Figure 1

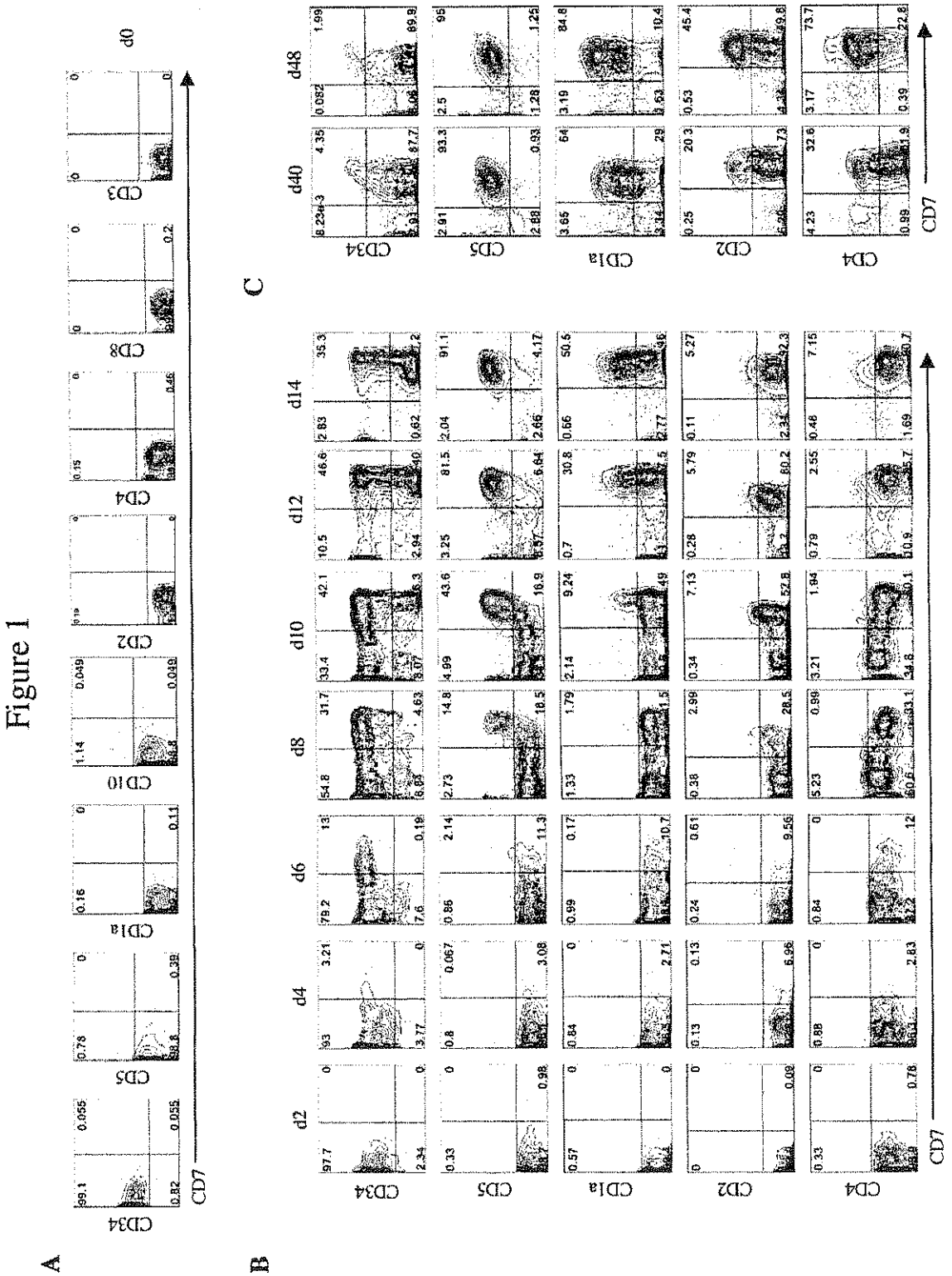

A
B
C
d0
d2
d4
d6
d8
d10
d12
d14
d40
d48
CD34
CD5
CD1a
CD10
CD2
CD4
CD8
CD3
CD7

Figure 2

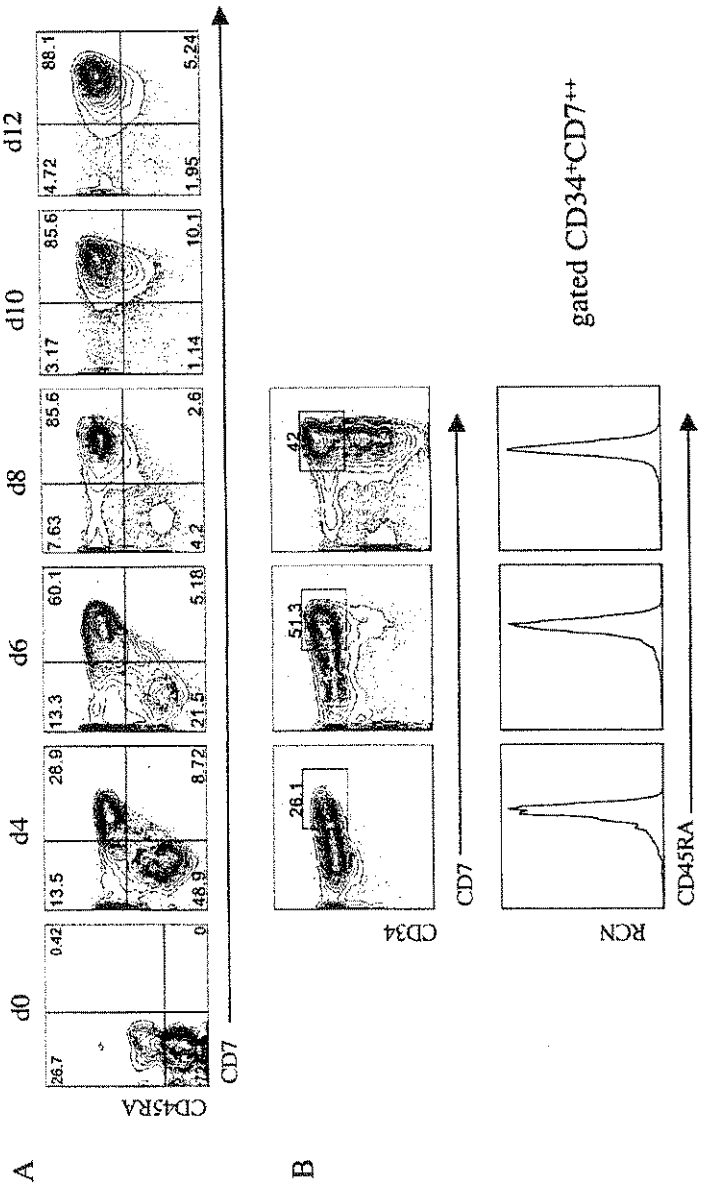
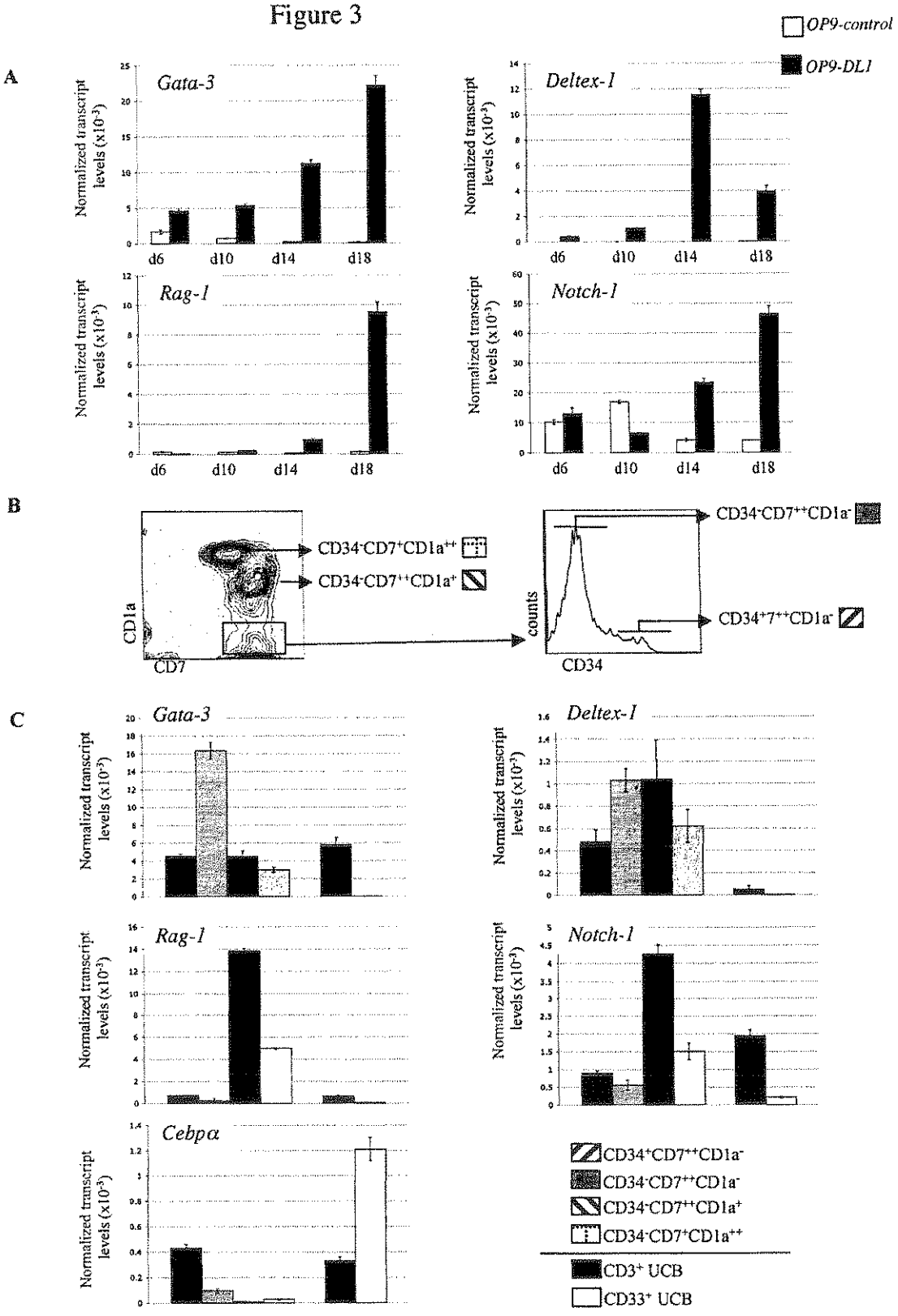

A
B
d0
d4
d6
d8
d10
d12
CD45RA
CD7
26.7
0.42
13.5
28.9
48.9
8.72
13.3
60.1
21.5
5.18
7.63
85.6
4.2
2.6
3.17
85.6
1.14
10.1
4.72
88.1
1.95
5.24
CD34
CD7
26.1
51.3
42
RCN
CD45RA
gated CD34+CD7++

Figure 3


A
OP9-control
OP9-DL1
Gata-3
Deltex-1
Rag-1
Notch-1
Normalized transcript levels (x10⁻³)
d6
d10
d14
d18
B
CD34⁻CD7⁺CD1a⁺⁺
CD34⁻CD7⁺⁺CD1a⁺
CD34⁻CD7⁺⁺CD1a⁻
CD34⁺7⁺⁺CD1a⁻
CD1a
CD7
counts
CD34
C
Gata-3
Deltex-1
Rag-1
Notch-1
Cebpα
CD34⁺CD7⁺⁺CD1a⁻
CD34⁻CD7⁺⁺CD1a⁻
CD34⁻CD7⁺⁺CD1a⁺
CD34⁻CD7⁺CD1a⁺⁺
CD3⁺ UCB
CD33⁺ UCB

