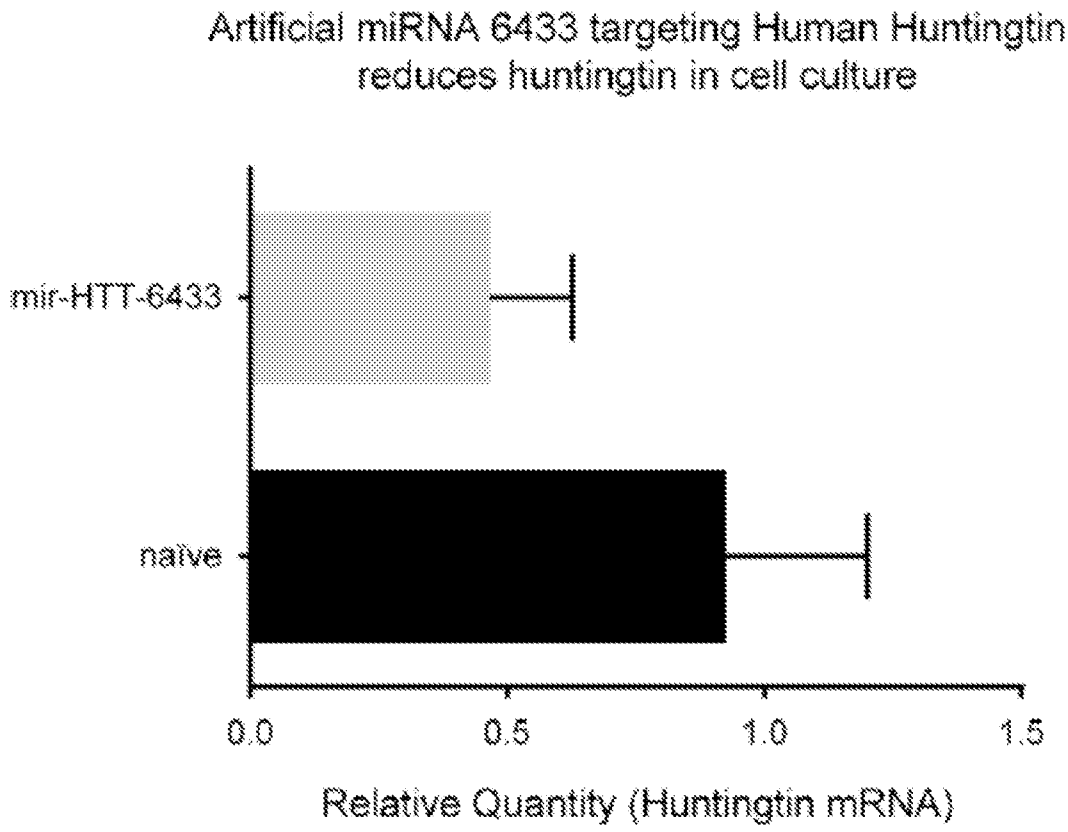

Artificial miRNA 6433 targeting Human Huntingtin
reduces huntingtin in cell culture
mir-HTT-6433
naive
0.0
0.5
1.0
1.5
Relative Quantity (Huntingtin mRNA)

FIG. 1

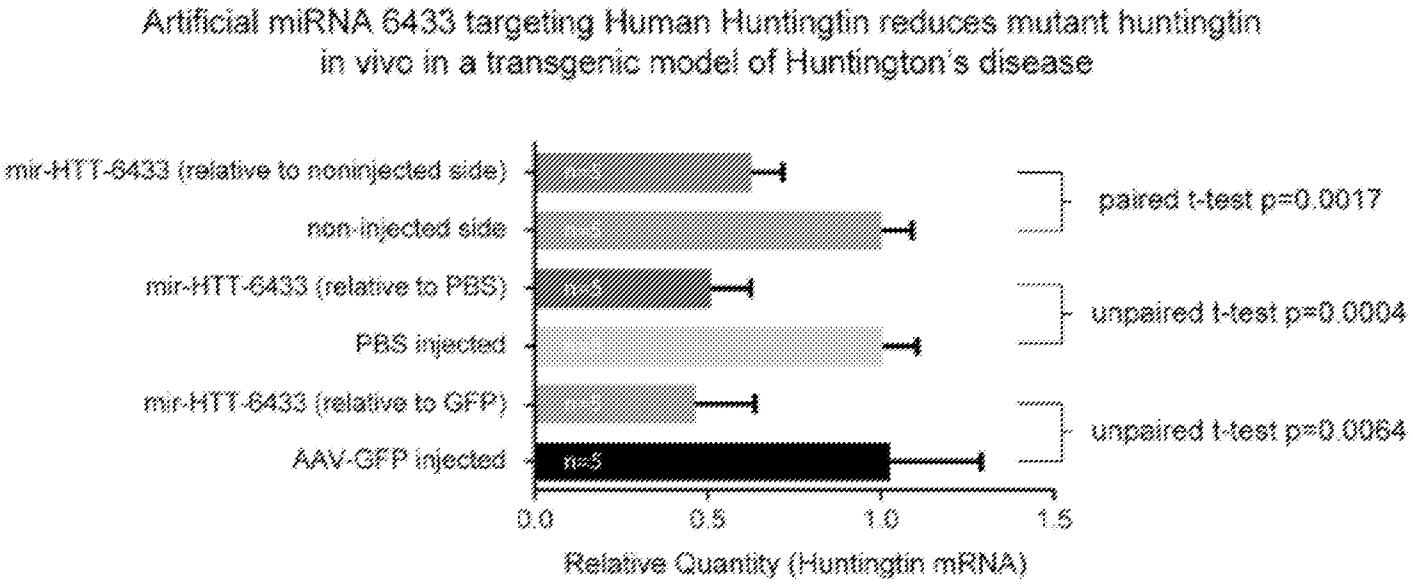

Artificial miRNA 6433 targeting Human Huntingtin reduces mutant huntingtin in vivo in a transgenic model of Huntington's disease
mir-HTT-6433 (relative to noninjected side)
non-injected side
mir-HTT-6433 (relative to PBS)
PBS injected
mir-HTT-6433 (relative to GFP)
AAV-GFP injected
n=5
paired t-test p=0.0017
unpaired t-test p=0.0004
unpaired t-test p=0.0064
0.0
0.5
1.0
1.5
Relative Quantity (Huntingtin mRNA)

FIG. 2

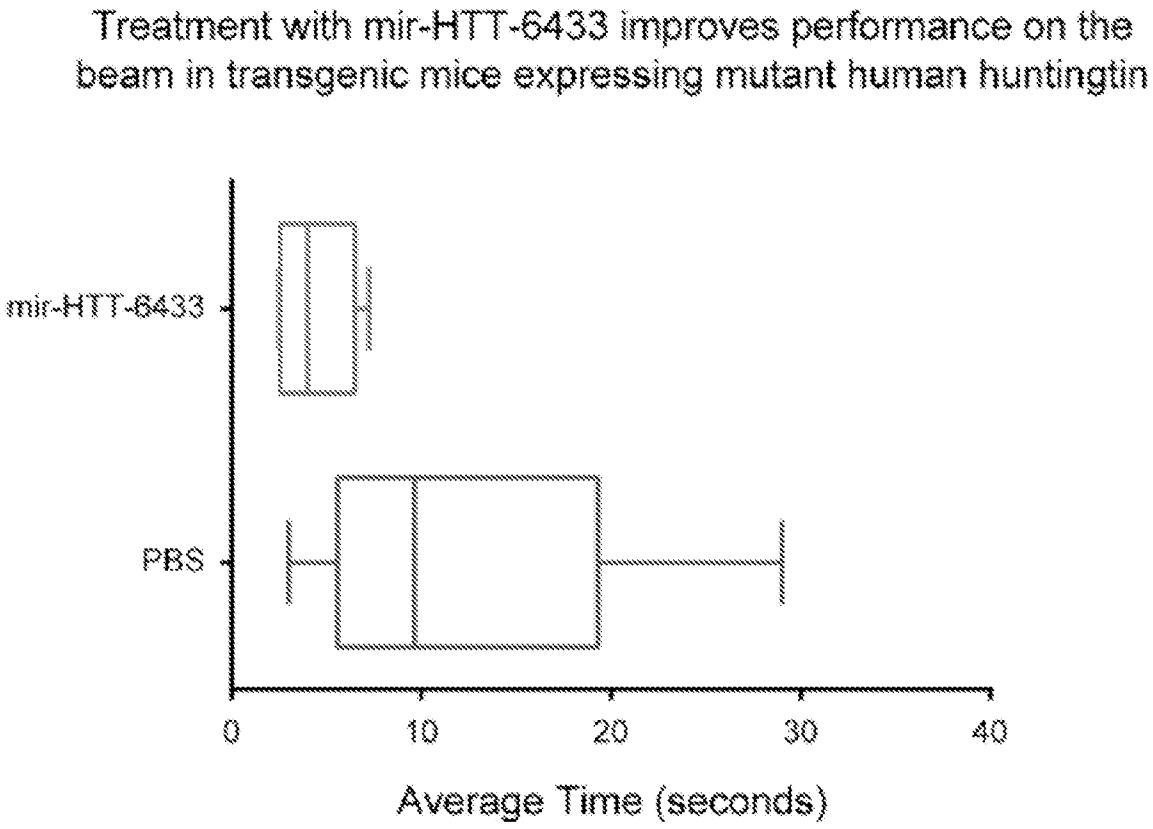

Treatment with mir-HTT-6433 improves performance on the beam in transgenic mice expressing mutant human huntingtin
mir-HTT-6433
PBS
0
10
20
30
40
Average Time (seconds)

FIG. 3

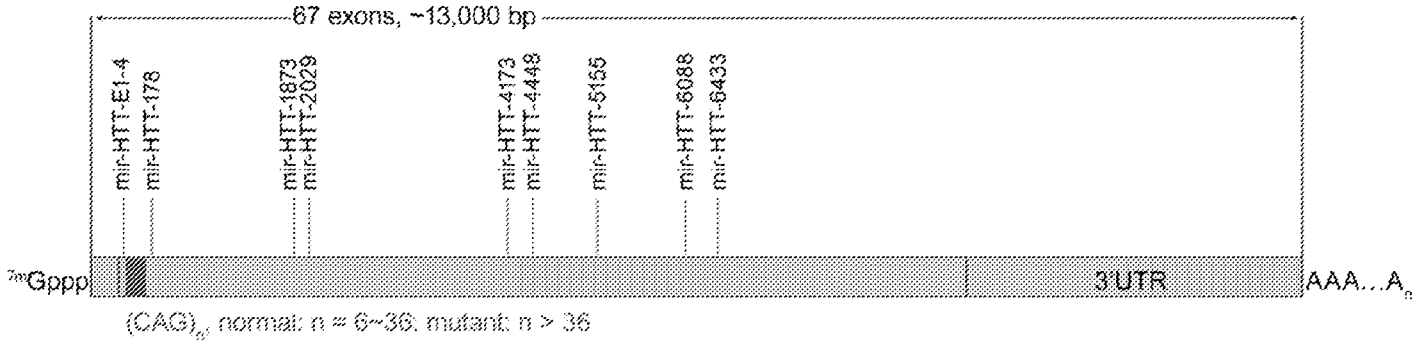

67 exons, ~13,000 bp
miR-HTT-E1-4
miR-HTT-178
miR-HTT-1873
miR-HTT-2029
miR-HTT-4173
miR-HTT-4449
miR-HTT-5155
miR-HTT-6088
miR-HTT-6433
7mGppp
3'UTR
AAA...An
(CAG)n normal: n = 6~36; mutant: n > 36

FIG. 4A

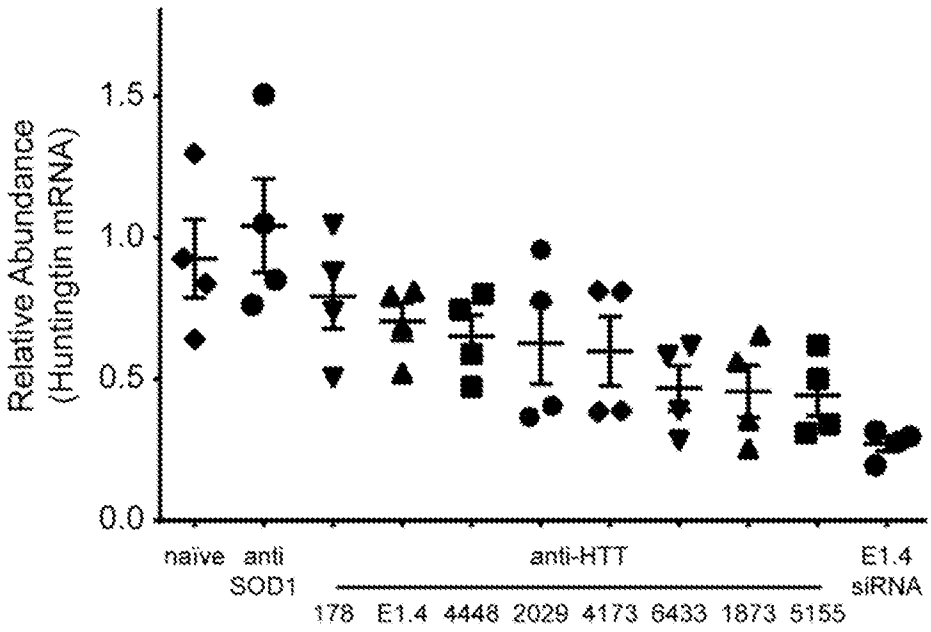

Relative Abundance
(Huntingtin mRNA)
1.5
1.0
0.5
0.0
naïve
anti
SOD1
anti-HTT
E1.4
siRNA
178
E1.4
4448
2029
4173
6433
1873
5155

FIG. 4B

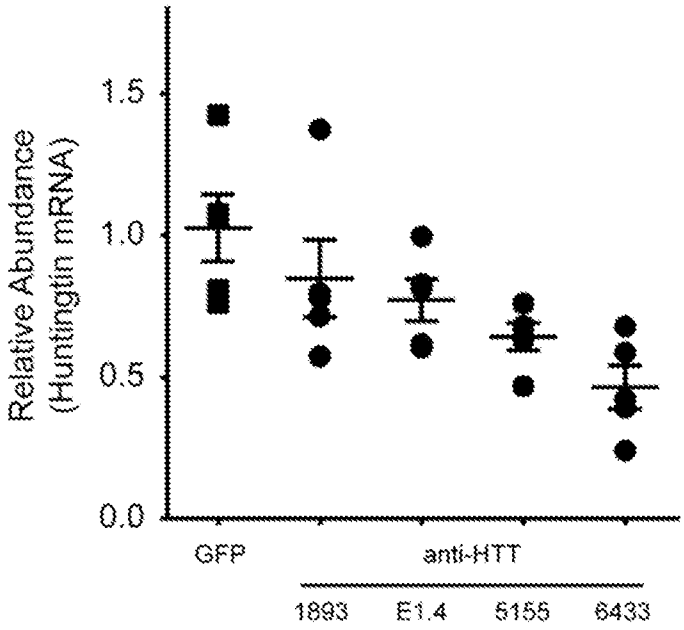

Relative Abundance
(Huntingtin mRNA)
1.5
1.0
0.5
0.0
GFP
anti-HTT
1893
E1.4
5155
6433

FIG. 4C

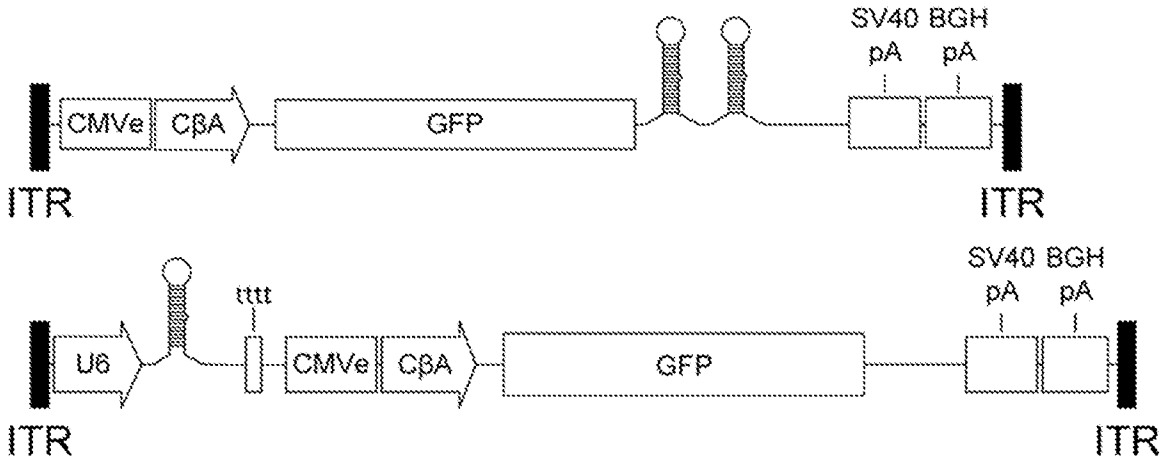

SV40 BGH
pA pA
CMVe CβA
GFP
ITR
ITR
SV40 BGH
pA pA
tttt
U6
CMVe CβA
GFP
ITR
ITR

FIG. 5A

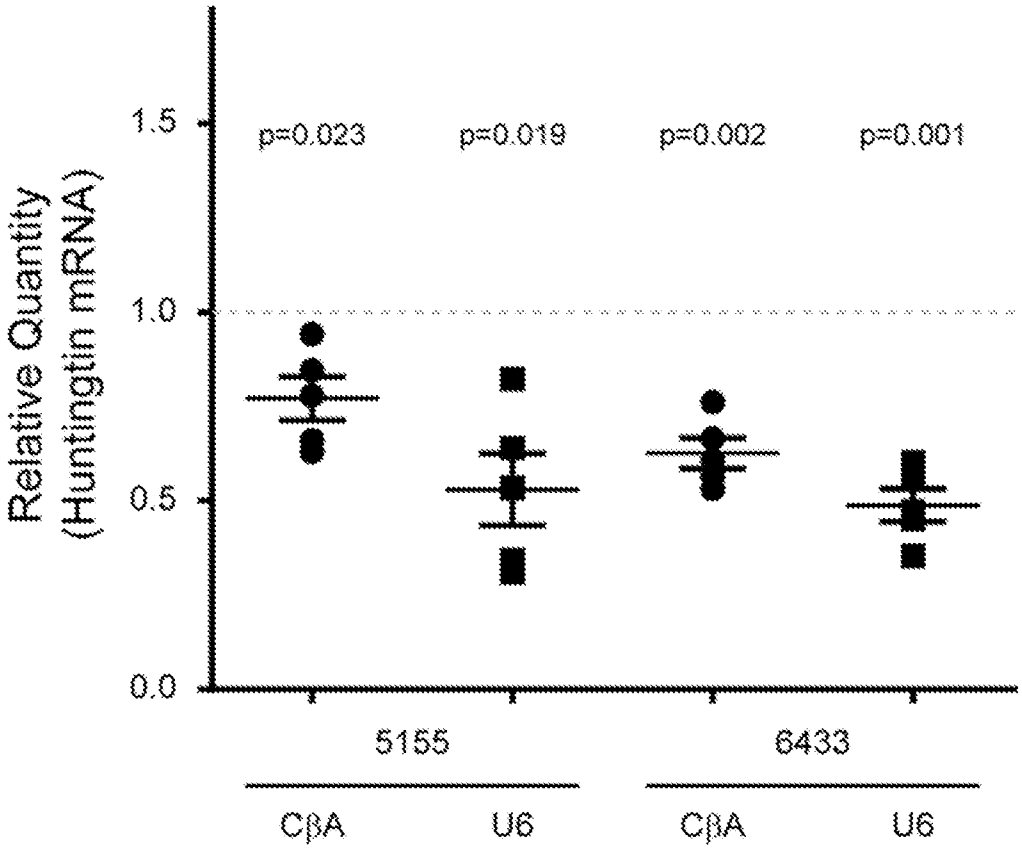

Relative Quantity
(Huntingtin mRNA)
1.5
1.0
0.5
0.0
p=0.023
p=0.019
p=0.002
p=0.001
5155
6433
CβA
U6
CβA
U6

