FIG. 1

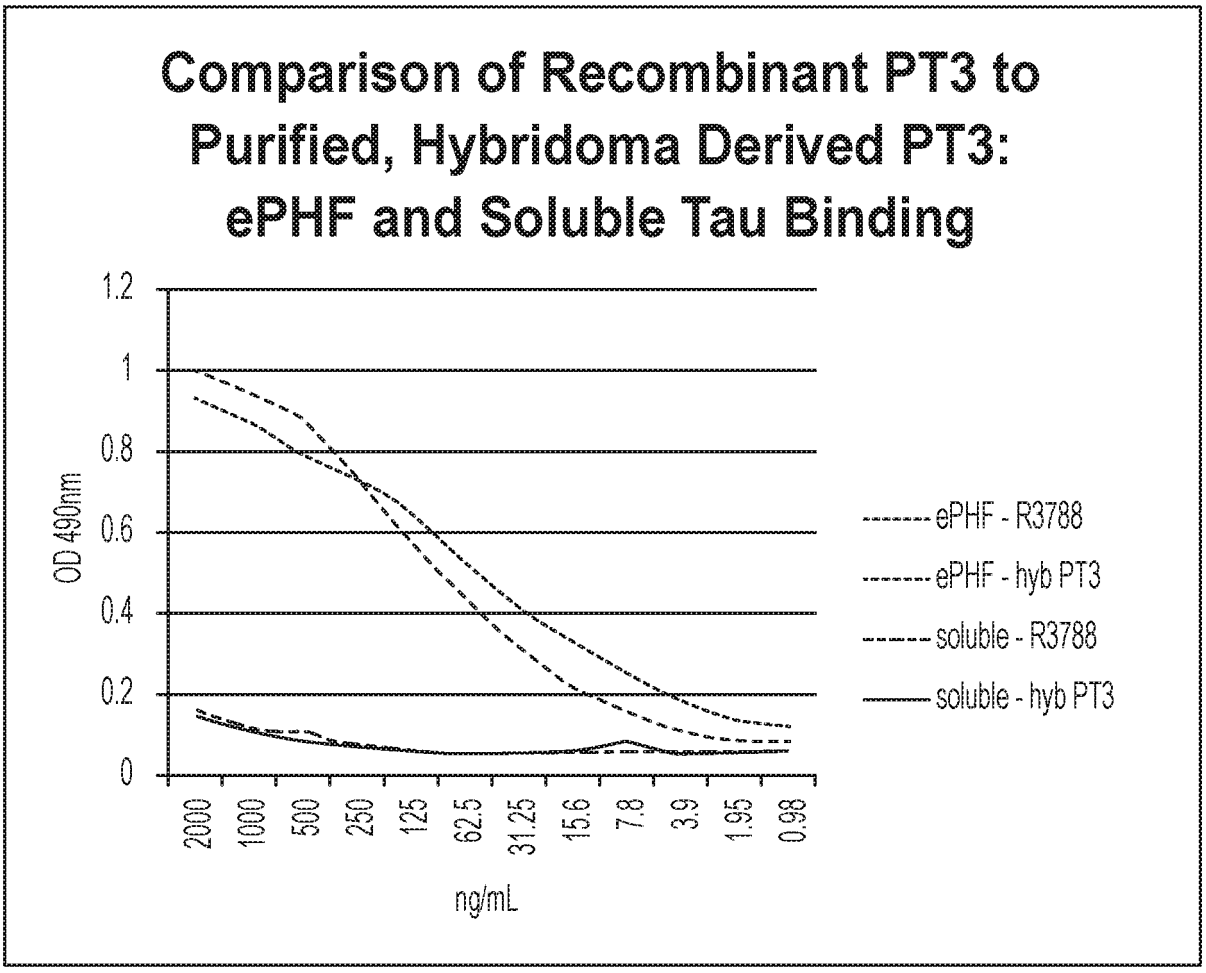

Comparison of Recombinant PT3 to Purified, Hybridoma Derived PT3: ePHF and Soluble Tau Binding
OD 490nm
1.2
1
0.8
0.6
0.4
0.2
0
2000
1000
500
250
125
62.5
31.25
15.6
7.8
3.9
1.95
0.98
ng/mL
ePHF - R3788
ePHF - hyb PT3
soluble - R3788
soluble - hyb PT3

