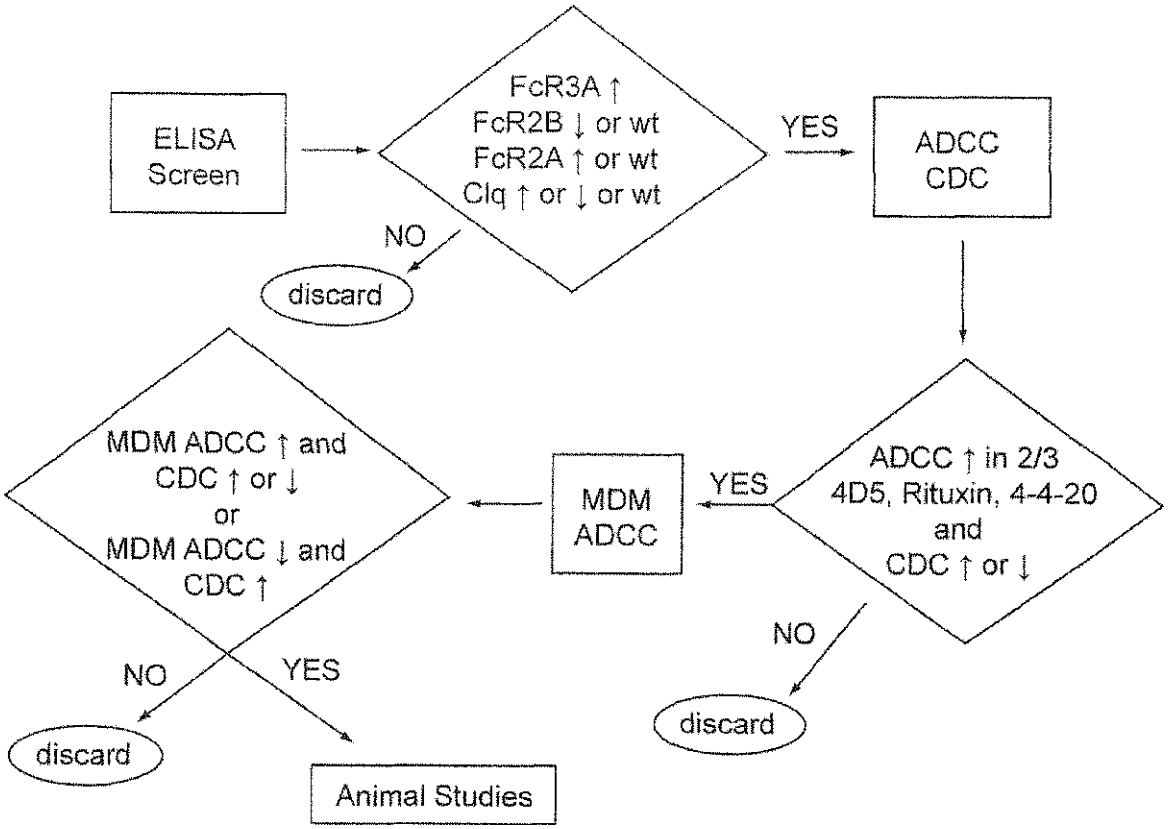

ELISA Screen
FcR3A ↑
FcR2B ↓ or wt
FcR2A ↑ or wt
Clq ↑ or ↓ or wt
NO
discard
YES
ADCC CDC
ADCC ↑ in 2/3 4D5, Rituxin, 4-4-20 and CDC ↑ or ↓
NO
discard
YES
MDM ADCC
MDM ADCC ↑ and CDC ↑ or ↓ or MDM ADCC ↓ and CDC ↑
NO
discard
YES
Animal Studies

FIG. 1

8B5.3.4 VH nucleotide/amino acid sequence

```
gaa gtg aag ctt gag gag tct gga gga ggc ttg gtg caa cct gga gga   48
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15

                                                      ┌──────
tcc atg aaa ctc tct tgt gaa gcc tct gga ttc act ttt agt gac gcc   96
Ser Met Lys Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Ser Asp Ala
            20                  25                  30

CDR1 ──────┐
tgg atg gac tgg gtc cgt cag tct cca gag aag ggg ctt gag tgg gtt  144
Trp Met Asp Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
         35                  40                  45

     ┌──────────────────────── CDR2 ──────────────────────────────
gct gaa att aga aac aaa gct aaa aat cat gca aca tac tat gct gag  192
Ala Glu Ile Arg Asn Lys Ala Lys Asn His Ala Thr Tyr Tyr Ala Glu
     50                  55                  60

──────────────────┐
tct gtg ata ggg agg ttc acc atc tca aga gat gat tcc aaa agt agt  240
Ser Val Ile Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser
 65                  70                  75                  80

gtc tac ctg caa atg aac agc tta aga gct gaa gac act ggc att tat  288
Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Gly Ile Tyr
                 85                  90                  95

       ┌──────── CDR3 ──────────┐
tac tgt ggg gct ctg ggc ctt gac tac tgg ggc caa ggc acc act ctc  336
Tyr Cys Gly Ala Leu Gly Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu
            100                 105                 110

aca gtc tcc tcg                                                 348
Thr Val Ser Ser
        115
```

FIG. 2

8B5.3.4 VL nucleotide/amino acid sequence

```
gac att cag atg aca cag tct cca tcc tcc cta ctt gcg gcg ctg gga   48
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Leu Ala Ala Leu Gly
 1               5                  10                  15

                                    ┌──────── CDR1 ────────┐
gaa aga gtc agt ctc act tgt cgg gca agt cag gaa att agt ggt tac   96
Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu Ile Ser Gly Tyr
            20                  25                  30

┌──────┐
tta agc tgg ctt cag cag aaa cca gat gga act att aaa cgc ctg atc  144
Leu Ser Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile Lys Arg Leu Ile
        35                  40                  45

    ┌──── CDR2 ────┐
tac gcc gca tcc act tta gat tct ggt gtc cca aaa agg ttc agt ggc  192
Tyr Ala Ala Ser Thr Leu Asp Ser Gly Val Pro Lys Arg Phe Ser Gly
    50                  55                  60

agt gag tct ggg tca gat tat tct ctc acc atc agc agt ctt gag tct  240
Ser Glu Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser
65                  70                  75                  80

                                ┌──── CDR3 ────┐
gaa gat ttt gca gac tat tac tgt cta caa tat ttt agt tat ccg ctc  288
Glu Asp Phe Ala Asp Tyr Tyr Cys Leu Gln Tyr Phe Ser Tyr Pro Leu
                85                  90                  95

┌──┐
acg ttc ggt gct ggg acc aag ctg gag ctg aaa                     321
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105
```

FIG. 3

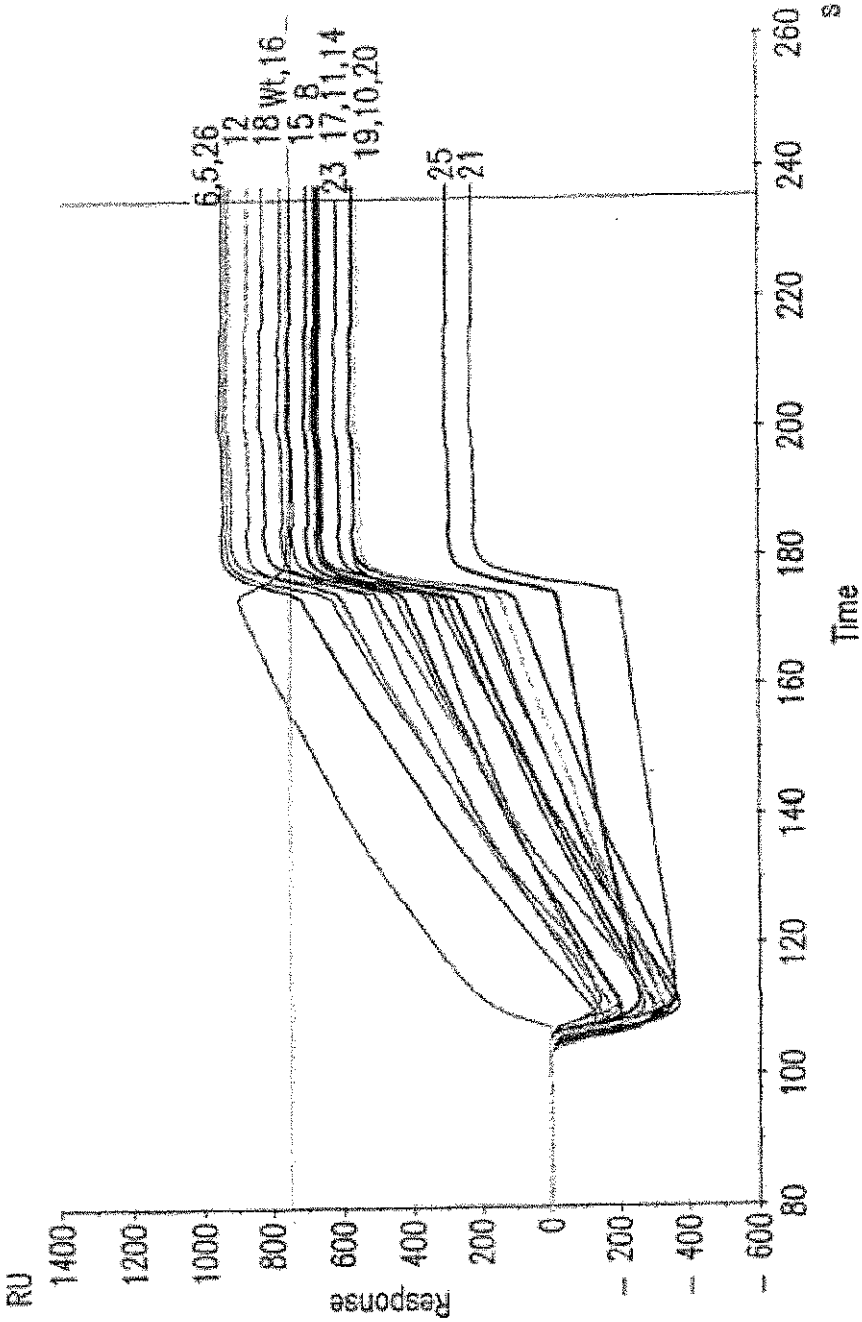

RU
1400
1200
1000
800
600
400
200
0
-200
-400
-600
Response
80
100
120
140
160
180
200
220
240
260
s
Time
6,5,26
12
18
Wt,16
15
8
17,11,14
19,10,20
23
25
21

FIG. 4

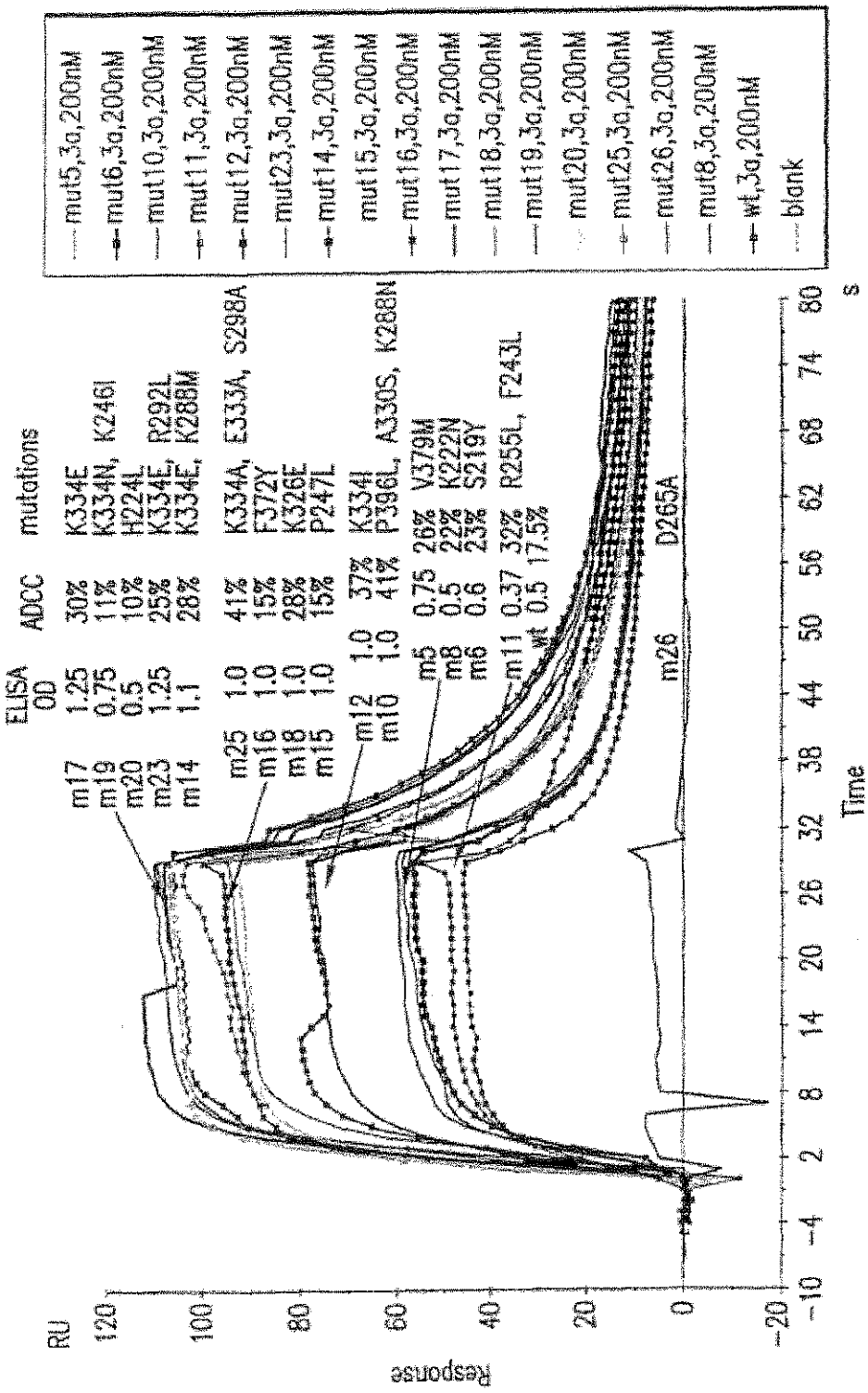

mut5,3a,200nM
mut6,3a,200nM
mut10,3a,200nM
mut11,3a,200nM
mut12,3a,200nM
mut23,3a,200nM
mut14,3a,200nM
mut15,3a,200nM
mut16,3a,200nM
mut17,3a,200nM
mut18,3a,200nM
mut19,3a,200nM
mut20,3a,200nM
mut25,3a,200nM
mut26,3a,200nM
mut8,3a,200nM
wt,3a,200nM
blank
ELISA OD
ADCC
mutations
m17 1.25 30% K334E
m19 0.75 11% K334N, K246I
m20 0.5 10% H224L
m23 1.25 25% K334E, R292L
m14 1.1 28% K334E, K288M
m25 1.0 41% K334A, E333A, S298A
m16 1.0 15% F372Y
m18 1.0 28% K326E
m15 1.0 15% P247L
m12 1.0 37% K334I
m10 1.0 41% P396L, A330S, K288N
m5 0.75 26% V379M
m8 0.5 22% K222N
m6 0.6 23% S219Y
m11 0.37 32% R255L, F243L
wt 0.5 17.5%
m26 D265A
RU
Response
Time
s

FIG. 5

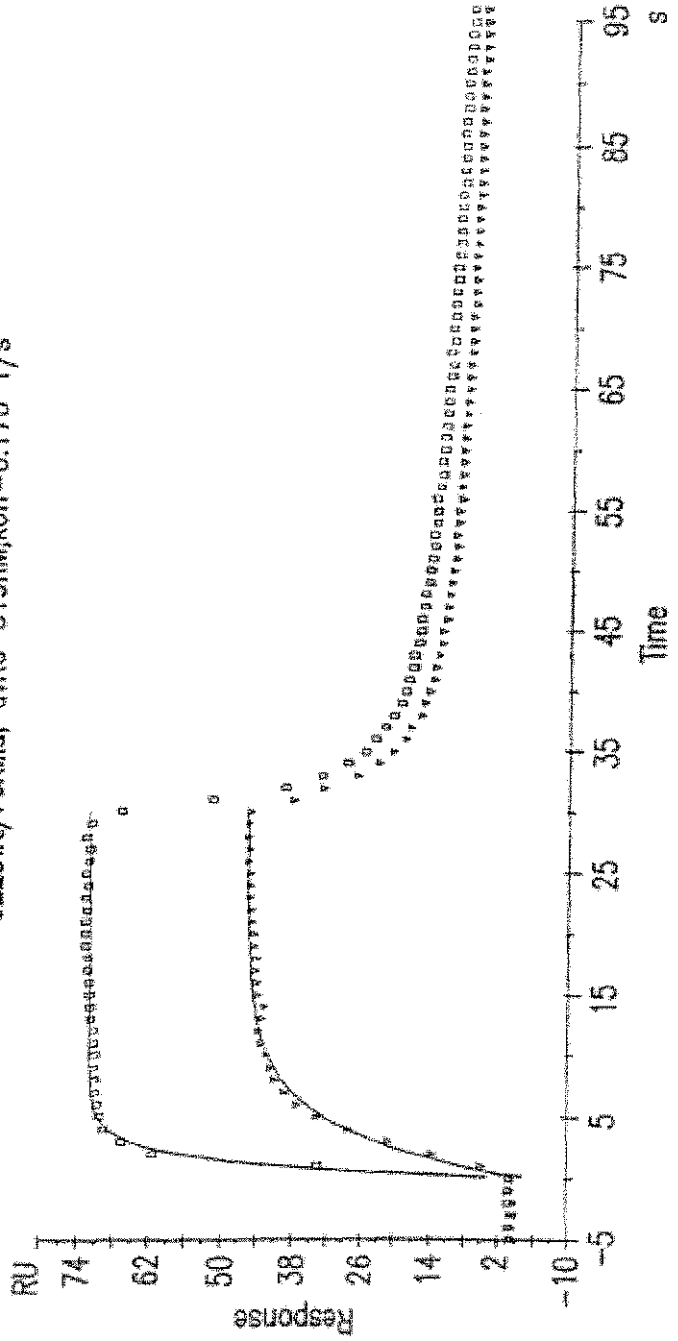

0225wt/FcRIIIa, avKd=319nM,koff=0.170 1/s
RU
74
62
50
38
26
14
2
Response
-10
-5
5
15
25
35
45
55
65
75
85
95
s
Time

FIG. 6A

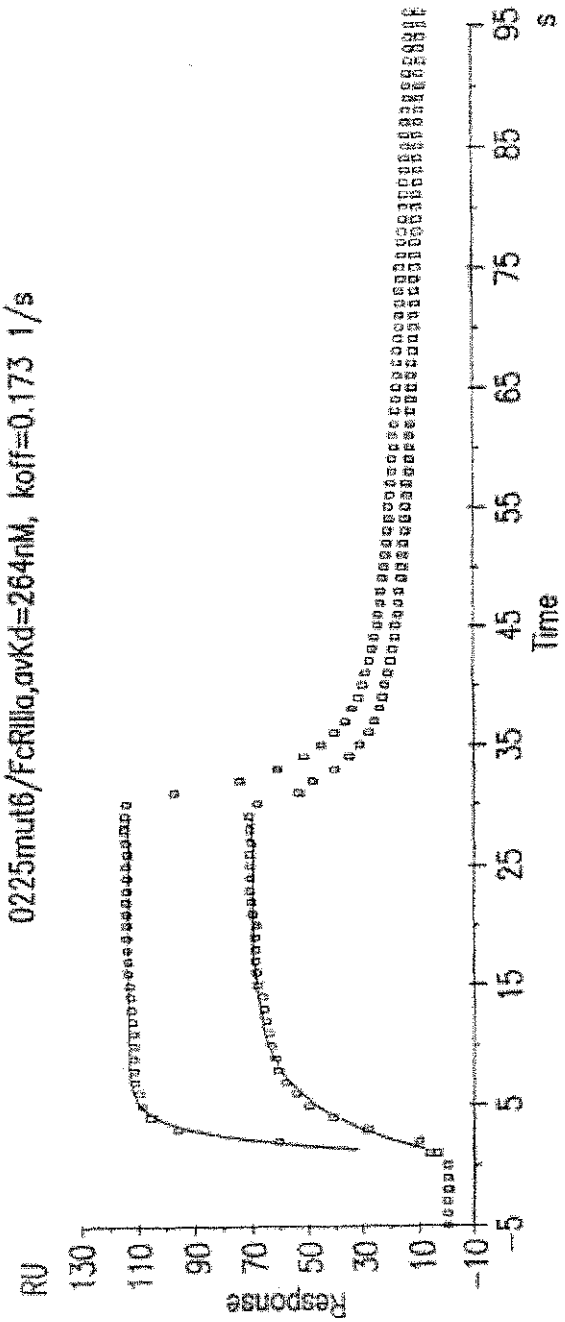

0225mut6/FcRIIIa,avKd=264nM, koff=0.173 1/s
RU
Response
130
110
90
70
50
30
10
-10
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 6B

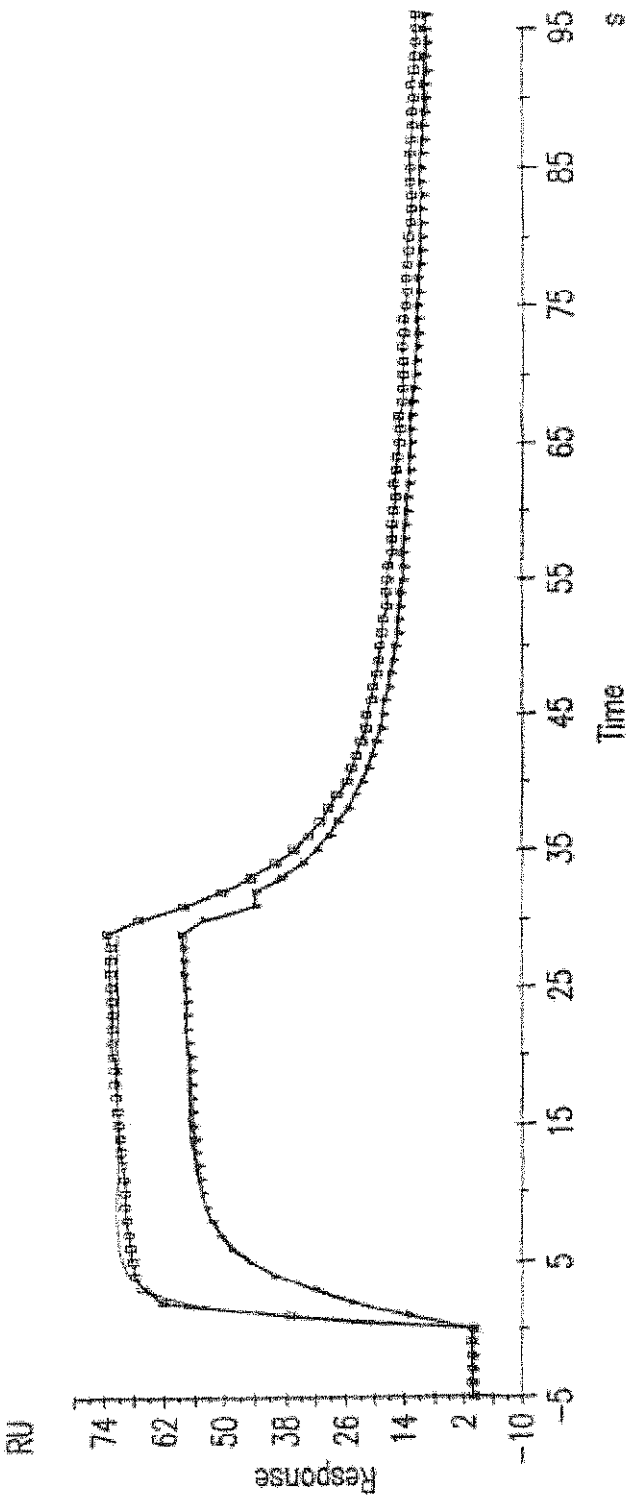

0225mut10/FcRIIIa,avKd=128nM,koff=0.115 1/s
RU
Response
74
62
50
38
26
14
2
-10
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 6C

