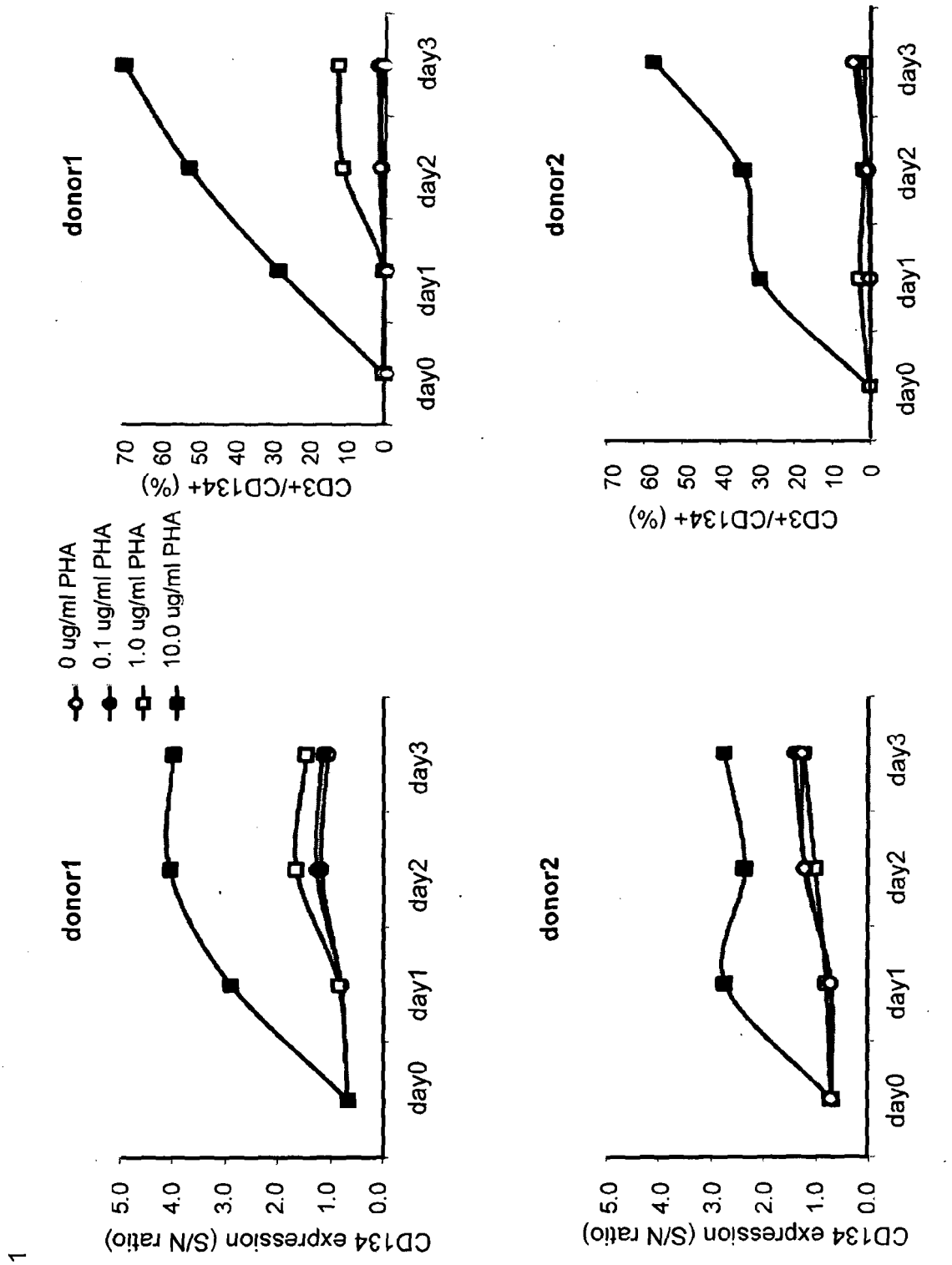

donor1
donor2
CD3+/CD134+ (%)
CD134 expression (S/N ratio)
day0
day1
day2
day3
0 ug/ml PHA
0.1 ug/ml PHA
1.0 ug/ml PHA
10.0 ug/ml PHA

