Figure 1:

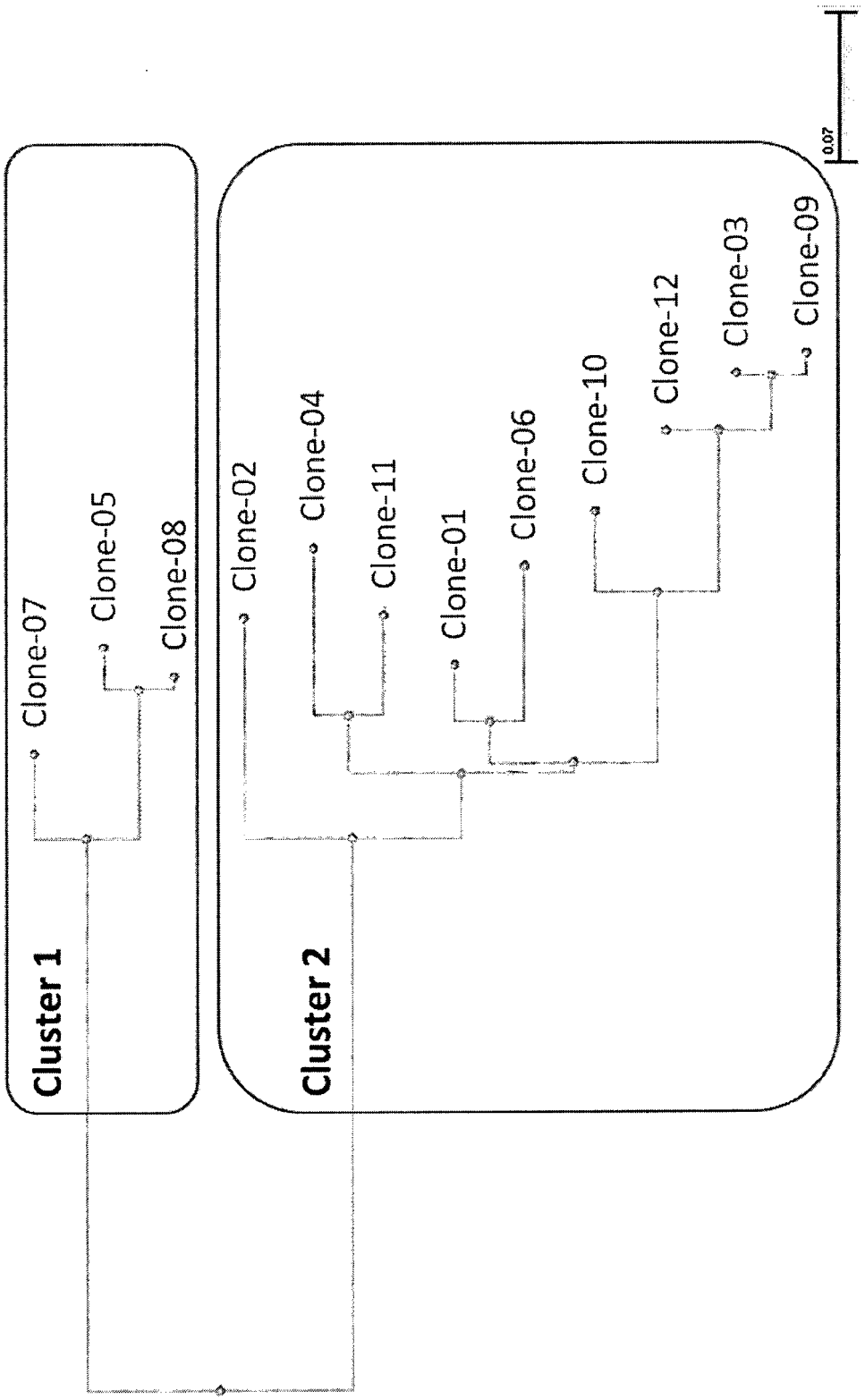

Cluster 1
Clone-07
Clone-05
Clone-08
Cluster 2
Clone-02
Clone-04
Clone-11
Clone-01
Clone-06
Clone-10
Clone-12
Clone-03
Clone-09
0.07

Figure 2:

