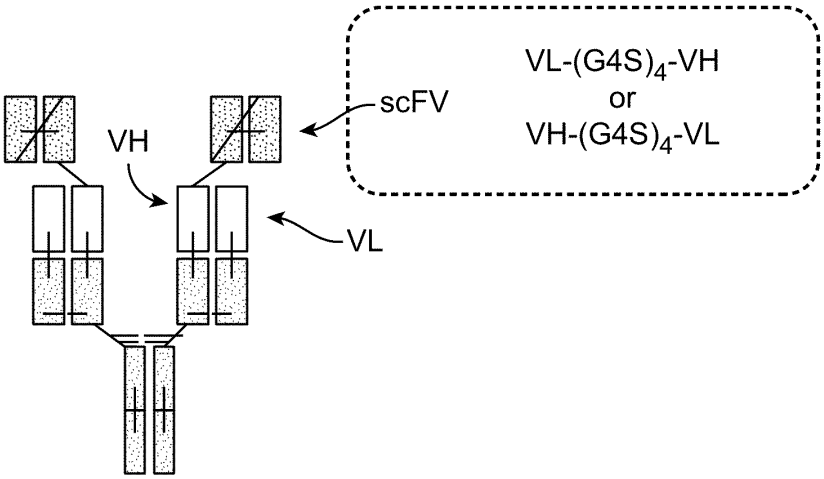

VH
scFV
VL
VL-(G4S)4-VH
or
VH-(G4S)4-VL

FIG. 1A

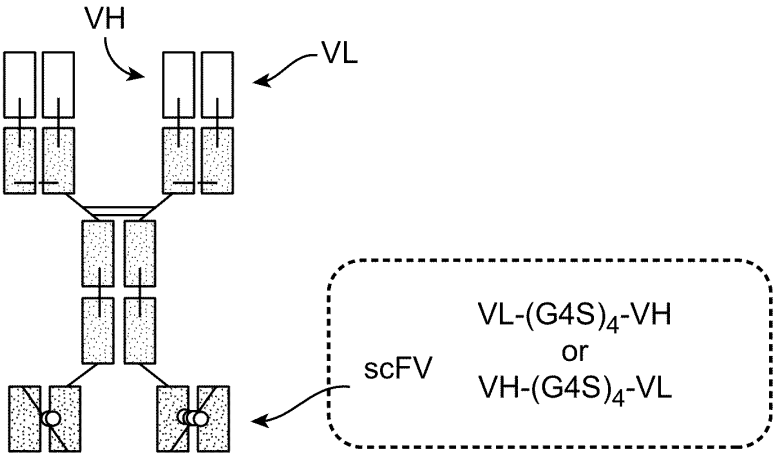

VH
VL
scFV
VL-(G4S)4-VH
or
VH-(G4S)4-VL

FIG. 1B

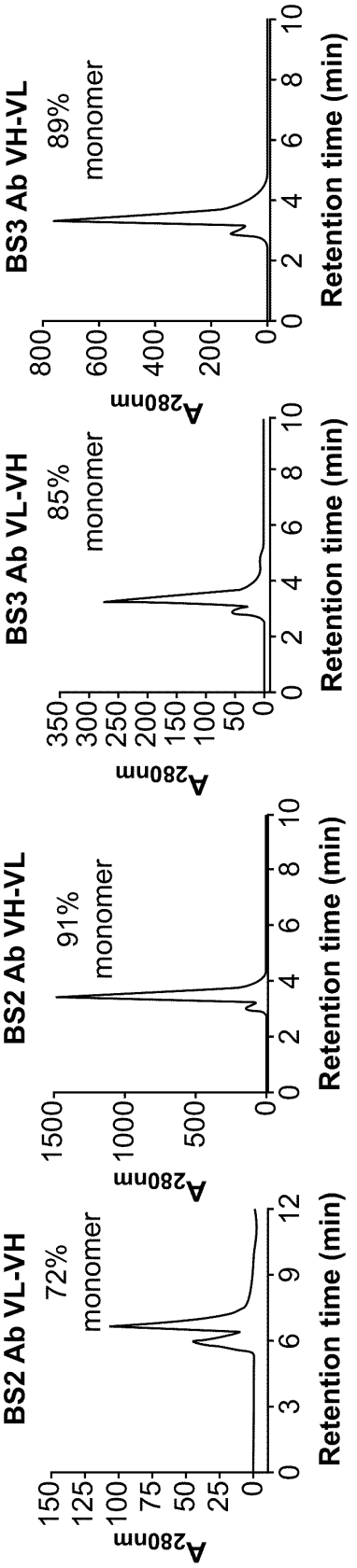


FIG. 2A

FIG. 2B

Test Molecule	After 10 days (mg/L)	% main peak after protein A
BS2 Ab VL-VH	180	72
BS2 Ab VH-VL	340	91
BS3 Ab VL-VH	130	85
BS3 Ab VH-VL	200	89

FIG. 2C

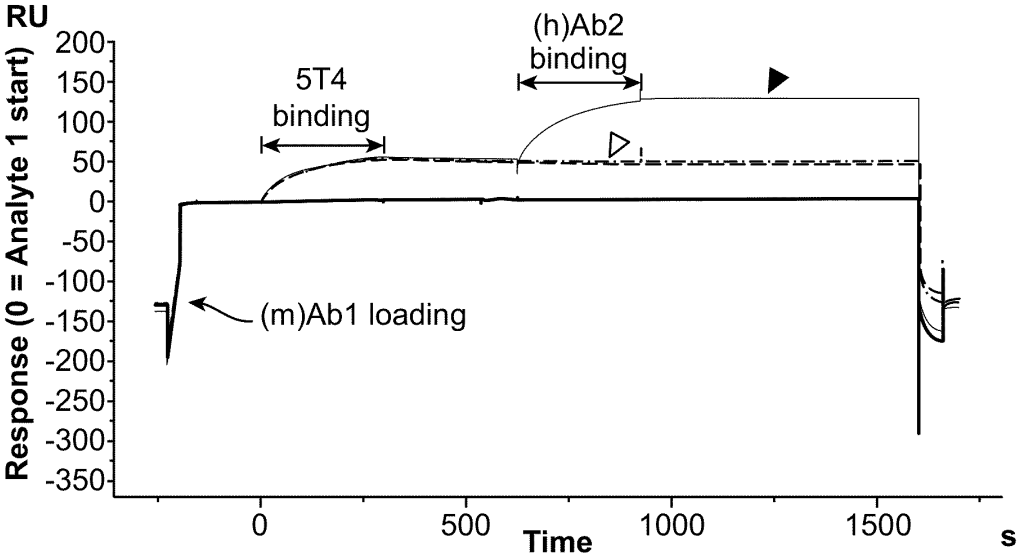


FIG. 3A

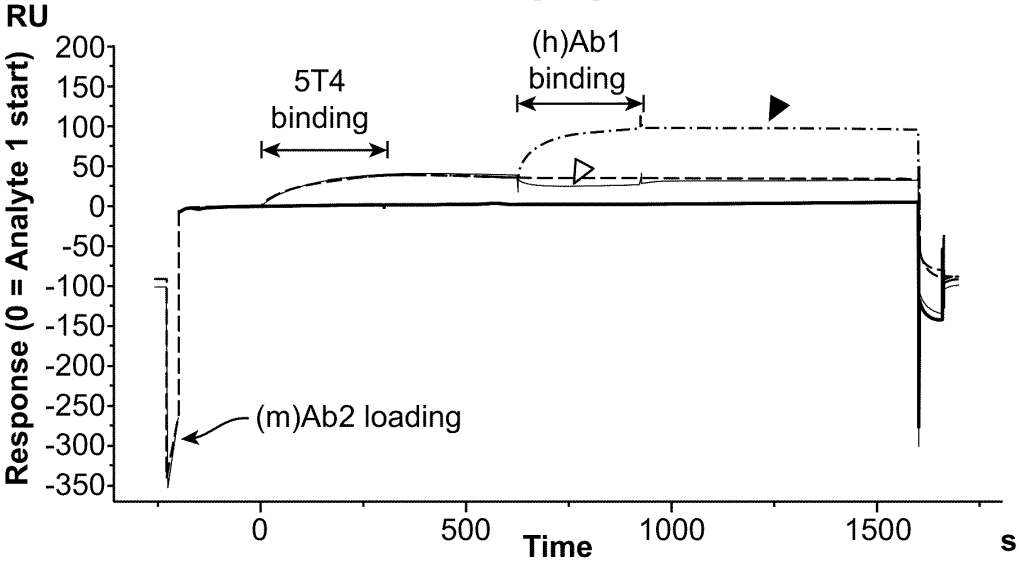


FIG. 3B

Curve	Injected Sample	
	Mouse Antibody binding	5T4 binding
Red	5T4	Human Ab1
Blue	5T4	Human Ab2
Yellow	5T4	Buffer
Green	Buffer	Buffer

FIG. 3C

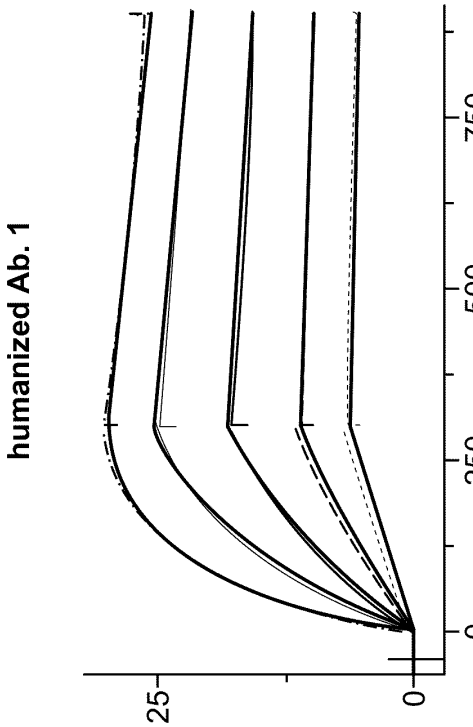

humanized Ab. 1
25
0
0
250
500
750
Group 1
Model
1:1 binding
ka(1/Ms) 1.25e+06 kd(1/s) 2.48e-04
Rmax(RU) 31.2 KD (M) 1.98e-10

FIG. 4A

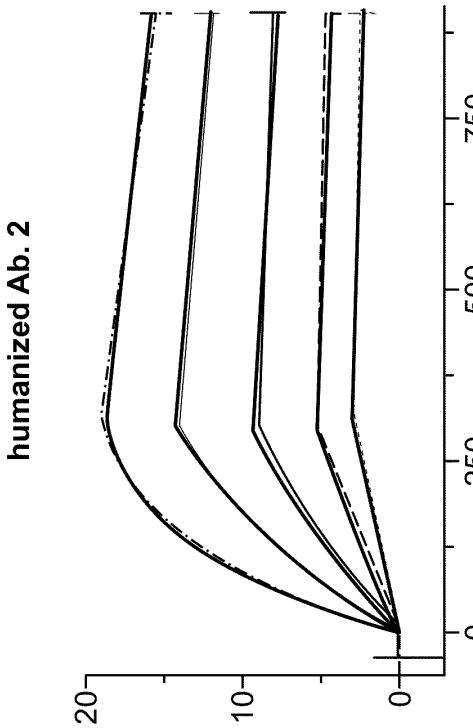

humanized Ab. 2
20
10
0
0
250
500
750
Group 2
Model
1:1 binding
ka(1/Ms) 8.84e+05 kd(1/s) 2.83e-04
Rmax(RU) 21.0 KD (M) 3.20e-10

