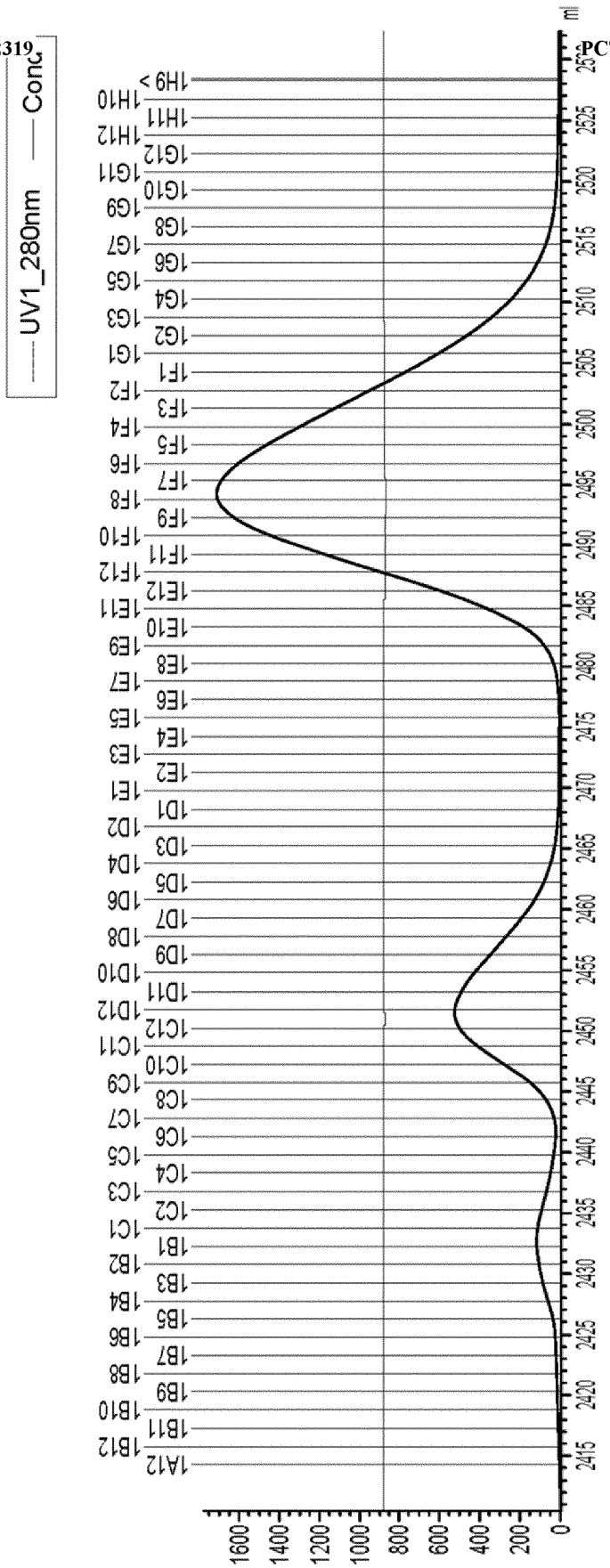
Fig. 1


UV1_280nm
Conc
ml
1600
1400
1200
1000
800
600
400
200
0
2415
2420
2425
2430
2435
2440
2445
2450
2455
2460
2465
2470
2475
2480
2485
2490
2495
2500
2505
2510
2515
2520
2525
1A12
1B12
1B11
1B10
1B9
1B8
1B7
1B6
1B5
1B4
1B3
1B2
1B1
1C1
1C2
1C3
1C4
1C5
1C6
1C7
1C8
1C9
1C10
1C11
1C12
1D12
1D11
1D10
1D9
1D8
1D7
1D6
1D5
1D4
1D3
1D2
1D1
1E1
1E2
1E3
1E4
1E5
1E6
1E7
1E8
1E9
1E10
1E11
1E12
1F12
1F11
1F10
1F9
1F8
1F7
1F6
1F5
1F4
1F3
1F2
1F1
1G1
1G2
1G3
1G4
1G5
1G6
1G7
1G8
1G9
1G10
1G11
1G12
1H12
1H11
1H10
1H9

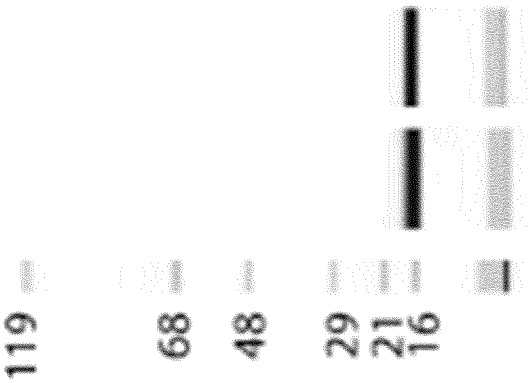


Fig. 2

Fig. 3A

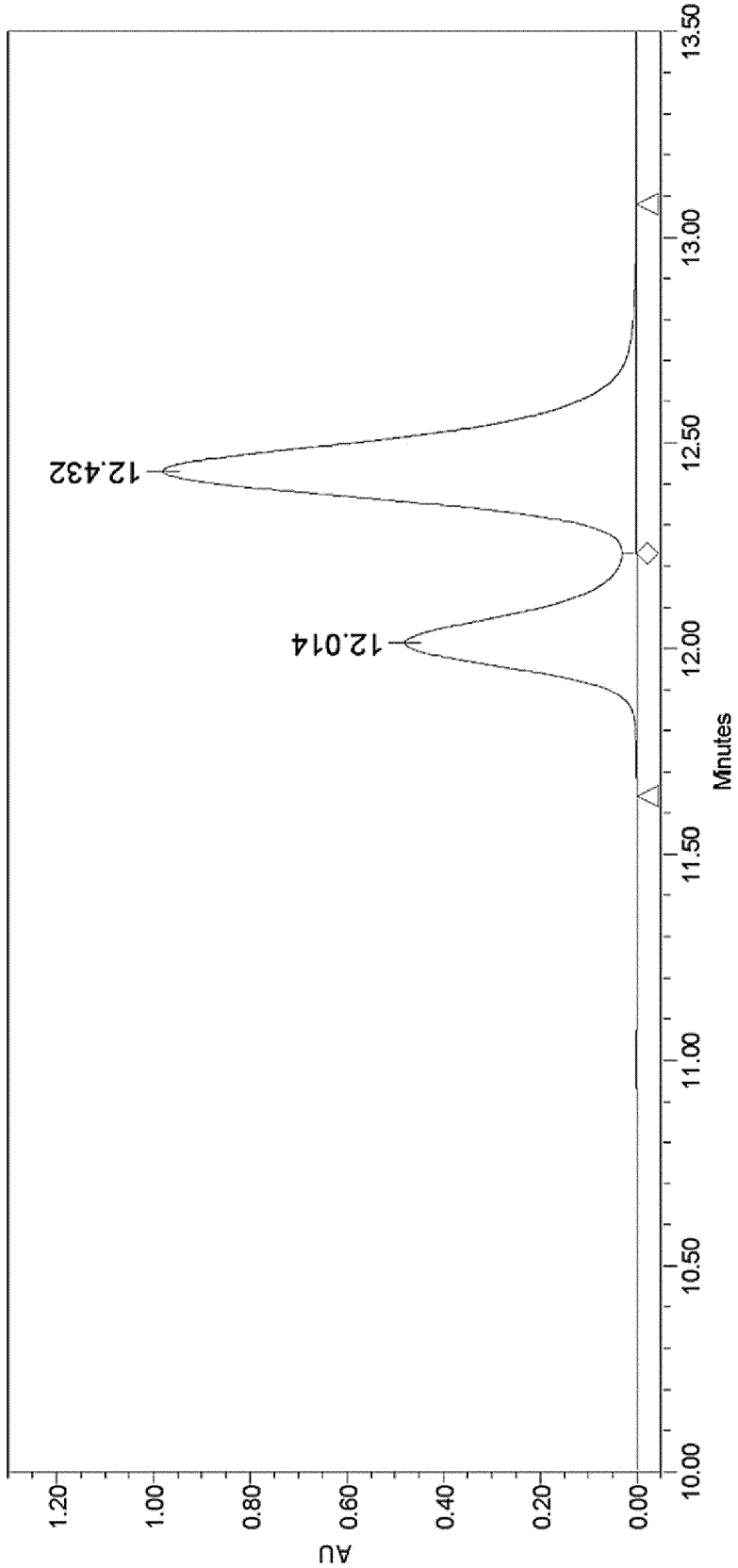

12.432
12.014
AU
Minutes
1.20
1.00
0.80
0.60
0.40
0.20
0.00
10.00
10.50
11.00
11.50
12.00
12.50
13.00
13.50

Fig. 3B

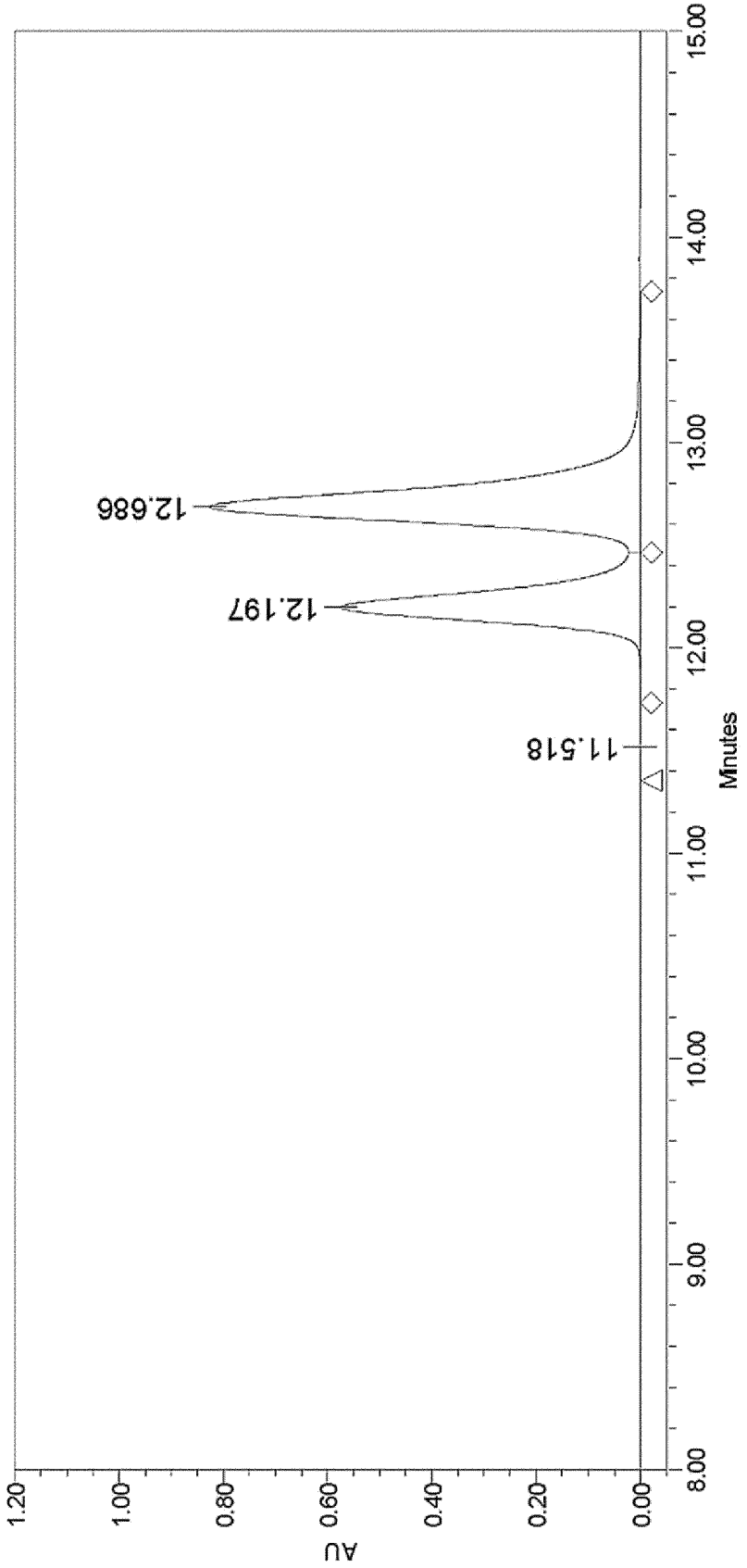

12.686
12.197
11.518
AU
Mnutes
1.20
1.00
0.80
0.60
0.40
0.20
0.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00

Fig. 4A

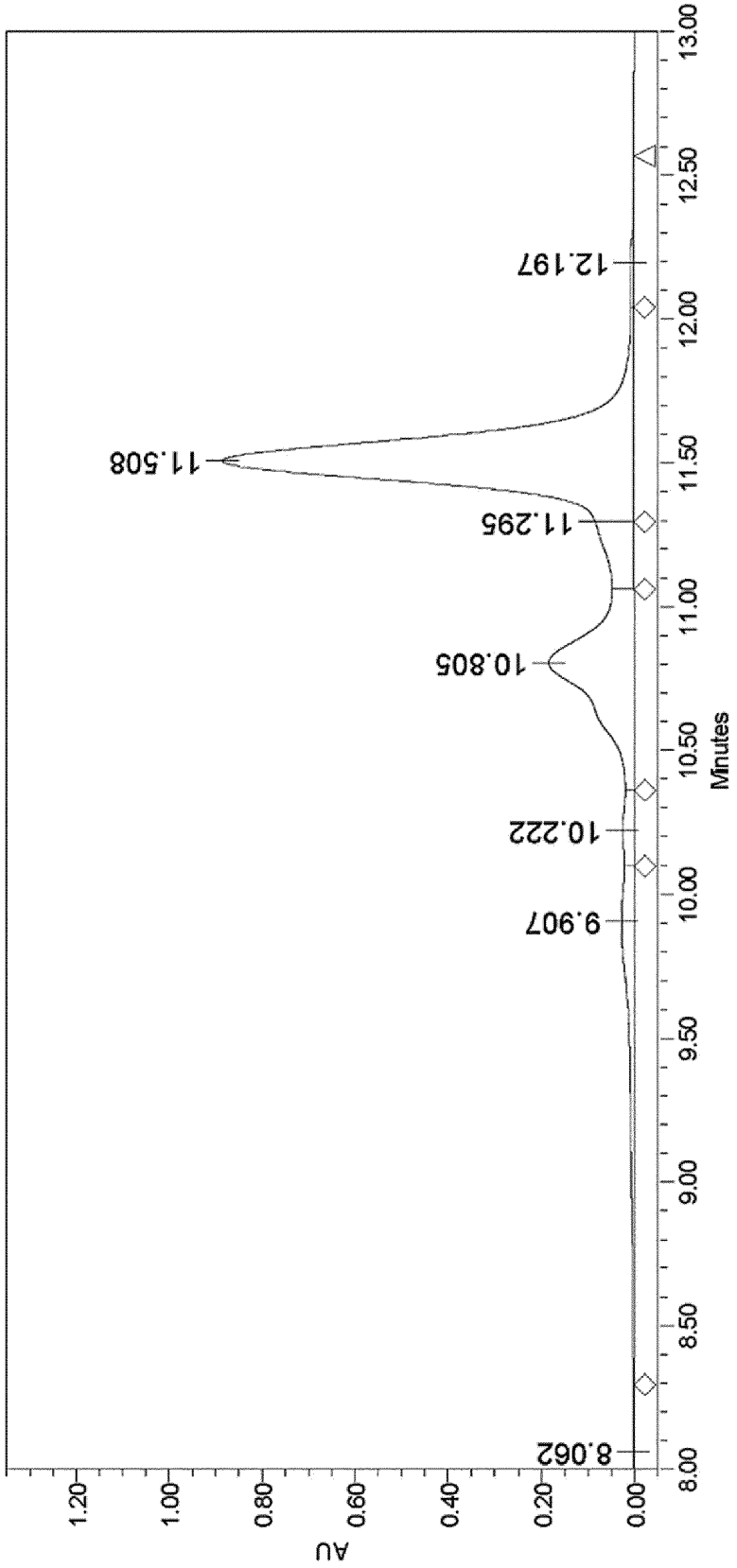
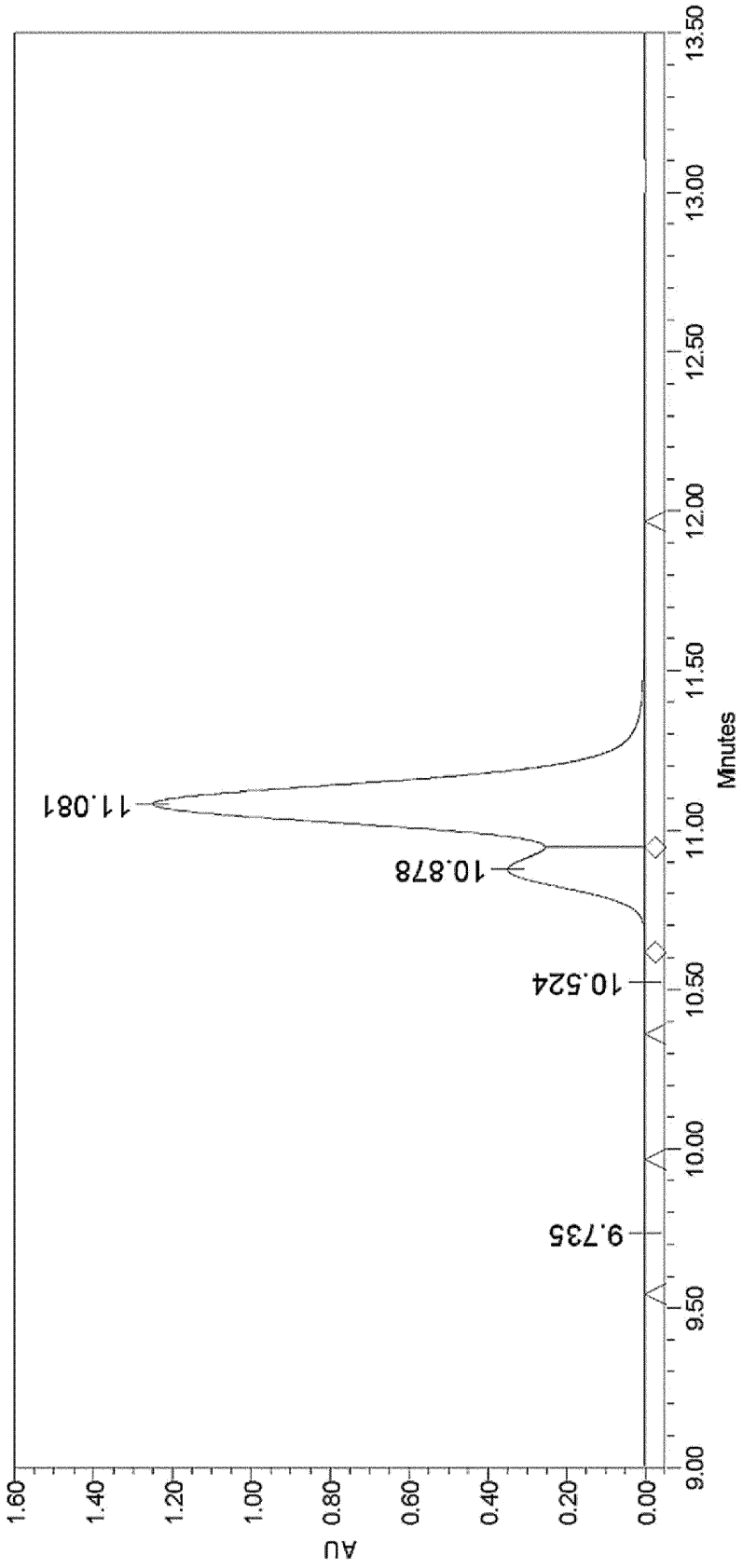

