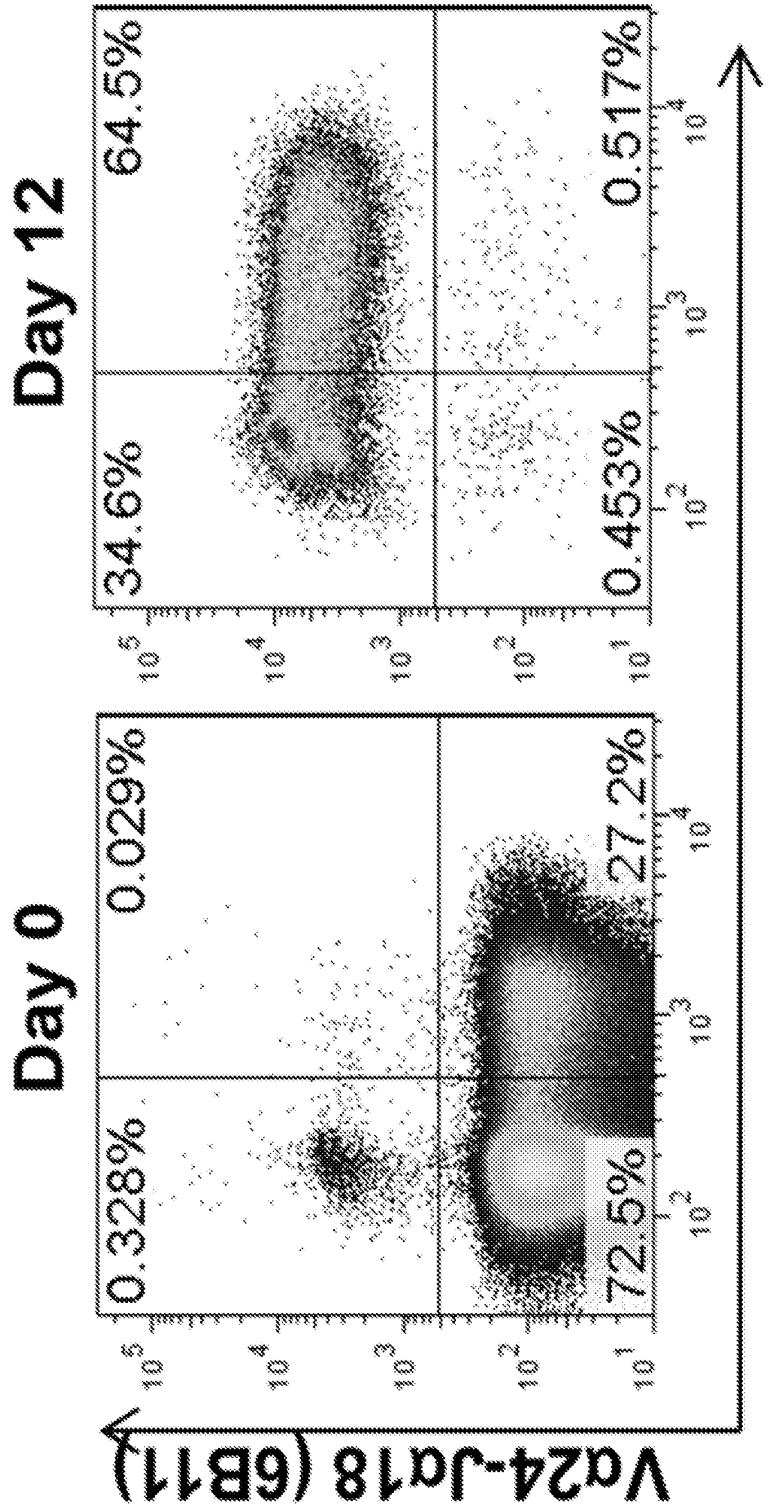
FIG. 1A


Day 0
Day 12
0.328%
0.029%
72.5%
27.2%
34.6%
64.5%
0.453%
0.517%
Vα24-Jα18 (6B11)
CD62L

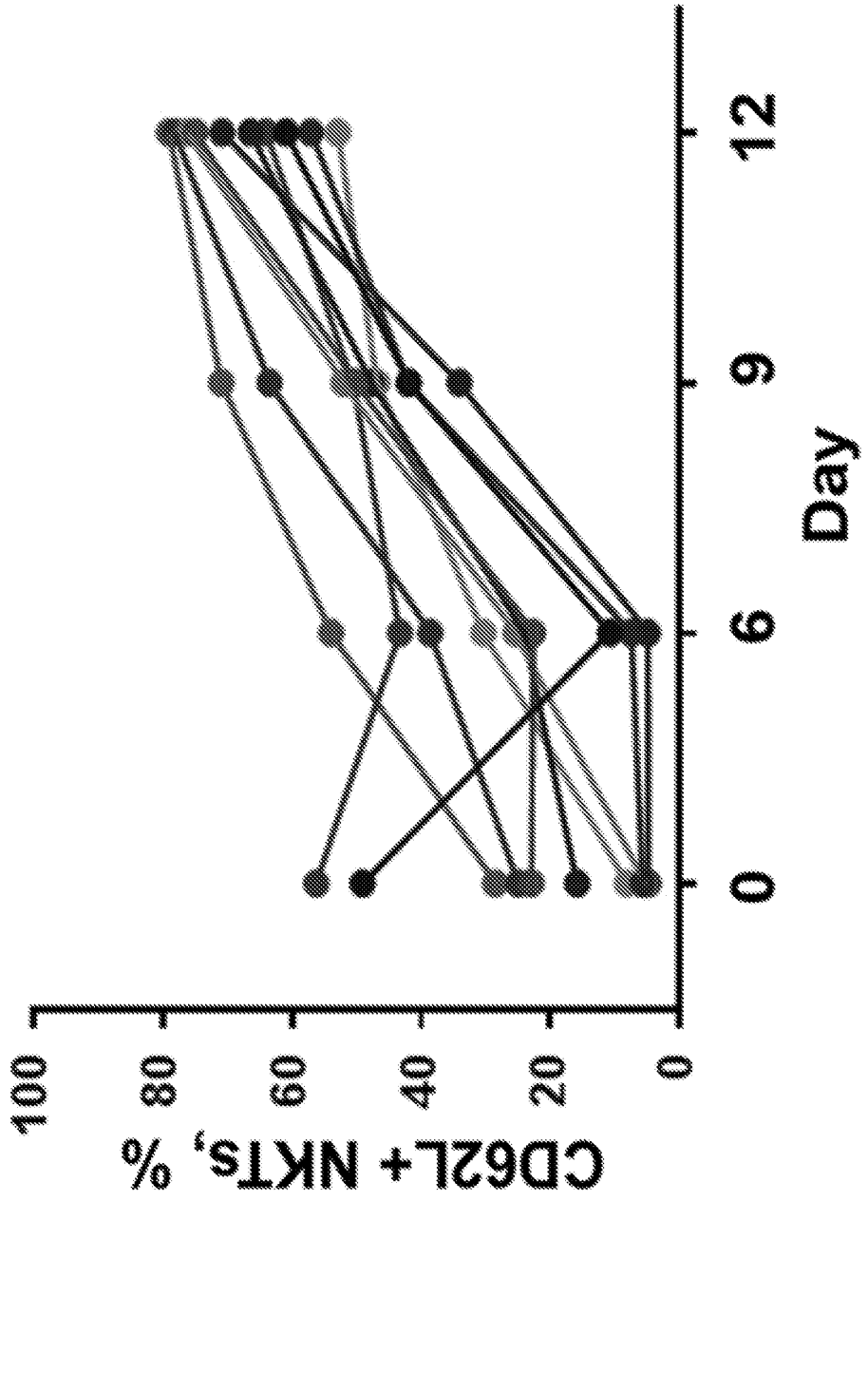



FIG. 1B

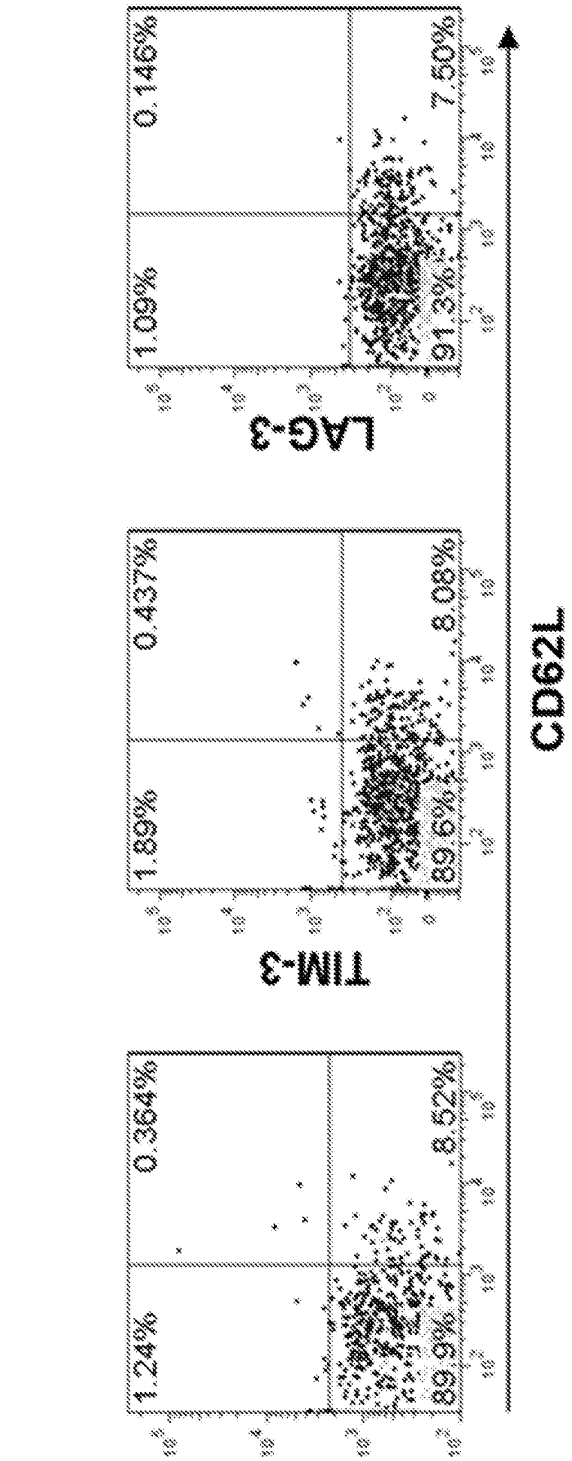

Day 0
PD-1
TIM-3
LAG-3
CD62L
1.24%
0.364%
89.9%
8.52%
1.89%
0.437%
89.6%
8.08%
1.09%
0.146%
91.3%
7.50%

FIG. 1C

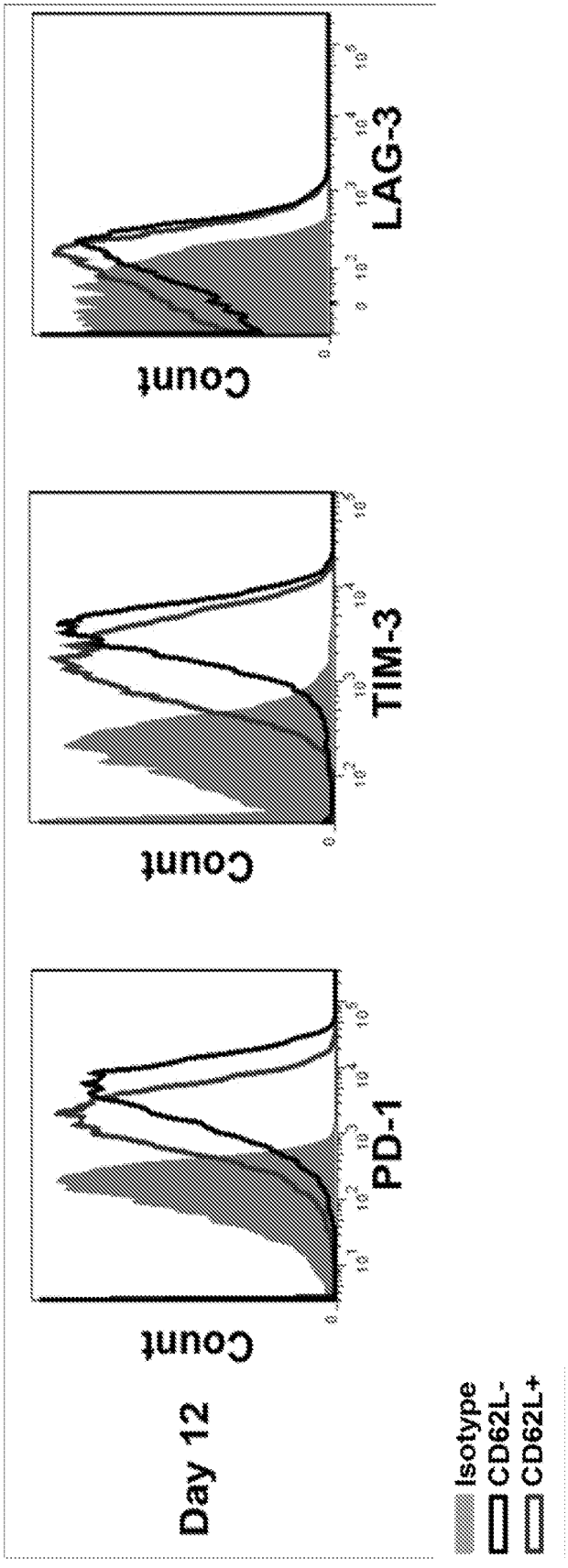



FIG. 1C

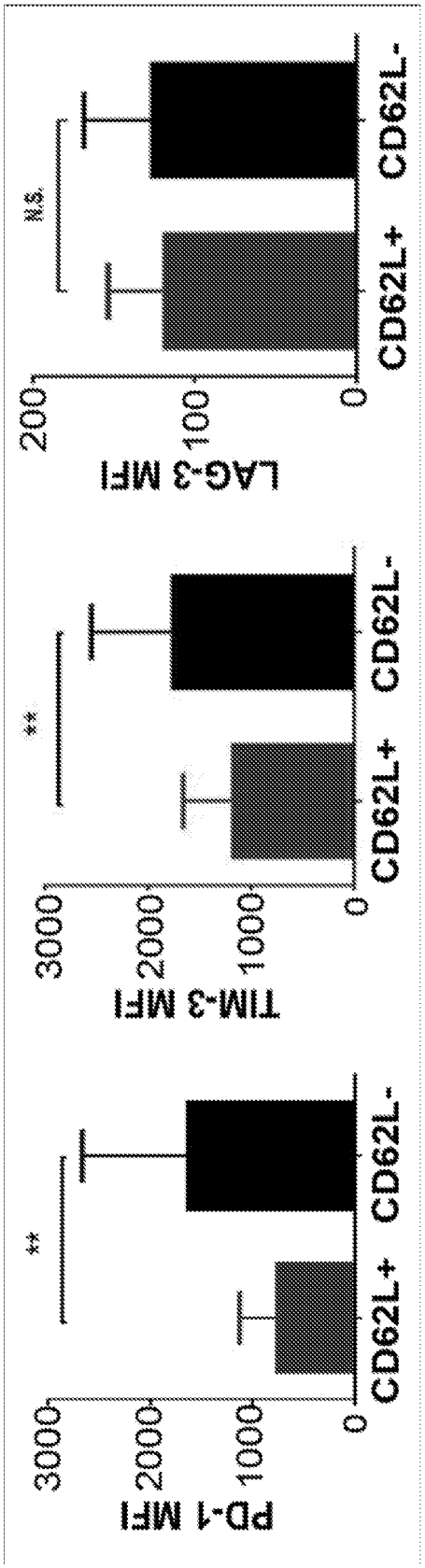
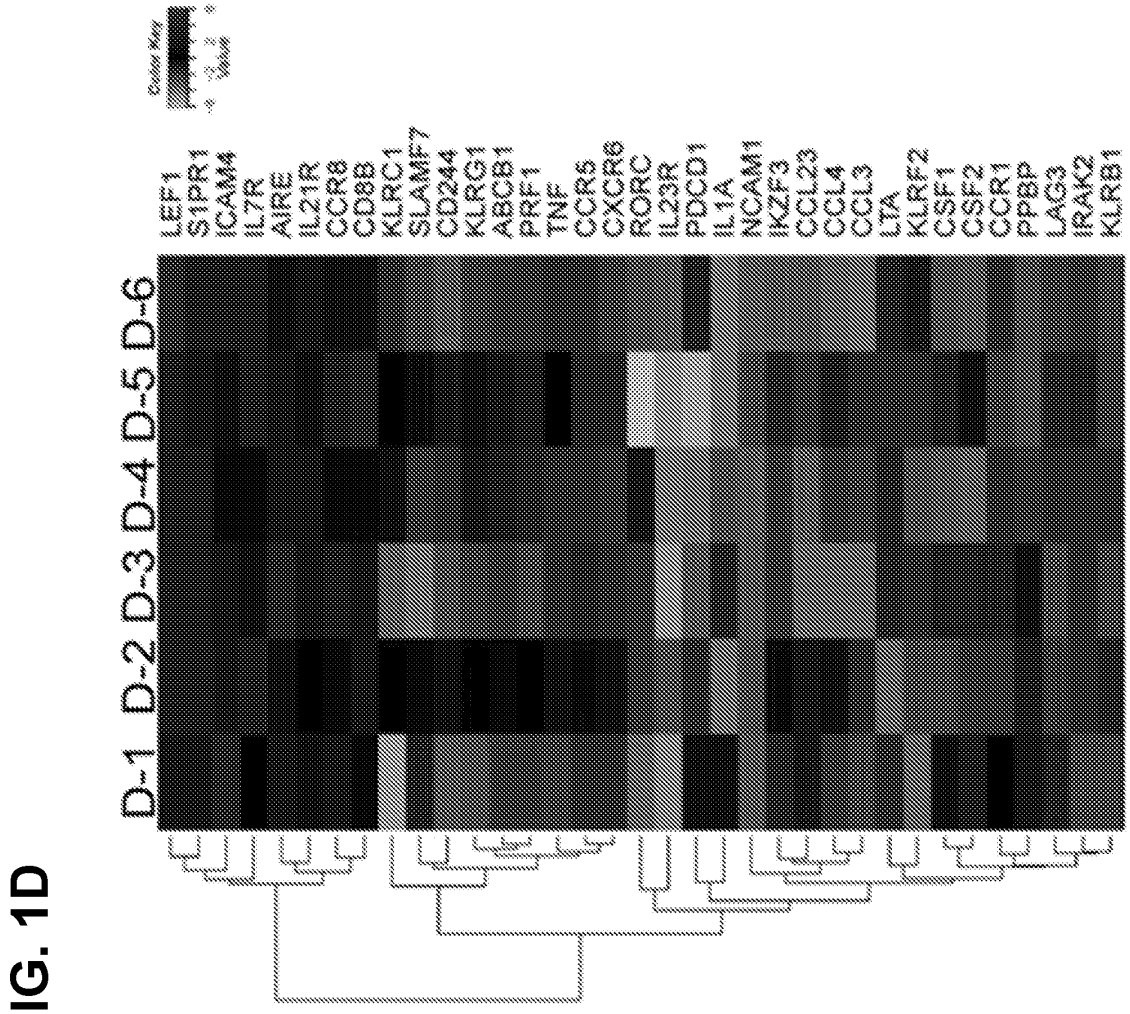

PD-1 MFI
3000
2000
1000
0
**
CD62L+
CD62L-
TIM-3 MFI
3000
2000
1000
0
**
CD62L+
CD62L-
LAG-3 MFI
200
100
0
N.S.
CD62L+
CD62L-

FIG. 1C




| Gene Name | Fold Change | P value |
|---|---|---|
| CD62L | 118.32 | 0.0001 |
| LEF1 | 6.48 | 0.0089 |
| S1PR1 | 4.69 | 0.0024 |
| ICAM4 | 3.67 | 0.0015 |
| IL7R | 2.85 | 0.0006 |
| AIRE | 3.31 | 0.0173 |
| IL21R | 2.19 | 0.0016 |
| CCR8 | 2.78 | 0.0050 |
| CD8B | 2.57 | 0.0175 |
| NCAM1(CD56) | -4.44 | 0.0014 |
| CCL3 | -4.89 | 0.0002 |
| PDCD1(PD-1) | -4.78 | 0.0207 |
| KLRB1(CD161) | -3.49 | 0.0001 |
| LAG-3 | -2.02 | 0.0027 |
| CD244 | -2.62 | 0.0026 |
| IRAK2 | -2.78 | 0.0001 |
| IKZF3 | -2.71 | 0.0038 |
| KLRC1 | -2.64 | 0.0026 |
| CXCR6 | -2.5 | 0.0028 |
| CCR1 | -2.37 | 0.0097 |
| HAVCR2(TIM-3) | -1.82 | 0.0011 |

FIG. 1D

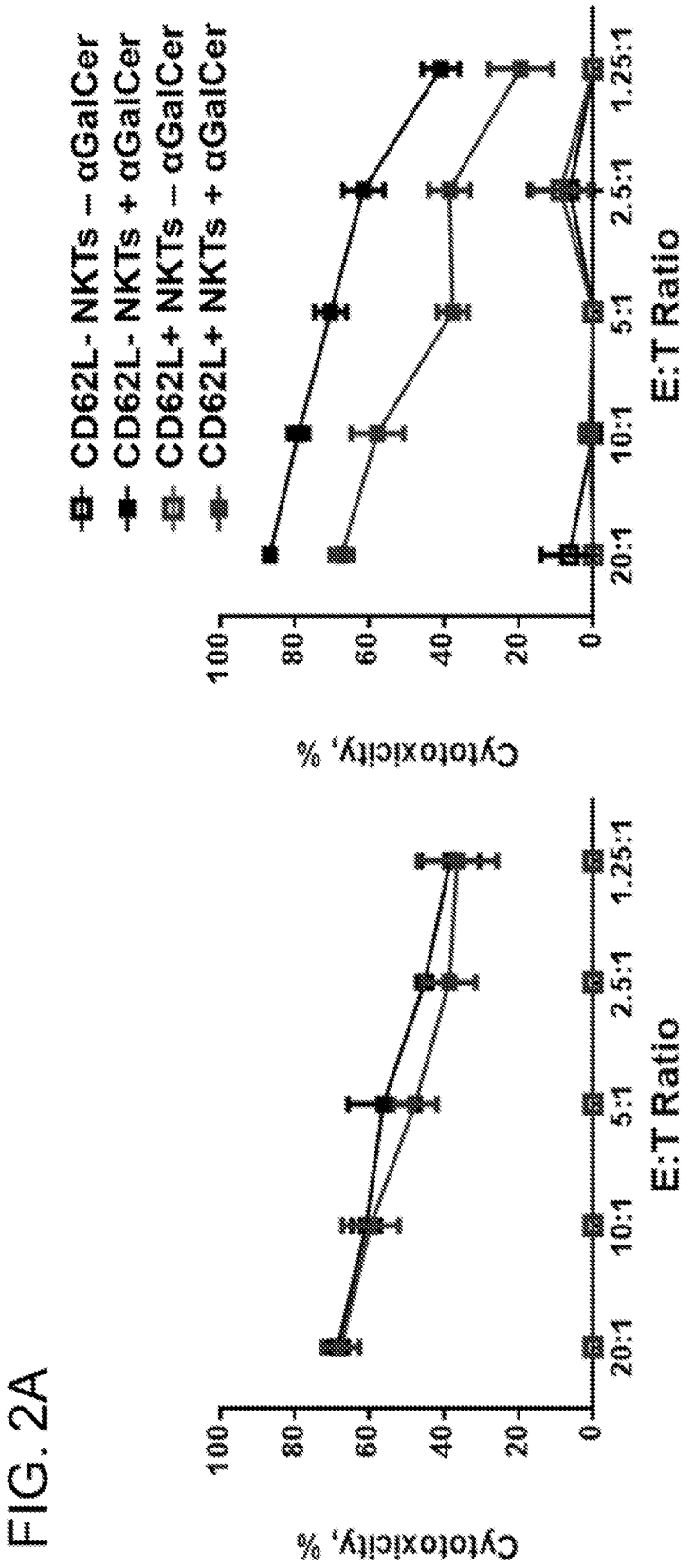

CD62L- NKTs – αGalCer
CD62L- NKTs + αGalCer
CD62L+ NKTs – αGalCer
CD62L+ NKTs + αGalCer
Cytotoxicity, %
100
80
60
40
20
0
20:1
10:1
5:1
2.5:1
1.25:1
E:T Ratio