FIG. 4B

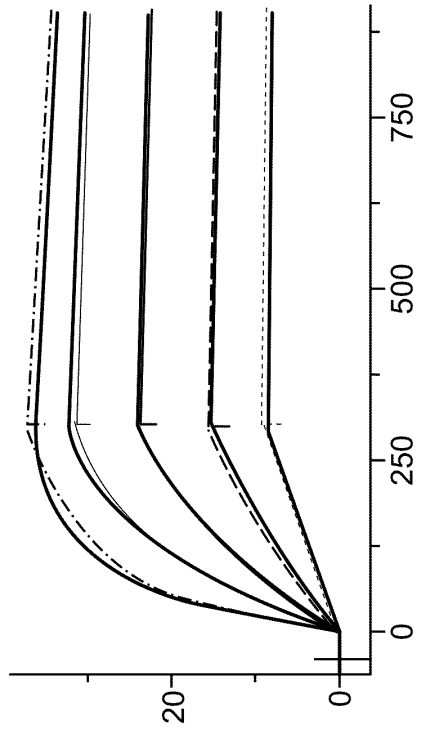

Bs3-HL
20
0
0
250
500
750
Group 5
Model
1:1 binding
ka(1/Ms) 1.50e+06 kd(1/s) 1.11e-04
Rmax(RU) 18.5 KD (M) 7.42e-11

FIG. 4C

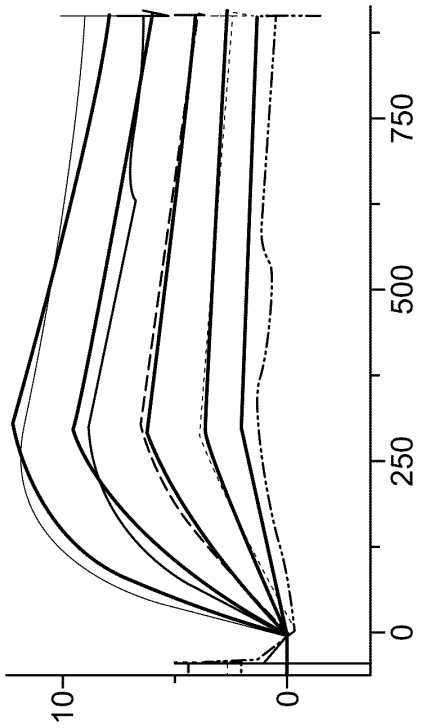

Bs3-HL-FCA
10
0
0
250
500
750
Group 4
Model
1:1 binding
ka(1/Ms) 9.41e+05 kd(1/s) 7.30e-04
Rmax(RU) 1.42 KD (M) 7.75e-10

FIG. 4D

Antibodies	Ka (1/Ms)	Kd (1/s)	KD (M)
Humanized No. 1	$1.25x10^{6}$	$2.48x10^{-4}$	$1.98x10^{-10}$
Humanized No. 2	$8.84x10^{5}$	$2.83x10^{-4}$	$3.20x10^{-10}$
Bs3-HL	$1.50x10^{6}$	$1.11x10^{-4}$	$7.42x10^{-11}$
Bs3-HL-FCA	$9.41x10^{5}$	$7.30x10^{-4}$	$7.75x10^{-10}$

FIG. 4E

	Mouse	Rat	Cynomolgus Monkey	Rhesus Monkey	Human
Accession Number	NP_001158264.1	NP_113995.2	NP_001306553.1	NP_001180630.1	NP_001159864.1
Extracellular Domain	79%	78%	94%	96%	100%
Full Length	82%	82%	96%	97%	100%

FIG. 5

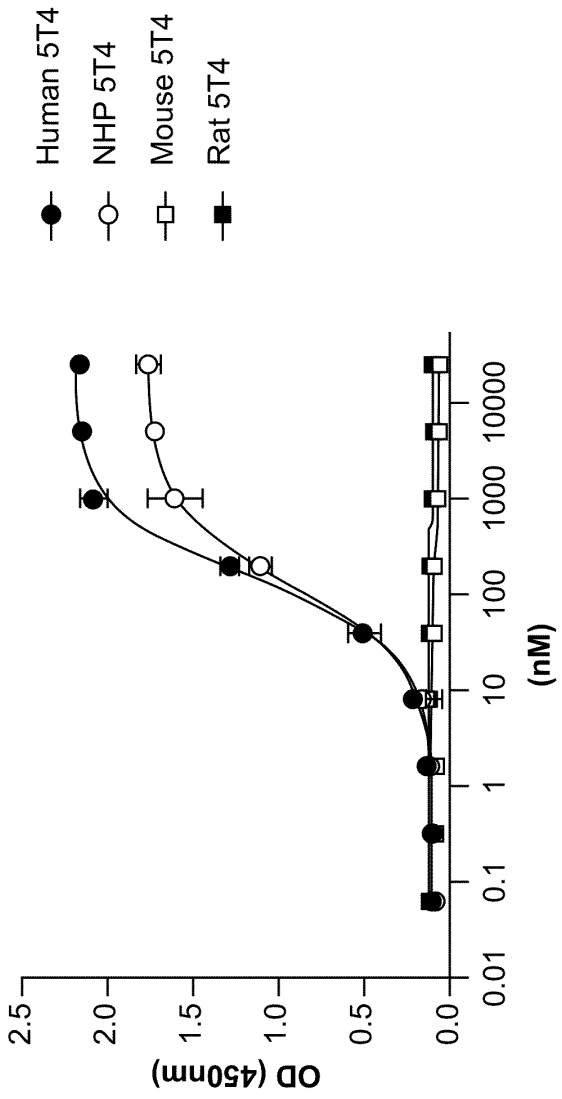

Human 5T4
NHP 5T4
Mouse 5T4
Rat 5T4
OD (450nm)
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
10000
(nM)

FIG. 6

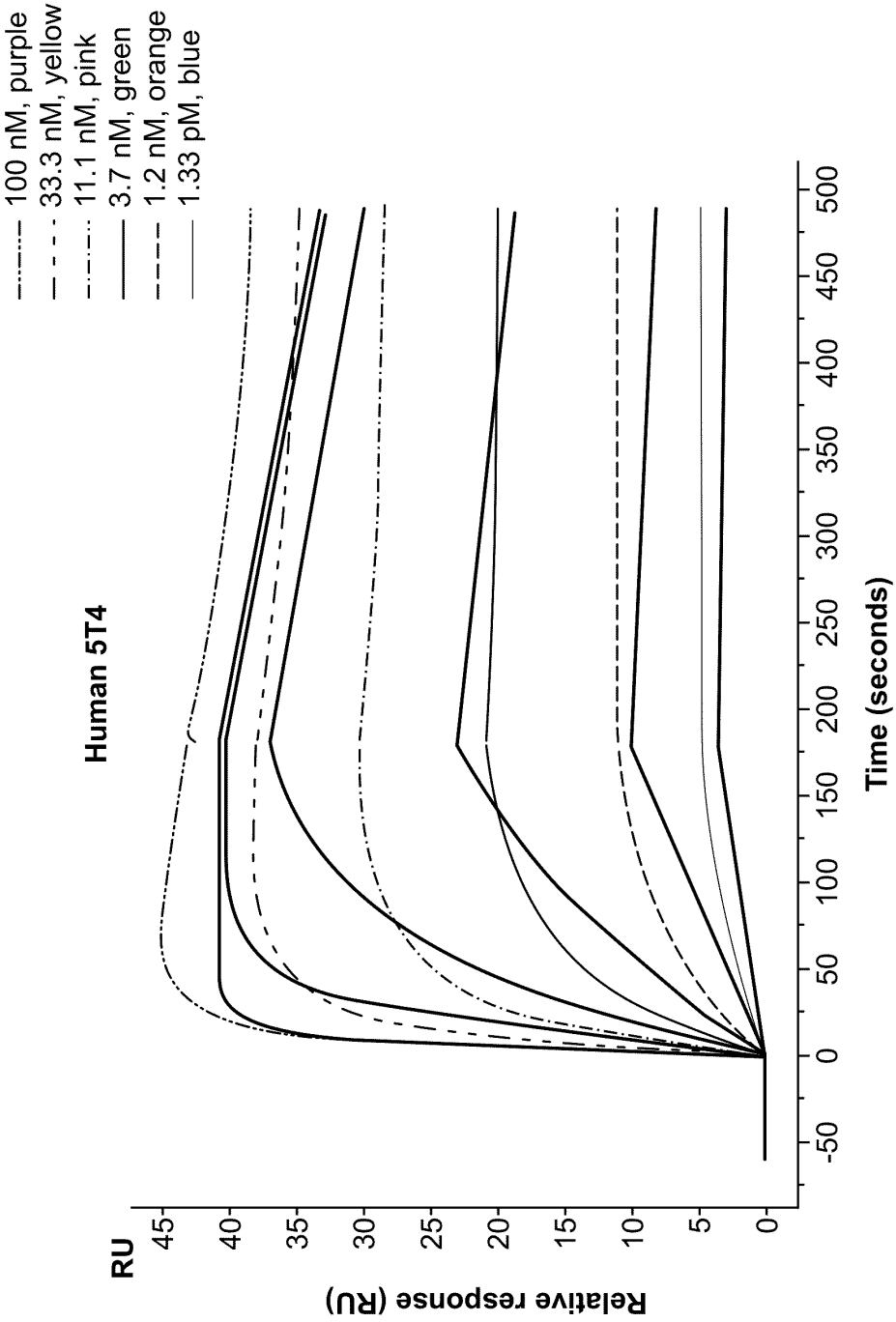

100 nM, purple
33.3 nM, yellow
11.1 nM, pink
3.7 nM, green
1.2 nM, orange
1.33 pM, blue
Human 5T4
RU
45
40
35
30
25
20
15
10
5
0
Relative response (RU)
-50
0
50
100
150
200
250
300
350
400
450
500
Time (seconds)