FIG. 5B

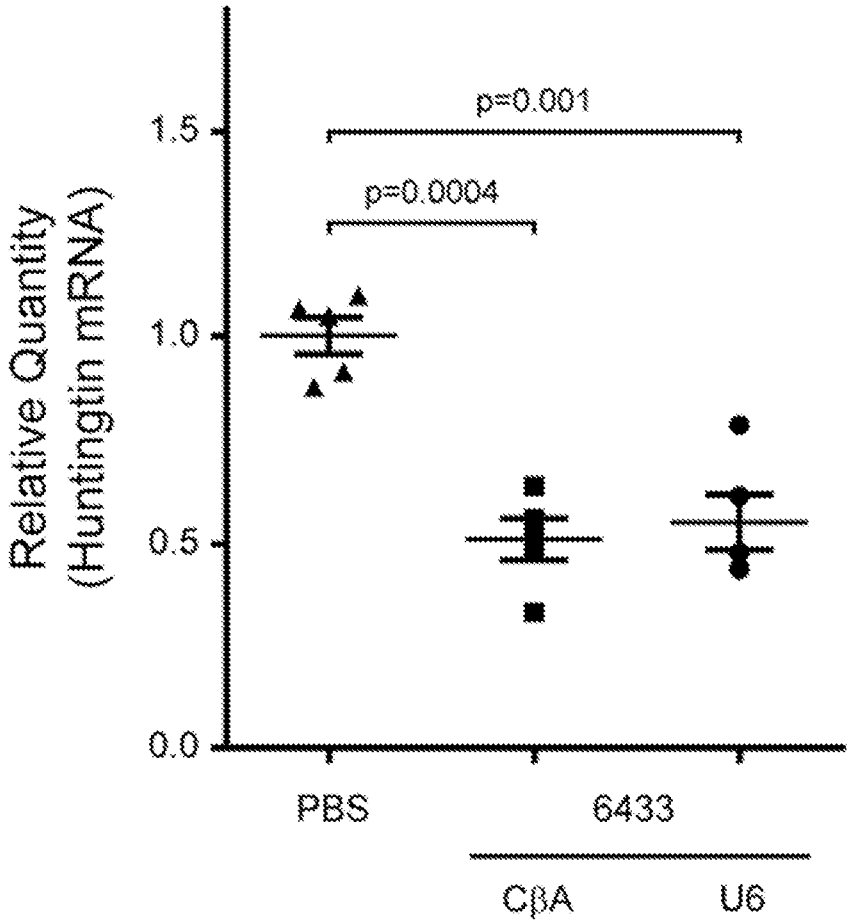

p=0.001
p=0.0004
Relative Quantity
(Huntingtin mRNA)
1.5
1.0
0.5
0.0
PBS
6433
CβA
U6

FIG. 5C

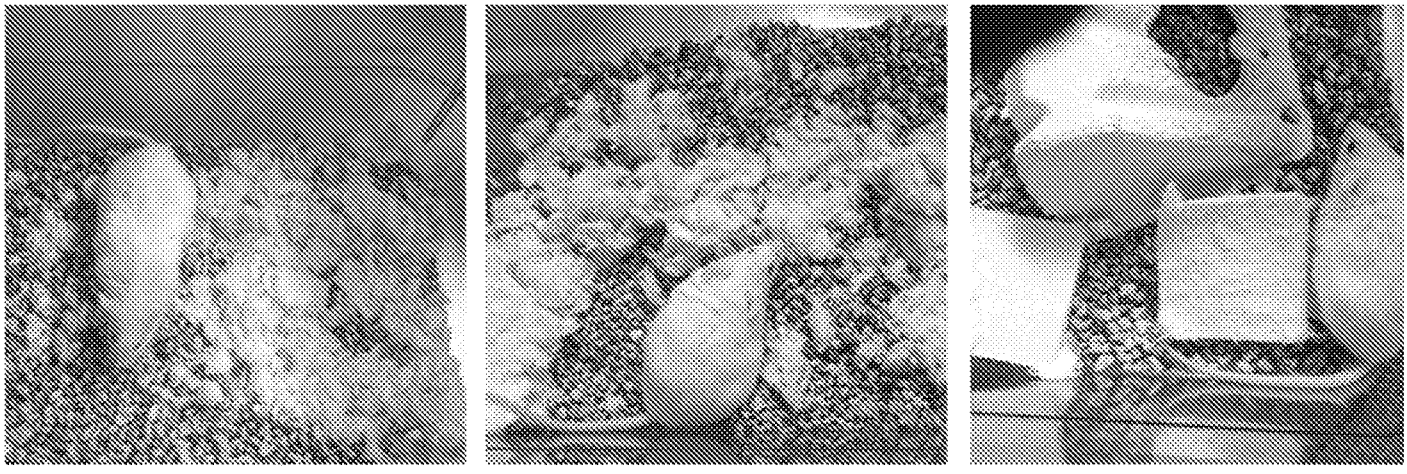


FIG. 6A

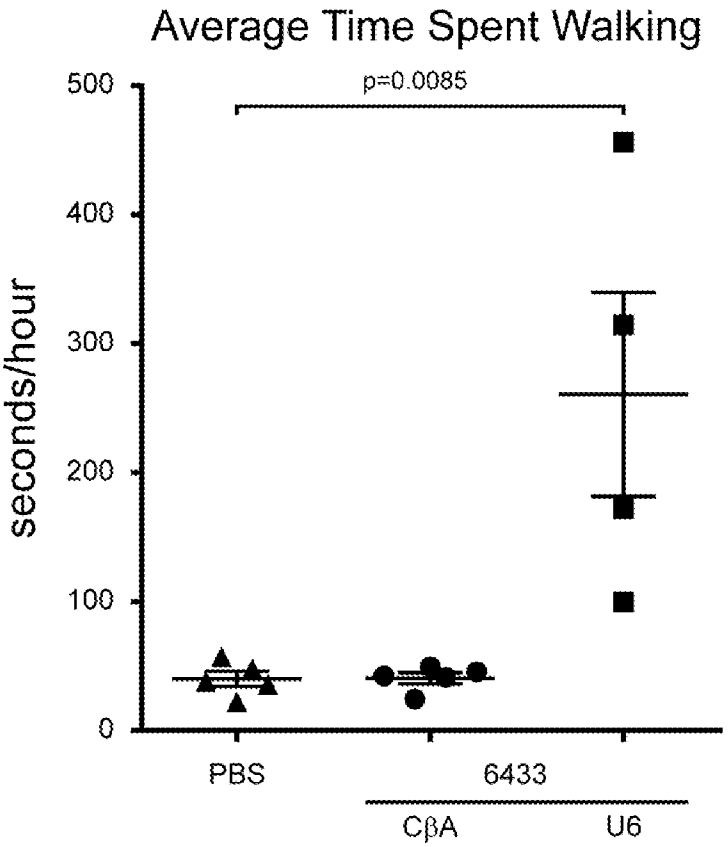

Average Time Spent Walking
p=0.0085
seconds/hour
500
400
300
200
100
0
PBS
6433
CβA
U6

FIG. 6B

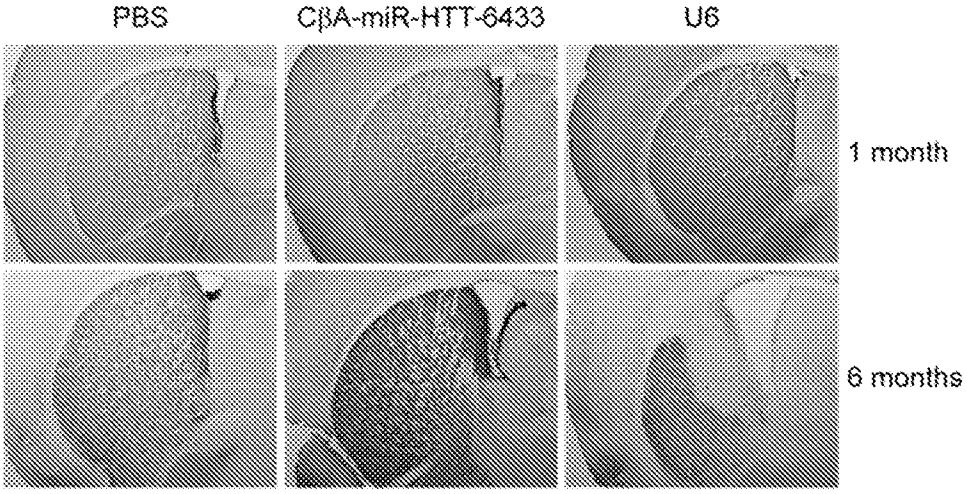

PBS
CβA-miR-HTT-6433
U6
1 month
6 months

FIG. 7A

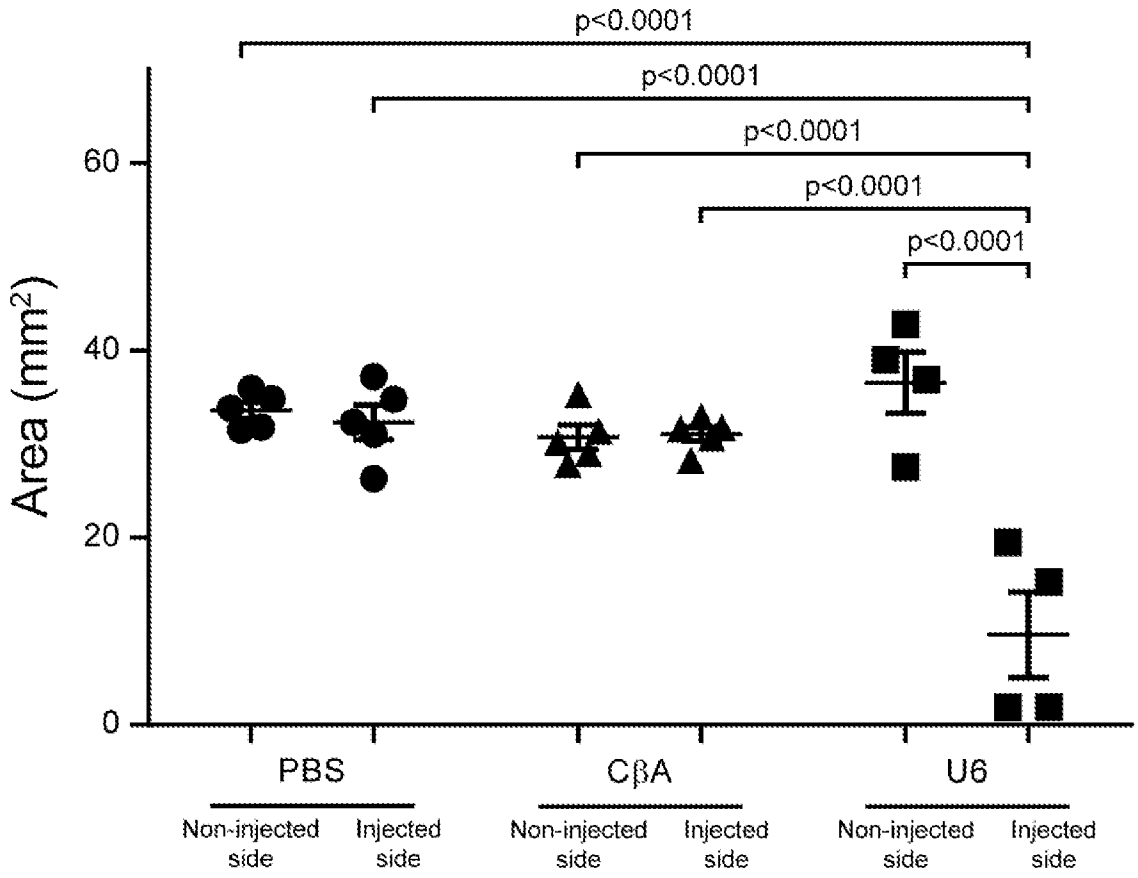

p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
Area (mm2)
60
40
20
0
PBS
CβA
U6
Non-injected side
Injected side
Non-injected side
Injected side
Non-injected side
Injected side

FIG. 7B

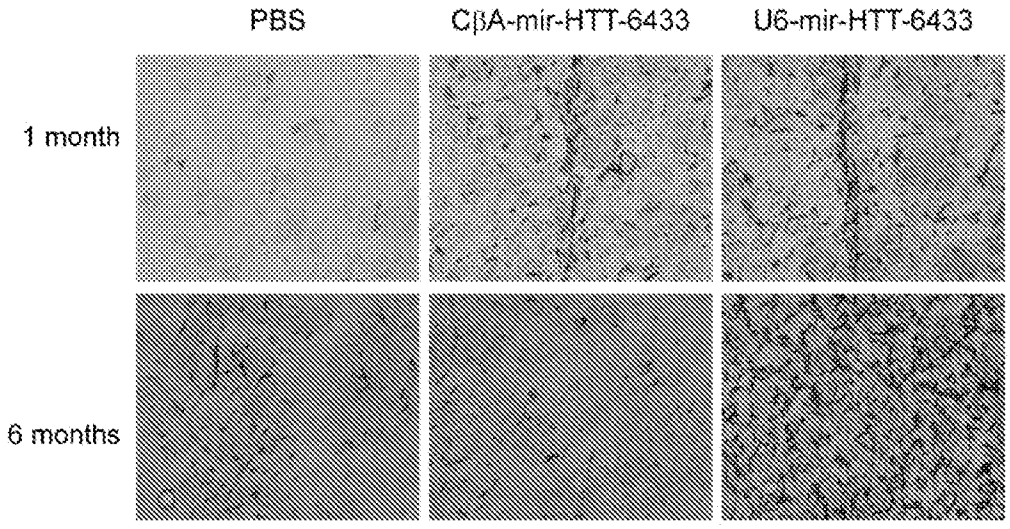

PBS
CβA-mir-HTT-6433
U6-mir-HTT-6433
1 month
6 months

FIG. 8A

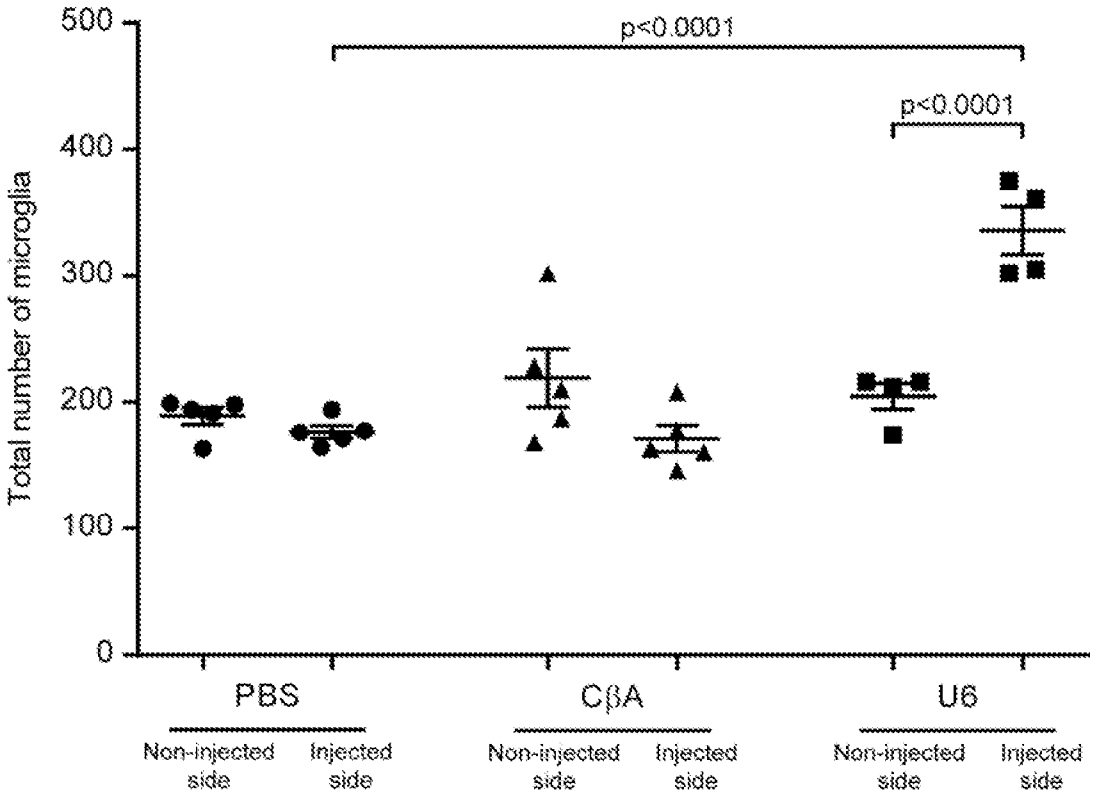

500
400
300
200
100
0
Total number of microglia
p<0.0001
p<0.0001
PBS
CβA
U6
Non-injected side
Injected side
Non-injected side
Injected side
Non-injected side
Injected side

FIG. 8B

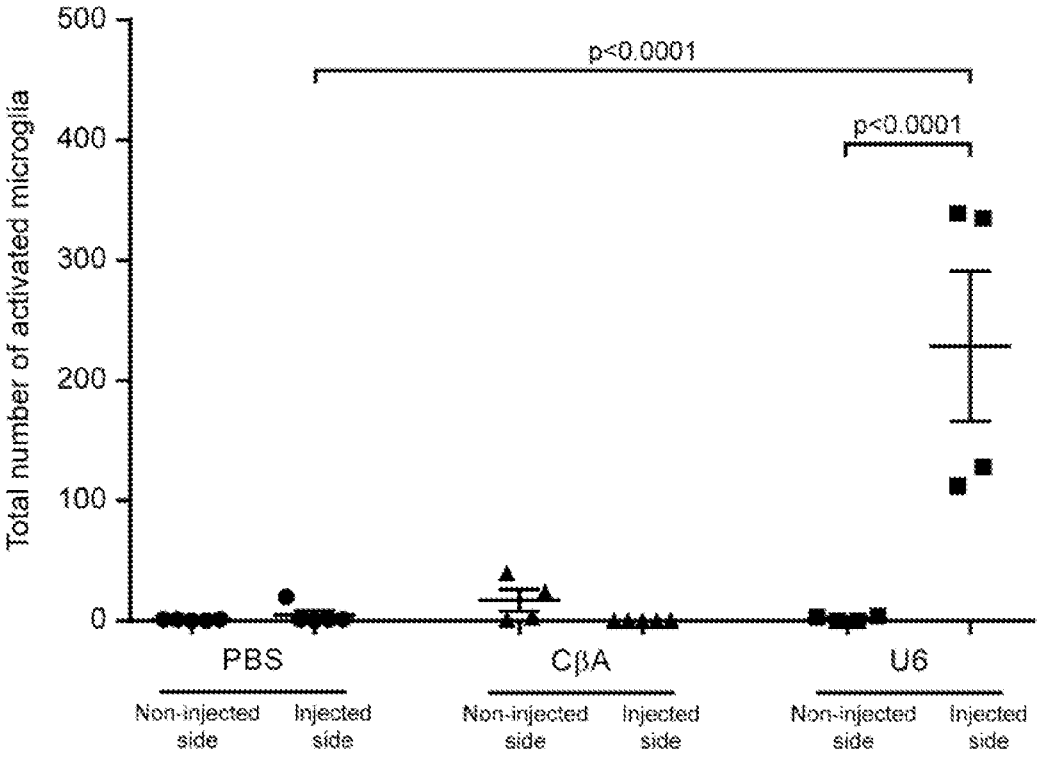

Total number of activated microglia
500
400
300
200
100
0
p<0.0001
p<0.0001
PBS
CβA
U6
Non-injected side
Injected side
Non-injected side
Injected side
Non-injected side
Injected side

FIG. 8C

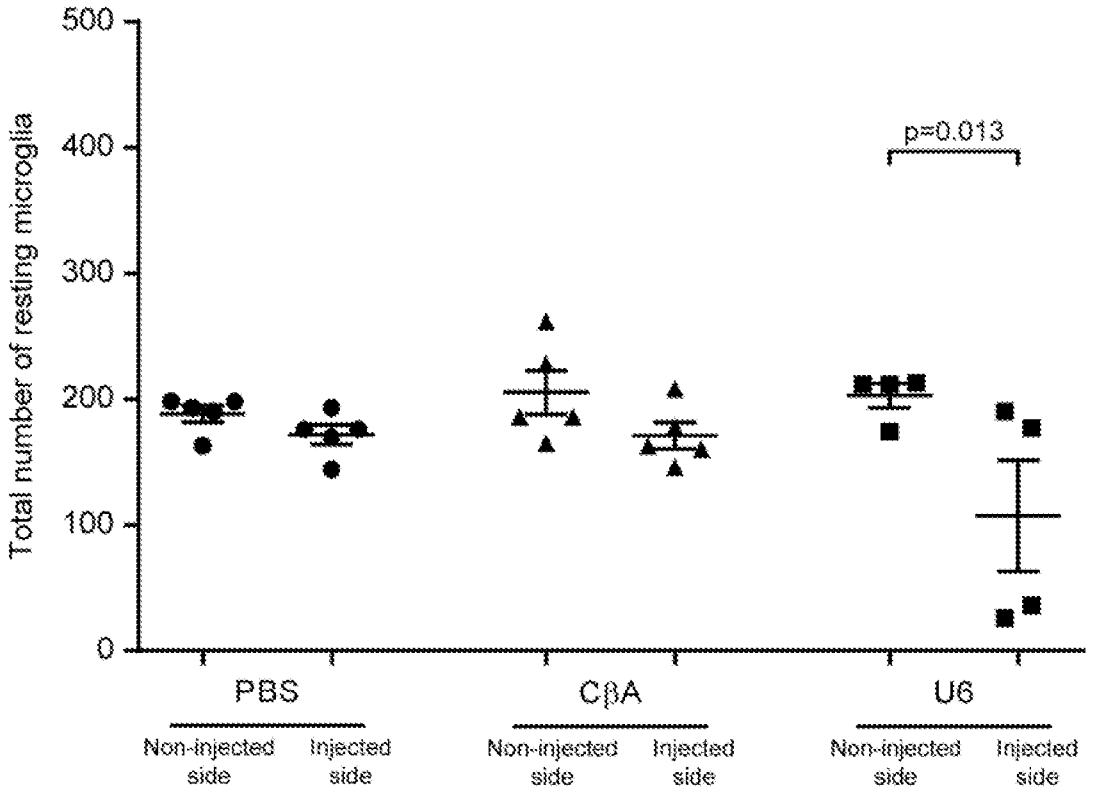

Total number of resting microglia
500
400
300
200
100
0
p=0.013
PBS
CβA
U6
Non-injected side
Injected side
Non-injected side
Injected side
Non-injected side
Injected side

