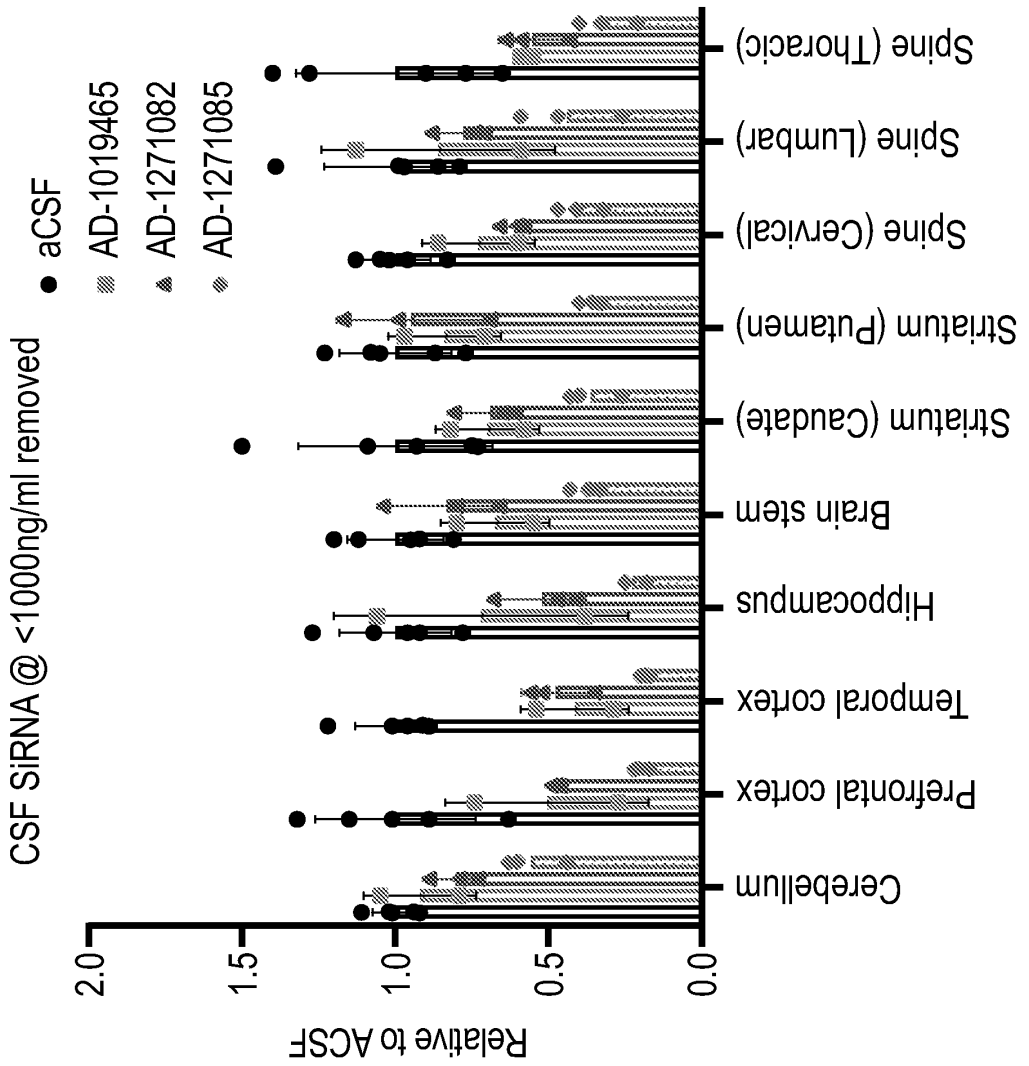
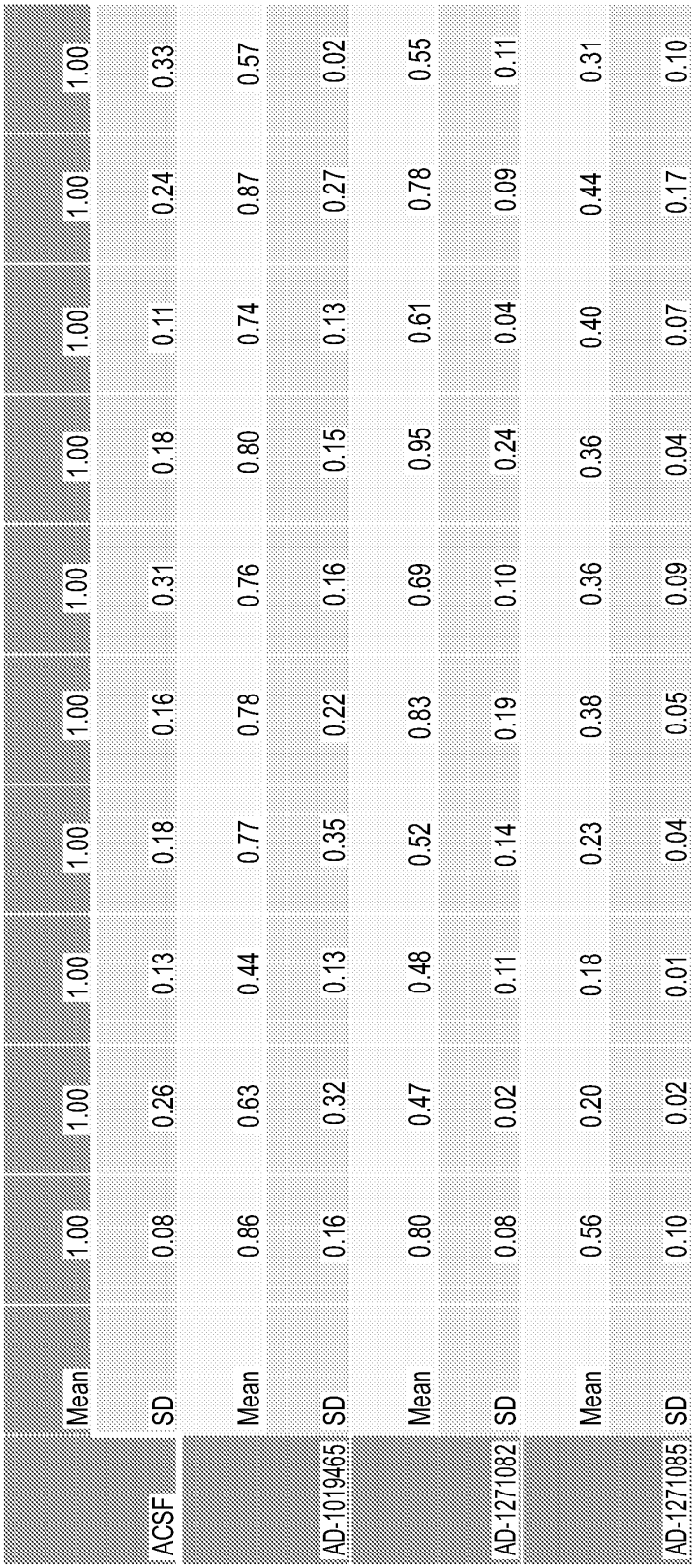

CSF SiRNA @ <1000ng/ml removed
aCSF
AD-1019465
AD-1271082
AD-1271085
Relative to ACSF
2.0
1.5
1.0
0.5
0.0
Cerebellum
Prefrontal cortex
Temporal cortex
Hippocampus
Brain stem
Striatum (Caudate)
Striatum (Putamen)
Spine (Cervical)
Spine (Lumbar)
Spine (Thoracic)

FIG. 1



	Mean	1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00		1.00	
ACSF	SD	0.08		0.26		0.13		0.18		0.16		0.31		0.18		0.11		0.24		0.33	
	Mean	0.86		0.63		0.44		0.77		0.78		0.76		0.80		0.74		0.87		0.57	
AD-1019465	SD	0.16		0.32		0.13		0.35		0.22		0.16		0.15		0.13		0.27		0.02	
	Mean	0.80		0.47		0.48		0.52		0.83		0.69		0.95		0.61		0.78		0.55	
AD-1271082	SD	0.08		0.02		0.11		0.14		0.19		0.10		0.24		0.04		0.09		0.11	
	Mean	0.56		0.20		0.18		0.23		0.38		0.36		0.36		0.40		0.44		0.31	
AD-1271085	SD	0.10		0.02		0.01		0.04		0.05		0.09		0.04		0.07		0.17		0.10	

FIG. 2

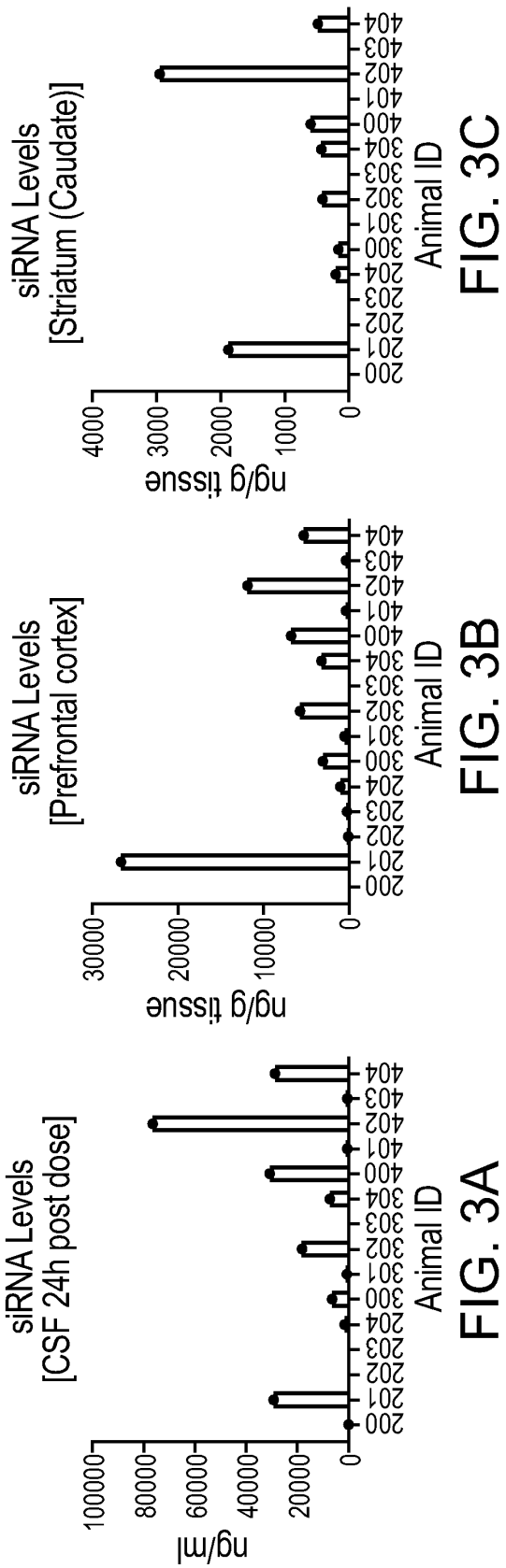


FIG. 3A

FIG. 3B

FIG. 3C

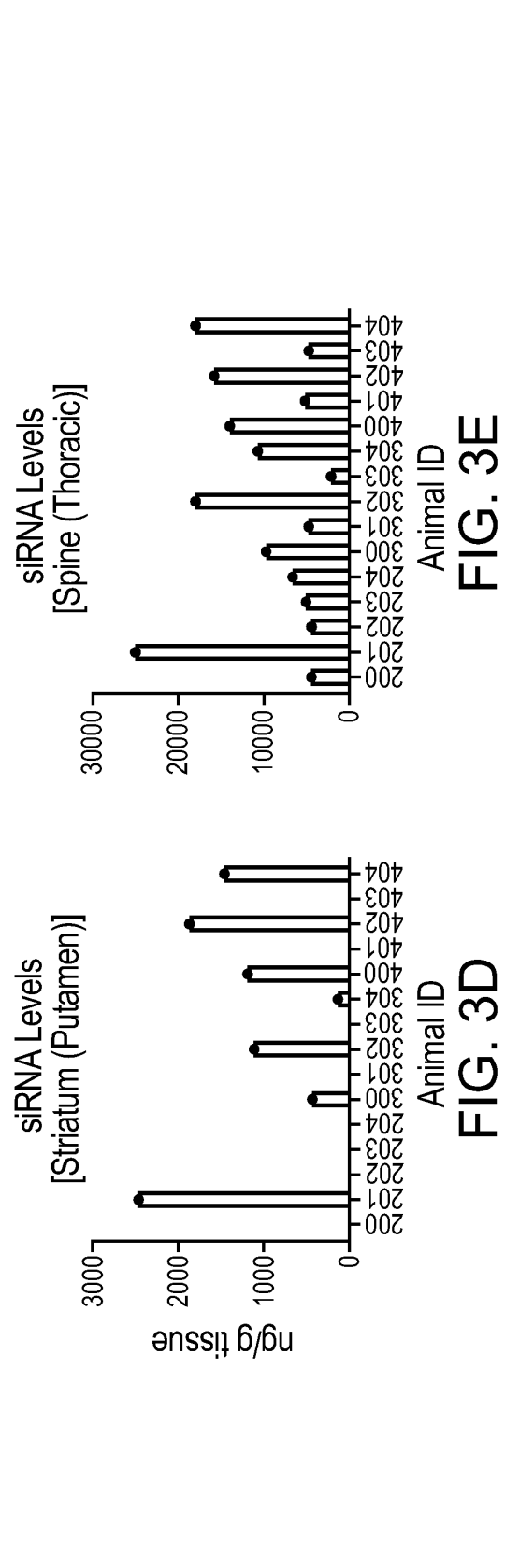


FIG. 3D

FIG. 3E

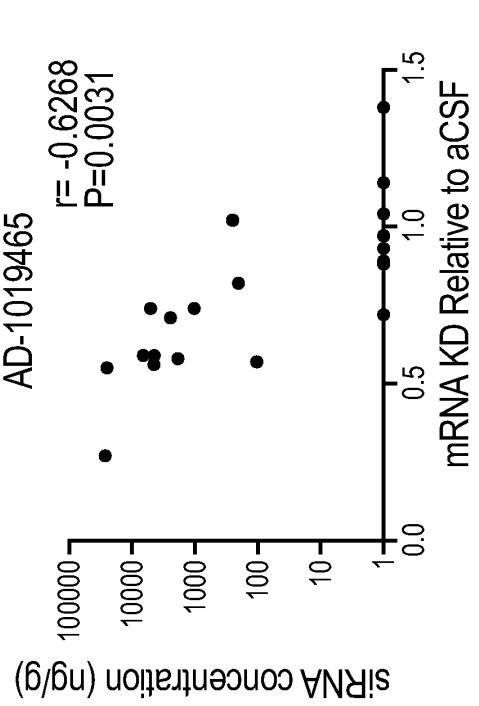


FIG. 4A

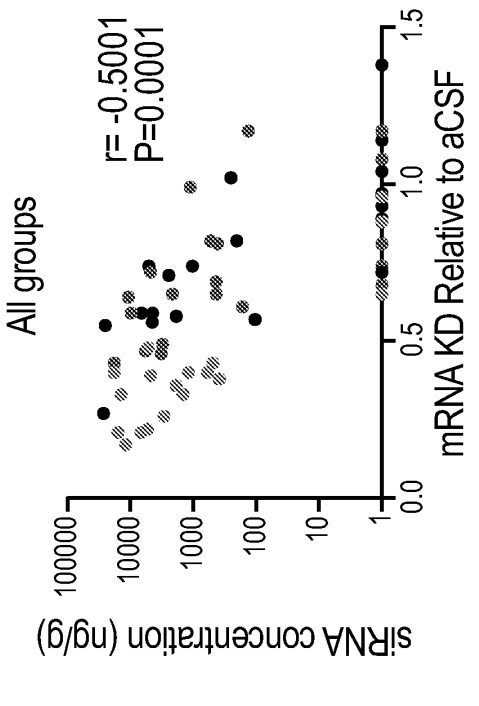


FIG. 4B

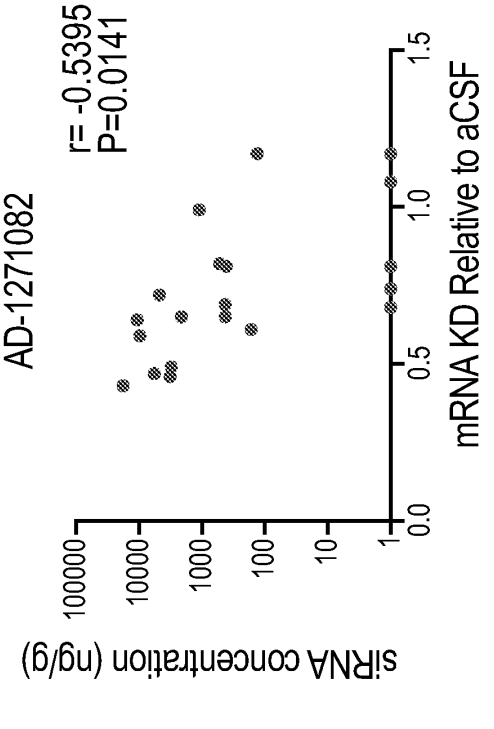


FIG. 4C

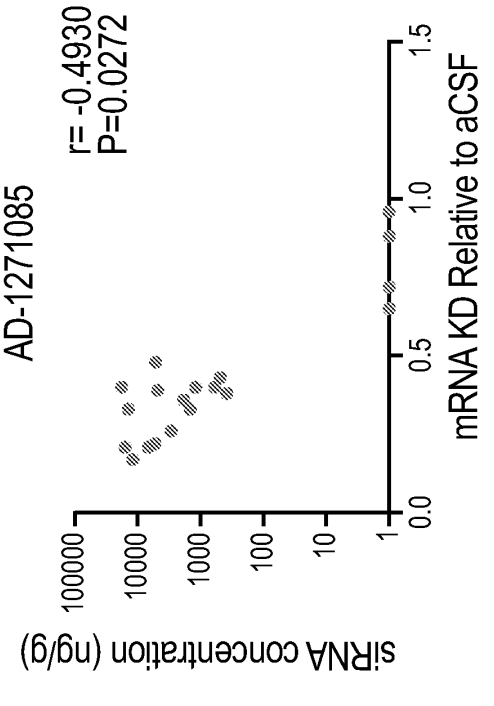


FIG. 4D

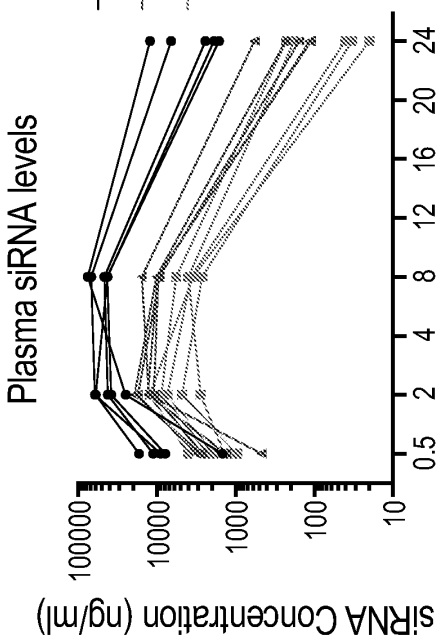

Plasma siRNA levels
AD-1019465 (Ex1)
AD-1271082 (Ex1)
AD-1271085 (FL)
siRNA Concentration (ng/ml)
100000
10000
1000
100
10
0.5
2
4
8
12
16
20
24
Time (hours)

FIG. 5A

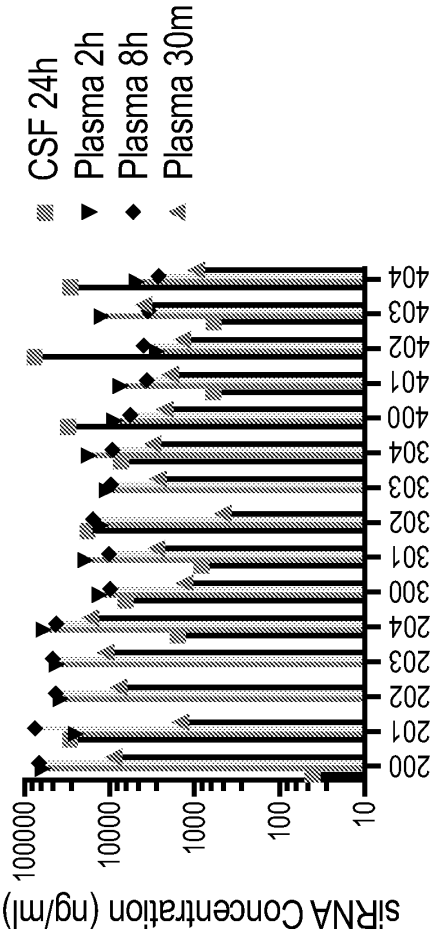

CSF 24h
Plasma 2h
Plasma 8h
Plasma 30m
siRNA Concentration (ng/ml)
100000
10000
1000
100
10
200
201
202
203
204
300
301
302
303
304
400
401
402
403
404
Animal ID

