Figure 1

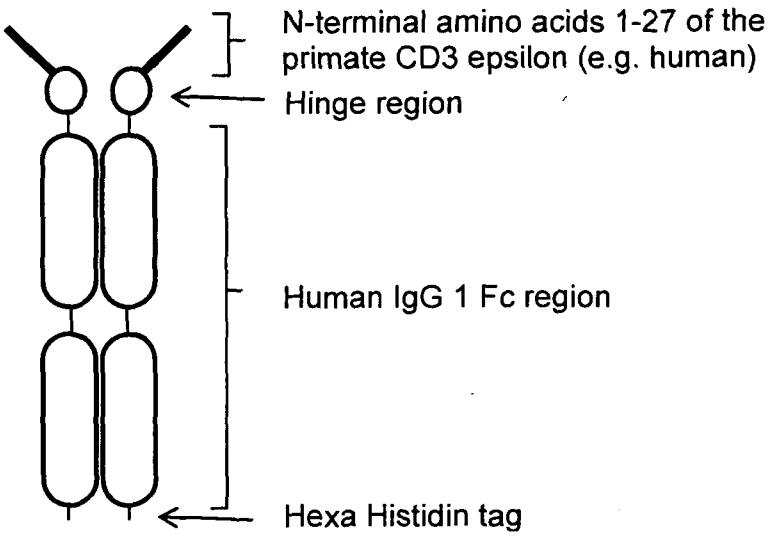
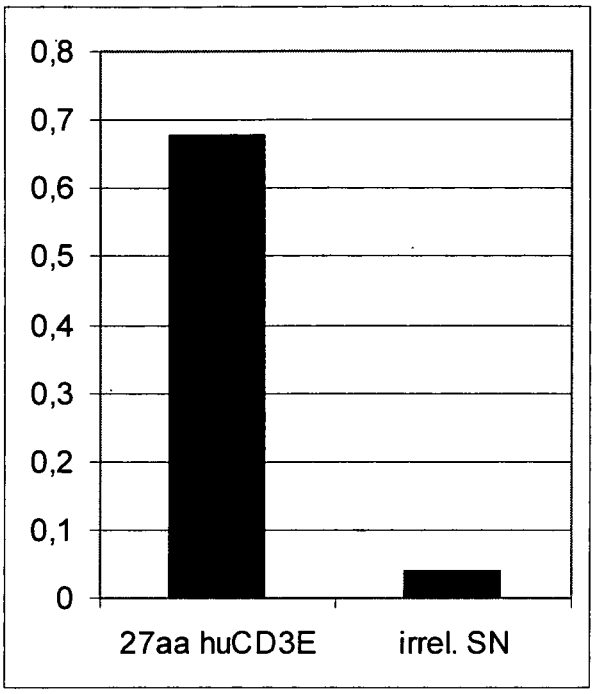

N-terminal amino acids 1-27 of the primate CD3 epsilon (e.g. human)
Hinge region
Human IgG 1 Fc region
Hexa Histidin tag

Figure 2



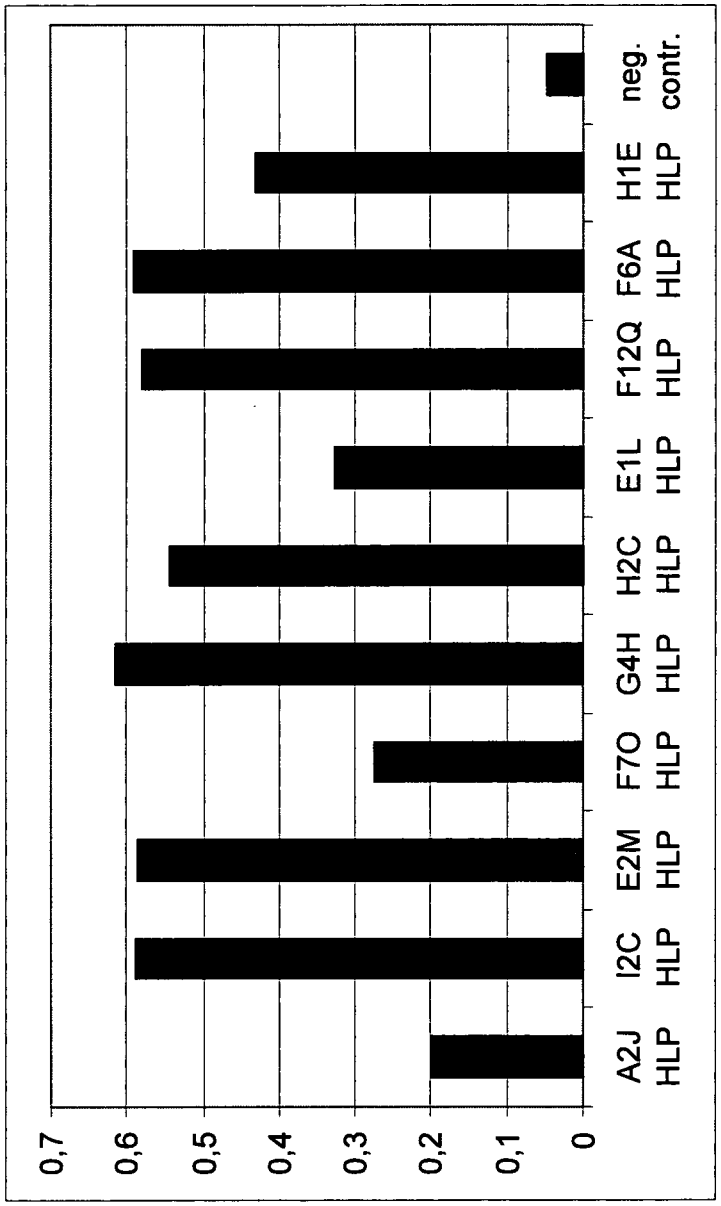

0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
A2J HLP
I2C HLP
E2M HLP
F7O HLP
G4H HLP
H2C HLP
E1L HLP
F12Q HLP
F6A HLP
H1E HLP
neg. contr.

Figure 3

Figure 4

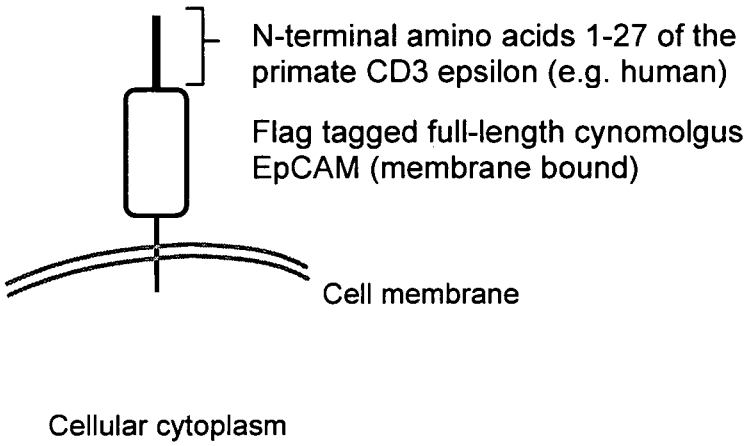

N-terminal amino acids 1-27 of the primate CD3 epsilon (e.g. human)
Flag tagged full-length cynomolgus EpCAM (membrane bound)
Cell membrane
Cellular cytoplasm

Figure 5

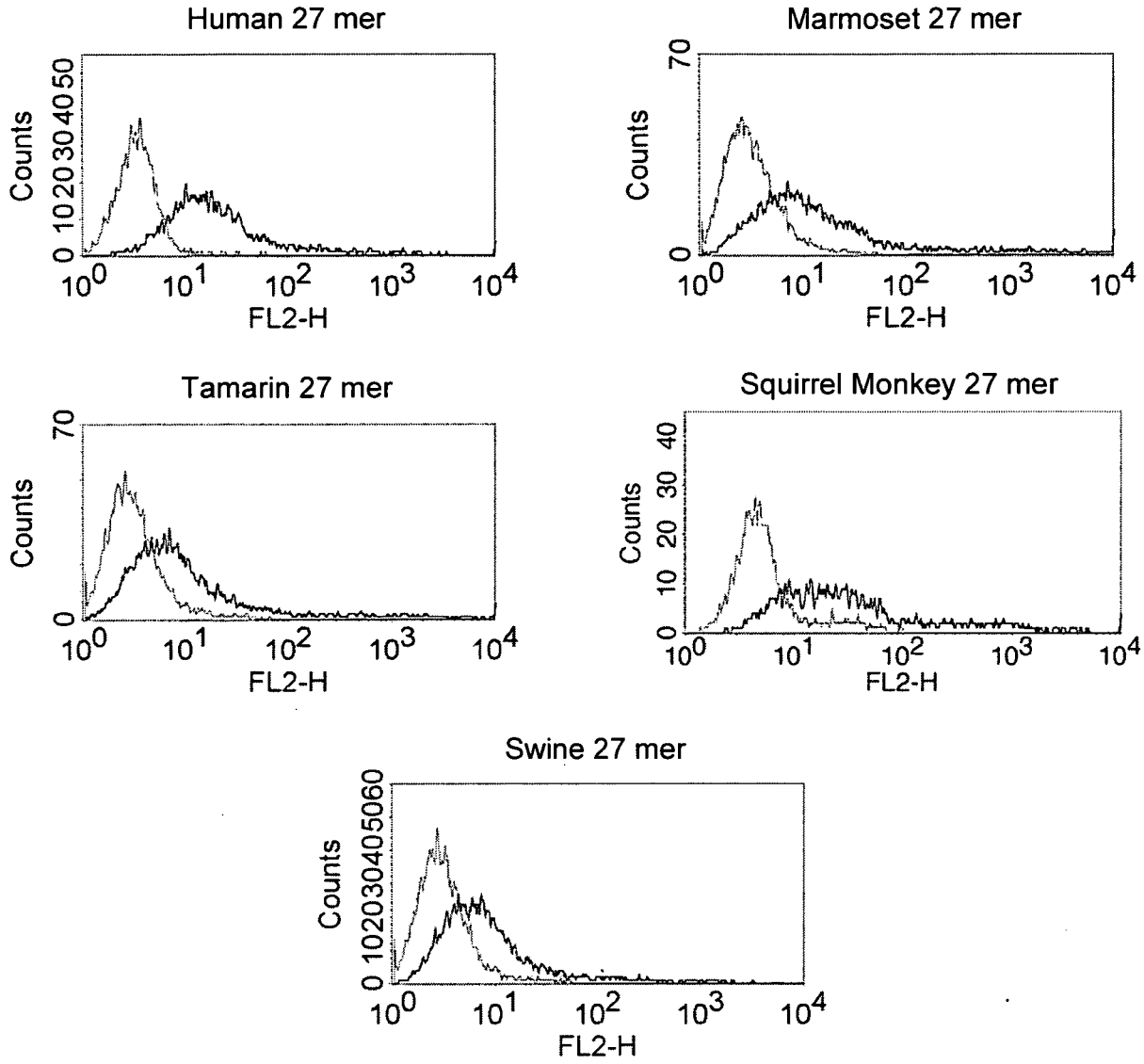
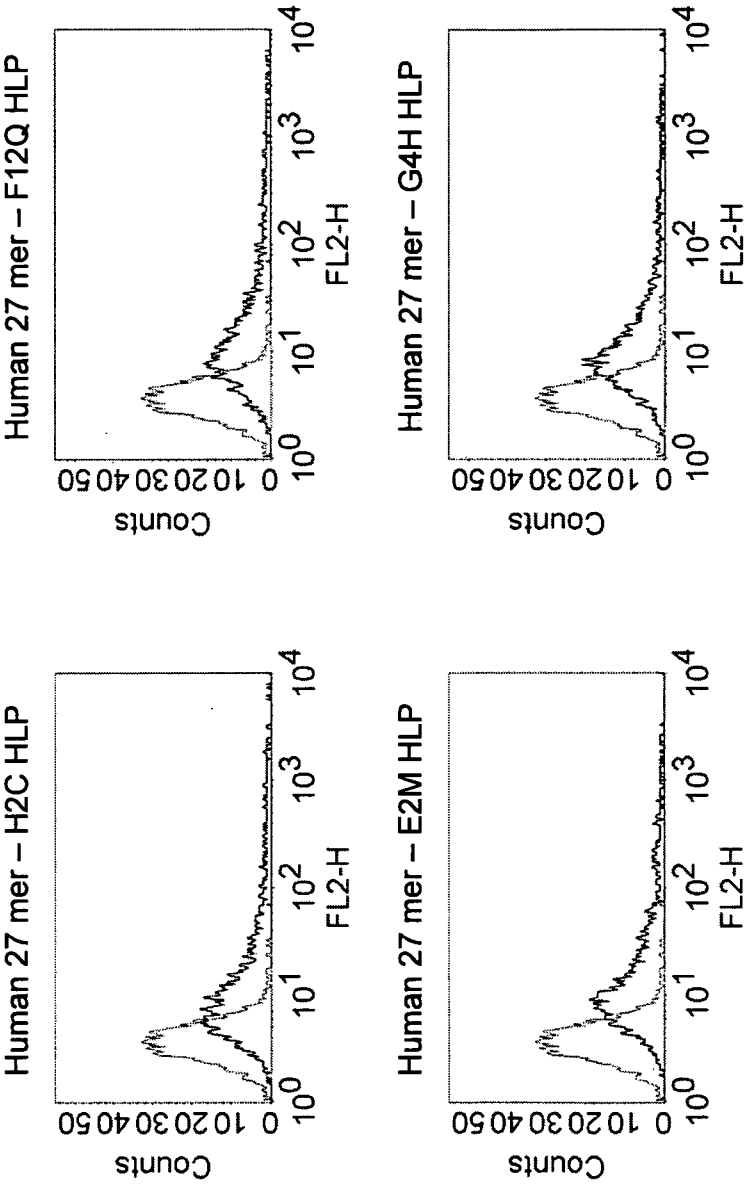

Human 27 mer
Counts
0 10 20 30 40 50
10^0 10^1 10^2 10^3 10^4
FL2-H
Marmoset 27 mer
Counts
0 70
10^0 10^1 10^2 10^3 10^4
FL2-H
Tamarin 27 mer
Counts
0 70
10^0 10^1 10^2 10^3 10^4
FL2-H
Squirrel Monkey 27 mer
Counts
0 10 20 30 40
10^0 10^1 10^2 10^3 10^4
FL2-H
Swine 27 mer
Counts
0 10 20 30 40 50 60
10^0 10^1 10^2 10^3 10^4
FL2-H

Figure 6A


Human 27 mer – F12Q HLP
Human 27 mer – G4H HLP
Human 27 mer – H2C HLP
Human 27 mer – E2M HLP
Counts
FL2-H
0 10 20 30 40 50
10^0 10^1 10^2 10^3 10^4

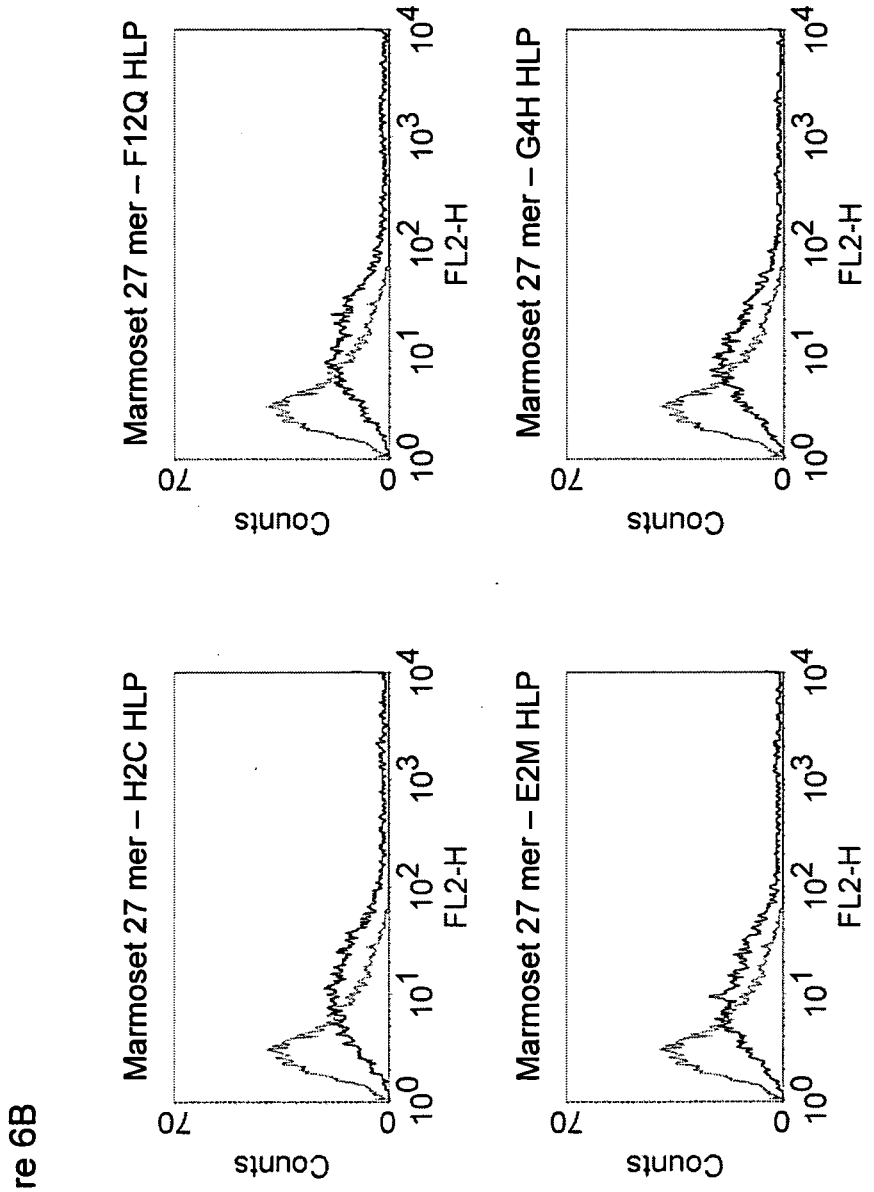

Marmoset 27 mer – F12Q HLP
Marmoset 27 mer – G4H HLP
Marmoset 27 mer – H2C HLP
Marmoset 27 mer – E2M HLP
Counts
FL2-H
0
70
10^0
10^1
10^2
10^3
10^4