AU
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Minutes
8.00
8.50
9.00
9.50
10.00
10.50
11.00
11.50
12.00
12.50
13.00
8.062
9.907
10.222
10.805
11.295
11.508
12.197

Fig. 4B


AU
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
9.00
9.50
10.00
10.50
11.00
11.50
12.00
12.50
13.00
13.50
Minutes
9.735
10.524
10.878
11.081

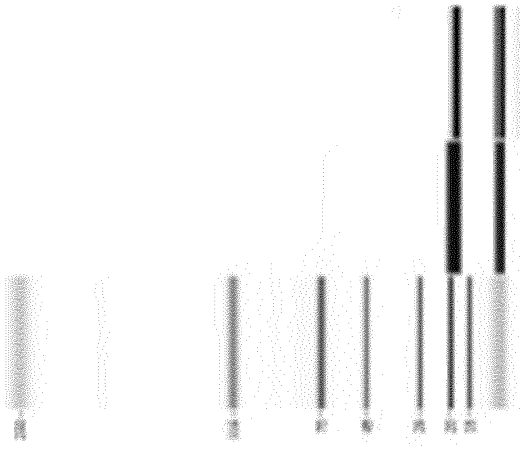


Fig. 5

Fig. 6A

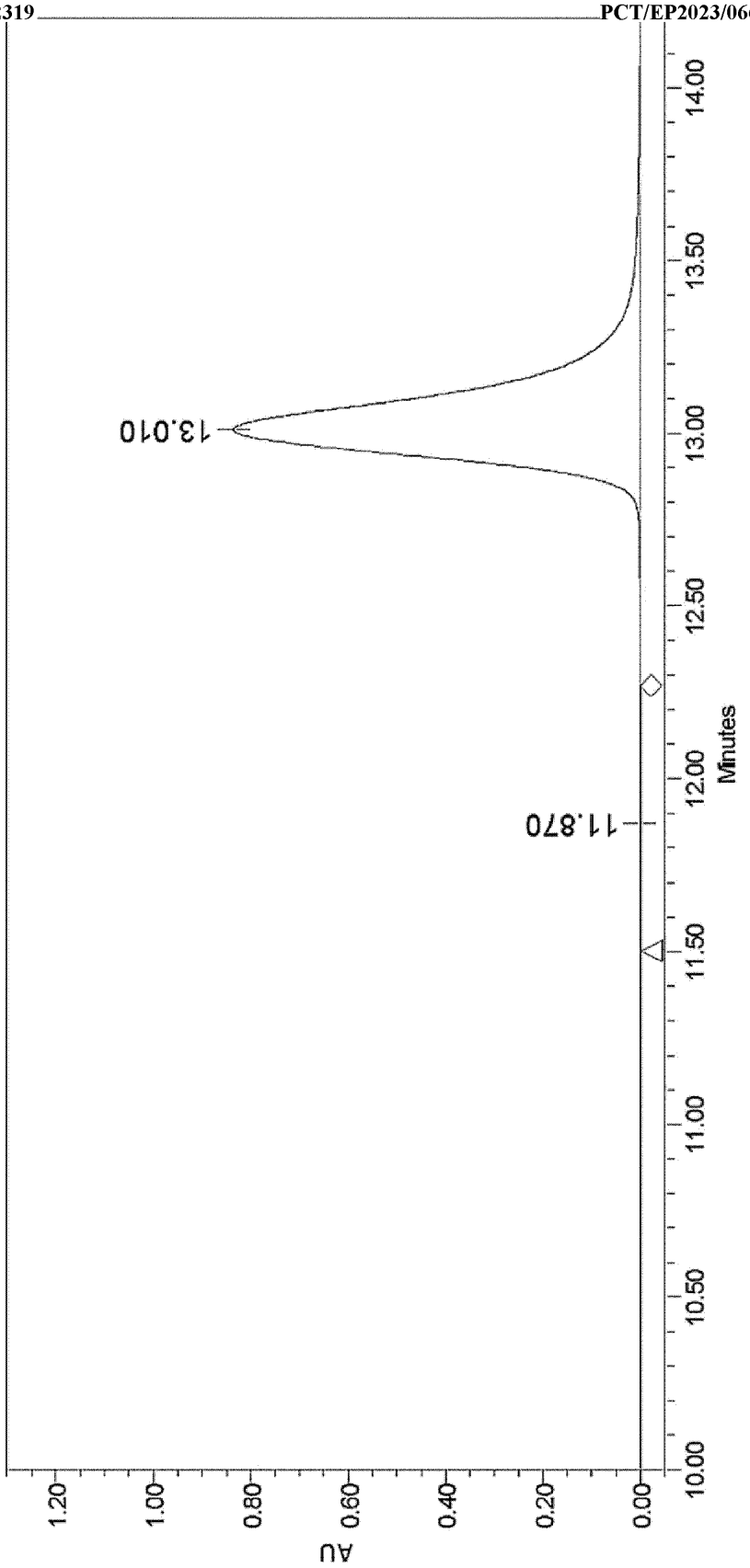

13.010
11.870
AU
Minutes
0.00
0.20
0.40
0.60
0.80
1.00
1.20
10.00
10.50
11.00
11.50
12.00
12.50
13.00
13.50
14.00

Fig. 6B

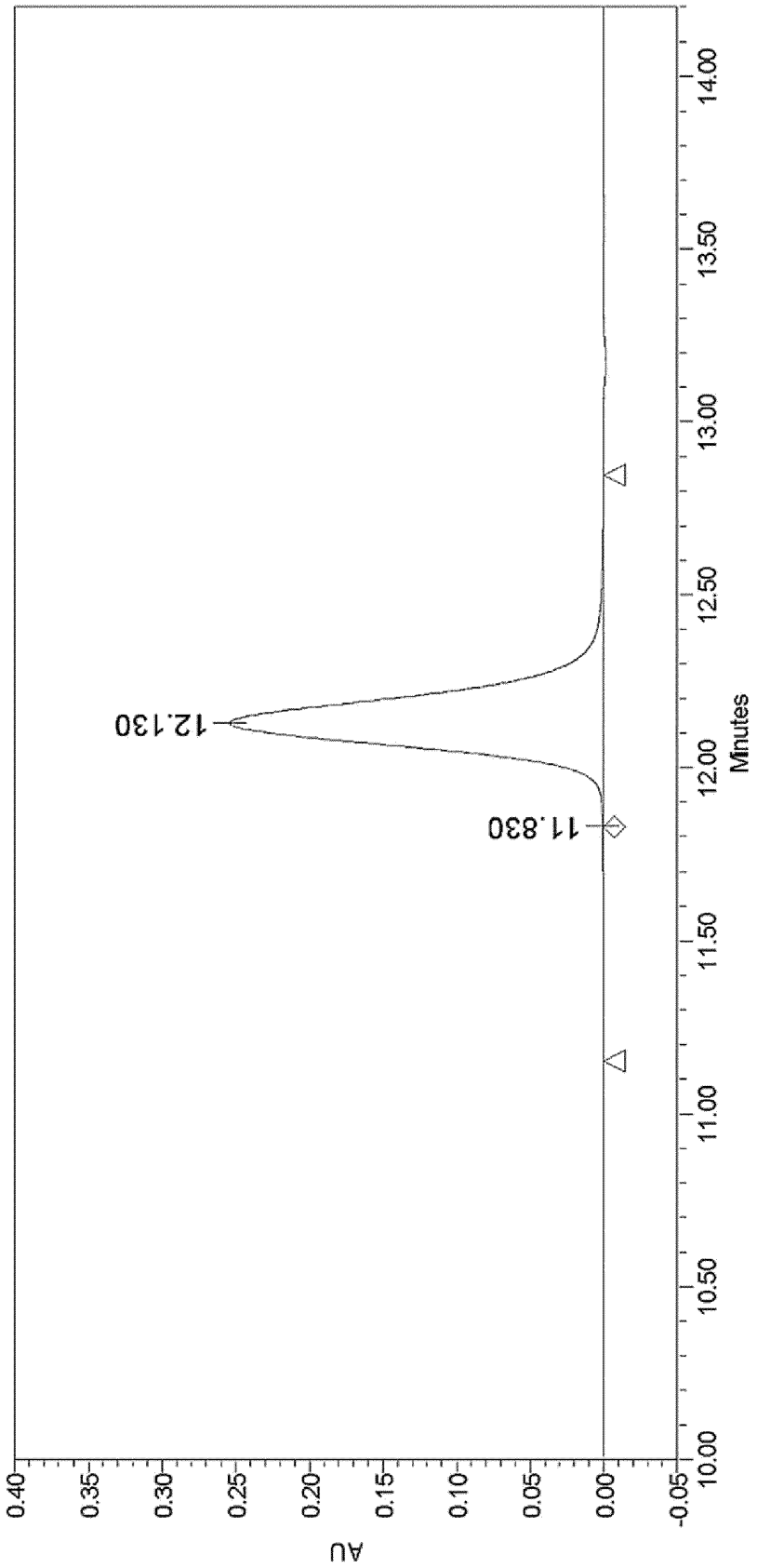

AU
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
10.00
10.50
11.00
11.50
12.00
12.50
13.00
13.50
14.00
Mnutes
11.830
12.130

Fig. 7A

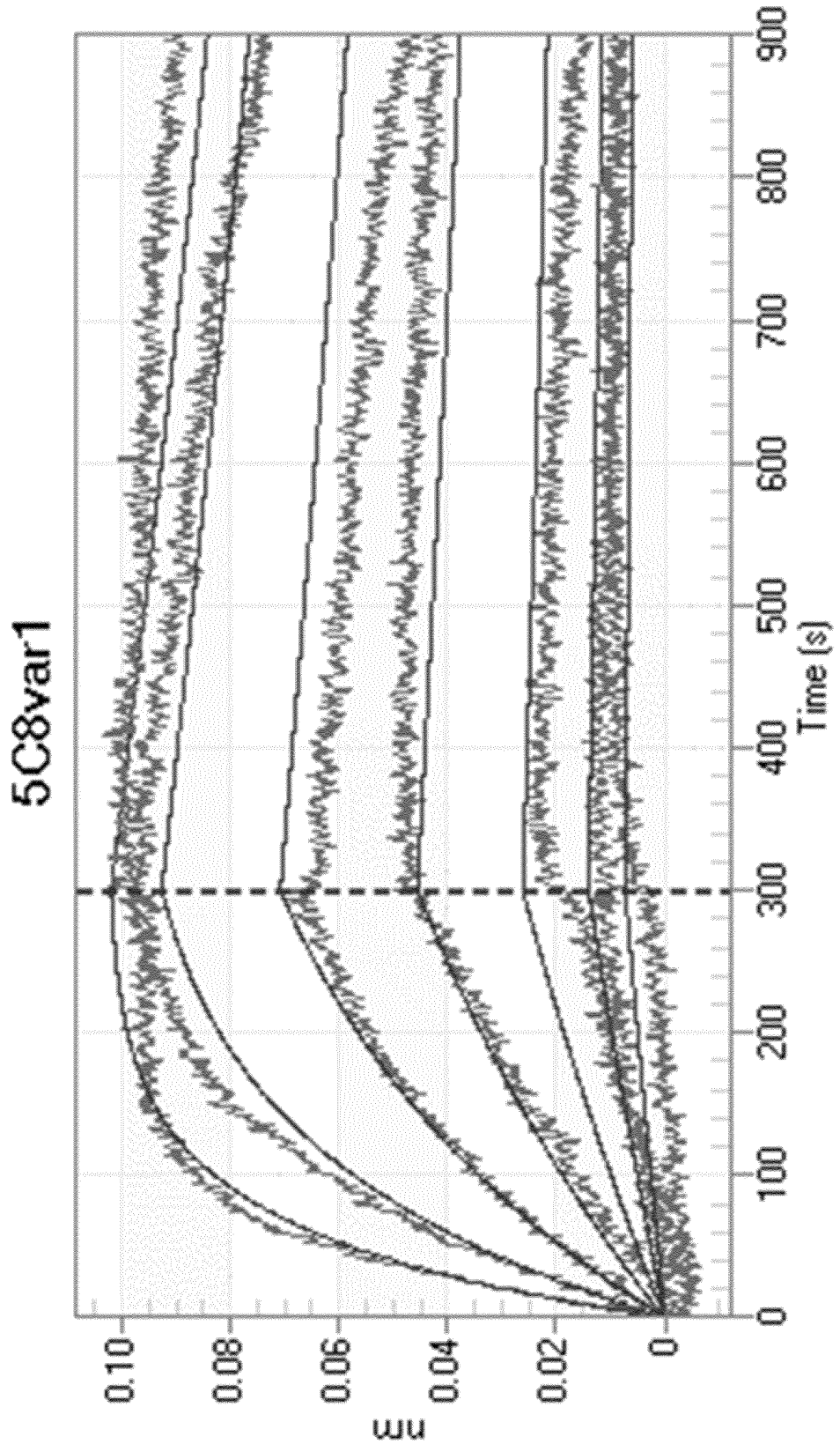

5C8var1
nm
0.10
0.08
0.06
0.04
0.02
0
0
100
200
300
400
500
600
700
800
900
Time (s)

