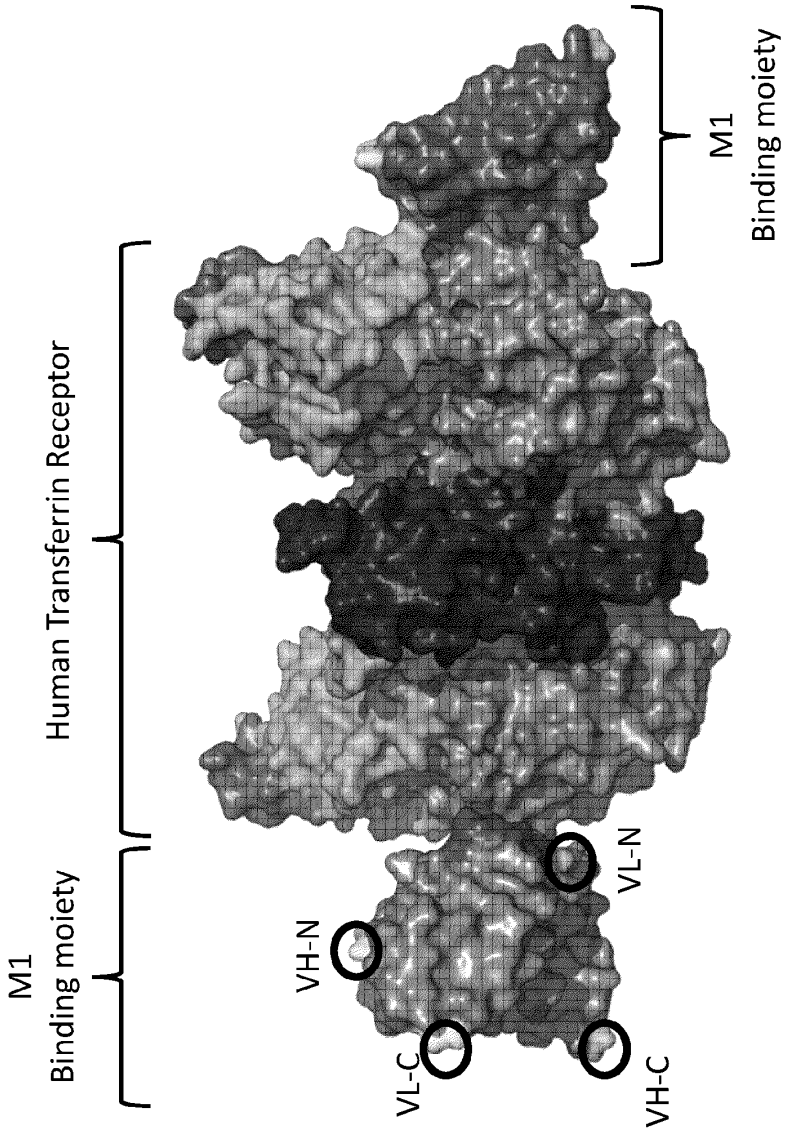
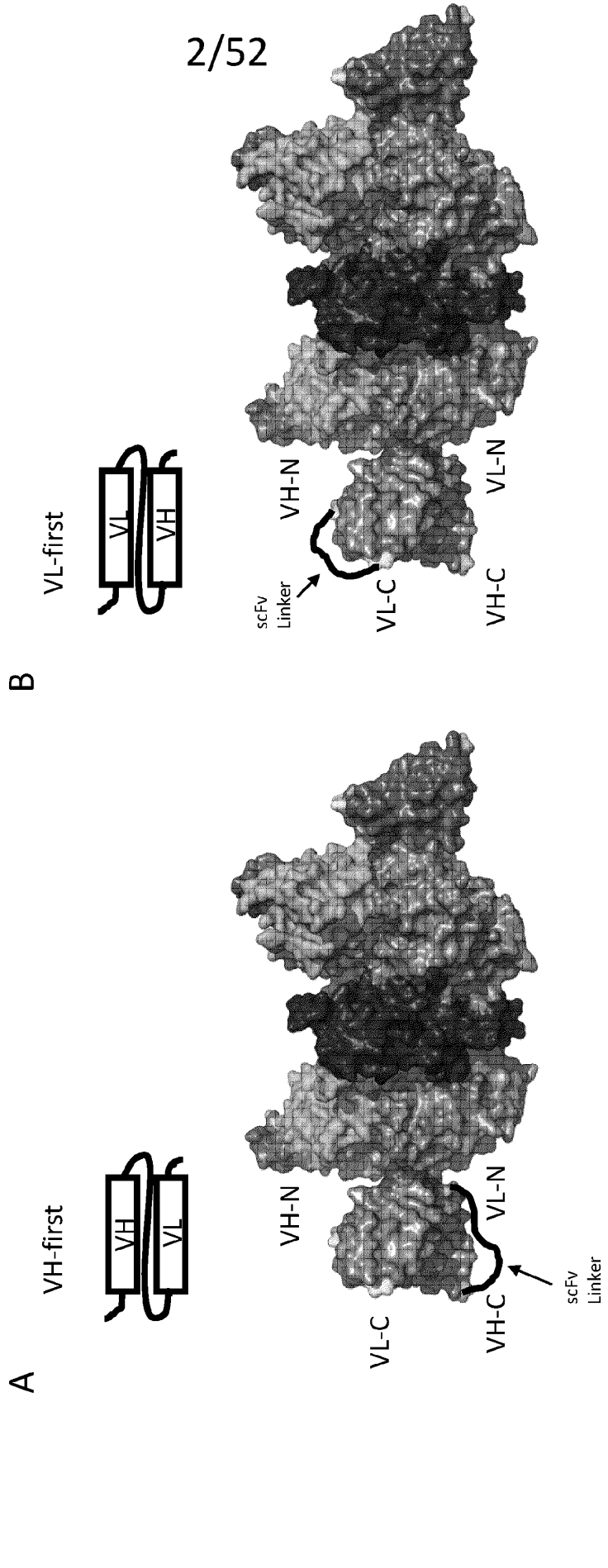

M1
Binding moiety
Human Transferrin Receptor
M1
Binding moiety
VH-N
VL-N
VL-C
VH-C

FIGURE 1

FIGURE 2


A
VH-first
VH
VL
VH-N
VL-N
VL-C
VH-C
scFv
Linker
B
VL-first
VL
VH
VH-N
VL-N
scFv
Linker
VL-C
VH-C

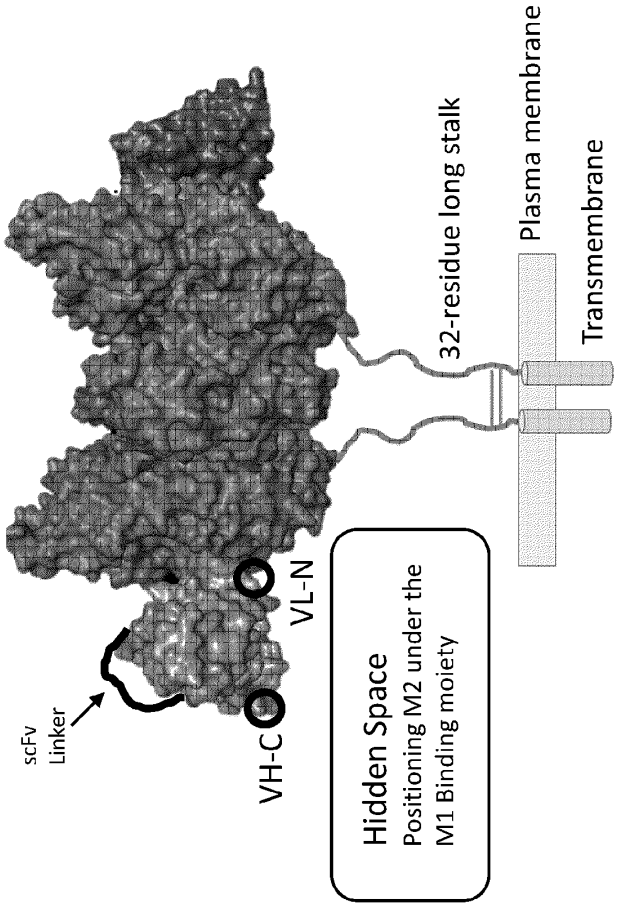

scFv
Linker
VH-C
VL-N
Hidden Space
Positioning M2 under the
M1 Binding moiety
32-residue long stalk
Plasma membrane
Transmembrane

FIGURE 3

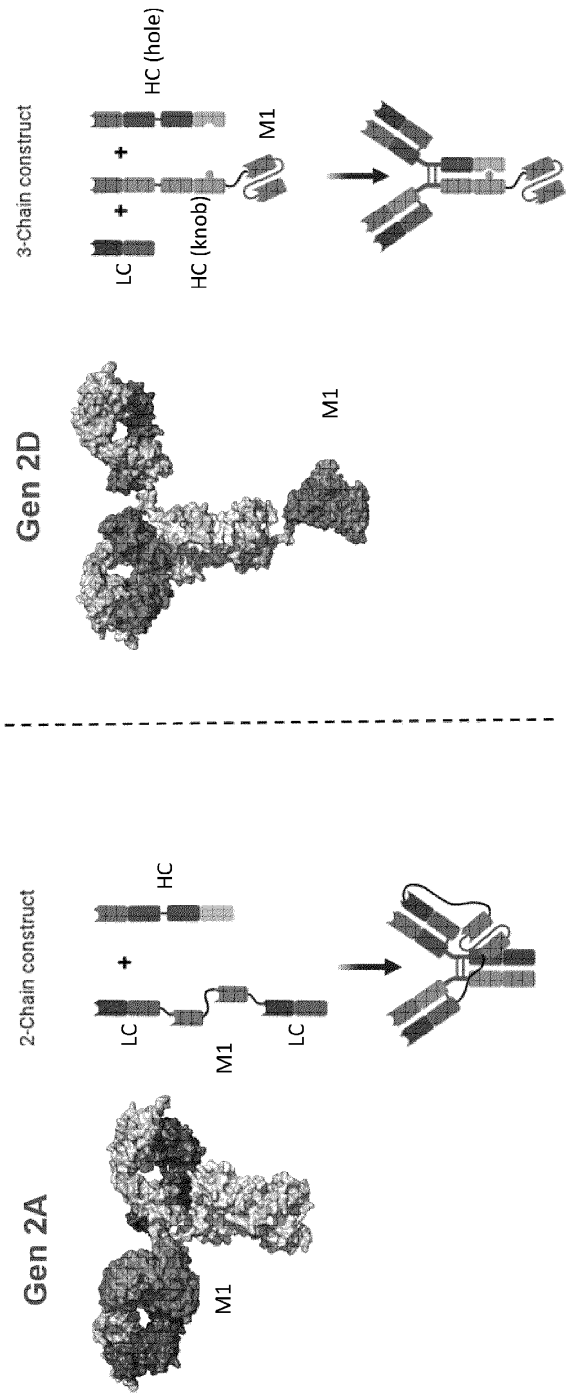

3-Chain construct
HC (hole)
M1
LC
HC (knob)
Gen 2D
M1
2-Chain construct
HC
LC
M1
LC
Gen 2A
M1

FIGURE 4

FIGURE 5

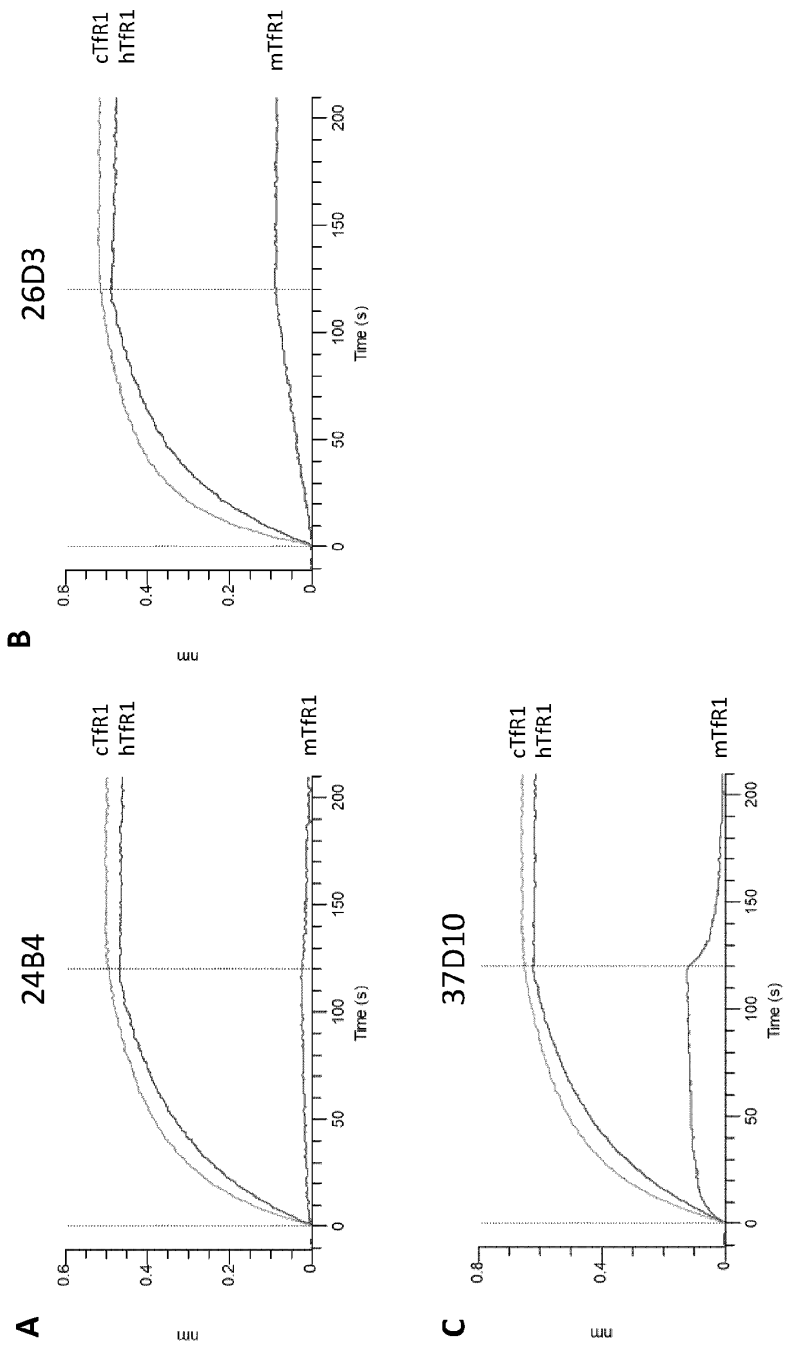

A
24B4
cTfR1
hTfR1
mTfR1
nm
Time (s)
B
26D3
cTfR1
hTfR1
mTfR1
nm
Time (s)
C
37D10
cTfR1
hTfR1
mTfR1
nm
Time (s)

FIGURE 6

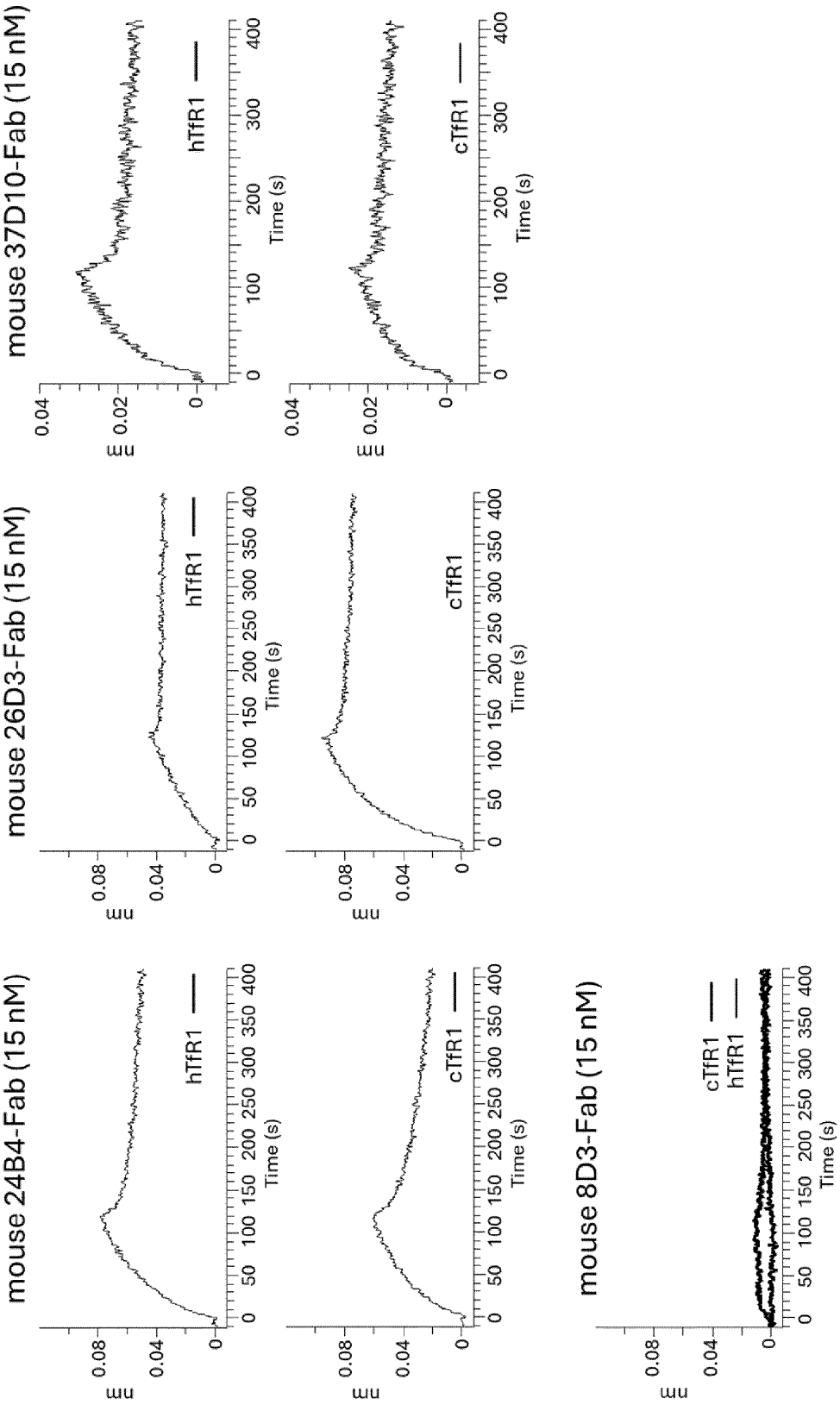
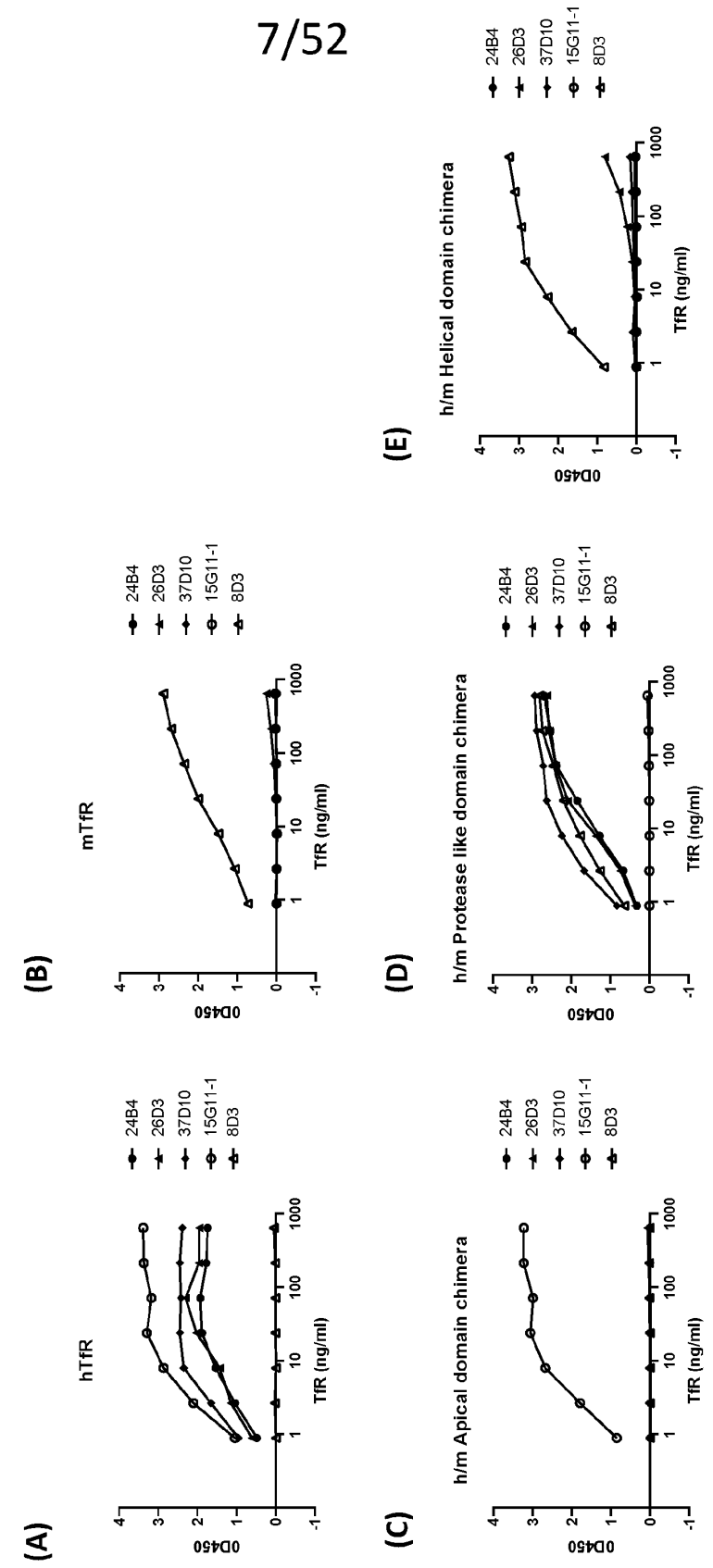

mouse 37D10-Fab (15 nM)
mouse 26D3-Fab (15 nM)
mouse 24B4-Fab (15 nM)
mouse 8D3-Fab (15 nM)
hTfR1
cTfR1
nm
Time (s)
0
0.02
0.04
0.08
0 50 100 150 200 250 300 350 400