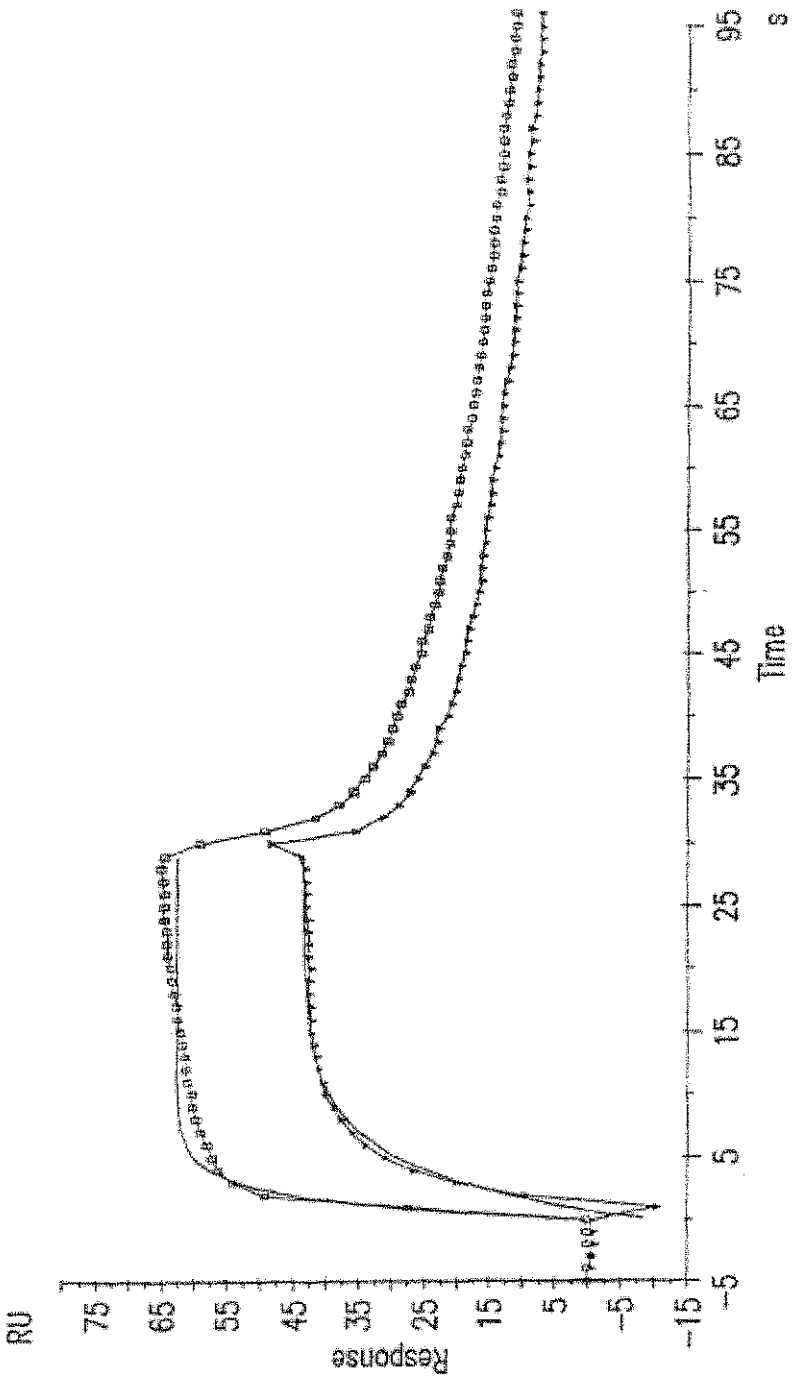

0225mut11FcRIIIa,avKd=91.8nM, koff=0.073 1/s
RU
Response
75
65
55
45
35
25
15
5
-5
-15
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 6D

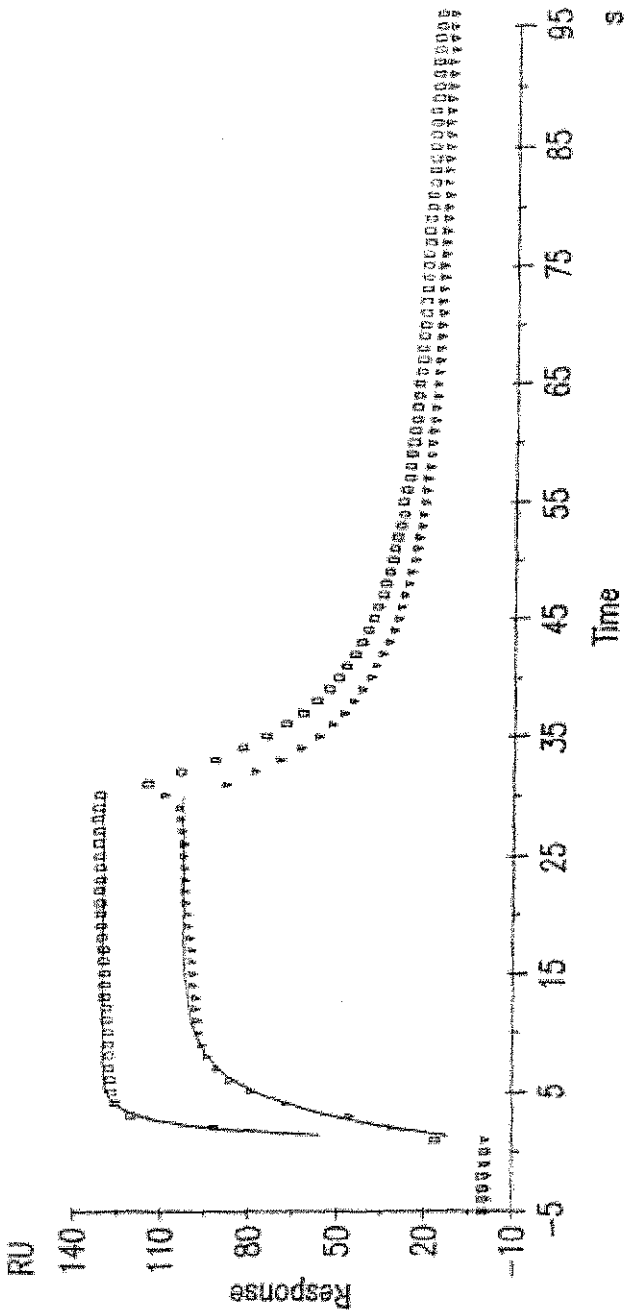

0225mut18/FcRIIIa,avKd=92nM, koff=0.112 1/s
RU
140
110
80
50
20
Response
-10
-5
5
15
25
35
45
55
65
75
85
95
s
Time

FIG. 6E

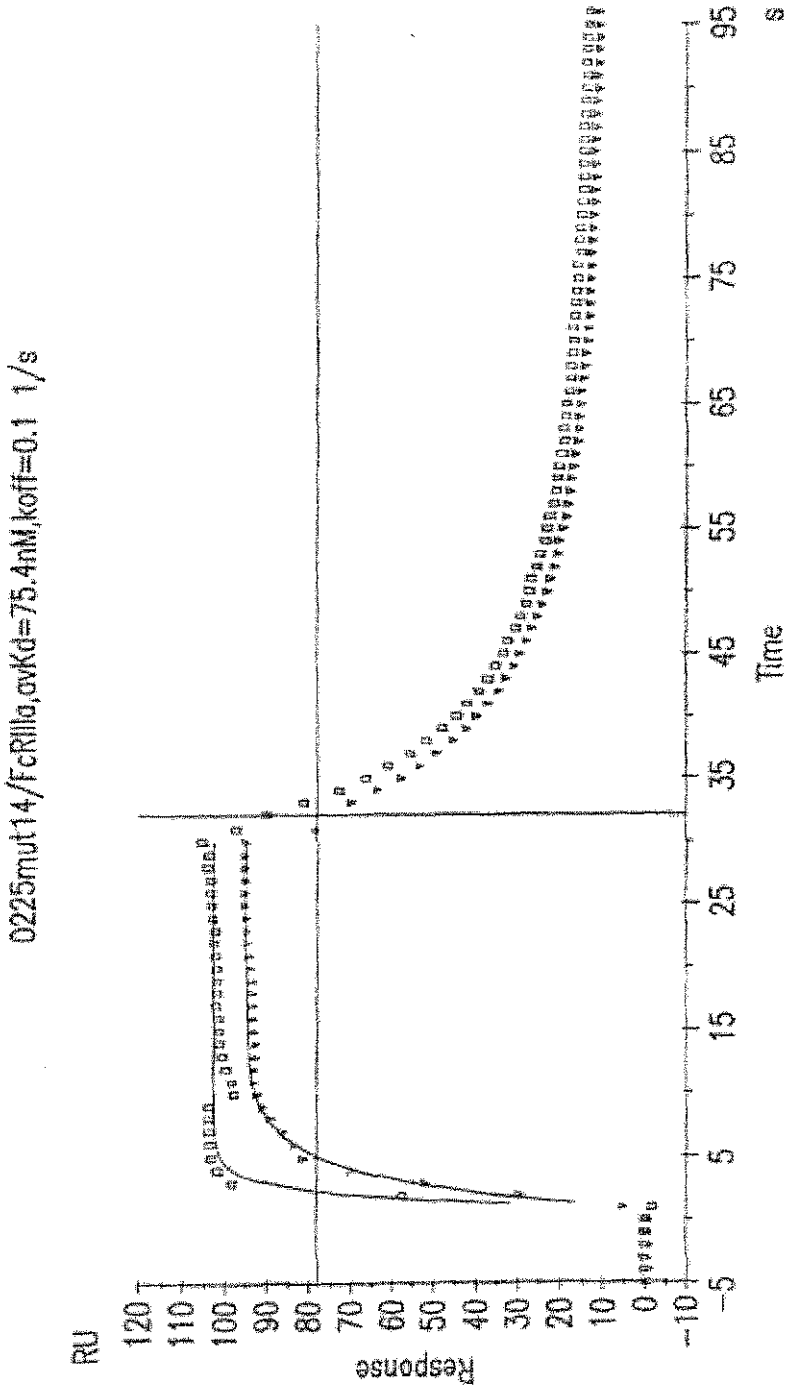


FIG. 6F

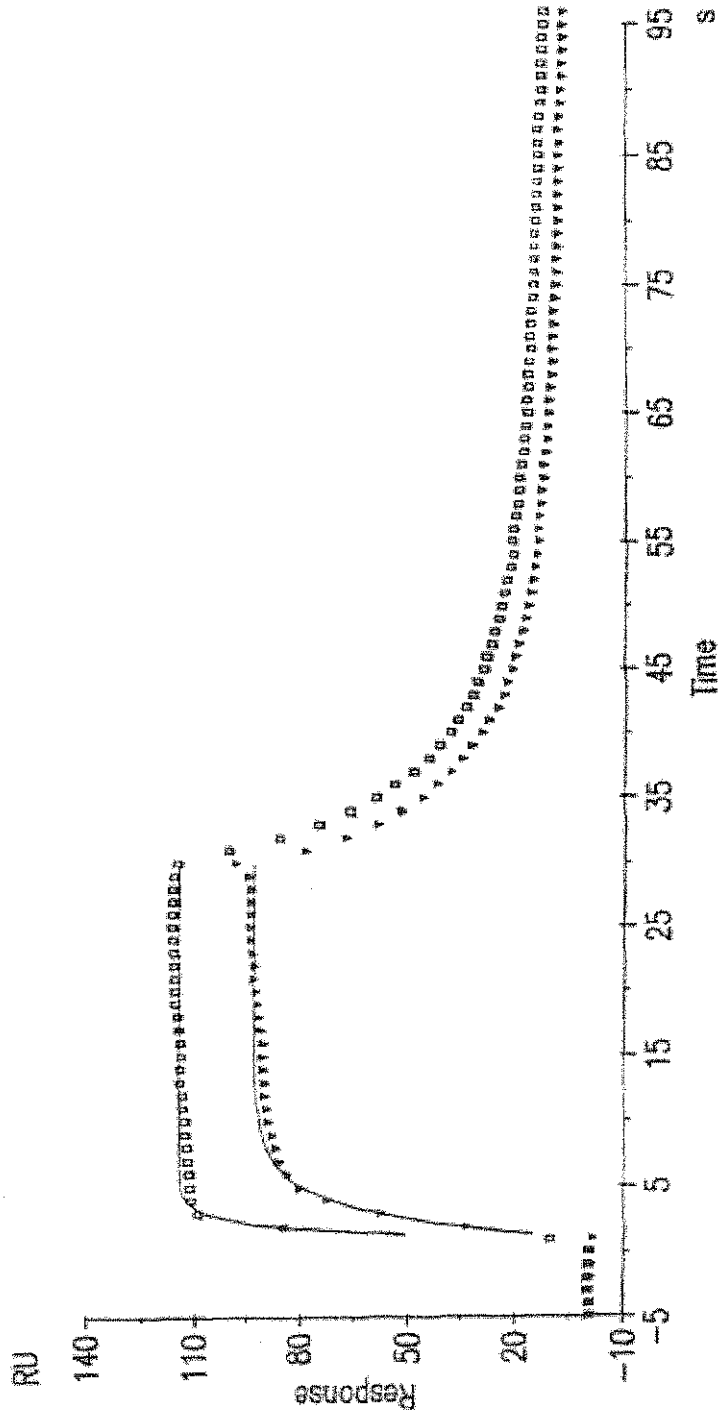

0225mut16/FcRIIIa,avKd=84.8nM,koff=0.133 1/s
RU
Response
140
110
80
50
20
-10
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 6G

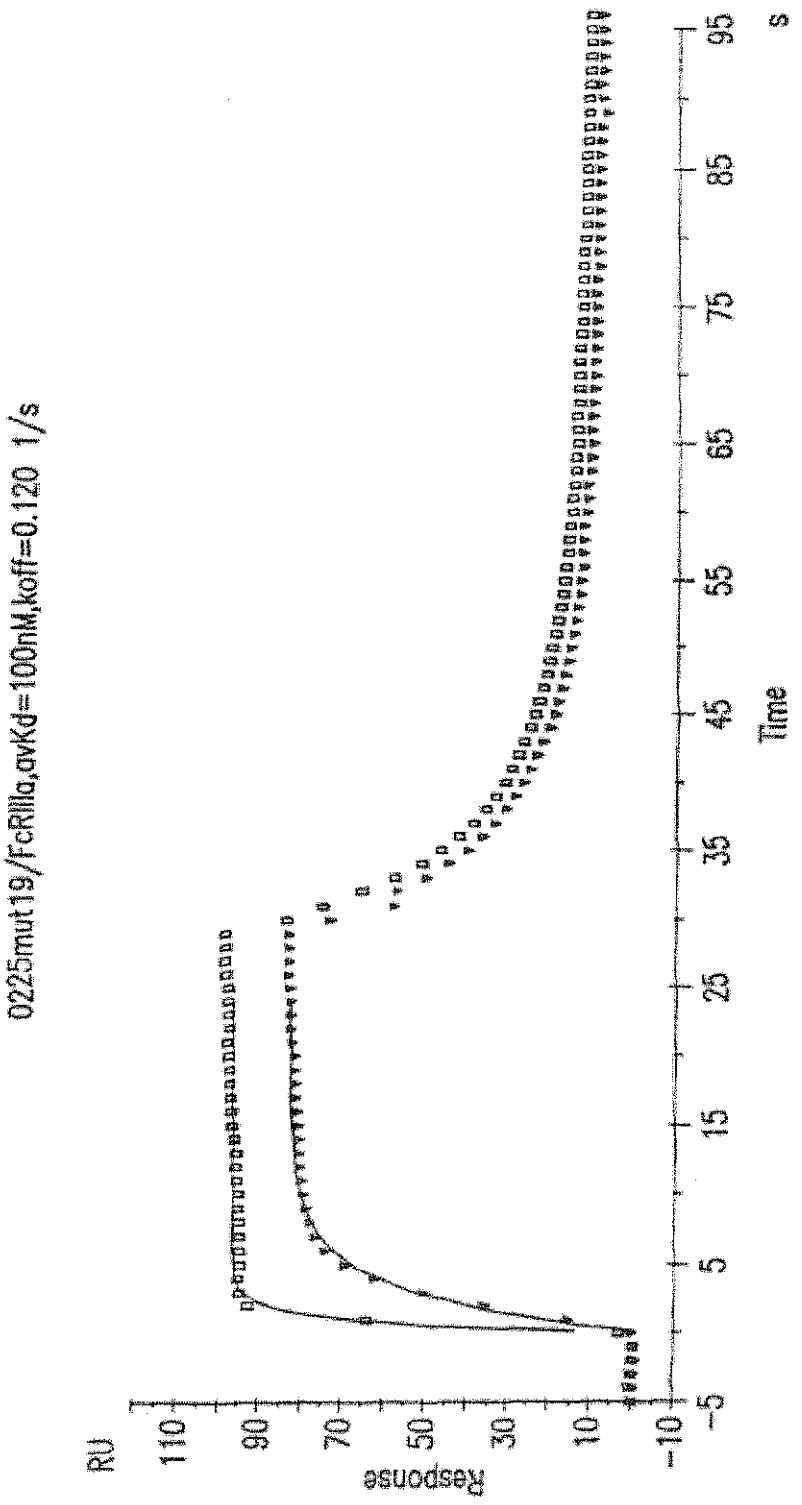

0225mut19/FcRIIIa,avKd=100nM,koff=0.120 1/s
RU
Response
110
90
70
50
30
10
-10
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 6H

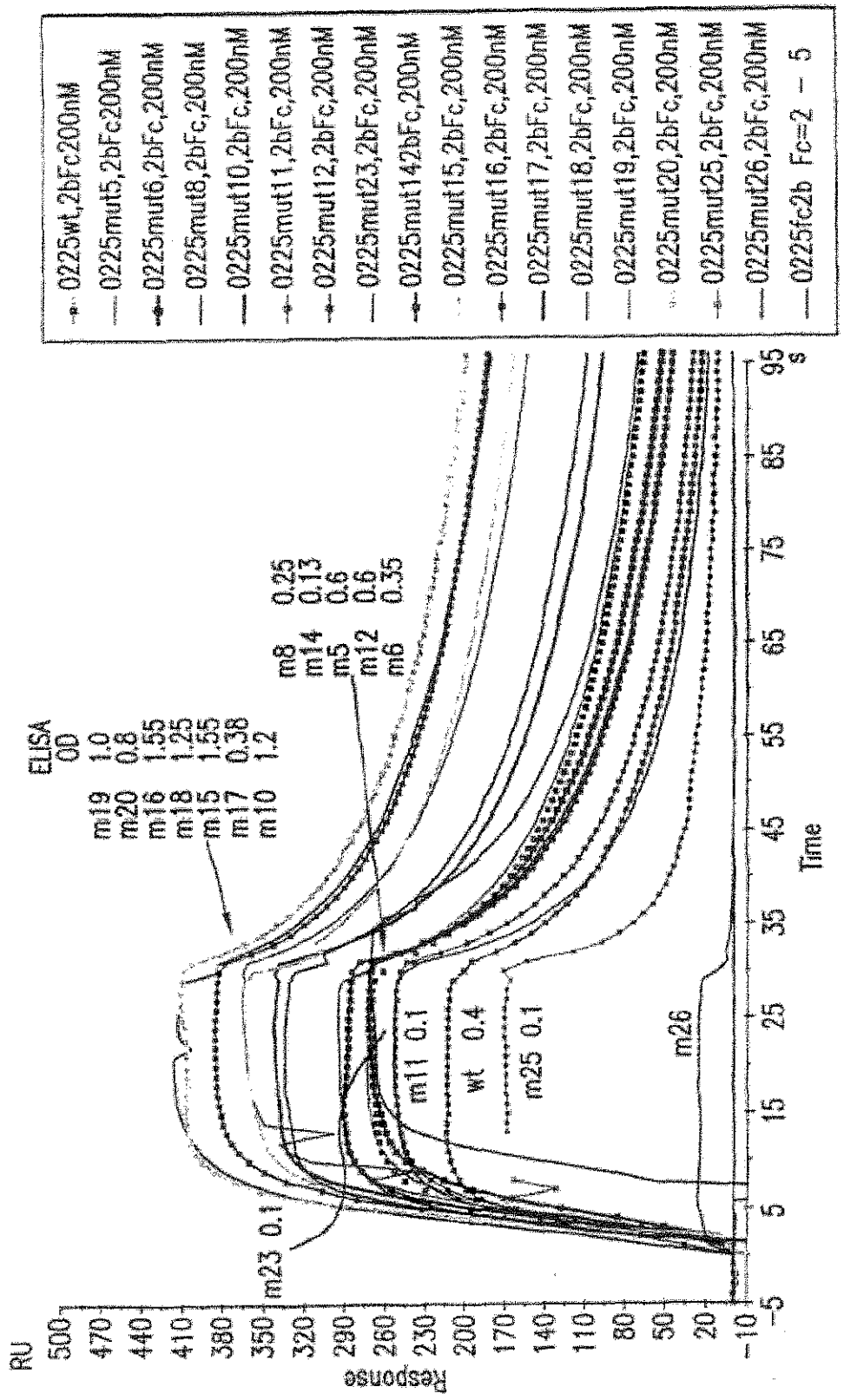

0225wt,2bFc200nM
0225mut5,2bFc200nM
0225mut6,2bFc,200nM
0225mut8,2bFc,200nM
0225mut10,2bFc,200nM
0225mut11,2bFc,200nM
0225mut12,2bFc,200nM
0225mut23,2bFc,200nM
0225mut142bFc,200nM
0225mut15,2bFc,200nM
0225mut16,2bFc,200nM
0225mut17,2bFc,200nM
0225mut18,2bFc,200nM
0225mut19,2bFc,200nM
0225mut20,2bFc,200nM
0225mut25,2bFc,200nM
0225mut26,2bFc,200nM
0225fc2b Fc=2 – 5
ELISA OD
m19 1.0
m20 0.8
m16 1.55
m18 1.25
m15 1.55
m17 0.38
m10 1.2
m8 0.25
m14 0.13
m5 0.6
m12 0.6
m6 0.35
m23 0.1
m11 0.1
wt 0.4
m25 0.1
m26
RU
Response
Time
s

FIG. 7

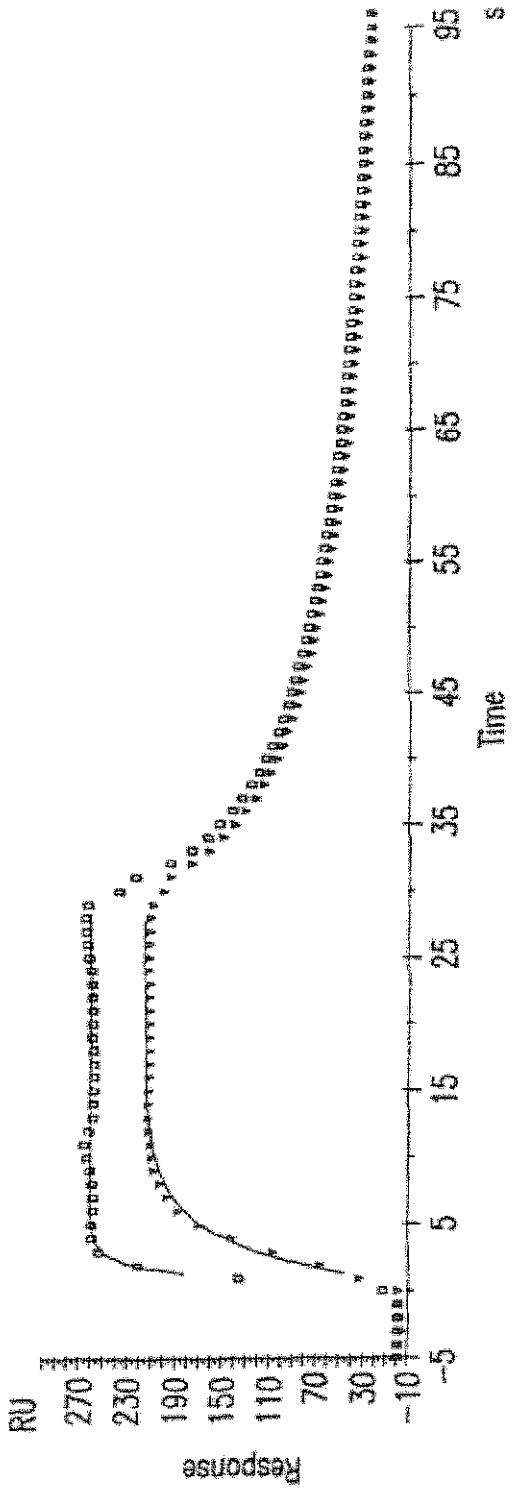

0225wt/FcRIIb,avKd=61.4nM, koff=0.0847 1/s
Response
RU
270
230
190
150
110
70
30
-10
-5
5
15
25
35
45
55
65
75
85
95
Time
s

