Figure 1A

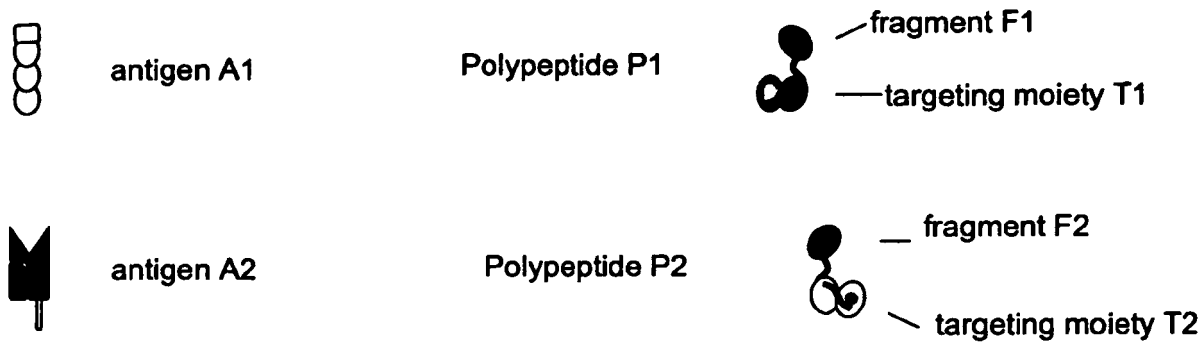


Figure 1B

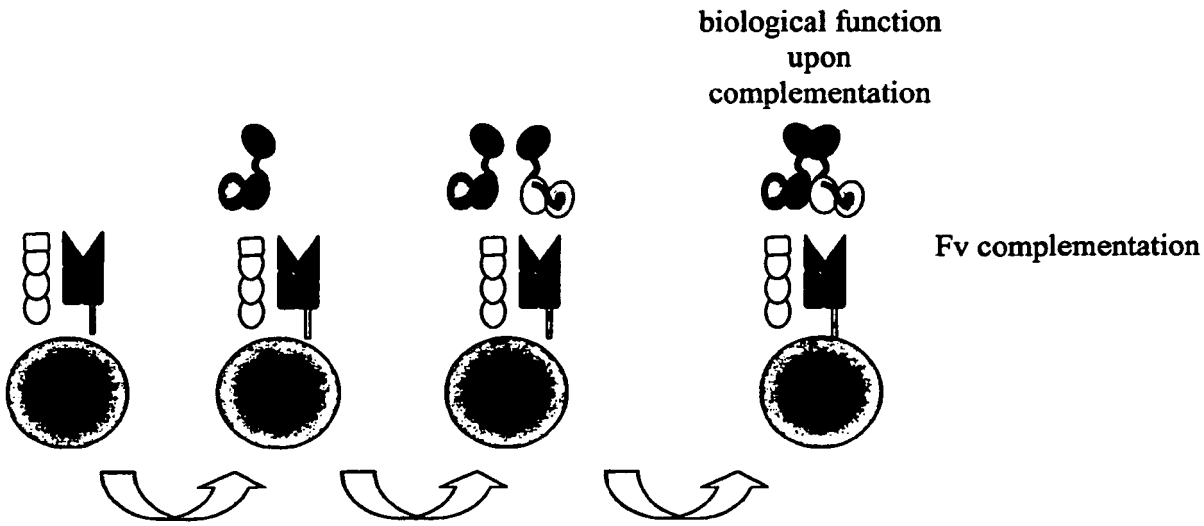


Figure 1C

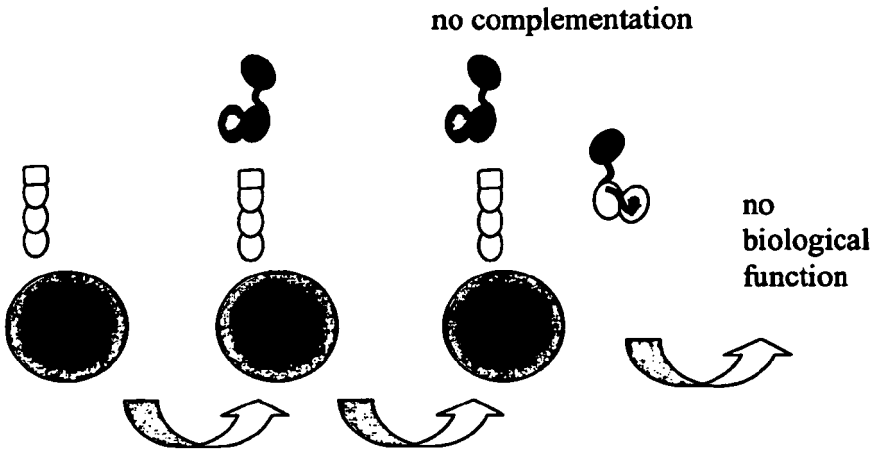


Figure 1D

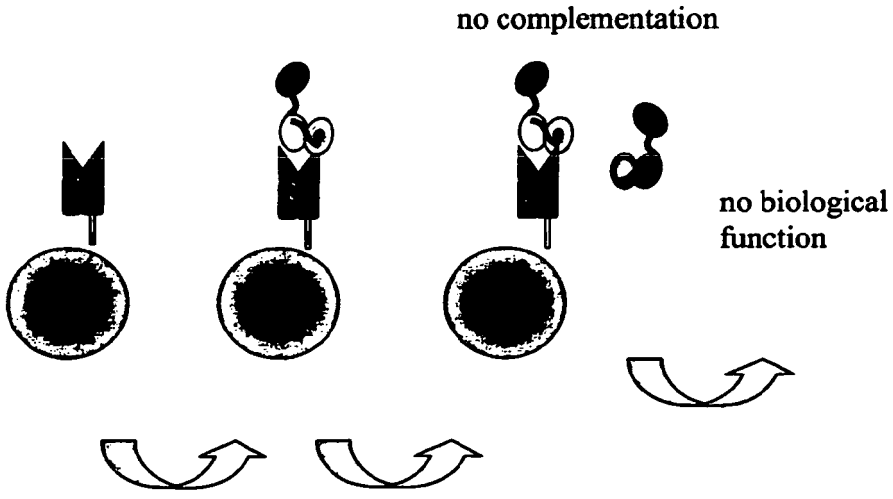


Figure 2

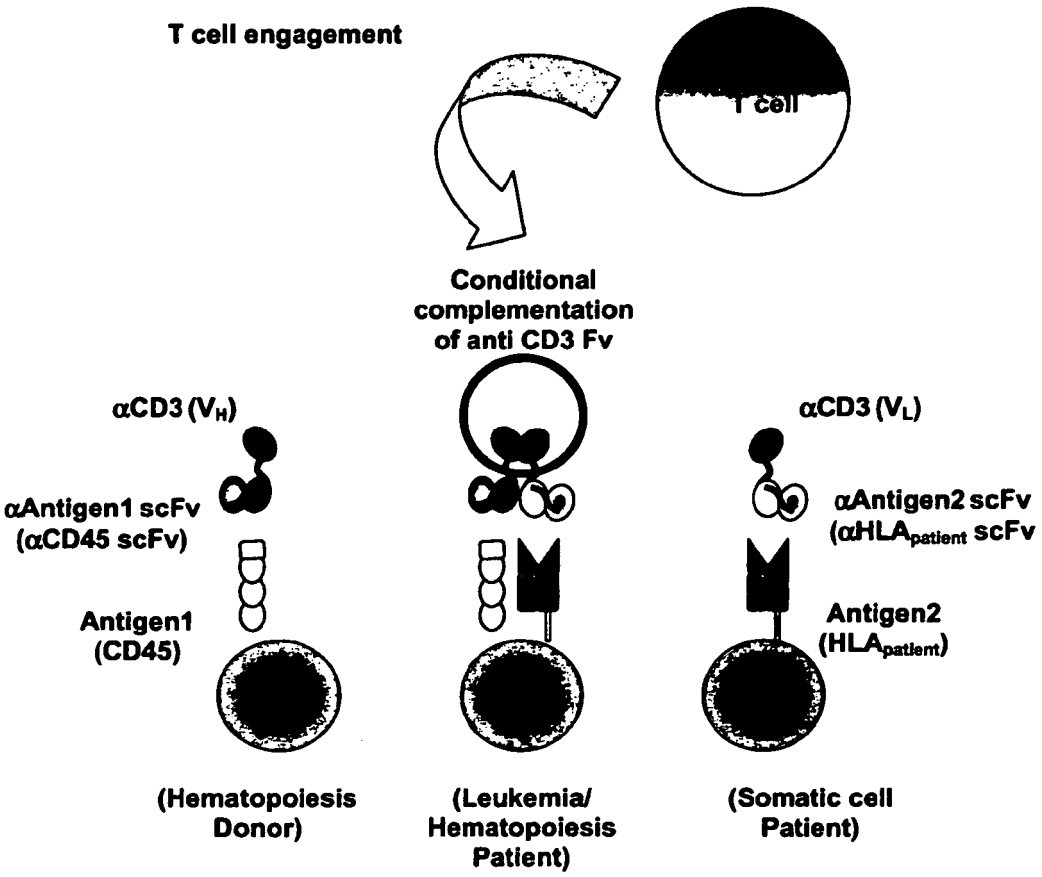
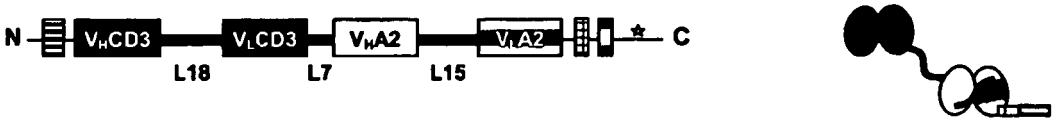
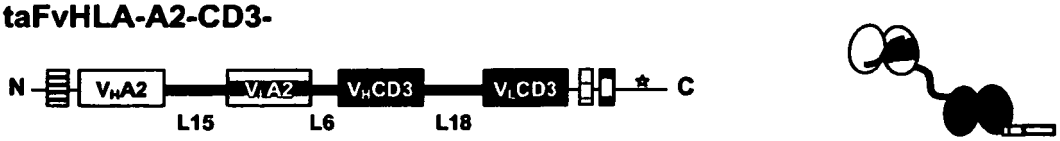

T cell engagement
T cell
Conditional complementation of anti CD3 Fv
αCD3 (VH)
αCD3 (VL)
αAntigen1 scFv (αCD45 scFv)
αAntigen2 scFv (αHLApatient scFv
Antigen1 (CD45)
Antigen2 (HLApatient)
(Hematopoiesis Donor)
(Leukemia/ Hematopoiesis Patient)
(Somatic cell Patient)

Figure 3A

85/71 **taFvCD3-HLA-A2-**

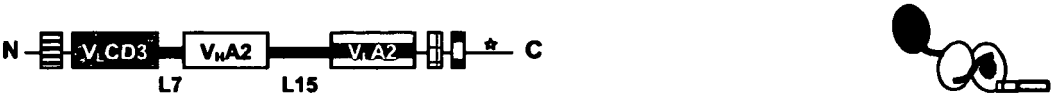


75/82 **taFvHLA-A2-CD3-**

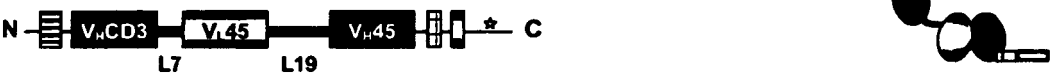




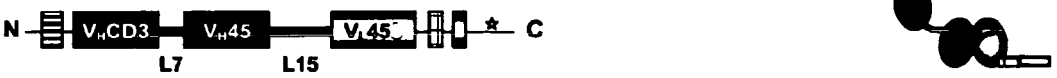
42 **V_LCD3-scFvHLA-A2-**



45 **V_HCD3-scFvCD45(V_L-V_H)-**



55 **V_HCD3-scFvCD45(V_H-V_L)-**



80/78 **V_HCD3-scFvHLA-A2-**

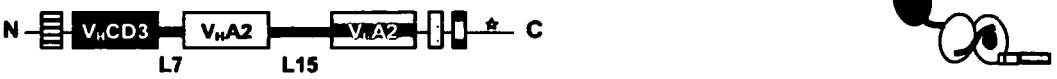




Figure 3B

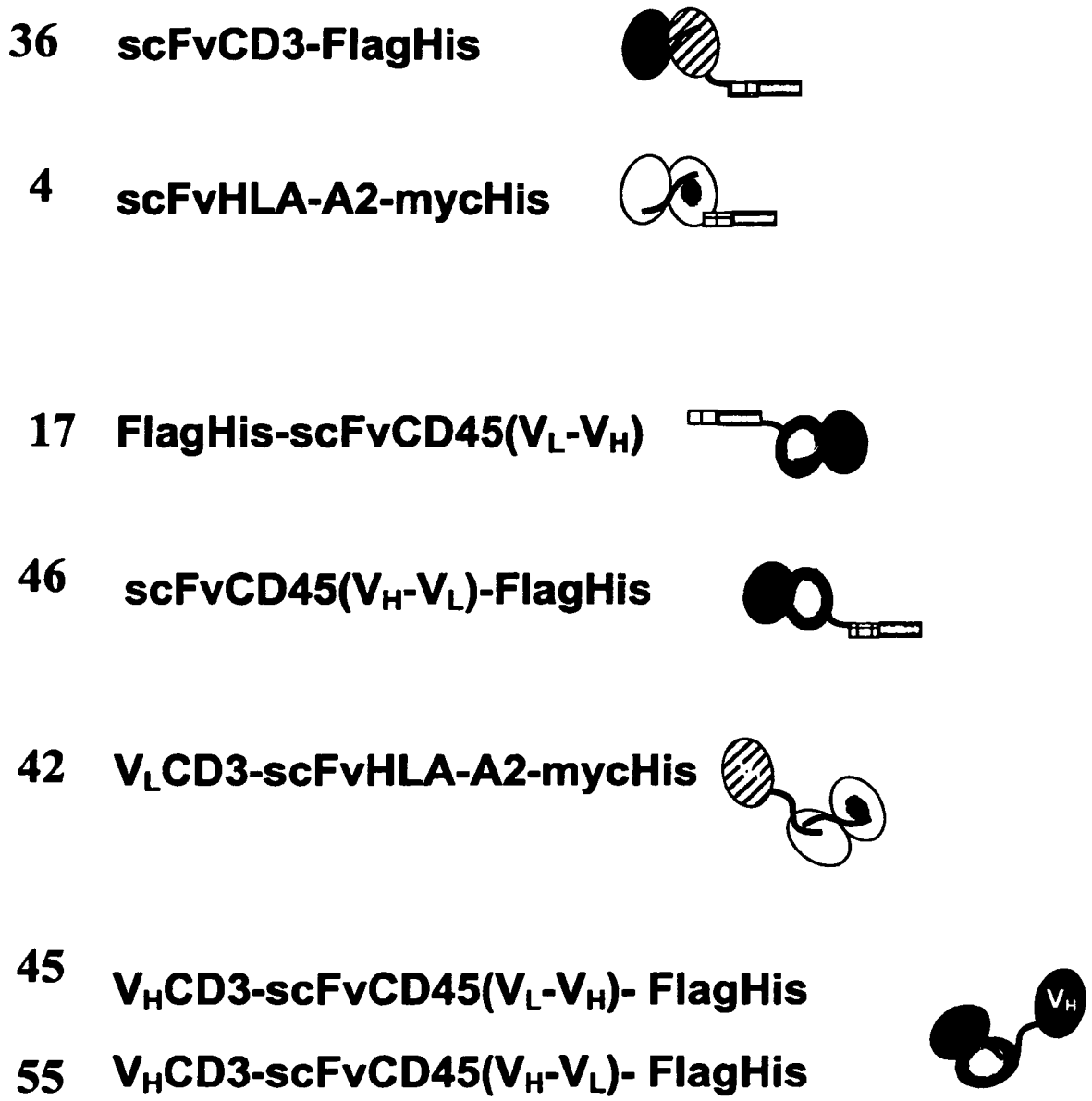

36 scFvCD3-FlagHis
4 scFvHLA-A2-mycHis
17 FlagHis-scFvCD45(V_L-V_H)
46 scFvCD45(V_H-V_L)-FlagHis
42 V_LCD3-scFvHLA-A2-mycHis
45 V_HCD3-scFvCD45(V_L-V_H)- FlagHis
55 V_HCD3-scFvCD45(V_H-V_L)- FlagHis
V_H

