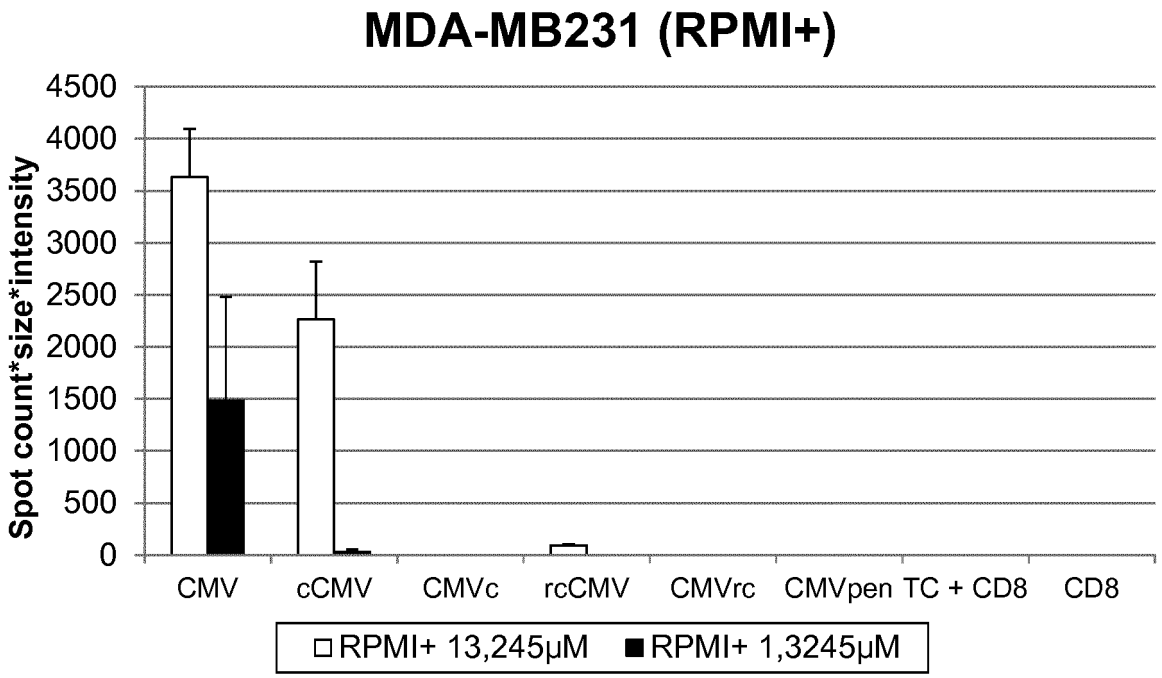

MDA-MB231 (RPMI+)
Spot count*size*intensity
4500
4000
3500
3000
2500
2000
1500
1000
500
0
CMV
cCMV
CMVc
rcCMV
CMVrc
CMVpen
TC + CD8
CD8
RPMI+ 13,245µM
RPMI+ 1,3245µM

Fig. 1A

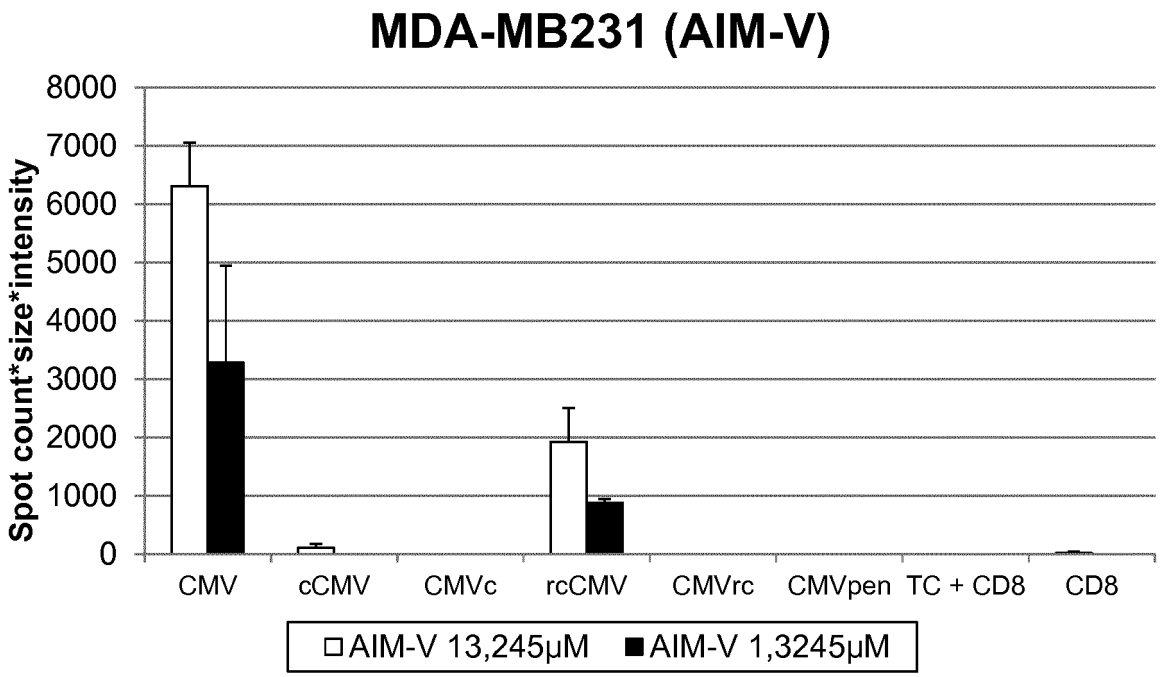

MDA-MB231 (AIM-V)
Spot count*size*intensity
8000
7000
6000
5000
4000
3000
2000
1000
0
CMV
cCMV
CMVc
rcCMV
CMVrc
CMVpen
TC + CD8
CD8
AIM-V 13,245µM
AIM-V 1,3245µM

Fig. 1B

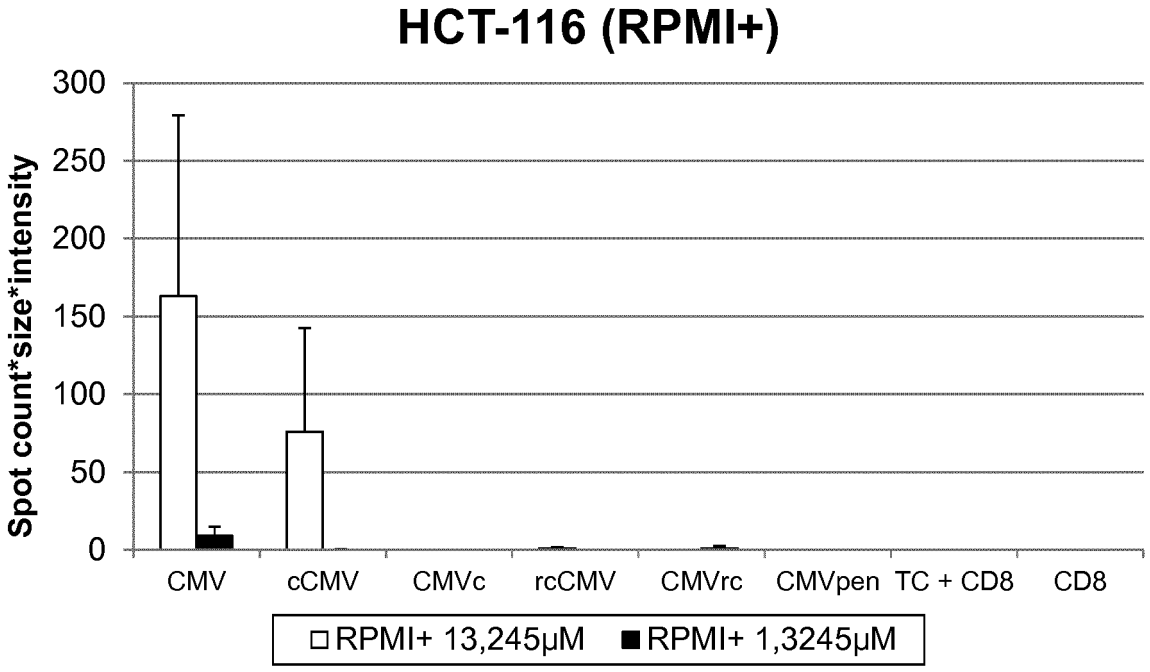

HCT-116 (RPMI+)
Spot count*size*intensity
300
250
200
150
100
50
0
CMV
cCMV
CMVc
rcCMV
CMVrc
CMVpen
TC + CD8
CD8
RPMI+ 13,245µM
RPMI+ 1,3245µM

Fig. 1C

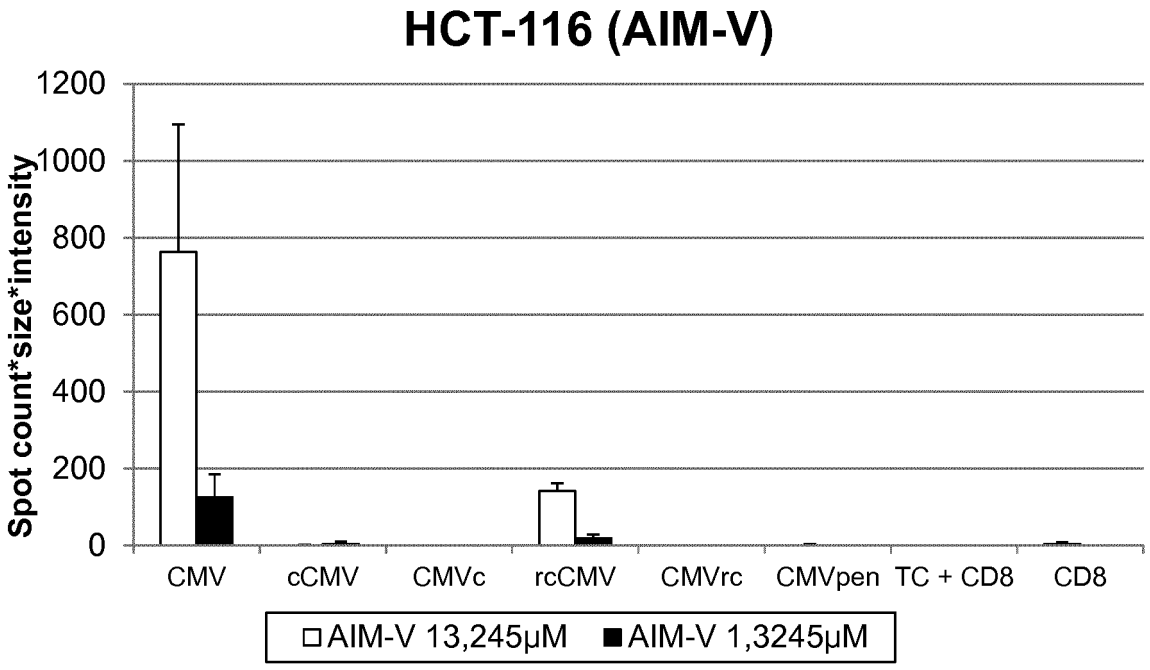

HCT-116 (AIM-V)
Spot count*size*intensity
1200
1000
800
600
400
200
0
CMV
cCMV
CMVc
rcCMV
CMVrc
CMVpen
TC + CD8
CD8
AIM-V 13,245µM
AIM-V 1,3245µM