FIG. 8D

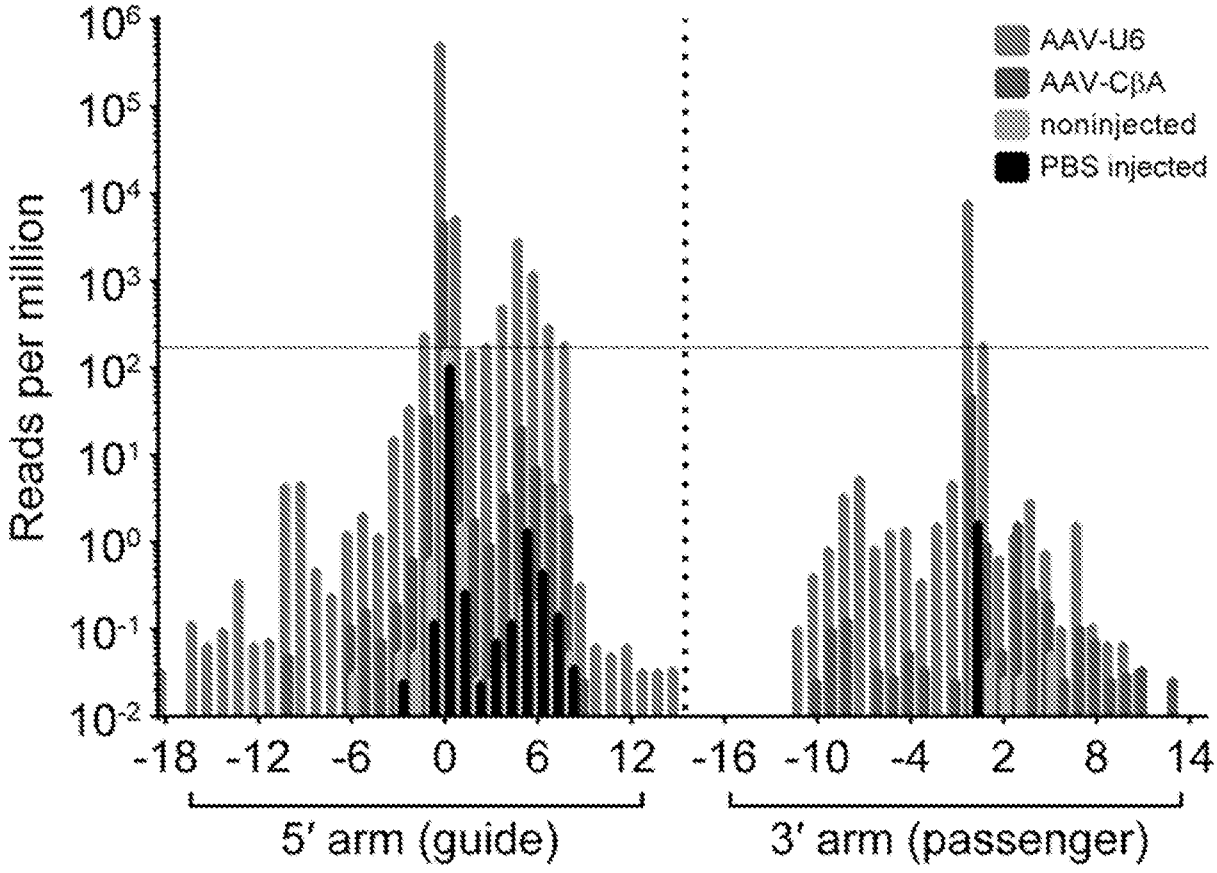

AAV-U6
AAV-CβA
noninjected
PBS injected
Reads per million
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
10^-2
-18 -12 -6 0 6 12
-16 -10 -4 2 8 14
5′ arm (guide)
3′ arm (passenger)

FIG. 9A

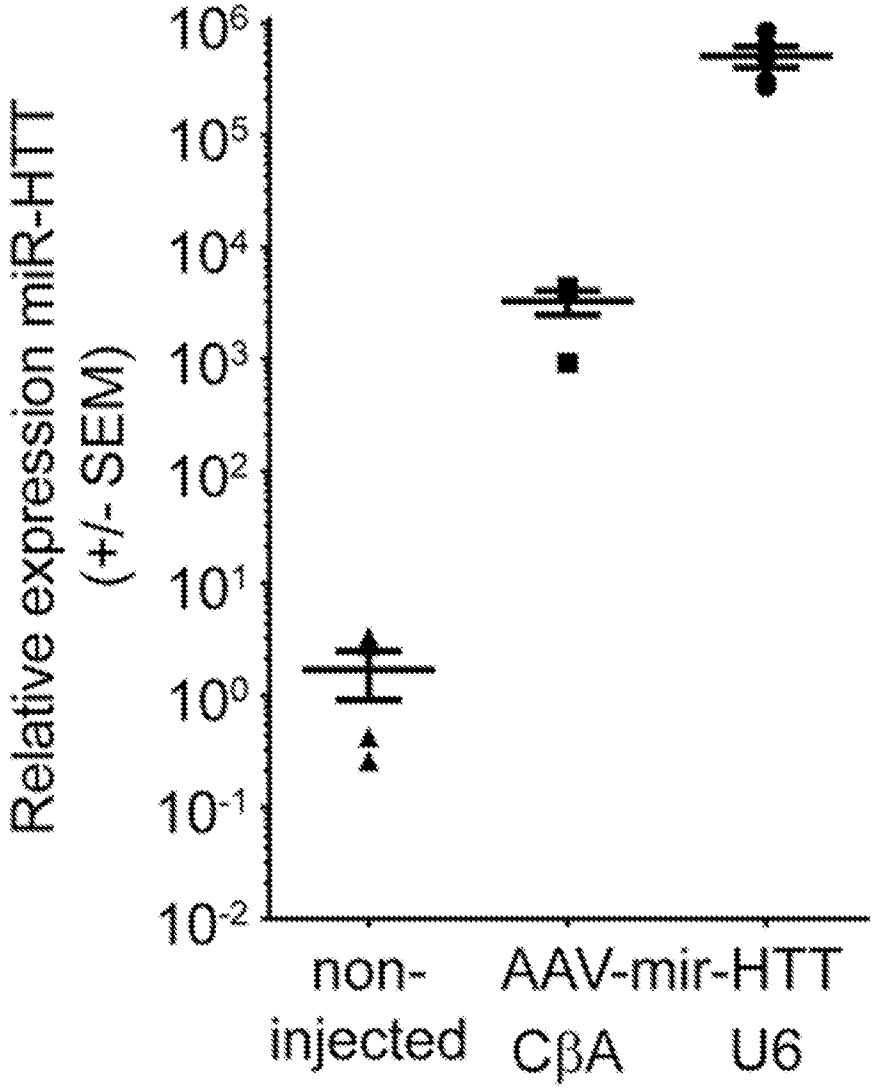

Relative expression miR-HTT
(+/- SEM)
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
10^-2
non-injected
AAV-mir-HTT
CβA
U6

FIG. 9B

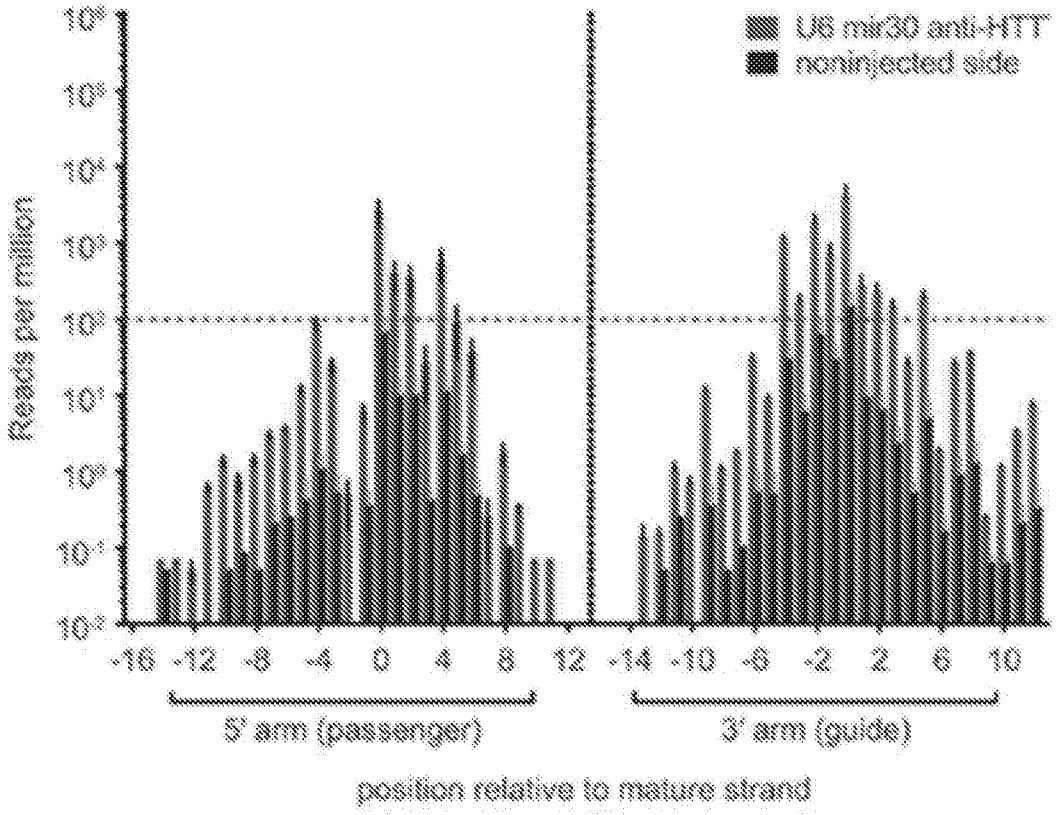

U6 mir30 anti-HTT
noninjected side
Reads per million
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^-1
10^-2
-16 -12 -8 -4 0 4 8 12 -14 -10 -6 -2 2 6 10
5' arm (passenger)
3' arm (guide)
position relative to mature strand

FIG. 9C

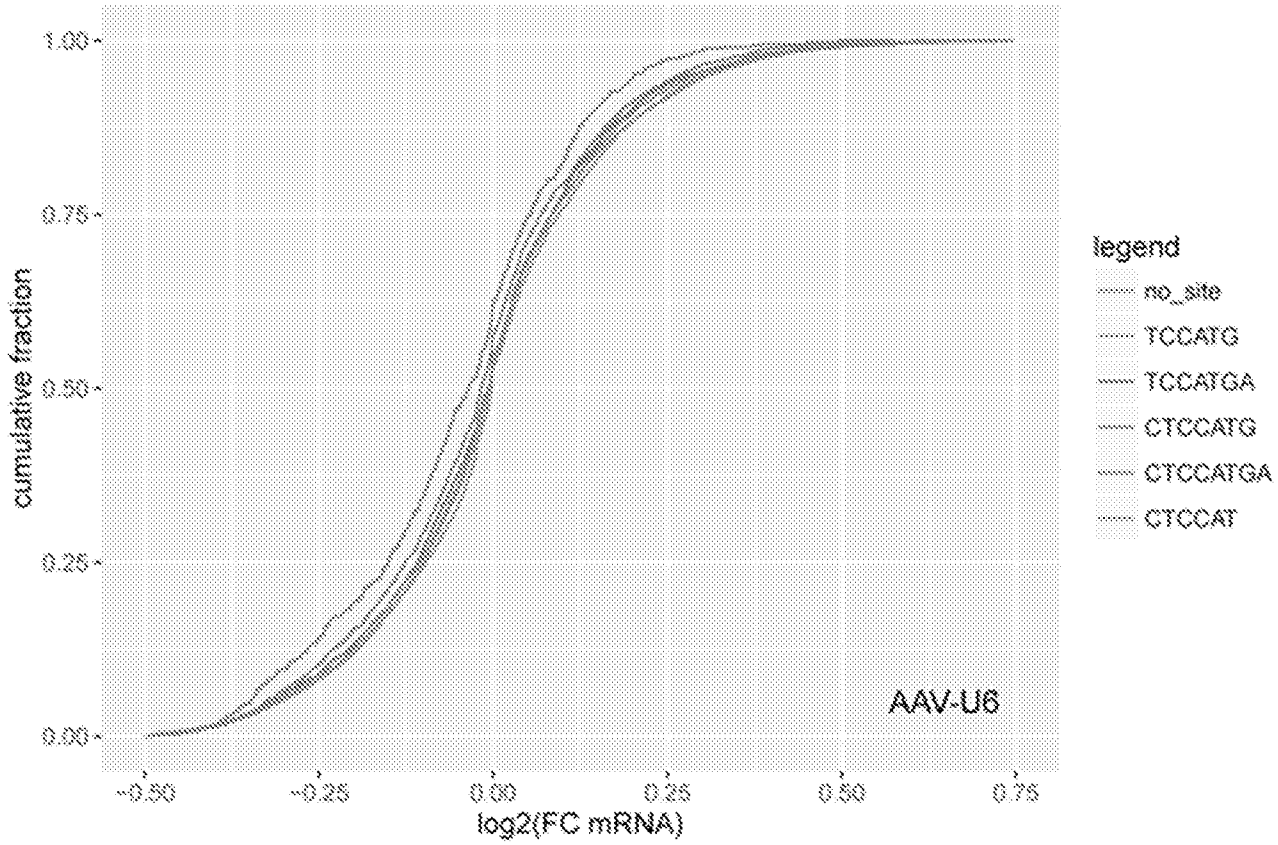

1.00
0.75
0.50
0.25
0.00
cumulative fraction
−0.50
−0.25
0.00
0.25
0.50
0.75
log2(FC mRNA)
AAV-U6
legend
no_site
TCCATG
TCCATGA
CTCCATG
CTCCATGA
CTCCAT

FIG. 10A

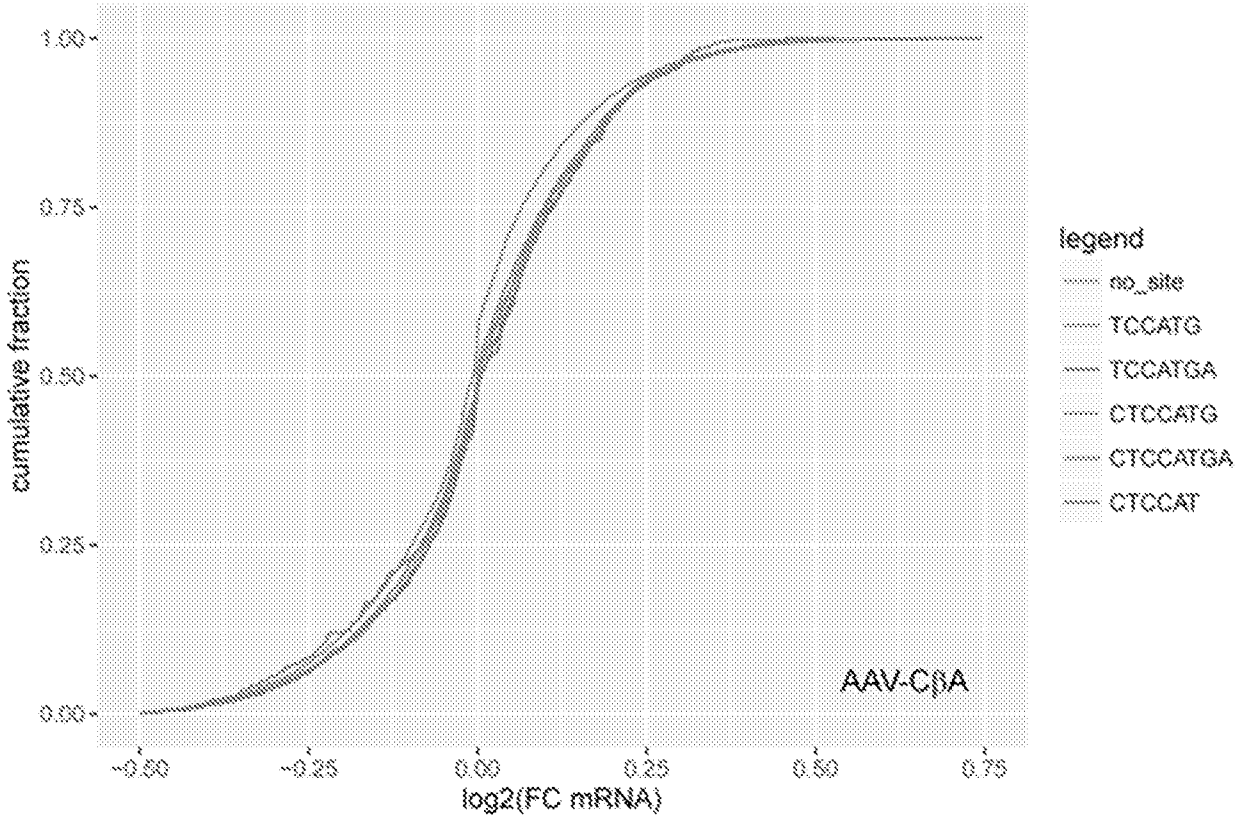

1.00
0.75
0.50
0.25
0.00
cumulative fraction
-0.50
-0.25
0.00
0.25
0.50
0.75
log2(FC mRNA)
AAV-CβA
legend
no_site
TCCATG
TCCATGA
CTCCATG
CTCCATGA
CTCCAT

