FIG. 1A

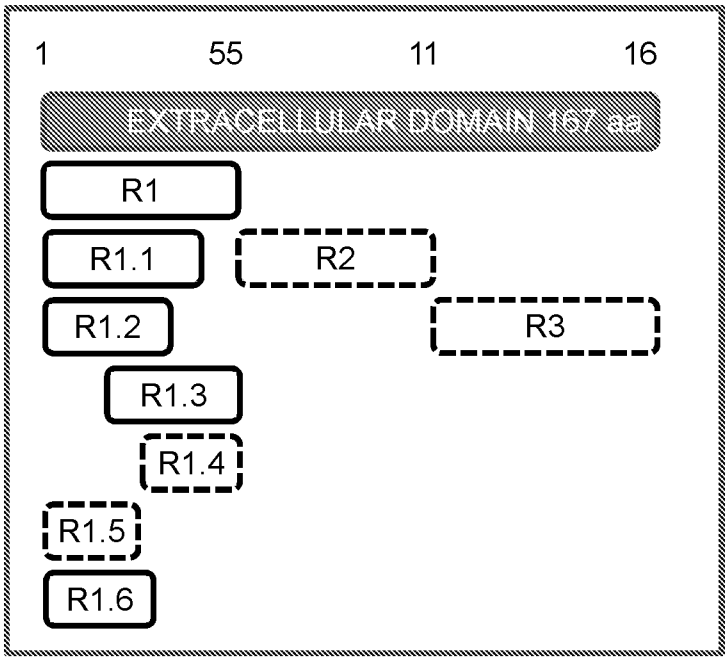

1
55
11
16
EXTRACELLULAR DOMAIN 167 aa
R1
R1.1
R2
R1.2
R3
R1.3
R1.4
R1.5
R1.6
ECD
R1 (1-55 aa)
R2 (56-110 aa)
R3 (110-167 aa)
R1.1 (1-45 aa)
R1.2 (1-35 aa)
R1.3 (11-55 aa)
R1.4 (21-55 aa)
R1.5 (1-25 aa)
R1.6 (1-30 aa)

