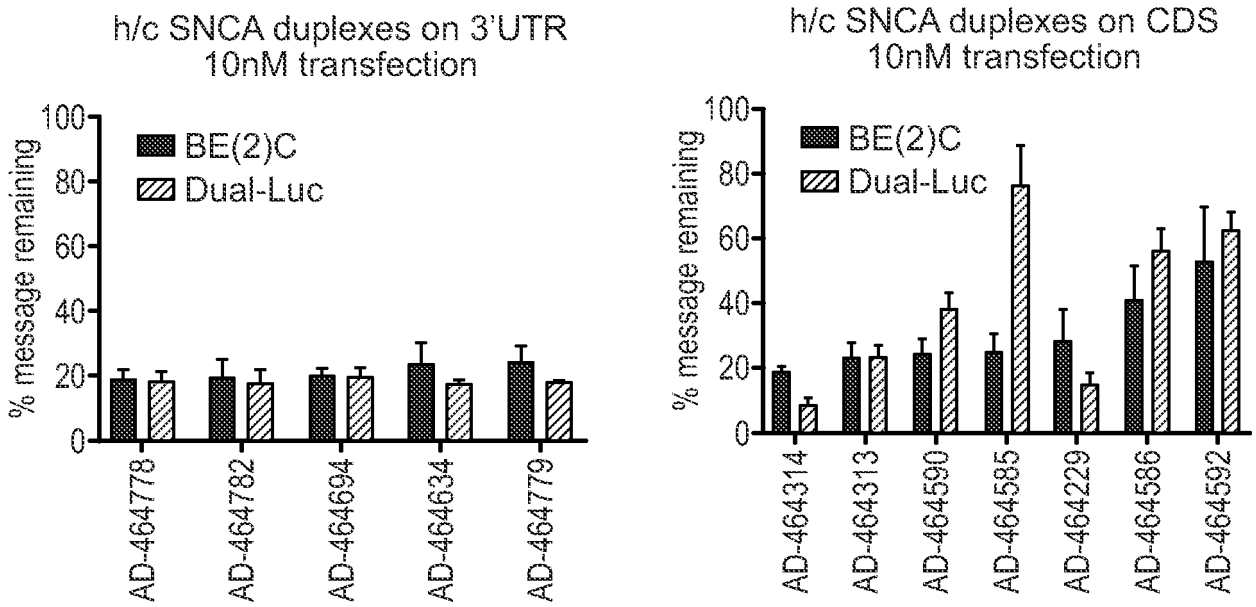

h/c SNCA duplexes on 3'UTR
10nM transfection
% message remaining
BE(2)C
Dual-Luc
AD-464778
AD-464782
AD-464694
AD-464634
AD-464779
h/c SNCA duplexes on CDS
10nM transfection
% message remaining
BE(2)C
Dual-Luc
AD-464314
AD-464313
AD-464590
AD-464585
AD-464229
AD-464586
AD-464592

FIG. 1A

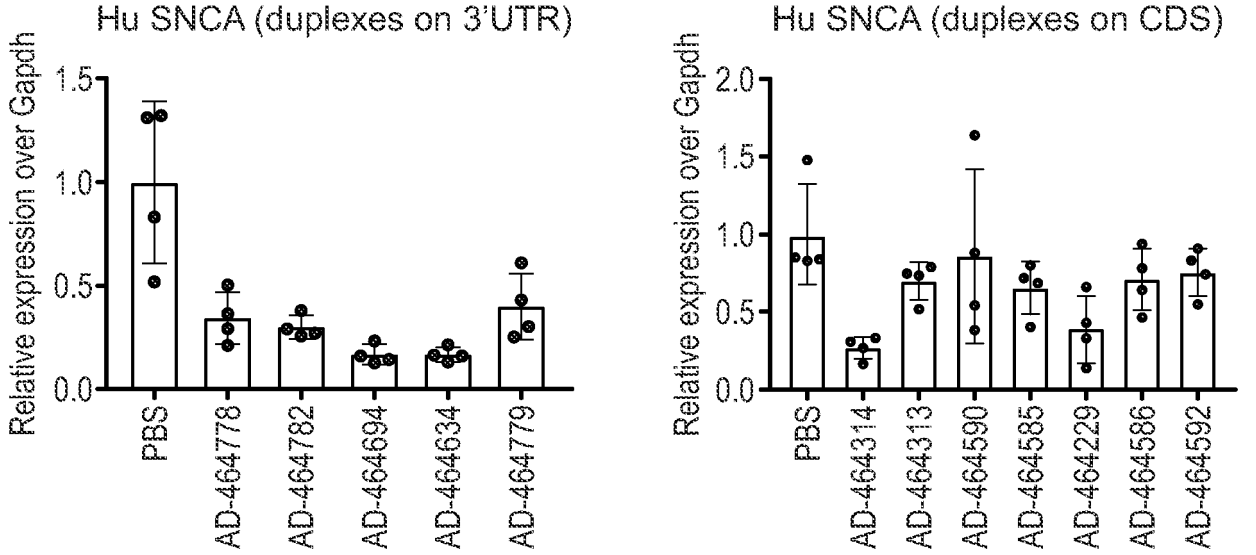

Hu SNCA (duplexes on 3'UTR)
Relative expression over Gapdh
PBS
AD-464778
AD-464782
AD-464694
AD-464634
AD-464779
Hu SNCA (duplexes on CDS)
Relative expression over Gapdh
PBS
AD-464314
AD-464313
AD-464590
AD-464585
AD-464229
AD-464586
AD-464592

FIG. 1B

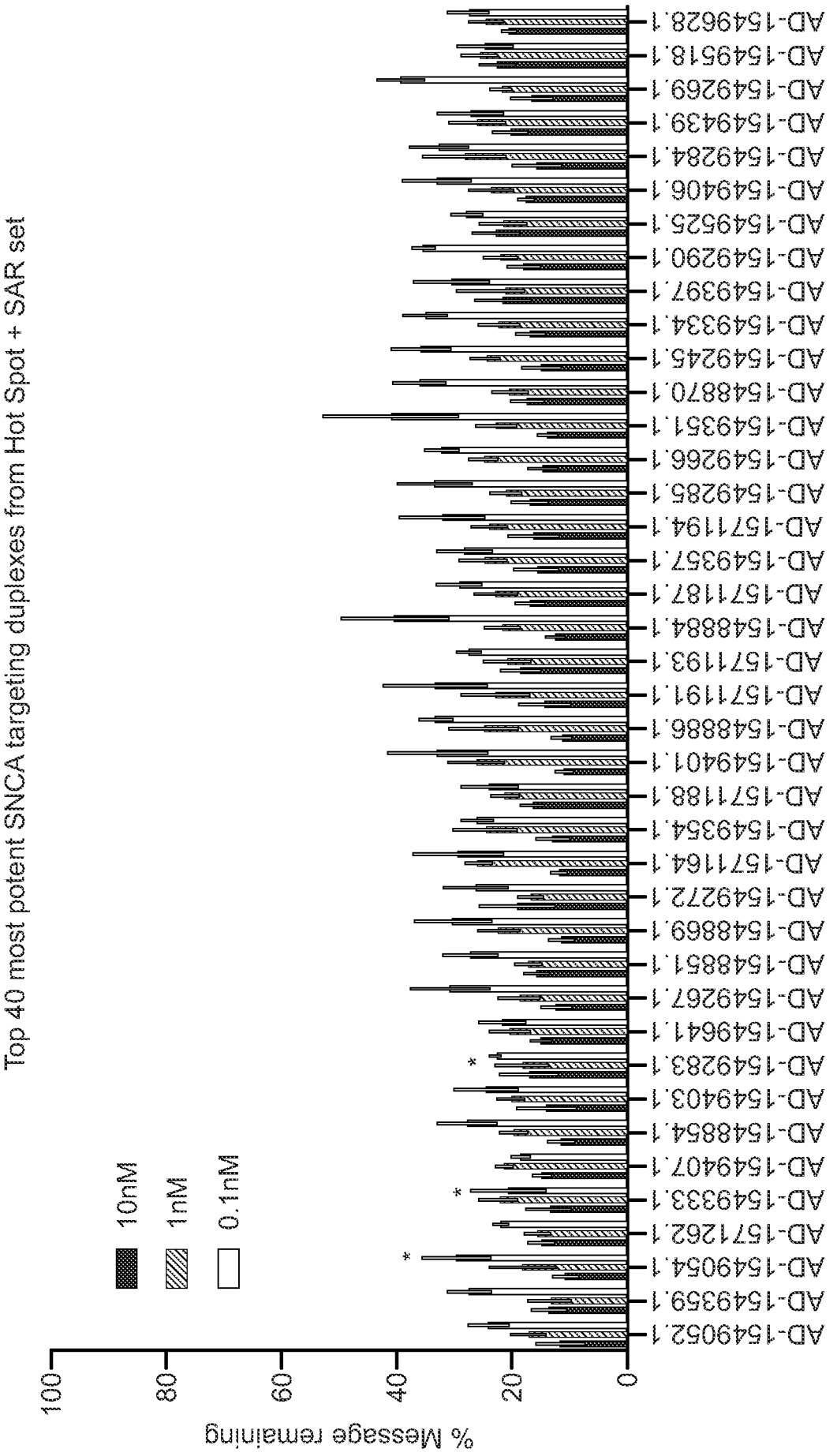

Top 40 most potent SNCA targeting duplexes from Hot Spot + SAR set
10nM
1nM
0.1nM
% Message remaining
100
80
60
40
20
0
AD-1549052.1
AD-1549359.1
AD-1549054.1
AD-1571262.1
AD-1549333.1
AD-1549407.1
AD-1548854.1
AD-1549403.1
AD-1549283.1
AD-1549641.1
AD-1549267.1
AD-1548851.1
AD-1548869.1
AD-1549272.1
AD-1571164.1
AD-1549354.1
AD-1571188.1
AD-1549401.1
AD-1548886.1
AD-1571191.1
AD-1571193.1
AD-1548884.1
AD-1571187.1
AD-1549357.1
AD-1571194.1
AD-1549285.1
AD-1549266.1
AD-1549351.1
AD-1548870.1
AD-1549245.1
AD-1549334.1
AD-1549397.1
AD-1549290.1
AD-1549525.1
AD-1549406.1
AD-1549284.1
AD-1549439.1
AD-1549269.1
AD-1549518.1
AD-1549628.1

FIG. 2

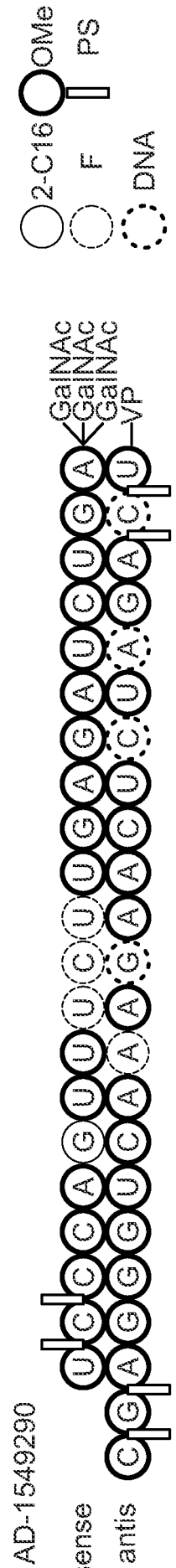

AD-1549290
sense
antis
2-C16
OMe
F
PS
DNA
GalNAc
GalNAc
GalNAc
VP

FIG. 3

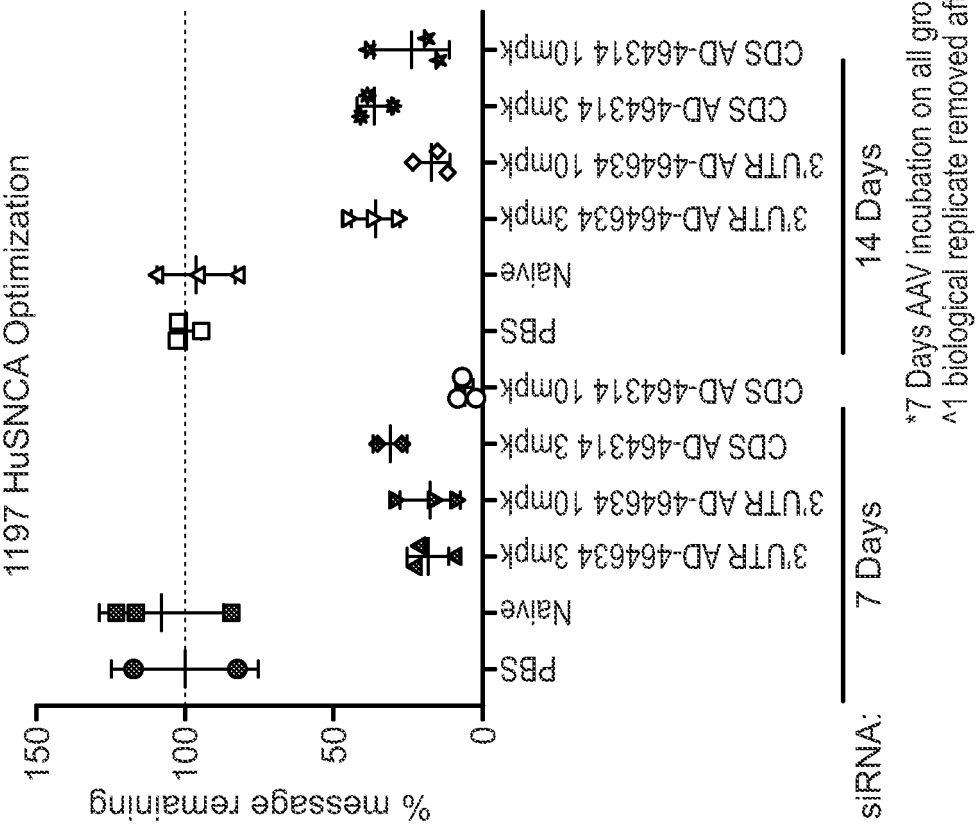
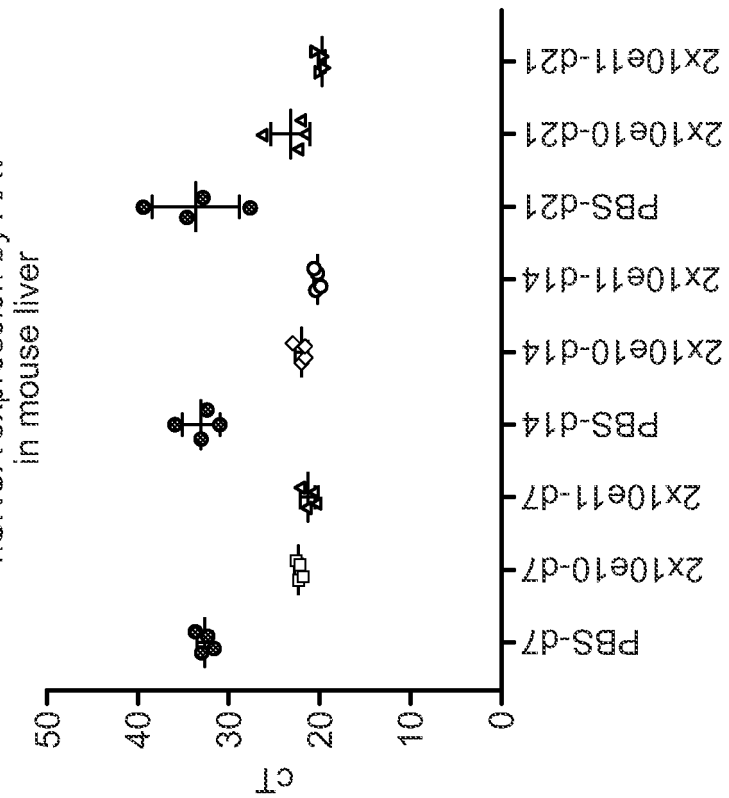

1197 HuSNCA Optimization
% message remaining
150
100
50
0
PBS
Naive
3'UTR AD-464634 3mpk
3'UTR AD-464634 10mpk
CDS AD-464314 3mpk
CDS AD-464314 10mpk
7 Days
PBS
Naive
3'UTR AD-464634 3mpk
3'UTR AD-464634 10mpk
CDS AD-464314 3mpk
CDS AD-464314 10mpk
14 Days
siRNA:
*7 Days AAV incubation on all group
^1 biological replicate removed after QC

FIG. 4


hSNCA expression by AAV
in mouse liver
cT
50
40
30
20
10
0
PBS-d7
2x10e10-d7
2x10e11-d7
PBS-d14
2x10e10-d14
2x10e11-d14
PBS-d21
2x10e10-d21
2x10e11-d21

