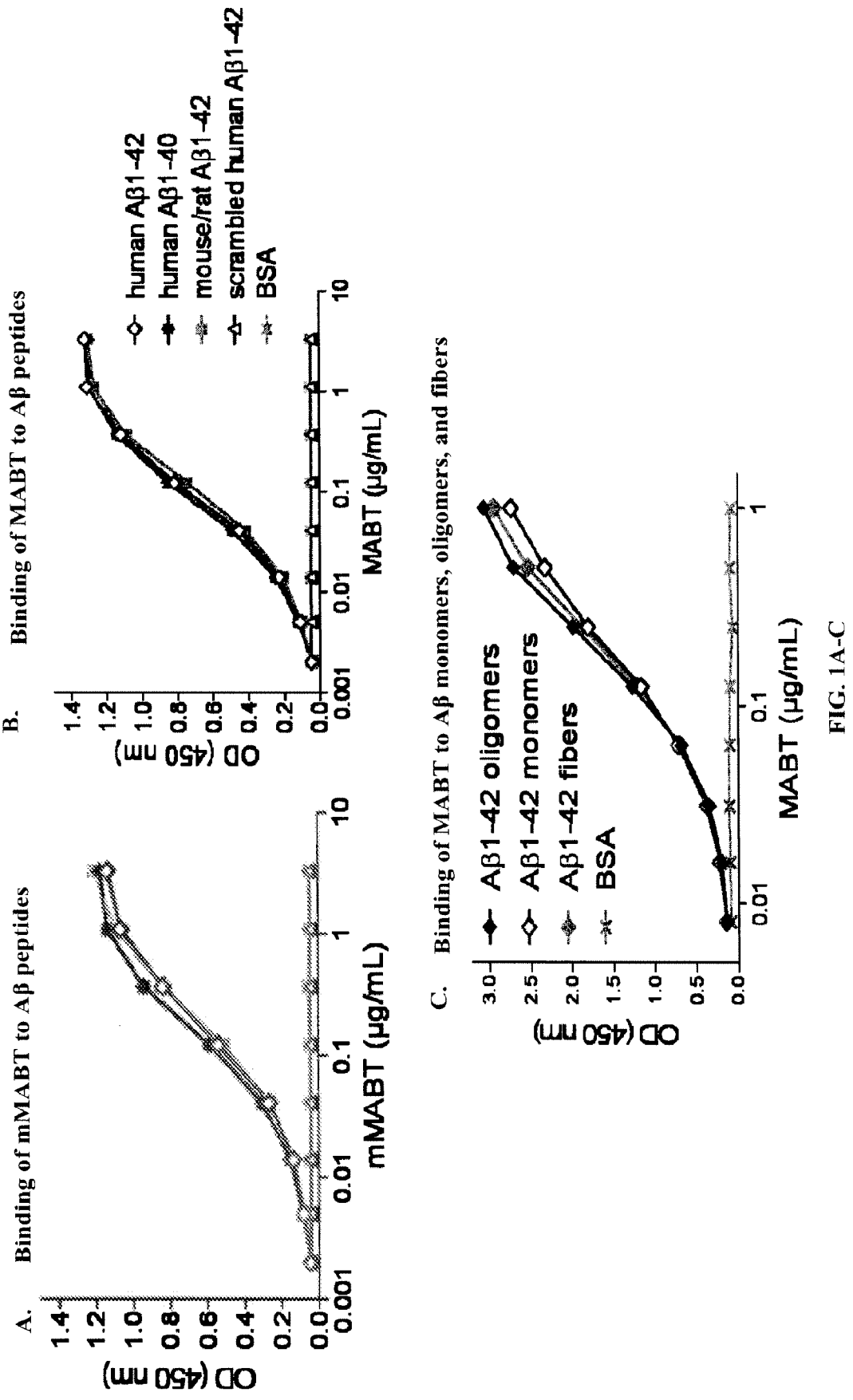

A. Binding of mMABT to Aβ peptides
OD (450 nm)
mMABT (μg/mL)
B. Binding of MABT to Aβ peptides
human Aβ1-42
human Aβ1-40
mouse/rat Aβ1-42
scrambled human Aβ1-42
BSA
OD (450 nm)
MABT (μg/mL)
C. Binding of MABT to Aβ monomers, oligomers, and fibers
Aβ1-42 oligomers
Aβ1-42 monomers
Aβ1-42 fibers
BSA
OD (450 nm)
MABT (μg/mL)