FIG. 10B

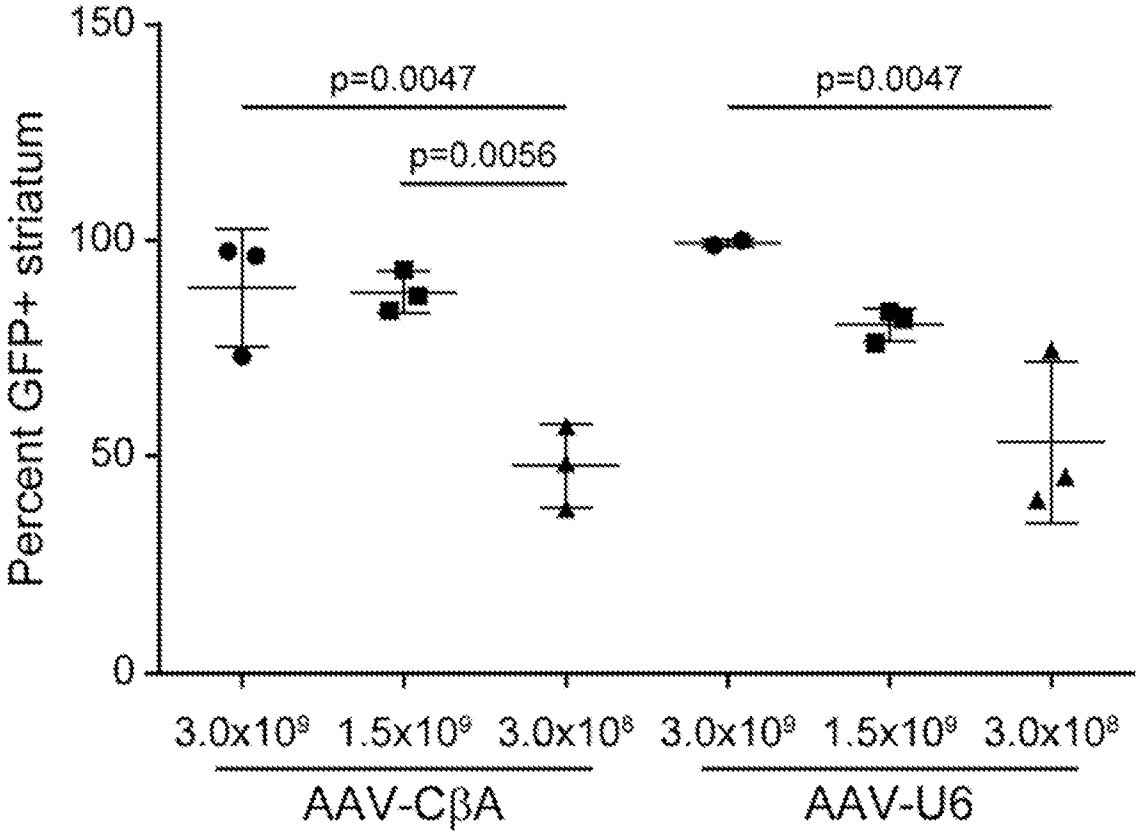


FIG. 11A

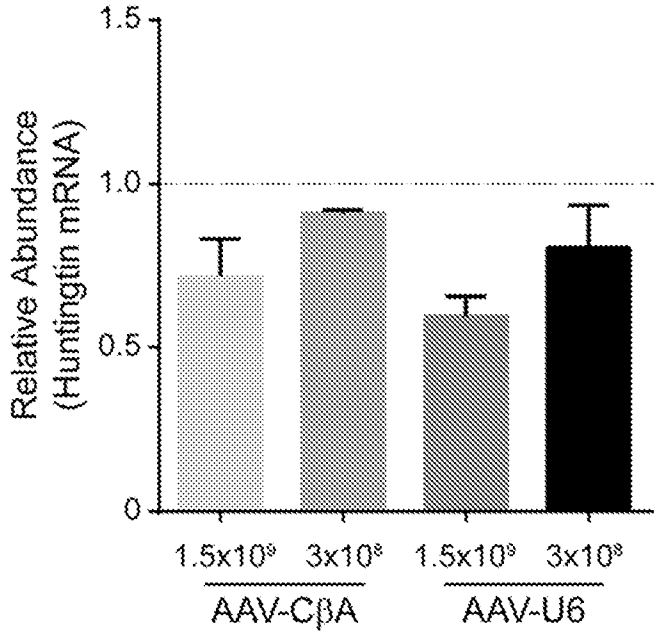


FIG. 11B

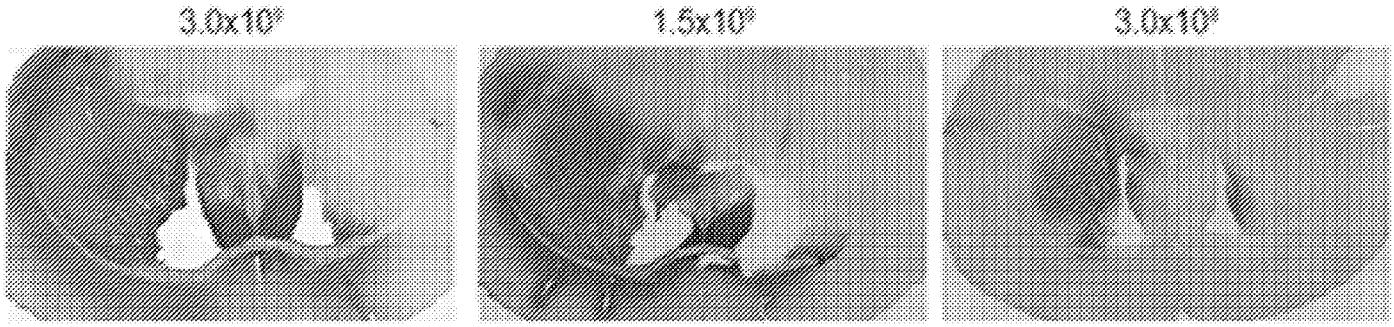


FIG. 11C

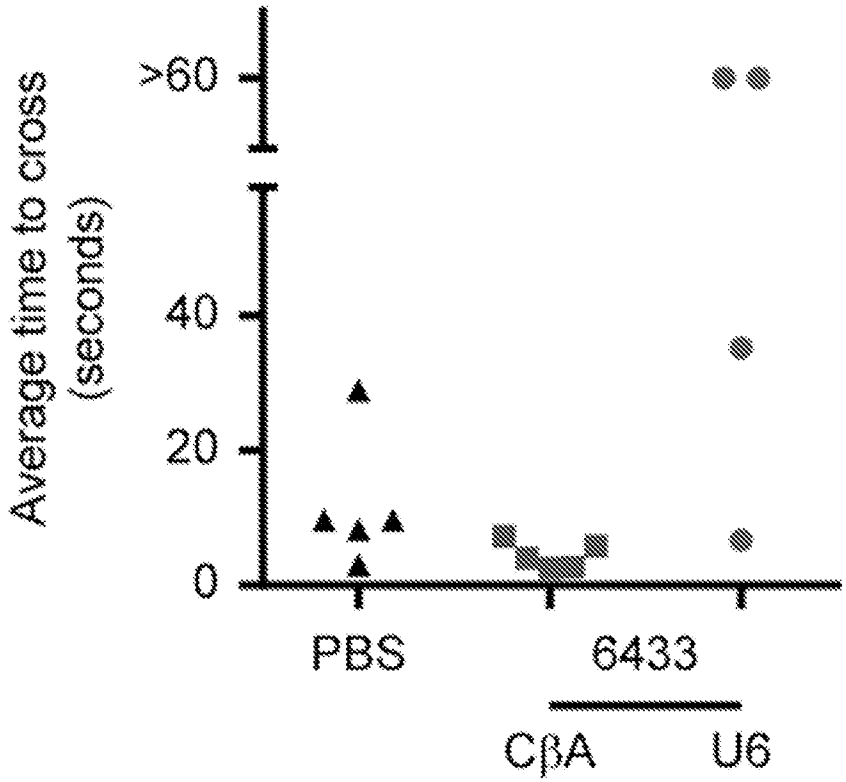


FIG. 12A

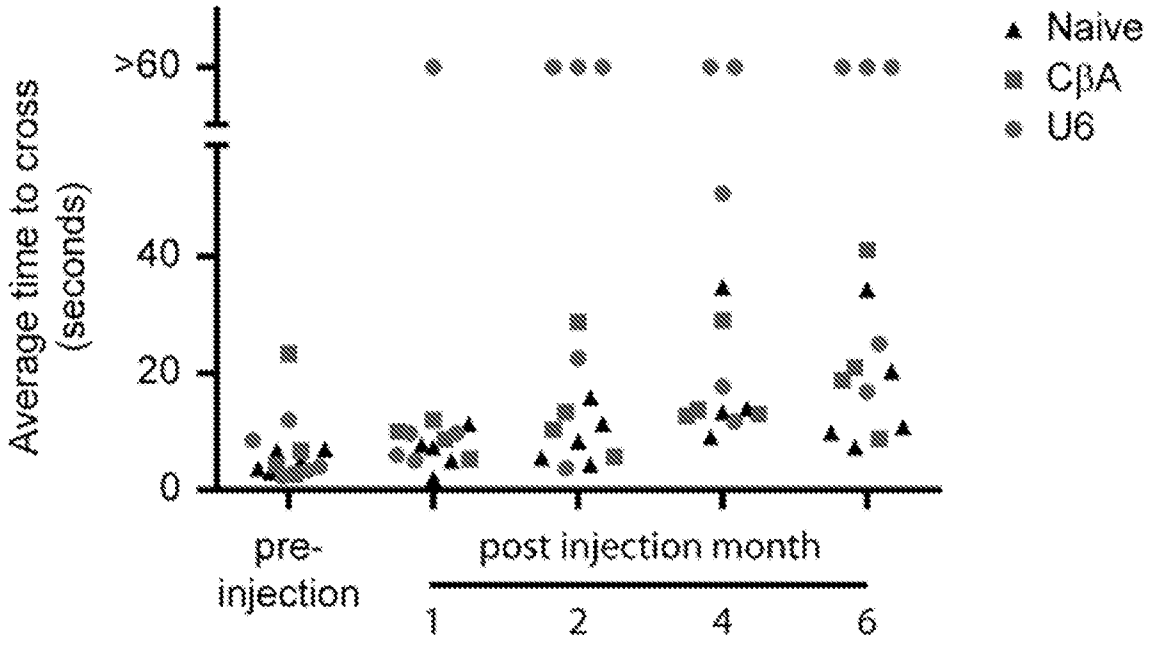


FIG. 12B

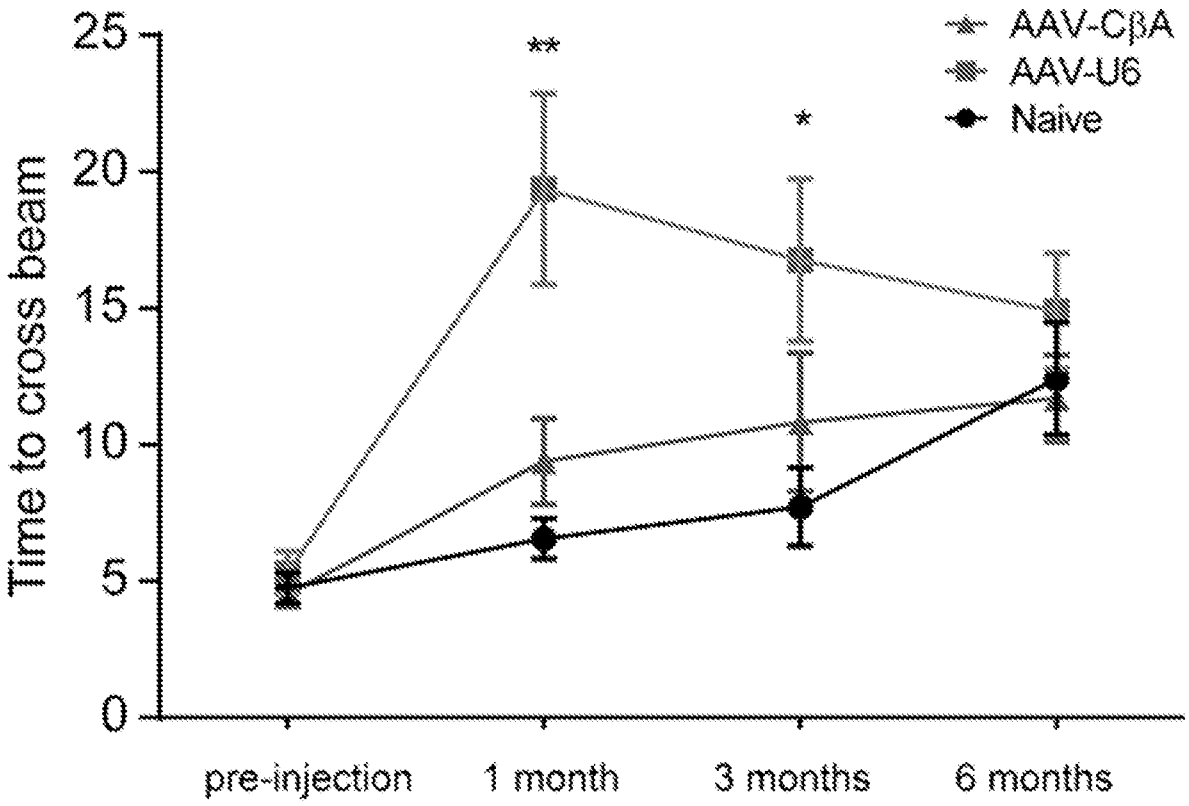

AAV-CβA
AAV-U6
Naive
**
*
Time to cross beam
25
20
15
10
5
0
pre-injection
1 month
3 months
6 months

FIG. 13A

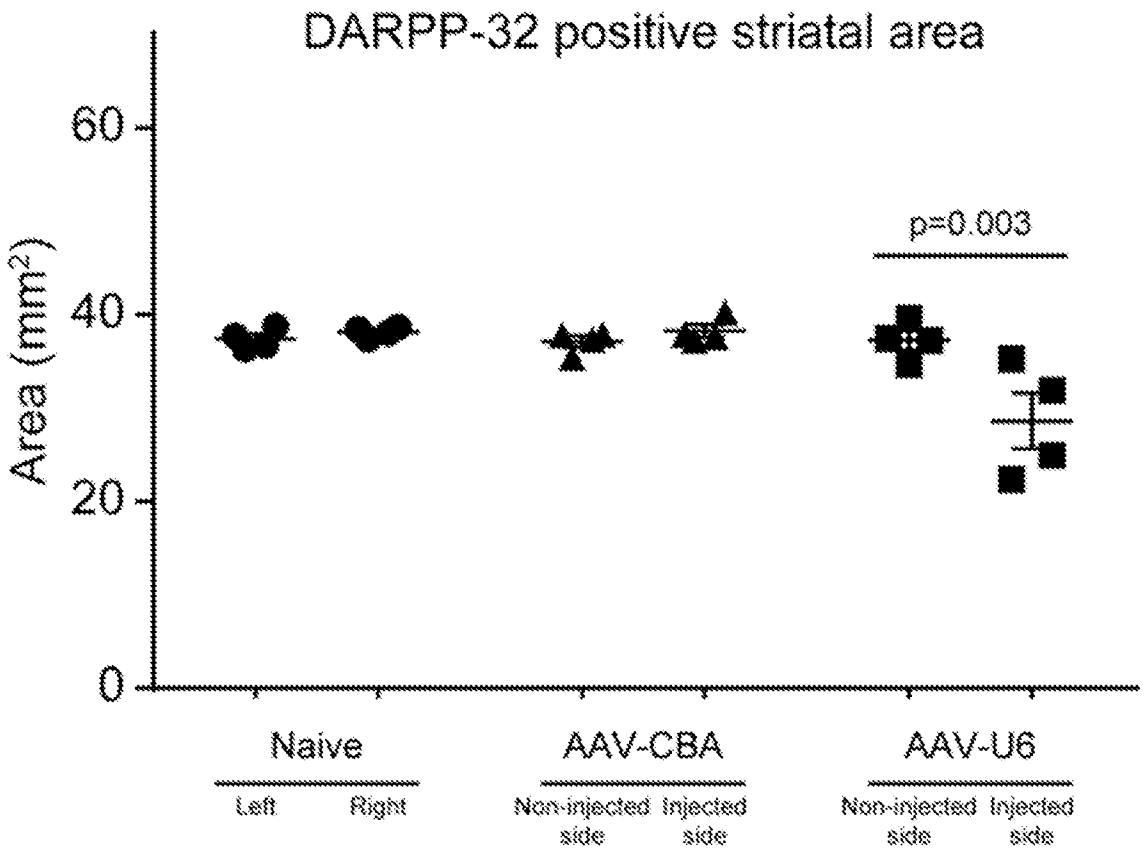

DARPP-32 positive striatal area
Area (mm2)
60
40
20
0
p=0.003
Naive
AAV-CBA
AAV-U6
Left
Right
Non-injected side
Injected side
Non-injected side
Injected side