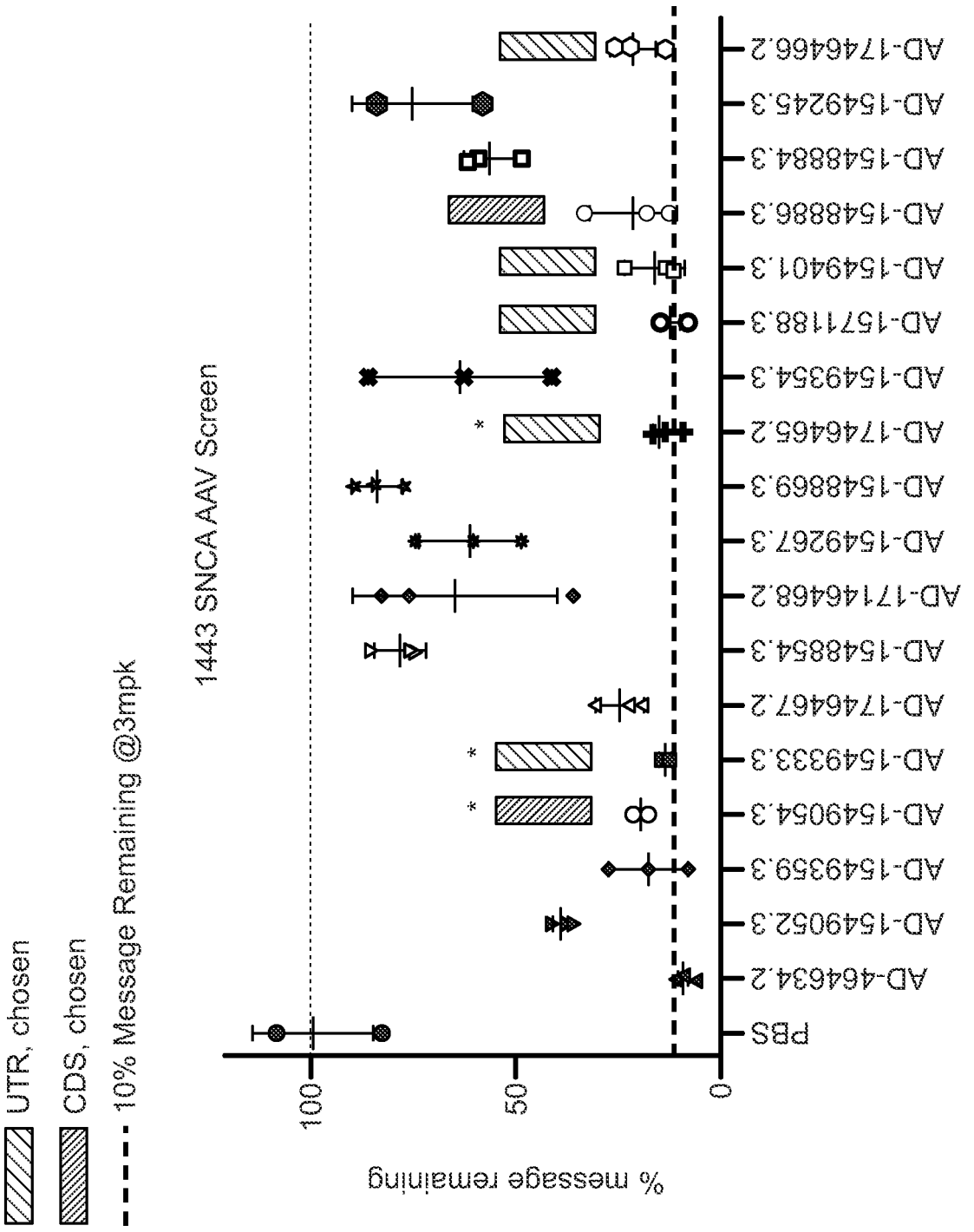

1443 SNCA AAV Screen
% message remaining
100
50
0
PBS
AD-464634.2
AD-154905 2.3
AD-154935 9.3
AD-154905 4.3
AD-154933 3.3
AD-174646 7.2
AD-154885 4.3
AD-171464 68.2
AD-154926 7.3
AD-154886 9.3
AD-174646 5.2
AD-154935 4.3
AD-157118 8.3
AD-154940 1.3
AD-154888 6.3
AD-154888 4.3
AD-154924 5.3
AD-174646 6.2
UTR, chosen
CDS, chosen
10% Message Remaining @3mpk

FIG. 5A

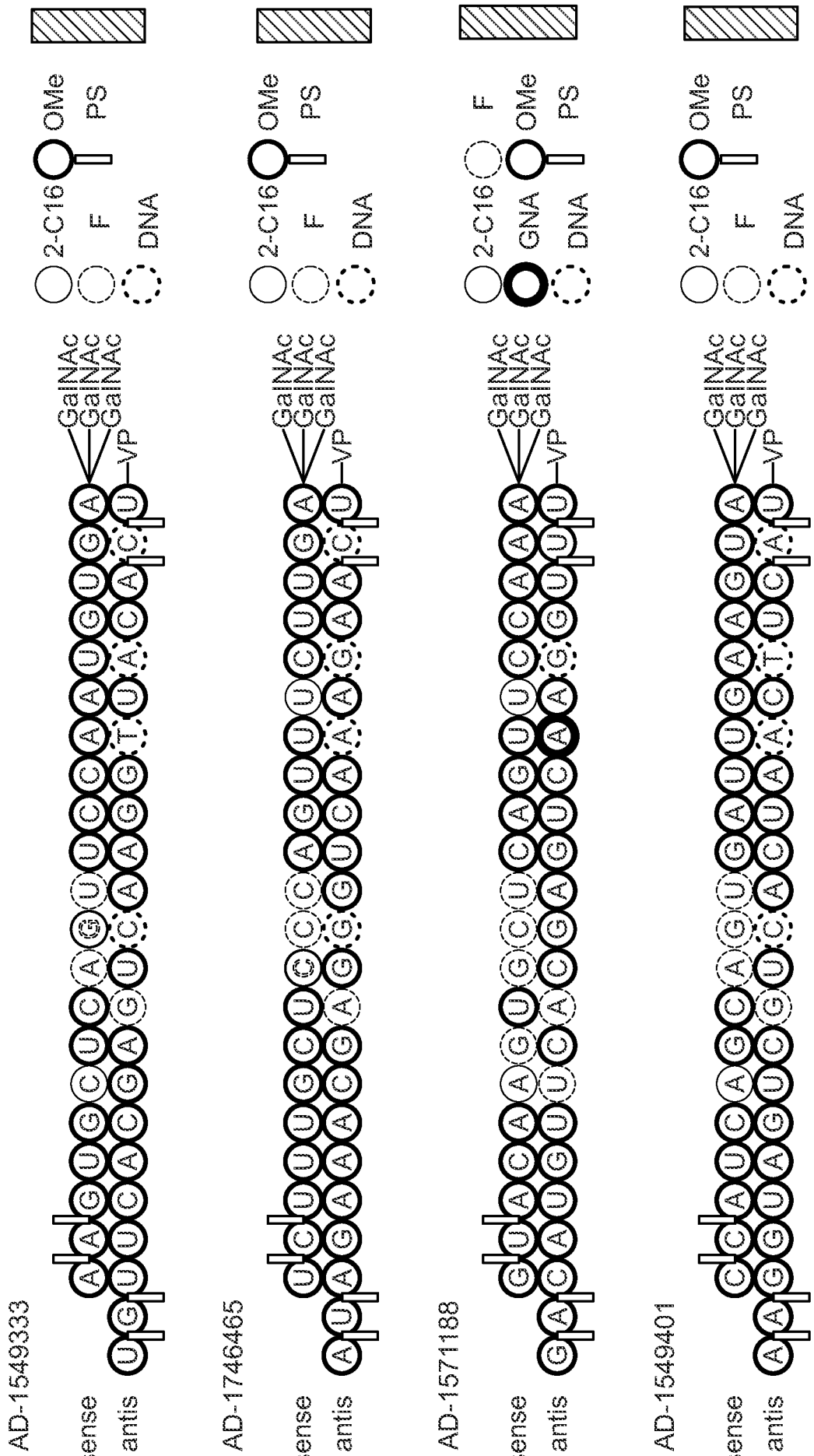

AD-1549333
sense
antis
AD-1746465
sense
antis
AD-1571188
sense
antis
AD-1549401
sense
antis
GalNAc
GalNAc
GalNAc
VP
2-C16
OMe
PS
F
DNA
GNA

FIG. 5B

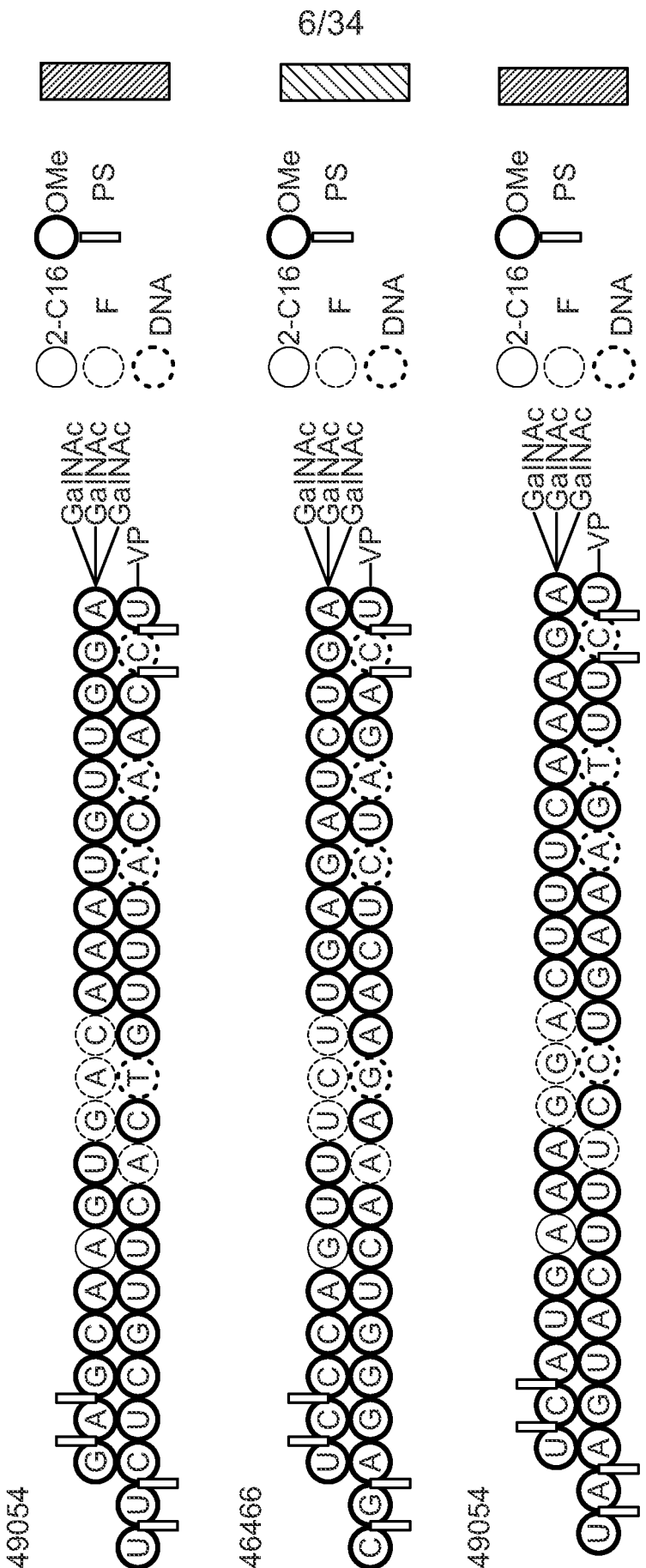

2-C16
OMe
F
PS
DNA
GalNAc
GalNAc
GalNAc
VP
AD-1549054
sense
antis
AD-1746466
sense
antis
AD-1549054
sense
antis

FIG. 5B (cont)

Identifier	Location	In vivo (% Message Remaining @3mpk)	ED_{80} (nM)
AD-1549333	UTR	9.06	0.235
AD-1746465	UTR	13.43	0.405
AD-1571188	UTR	12.37	1.075
AD-1549401	UTR	16.03	1.216
AD-1549054	CDS	18.62	0.504
AD-1746466	UTR	21.41	2.871
AD-1548886	CDS	21.49	1.210

FIG. 6A

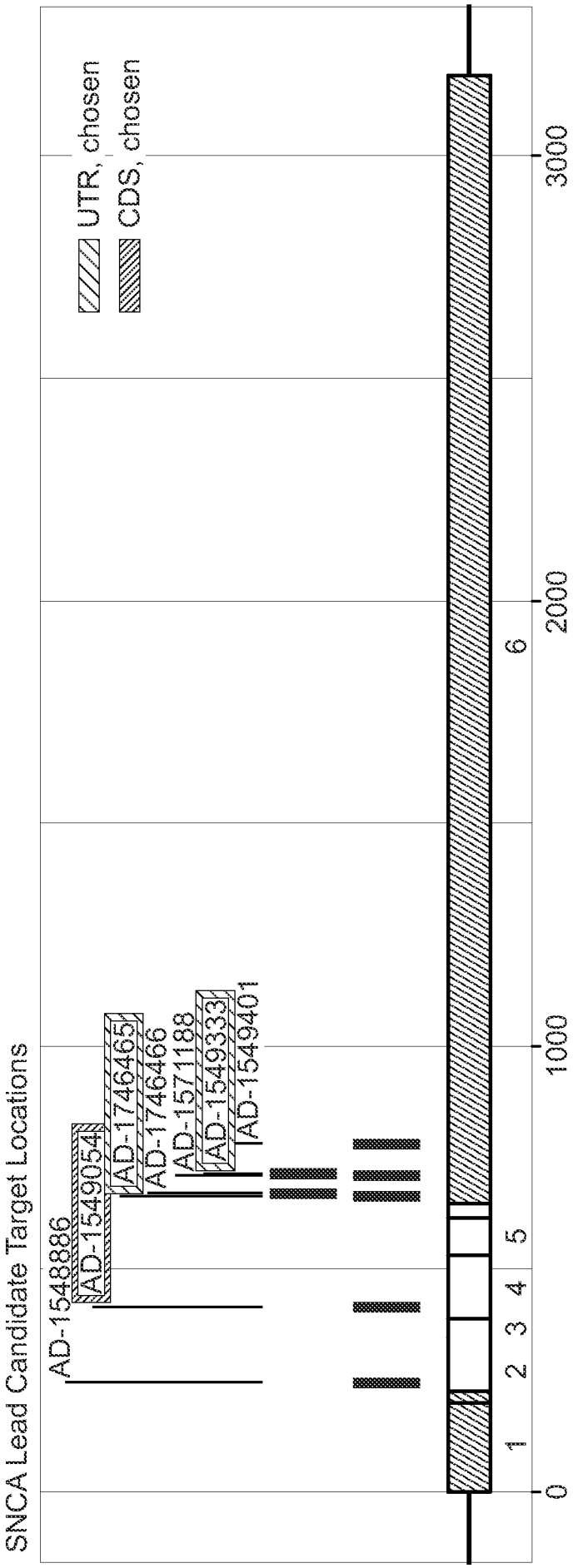

SNCA Lead Candidate Target Locations
AD-1548886
AD-1549054
AD-1746465
AD-1746466
AD-1571188
AD-1549333
AD-1549401
UTR, chosen
CDS, chosen
0
1000
2000
3000
1
2
3
4
5
6

FIG. 6B

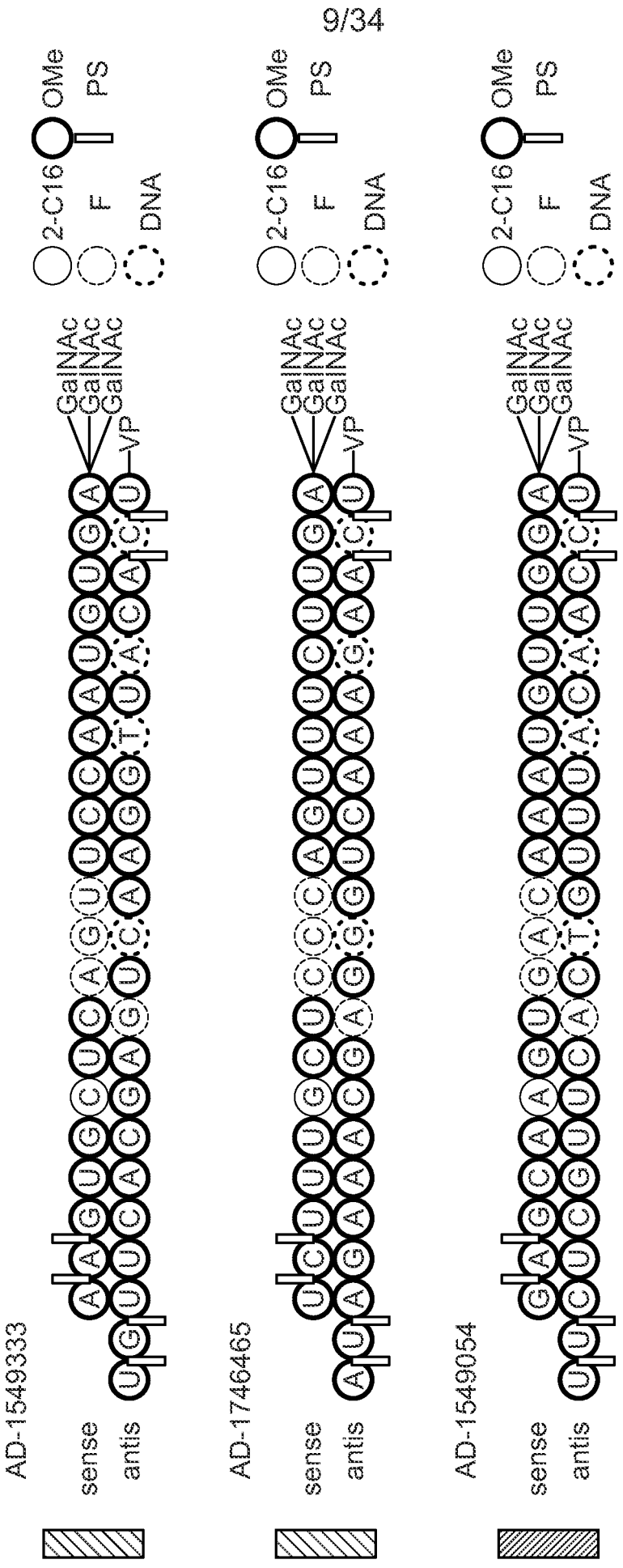

AD-1549333
sense
antis
AD-1746465
sense
antis
AD-1549054
sense
antis
GalNAc
GalNAc
GalNAc
VP
2-C16
OMe
F
PS
DNA