Figure 1

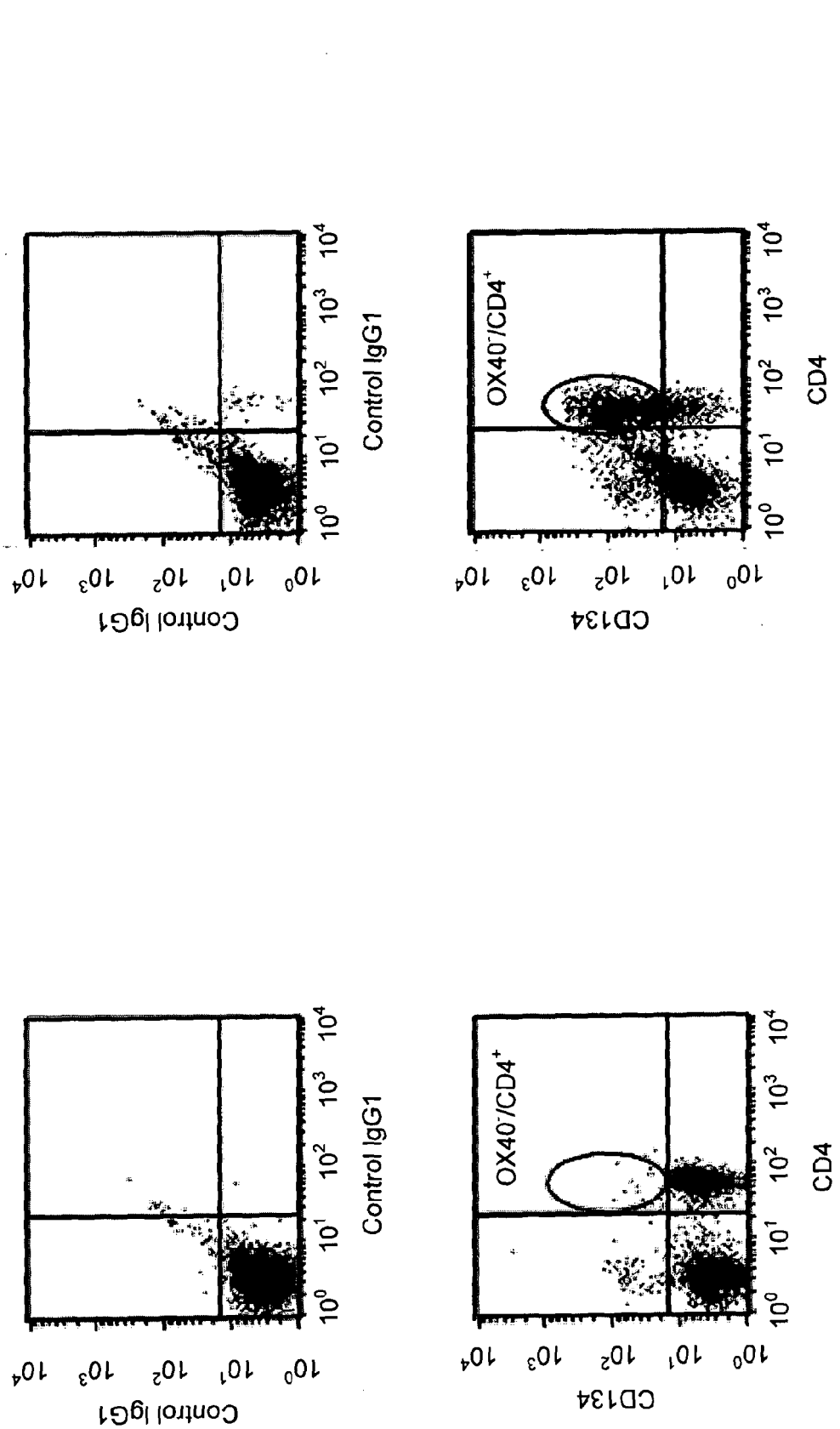

Resting CD4 T cells
PHA-stimulated/activated CD4 T cells
Control IgG1
Control IgG1
CD134
OX40+/CD4+
CD4
CD134
OX40+/CD4+
CD4
10^0
10^1
10^2
10^3
10^4

Figure 2

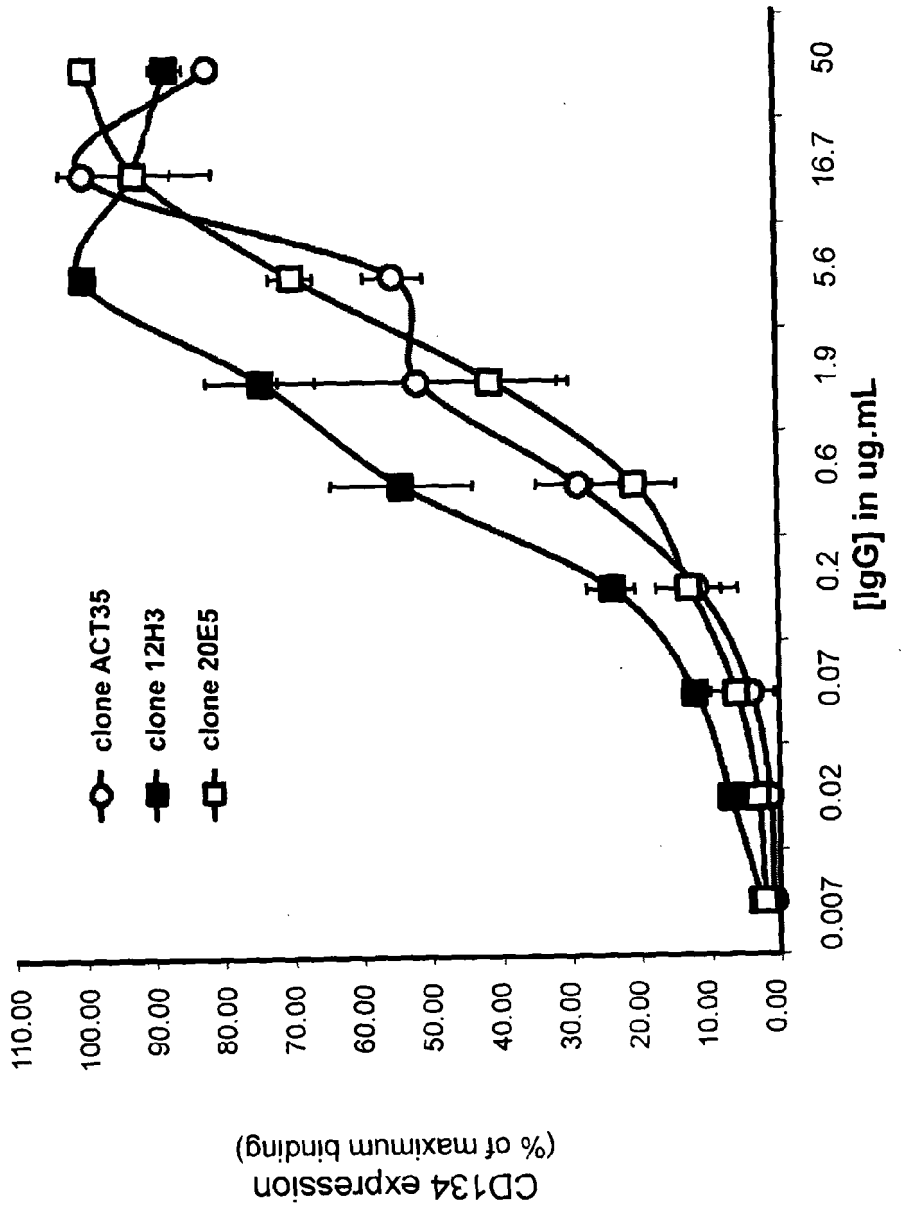

CD134 expression
(% of maximum binding)
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.007
0.02
0.07
0.2
0.6
1.9
5.6
16.7
50
[IgG] in ug.mL
clone ACT35
clone 12H3
clone 20E5