FIG. 8A

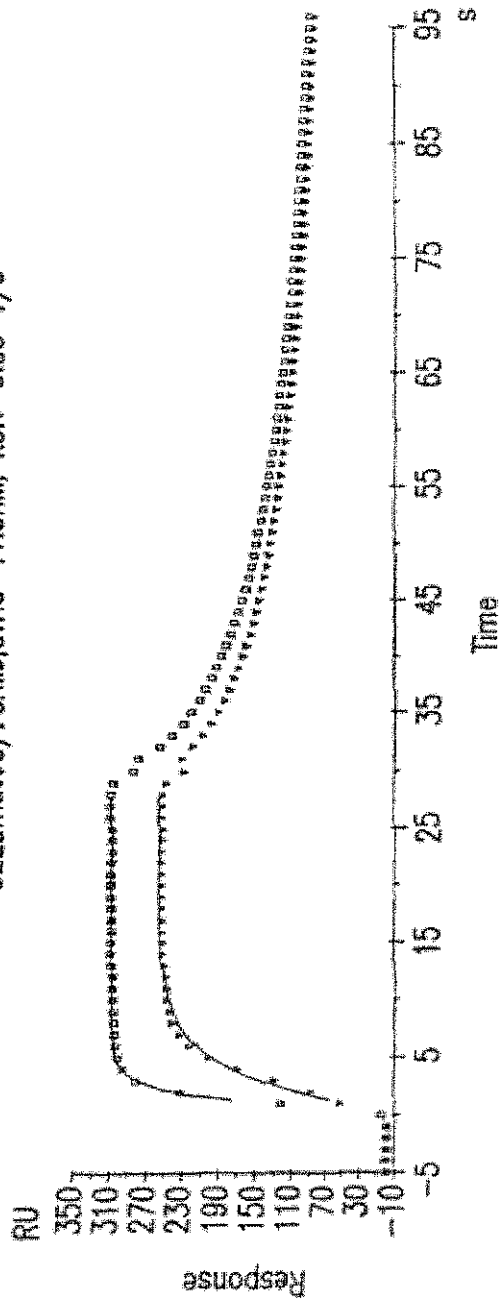

0225mut10/FcRIIb,avKd=41.3nM, koff=0.05 1/s
Response
RU
350
310
270
230
190
150
110
70
30
-10
-5
5
15
25
35
45
55
65
75
85
95
s
Time

FIG. 8B

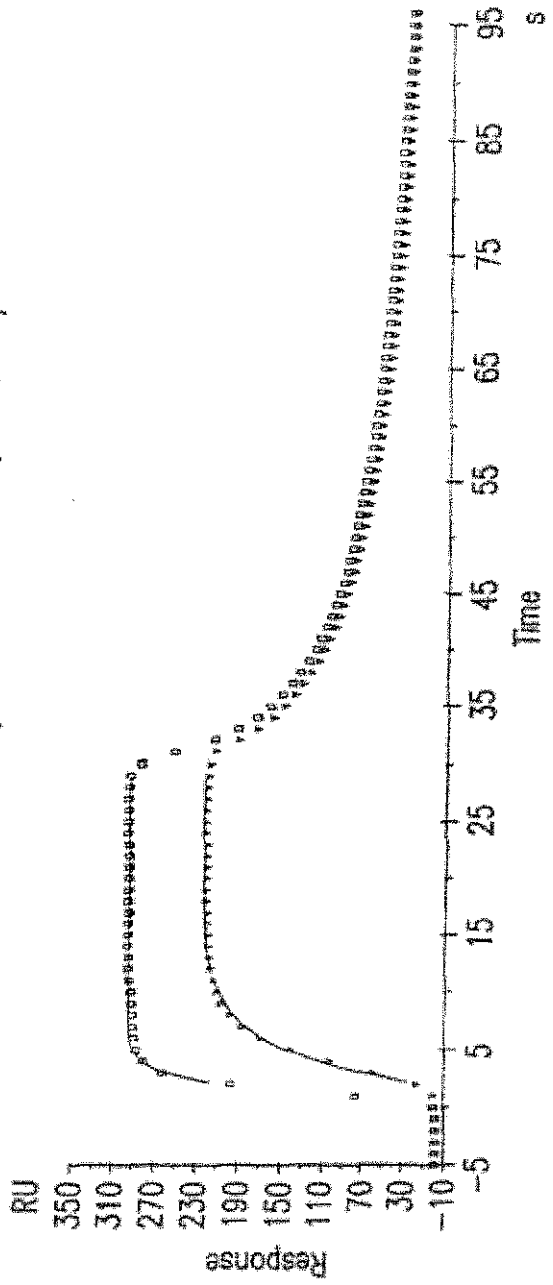

0225mut11/FcRIIb,avKd=82.3nM,koff=0.1 1/s
RU
350
310
270
230
190
150
110
70
30
−10
Response
−5
5
15
25
35
45
55
65
75
85
95
s
Time

FIG. 8C

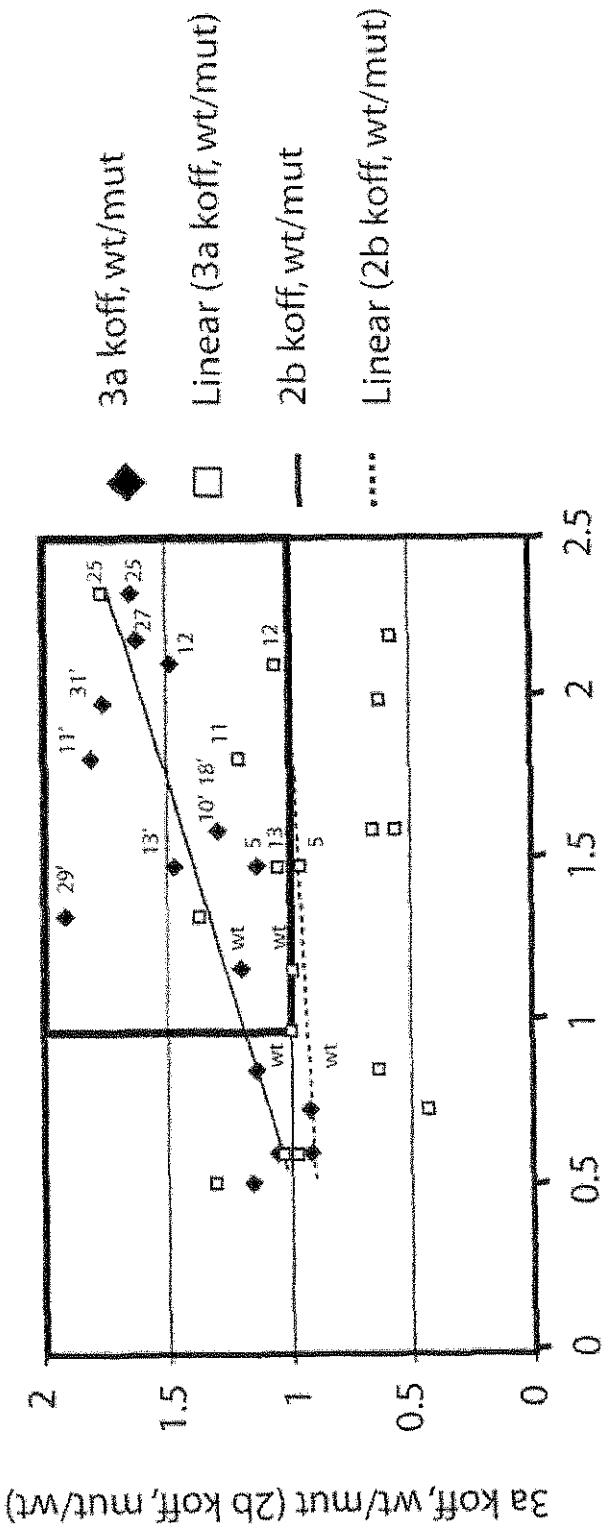

3a koff, wt/mut
Linear (3a koff, wt/mut)
2b koff, wt/mut
Linear (2b koff, wt/mut)
3a koff, wt/mut (2b koff, mut/wt)
ADCC mut/wt
0
0.5
1
1.5
2
2.5
29'
31'
11'
13'
10'
18'
11
5
13
12
27
25
wt

FIG. 9

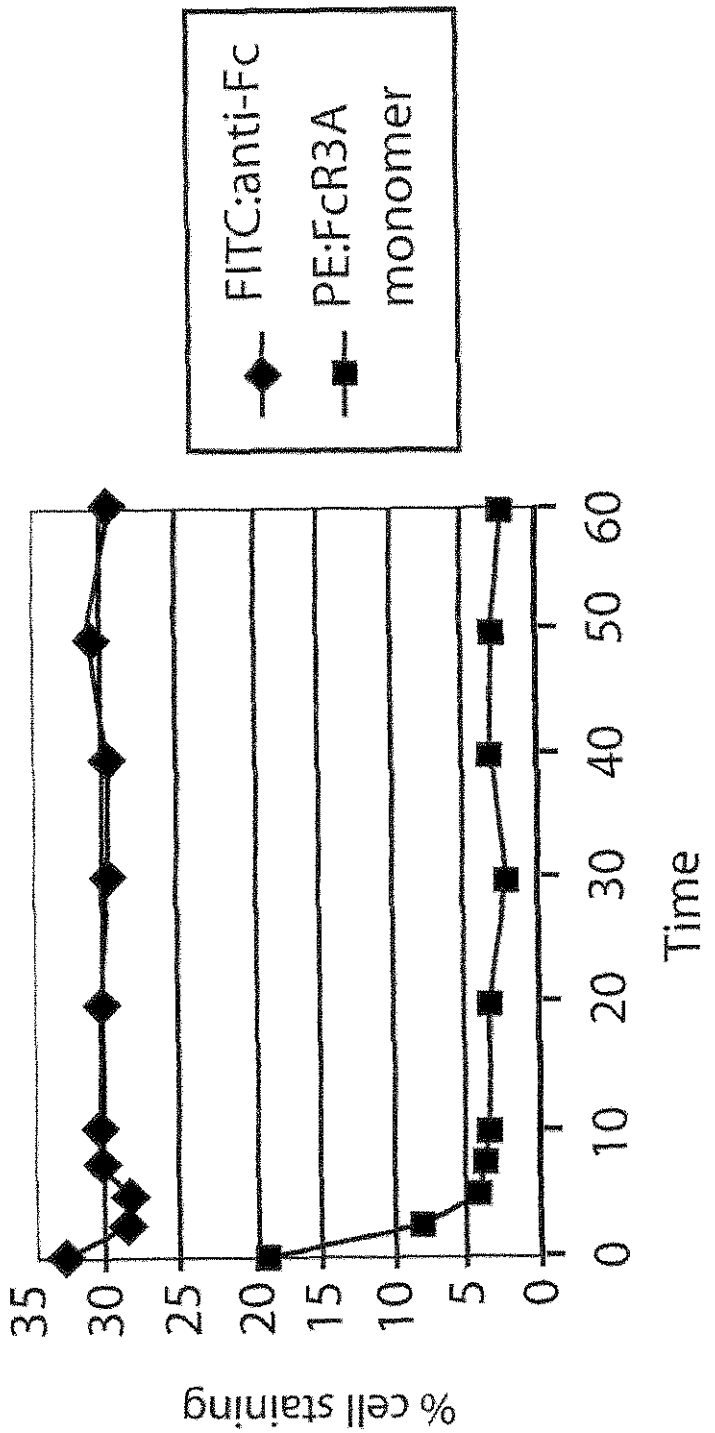

FITC:anti-Fc
PE:FcR3A monomer
% cell staining
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time