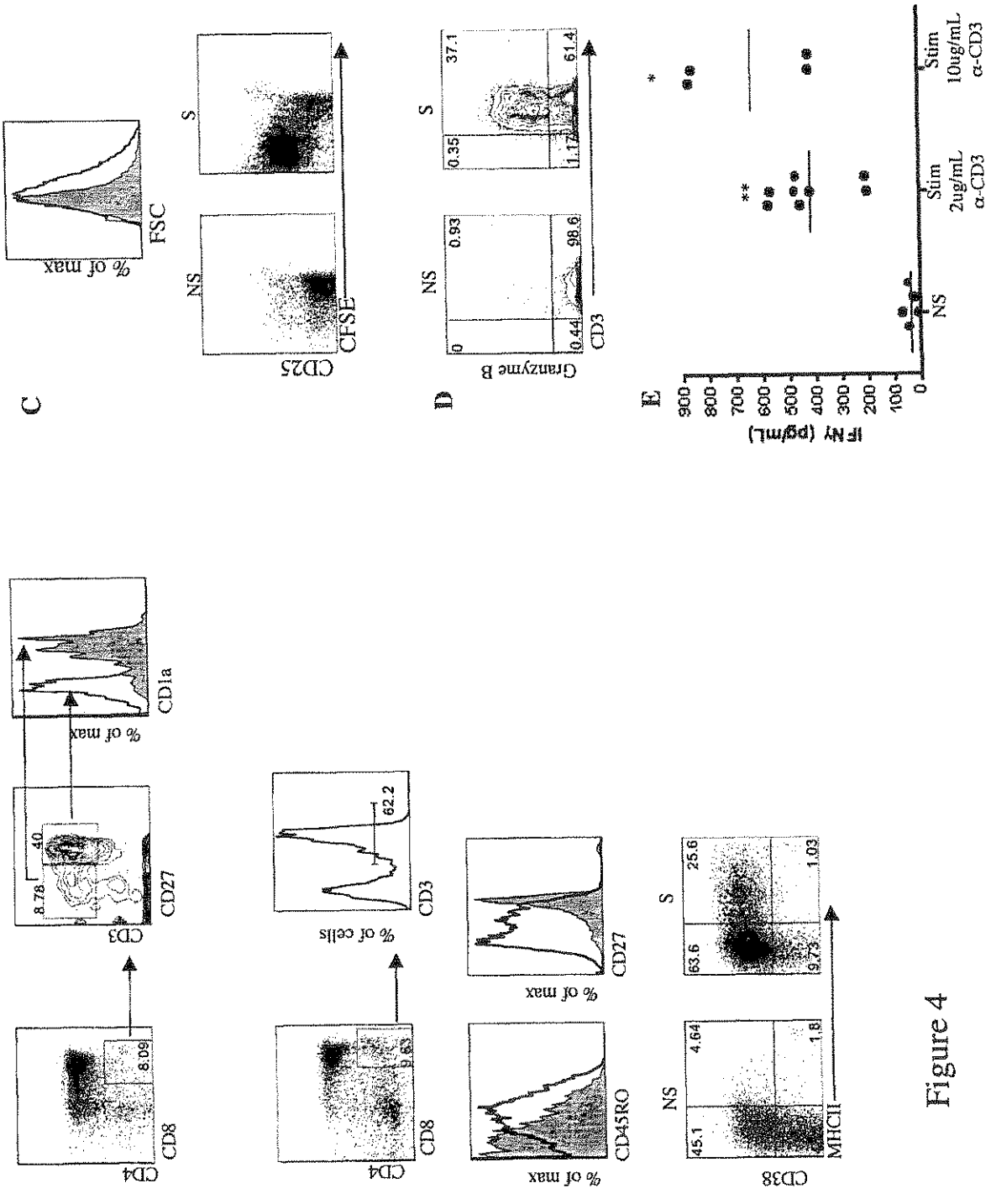

A
CD4
CD8
8.09
CD3
CD27
8.78
40
% of max
CD1a
B
CD4
CD8
9.63
% of cells
62.2
CD3
% of max
CD27
% of max
CD45RO
NS
45.1
4.64
1.8
S
25.6
63.6
9.73
1.03
CD38
MHCII
C
% of max
FSC
NS
S
CD25
CFSE
D
NS
0
0.93
0.44
98.6
S
0.35
37.1
1.17
61.4
Granzyme B
CD3
E
IFNγ (pg/mL)
900
800
700
600
500
400
300
200
100
0
*
**
NS
Stim 2ug/mL α-CD3
Stim 10ug/mL α-CD3

Figure 4

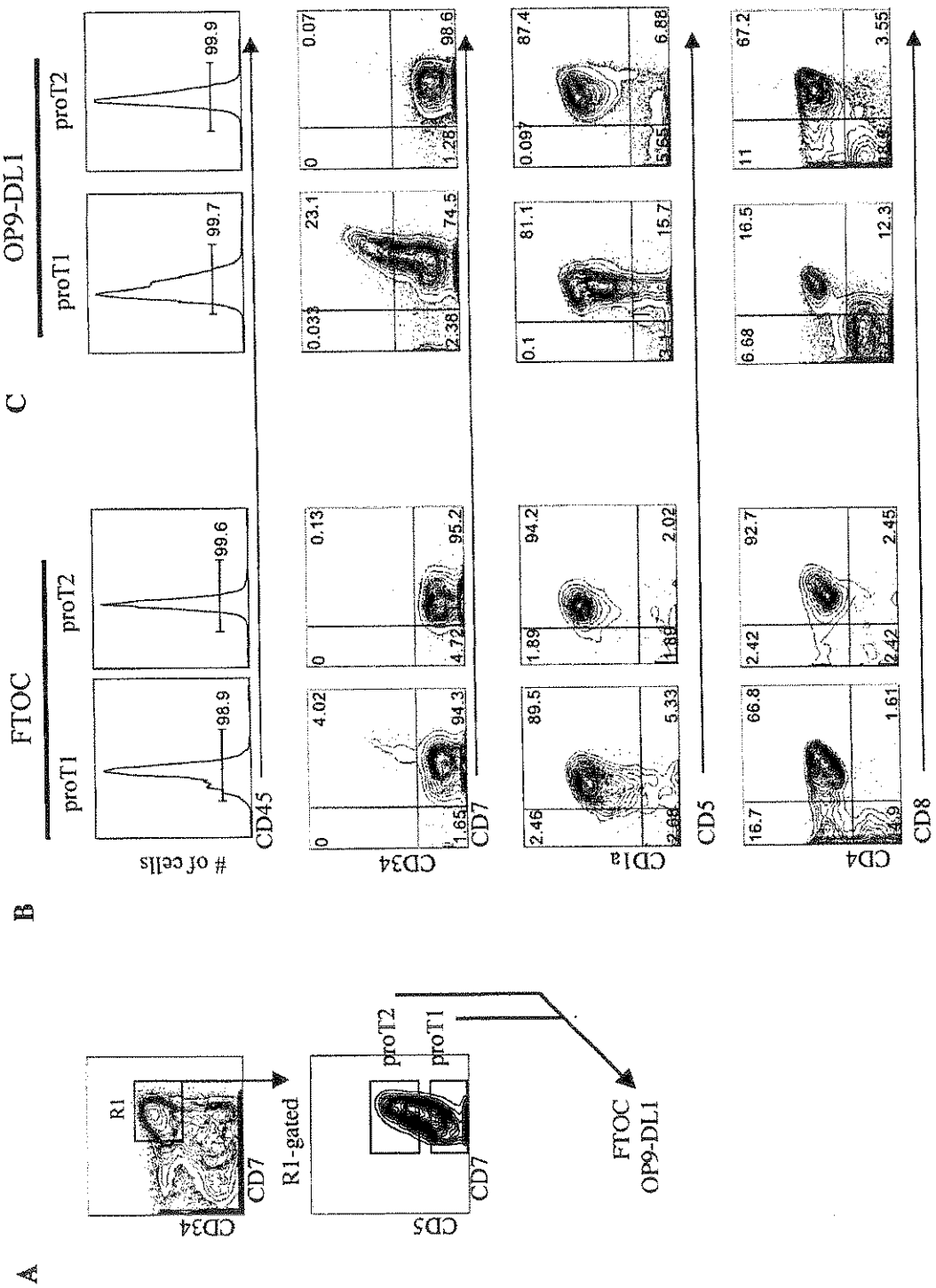

A
CD34
CD7
R1
R1-gated
CD5
CD7
proT2
proT1
FTOC
OP9-DL1
B
FTOC
proT1
proT2
# of cells
CD45
98.9
99.6
CD34
CD7
4.02
94.3
0
0.13
95.2
4.72
CD1a
CD5
89.5
5.33
94.2
2.02
CD4
CD8
66.8
1.61
92.7
2.45
C
OP9-DL1
proT1
proT2
99.7
99.9
CD34
CD7
0.033
23.1
74.5
0.07
98.6
CD1a
CD5
0.1
81.1
15.7
0.097
87.4
6.88
CD4
CD8
6.68
16.5
12.3
11
67.2
3.55

Figure 5

Figure 6

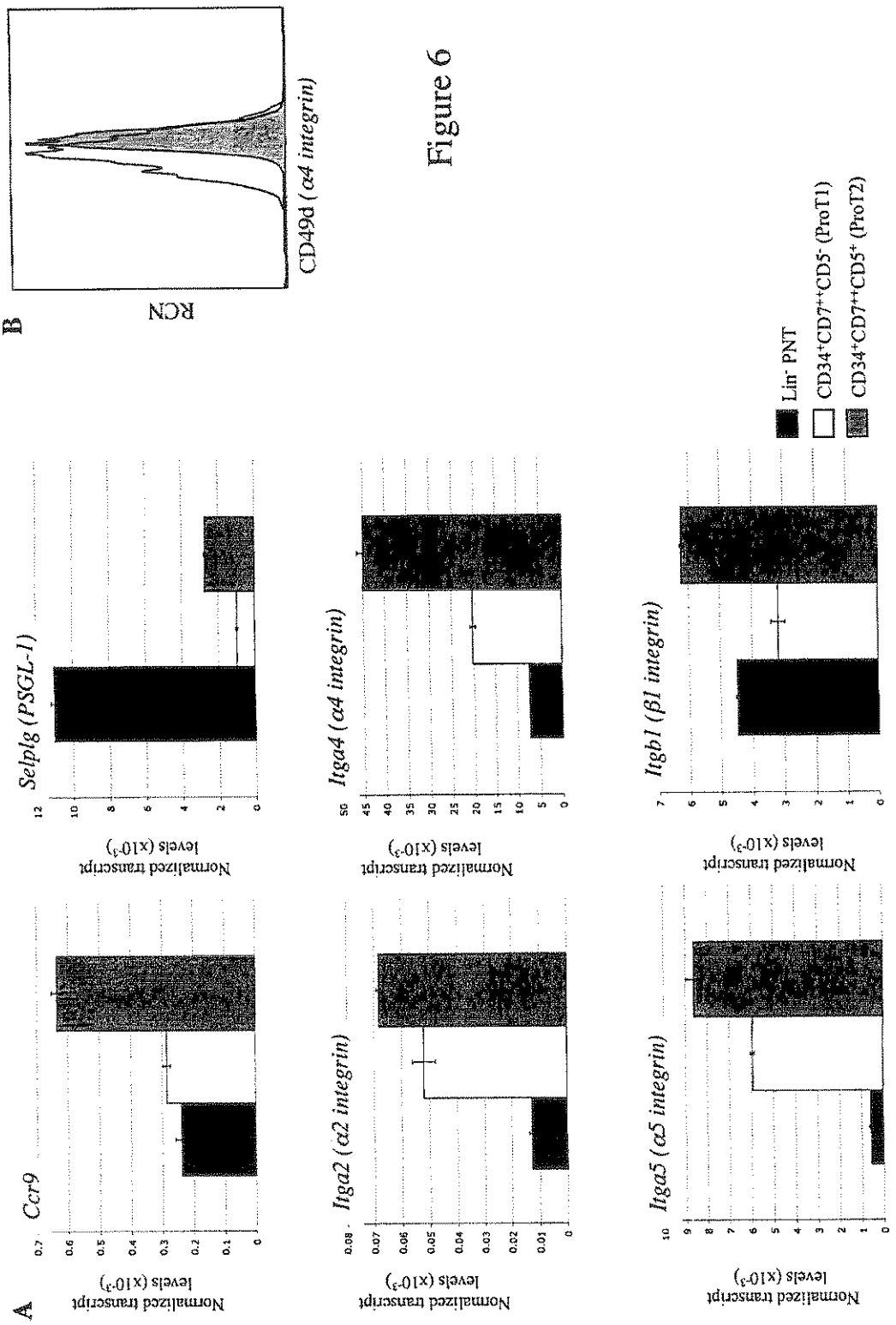

A
B
RCN
CD49d (α4 integrin)
Lin⁻ PNT
CD34⁺CD7⁺⁺CD5⁻ (ProT1)
CD34⁺CD7⁺⁺CD5⁺ (ProT2)
Selplg (PSGL-1)
Itga4 (α4 integrin)
Itgb1 (β1 integrin)
Ccr9
Itga2 (α2 integrin)
Itga5 (α5 integrin)
Normalized transcript levels (x10⁻³)