Figure 4

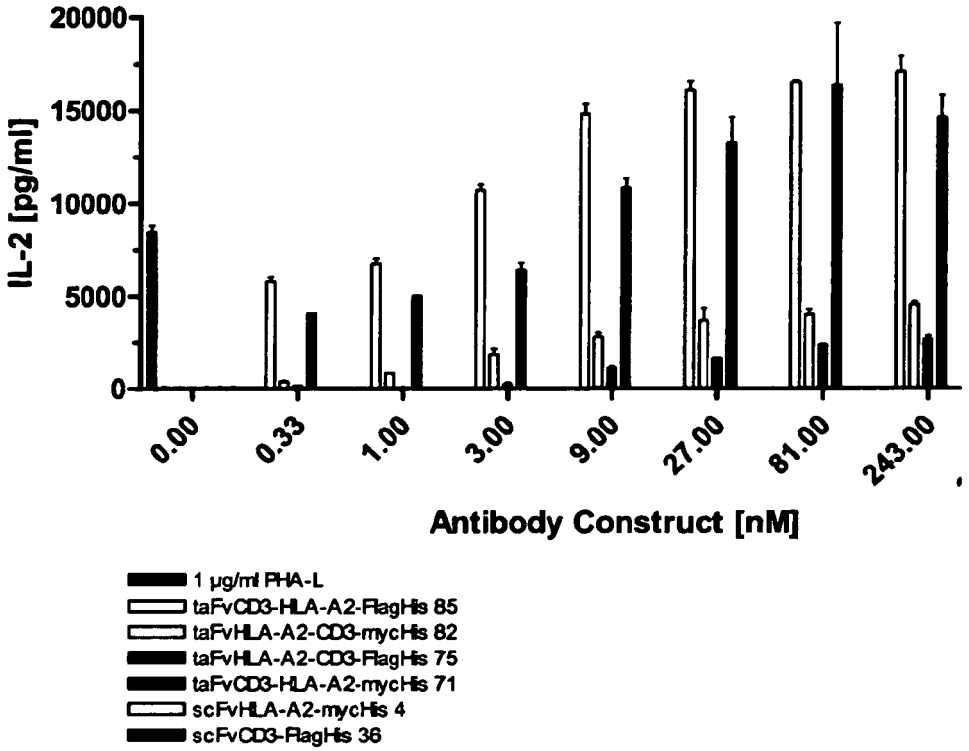


Figure 5

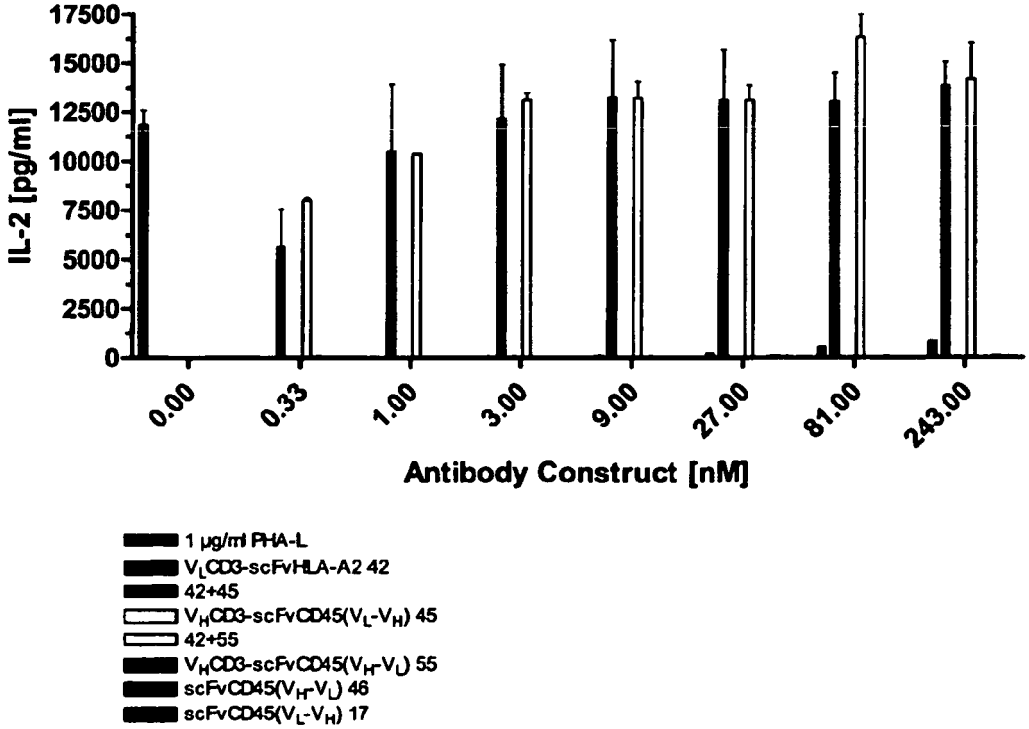


Figure 6

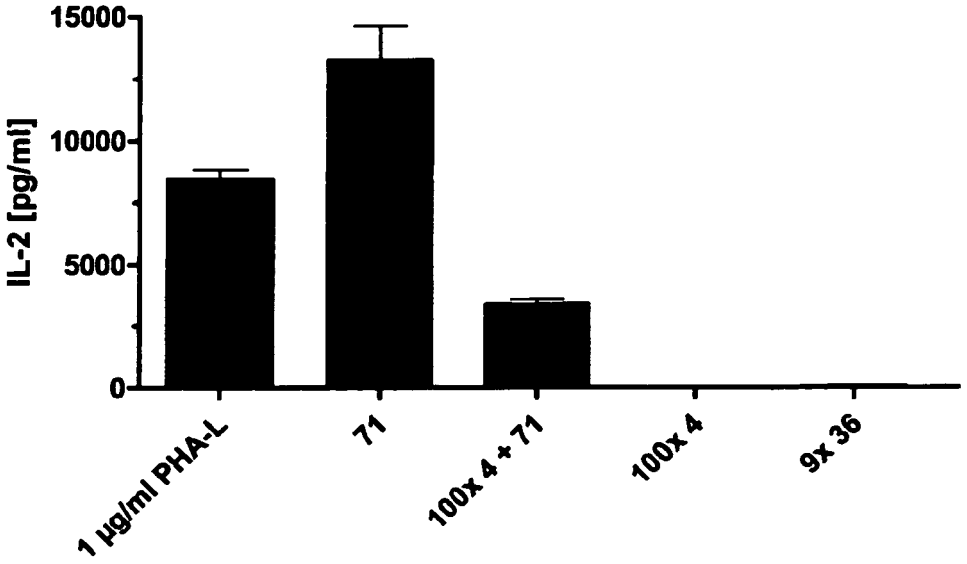


Figure 7

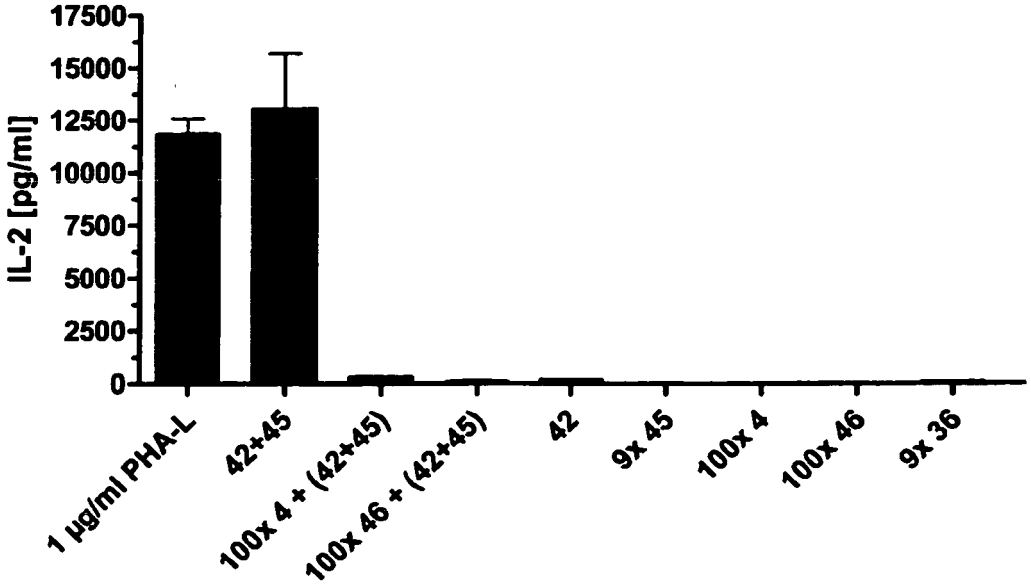


Figure 8

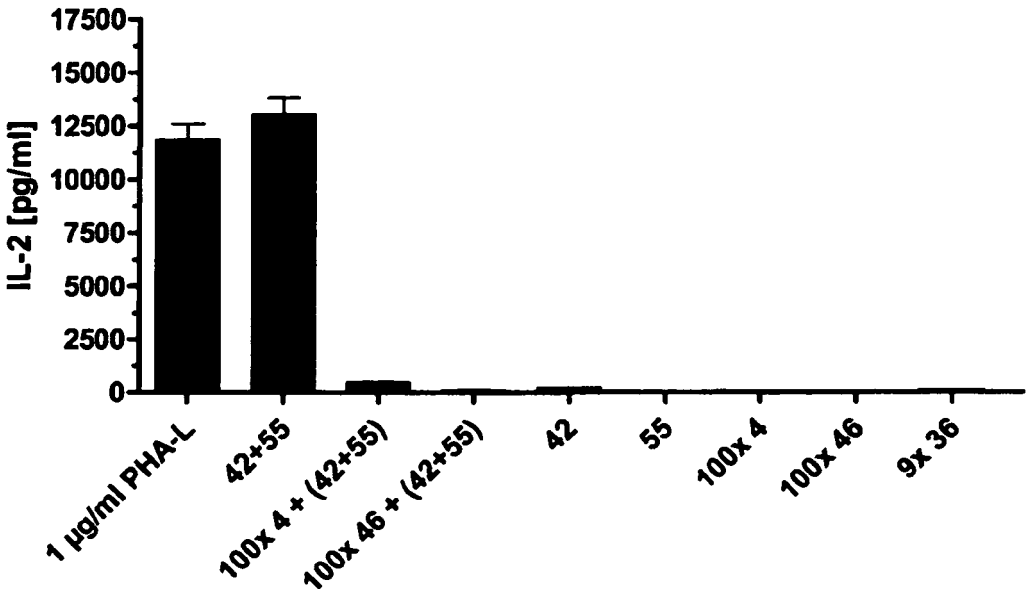


Figure 9

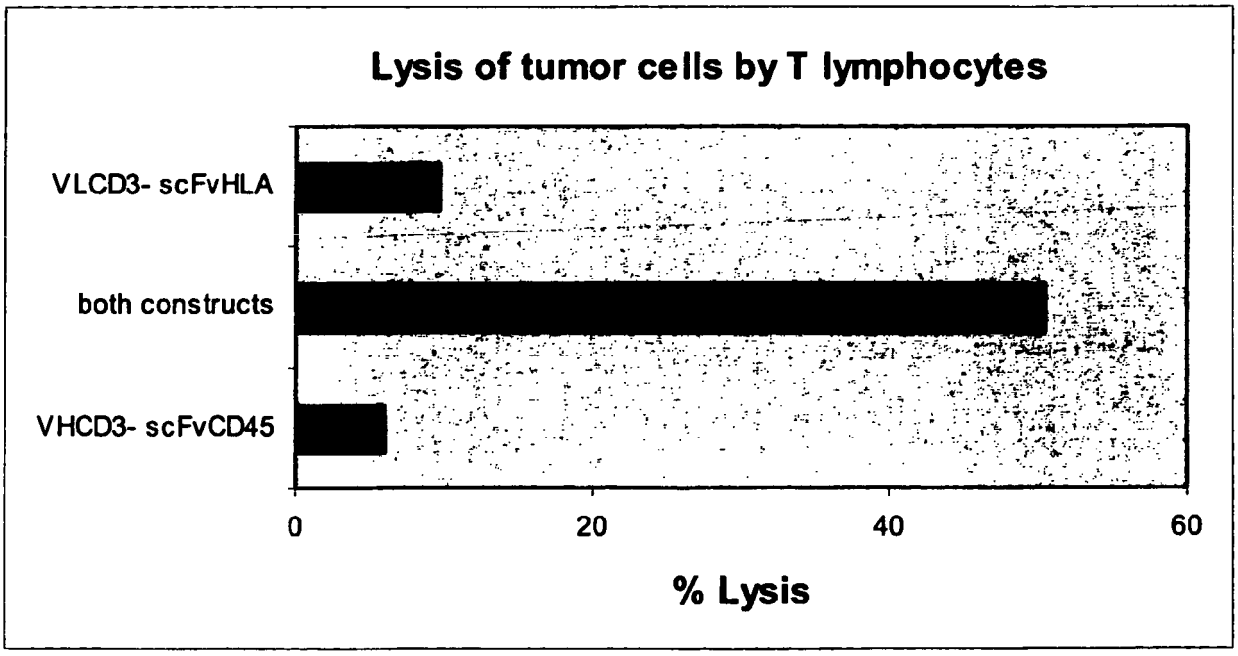


Figure 10

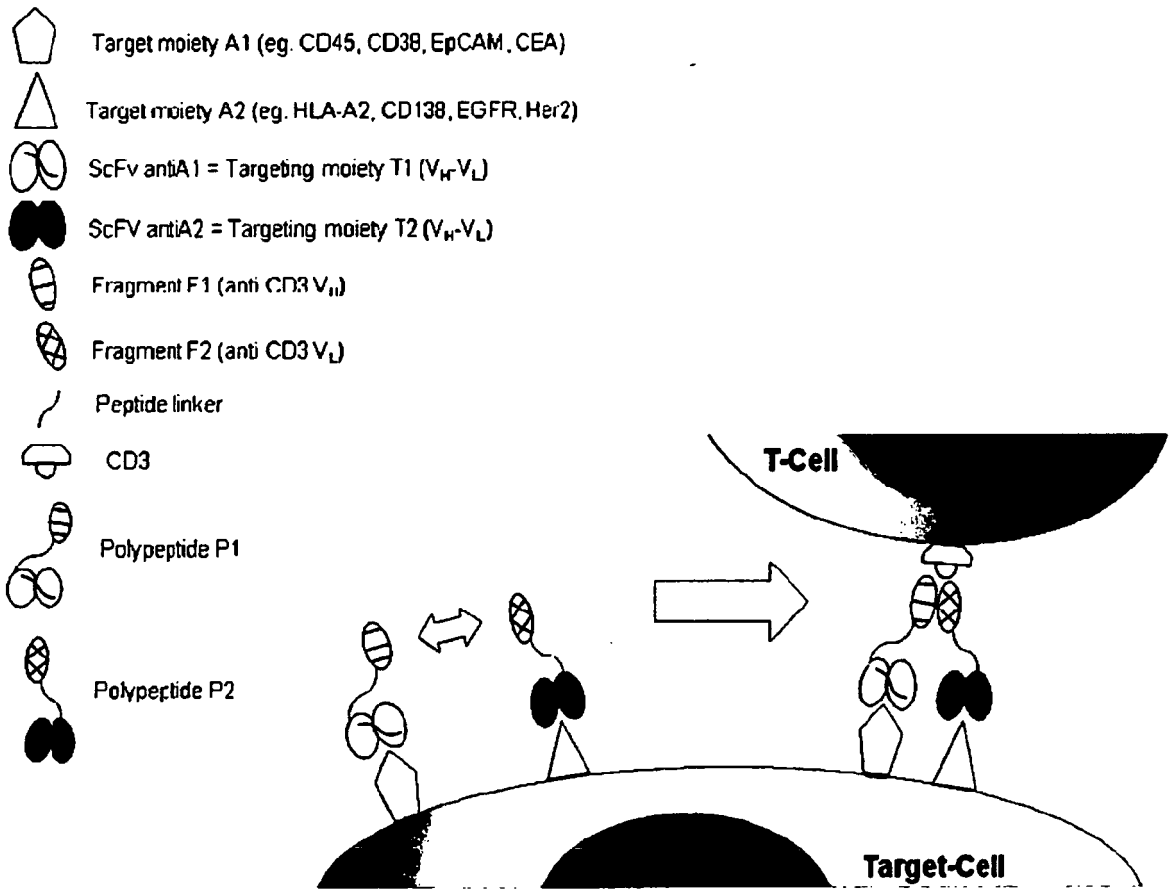

Target moiety A1 (eg. CD45, CD38, EpCAM, CEA)
Target moiety A2 (eg. HLA-A2, CD138, EGFR, Her2)
ScFv antiA1 = Targeting moiety T1 (V_H-V_L)
ScFV antiA2 = Targeting moiety T2 (V_H-V_L)
Fragment F1 (anti CD3 V_H)
Fragment F2 (anti CD3 V_L)
Peptide linker
CD3
Polypeptide P1
Polypeptide P2
T-Cell
Target-Cell

Figure 11

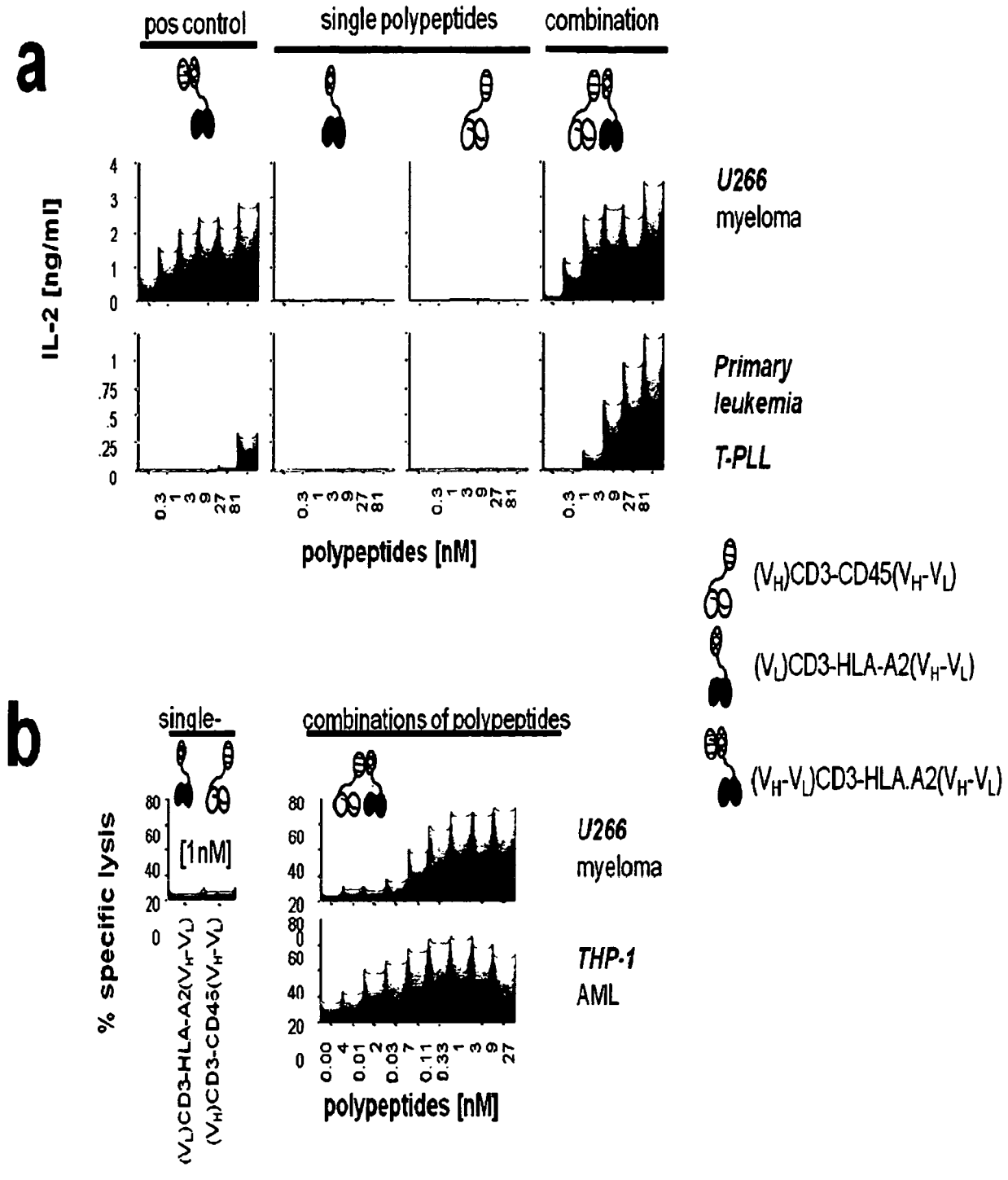

a
pos control
single polypeptides
combination
IL-2 [ng/ml]
U266
myeloma
Primary
leukemia
T-PLL