FIG. 1B

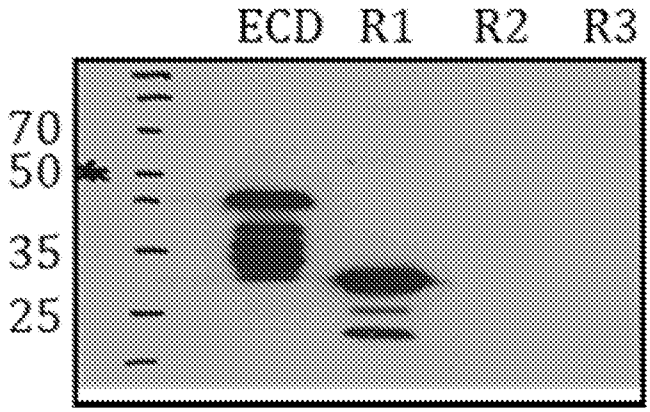

ECD
R1
R2
R3
70
50
35
25

FIG. 2A

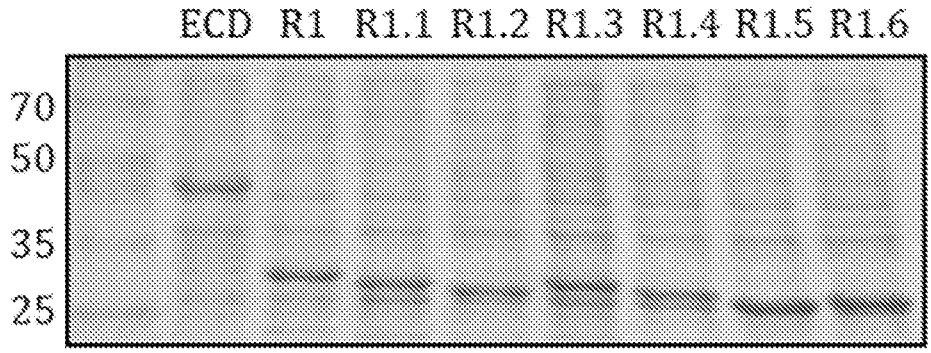

ECD R1 R1.1 R1.2 R1.3 R1.4 R1.5 R1.6
70
50
35
25

FIG. 2B

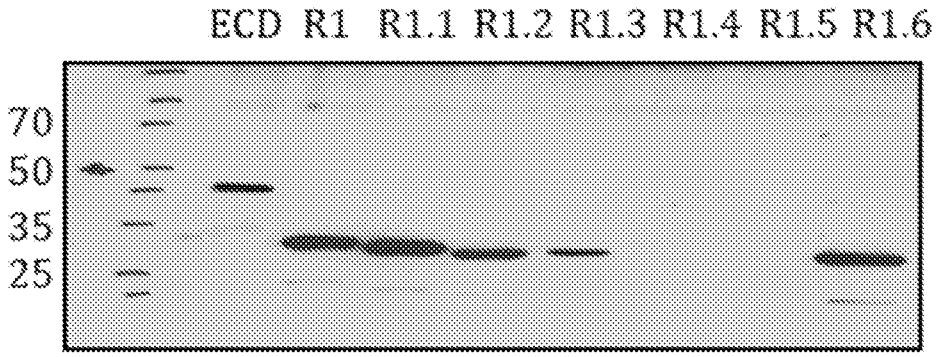

ECD R1 R1.1 R1.2 R1.3 R1.4 R1.5 R1.6
70
50
35
25

FIG. 3

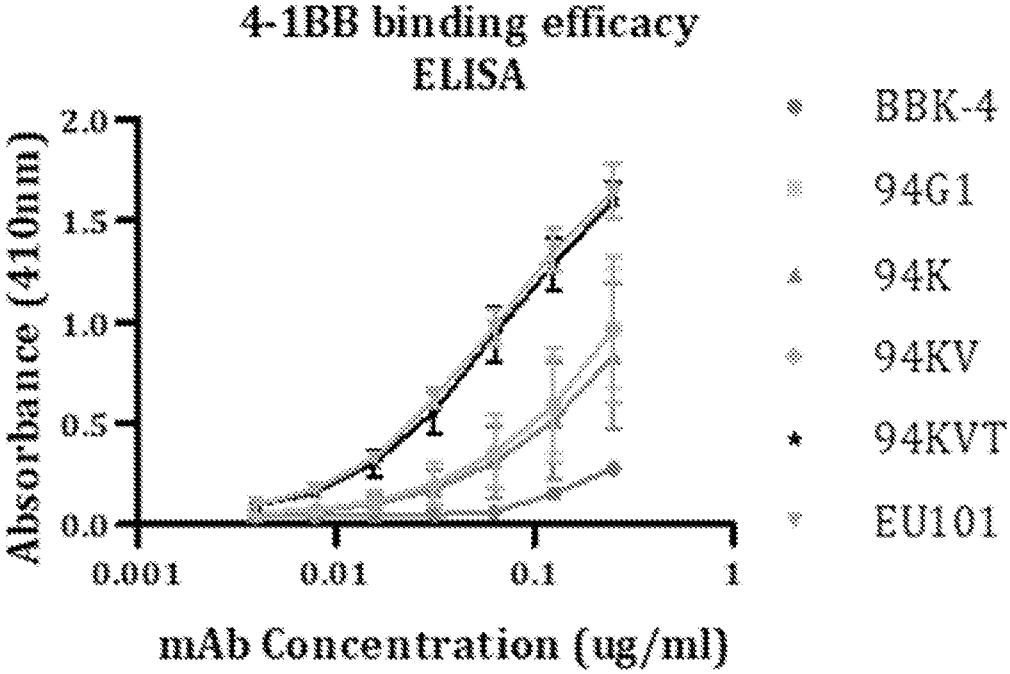

4-1BB binding efficacy
ELISA
Absorbance (410nm)
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
mAb Concentration (ug/ml)
BBK-4
94G1
94K
94KV
94KVT
EU101

FIG. 4

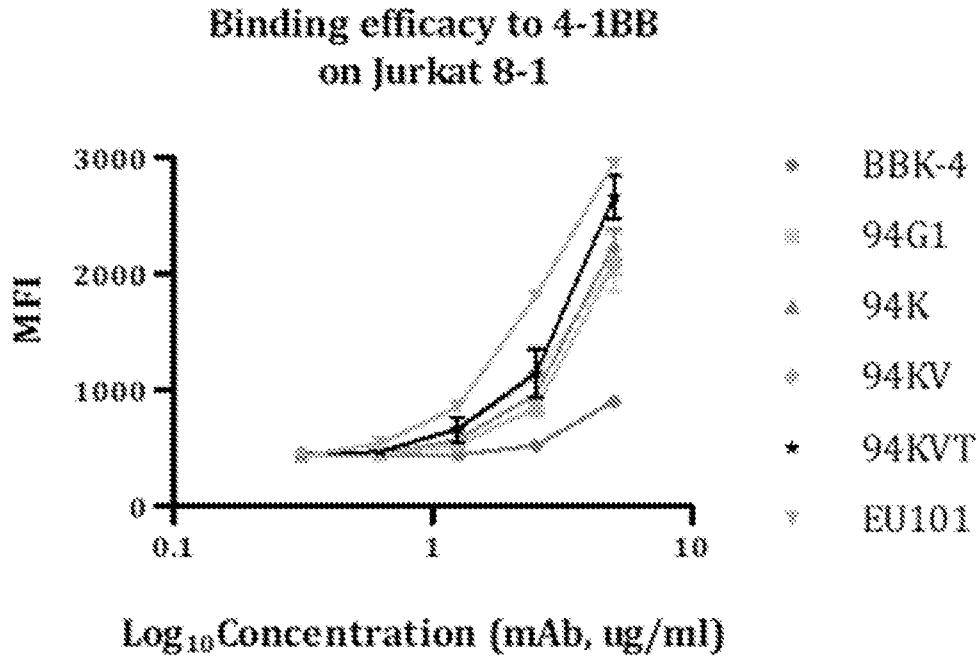
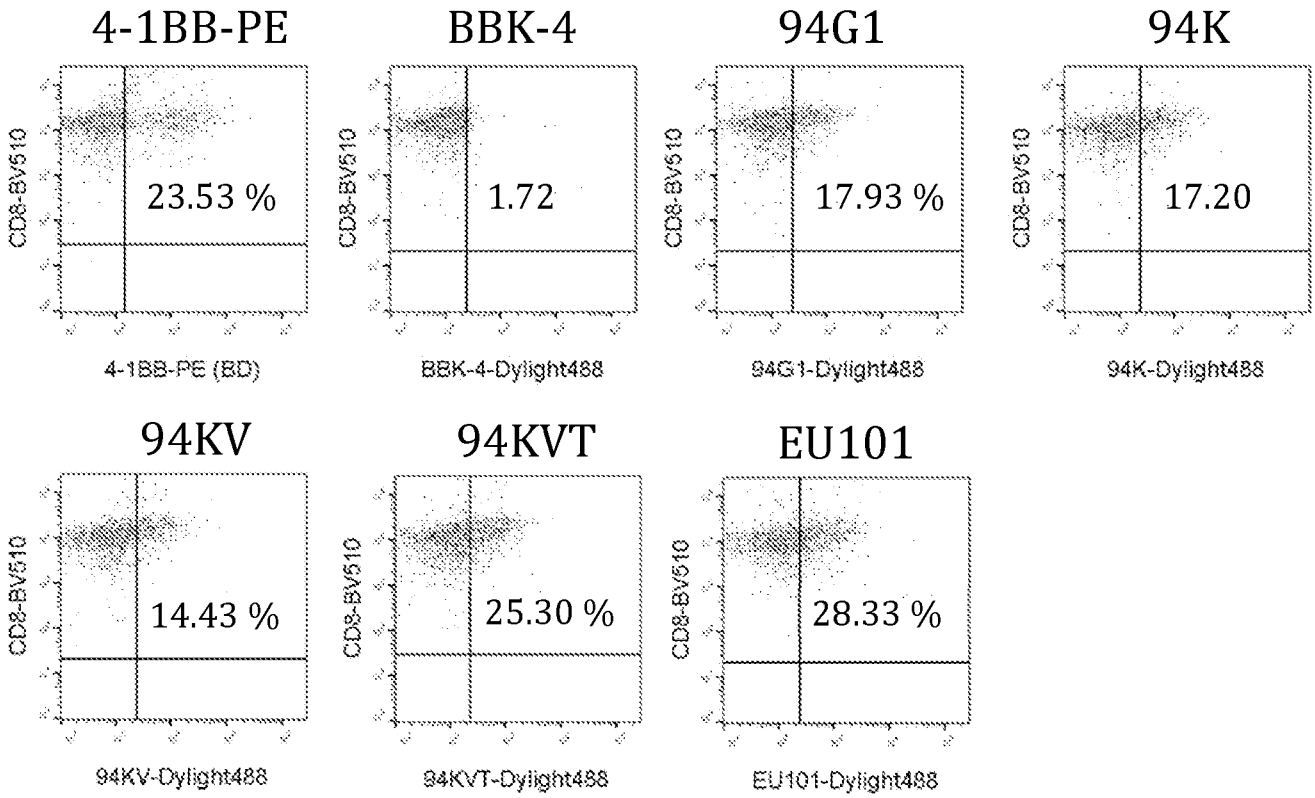

