FIG. 1A

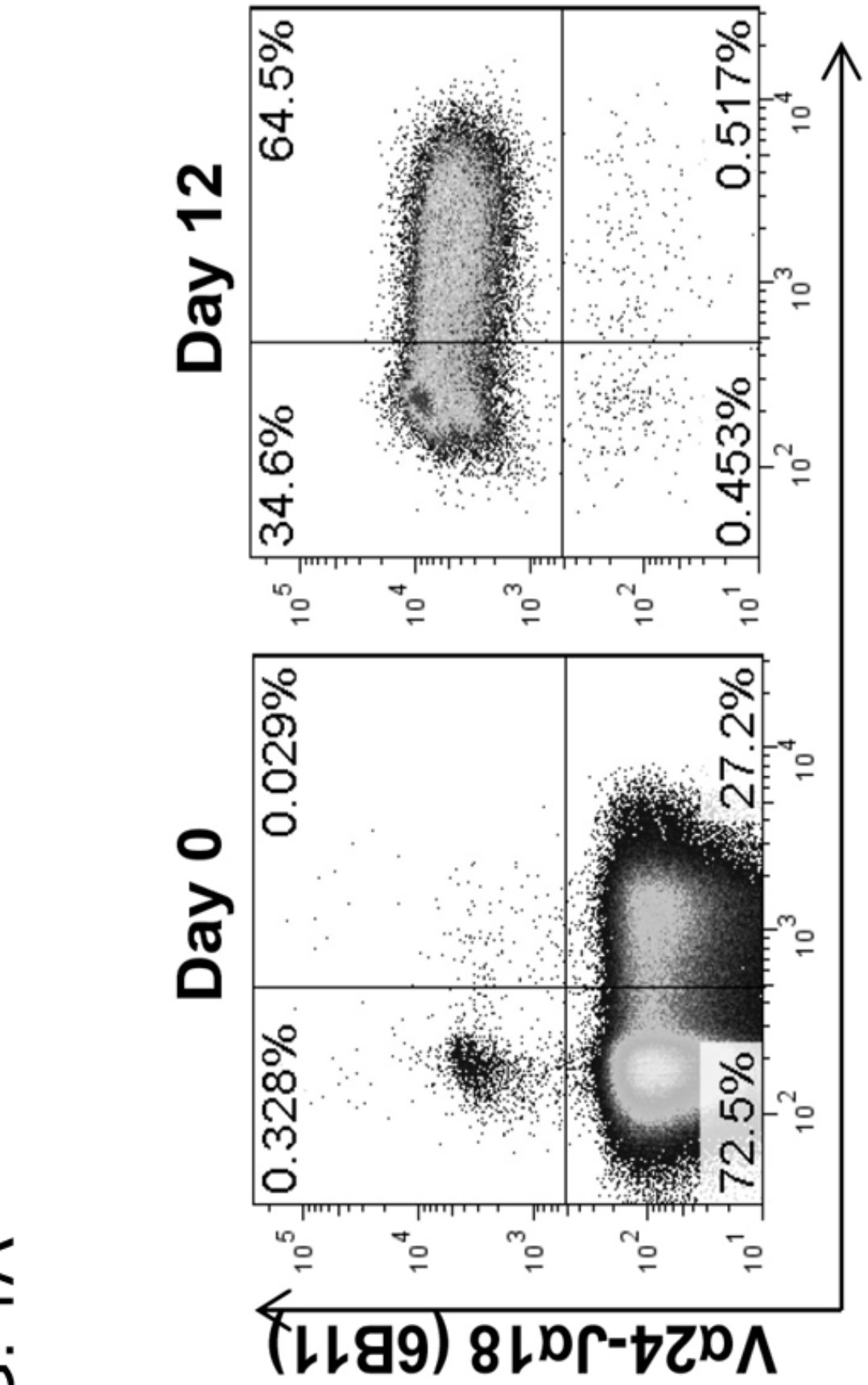
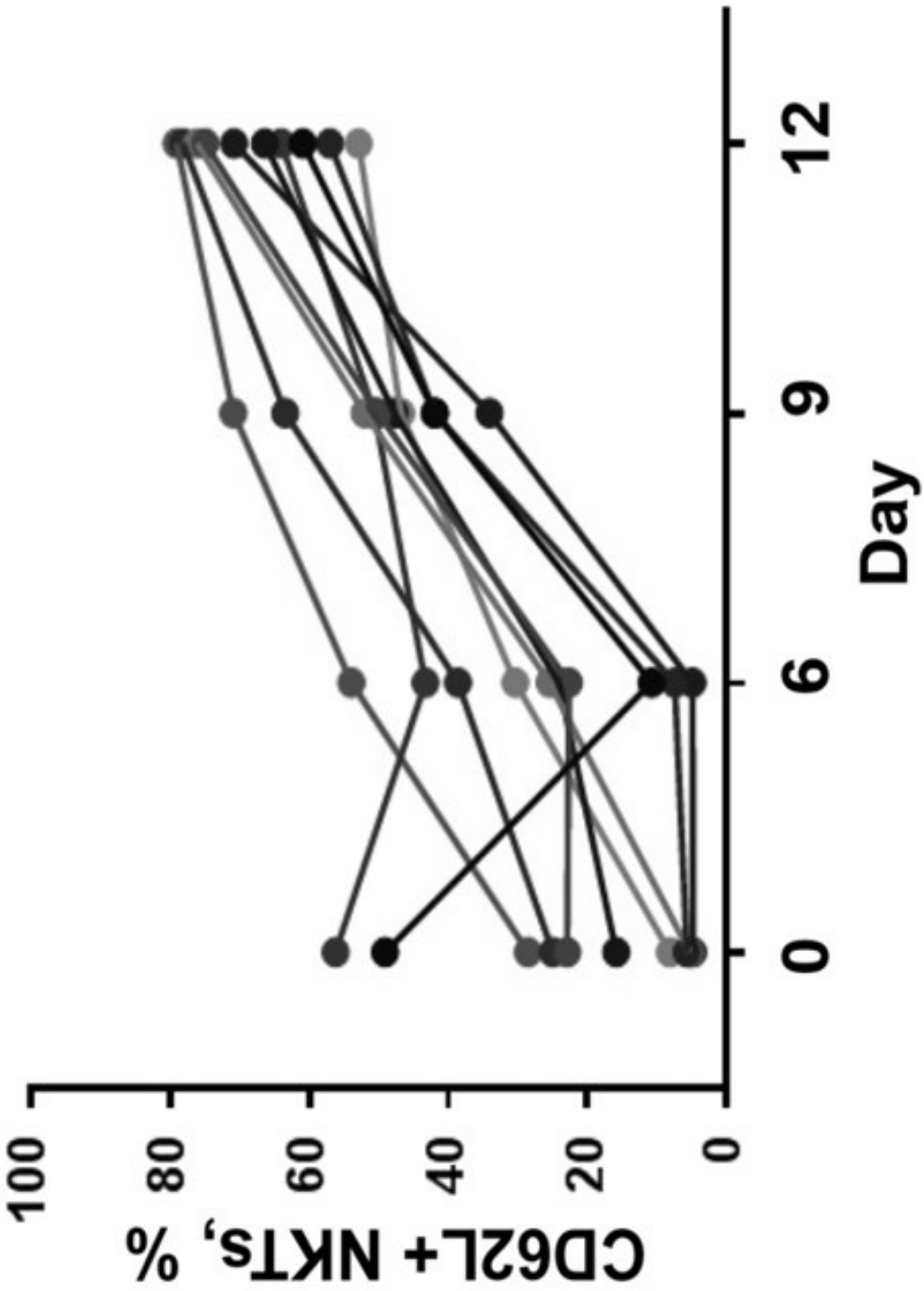

Day 0
Day 12
0.328%
0.029%
72.5%
27.2%
34.6%
64.5%
0.453%
0.517%
Vα24-Jα18 (6B11)
CD62L

FIG. 1B



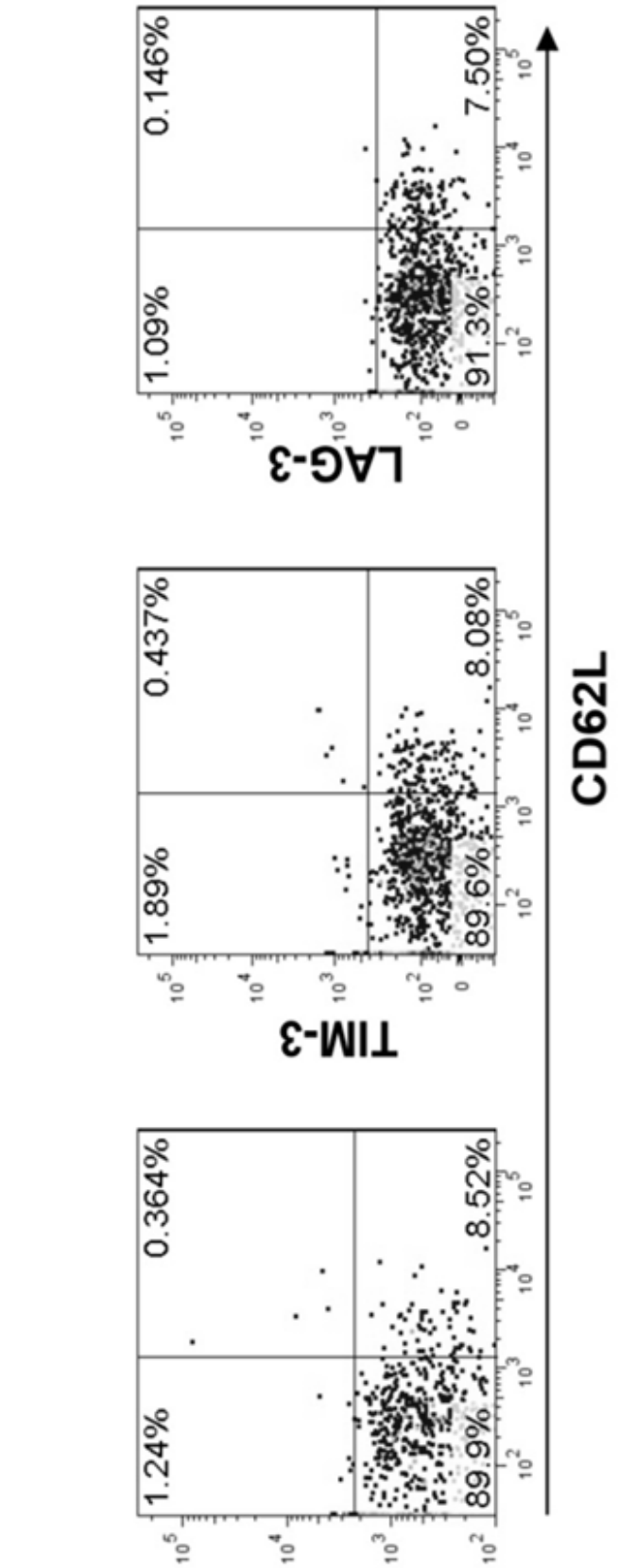

Day 0
PD-1
1.24%
0.364%
89.9%
8.52%
TIM-3
1.89%
0.437%
89.6%
8.08%
LAG-3
1.09%
0.146%
91.3%
7.50%
CD62L

FIG. 1C

FIG. 1C (continued)

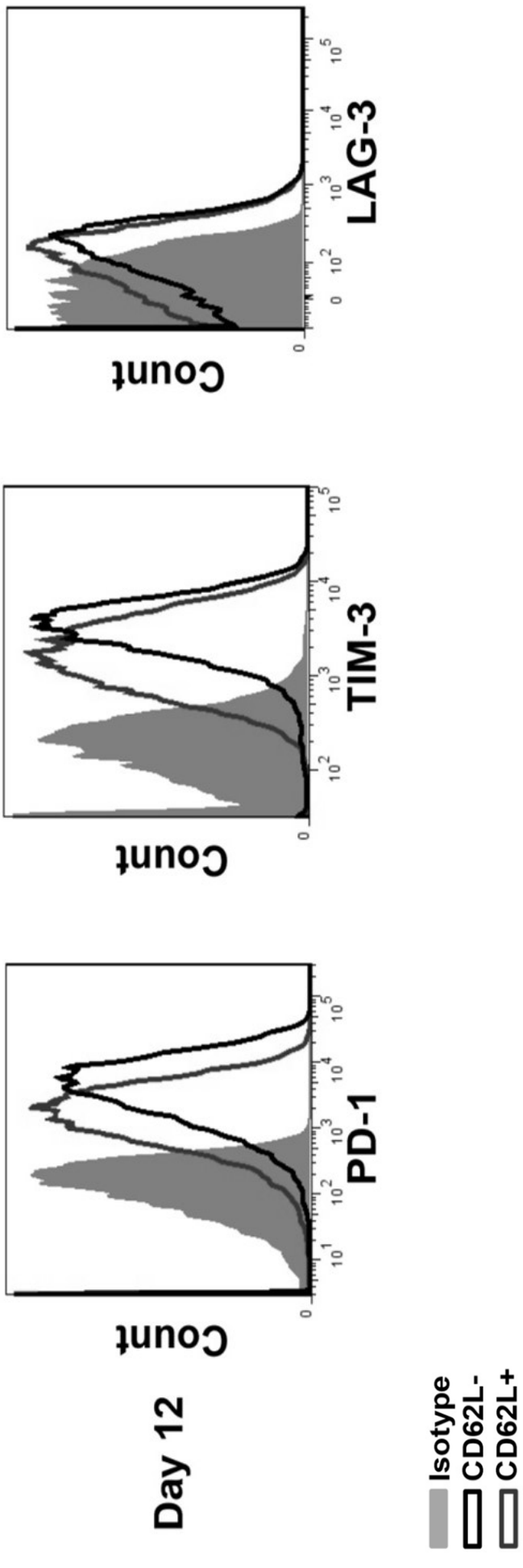


FIG. 1C (continued)

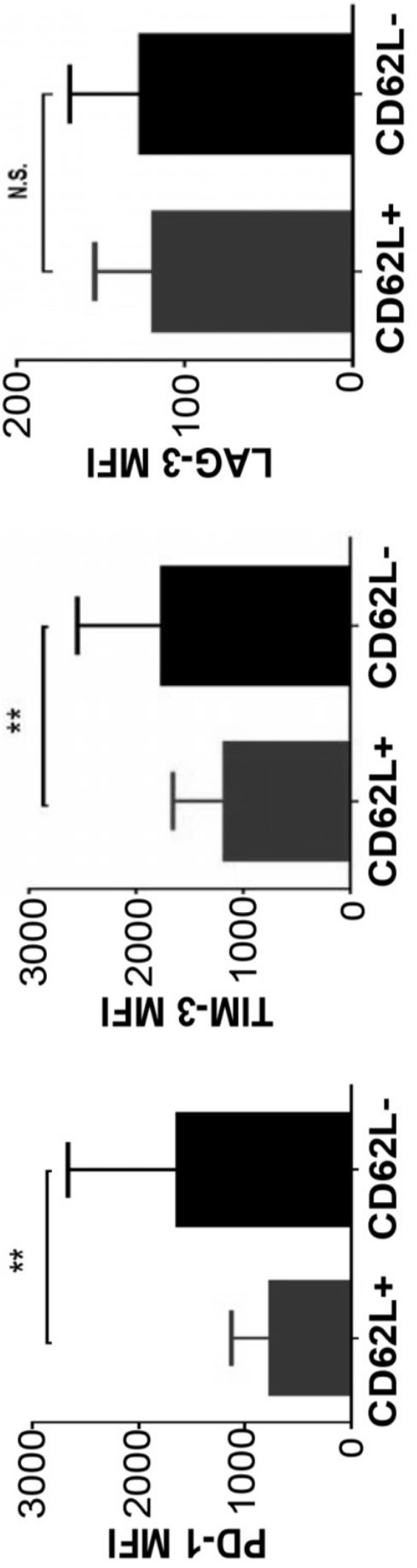
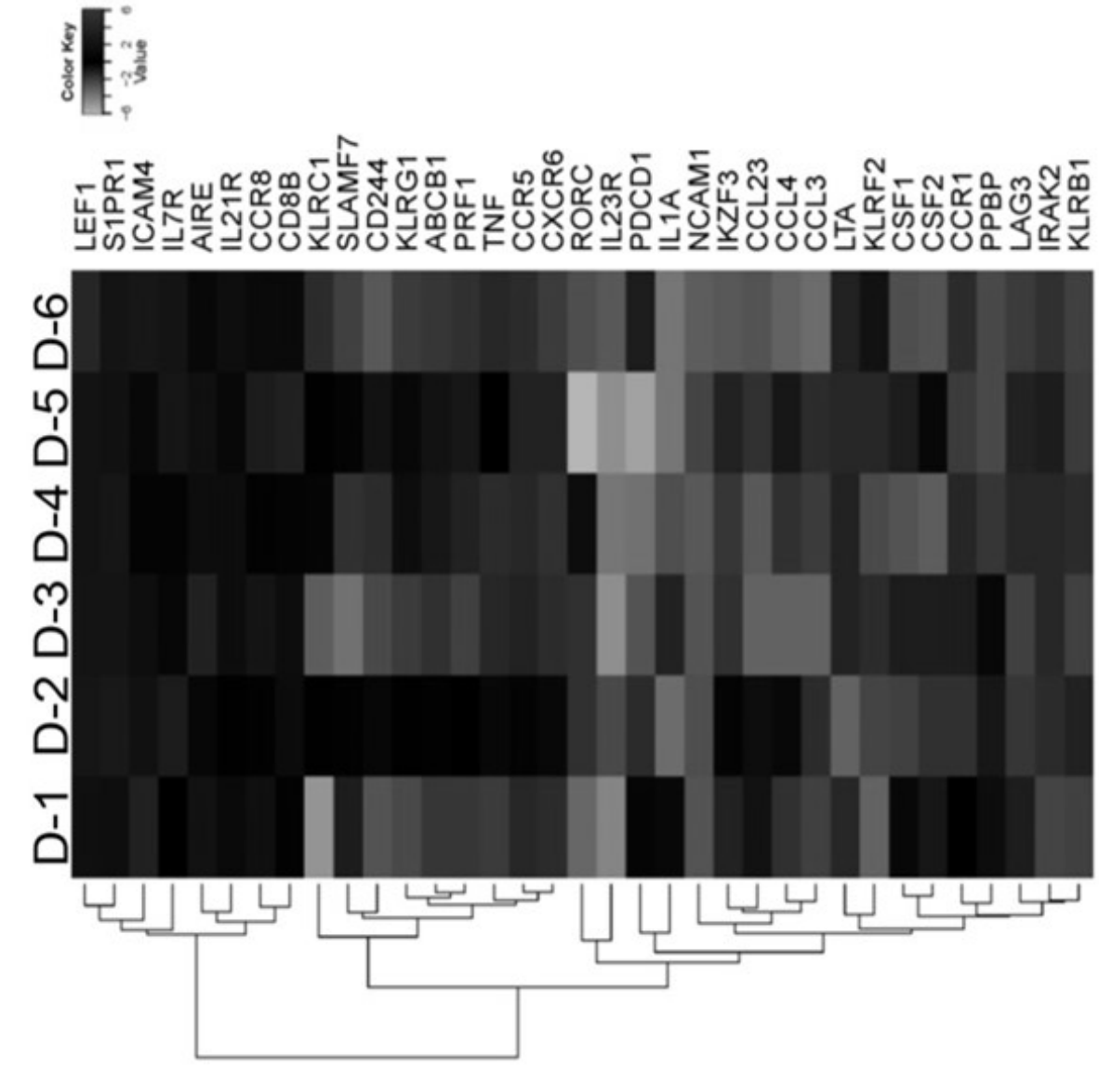


FIG. 1D



Gene Name	Fold Change	P value
CD62L	118.32	0.0001
LEF1	5.46	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)	-6.44	0.0014
CCL3	-4.89	0.0002
PDCD1(PD-1)	-4.78	0.0207
KLRB1(CD161)	-3.49	0.0001
LAG-3	-2.92	0.0027
CD244	-2.92	0.0025
IRAK2	-2.76	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. 2A

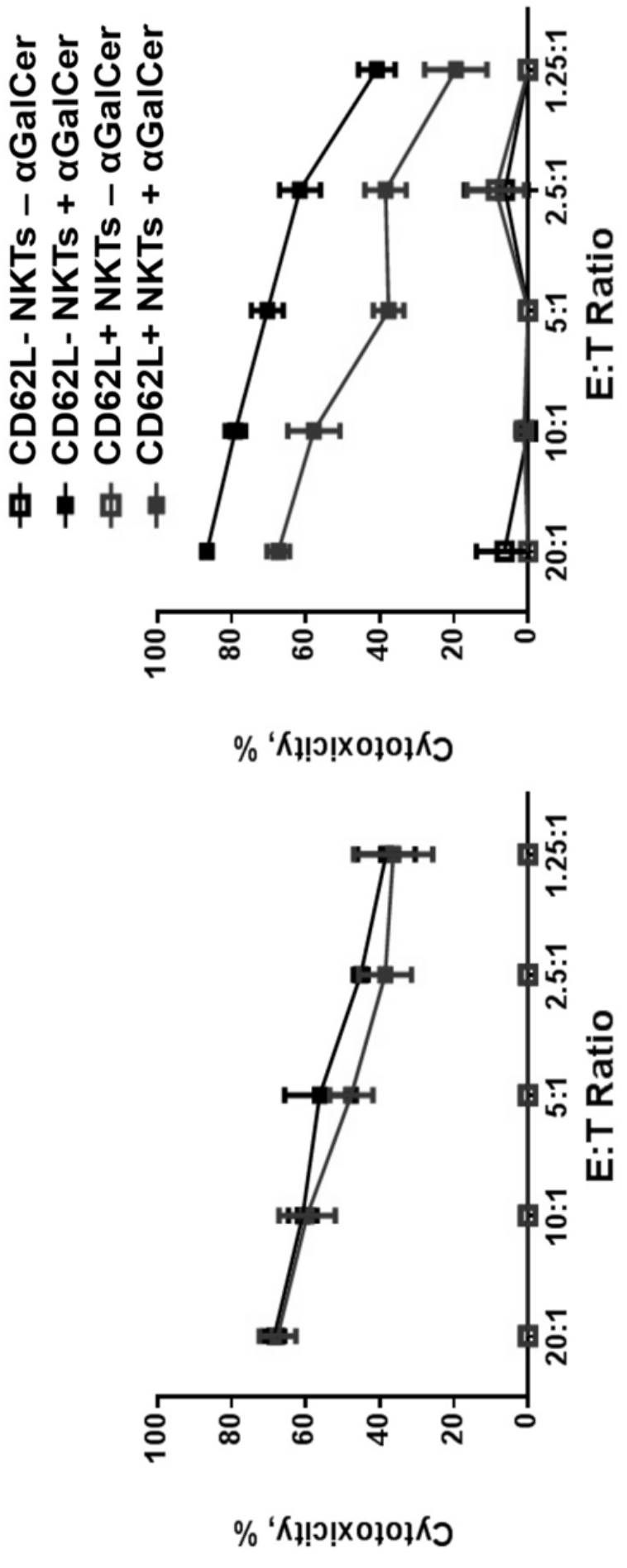

CD62L- NKTs – αGalCer
CD62L- NKTs + αGalCer
CD62L+ NKTs – αGalCer
CD62L+ NKTs + αGalCer
Cytotoxicity, %
100
80
60
40
20
0
20:1
10:1
5:1
2.5:1
1.25:1
E:T Ratio
Cytotoxicity, %
100
80
60
40
20
0
20:1
10:1
5:1
2.5:1
1.25:1
E:T Ratio