FIG. 5B

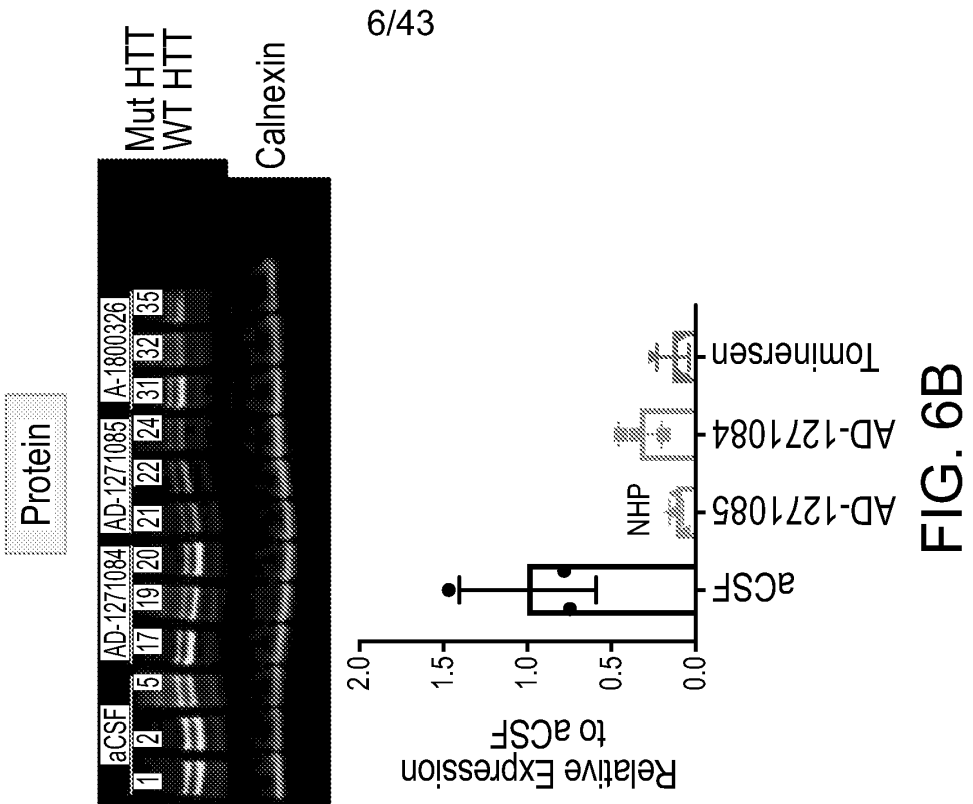

Protein
Mut HTT
WT HTT
Calnexin
aCSF
AD-1271084
AD-1271085
A-1800326
1
2
5
17
19
20
21
22
24
31
32
35
Relative Expression to aCSF
2.0
1.5
1.0
0.5
0.0
aCSF
NHP
AD-1271085
AD-1271084
Tominersen

FIG. 6B

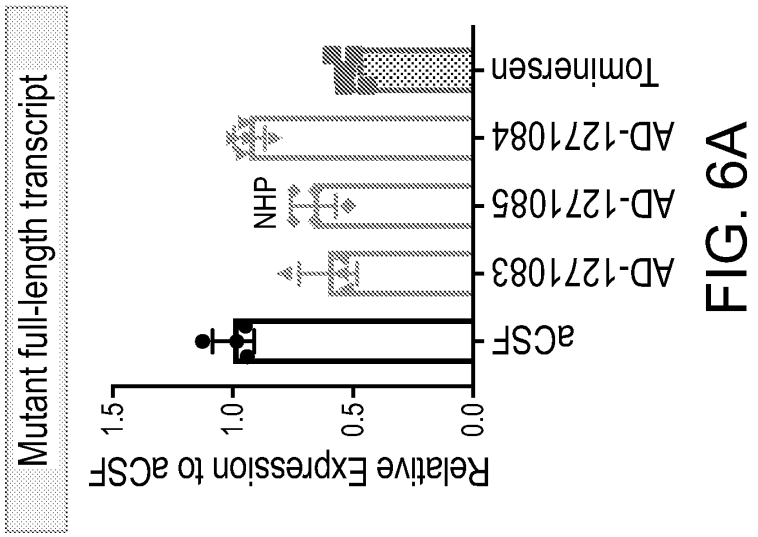

Mutant full-length transcript
Relative Expression to aCSF
1.5
1.0
0.5
0.0
aCSF
AD-1271083
NHP
AD-1271085
AD-1271084
Tominersen

FIG. 6A

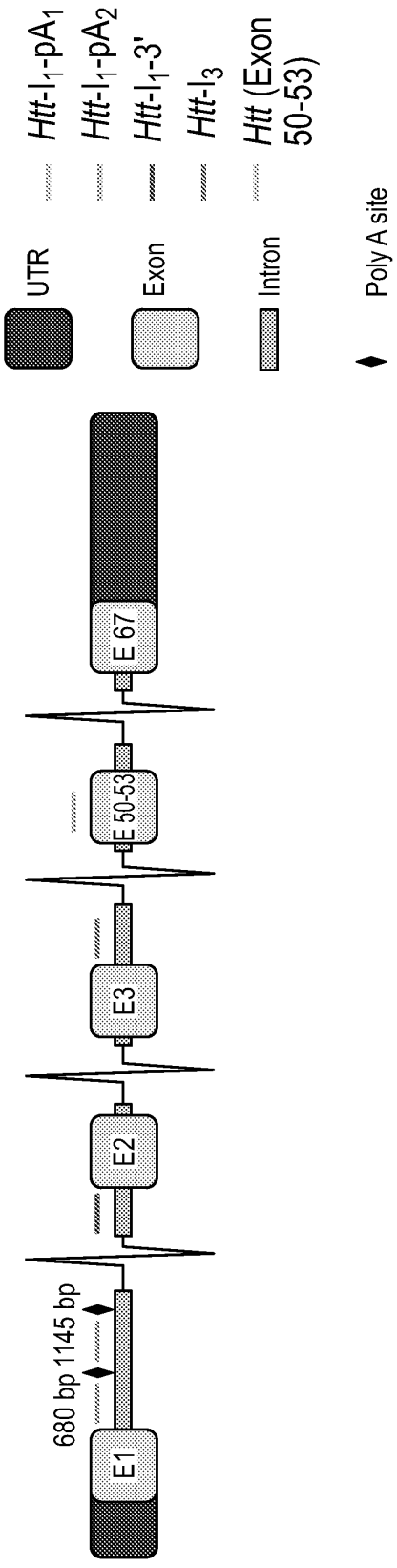

Htt-I1-pA1
Htt-I1-pA2
Htt-I1-3'
Htt-I3
Htt (Exon 50-53)
UTR
Exon
Intron
Poly A site
E1
E2
E3
E 50-53
E 67
680 bp
1145 bp

FIG. 7

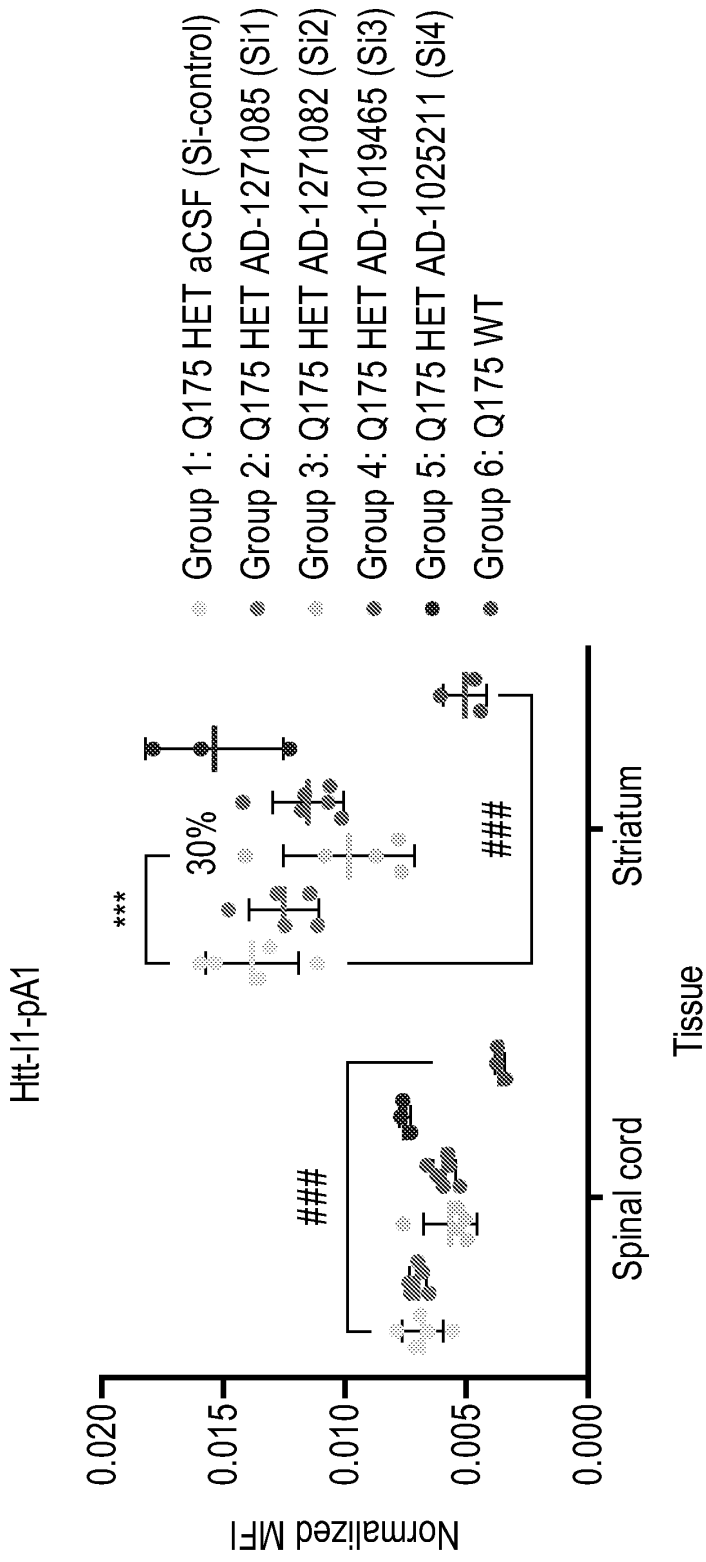

Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Group 6: Q175 WT
Htt-I1-pA1
Normalized MFI
0.020
0.015
0.010
0.005
0.000
***
30%
###
###
Spinal cord
Striatum
Tissue

FIG. 8A

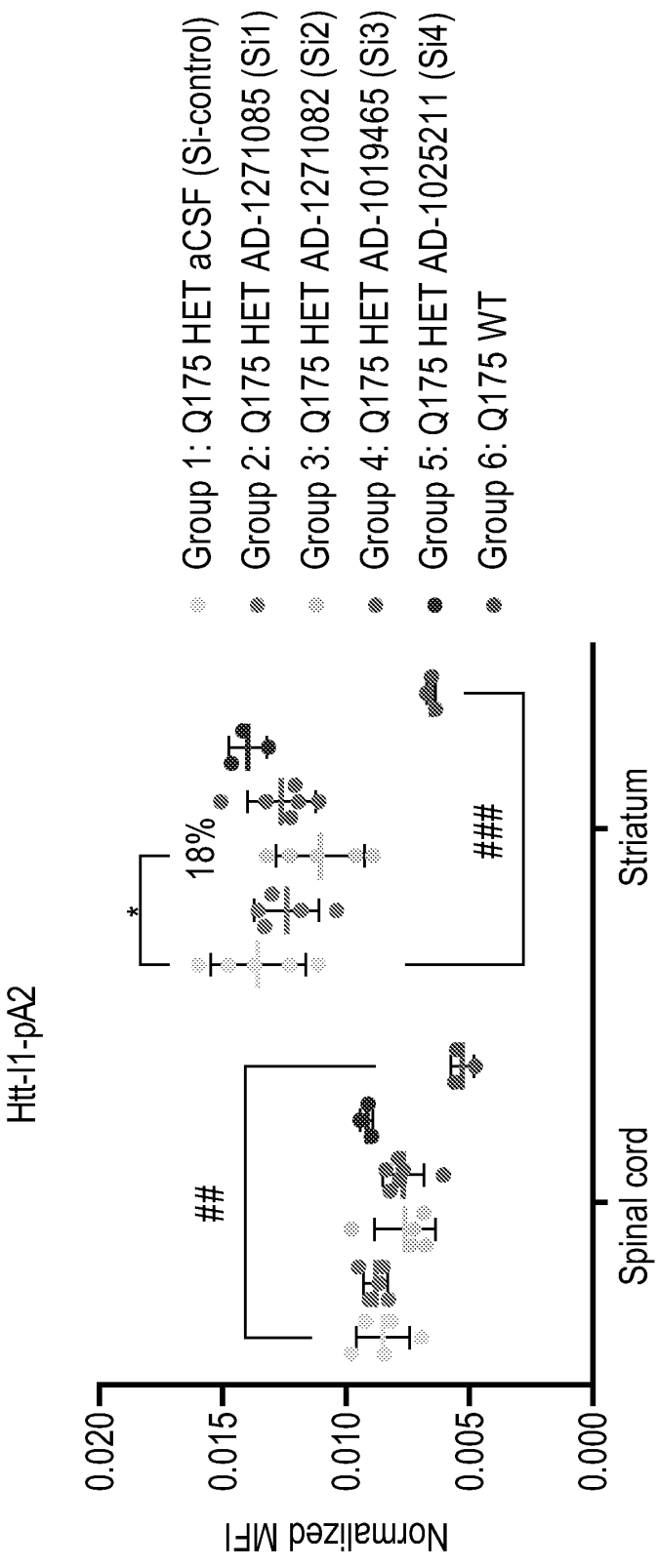

Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Group 6: Q175 WT
Htt-I1-pA2
Normalized MFI
0.020
0.015
0.010
0.005
0.000
##
*
18%
###
Spinal cord
Striatum
Tissue

FIG. 8B

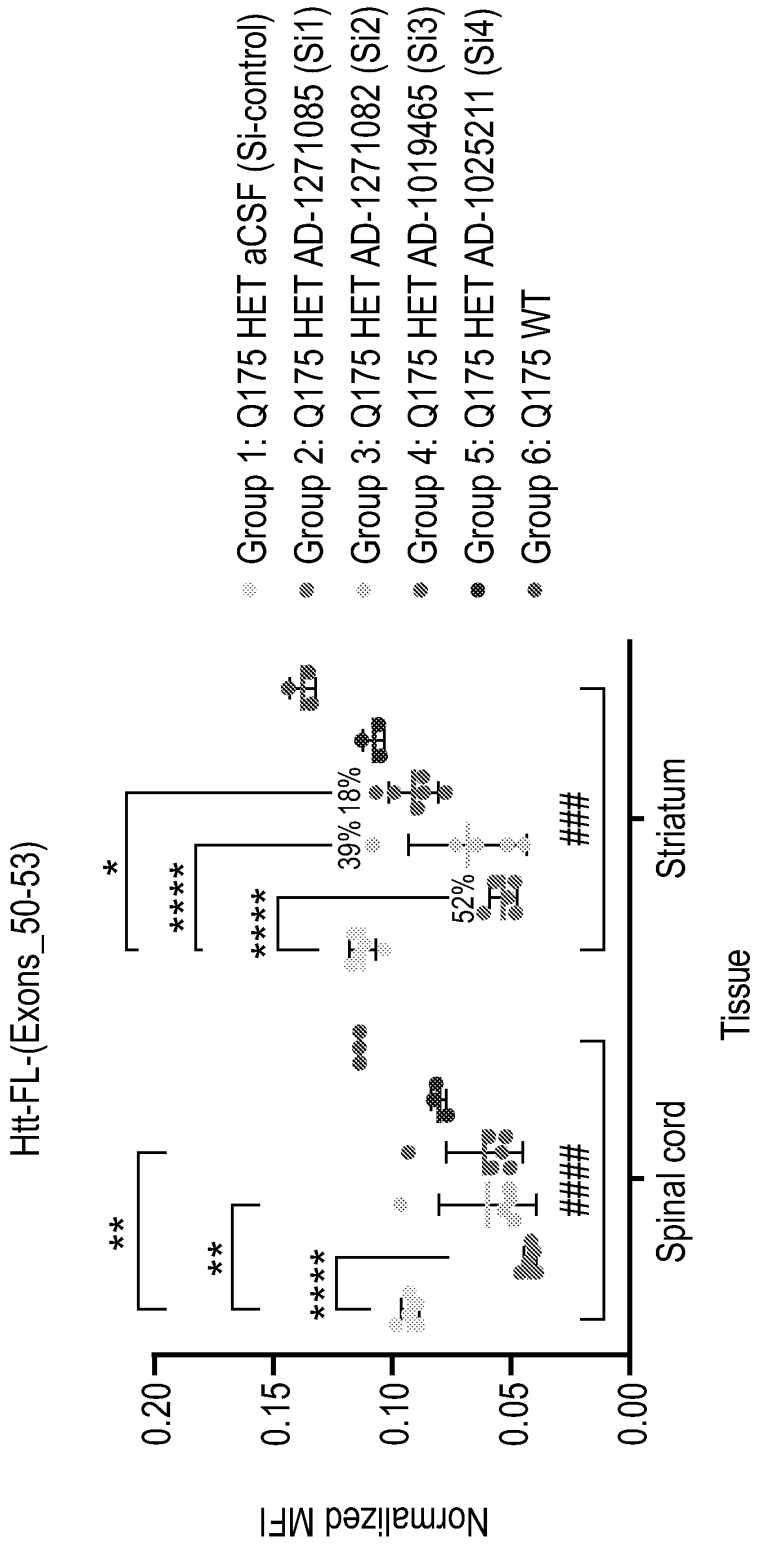

Htt-FL-(Exons_50-53)
Normalized MFI
0.20
0.15
0.10
0.05
0.00
Tissue
Spinal cord
Striatum
Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Group 6: Q175 WT
**
**
****
#####
****
*
****
###
39%
18%
52%

FIG. 8C

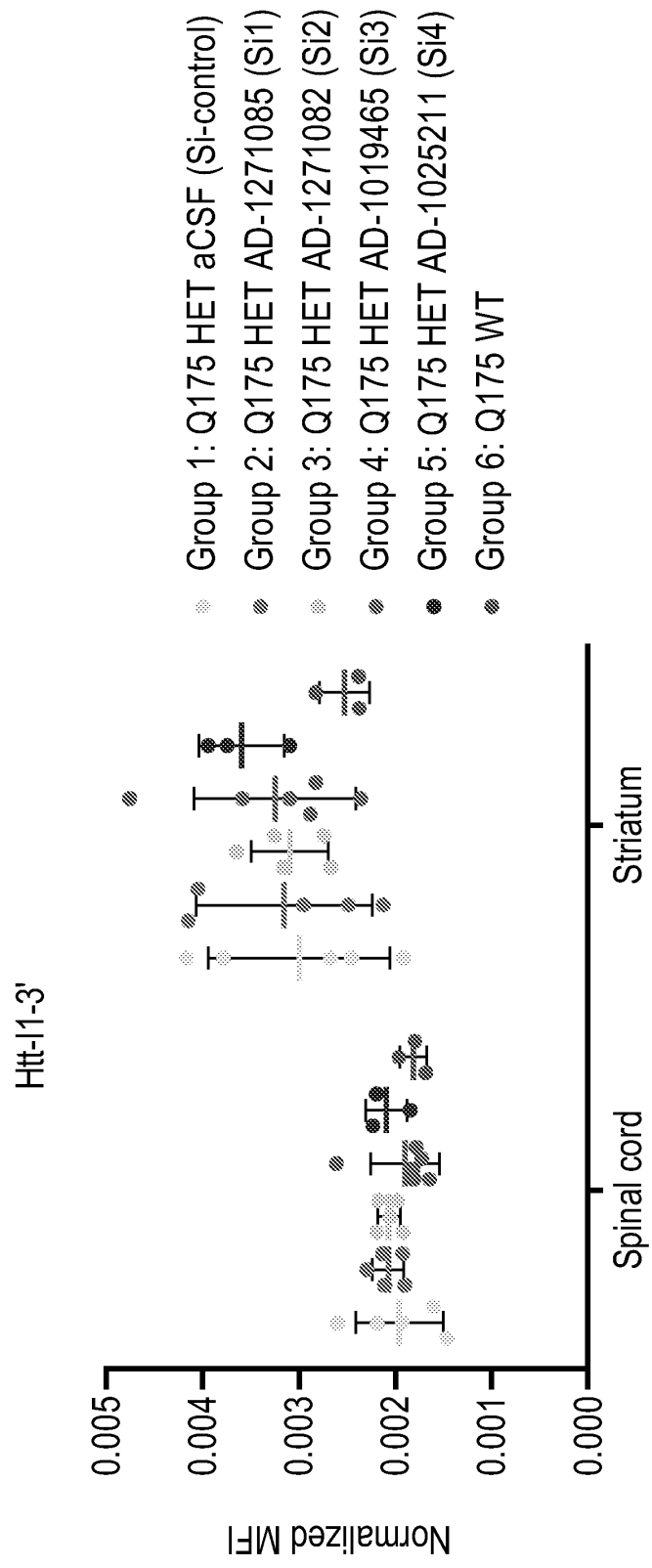

Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Group 6: Q175 WT
Htt-I1-3'
Normalized MFI
0.005
0.004
0.003
0.002
0.001
0.000
Spinal cord
Striatum
Tissue

