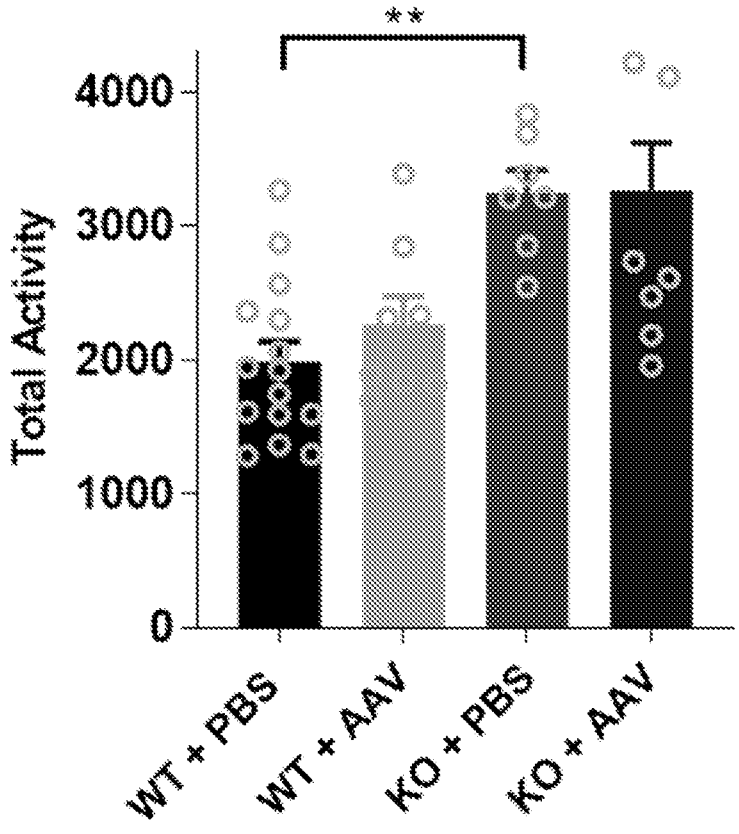


FIG 12

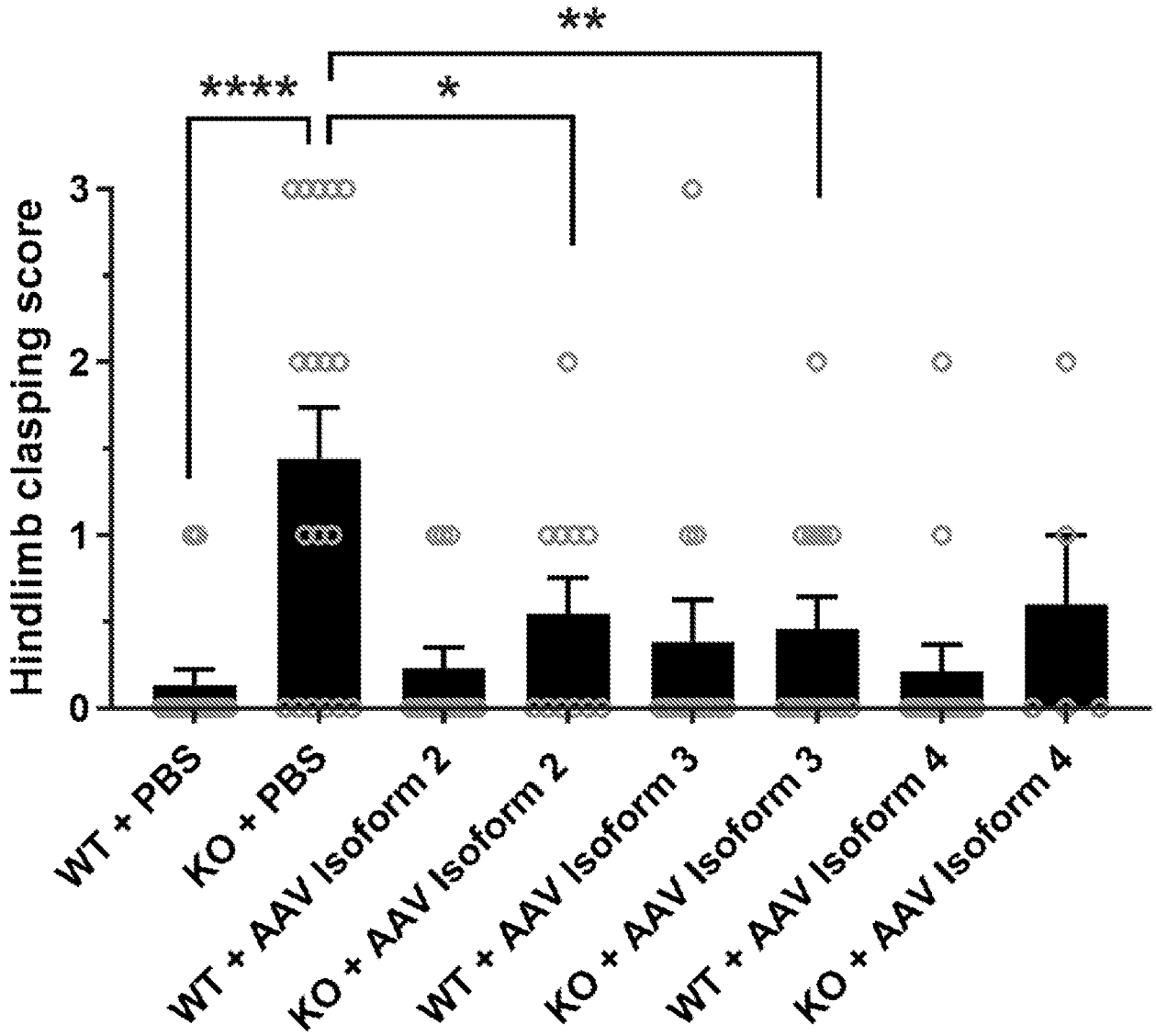

**
****
*
Hindlimb clasping score
3
2
1
0
WT + PBS
KO + PBS
WT + AAV Isoform 2
KO + AAV Isoform 2
WT + AAV Isoform 3
KO + AAV Isoform 3
WT + AAV Isoform 4
KO + AAV Isoform 4

FIG 13A

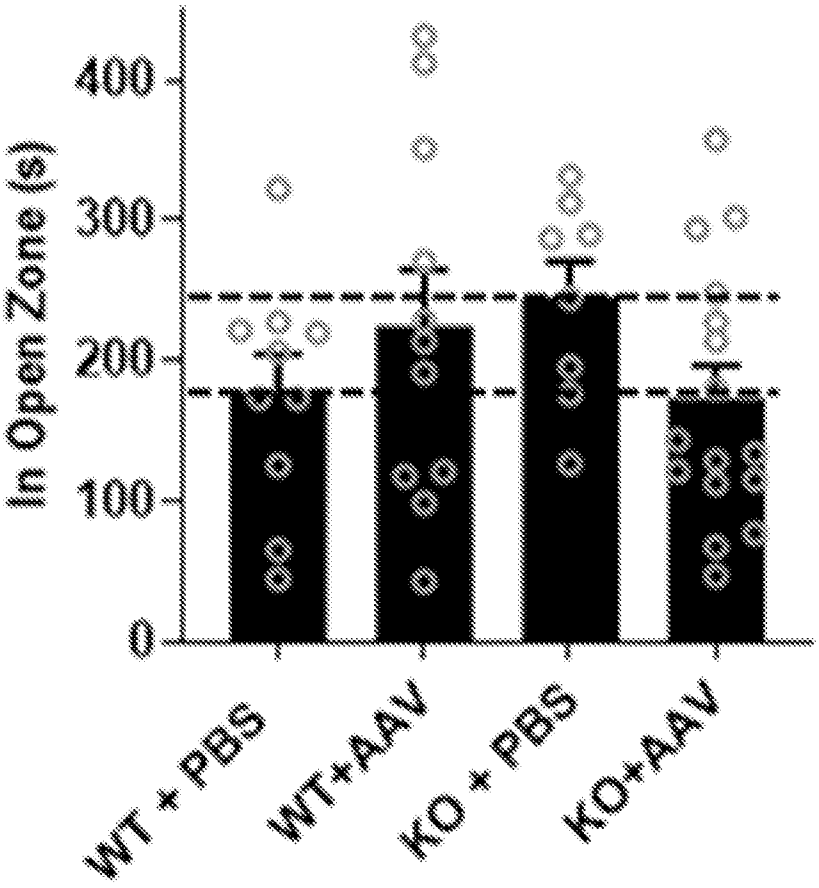


FIG 13B

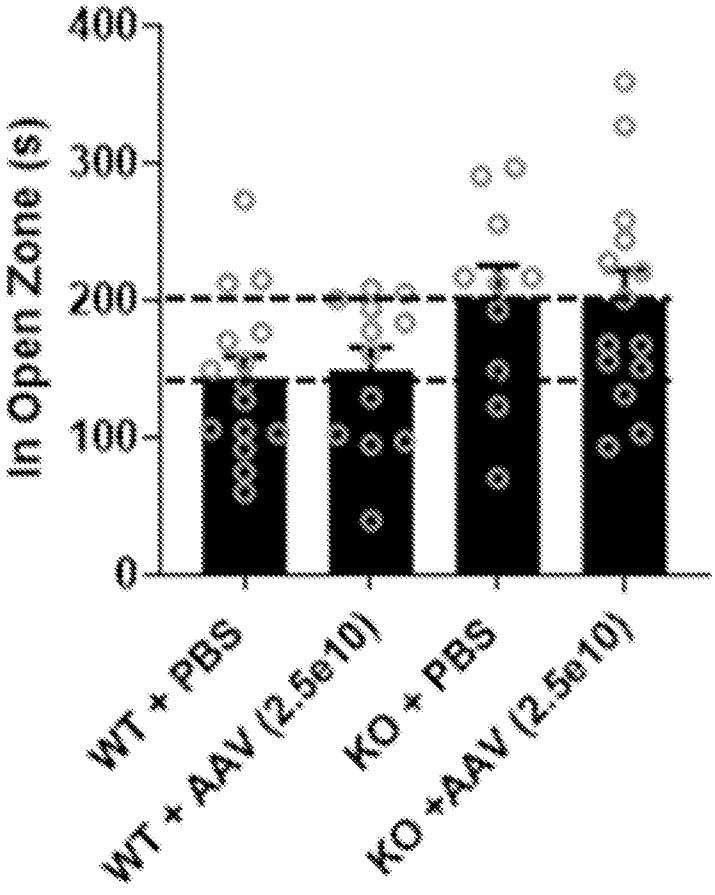


FIG 13C

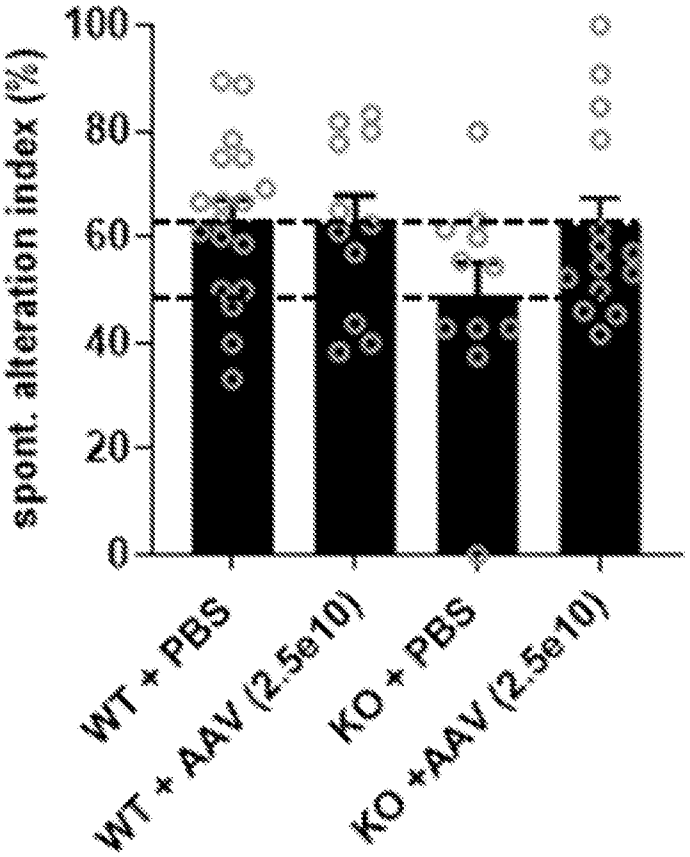


FIG 13D

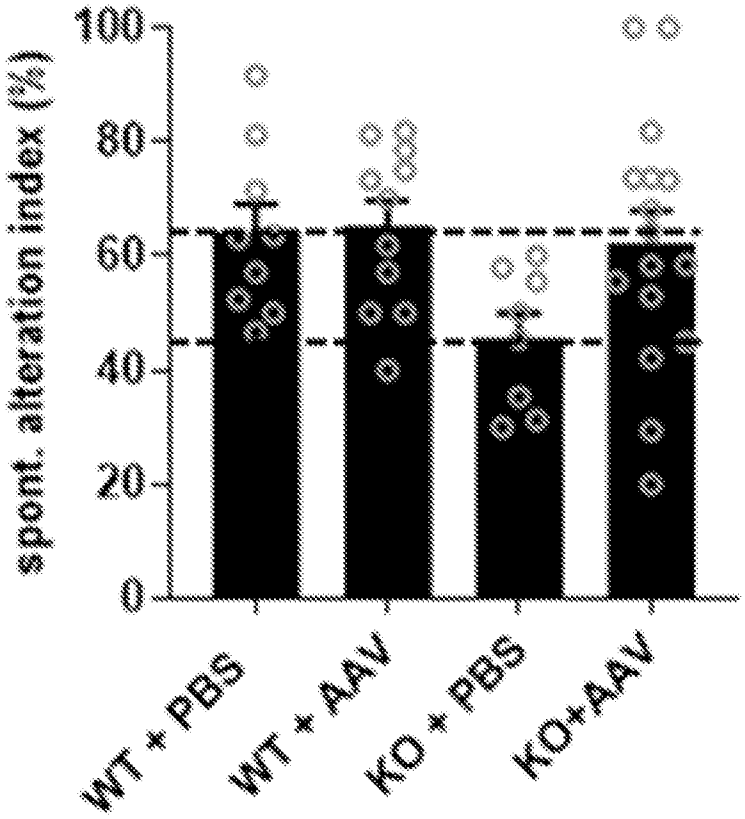


FIG 14B

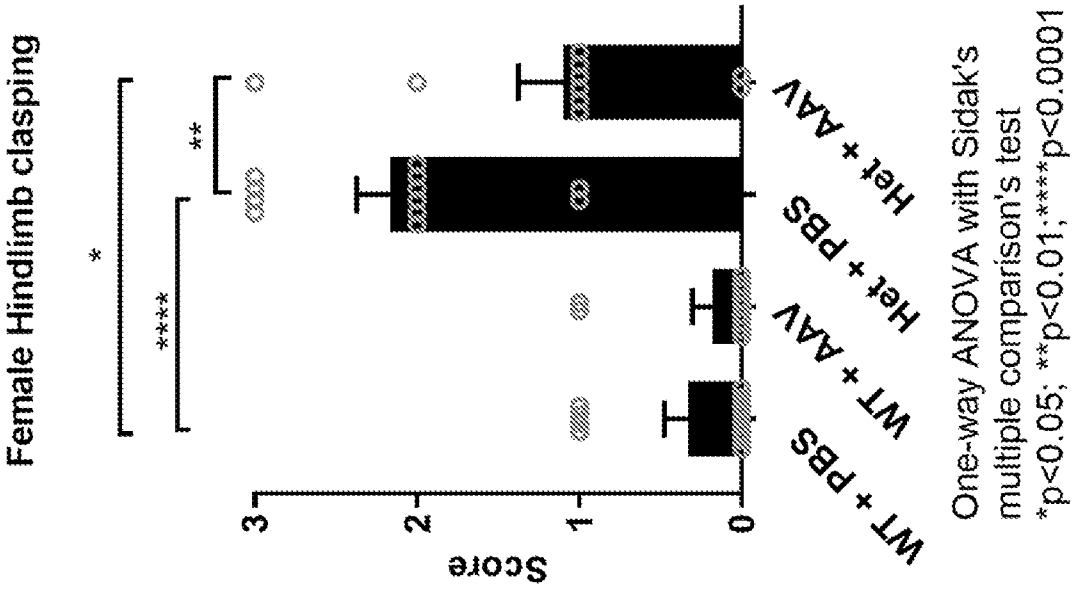


FIG 14A

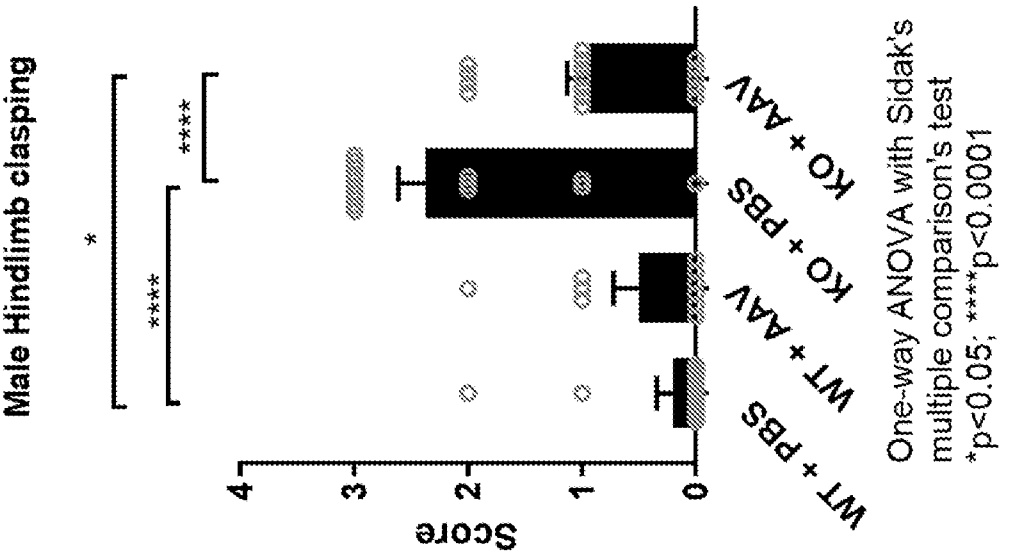


FIG 14C

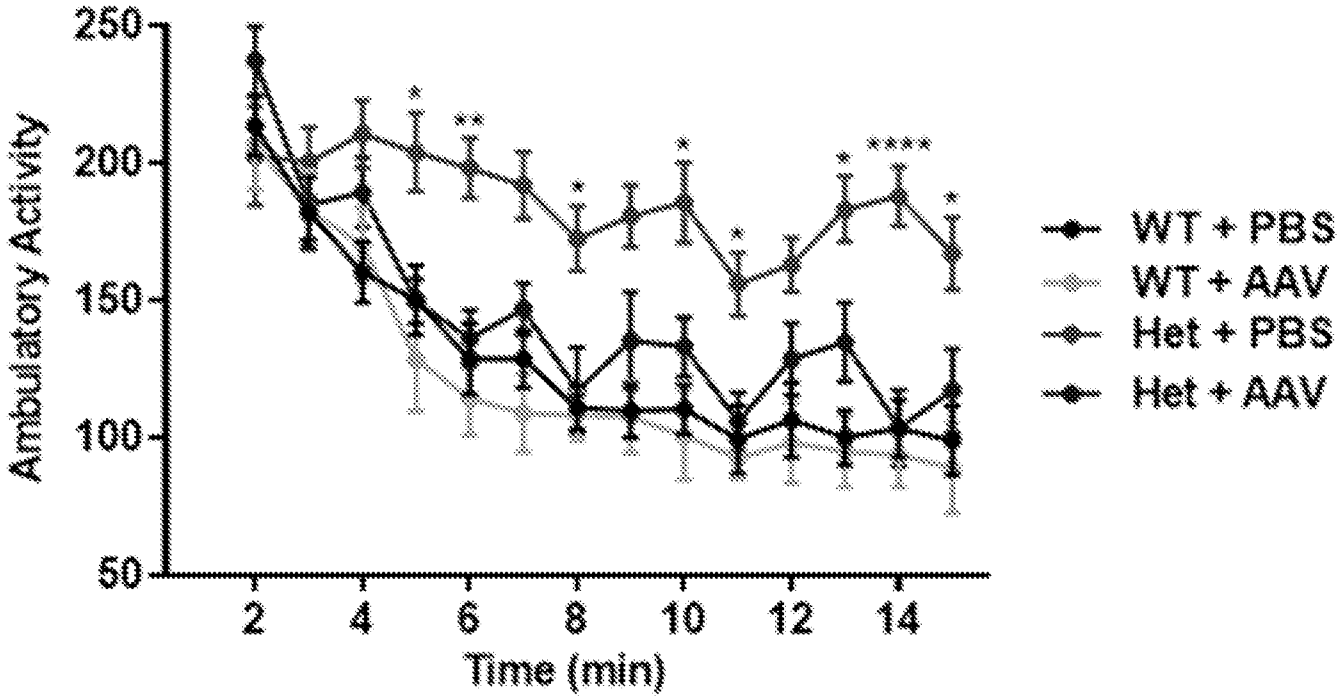

Ambulatory Activity
250
200
150
100
50
2
4
6
8
10
12
14
Time (min)
WT + PBS
WT + AAV
Het + PBS
Het + AAV