polypeptides [nM]
(VH)CD3-CD45(VH-VL)
(VL)CD3-HLA-A2(VH-VL)
(VH-VL)CD3-HLA.A2(VH-VL)
b
single-
combinations of polypeptides
% specific lysis
[1nM]
(VL)CD3-HLA-A2(VH-VL)
(VH)CD3-CD45(VH-VL)
U266
myeloma
THP-1
AML
polypeptides [nM]

Figure 11

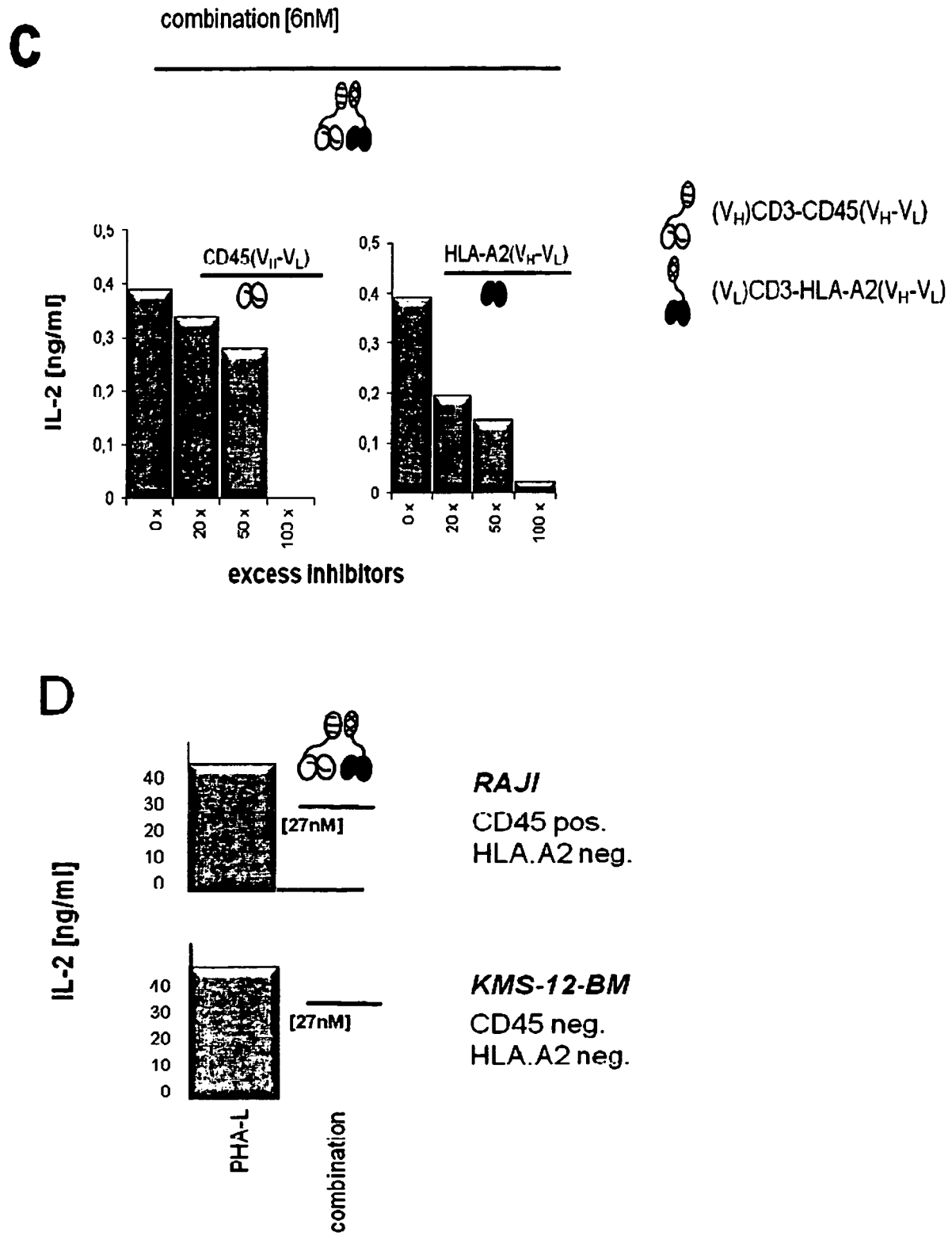

C
combination [6nM]
(VH)CD3-CD45(VH-VL)
(VL)CD3-HLA-A2(VH-VL)
CD45(VH-VL)
HLA-A2(VH-VL)
IL-2 [ng/ml]
0,5
0,4
0,3
0,2
0,1
0
0x
20x
50x
100x
excess inhibitors
D
IL-2 [ng/ml]
40
30
20
10
0
[27nM]
RAJI
CD45 pos.
HLA.A2 neg.
KMS-12-BM
CD45 neg.
HLA.A2 neg.
PHA-L
combination

Figure 12A

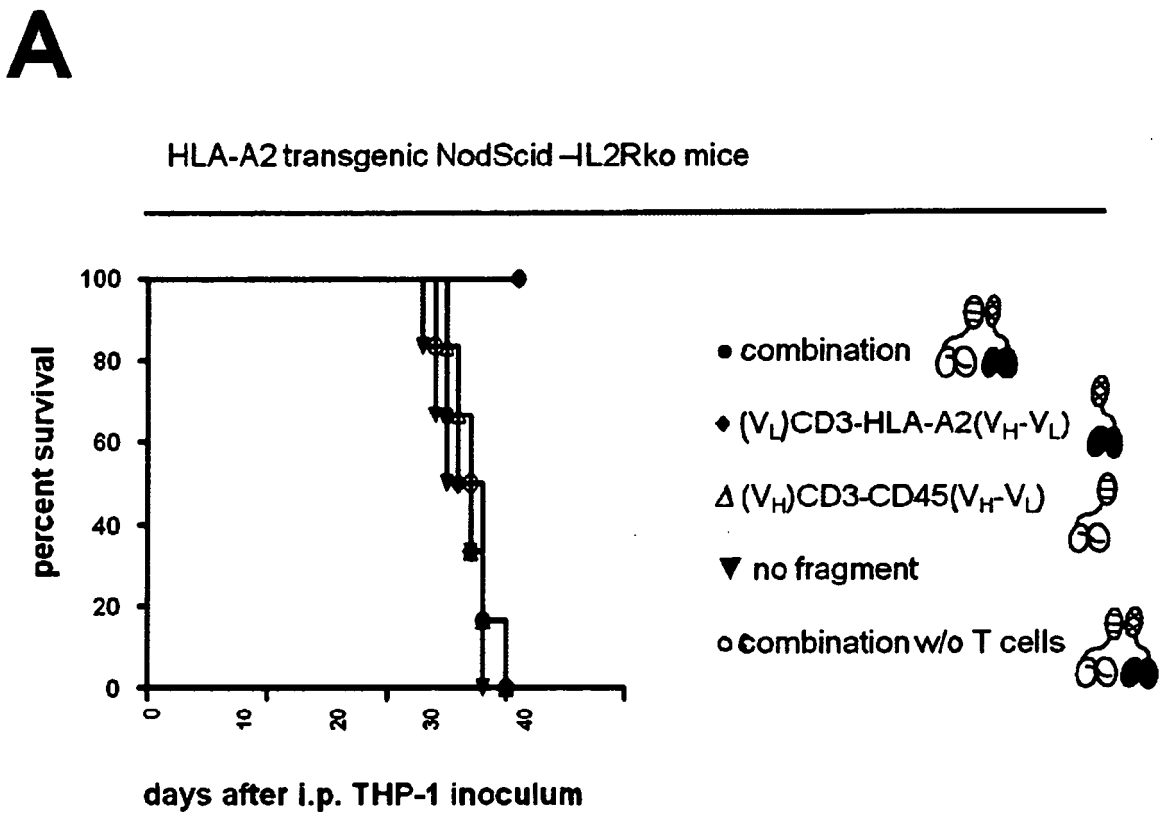

A
HLA-A2 transgenic NodScid –IL2Rko mice
percent survival
100
80
60
40
20
0
0
10
20
30
40
days after i.p. THP-1 inoculum
• combination
♦ (VL)CD3-HLA-A2(VH-VL)
Δ (VH)CD3-CD45(VH-VL)
▼ no fragment
o combination w/o T cells