FIG. 8D

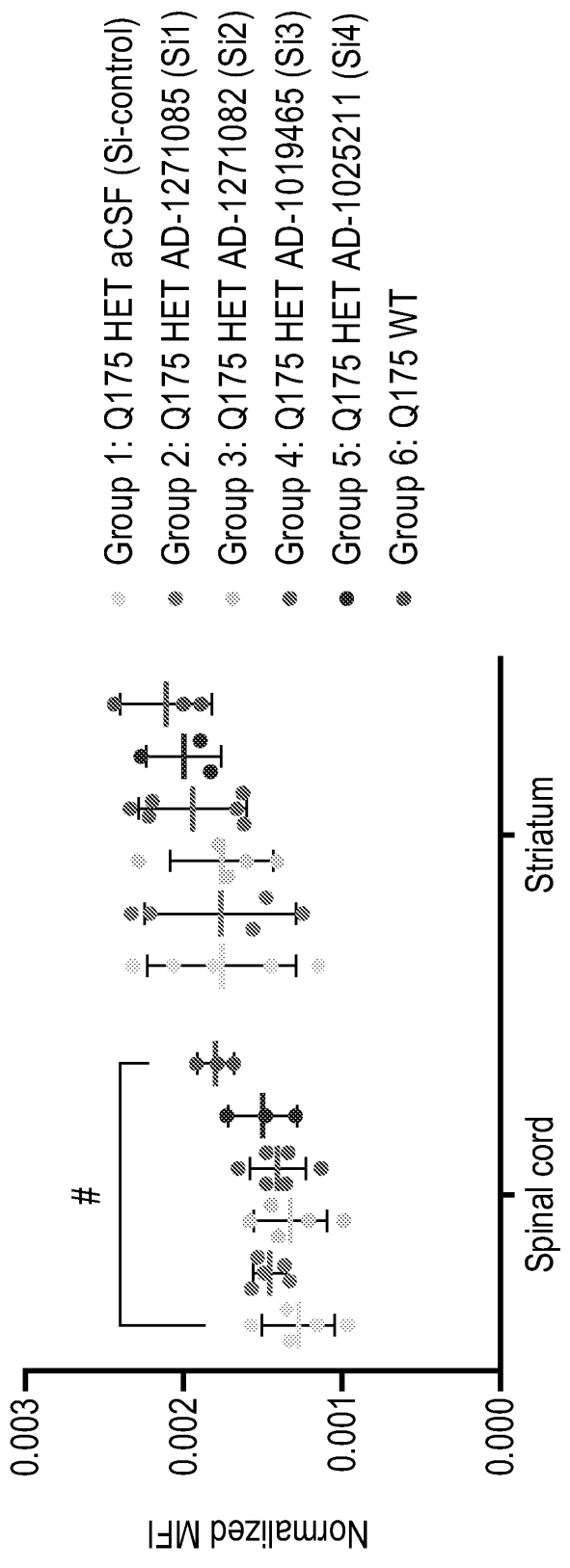

Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Group 6: Q175 WT
Htt-I3
#
0.003
0.002
0.001
0.000
Normalized MFI
Spinal cord
Striatum
Tissue

FIG. 8E

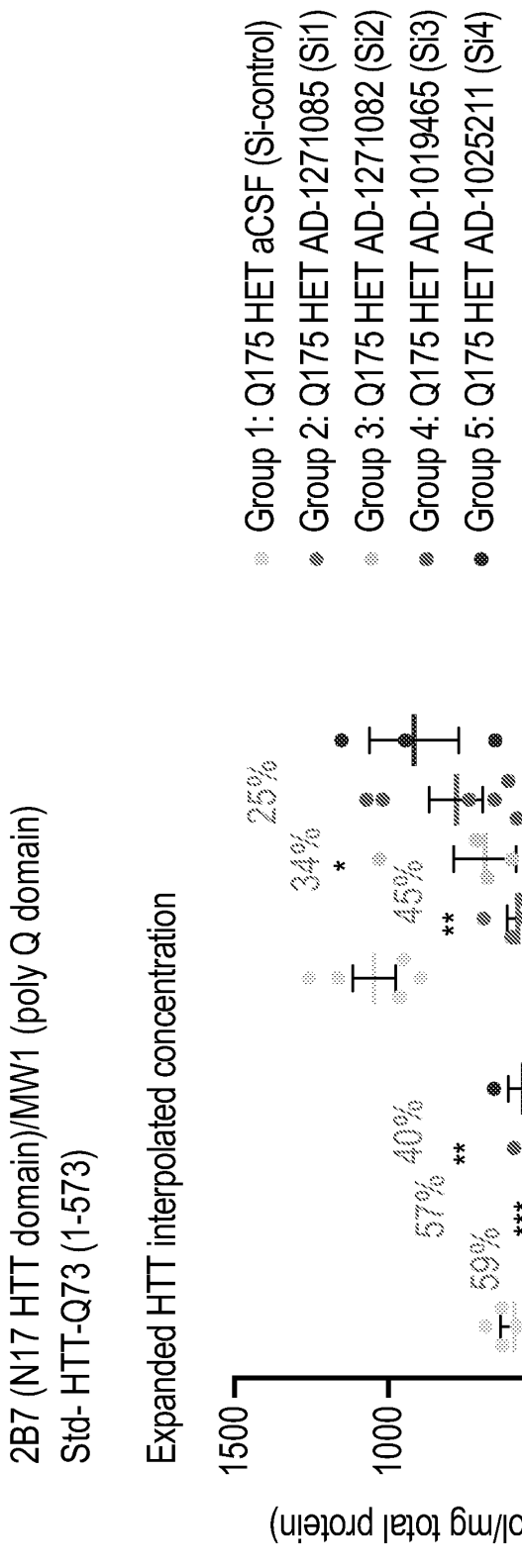

2B7 (N17 HTT domain)/MW1 (poly Q domain)
Std- HTT-Q73 (1-573)
Expanded HTT interpolated concentration
Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Conc. (fmol/mg total protein)
1500
1000
500
0
25%
34%
46%
40%
57%
59%
*
**
**
***
****
Spinal cord
Striatum
Tissue

FIG. 8F

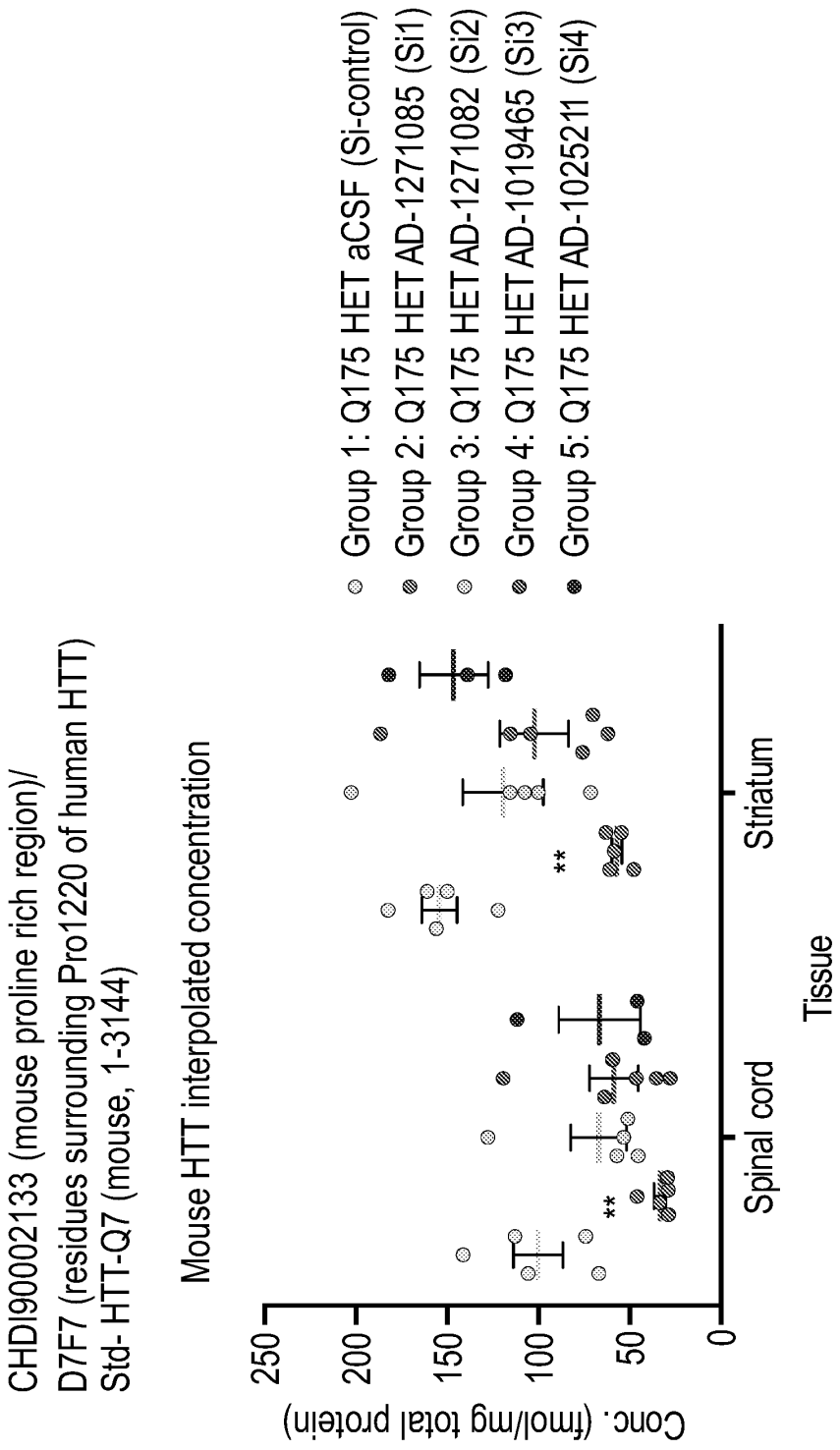
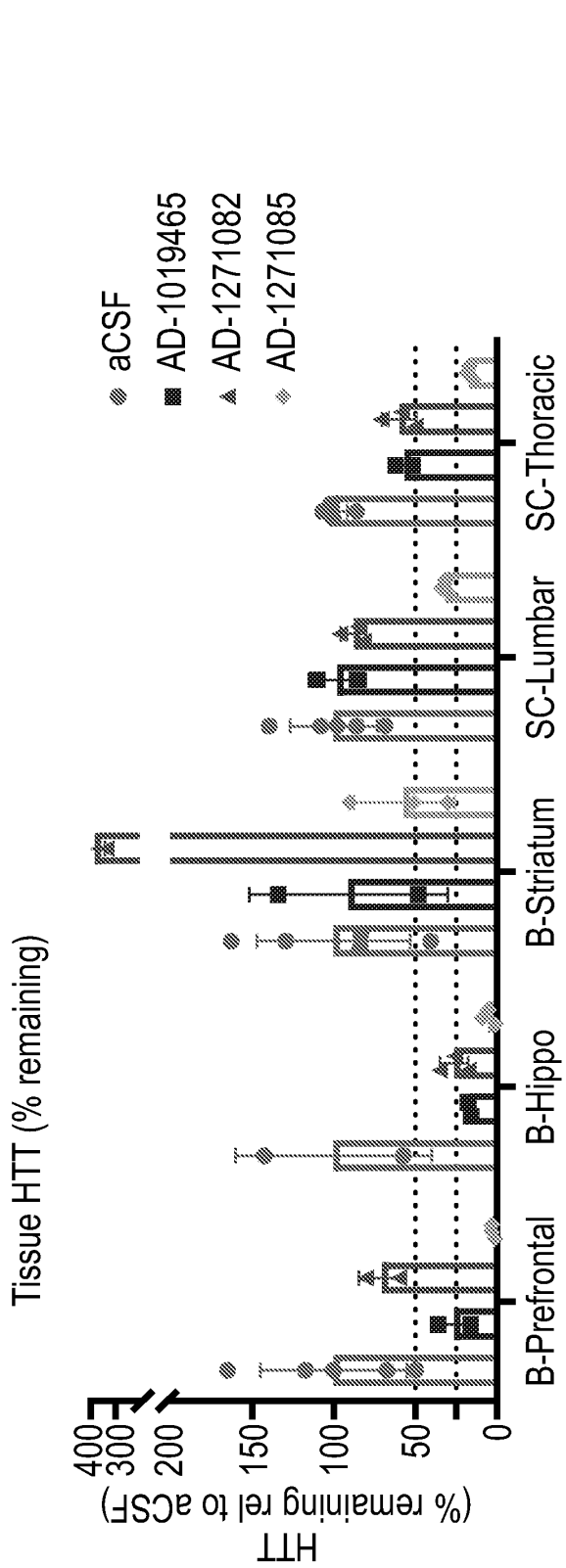

CHDI90002133 (mouse proline rich region)/
D7F7 (residues surrounding Pro1220 of human HTT)
Std- HTT-Q7 (mouse, 1-3144)
Mouse HTT interpolated concentration
Group 1: Q175 HET aCSF (Si-control)
Group 2: Q175 HET AD-1271085 (Si1)
Group 3: Q175 HET AD-1271082 (Si2)
Group 4: Q175 HET AD-1019465 (Si3)
Group 5: Q175 HET AD-1025211 (Si4)
Conc. (fmol/mg total protein)
250
200
150
100
50
0
**
**
Spinal cord
Striatum
Tissue

FIG. 8G



		Brain-Prefrontal			Brain-Hippocampus			Brain-Striatum Caudate			Spine-Lumbar			Spine-Thoracic		
Treatment	Study dose (mg)	Mean HTT (ng/mg)	% K/D	SD	Mean HTT (ng/mg)	% K/D	SD	Mean HTT (ng/mg)	% K/D	SD	Mean HTT (ng/mg)	% K/D	SD	Mean HTT (ng/mg)	% K/D	SD
aCSF	0	35.91	0.000	44.96	39.13	0.000	60.01	50.85	0.000	47.02	19.61	0.000	26.53	27.85	0.000	8.20
AD-1019465	60	9.36	73.935	14.10	6.56	83.241	1.27	46.35	8.862	60.69	19.17	2.246	17.31	15.81	43.223	7.52
AD-1271082	60	25.25	29.687	14.38	10.25	73.800	8.68	194.83	-283.104	39.25	17.23	12.167	7.59	16.68	40.108	10.50
AD-1271085	60	0.97	97.307	0.93	2.04	94.774	3.99	29.01	42.964	30.91	6.18	68.483	2.40	4.69	83.147	2.04

FIG. 9

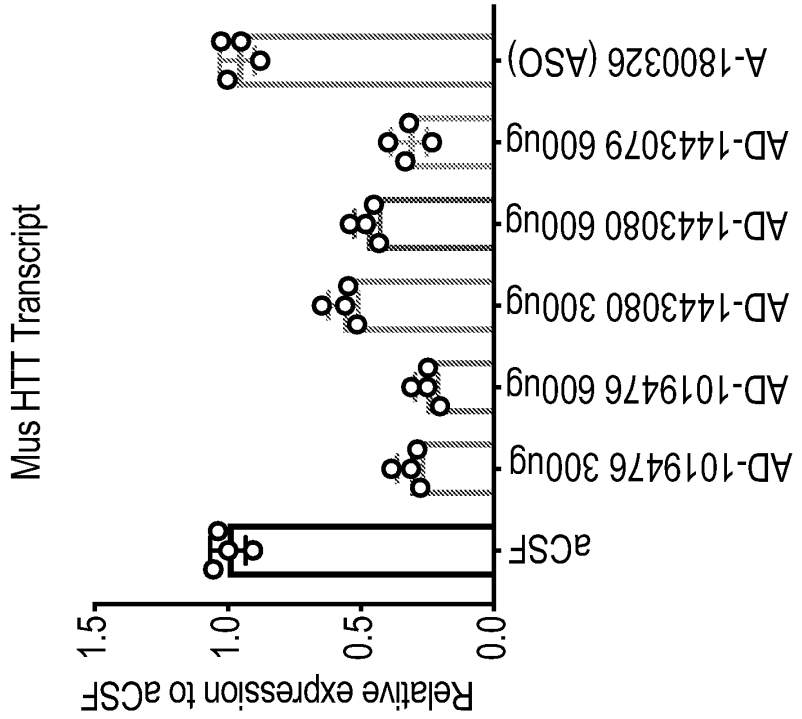


FIG. 10B

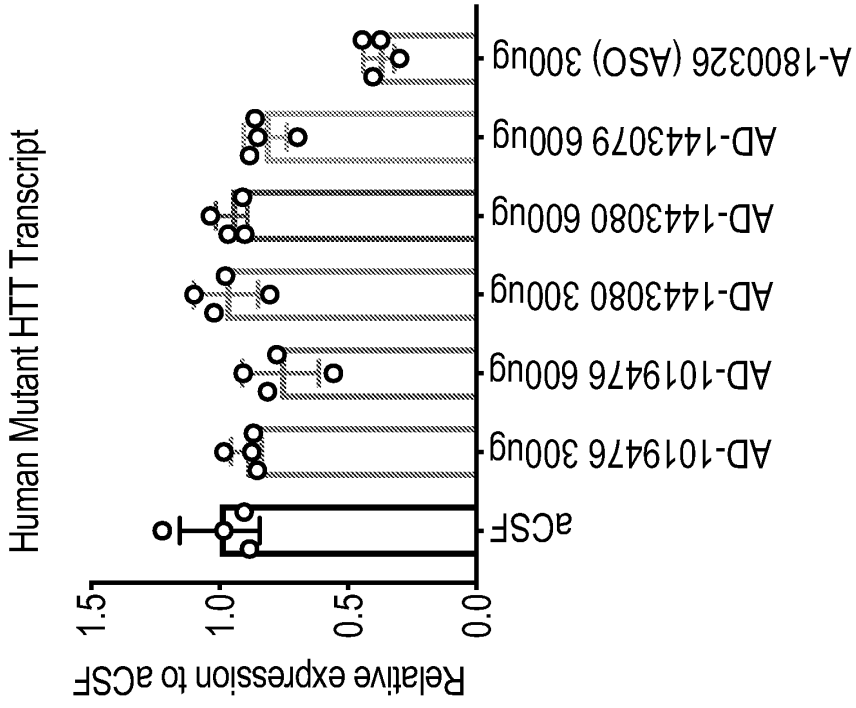


FIG. 10A

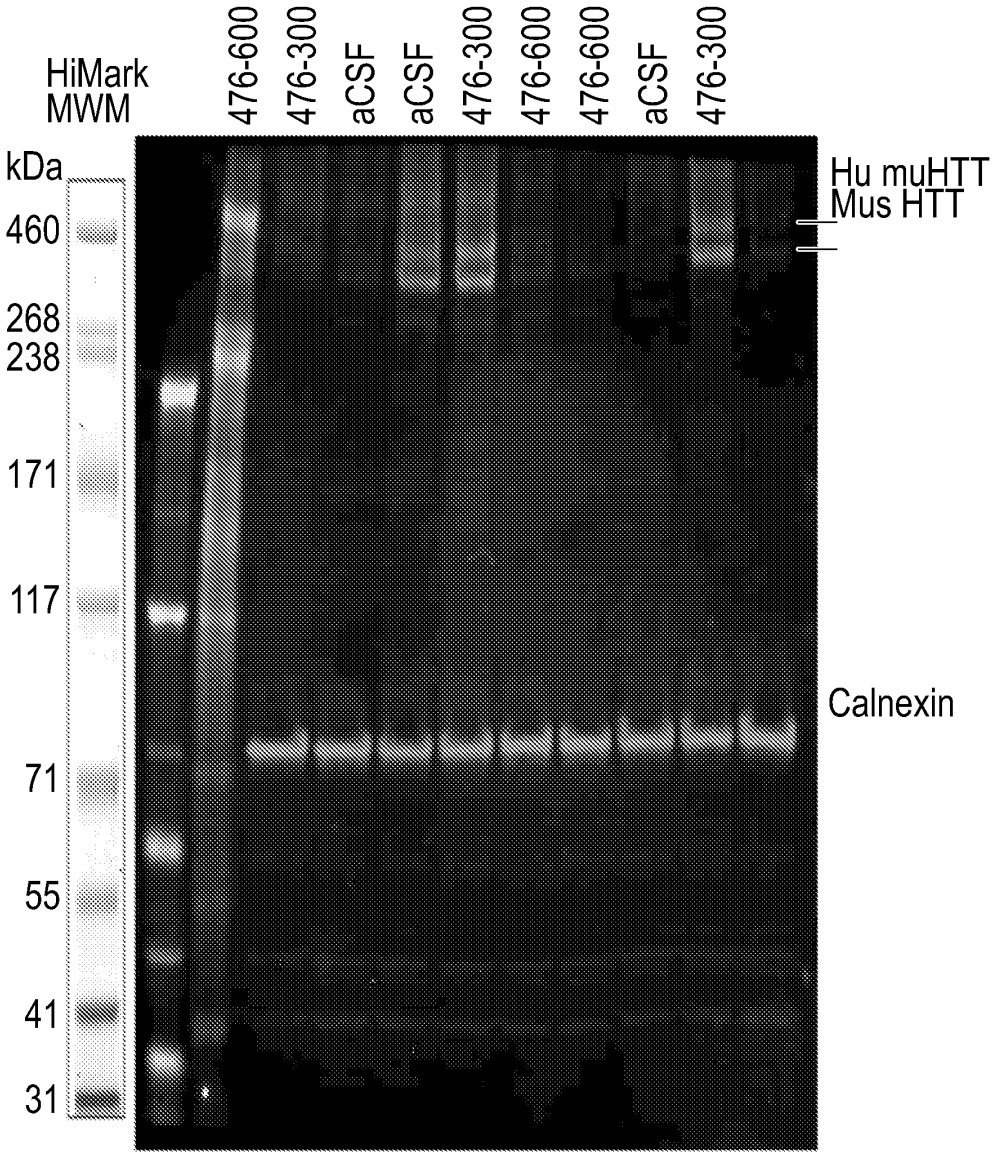

HiMark MWM
476-600
476-300
aCSF
aCSF
476-300
476-600
476-600
aCSF
476-300
kDa
460
268
238
171
117
71
55
41
31
Hu muHTT
Mus HTT
Calnexin