Fig. 7B

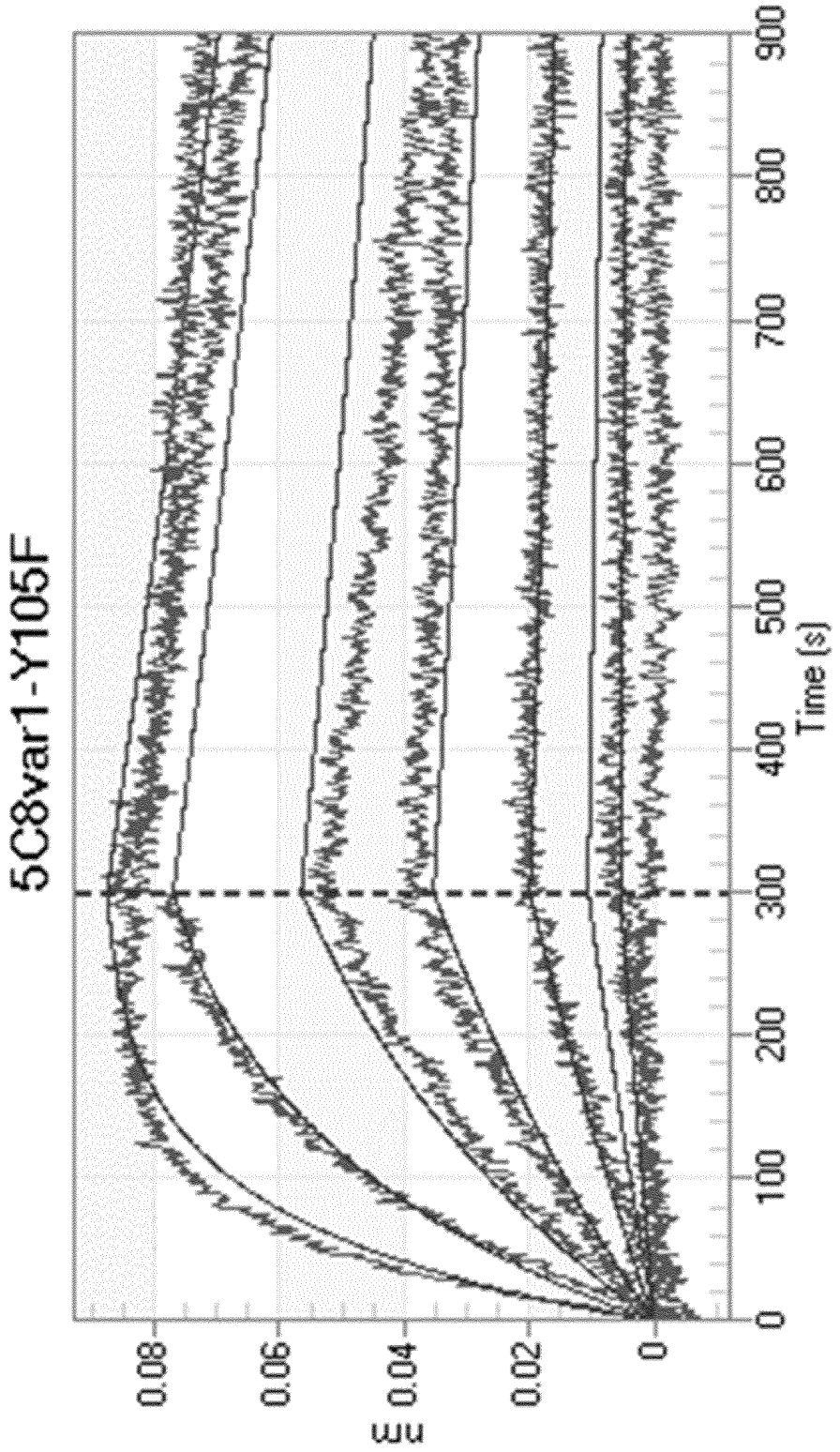

5C8var1-Y105F
nm
0.08
0.06
0.04
0.02
0
0
100
200
300
400
500
600
700
800
900
Time (s)

Fig. 7C

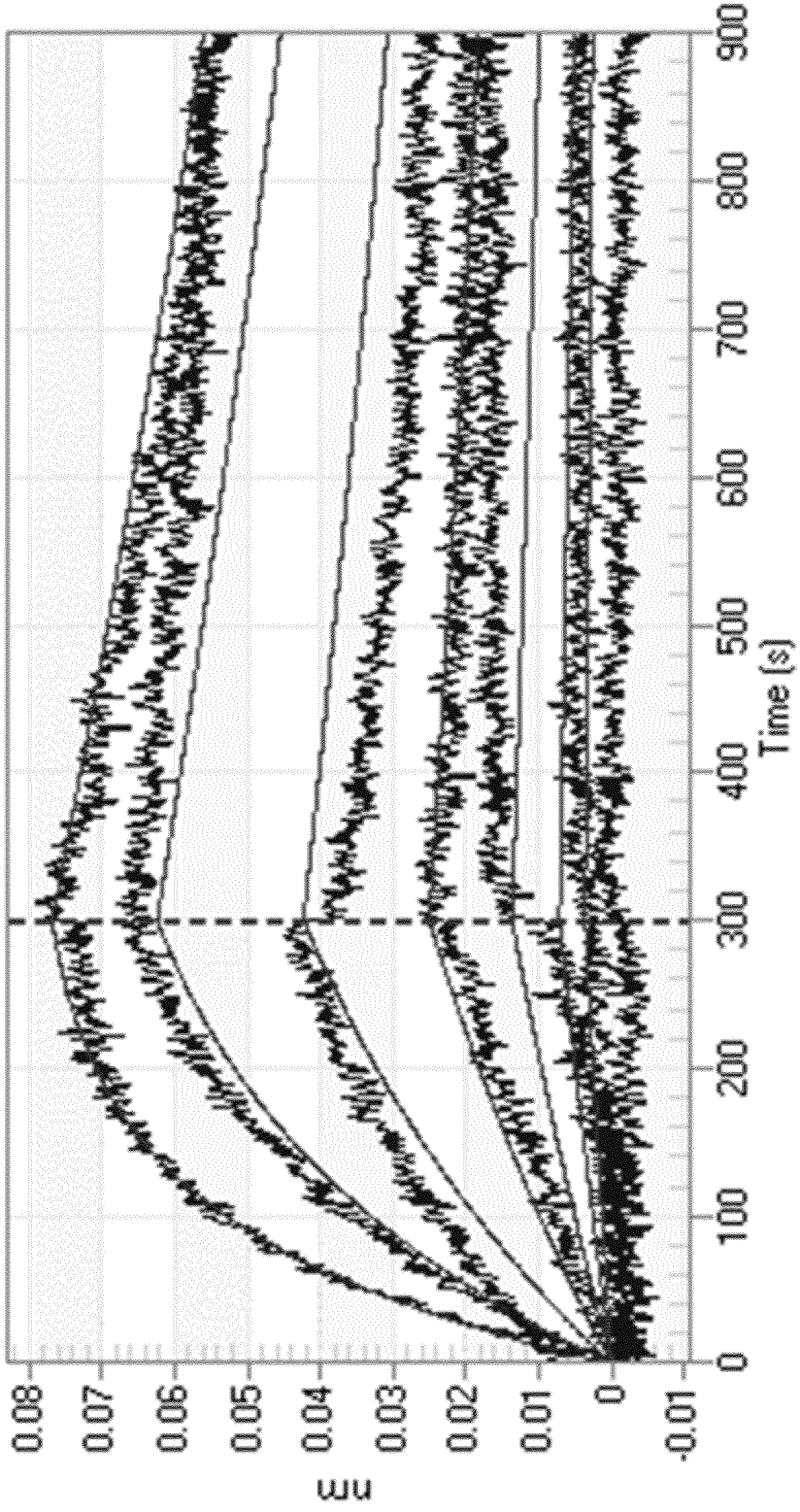
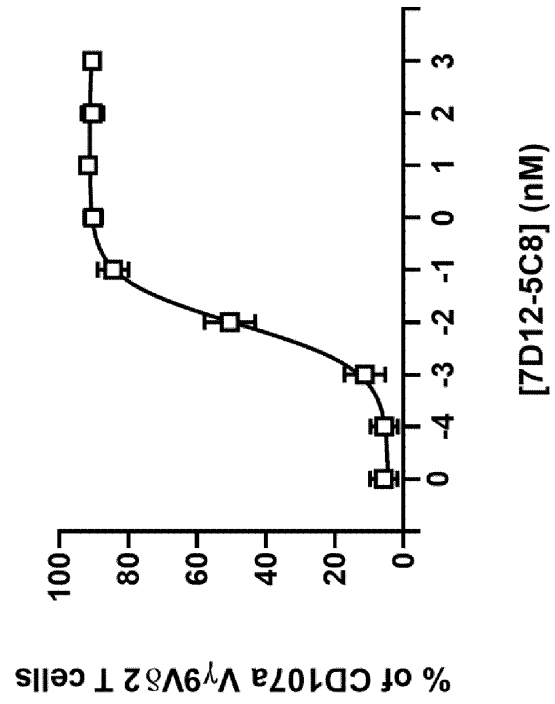

5C8var1-Y105S
nm
Time (s)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
0
100
200
300
400
500
600
700
800
900

Fig. 8A


% of CD107a Vγ9Vδ2 T cells
100
80
60
40
20
0
0 -4 -3 -2 -1 0 1 2 3
[7D12-5C8] (nM)

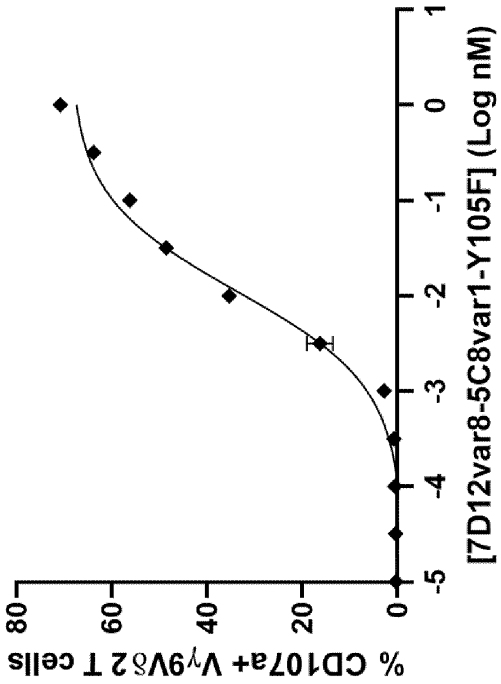
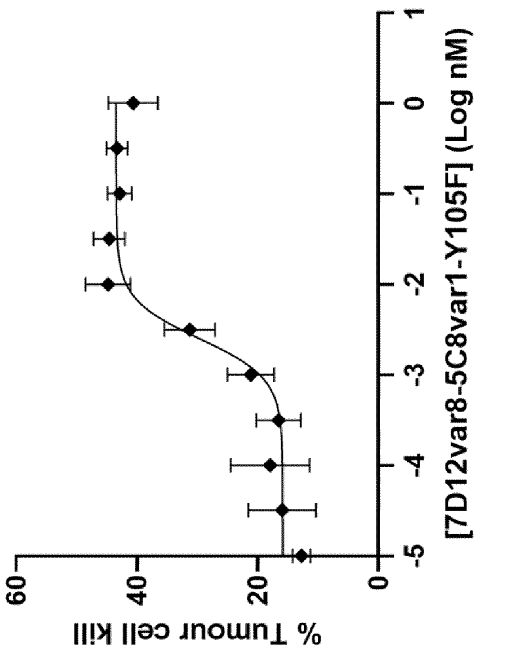

% CD107a+ Vγ9Vδ2 T cells
80
60
40
20
0
-5 -4 -3 -2 -1 0 1
[7D12var8-5C8var1-Y105F] (Log nM)

Fig. 8B


% Tumour cell kill
60
40
20
0
-5
-4
-3
-2
-1
0
1
[7D12var8-5C8var1-Y105F] (Log nM)

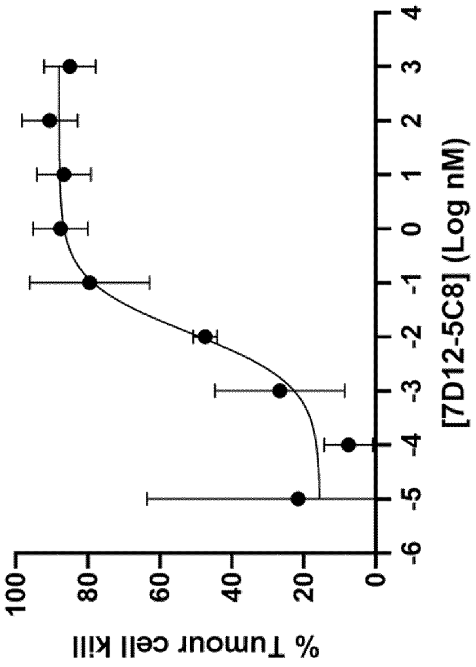

% Tumour cell kill
100
80
60
40
20
0
-6
-5
-4
-3
-2
-1
0
1
2
3
4
[7D12-5C8] (Log nM)

Fig. 9

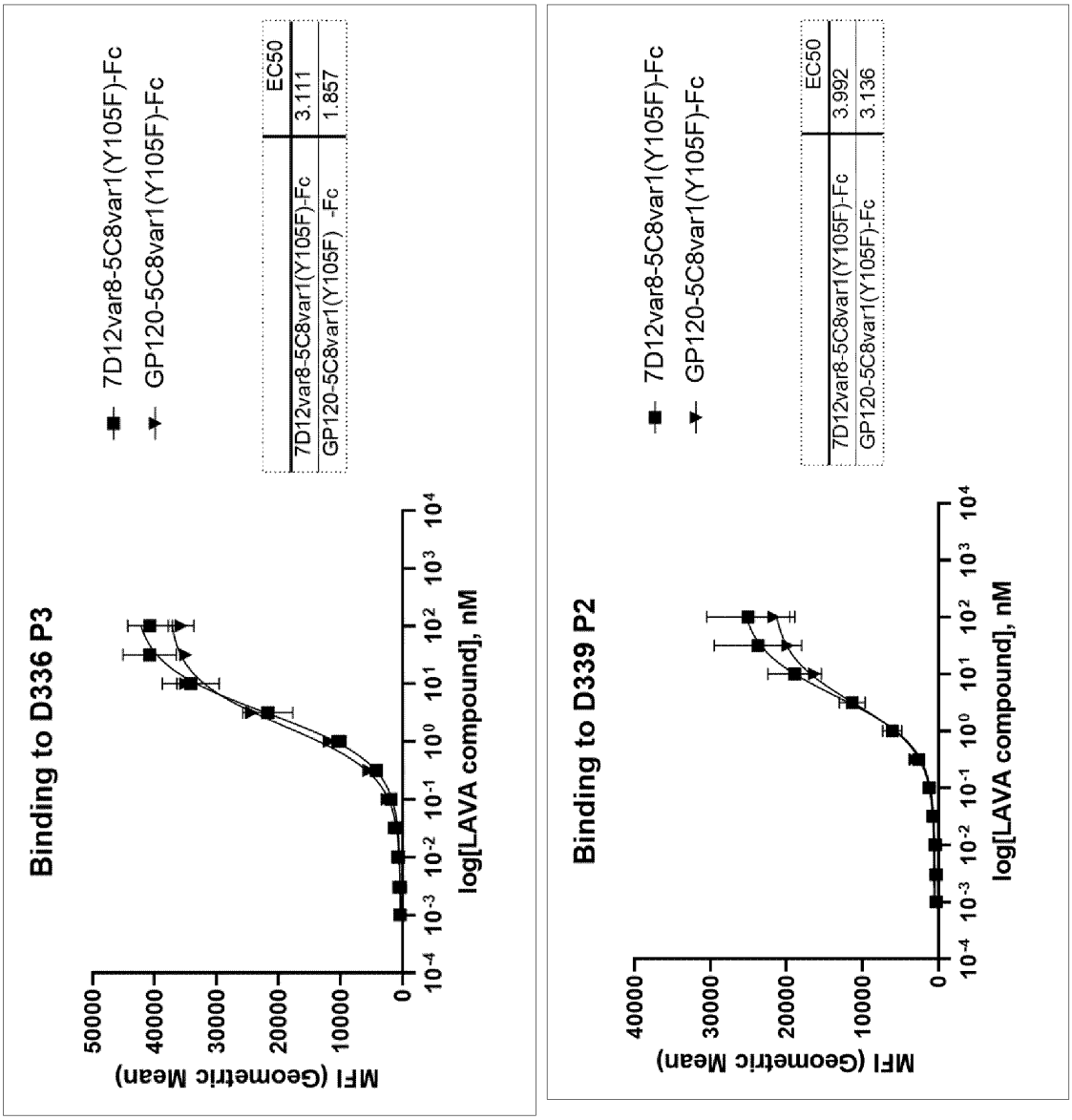

Binding to D336 P3
7D12var8-5C8var1(Y105F)-Fc
GP120-5C8var1(Y105F)-Fc
EC50
7D12var8-5C8var1(Y105F)-Fc 3.111
GP120-5C8var1(Y105F) -Fc 1.857
MFI (Geometric Mean)
log[LAVA compound], nM
Binding to D339 P2
7D12var8-5C8var1(Y105F)-Fc
GP120-5C8var1(Y105F)-Fc
EC50
7D12var8-5C8var1(Y105F)-Fc 3.992
GP120-5C8var1(Y105F)-Fc 3.136
MFI (Geometric Mean)
log[LAVA compound], nM

Fig. 10A

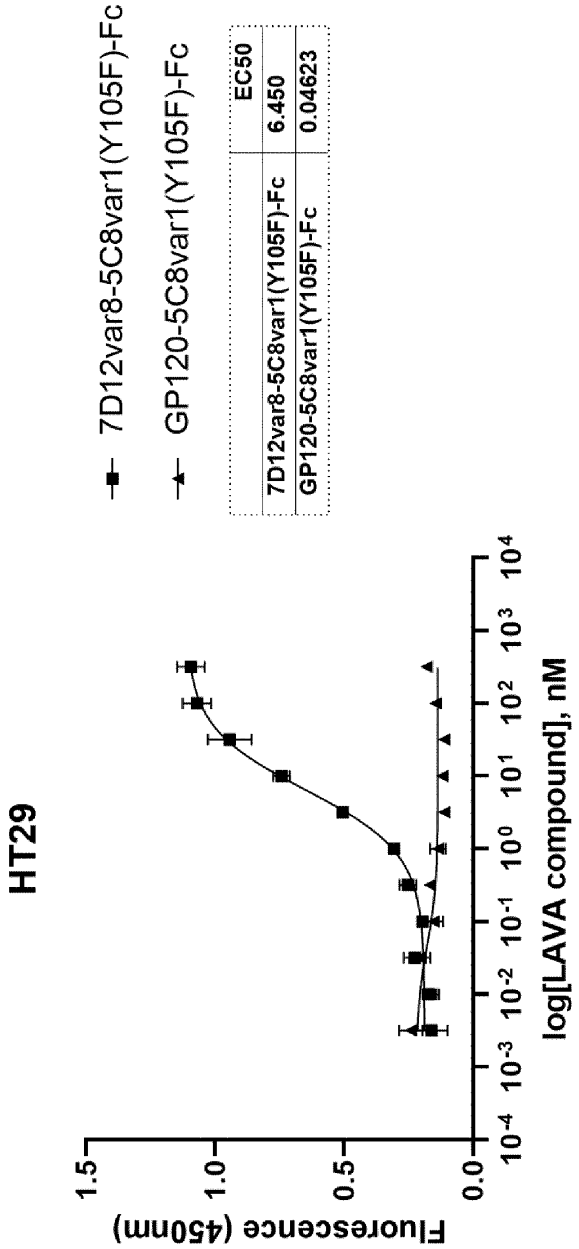

HT29
7D12var8-5C8var1(Y105F)-Fc
GP120-5C8var1(Y105F)-Fc
EC50
7D12var8-5C8var1(Y105F)-Fc 6.450
GP120-5C8var1(Y105F)-Fc 0.04623
Fluorescence (450nm)
1.5
1.0
0.5
0.0
10-4 10-3 10-2 10-1 100 101 102 103 104
log[LAVA compound], nM