FIG. 6C

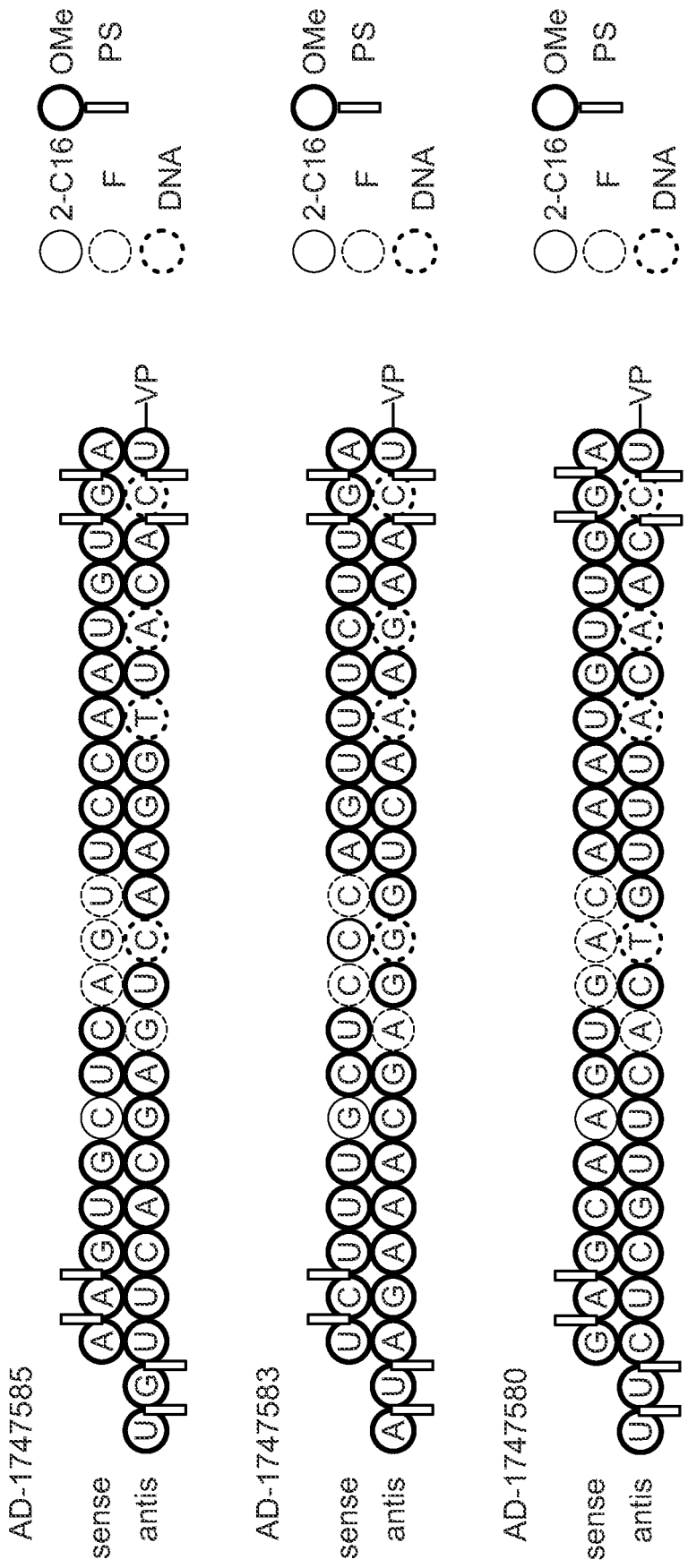

2-C16
OMe
F
PS
DNA
AD-1747585
sense
antis
AD-1747583
sense
antis
AD-1747580
sense
antis
VP

FIG. 7

Identifier	Location	In vivo (% Message Remaining @3mpk)	ED_{80} (nM)
AD-1747585	UTR	9.06	0.235
AD-1747583	UTR	13.43	0.405
AD-1747580	CDS	18.62	0.504

FIG. 8A

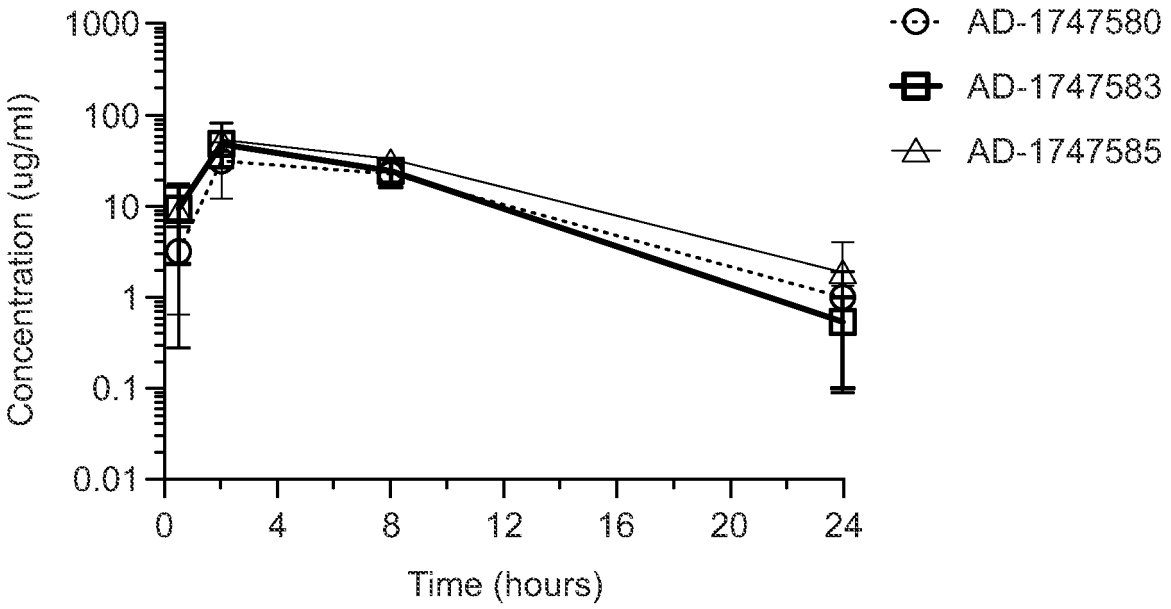


FIG. 8B

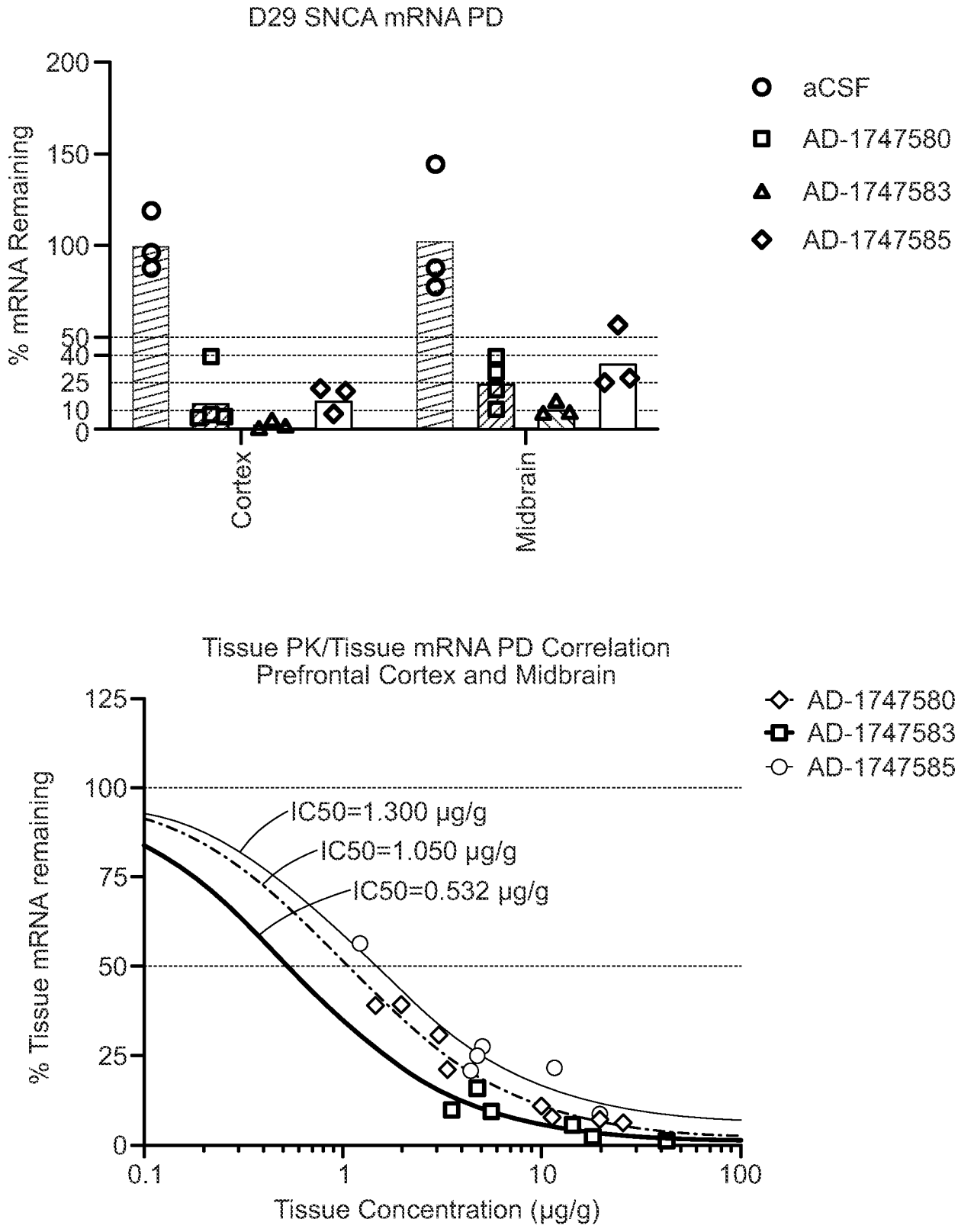

D29 SNCA mRNA PD
% mRNA Remaining
200
150
100
50
40
25
10
0
Cortex
Midbrain
aCSF
AD-1747580
AD-1747583
AD-1747585
Tissue PK/Tissue mRNA PD Correlation
Prefrontal Cortex and Midbrain
% Tissue mRNA remaining
125
100
75
50
25
0
IC50=1.300 µg/g
IC50=1.050 µg/g
IC50=0.532 µg/g
0.1
1
10
100
Tissue Concentration (µg/g)
AD-1747580
AD-1747583
AD-1747585

FIG. 9A

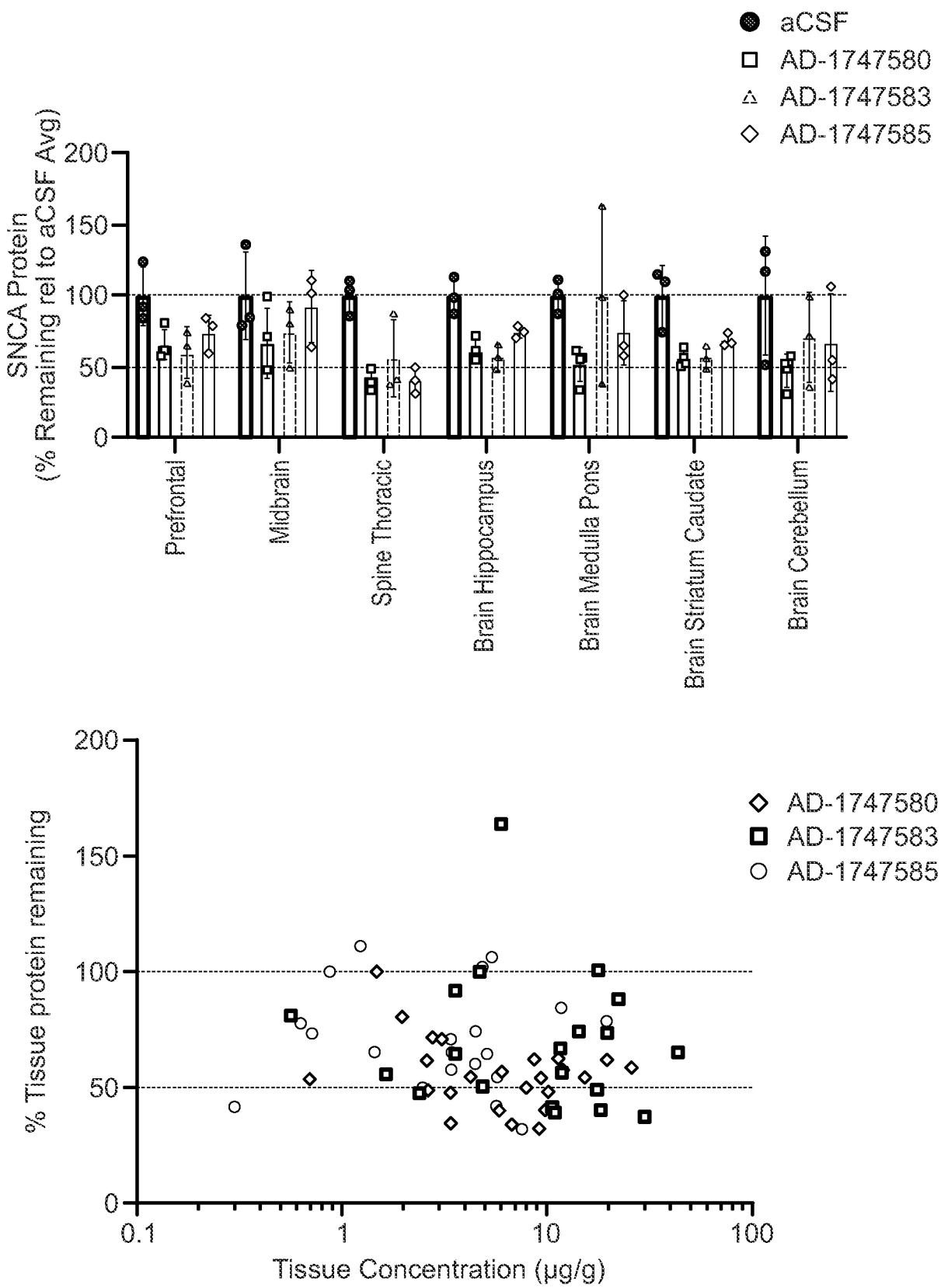

aCSF
AD-1747580
AD-1747583
AD-1747585
SNCA Protein
(% Remaining rel to aCSF Avg)
200
150
100
50
0
Prefrontal
Midbrain
Spine Thoracic
Brain Hippocampus
Brain Medulla Pons
Brain Striatum Caudate
Brain Cerebellum
% Tissue protein remaining
Tissue Concentration (µg/g)
0.1
1
10
100

FIG. 9B

Identifier	Location	Average %NHP mRNA knockdown Cortex	Average %NHP mRNA knockdown Midbrain	Average %NHP CSP protein knockdown D29	Average %NHP tissue protein knockdown Cortex	Average %NHP tissue protein knockdown Midbrain
AD-1747585	UTR	83.1	63.80	N/A	25.73	7.67
AD-1747583	UTR	97.09	88.42	64.72	40.17	25.73
AD-1747580	CDS	84.98	74.57	67.08	34.25	33.3