Fig. 1D

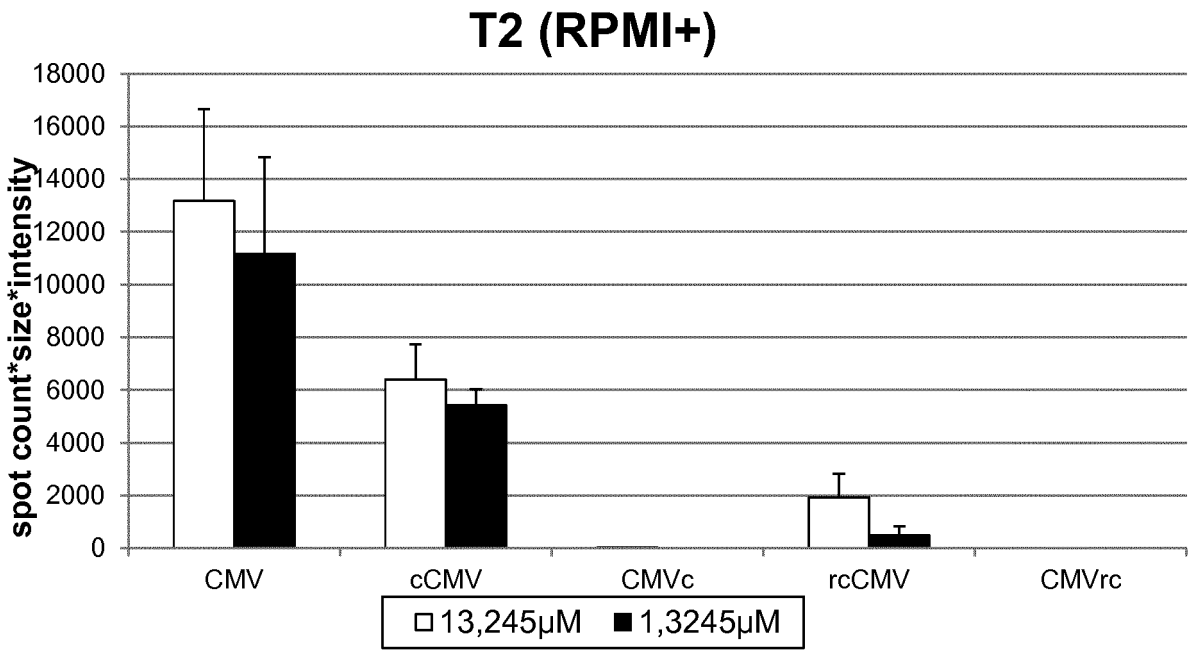

T2 (RPMI+)
spot count*size*intensity
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
CMV
cCMV
CMVc
rcCMV
CMVrc
13,245µM
1,3245µM

Fig. 1E

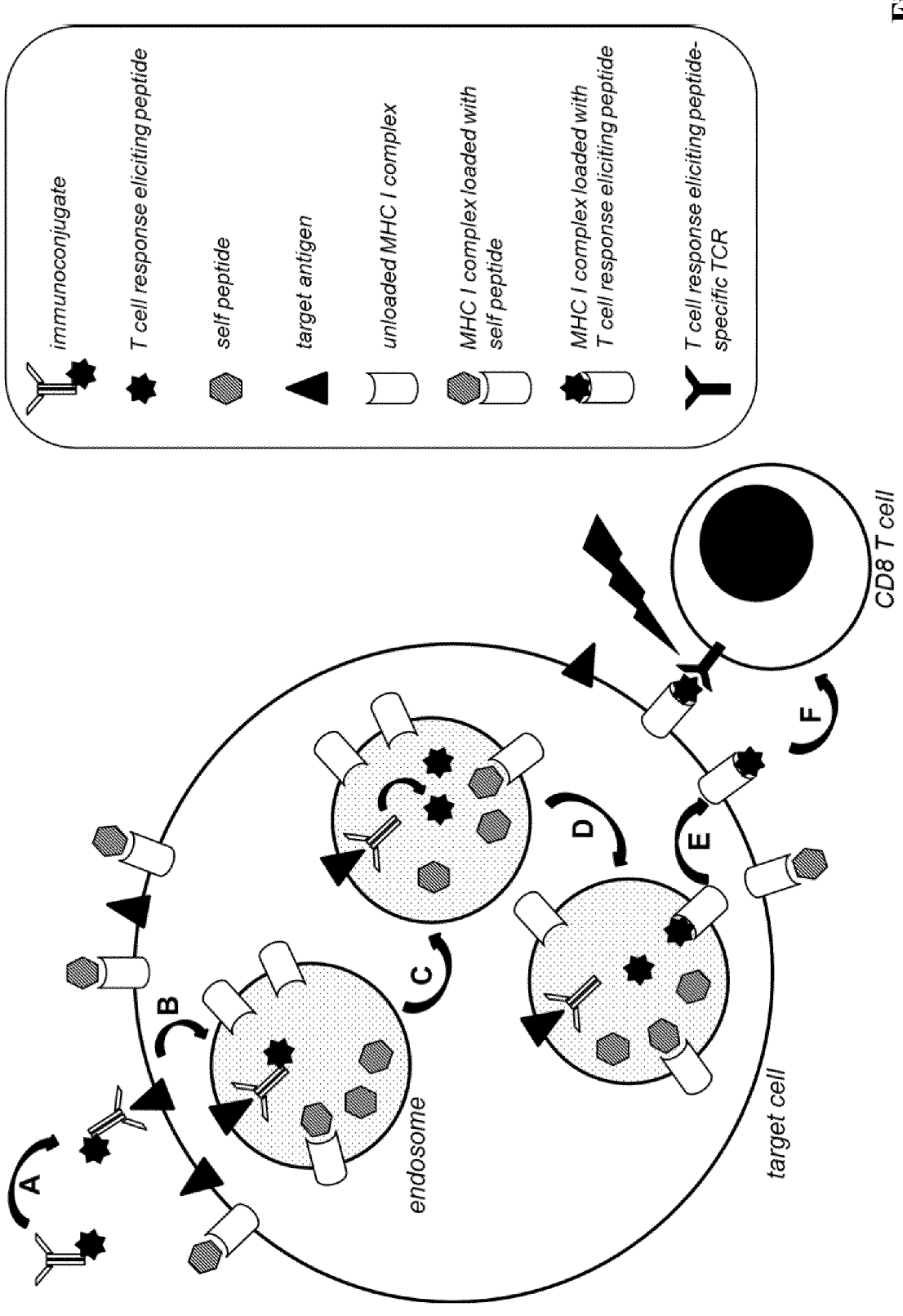

immunoconjugate
T cell response eliciting peptide
self peptide
target antigen
unloaded MHC I complex
MHC I complex loaded with self peptide
MHC I complex loaded with T cell response eliciting peptide
T cell response eliciting peptide-specific TCR
A
B
C
D
E
F
endosome
target cell
CD8 T cell

Fig. 2

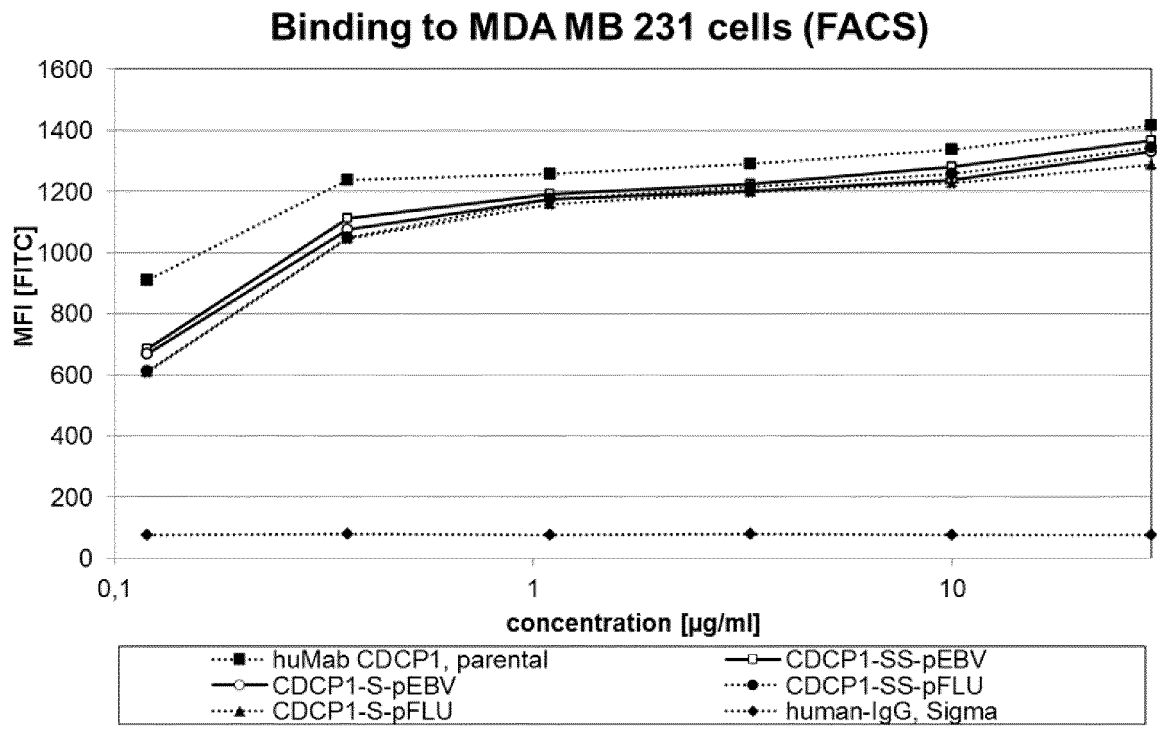

Binding to MDA MB 231 cells (FACS)
MFI [FITC]
1600
1400
1200
1000
800
600
400
200
0
0,1
1
10
concentration [µg/ml]
huMab CDCP1, parental
CDCP1-S-pEBV
CDCP1-S-pFLU
CDCP1-SS-pEBV
CDCP1-SS-pFLU
human-IgG, Sigma