FIG. 10

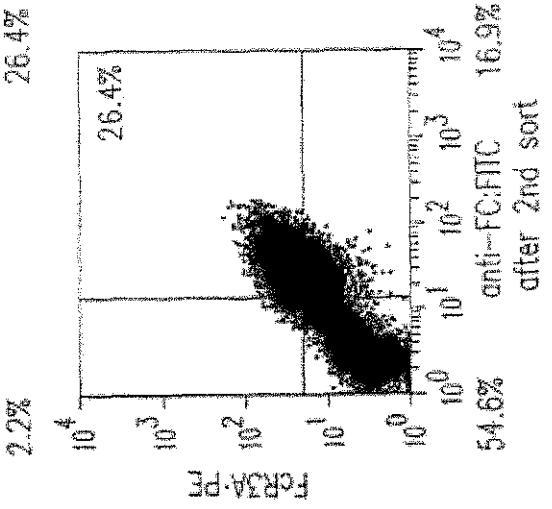


FIG. 11C

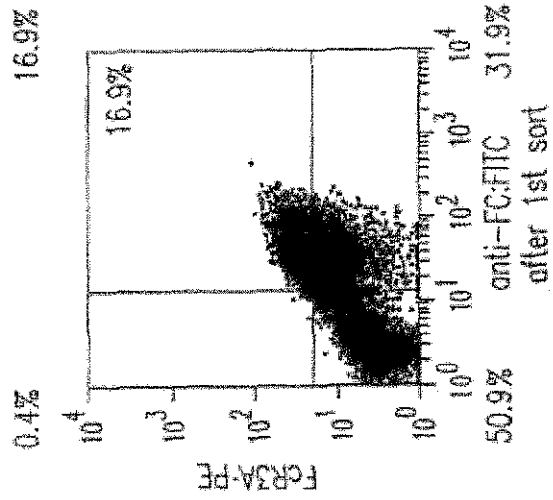


FIG. 11B

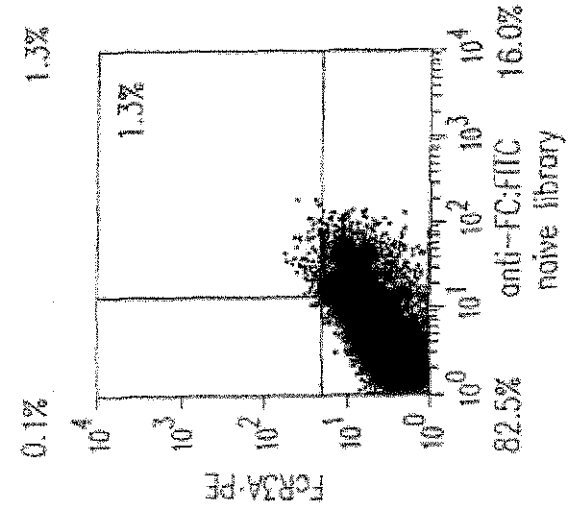


FIG. 11A

3aLAVI(V158) P051

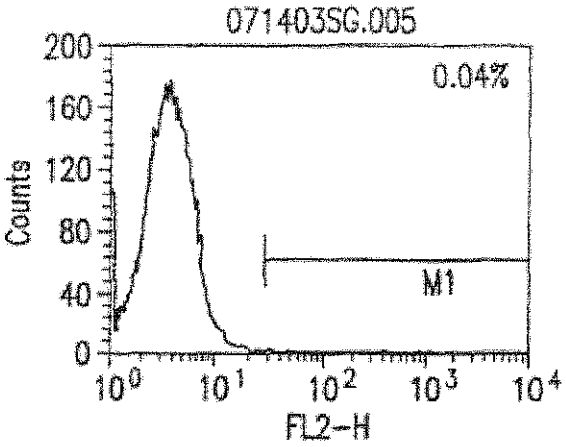


FIG. 12A-1

3aLAVI(V158) P051

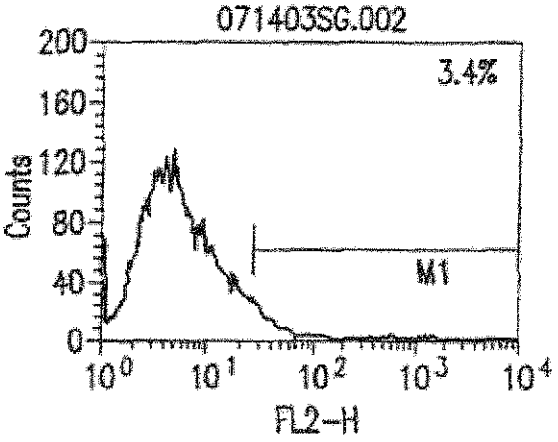


FIG. 12A-2

3aLAVI(V158) P051

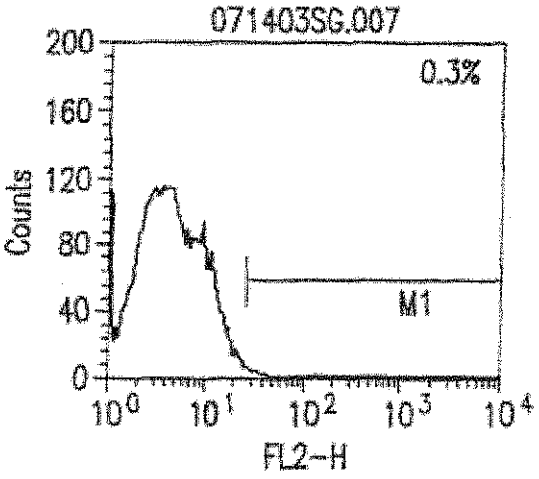


FIG. 12A-3

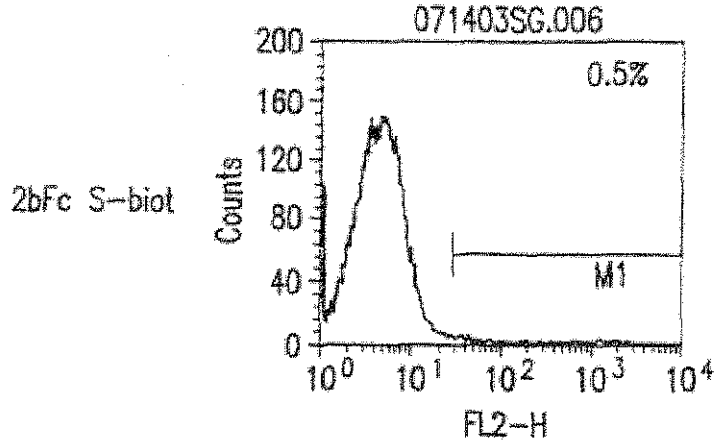


FIG. 12A-4

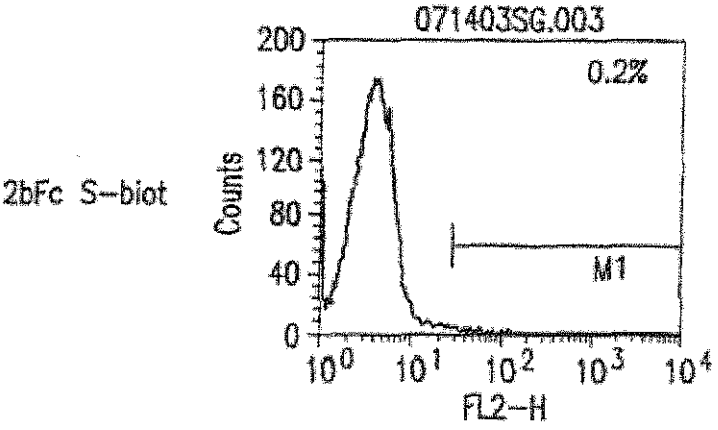


FIG. 12A-5

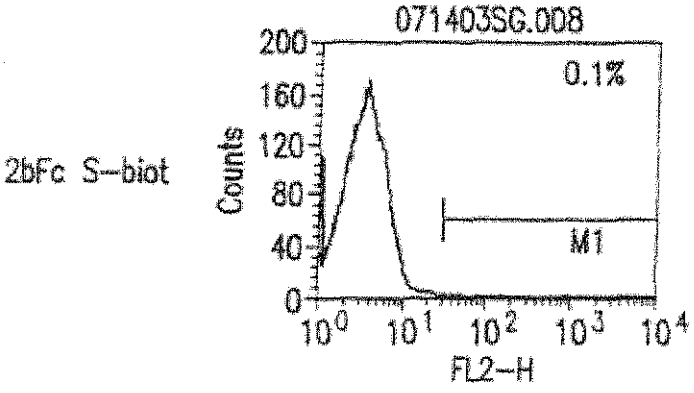


FIG. 12A-6

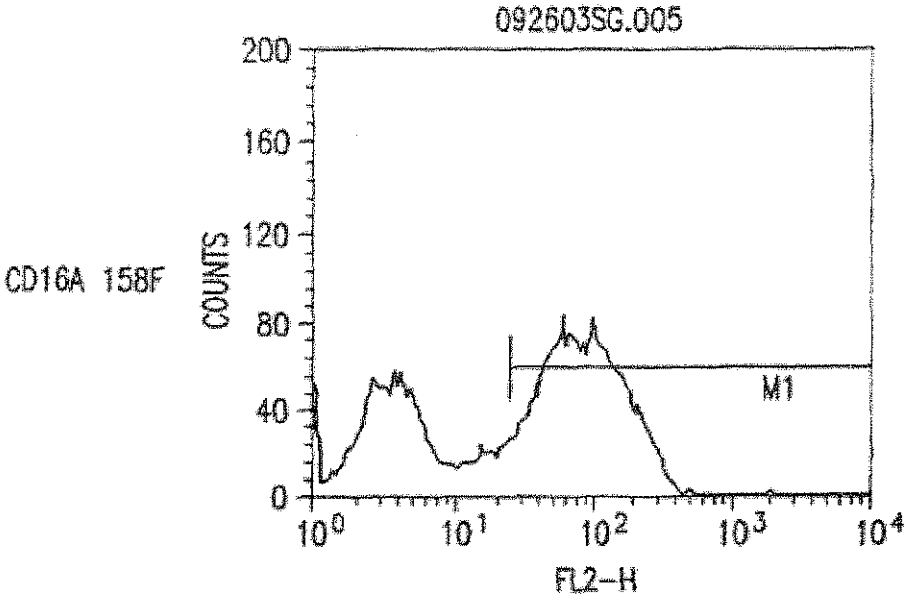


FIG. 12B-1

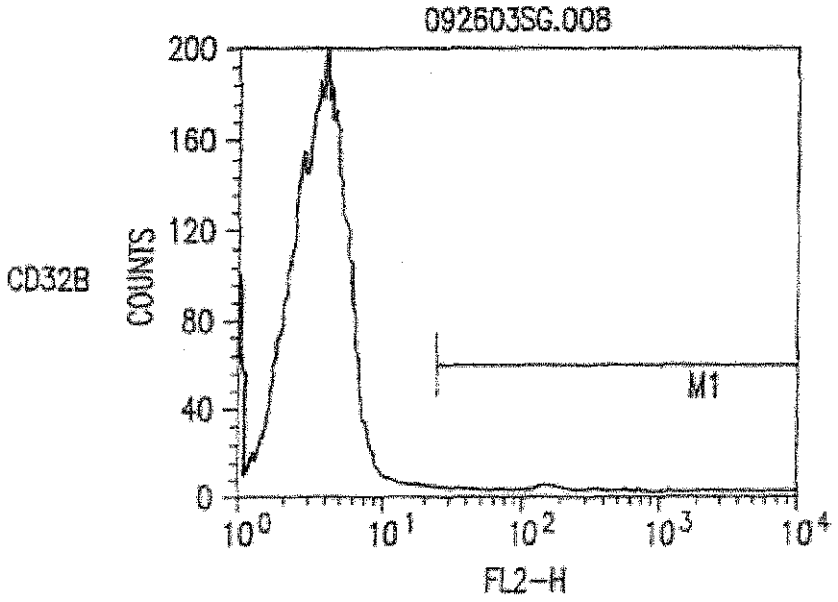


FIG. 12B-2

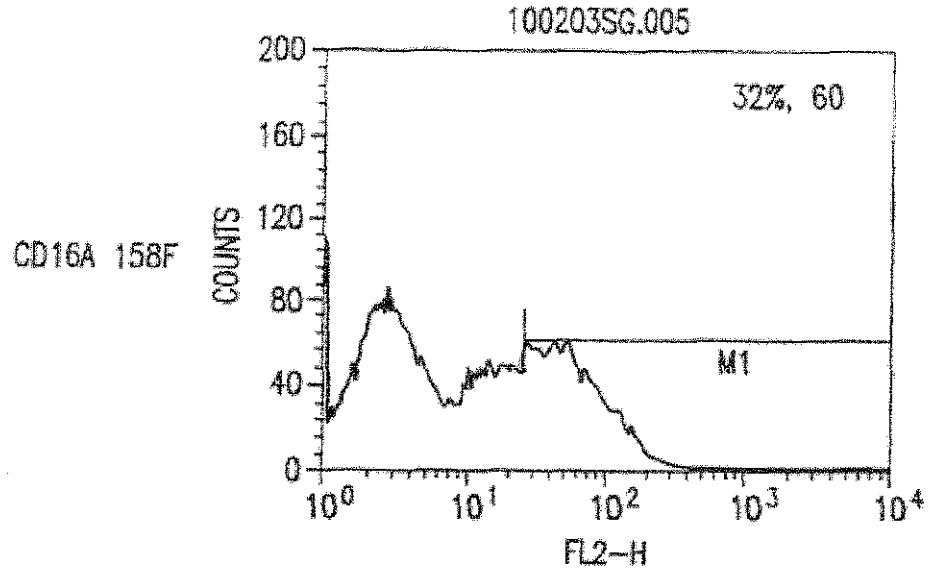


FIG. 12B-3

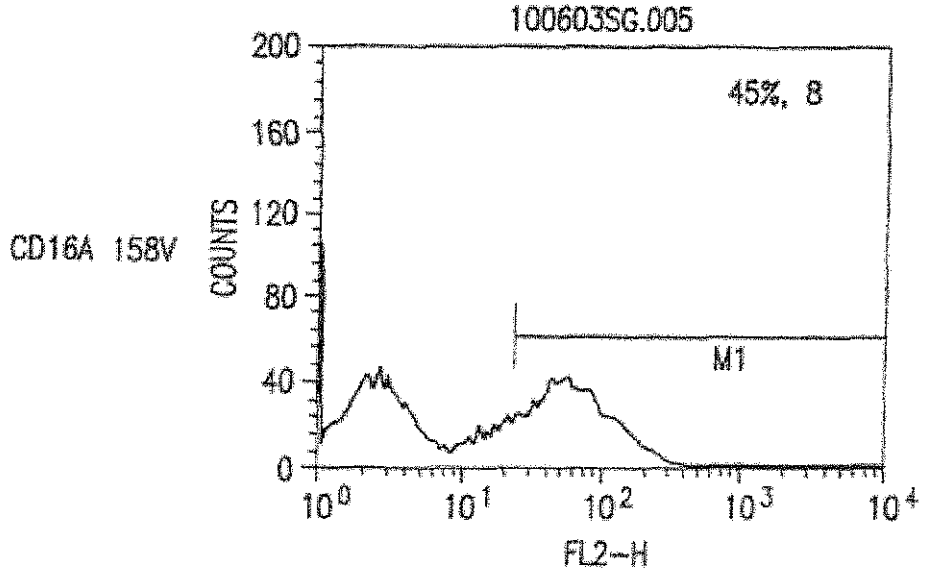


FIG. 12B-4

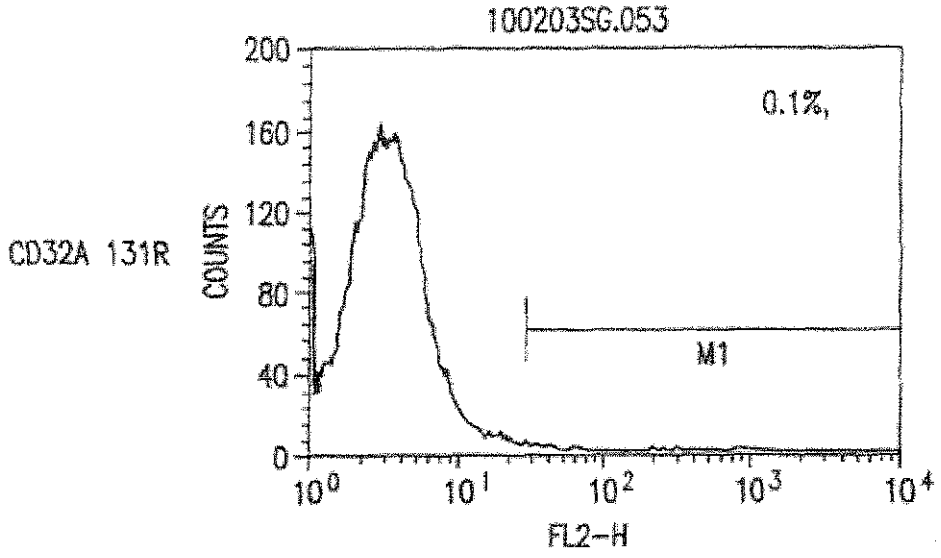


FIG. 12B-5

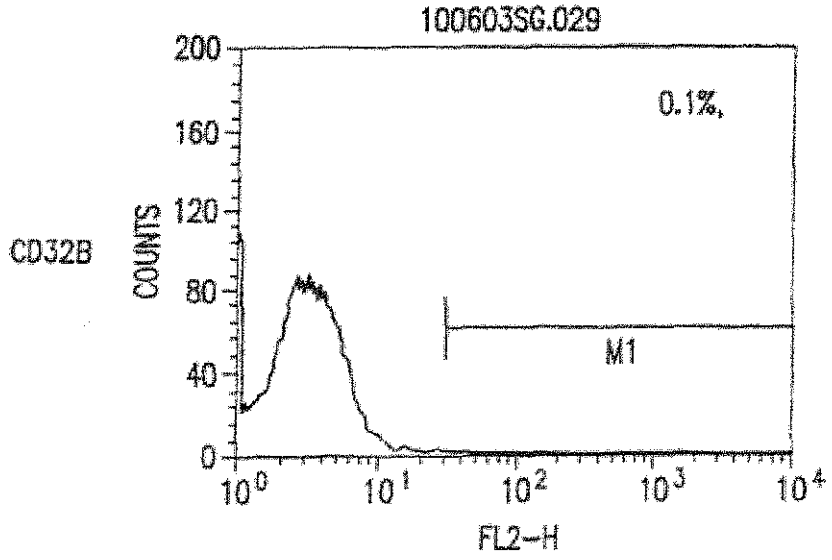


FIG. 12B-6

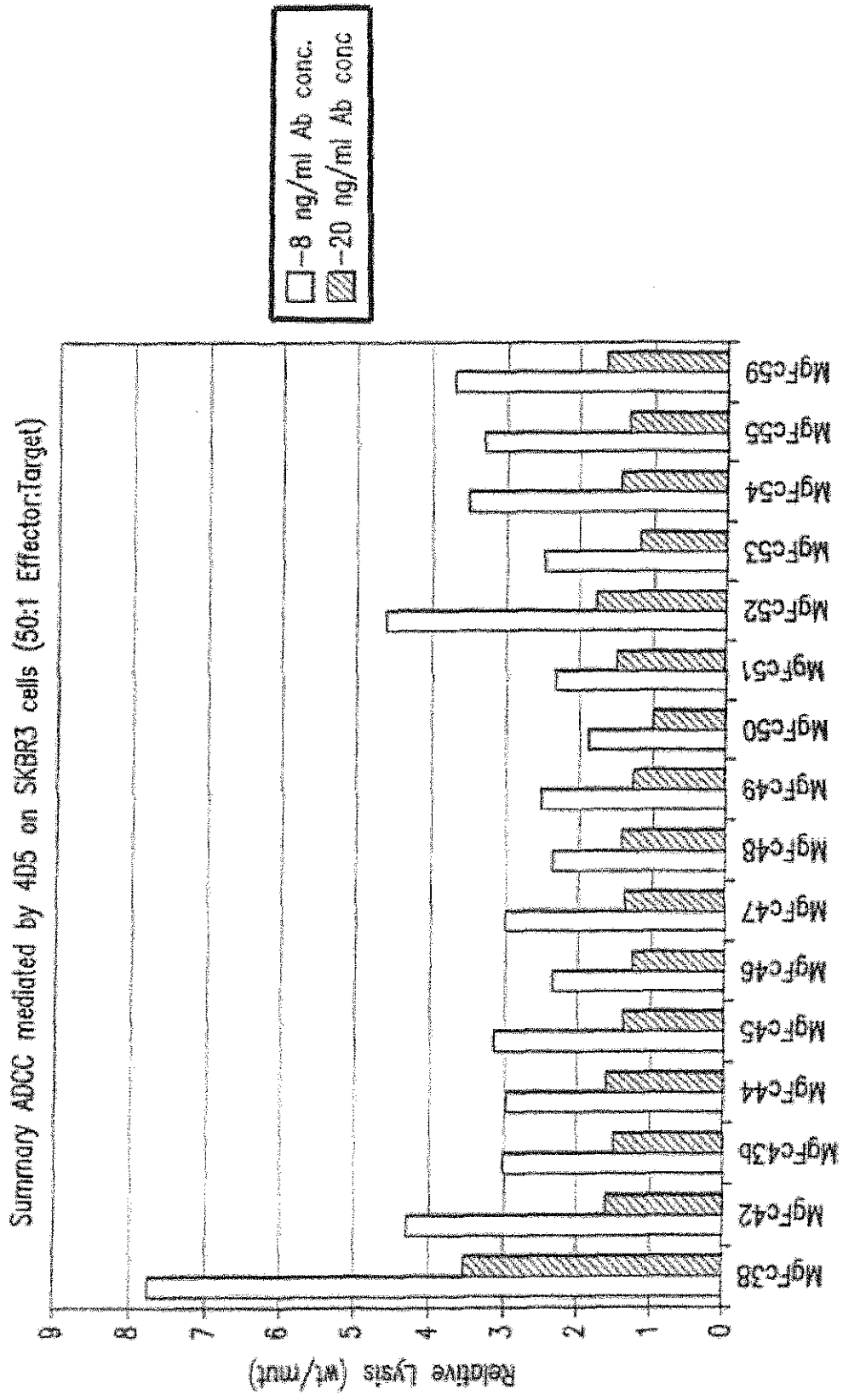

Summary ADCC mediated by 4D5 on SKBR3 cells (50:1 Effector:Target)
Relative Lysis (wt/mut)
0
1
2
3
4
5
6
7
8
9
8 ng/ml Ab conc.
20 ng/ml Ab conc
MgFc38
MgFc42
MgFc43b
MgFc44
MgFc45
MgFc46
MgFc47
MgFc48
MgFc49
MgFc50
MgFc51
MgFc52
MgFc53
MgFc54
MgFc55
MgFc59

FIG. 13

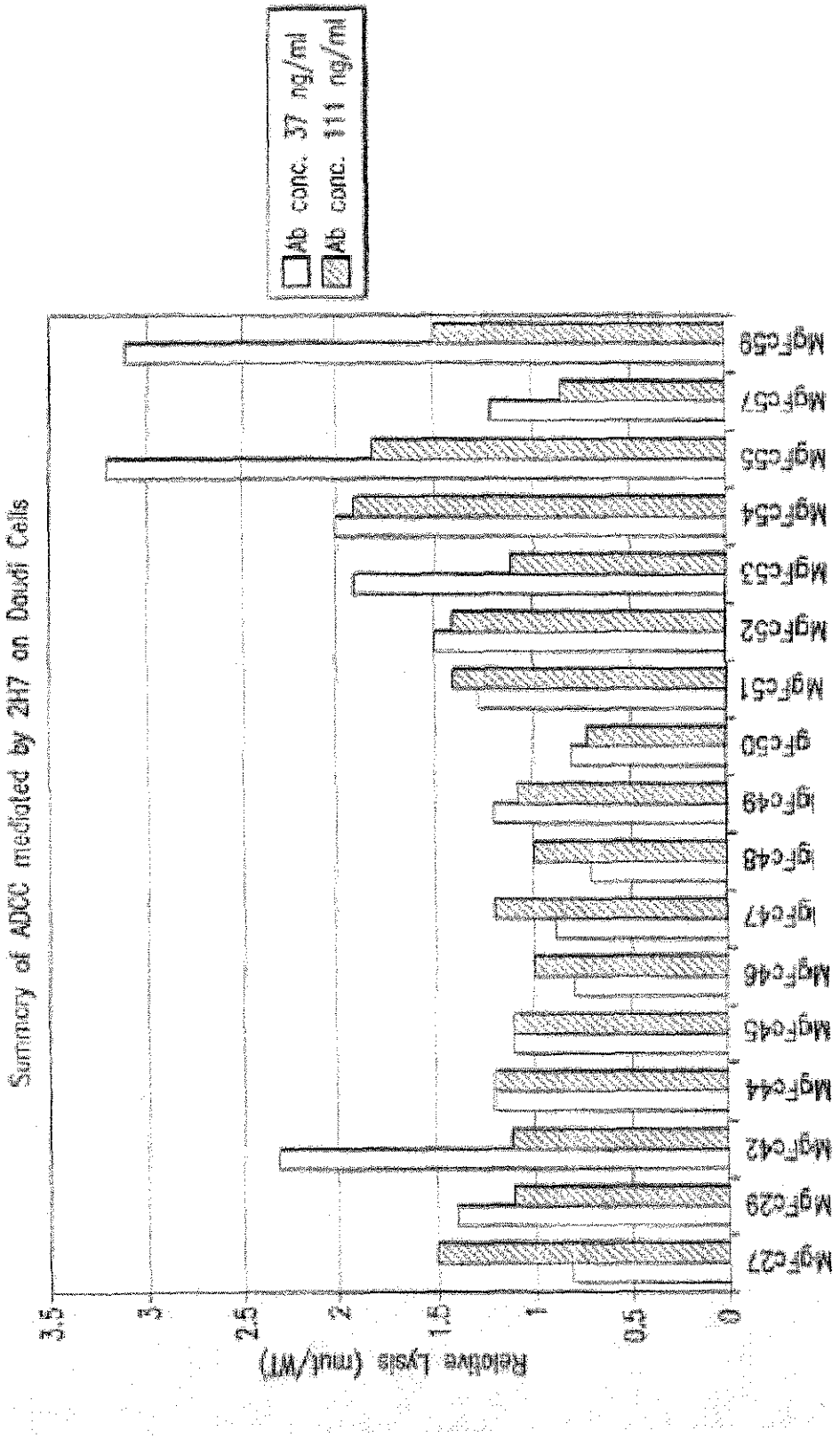
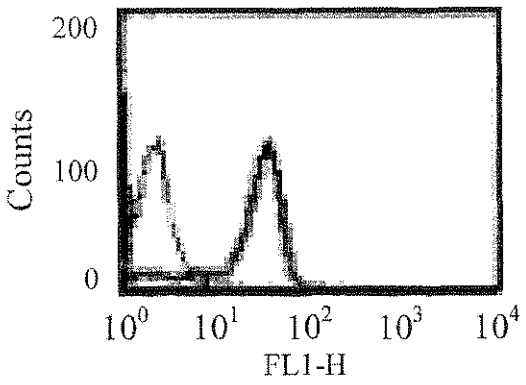
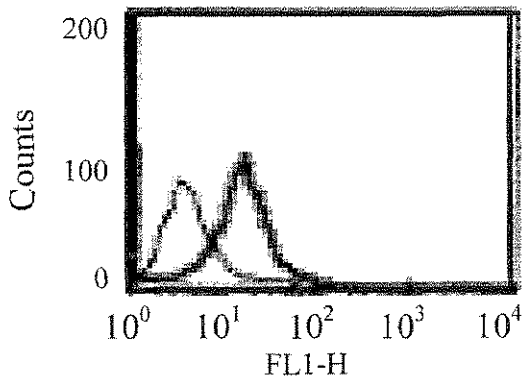

Summary of ADCC mediated by 2H7 on Daudi Cells
Relative Lysis (mut/WT)
3.5
3
2.5
2
1.5
1
0.5
0
Ab conc. 37 ng/ml
Ab conc. 111 ng/ml
MgFc27
MgFc29
MgFc42
MgFc44
MgFc45
MgFc46
IgFc47
IgFc48
IgFc49
gFc50
MgFc51
MgFc52
MgFc53
MgFc54
MgFc55
MgFc57
MgFc59

FIG. 14

CD64 Day 0



CD64 Day 8
Condition 1



CD64 Day 8
Condition 2

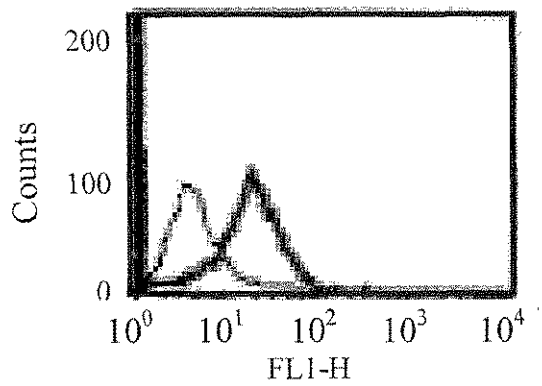
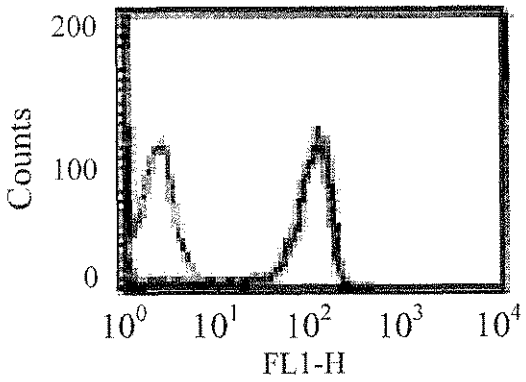
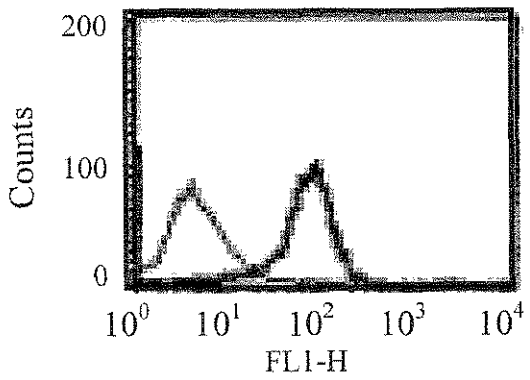


FIG. 15, Panel A

CD32A Day 0



CD32A Day 8
Condition 1



CD32A Day 8
Condition 2

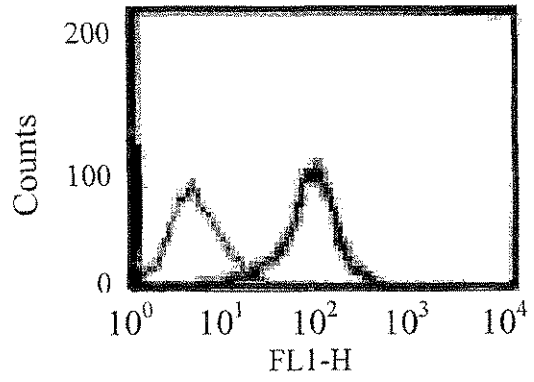
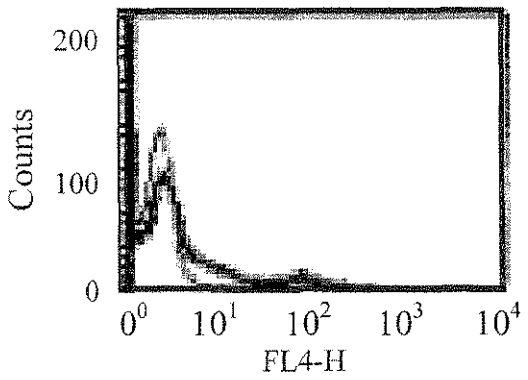
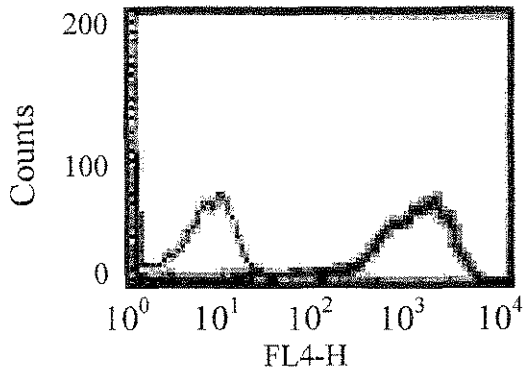


FIG. 15, Panel B

CD32B Day 0



CD32B Day 8
Condition 1



CD32B Day 8
Condition 2

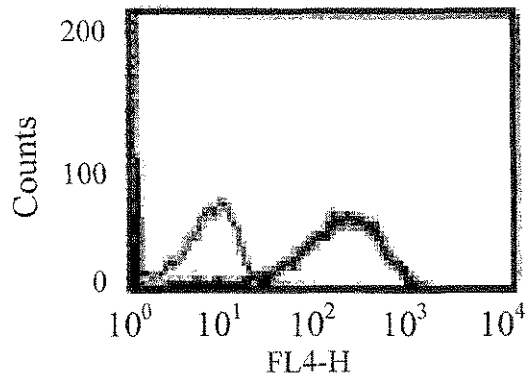
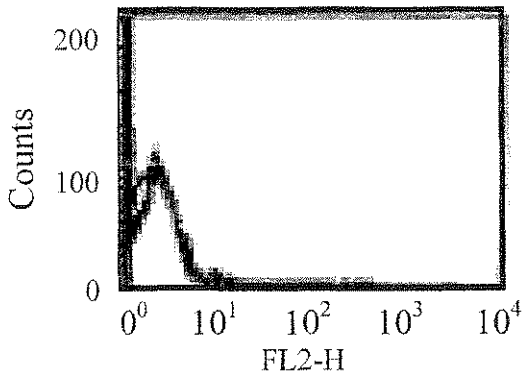
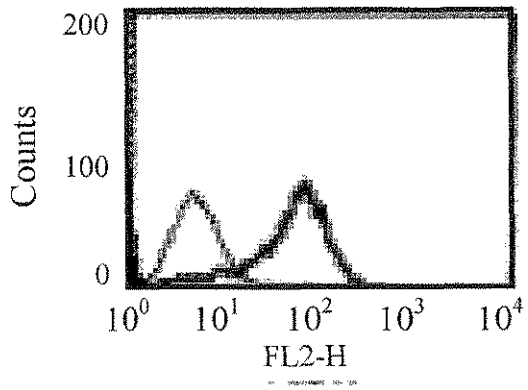


FIG. 15, Panel C

CD16 Day 0



CD16 Day 8
Condition 1



CD16 Day 8
Condition 2

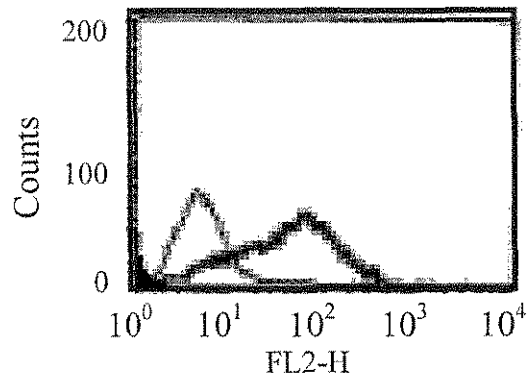
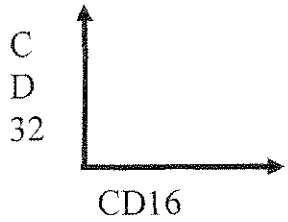
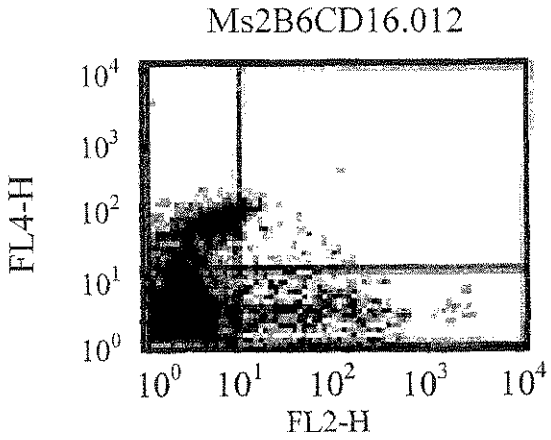


