FIG. 1A

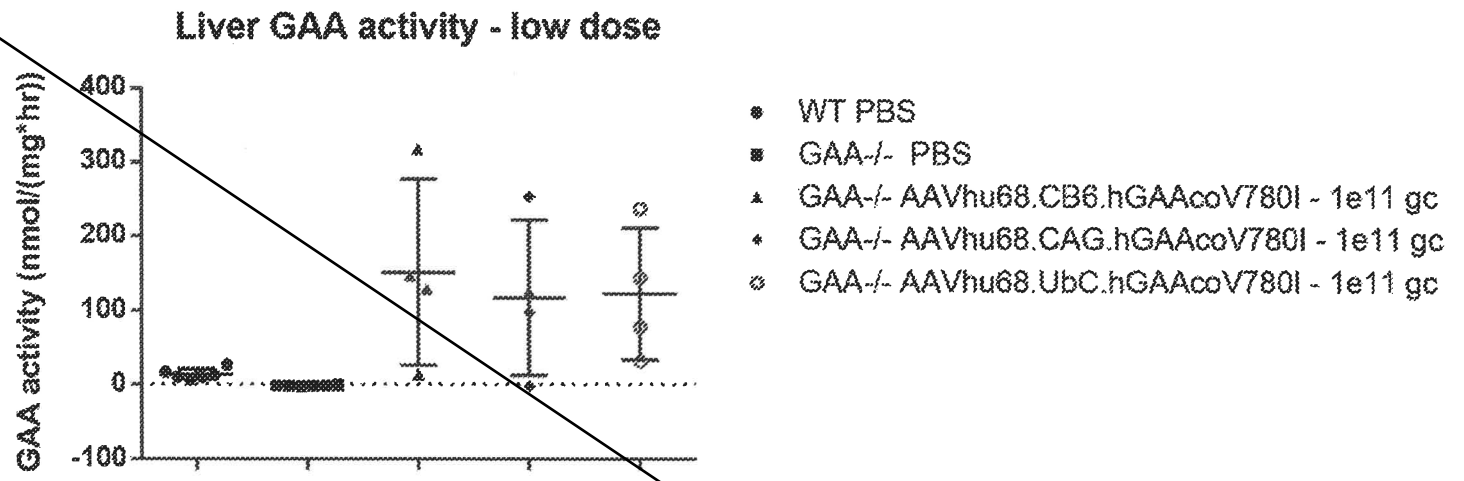

Liver GAA activity - low dose
GAA activity (nmol/(mg*hr))
400
300
200
100
0
-100
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780l - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780l - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780l - 1e11 gc

FIG. 1B

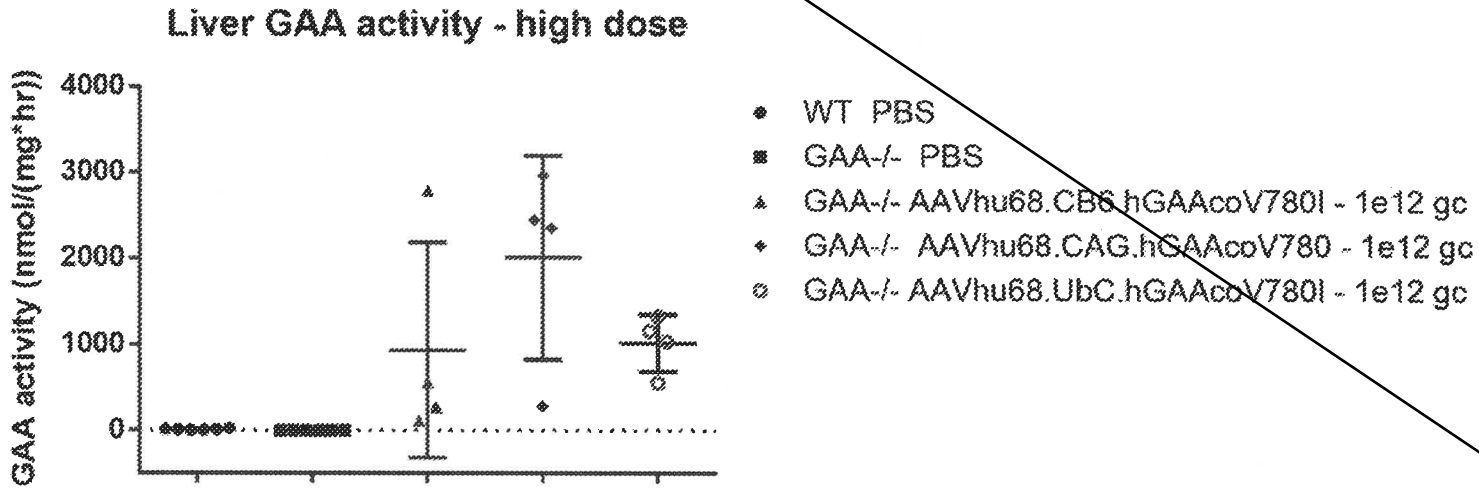

Liver GAA activity - high dose
GAA activity (nmol/(mg*hr))
4000
3000
2000
1000
0
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780l - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780 - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780l - 1e12 gc

FIG. 2A

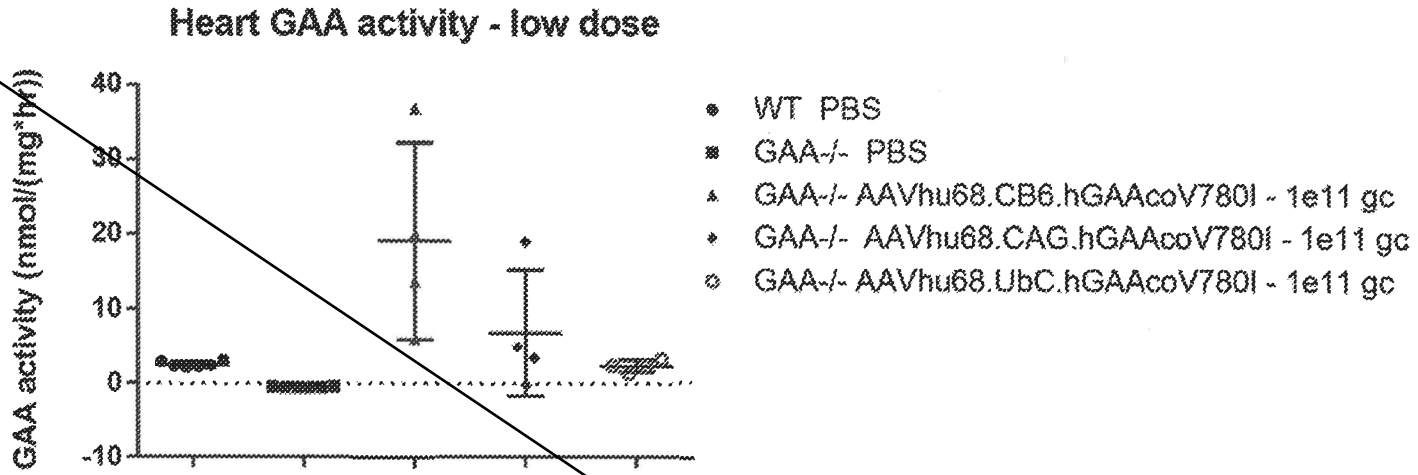

Heart GAA activity - low dose
GAA activity (nmol/(mg*hr))
40
30
20
10
0
-10
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 2B

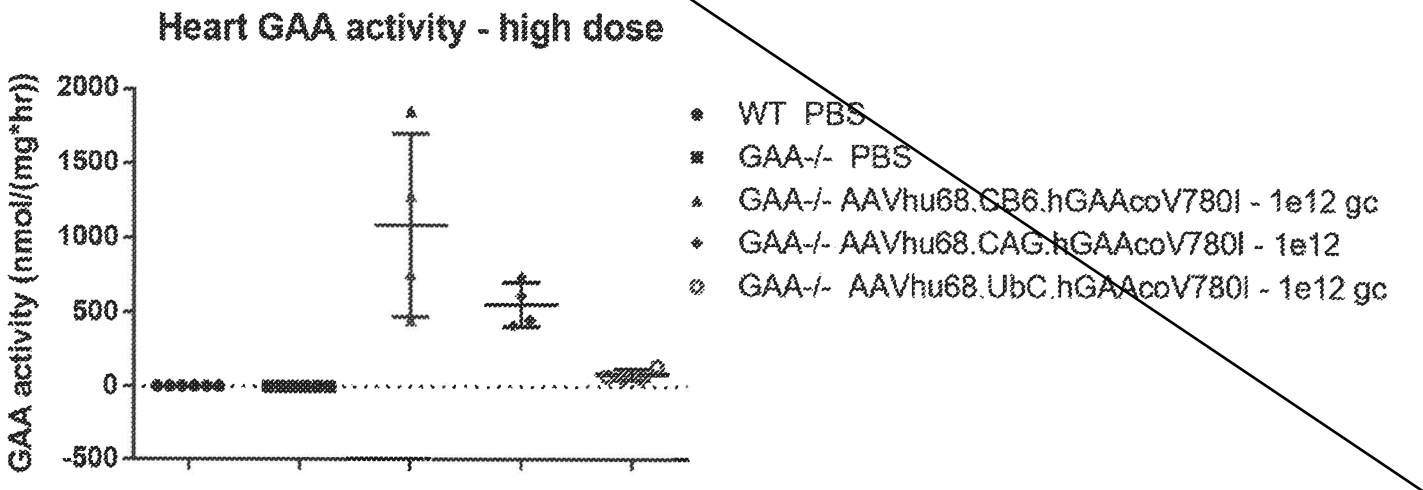

Heart GAA activity - high dose
GAA activity (nmol/(mg*hr))
2000
1500
1000
500
0
-500
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e12
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

FIG. 3A

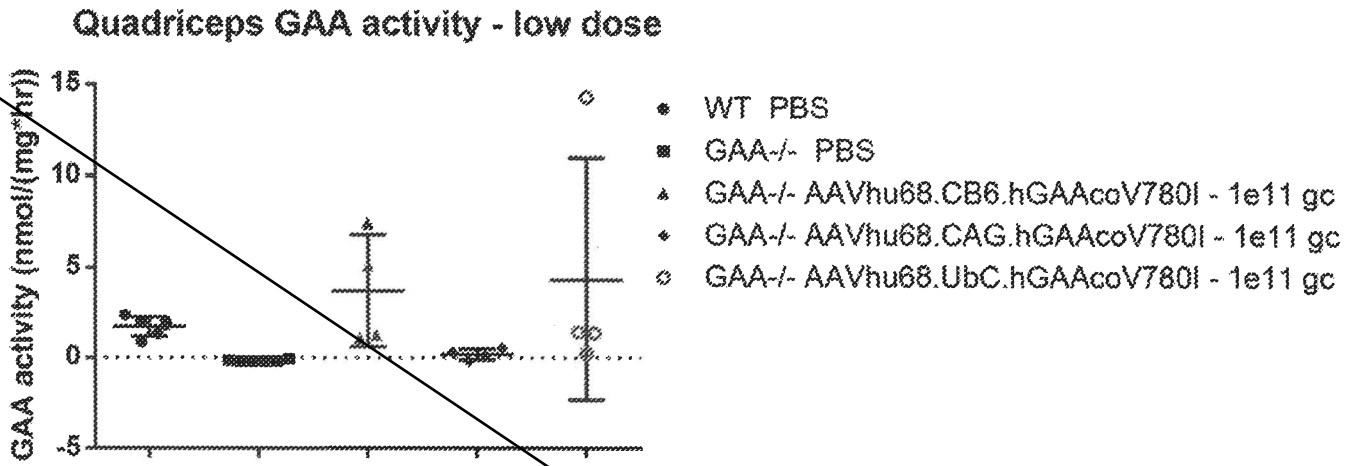

Quadriceps GAA activity - low dose
GAA activity (nmol/(mg*hr))
15
10
5
0
-5
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 3B

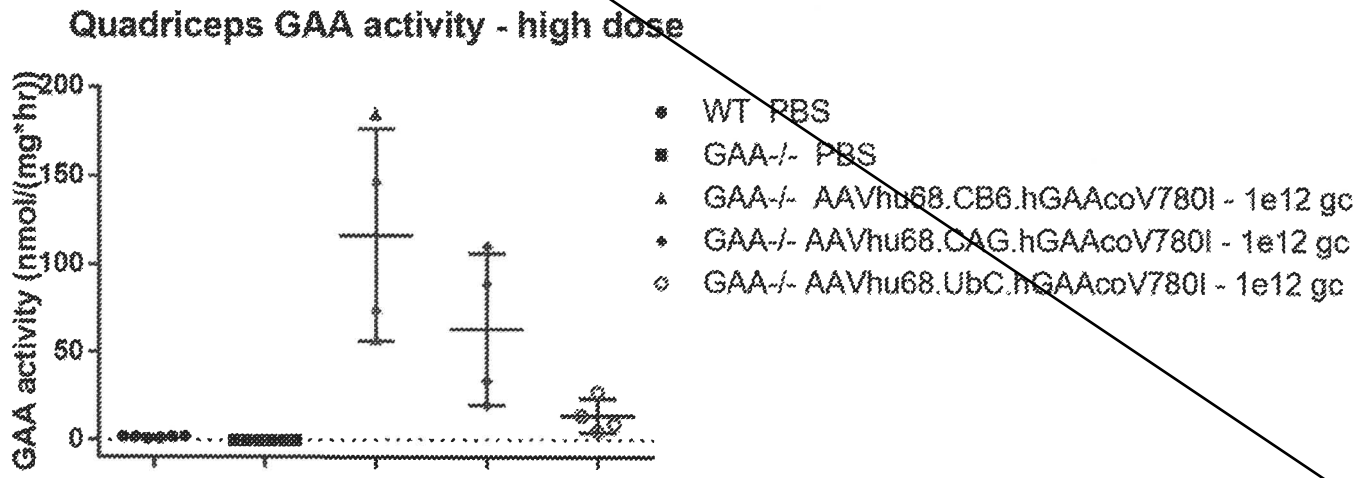

Quadriceps GAA activity - high dose
GAA activity (nmol/(mg*hr))
200
150
100
50
0
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

FIG. 4A

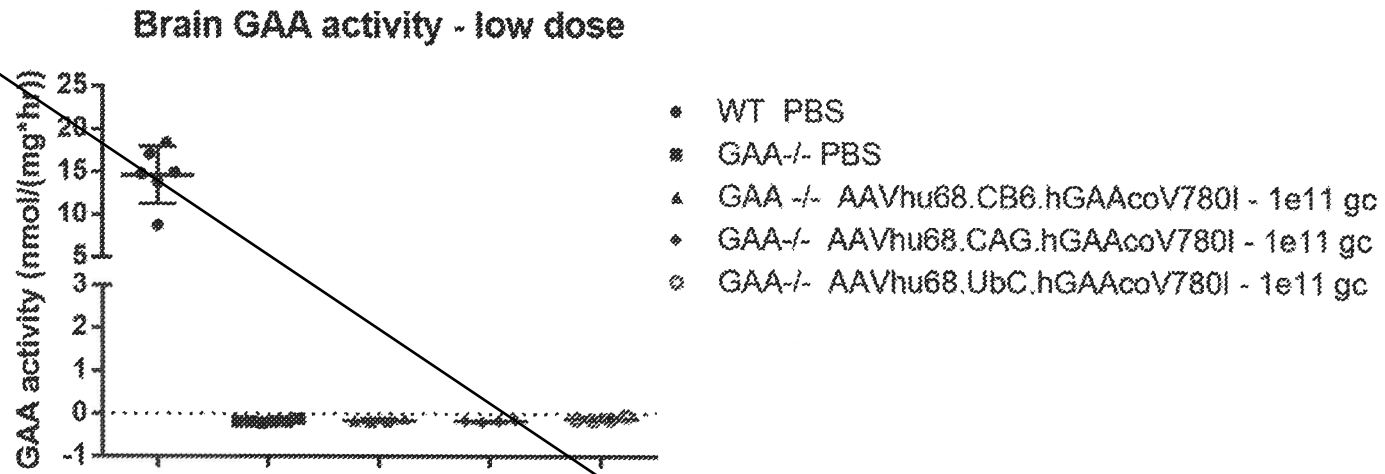

Brain GAA activity - low dose
GAA activity (nmol/(mg*hr))
25
20
15
10
5
3
2
1
0
-1
WT PBS
GAA-/- PBS
GAA -/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 4B

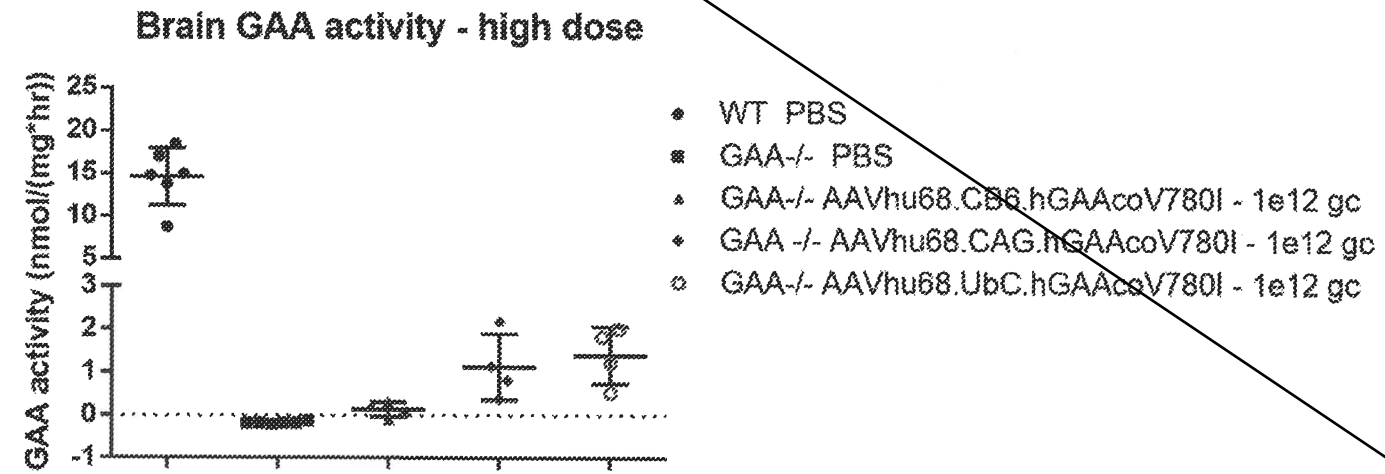

Brain GAA activity - high dose
GAA activity (nmol/(mg*hr))
25
20
15
10
5
3
2
1
0
-1
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA -/- AAVhu68.CAG.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

FIG. 5A

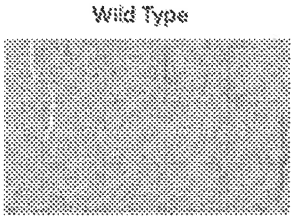


FIG. 5B

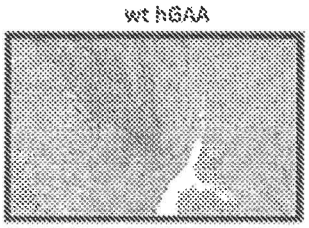


FIG. 5C

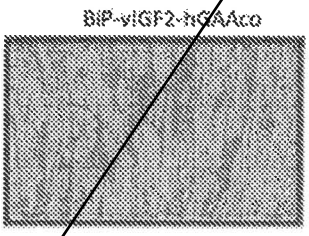


FIG. 5D

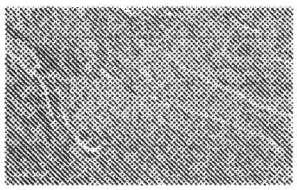


FIG. 5E

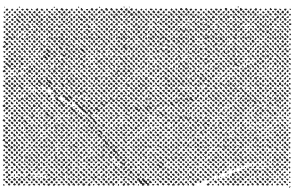


FIG. 5F

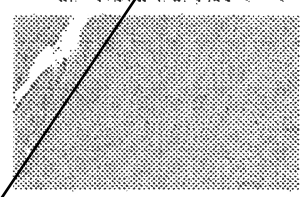


FIG. 5G

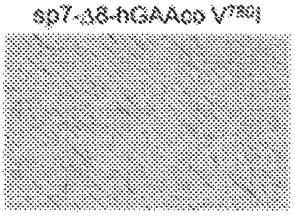


FIG. 5H

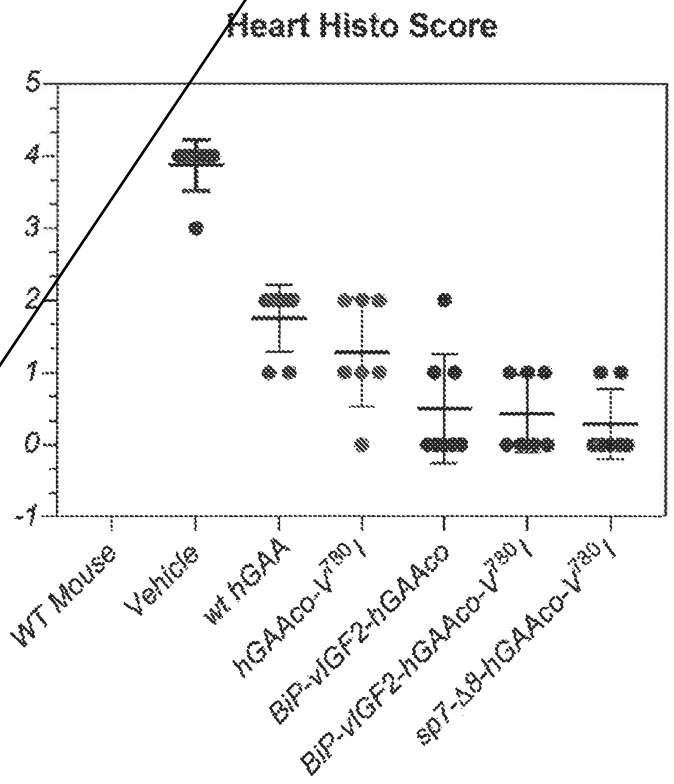


FIG. 6A

+/+ PBS

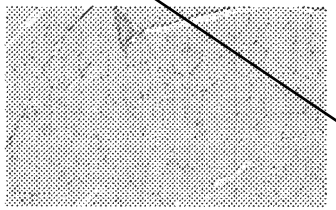


FIG. 6B

-/- hGAA

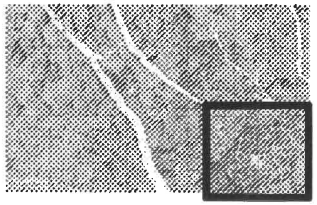


FIG. 6C

-/- BiP.vIGF2.hGAAco

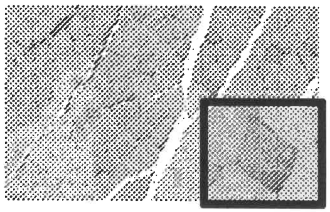


FIG. 6D

-/- PBS

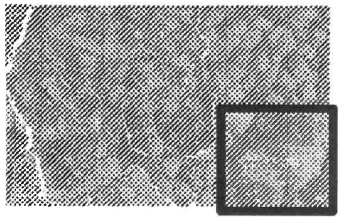


FIG. 6E

-/- hGAAcoV780I

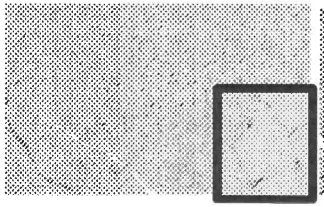


FIG. 6F

-/- Sp7.Δ8.hGAAcoV780I

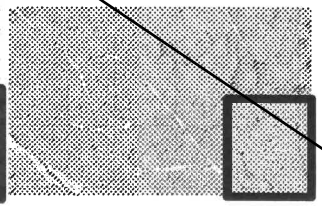


FIG. 6G

-/- BiP.vIGF2.hGAAcoV780I

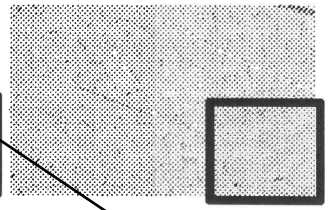
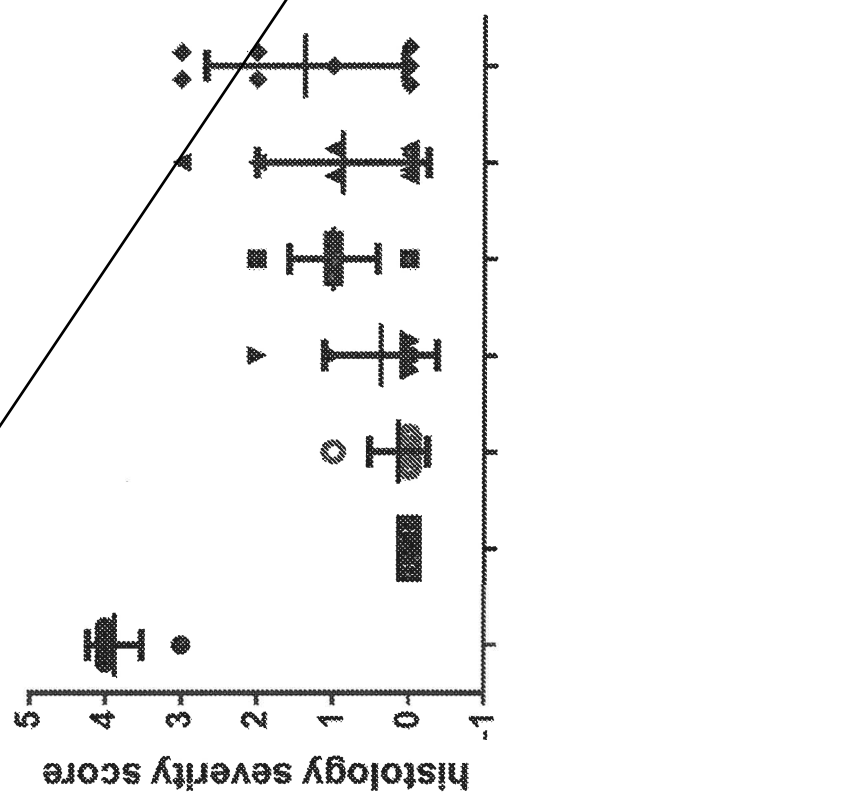


FIG. 6H


Quad histo score
histology severity score
5
4
3
2
1
0
-1
-/- PBS
+/+ PBS
hGAAcoV780I
BiP.vIGF2.hGAAcoV780I
sp7-delta8.hGAAcoV780I
hGAA
BiP.vIGF2.hGAAco

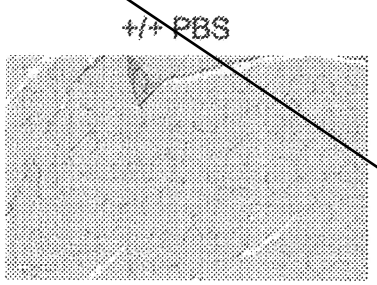


FIG. 7A

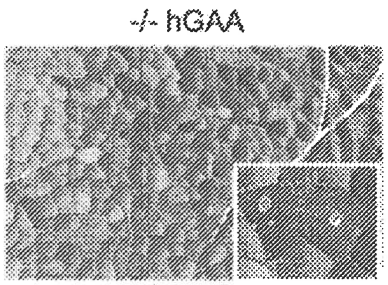


FIG. 7B

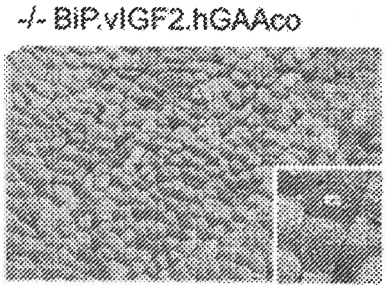


FIG. 7C

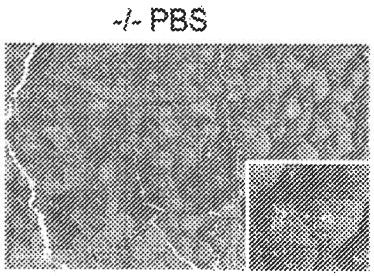


FIG. 7D

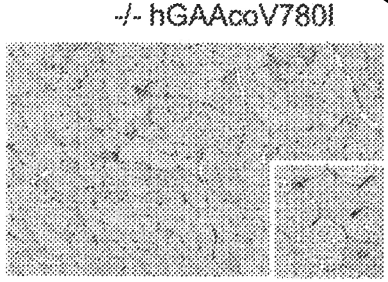


FIG. 7E

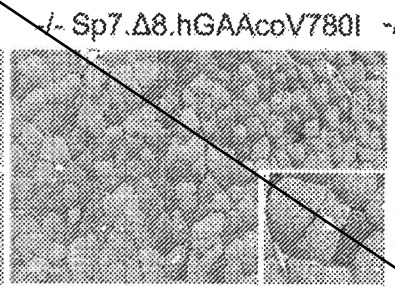


FIG. 7F

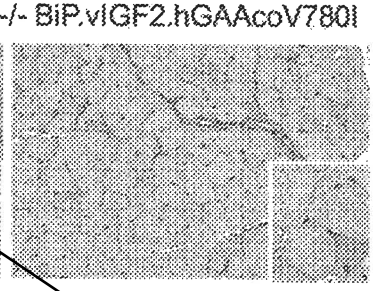


FIG. 7G

FIG. 7H

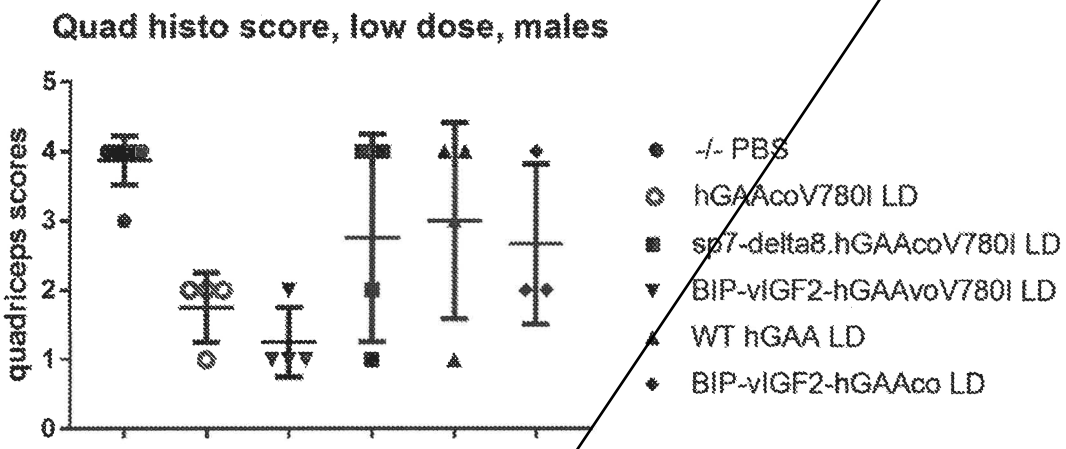

Quad histo score, low dose, males
quadriceps scores
0
1
2
3
4
5
-/- PBS
hGAAcoV780I LD
sp7-delta8.hGAAcoV780I LD
BIP-vIGF2-hGAAvoV780I LD
WT hGAA LD
BIP-vIGF2-hGAAco LD

FIG. 8

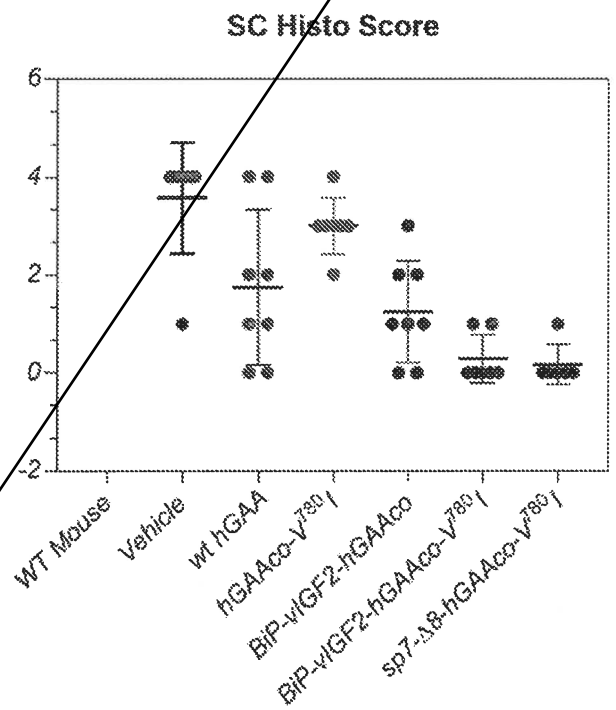

SC Histo Score
6
4
2
0
-2
WT Mouse
Vehicle
wt hGAA
hGAAco-V780I
BIP-vIGF2-hGAAco
BIP-vIGF2-hGAAco-V780I
sp7-Δ8-hGAAco-V780I

FIG. 9A

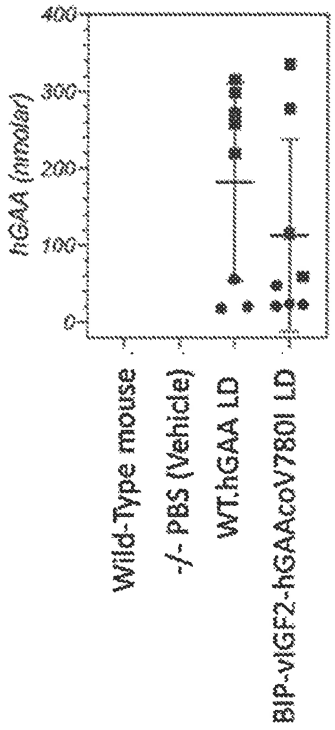


FIG. 9B

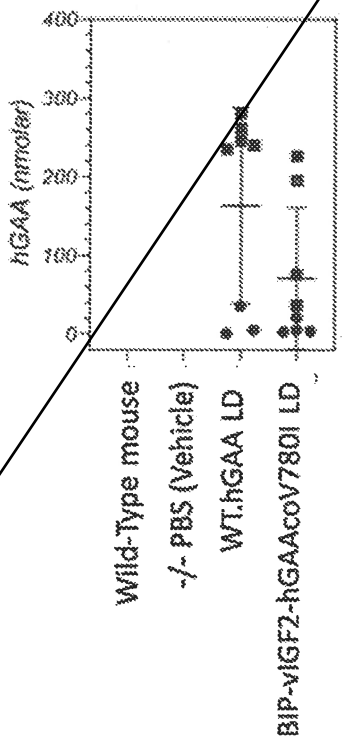


FIG. 9C

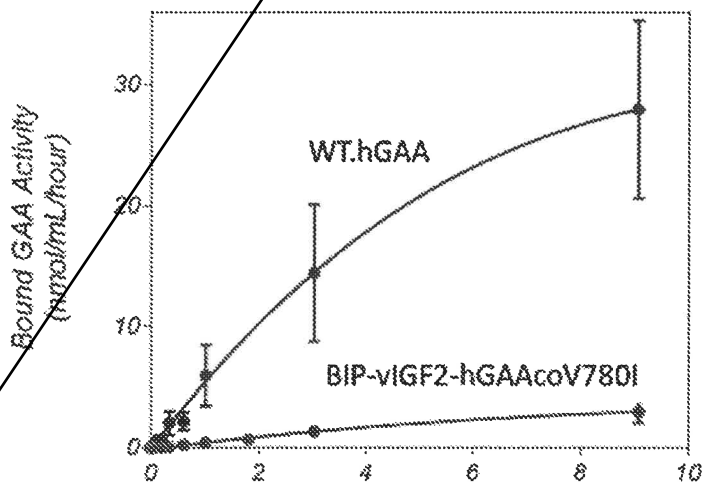


FIG. 10

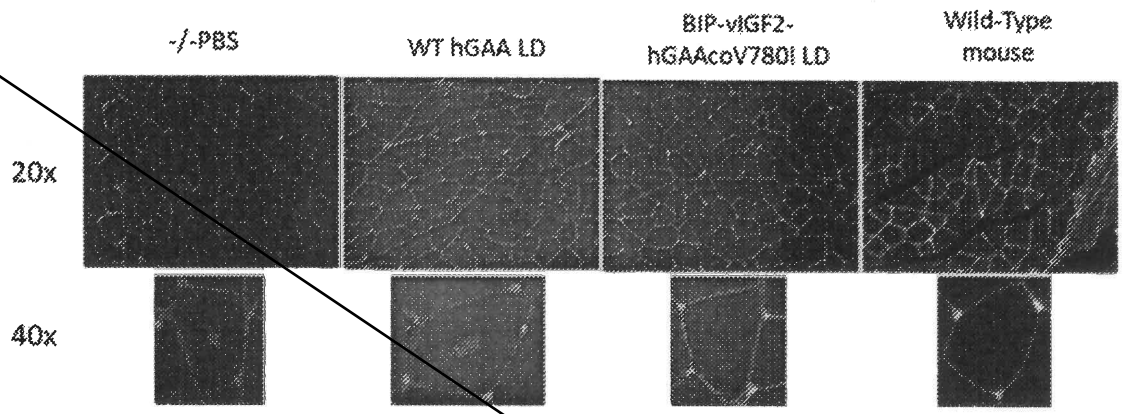

LC3b (autophagic vacuole marker) and lectin (membrane)
-/-PBS
WT hGAA LD
BIP-vIGF2-hGAAcoV780I LD
Wild-Type mouse
20x
40x

FIG. 11

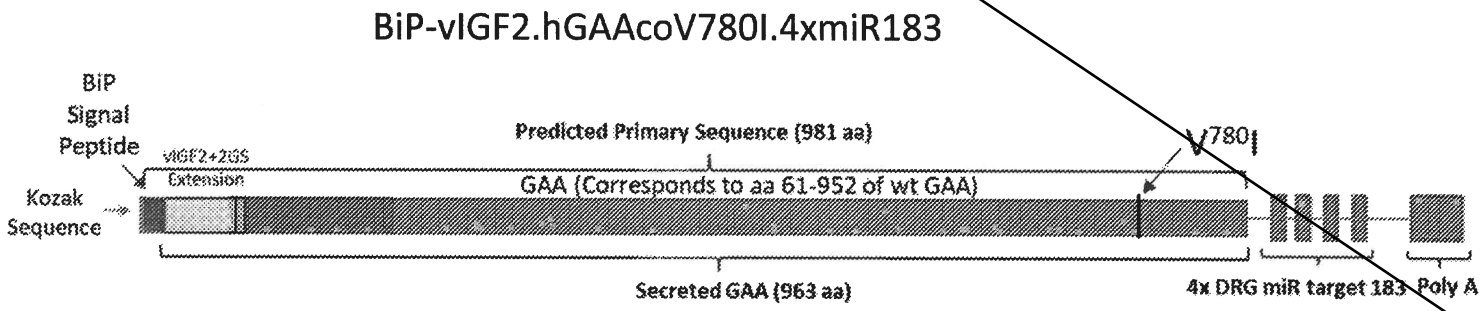

BiP-vIGF2.hGAAcoV780I.4xmiR183
BiP Signal Peptide
vIGF2+2GS Extension
Predicted Primary Sequence (981 aa)
V780I
Kozak Sequence
GAA (Corresponds to aa 61-952 of wt GAA)
Secreted GAA (963 aa)
4x DRG miR target 183
Poly A

FIG. 12

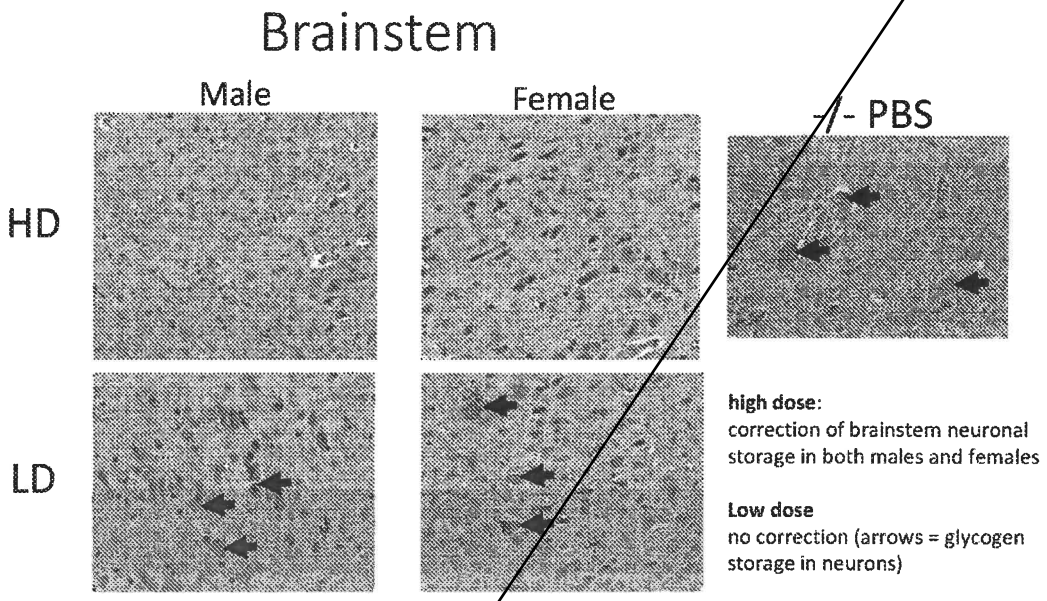

Brainstem
Male
Female
-/- PBS
HD
LD
high dose:
correction of brainstem neuronal storage in both males and females
Low dose
no correction (arrows = glycogen storage in neurons)

FIG. 13

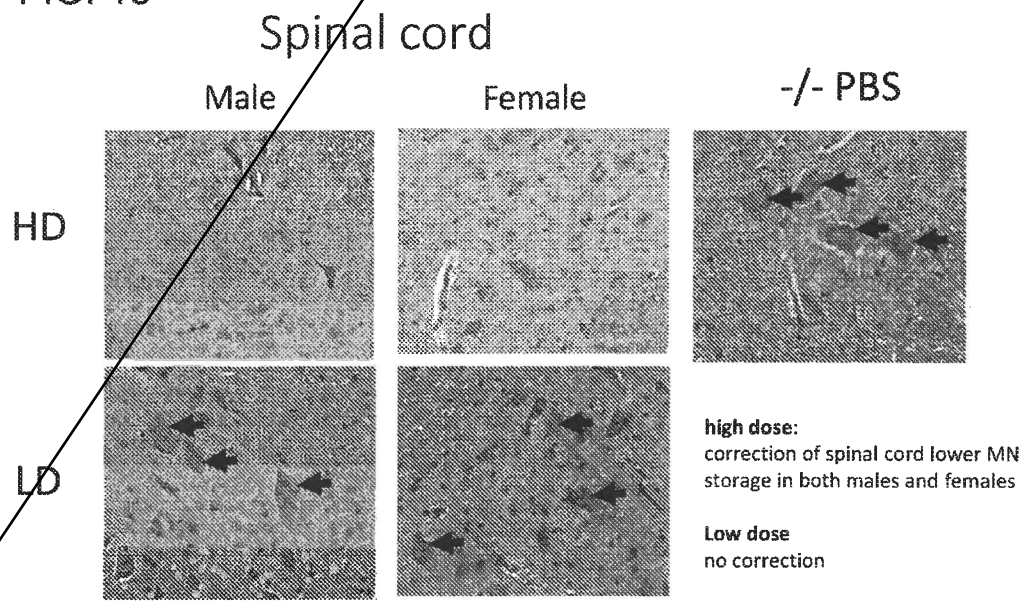

Spinal cord
Male
Female
-/- PBS
HD
LD
high dose:
correction of spinal cord lower MN storage in both males and females
Low dose
no correction

