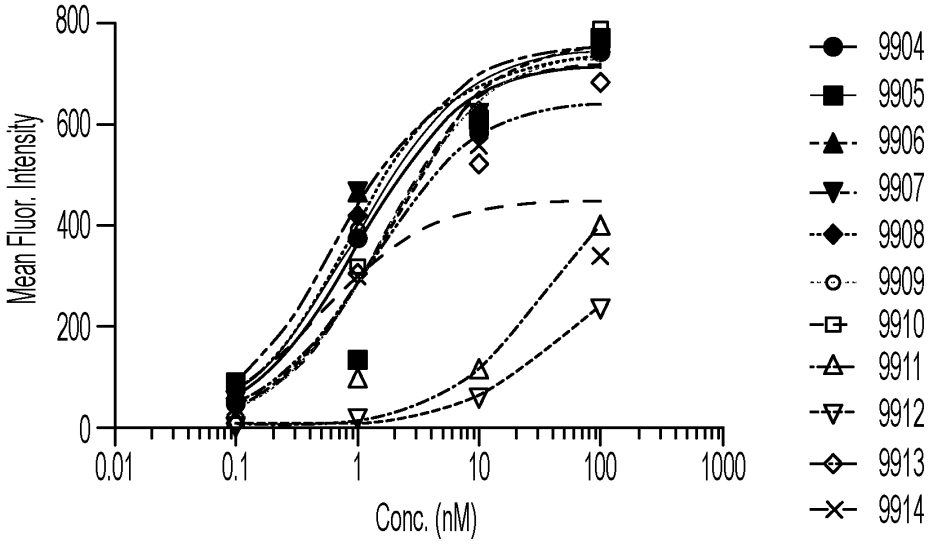
800
600
400
200
0
Mean Fluor. Intensity
0.01
0.1
1
10
100
1000
Conc. (nM)
9904
9905
9906
9907
9908
9909
9910
9911
9912
9913
9914

FIG. 1A

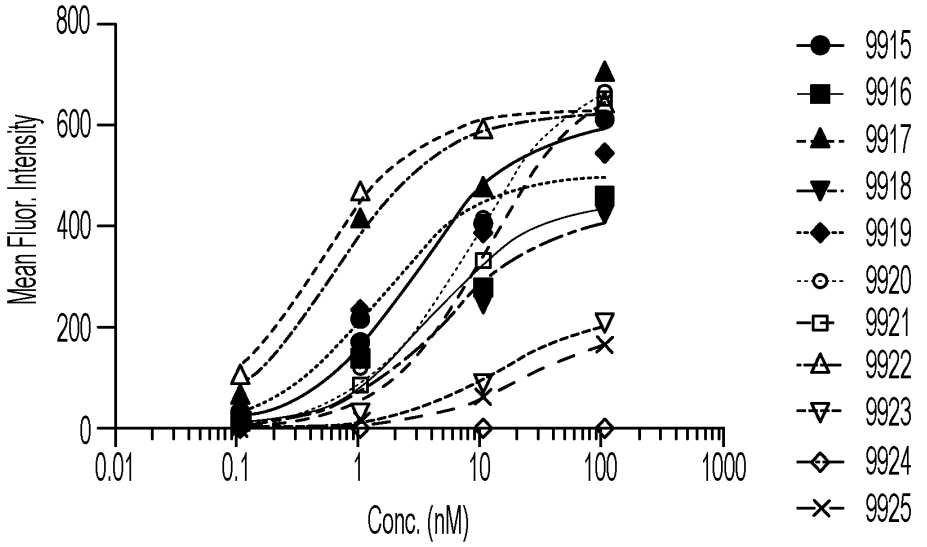
800
600
400
200
0
Mean Fluor. Intensity
0.01
0.1
1
10
100
1000
Conc. (nM)
9915
9916
9917
9918
9919
9920
9921
9922
9923
9924
9925

FIG. 1B

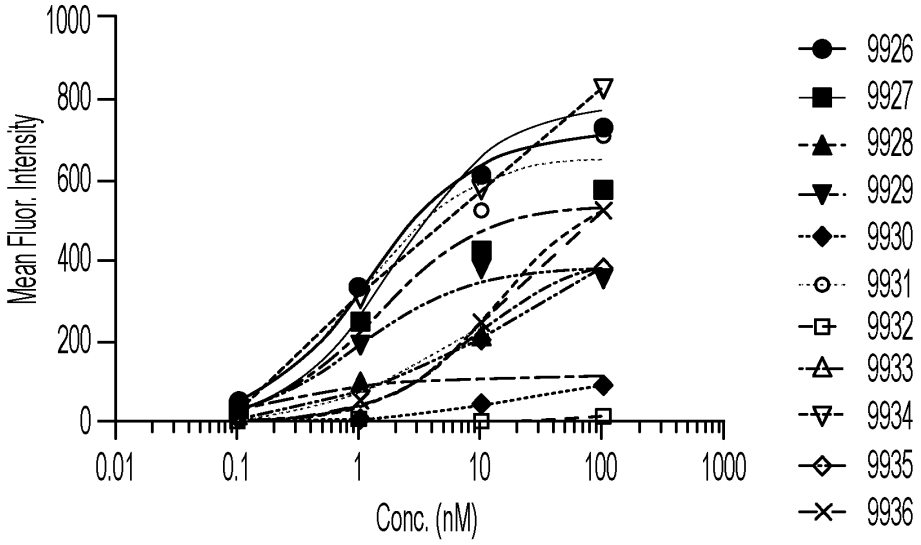
1000
800
600
400
200
0
Mean Fluor. Intensity
0.01
0.1
1
10
100
1000
Conc. (nM)
9926
9927
9928
9929
9930
9931
9932
9933
9934
9935
9936

