FIG. 1A

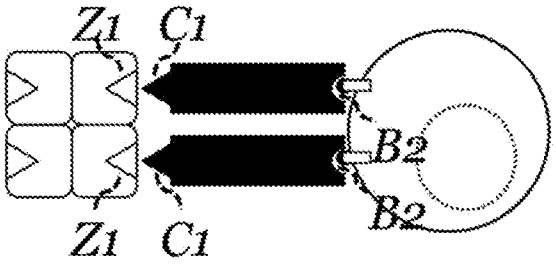


FIG. 1B

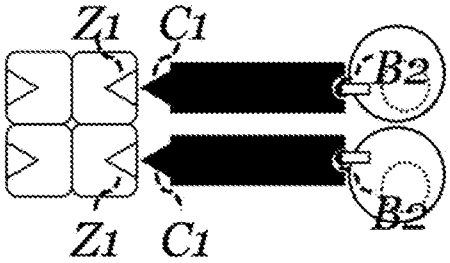


FIG. 1C

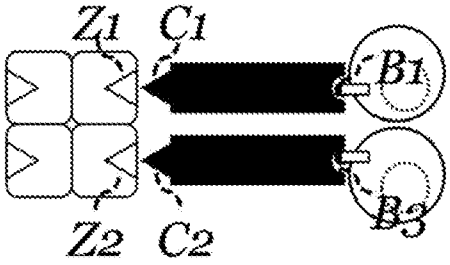


FIG. 1D

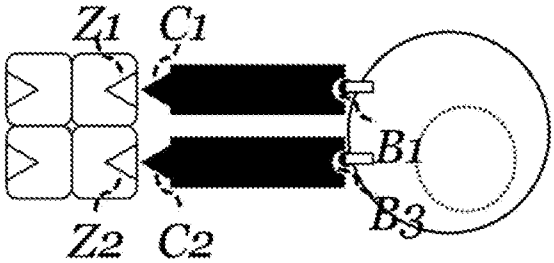


FIG. 1E

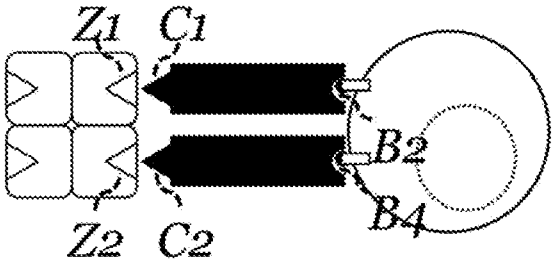


FIG. 2A

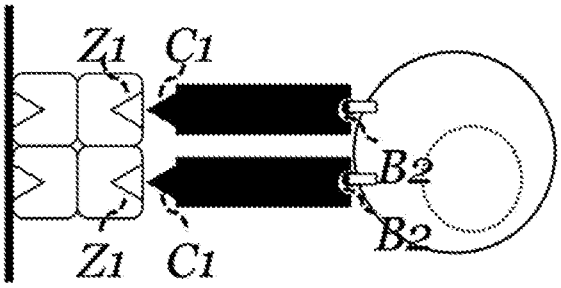


FIG. 2B

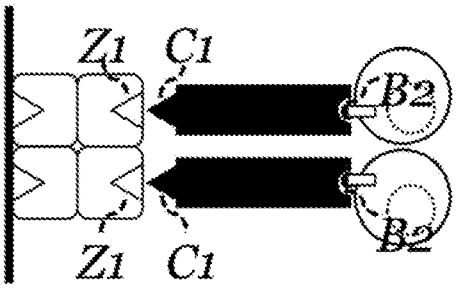


FIG. 2C

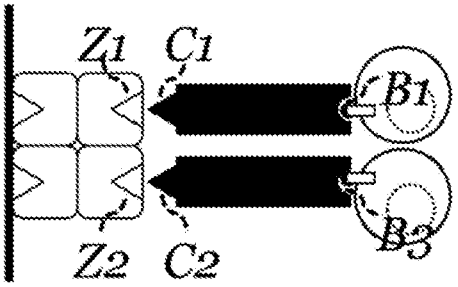


FIG. 2D

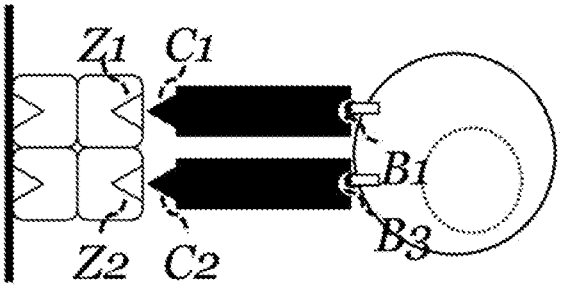


FIG. 2E

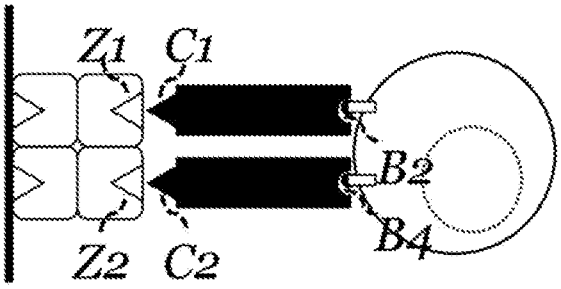


FIG. 3

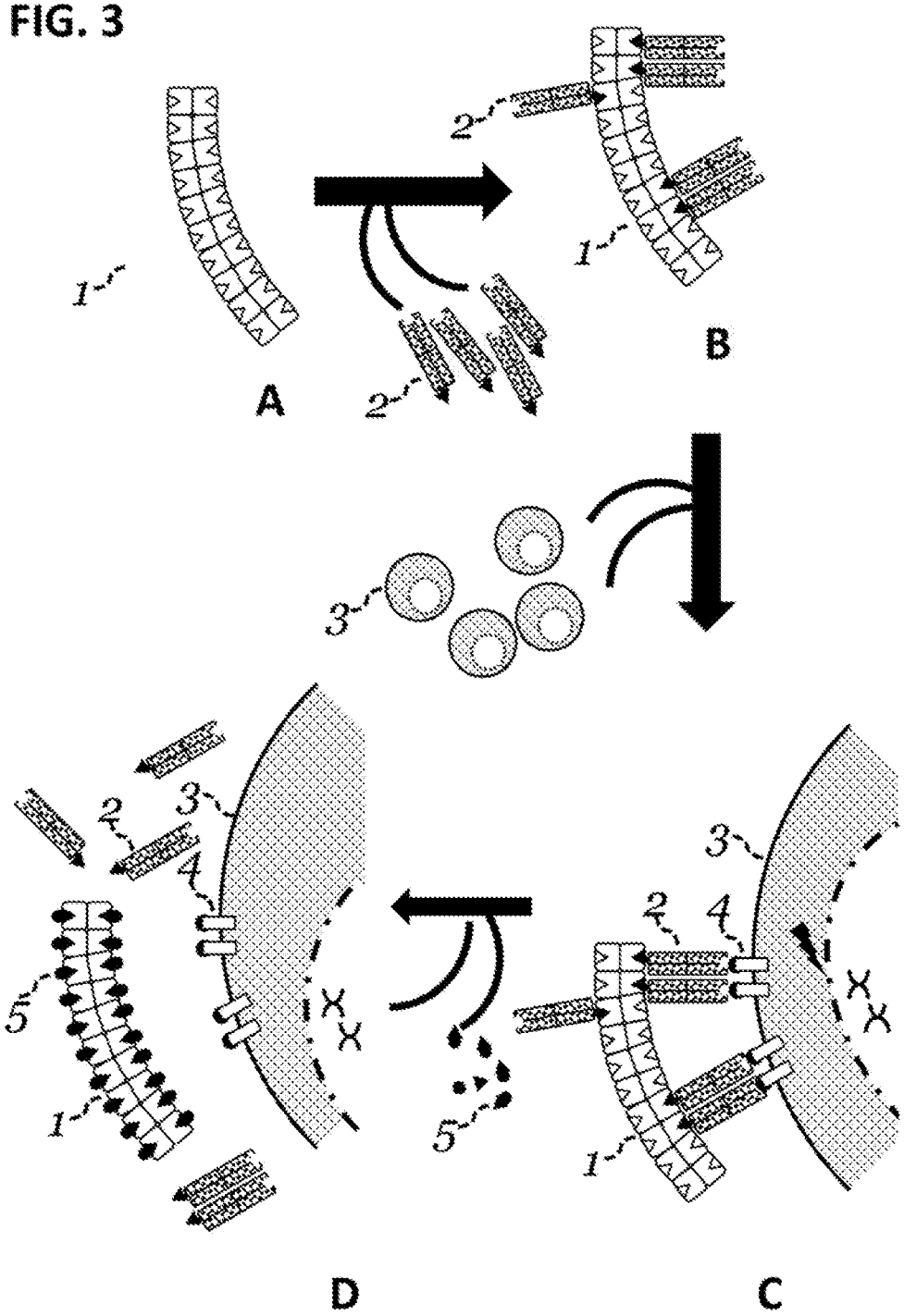


FIG. 4

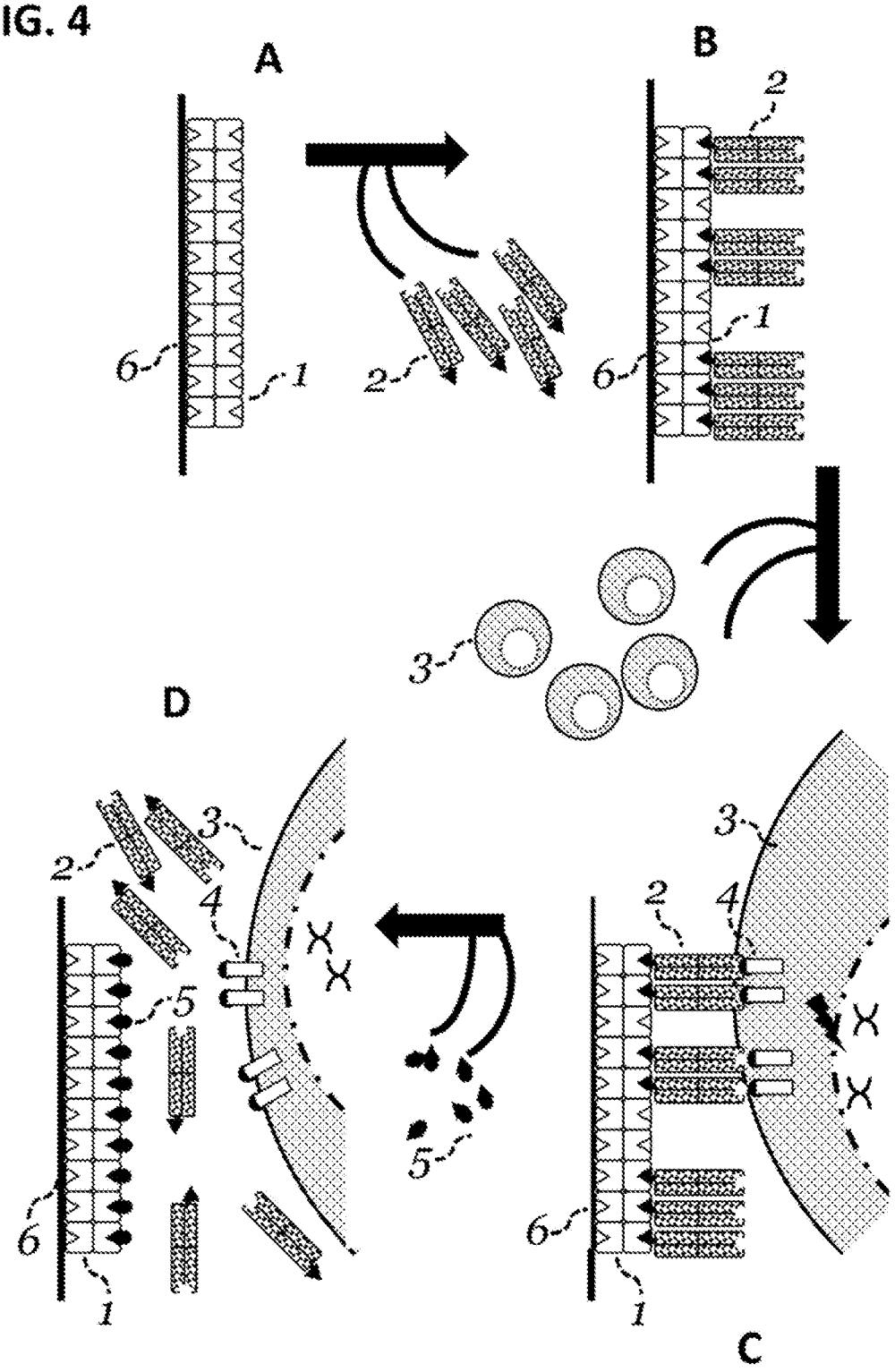


FIG. 5A

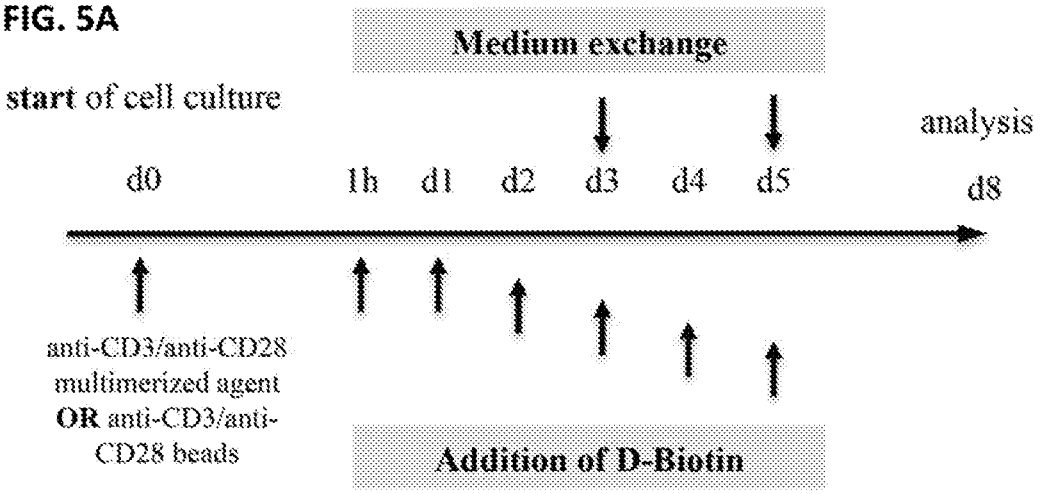


FIG. 5B

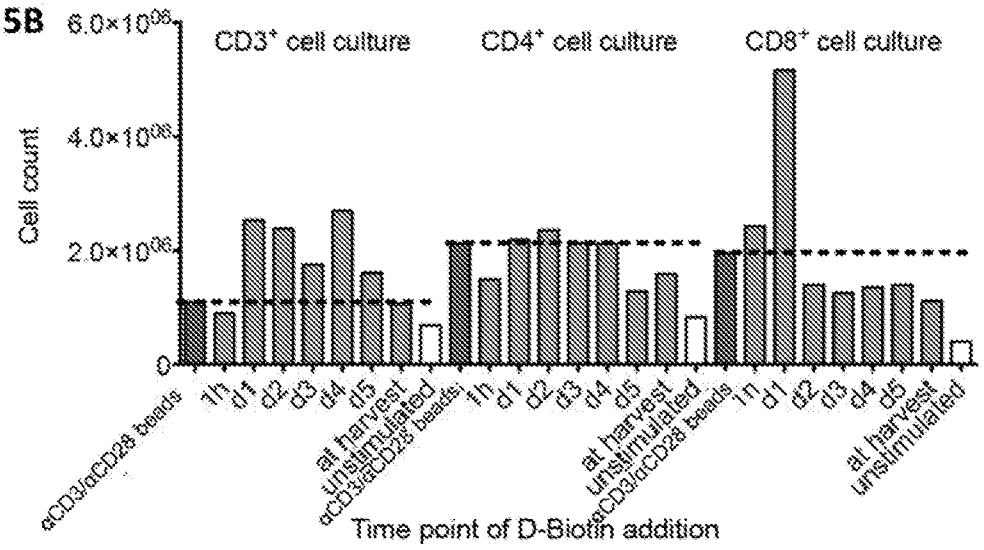


FIG. 5C

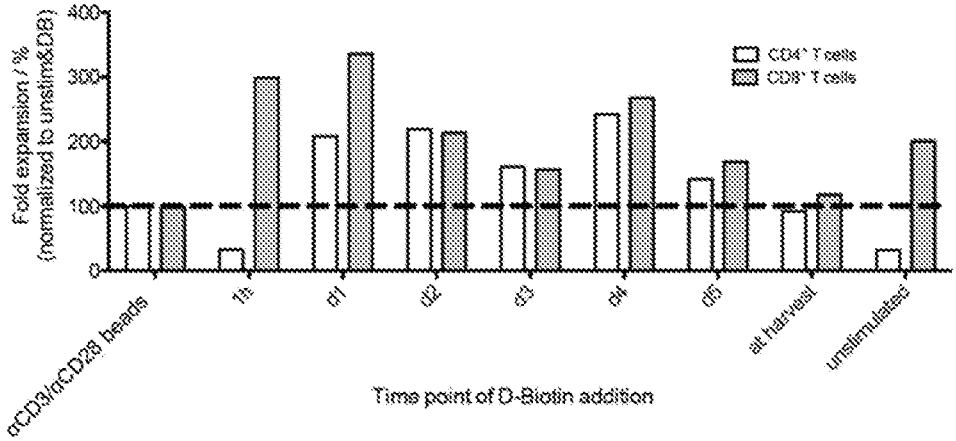


FIG. 6A

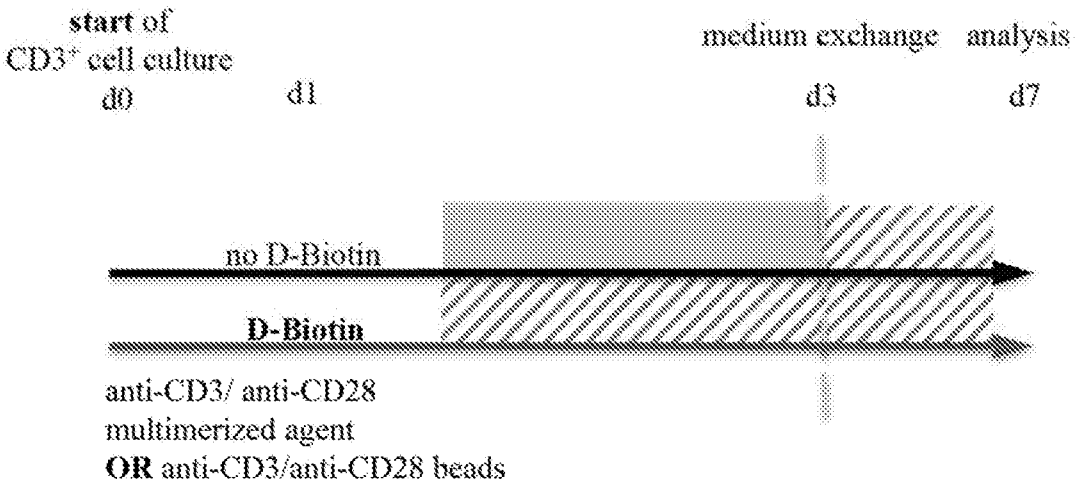

start of
CD3+ cell culture
d0
d1
medium exchange
d3
analysis
d7
no D-Biotin
D-Biotin
anti-CD3/ anti-CD28
multimerized agent
OR anti-CD3/anti-CD28 beads

FIG . 6B

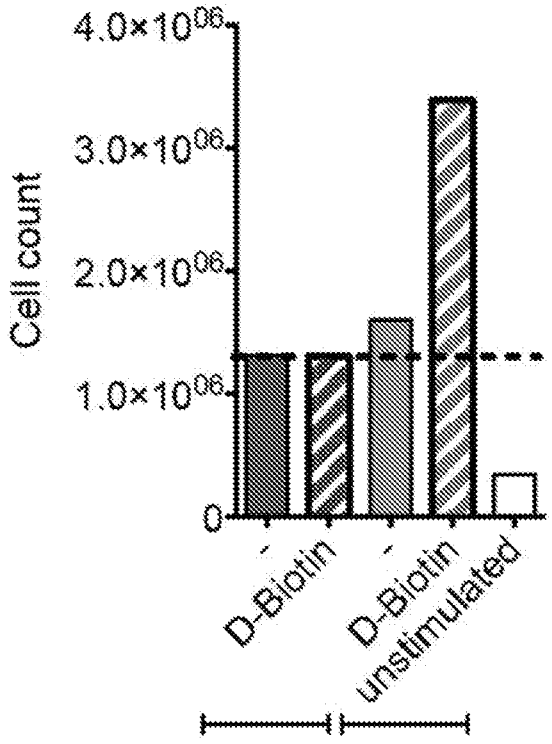

4.0×10^06
3.0×10^06
2.0×10^06
1.0×10^06
0
Cell count
-
D-Biotin
-
D-Biotin
unstimulated
Anti-CD3/anti-CD28 beads
Multimerized agent

