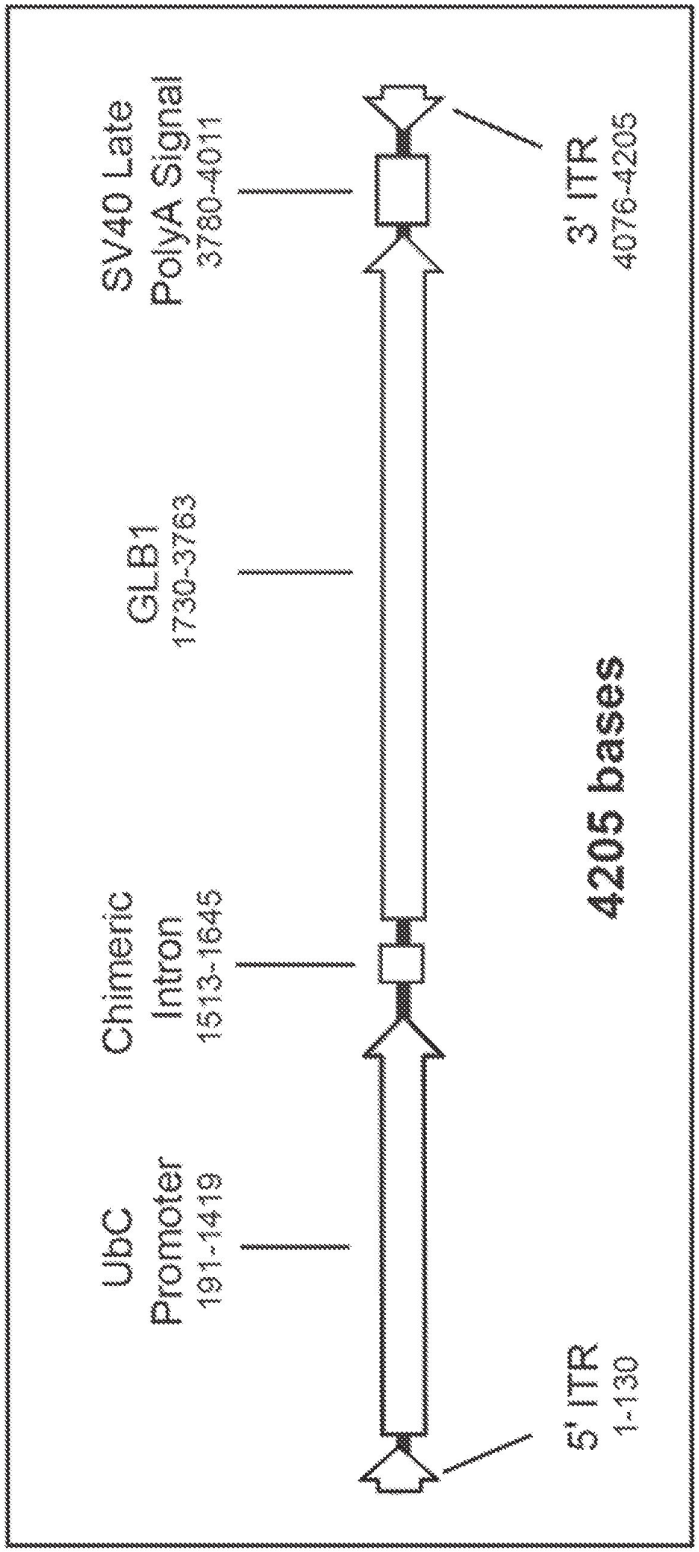
FIG 1A


UbC
Promoter
191-1419
Chimeric
Intron
1513-1645
GLB1
1730-3763
SV40 Late
PolyA Signal
3780-4011
5' ITR
1-130
4205 bases
3' ITR
4076-4205

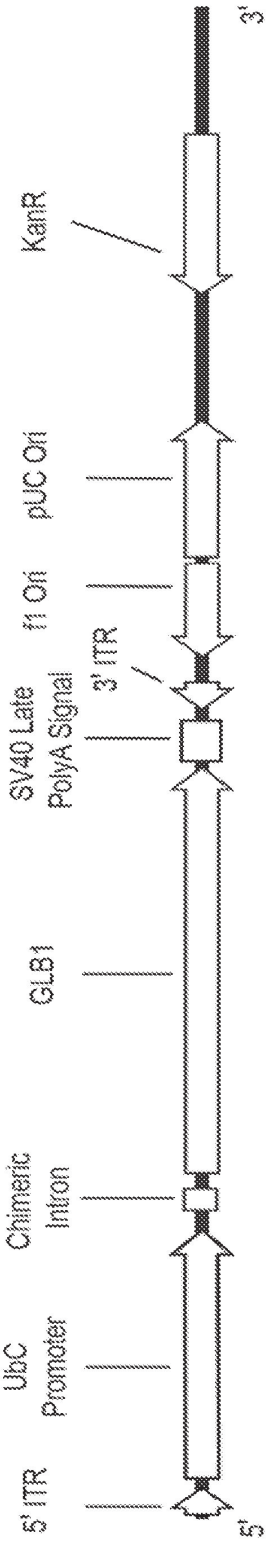

5' ITR
UbC Promoter
Chimeric Intron
GLB1
SV40 Late PolyA Signal
3' ITR
f1 Ori
pUC Ori
KanR
5'
3'

FIG 1B

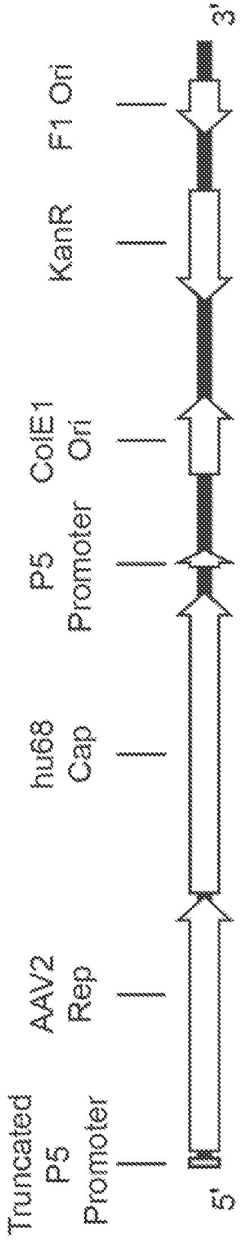

Truncated P5 Promoter
AAV2 Rep
hu68 Cap
P5 Promoter
ColE1 Ori
KanR
F1 Ori
5'
3'

FIG 1C

FIG 2A

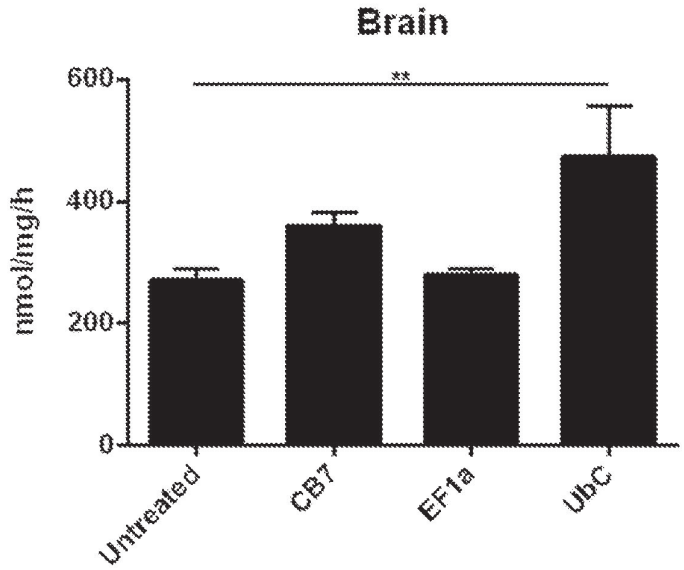

Brain
**
600
400
200
0
nmol/mg/h
Untreated
CB7
EF1a
UbC

FIG 2B

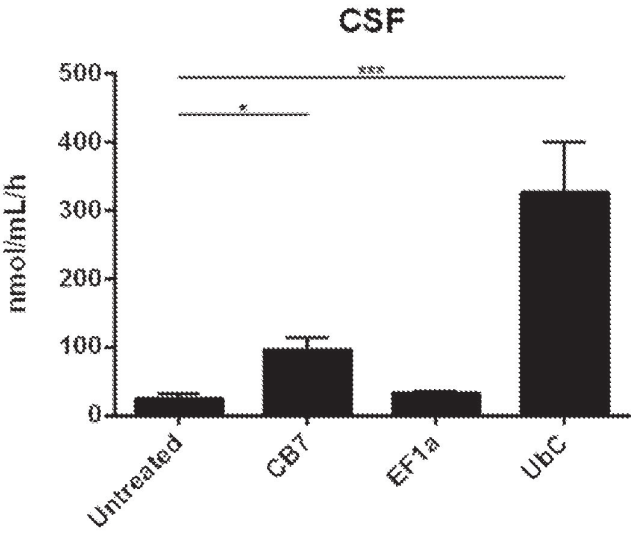

CSF
***
*
500
400
300
200
100
0
nmol/mL/h
Untreated
CB7
EF1a
UbC

FIG 3A

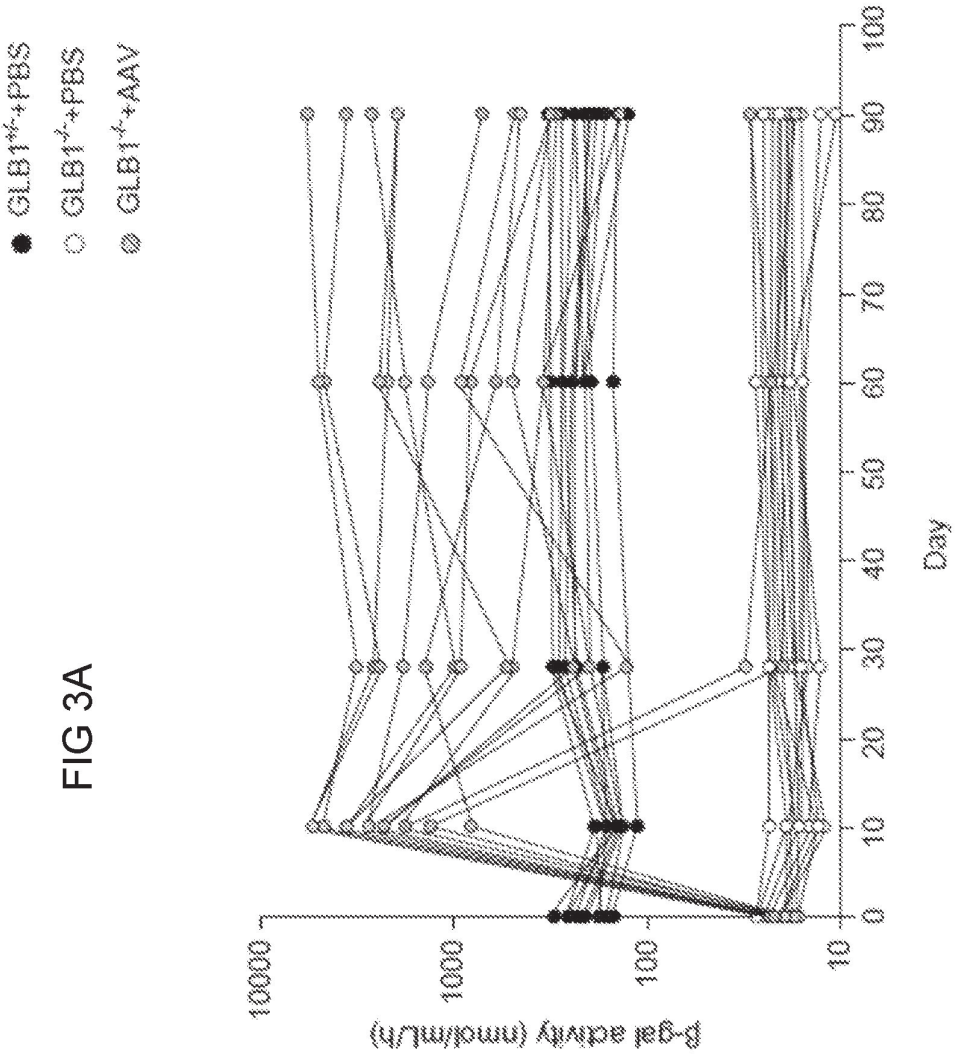

GLB1+/+ +PBS
GLB1-/- +PBS
GLB1-/- +AAV
β-gal activity (nmol/mL/h)
10000
1000
100
10
0
10
20
30
40
50
60
70
80
90
100
Day

FIG 3B

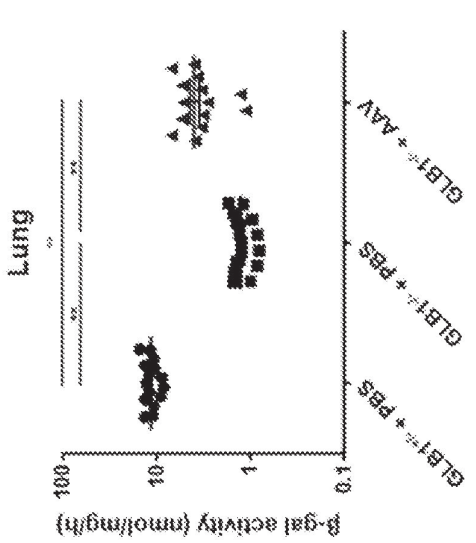


FIG 3C

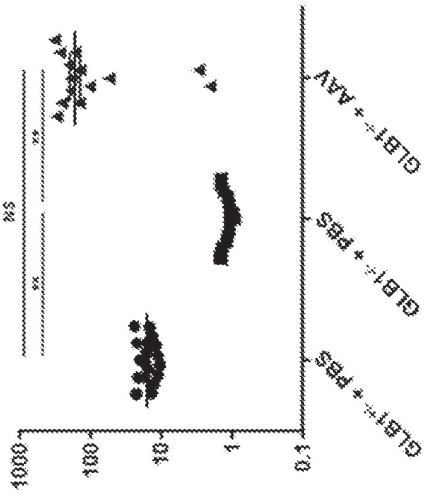


FIG 3D

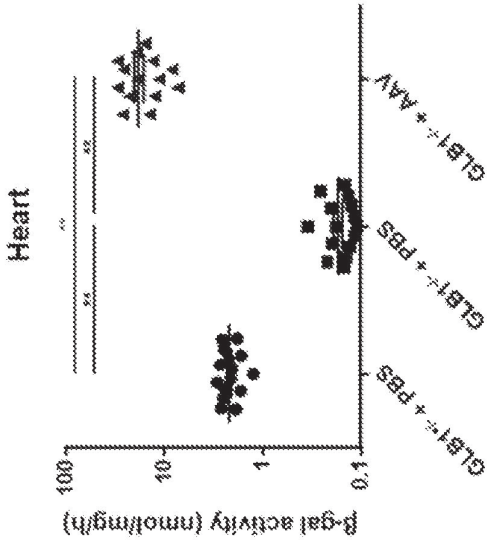


FIG 3E

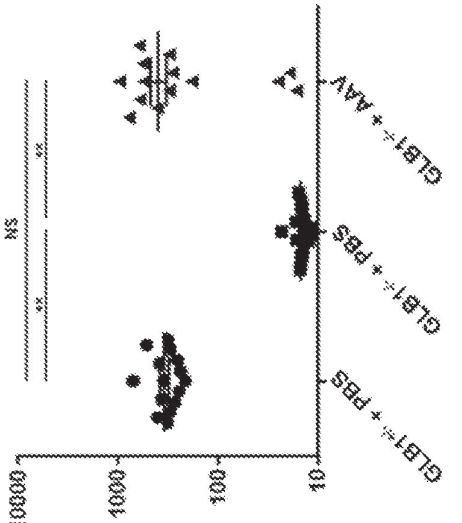


FIG 4A

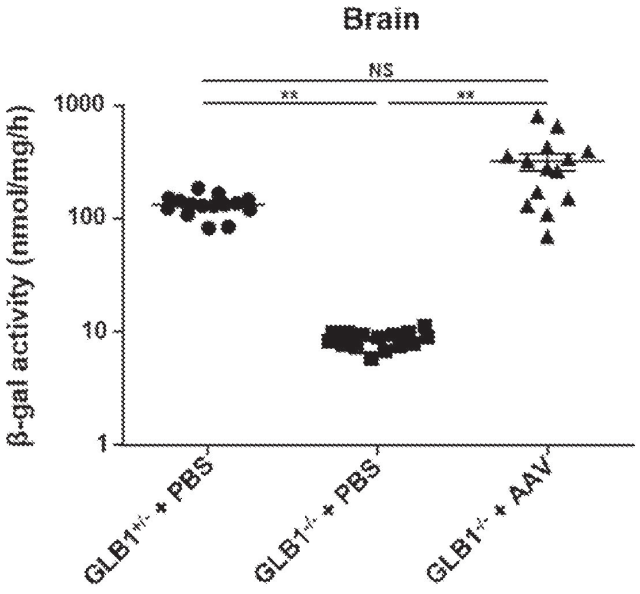

Brain
NS
**
**
1000
100
10
1
β-gal activity (nmol/mg/h)
GLB1+/- + PBS
GLB1-/- + PBS
GLB1-/- + AAV