FIG 15A

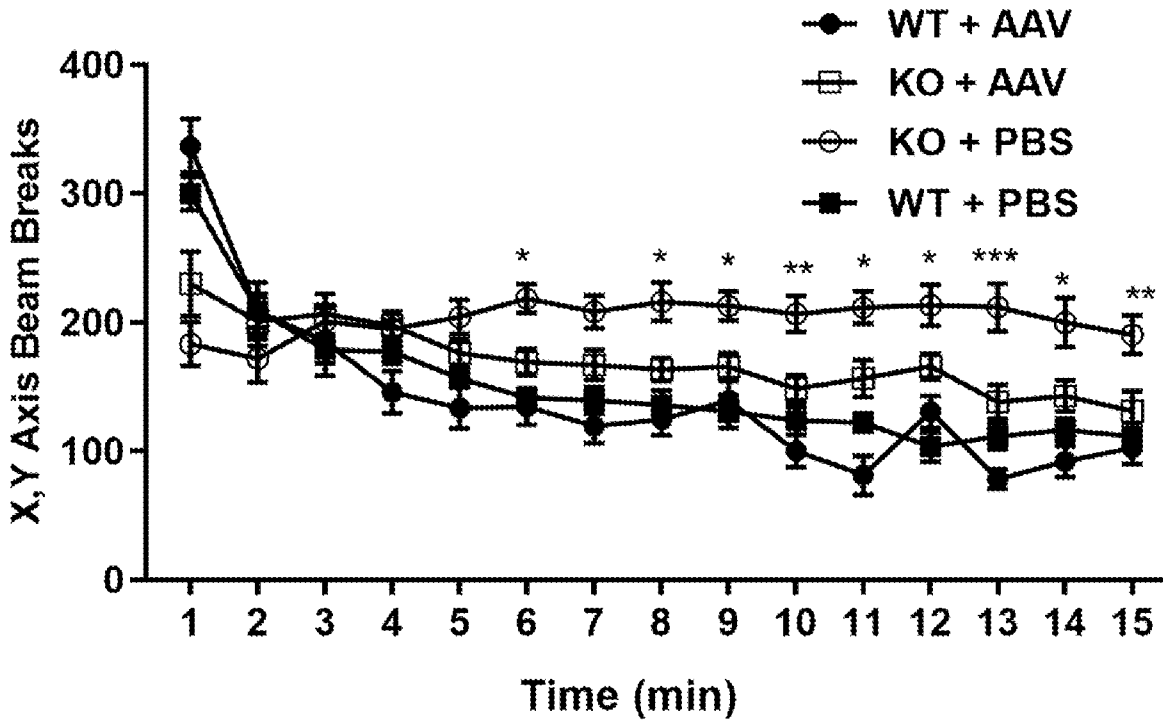


FIG 15B

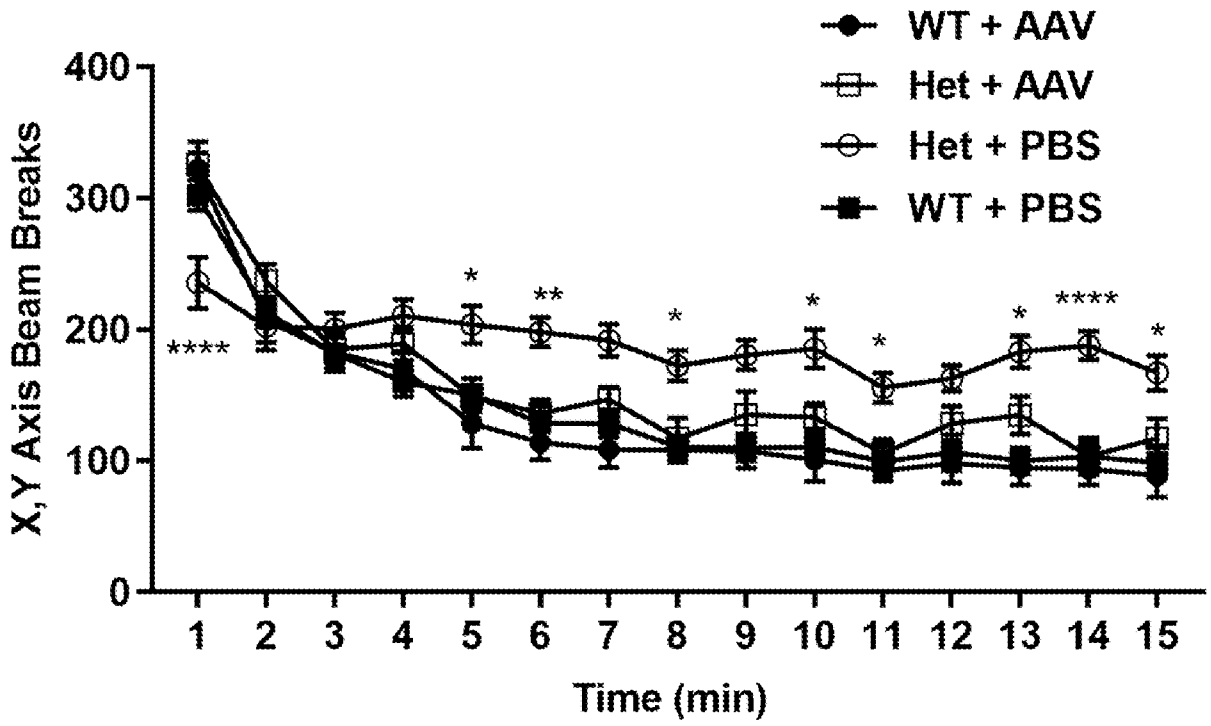


FIG 15C

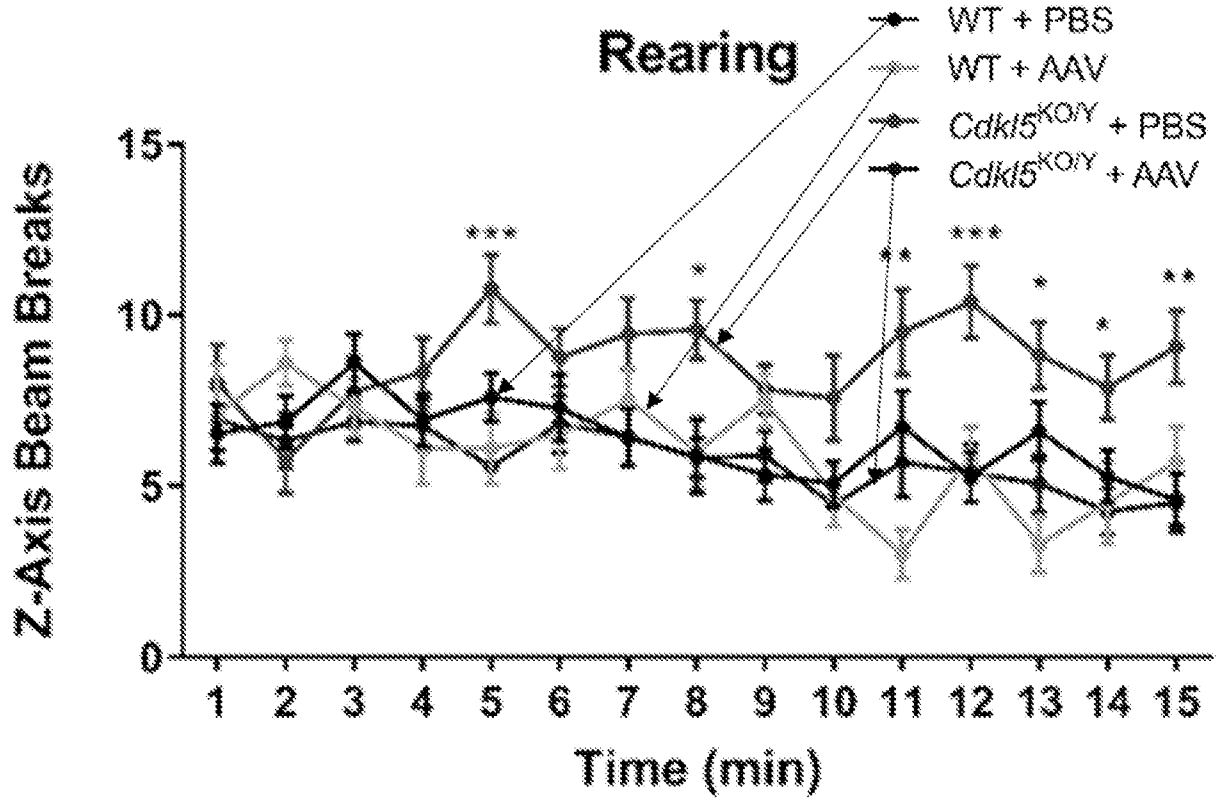


FIG 15D

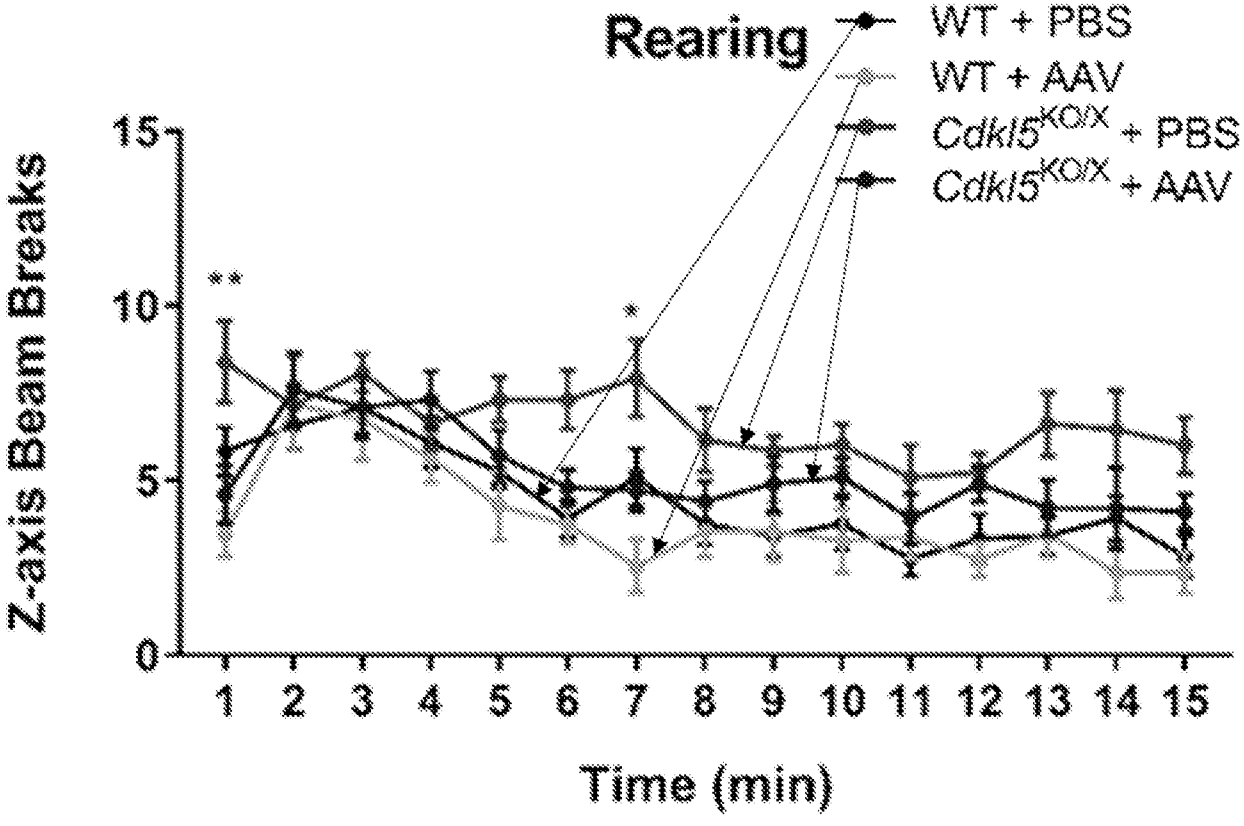


FIG 15E

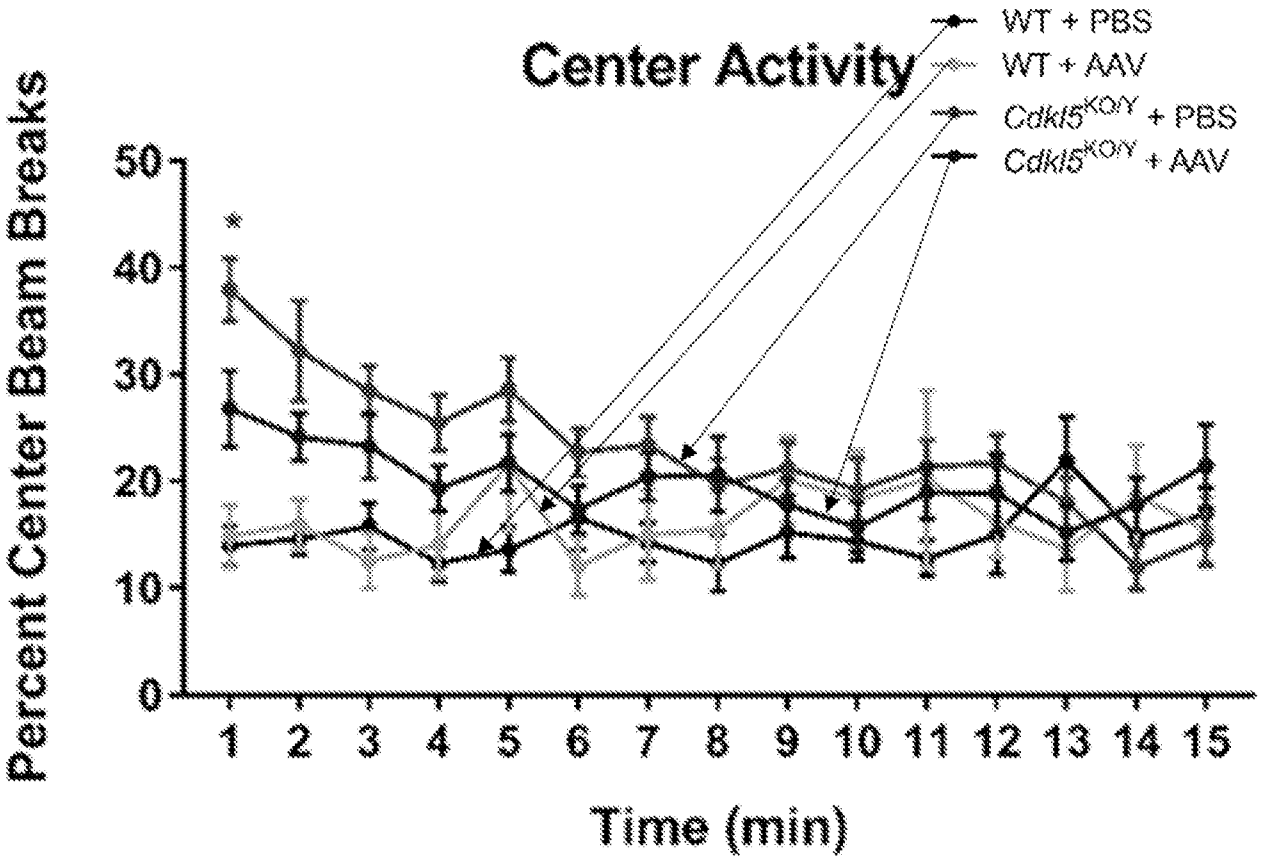


FIG 15F

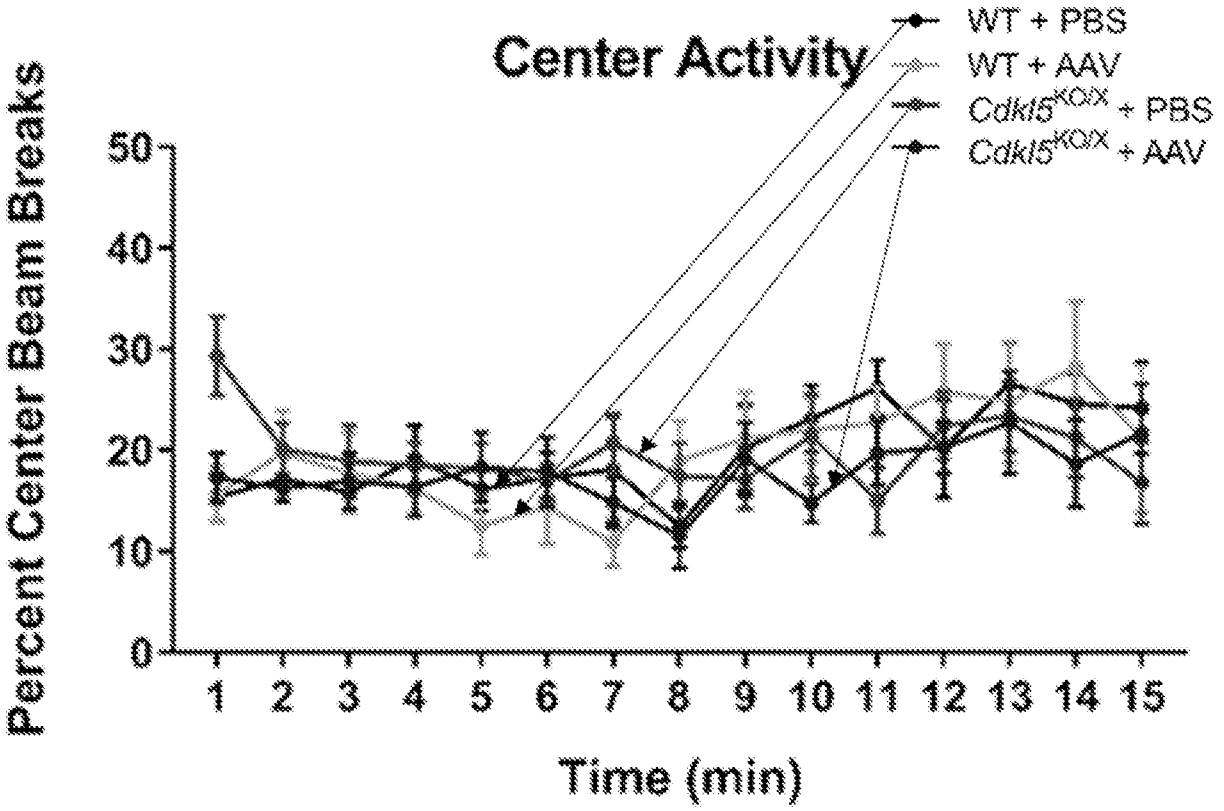


FIG 16A

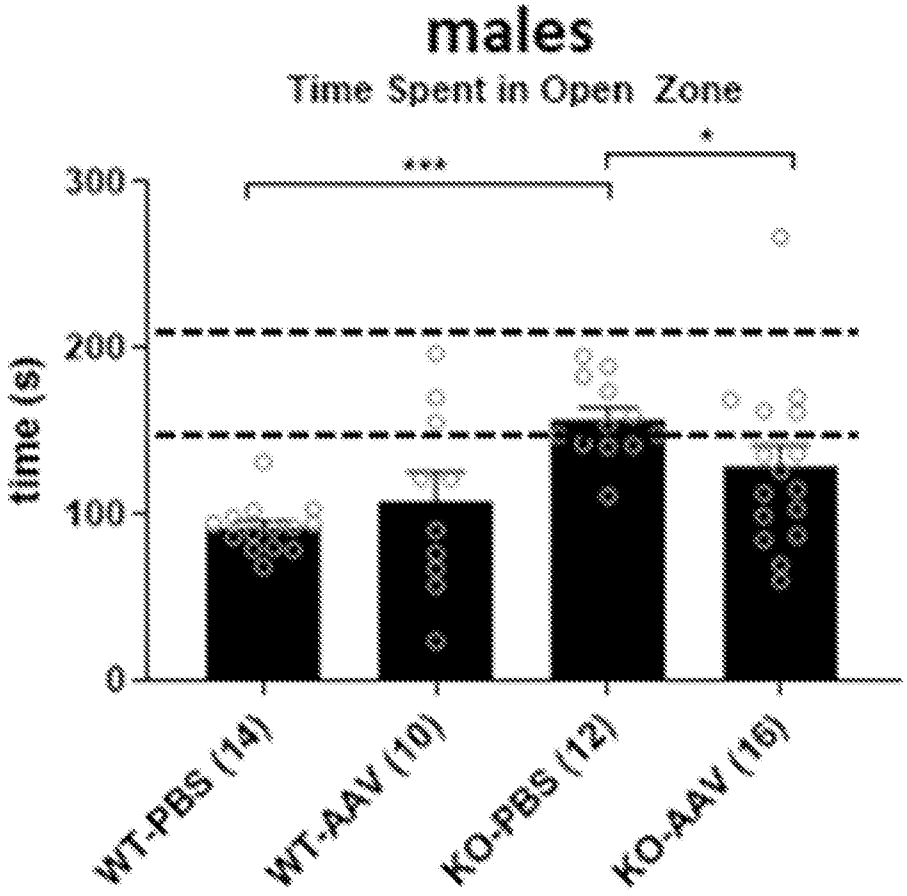


FIG 16B

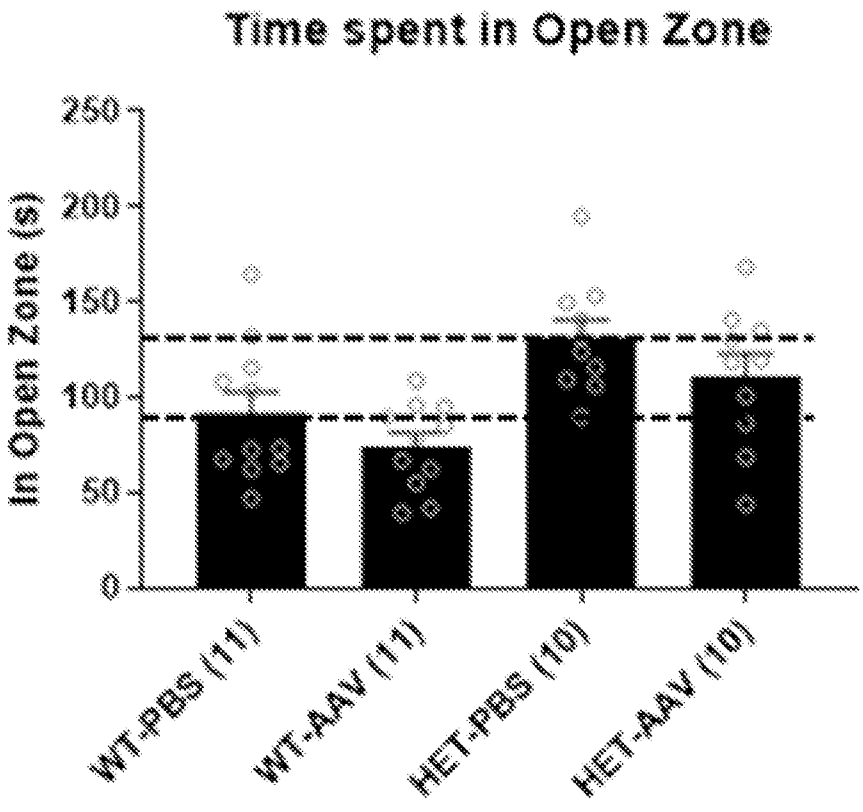


FIG 17

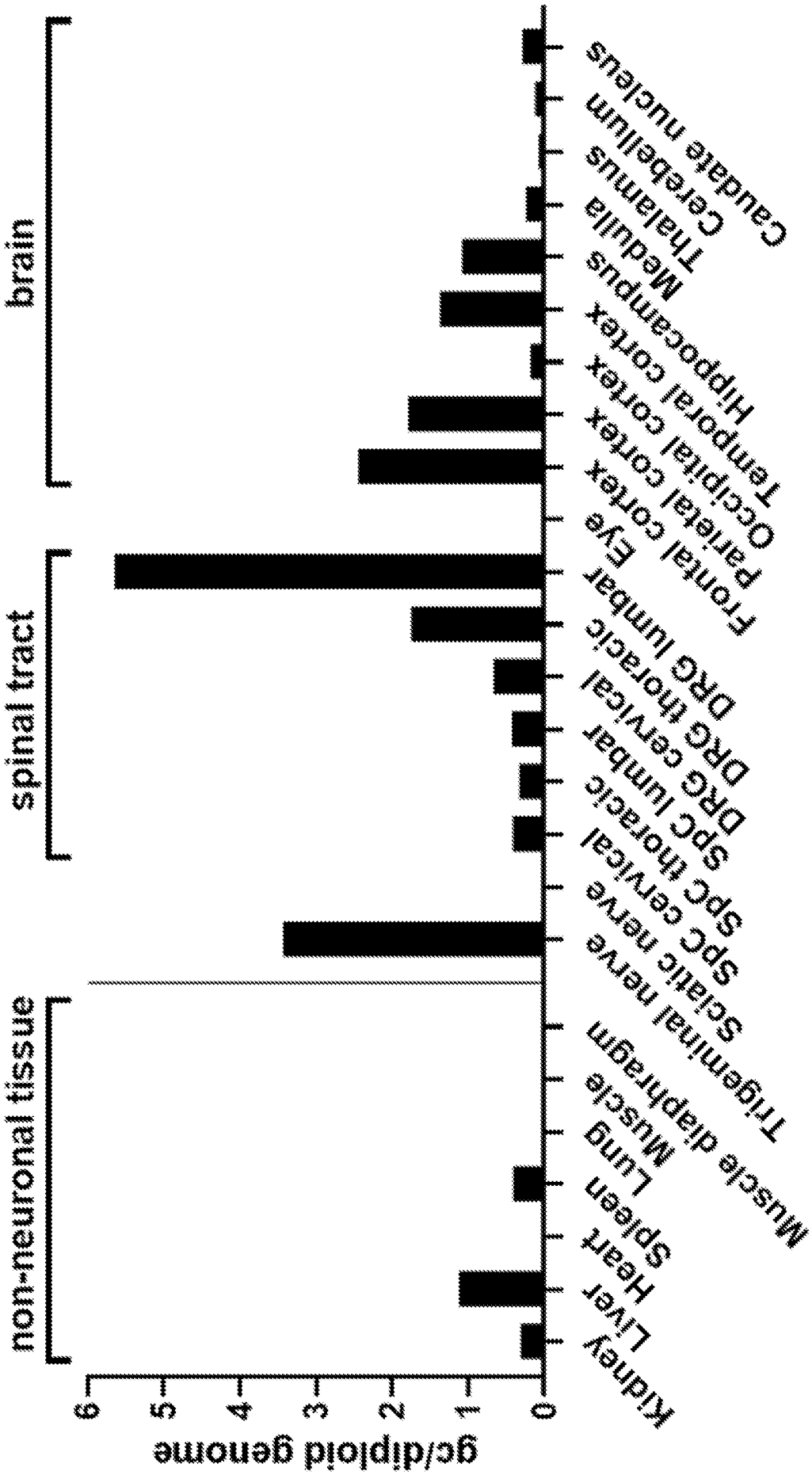

gc/diploid genome
0
1
2
3
4
5
6
non-neuronal tissue
spinal tract
brain
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SPC cervical
SPC thoracic
SPC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 18