FIG. 2B

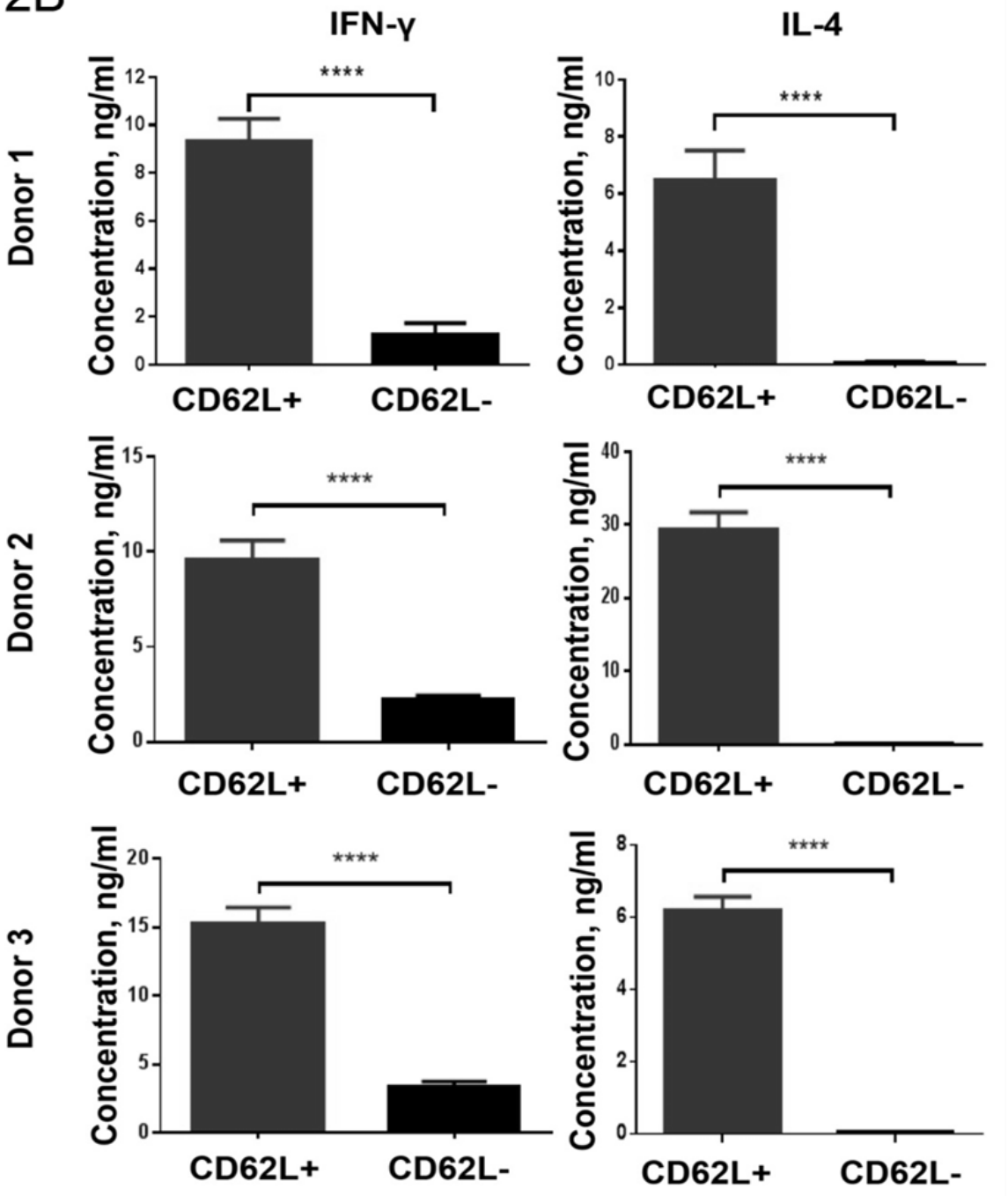

IFN-γ
IL-4
Donor 1
Donor 2
Donor 3
Concentration, ng/ml
****
CD62L+
CD62L-

FIG. 2C

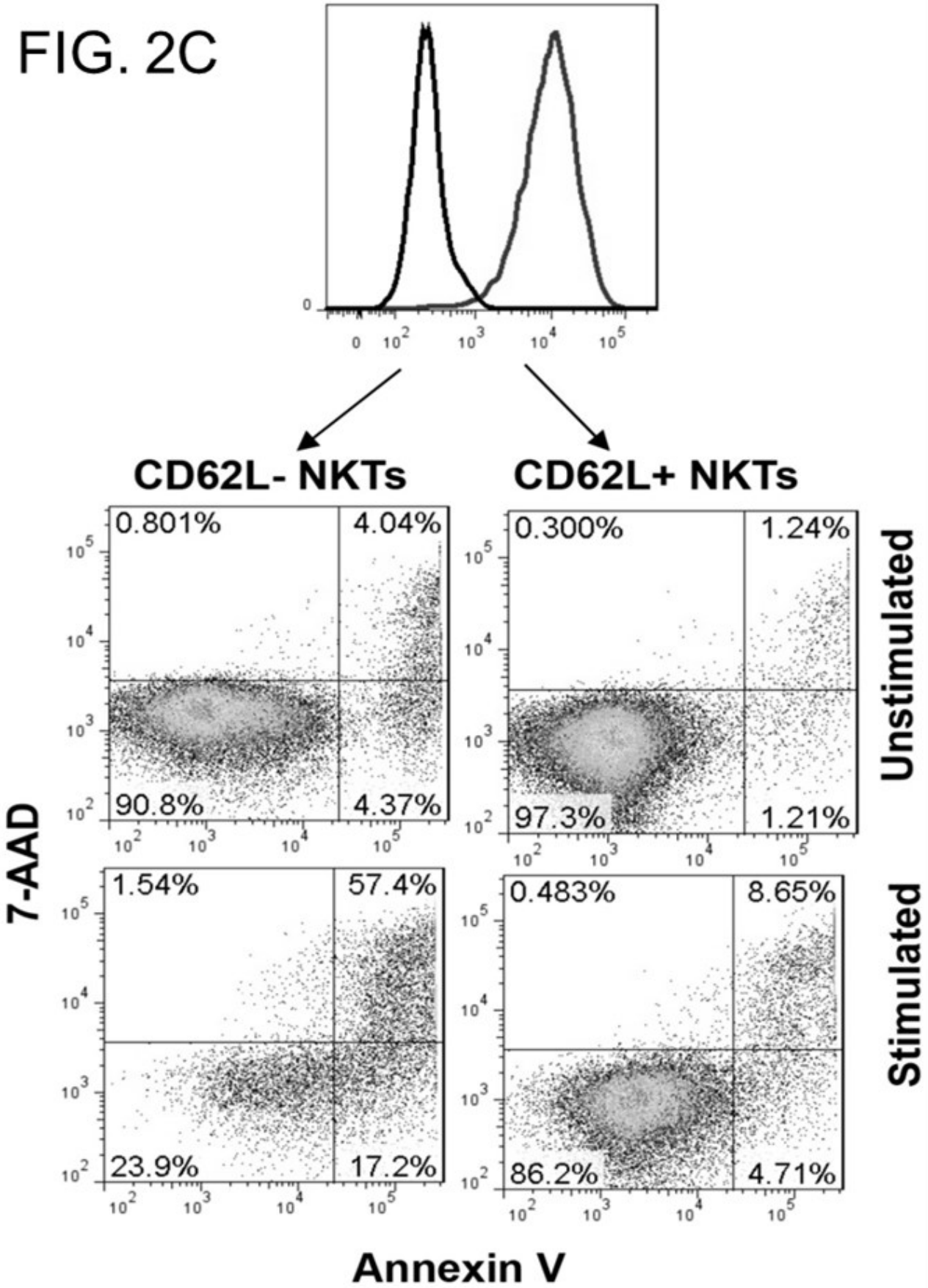
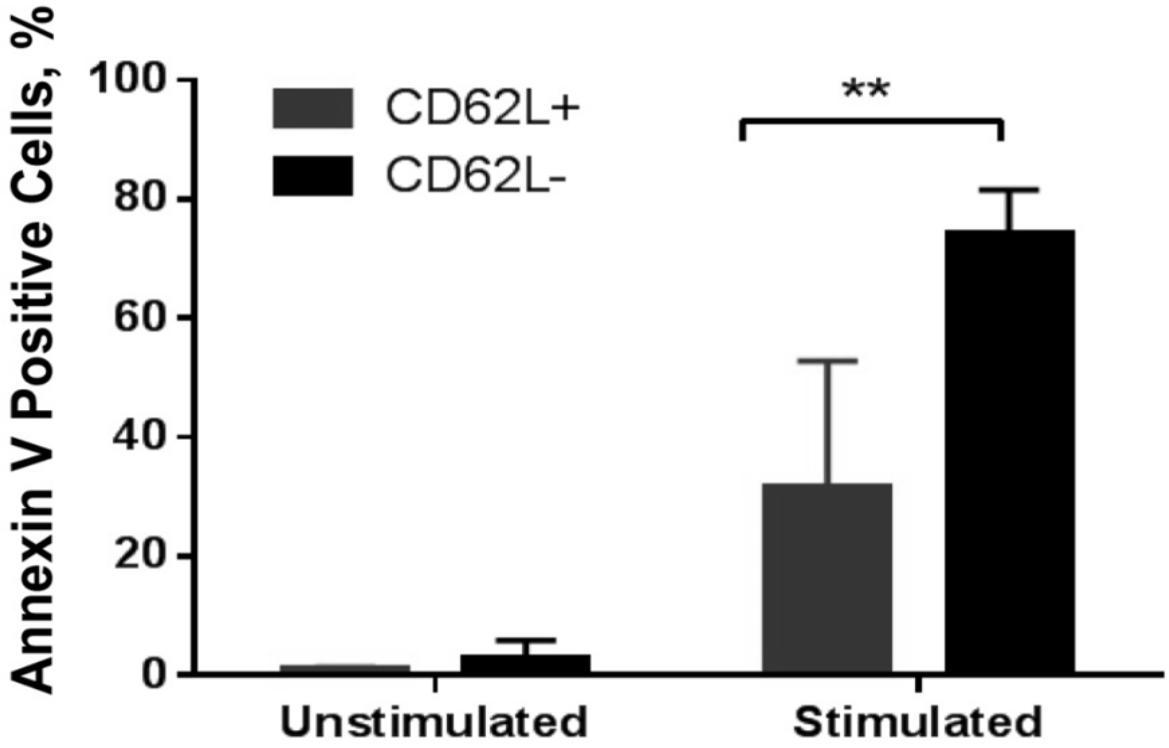

CD62L- NKTs
CD62L+ NKTs
0.801%
4.04%
90.8%
4.37%
0.300%
1.24%
97.3%
1.21%
1.54%
57.4%
23.9%
17.2%
0.483%
8.65%
86.2%
4.71%
Unstimulated
Stimulated
7-AAD
Annexin V

FIG. 2C (continued)



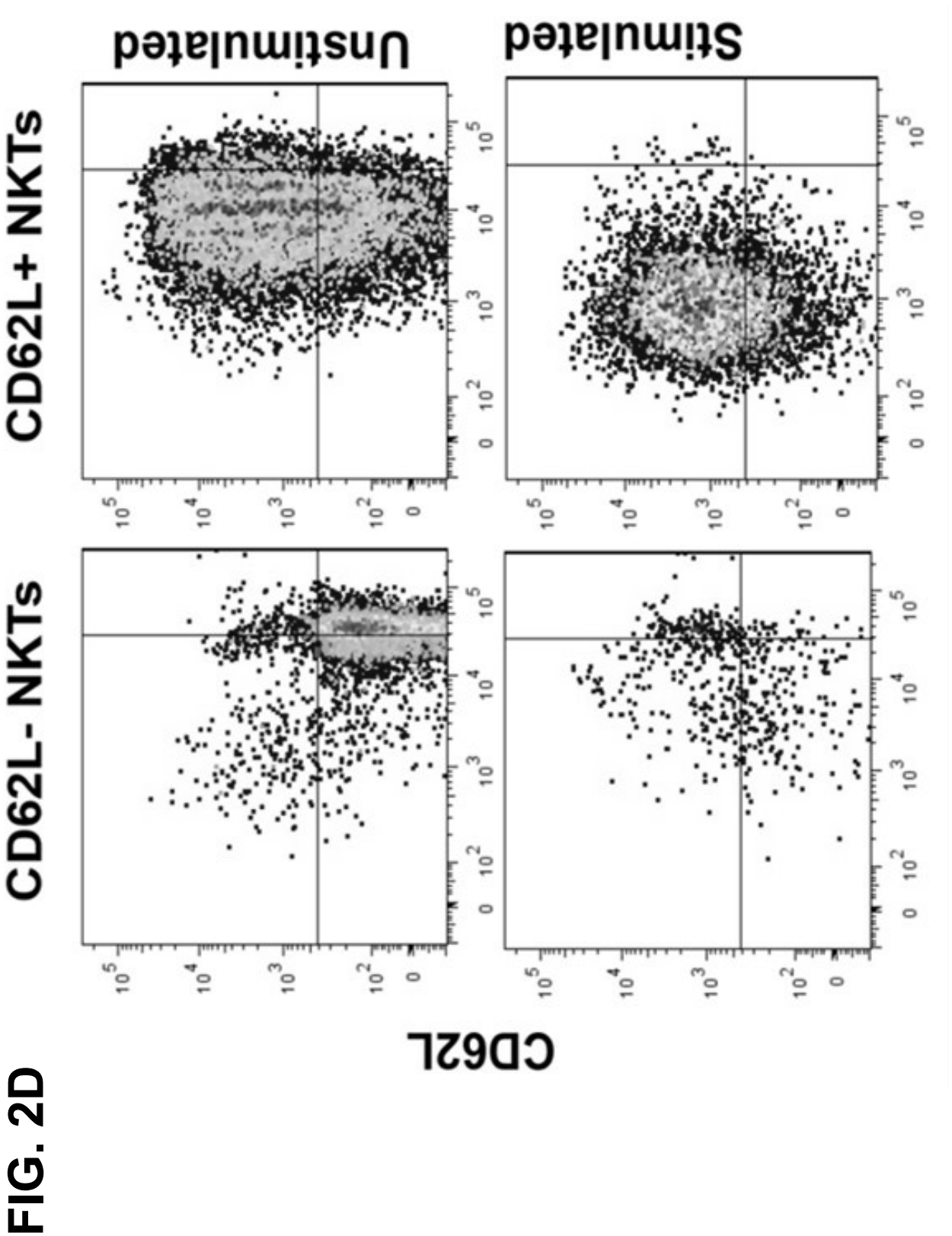


FIG. 2D

FIG. 2D (continued)

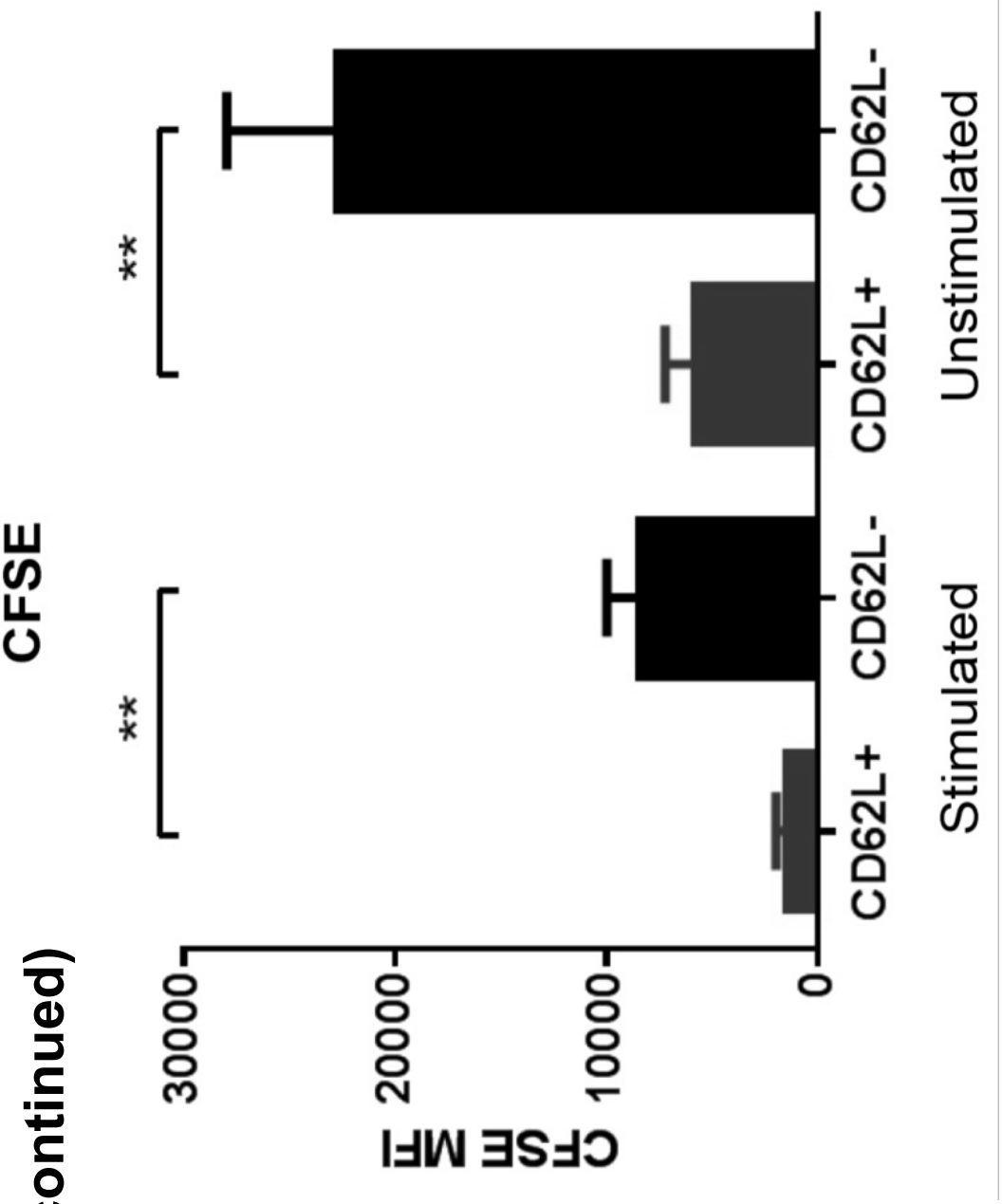
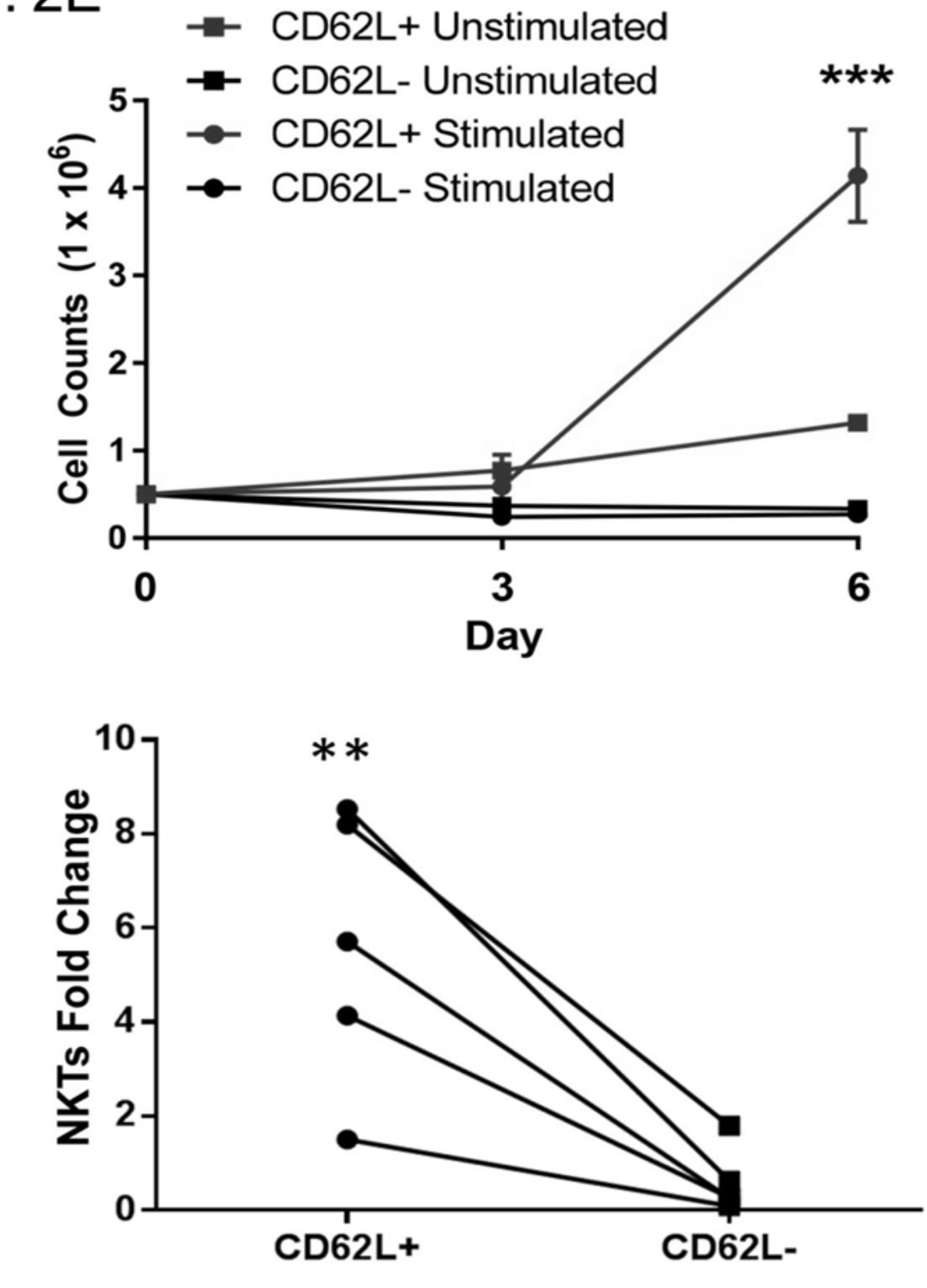