FIG . 6C

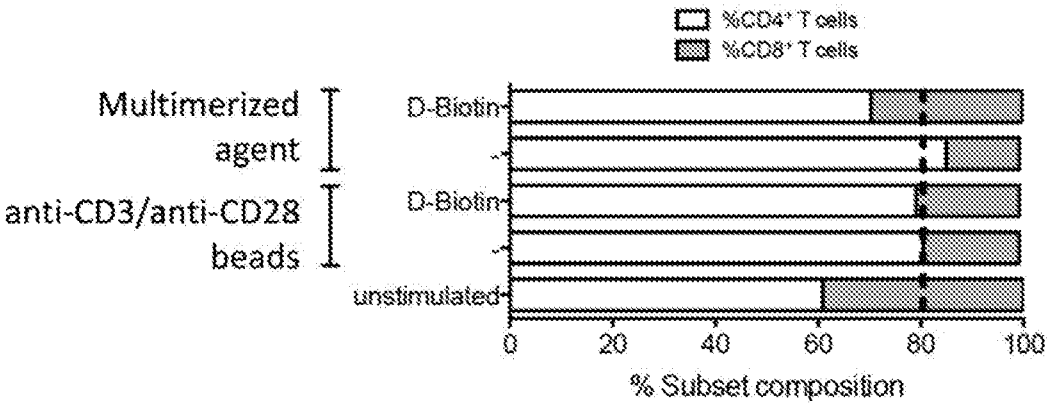

%CD4+ T cells
%CD8+ T cells
Multimerized agent
anti-CD3/anti-CD28 beads
D-Biotin
-
D-Biotin
-
unstimulated
0
20
40
60
80
100
% Subset composition

FIG . 6D

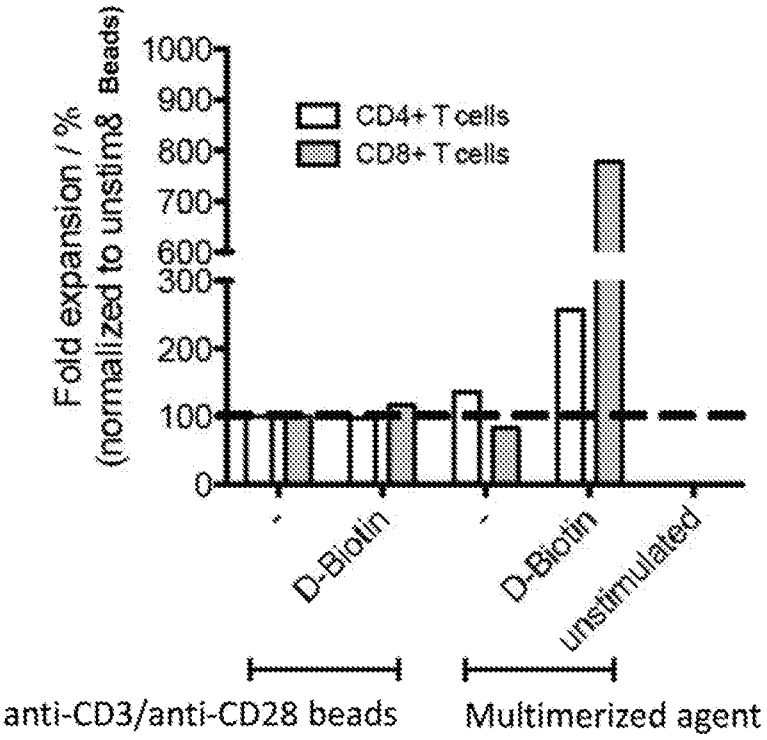

Fold expansion / % (normalized to unstim8 Beads)
1000
900
800
700
600
300
200
100
0
CD4+ T cells
CD8+ T cells
-
D-Biotin
-
D-Biotin
unstimulated
anti-CD3/anti-CD28 beads
Multimerized agent

FIG . 6E

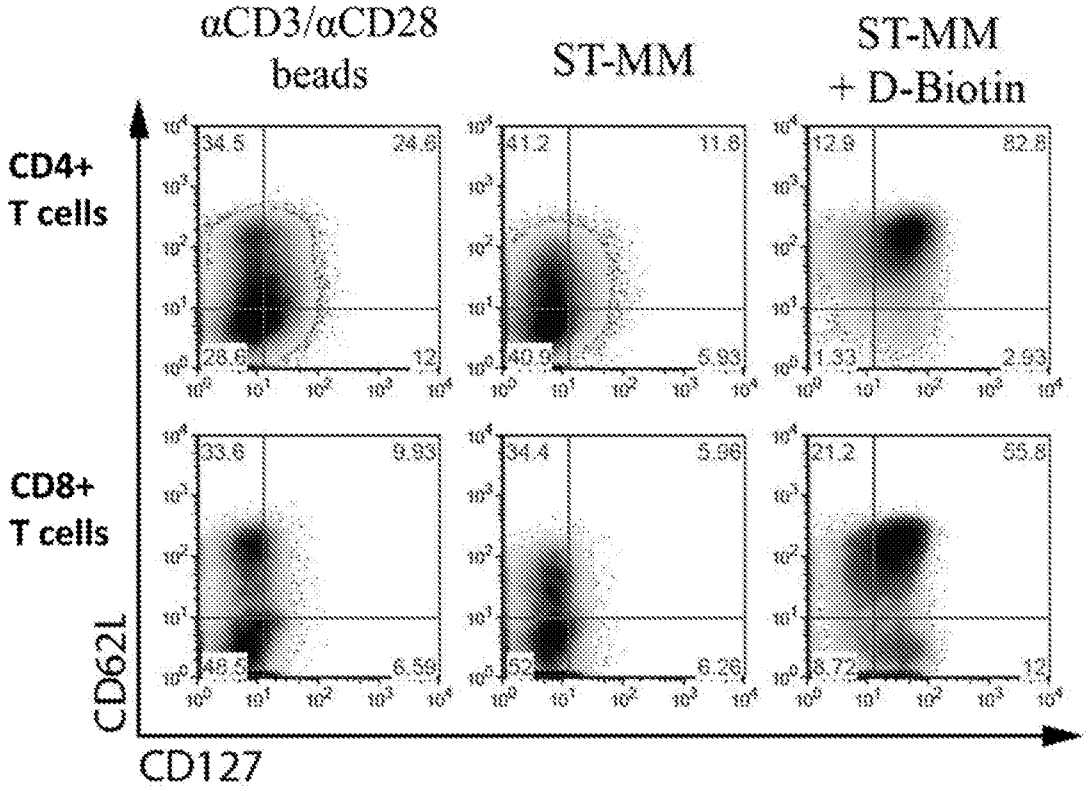

αCD3/αCD28 beads
ST-MM
ST-MM + D-Biotin
CD4+ T cells
CD8+ T cells
CD62L
CD127
34.5
24.8
28.6
12
51.2
11.6
40.9
5.93
12.9
82.8
1.33
2.93
33.6
9.93
49.5
6.56
34.4
5.86
52
6.26
21.2
55.8
8.72
12

FIG . 6F

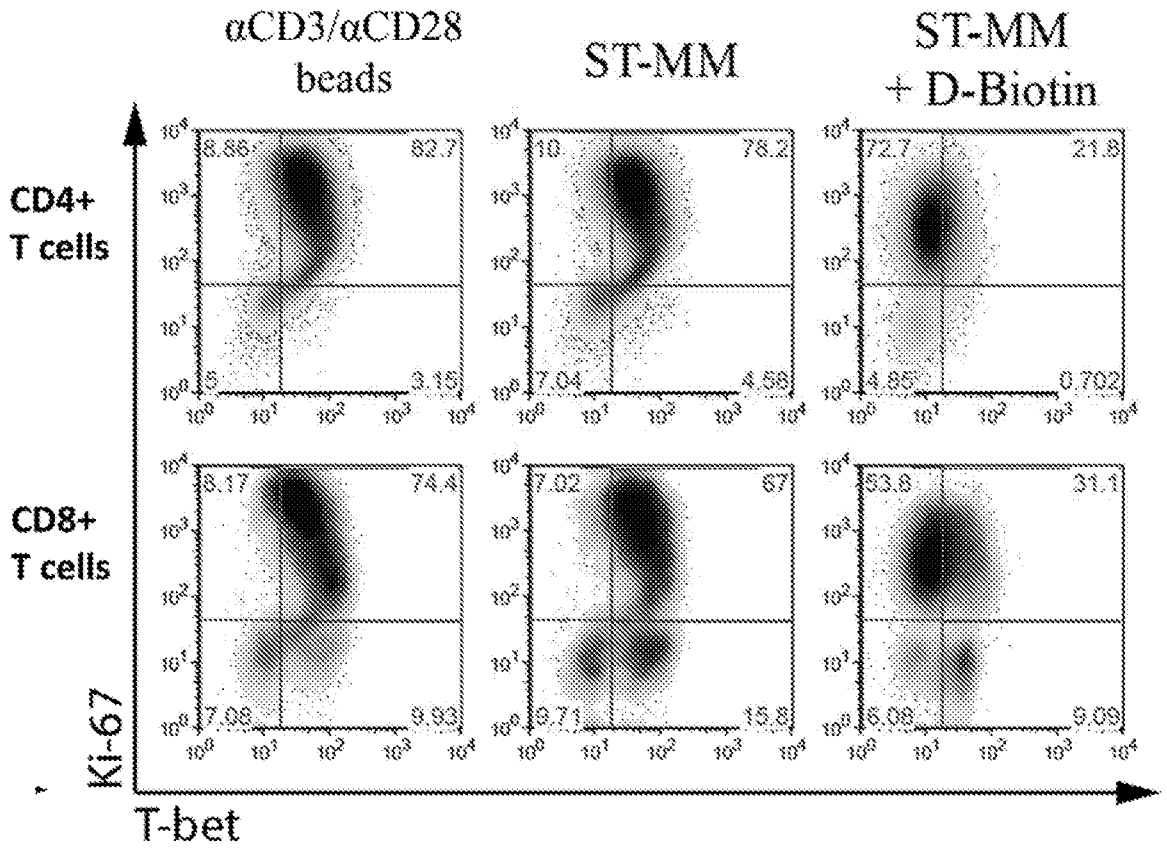

αCD3/αCD28 beads
ST-MM
ST-MM + D-Biotin
CD4+ T cells
CD8+ T cells
Ki-67
T-bet
8.86
82.7
5
3.15
10
78.2
7.04
4.58
72.7
21.8
4.85
0.702
8.17
74.4
7.08
9.93
7.02
67
9.71
15.8
53.8
31.1
6.08
9.09

FIG . 7A

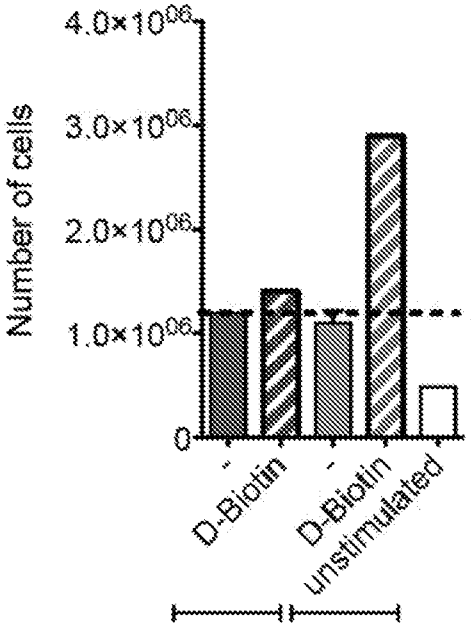

4.0×10^06
3.0×10^06
2.0×10^06
1.0×10^06
0
Number of cells
-
D-Biotin
-
D-Biotin
unstimulated
anti-CD3/anti-CD28 beads
Multimerized agent

FIG . 7B

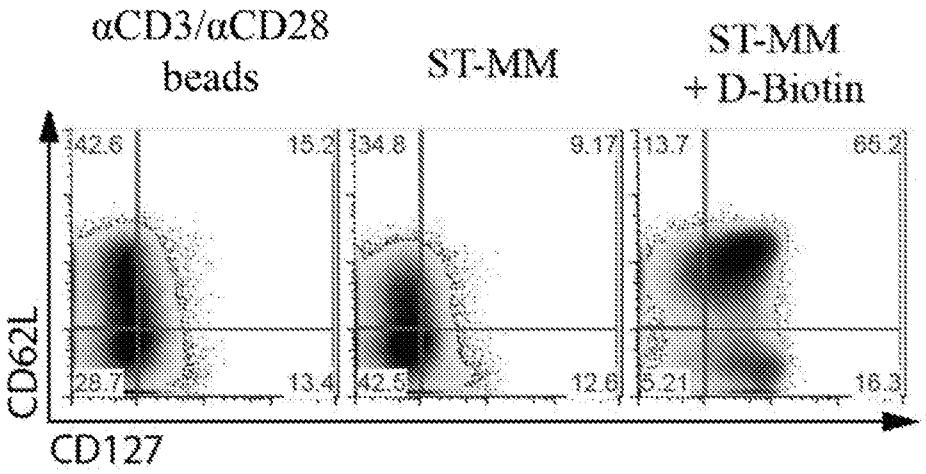

αCD3/αCD28
beads
ST-MM
ST-MM
+ D-Biotin
42.6
15.2
28.7
13.4
34.8
9.17
42.5
12.6
13.7
65.2
5.21
16.3
CD62L
CD127

FIG . 7C

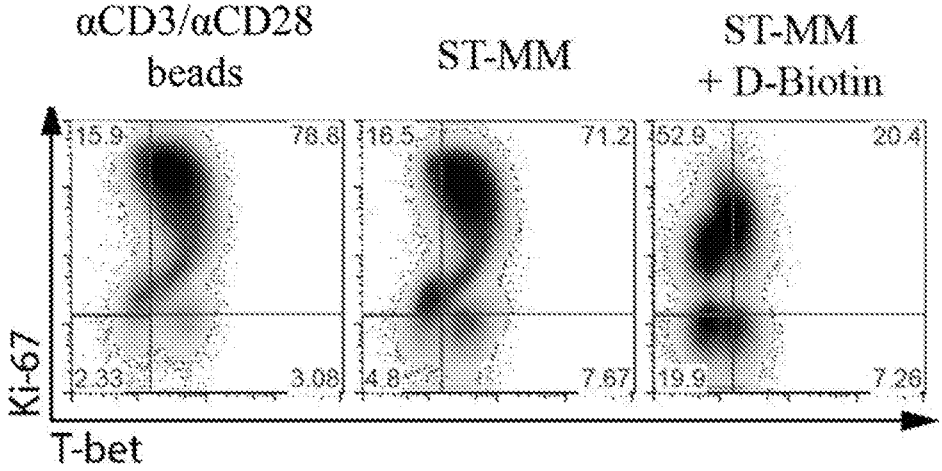

αCD3/αCD28
beads
ST-MM
ST-MM
+ D-Biotin
15.9
78.6
2.33
3.08
16.5
71.2
4.8
7.67
52.9
20.4
19.9
7.26
Ki-67
T-bet

FIG . 8A

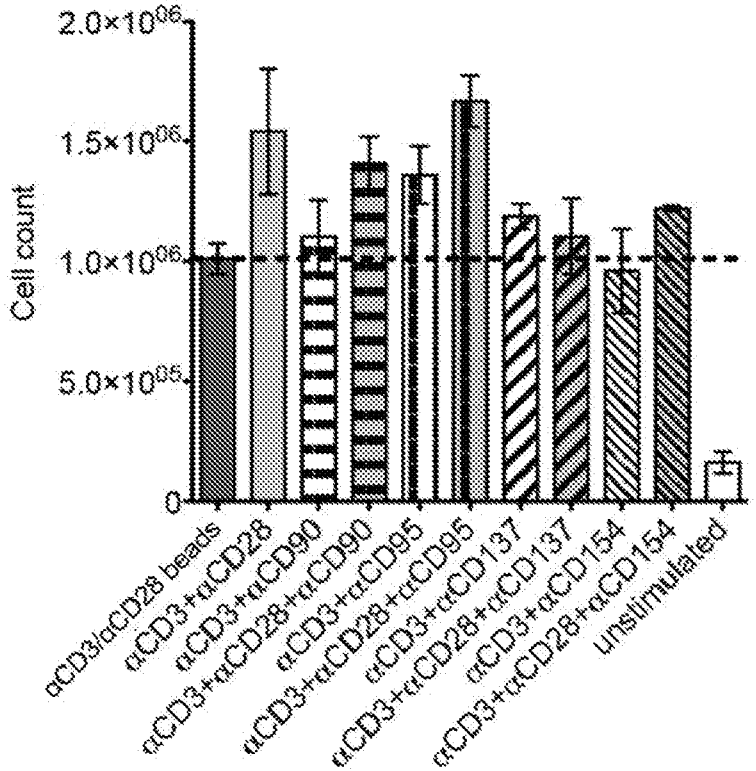

2.0×10^06
1.5×10^06
1.0×10^06
5.0×10^05
0
Cell count
αCD3/αCD28 beads
αCD3+αCD28
αCD3+αCD90
αCD3+αCD28+αCD90
αCD3+αCD95
αCD3+αCD28+αCD95
αCD3+αCD137
αCD3+αCD28+αCD137
αCD3+αCD154
αCD3+αCD28+αCD154
unstimulated

FIG . 8B

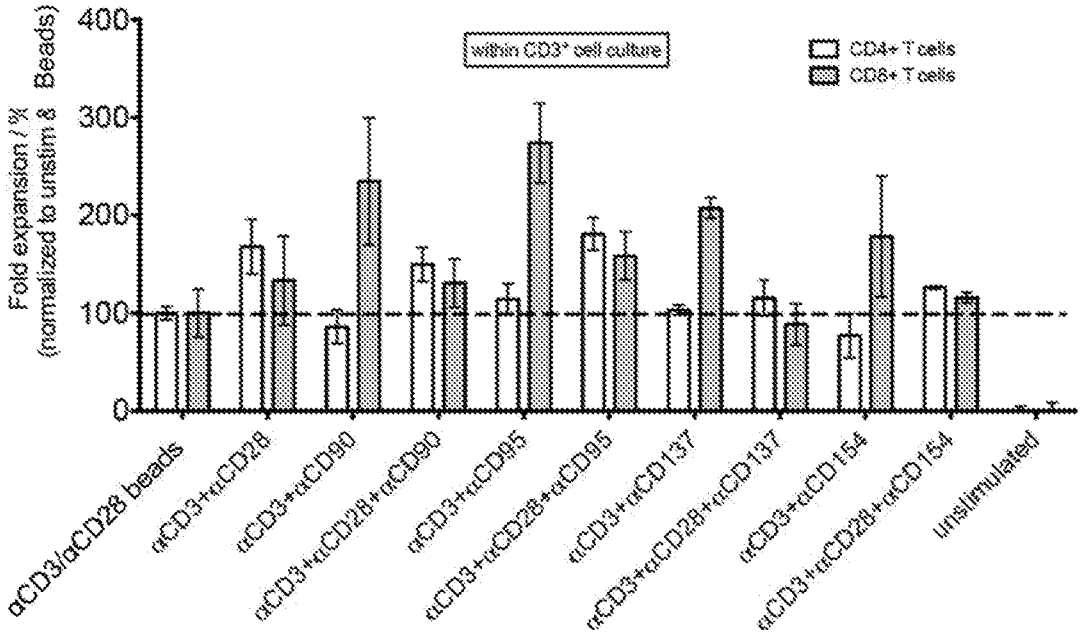

400
300
200
100
0
Fold expansion / % (normalized to unstim & Beads)
within CD3+ cell culture
CD4+ T cells
CD8+ T cells
αCD3/αCD28 beads
αCD3+αCD28
αCD3+αCD90
αCD3+αCD28+αCD90
αCD3+αCD95
αCD3+αCD28+αCD95
αCD3+αCD137
αCD3+αCD28+αCD137
αCD3+αCD154
αCD3+αCD28+αCD154
unstimulated