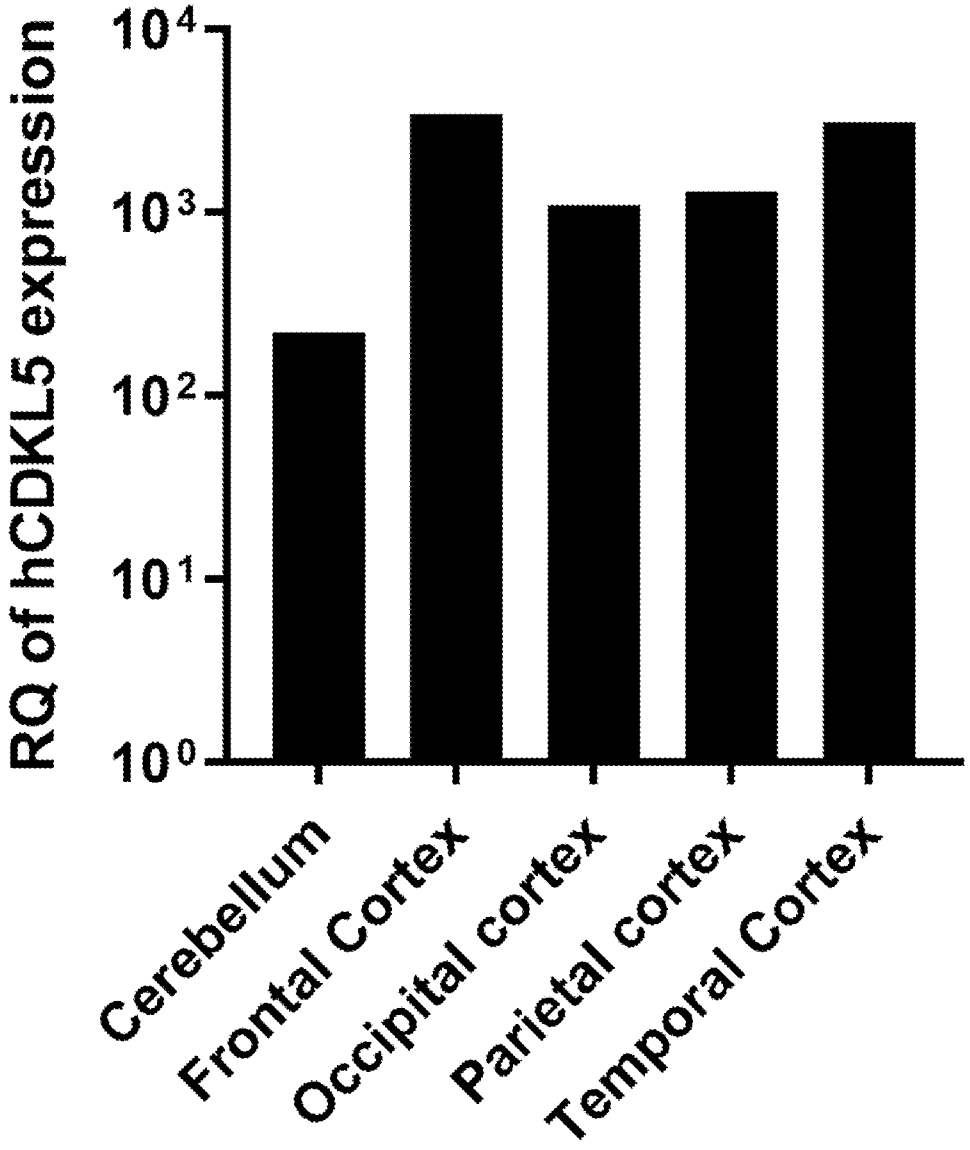

RQ of hCDKL5 expression
10^4
10^3
10^2
10^1
10^0
Cerebellum
Frontal Cortex
Occipital cortex
Parietal cortex
Temporal Cortex

FIG 19A

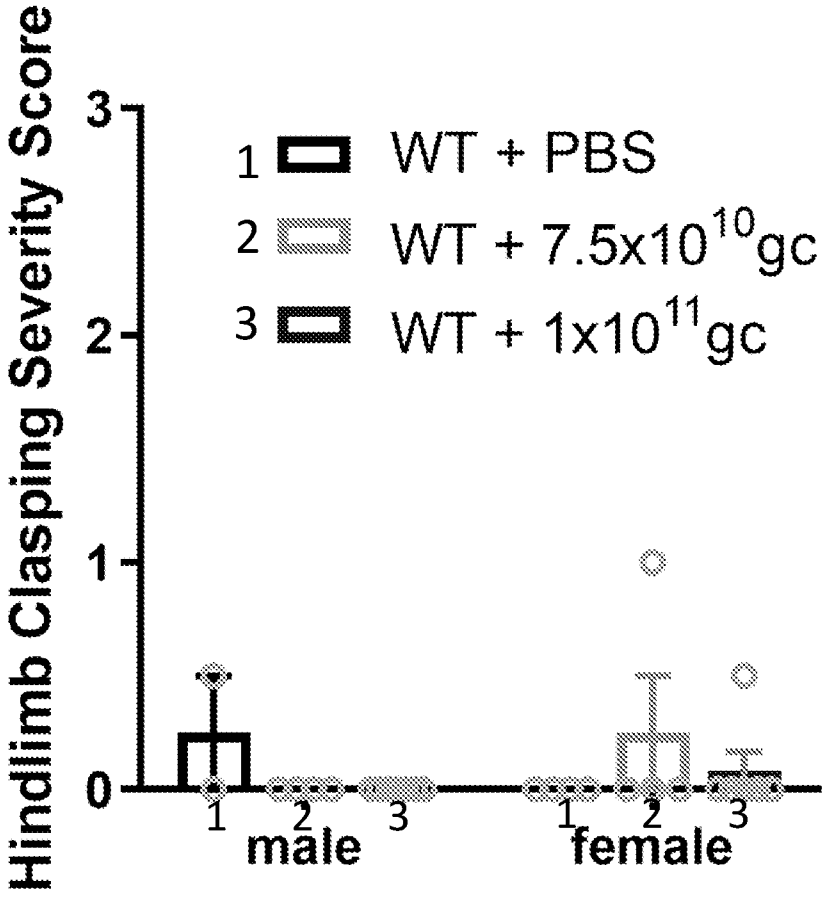


FIG 19B

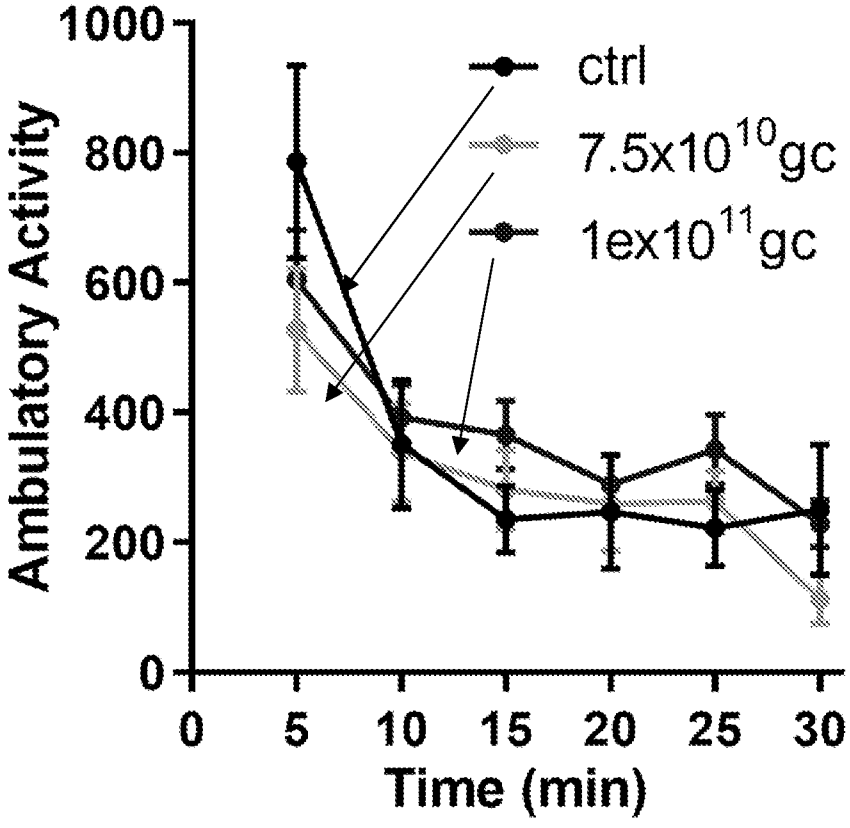


FIG 20

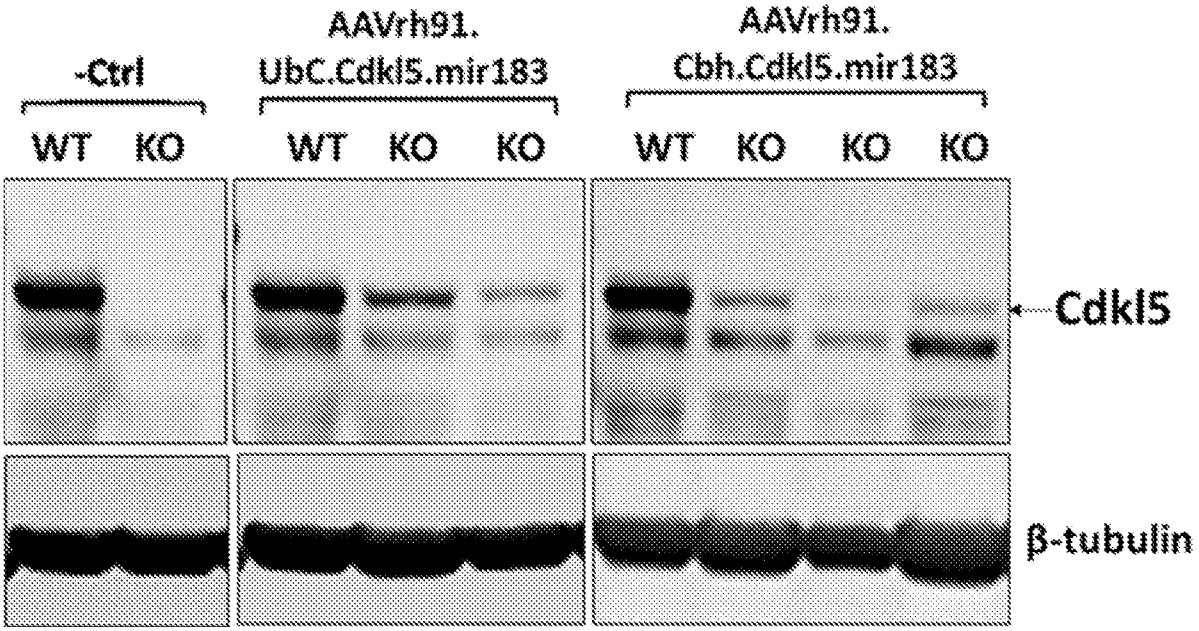

-Ctrl
AAVrh91.
UbC.Cdkl5.mir183
AAVrh91.
Cbh.Cdkl5.mir183
WT
KO
WT
KO
KO
WT
KO
KO
KO
Cdkl5
β-tubulin

FIG 21A

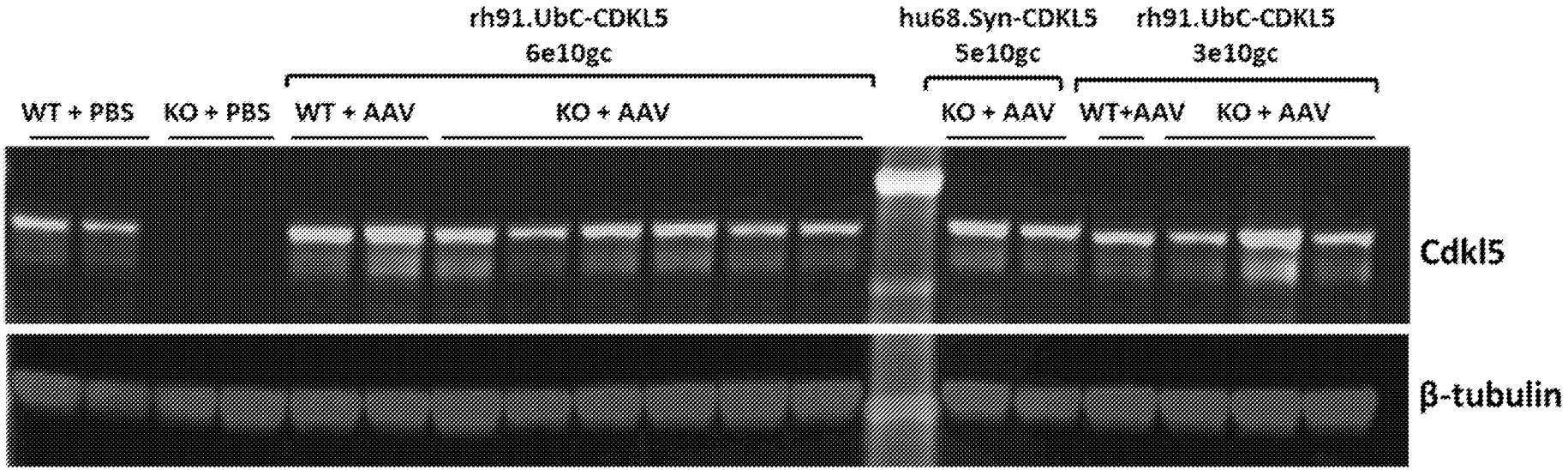


FIG 21B

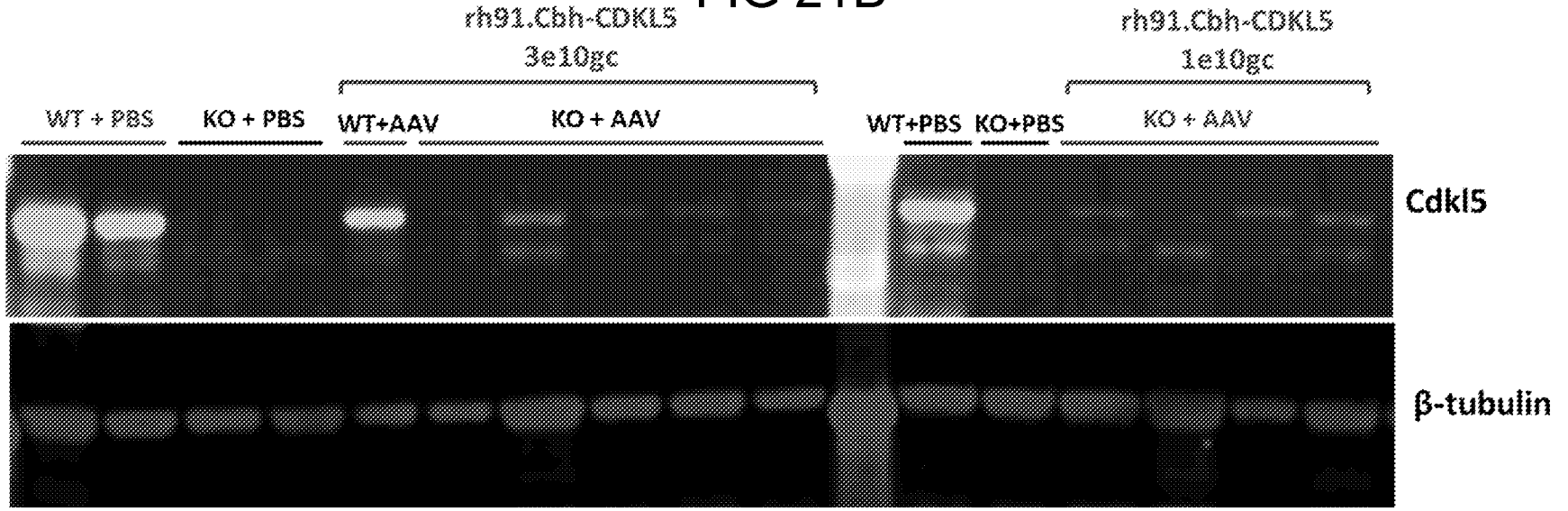


FIG 21C

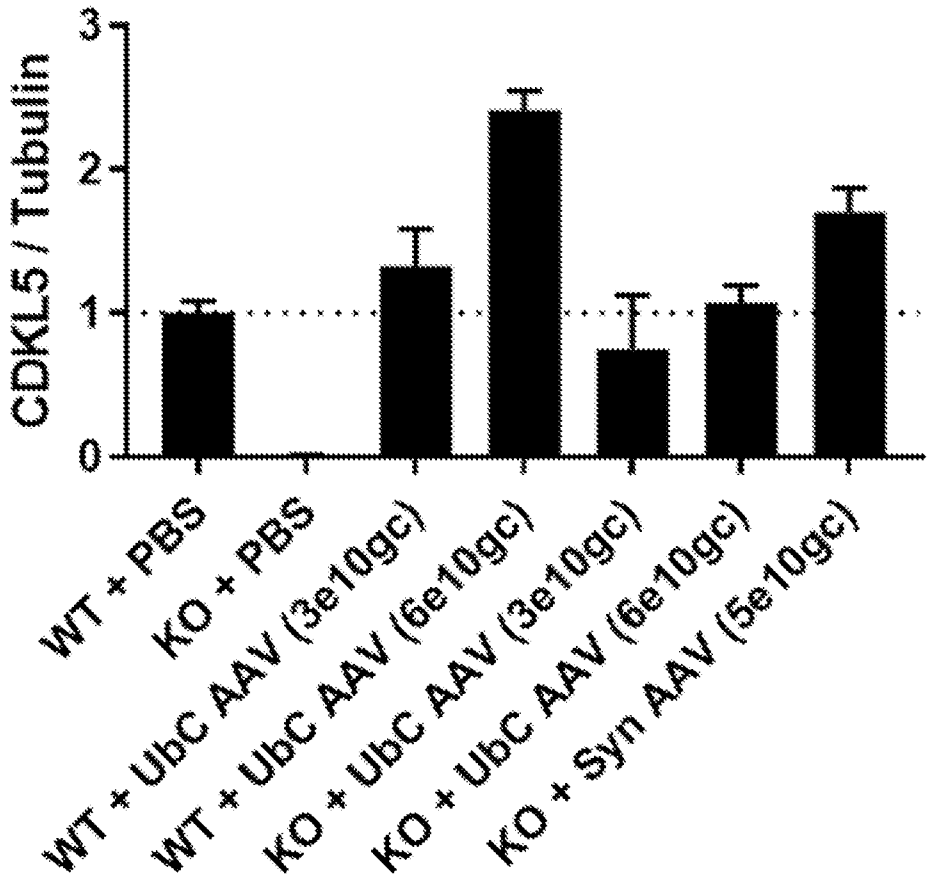

CDKL5 / Tubulin
3
2
1
0
WT + PBS
KO + PBS
WT + UbC AAV (3e10gc)
WT + UbC AAV (6e10gc)
KO + UbC AAV (3e10gc)
KO + UbC AAV (6e10gc)
KO + Syn AAV (5e10gc)

FIG 22A

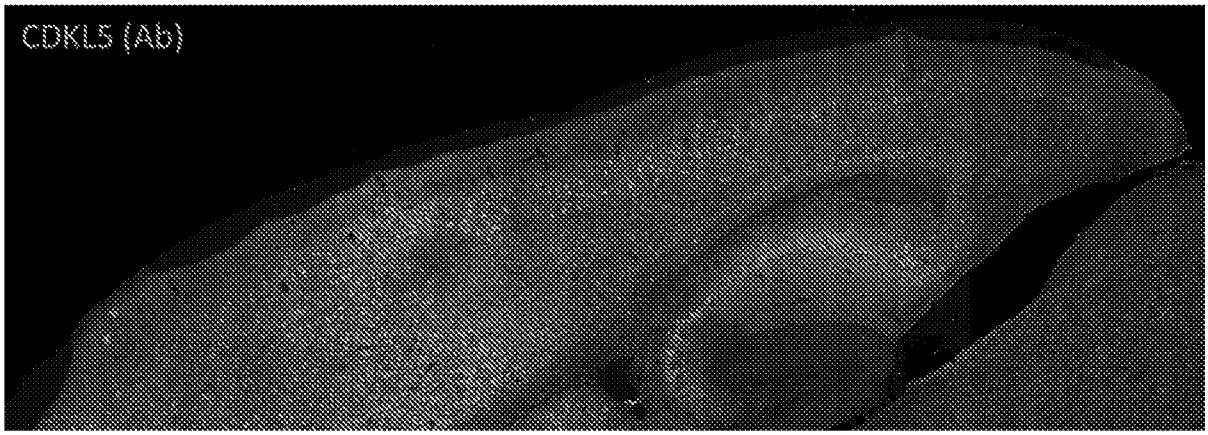


FIG 22B

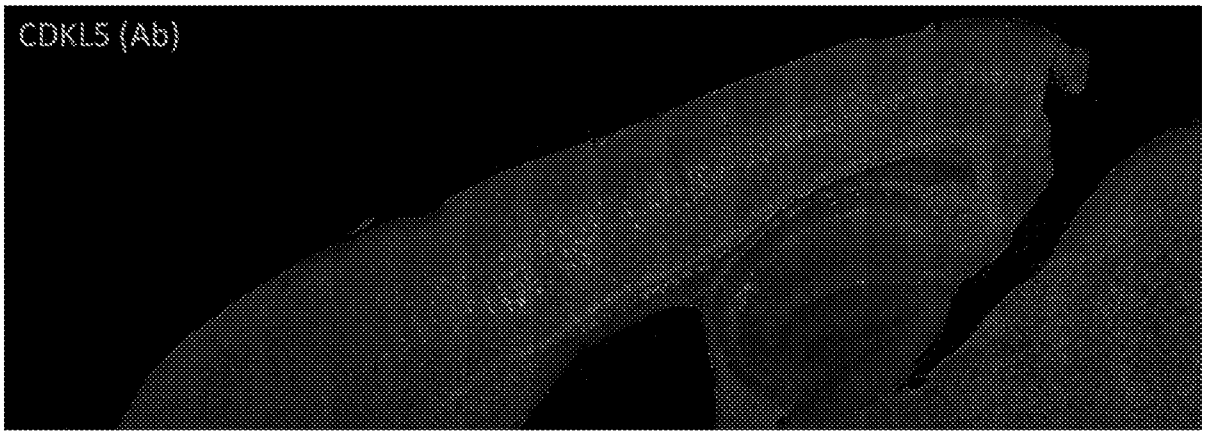
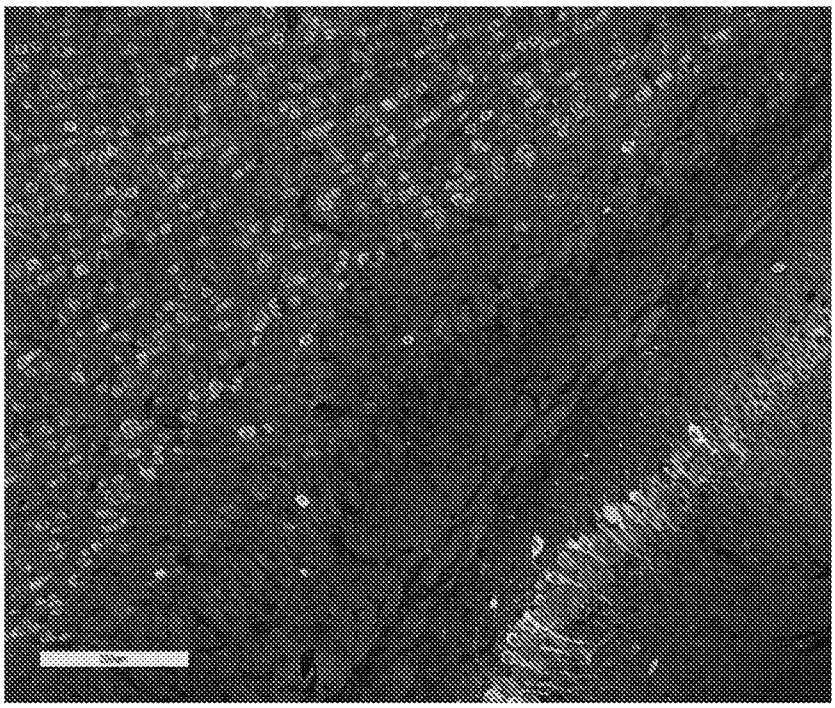


FIG 23A

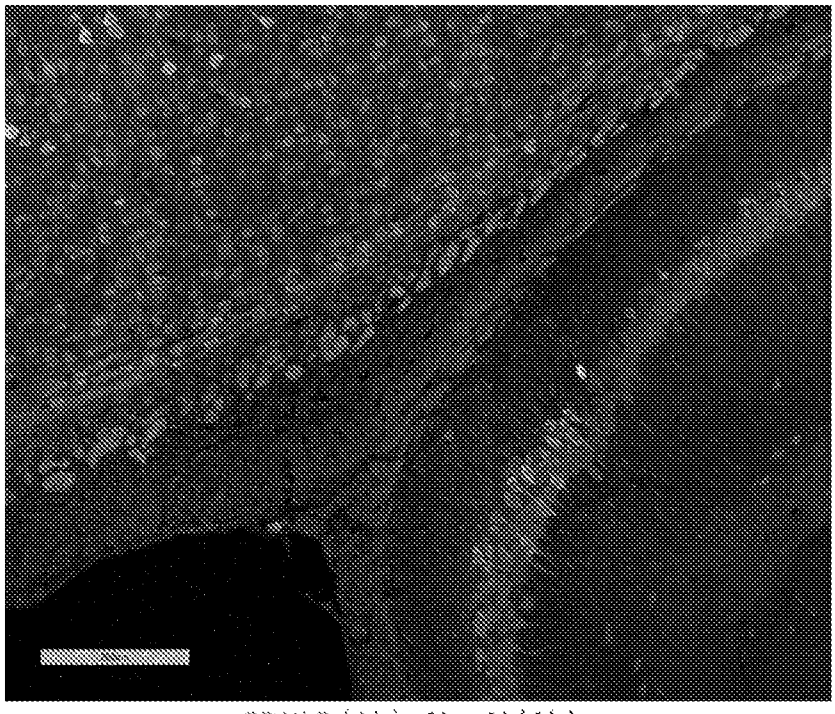
AAV**rh91**.UbC.CDKL5-1co.miR183



CDKL5 (Ab) NeuN (Ab)