FIG. 1A-C

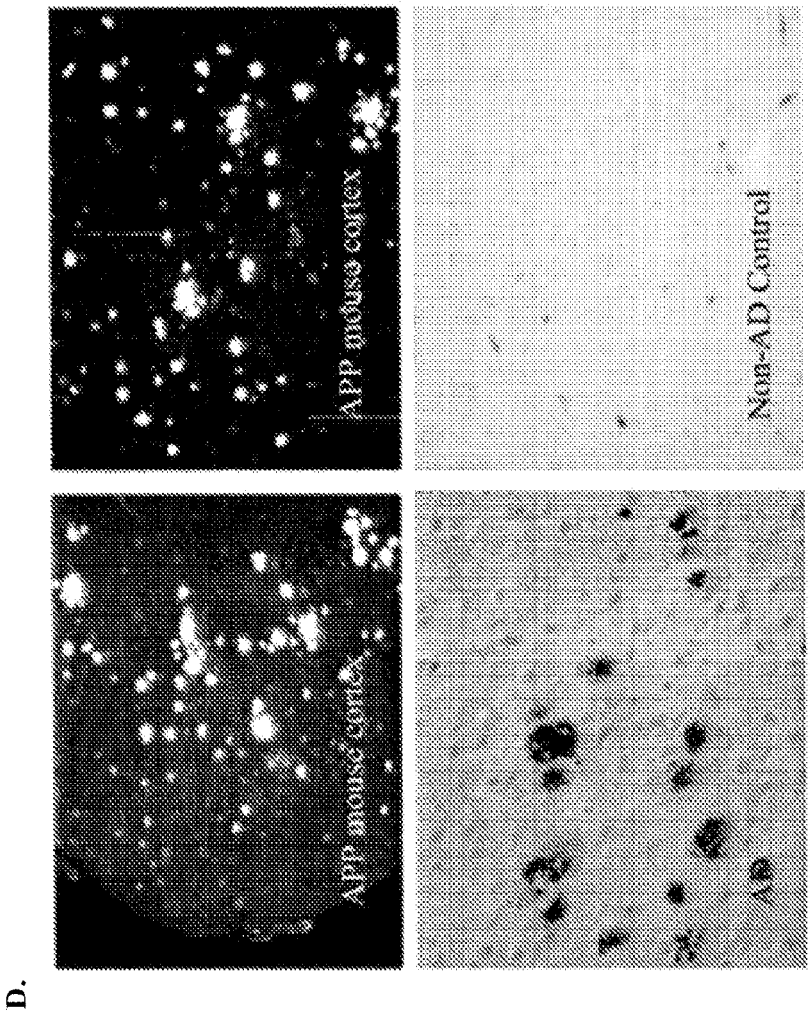


FIG. 1D

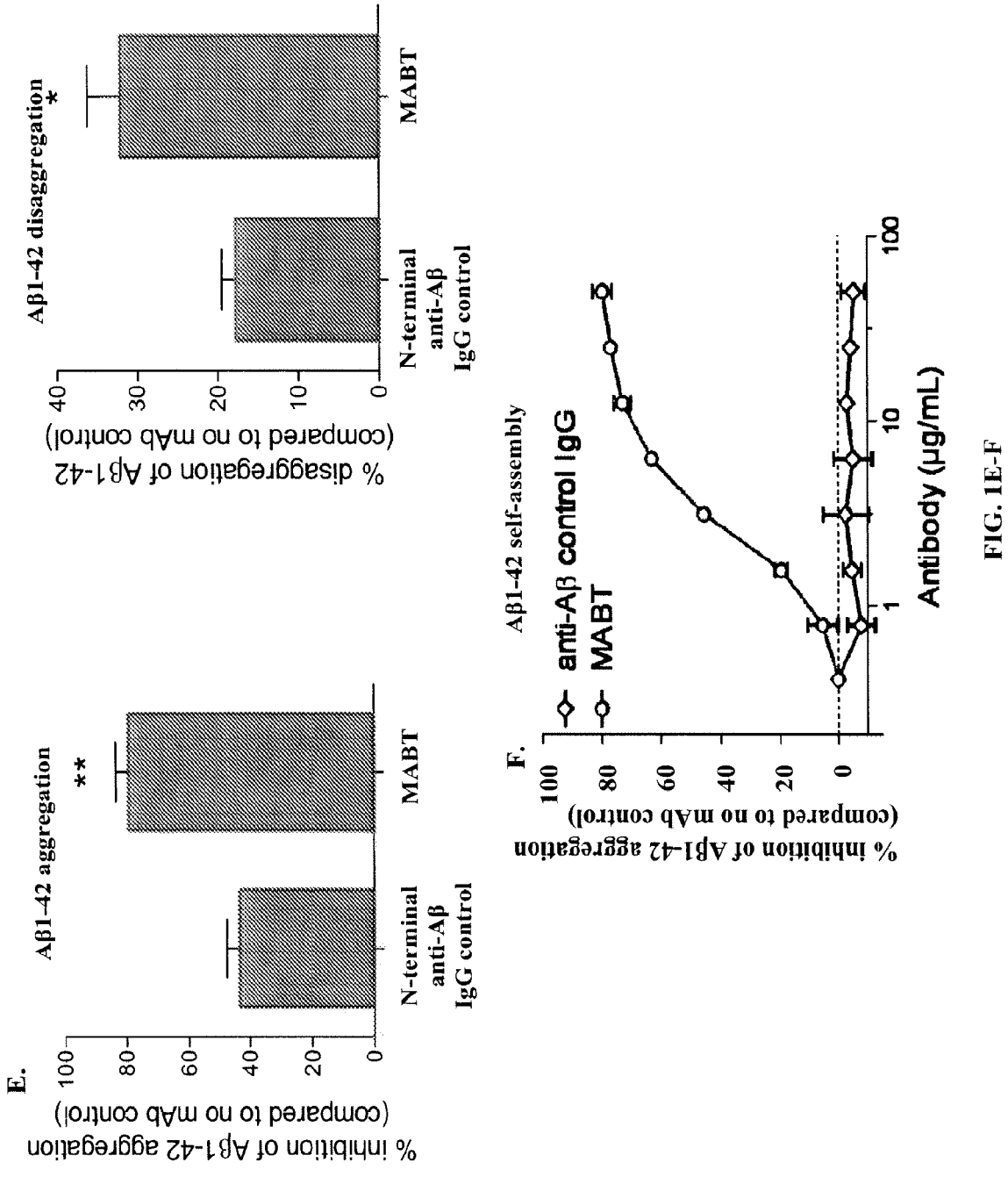

E.
% inhibition of Aβ1-42 aggregation
(compared to no mAb control)
Aβ1-42 aggregation
**
N-terminal anti-Aβ
IgG control
MABT
% disaggregation of Aβ1-42
(compared to no mAb control)
Aβ1-42 disaggregation
*
F.
Aβ1-42 self-assembly
anti-Aβ control IgG
MABT
% inhibition of Aβ1-42 aggregation
(compared to no mAb control)
Antibody (μg/mL)

FIG. 1E-F

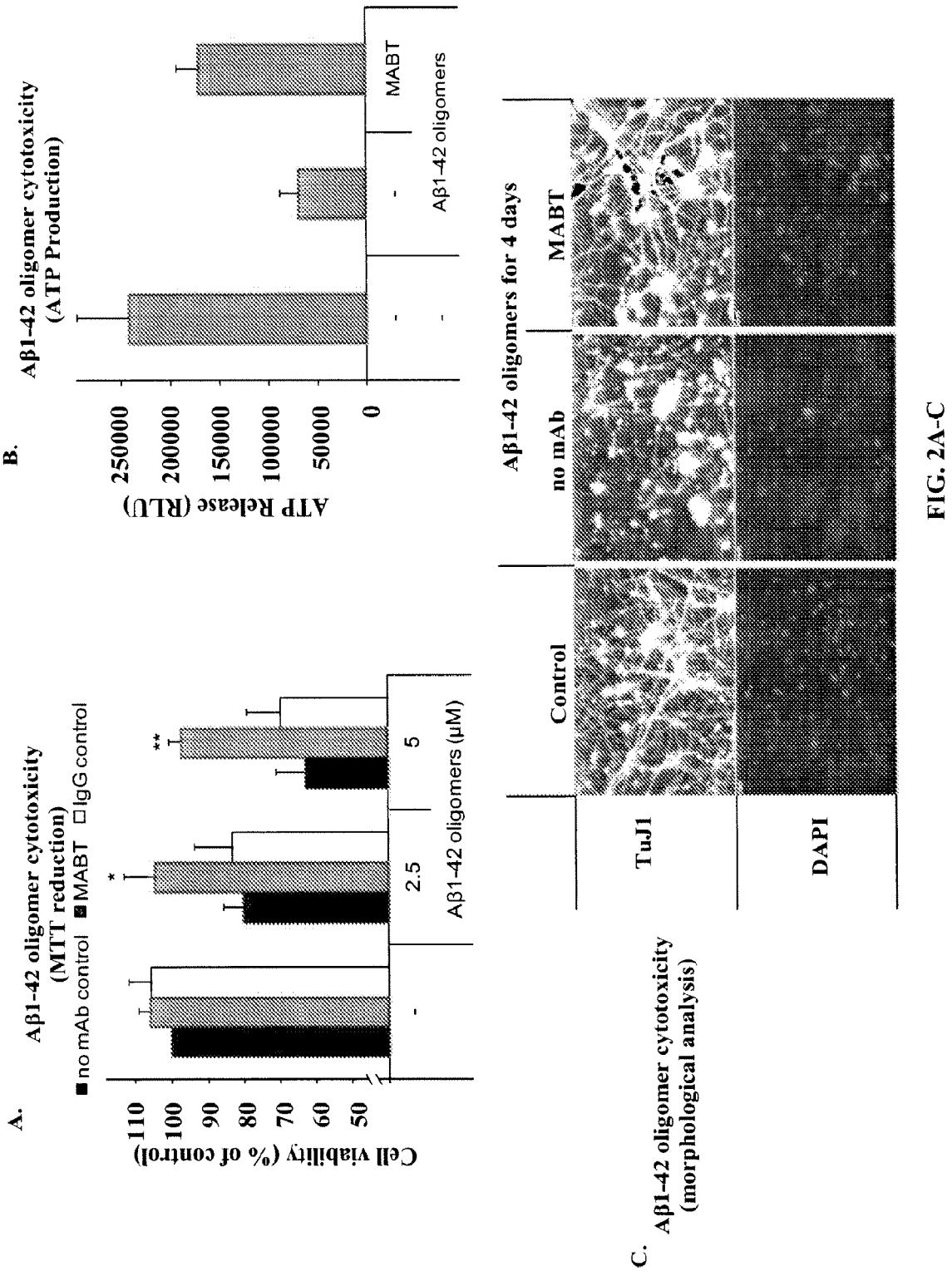
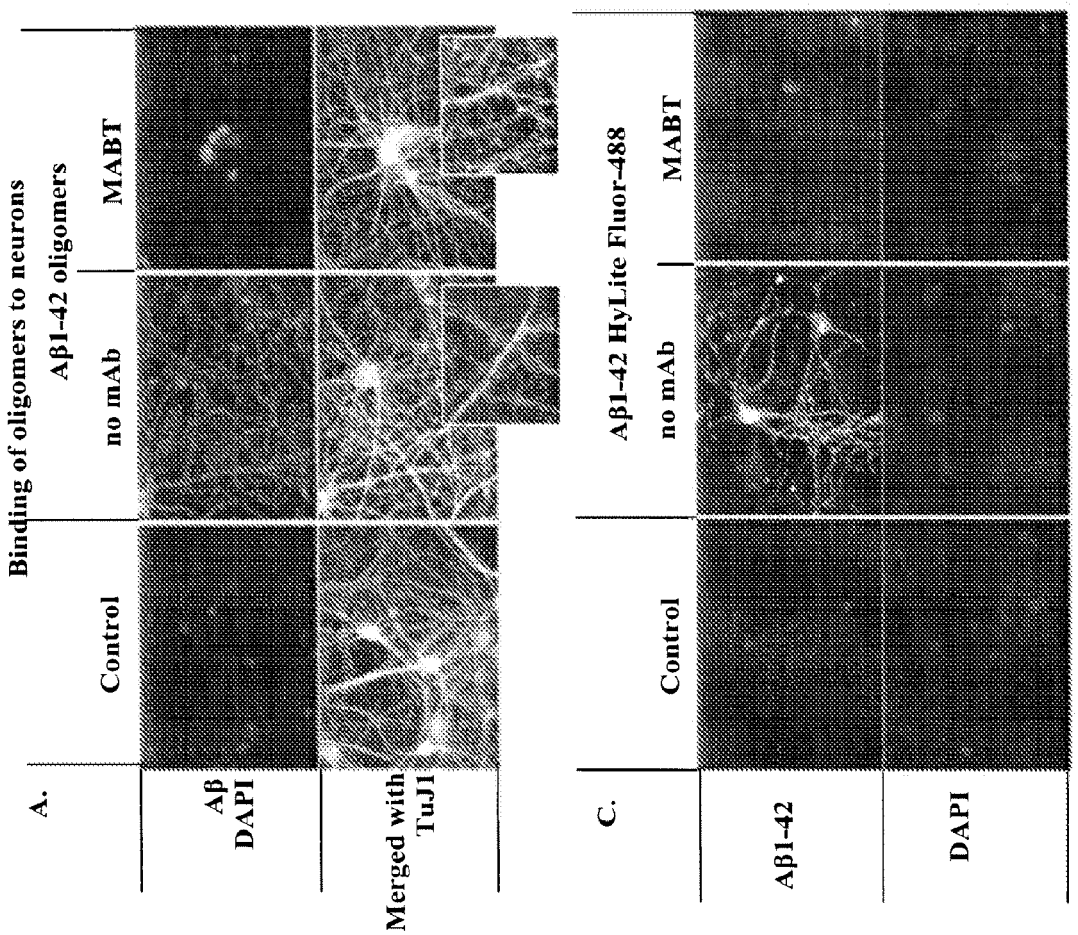
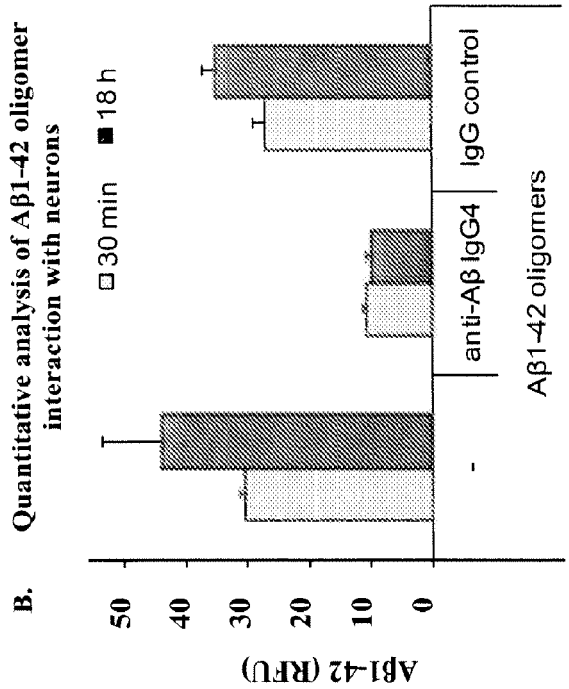