Fig. 3

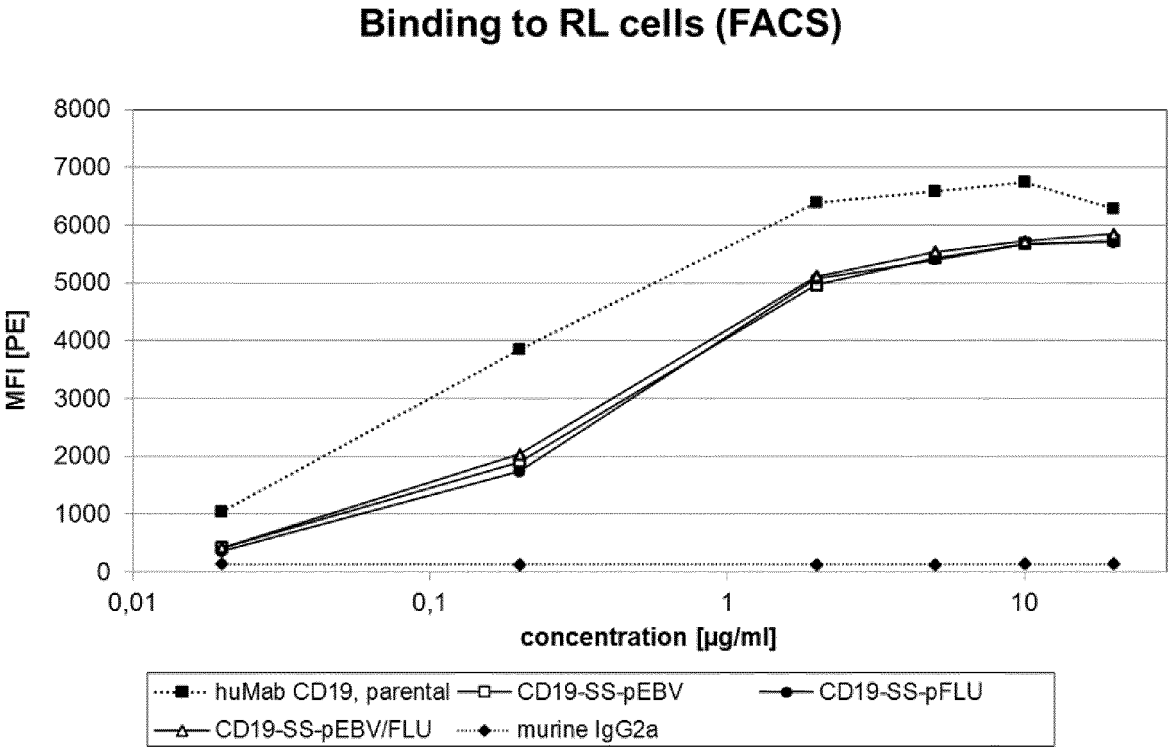

Binding to RL cells (FACS)
MFI [PE]
8000
7000
6000
5000
4000
3000
2000
1000
0
0,01
0,1
1
10
concentration [µg/ml]
huMab CD19, parental
CD19-SS-pEBV
CD19-SS-pFLU
CD19-SS-pEBV/FLU
murine IgG2a

Fig. 4

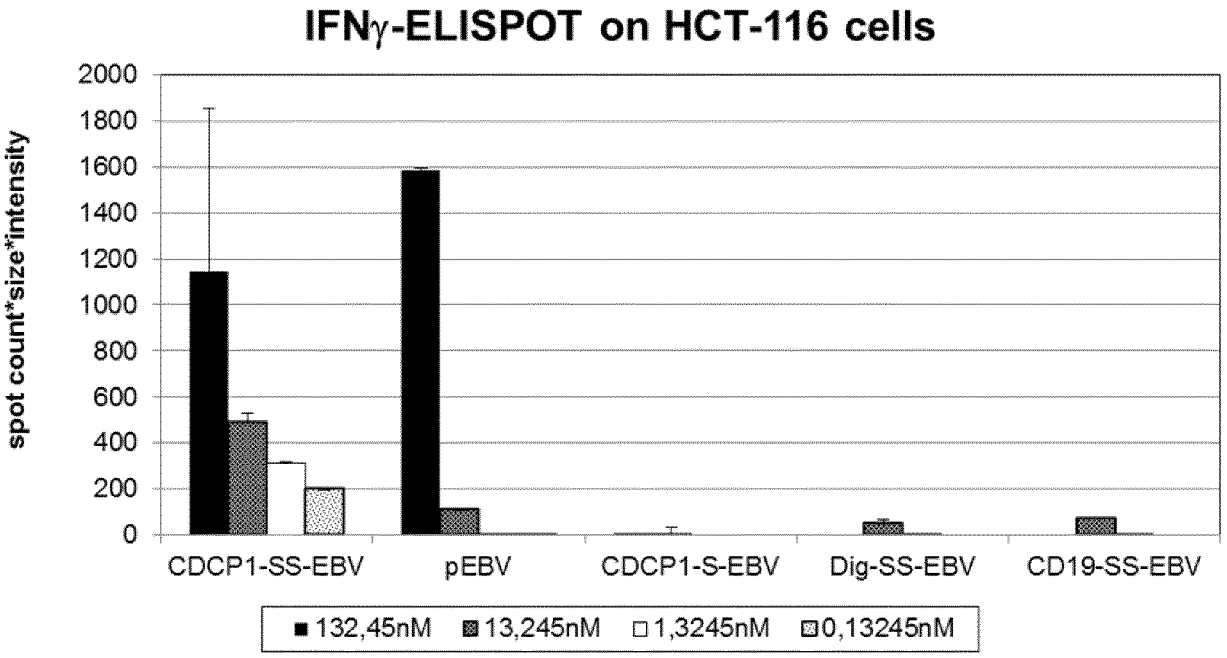

IFNγ-ELISPOT on HCT-116 cells
spot count*size*intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
CDCP1-SS-EBV
pEBV
CDCP1-S-EBV
Dig-SS-EBV
CD19-SS-EBV
132,45nM
13,245nM
1,3245nM
0,13245nM

Fig. 5A

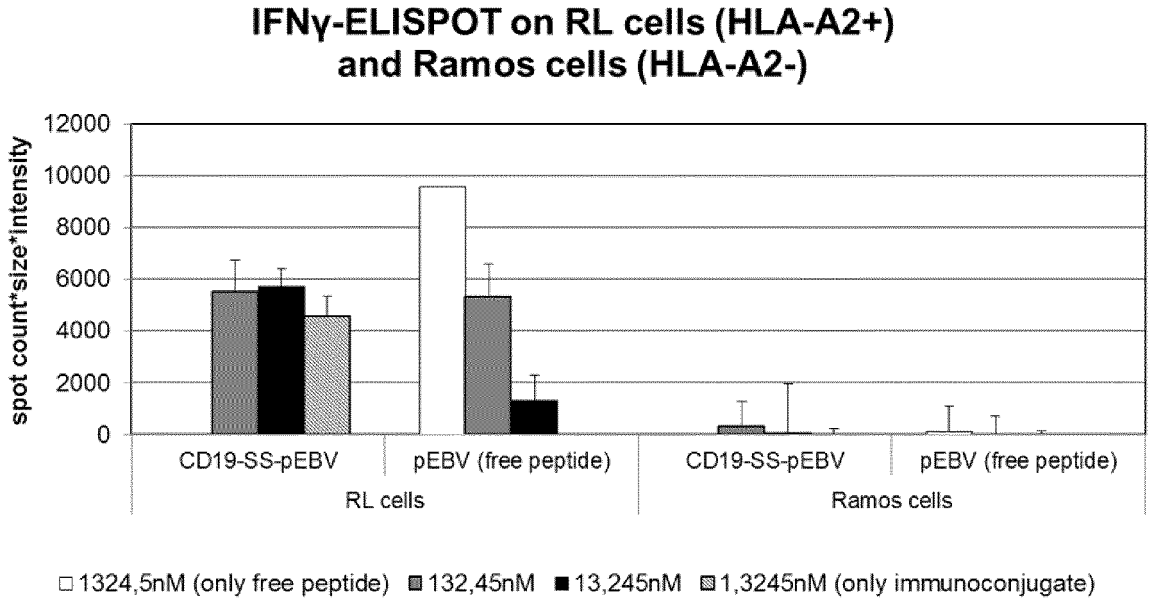

IFNγ-ELISPOT on RL cells (HLA-A2+)
and Ramos cells (HLA-A2-)
spot count*size*intensity
12000
10000
8000
6000
4000
2000
0
CD19-SS-pEBV
pEBV (free peptide)
CD19-SS-pEBV
pEBV (free peptide)
RL cells
Ramos cells
1324,5nM (only free peptide)
132,45nM
13,245nM
1,3245nM (only immunoconjugate)