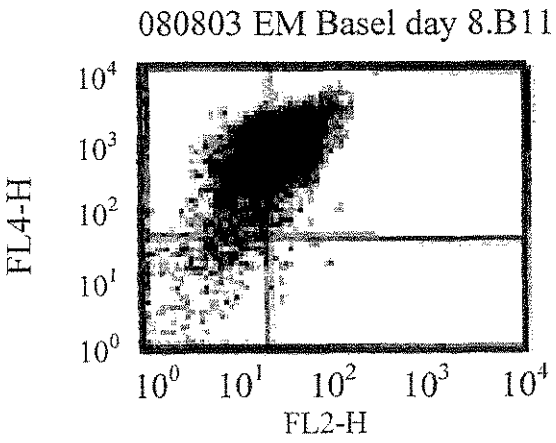
FIG. 15, Panel D



Day 0



Day 8
Condition 1



Day 8
Condition 2

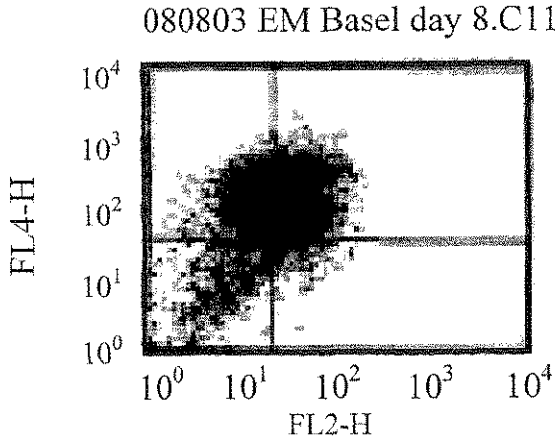


FIG. 15, Panel E

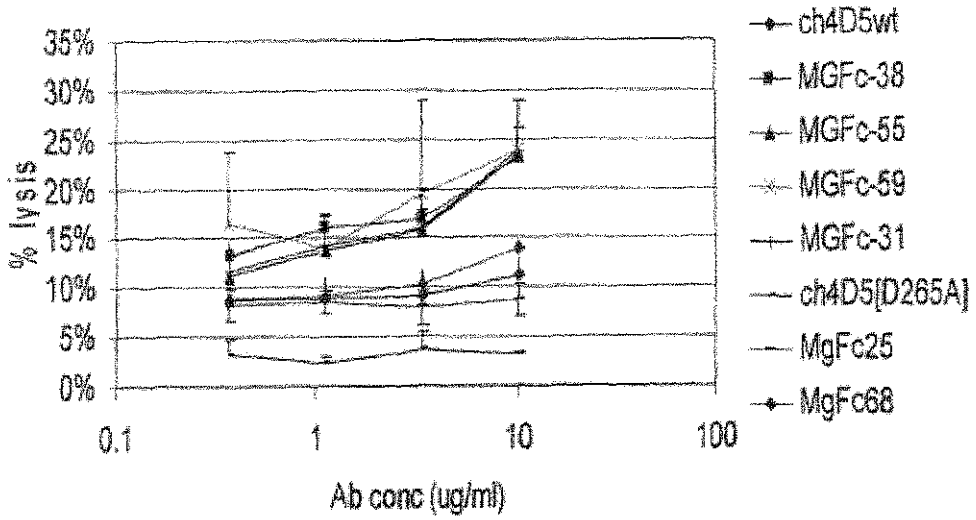

35%
30%
25%
20%
15%
10%
5%
0%
% lysis
0.1
1
10
100
Ab conc (ug/ml)
ch4D5wt
MGFc-38
MGFc-55
MGFc-59
MGFc-31
ch4D5[D265A]
MgFc25
MgFc68

FIG. 16

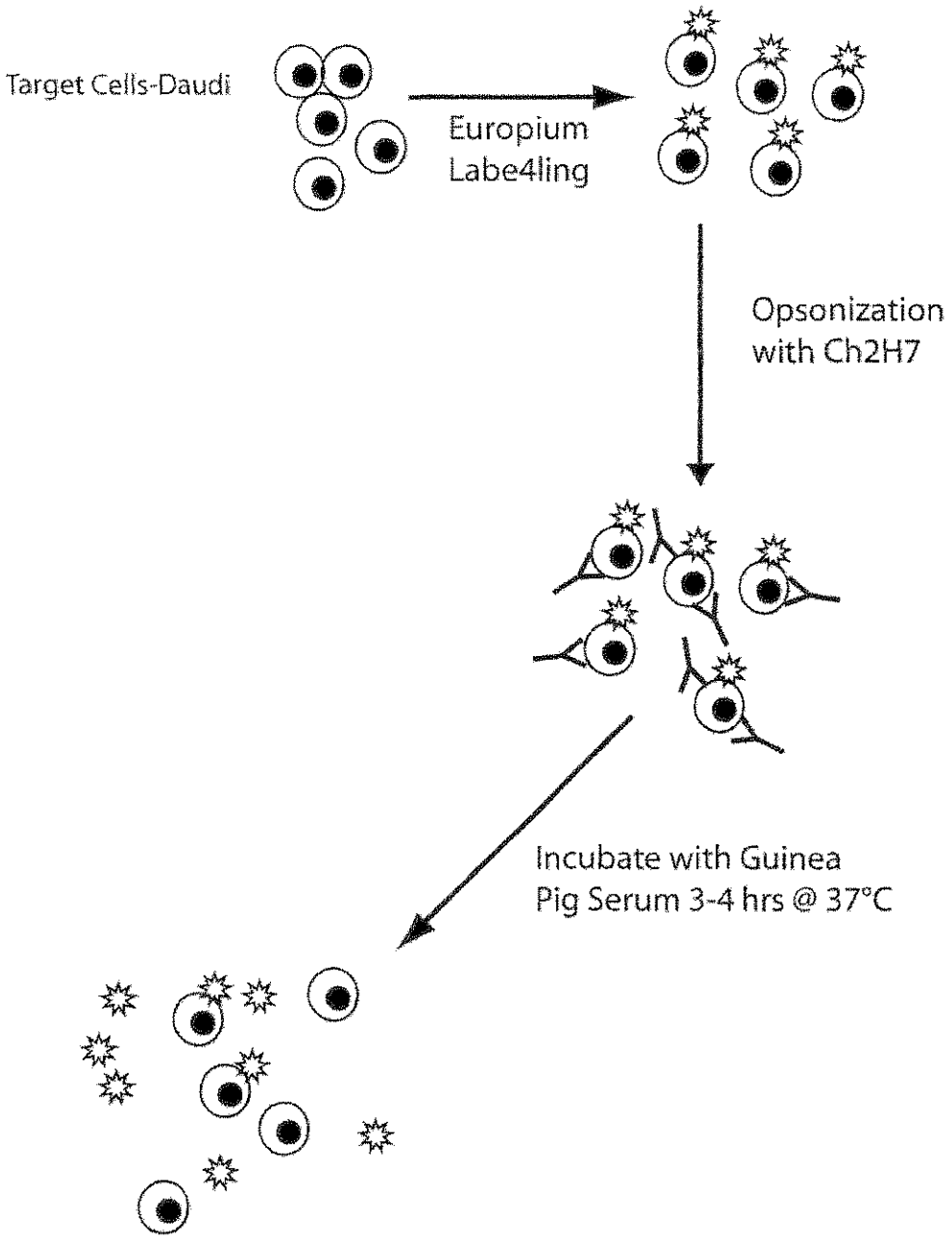
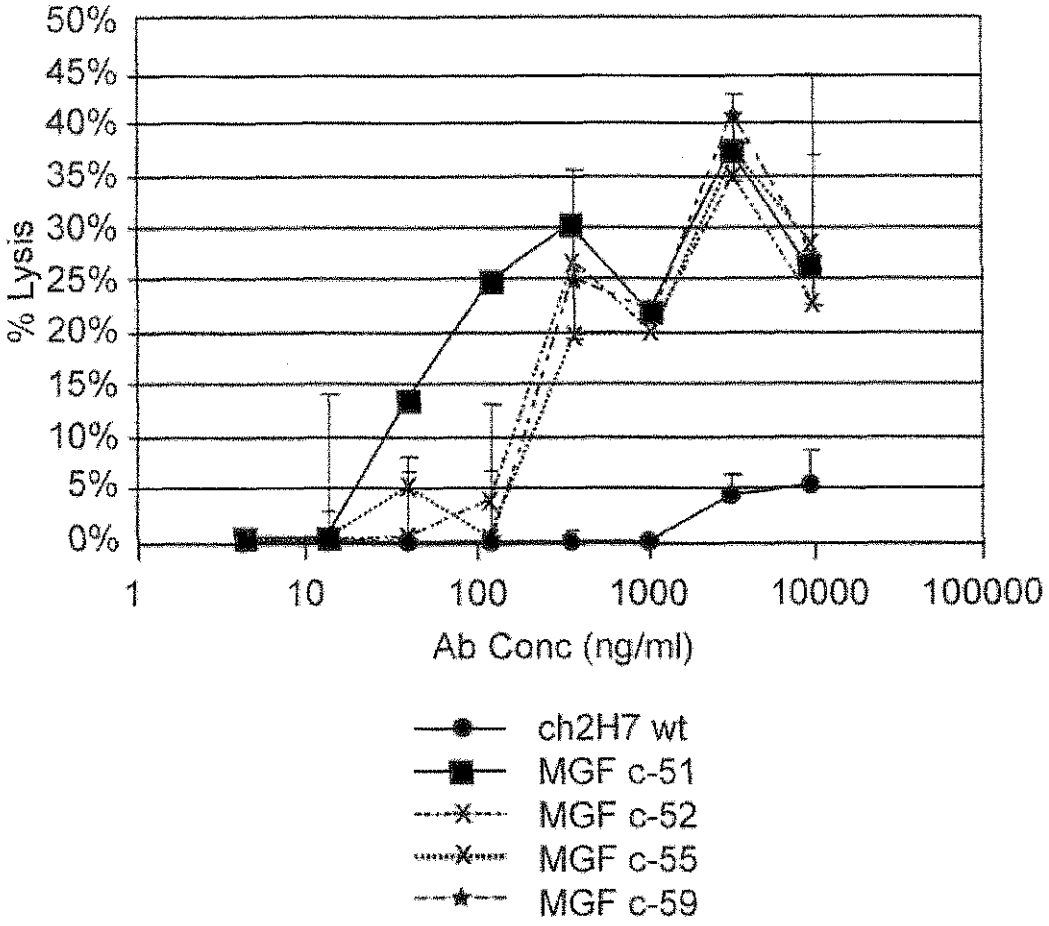

Target Cells-Daudi
Europium
Labe4ling
Opsonization
with Ch2H7
Incubate with Guinea
Pig Serum 3-4 hrs @ 37°C

FIG. 17

FIG. 18


50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
% Lysis
1
10
100
1000
10000
100000
Ab Conc (ng/ml)
ch2H7 wt
MGF c-51
MGF c-52
MGF c-55
MGF c-59

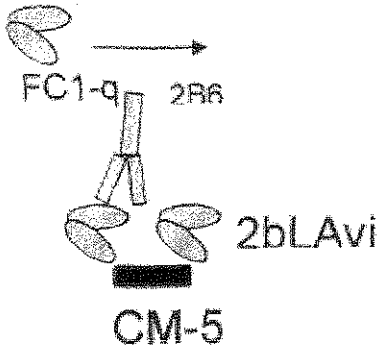


FIG. 19A

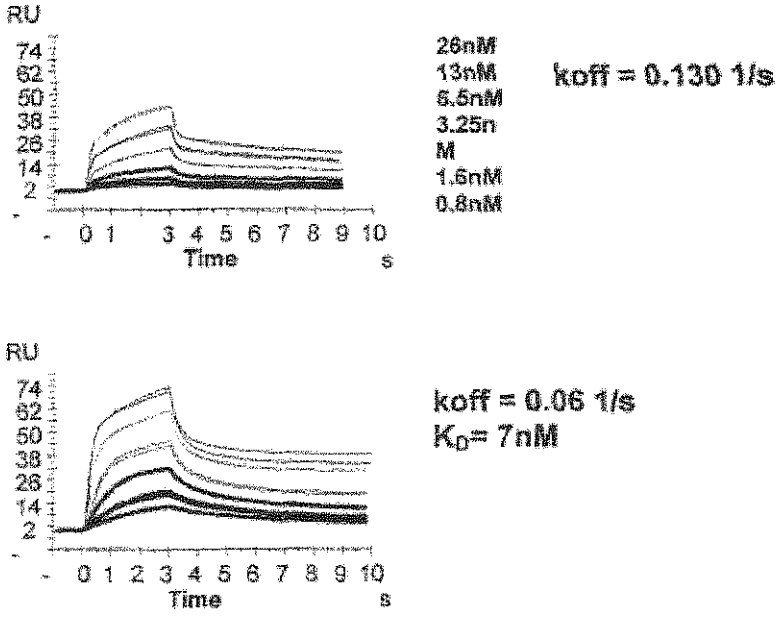


FIG. 19B

FIG. 20A

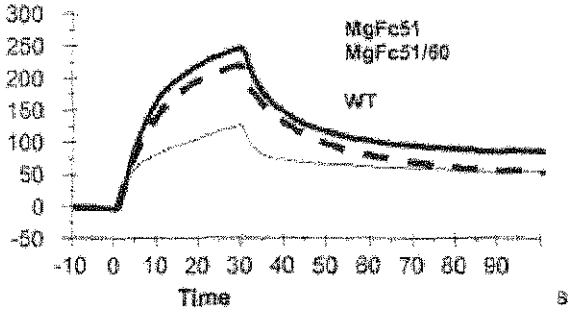


FIG. 20B

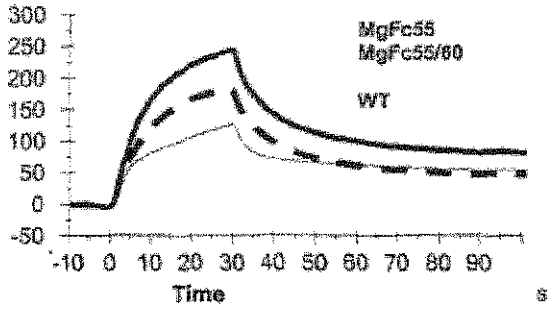


FIG. 20C

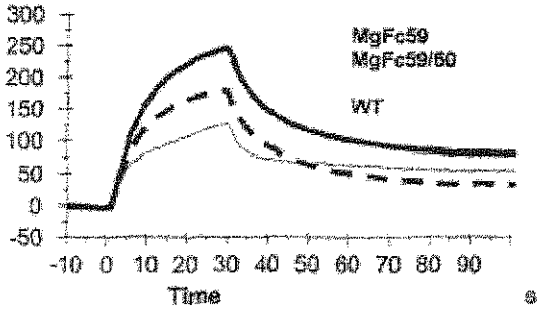


FIG. 20D

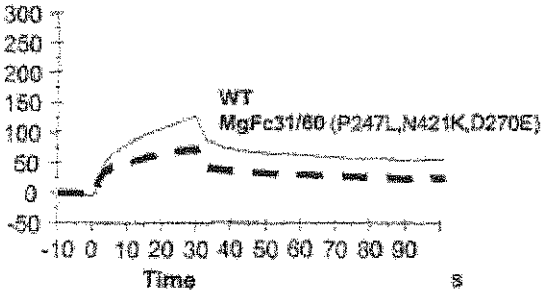


FIG. 21A

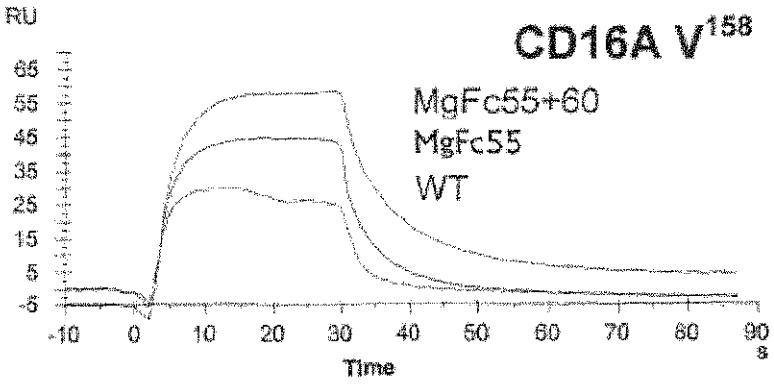


FIG. 21B

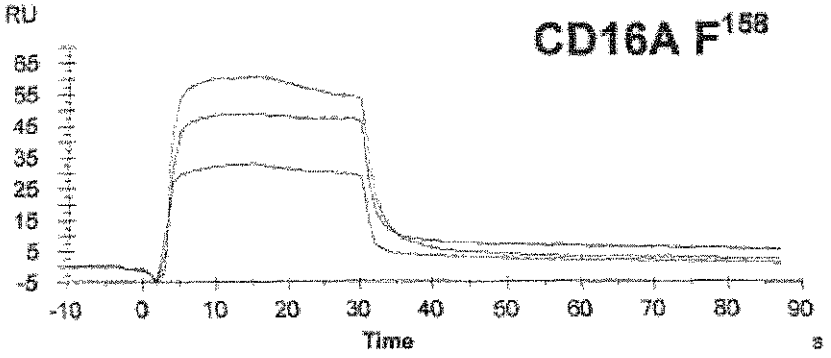


FIG. 21C

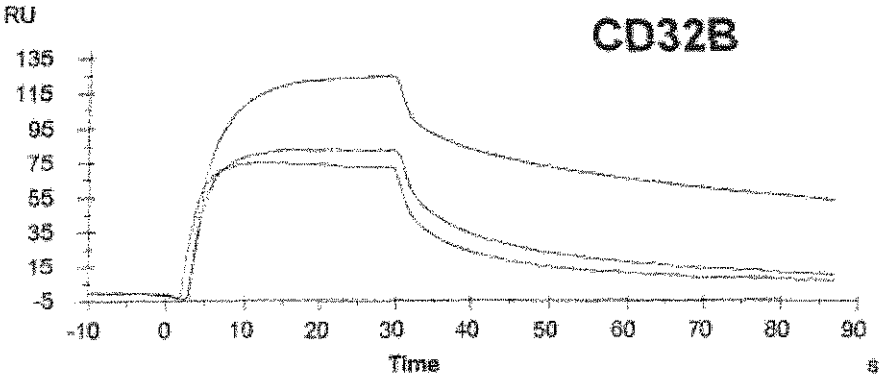


FIG. 21D

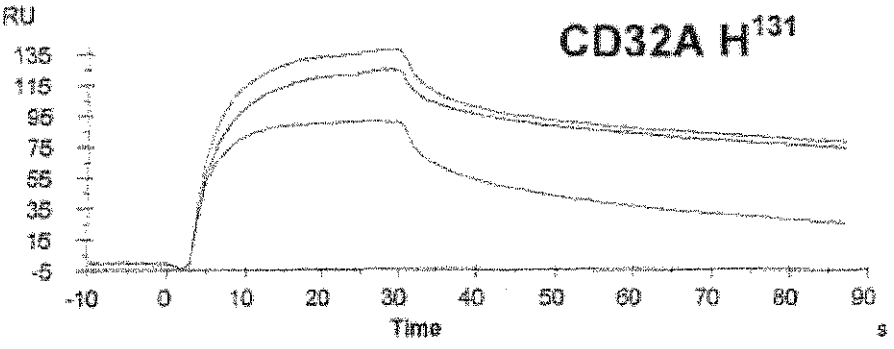


FIG. 22A

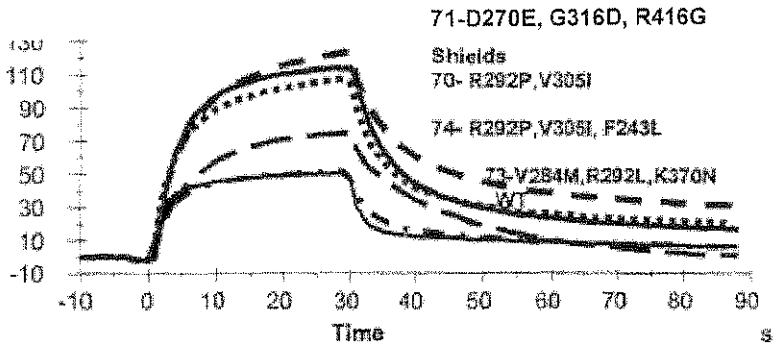


FIG. 22B

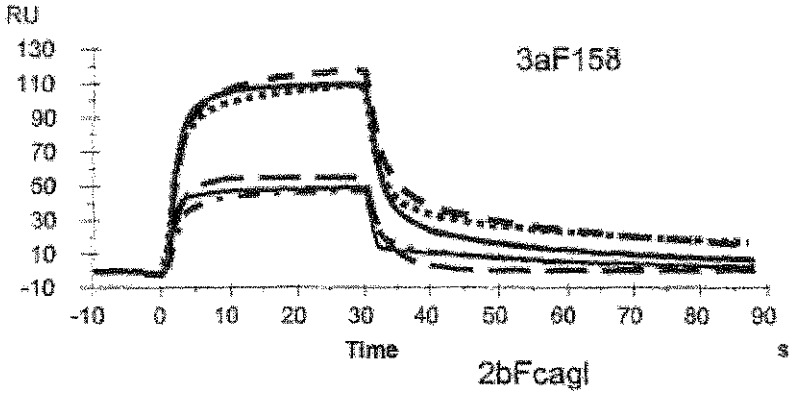


FIG. 22C

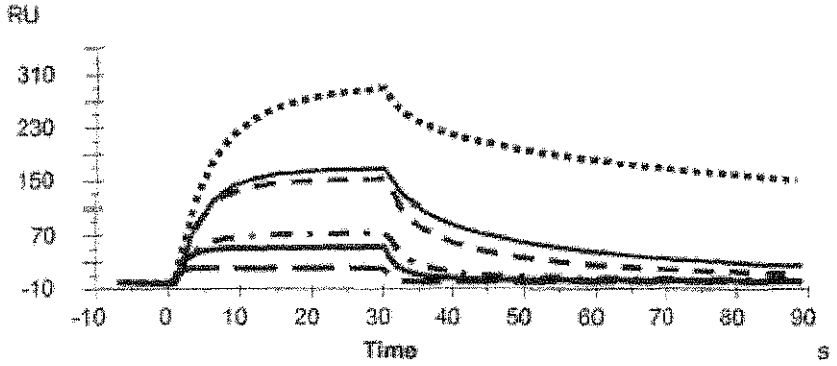
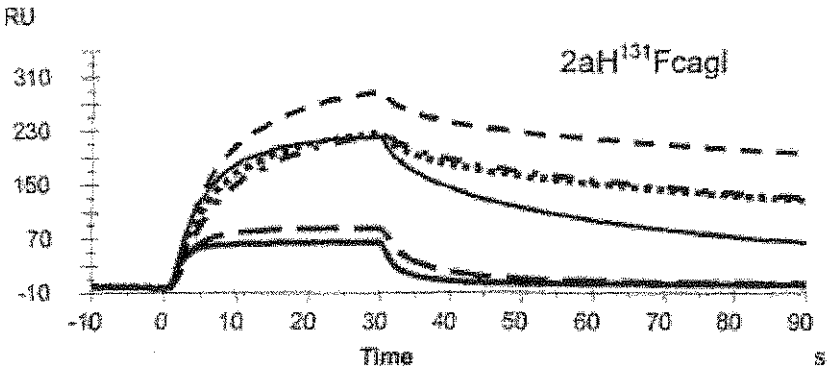


FIG. 22D



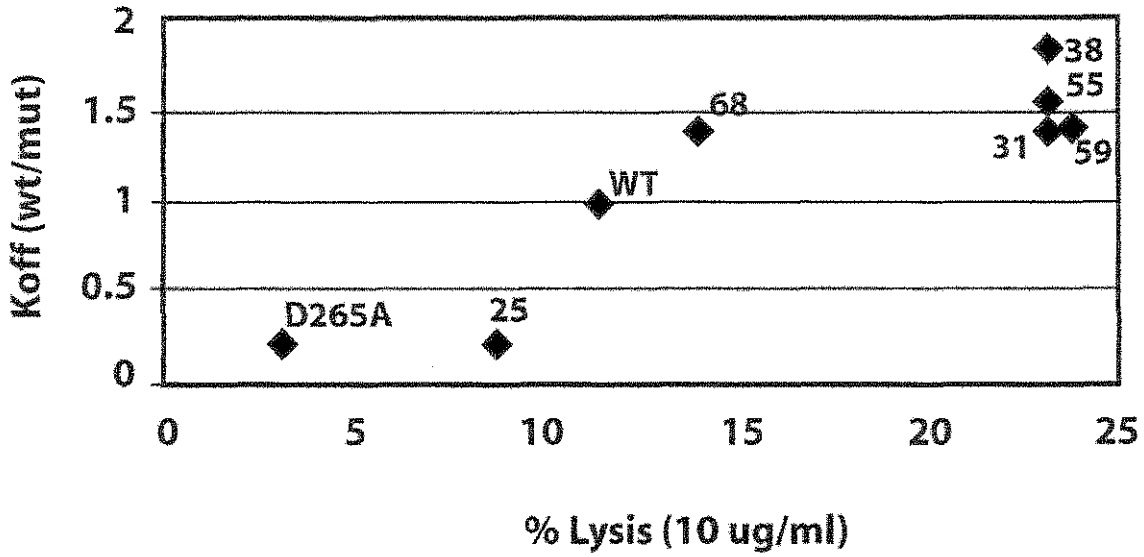

Koff (wt/mut)
2
1.5
1
0.5
0
0
5
10
15
20
25
% Lysis (10 ug/ml)
38
55
68
31
59
WT
D265A
25