A.
Aβ1-42 oligomer cytotoxicity
(MTT reduction)
no mAb control
MABT
IgG control
Cell viability (% of control)
110
100
90
80
70
60
50
*
**
-
2.5
5
Aβ1-42 oligomers (μM)
B.
Aβ1-42 oligomer cytotoxicity
(ATP Production)
ATP Release (RLU)
250000
200000
150000
100000
50000
0
-
-
MABT
-
-
Aβ1-42 oligomers
C.
Aβ1-42 oligomer cytotoxicity
(morphological analysis)
Control
Aβ1-42 oligomers for 4 days
no mAb
MABT
TuJ1
DAPI

FIG. 2A-C


A.
Binding of oligomers to neurons
Control
Aβ1-42 oligomers
no mAb
MABT
Aβ
DAPI
Merged with TuJ1
C.
Aβ1-42 HyLite Fluor-488
Control
no mAb
MABT
Aβ1-42
DAPI


B.
Quantitative analysis of Aβ1-42 oligomer interaction with neurons
30 min
18 h
Aβ1-42 (RFU)
50
40
30
20
10
0
-
anti-Aβ IgG4
IgG control
Aβ1-42 oligomers

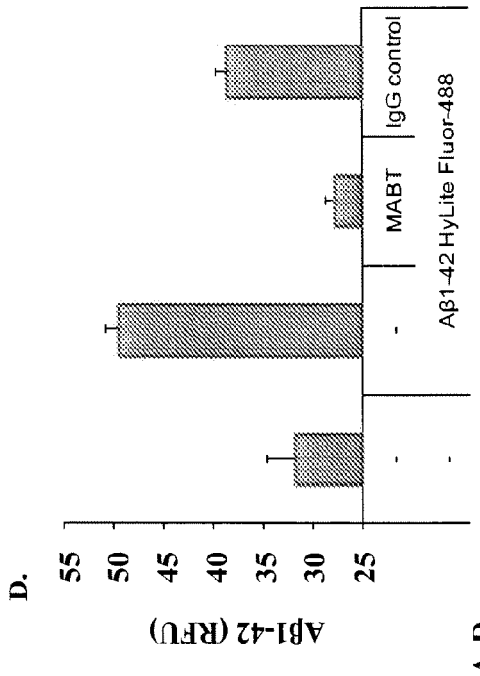
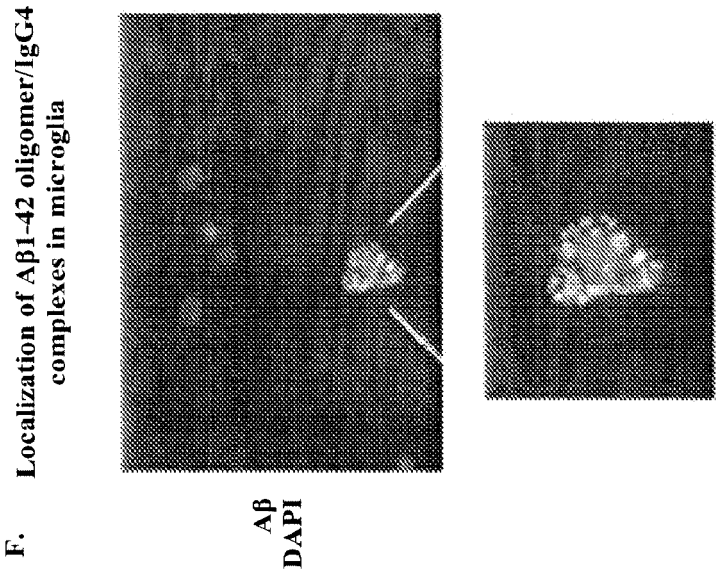