Figure 12B

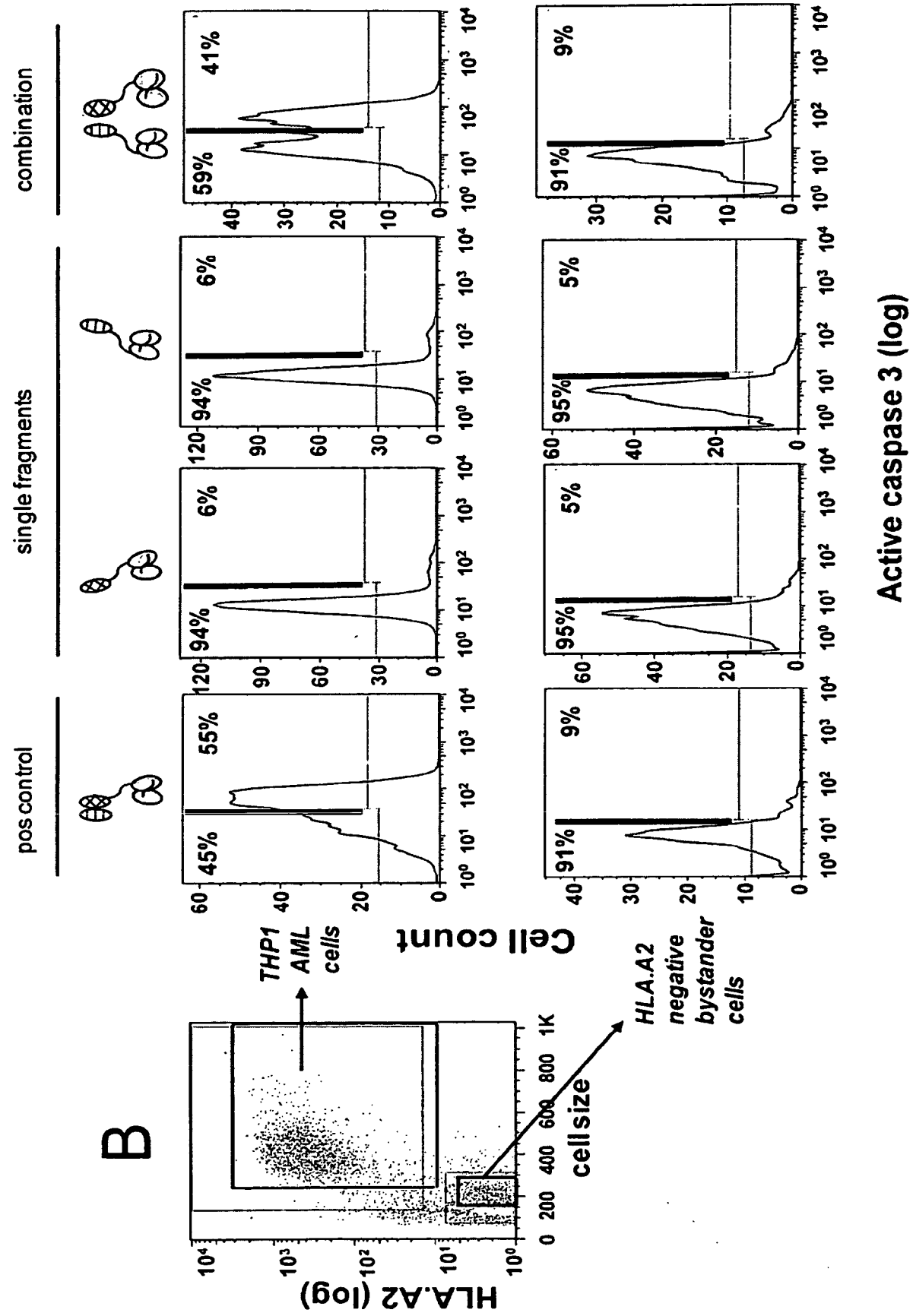

B
HLA.A2 (log)
cell size
THP1 AML cells
HLA.A2 negative bystander cells
Cell count
Active caspase 3 (log)
pos control
single fragments
combination
45%
55%
94%
6%
59%
41%
91%
9%
95%
5%

Figure 13

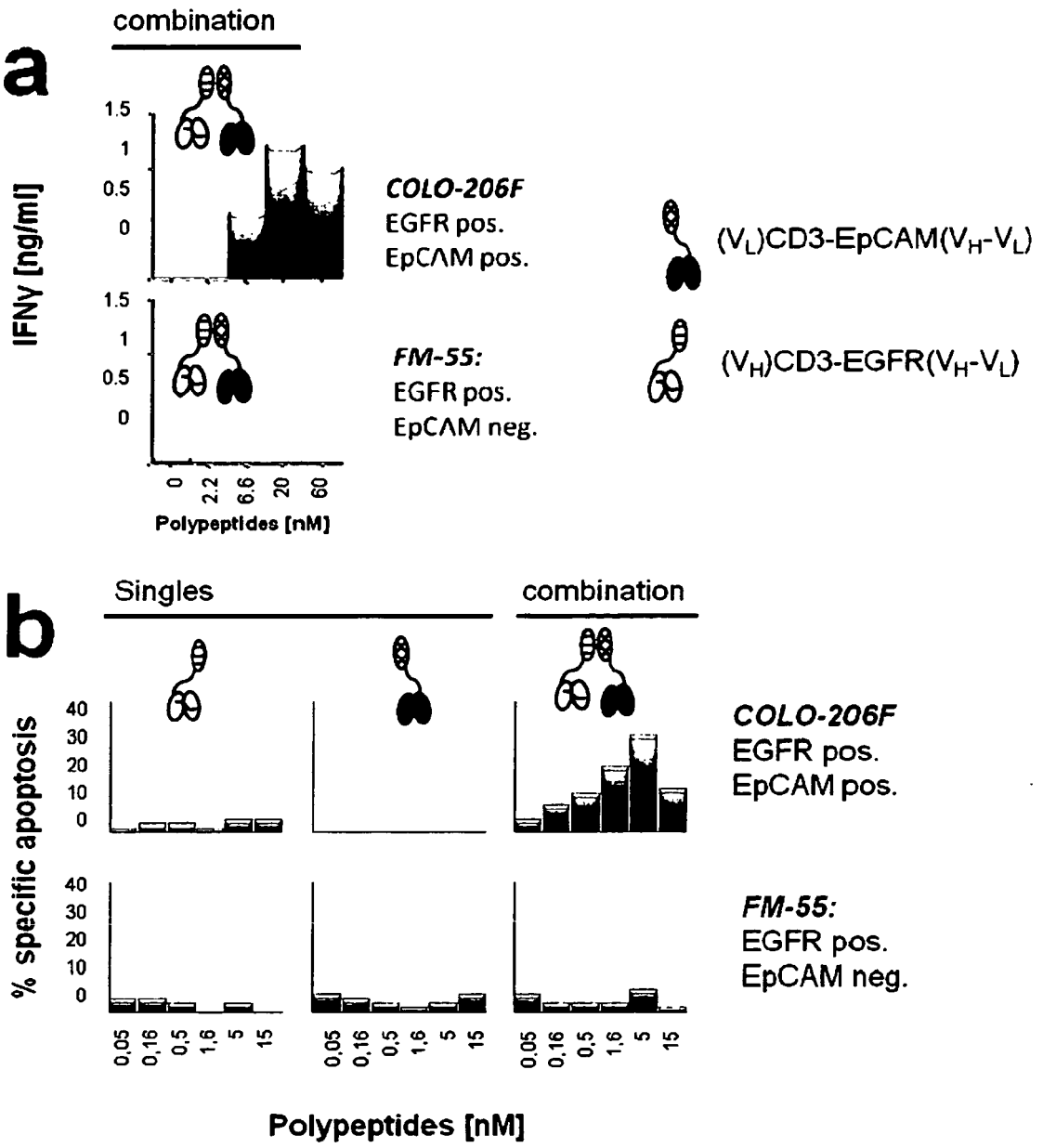

a
combination
IFNγ [ng/ml]
1.5
1
0.5
0
COLO-206F
EGFR pos.
EpCAM pos.
FM-55:
EGFR pos.
EpCAM neg.
0
2.2
6.6
20
60
Polypeptides [nM]
(VL)CD3-EpCAM(VH-VL)
(VH)CD3-EGFR(VH-VL)
b
Singles
combination
% specific apoptosis
40
30
20
10
0
COLO-206F
EGFR pos.
EpCAM pos.
FM-55:
EGFR pos.
EpCAM neg.
0,05
0,16
0,5
1,6
5
15
Polypeptides [nM]

Figure 14

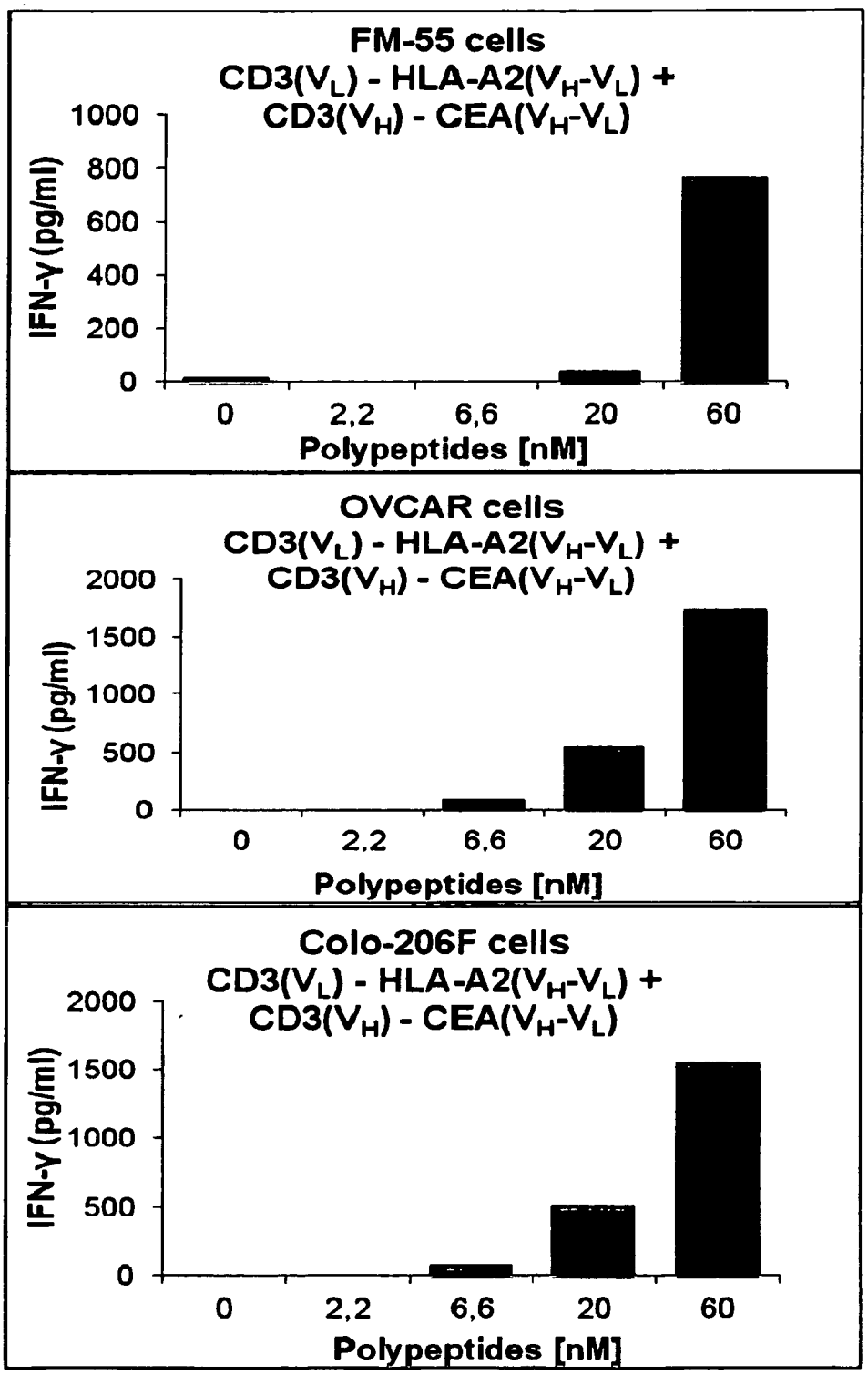

FM-55 cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CEA(VH-VL)
IFN-γ (pg/ml)
1000
800
600
400
200
0
0
2,2
6,6
20
60
Polypeptides [nM]
OVCAR cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CEA(VH-VL)
IFN-γ (pg/ml)
2000
1500
1000
500
0
0
2.2
6.6
20
60
Polypeptides [nM]
Colo-206F cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CEA(VH-VL)
IFN-γ (pg/ml)
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]