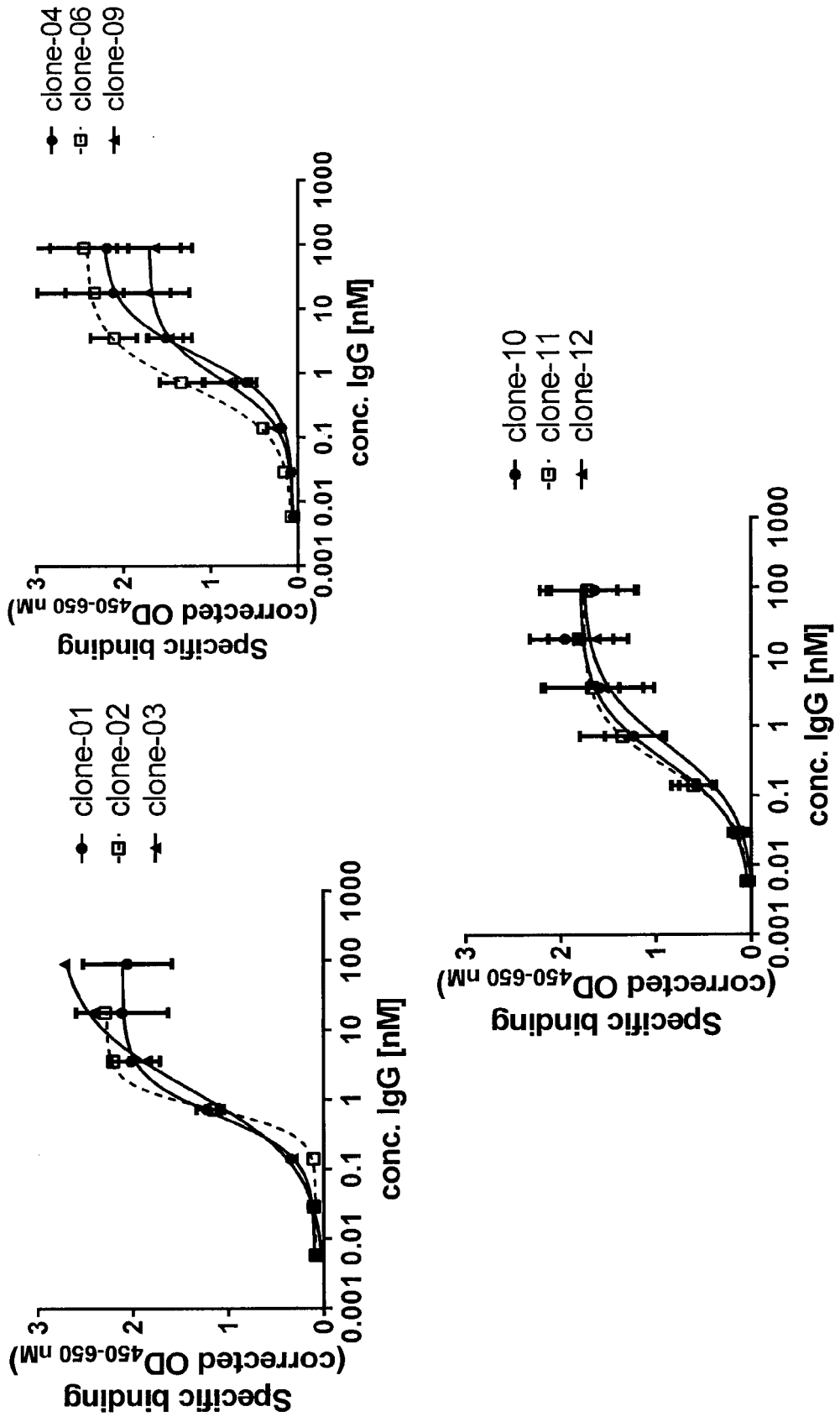

clone-01
clone-02
clone-03
clone-04
clone-06
clone-09
clone-10
clone-11
clone-12
Specific binding
(corrected OD450-650 nM)
conc. IgG [nM]
0.001
0.01
0.1
1
10
100
1000
0
1
2
3

Figure 3:

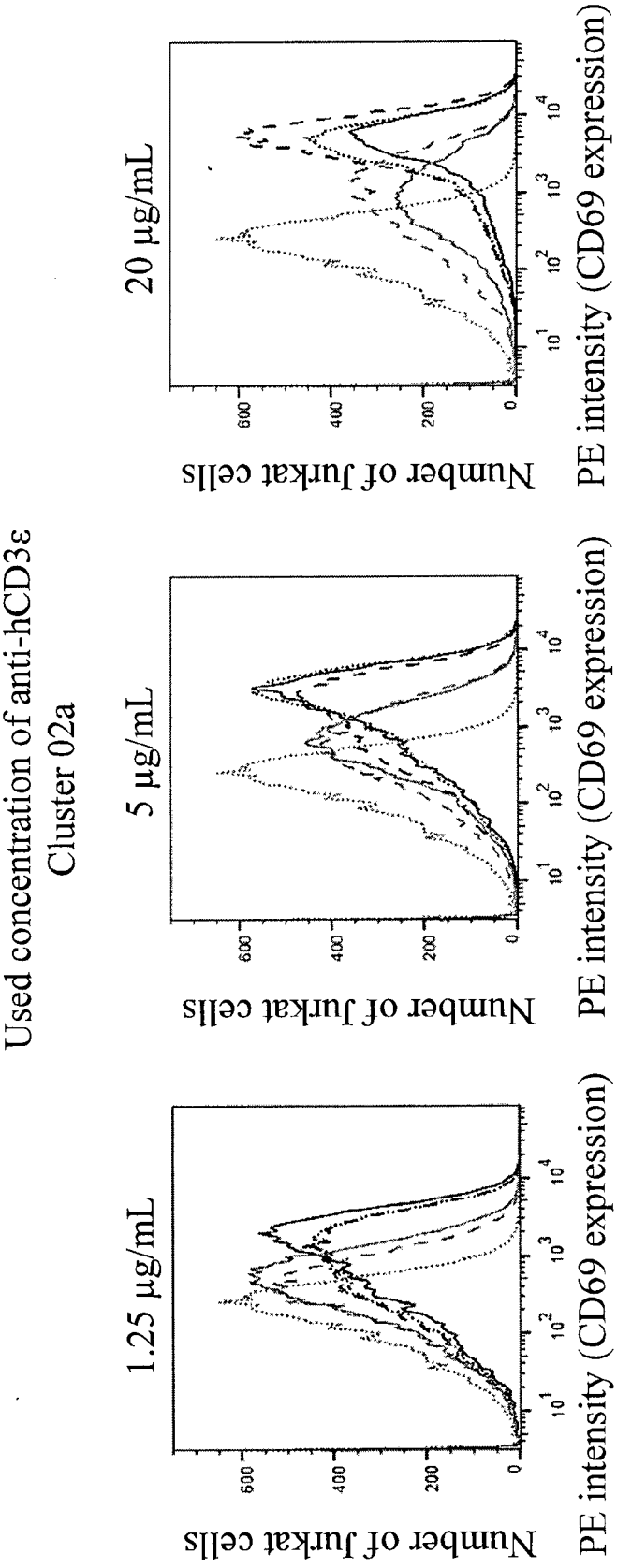

Used concentration of anti-hCD3ε
Cluster 02a
1.25 µg/mL
5 µg/mL
20 µg/mL
Number of Jurkat cells
PE intensity (CD69 expression)
Untreated
clone-03
TR66
clone-04
OKT3
clone-02

Figure 3 (contd.):

