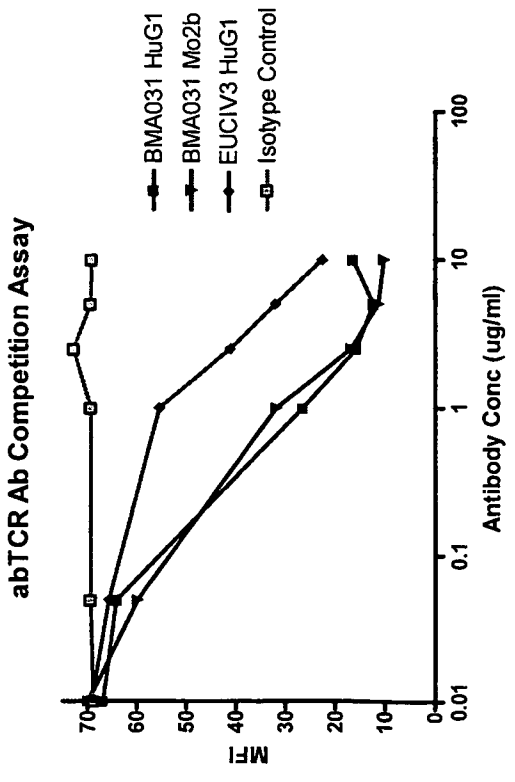

abTCR Ab Competition Assay
BMA031 HuG1
BMA031 Mo2b
EUCIV3 HuG1
Isotype Control
MFI
0
10
20
30
40
50
60
70
0.01
0.1
1
10
100
Antibody Conc (ug/ml)

Figure 1

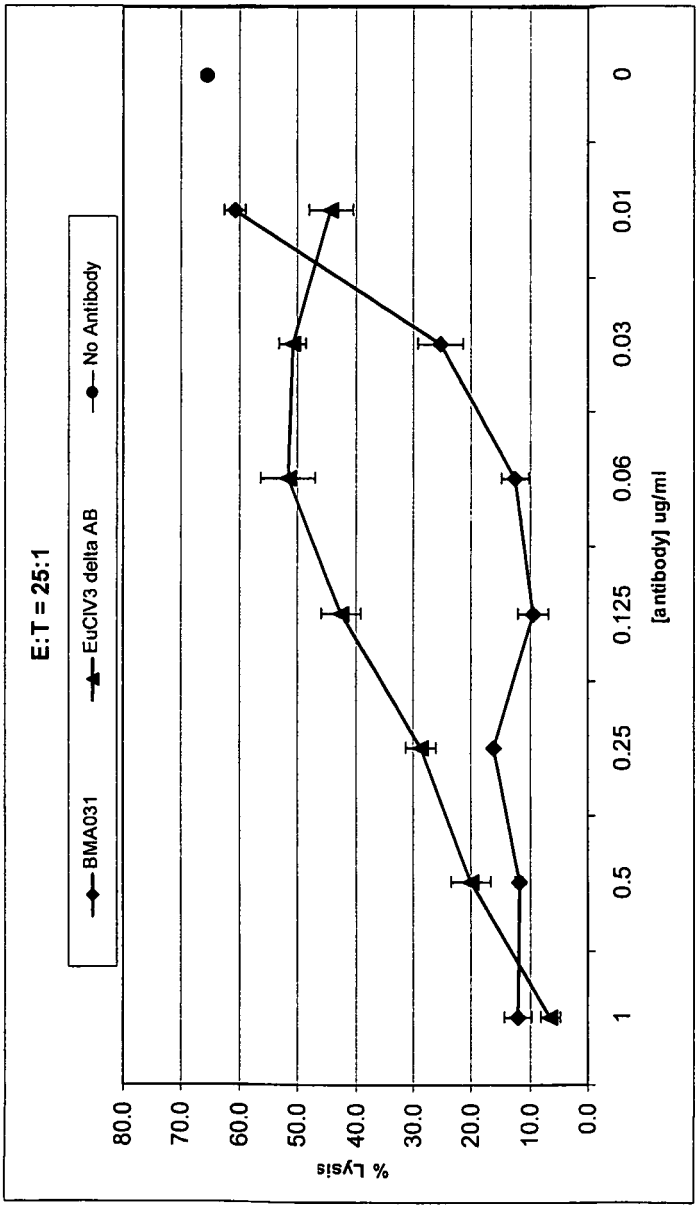

E:T = 25:1
BMA031
EuCIV3 delta AB
No Antibody
% Lysis
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
0.5
0.25
0.125
0.06
0.03
0.01
0
[antibody] ug/ml

Figure 2

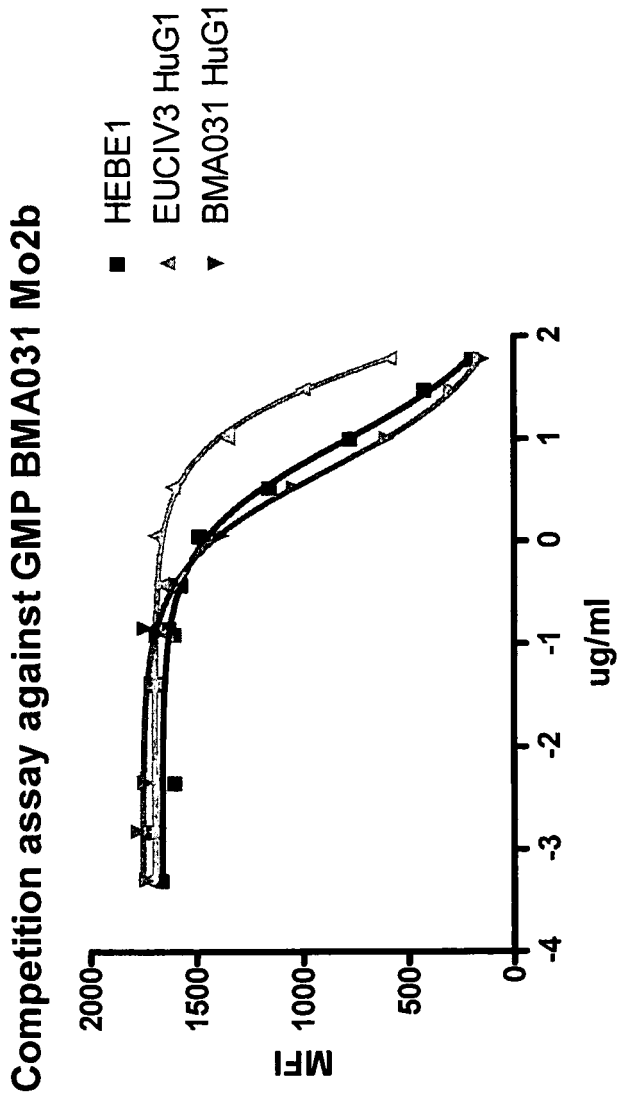

Competition assay against GMP BMA031 Mo2b
HEBE1
EUCIV3 HuG1
BMA031 HuG1
MFI
2000
1500
1000
500
0
-4
-3
-2
-1
0
1
2
ug/ml

Figure 3

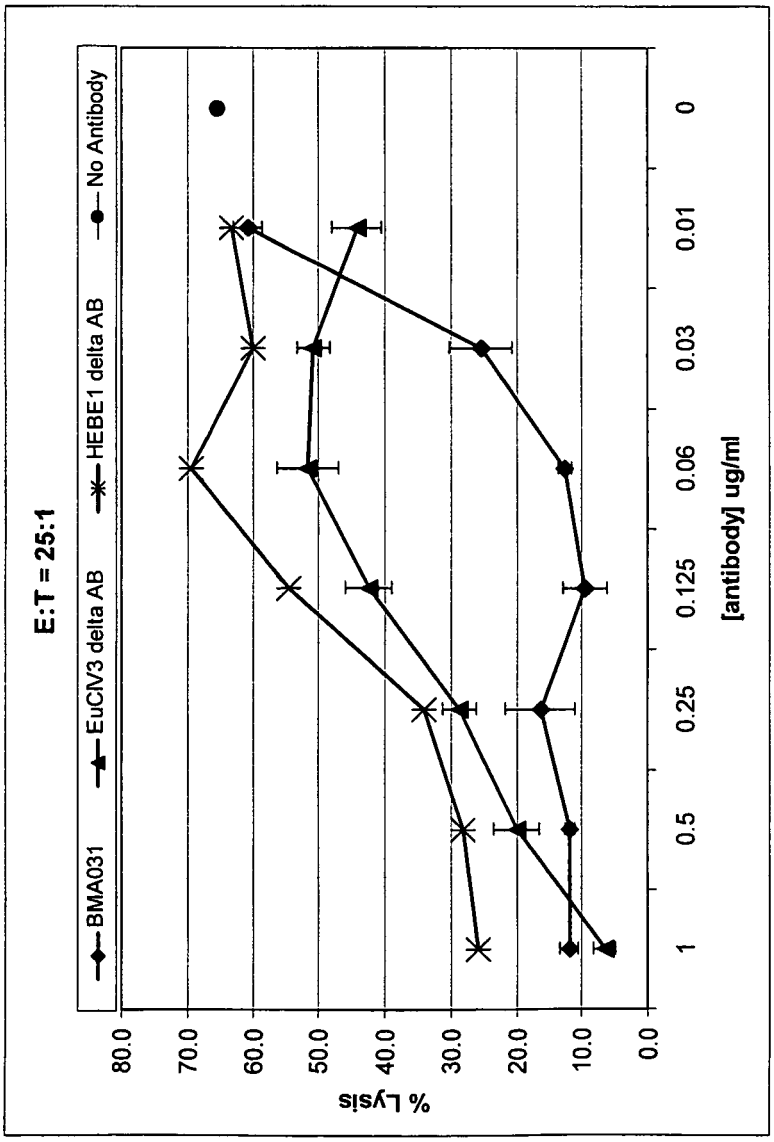

E:T = 25:1
BMA031
EuCIV3 delta AB
HEBE1 delta AB
No Antibody
% Lysis
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
0.5
0.25
0.125
0.06
0.03
0.01
0
[antibody] ug/ml