FIG. 2E


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-

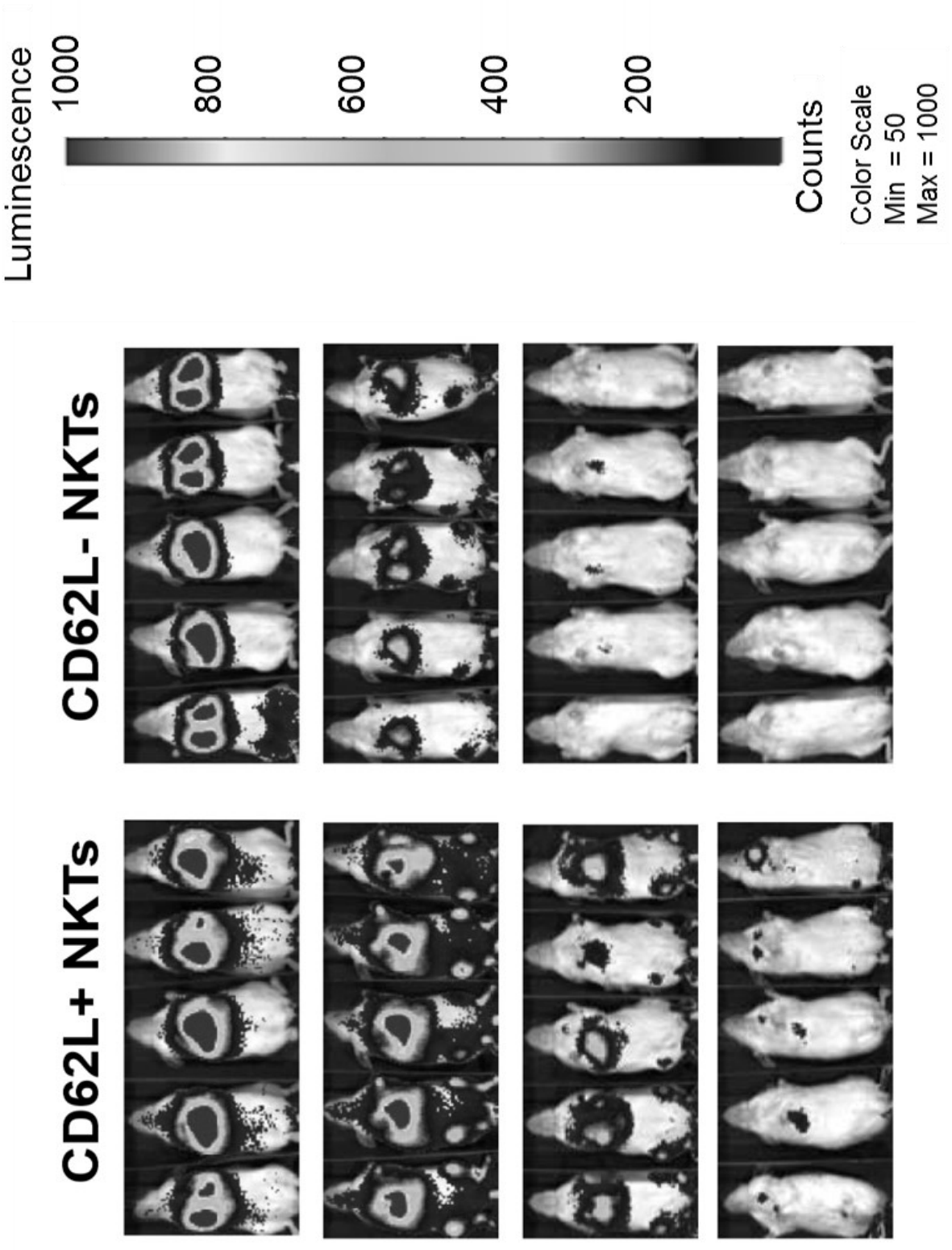

Luminescence
1000
800
600
400
200
Counts
Color Scale
Min = 50
Max = 1000
CD62L+ NKTs
CD62L- NKTs
Days
0
2
4
10

FIG. 3A

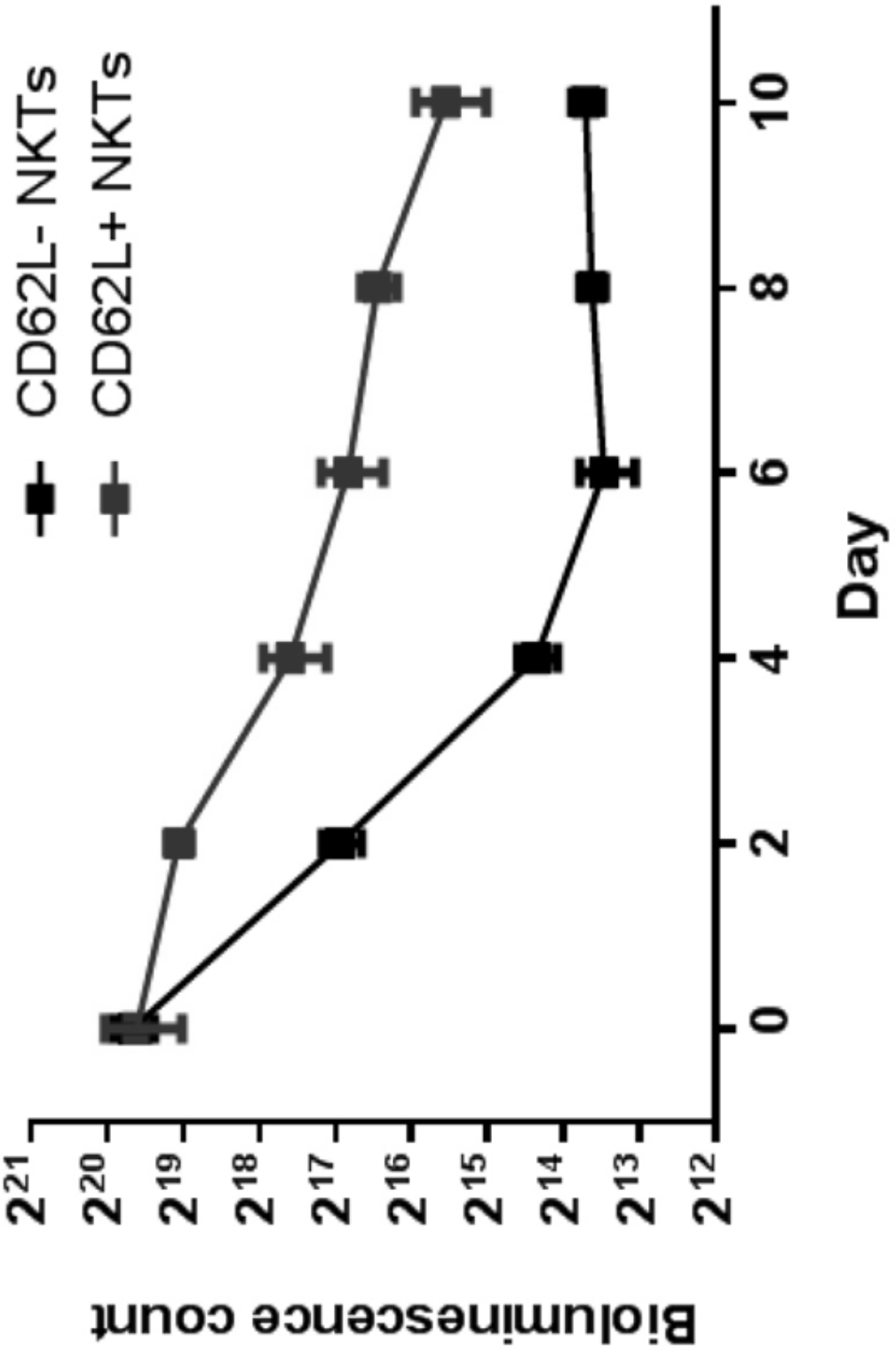

CD62L- NKTs
CD62L+ NKTs
Bioluminescence count
2^{21}
2^{20}
2^{19}
2^{18}
2^{17}
2^{16}
2^{15}
2^{14}
2^{13}
2^{12}
0
2
4
6
8
10
Day

FIG. 3B

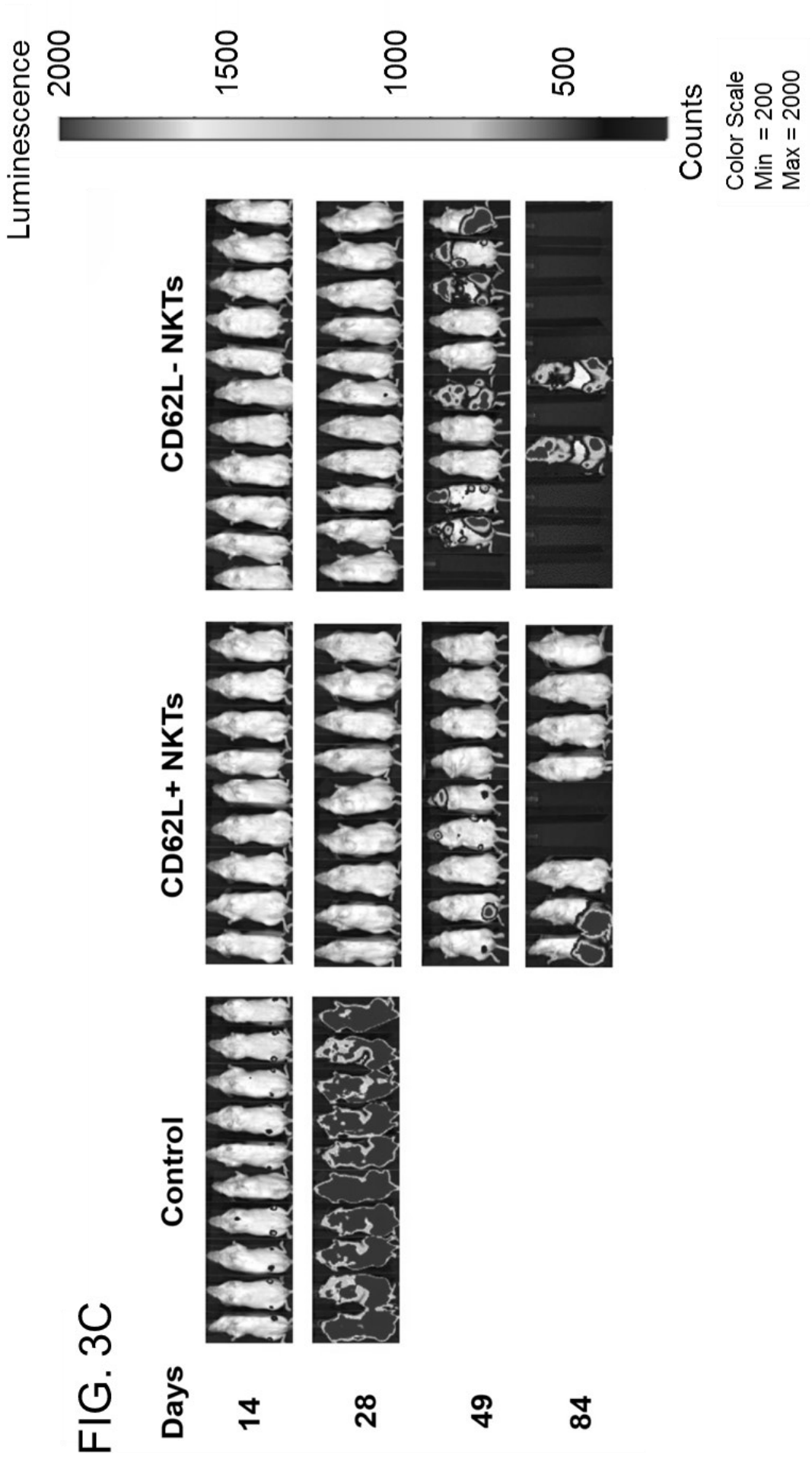

Luminescence
2000
1500
1000
500
Counts
Color Scale
Min = 200
Max = 2000
CD62L- NKTs
CD62L+ NKTs
Control
Days
14
28
49
84

FIG. 3C

FIG. 3D

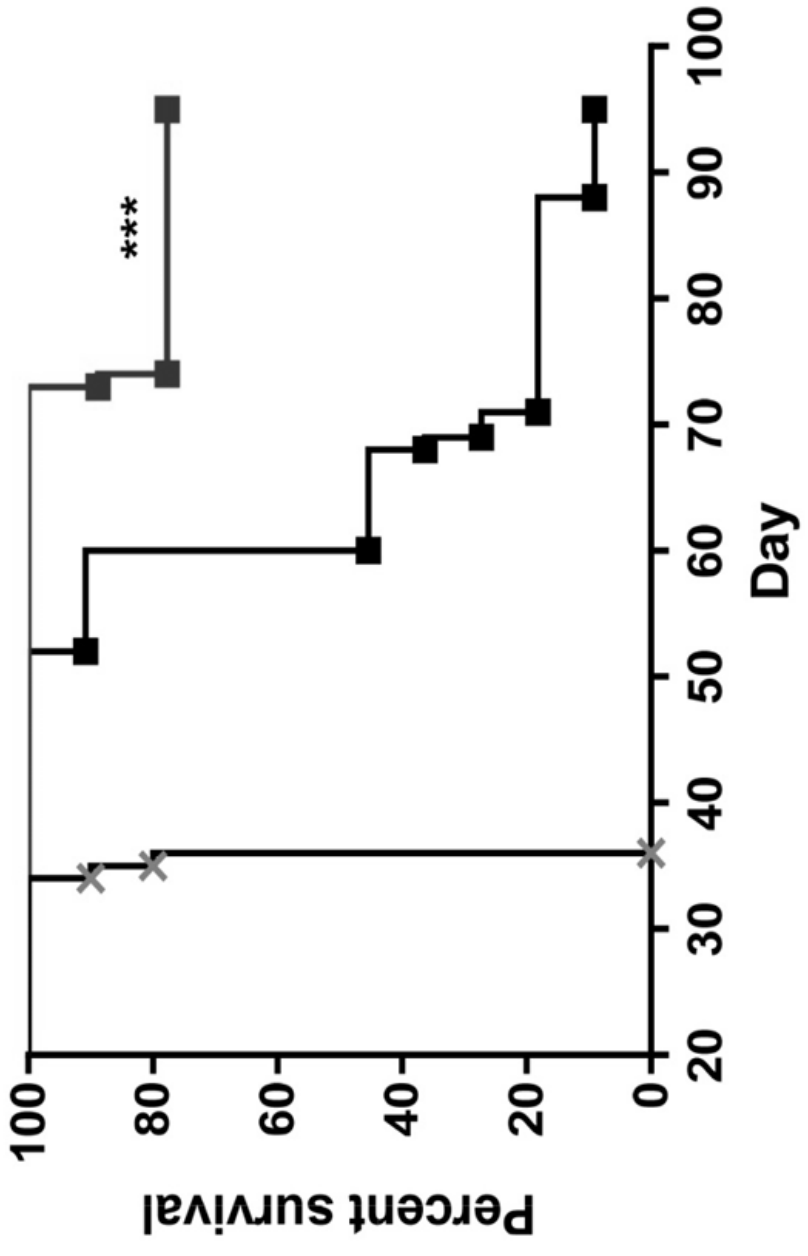

Control
CD62L+ NKTs
CD62L- NKTs
***
Percent survival
100
80
60
40
20
0
20
30
40
50
60
70
80
90
100
Day

FIG. 4A

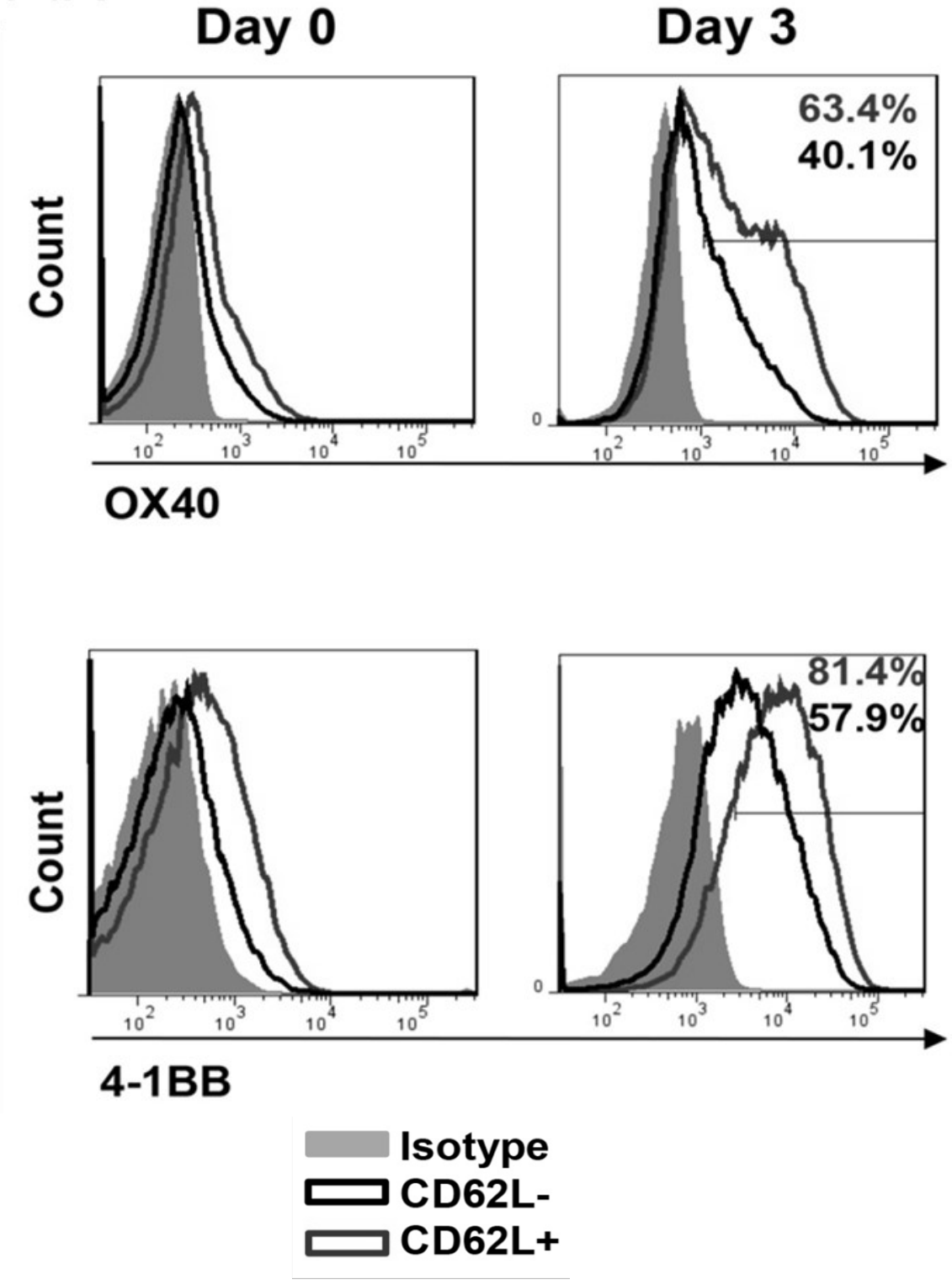
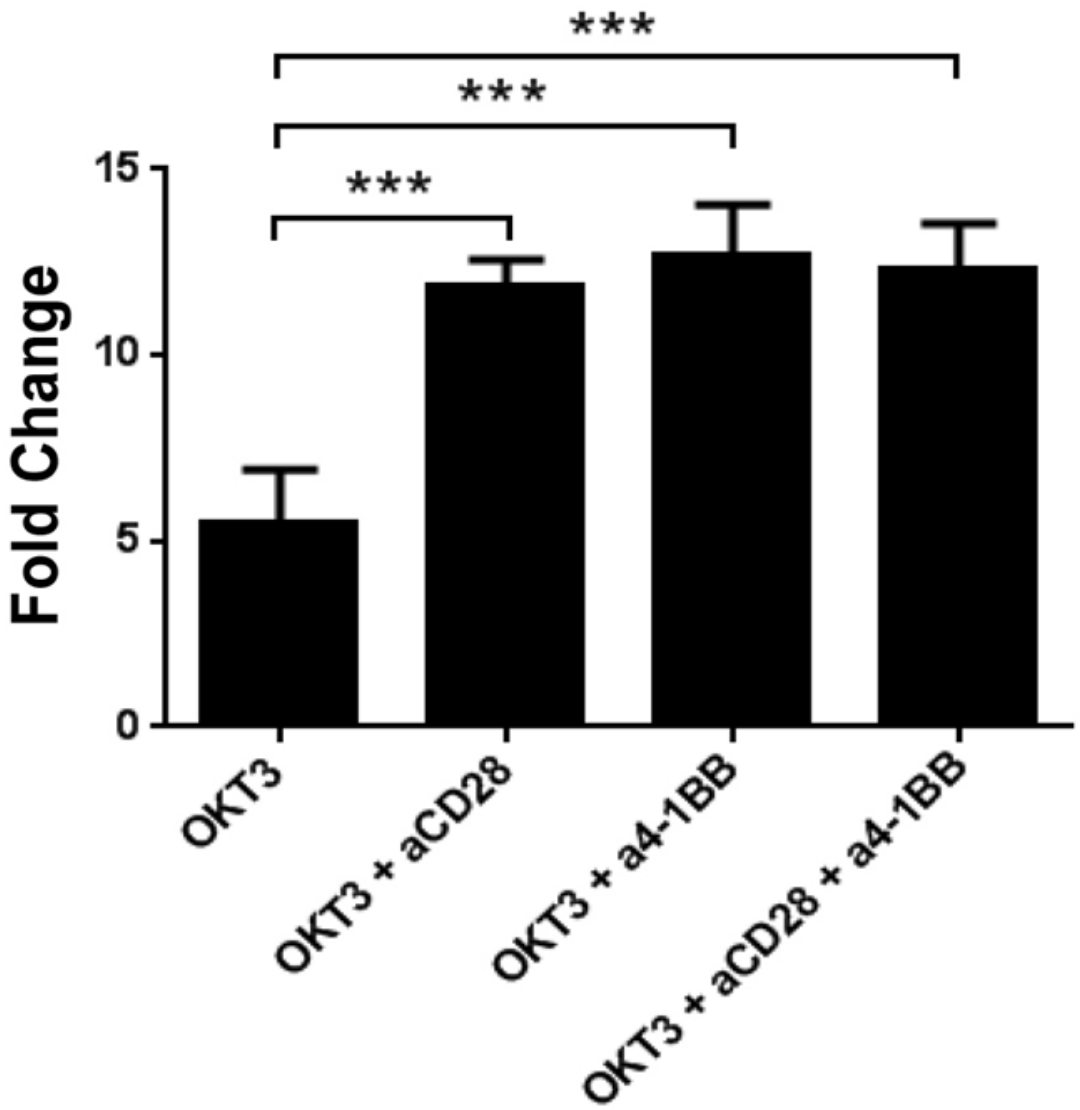

Day 0
Day 3
63.4%
40.1%
Count
OX40
81.4%
57.9%
Count
4-1BB
Isotype
CD62L-
CD62L+

FIG. 4B


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

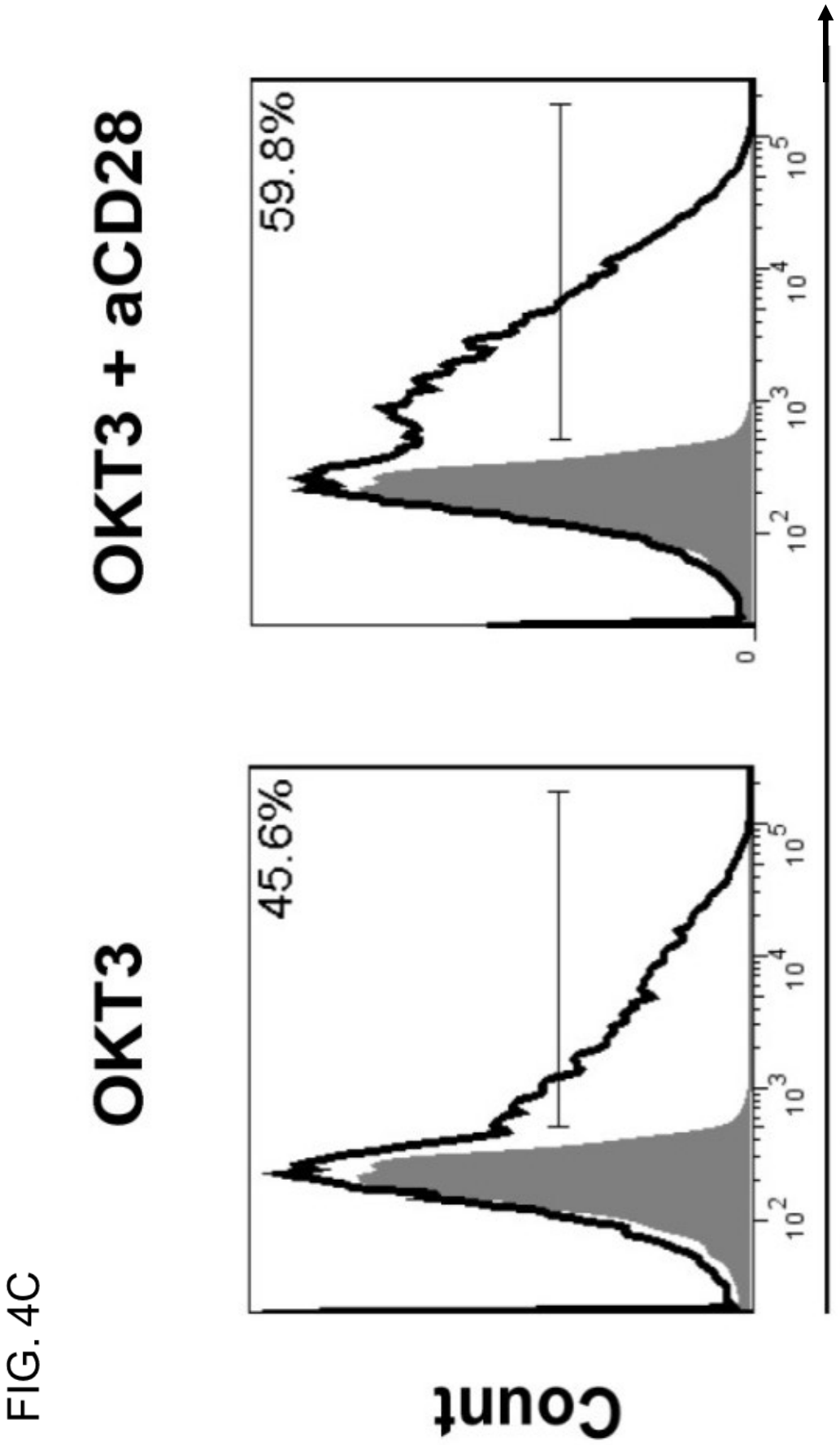


FIG. 4C

FIG. 4C (continued)