FIG . 8C

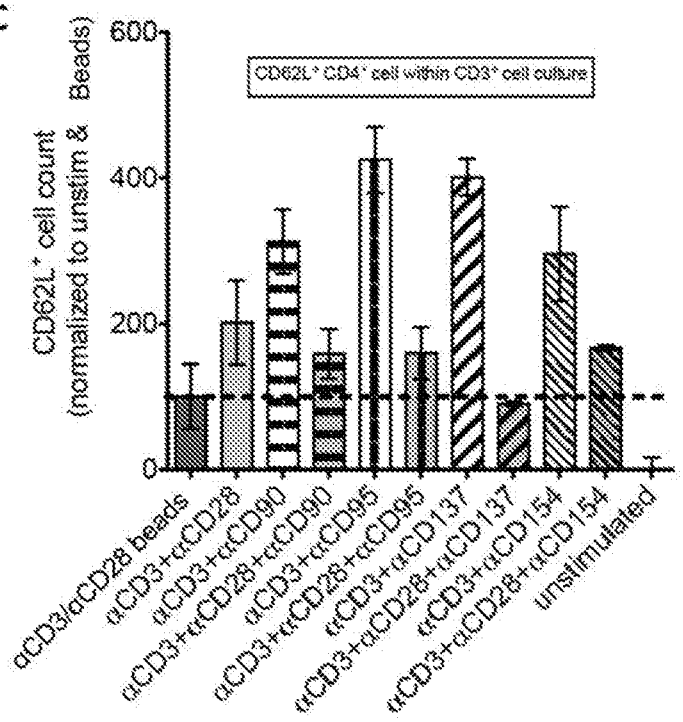

CD62L+ CD4+ cell within CD3+ cell culture
CD62L+ cell count (normalized to unstim & Beads)
600
400
200
0
αCD3/αCD28 beads
αCD3+αCD28
αCD3+αCD90
αCD28+αCD90
αCD3+αCD95
αCD28+αCD95
αCD3+αCD137
αCD28+αCD137
αCD3+αCD154
αCD28+αCD154
unstimulated

FIG . 8D

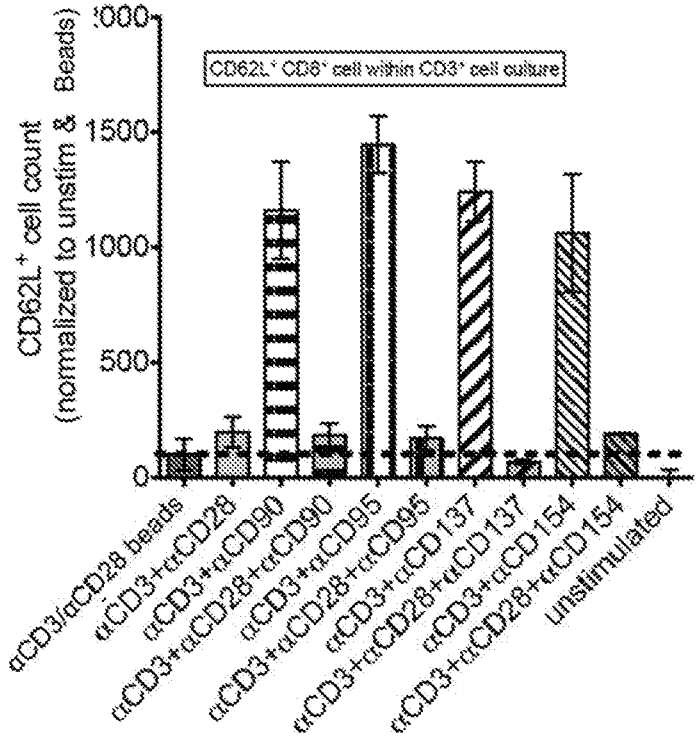

CD62L+ CD8+ cell within CD3+ cell culture
CD62L+ cell count (normalized to unstim & Beads)
2000
1500
1000
500
0
αCD3/αCD28 beads
αCD3+αCD28
αCD3+αCD90
αCD28+αCD90
αCD3+αCD95
αCD28+αCD95
αCD3+αCD137
αCD28+αCD137
αCD3+αCD154
αCD28+αCD154
unstimulated

FIG . 8E

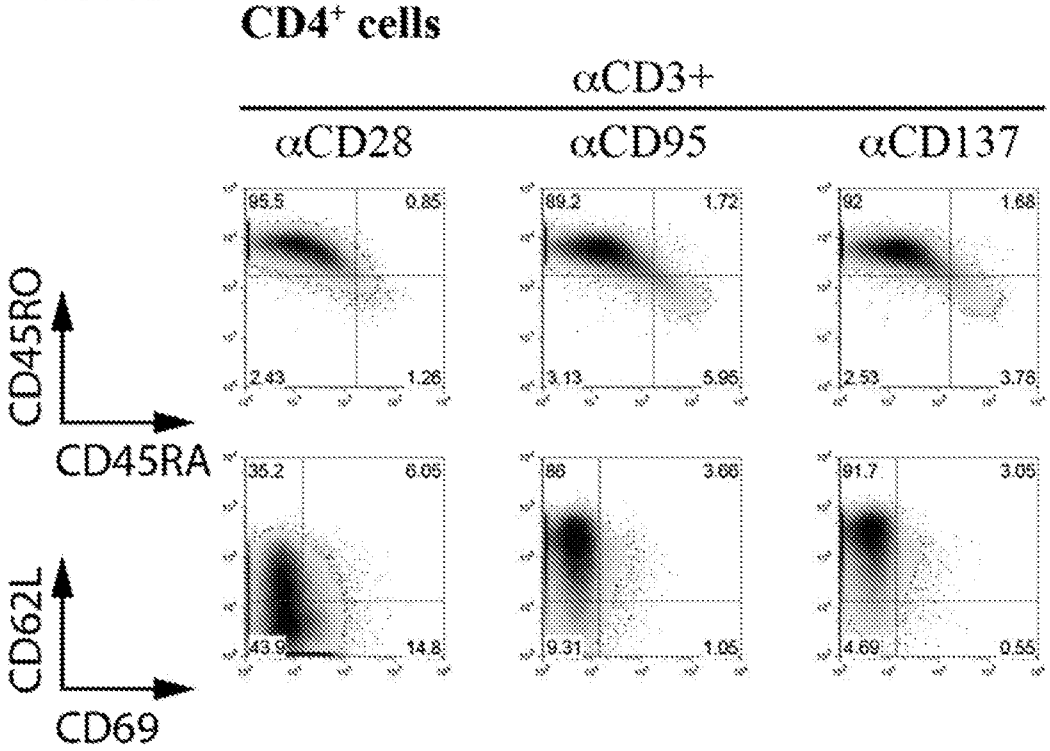

CD4+ cells
αCD3+
αCD28
αCD95
αCD137
CD45RO
CD45RA
CD62L
CD69
96.5
0.85
2.43
1.26
89.2
1.72
3.13
5.96
92
1.68
2.53
3.76
35.2
6.05
43.9
14.8
88
3.66
9.31
1.05
91.7
3.05
4.69
0.55

FIG . 8F

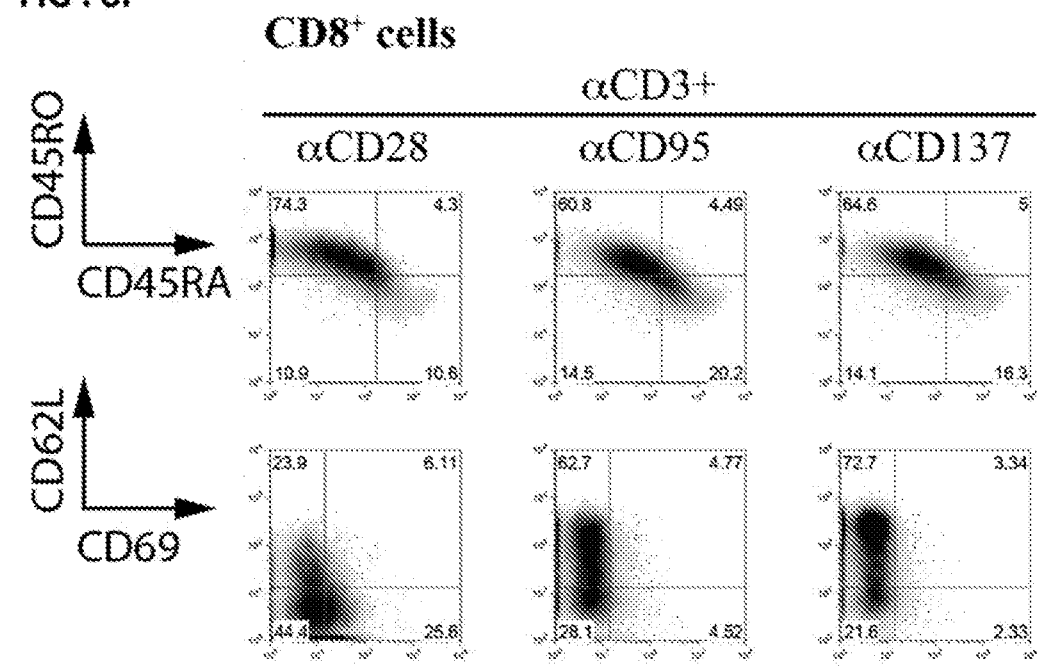

CD8+ cells
αCD3+
αCD28
αCD95
αCD137
CD45RO
CD45RA
CD62L
CD69
74.3
4.3
10.9
10.6
60.8
4.49
14.5
20.2
84.6
5
14.1
16.3
23.9
6.11
44.4
25.6
82.7
4.77
28.1
4.52
73.7
3.34
21.6
2.33

FIG . 9A

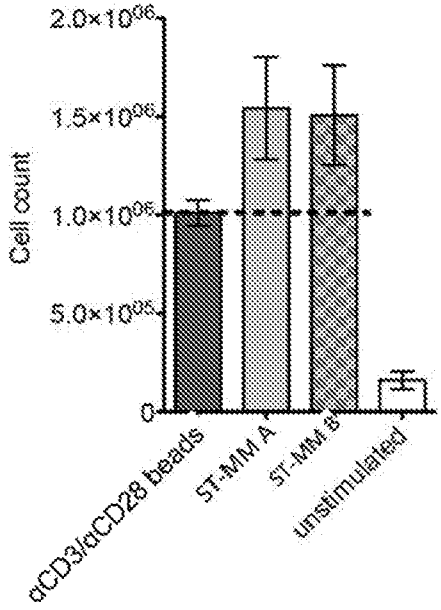

2.0×10^06
1.5×10^06
1.0×10^06
5.0×10^05
0
Cell count
αCD3/αCD28 beads
ST-MM A
ST-MM B
unstimulated

FIG . 9B

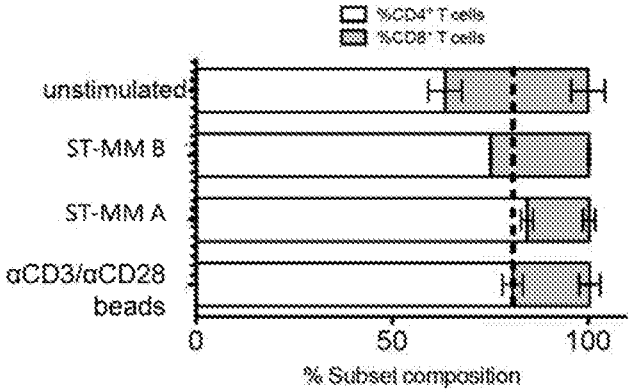

%CD4+ T cells
%CD8+ T cells
unstimulated
ST-MM B
ST-MM A
αCD3/αCD28 beads
0
50
100
% Subset composition

FIG . 9C

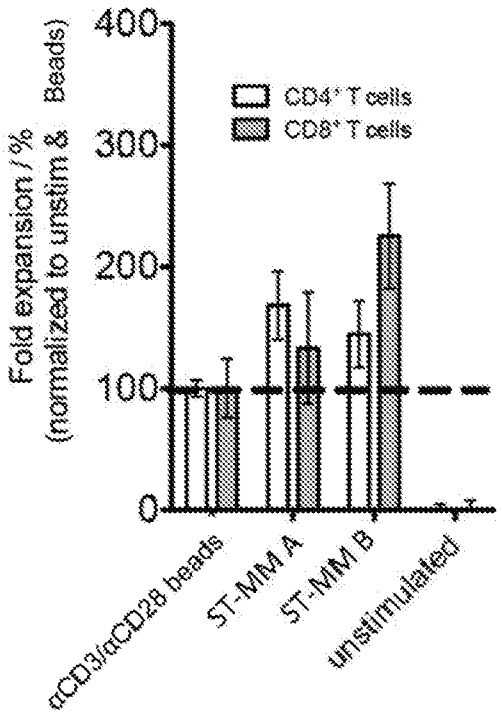

Fold expansion / % (normalized to unstim & Beads)
400
300
200
100
0
CD4+ T cells
CD8+ T cells
αCD3/αCD28 beads
ST-MM A
ST-MM B
unstimulated

FIG . 9D

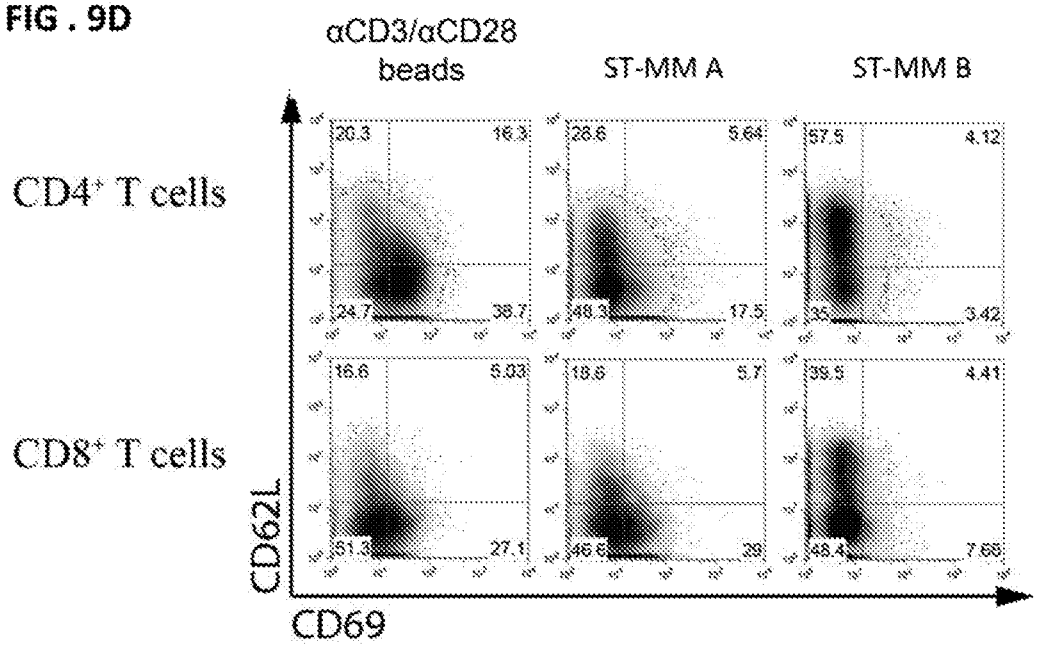

αCD3/αCD28 beads
ST-MM A
ST-MM B
CD4+ T cells
CD8+ T cells
CD62L
CD69
20.3
16.3
24.7
38.7
28.6
5.64
48.3
17.5
57.5
4.12
35
3.42
16.6
5.03
51.3
27.1
18.6
5.7
46.6
29
39.5
4.41
48.4
7.68

FIG . 10A

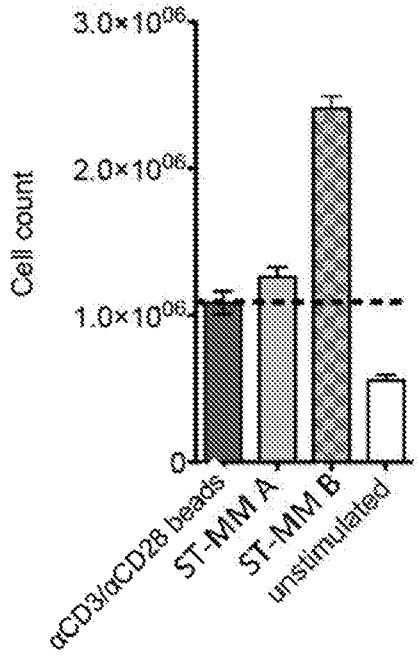


FIG . 10B

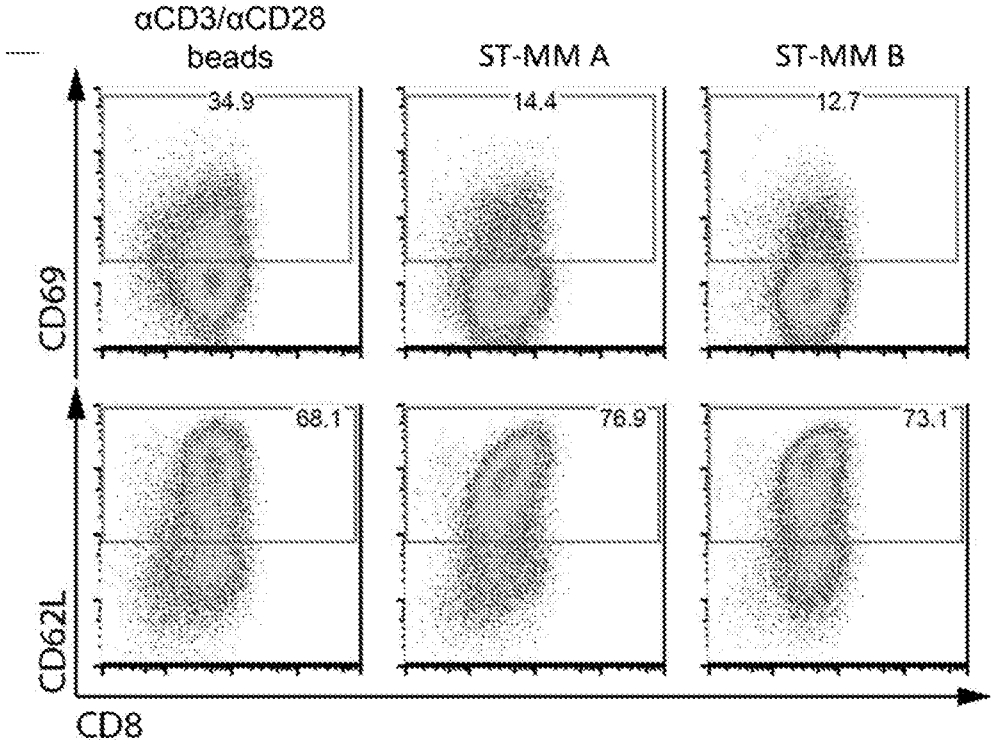
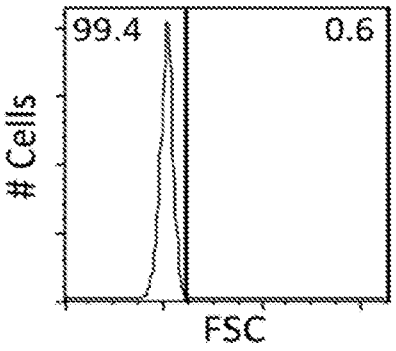
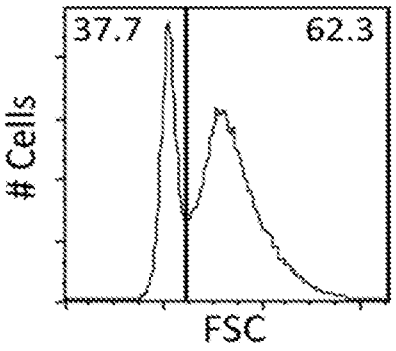