FIG. 10C

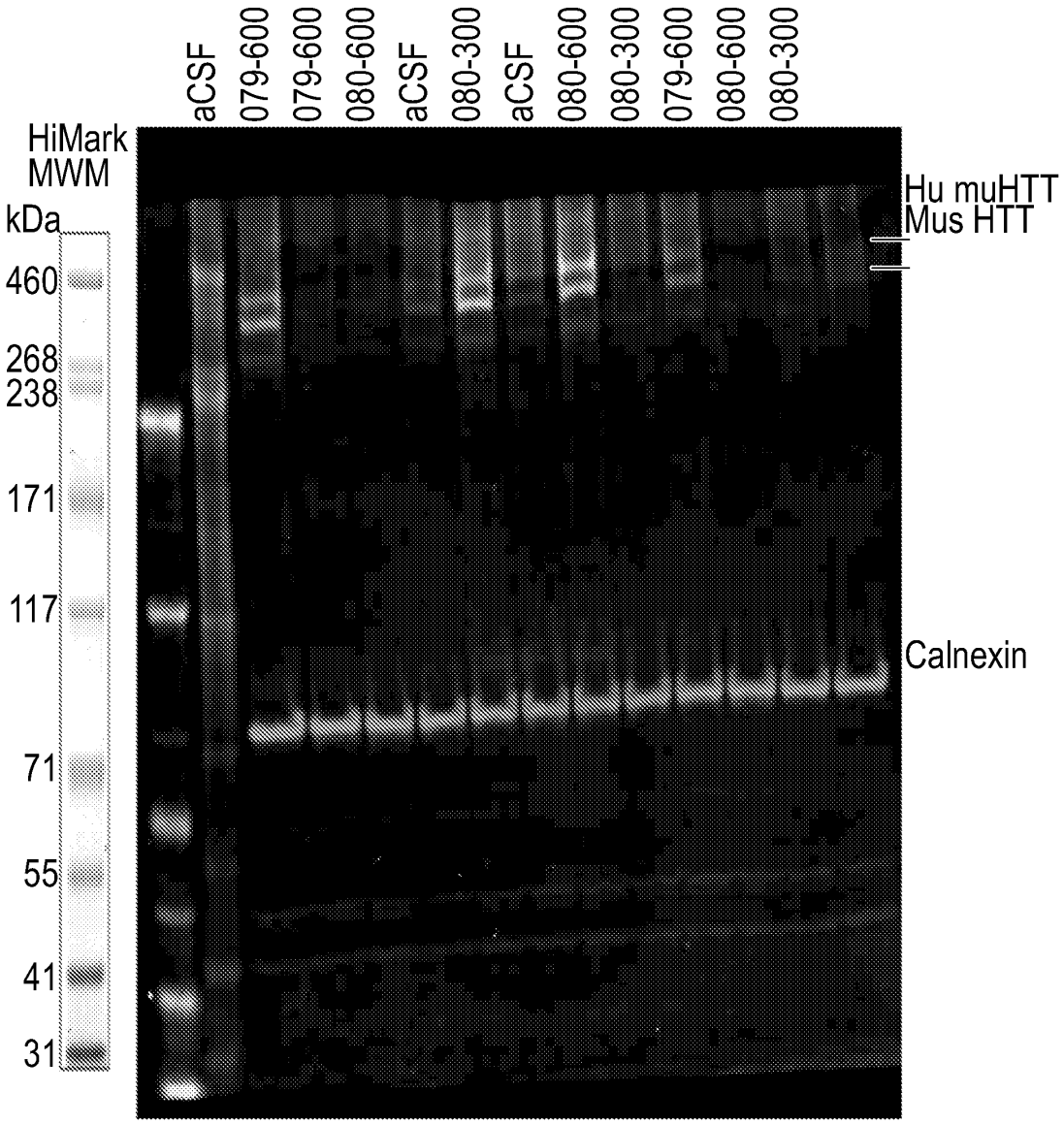

aCSF
079-600
079-600
080-600
aCSF
080-300
aCSF
080-600
080-300
079-600
080-600
080-300
HiMark
MWM
kDa
460
268
238
171
117
71
55
41
31
Hu muHTT
Mus HTT
Calnexin

FIG. 10D

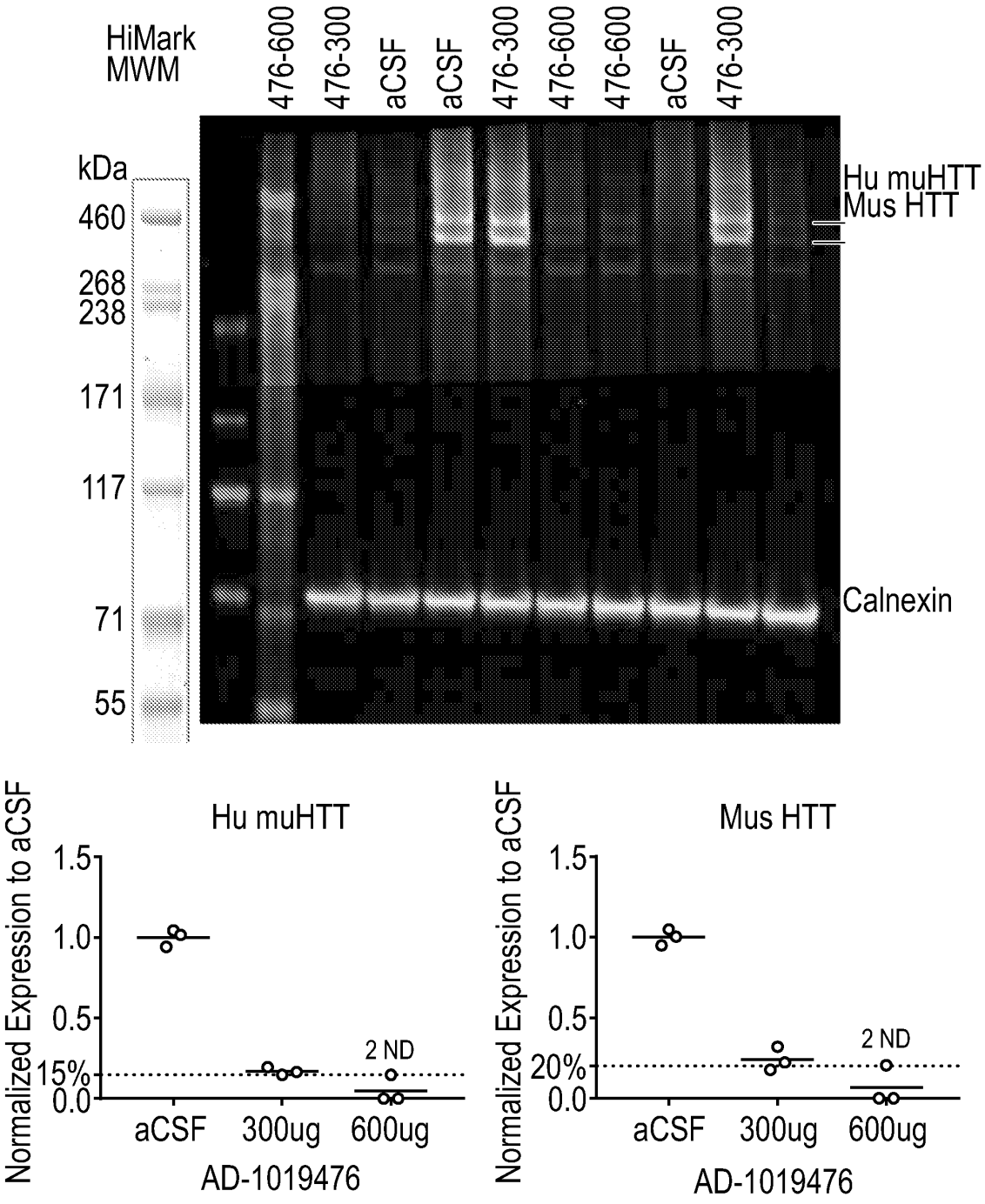

HiMark MWM
476-600
476-300
aCSF
aCSF
476-300
476-600
476-600
aCSF
476-300
kDa
460
268
238
171
117
71
55
Hu muHTT
Mus HTT
Calnexin
Hu muHTT
Normalized Expression to aCSF
1.5
1.0
0.5
15%
0.0
2 ND
aCSF
300ug
600ug
AD-1019476
Mus HTT
Normalized Expression to aCSF
1.5
1.0
0.5
20%
0.0
2 ND
aCSF
300ug
600ug
AD-1019476

FIG. 10E

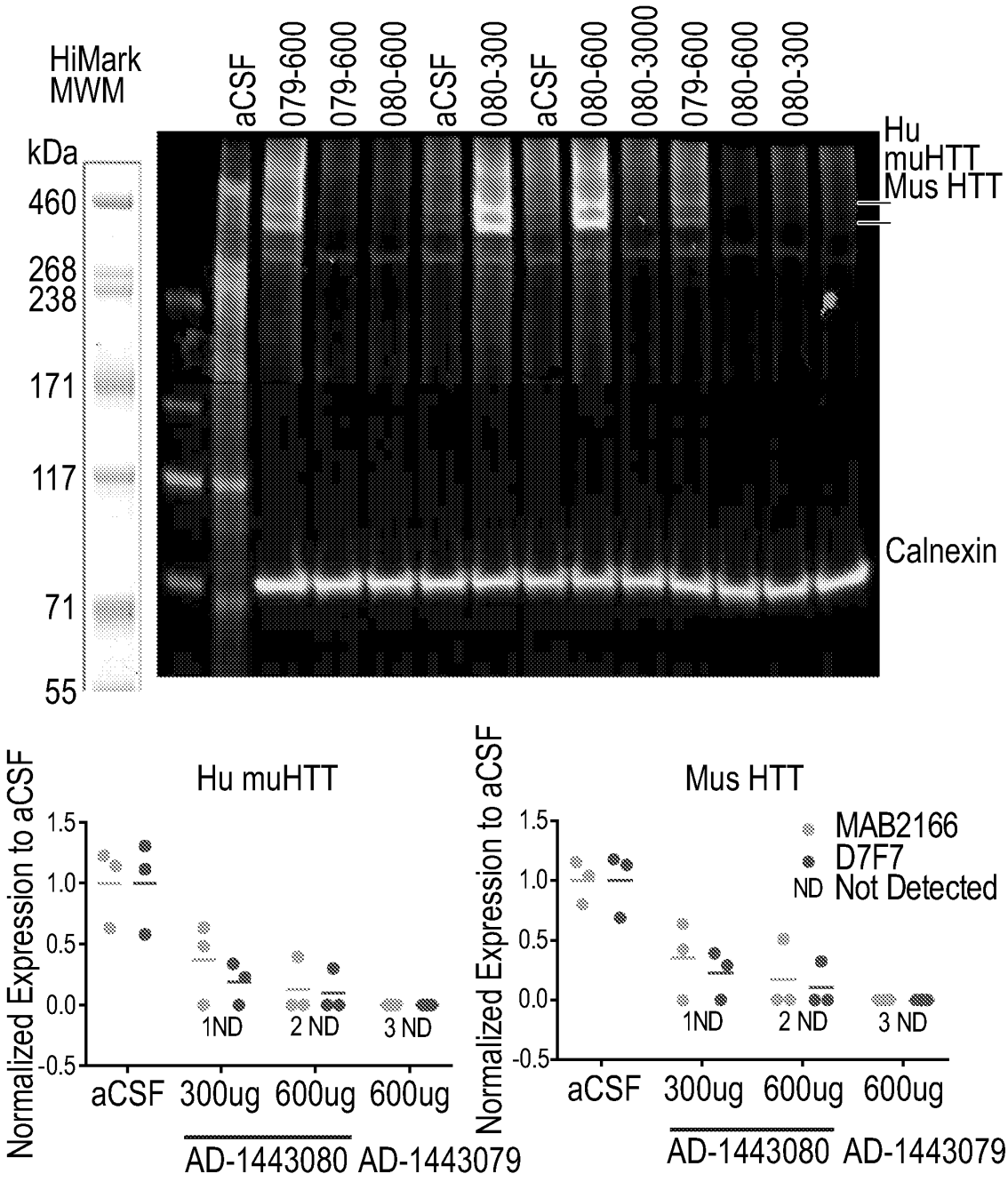

HiMark MWM
aCSF
079-600
079-600
080-600
aCSF
080-300
aCSF
080-600
080-3000
079-600
080-600
080-300
kDa
460
268
238
171
117
71
55
Hu muHTT
Mus HTT
Calnexin
Hu muHTT
Mus HTT
Normalized Expression to aCSF
1.5
1.0
0.5
0.0
-0.5
1ND
2 ND
3 ND
aCSF
300ug
600ug
600ug
AD-1443080
AD-1443079
MAB2166
D7F7
ND Not Detected

FIG. 10F

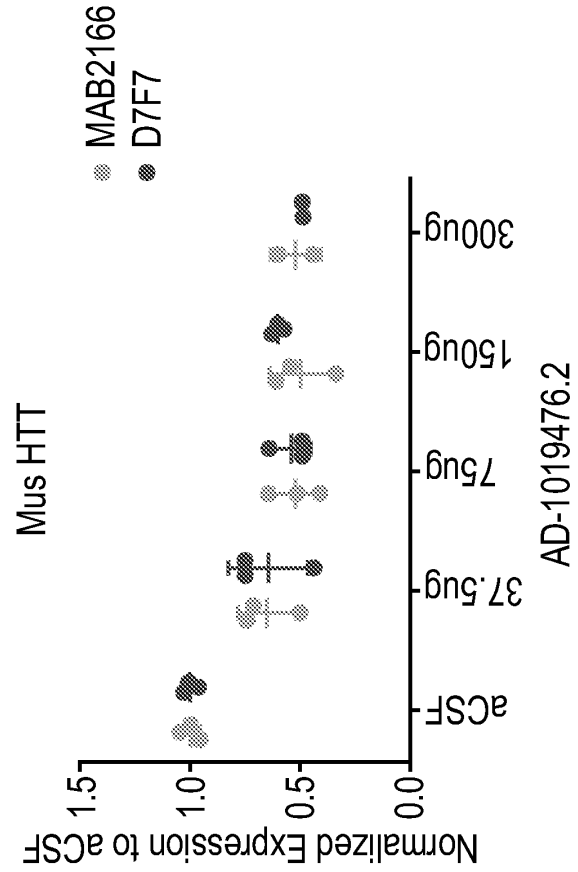


FIG. 11B

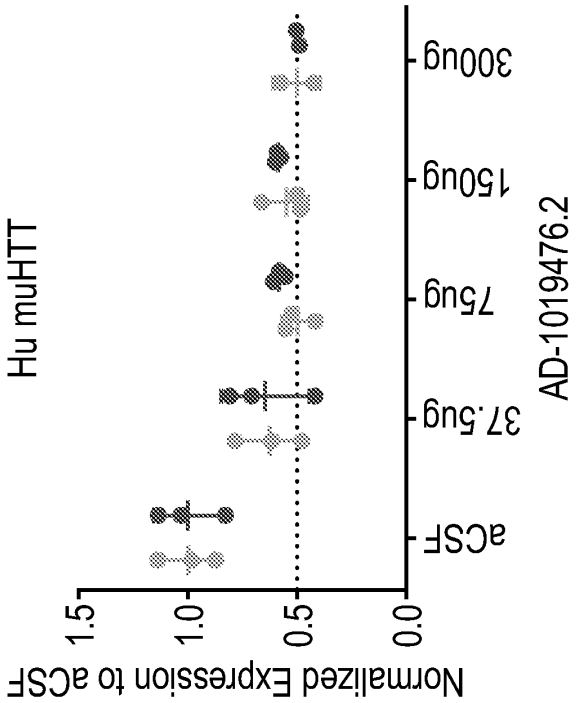


FIG. 11A

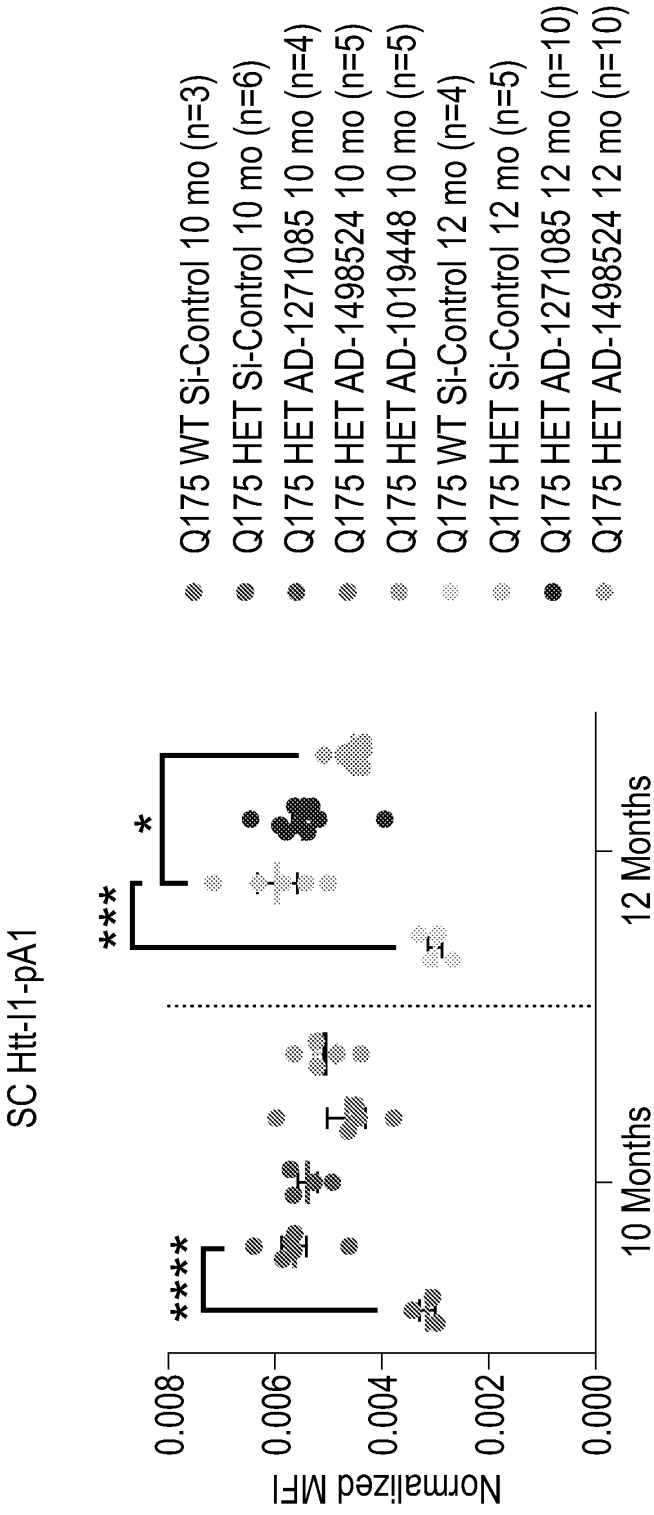

Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)
SC Htt-I1-pA1
Normalized MFI
0.008
0.006
0.004
0.002
0.000
****
***
*
10 Months
12 Months
Endpoint