FIG. 2A

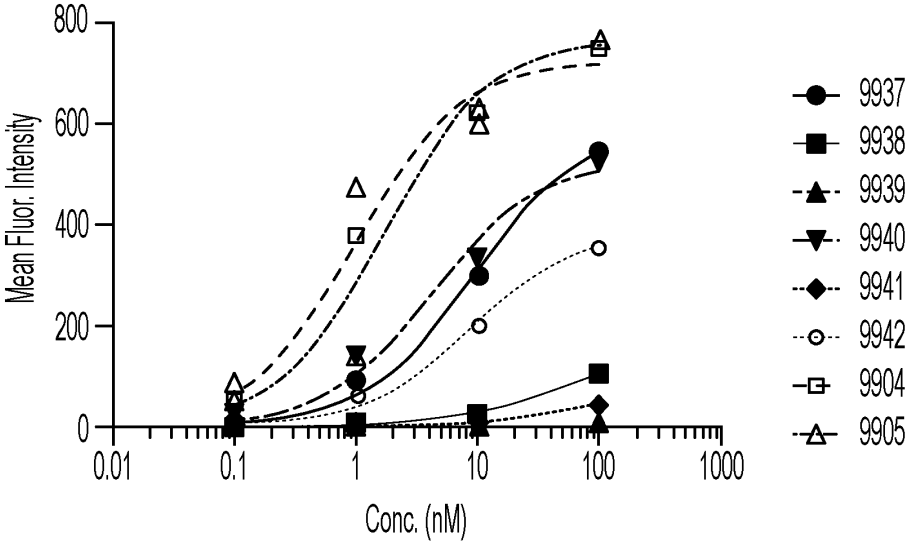
800
600
400
200
0
Mean Fluor. Intensity
0.01
0.1
1
10
100
1000
Conc. (nM)
9937
9938
9939
9940
9941
9942
9904
9905

FIG. 2B

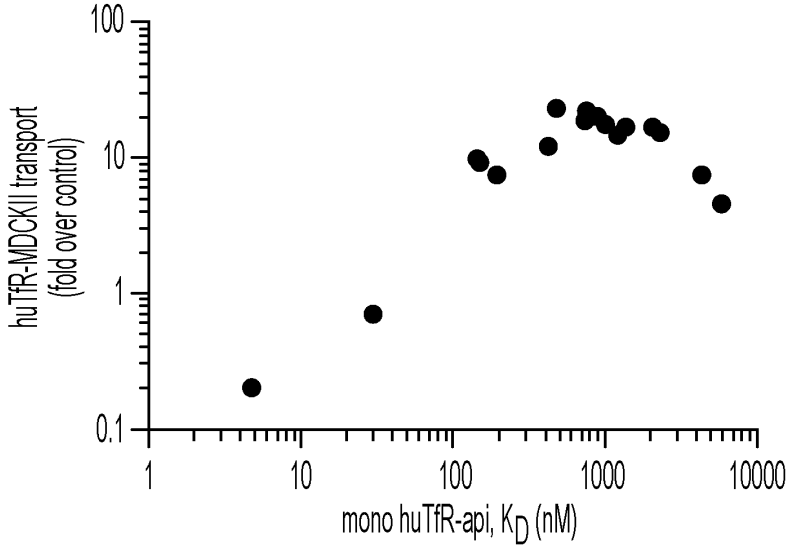
huTfR-MDCKII transport
(fold over control)
100
10
1
0.1
1
10
100
1000
10000
mono huTfR-api, K_D (nM)

FIG. 3

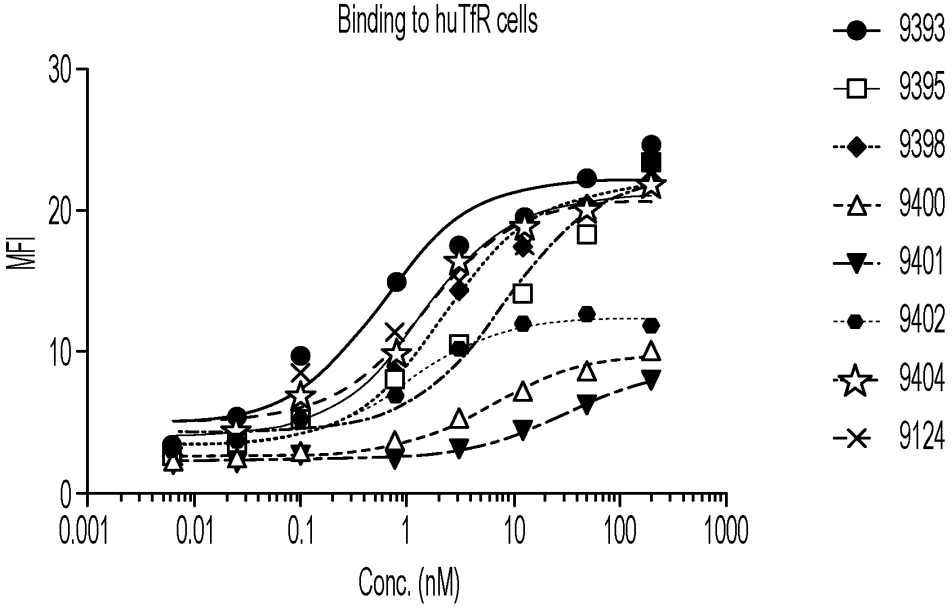
Binding to huTfR cells
MFI
30
20
10
0
0.001
0.01
0.1
1
10
100
1000
Conc. (nM)
9393
9395
9398
9400
9401
9402
9404
9124