Figure 3

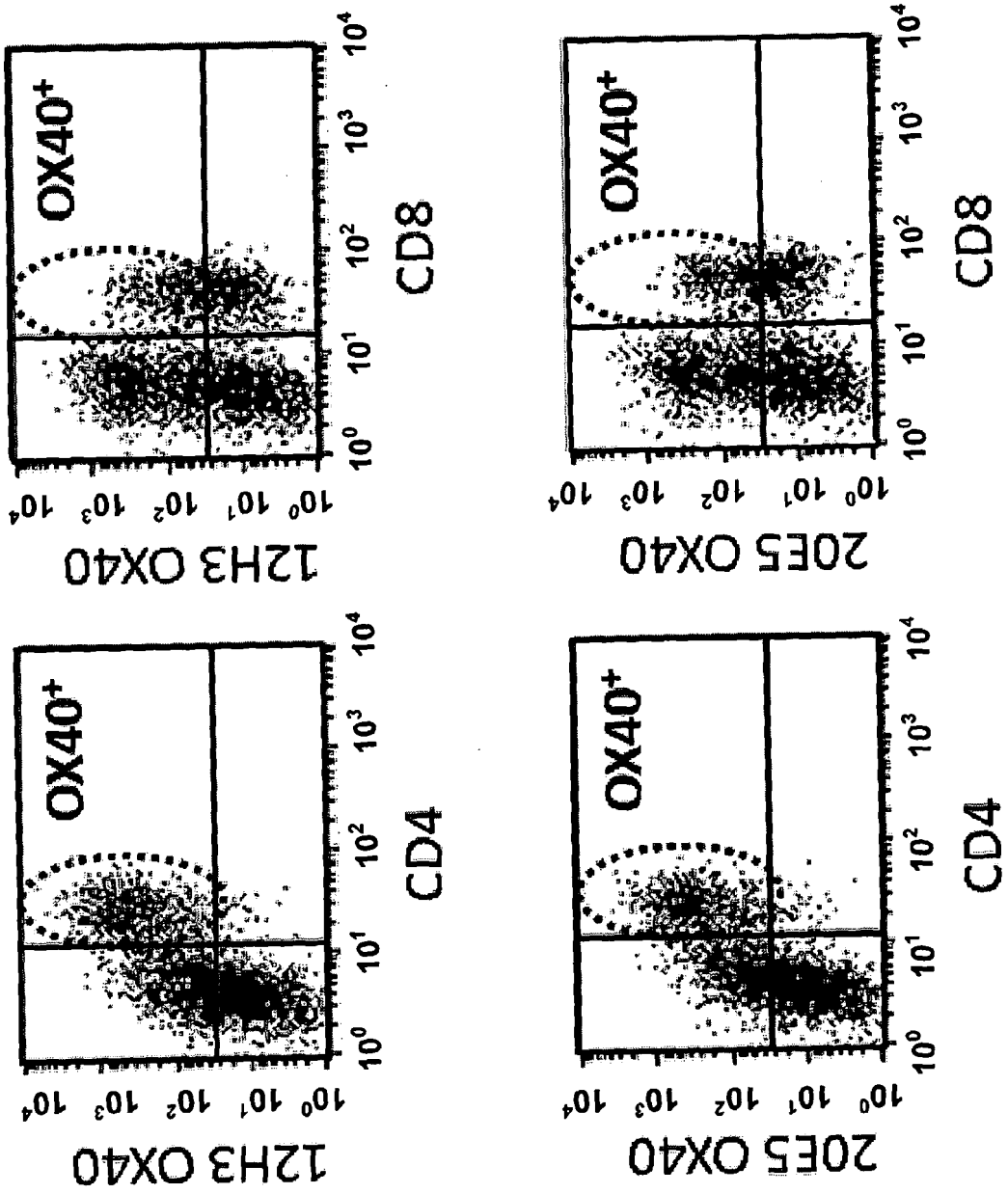

12H3 OX40
OX40+
CD8
20E5 OX40
OX40+
CD8
12H3 OX40
OX40+
CD4
20E5 OX40
OX40+
CD4

Figure 4

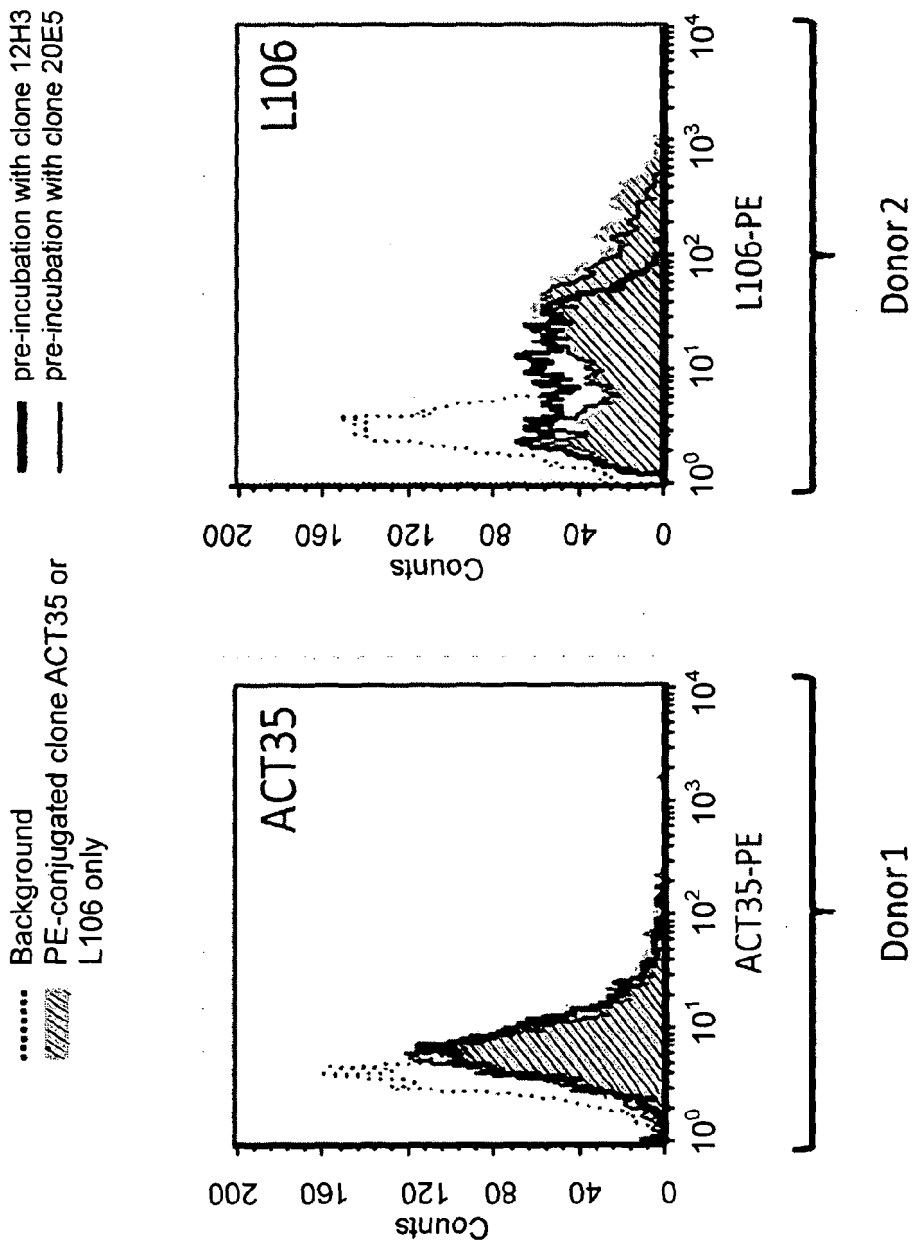

pre-incubation with clone 12H3
pre-incubation with clone 20E5
Background
PE-conjugated clone ACT35 or L106 only
ACT35
L106
Counts
0
40
80
120
160
200
10^0
10^1
10^2
10^3
10^4
ACT35-PE
L106-PE
Donor 1
Donor 2