FIG. 12A

FIG. 12B

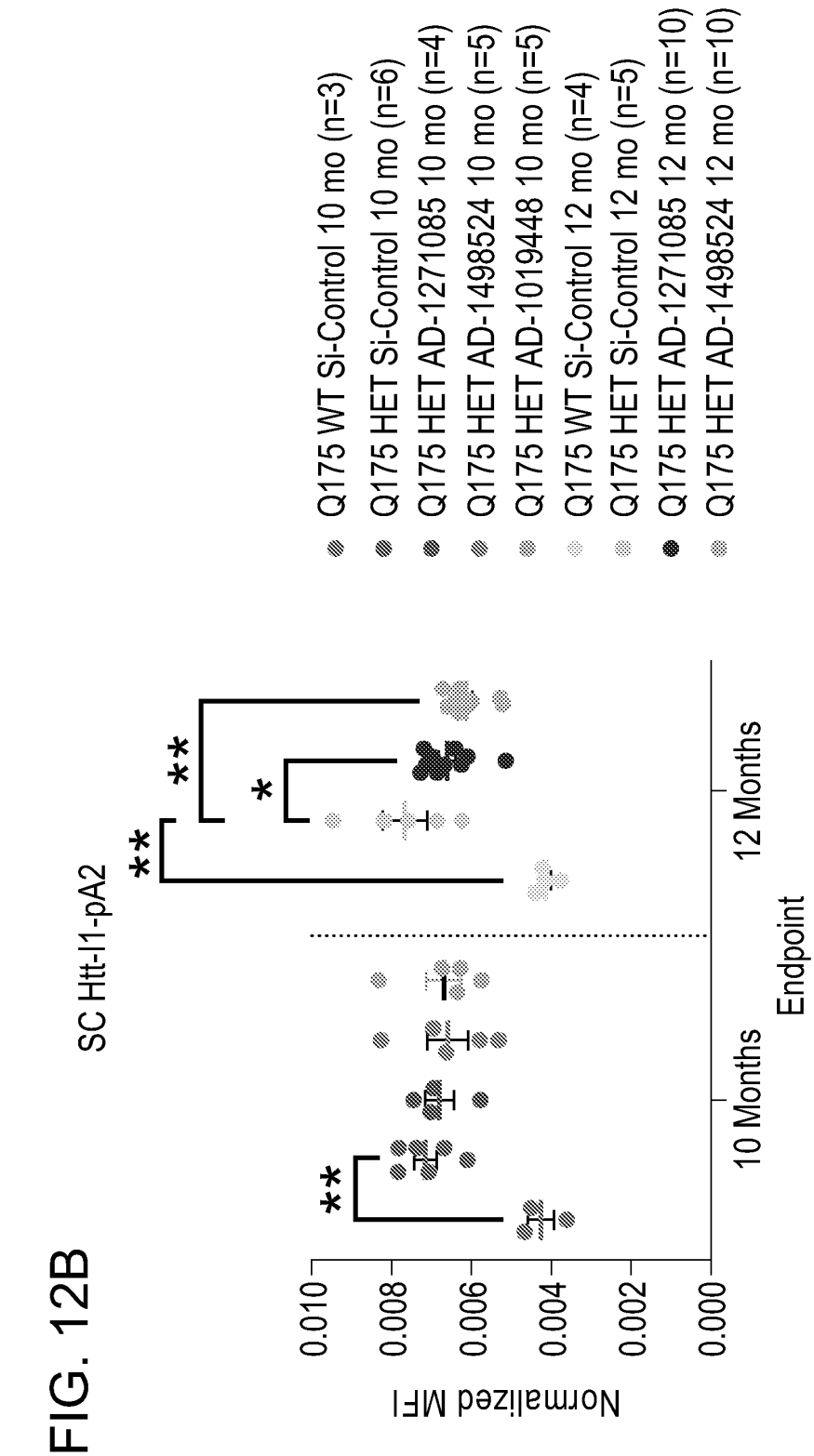

SC Htt-I1-pA2
Normalized MFI
0.010
0.008
0.006
0.004
0.002
0.000
10 Months
12 Months
Endpoint
**
**
*
**
Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)

FIG. 12C

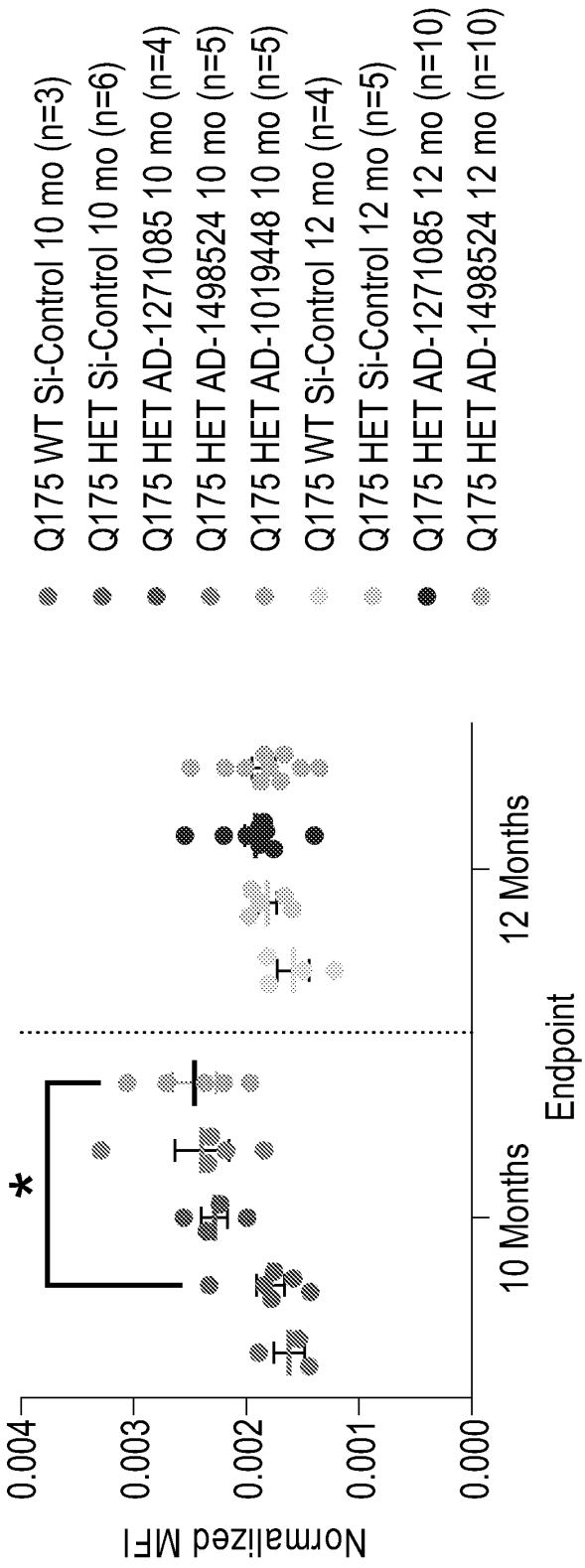

Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)
SC Htt-I1-3'
*
Normalized MFI
0.004
0.003
0.002
0.001
0.000
10 Months
12 Months
Endpoint

FIG. 12D

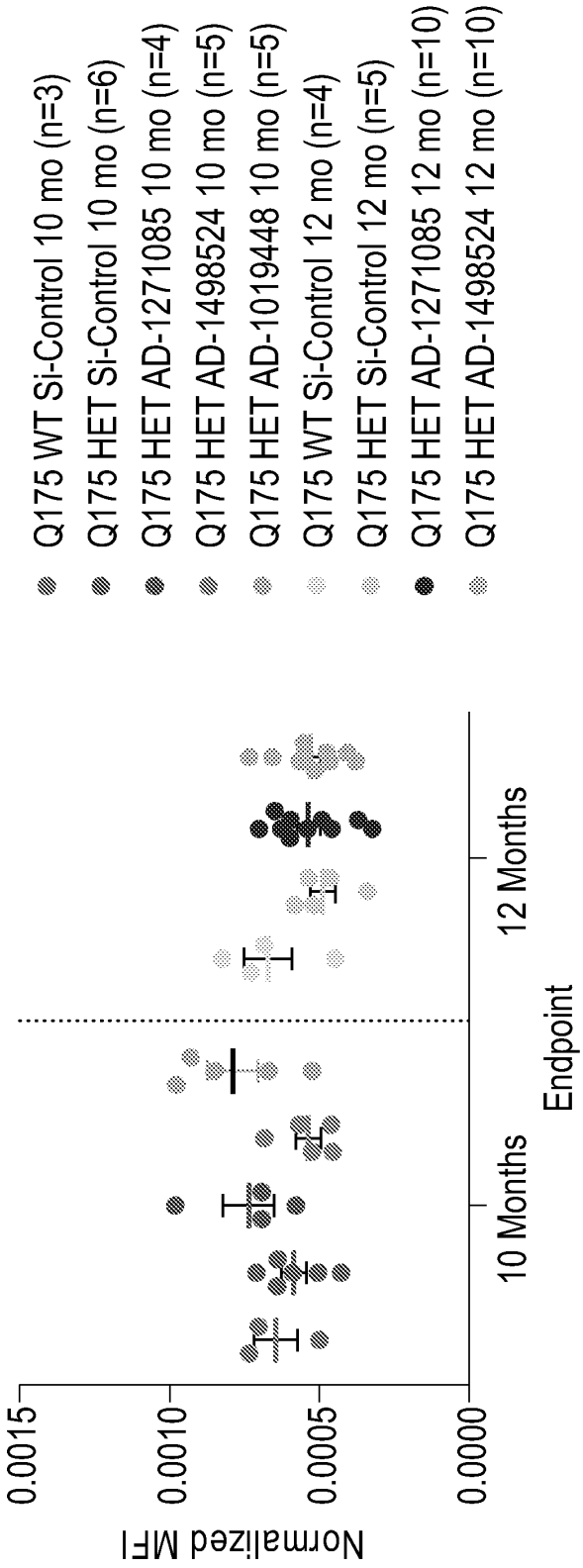

Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)
SC Htt-I3
Normalized MFI
0.0015
0.0010
0.0005
0.0000
10 Months
12 Months
Endpoint

FIG. 12E

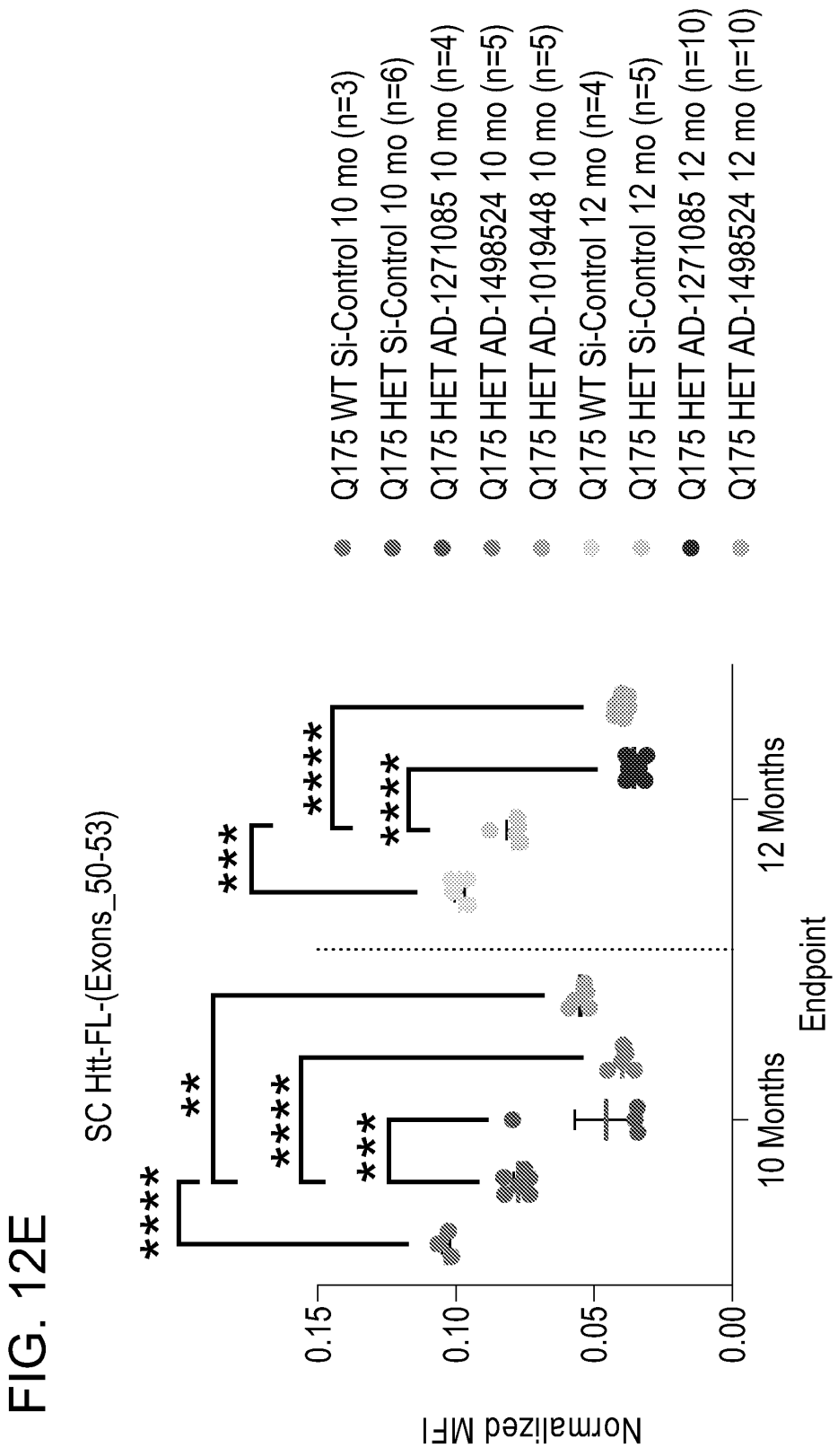

SC Htt-FL-(Exons_50-53)
Normalized MFI
0.15
0.10
0.05
0.00
10 Months
12 Months
Endpoint
****
**
****
***
***
****
Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)

FIG. 12F

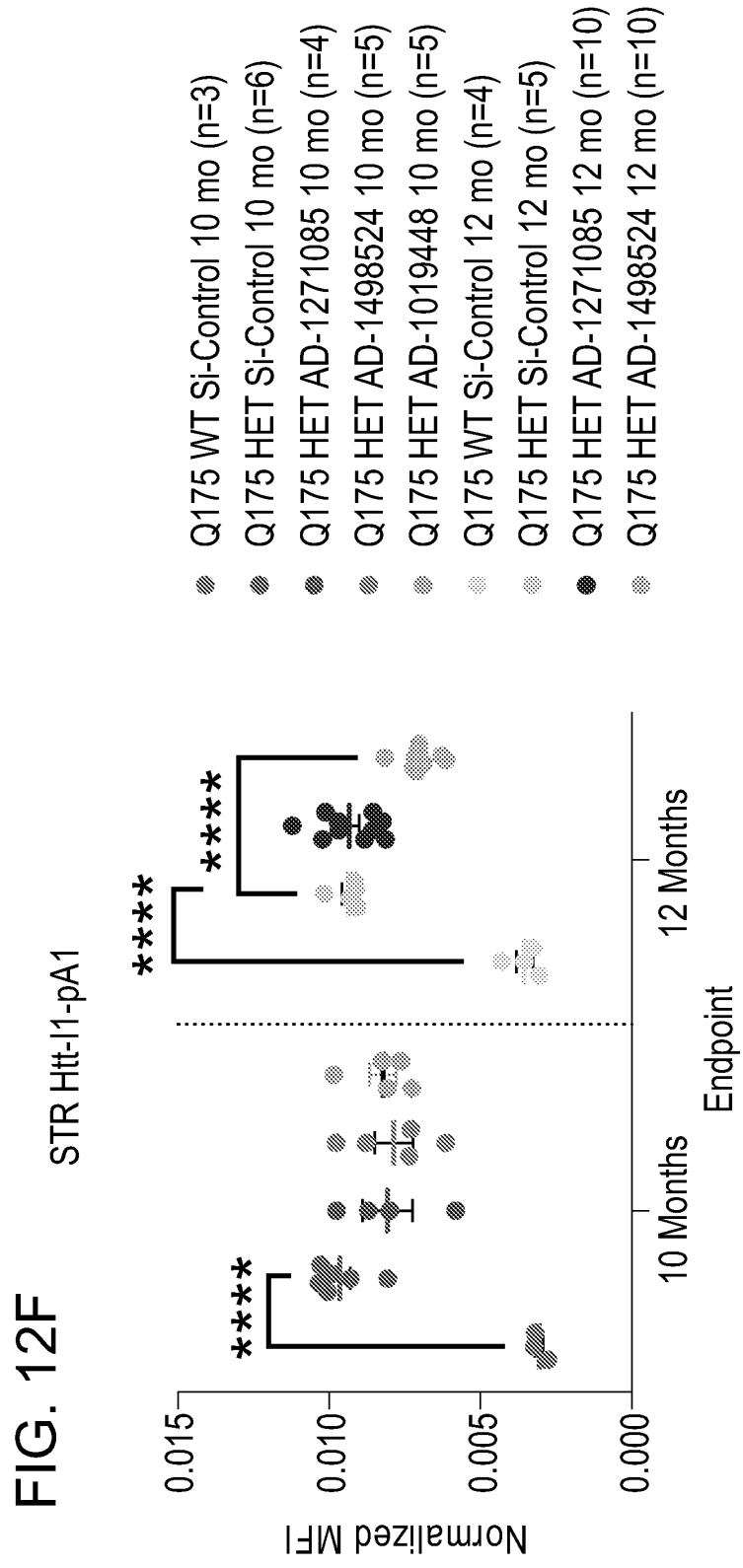

STR Htt-I1-pA1
Normalized MFI
0.015
0.010
0.005
0.000
10 Months
12 Months
Endpoint
****
****
****
Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)

FIG. 12G

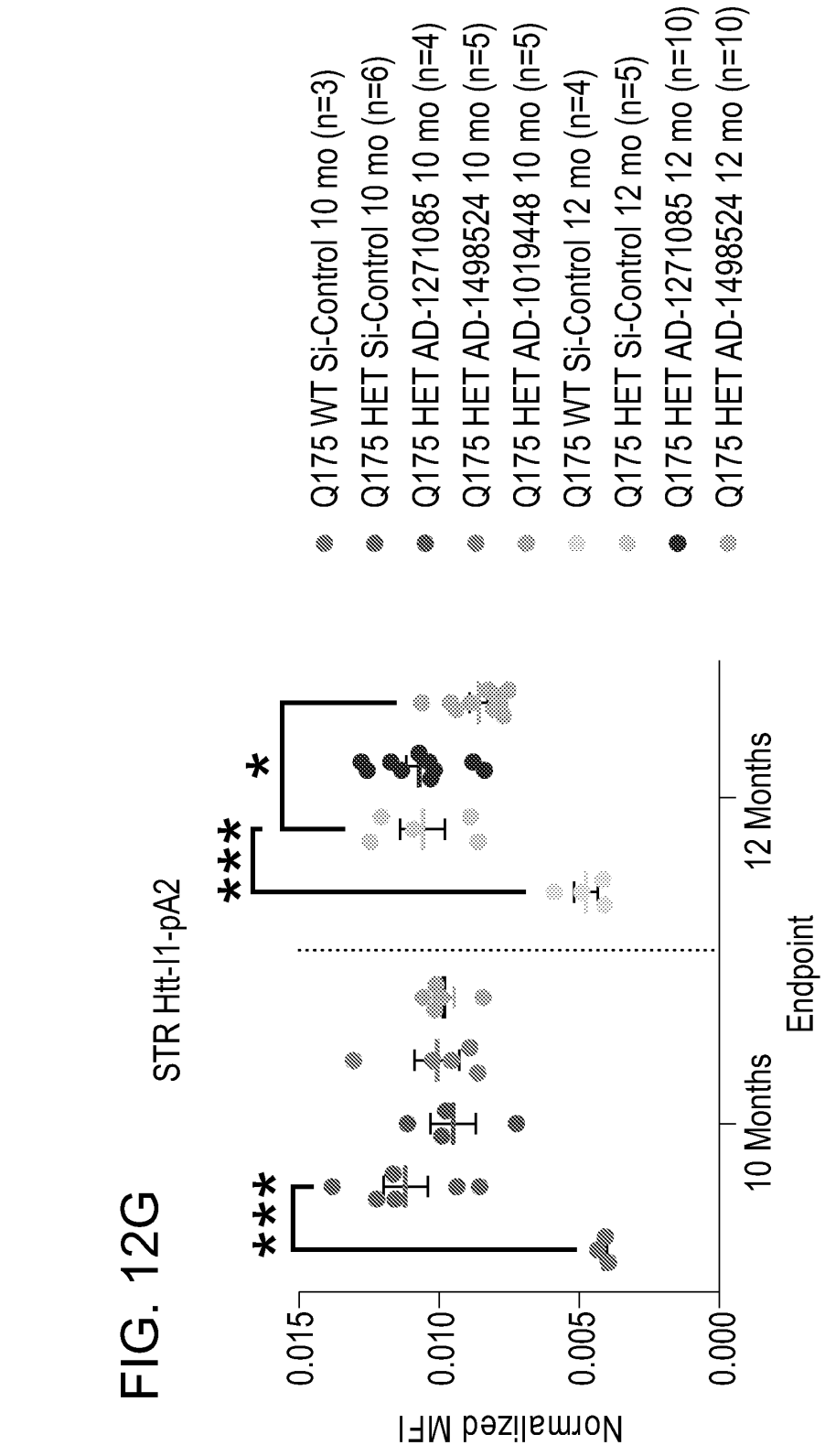

STR Htt-I1-pA2
Normalized MFI
0.015
0.010
0.005
0.000
***
***
*
10 Months
12 Months
Endpoint
Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)