FIG. 13B

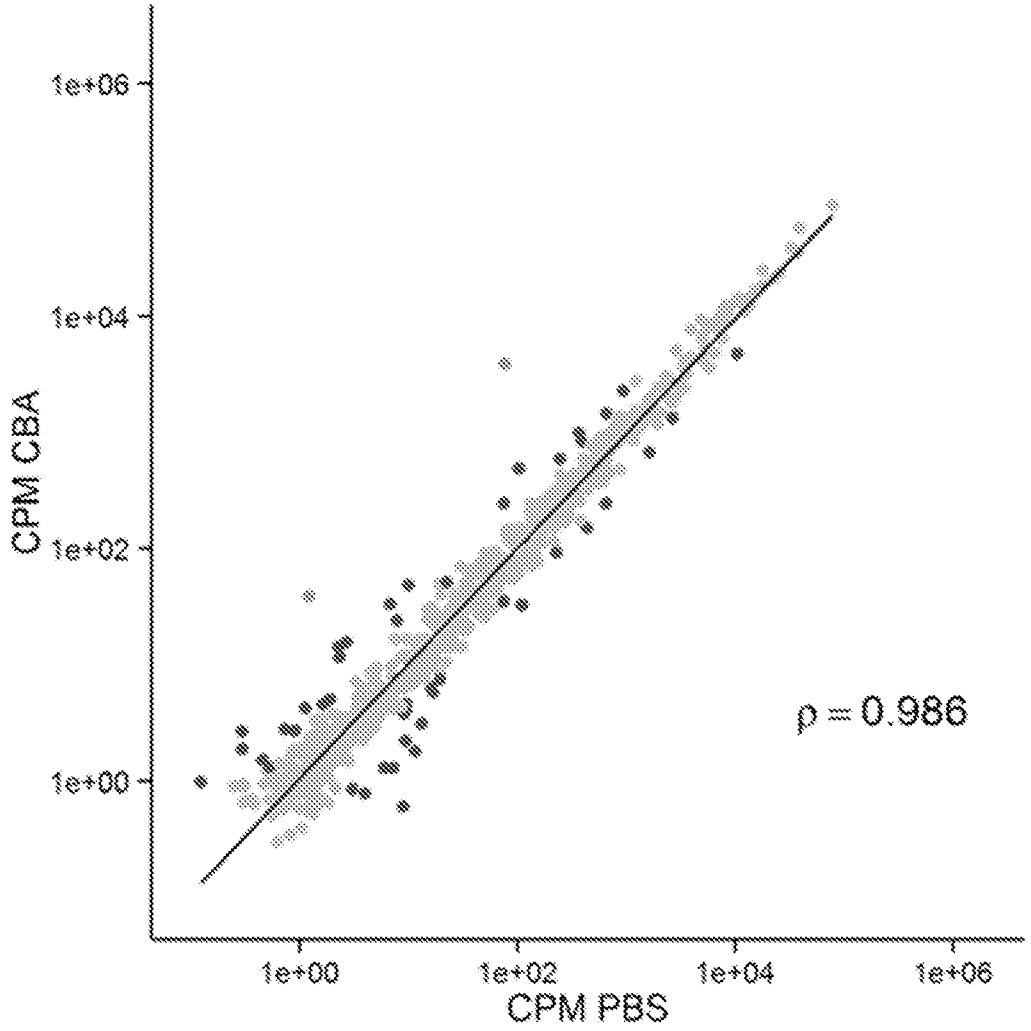


FIG. 14A

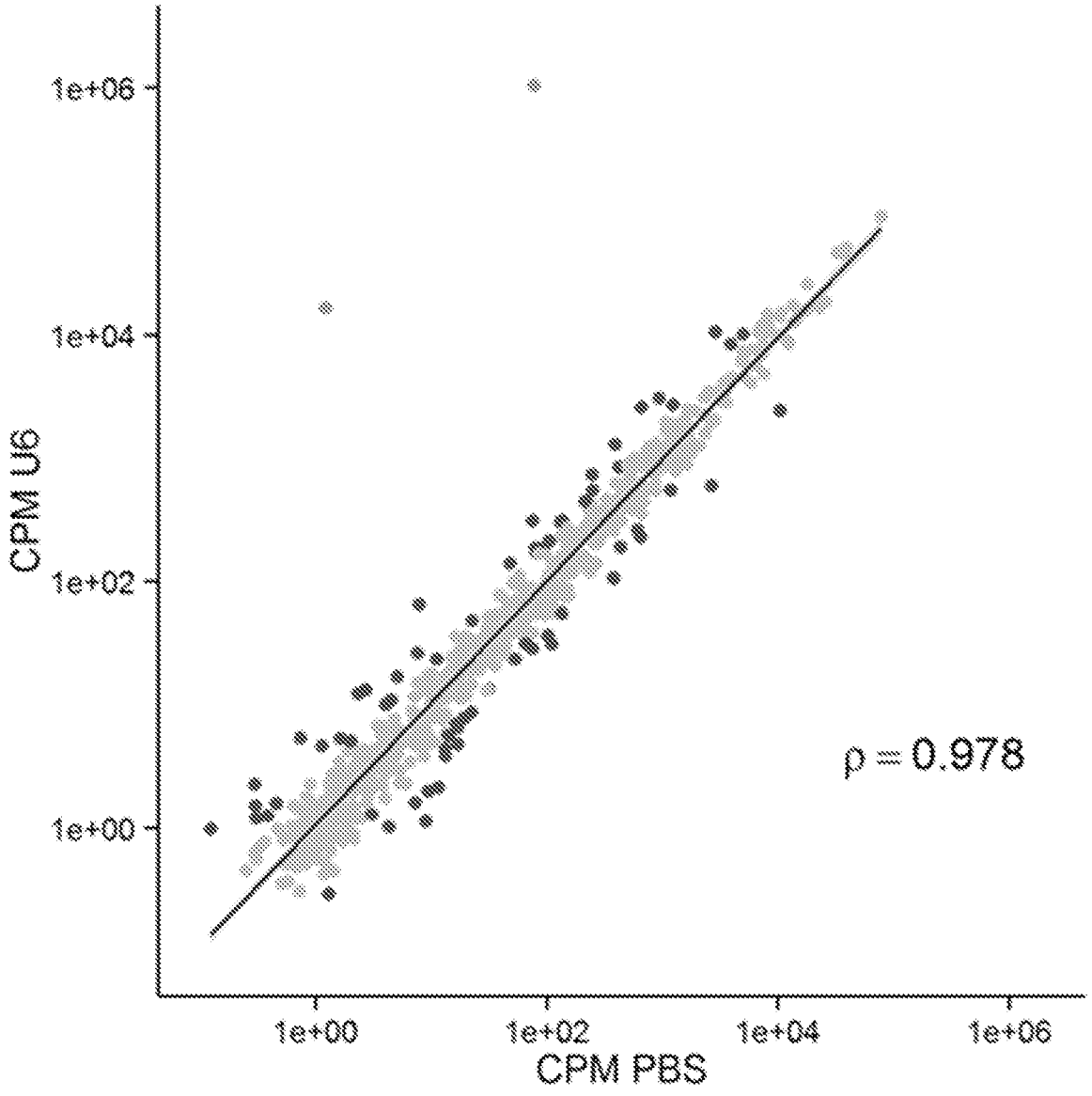


FIG. 14B

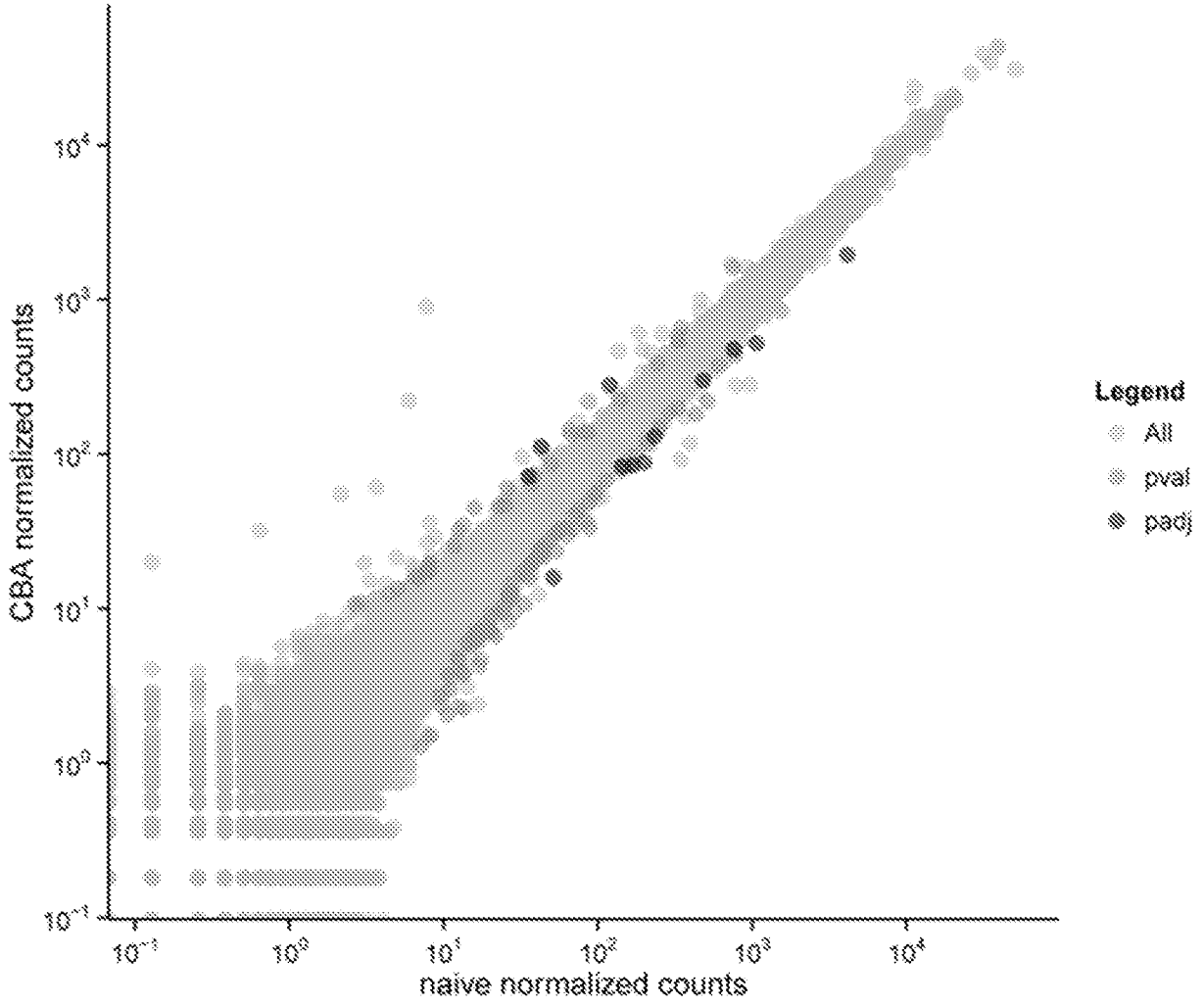

CBA normalized counts
naive normalized counts
Legend
All
pval
padj

FIG. 15A

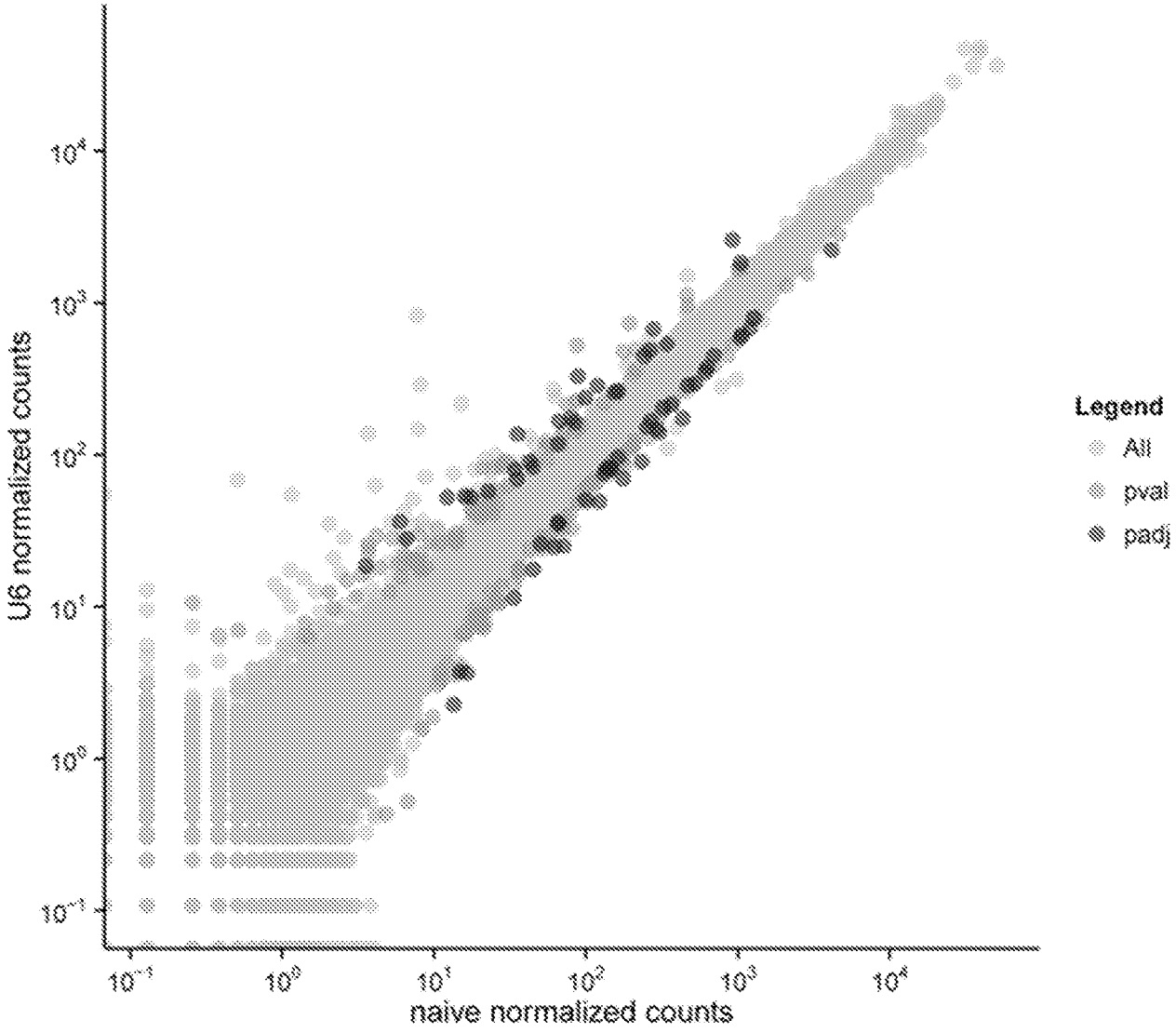

U6 normalized counts
naive normalized counts
Legend
All
pval
padj

FIG. 15B

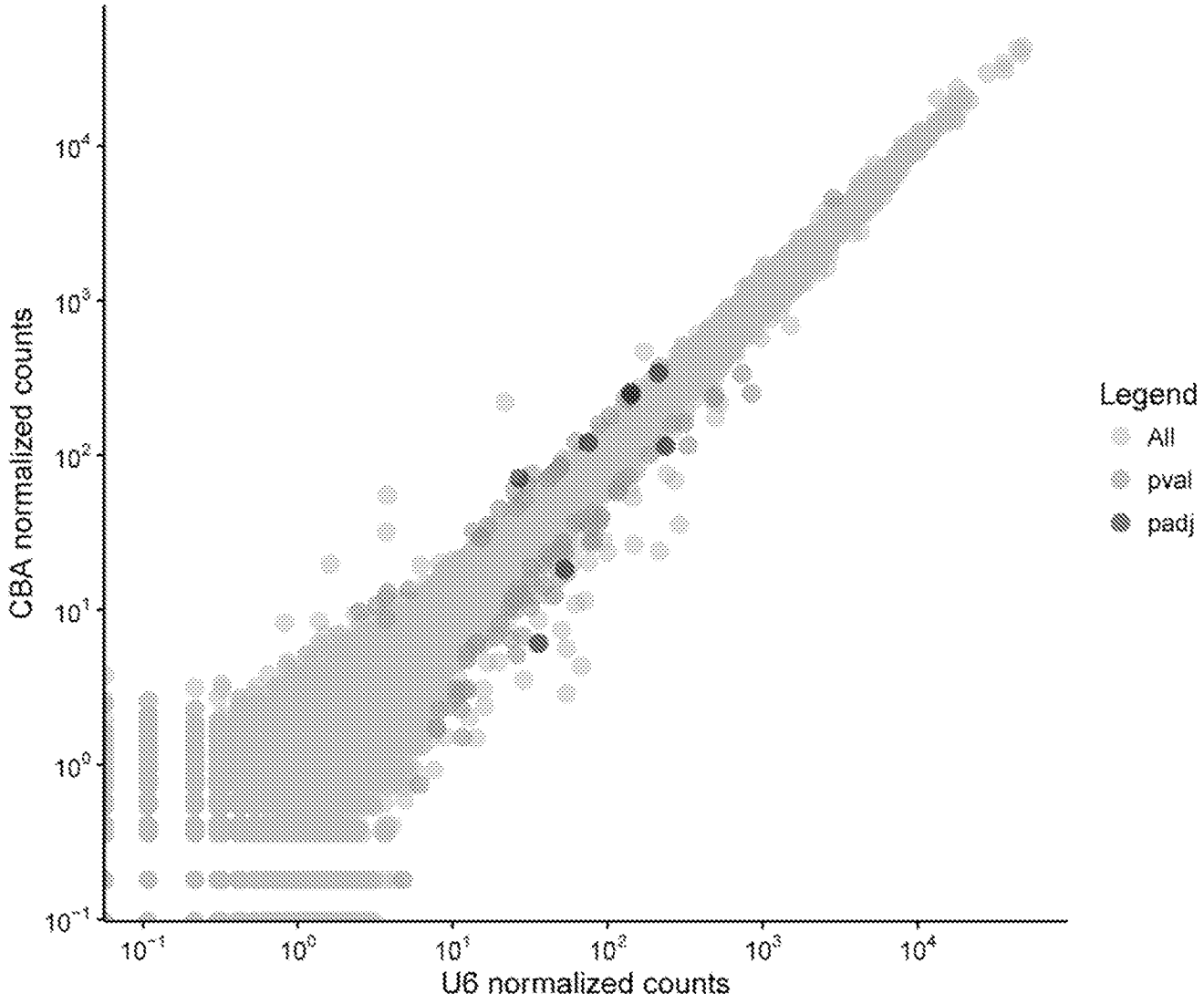

CBA normalized counts
U6 normalized counts
Legend
All
pval
padj

FIG. 15C

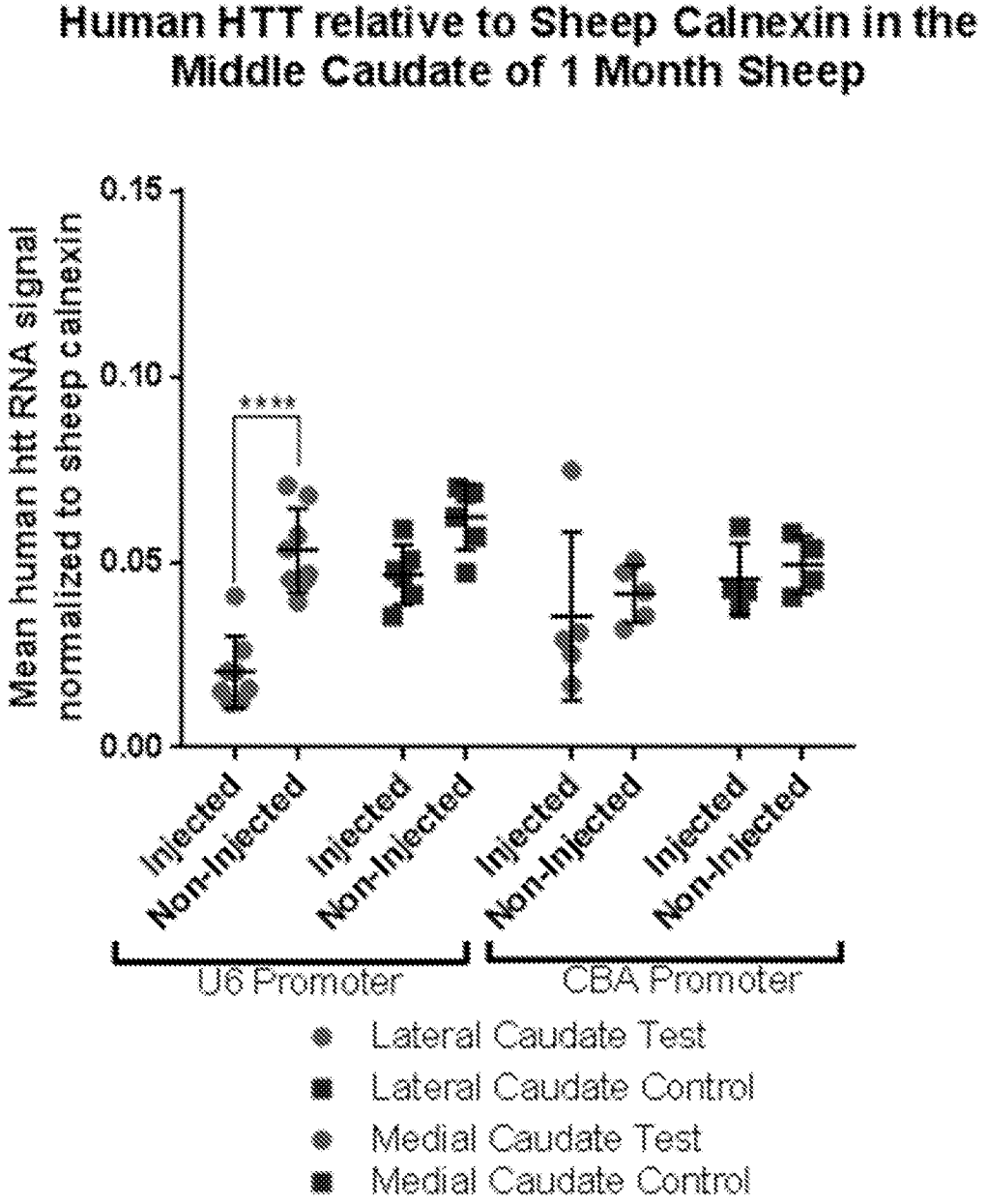

Human HTT relative to Sheep Calnexin in the Middle Caudate of 1 Month Sheep
Mean human htt RNA signal normalized to sheep calnexin
0.15
0.10
0.05
0.00
****
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
U6 Promoter
CBA Promoter
Lateral Caudate Test
Lateral Caudate Control
Medial Caudate Test
Medial Caudate Control

FIG. 16

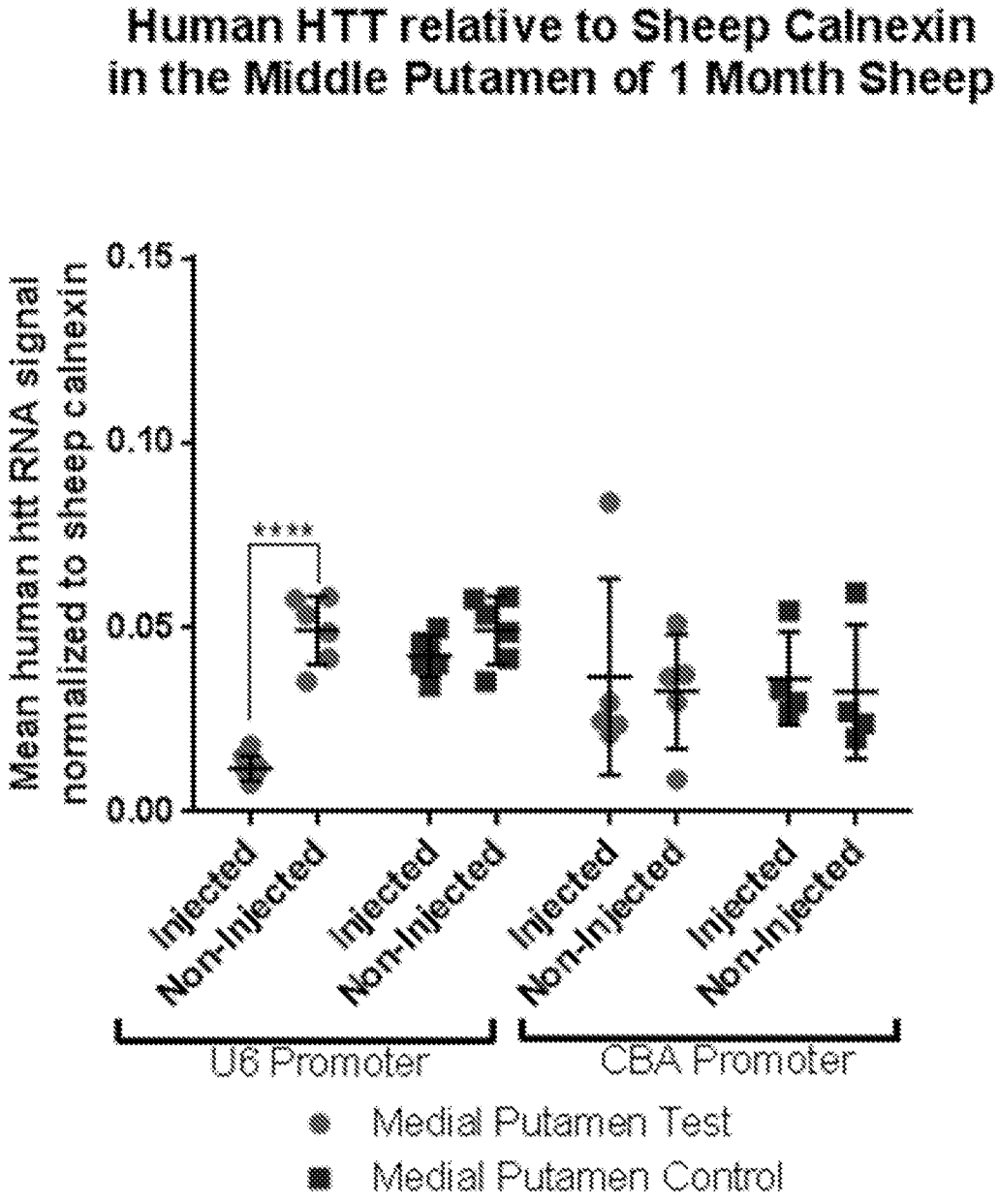

Human HTT relative to Sheep Calnexin
in the Middle Putamen of 1 Month Sheep
Mean human htt RNA signal normalized to sheep calnexin
0.15
0.10
0.05
0.00
****
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
U6 Promoter
CBA Promoter
Medial Putamen Test
Medial Putamen Control