FIGURE 7


(A)
hTfR
(B)
mTfR
(C)
h/m Apical domain chimera
(D)
h/m Protease like domain chimera
(E)
h/m Helical domain chimera
24B4
26D3
37D10
15G11-1
8D3
OD450
TfR (ng/ml)

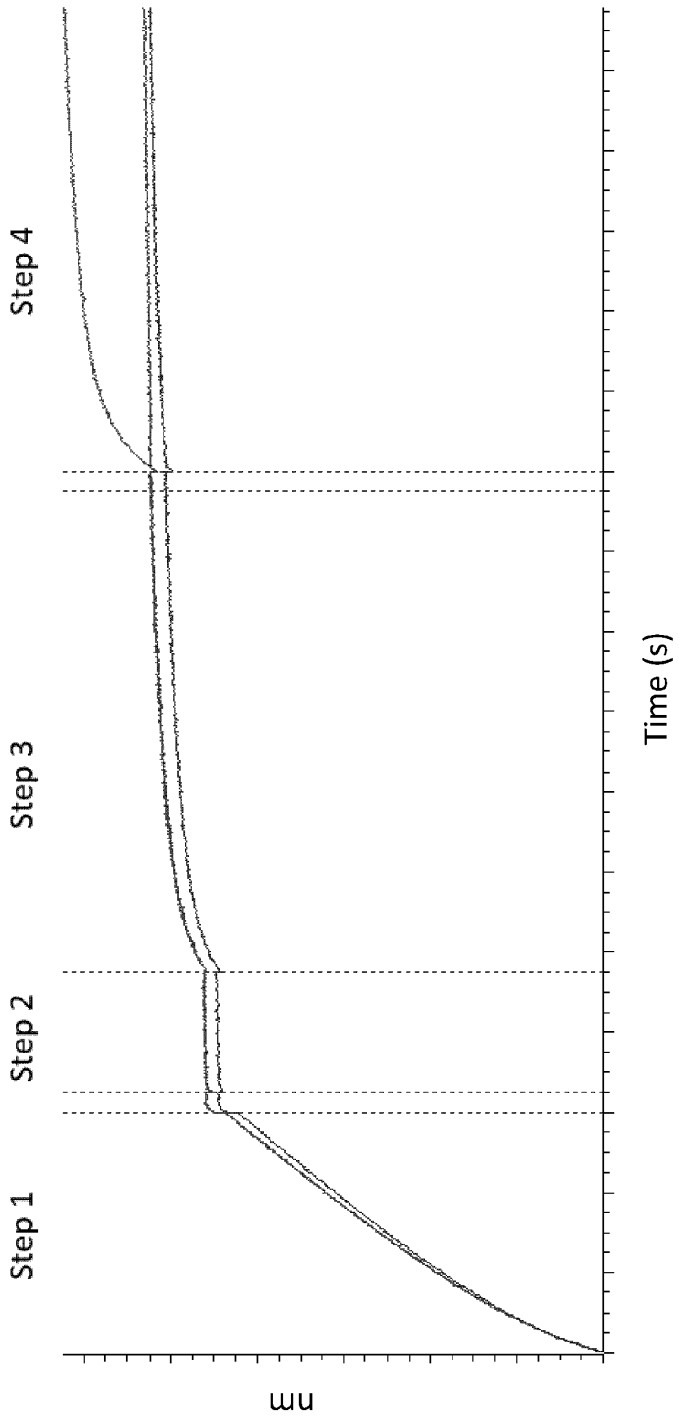


FIGURE 8

FIGURE 9A

A

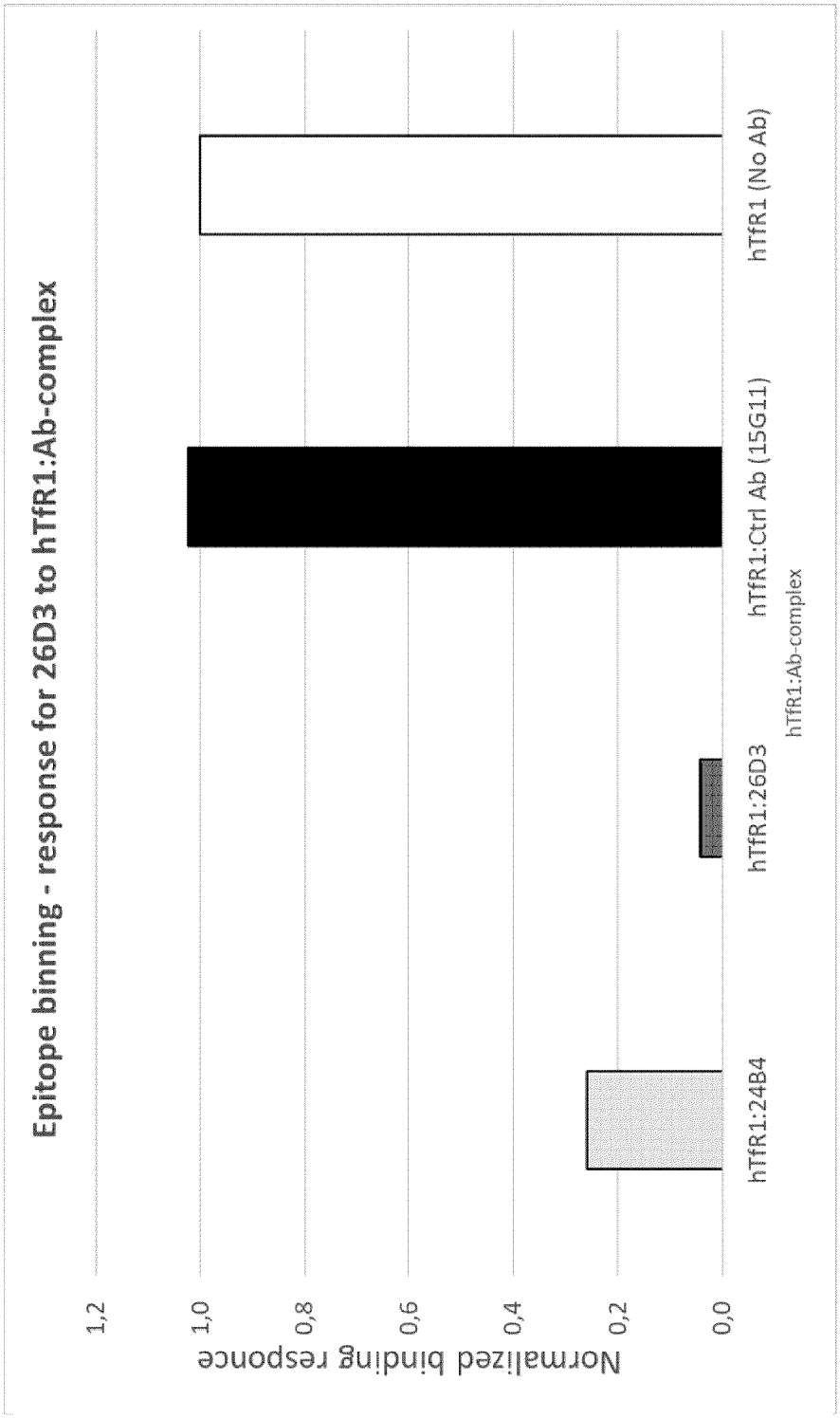
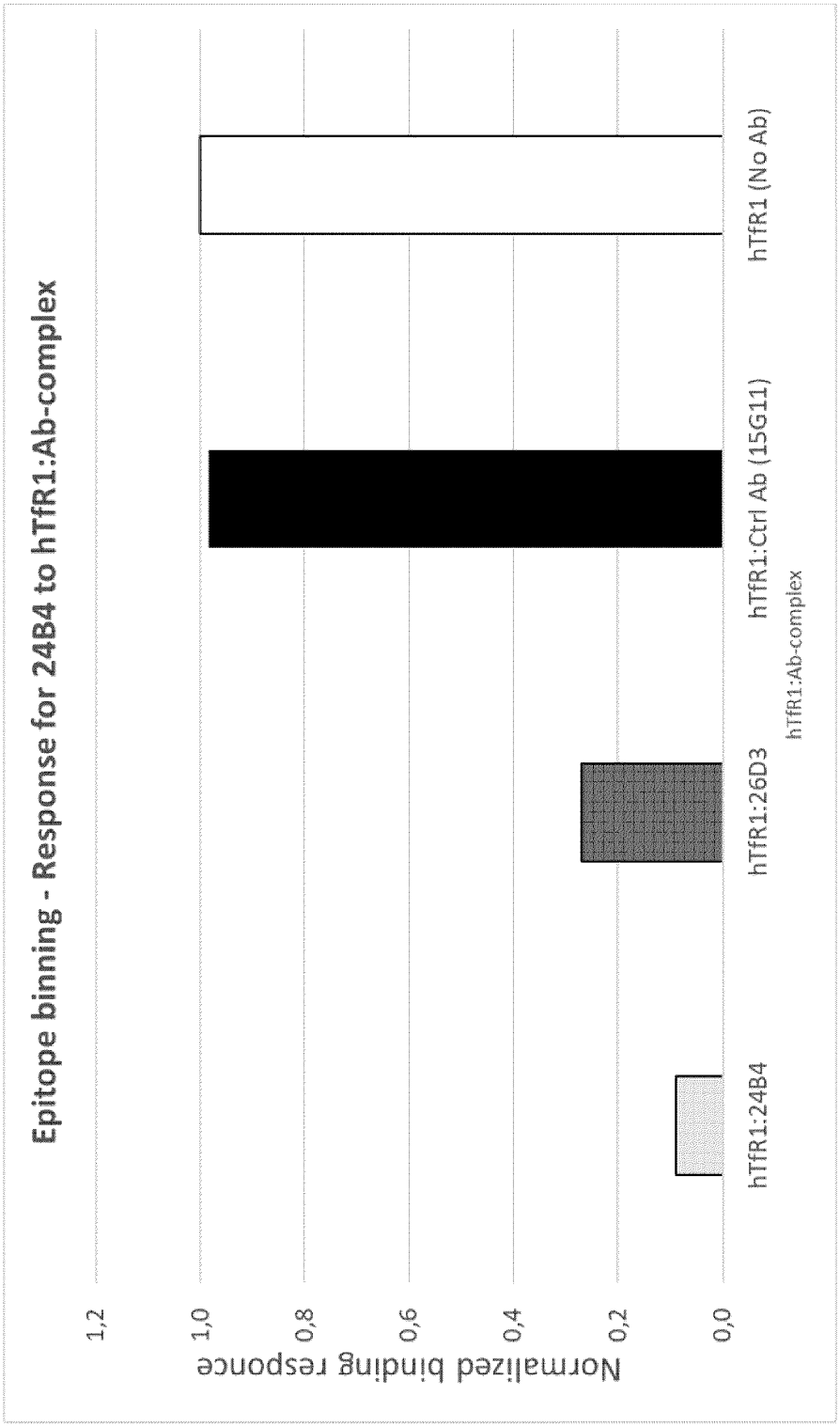
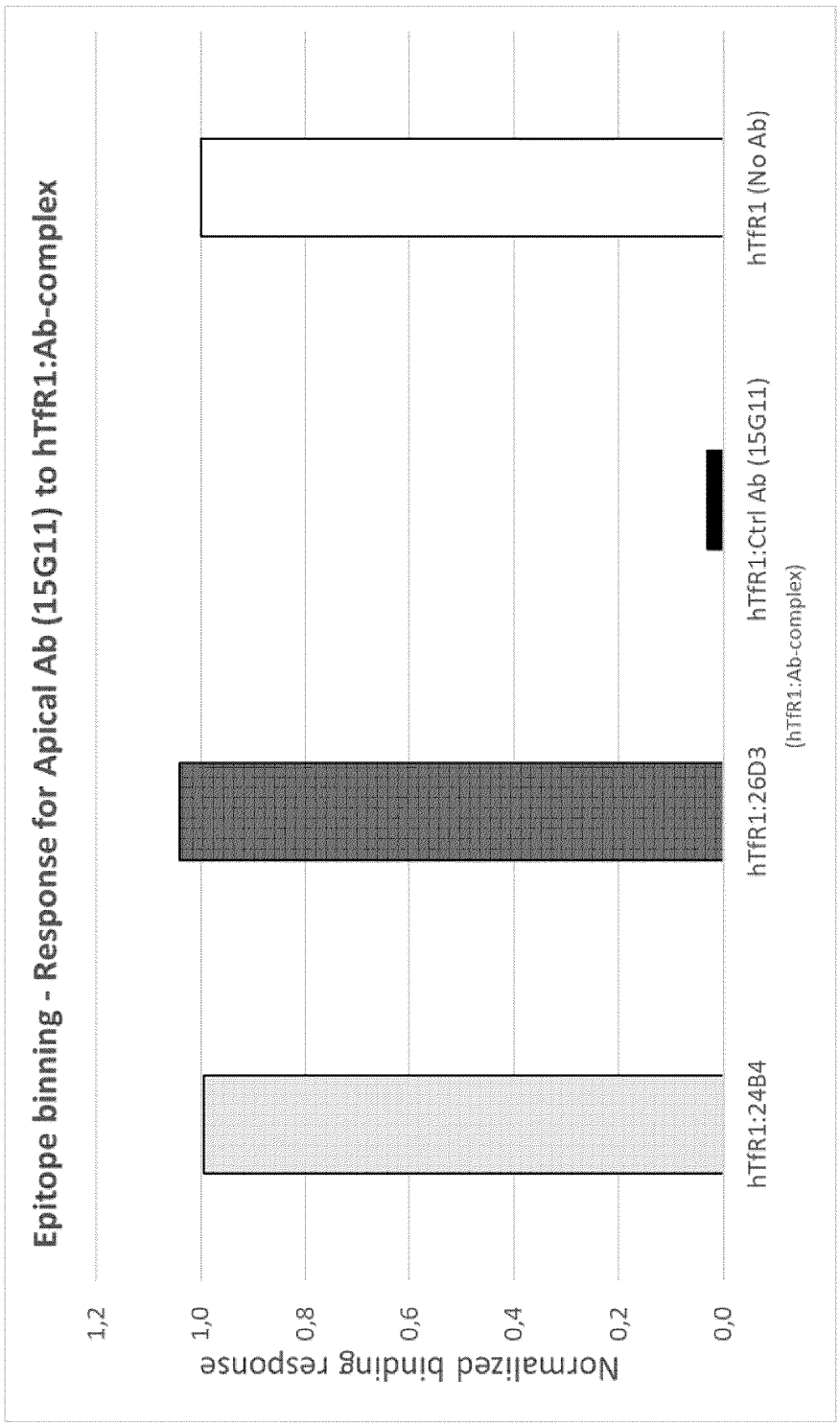

Epitope binning - response for 26D3 to hTfR1:Ab-complex
Normalized binding responce
1,2
1,0
0,8
0,6
0,4
0,2
0,0
hTfR1:24B4
hTfR1:26D3
hTfR1:Ctrl Ab (15G11)
hTfR1 (No Ab)
hTfR1:Ab-complex

FIGURE 9B

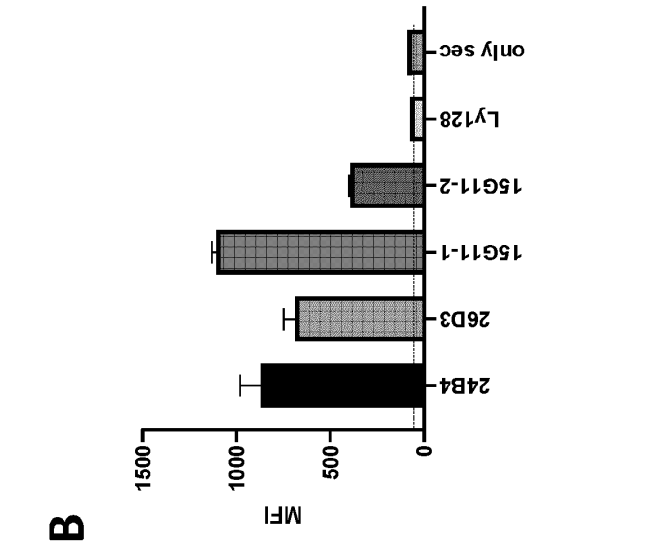
B


Epitope binning - Response for 24B4 to hTfR1:Ab-complex
Normalized binding responce
1,2
1,0
0,8
0,6
0,4
0,2
0,0
hTfR1:24B4
hTfR1:26D3
hTfR1:Ctrl Ab (15G11)
hTfR1 (No Ab)
hTfR1:Ab-complex


Epitope binning - Response for Apical Ab (15G11) to hTfR1:Ab-complex
Normalized binding response
1,2
1,0
0,8
0,6
0,4
0,2
0,0
hTfR1:24B4
hTfR1:26D3
hTfR1:Ctrl Ab (15G11)
(hTfR1:Ab-complex)
hTfR1 (No Ab)

C

FIGURE 9C


B
MFI
1500
1000
500
0
24B4
26D3
15G11-1
15G11-2
Ly128
only sec

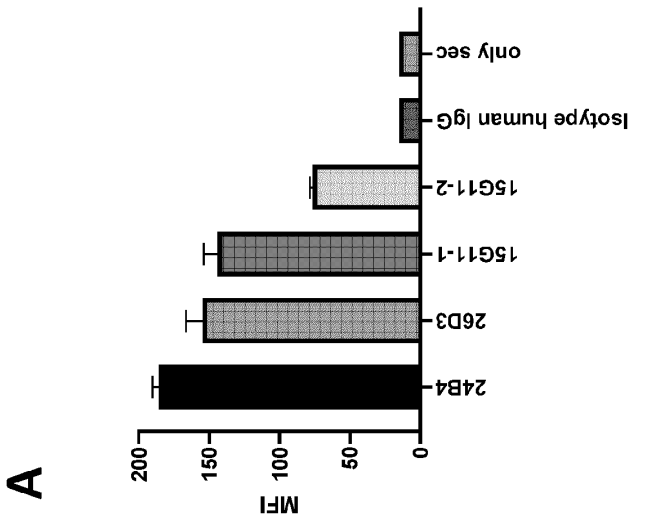
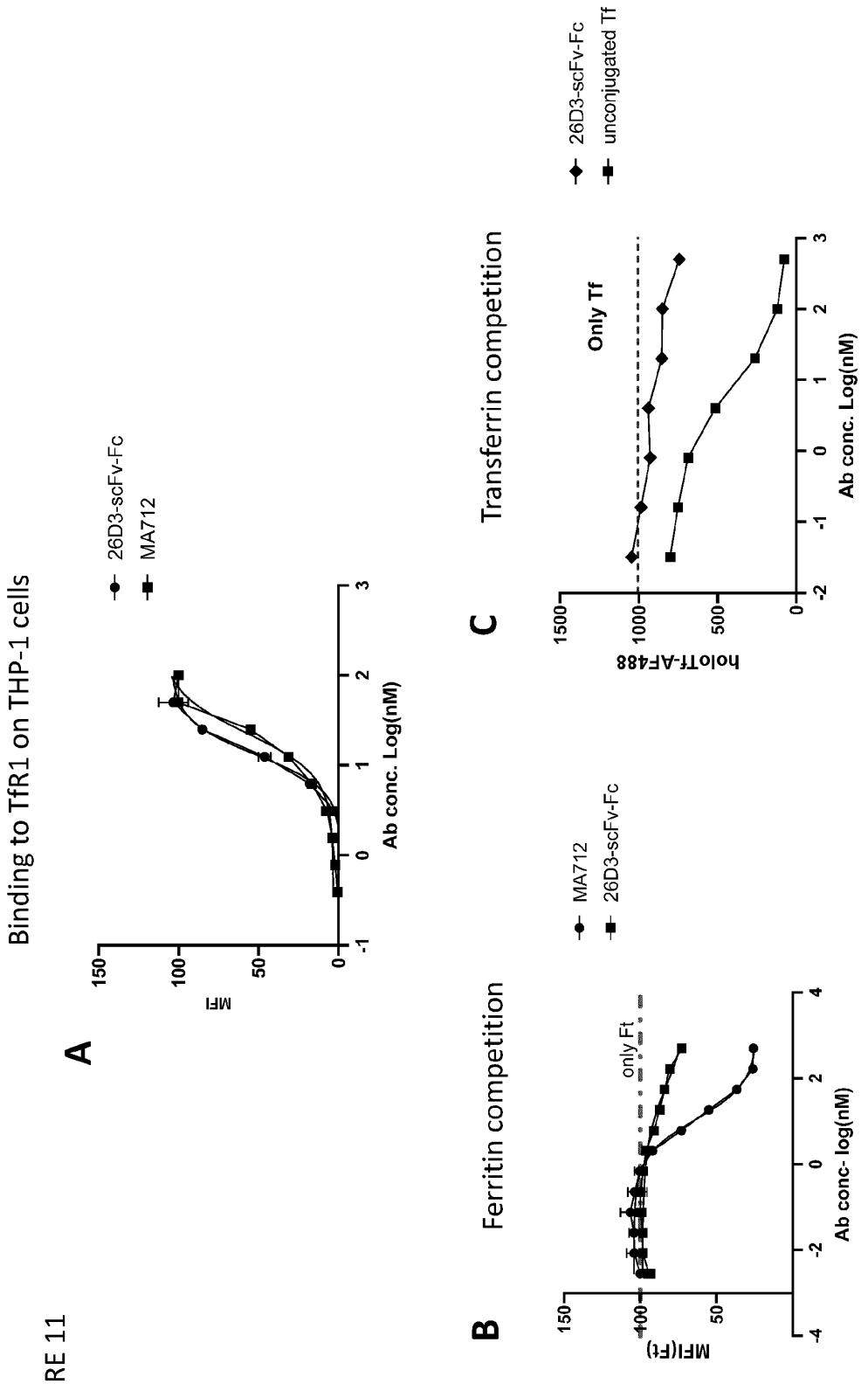