FIG 23B

AAV**rh91**.CBh.CDKL5-1co.miR183



CDKL5 (Ab) NeuN (Ab)

FIG 24

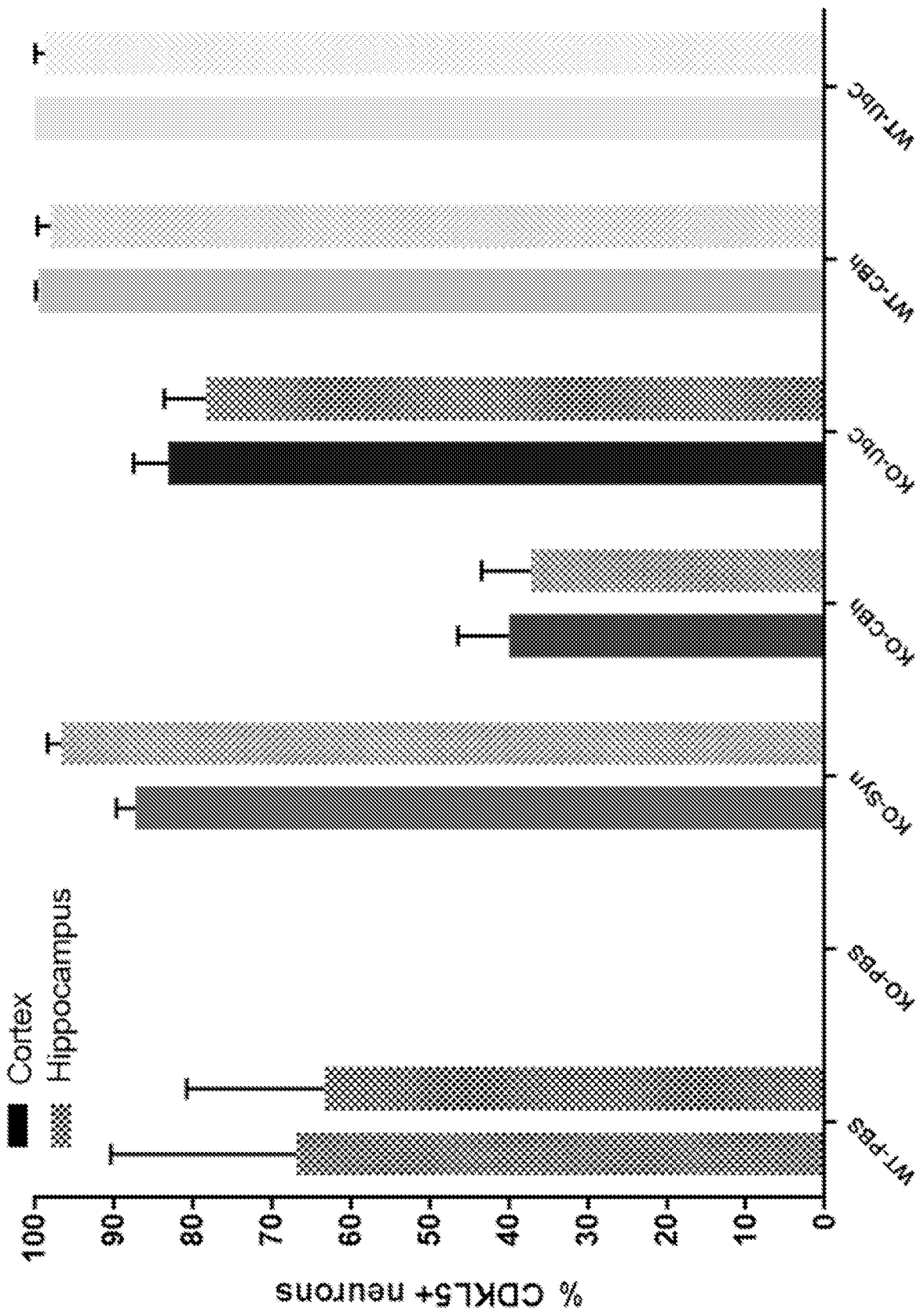

% CDKL5+ neurons
100
90
80
70
60
50
40
30
20
10
0
Cortex
Hippocampus
WT-PBS
KO-PBS
KO-Syn
KO-CBh
KO-UbC
WT-CBh
WT-UbC

FIG 25A

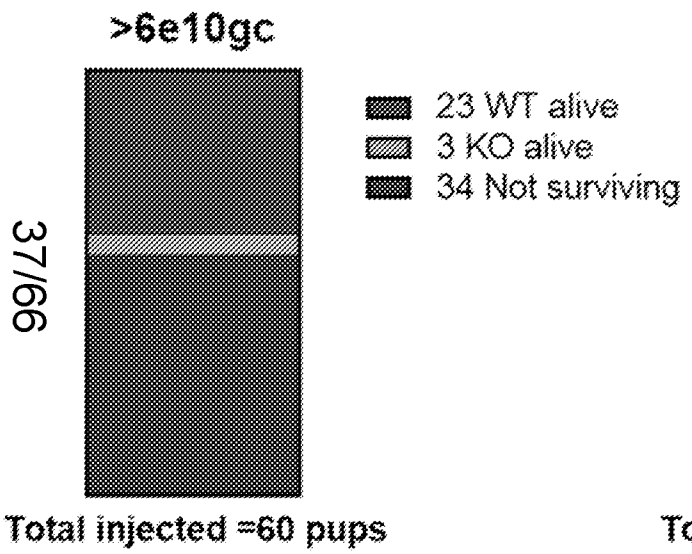


FIG 25B

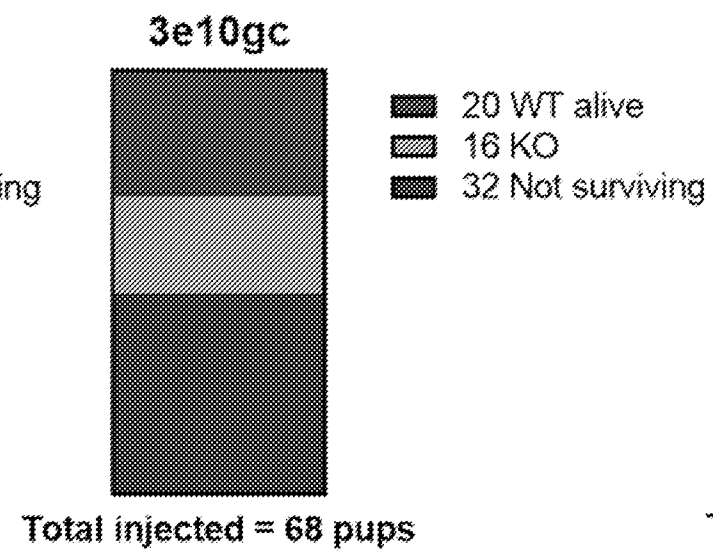


FIG 25C

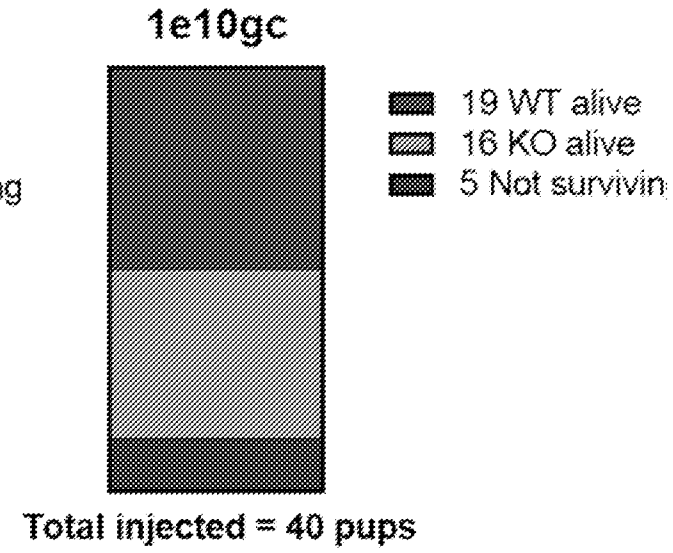


FIG 26A

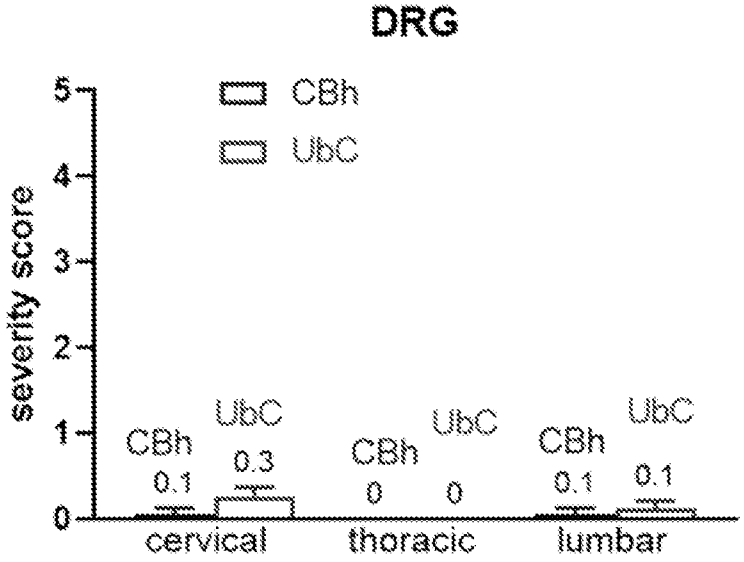


FIG 26B

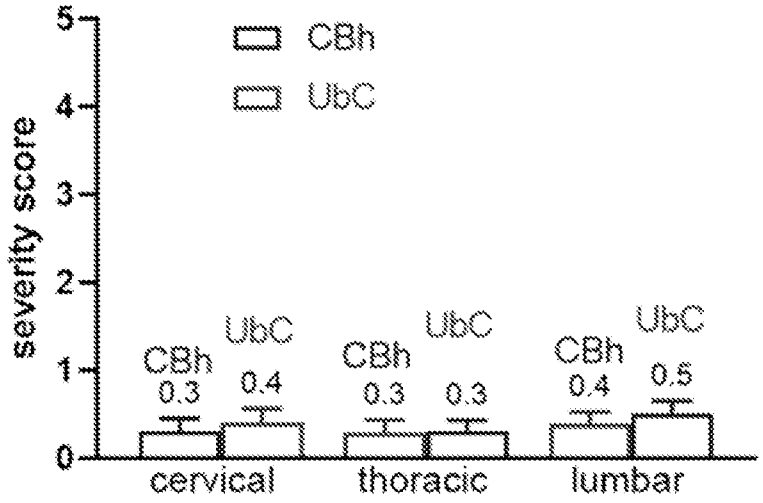


FIG 26C

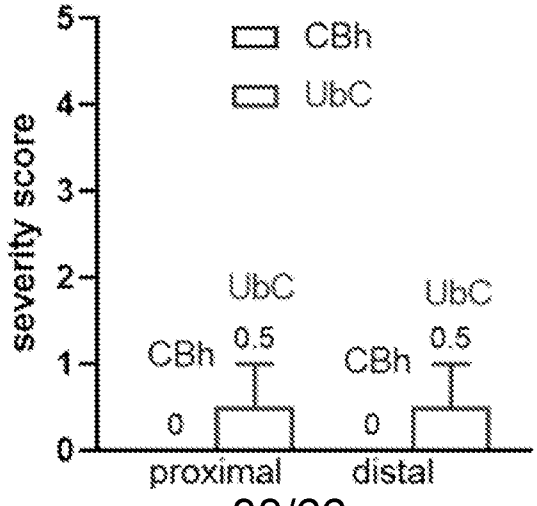


FIG 27

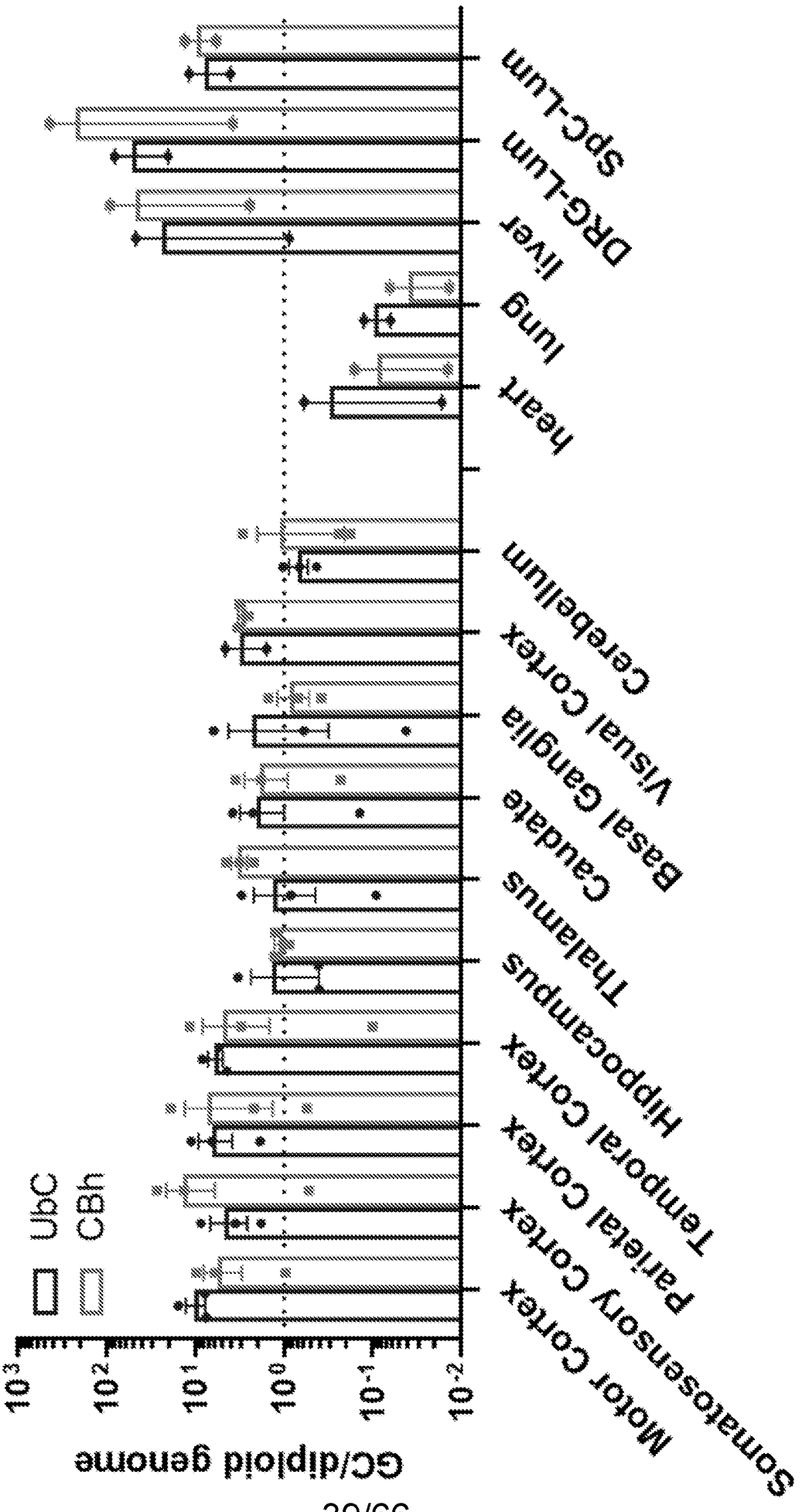

UbC
CBh
GC/diploid genome
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
Motor Cortex
Somatosensory Cortex
Parietal Cortex
Temporal Cortex
Hippocampus
Thalamus
Caudate
Basal Ganglia
Visual Cortex
Cerebellum
Heart
Lung
Liver
DRG-Lum
SpC-Lum

FIG 28

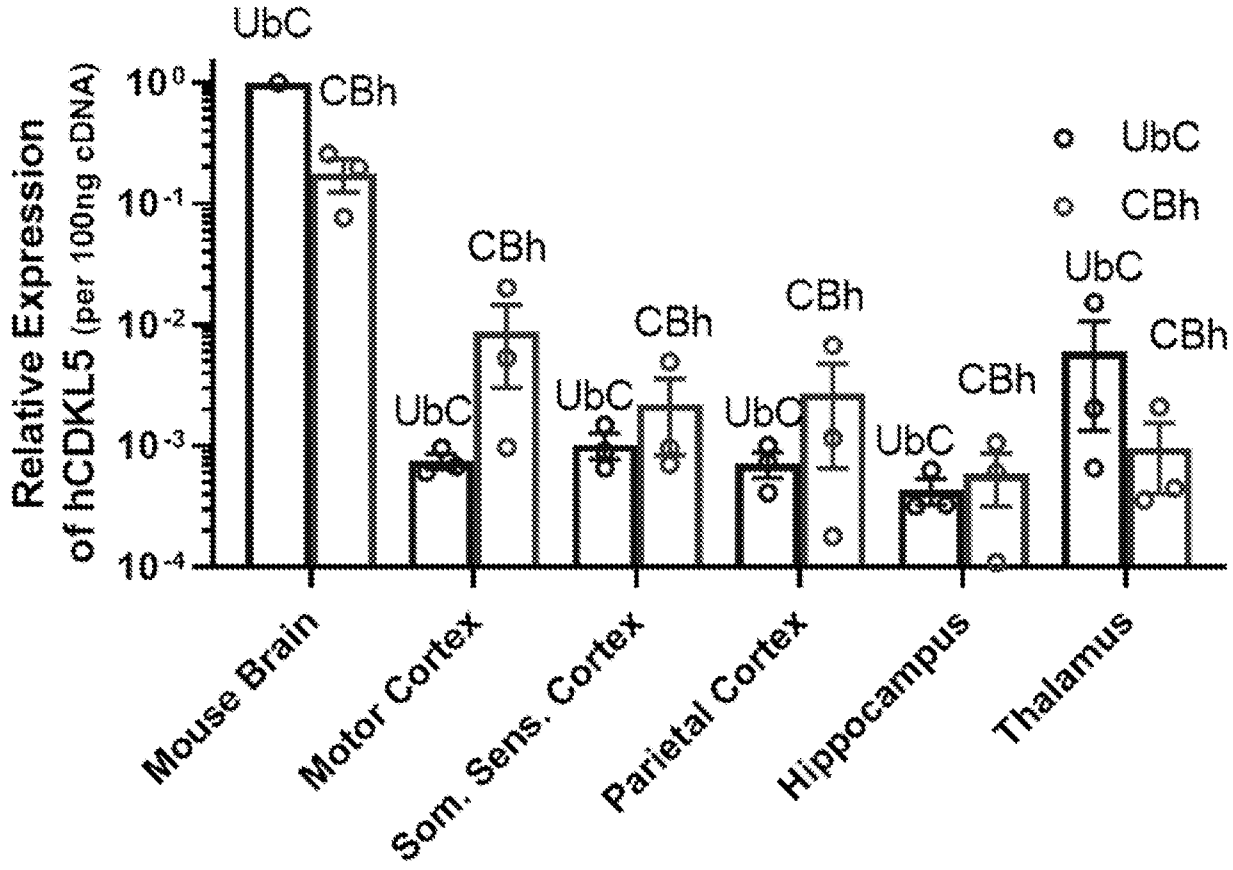

Relative Expression
of hCDKL5 (per 100ng cDNA)
10^0
10^-1
10^-2
10^-3
10^-4
UbC
CBh
UbC
CBh
Mouse Brain
Motor Cortex
Som. Sens. Cortex
Parietal Cortex
Hippocampus
Thalamus