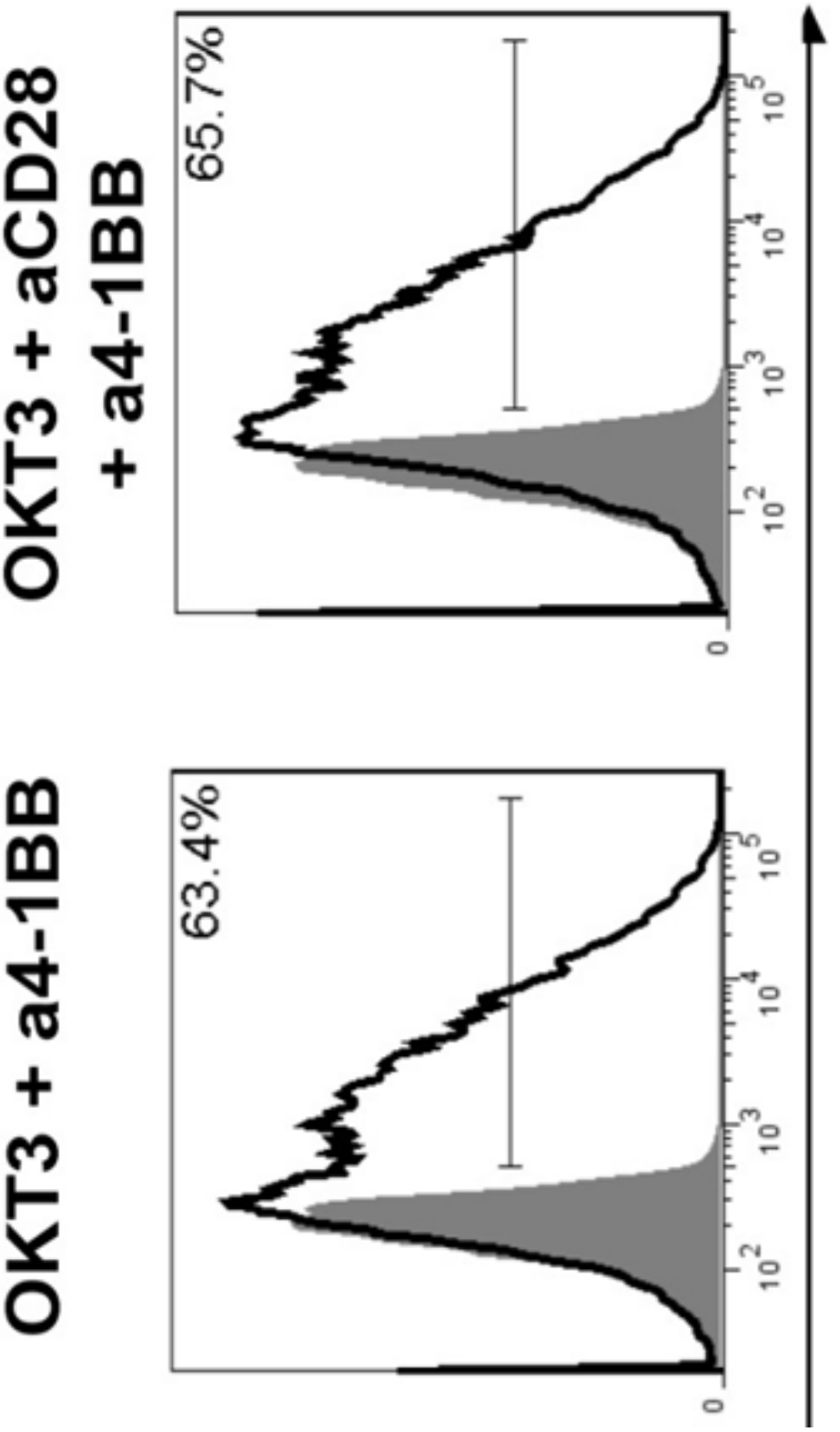
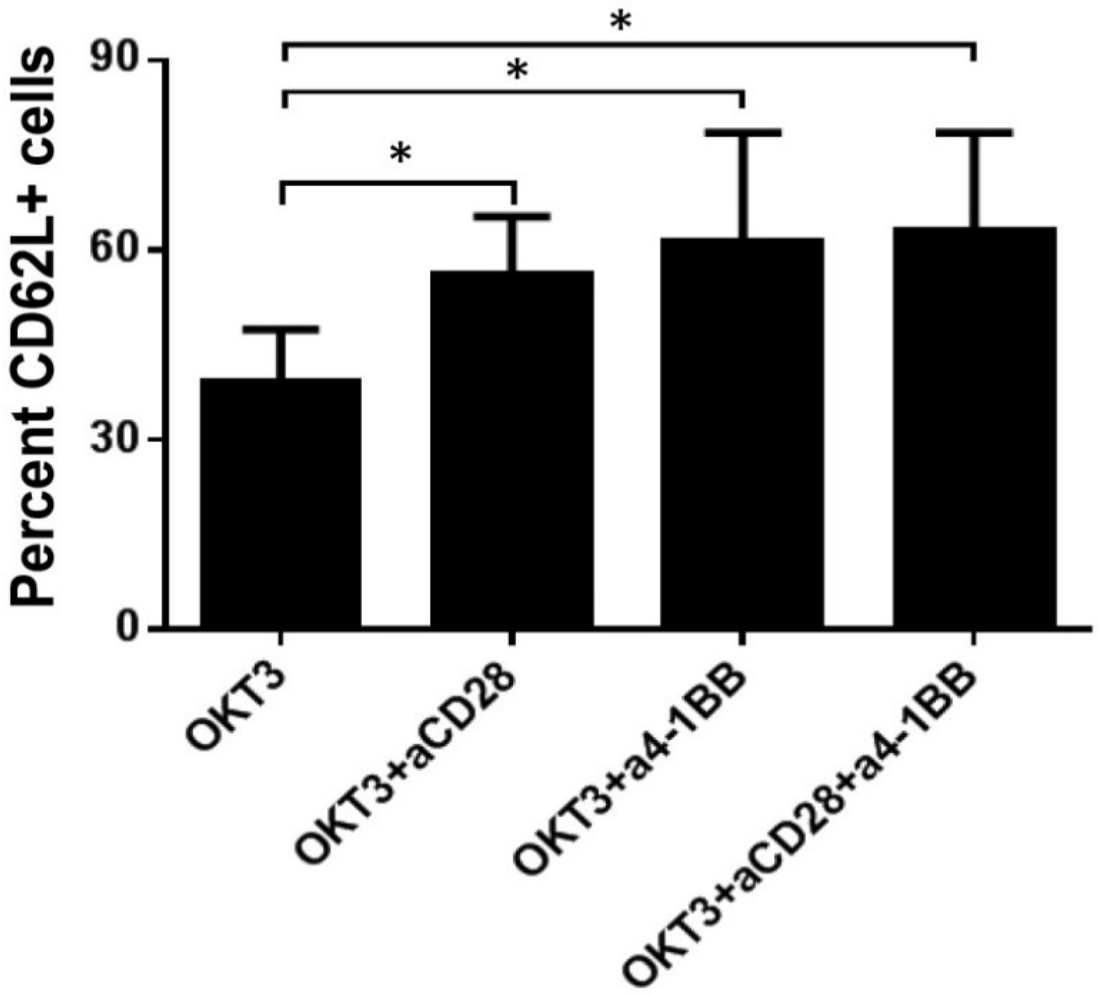


FIG. 4C (continued)



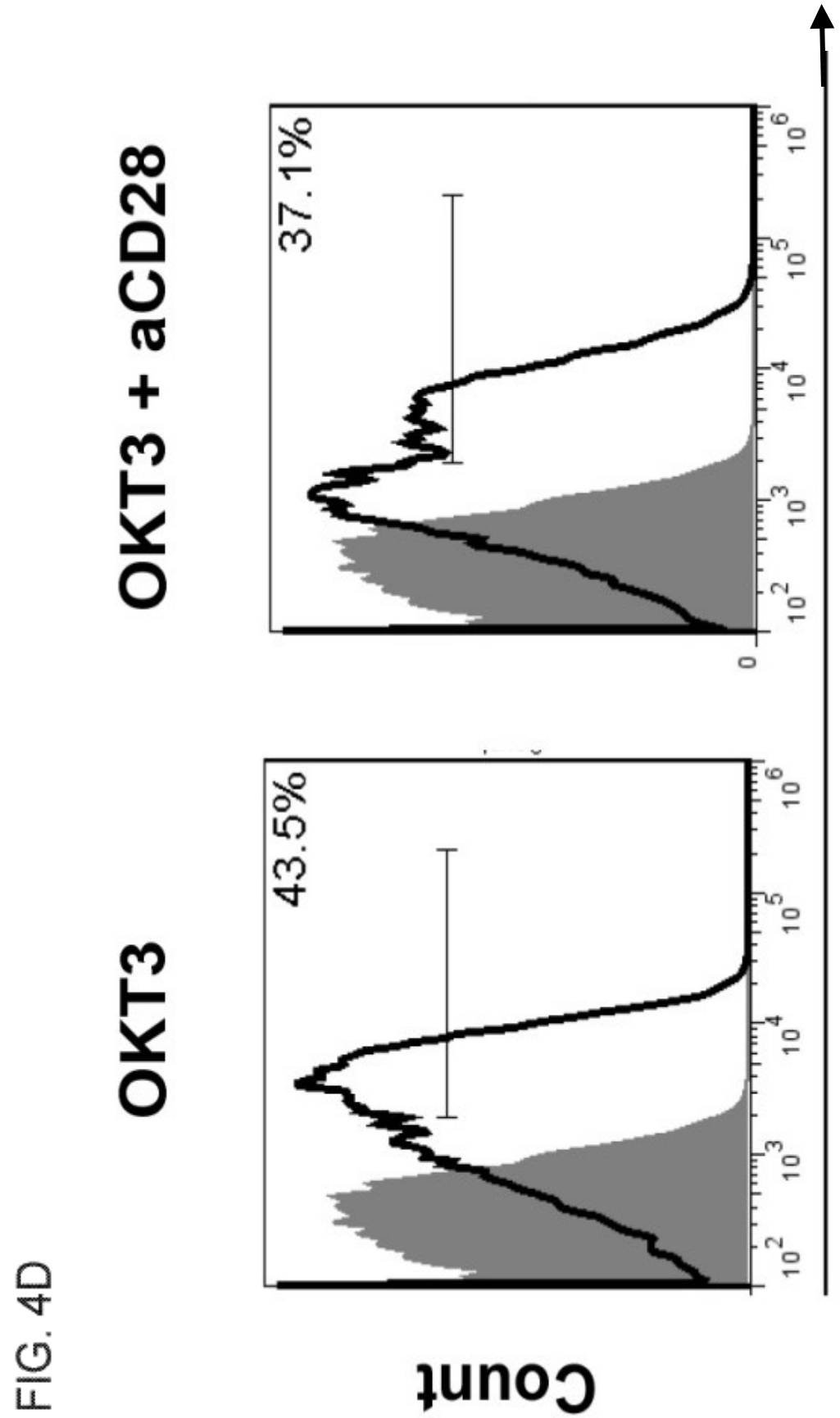


FIG. 4D

FIG. 4D (continued)