Figure 4

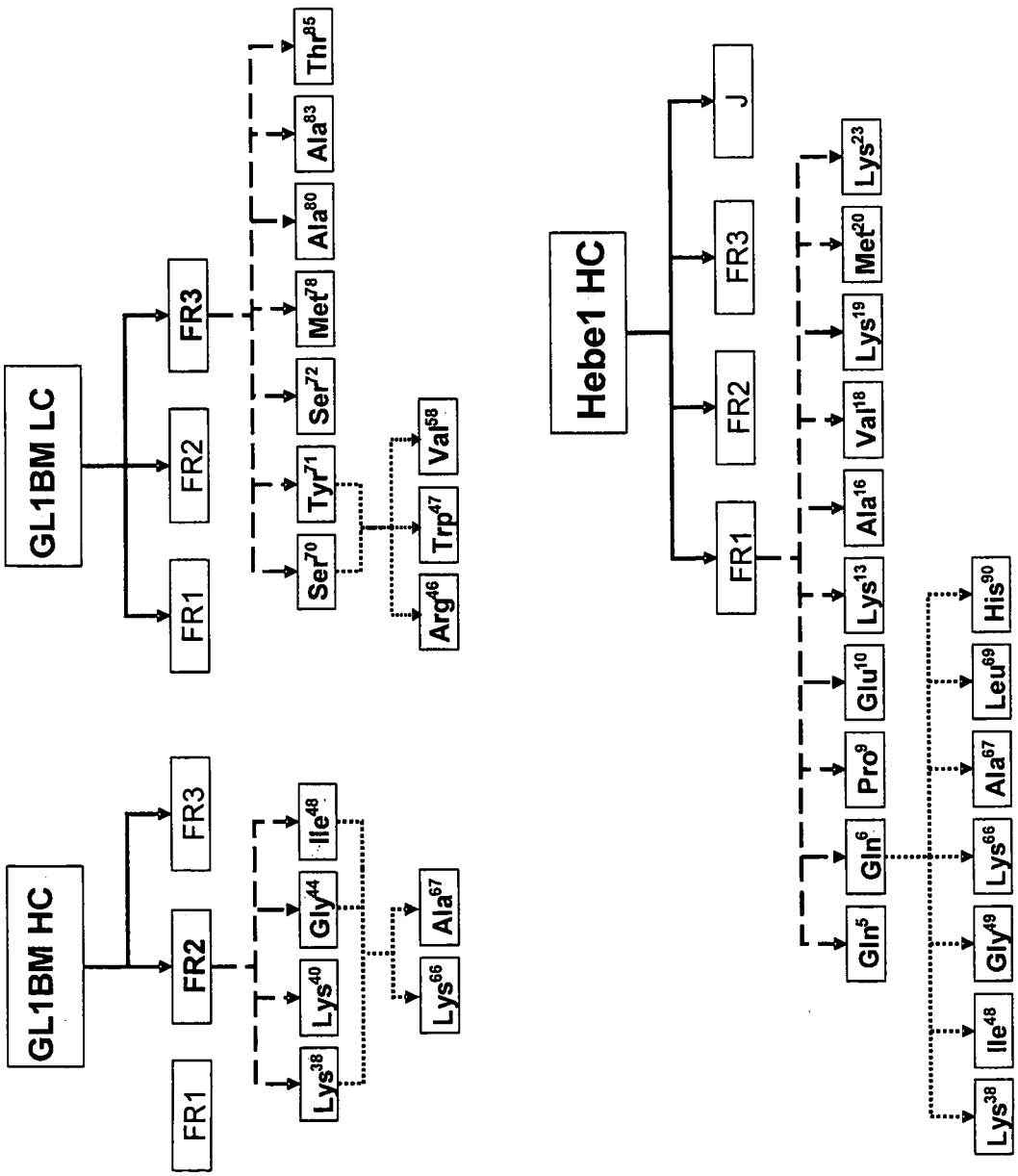

GL1BM HC
FR1
FR2
FR3
Lys38
Lys40
Gly44
Ile48
Lys66
Ala67
GL1BM LC
FR1
FR2
FR3
Ser70
Tyr71
Ser72
Met78
Ala80
Ala83
Thr85
Arg46
Trp47
Val58
Hebe1 HC
FR1
FR2
FR3
J
Gln5
Gln6
Pro9
Glu10
Lys13
Ala16
Val18
Lys19
Met20
Lys23
Lys38
Ile48
Gly49
Lys66
Ala67
Leu69
His90

Figure 5

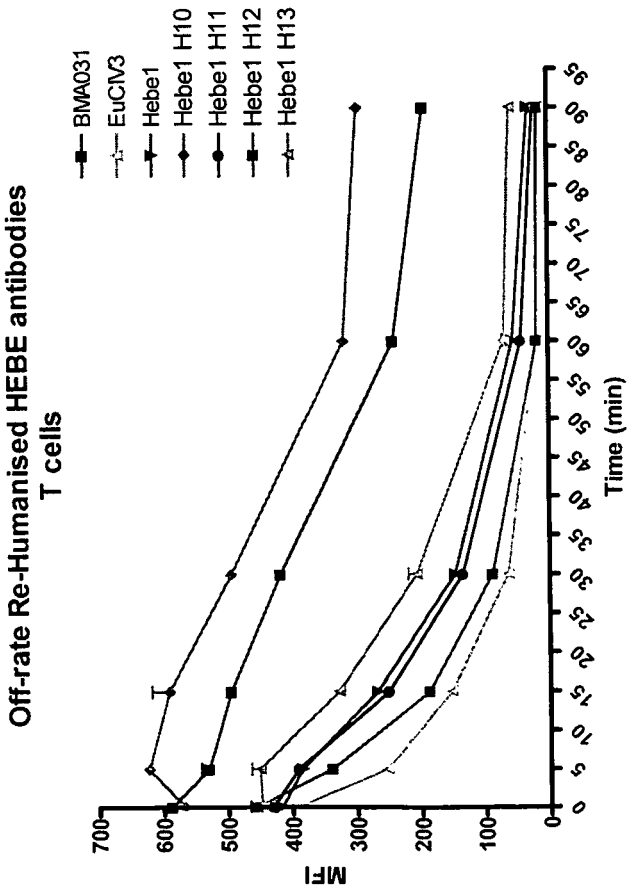

Off-rate Re-Humanised HEBE antibodies
T cells
BMA031
EuCIV3
Hebe1
Hebe1 H10
Hebe1 H11
Hebe1 H12
Hebe1 H13
MFI
Time (min)

Figure 6

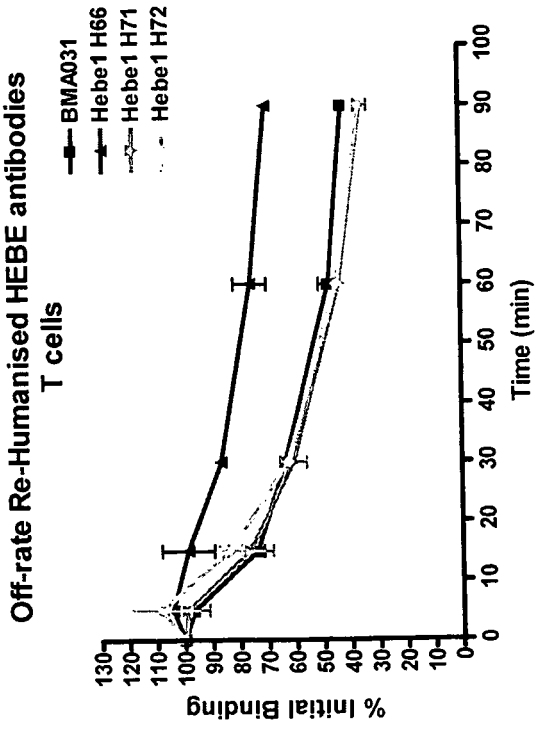

Off-rate Re-Humanised HEBE antibodies
T cells
BMA031
Hebe1 H66
Hebe1 H71
Hebe1 H72
% Initial Binding
Time (min)
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100

Figure 7

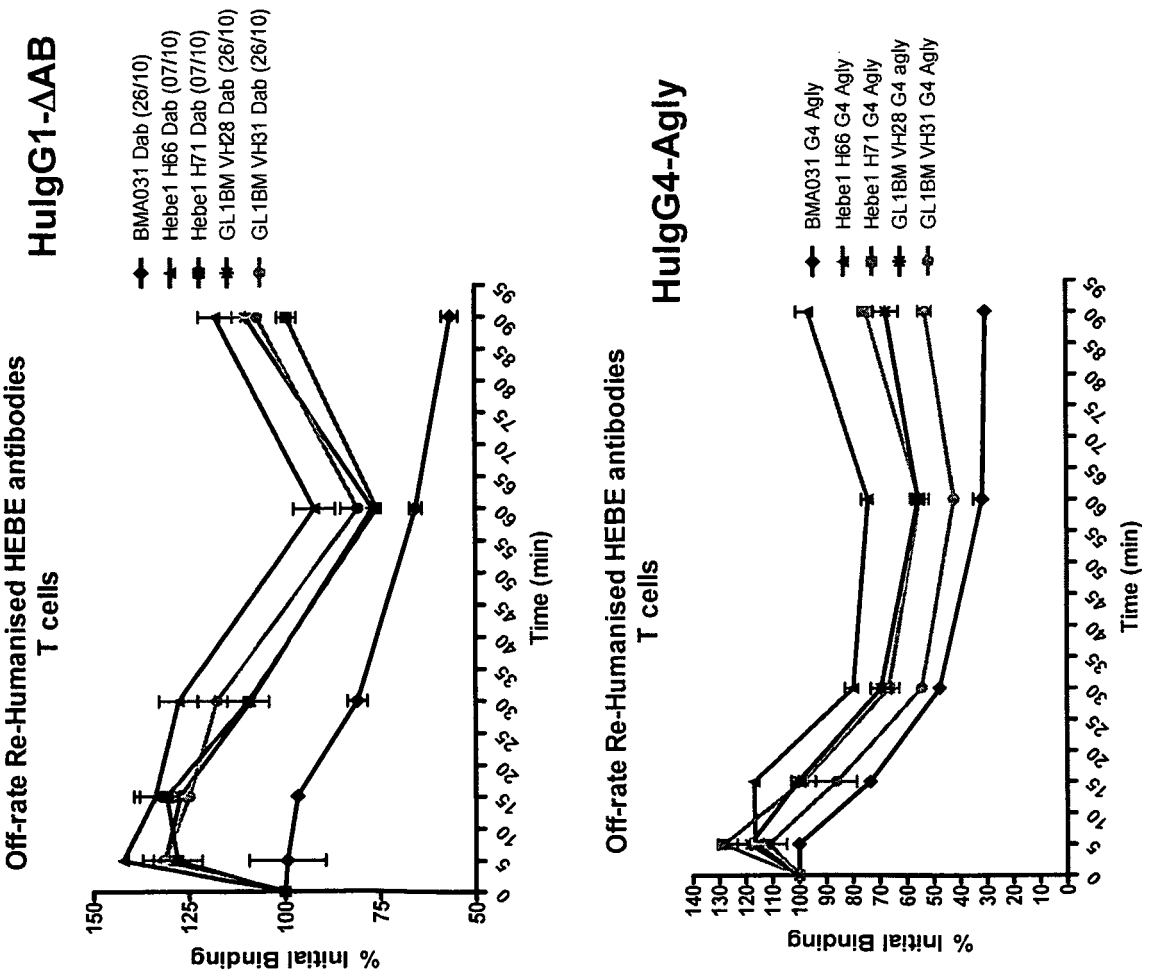

Off-rate Re-Humanised HEBE antibodies
T cells
HuIgG1-ΔAB
BMA031 Dab (26/10)
Hebe1 H66 Dab (07/10)
Hebe1 H71 Dab (07/10)
GL1BM VH28 Dab (26/10)
GL1BM VH31 Dab (26/10)
% Initial Binding
Time (min)
Off-rate Re-Humanised HEBE antibodies
T cells
HuIgG4-Agly
BMA031 G4 Agly
Hebe1 H66 G4 Agly
Hebe1 H71 G4 Agly
GL1BM VH28 G4 agly
GL1BM VH31 G4 Agly
% Initial Binding
Time (min)