FIG. 23

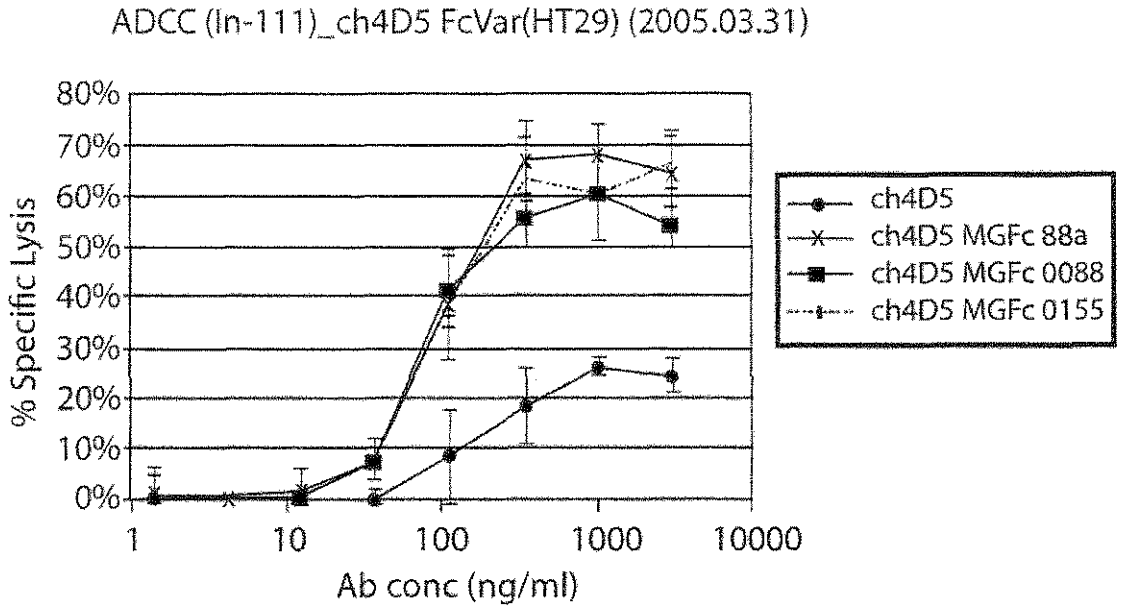


FIG. 24A

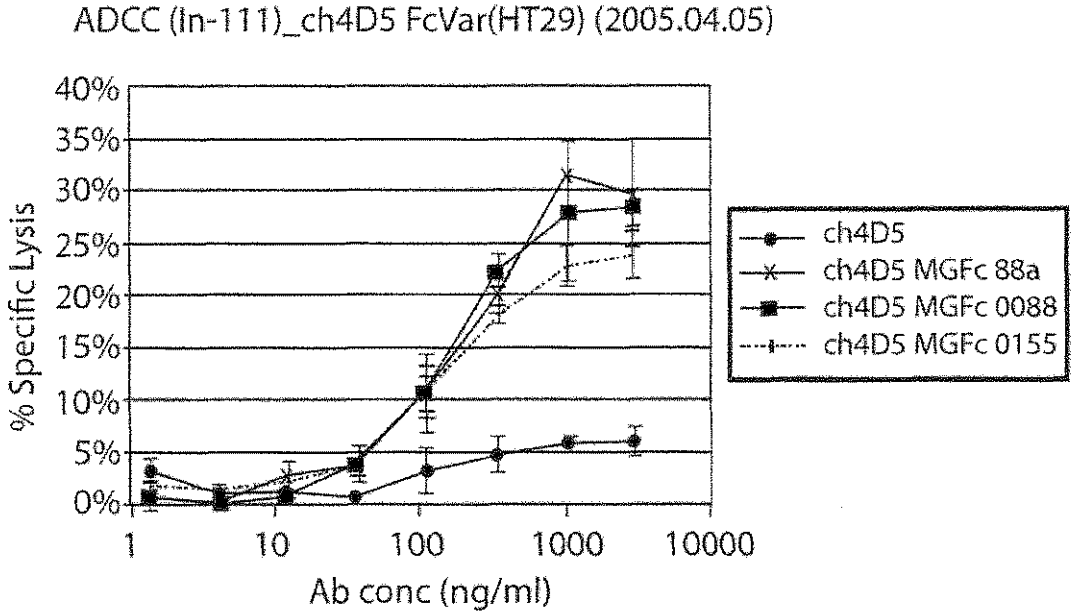


FIG. 24B

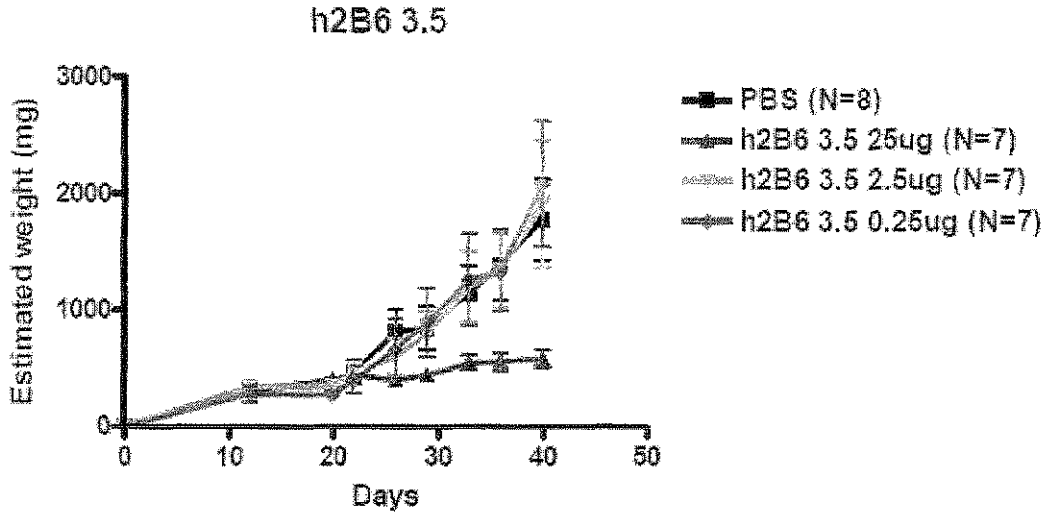


FIG. 25A

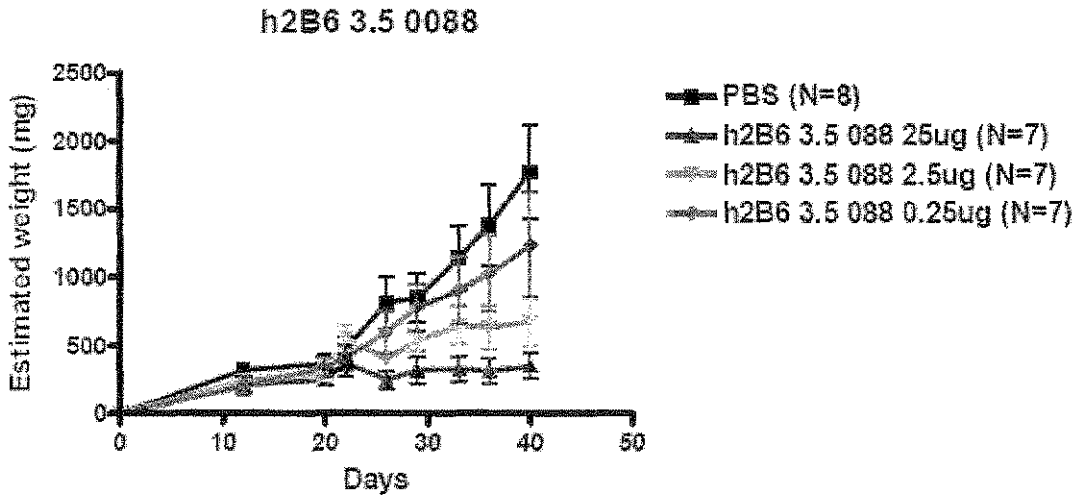


FIG. 25B

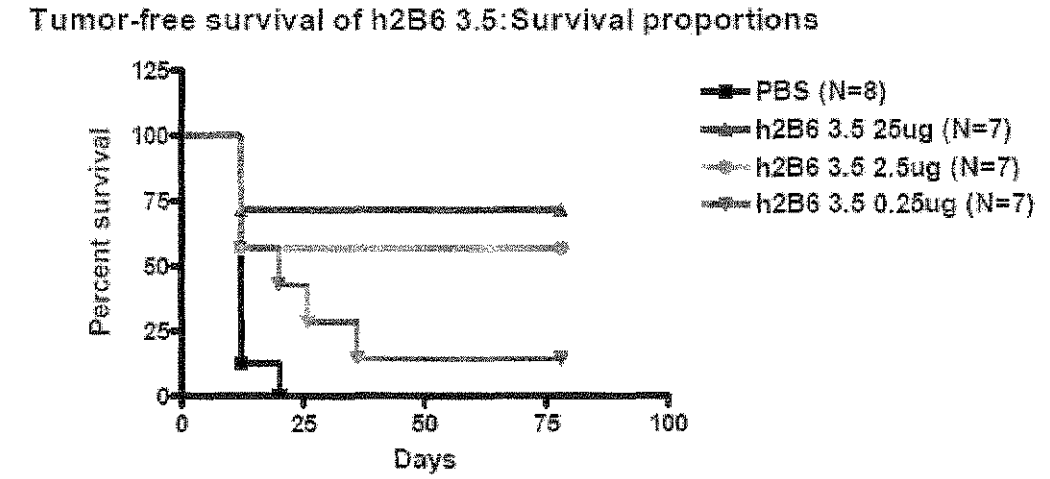


FIG. 26A

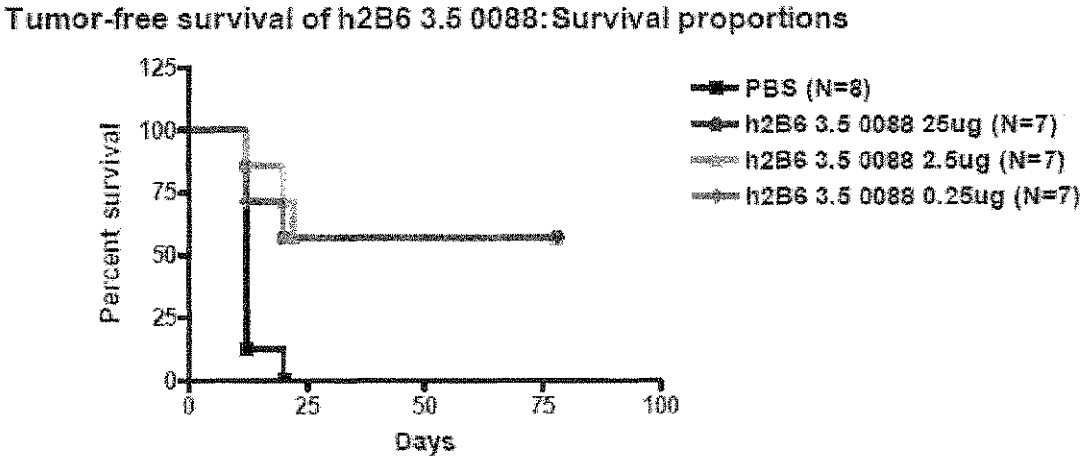


FIG. 26B

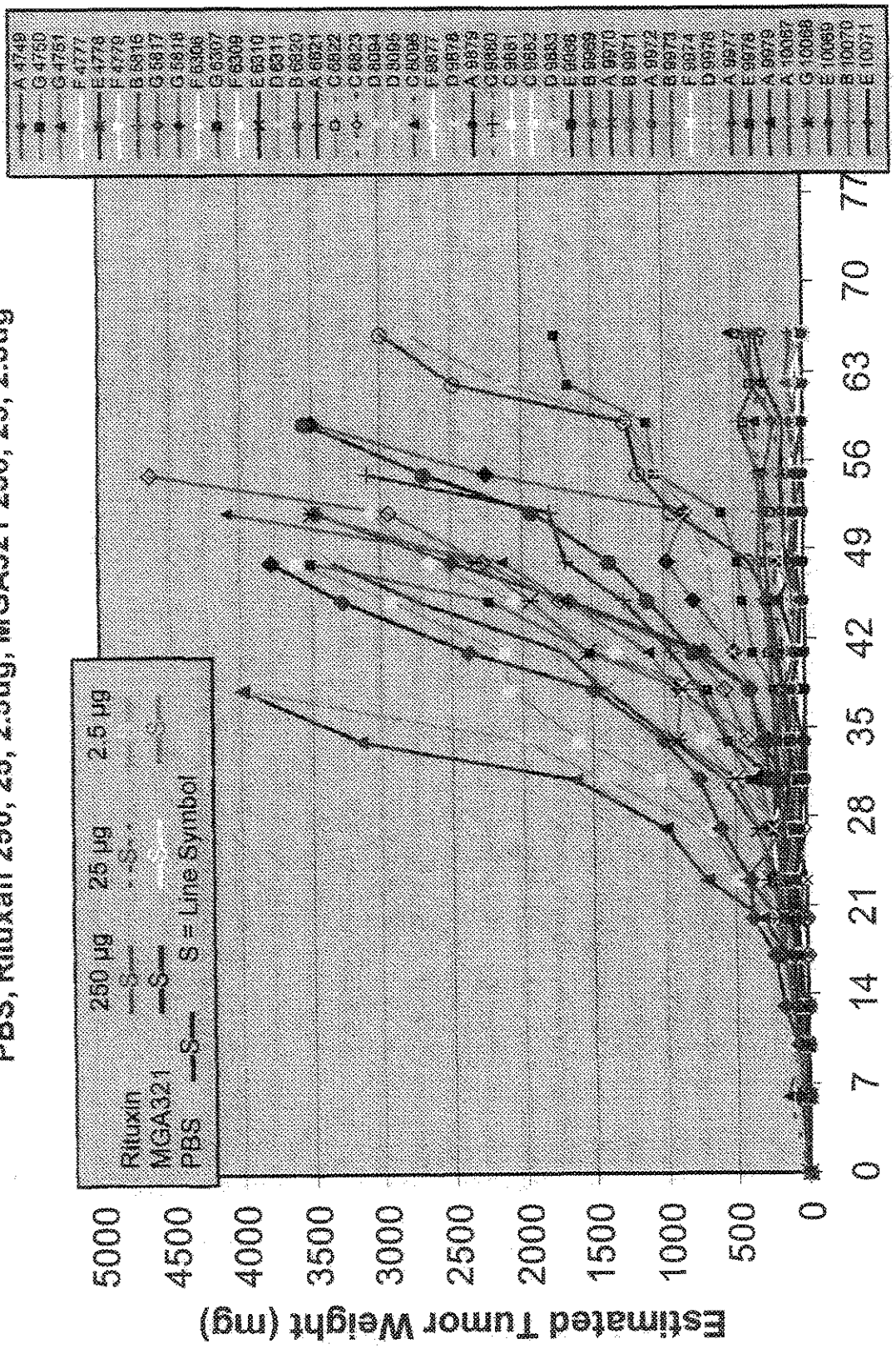

060609 - Raji - mCD16-/- hCD16A+ RAG1-/- B6
PBS, Rituxan 250, 25, 2.5ug, MGA321 250, 25, 2.5ug
Rituxin
MGA321
PBS
250 µg 25 µg 2.5 µg
S = Line Symbol
Estimated Tumor Weight (mg)
Days
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
0
7
14
21
28
35
42
49
56
63
70
77

FIG. 27

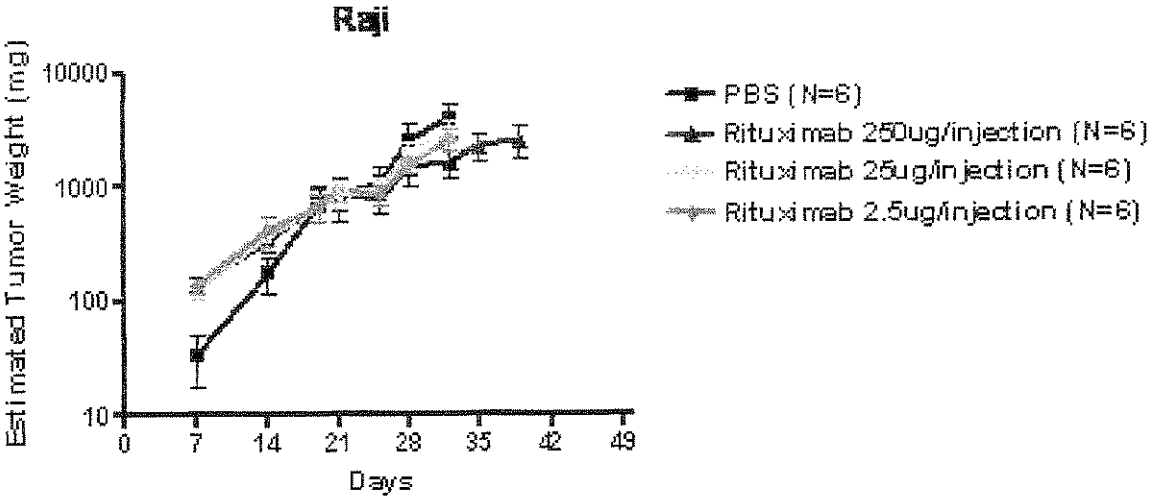


FIG. 28 A

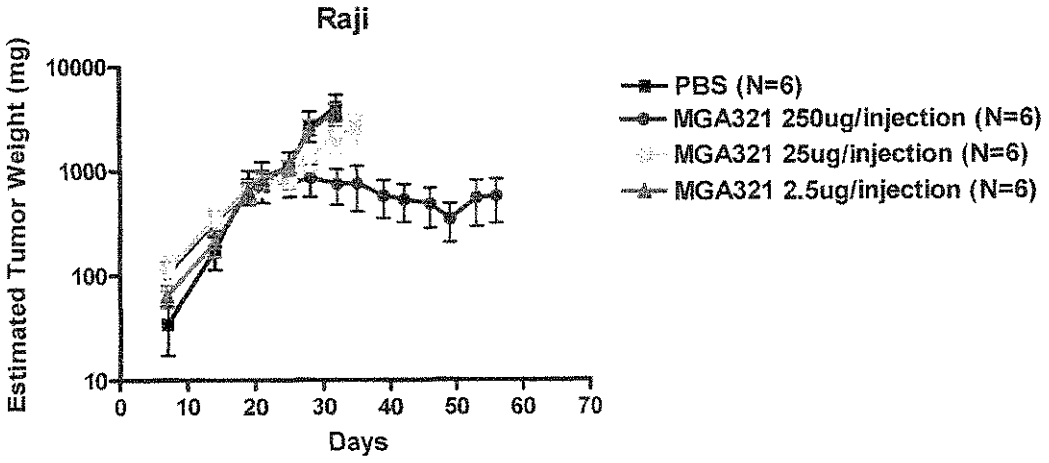


FIG. 28B

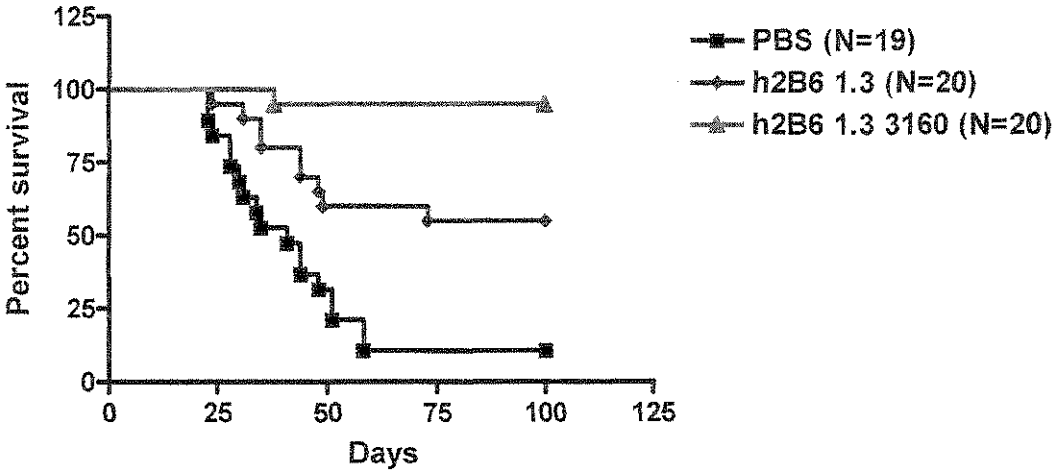

Percent survival
125
100
75
50
25
0
0
25
50
75
100
125
Days
PBS (N=19)
h2B6 1.3 (N=20)
h2B6 1.3 3160 (N=20)

FIG. 29

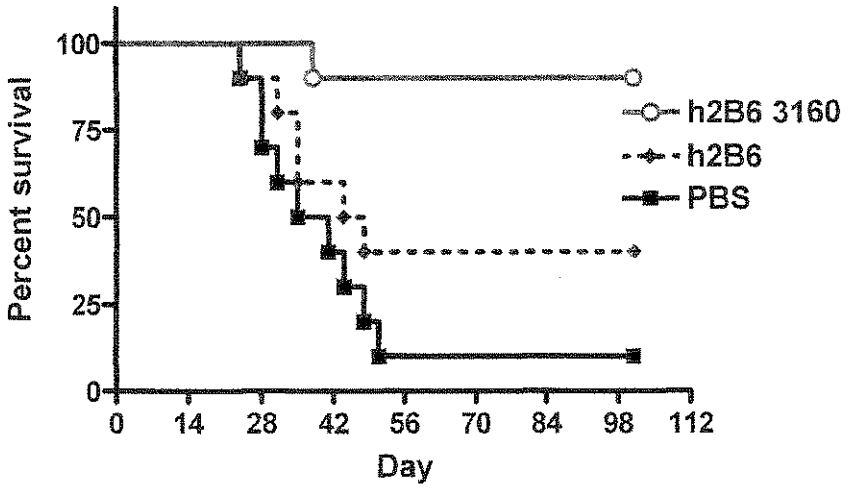

Percent survival
100
75
50
25
0
0
14
28
42
56
70
84
98
112
Day
h2B6 3160
h2B6
PBS

FIG. 30

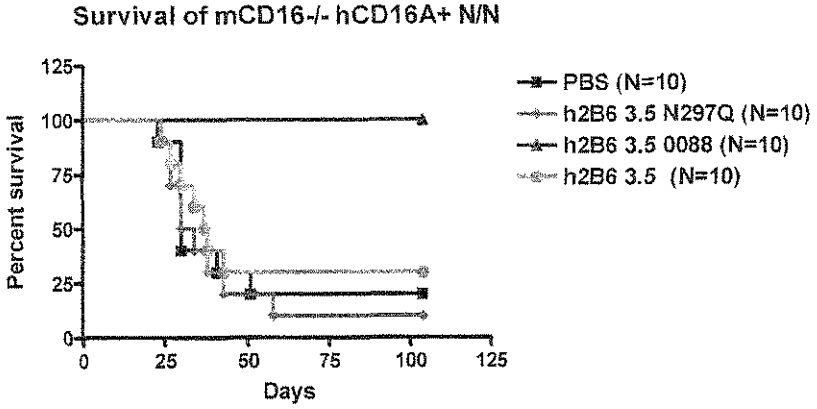


FIG. 31A

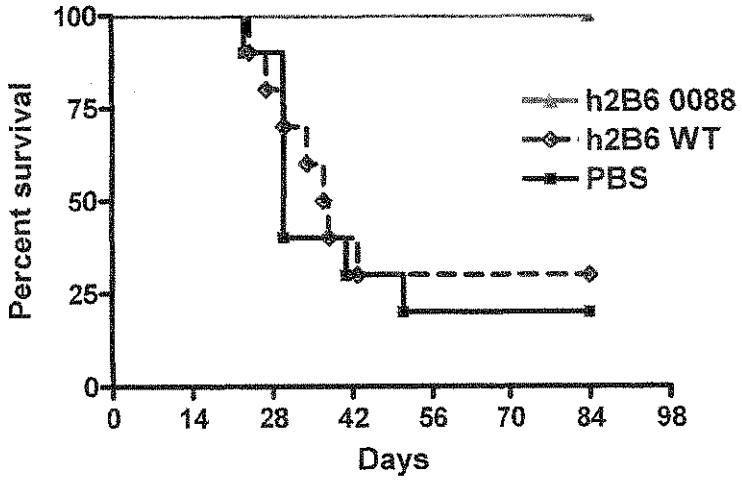


FIG. 31B

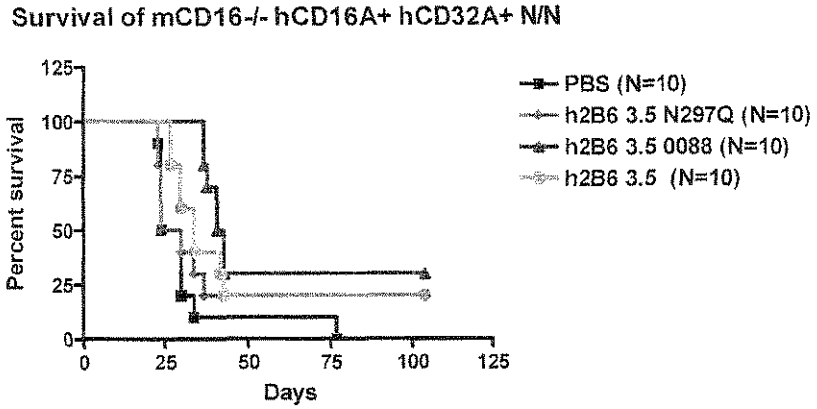

Survival of mCD16-/- hCD16A+ hCD32A+ N/N
Percent survival
125
100
75
50
25
0
0
25
50
75
100
125
Days
PBS (N=10)
h2B6 3.5 N297Q (N=10)
h2B6 3.5 0088 (N=10)
h2B6 3.5 (N=10)