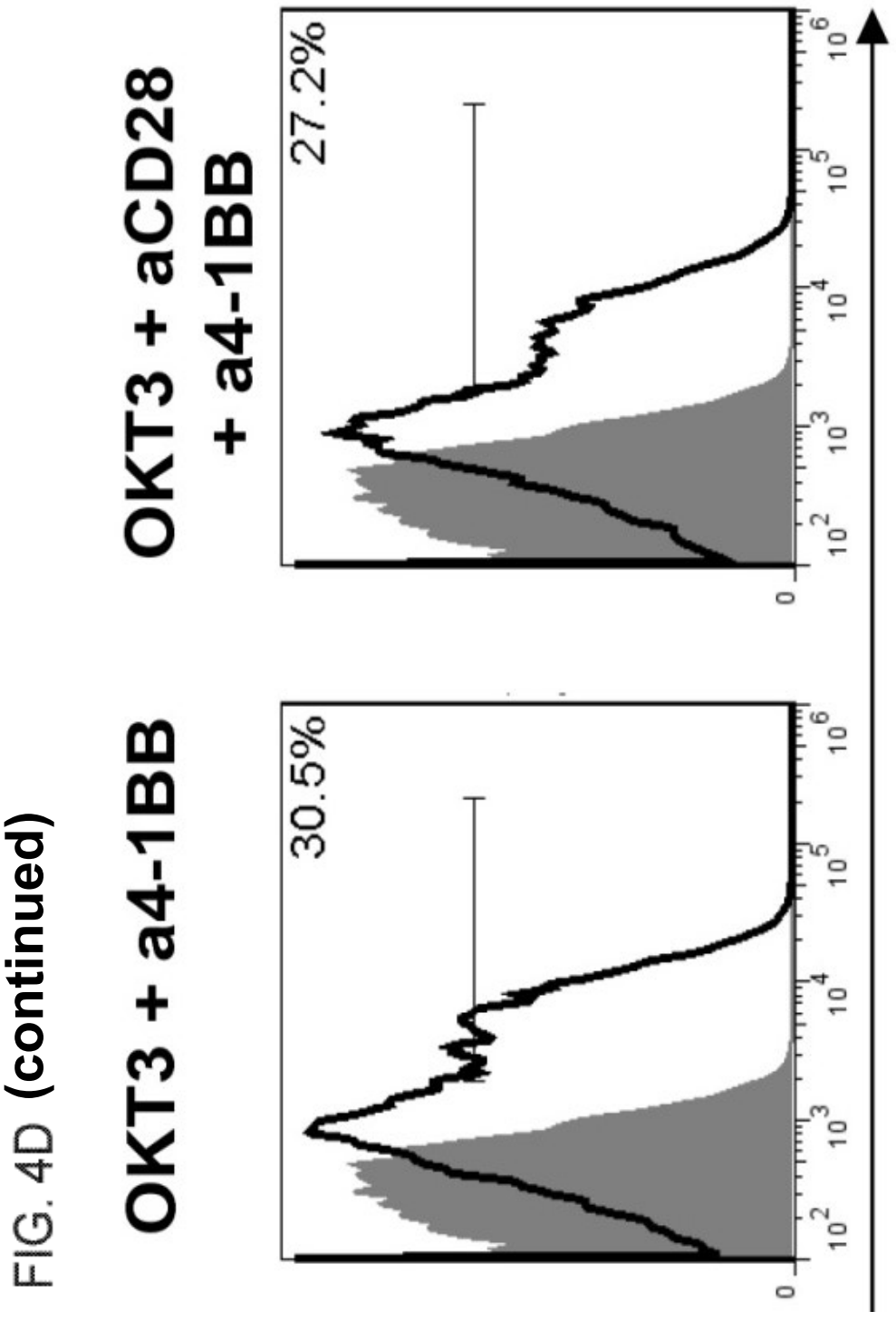


FIG. 4D **(continued)**

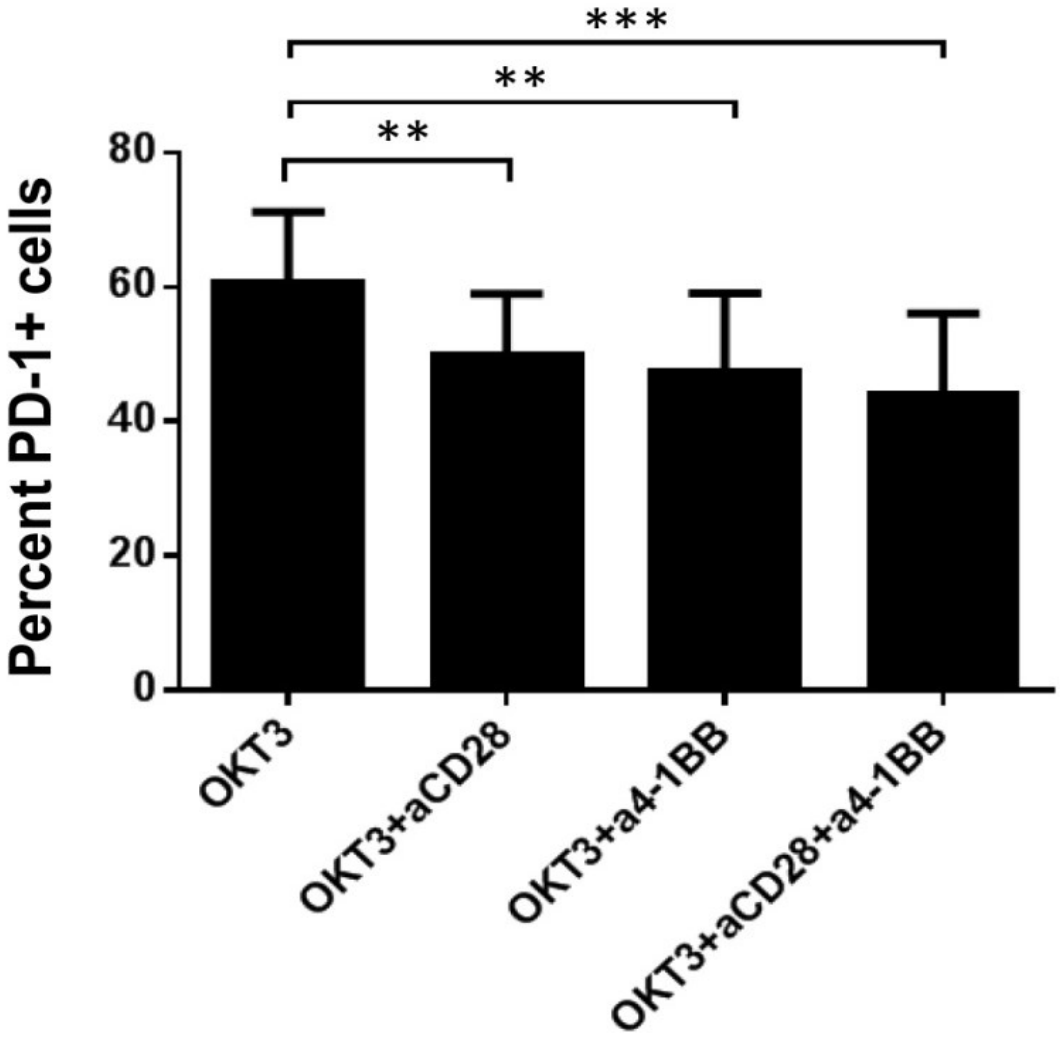


FIG. 5A

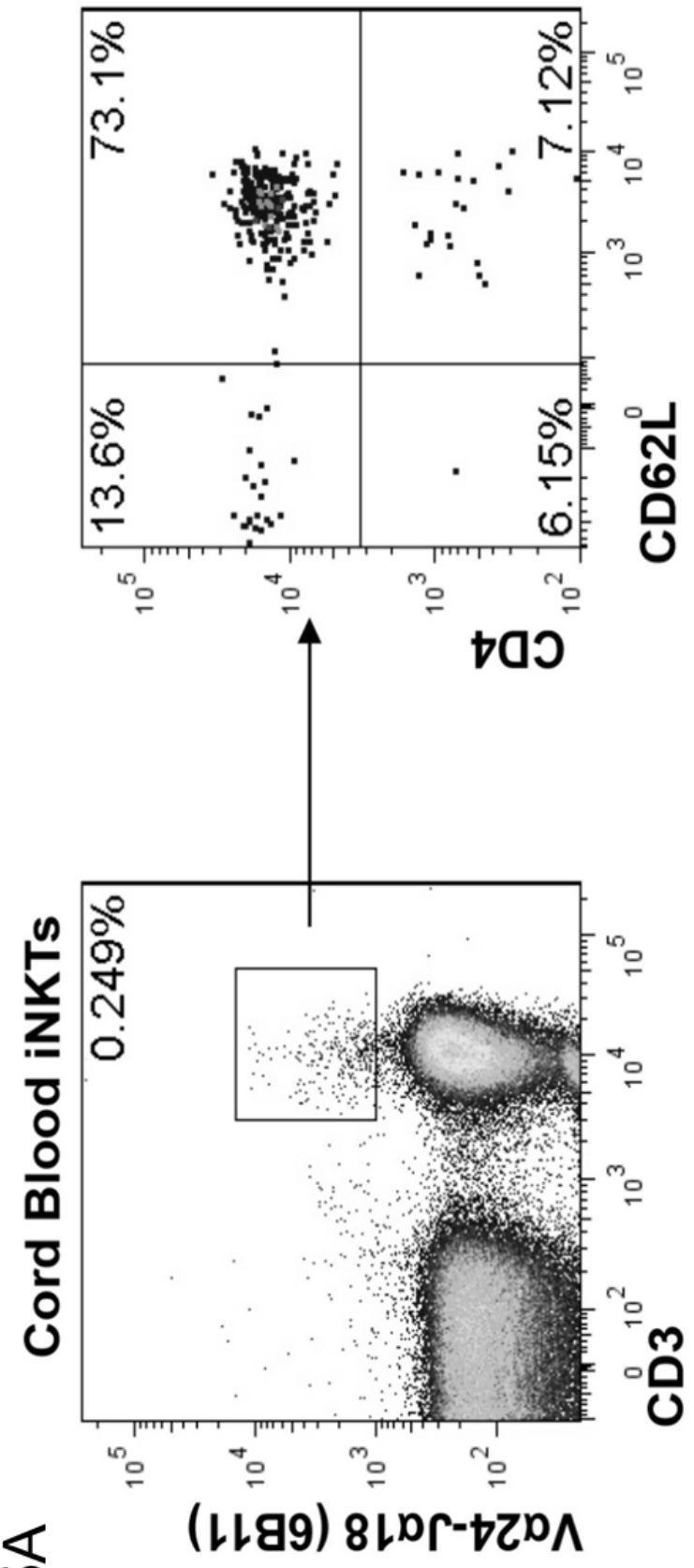

Cord Blood iNKTs
0.249%
Vα24-Jα18 (6B11)
CD3
13.6%
73.1%
6.15%
7.12%
CD4
CD62L

FIG. 5B

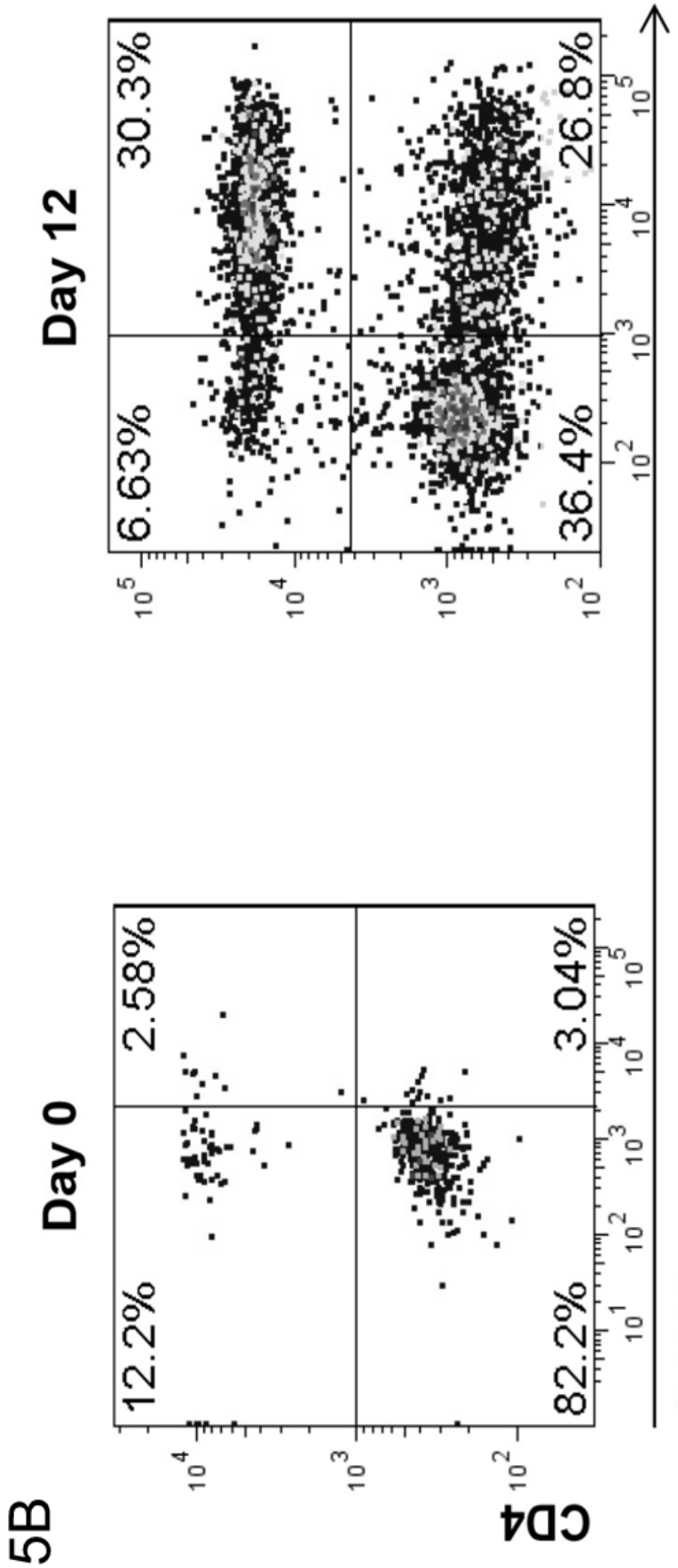


FIG. 5C

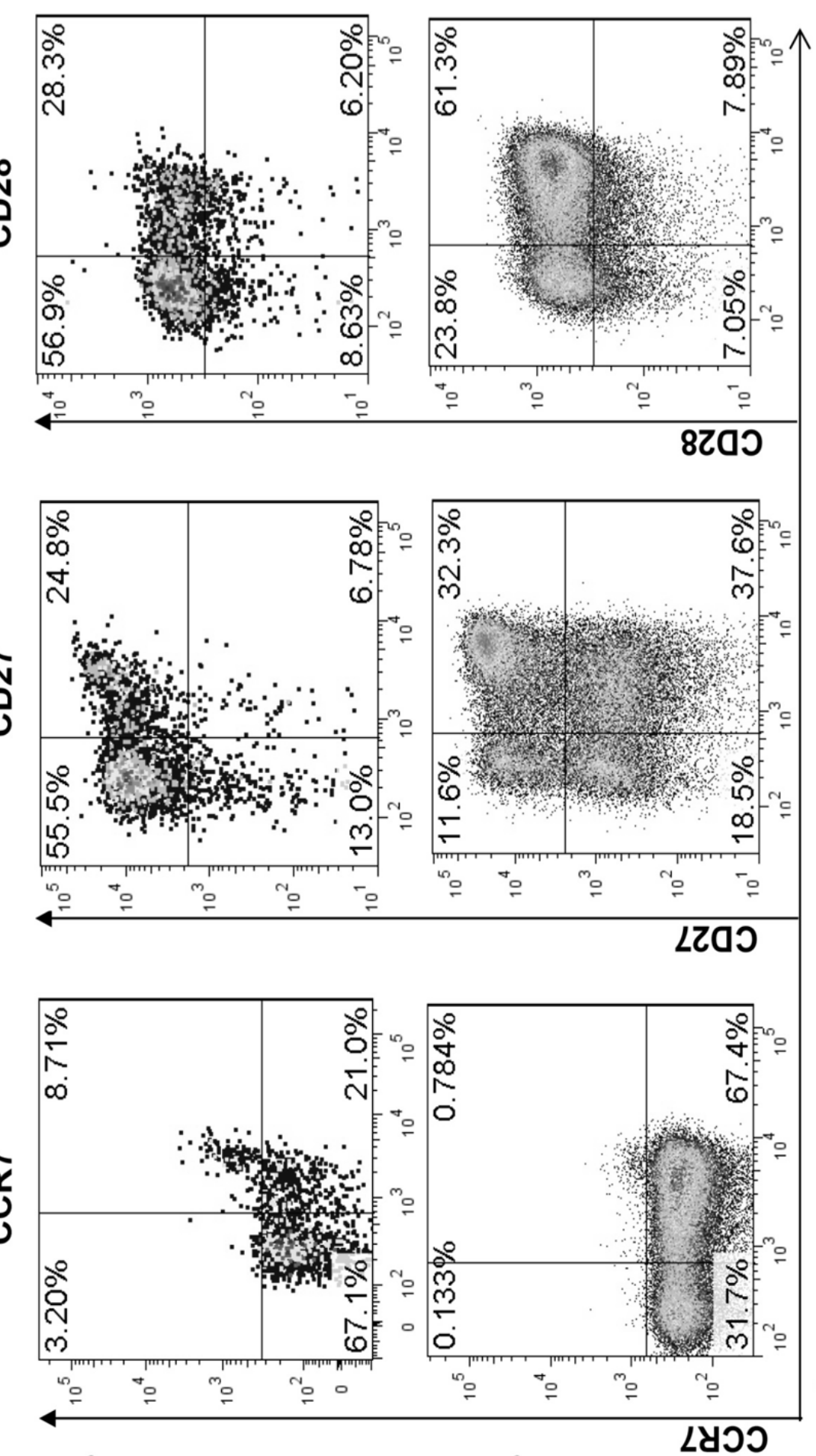
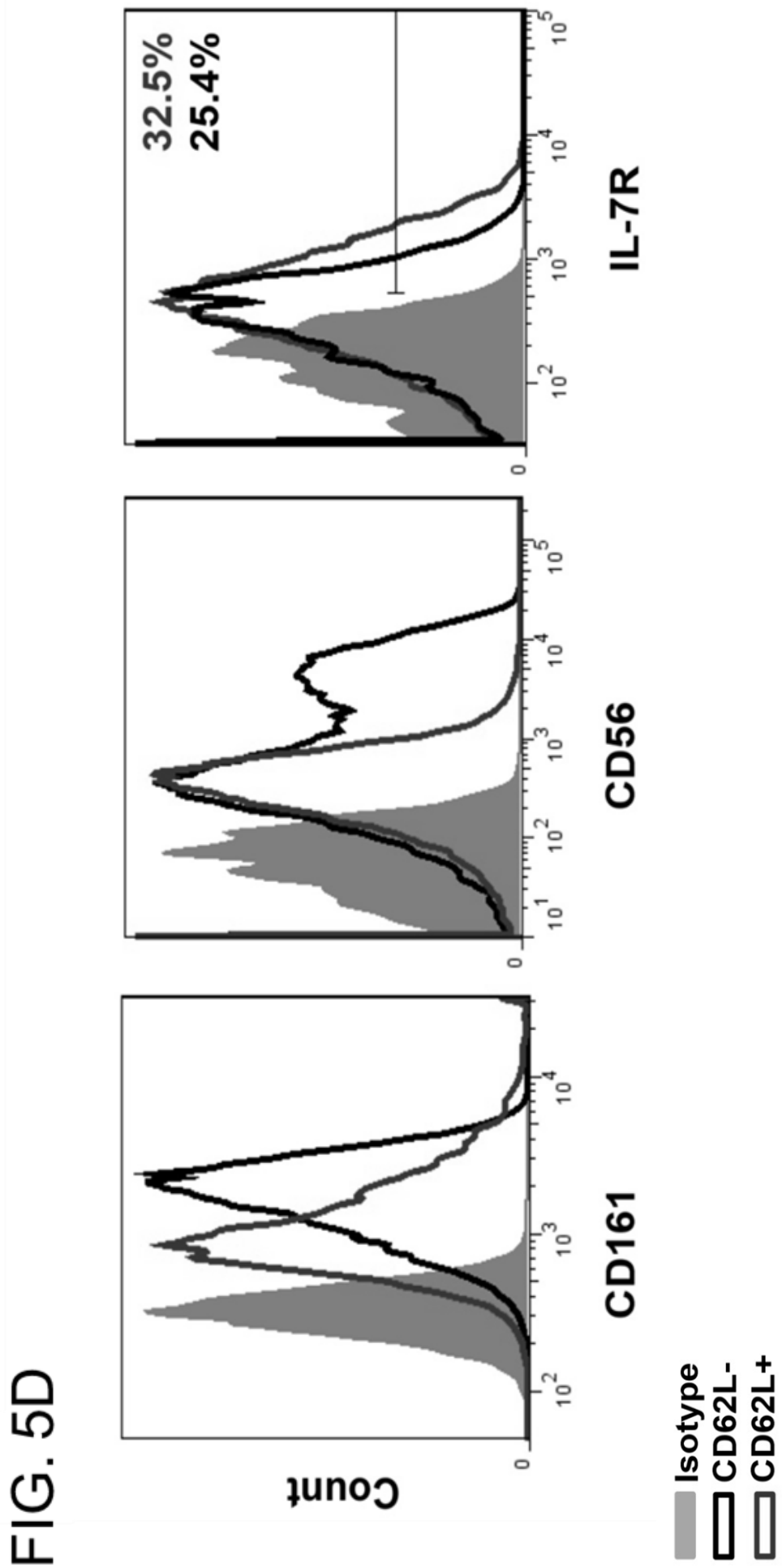

CCR7
CD27
CD28
Day 0
Day 12
CCR7
CD27
CD28
CD62L
3.20%
8.71%
67.1%
21.0%
0.133%
0.784%
31.7%
67.4%
55.5%
24.8%
13.0%
6.78%
11.6%
32.3%
18.5%
37.6%
56.9%
28.3%
8.63%
6.20%
23.8%
61.3%
7.05%
7.89%

FIG. 5D



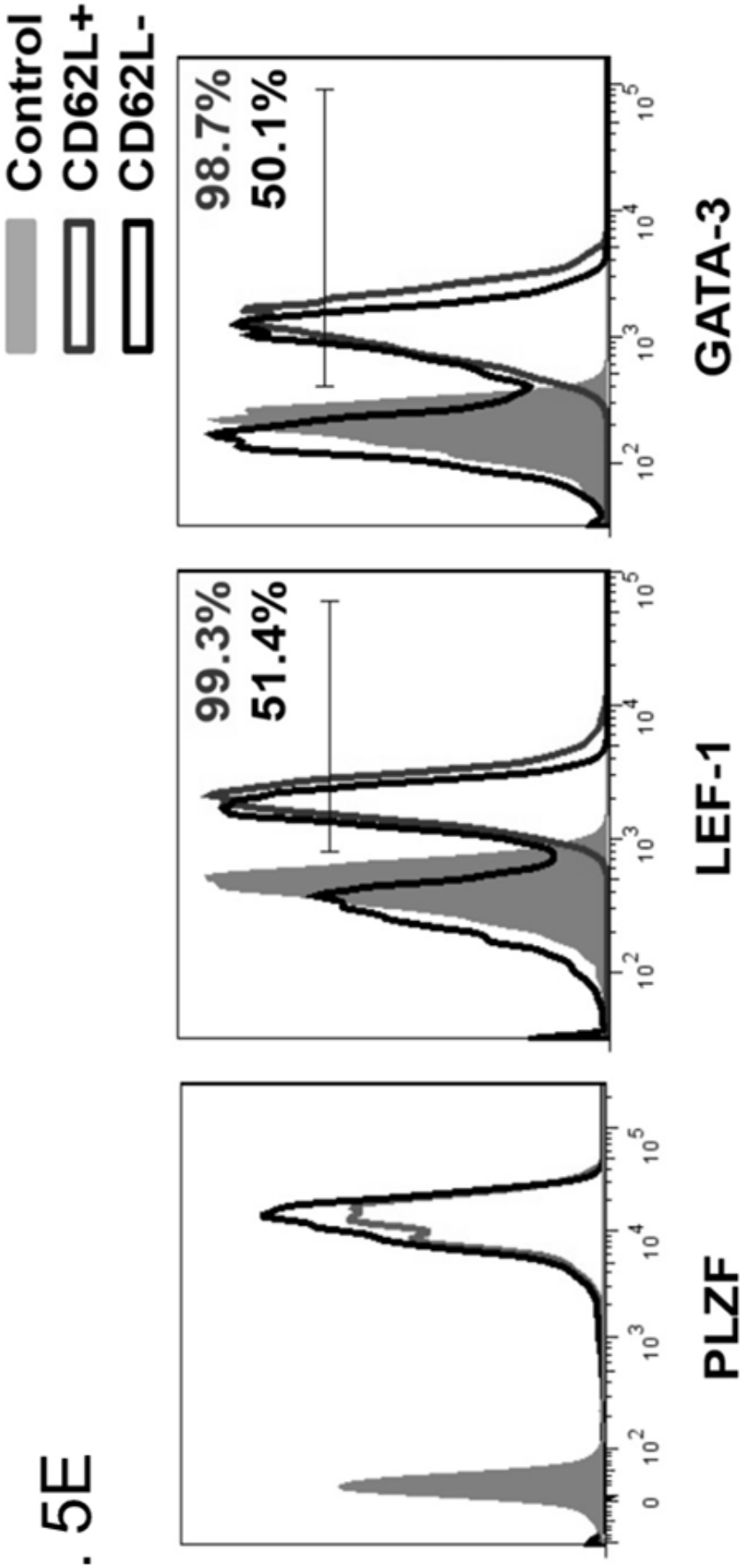


FIG. 5E

FIG. 5E (continued)

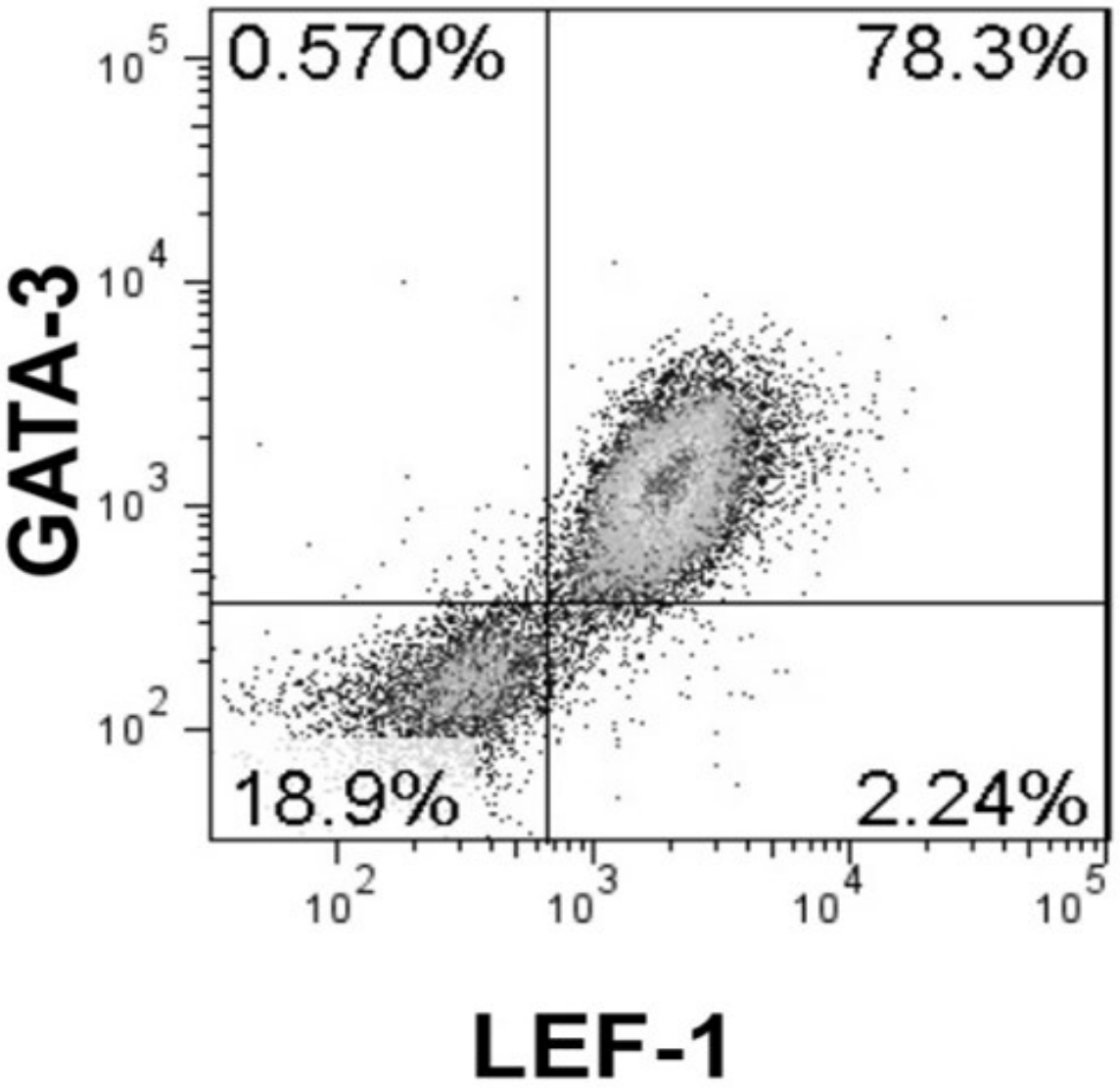


FIG. 6A

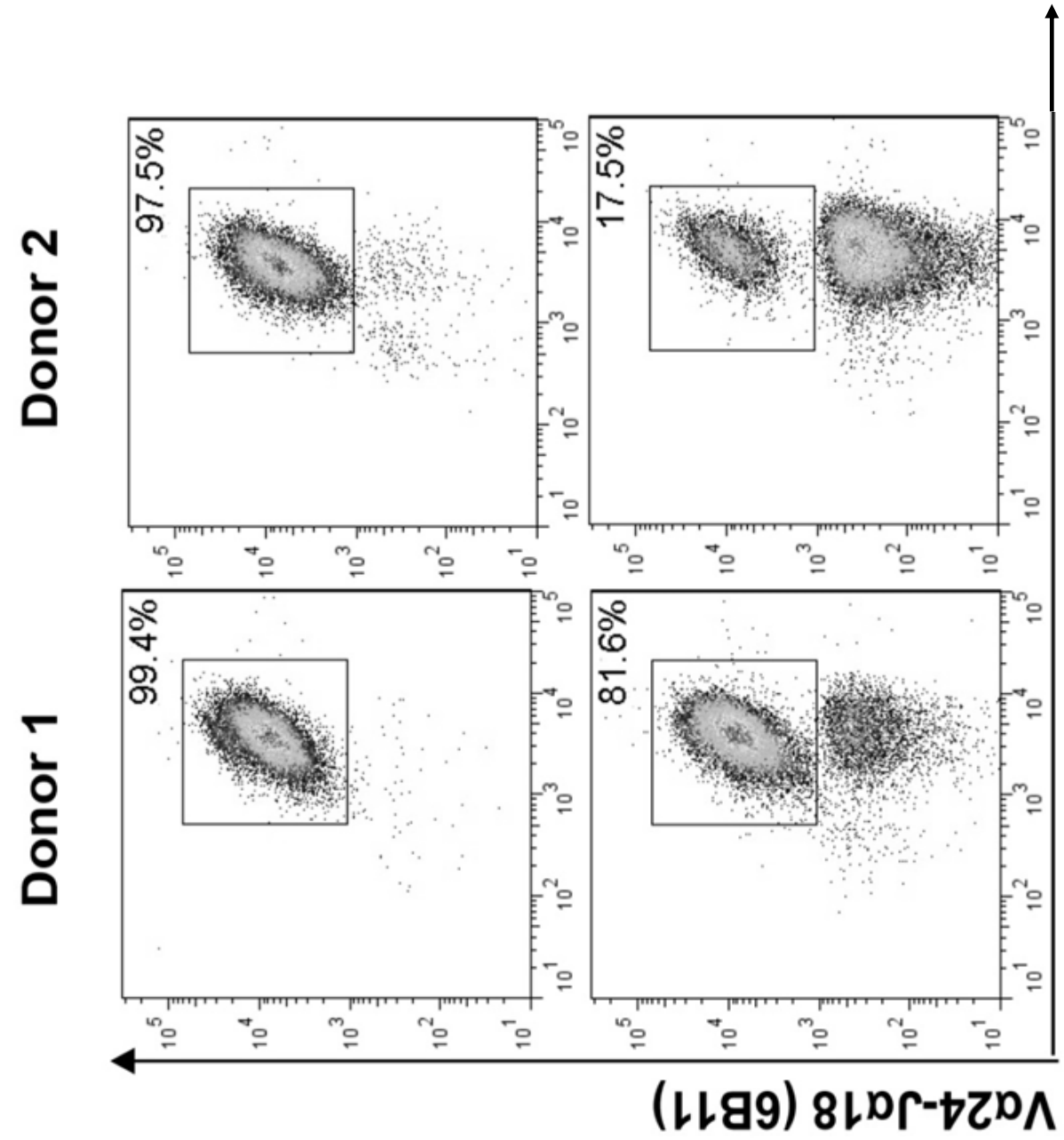
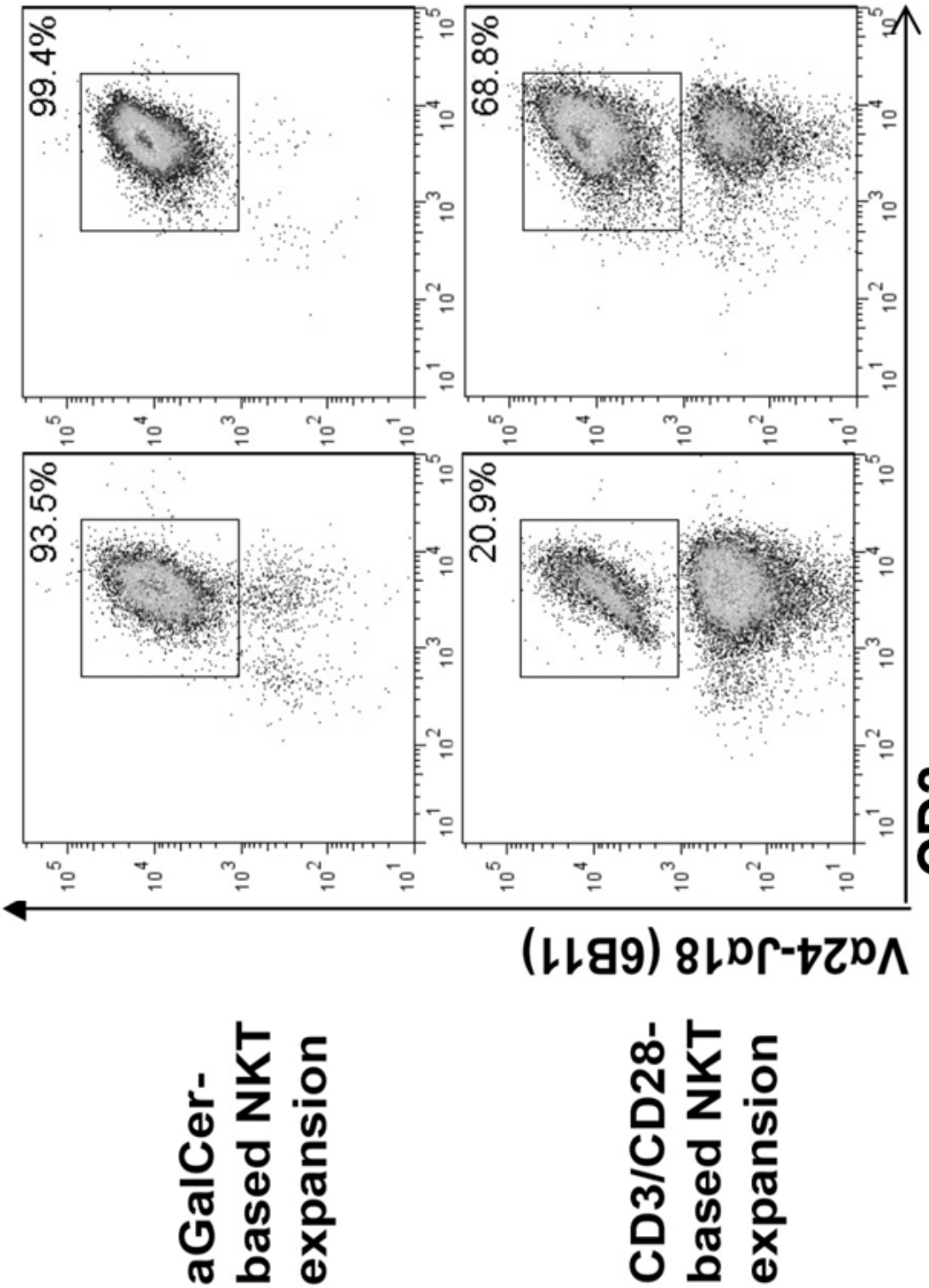