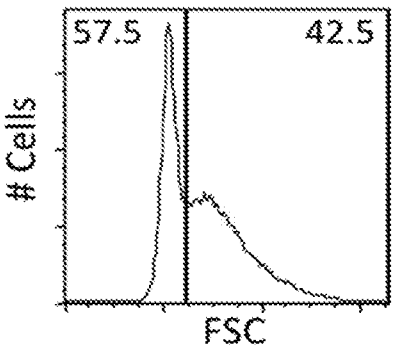
FIG. 11A



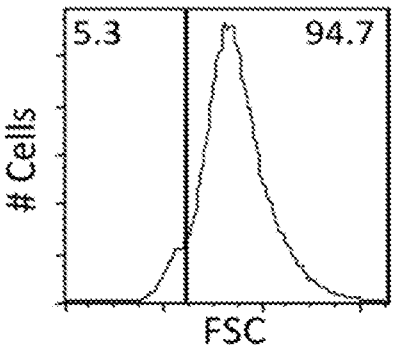
beads only



beads 0.5µg αCD3 Fab



beads 0.5µg αCD28 Fab



beads 0.5µg αCD3 +
0.5µg αCD28 Fab

FIG. 11B

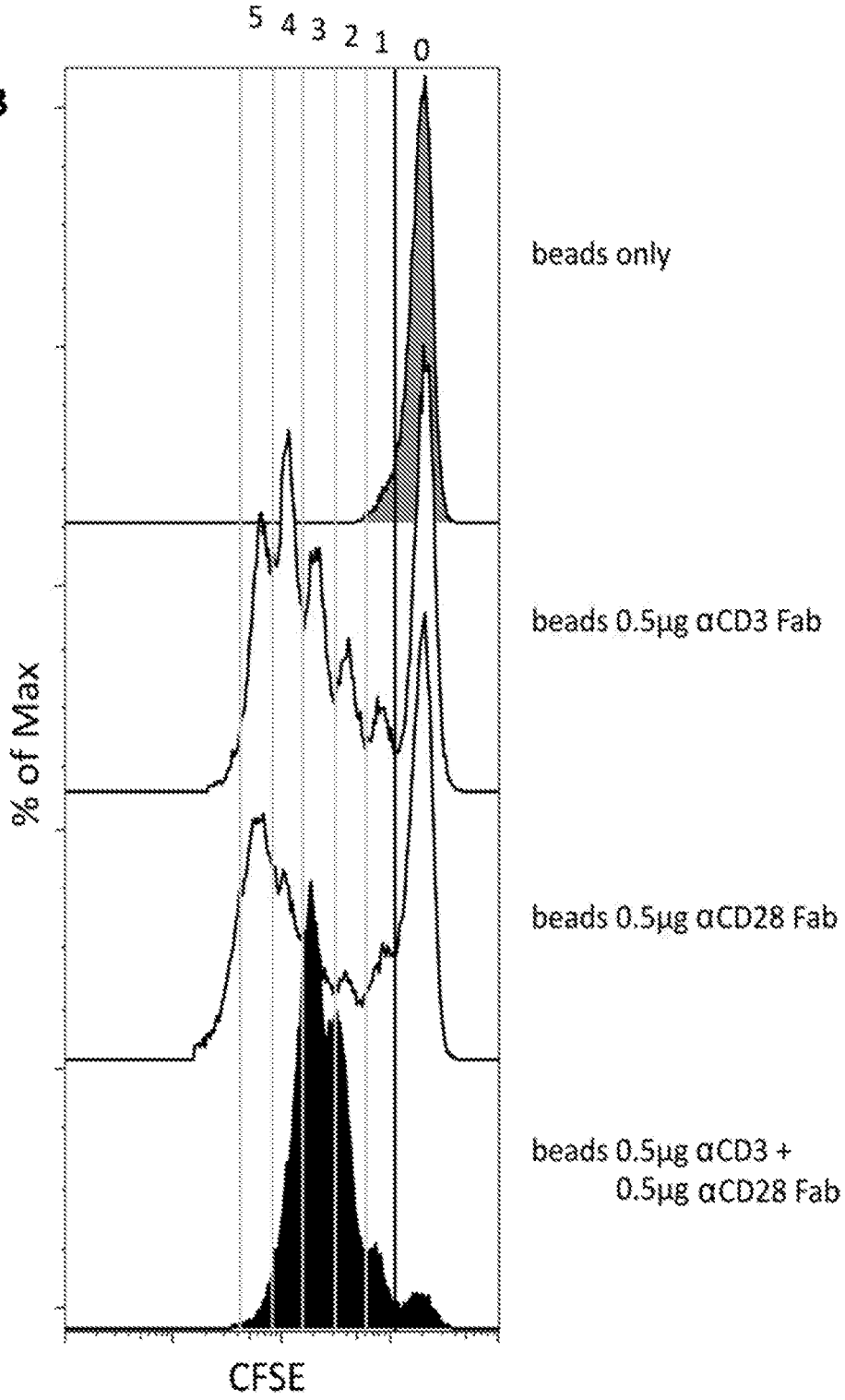

5 4 3 2 1 0
% of Max
CFSE
beads only
beads 0.5µg αCD3 Fab
beads 0.5µg αCD28 Fab
beads 0.5µg αCD3 +
0.5µg αCD28 Fab

FIG. 11C

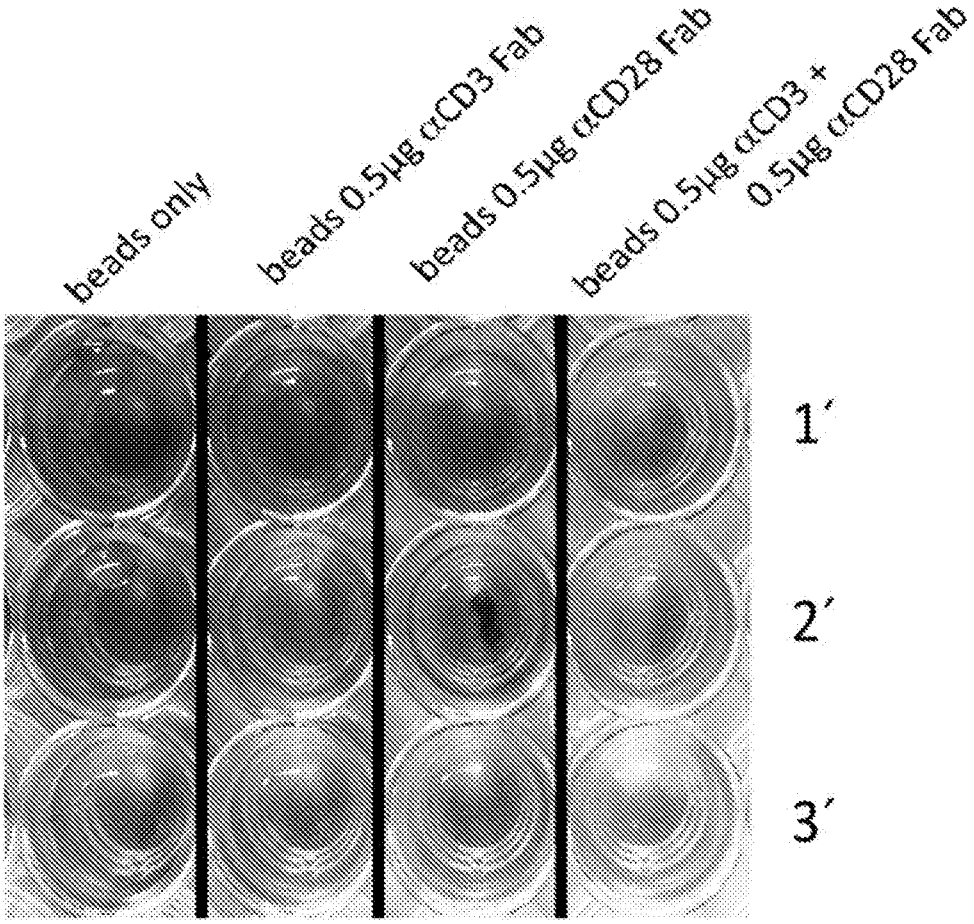

beads only
beads 0.5µg αCD3 Fab
beads 0.5µg αCD28 Fab
beads 0.5µg αCD3 + 0.5µg αCD28 Fab
1´
2´
3´

FIG. 12A

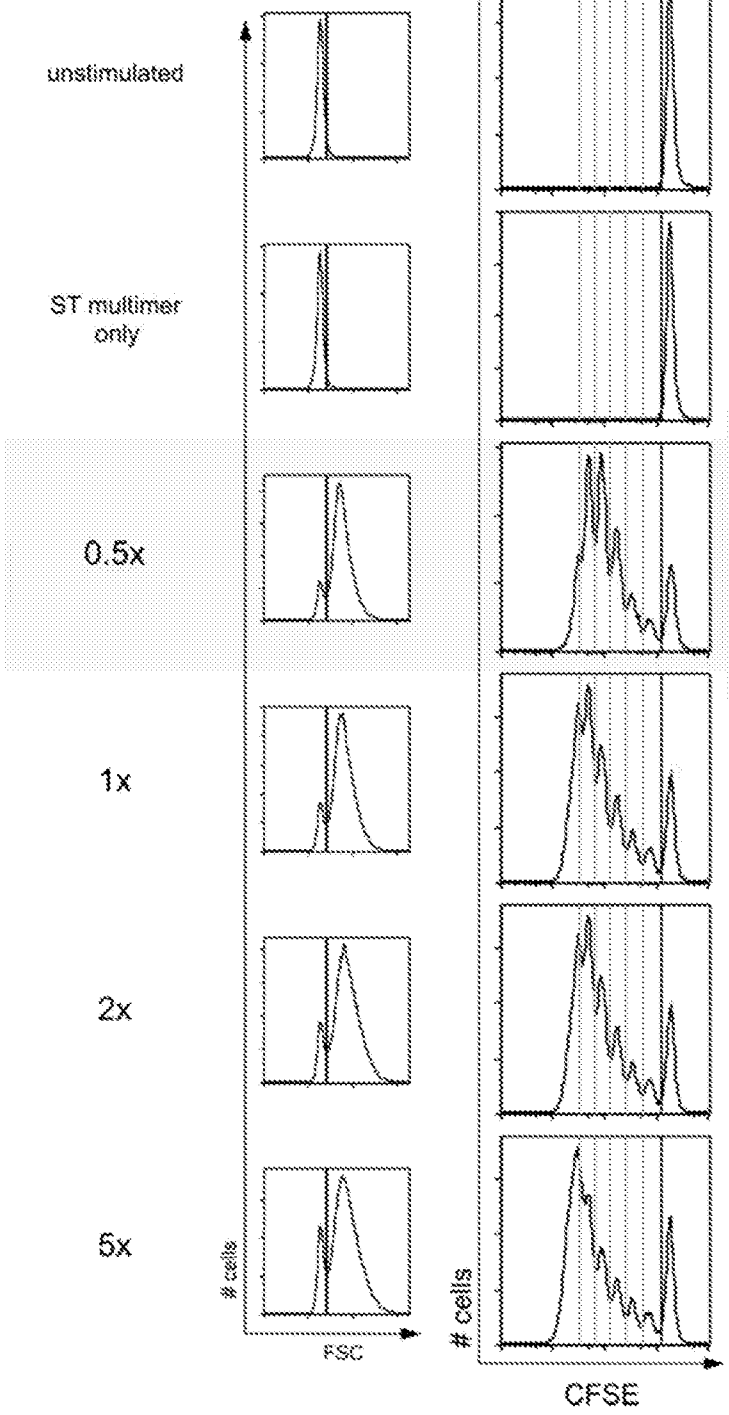


FIG. 12B

FIG. 12C

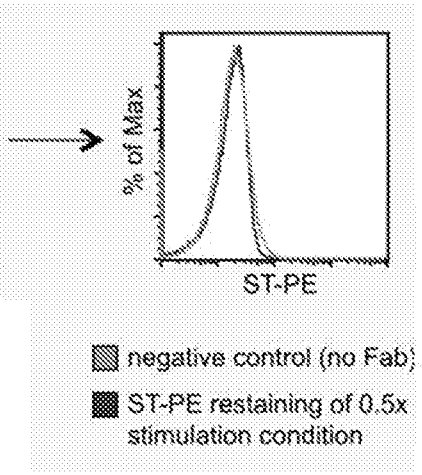


FIG. 12D

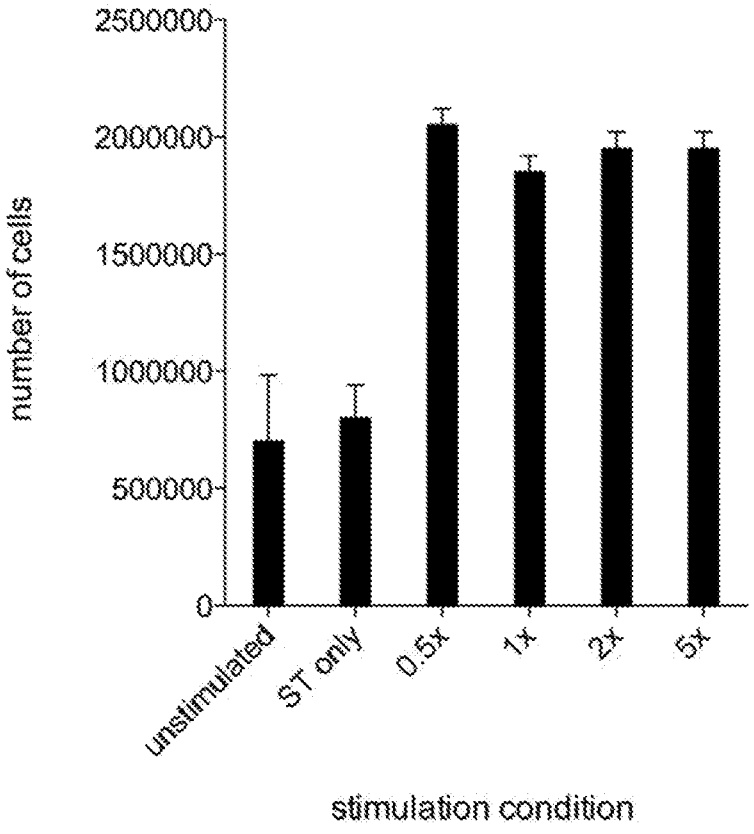

2500000
2000000
1500000
1000000
500000
0
number of cells
unstimulated
ST only
0.5x
1x
2x
5x
stimulation condition