FIG. 14

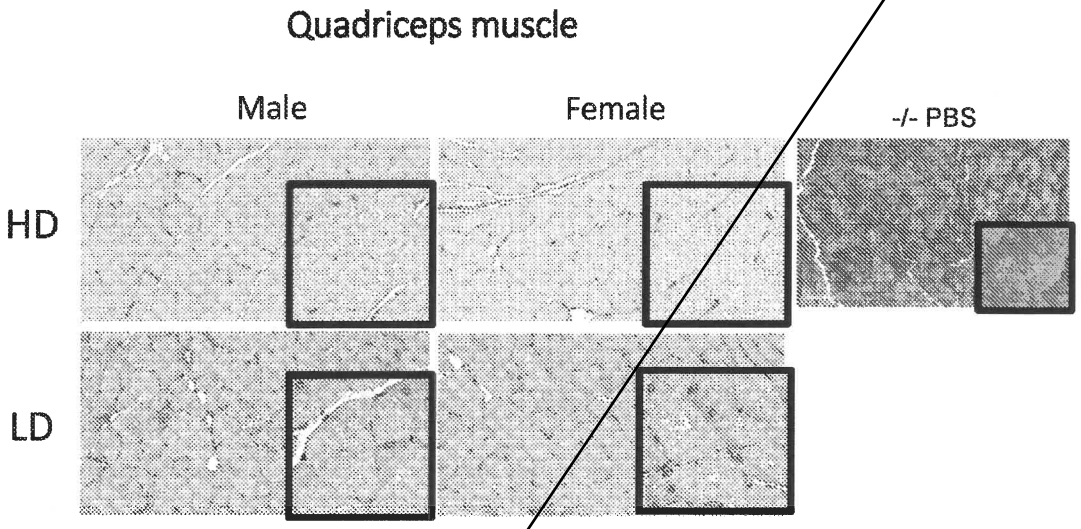
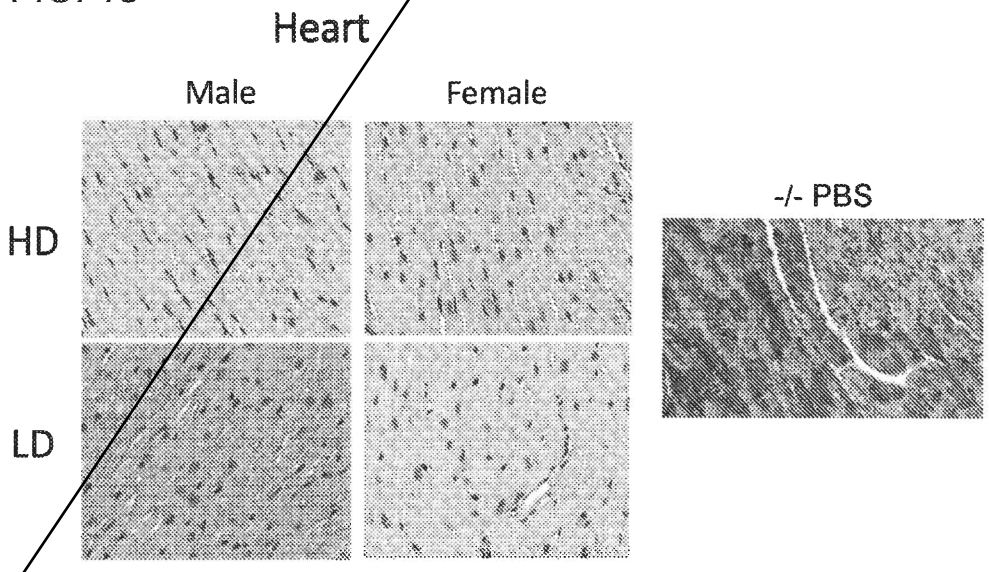

Quadriceps muscle
Male
Female
-/- PBS
HD
LD

FIG. 15


Heart
Male
Female
-/- PBS
HD
LD

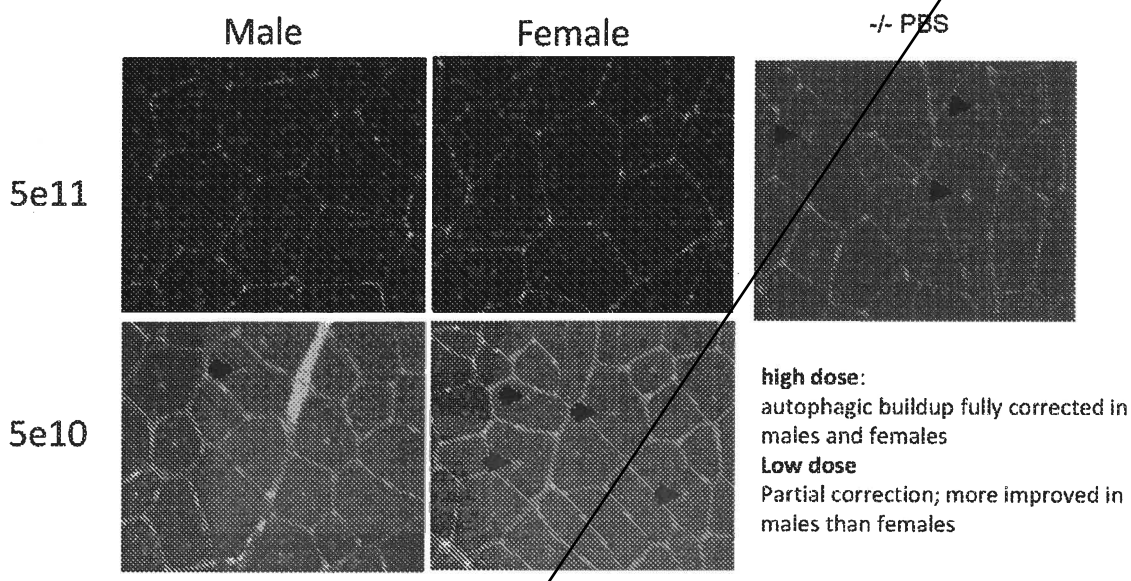

Male
Female
-/- PBS
5e11
5e10
high dose:
autophagic buildup fully corrected in males and females
Low dose
Partial correction; more improved in males than females

FIG. 16

FIG. 17

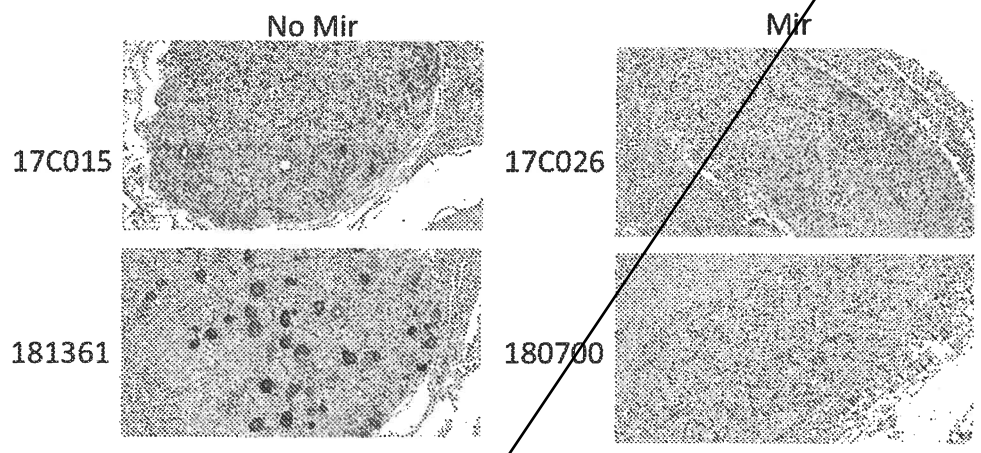

Cervical DRG hGAA IHC
No Mir
Mir
17C015
17C026
181361
180700

FIG. 18

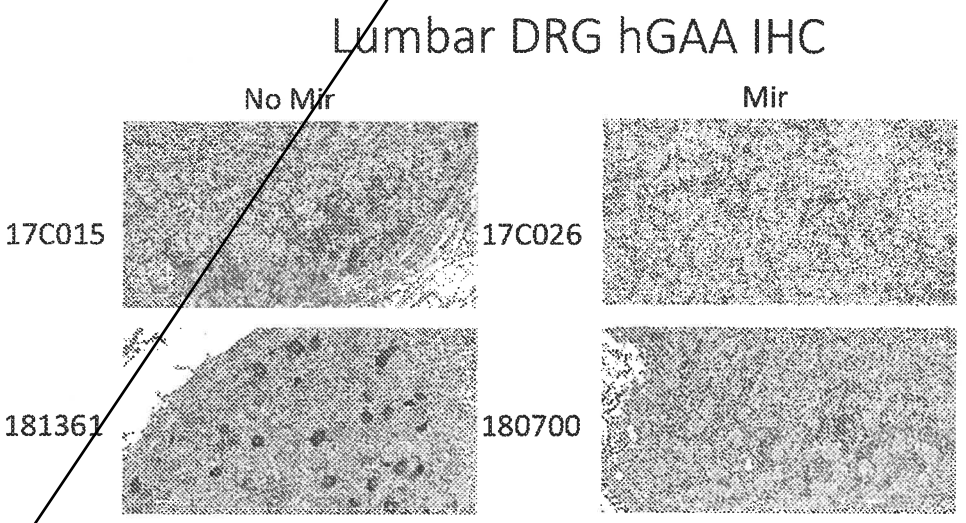

Lumbar DRG hGAA IHC
No Mir
Mir
17C015
17C026
181361
180700

FIG. 19

cervical spinal cord
lower MN hGAA IHC

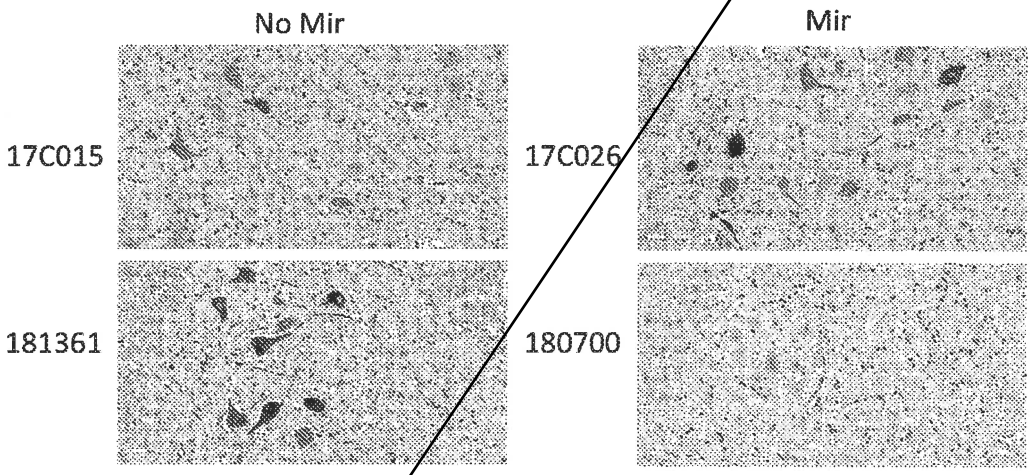


FIG. 20

heart hGAA IHC

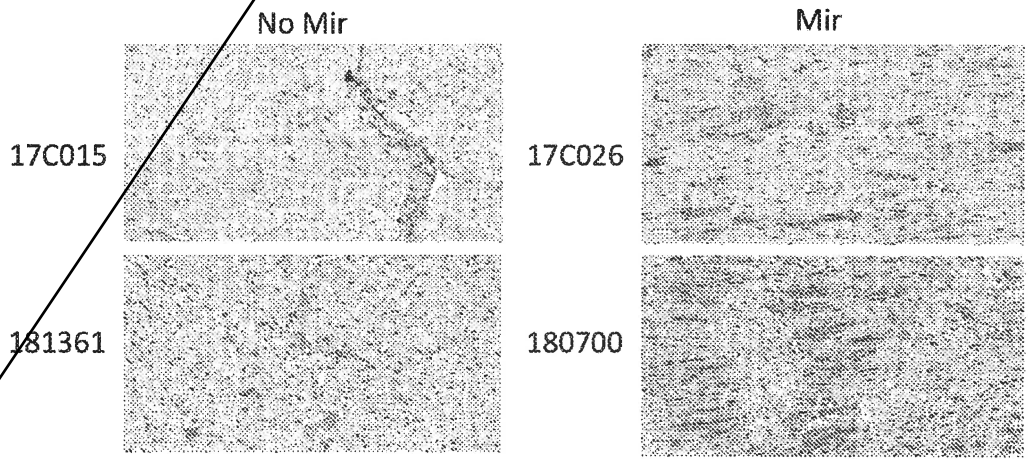


FIG. 21A

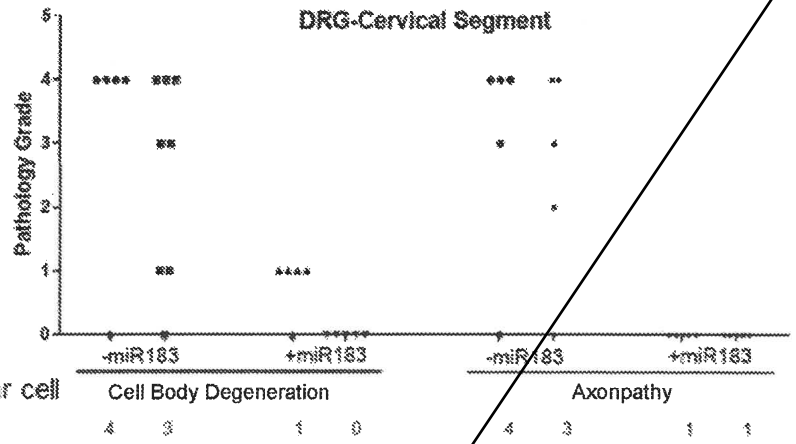


FIG. 21B

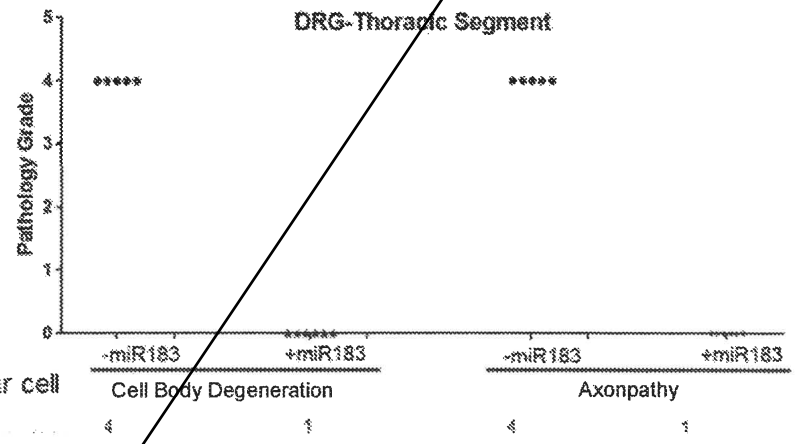


FIG. 21C

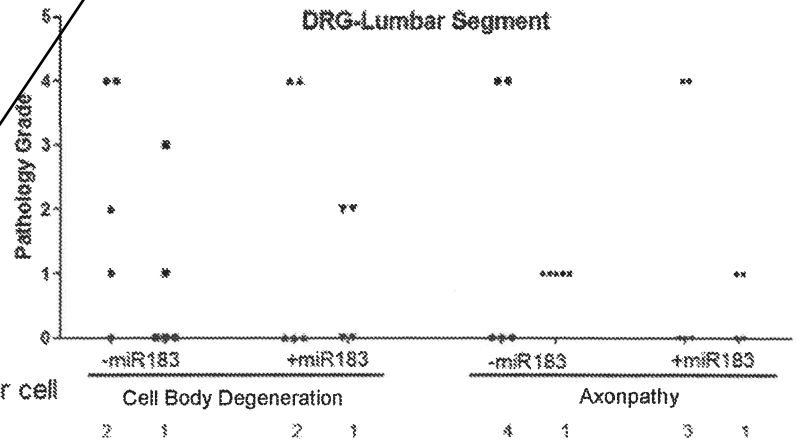


FIG. 22A

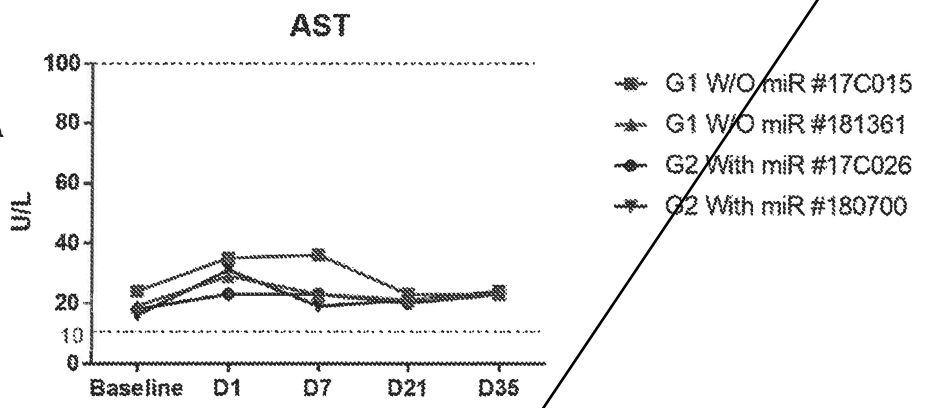


FIG. 22B

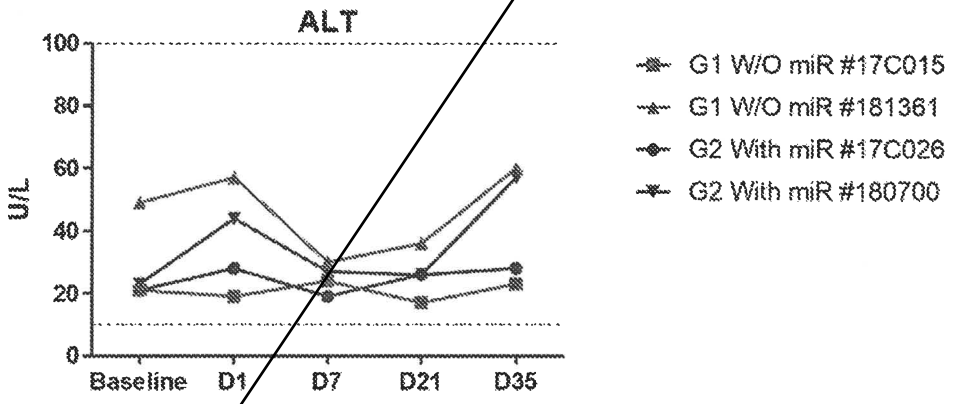


FIG. 22C

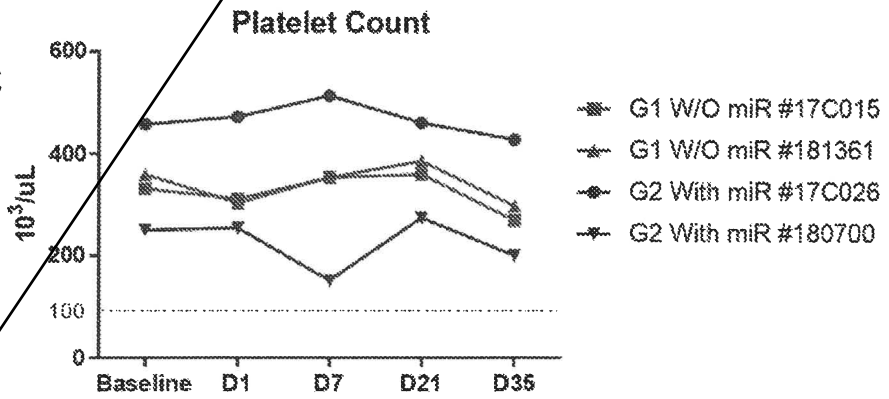


FIG. 23

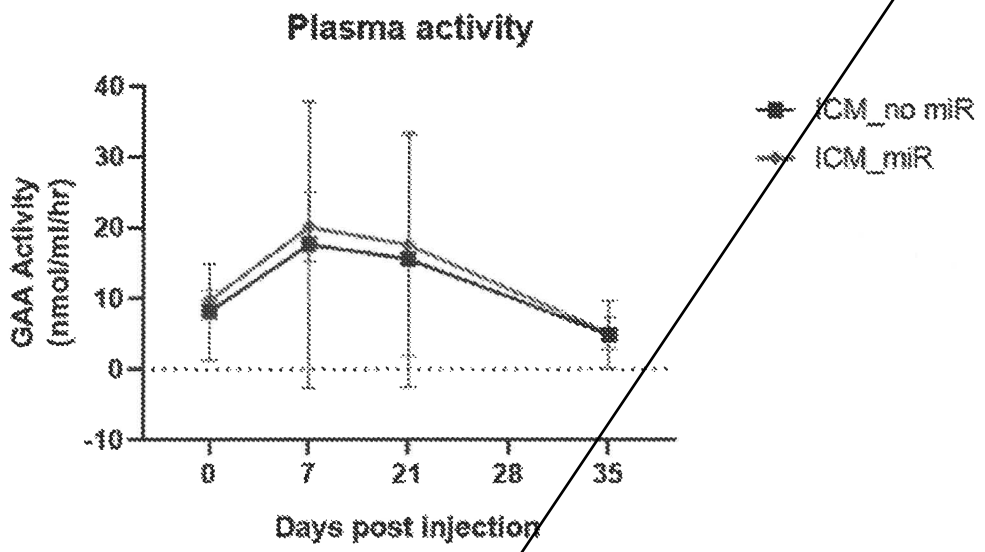

Plasma activity
GAA Activity (nmol/ml/hr)
40
30
20
10
0
-10
0
7
21
28
35
Days post injection
ICM_no miR
ICM_miR

FIG. 24A

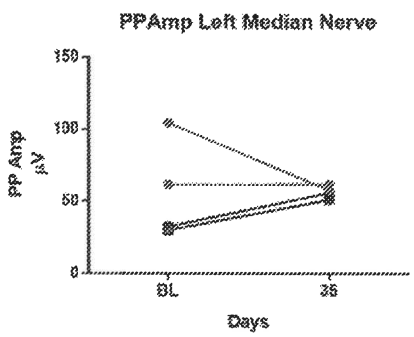


FIG. 24B

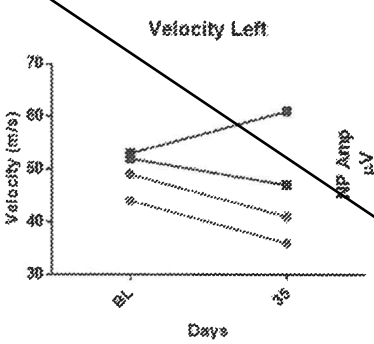


FIG. 24C

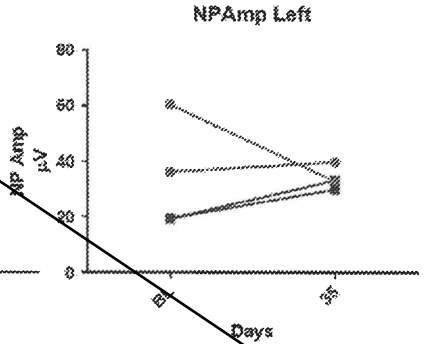
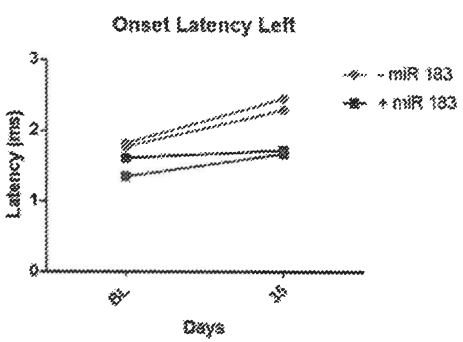


FIG. 24D



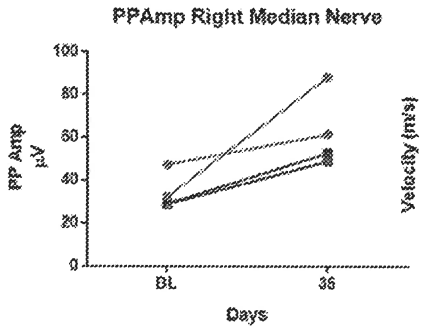


FIG. 24E

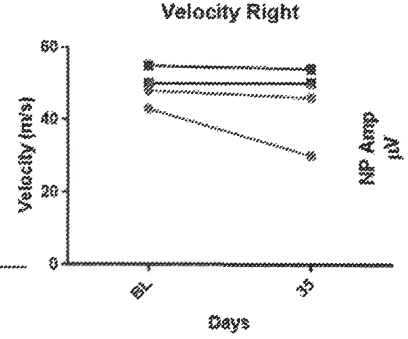


FIG. 24F

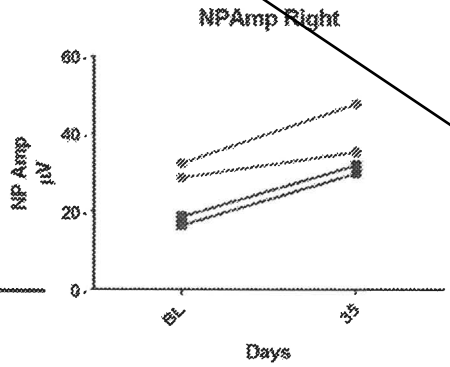


FIG. 24G

FIG. 25A

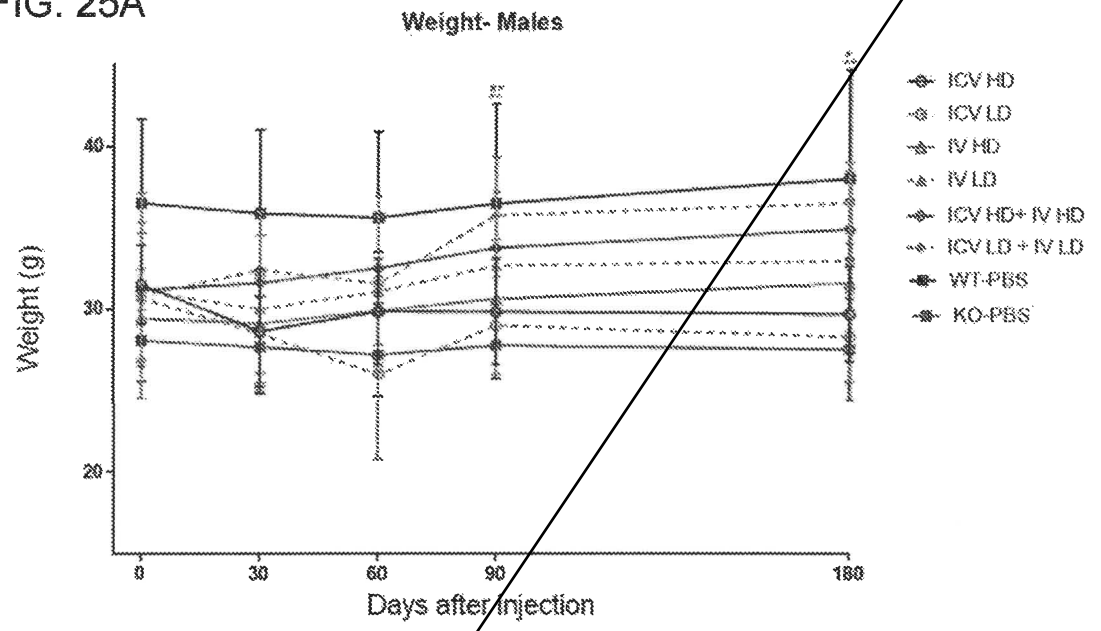


FIG. 25B

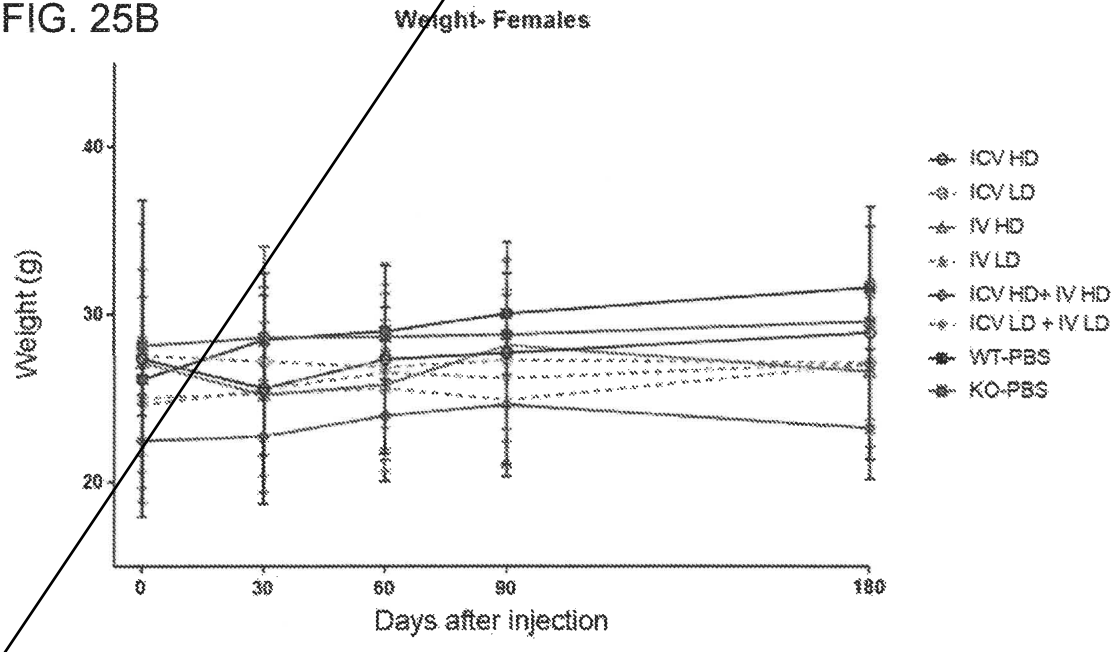


FIG. 26

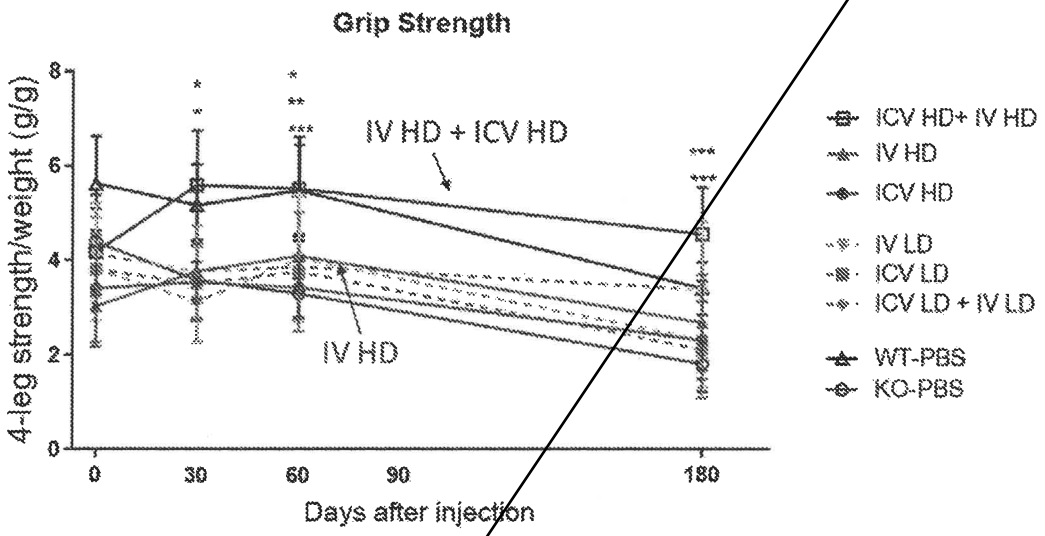

Grip Strength
4-leg strength/weight (g/g)
0
2
4
6
8
0
30
60
90
180
Days after injection
IV HD + ICV HD
IV HD
ICV HD+ IV HD
IV HD
ICV HD
IV LD
ICV LD
ICV LD + IV LD
WT-PBS
KO-PBS

FIG. 27

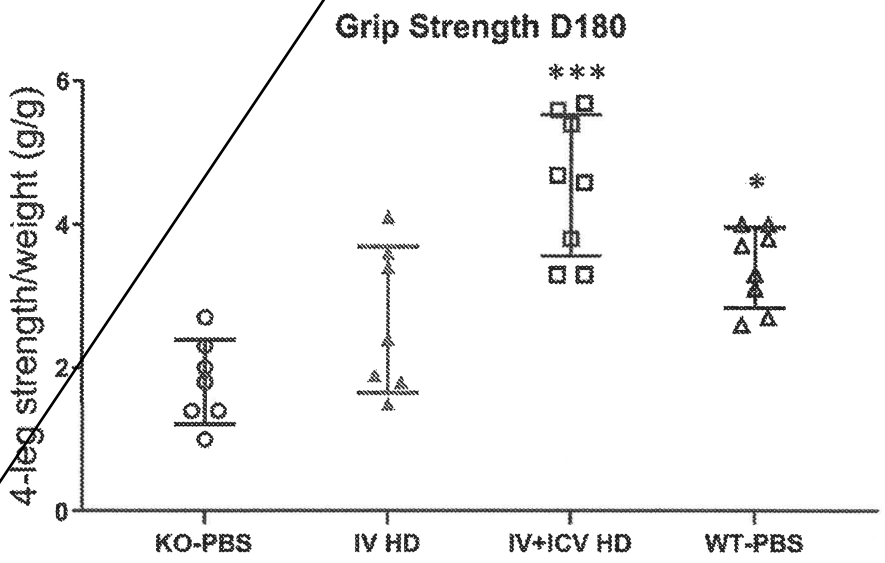

Grip Strength D180
4-leg strength/weight (g/g)
0
2
4
6
***
*
KO-PBS
IV HD
IV+ICV HD
WT-PBS

FIG. 28

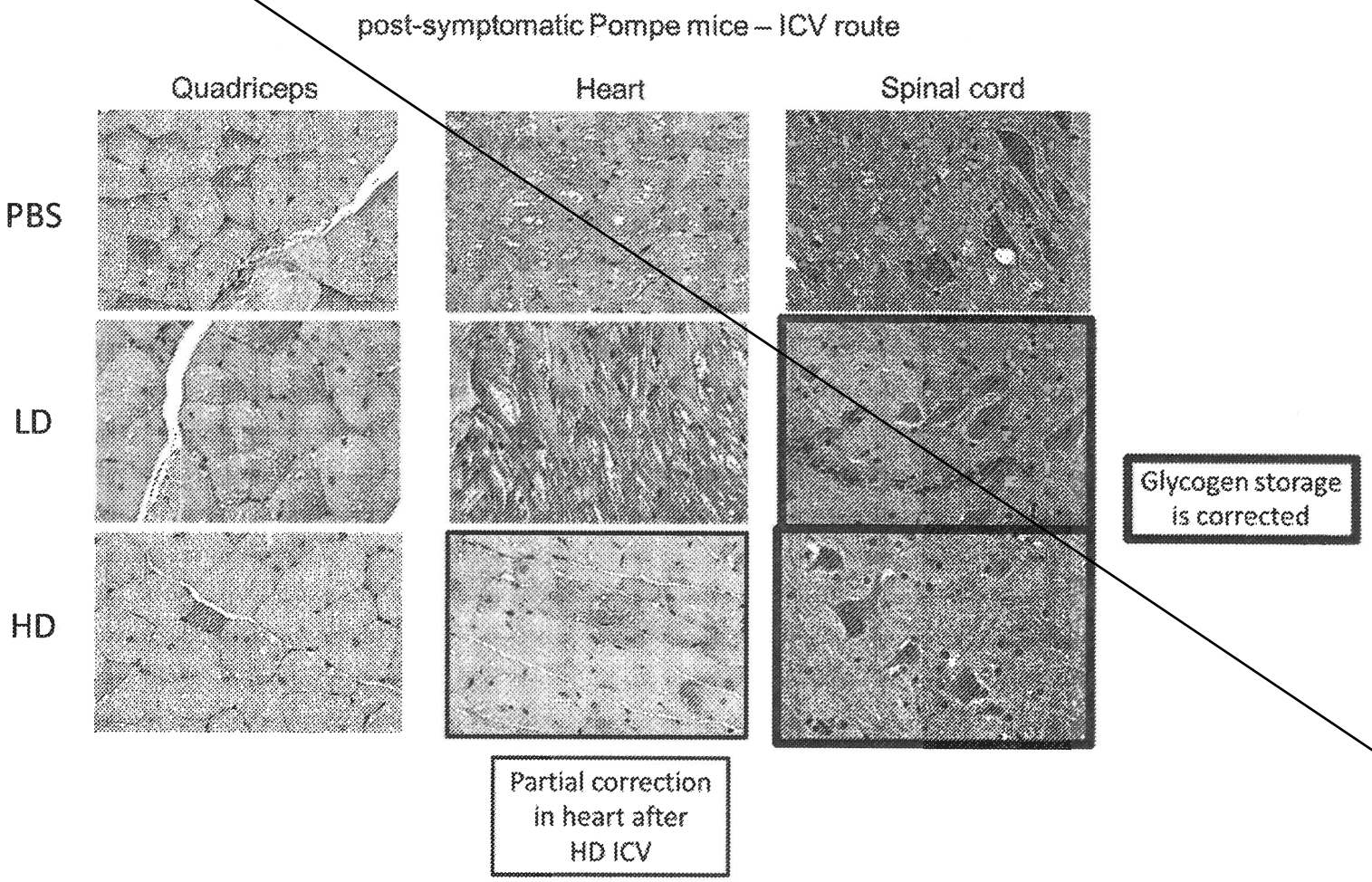

post-symptomatic Pompe mice – ICV route
Quadriceps
Heart
Spinal cord
PBS
LD
HD
Glycogen storage is corrected
Partial correction in heart after HD ICV

FIG. 29

post-symptomatic aged Pompe mice – IV route

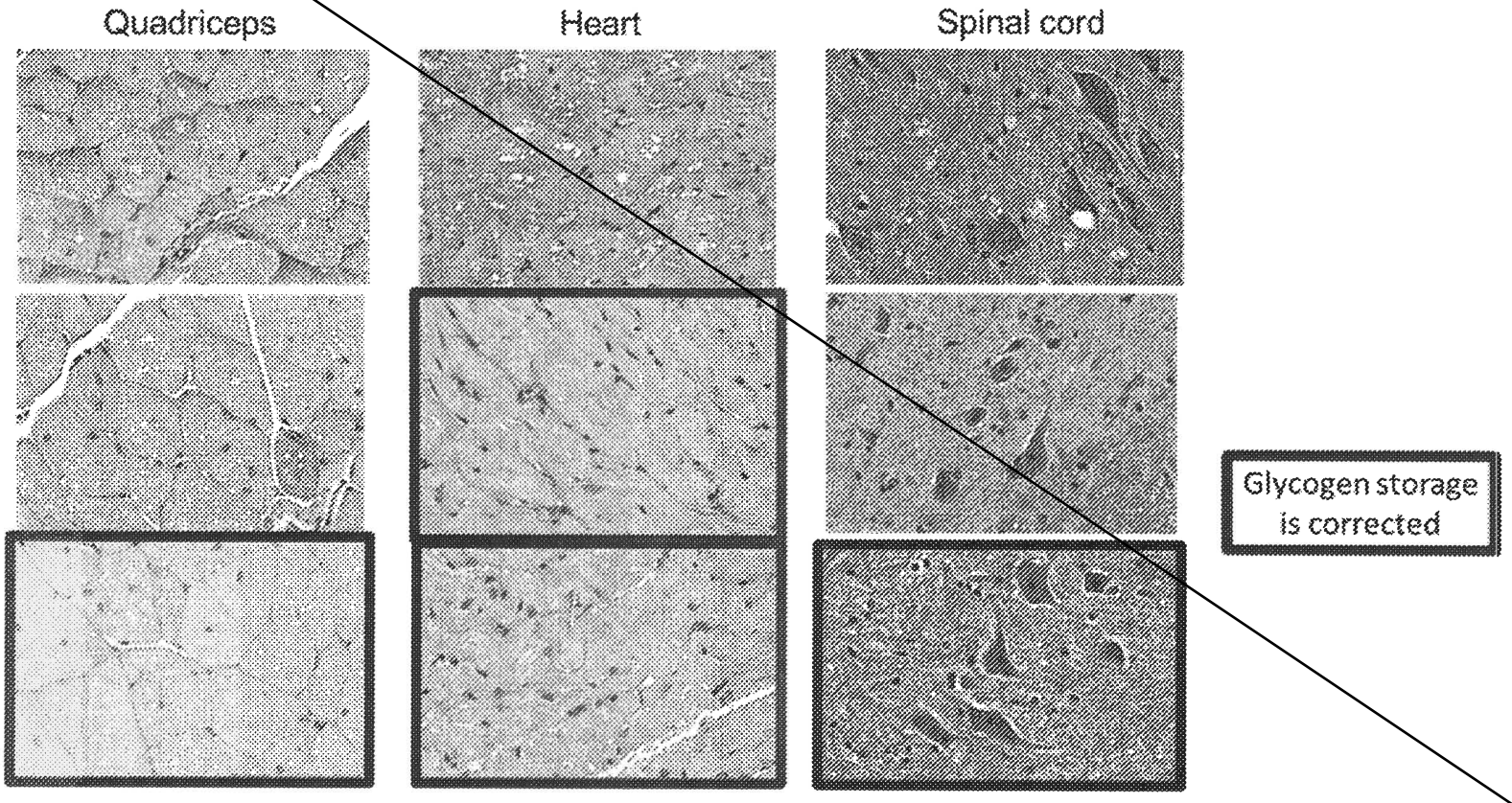


FIG. 30A

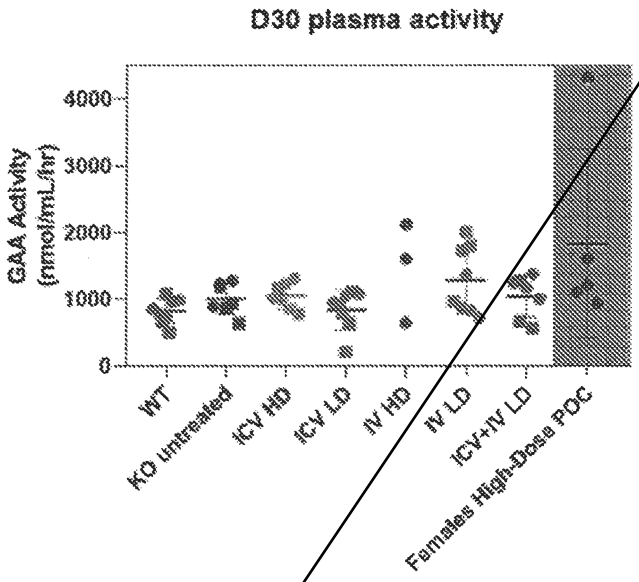


FIG. 30B

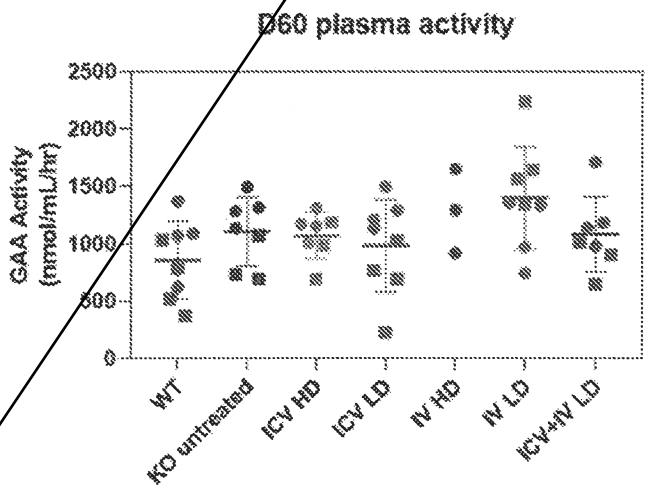


FIG. 30C

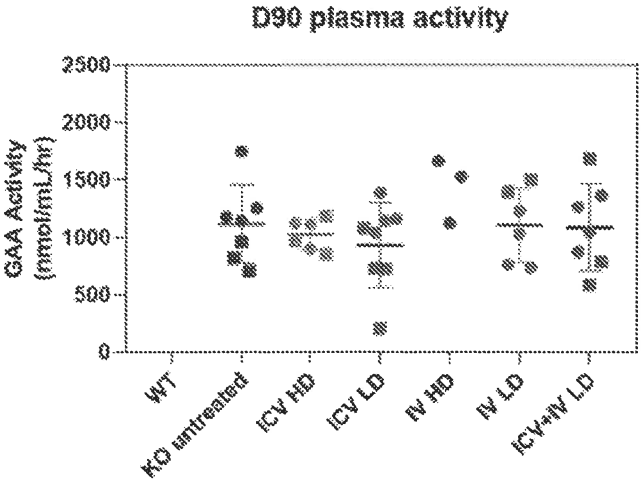


FIG. 31

Day								
-21	-10 (approx.)	0	3	7	21	29	35	60
Baseline Clin Path NAB Neuro Exam	Pred	Vector dosing Clinpath Plasma / CSF NAB / PBMC	Clinpath Plasma	Clinpath Plasma / CSF	Clinpath Plasma / CSF	Pred Taper	Clinpath Plasma / CSF Neuro exam	Necropsy Clinpath Plasma / CSF NAB / PBMC Neuro exam