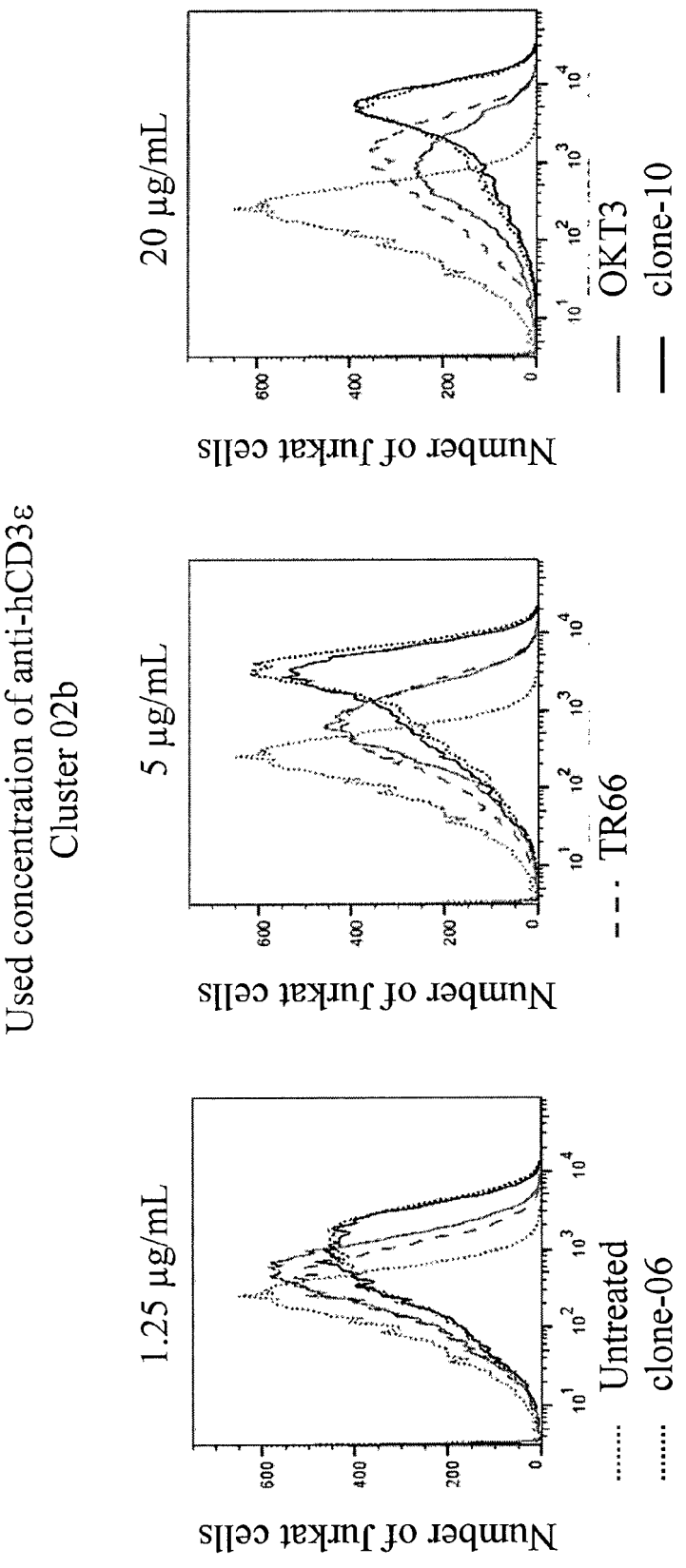
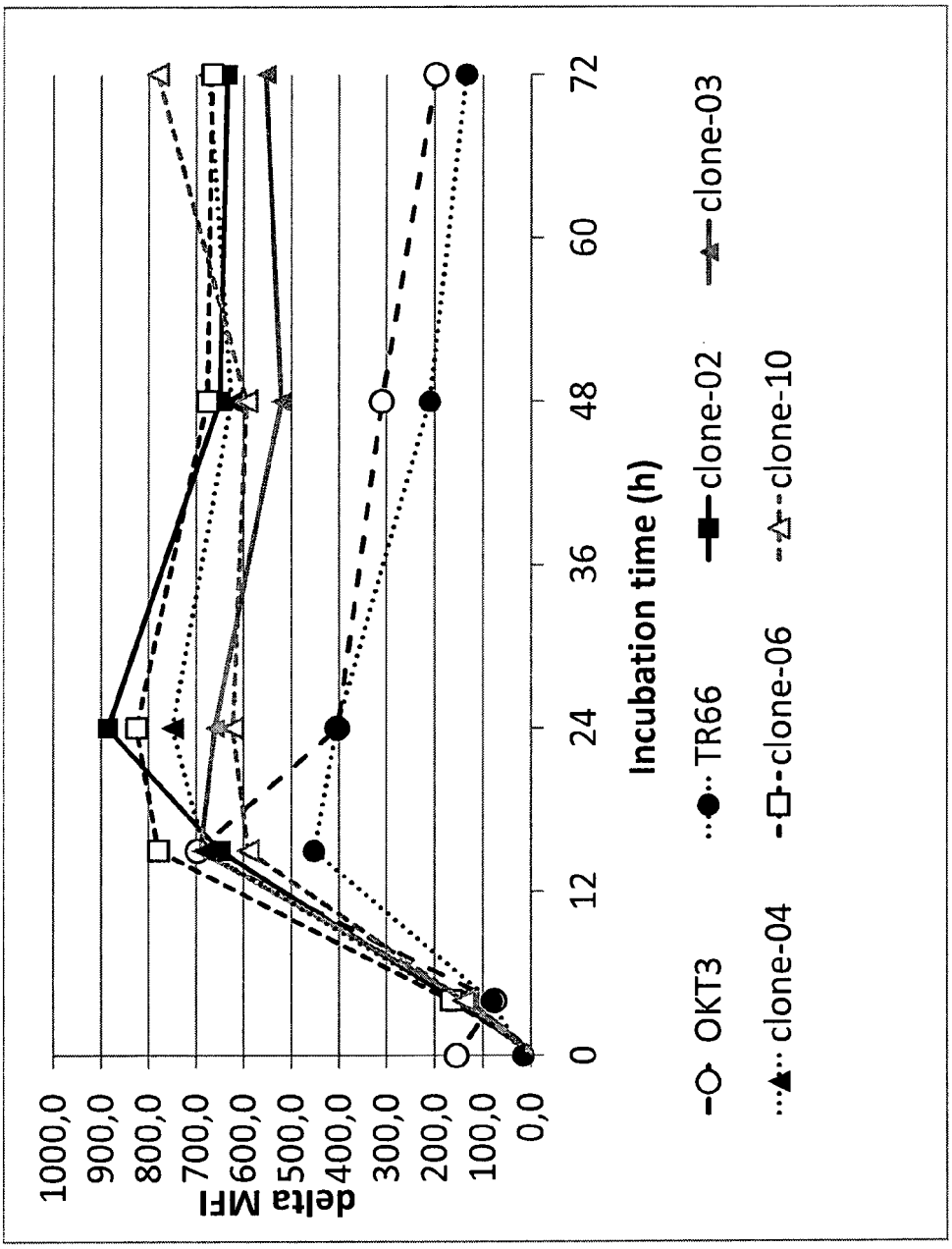