FIG. 7A

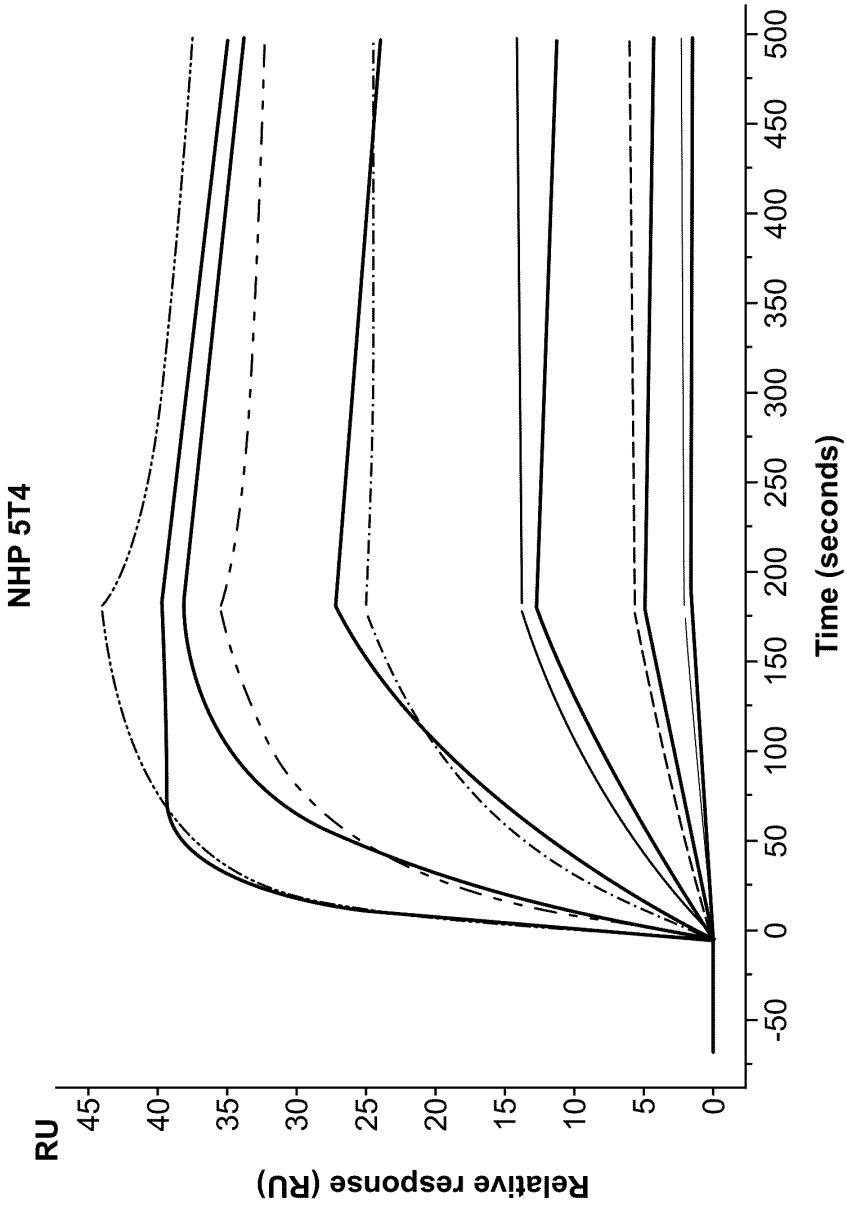

NHP 5T4
RU
45
40
35
30
25
20
15
10
5
0
Relative response (RU)
-50
0
50
100
150
200
250
300
350
400
450
500
Time (seconds)

FIG. 7B

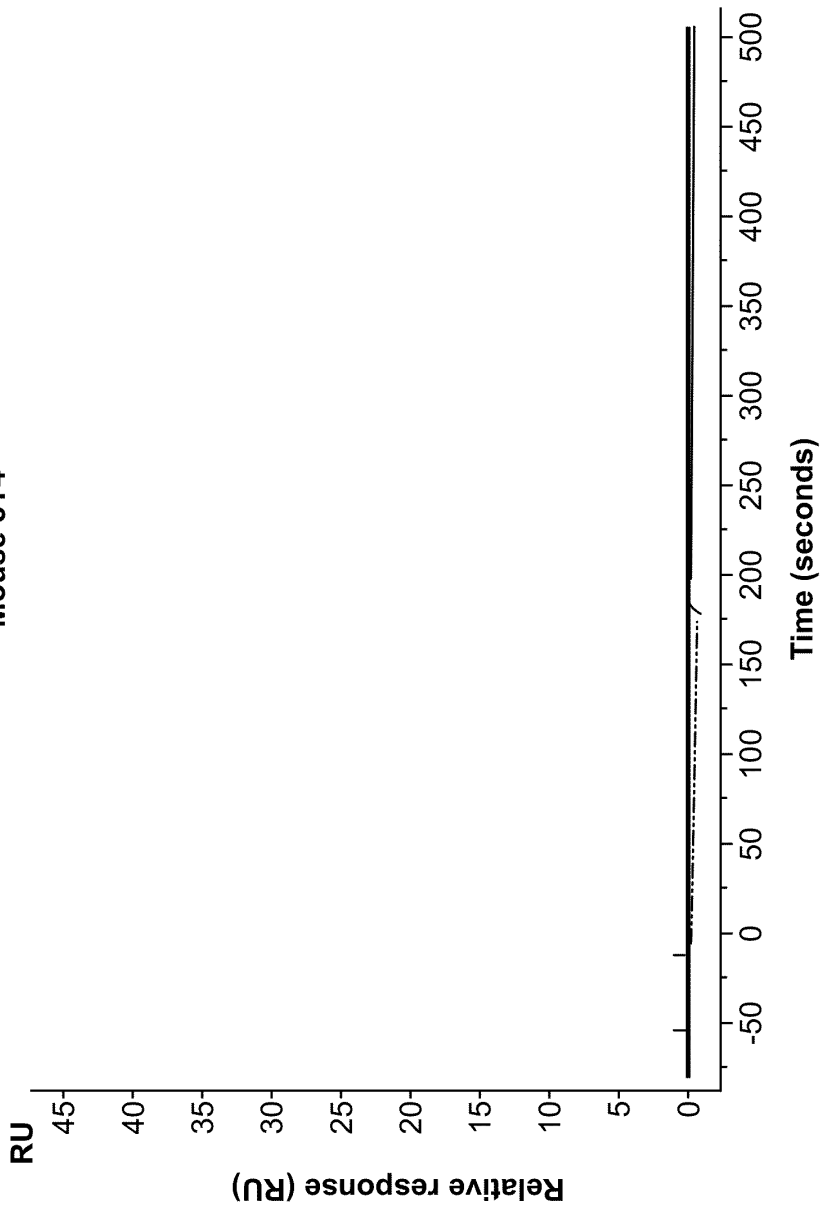

Mouse 5T4
RU
45
40
35
30
25
20
15
10
5
0
Relative response (RU)
-50
0
50
100
150
200
250
300
350
400
450
500
Time (seconds)

FIG. 7C

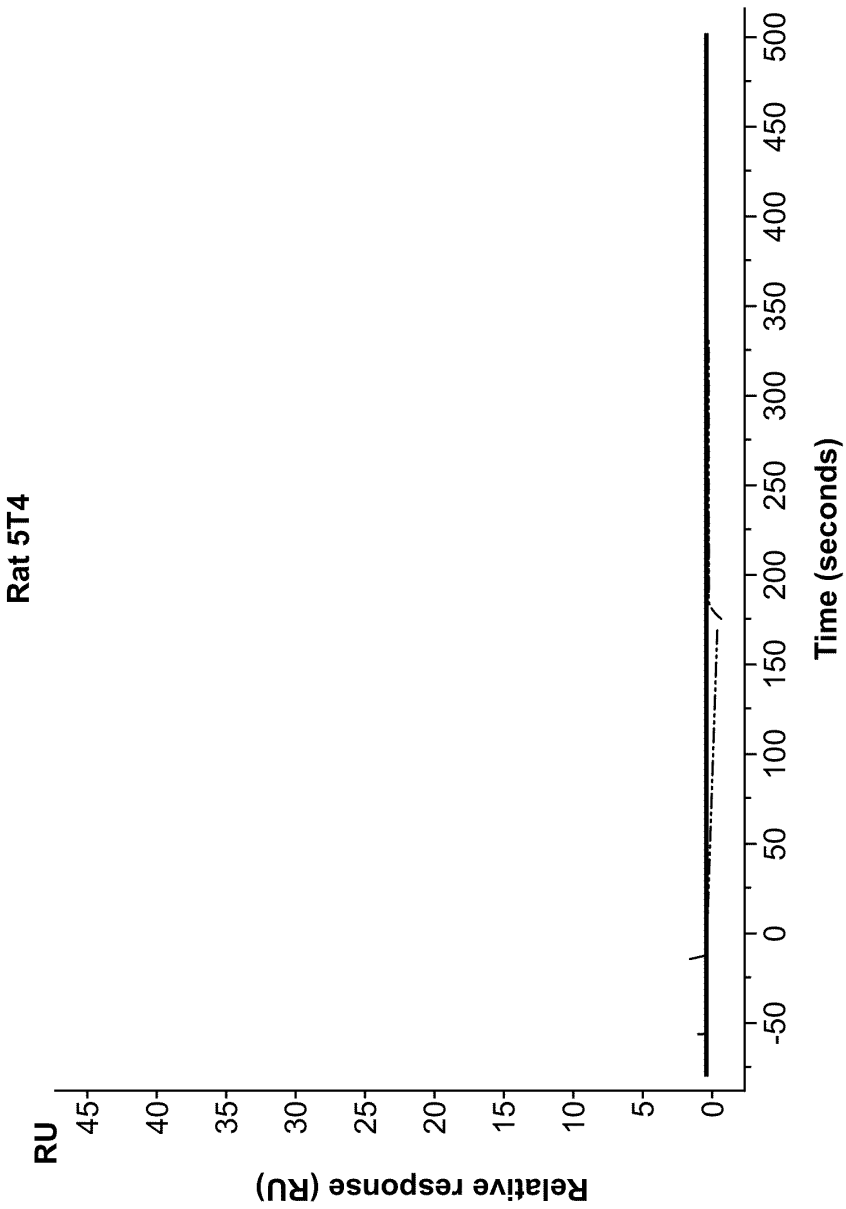

Rat 5T4
RU
45
40
35
30
25
20
15
10
5
0
Relative response (RU)
-50
0
50
100
150
200
250
300
350
400
450
500
Time (seconds)

FIG. 7D

Fc Receptor	KD	
	5T4-Bs3- FCA	Parental Ab1 without Fc mutations
FcγRI	3.96E-08	3.73E-09
FcγRIIa	9.15E-08	9.86E-08
FcγRIIb	1.16E-07	1.11E-07
FcγRIIIa	No binding	5.05E-08
FcγRIIIb	No binding	No binding