FIG. 2A

FIG. 2B

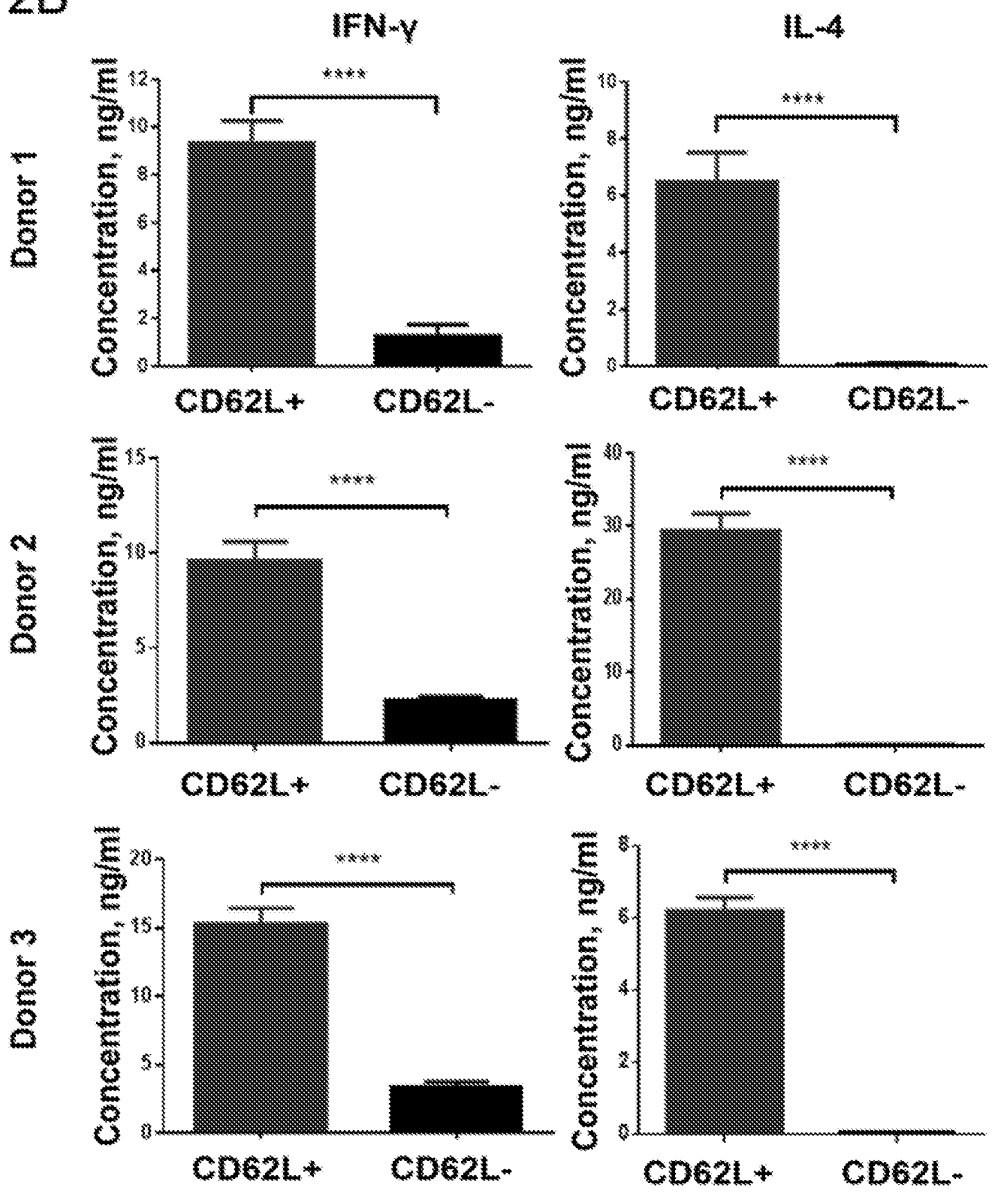

IFN-γ
IL-4
Donor 1
Donor 2
Donor 3
Concentration, ng/ml
****
CD62L+
CD62L-

FIG. 2C

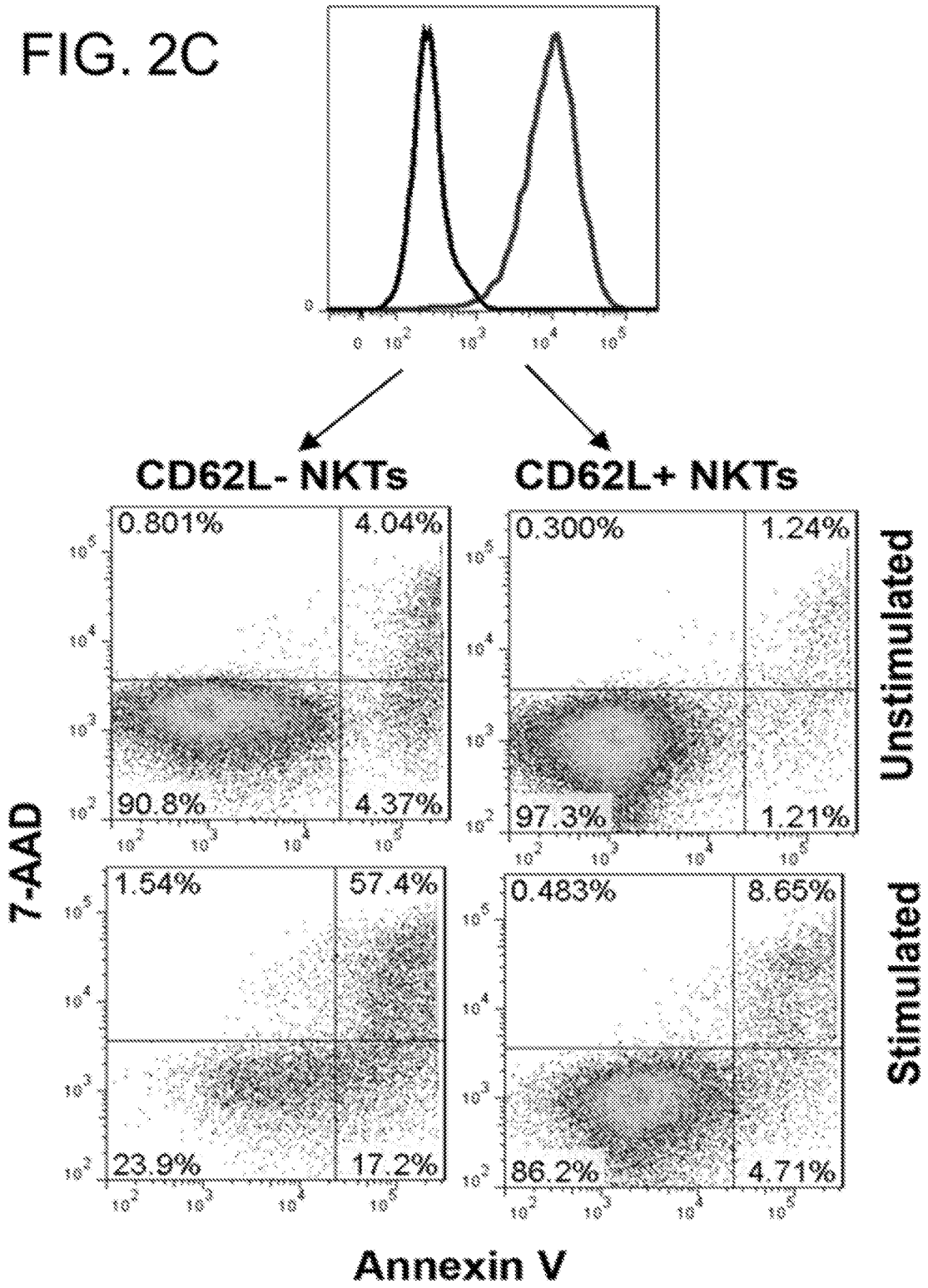
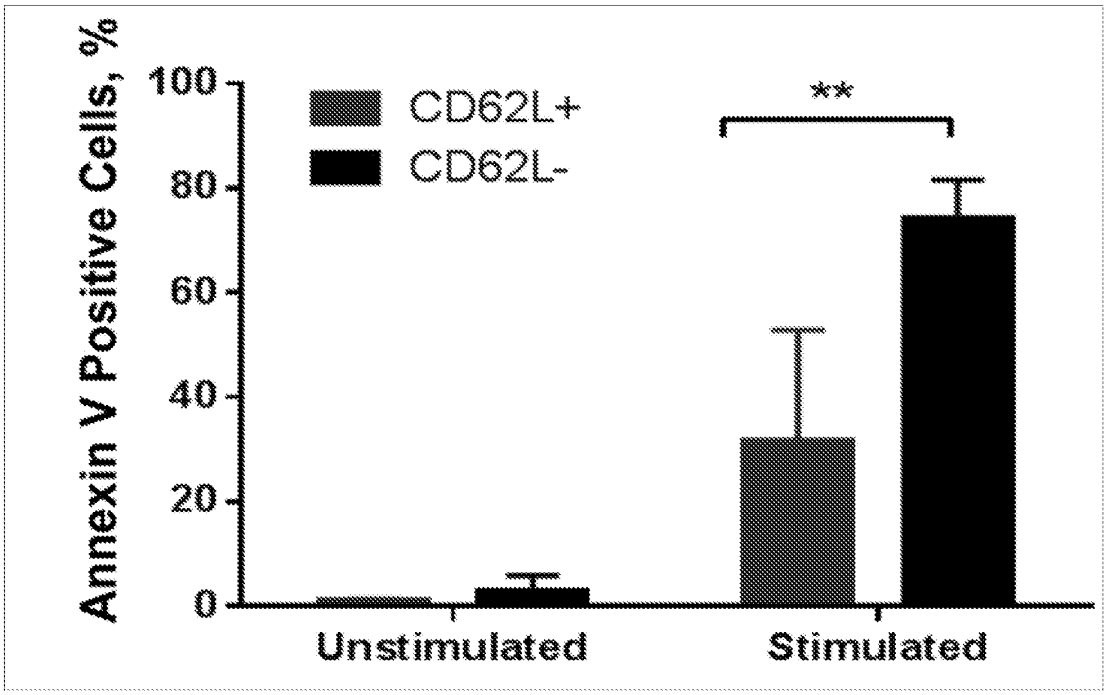

CD62L- NKTs
CD62L+ NKTs
0.801%
4.04%
90.8%
4.37%
0.300%
1.24%
97.3%
1.21%
1.54%
57.4%
23.9%
17.2%
0.483%
8.65%
86.2%
4.71%
Unstimulated
Stimulated
7-AAD
Annexin V

FIG. 2C


Annexin V Positive Cells, %
100
80
60
40
20
0
CD62L+
CD62L-
**
Unstimulated
Stimulated

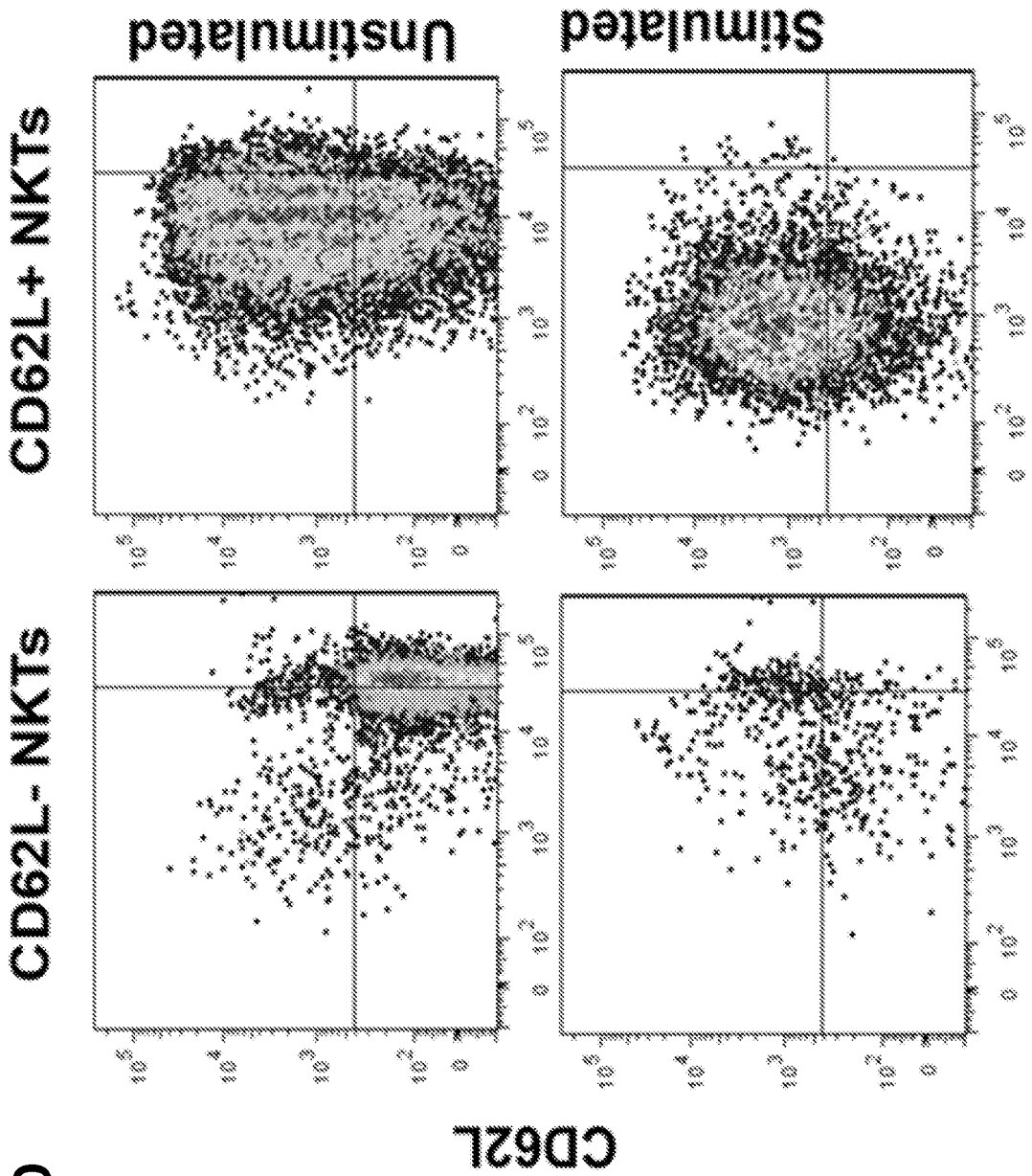



FIG. 2D

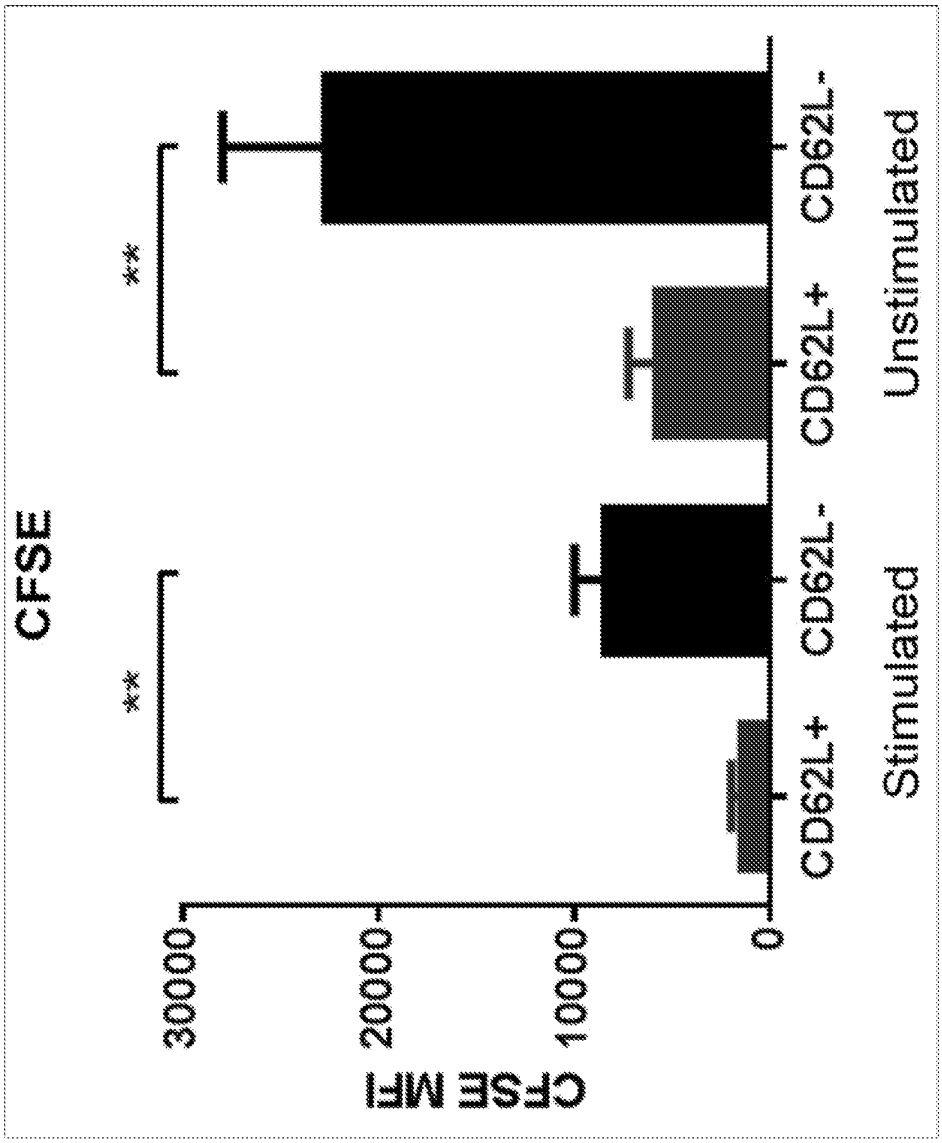
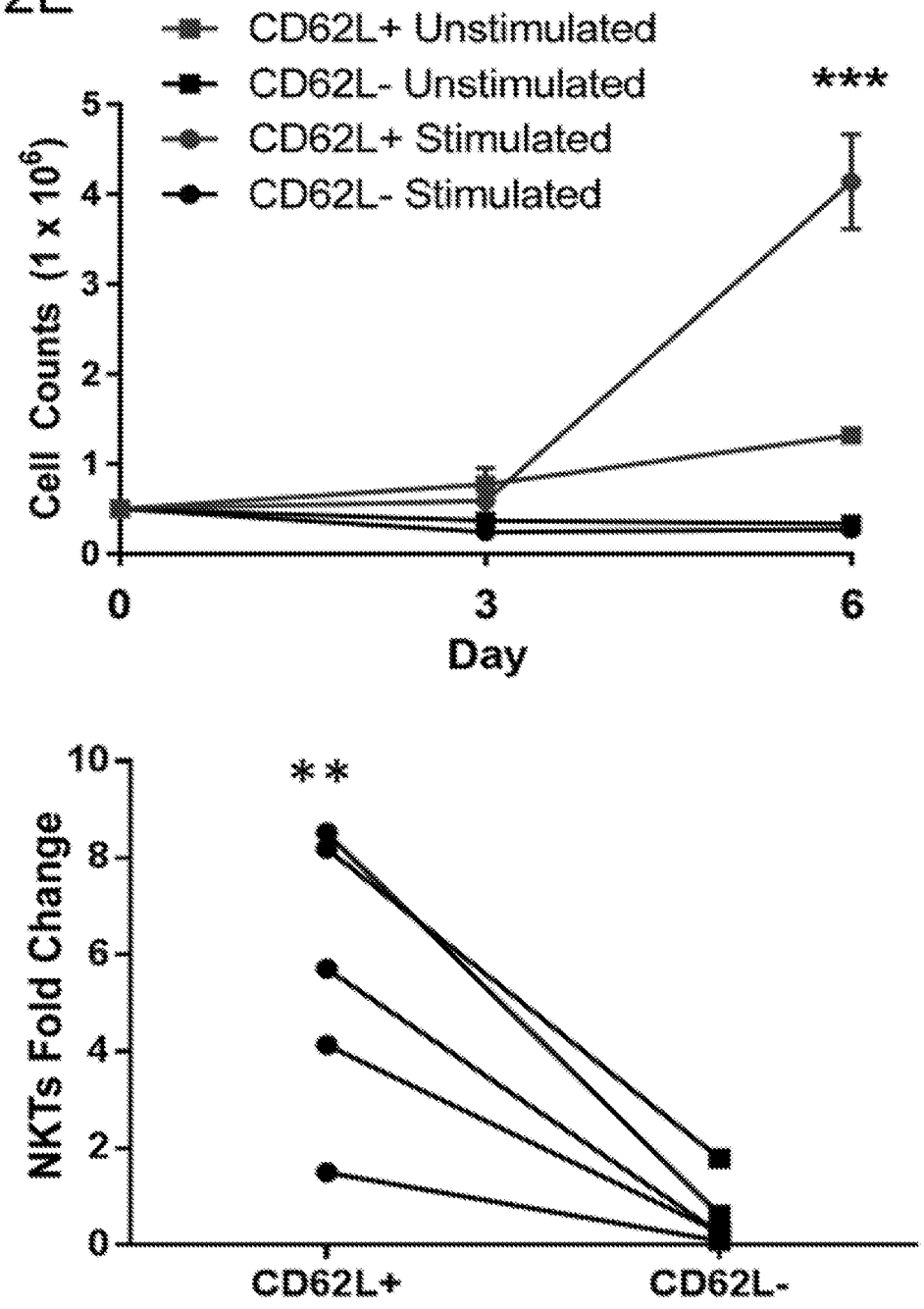

CFSE
**
**
CFSE MFI
30000
20000
10000
0
CD62L+
CD62L-
CD62L+
CD62L-
Stimulated
Unstimulated

FIG. 2D

FIG. 2E


CD62L+ Unstimulated
CD62L- Unstimulated
CD62L+ Stimulated
CD62L- Stimulated
***
Cell Counts (1 x 10^6)
0
1
2
3
4
5
0
3
6
Day
**
NKTs Fold Change
0
2
4
6
8
10
CD62L+
CD62L-