Group Designation	1	2	3	4	5	6*
N / Group	2	2	2	2	2	2
Route of administration	IV LD	IV HD	ICM LD	ICM HD	IV HD ICM HD	IV HHD ICM LD
Vector Dose	1e13 GC/kg	5e13 GC/kg	1e13 GC	3e13 GC	5E13 GC/kg 3e13 GC	1E14 GC/kg 1e13 GC

FIG. 32A

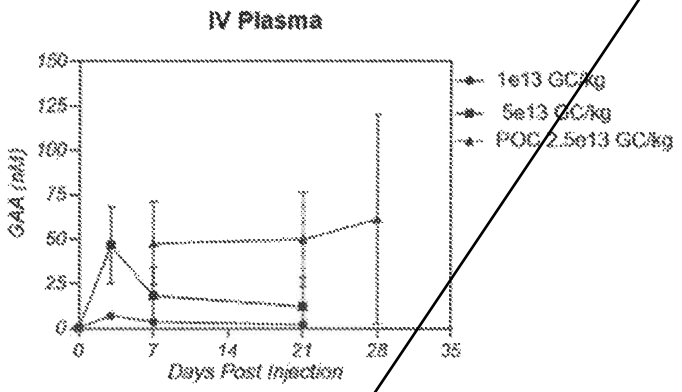


FIG. 32B

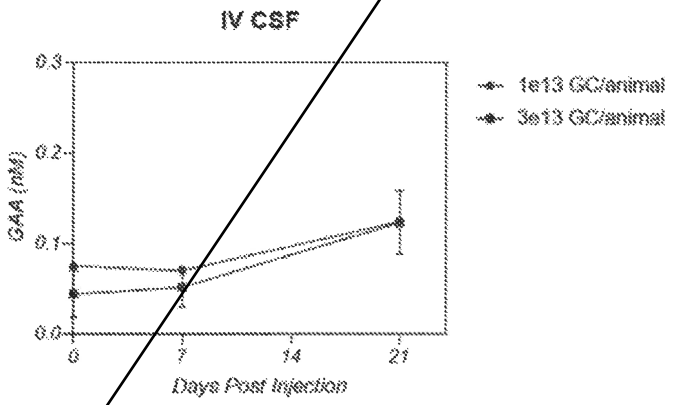


FIG. 32C

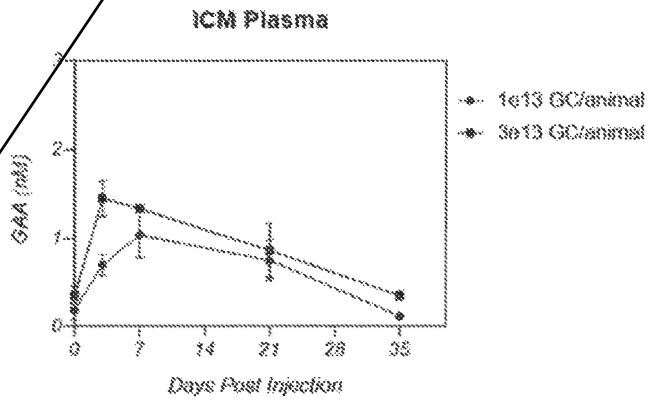


FIG. 32D

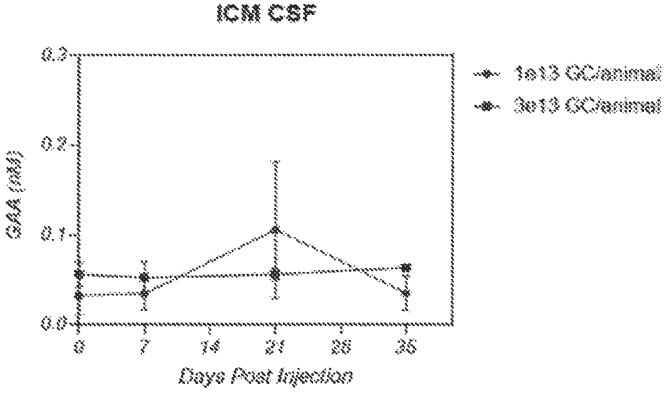


FIG. 32E

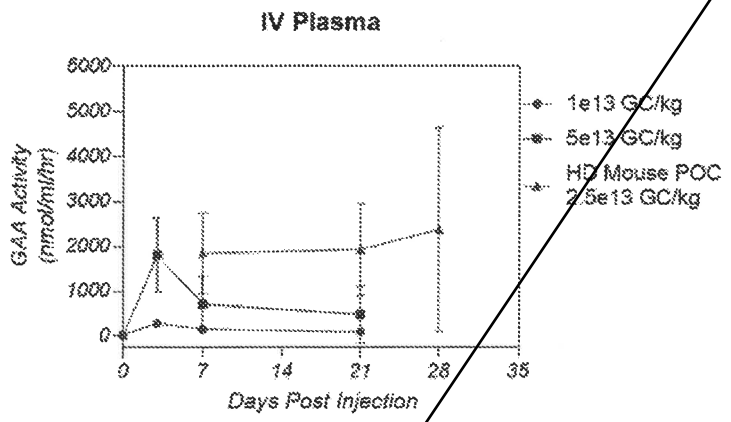


FIG. 32F

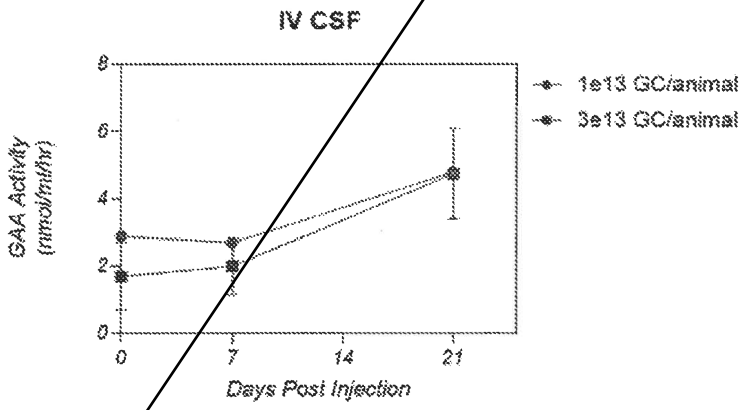


FIG. 32G

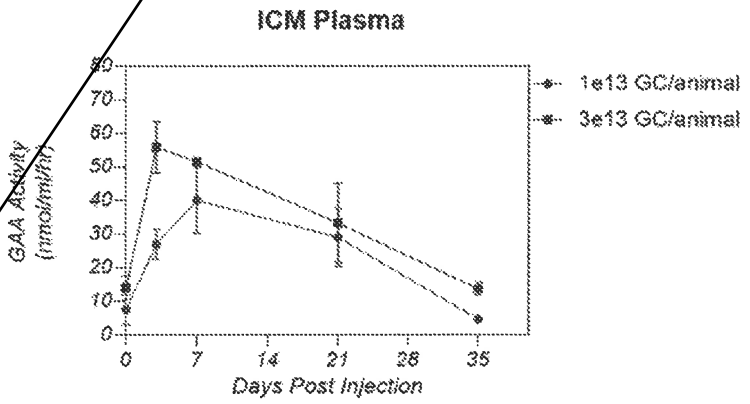


FIG. 32H

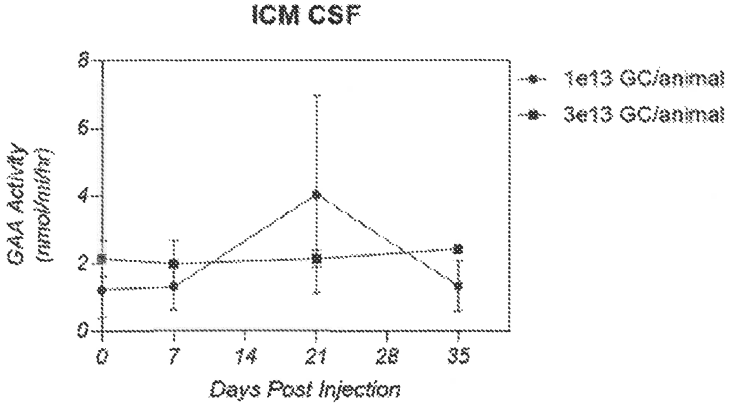


FIG. 33A

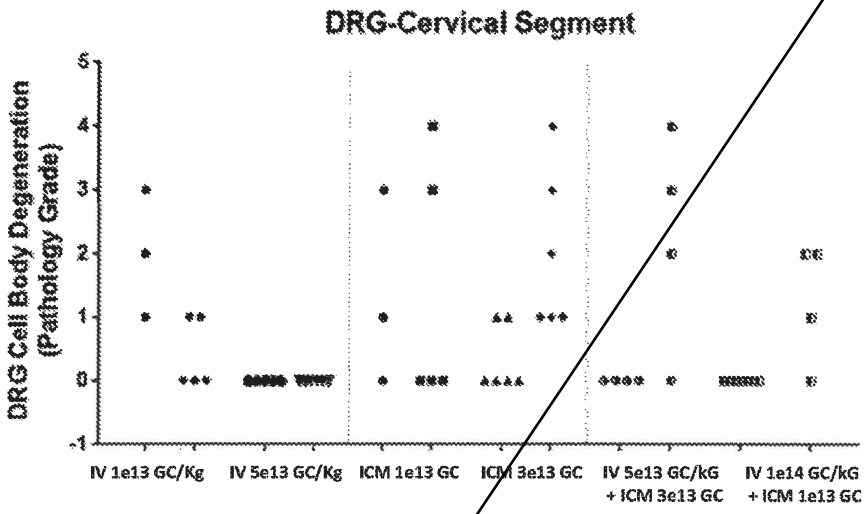


FIG. 33B

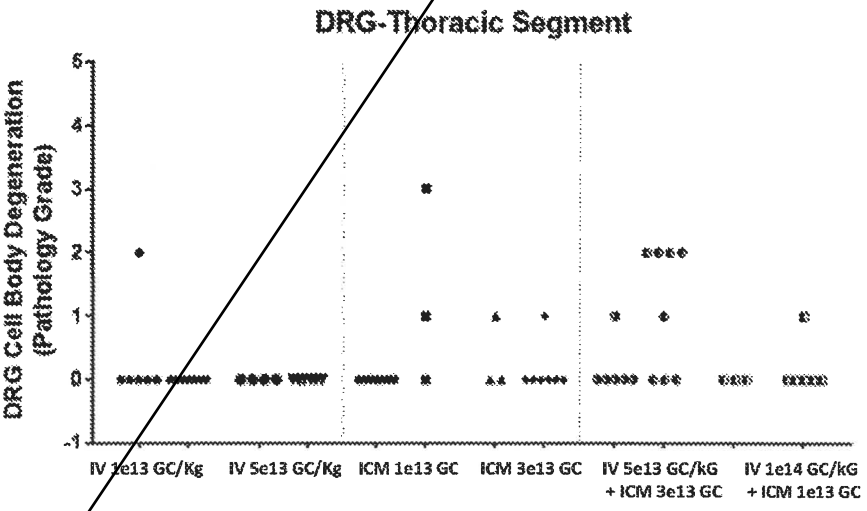


FIG. 33C

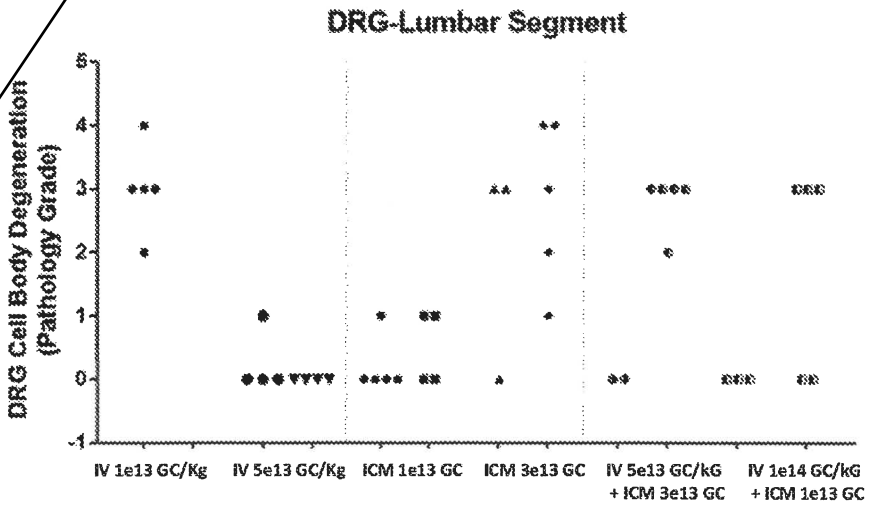


FIG. 33D

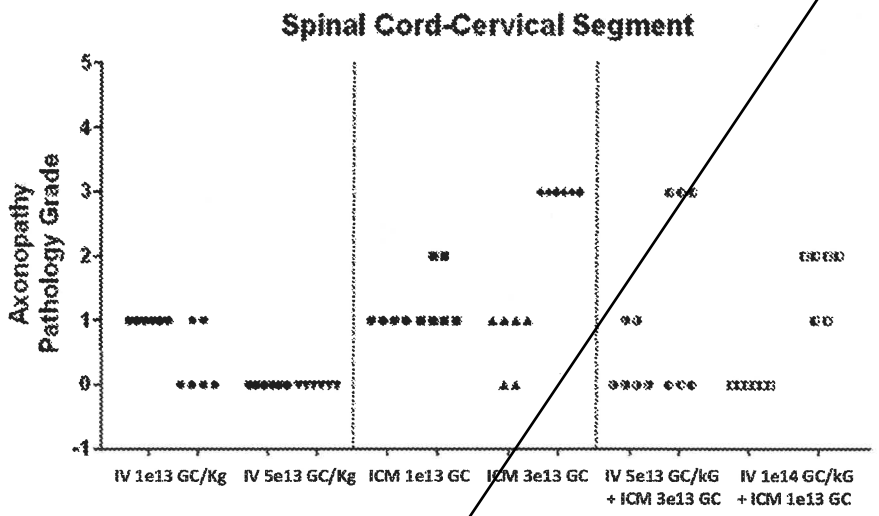


FIG. 33E

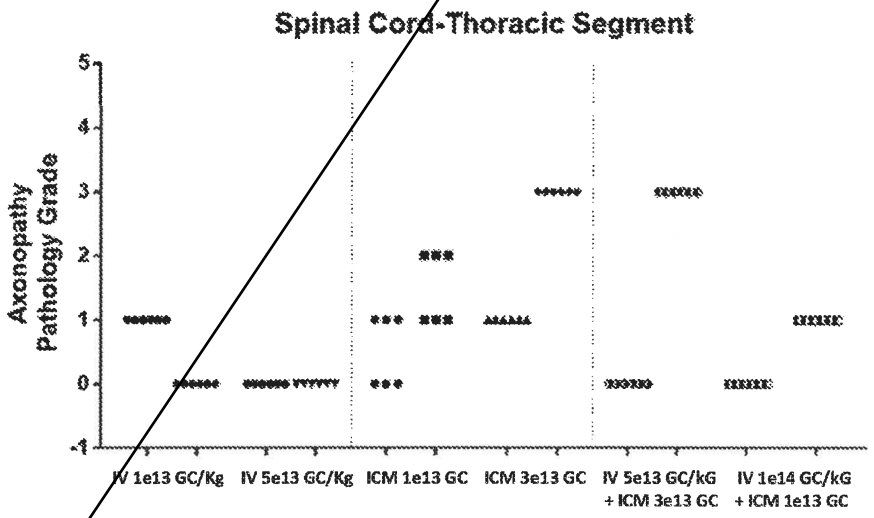


FIG. 33F

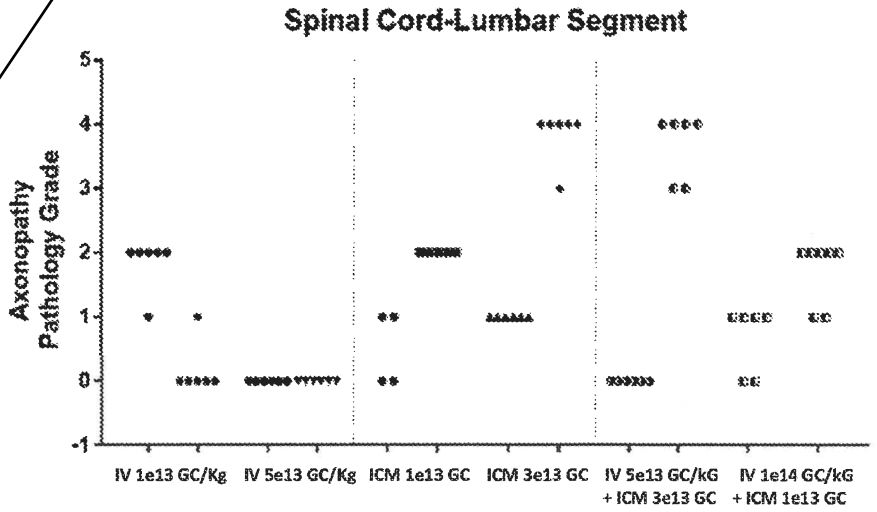


FIG. 34

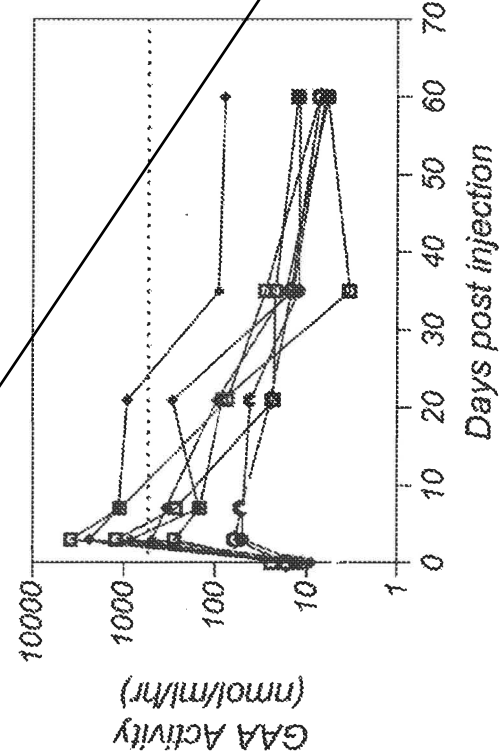

Plasma GAA Activity
IV LD (#RA3607)
IV LD (#180268)
IV HD (#RA3679)
IV HD (#181278)
IV HD+ICM HD (#180672)
IV HD+ICM HD (#180717)
ICM LD (#180637)
ICM LD (#13-2251R/7006)
ICM HD (#12-1695R/7022)
ICM HD (#808041/6468)
GAA Activity (nmol/ml/hr)
10000
1000
100
10
1
0
10
20
30
40
50
60
70
Days post injection

FIG. 35A

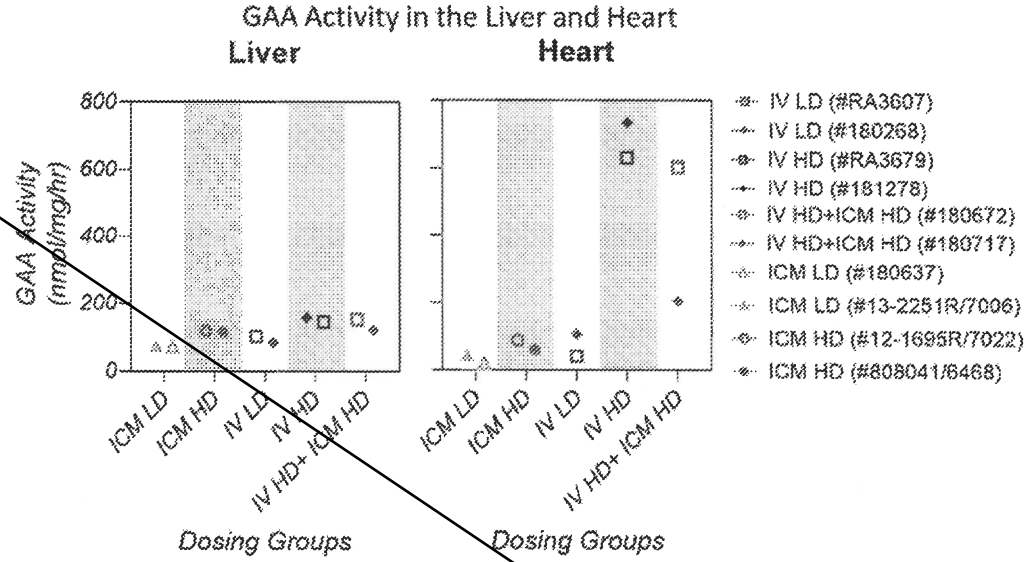


FIG. 35B

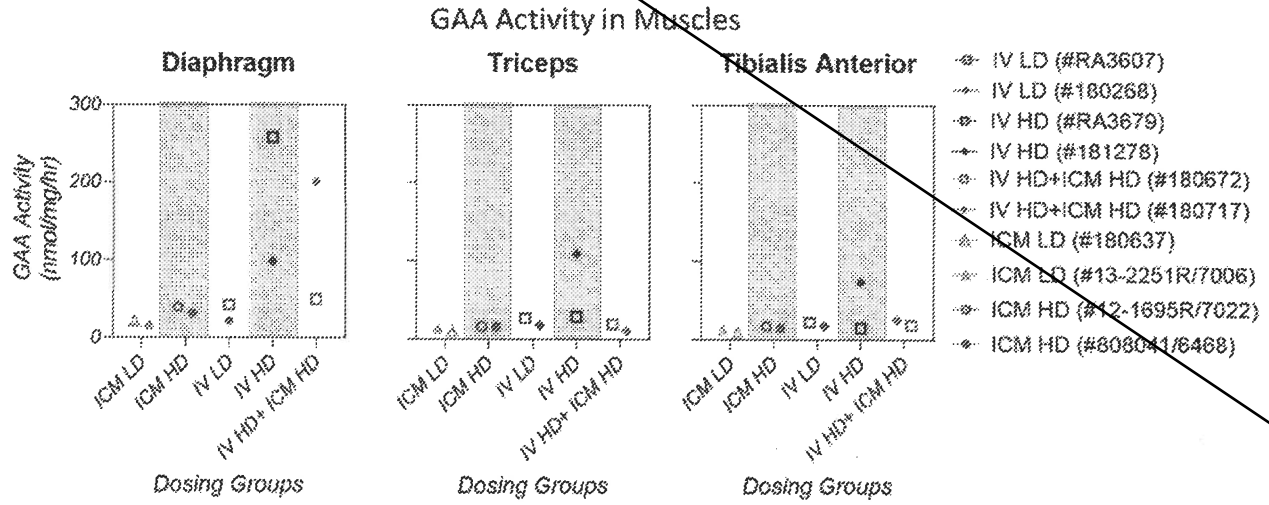


FIG. 35C

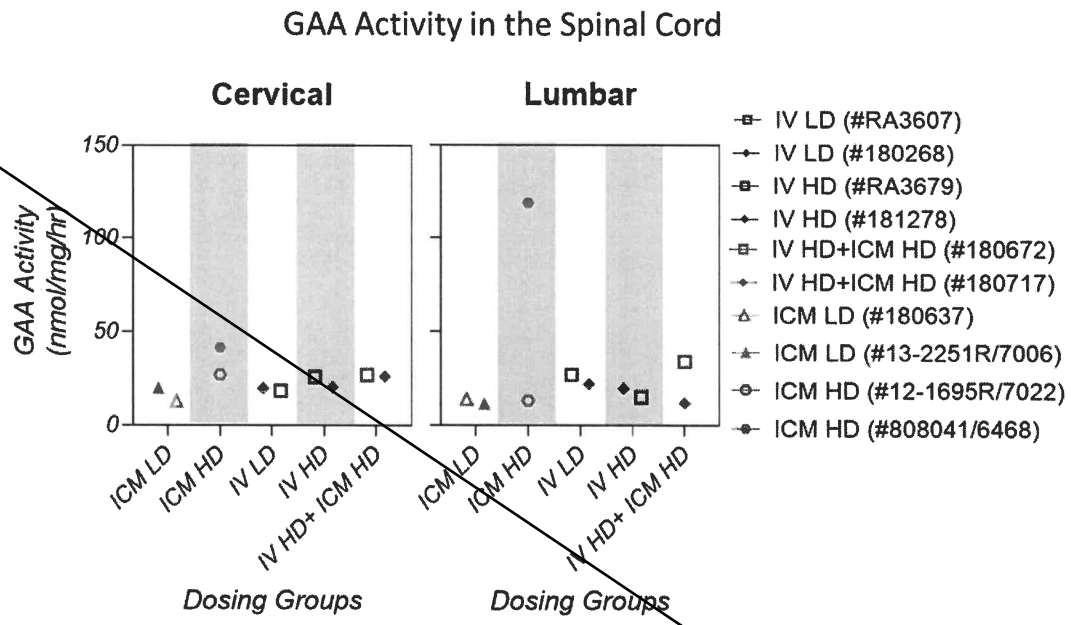


FIG. 35D

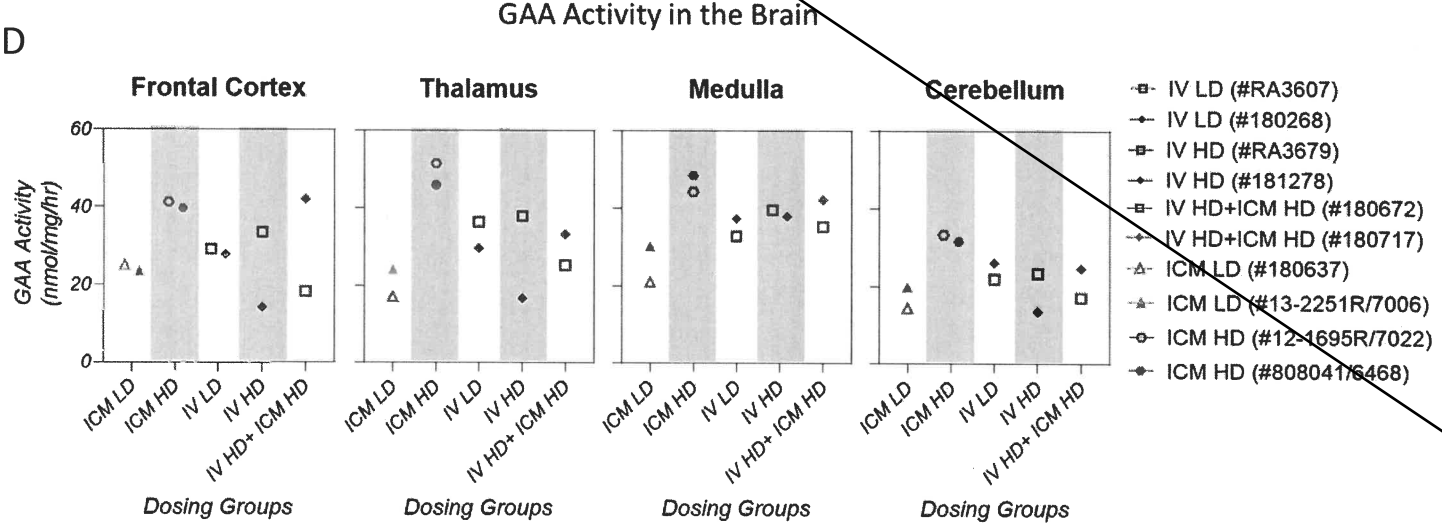


FIG. 36

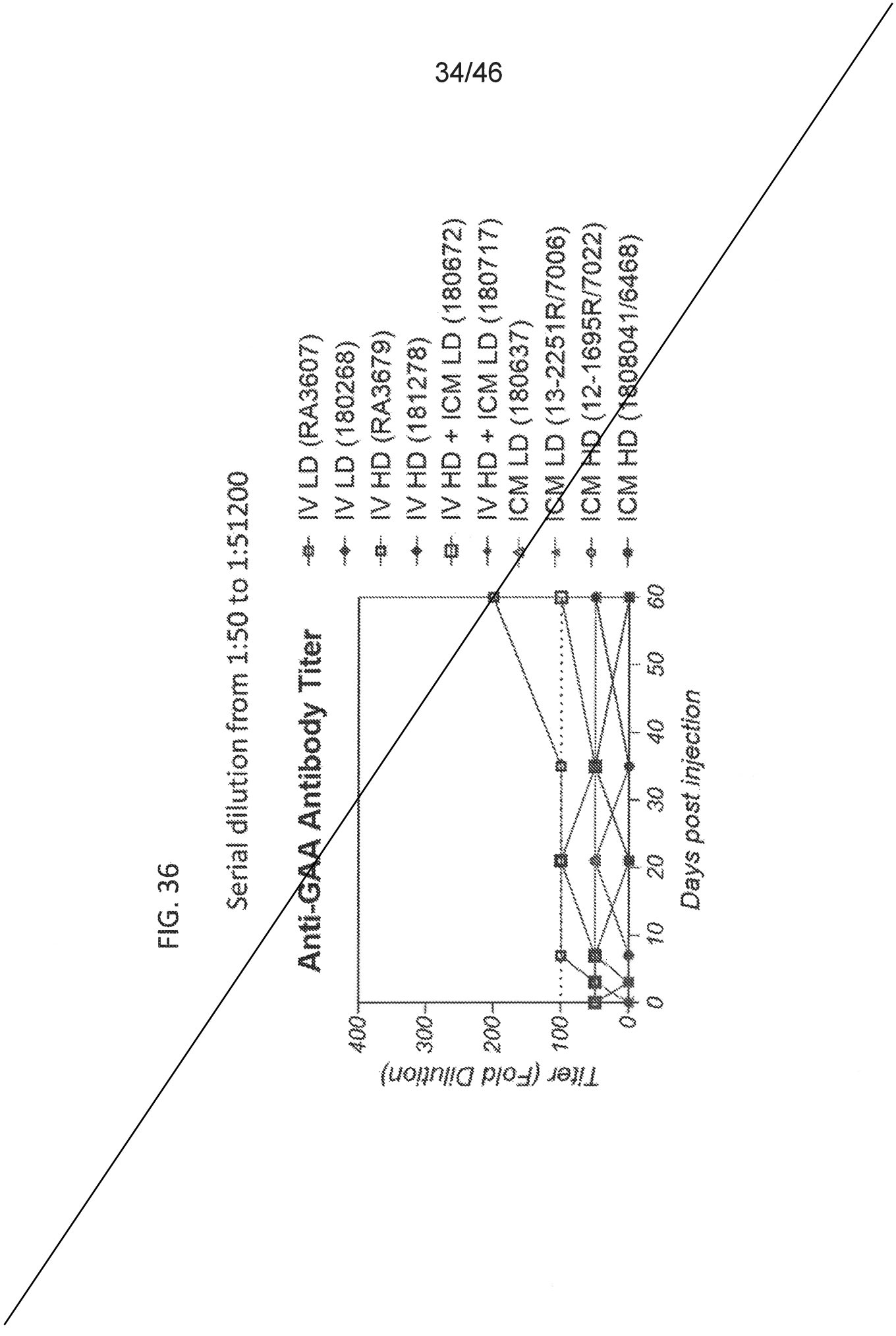
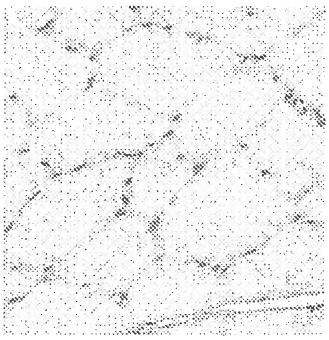
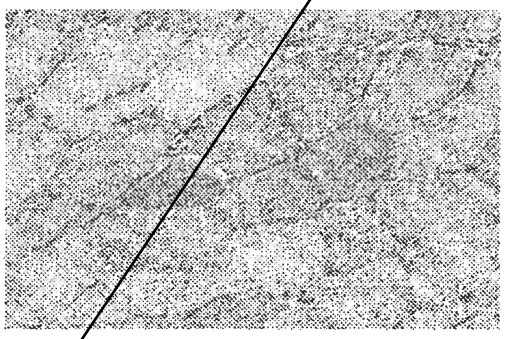

Serial dilution from 1:50 to 1:51200
Anti-GAA Antibody Titer
IV LD (RA3607)
IV LD (180268)
IV HD (RA3679)
IV HD (181278)
IV HD + ICM LD (180672)
IV HD + ICM LD (180717)
ICM LD (180637)
ICM LD (13-2251R/7006)
ICM HD (12-1695R/7022)
ICM HD (1808041/6468)
Titer (Fold Dilution)
400
300
200
100
0
0
10
20
30
40
50
60
Days post injection

FIG. 37

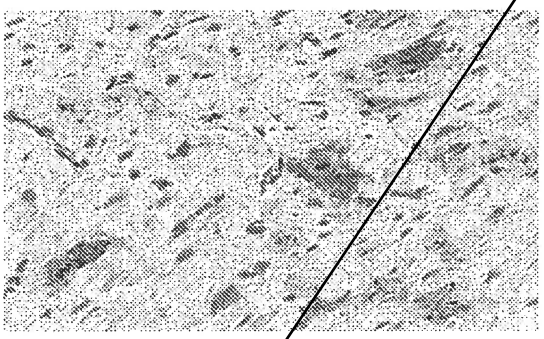
Quadriceps
Non injected NHP



Quadriceps
IV 1e13 GC/Kg



Heart
IV 1e13 GC/Kg



Spinal cord
ICM 1e13 GC

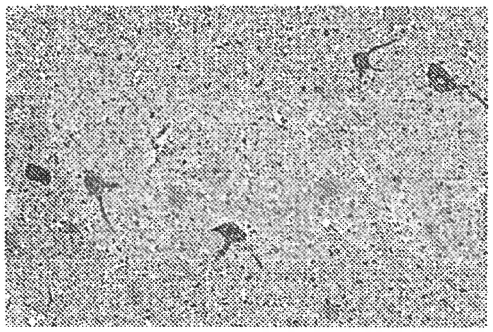


FIG. 38

Glycogen PAS
Vehicle
AAV.hGAA nat
AAV.hGAA eng
WT
quadriceps
heart
Quadriceps GAA activity
Heart GAA activity
WT Mouse
Vehicle
AAV.hGAA nat
AAV.hGAA eng
LD 2.5e12 GC/Kg:
Muscle: Storage and autophagic buildup correction with hGAA eng only
Heart: Good correction with both hGAA constructs
Short term study: rapid glycogen clearance

FIG. 39

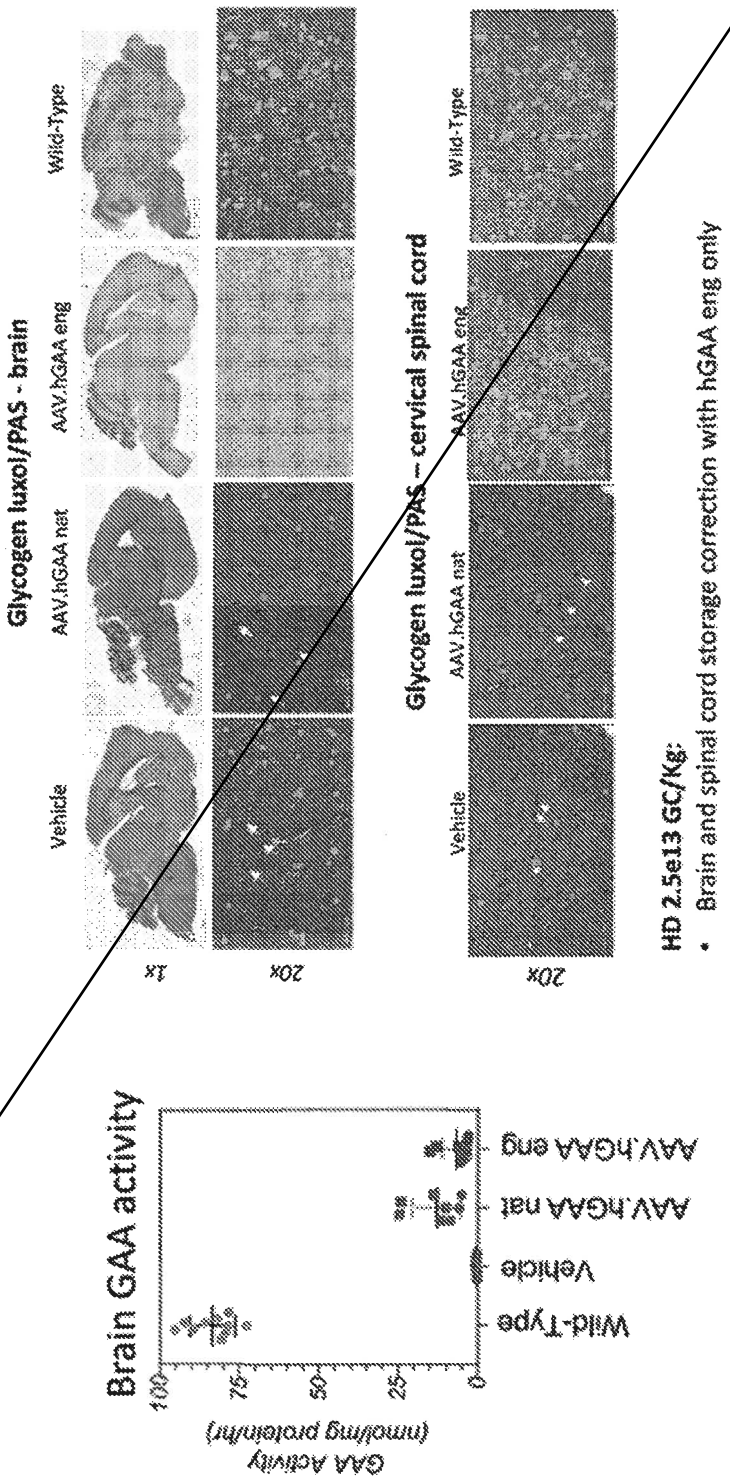

Glycogen luxol/PAS - brain
Vehicle
AAV.hGAA nat
AAV.hGAA eng
Wild-Type
1x
20x
Glycogen luxol/PAS – cervical spinal cord
Vehicle
AAV.hGAA nat
AAV.hGAA eng
Wild-Type
20x
HD 2.5e13 GC/Kg:
• Brain and spinal cord storage correction with hGAA eng only
Brain GAA activity
GAA Activity
(nmol/mg protein/hr)
100
75
50
25
0
Wild-Type
Vehicle
AAV.hGAA nat
AAV.hGAA eng

FIG. 40

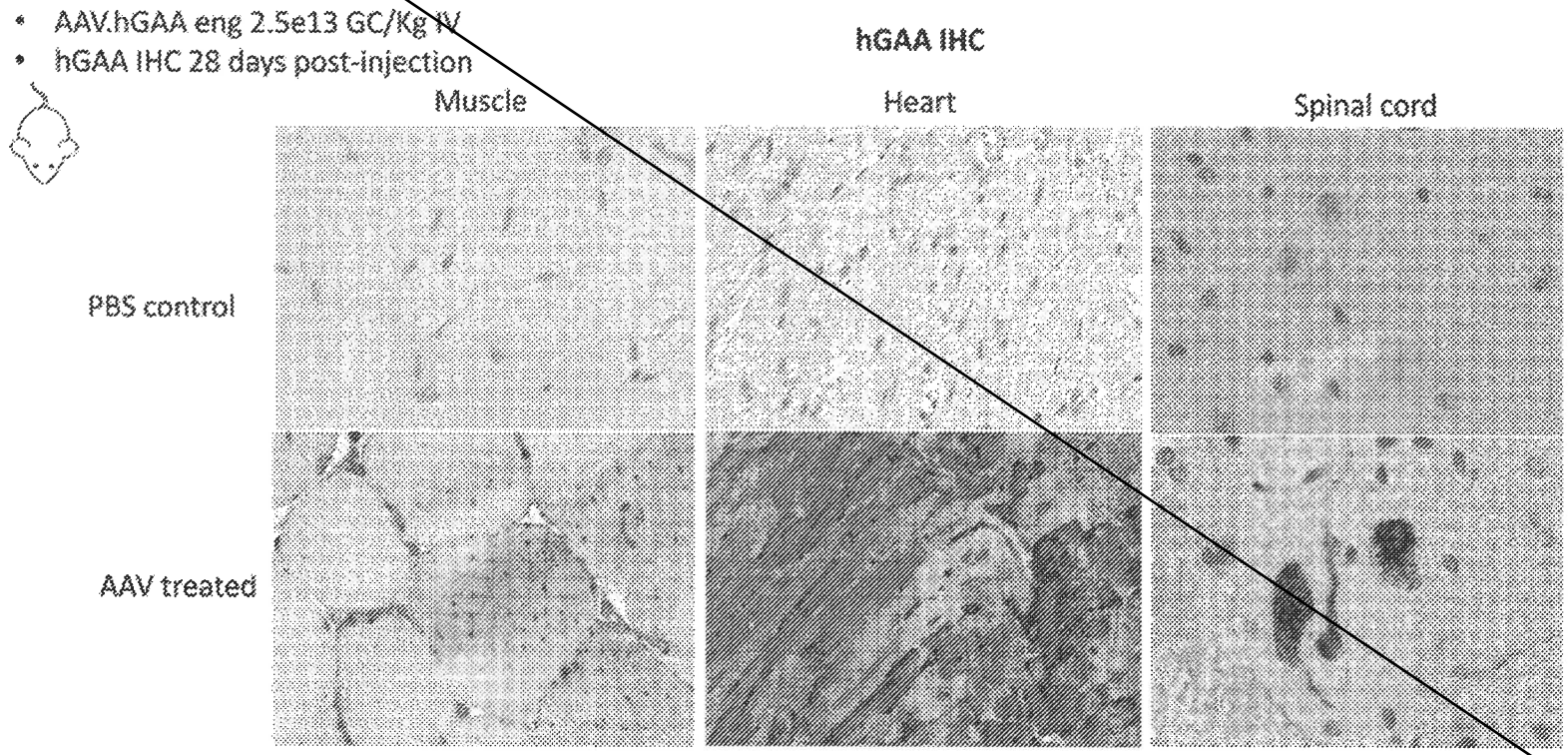

• AAV.hGAA eng 2.5e13 GC/Kg IV
• hGAA IHC 28 days post-injection
hGAA IHC
Muscle
Heart
Spinal cord
PBS control
AAV treated