Binding efficacy to 4-1BB
on Jurkat 8-1
MFI
3000
2000
1000
0
0.1
1
10
Log10Concentration (mAb, ug/ml)
BBK-4
94G1
94K
94KV
94KVT
EU101

FIG. 5

Antibody	Ka(1/Ms)	Kd(1/s)	K_D (M)
EU101	1.57e10	1.8e-5	6.36e-11
94G1	1.66e9	1.18e-4	6.02e-10

FIG. 6


4-1BB-PE
BBK-4
94G1
94K
94KV
94KVT
EU101
CD8-BV510
23.53 %
1.72
17.93 %
17.20
14.43 %
25.30 %
28.33 %
4-1BB-PE (BD)
BBK-4-Dylight488
94G1-Dylight488
94K-Dylight488
94KV-Dylight488
94KVT-Dylight488
EU101-Dylight488

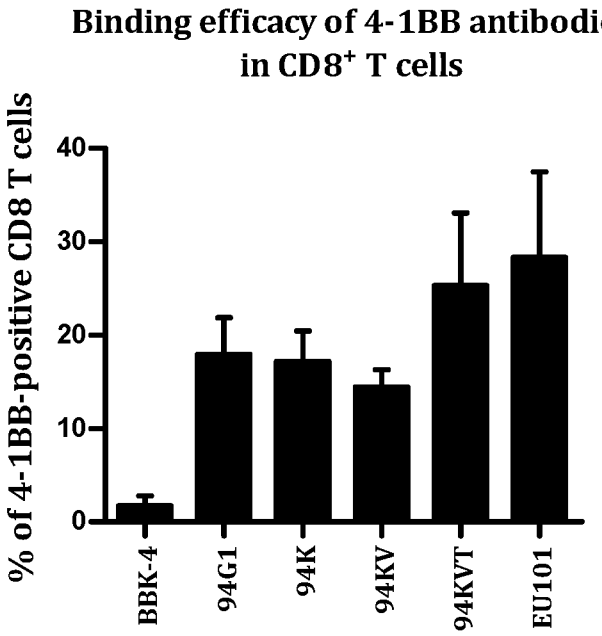

Binding efficacy of 4-1BB antibodies in CD8+ T cells
% of 4-1BB-positive CD8 T cells
40
30
20
10
0
BBK-4
94G1
94K
94KV
94KVT
EU101

FIG. 7

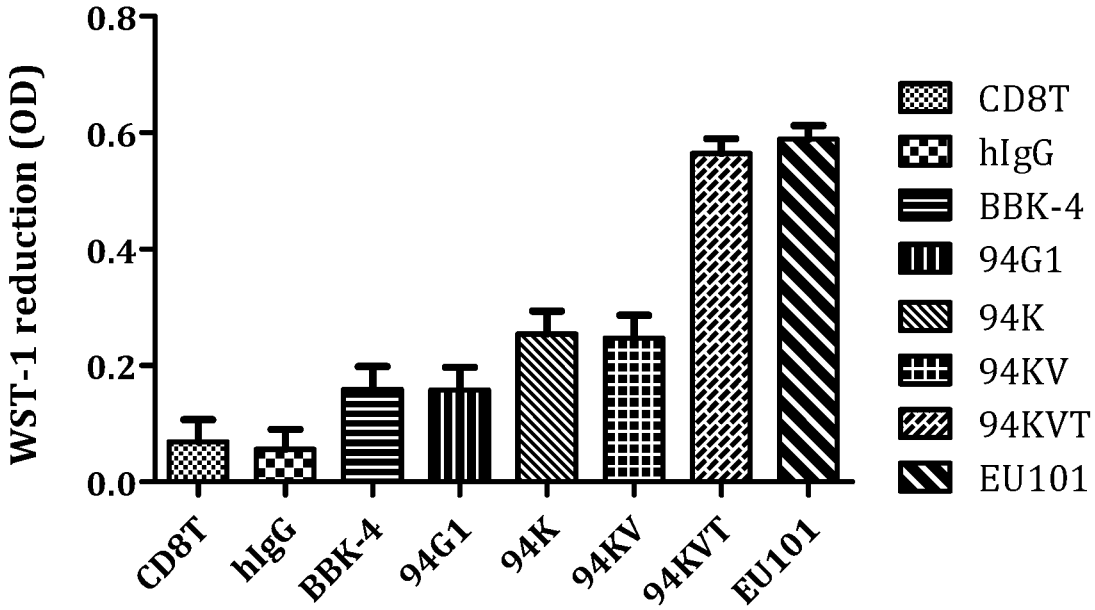

WST-1 reduction (OD)
0.8
0.6
0.4
0.2
0.0
CD8T
hIgG
BBK-4
94G1
94K
94KV
94KVT
EU101