FIG. 8

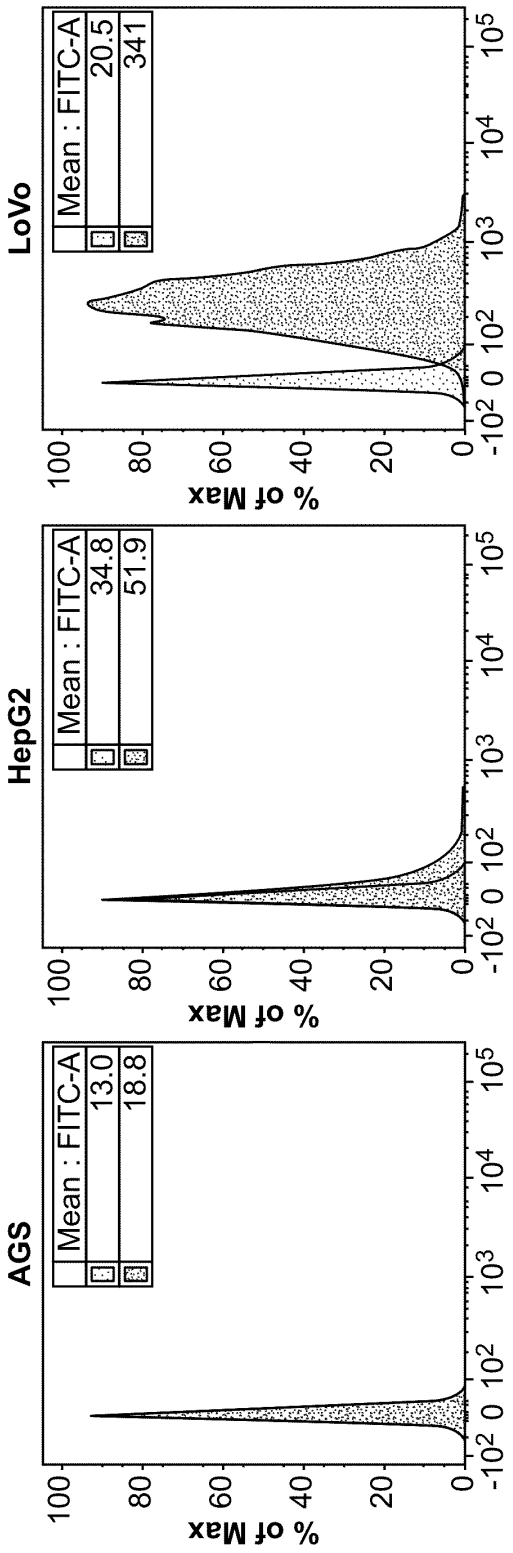


FIG. 9A

FIG. 9B

FIG. 9C

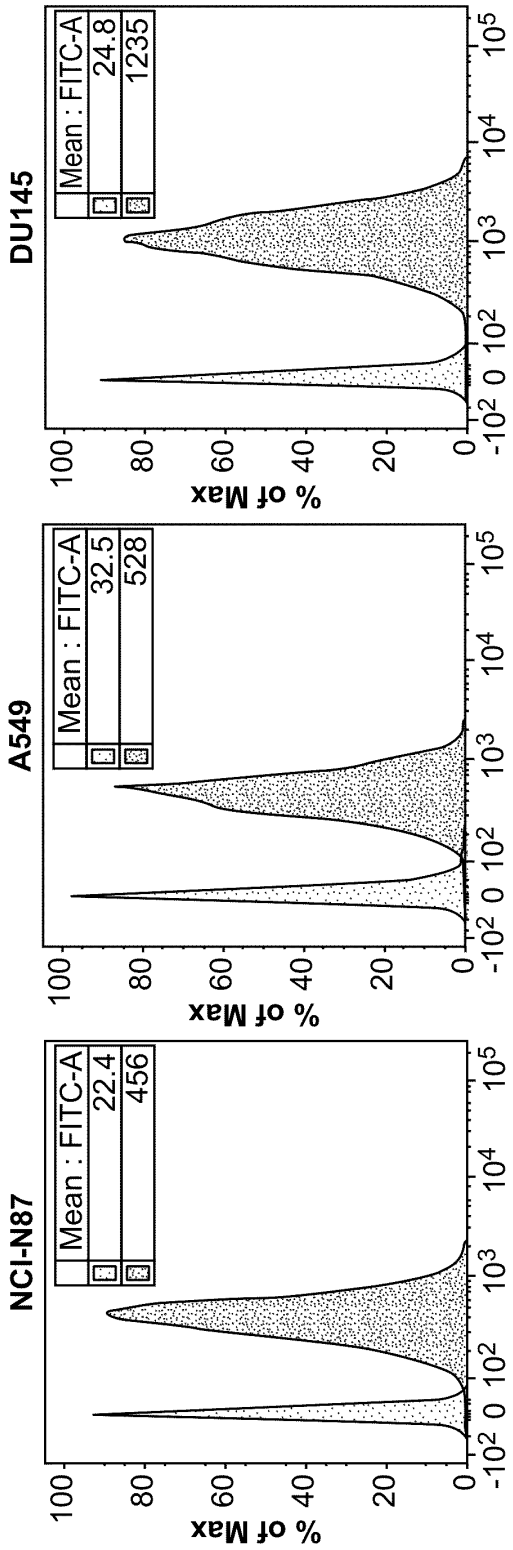


FIG. 9D

FIG. 9E

FIG. 9F

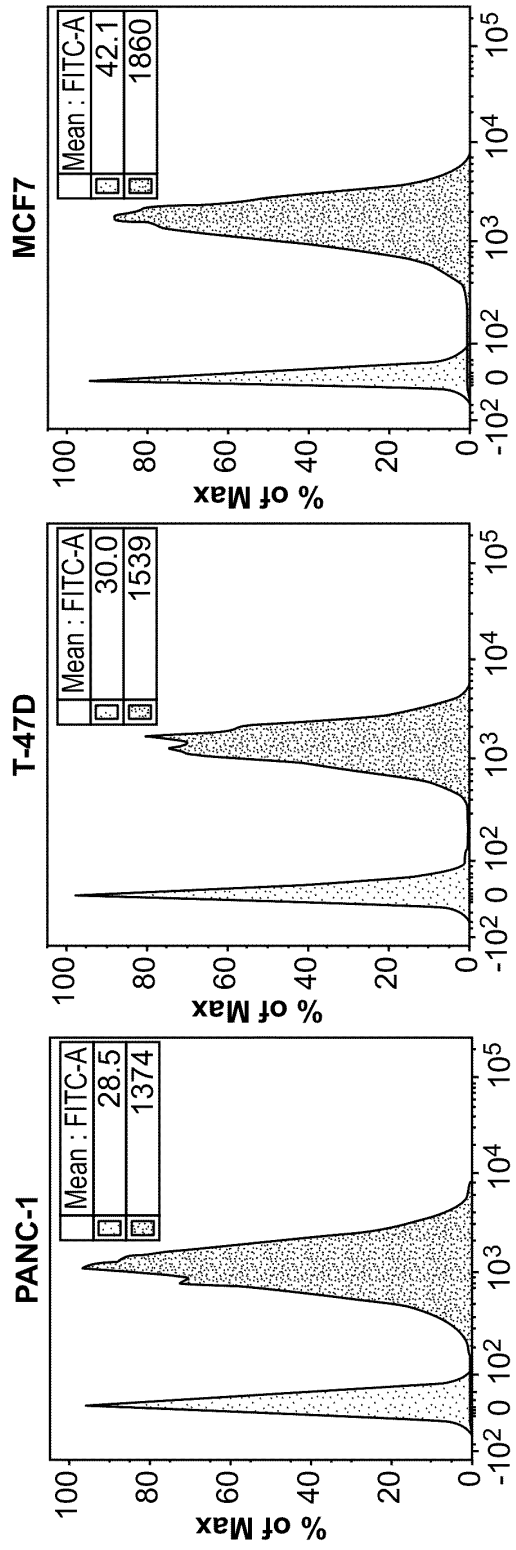


FIG. 9G

FIG. 9H

FIG. 9I

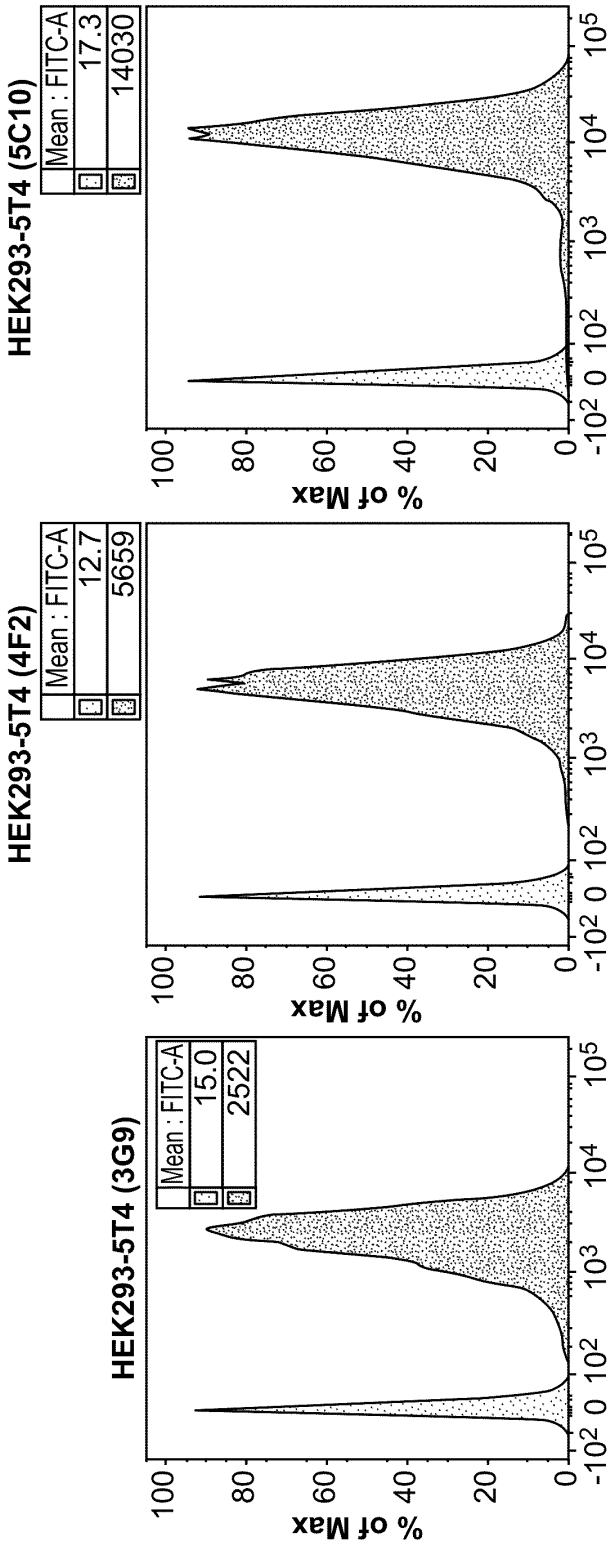


FIG. 9J

FIG. 9K

FIG. 9L

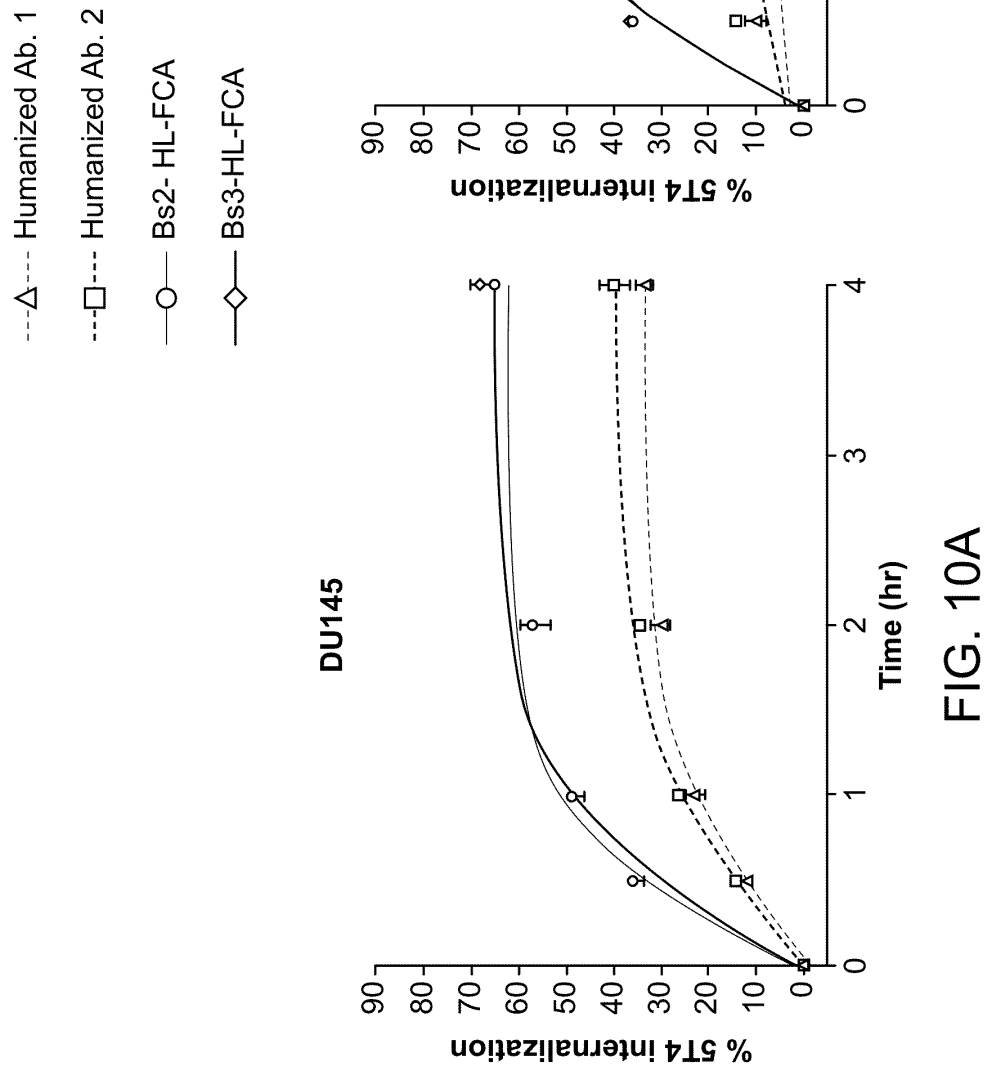


FIG. 10A

FIG. 10B

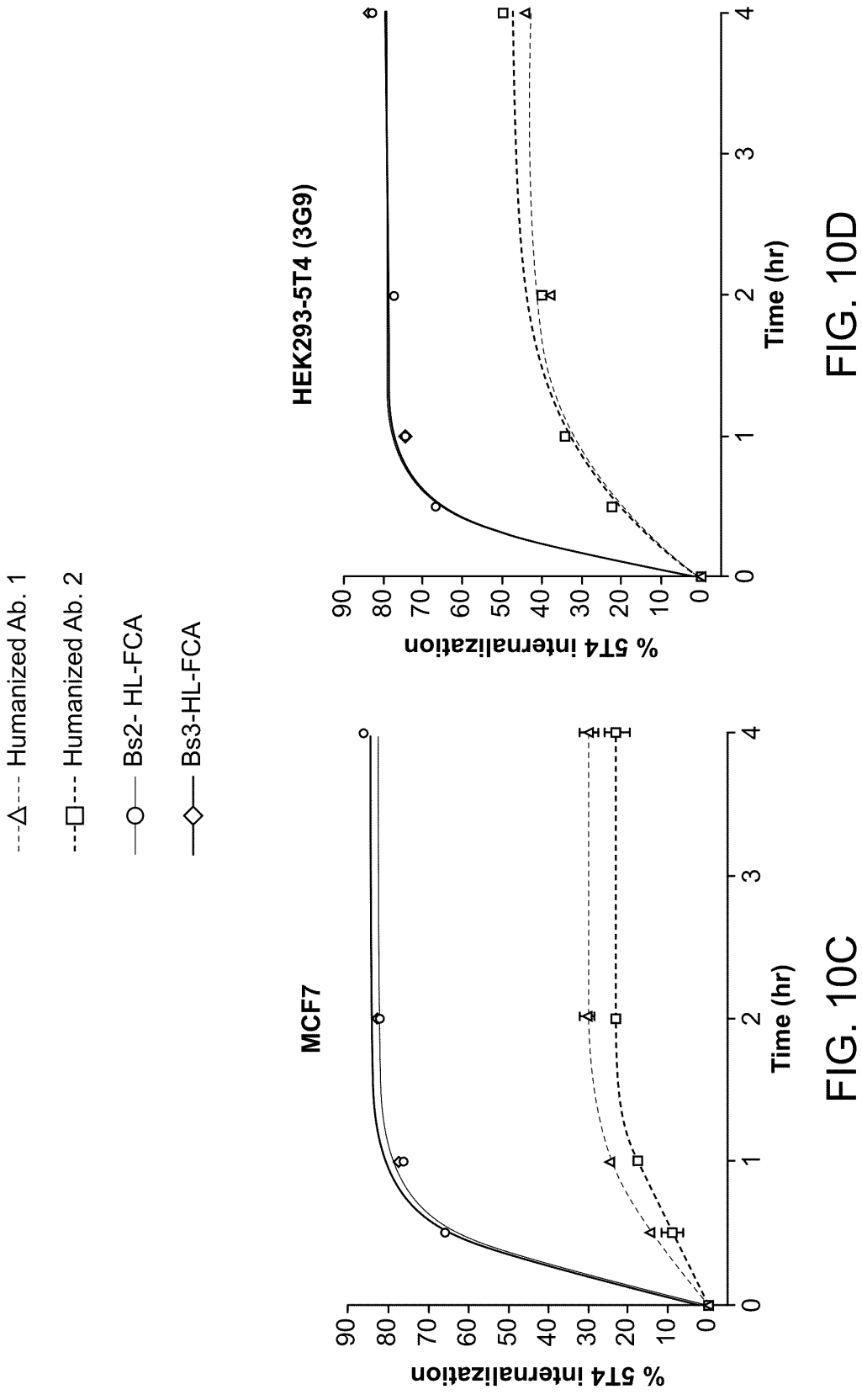


FIG. 10C

FIG. 10D