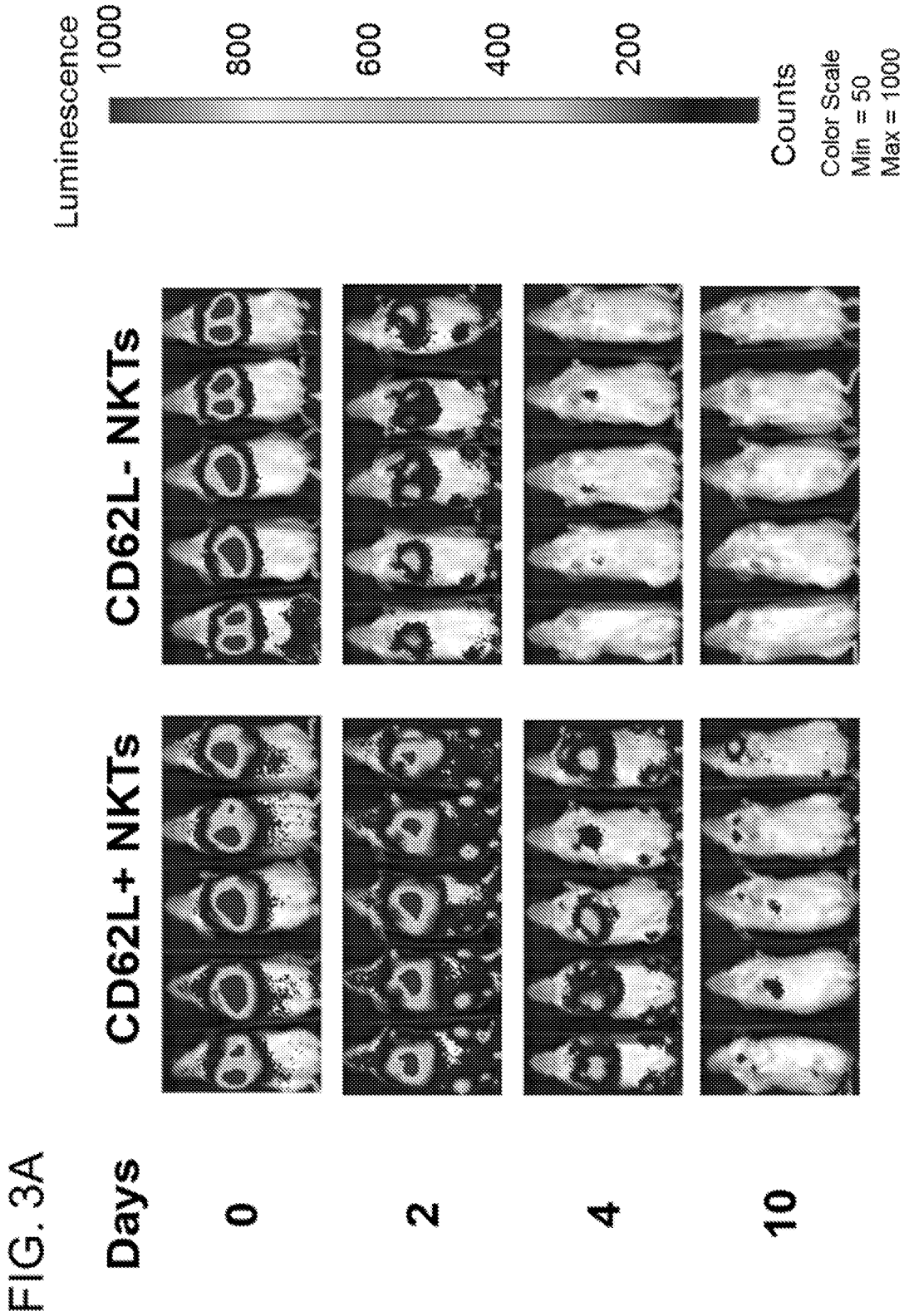

Luminescence
1000
800
600
400
200
Counts
Color Scale
Min = 50
Max = 1000
CD62L+ NKTs
CD62L- NKTs
Days
0
2
4
10

FIG. 3A

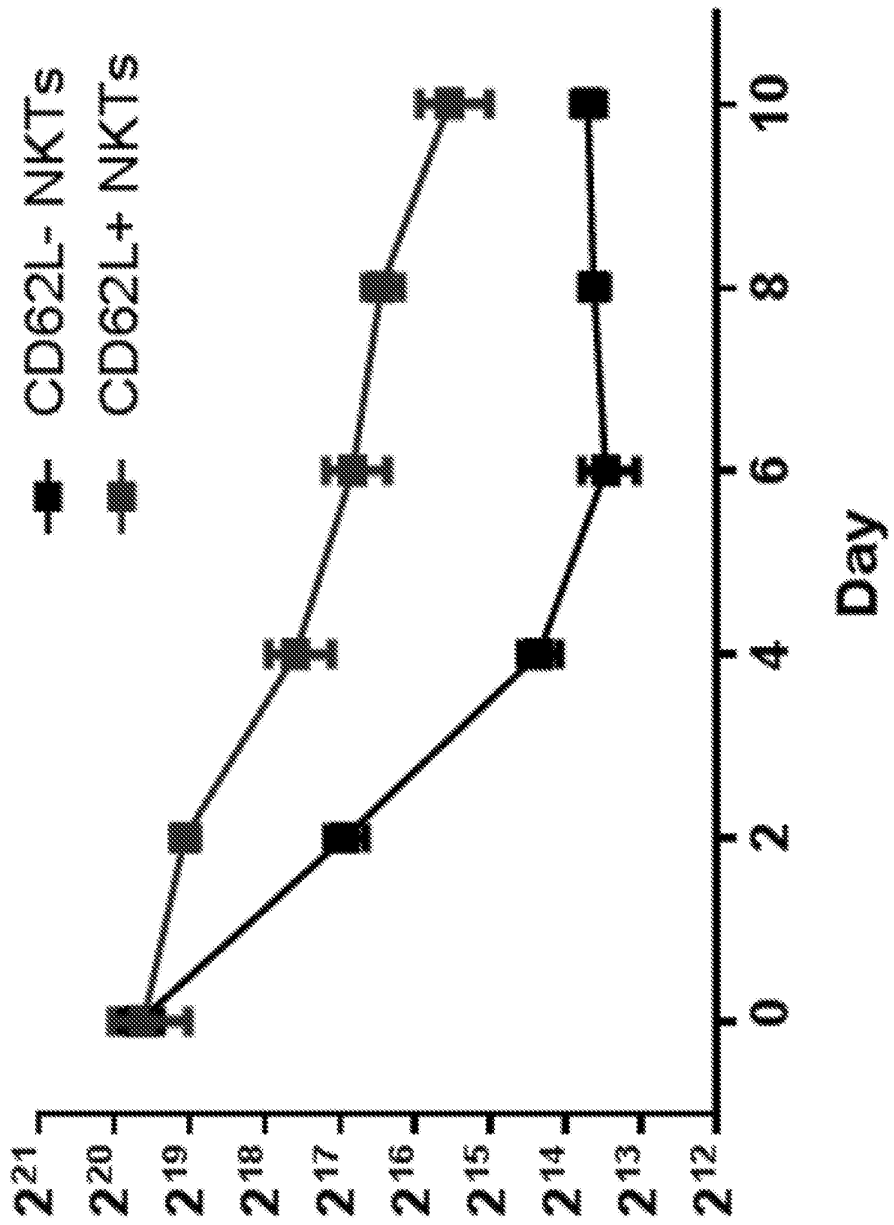

CD62L- NKTs
CD62L+ NKTs
Bioluminescence count
$2^{21}$
$2^{20}$
$2^{19}$
$2^{18}$
$2^{17}$
$2^{16}$
$2^{15}$
$2^{14}$
$2^{13}$
$2^{12}$
0
2
4
6
8
10
Day

FIG. 3B

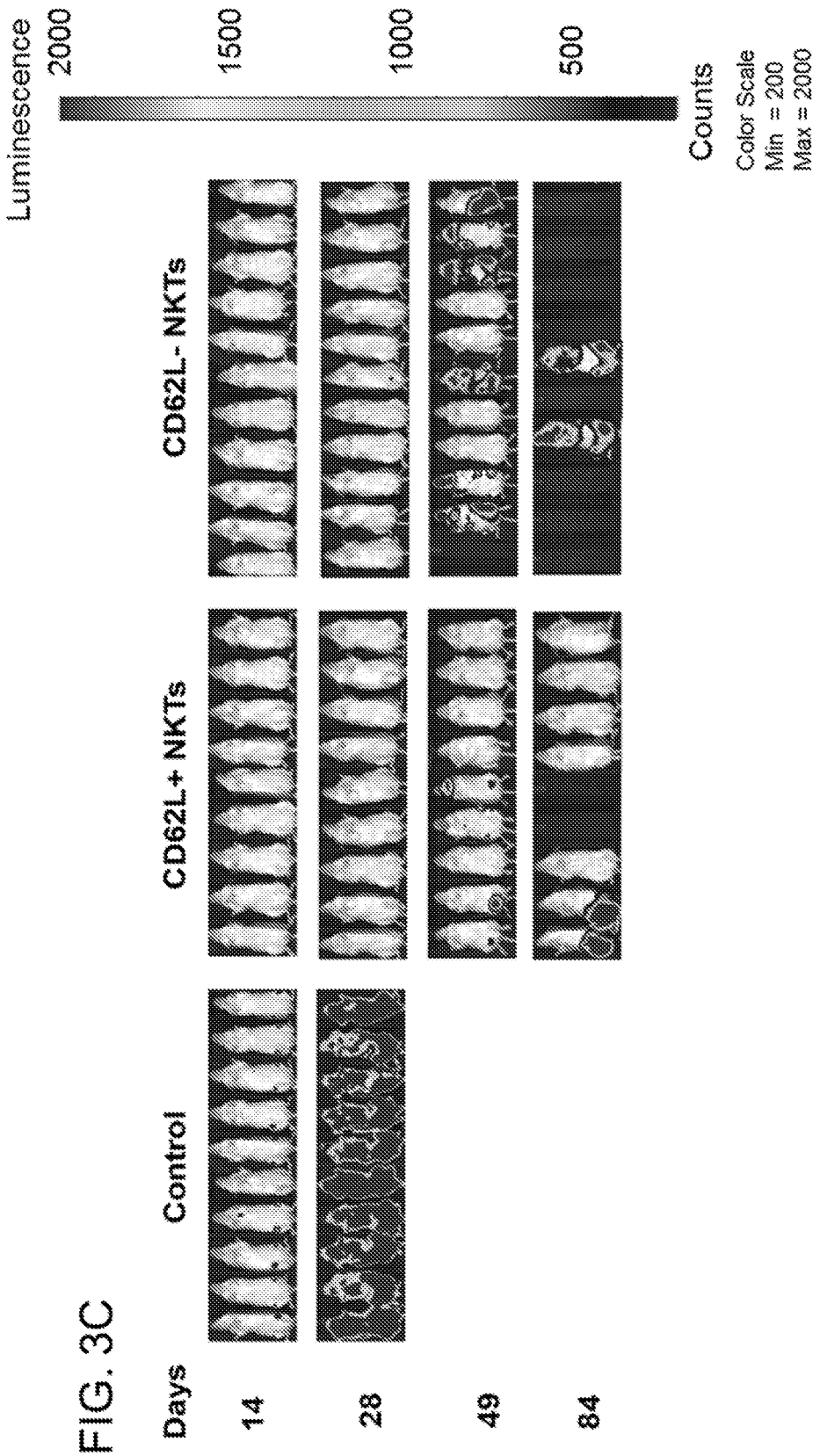

Luminescence
2000
1500
1000
500
Counts
Color Scale
Min = 200
Max = 2000
CD62L- NKTs
CD62L+ NKTs
Control
Days
14
28
49
84

FIG. 3C

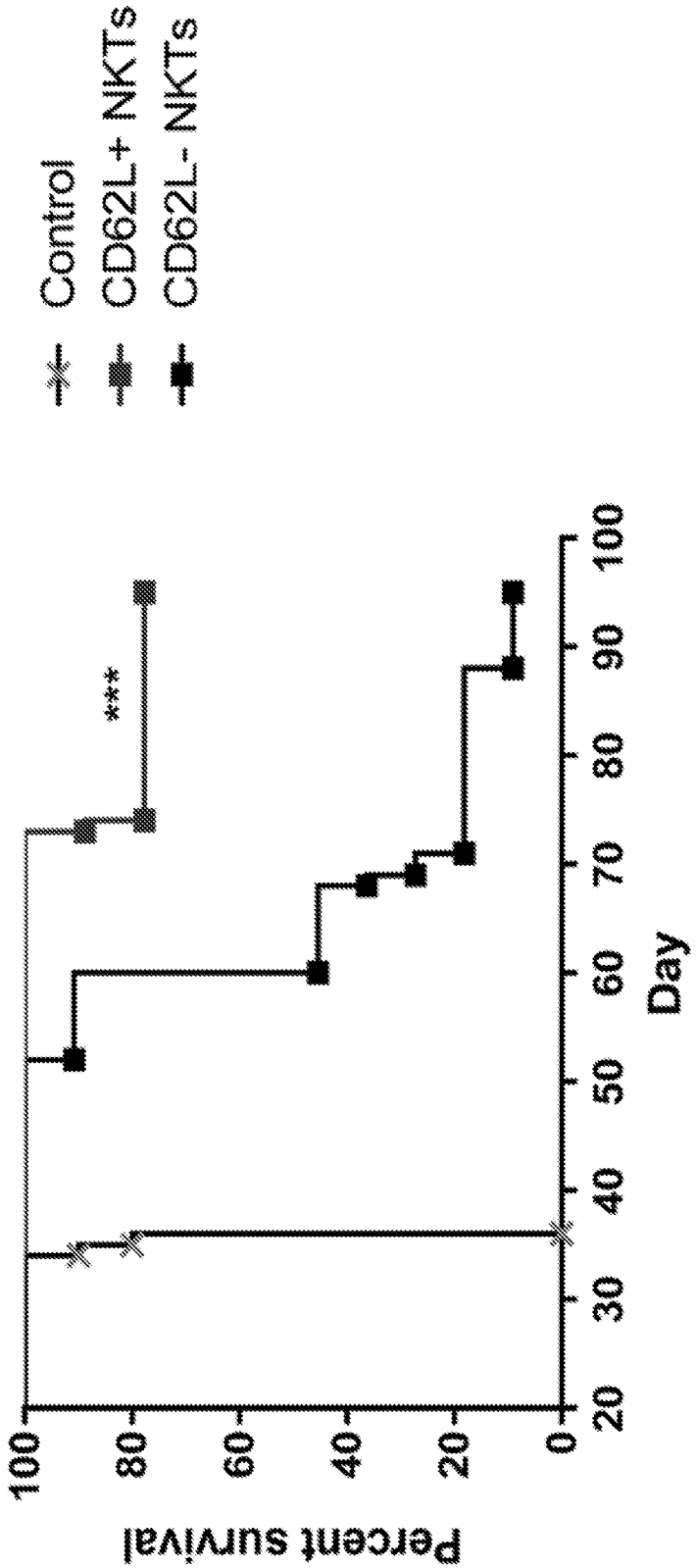

Control
CD62L+ NKTs
CD62L- NKTs
***
Percent survival
0
20
40
60
80
100
20
30
40
50
60
70
80
90
100
Day

FIG. 3D

FIG. 4A

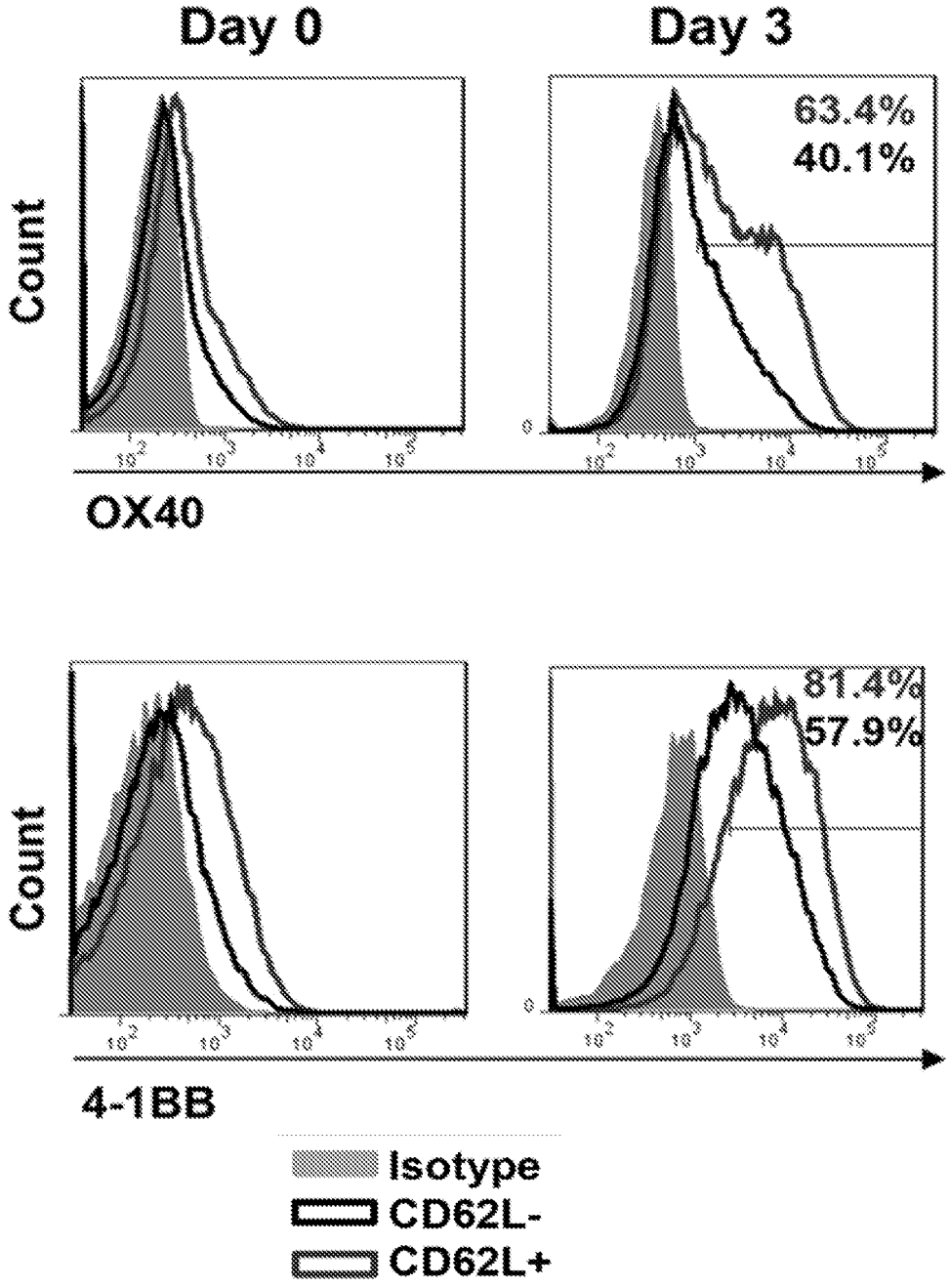
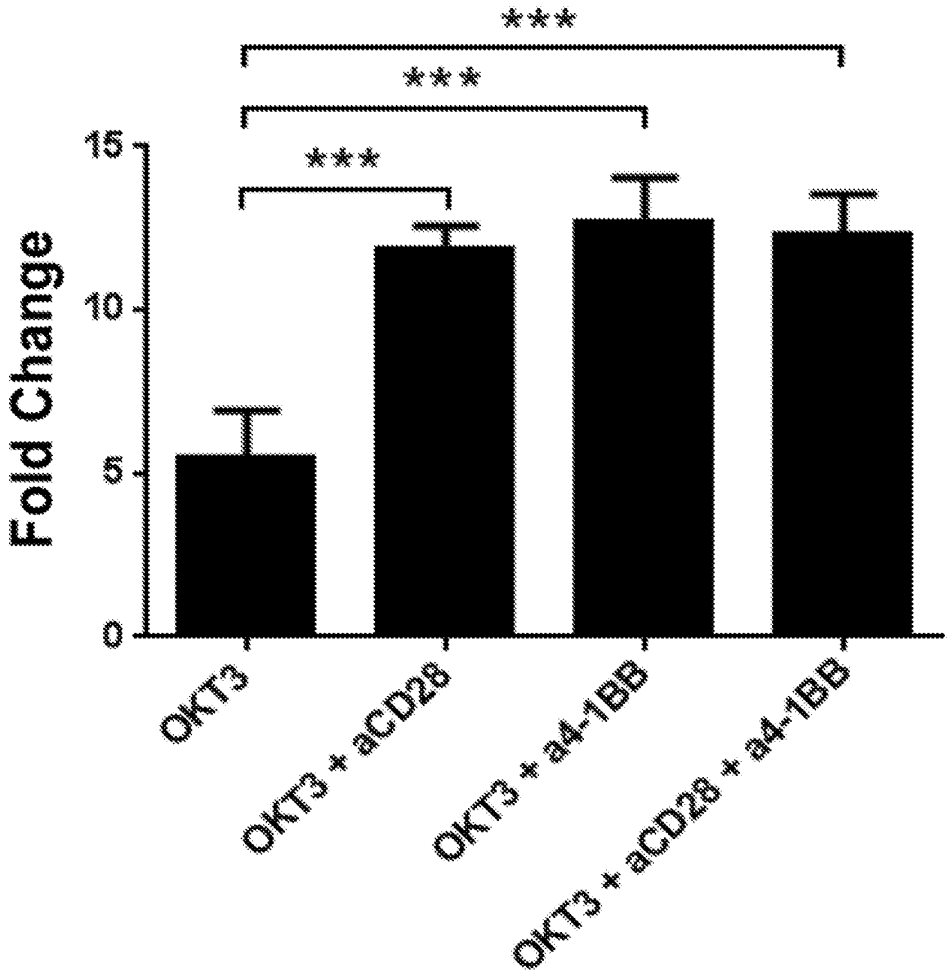