FIG 29A

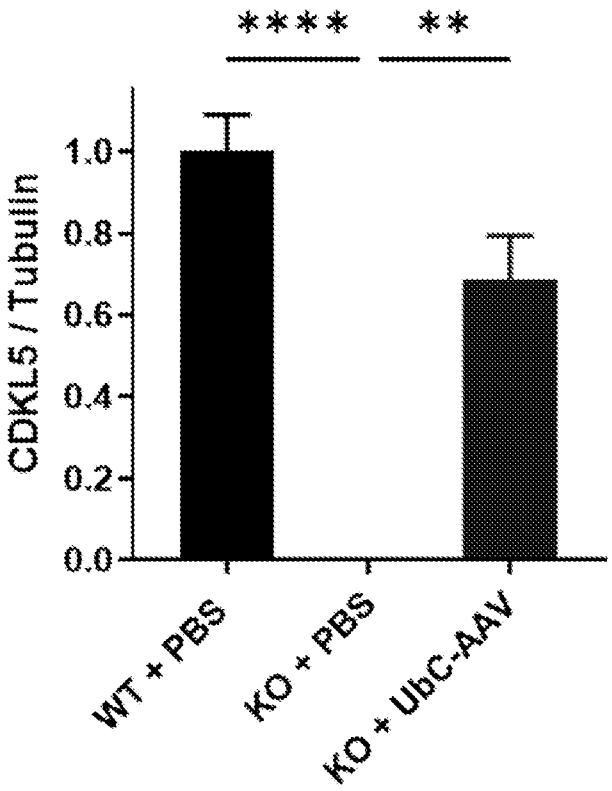


FIG 29B

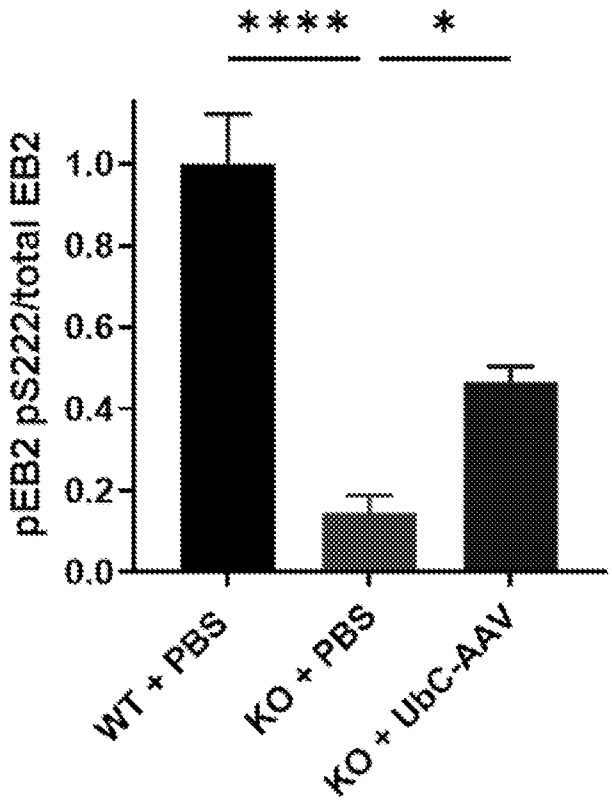


FIG 30

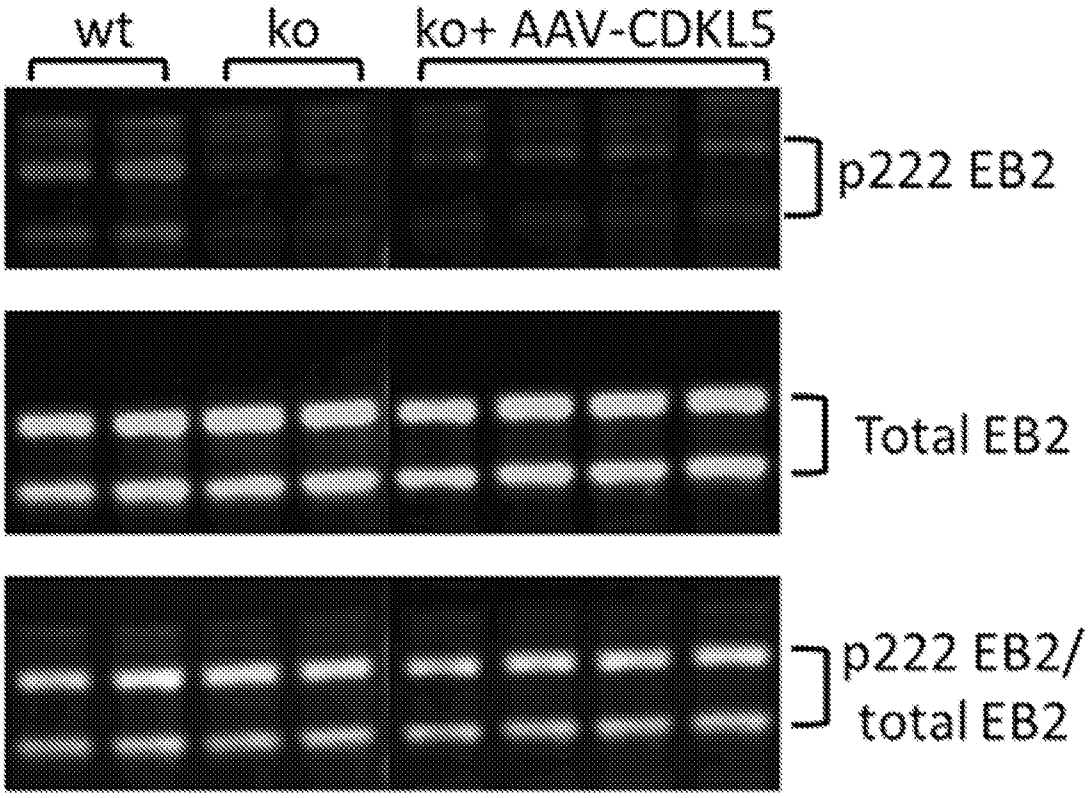


FIG 31A

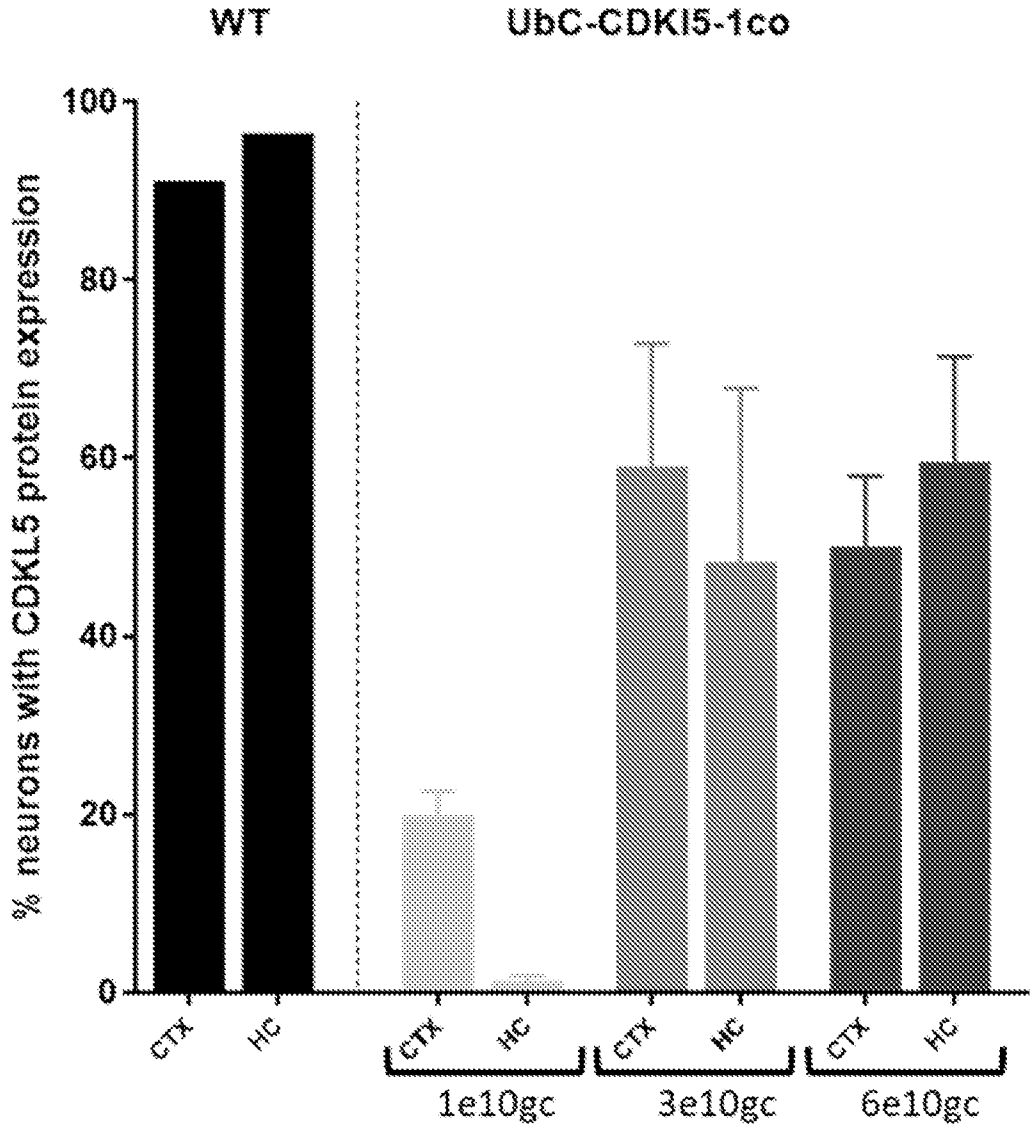


FIG 31B

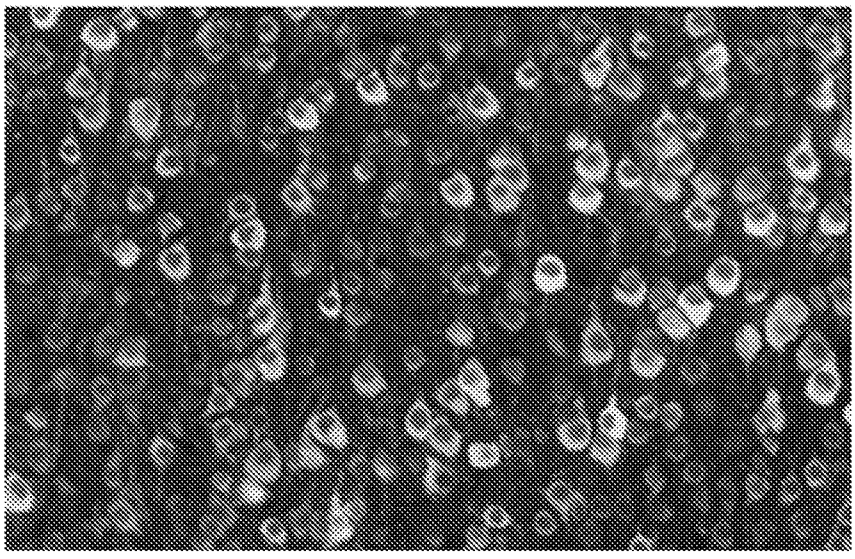


FIG 32

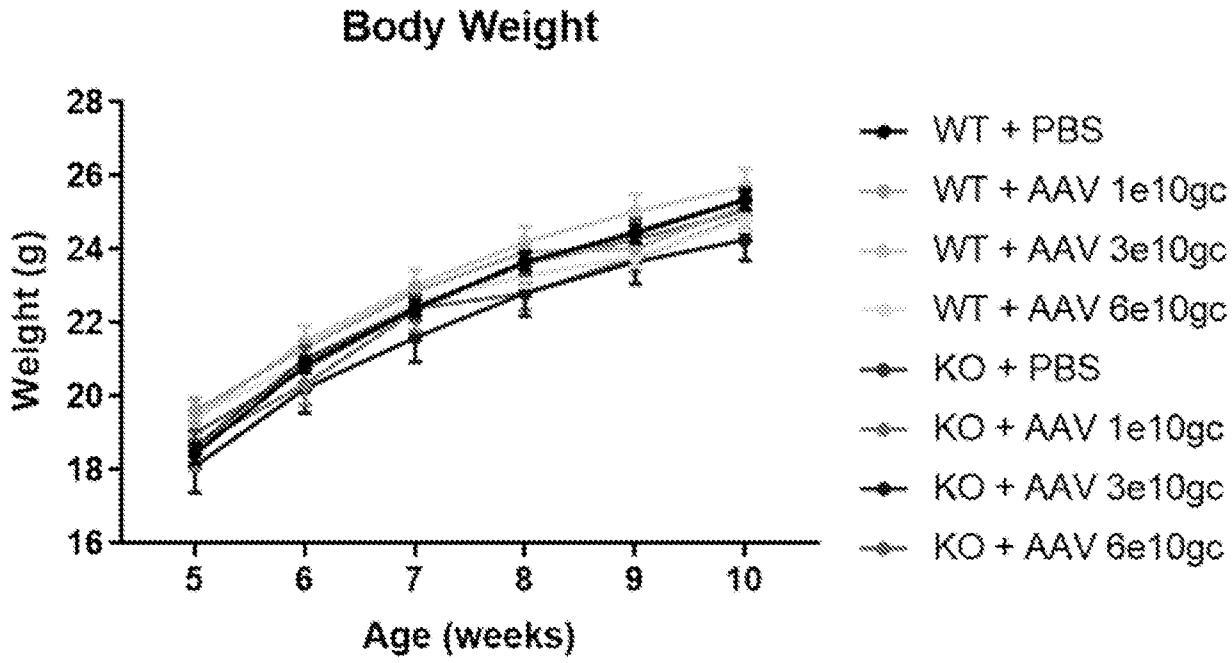

Body Weight
Weight (g)
28
26
24
22
20
18
16
5
6
7
8
9
10
Age (weeks)
WT + PBS
WT + AAV 1e10gc
WT + AAV 3e10gc
WT + AAV 6e10gc
KO + PBS
KO + AAV 1e10gc
KO + AAV 3e10gc
KO + AAV 6e10gc

FIG 33A

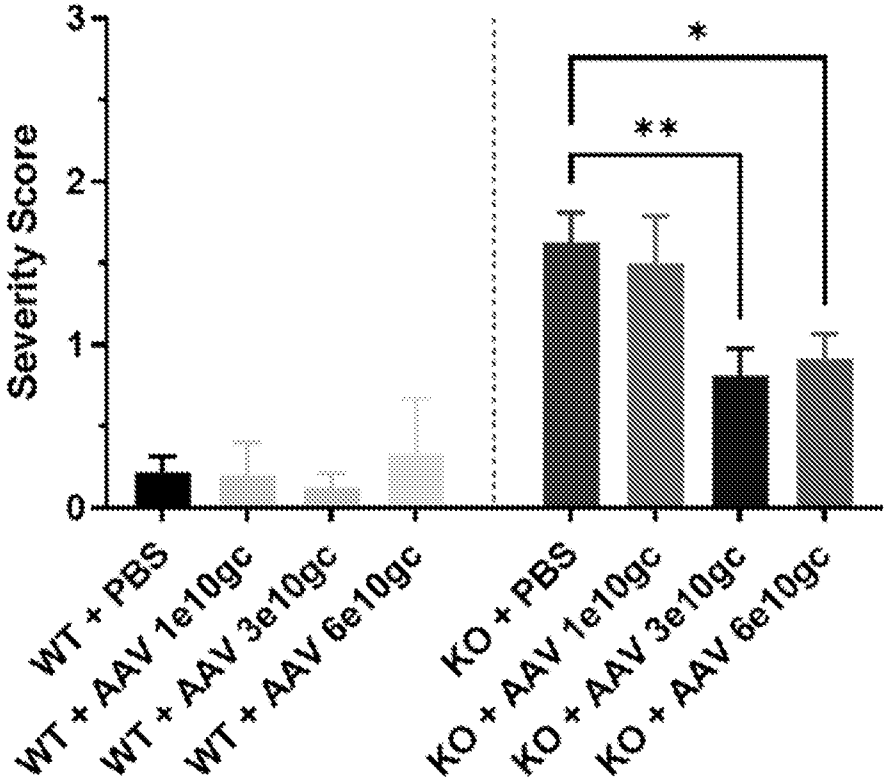


FIG 33B

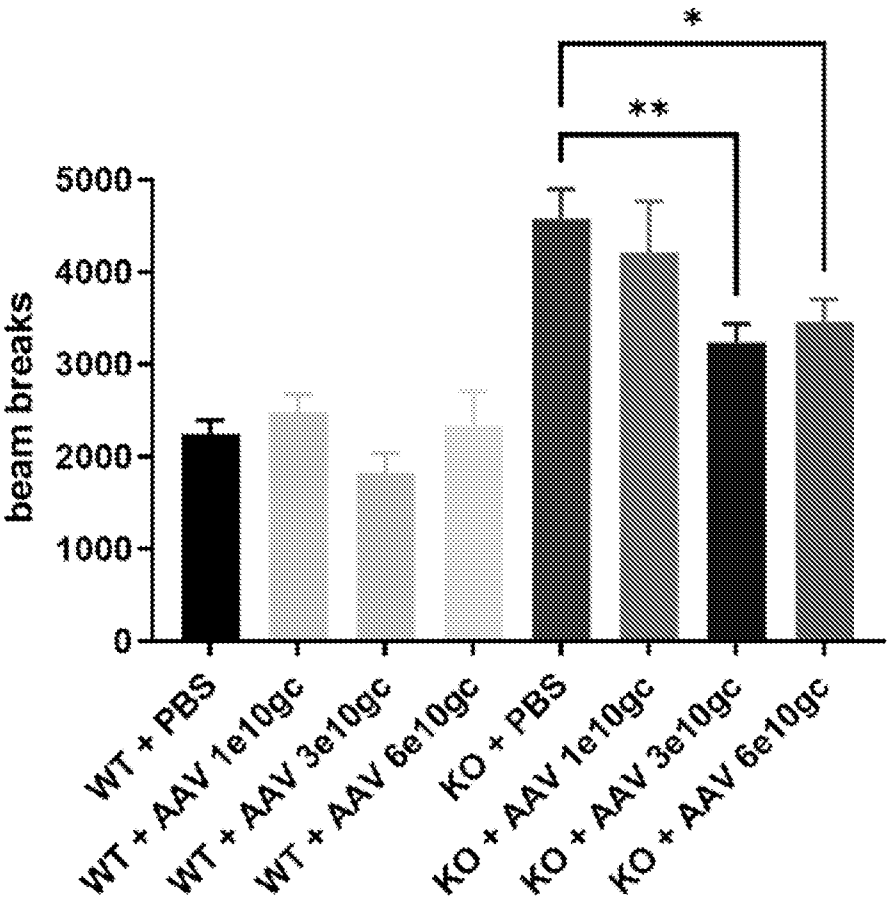


FIG 34A

Low dose

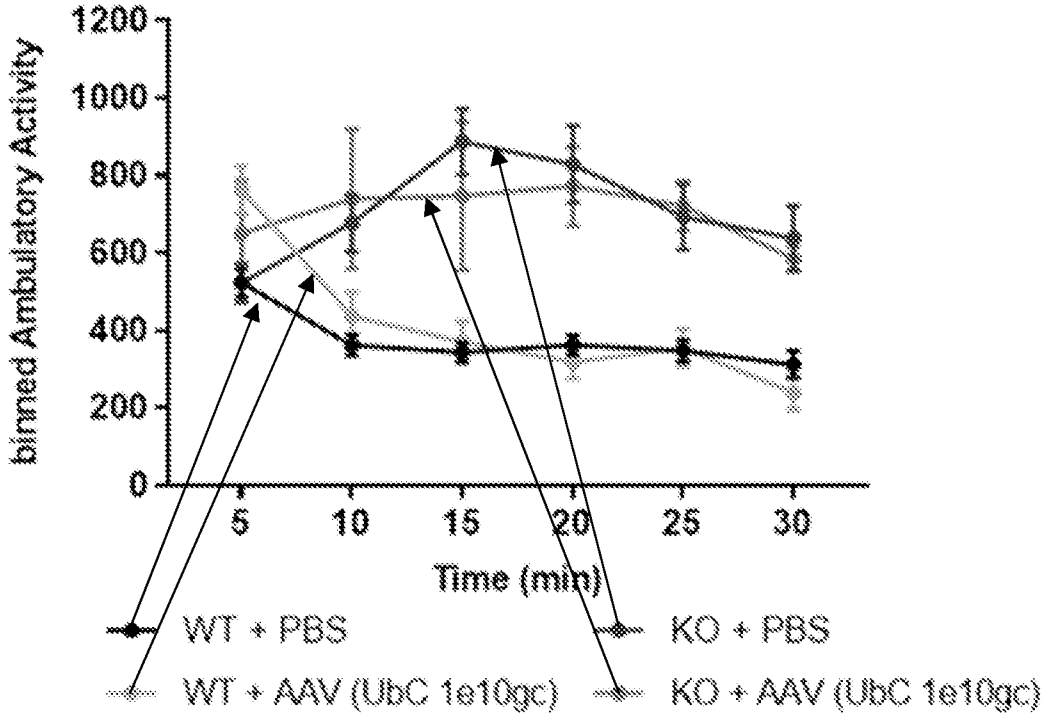


FIG 34B

Medium dose

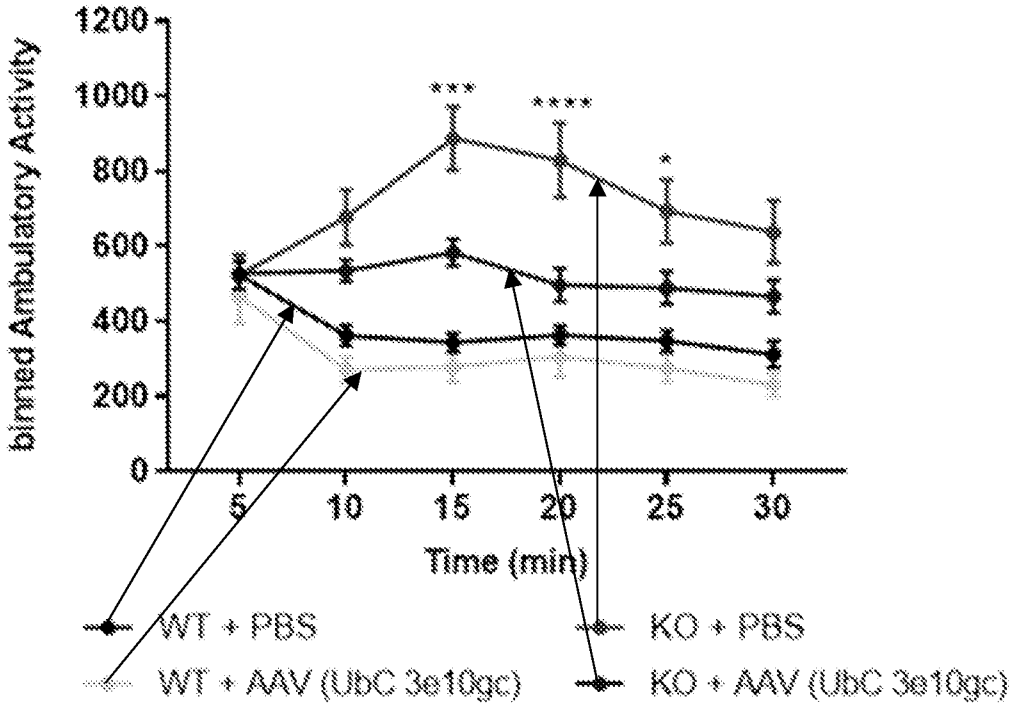


FIG 34C

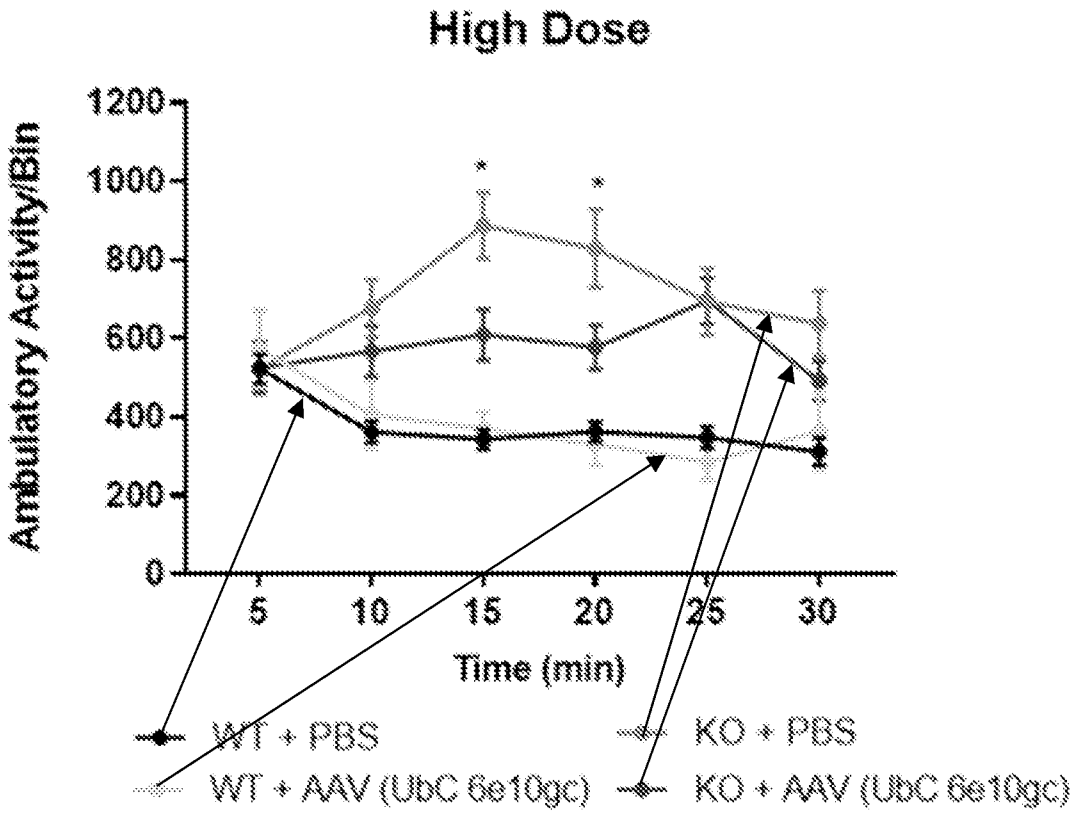

High Dose
Ambulatory Activity/Bin
1200
1000
800
600
400
200
0
5
10
15
20
25
30
Time (min)
WT + PBS
WT + AAV (UbC 6e10gc)
KO + PBS
KO + AAV (UbC 6e10gc)

FIG 35

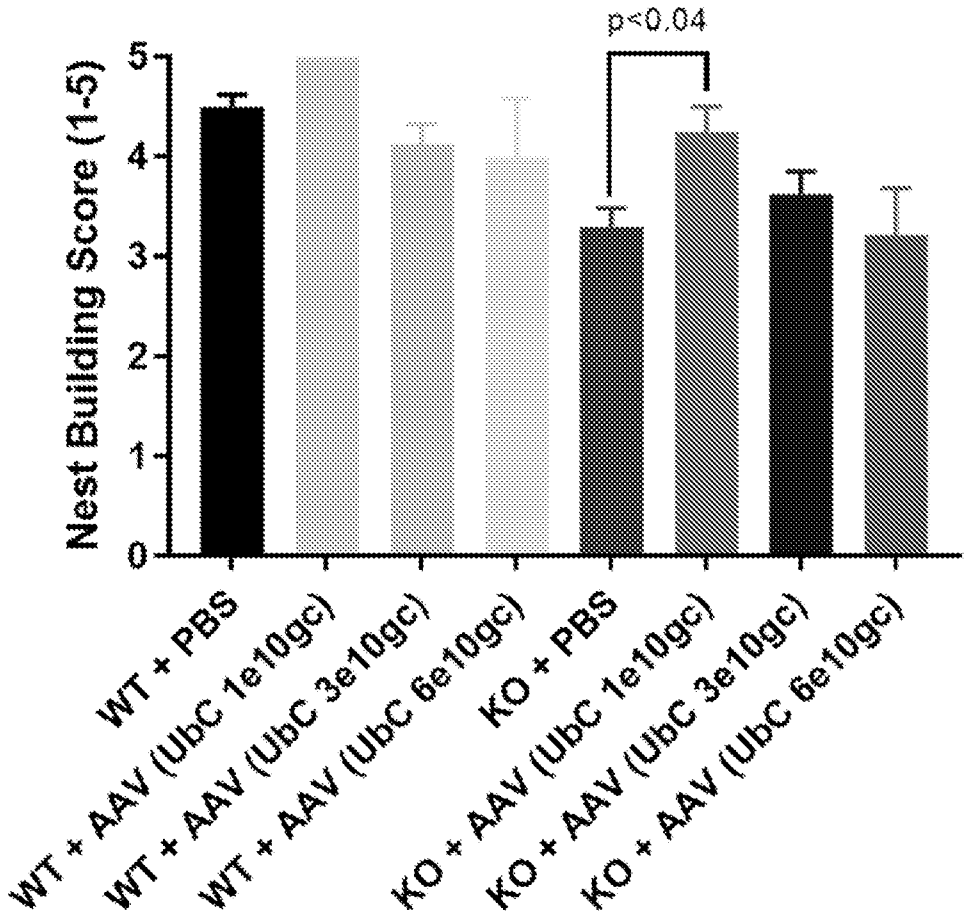

p<0.04
Nest Building Score (1-5)
0
1
2
3
4
5
WT + PBS
WT + AAV (UbC 1e10gc)
WT + AAV (UbC 3e10gc)
WT + AAV (UbC 6e10gc)
KO + PBS
KO + AAV (UbC 1e10gc)
KO + AAV (UbC 3e10gc)
KO + AAV (UbC 6e10gc)