Donor 1
Donor 2
99.4%
97.5%
81.6%
17.5%
aGalCer-based NKT expansion
CD3/CD28-based NKT expansion
Vα24-Jα18 (6B11)
CD3

FIG. 6A (continued)



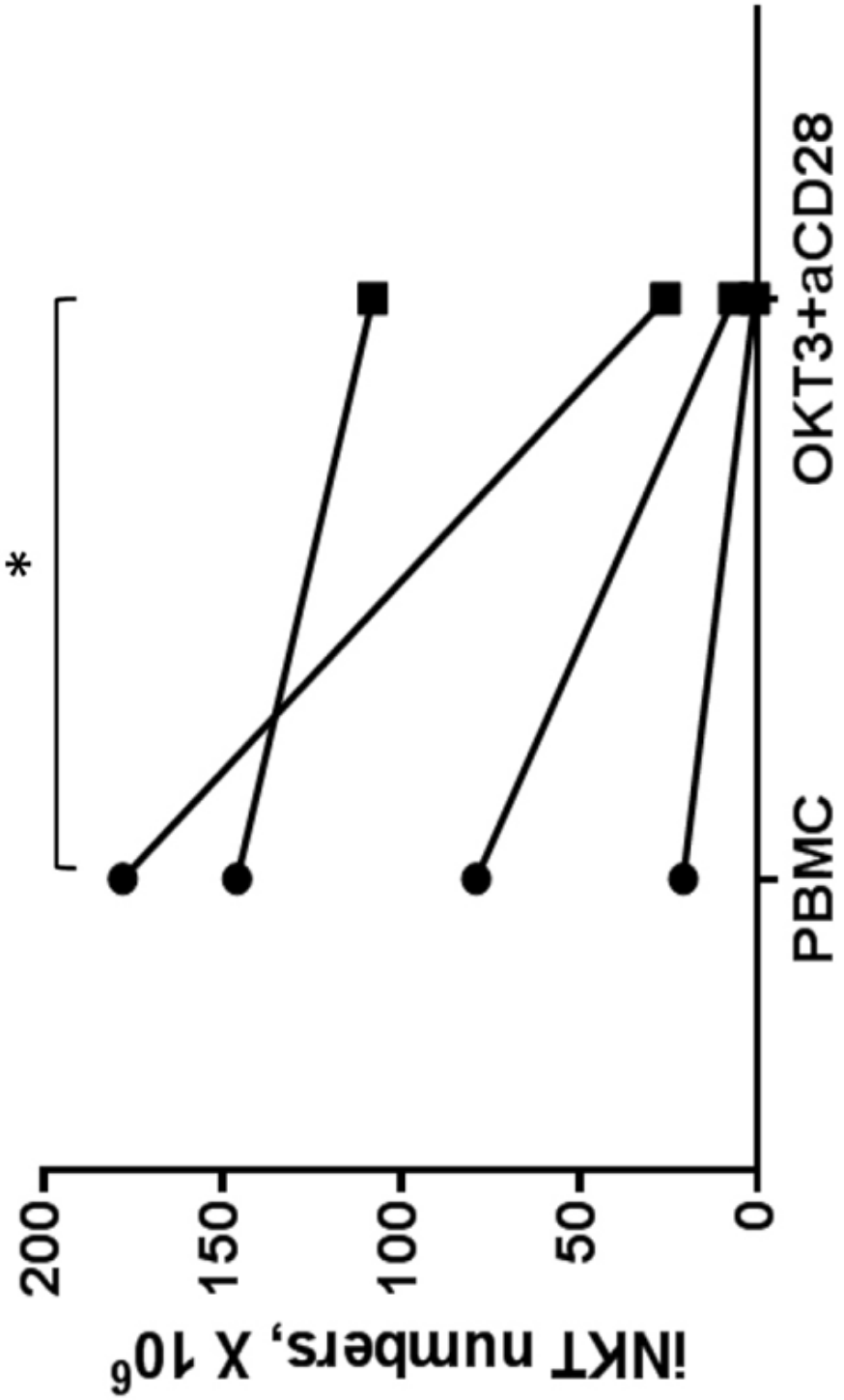


FIG. 6B

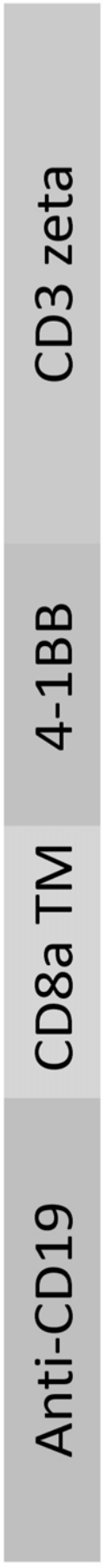


FIG. 7A

FIG. 7B

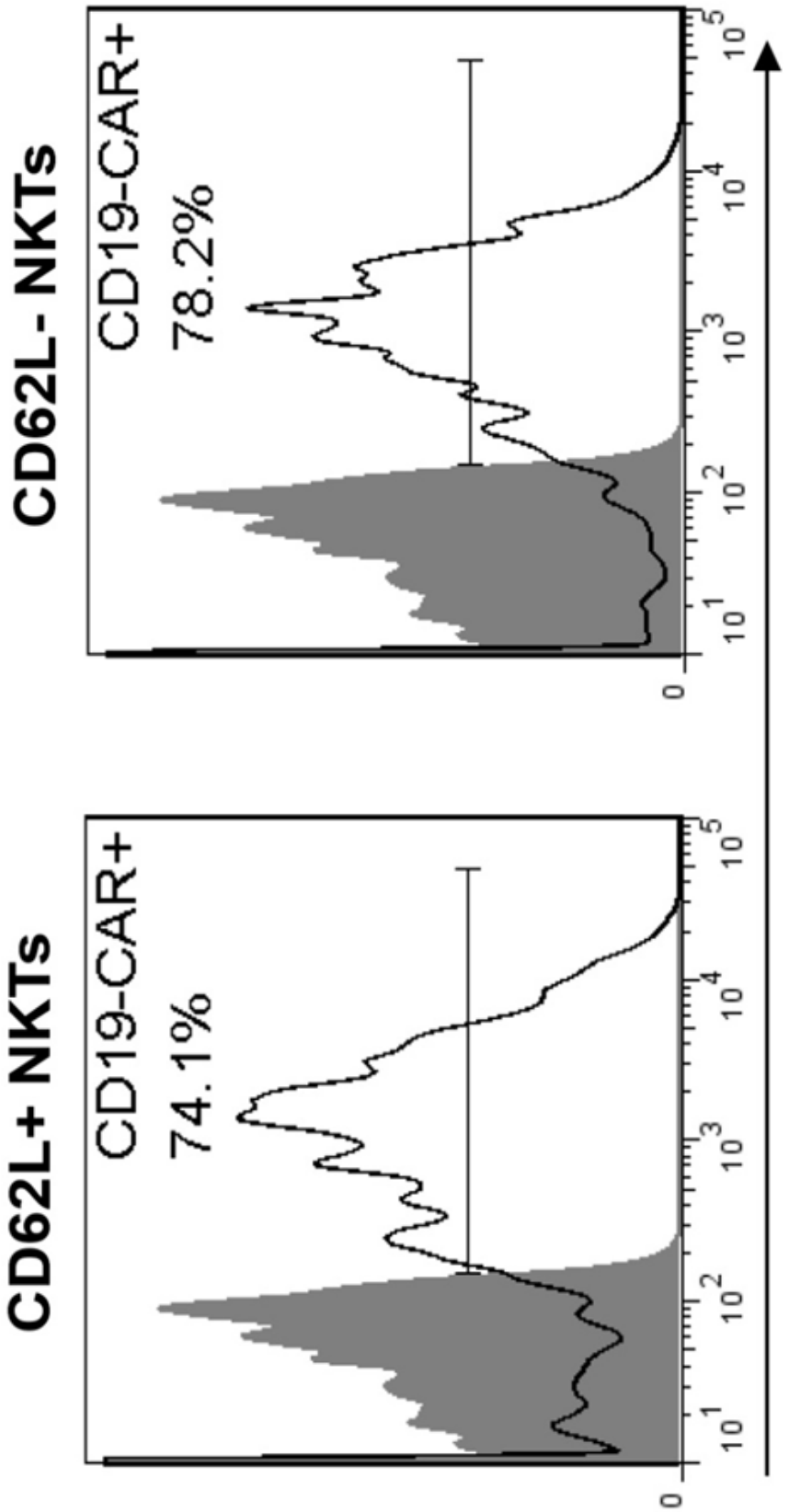

CD62L+ NKTs
CD19-CAR+
74.1%
CD62L- NKTs
CD19-CAR+
78.2%
Count
CAR.CD19
0
10^1
10^2
10^3
10^4
10^5

FIG. 8A

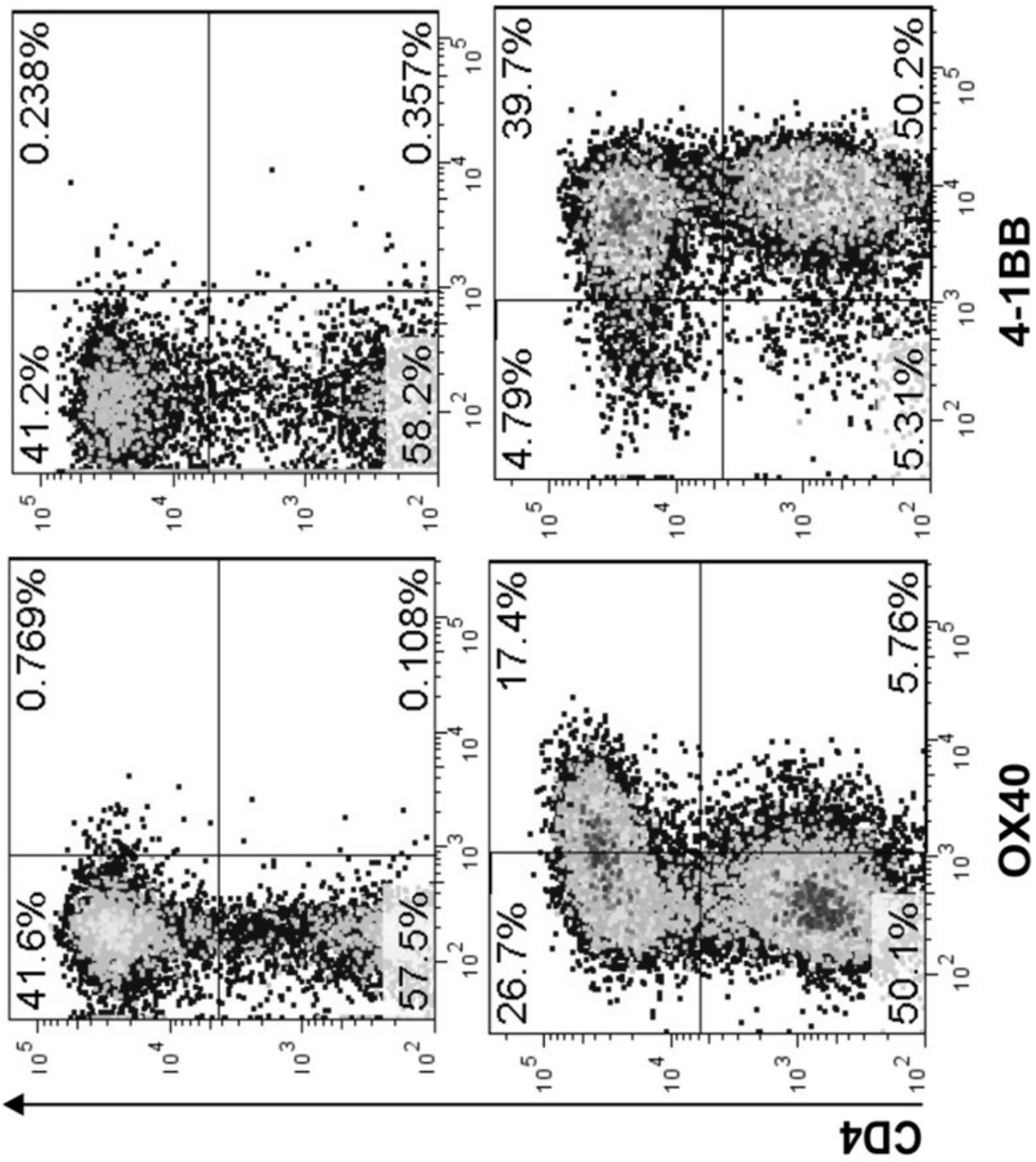

Day 0
Day 3
CD4
OX40
4-1BB
41.6%
0.769%
57.5%
0.108%
41.2%
0.238%
58.2%
0.357%
26.7%
17.4%
50.1%
5.76%
4.79%
39.7%
5.31%
50.2%

FIG. 8B

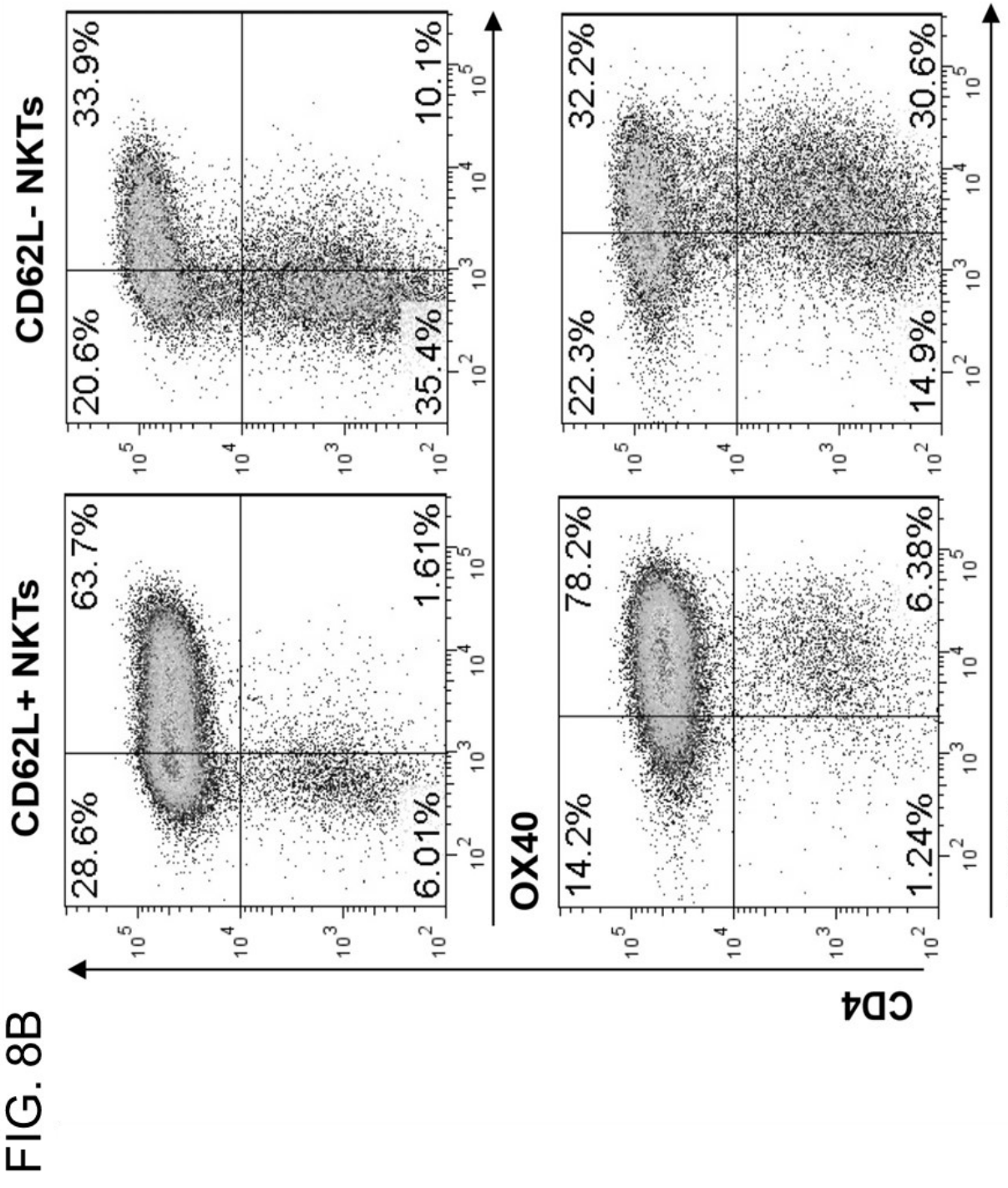

CD62L+ NKTs
CD62L- NKTs
28.6%
63.7%
6.01%
1.61%
20.6%
33.9%
35.4%
10.1%
22.3%
32.2%
14.9%
30.6%
14.2%
78.2%
1.24%
6.38%
OX40
4-1BB
CD4

FIG. 9

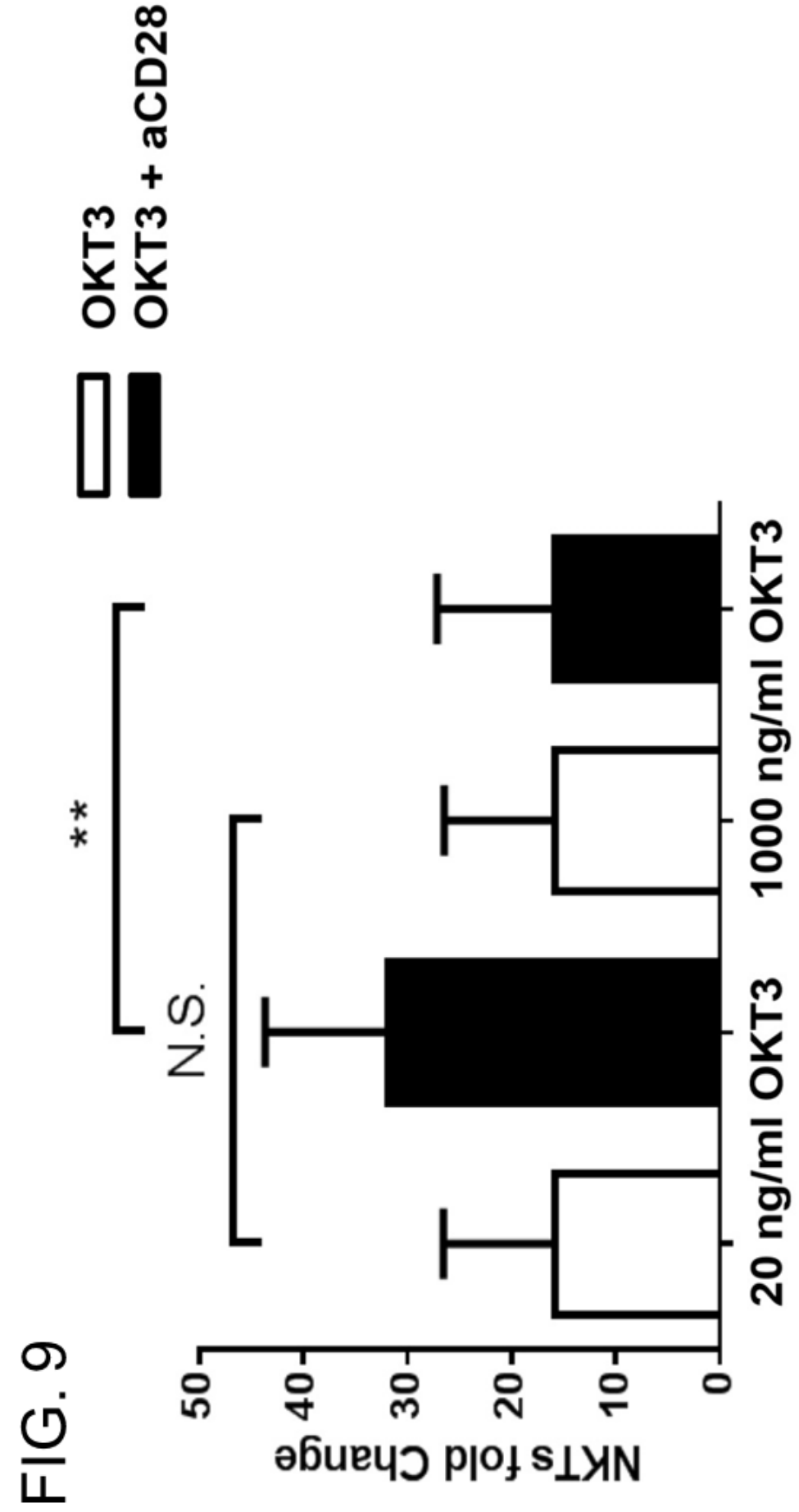

OKT3
OKT3 + aCD28
**
N.S.
NKTs fold Change
0
10
20
30
40
50
20 ng/ml OKT3
1000 ng/ml OKT3