Figure 5

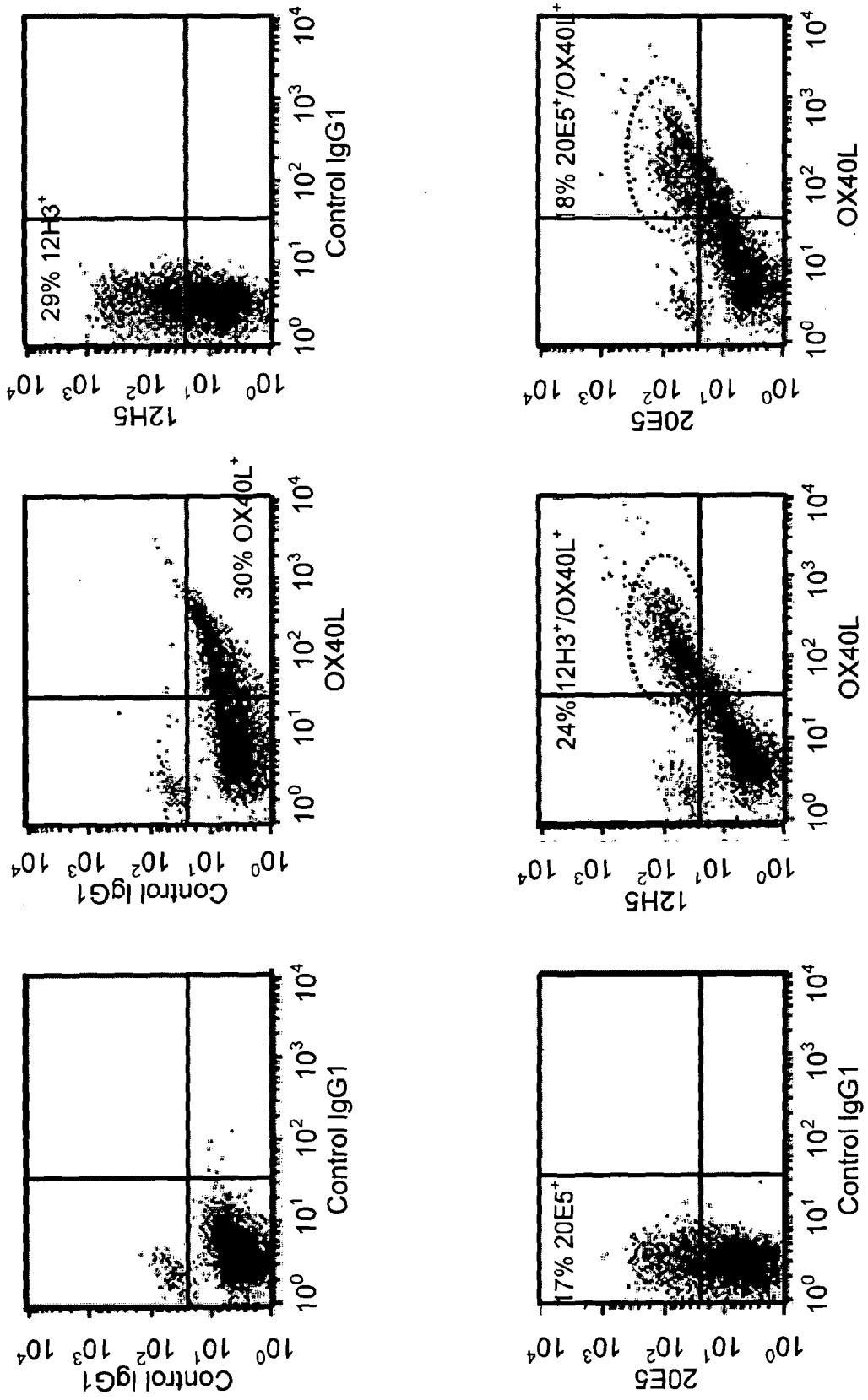

29% 12H3+
Control IgG1
12H5
18% 20E5+/OX40L+
OX40L
20E5
30% OX40L+
24% 12H3+/OX40L+
17% 20E5+

Figure 6

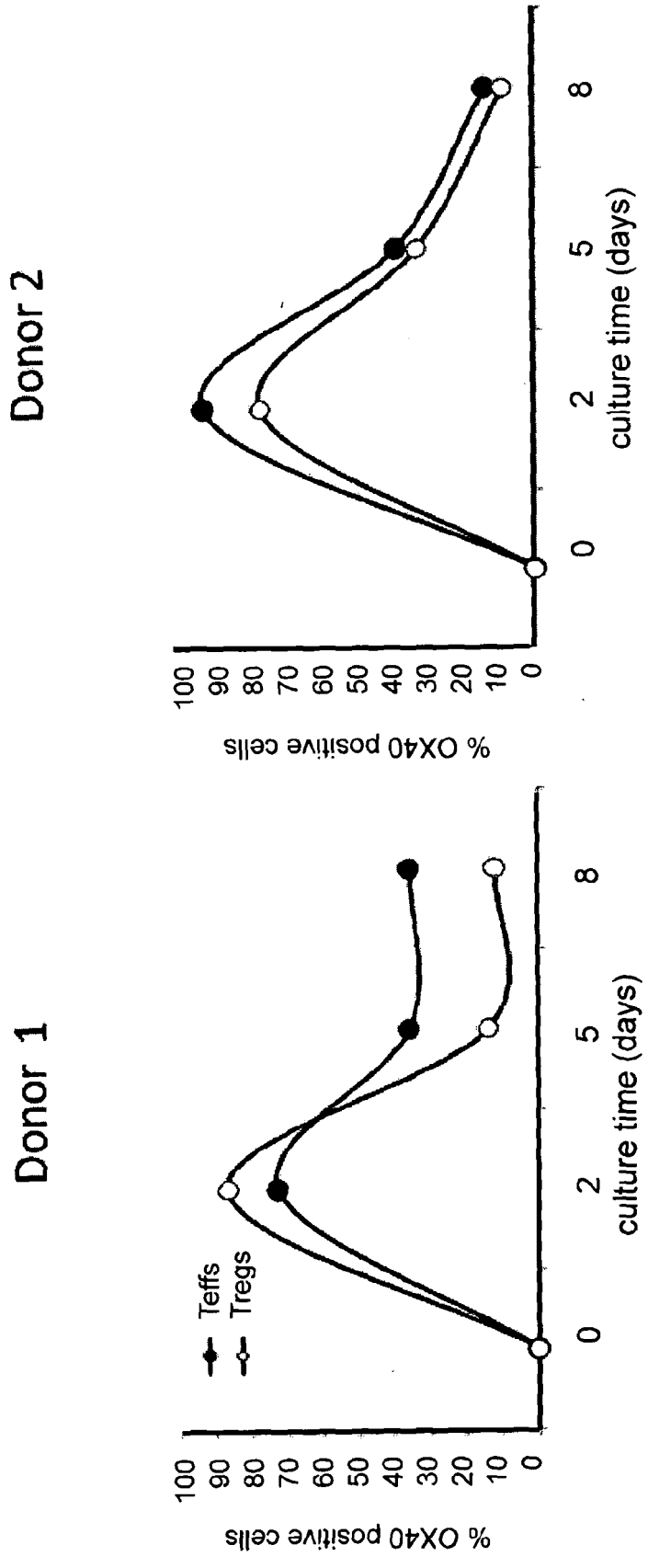

Donor 2
% OX40 positive cells
100
90
80
70
60
50
40
30
20
10
0
0
2
5
8
culture time (days)
Donor 1
Teffs
Tregs
% OX40 positive cells
100
90
80
70
60
50
40
30
20
10
0
0
2
5
8
culture time (days)