FIG. 4

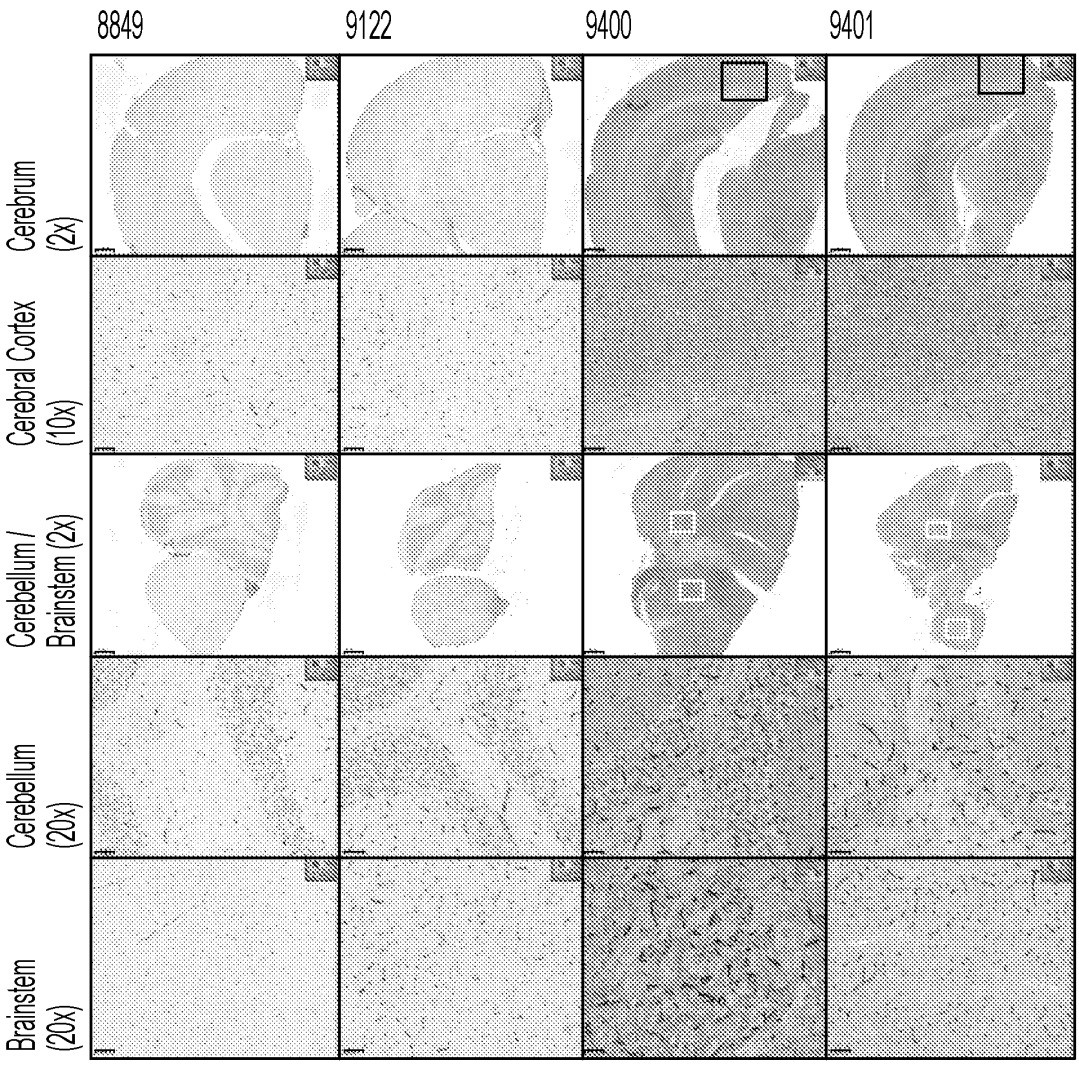
8849
9122
9400
9401
Cerebrum (2x)
Cerebral Cortex (10x)
Cerebellum / Brainstem (2x)
Cerebellum (20x)
Brainstem (20x)

FIG. 5

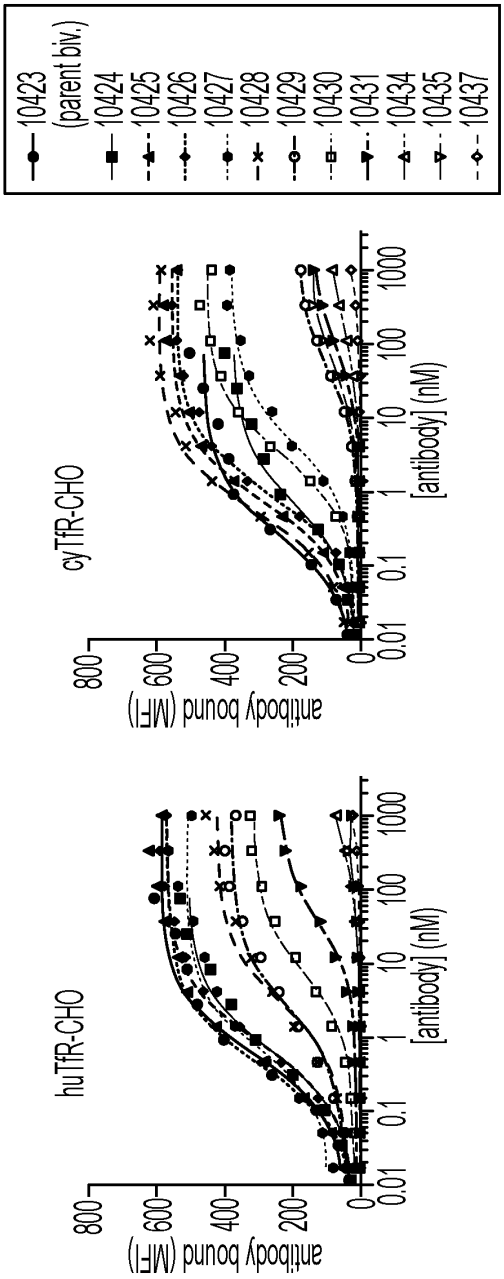
10423 (parent biv.)
10424
10425
10426
10427
10428
10429
10430
10431
10434
10435
10437
huTfR-CHO
cynTfR-CHO
antibody bound (MFI)
0
200
400
600
800
0.01
0.1
1
10
100
1000
[antibody] (nM)