FIG. 2

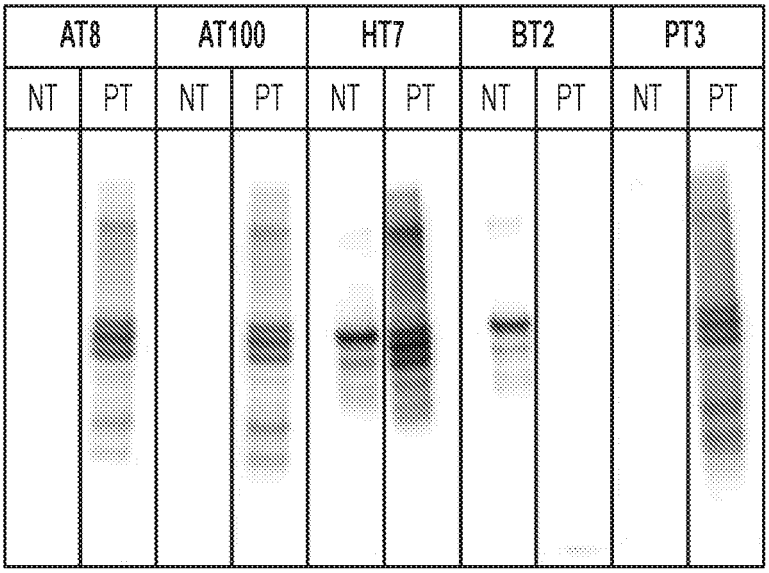


FIG. 3

FIG. 3A

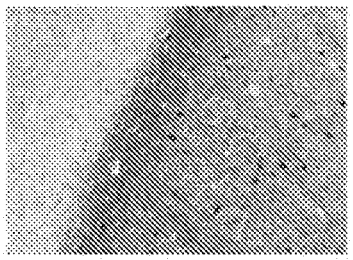


FIG. 3B

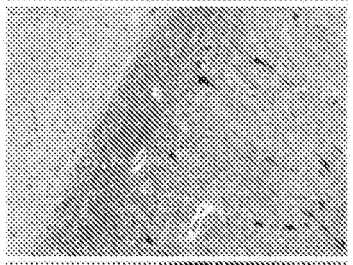


FIG. 3C

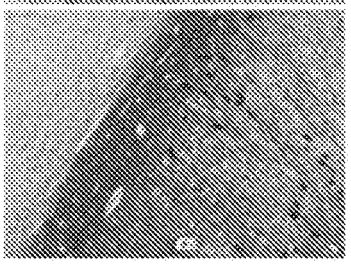


FIG. 3D

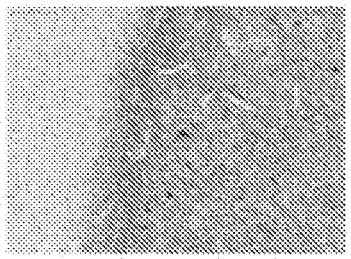


FIG. 3E

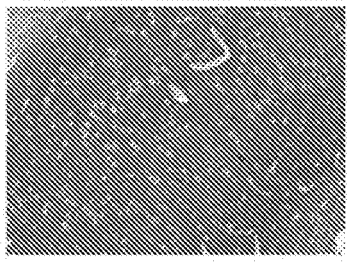


FIG. 4

FIG. 4A

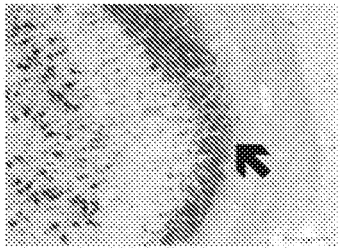


FIG. 4B

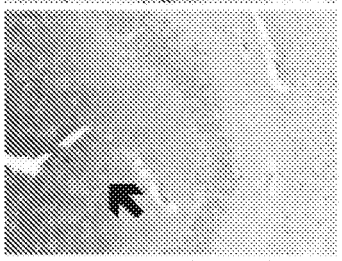


FIG. 4C

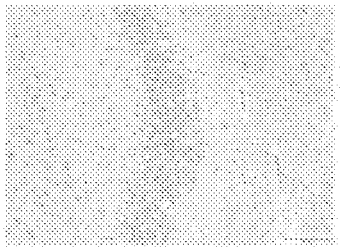


FIG. 4D

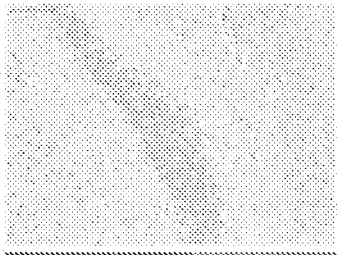


FIG. 4E

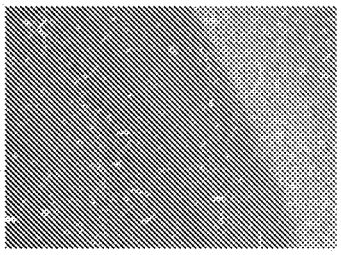


FIG. 5

FIG. 5A



FIG. 5B

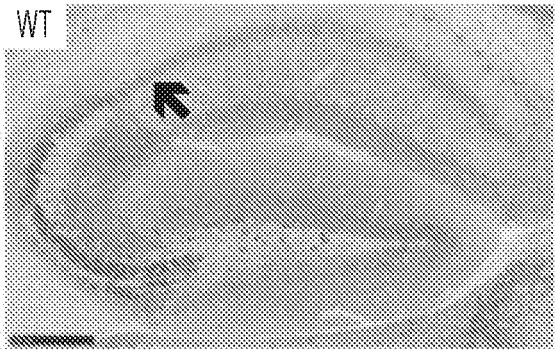


FIG. 6

FIG. 6A



FIG. 6B

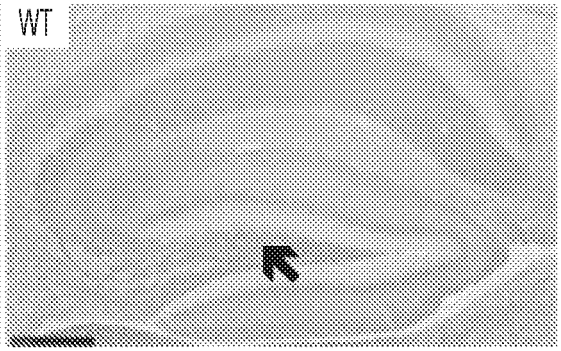


FIG. 7

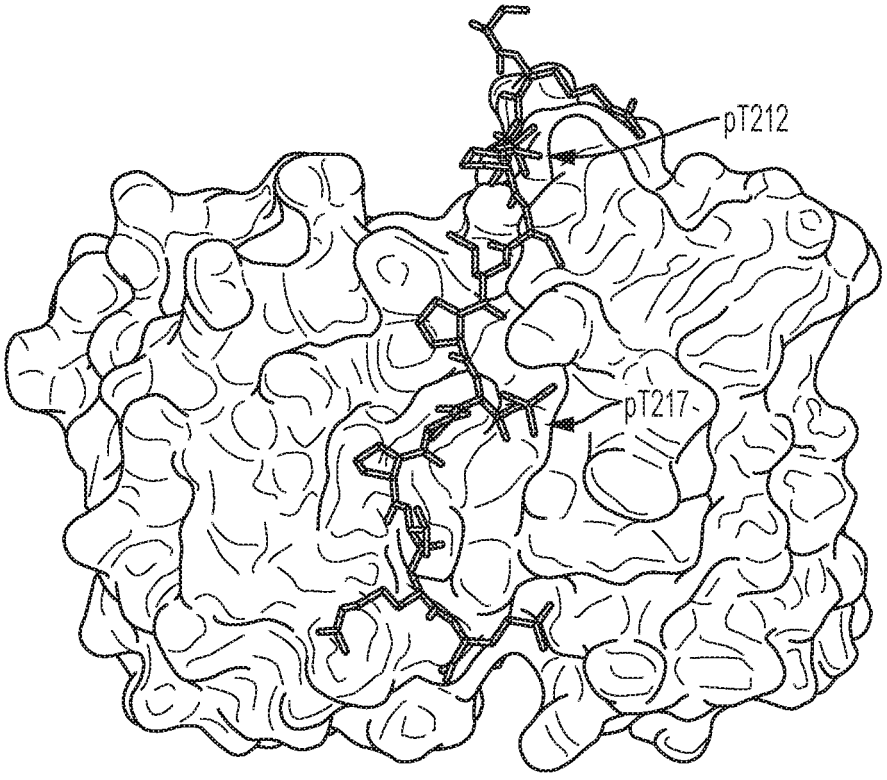


FIG. 8

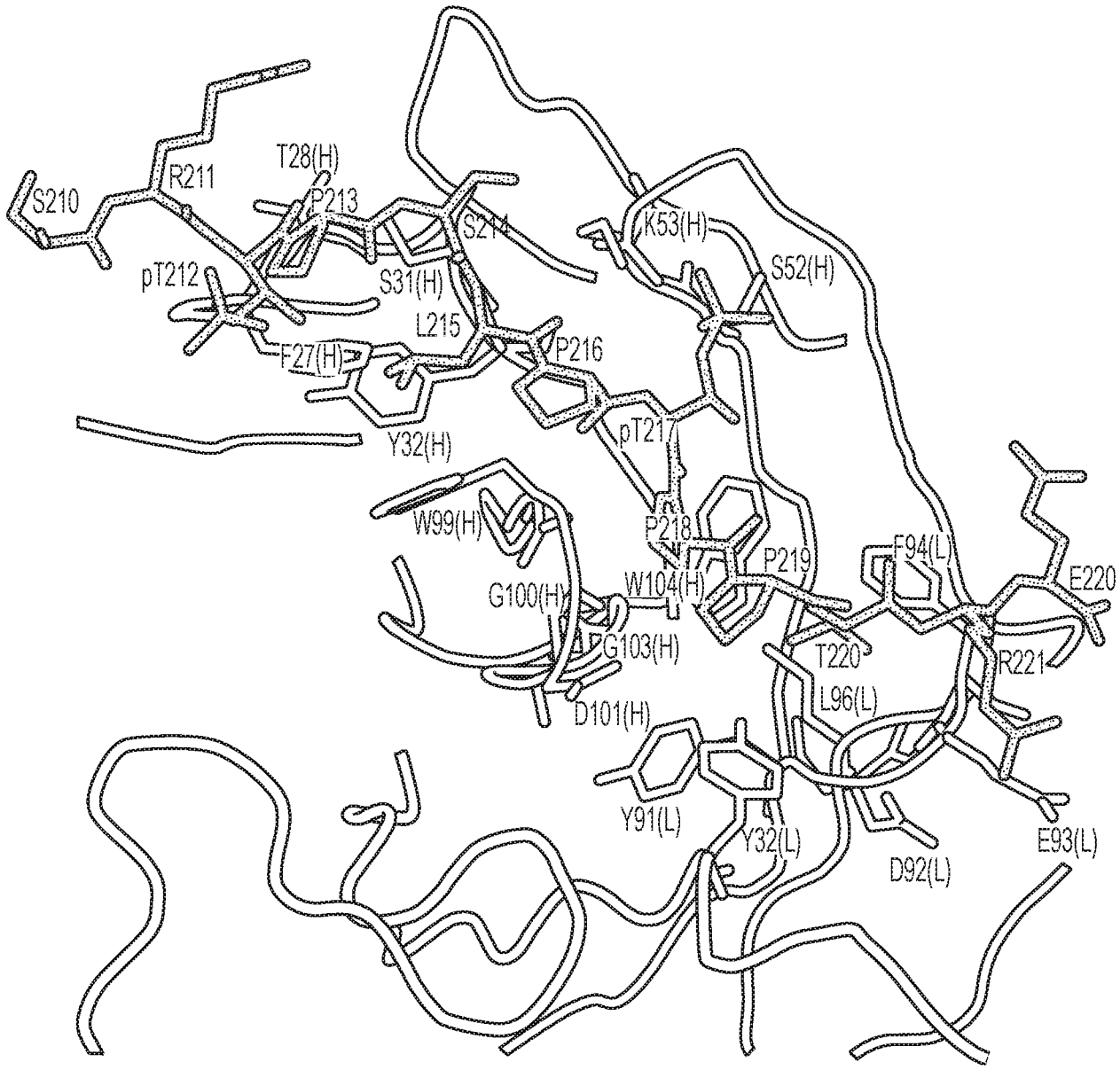

T28(H)
R211
S210
P213
S214
K53(H)
pT212
S31(H)
S52(H)
L215
F27(H)
P216
pT217
Y32(H)
W99(H)
P218
P219
F94(L)
E220
G100(H)
W104(H)
G103(H)
T220
R221
L96(L)
D101(H)
Y91(L)
Y32(L)
E93(L)
D92(L)