Fig. 5B

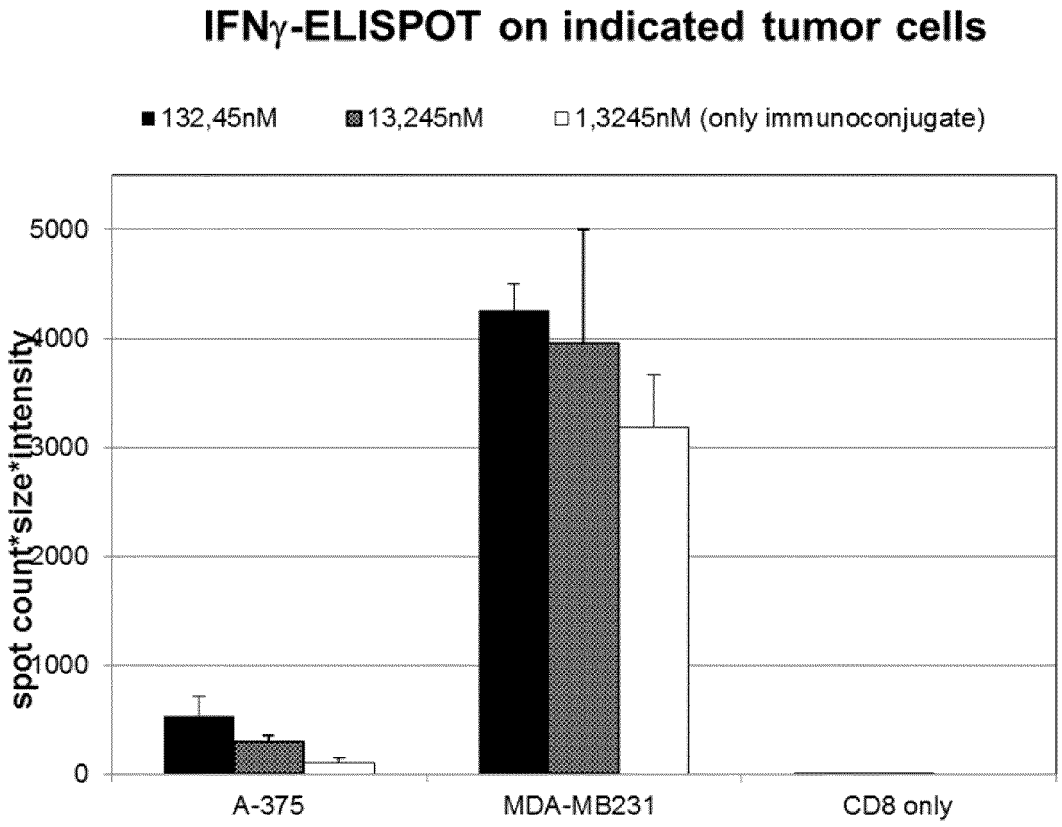

IFNγ-ELISPOT on indicated tumor cells
132,45nM
13,245nM
1,3245nM (only immunoconjugate)
spot count*size*intensity
5000
4000
3000
2000
1000
0
A-375
MDA-MB231
CD8 only

Fig. 5C

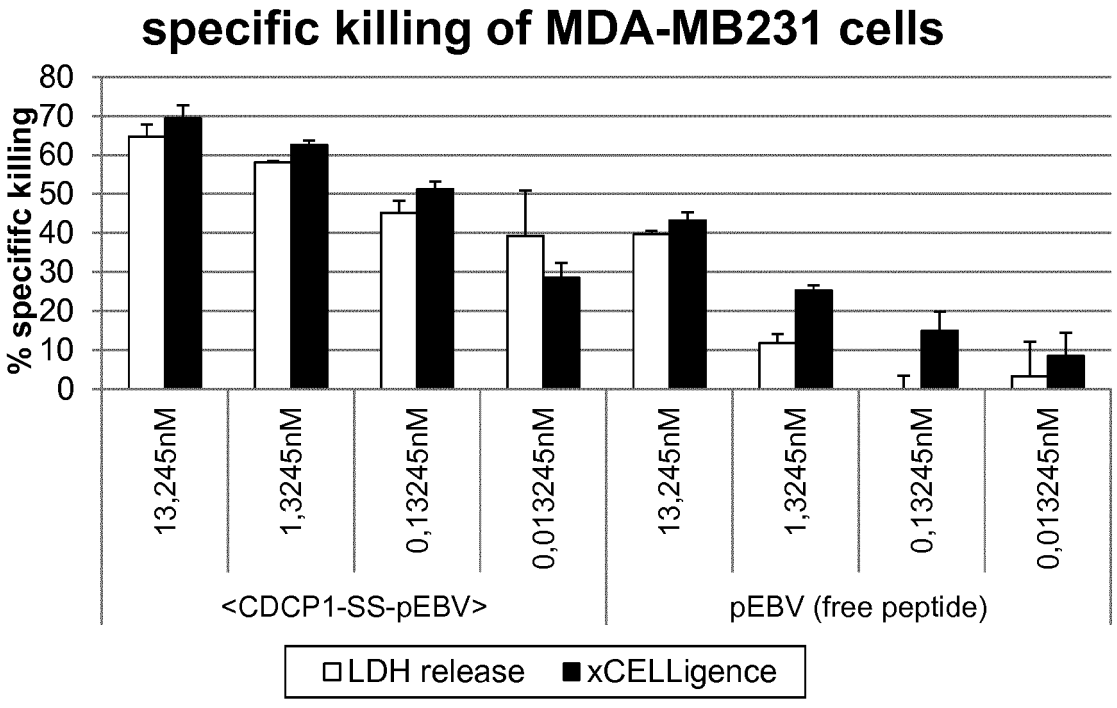

specific killing of MDA-MB231 cells
% specific killing
80
70
60
50
40
30
20
10
0
13,245nM
1,3245nM
0,13245nM
0,013245nM
13,245nM
1,3245nM
0,13245nM
0,013245nM
<CDCP1-SS-pEBV>
pEBV (free peptide)
LDH release
xCELLigence

Fig. 6A

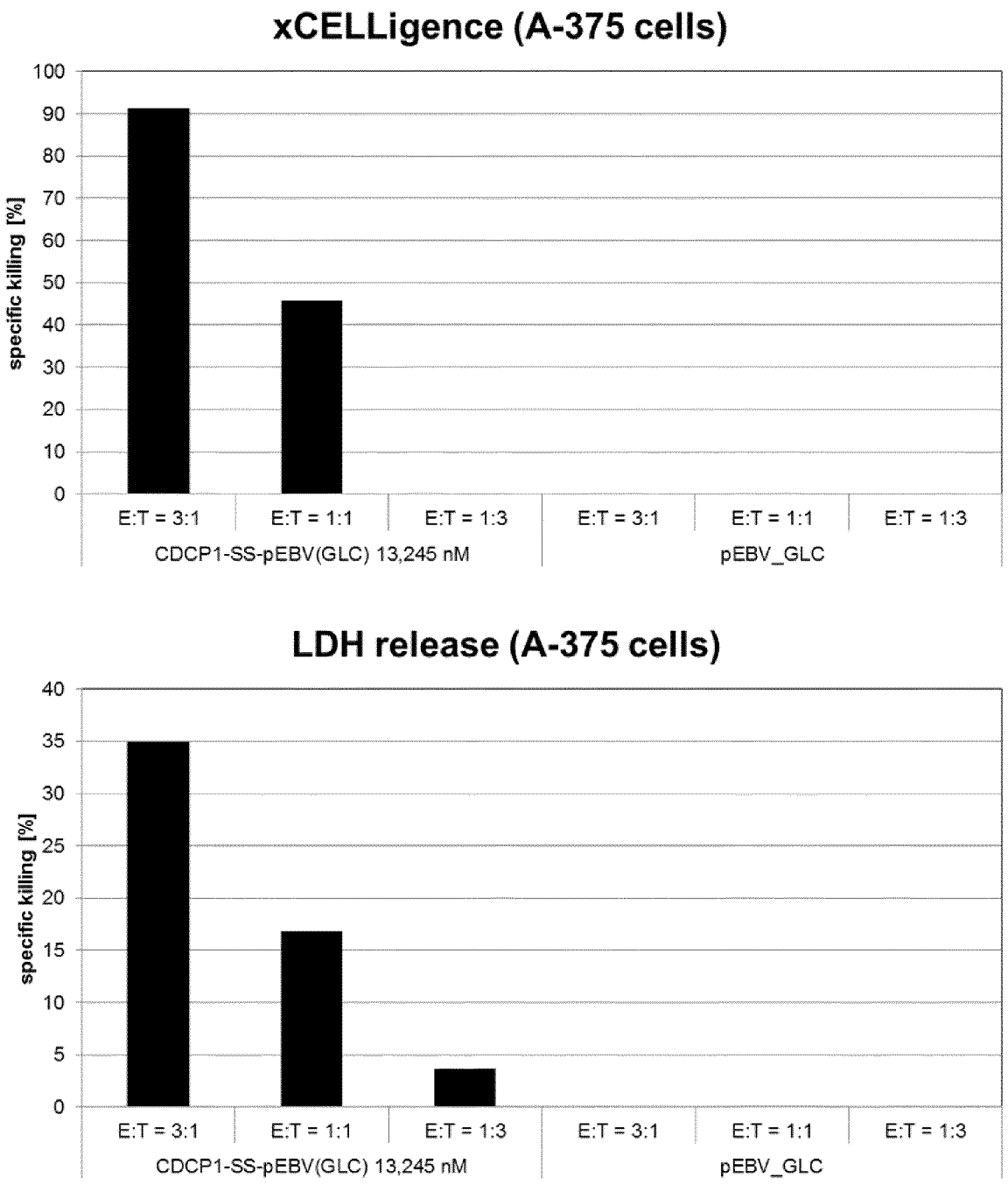
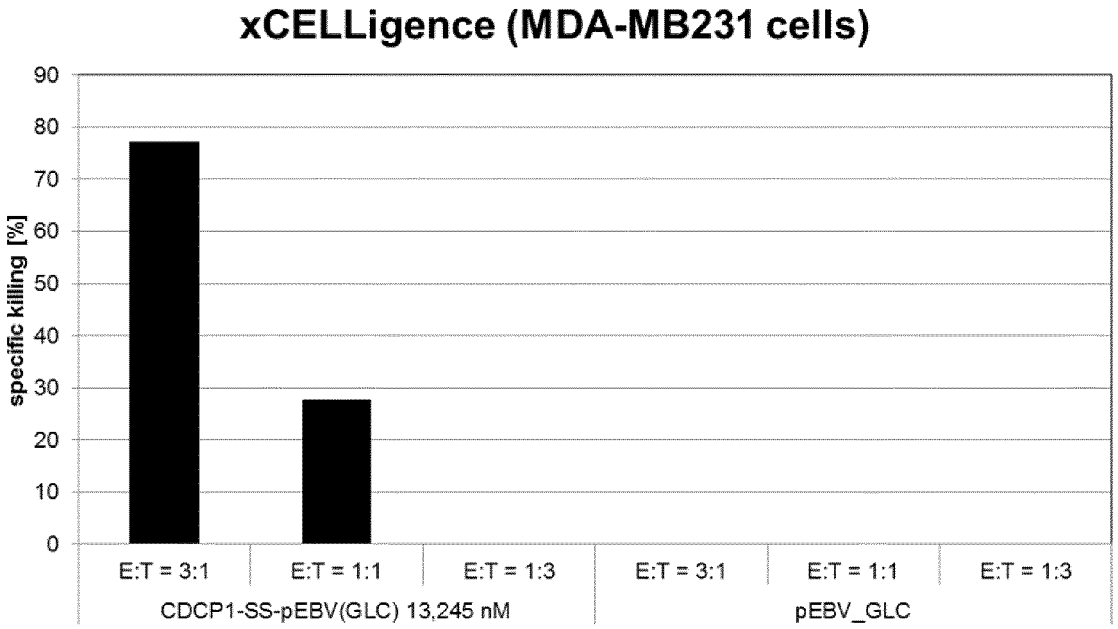