FIG. 6A

FIG. 6B

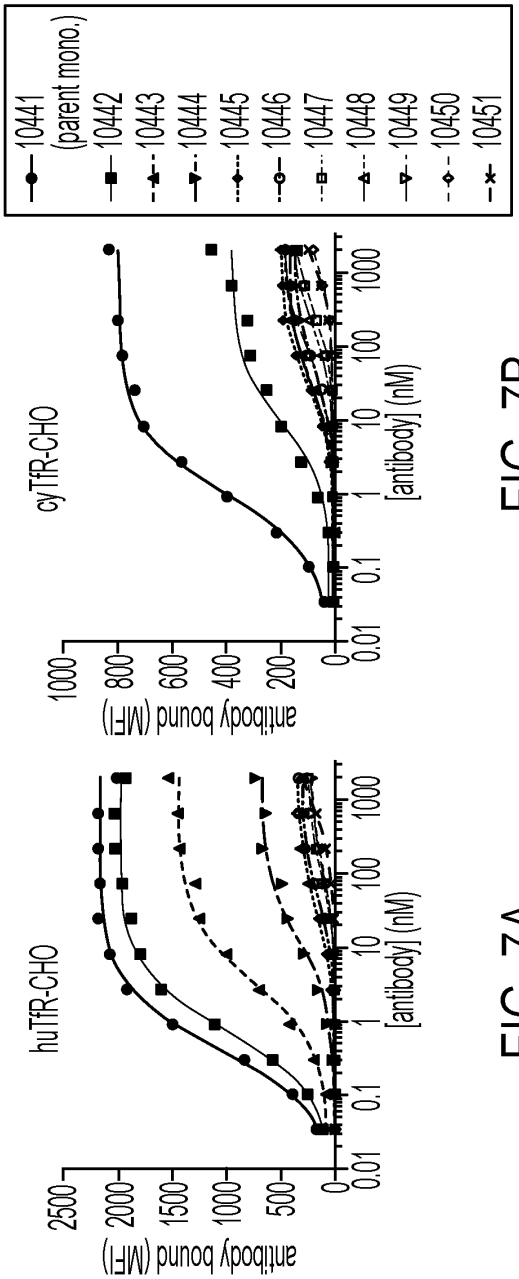
10441 (parent mono.)
10442
10443
10444
10445
10446
10447
10448
10449
10450
10451
cynTfR-CHO
huTfR-CHO
antibody bound (MFI)
[antibody] (nM)
0
200
400
600
800
1000
500
1000
1500
2000
2500
0.01
0.1
1
10
100
1000

FIG. 7B

FIG. 7A

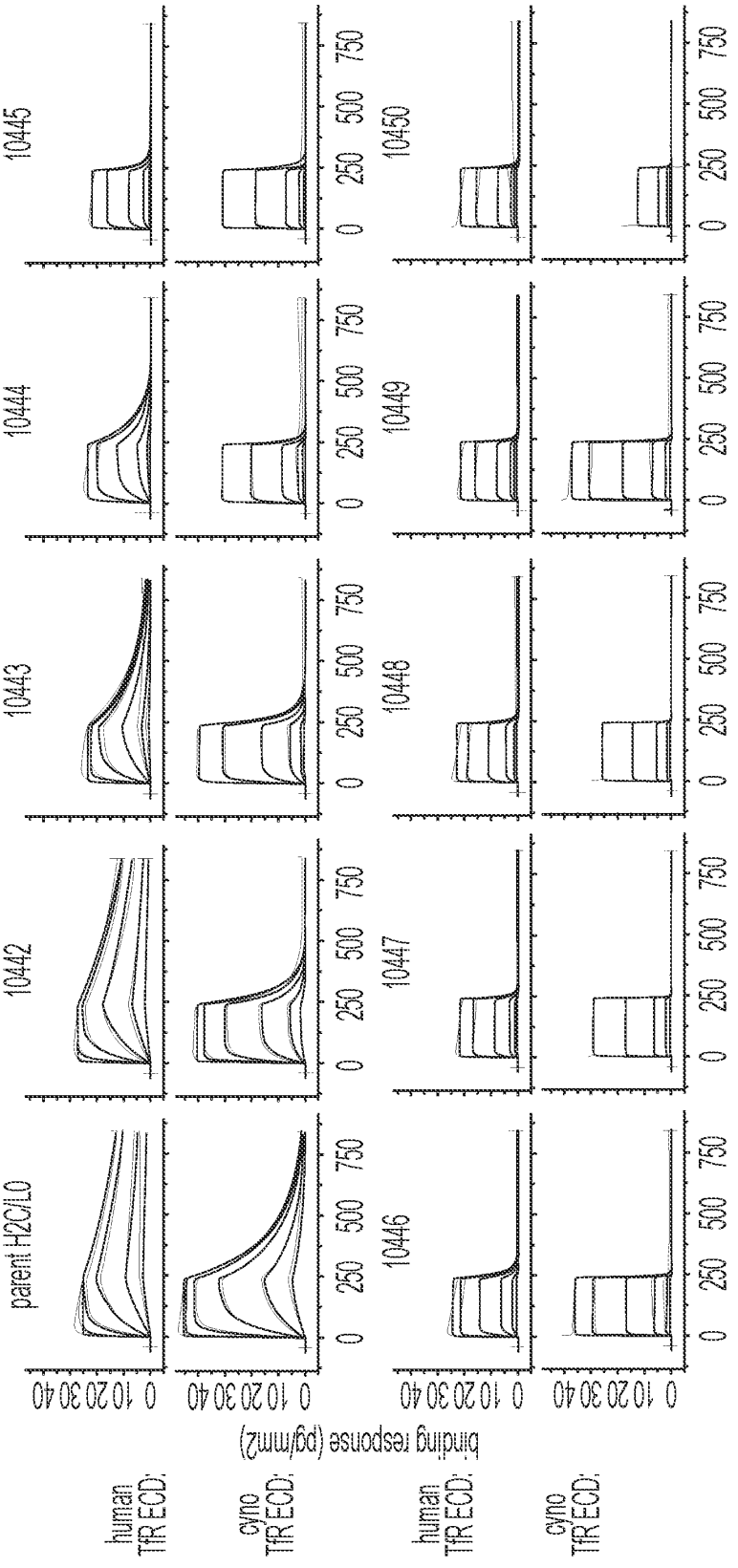
parent H2C/L0
10442
10443
10444
10445
10446
10447
10448
10449
10450
human TfR ECD:
cyno TfR ECD:
human TfR ECD:
cyno TfR ECD:
binding response (pg/mm2)
0 10 20 30 40
0 250 500 750
time (s)