Figure 6B

Figure 6C

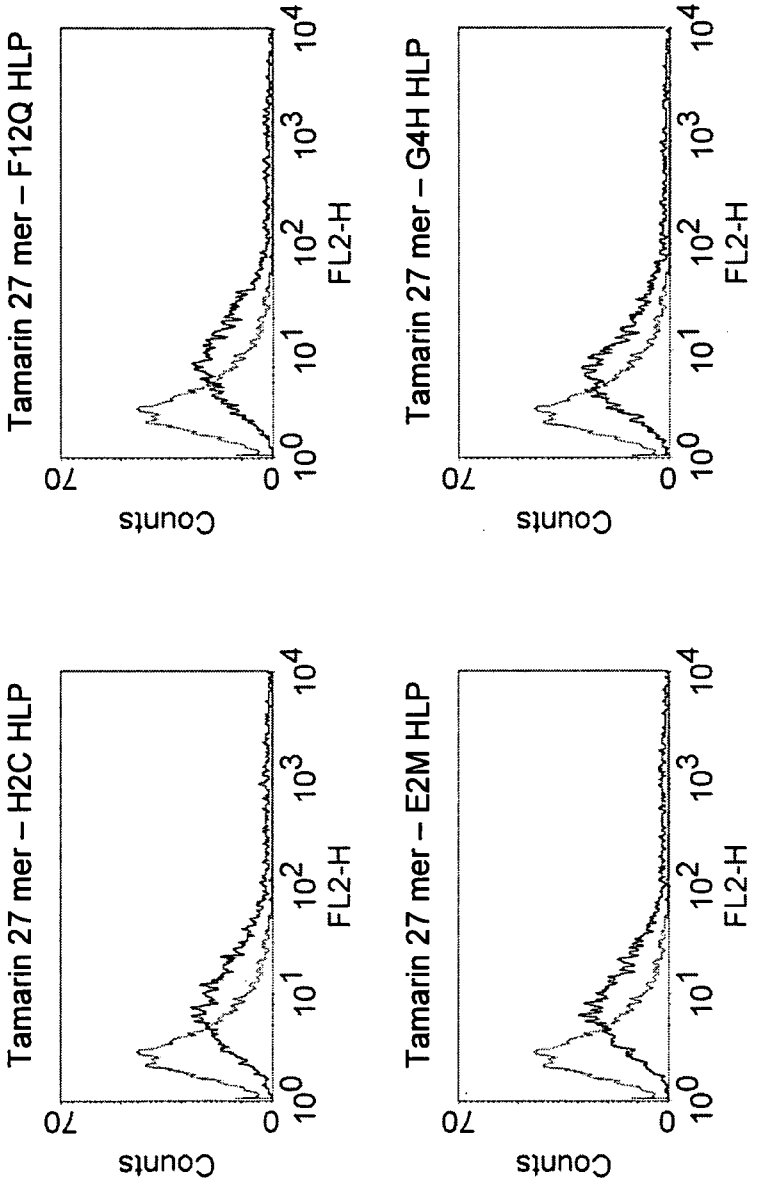

Tamarin 27 mer – F12Q HLP
Tamarin 27 mer – G4H HLP
Tamarin 27 mer – H2C HLP
Tamarin 27 mer – E2M HLP
Counts
FL2-H
0
70
10^0
10^1
10^2
10^3
10^4

Figure 6D

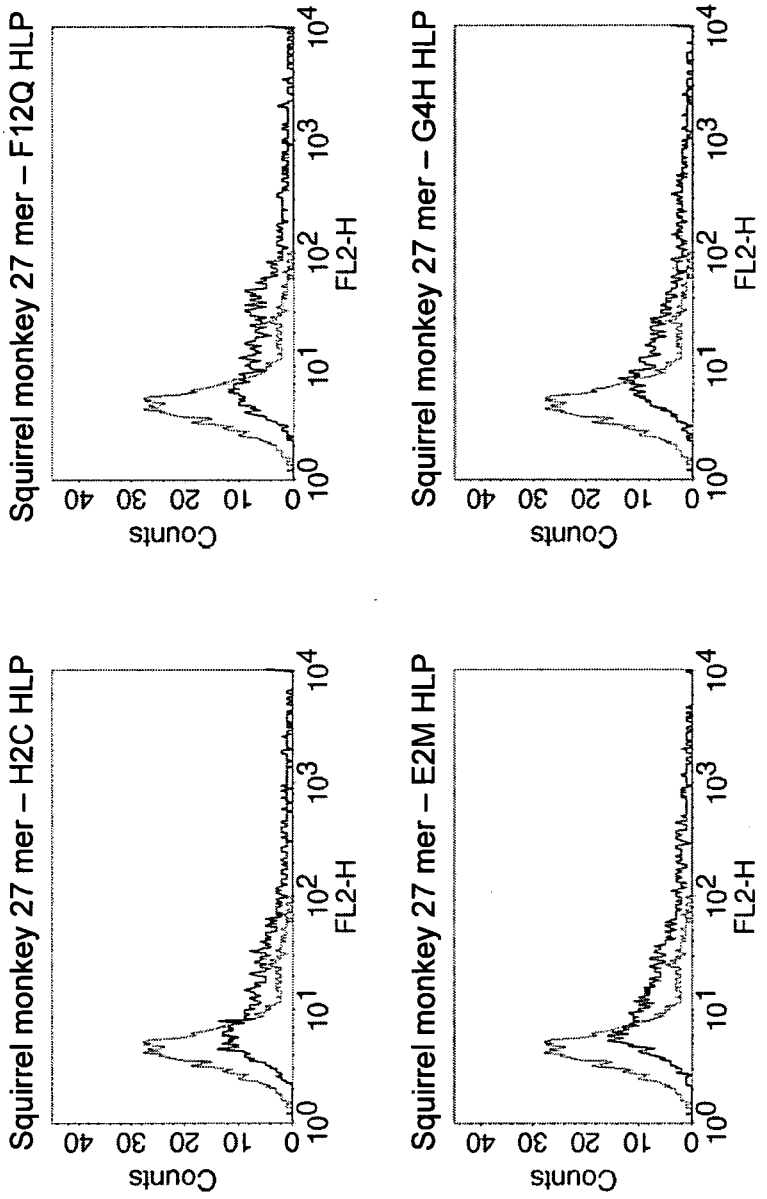

Squirrel monkey 27 mer – F12Q HLP
Squirrel monkey 27 mer – G4H HLP
Squirrel monkey 27 mer – H2C HLP
Squirrel monkey 27 mer – E2M HLP
Counts
FL2-H
0
10
20
30
40
10^0
10^1
10^2
10^3
10^4

Figure 6E

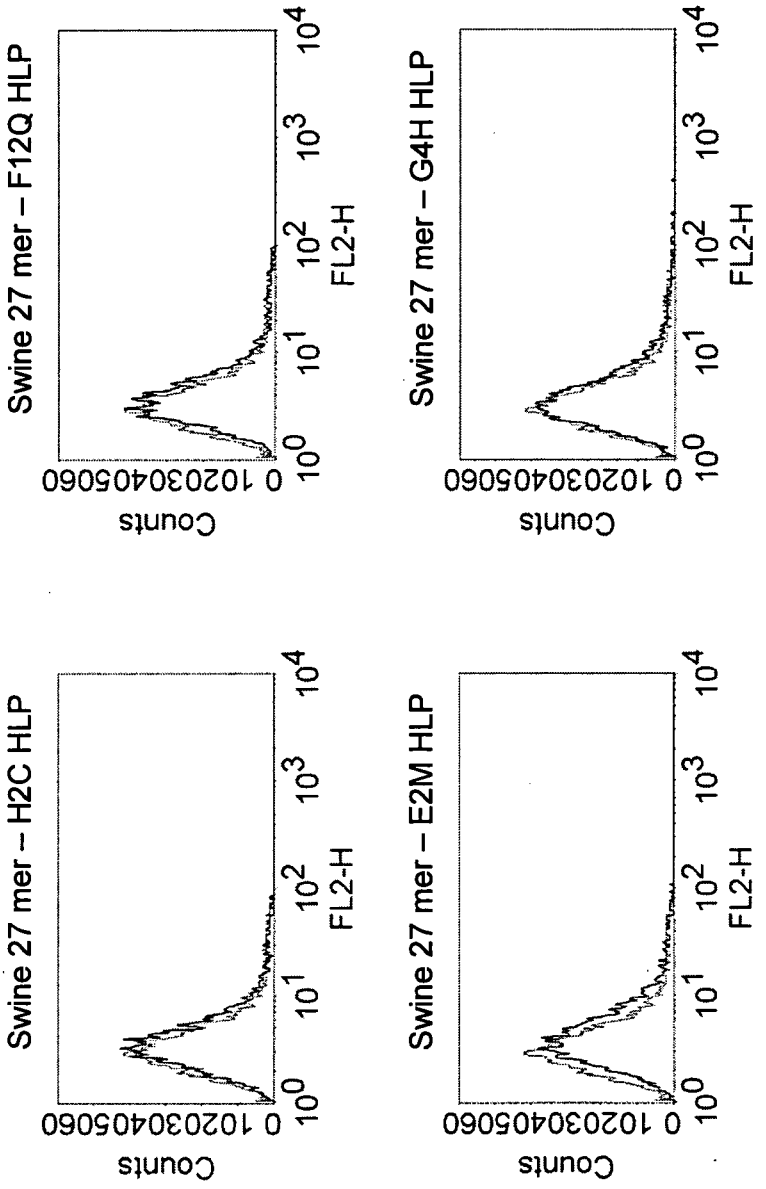
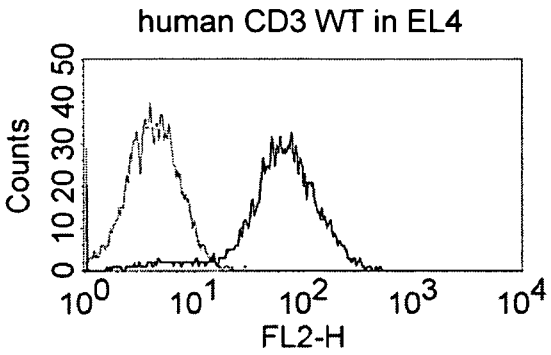

Swine 27 mer – F12Q HLP
Swine 27 mer – G4H HLP
Swine 27 mer – H2C HLP
Swine 27 mer – E2M HLP
Counts
FL2-H
0
10
20
30
40
50
60
10^0
10^1
10^2
10^3
10^4