FIG. 8

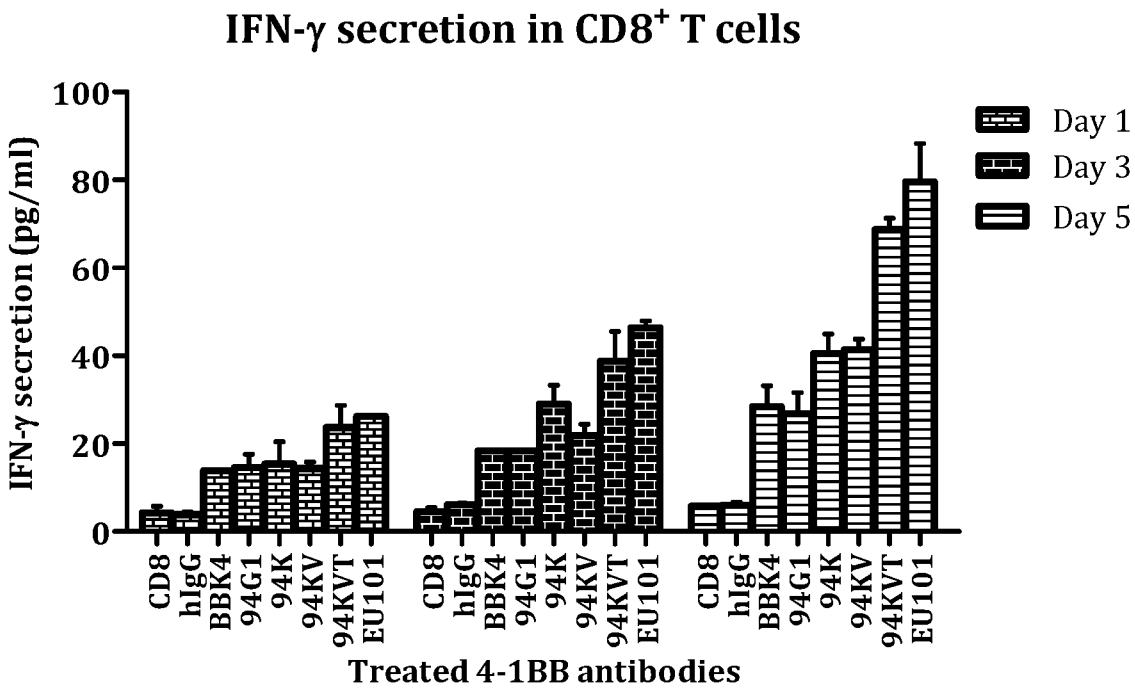

IFN-γ secretion in CD8+ T cells
IFN-γ secretion (pg/ml)
100
80
60
40
20
0
Day 1
Day 3
Day 5
CD8
hIgG
BBK4
94G1
94K
94KV
94KVT
EU101
Treated 4-1BB antibodies

FIG. 9

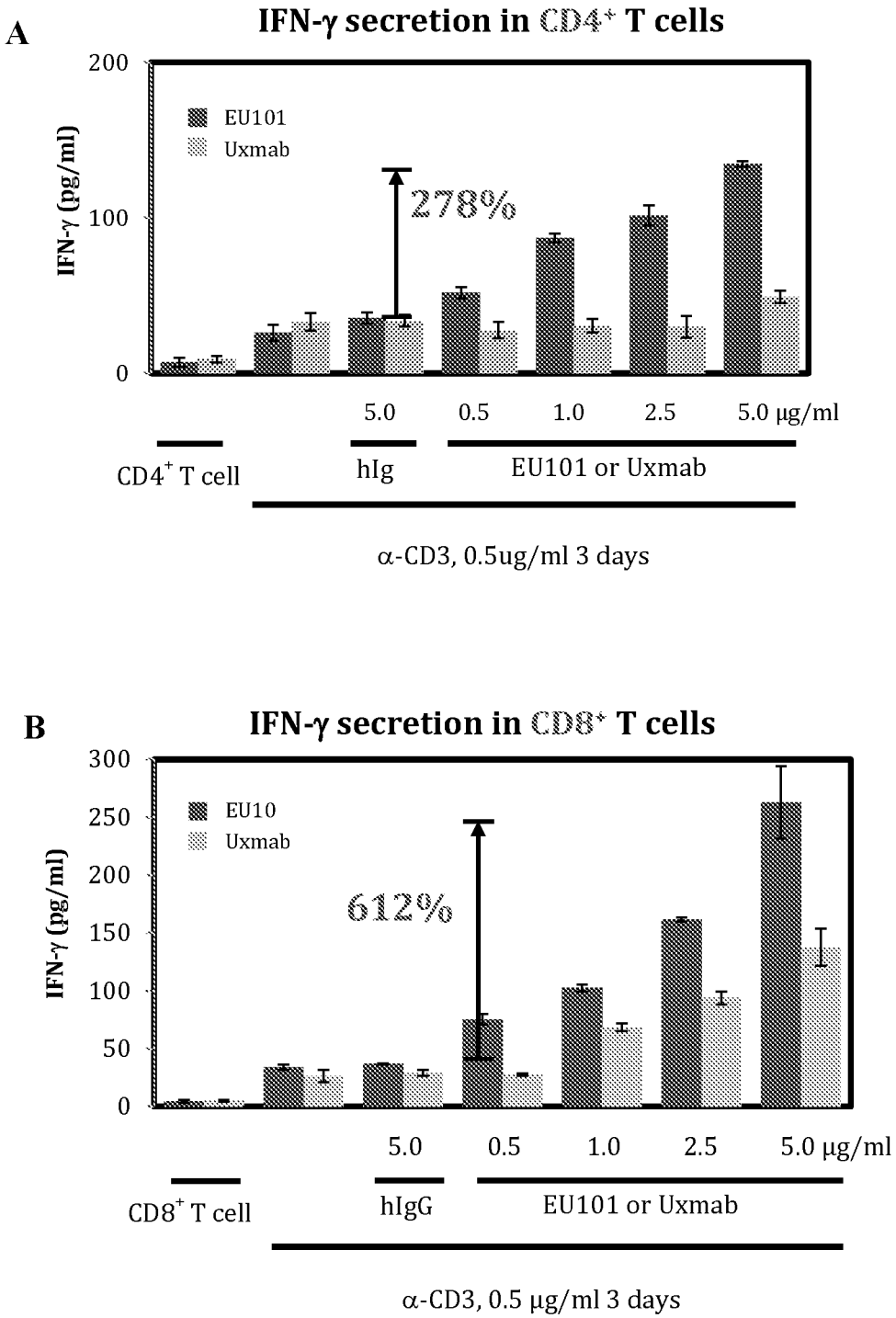
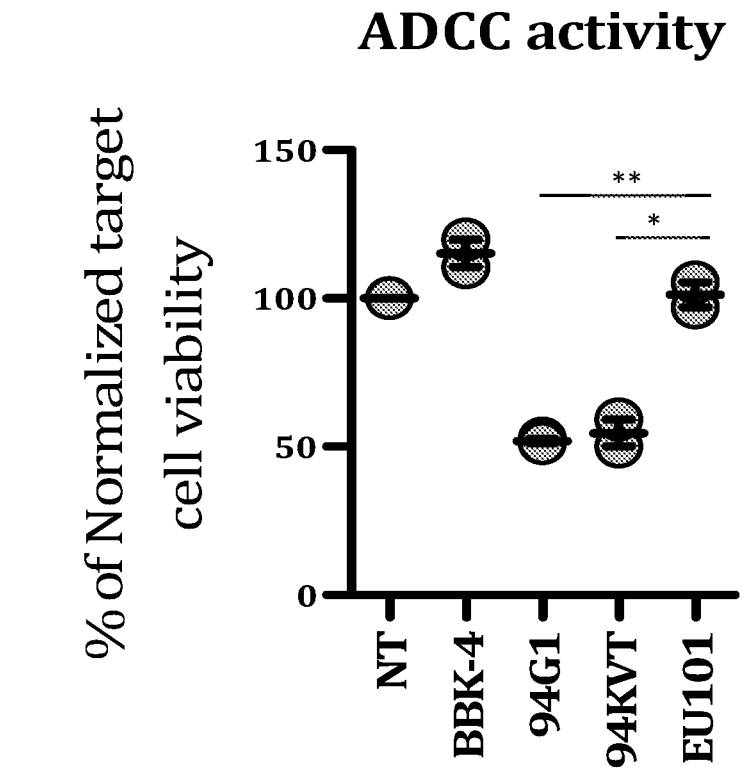