Fig. 10B

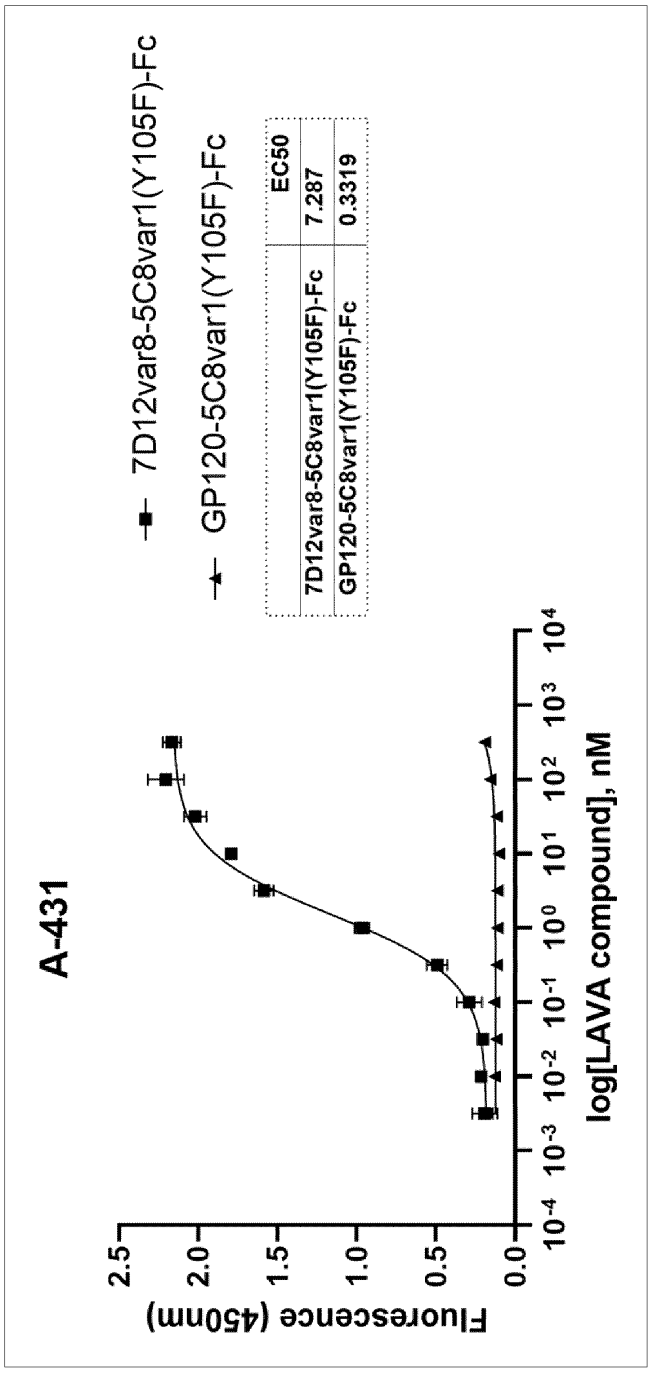

A-431
7D12var8-5C8var1(Y105F)-Fc
GP120-5C8var1(Y105F)-Fc
EC50
7D12var8-5C8var1(Y105F)-Fc 7.287
GP120-5C8var1(Y105F)-Fc 0.3319
Fluorescence (450nm)
log[LAVA compound], nM

Fig. 10C

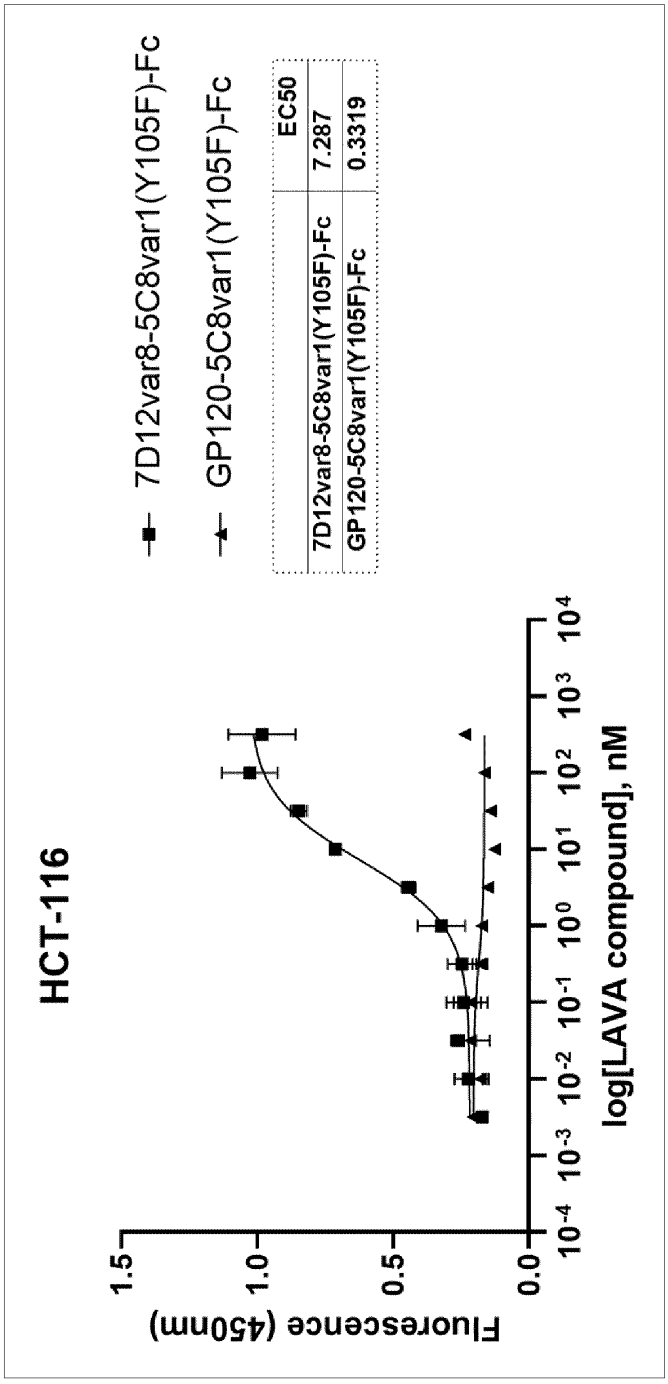
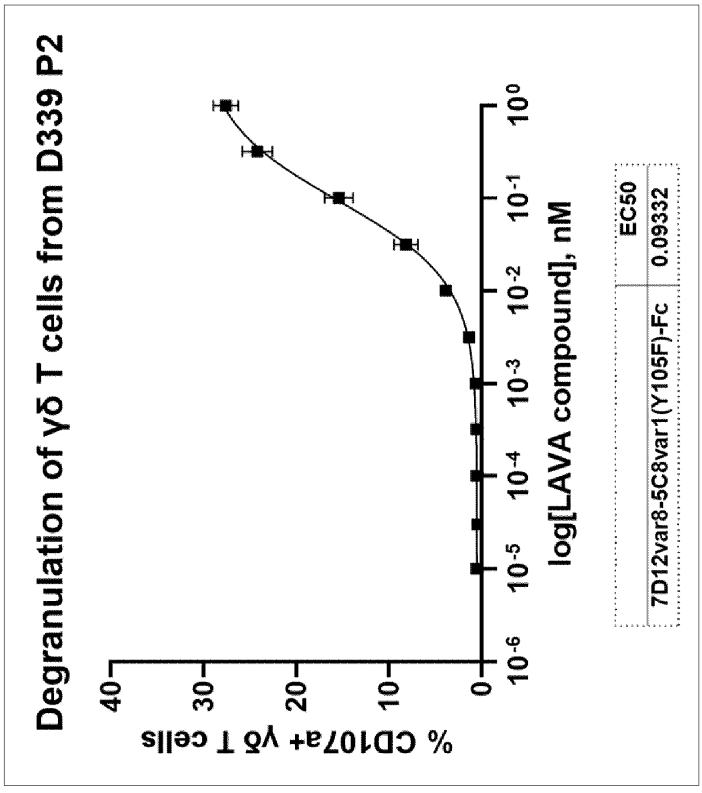

HCT-116
7D12var8-5C8var1(Y105F)-Fc
GP120-5C8var1(Y105F)-Fc
EC50
7D12var8-5C8var1(Y105F)-Fc 7.287
GP120-5C8var1(Y105F)-Fc 0.3319
Fluorescence (450nm)
1.5
1.0
0.5
0.0
10-4 10-3 10-2 10-1 100 101 102 103 104
log[LAVA compound], nM

Fig. 11


Degranulation of γδ T cells from D339 P2
% CD107a+ γδ T cells
log[LAVA compound], nM
EC50
7D12var8-5C8var1(Y105F)-Fc 0.09332

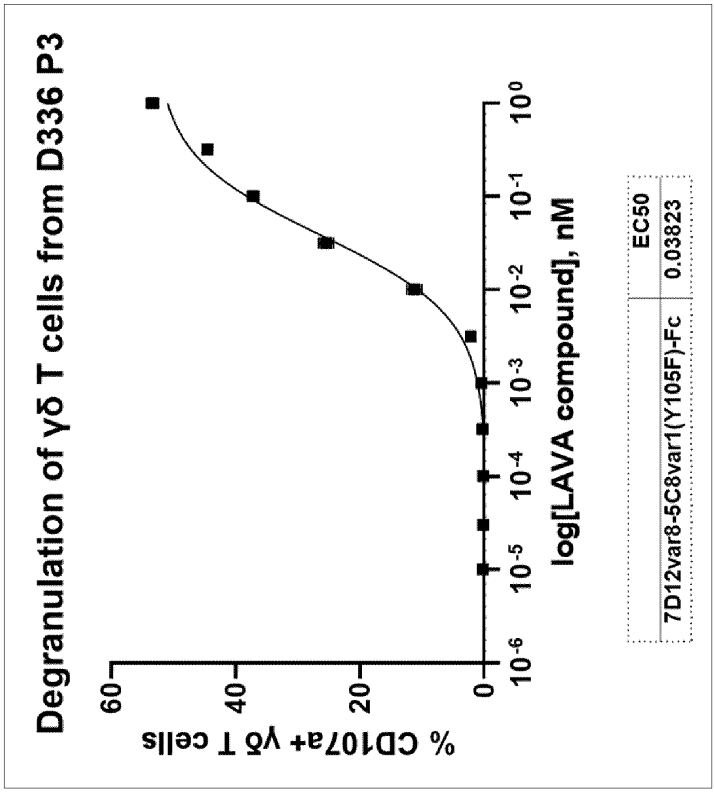

Degranulation of γδ T cells from D336 P3
% CD107a+ γδ T cells
log[LAVA compound], nM
EC50
7D12var8-5C8var1(Y105F)-Fc 0.03823

Fig. 12

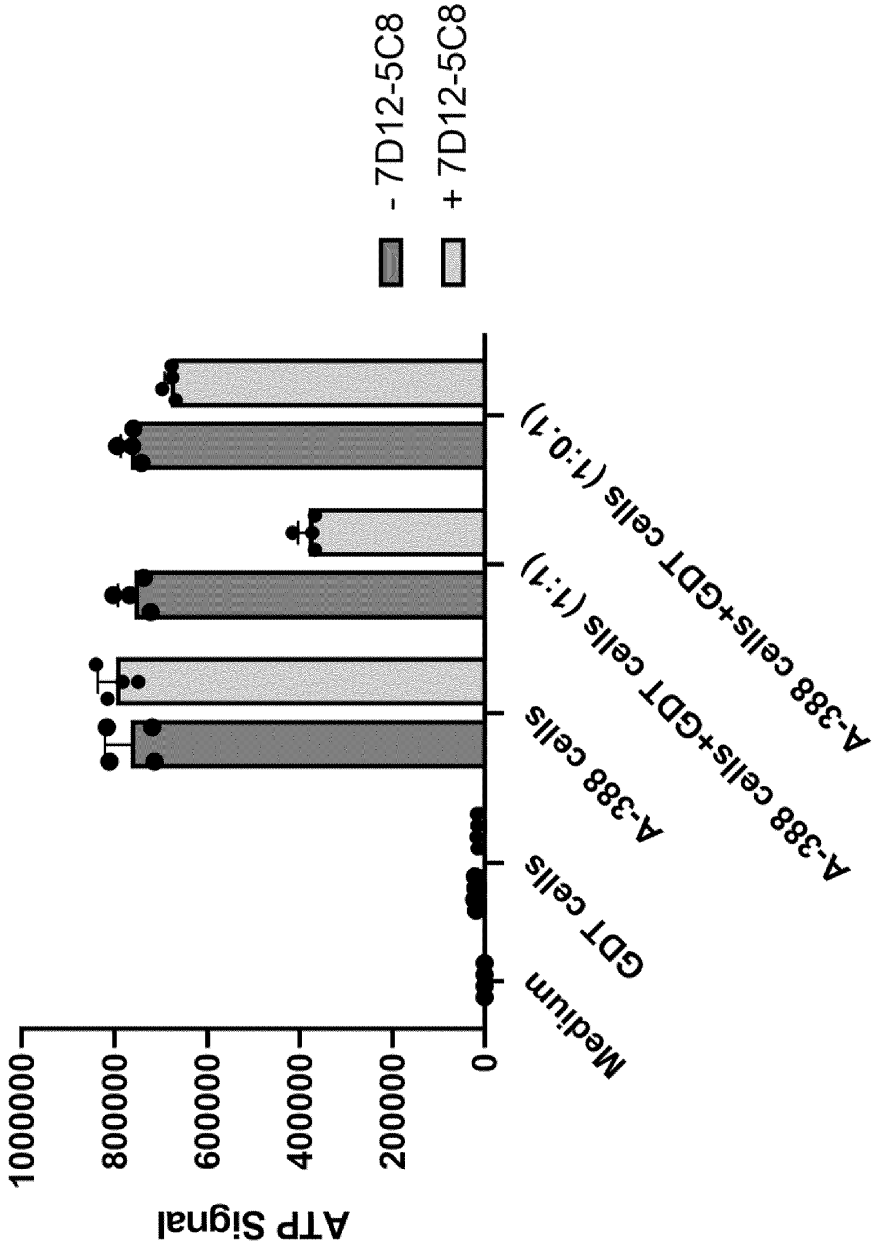

- 7D12-5C8
+ 7D12-5C8
ATP Signal
1000000
800000
600000
400000
200000
0
Medium
GDT cells
A-388 cells
A-388 cells+GDT cells (1:1)
A-388 cells+GDT cells (1:0.1)