Figure 7


human CD3 WT in EL4
Counts
0 10 20 30 40 50
10^0 10^1 10^2 10^3 10^4
FL2-H

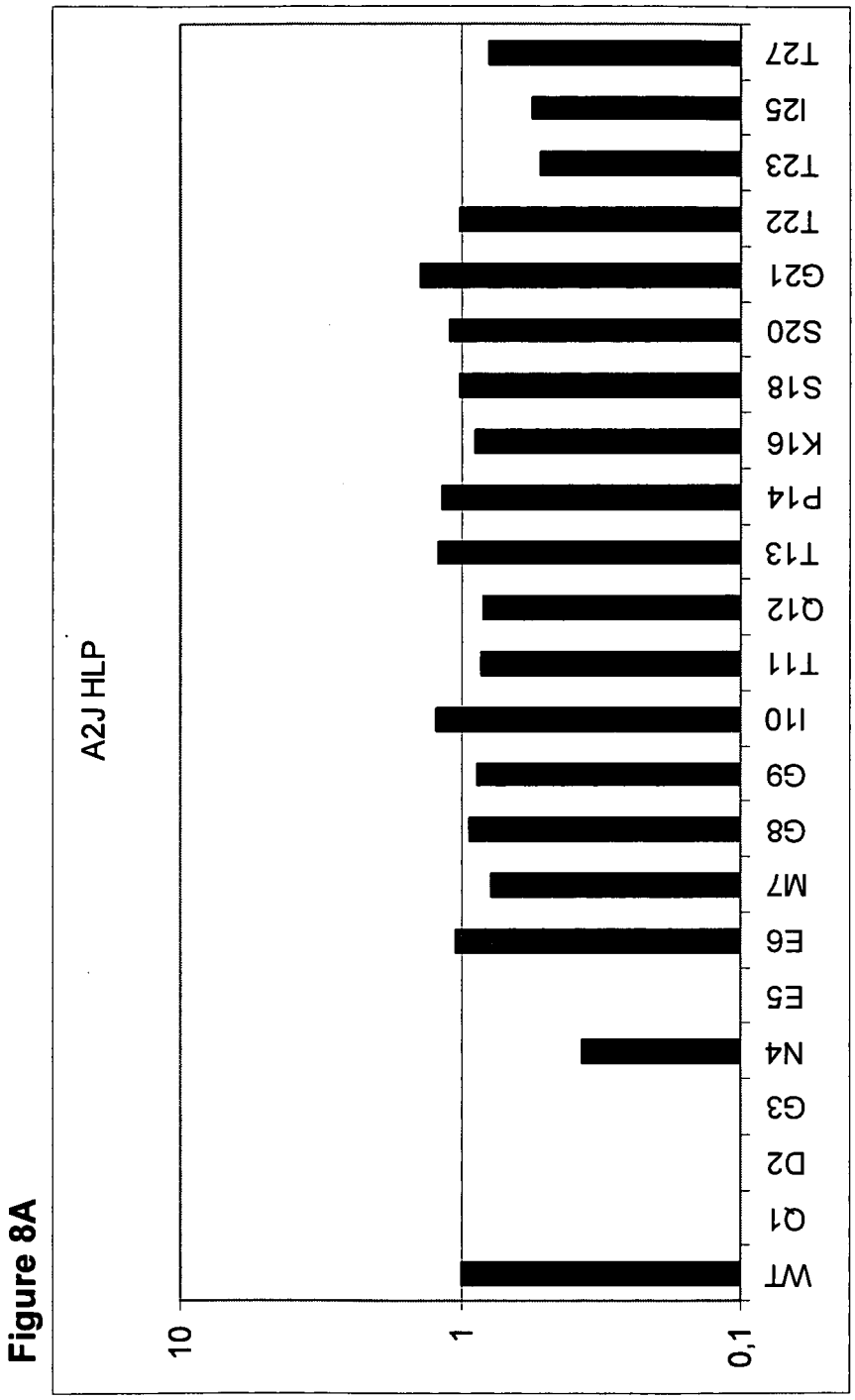

A2J HLP
10
1
0,1
WT
Q1
D2
G3
N4
E5
E6
M7
G8
G9
I10
T11
Q12
T13
P14
K16
S18
S20
G21
T22
T23
I25
T27

Figure 8A

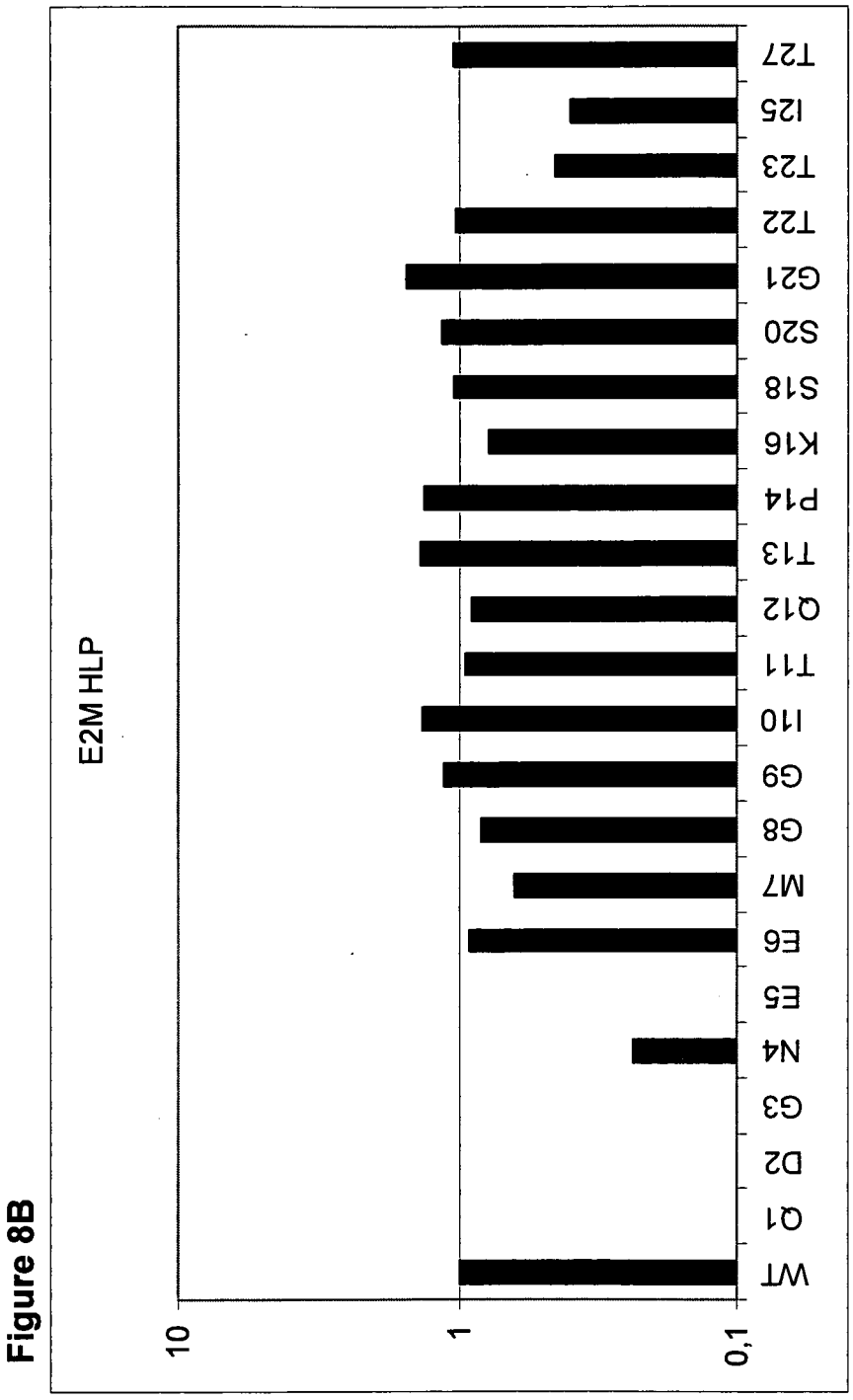

E2M HLP
10
1
0,1
WT
Q1
D2
G3
N4
E5
E6
M7
G8
G9
I10
T11
Q12
T13
P14
K16
S18
S20
G21
T22
T23
I25
T27

Figure 8B

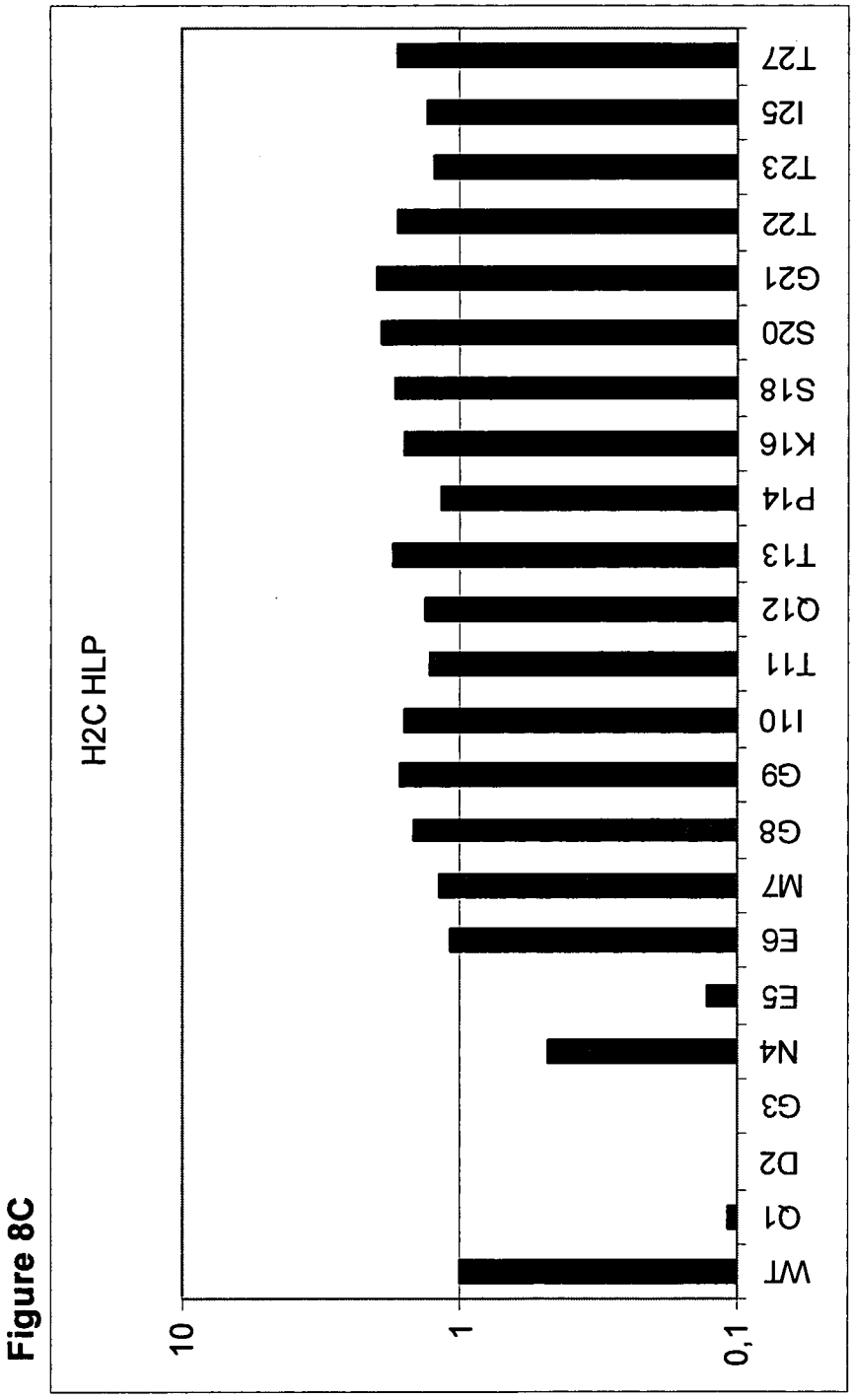

H2C HLP
10
1
0,1
WT
Q1
D2
G3
N4
E5
E6
M7
G8
G9
I10
T11
Q12
T13
P14
K16
S18
S20
G21
T22
T23
I25
T27

Figure 8C

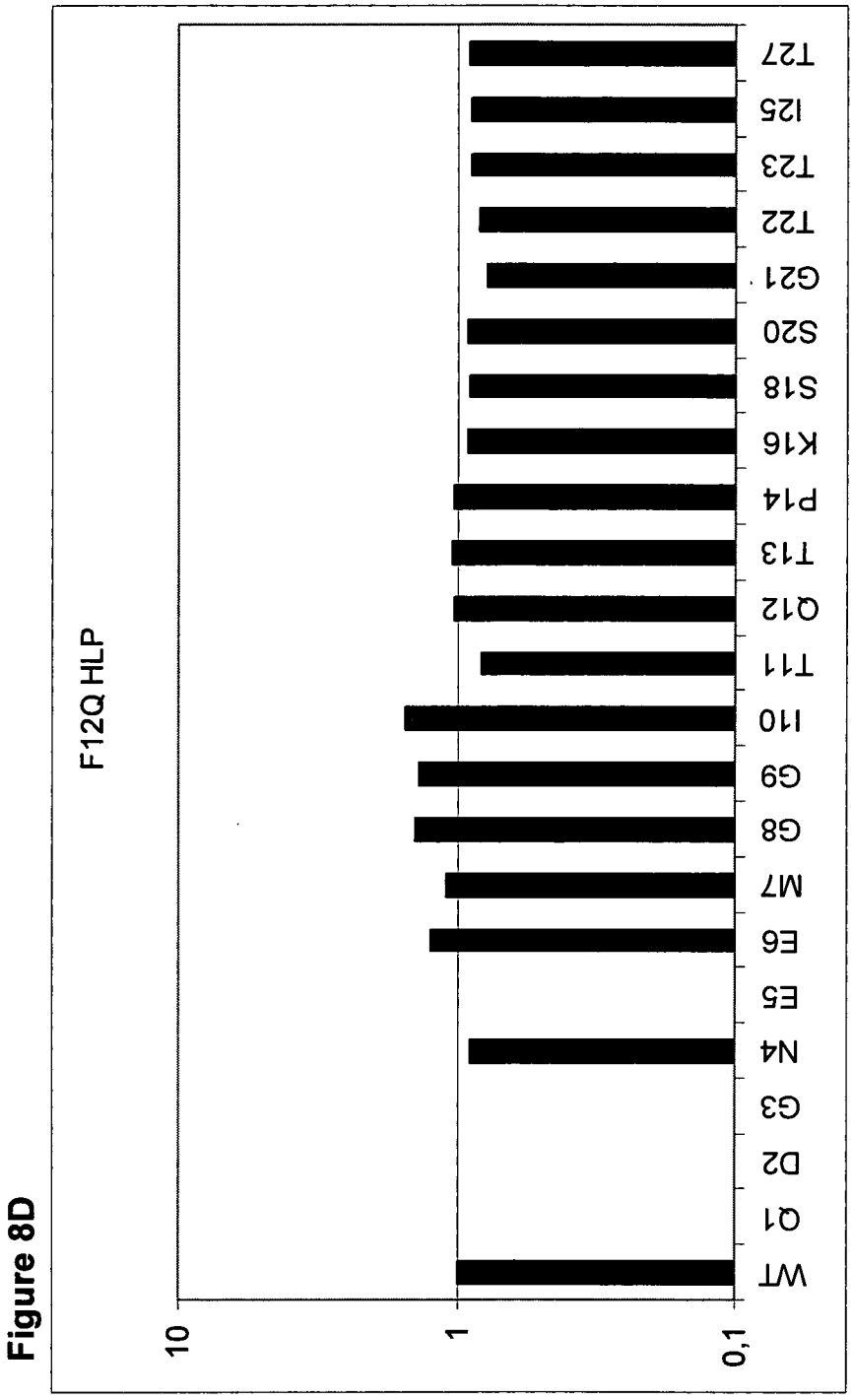

F12Q HLP
10
1
0,1
WT
Q1
D2
G3
N4
E5
E6
M7
G8
G9
I10
T11
Q12
T13
P14
K16
S18
S20
G21
T22
T23
I25
T27

Figure 8D

Figure 9

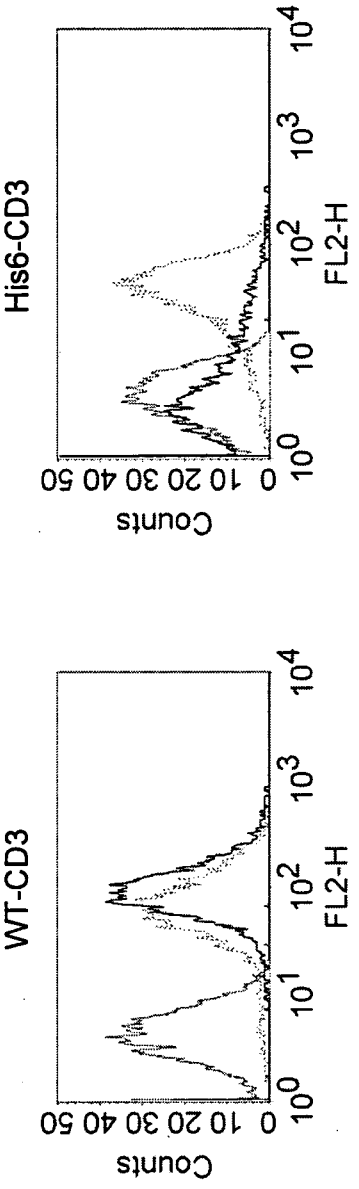

His6-CD3
Counts
0 10 20 30 40 50
FL2-H
10^0 10^1 10^2 10^3 10^4
WT-CD3
Counts
0 10 20 30 40 50
FL2-H
10^0 10^1 10^2 10^3 10^4

Figure 10

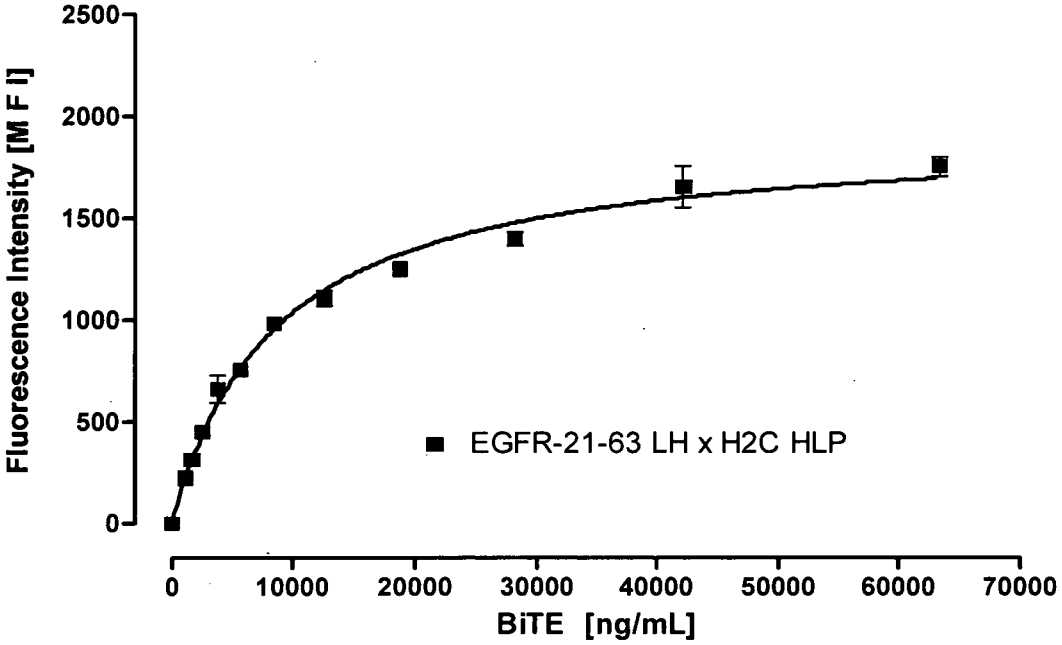
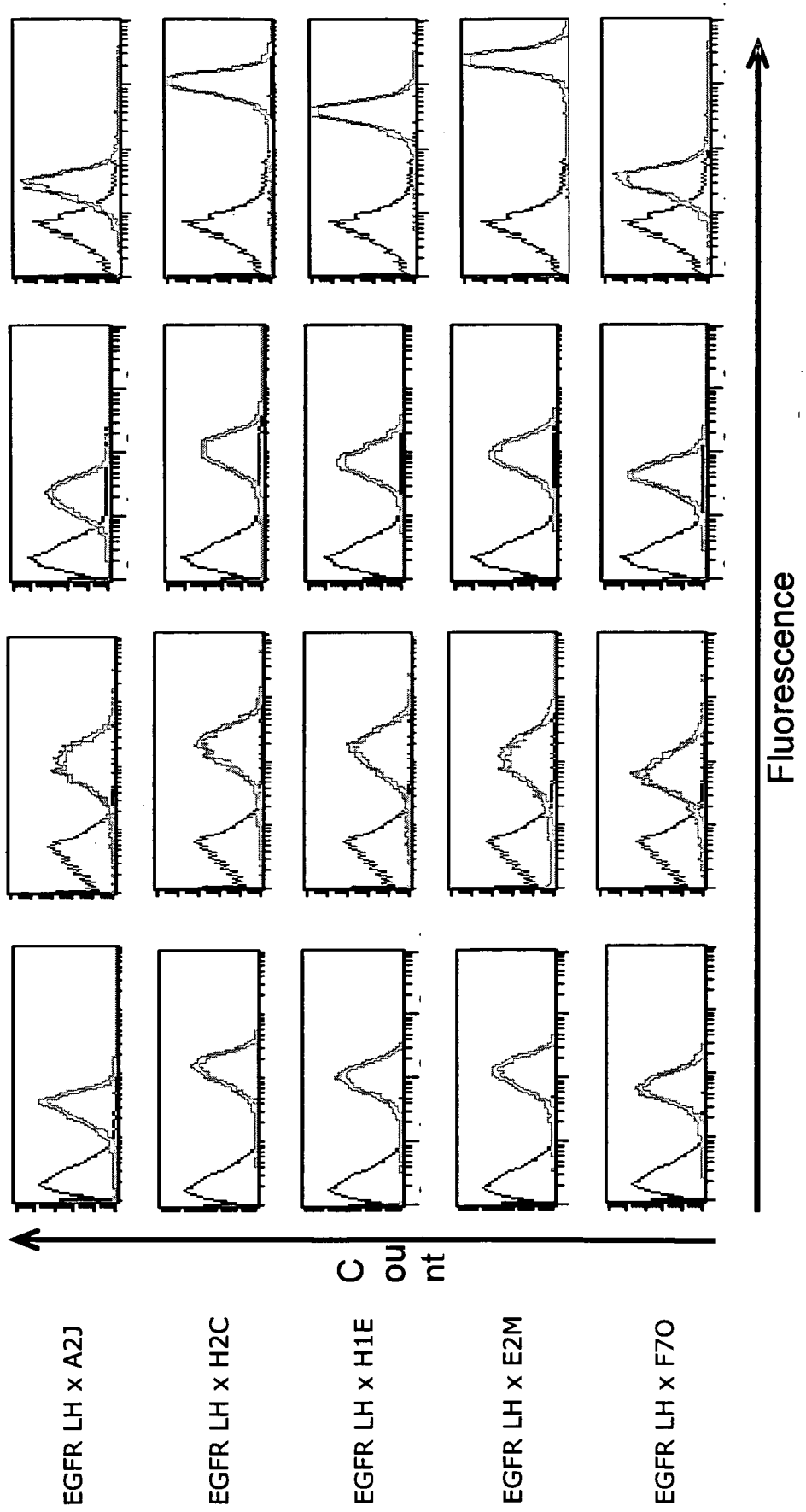

Fluorescence Intensity [M F I]
2500
2000
1500
1000
500
0
EGFR-21-63 LH x H2C HLP
0
10000
20000
30000
40000
50000
60000
70000
BiTE [ng/mL]

Figure 11 a


CHO human EGFR
HPB-ALL (human)
CHO cyno EGFR
4119
EGFR LH x A2J
EGFR LH x H2C
EGFR LH x H1E
EGFR LH x E2M
EGFR LH x F7O
Count
Fluorescence