FIG. 9

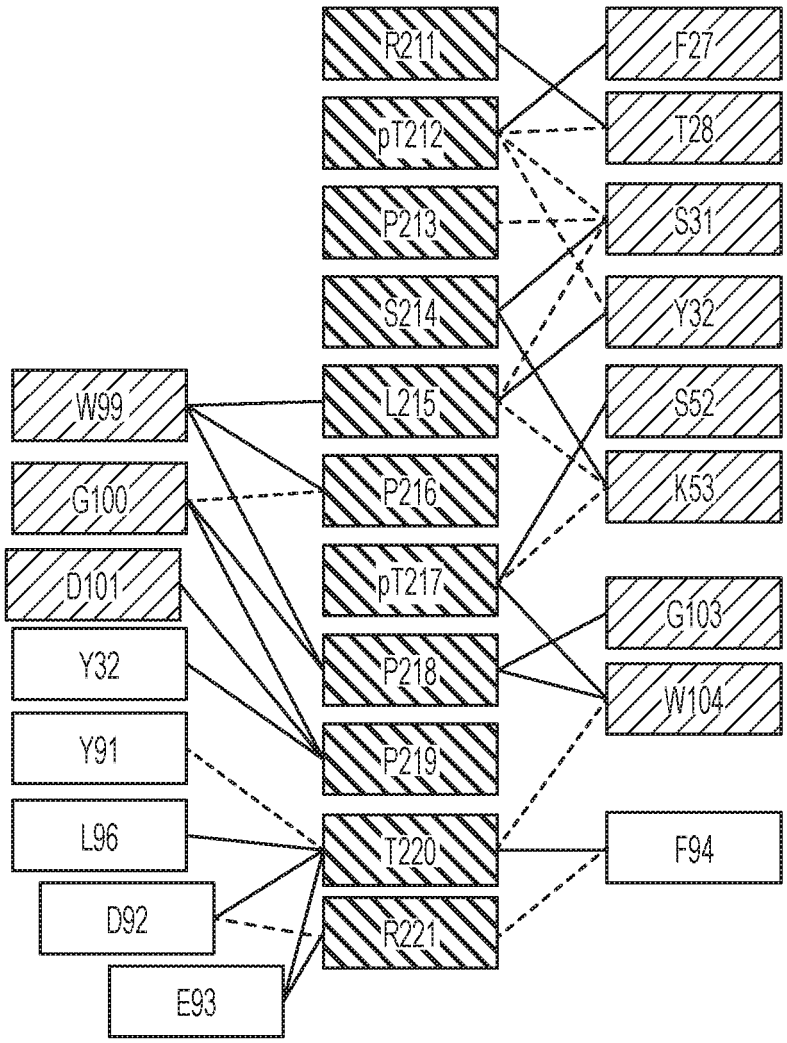

R211
pT212
P213
S214
L215
P216
pT217
P218
P219
T220
R221
F27
T28
S31
Y32
S52
K53
G103
W104
F94
W99
G100
D101
Y32
Y91
L96
D92
E93

FIG. 10

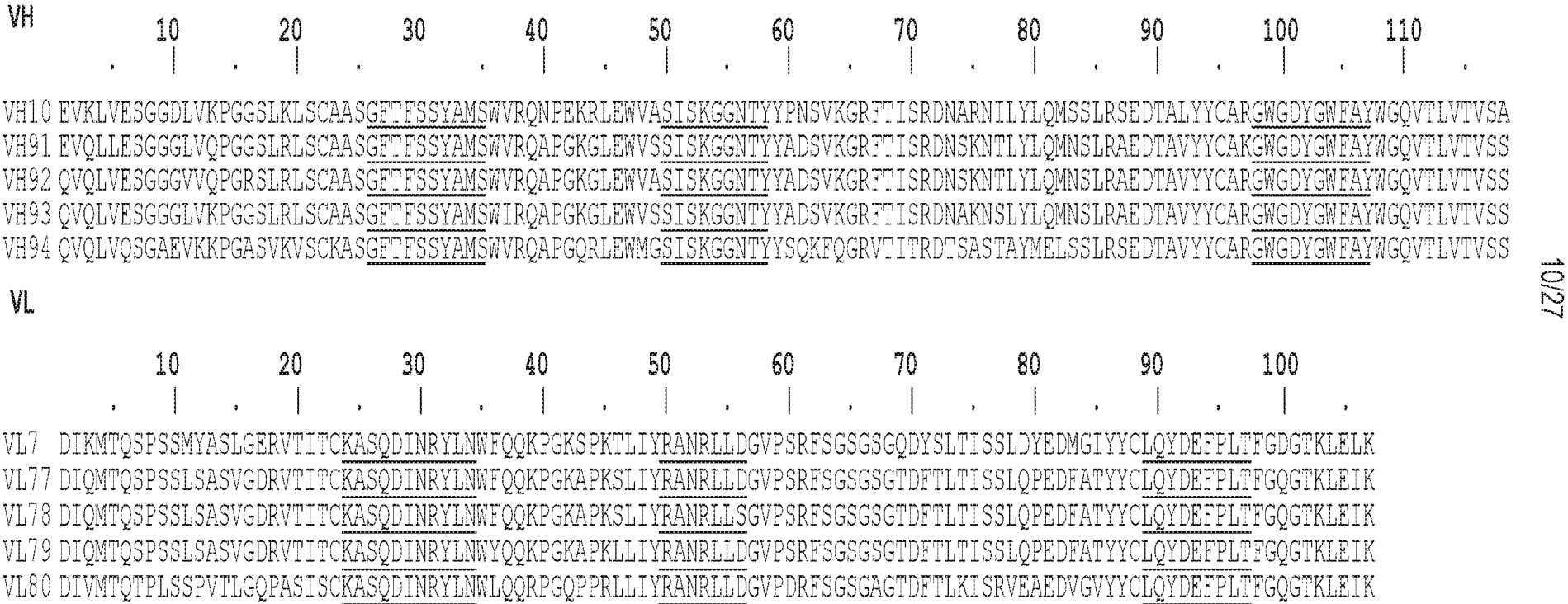

VH
10 20 30 40 50 60 70 80 90 100 110
VH10 EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYAMSWVRQNPEKRLEWVASISKGGNTYYPNSVKGRFTISRDNARNILYLQMSSLRSEDTALYYCARGWGDYGWFAYWGQVTLVTVSA
VH91 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSISKGGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWGDYGWFAYWGQVTLVTVSS
VH92 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVASISKGGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGWGDYGWFAYWGQVTLVTVSS
VH93 QVQLVESGGGLVKPGGSLRLSCAASGFTFSSYAMSWIRQAPGKGLEWVSSISKGGNTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGWGDYGWFAYWGQVTLVTVSS
VH94 QVQLVQSGAEVKKPGASVKVSCKASGFTFSSYAMSWVRQAPGQRLEWMGSISKGGNTYYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCARGWGDYGWFAYWGQVTLVTVSS
VL
10 20 30 40 50 60 70 80 90 100
VL7 DIKMTQSPSSMYASLGERVTITCKASQDINRYLNWFQQKPGKSPKTLIYRANRLLDGVPSRFSGSGSGQDYSLTISSLDYEDMGIYYCLQYDEFPLTFGDGTKLELK
VL77 DIQMTQSPSSLSASVGDRVTITCKASQDINRYLNWFQQKPGKAPKSLIYRANRLLDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYDEFPLTFGQGTKLEIK
VL78 DIQMTQSPSSLSASVGDRVTITCKASQDINRYLNWFQQKPGKAPKSLIYRANRLLSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYDEFPLTFGQGTKLEIK
VL79 DIQMTQSPSSLSASVGDRVTITCKASQDINRYLNWYQQKPGKAPKLLIYRANRLLDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQYDEFPLTFGQGTKLEIK
VL80 DIVMTQTPLSSPVTLGQPASISCKASQDINRYLNWLQQRPGQPPRLLIYRANRLLDGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQYDEFPLTFGQGTKLEIK

FIG. 11



FIG. 12

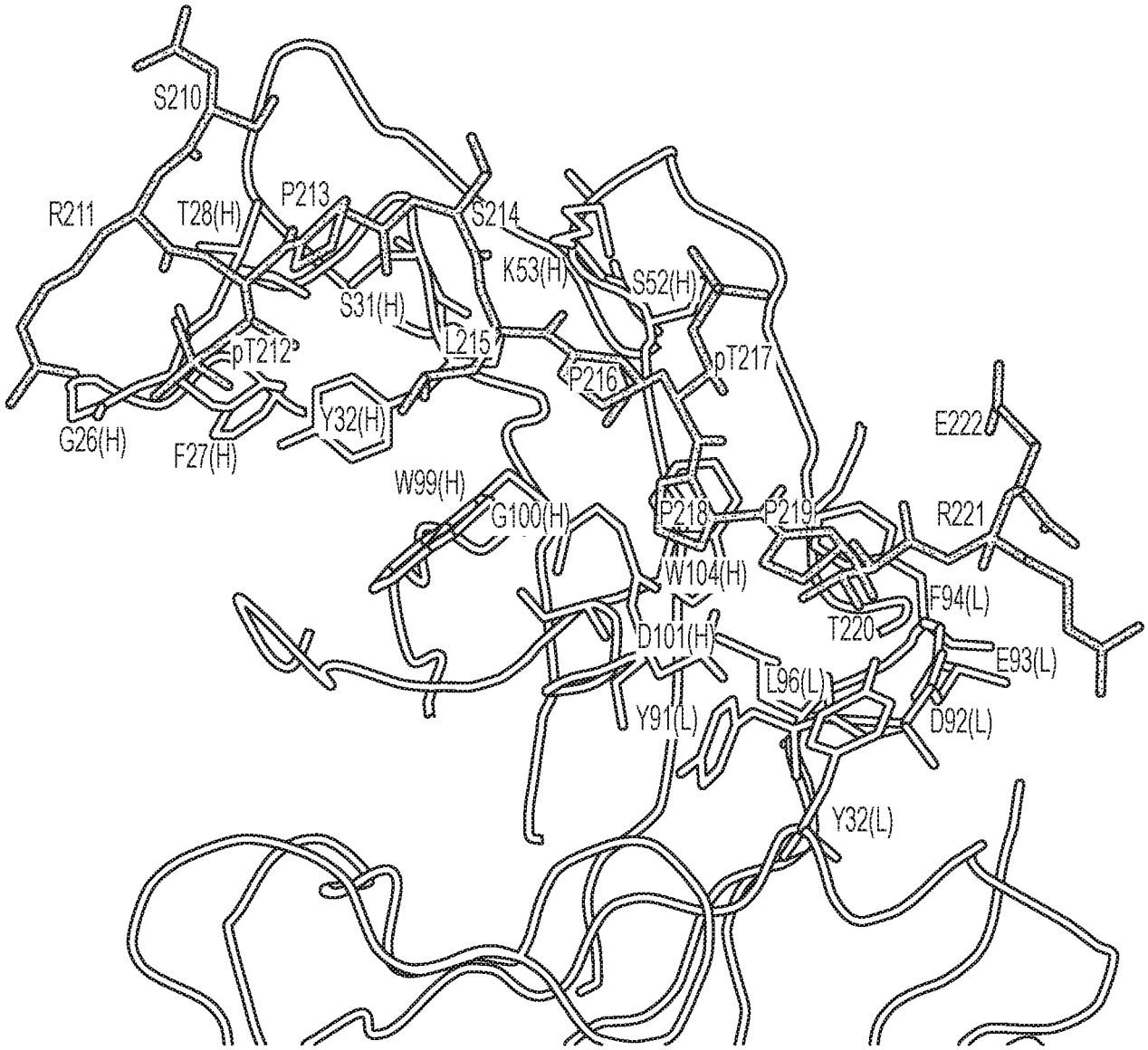

S210
R211
T28(H)
P213
S214
K53(H)
S52(H)
S31(H)
L215
pT212
P216
pT217
Y32(H)
G26(H)
F27(H)
E222
W99(H)
G100(H)
P218
P219
R221
W104(H)
F94(L)
T220
D101(H)
E93(L)
L96(L)
Y91(L)
D92(L)
Y32(L)

FIG. 13

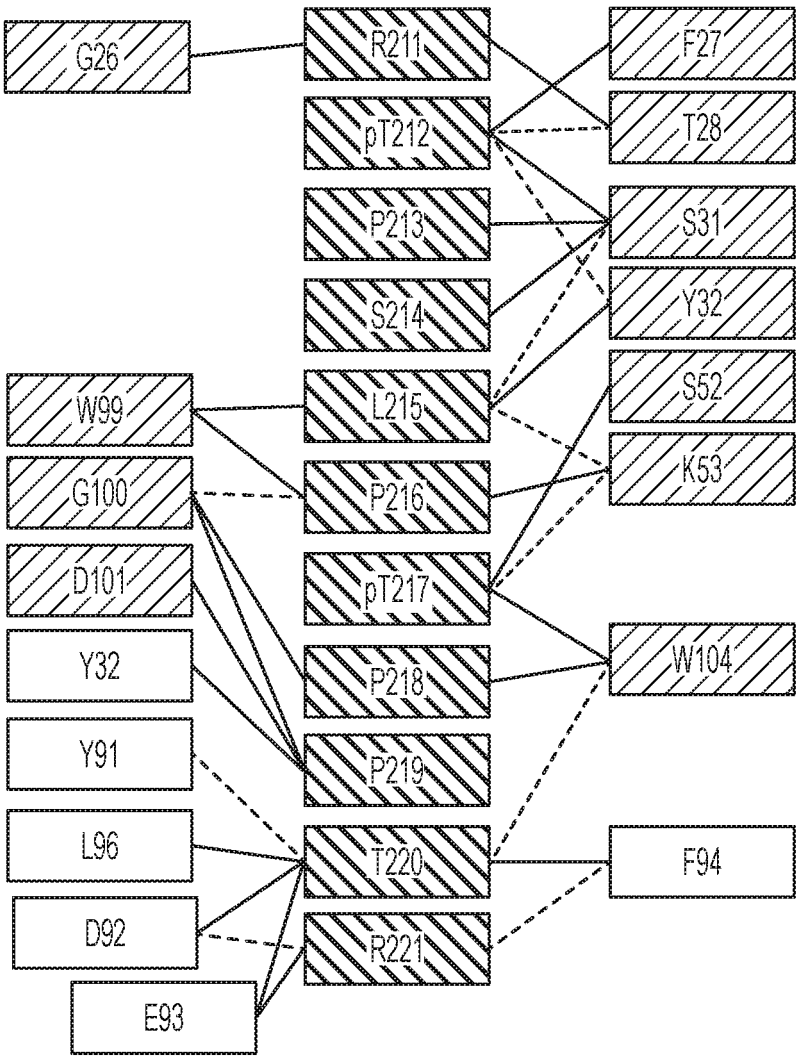

G26
R211
F27
pT212
T28
P213
S31
S214
Y32
W99
L215
S52
G100
P216
K53
D101
pT217
Y32
P218
W104
Y91
P219
L96
T220
F94
D92
R221
E93