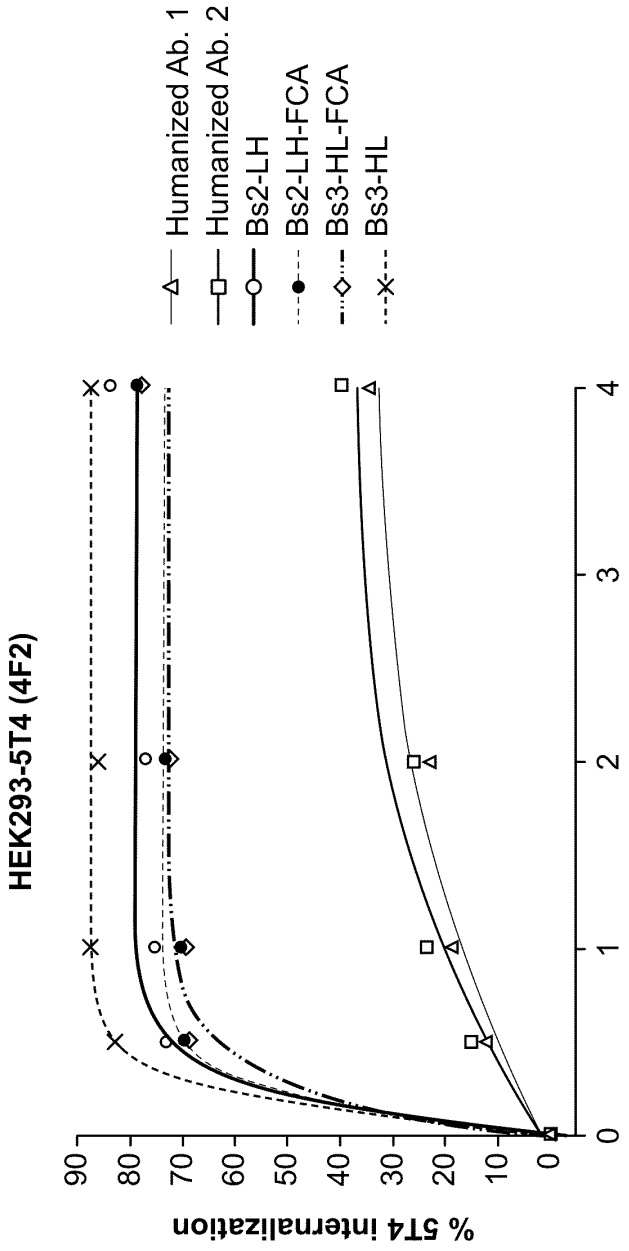

Humanized Ab. 1
Humanized Ab. 2
Bs2-LH
Bs2-LH-FCA
Bs3-HL-FCA
Bs3-HL
HEK293-5T4 (4F2)
% 5T4 internalization
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
Time (hr)

FIG. 10E

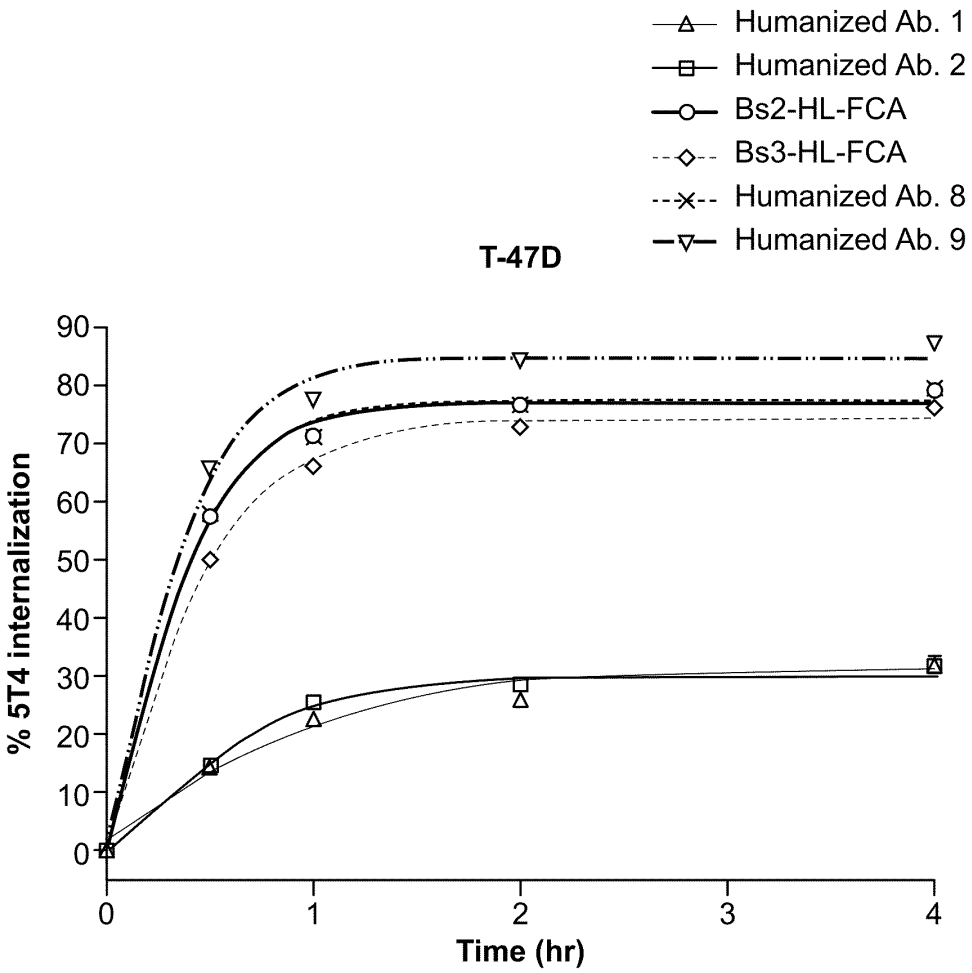

Humanized Ab. 1
Humanized Ab. 2
Bs2-HL-FCA
Bs3-HL-FCA
Humanized Ab. 8
Humanized Ab. 9
T-47D
% 5T4 internalization
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
Time (hr)