FIG. 10

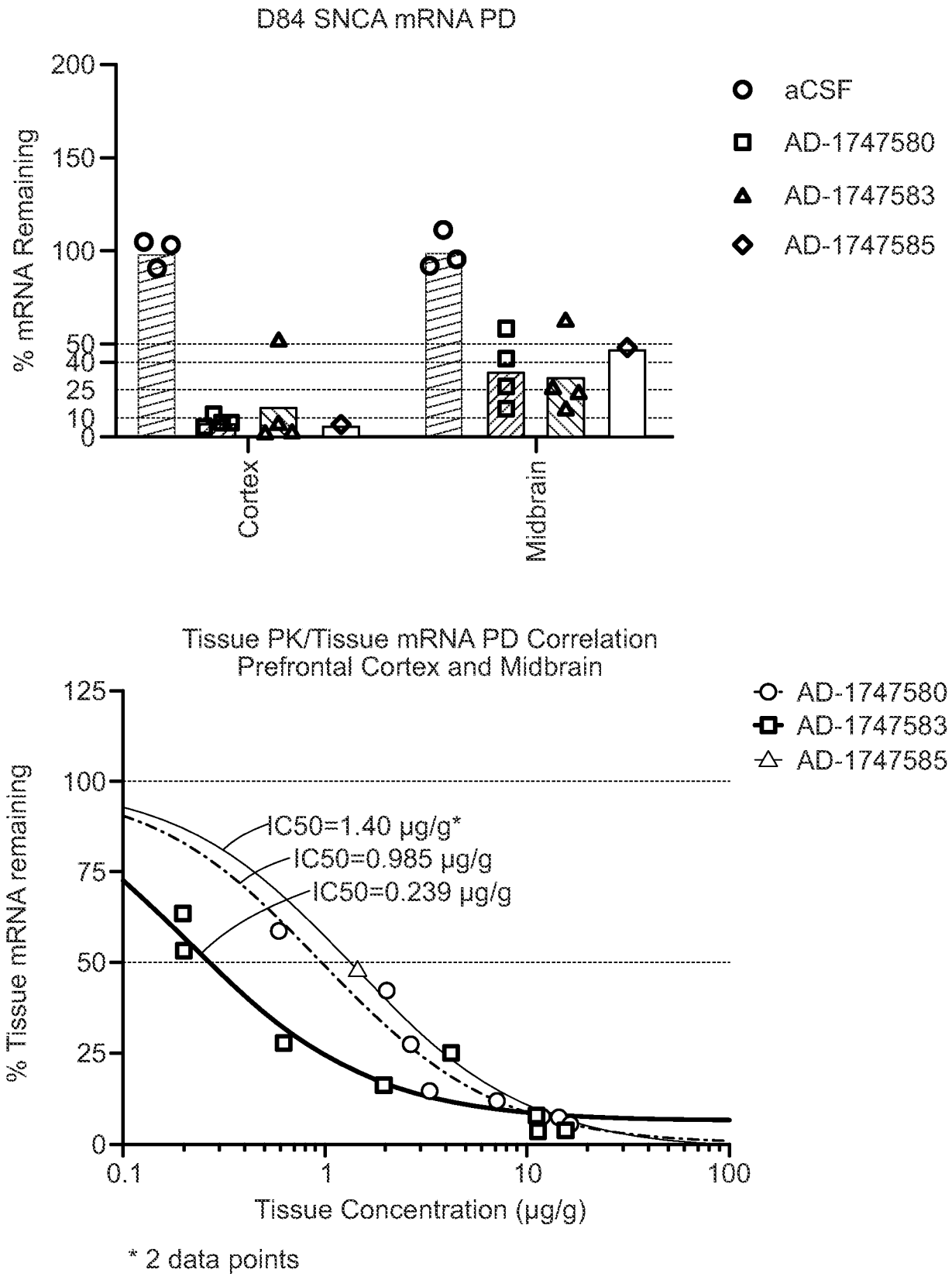

D84 SNCA mRNA PD
% mRNA Remaining
200
150
100
50
40
25
10
0
Cortex
Midbrain
aCSF
AD-1747580
AD-1747583
AD-1747585
Tissue PK/Tissue mRNA PD Correlation
Prefrontal Cortex and Midbrain
% Tissue mRNA remaining
125
100
75
50
25
0
IC50=1.40 µg/g*
IC50=0.985 µg/g
IC50=0.239 µg/g
0.1
1
10
100
Tissue Concentration (µg/g)
AD-1747580
AD-1747583
AD-1747585
* 2 data points

FIG. 11

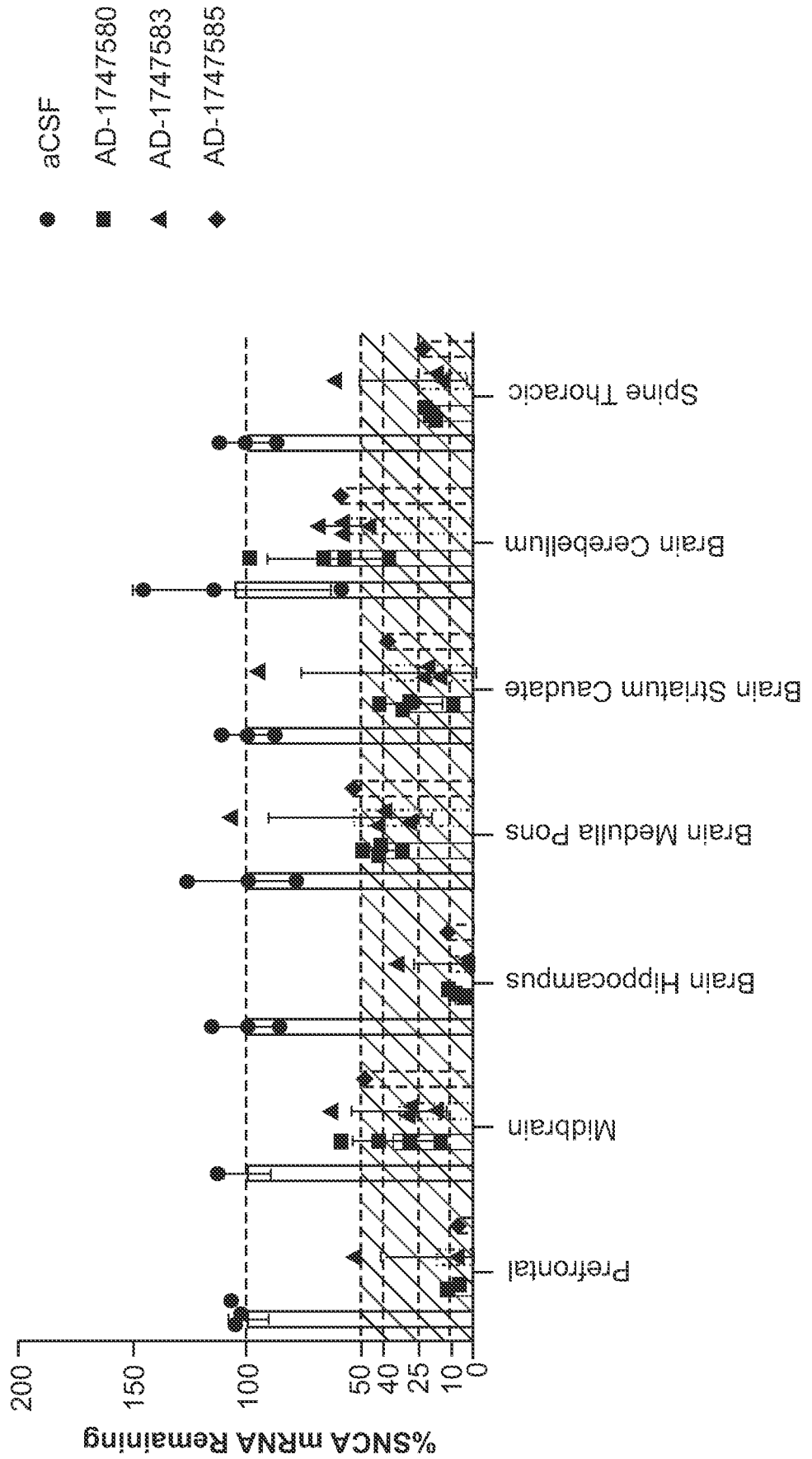

aCSF
AD-1747580
AD-1747583
AD-1747585
%SNCA mRNA Remaining
200
150
100
50
40
25
10
0
Prefrontal
Midbrain
Brain Hippocampus
Brain Medulla Pons
Brain Striatum Caudate
Brain Cerebellum
Spine Thoracic

FIG. 12A

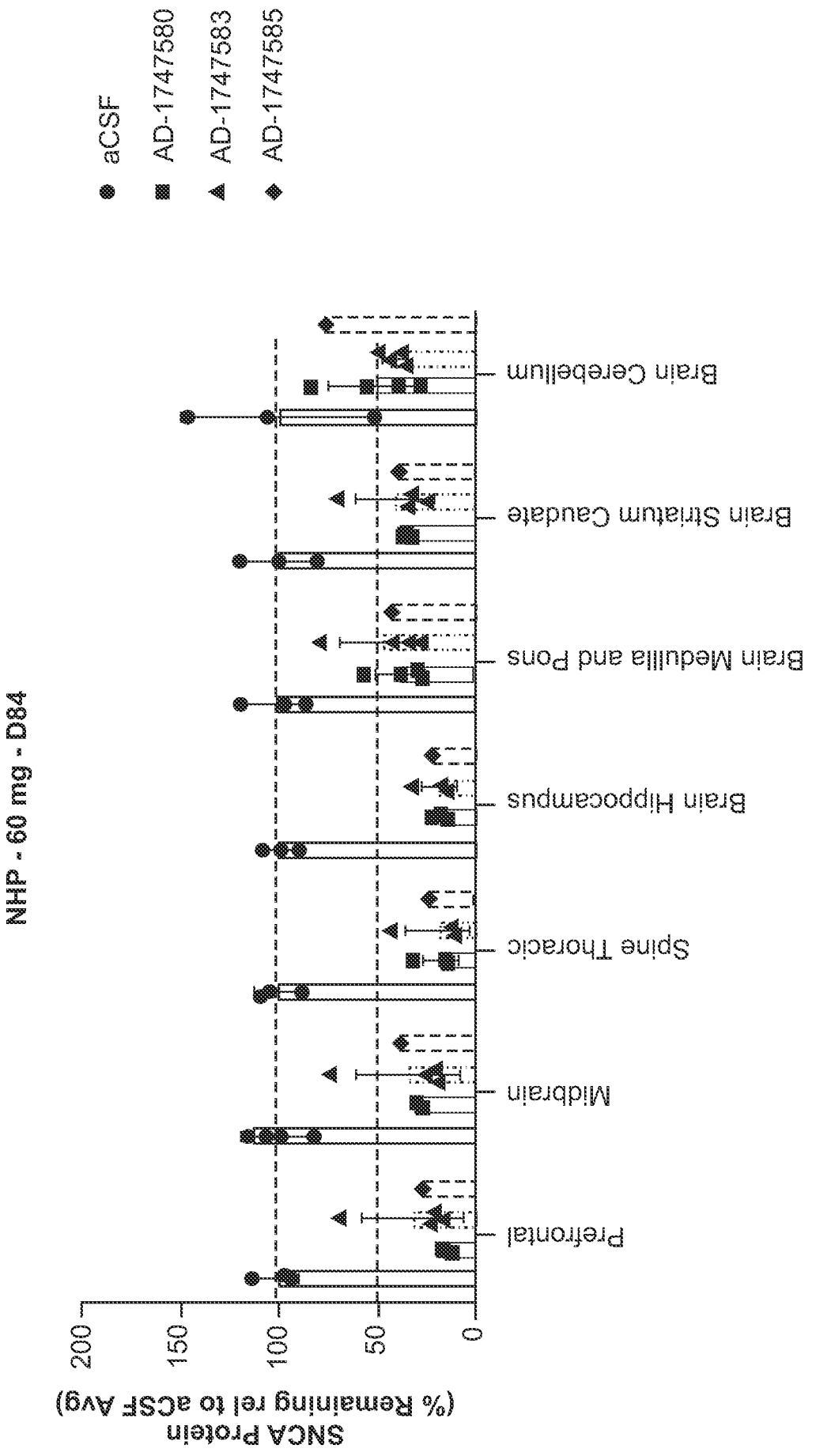
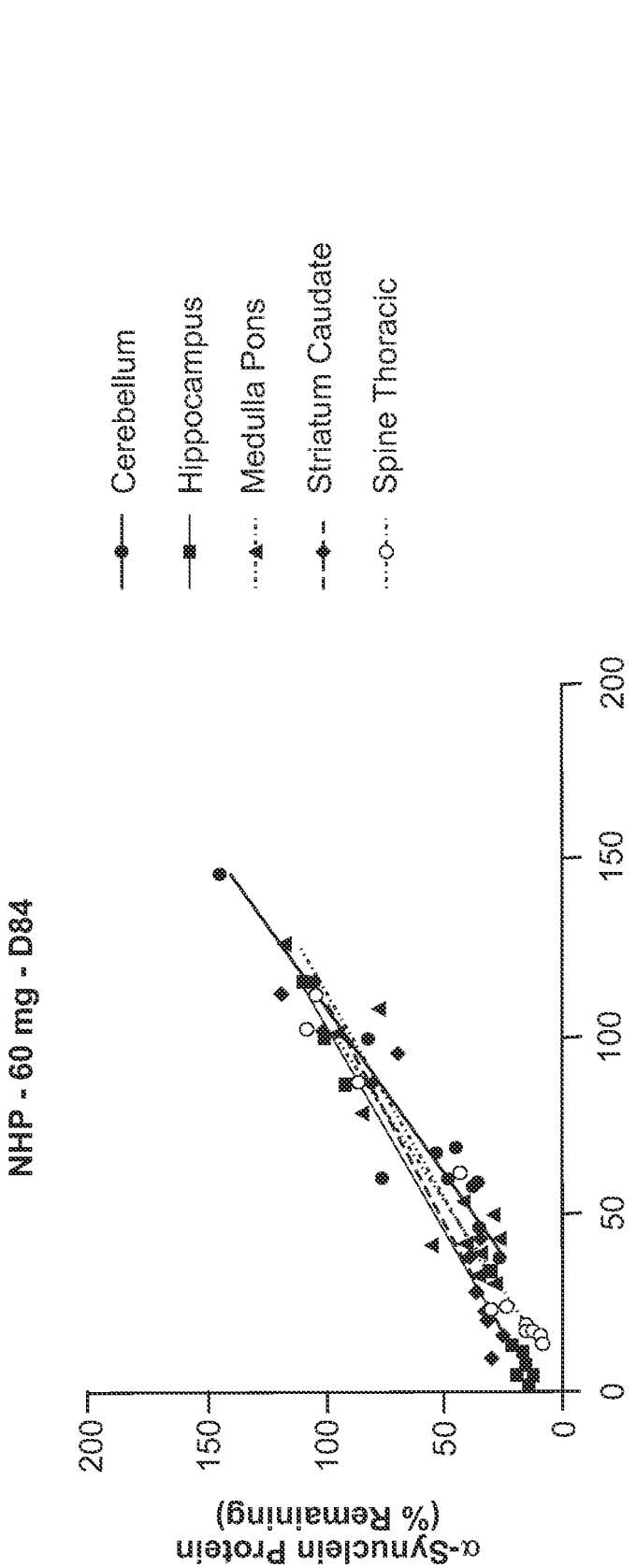

aCSF
AD-1747580
AD-1747583
AD-1747585
NHP - 60 mg - D84
SNCA Protein
(% Remaining rel to aCSF Avg)
200
150
100
50
0
Prefrontal
Midbrain
Spine Thoracic
Brain Hippocampus
Brain Medulla and Pons
Brain Striatum Caudate
Brain Cerebellum

FIG. 12B



	Cerebellum	Hippocampus	Medulla Pons	Striatum Caudate	Spine Thoracic
R squared	0.9075	0.9872	0.8608	0.9083	0.9679
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