Figure 7

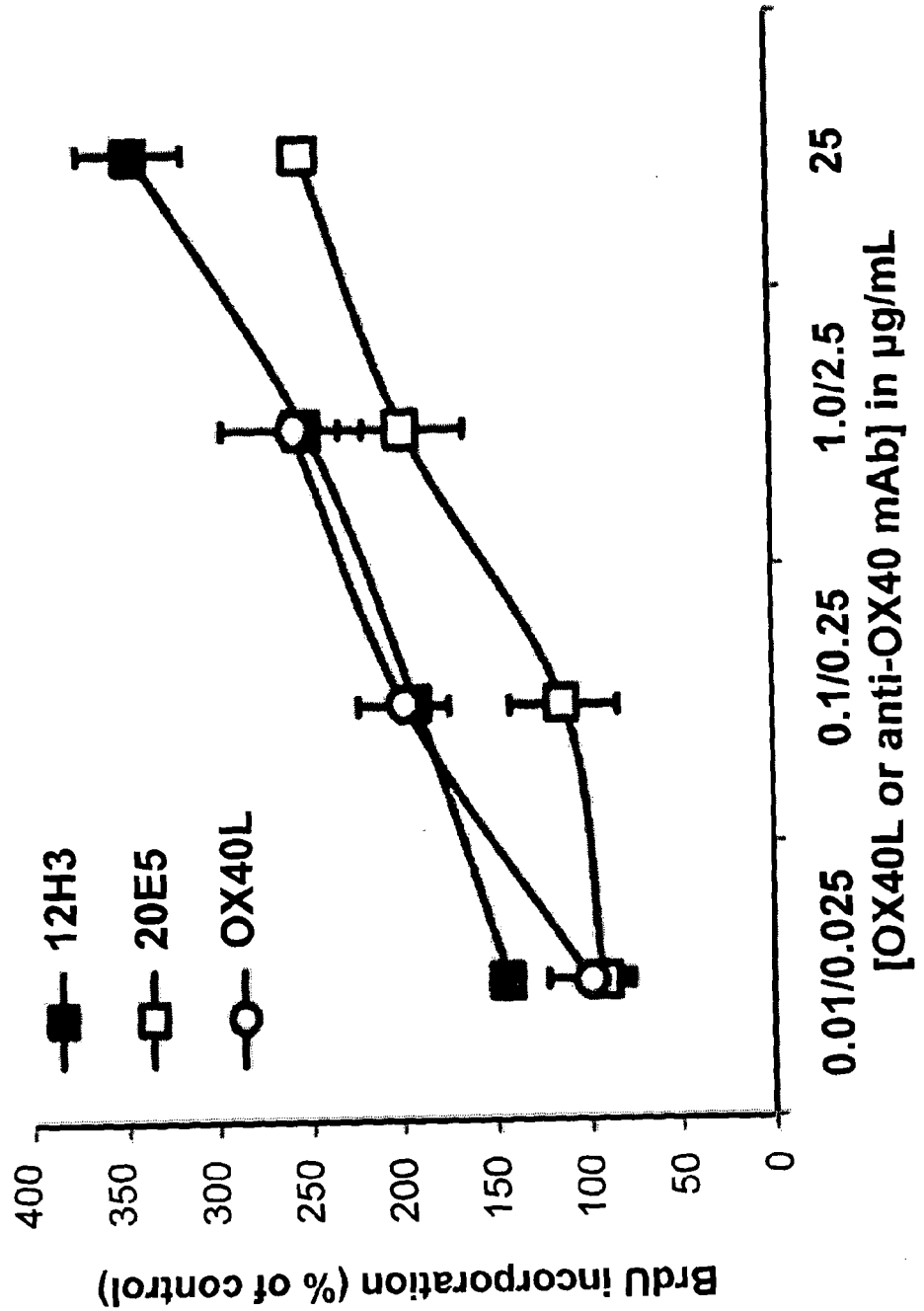

BrdU incorporation (% of control)
400
350
300
250
200
150
100
50
0
12H3
20E5
OX40L
0.01/0.025
0.1/0.25
1.0/2.5
25
[OX40L or anti-OX40 mAb] in µg/mL