FIG. 10F

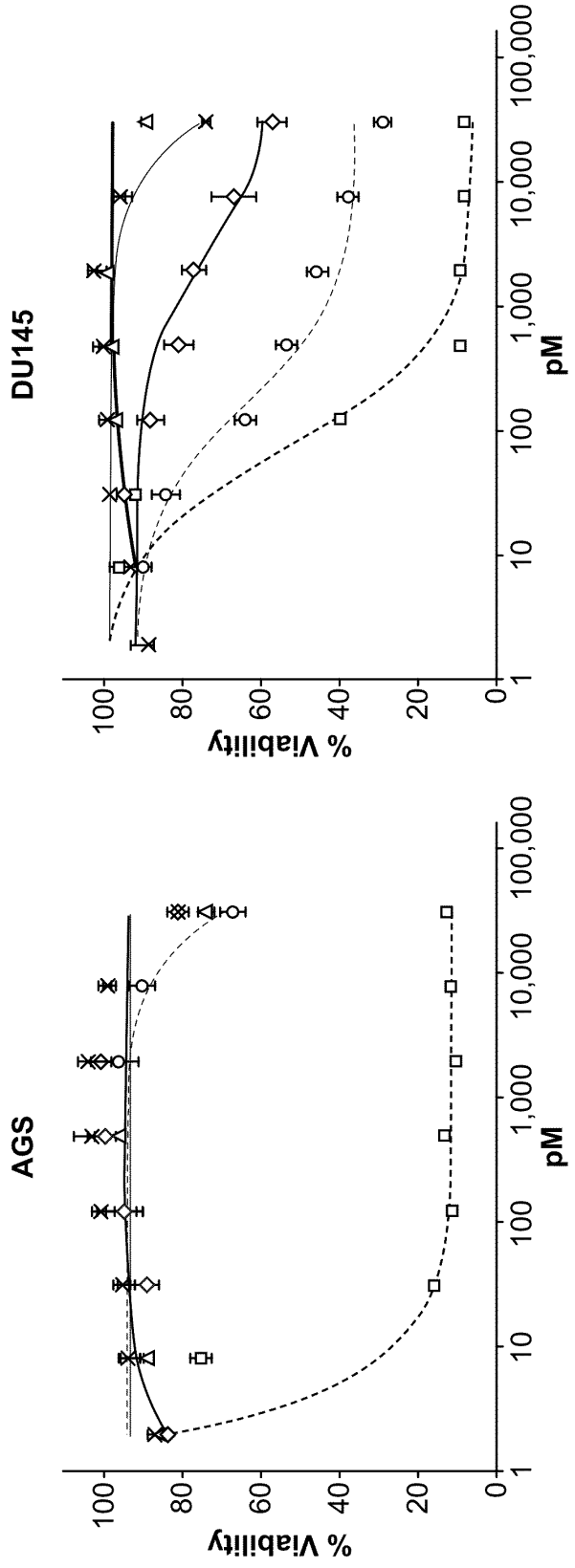


FIG. 11A

FIG. 11B

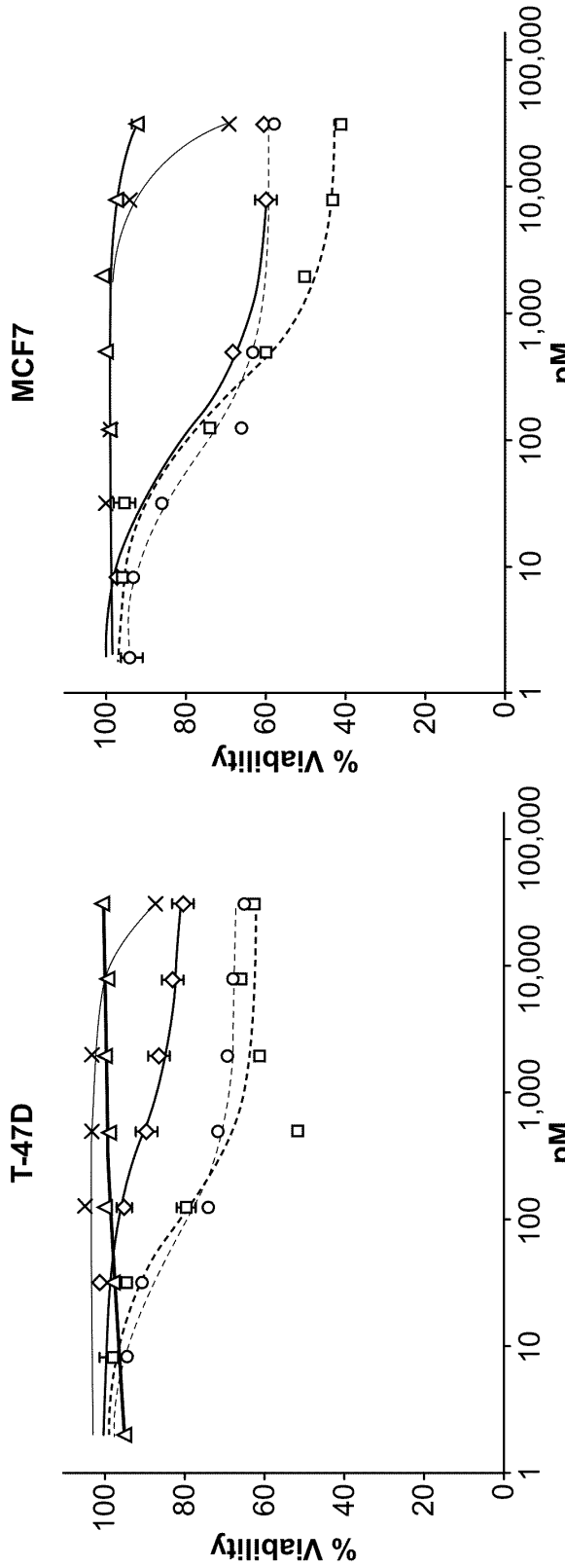


FIG. 11C

FIG. 11D

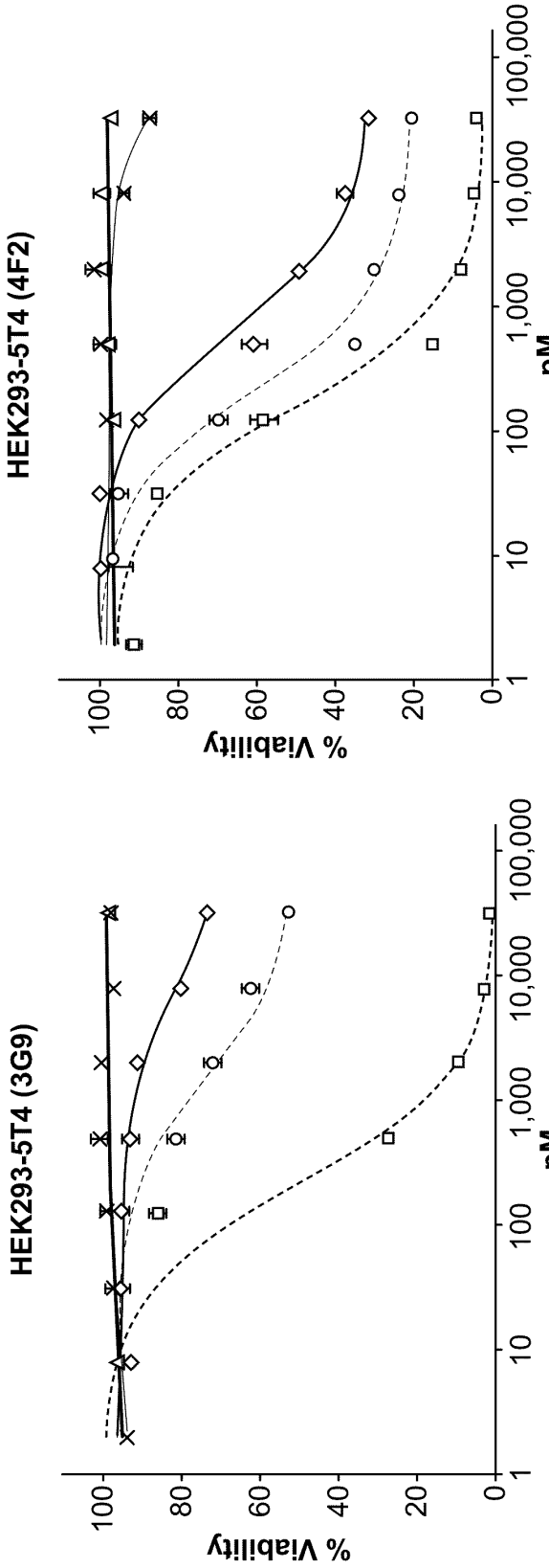


FIG. 11E

FIG. 11F

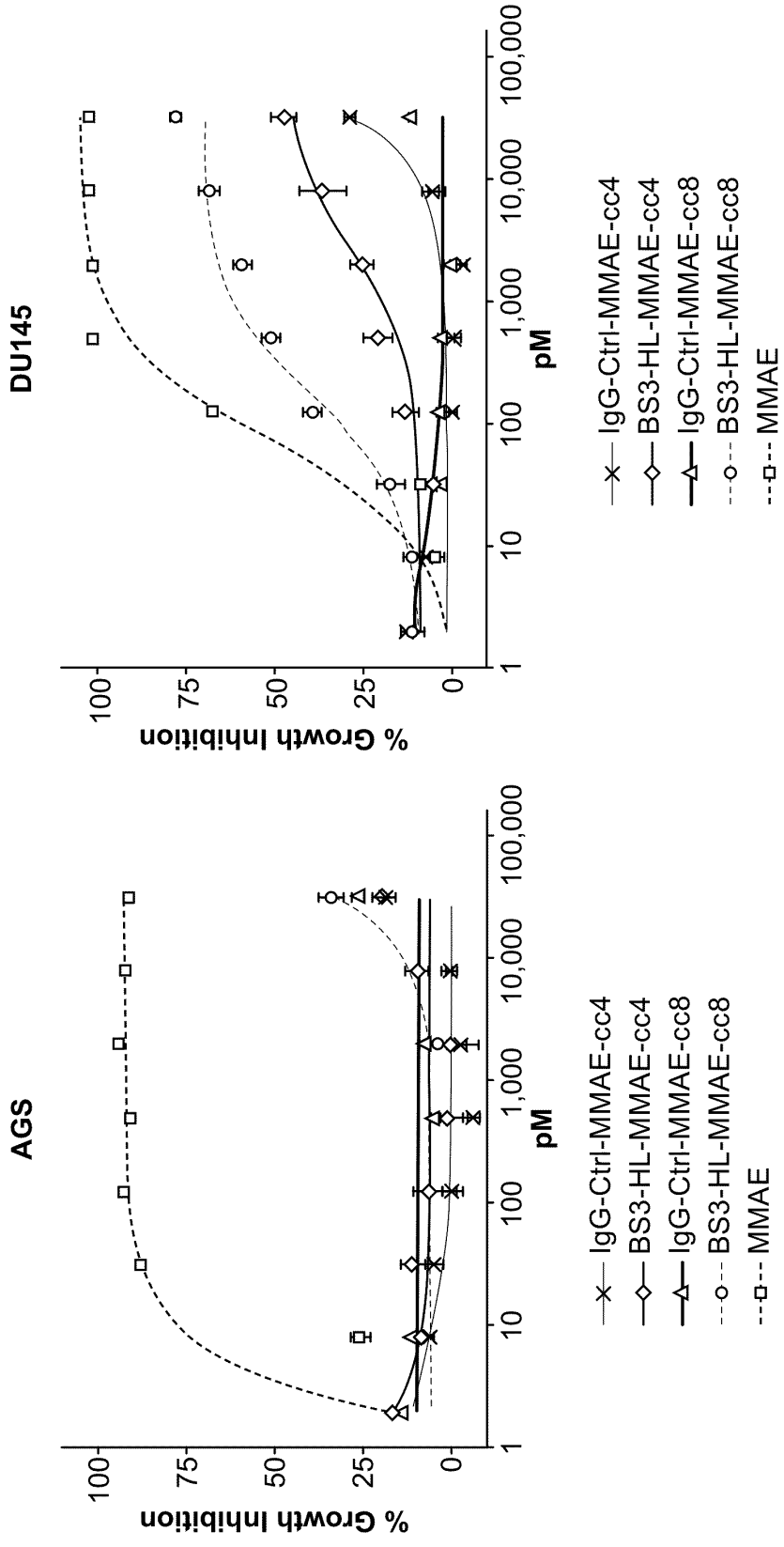


FIG. 12B

FIG. 12A

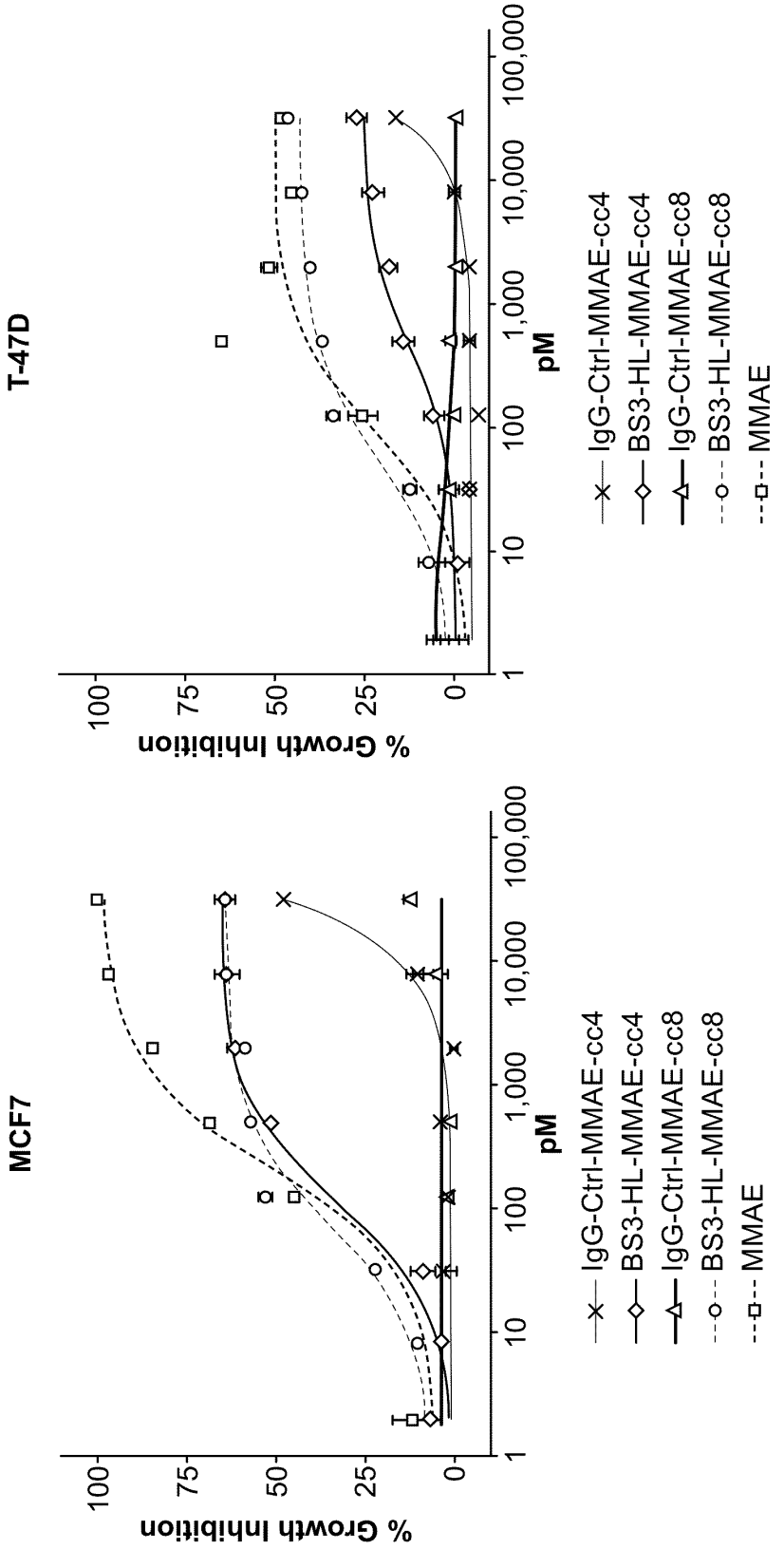


FIG. 12C

FIG. 12D

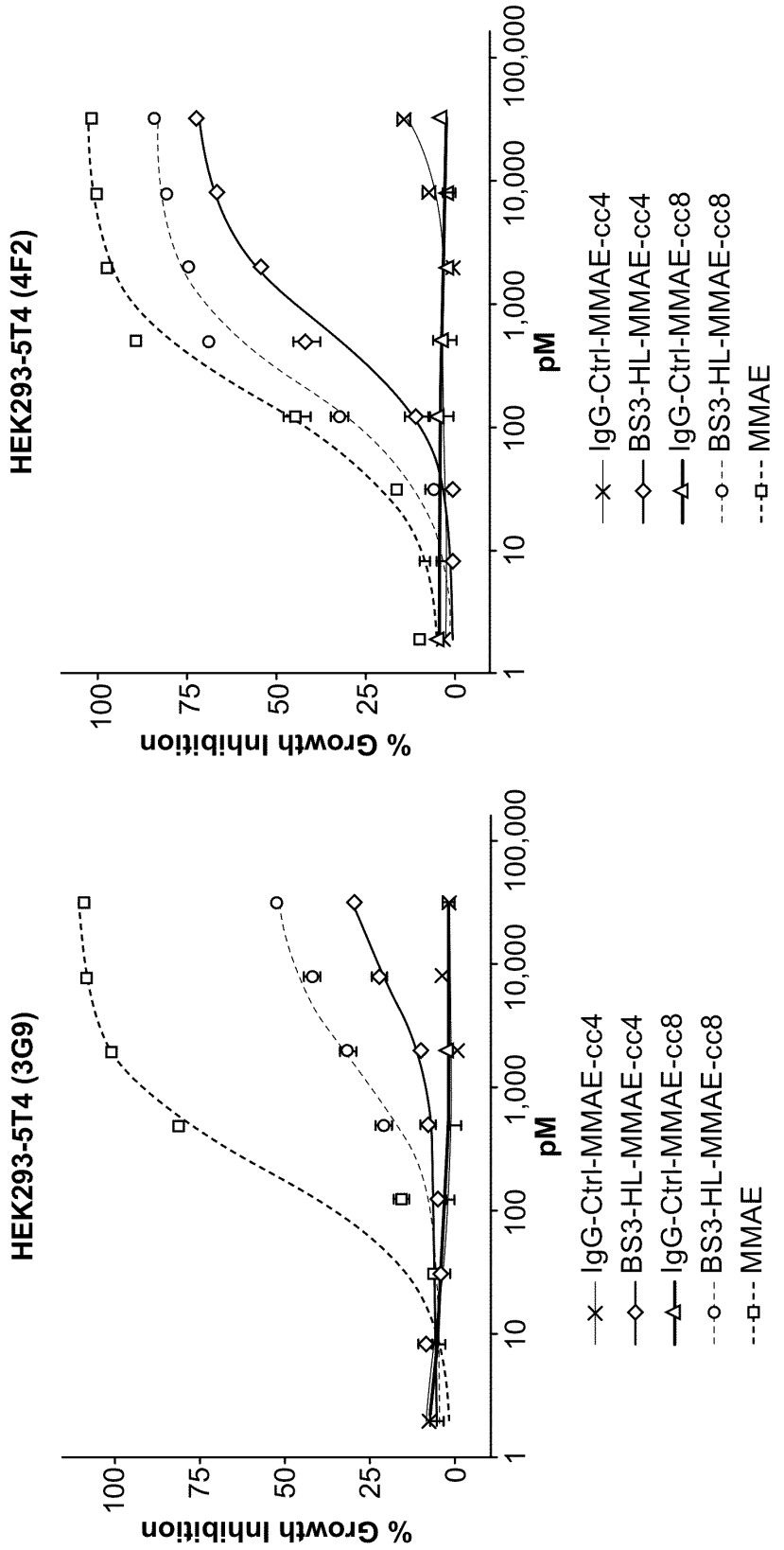


FIG. 12E