FIG. 41

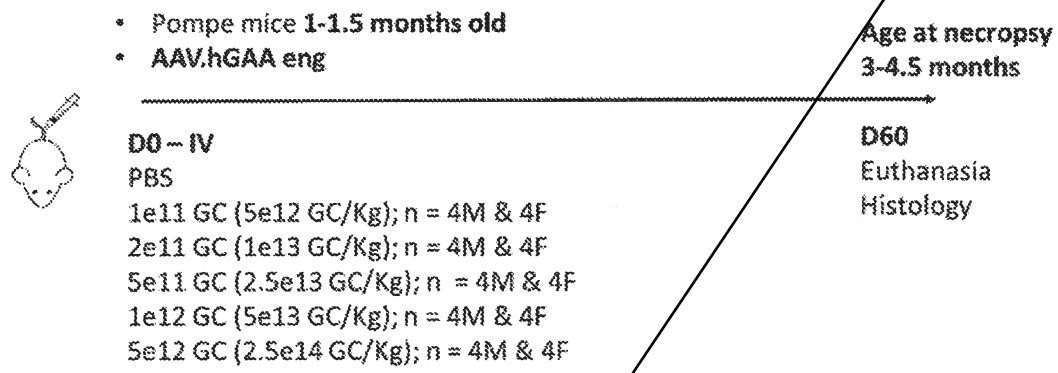
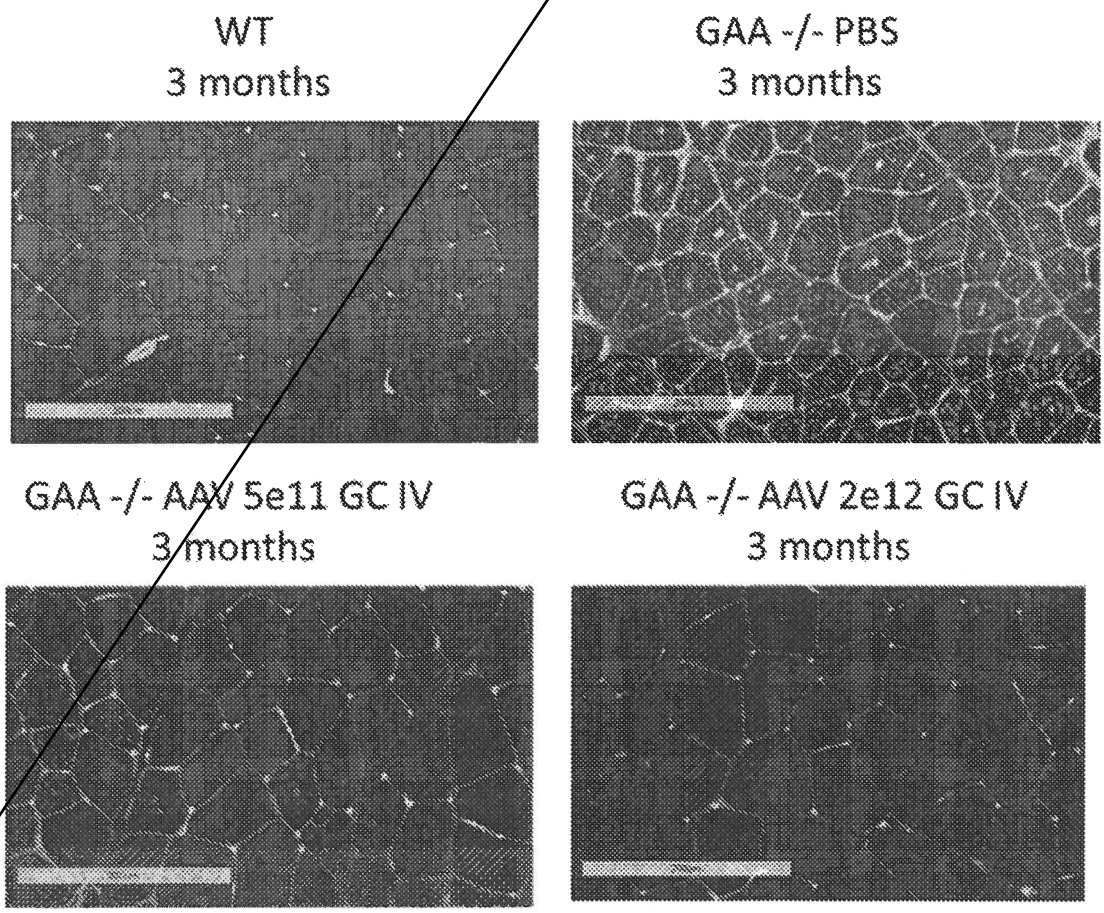

• Pompe mice 1-1.5 months old
• AAV.hGAA eng
Age at necropsy
3-4.5 months
D0 – IV
PBS
1e11 GC (5e12 GC/Kg); n = 4M & 4F
2e11 GC (1e13 GC/Kg); n = 4M & 4F
5e11 GC (2.5e13 GC/Kg); n = 4M & 4F
1e12 GC (5e13 GC/Kg); n = 4M & 4F
5e12 GC (2.5e14 GC/Kg); n = 4M & 4F
D60
Euthanasia
Histology

FIG. 42


WT
3 months
GAA -/- PBS
3 months
GAA -/- AAV 5e11 GC IV
3 months
GAA -/- AAV 2e12 GC IV
3 months

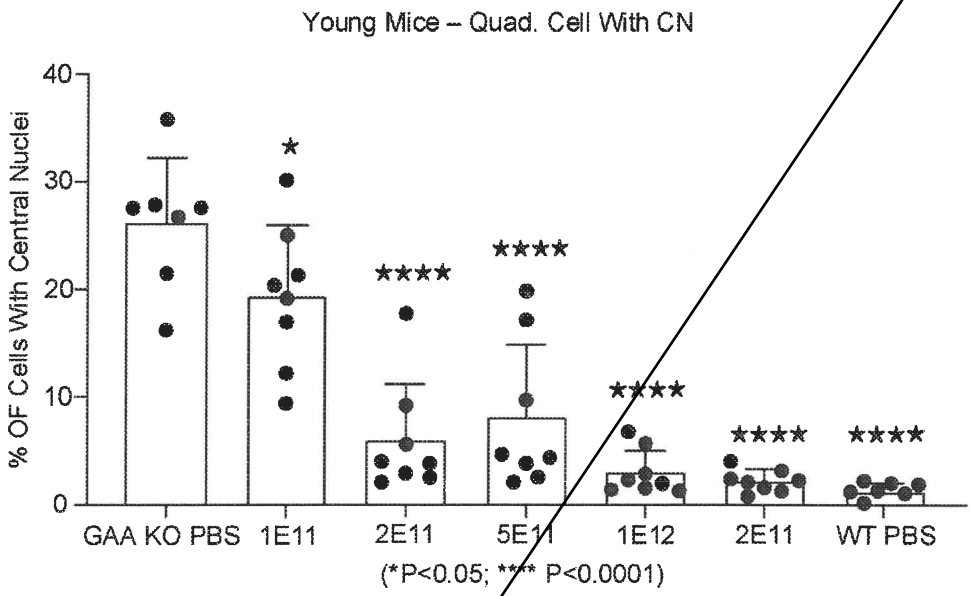

Young Mice – Quad. Cell With CN
% OF Cells With Central Nuclei
40
30
20
10
0
*
****
****
****
****
****
GAA KO PBS
1E11
2E11
5E11
1E12
2E11
WT PBS
(*P<0.05; **** P<0.0001)

FIG.43

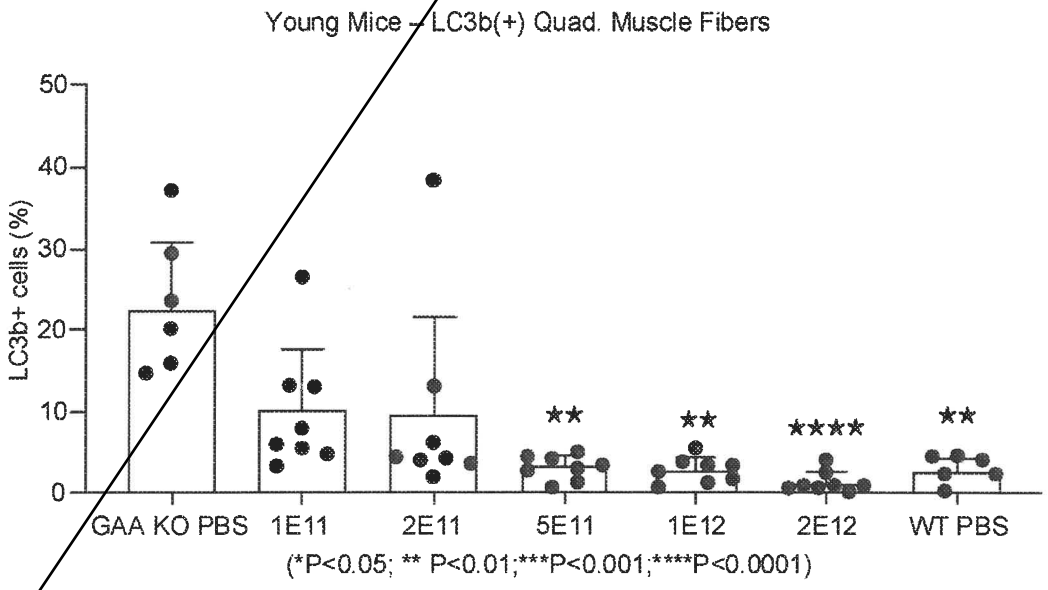

Young Mice – LC3b(+) Quad. Muscle Fibers
LC3b+ cells (%)
50
40
30
20
10
0
**
**
****
**
GAA KO PBS
1E11
2E11
5E11
1E12
2E12
WT PBS
(*P<0.05; ** P<0.01;***P<0.001;****P<0.0001)

FIG.44

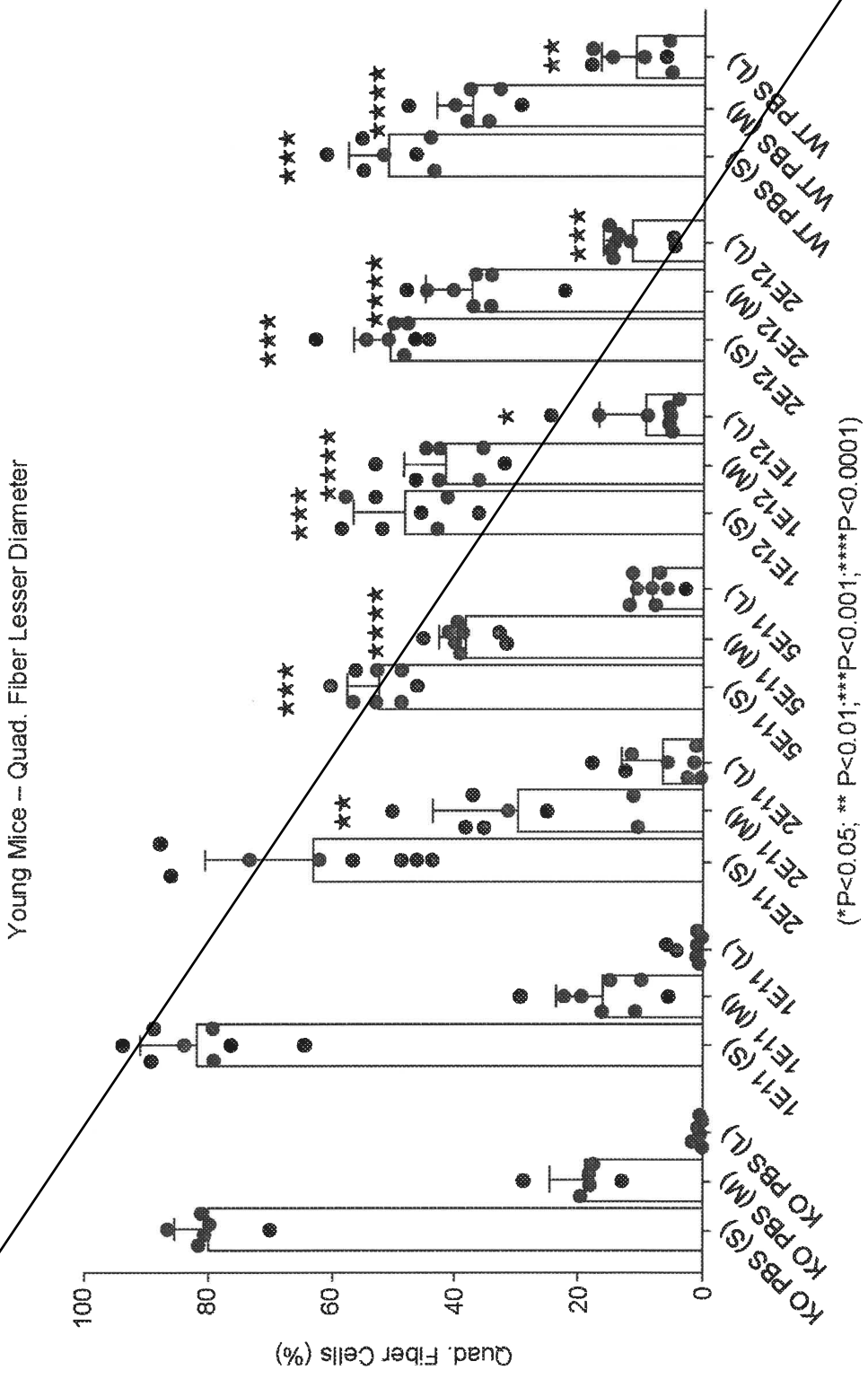

Young Mice -- Quad. Fiber Lesser Diameter
Quad. Fiber Cells (%)
0
20
40
60
80
100
KO PBS (S)
KO PBS (M)
KO PBS (L)
1E11 (S)
1E11 (M)
1E11 (L)
2E11 (S)
2E11 (M)
2E11 (L)
5E11 (S)
5E11 (M)
5E11 (L)
1E12 (S)
1E12 (M)
1E12 (L)
2E12 (S)
2E12 (M)
2E12 (L)
WT PBS (S)
WT PBS (M)
WT PBS (L)
(*P<0.05; ** P<0.01;***P<0.001;****P<0.0001)

FIG.45

FIG. 46A

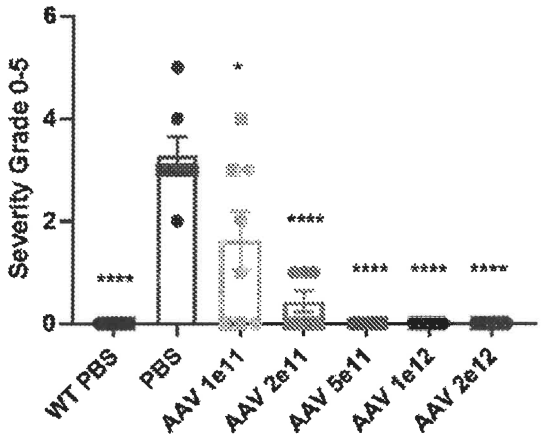


FIG. 46B

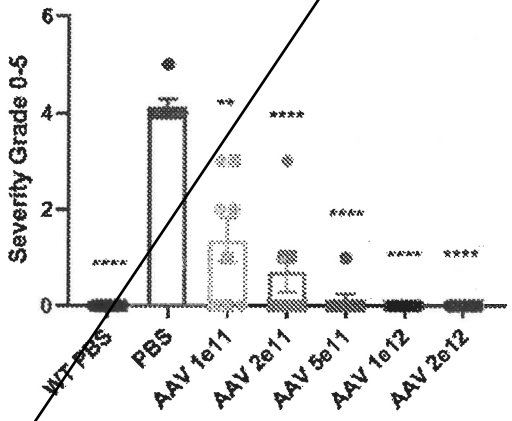


FIG. 46C

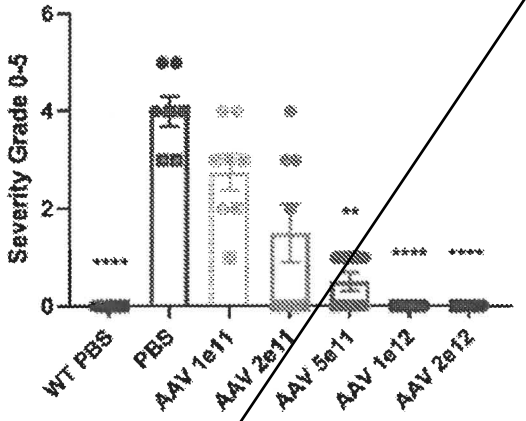


FIG. 46D

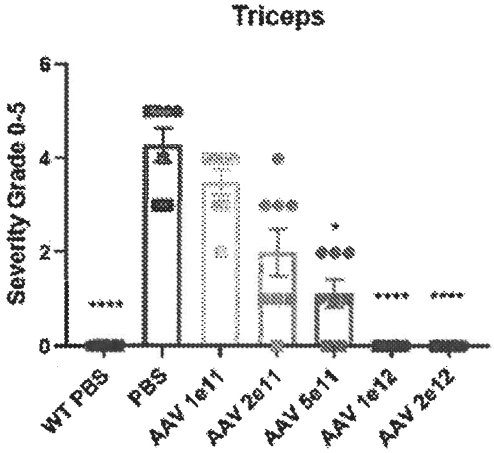


FIG. 46E

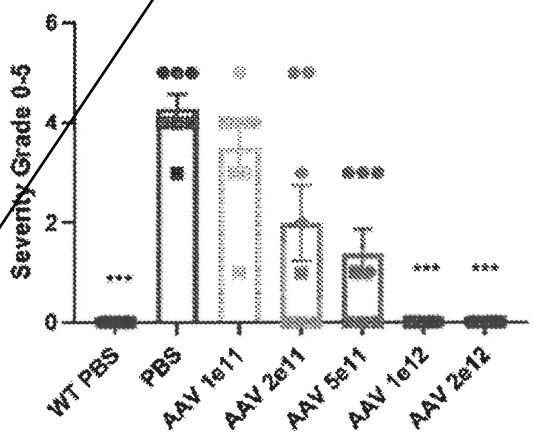


FIG. 46F

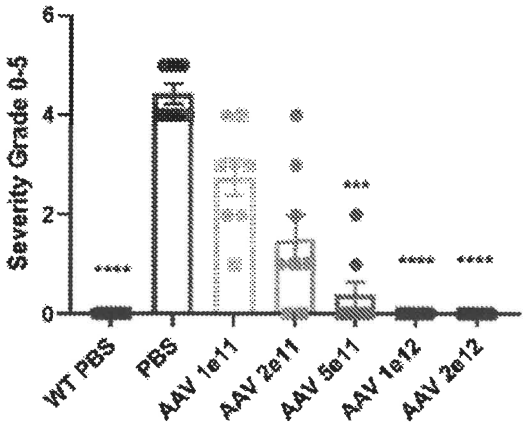


FIG. 47

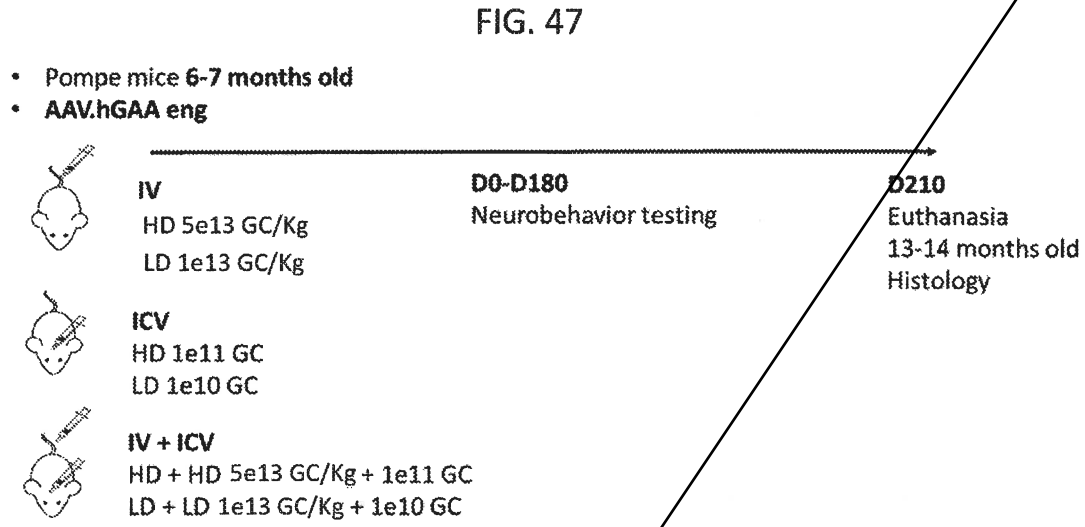
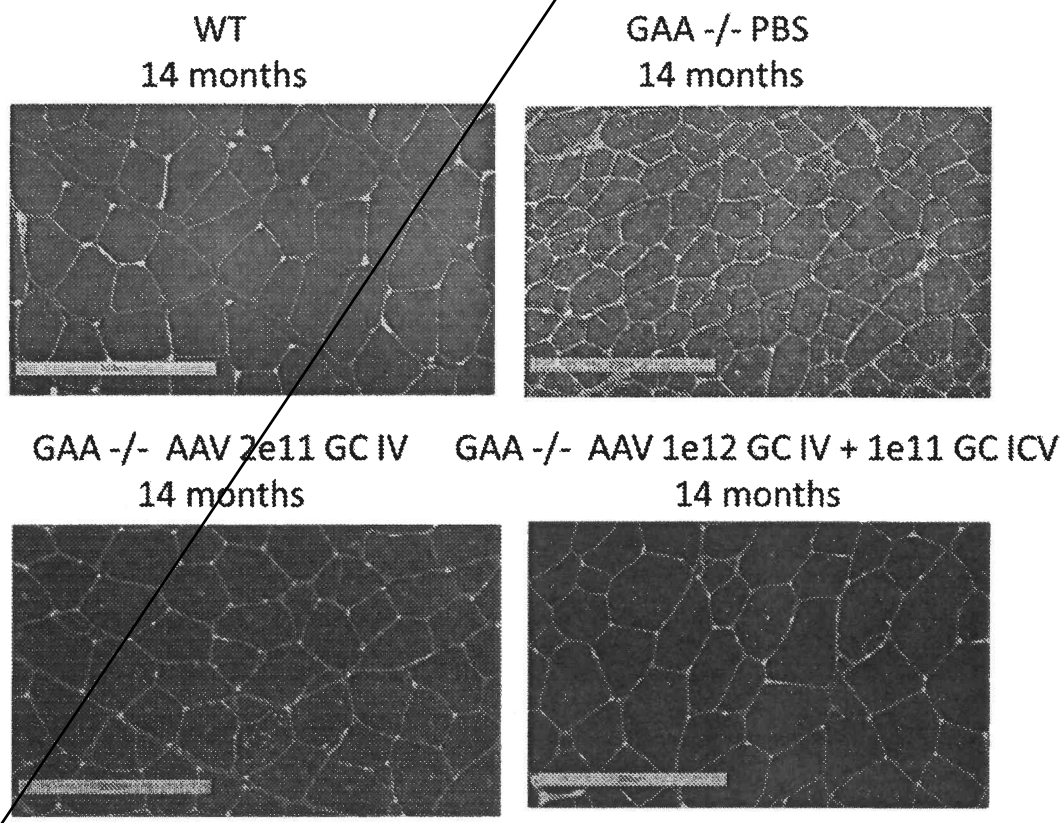

• Pompe mice 6-7 months old
• AAV.hGAA eng
IV
HD 5e13 GC/Kg
LD 1e13 GC/Kg
ICV
HD 1e11 GC
LD 1e10 GC
IV + ICV
HD + HD 5e13 GC/Kg + 1e11 GC
LD + LD 1e13 GC/Kg + 1e10 GC
D0-D180
Neurobehavior testing
D210
Euthanasia
13-14 months old
Histology

FIG. 48


WT
14 months
GAA -/- PBS
14 months
GAA -/- AAV 2e11 GC IV
14 months
GAA -/- AAV 1e12 GC IV + 1e11 GC ICV
14 months

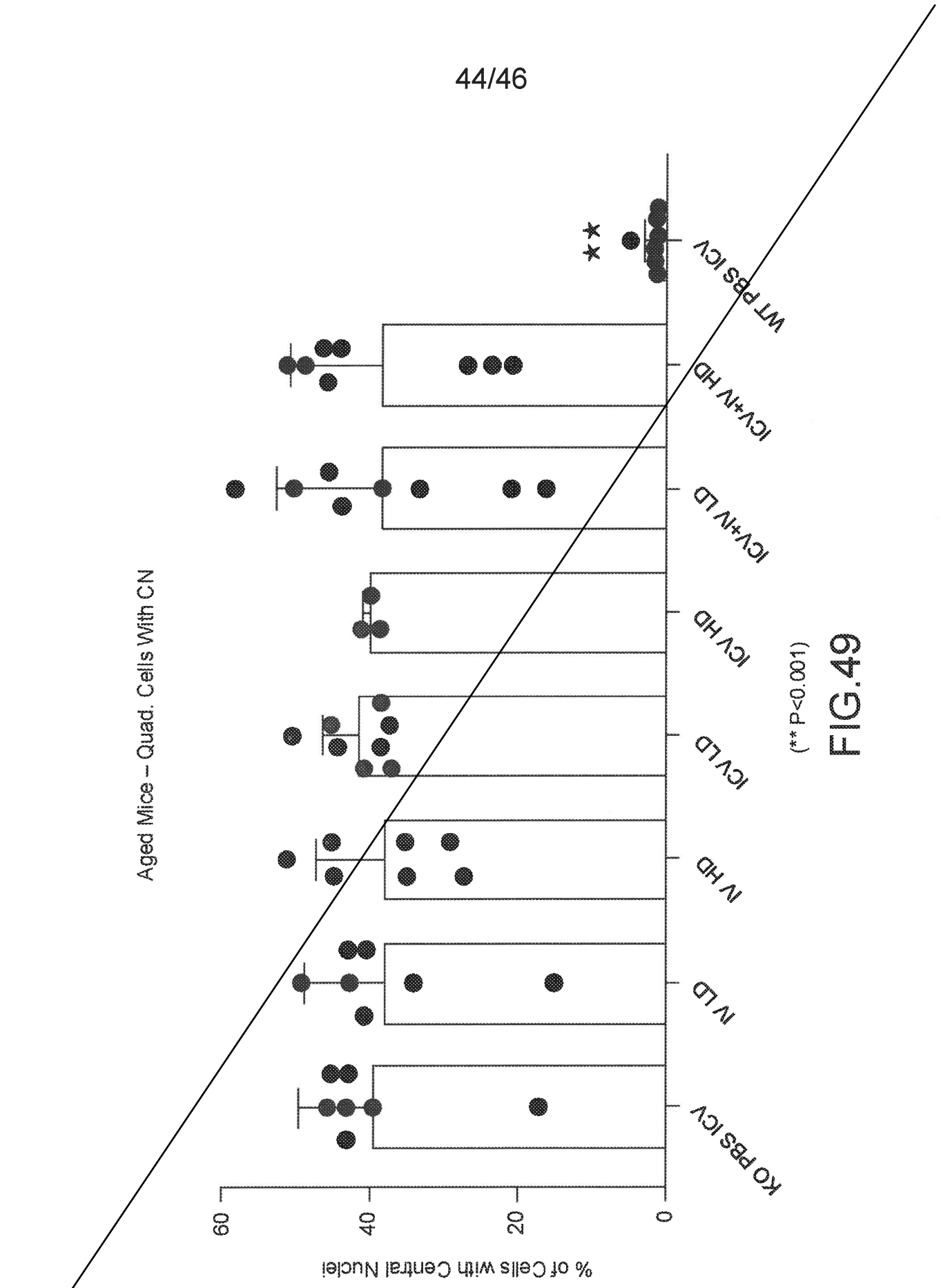

Aged Mice – Quad. Cells With CN
% of Cells with Central Nuclei
60
40
20
0
KO PBS ICV
IV LD
IV HD
ICV LD
ICV HD
ICV+IV LD
ICV+IV HD
WT PBS ICV
**
(** P<0.001)

FIG.49

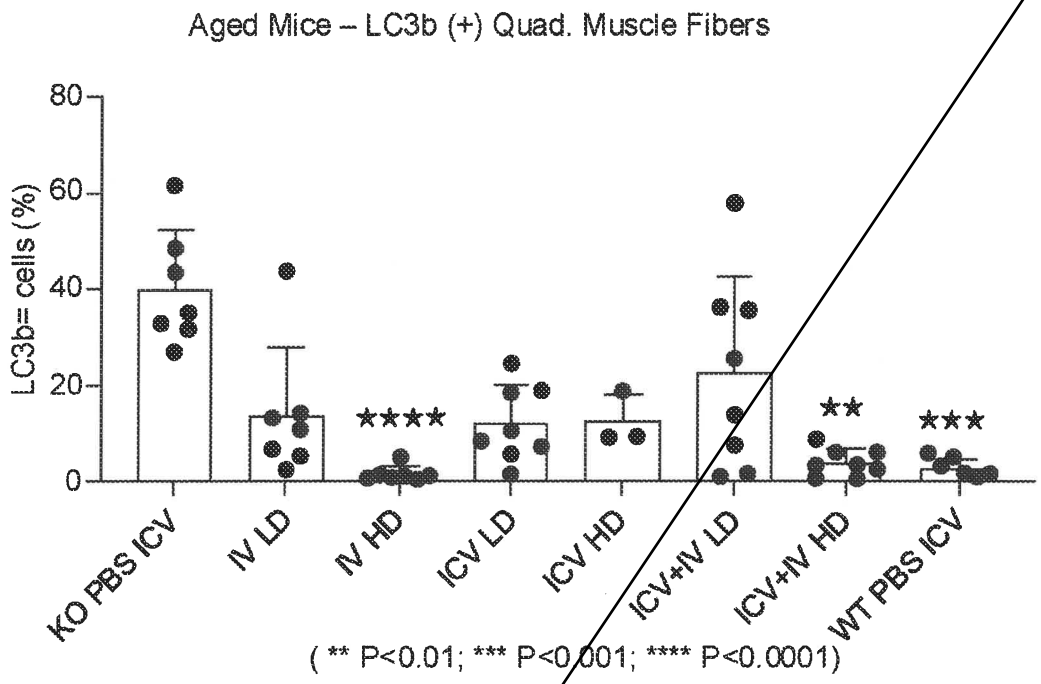

Aged Mice – LC3b (+) Quad. Muscle Fibers
LC3b= cells (%)
80
60
40
20
0
****
**
***
KO PBS ICV
IV LD
IV HD
ICV LD
ICV HD
ICV+IV LD
ICV+IV HD
WT PBS ICV
(** P<0.01; *** P<0.001; **** P<0.0001)

FIG.50

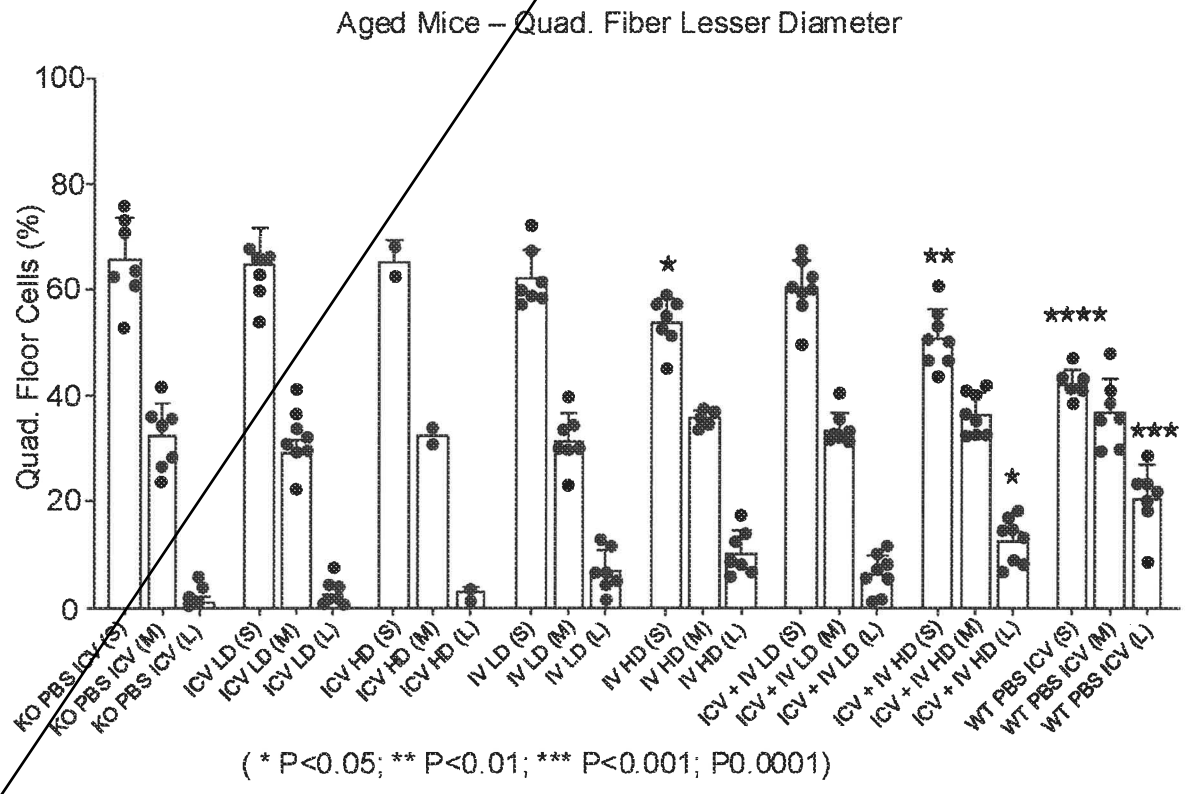

Aged Mice – Quad. Fiber Lesser Diameter
Quad. Floor Cells (%)
100
80
60
40
20
0
*
**
****
***
KO PBS ICV (S)
KO PBS ICV (M)
KO PBS ICV (L)
ICV LD (S)
ICV LD (M)
ICV LD (L)
ICV HD (S)
ICV HD (M)
ICV HD (L)
IV LD (S)
IV LD (M)
IV LD (L)
IV HD (S)
IV HD (M)
IV HD (L)
ICV + IV LD (S)
ICV + IV LD (M)
ICV + IV LD (L)
ICV + IV HD (S)
ICV + IV HD (M)
ICV + IV HD (L)
WT PBS ICV (S)
WT PBS ICV (M)
WT PBS ICV (L)
(* P<0.05; ** P<0.01; *** P<0.001; P0.0001)

FIG.51

FIG. 52

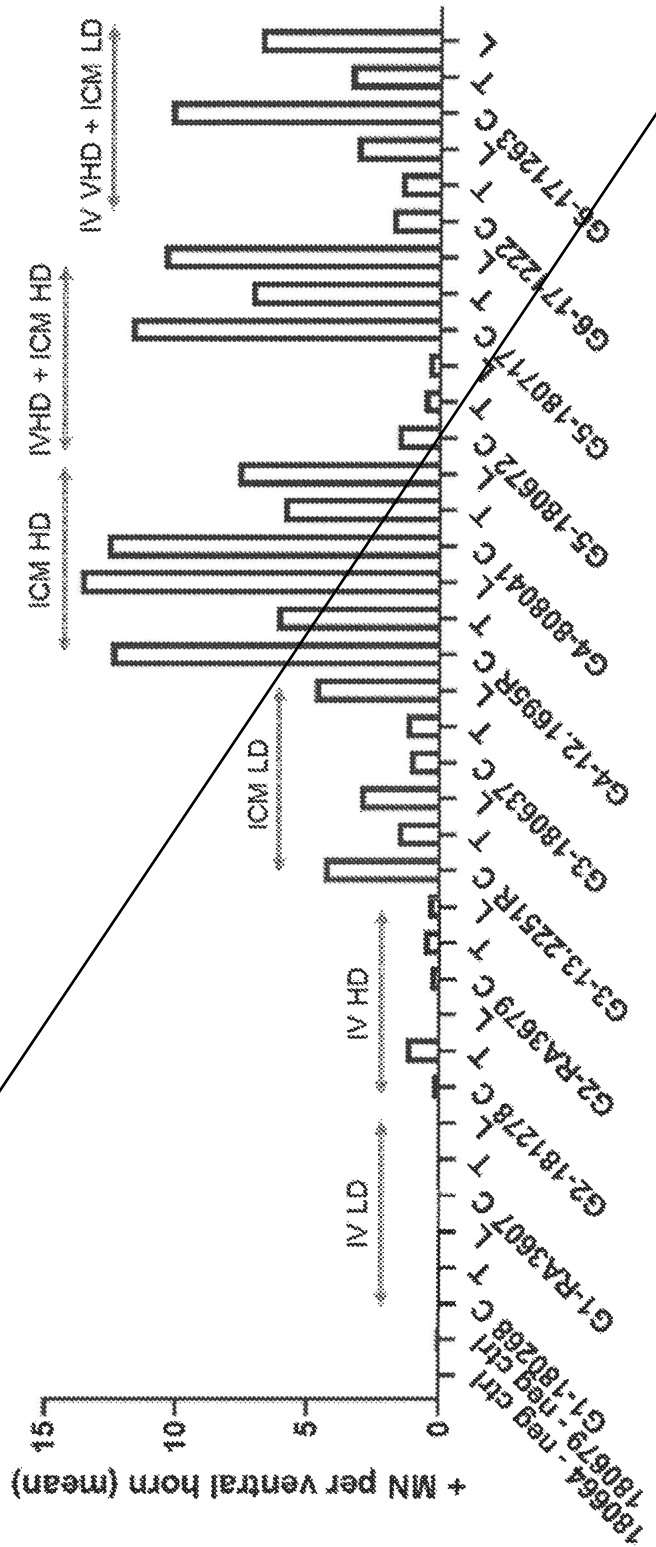

hGAA IHC motor neuron count
+ MN per ventral horn (mean)
0
5
10
15
ICM HD
IVHD + ICM HD
IV VHD + ICM LD
ICM LD
IV HD
IV LD
180664 - neg ctrl
180679 - neg ctrl
G1-180268
G1-RA360T
G2-180278
G2-RA367G
G3-13-225IR
G3-180637
G4-12-1695R
G4-808041
G5-180673
G5-180771
G6-177222
G6-177263

FIG. 1A

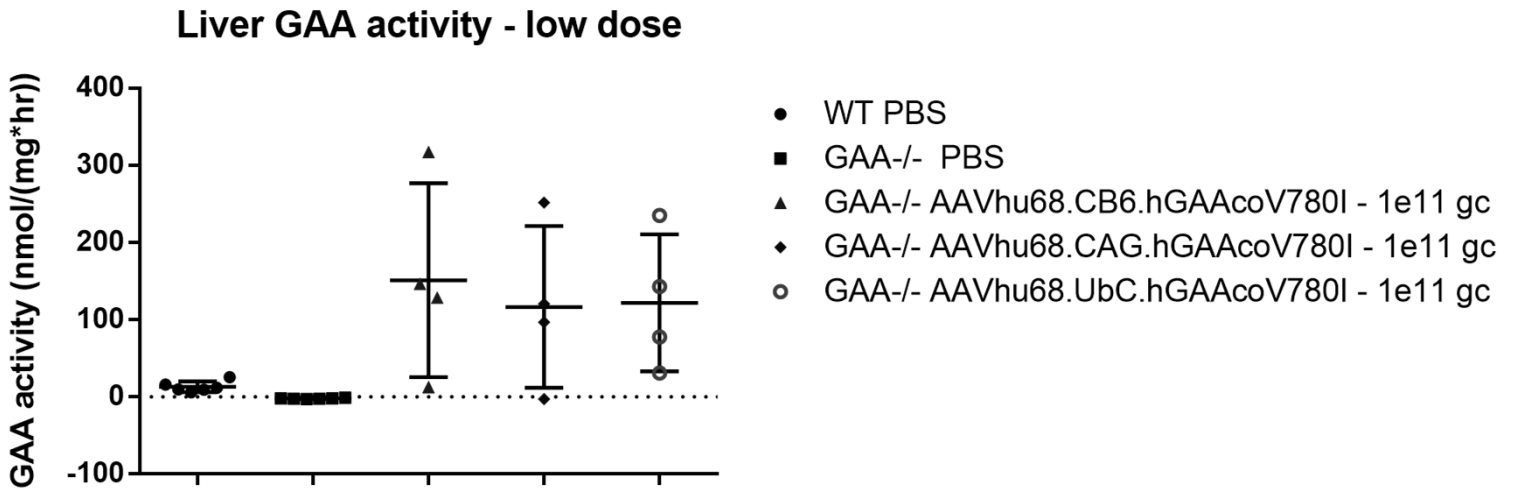
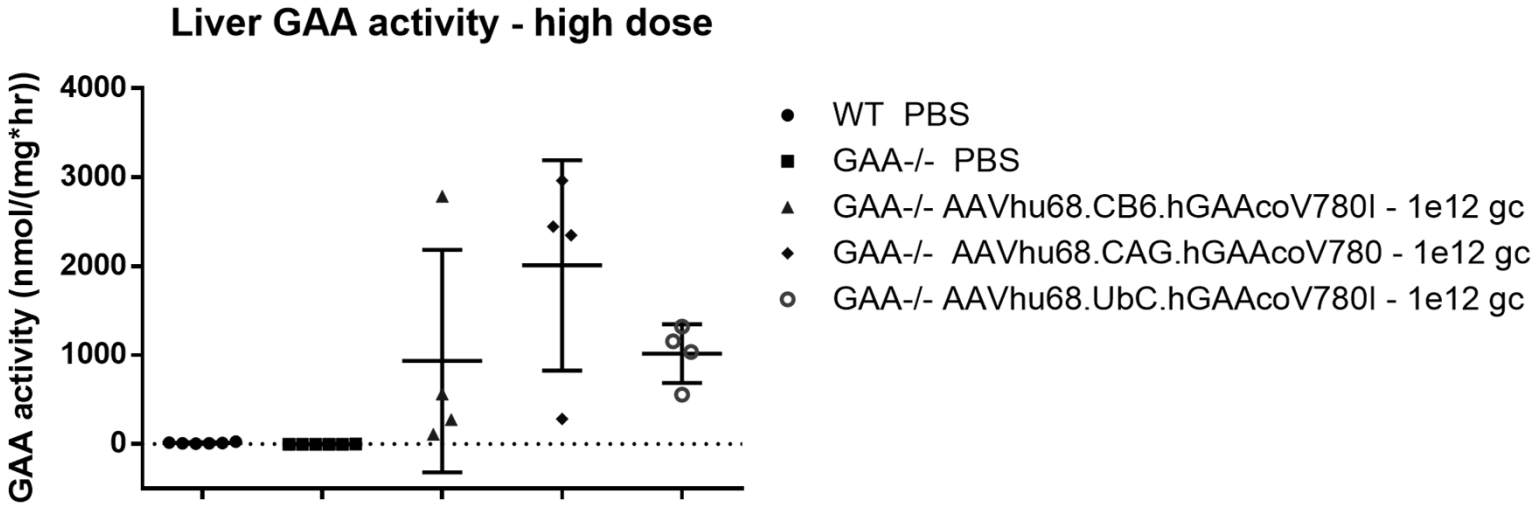

Liver GAA activity - low dose
GAA activity (nmol/(mg*hr))
400
300
200
100
0
-100
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 1B


Liver GAA activity - high dose
GAA activity (nmol/(mg*hr))
4000
3000
2000
1000
0
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780 - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

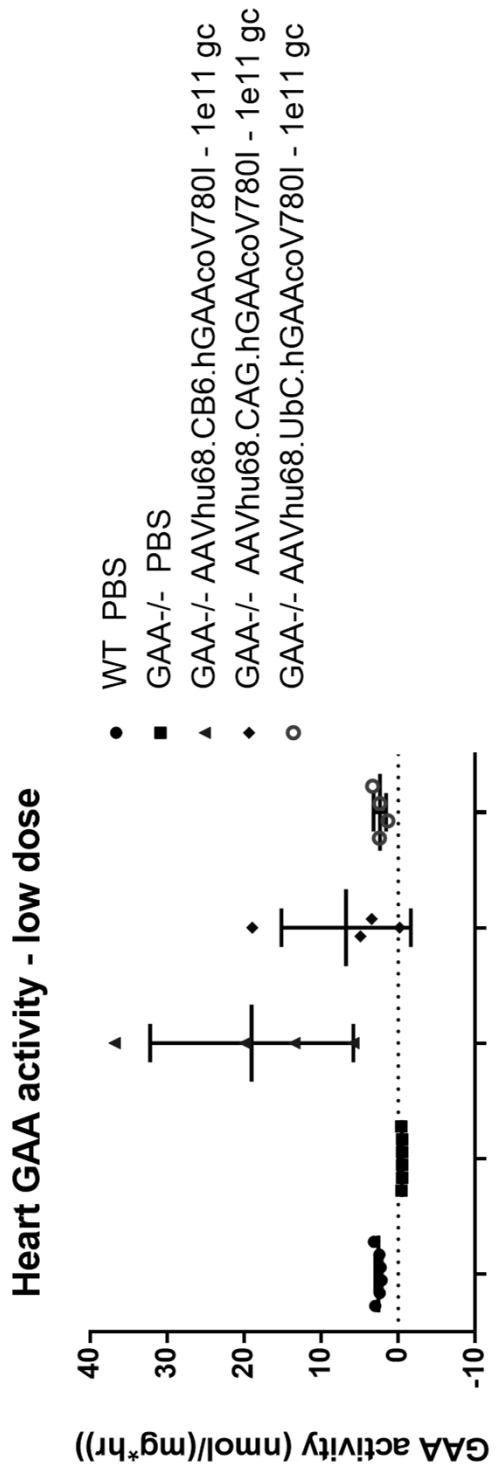

Heart GAA activity - low dose
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc
GAA activity (nmol/(mg*hr))
40
30
20
10
0
-10