Fig. 13

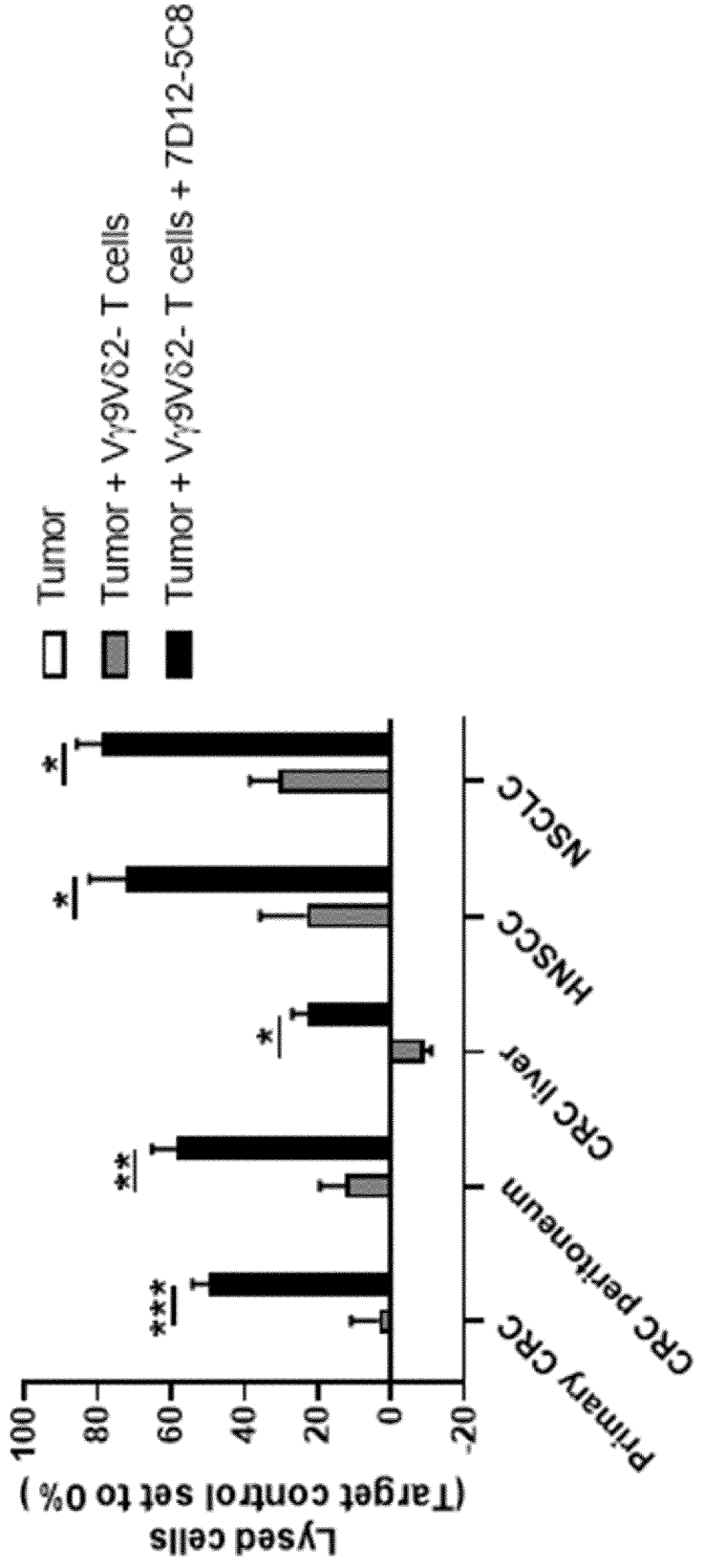
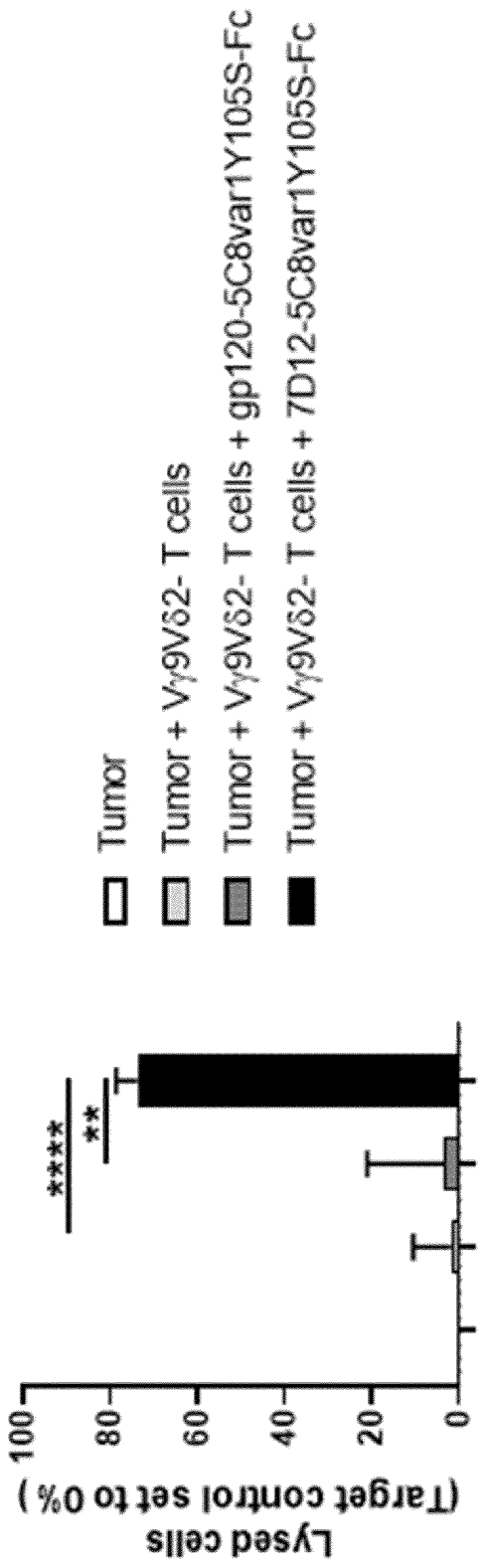

Tumor
Tumor + Vγ9Vδ2- T cells
Tumor + Vγ9Vδ2- T cells + 7D12-5C8
Lysed cells
(Target control set to 0%)
100
80
60
40
20
0
-20
***
**
*
*
*
Primary CRC
CRC peritoneum
CRC liver
HNSCC
NSCLC

Fig. 14


Tumor
Tumor + Vγ9Vδ2- T cells
Tumor + Vγ9Vδ2- T cells + gp120-5C8var1Y105S-Fc
Tumor + Vγ9Vδ2- T cells + 7D12-5C8var1Y105S-Fc
Lysed cells
(Target control set to 0%)
100
80
60
40
20
0
****
**

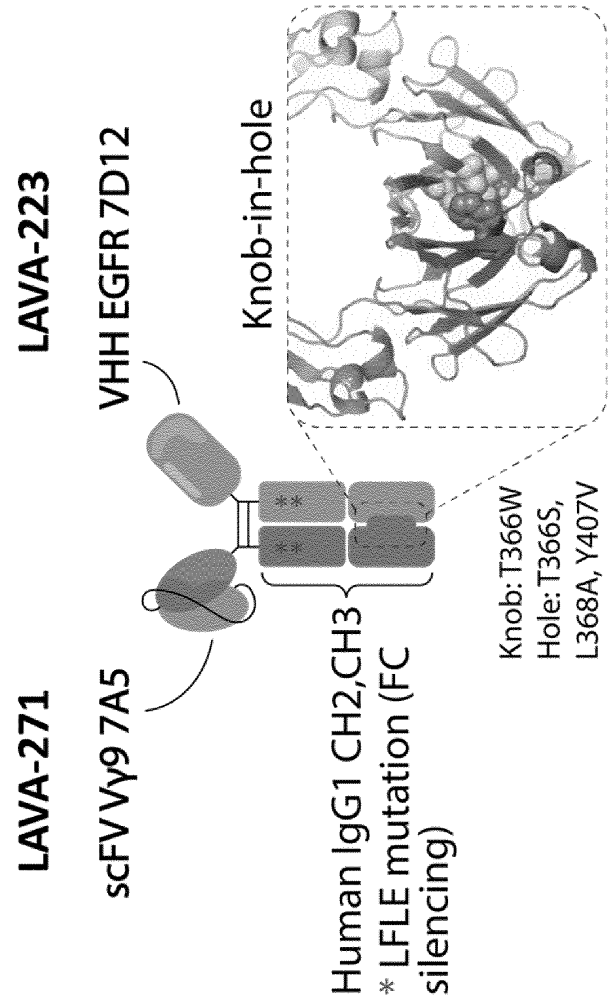
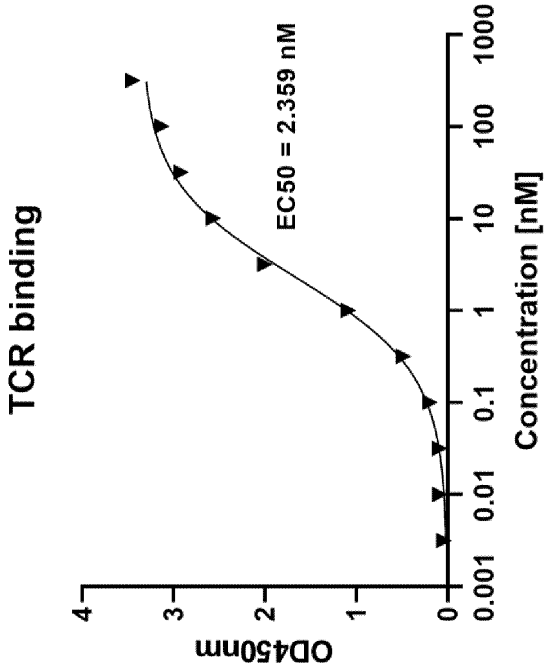

LAVA-271
LAVA-223
scFV Vγ9 7A5
VHH EGFR 7D12
Knob-in-hole
Human IgG1 CH2,CH3
* LFLE mutation (FC silencing)
Knob: T366W
Hole: T366S, L368A, Y407V

Fig. 15

Fig. 16


TCR binding
EC50 = 2.359 nM
OD450nm
0
1
2
3
4
0.001
0.01
0.1
1
10
100
1000
Concentration [nM]

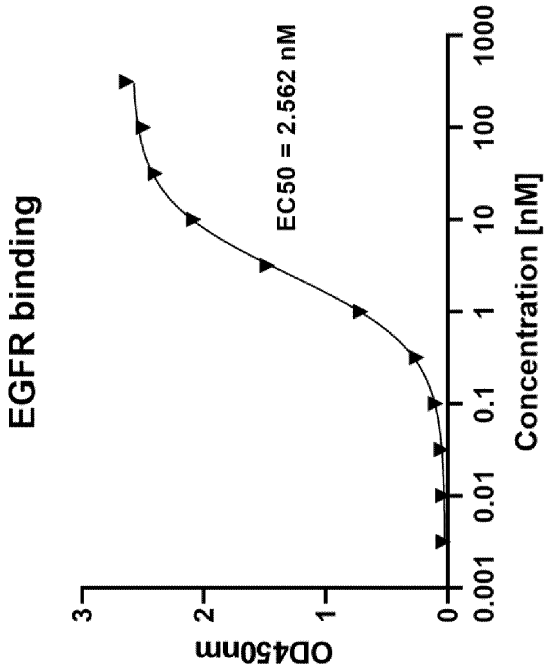
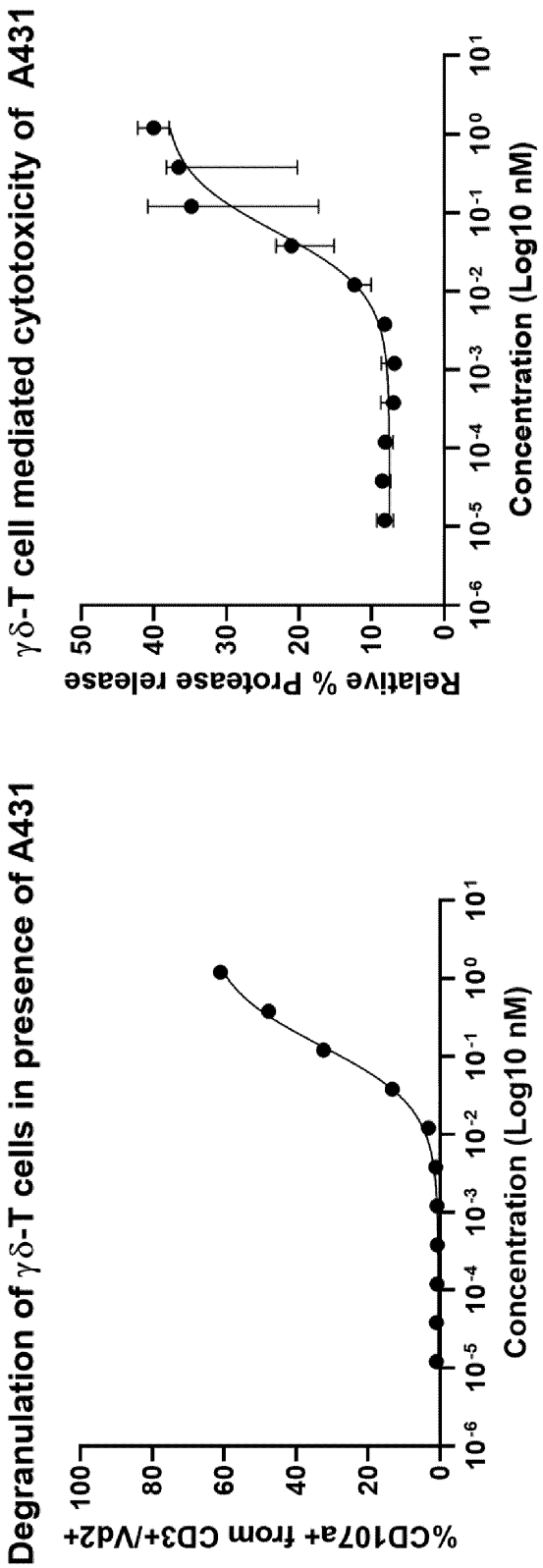

EGFR binding
EC50 = 2.562 nM
OD450nm
0
1
2
3
0.001
0.01
0.1
1
10
100
1000
Concentration [nM]

Fig. 17


Degranulation of γδ-T cells in presence of A431
%CD107a+ from CD3+/Vd2+
100
80
60
40
20
0
10-6 10-5 10-4 10-3 10-2 10-1 100 101
Concentration (Log10 nM)
γδ-T cell mediated cytotoxicity of A431
Relative % Protease release
50
40
30
20
10
0
10-6 10-5 10-4 10-3 10-2 10-1 100 101
Concentration (Log10 nM)

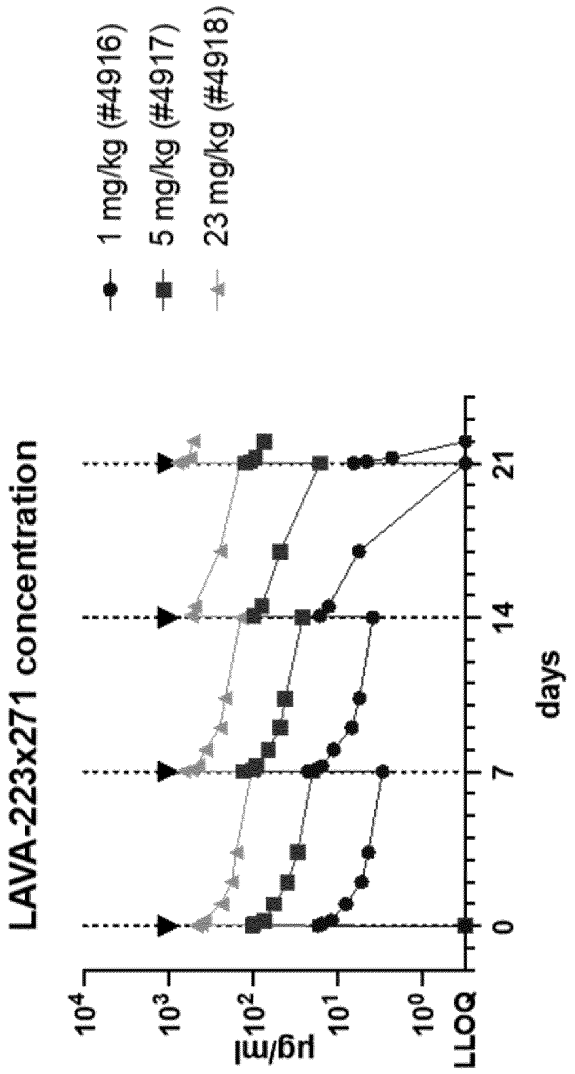

LAVA-223x271 concentration
1 mg/kg (#4916)
5 mg/kg (#4917)
23 mg/kg (#4918)
10^4
10^3
10^2
10^1
10^0
LLOQ
µg/ml
0
7
14
21
days