FIG. 17

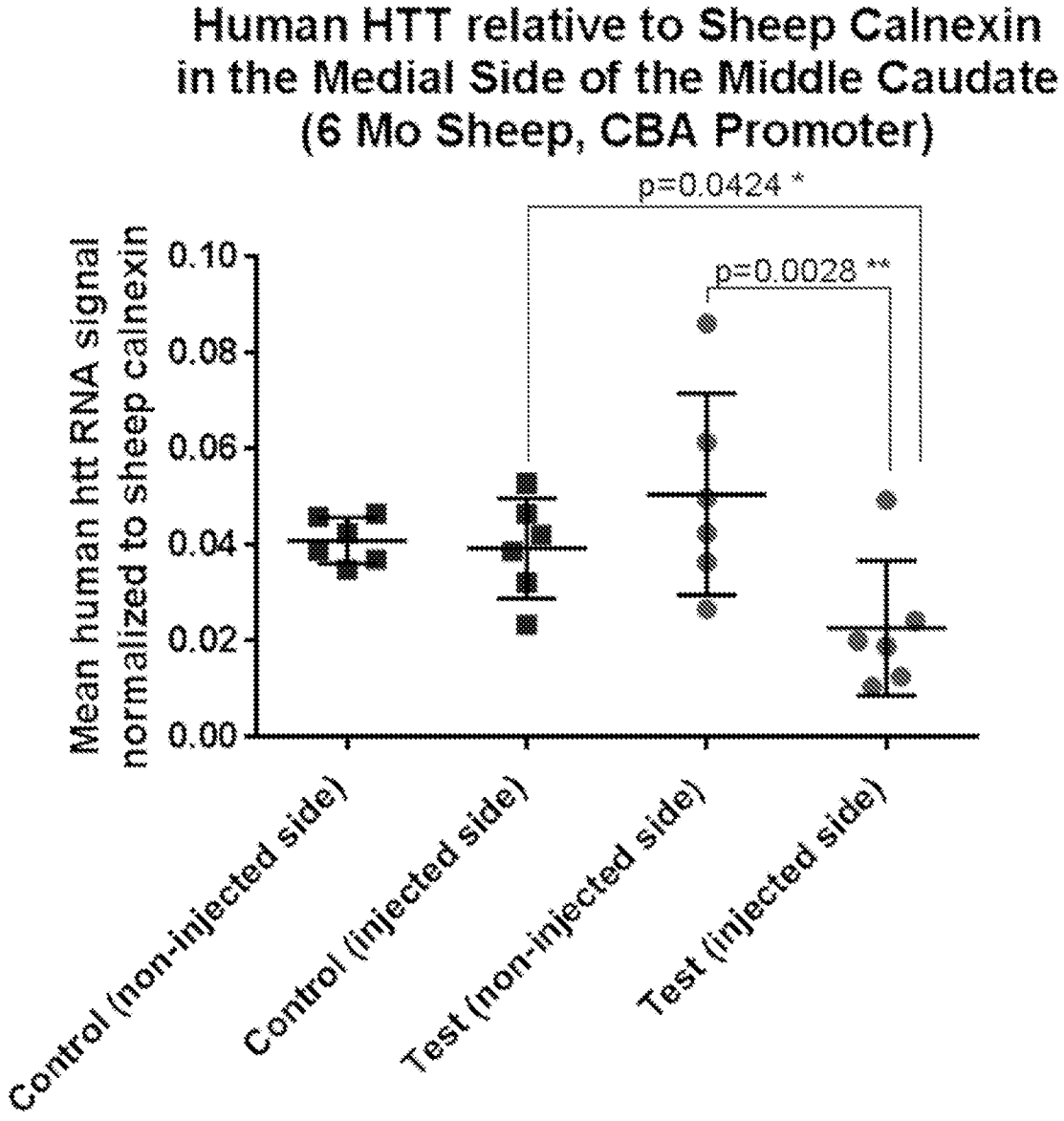

Human HTT relative to Sheep Calnexin
in the Medial Side of the Middle Caudate
(6 Mo Sheep, CBA Promoter)
p=0.0424 *
p=0.0028 **
Mean human htt RNA signal
normalized to sheep calnexin
0.10
0.08
0.06
0.04
0.02
0.00
Control (non-injected side)
Control (injected side)
Test (non-injected side)
Test (injected side)

FIG. 18

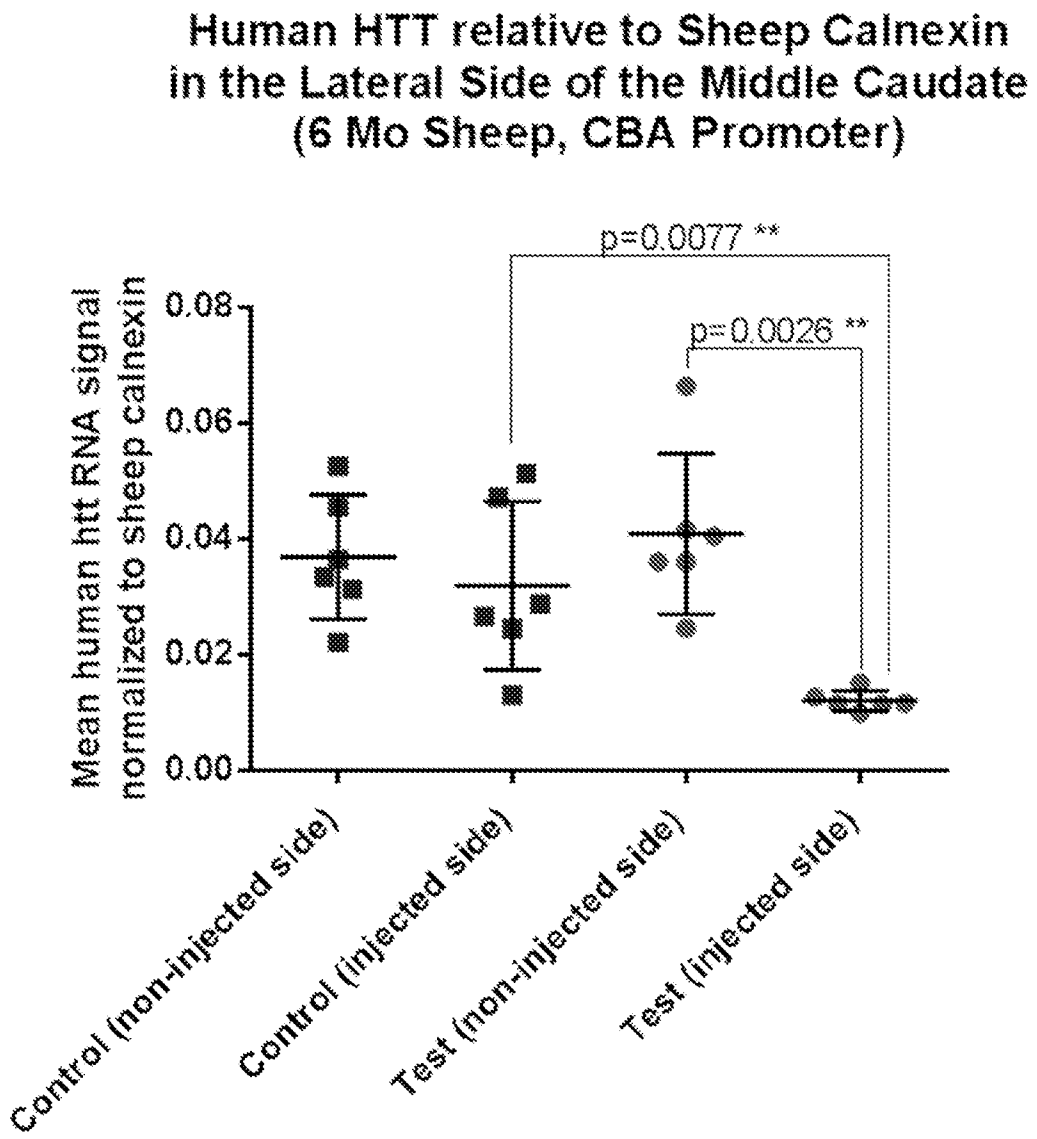

Human HTT relative to Sheep Calnexin
in the Lateral Side of the Middle Caudate
(6 Mo Sheep, CBA Promoter)
p=0.0077 **
p=0.0026 **
Mean human htt RNA signal
normalized to sheep calnexin
0.08
0.06
0.04
0.02
0.00
Control (non-injected side)
Control (injected side)
Test (non-injected side)
Test (injected side)

FIG. 19

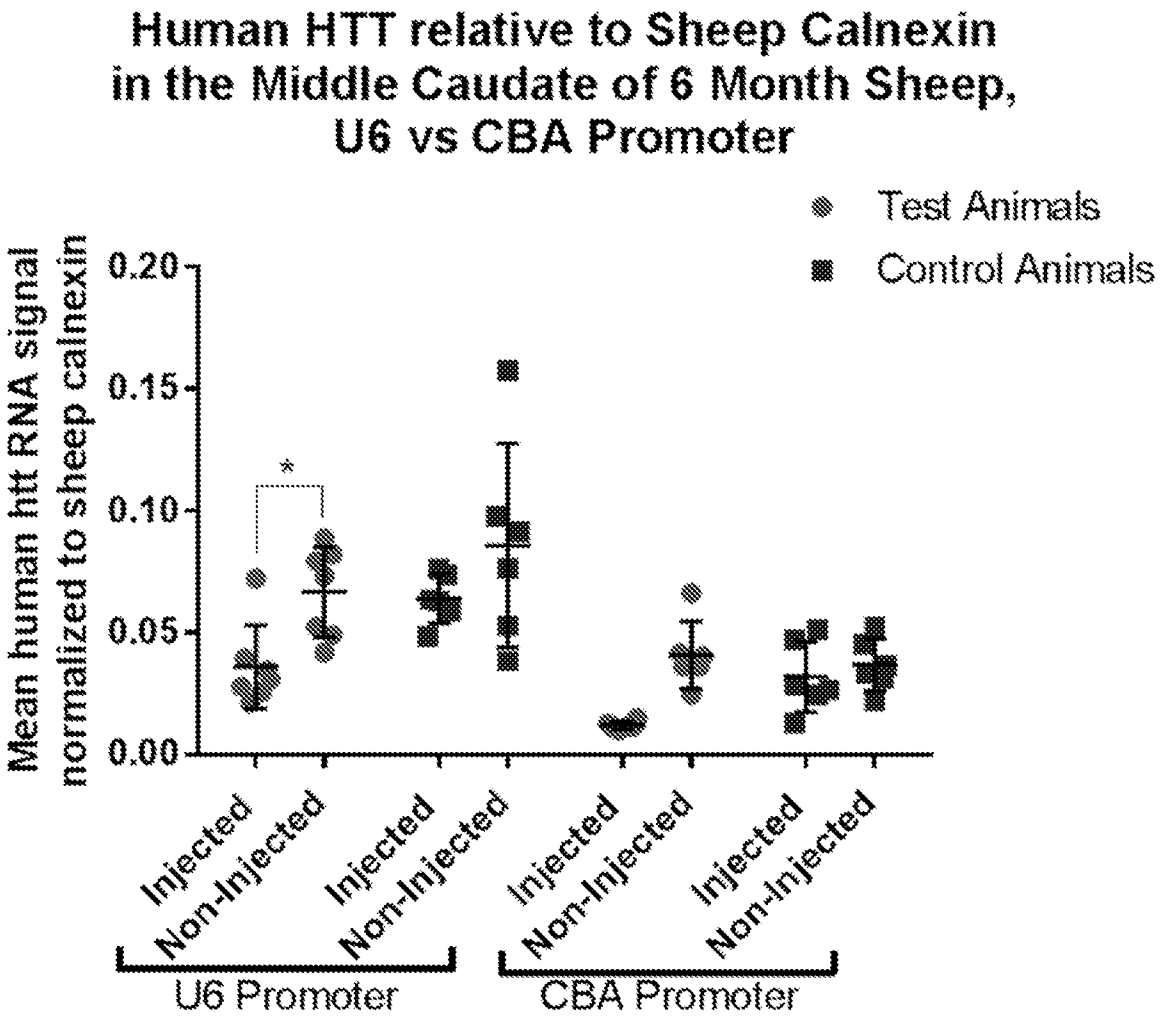

Human HTT relative to Sheep Calnexin
in the Middle Caudate of 6 Month Sheep,
U6 vs CBA Promoter
Test Animals
Control Animals
Mean human htt RNA signal
normalized to sheep calnexin
0.20
0.15
0.10
0.05
0.00
*
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
U6 Promoter
CBA Promoter

FIG. 20

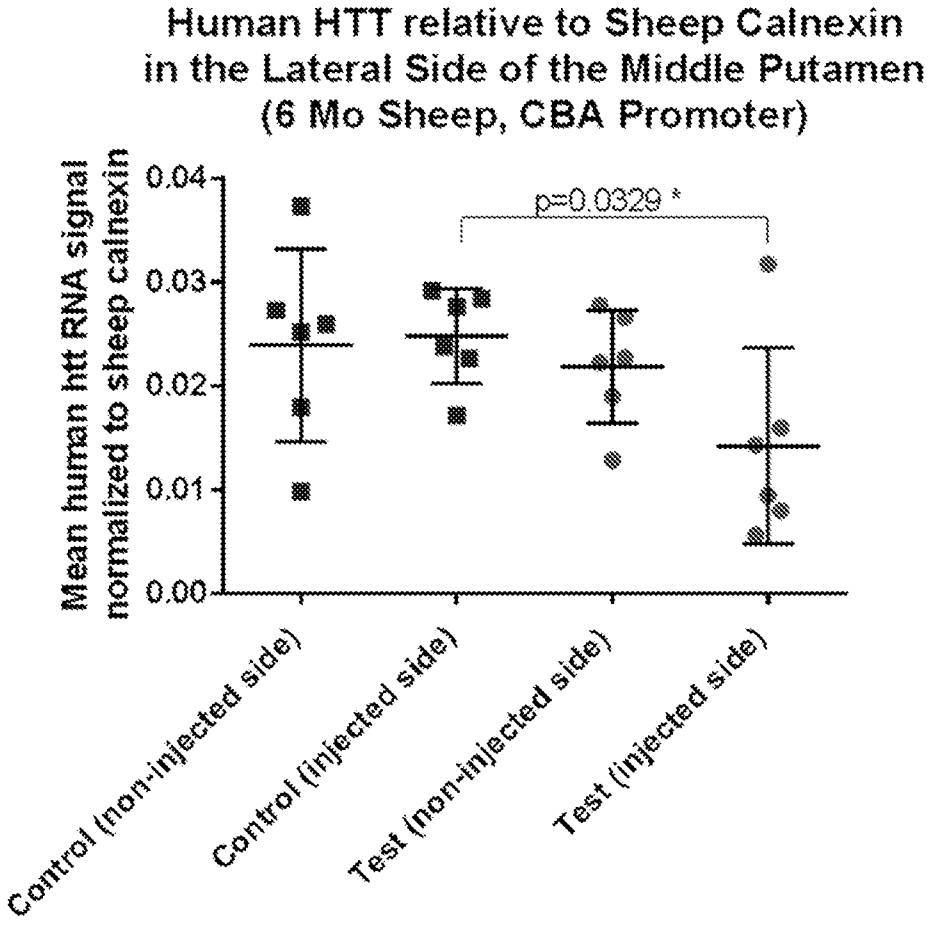

Human HTT relative to Sheep Calnexin
in the Lateral Side of the Middle Putamen
(6 Mo Sheep, CBA Promoter)
Mean human htt RNA signal
normalized to sheep calnexin
0.04
0.03
0.02
0.01
0.00
p=0.0329 *
Control (non-injected side)
Control (injected side)
Test (non-injected side)
Test (injected side)

FIG. 21

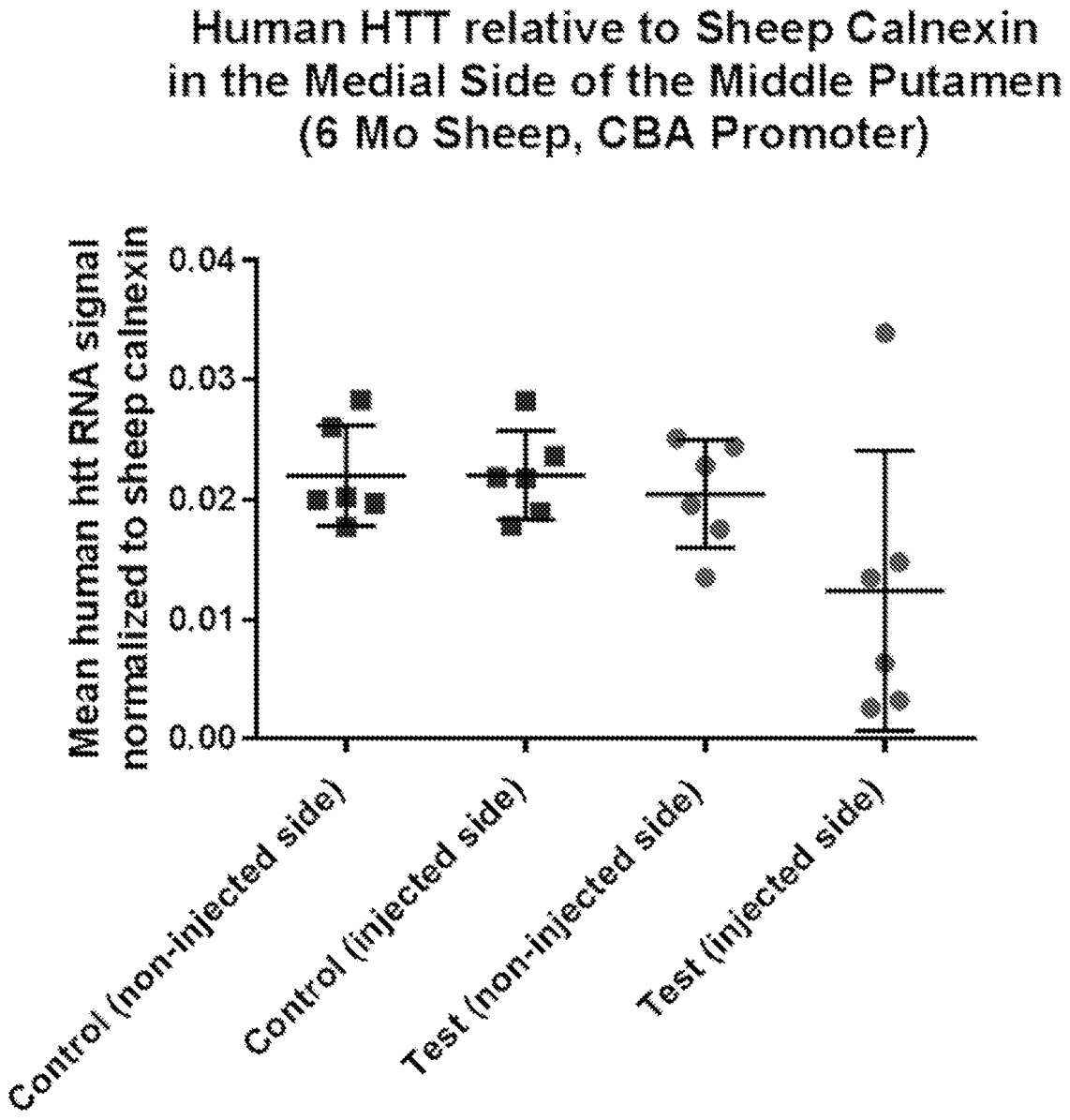

Human HTT relative to Sheep Calnexin
in the Medial Side of the Middle Putamen
(6 Mo Sheep, CBA Promoter)
Mean human htt RNA signal
normalized to sheep calnexin
0.04
0.03
0.02
0.01
0.00
Control (non-injected side)
Control (injected side)
Test (non-injected side)
Test (injected side)