Figure 8

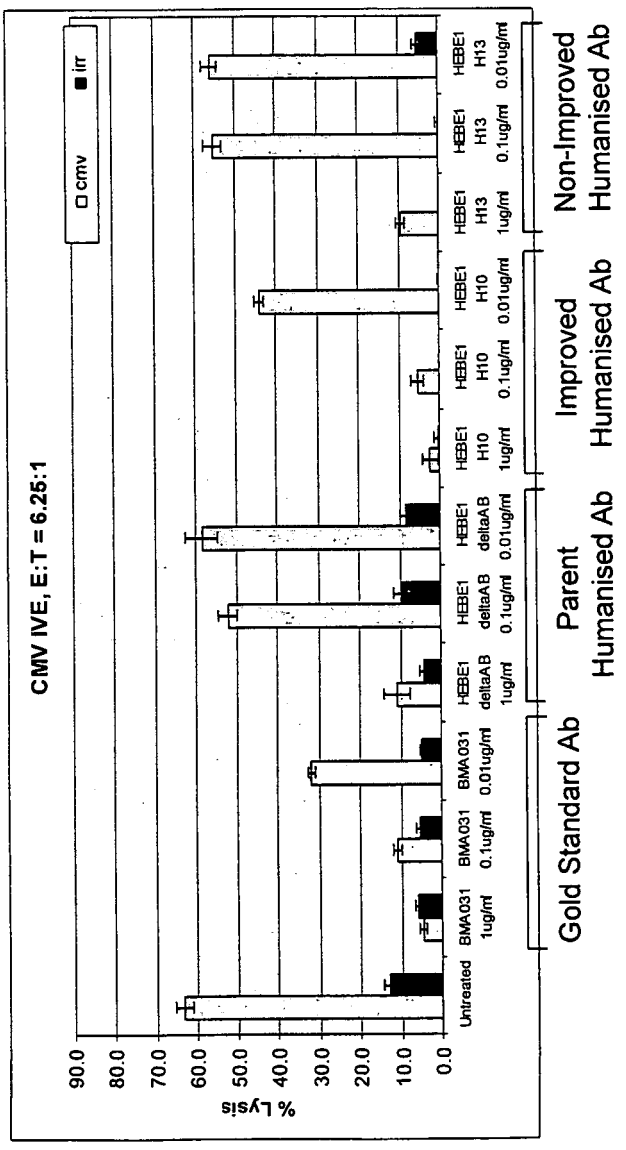

CMV IVE, E:T = 6.25:1
cmv
irr
% Lysis
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Untreated
BMA031 1ug/ml
BMA031 0.1ug/ml
BMA031 0.01ug/ml
HEBE1 deltaAB 1ug/ml
HEBE1 deltaAB 0.1ug/ml
HEBE1 deltaAB 0.01ug/ml
HEBE1 H10 1ug/ml
HEBE1 H10 0.1ug/ml
HEBE1 H10 0.01ug/ml
HEBE1 H13 1ug/ml
HEBE1 H13 0.1ug/ml
HEBE1 H13 0.01ug/ml
Gold Standard Ab
Parent Humanised Ab
Improved Humanised Ab
Non-Improved Humanised Ab

Figure 9

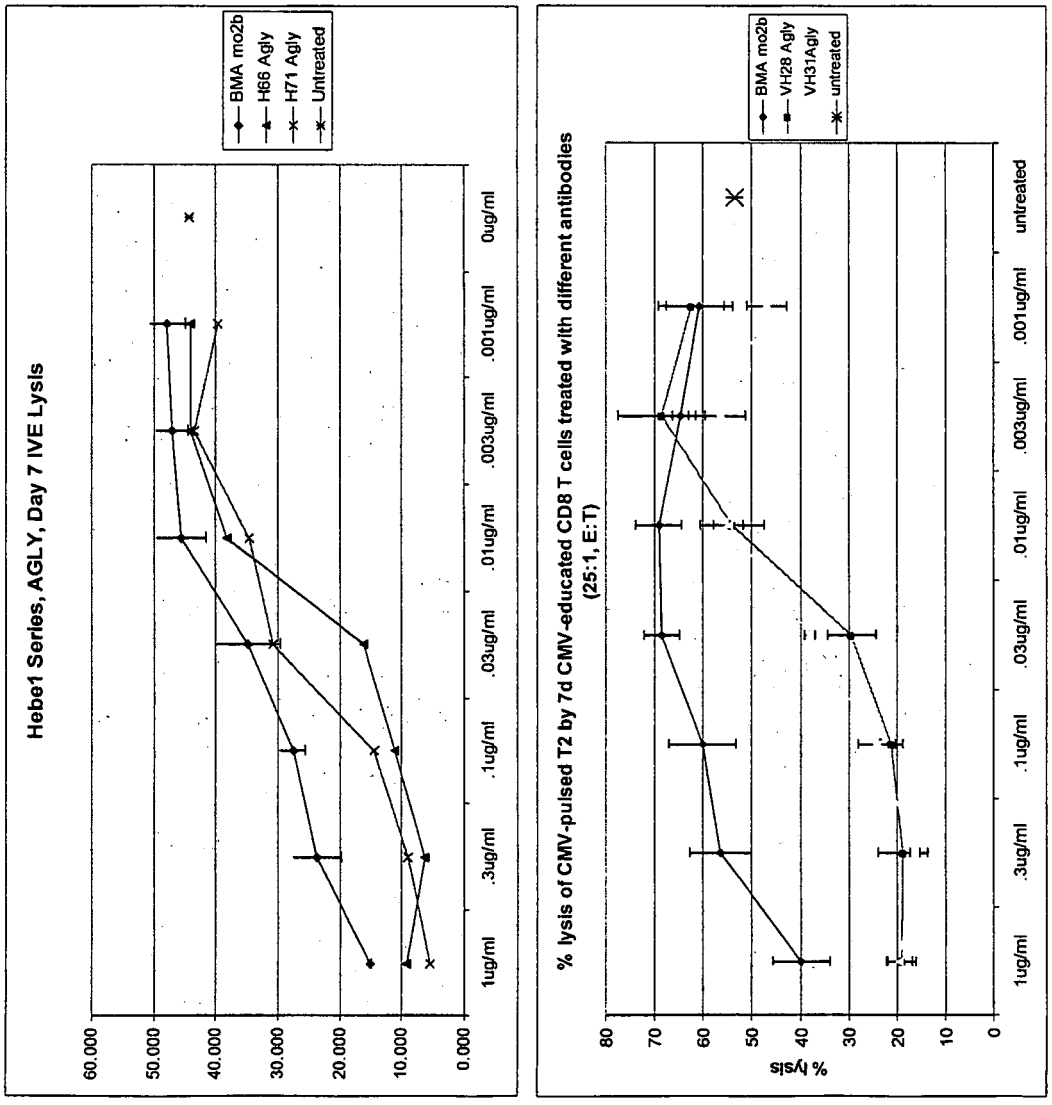

Hebe1 Series, AGLY, Day 7 IVE Lysis
BMA mo2b
H66 Agly
H71 Agly
Untreated
60.000
50.000
40.000
30.000
20.000
10.000
0.000
1ug/ml
.3ug/ml
.1ug/ml
.03ug/ml
.01ug/ml
.003ug/ml
.001ug/ml
0ug/ml
% lysis of CMV-pulsed T2 by 7d CMV-educated CD8 T cells treated with different antibodies (25:1, E:T)
BMA mo2b
VH28 Agly
VH31Agly
untreated
% lysis
80
70
60
50
40
30
20
10
0
1ug/ml
.3ug/ml
.1ug/ml
.03ug/ml
.01ug/ml
.003ug/ml
.001ug/ml
untreated

Figure 10

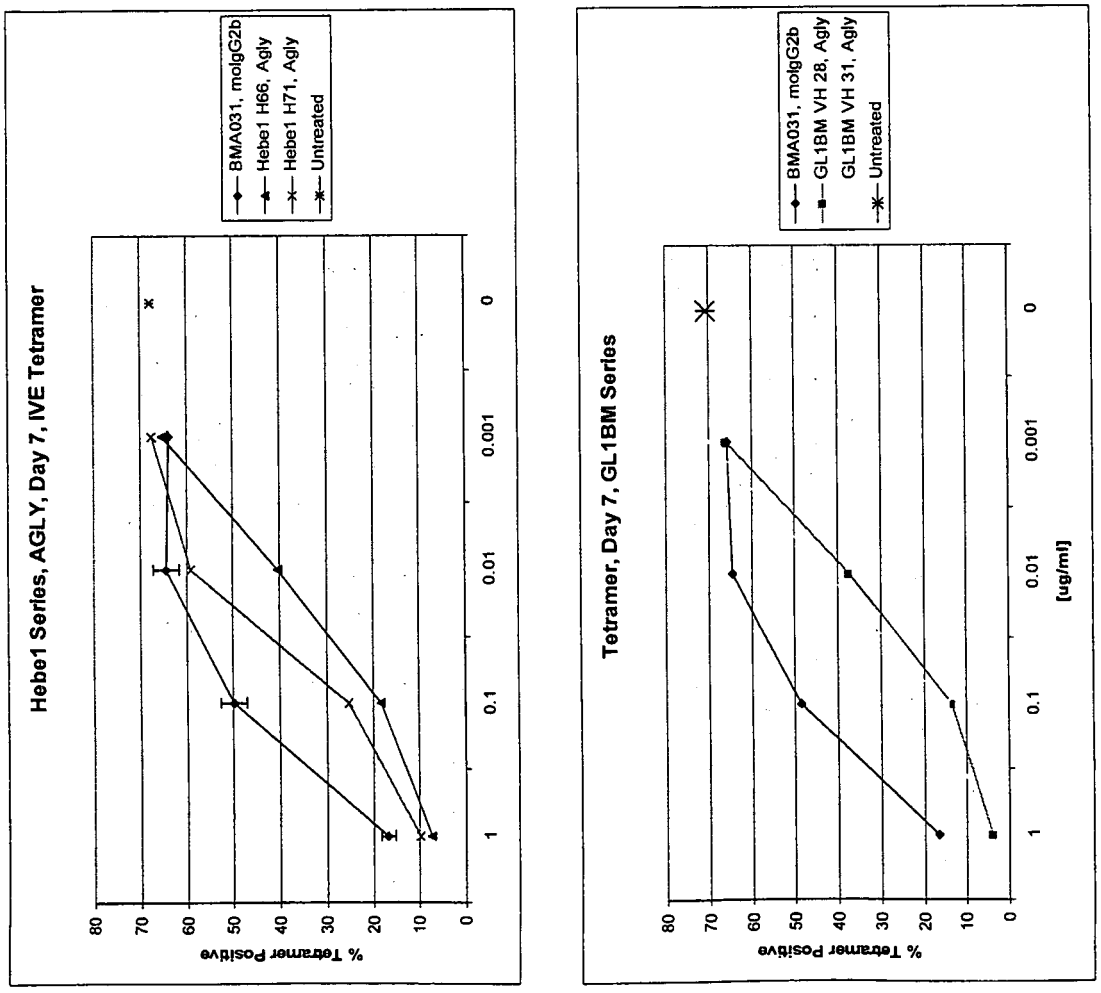

Hebe1 Series, AGLY, Day 7, IVE Tetramer
BMA031, molgG2b
Hebe1 H66, Agly
Hebe1 H71, Agly
Untreated
% Tetramer Positive
80
70
60
50
40
30
20
10
0
1
0.1
0.01
0.001
0
Tetramer, Day 7, GL1BM Series
BMA031, molgG2b
GL1BM VH 28, Agly
GL1BM VH 31, Agly
Untreated
% Tetramer Positive
[ug/ml]