FIG. 8

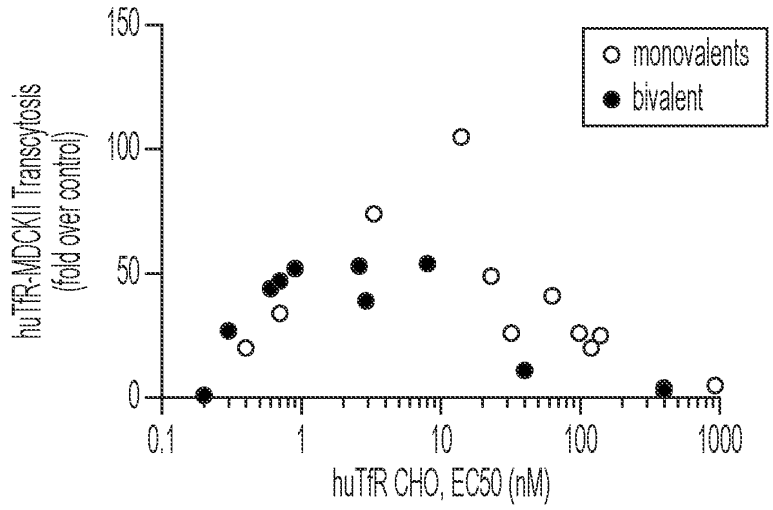
150
100
50
0
0.1
1
10
100
1000
huTfR-MDCKII Transcytosis
(fold over control)
huTfR CHO, EC50 (nM)
monovalents
bivalent

FIG. 9A

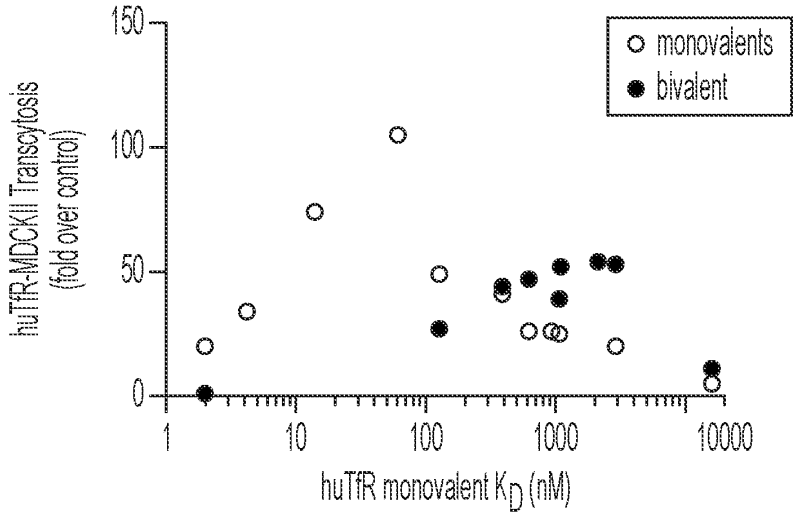
150
100
50
0
1
10
100
1000
10000
huTfR-MDCKII Transcytosis
(fold over control)
huTfR monovalent K_D (nM)
monovalents
bivalent