Figure 7

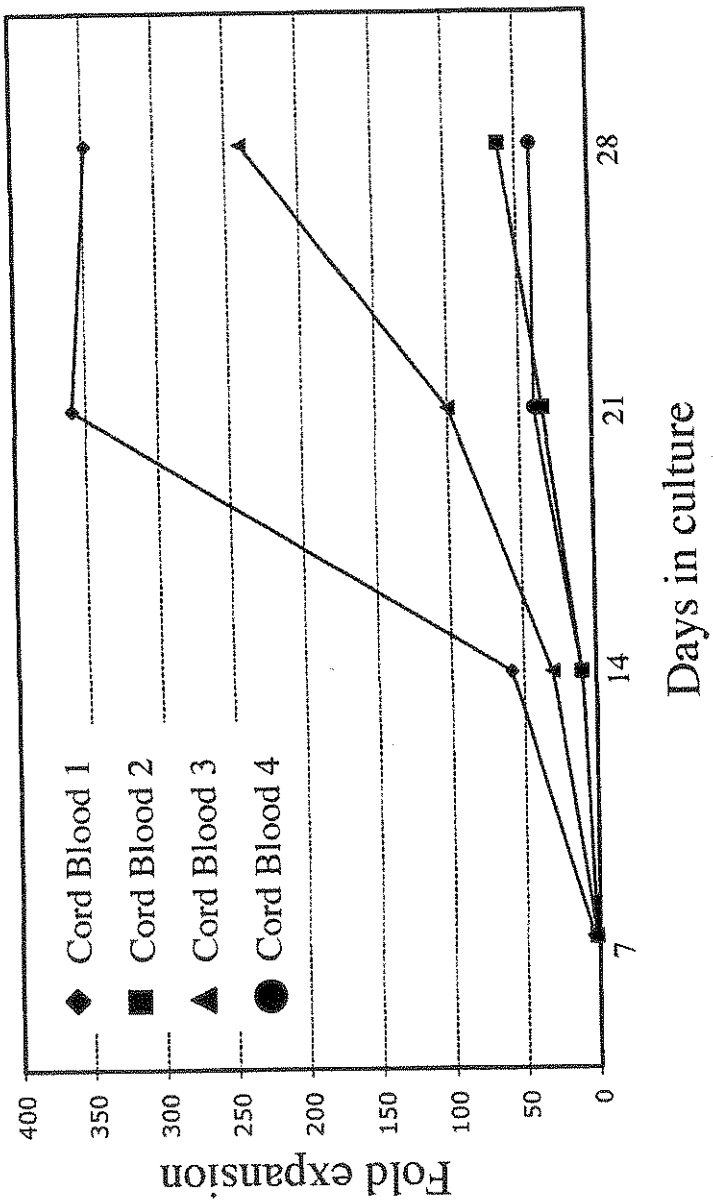

Fold expansion
400
350
300
250
200
150
100
50
0
Cord Blood 1
Cord Blood 2
Cord Blood 3
Cord Blood 4
7
14
21
28
Days in culture

Figure 8

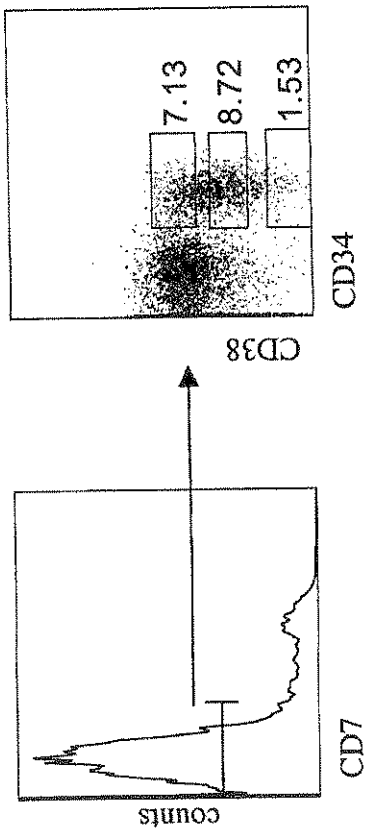


Figure 9

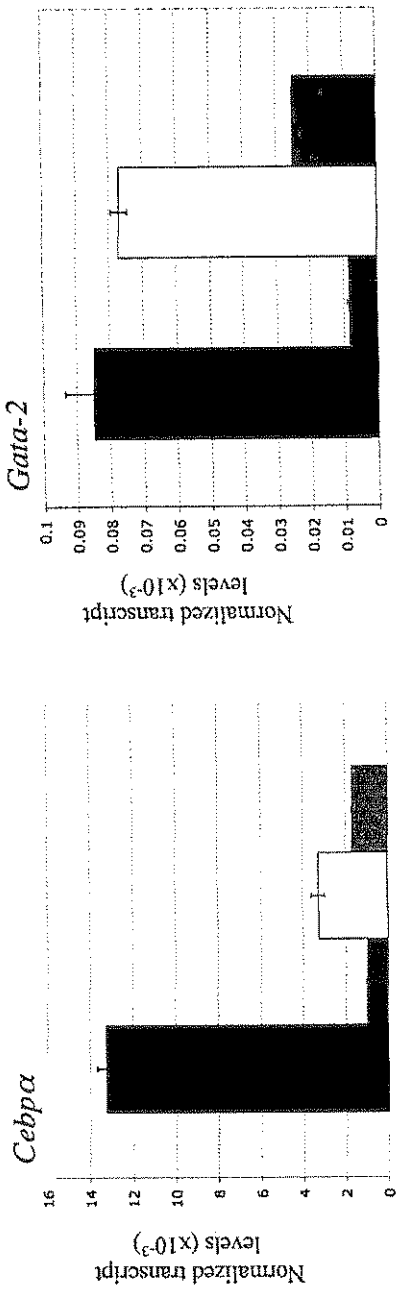
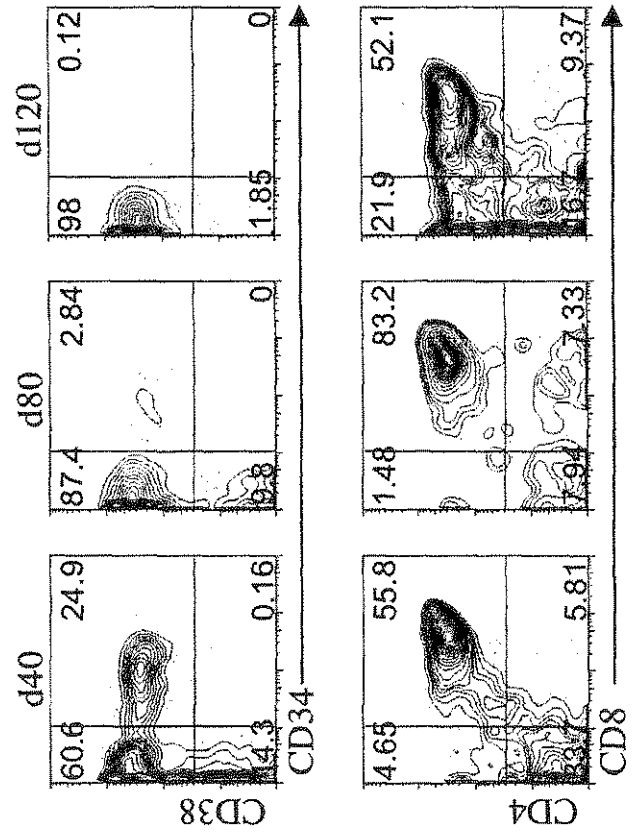

CD33+ UCB
PNT
CD34+CD7++CD5- (ProT1)
CD34+CD7++CD5+ (ProT2)
Gata-2
Normalized transcript levels (x10-3)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Cebpa
Normalized transcript levels (x10-3)
16
14
12
10
8
6
4
2
0

Figure 10


d40
d80
d120
60.6
24.9
4.3
0.16
87.4
2.84
9.8
0
98
0.12
1.85
0
CD38
CD34
4.65
55.8
33.7
5.81
1.48
83.2
7.94
7.33
21.9
52.1
9.37
CD4
CD8

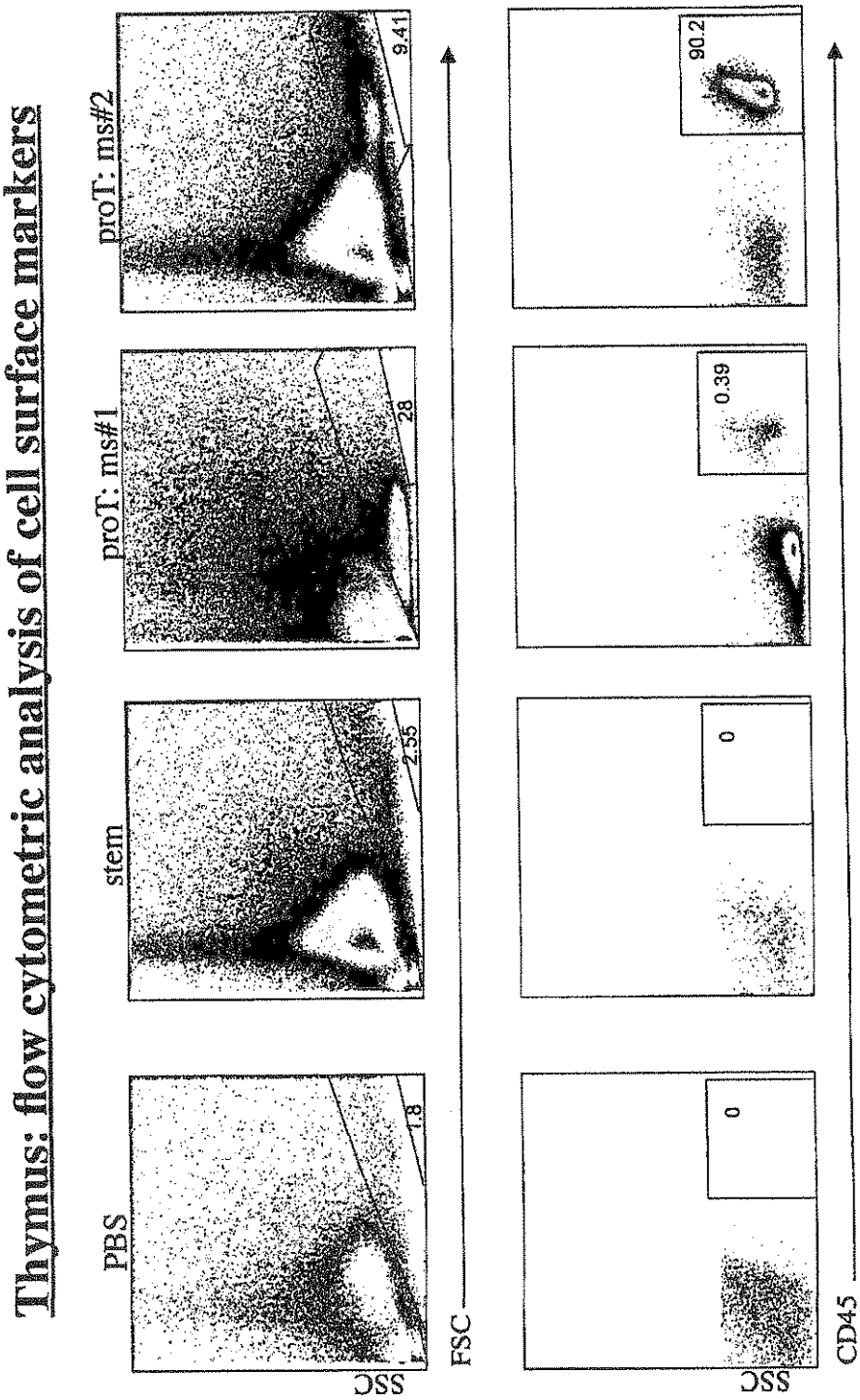

Thymus: flow cytometric analysis of cell surface markers
PBS
stem
proT: ms#1
proT: ms#2
1.8
2.55
28
9.41
0
0
0.39
90.2
SSC
FSC
SSC
CD45