FIG. 12H

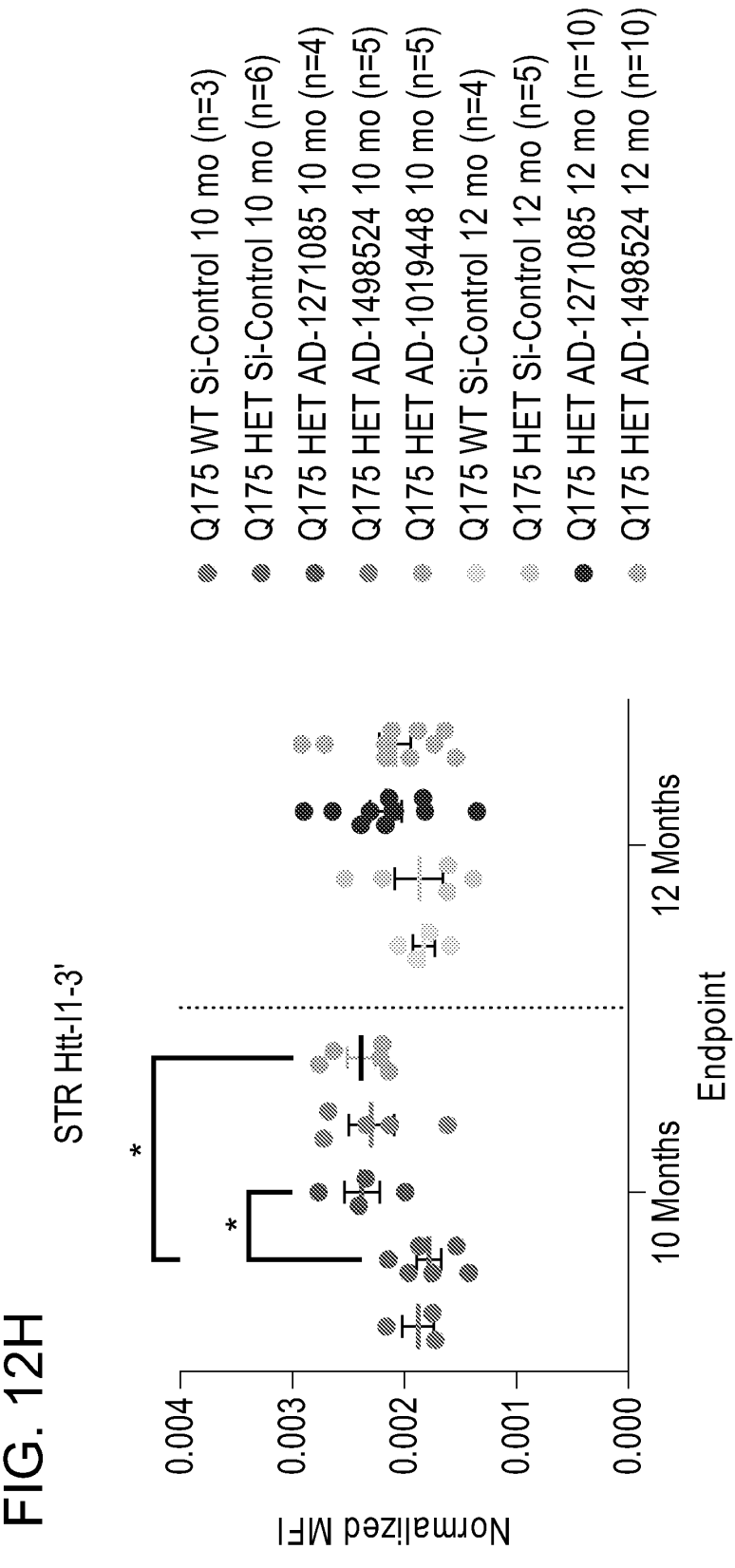

Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)
STR Htt-I1-3'
Normalized MFI
0.004
0.003
0.002
0.001
0.000
*
*
10 Months
12 Months
Endpoint

FIG. 12I

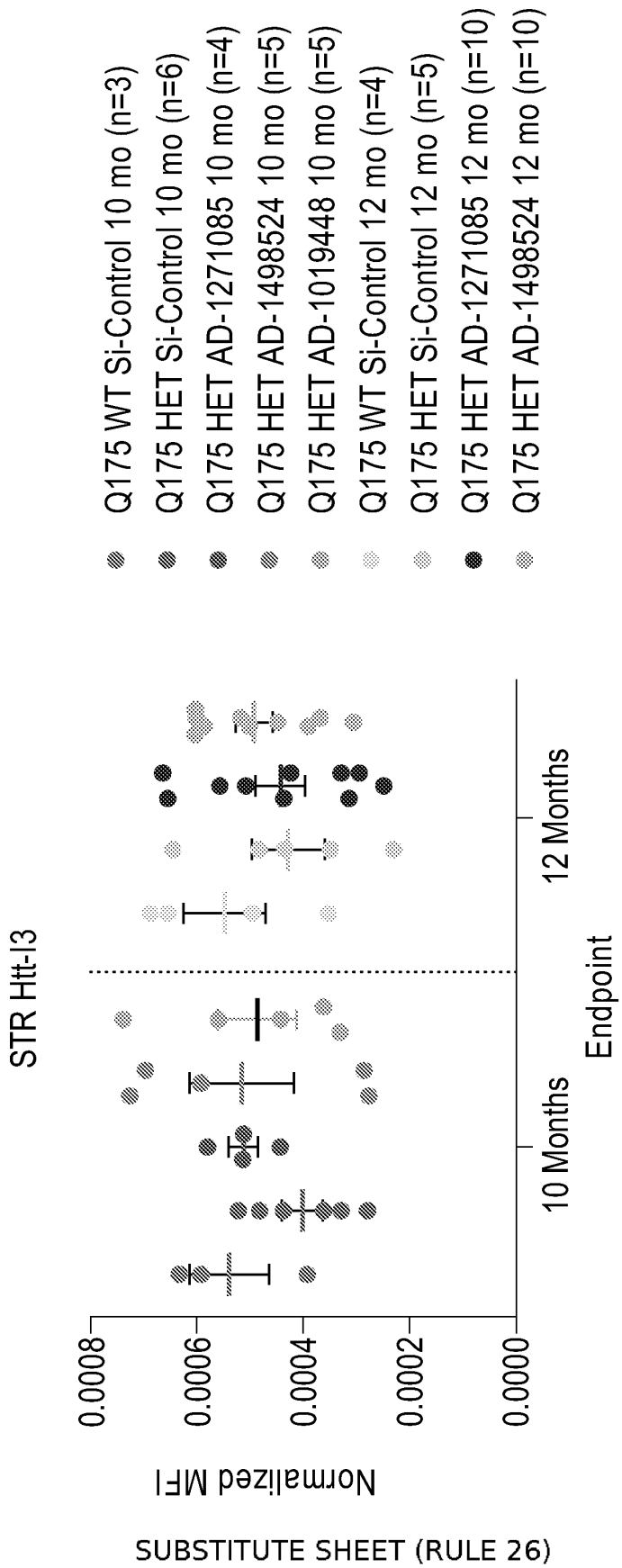

Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)
STR Htt-I3
Normalized MFI
0.0008
0.0006
0.0004
0.0002
0.0000
10 Months
12 Months
Endpoint

FIG. 12J

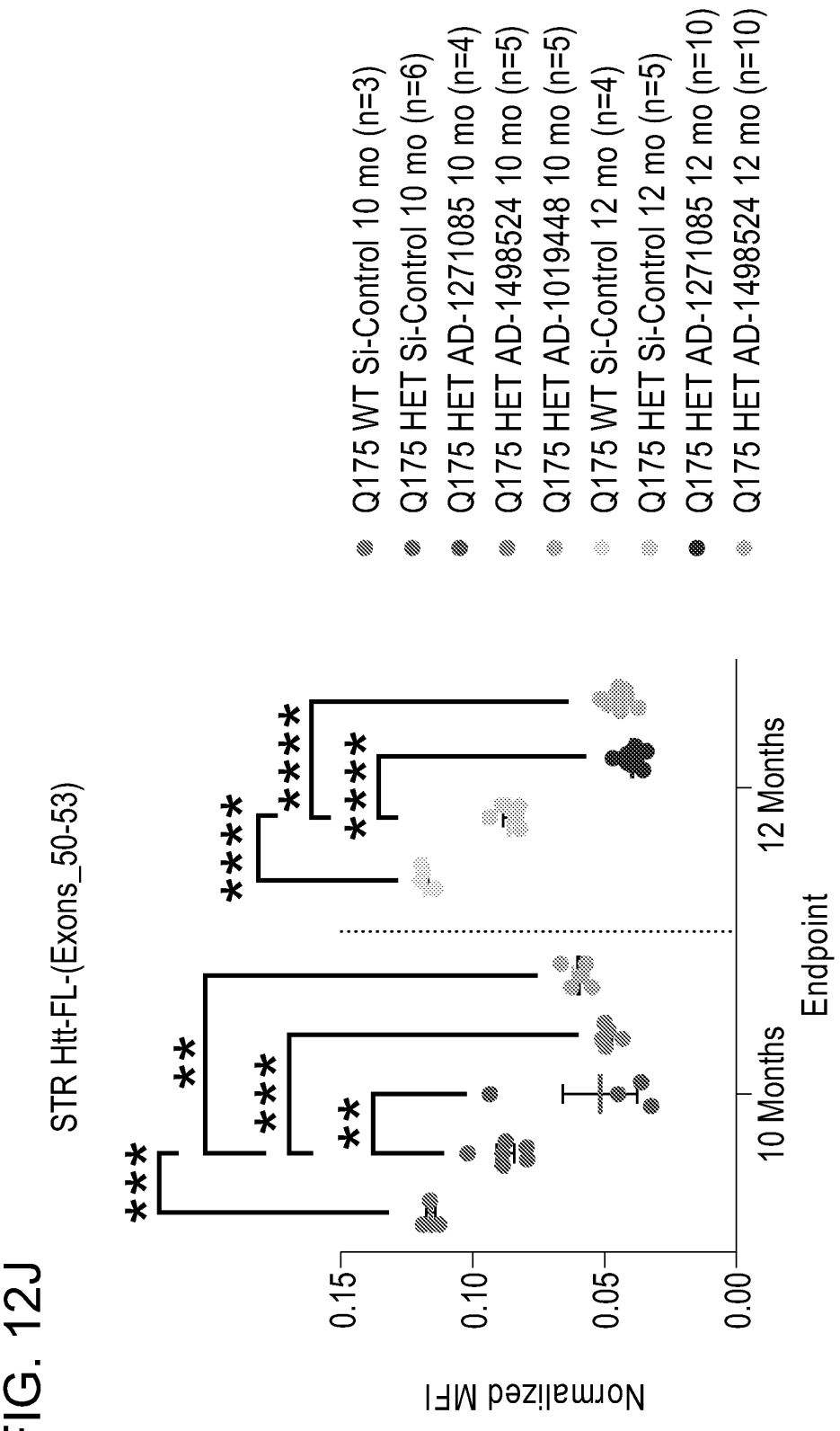
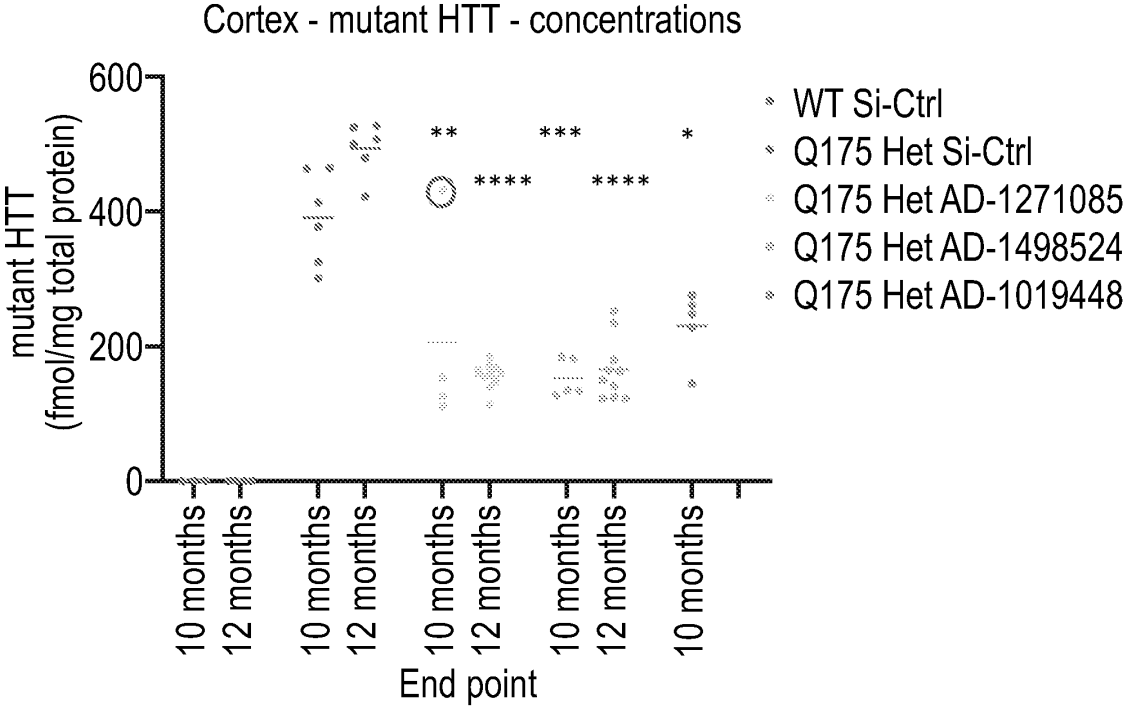
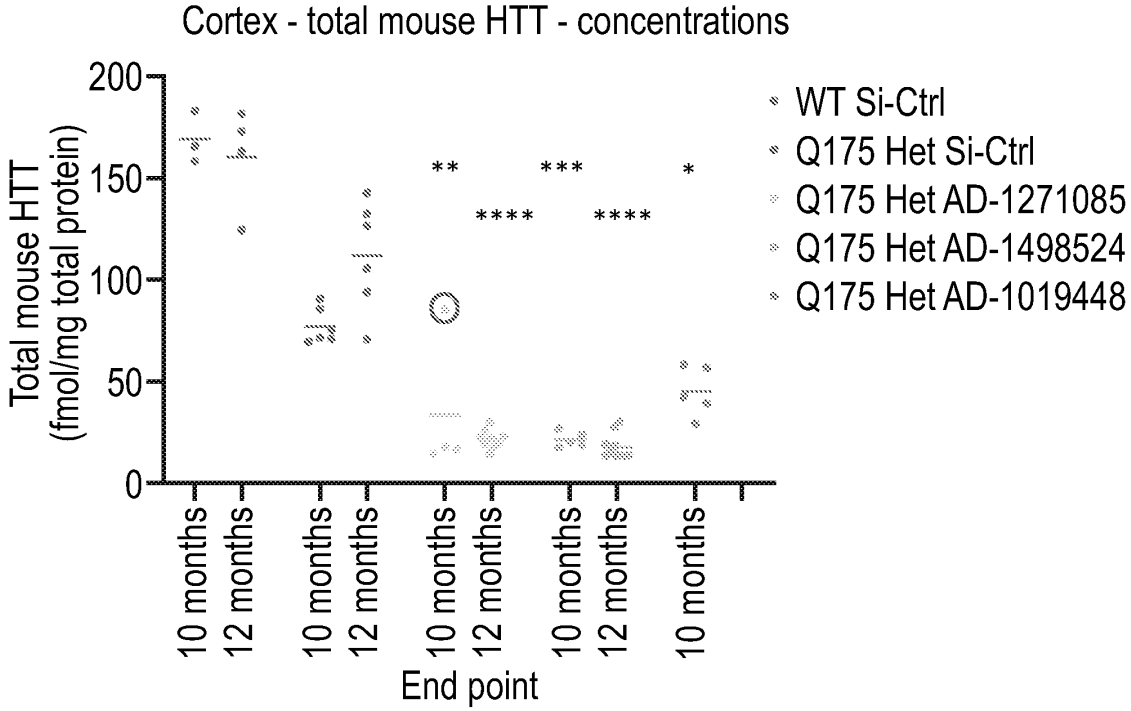

STR Htt-FL-(Exons_50-53)
Normalized MFI
0.15
0.10
0.05
0.00
10 Months
12 Months
Endpoint
****
**
***
**
*****
*****
Q175 WT Si-Control 10 mo (n=3)
Q175 HET Si-Control 10 mo (n=6)
Q175 HET AD-1271085 10 mo (n=4)
Q175 HET AD-1498524 10 mo (n=5)
Q175 HET AD-1019448 10 mo (n=5)
Q175 WT Si-Control 12 mo (n=4)
Q175 HET Si-Control 12 mo (n=5)
Q175 HET AD-1271085 12 mo (n=10)
Q175 HET AD-1498524 12 mo (n=10)

FIG. 12K





Q175 Het Si-Control vs treatments: One-way ANOVA followed by Dunnett's multiple comparisons test compared at the respective timepoint

*p ≤0.05 ***p <0.001

**p <0.01 ****p<0.0001

FIG. 12L

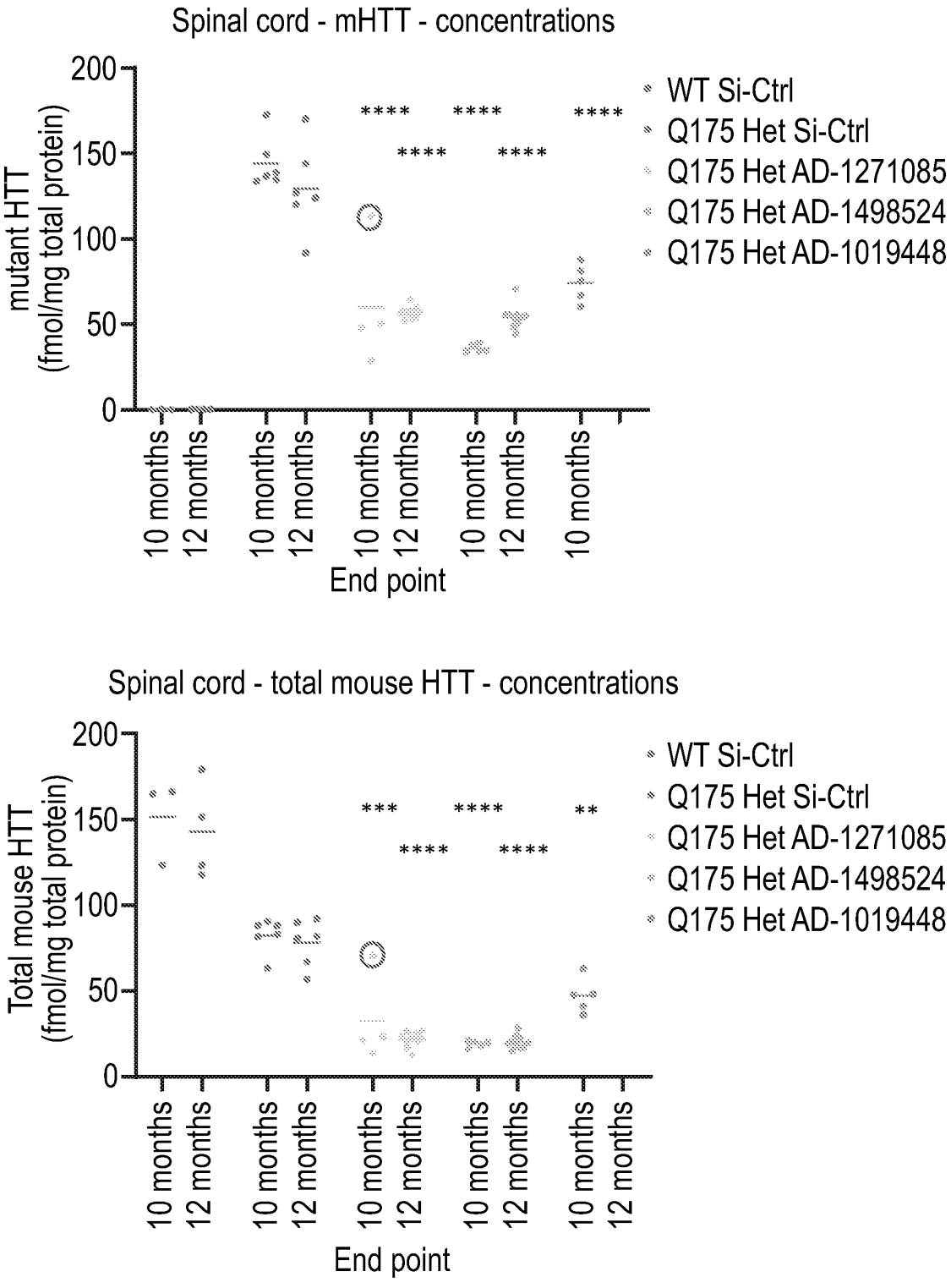

Spinal cord - mHTT - concentrations
mutant HTT (fmol/mg total protein)
200
150
100
50
0
****
****
****
****
****
10 months
12 months
10 months
12 months
10 months
12 months
10 months
12 months
10 months
End point
WT Si-Ctrl
Q175 Het Si-Ctrl
Q175 Het AD-1271085
Q175 Het AD-1498524
Q175 Het AD-1019448
Spinal cord - total mouse HTT - concentrations
Total mouse HTT (fmol/mg total protein)
200
150
100
50
0
***
****
**
****
****
10 months
12 months
10 months
12 months
10 months
12 months
10 months
12 months
10 months
12 months
End point
WT Si-Ctrl
Q175 Het Si-Ctrl
Q175 Het AD-1271085
Q175 Het AD-1498524
Q175 Het AD-1019448