Figure 15

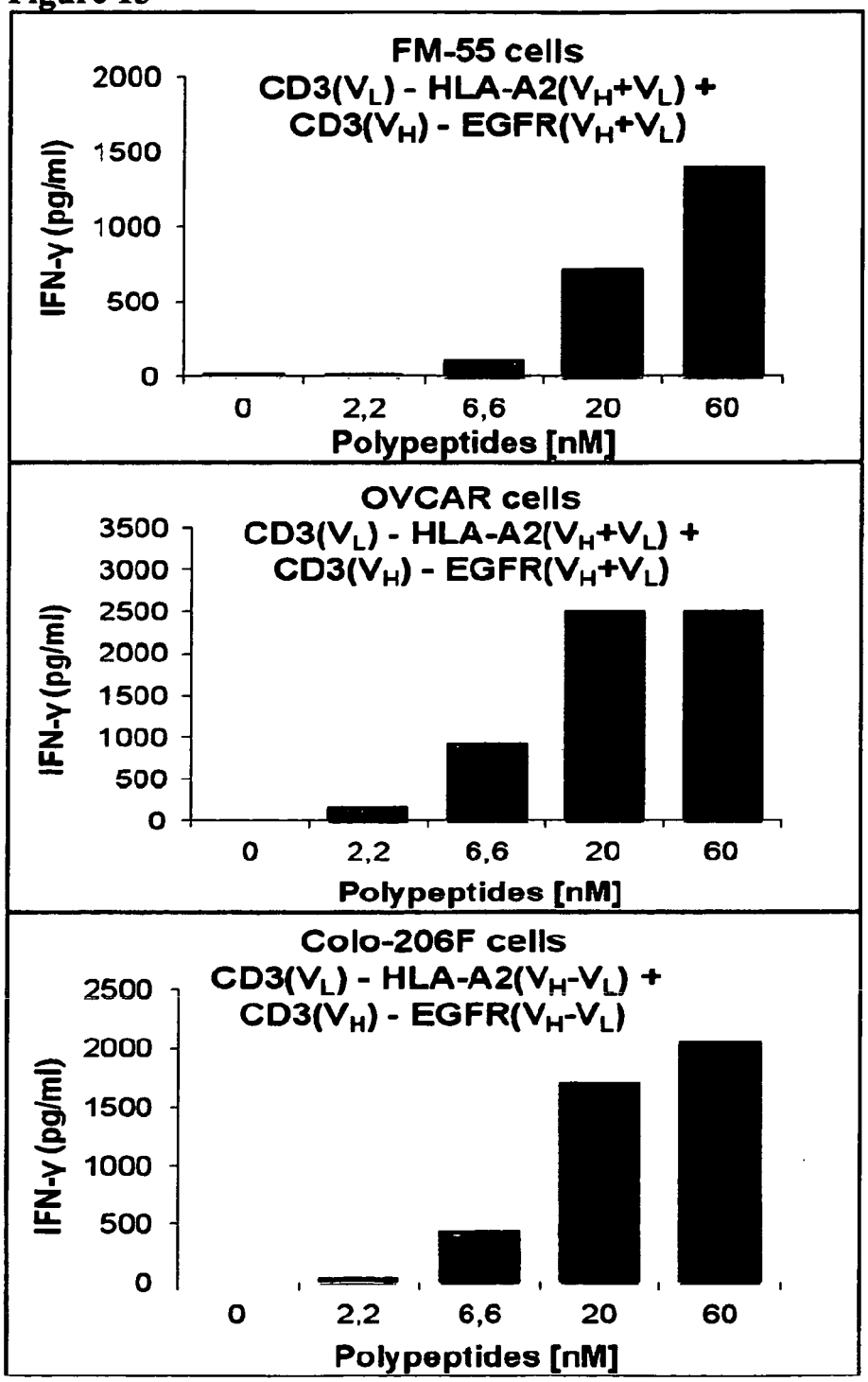

FM-55 cells
CD3(VL) - HLA-A2(VH+VL) +
CD3(VH) - EGFR(VH+VL)
IFN-γ (pg/ml)
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
OVCAR cells
CD3(VL) - HLA-A2(VH+VL) +
CD3(VH) - EGFR(VH+VL)
IFN-γ (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
Colo-206F cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - EGFR(VH-VL)
IFN-γ (pg/ml)
2500
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]

Figure 16

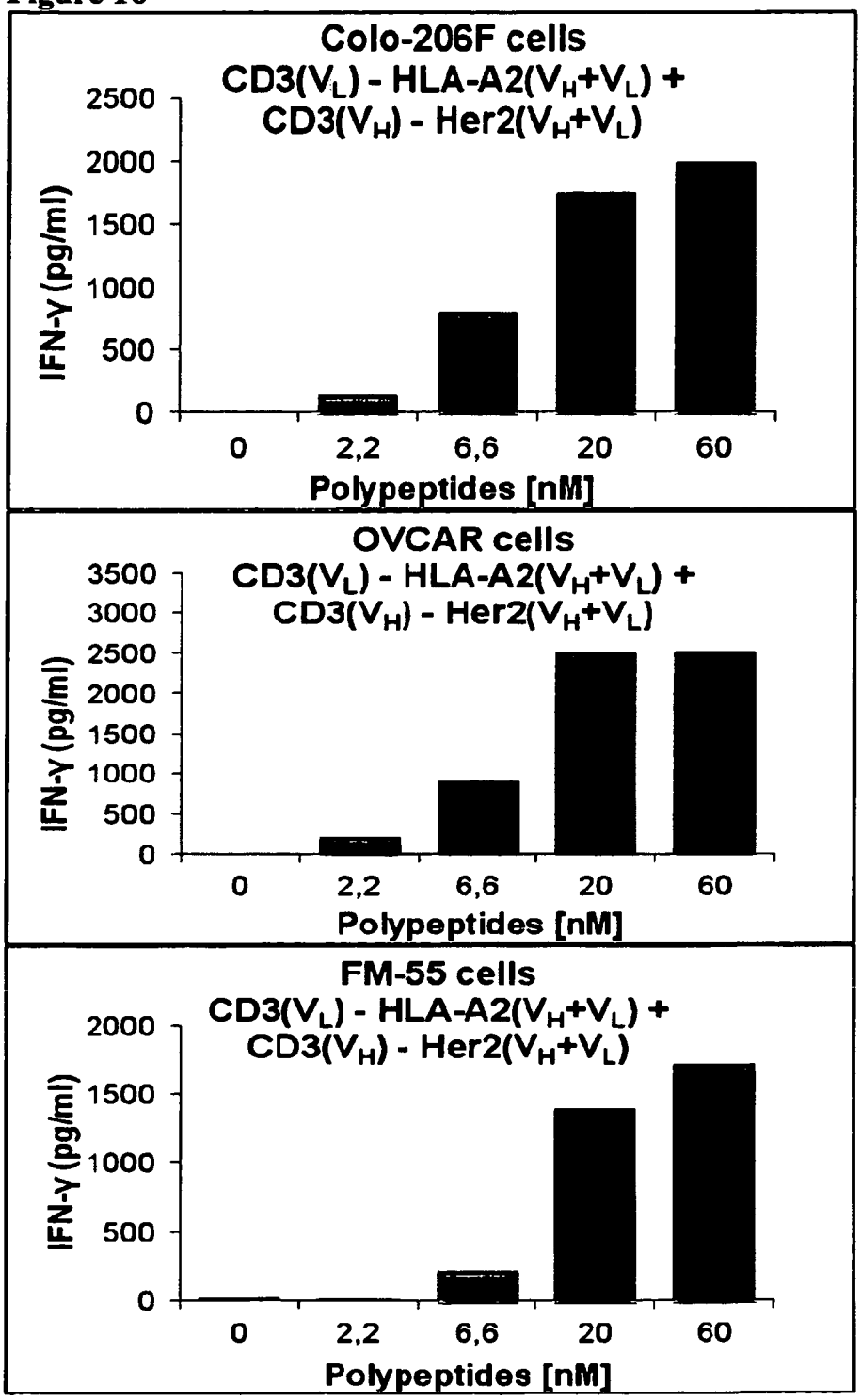

Colo-206F cells
CD3(VL) - HLA-A2(VH+VL) +
CD3(VH) - Her2(VH+VL)
IFN-γ (pg/ml)
2500
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
OVCAR cells
CD3(VL) - HLA-A2(VH+VL) +
CD3(VH) - Her2(VH+VL)
IFN-γ (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
FM-55 cells
CD3(VL) - HLA-A2(VH+VL) +
CD3(VH) - Her2(VH+VL)
IFN-γ (pg/ml)
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]

Figure 17

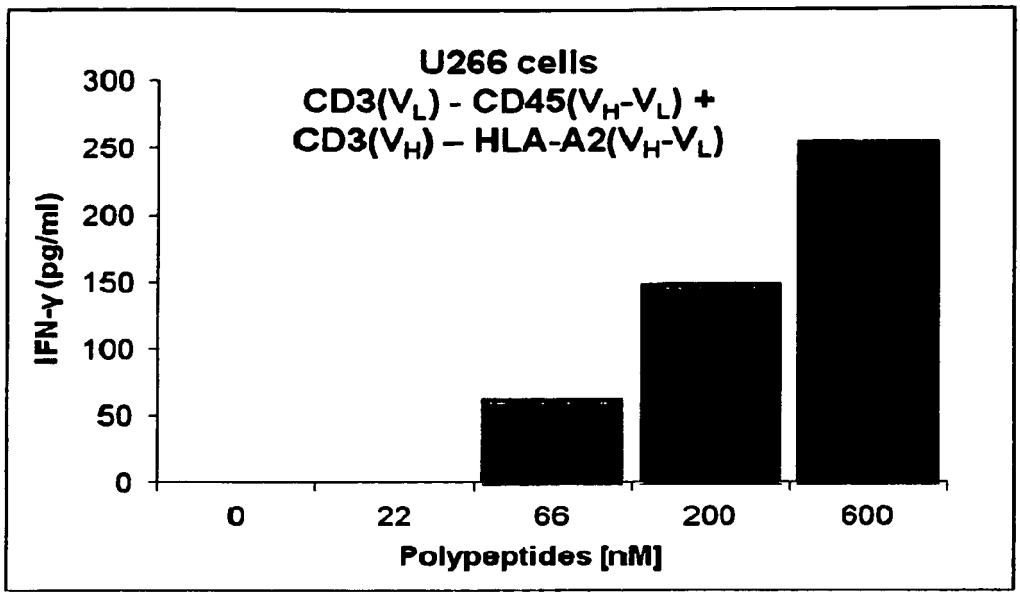

U266 cells
CD3(V_L) - CD45(V_H-V_L) +
CD3(V_H) – HLA-A2(V_H-V_L)
IFN-γ (pg/ml)
300
250
200
150
100
50
0
0
22
66
200
600
Polypeptides [nM]

Figure 18

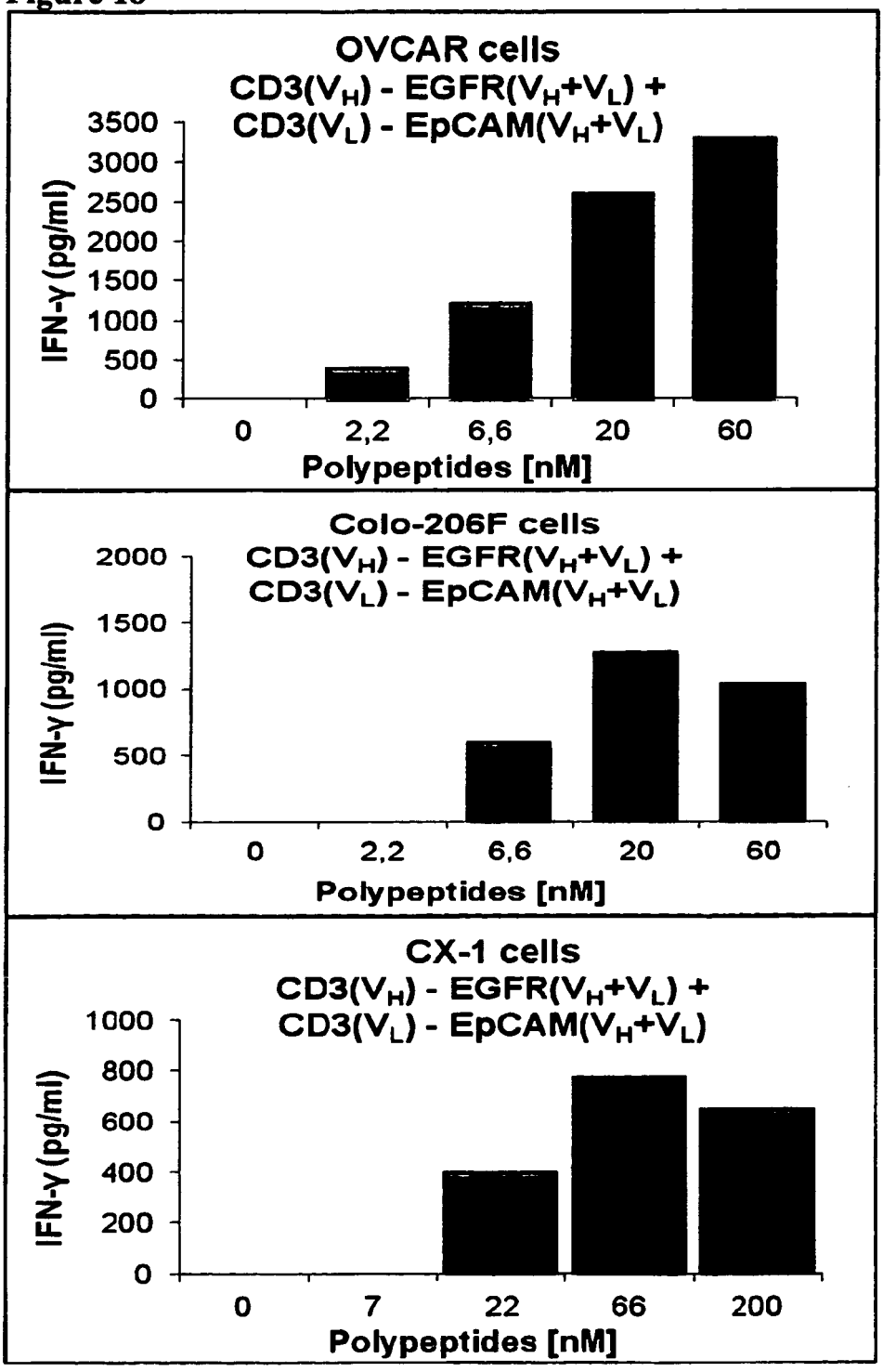

OVCAR cells
CD3(VH) - EGFR(VH+VL) +
CD3(VL) - EpCAM(VH+VL)
IFN-γ (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
Colo-206F cells
CD3(VH) - EGFR(VH+VL) +
CD3(VL) - EpCAM(VH+VL)
IFN-γ (pg/ml)
2000
1500
1000
500
0
0
2,2
6,6
20
60
Polypeptides [nM]
CX-1 cells
CD3(VH) - EGFR(VH+VL) +
CD3(VL) - EpCAM(VH+VL)
IFN-γ (pg/ml)
1000
800
600
400
200
0
0
7
22
66
200
Polypeptides [nM]

Figure 19

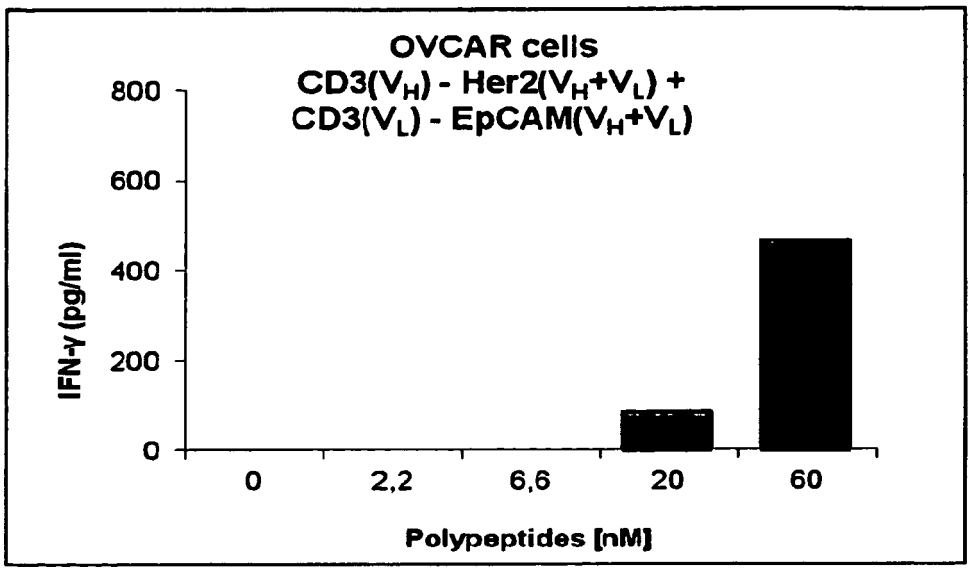

OVCAR cells
CD3(VH) - Her2(VH+VL) +
CD3(VL) - EpCAM(VH+VL)
800
600
400
200
0
IFN-γ (pg/ml)
0
2,2
6,6
20
60
Polypeptides [nM]