FIG. 2A

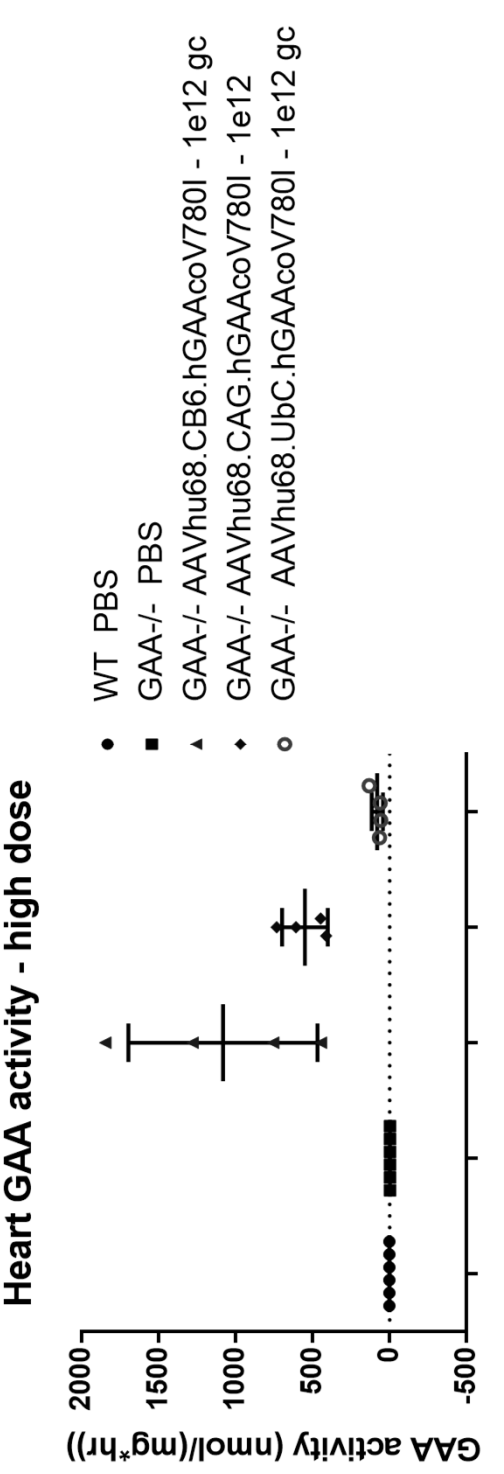

Heart GAA activity - high dose
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e12
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc
GAA activity (nmol/(mg*hr))
2000
1500
1000
500
0
-500

FIG. 2B

FIG. 3A

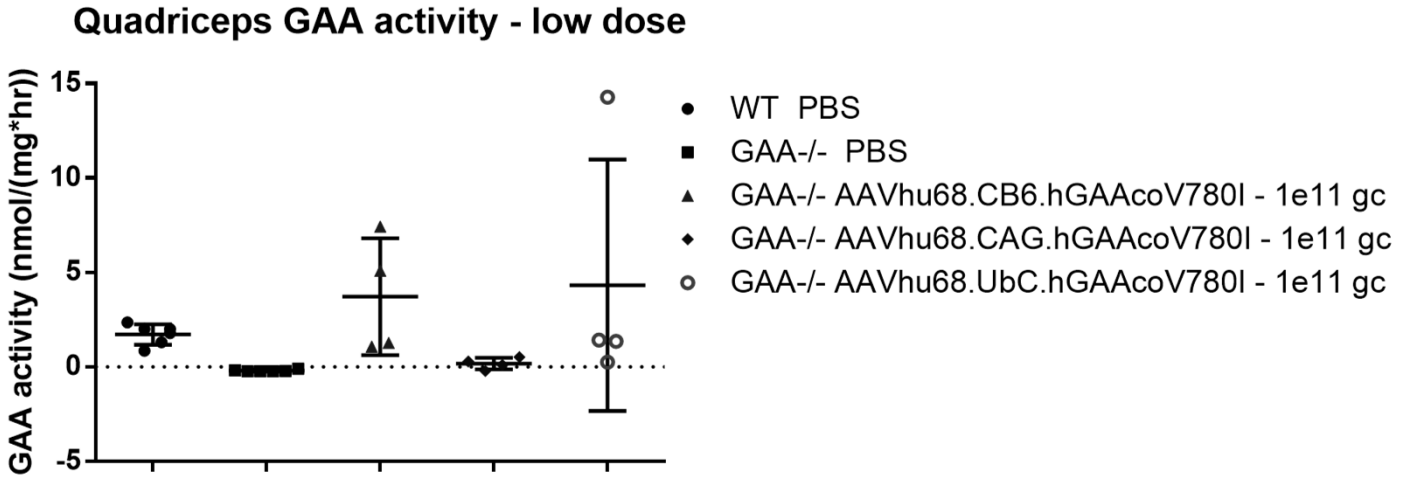

Quadriceps GAA activity - low dose
GAA activity (nmol/(mg*hr))
15
10
5
0
-5
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 3B

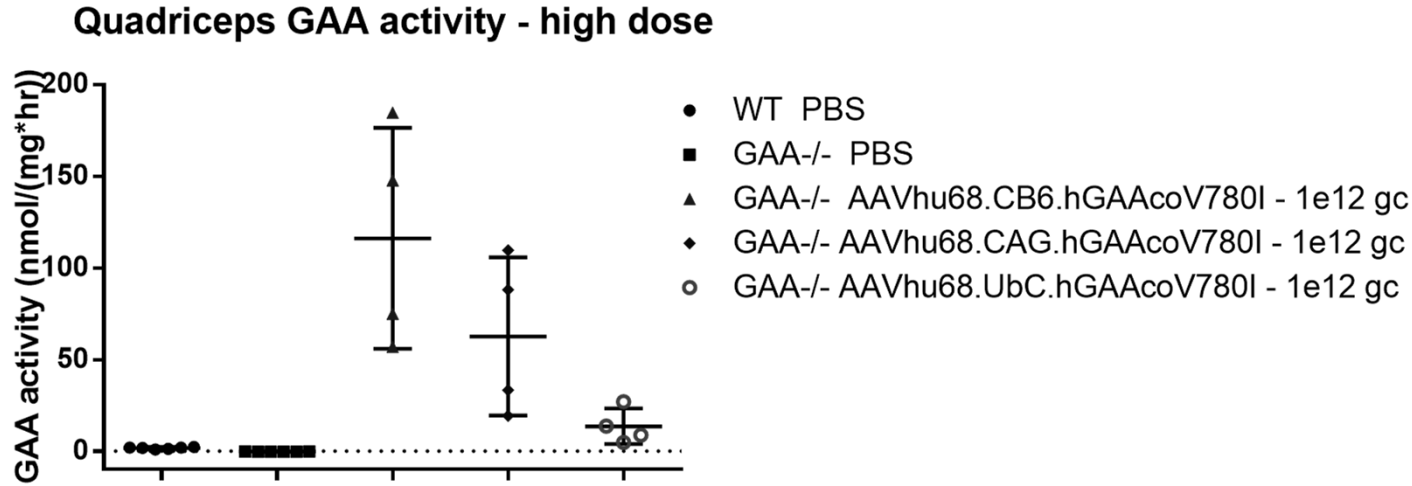

Quadriceps GAA activity - high dose
GAA activity (nmol/(mg*hr))
200
150
100
50
0
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

FIG. 4A

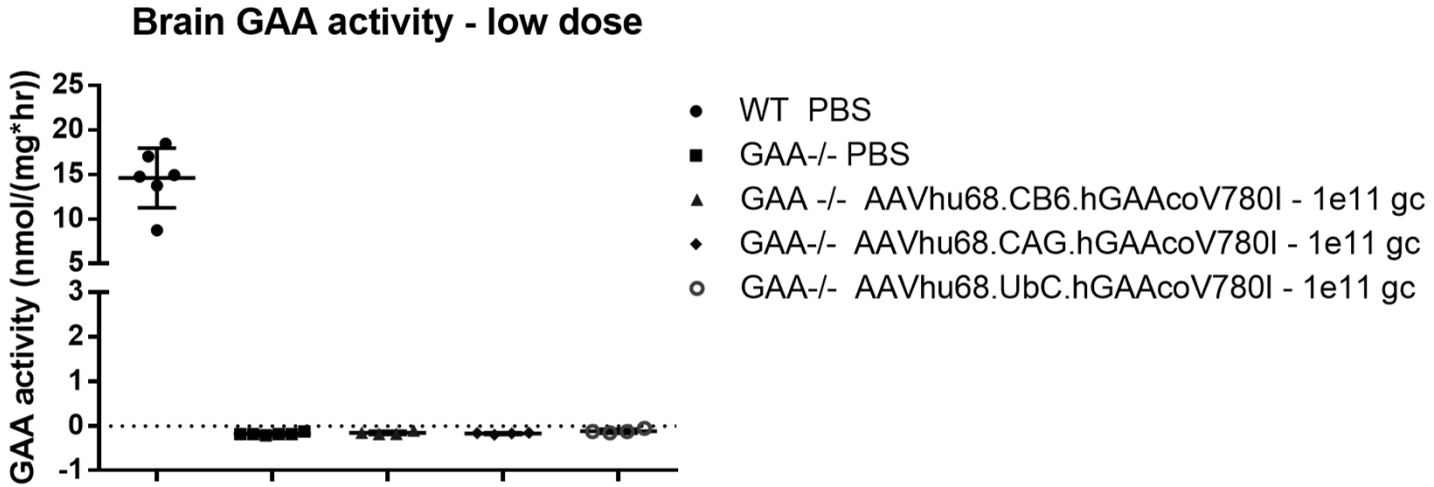

Brain GAA activity - low dose
GAA activity (nmol/(mg*hr))
25
20
15
10
5
3
2
1
0
-1
WT PBS
GAA-/- PBS
GAA -/- AAVhu68.CB6.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.CAG.hGAAcoV780I - 1e11 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e11 gc

FIG. 4B

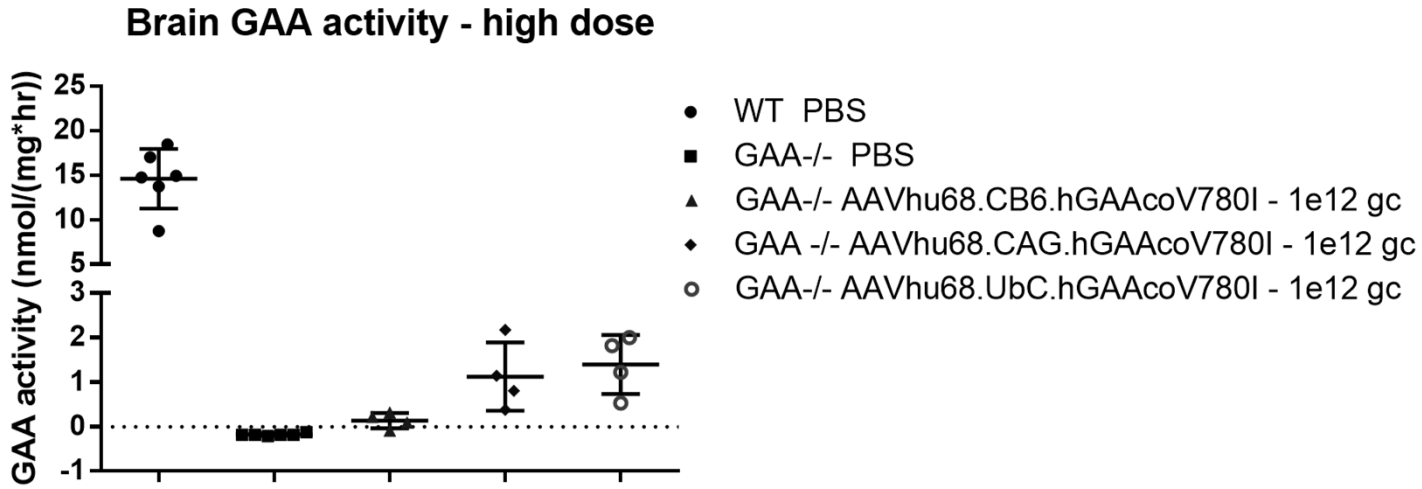

Brain GAA activity - high dose
GAA activity (nmol/(mg*hr))
25
20
15
10
5
3
2
1
0
-1
WT PBS
GAA-/- PBS
GAA-/- AAVhu68.CB6.hGAAcoV780I - 1e12 gc
GAA -/- AAVhu68.CAG.hGAAcoV780I - 1e12 gc
GAA-/- AAVhu68.UbC.hGAAcoV780I - 1e12 gc

FIG. 5A

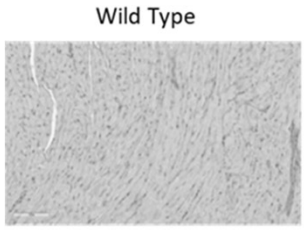


FIG. 5B

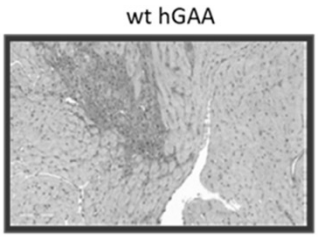


FIG. 5C

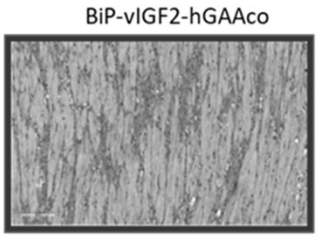


FIG. 5D

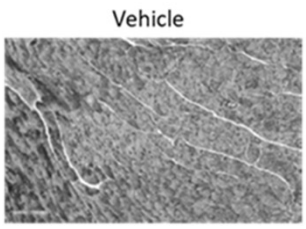


FIG. 5E

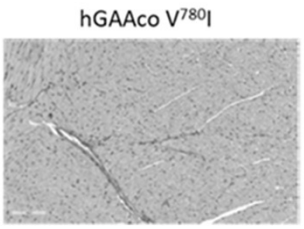


FIG. 5F

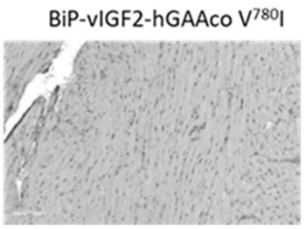


FIG. 5G

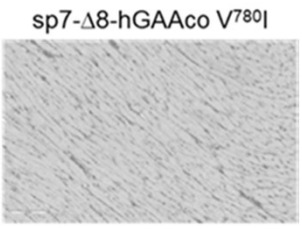


FIG. 5H

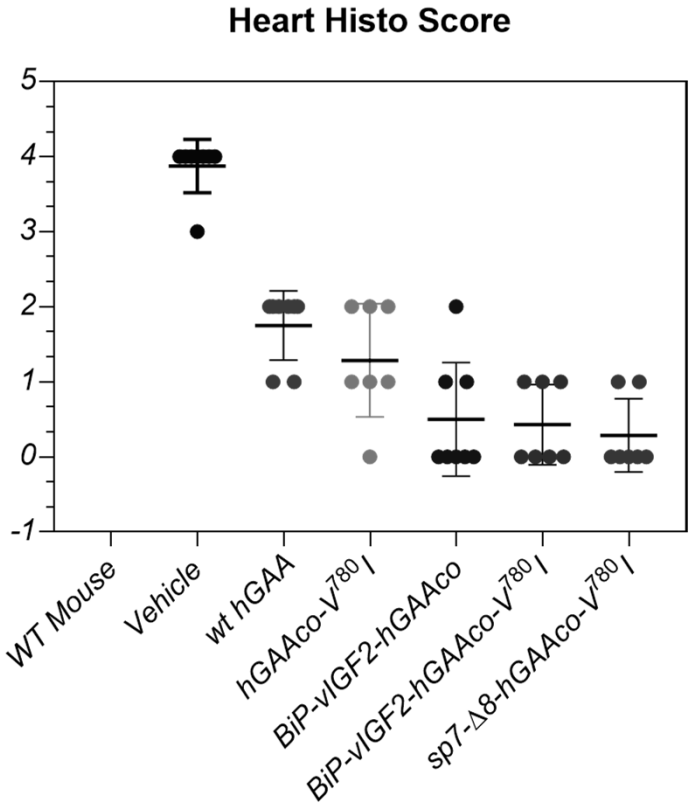


FIG. 6A

+/+ PBS

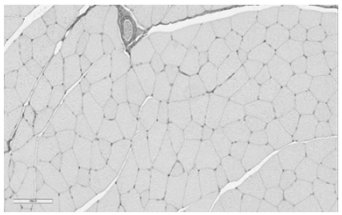


FIG. 6B

-/- hGAA

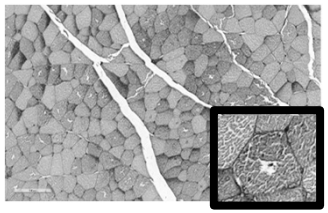


FIG. 6C

-/- BiP.vIGF2.hGAAco

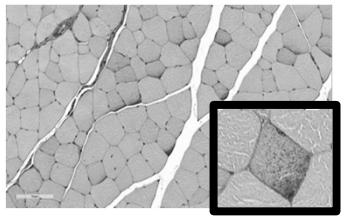


FIG. 6D

-/- PBS

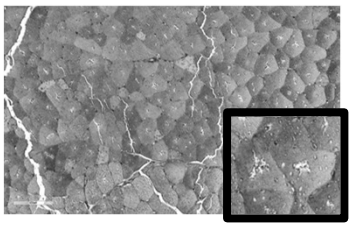


FIG. 6E

-/- hGAAcoV780I

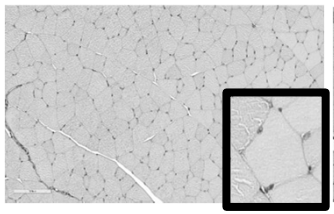


FIG. 6F

-/- Sp7.Δ8.hGAAcoV780I

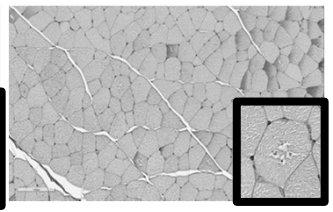


FIG. 6G

-/- BiP.vIGF2.hGAAcoV780I

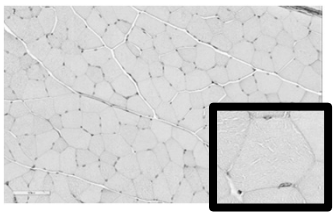


FIG. 6H

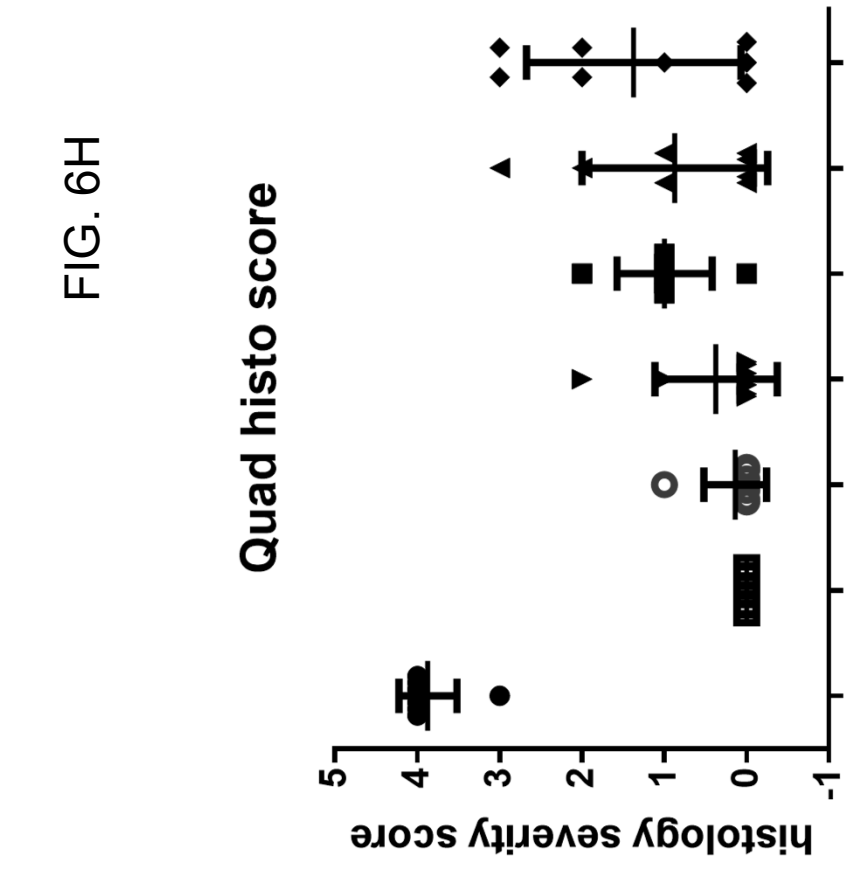

Quad histo score
histology severity score
5
4
3
2
1
0
-1
-/- PBS
+/+ PBS
hGAAcoV780I
BiP.vIGF2.hGAAcoV780I
sp7-delta8.hGAAcoV780I
hGAA
BiP.vIGF2.hGAAco

FIG. 7A

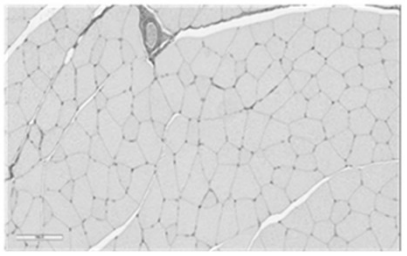


FIG. 7B

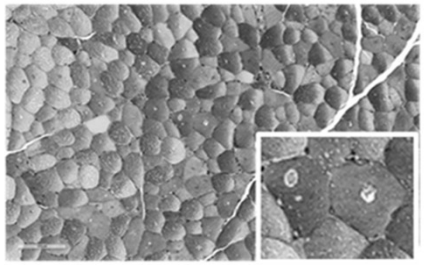
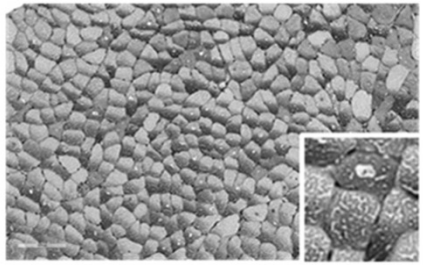


FIG. 7C



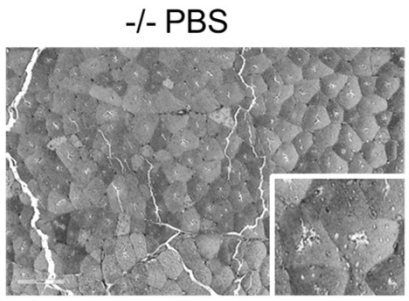


FIG. 7D

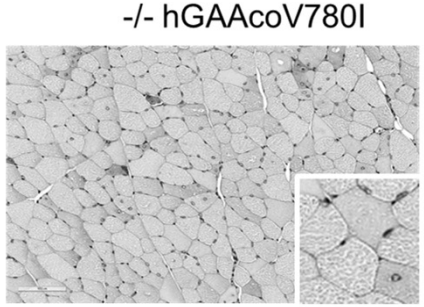


FIG. 7E

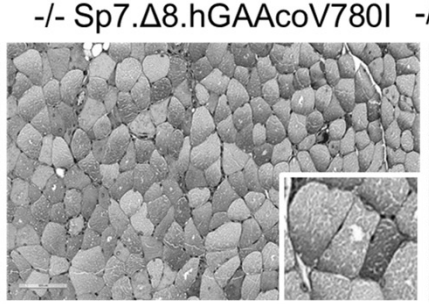


FIG. 7F

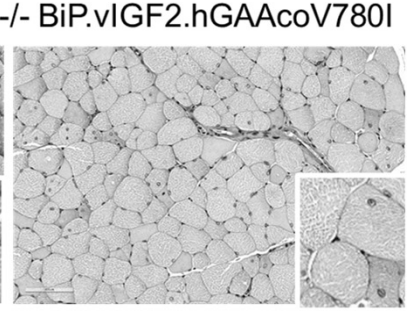


FIG. 7G

FIG. 7H

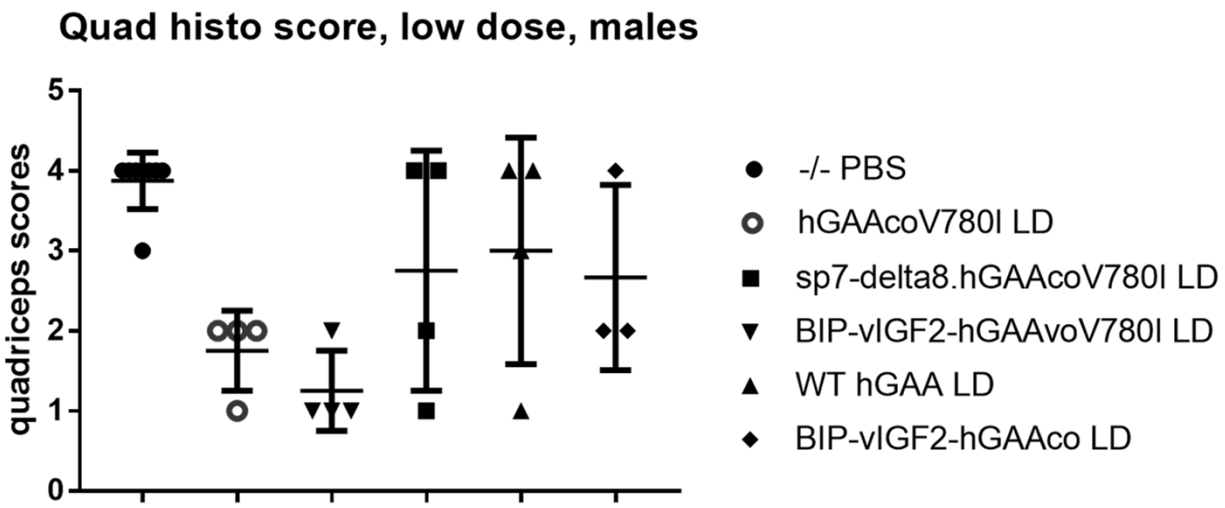

Quad histo score, low dose, males
quadriceps scores
5
4
3
2
1
0
-/- PBS
hGAAcoV780I LD
sp7-delta8.hGAAcoV780I LD
BIP-vIGF2-hGAAvoV780I LD
WT hGAA LD
BIP-vIGF2-hGAAco LD

FIG. 8

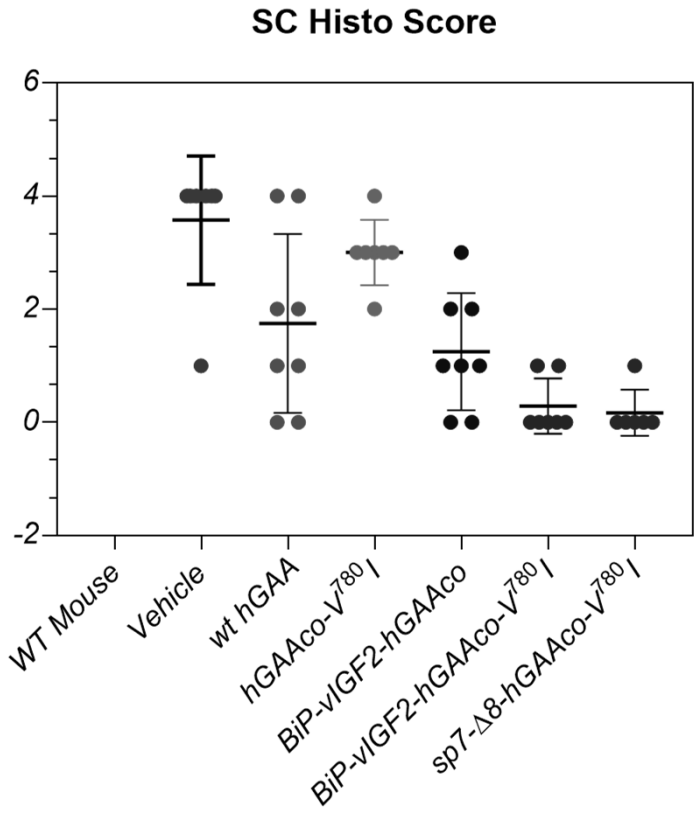

SC Histo Score
6
4
2
0
-2
WT Mouse
Vehicle
wt hGAA
hGAAco-V780I
BiP-vIGF2-hGAAco
BiP-vIGF2-hGAAco-V780I
sp7-Δ8-hGAAco-V780I

FIG. 9A

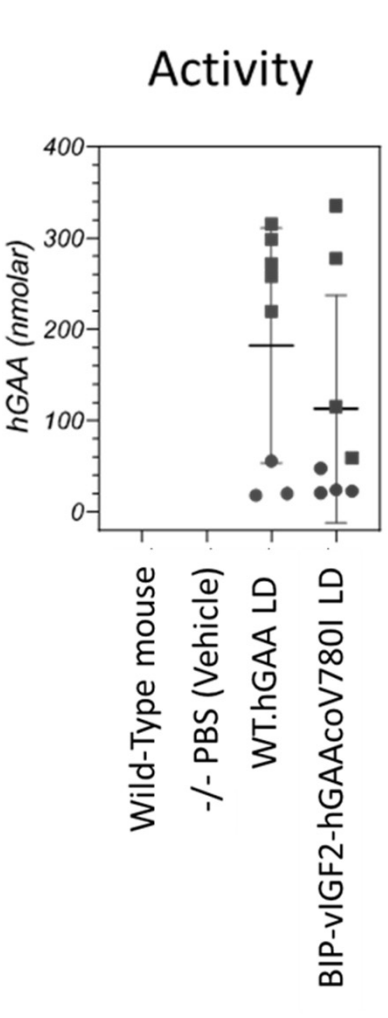


FIG. 9B

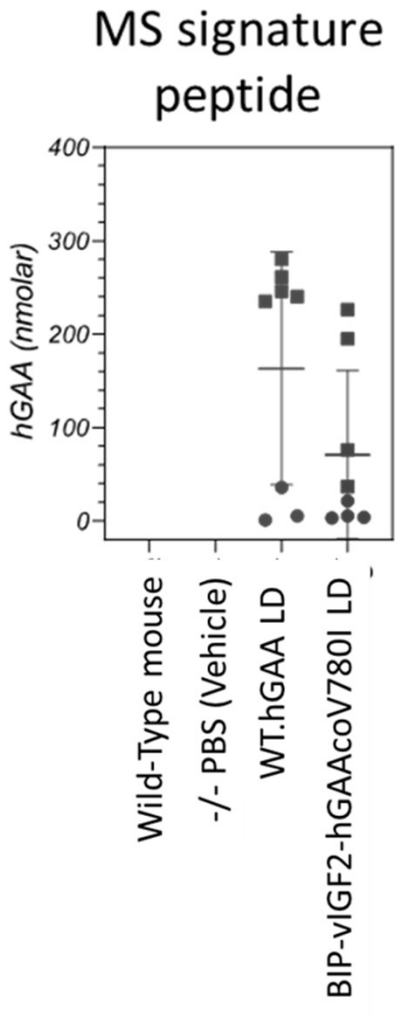


FIG. 9C

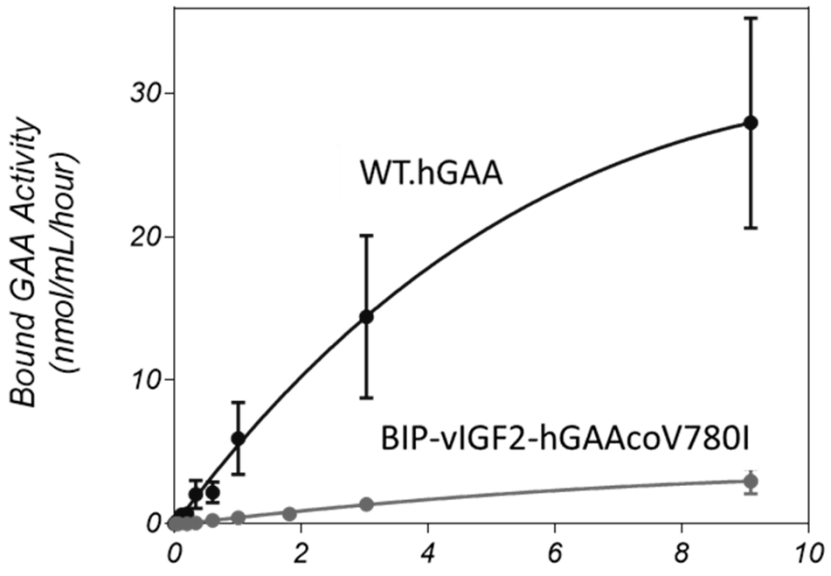


FIG. 10

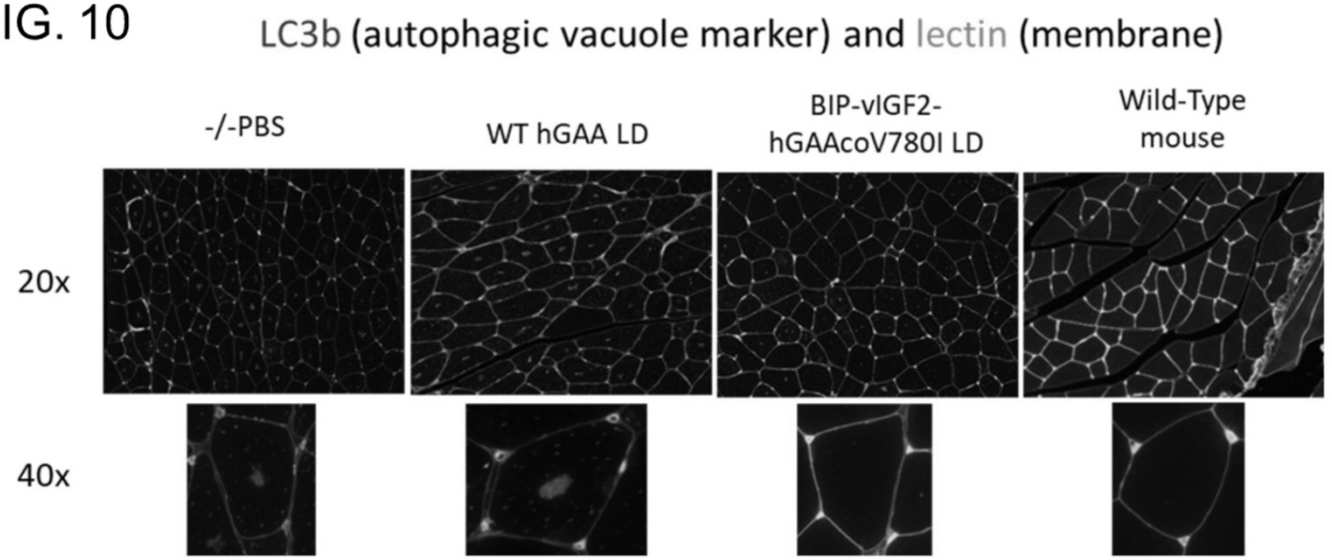


FIG. 11

BiP-vIGF2.hGAAcoV780I.4xmiR183

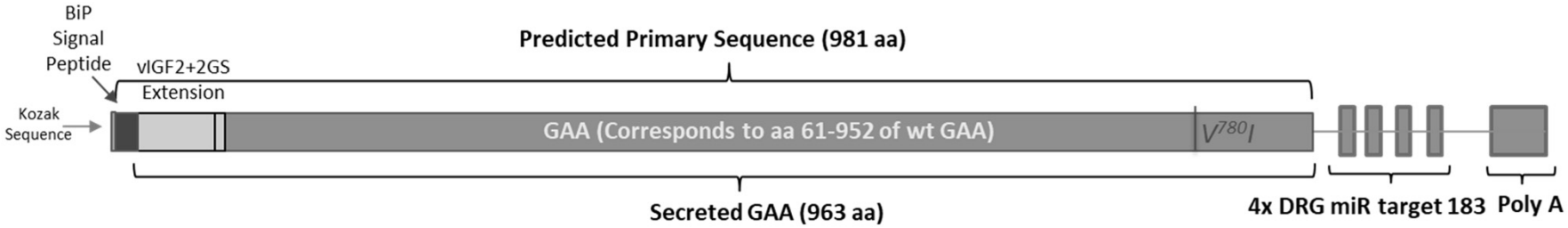


FIG. 12

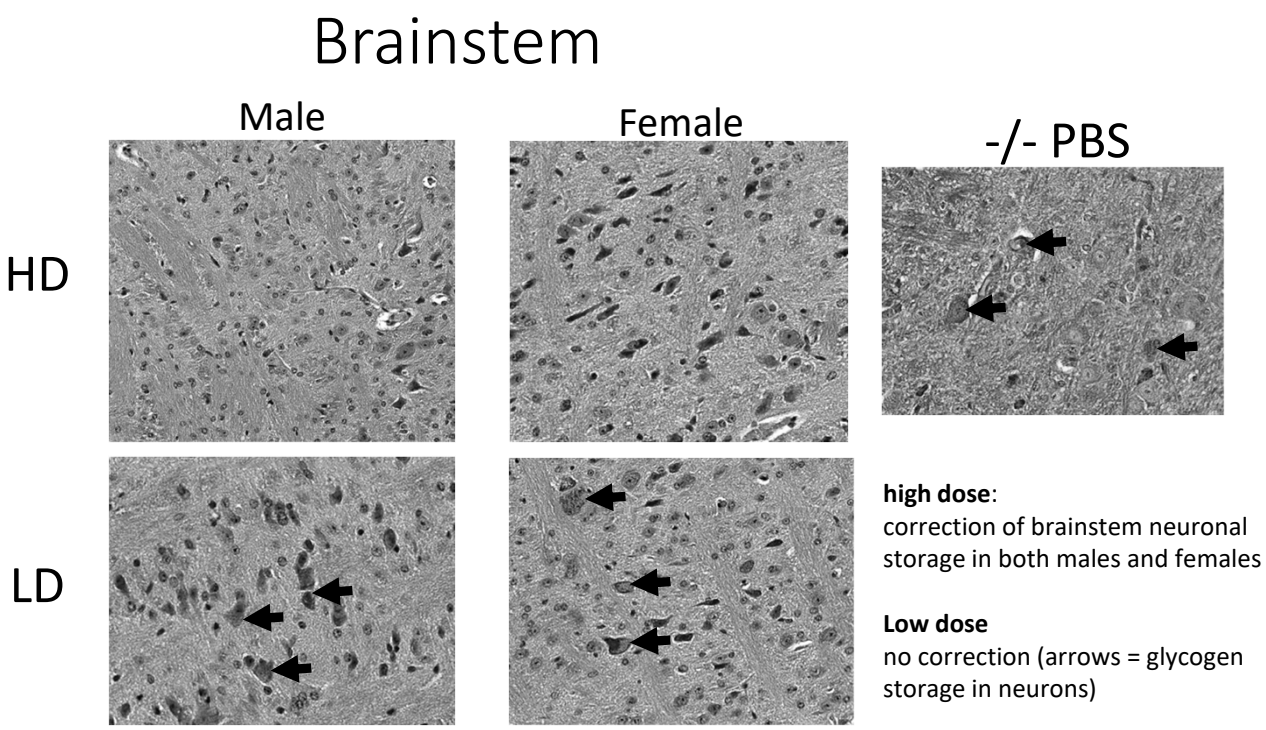

Brainstem
Male
Female
-/- PBS
HD
LD
high dose:
correction of brainstem neuronal storage in both males and females
Low dose
no correction (arrows = glycogen storage in neurons)

FIG. 13

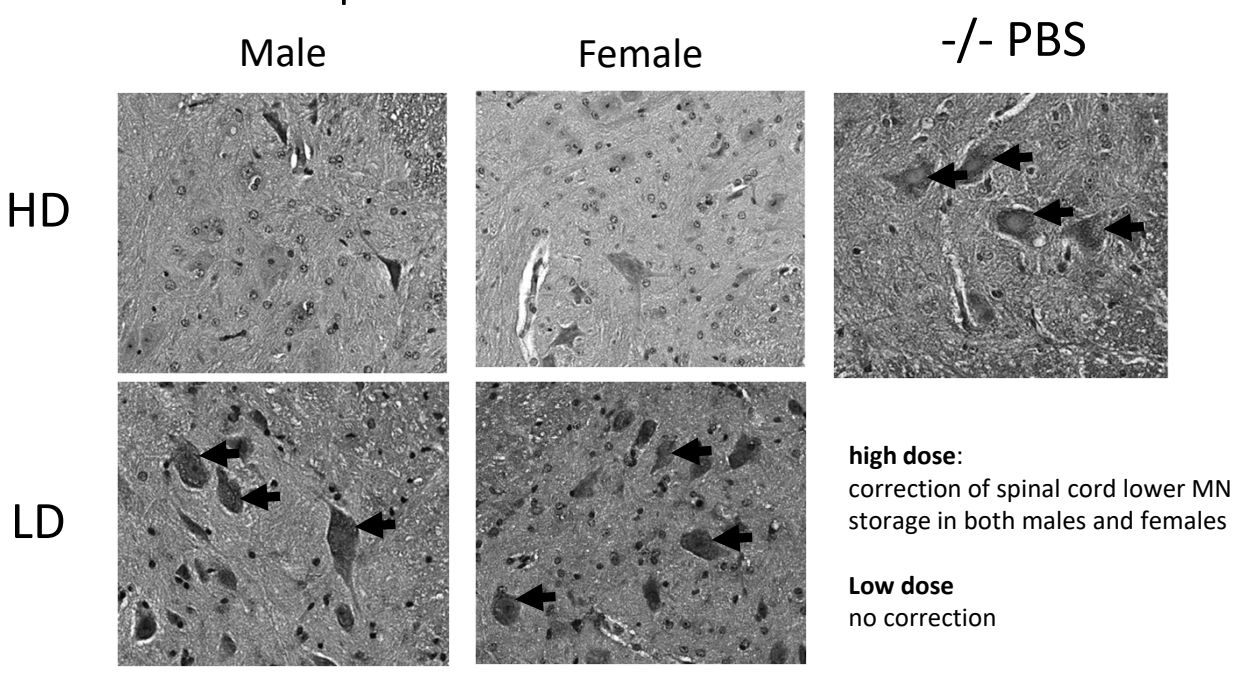

Spinal cord
Male
Female
-/- PBS
HD
LD
high dose:
correction of spinal cord lower MN storage in both males and females
Low dose
no correction