FIG. 12E



FIG. 13A

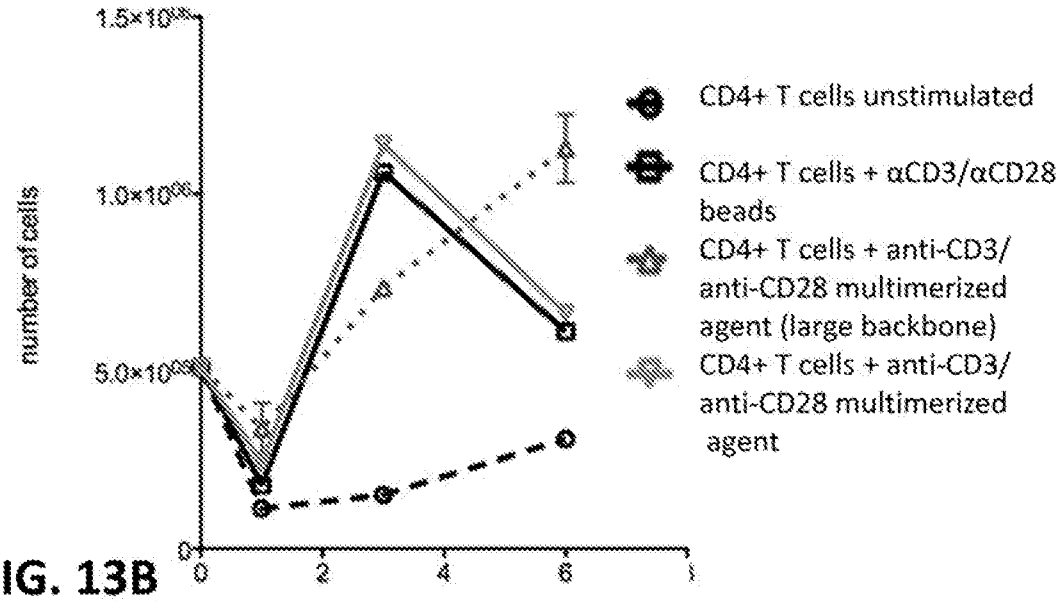


FIG. 13B

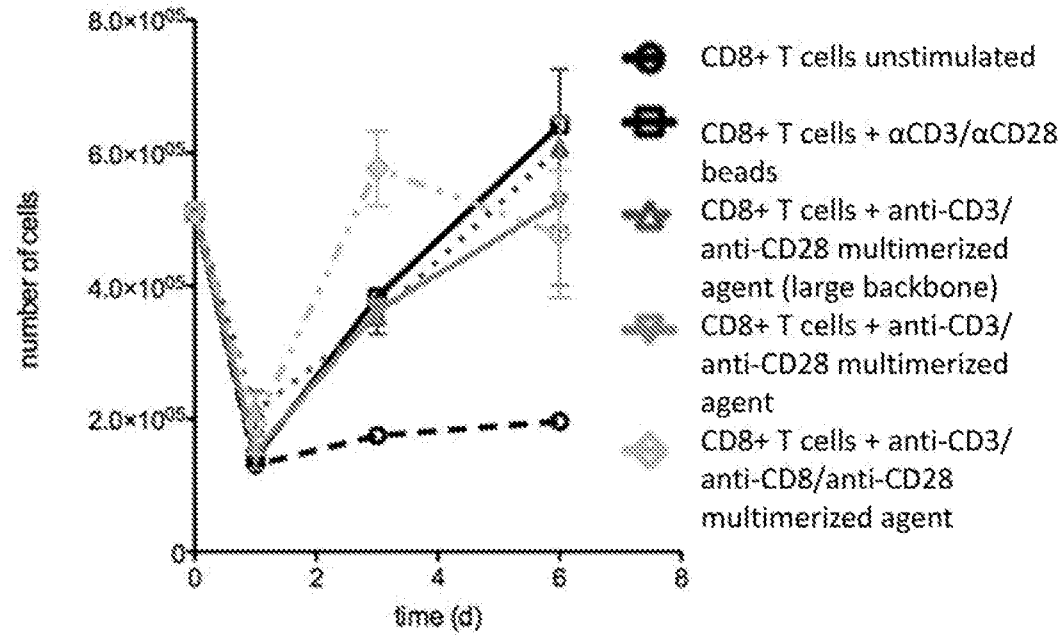


FIG. 14A

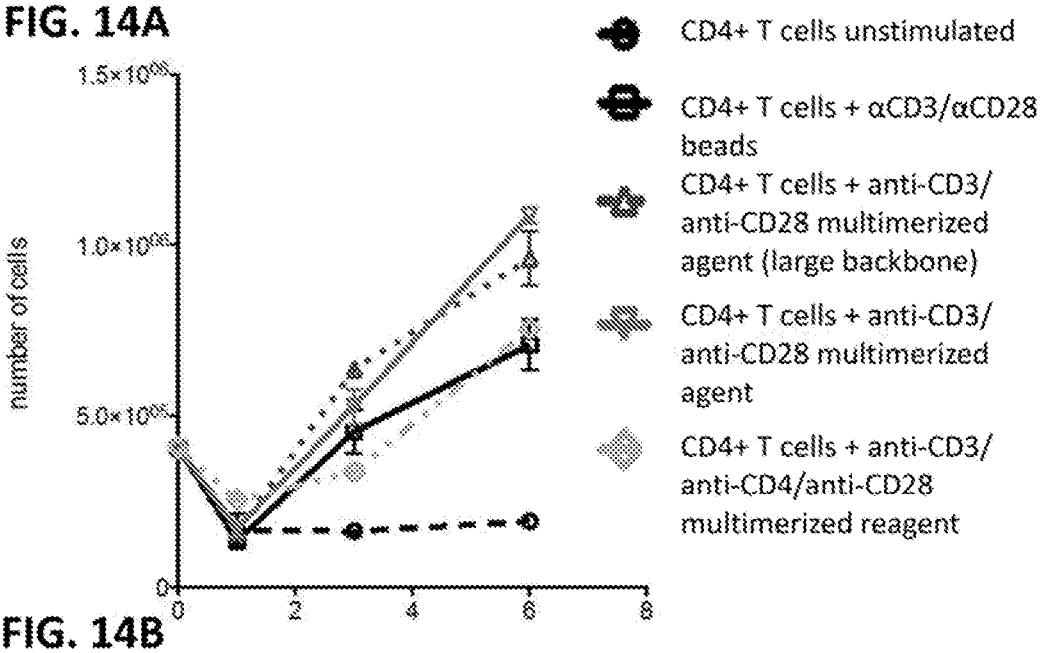


FIG. 14B

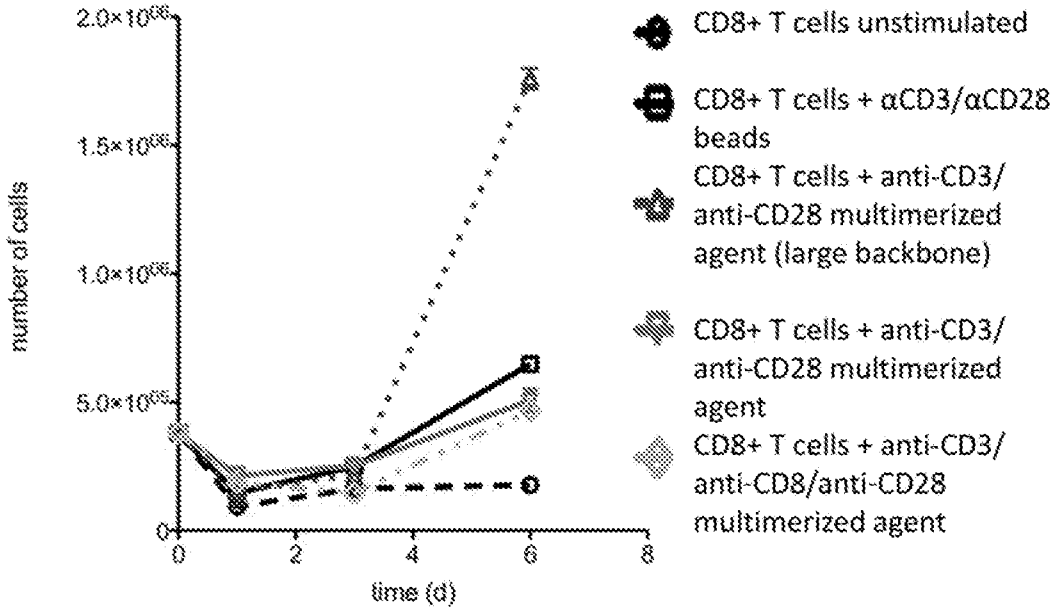


FIG. 15A

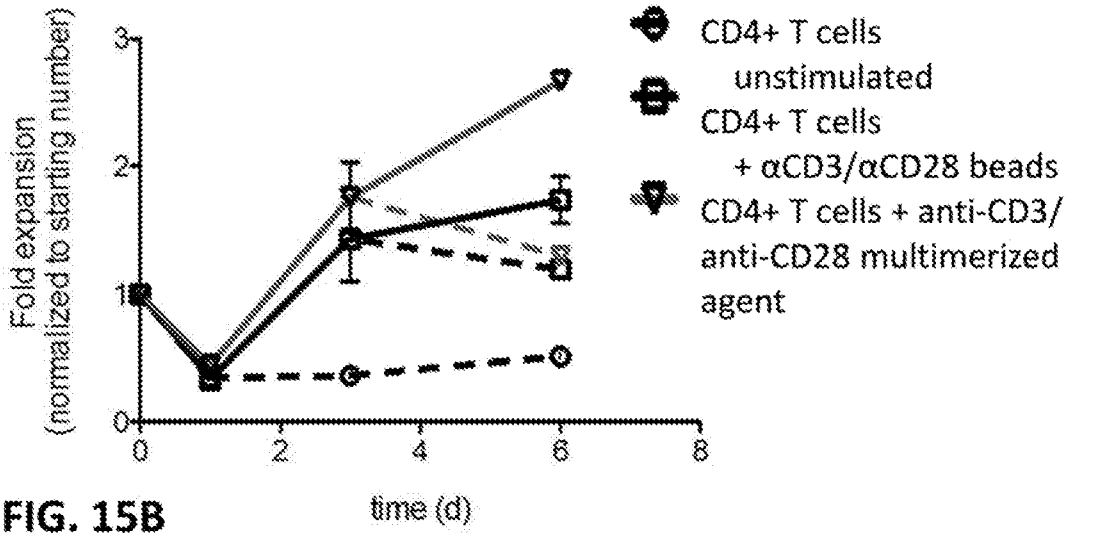


FIG. 15B

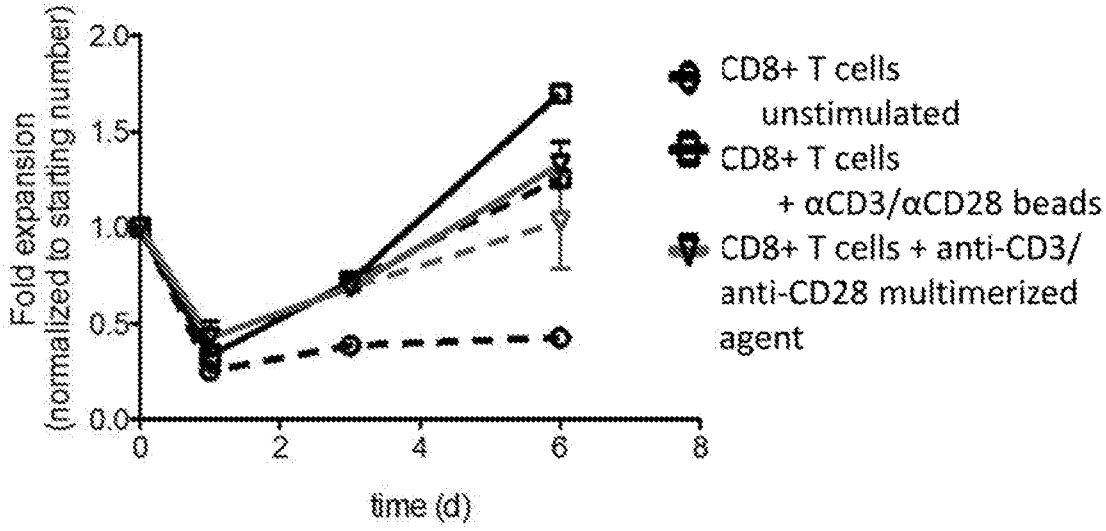


FIG. 16A

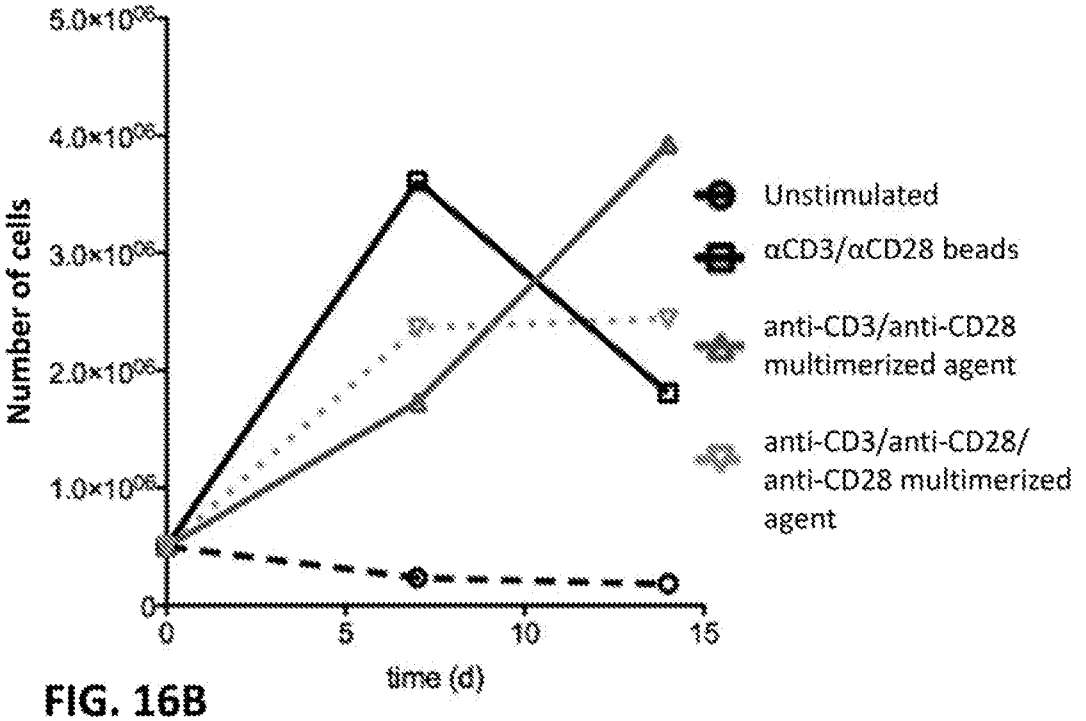


FIG. 16B

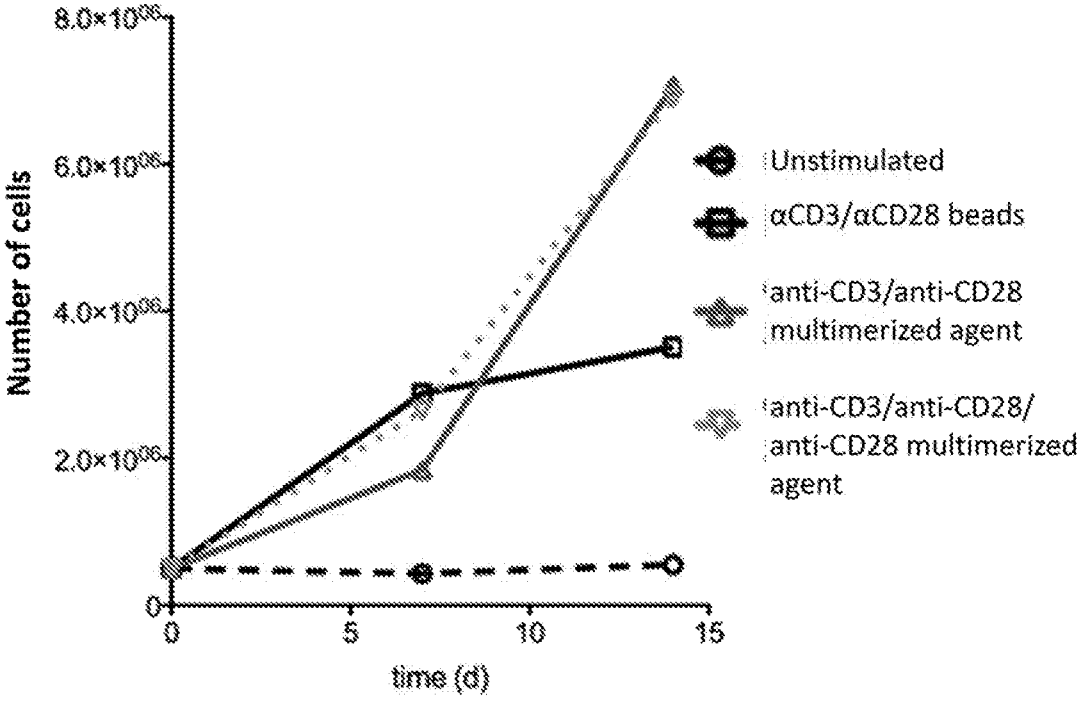


FIG. 16C

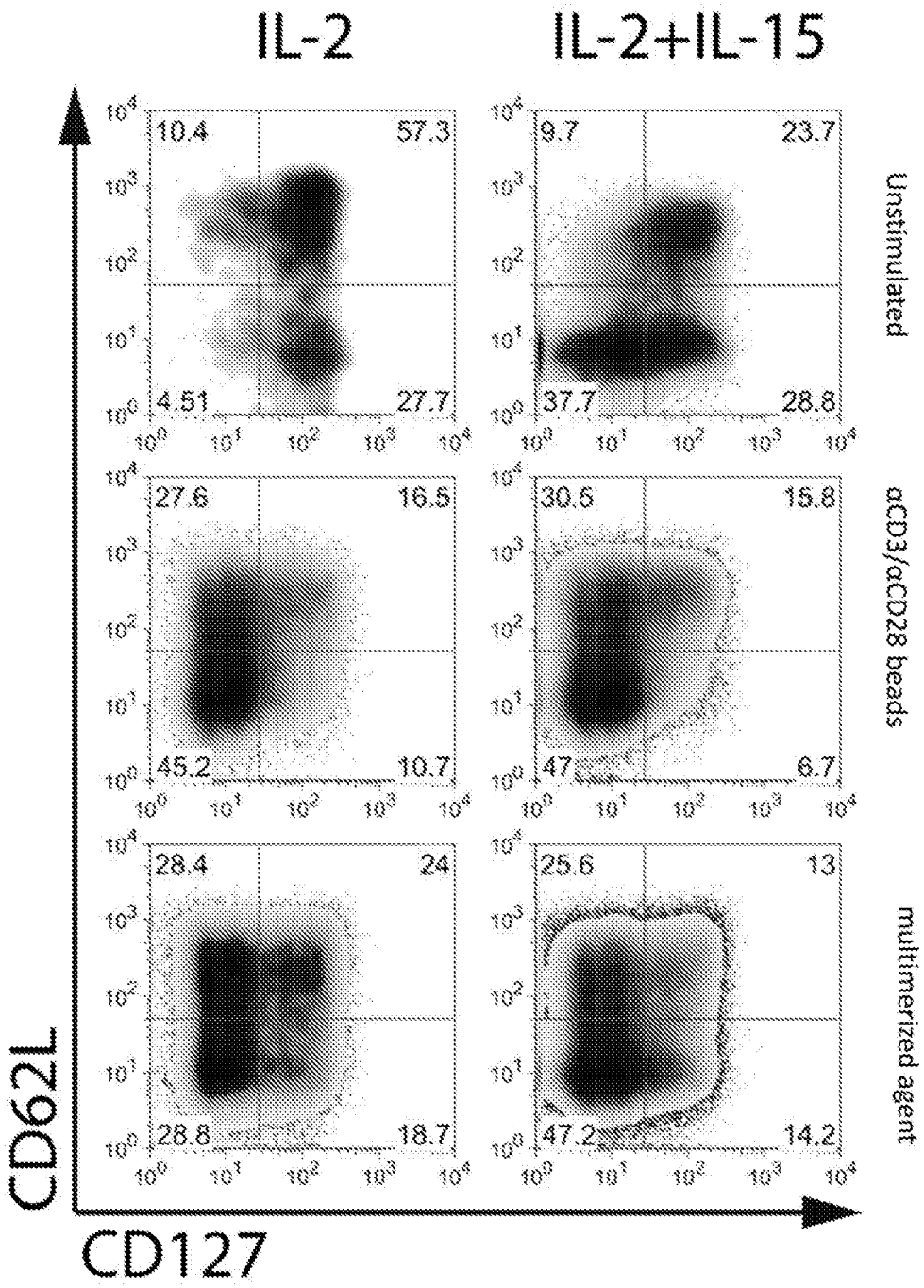

IL-2
IL-2+IL-15
Unstimulated
αCD3/αCD28 beads
multimerized agent
CD62L
CD127
10.4
57.3
4.51
27.7
9.7
23.7
37.7
28.8
27.6
16.5
45.2
10.7
30.5
15.8
47
6.7
28.4
24
28.8
18.7
25.6
13
47.2
14.2

FIG. 17A

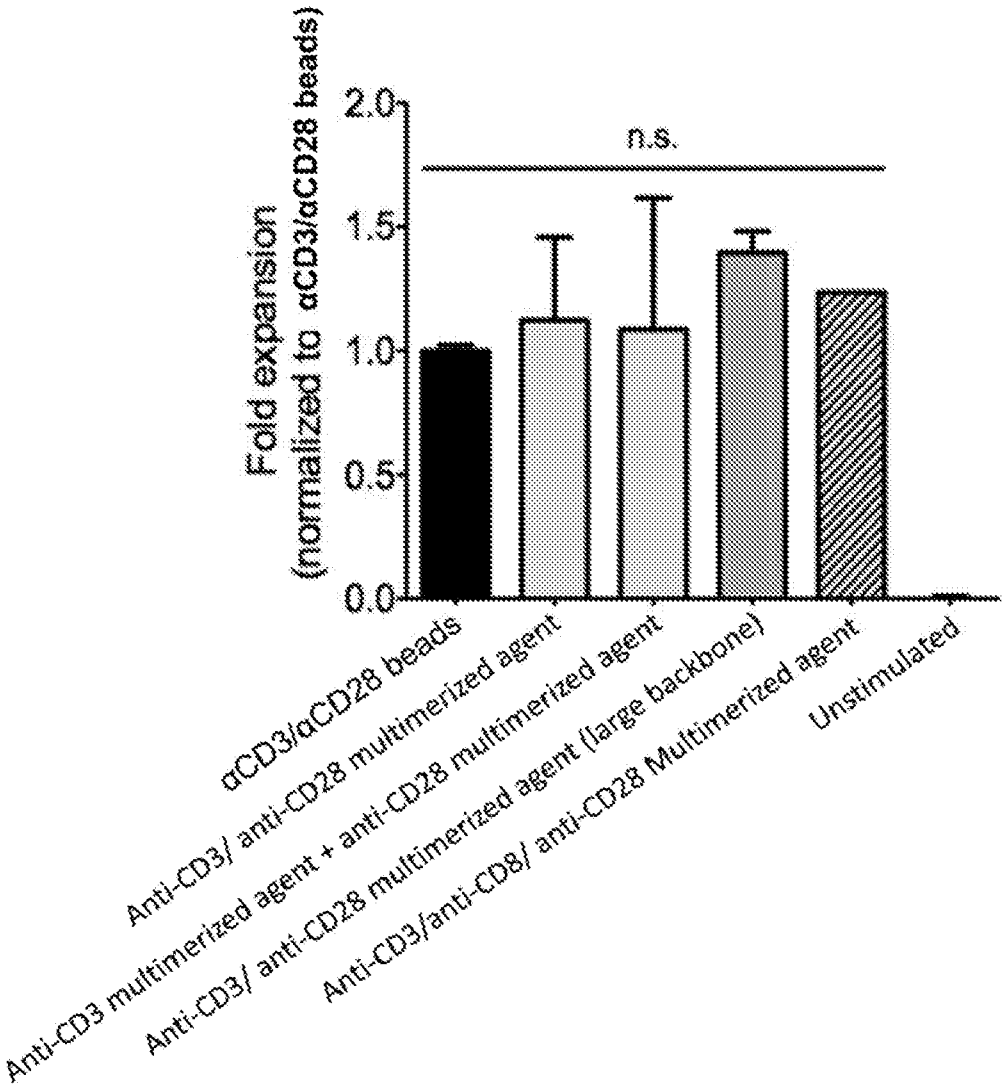

Fold expansion
(normalized to αCD3/αCD28 beads)
2.0
1.5
1.0
0.5
0.0
n.s.
αCD3/αCD28 beads
Anti-CD3/ anti-CD28 multimerized agent
Anti-CD3 multimerized agent + anti-CD28 multimerized agent
Anti-CD3/ anti-CD28 multimerized agent (large backbone)
Anti-CD3/anti-CD8/ anti-CD28 Multimerized agent
Unstimulated

FIG. 17B

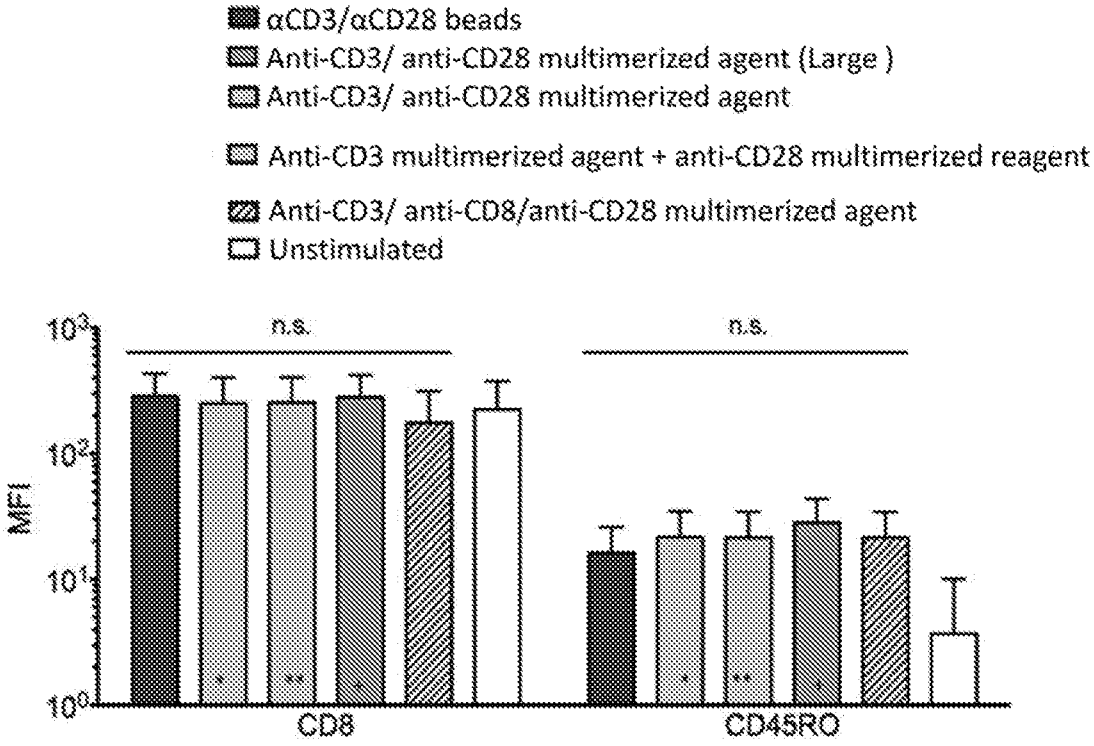

αCD3/αCD28 beads
Anti-CD3/ anti-CD28 multimerized agent (Large)
Anti-CD3/ anti-CD28 multimerized agent
Anti-CD3 multimerized agent + anti-CD28 multimerized reagent
Anti-CD3/ anti-CD8/anti-CD28 multimerized agent
Unstimulated
MFI
10^3
10^2
10^1
10^0
n.s.
n.s.
*
**
*
*
*
**
*
CD8
CD45RO