Figure 20

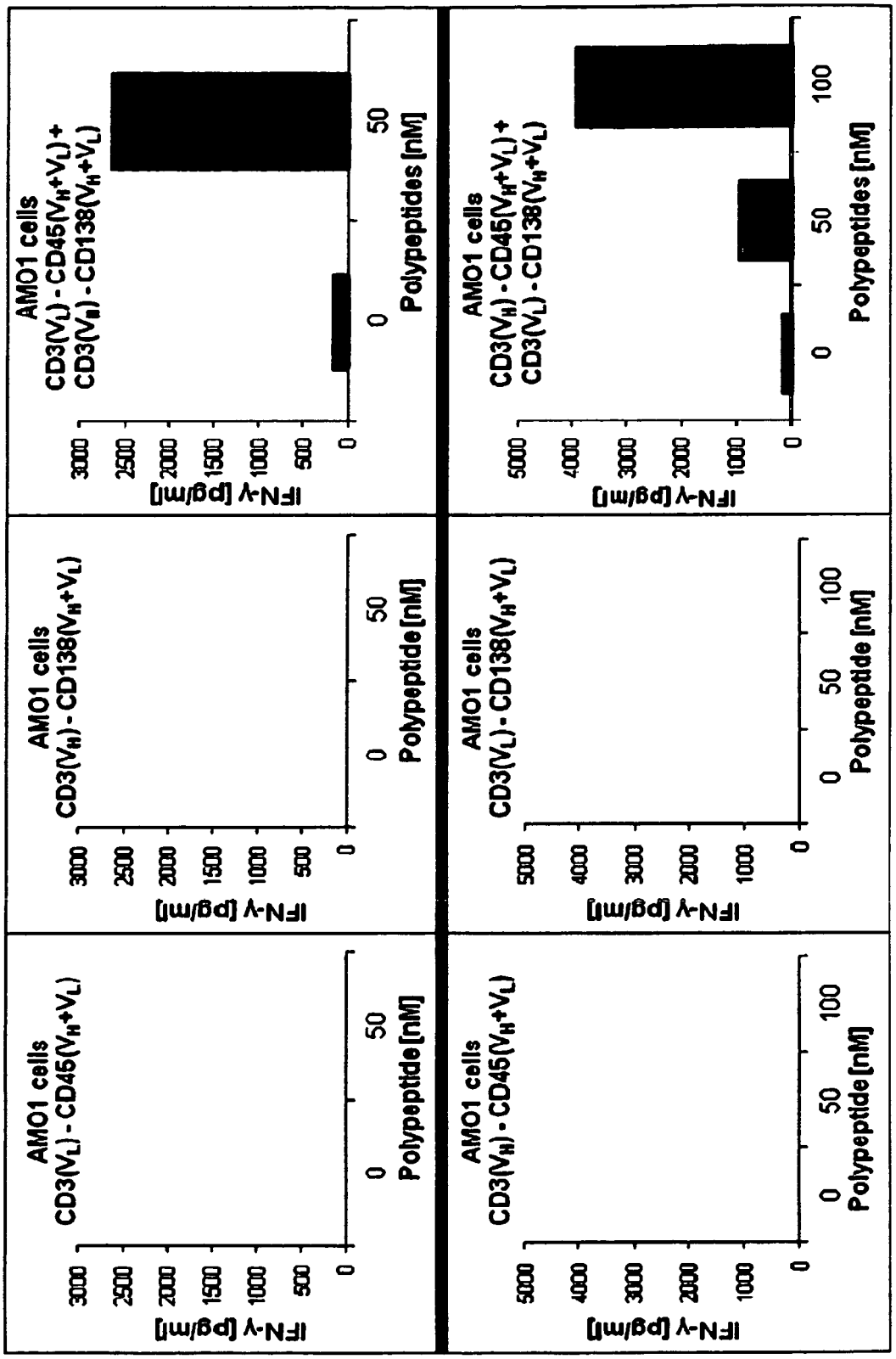

AMO1 cells
CD3(VL) - CD45(VH+VL) +
CD3(VH) - CD138(VH+VL)
IFN-γ [pg/ml]
Polypeptides [nM]
AMO1 cells
CD3(VH) - CD45(VH+VL) +
CD3(VL) - CD138(VH+VL)
IFN-γ [pg/ml]
Polypeptides [nM]
AMO1 cells
CD3(VH) - CD138(VH+VL)
IFN-γ [pg/ml]
Polypeptide [nM]
AMO1 cells
CD3(VL) - CD138(VH+VL)
IFN-γ [pg/ml]
Polypeptide [nM]
AMO1 cells
CD3(VL) - CD45(VH+VL)
IFN-γ [pg/ml]
Polypeptide [nM]
AMO1 cells
CD3(VH) - CD45(VH+VL)
IFN-γ [pg/ml]
Polypeptide [nM]

Figure 21

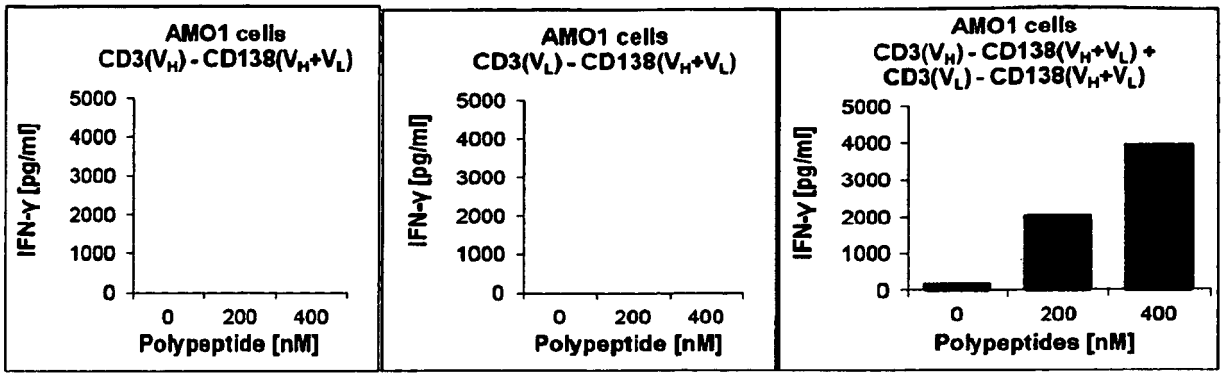


Figure 22

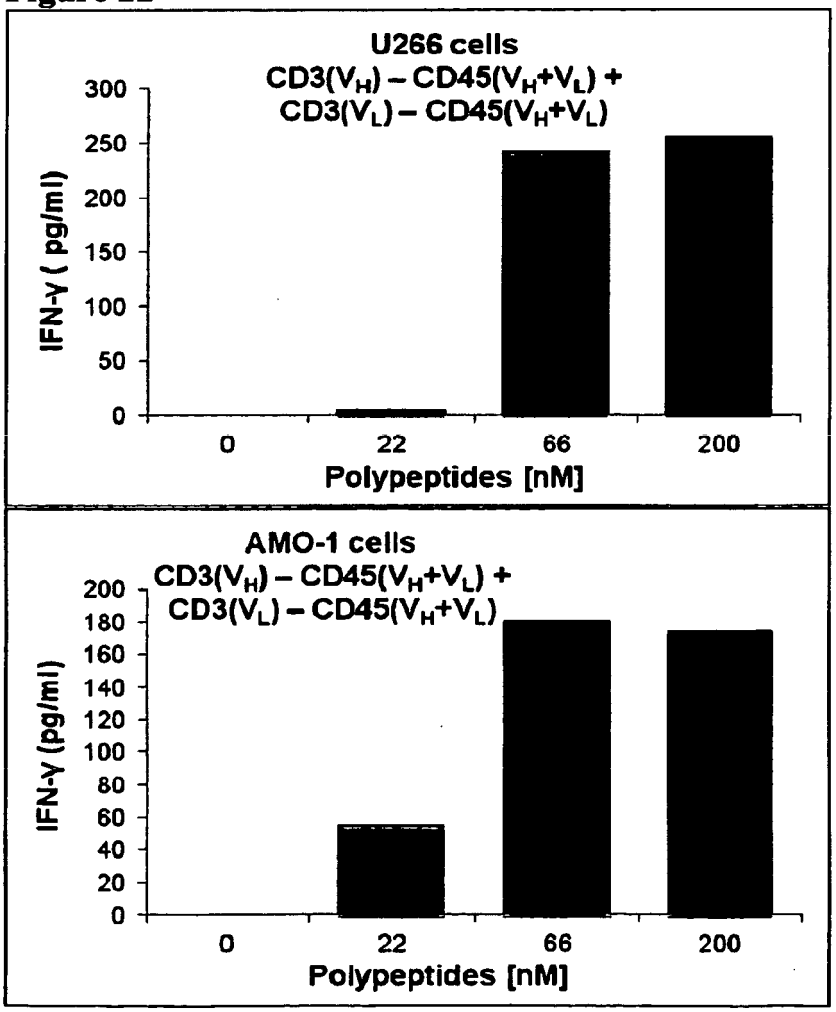


Figure 23

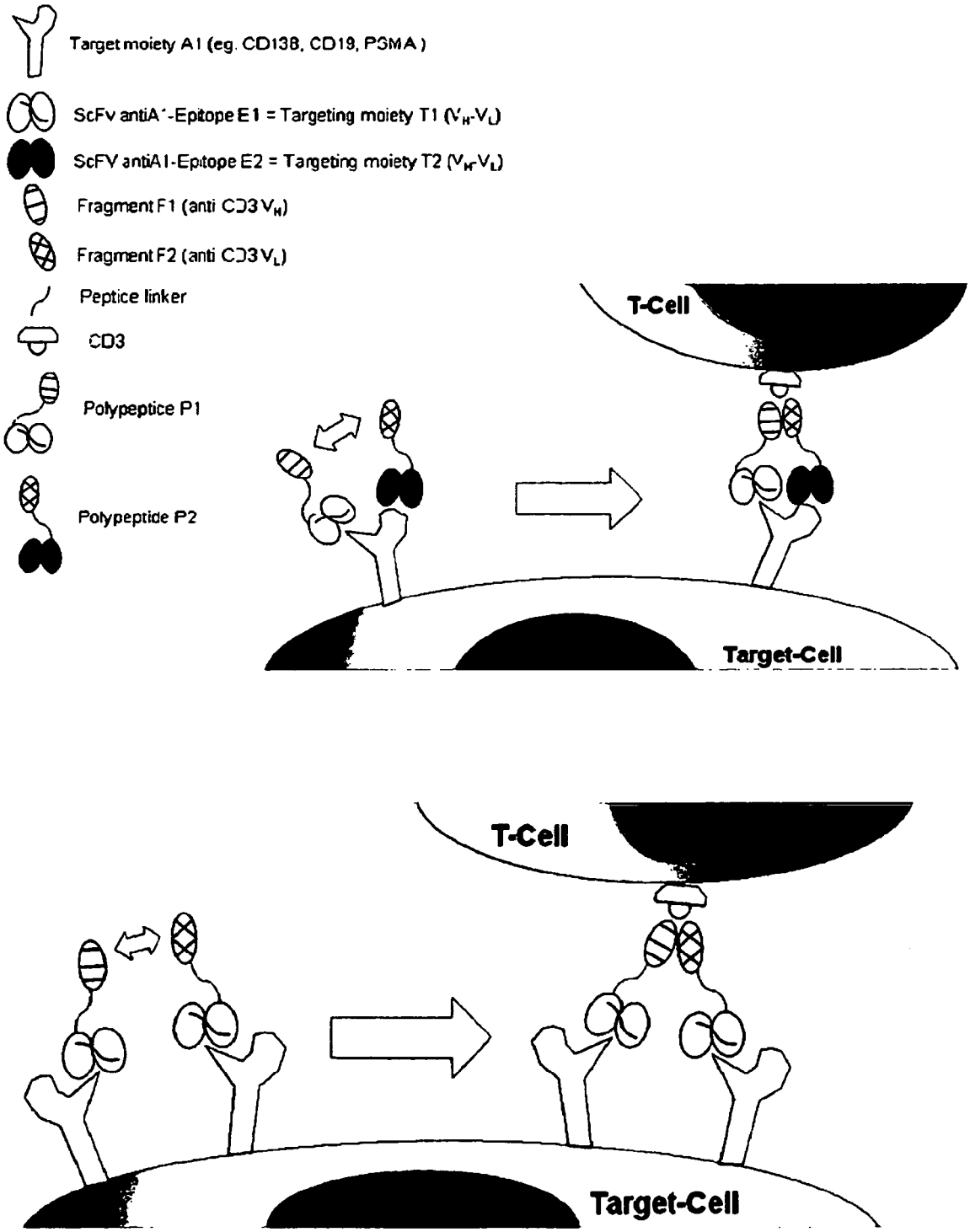

Target moiety A1 (eg. CD138, CD19, PSMA)
ScFv antiA'-Epitope E1 = Targeting moiety T1 (V_H-V_L)
ScFV antiA1-Epitope E2 = Targeting moiety T2 (V_H-V_L)
Fragment F1 (anti CD3 V_H)
Fragment F2 (anti CD3 V_L)
Peptice linker
CD3
Polypeptice P1
Polypeptide P2
T-Cell
Target-Cell
T-Cell
Target-Cell