Figure 11

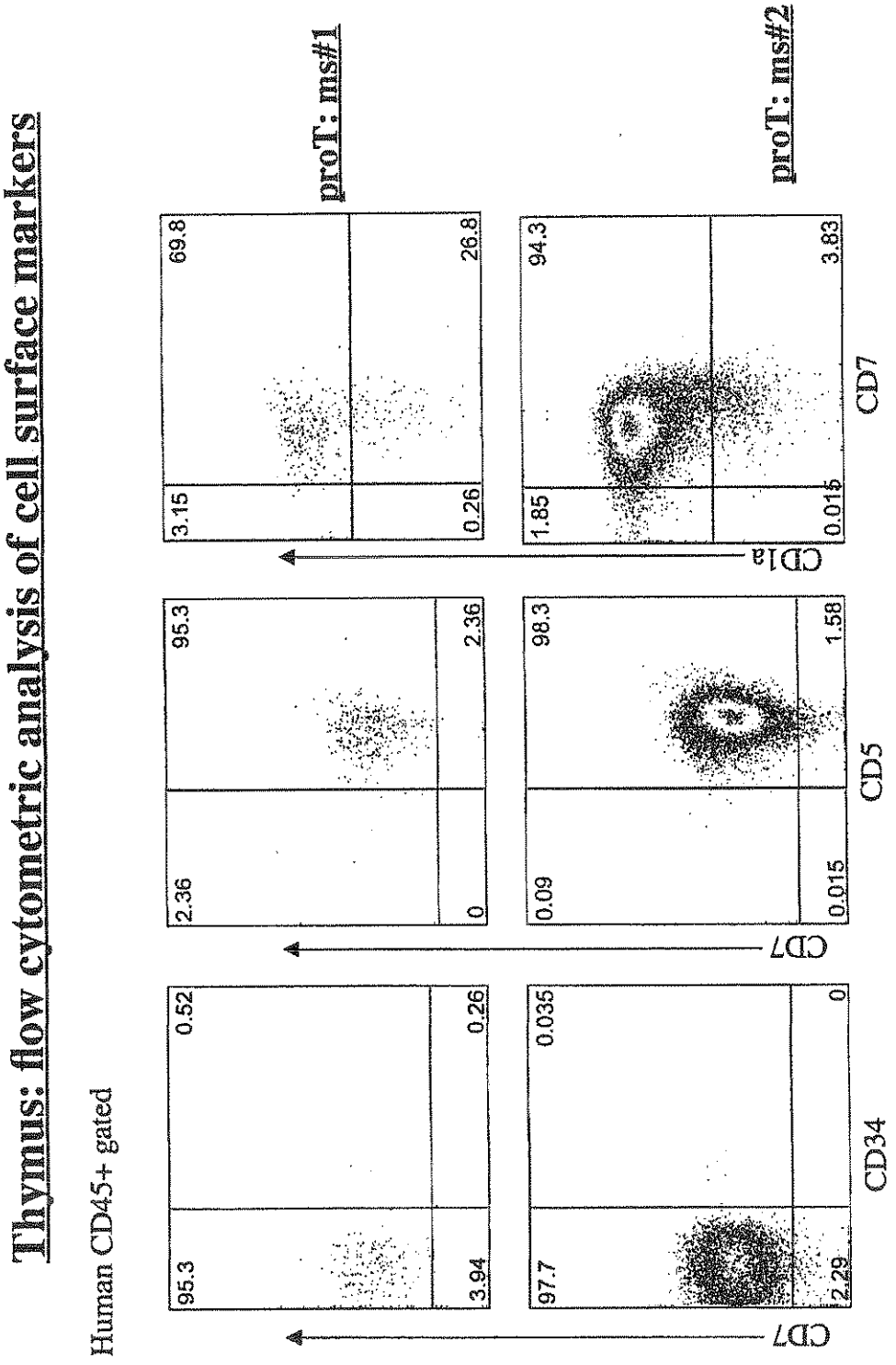

Thymus: flow cytometric analysis of cell surface markers
Human CD45+ gated
proT: ms#1
proT: ms#2
CD34
CD5
CD7
CD1a
95.3
0.52
3.94
0.26
97.7
0.035
2.29
0
2.36
95.3
0
2.36
0.09
98.3
0.015
1.58
3.15
69.8
0.26
26.8
1.85
94.3
0.016
3.83

Figure 12

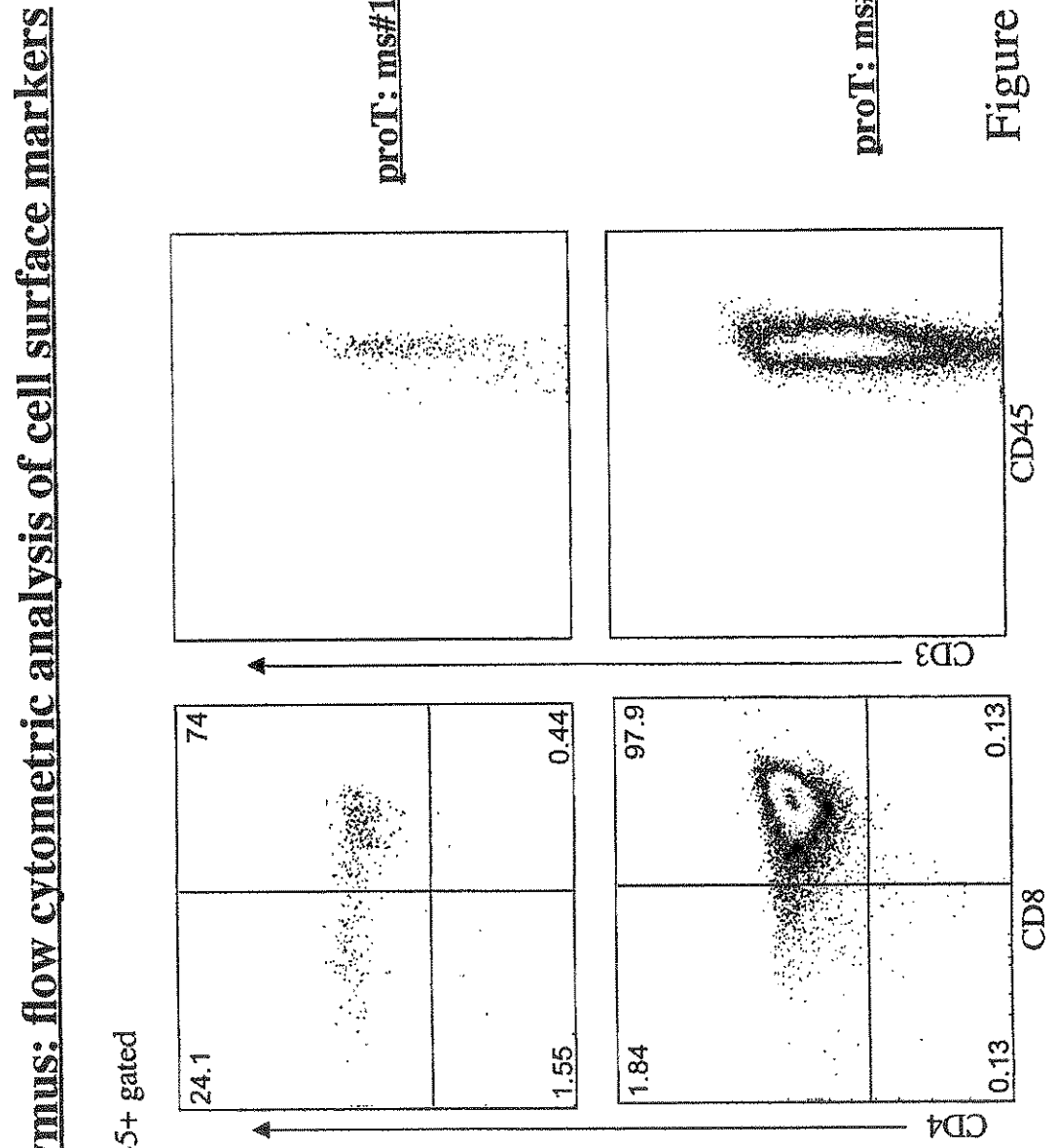

Thymus: flow cytometric analysis of cell surface markers
Human CD45+ gated
proT: ms#1
proT: ms#2
CD3
CD45
CD4
CD8
24.1
74
1.55
0.44
1.84
97.9
0.13
0.13