xCELLigence (A-375 cells)
specific killing [%]
100
90
80
70
60
50
40
30
20
10
0
E:T = 3:1
E:T = 1:1
E:T = 1:3
E:T = 3:1
E:T = 1:1
E:T = 1:3
CDCP1-SS-pEBV(GLC) 13,245 nM
pEBV_GLC
LDH release (A-375 cells)
specific killing [%]
40
35
30
25
20
15
10
5
0
E:T = 3:1
E:T = 1:1
E:T = 1:3
E:T = 3:1
E:T = 1:1
E:T = 1:3
CDCP1-SS-pEBV(GLC) 13,245 nM
pEBV_GLC

Fig. 6B


xCELLigence (MDA-MB231 cells)
specific killing [%]
90
80
70
60
50
40
30
20
10
0
E:T = 3:1
E:T = 1:1
E:T = 1:3
E:T = 3:1
E:T = 1:1
E:T = 1:3
CDCP1-SS-pEBV(GLC) 13,245 nM
pEBV_GLC

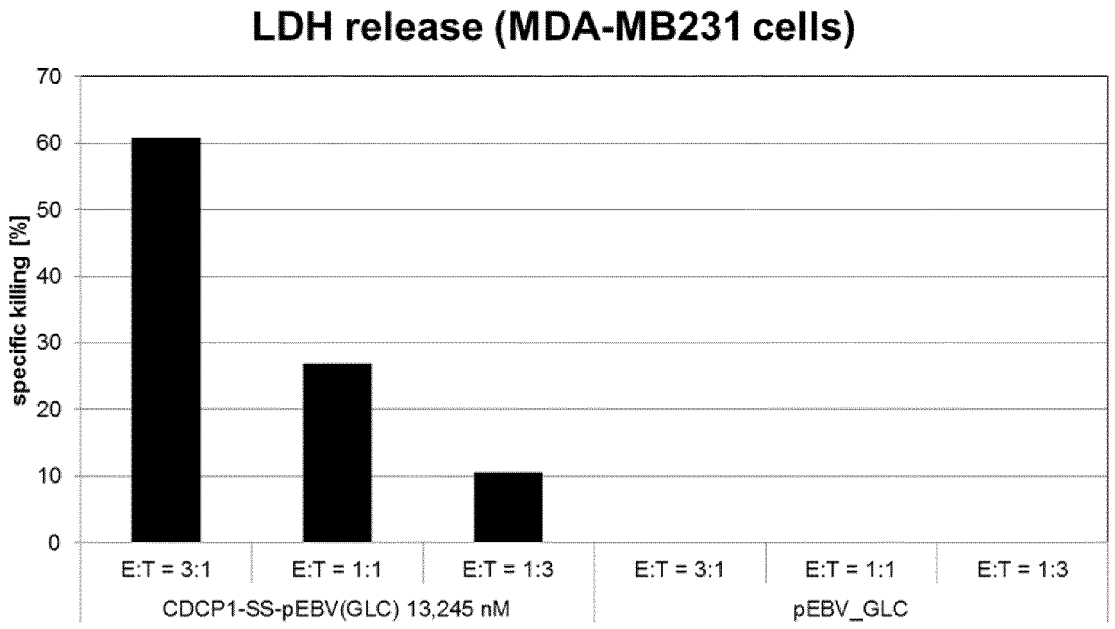

LDH release (MDA-MB231 cells)
specific killing [%]
70
60
50
40
30
20
10
0
E:T = 3:1
E:T = 1:1
E:T = 1:3
E:T = 3:1
E:T = 1:1
E:T = 1:3
CDCP1-SS-pEBV(GLC) 13,245 nM
pEBV_GLC

Fig. 6C

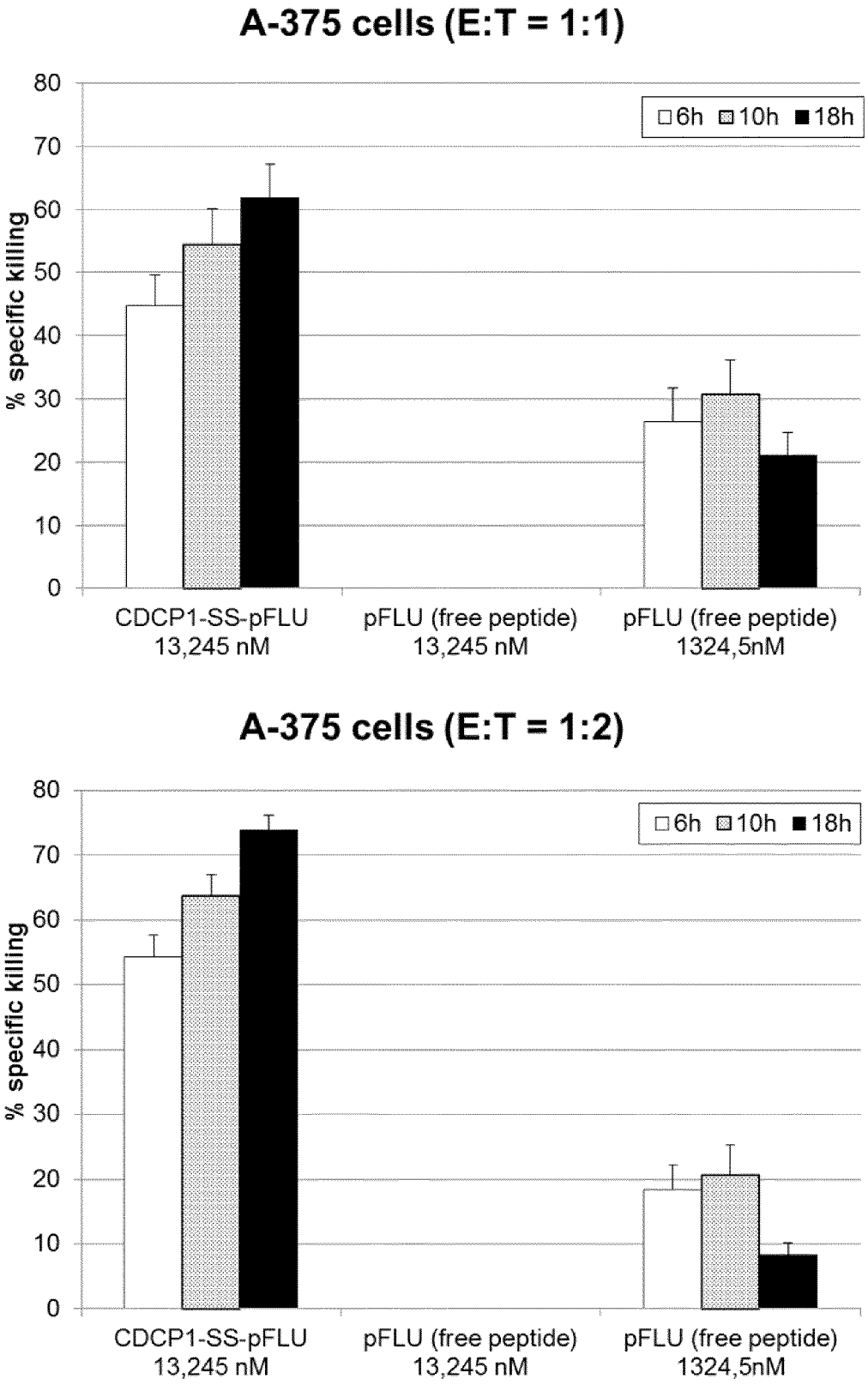

A-375 cells (E:T = 1:1)
% specific killing
80
70
60
50
40
30
20
10
0
6h
10h
18h
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM
A-375 cells (E:T = 1:2)
% specific killing
80
70
60
50
40
30
20
10
0
6h
10h
18h
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