Day 0
Day 3
63.4%
40.1%
Count
OX40
81.4%
57.9%
Count
4-1BB
Isotype
CD62L-
CD62L+

FIG. 4B


***
***
***
15
10
5
0
Fold Change
OKT3
OKT3 + aCD28
OKT3 + a4-1BB
OKT3 + aCD28 + a4-1BB

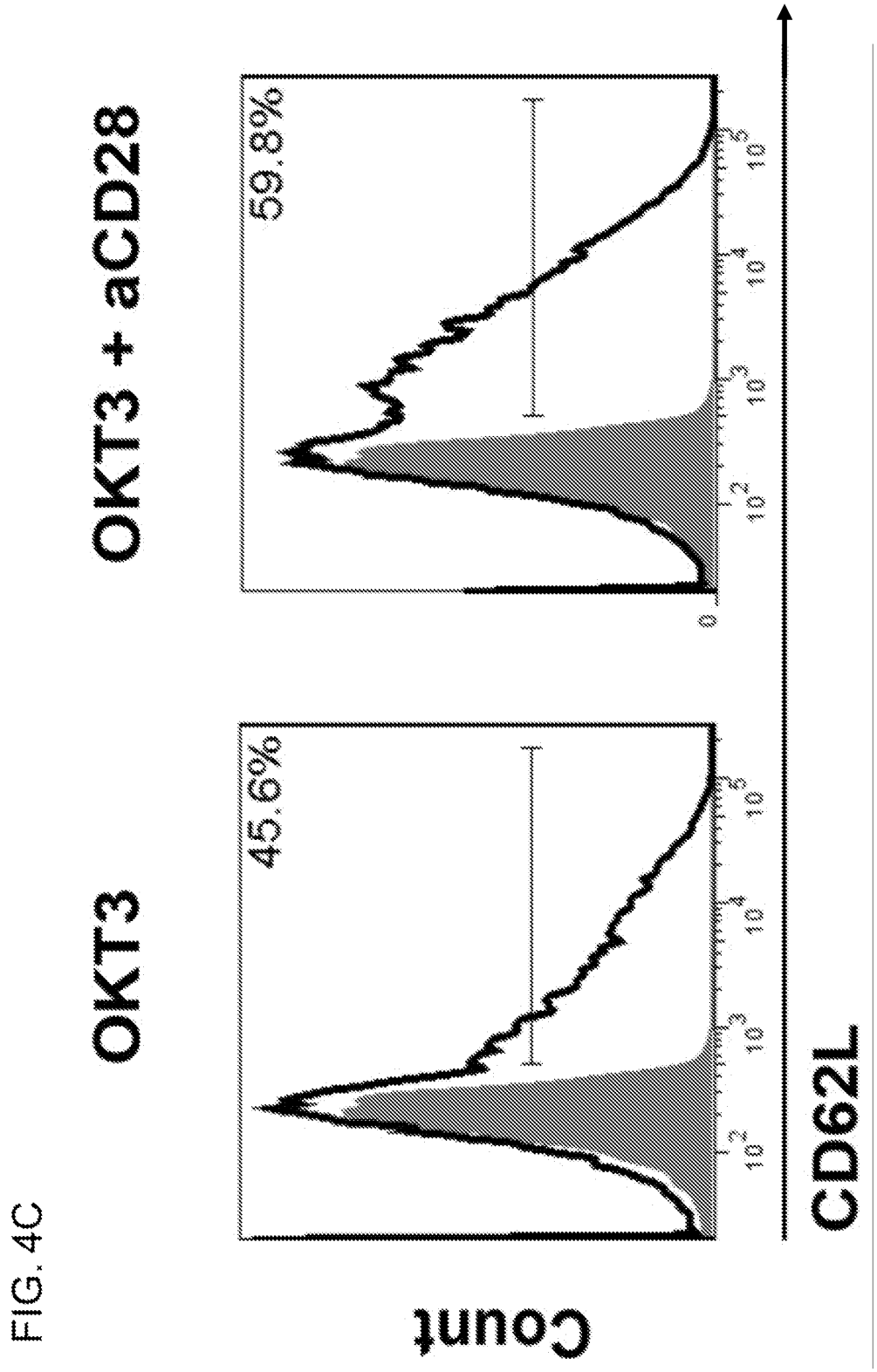



FIG. 4C

FIG. 4C

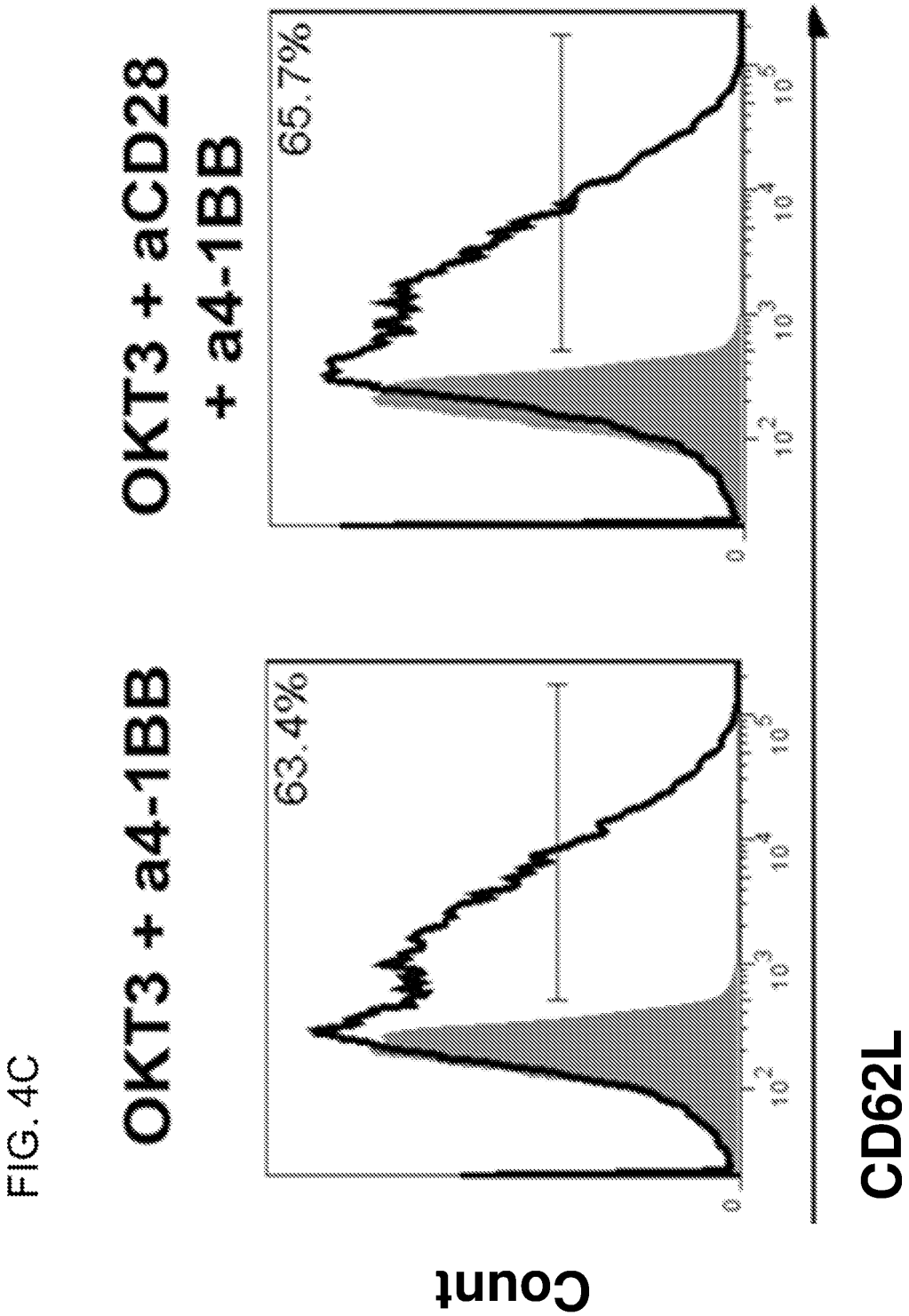
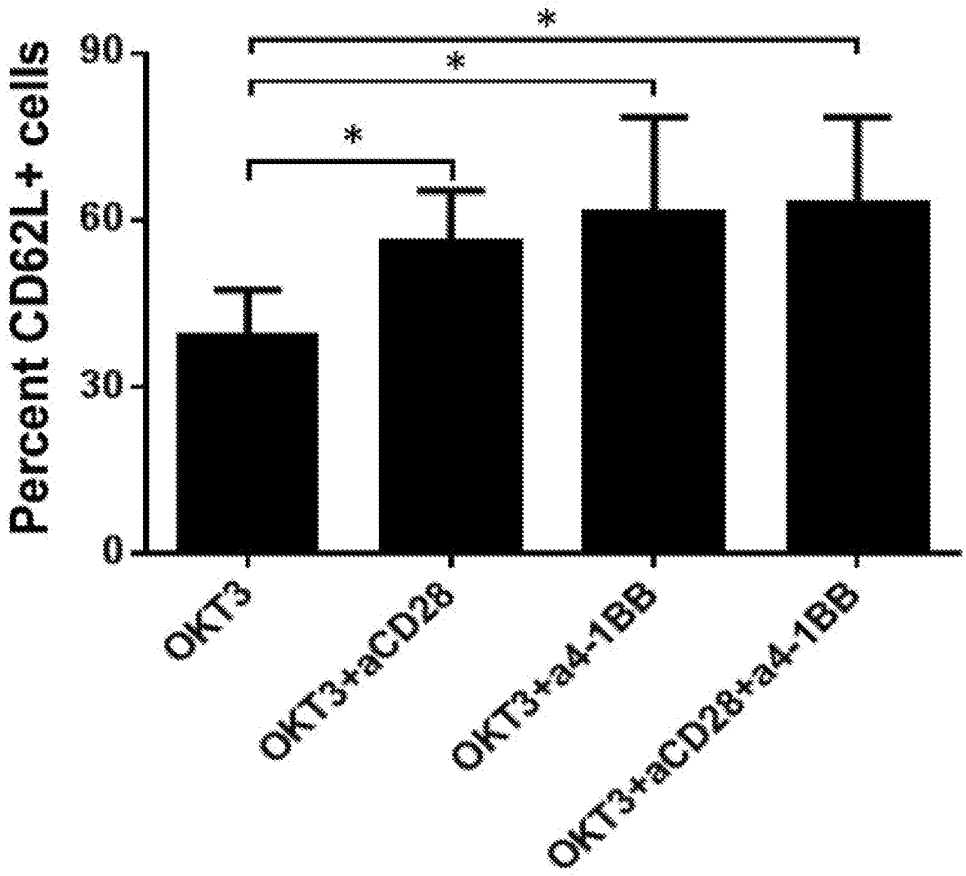

OKT3 + a4-1BB
63.4%
OKT3 + aCD28
+ a4-1BB
65.7%
Count
CD62L
0
10^2
10^3
10^4
10^5
10^6

FIG. 4C


Percent CD62L+ cells
90
60
30
0
*
*
*
OKT3
OKT3+aCD28
OKT3+a4-1BB
OKT3+aCD28+a4-1BB

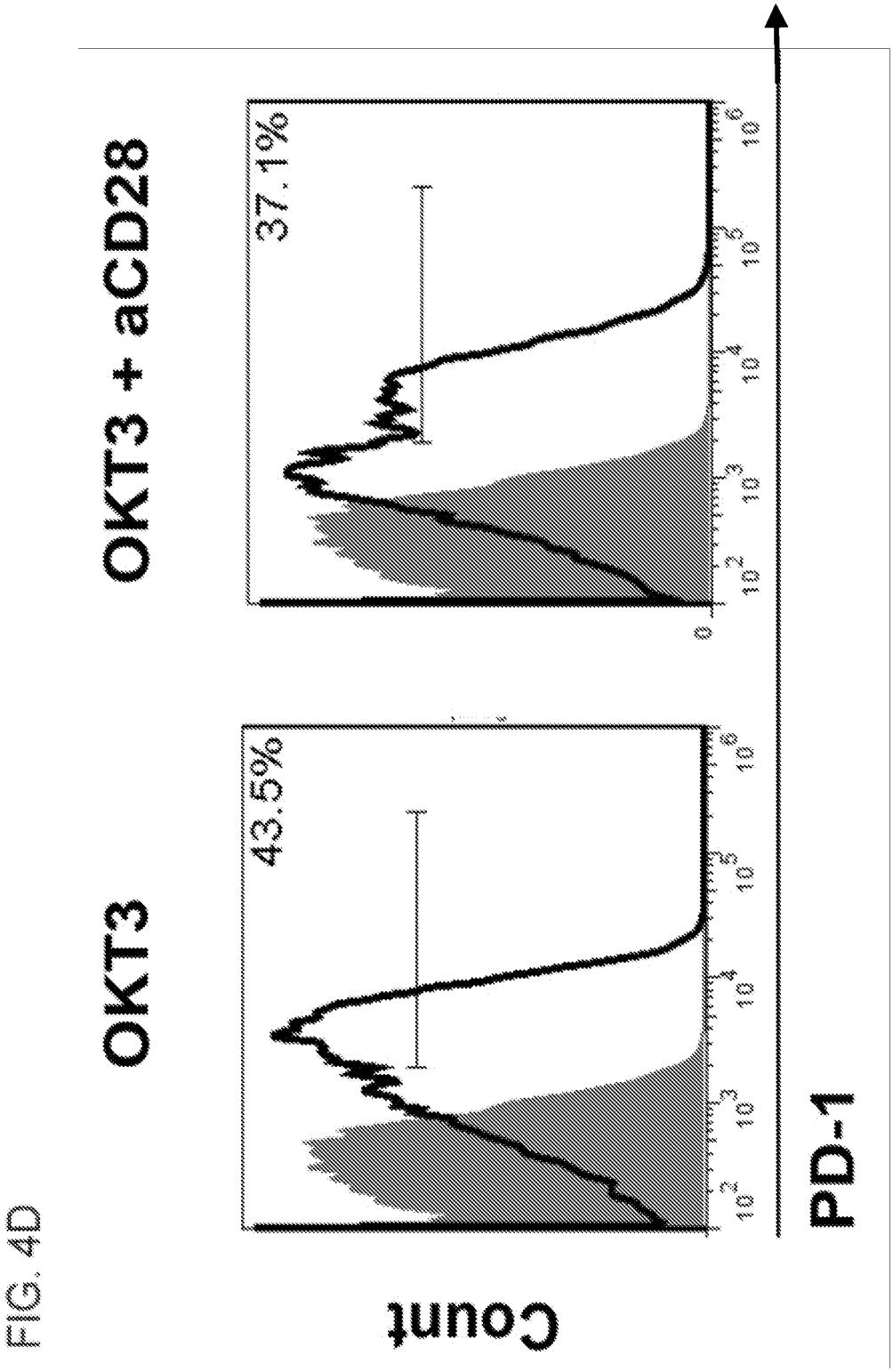



FIG. 4D

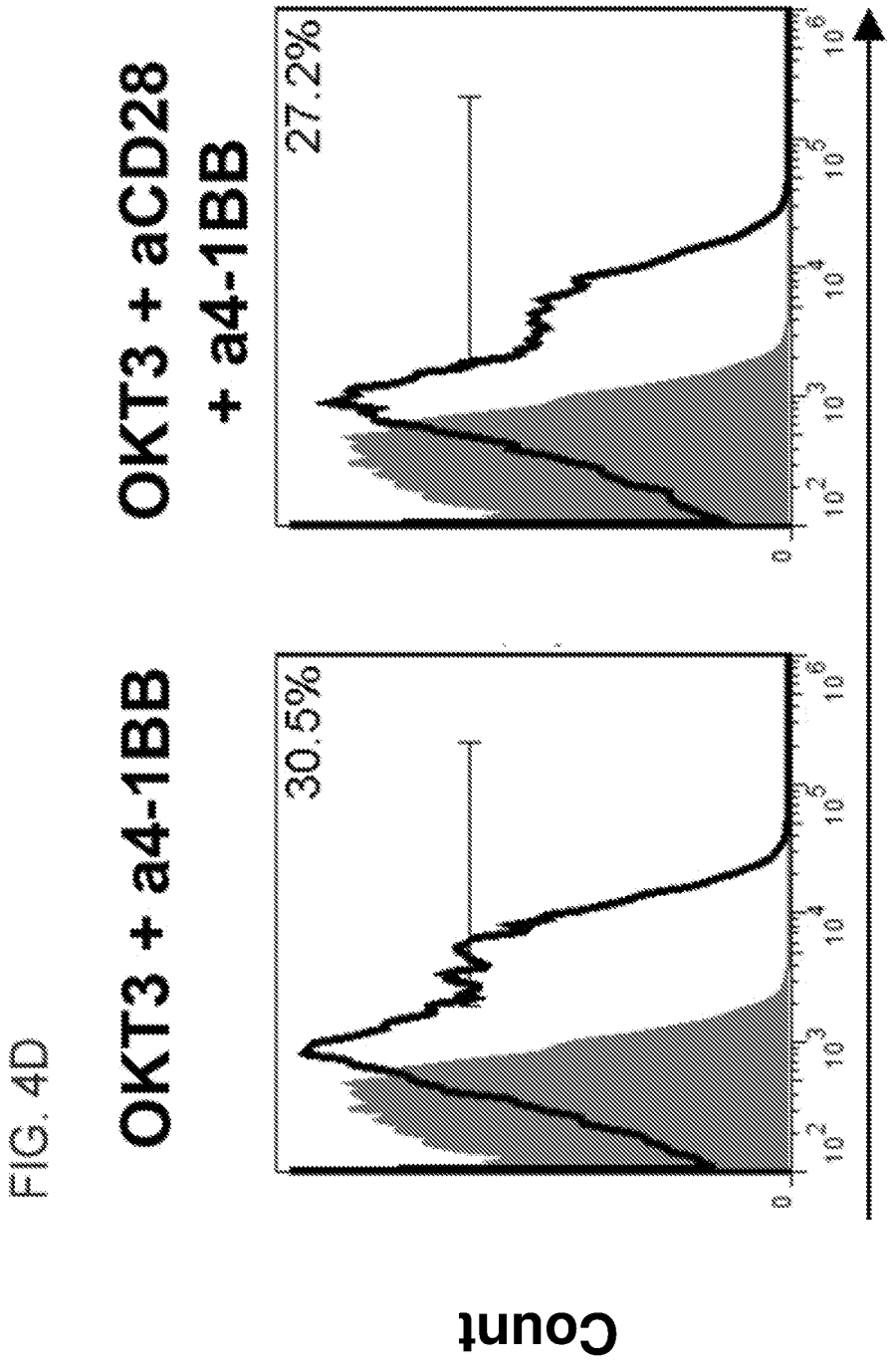
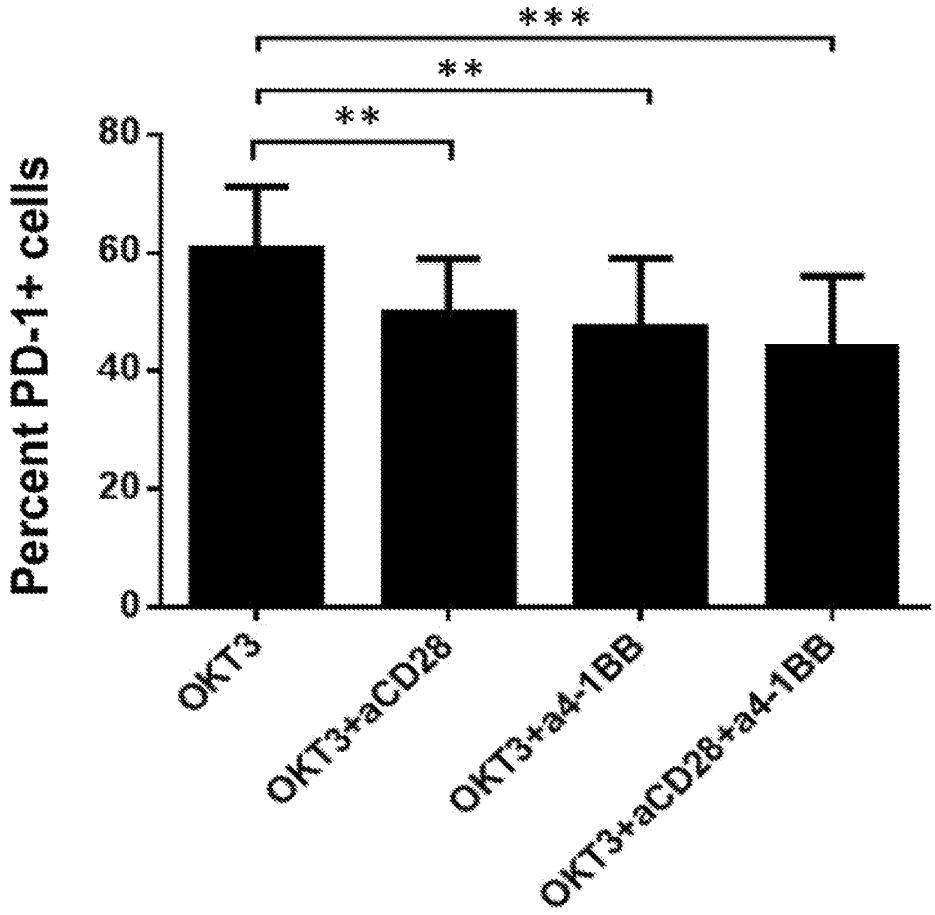