FIG 4B

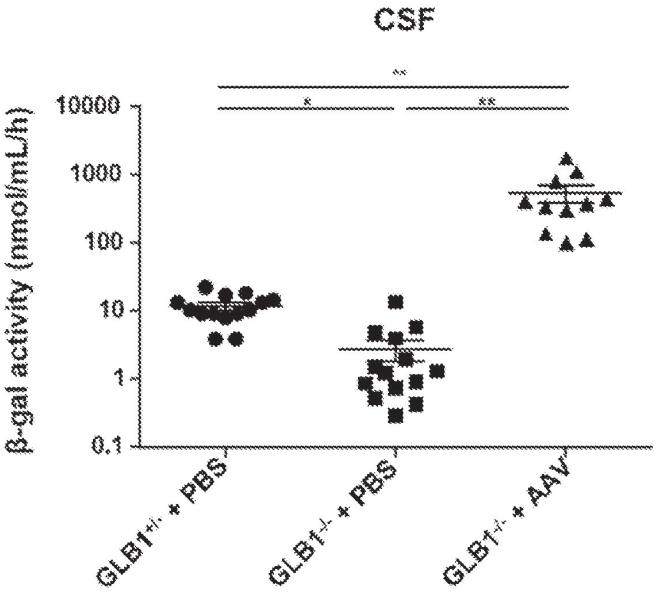

CSF
**
*
**
10000
1000
100
10
1
0.1
β-gal activity (nmol/mL/h)
GLB1+/- + PBS
GLB1-/- + PBS
GLB1-/- + AAV

FIG 5

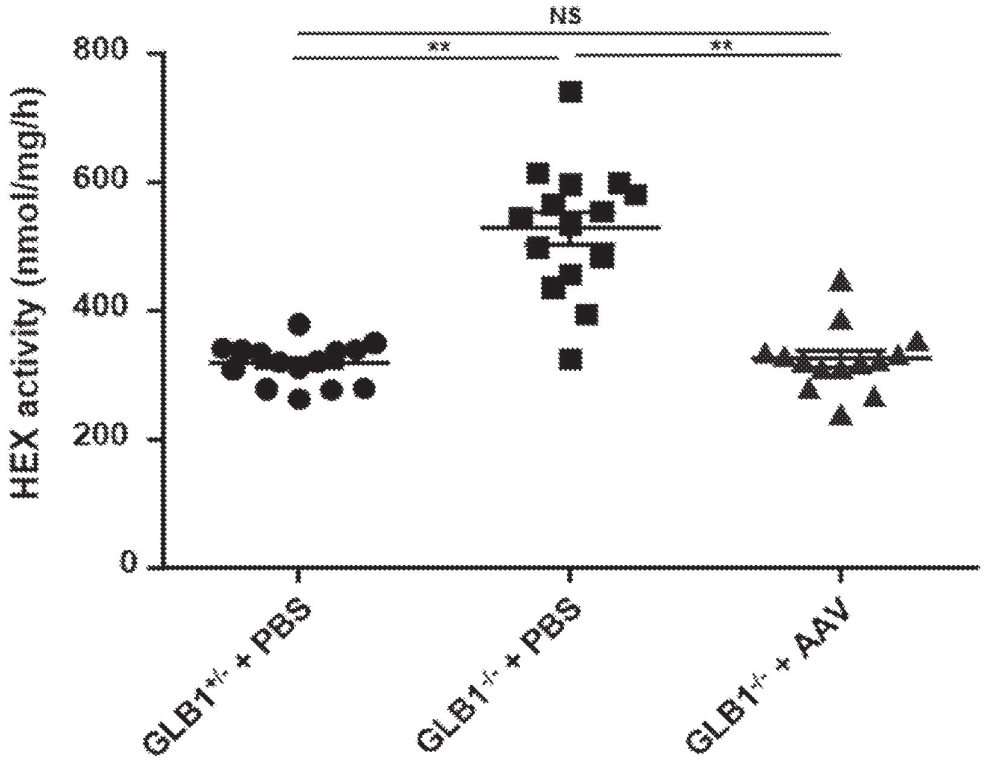
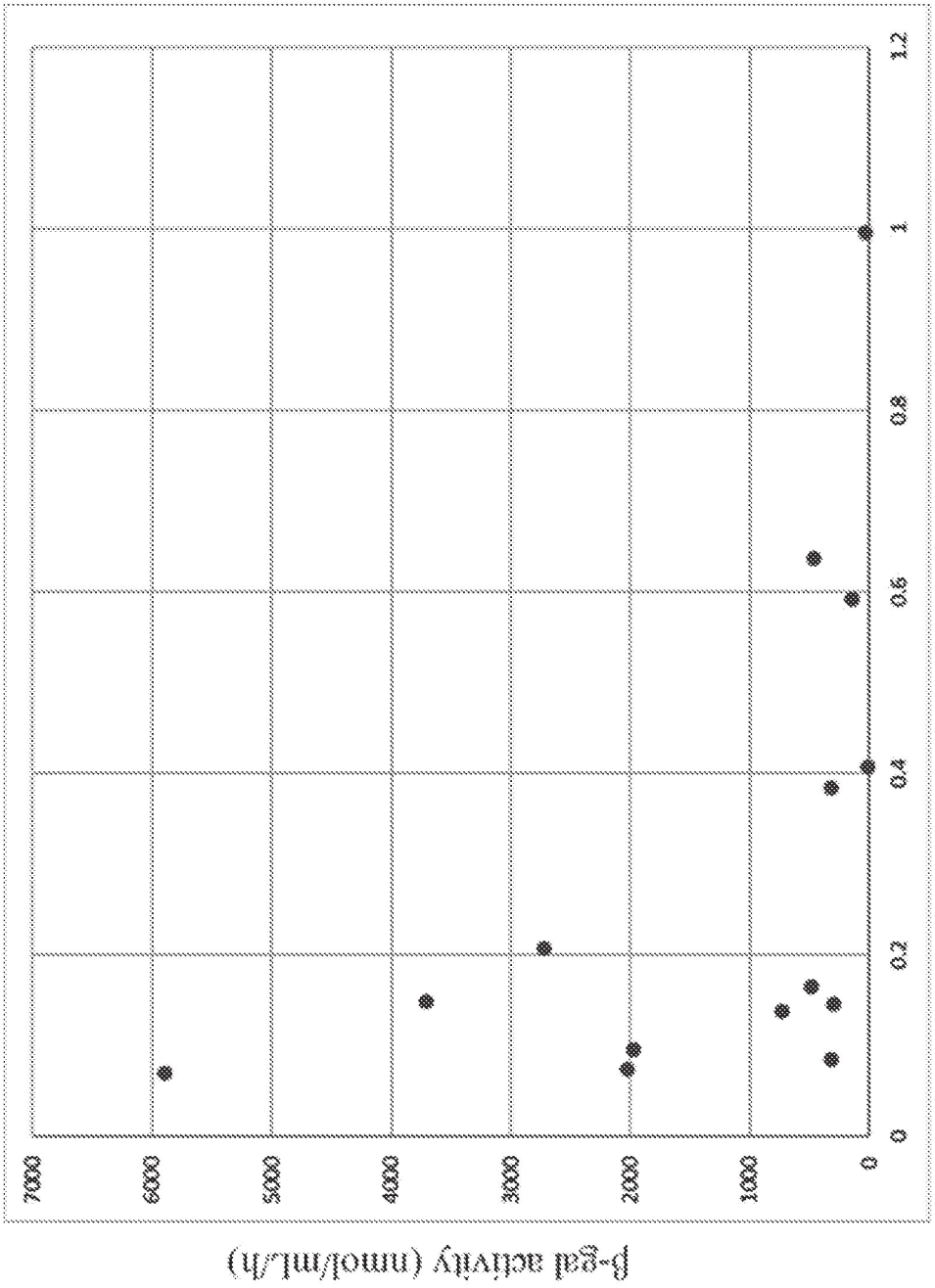

NS
**
**
800
600
400
200
0
HEX activity (nmol/mg/h)
GLB1+/- + PBS
GLB1-/- + PBS
GLB1-/- + AAV

FIG 6


β-gal activity (nmol/mL/h)
7000
6000
5000
4000
3000
2000
1000
0
0
0.2
0.4
0.6
0.8
1
1.2
β-gal antibodies (A_{450})