Fig. 7A

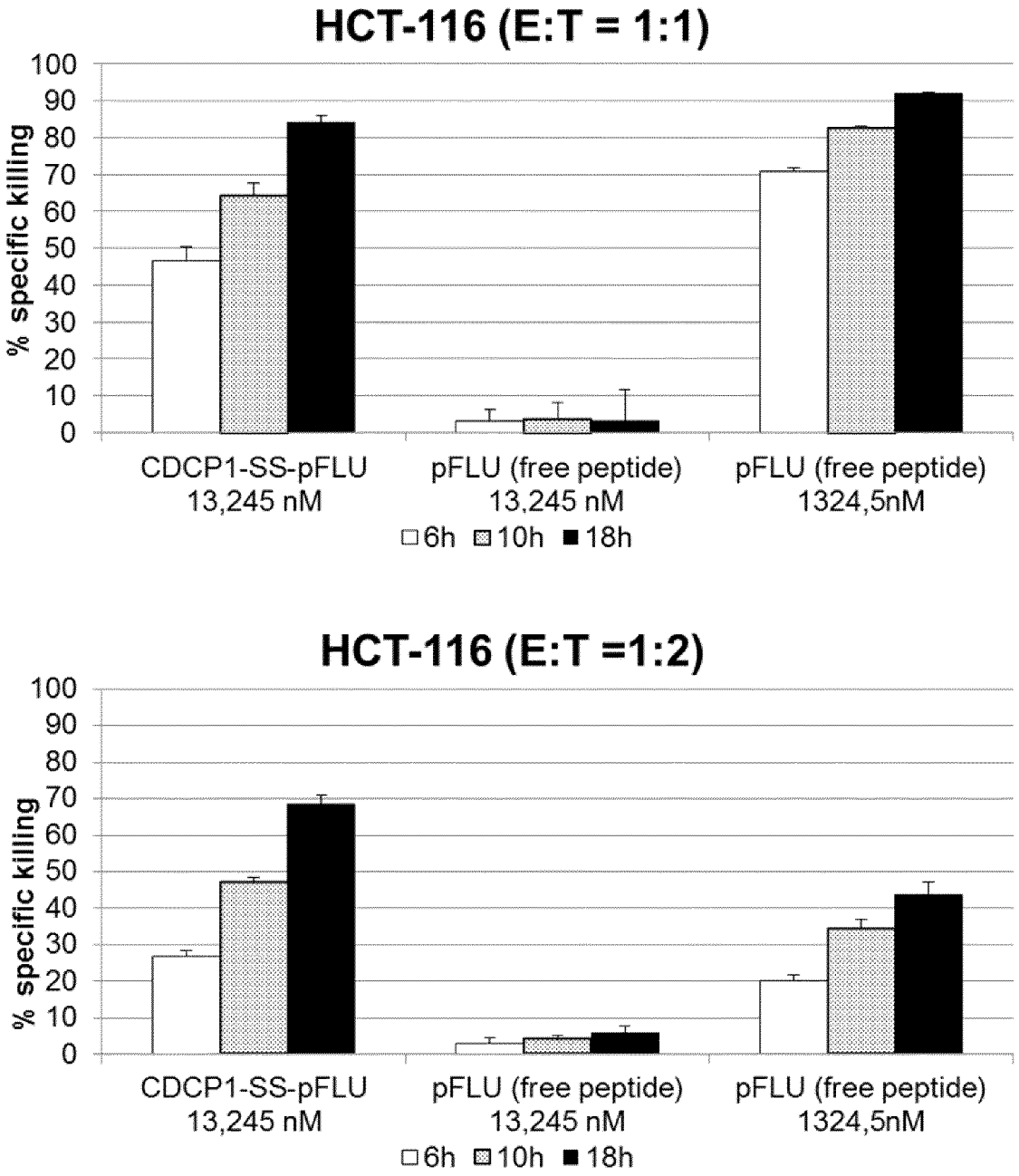

HCT-116 (E:T = 1:1)
% specific killing
100
90
80
70
60
50
40
30
20
10
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM
6h
10h
18h
HCT-116 (E:T =1:2)
% specific killing
100
90
80
70
60
50
40
30
20
10
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM
6h
10h
18h

Fig. 7B

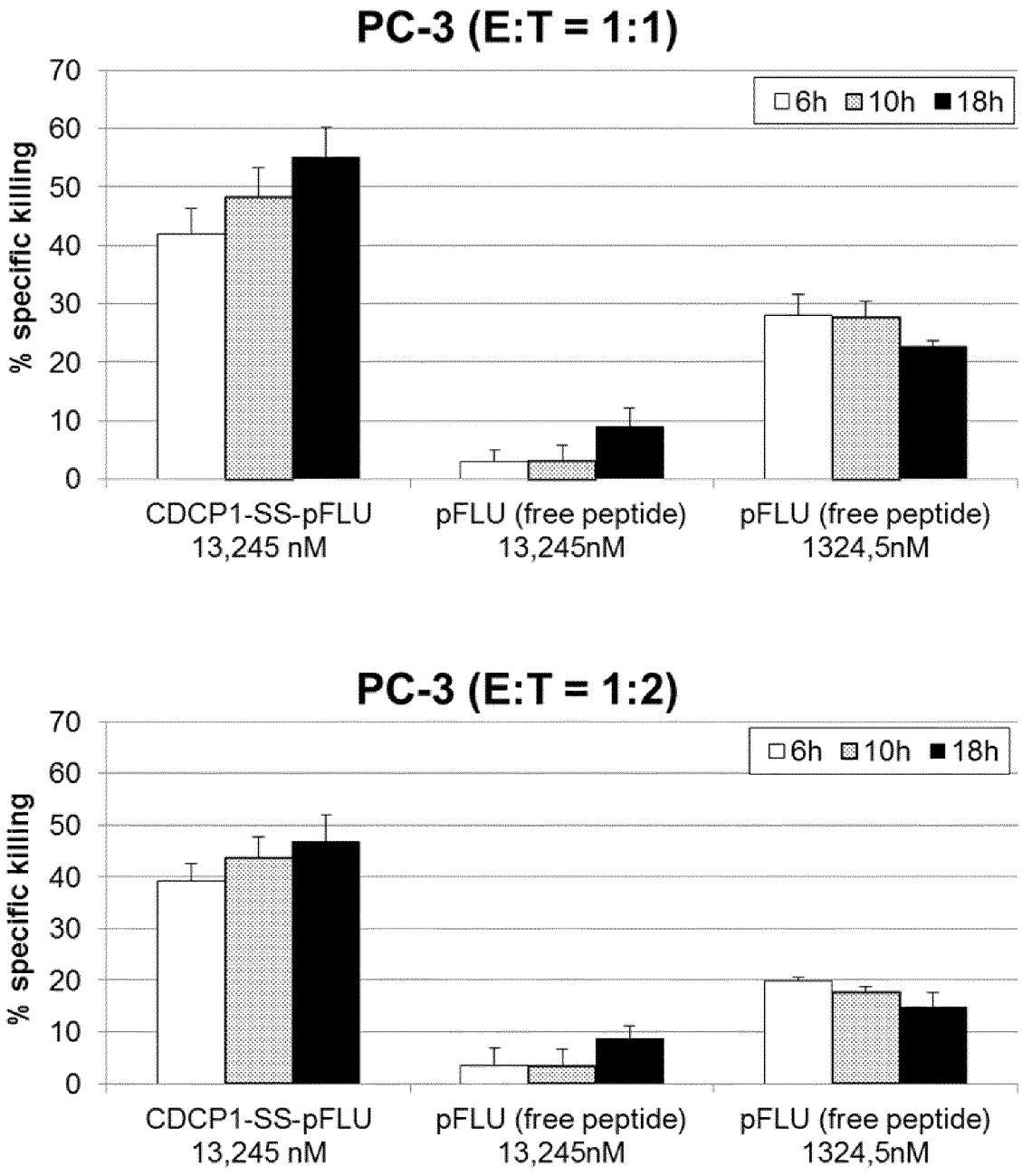
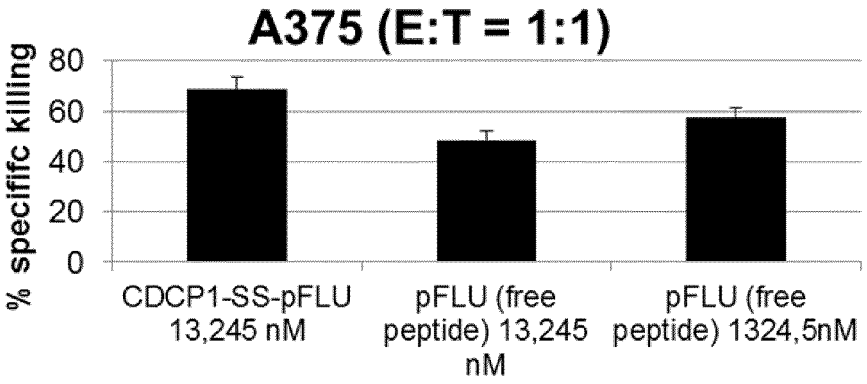

PC-3 (E:T = 1:1)
% specific killing
70
60
50
40
30
20
10
0
6h
10h
18h
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245nM
pFLU (free peptide) 1324,5nM
PC-3 (E:T = 1:2)
% specific killing
70
60
50
40
30
20
10
0
6h
10h
18h
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245nM
pFLU (free peptide) 1324,5nM

Fig. 7C


A375 (E:T = 1:1)
% specifc killing
80
60
40
20
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

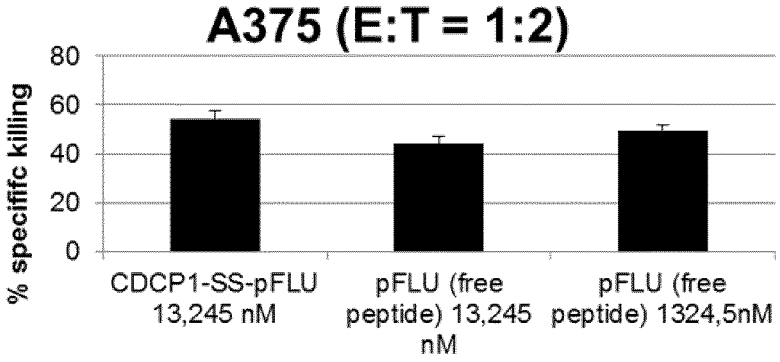
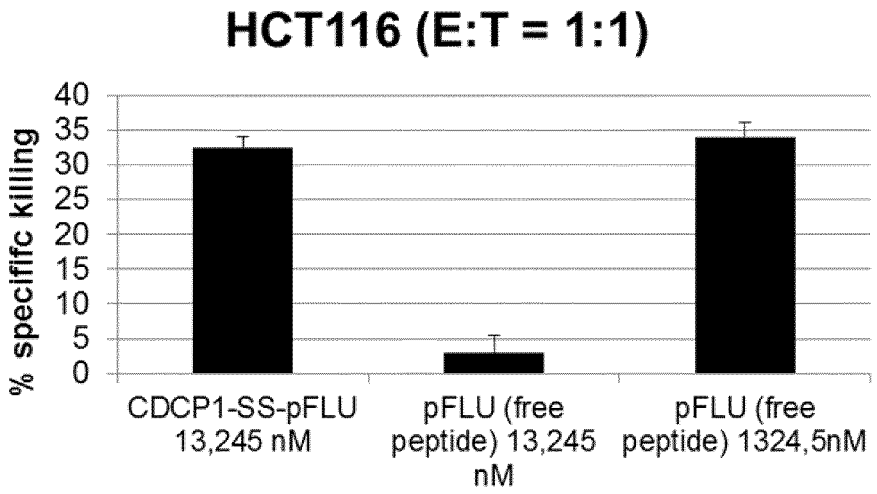