FIG. 9B

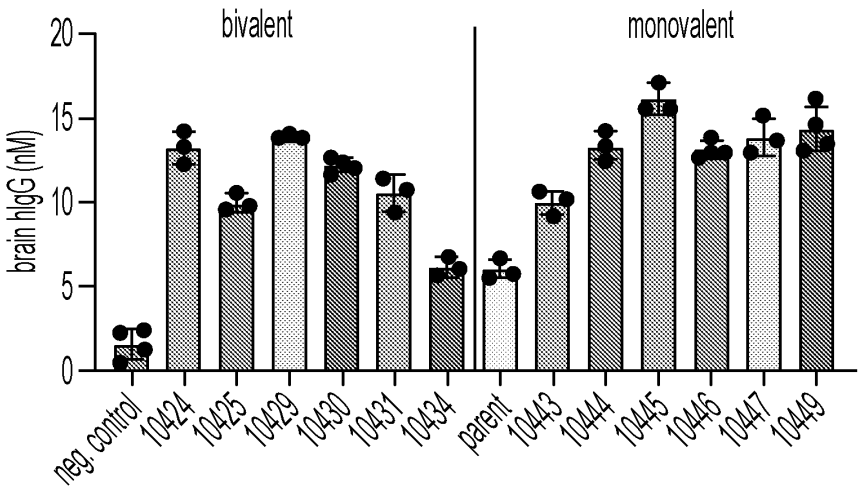

FIG. 10A

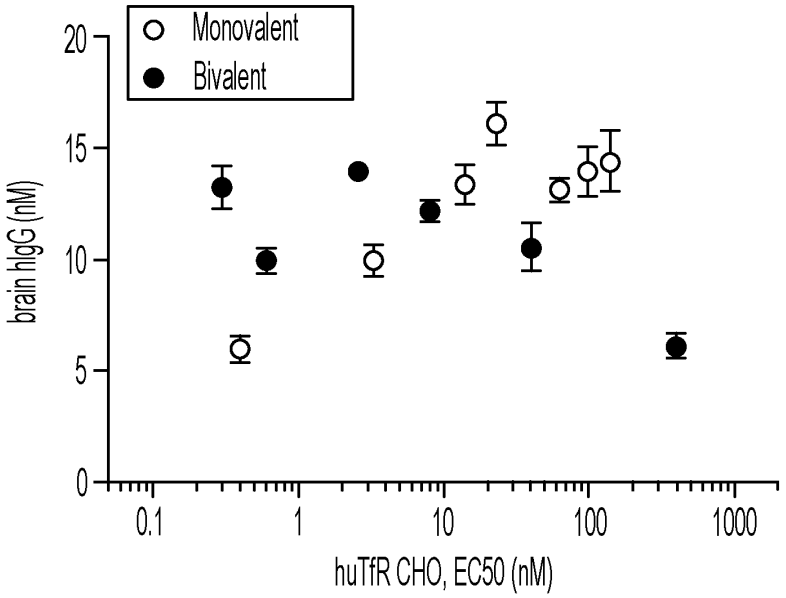

FIG. 10B

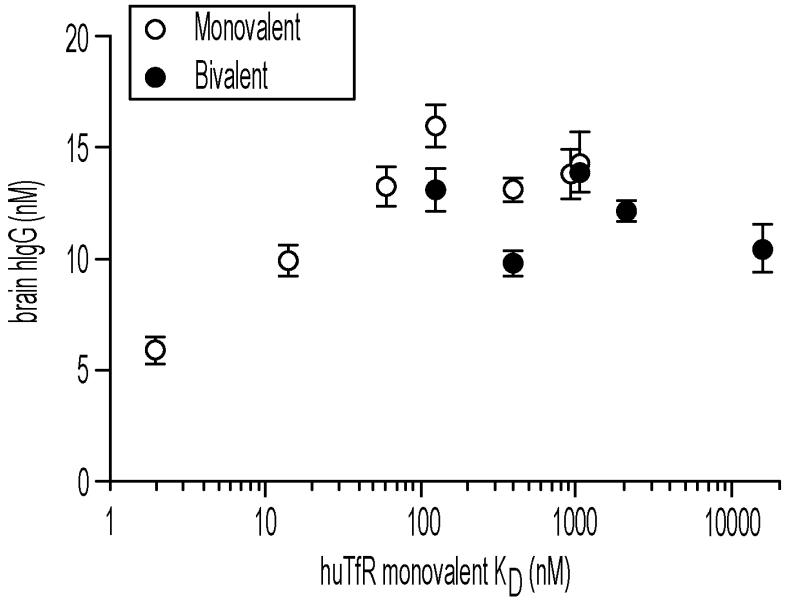

FIG. 10C

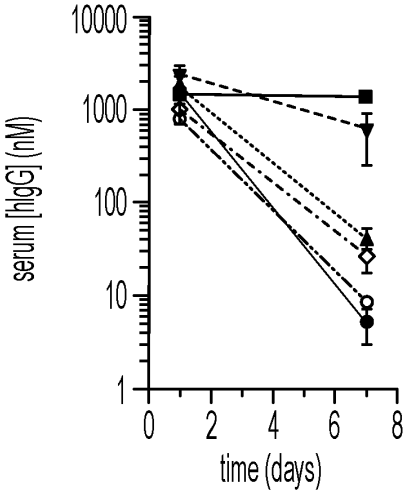

FIG. 11A

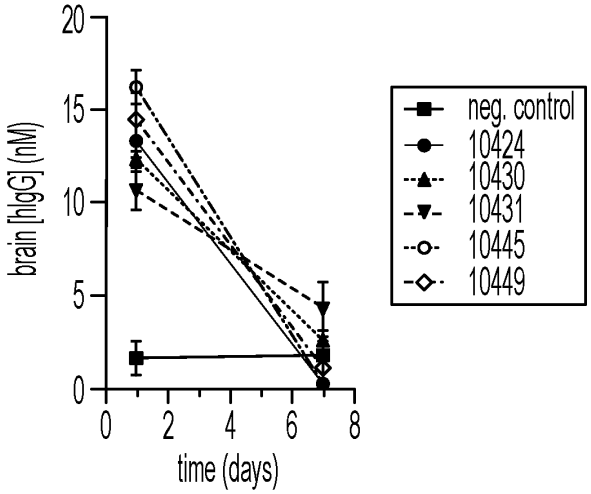

FIG. 11B

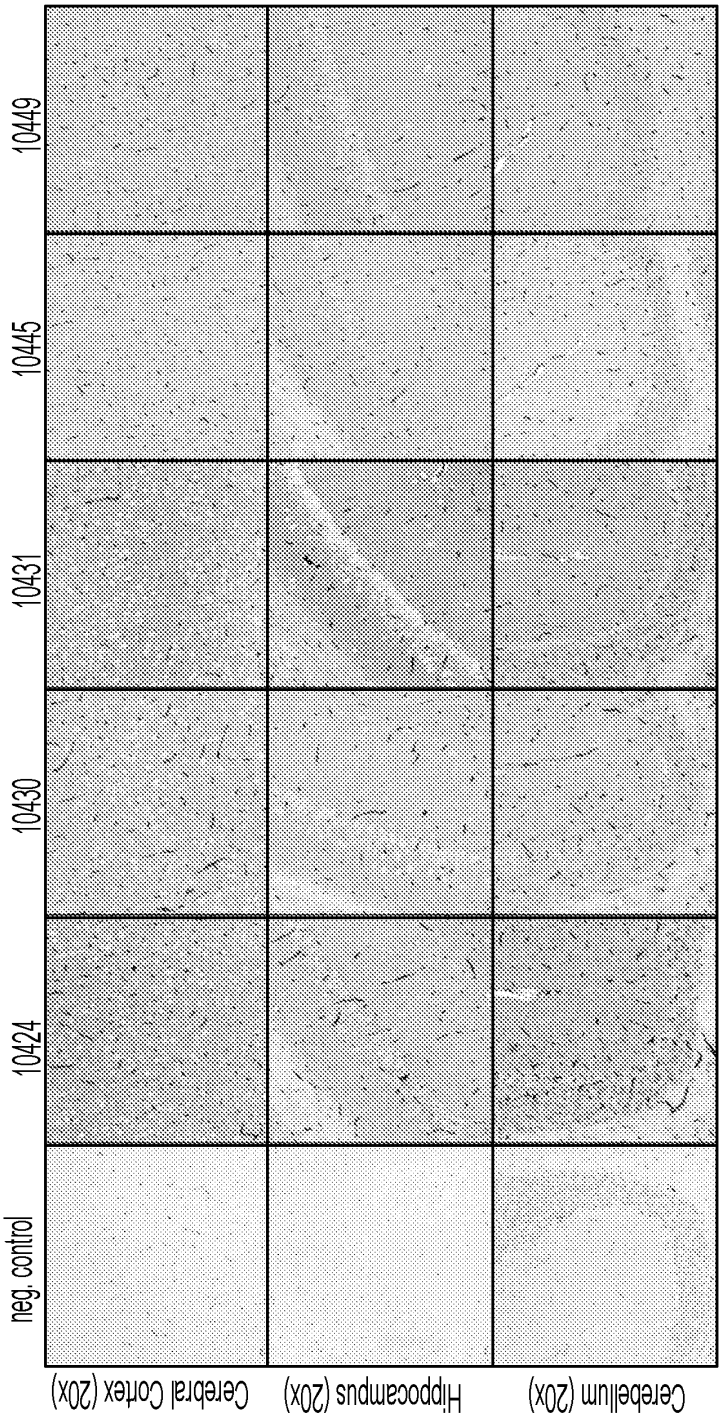
neg. control
10424
10430
10431
10445
10449
Cerebellum (20x)
Hippocampus (20x)
Cerebral Cortex (20x)