D.
Aβ1-42 (RFU)
55
50
45
40
35
30
25
-
-
MABT
IgG control
-
Aβ1-42 HyLite Fluor-488

FIG. 3A-D


F.
Localization of Aβ1-42 oligomer/IgG4 complexes in microglia
Aβ
DAPI

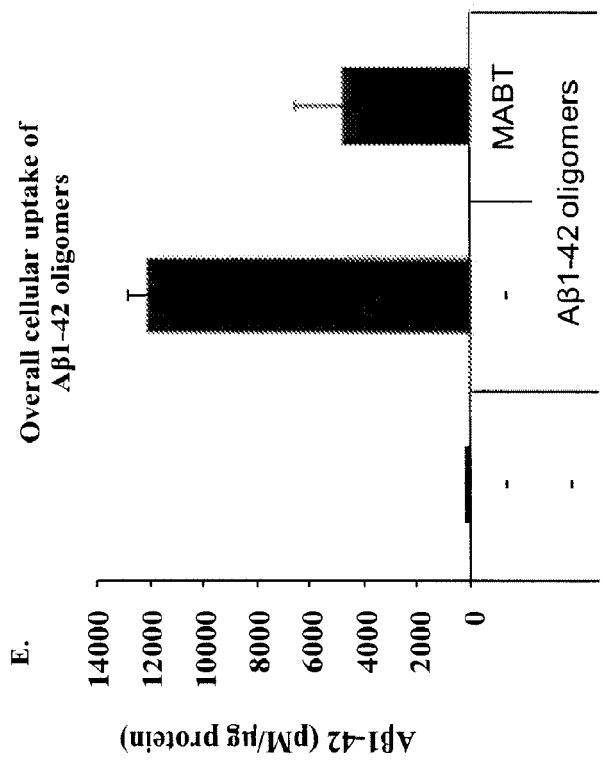
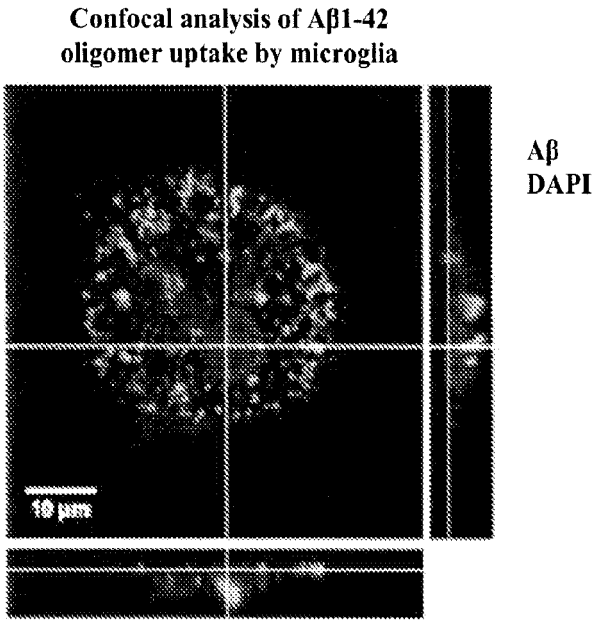
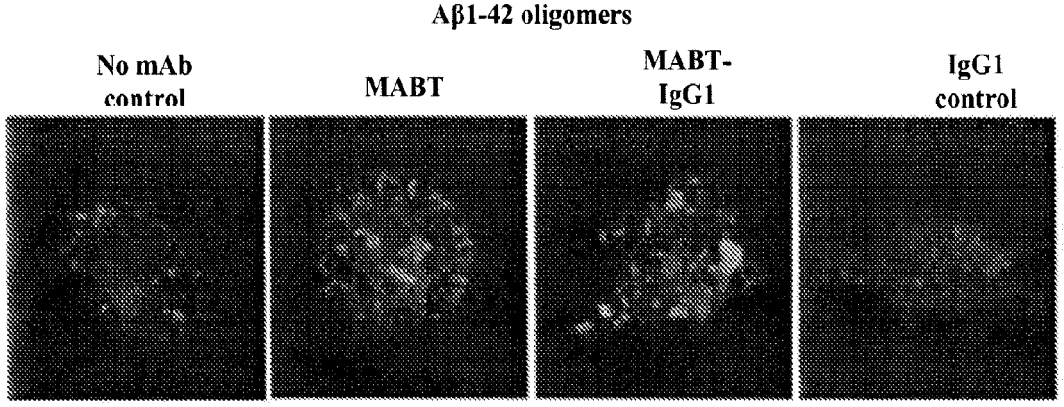

E.
Overall cellular uptake of Aβ1-42 oligomers
Aβ1-42 (pM/μg protein)
14000
12000
10000
8000
6000
4000
2000
0
-
-
-
MABT
Aβ1-42 oligomers

FIG. 3E-F

A.


Confocal analysis of Aβ1-42 oligomer uptake by microglia
Aβ
DAPI
10 μm

B.


Aβ1-42 oligomers
No mAb control
MABT
MABT-IgG1
IgG1 control
Aβ, DAPI

C.

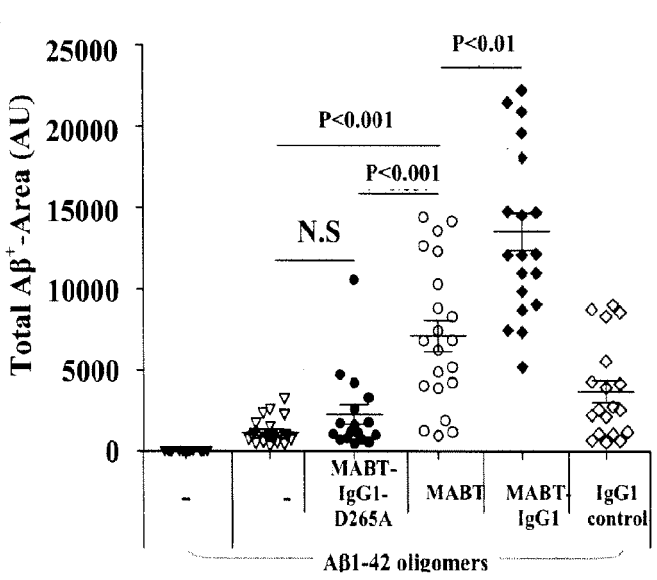
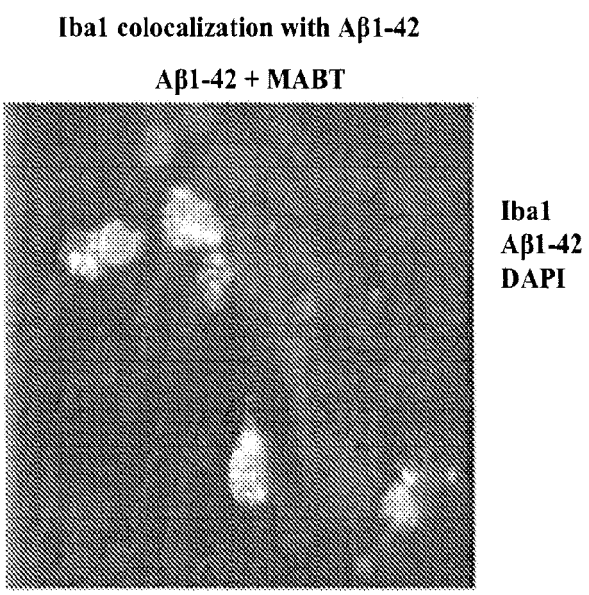
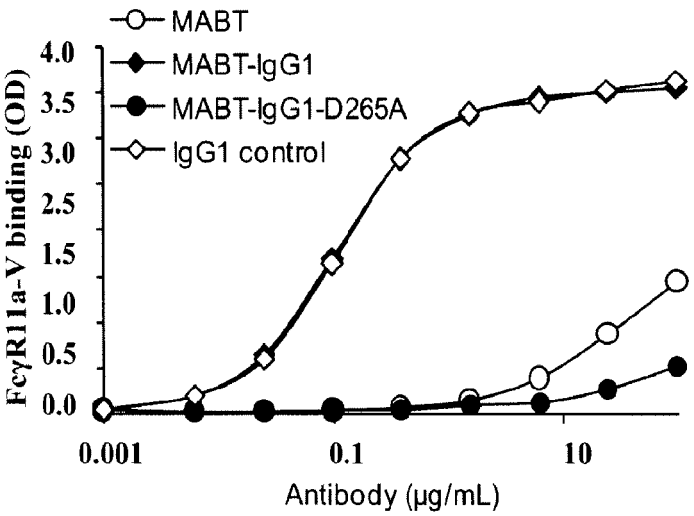

Total Aβ+-Area (AU)
25000
20000
15000
10000
5000
0
P<0.01
P<0.001
P<0.001
N.S
-
-
MABT-IgG1-D265A
MABT
MABT-IgG1
IgG1 control
Aβ1-42 oligomers

FIG. 4A-C

D.


Iba1 colocalization with Aβ1-42
Aβ1-42 + MABT
Iba1
Aβ1-42
DAPI

E.


Binding of IgG variants to FcγRIIIa-V158
MABT
MABT-IgG1
MABT-IgG1-D265A
IgG1 control
FcγR11a-V binding (OD)
4.0
3.5
3.0
2.5
1.5
1.0
0.5
0.0
0.001
0.1
10
Antibody (μg/mL)

F.