Figure 24

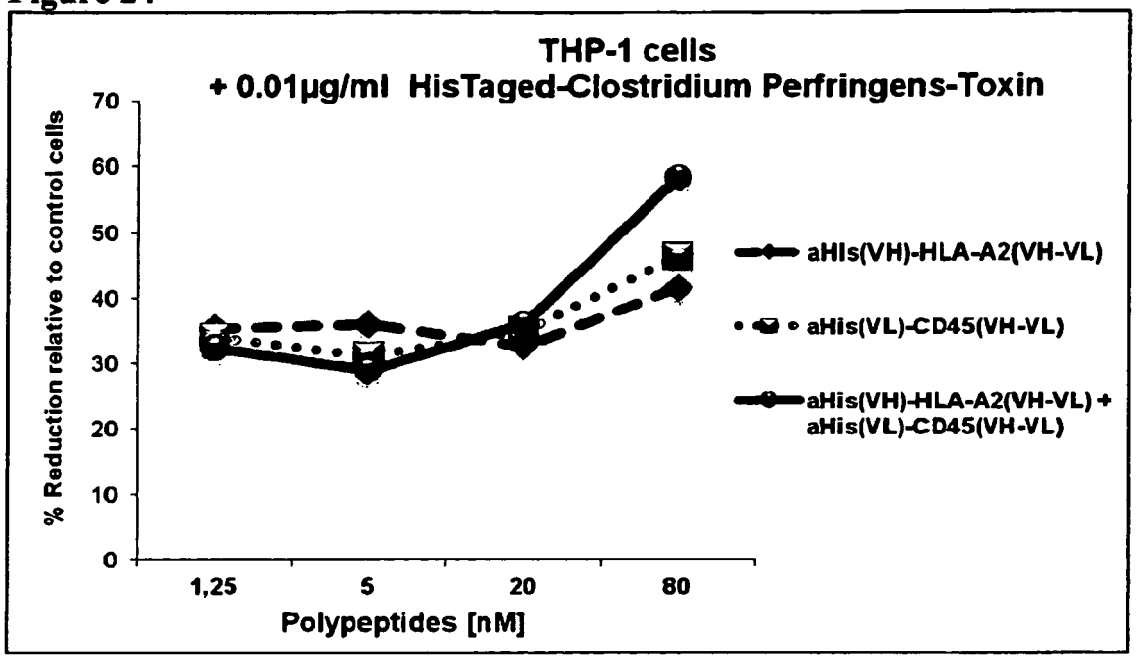


Figure 25

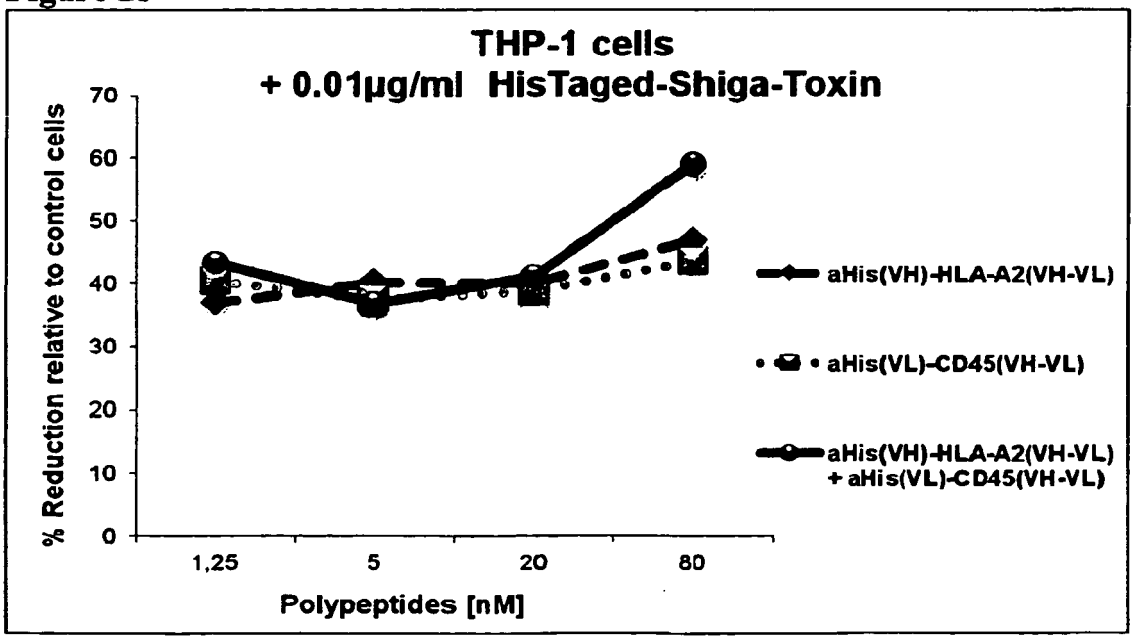


Figure 26

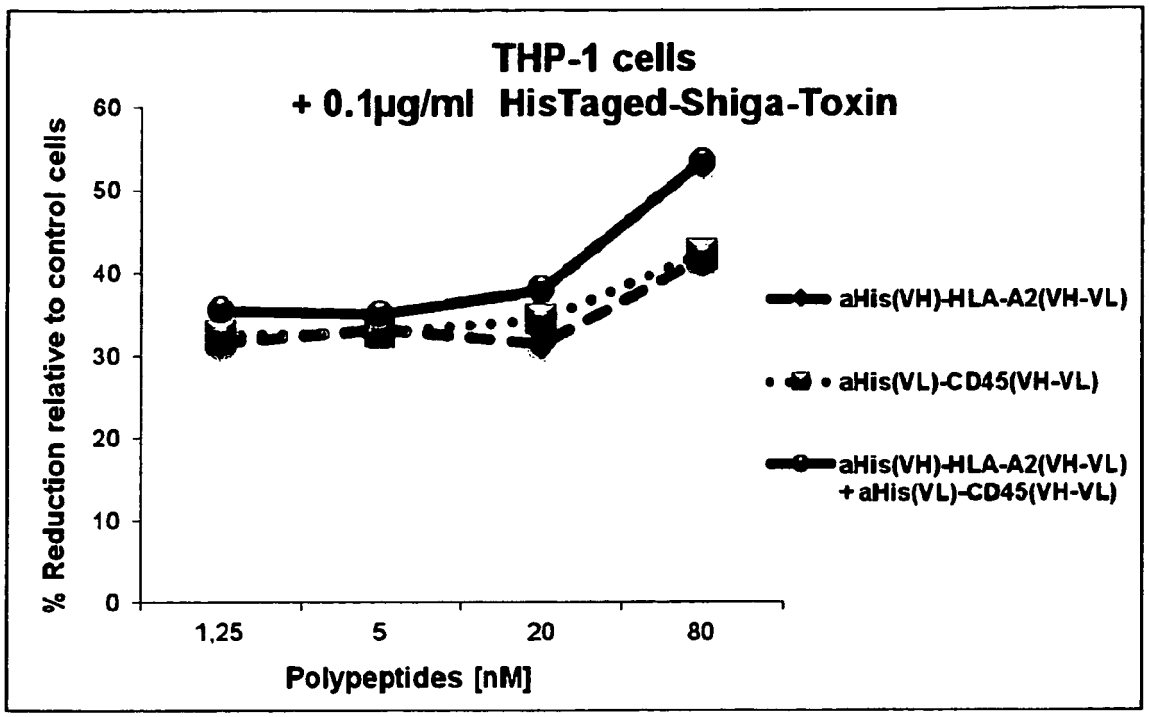

THP-1 cells
+ 0.1µg/ml HisTaged-Shiga-Toxin
% Reduction relative to control cells
60
50
40
30
20
10
0
1,25
5
20
80
Polypeptides [nM]
aHis(VH)-HLA-A2(VH-VL)
aHis(VL)-CD45(VH-VL)
aHis(VH)-HLA-A2(VH-VL)
+aHis(VL)-CD45(VH-VL)

Figure 27

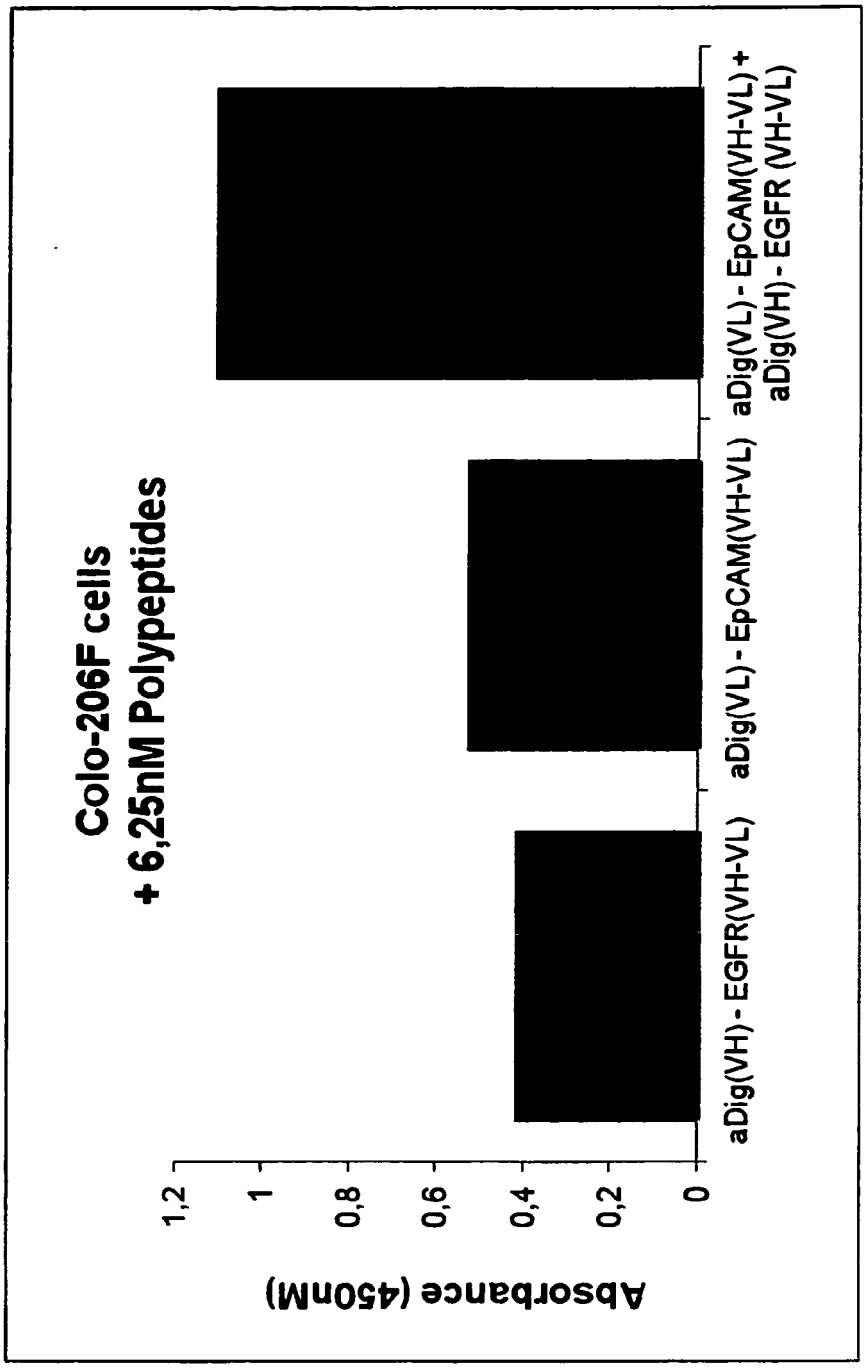

Colo-206F cells
+ 6,25nM Polypeptides
Absorbance (450nM)
1,2
1
0,8
0,6
0,4
0,2
0
aDig(VH) - EGFR(VH-VL)
aDig(VL) - EpCAM(VH-VL)
aDig(VL) - EpCAM(VH-VL) + aDig(VH) - EGFR (VH-VL)

Figure 28

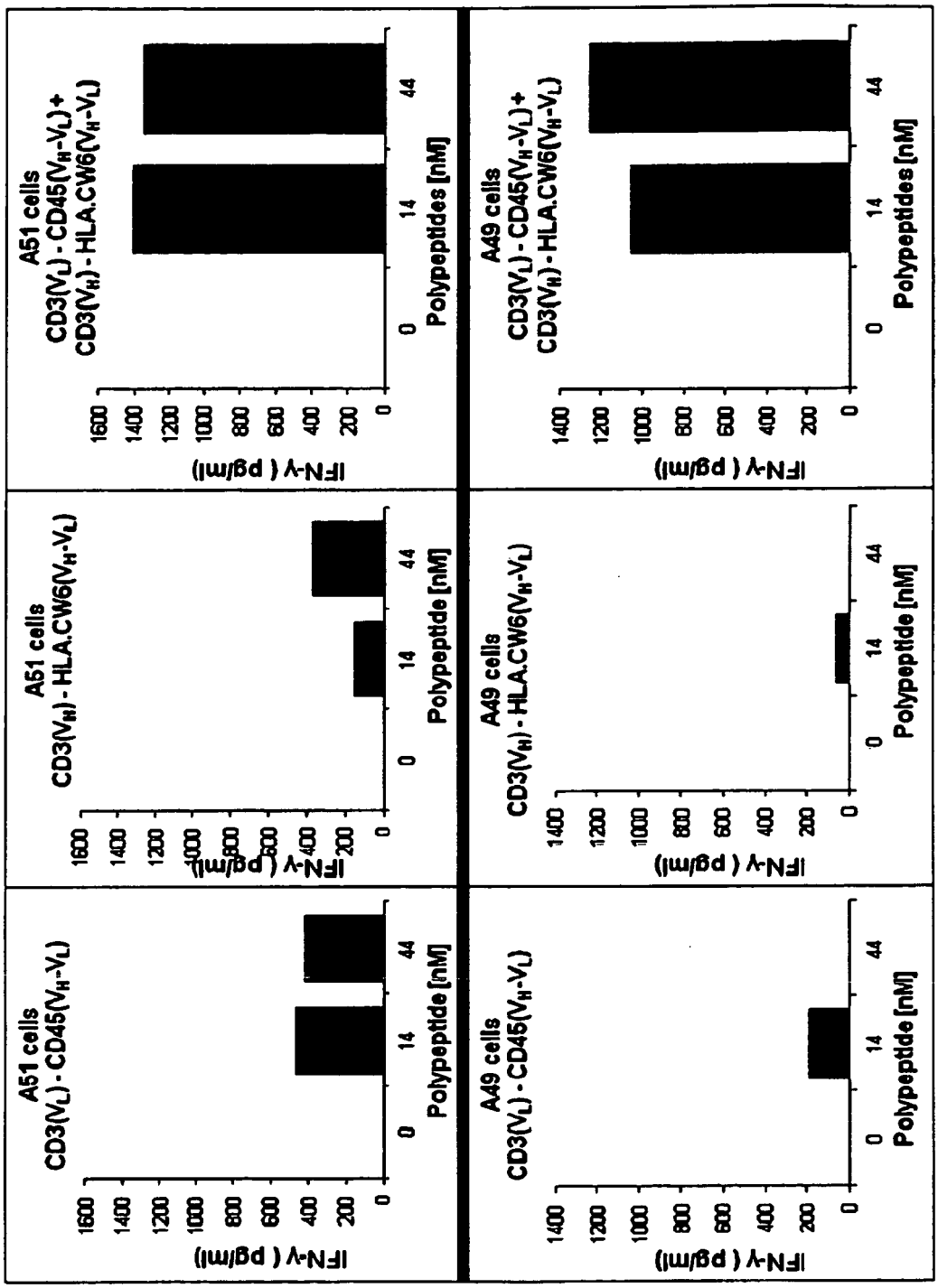

A51 cells
CD3(VL) - CD45(VH-VL)
A51 cells
CD3(VH) - HLA.CW6(VH-VL)
A51 cells
CD3(VL) - CD45(VH-VL)+
CD3(VH) - HLA.CW6(VH-VL)
A49 cells
CD3(VL) - CD45(VH-VL)
A49 cells
CD3(VH) - HLA.CW6(VH-VL)
A49 cells
CD3(VL) - CD45(VH-VL)+
CD3(VH) - HLA.CW6(VH-VL)
IFN-γ (pg/ml)
Polypeptide [nM]
0
14
44
200
400
600
800
1000
1200
1400
1600