FIG. 18A

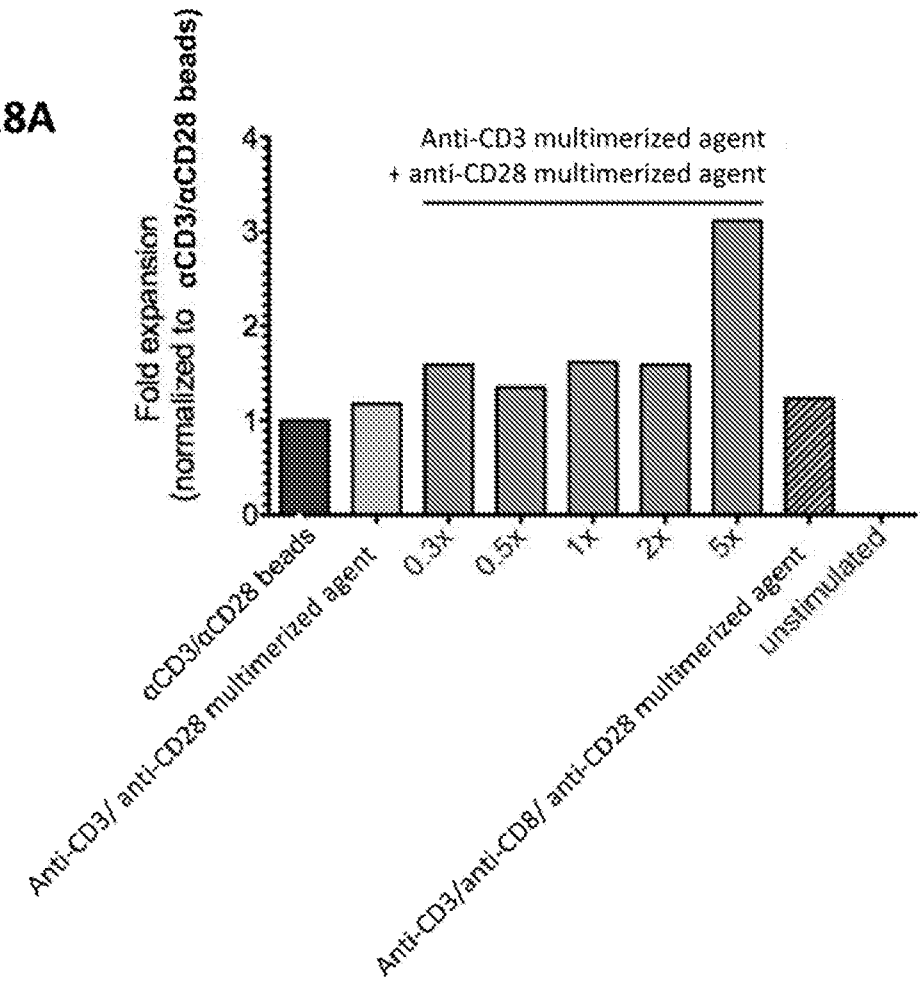

Fold expansion
(normalized to αCD3/αCD28 beads)
0
1
2
3
4
Anti-CD3 multimerized agent
+ anti-CD28 multimerized agent
αCD3/αCD28 beads
Anti-CD3/ anti-CD28 multimerized agent
0.3x
0.5x
1x
2x
5x
Anti-CD3/anti-CD8/ anti-CD28 multimerized agent
Unstimulated

FIG. 18B

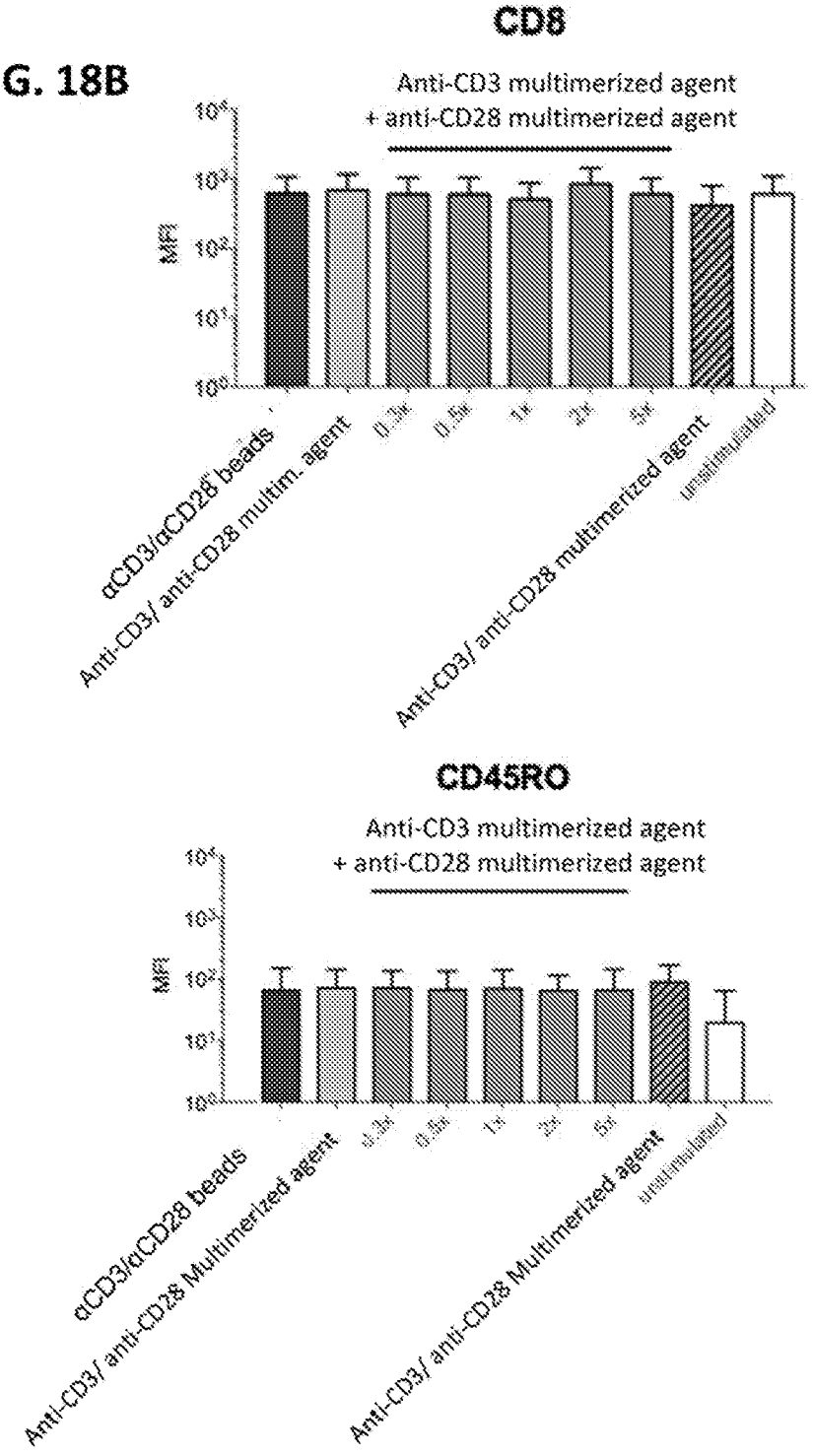

CD8
Anti-CD3 multimerized agent
+ anti-CD28 multimerized agent
MFI
αCD3/αCD28 beads
Anti-CD3/ anti-CD28 multim. agent
Anti-CD3/ anti-CD28 multimerized agent
CD45RO
Anti-CD3 multimerized agent
+ anti-CD28 multimerized agent
MFI
αCD3/αCD28 beads
Anti-CD3/ anti-CD28 Multimerized agent
Anti-CD3/ anti-CD28 Multimerized agent

FIG. 19A

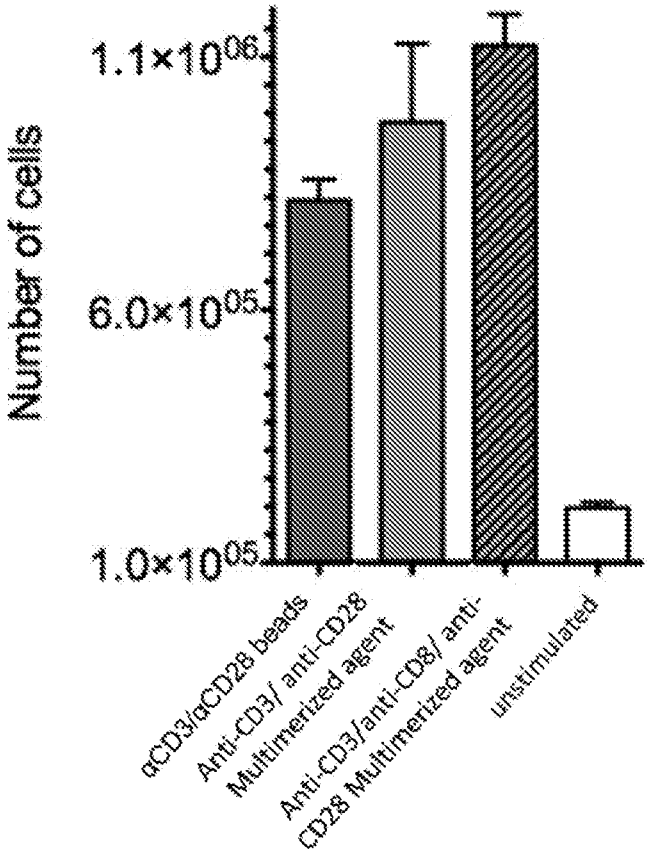


FIG. 19B

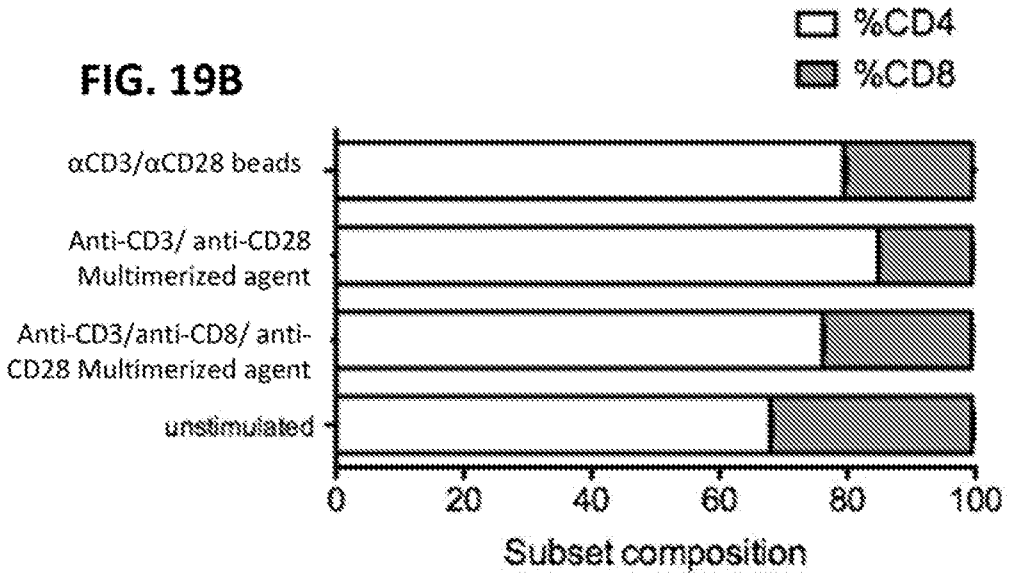


FIG. 20A

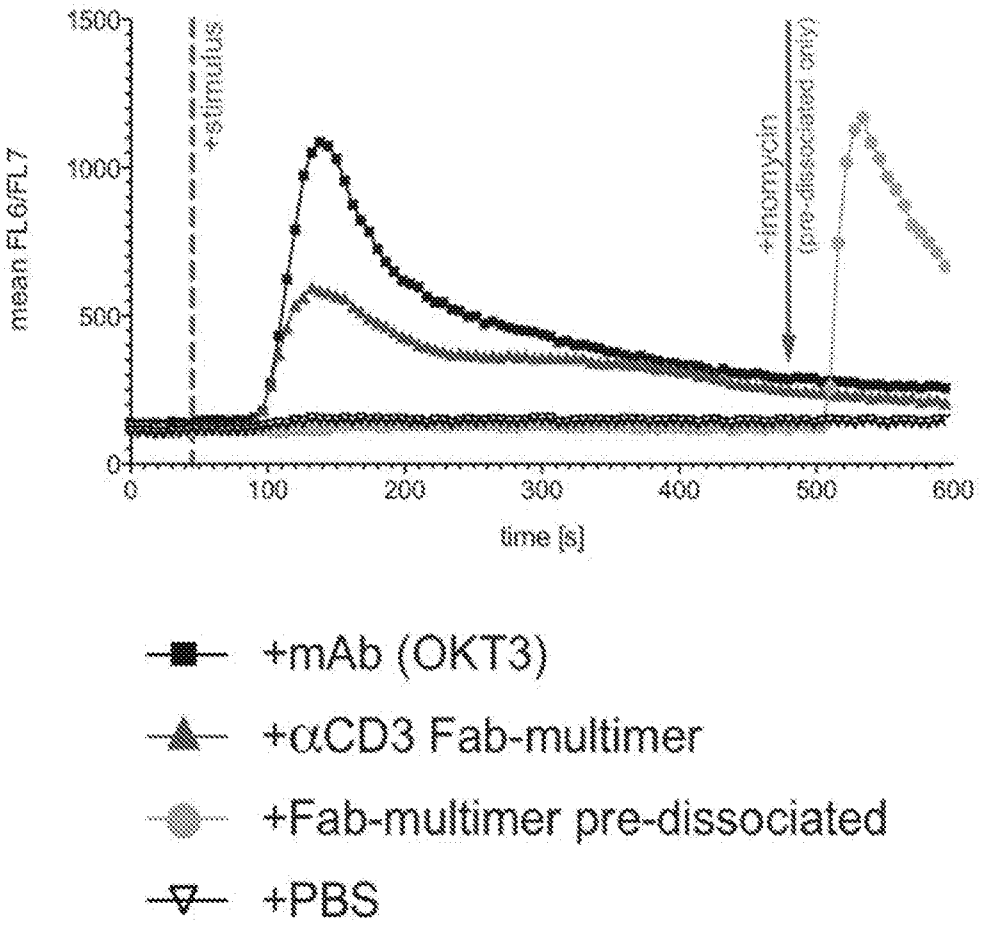

1500
1000
500
0
mean FL6/FL7
+stimulus
+Ionomycin
(pre-dissociated only)
0
100
200
300
400
500
600
time [s]
+mAb (OKT3)
+αCD3 Fab-multimer
+Fab-multimer pre-dissociated
+PBS

FIG. 20B

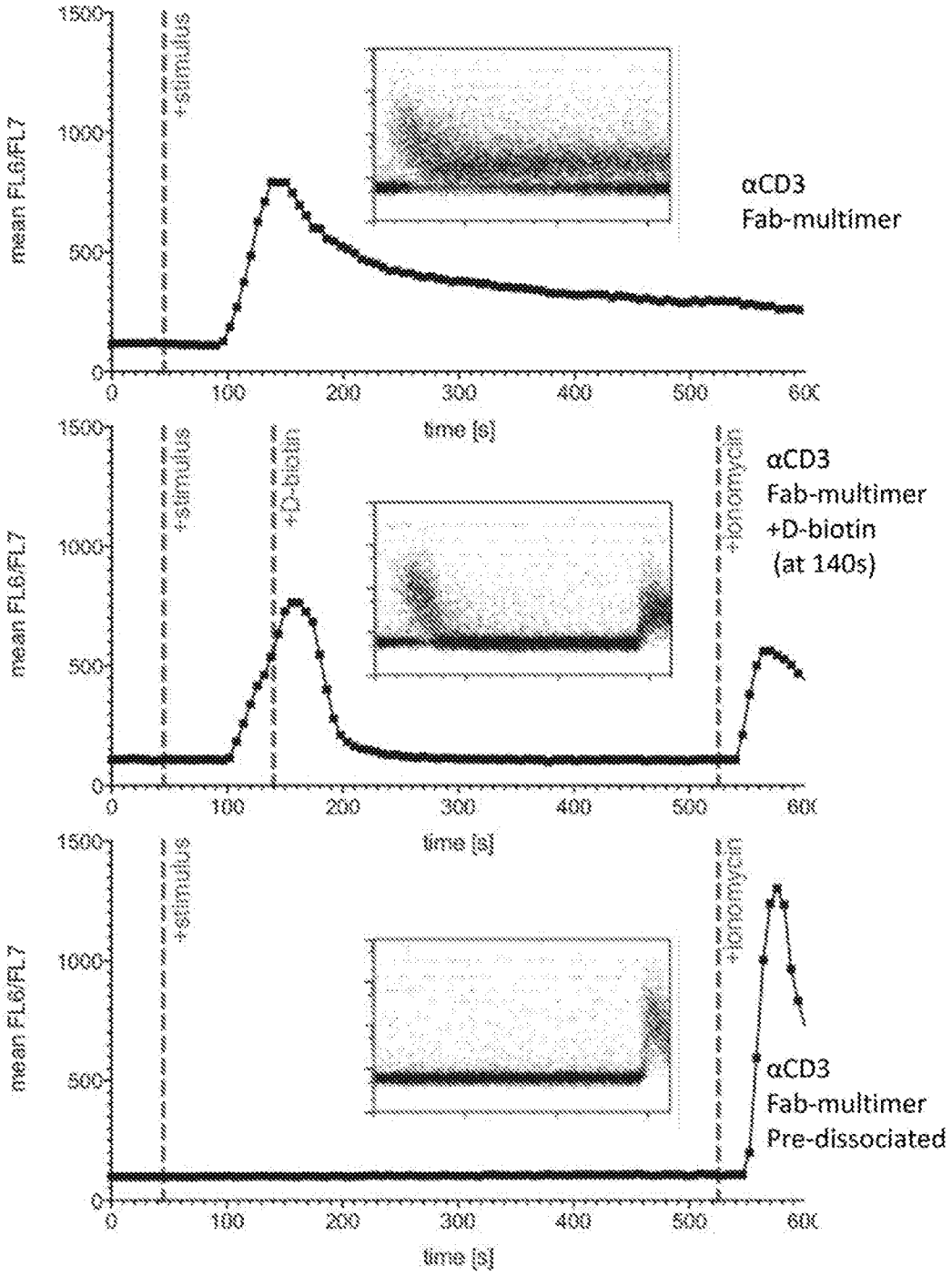

mean FL6/FL7
1500
1000
500
0
+stimulus
αCD3
Fab-multimer
0
100
200
300
400
500
600
time [s]
+stimulus
+D-biotin
+ionomycin
αCD3
Fab-multimer
+D-biotin
(at 140s)
time [s]
+stimulus
+ionomycin
αCD3
Fab-multimer
Pre-dissociated
time [s]