A
IFN-γ secretion in CD4+ T cells
IFN-γ (pg/ml)
200
100
0
EU101
Uxmab
278%
5.0
0.5
1.0
2.5
5.0 μg/ml
CD4+ T cell
hIg
EU101 or Uxmab
α-CD3, 0.5ug/ml 3 days
B
IFN-γ secretion in CD8+ T cells
IFN-γ (pg/ml)
300
250
200
150
100
50
0
EU10
Uxmab
612%
5.0
0.5
1.0
2.5
5.0 μg/ml
CD8+ T cell
hIgG
EU101 or Uxmab
α-CD3, 0.5 μg/ml 3 days

FIG. 10 A



ns P > 0.05
* P ≤ 0.05
** P ≤ 0.01

BBK-4: Mouse IgG2a
94G1, 94KVT: Human IgG1
EU101: engineered IgG1

FIG. 10B

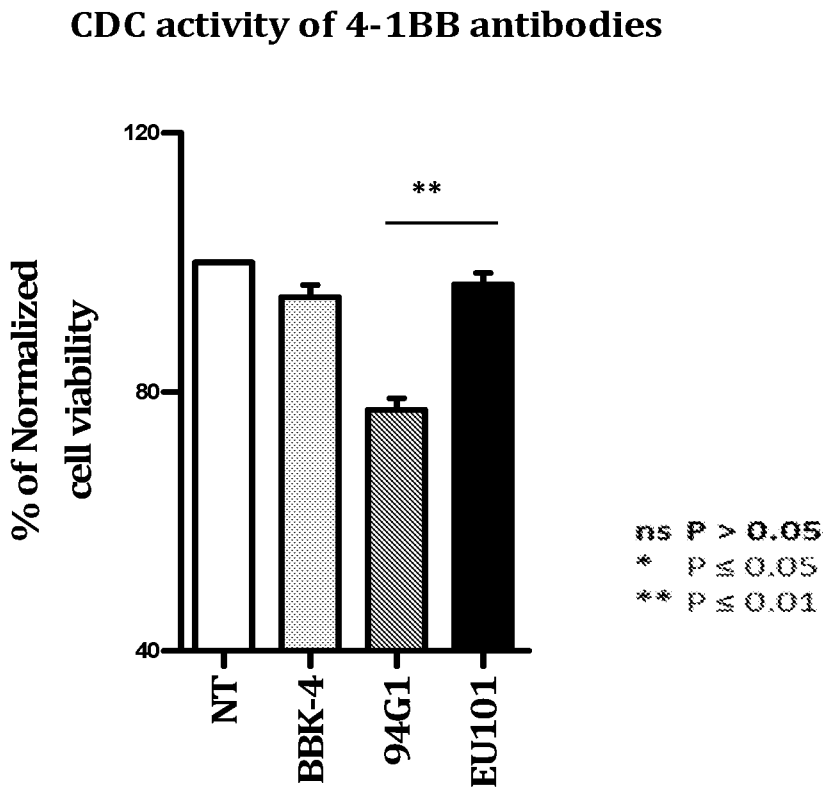

CDC activity of 4-1BB antibodies
% of Normalized cell viability
120
80
40
**
NT
BBK-4
94G1
EU101
ns P > 0.05
* P ≤ 0.05
** P ≤ 0.01