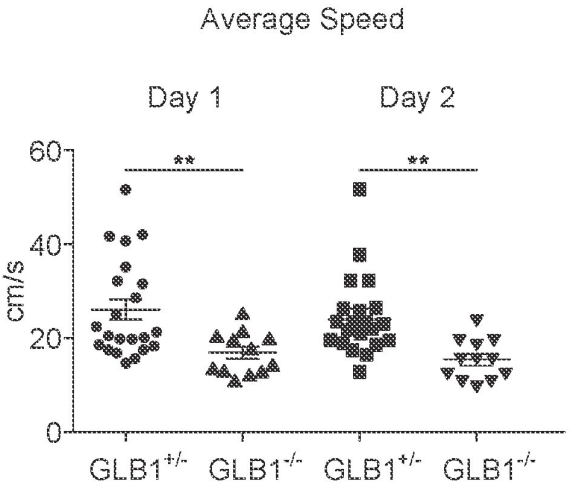


FIG. 7A

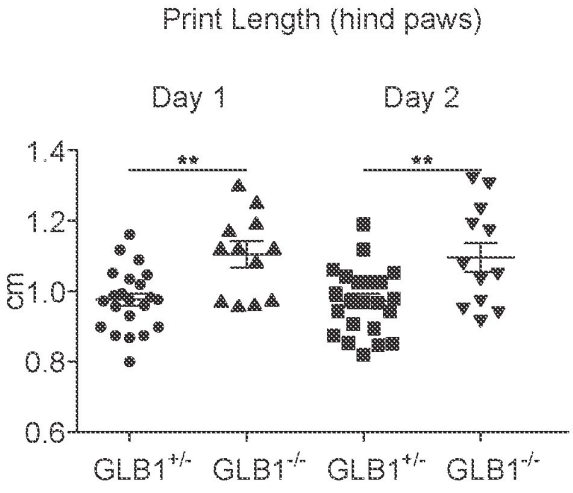


FIG. 7B

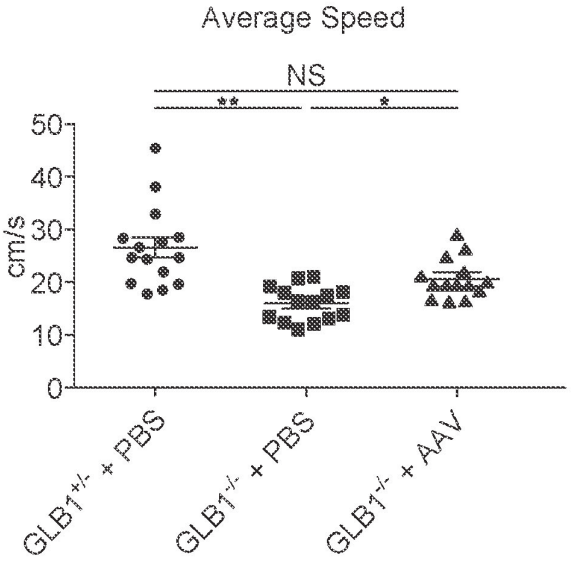


FIG. 7C

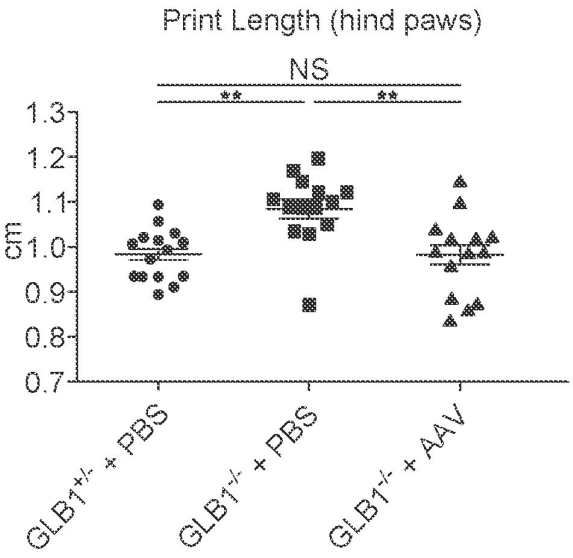


FIG. 7D

FIG 7E

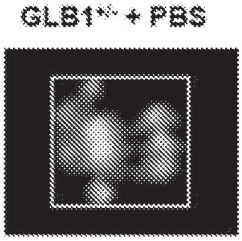


FIG 7F



FIG 7G

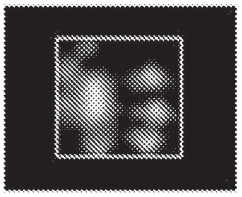


FIG 8A

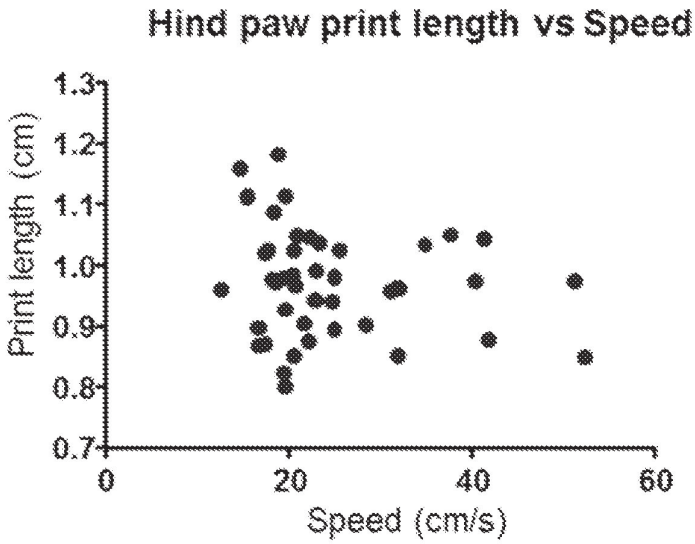


FIG 8B

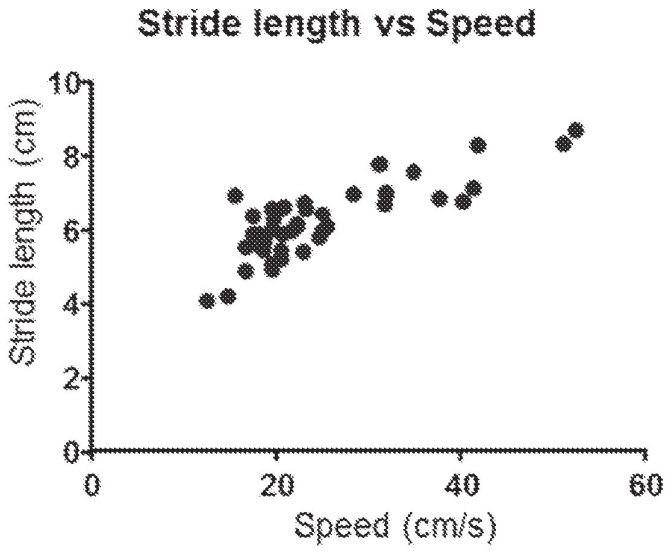


FIG 9A

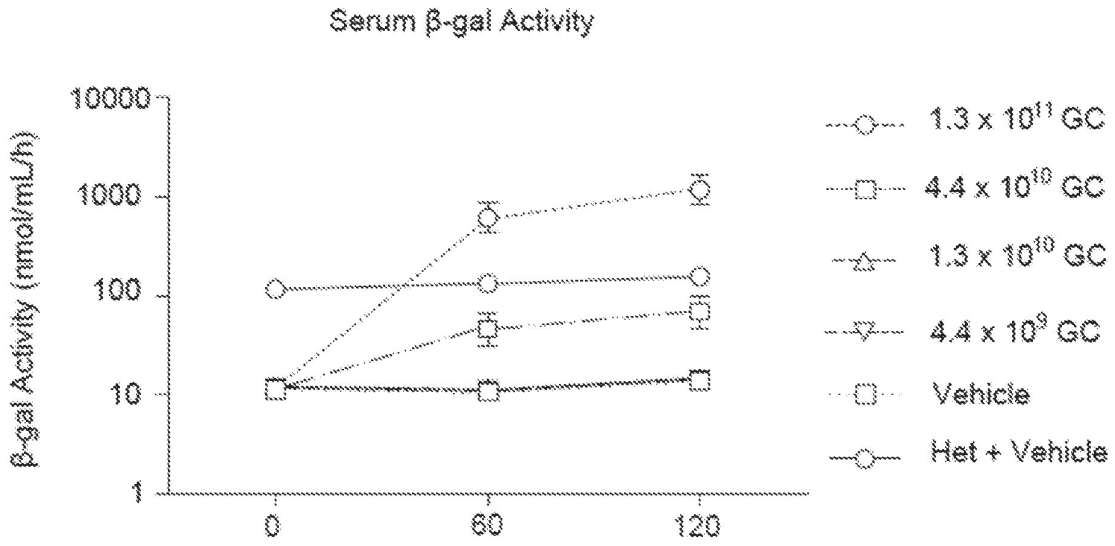

Serum β-gal Activity
β-gal Activity (nmol/mL/h)
10000
1000
100
10
1
0
60
120
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9B

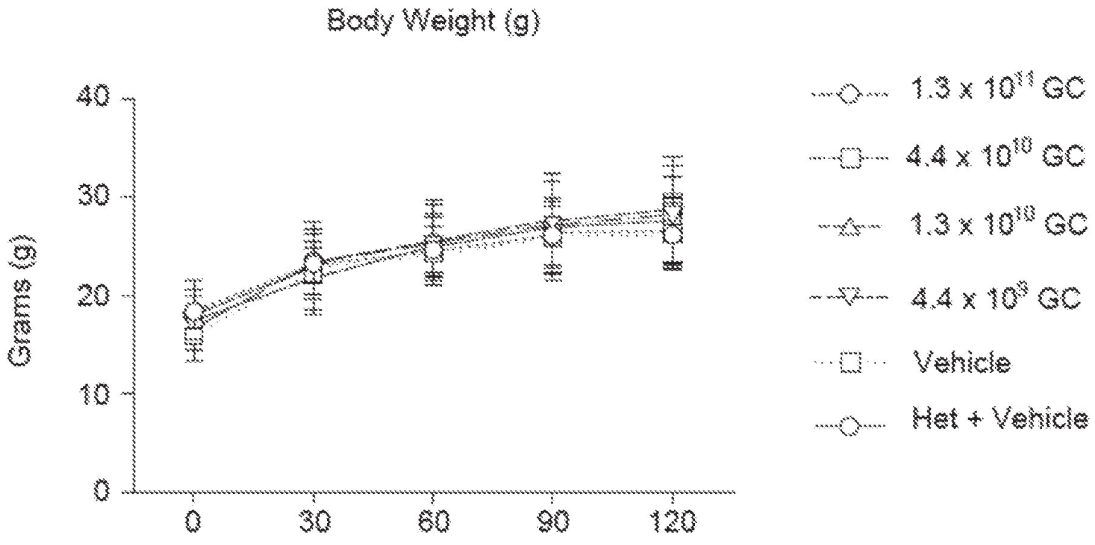

Body Weight (g)
Grams (g)
40
30
20
10
0
0
30
60
90
120
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9C

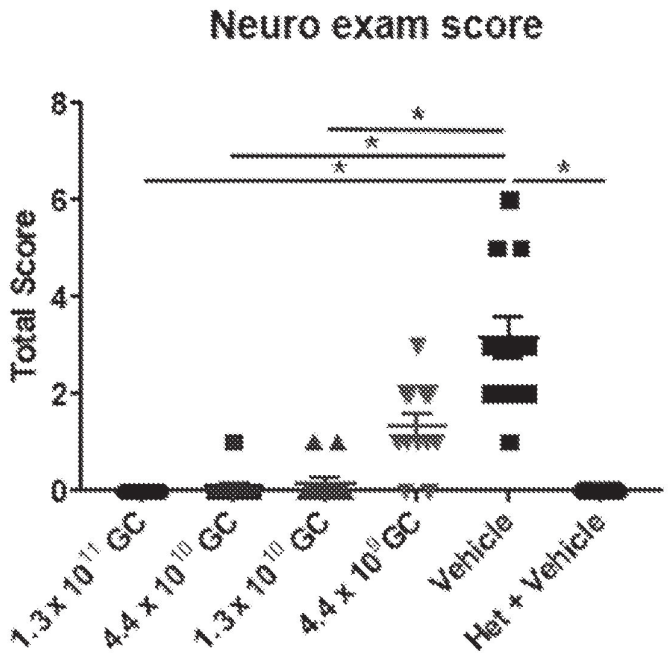

Neuro exam score
Total Score
0
2
4
6
8
*
*
*
*
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9D

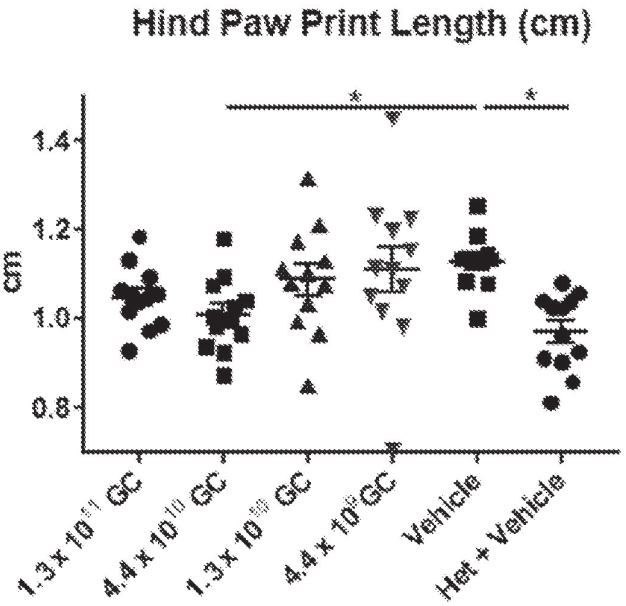

Hind Paw Print Length (cm)
cm
0.8
1.0
1.2
1.4
*
*
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9E

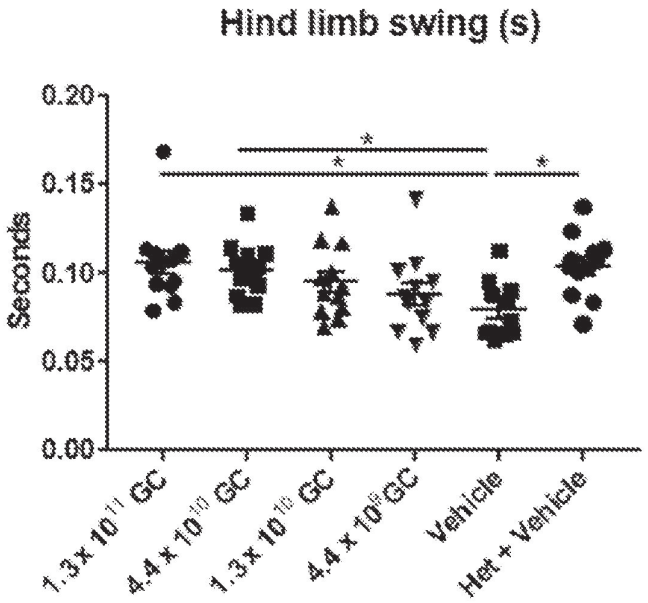

Hind limb swing (s)
Seconds
0.20
0.15
0.10
0.05
0.00
*
*
*
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9F

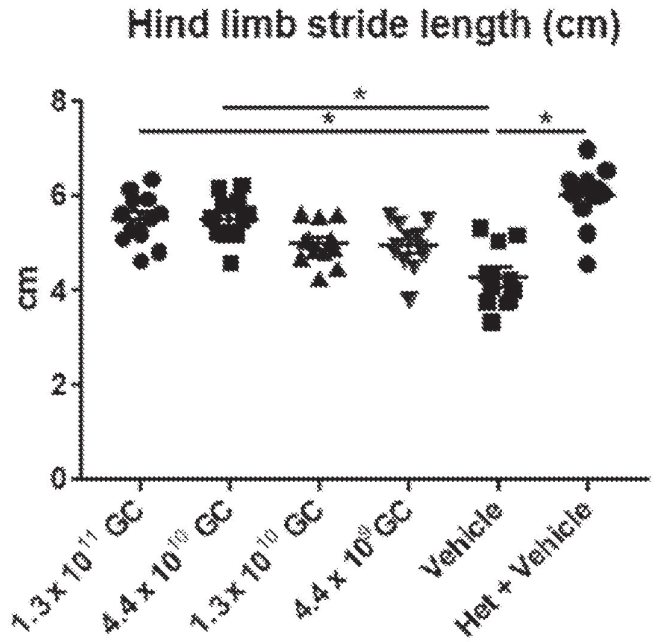
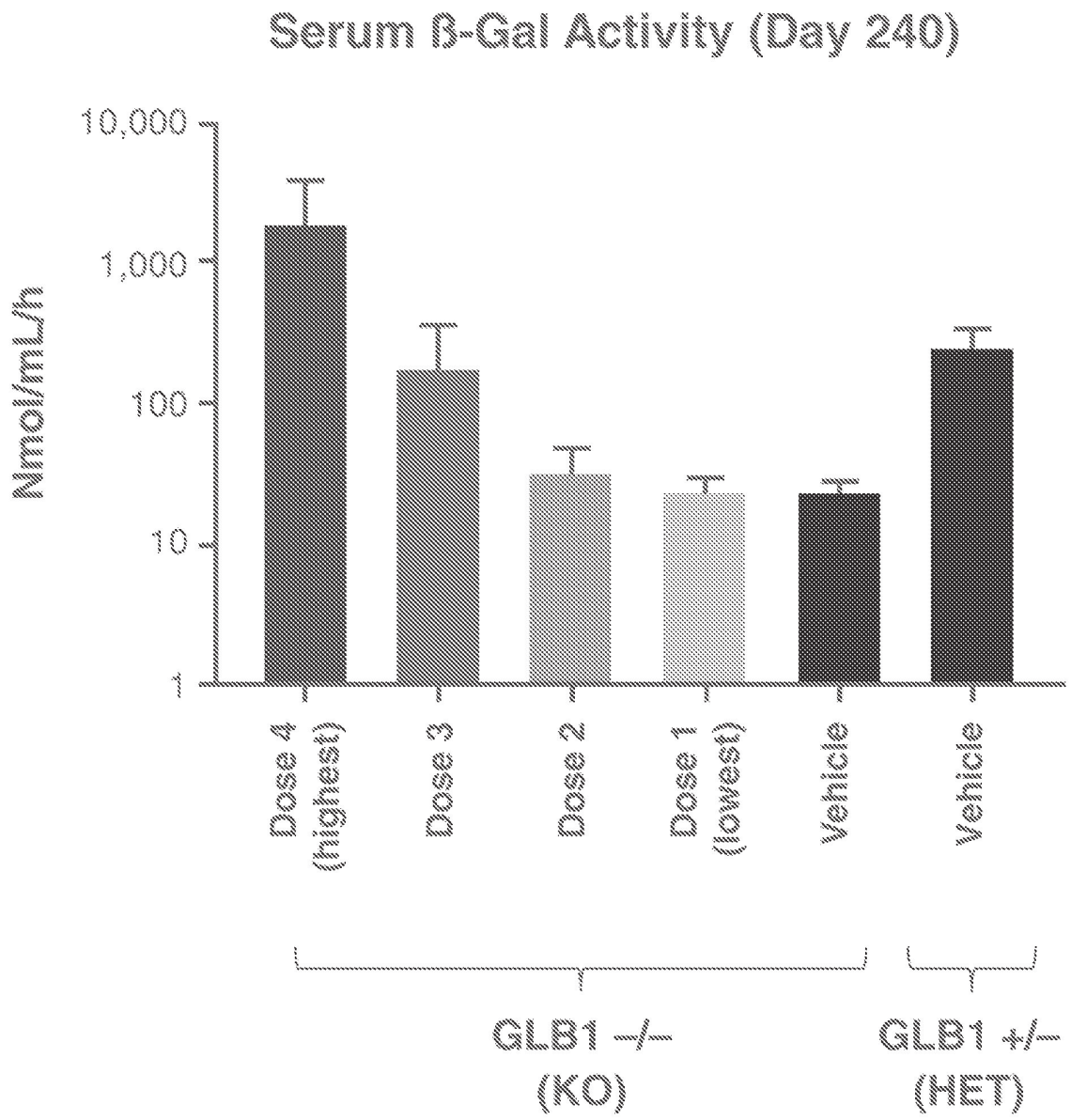