FIG. 4D

FIG. 4D


***
**
**
Percent PD-1+ cells
80
60
40
20
0
OKT3
OKT3+aCD28
OKT3+a4-1BB
OKT3+aCD28+a4-1BB

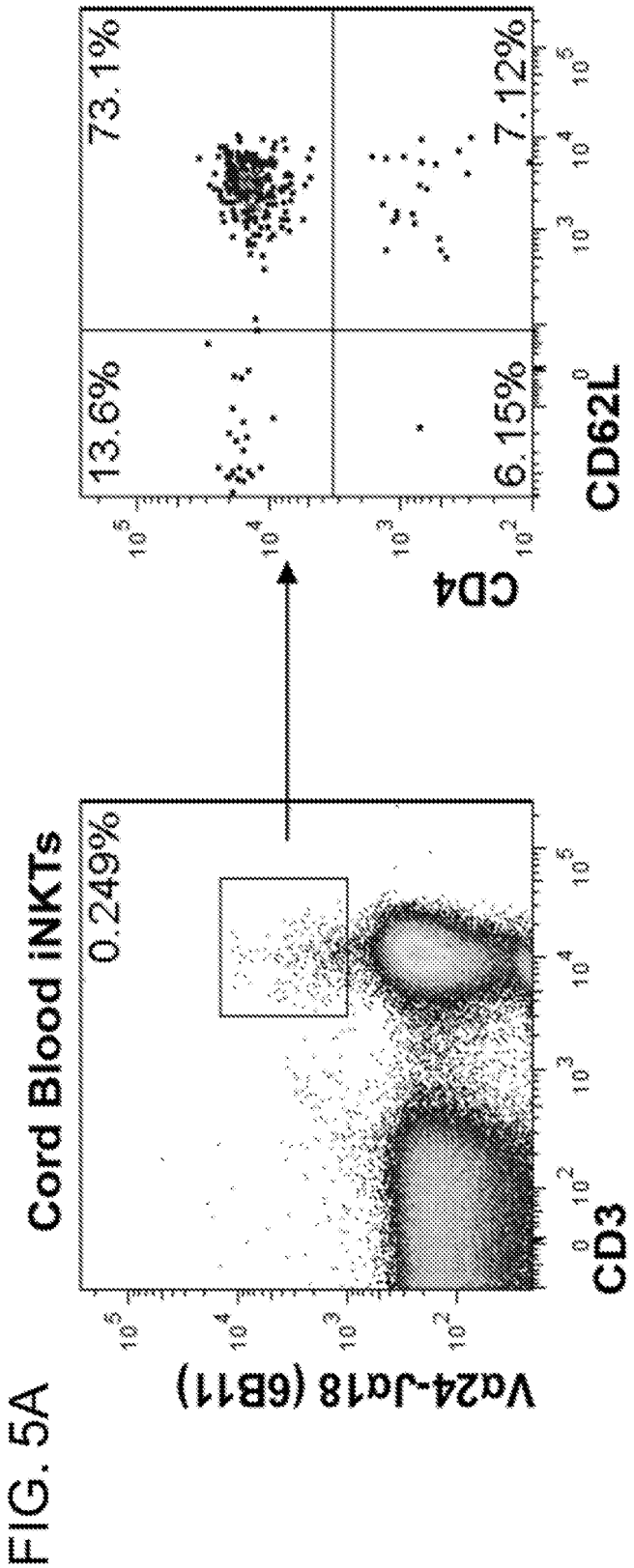

Cord Blood iNKTs
0.249%
Vα24-Jα18 (6B11)
CD3
CD4
CD62L
73.1%
7.12%
13.6%
6.15%

FIG. 5A

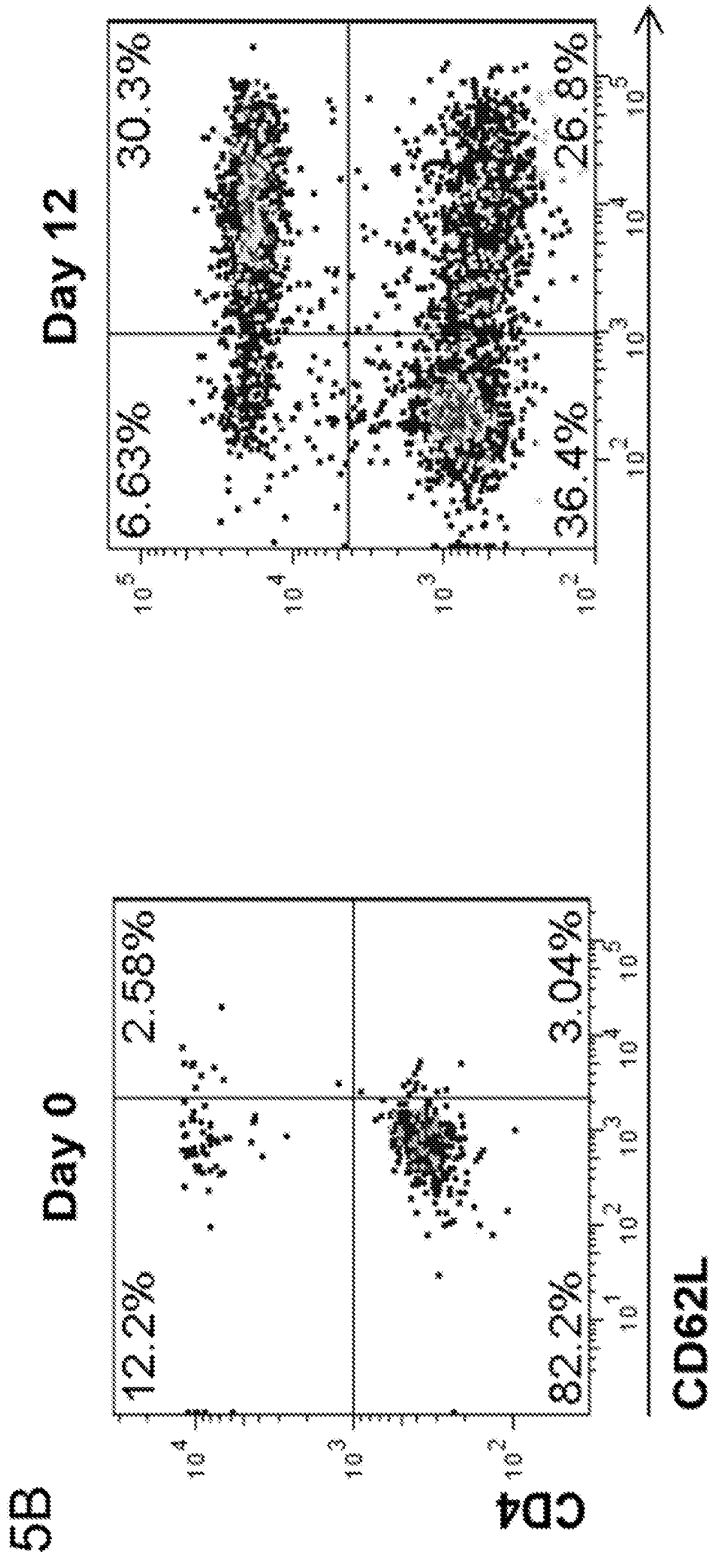
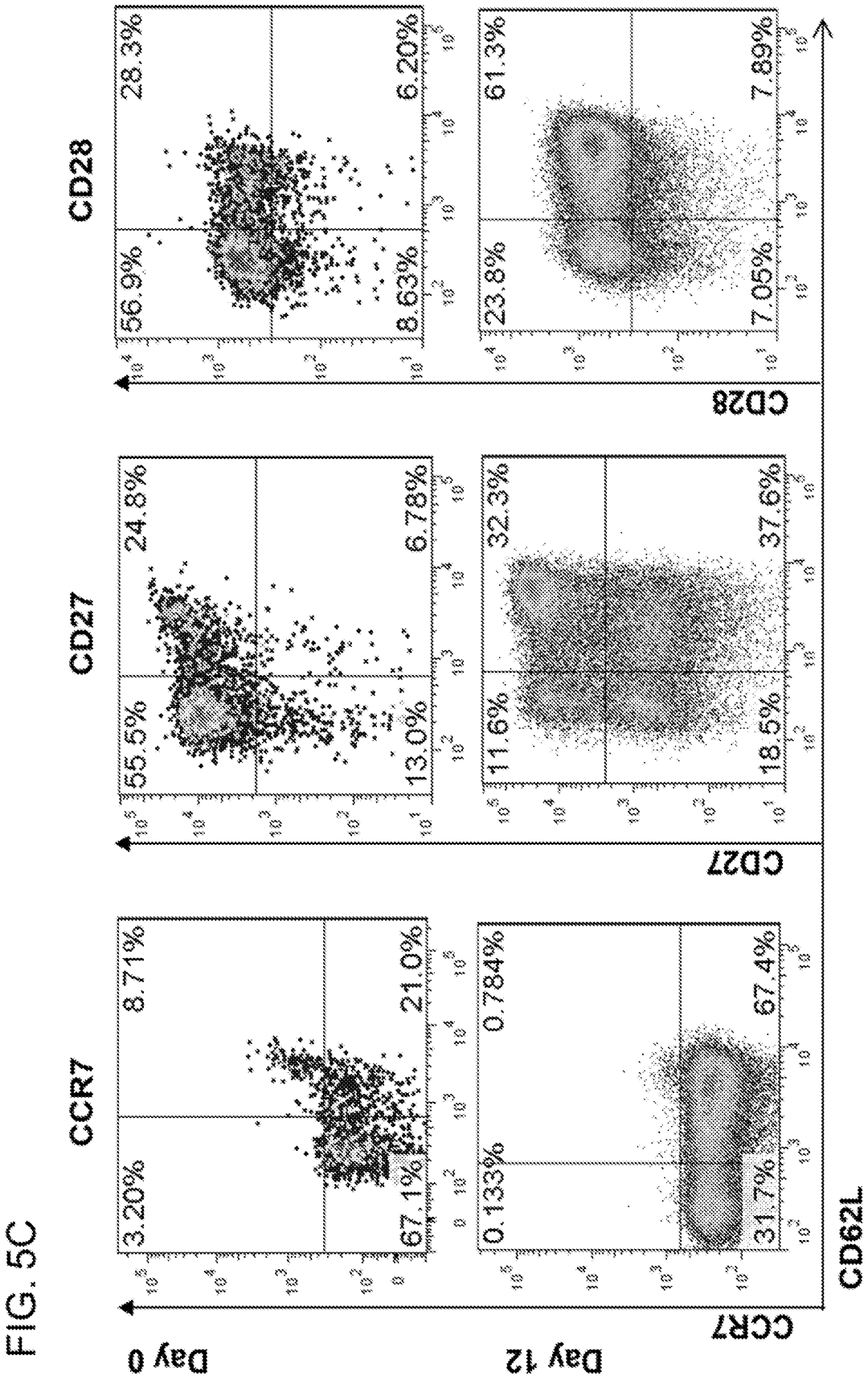



FIG. 5B

FIG. 5C


Day 0
Day 12
CCR7
CD62L
CD27
CD28
3.20%
8.71%
67.1%
21.0%
0.133%
0.784%
31.7%
67.4%
55.5%
24.8%
13.0%
6.78%
11.6%
32.3%
18.5%
37.6%
56.9%
28.3%
8.63%
6.20%
23.8%
61.3%
7.05%
7.89%

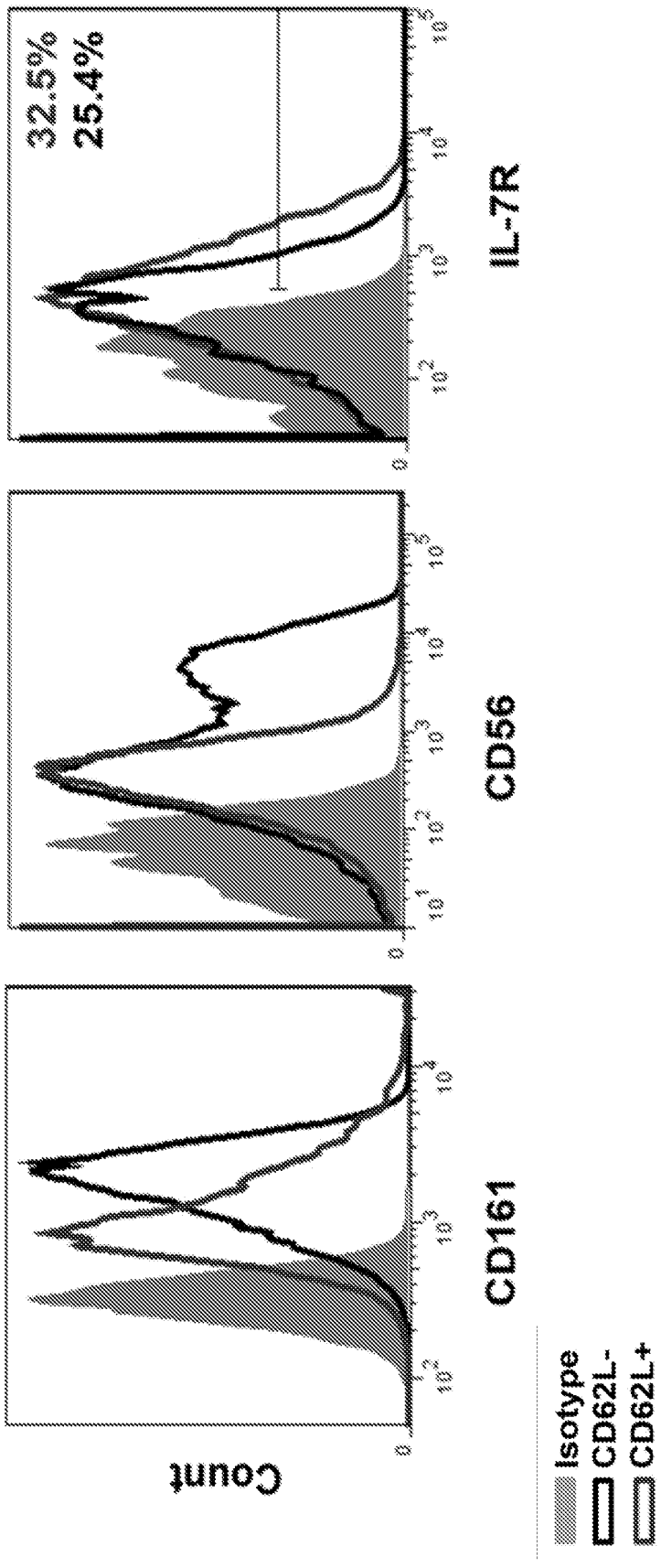



FIG. 5D

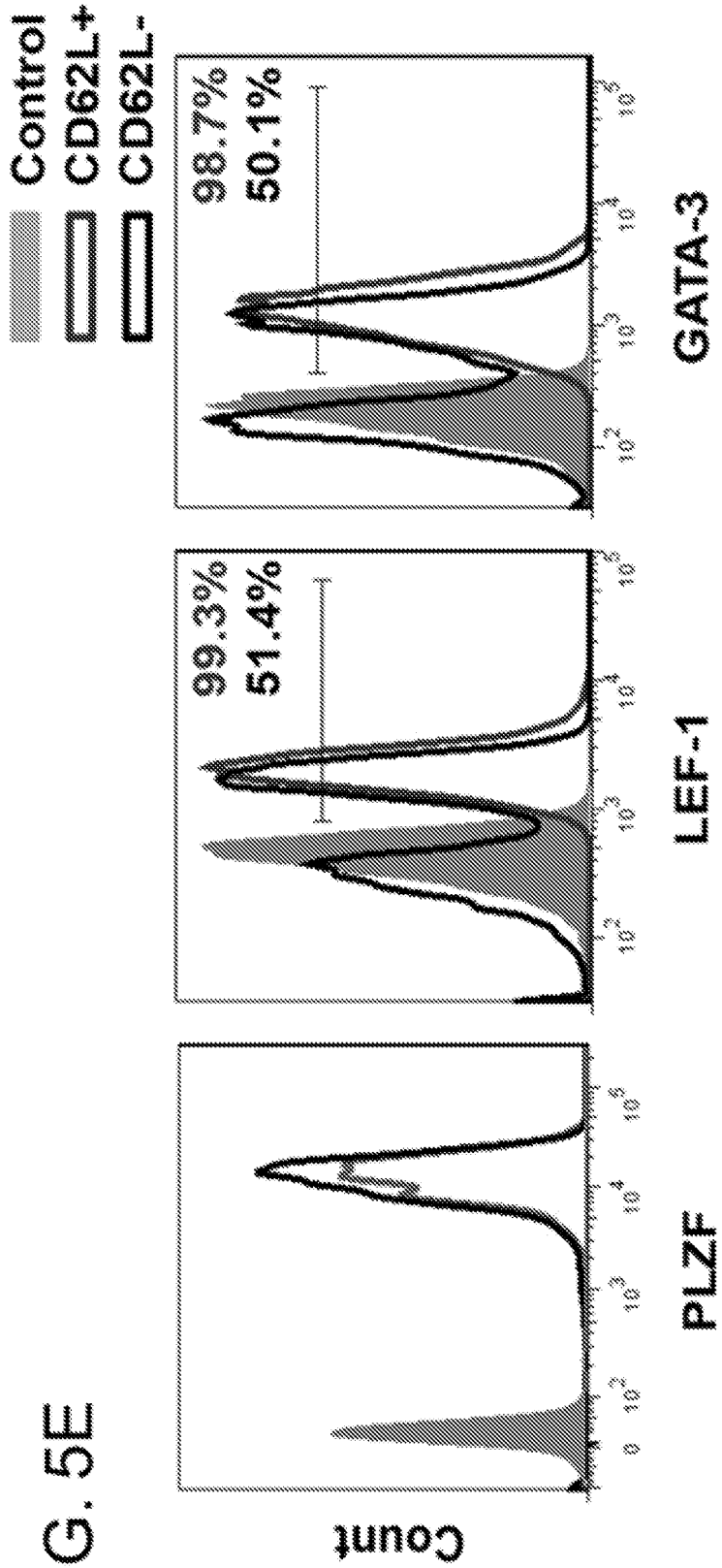
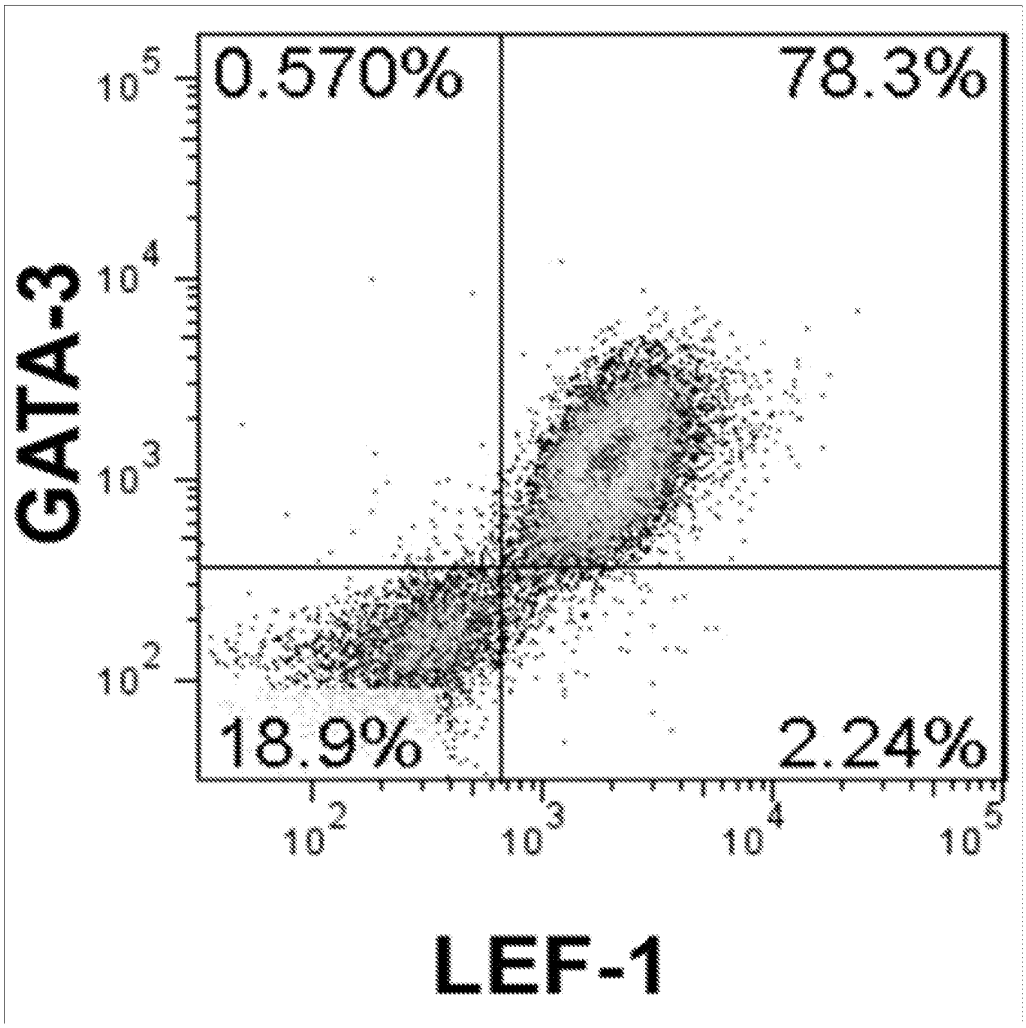



FIG. 5E

FIG. 5E



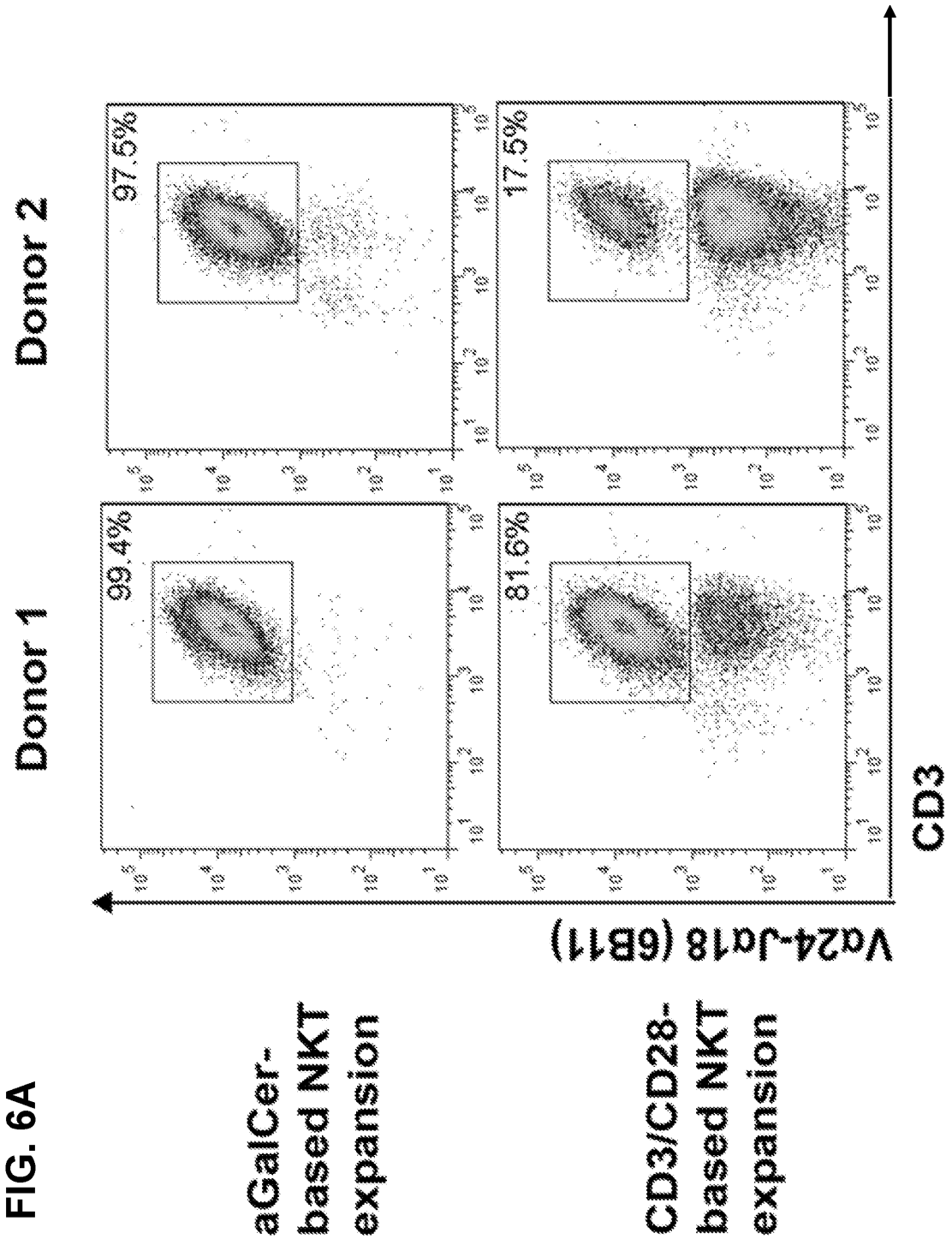

Donor 2
Donor 1
97.5%
17.5%
99.4%
81.6%
Vα24-Jα18 (6B11)
CD3
aGalCer-based NKT expansion
CD3/CD28-based NKT expansion