Fig. 18

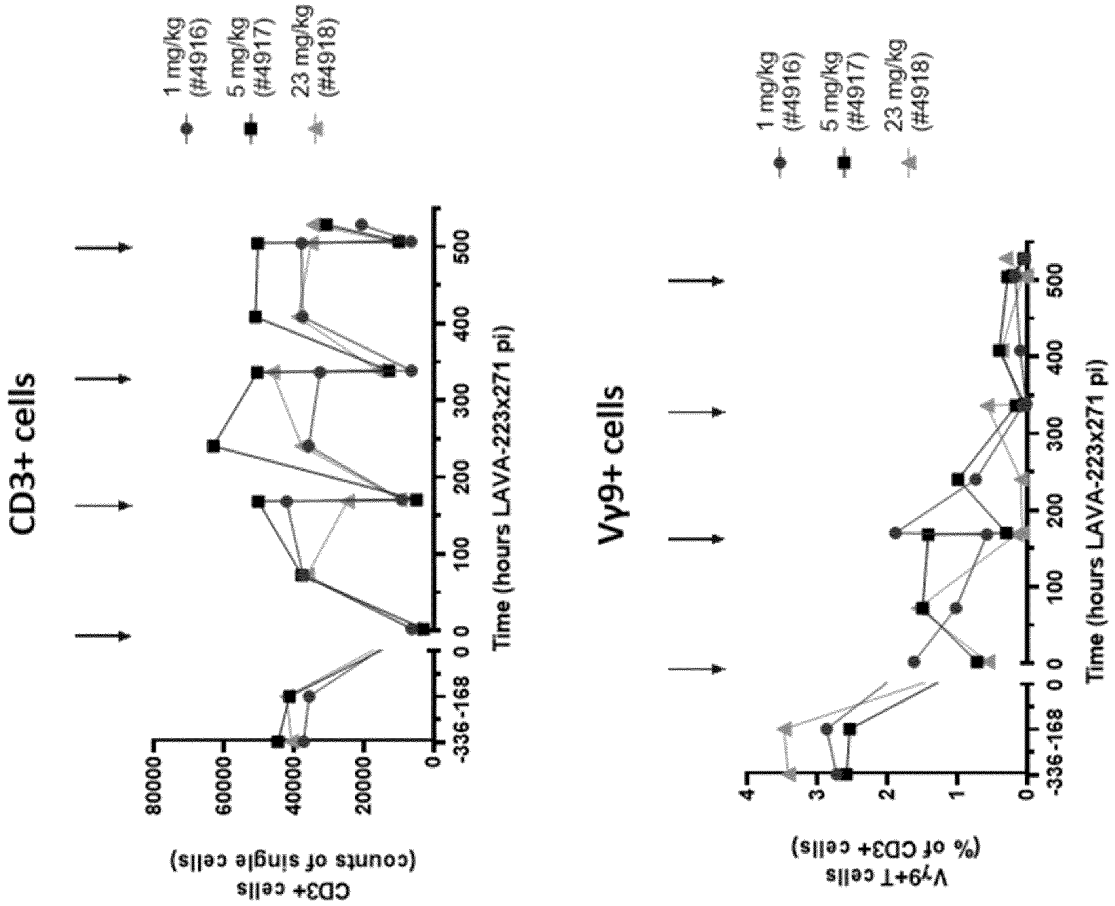

CD3+ cells
CD3+ cells
(counts of single cells)
80000
60000
40000
20000
0
-336 -168 0 0 100 200 300 400 500
Time (hours LAVA-223x271 pi)
1 mg/kg (#4916)
5 mg/kg (#4917)
23 mg/kg (#4918)
Vγ9+ cells
Vγ9+T cells
(% of CD3+ cells)
4
3
2
1
0
-336 -168 0 0 100 200 300 400 500
Time (hours LAVA-223x271 pi)
1 mg/kg (#4916)
5 mg/kg (#4917)
23 mg/kg (#4918)

Fig. 19

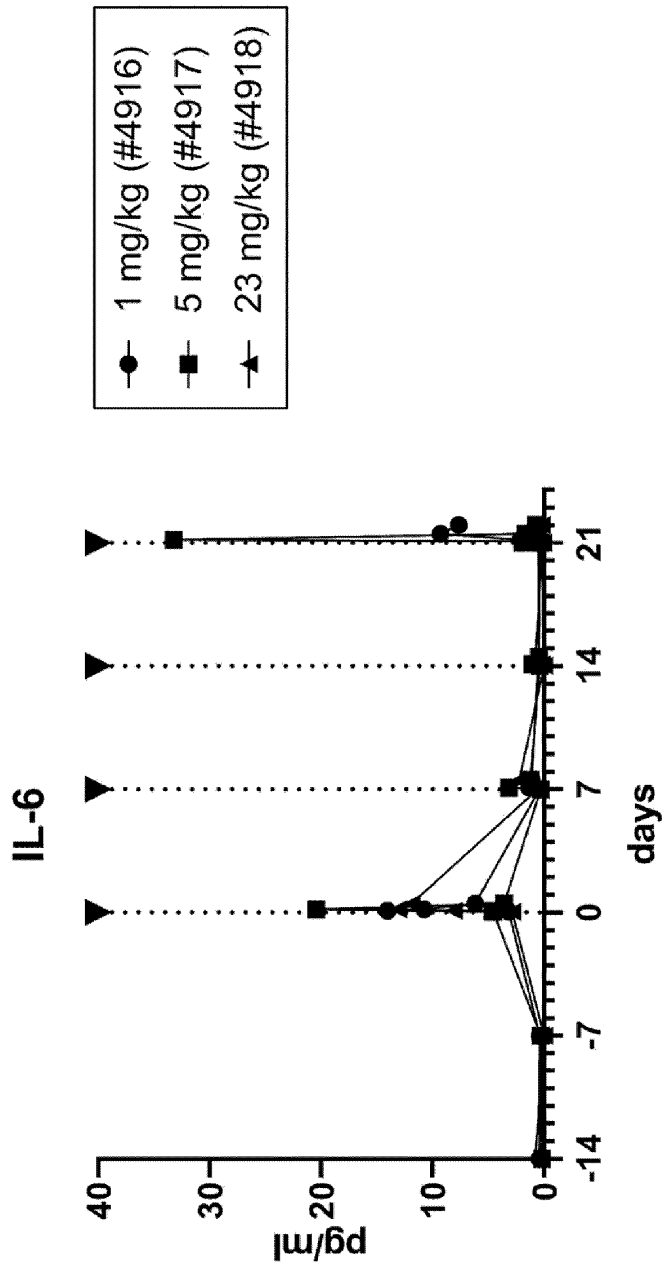

1 mg/kg (#4916)
5 mg/kg (#4917)
23 mg/kg (#4918)
IL-6
pg/ml
0
10
20
30
40
-14
-7
0
7
14
21
days

Fig. 20

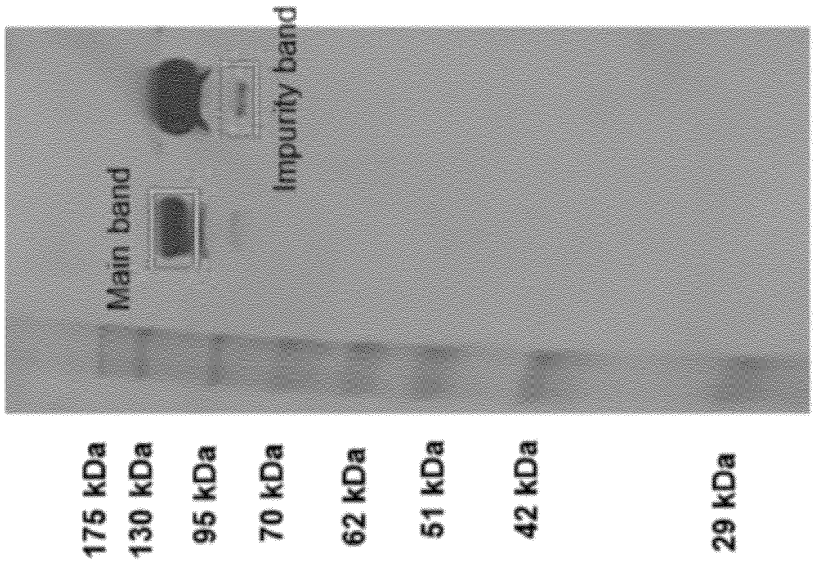

Main band
Impurity band
175 kDa
130 kDa
95 kDa
70 kDa
62 kDa
51 kDa
42 kDa
29 kDa

Fig. 21

Fig. 22

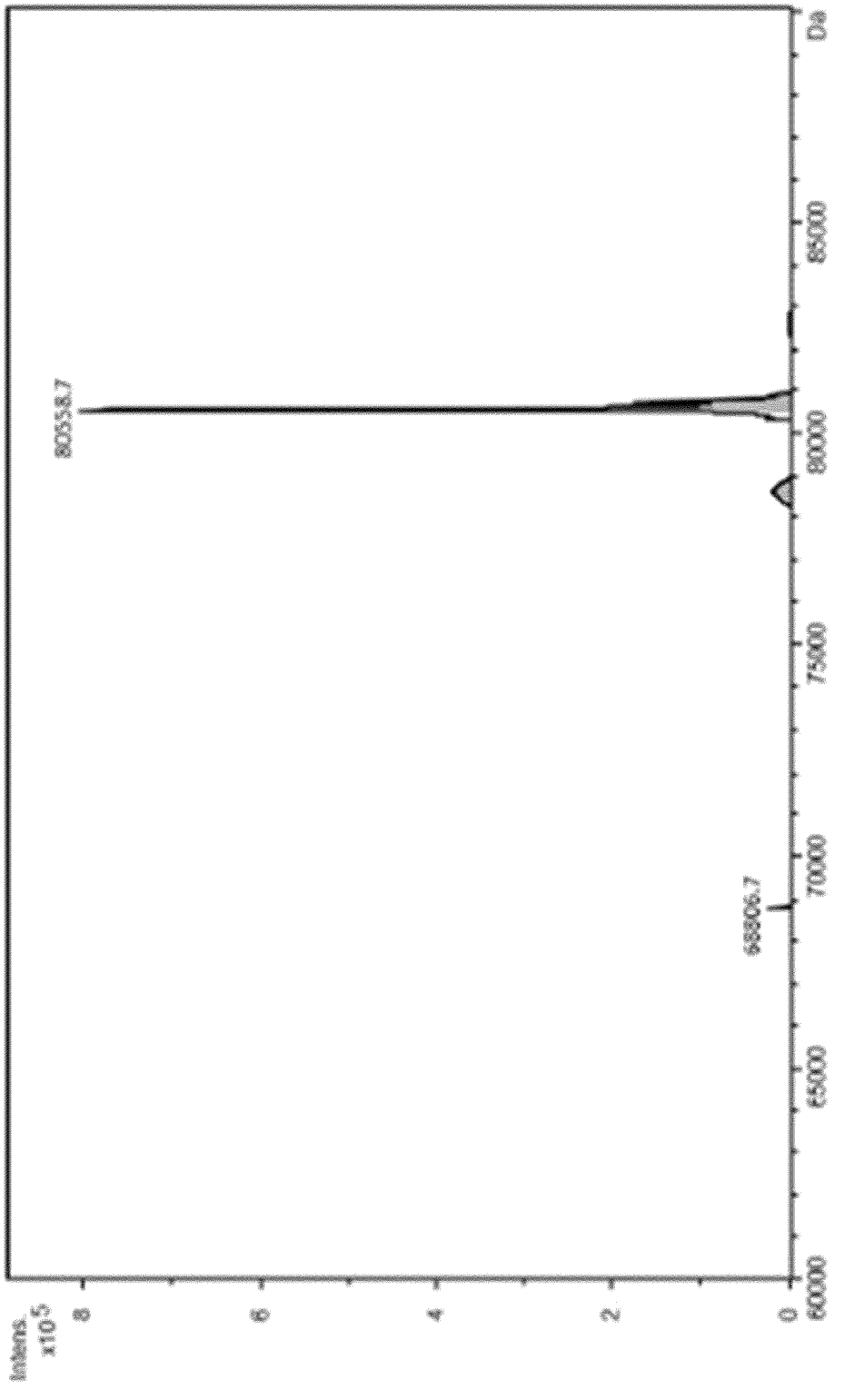

Intens.
x10^5
8
6
4
2
0
60000
65000
70000
75000
80000
85000
Da
80558.7
68806.7

Fig. 23

	105 109
5C8 CDR3:	**QFSGADYGFGRLGIRGYEYDY**
Y105F	**F**
+G108A	**F A**
+R109A	**F A**
+R109K	**F K**
Truncated	**QFSGADFGFGAGYEYDY**

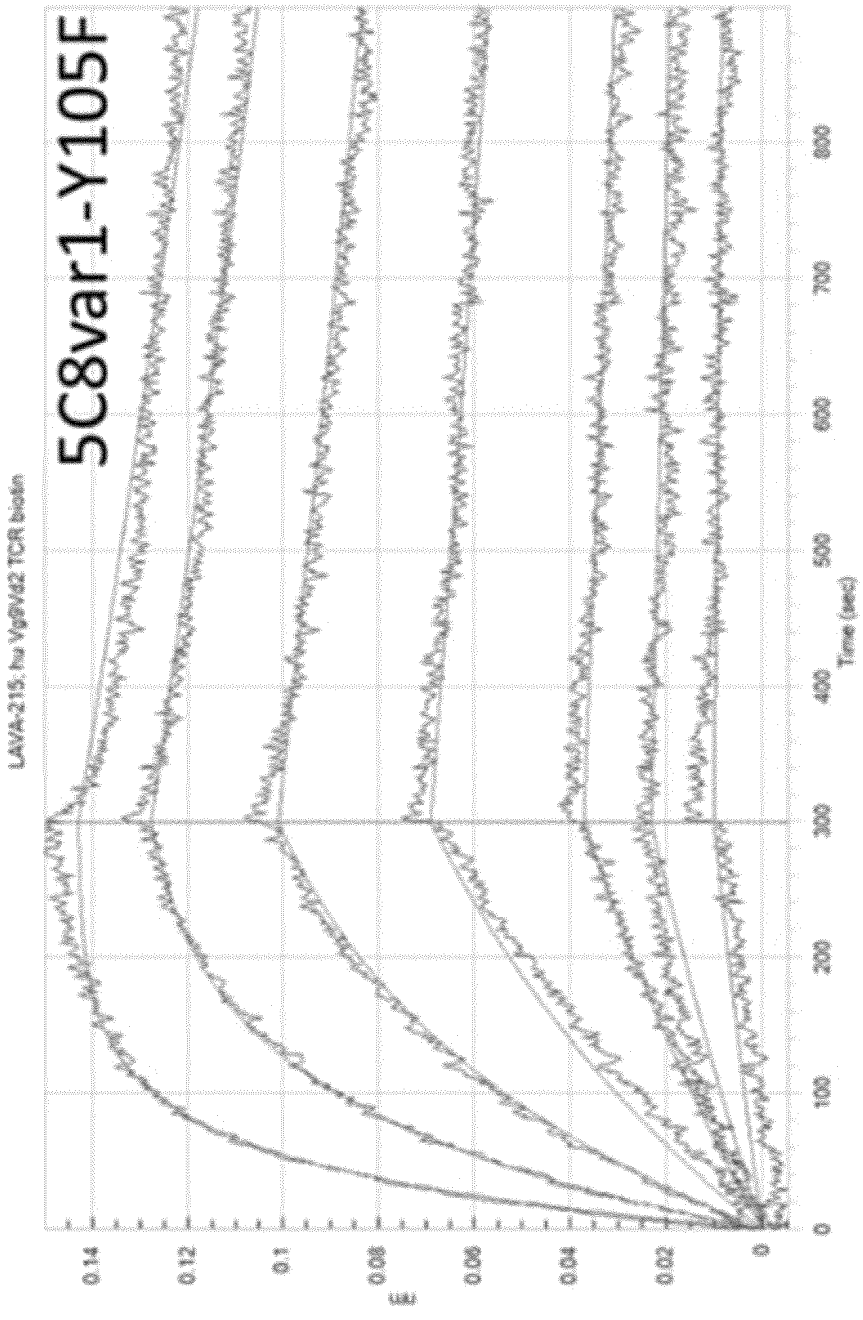

5C8var1-Y105F
LAVA-215: hu Vg9Vd2 TCR biotin
Time (sec)
nm
0
100
200
300
400
500
600
700
800
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0

Fig. 24A

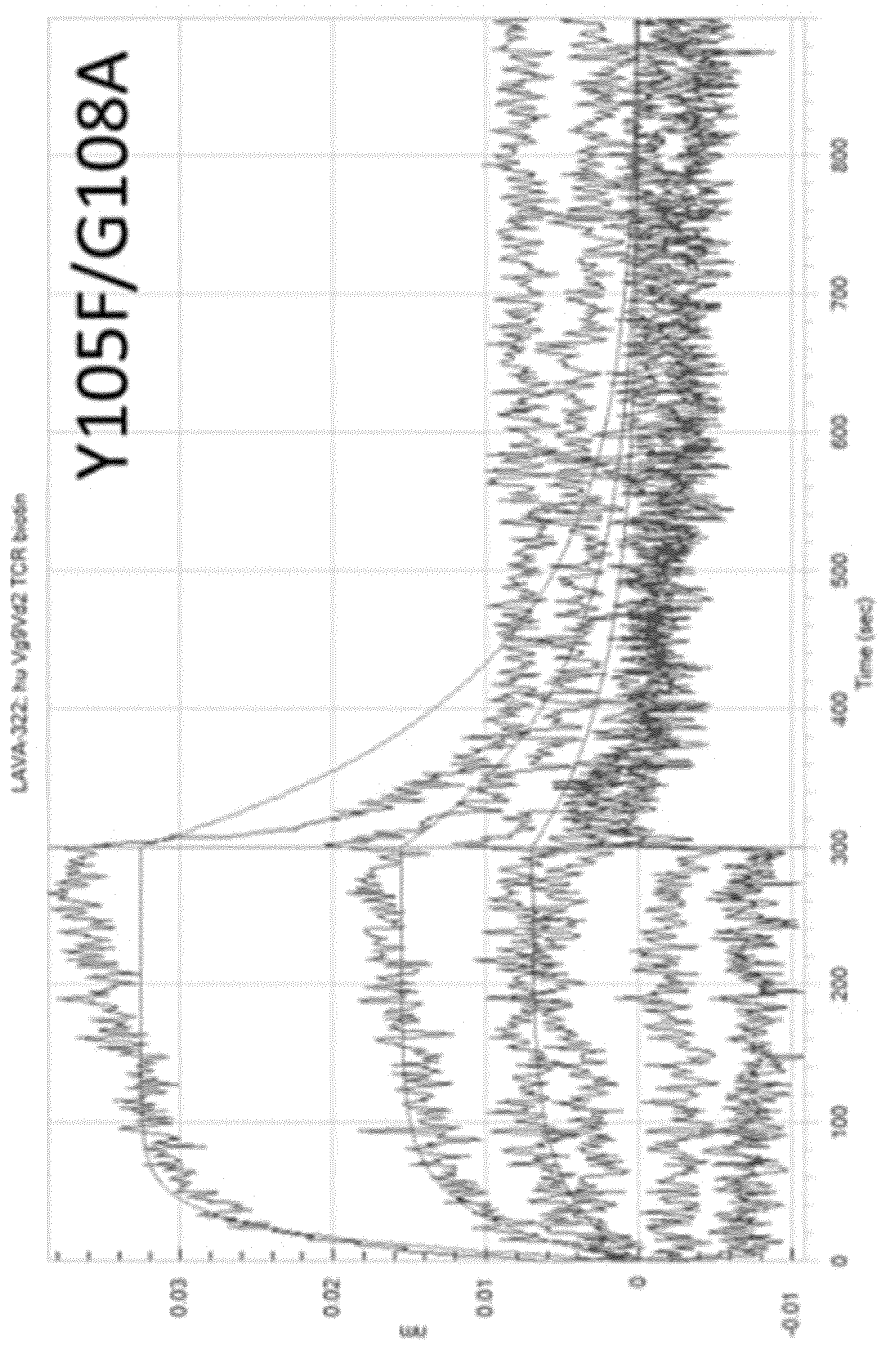

Y105F/G108A
LAVA-322: hu Vg9Vd2 TCR biotin
nm
0.03
0.02
0.01
0
-0.01
0
100
200
300
400
500
600
700
800
Time (sec)