FIG. 13A

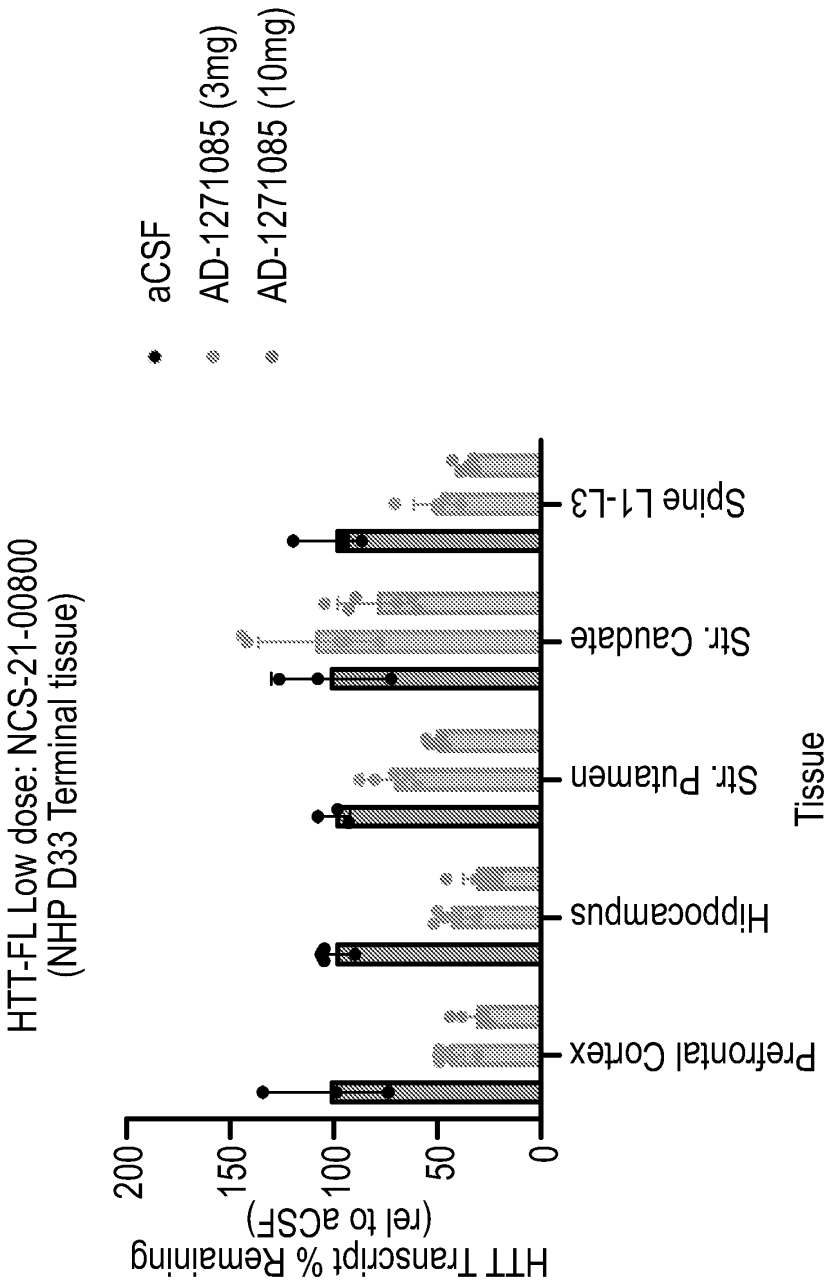
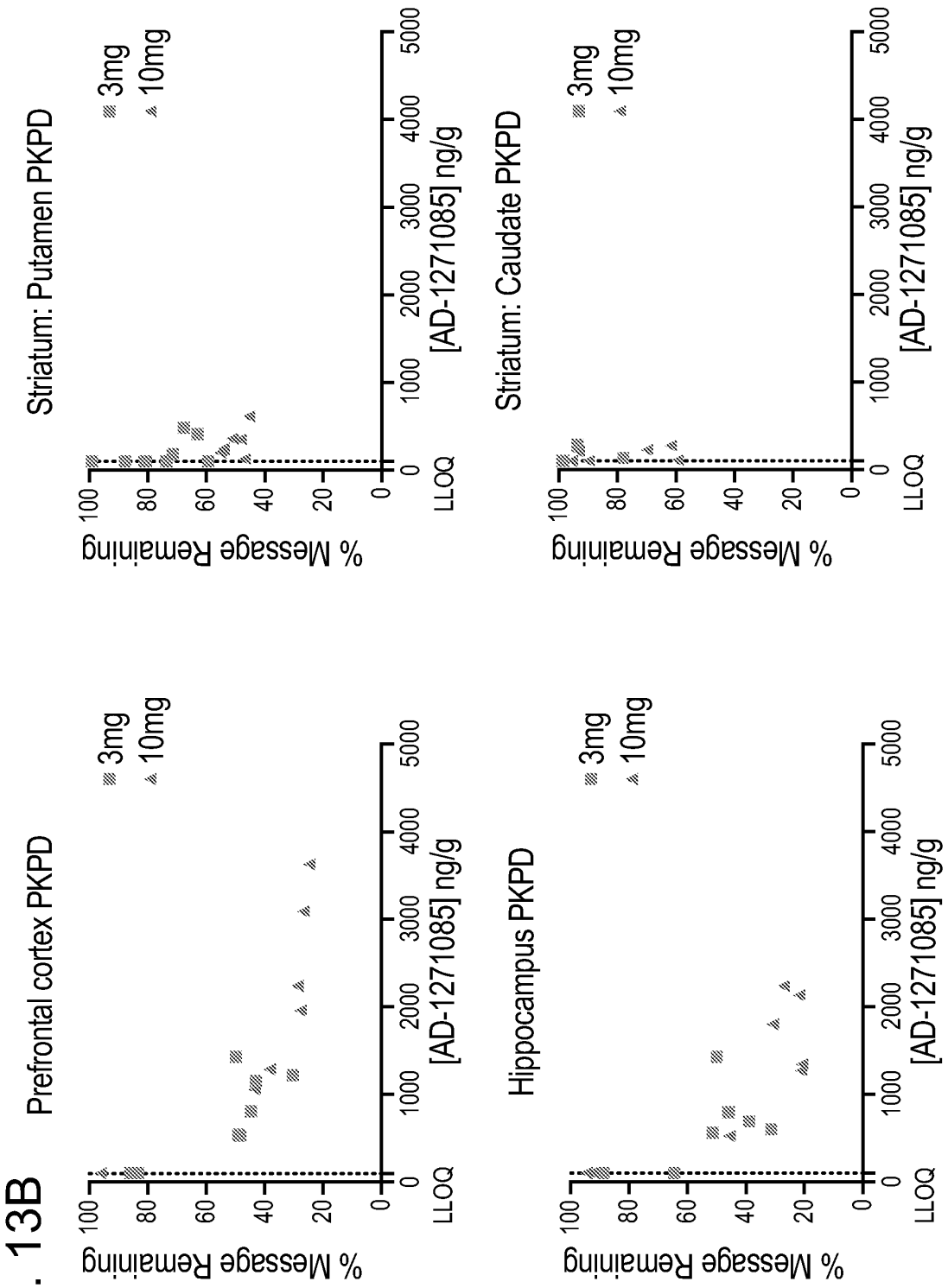

HTT-FL Low dose: NCS-21-00800
(NHP D33 Terminal tissue)
aCSF
AD-1271085 (3mg)
AD-1271085 (10mg)
HTT Transcript % Remaining
(rel to aCSF)
200
150
100
50
0
Prefrontal Cortex
Hippocampus
Str. Putamen
Str. Caudate
Spine L1-L3
Tissue

FIG. 13B


Striatum: Putamen PKPD
Striatum: Caudate PKPD
Prefrontal cortex PKPD
Hippocampus PKPD
3mg
10mg
% Message Remaining
[AD-1271085] ng/g
LLOQ
0
20
40
60
80
100
1000
2000
3000
4000
5000

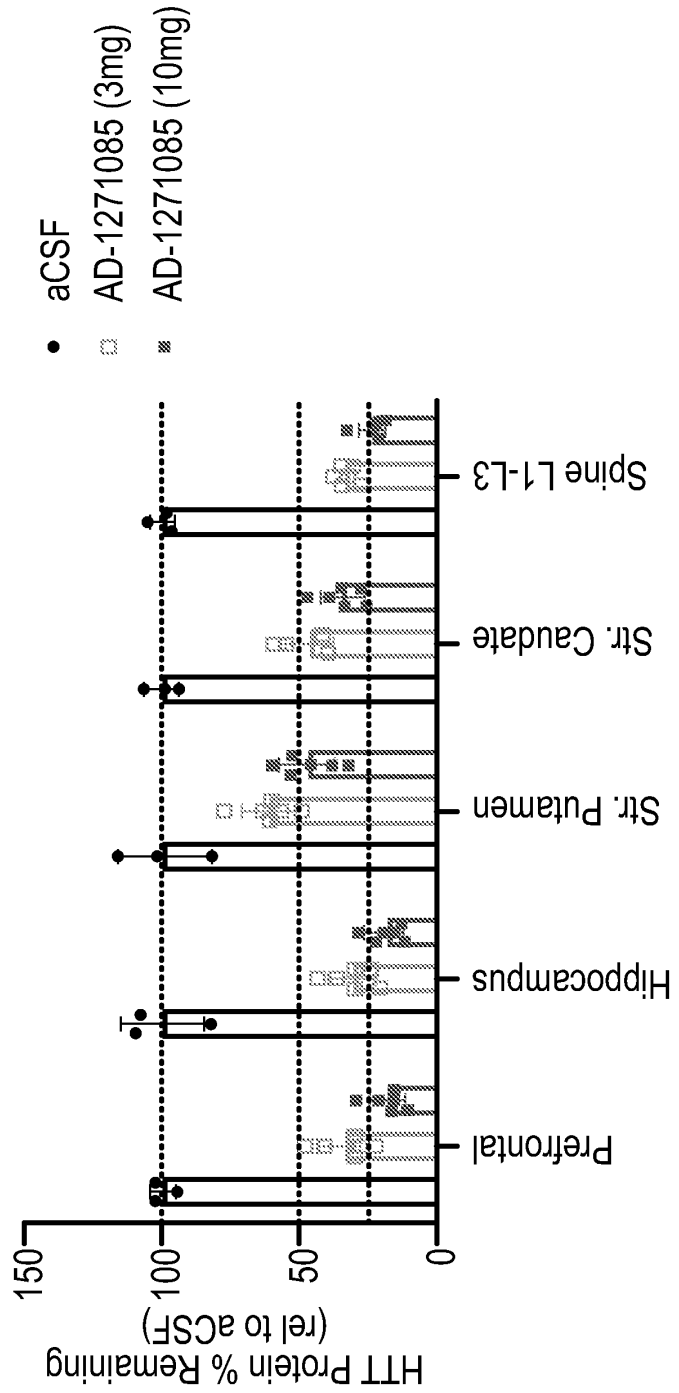

HTT-FL Low dose: NCS-21-00800
(NHP D33 Terminal tissue)
aCSF
AD-1271085 (3mg)
AD-1271085 (10mg)
HTT Protein % Remaining
(rel to aCSF)
150
100
50
0
Prefrontal
Hippocampus
Str. Putamen
Str. Caudate
Spine L1-L3

FIG. 13C

FIG. 13D

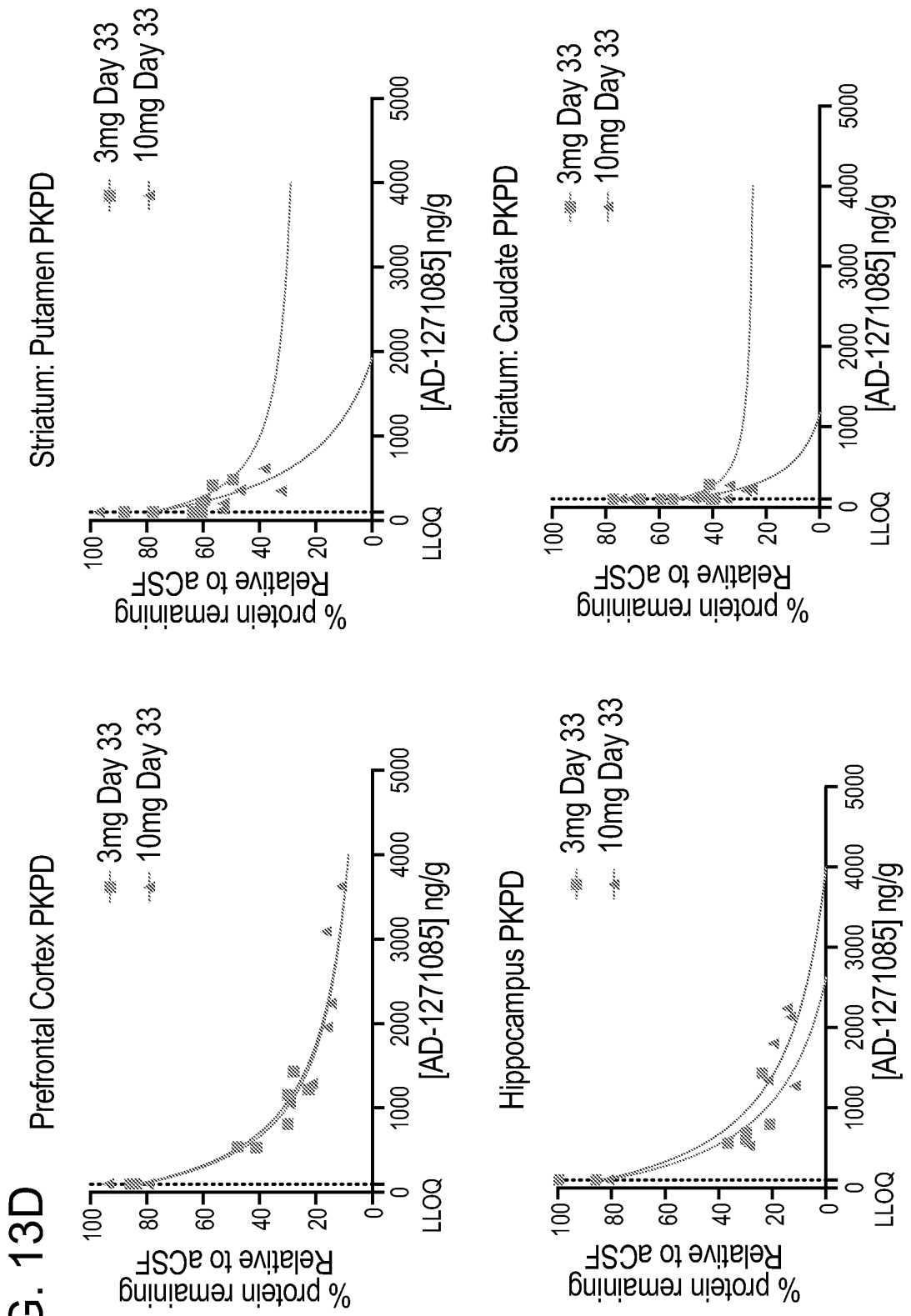
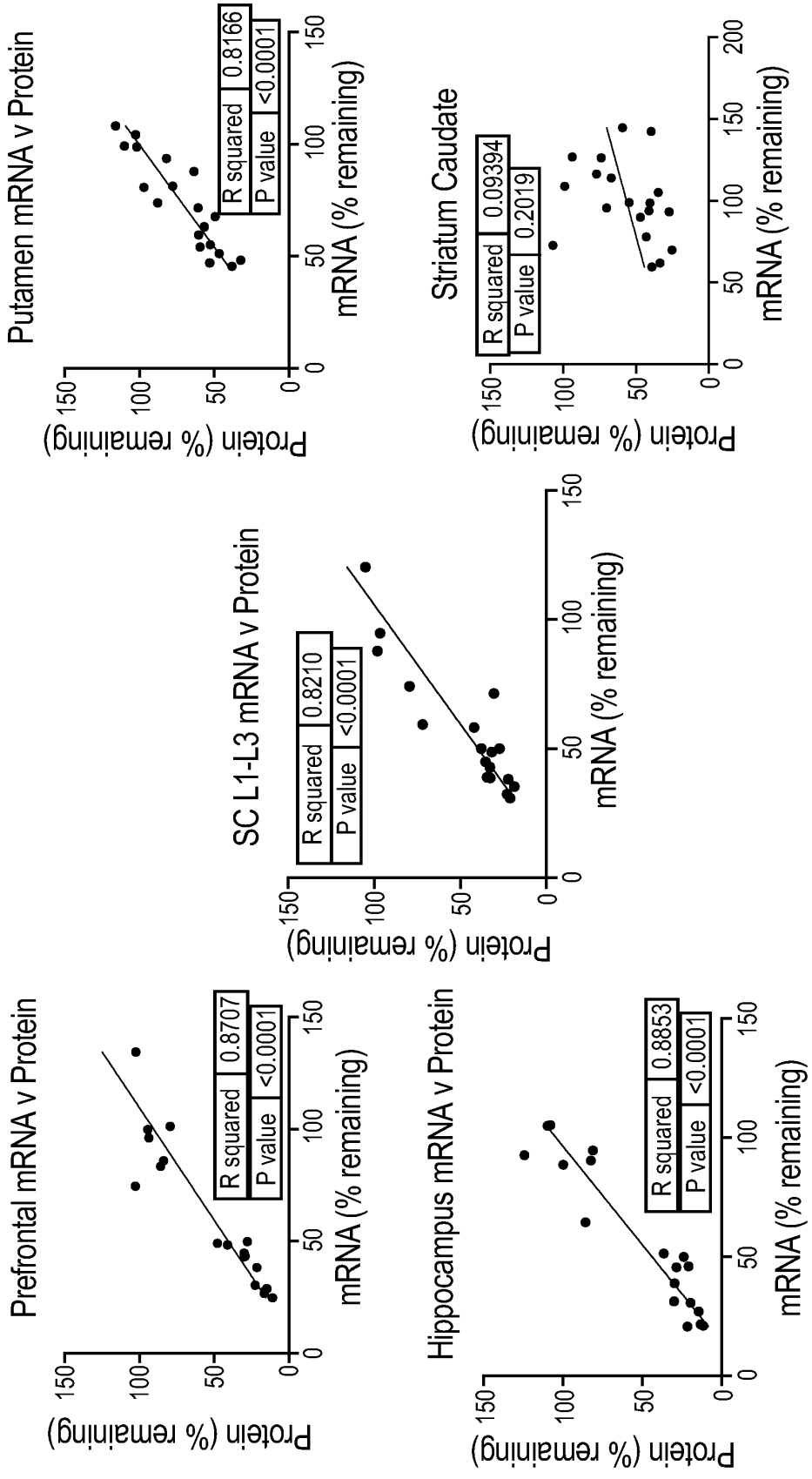