FIG. 11

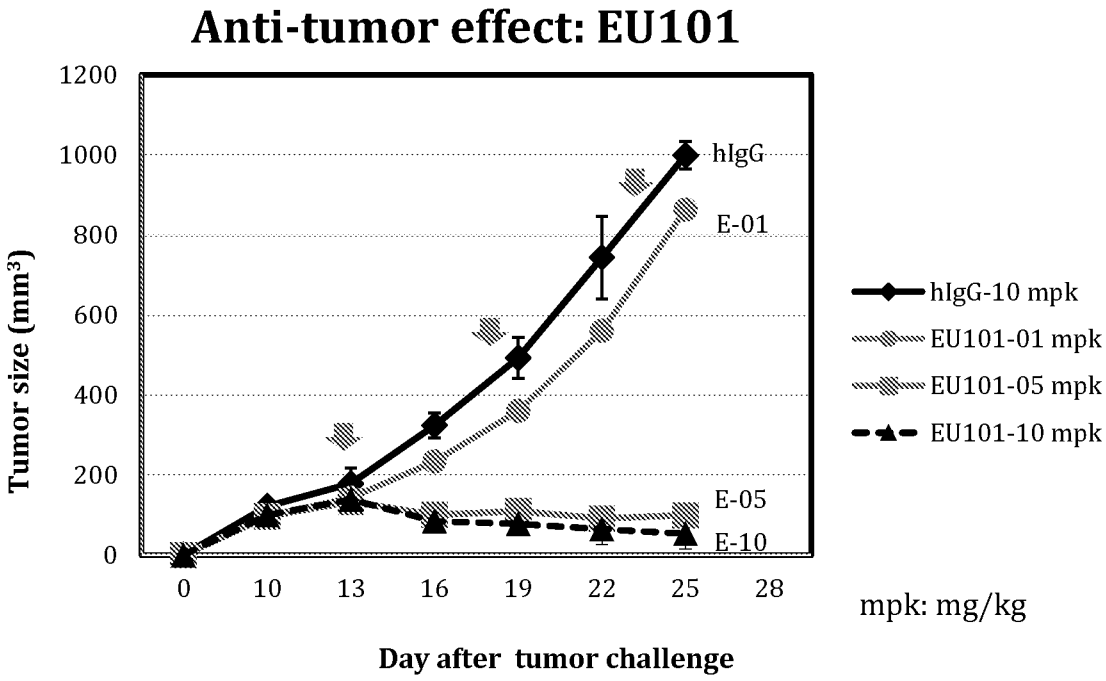

Anti-tumor effect: EU101
Tumor size (mm3)
1200
1000
800
600
400
200
0
0
10
13
16
19
22
25
28
Day after tumor challenge
hIgG
E-01
E-05
E-10
hIgG-10 mpk
EU101-01 mpk
EU101-05 mpk
EU101-10 mpk
mpk: mg/kg

FIG. 12

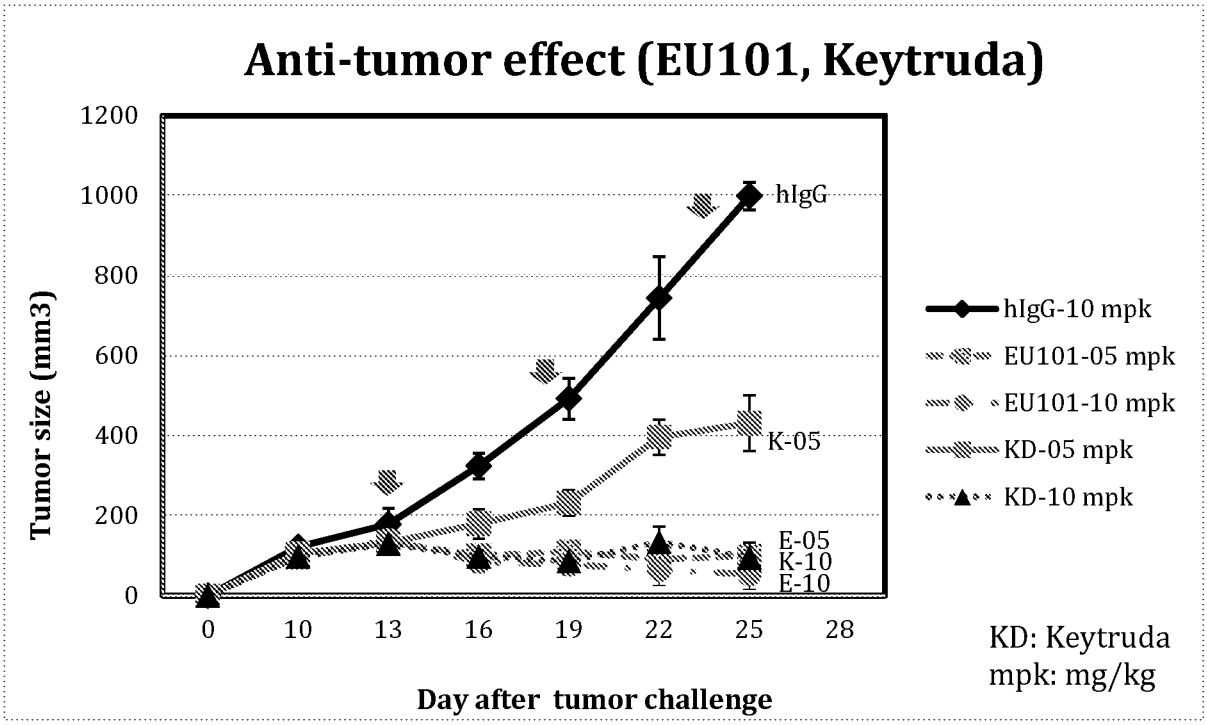

Anti-tumor effect (EU101, Keytruda)
Tumor size (mm3)
1200
1000
800
600
400
200
0
0
10
13
16
19
22
25
28
Day after tumor challenge
hIgG
K-05
E-05
K-10
E-10
hIgG-10 mpk
EU101-05 mpk
EU101-10 mpk
KD-05 mpk
KD-10 mpk
KD: Keytruda
mpk: mg/kg