A375 (E:T = 1:2)
% specifc killing
80
60
40
20
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

Fig. 7D


HCT116 (E:T = 1:1)
% specifc killing
40
35
30
25
20
15
10
5
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

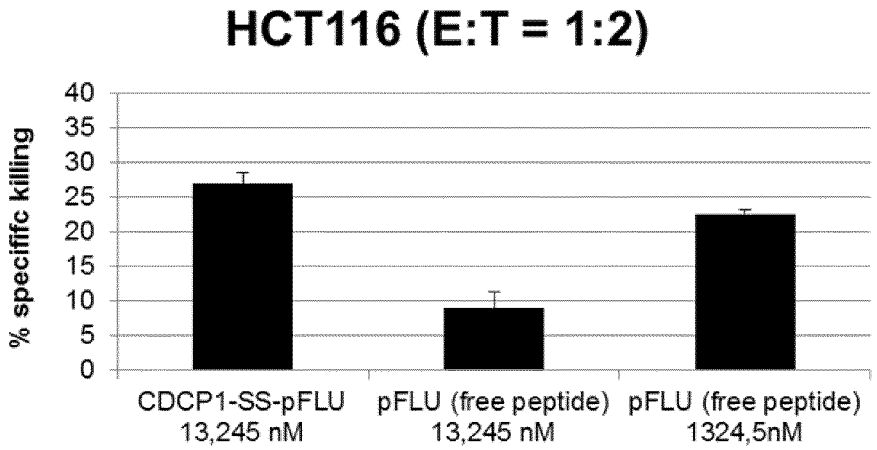
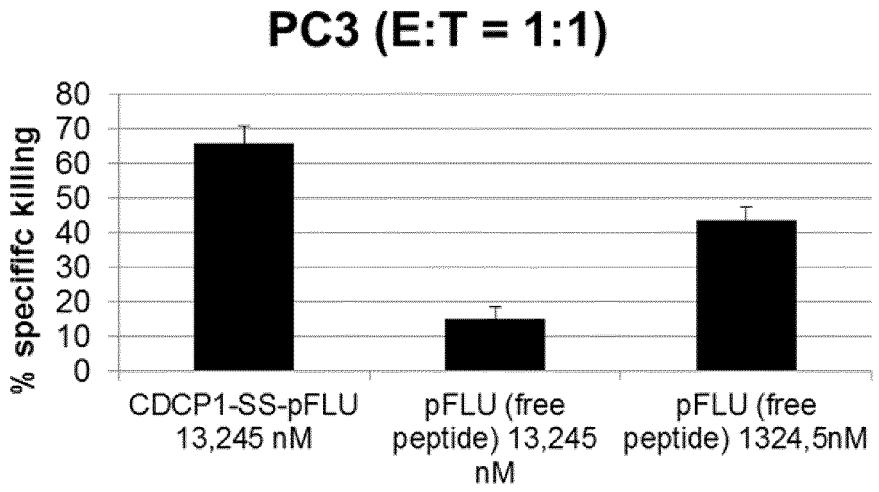

HCT116 (E:T = 1:2)
% specifc killing
40
35
30
25
20
15
10
5
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

Fig. 7E


PC3 (E:T = 1:1)
% specififc killing
80
70
60
50
40
30
20
10
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

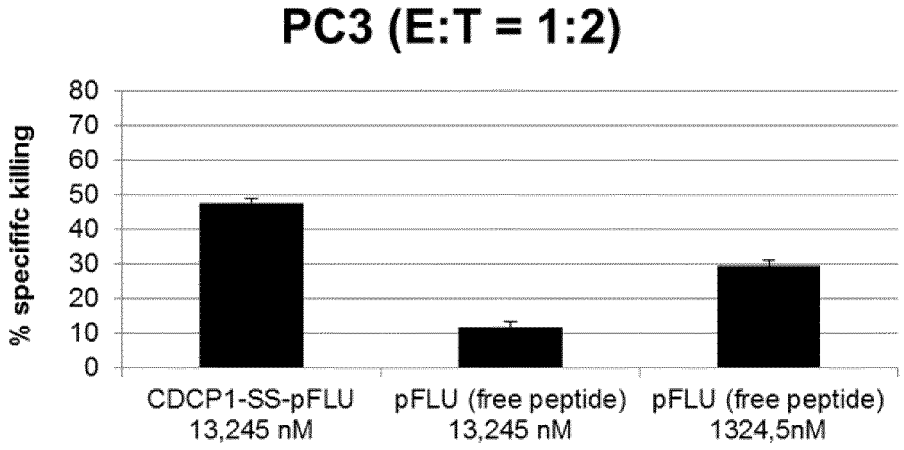

PC3 (E:T = 1:2)
% specififc killing
80
70
60
50
40
30
20
10
0
CDCP1-SS-pFLU 13,245 nM
pFLU (free peptide) 13,245 nM
pFLU (free peptide) 1324,5nM

Fig. 7F

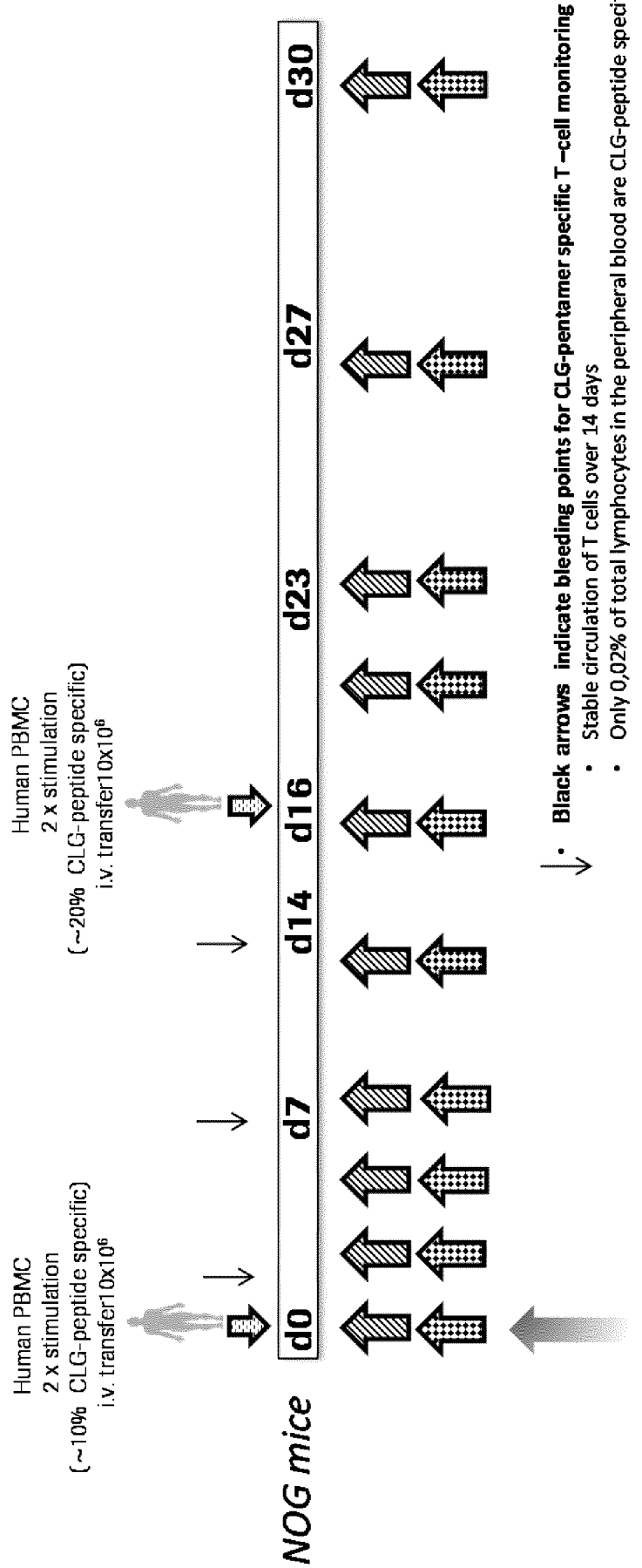

Human PBMC
2 x stimulation
(~10% CLG-peptide specific)
i.v. transfer10x10⁶
Human PBMC
2 x stimulation
(~20% CLG-peptide specific)
i.v. transfer10x10⁶
NOG mice
d0
d7
d14
d16
d23
d27
d30
Black arrows indicate bleeding points for CLG-pentamer specific T-cell monitoring
Stable circulation of T cells over 14 days
Only 0,02% of total lymphocytes in the peripheral blood are CLG-peptide specific
Human tumor cells injection s.c.
(5x10⁶ Mio MDA-MB231)
ATTP: <CDCP1>-IgG4-SS-CLG Peptide 5 mg/kg (on day: 0, 3, 6, 9, 13, 16, 20, 23, 27, 30)
Control AB: <CDCP1>-IgG4 5 mg/kg (on day: 0, 3, 6, 9, 13, 16, 20, 23, 27, 30)