FIG. 14

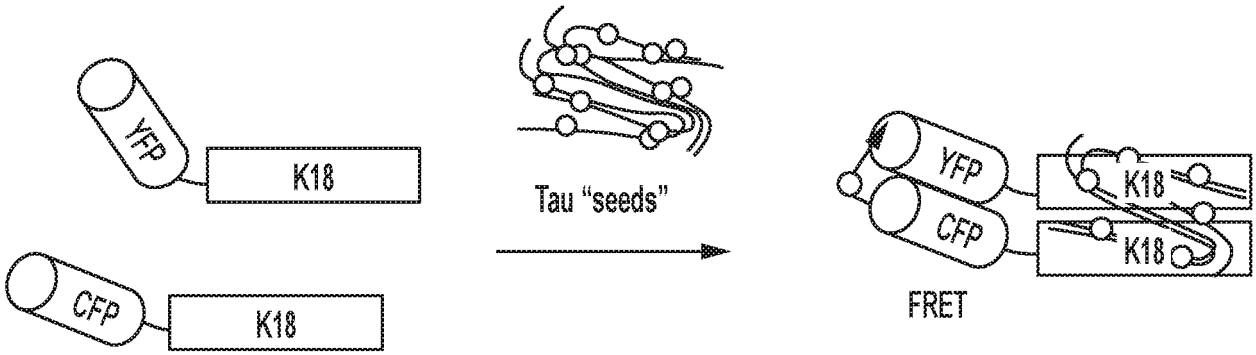


FIG. 15

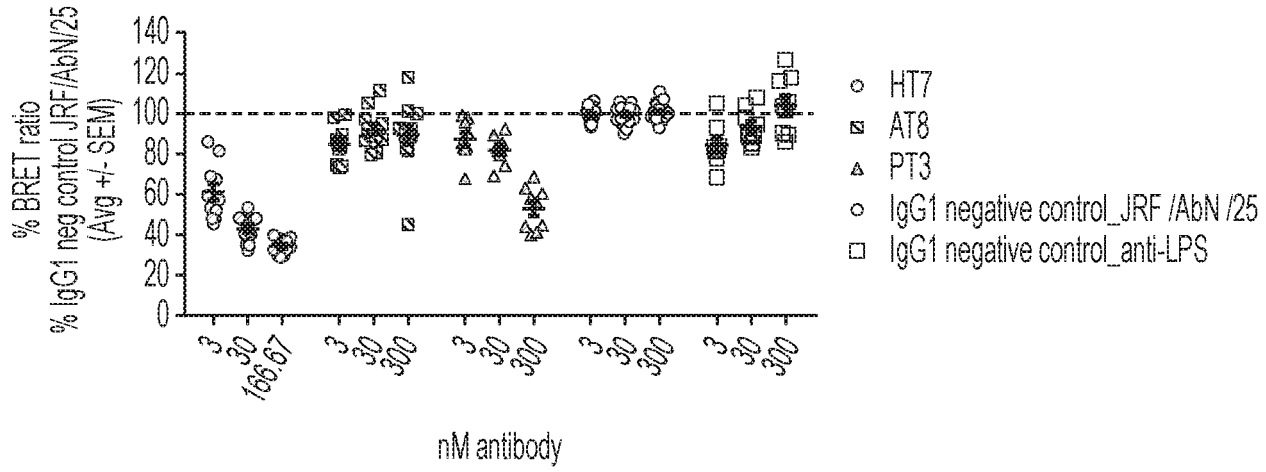

% BRET ratio
% IgG1 neg control JRF/AbN/25
(Avg +/- SEM)
140
120
100
80
60
40
20
0
3
30
166.67
3
30
300
3
30
300
3
30
300
3
30
300
nM antibody
HT7
AT8
PT3
IgG1 negative control_JRF /AbN /25
IgG1 negative control_anti-LPS

FIG. 16

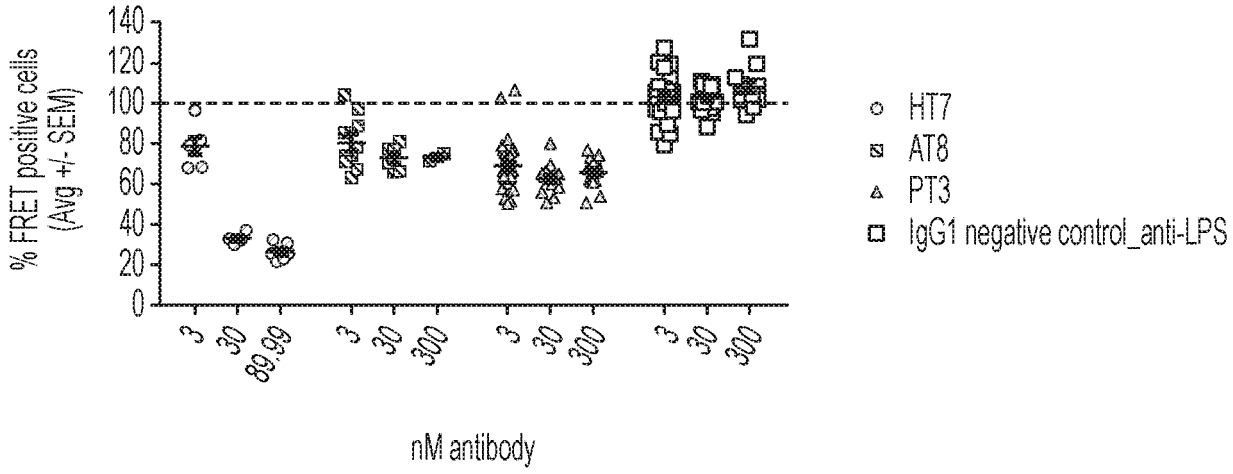

% FRET positive cells
(Avg +/- SEM)
140
120
100
80
60
40
20
0
3
30
89.99
3
30
300
3
30
300
3
30
300
nM antibody
HT7
AT8
PT3
IgG1 negative control_anti-LPS

FIG. 17

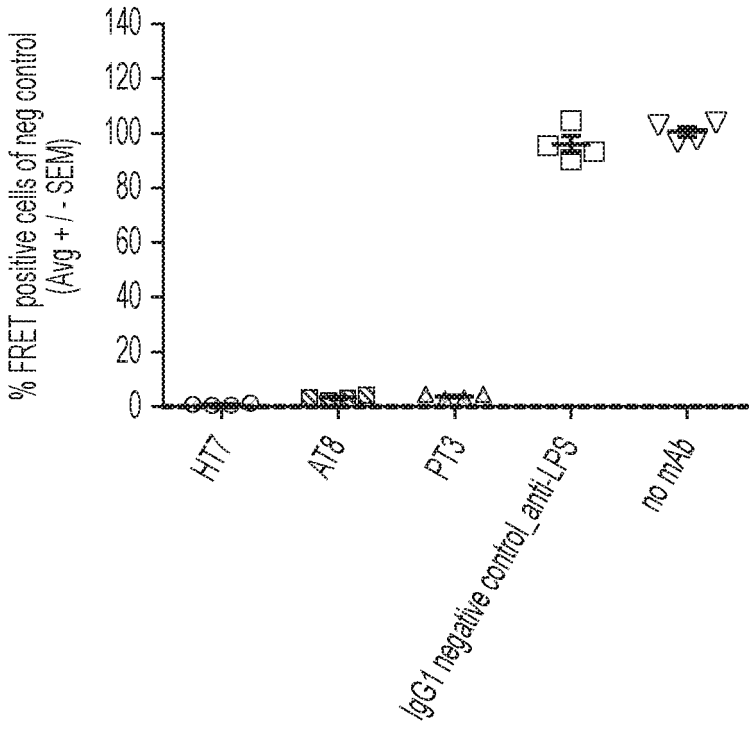

% FRET positive cells of neg control
(Avg + / - SEM)
140
120
100
80
60
40
20
0
HT7
AT8
PT3
IgG1 negative control_anti-LPS
no mAb

FIG. 18

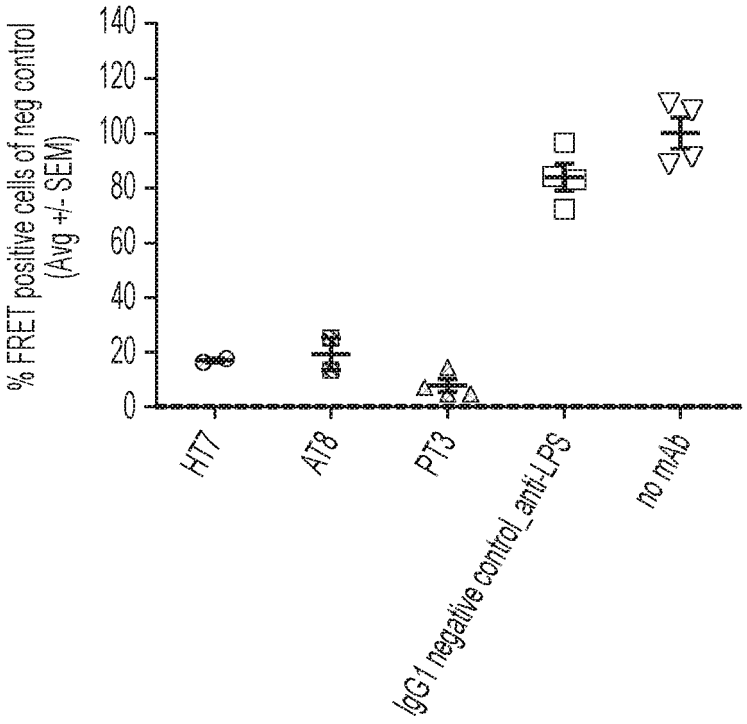
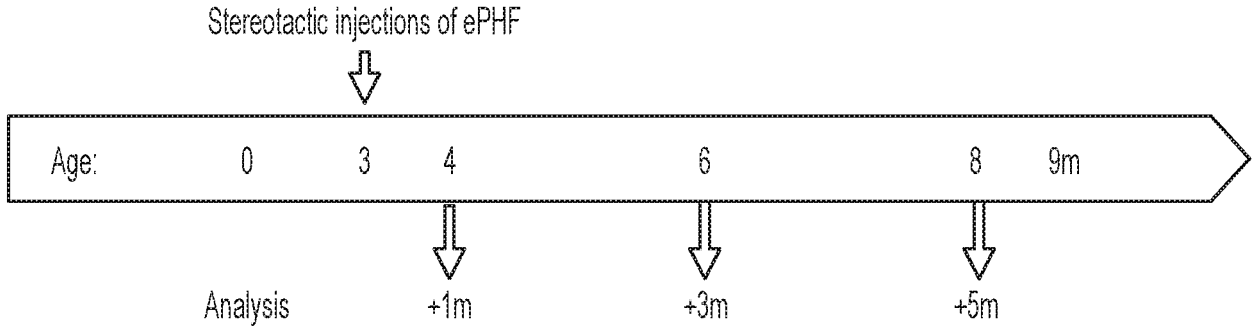