FIG. 12F

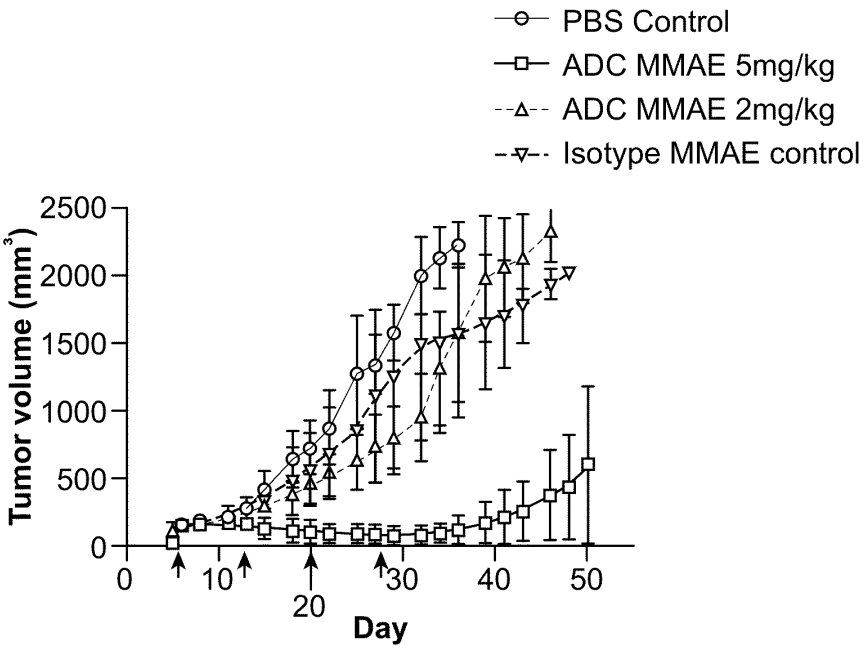


FIG. 13A

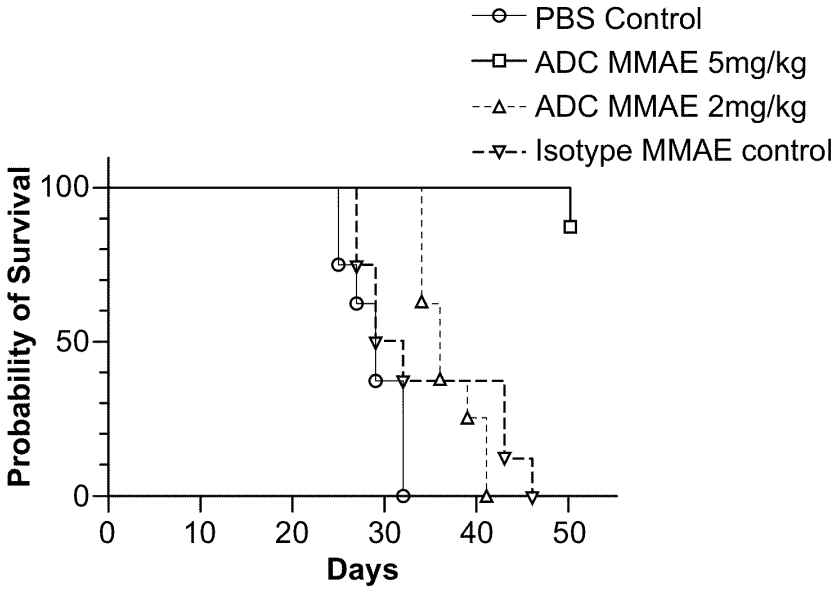


FIG. 13B

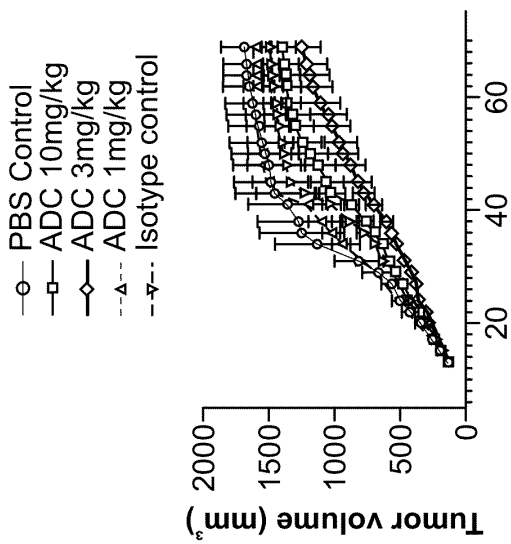


FIG. 14C

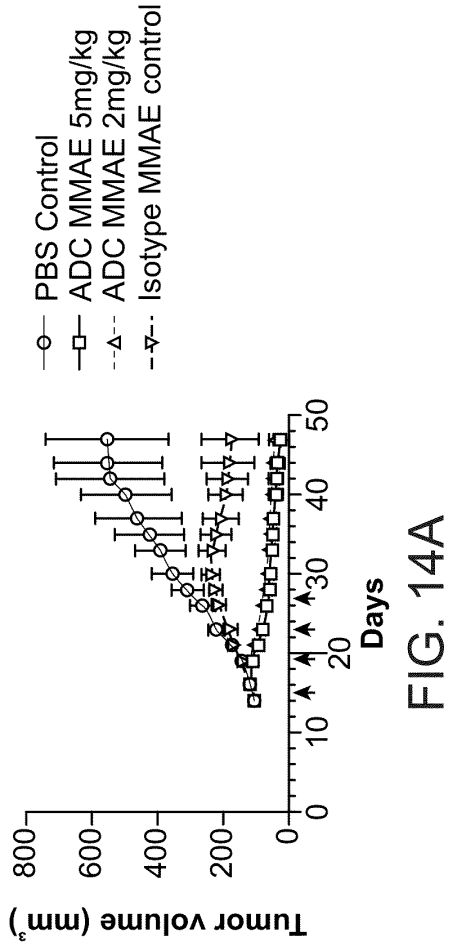


FIG. 14A

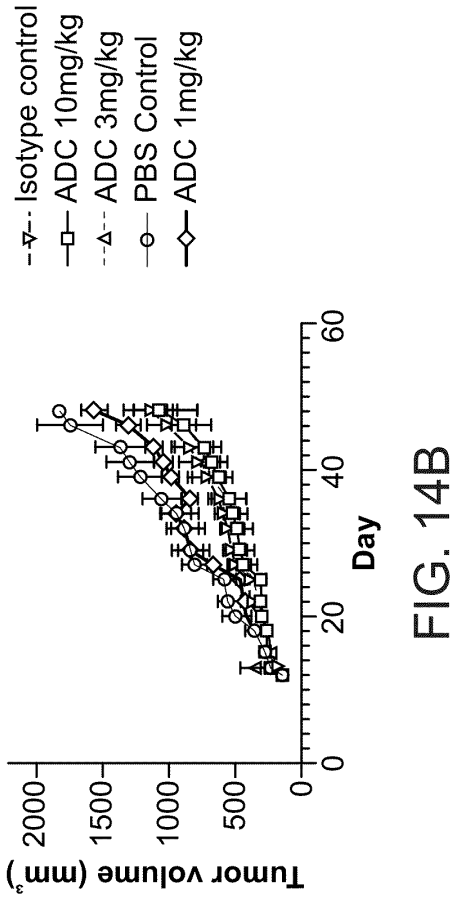


FIG. 14B

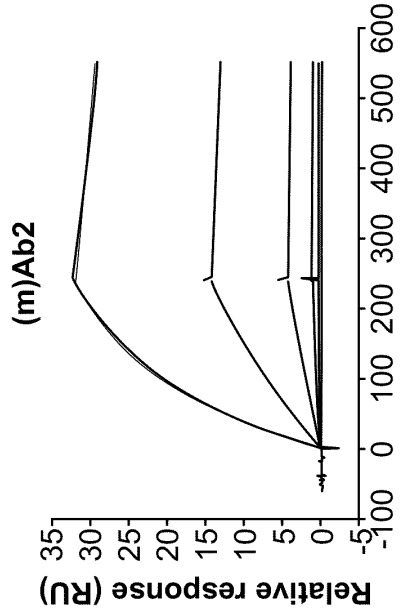


FIG. 15A

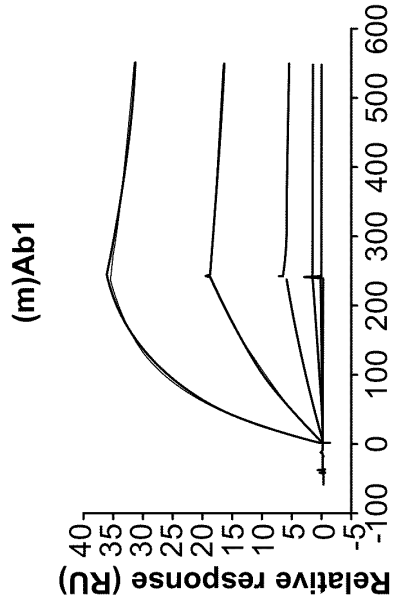