Hind limb stride length (cm)
cm
8
6
4
2
0
*
*
*
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Het + Vehicle

FIG 9G


Serum ß-Gal Activity (Day 240)
Nmol/mL/h
10,000
1,000
100
10
1
Dose 4 (highest)
Dose 3
Dose 2
Dose 1 (lowest)
Vehicle
Vehicle
GLB1 –/– (KO)
GLB1 +/– (HET)


1 100
AAV9 (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
hu.68.VP1 (1) MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
hu.31 (1) MAADGYLPDWLEDTLSEGIRBWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
hu.32 (1) MAADGYLPDWLEDTLSEGIRBWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEF
101 200
AAV9 (101) QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGS
hu.68.VP1 (101) QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSVGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGS
hu.31 (101) QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGSQPAKKKLNFGQTGDTESVPDPQPIGEPPAAPSGVGS
hu.32 (101) QERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGSQPAKKKLNFGQTGDTESVPDPQPIGEPPAAPSGVGS
201 300
AAV9 (201) LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR
hu.68.VP1 (201) LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR
hu.31 (201) LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR
hu.32 (201) LTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR
301 400
AAV9 (301) LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYF
hu.68.VP1 (301) LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYF
hu.31 (301) LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGGQAVGRSSFYCLEYF
hu.32 (301) LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYF
401 500
AAV9 (401) PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSE
hu.68.VP1 (401) PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSE
hu.31 (401) PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSE
hu.32 (401) PSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSE

FIG 10A

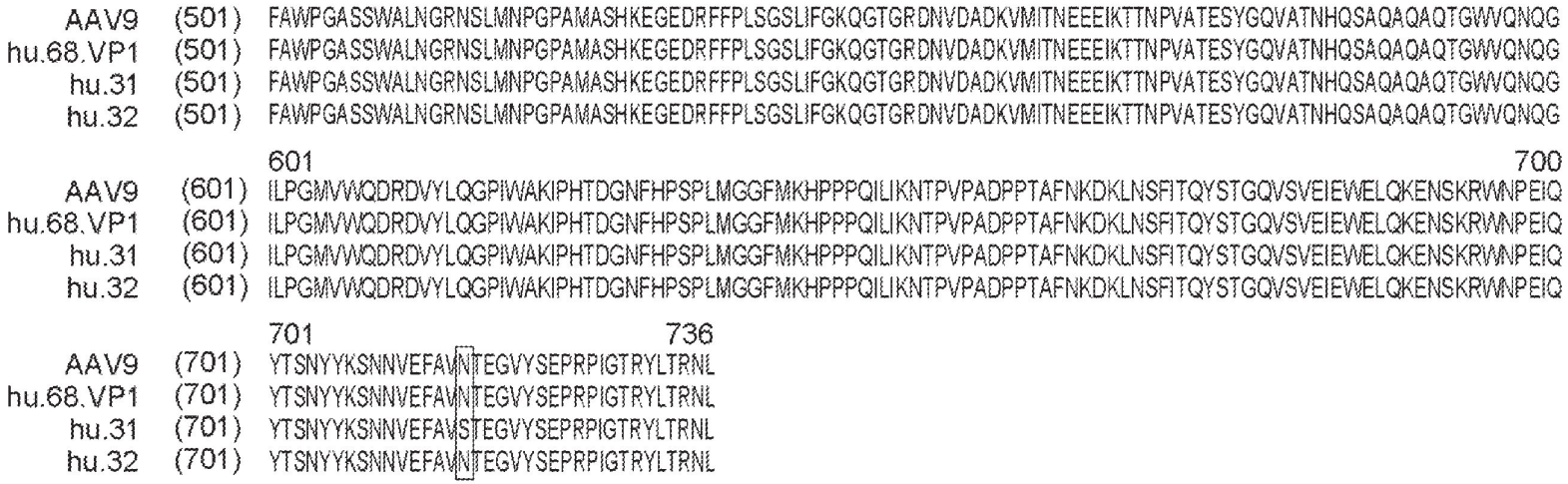

AAV9 (501) FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG
hu.68.VP1 (501) FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG
hu.31 (501) FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG
hu.32 (501) FAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQG
601 700
AAV9 (601) ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
hu.68.VP1 (601) ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
hu.31 (601) ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
hu.32 (601) ILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQ
701 736
AAV9 (701) YTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL
hu.68.VP1 (701) YTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL
hu.31 (701) YTSNYYKSNNVEFAVSTEGVYSEPRPIGTRYLTRNL
hu.32 (701) YTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

FIG 10B


1 100
AAV9 (1) ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACCTTAGTGAAGGAATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCCAAGG
hu.68.VP1 (1) ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCAGTGAAGGCATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCCAAGG
hu.31 (1) ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCCCAACCACCAAAGC
hu.32 (1) ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCAACCACCAAAGC
101 200
AAV9 (101) CAAATCAACAACATCAAGACAACGCTCGAGGTCTTGTGCTTCCGGGTTACAATACCTTGGACCCGGCAACGGACTCGACAAGGGGGAGCCGGTCAACGC
hu.68.VP1 (101) CAAATCAACAACATCAAGACAACGCTCGGGGTCTTGTGCTTCCGGGTTACAATACCTTGGACCCGGCAACGGACTCGACAAGGGGGAGCCGGTCAACGA
hu.31 (101) CCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACCCGGCAACGGACTCGACAAGGGGGAGCCGGTCAACGC
hu.32 (101) CCGCAGAGCGGCATAAGGACGACAGCAGCGGGTCTTGTGCTTCCTGGGTACAAGTACCTCGGACCCGGCAACGGACTCGACAAGGGGGAGCCGGTCAACGC
201 300
AAV9 (201) AGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC
hu.68.VP1 (201) AGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC
hu.31 (201) AGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC
hu.32 (201) AGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCCGAGTTC
301 400
AAV9 (301) CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGG
hu.68.VP1 (301) CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGG
hu.31 (301) CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGG
hu.32 (301) CAGGAGCGGCTCAAAGAAGATACGTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAAGAGGCTTCTTGAACCTCTTGGTCTGGTTGAGG
401 500
AAV9 (401) AAGCGGCTAAGACGGCTCCTGGAAAGAAGAGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGGTATTGGCAAATCGGGTTGCACAGCCCGC
hu.68.VP1 (401) AAGCGGCTAAGACGGCTCCTGGAAAGAAGAGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGTGGGTATTGGCAAATCGGGTTGCACAGCCCGC
hu.31 (401) AAGCGGCTAAGACGGCTCCTGGAAAGAAGAGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGGTATTGGCAAATCGGGTTCACAGCCCGC
hu.32 (401) AAGCGGCTAAGACGGCTCCTGGAAAGAAGAGGCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGGTATTGGCAAATCGGGTTCACAGCCCGC

FIG 11A


501 600
AAV9 (501) TAAAAAGAGACTCAATTTCGGTCAGACTGGCGACACAGAGTCAGTCCCAGACCCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT
hu.68.VP1 (501) TAAAAAGAGACTCAATTTCGGTCAGACTGGCGACACAGAGTCAGTCCCCGACCCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT
hu.31 (501) TAAAAAGAAACTCAATTTCGGTCAGACTGGCGACACAGAGTCAGTCCCCGACCCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT
hu.32 (501) TAAAAAGAAACTCAATTTCGGTCAGACTGGCGACACAGAGTCAGTCCCCGACCCTCAACCAATCGGAGAACCTCCCGCAGCCCCCTCAGGTGTGGGATCT
601 700
AAV9 (601) CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAAT
hu.68.VP1 (601) CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAAT
hu.31 (601) CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAAT
hu.32 (601) CTTACAATGGCTTCAGGTGGTGGCGCACCAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTAGTTCCTCGGGAAATTGGCATTGCGATTCCCAAT
701 800
AAV9 (701) GGCTGGGGGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAATCACCTCTACAAGCAAATCTCCAACAGCACATCTGGAGG
hu.68.VP1 (701) GGCTGGGGGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAATCACCTCTACAAGCAAATCTCCAACAGCACATCTGGAGG
hu.31 (701) GGCTGGGGGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAATCACCTCTACAAGCAAATCTCCAACAGCACATCTGGAGG
hu.32 (701) GGCTGGGGGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCCACCTACAACAATCACCTCTACAAGCAAATCTCCAACAGCACATCTGGAGG
801 900
AAV9 (801) ATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA
hu.68.VP1 (801) ATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAAAGA
hu.31 (801) ATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA
hu.32 (801) ATCTTCAAATGACAACGCCTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGA
901 1000
AAV9 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.68.VP1 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.31 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.32 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA

FIG 11B


901 1000
AAV9 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.68.VP1 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.31 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
hu.32 (901) CTCATCAACAACAACTGGGGATTCCGGCCTAAGCGACTCAACTTCAAGCTCTTCAACATTCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCA
1001 1100
AAV9 (1001) TCGCCAATAACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCCGTT
hu.68.VP1 (1001) TCGCTAATAACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCCGTT
hu.31 (1001) TCGCCAATAACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCCGTT
hu.32 (1001) TCGCCAATAACCTTACCAGCACGGTCCAGGTCTTCACGGACTCAGACTATCAGCTCCCGTACGTGCTCGGGTCGGCTCACGAGGGCTGCCTCCCGCCGTT
1101 1200
AAV9 (1101) CCCAGCGGACGTTTTCATGATTCCTCAGTACGGGTATCTGACGCTTAATGATGGAAGCCAGGCCGTGGGTCGTTCGTCCTTTTACTGCCTGGAATATTTC
hu.68.VP1 (1101) CCCAGCGGACGTTTTCATGATTCCTCAGTACGGGTATCTAACGCTTAATGATGGAAGCCAAGCCGTGGGTCGTTCGTCCTTTTACTGCCTGGAATATTTC
hu.31 (1101) CCCAGCGGACGTTTTCATGATTCCTCAGTACGGGTATCTGACGCTTAATGATGGAGGCCAGGCCGTGGGTCGTTCGTCCTTTTACTGCCTGGAATATTTC
hu.32 (1101) CCCAGCGGACGTTTTCATGATTCCTCAGTACGGGTATCTGACGCTTAATGATGGCAGCCAGGCCGTGGGTCGTTCGTCCTTTTACTGCCTGGAATATTTC
1201 1300
AAV9 (1201) CCGTCGCAAATGCTAAGAACGGGTAACAACTTCCAGTTCAGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACC
hu.68.VP1 (1201) CCGTCGCAAATGCTAAGAACGGGTAACAACTTCCAGTTCAGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTATGCTCACAGCCAAAGCCTGGACC
hu.31 (1201) CCGTCGCAAATGCTAAGAACGGGTAACAACTTCCAGTTCAGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACC
hu.32 (1201) CCGTCGCAAATGCTAAGAACGGGTAACAACTTCCAGTTCAGCTACGAGTTTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGGACC
1301 1400
AAV9 (1301) GACTAATGAATCCACTCATCGACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAGTGTGGCCGG
hu.68.VP1 (1301) GACTCATGAATCCACTCATCGACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAGTGTGGCCGG
hu.31 (1301) GACTAATGAATCCACTCATCGACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAGTGTGGCCGG
hu.32 (1301) GACTAATGAATCCACTCATCGACCAATACTTGTACTATCTCTCAAAGACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAGCGTGGCCGG

FIG 11C


1401 1500
AAV9 (1401) ACCCAGCAACATGGCTGTCCAGGGAAGAAACTACATACCTGGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA
hu.68.VP1 (1401) ACCCAGCAACATGGCTGTCCAGGGAAGAAACTACATACCTGGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA
hu.31 (1401) ACCCAGCAACATGGCTGTCCAGGGAAGAAACTACATACCTGGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA
hu.32 (1401) ACCCAGCAACATGGCTGTCCAGGGAAGAAACTACATACCTGGACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAAACAACAACAGCGAA
1501 1600
AAV9 (1501) TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTT
hu.68.VP1 (1501) TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTT
hu.31 (1501) TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTT
hu.32 (1501) TTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGGACGTAATAGCTTGATGAATCCTGGACCTGCTATGGCCAGCCACAAAGAAGGAGAGGACCGTT
1601 1700
AAV9 (1601) TCTTTCCTTTGTCTGGATCTTTAATTTTTGGCAAACAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAACCAACGAAGAAGAAATTAA
hu.68.VP1 (1601) TCTTTCCTTTGTCTGGATCTTTAATTTTTGGCAAACAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAACCAACGAAGAAGAAATTAA
hu.31 (1601) TCTTTCCTTTGTCGGATCTTTAATTTTTGGCAAACAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAACCAACGAAGAAGAAATTAA
hu.32 (1601) TCTTTCCTTTGTCTGGATCTTTAATTTTTGGCAAACAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAACCAACGAAGAAGAAATTAA
1701 1800
AAV9 (1701) AACTACTAACCCGGTAGCAACGGAGTCCTATGGACAAGTGGCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAAACCAAGGA
hu.68.VP1 (1701) AACTACCAACCCAGTAGCAACGGAGTCCTATGGACAAGTGGCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAAACCAAGGA
hu.31 (1701) AACTACTAACCCGGTAGCAACGGAGTCCTATGGACAAGTGGCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAAACCAAGGA
hu.32 (1701) AACTACTAACCCGGTAGCAACGGAGTCCTATGGACAAGTGGCCACAAACCACCAGAGTGCCCAAGCACAGGCGCAGACCGGCTGGGTTCAAAACCAAGGA