FIG. 12C

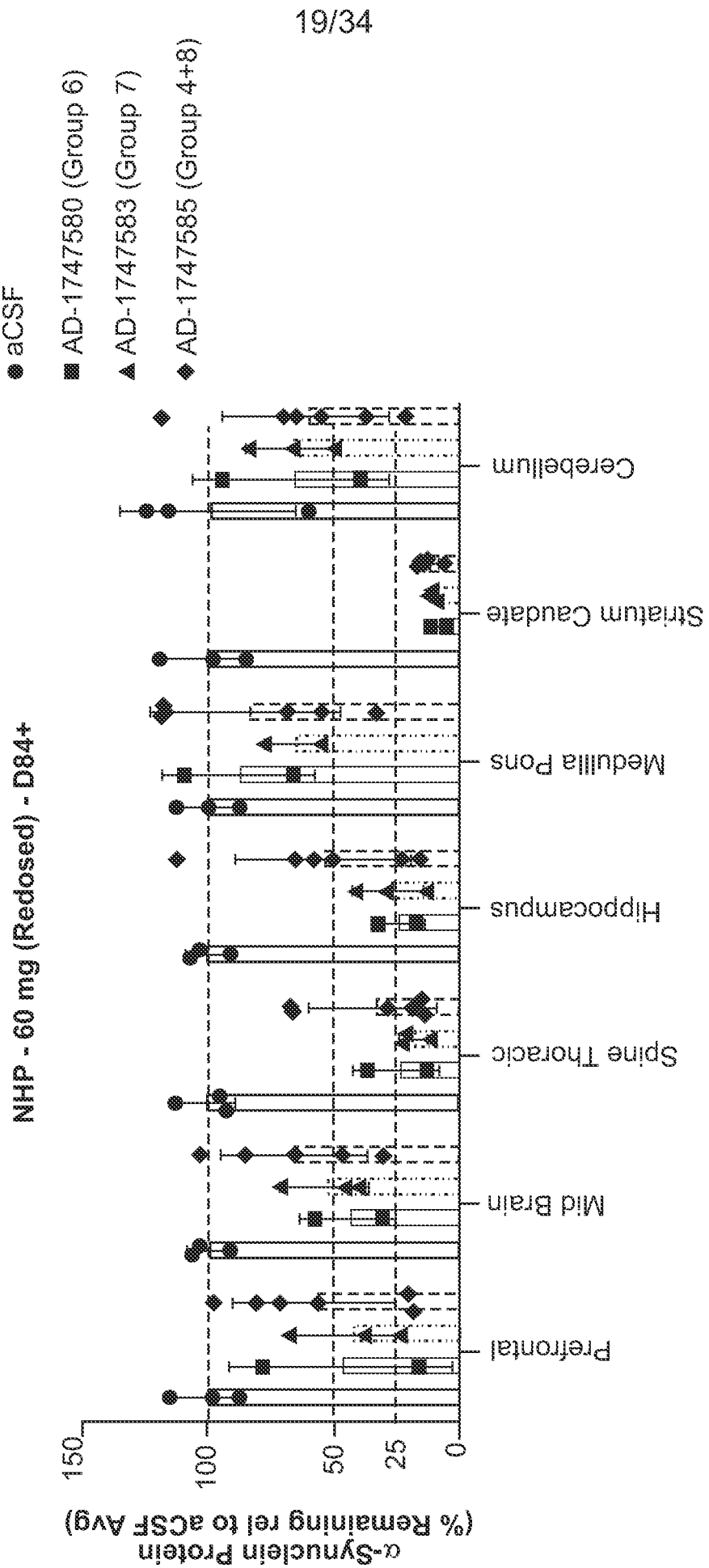

aCSF
AD-1747580 (Group 6)
AD-1747583 (Group 7)
AD-1747585 (Group 4+8)
NHP - 60 mg (Redosed) - D84+
α-Synuclein Protein
(% Remaining rel to aCSF Avg)
150
100
50
25
0
Prefrontal
Mid Brain
Spine Thoracic
Hippocampus
Medulla Pons
Striatum Caudate
Cerebellum

FIG. 12D

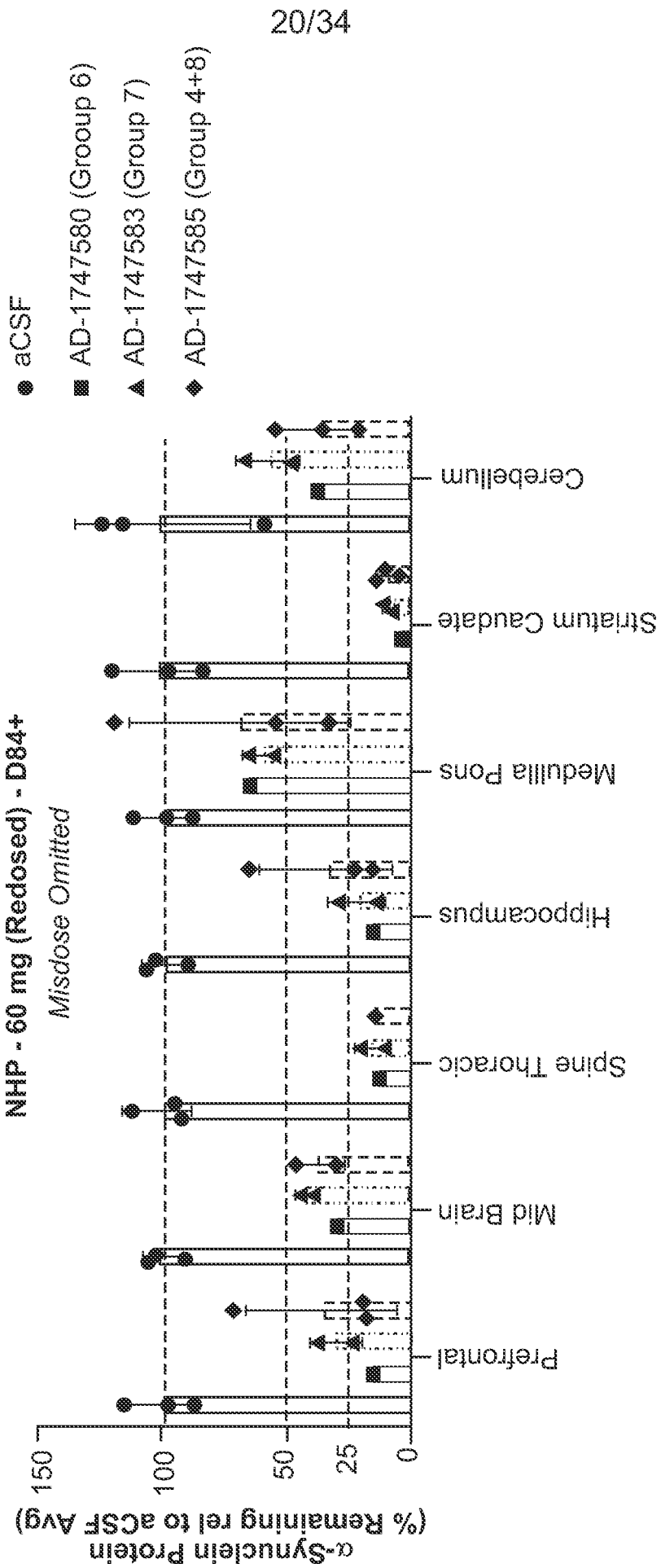
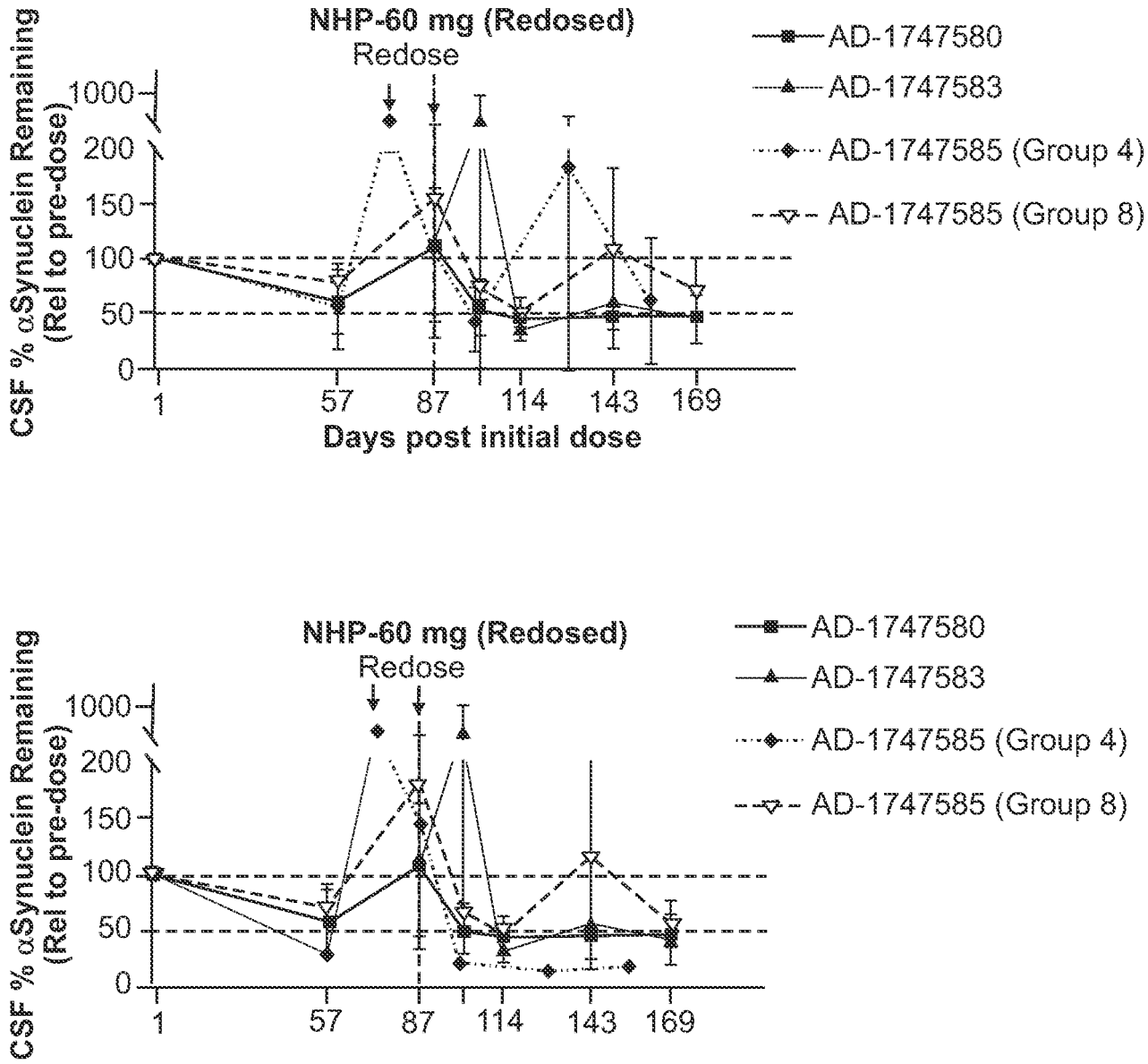

aCSF
AD-1747580 (Grooup 6)
AD-1747583 (Group 7)
AD-1747585 (Group 4+8)
NHP - 60 mg (Redosed) - D84+
Misdose Omitted
α-Synuclein Protein
(% Remaining rel to aCSF Avg)
150
100
50
25
0
Prefrontal
Mid Brain
Spine Thoracic
Hippocampus
Medulla Pons
Striatum Caudate
Cerebellum

FIG. 12D (CONT)



Animals with misdose omitted based on 24H CSF [ng/mL]

FIG. 12E

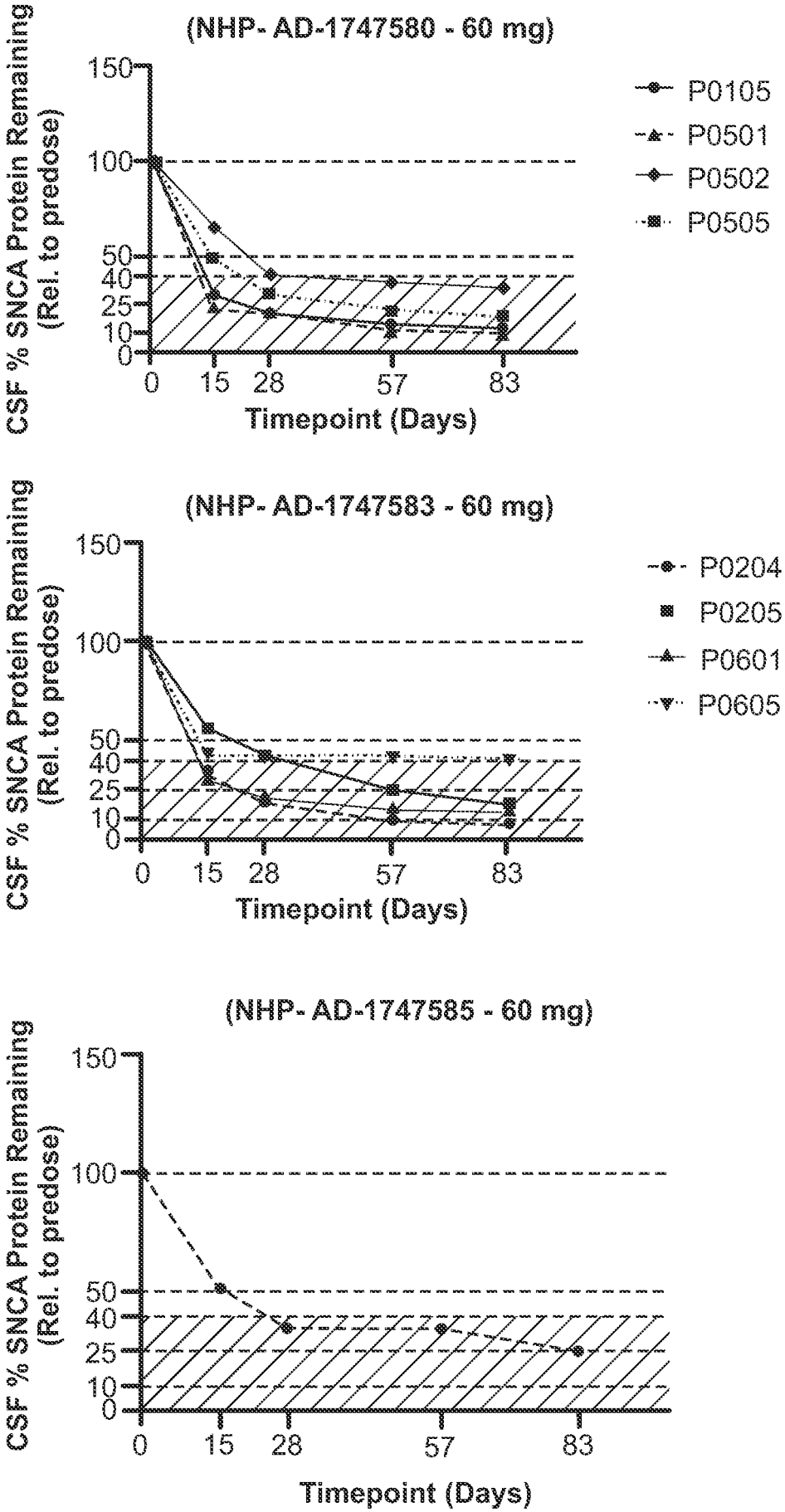

(NHP- AD-1747580 - 60 mg)
CSF % SNCA Protein Remaining (Rel. to predose)
150
100
50
40
25
10
0
0
15
28
57
83
Timepoint (Days)
P0105
P0501
P0502
P0505
(NHP- AD-1747583 - 60 mg)
CSF % SNCA Protein Remaining (Rel. to predose)
Timepoint (Days)
P0204
P0205
P0601
P0605
(NHP- AD-1747585 - 60 mg)
CSF % SNCA Protein Remaining (Rel. to predose)
Timepoint (Days)