FIG. 6A

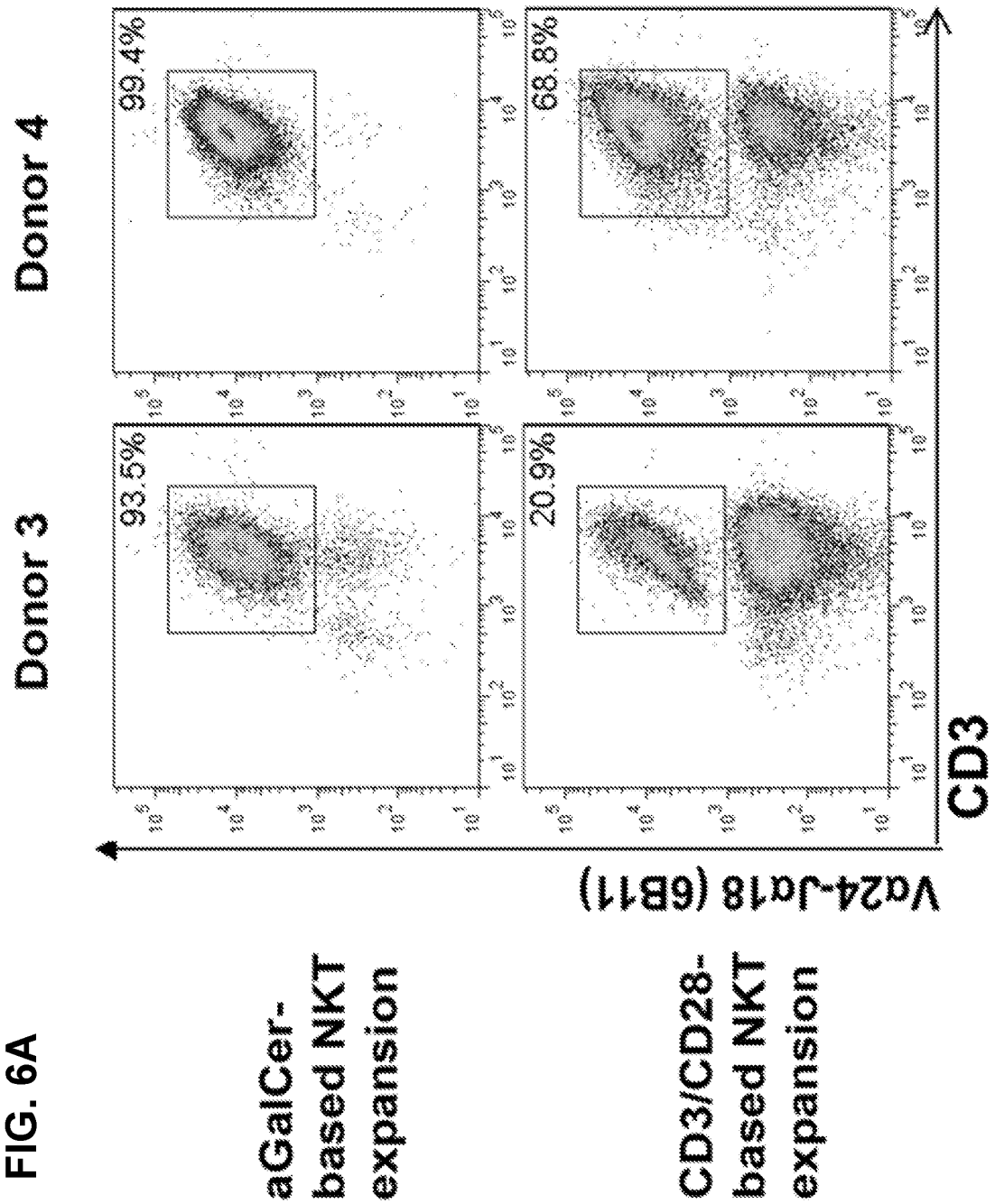

Donor 4
Donor 3
99.4%
68.8%
93.5%
20.9%
Vα24-Jα18 (6B11)
CD3
aGalCer-based NKT expansion
CD3/CD28-based NKT expansion

FIG. 6A

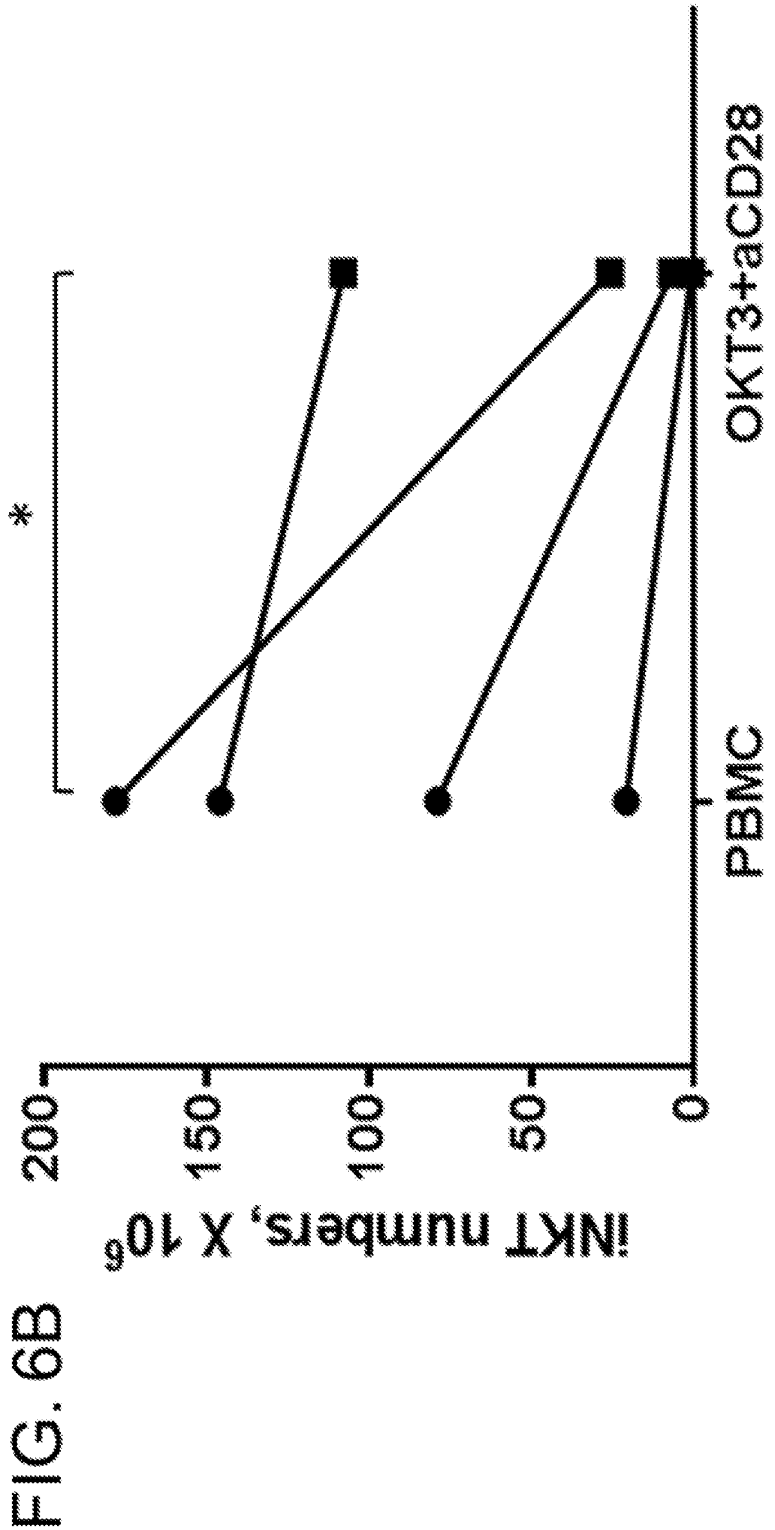



FIG. 6B

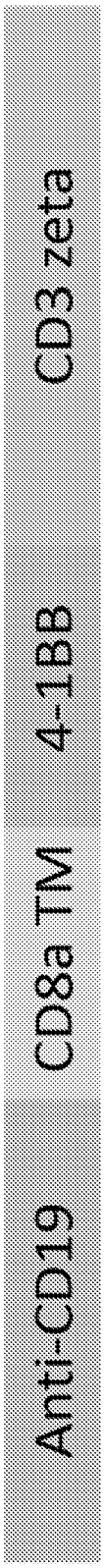



FIG. 7A

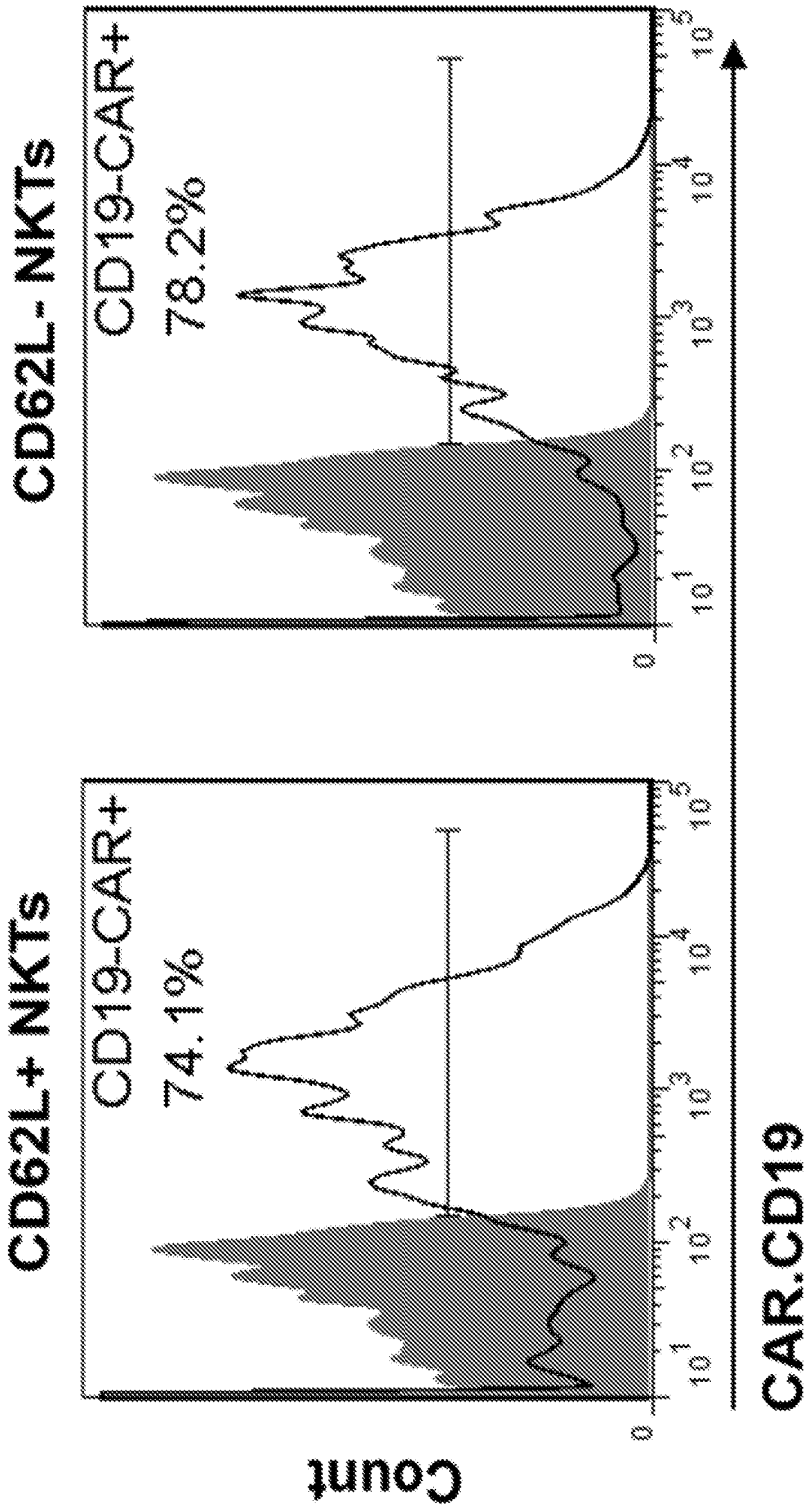

CD62L- NKTs
CD19-CAR+
78.2%
CD62L+ NKTs
CD19-CAR+
74.1%
Count
CAR.CD19
0
10^1
10^2
10^3
10^4
10^5

FIG. 7B

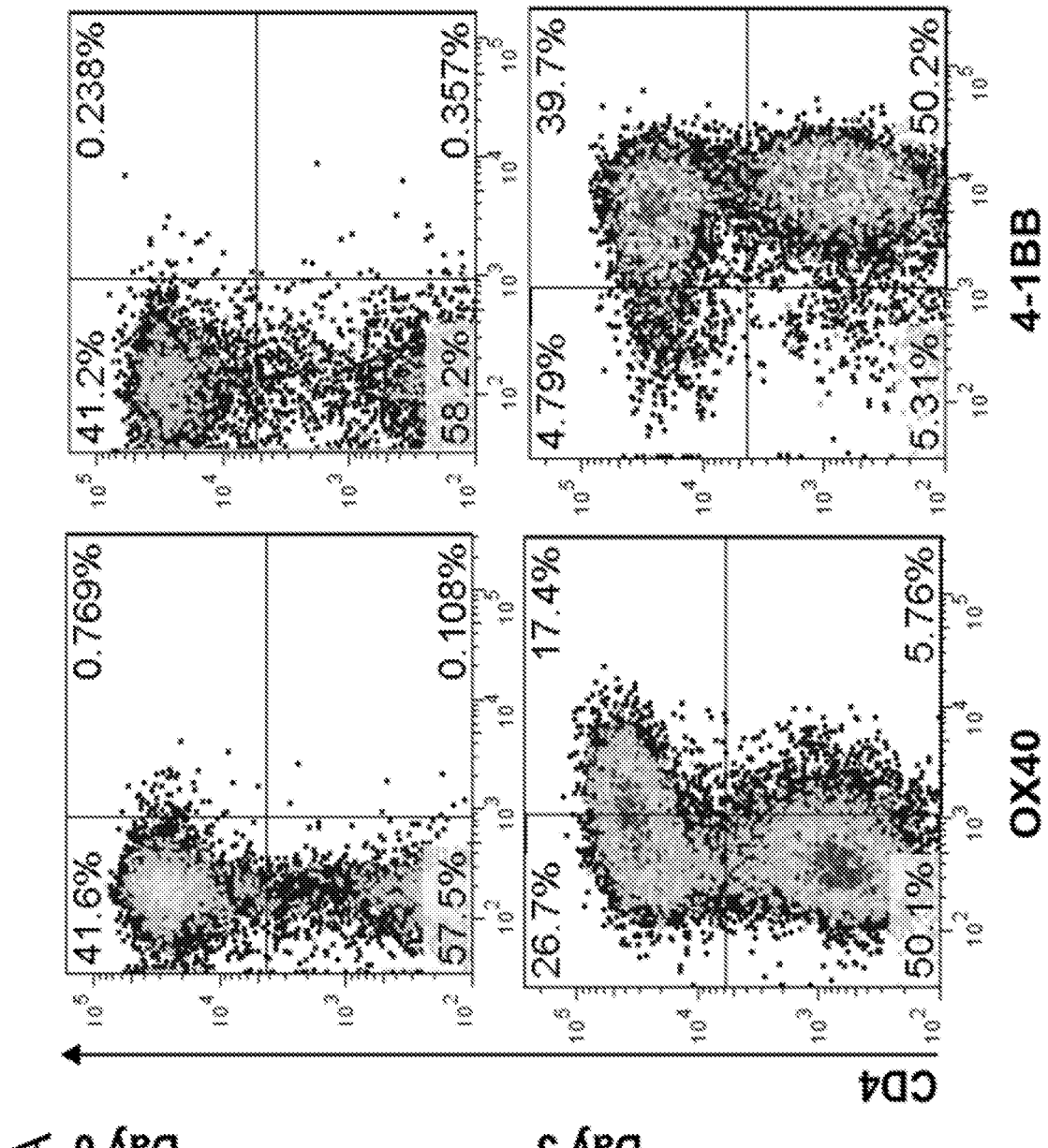
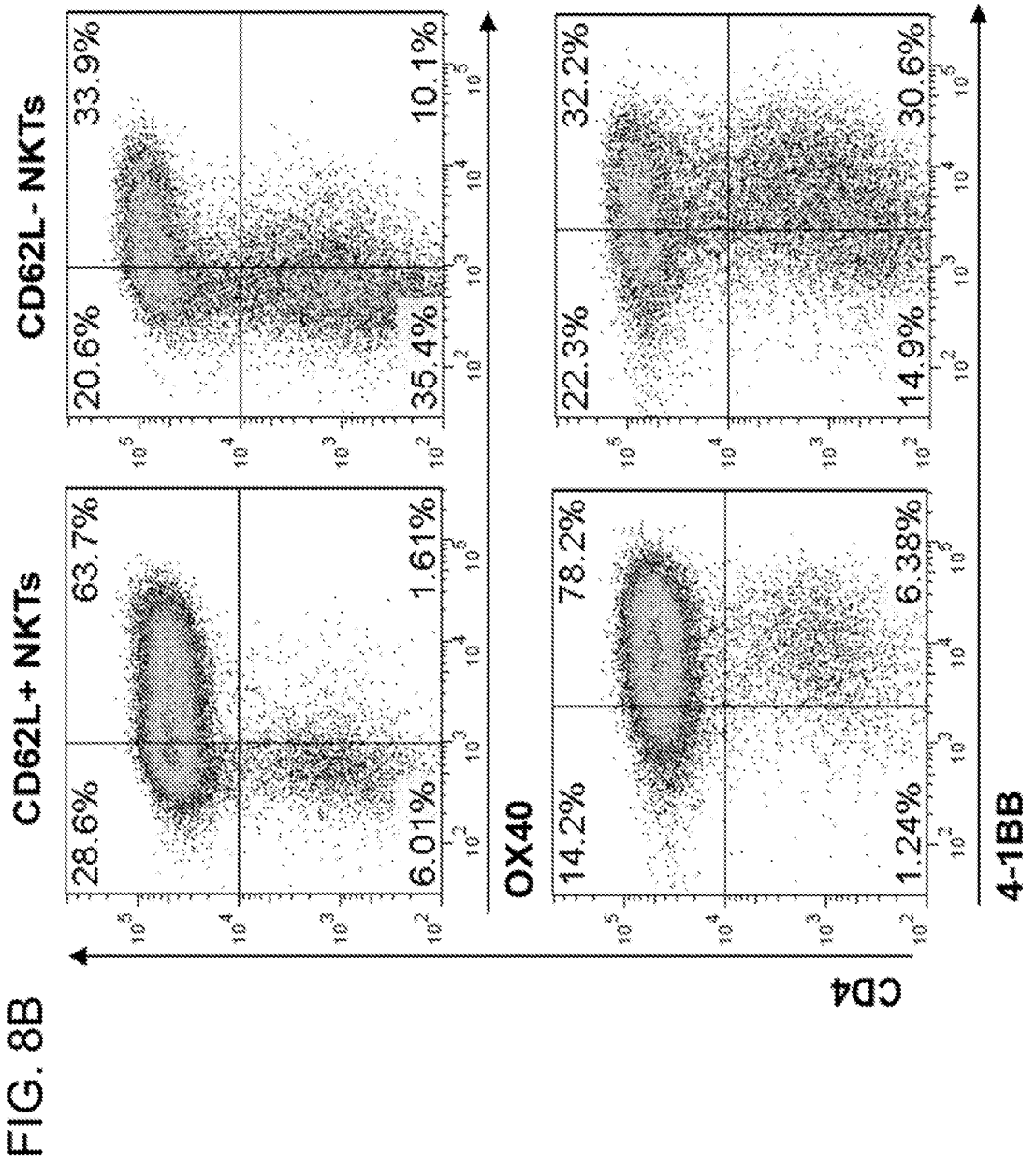

Day 0
Day 3
CD4
OX40
4-1BB
0.238%
0.357%
41.2%
68.2%
39.7%
50.2%
4.79%
6.31%
0.769%
0.108%
41.6%
57.5%
17.4%
5.76%
26.7%
50.1%

FIG. 8A

FIG. 8B


CD62L+ NKTs
CD62L- NKTs
28.6%
63.7%
6.01%
1.61%
20.6%
33.9%
35.4%
10.1%
14.2%
78.2%
1.24%
6.38%
22.3%
32.2%
14.9%
30.6%
OX40
4-1BB
CD4

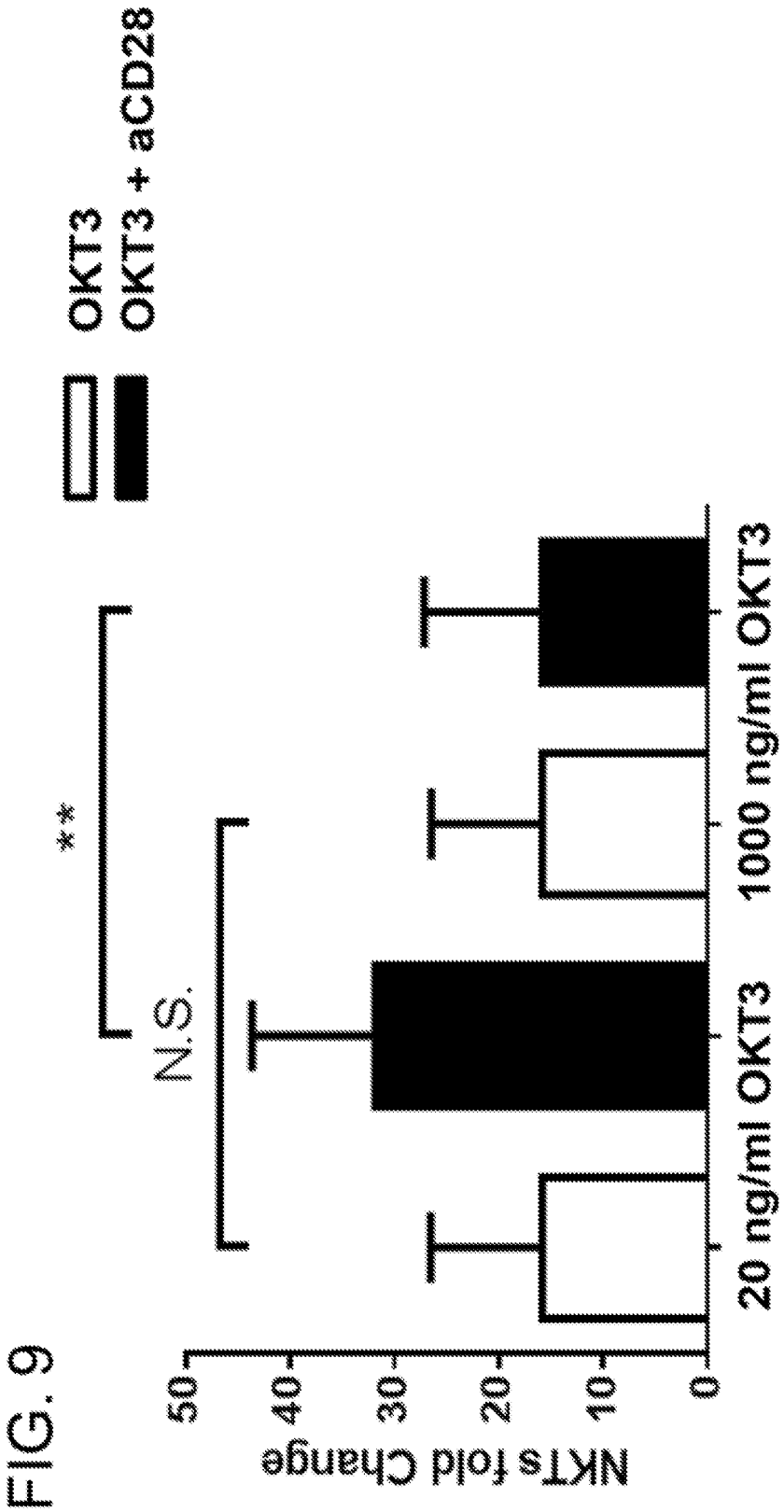

OKT3
OKT3 + aCD28
**
N.S.
NKTs fold Change
50
40
30
20
10
0
20 ng/ml OKT3
1000 ng/ml OKT3