Fig. 24B

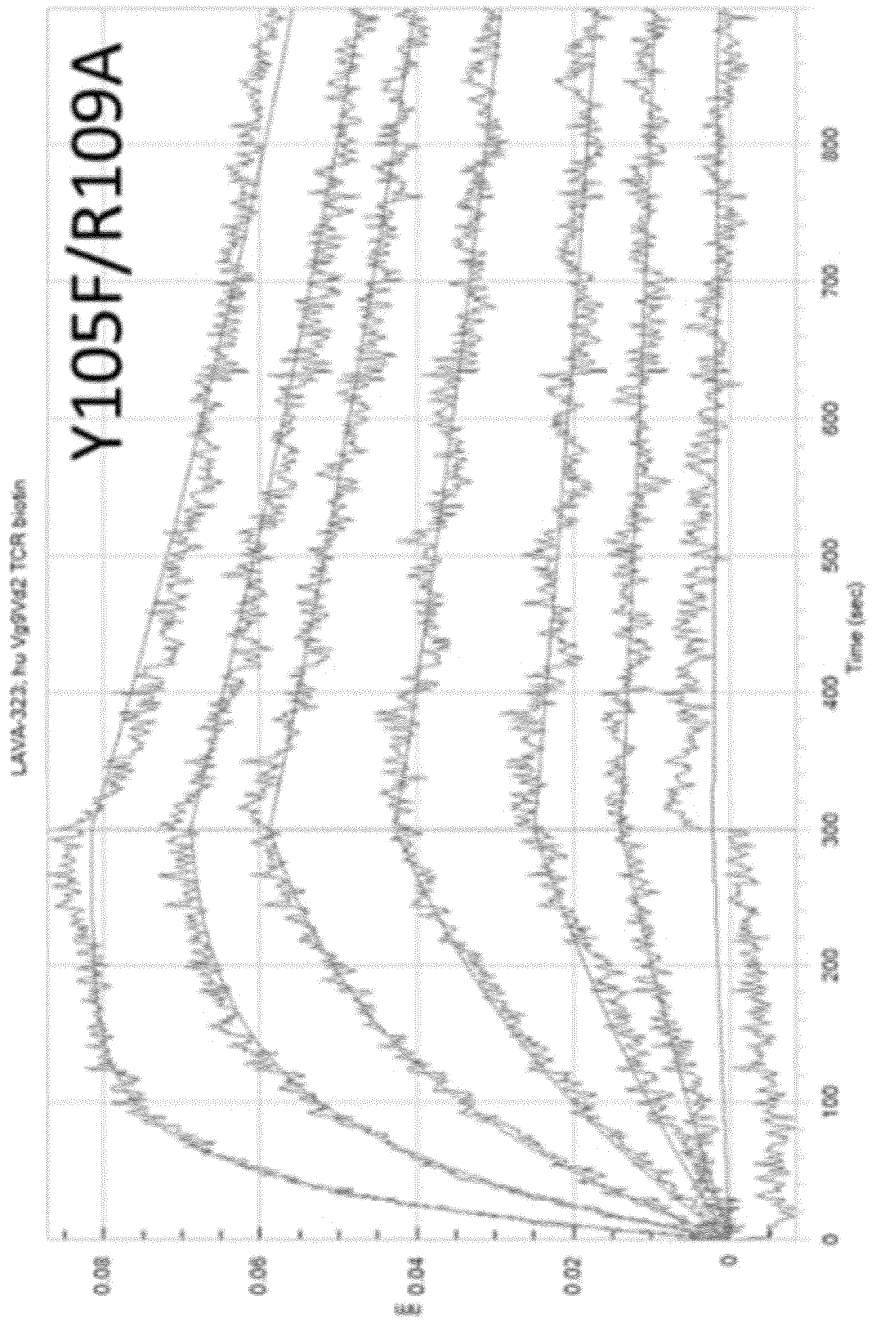
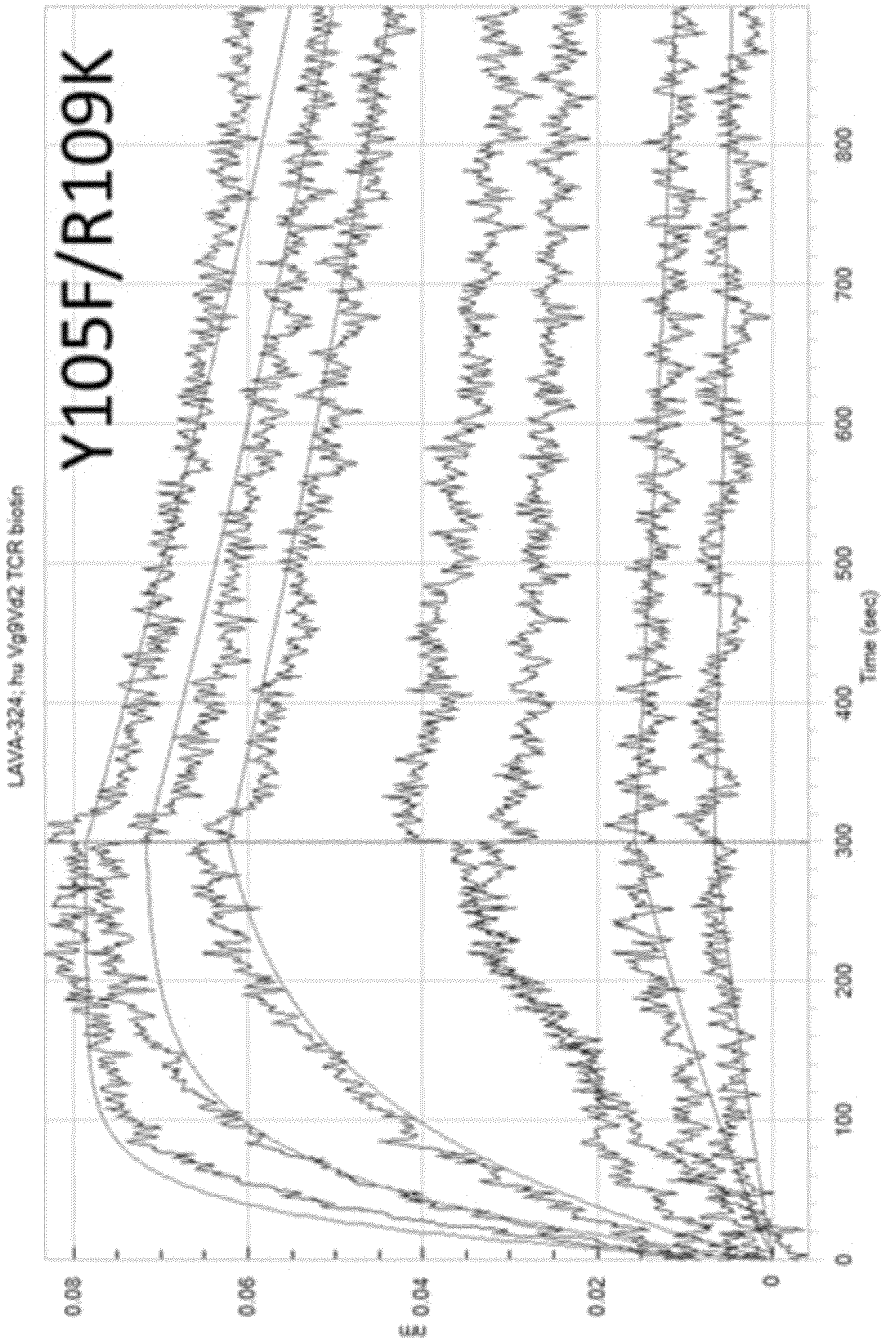

Y105F/R109A
LAVA-323, hu Vg9Vd2 TCR biotin
Time (sec)
0
100
200
300
400
500
600
700
800
0.08
0.06
0.04
0.02
0

Fig. 24C

Fig. 24D


Y105F/R109K
LAVA-324; hu Vg9Vd2 TCR biotin
0.08
0.06
0.04
0.02
0
E
0
100
200
300
400
500
600
700
800
Time (sec)

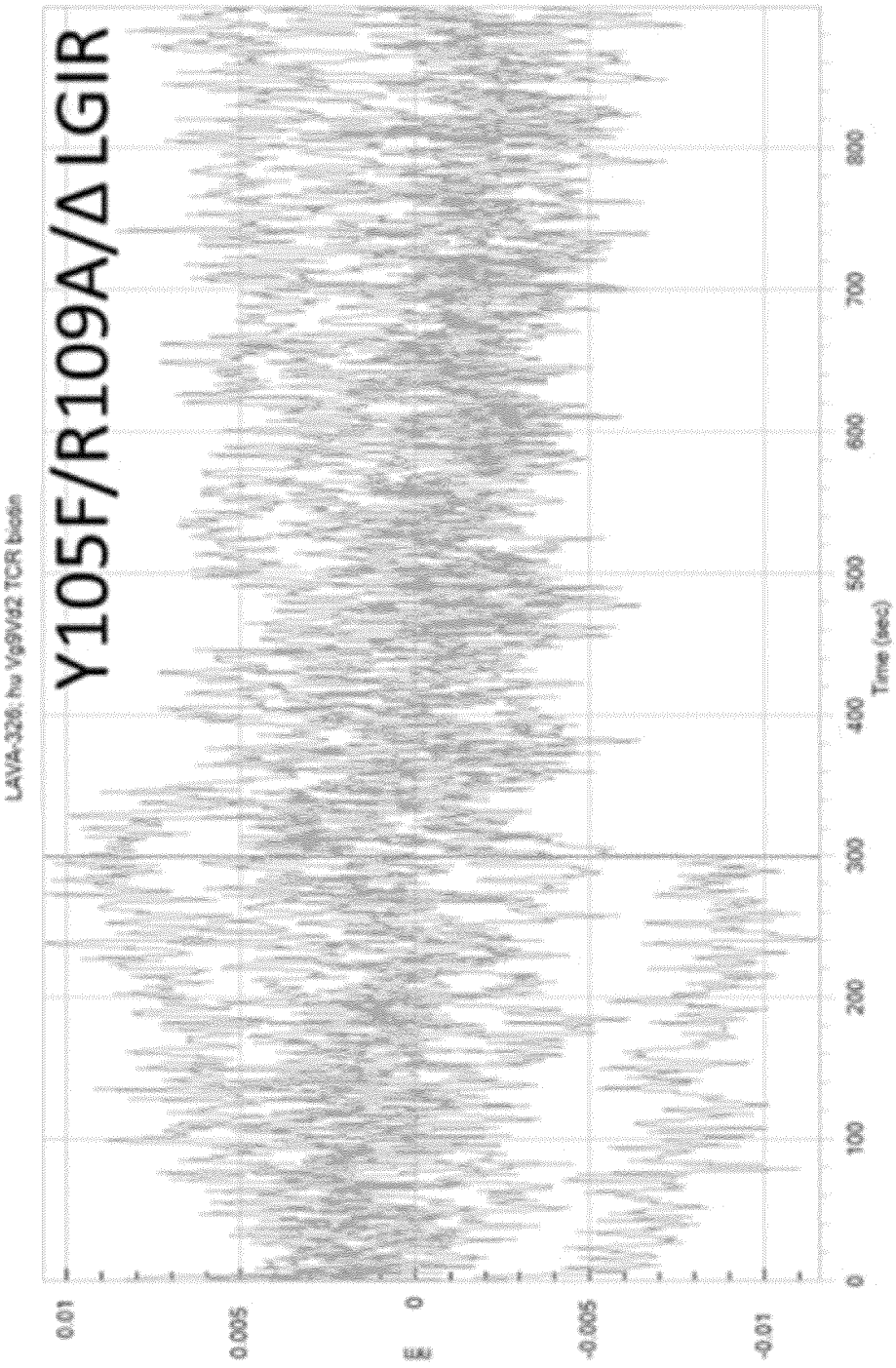

Y105F/R109A/Δ LGIR
LAVA-326: hu Vgδ9/Vδ2 TCR biotin
0.01
0.005
0
-0.005
-0.01
EU
0
100
200
300
400
500
600
700
800
Time (sec)

Fig. 24E

Fig. 25

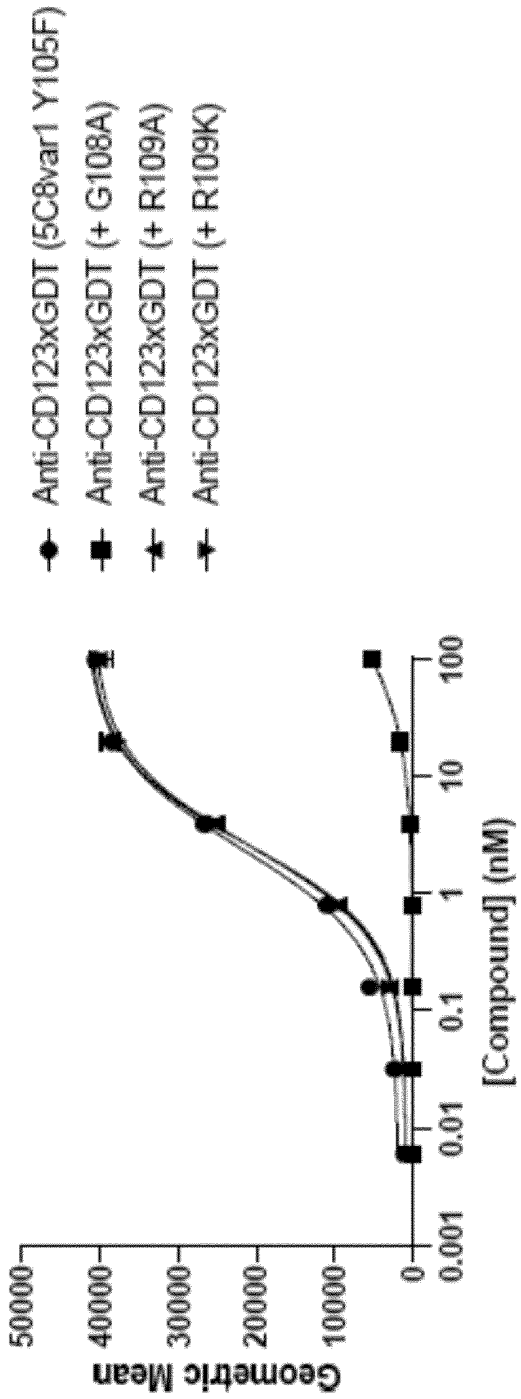

Anti-CD123xGDT (5C8var1 Y105F)
Anti-CD123xGDT (+ G108A)
Anti-CD123xGDT (+ R109A)
Anti-CD123xGDT (+ R109K)
Geometric Mean
50000
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
[Compound] (nM)