Figure 11

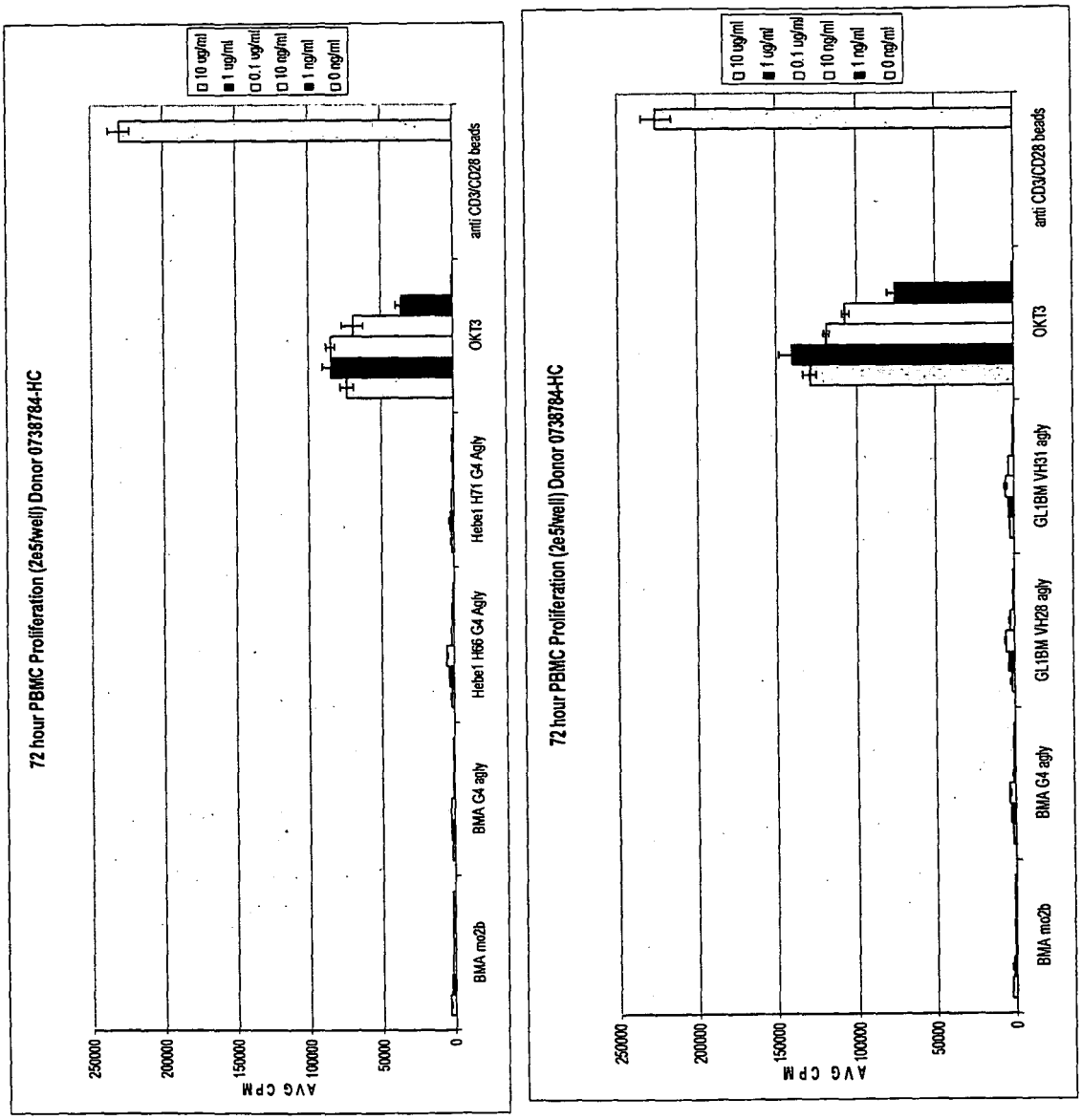

72 hour PBMC Proliferation (2e5/well) Donor 0738784-HC
10 ug/ml
1 ug/ml
0.1 ug/ml
10 ng/ml
1 ng/ml
0 ng/ml
AVG CPM
250000
200000
150000
100000
50000
0
BMA mo2b
BMA G4 agly
Hebe1 H66 G4 Agly
Hebe1 H71 G4 Agly
OKT3
anti CD3/CD28 beads
72 hour PBMC Proliferation (2e5/well) Donor 0738784-HC
10 ug/ml
1 ug/ml
0.1 ug/ml
10 ng/ml
1 ng/ml
0 ng/ml
AVG CPM
250000
200000
150000
100000
50000
0
BMA mo2b
BMA G4 agly
GL1BM VH28 agly
GL1BM VH31 agly
OKT3
anti CD3/CD28 beads

Figure 12

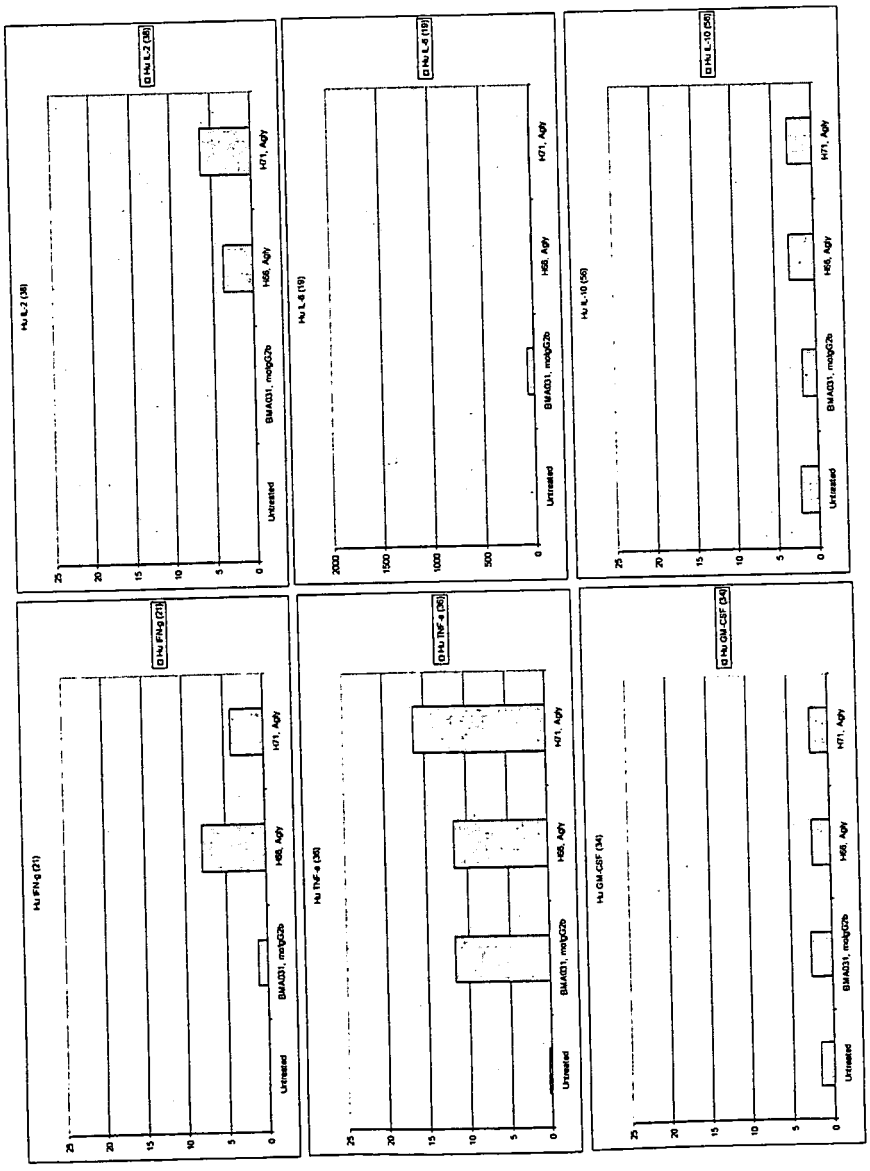
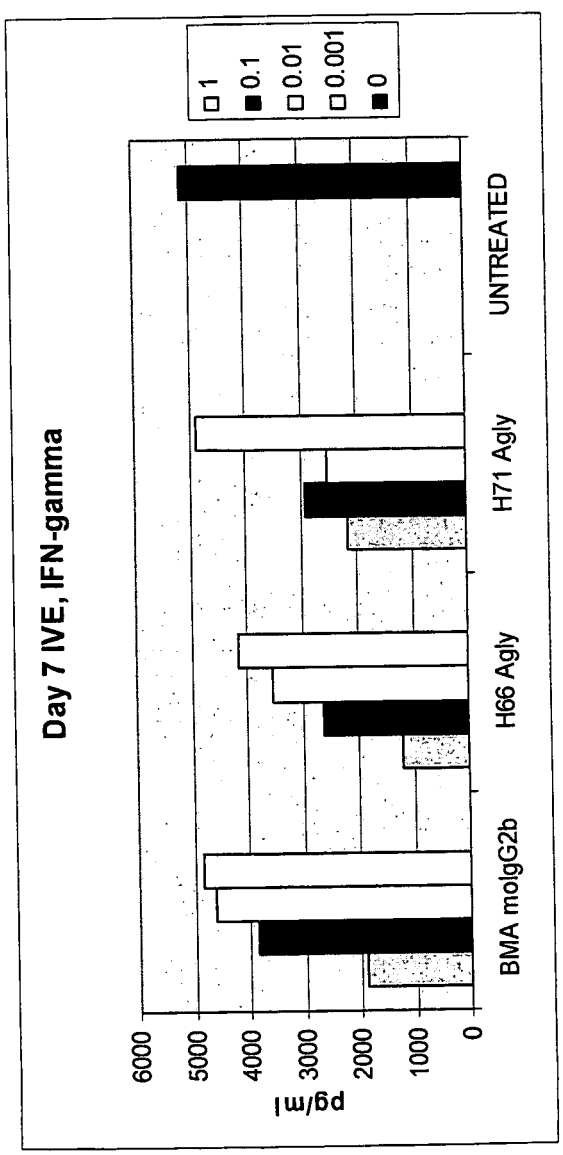