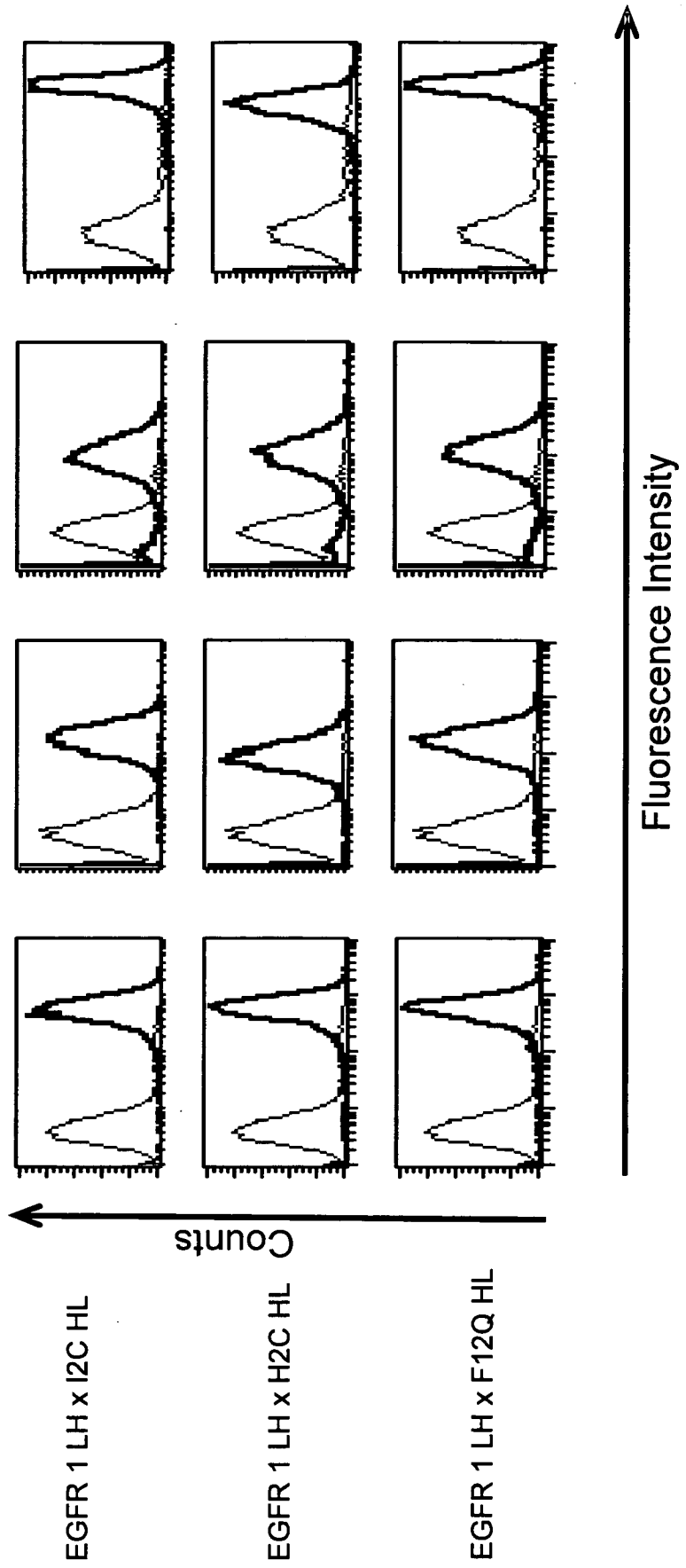

CHO human EGFR
HPB-ALL (human)
CHO cyno EGFR
4119 LnPx (cyno)
Counts
Fluorescence Intensity
EGFR 1 LH x I2C HL
EGFR 1 LH x H2C HL
EGFR 1 LH x F12Q HL

Figure: 11b

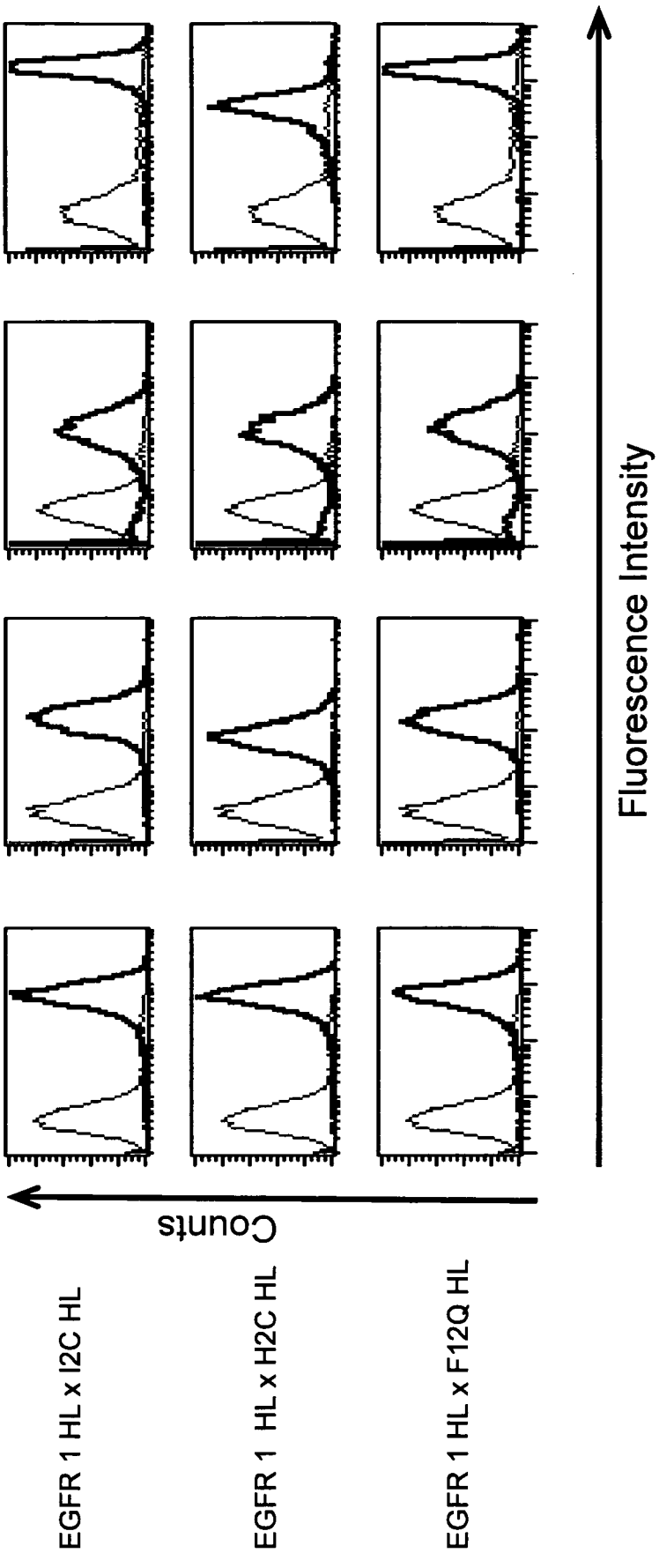

CHO human EGFR
HPB-ALL (human)
CHO cyno EGFR
4119 LnPx (cyno)
Counts
Fluorescence Intensity
EGFR 1 HL x I2C HL
EGFR 1 HL x H2C HL
EGFR 1 HL x F12Q HL

Figure: 11 c

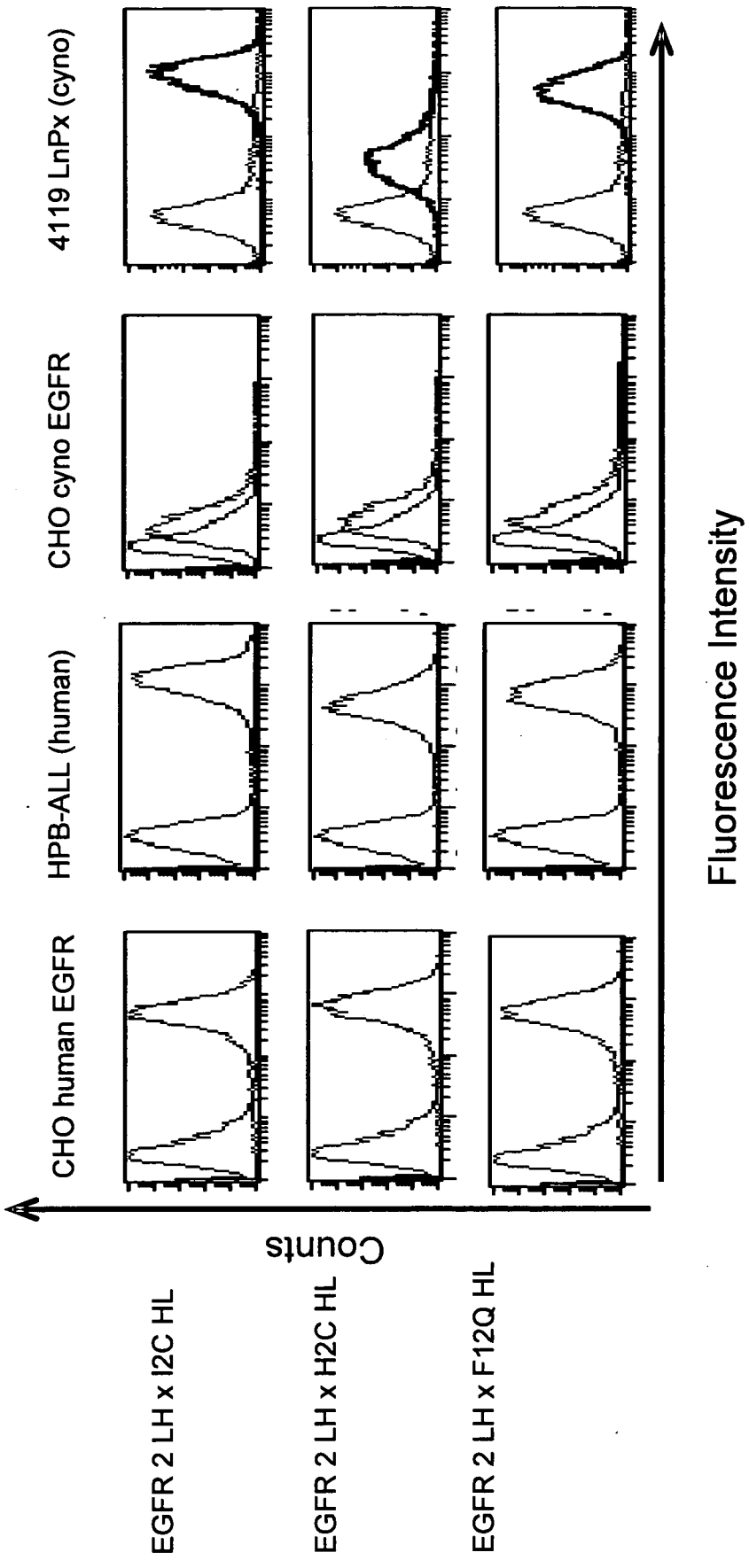
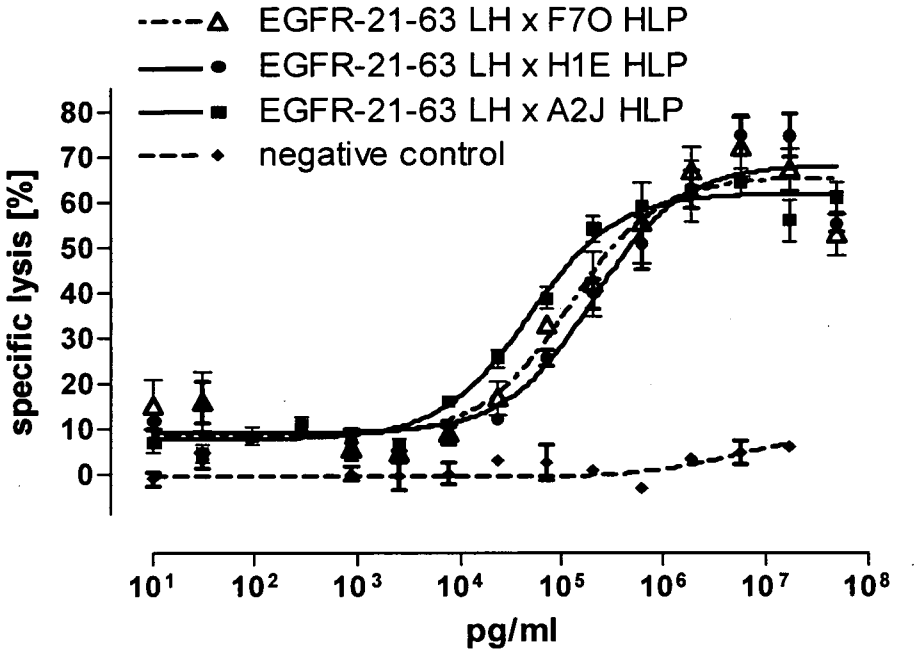

CHO human EGFR
HPB-ALL (human)
CHO cyno EGFR
4119 LnPx (cyno)
Counts
Fluorescence Intensity
EGFR 2 LH x I2C HL
EGFR 2 LH x H2C HL
EGFR 2 LH x F12Q HL

Figure: 11 d

Figure 12

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human EGFR



B)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human EGFR

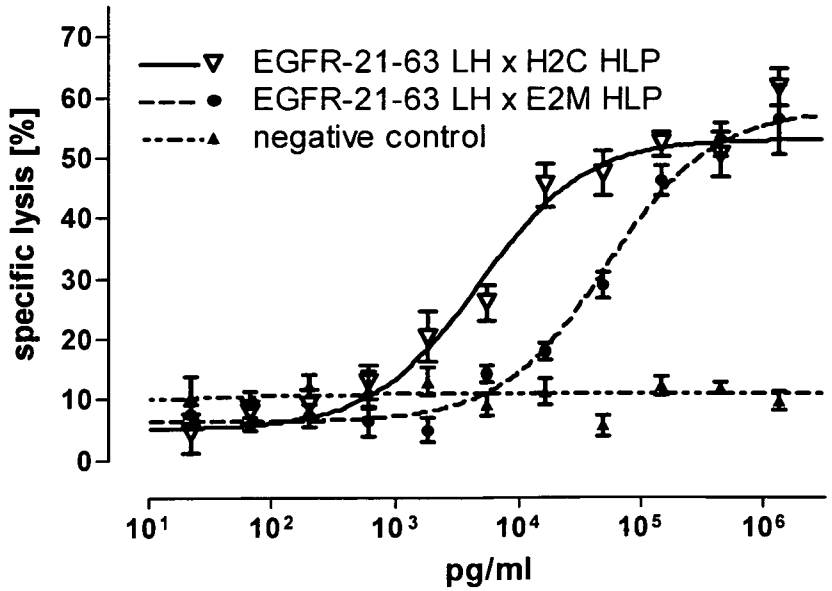
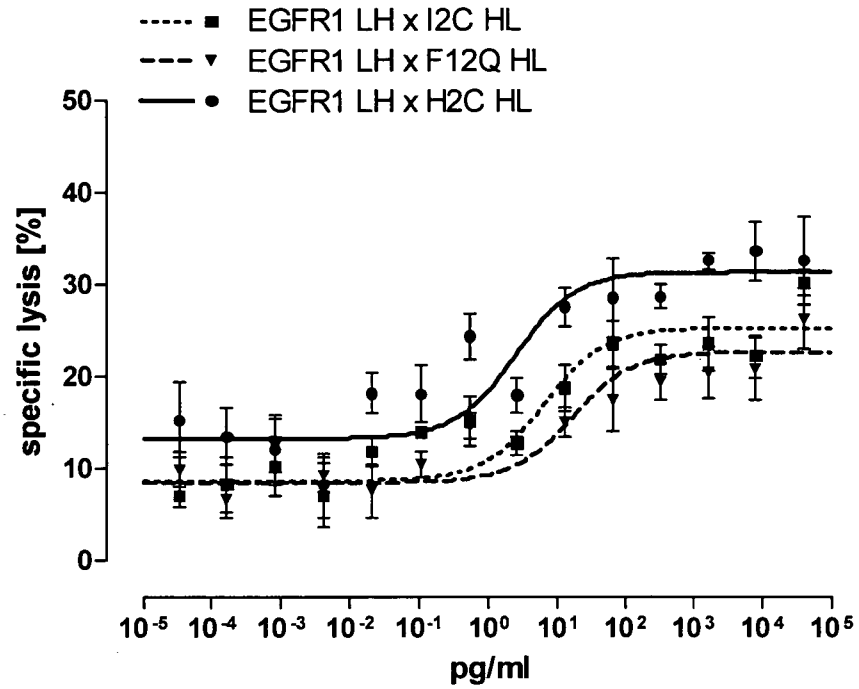


Figure: 12 b

A) Effector cells: CD56 depleted unstimulated human PBMC
Target cells: CHO transfected with human EGFR