FIG. 13

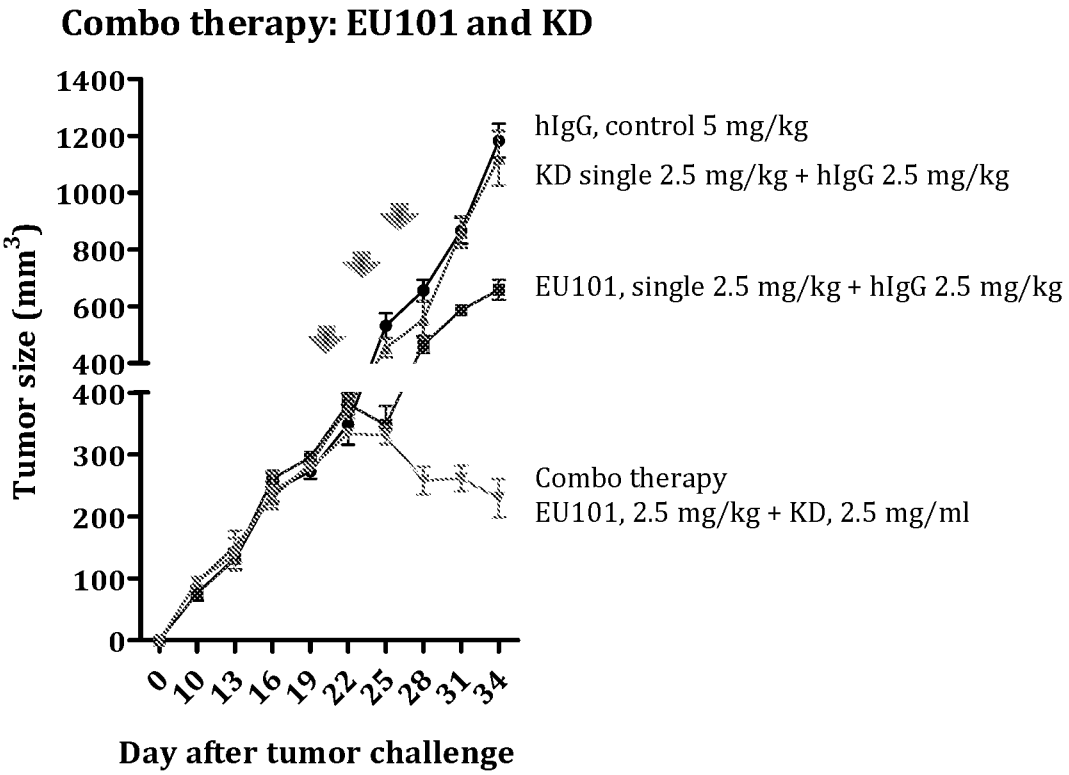

Combo therapy: EU101 and KD
Tumor size (mm3)
1400
1200
1000
800
600
400
400
300
200
100
0
0 10 13 16 19 22 25 28 31 34
Day after tumor challenge
hIgG, control 5 mg/kg
KD single 2.5 mg/kg + hIgG 2.5 mg/kg
EU101, single 2.5 mg/kg + hIgG 2.5 mg/kg
Combo therapy
EU101, 2.5 mg/kg + KD, 2.5 mg/ml