Hu IL-2 (38)
Hu IL-6 (19)
Hu IL-10 (56)
Hu IFN-g (21)
Hu TNF-a (36)
Hu GM-CSF (34)
Untreated
BMA031, mutG2b
H66, Agly
H71, Agly

Figure 13


Day 7 IVE, IFN-gamma
1
0.1
0.01
0.001
0
pg/ml
6000
5000
4000
3000
2000
1000
0
BMA molgG2b
H66 Agly
H71 Agly
UNTREATED

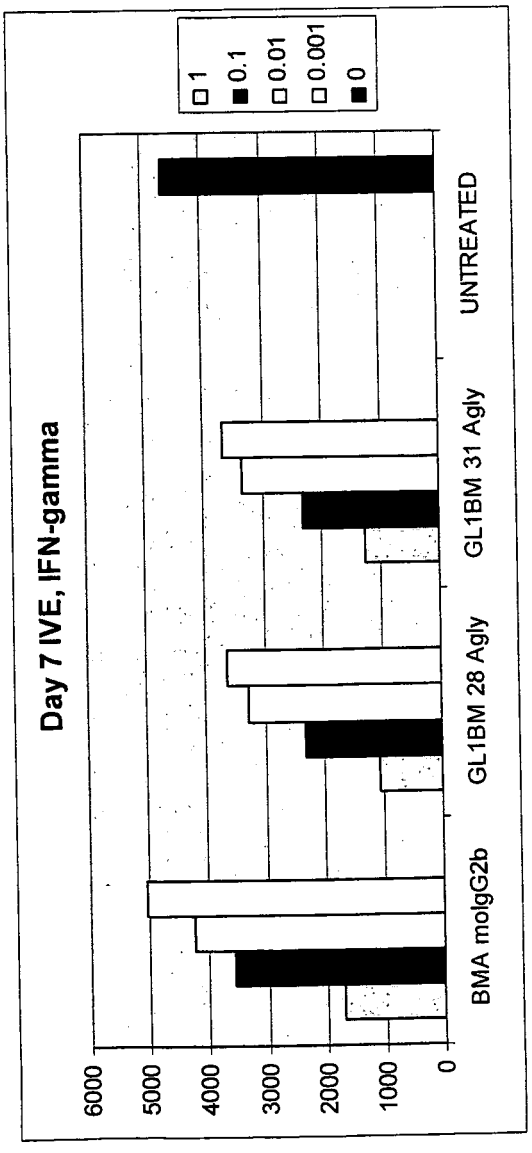

Day 7 IVE, IFN-gamma
1
0.1
0.01
0.001
0
6000
5000
4000
3000
2000
1000
0
BMA molgG2b
GL1BM 28 Agly
GL1BM 31 Agly
UNTREATED

Figure 14

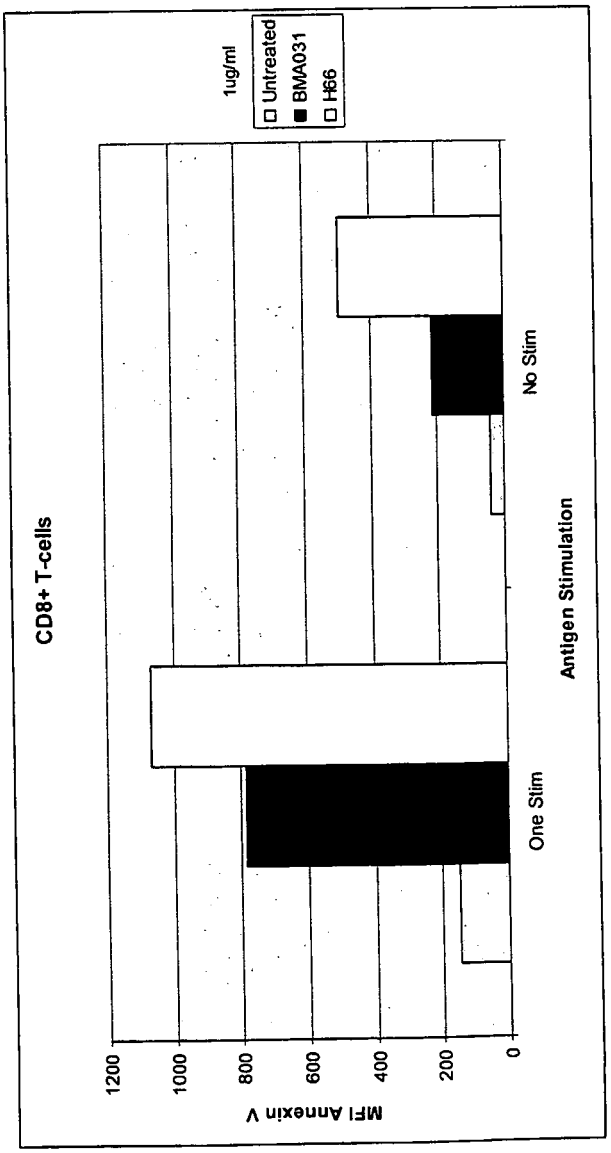

CD8+ T-cells
1ug/ml
Untreated
BMA031
H56
MFI Annexin V
1200
1000
800
600
400
200
0
No Stim
One Stim
Antigen Stimulation

Figure 15

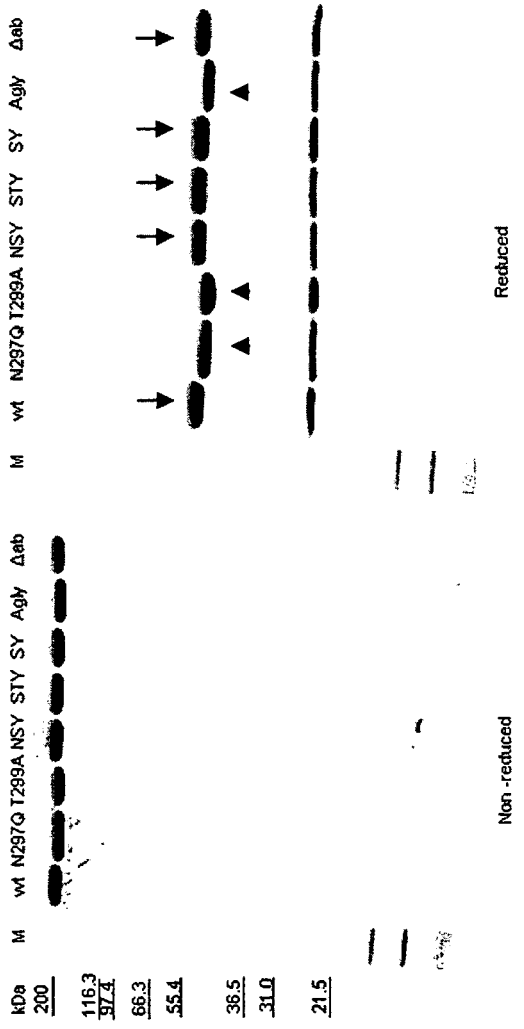

kDa
200
116.3
97.4
66.3
55.4
36.5
31.0
21.5
M
wt
N297Q
T299A
NSY
STY
SY
Agly
Δab
Non -reduced
Reduced

Figure 16

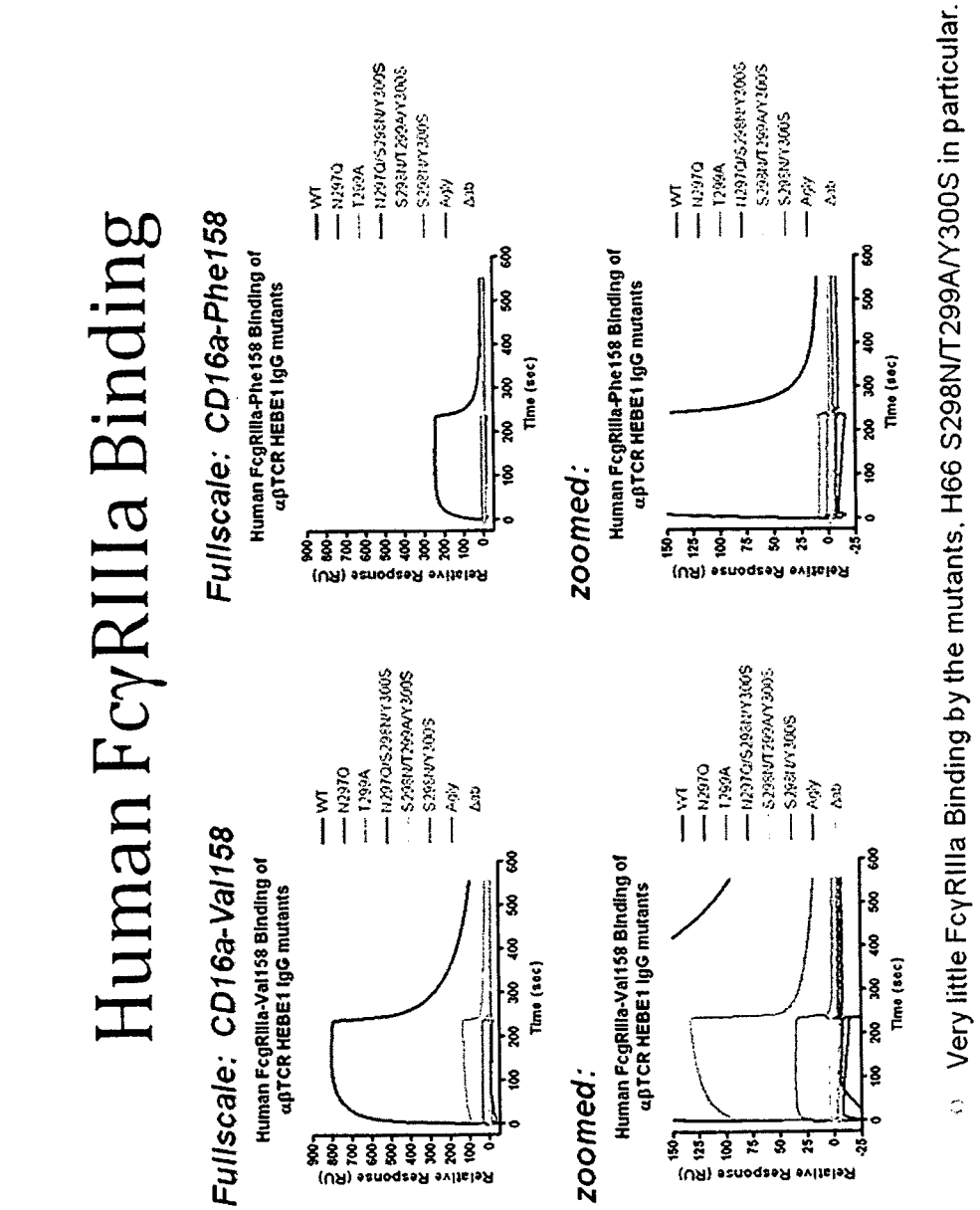
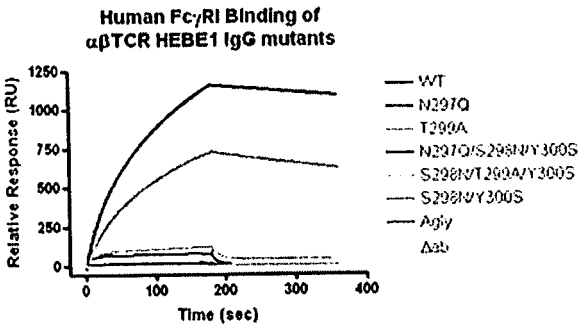