FIG. 13

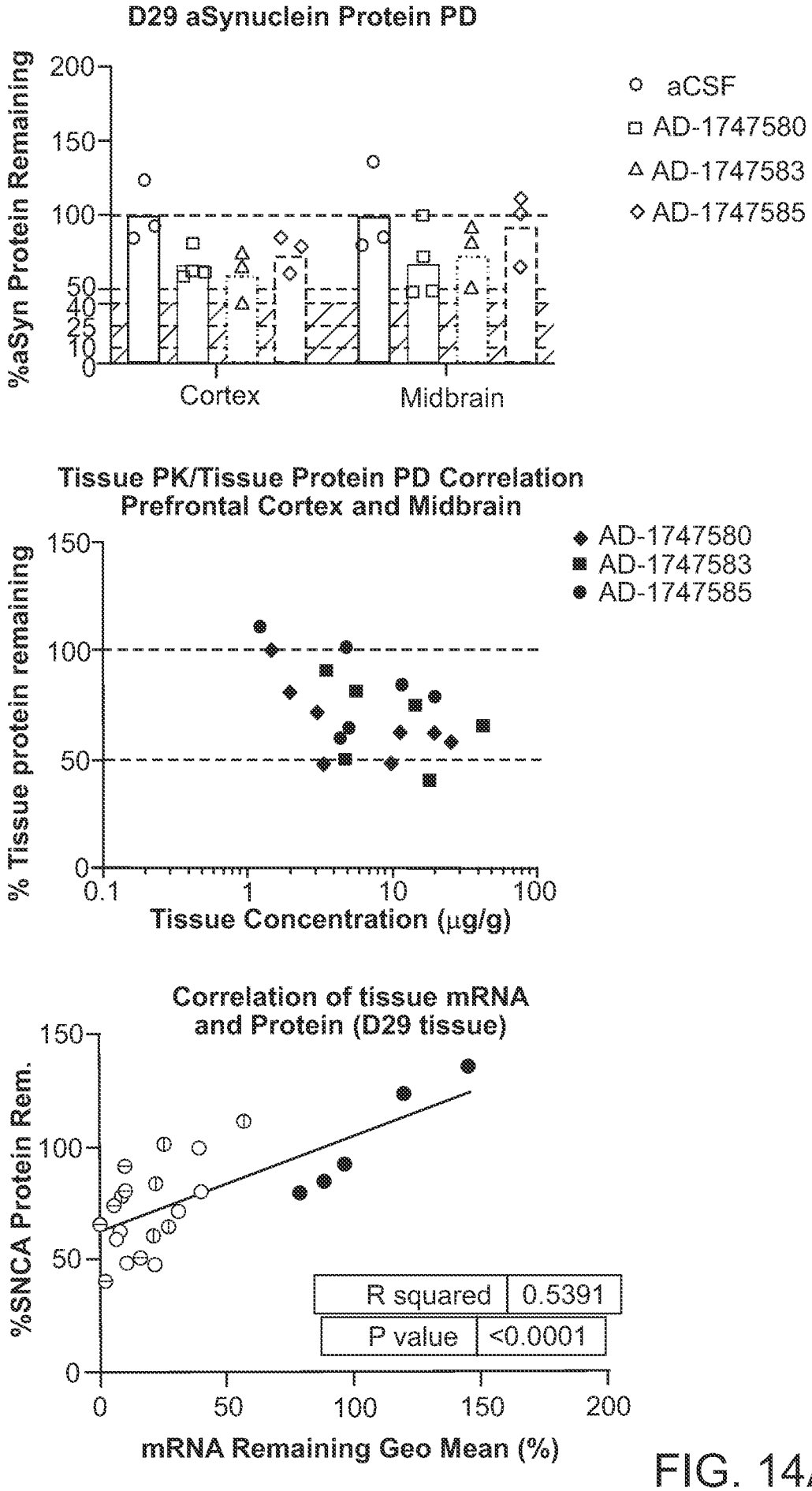
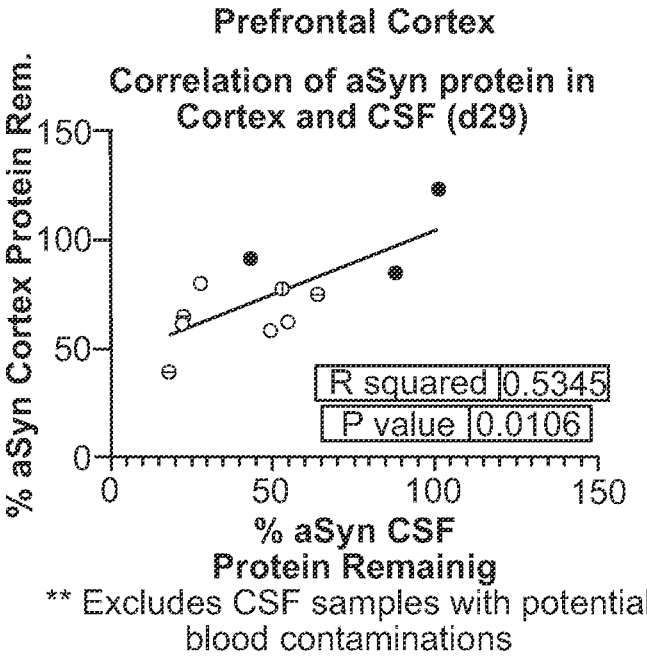
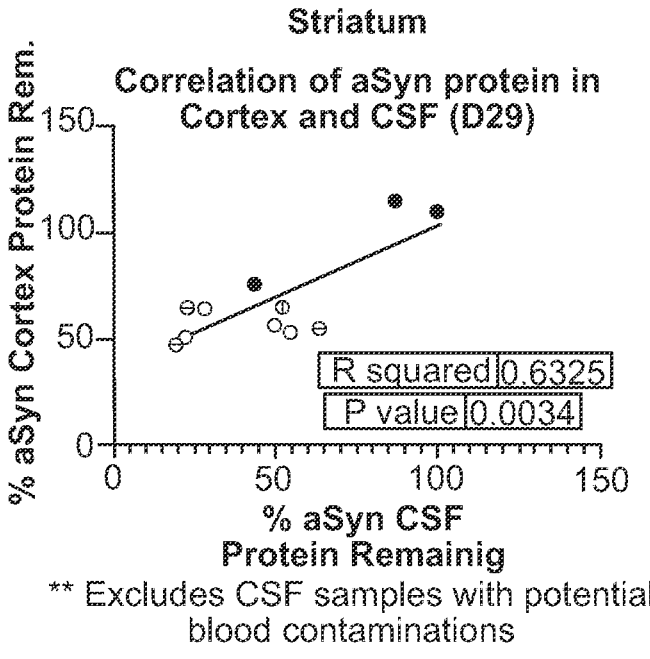

D29 aSynuclein Protein PD
%aSyn Protein Remaining
200
150
100
50
40
25
10
0
Cortex
Midbrain
aCSF
AD-1747580
AD-1747583
AD-1747585
Tissue PK/Tissue Protein PD Correlation
Prefrontal Cortex and Midbrain
% Tissue protein remaining
150
100
50
0
0.1
1
10
100
Tissue Concentration (µg/g)
AD-1747580
AD-1747583
AD-1747585
Correlation of tissue mRNA
and Protein (D29 tissue)
%SNCA Protein Rem.
150
100
50
0
0
50
100
150
200
mRNA Remaining Geo Mean (%)
R squared 0.5391
P value <0.0001

FIG. 14A





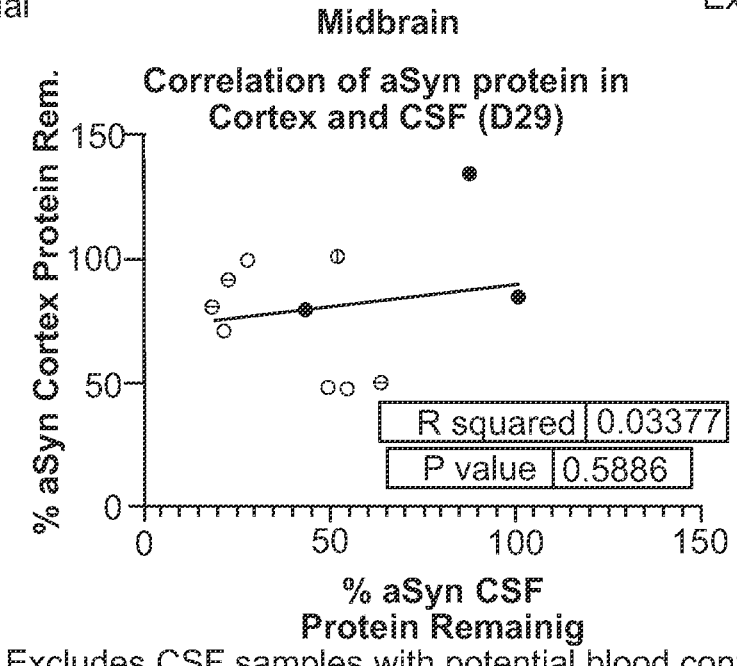


FIG. 14B

SUBSTITUTE SHEET (RULE 26)

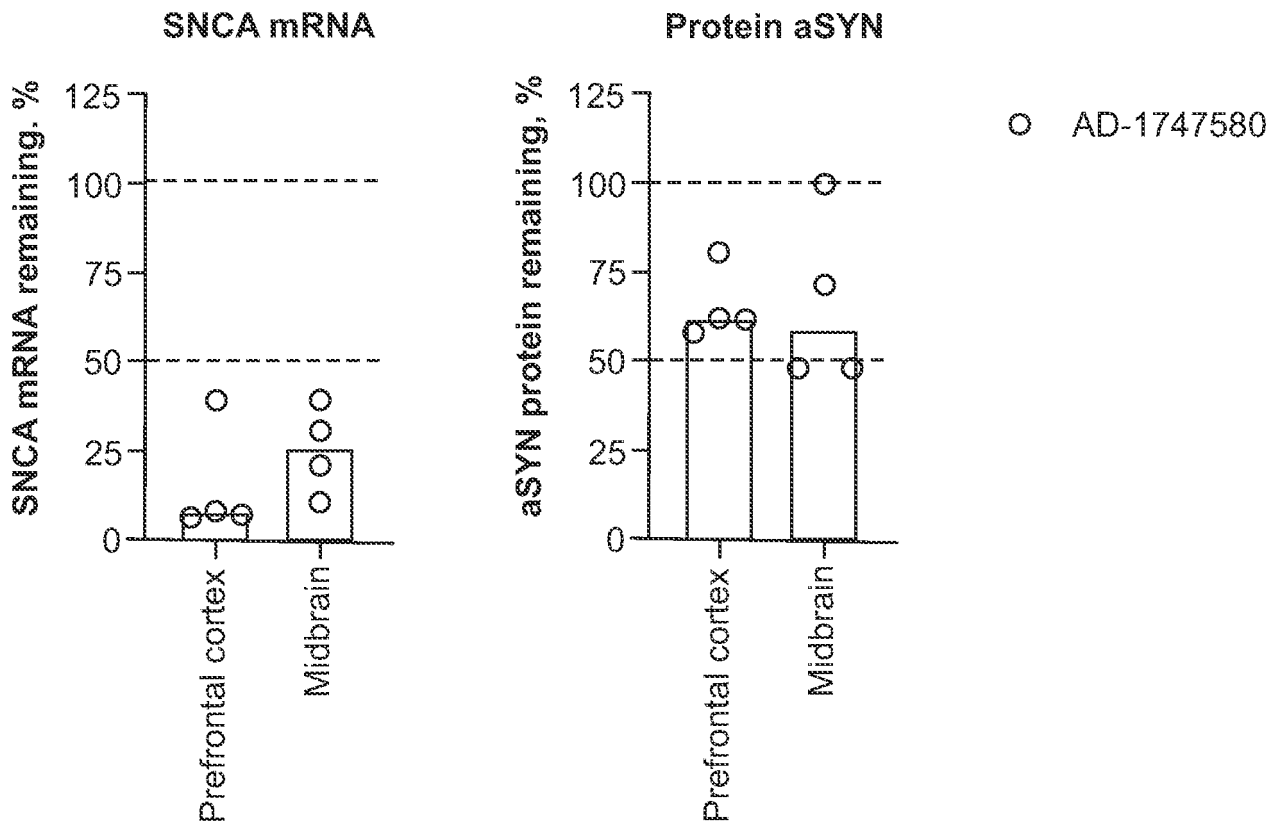
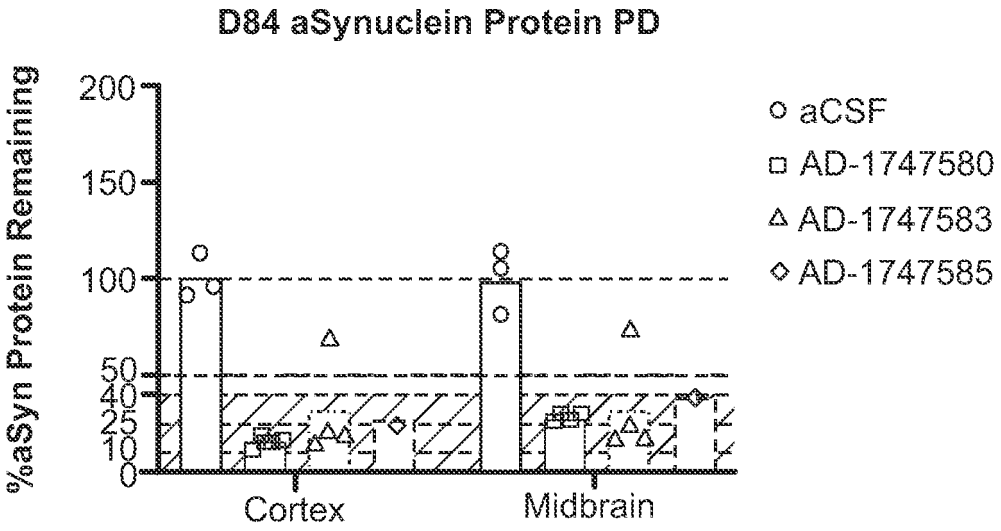
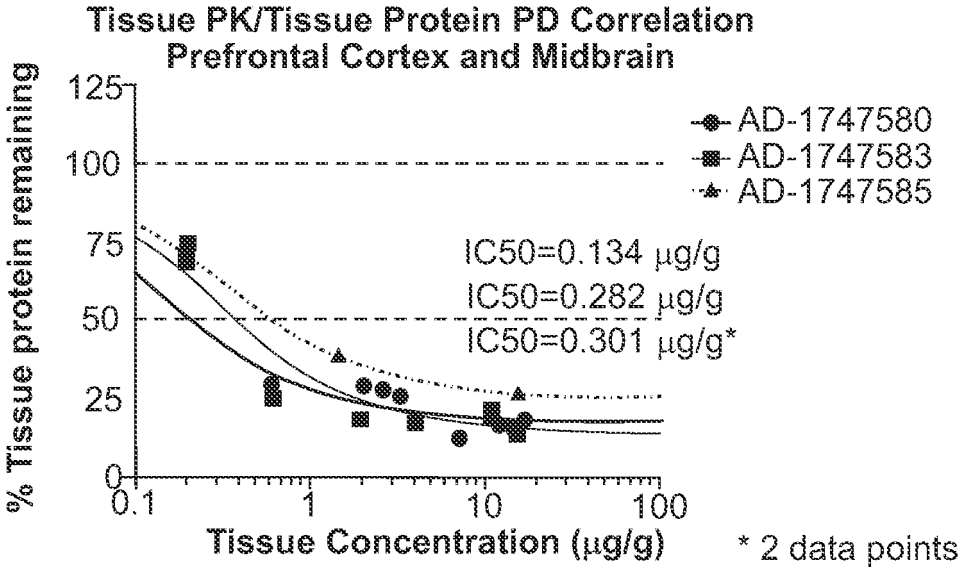
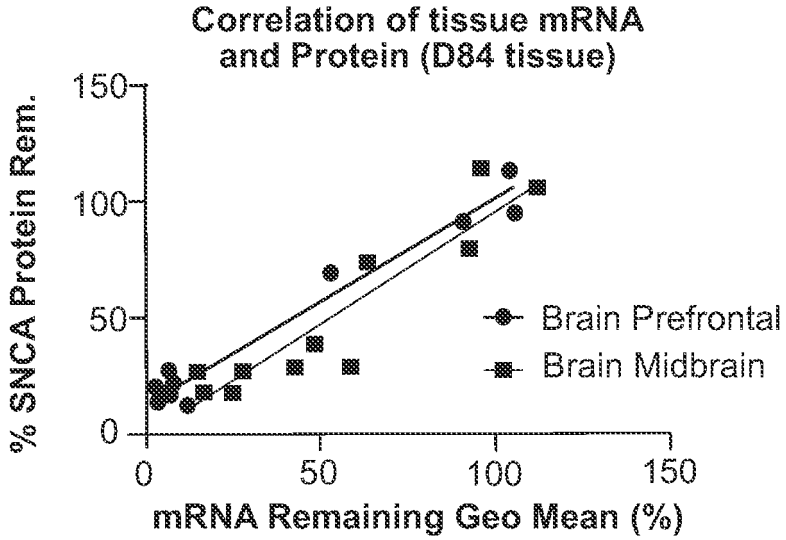

SNCA mRNA
SNCA mRNA remaining, %
125
100
75
50
25
0
Prefrontal cortex
Midbrain
Protein aSYN
aSYN protein remaining, %
125
100
75
50
25
0
Prefrontal cortex
Midbrain
AD-1747580