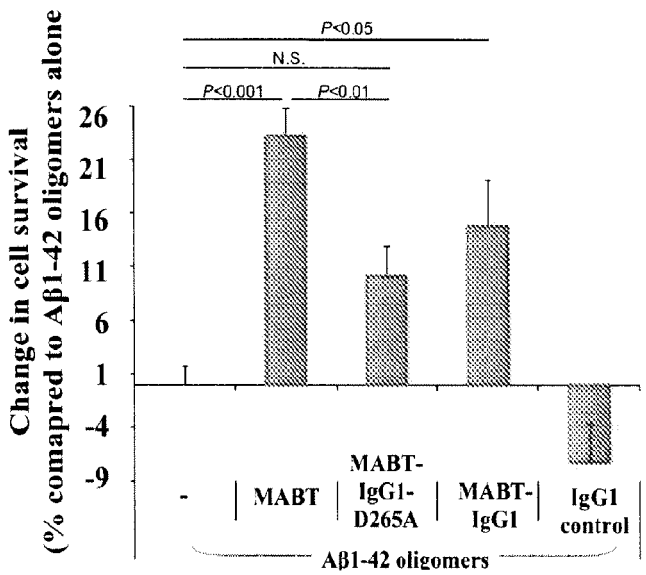

P<0.05
N.S.
P<0.001
P<0.01
Change in cell survival
(% comapred to Aβ1-42 oligomers alone
26
21
16
11
6
1
-4
-9
-
MABT
MABT-IgG1-D265A
MABT-IgG1
IgG1 control
Aβ1-42 oligomers

FIG. 4D-F

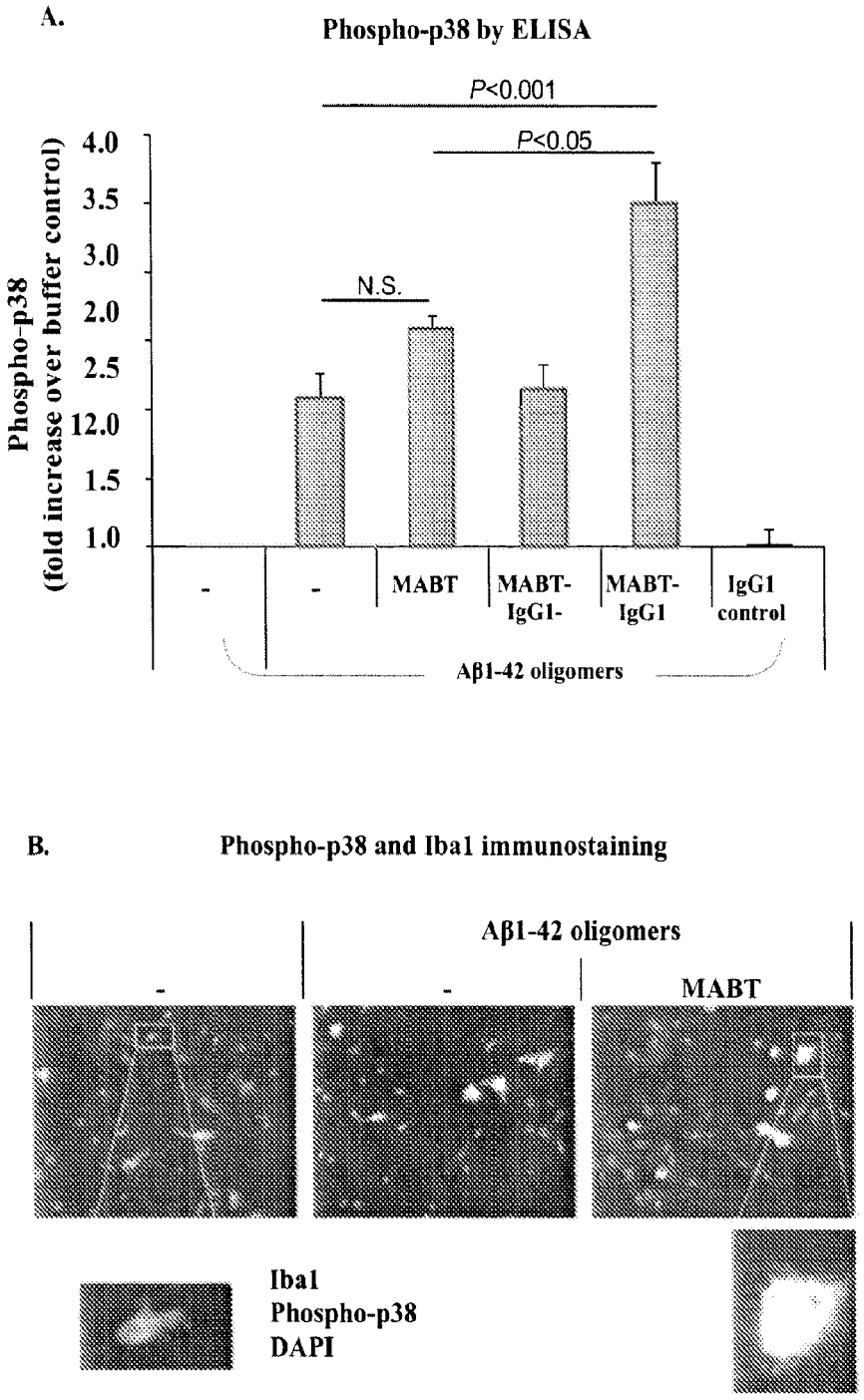
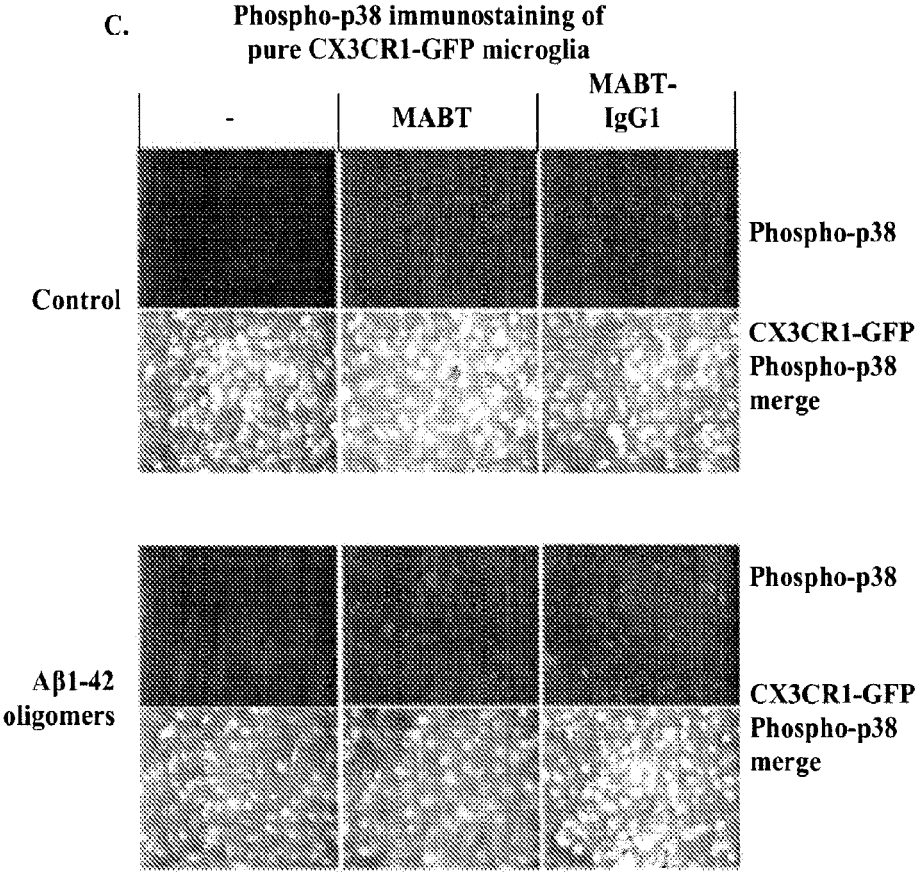

A.
Phospho-p38 by ELISA
P<0.001
P<0.05
N.S.
Phospho-p38
(fold increase over buffer control)
4.0
3.5
3.0
2.0
2.5
12.0
1.5
1.0
-
-
MABT
MABT-IgG1-
MABT-IgG1
IgG1 control
Aβ1-42 oligomers
B.
Phospho-p38 and Iba1 immunostaining
Aβ1-42 oligomers
-
-
MABT
Iba1
Phospho-p38
DAPI