FIG. 10

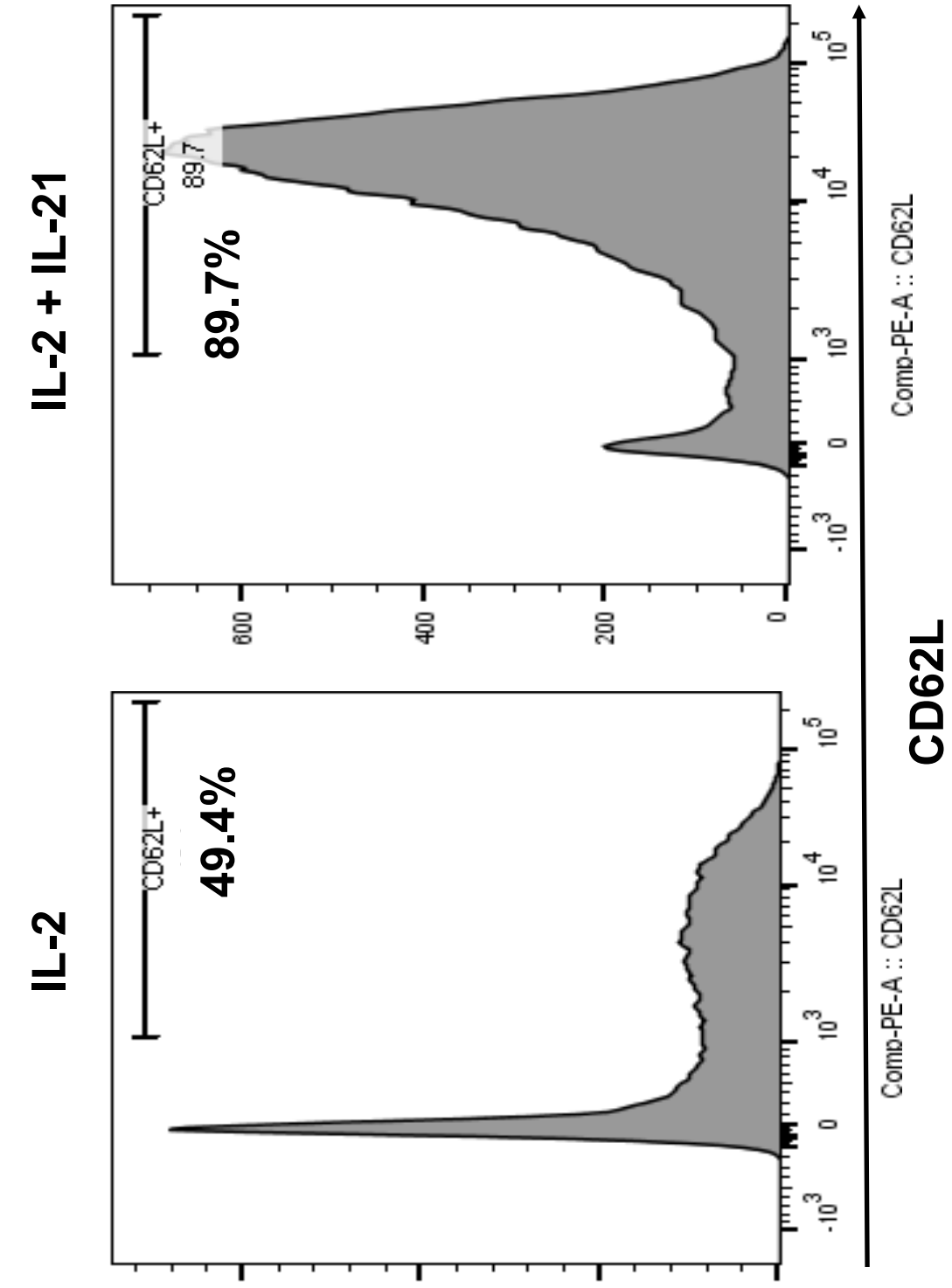

IL-2
IL-2 + IL-21
Donor 1
Counts
CD62L+
49.4%
89.7%
89.7
1.5K
1.0K
500
0
600
400
200
-10^3
0
10^3
10^4
10^5
Comp-PE-A :: CD62L
CD62L

FIG. 10 (continued)

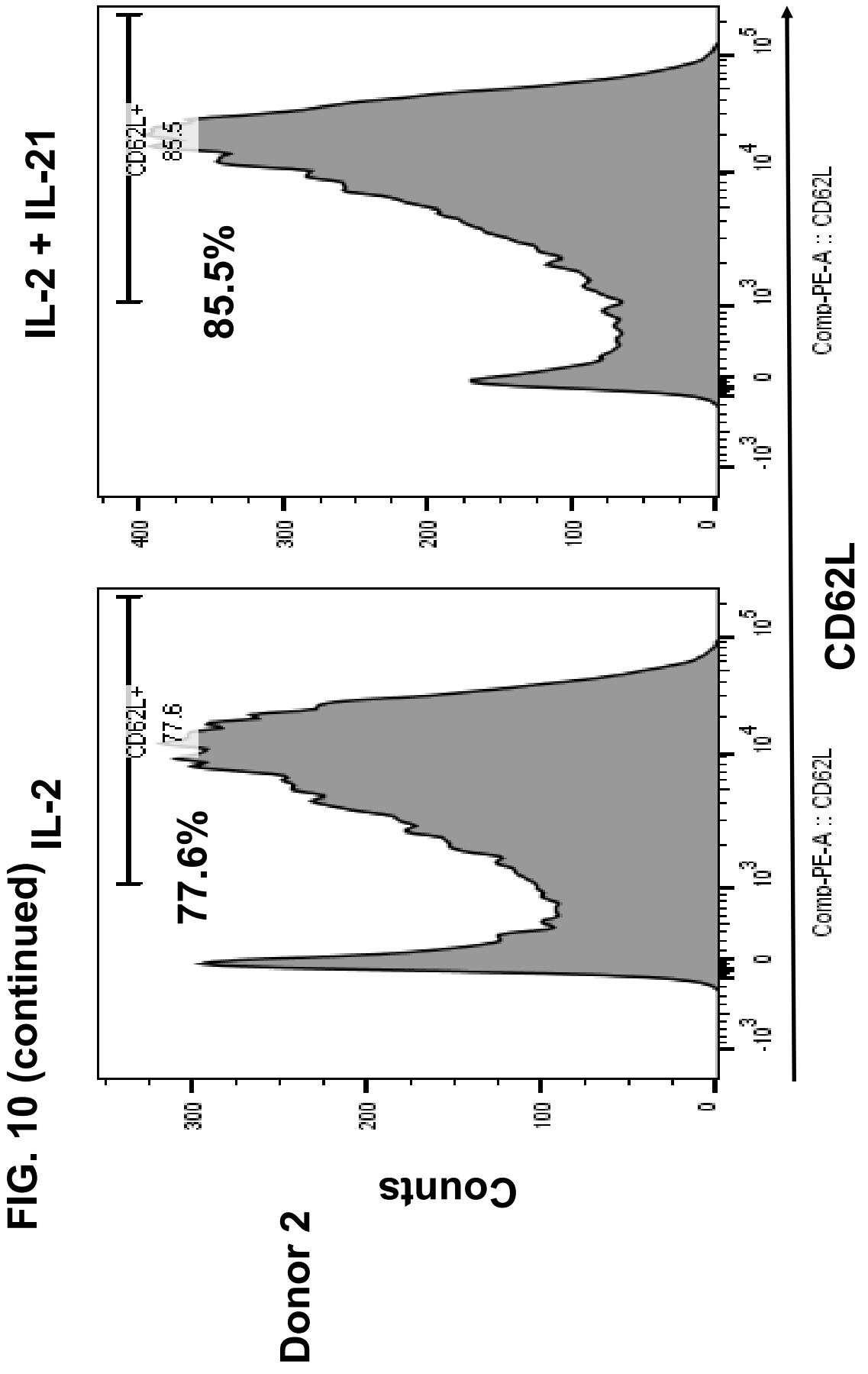


FIG. 10 (continued)

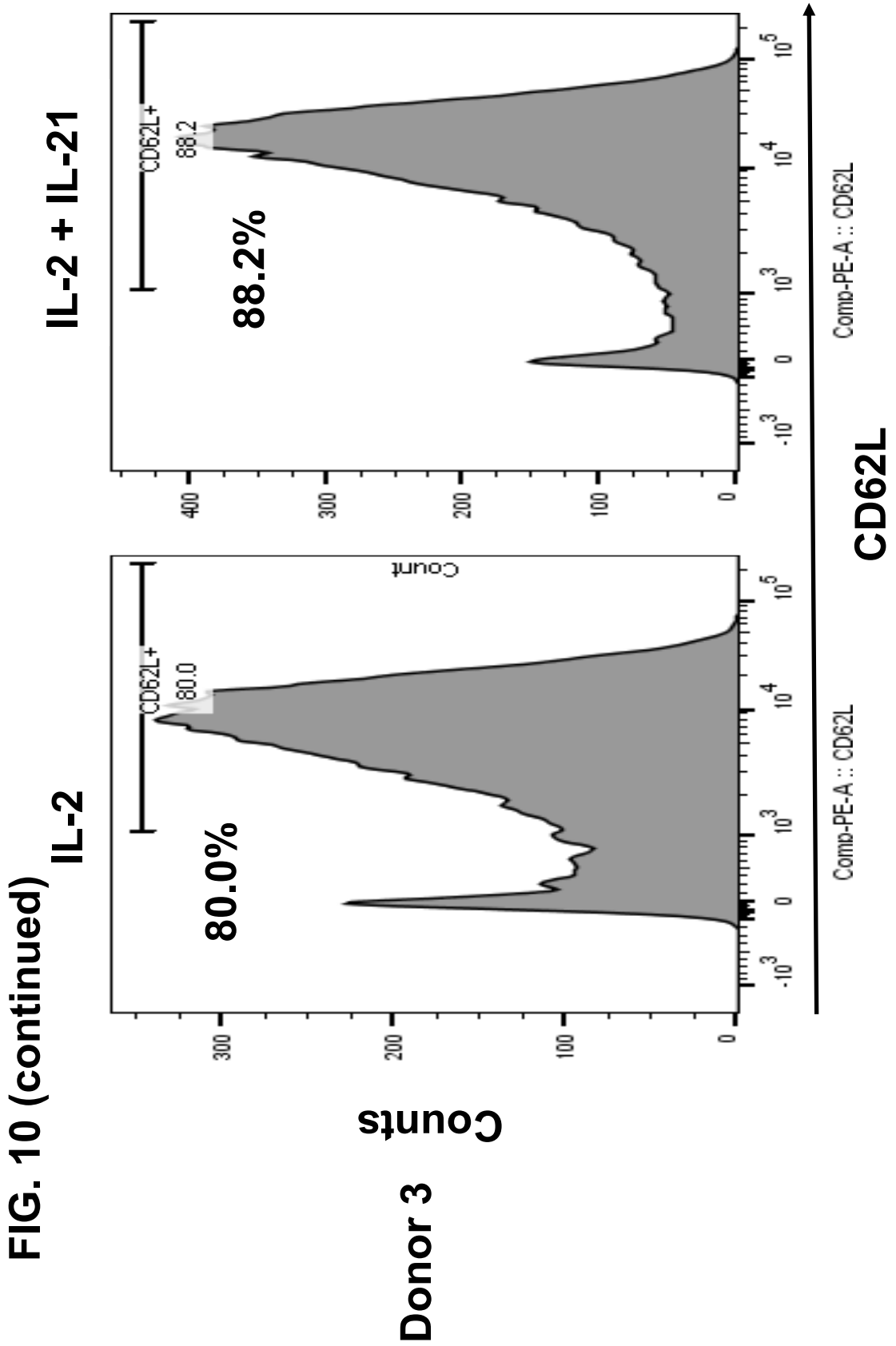


FIG. 11

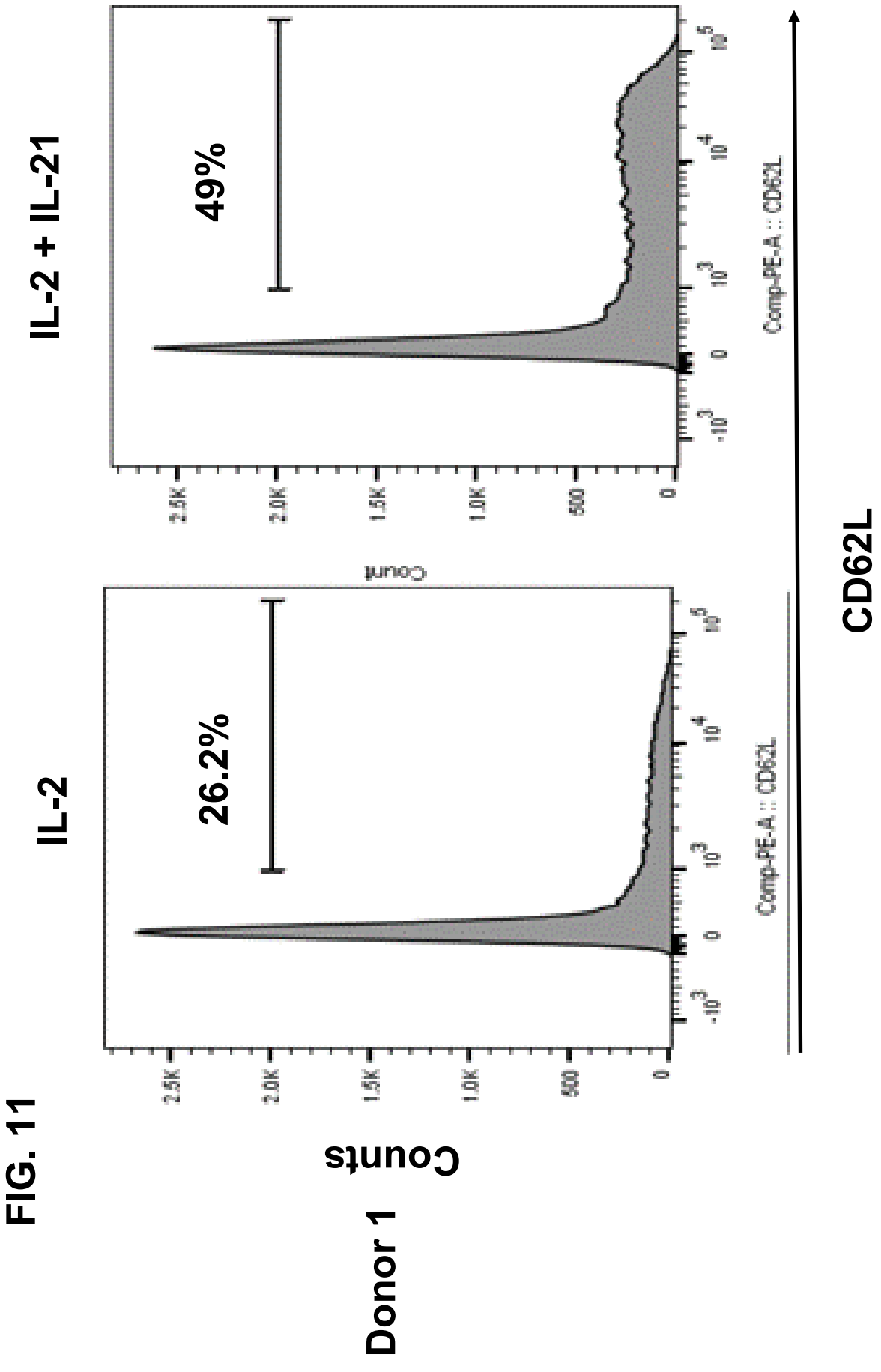

IL-2
IL-2 + IL-21
26.2%
49%
Donor 1
Counts
Count
2.5K
2.0K
1.5K
1.0K
500
0
-10^3
0
10^3
10^4
10^5
Comp-PE-A :: CD62L
CD62L

FIG. 11 (continued)

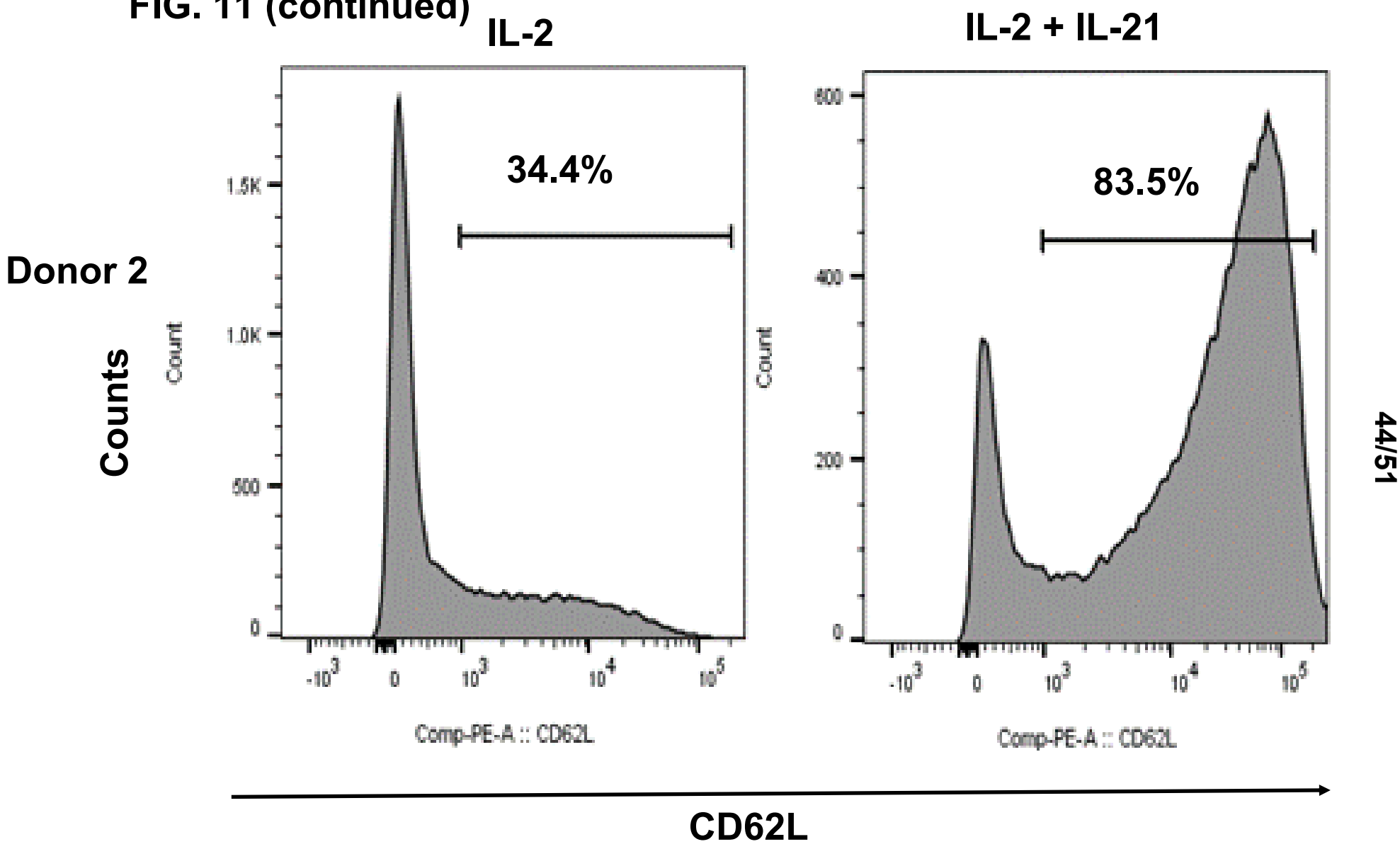
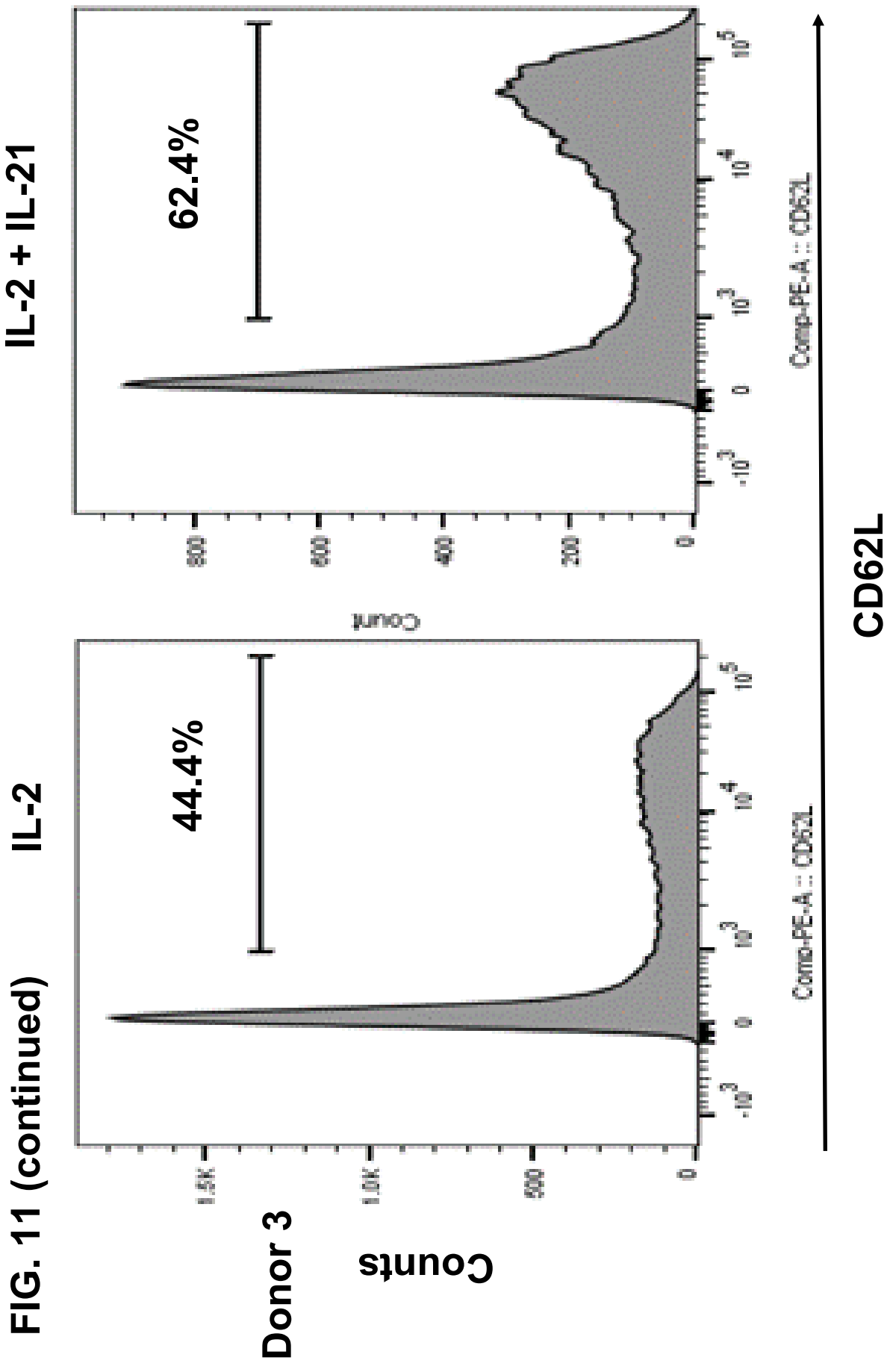