FIG. 14

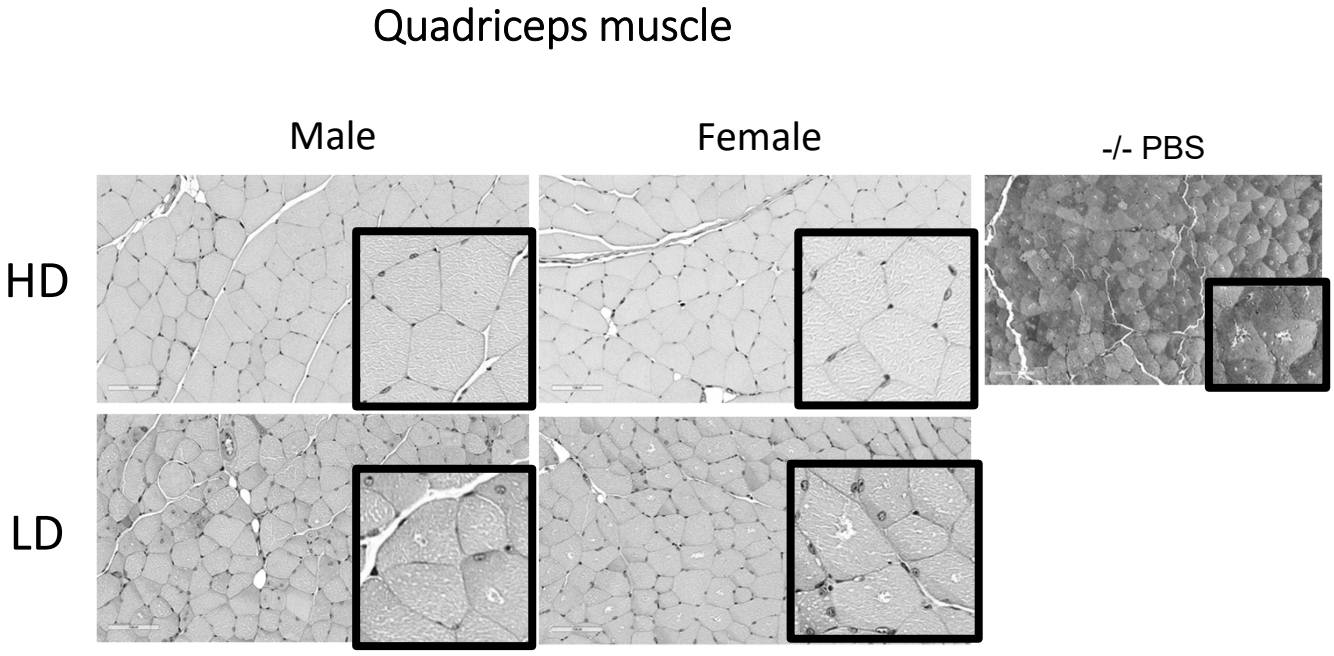
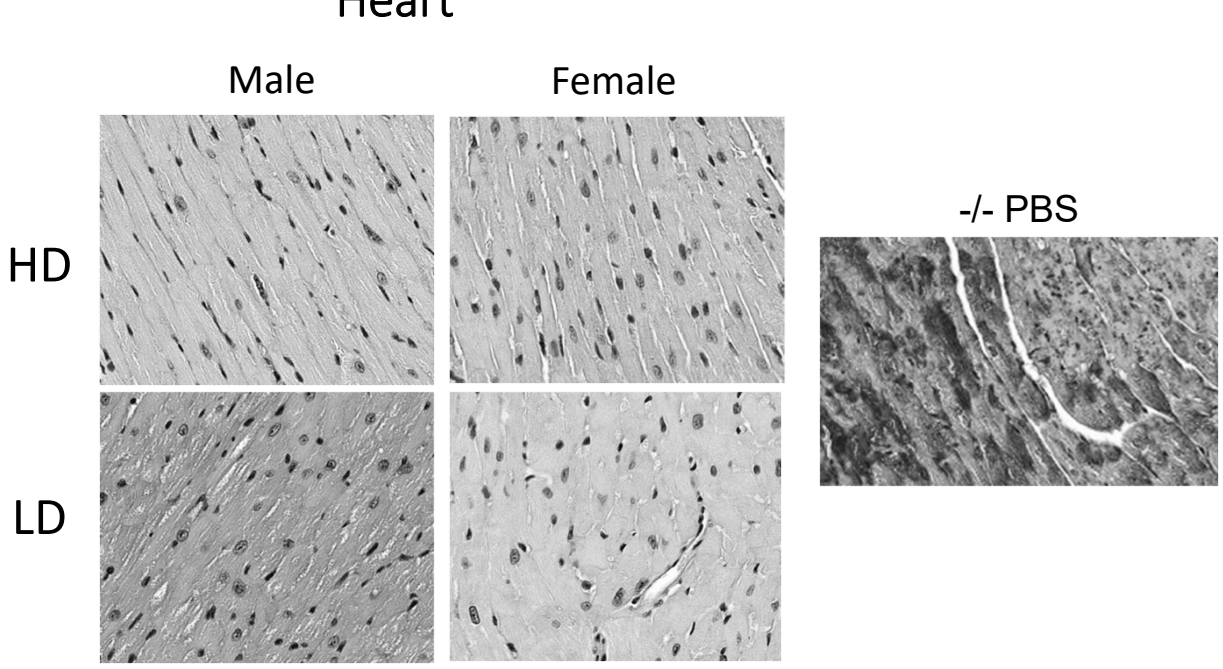

Quadriceps muscle
Male
Female
-/- PBS
HD
LD

FIG. 15


Heart
Male
Female
-/- PBS
HD
LD

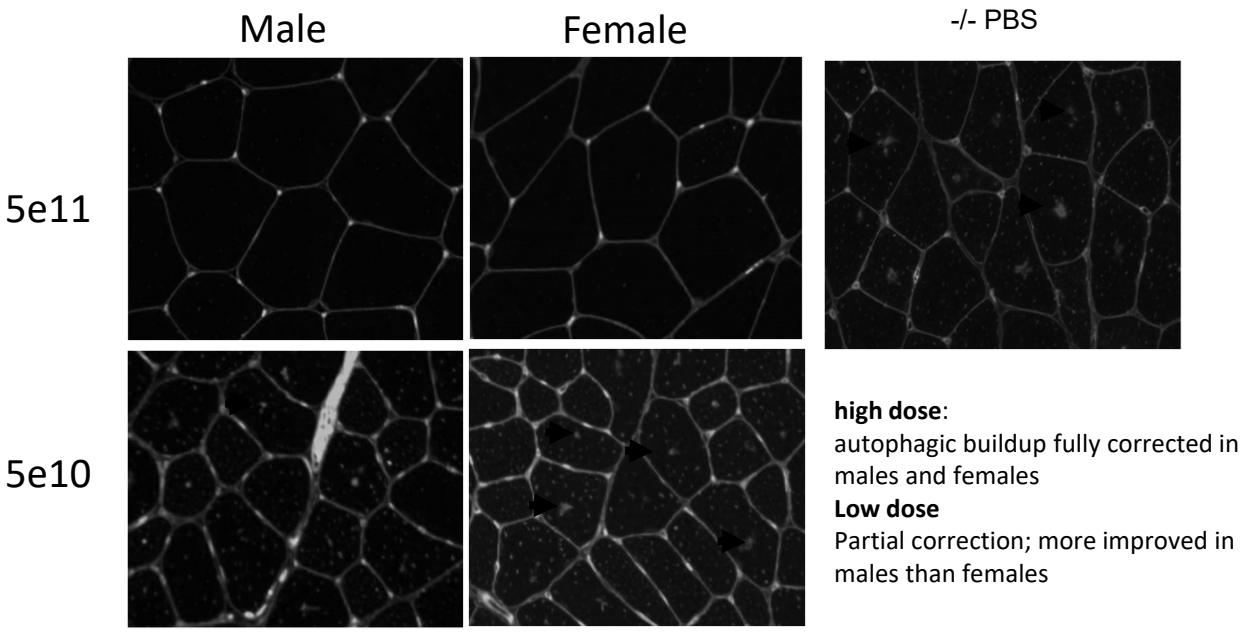

Male
Female
-/- PBS
5e11
5e10
high dose:
autophagic buildup fully corrected in males and females
Low dose
Partial correction; more improved in males than females

FIG. 16

FIG. 17

Cervical DRG hGAA IHC

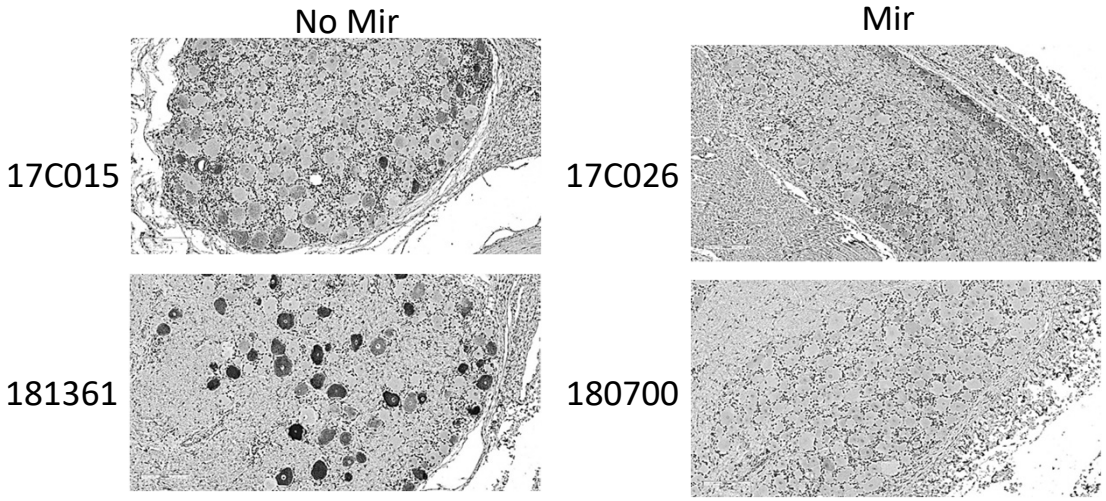


FIG. 18

Lumbar DRG hGAA IHC

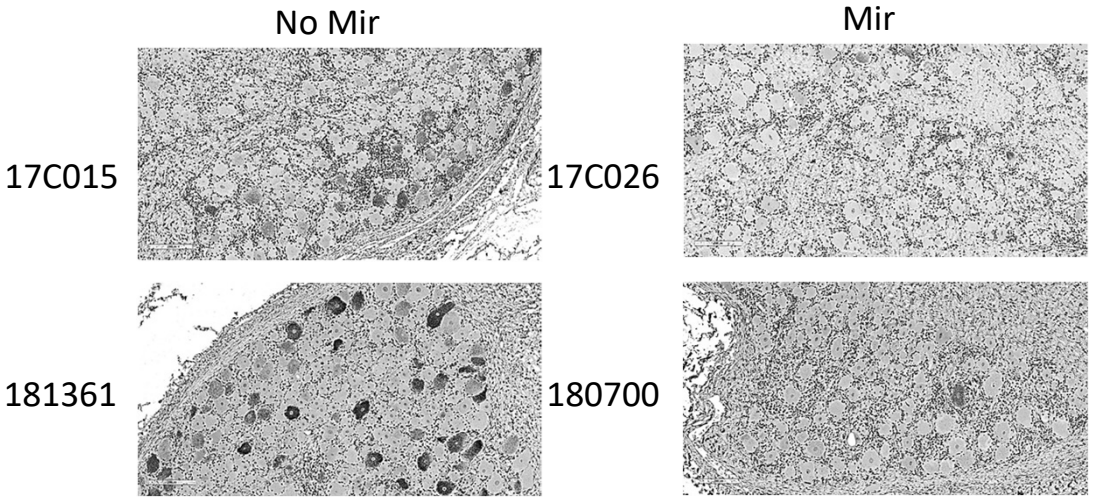


FIG. 19

cervical spinal cord
lower MN hGAA IHC

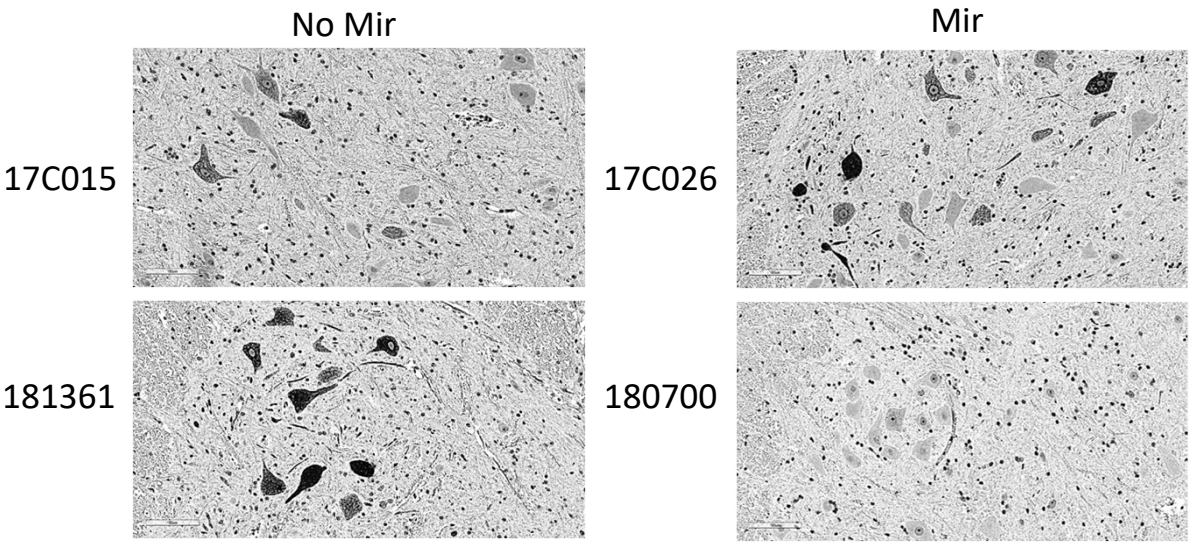


FIG. 20

heart hGAA IHC

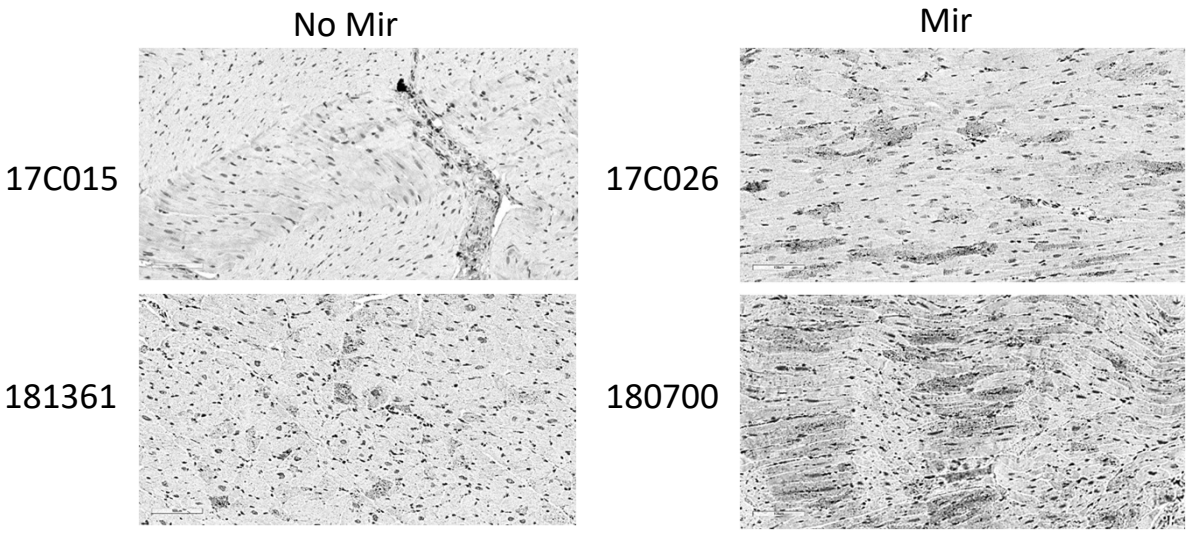


FIG. 21A

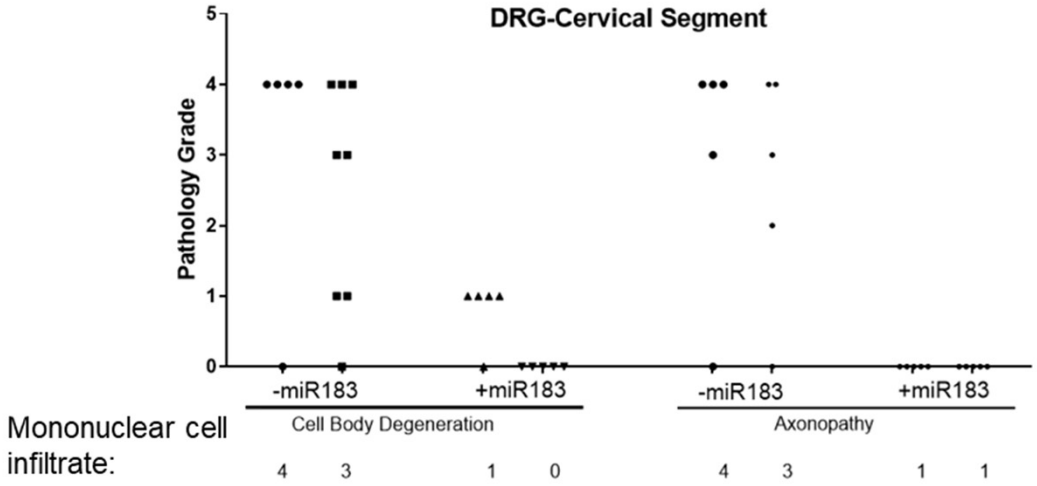


FIG. 21B

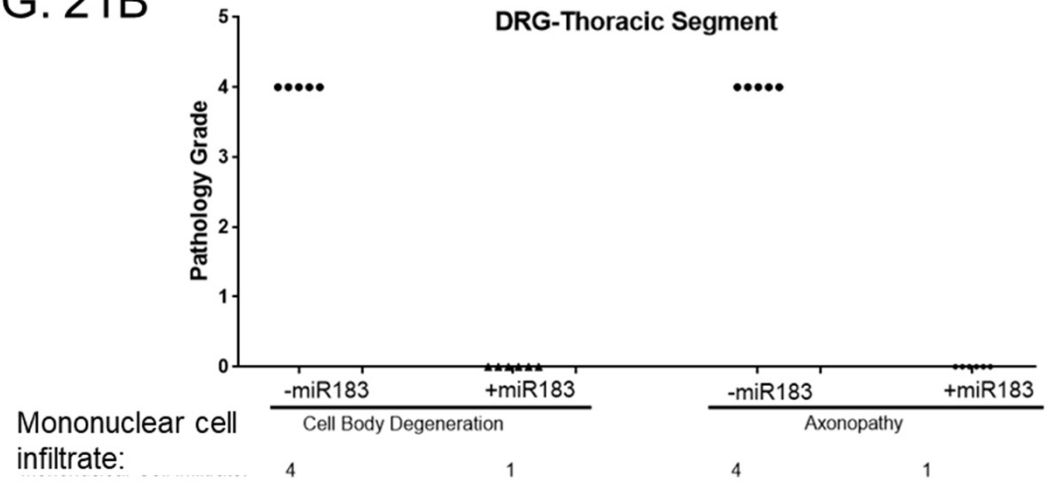


FIG. 21C

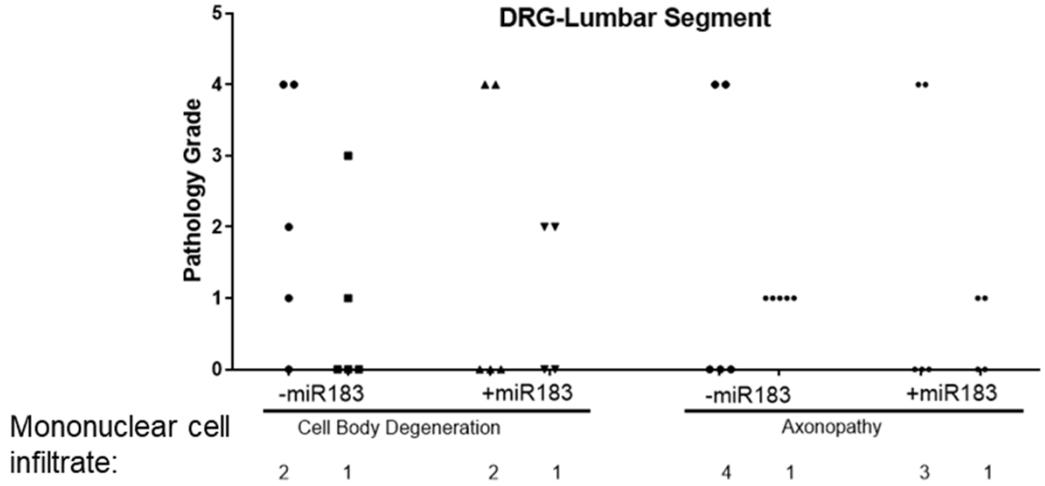


FIG. 22A

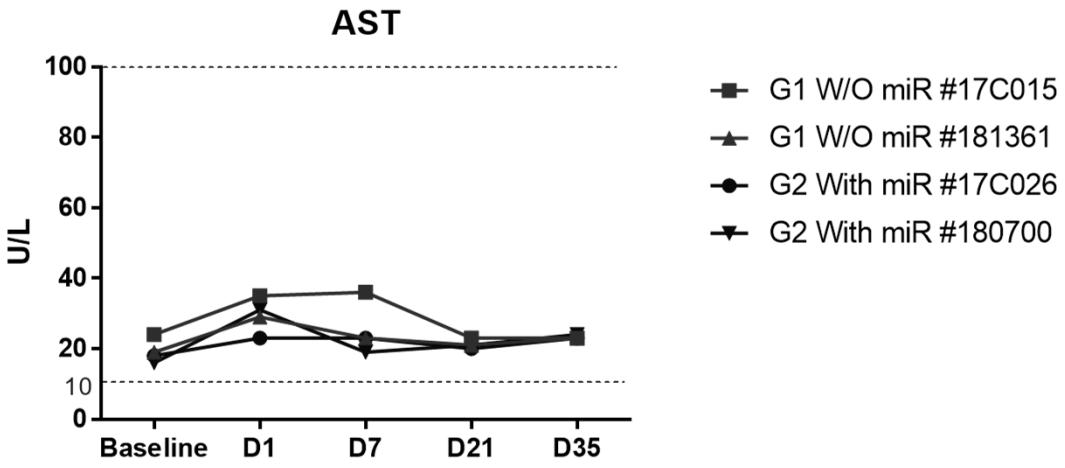


FIG. 22B

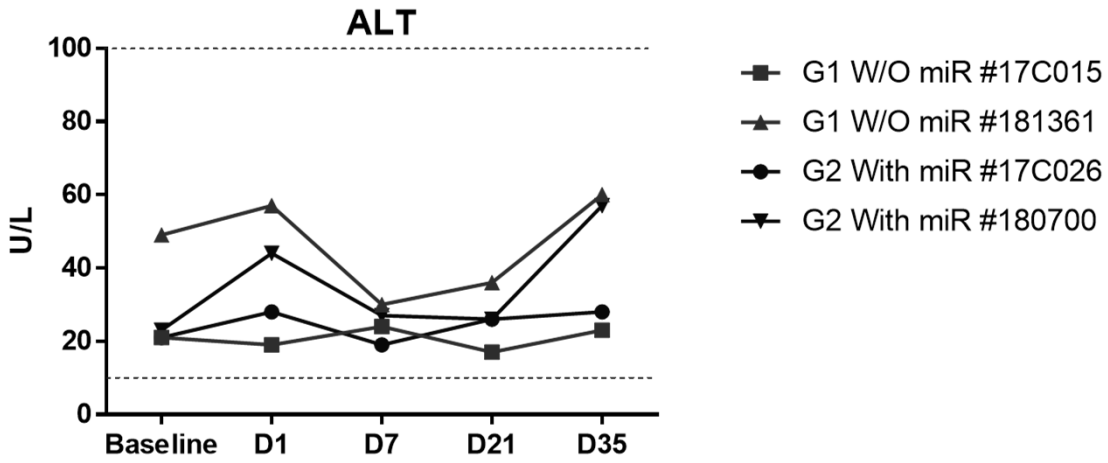


FIG. 22C

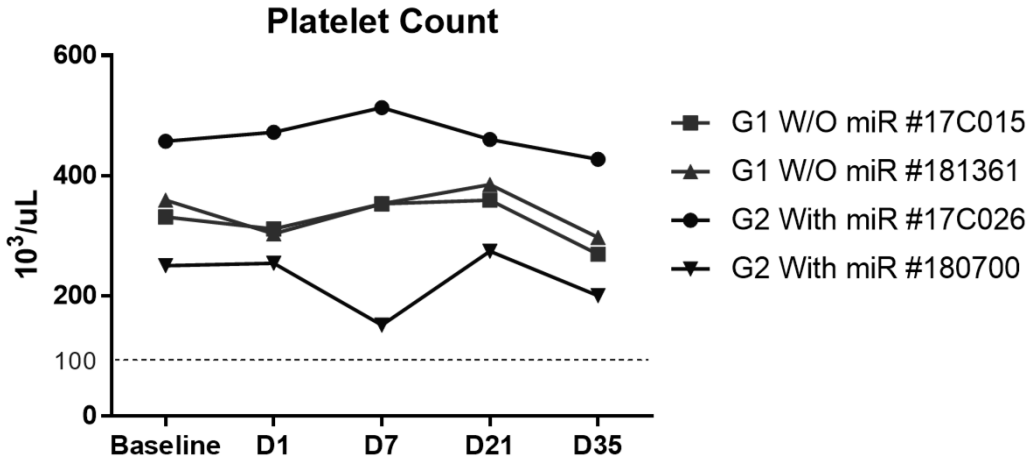
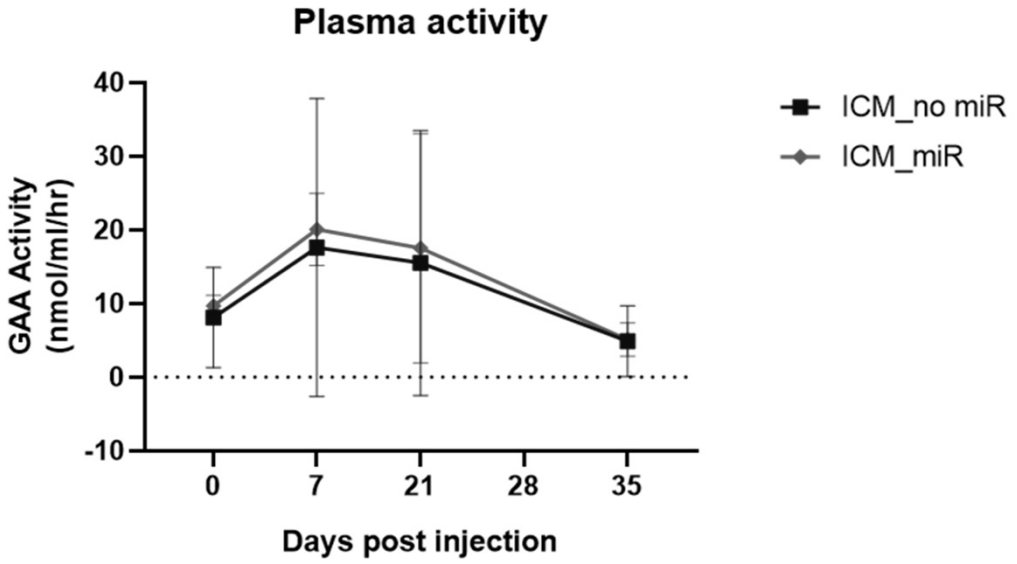


FIG. 23


Plasma activity
GAA Activity (nmol/ml/hr)
40
30
20
10
0
-10
0
7
21
28
35
Days post injection
ICM_no miR
ICM_miR

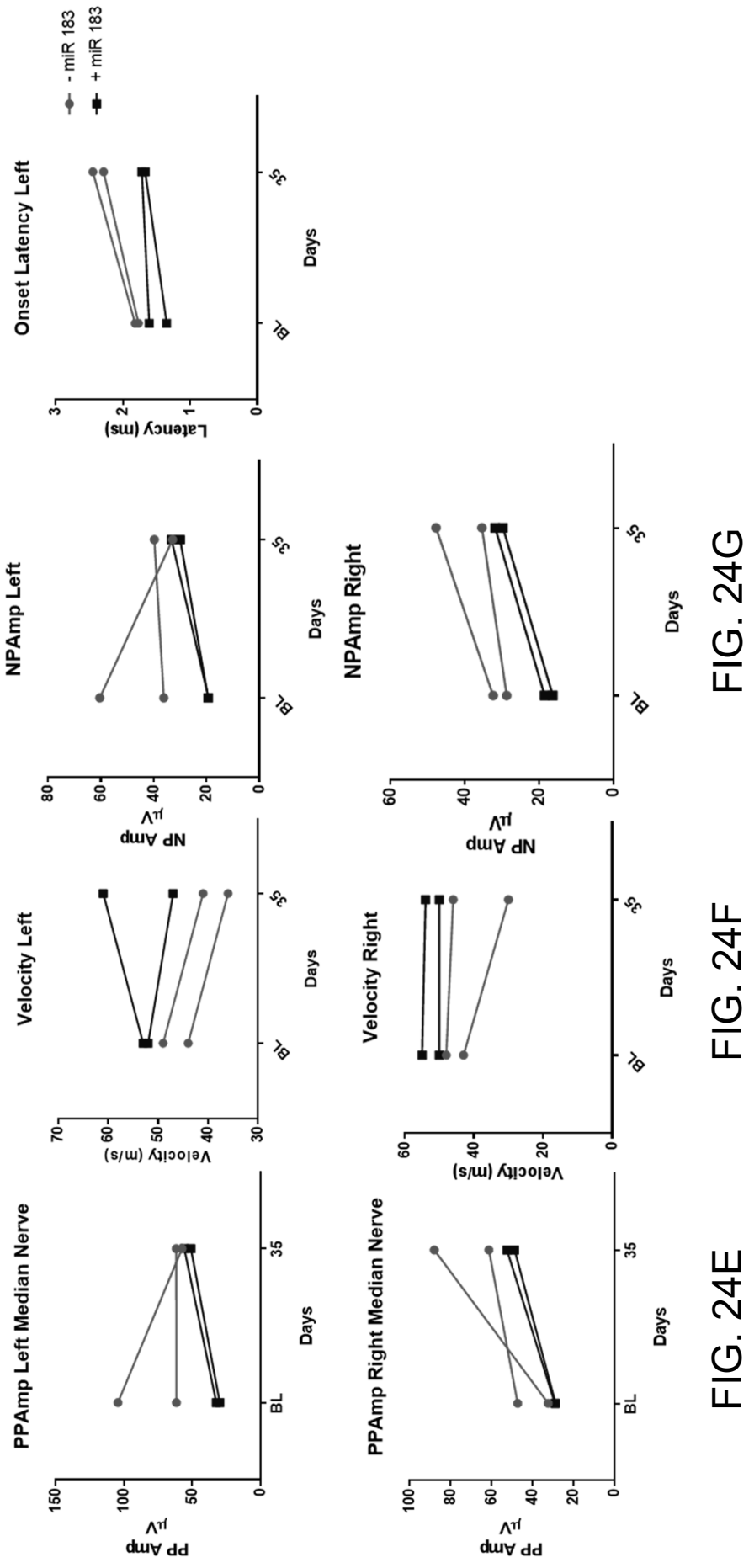


FIG. 24A FIG. 24B FIG. 24C FIG. 24D

FIG. 24E FIG. 24F FIG. 24G

FIG. 25A

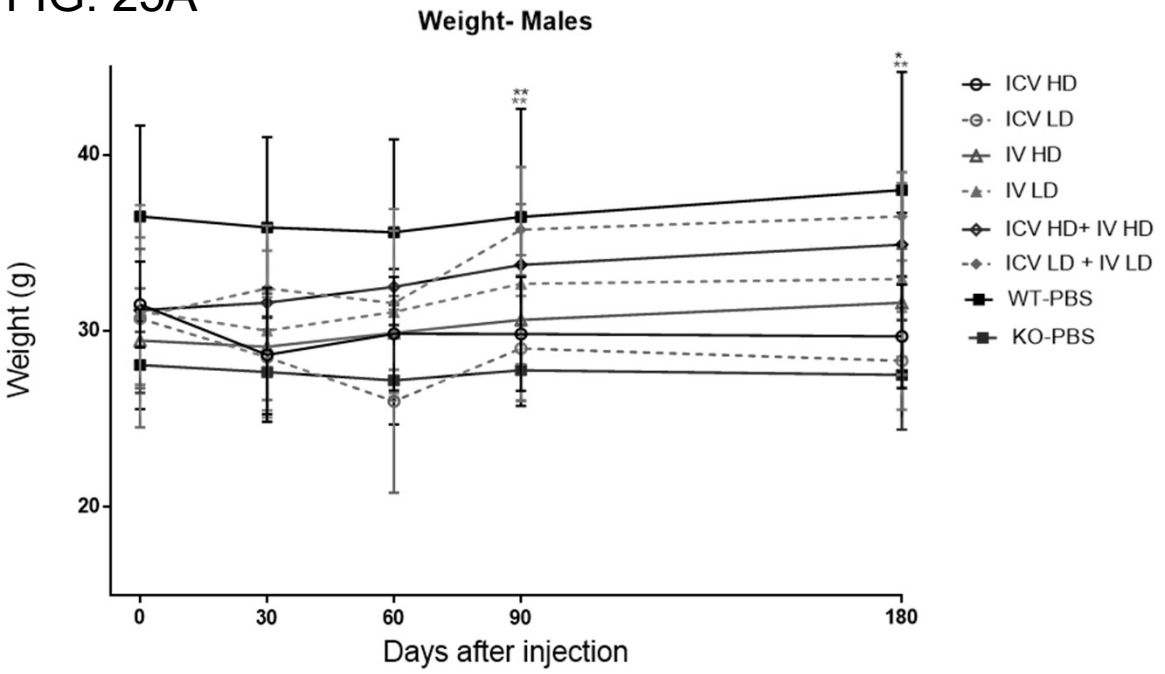


FIG. 25B

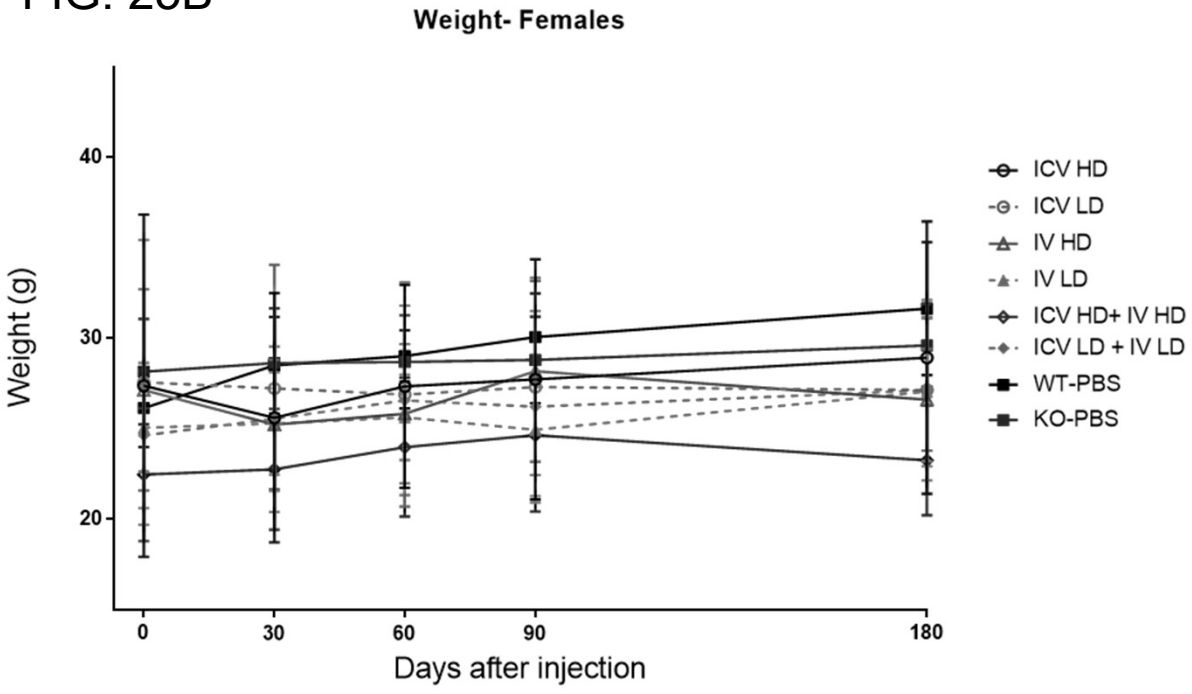


FIG. 26

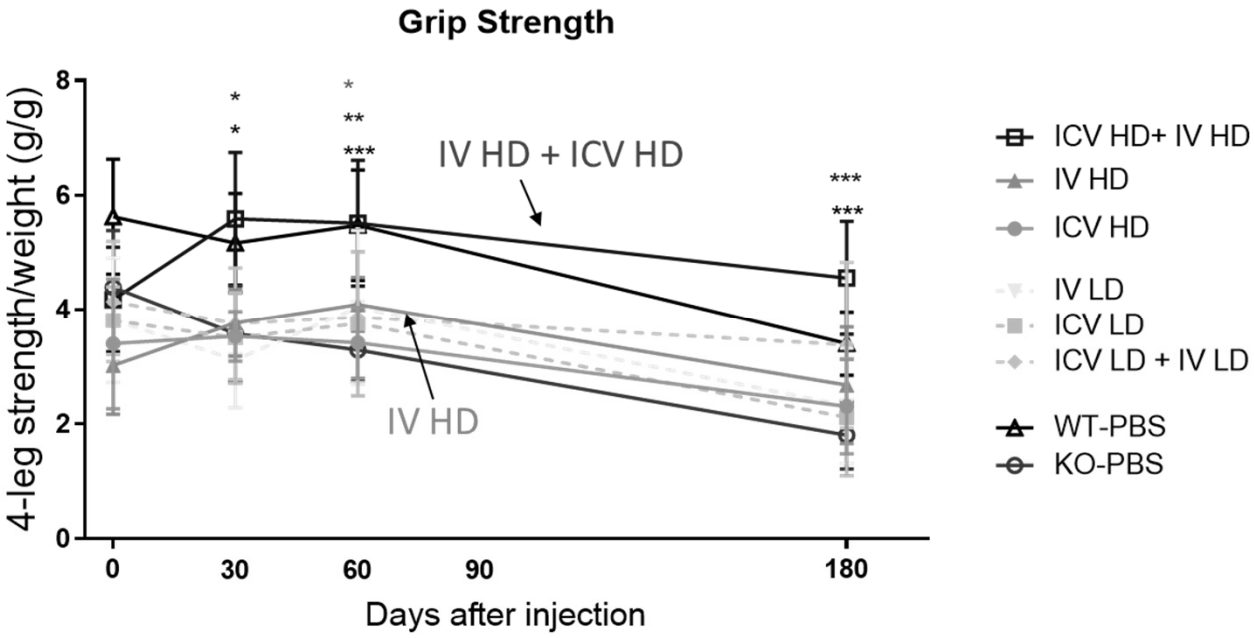

Grip Strength
4-leg strength/weight (g/g)
Days after injection
IV HD + ICV HD
IV HD
ICV HD+ IV HD
IV HD
ICV HD
IV LD
ICV LD
ICV LD + IV LD
WT-PBS
KO-PBS

FIG. 27

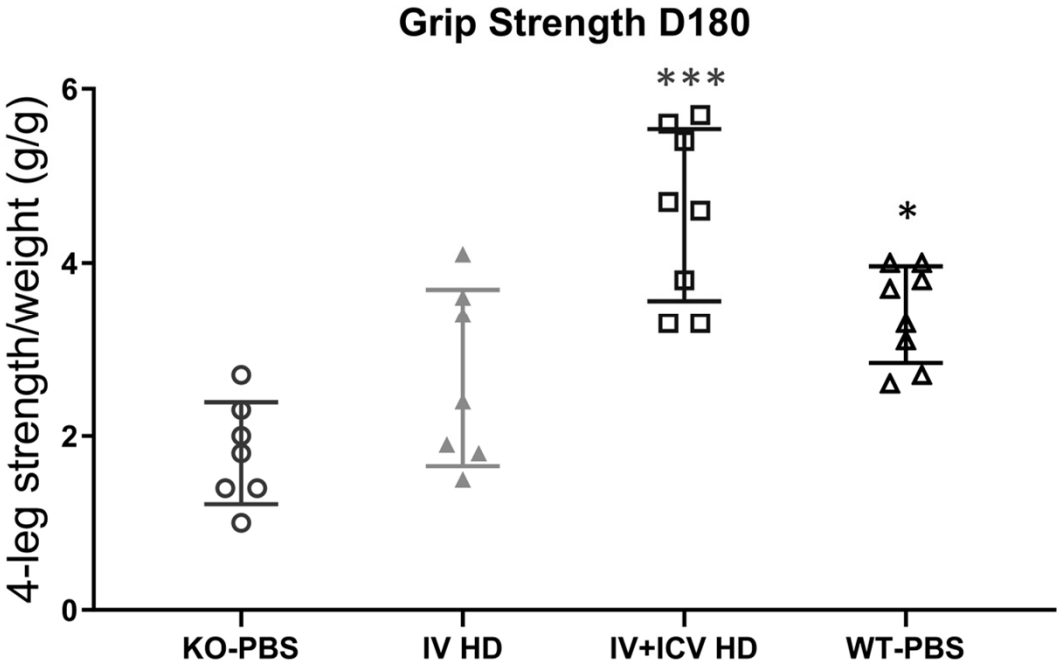

Grip Strength D180
4-leg strength/weight (g/g)
KO-PBS
IV HD
IV+ICV HD
WT-PBS

FIG. 28

post-symptomatic Pompe mice – ICV route

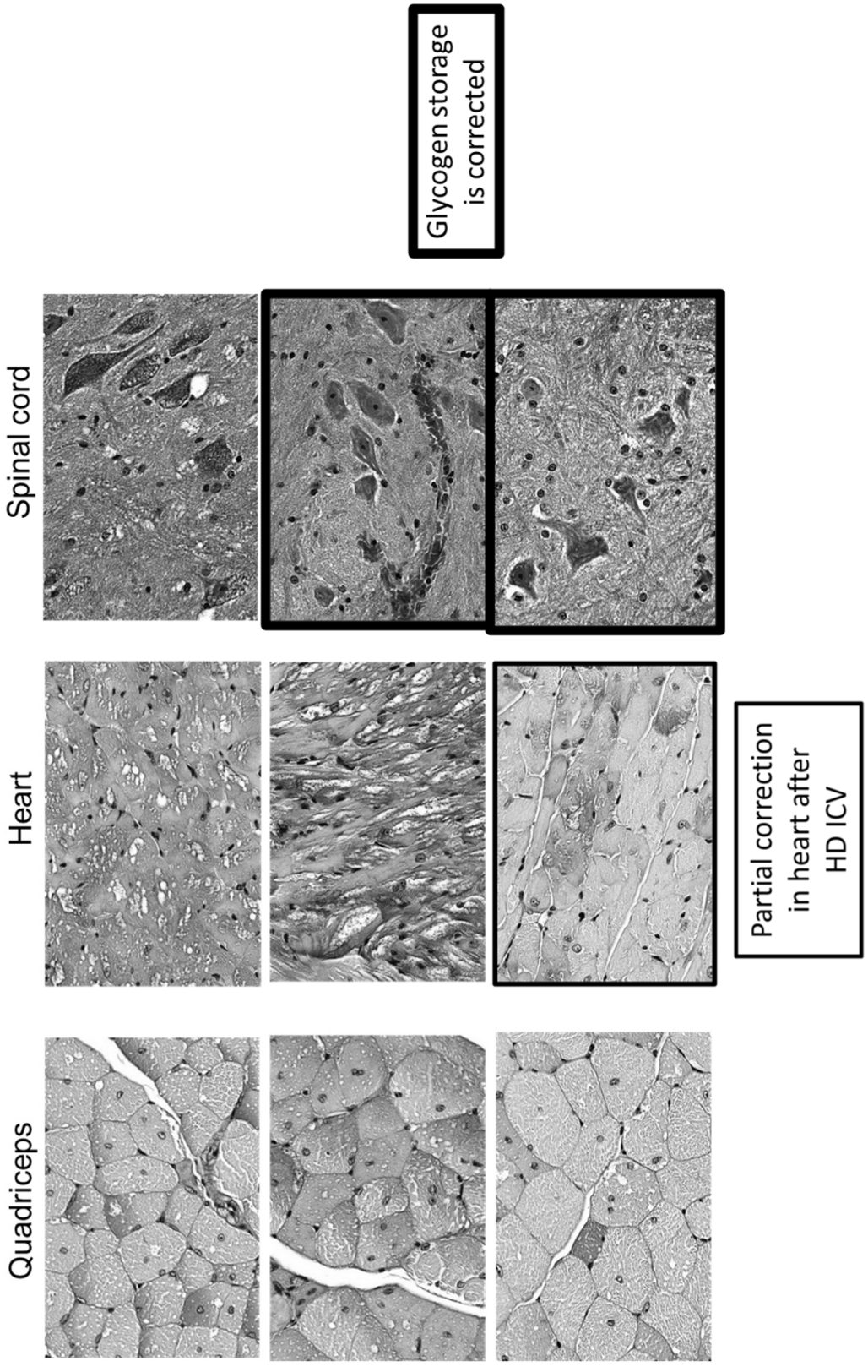


FIG. 29

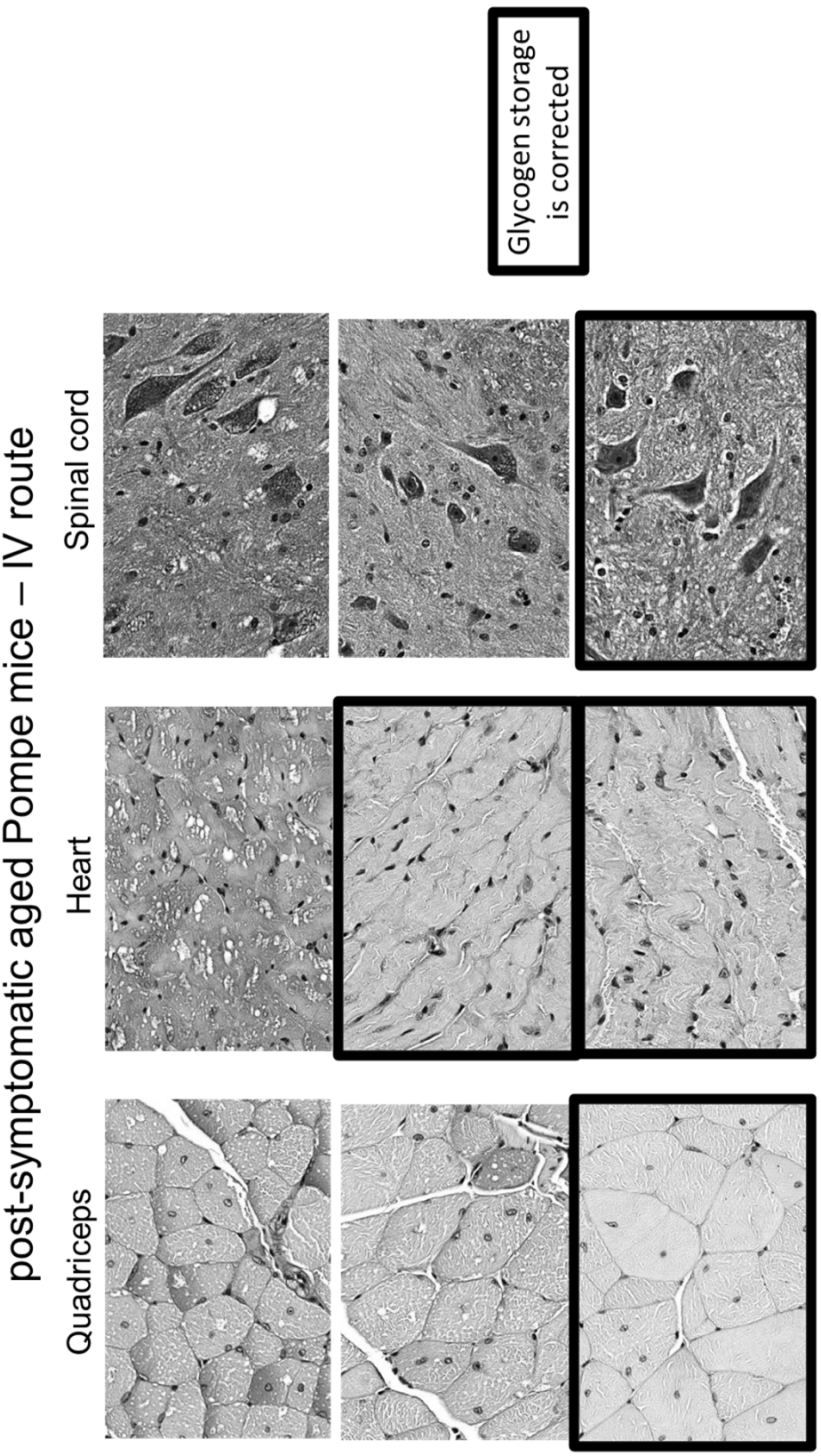

post-symptomatic aged Pompe mice – IV route
Quadriceps
Heart
Spinal cord
Glycogen storage is corrected