FIG. 5A-B


C.
Phospho-p38 immunostaining of pure CX3CR1-GFP microglia
-
MABT
MABT-IgG1
Control
Phospho-p38
CX3CR1-GFP
Phospho-p38
merge
Aβ1-42 oligomers
Phospho-p38
CX3CR1-GFP
Phospho-p38
merge

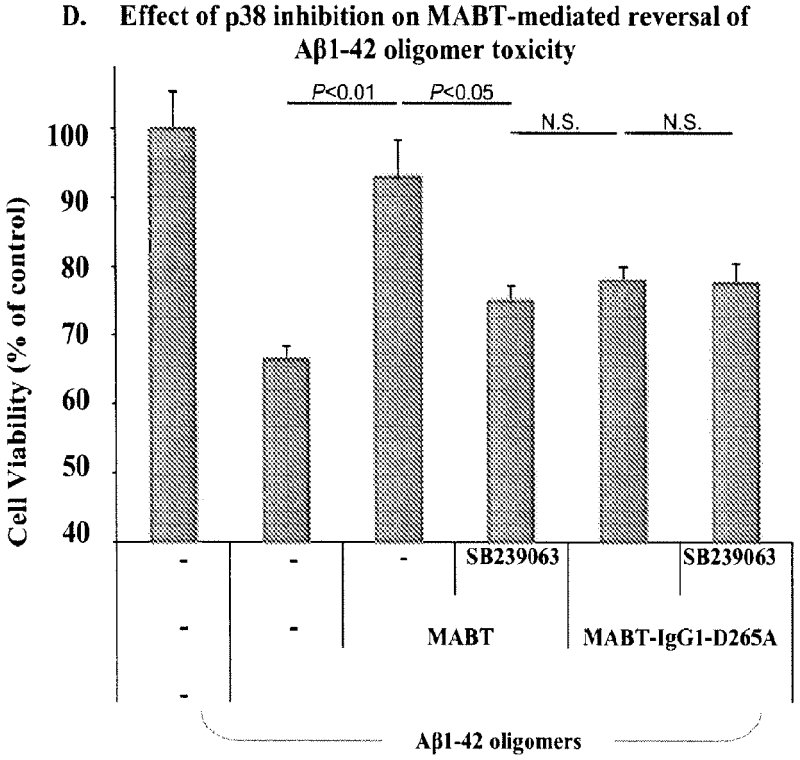

D. Effect of p38 inhibition on MABT-mediated reversal of Aβ1-42 oligomer toxicity
P<0.01
P<0.05
N.S.
N.S.
Cell Viability (% of control)
100
90
80
70
60
50
40
SB239063
SB239063
MABT
MABT-IgG1-D265A
Aβ1-42 oligomers

FIG. 5C-D

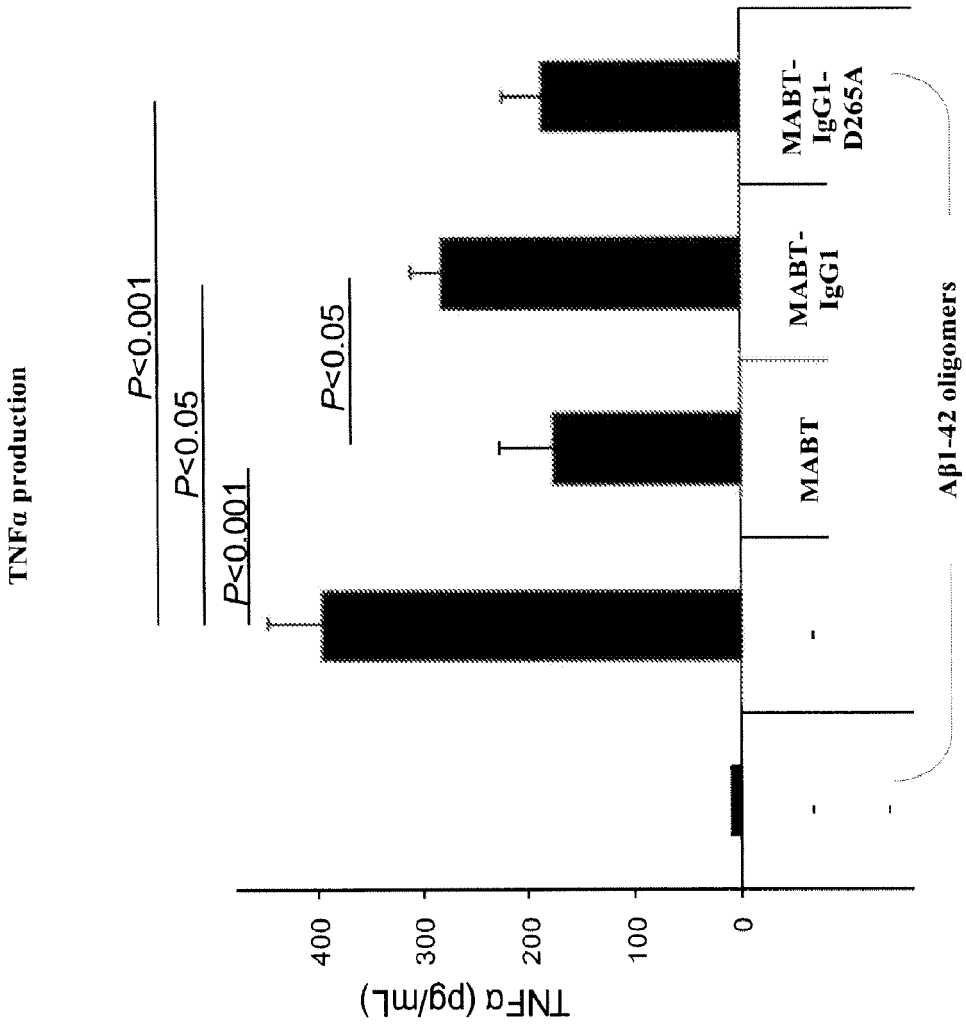

TNFα production
P<0.001
P<0.05
P<0.001
P<0.05
TNFα (pg/mL)
400
300
200
100
0
-
-
MABT
MABT-IgG1
MABT-IgG1-D265A
Aβ1-42 oligomers