Figure 29

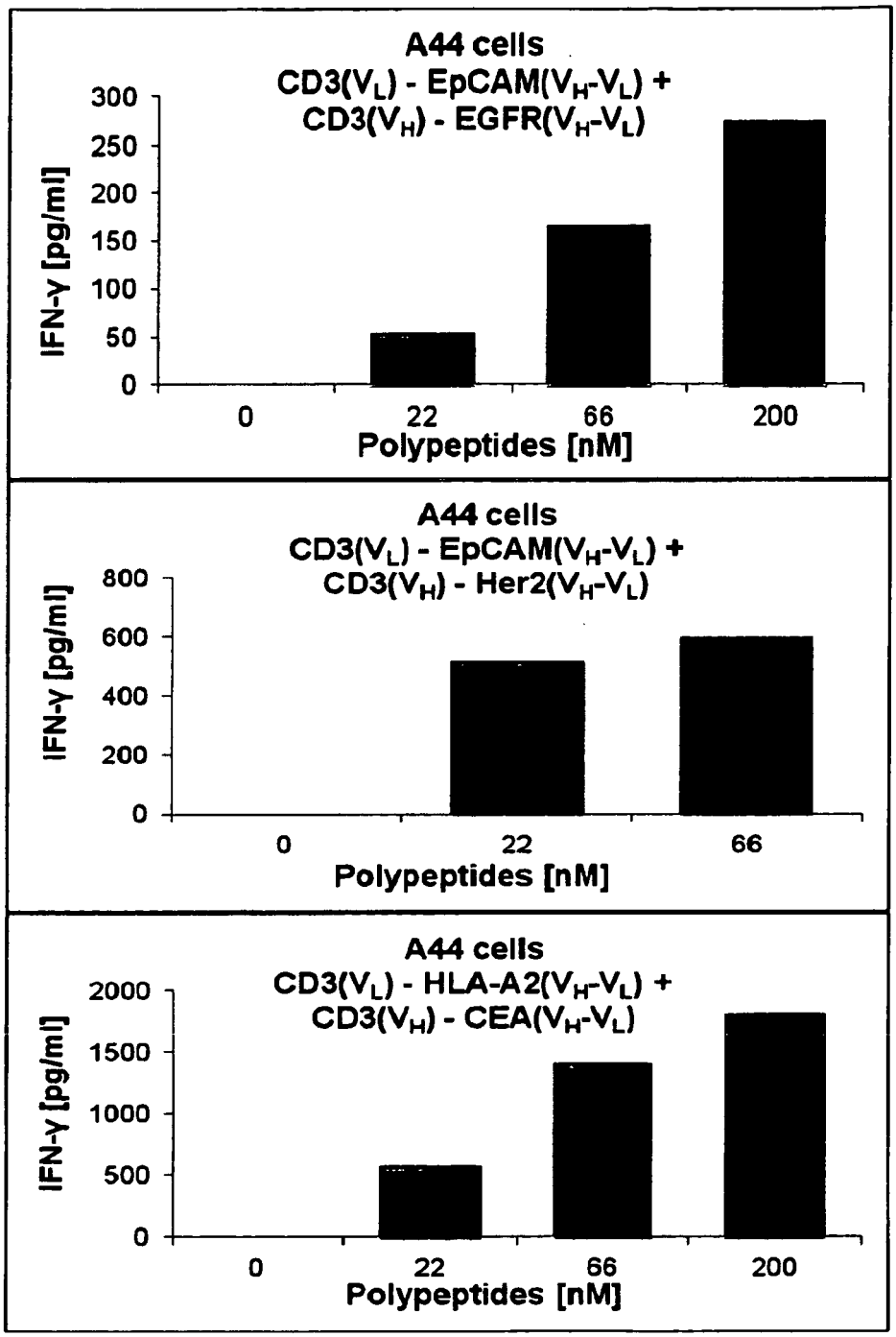

A44 cells
CD3(VL) - EpCAM(VH-VL) +
CD3(VH) - EGFR(VH-VL)
IFN-γ [pg/ml]
300
250
200
150
100
50
0
0
22
66
200
Polypeptides [nM]
A44 cells
CD3(VL) - EpCAM(VH-VL) +
CD3(VH) - Her2(VH-VL)
IFN-γ [pg/ml]
800
600
400
200
0
0
22
66
Polypeptides [nM]
A44 cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CEA(VH-VL)
IFN-γ [pg/ml]
2000
1500
1000
500
0
0
22
66
200
Polypeptides [nM]

Figure 30

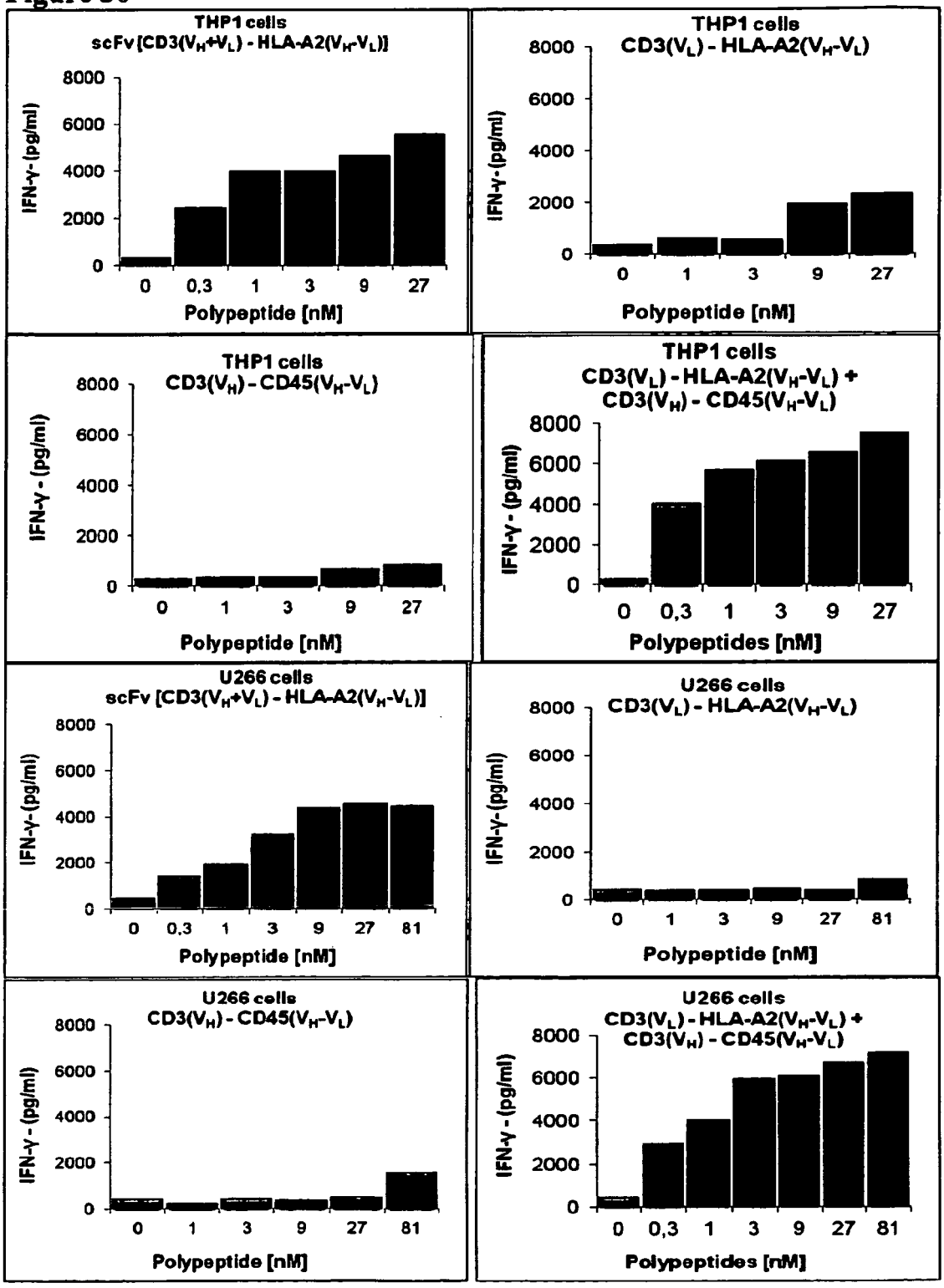
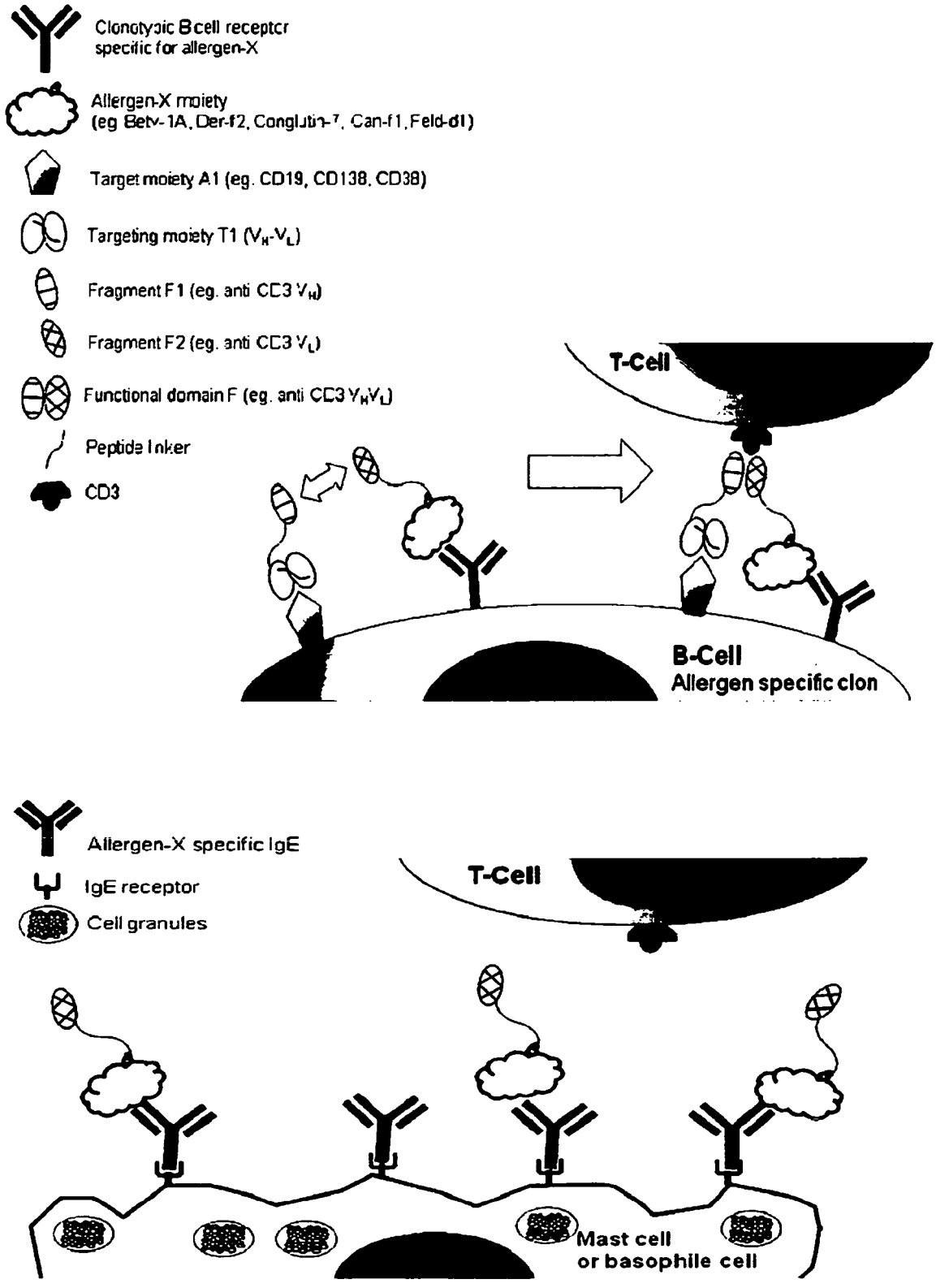

THP1 cells
scFv [CD3(VH+VL) - HLA-A2(VH-VL)]
IFN-γ- (pg/ml)
8000
6000
4000
2000
0
0 0,3 1 3 9 27
Polypeptide [nM]
THP1 cells
CD3(VL) - HLA-A2(VH-VL)
IFN-γ- (pg/ml)
8000
6000
4000
2000
0
0 1 3 9 27
Polypeptide [nM]
THP1 cells
CD3(VH) - CD45(VH-VL)
IFN-γ - (pg/ml)
8000
6000
4000
2000
0
0 1 3 9 27
Polypeptide [nM]
THP1 cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CD45(VH-VL)
IFN-γ - (pg/ml)
8000
6000
4000
2000
0
0 0,3 1 3 9 27
Polypeptides [nM]
U266 cells
scFv [CD3(VH+VL) - HLA-A2(VH-VL)]
IFN-γ- (pg/ml)
8000
6000
4000
2000
0
0 0,3 1 3 9 27 81
Polypeptide [nM]
U266 cells
CD3(VL) - HLA-A2(VH-VL)
IFN-γ- (pg/ml)
8000
6000
4000
2000
0
0 1 3 9 27 81
Polypeptide [nM]
U266 cells
CD3(VH) - CD45(VH-VL)
IFN-γ - (pg/ml)
8000
6000
4000
2000
0
0 1 3 9 27 81
Polypeptide [nM]
U266 cells
CD3(VL) - HLA-A2(VH-VL) +
CD3(VH) - CD45(VH-VL)
IFN-γ - (pg/ml)
8000
6000
4000
2000
0
0 0,3 1 3 9 27 81
Polypeptides [nM]

Figure 31


Clonotypic B cell receptor specific for allergen-X
Allergen-X moiety (eg Betv-1A, Der-f2, Conglutin-7, Can-f1, Feld-d1)
Target moiety A1 (eg. CD19, CD138, CD38)
Targeting moiety T1 (V_H-V_L)
Fragment F1 (eg. anti CD3 V_H)
Fragment F2 (eg. anti CD3 V_L)
Functional domain F (eg. anti CD3 $V_H V_L$)
Peptide linker
CD3
T-Cell
B-Cell
Allergen specific clon
Allergen-X specific IgE
IgE receptor
Cell granules
T-Cell
Mast cell
or basophile cell