FIG 11D



```
                1801                                                                                                1900
AAV9      (1801) ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGC
hu.68.VP1 (1801) ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGC
hu.31     (1801) ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGC
hu.32     (1801) ATACTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCCATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGC

                1901                                                                                                2000
AAV9      (1901) TGATGGGAGGGTTTGGAATGAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTCCAACGGCCTTCAACAAGGACAAGCT
hu.68.VP1 (1901) TGATGGGAGGGTTTGGAATGAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTCCAACGGCTTTCAACAAGGACAAGCT
hu.31     (1901) TAATGGGAGGGTTTGGAATGAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTCCAACGGCTTTCAATAAGGACAAGCT
hu.32     (1901) TAATGGGAGGGTTTGGAATGAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACACCTGTACCTGCGGATCCTCCAACGGCTTTCAATAAGGACAAGCT

                2001                                                                                                2100
AAV9      (2001) GAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG
hu.68.VP1 (2001) GAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGCGTGGAGATTGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG
hu.31     (2001) GAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGCGTGGAGATTGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG
hu.32     (2001) GAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGCGTGGAGATTGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCGGAGATCCAG

                2101                                                                                                2200
AAV9      (2101) TACACTTCCAACTATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCAGATACCTGACTC
hu.68.VP1 (2101) TACACTTCCAACTATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTTTATTCTGAACCCCGCCCCATTGGCACCAGATACCTGACTC
hu.31     (2101) TACACTTCCAACTATTACAAGTCTAATAATGTTGAATTTGCTGTTAGTACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCAGATACCTGACTC
hu.32     (2101) TACACTTCCAACTATTACAAGTCTAATAATGTTGAATTTGCTGTTAATACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCAGATACCTGACTC

                2201 2211
AAV9      (2201) GTAATCTGTAA
hu.68.VP1 (2201) GTAATCTGTAA
hu.31     (2201) GTAATCTGTAA
hu.32     (2201) GTAATCTGTAA
```

FIG 11E

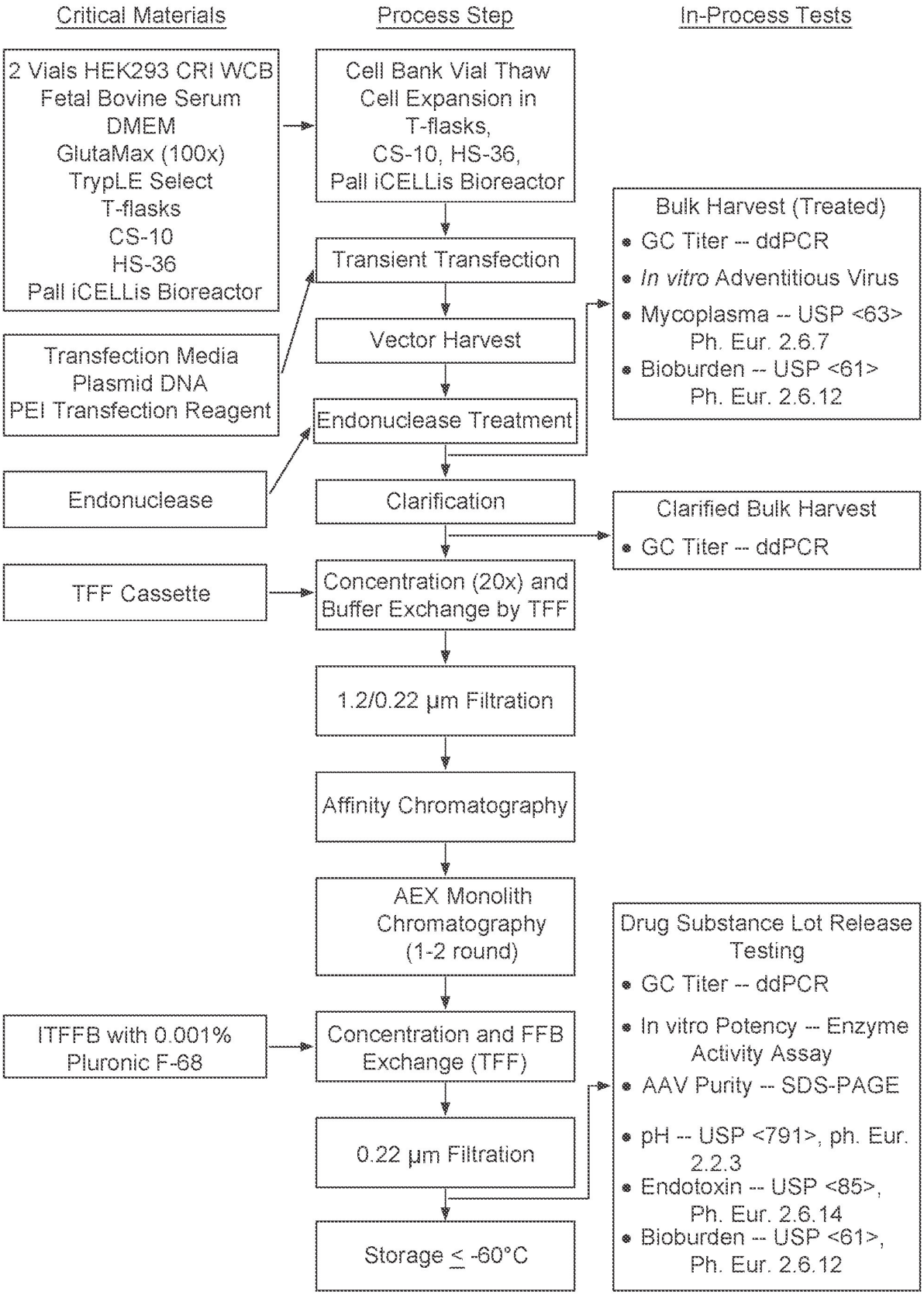

Critical Materials
Process Step
In-Process Tests
2 Vials HEK293 CRI WCB
Fetal Bovine Serum
DMEM
GlutaMax (100x)
TrypLE Select
T-flasks
CS-10
HS-36
Pall iCELLis Bioreactor
Cell Bank Vial Thaw
Cell Expansion in
T-flasks,
CS-10, HS-36,
Pall iCELLis Bioreactor
Transfection Media
Plasmid DNA
PEI Transfection Reagent
Transient Transfection
Vector Harvest
Endonuclease Treatment
Endonuclease
Clarification
TFF Cassette
Concentration (20x) and
Buffer Exchange by TFF
1.2/0.22 µm Filtration
Affinity Chromatography
AEX Monolith
Chromatography
(1-2 round)
ITFFB with 0.001%
Pluronic F-68
Concentration and FFB
Exchange (TFF)
0.22 µm Filtration
Storage ≤ -60°C
Bulk Harvest (Treated)
GC Titer -- ddPCR
In vitro Adventitious Virus
Mycoplasma -- USP <63>
Ph. Eur. 2.6.7
Bioburden -- USP <61>
Ph. Eur. 2.6.12
Clarified Bulk Harvest
GC Titer -- ddPCR
Drug Substance Lot Release
Testing
GC Titer -- ddPCR
In vitro Potency -- Enzyme
Activity Assay
AAV Purity -- SDS-PAGE
pH -- USP <791>, ph. Eur.
2.2.3
Endotoxin -- USP <85>,
Ph. Eur. 2.6.14
Bioburden -- USP <61>,
Ph. Eur. 2.6.12

FIG. 12A

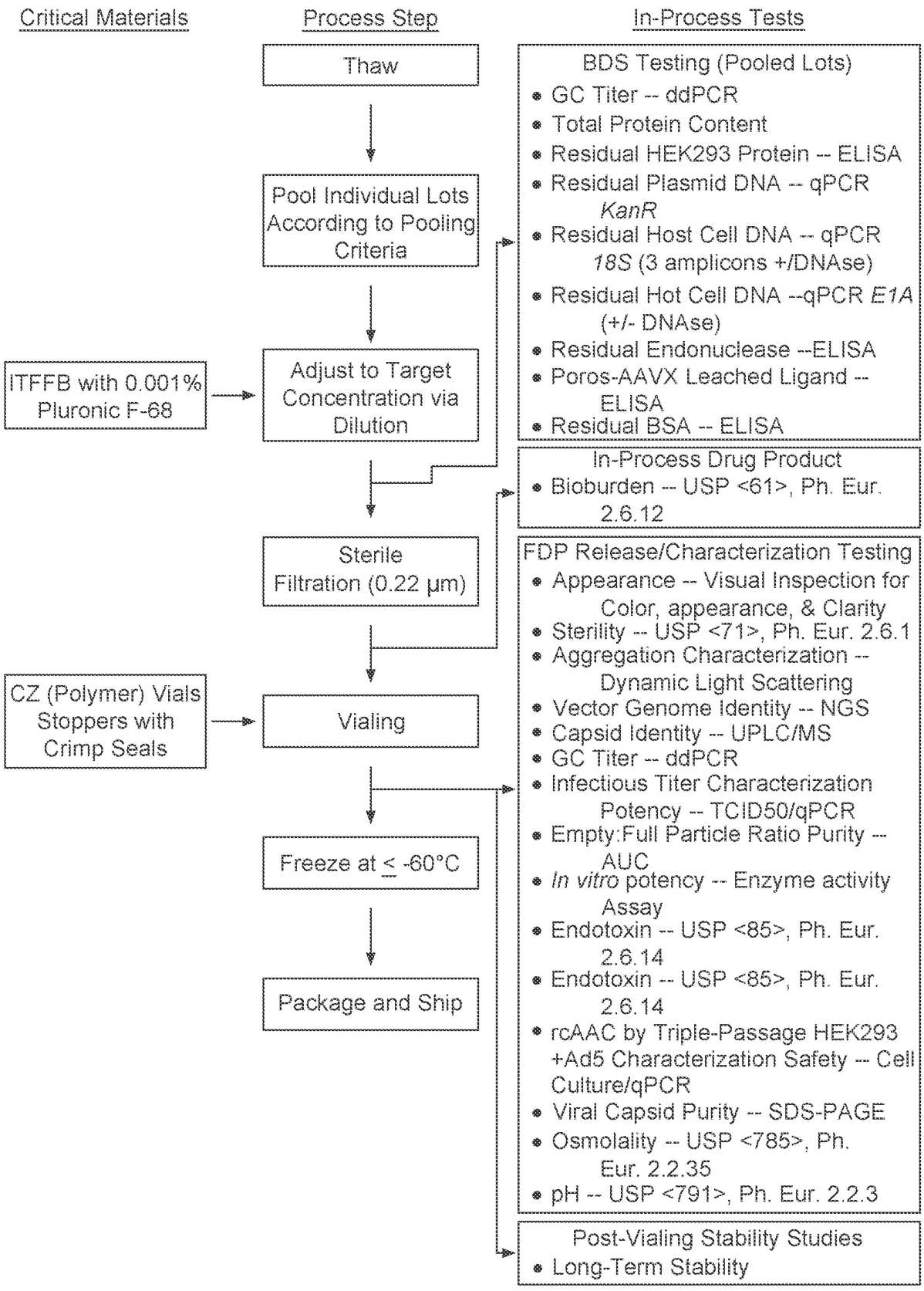

Critical Materials
Process Step
In-Process Tests
Thaw
Pool Individual Lots According to Pooling Criteria
ITFFB with 0.001% Pluronic F-68
Adjust to Target Concentration via Dilution
Sterile Filtration (0.22 µm)
CZ (Polymer) Vials Stoppers with Crimp Seals
Vialing
Freeze at ≤ -60°C
Package and Ship
BDS Testing (Pooled Lots)
GC Titer -- ddPCR
Total Protein Content
Residual HEK293 Protein -- ELISA
Residual Plasmid DNA -- qPCR KanR
Residual Host Cell DNA -- qPCR 18S (3 amplicons +/DNAse)
Residual Hot Cell DNA --qPCR E1A (+/- DNAse)
Residual Endonuclease --ELISA
Poros-AAVX Leached Ligand -- ELISA
Residual BSA -- ELISA
In-Process Drug Product
Bioburden -- USP <61>, Ph. Eur. 2.6.12
FDP Release/Characterization Testing
Appearance -- Visual Inspection for Color, appearance, & Clarity
Sterility -- USP <71>, Ph. Eur. 2.6.1
Aggregation Characterization -- Dynamic Light Scattering
Vector Genome Identity -- NGS
Capsid Identity -- UPLC/MS
GC Titer -- ddPCR
Infectious Titer Characterization Potency -- TCID50/qPCR
Empty:Full Particle Ratio Purity -- AUC
In vitro potency -- Enzyme activity Assay
Endotoxin -- USP <85>, Ph. Eur. 2.6.14
Endotoxin -- USP <85>, Ph. Eur. 2.6.14
rcAAC by Triple-Passage HEK293 +Ad5 Characterization Safety -- Cell Culture/qPCR
Viral Capsid Purity -- SDS-PAGE
Osmolality -- USP <785>, Ph. Eur. 2.2.35
pH -- USP <791>, Ph. Eur. 2.2.3
Post-Vialing Stability Studies
Long-Term Stability

FIG. 12B

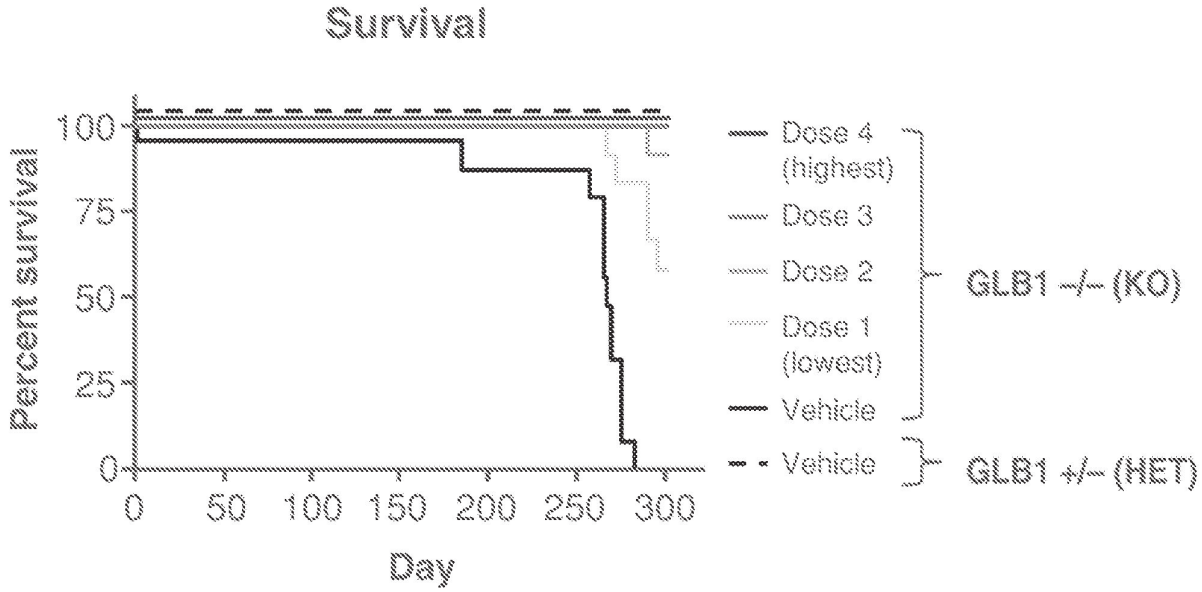

Survival
Percent survival
100
75
50
25
0
0
50
100
150
200
250
300
Day
Dose 4 (highest)
Dose 3
Dose 2
Dose 1 (lowest)
Vehicle
GLB1 -/- (KO)
Vehicle
GLB1 +/- (HET)