Figure 13

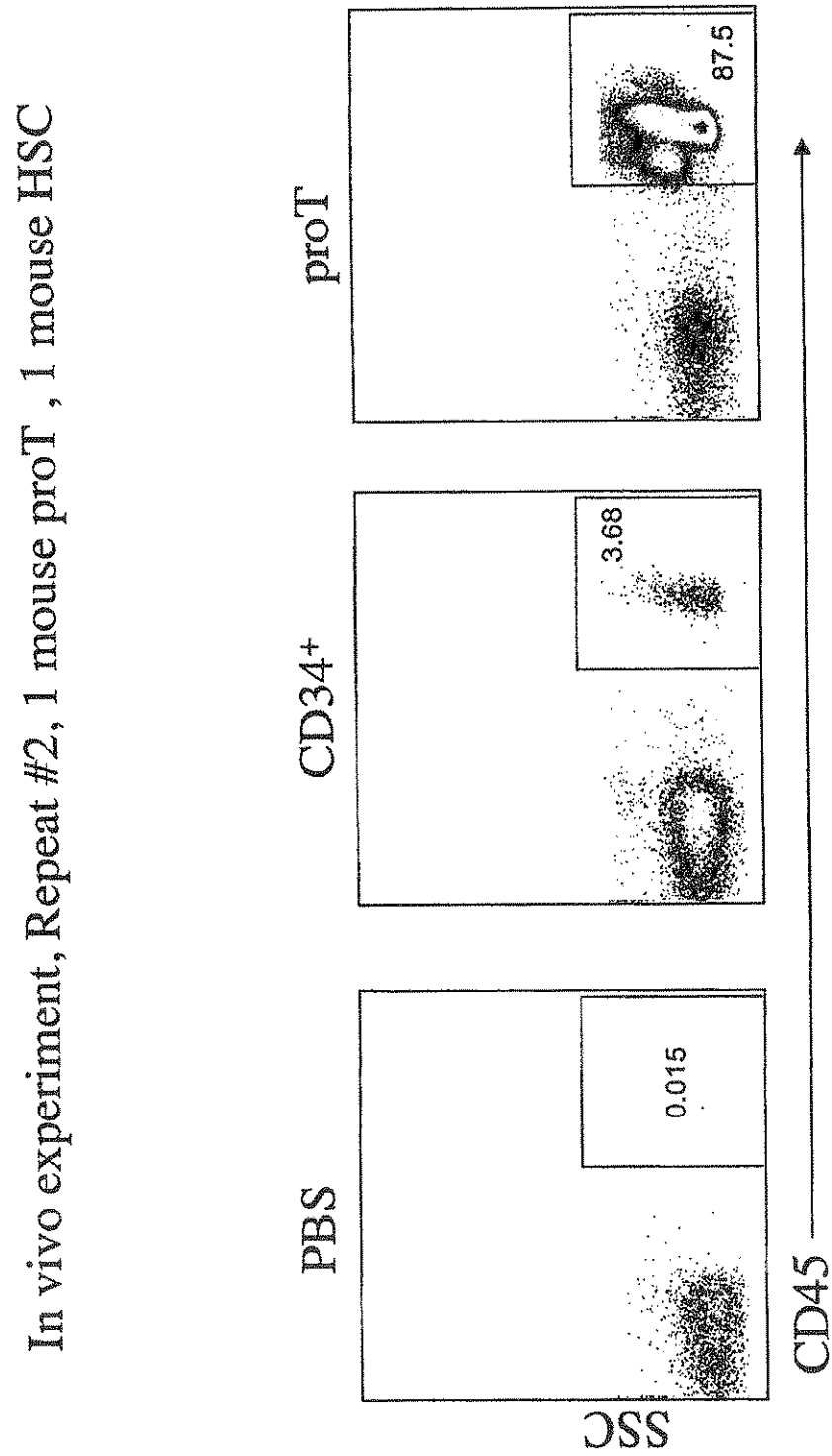
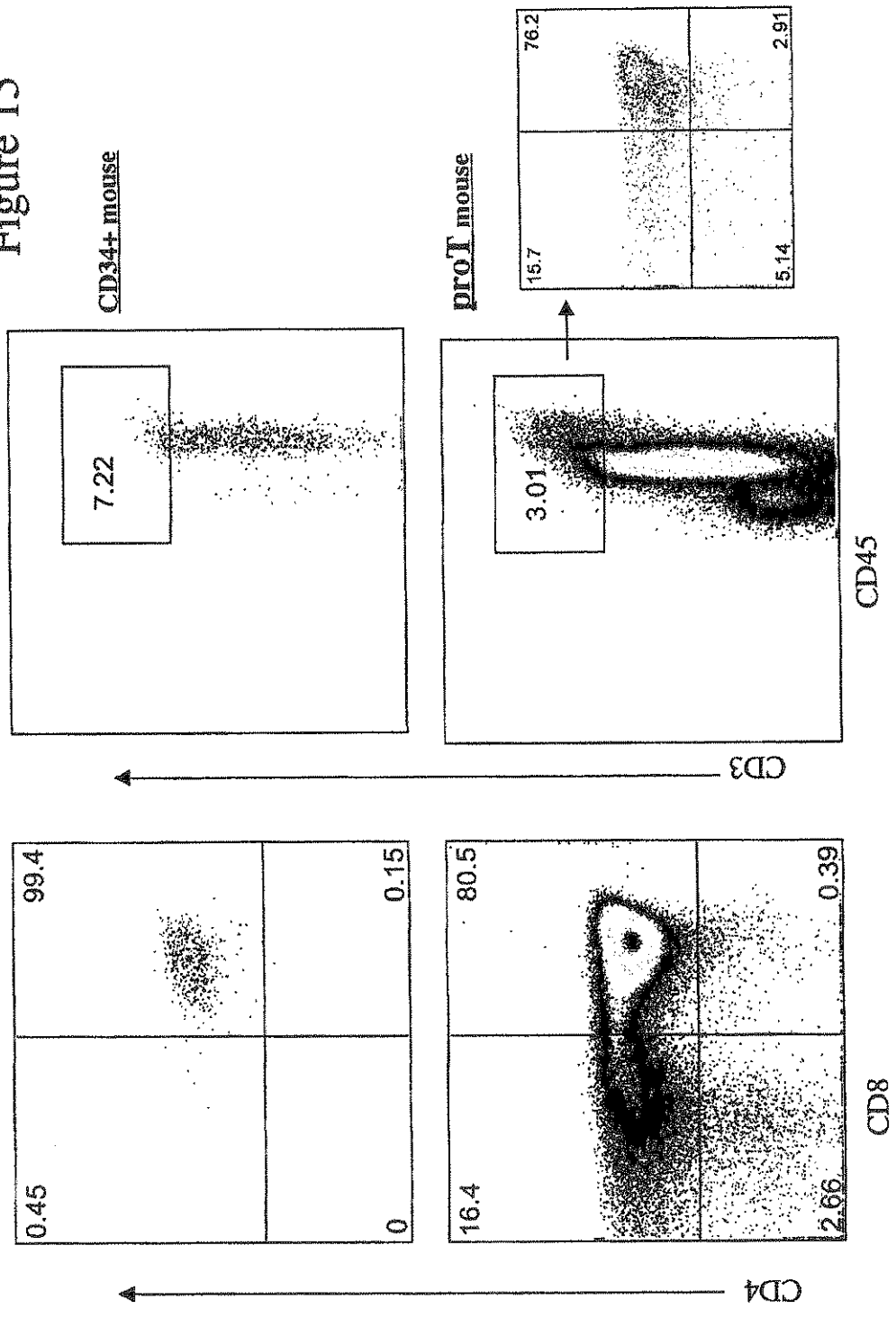

In vivo experiment, Repeat #2, 1 mouse proT , 1 mouse HSC
PBS
CD34+
proT
0.015
3.68
87.5
SSC
CD45

Figure 14

Figure 15


CD34+ mouse
proT mouse
7.22
3.01
76.2
2.91
15.7
5.14
CD3
CD45
99.4
0.15
0.45
0
80.5
0.39
16.4
2.66
CD4
CD8

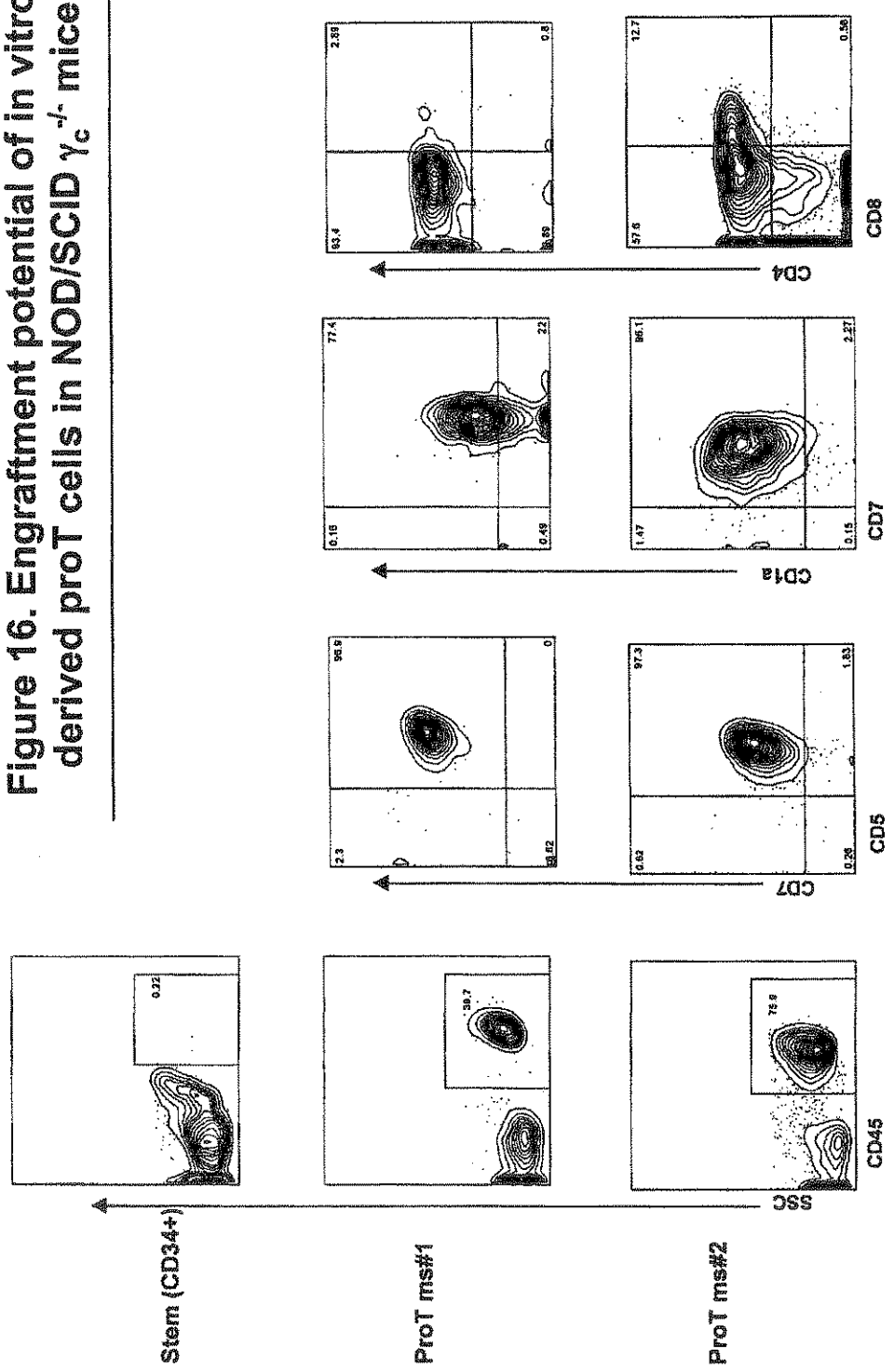


Figure 16. Engraftment potential of in vitro-derived proT cells in NOD/SCID $\gamma_c^{-/-}$ mice

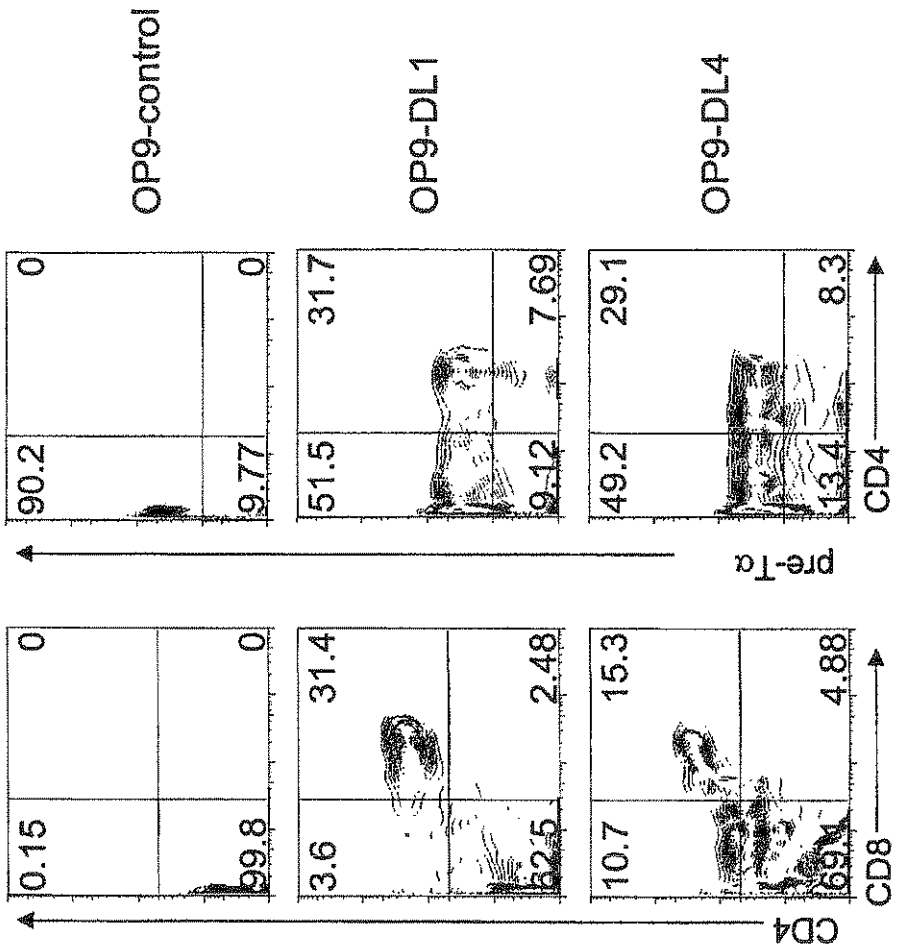

OP9-control
OP9-DL1
OP9-DL4
pre-Tα
CD4
CD8
0
90.2
9.77
0
0
51.5
9.12
31.7
7.69
0
49.2
13.4
29.1
8.3
0
0.15
99.8
0
0
3.6
62.5
31.4
2.48
0
10.7
69.1
15.3
4.88