FIG. 22

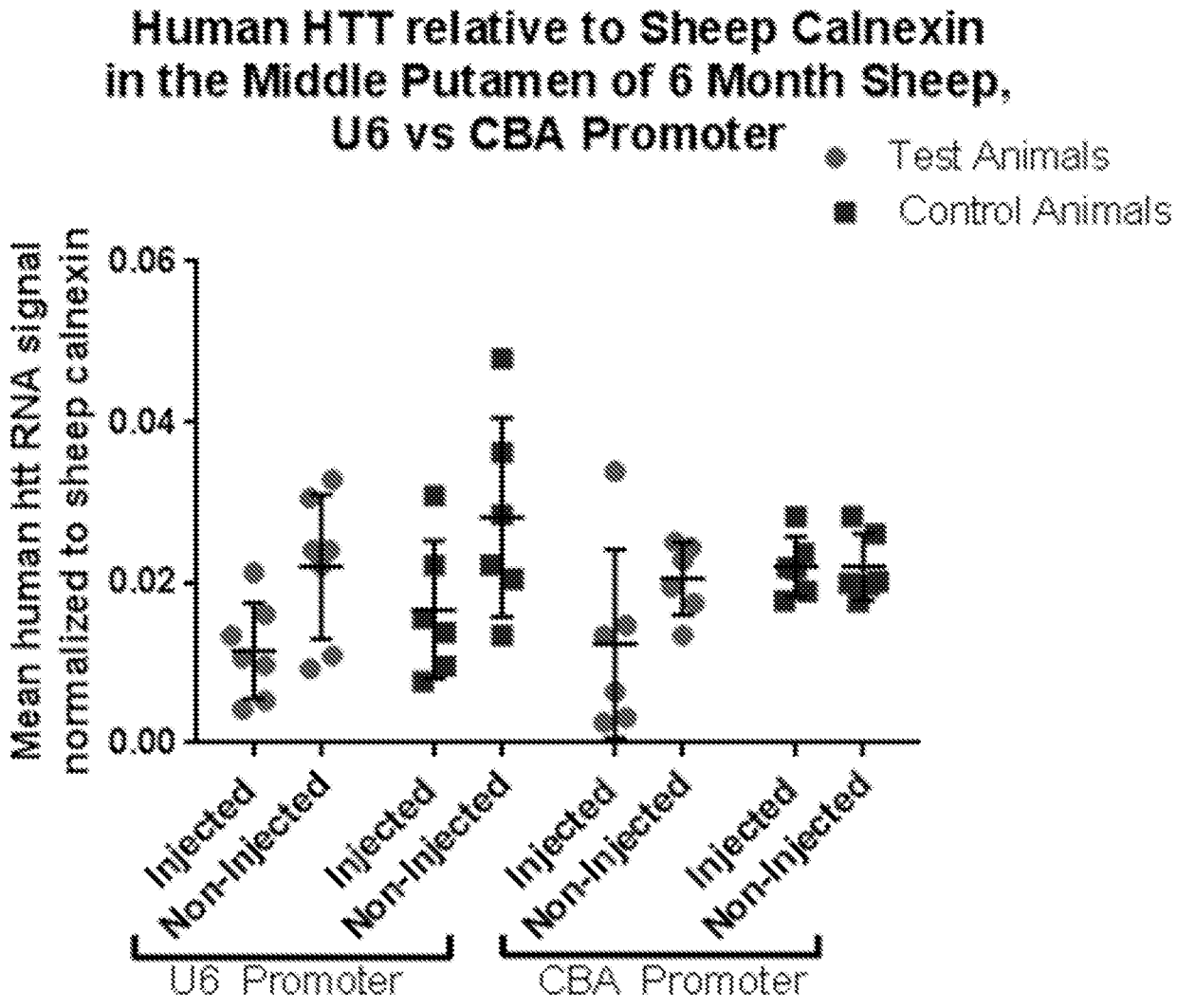

Human HTT relative to Sheep Calnexin
in the Middle Putamen of 6 Month Sheep,
U6 vs CBA Promoter
Test Animals
Control Animals
Mean human htt RNA signal
normalized to sheep calnexin
0.06
0.04
0.02
0.00
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
U6 Promoter
CBA Promoter

FIG. 23

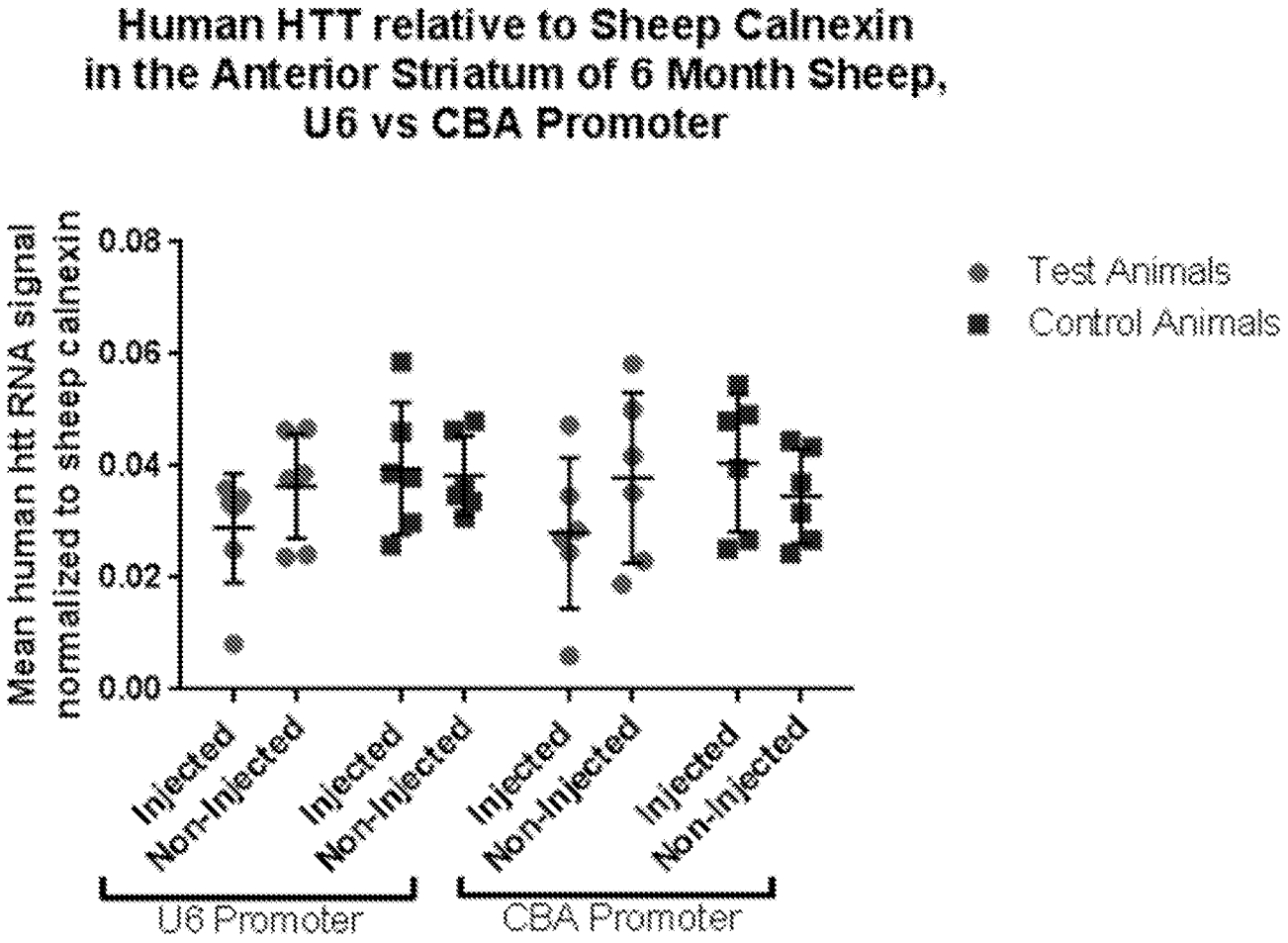

Human HTT relative to Sheep Calnexin
in the Anterior Striatum of 6 Month Sheep,
U6 vs CBA Promoter
Mean human htt RNA signal
normalized to sheep calnexin
0.08
0.06
0.04
0.02
0.00
Test Animals
Control Animals
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
Injected
Non-Injected
U6 Promoter
CBA Promoter

FIG. 24

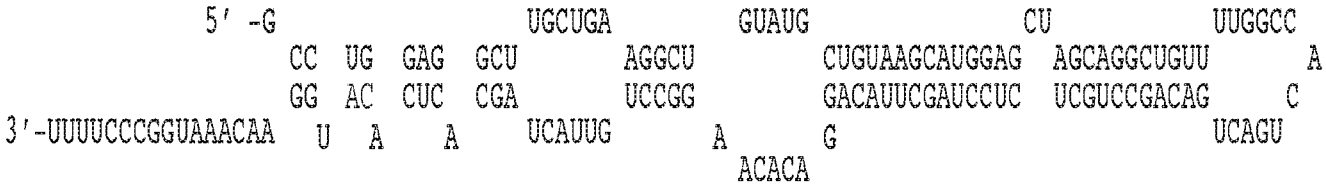


FIG. 25A

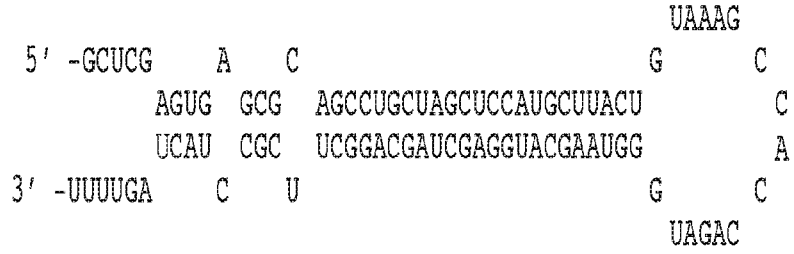


FIG. 25B

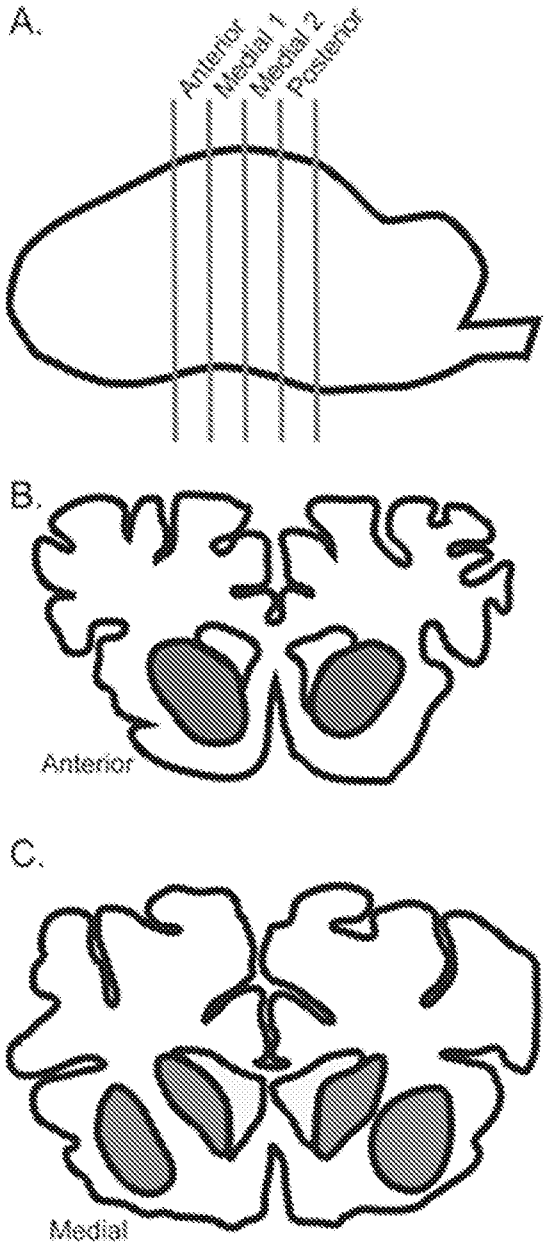


FIG. 26A

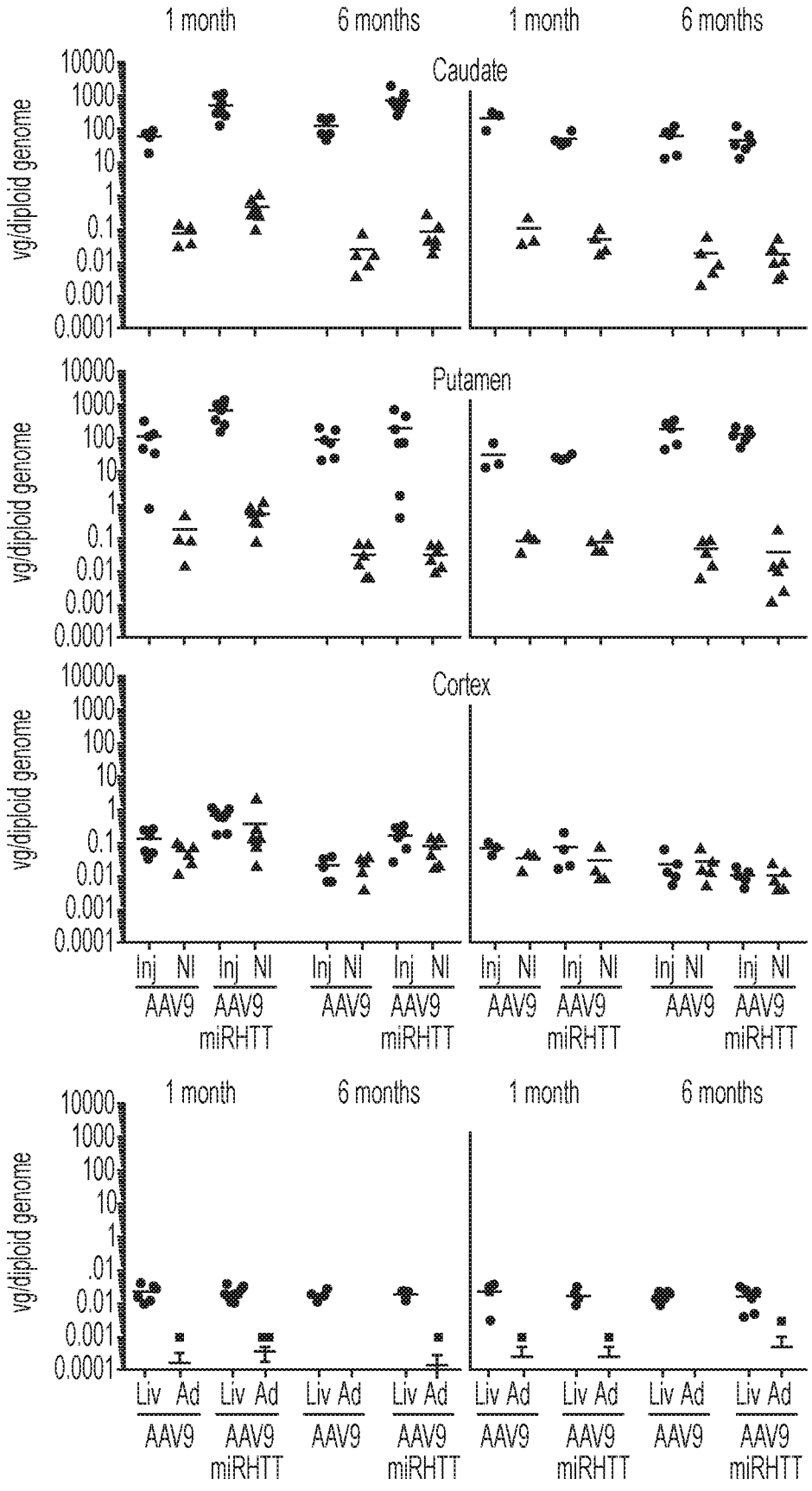

1 month
6 months
1 month
6 months
Caudate
Putamen
Cortex
vg/diploid genome
10000
1000
100
10
1
0.1
0.01
0.001
0.0001
Inj NI Inj NI Inj NI Inj NI
AAV9 AAV9 miRHTT AAV9 AAV9 miRHTT
Liv Ad Liv Ad Liv Ad Liv Ad
AAV9 AAV9 miRHTT AAV9 AAV9 miRHTT

FIG. 26B

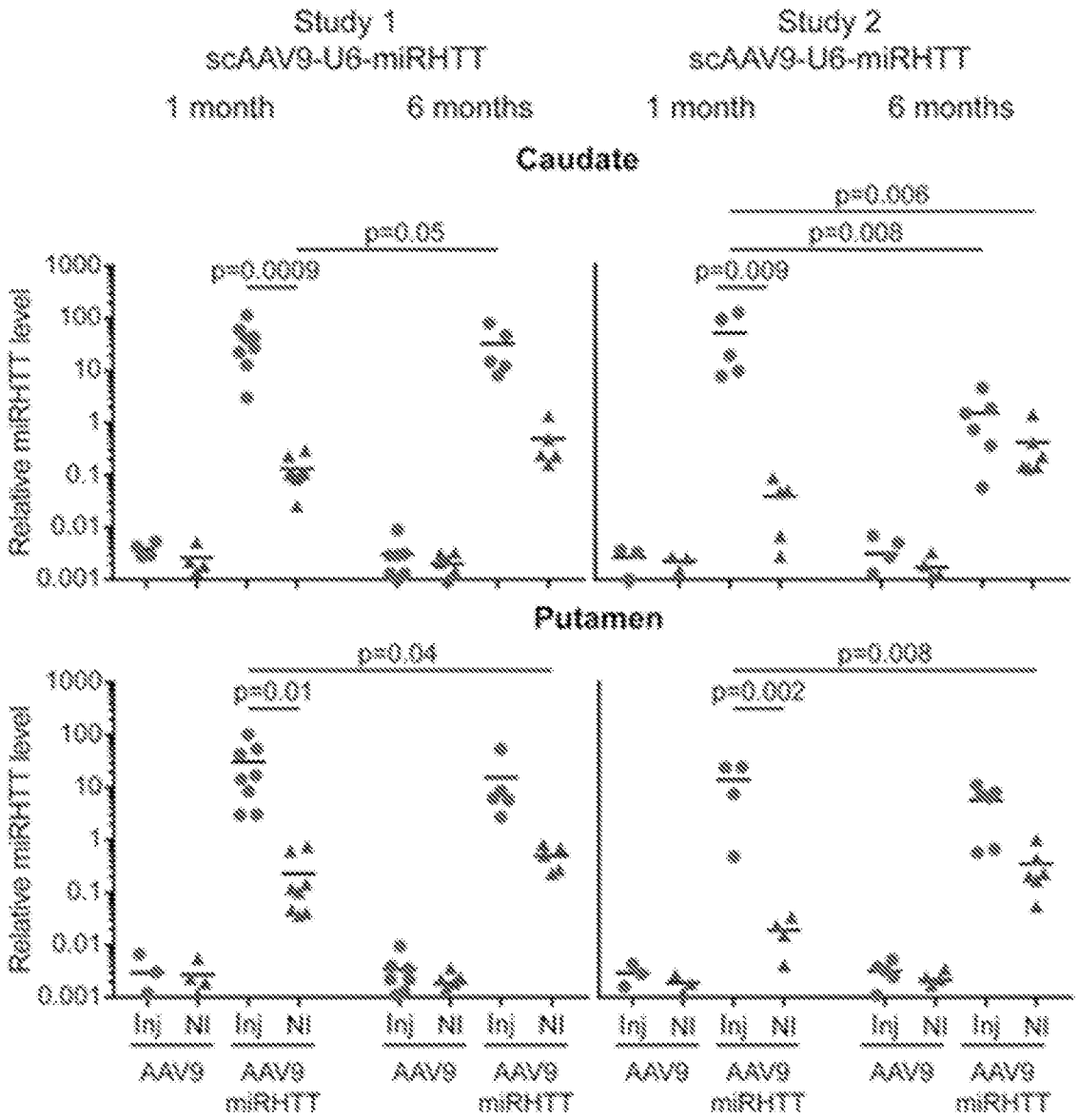

Study 1
scAAV9-U6-miRHTT
1 month
6 months
Study 2
scAAV9-U6-miRHTT
1 month
6 months
Caudate
Putamen
Relative miRHTT level
1000
100
10
1
0.1
0.01
0.001
p=0.05
p=0.0009
p=0.006
p=0.008
p=0.009
p=0.04
p=0.01
p=0.008
p=0.002
Inj
NI
AAV9
AAV9 miRHTT

FIG. 27

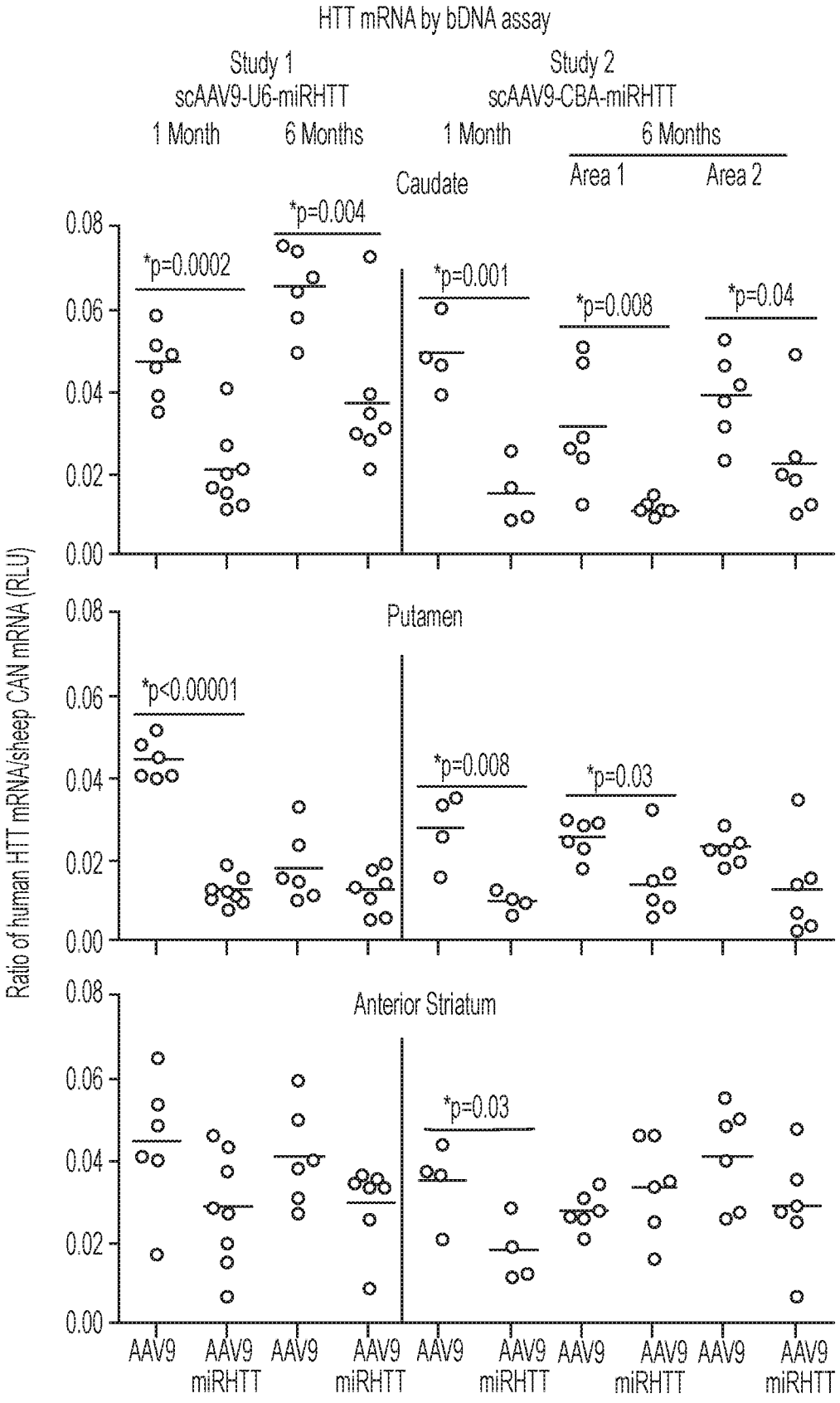

HTT mRNA by bDNA assay
Study 1
scAAV9-U6-miRHTT
1 Month
6 Months
Study 2
scAAV9-CBA-miRHTT
1 Month
6 Months
Area 1
Area 2
Caudate
Putamen
Anterior Striatum
*p=0.0002
*p=0.004
*p=0.001
*p=0.008
*p=0.04
*p<0.00001
*p=0.008
*p=0.03
*p=0.03
Ratio of human HTT mRNA/sheep CAN mRNA (RLU)
0.00
0.02
0.04
0.06
0.08
AAV9
AAV9 miRHTT