B) Effector cells: 4119LnPx
Target cells: CHO transfected with cyno EGFR

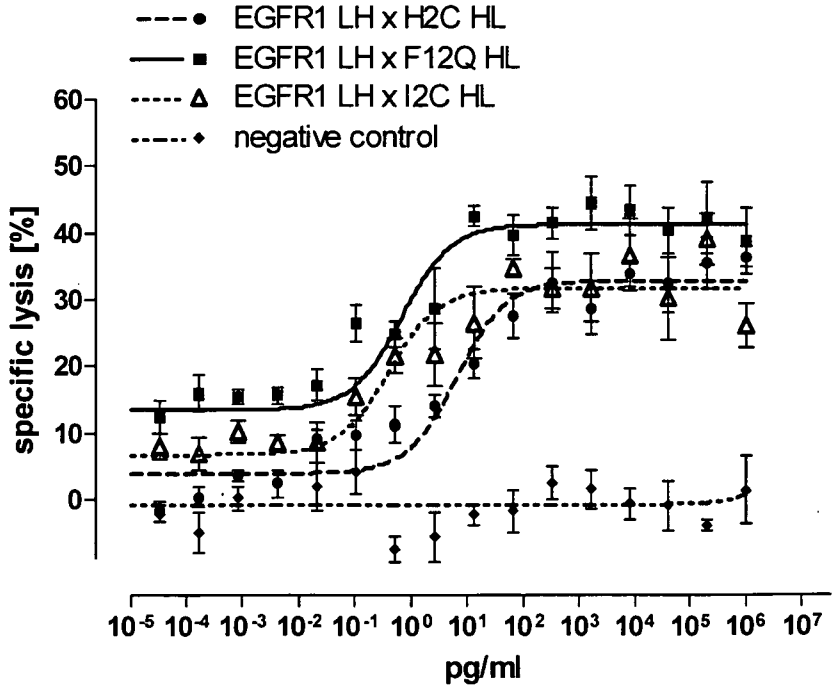
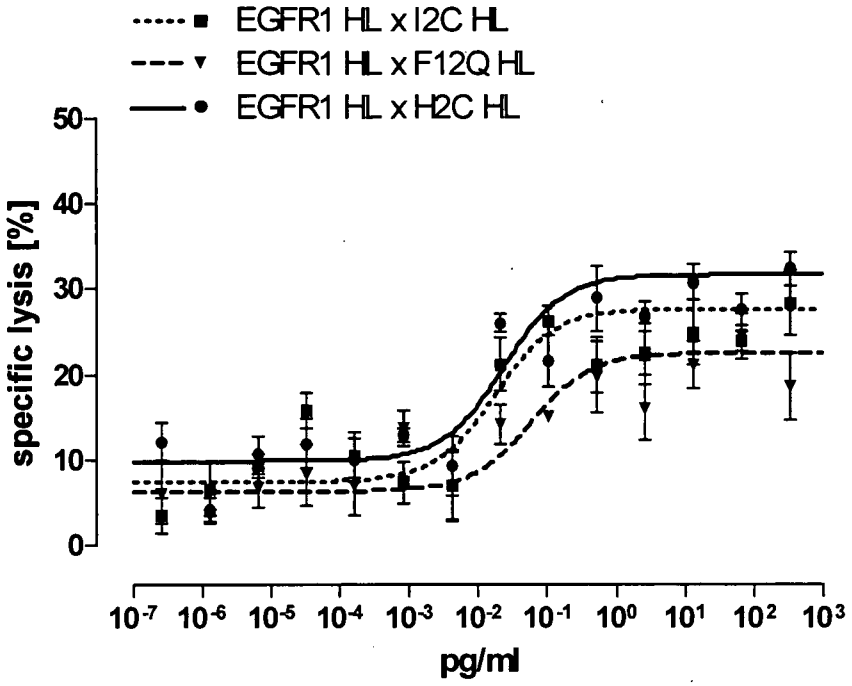


Figure: 12 c

A) Effector cells: CD56 depleted unstimulated human PBMC
Target cells: CHO transfected with human EGFR



B) Effector cells: 4119LnPx
Target cells: CHO transfected with cyno EGFR

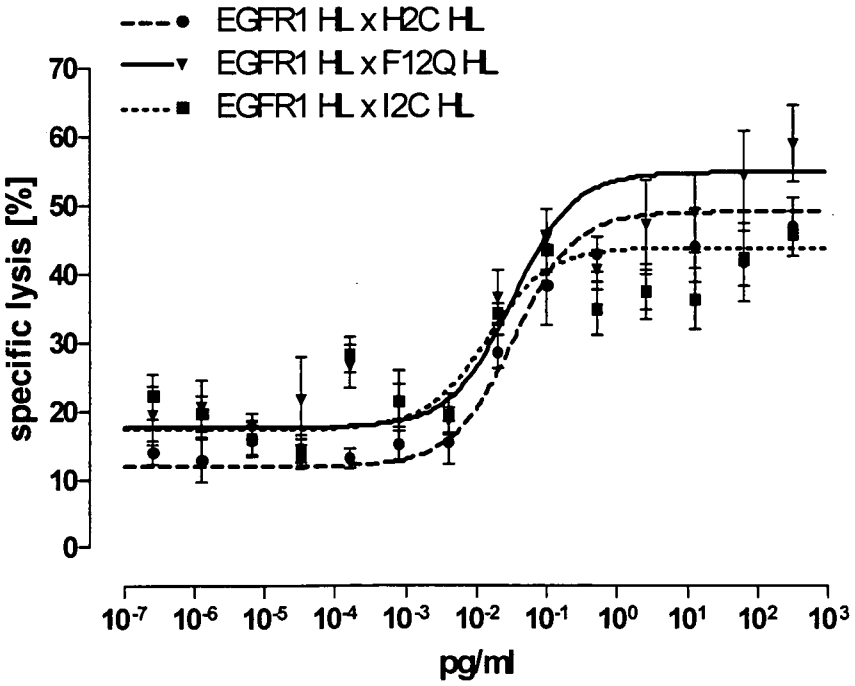
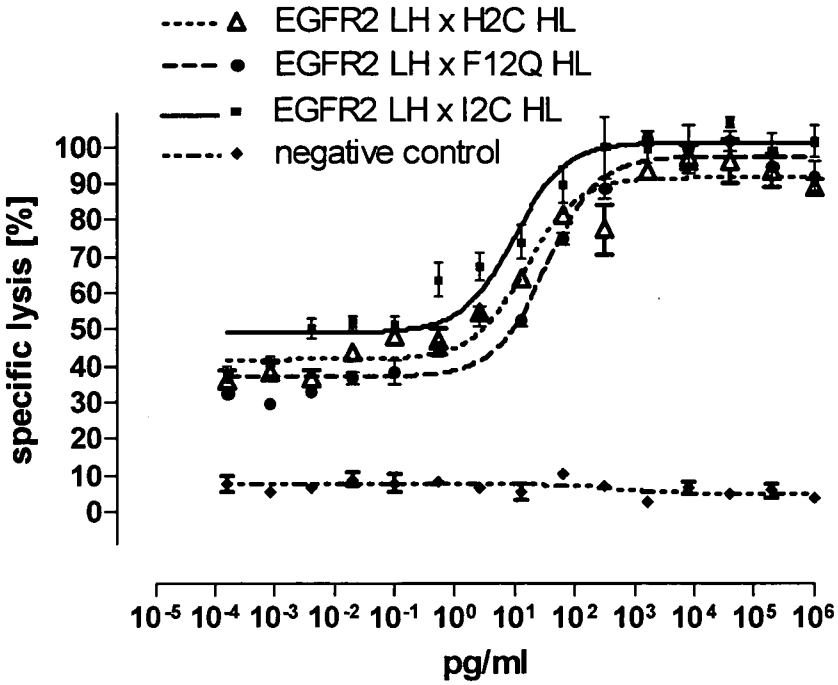


Figure: 12 d

A) Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human EGFR



B) Effector cells: 4119LnPx
Target cells: CHO transfected with cyno EGFR

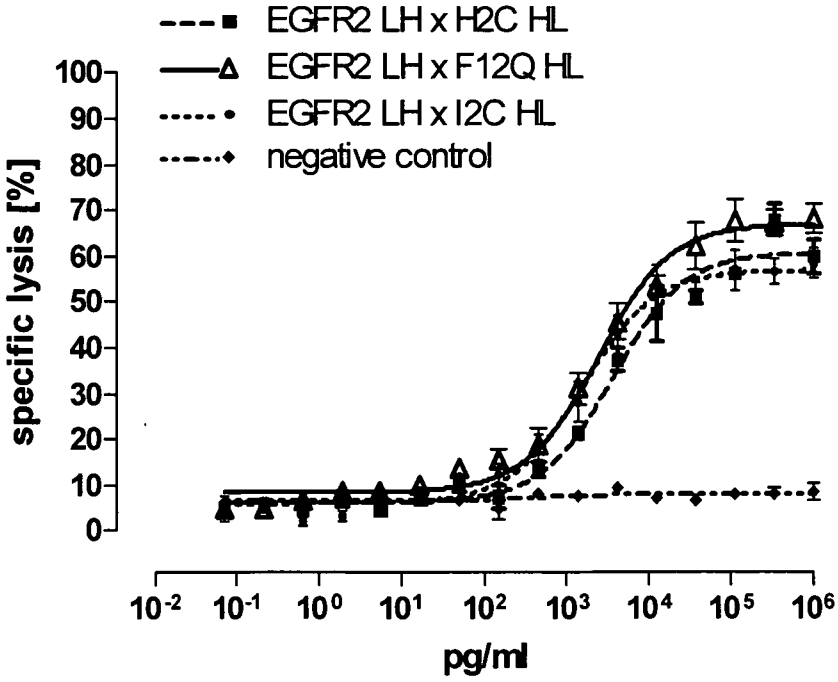
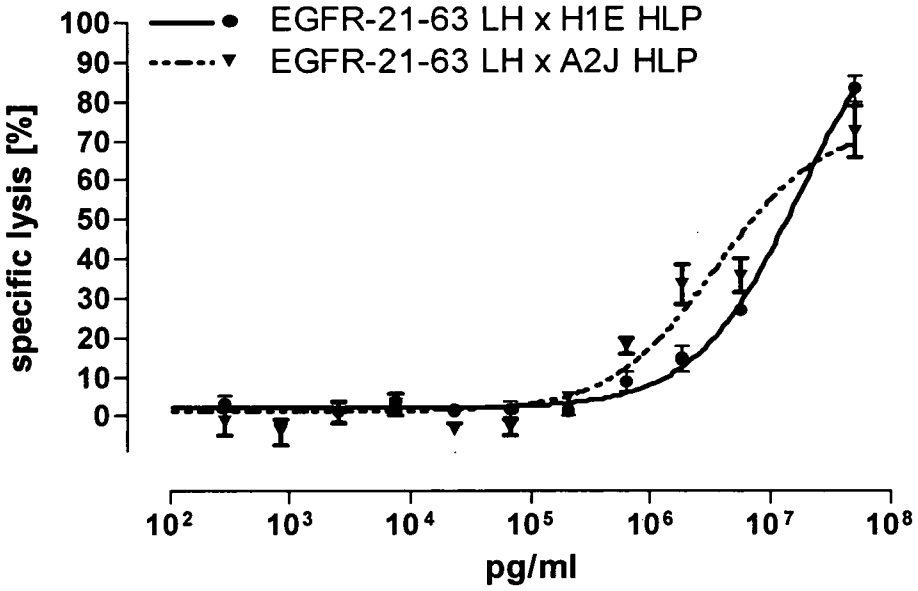


Figure 13

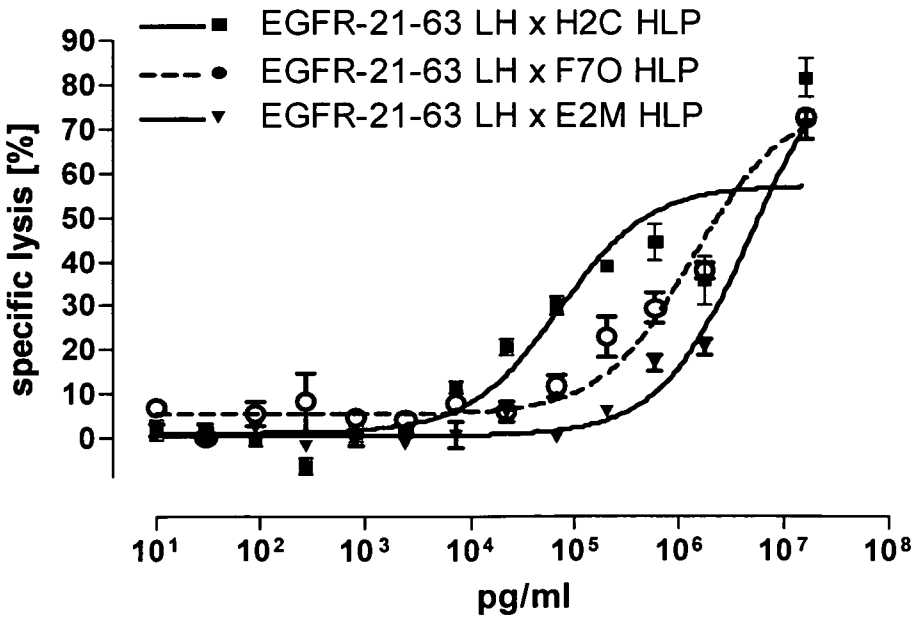
A)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus EGFR



B)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus EGFR



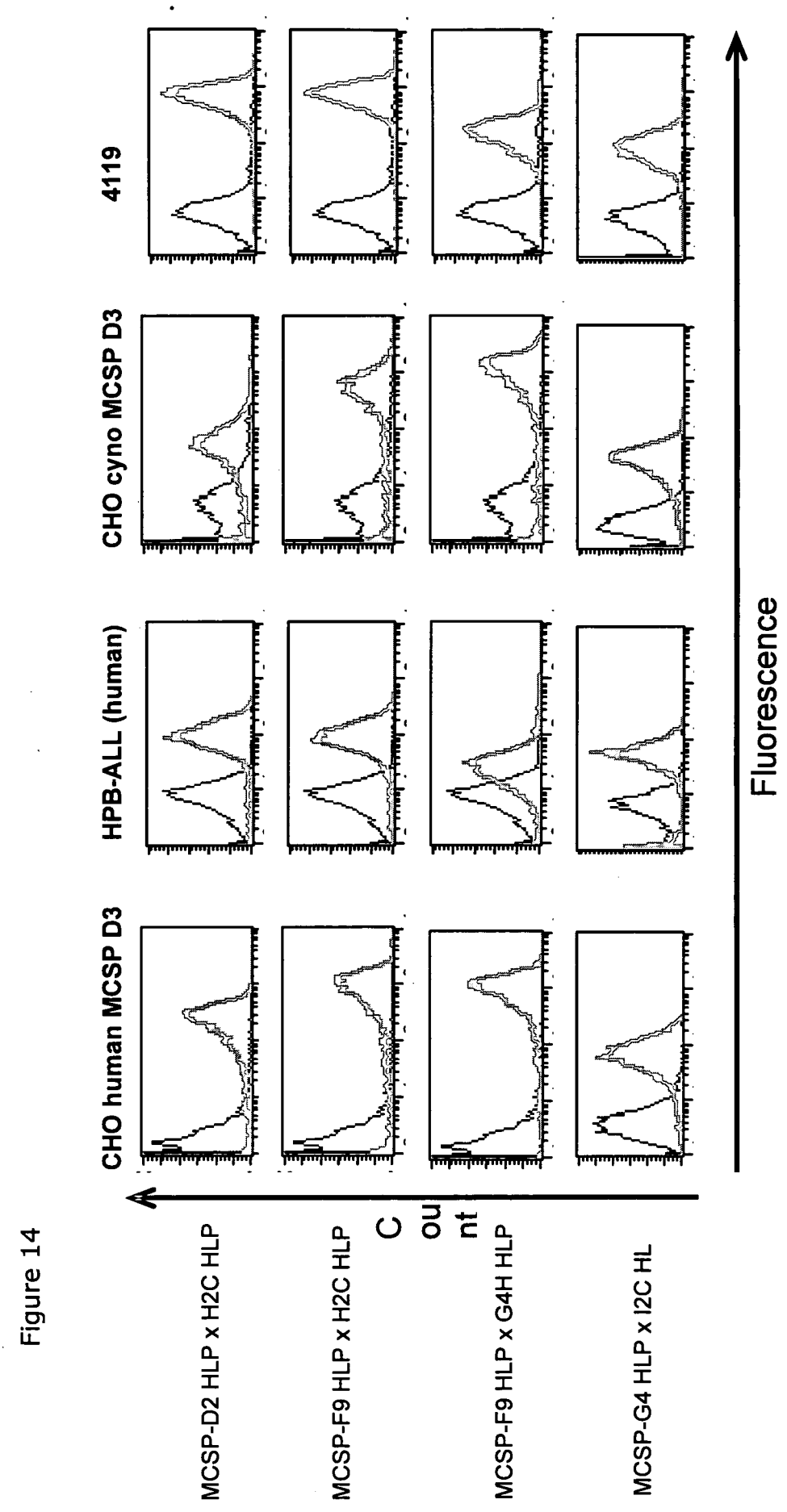

CHO human MCSP D3
HPB-ALL (human)
CHO cyno MCSP D3
4119
MCSP-D2 HLP x H2C HLP
MCSP-F9 HLP x H2C HLP
MCSP-F9 HLP x G4H HLP
MCSP-G4 HLP x I2C HL
Count
Fluorescence