A
MFI
200
150
100
50
0
24B4
26D3
15G11-1
15G11-2
Isotype human IgG
only sec

FIGURE 10

FIGURE 11


A
Binding to TfR1 on THP-1 cells
26D3-scFv-Fc
MA712
MFI
Ab conc. Log(nM)
B
Ferritin competition
MA712
26D3-scFv-Fc
only Ft
MFI(Ft)
Ab conc- log(nM)
C
Transferrin competition
26D3-scFv-Fc
unconjugated Tf
Only Tf
holoTf-AF488
Ab conc. Log(nM)

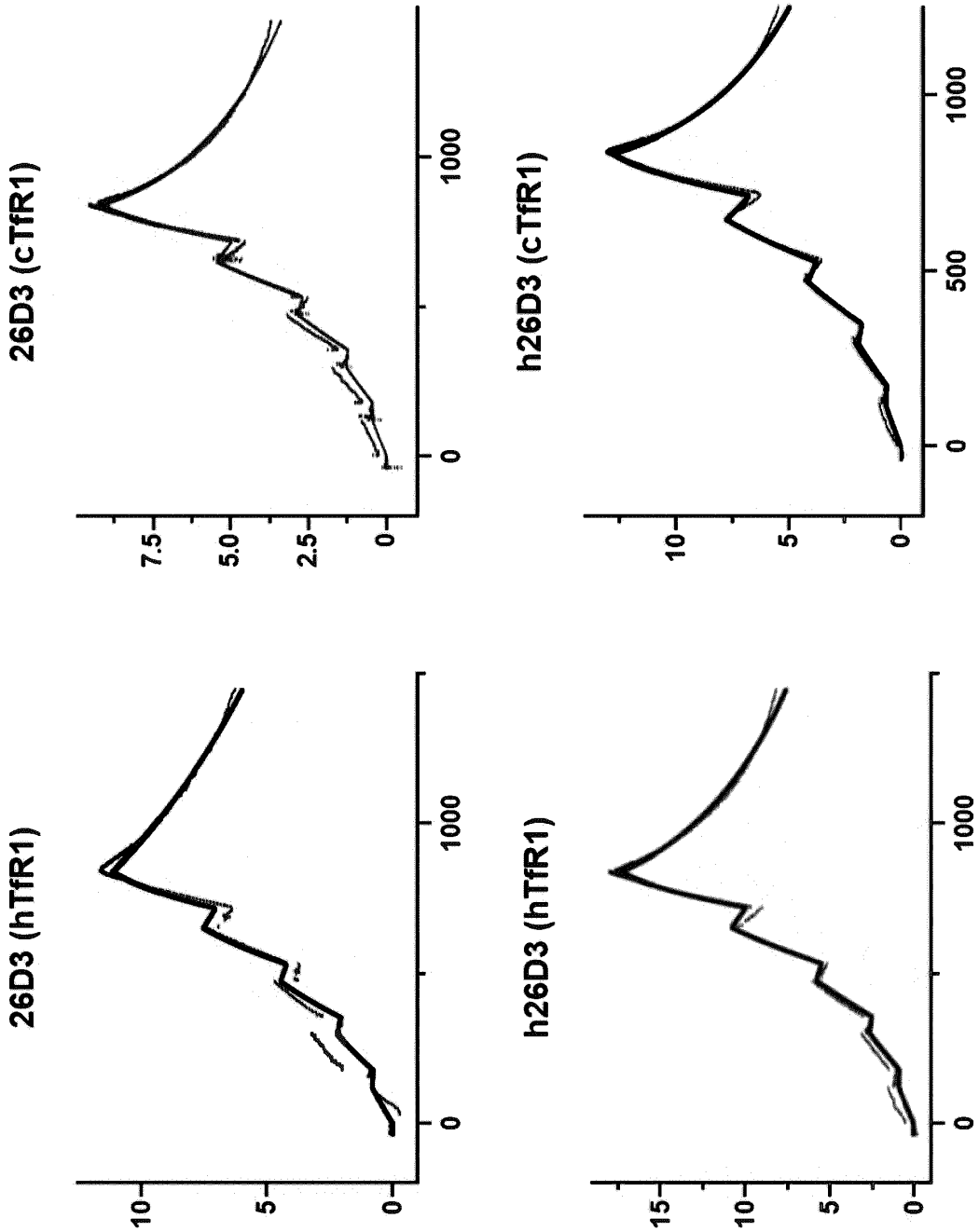

26D3 (cTfR1)
h26D3 (cTfR1)
26D3 (hTfR1)
h26D3 (hTfR1)
0
1000
500
2.5
5.0
7.5
5
10
15

FIGURE 12

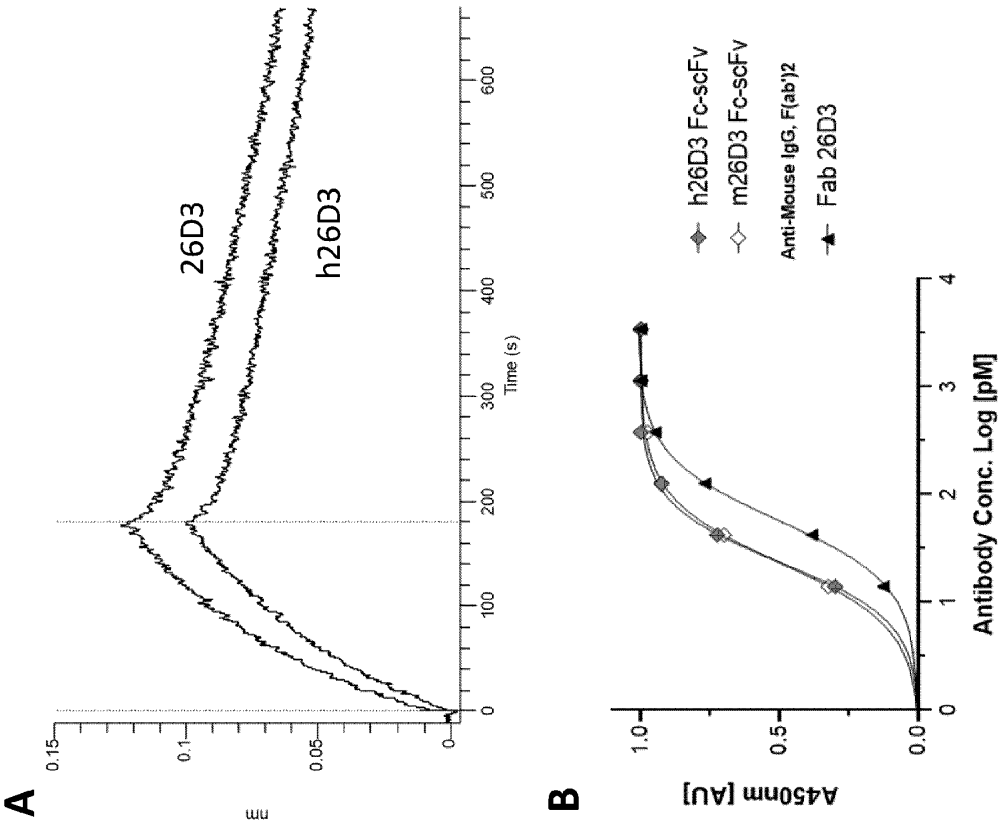

A
26D3
h26D3
nm
Time (s)
0.15
0.1
0.05
0
0
100
200
300
400
500
600
B
h26D3 Fc-scFv
m26D3 Fc-scFv
Anti-Mouse IgG, F(ab')2
Fab 26D3
A450nm [AU]
Antibody Conc. Log [pM]
1.0
0.5
0.0
0
1
2
3
4

FIGURE 13

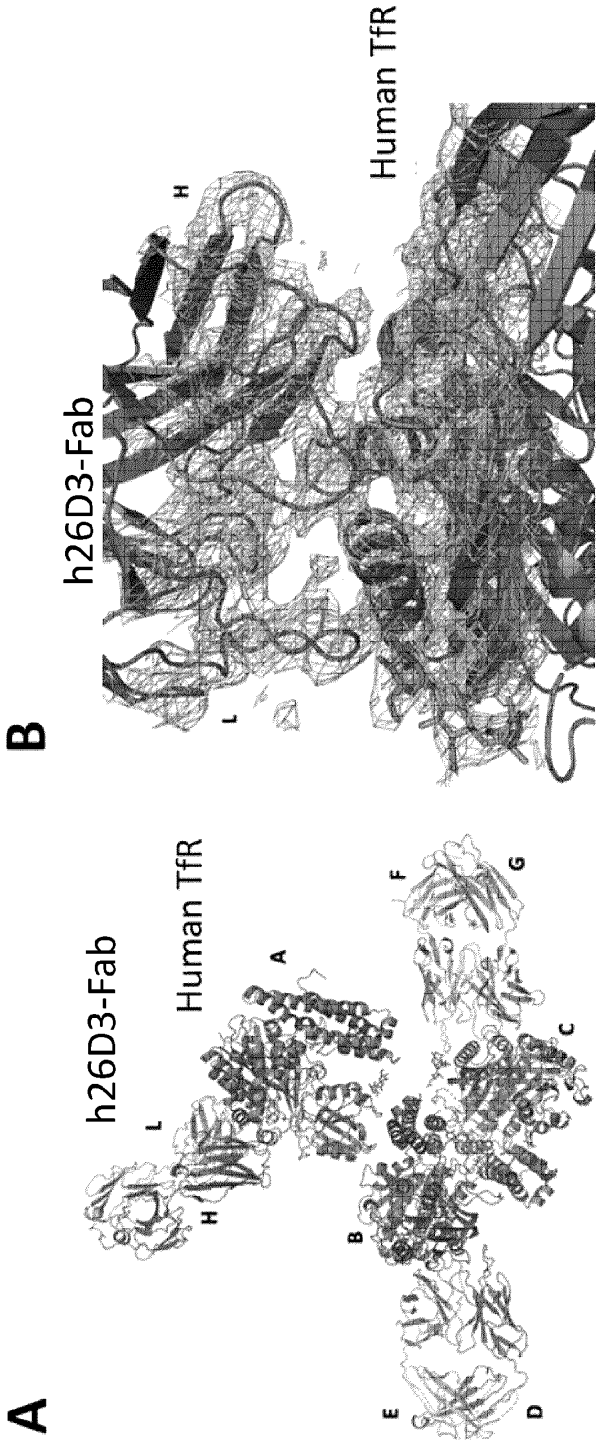


FIGURE 14

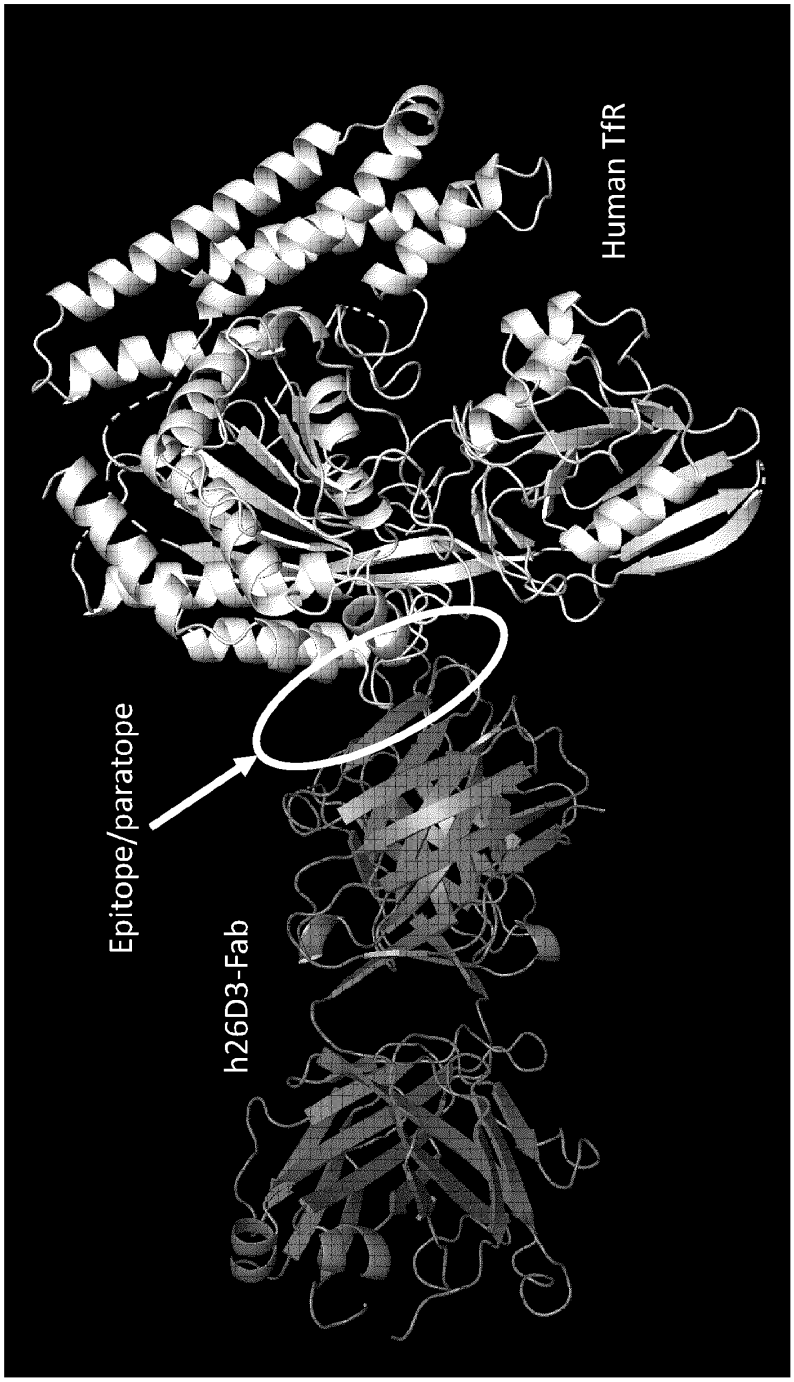


FIGURE 15

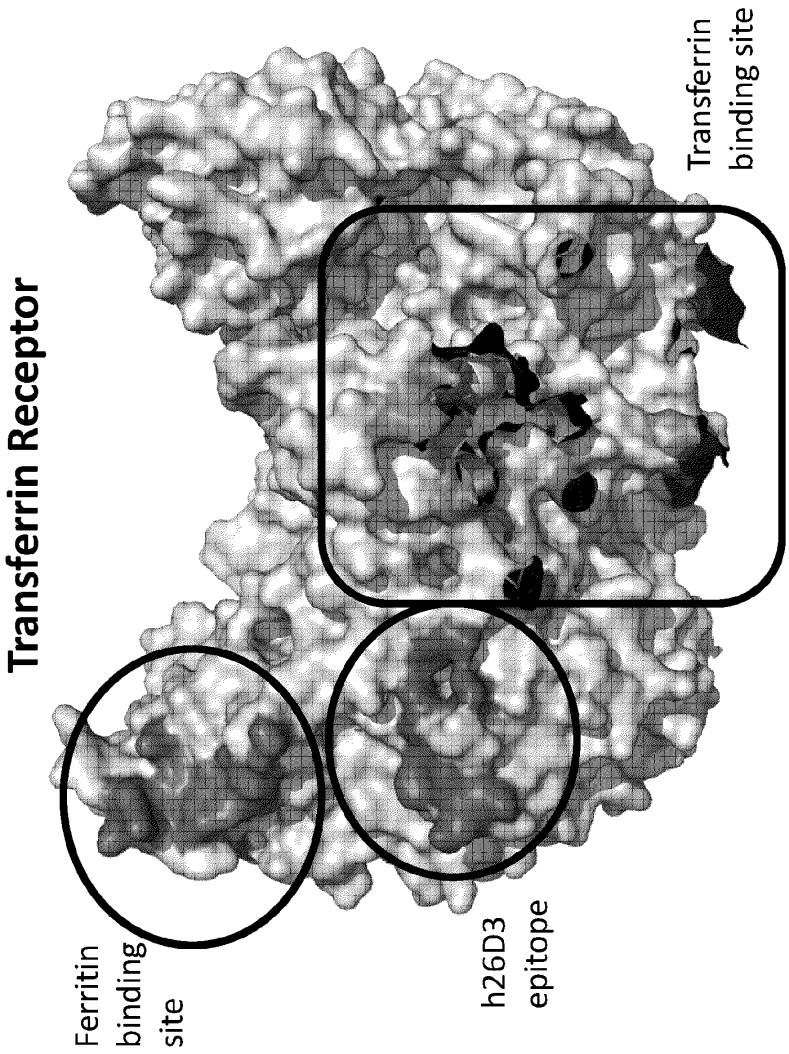
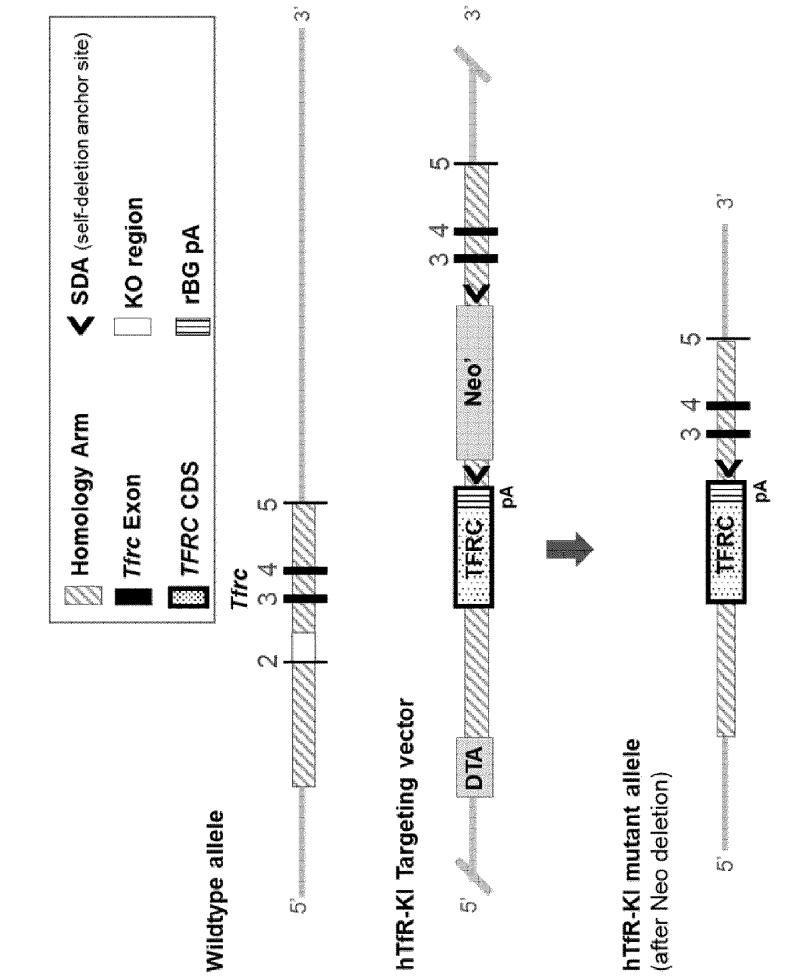