Figure 4:


delta MFI
1000,0
900,0
800,0
700,0
600,0
500,0
400,0
300,0
200,0
100,0
0,0
0
12
24
36
48
60
72
Incubation time (h)
OKT3
TR66
clone-02
clone-03
clone-04
clone-06
clone-10

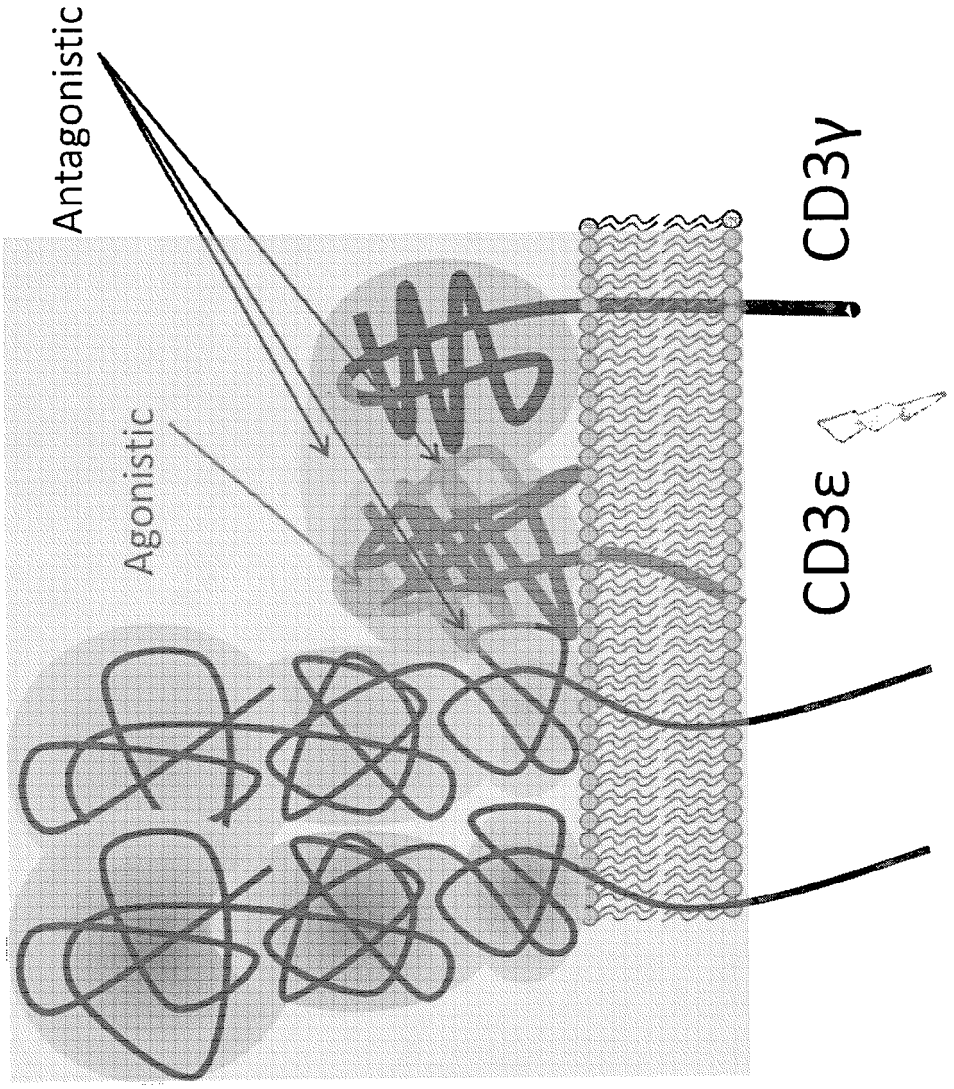
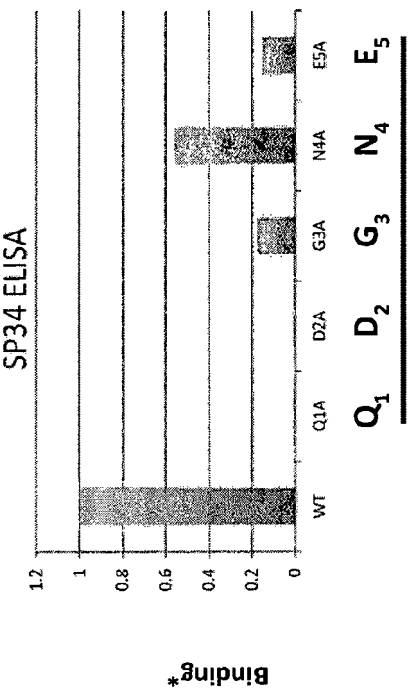


Figure 5:

Figure 6:

(a)


SP34 ELISA
Binding*
1.2
1
0.8
0.6
0.4
0.2
0
WT
Q1A
D2A
G3A
N4A
E5A
Q1
D2
G3
N4
E5

(b)