FIG. 14C







	Brain Prefrontal	Brain Midbrain
R squared	0.9685	0.8606

FIG. 14D

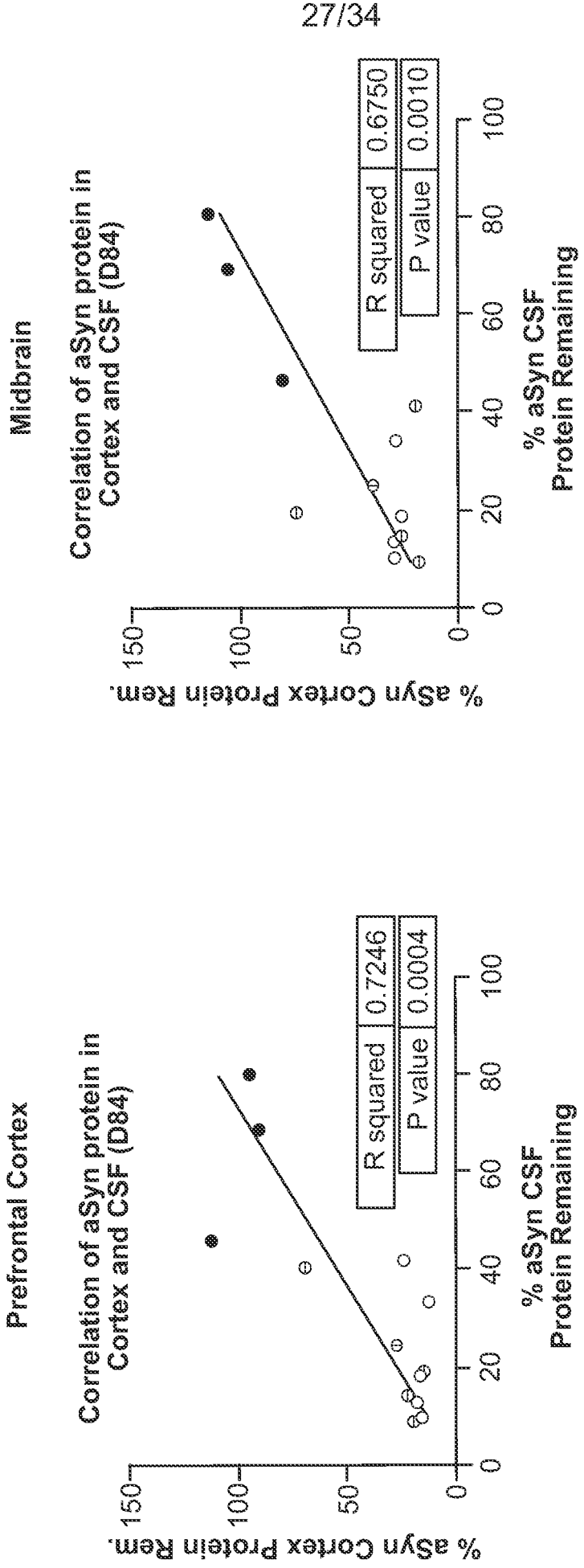

Midbrain
Correlation of aSyn protein in Cortex and CSF (D84)
% aSyn Cortex Protein Rem.
% aSyn CSF Protein Remaining
R squared 0.6750
P value 0.0010
Prefrontal Cortex
Correlation of aSyn protein in Cortex and CSF (D84)
% aSyn Cortex Protein Rem.
% aSyn CSF Protein Remaining
R squared 0.7246
P value 0.0004

FIG. 15

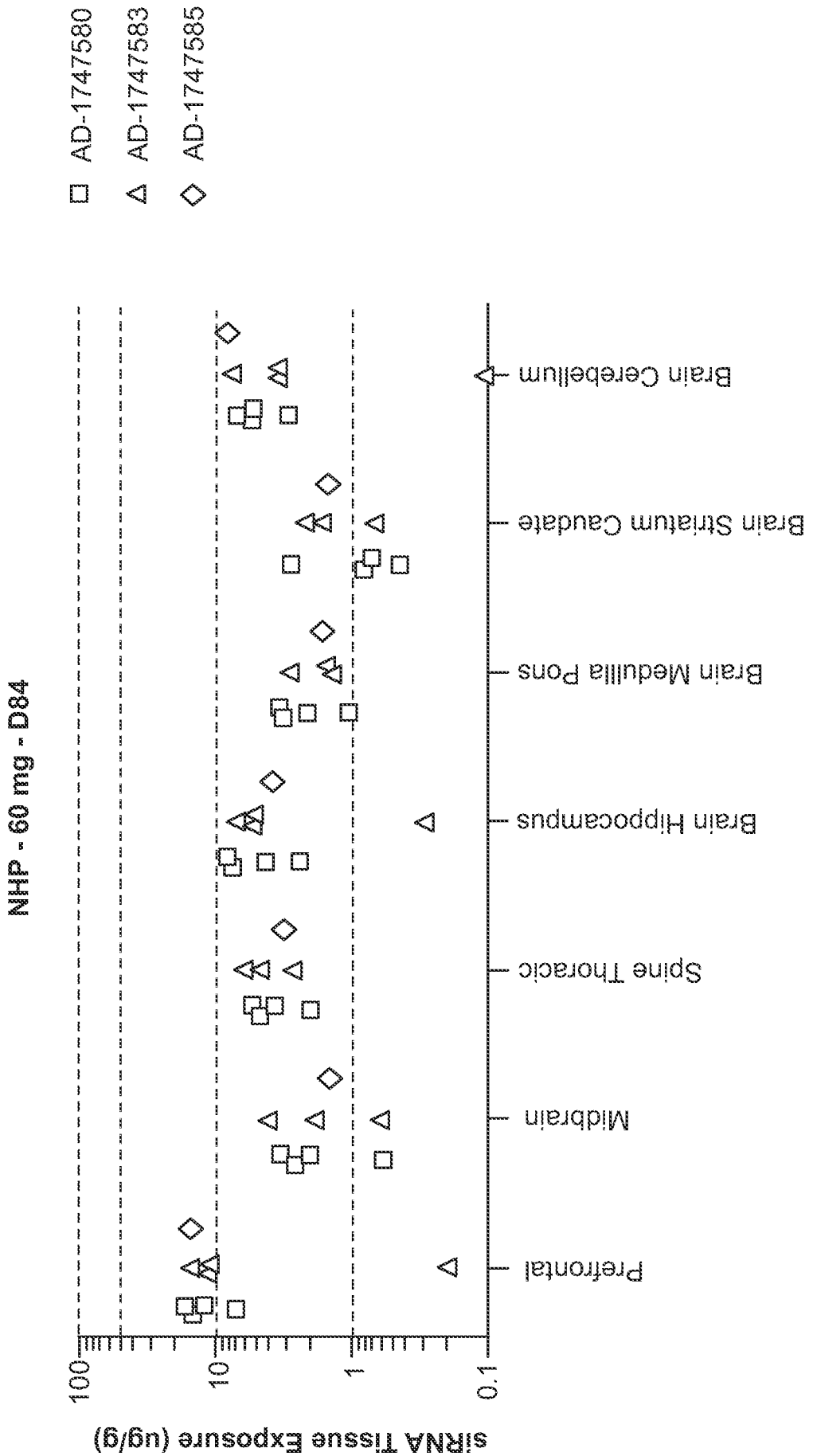
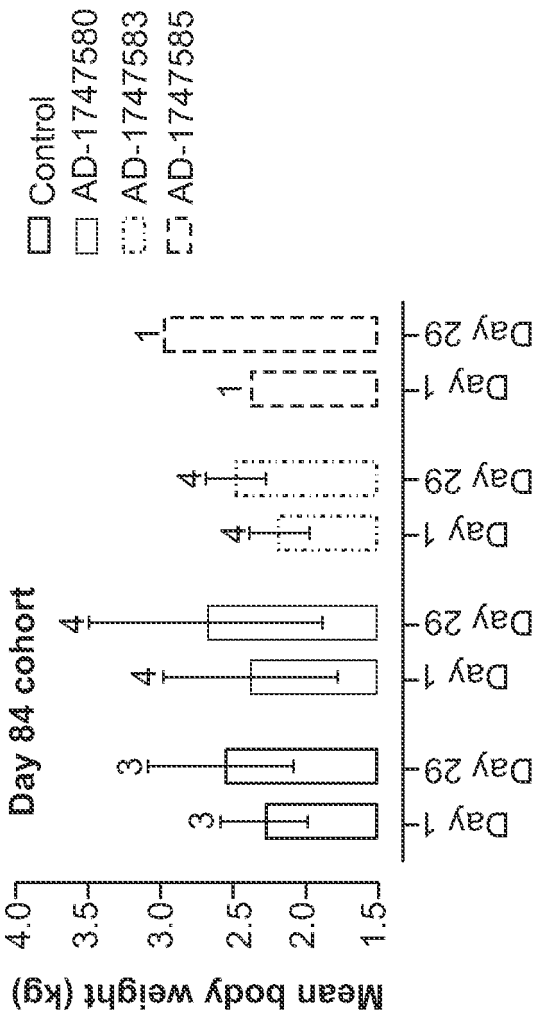

AD-1747580
AD-1747583
AD-1747585
NHP - 60 mg - D84
siRNA Tissue Exposure (ug/g)
100
10
1
0.1
Prefrontal
Midbrain
Spine Thoracic
Brain Hippocampus
Brain Medulla Pons
Brain Striatum Caudate
Brain Cerebellum

FIG. 16


Control
AD-1747580
AD-1747583
AD-1747585
Day 84 cohort
Mean body weight (kg)
4.0
3.5
3.0
2.5
2.0
1.5
Day 1
Day 29

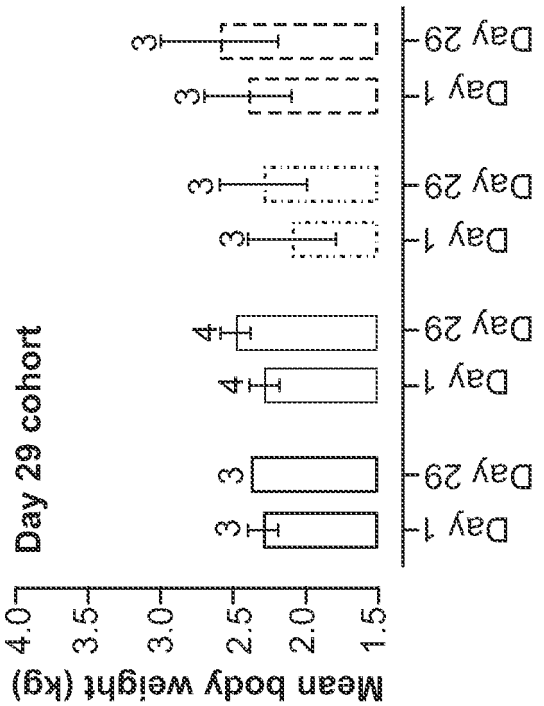

Day 29 cohort
Mean body weight (kg)
4.0
3.5
3.0
2.5
2.0
1.5
Day 1
Day 29

FIG. 17

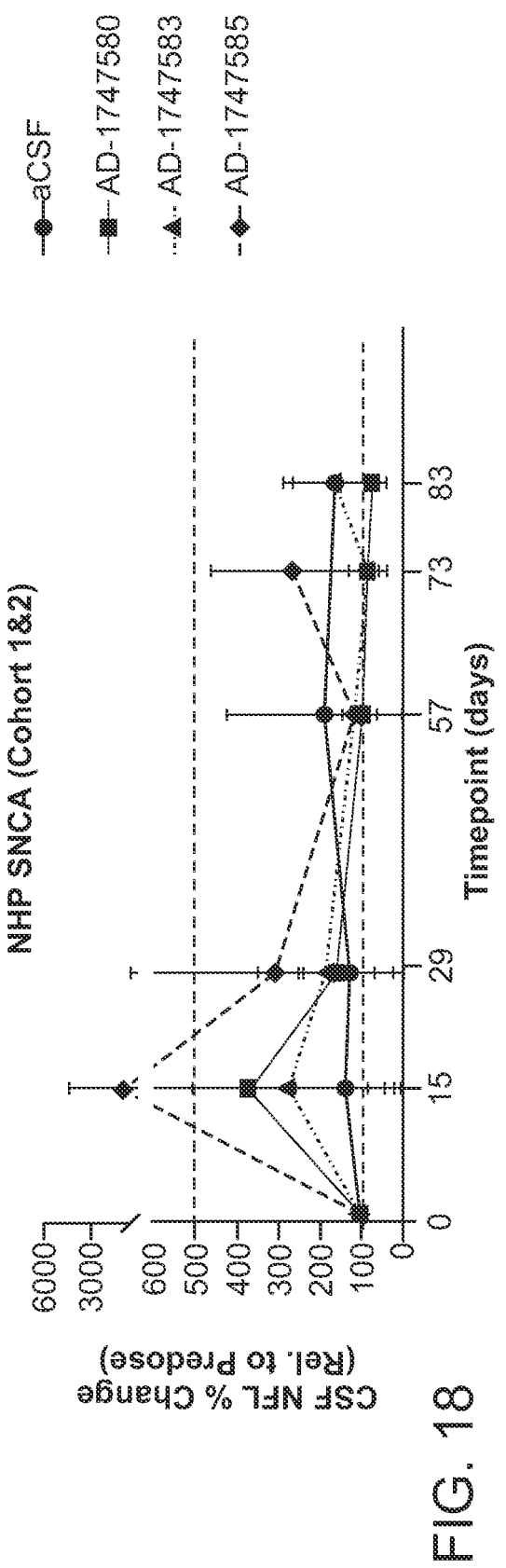

aCSF
AD-1747580
AD-1747583
AD-1747585
NHP SNCA (Cohort 1&2)
CSF NFL % Change
(Rel. to Predose)
6000
3000
600
500
400
300
200
100
0
0
15
29
57
73
83
Timepoint (days)

FIG. 18

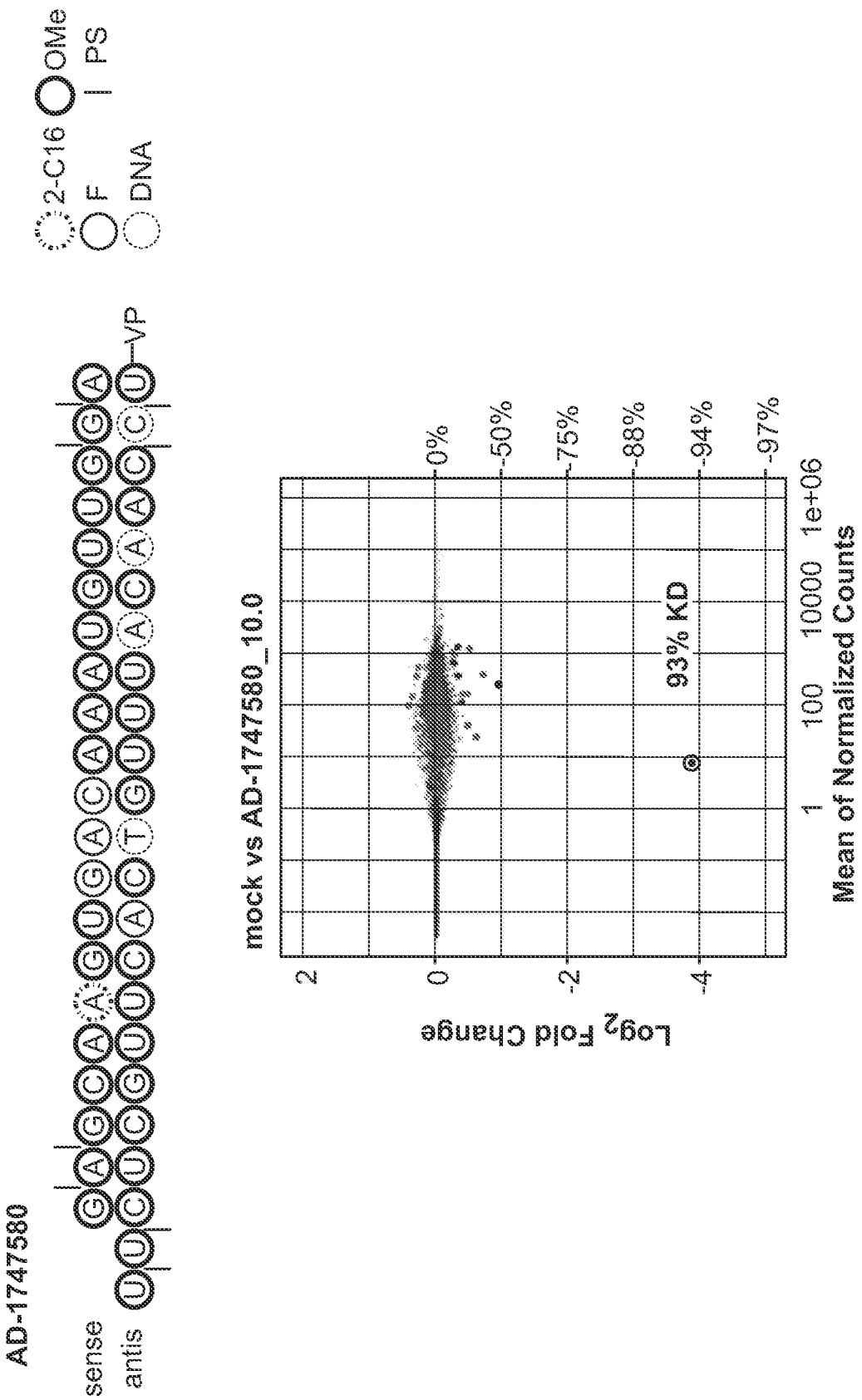

AD-1747580
sense
antis
2-C16
OMe
F
PS
DNA
VP
mock vs AD-1747580_10.0
Log2 Fold Change
Mean of Normalized Counts
93% KD
0%
-50%
-75%
-88%
-94%
-97%
2
0
-2
-4
1
100
10000
1e+06