FIG. 32

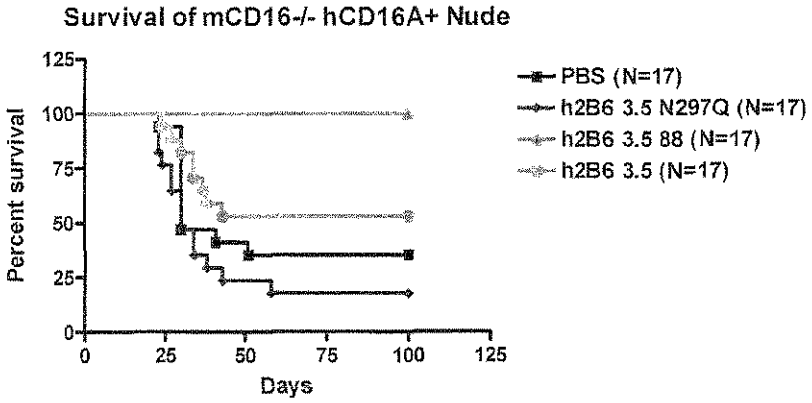


FIG. 33A

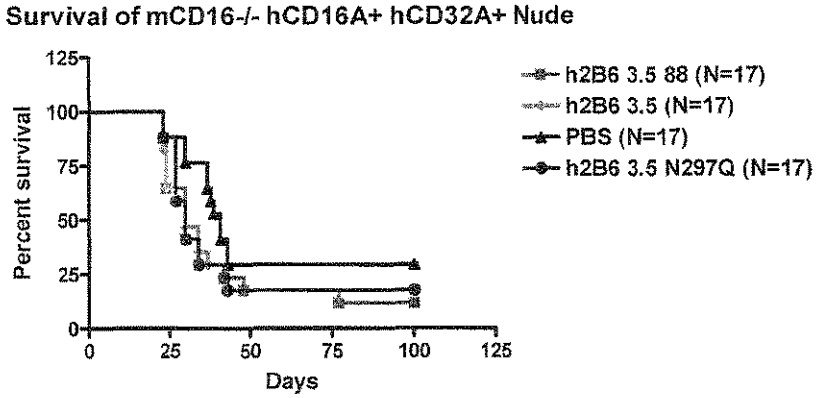


FIG. 33B

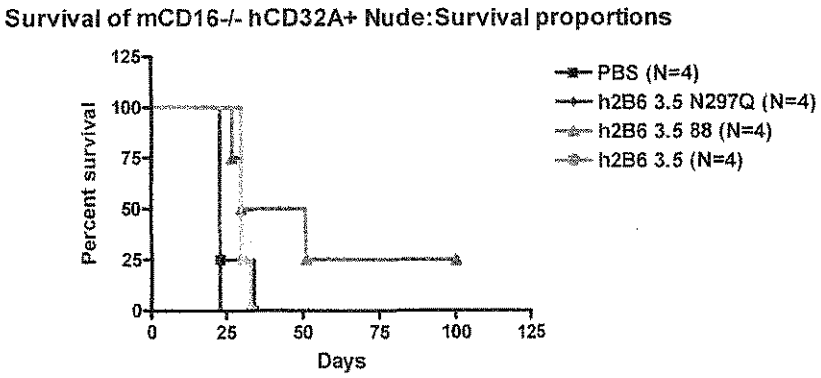


FIG. 33C

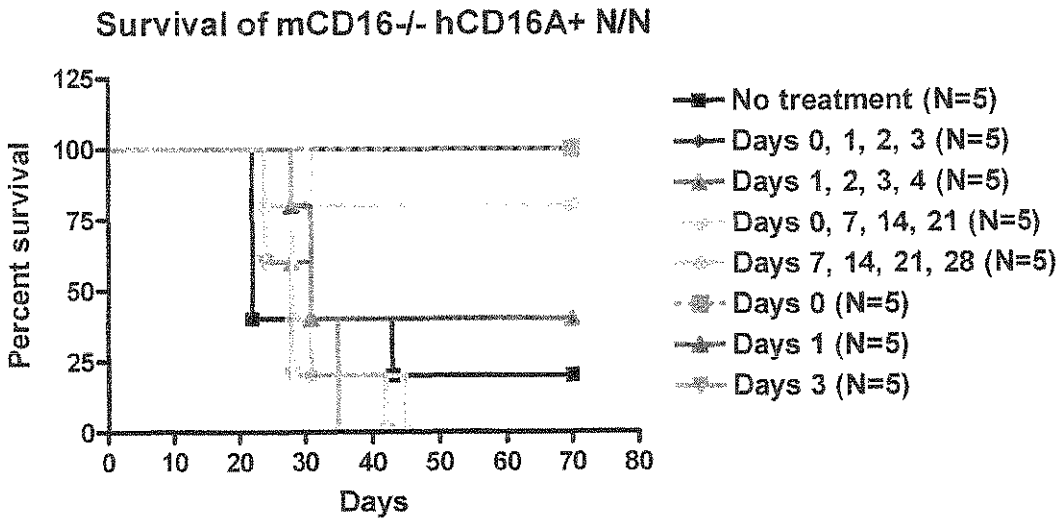

Survival of mCD16-/- hCD16A+ N/N
Percent survival
125
100
75
50
25
0
0
10
20
30
40
50
60
70
80
Days
No treatment (N=5)
Days 0, 1, 2, 3 (N=5)
Days 1, 2, 3, 4 (N=5)
Days 0, 7, 14, 21 (N=5)
Days 7, 14, 21, 28 (N=5)
Days 0 (N=5)
Days 1 (N=5)
Days 3 (N=5)

FIG. 34

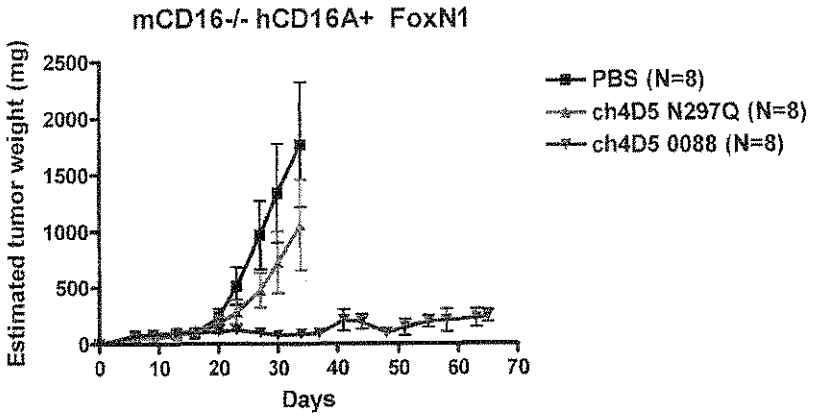


FIG. 35A

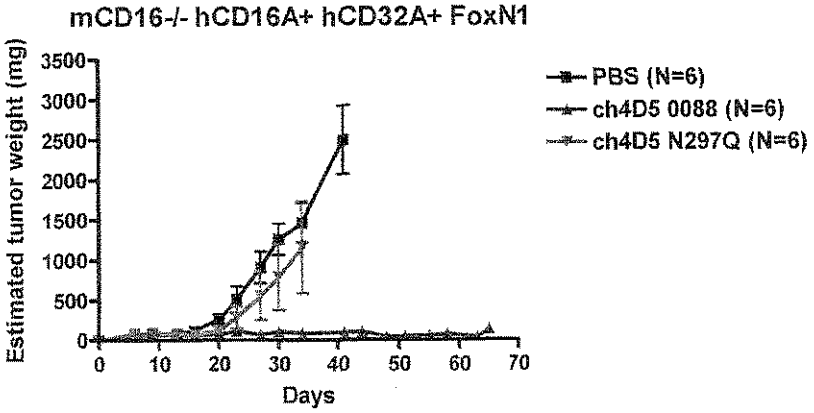


FIG. 35B

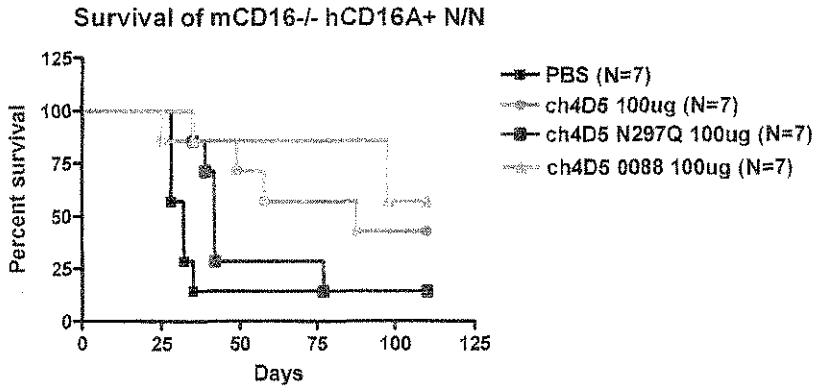


FIG. 36A

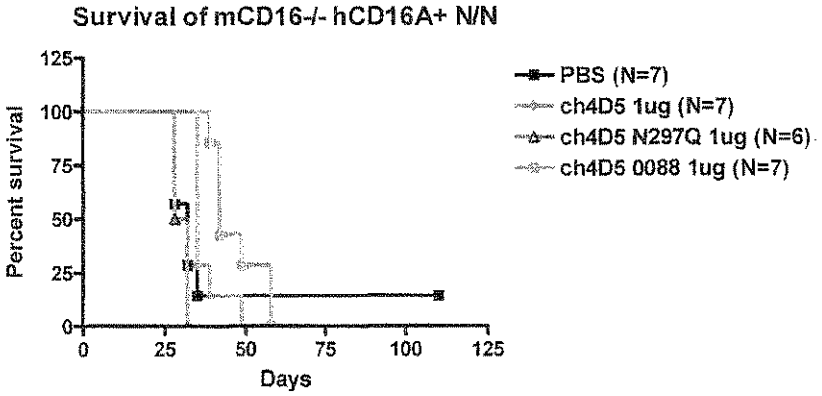


FIG. 36B

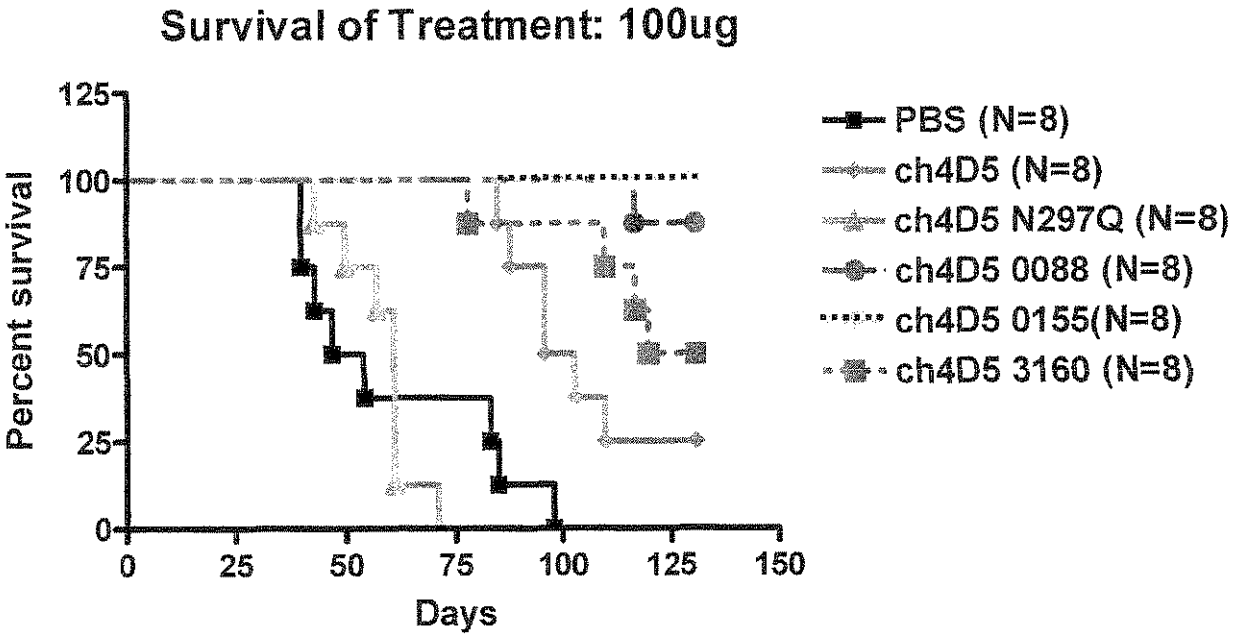


FIG. 37A

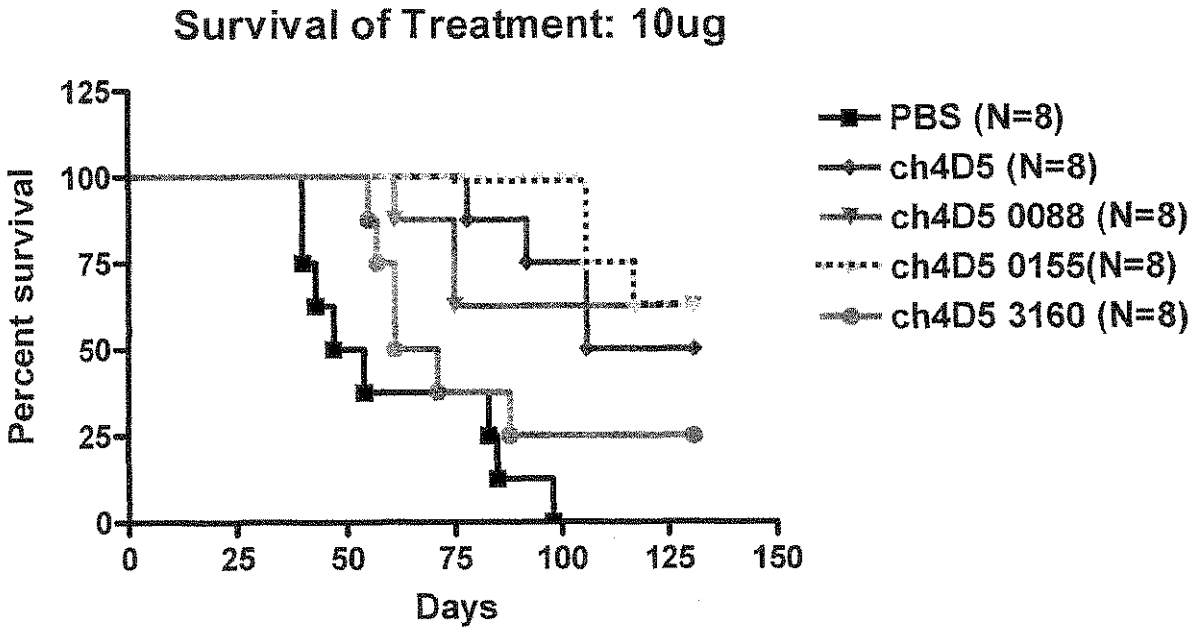


FIG. 37B

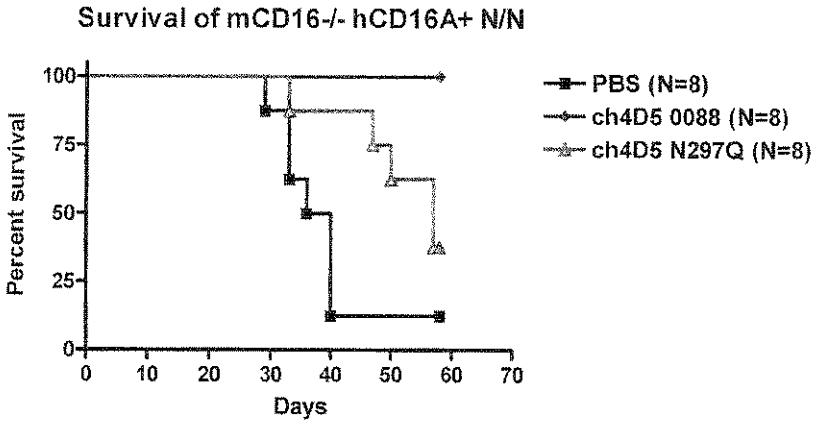


FIG. 38A

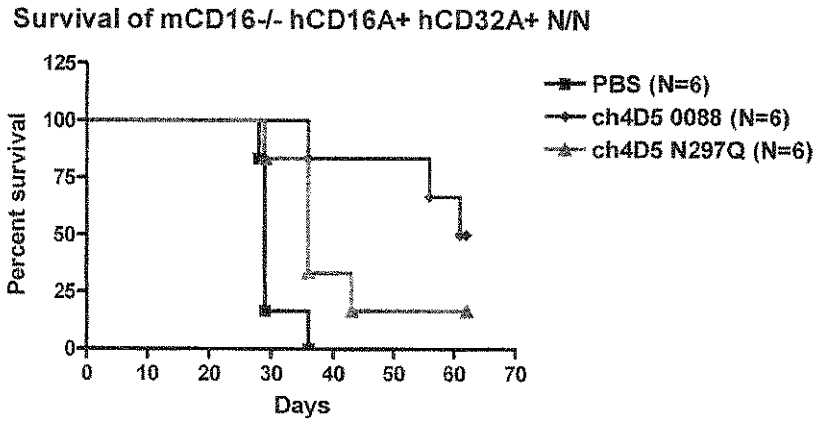
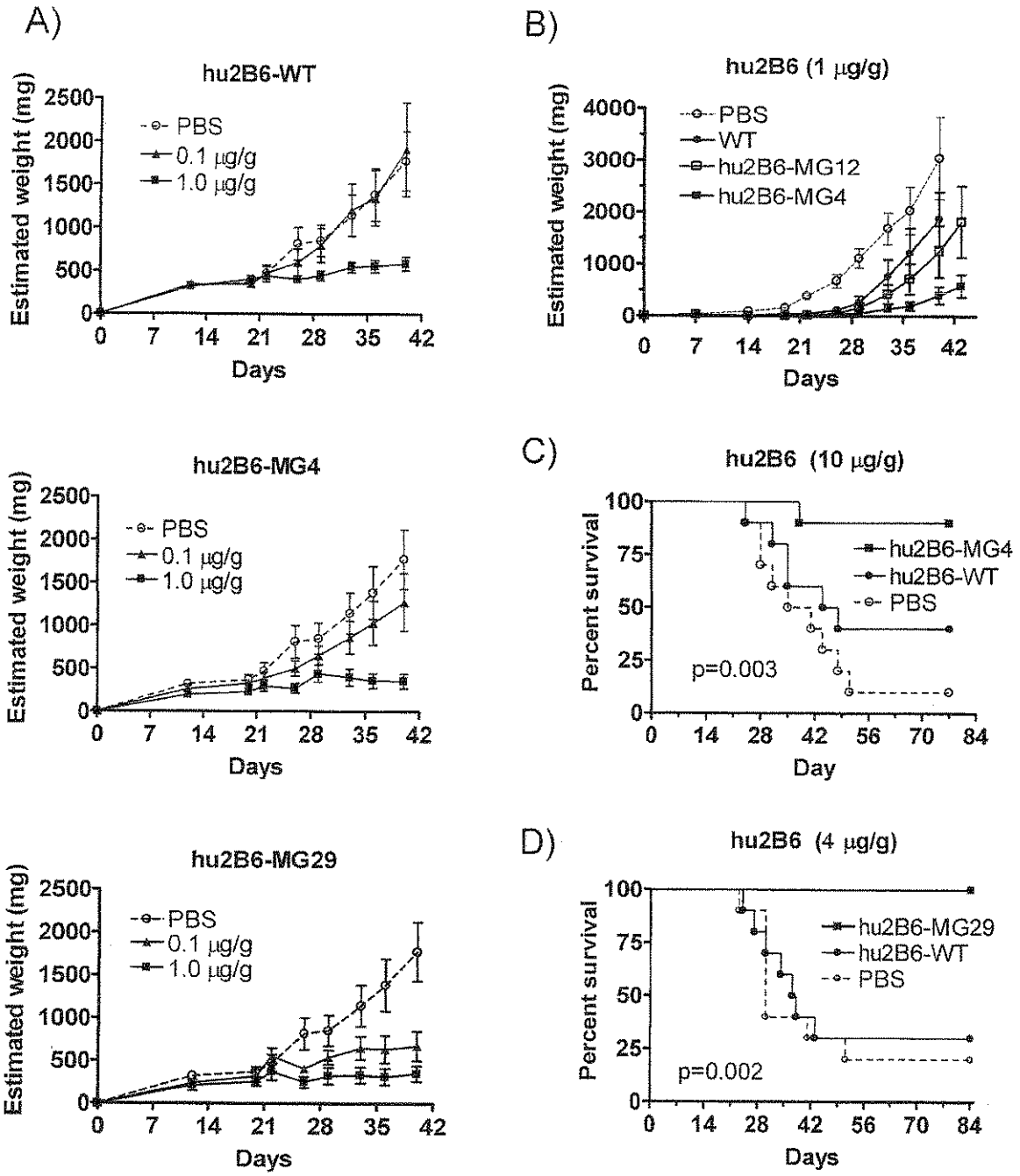


FIG. 38B

Figure 39


A)
hu2B6-WT
Estimated weight (mg)
PBS
0.1 μg/g
1.0 μg/g
Days
B)
hu2B6 (1 μg/g)
Estimated weight (mg)
PBS
WT
hu2B6-MG12
hu2B6-MG4
Days
hu2B6-MG4
Estimated weight (mg)
PBS
0.1 μg/g
1.0 μg/g
Days
C)
hu2B6 (10 μg/g)
Percent survival
hu2B6-MG4
hu2B6-WT
PBS
p=0.003
Day
hu2B6-MG29
Estimated weight (mg)
PBS
0.1 μg/g
1.0 μg/g
Days
D)
hu2B6 (4 μg/g)
Percent survival
hu2B6-MG29
hu2B6-WT
PBS
p=0.002
Days