FIG 36A

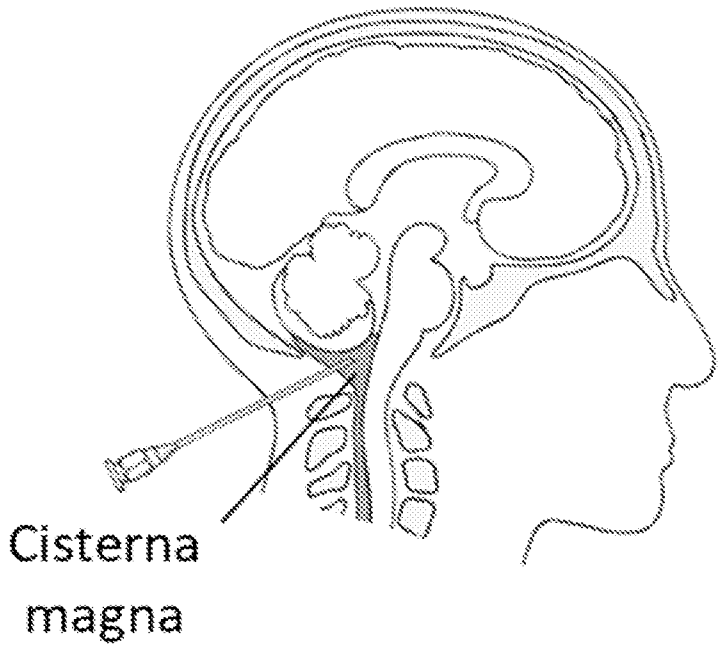
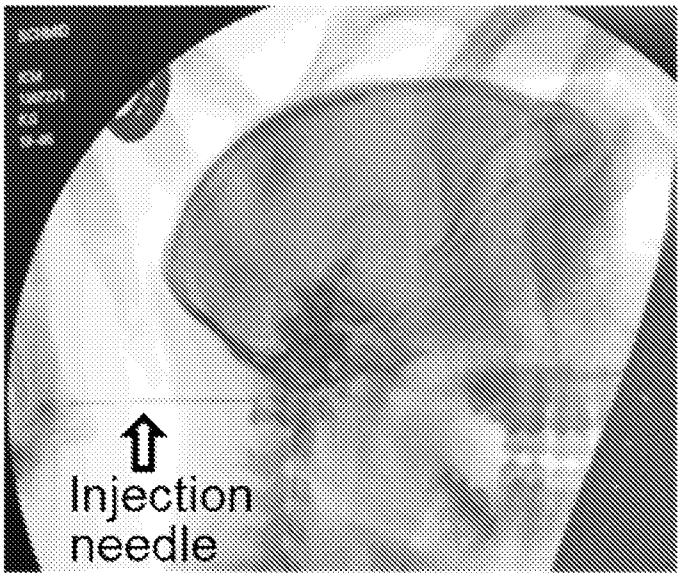


FIG 36B



Fluoroscopy-guided
procedure

FIG 37A

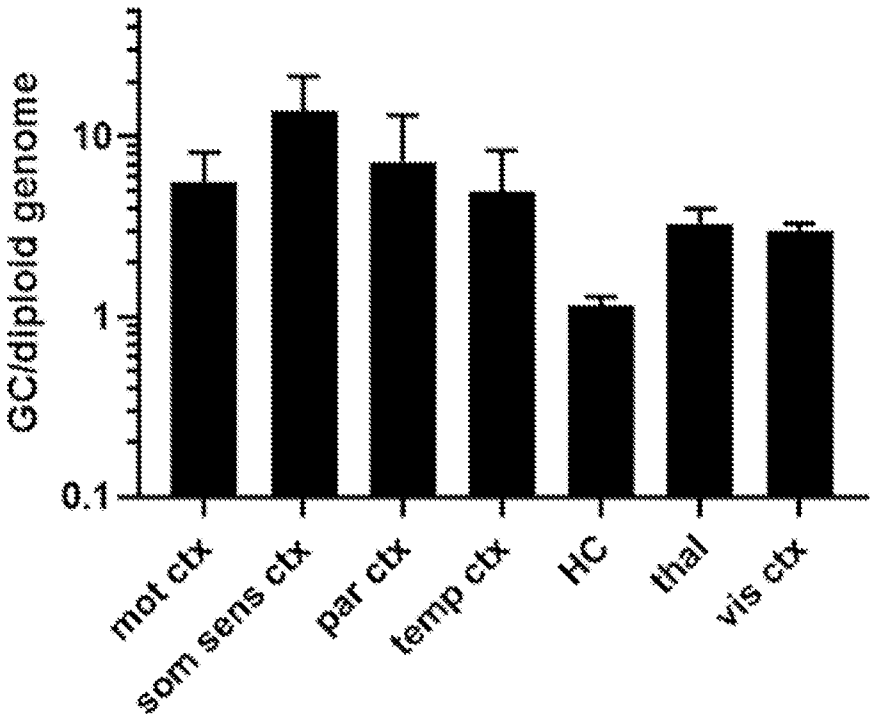
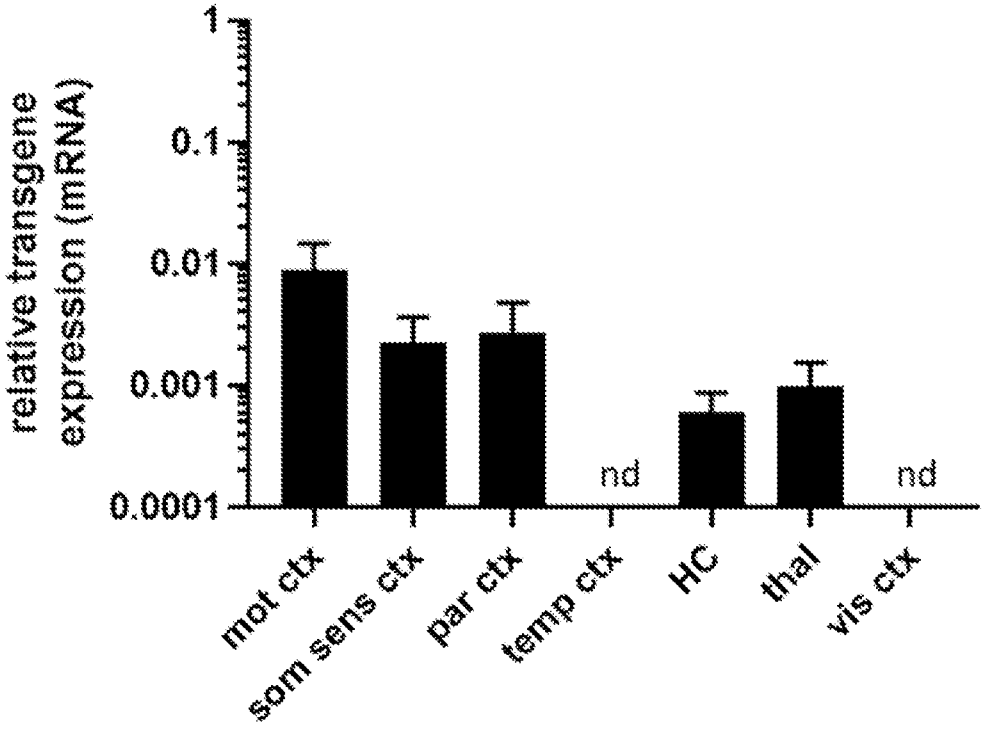


FIG 37B



nd = not determined

Expression relative to mouse brain (3e10gc dose)