Transferrin Receptor
Transferrin binding site
Ferritin binding site
h26D3 epitope

FIGURE 16

FIGURE 17A


A
Homology Arm
SDA (self-deletion anchor site)
Tfrc Exon
KO region
TFRC CDS
rBG pA
Wildtype allele
Tfrc
2
3
4
5
5'
3'
hTfR-KI Targeting vector
DTA
TFRC
pA
Neo'
hTfR-KI mutant allele
(after Neo deletion)

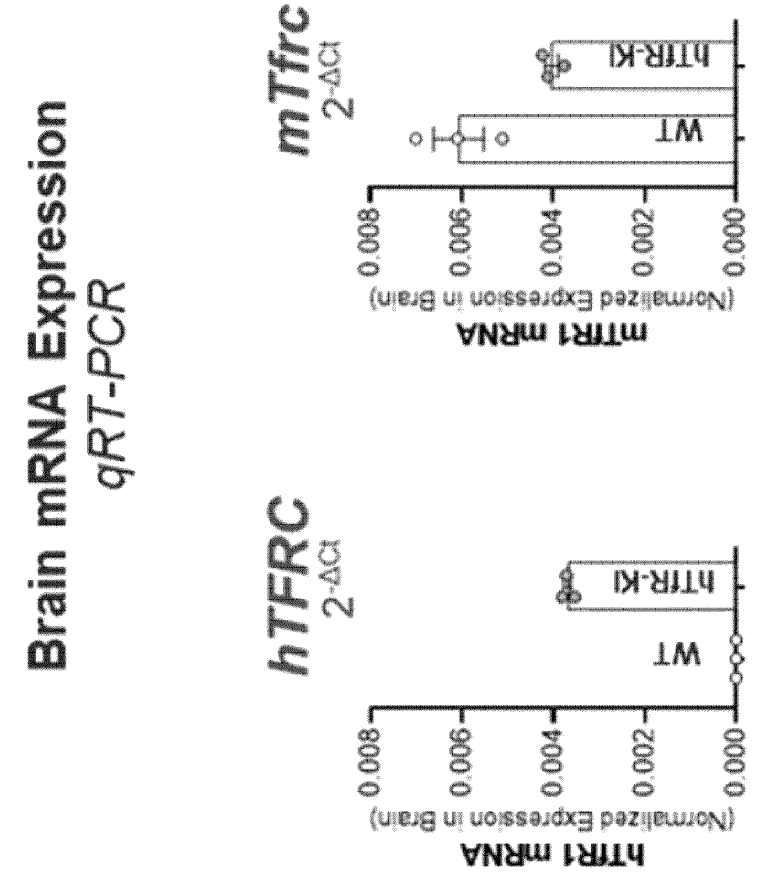

B
Brain mRNA Expression
qRT-PCR
hTFRC
2-ΔCt
hTfR1 mRNA
(Normalized Expression in Brain)
0.008
0.006
0.004
0.002
0.000
WT
hTfR-KI
mTfrc
2-ΔCt
mTfR1 mRNA
(Normalized Expression in Brain)
0.008
0.006
0.004
0.002
0.000
WT
hTfR-KI

FIGURE 17B

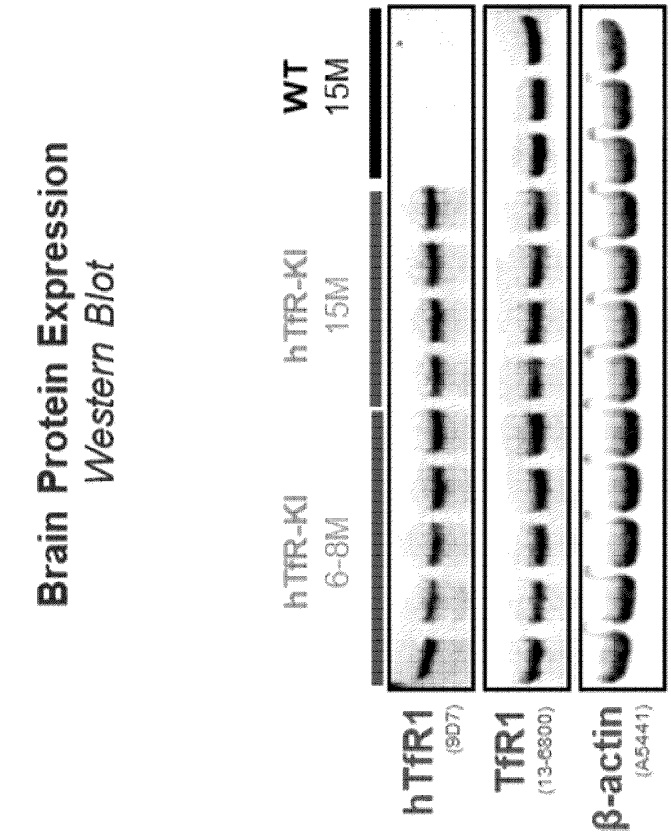
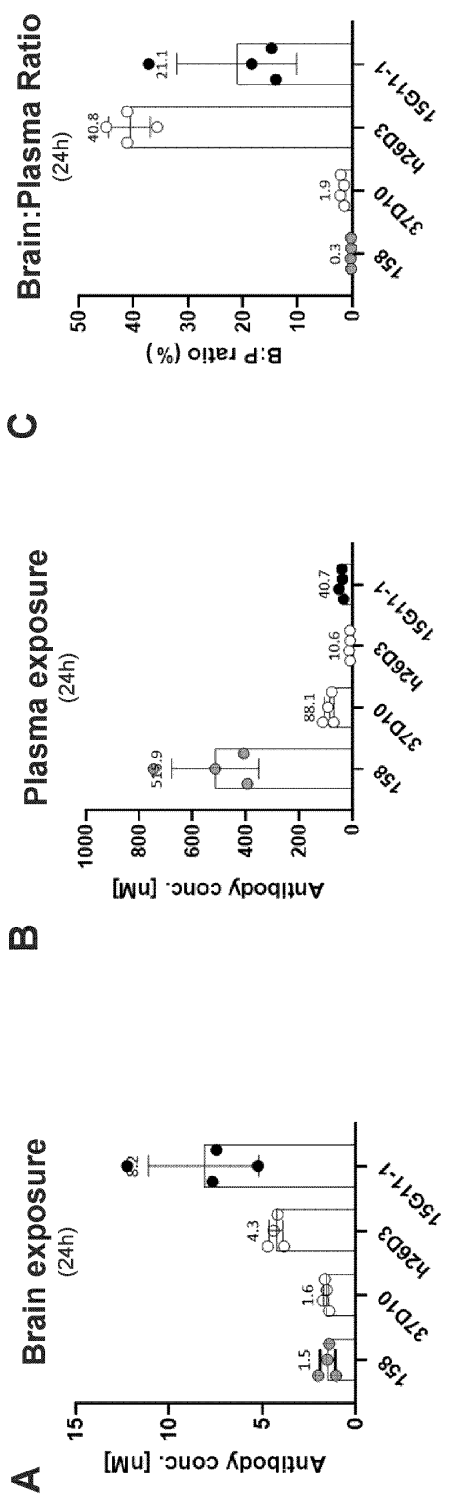

C
Brain Protein Expression
Western Blot
hTfR-KI
6-8M
hTfR-KI
15M
WT
15M
hTfR1
(9D7)
TfR1
(13-6800)
β-actin
(A5441)

FIGURE 17C


A
Brain exposure
(24h)
Antibody conc. [nM]
15
10
5
0
1.5
1.6
4.3
15
15G11-1
h26D3
37D10
15B

B
Plasma exposure
(24h)
Antibody conc. [nM]
1000
800
600
400
200
0
519.9
88.1
10.6
40.7
15B
37D10
h26D3
15G11-1

C
Brain:Plasma Ratio
(24h)
B:P ratio (%)
50
40
30
20
10
0
0.3
1.9
40.8
21.1
15B
37D10
h26D3
15G11-1

FIGURE 18

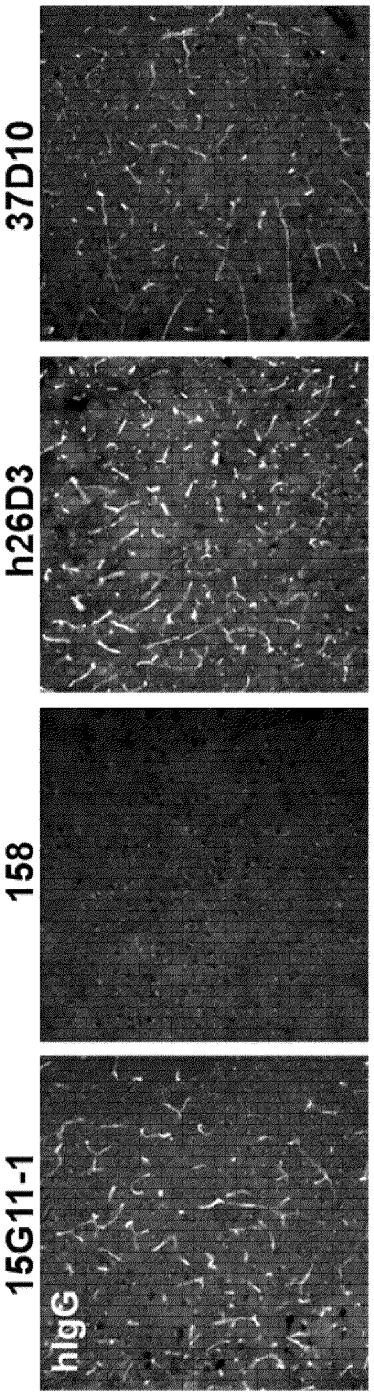
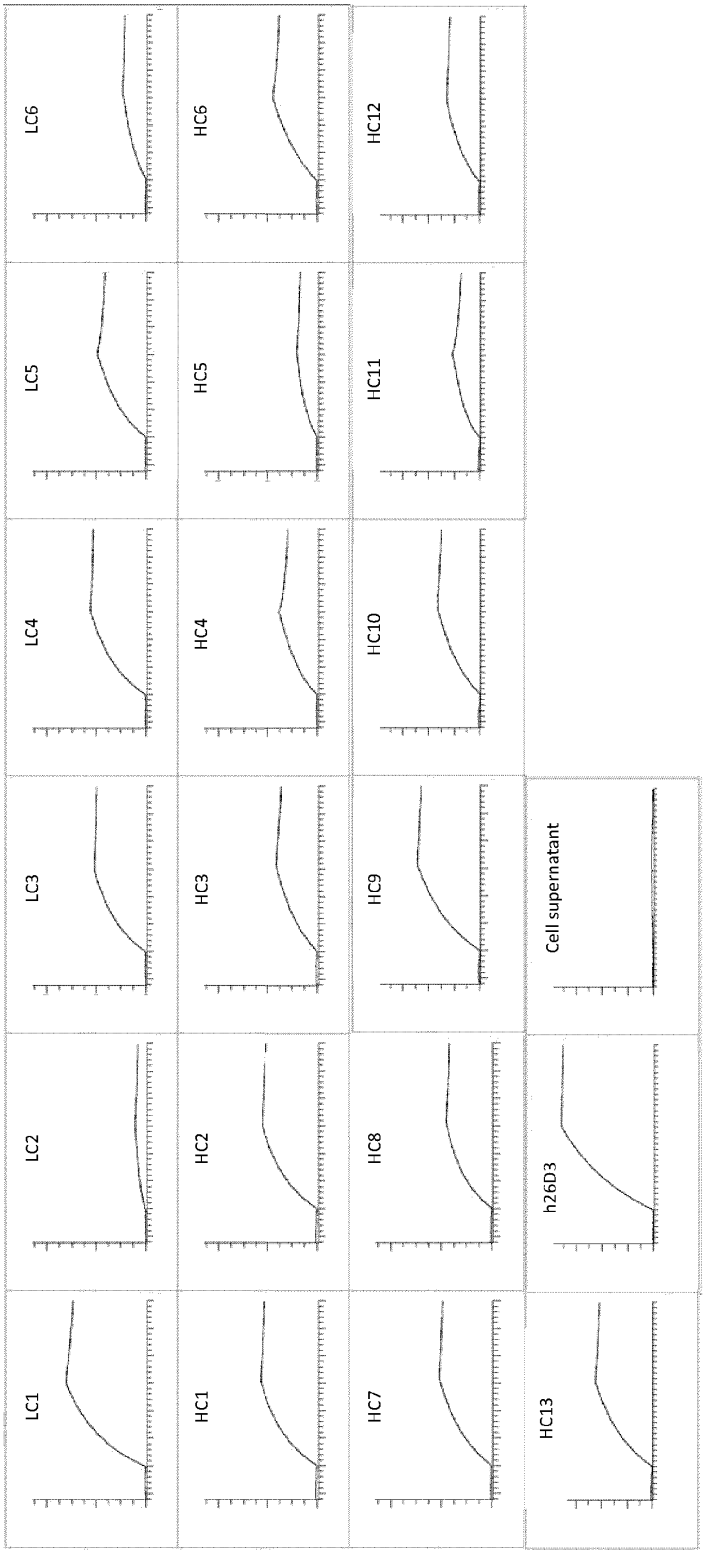


FIGURE 19

FIGURE 20


LC6
HC6
HC12
LC5
HC5
HC11
LC4
HC4
HC10
LC3
HC3
HC9
Cell supernatant
LC2
HC2
HC8
h26D3
LC1
HC1
HC7
HC13

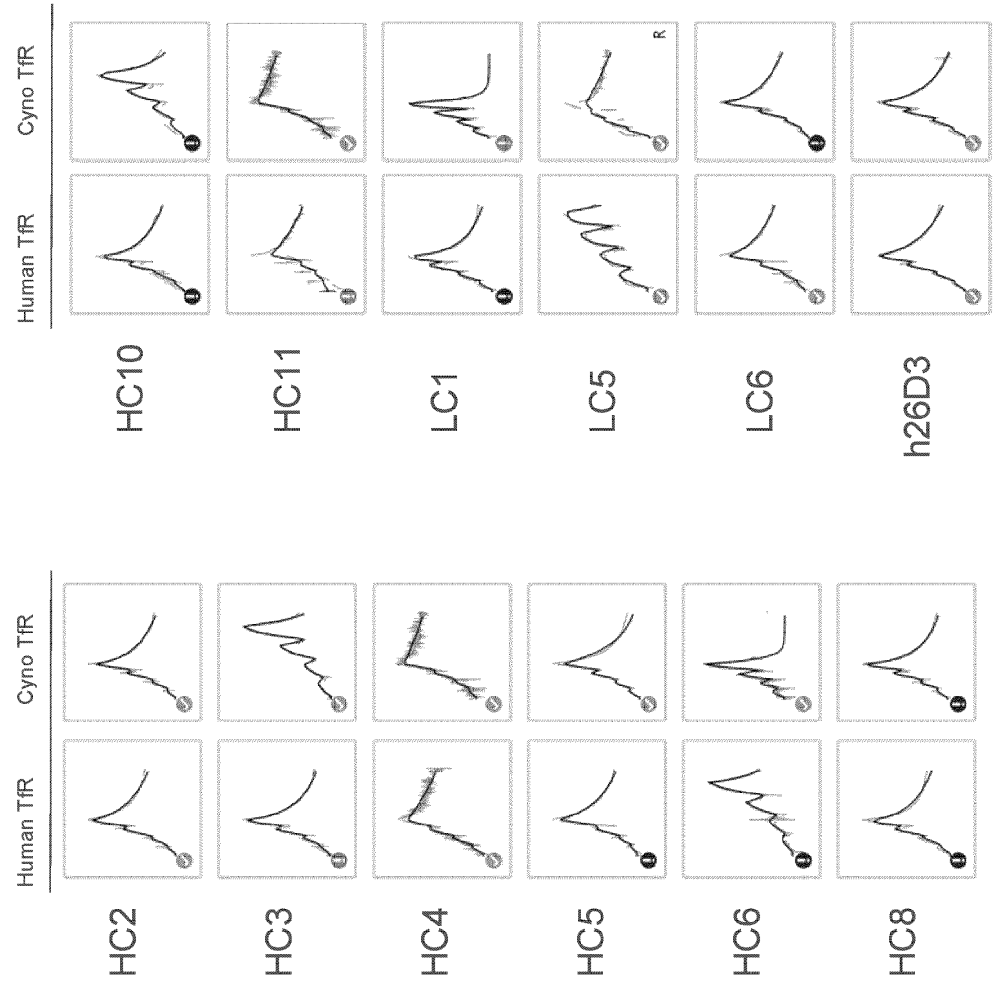
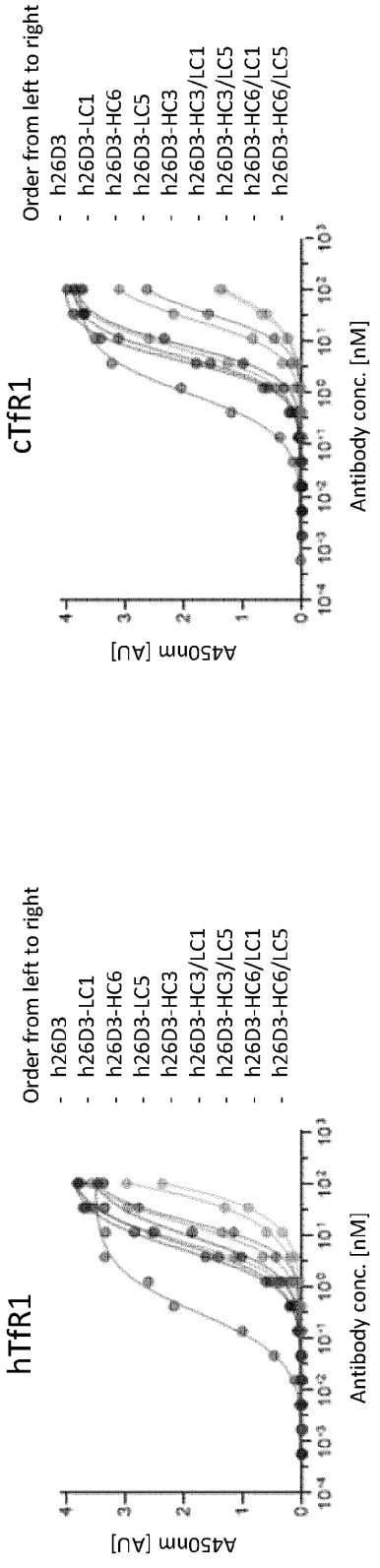
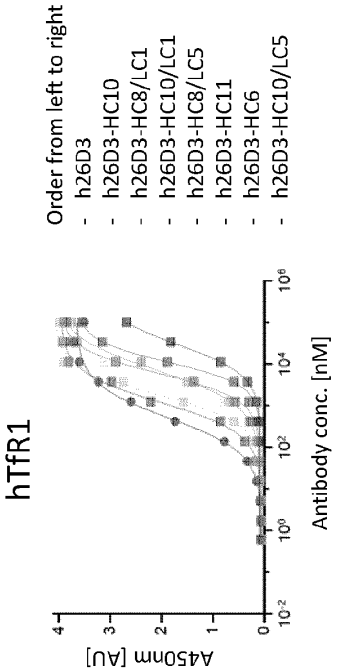

Human TfR
Cyno TfR
HC10
HC11
LC1
LC5
LC6
h26D3
HC2
HC3
HC4
HC5
HC6
HC8

FIGURE 21

FIGURE 22


hTfR1
A450nm [AU]
Antibody conc. [nM]
Order from left to right
- h26D3
- h26D3-LC1
- h26D3-HC6
- h26D3-LC5
- h26D3-HC3
- h26D3-HC3/LC1
- h26D3-HC3/LC5
- h26D3-HC6/LC1
- h26D3-HC6/LC5

cTfR1
A450nm [AU]
Antibody conc. [nM]
Order from left to right
- h26D3
- h26D3-LC1
- h26D3-HC6
- h26D3-LC5
- h26D3-HC3
- h26D3-HC3/LC1
- h26D3-HC3/LC5
- h26D3-HC6/LC1
- h26D3-HC6/LC5


hTfR1
A450nm [AU]
Antibody conc. [nM]
Order from left to right
- h26D3
- h26D3-HC10
- h26D3-HC8/LC1
- h26D3-HC10/LC1
- h26D3-HC8/LC5
- h26D3-HC11
- h26D3-HC6
- h26D3-HC10/LC5