FIG. 6

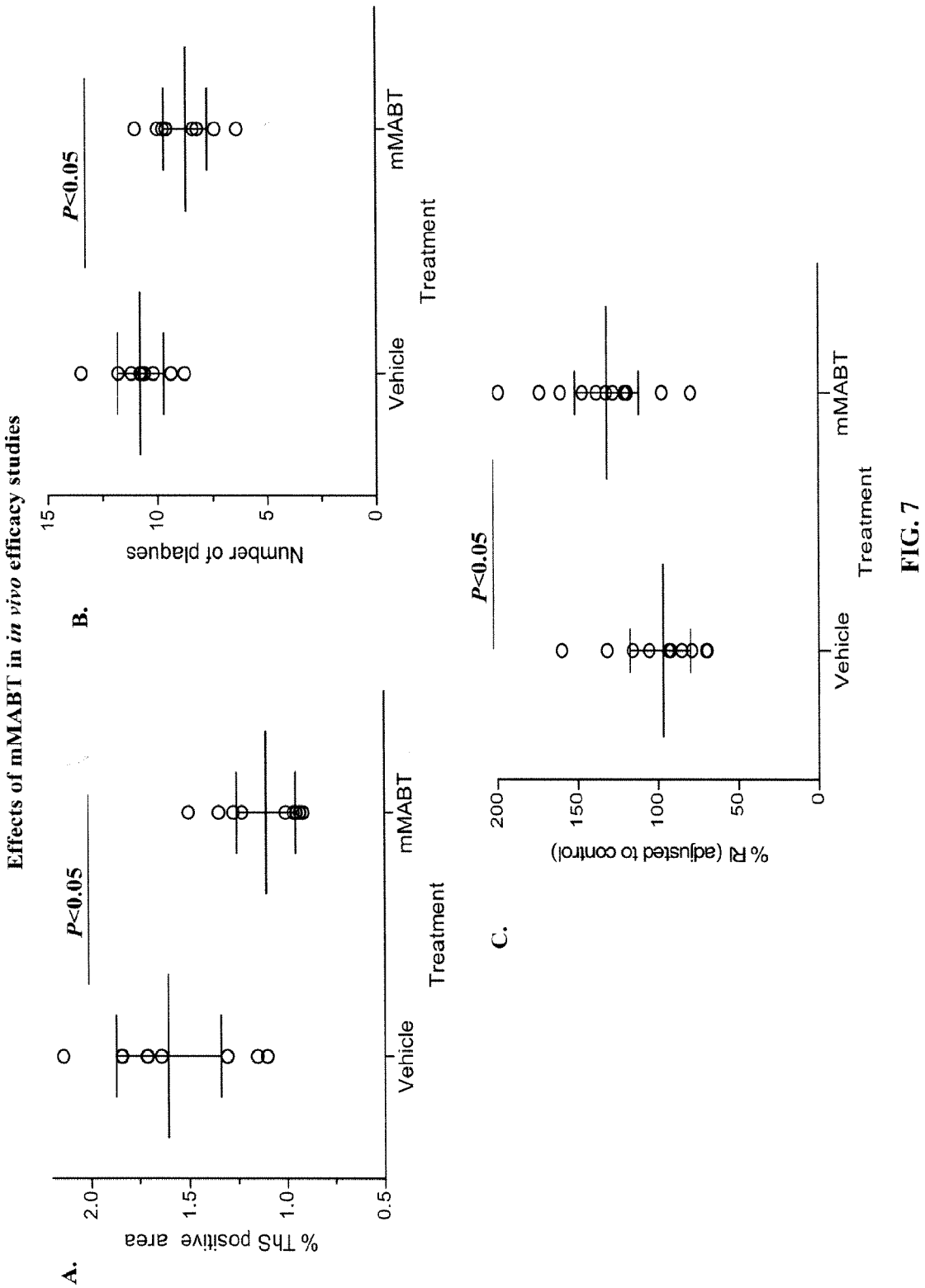

Effects of mMABT in in vivo efficacy studies
A.
% ThS positive area
2.0
1.5
1.0
0.5
P<0.05
Vehicle
mMABT
Treatment
B.
Number of plaques
15
10
5
0
P<0.05
Vehicle
mMABT
Treatment
C.
% RI (adjusted to control)
200
150
100
50
0
P<0.05
Vehicle
mMABT
Treatment

FIG. 7

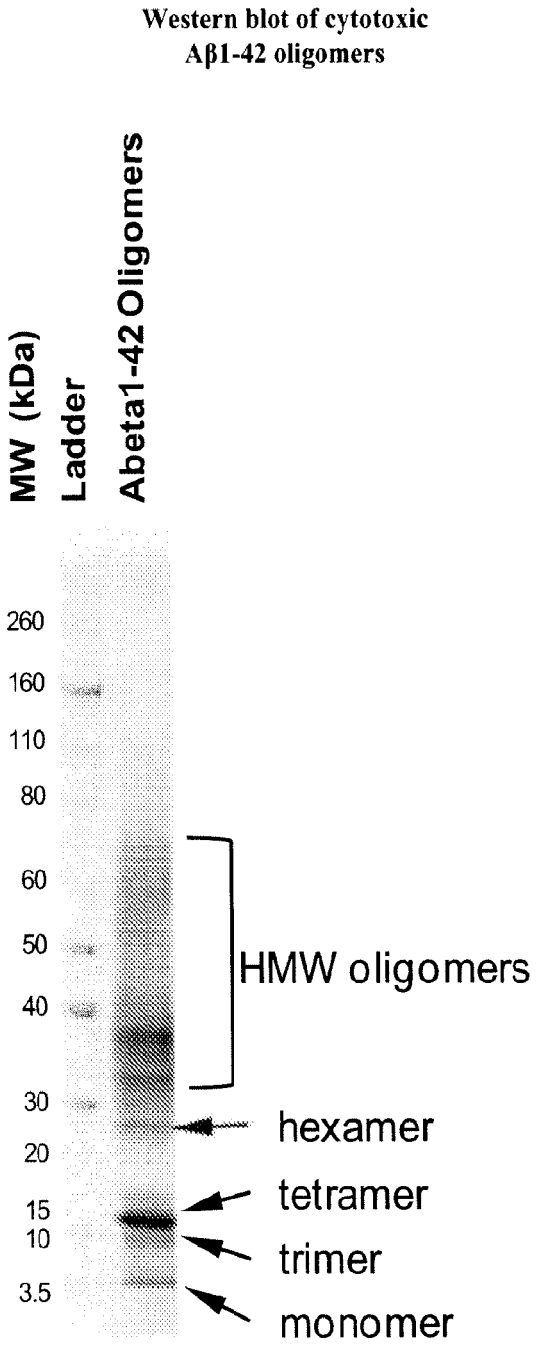

Western blot of cytotoxic
Aβ1-42 oligomers
MW (kDa)
Ladder
Abeta1-42 Oligomers
260
160
110
80
60
50
40
30
20
15
10
3.5
HMW oligomers
hexamer
tetramer
trimer
monomer

FIG. 8

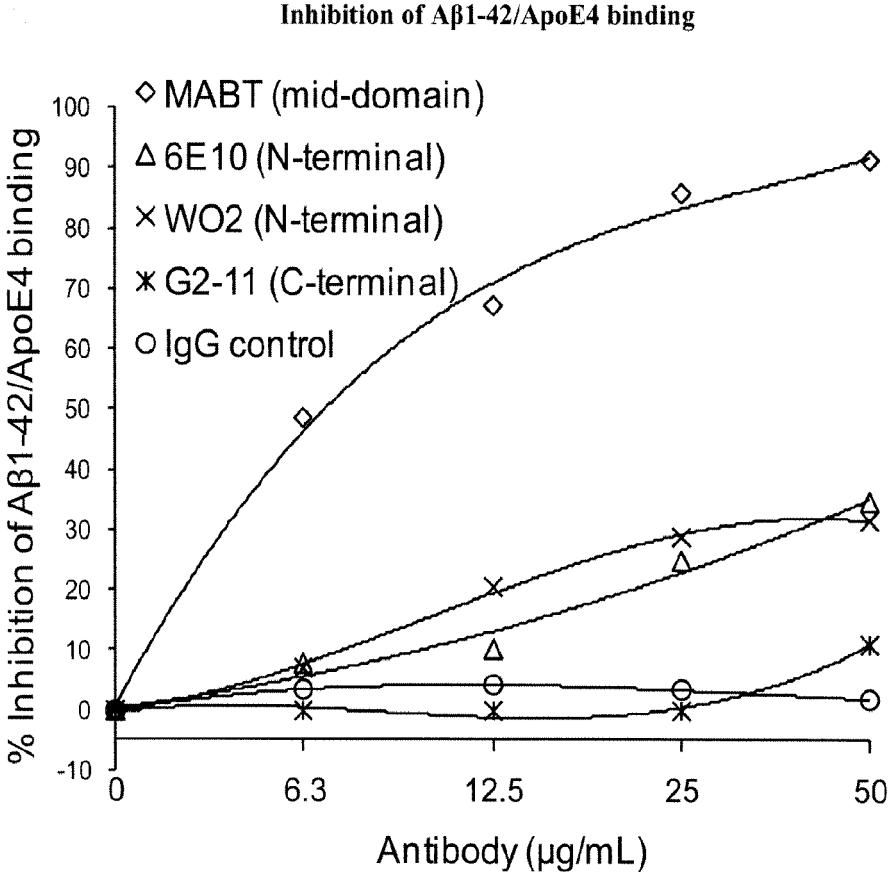

Inhibition of Aβ1-42/ApoE4 binding
MABT (mid-domain)
6E10 (N-terminal)
WO2 (N-terminal)
G2-11 (C-terminal)
IgG control
% Inhibition of Aβ1-42/ApoE4 binding
100
90
80
70
60
50
40
30
20
10
0
-10
0
6.3
12.5
25
50
Antibody (µg/mL)