Prefrontal Cortex PKPD
Striatum: Putamen PKPD
Hippocampus PKPD
Striatum: Caudate PKPD
3mg Day 33
10mg Day 33
% protein remaining
Relative to aCSF
[AD-1271085] ng/g
LLOQ
0
20
40
60
80
100
0
1000
2000
3000
4000
5000

FIG. 13E


Putamen mRNA v Protein
R squared 0.8166
P value <0.0001
Protein (% remaining)
mRNA (% remaining)
Striatum Caudate
R squared 0.09394
P value 0.2019
SC L1-L3 mRNA v Protein
R squared 0.8210
P value <0.0001
Prefrontal mRNA v Protein
R squared 0.8707
P value <0.0001
Hippocampus mRNA v Protein
R squared 0.8853
P value <0.0001

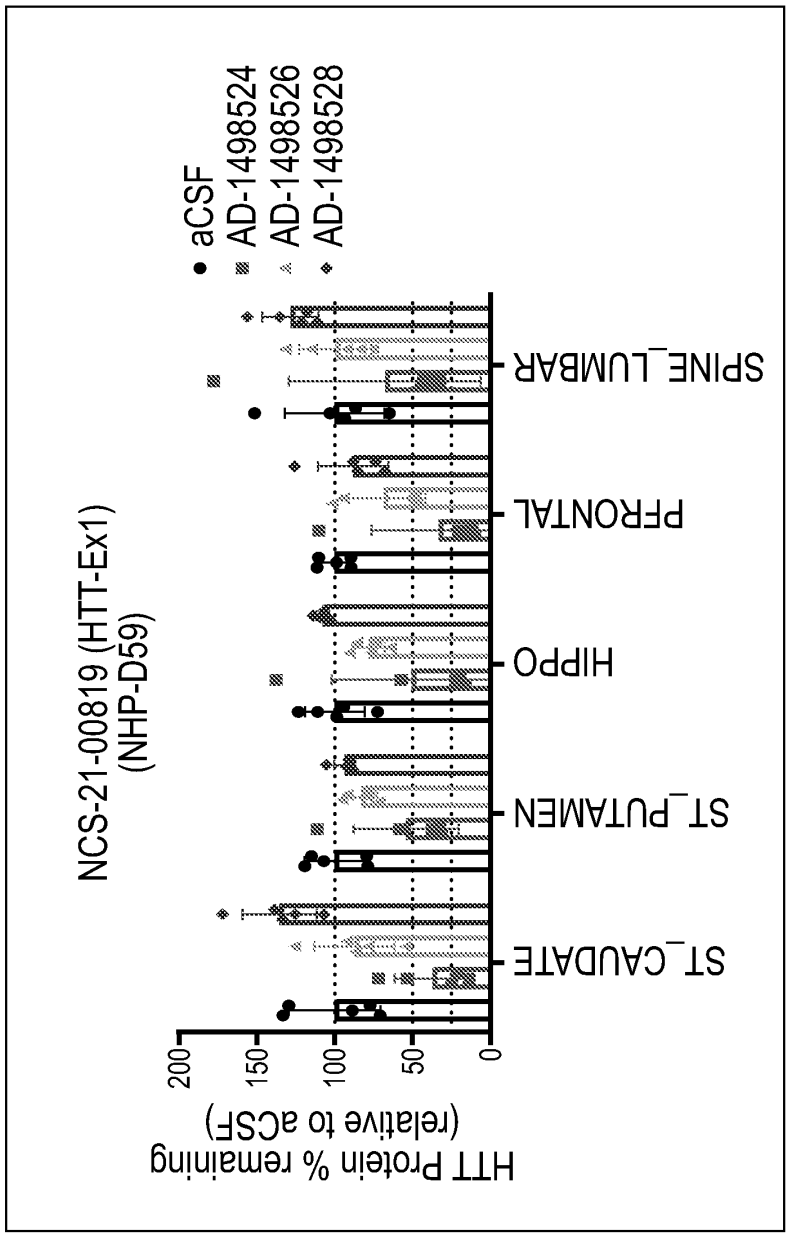
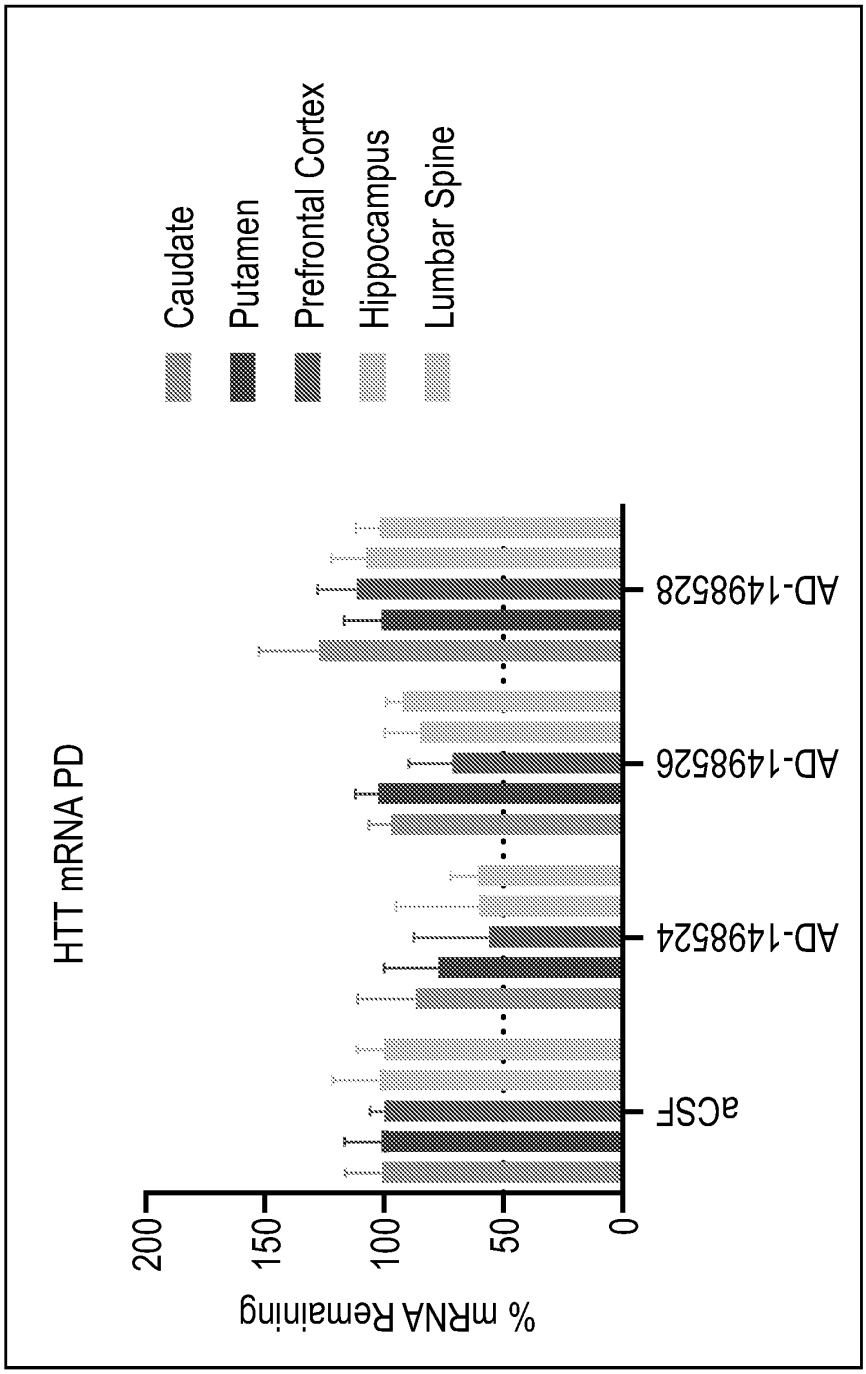

NCS-21-00819 (HTT-Ex1)
(NHP-D59)
aCSF
AD-1498524
AD-1498526
AD-1498528
HTT Protein % remaining
(relative to aCSF)
200
150
100
50
0
ST_CAUDATE
ST_PUTAMEN
HIPPO
PFRONTAL
SPINE_LUMBAR

FIG. 14A

FIG. 14B


HTT mRNA PD
Caudate
Putamen
Prefrontal Cortex
Hippocampus
Lumbar Spine
% mRNA Remaining
200
150
100
50
0
aCSF
AD-1498524
AD-1498526
AD-1498528

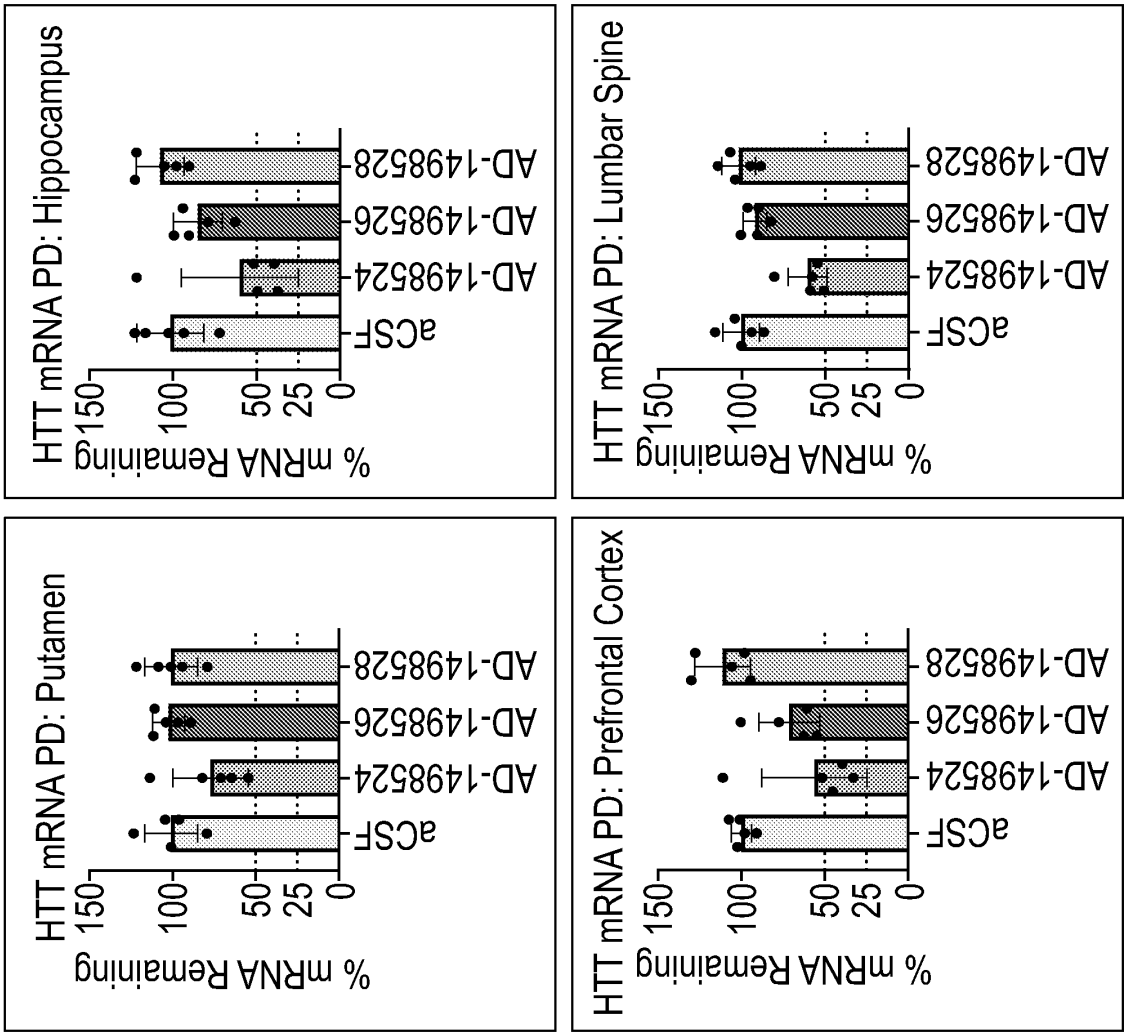

HTT mRNA PD: Hippocampus
% mRNA Remaining
150
100
50
25
0
aCSF
AD-1498524
AD-1498526
AD-1498528
HTT mRNA PD: Lumbar Spine
% mRNA Remaining
150
100
50
25
0
aCSF
AD-1498524
AD-1498526
AD-1498528
HTT mRNA PD: Putamen
% mRNA Remaining
150
100
50
25
0
aCSF
AD-1498524
AD-1498526
AD-1498528
HTT mRNA PD: Prefrontal Cortex
% mRNA Remaining
150
100
50
25
0
aCSF
AD-1498524
AD-1498526
AD-1498528

FIG. 14C

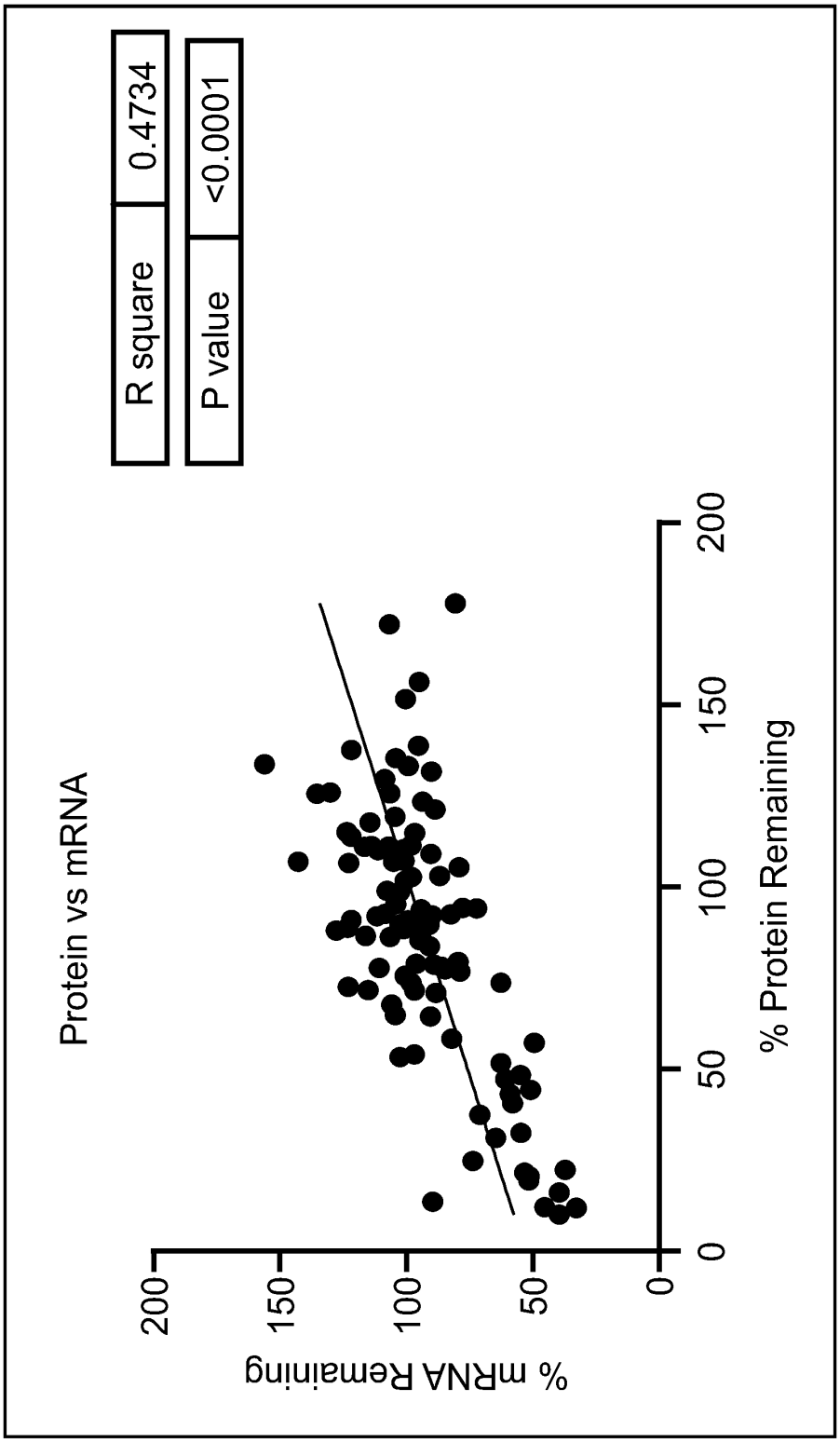
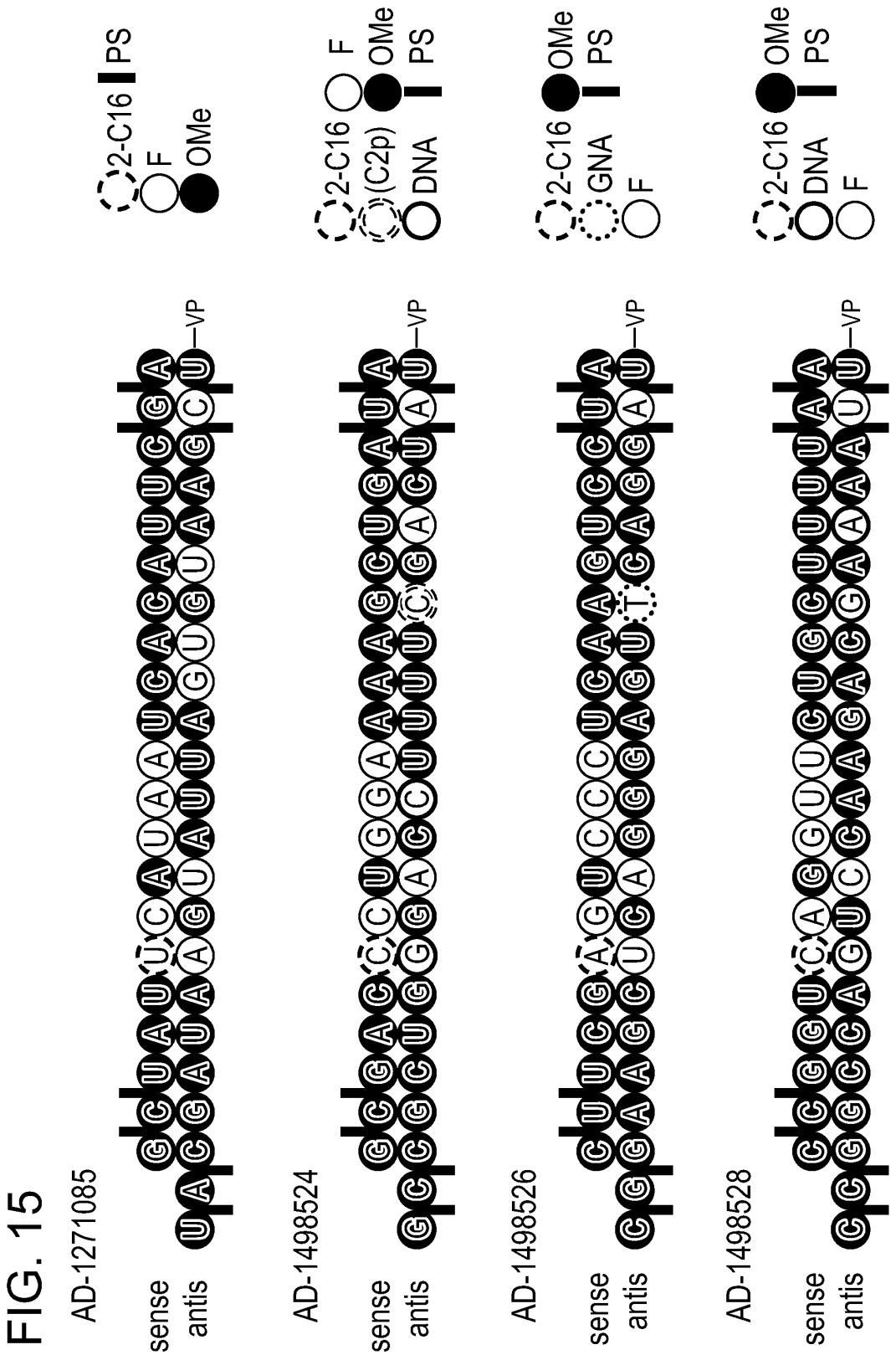

Protein vs mRNA
R square
0.4734
P value
<0.0001
% mRNA Remaining
200
150
100
50
0
0
50
100
150
200
% Protein Remaining

FIG. 14D

FIG. 15


AD-1271085
sense
antis
VP
2-C16
PS
F
OMe
AD-1498524
(C2p)
DNA
AD-1498526
GNA
AD-1498528