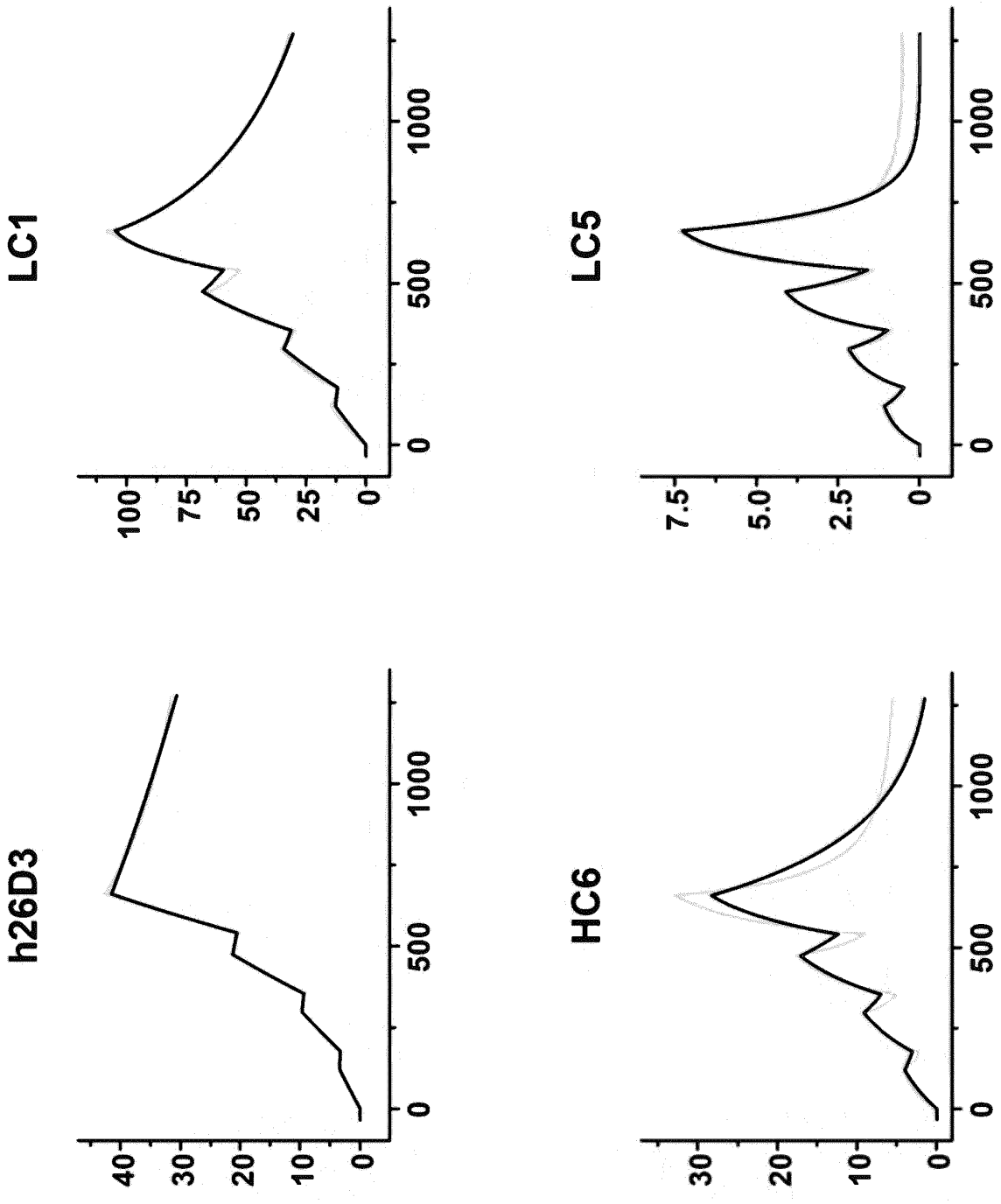

LC1
100
75
50
25
0
0
500
1000
LC5
7.5
5.0
2.5
0
0
500
1000
h26D3
40
30
20
10
0
0
500
1000
HC6
30
20
10
0
0
500
1000

FIGURE 23

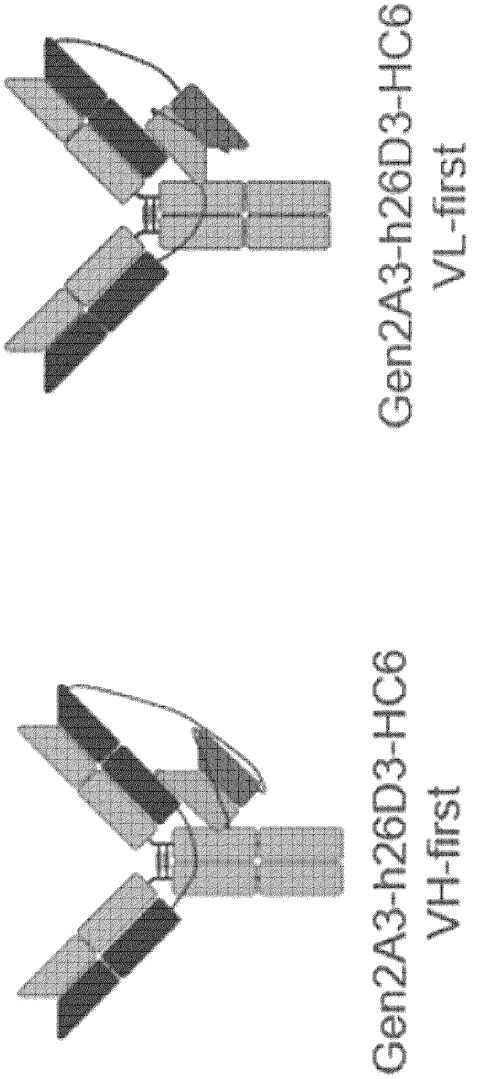


FIGURE 24

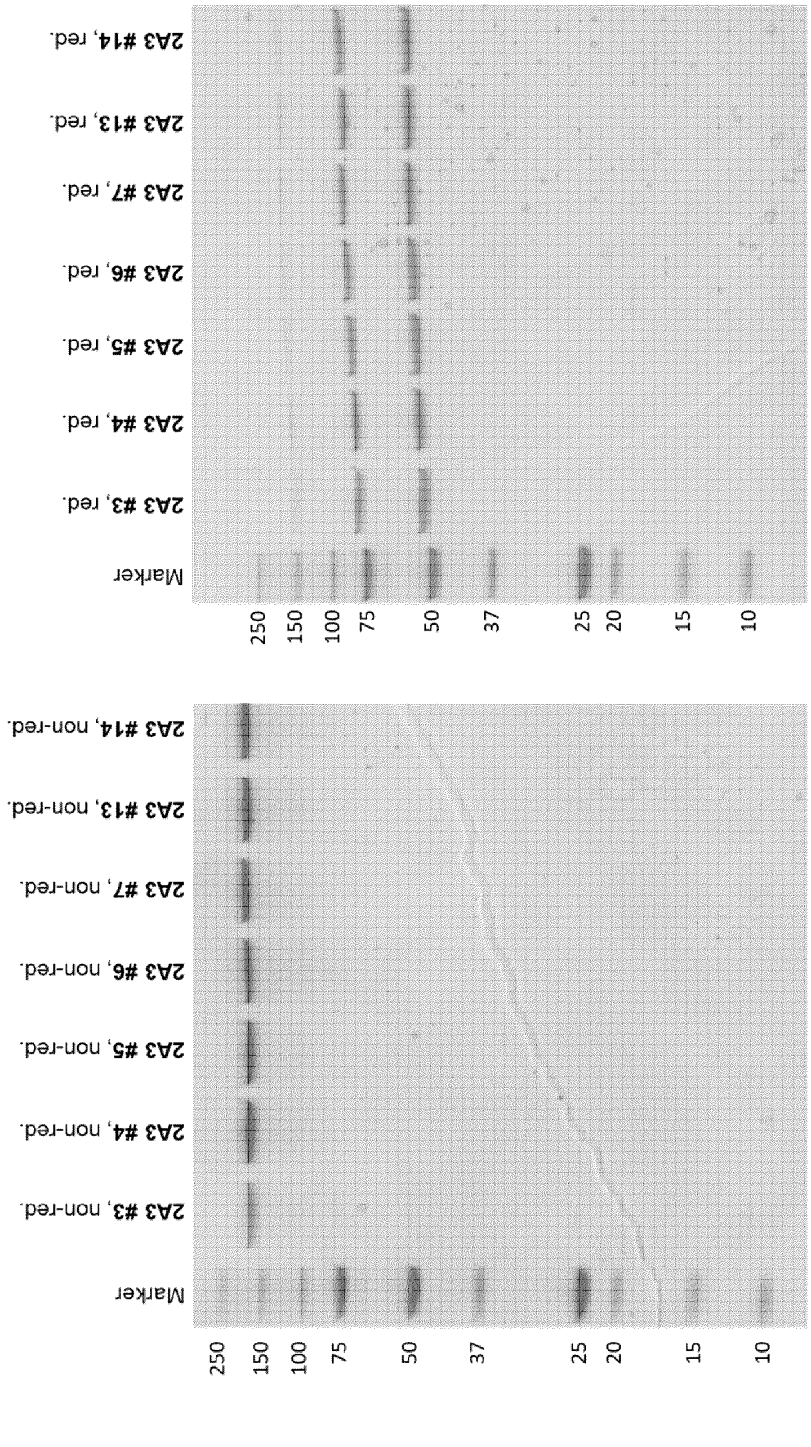
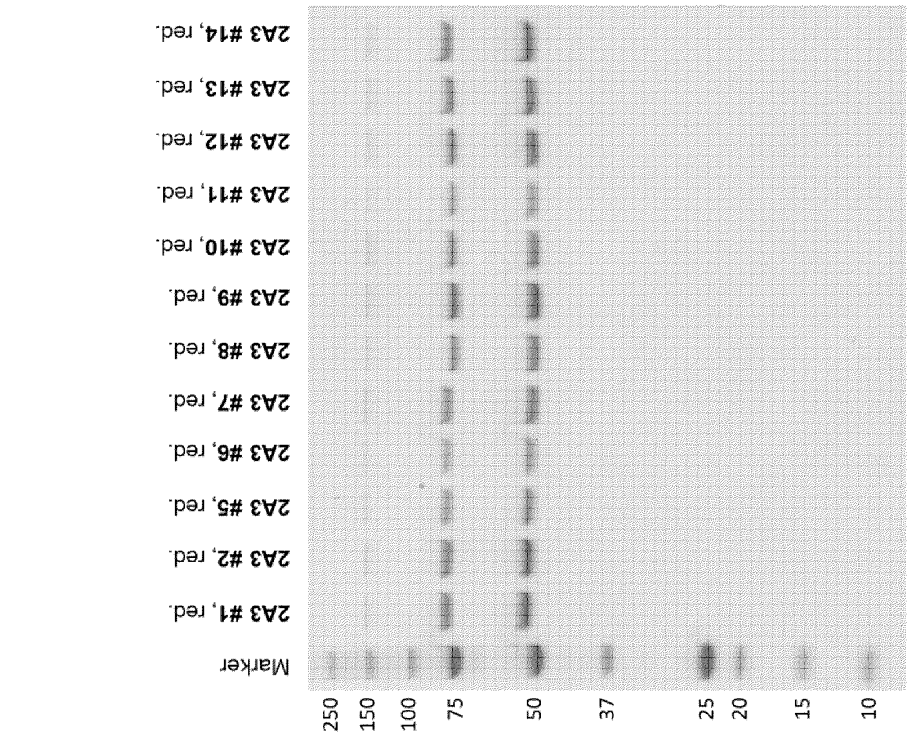

Marker
2A3 #3, non-red.
2A3 #4, non-red.
2A3 #5, non-red.
2A3 #6, non-red.
2A3 #7, non-red.
2A3 #13, non-red.
2A3 #14, non-red.
250
150
100
75
50
37
25
20
15
10
Marker
2A3 #3, red.
2A3 #4, red.
2A3 #5, red.
2A3 #6, red.
2A3 #7, red.
2A3 #13, red.
2A3 #14, red.
250
150
100
75
50
37
25
20
15
10

FIGURE 25



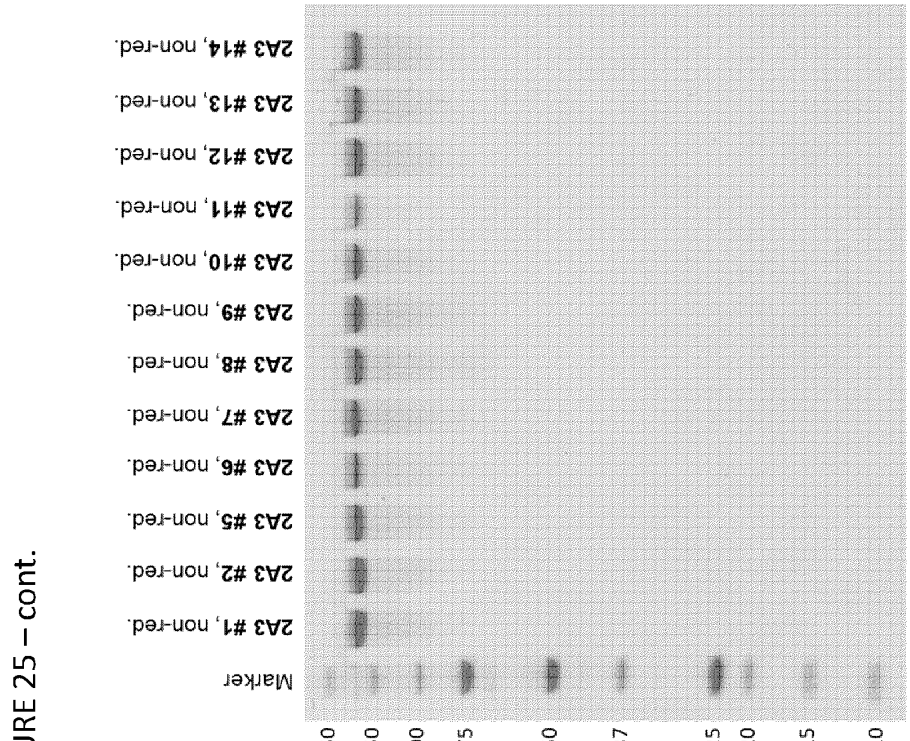
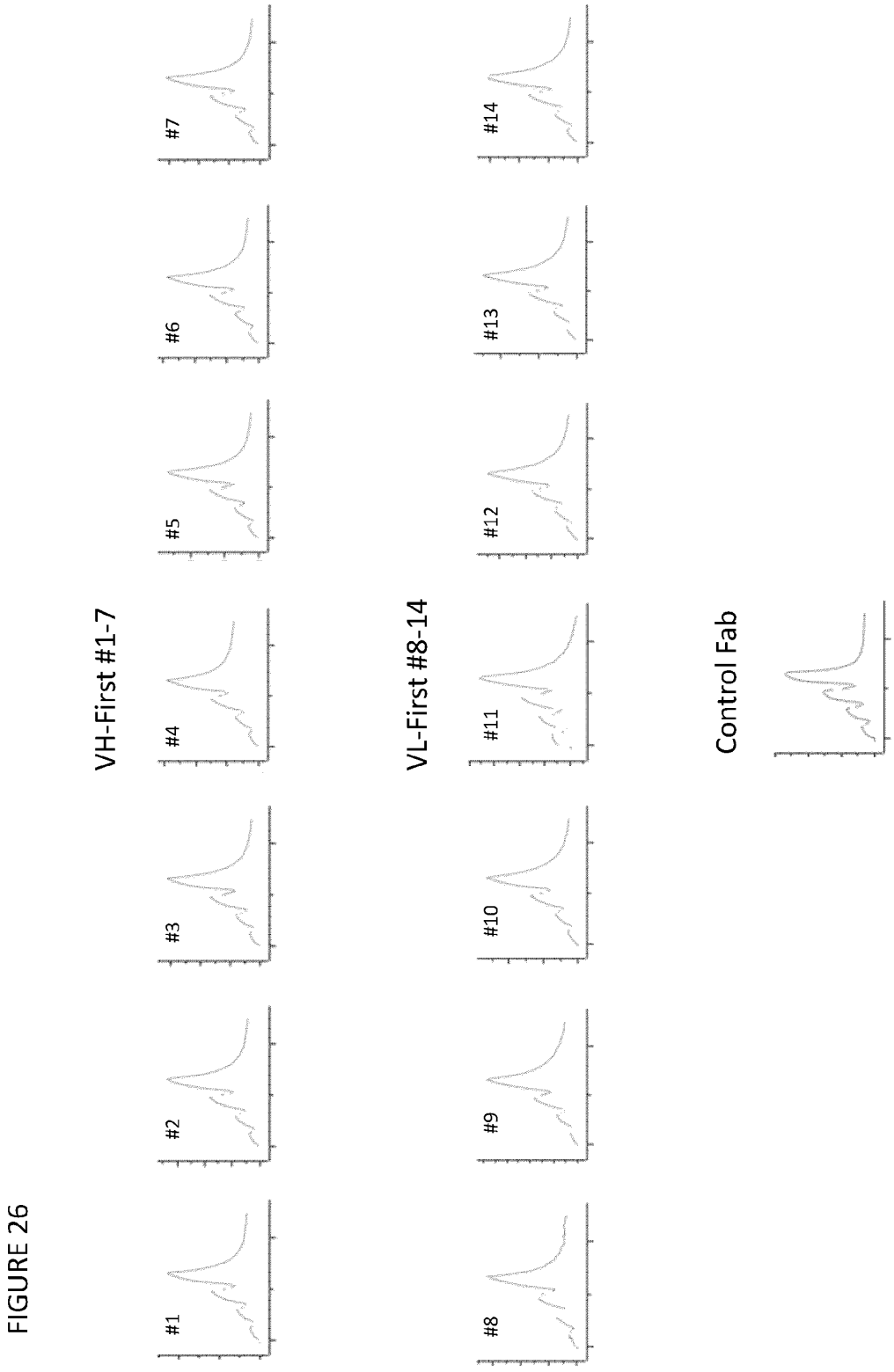


FIGURE 25 – cont.

FIGURE 26



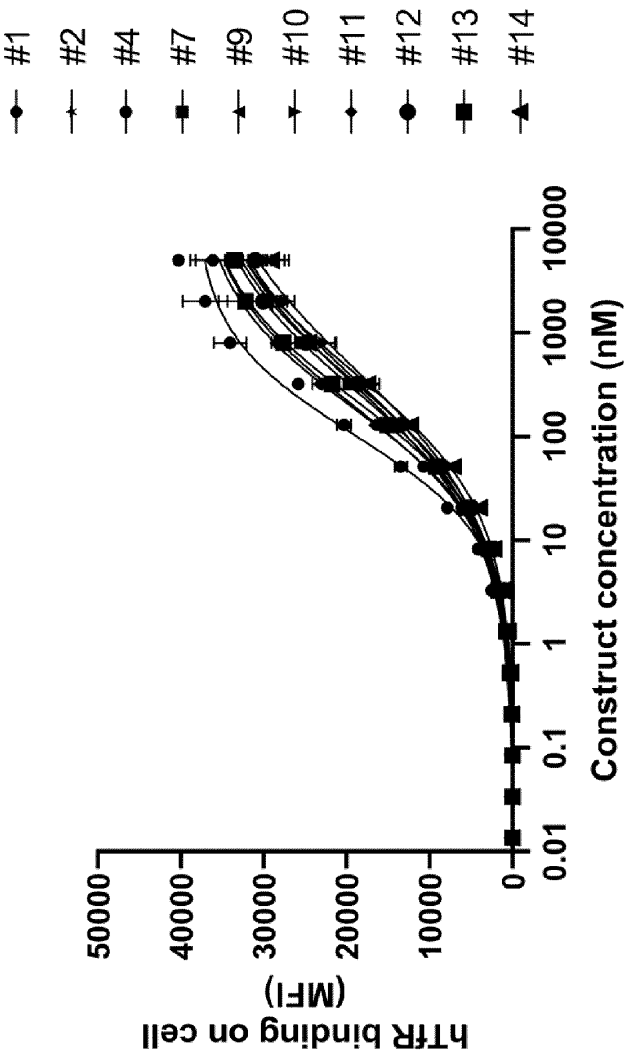

#1
#2
#4
#7
#9
#10
#11
#12
#13
#14
hTfR binding on cell (MFI)
50000
40000
30000
20000
10000
0
0.01
0.1
1
10
100
1000
10000
Construct concentration (nM)