FIG. 21

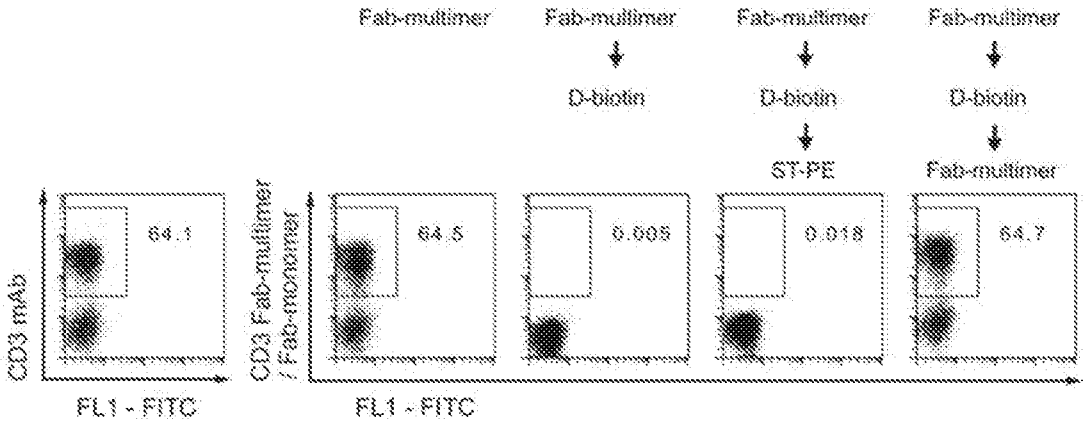

Fab-multimer
Fab-multimer
Fab-multimer
Fab-multimer
D-biotin
D-biotin
D-biotin
ST-PE
Fab-multimer
CD3 mAb
64.1
FL1 - FITC
CD3 Fab-multimer / Fab-monomer
64.5
0.005
0.018
64.7
FL1 - FITC

FIG. 22

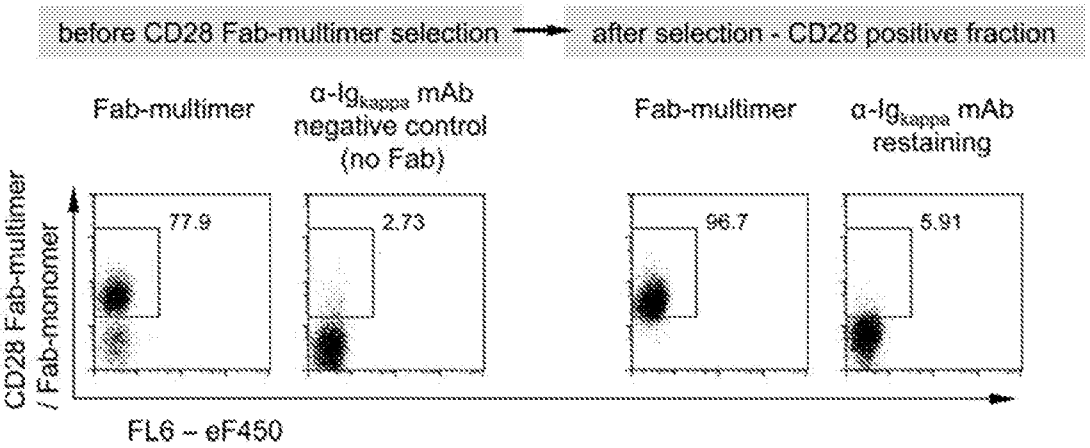

before CD28 Fab-multimer selection
after selection - CD28 positive fraction
Fab-multimer
α-Ig$_{kappa}$ mAb negative control (no Fab)
Fab-multimer
α-Ig$_{kappa}$ mAb restaining
CD28 Fab-multimer / Fab-monomer
77.9
2.73
96.7
5.91
FL6 – eF450

FIG. 23A

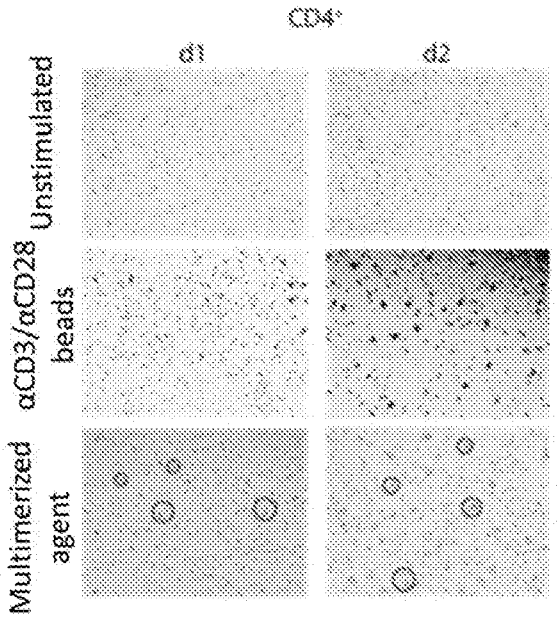


FIG. 23B

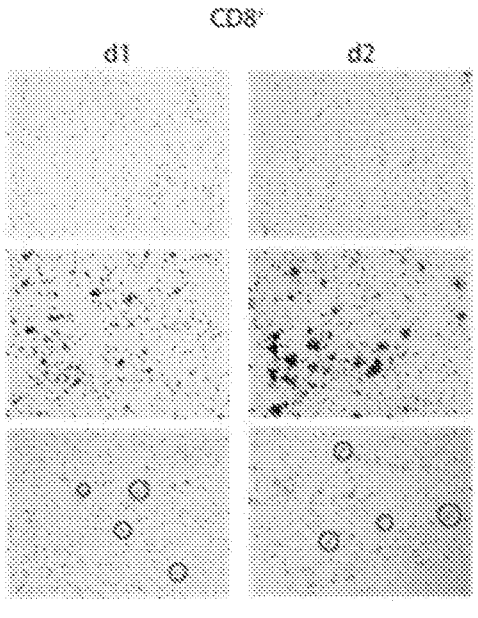


FIG. 24A

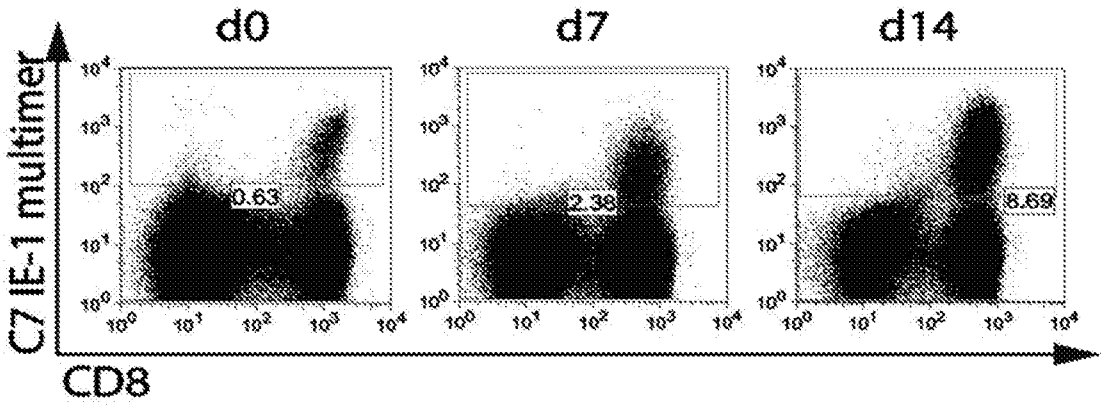


FIG. 24B

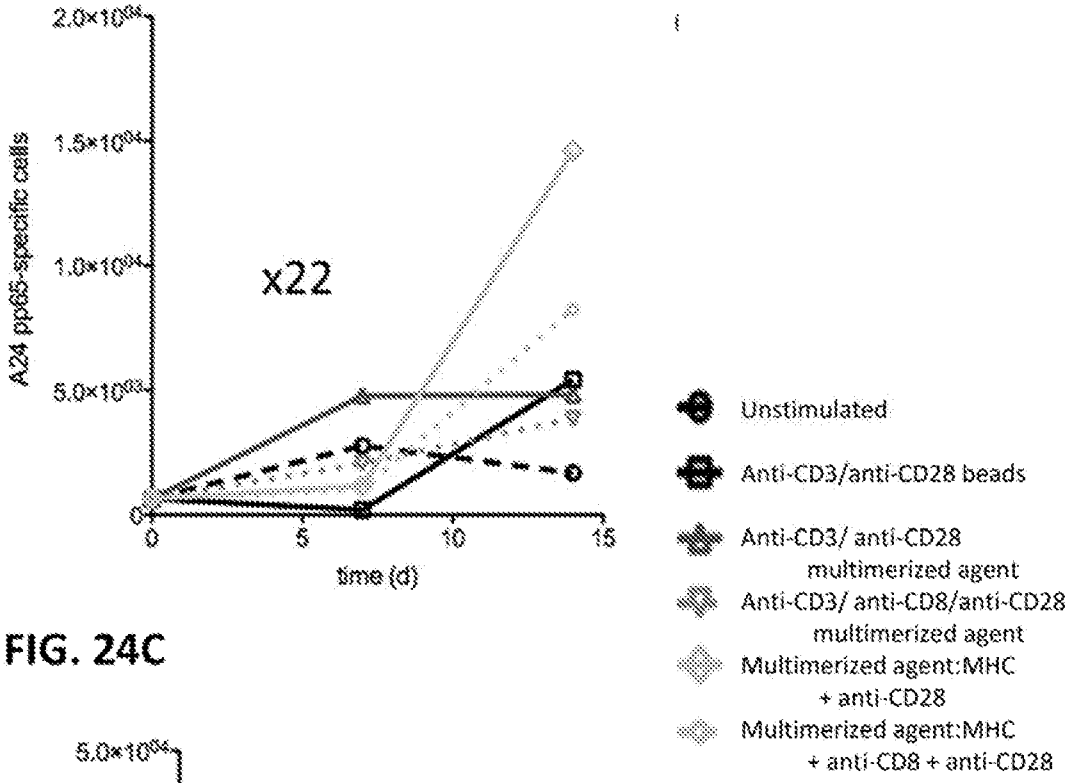


FIG. 24C

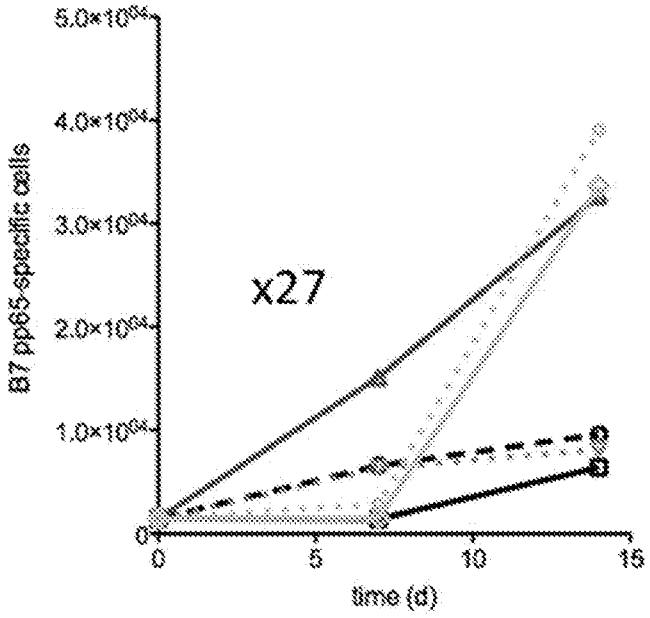


FIG. 24D

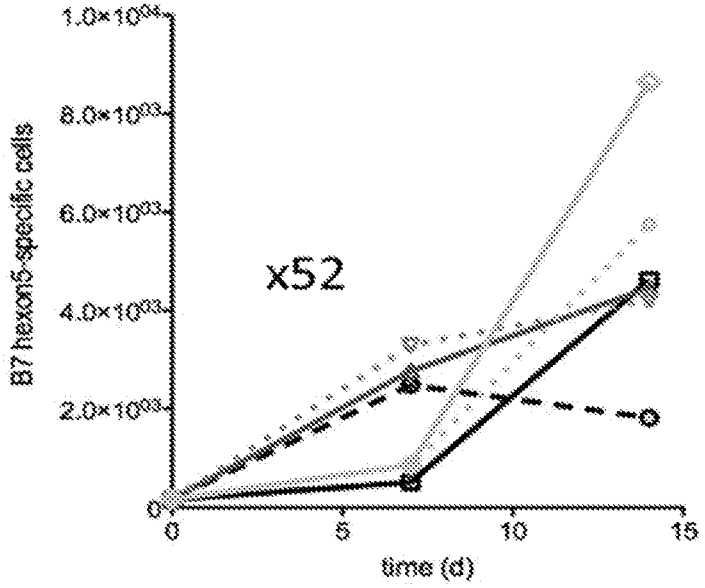


FIG. 24E

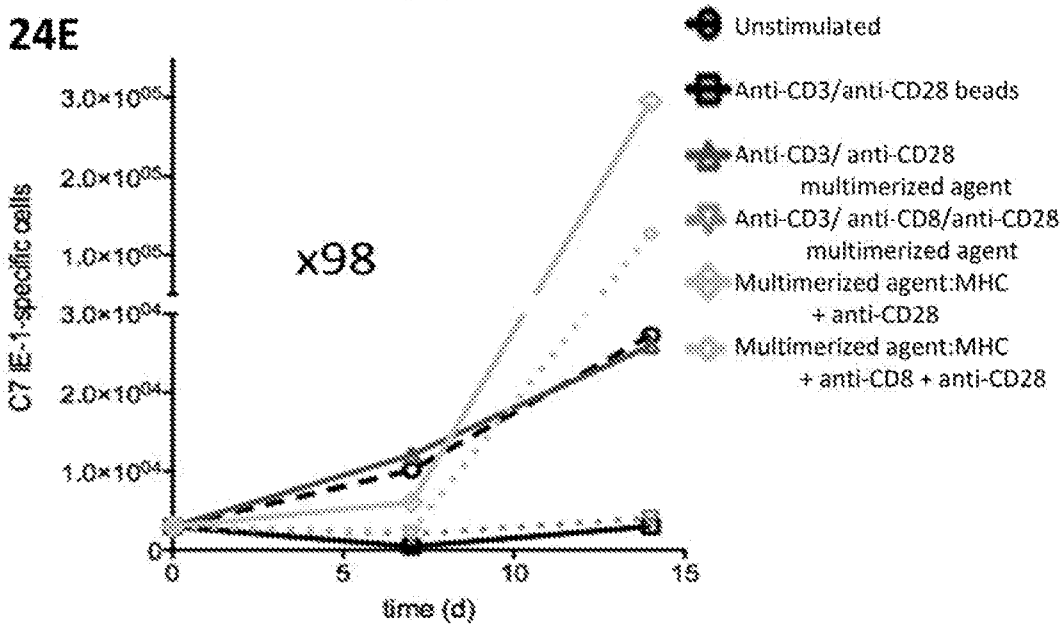


FIG. 24F

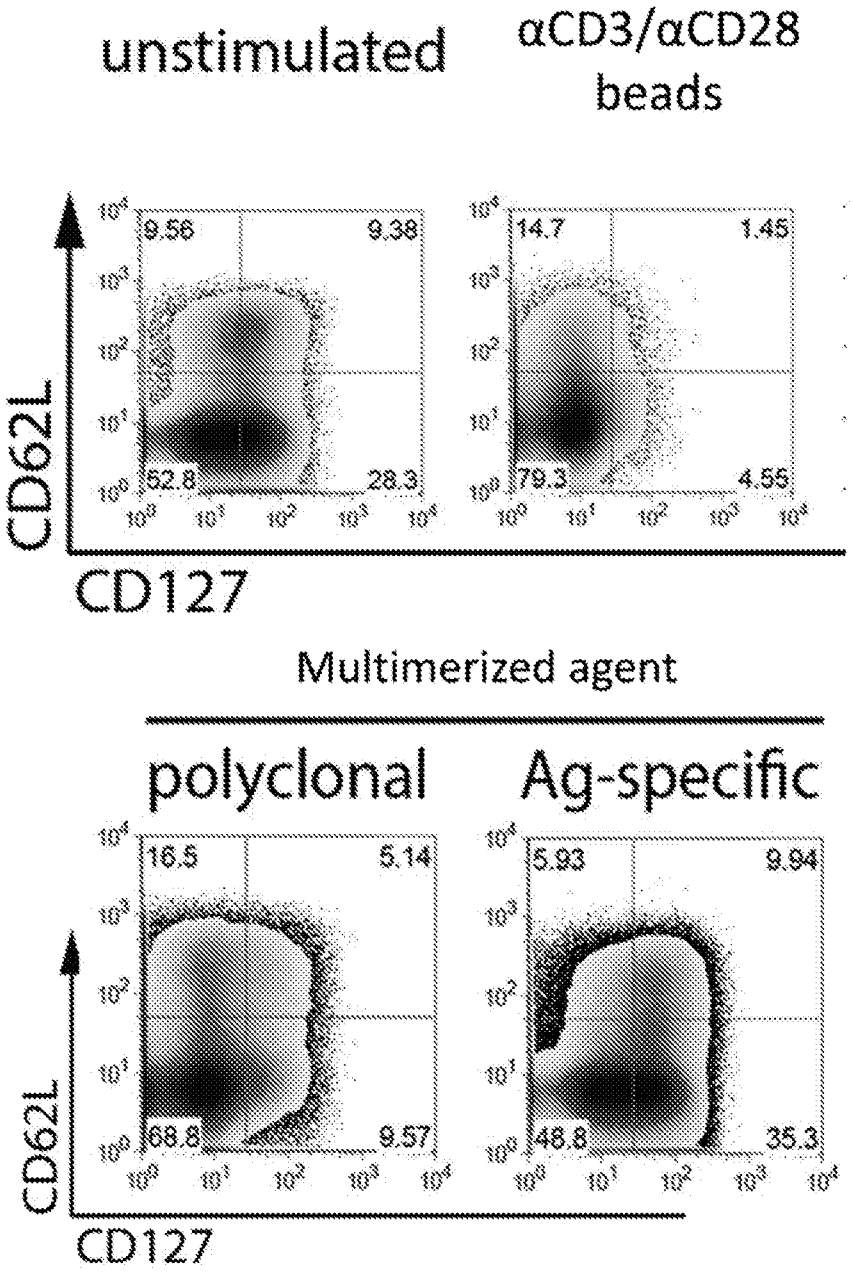

unstimulated
αCD3/αCD28 beads
9.56
9.38
52.8
28.3
14.7
1.45
79.3
4.55
CD62L
CD127
Multimerized agent
polyclonal
Ag-specific
16.5
5.14
68.8
9.57
5.93
9.94
48.8
35.3
CD62L
CD127