Figure 14

Figure 15

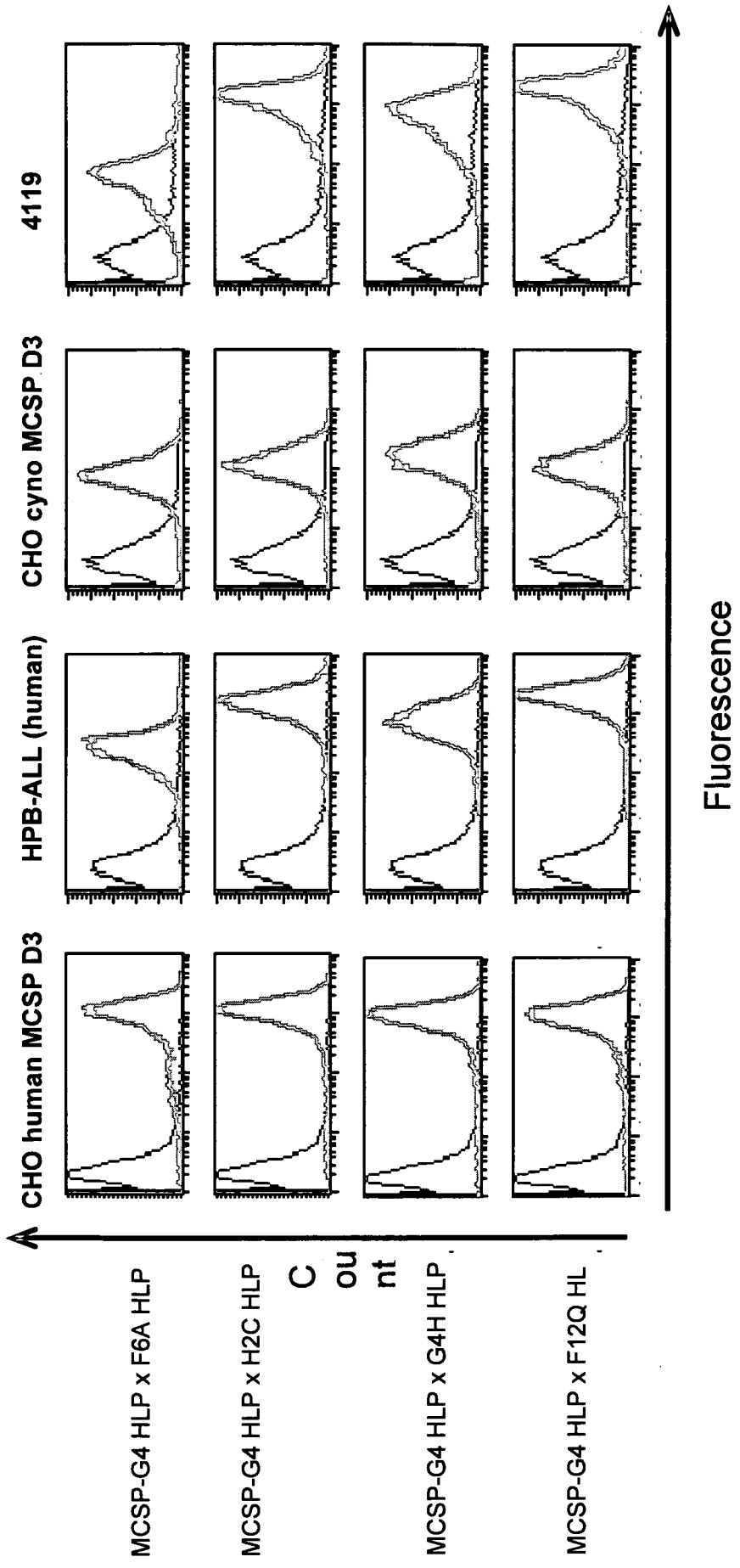

CHO human MCSP D3
HPB-ALL (human)
CHO cyno MCSP D3
4119
MCSP-G4 HLP x F6A HLP
MCSP-G4 HLP x H2C HLP
MCSP-G4 HLP x G4H HLP
MCSP-G4 HLP x F12Q HL
Count
Fluorescence

Figure 16

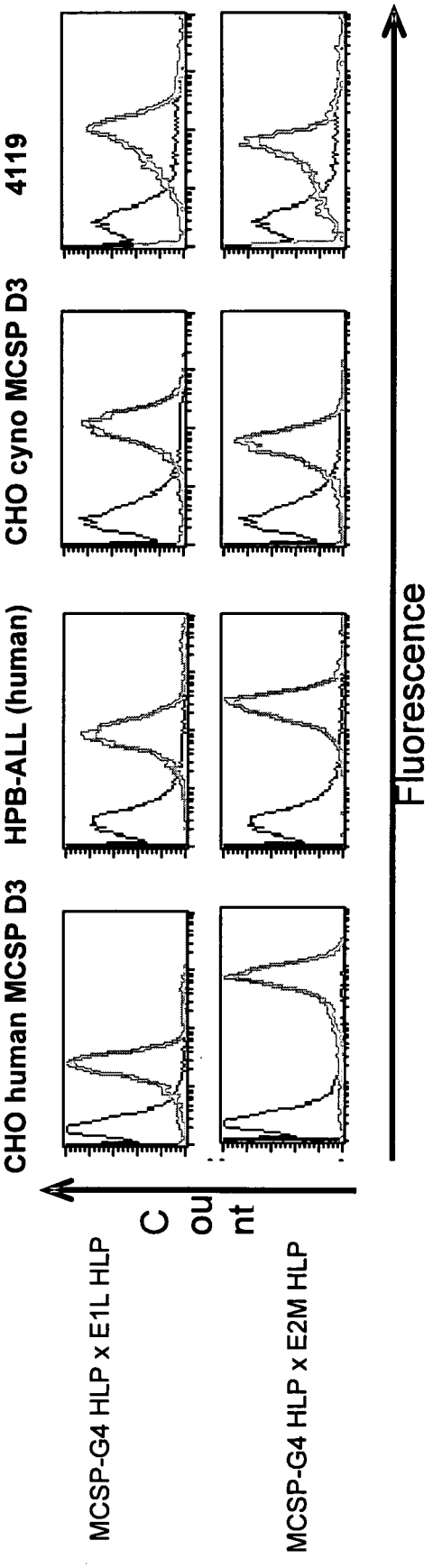
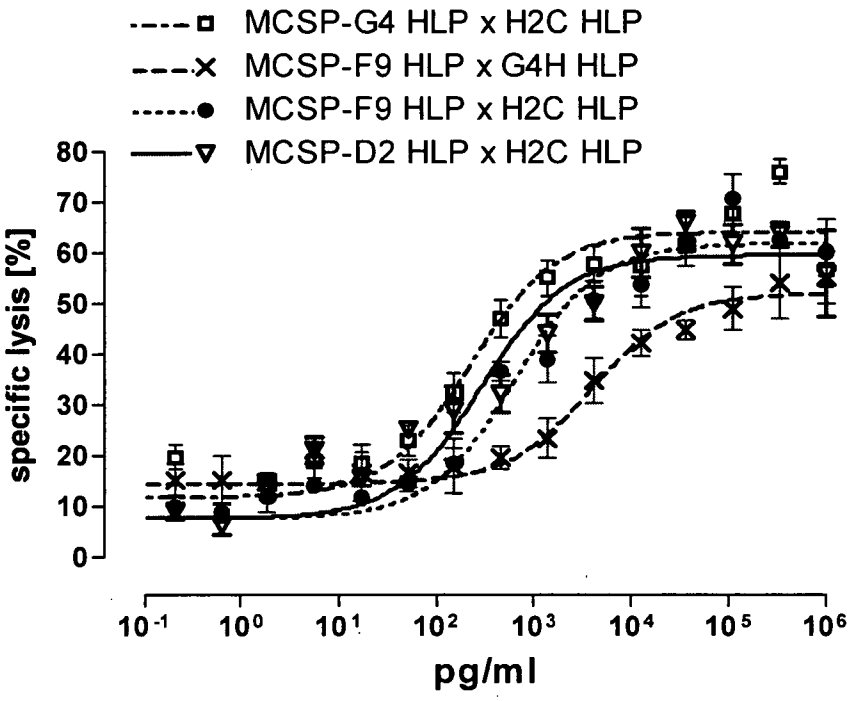

CHO human MCSP D3
HPB-ALL (human)
CHO cyno MCSP D3
4119
MCSP-G4 HLP x E1L HLP
MCSP-G4 HLP x E2M HLP
Count
Fluorescence

Figure 17

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human MCSP D3



B)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus MCSP D3

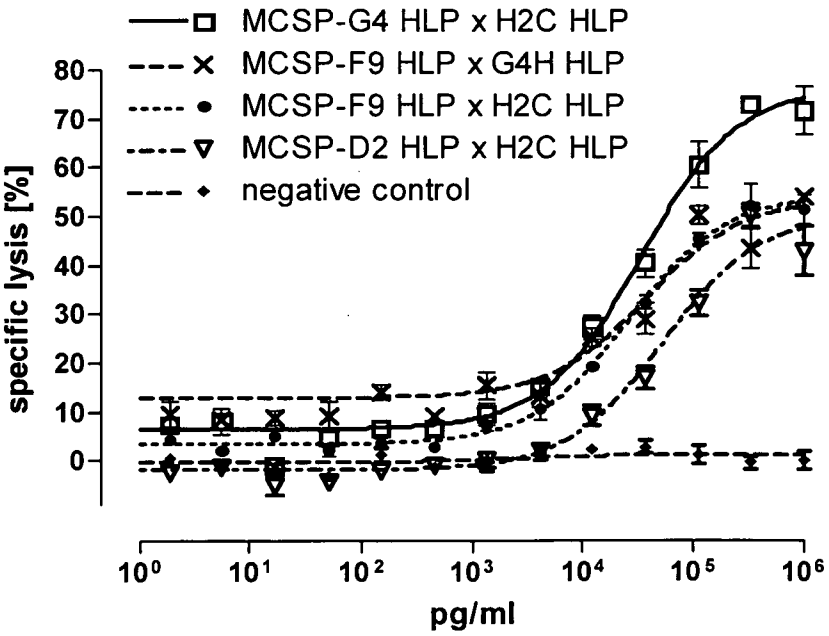
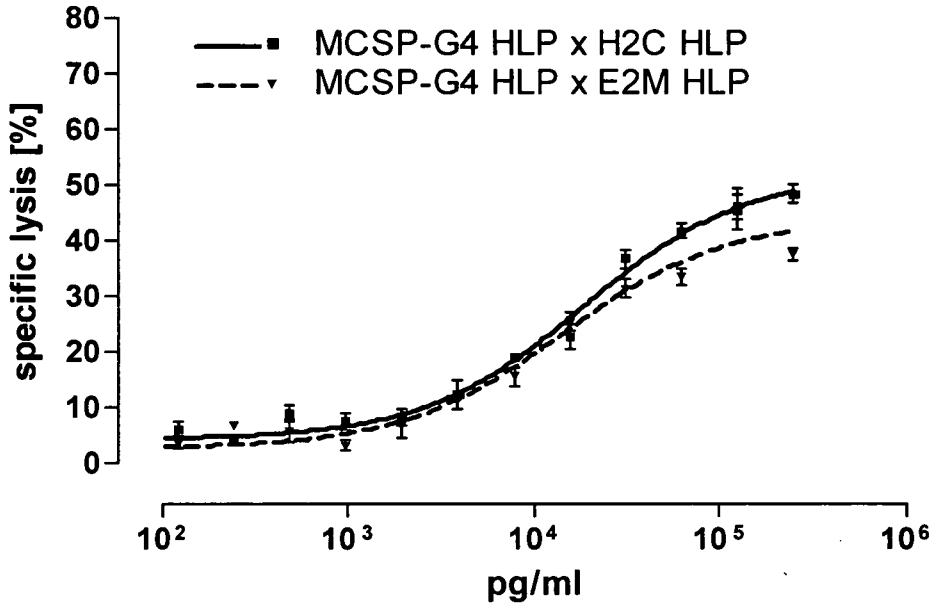


Figure 18

A)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus MCSP D3



B)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus MCSP D3

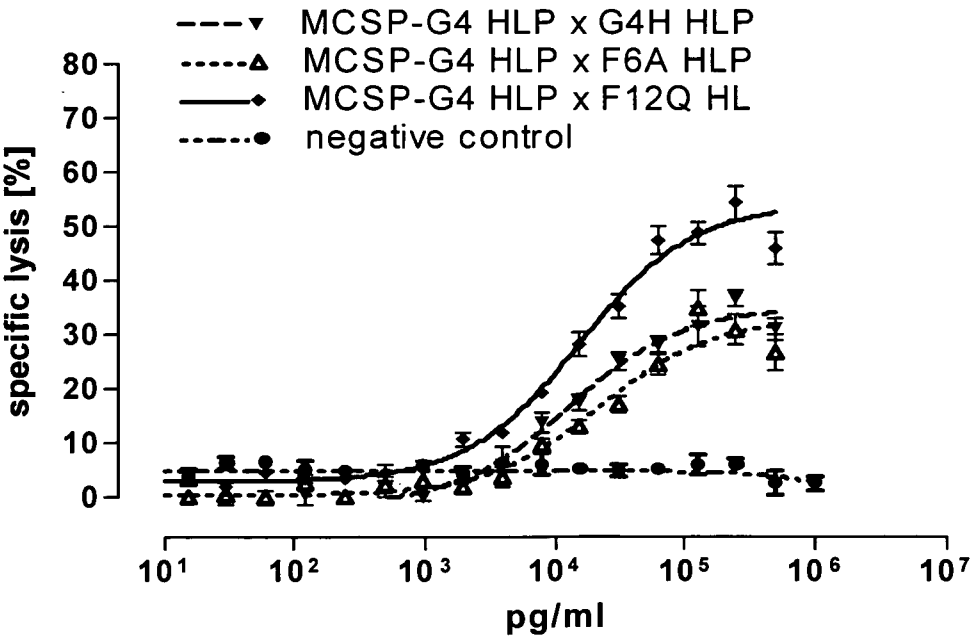
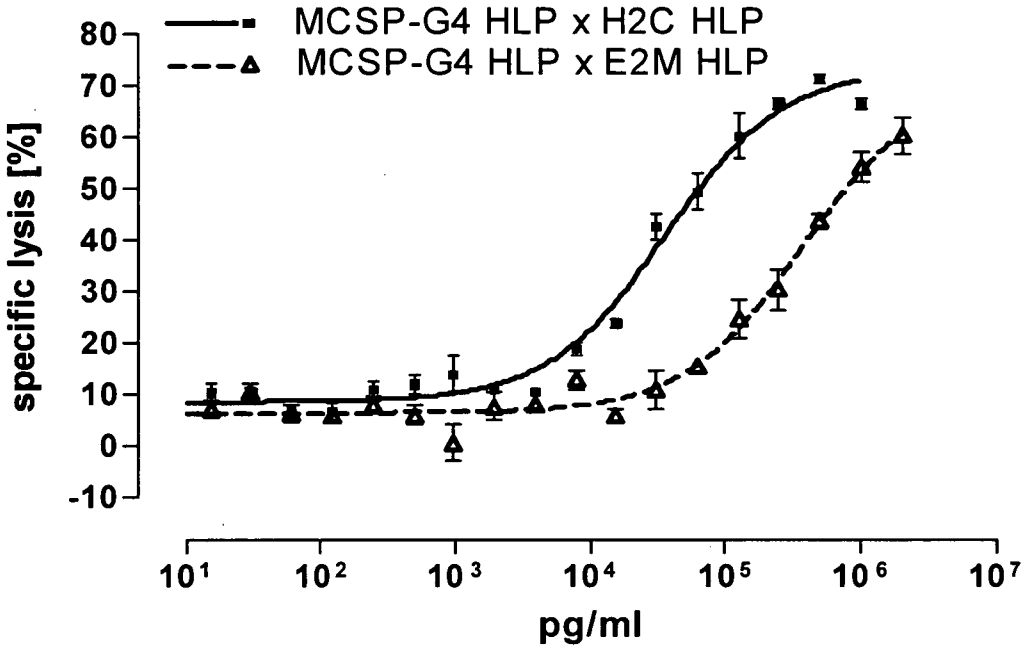


Figure 19

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human MCSP D3



B)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human MCSP D3

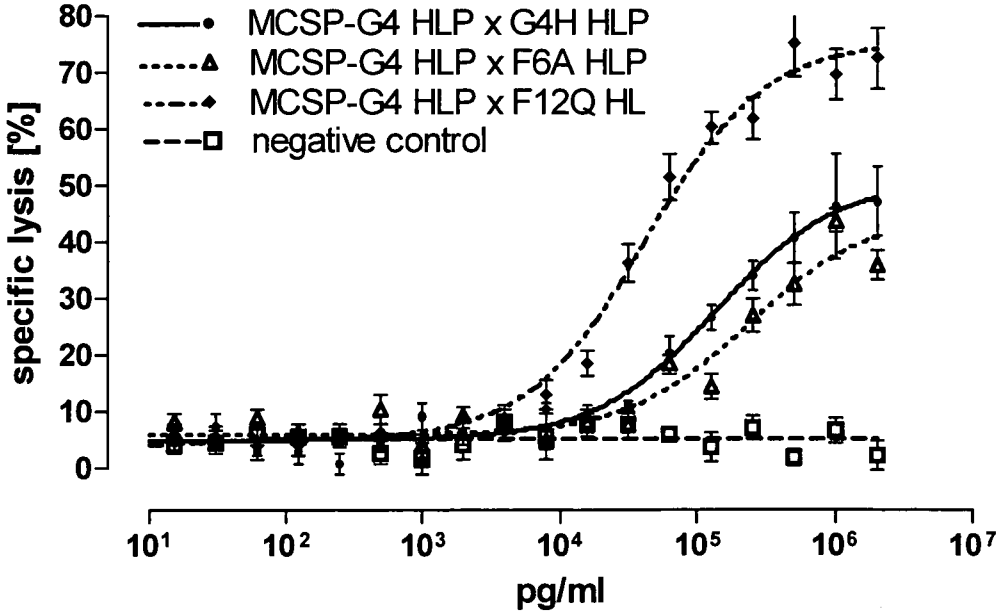
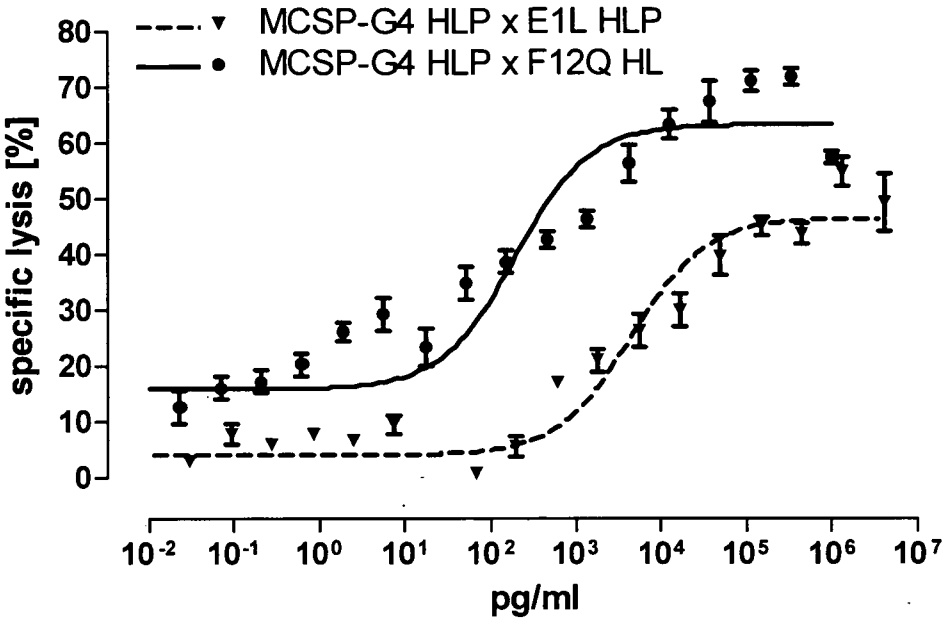