FIG. 9

FIG. 10

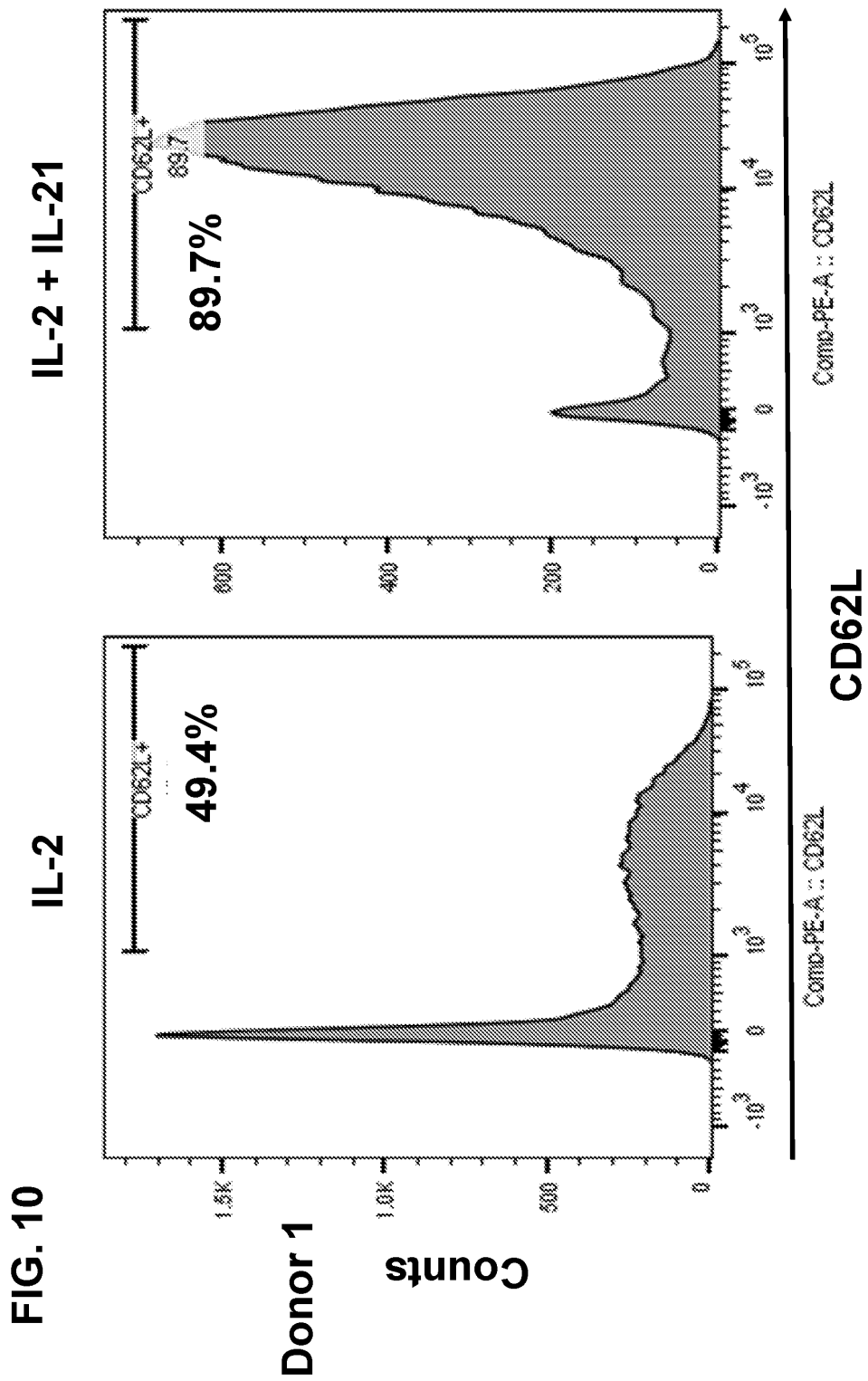
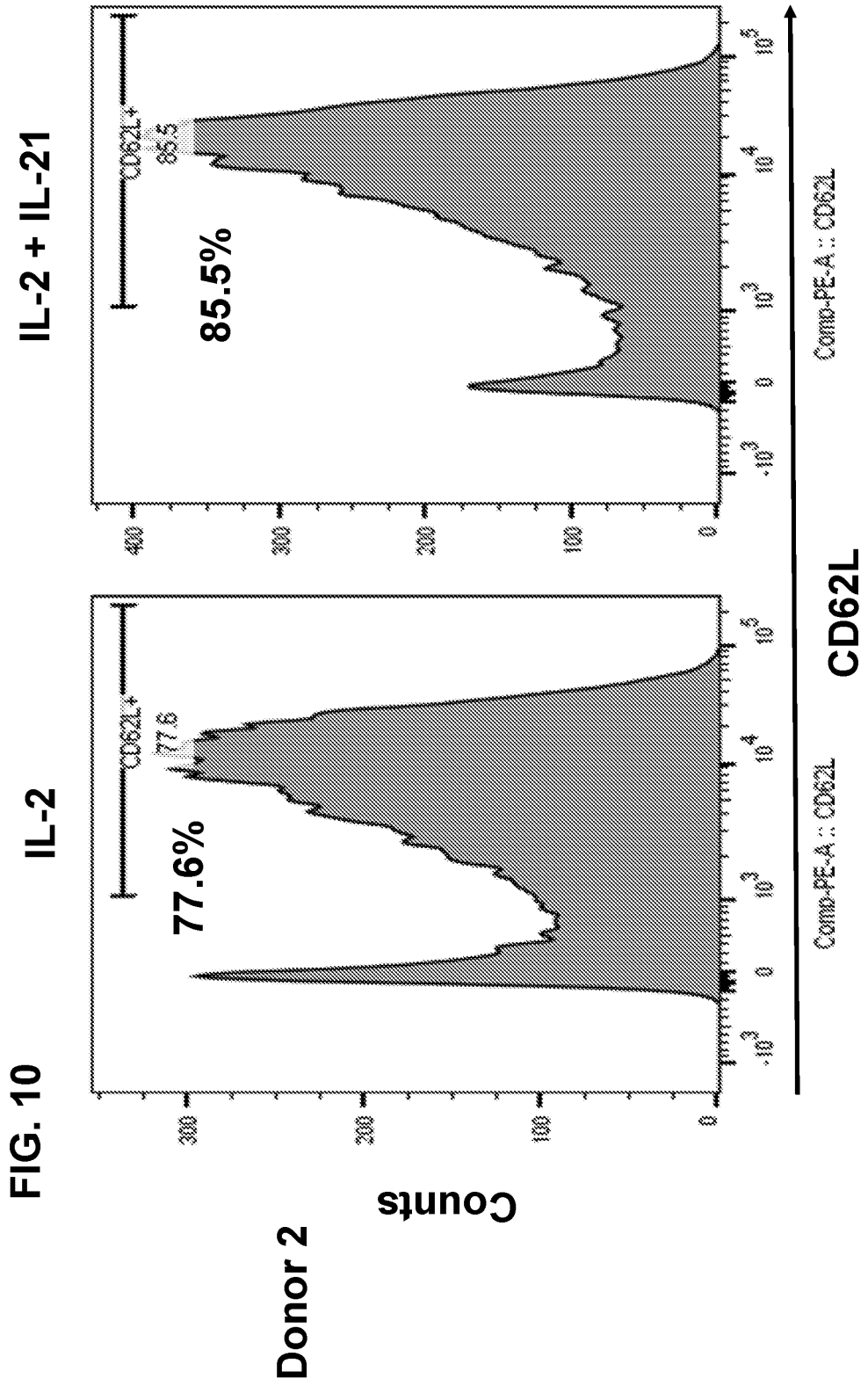

IL-2
IL-2 + IL-21
Donor 1
Counts
CD62L
CD62L+
49.4%
89.7%
89.7
Comb-PE-A :: CD62L
Comb-PE-A :: CD62L
0
500
1.0K
1.5K
200
400
600
-10^3
0
10^3
10^4
10^5

FIG. 10


IL-2
IL-2 + IL-21
Donor 2
Counts
CD62L
CD62L+
77.6
77.6%
85.5
85.5%
Comp-PE-A :: CD62L

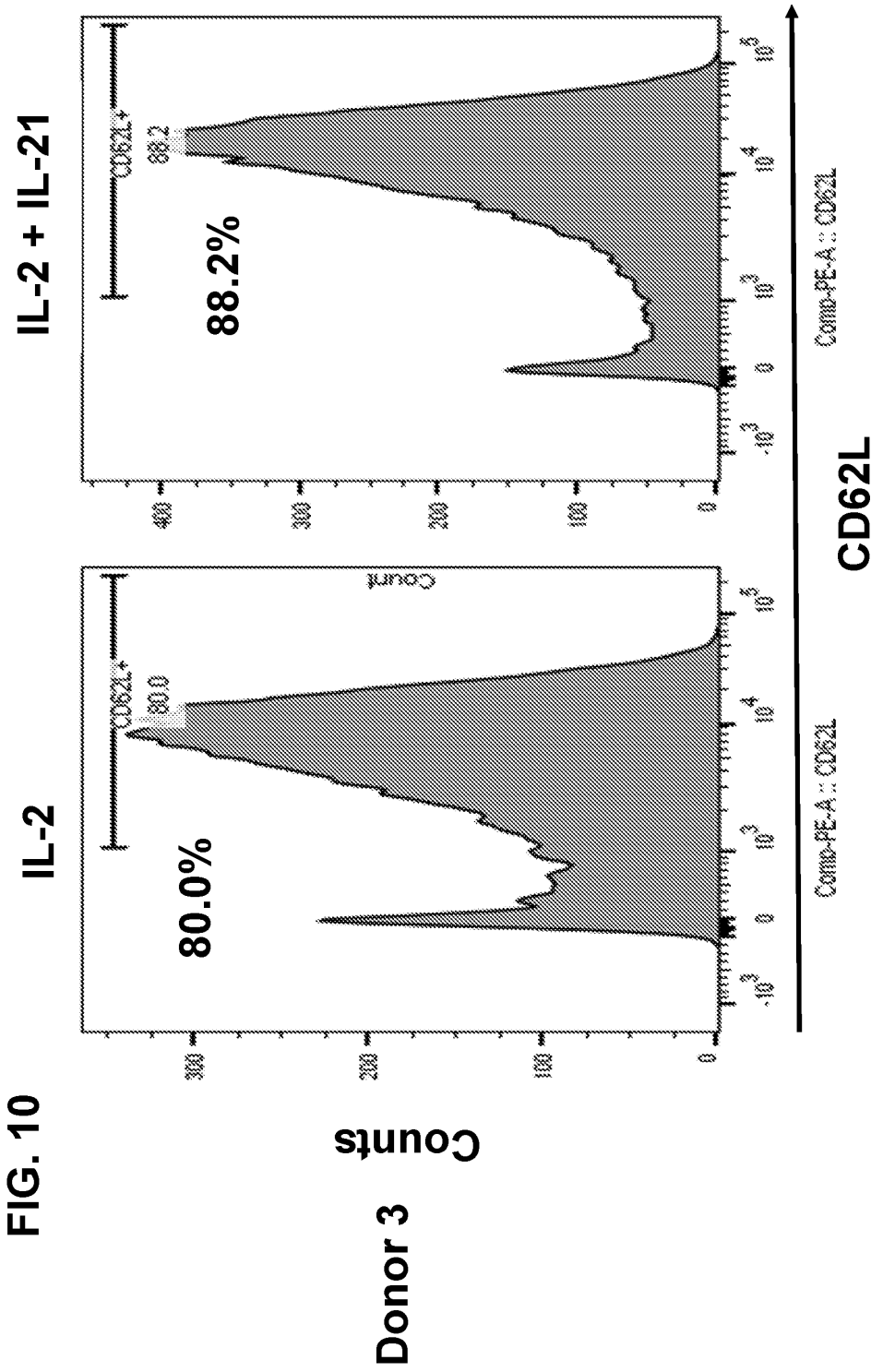

IL-2
IL-2 + IL-21
80.0%
88.2%
CD62L+
80.0
88.2
Count
Comp-PE-A :: CD62L
CD62L
Counts
Donor 3

FIG. 10

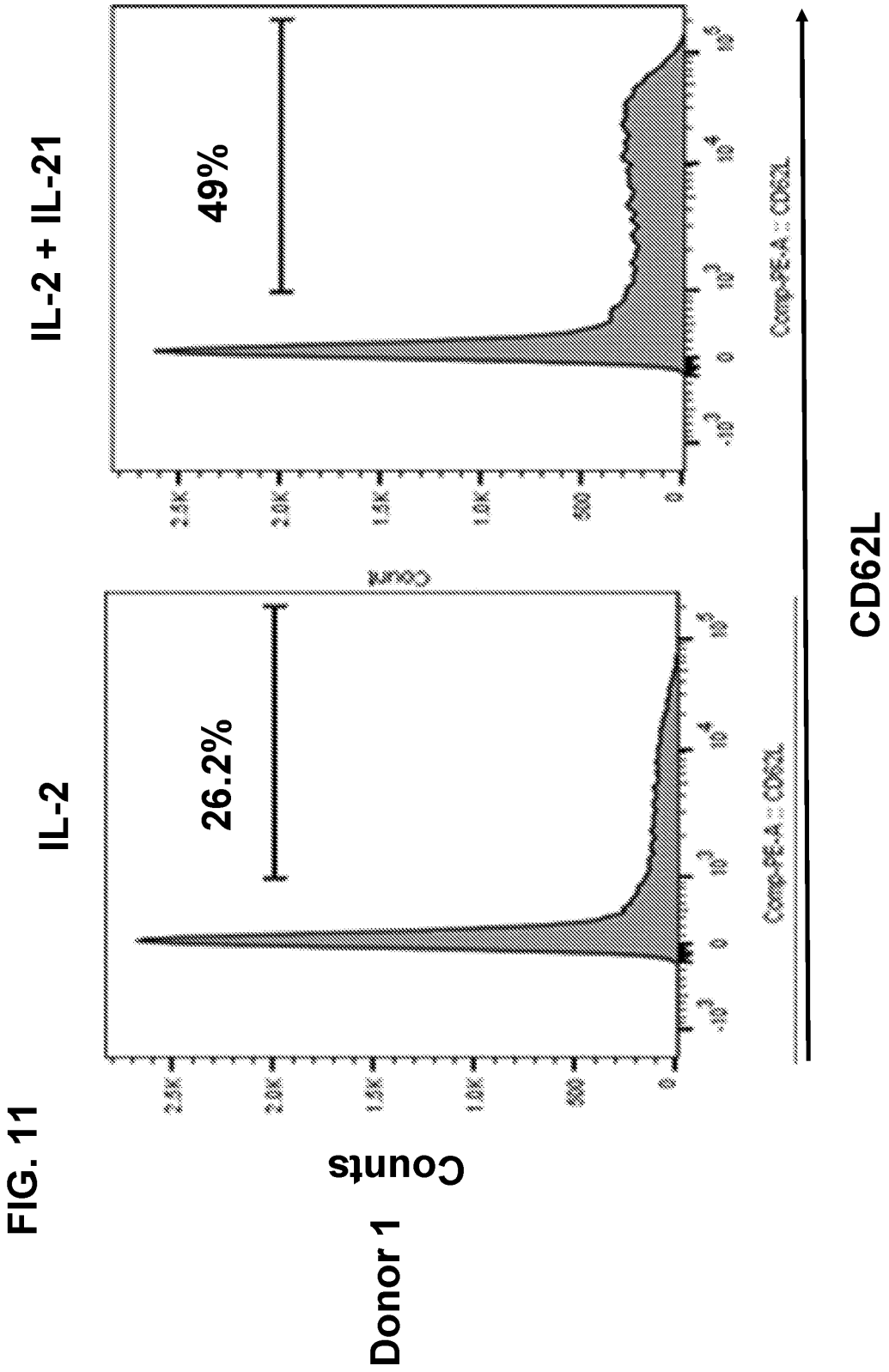



FIG. 11

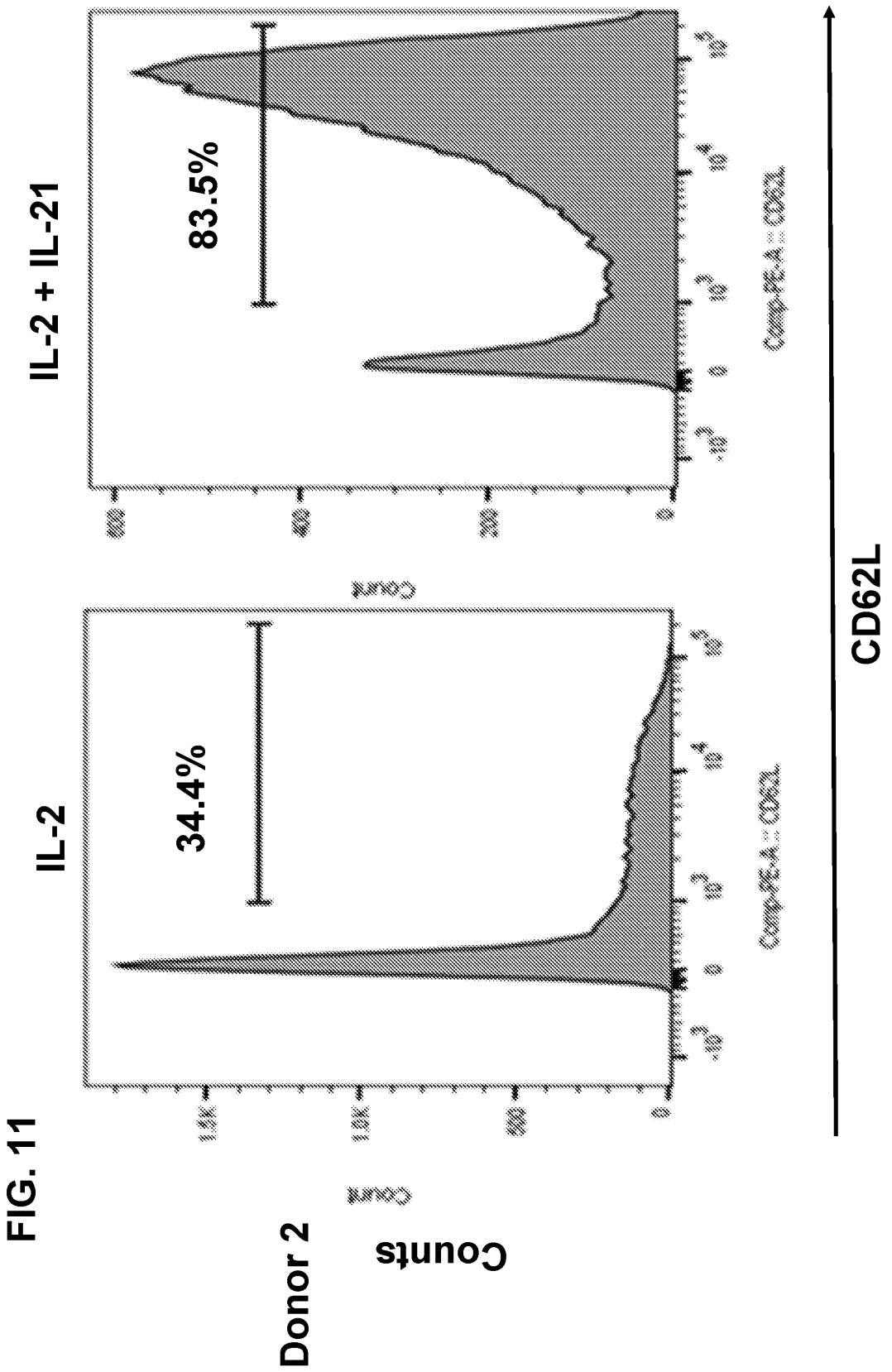
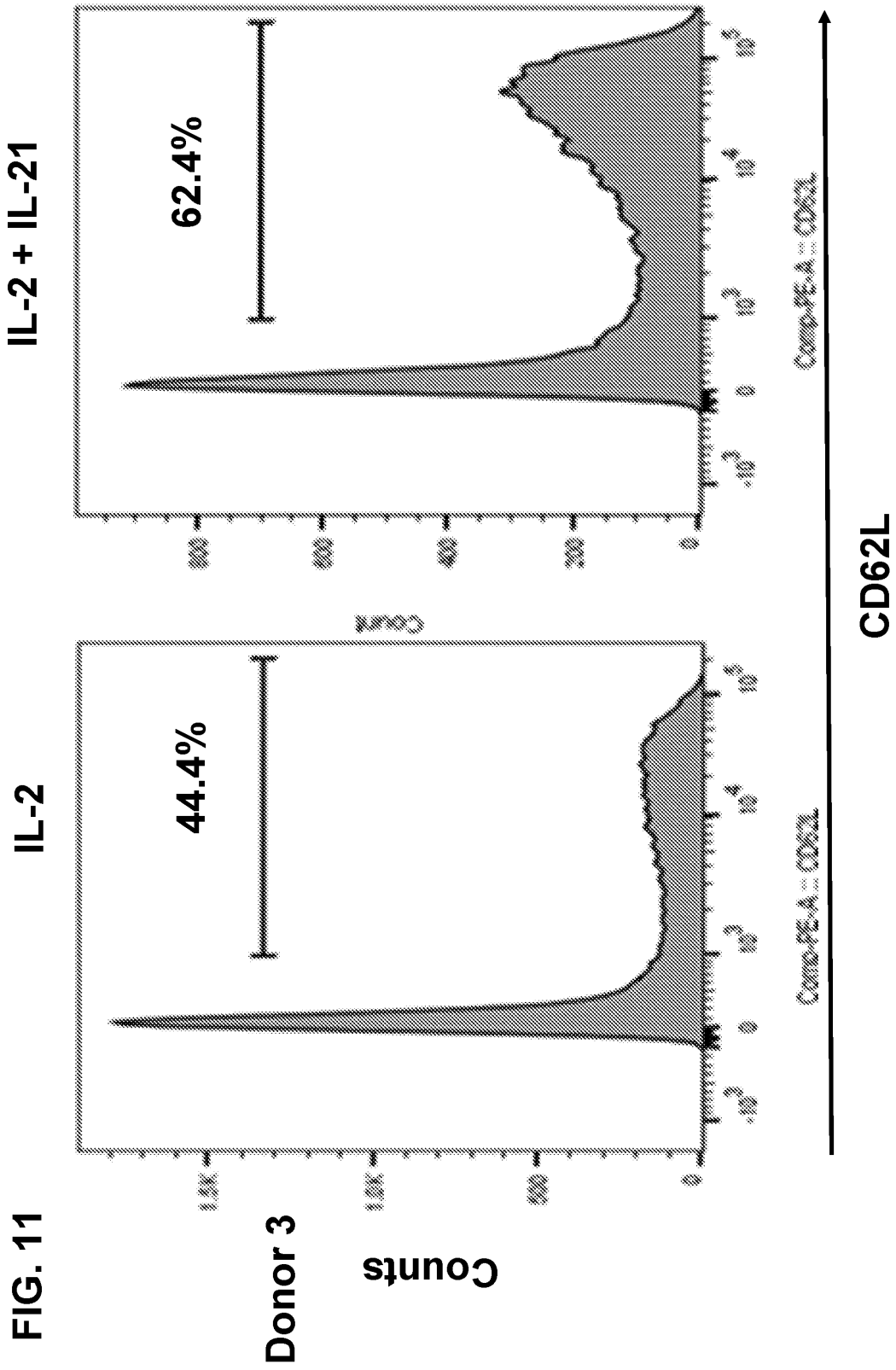