FIGURE 27

FIGURE 28

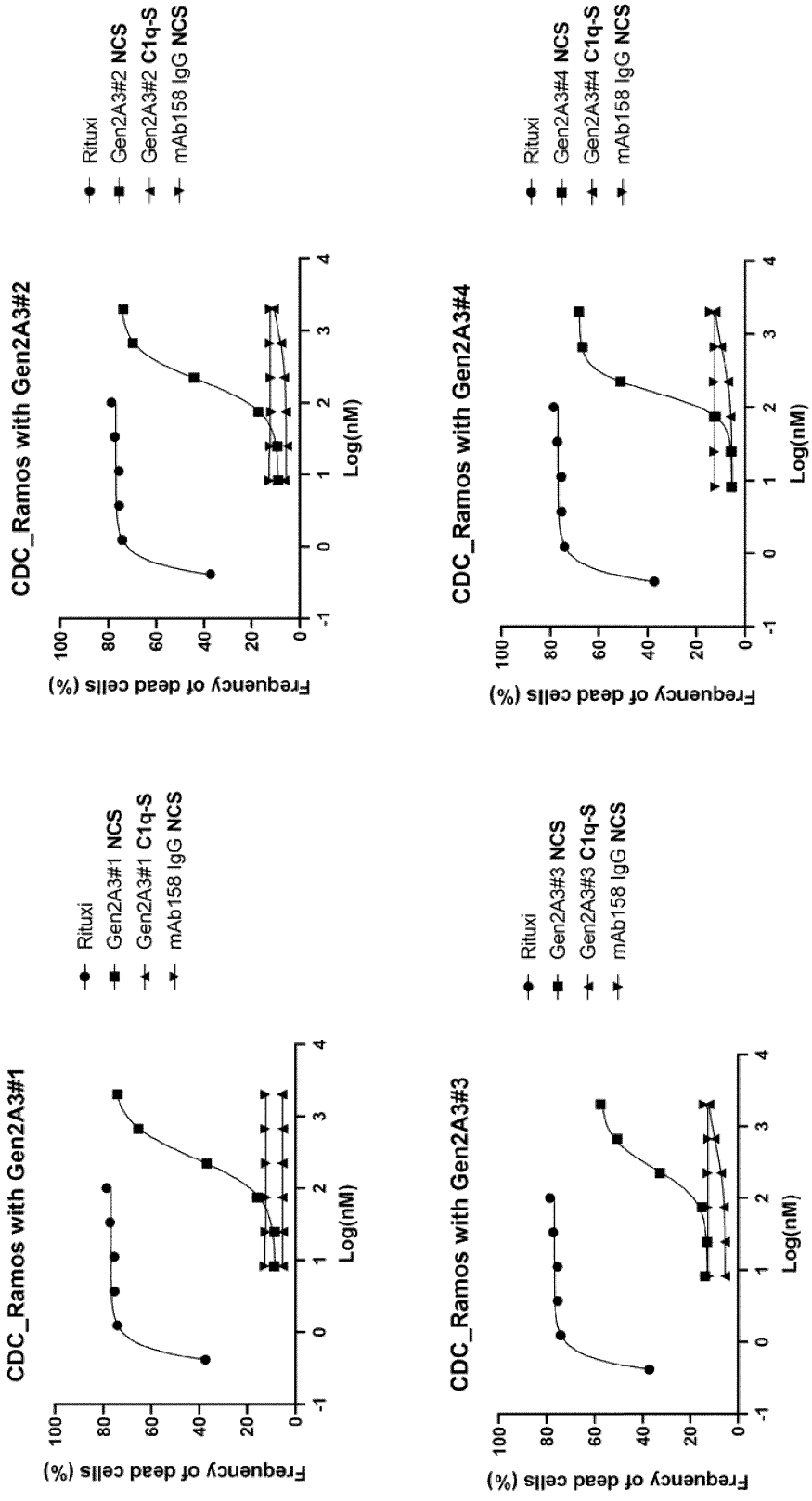

CDC_Ramos with Gen2A3#1
CDC_Ramos with Gen2A3#2
CDC_Ramos with Gen2A3#3
CDC_Ramos with Gen2A3#4
Rituxi
Gen2A3#1 NCS
Gen2A3#2 NCS
Gen2A3#3 NCS
Gen2A3#4 NCS
Gen2A3#1 C1q-S
Gen2A3#2 C1q-S
Gen2A3#3 C1q-S
Gen2A3#4 C1q-S
mAb158 IgG NCS
Frequency of dead cells (%)
Log(nM)

FIGURE 28 – cont.

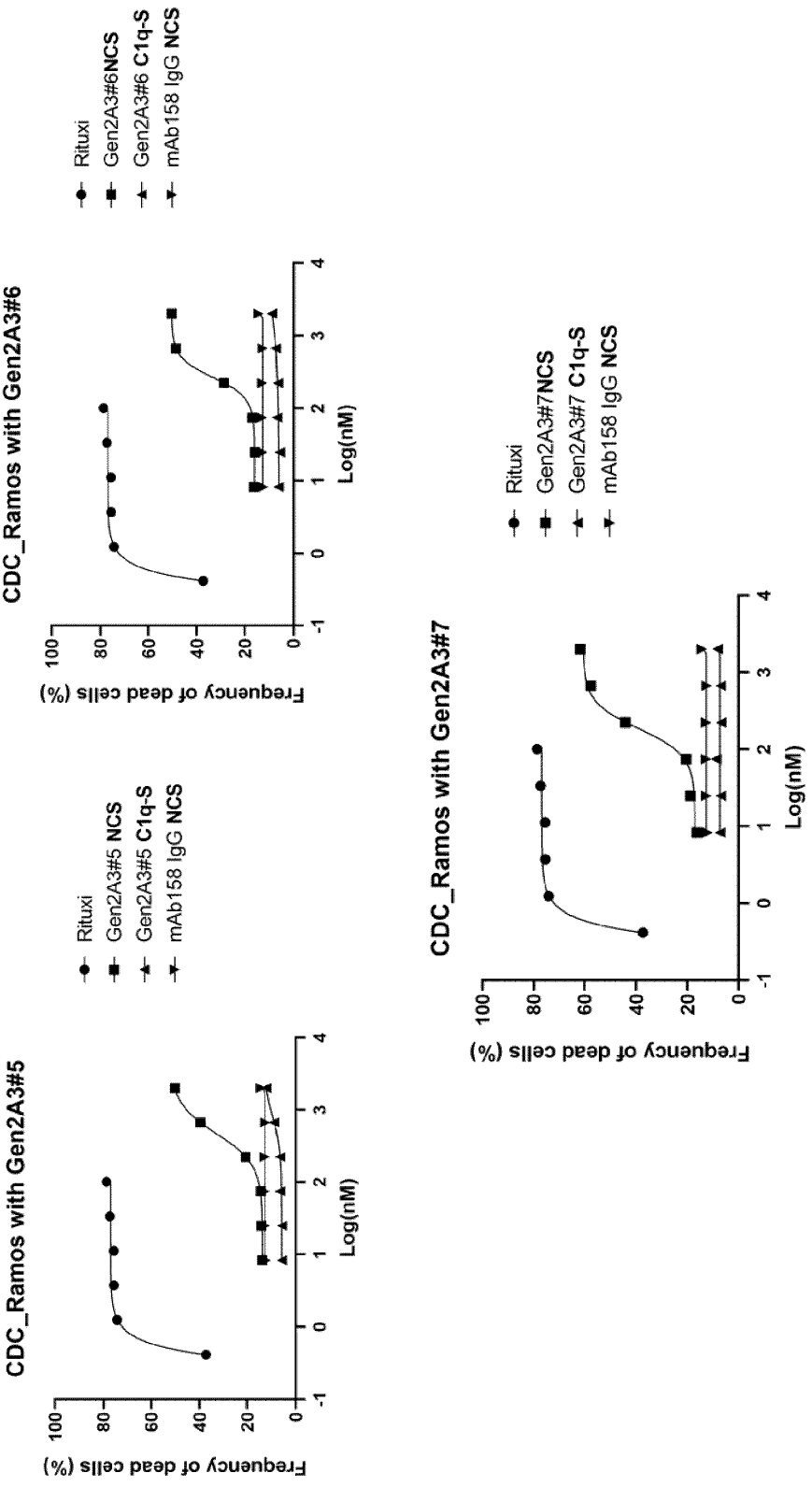


FIGURE 29

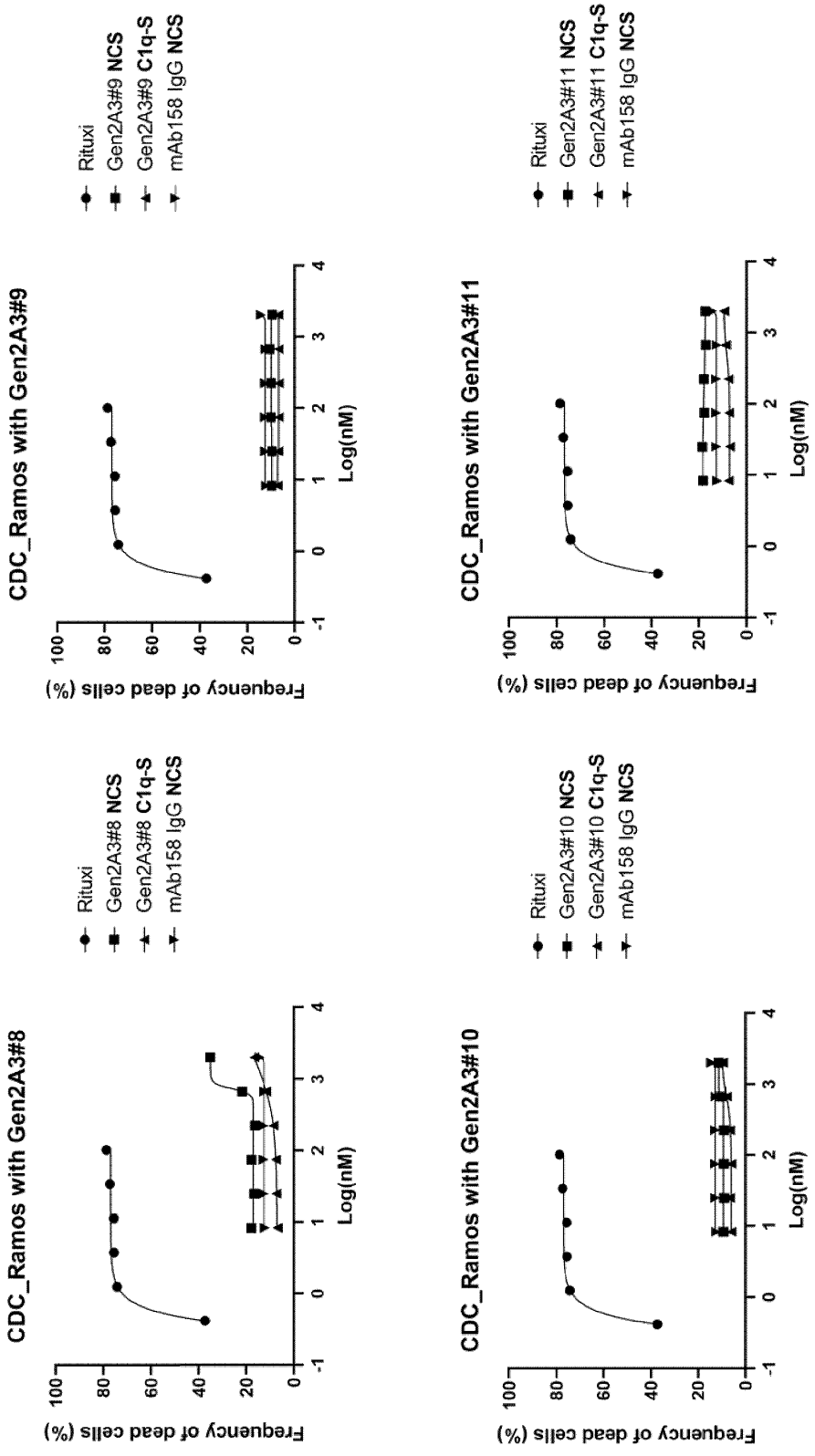
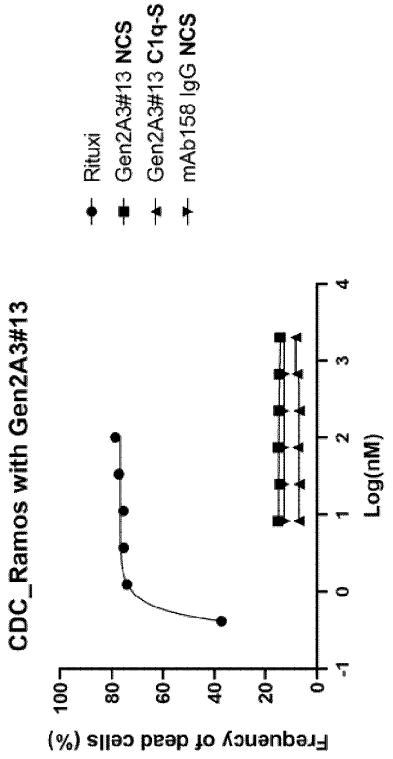
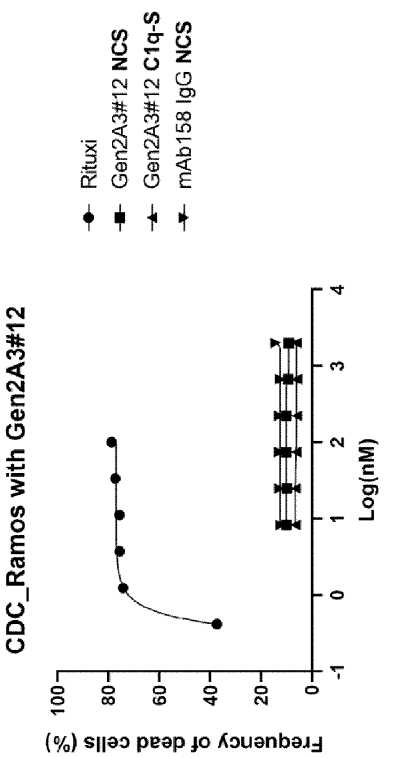

CDC_Ramos with Gen2A3#9
Rituxi
Gen2A3#9 NCS
Gen2A3#9 C1q-S
mAb158 IgG NCS
Frequency of dead cells (%)
Log(nM)
CDC_Ramos with Gen2A3#11
Rituxi
Gen2A3#11 NCS
Gen2A3#11 C1q-S
mAb158 IgG NCS
Frequency of dead cells (%)
Log(nM)
CDC_Ramos with Gen2A3#8
Rituxi
Gen2A3#8 NCS
Gen2A3#8 C1q-S
mAb158 IgG NCS
Frequency of dead cells (%)
Log(nM)
CDC_Ramos with Gen2A3#10
Rituxi
Gen2A3#10 NCS
Gen2A3#10 C1q-S
mAb158 IgG NCS
Frequency of dead cells (%)
Log(nM)

FIGURE 29 – cont.





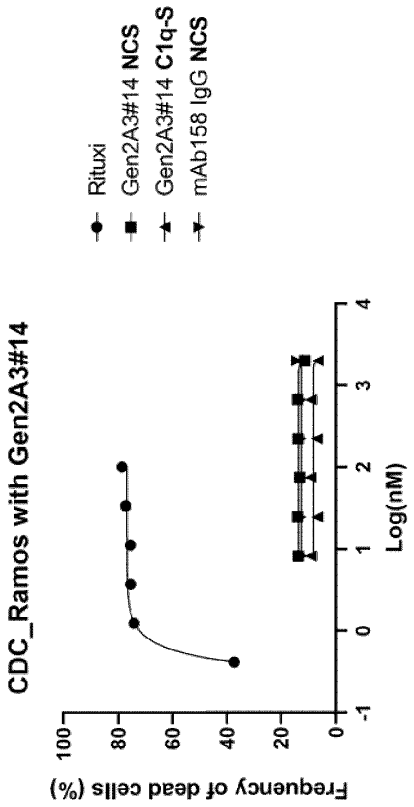
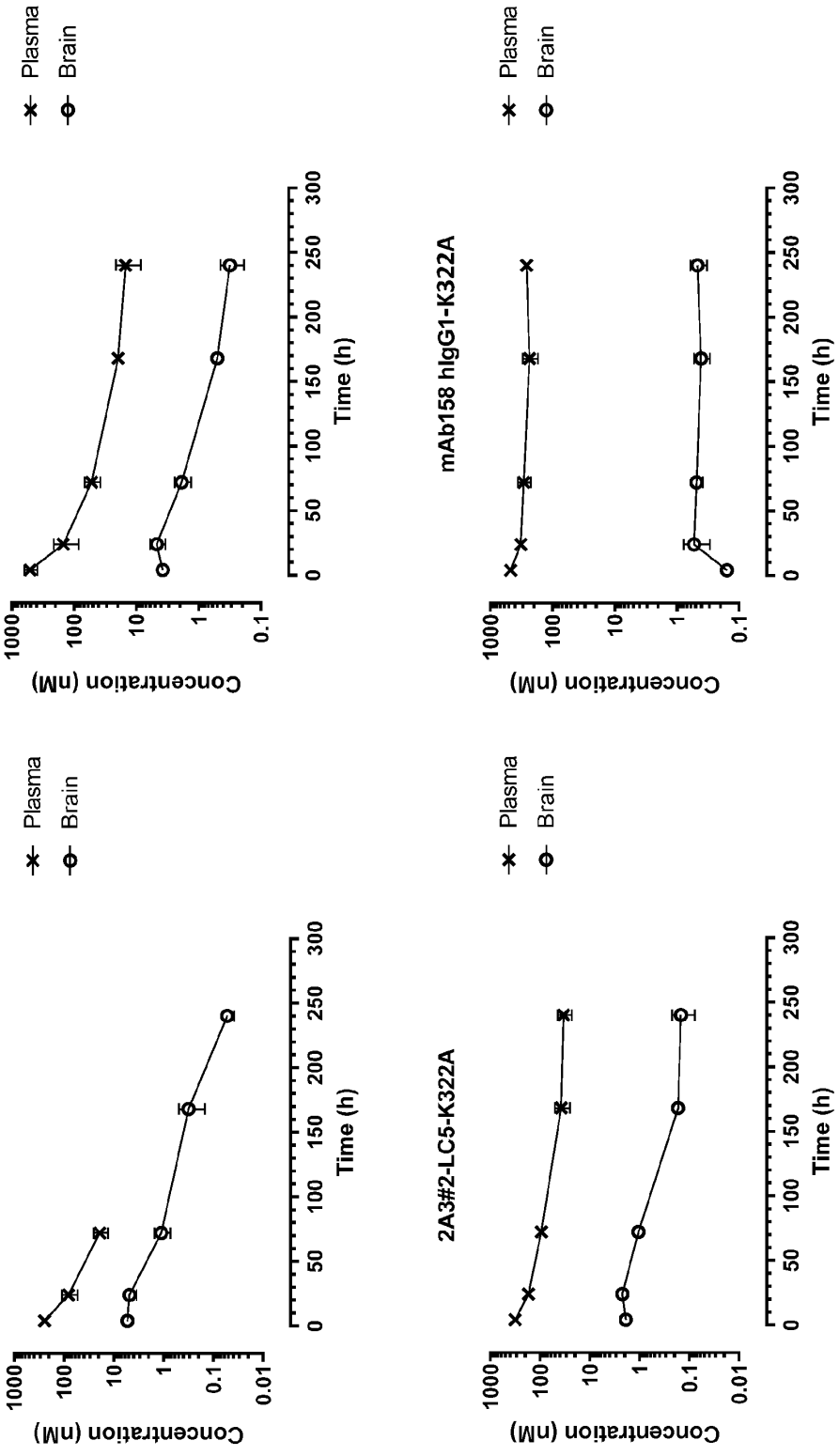


FIGURE 30


2A3#2-LC1-K322A
2A3#2-HC6-K322A
mAb158 hIgG1-K322A
2A3#2-LC5-K322A
Plasma
Brain
Concentration (nM)
Time (h)
0.01
0.1
1
10
100
1000
0
50
100
150
200
250
300

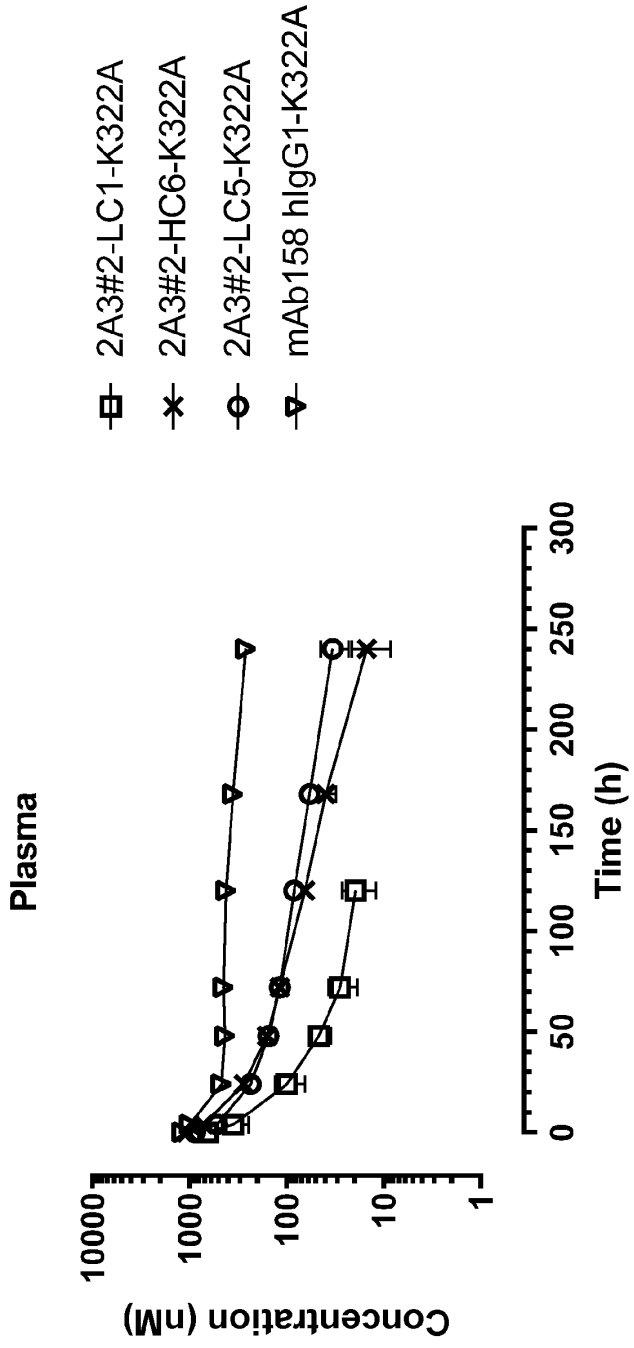

2A3#2-LC1-K322A
2A3#2-HC6-K322A
2A3#2-LC5-K322A
mAb158 hIgG1-K322A
Plasma
Concentration (nM)
10000
1000
100
10
1
0
50
100
150
200
250
300
Time (h)