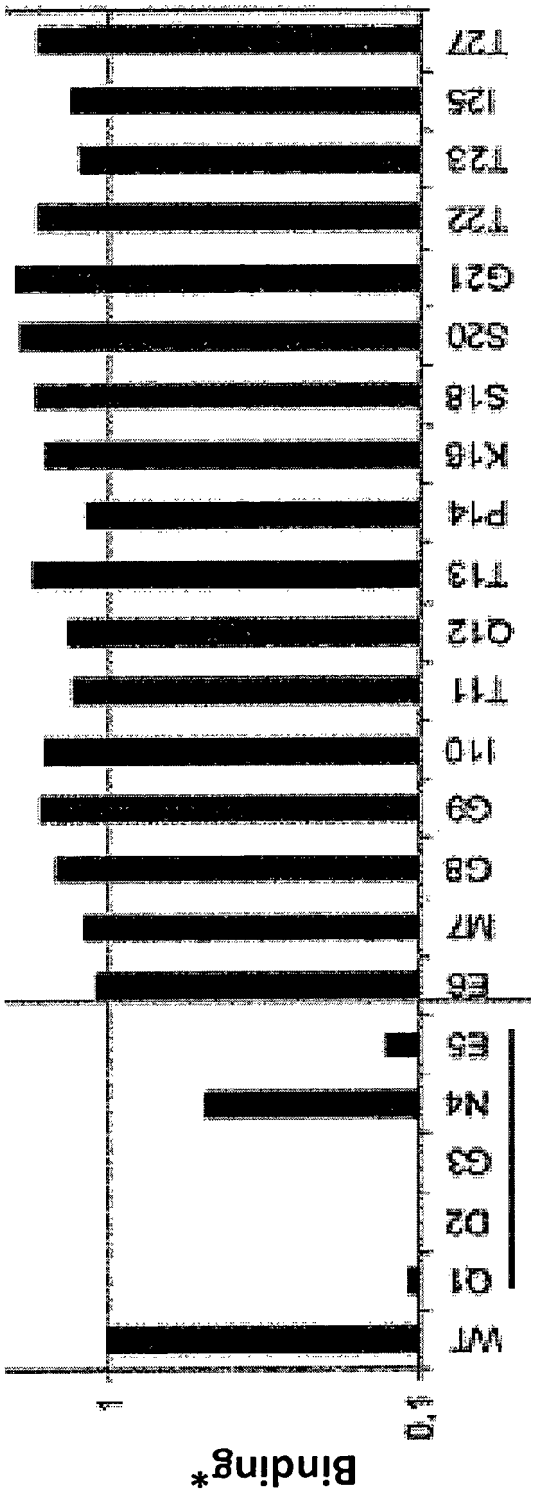
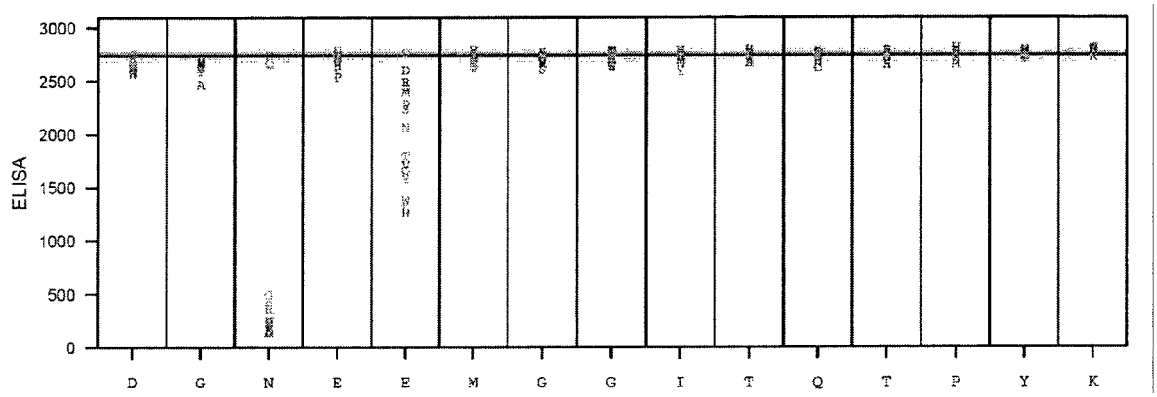
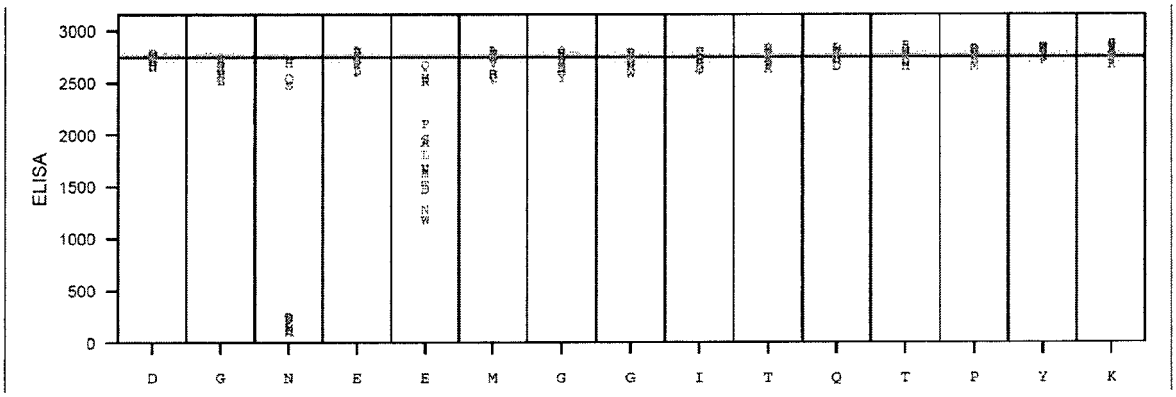

Binding*
WT
Q1
D2
G3
N4
E5
E6
M7
G8
G9
I10
T11
Q12
T13
P14
K15
S18
S20
G21
T22
T23
I25
T27

Figure 7A:

Clone 6



Clone 2



Clone 3

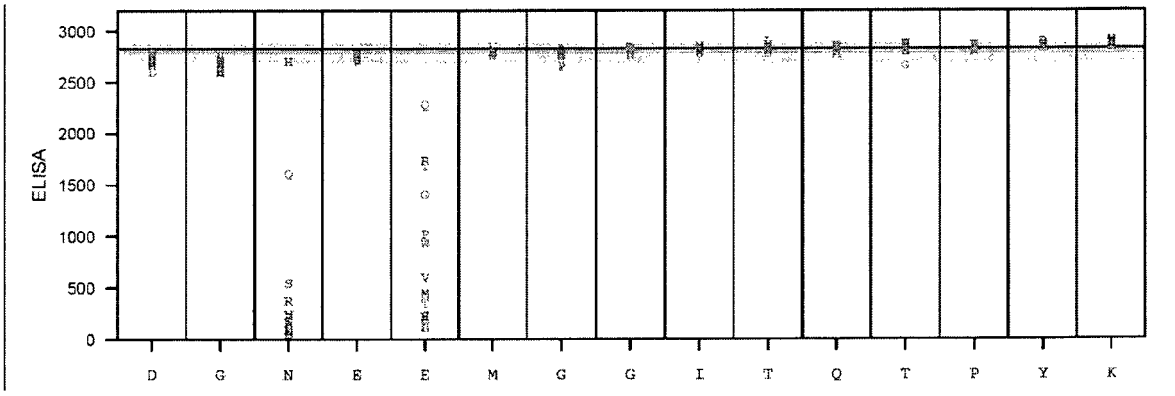
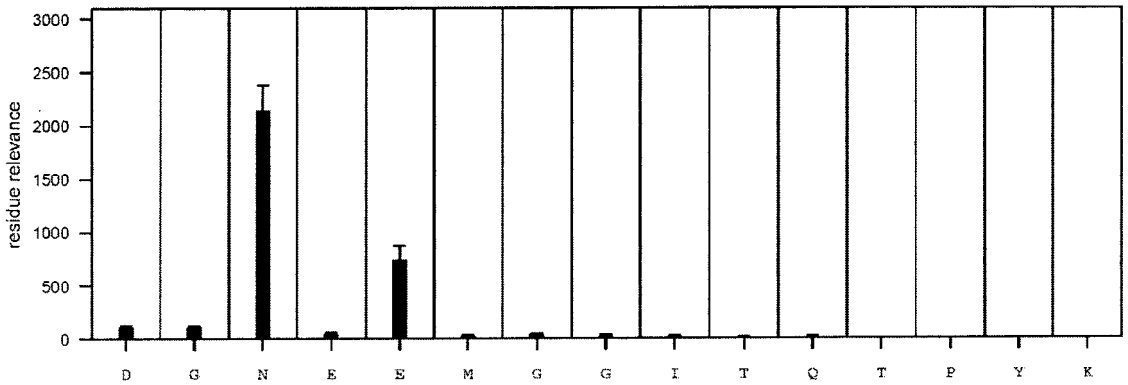
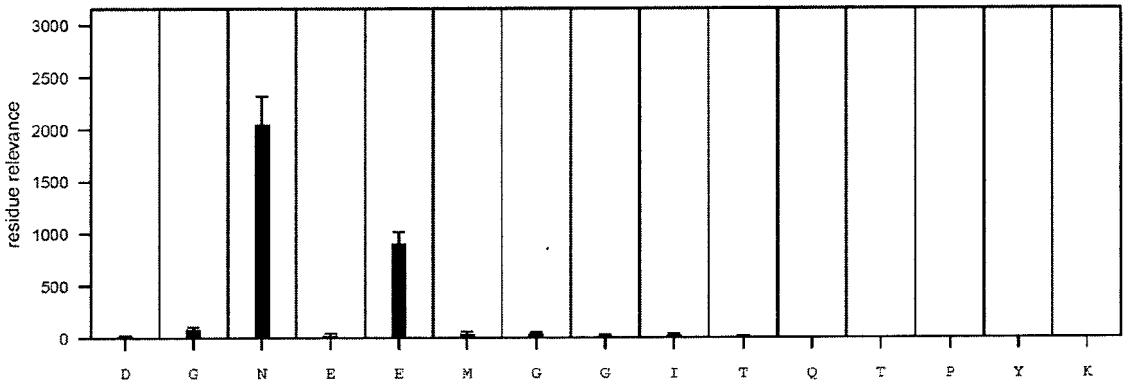


Figure 7B

Clone 6



Clone 2



Clone 3

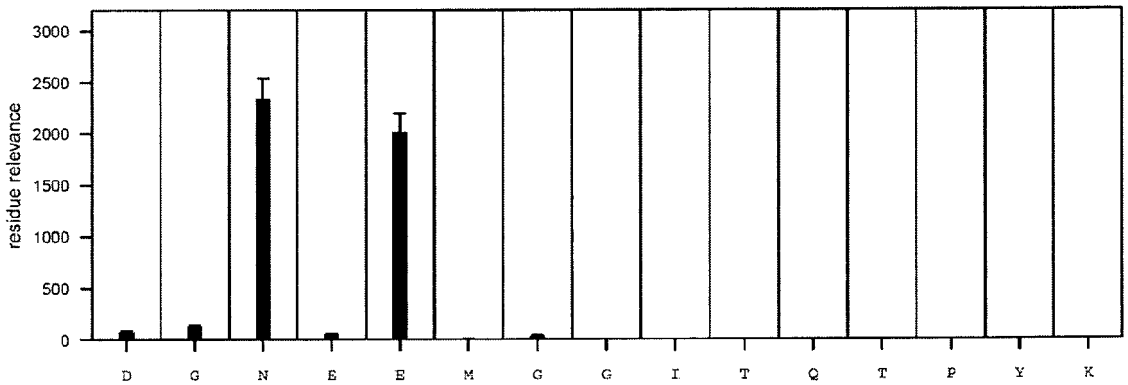


Figure 8:

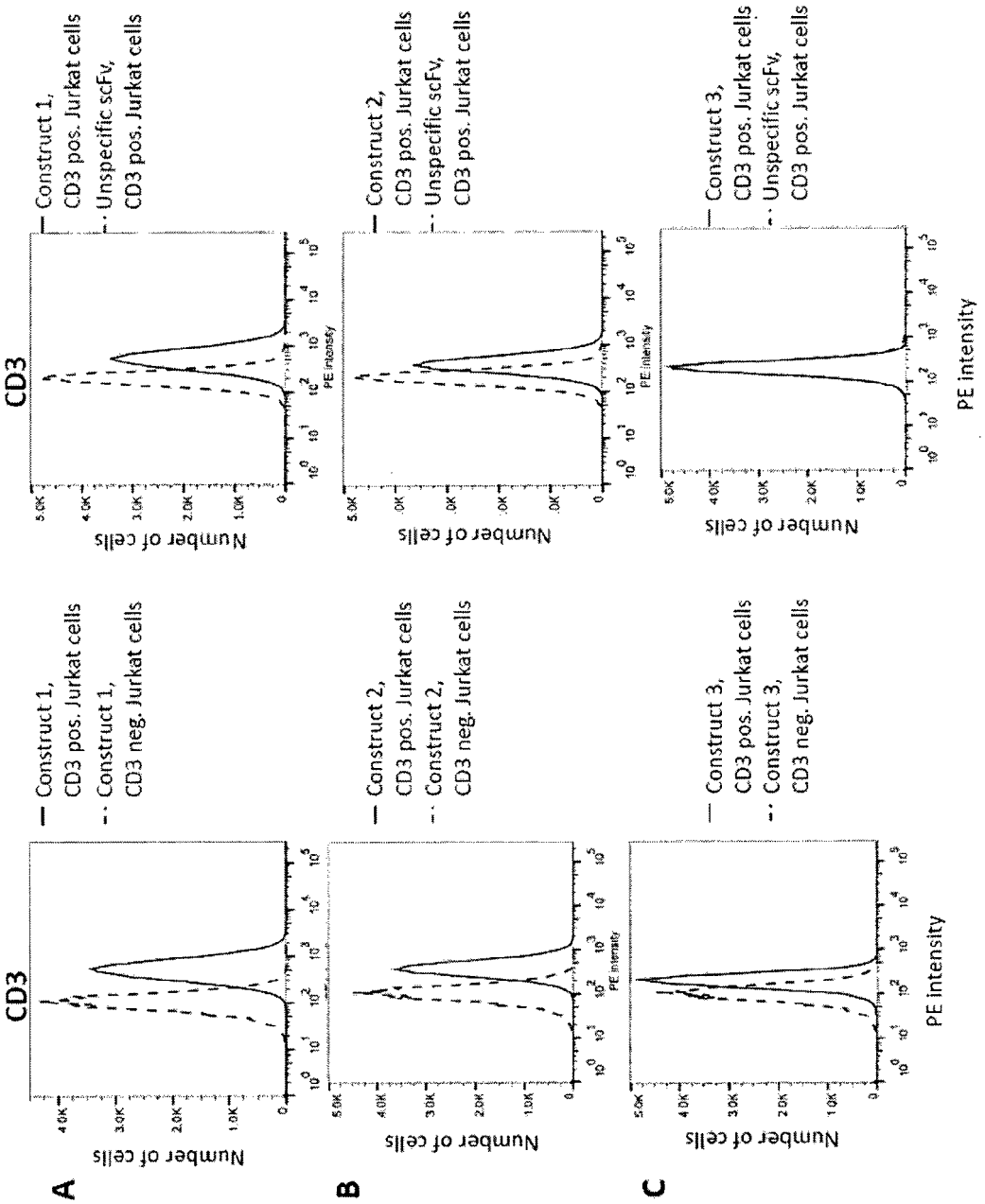

A
B
C
CD3
Construct 1, CD3 pos. Jurkat cells
Construct 1, CD3 neg. Jurkat cells
Construct 2, CD3 pos. Jurkat cells
Construct 2, CD3 neg. Jurkat cells
Construct 3, CD3 pos. Jurkat cells
Construct 3, CD3 neg. Jurkat cells
Unspecific scFv, CD3 pos. Jurkat cells
Number of cells
PE intensity

Figure 8 (contd.):

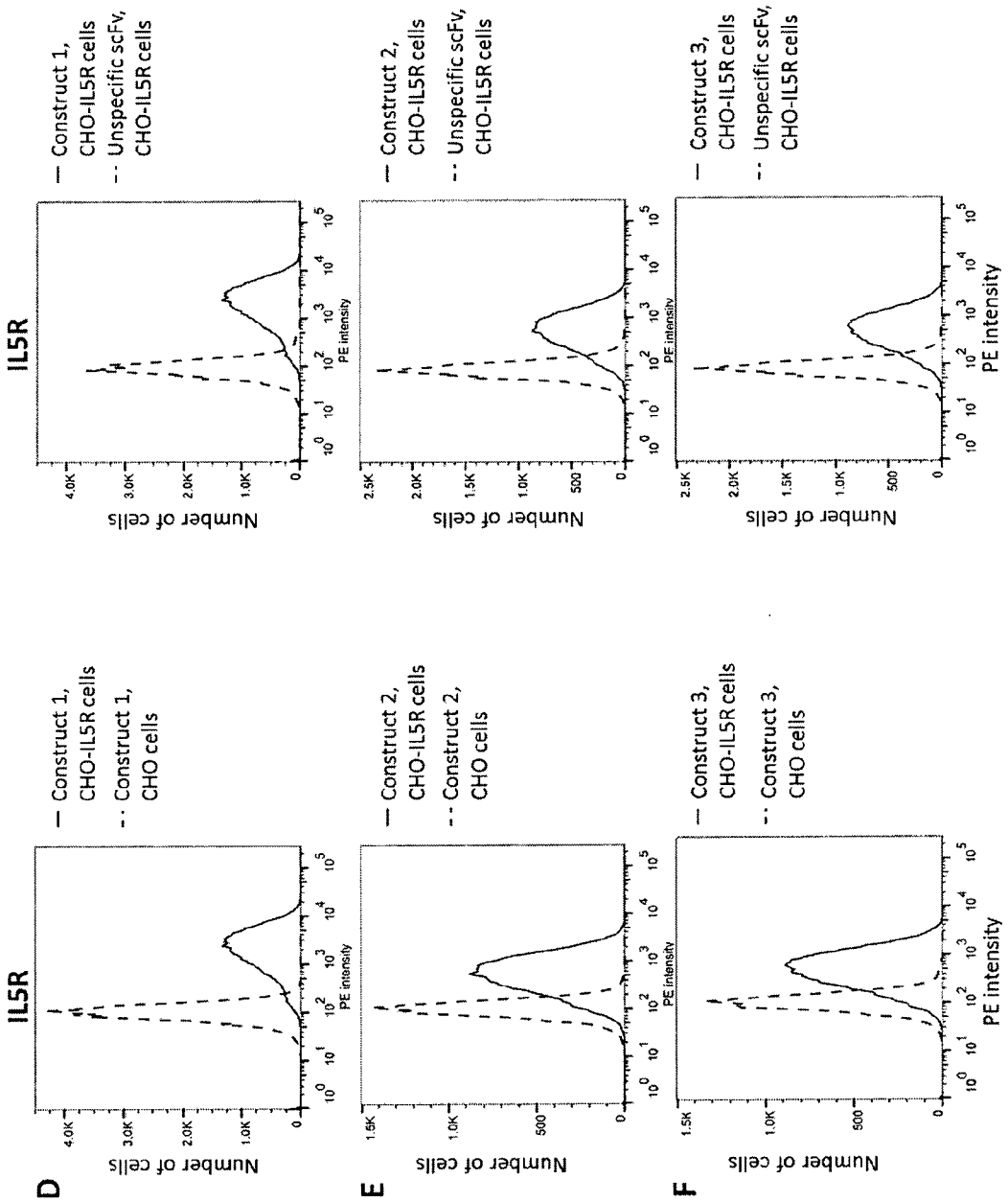


Figure 9:

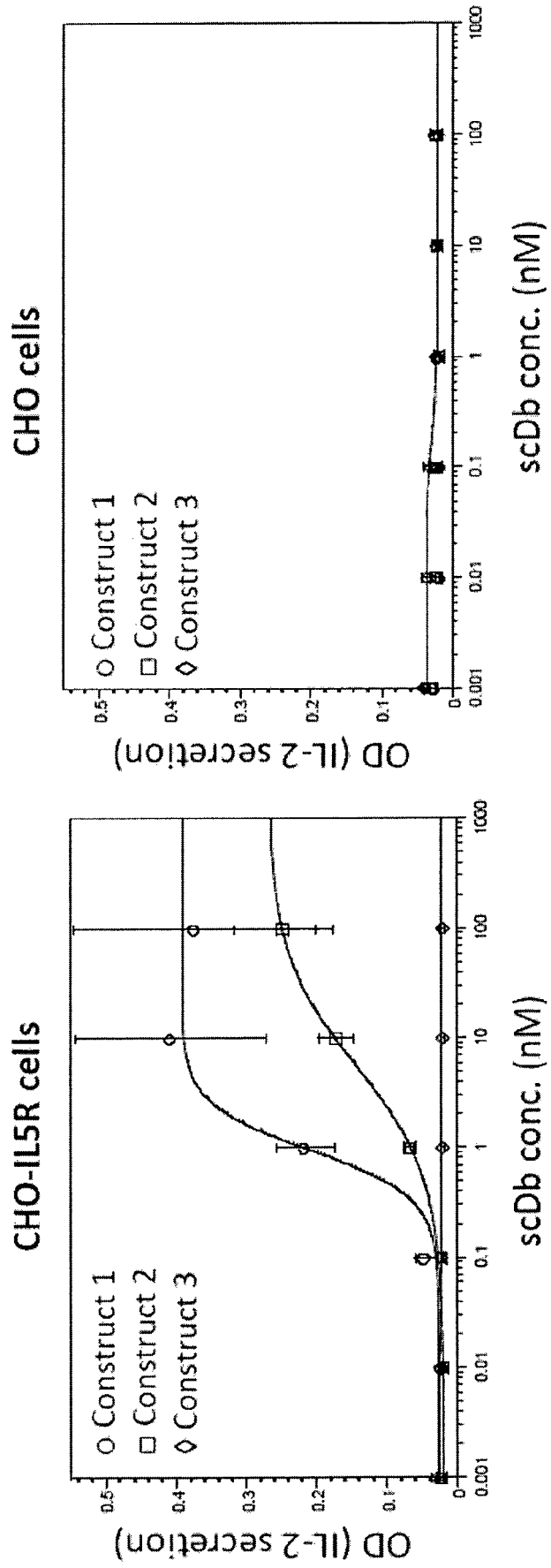
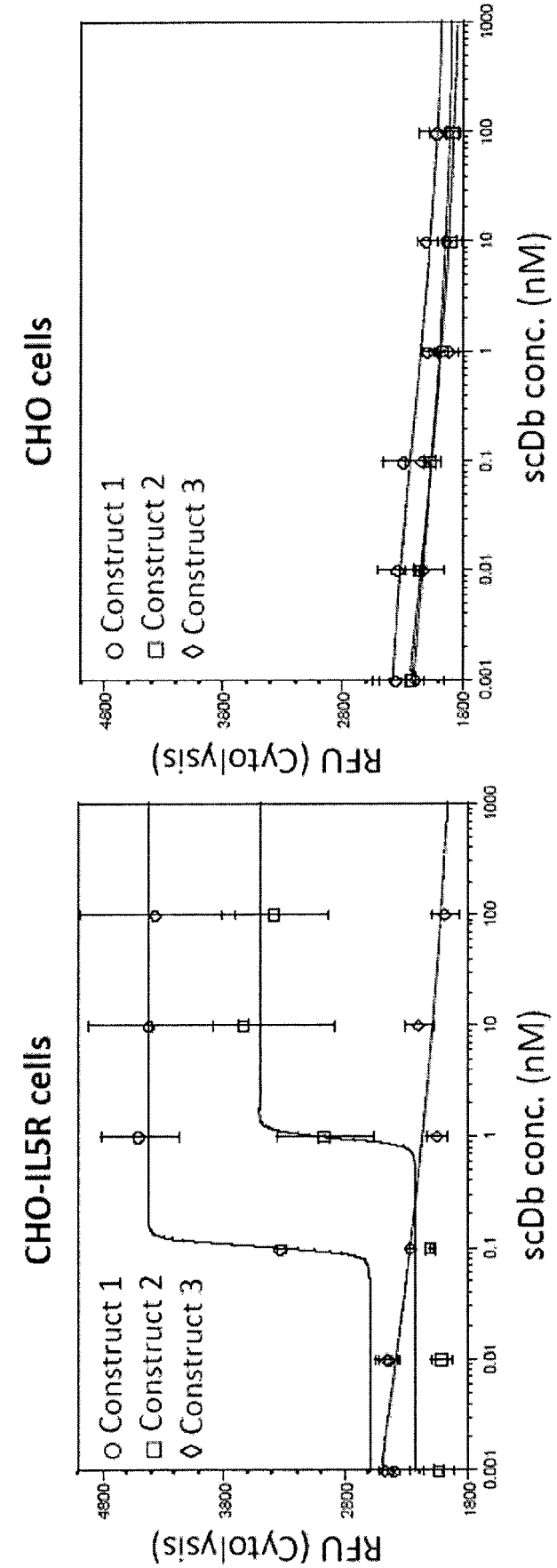

CHO-IL5R cells
CHO cells
○ Construct 1
□ Construct 2
◊ Construct 3
OD (IL-2 secretion)
scDb conc. (nM)
0.001
0.01
0.1
1
10
100
1000
0
0.1
0.2
0.3
0.4
0.5

Figure 10:


CHO-IL5R cells
CHO cells
Construct 1
Construct 2
Construct 3
RFU (Cytolysis)
scDb conc. (nM)
4800
3800
2800
1800
0.001
0.01
0.1
1
10
100
1000