Fig. 8A

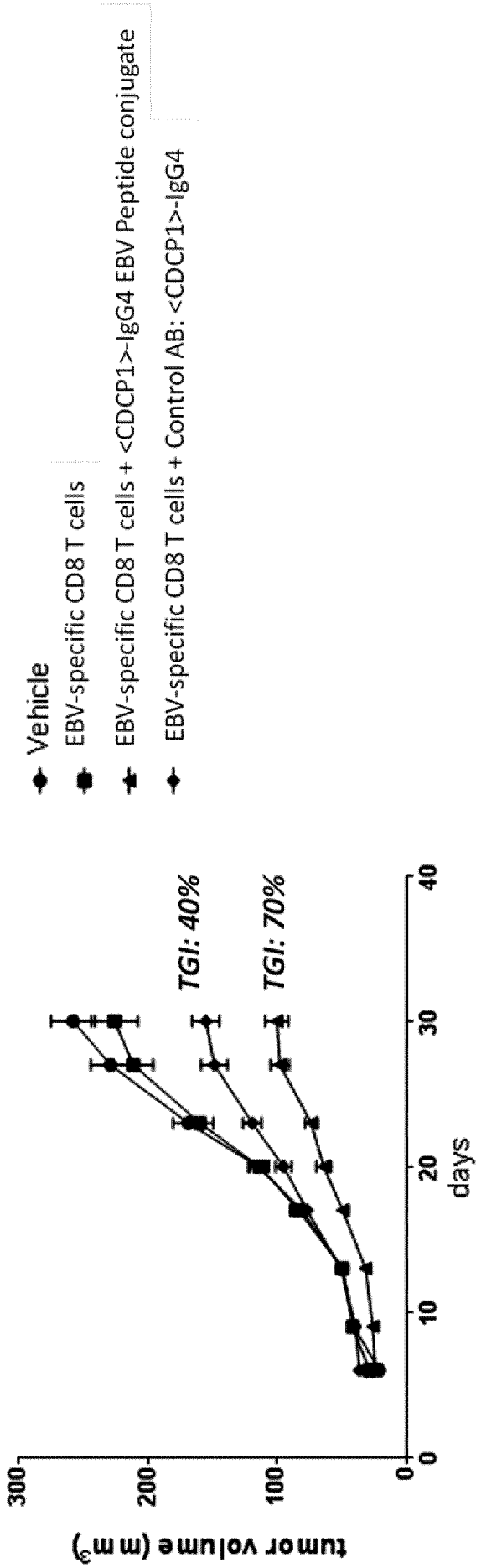
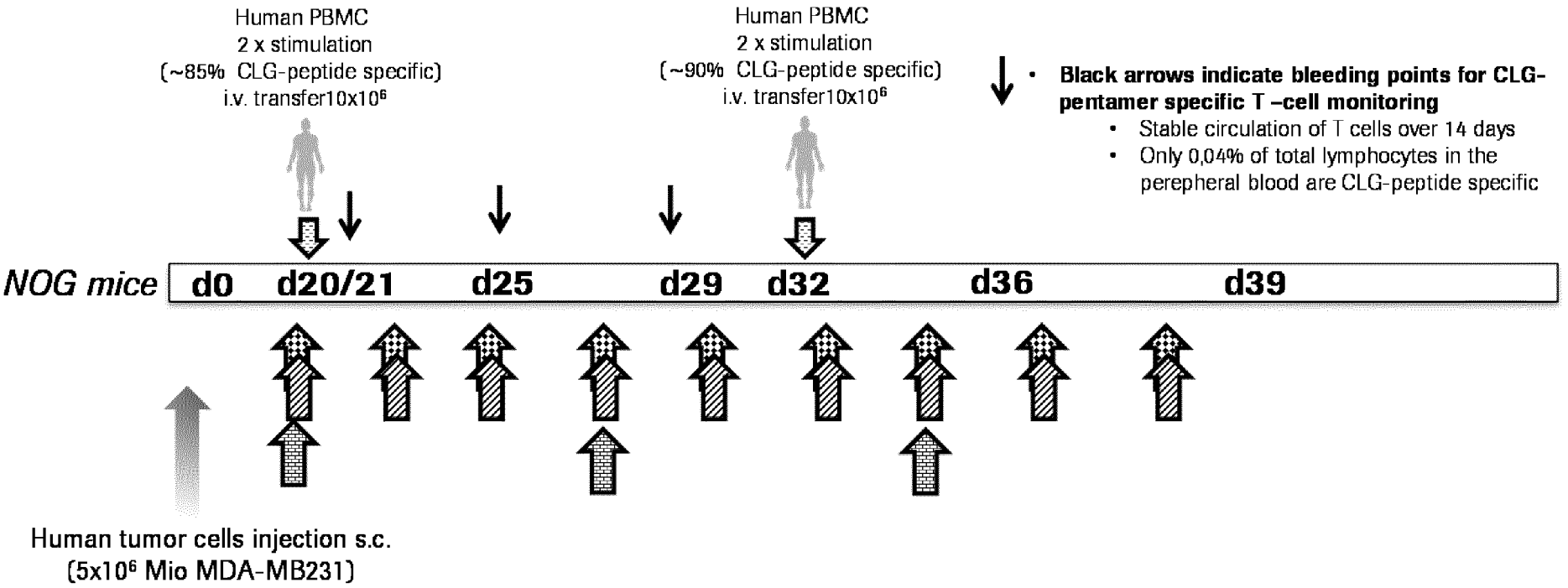

Vehicle
EBV-specific CD8 T cells
EBV-specific CD8 T cells + <CDCP1>-IgG4 EBV Peptide conjugate
EBV-specific CD8 T cells + Control AB: <CDCP1>-IgG4
TGI: 40%
TGI: 70%
tumor volume (mm³)
300
200
100
0
0
10
20
30
40
days

Fig. 8B



Treatment groups:

ATTP: <CDCP1>-IgG1_PG LALA-SS-CLG Peptide

Control AB: <CDCP1>-IgG1_PG LALA

<PD1>-IgG1_PG LALA

	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Treatment 1 (ATPP/AB, mg/kg)	2,5	2,5	5	5	10	10	15	15	20
Treatment 2 (PD1, mg/kg)		5			5			5	

Fig. 9A

		EBV-specific CD8+ T cells				
Tumor growth inhibition	CD8+ T cells only	**ATTP conjugate** i.p.	**ATTP conjugate + <PD1>** i.p.	Control antibody i.p.	Control antibody + <PD1> i.p.	**ATTP conjugate** p.t.
TGI in % day 29	3	41	**60**	2	4	59
TGI in % day 32	9	47	**63**	18	8	52
TGI in % day 36	-6	22	**58**	4	4	38
TGI in % day 39	-5	16	**56**	4	0	41

Fig. 9B

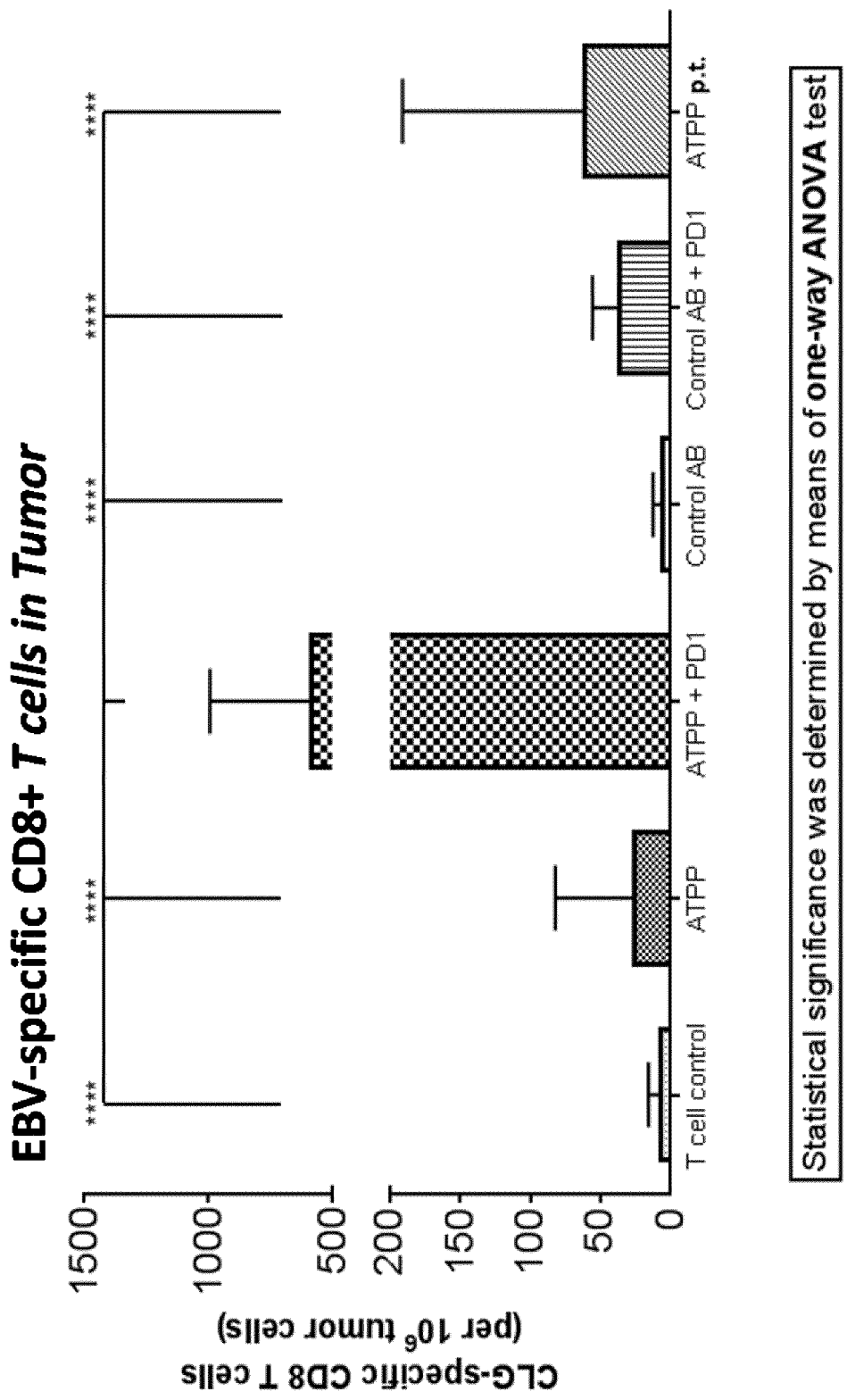

EBV-specific CD8+ T cells in Tumor
CLG-specific CD8 T cells
(per 10^6 tumor cells)
1500
1000
500
200
150
100
50
0
****
****
****
****
****
T cell control
ATPP
ATPP + PD1
Control AB
Control AB + PD1
ATPP p.t.
Statistical significance was determined by means of one-way ANOVA test

Fig. 9C