FIGURE 31

FIGURE 32

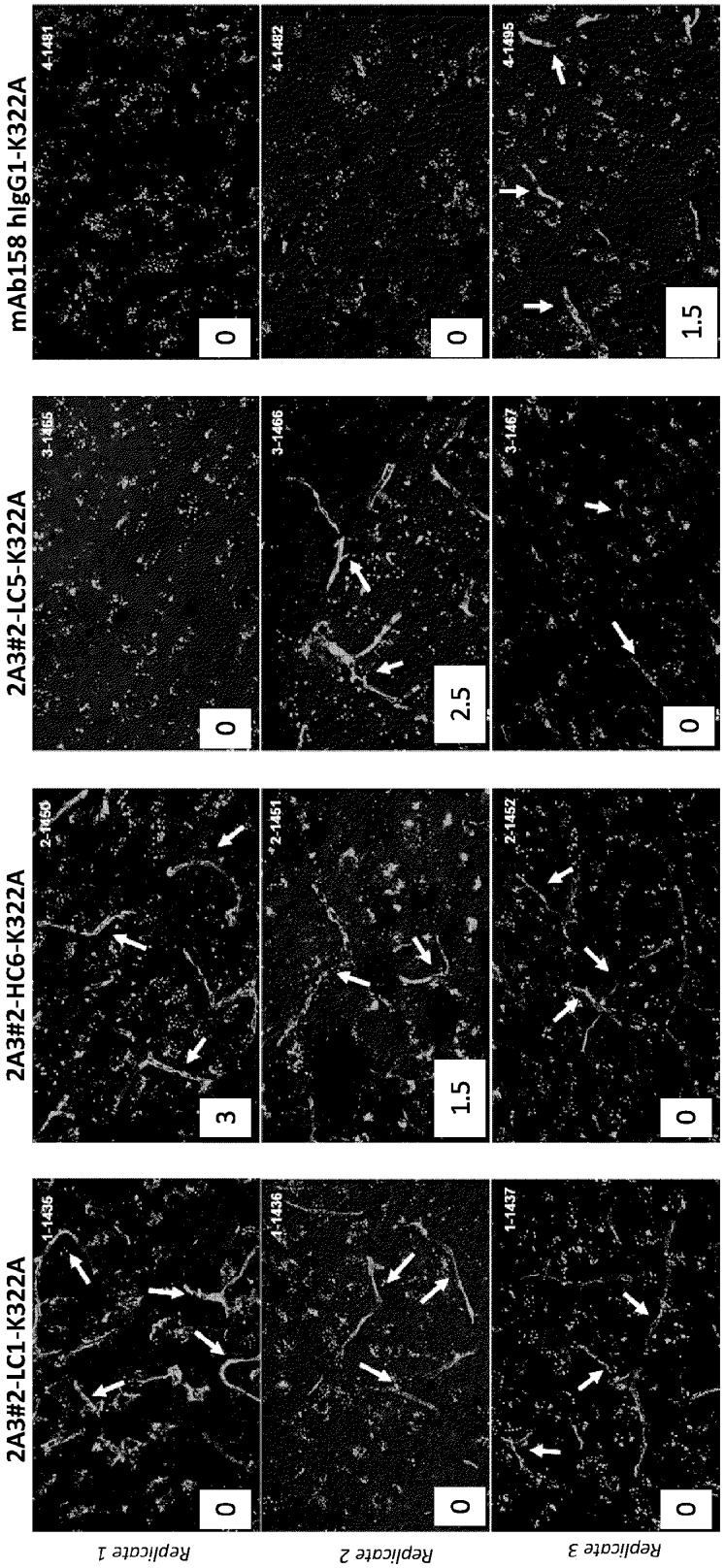
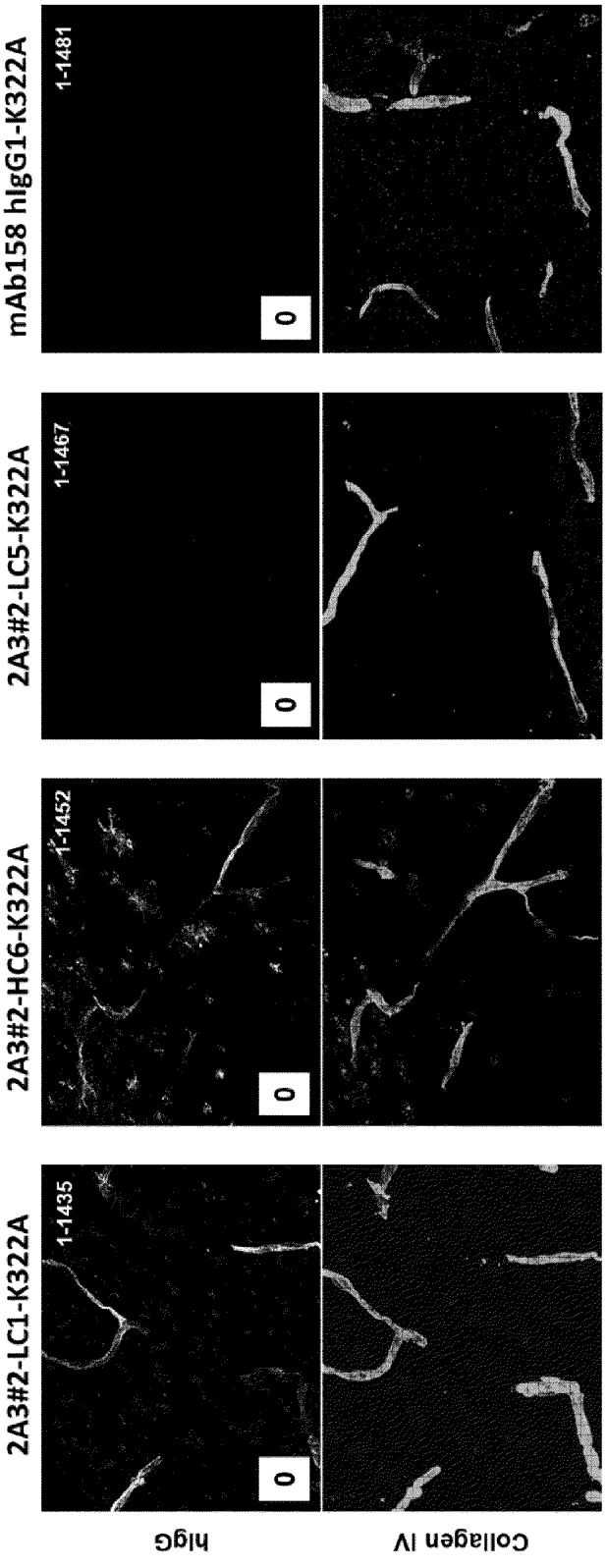

2A3#2-LC1-K322A
2A3#2-HC6-K322A
2A3#2-LC5-K322A
mAb158 hIgG1-K322A
Replicate 1
Replicate 2
Replicate 3
1-1436
1-1437
2-1450
2-1451
2-1452
3-1466
3-1467
4-1481
4-1482
4-1495
0
3
1.5
2.5
1.5

FIGURE 33


2A3#2-LC1-K322A
1-1435
2A3#2-HC6-K322A
1-1452
2A3#2-LC5-K322A
1-1467
mAb158 hIgG1-K322A
1-1481
0
hIgG
Collagen IV

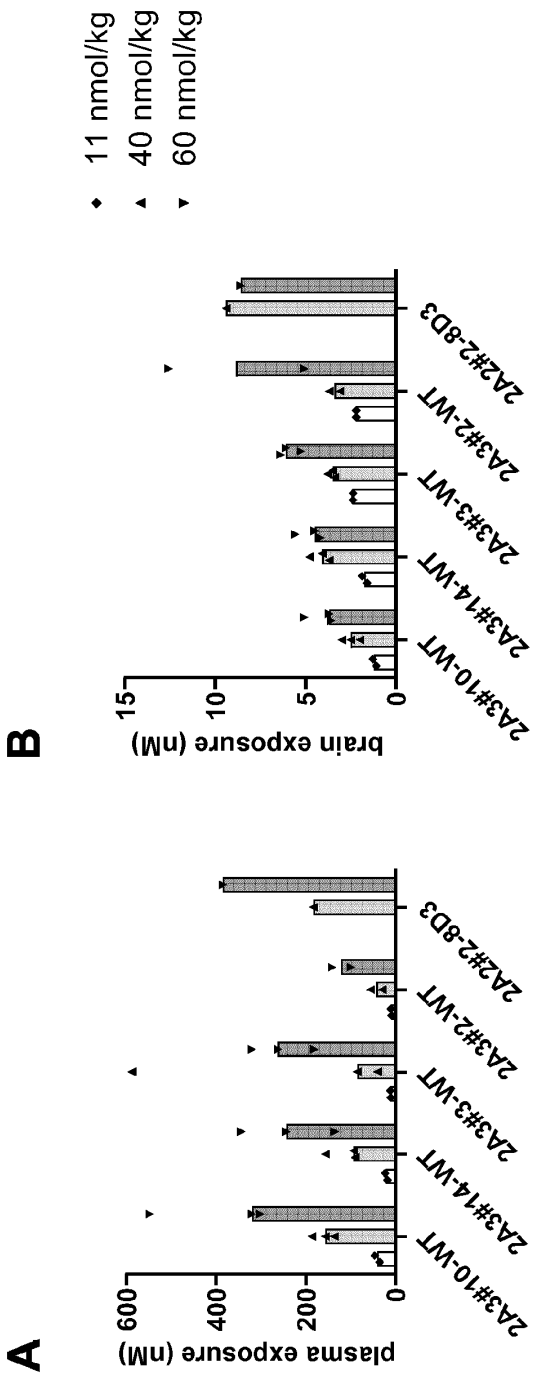

A
plasma exposure (nM)
0
200
400
600
2A3#10-WT
2A3#14-WT
2A3#3-WT
2A3#2-WT
2A2#2-8D3
B
11 nmol/kg
40 nmol/kg
60 nmol/kg
brain exposure (nM)
0
5
10
15
2A3#10-WT
2A3#14-WT
2A3#3-WT
2A3#2-WT
2A2#2-8D3

FIGURE 34

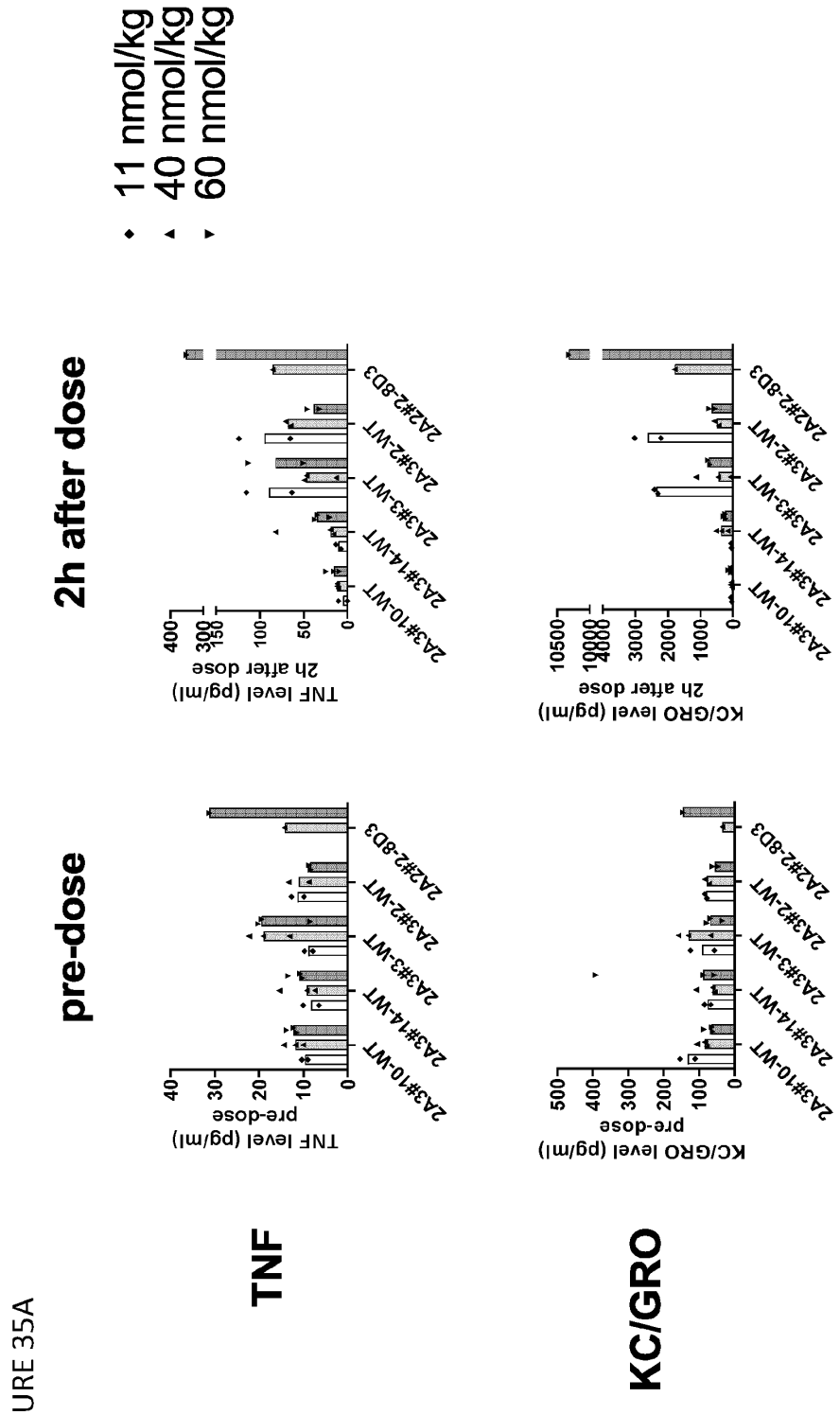

11 nmol/kg
40 nmol/kg
60 nmol/kg
2h after dose
pre-dose
TNF
KC/GRO
TNF level (pg/ml)
pre-dose
TNF level (pg/ml)
2h after dose
KC/GRO level (pg/ml)
pre-dose
KC/GRO level (pg/ml)
2h after dose
2A3#10-WT
2A3#14-WT
2A3#3-WT
2A3#2-WT
2A2#2-8D3

FIGURE 35A

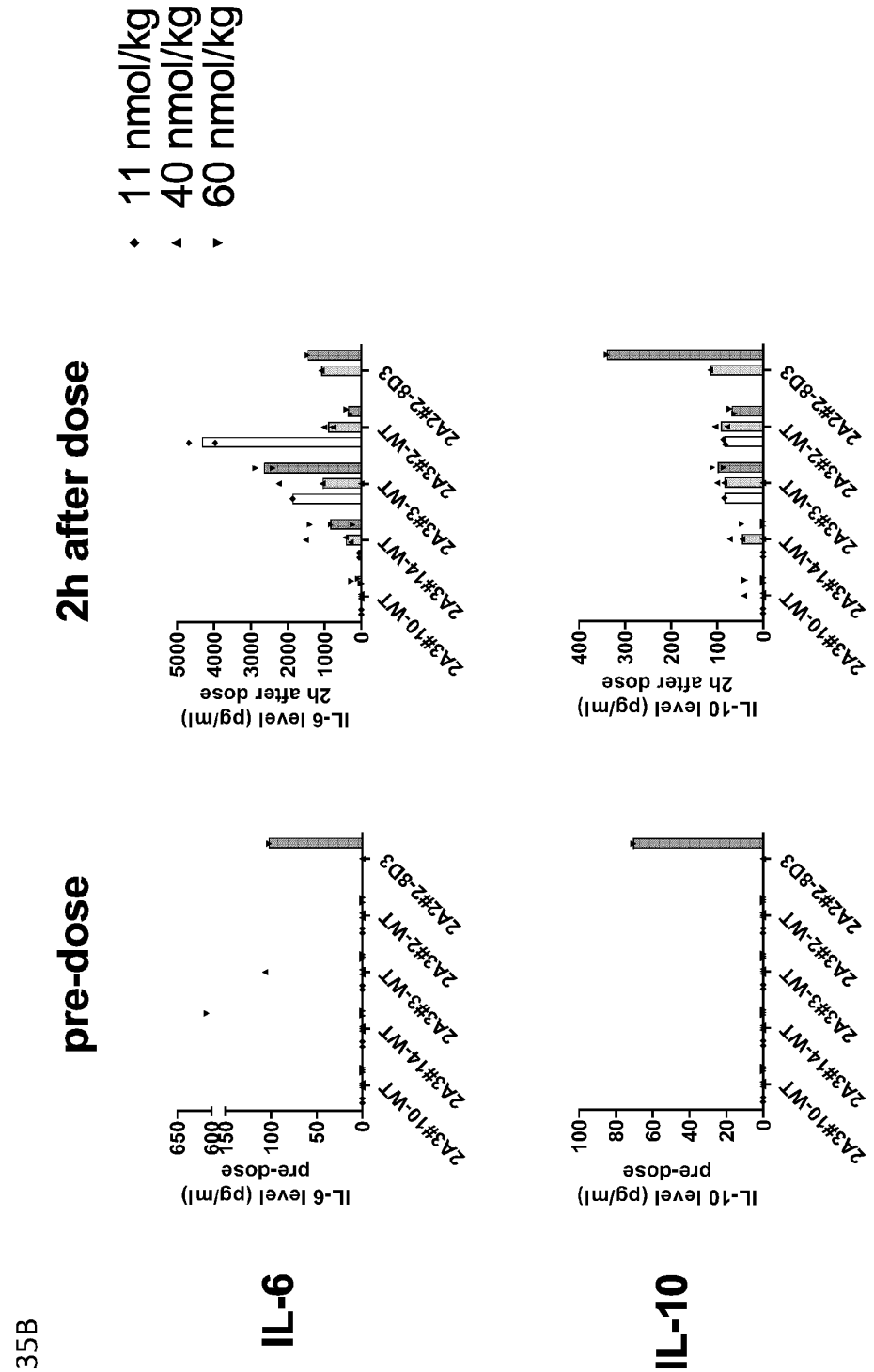

11 nmol/kg
40 nmol/kg
60 nmol/kg
2h after dose
pre-dose
IL-6
IL-10
IL-6 level (pg/ml)
2h after dose
IL-10 level (pg/ml)
pre-dose
2A3#10-WT
2A3#14-WT
2A3#3-WT
2A3#2-WT
2A2#2-8D3
0
1000
2000
3000
4000
5000
100
200
300
400
20
40
60
80
50
600
650