Figure 17

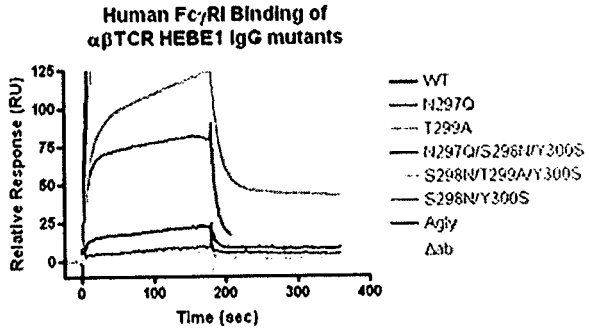
) Very little FcγRIIIa Binding by the mutants, H66 S298N/T299A/Y300S in particular.

Human FcγRI Binding

Fullscale:



zoomed in:



- Very little FcγRI Binding by the mutants, H66 S298N/T299A/Y300S in particular.

Figure 18

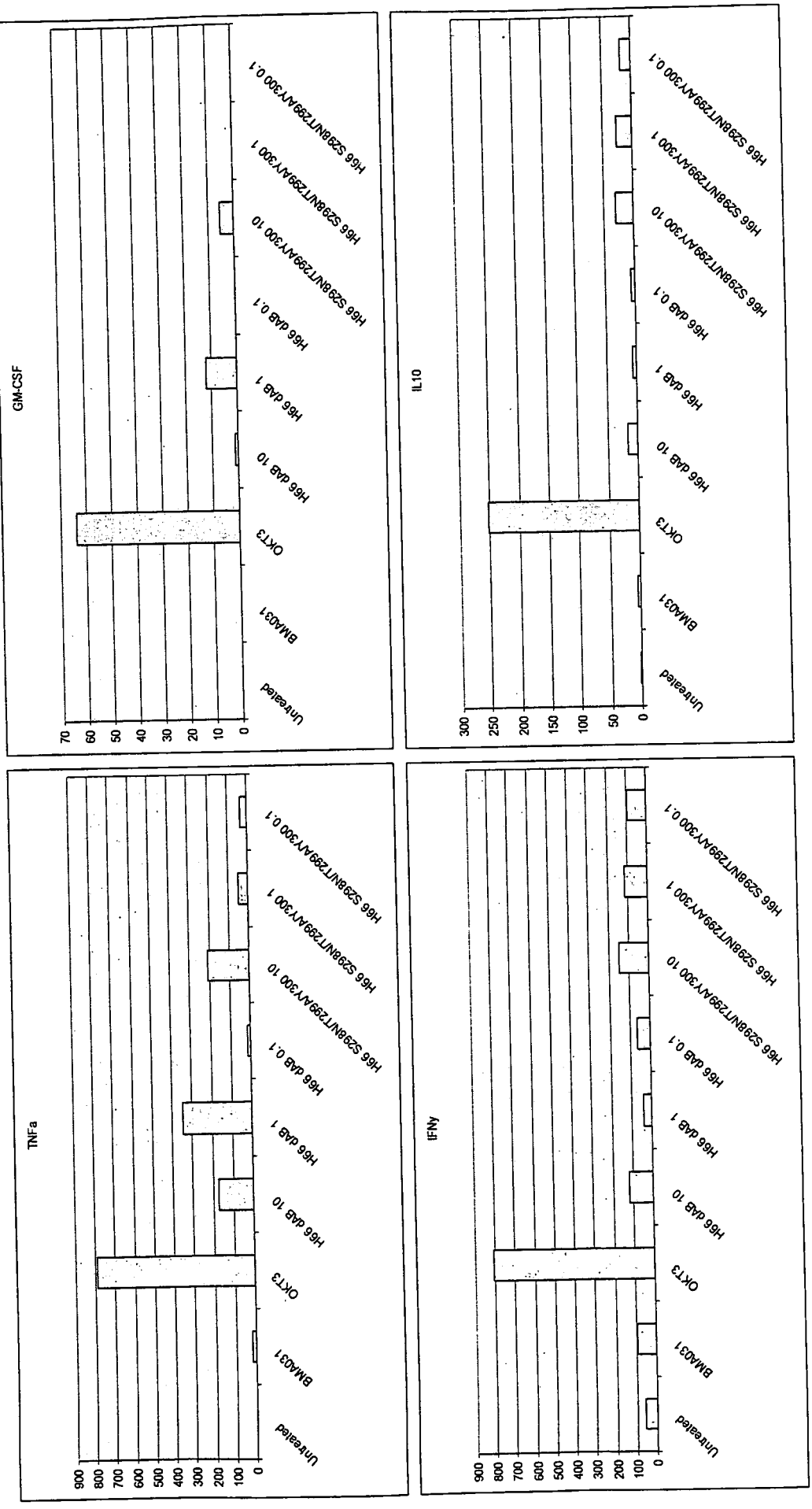

GM-CSF
0
10
20
30
40
50
60
70
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298NT299AV300 10
H66 S298NT299AV300 1
H66 S298NT299AV300 0,1
IL10
0
50
100
150
200
250
300
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298NT299AV300 10
H66 S298NT299AV300 1
H66 S298NT299AV300 0,1
TNFa
0
100
200
300
400
500
600
700
800
900
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298NT299AV300 10
H66 S298NT299AV300 1
H66 S298NT299AV300 0,1
IFNγ
0
100
200
300
400
500
600
700
800
900
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298NT299AV300 10
H66 S298NT299AV300 1
H66 S298NT299AV300 0,1

Figure 19

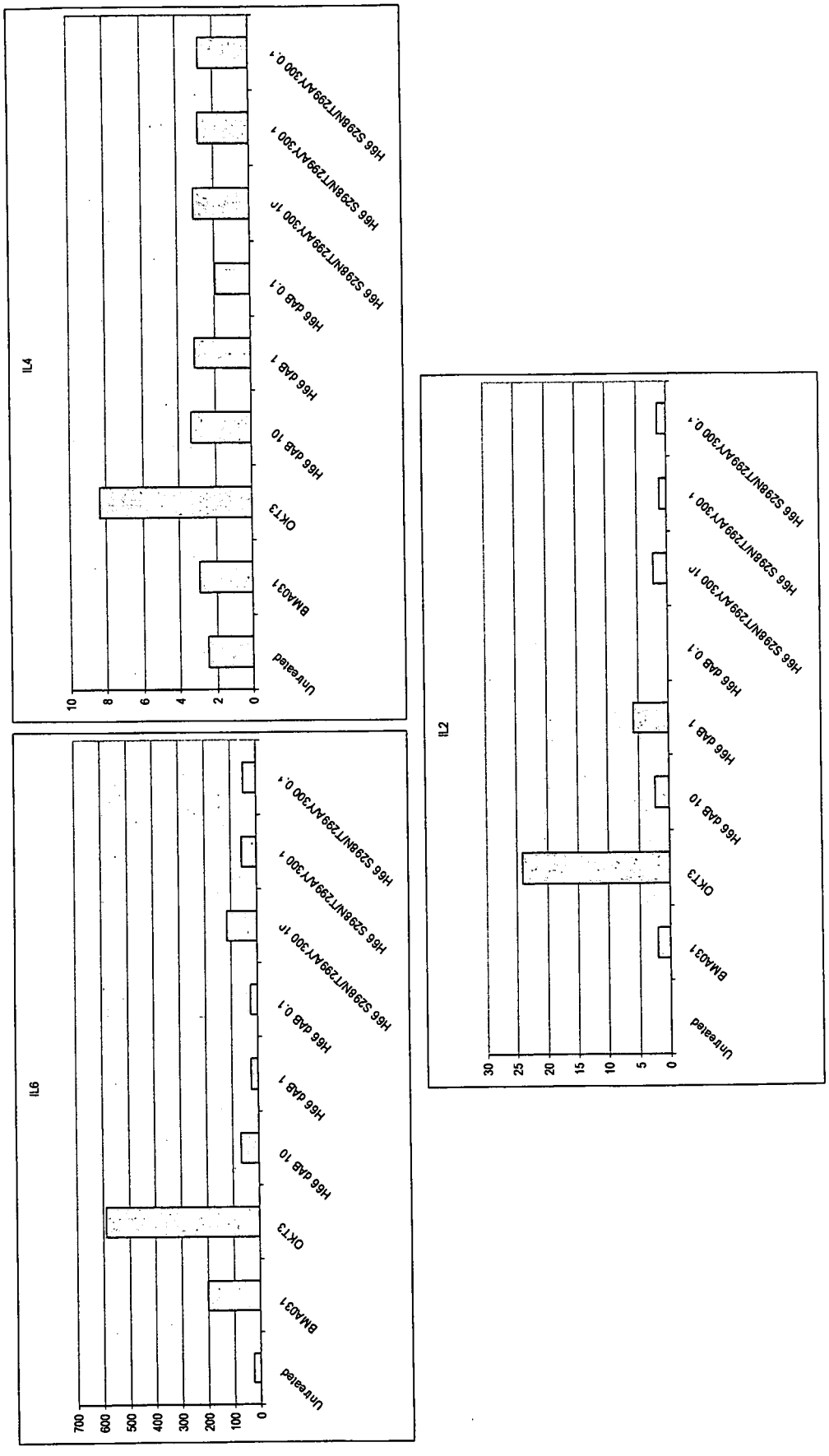

IL4
10
8
6
4
2
0
H66 S298N/T299A/Y300 0,1
H66 S298N/T299A/Y300 1
H66 S298N/T299A/Y300 1r
H66 dAb 0,1
H66 dAb 1
H66 dAb 10
OKT3
BMA031
untreated
IL2
30
25
20
15
10
5
0
H66 S298N/T299A/Y300 0,1
H66 S298N/T299A/Y300 1
H66 S298N/T299A/Y300 1r
H66 dAb 0,1
H66 dAb 1
H66 dAb 10
OKT3
BMA031
untreated
IL6
700
600
500
400
300
200
100
0
H66 S298N/T299A/Y300 0,1
H66 S298N/T299A/Y300 1
H66 S298N/T299A/Y300 1r
H66 dAb 0,1
H66 dAb 1
H66 dAb 10
OKT3
BMA031
untreated