FIG 38A

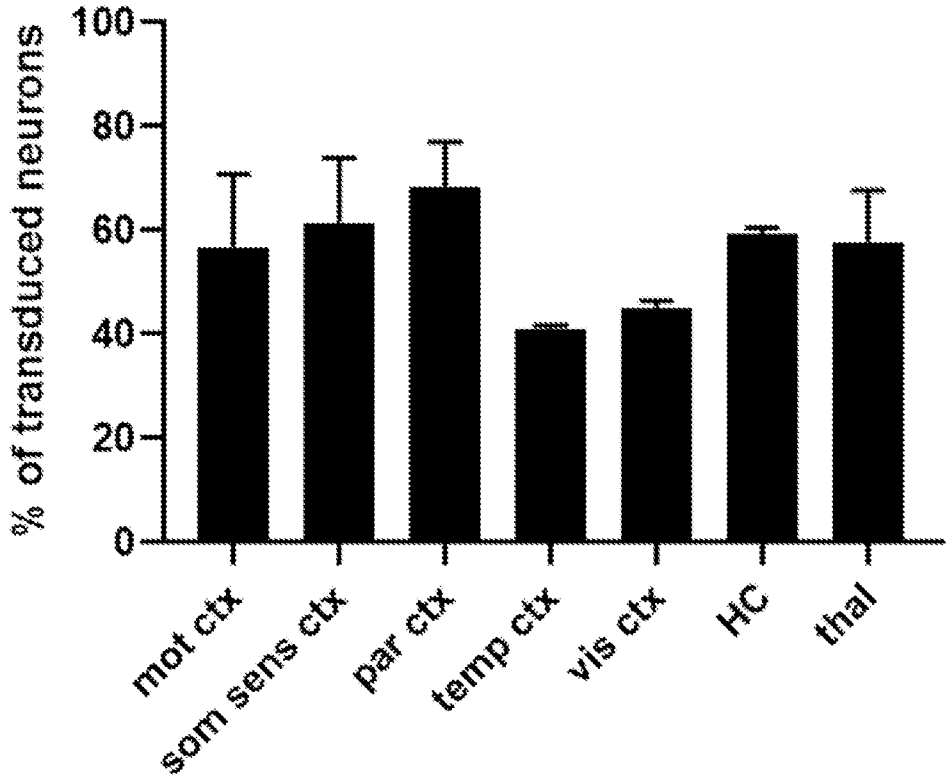


FIG 38B

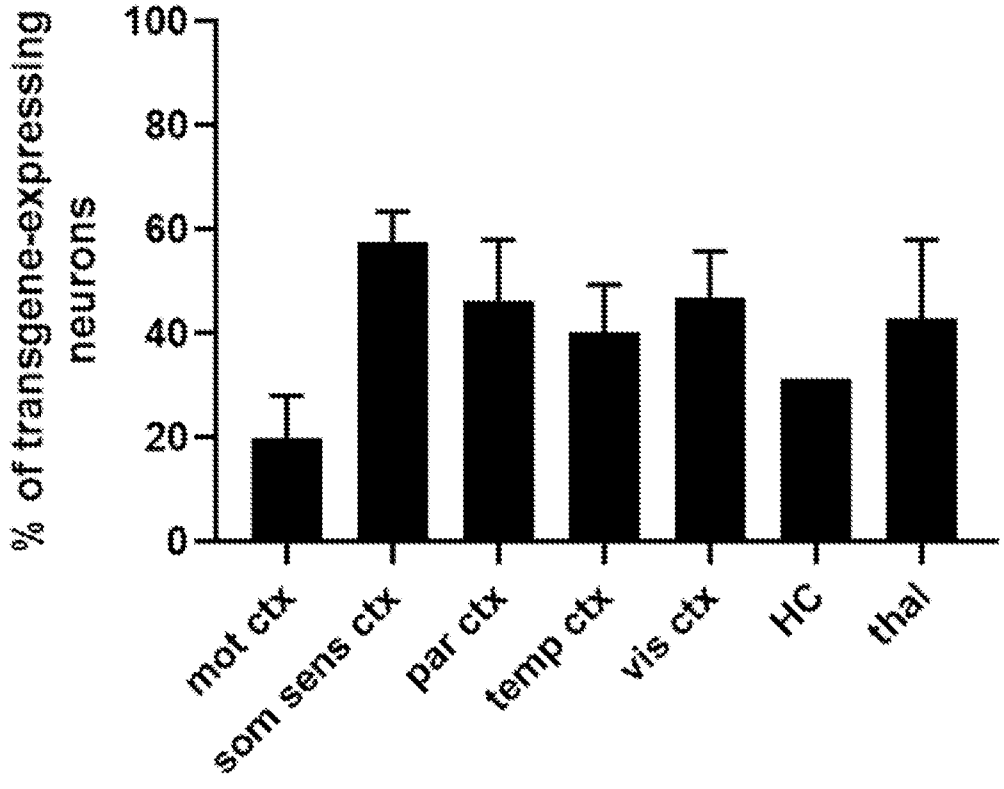


FIG 39A

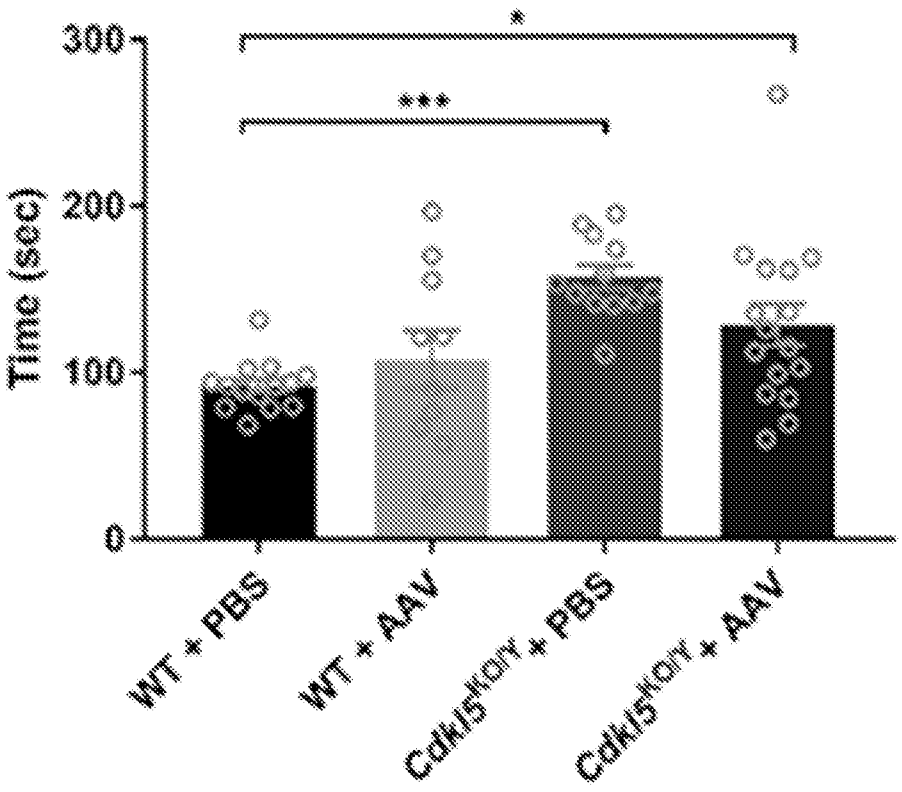


FIG 39B

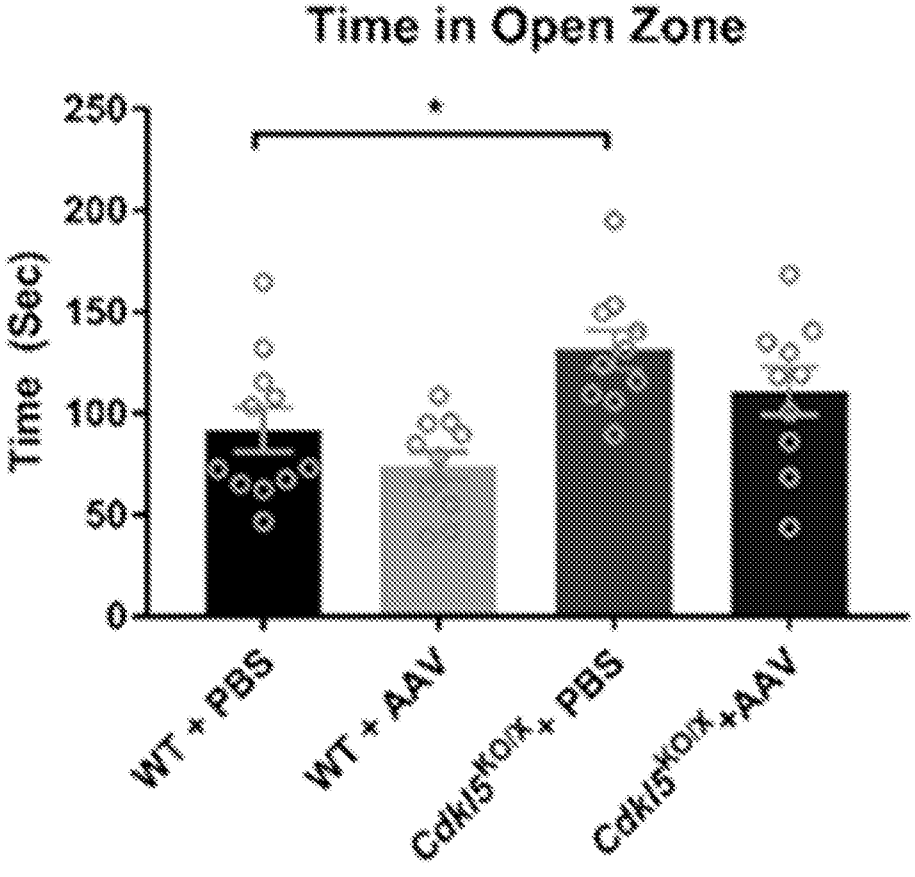


FIG 40A

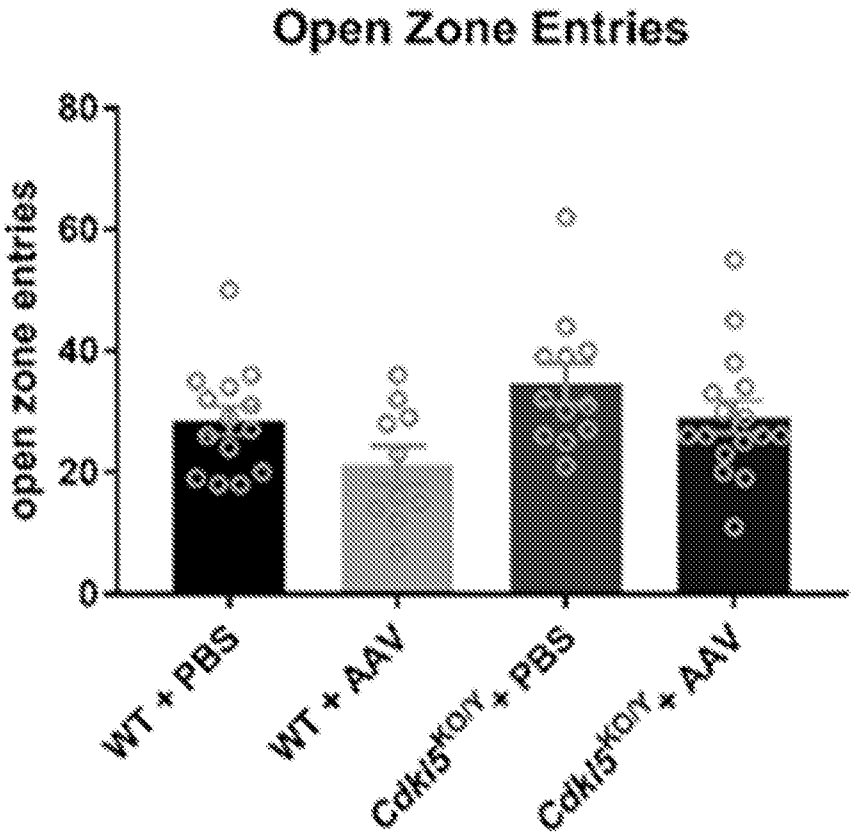


FIG 40B

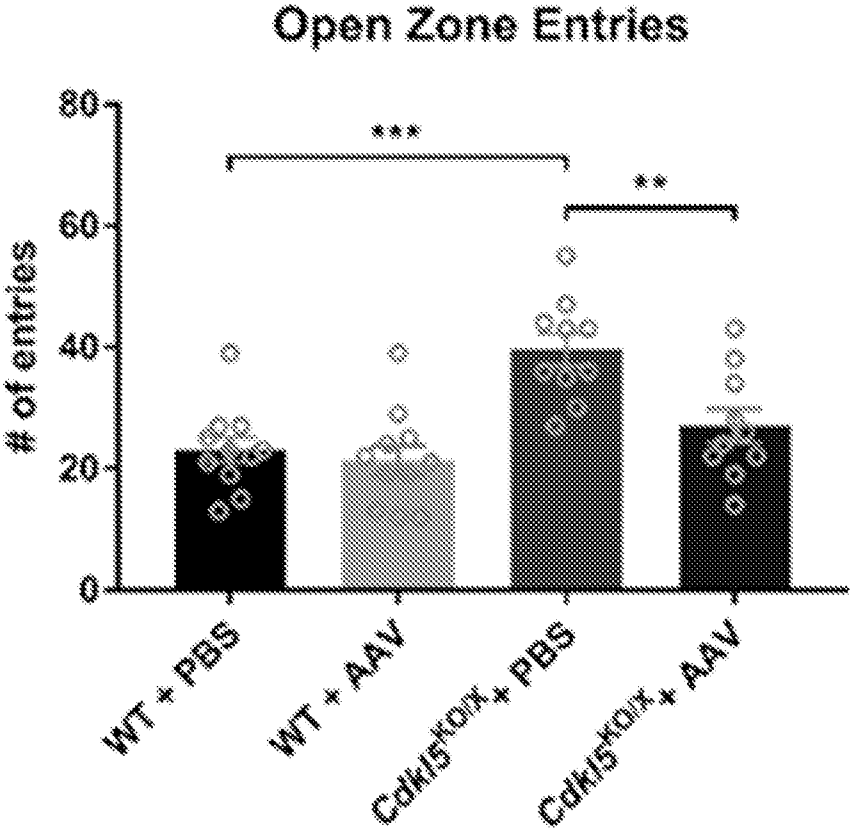


FIG 41A

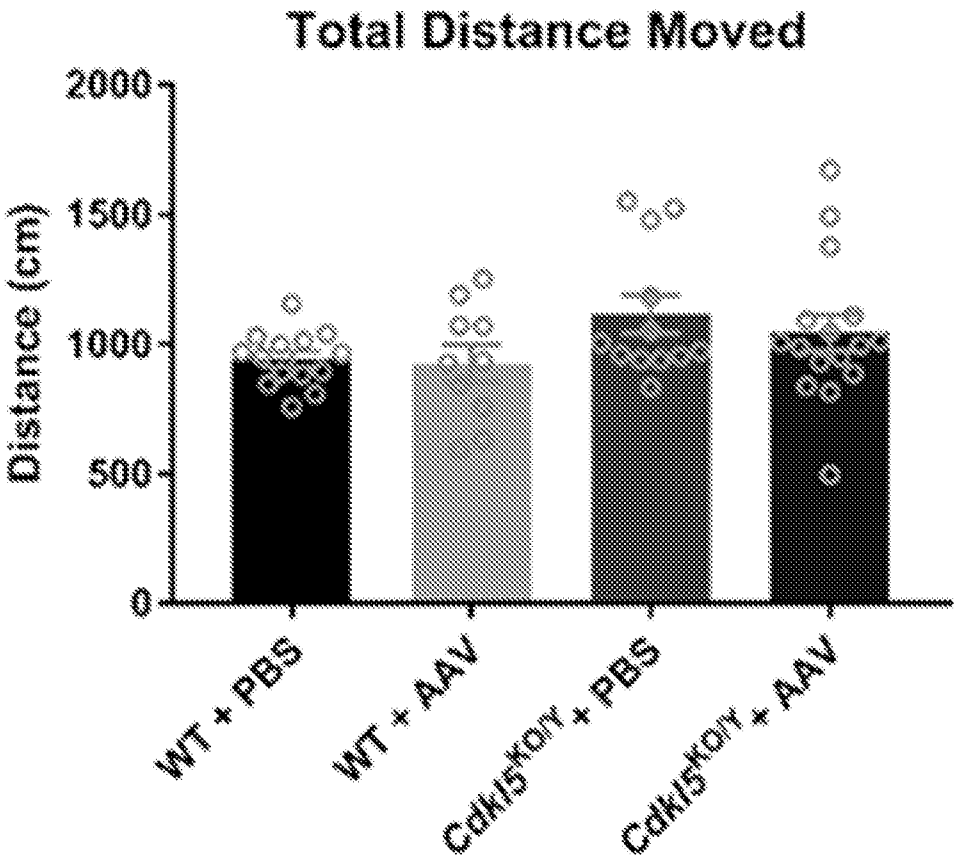


FIG 41B

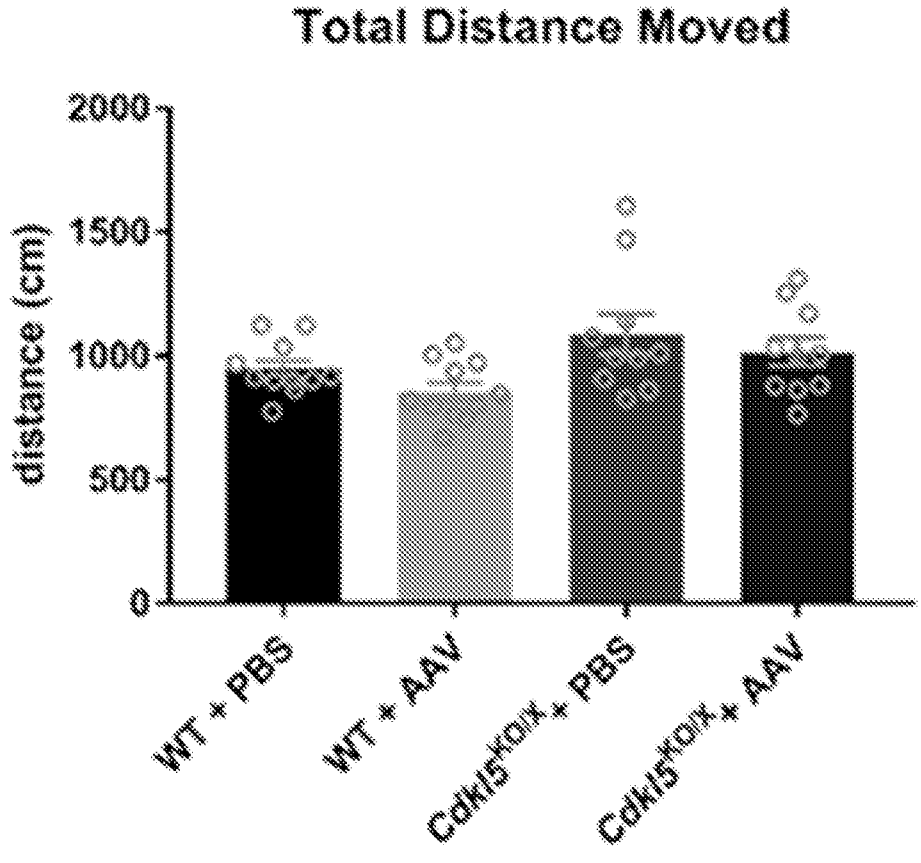


FIG 42A

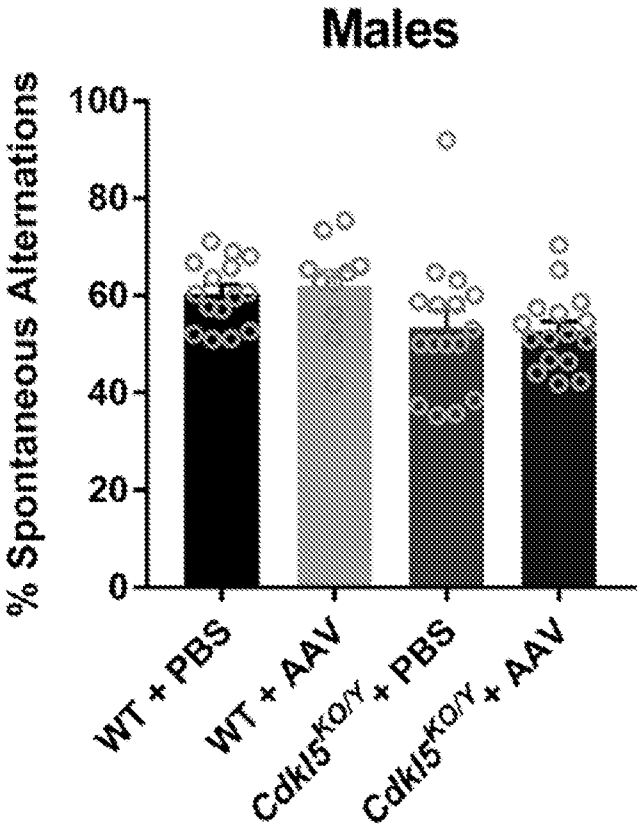


FIG 42B

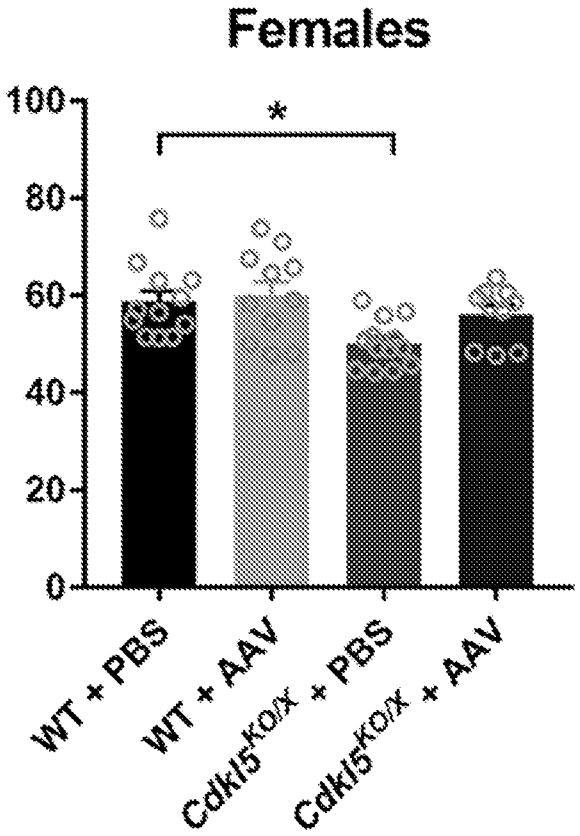


FIG 43A

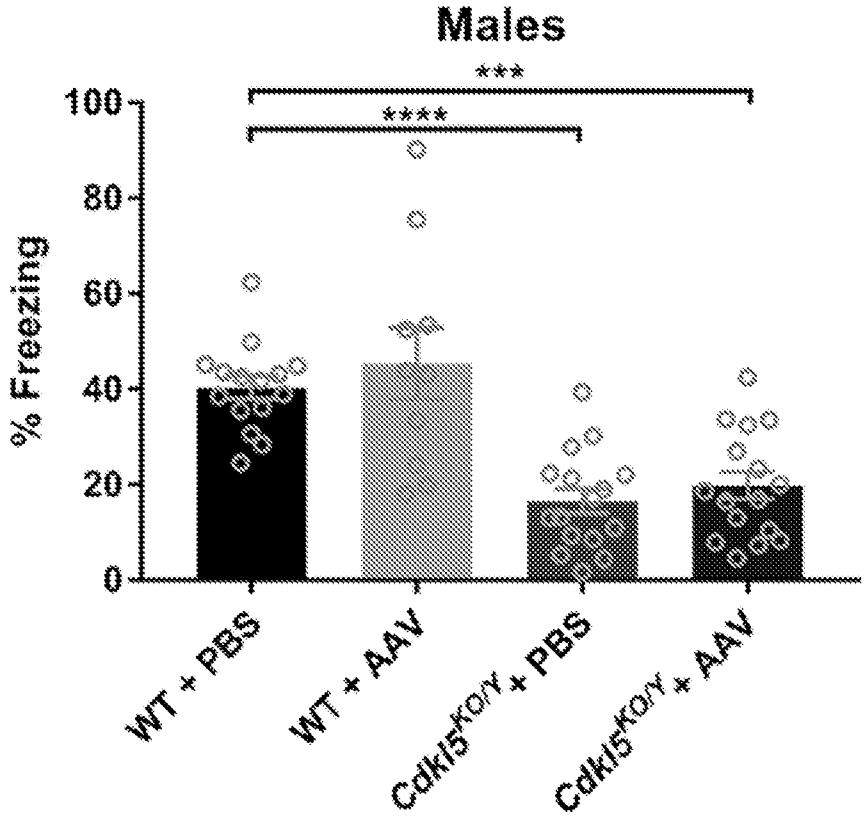


FIG 43B

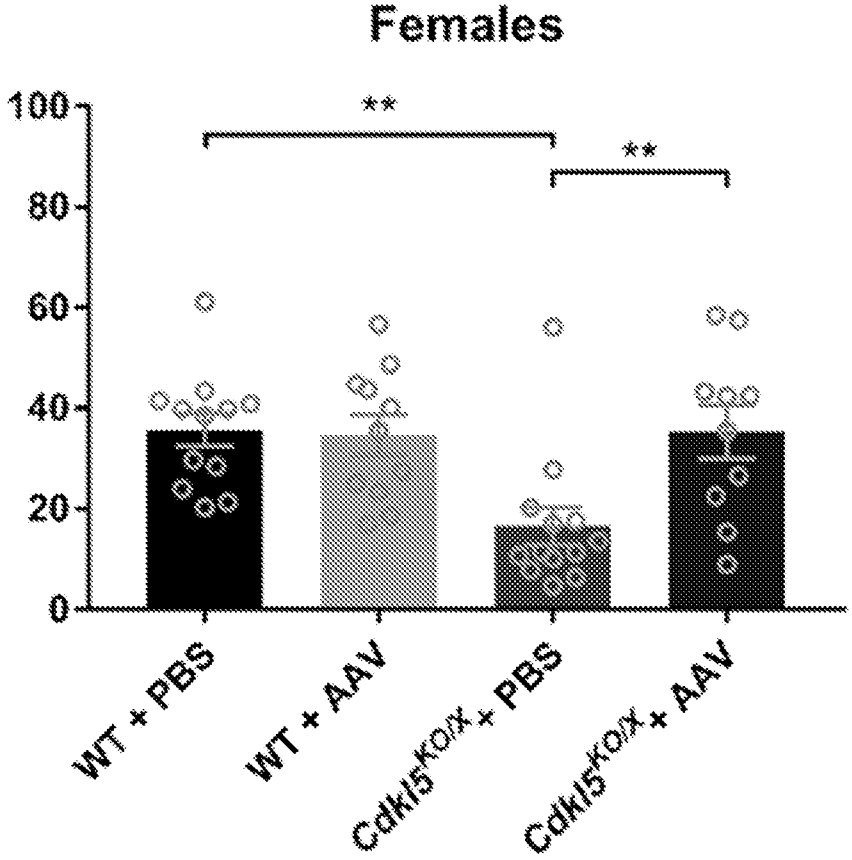


FIG 44A

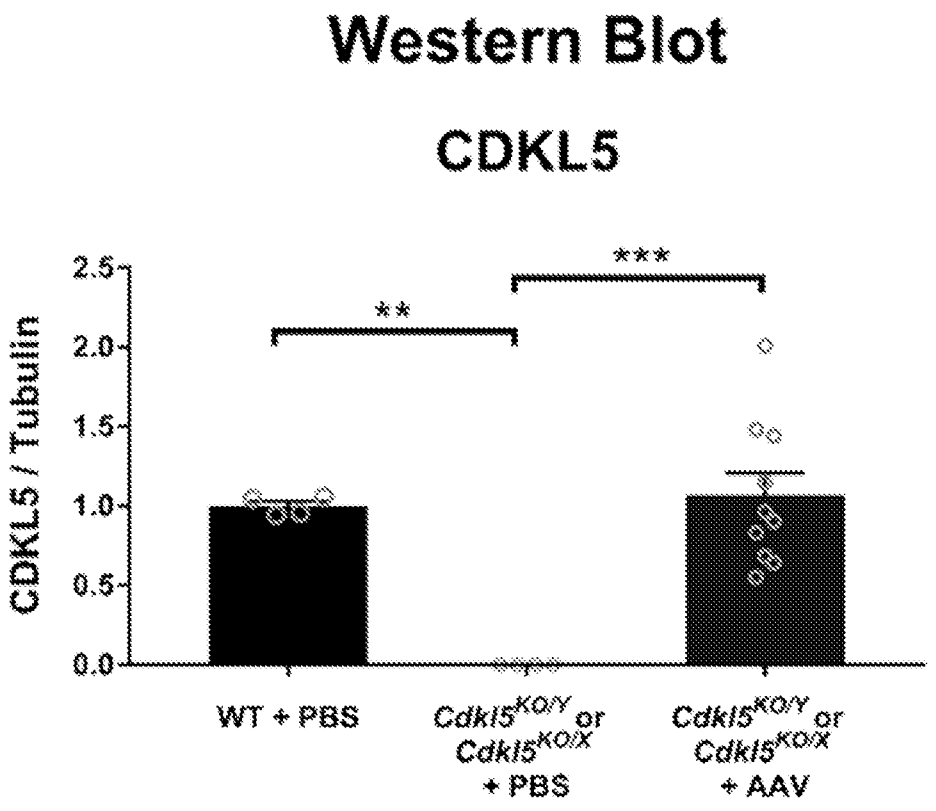


FIG 44B

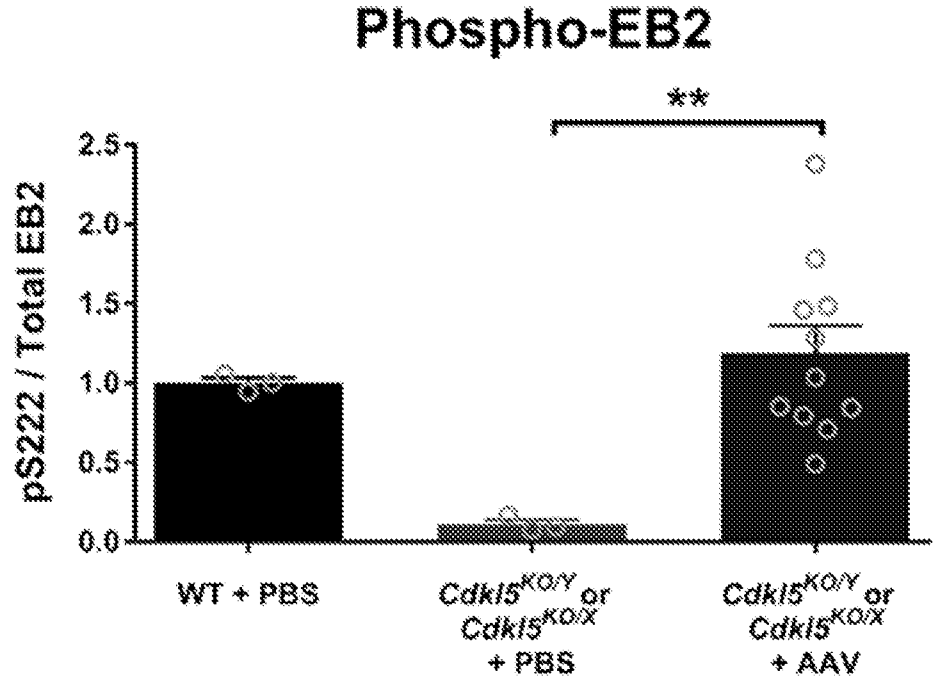


FIG 45A

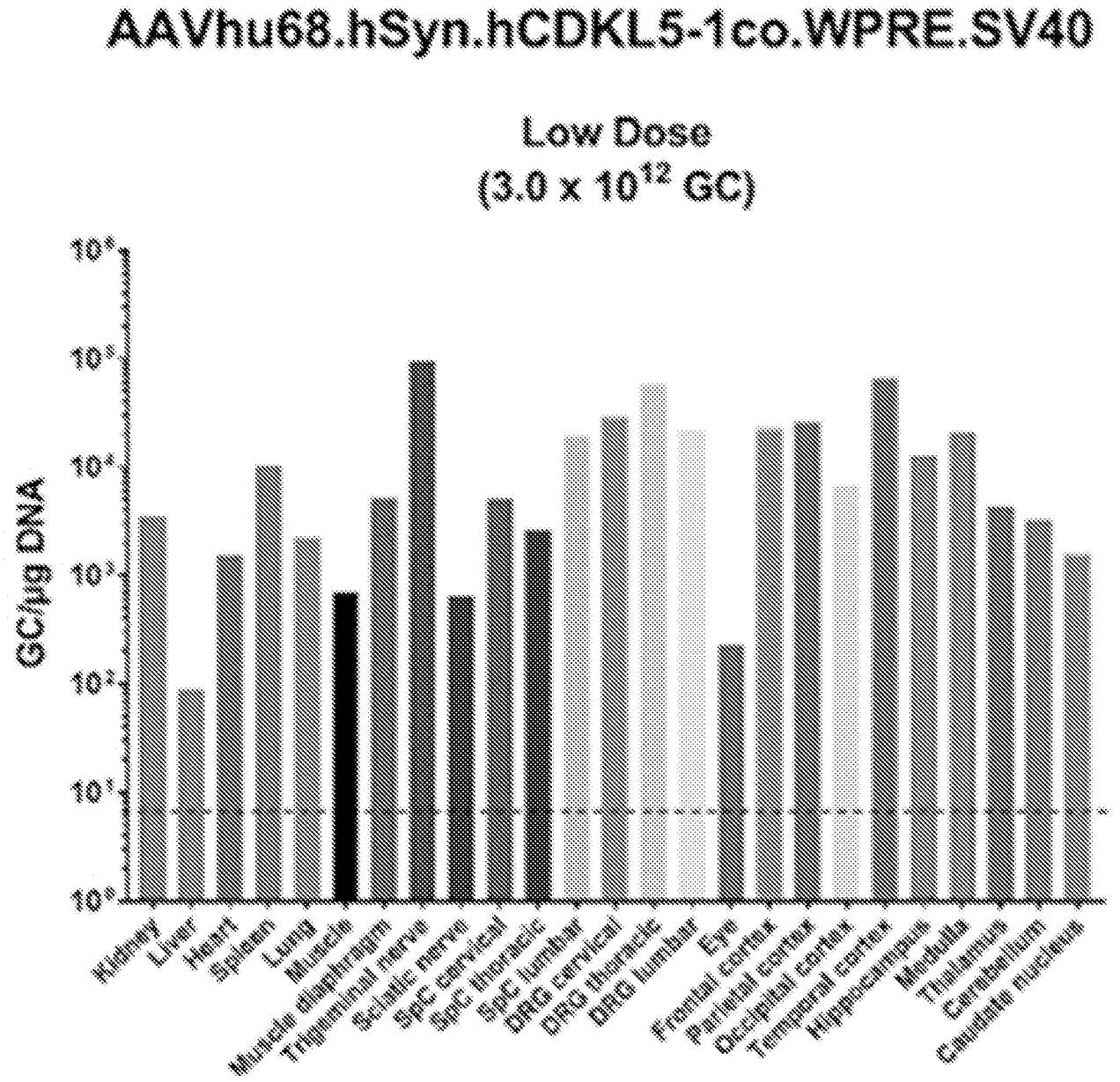

AAVhu68.hSyn.hCDKL5-1co.WPRE.SV40
Low Dose
(3.0 x 10^12 GC)
GC/µg DNA
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 45B

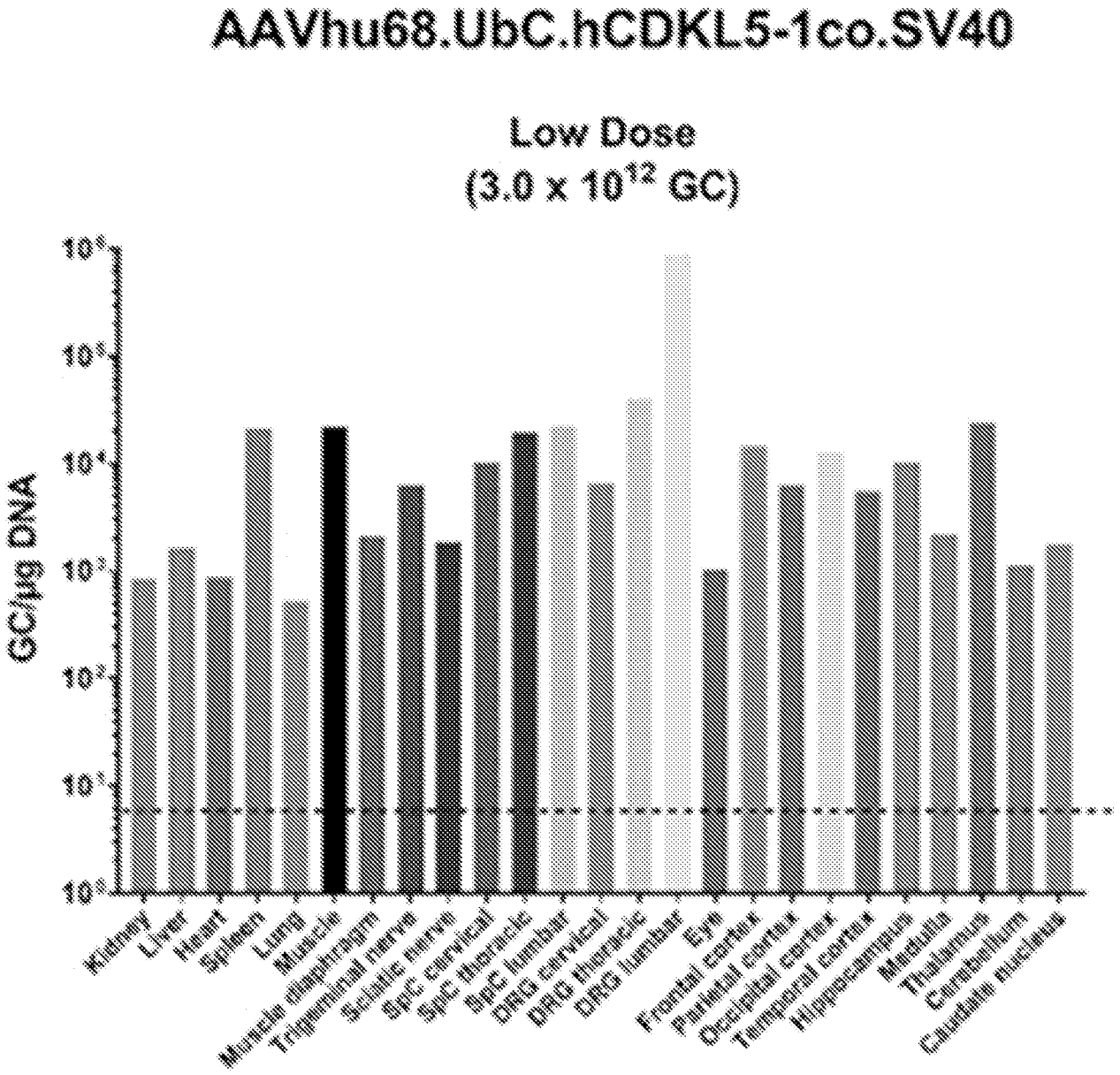

AAVhu68.UbC.hCDKL5-1co.SV40
Low Dose
(3.0 x 10^12 GC)
GC/µg DNA
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 45C

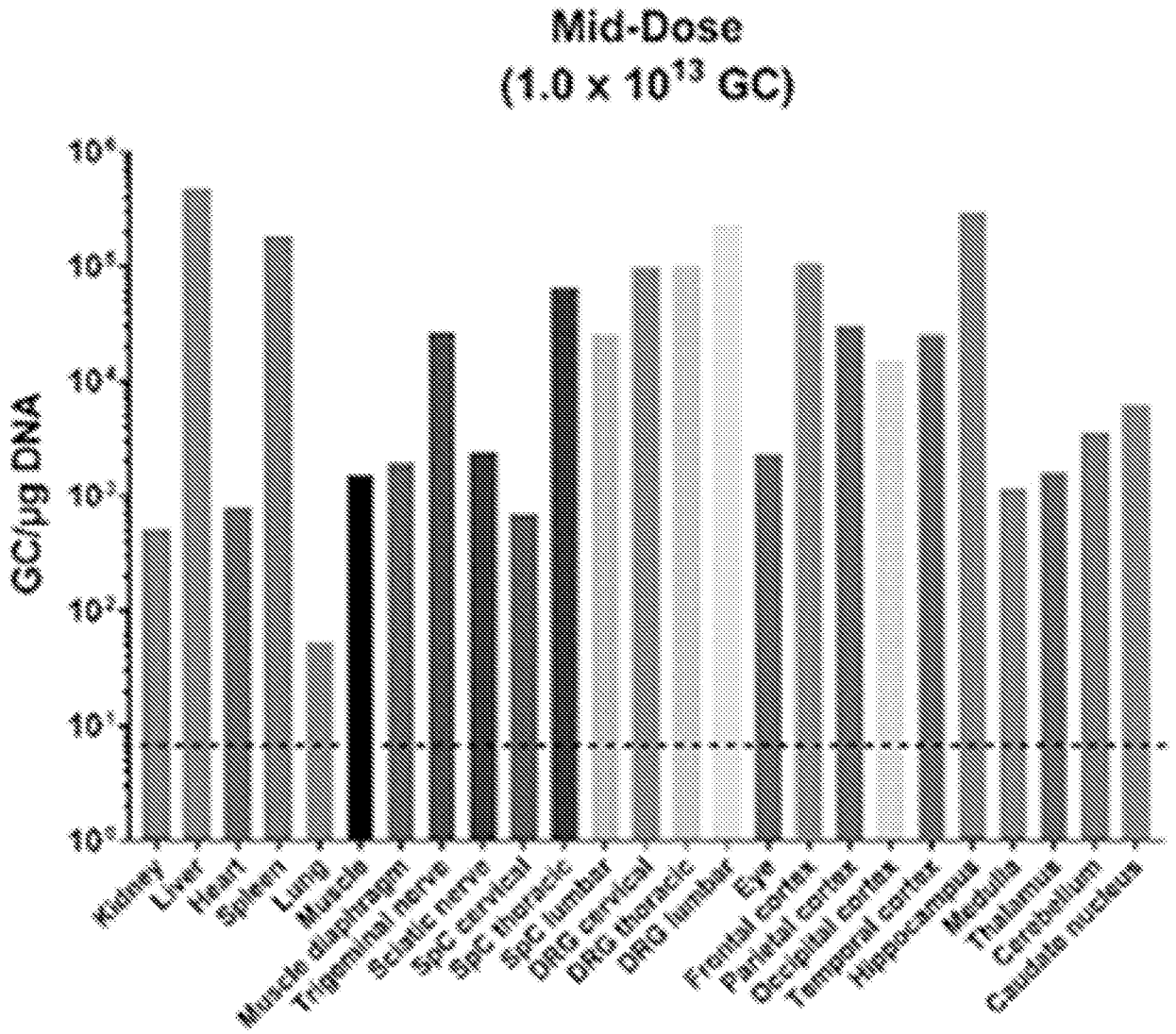

Mid-Dose
(1.0 x 10^13 GC)
GC/µg DNA
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 45D