FIG. 28

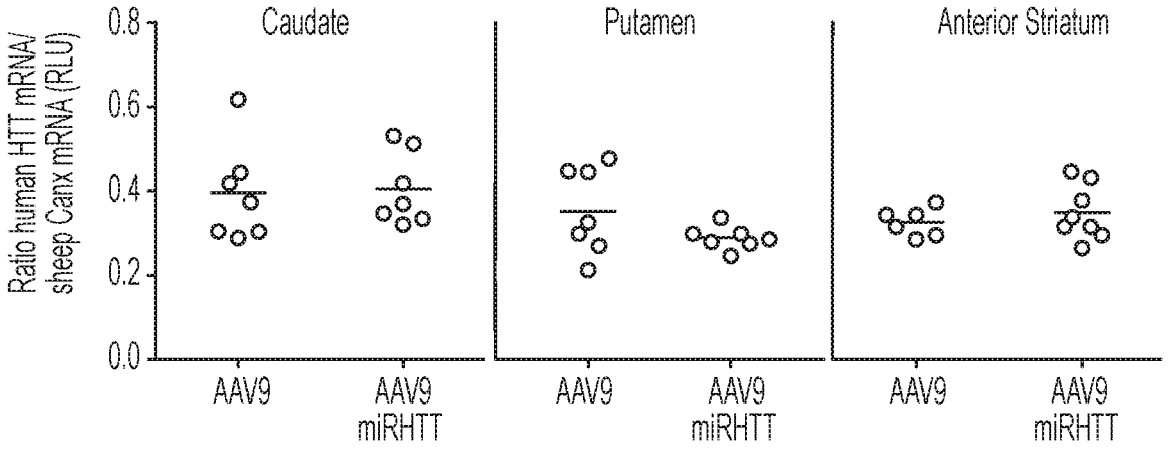


FIG. 29A

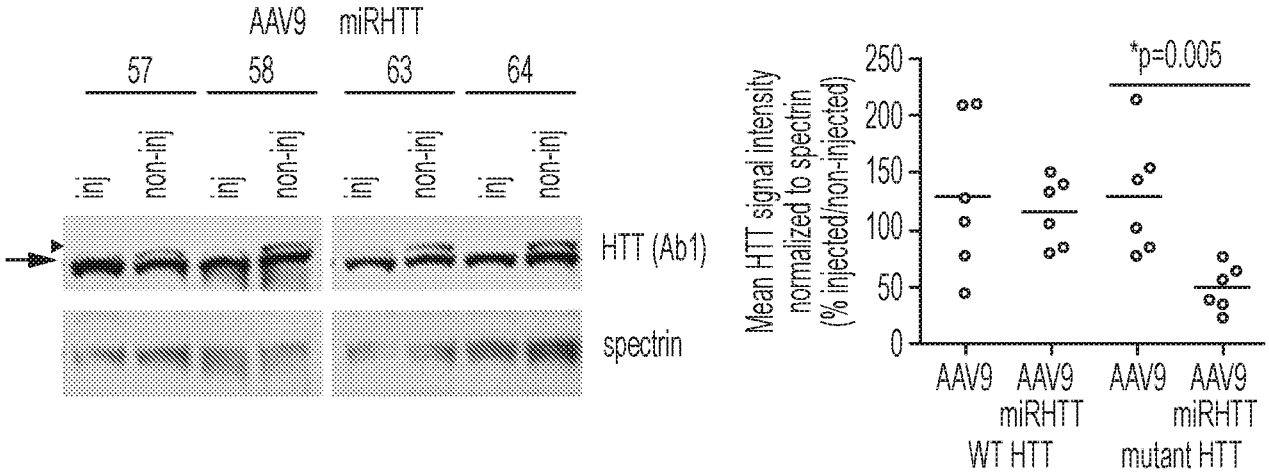


FIG. 29B

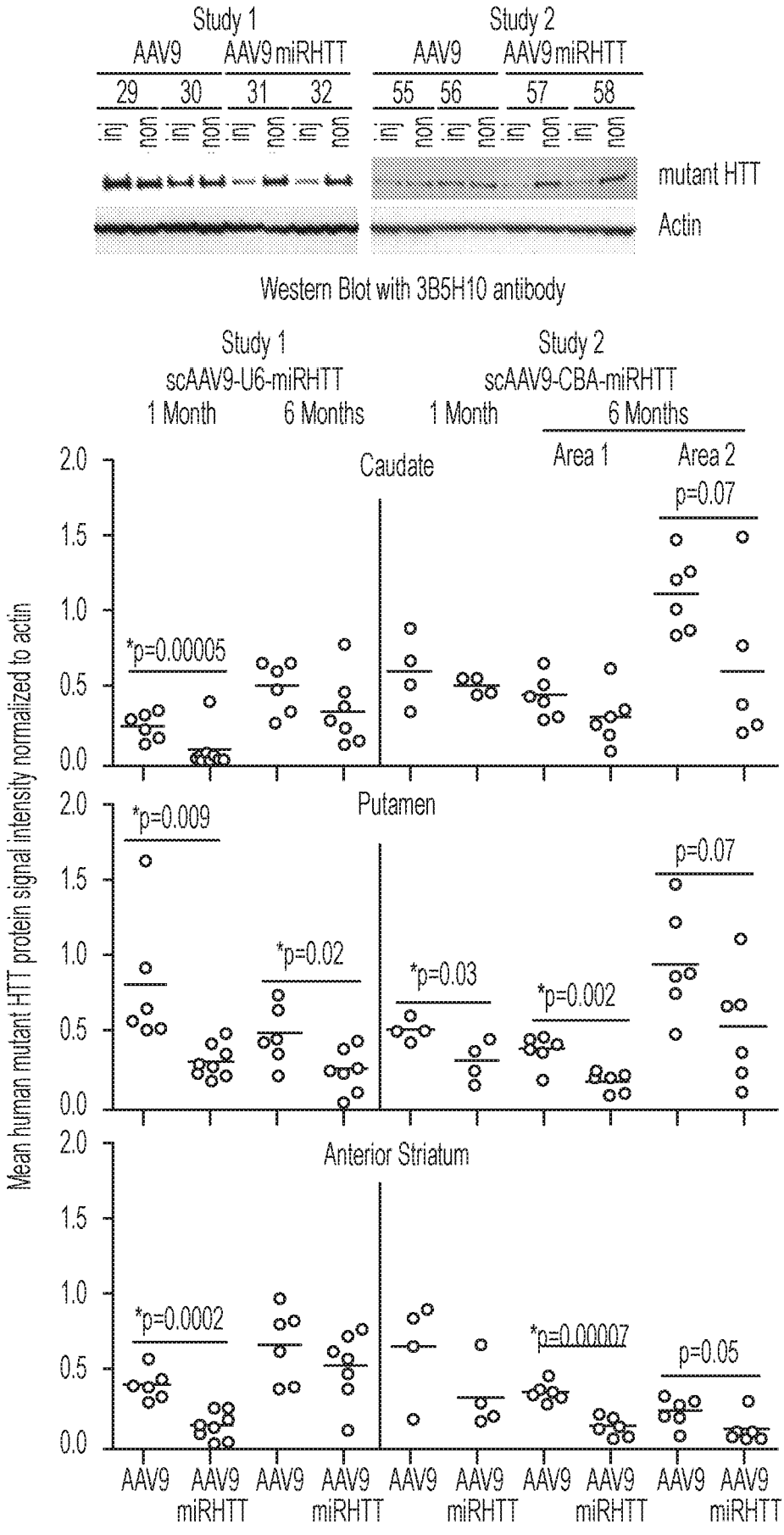

Study 1
AAV9
AAV9 miRHTT
29
30
31
32
Study 2
AAV9
AAV9 miRHTT
55
56
57
58
inj
non
mutant HTT
Actin
Western Blot with 3B5H10 antibody
Study 1
scAAV9-U6-miRHTT
1 Month
6 Months
Study 2
scAAV9-CBA-miRHTT
1 Month
6 Months
Area 1
Area 2
Caudate
Putamen
Anterior Striatum
Mean human mutant HTT protein signal intensity normalized to actin
2.0
1.5
1.0
0.5
0.0
*p=0.00005
p=0.07
*p=0.009
*p=0.02
*p=0.03
*p=0.002
p=0.07
*p=0.0002
*p=0.00007
p=0.05
AAV9
AAV9 miRHTT

FIG. 30

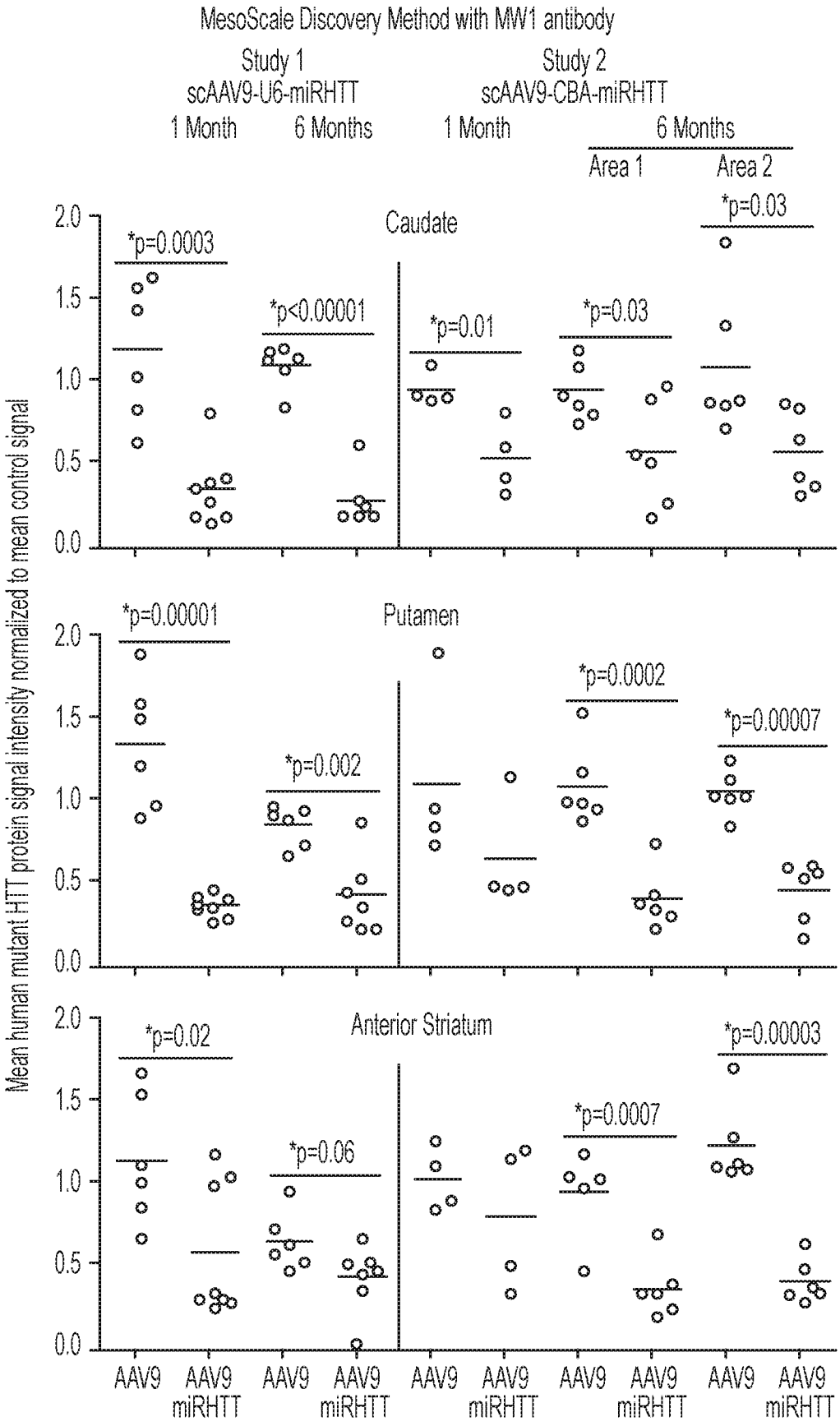

MesoScale Discovery Method with MW1 antibody
Study 1
scAAV9-U6-miRHTT
1 Month
6 Months
Study 2
scAAV9-CBA-miRHTT
1 Month
6 Months
Area 1
Area 2
Caudate
*p=0.0003
*p<0.00001
*p=0.01
*p=0.03
*p=0.03
Putamen
*p=0.00001
*p=0.002
*p=0.0002
*p=0.00007
Anterior Striatum
*p=0.02
*p=0.06
*p=0.0007
*p=0.00003
Mean human mutant HTT protein signal intensity normalized to mean control signal
2.0
1.5
1.0
0.5
0.0
AAV9
AAV9 miRHTT

FIG. 31

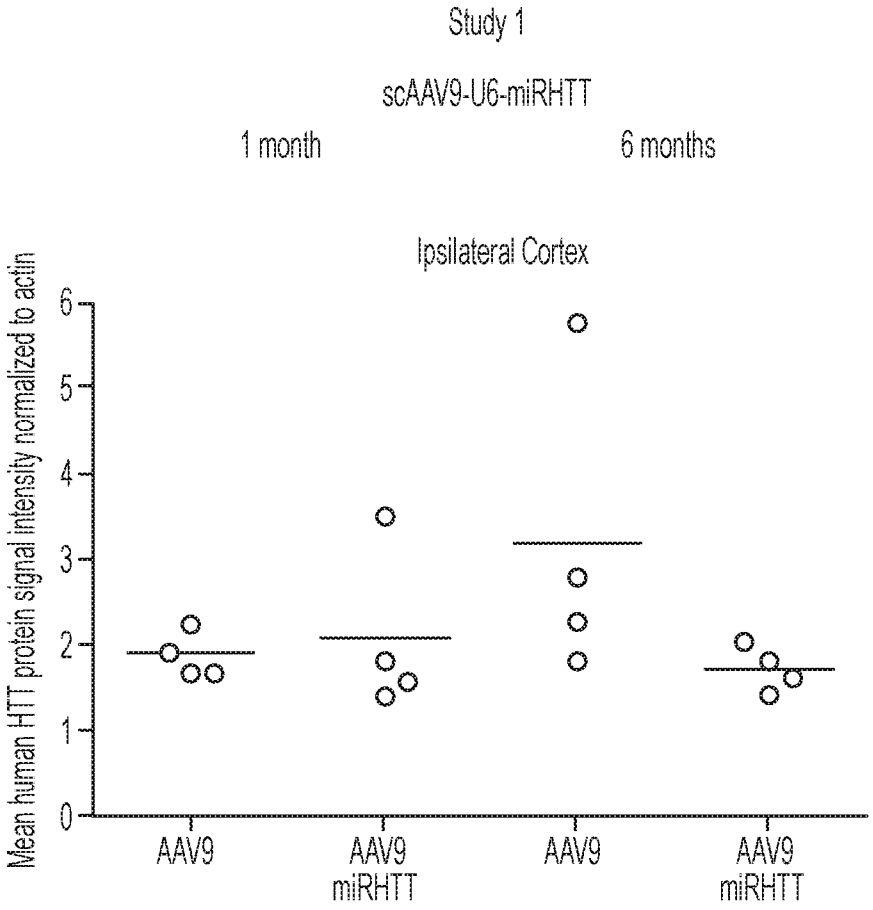

Study 1
scAAV9-U6-miRHTT
1 month
6 months
Ipsilateral Cortex
Mean human HTT protein signal intensity normalized to actin
0
1
2
3
4
5
6
AAV9
AAV9 miRHTT
AAV9
AAV9 miRHTT

FIG. 32A

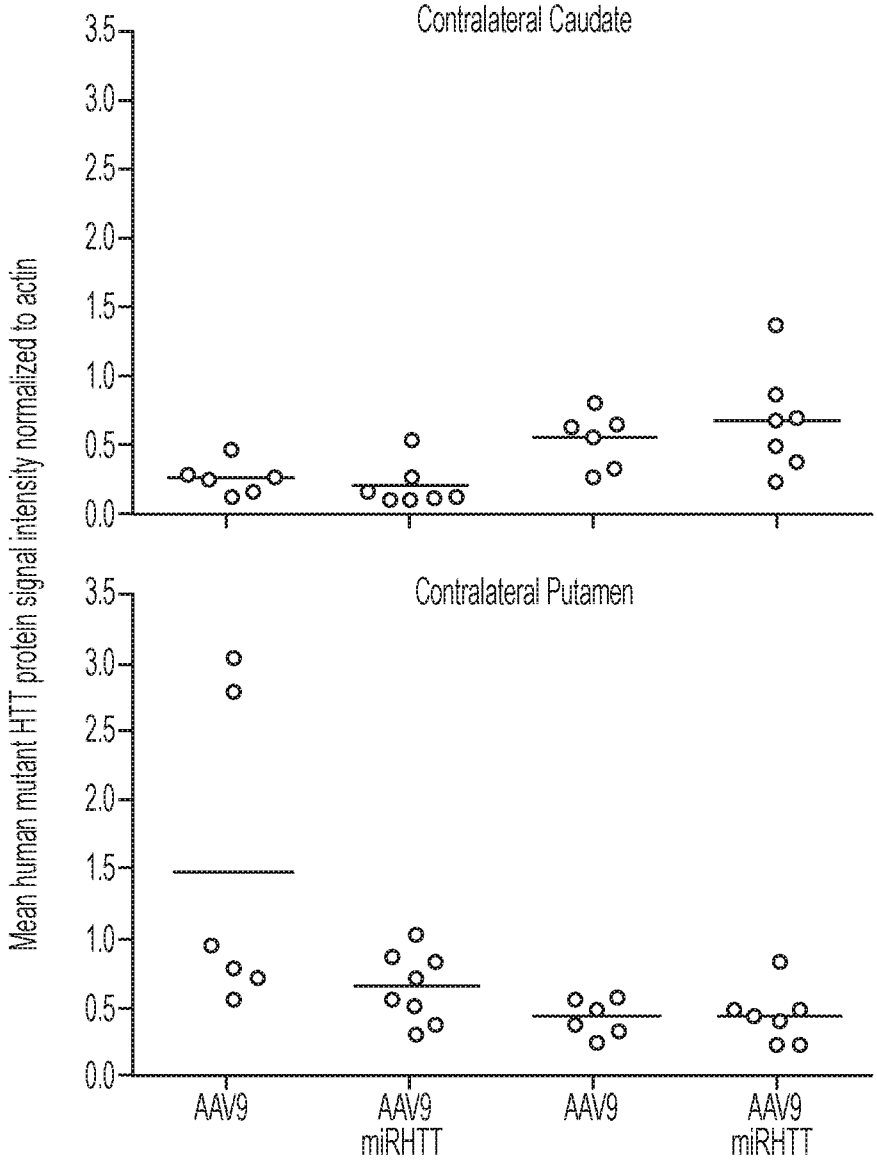

Contralateral Caudate
Contralateral Putamen
Mean human mutant HTT protein signal intensity normalized to actin
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
AAV9
AAV9 miRHTT
AAV9
AAV9 miRHTT

FIG. 32B