FIG 13

FIG 14A

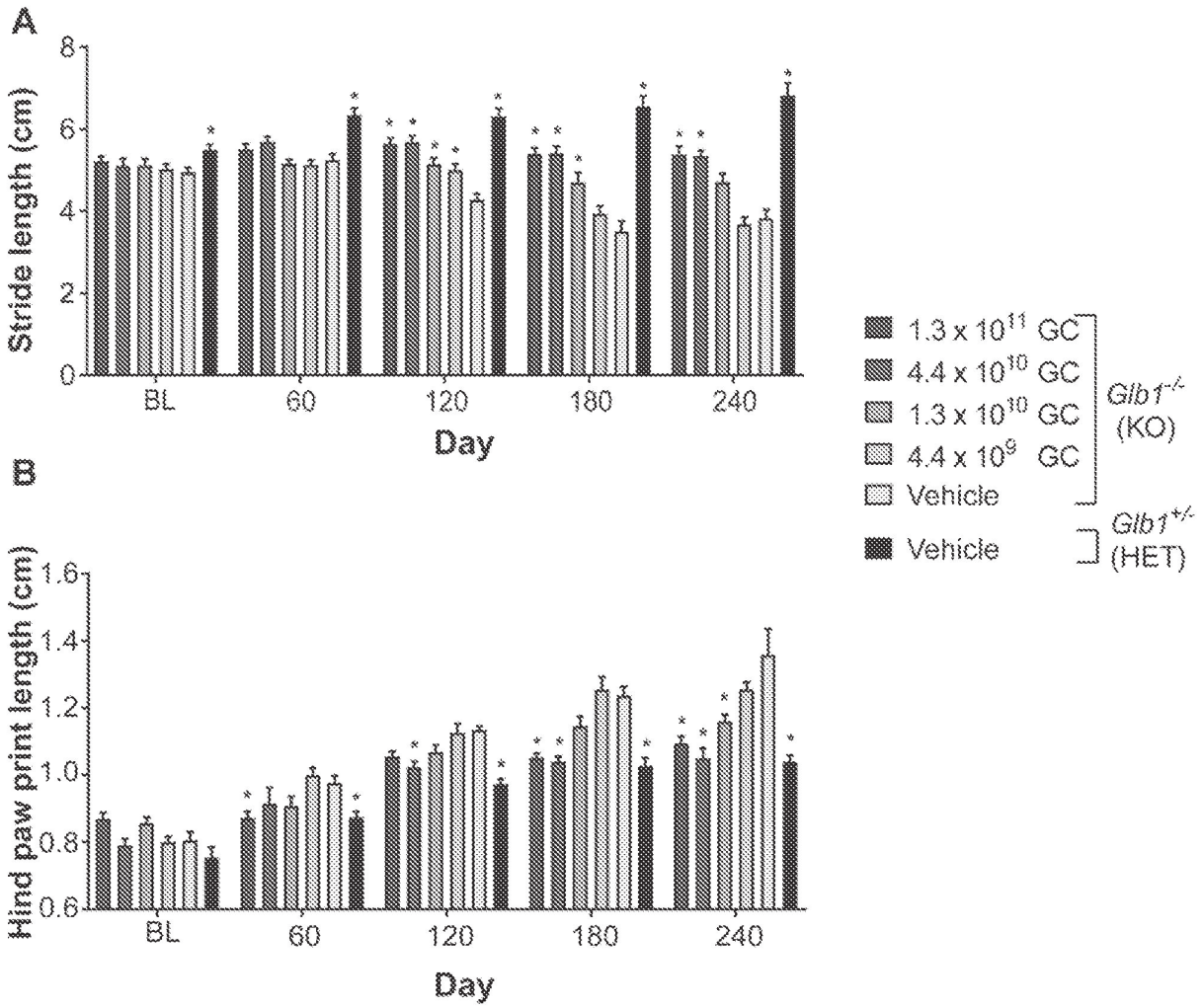


FIG 14B

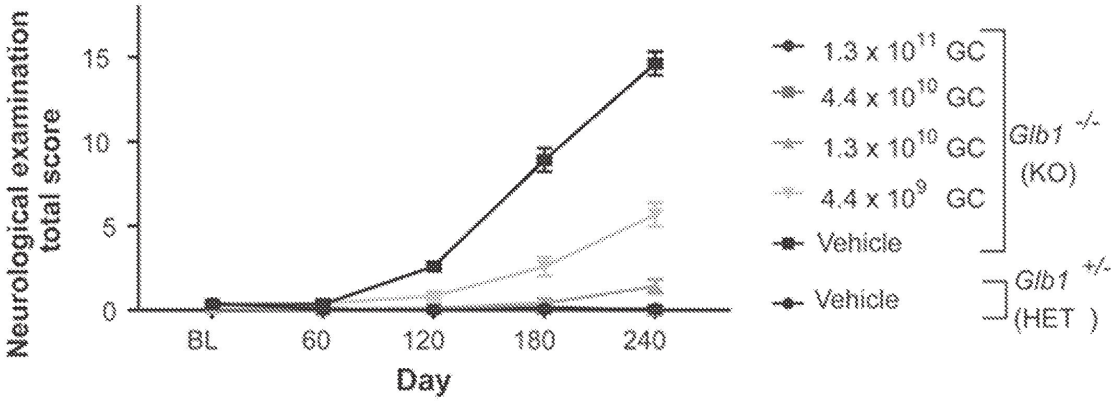

Neurological examination total score
15
10
5
0
BL
60
120
180
240
Day
1.3 x 10^11 GC
4.4 x 10^10 GC
1.3 x 10^10 GC
4.4 x 10^9 GC
Vehicle
Vehicle
Glb1 -/- (KO)
Glb1 +/- (HET)

FIG 14C

FIG 15A

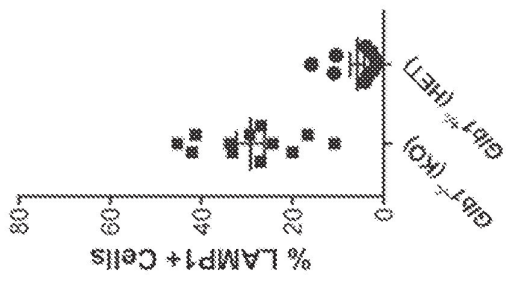


FIG 15B

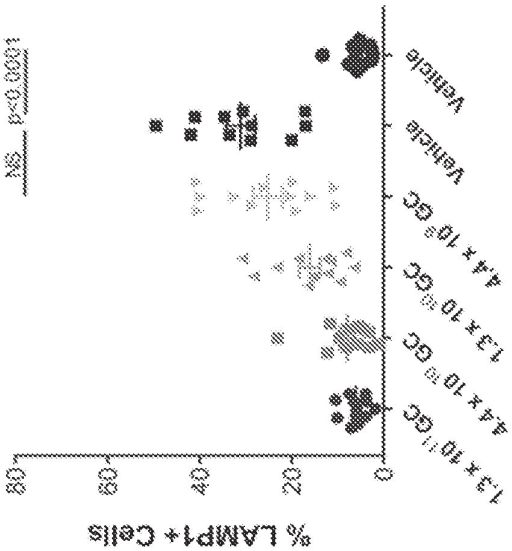


FIG 15C

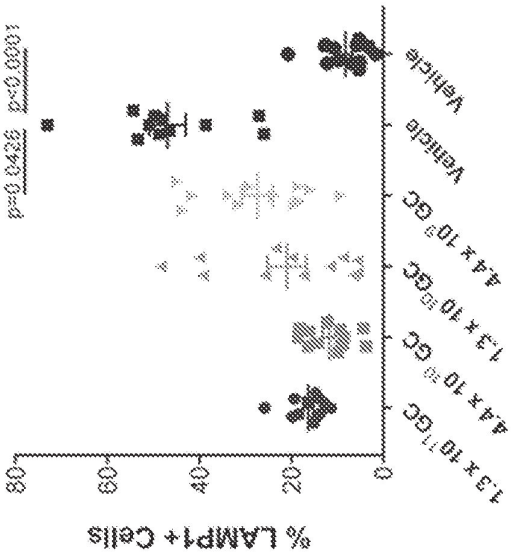
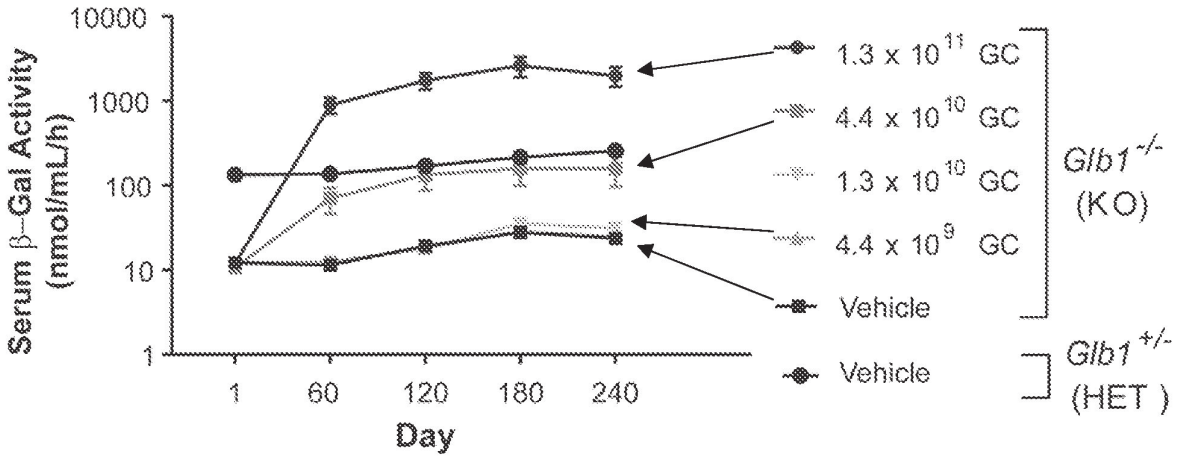