Figure 8

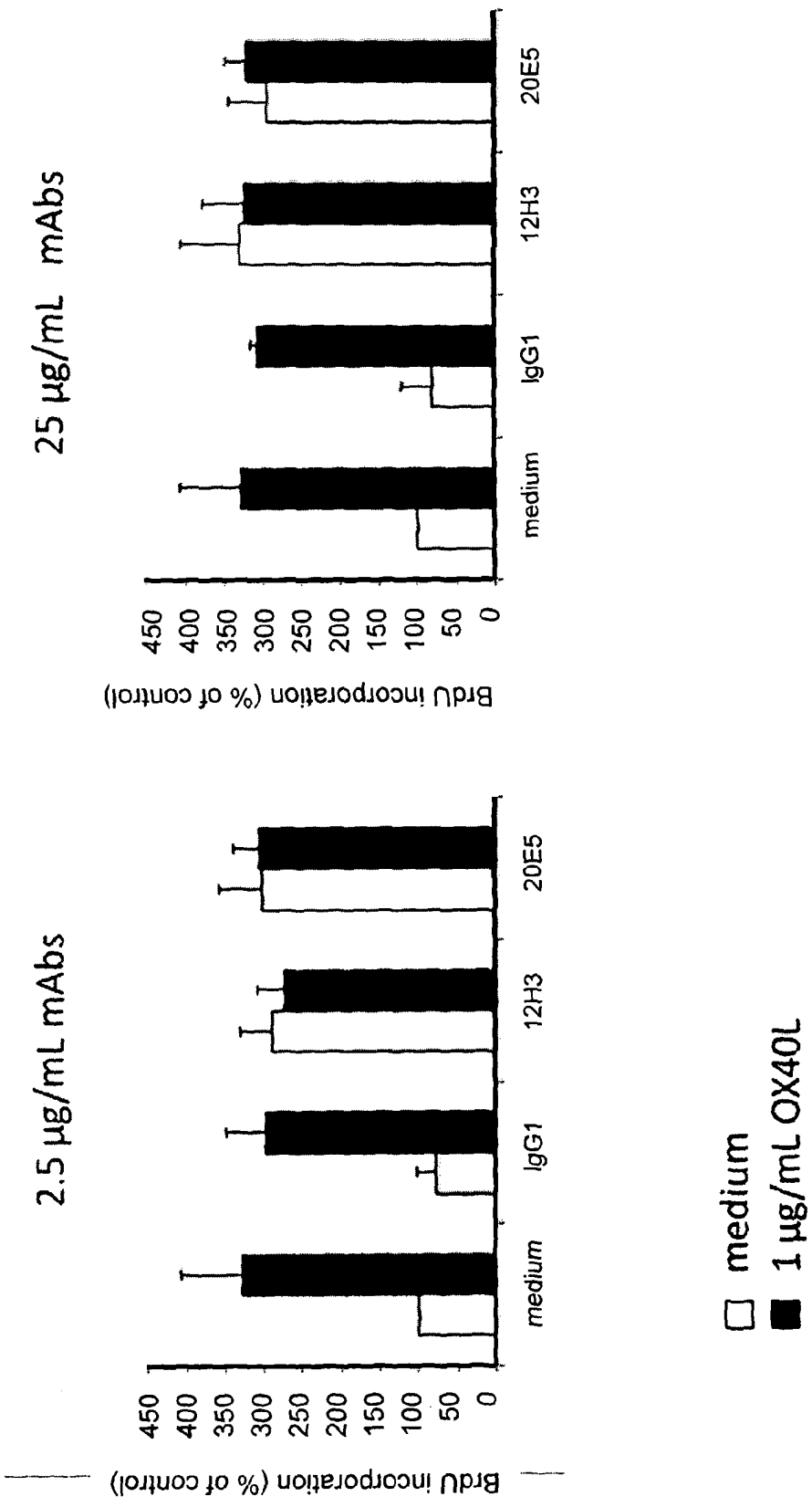

2.5 µg/mL mAbs
25 µg/mL mAbs
BrdU incorporation (% of control)
450
400
350
300
250
200
150
100
50
0
medium
IgG1
12H3
20E5
medium
1 µg/mL OX40L

Figure 9

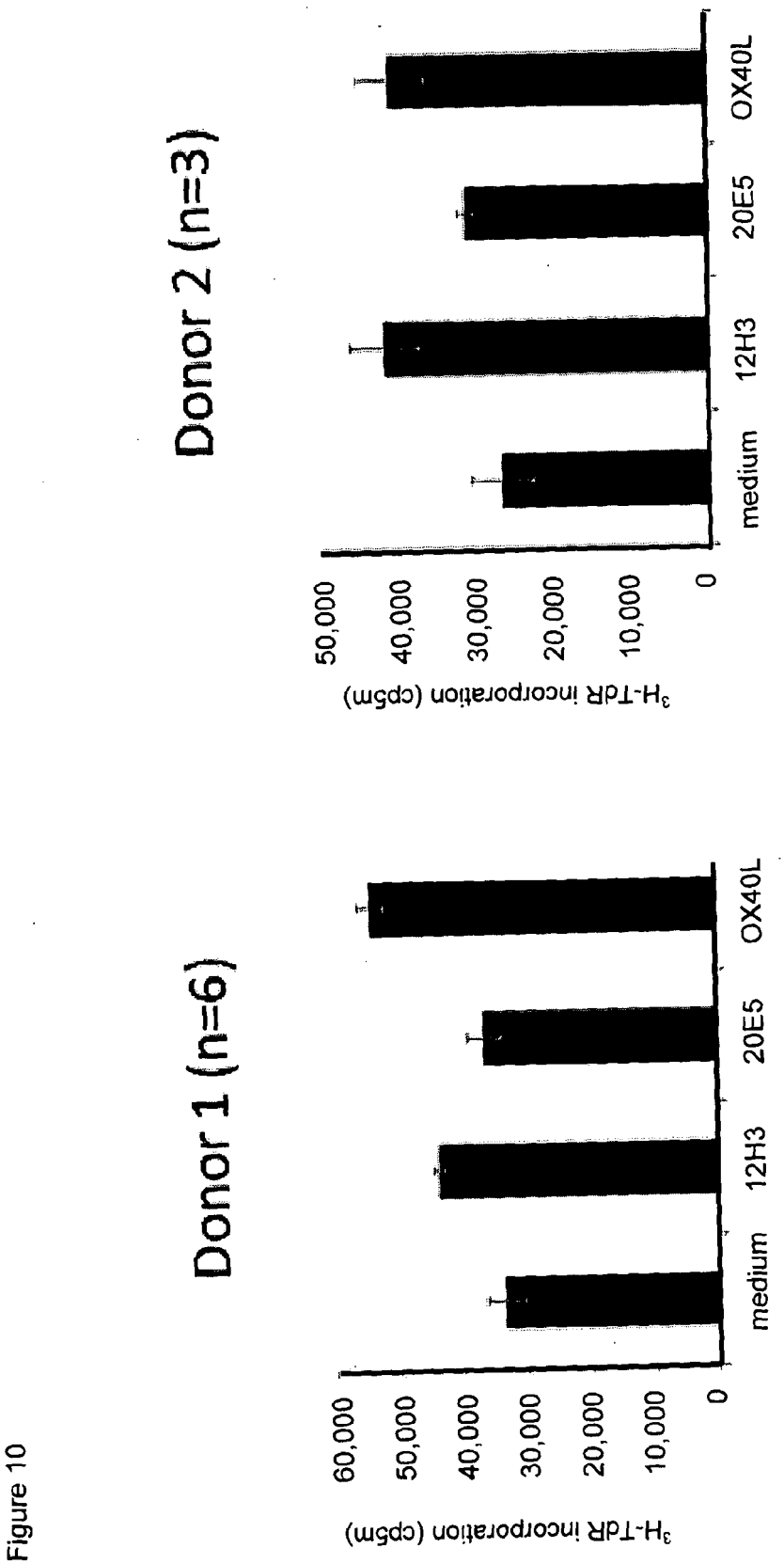

Donor 1 (n=6)
^{3}H-TdR incorporation (cpδm)
60,000
50,000
40,000
30,000
20,000
10,000
0
medium
12H3
20E5
OX40L
Donor 2 (n=3)
^{3}H-TdR incorporation (cpδm)
50,000
40,000
30,000
20,000
10,000
0
medium
12H3
20E5
OX40L