FIG. 25

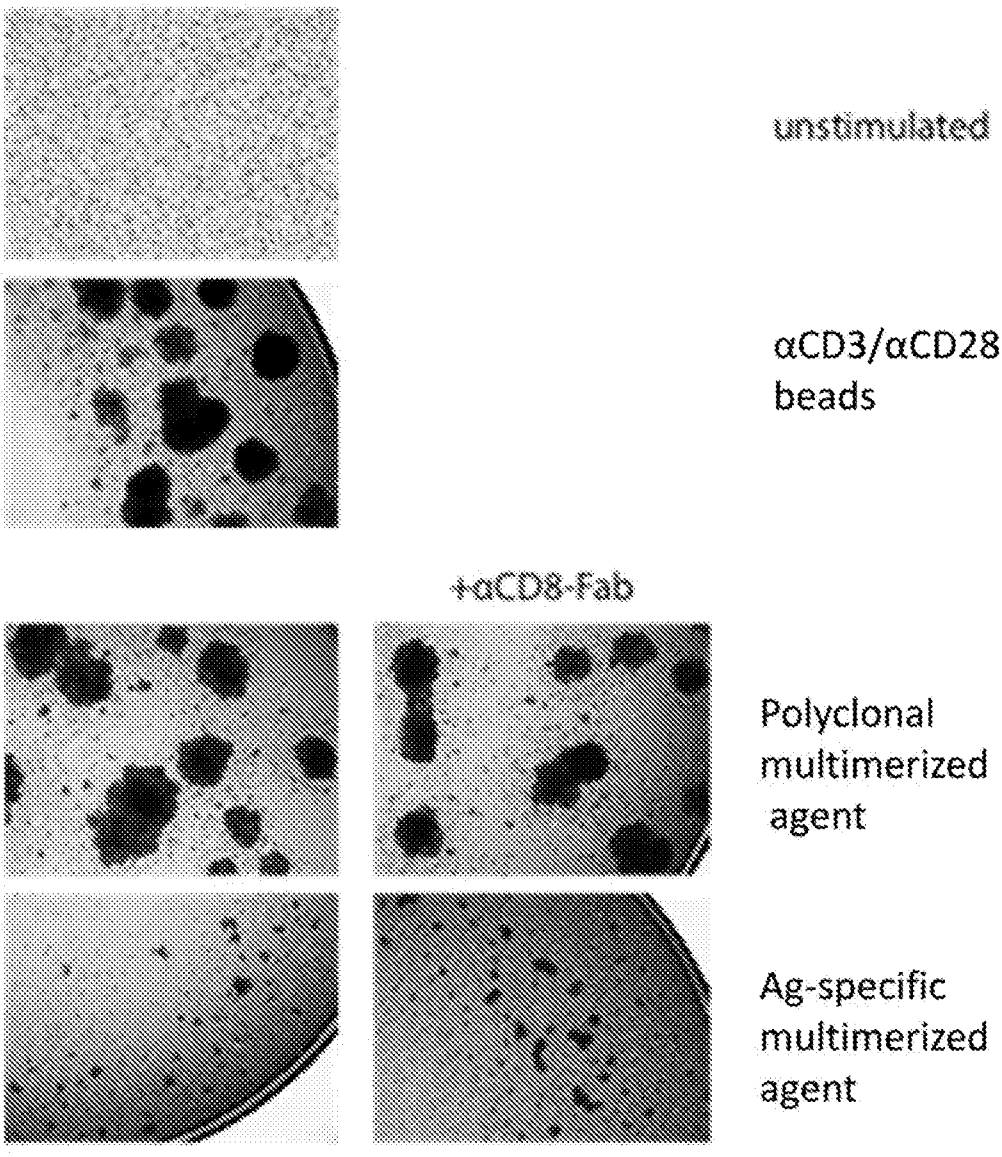

unstimulated
αCD3/αCD28 beads
+αCD8-Fab
Polyclonal multimerized agent
Ag-specific multimerized agent

FIG. 26A

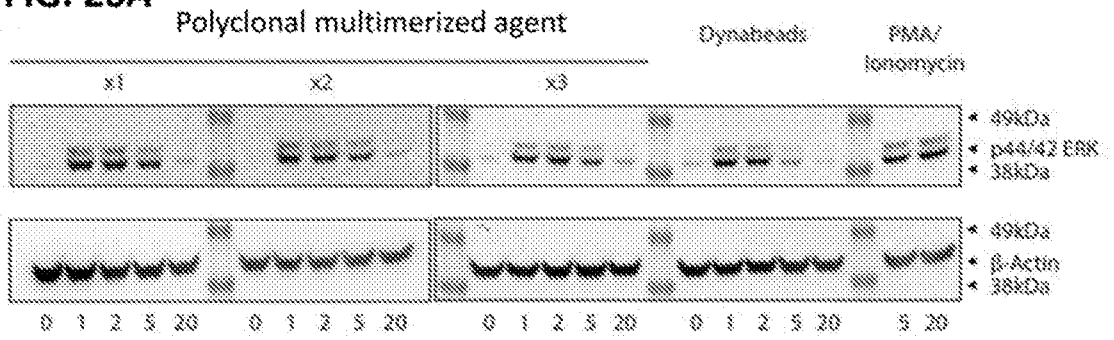


FIG. 26B

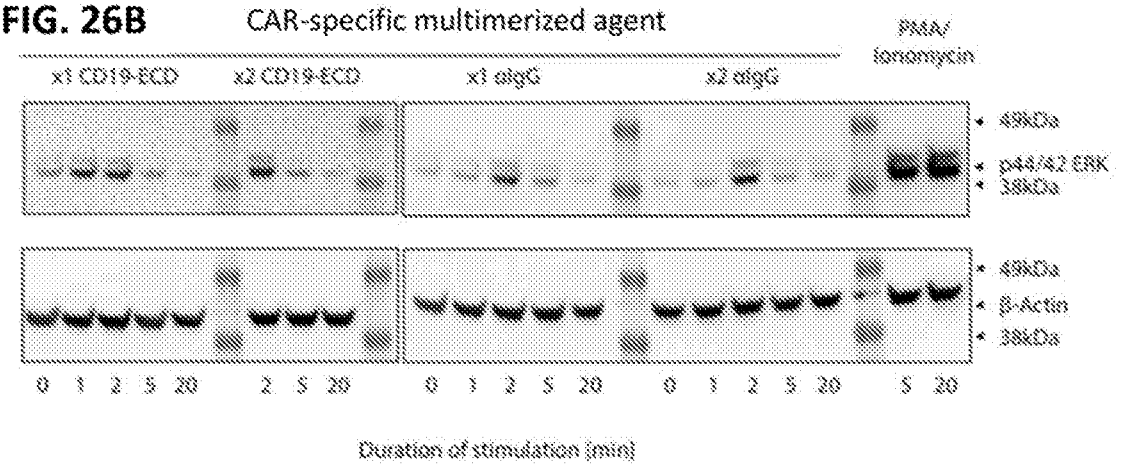


FIG 27

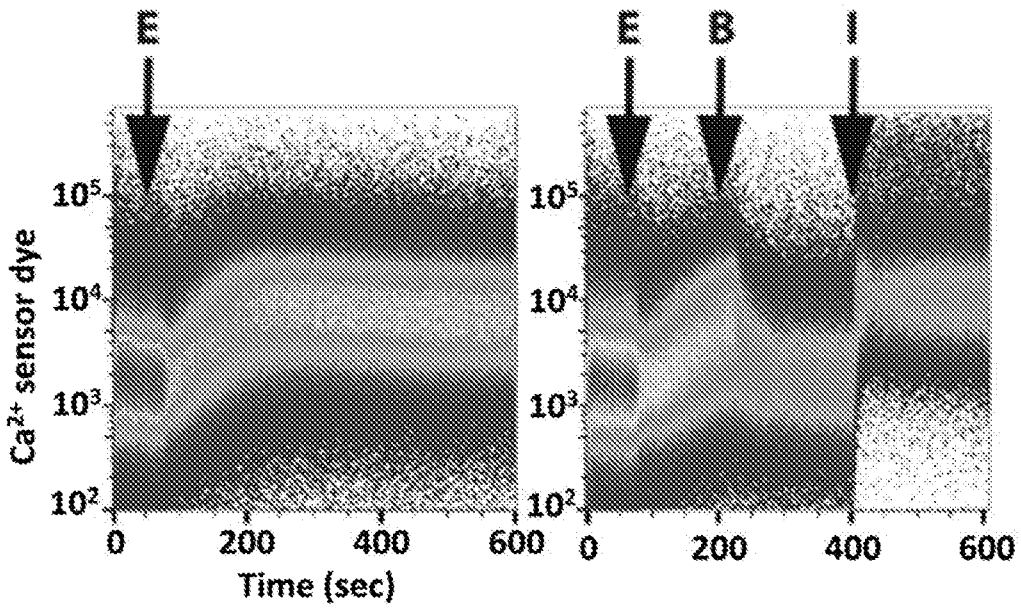


FIG. 28A

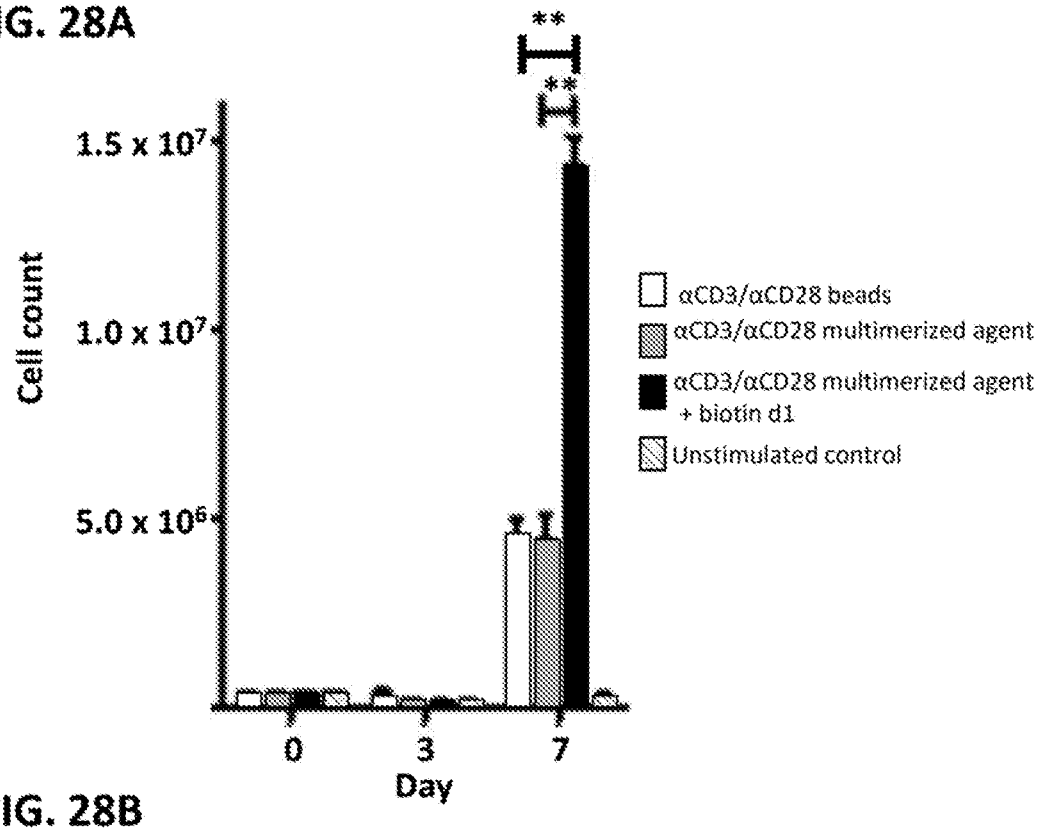


FIG. 28B

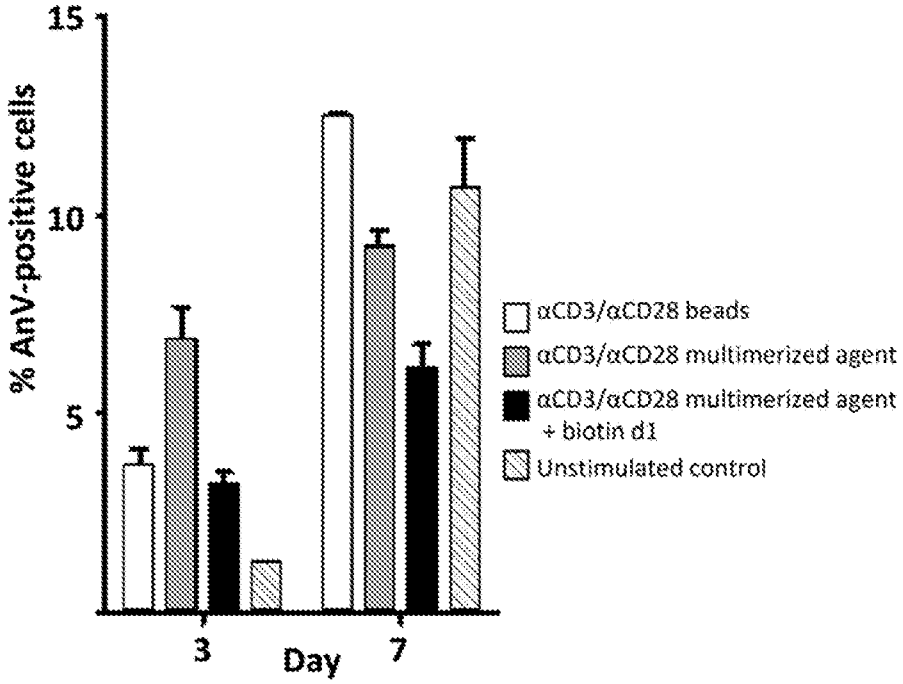
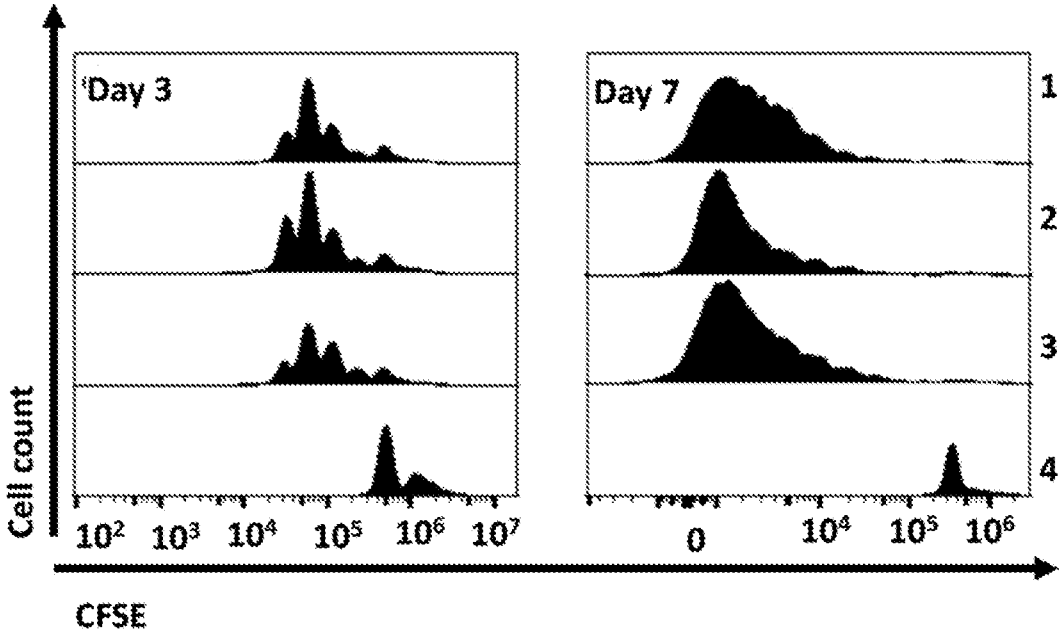


FIG. 29



1: αCD3/αCD28 beads
2: αCD3/αCD28 multimerized agent + biotin d2
3: αCD3/αCD28 multimerized agent
4: unstimulated control

FIG. 30

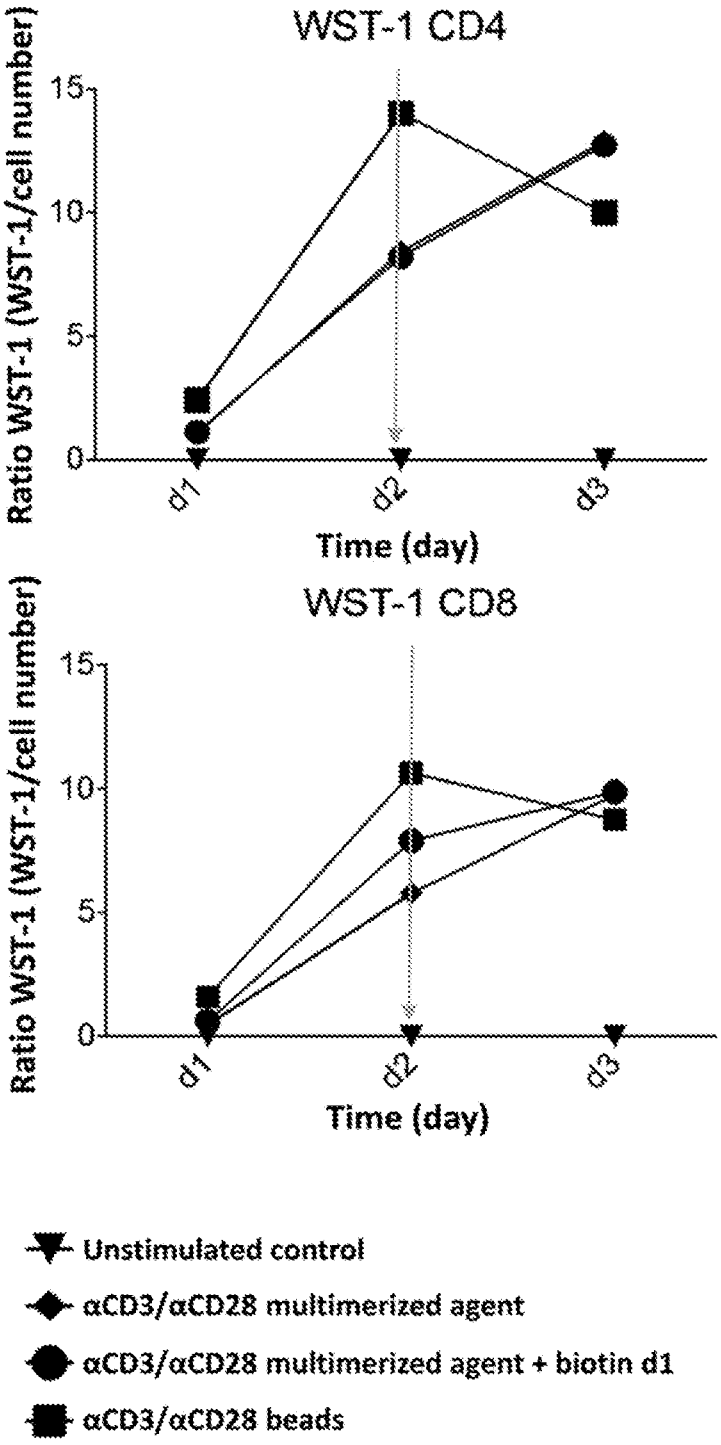
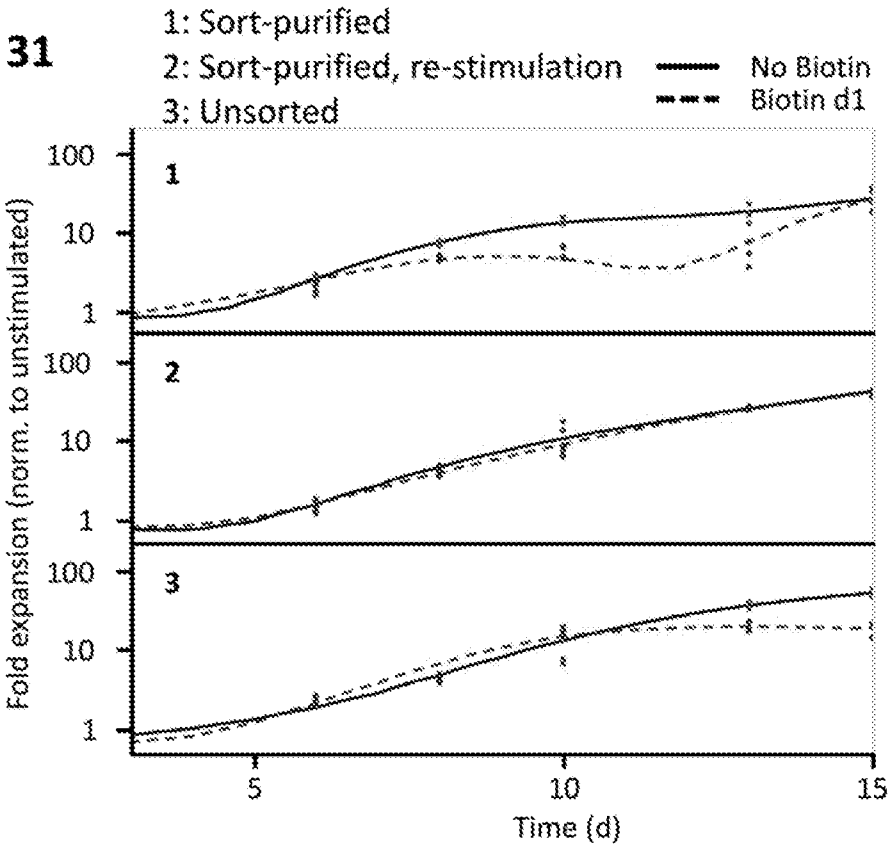

WST-1 CD4
Ratio WST-1 (WST-1/cell number)
15
10
5
0
d1
d2
d3
Time (day)
WST-1 CD8
Ratio WST-1 (WST-1/cell number)
15
10
5
0
d1
d2
d3
Time (day)
Unstimulated control
αCD3/αCD28 multimerized agent
αCD3/αCD28 multimerized agent + biotin d1
αCD3/αCD28 beads

FIG. 31


1: Sort-purified
2: Sort-purified, re-stimulation
3: Unsorted
No Biotin
Biotin d1
Fold expansion (norm. to unstimulated)
100
10
1
1
2
3
5
10
15
Time (d)