% FRET positive cells of neg control
(Avg +/- SEM)
140
120
100
80
60
40
20
0
HT7
AT8
PT3
IgG1 negative control_anti-LPS
no mAb

FIG. 19



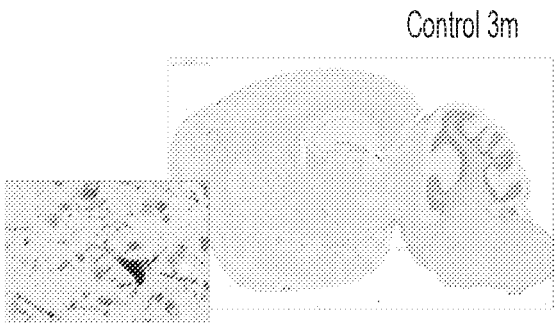


FIG. 19A

FIG. 19B

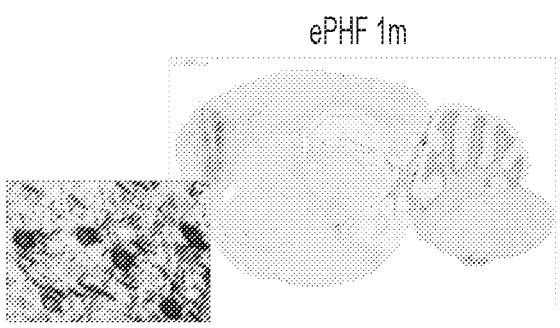


FIG. 19C

FIG. 19D

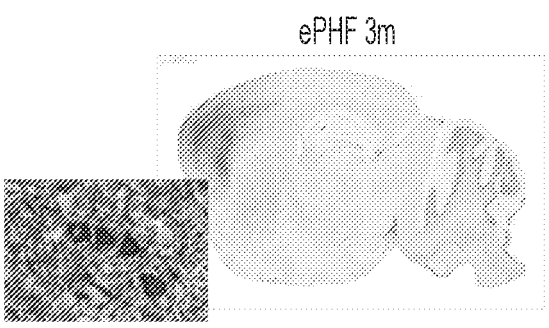


FIG. 19E

FIG. 19F

FIG. 19G

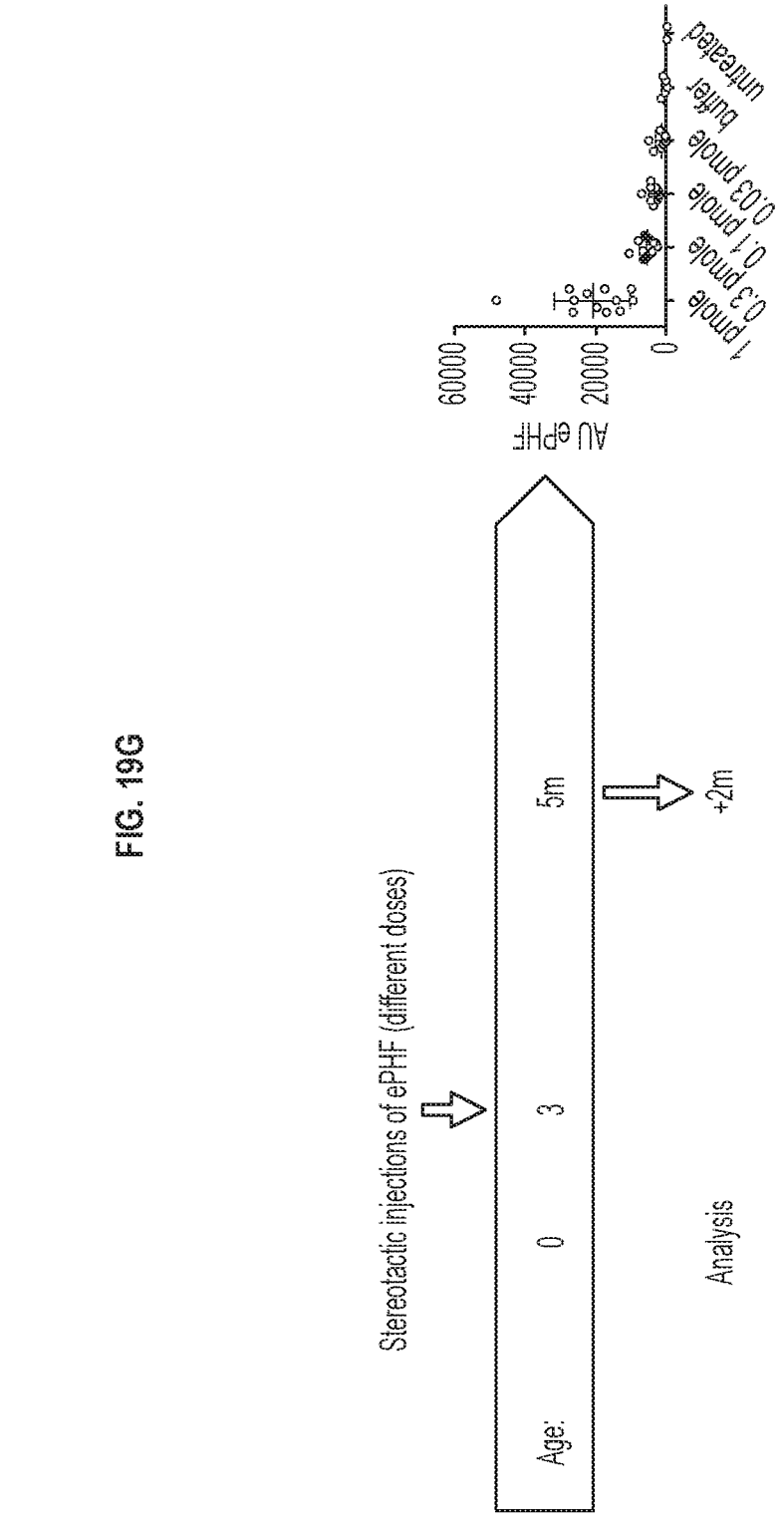
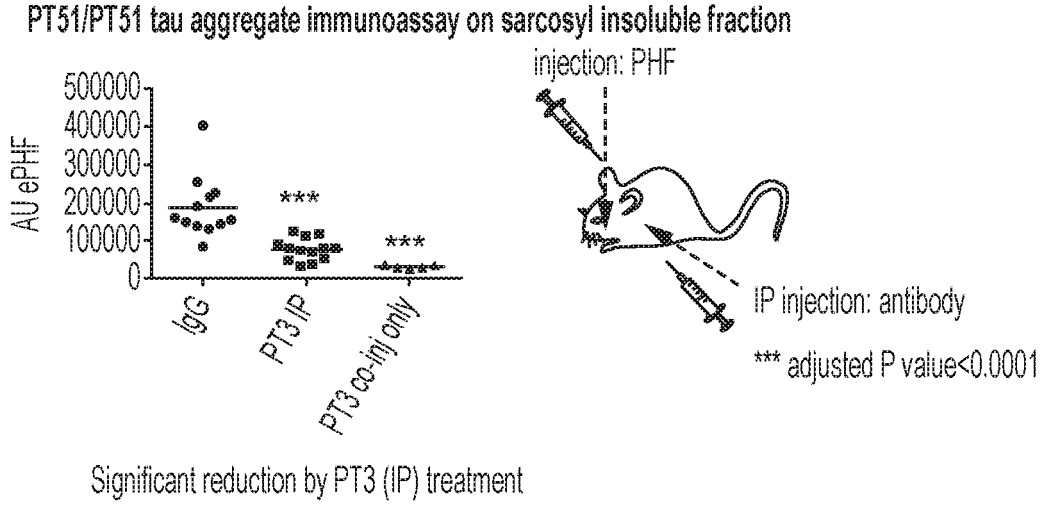