FIG. 30A

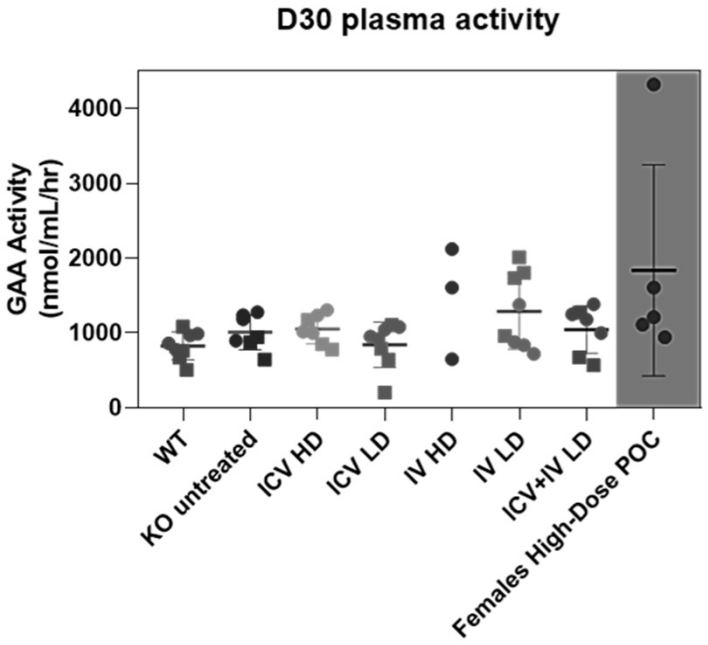


FIG. 30B

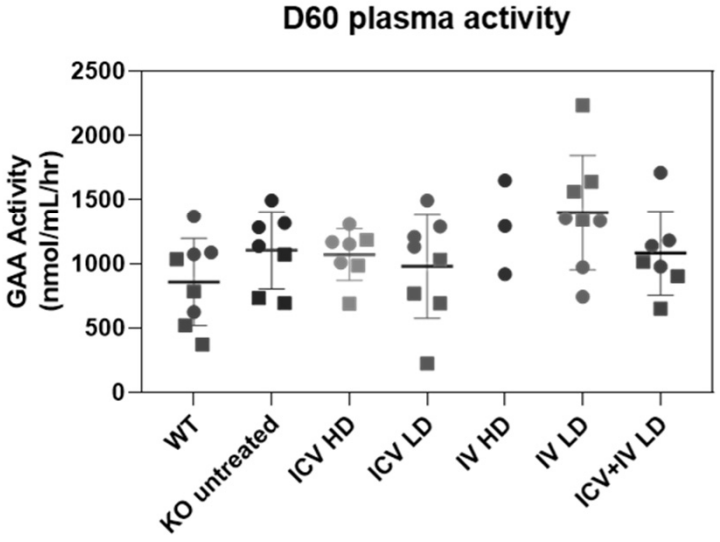


FIG. 30C

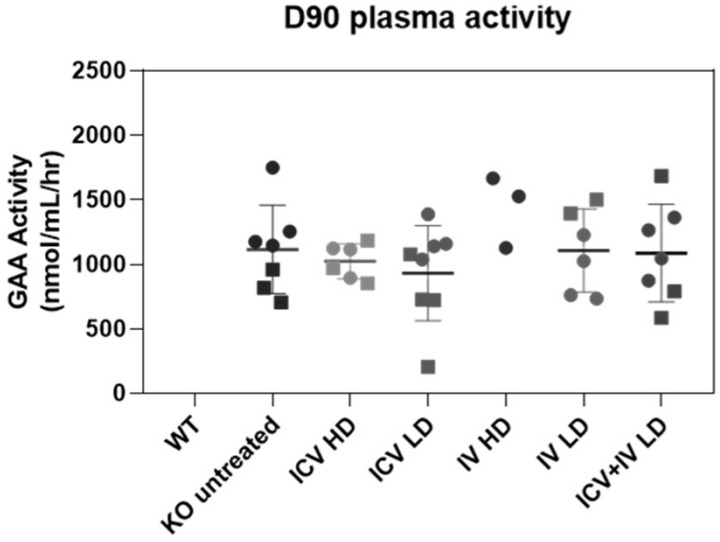


FIG. 31

Day								
-21	-10 (approx.)	0	3	7	21	29	35	60
Baseline Clin Path NAB Neuro Exam	Pred	Vector dosing Clinpath Plasma / CSF NAB / PBMC	Clinpath Plasma	Clinpath Plasma / CSF	Clinpath Plasma / CSF	Pred Taper	Clinpath Plasma / CSF Neuro exam	Necropsy Clinpath Plasma / CSF NAB / PBMC Neuro exam

Group Designation	1	2	3	4	5	6*
N / Group	2	2	2	2	2	2
Route of administration	IV LD	IV HD	ICM LD	ICM HD	IV HD ICM HD	IV HHD ICM LD
Vector Dose	1e13 GC/kg	5e13 GC/kg	1e13 GC	3e13 GC	5E13 GC/kg 3e13 GC	1E14 GC/kg 1e13 GC

FIG. 32A

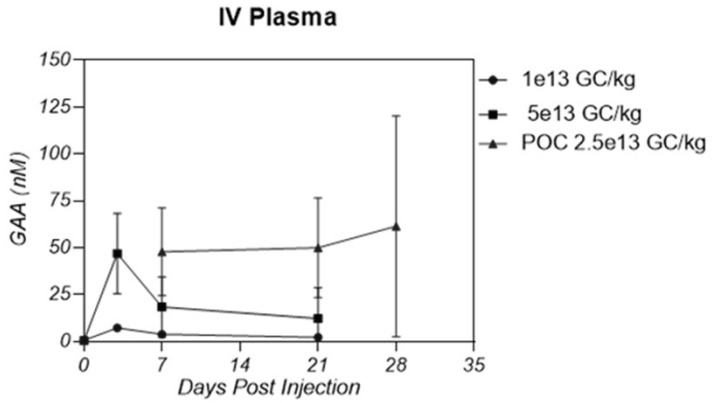


FIG. 32B

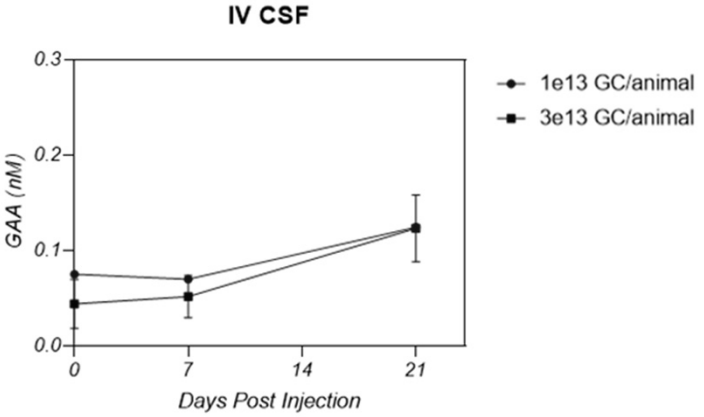


FIG. 32C

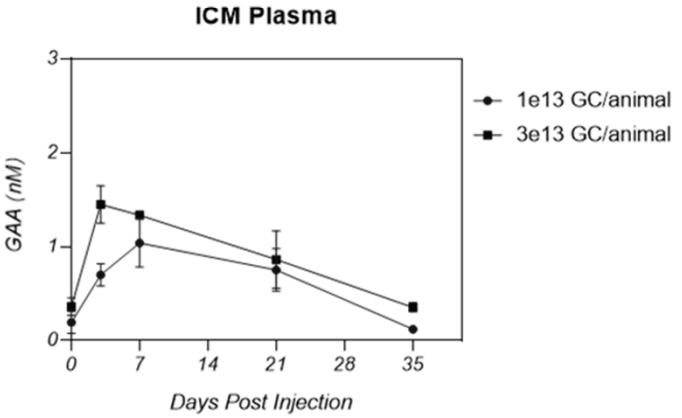


FIG. 32D

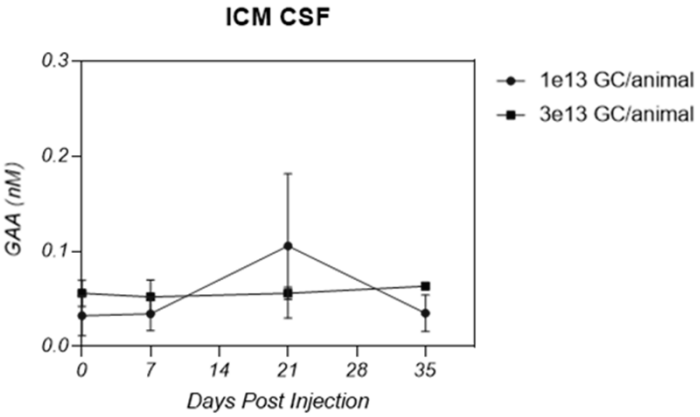


FIG. 32E

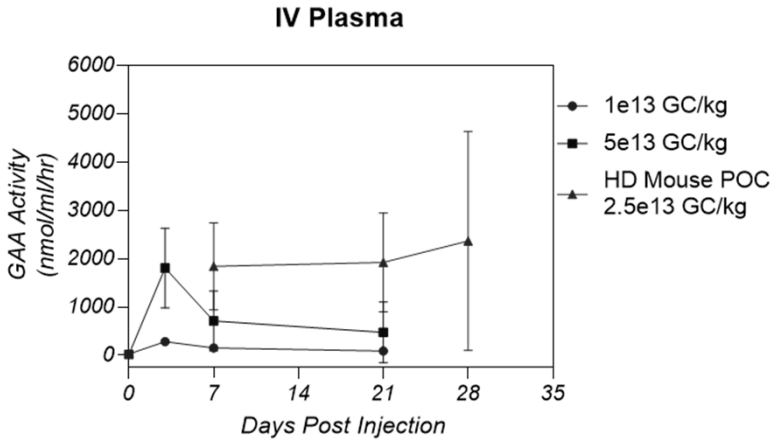


FIG. 32F

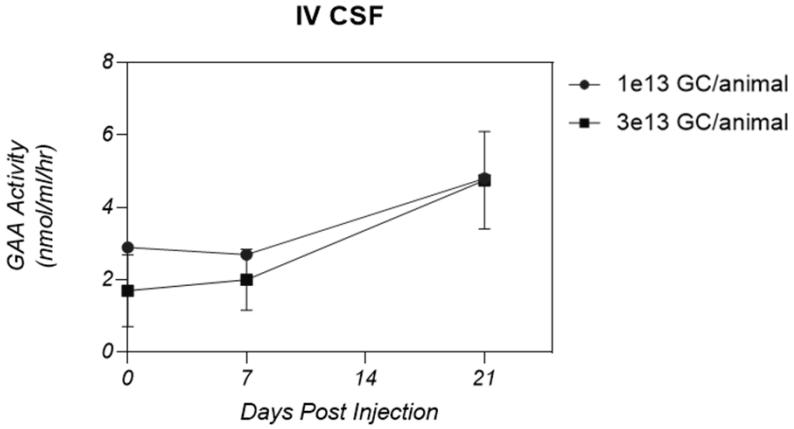


FIG. 32G

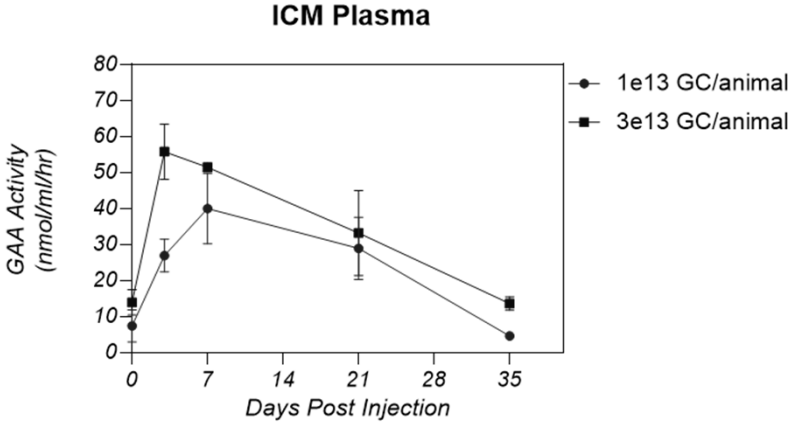


FIG. 32H

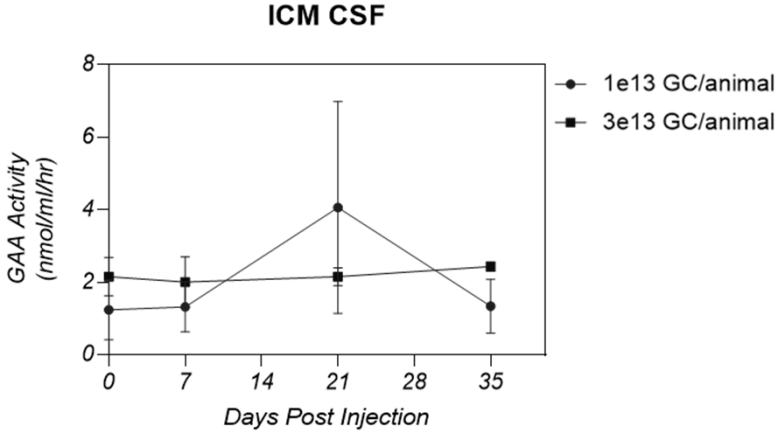


FIG. 33A

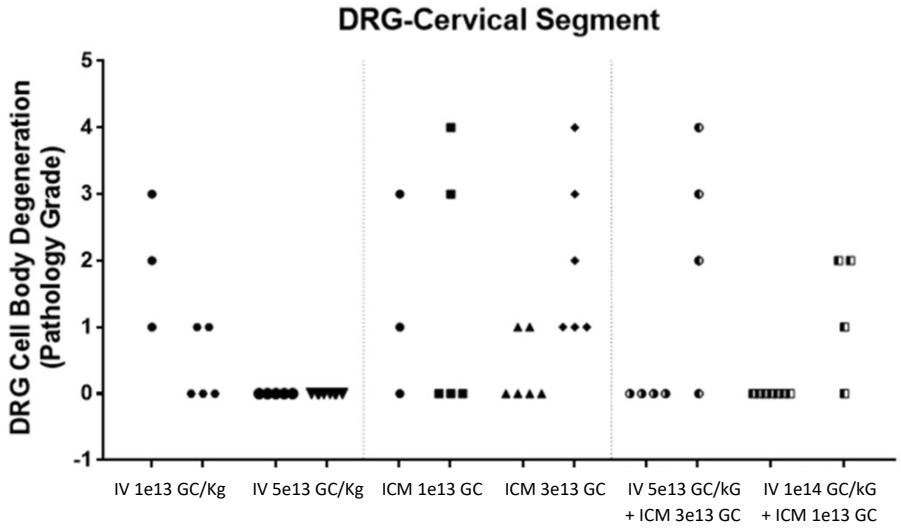


FIG. 33B

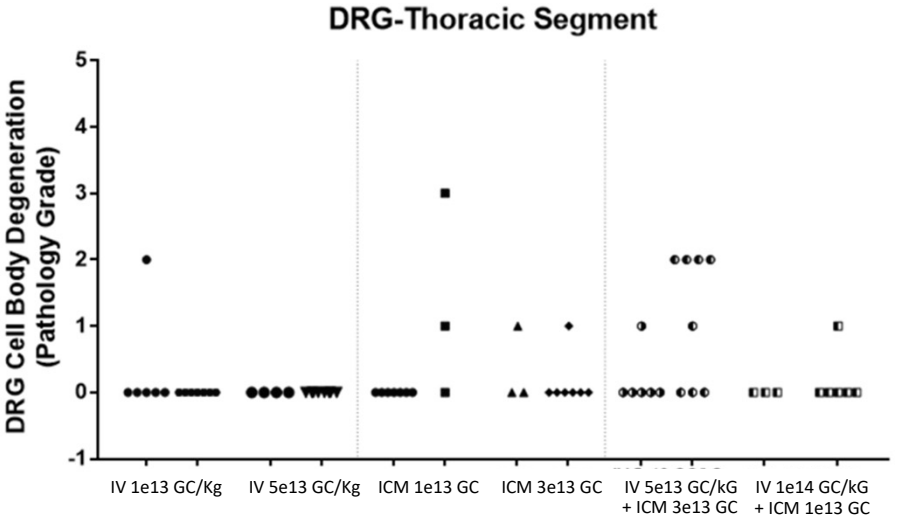


FIG. 33C

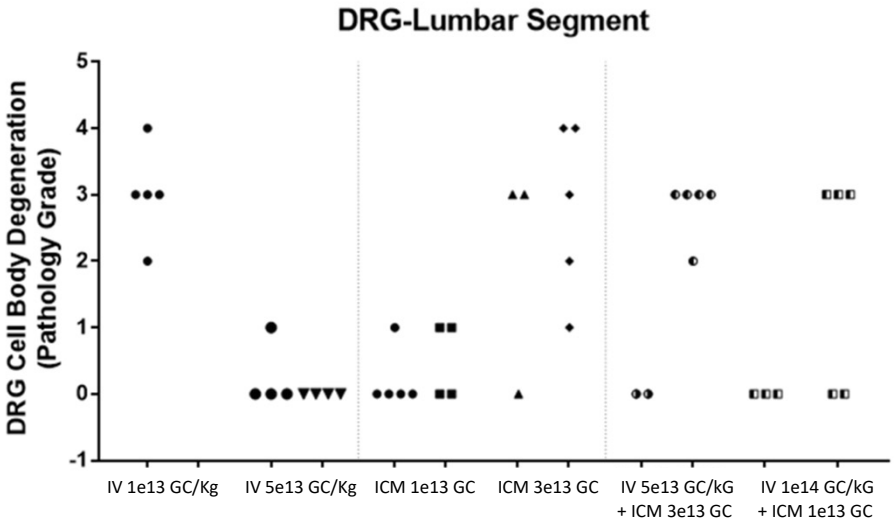


FIG. 33D

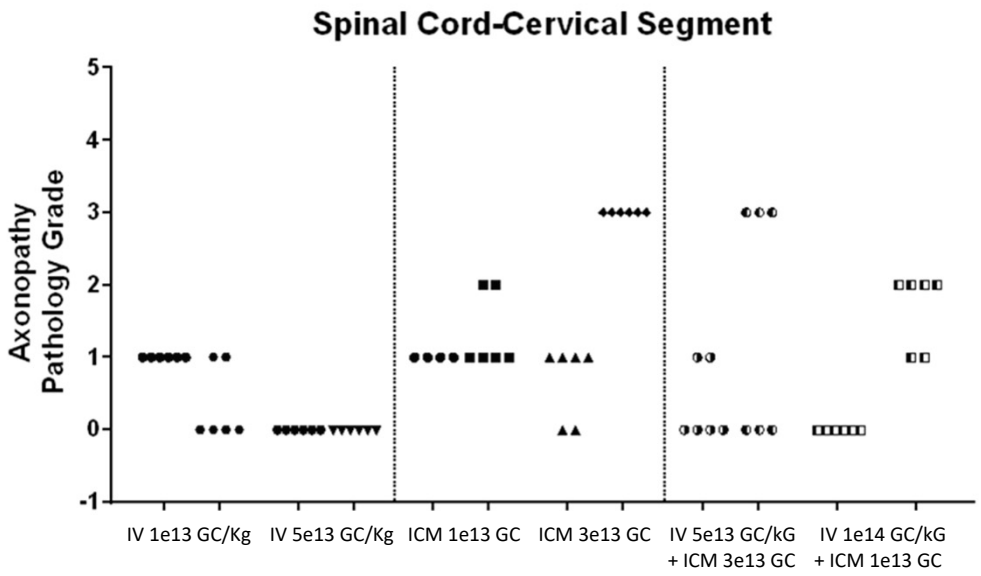


FIG. 33E

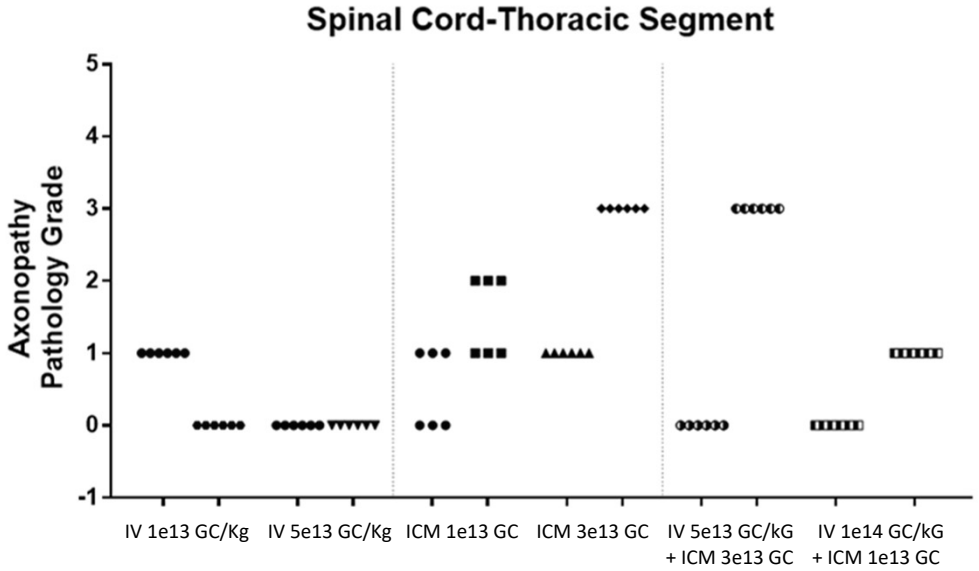


FIG. 33F

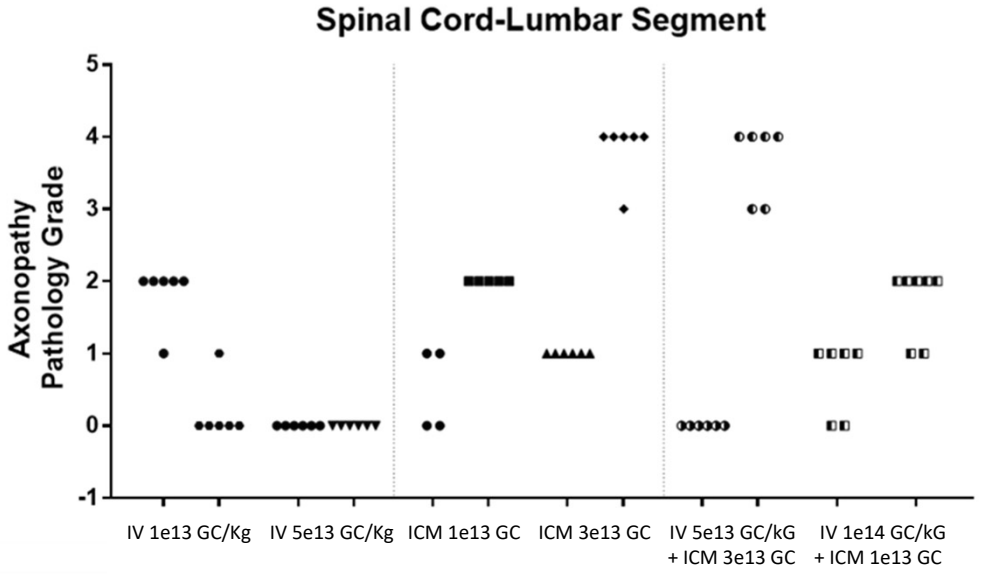
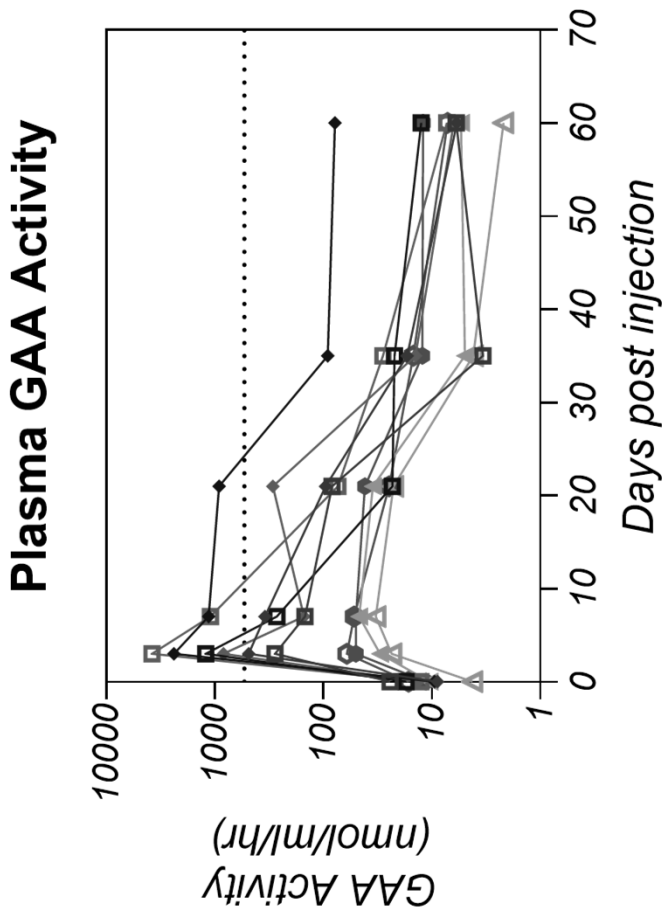


FIG. 34


Plasma GAA Activity
GAA Activity
(nmol/ml/hr)
10000
1000
100
10
1
0
10
20
30
40
50
60
70
Days post injection
IV LD (#RA3607)
IV LD (#180268)
IV HD (#RA3679)
IV HD (#181278)
IV HD+ICM HD (#180672)
IV HD+ICM HD (#180717)
ICM LD (#180637)
ICM LD (#13-2251R/7006)
ICM HD (#12-1695R/7022)
ICM HD (#808041/6468)

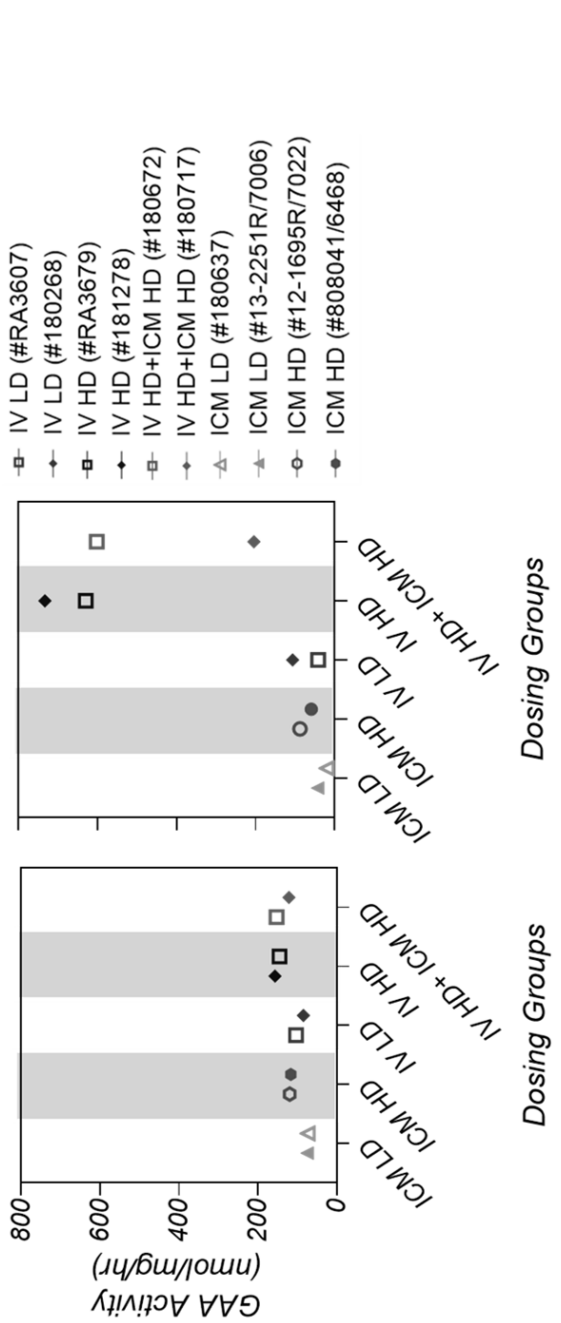


FIG. 35A

FIG. 35B

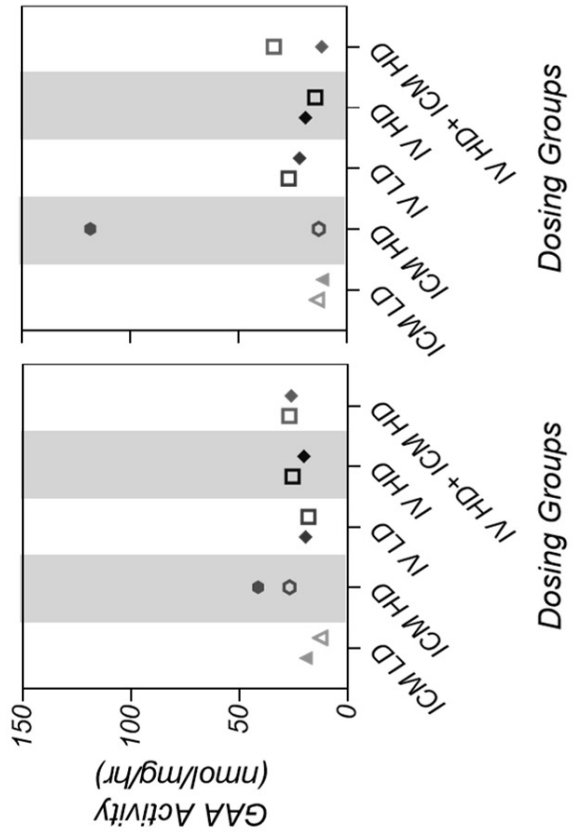


FIG. 35C

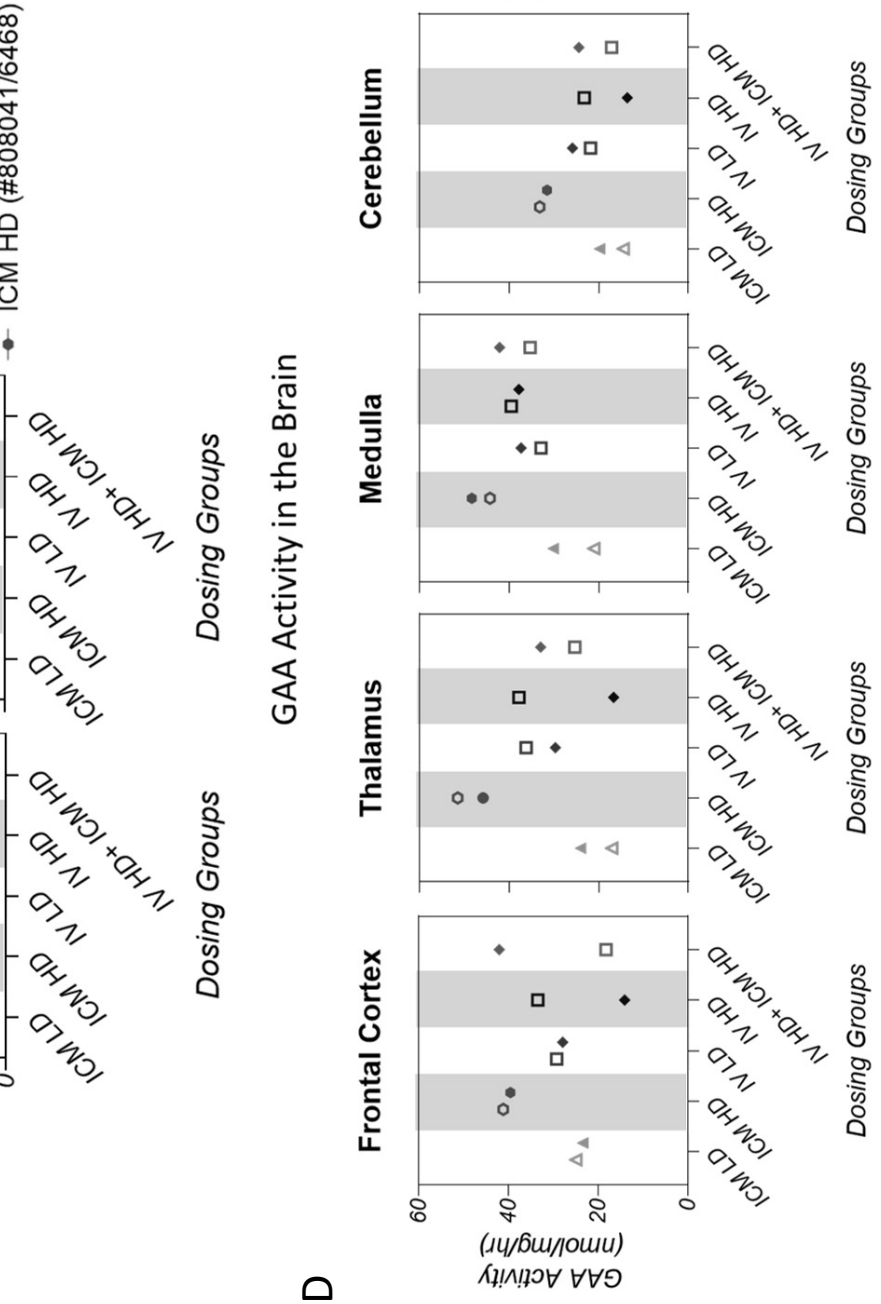


FIG. 35D

FIG. 36

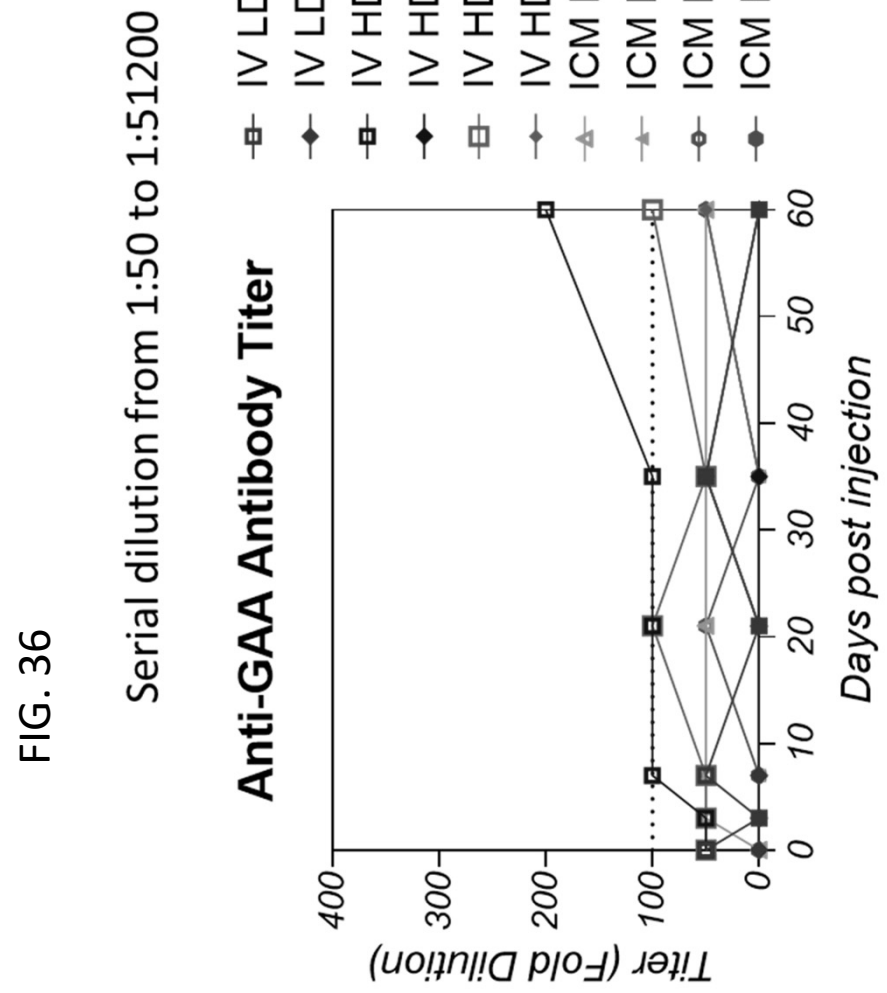

Serial dilution from 1:50 to 1:51200
Anti-GAA Antibody Titer
IV LD (RA3607)
IV LD (180268)
IV HD (RA3679)
IV HD (181278)
IV HD + ICM LD (180672)
IV HD + ICM LD (180717)
ICM LD (180637)
ICM LD (13-2251R/7006)
ICM HD (12-1695R/7022)
ICM HD (1808041/6468)
Titer (Fold Dilution)
400
300
200
100
0
0
10
20
30
40
50
60
Days post injection