Fig. 26A

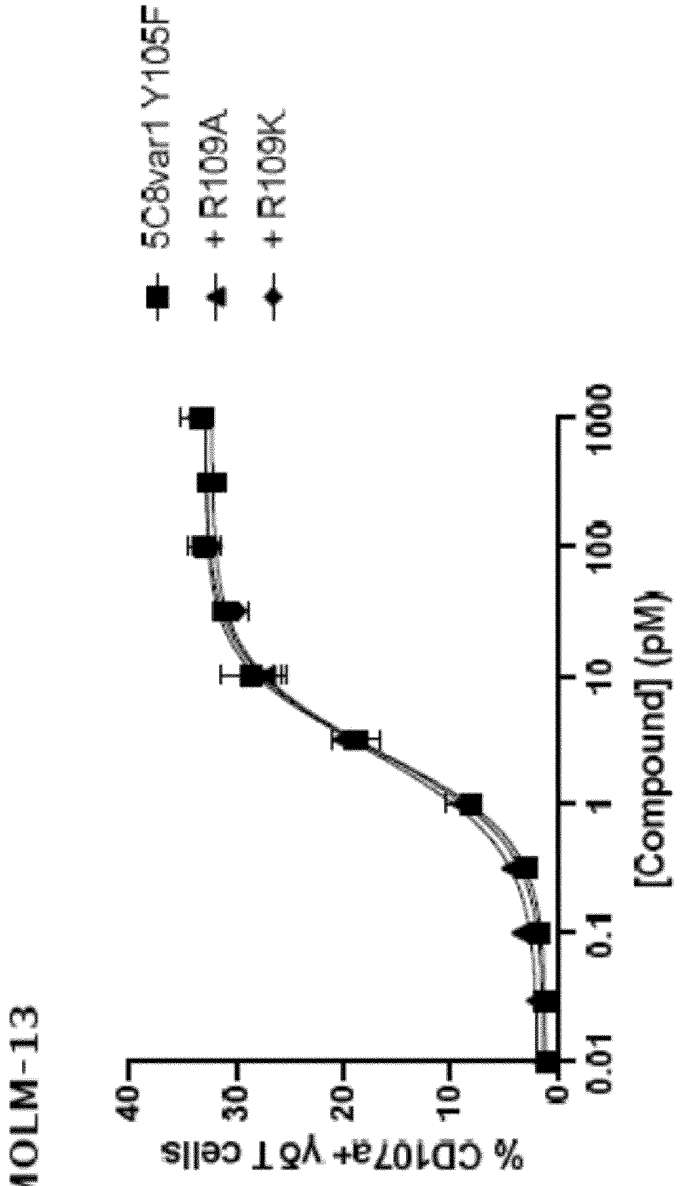

MOLM-13
% CD107a+ γδ T cells
40
30
20
10
0
0.01
0.1
1
10
100
1000
[Compound] (pM)
5C8var1 Y105F
+ R109A
+ R109K

Fig. 26B

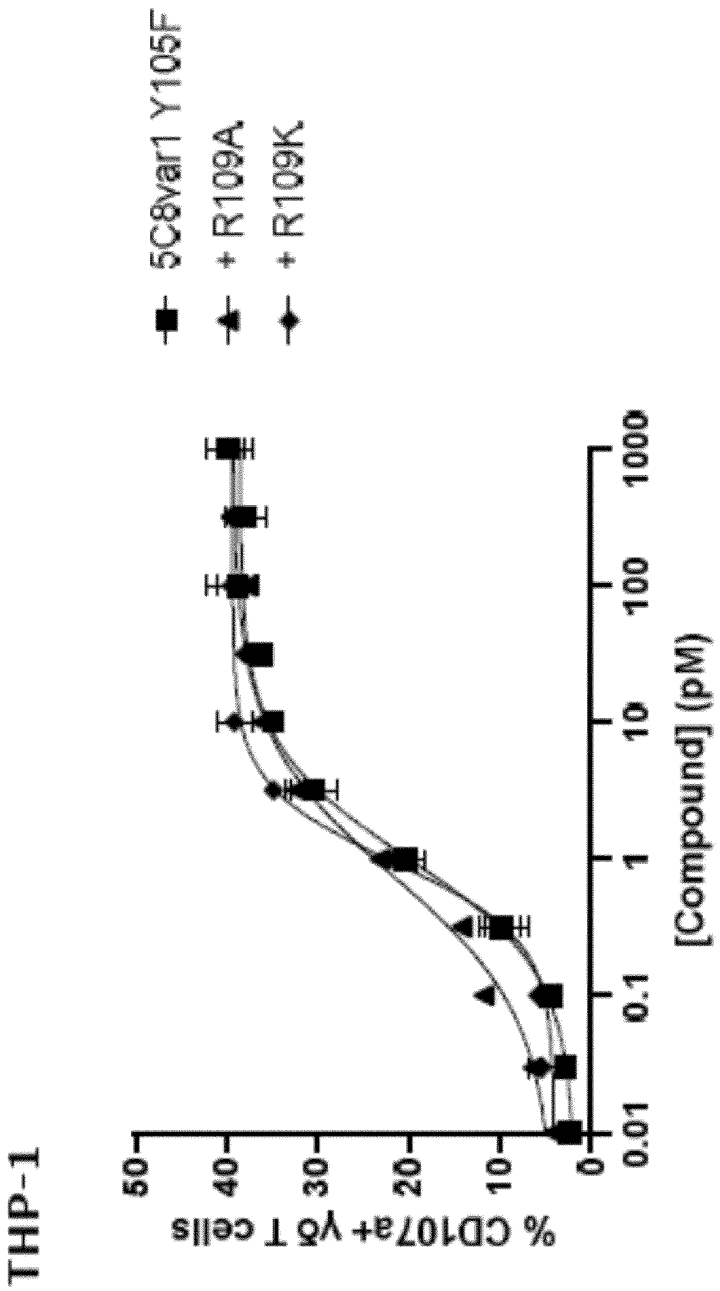

THP-1
% CD107a+ γδ T cells
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
[Compound] (pM)
5C8var1 Y105F
+ R109A
+ R109K

Fig. 27A

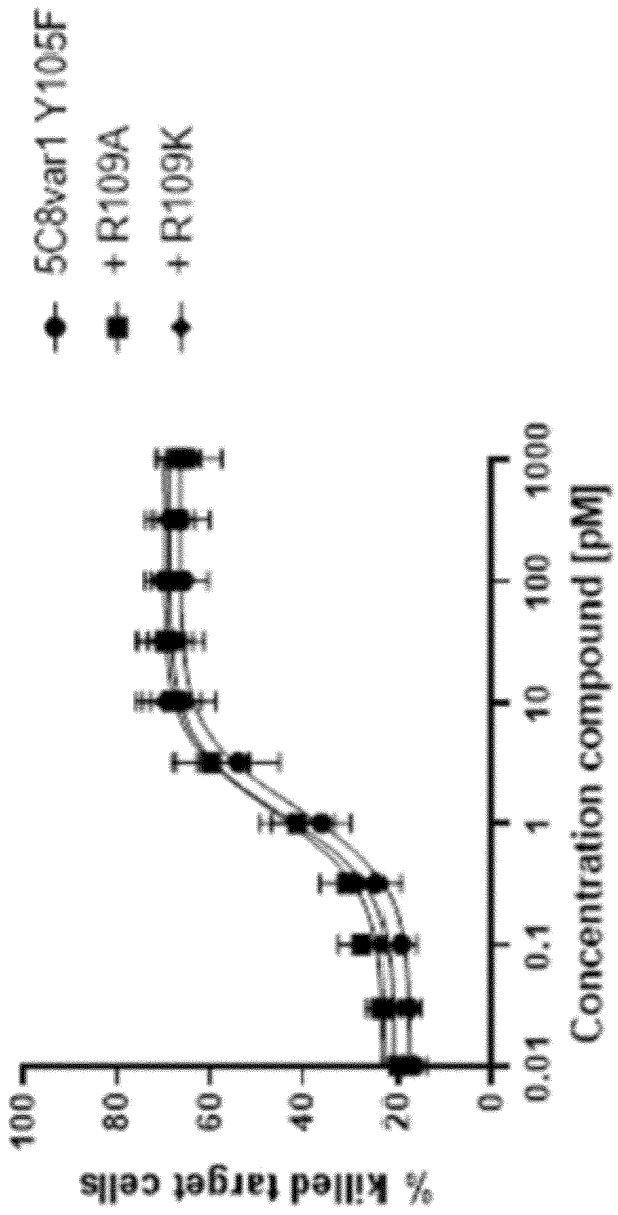
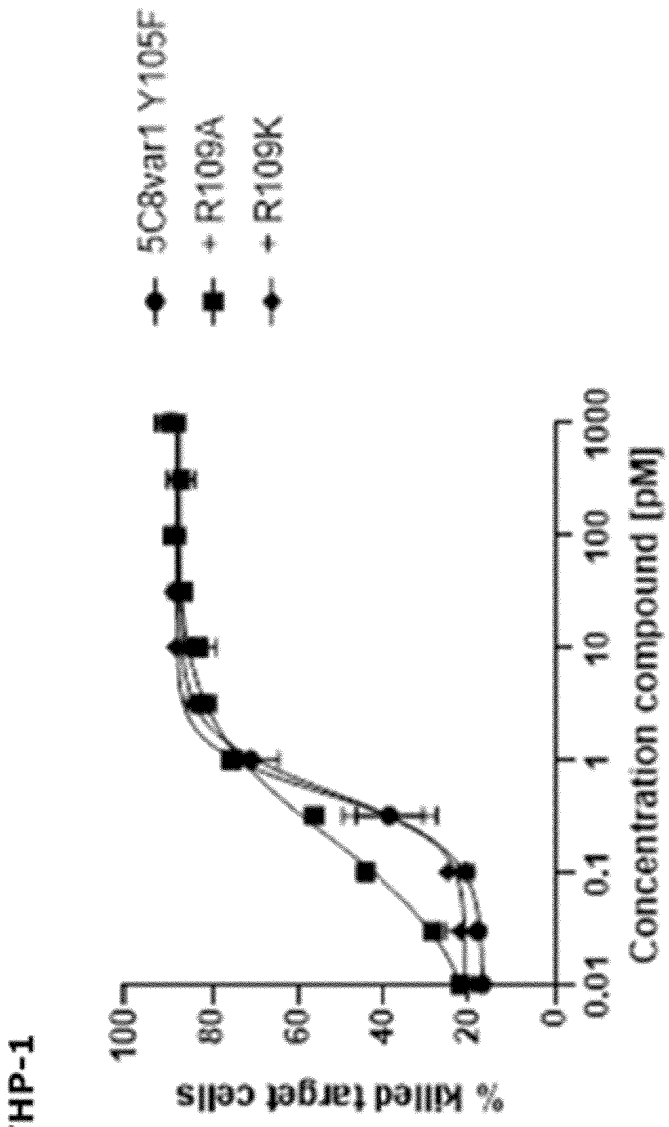

5C8var1 Y105F
+ R109A
+ R109K
% killed target cells
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration compound [pM]

Fig. 27B


THP-1
% killed target cells
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
Concentration compound [pM]
5C8var1 Y105F
R109A
R109K

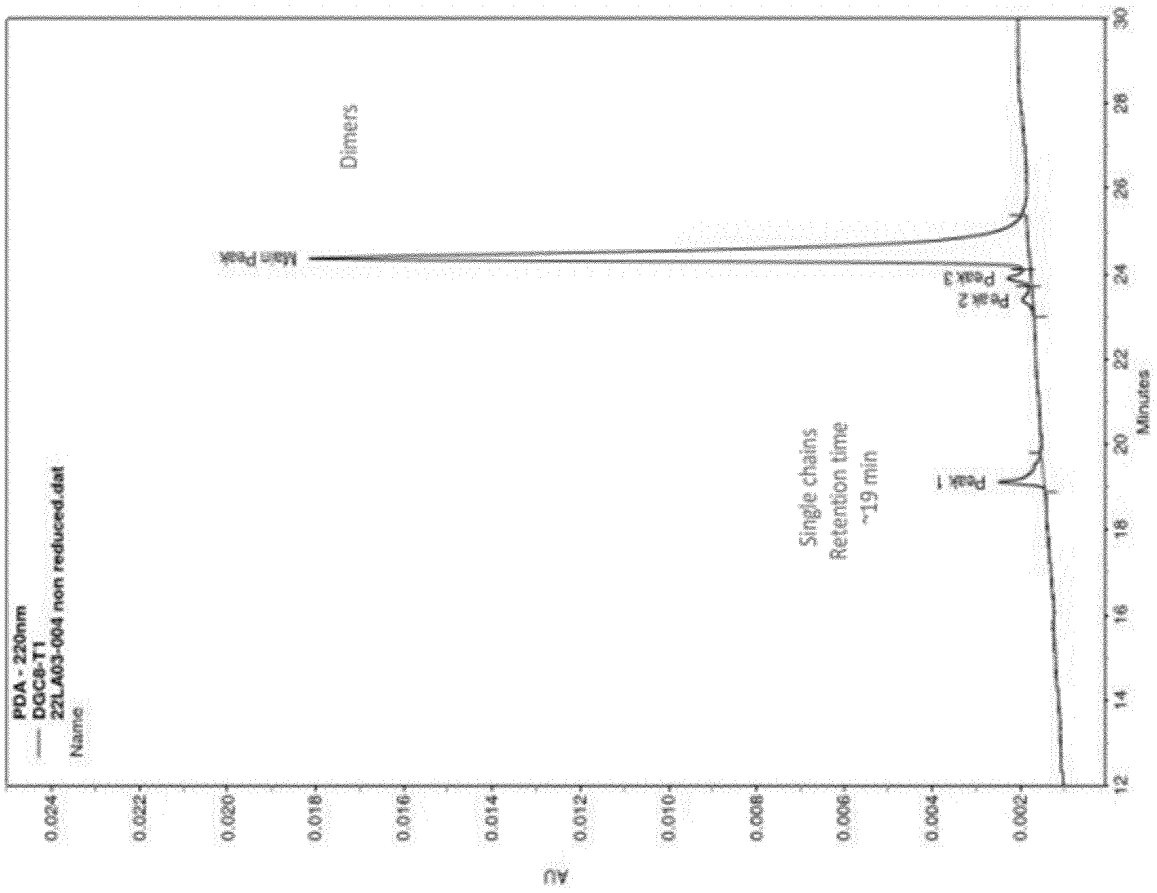

PDA - 220nm
DGC8-T1
22LA03-004 non reduced.dat
Name
Dimers
Main Peak
Peak 1
Peak 2
Peak 3
Single chains
Retention time
~19 min
AU
Minutes
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
12
14
16
18
20
22
24
26
28
30

Fig. 28A

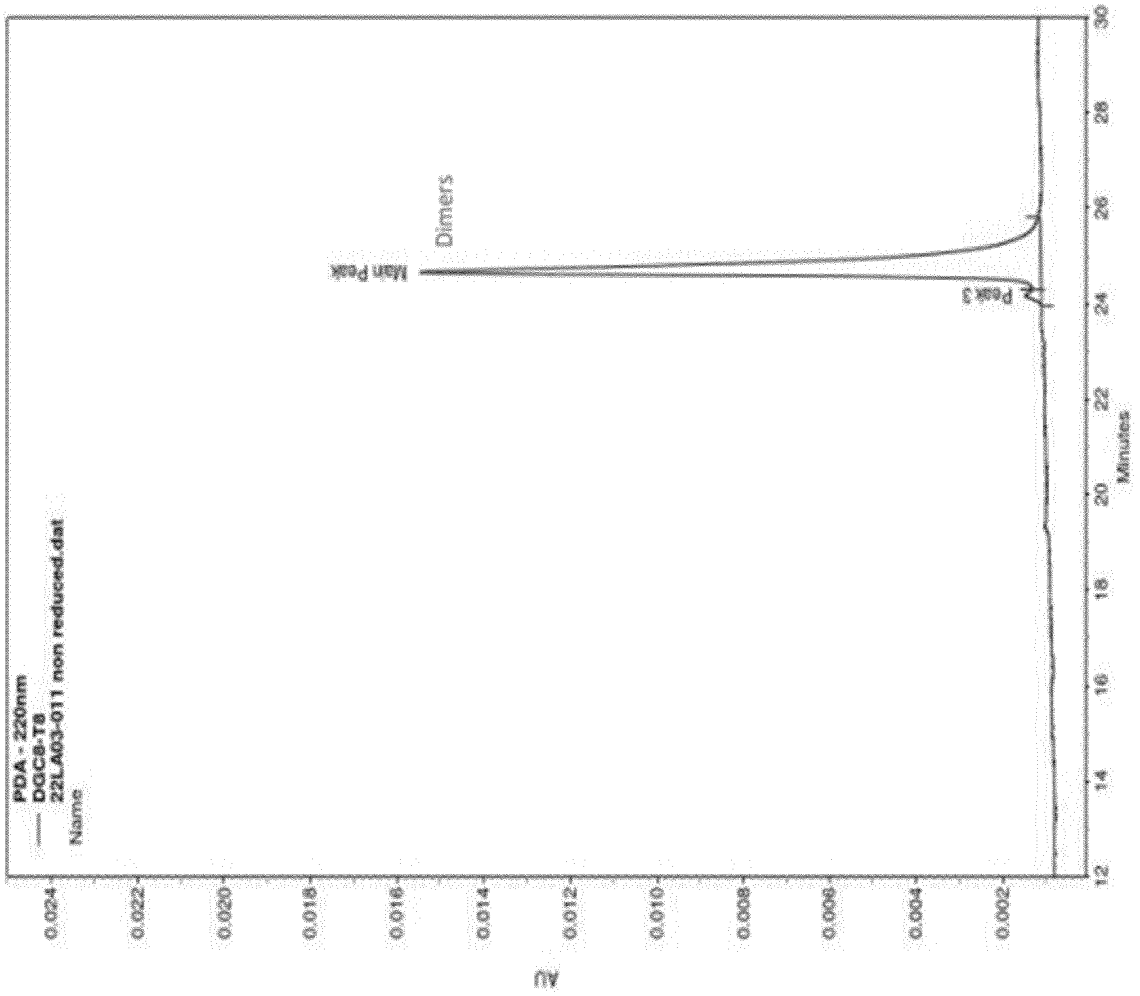

PDA - 220nm
DGCB-T8
22LA03-011 non reduced.dat
Name
Main Peak
Dimers
Peak 3
AU
Minutes
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
12
14
16
18
20
22
24
26
28
30

Fig. 28B