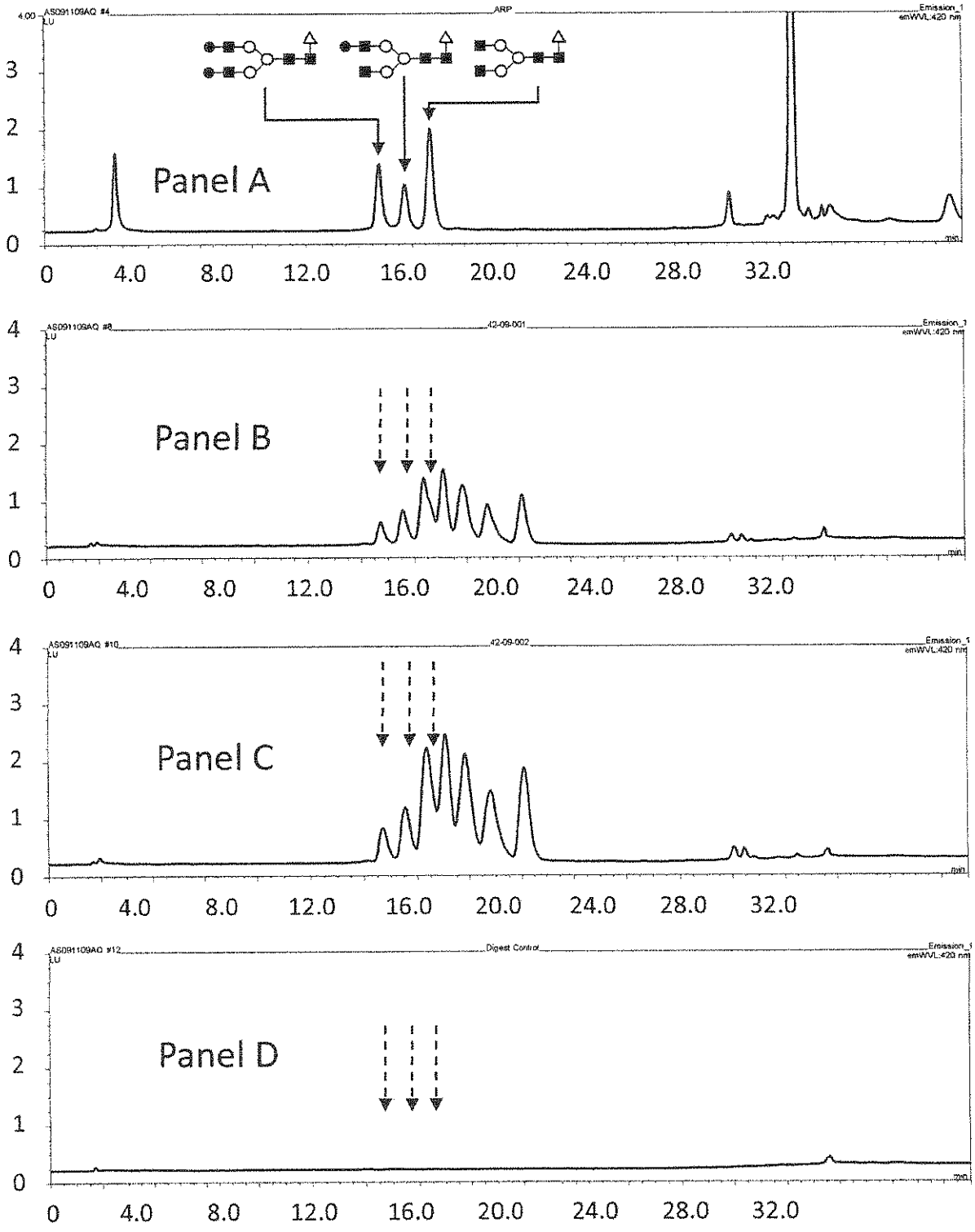


Figure 40

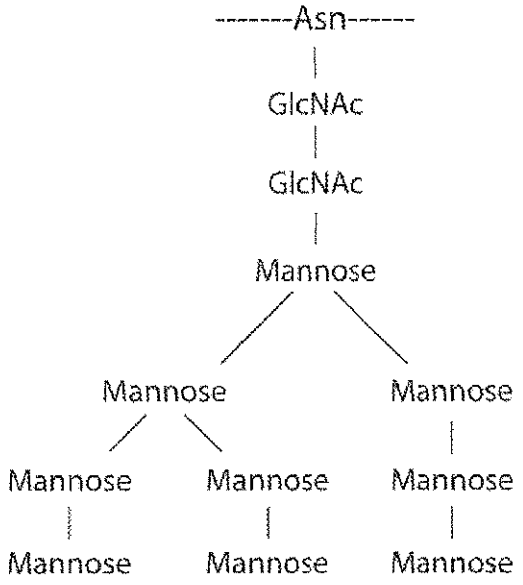


Figure 41

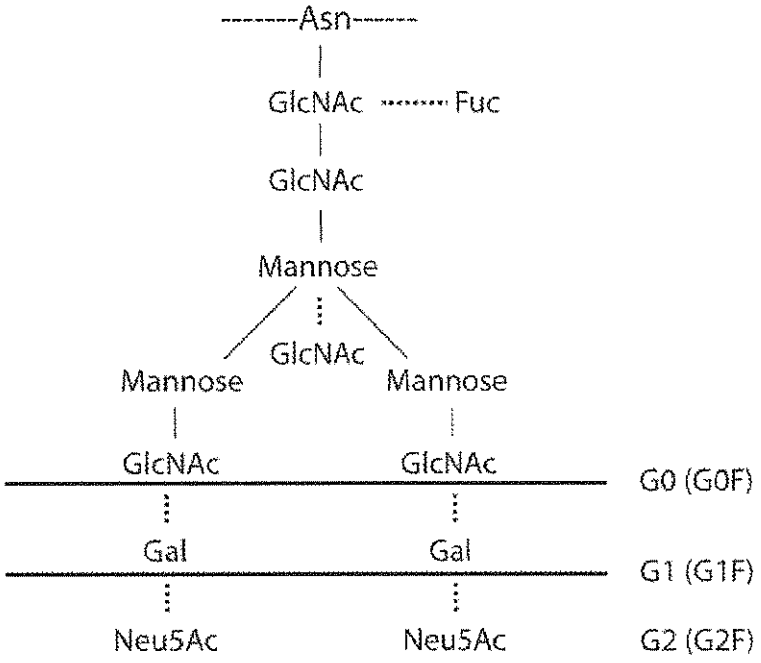


Figure 42

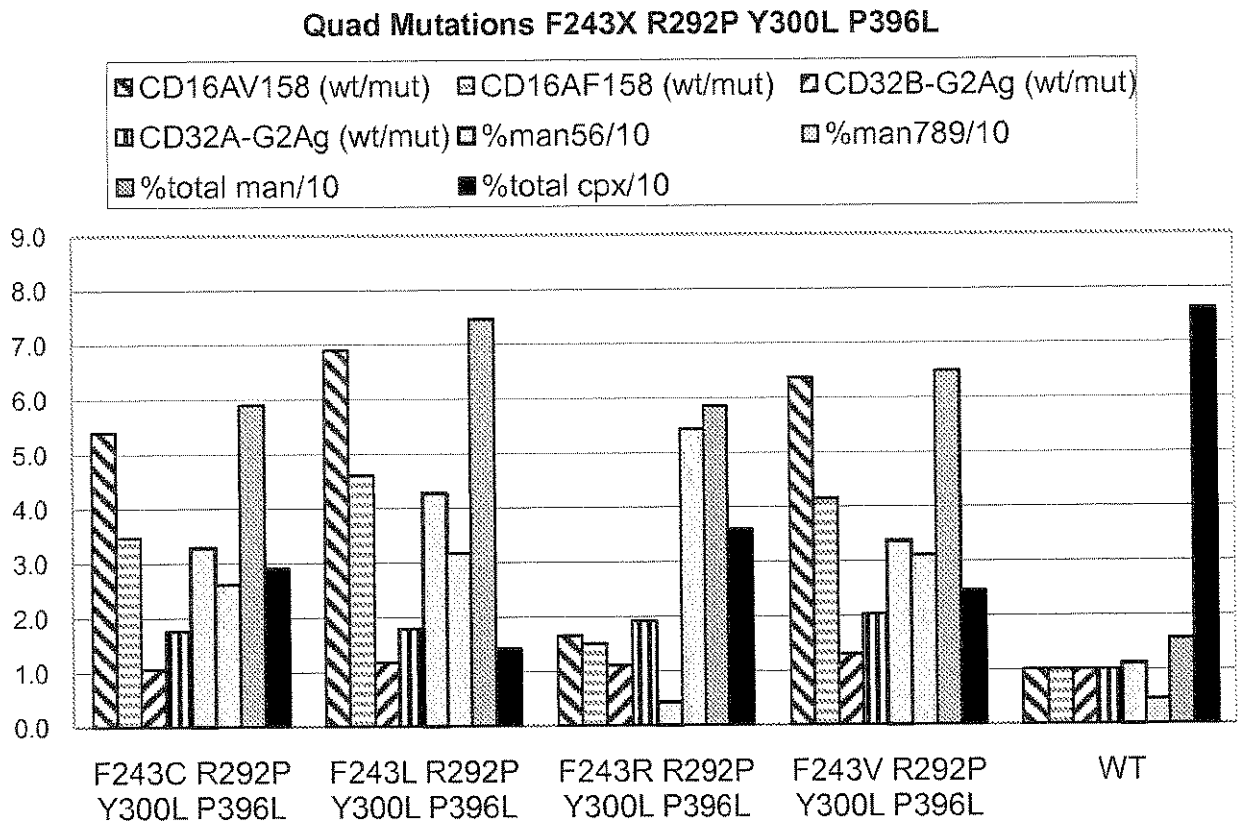

Quad Mutations F243X R292P Y300L P396L
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243C R292P Y300L P396L
F243L R292P Y300L P396L
F243R R292P Y300L P396L
F243V R292P Y300L P396L
WT

Figure 43

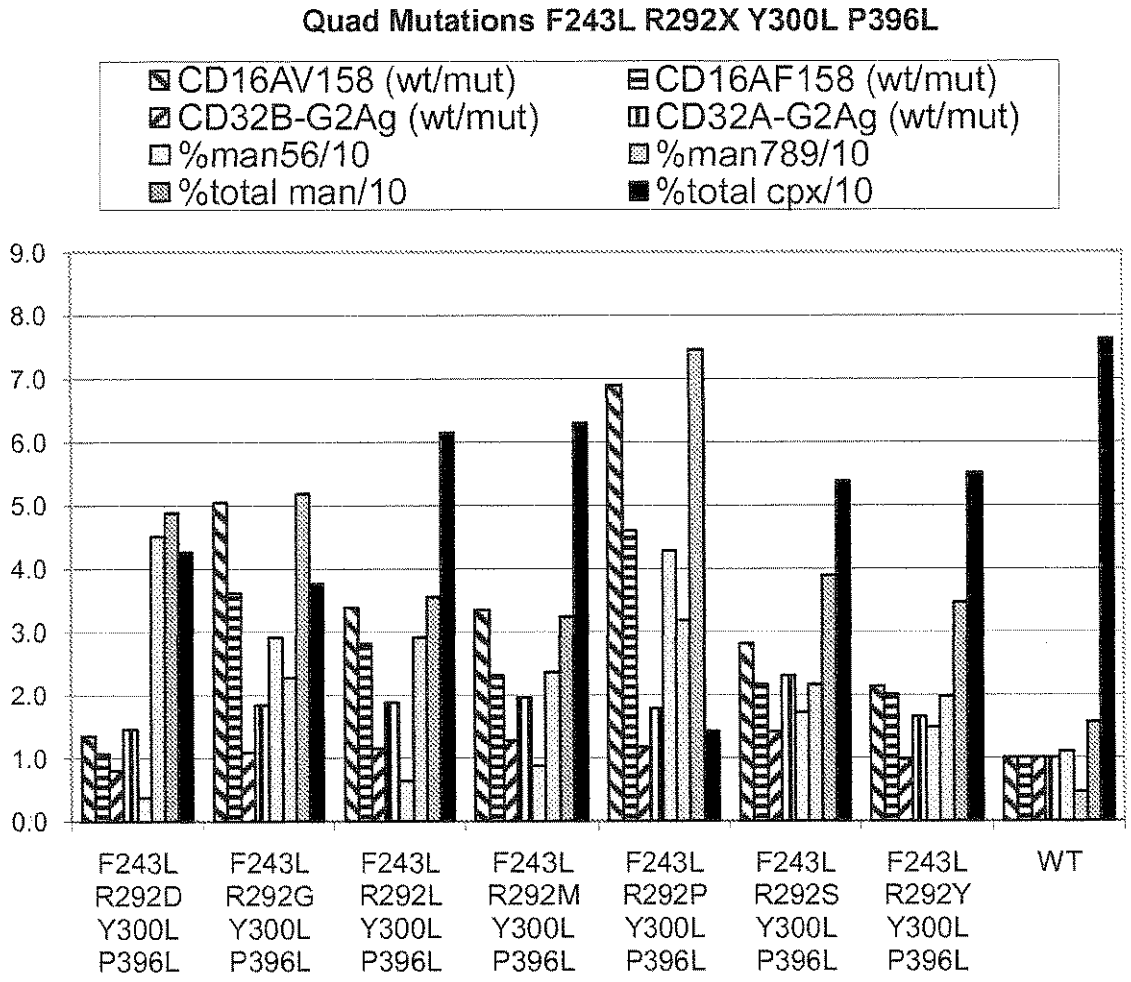

Quad Mutations F243L R292X Y300L P396L
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243L R292D Y300L P396L
F243L R292G Y300L P396L
F243L R292L Y300L P396L
F243L R292M Y300L P396L
F243L R292P Y300L P396L
F243L R292S Y300L P396L
F243L R292Y Y300L P396L
WT

Figure 44

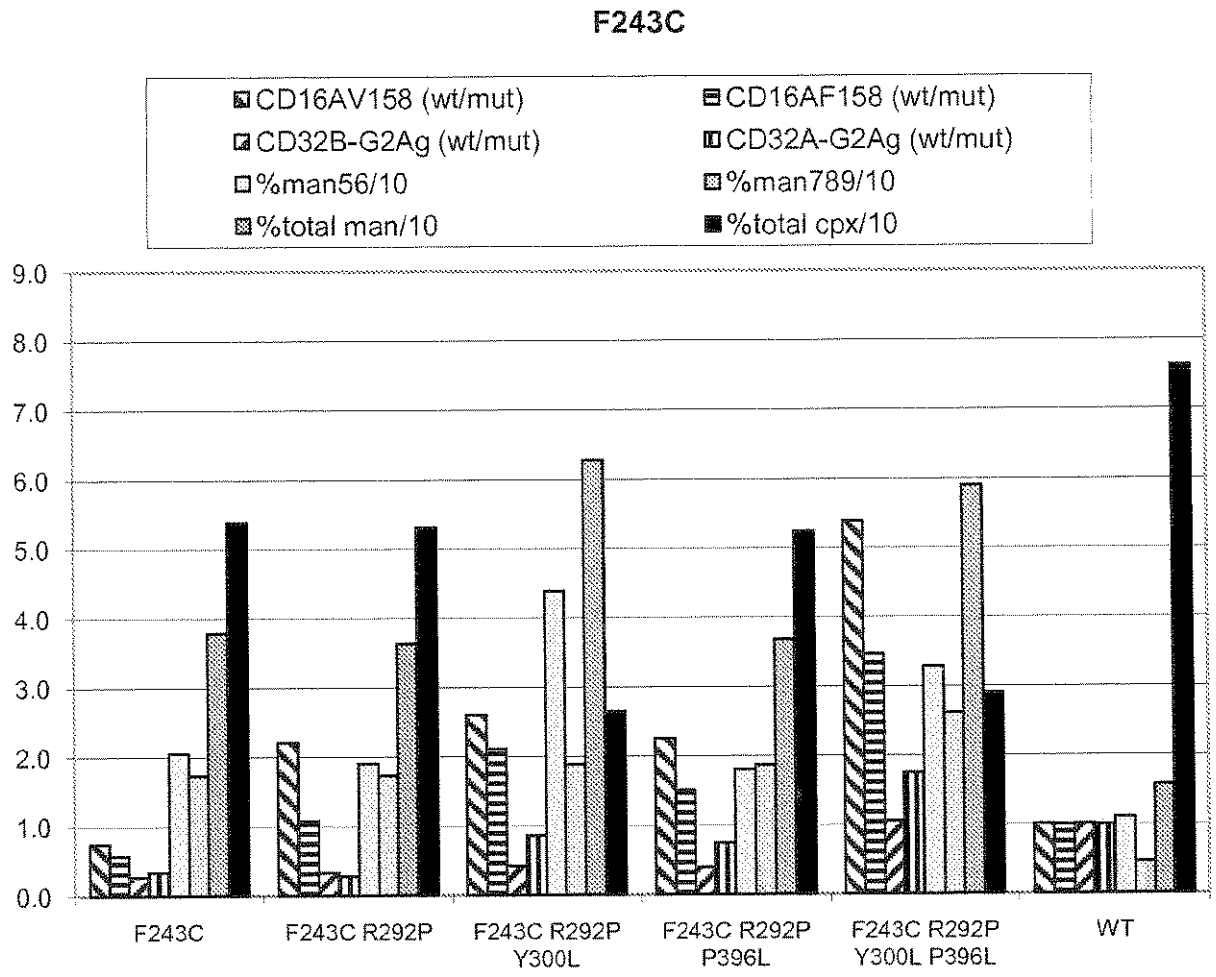

F243C
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243C
F243C R292P
F243C R292P Y300L
F243C R292P P396L
F243C R292P Y300L P396L
WT

Figure 45

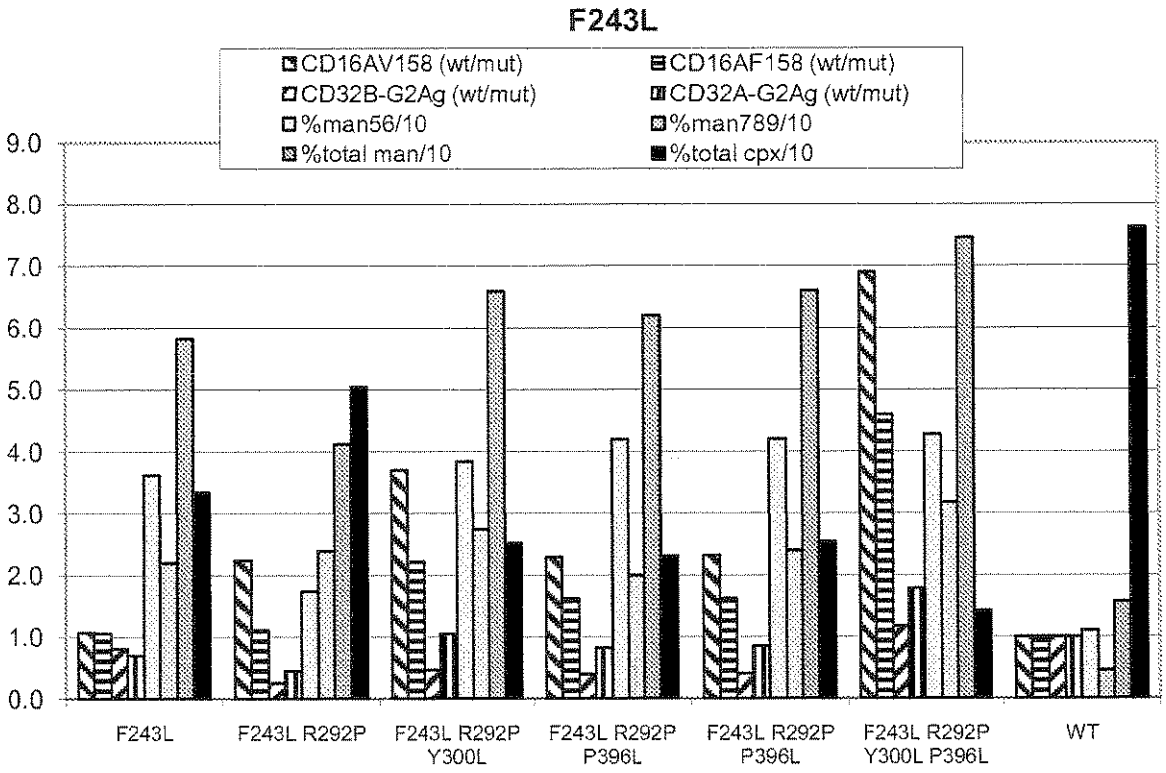

F243L
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243L
F243L R292P
F243L R292P Y300L
F243L R292P P396L
F243L R292P P396L
F243L R292P Y300L P396L
WT

Figure 46

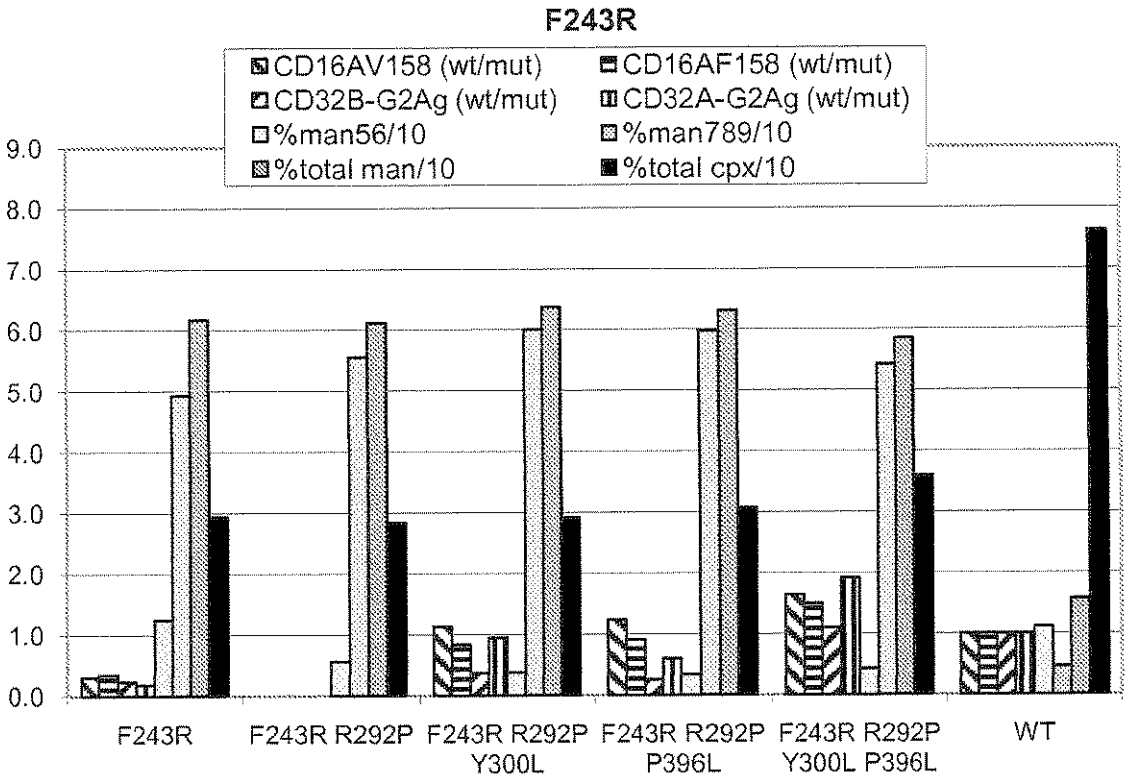

F243R
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243R
F243R R292P
F243R R292P Y300L
F243R R292P P396L
F243R R292P Y300L P396L
WT

Figure 47

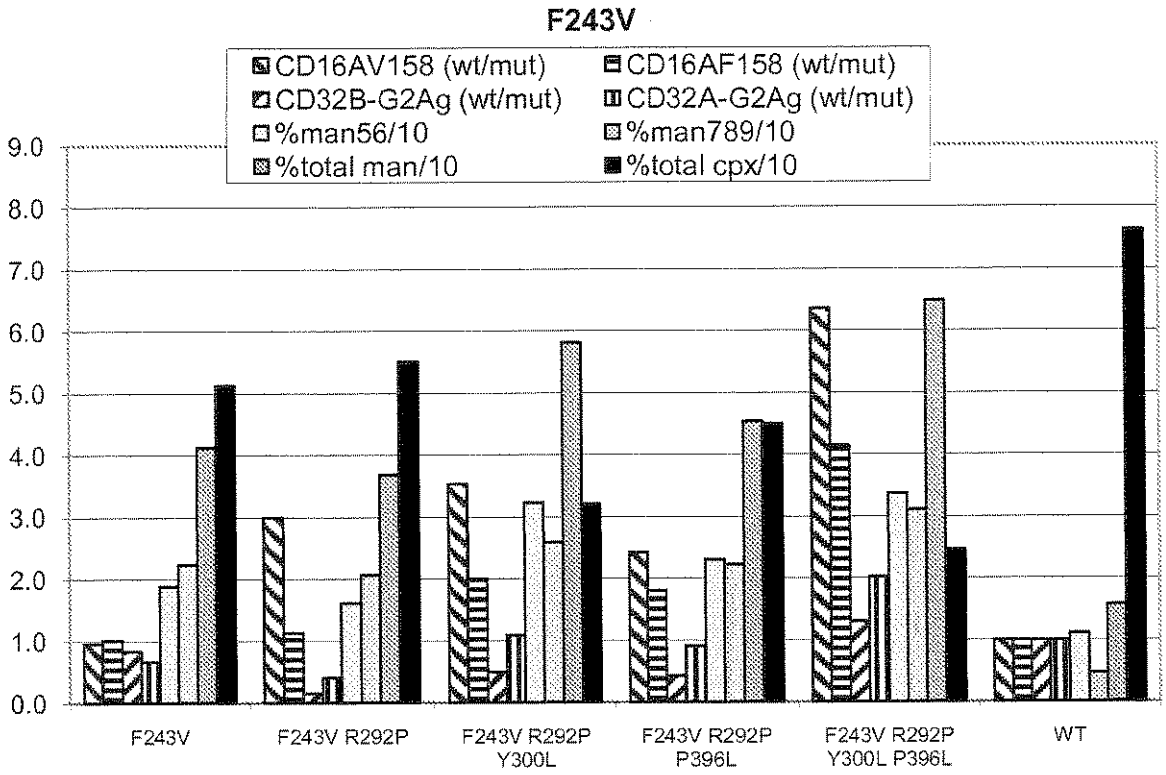

F243V
CD16AV158 (wt/mut)
CD16AF158 (wt/mut)
CD32B-G2Ag (wt/mut)
CD32A-G2Ag (wt/mut)
%man56/10
%man789/10
%total man/10
%total cpx/10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
F243V
F243V R292P
F243V R292P Y300L
F243V R292P P396L
F243V R292P Y300L P396L
WT

Figure 48