FIG. 19A

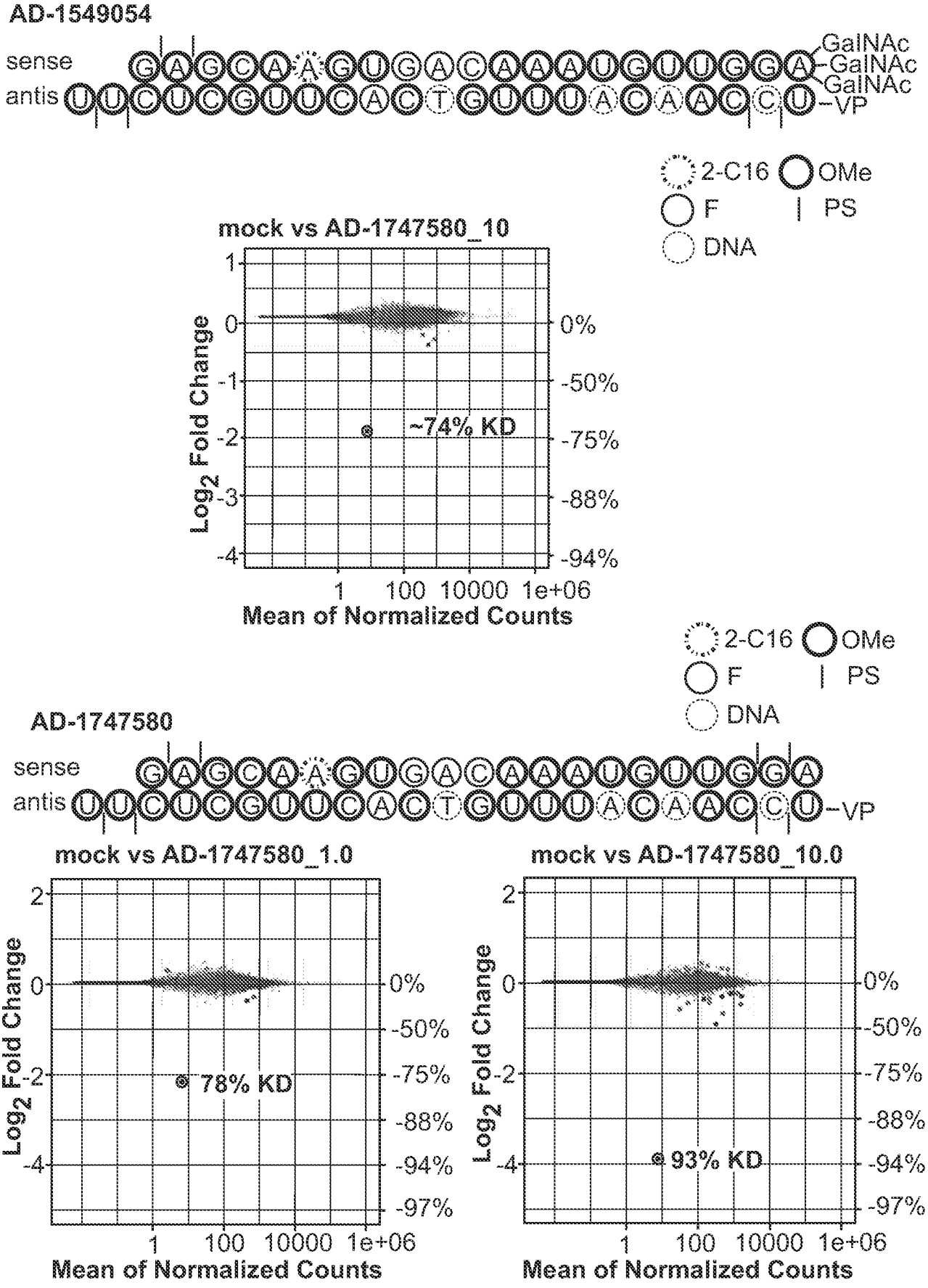

AD-1549054
sense
antis
GalNAc
GalNAc
GalNAc
VP
2-C16
OMe
F
PS
DNA
mock vs AD-1747580_10
Log2 Fold Change
~74% KD
0%
-50%
-75%
-88%
-94%
Mean of Normalized Counts
AD-1747580
mock vs AD-1747580_1.0
78% KD
mock vs AD-1747580_10.0
93% KD
-97%

FIG. 19B

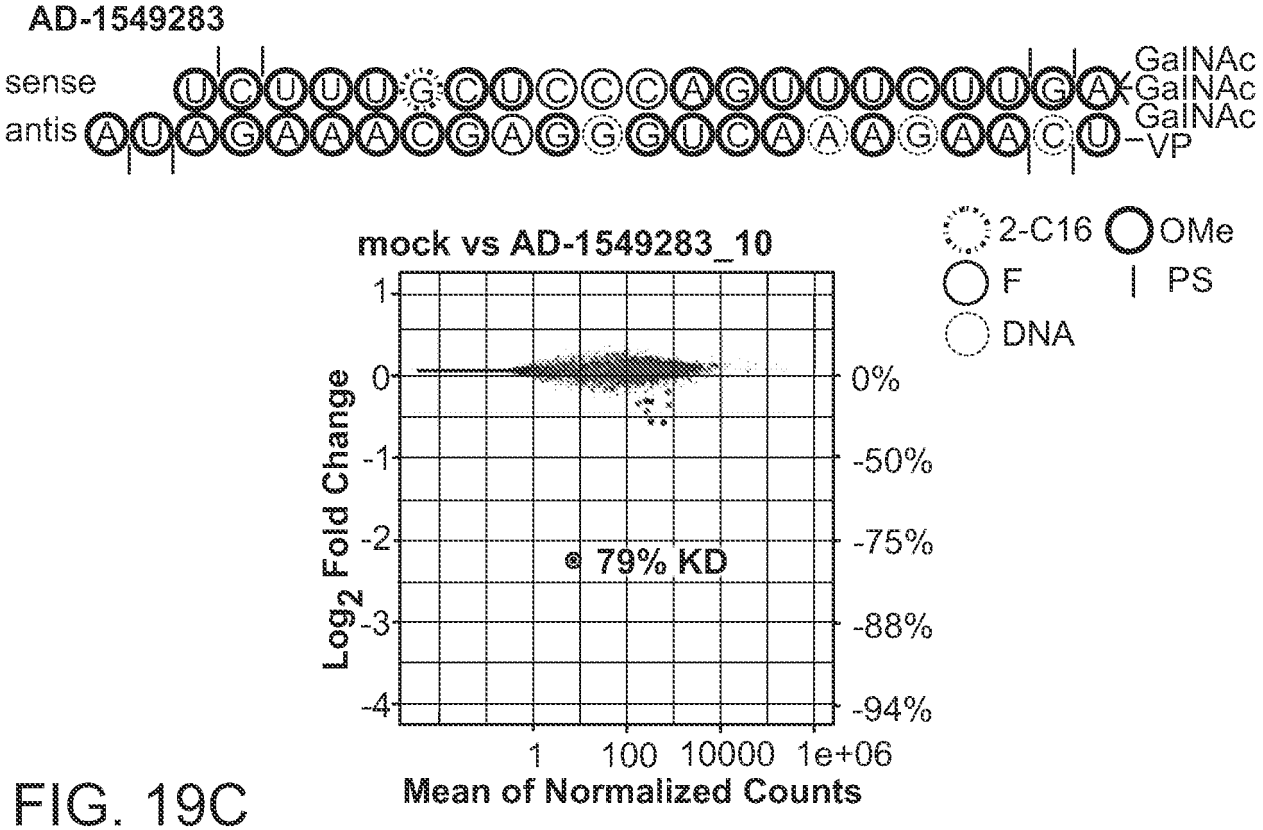

AD-1549283
sense
antis
GalNAc
GalNAc
GalNAc
VP
2-C16
OMe
F
PS
DNA
mock vs AD-1549283_10
Log2 Fold Change
1
0
-1
-2
-3
-4
0%
-50%
-75%
-88%
-94%
79% KD
1
100
10000
1e+06
Mean of Normalized Counts

FIG. 19C

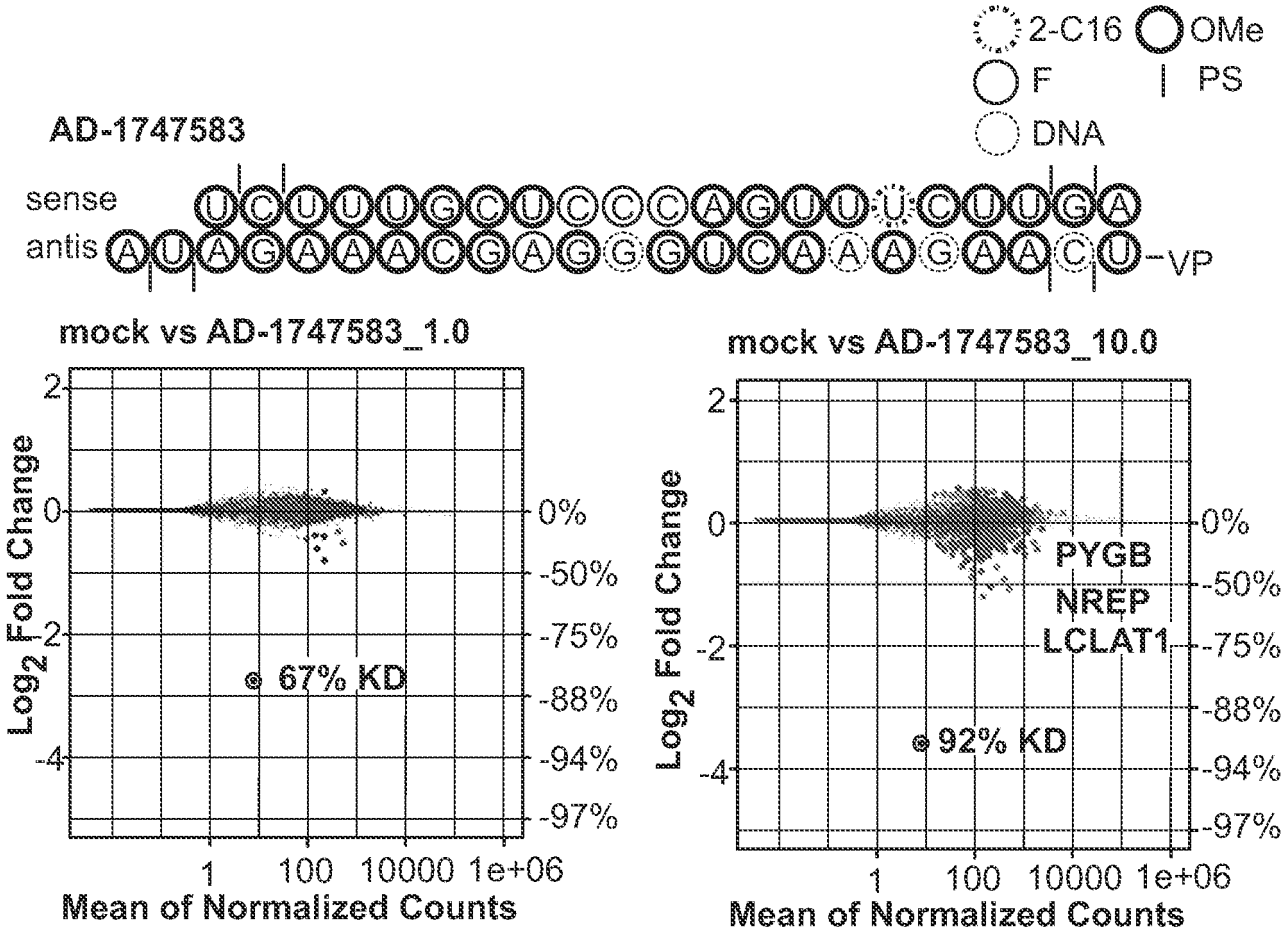
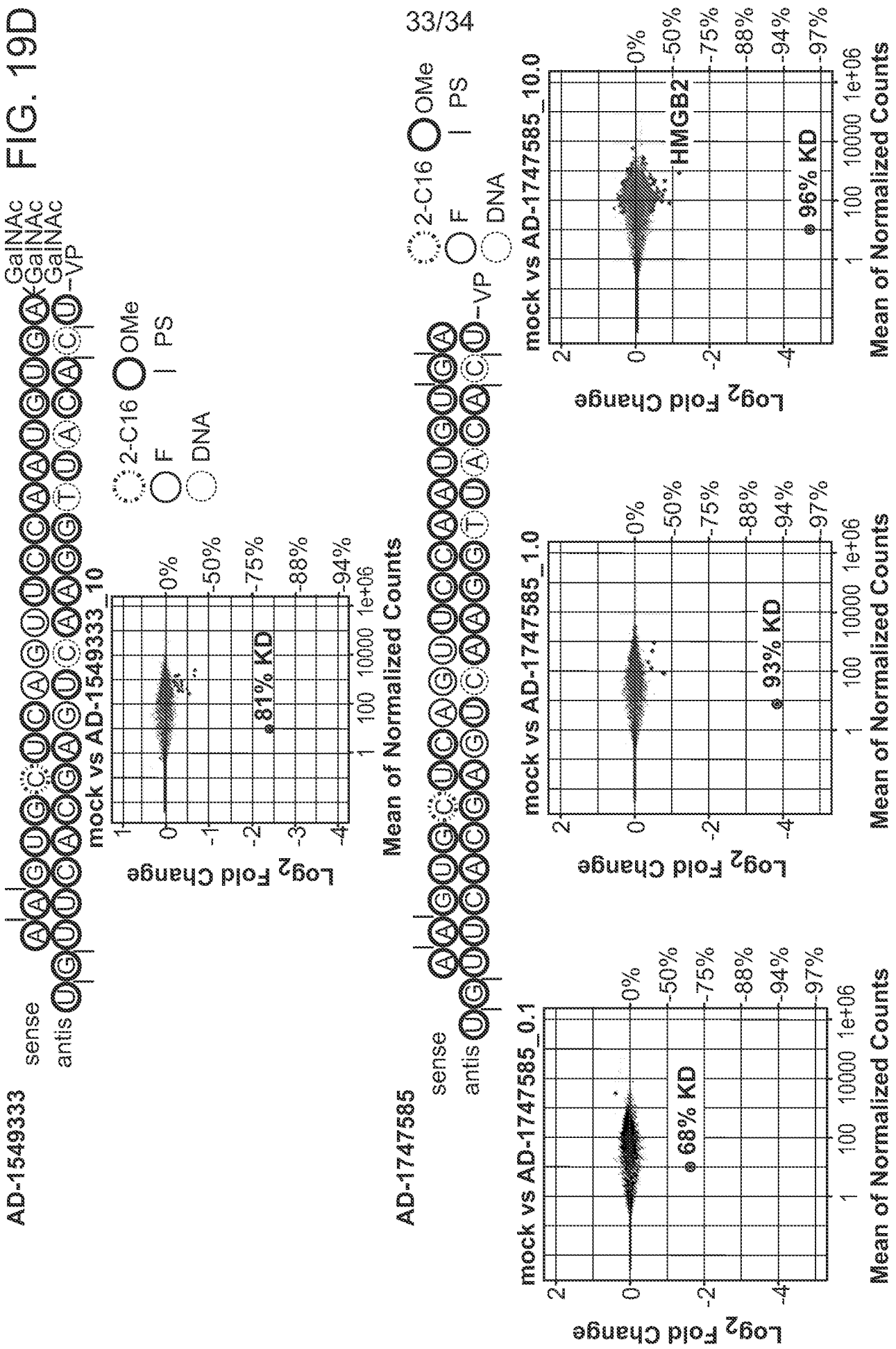

2-C16
OMe
F
PS
DNA
AD-1747583
sense
antis
VP
mock vs AD-1747583_1.0
Log2 Fold Change
2
0
-2
-4
0%
-50%
-75%
-88%
-94%
-97%
67% KD
1
100
10000
1e+06
Mean of Normalized Counts
mock vs AD-1747583_10.0
PYGB
NREP
LCLAT1
92% KD

FIG. 19D


AD-1549333
sense
antis
GalNAc
GalNAc
GalNAc
-VP
2-C16
OMe
F
PS
DNA
mock vs AD-1549333_10
81% KD
Log2 Fold Change
Mean of Normalized Counts
AD-1747585
mock vs AD-1747585_0.1
68% KD
mock vs AD-1747585_1.0
93% KD
mock vs AD-1747585_10.0
HMGB2
96% KD
0%
-50%
-75%
-88%
-94%
-97%

		mRNA		Protein		Protein (redosed)		RNAseq	
Identifier	Target sequence Location	Maximal % knockdown **Cortex** D84	Maximal % knockdown **Midbrain** D84	Maximal % KD **CSF** D83/D85	Mean % KD **Cortex** D84	Mean % KD **Midbrain** D84	Mean % KD** **Cortex** D85	Mean % KD** **Midbrain** D85	Dysregulated Genes >50%
AD-1747585	UTR	93.1	51.5	75.5	72.7	61.3	64.0	62.0	1
AD-1747583	UTR	96.6	83.7	91.2	98.5	66.0	70.0	57.3	3
AD-1747580	CDS	**94.4**	**85.4**	**90.4**	**84.1**	**71.9**	**84.9**	**69.5**	0

FIG. 20