Fig 17

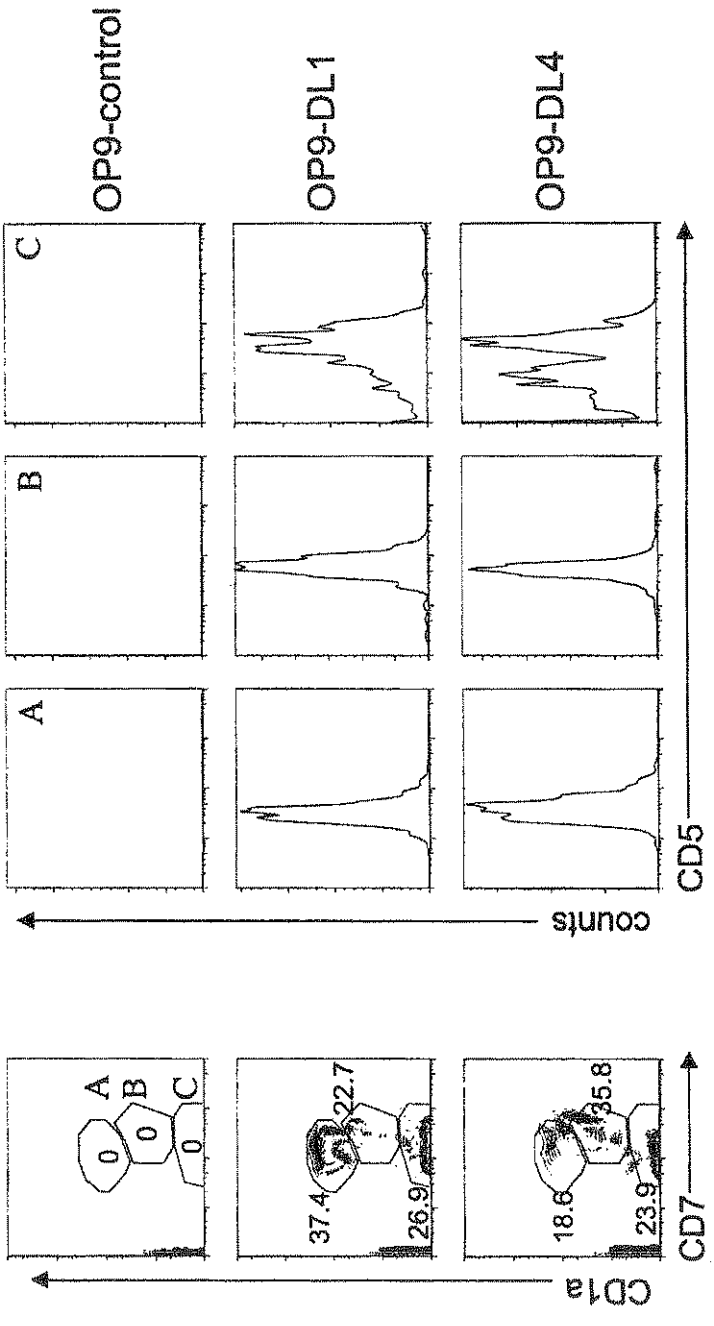

OP9-control
OP9-DL1
OP9-DL4
A
B
C
counts
CD5
CD1a
CD7
37.4
22.7
26.9
18.6
35.8
23.9

Fig 18

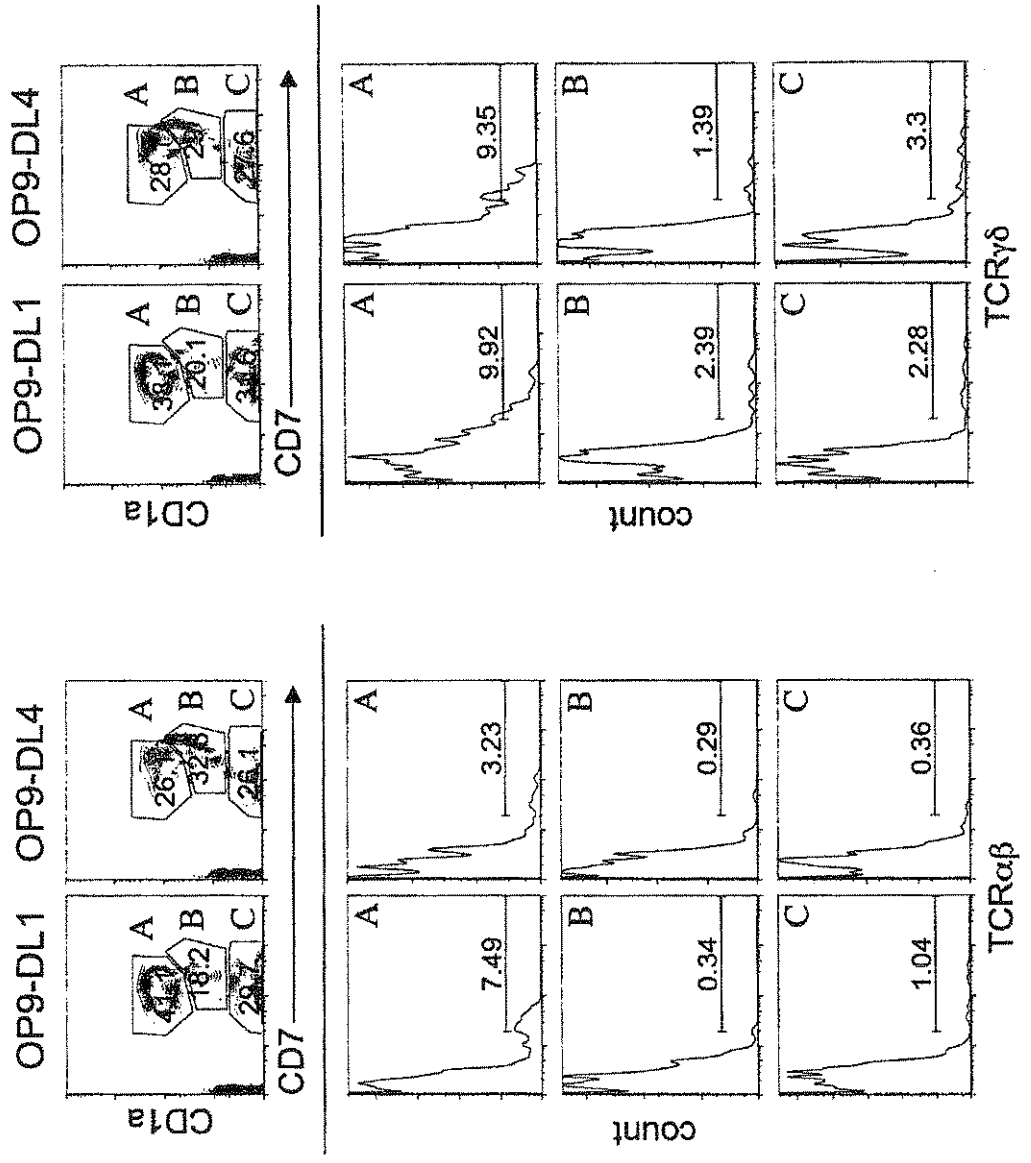

OP9-DL1
OP9-DL4
CD1a
CD7
A
B
C
18.2
29.7
26.1
32.8
26.1
count
7.49
0.34
1.04
3.23
0.29
0.36
TCRαβ
OP9-DL1
OP9-DL4
CD1a
CD7
A
B
C
36.4
20.1
34.6
28
27.6
count
9.92
2.39
2.28
9.35
1.39
3.3
TCRγδ

Fig 19

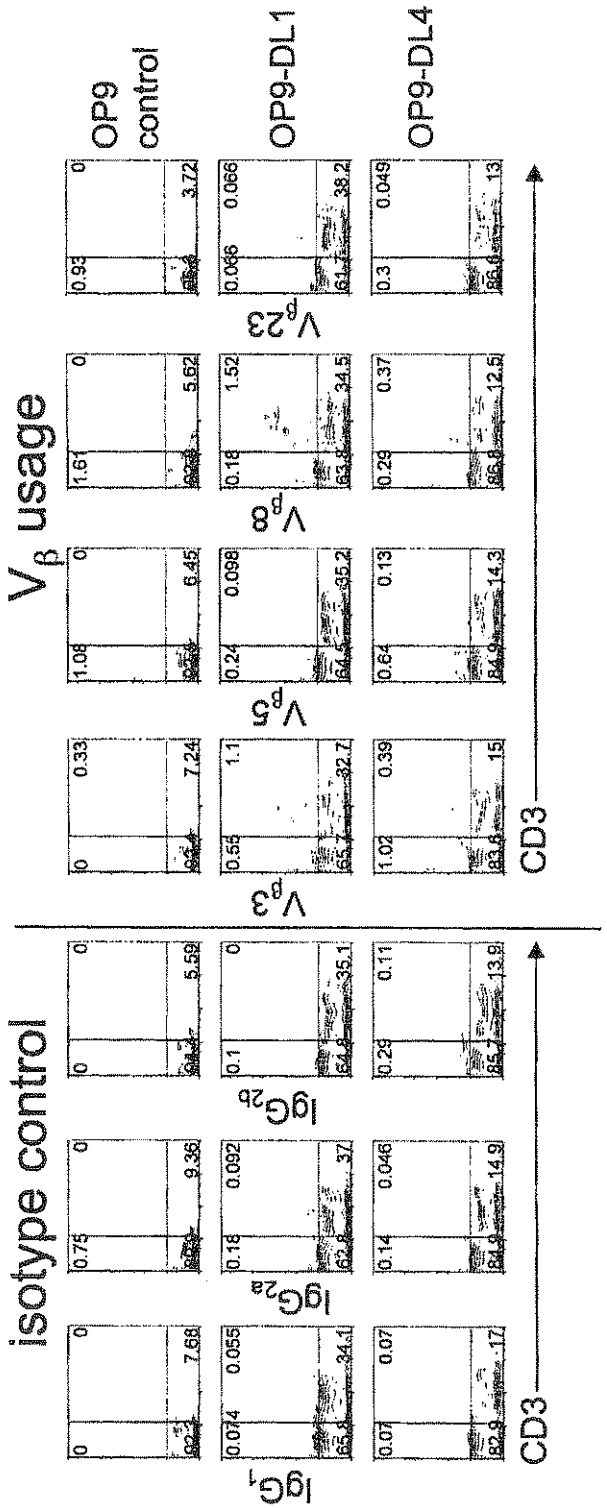

isotype control
OP9 control
OP9-DL1
OP9-DL4
V_β usage
$V_\beta 3$
$V_\beta 5$
$V_\beta 8$
$V_\beta 23$
IgG_1
IgG_{2a}
IgG_{2b}
CD3
CD3