Figure 10

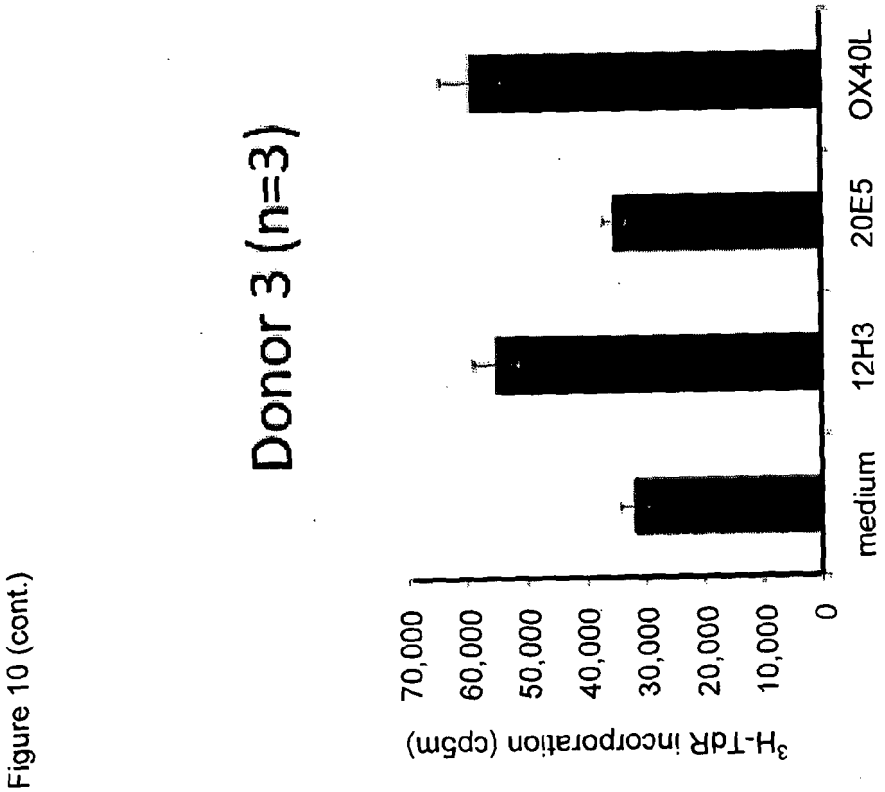


Figure 10 (cont.)

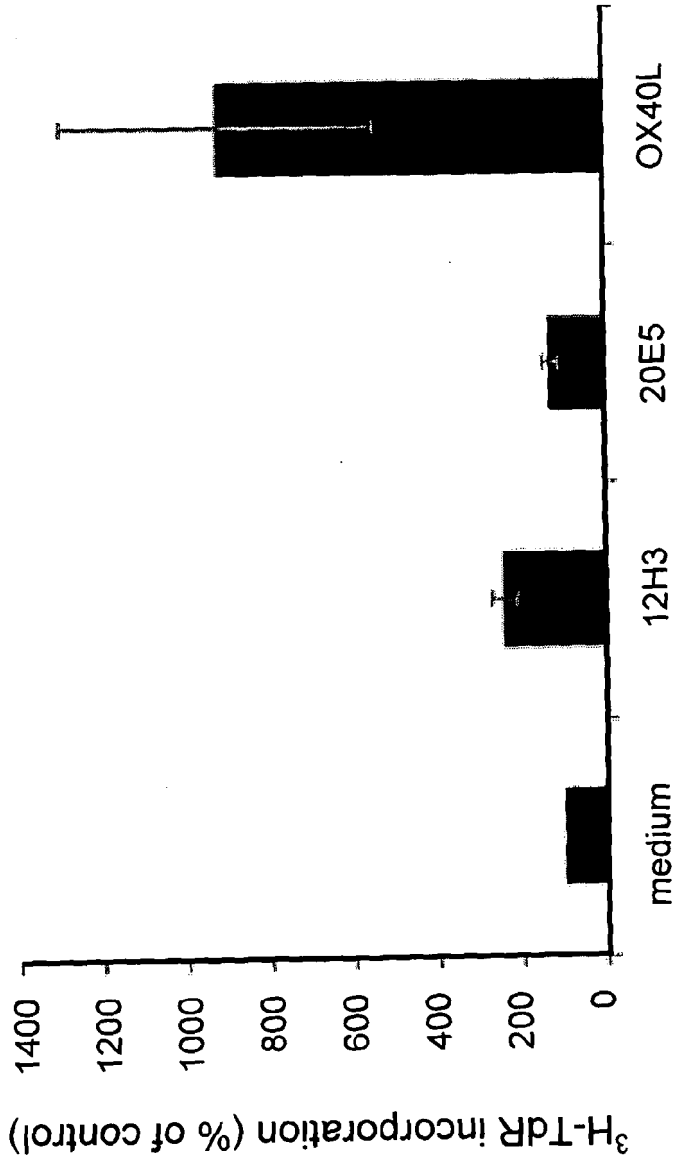

^{3}H-TdR incorporation (% of control)
0
200
400
600
800
1000
1200
1400
medium
12H3
20E5
OX40L