Figure 20

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human MCSP D3



B)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus MCSP D

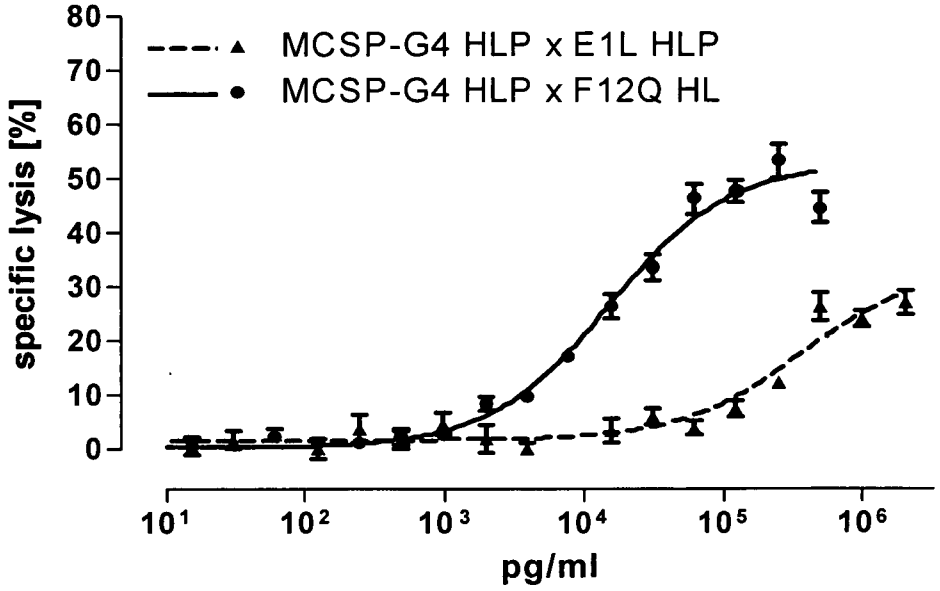
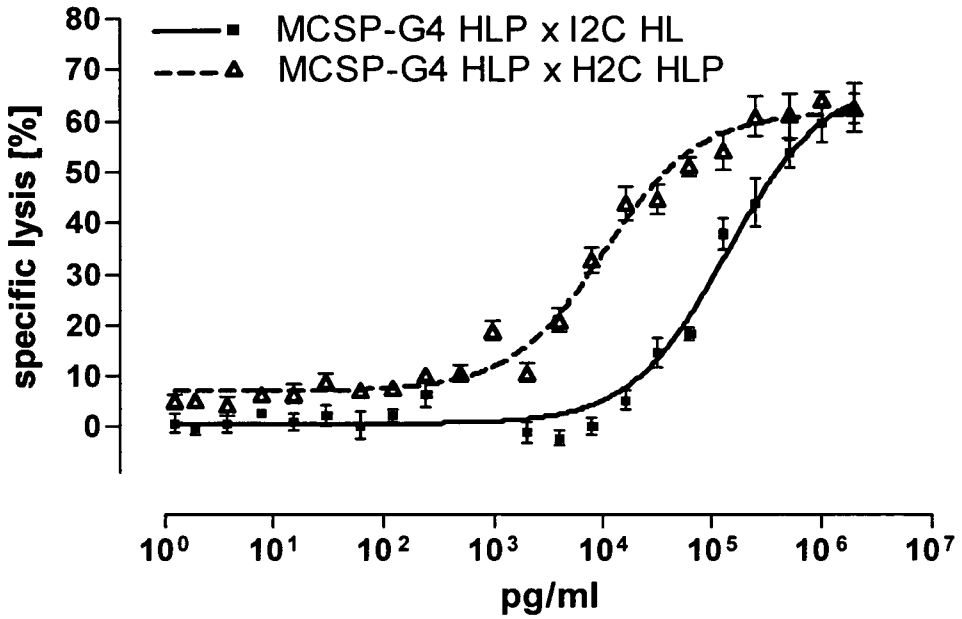


Figure 21

A)
Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human MCSP D3



B)

Effector cells: macaque T cell line 4119 LnPx
Target cells: CHO transfected with cynomolgus MCSP D3

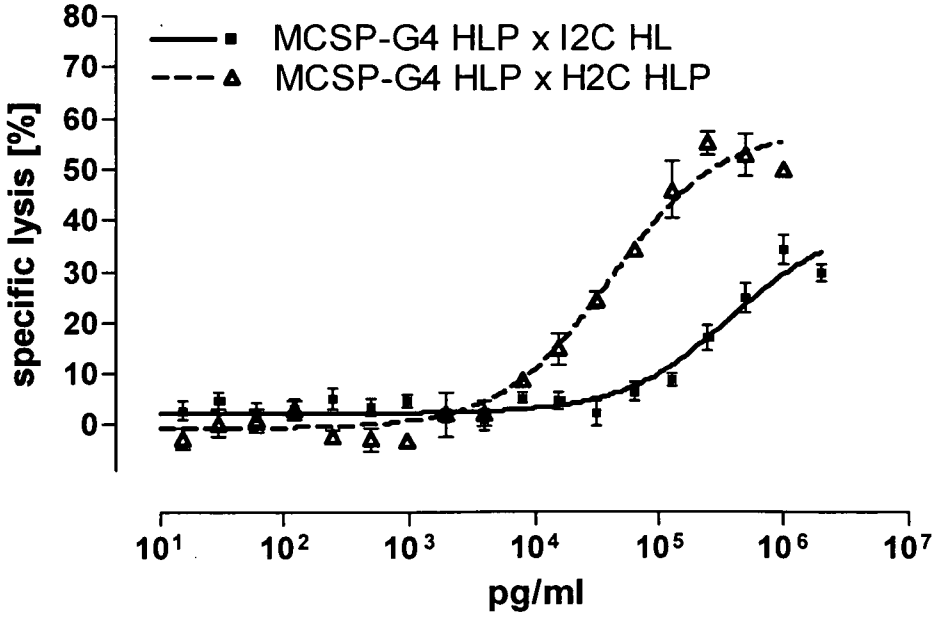


Figure 22 (1)

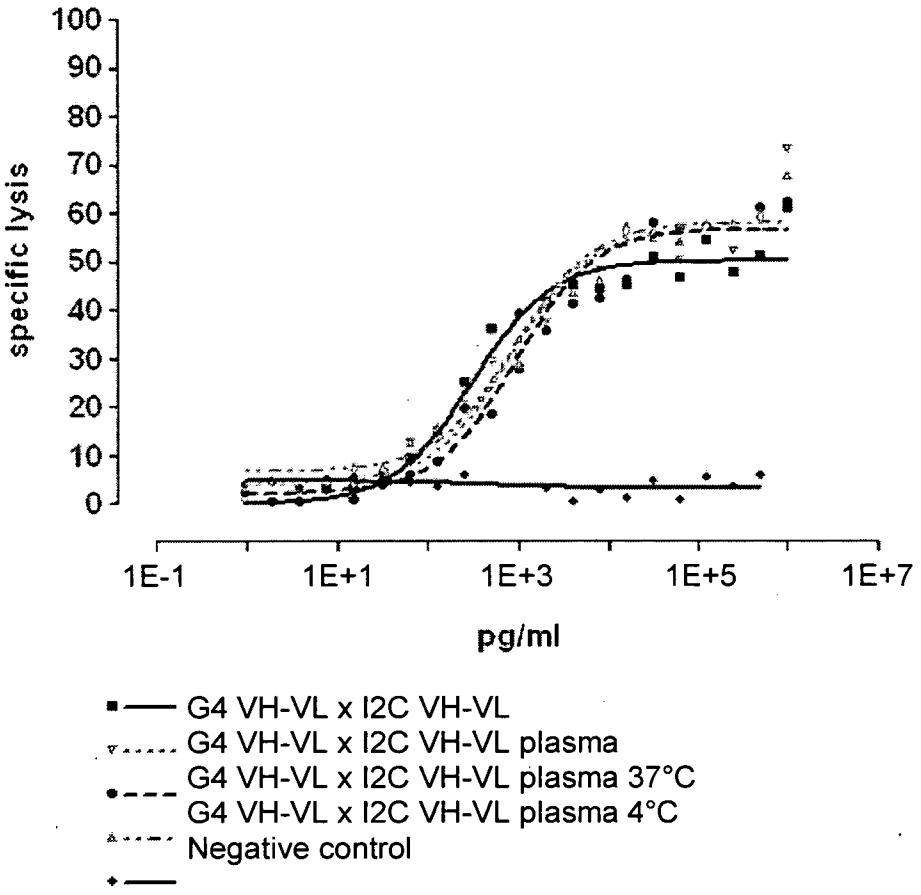

100
90
80
70
60
50
40
30
20
10
0
specific lysis
1E-1
1E+1
1E+3
1E+5
1E+7
pg/ml
G4 VH-VL x I2C VH-VL
G4 VH-VL x I2C VH-VL plasma
G4 VH-VL x I2C VH-VL plasma 37°C
G4 VH-VL x I2C VH-VL plasma 4°C
Negative control

Figure 22 (2)

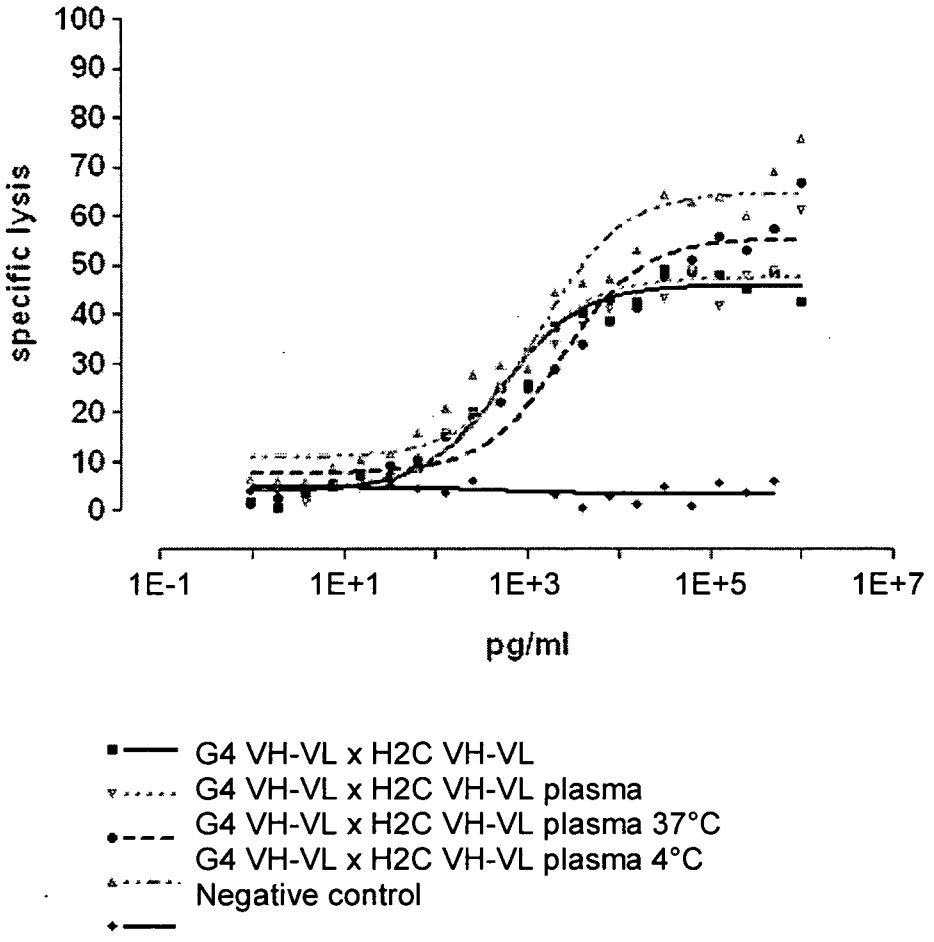
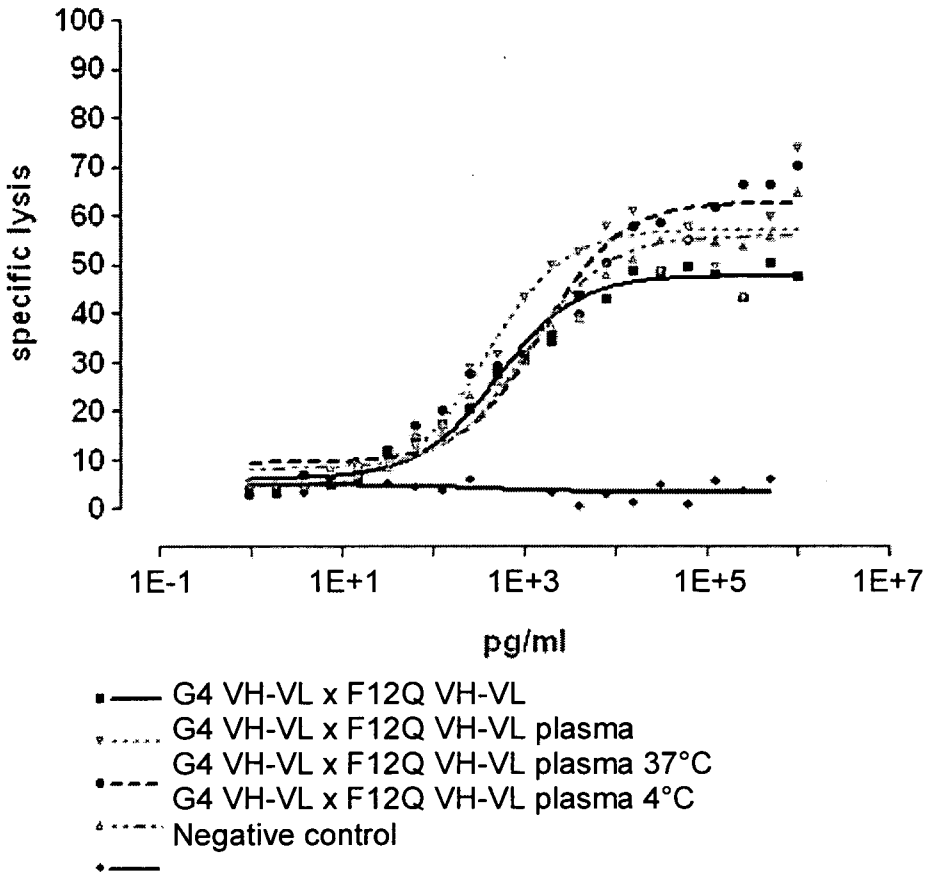

100
90
80
70
60
50
40
30
20
10
0
specific lysis
1E-1
1E+1
1E+3
1E+5
1E+7
pg/ml
G4 VH-VL x H2C VH-VL
G4 VH-VL x H2C VH-VL plasma
G4 VH-VL x H2C VH-VL plasma 37°C
G4 VH-VL x H2C VH-VL plasma 4°C
Negative control

Figure 22 (3)


100
90
80
70
60
50
40
30
20
10
0
specific lysis
1E-1
1E+1
1E+3
1E+5
1E+7
pg/ml
G4 VH-VL x F12Q VH-VL
G4 VH-VL x F12Q VH-VL plasma
G4 VH-VL x F12Q VH-VL plasma 37°C
G4 VH-VL x F12Q VH-VL plasma 4°C
Negative control