Fig 20

Figure 21

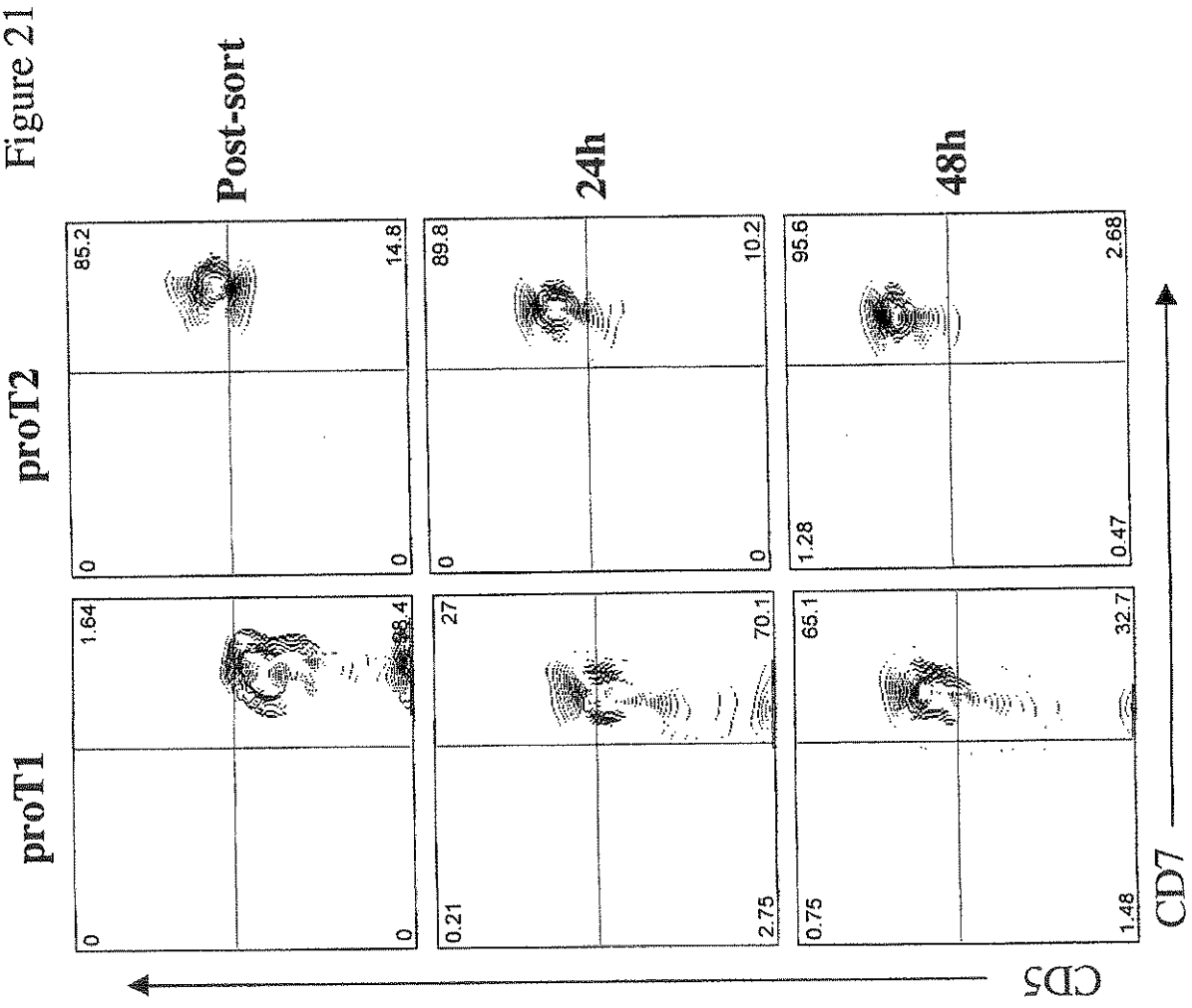
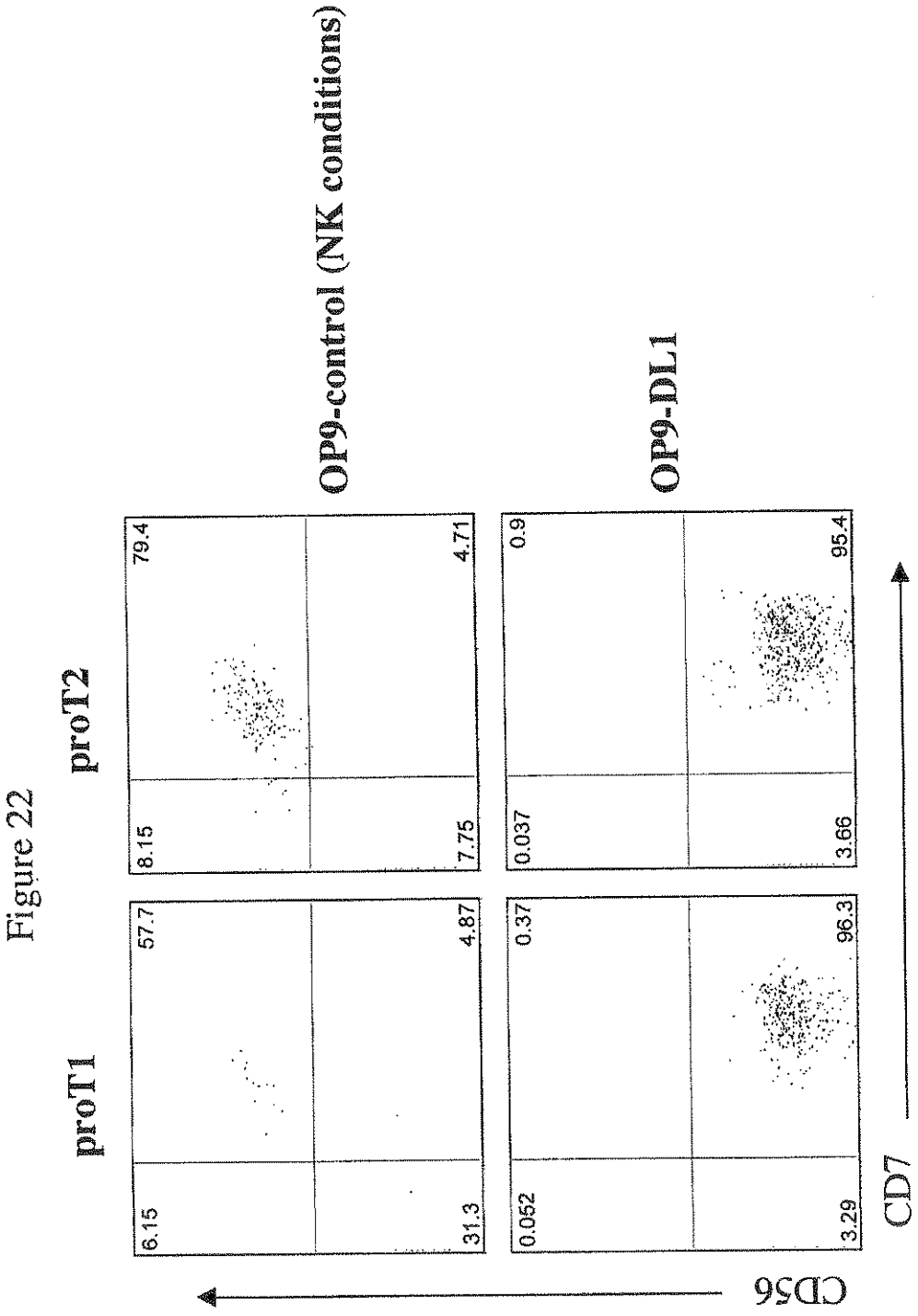

proT1
proT2
Post-sort
24h
48h
CD5
CD7
1.64
88.4
0
0
85.2
14.8
0
0
27
70.1
0.21
2.75
89.8
10.2
0
0
65.1
32.7
0.75
1.48
95.6
2.68
1.28
0.47

Figure 22


proT1
proT2
OP9-control (NK conditions)
OP9-DL1
6.15
57.7
4.87
31.3
8.15
79.4
4.71
7.75
0.052
0.37
96.3
3.29
0.037
0.9
95.4
3.66
CD56
CD7

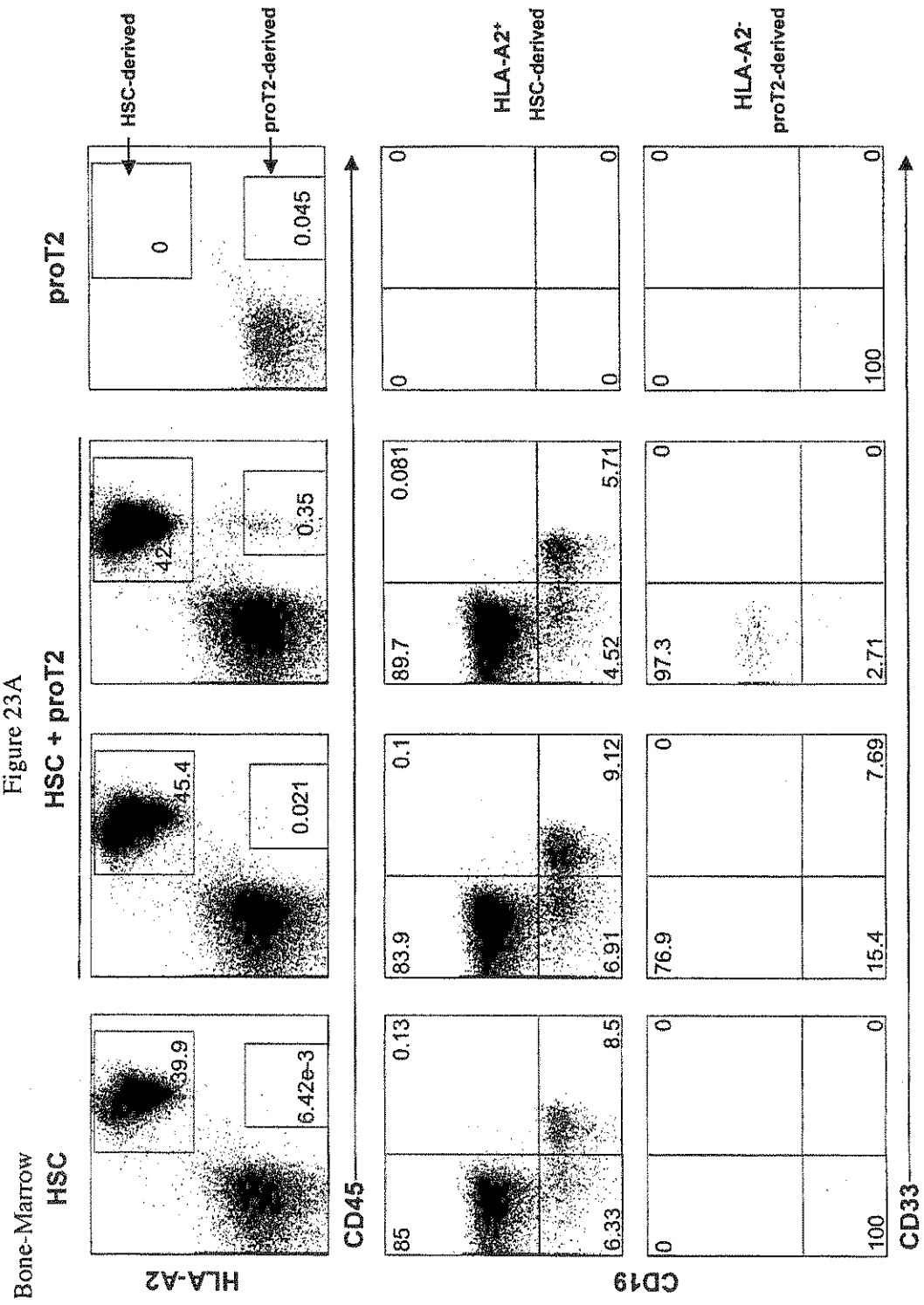

Bone-Marrow
HSC
HSC + proT2
proT2
HSC-derived
proT2-derived
HLA-A2+
HSC-derived
HLA-A2-
proT2-derived
HLA-A2
CD45
CD19
CD33
39.9
6.42e-3
45.4
0.021
42
0.35
0
0.045
0.13
8.5
85
6.33
0.1
9.12
83.9
6.91
0
0
76.9
15.4
7.69
100
0
0.081
5.71
89.7
4.52
0
0
97.3
2.71
0
0
0
0
0
0
0
100