Figure 20

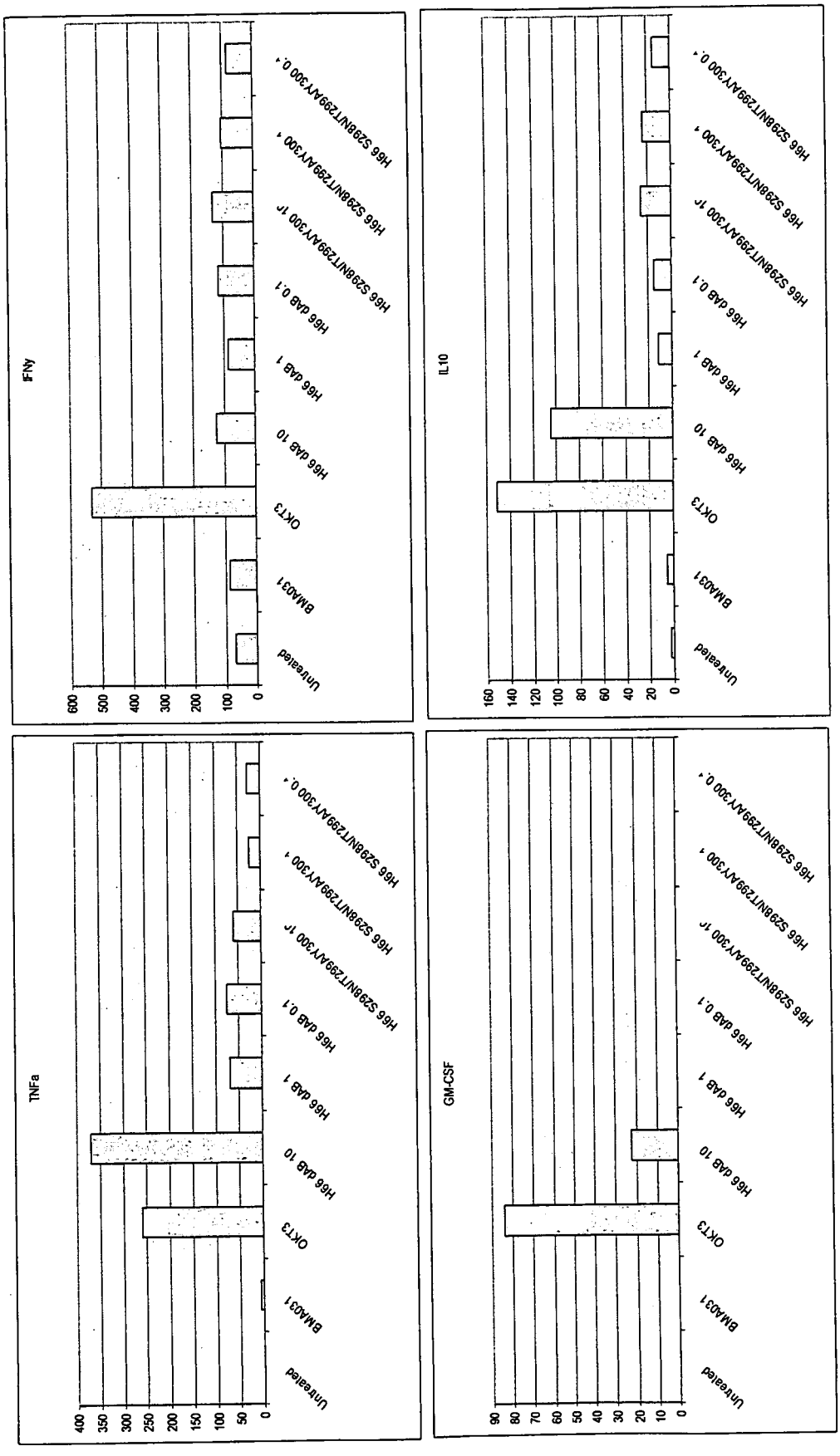

IFNγ
IL10
TNFa
GM-CSF
Untreated
BMA031
OKT3
H66 dAb 10
H66 dAb 1
H66 dAb 0.1
H66 S298NT299AY300 1F
H66 S298NT299AY300 1
H66 S298NT299AY300 0.1

Figure 21

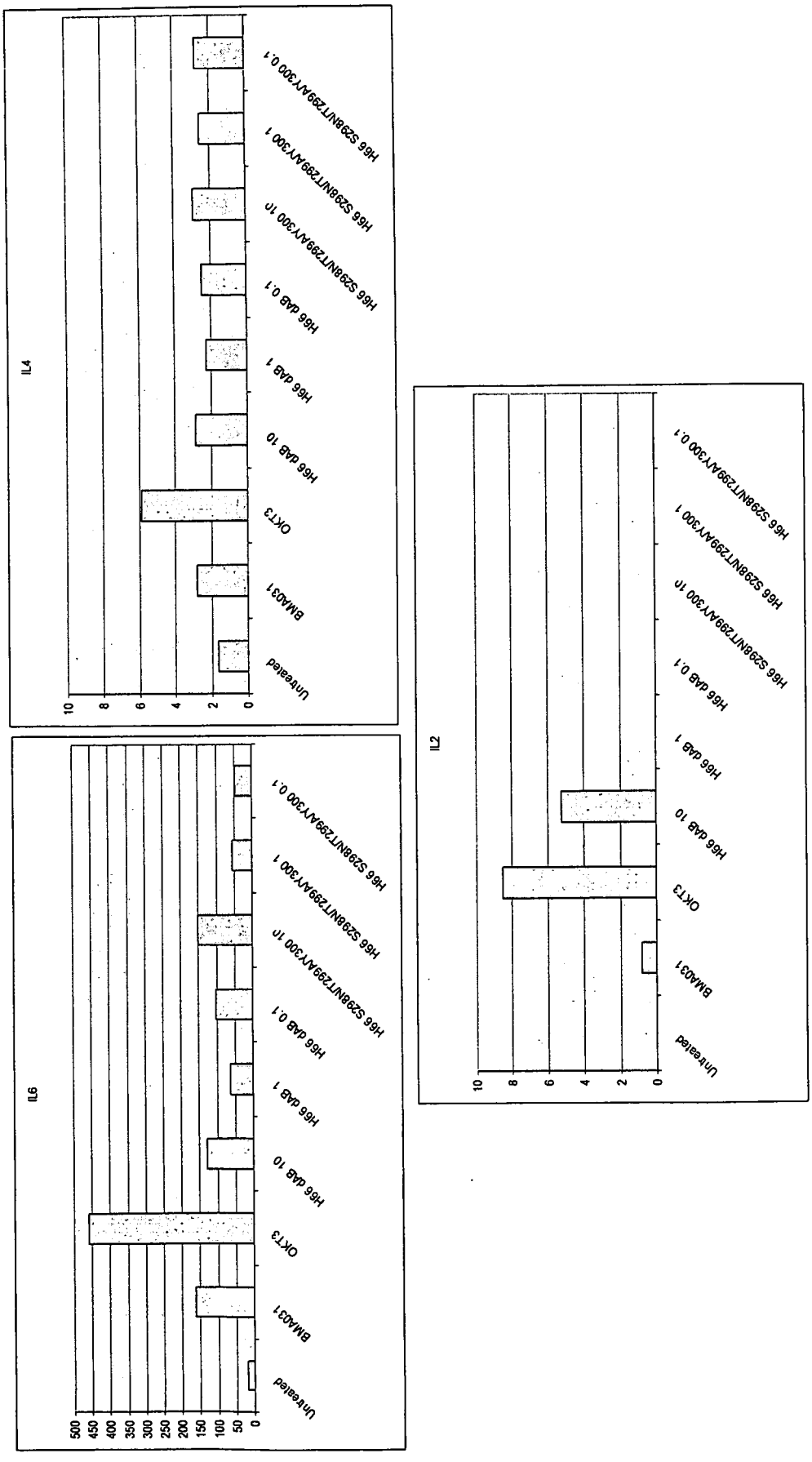

IL4
IL2
IL6
Untreated
BMA031
OKT3
H66 dAg 10
H66 dAg 1
H66 dAg 0,1
H66 S298NT299AY300 1n
H66 S298NT299AY300 1
H66 S298NT299AY300 0,1