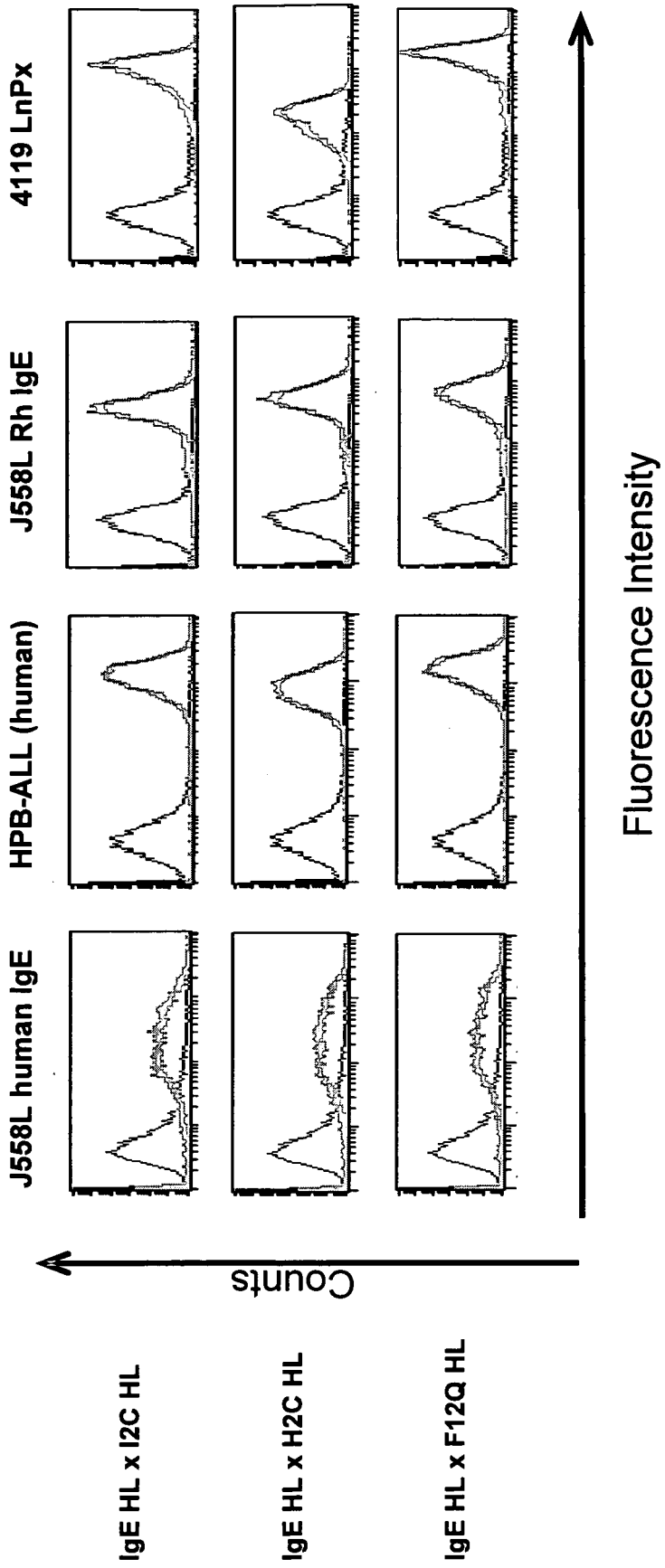

J558L human IgE
HPB-ALL (human)
J558L Rh IgE
4119 LnPx
IgE HL x I2C HL
IgE HL x H2C HL
IgE HL x F12Q HL
Counts
Fluorescence Intensity

Figure: 23 a

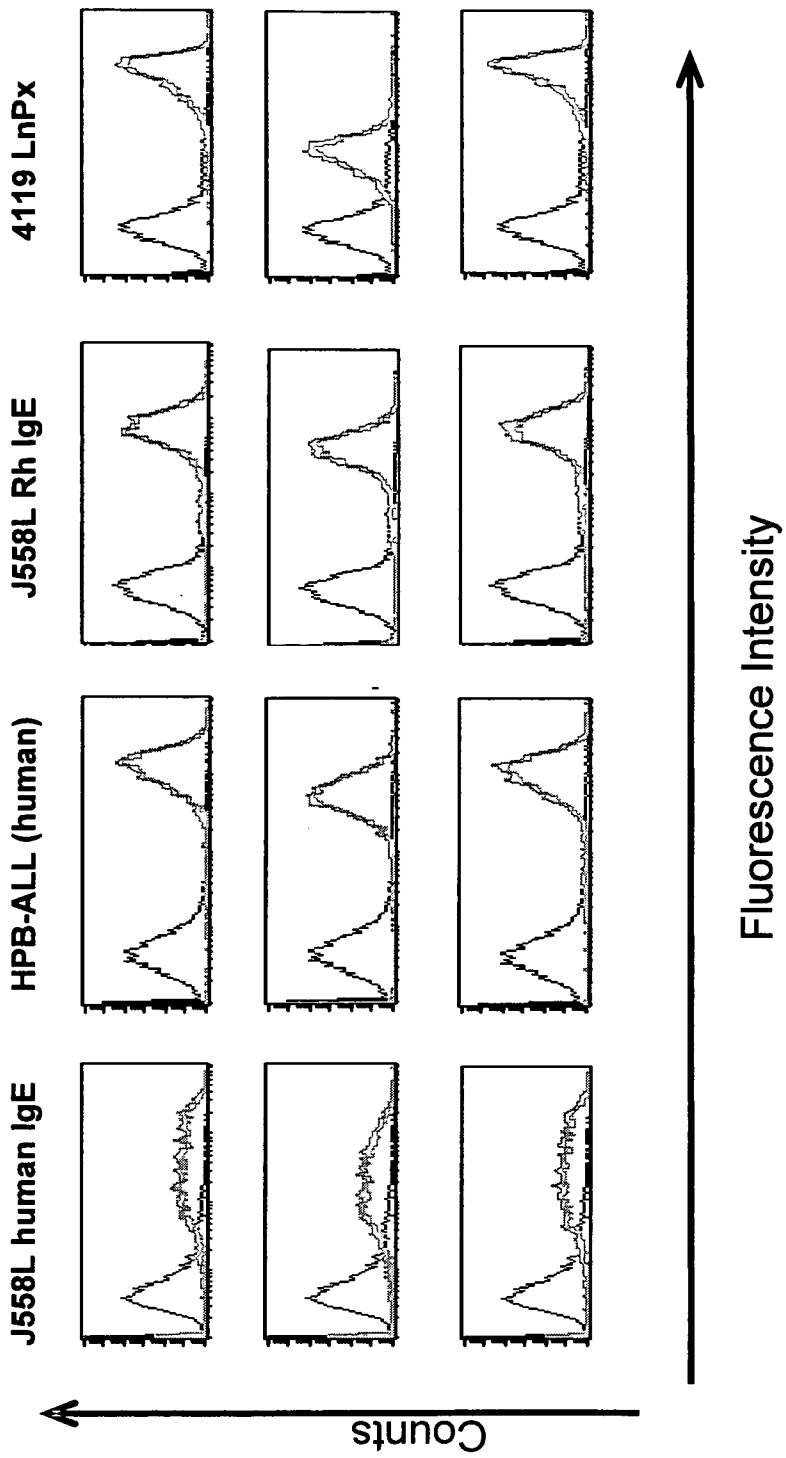
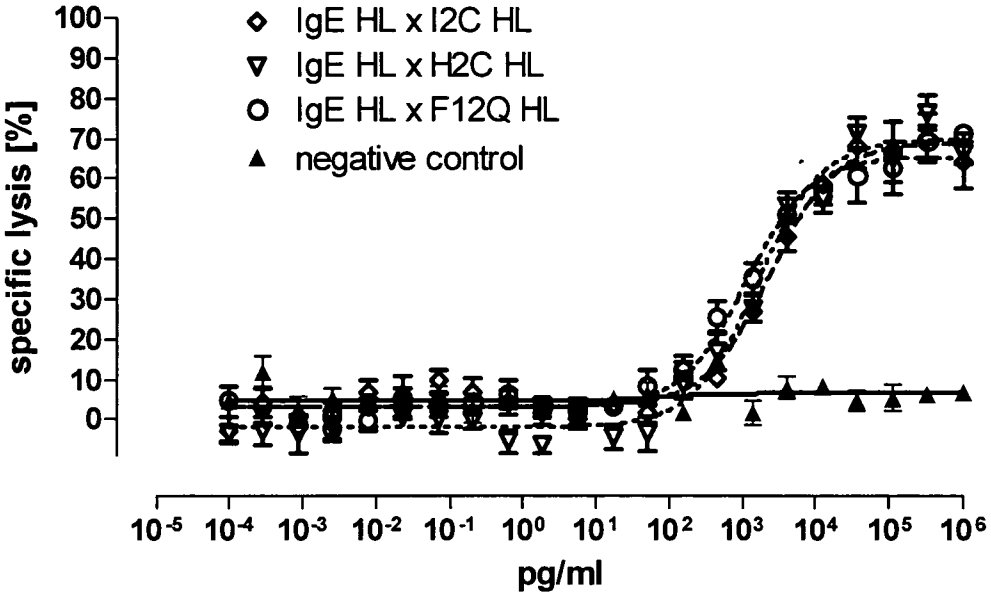

J558L human IgE
HPB-ALL (human)
J558L Rh IgE
4119 LnPx
IgE LH x I2C HL
IgE LH x H2C HL
IgE LH x F12Q HL
Counts
Fluorescence Intensity

Figure: 23 b

Figure 24 a

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: J558L cells transfected with Human IgE



B)

Effector cells: 4119 LnPx
Target cells: J558L cells transfected with macaque IgE

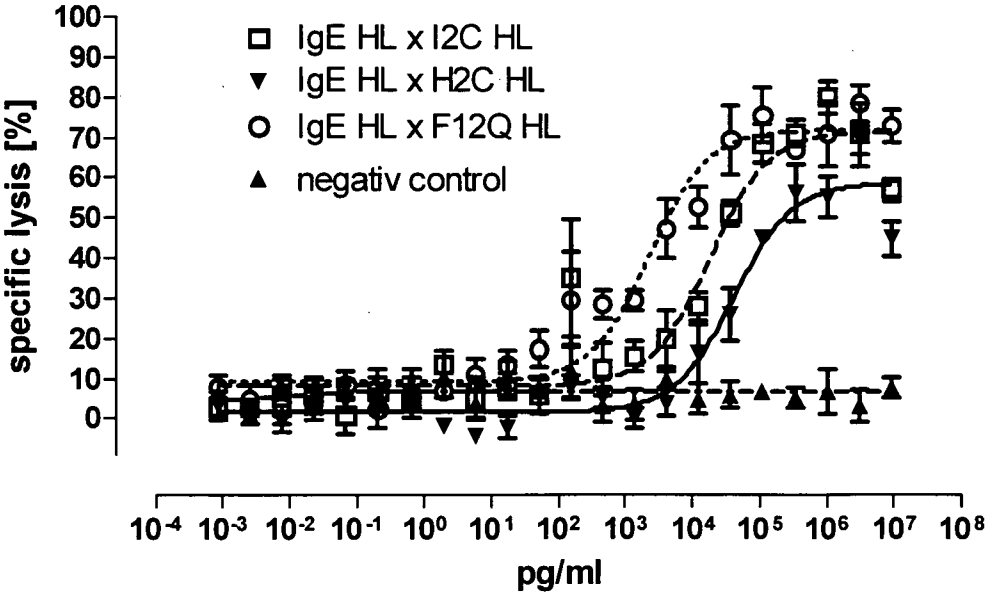
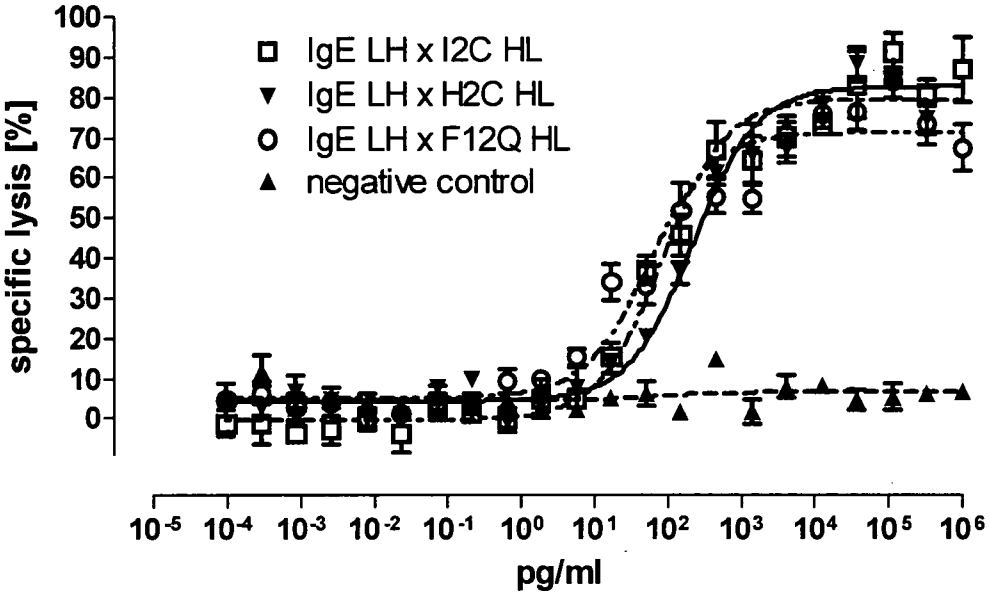


Figure 24 b

A)

Effector cells: stimulated CD4/CD56 depleted human PBMC
Target cells: J558L cells transfected with Human IgE



B)

Effector cells: 4119 LnPx
Target cells: J558L cells transfected with macaque IgE

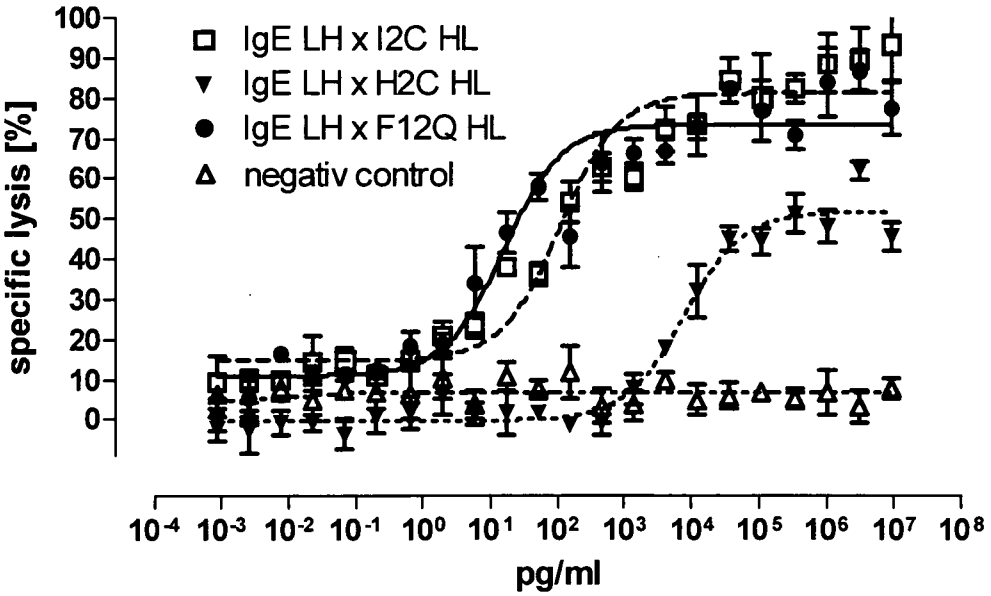


Figure 25

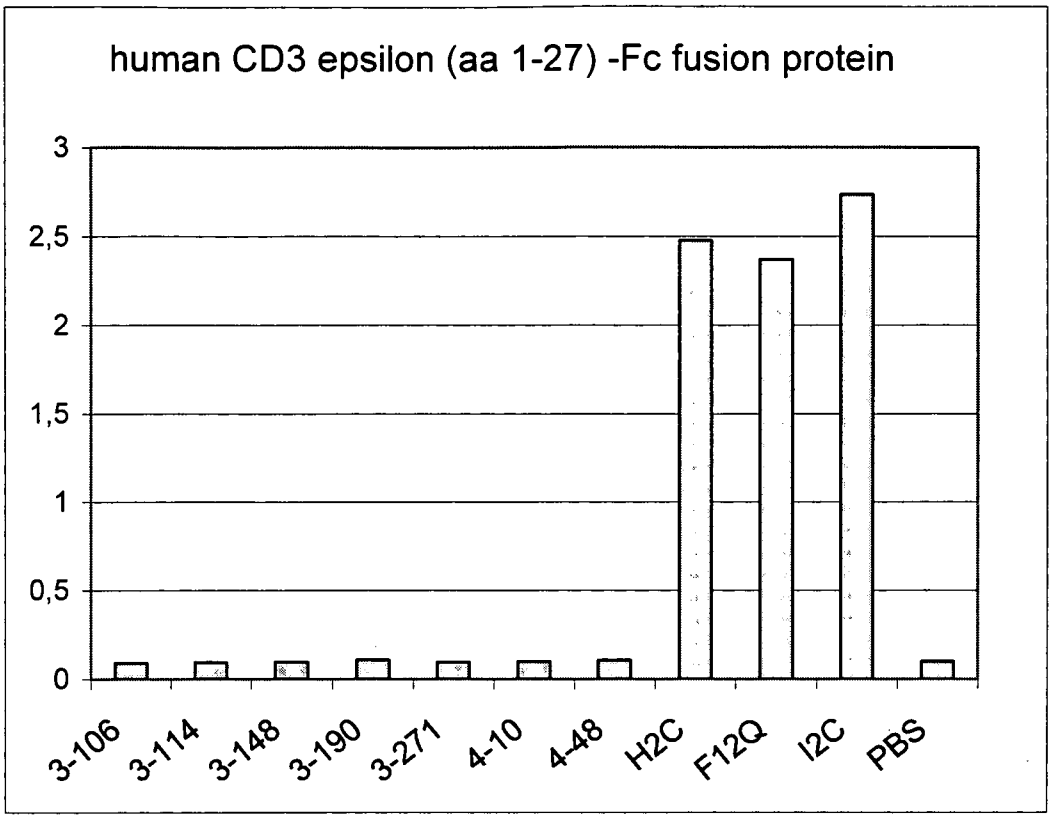


Figure 26