FIG. 12

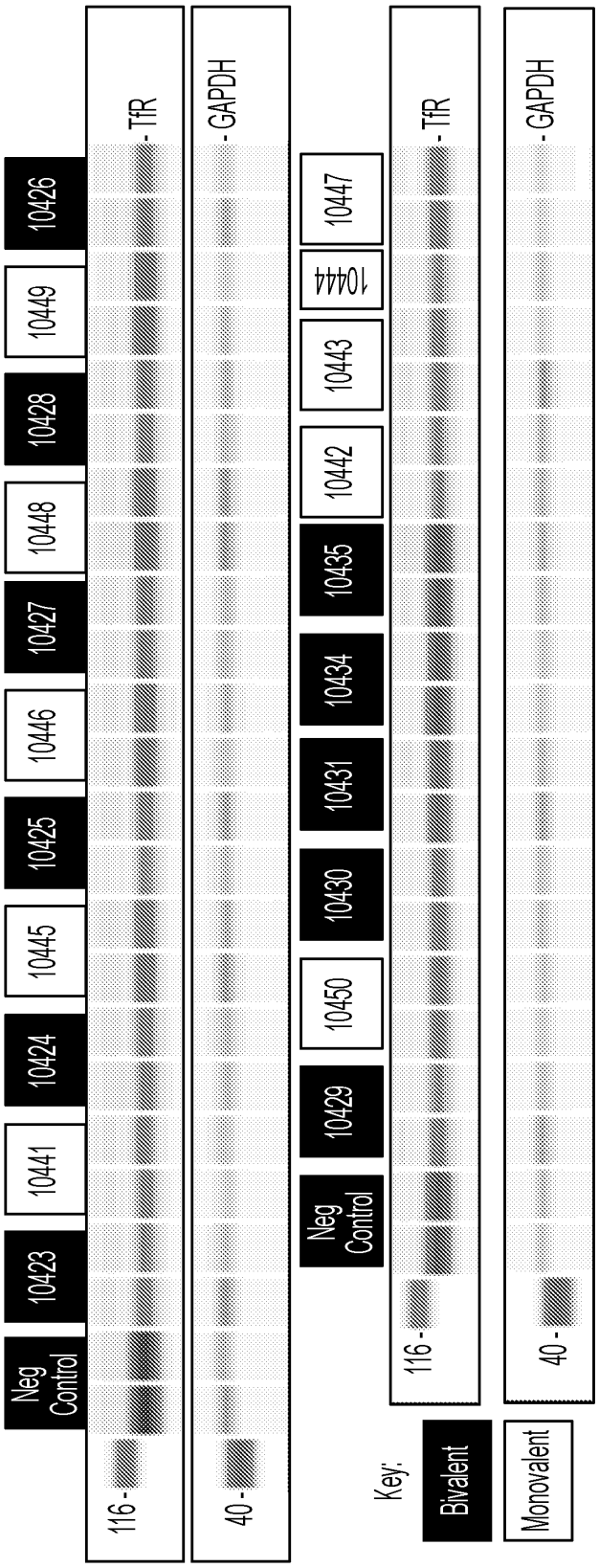
Neg Control
10423
10441
10424
10445
10425
10446
10427
10448
10428
10449
10426
116 -
- TfR
40 -
- GAPDH
Neg Control
10429
10450
10430
10431
10434
10435
10442
10443
10444
10447
116 -
- TfR
40 -
- GAPDH
Key:
Bivalent
Monovalent

FIG. 13A

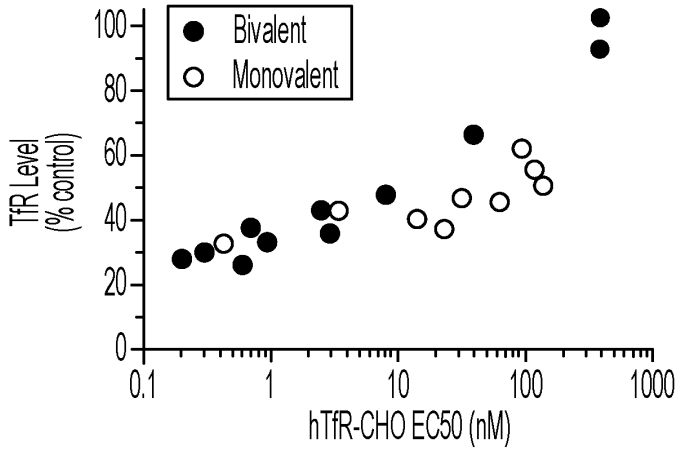
Bivalent
Monovalent
TfR Level
(% control)
100
80
60
40
20
0
0.1
1
10
100
1000
hTfR-CHO EC50 (nM)