Stereotactic injections of ePHF (different doses)
Age:
0
3
5m
+2m
Analysis
AU ePHF
60000
40000
20000
0
1 pmole
0.3 pmole
0.1 pmole
0.03 pmole
buffer
untreated

FIG. 20



Significant reduction by PT3 (IP) treatment

FIG. 21

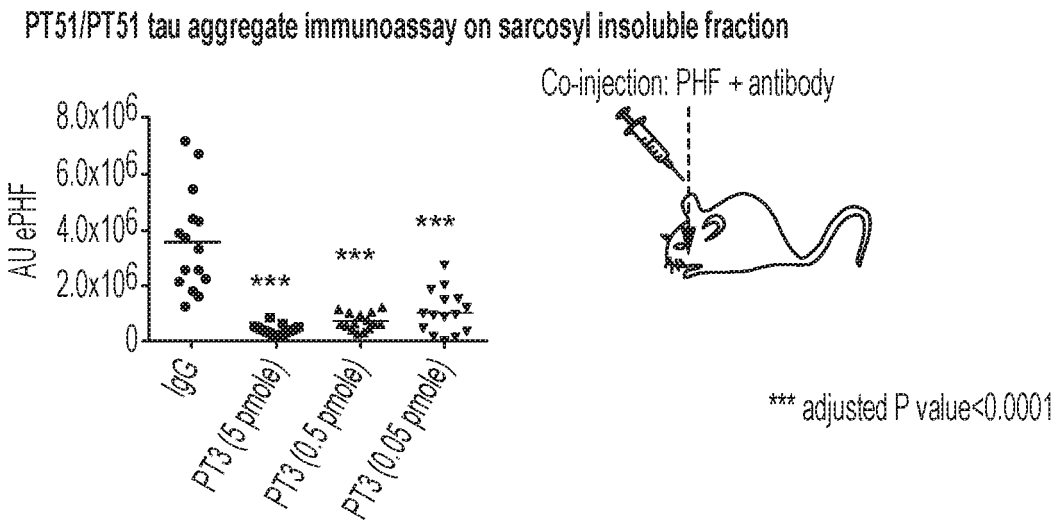

PT51/PT51 tau aggregate immunoassay on sarcosyl insoluble fraction
AU ePHF
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0
***
***
***
IgG
PT3 (5 pmole)
PT3 (0.5 pmole)
PT3 (0.05 pmole)
Co-injection: PHF + antibody
*** adjusted P value<0.0001

FIG. 22

FIG. 22A

Group	Co-injection	IP (20 mg/kg, 2x/week)	n
1	IgG1	IgG1	19
2	PT3(G1)	PT3(G1)	16
3	PT3(G2a)	PT3(G2a)	18
4	PT3(G2a-HFA)	PT3(G2a-HFA)	15

Inject	Amount	Total volume (μL)	Concentration (μM)
2.5 μL ePHF	1 pmole Tau	5	0.2
2.5 mL antibody	25 pmole ab	5	5