FIG. 15B

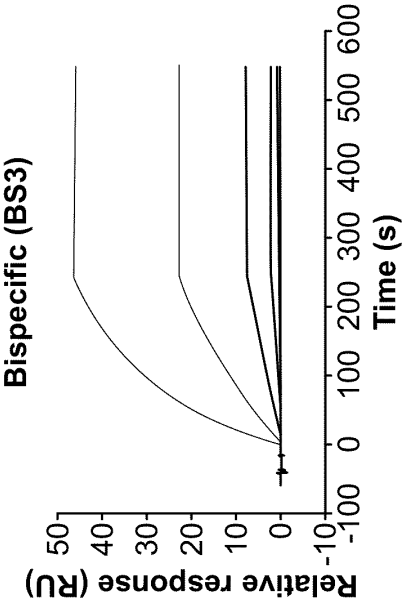


FIG. 15C

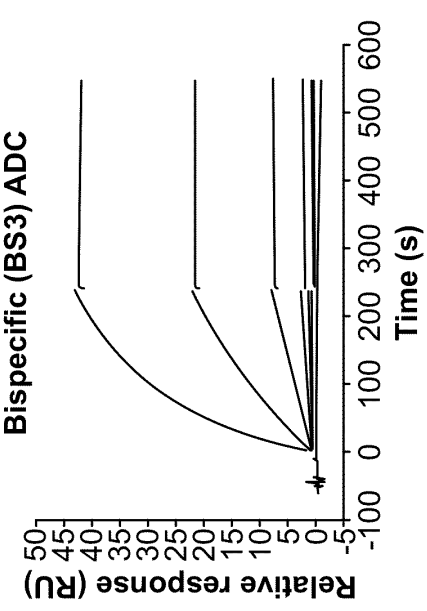


FIG. 15D

Antibodies	Ka (1/Ms)	Kd (1/s)	KD (M)
(m)Ab1	$2.59x10^{5}$	$4.21x10^{-4}$	$1.43x10^{-9}$
(m)Ab2	$2.03x10^{5}$	$2.73x10^{-4}$	$1.34x10^{-9}$
BsAb (BS3)	$4.61x10^{5}$	$1.67x10^{-6}$	$3.63x10^{-12}$
BsAb (BS3) ADC	$3.76x10^{5}$	$5.99x10^{-6}$	$1.59x10^{-11}$

FIG. 16