FIG. 13B

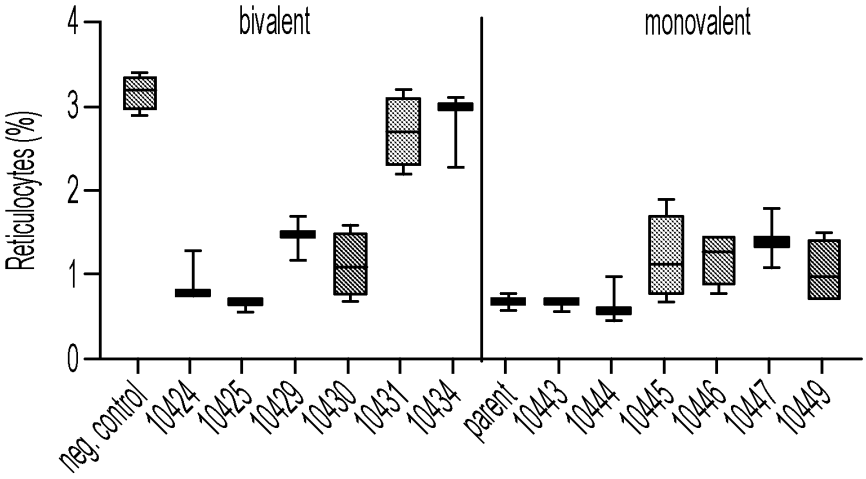

FIG. 14A

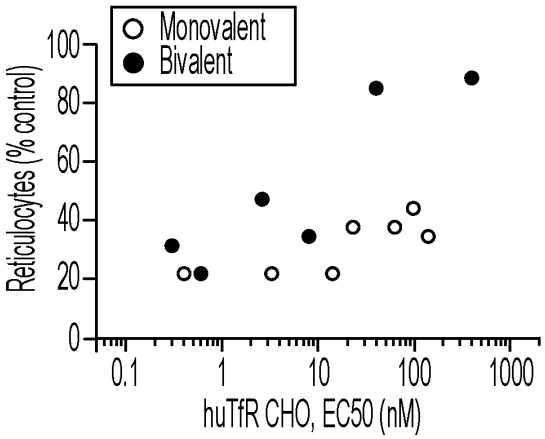

FIG. 14B

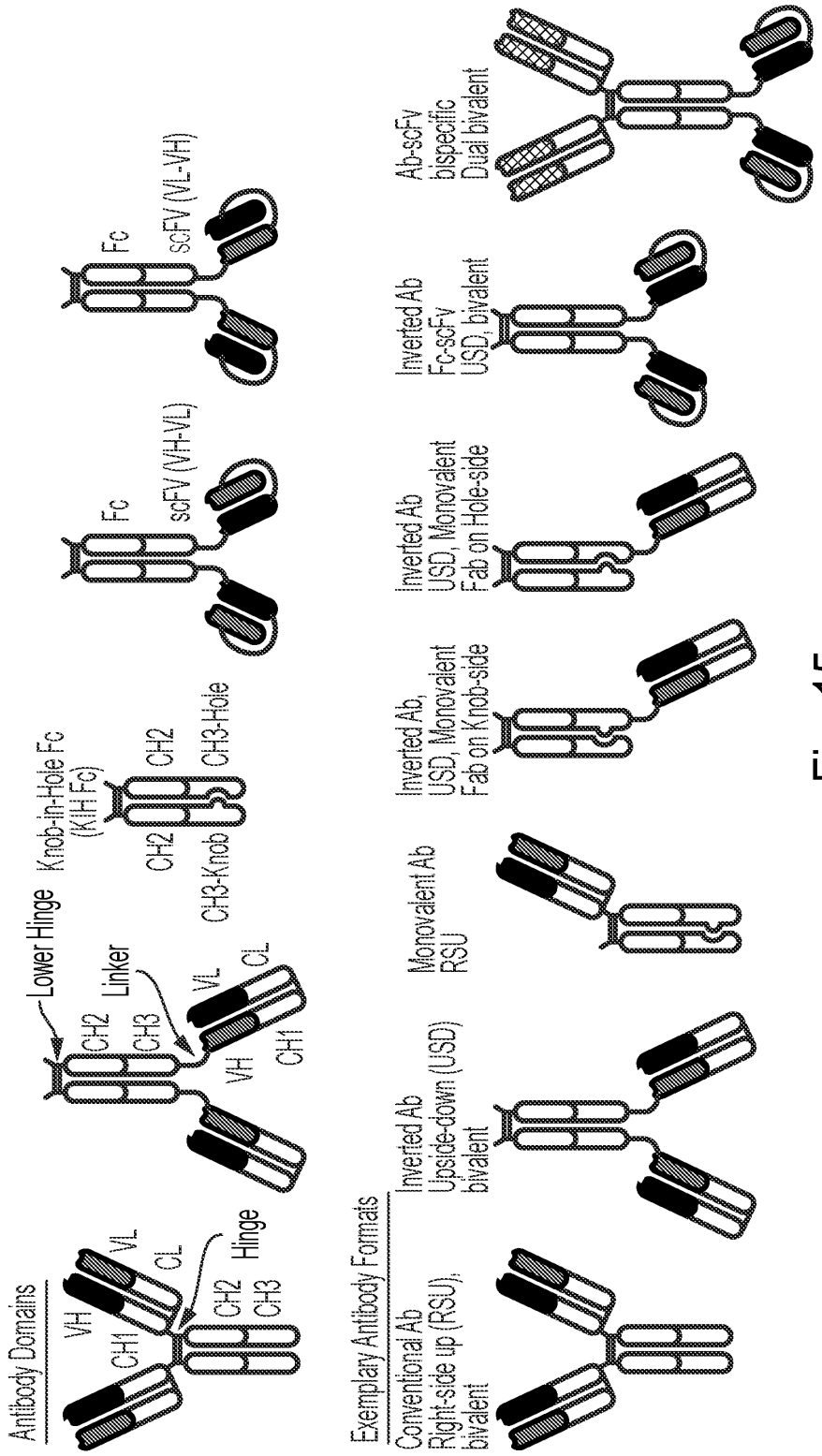
Antibody Domains
VH
VL
CH1
CL
CH2
CH3
Hinge
Lower Hinge
Linker
Knob-in-Hole Fc (KIH Fc)
CH3-Knob
CH3-Hole
Fc
scFV (VH-VL)
scFV (VL-VH)
Exemplary Antibody Formats
Conventional Ab Right-side up (RSU), bivalent
Inverted Ab Upside-down (USD) bivalent
Monovalent Ab RSU
Inverted Ab, USD, Monovalent Fab on Knob-side
Inverted Ab USD, Monovalent Fab on Hole-side
Inverted Ab Fc-scFv USD, bivalent
Ab-scFv bispecific Dual bivalent

Fig. 15