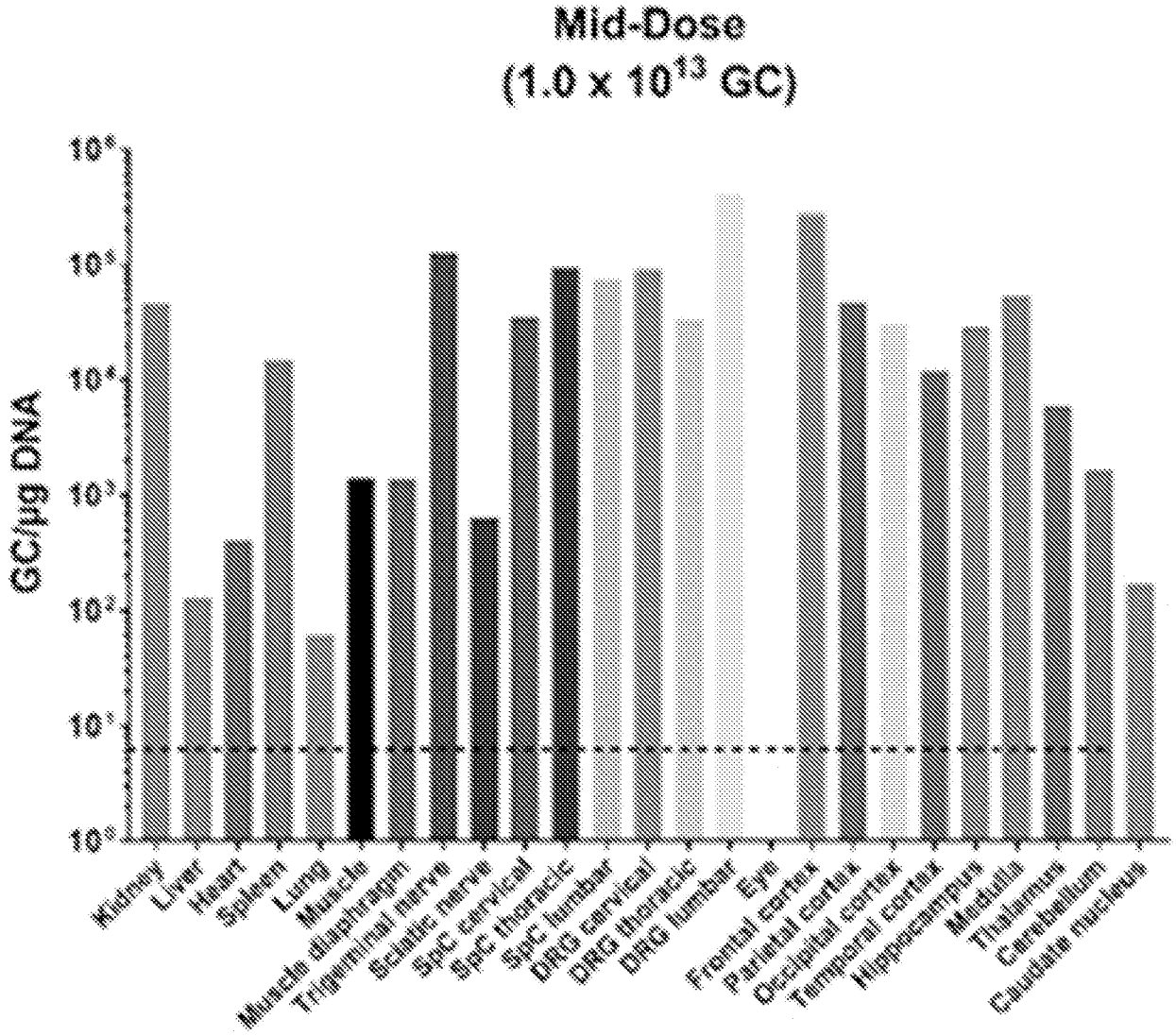

Mid-Dose
(1.0 x 10^13 GC)
GC/µg DNA
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 45E

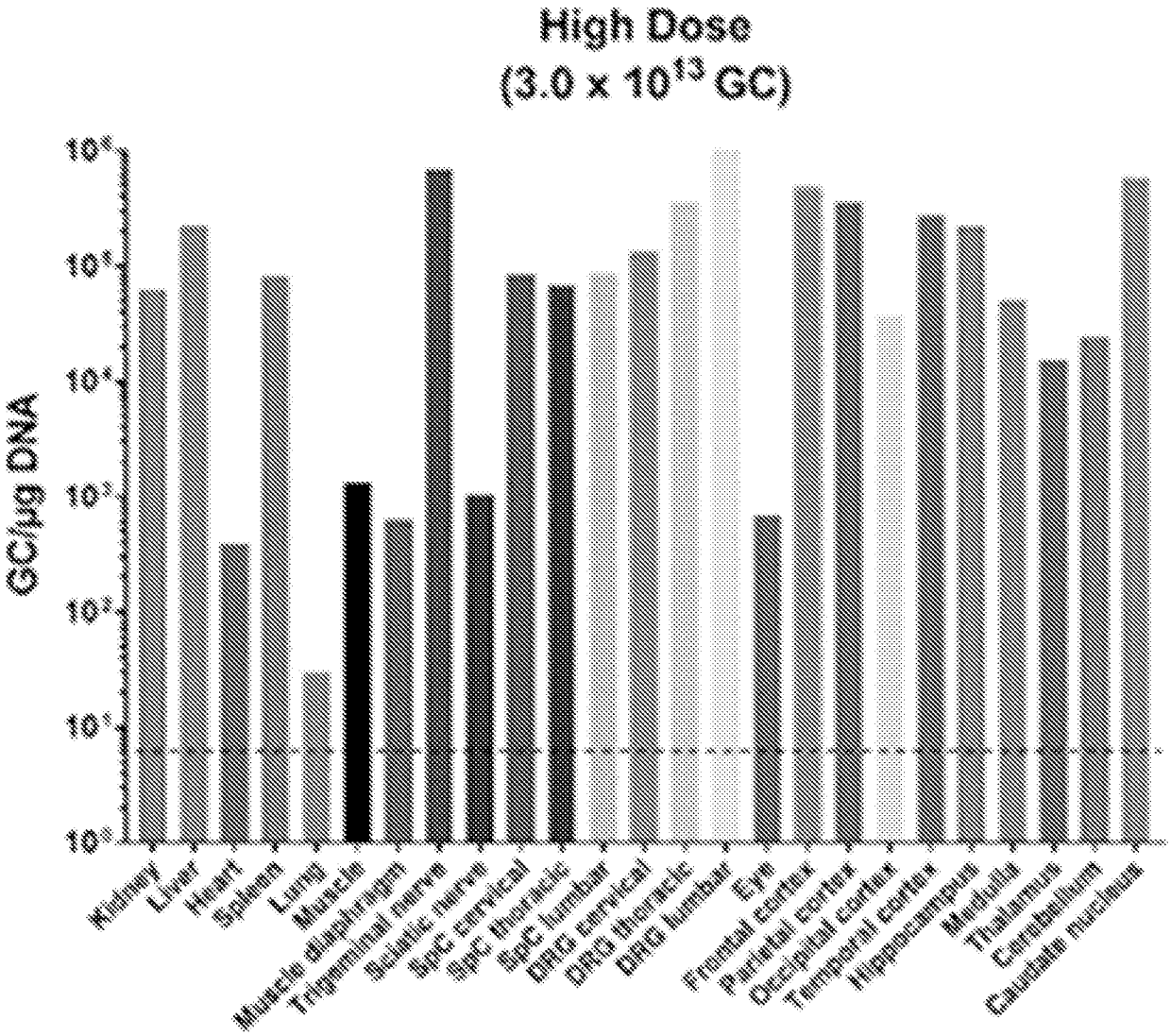

High Dose
(3.0 x 10^13 GC)
GC/μg DNA
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal Cortex
Parietal Cortex
Occipital Cortex
Temporal Cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

FIG 45F

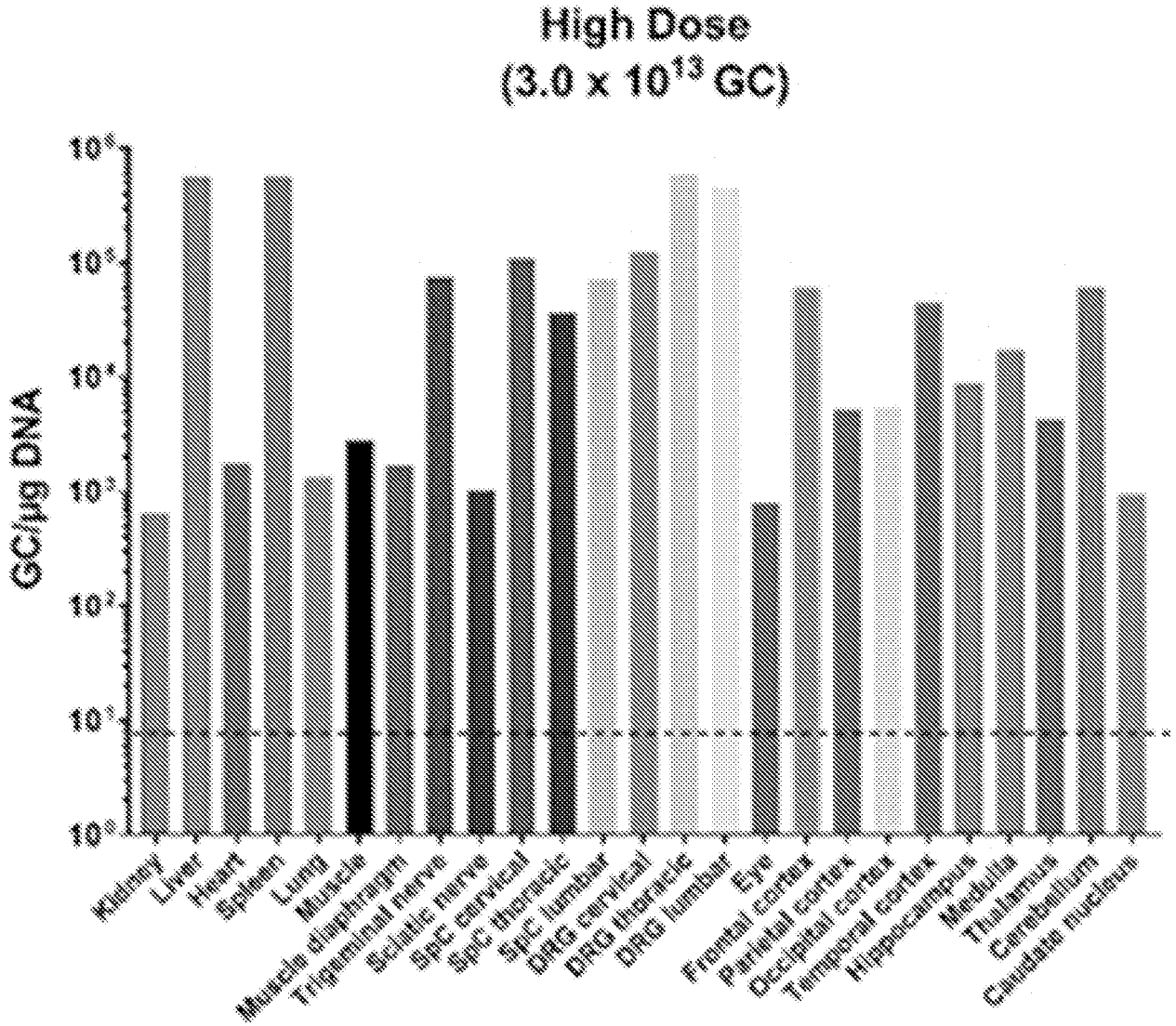
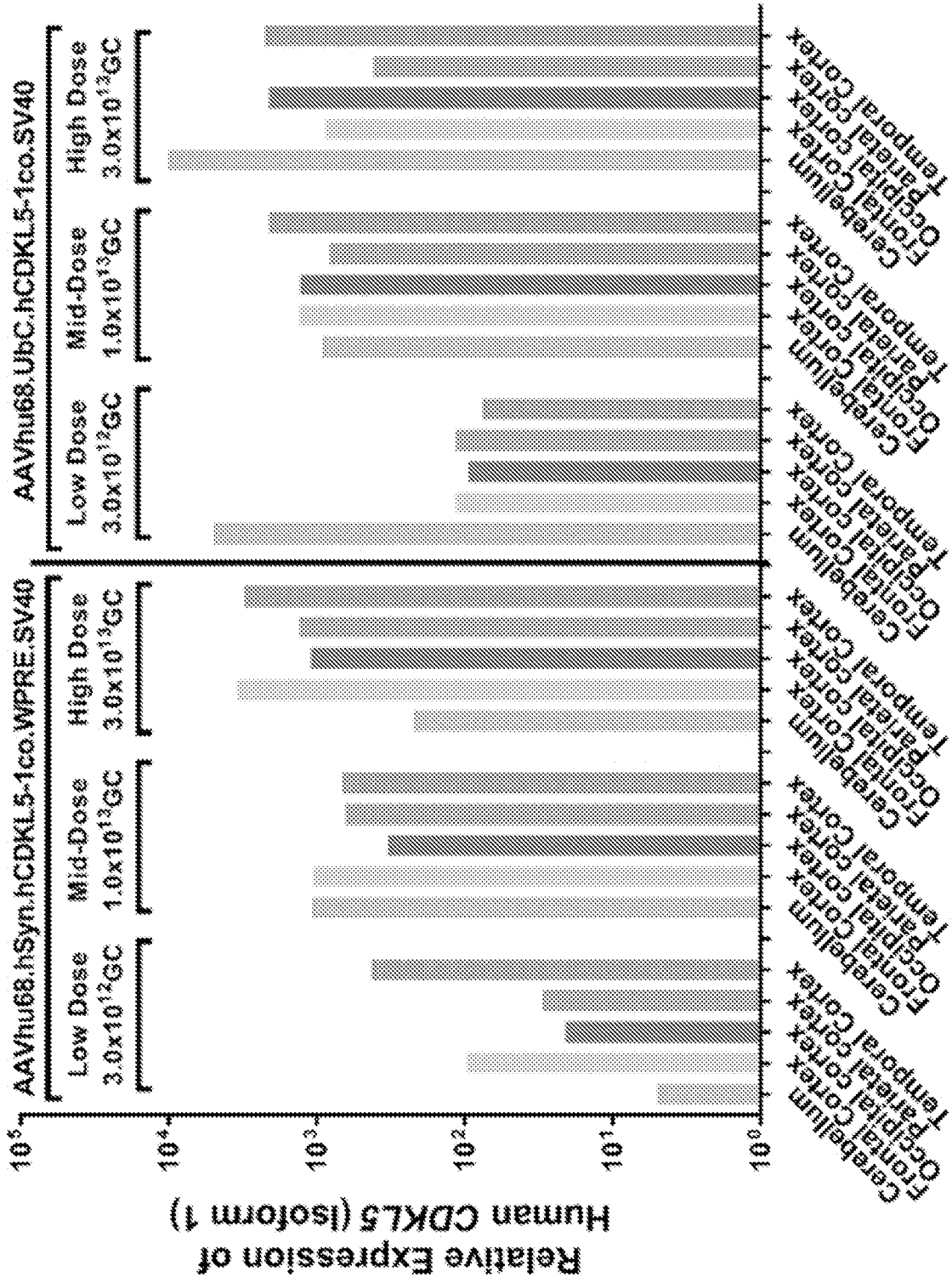
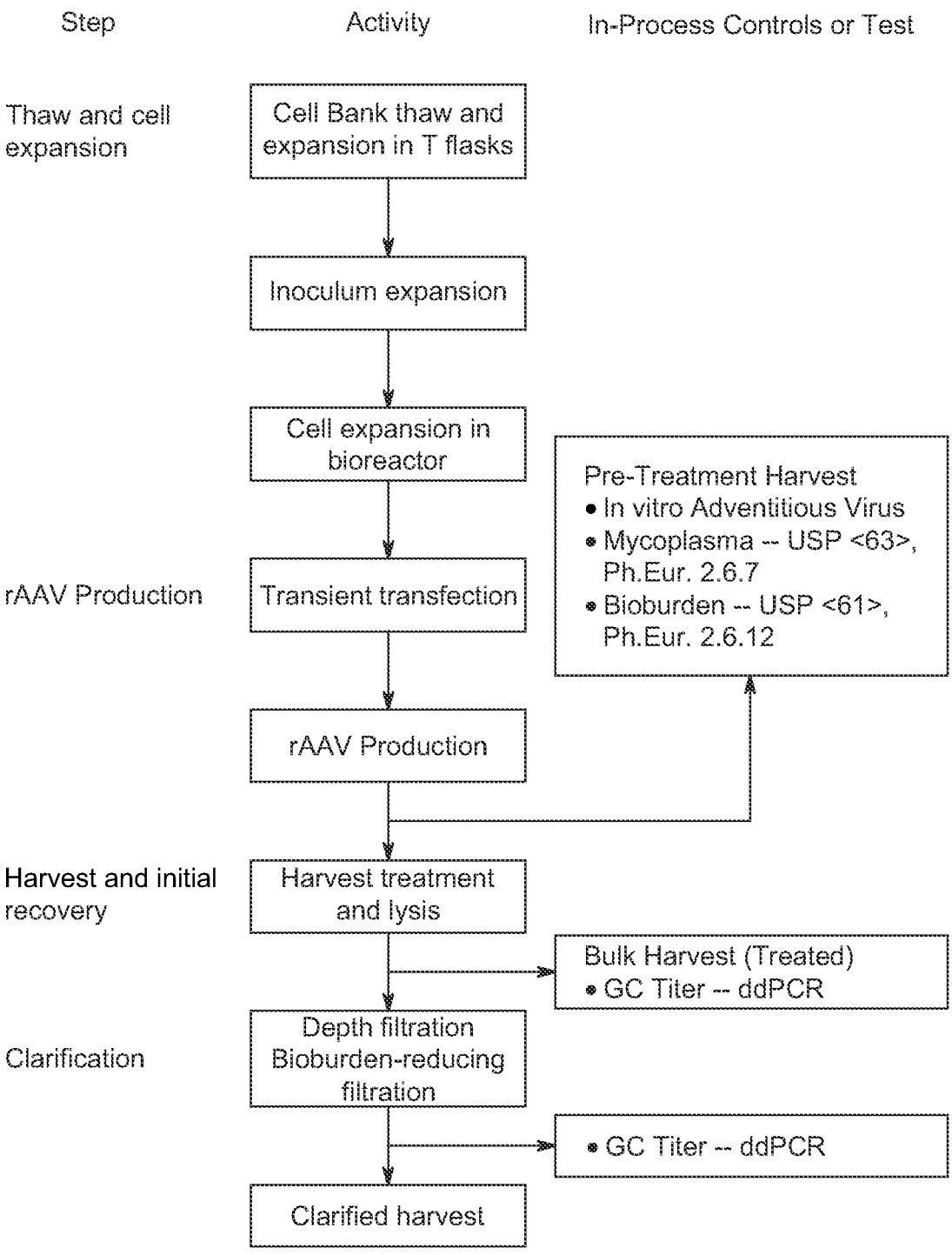

High Dose
(3.0 x 10^13 GC)
GC/µg DNA
10^5
10^4
10^3
10^2
10^1
10^0
Kidney
Liver
Heart
Spleen
Lung
Muscle
Muscle diaphragm
Trigeminal nerve
Sciatic nerve
SpC cervical
SpC thoracic
SpC lumbar
DRG cervical
DRG thoracic
DRG lumbar
Eye
Frontal cortex
Parietal cortex
Occipital cortex
Temporal cortex
Hippocampus
Medulla
Thalamus
Cerebellum
Caudate nucleus

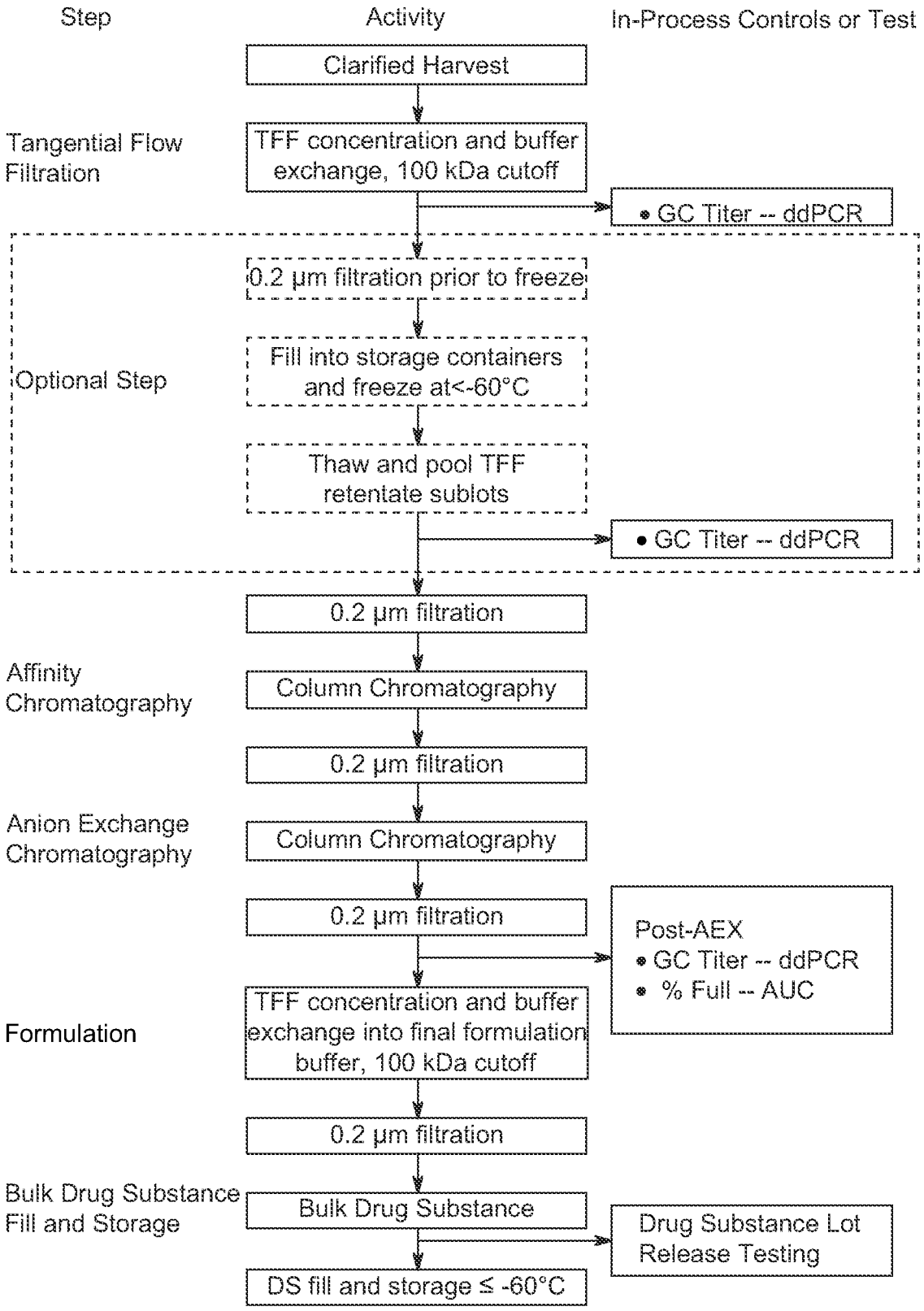
FIG 46


Relative Expression of
Human CDKL5 (isoform 1)
10^5
10^4
10^3
10^2
10^1
10^0
AAVhu68.hSyn.hCDKL5-1co.WPRE.SV40
Low Dose 3.0x10^12GC
Mid-Dose 1.0x10^13GC
High Dose 3.0x10^13GC
AAVhu68.UbC.hCDKL5-1co.SV40
Low Dose 3.0x10^12GC
Mid-Dose 1.0x10^13GC
High Dose 3.0x10^13GC
Cerebellum
Frontal Cortex
Occipital Cortex
Parietal Cortex
Temporal Cortex



Abbreviations: ddPCR, droplet digital polymerase chain reaction; GC, genome copies; Ph. Eur., European Pharmacopoeia; rAAV, recombinant adeno-associated virus; USP, United States Pharmacopeia.

FIG. 47A



Abbreviations: AEX, anion exchange; ddPCR, droplet digital polymerase chain reaction; DS, drug substance; GC, genome copies; TFF, tangential flow filtration.

FIG. 47B