FIG 16A



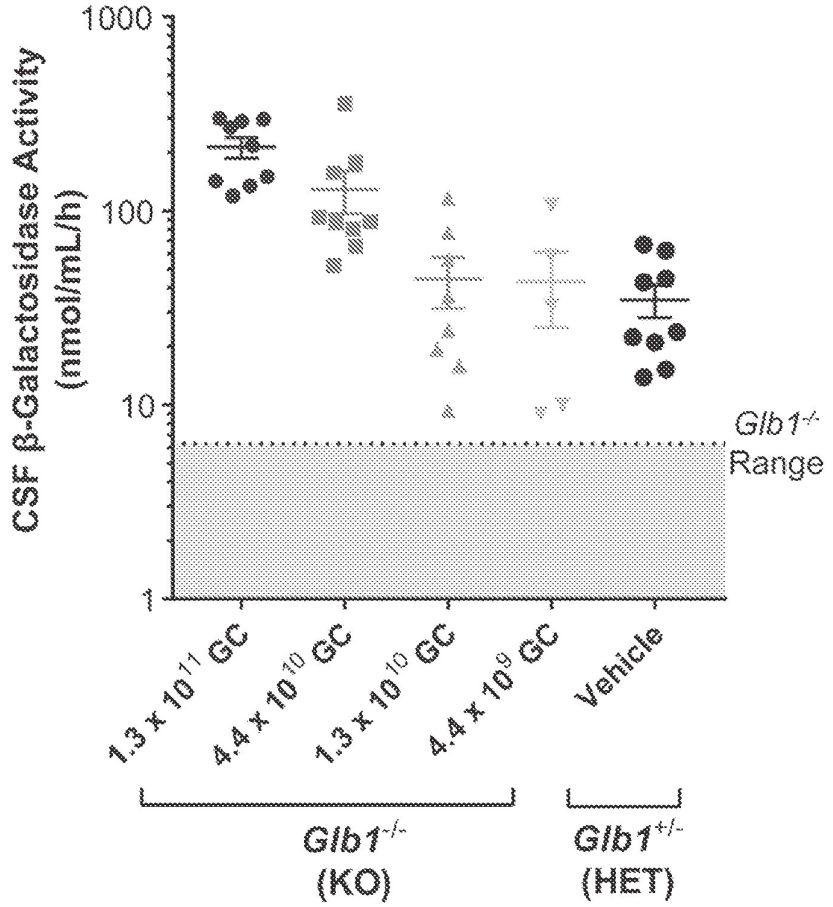


FIG 16B

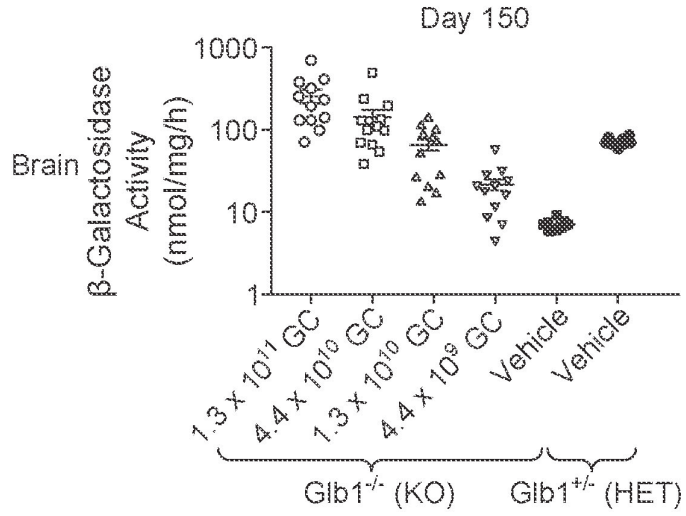


FIG. 17A

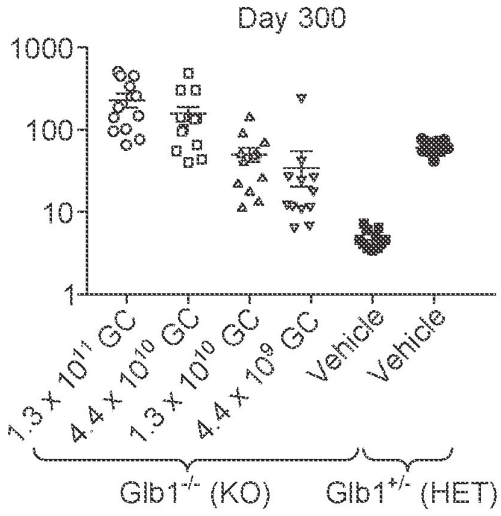


FIG. 17B

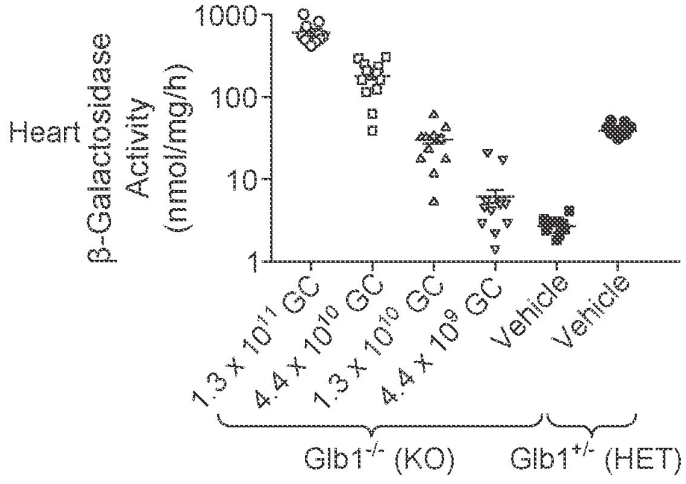


FIG. 17C

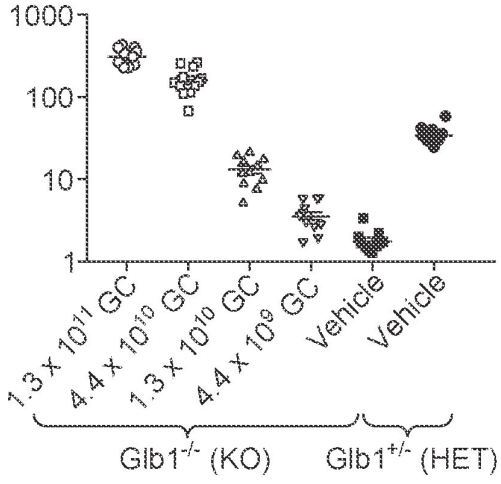


FIG. 17D

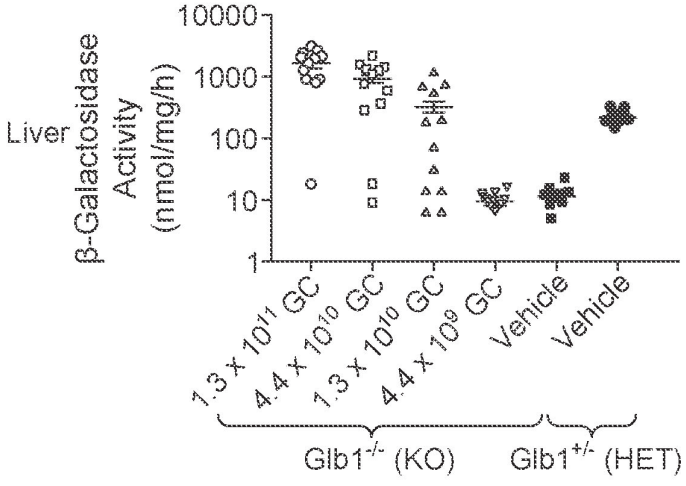


FIG. 17E

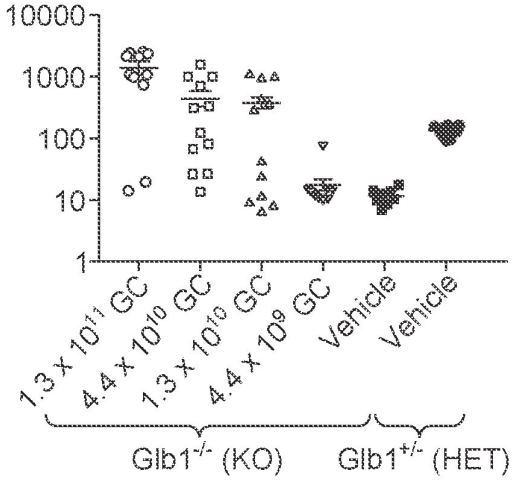


FIG. 17F

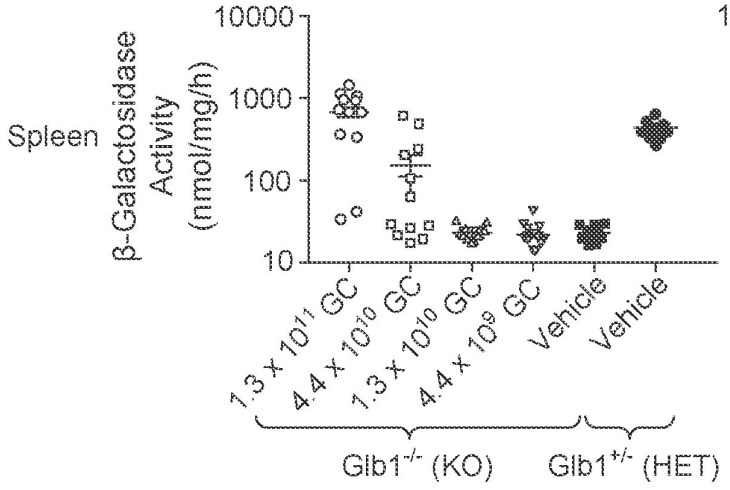


FIG. 17G

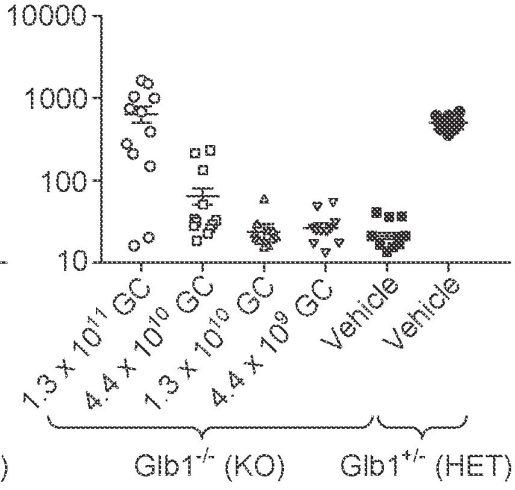


FIG. 17H

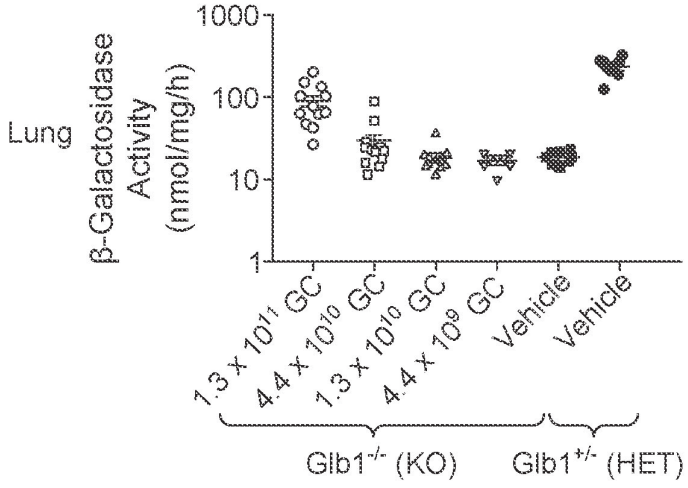


FIG. 17I

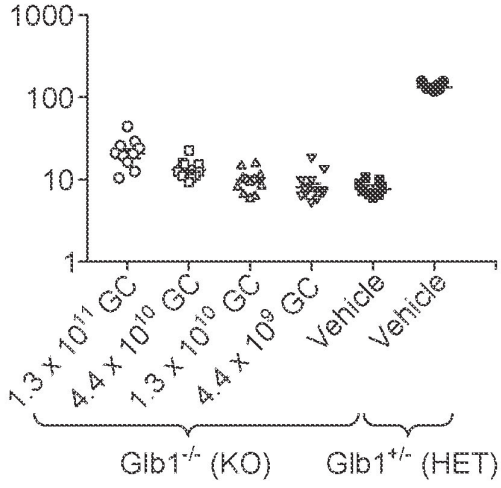


FIG. 17J

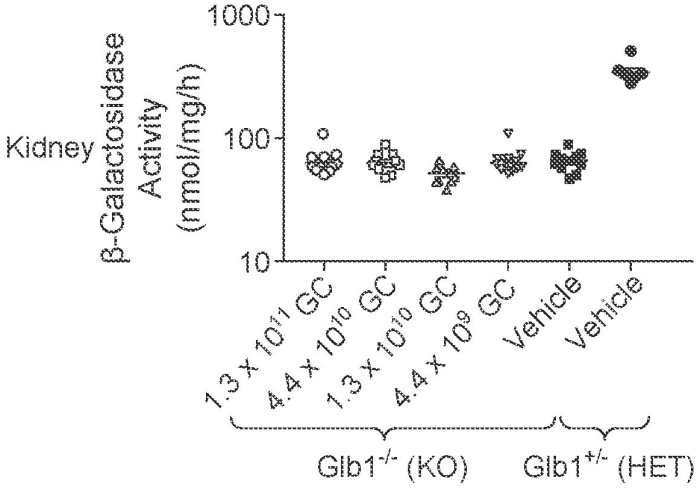


FIG. 17K

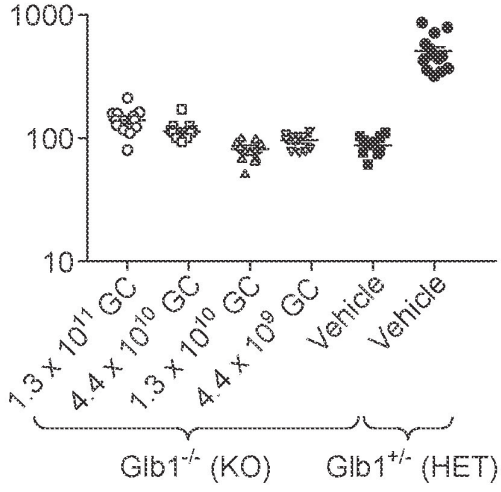


FIG. 17L

FIG 18A

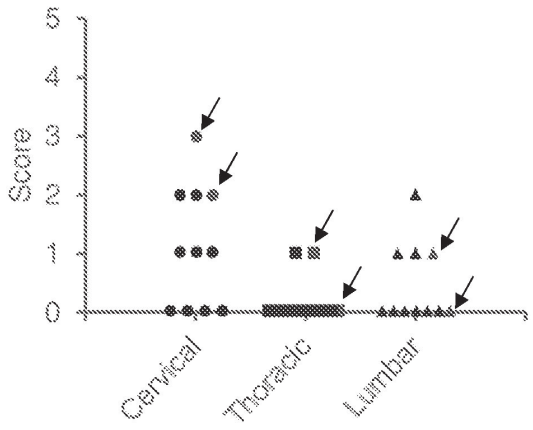


FIG 18B

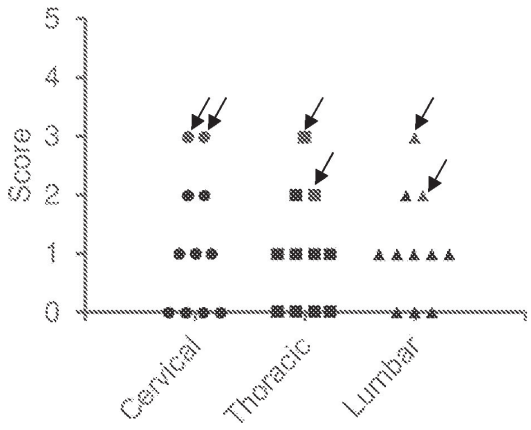


FIG 19A

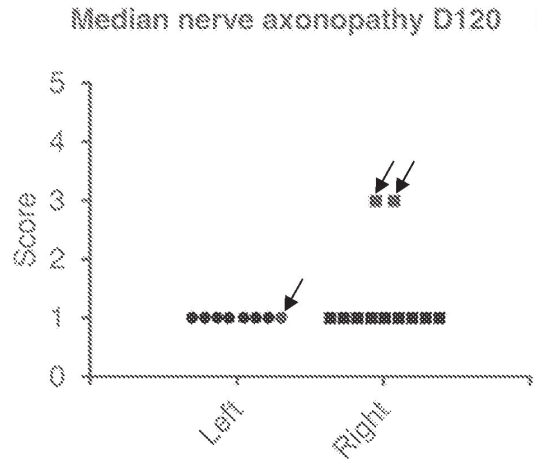
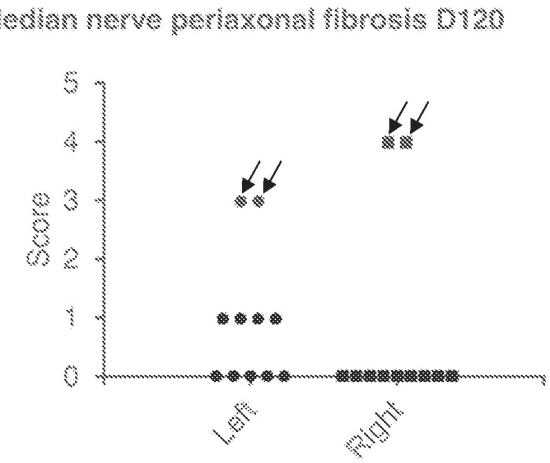