FIG. 9

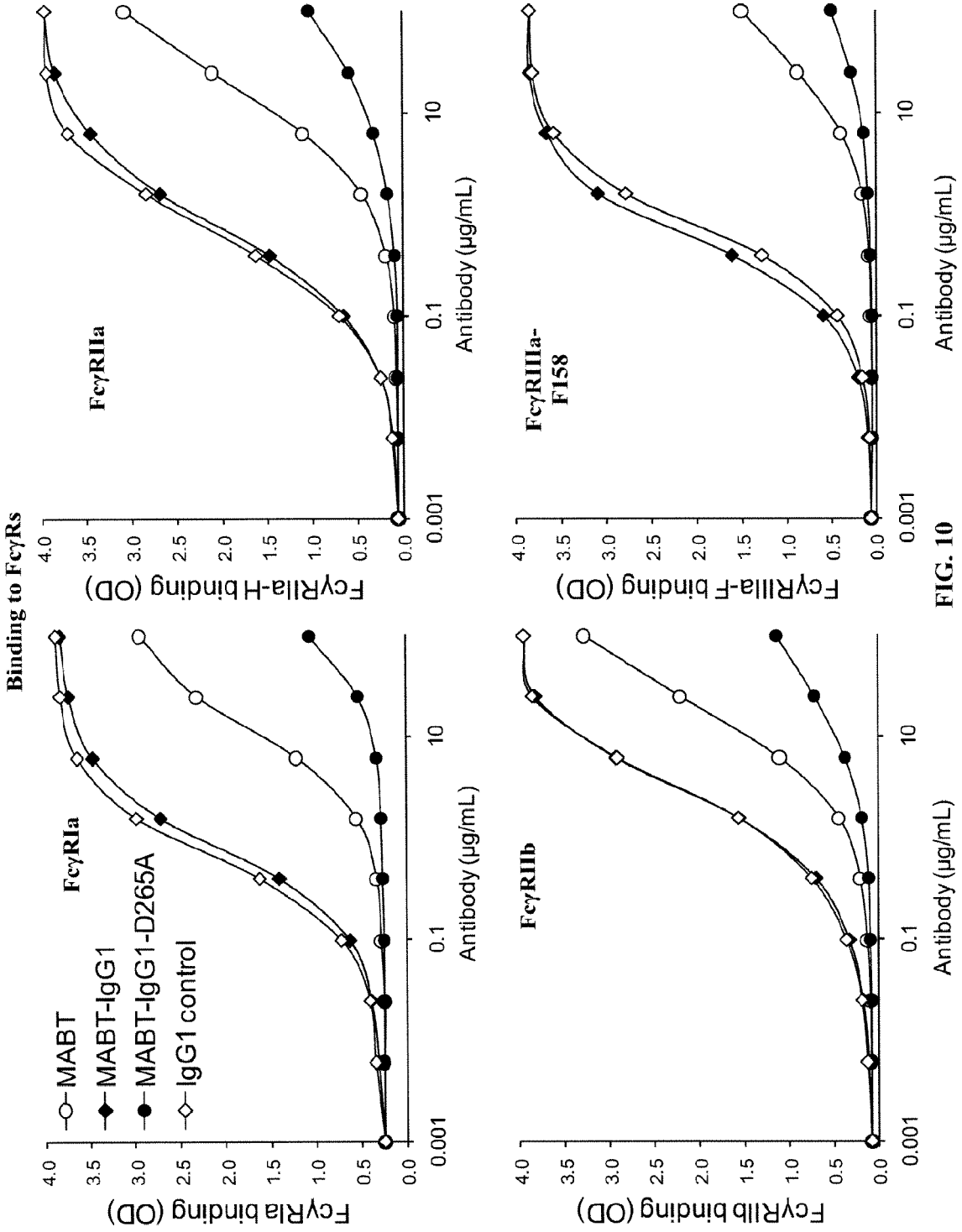

Binding to FcγRs
FcγRIa
FcγRIIa
FcγRIIb
FcγRIIIa-F158
MABT
MABT-IgG1
MABT-IgG1-D265A
IgG1 control
FcγRIa binding (OD)
FcγRIIa-H binding (OD)
FcγRIIb binding (OD)
FcγRIIIa-F binding (OD)
Antibody (µg/mL)
0.001
0.1
10
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

FIG. 10

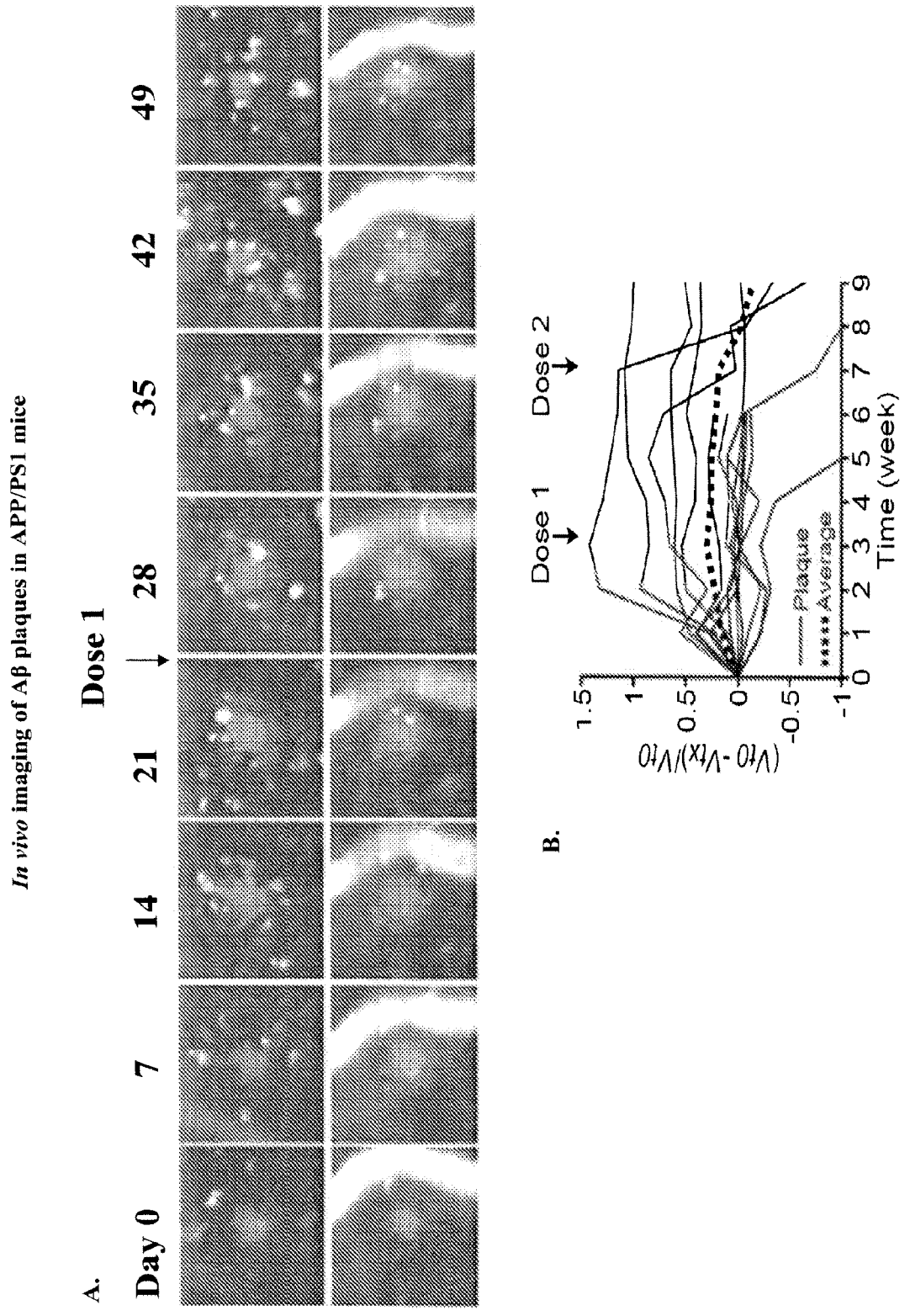

A.
Day 0
7
14
21
Dose 1
28
35
42
49
In vivo imaging of Aβ plaques in APP/PS1 mice
B.
Dose 1
Dose 2
(Vt0 - Vtx)/Vt0
1.5
1
0.5
0
-0.5
-1
0 1 2 3 4 5 6 7 8 9
Time (week)
Plaque
Average

FIG. 11