FIG. 11 (continued)



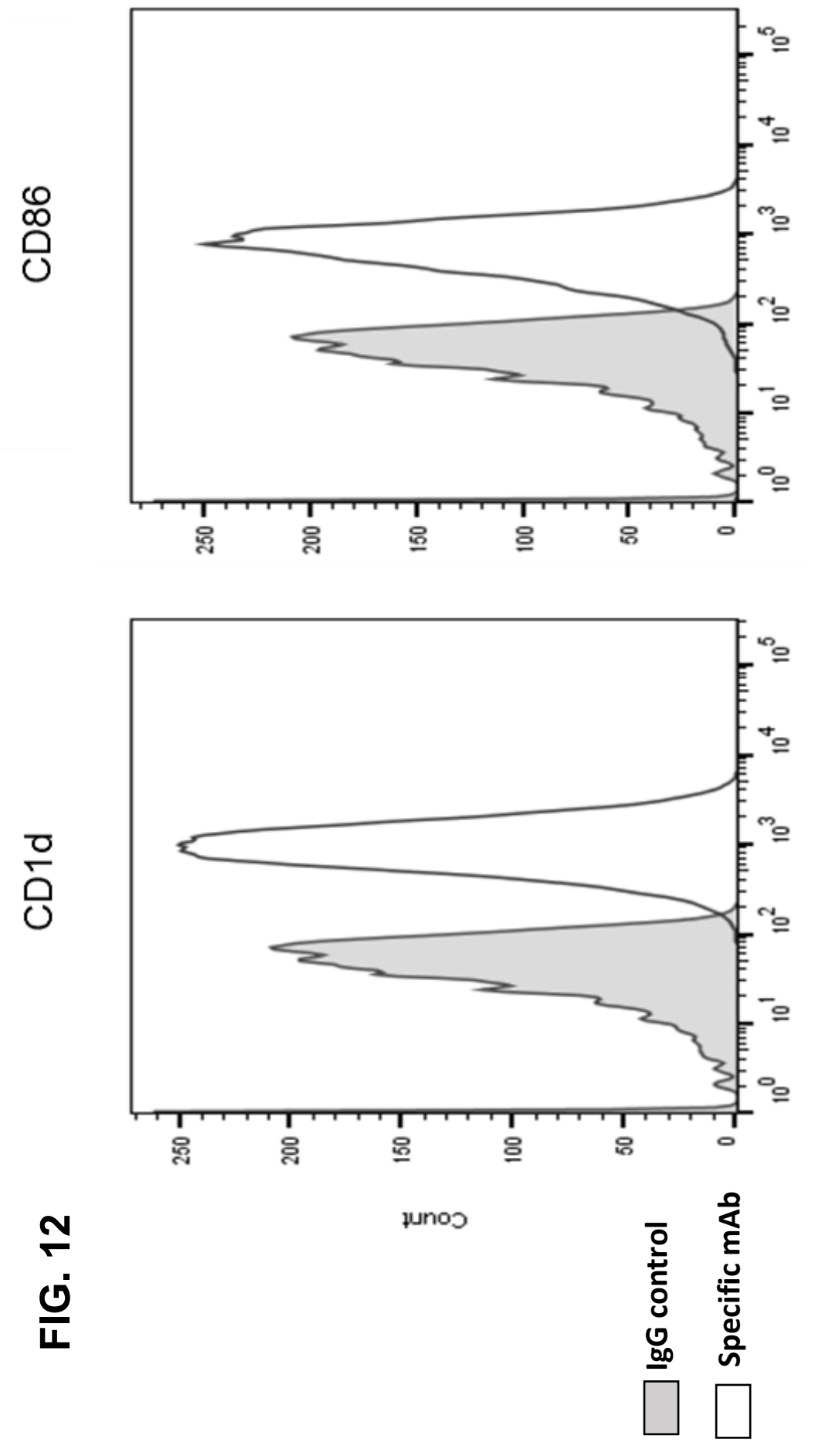

CD86
CD1d
Count
0
50
100
150
200
250
10^0
10^1
10^2
10^3
10^4
10^5
IgG control
Specific mAb

FIG. 12

FIG. 12 (continued)

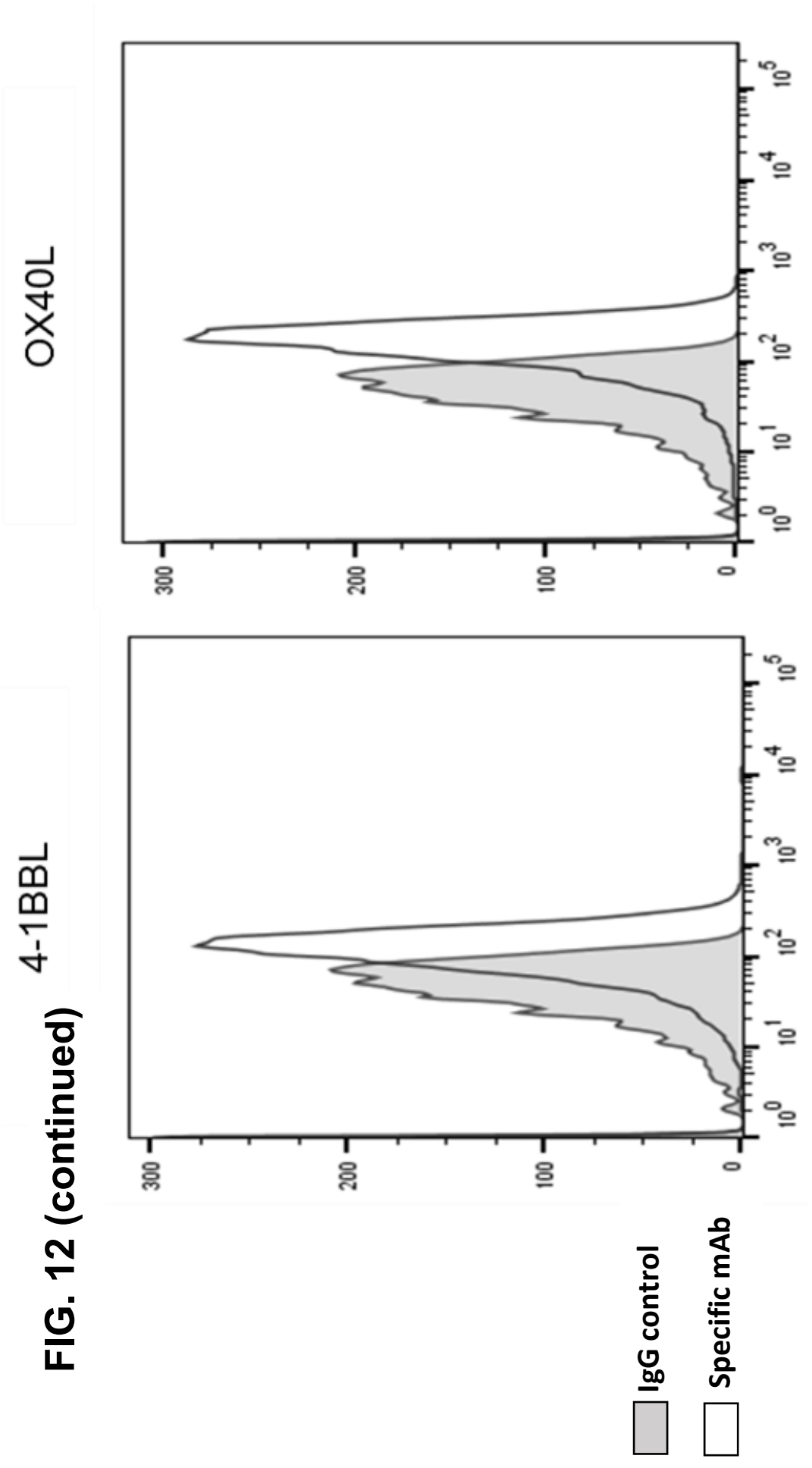
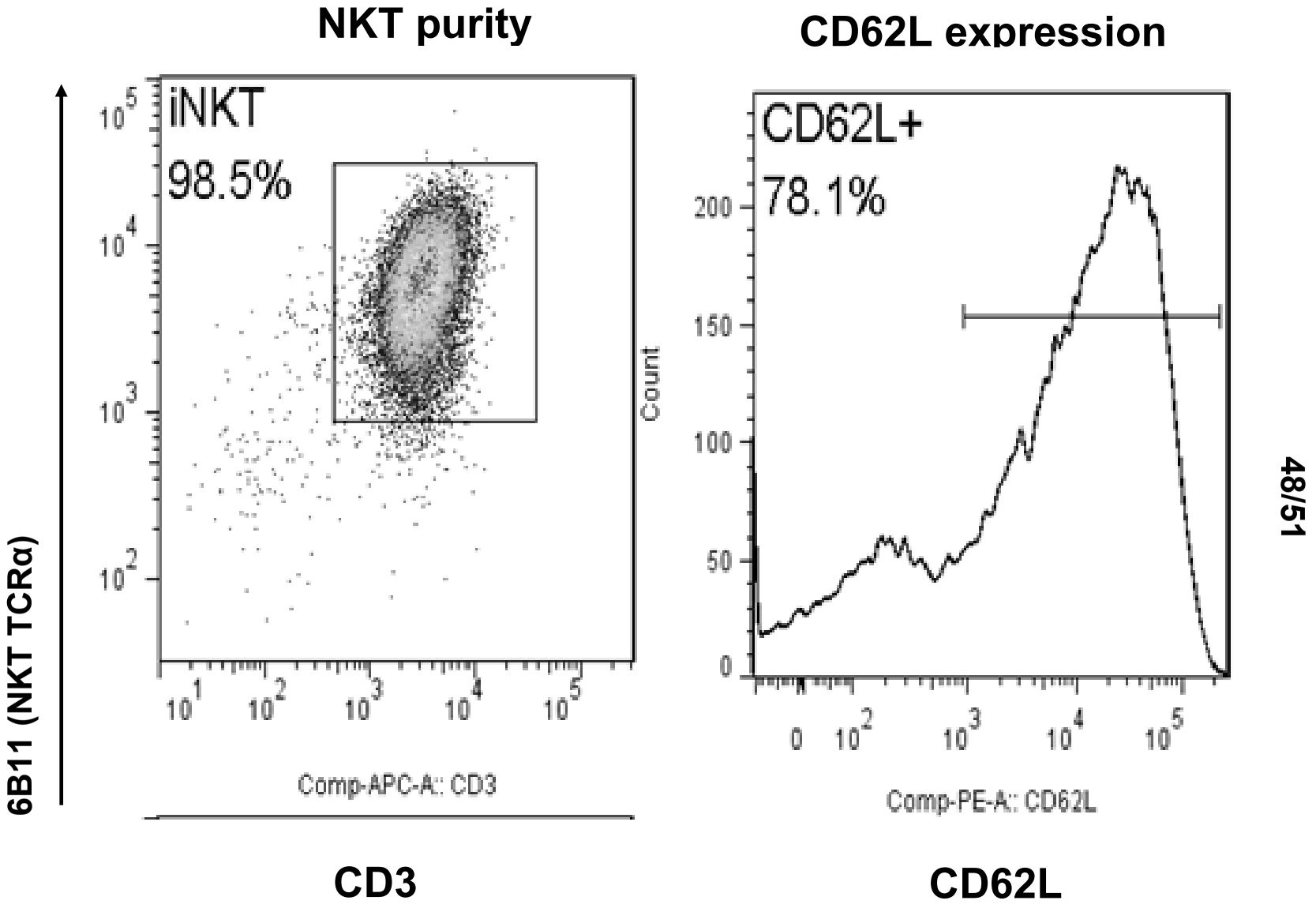


FIG. 13

Donor 1



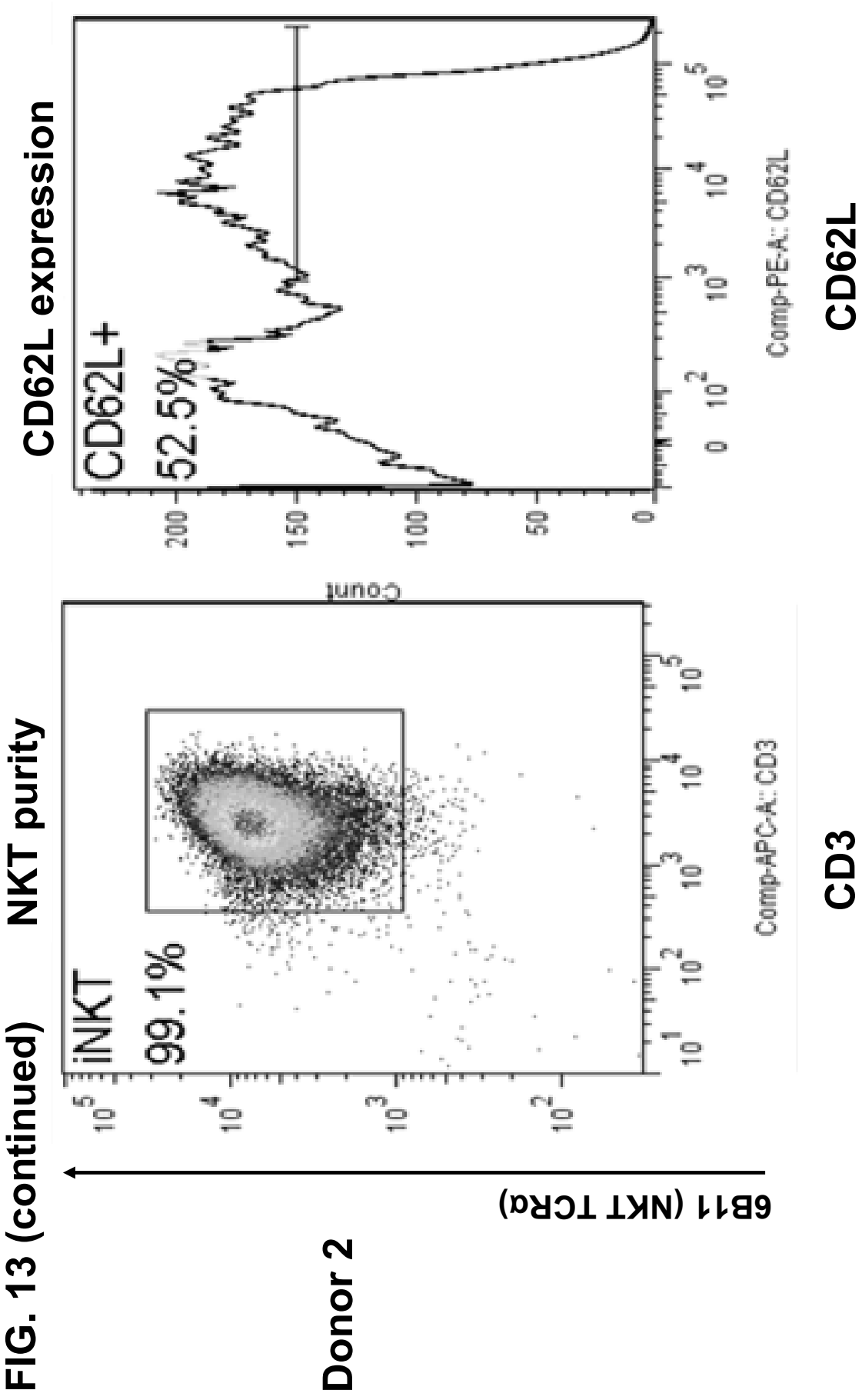


FIG. 13 (continued)
NKT purity
Donor 2
iNKT
99.1%
6B11 (NKT TCRα)
CD3
Comp-APC-A:: CD3
Count
CD62L expression
CD62L+
52.5%
CD62L
Comp-PE-A:: CD62L

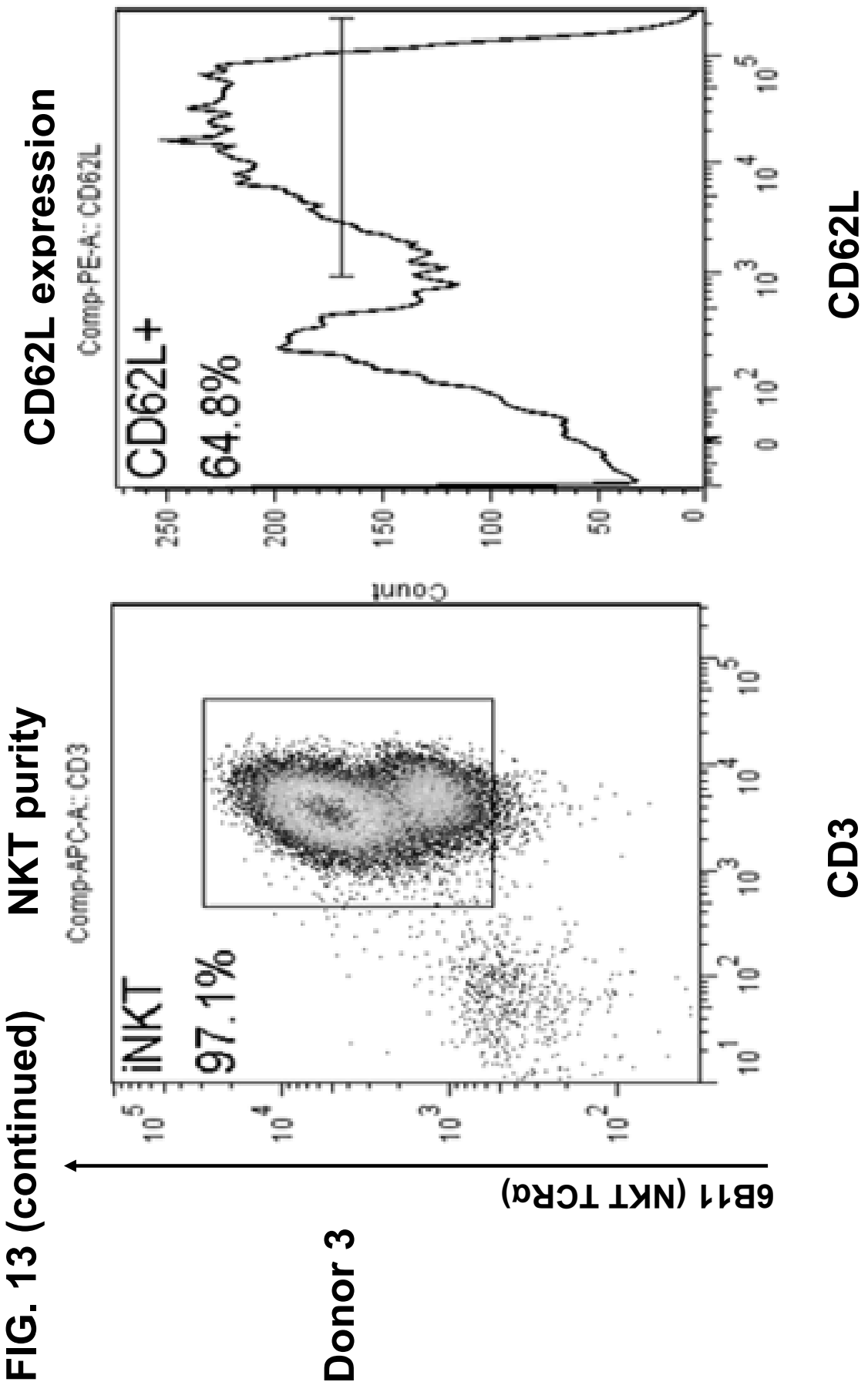


FIG. 13 (continued)
NKT purity
Donor 3
iNKT
97.1%
6B11 (NKT TCRα)
CD3
CD62L expression
CD62L+
64.8%
Count
Comp-PE-A:: CD62L
CD62L

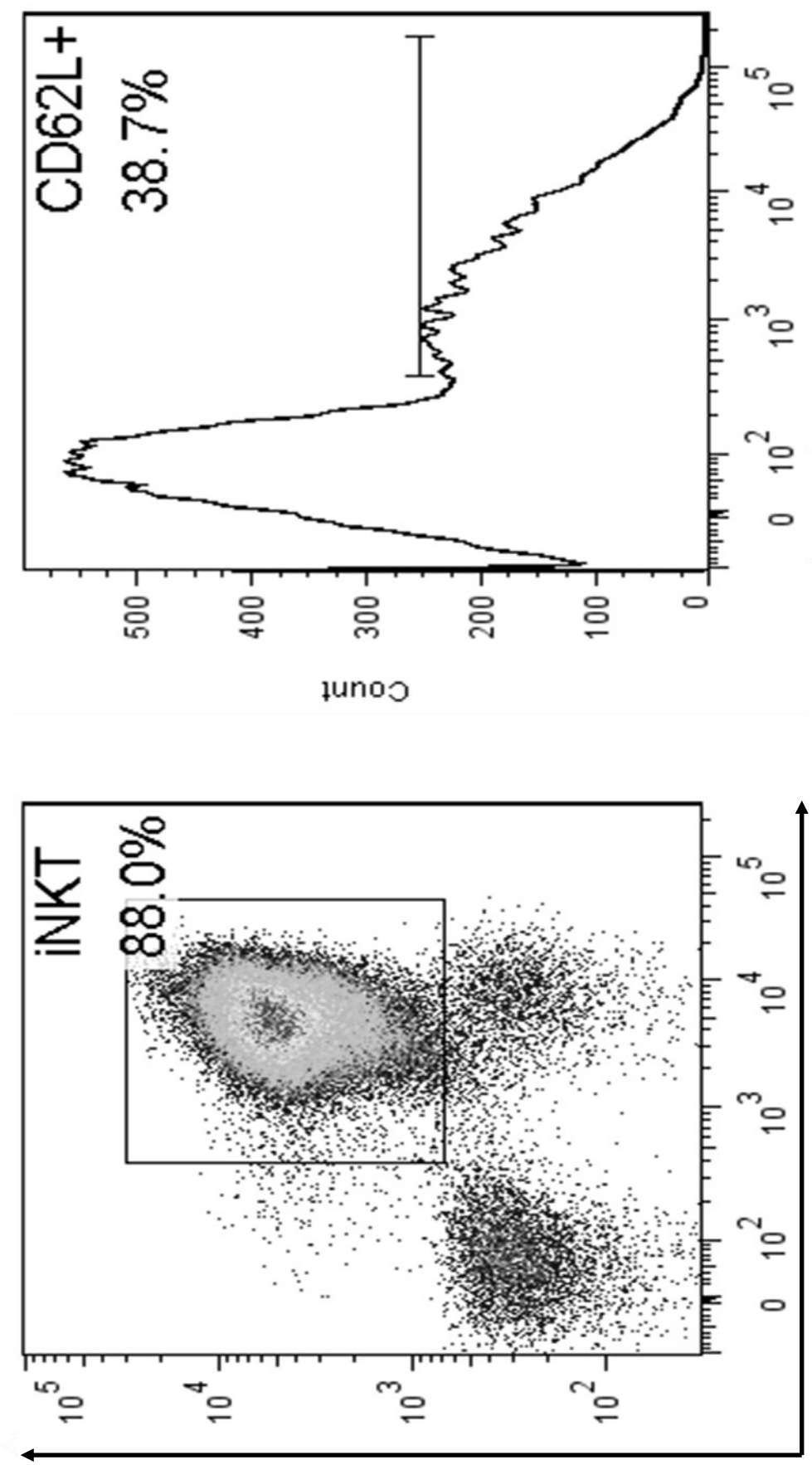


FIG. 14