FIG. 11


FIG. 11
IL-2
IL-2 + IL-21
Donor 3
Counts
44.4%
62.4%
CD62L
Comp-PE-A :: CD62L

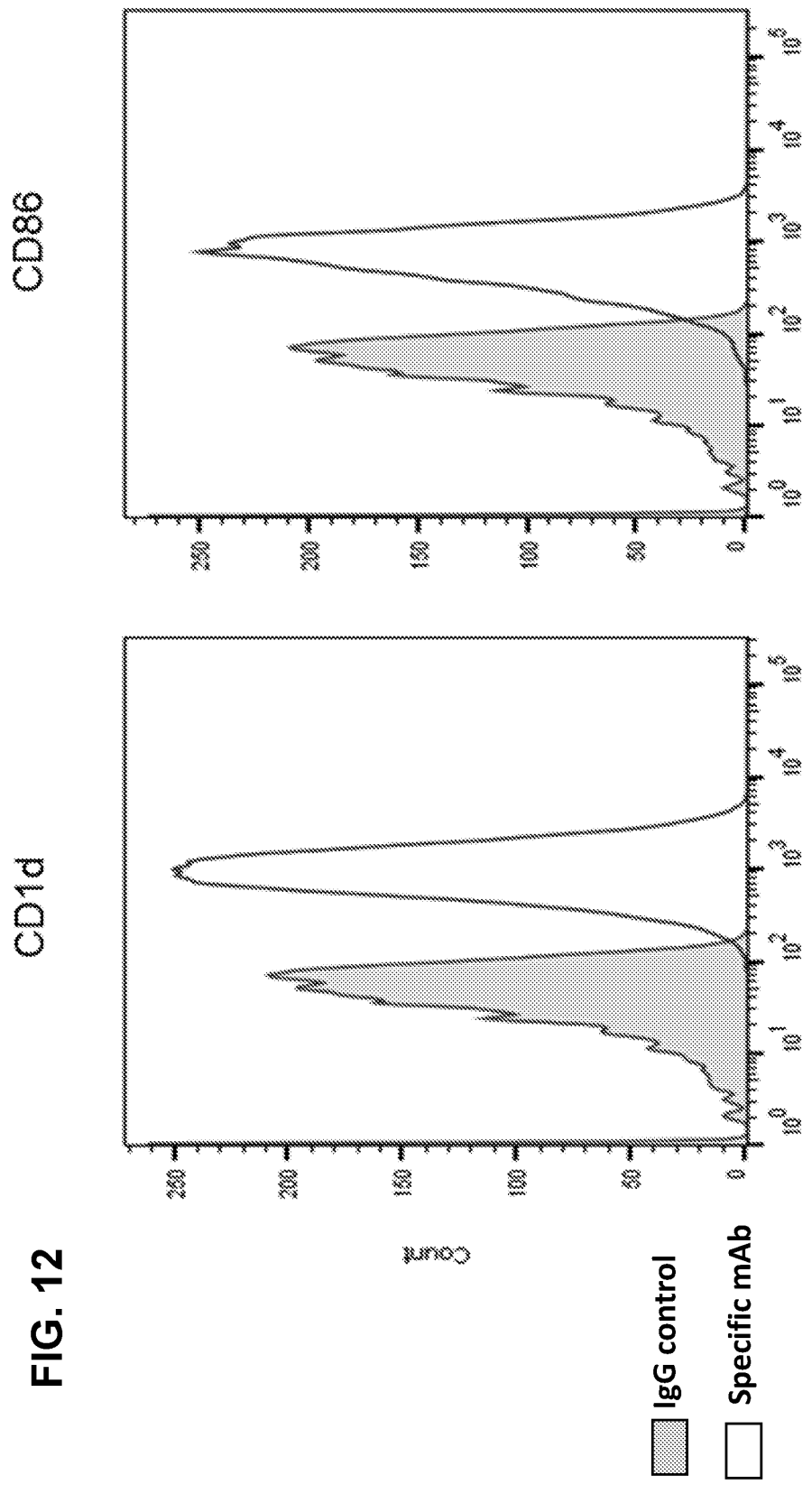



FIG. 12

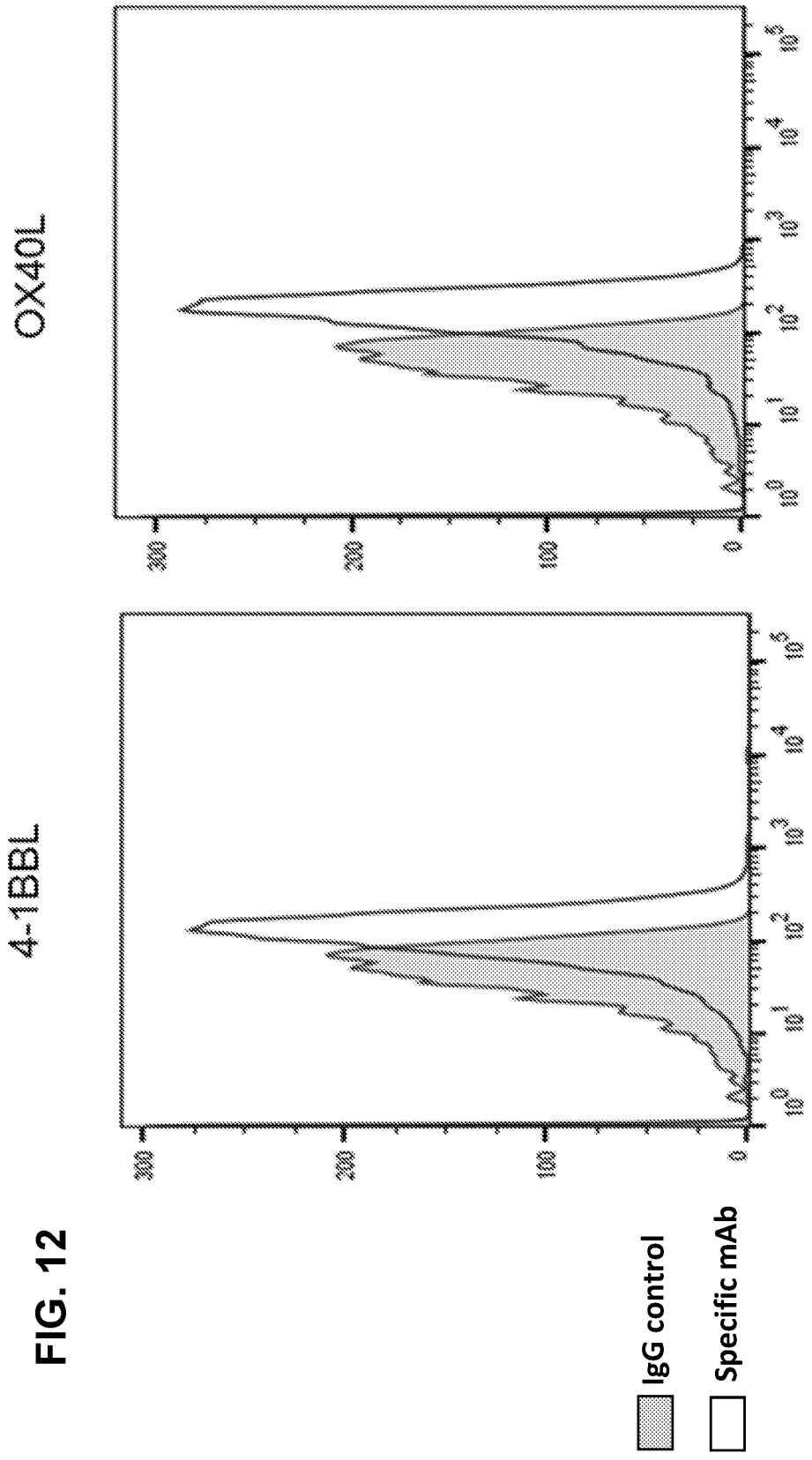

OX40L
4-1BBL
300
200
100
0
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$
$10^5$
IgG control
Specific mAb

FIG. 12

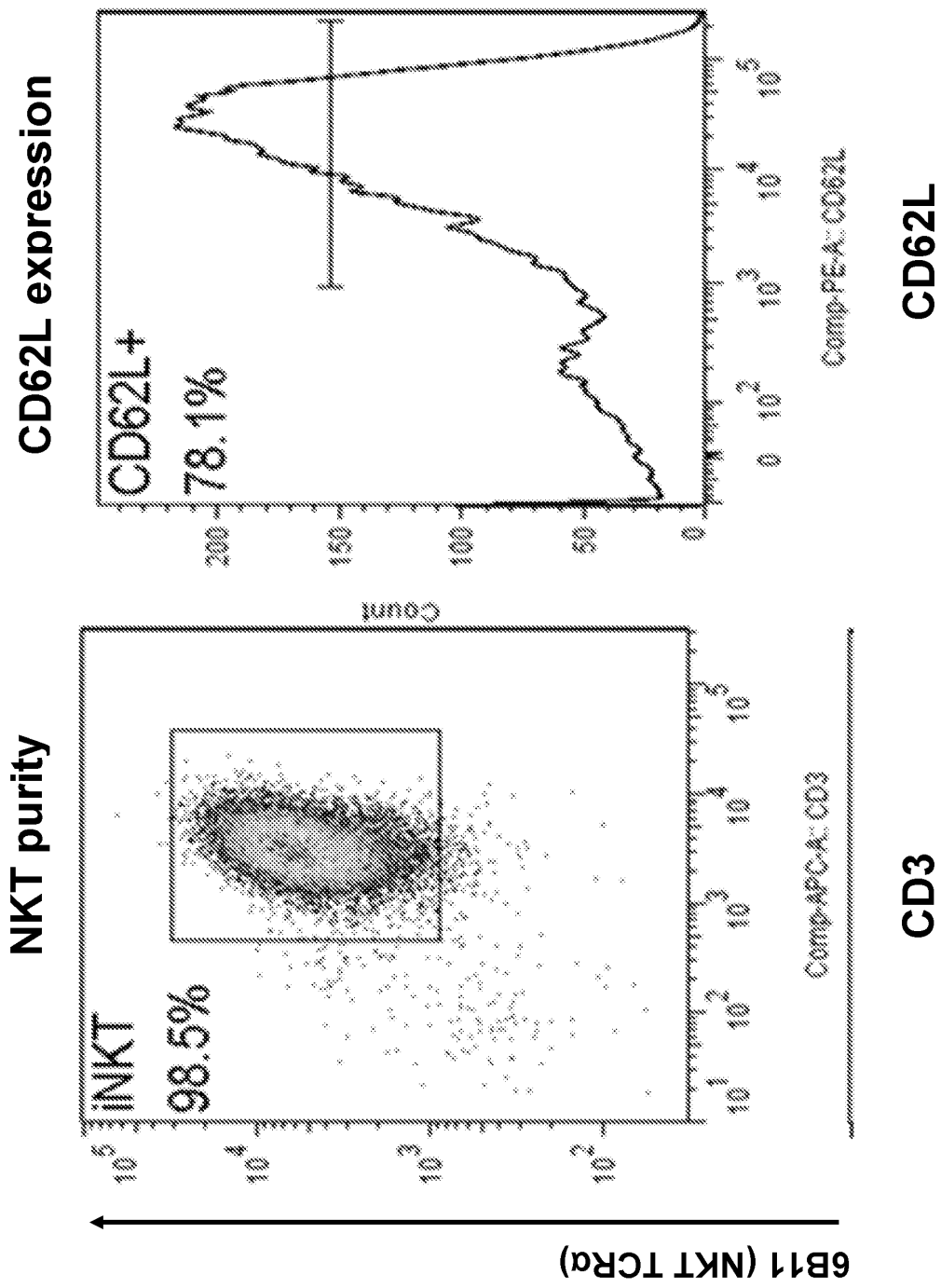

CD62L expression
CD62L+
78.1%
Count
Comp-PE-A: CD62L
CD62L
NKT purity
iNKT
98.5%
Comp-APC-A: CD3
CD3
6B11 (NKT TCRα)
Donor 1

FIG. 13

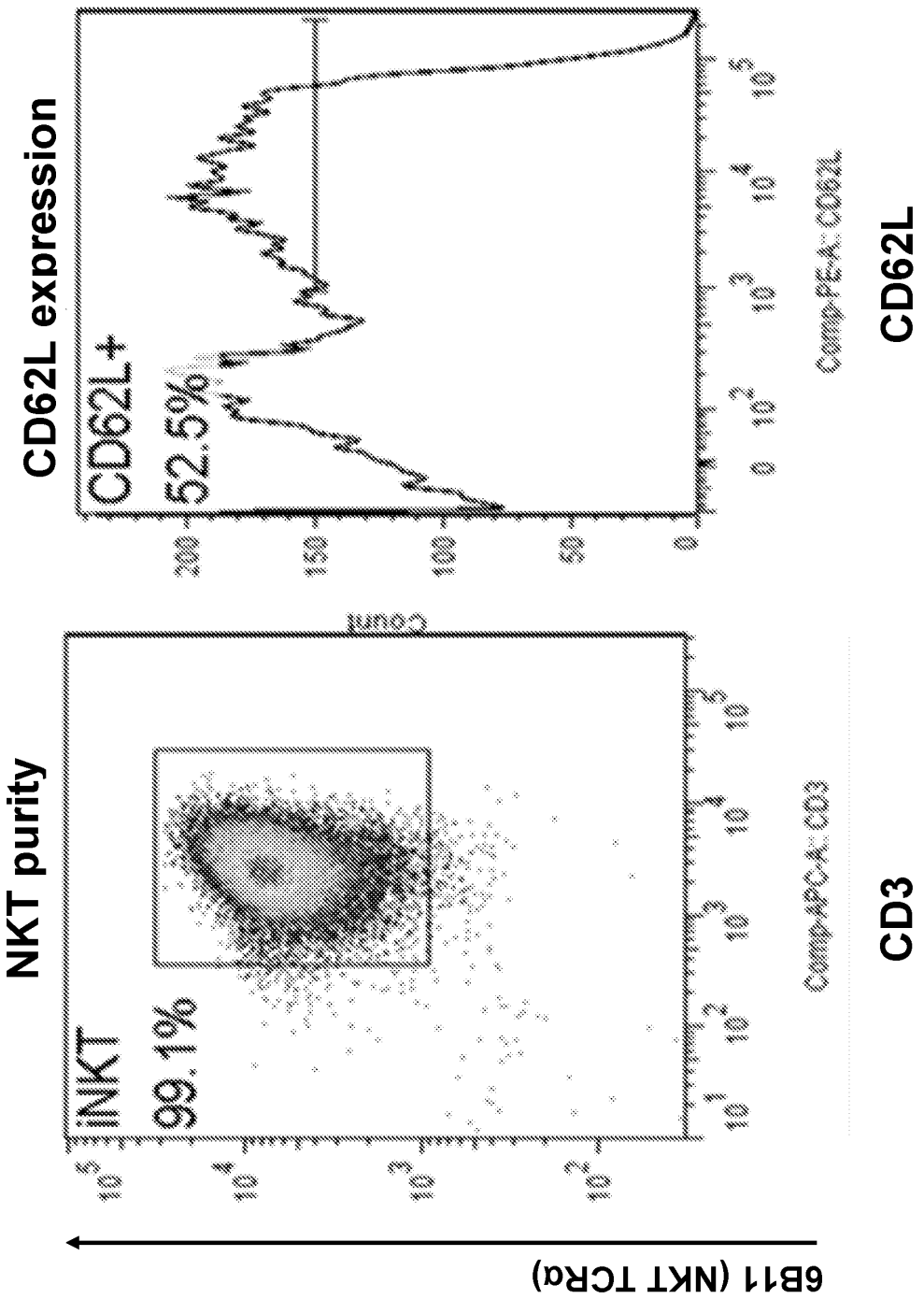

CD62L expression
CD62L+
52.5%
Count
Comp-PE-A:: CD62L
CD62L
NKT purity
iNKT
99.1%
Comp-APC-A:: CD3
CD3
6B11 (NKT TCRα)
Donor 2

FIG. 13

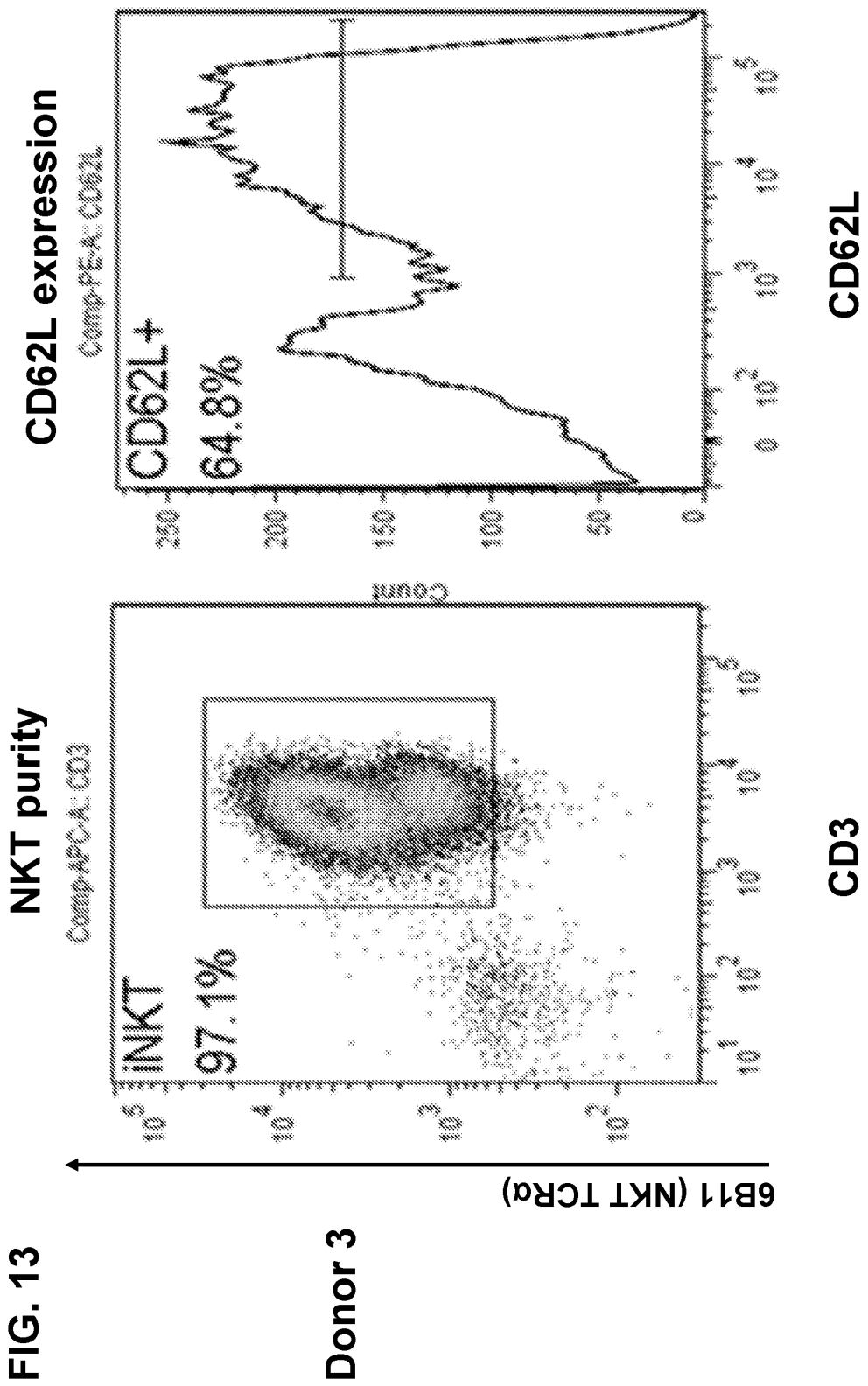

CD62L expression
Comp-PE-A: CD62L
CD62L+
64.8%
Count
CD62L
NKT purity
Comp-APC-A: CD3
iNKT
97.1%
6B11 (NKT TCRα)
CD3
Donor 3

FIG. 13

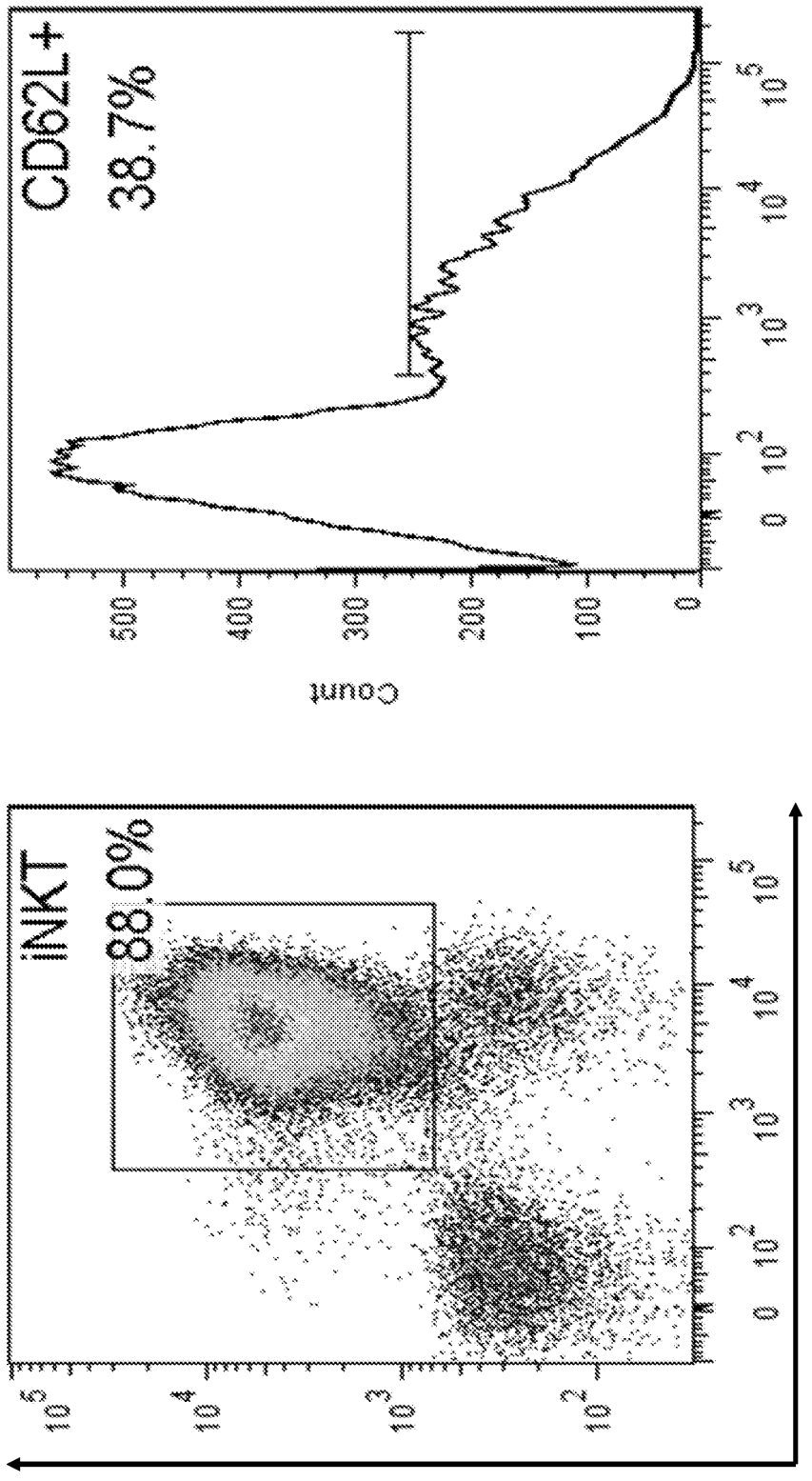

CD62L+
38.7%
Count
500
400
300
200
100
0
0
$10^2$
$10^3$
$10^4$
$10^5$
iNKT
88.0%
$10^6$
$10^4$
$10^3$
$10^2$

FIG. 14