FIG. 37

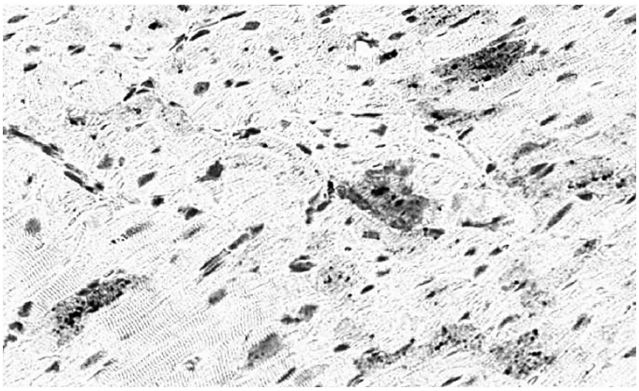
Quadriceps
Non injected NHP



Quadriceps
IV 1e13 GC/Kg



Heart
IV 1e13 GC/Kg



Spinal cord
ICM 1e13 GC

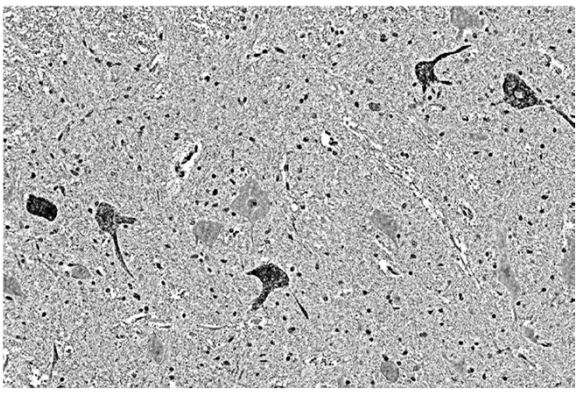


FIG. 38

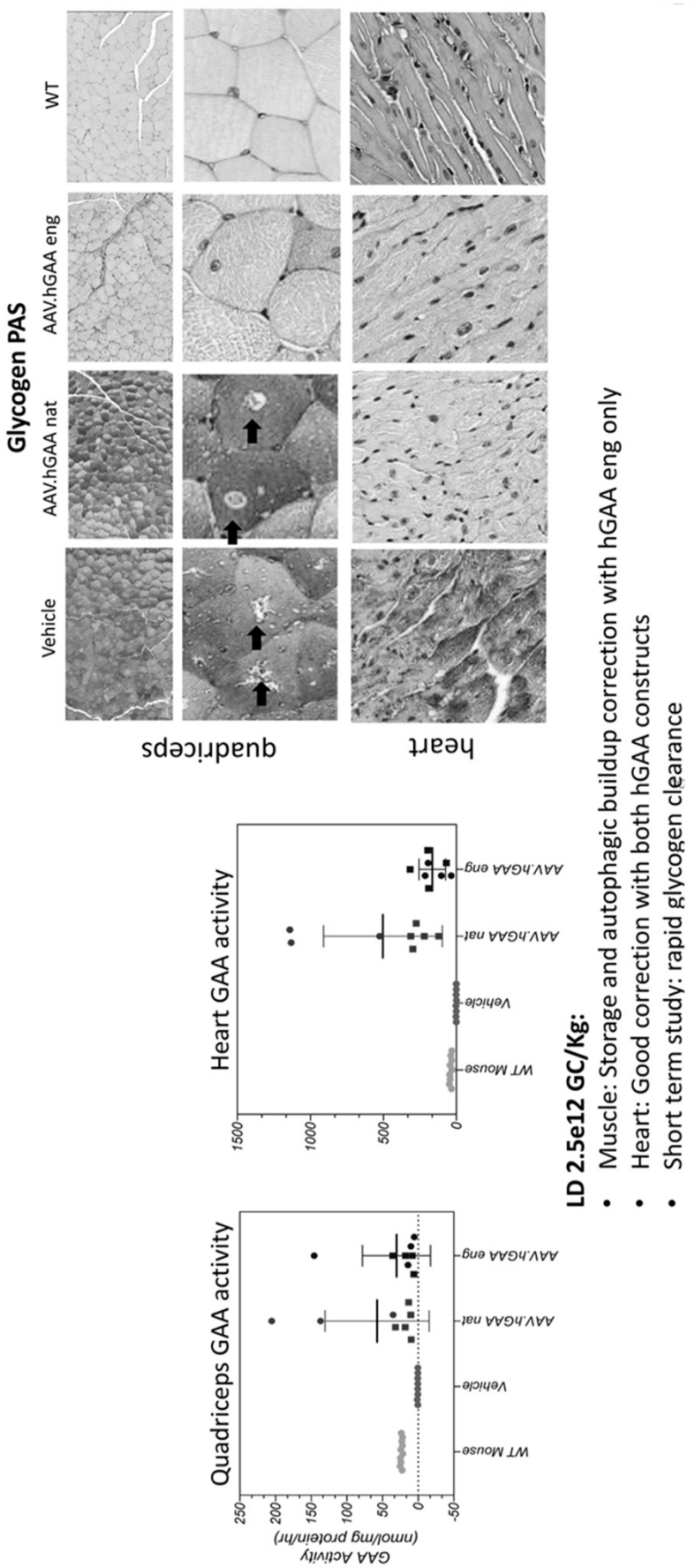
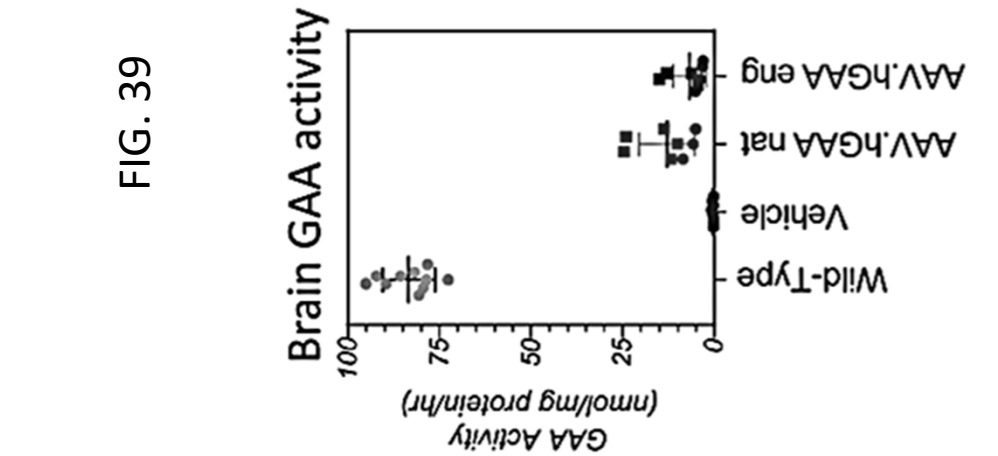
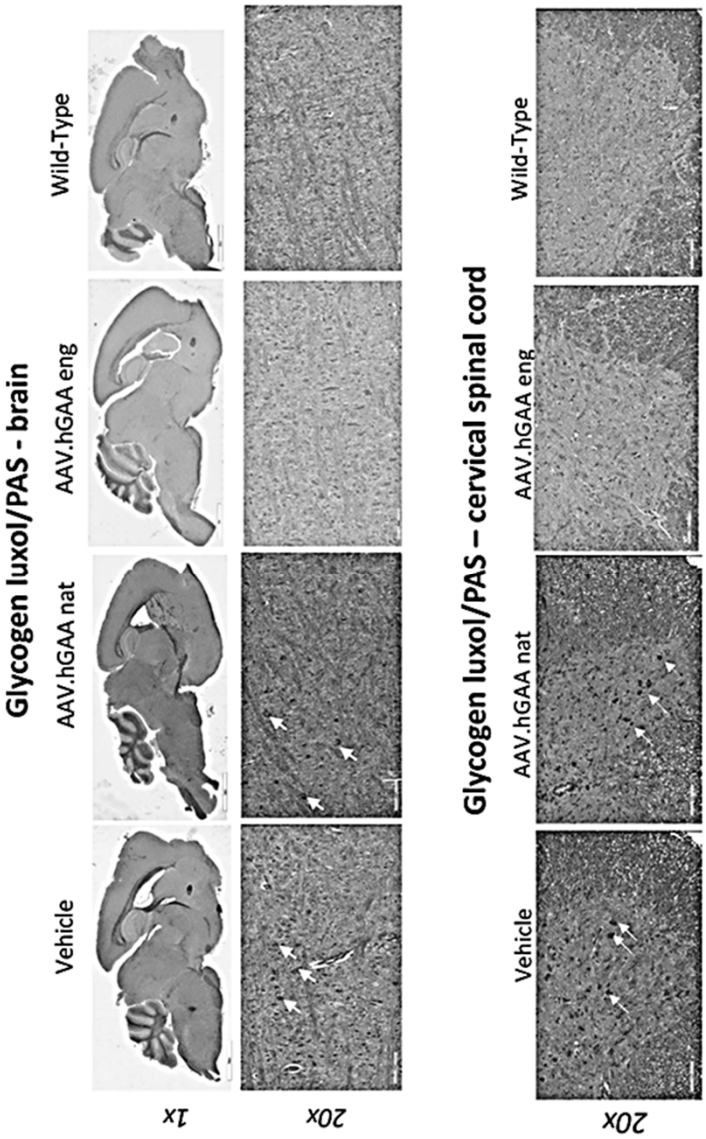

Glycogen PAS
Vehicle
AAV.hGAA nat
AAV.hGAA eng
WT
quadriceps
heart
Heart GAA activity
Quadriceps GAA activity
GAA Activity
(nmol/mg protein/hr)
WT Mouse
Vehicle
AAV.hGAA nat
AAV.hGAA eng
LD 2.5e12 GC/Kg:
• Muscle: Storage and autophagic buildup correction with hGAA eng only
• Heart: Good correction with both hGAA constructs
• Short term study: rapid glycogen clearance

FIG. 39





HD 2.5e13 GC/Kg:

- Brain and spinal cord storage correction with hGAA eng only

FIG. 40

- AAV.hGAA eng 2.5e13 GC/Kg IV
- hGAA IHC 28 days post-injection

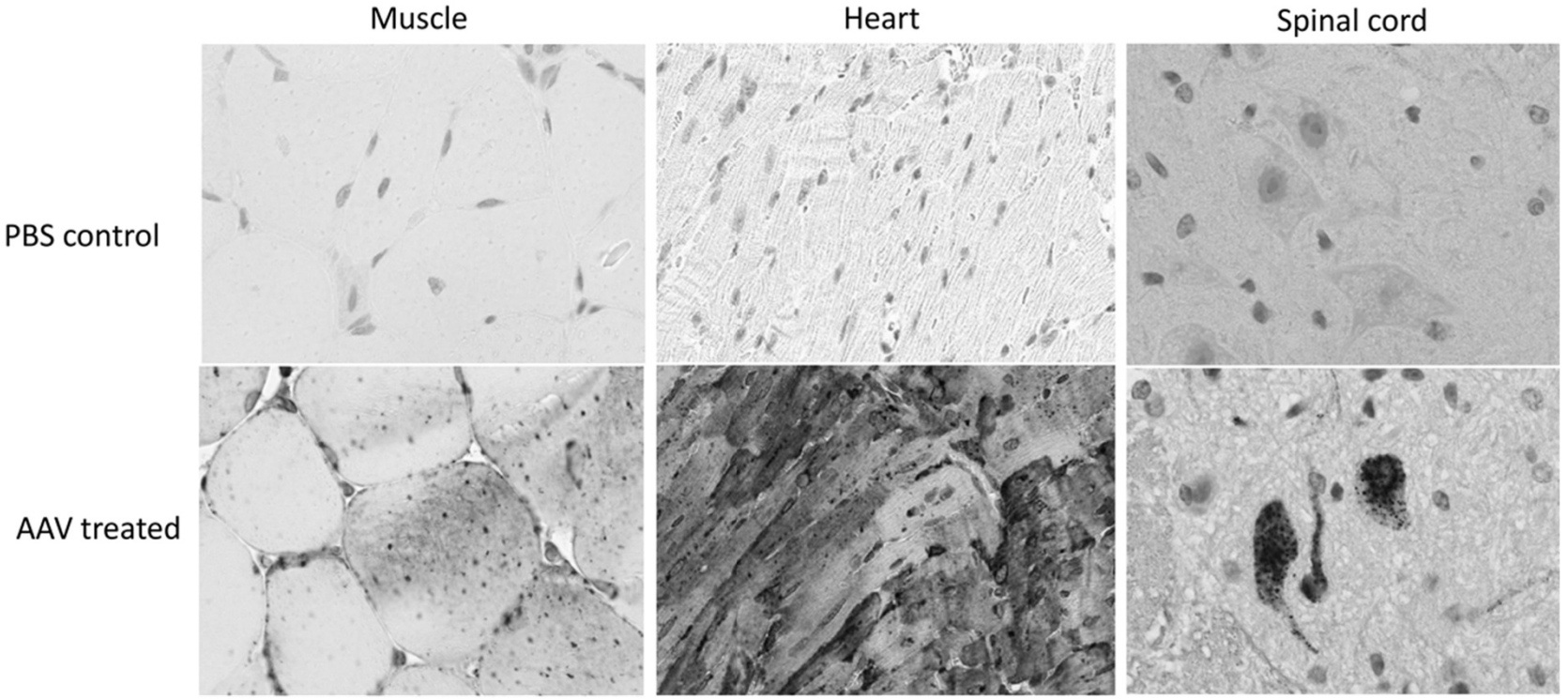


FIG. 41

- Pompe mice **1-1.5 months old**
- **AAV.hGAA eng**

Age at necropsy
3-4.5 months

D0 – IV
PBS
1e11 GC (5e12 GC/Kg); n = 4M & 4F
2e11 GC (1e13 GC/Kg); n = 4M & 4F
5e11 GC (2.5e13 GC/Kg); n = 4M & 4F
1e12 GC (5e13 GC/Kg); n = 4M & 4F
5e12 GC (2.5e14 GC/Kg); n = 4M & 4F

D60
Euthanasia
Histology

FIG. 42

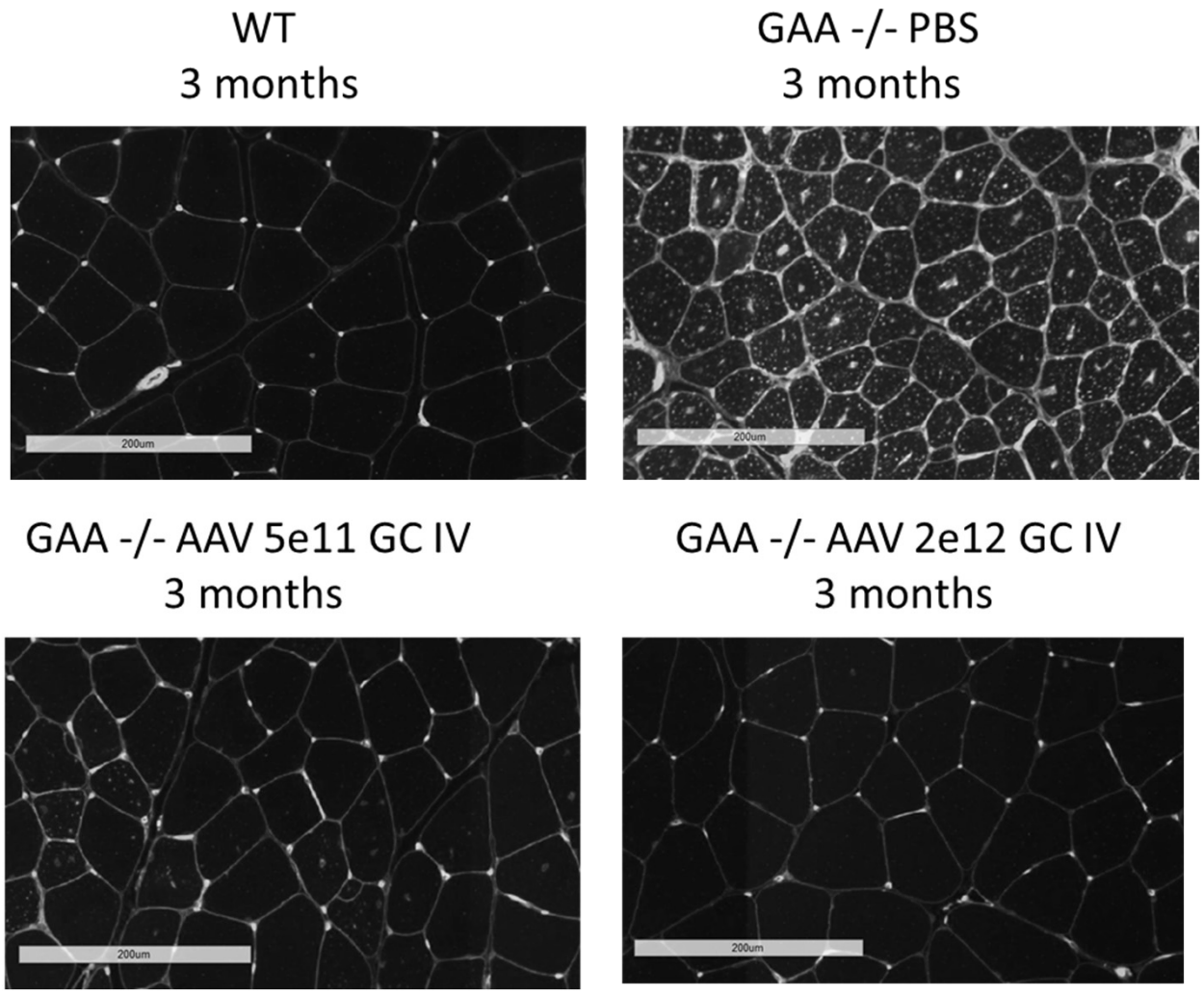


FIG. 43

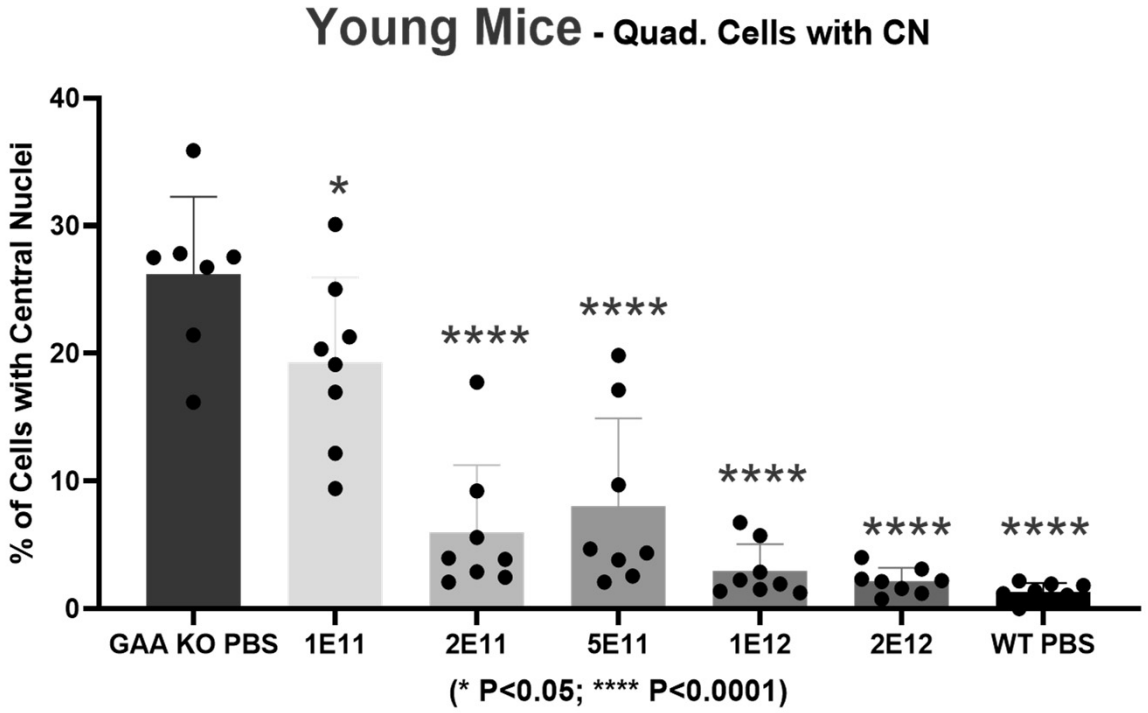

Young Mice - Quad. Cells with CN
% of Cells with Central Nuclei
40
30
20
10
0
*
****
****
****
****
****
GAA KO PBS
1E11
2E11
5E11
1E12
2E12
WT PBS
(* P<0.05; **** P<0.0001)

FIG. 44

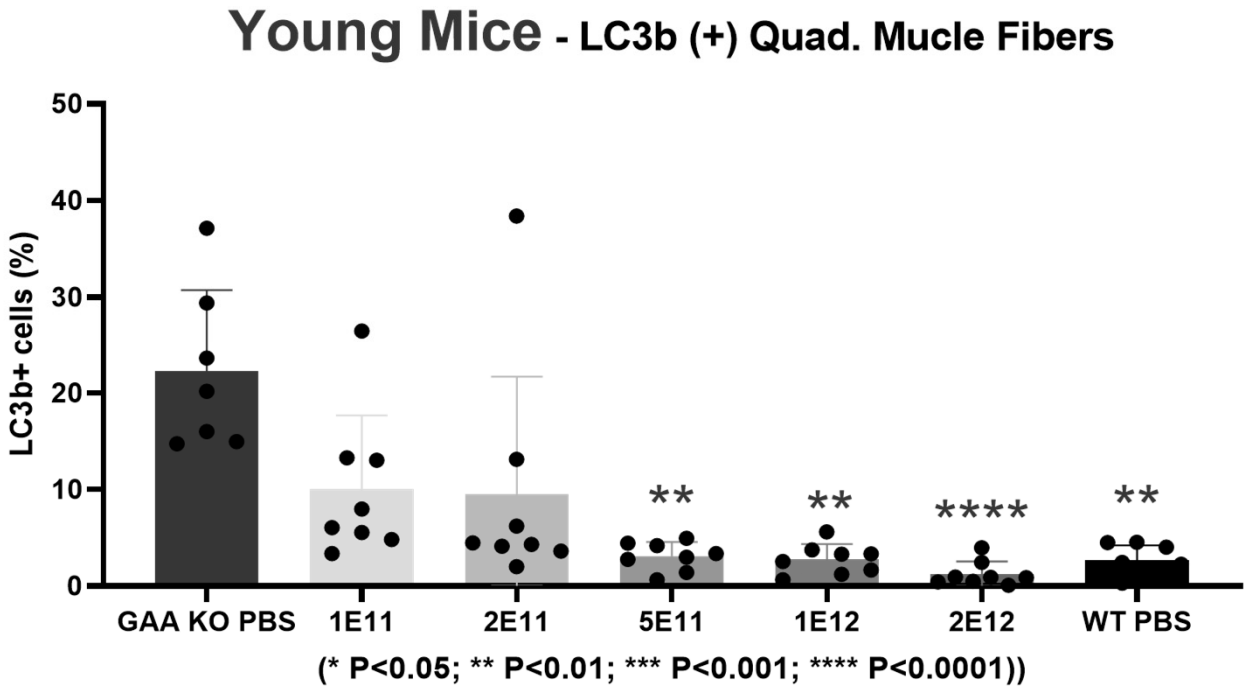

Young Mice - LC3b (+) Quad. Mucle Fibers
LC3b+ cells (%)
50
40
30
20
10
0
**
**
****
**
GAA KO PBS
1E11
2E11
5E11
1E12
2E12
WT PBS
(* P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001))

FIG. 45

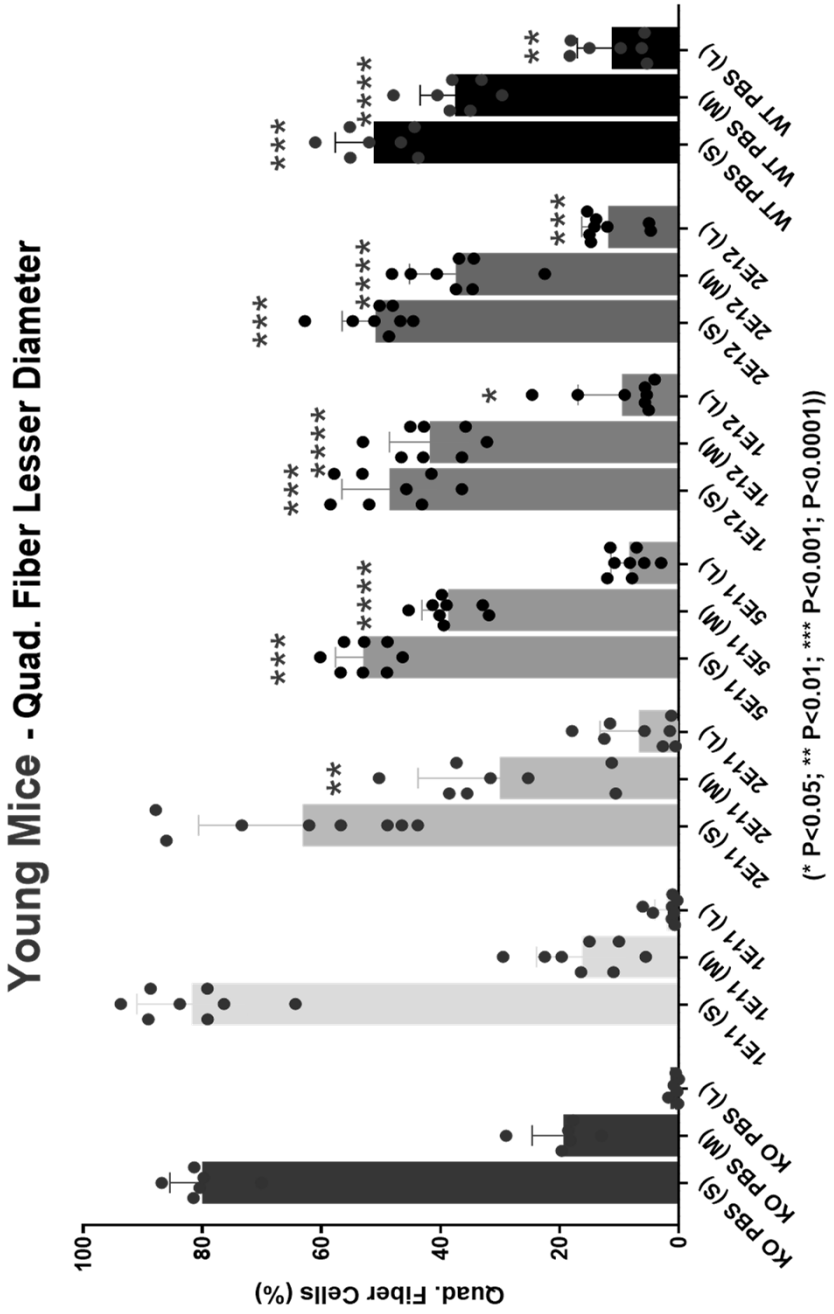

Young Mice - Quad. Fiber Lesser Diameter
Quad. Fiber Cells (%)
0
20
40
60
80
100
KO PBS (S)
KO PBS (M)
KO PBS (L)
1E11 (S)
1E11 (M)
1E11 (L)
2E11 (S)
2E11 (M)
2E11 (L)
5E11 (S)
5E11 (M)
5E11 (L)
1E12 (S)
1E12 (M)
1E12 (L)
2E12 (S)
2E12 (M)
2E12 (L)
WT PBS (S)
WT PBS (M)
WT PBS (L)
(* P<0.05; ** P<0.01; *** P<0.001; P<0.0001))

FIG. 46A

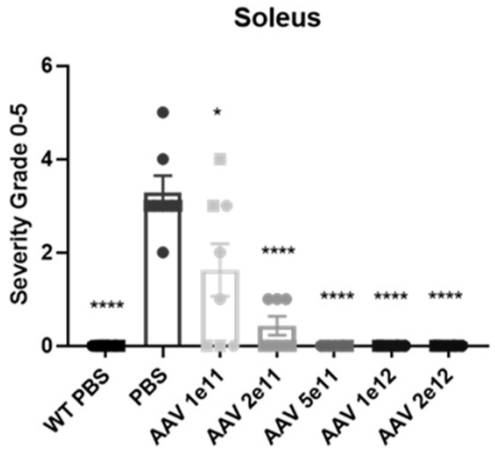


FIG. 46B

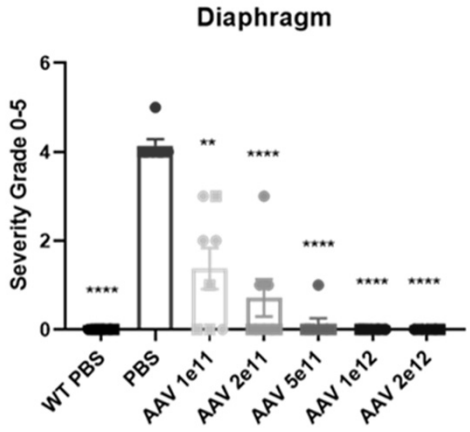


FIG. 46C

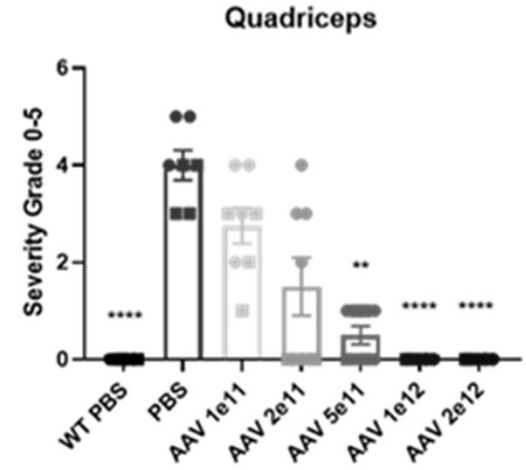


FIG. 46D

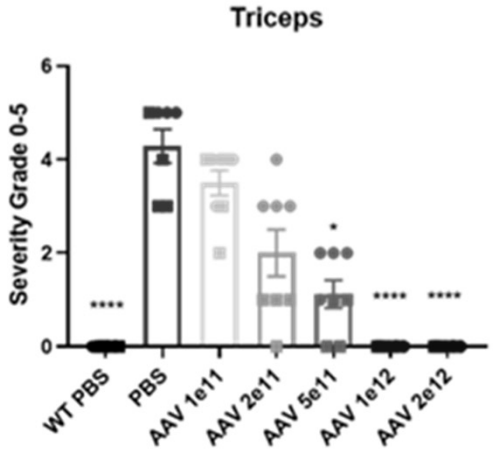


FIG. 46E

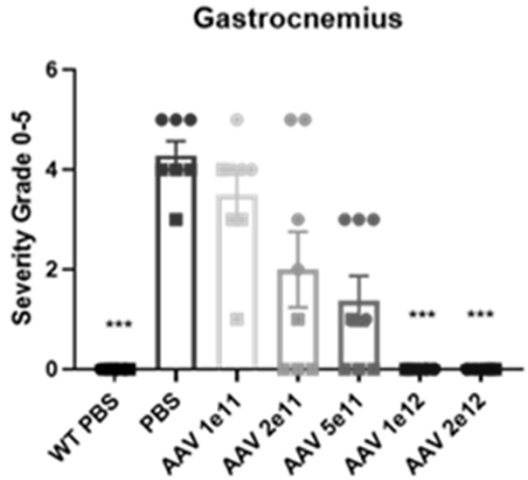


FIG. 46F

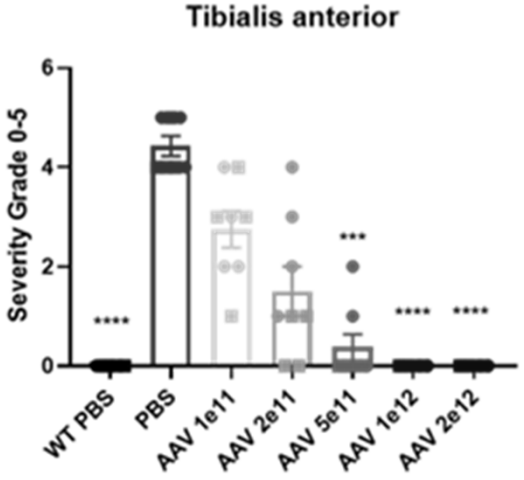


FIG. 47

- Pompe mice **6-7 months old**
- **AAV.hGAA eng**

IV
HD 5e13 GC/Kg
LD 1e13 GC/Kg

ICV
HD 1e11 GC
LD 1e10 GC

IV + ICV
HD + HD 5e13 GC/Kg + 1e11 GC
LD + LD 1e13 GC/Kg + 1e10 GC

D0-D180
Neurobehavior testing

D210
Euthanasia
13-14 months old
Histology

FIG. 48

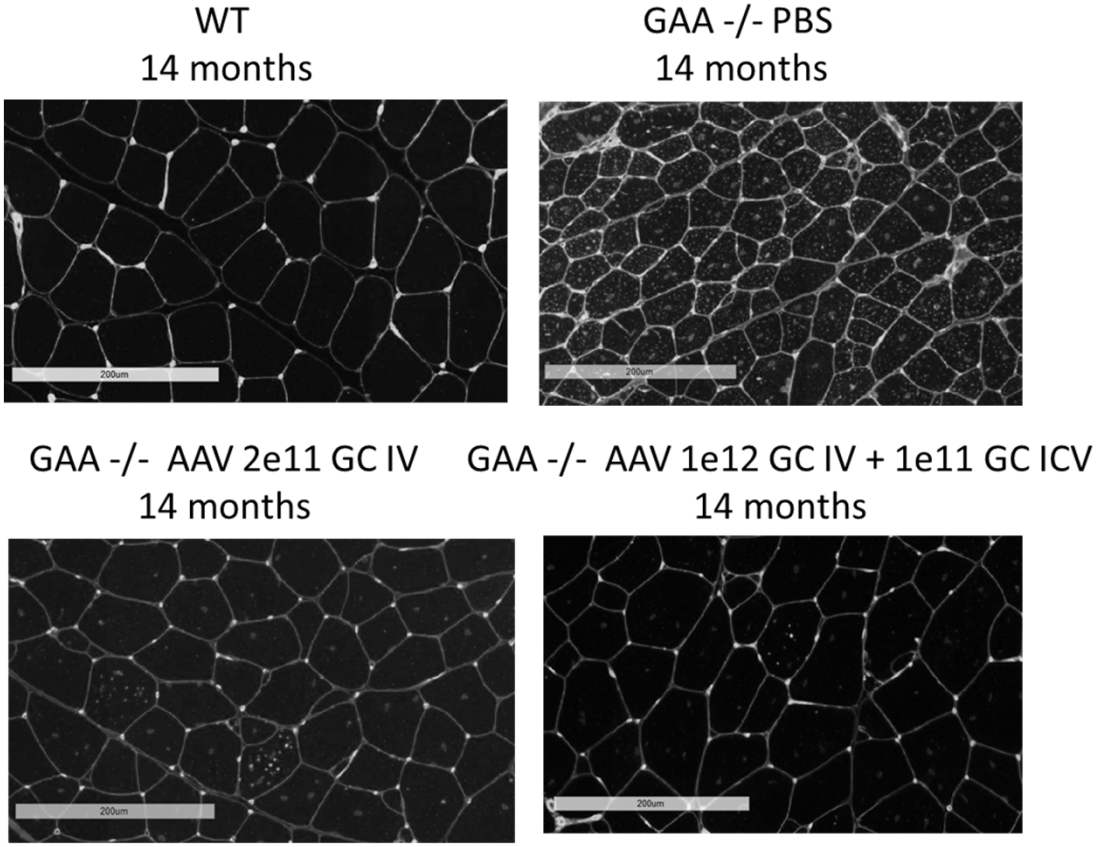


FIG. 49

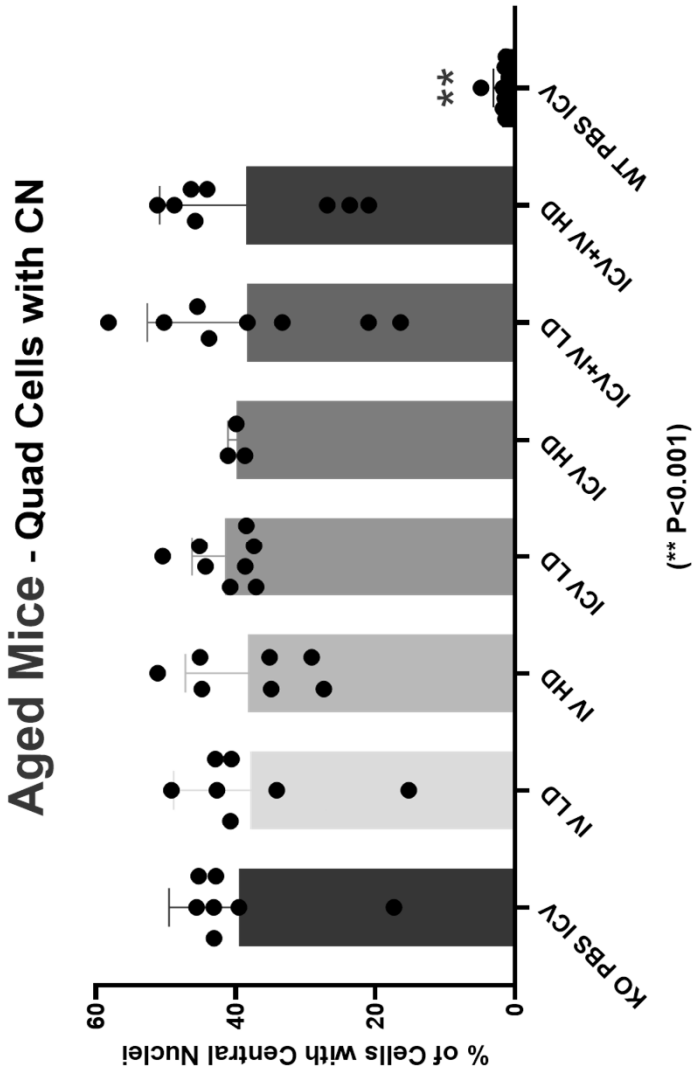

Aged Mice - Quad Cells with CN
% of Cells with Central Nuclei
60
40
20
0
**
KO PBS ICV
IV LD
IV HD
ICV LD
ICV HD
ICV+IV LD
ICV+IV HD
WT PBS ICV
(** P<0.001)

FIG. 50

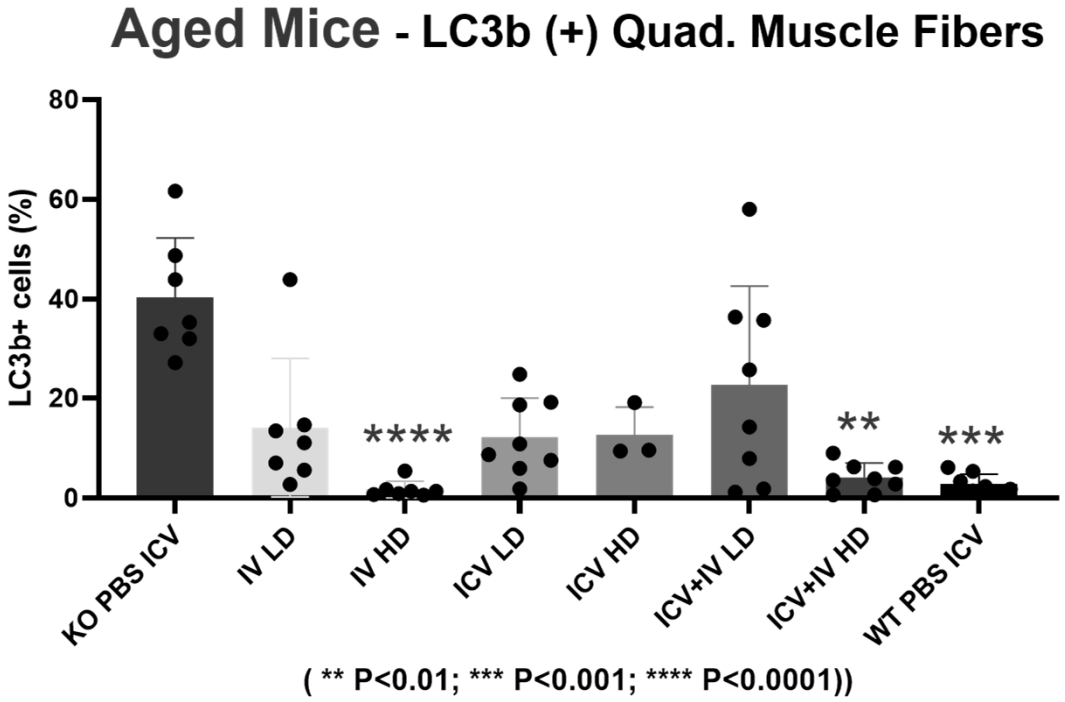

Aged Mice - LC3b (+) Quad. Muscle Fibers
LC3b+ cells (%)
80
60
40
20
0
****
**
***
KO PBS ICV
IV LD
IV HD
ICV LD
ICV HD
ICV+IV LD
ICV+IV HD
WT PBS ICV
(** P<0.01; *** P<0.001; **** P<0.0001))

FIG. 51

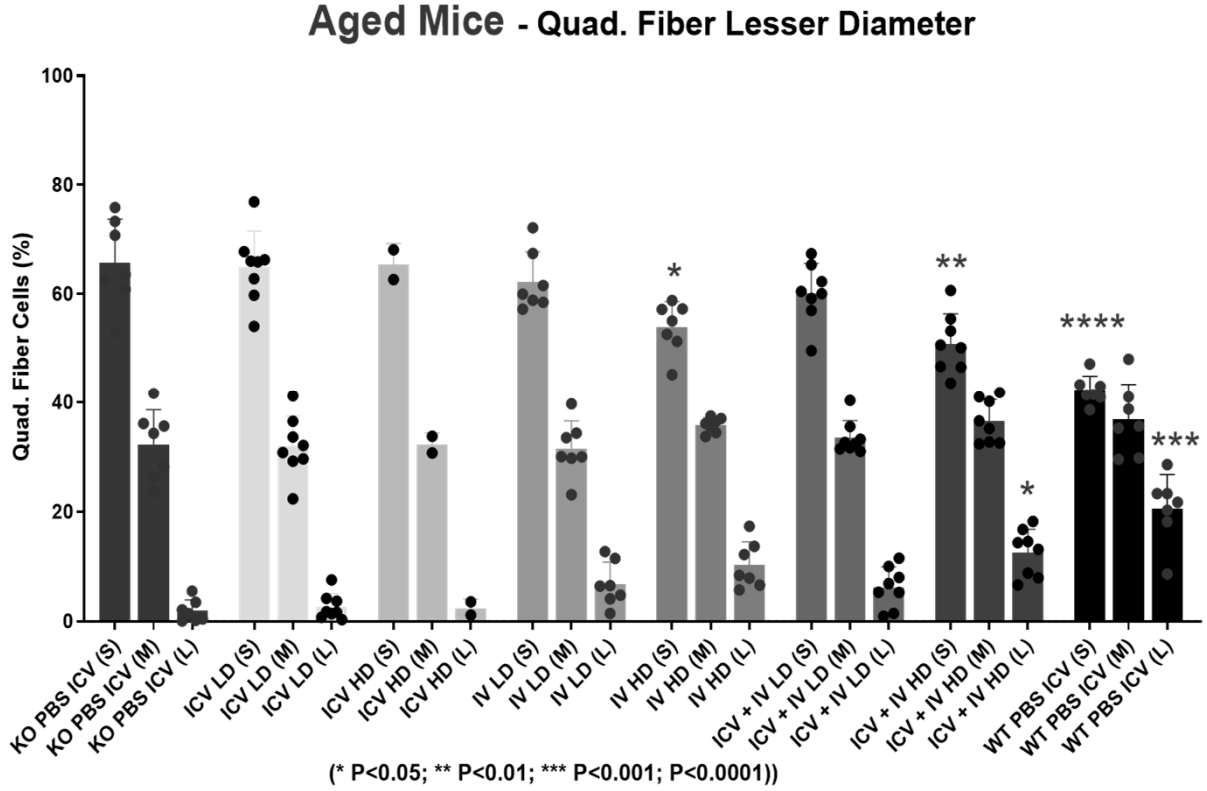
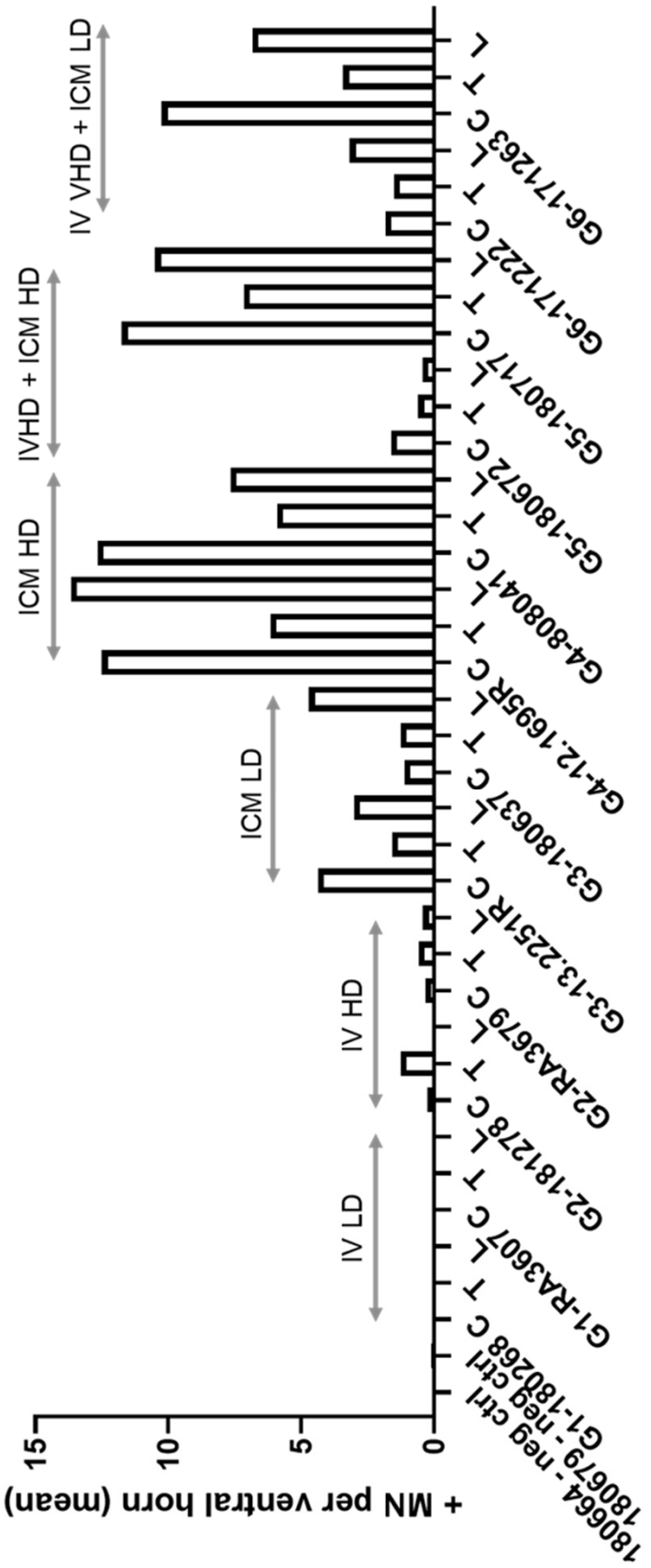

Aged Mice - Quad. Fiber Lesser Diameter
Quad. Fiber Cells (%)
100
80
60
40
20
0
*
**
****
***
*
KO PBS ICV (S)
KO PBS ICV (M)
KO PBS ICV (L)
ICV LD (S)
ICV LD (M)
ICV LD (L)
ICV HD (S)
ICV HD (M)
ICV HD (L)
IV LD (S)
IV LD (M)
IV LD (L)
IV HD (S)
IV HD (M)
IV HD (L)
ICV + IV LD (S)
ICV + IV LD (M)
ICV + IV LD (L)
ICV + IV HD (S)
ICV + IV HD (M)
ICV + IV HD (L)
WT PBS ICV (S)
WT PBS ICV (M)
WT PBS ICV (L)
(* P<0.05; ** P<0.01; *** P<0.001; P<0.0001))

FIG. 52


hGAA IHC motor neuron count
+ MN per ventral horn (mean)
0
5
10
15
IV LD
IV HD
ICM LD
ICM HD
IVHD + ICM HD
IV VHD + ICM LD
180664 - neg ctrl
180679 - neg ctrl
G1-180268 C T
G1-RA3607 C T
G2-181278 C T
G2-RA3679 C T
G3-13.2251R C T
G3-180637 C T
G4-12.1695R C T
G4-808041 C T
G5-180672 C T
G5-180717 C T
G6-171222 C T
G6-171263 C T