Figure 22

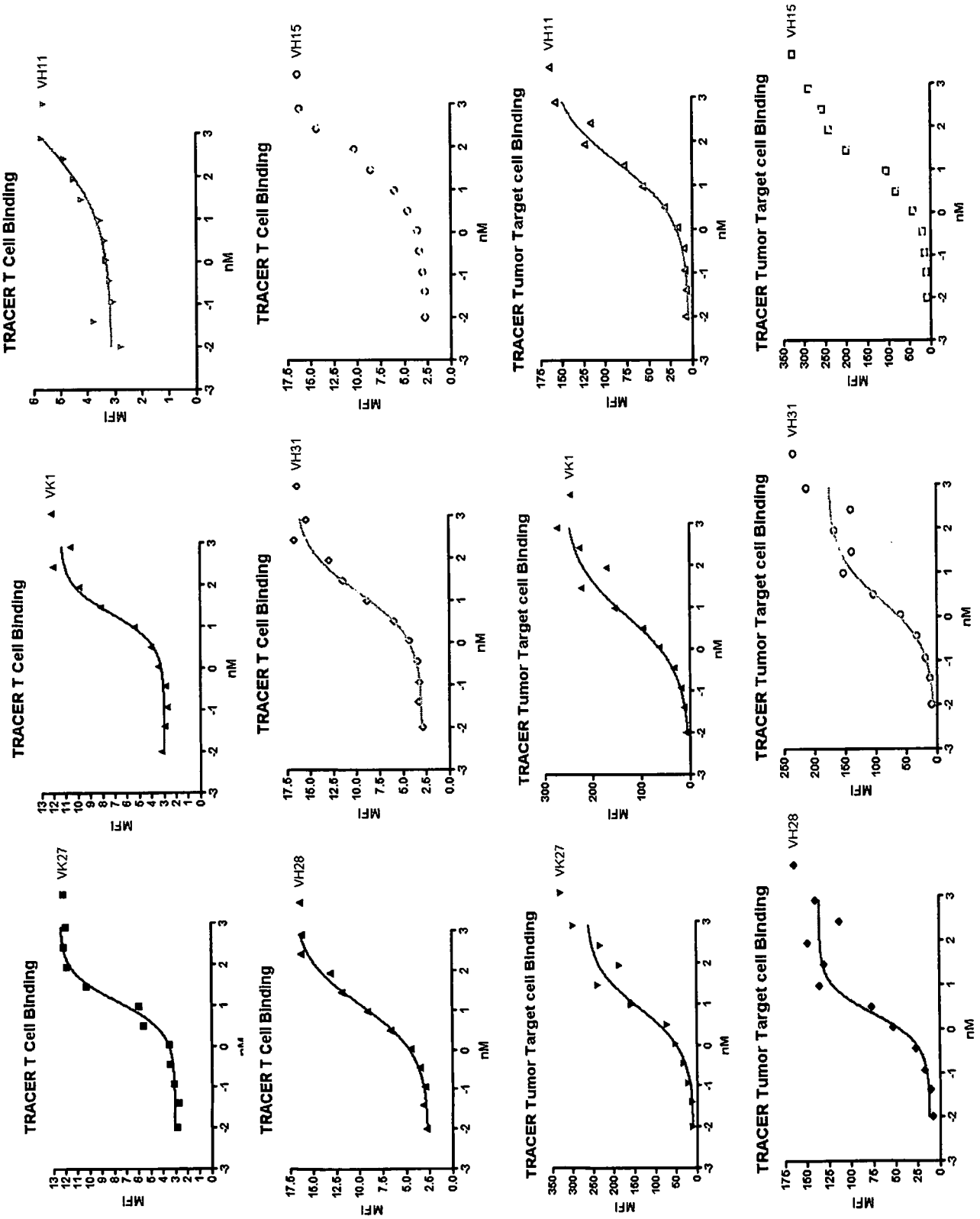

TRACER T Cell Binding
VH11
VK1
VH15
VH31
VK27
VH28
TRACER Tumor Target cell Binding
MFI
nM

Figure 23

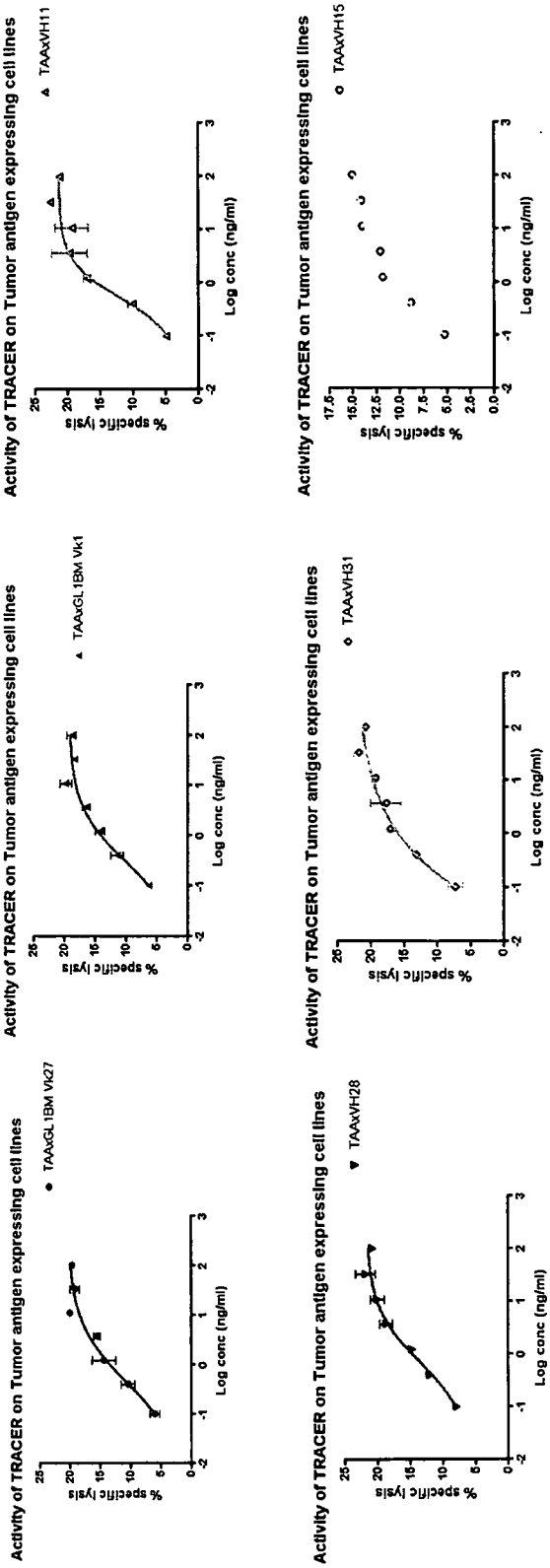

Activity of TRACER on Tumor antigen expressing cell lines
TAAxVH11
% specific lysis
Log conc (ng/ml)
Activity of TRACER on Tumor antigen expressing cell lines
TAAxGL1BM Vk1
% specific lysis
Log conc (ng/ml)
Activity of TRACER on Tumor antigen expressing cell lines
TAAxVH15
% specific lysis
Log conc (ng/ml)
Activity of TRACER on Tumor antigen expressing cell lines
TAAxGL1BM Vk27
% specific lysis
Log conc (ng/ml)
Activity of TRACER on Tumor antigen expressing cell lines
TAAxVH31
% specific lysis
Log conc (ng/ml)
Activity of TRACER on Tumor antigen expressing cell lines
TAAxVH28
% specific lysis
Log conc (ng/ml)

Figure 24

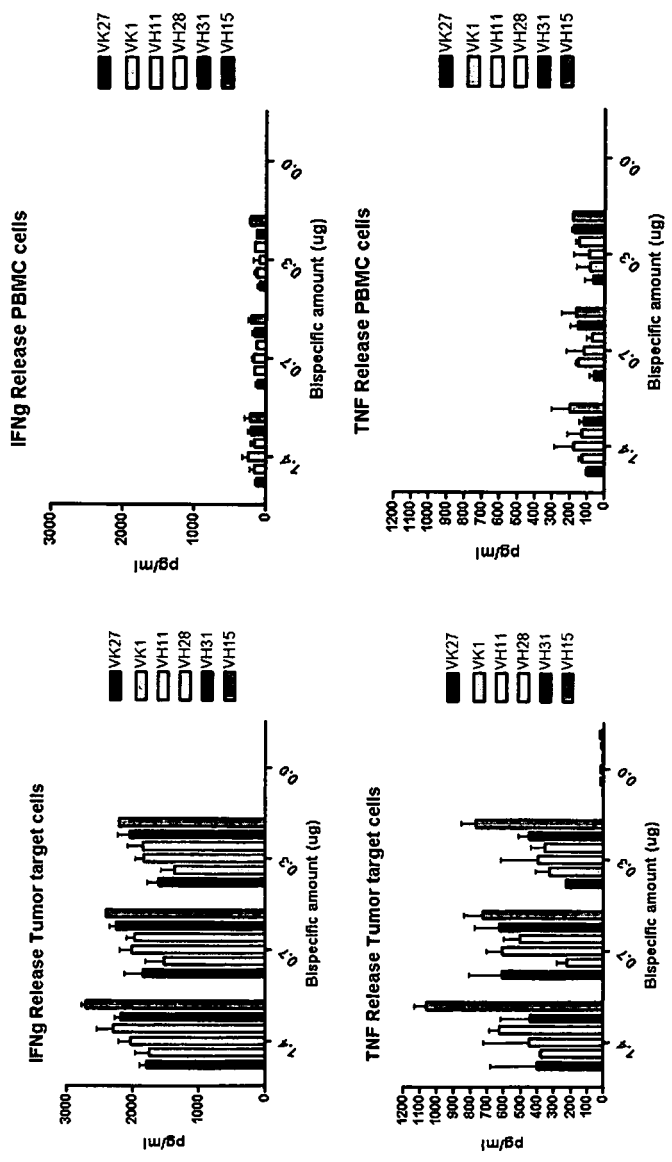
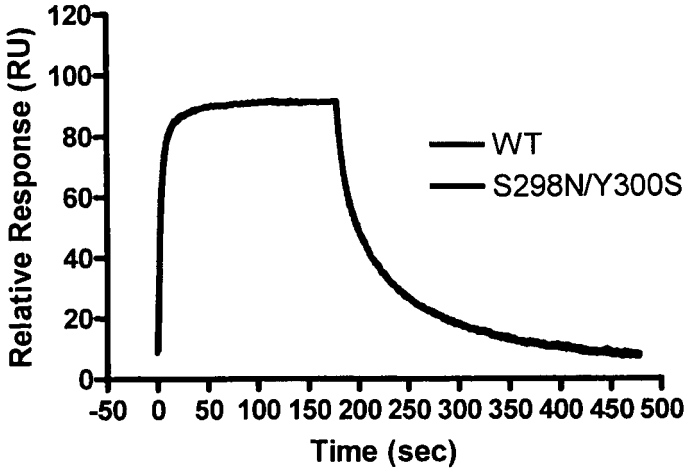
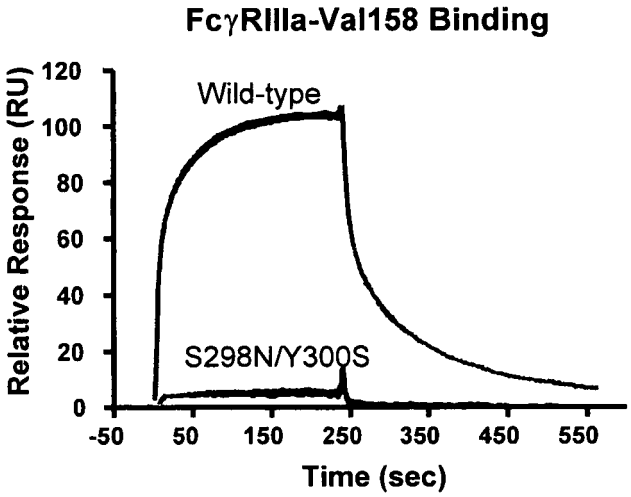

IFNg Release PBMC cells
TNF Release PBMC cells
IFNg Release Tumor target cells
TNF Release Tumor target cells
pg/ml
Bispecific amount (ug)
VK27
VK1
VH11
VH28
VH31
VH15

Figure 25

A.



B.



C.

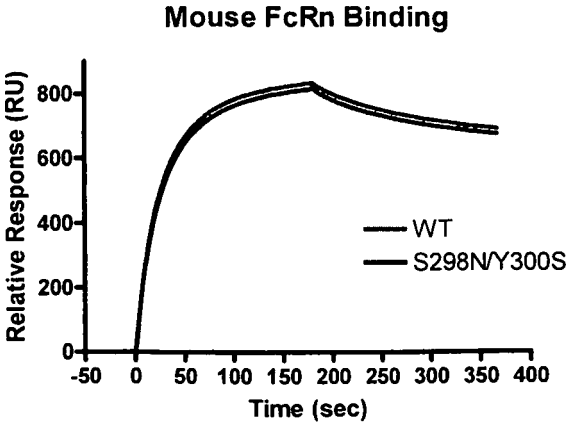


Figure 26