FIG 19B



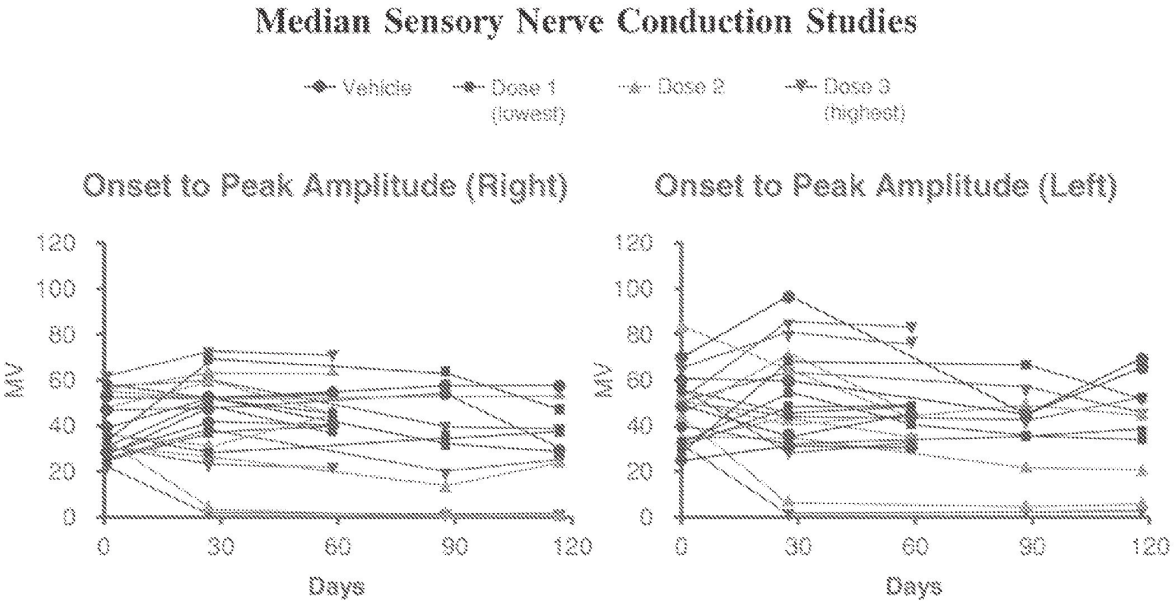


FIG 20A

FIG 20B

FIG 21A

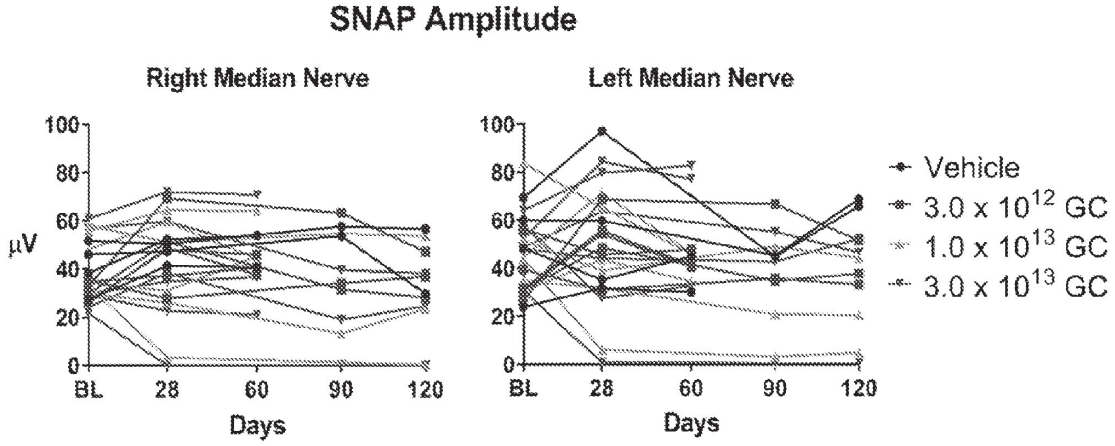


FIG 21B

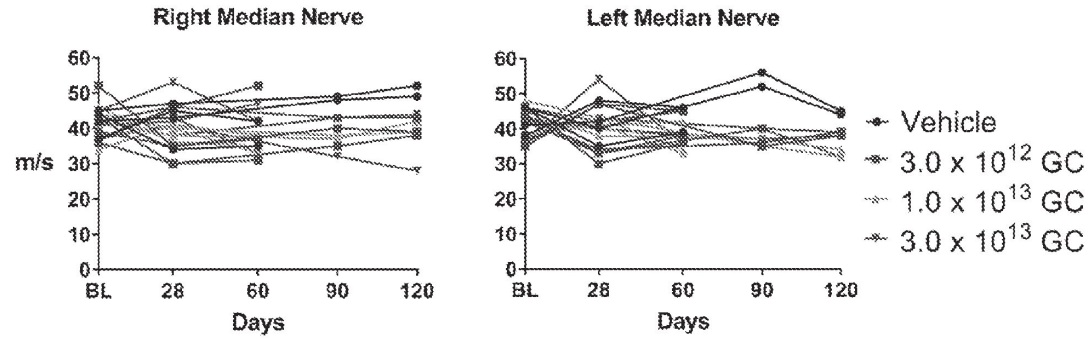


FIG 22A

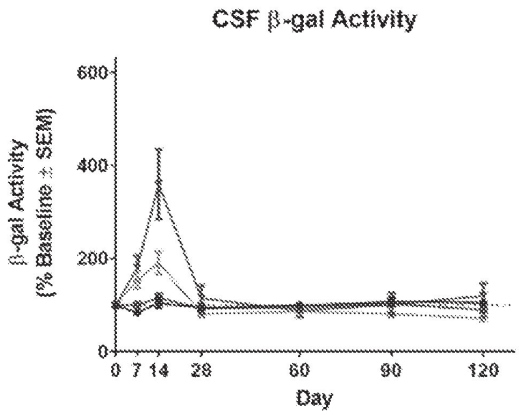


FIG 22B

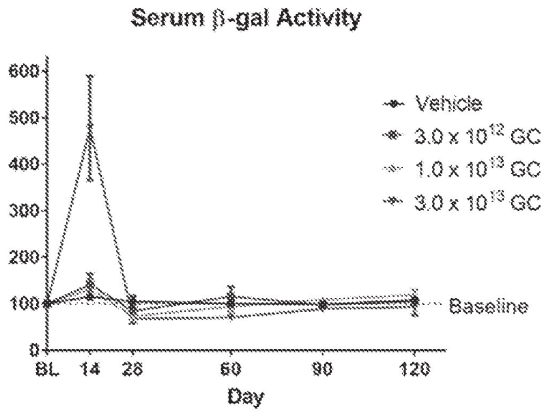


FIG 22C

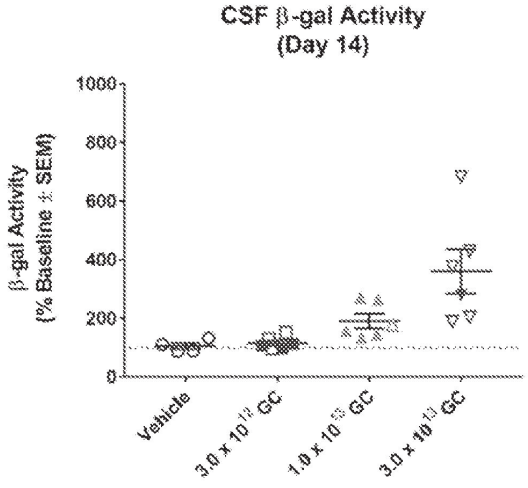
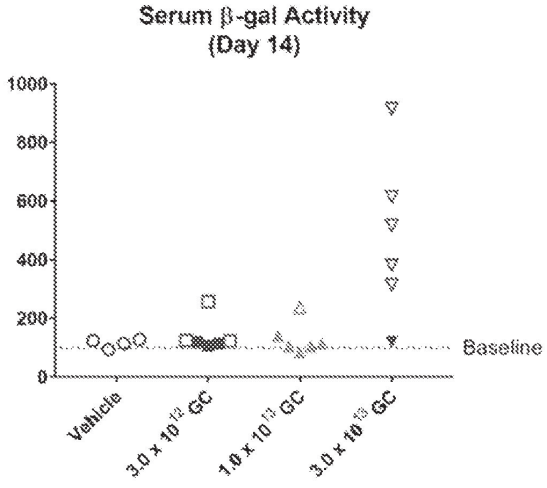


FIG 22D



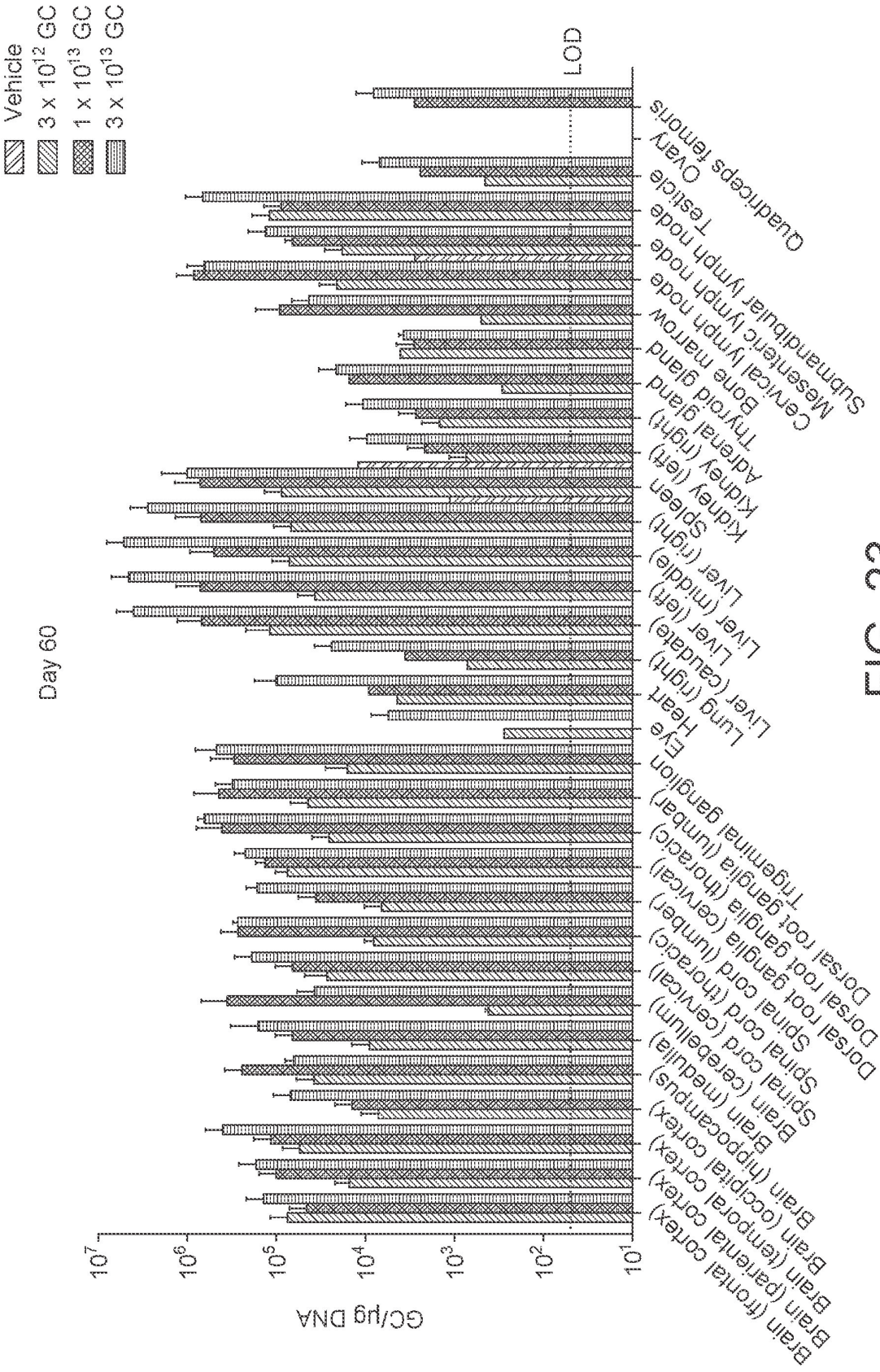

Vehicle
3 x 10^12 GC
1 x 10^13 GC
3 x 10^13 GC
Day 60
GC/µg DNA
10^7
10^6
10^5
10^4
10^3
10^2
10^1
LOD
Brain (frontal cortex)
Brain (parietal cortex)
Brain (temporal cortex)
Brain (occipital cortex)
Brain (hippocampus)
Brain (cerebellum)
Brain (medulla)
Spinal cord (cervical)
Spinal cord (thoracic)
Spinal cord (lumbar)
Dorsal root ganglia (cervical)
Dorsal root ganglia (thoracic)
Dorsal root ganglia (lumbar)
Trigeminal ganglion
Eye
Heart
Lung (right)
Liver (caudate)
Liver (left)
Liver (middle)
Liver (right)
Spleen
Kidney (left)
Kidney (right)
Adrenal gland
Thyroid gland
Bone marrow
Cervical lymph node
Mesenteric lymph node
Submandibular lymph node
Testicle
Ovary
Quadriceps femoris

FIG. 23

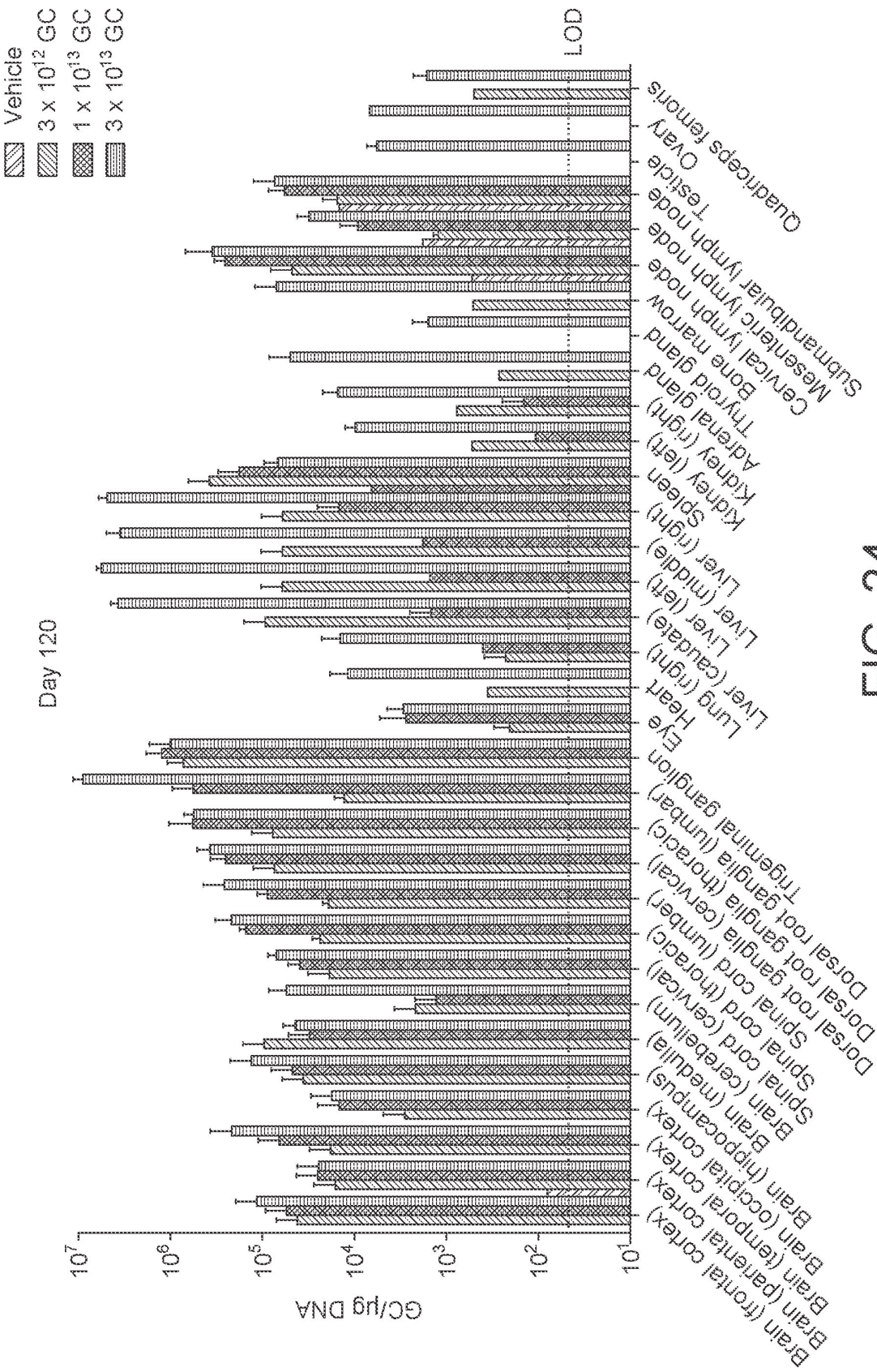

Vehicle
3 x 10^12 GC
1 x 10^13 GC
3 x 10^13 GC
LOD
Day 120
GC/µg DNA
10^7
10^6
10^5
10^4
10^3
10^2
10^1
Brain (frontal cortex)
Brain (parietal cortex)
Brain (temporal cortex)
Brain (occipital cortex)
Brain (hippocampus)
Brain (medulla)
Brain (cerebellum)
Spinal cord (cervical)
Spinal cord (thoracic)
Spinal cord (lumbar)
Dorsal root ganglia (cervical)
Dorsal root ganglia (thoracic)
Dorsal root ganglia (lumbar)
Trigeminal ganglion
Eye
Heart
Lung (right)
Liver (caudate)
Liver (left)
Liver (middle)
Liver (right)
Spleen
Kidney (left)
Kidney (right)
Adrenal gland
Thyroid gland
Bone marrow
Cervical lymph node
Mesenteric lymph node
Submandibular lymph node
Testicle
Ovary
Quadriceps femoris

FIG. 24