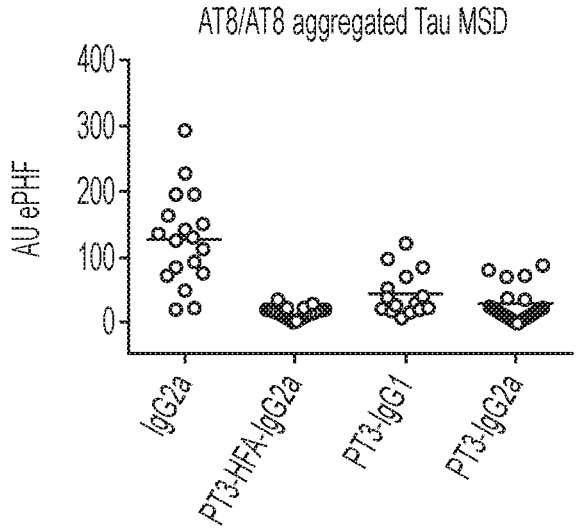


FIG. 22B

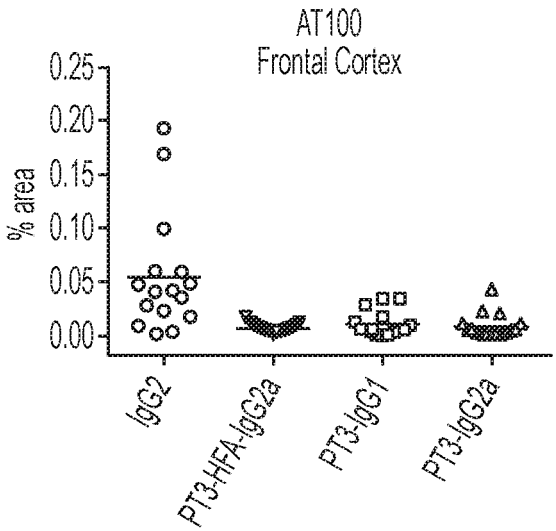


FIG. 22C

FIG. 23

FIG. 23A

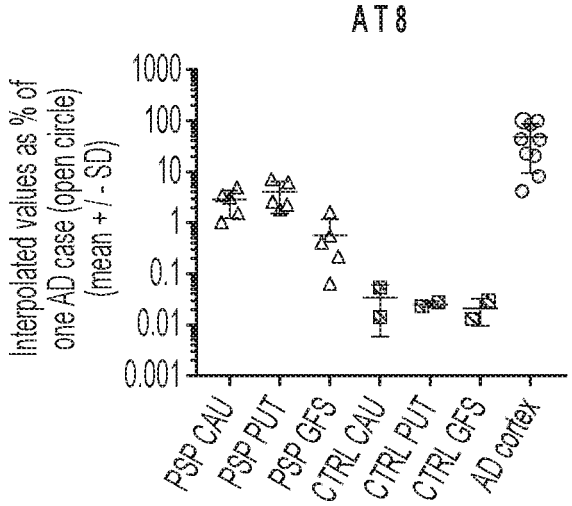


FIG. 23B

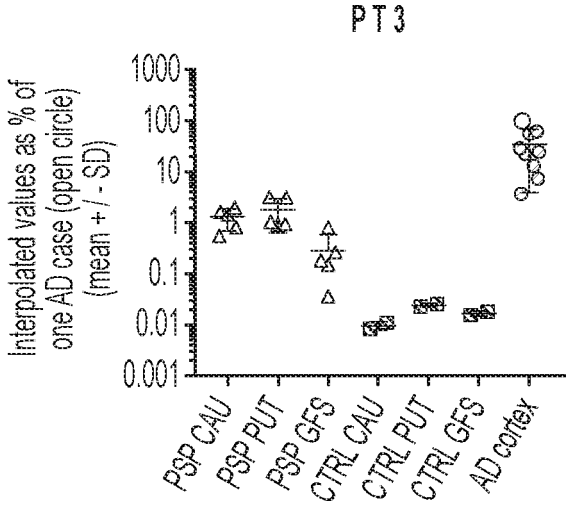


FIG. 24

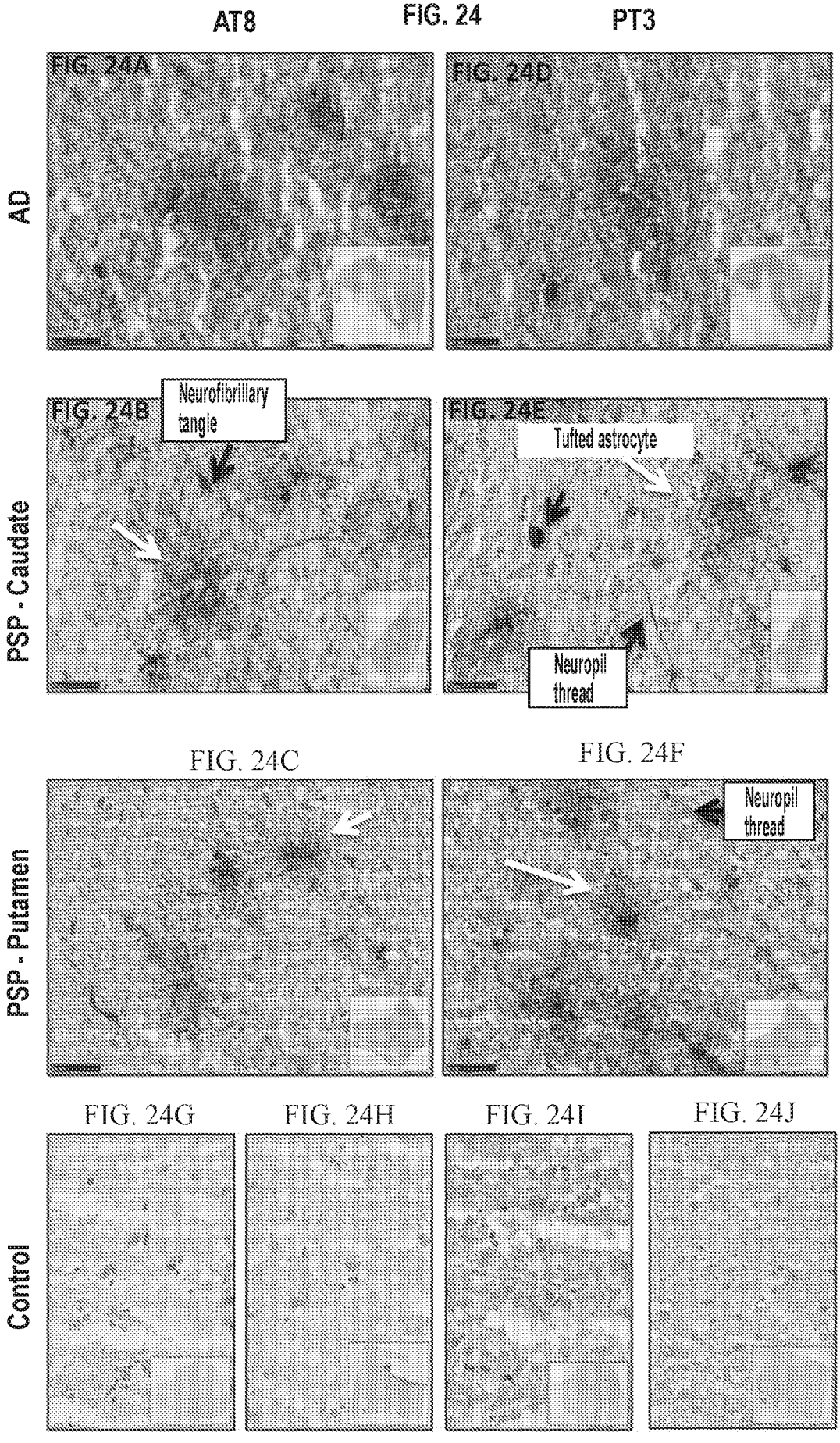

AT8
PT3
AD
FIG. 24A
FIG. 24D
PSP - Caudate
FIG. 24B
Neurofibrillary tangle
FIG. 24E
Tufted astrocyte
Neuropil thread
FIG. 24C
FIG. 24F
PSP - Putamen
Neuropil thread
FIG. 24G
FIG. 24H
FIG. 24I
FIG. 24J
Control

FIG. 25

FIG. 25A FIG. 25B FIG. 25C FIG. 25D

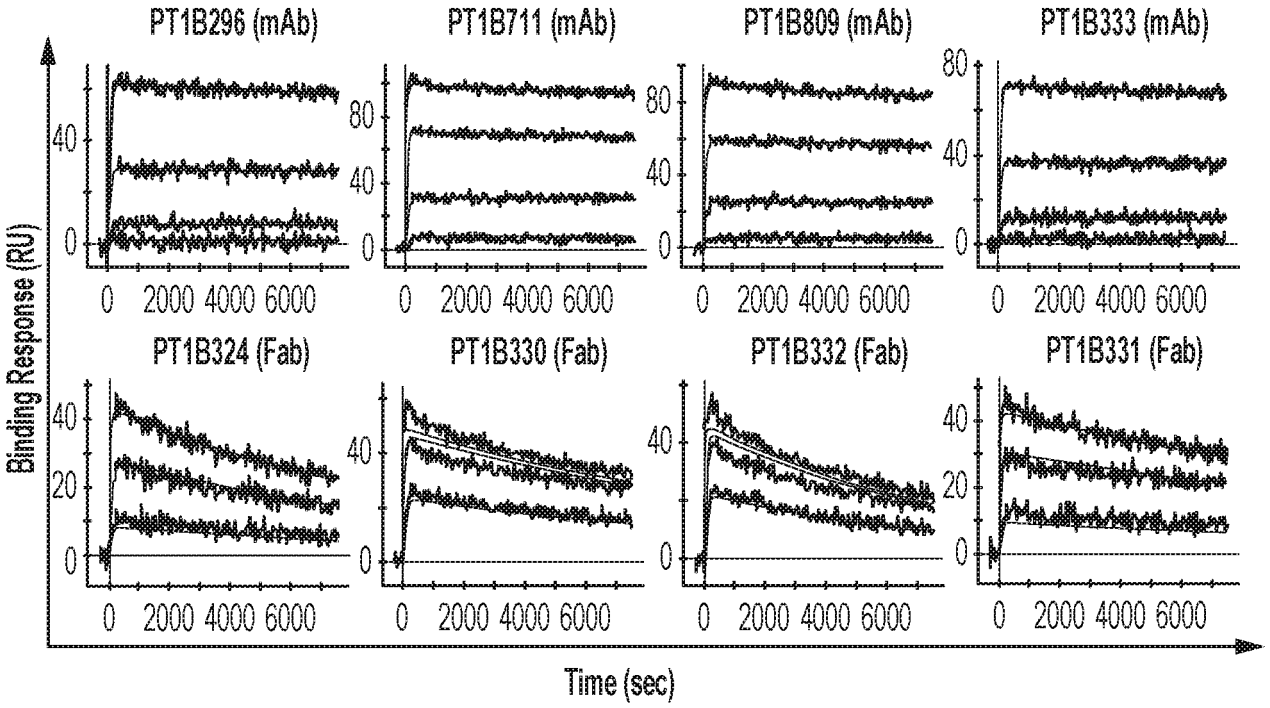


FIG. 25E FIG. 25F FIG. 25G FIG. 25H

FIG. 26

FIG. 26A

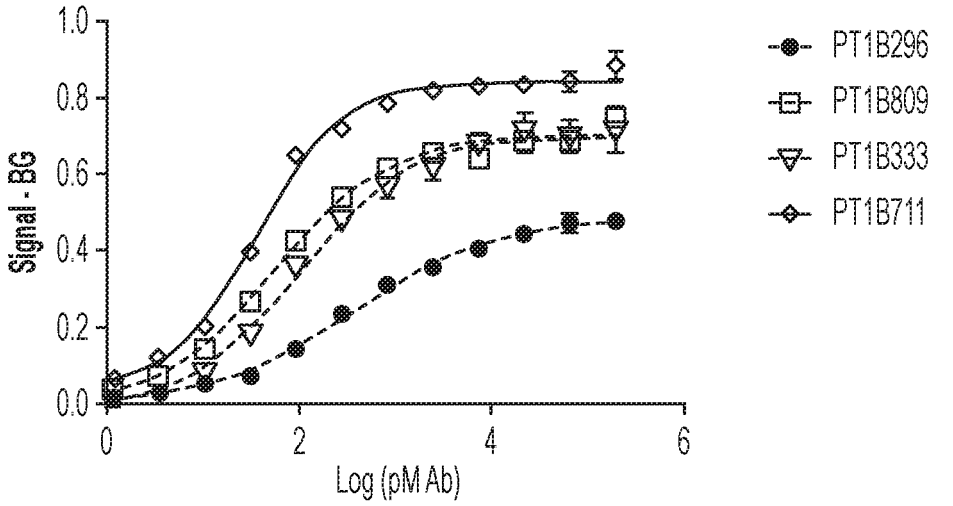


FIG. 26B