FIG. 14A

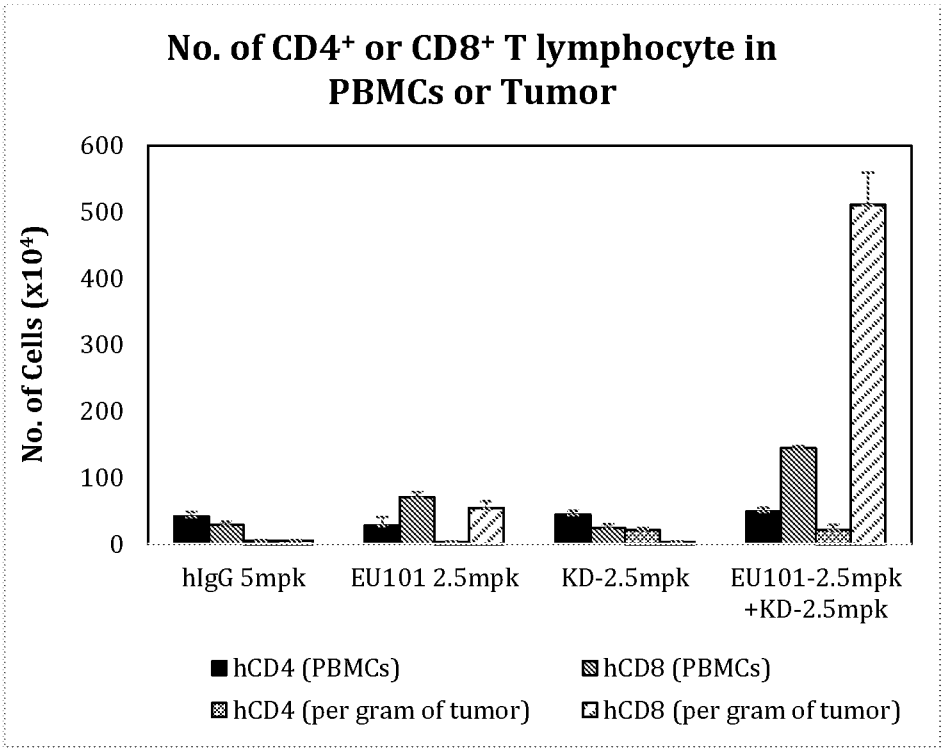


FIG. 14B

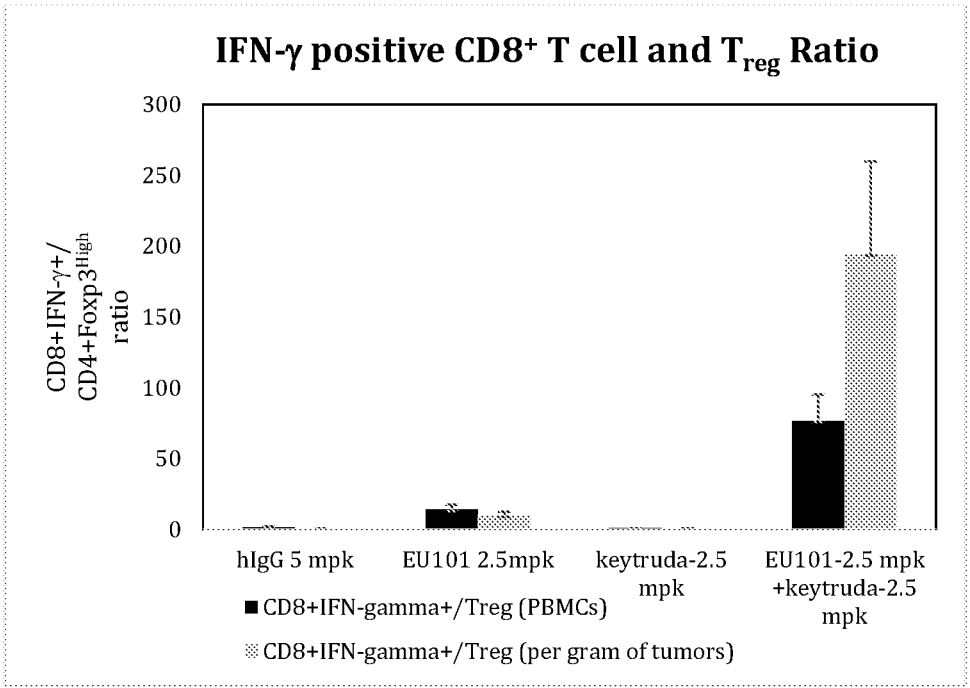


FIG. 15A

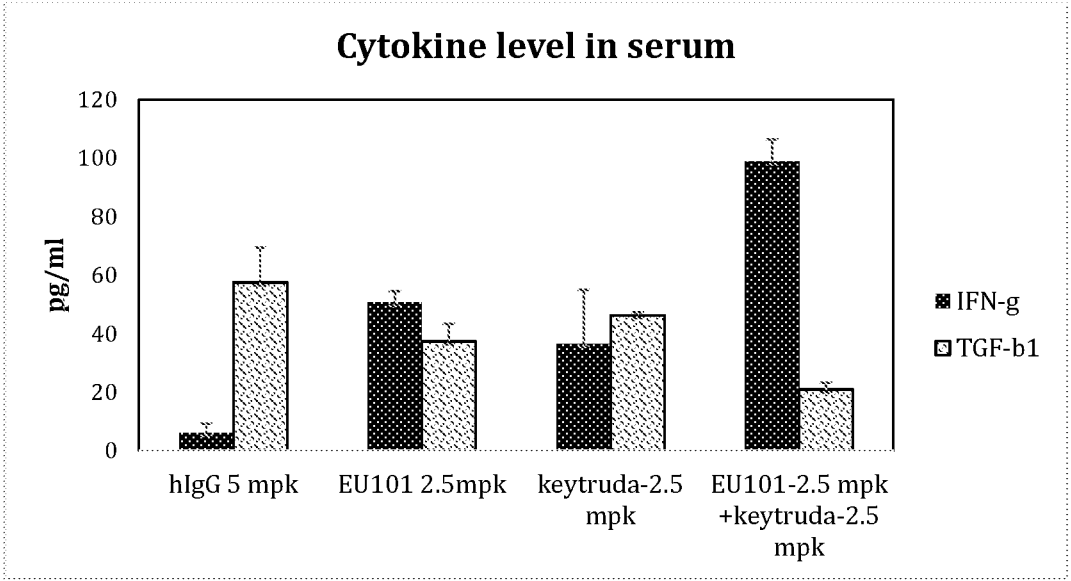


FIG. 15B

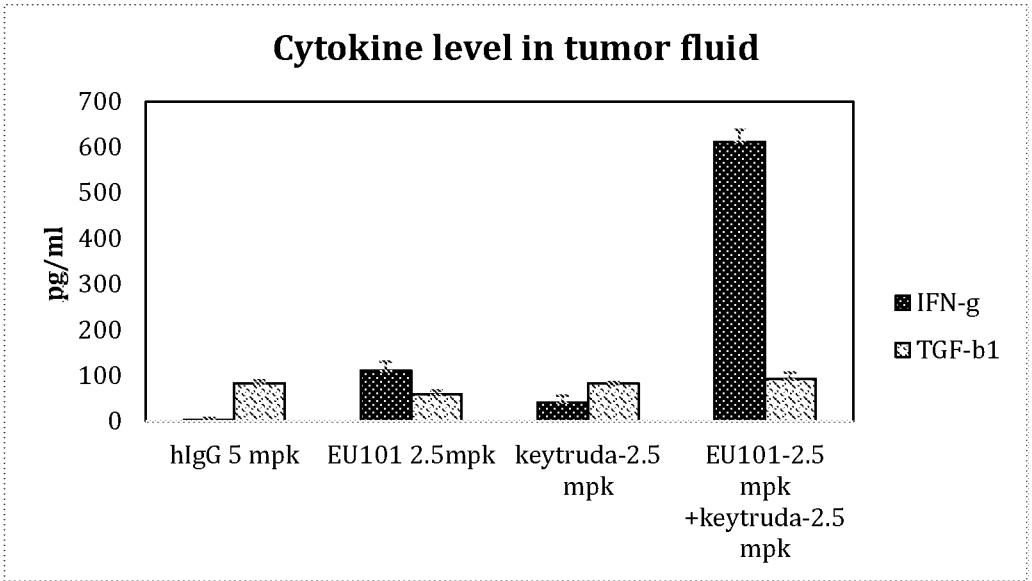


FIG. 16A

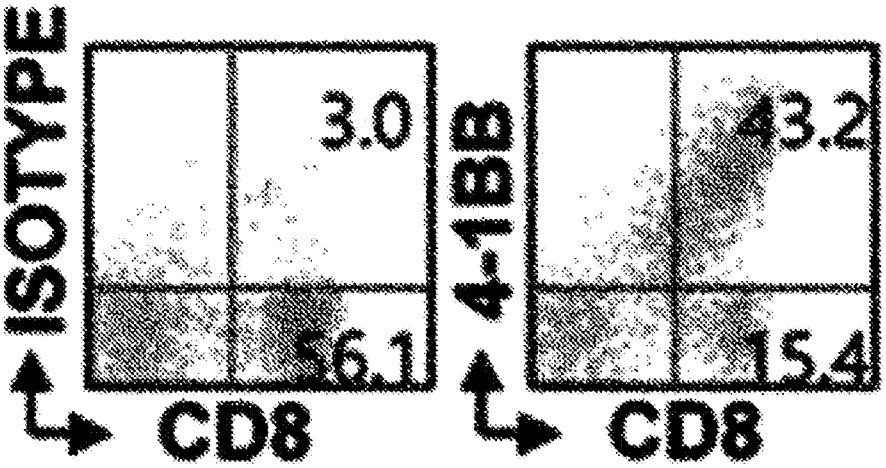


FIG. 16B