Figure 23A

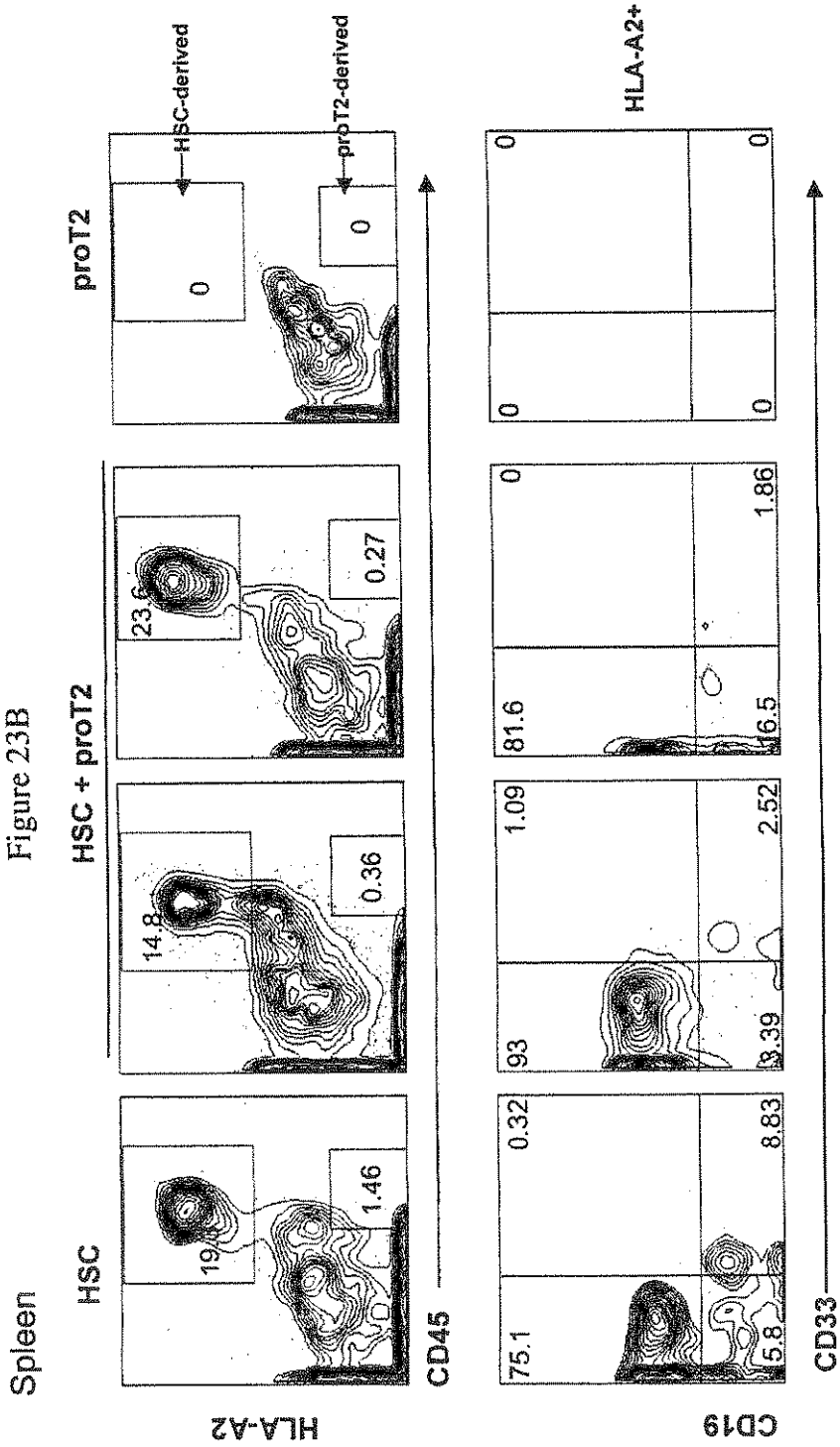
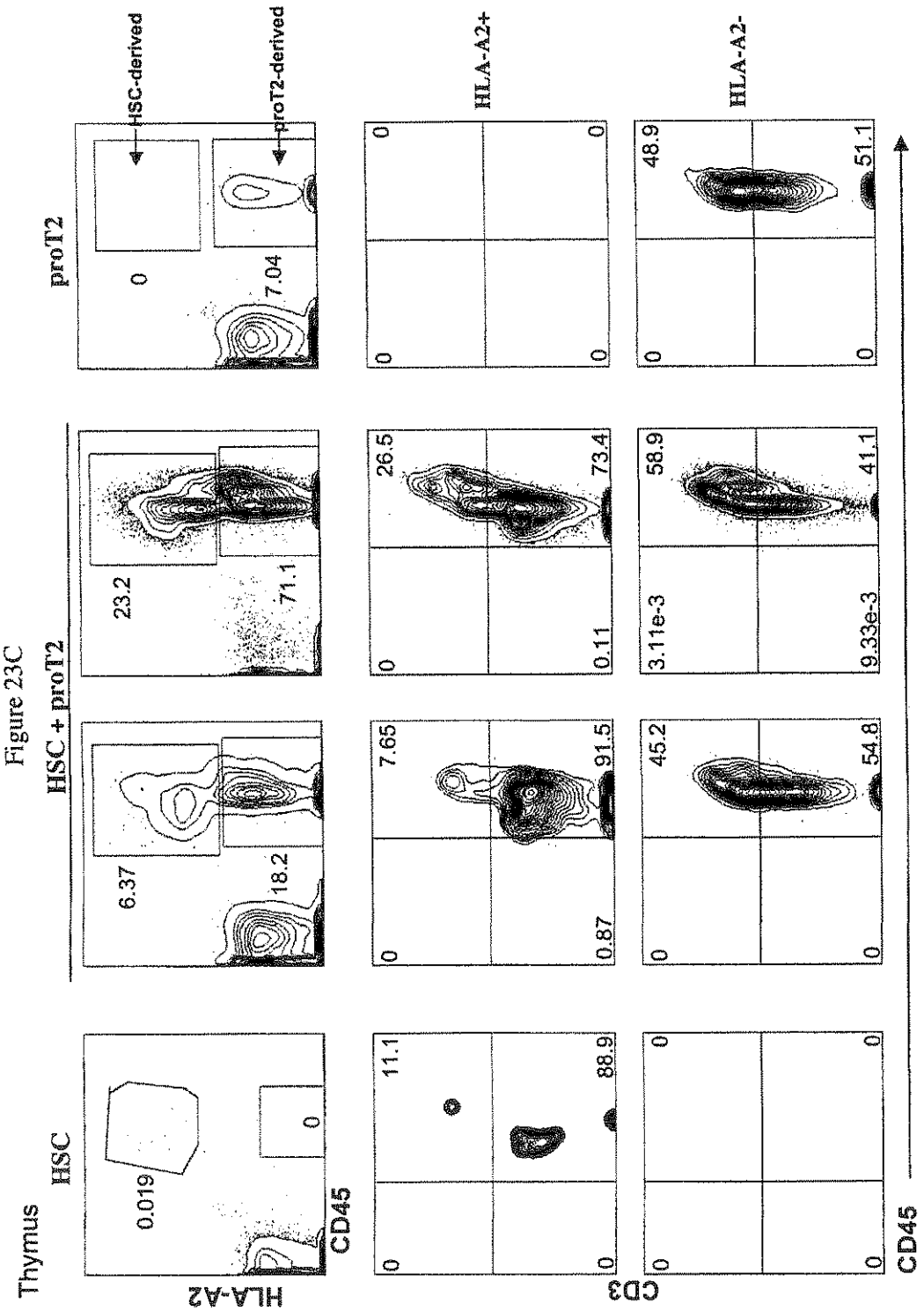

Spleen
HSC
HSC + proT2
proT2
HSC-derived
proT2-derived
HLA-A2
CD45
CD19
CD33
HLA-A2+
19
1.46
14.8
0.36
23.6
0.27
0
0
0.32
8.83
75.1
5.8
1.09
2.52
93
3.39
0
1.86
81.6
16.5
0
0
0
0

Figure 23B


Figure 23C
Thymus
HSC
HSC + proT2
proT2
HSC-derived
proT2-derived
HLA-A2+
HLA-A2-
HLA-A2
CD45
CD3
0.019
6.37
18.2
23.2
71.1
7.04
7.65
91.5
0.87
26.5
73.4
0.11
45.2
54.8
11.1
88.9
58.9
41.1
3.11e-3
9.33e-3
48.9
51.1

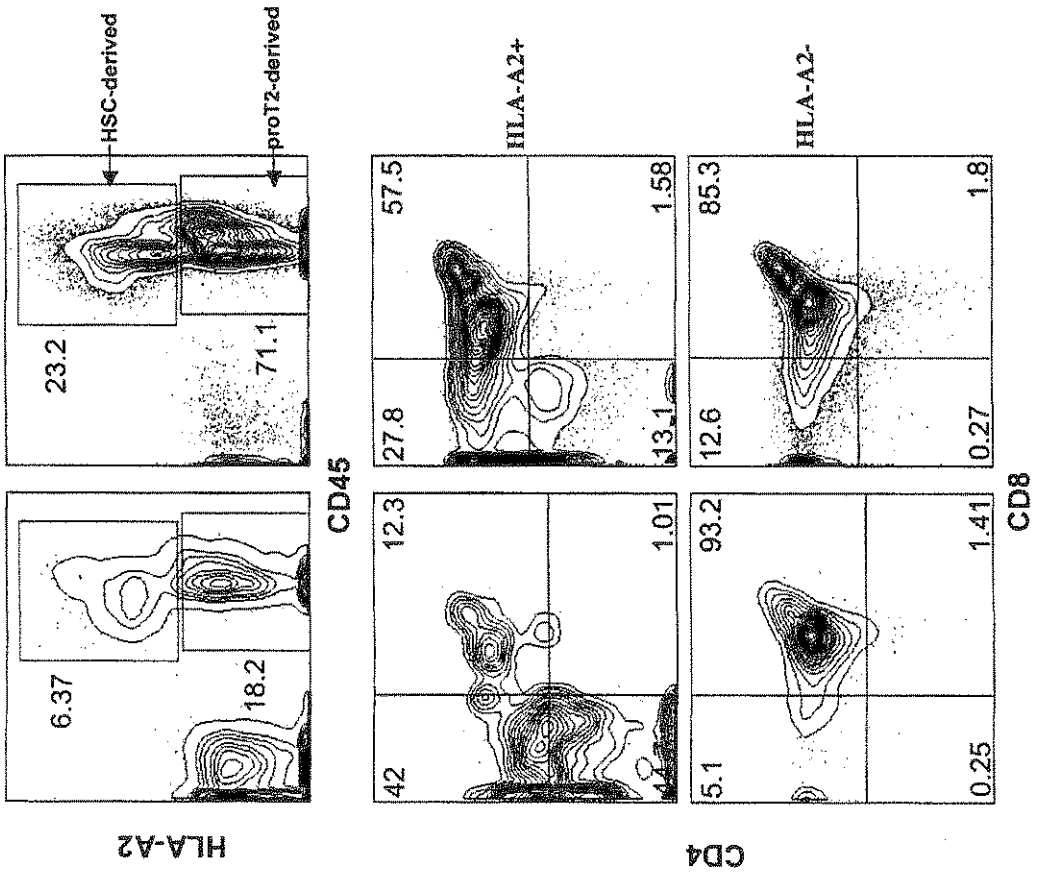
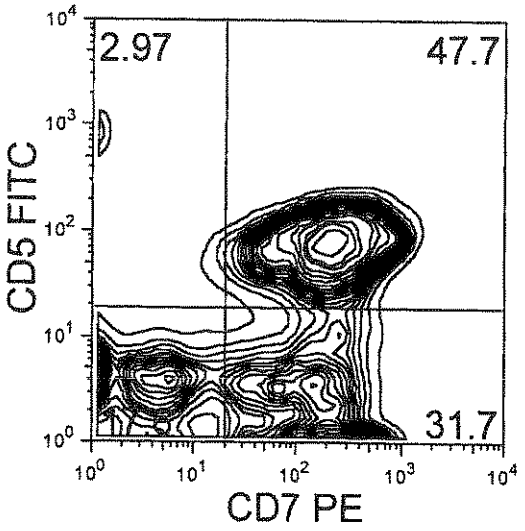


Figure 23D

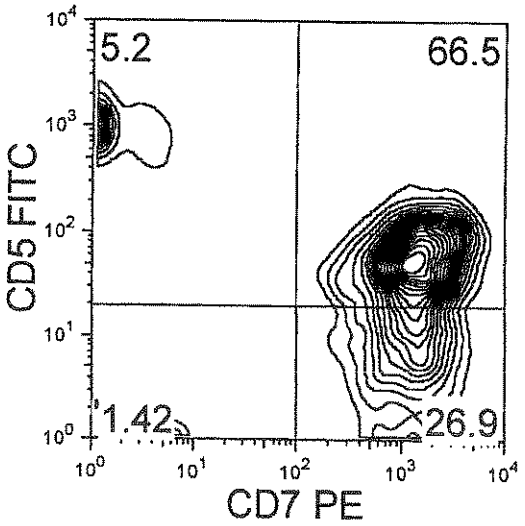
Thymus

Figure 24

A


Day 20 CD34+ hEB/HES-1 OP9-DL1
2.97
47.7
31.7
CD5 FITC
CD7 PE
10^0
10^1
10^2
10^3
10^4

B


Day 22 CD34+ hEB/hiPSC OP9-DL4
5.2
66.5
1.42
26.9
CD5 FITC
CD7 PE
10^0
10^1
10^2
10^3
10^4