FIGURE 35B

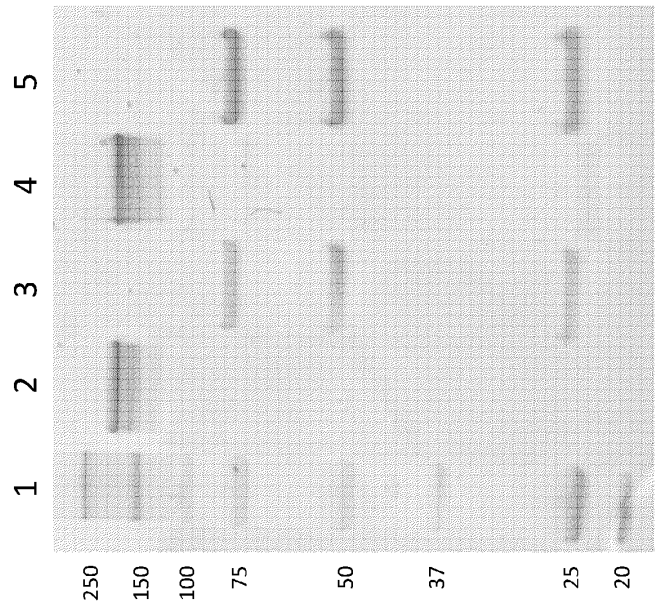


FIGURE 36

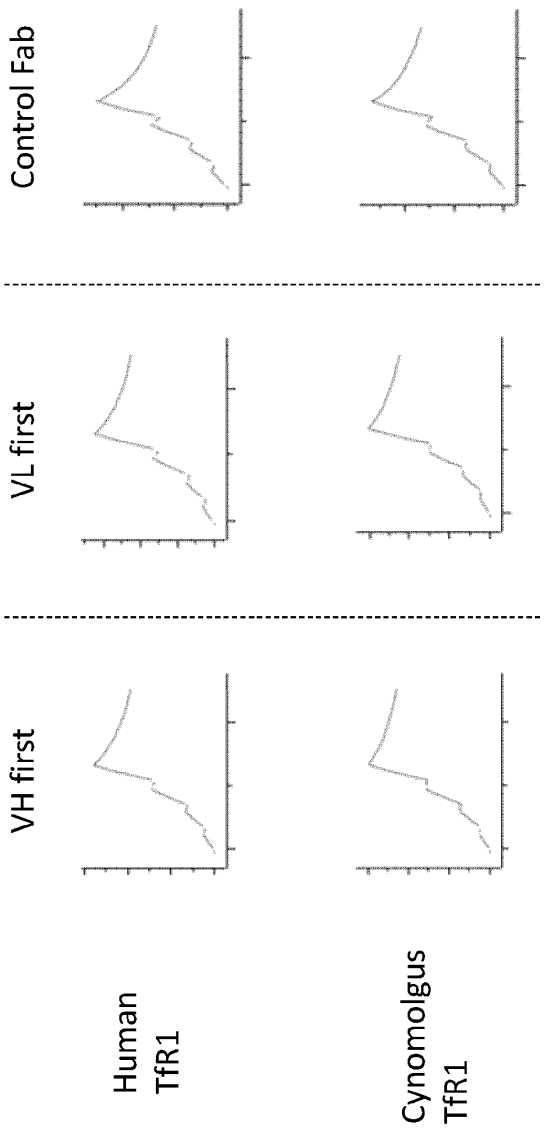


FIGURE 37

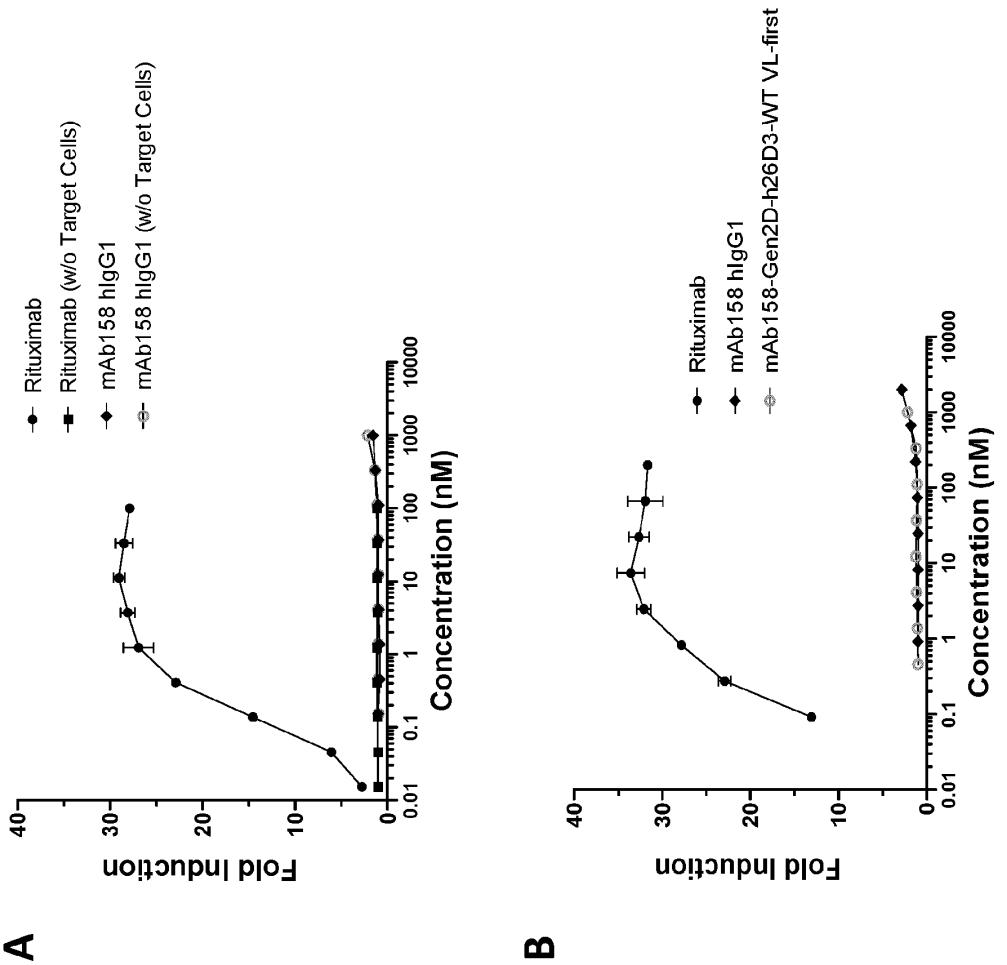

A
Rituximab
Rituximab (w/o Target Cells)
mAb158 hIgG1
mAb158 hIgG1 (w/o Target Cells)
Fold Induction
Concentration (nM)
B
Rituximab
mAb158 hIgG1
mAb158-Gen2D-h26D3-WT VL-first
Fold Induction
Concentration (nM)

FIGURE 38

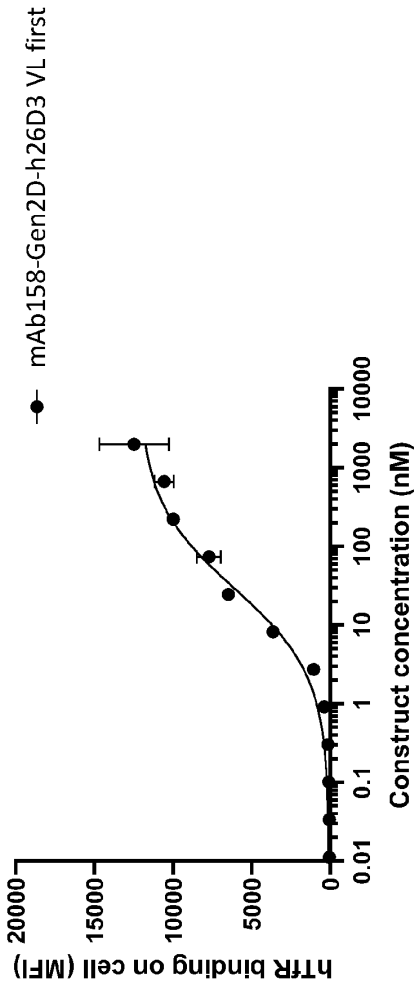
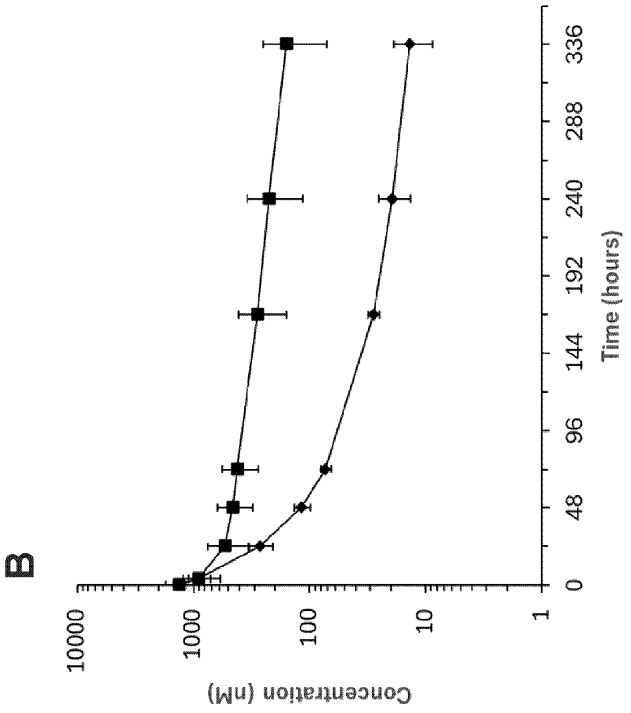

mAb158-Gen2D-h26D3 VL first
hTfR binding on cell (MFI)
20000
15000
10000
5000
0
0.01
0.1
1
10
100
1000
10000
Construct concentration (nM)

FIGURE 39


B
Concentration (nM)
10000
1000
100
10
1
0
48
96
144
192
240
288
336
Time (hours)

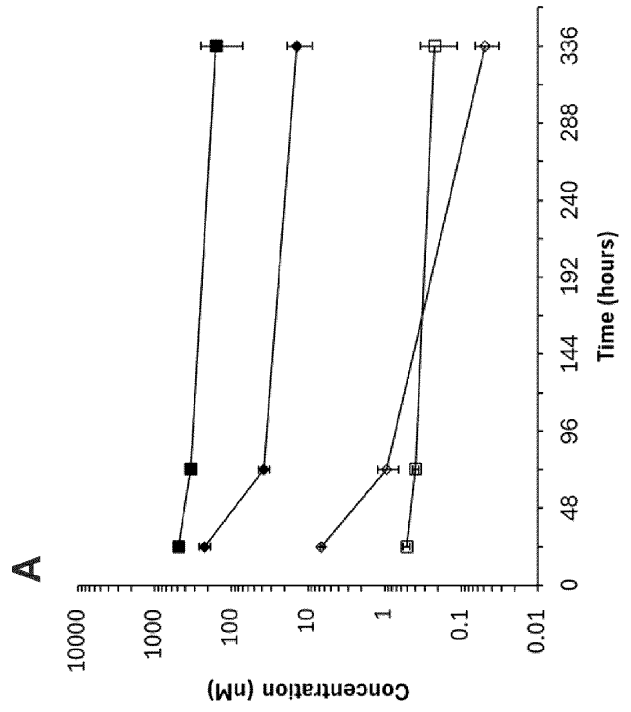

A
Concentration (nM)
10000
1000
100
10
1
0.1
0.01
0
48
96
144
192
240
288
336
Time (hours)

FIGURE 40

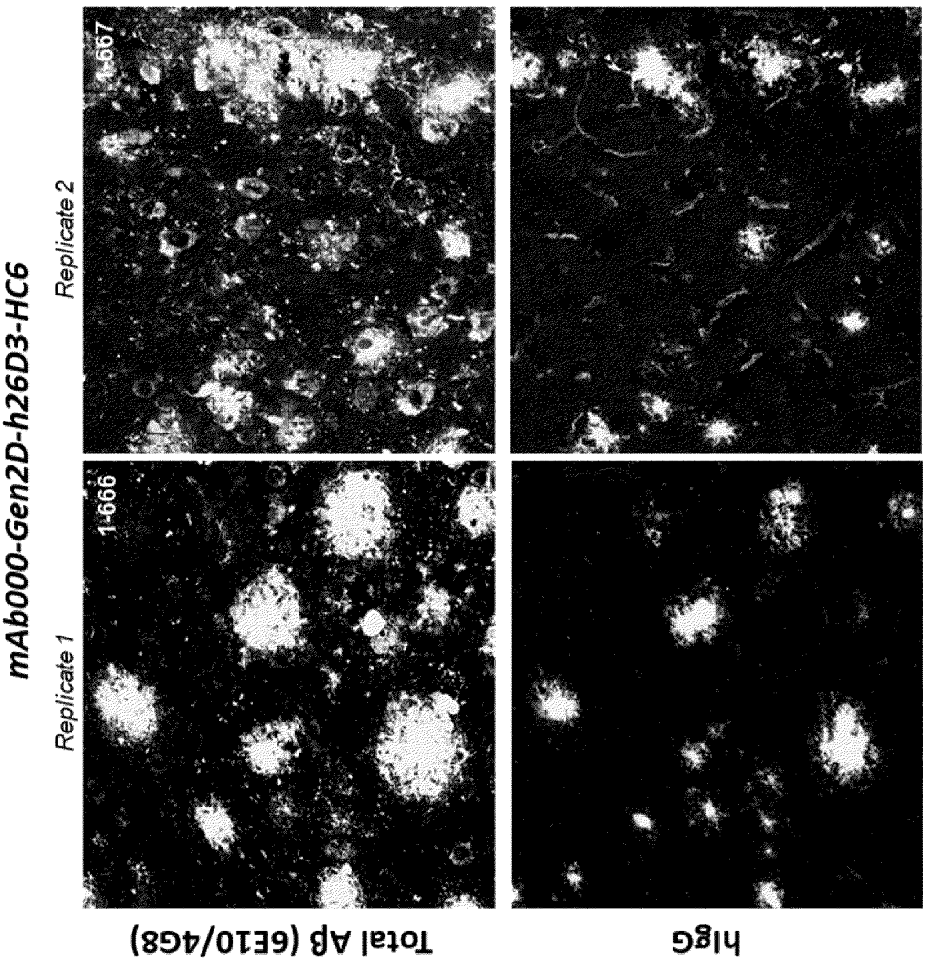

mAb000-Gen2D-h26D3-HC6
Replicate 1
Replicate 2
1-666
1-667
Total Aβ (6E10/4G8)
hIgG

FIGURE 41A

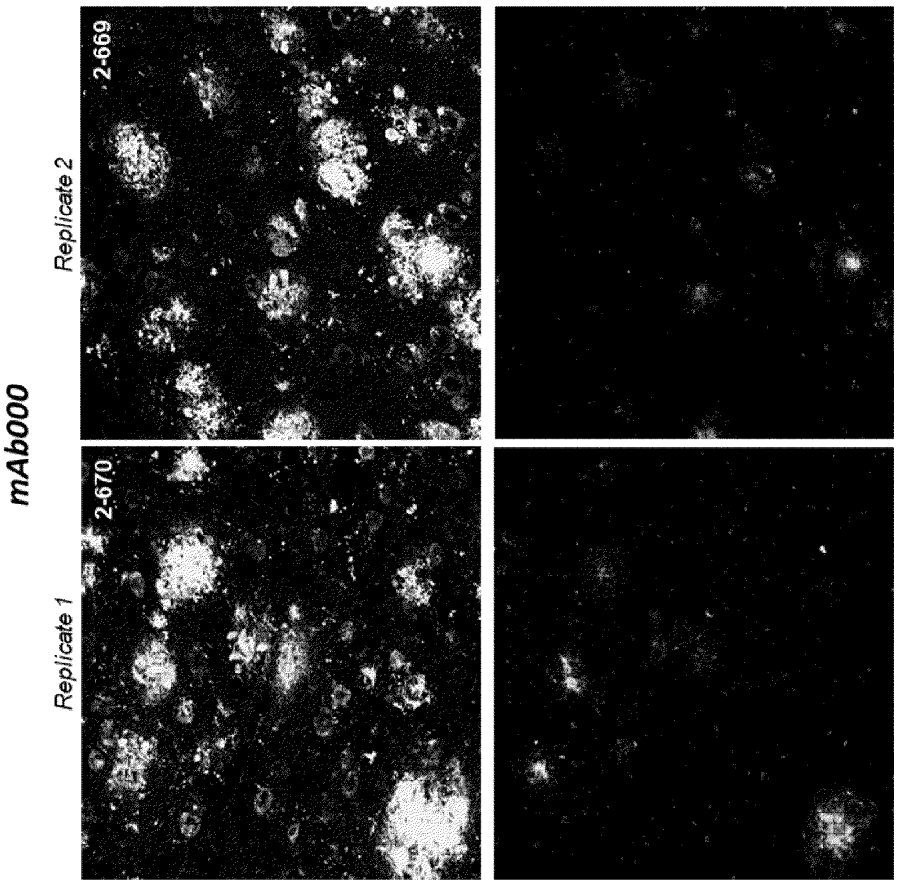

mAb000
Replicate 1
Replicate 2
2-670
2-669
Total Aβ (6E10/4G8)
hIgG

FIGURE 41B

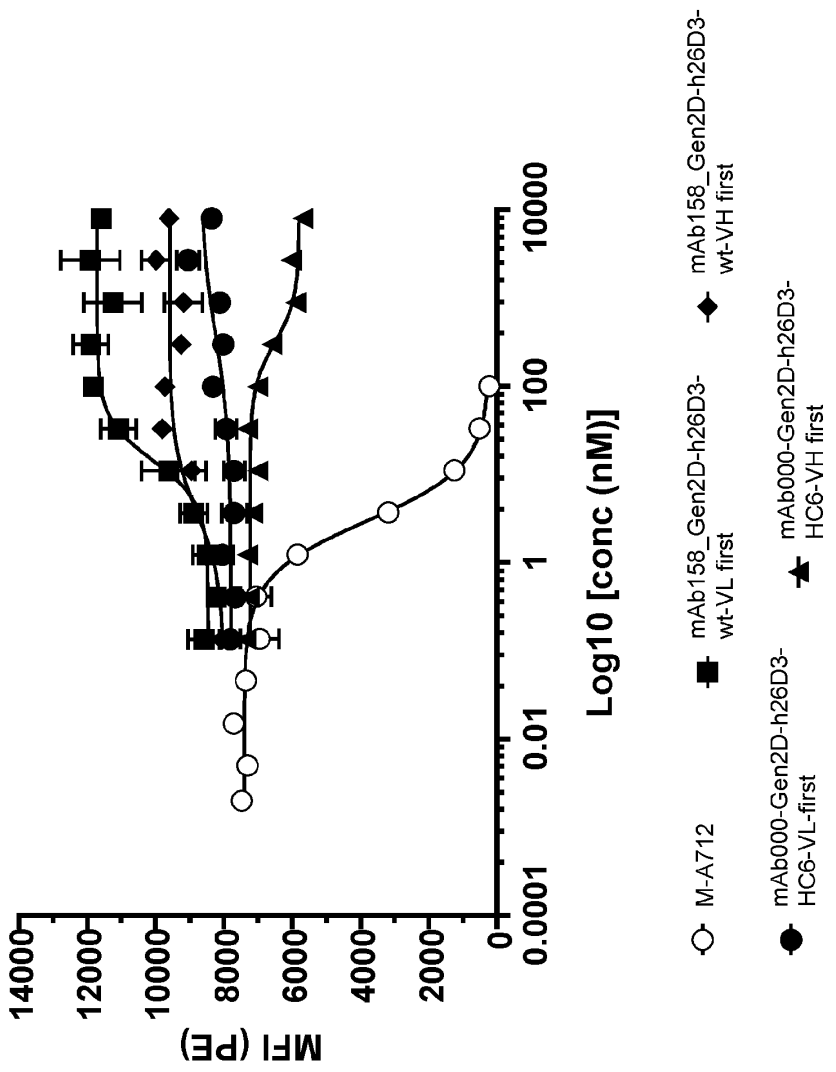

MFI (PE)
14000
12000
10000
8000
6000
4000
2000
0
0.0001
0.01
1
100
10000
Log10 [conc (nM)]
M-A712
mAb158_Gen2D-h26D3-wt-VL first
mAb158_Gen2D-h26D3-wt-VH first
mAb000-Gen2D-h26D3-HC6-VL-first
mAb000-Gen2D-h26D3-HC6-VH first

FIGURE 42

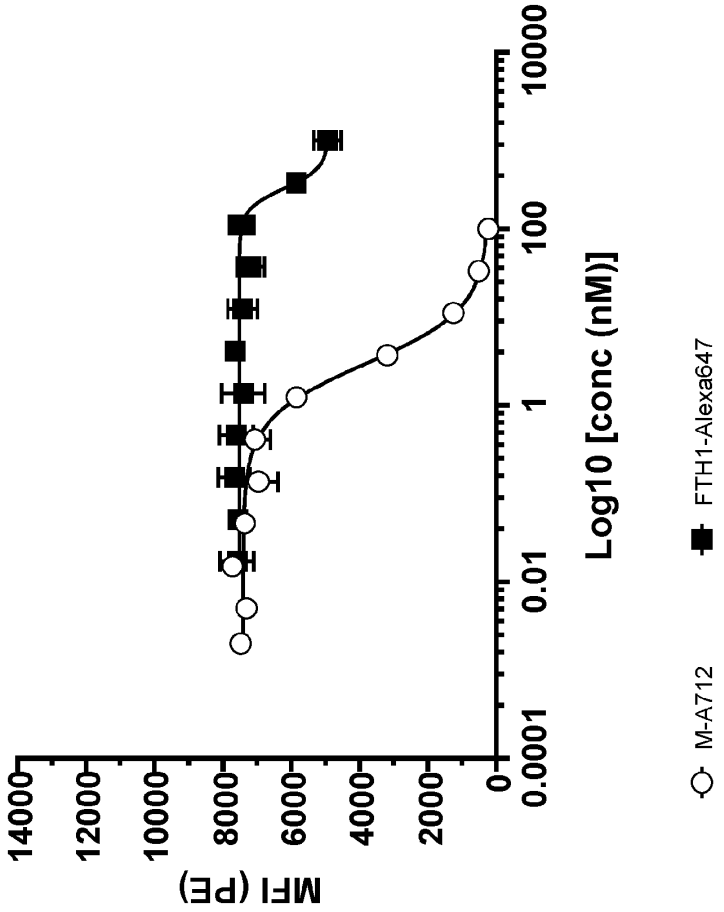

MFI (PE)
14000
12000
10000
8000
6000
4000
2000
0
0.0001
0.01
1
100
10000
Log10 [conc (nM)]
M-A712
FTH1-Alexa647

FIGURE 43