Figure 11

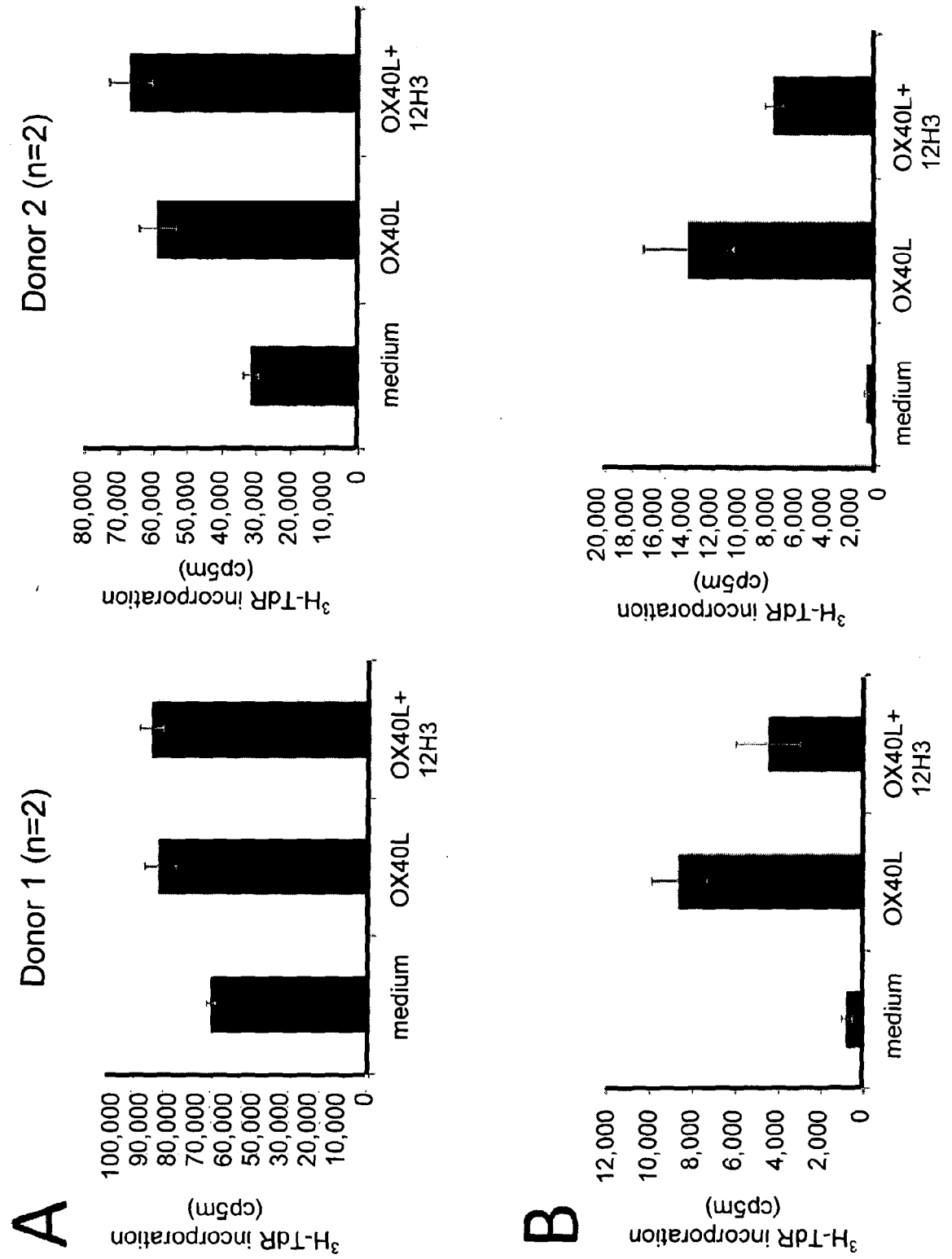
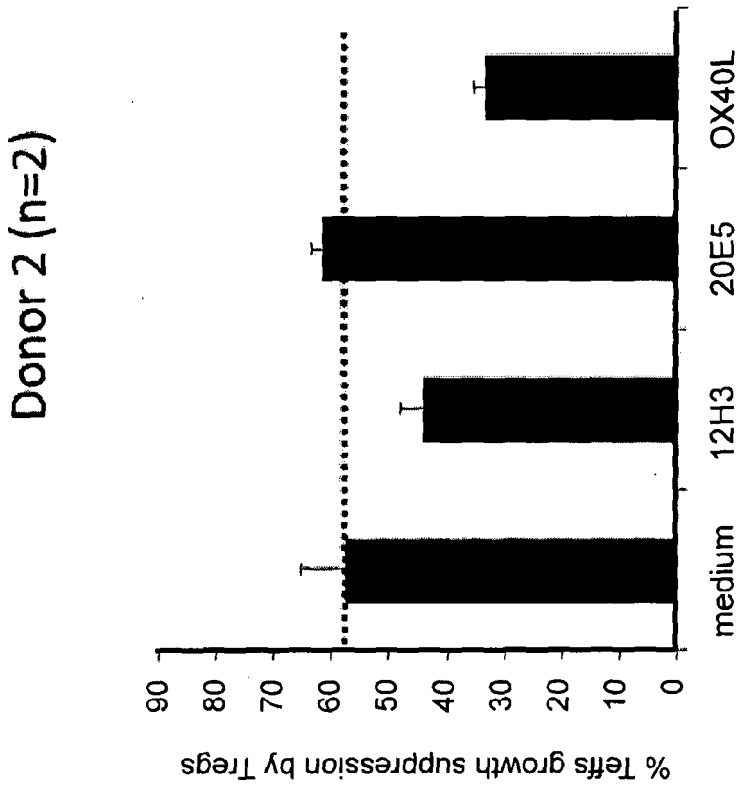

A
Donor 1 (n=2)
^{3}H-TdR incorporation (cpδm)
100,000
90,000
80,000
70,000
60,000
50,000
30,000
20,000
10,000
0
medium
OX40L
OX40L+ 12H3
Donor 2 (n=2)
^{3}H-TdR incorporation (cpδm)
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
medium
OX40L
OX40L+ 12H3
B
^{3}H-TdR incorporation (cpδm)
12,000
10,000
8,000
6,000
4,000
2,000
0
medium
OX40L
OX40L+ 12H3
^{3}H-TdR incorporation (cpδm)
20,000
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
medium
OX40L
OX40L+ 12H3

Figure 12


Donor 2 (n=2)
% Teffs growth suppression by Tregs
0
10
20
30
40
50
60
70
80
90
medium
12H3
20E5
OX40L

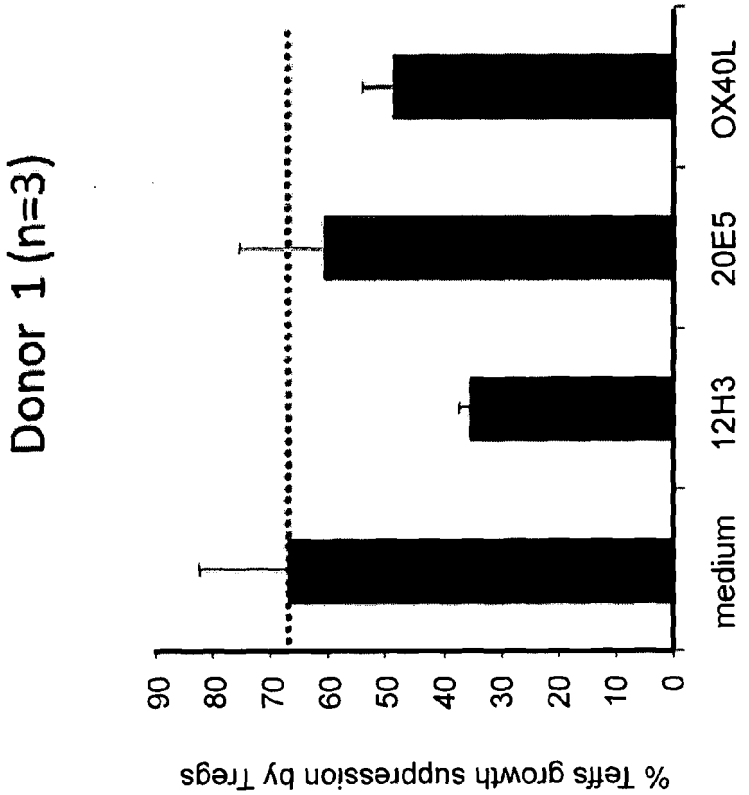

Donor 1 (n=3)
% Teffs growth suppression by Tregs
0
10
20
30
40
50
60
70
80
90
medium
12H3
20E5
OX40L

Figure 13

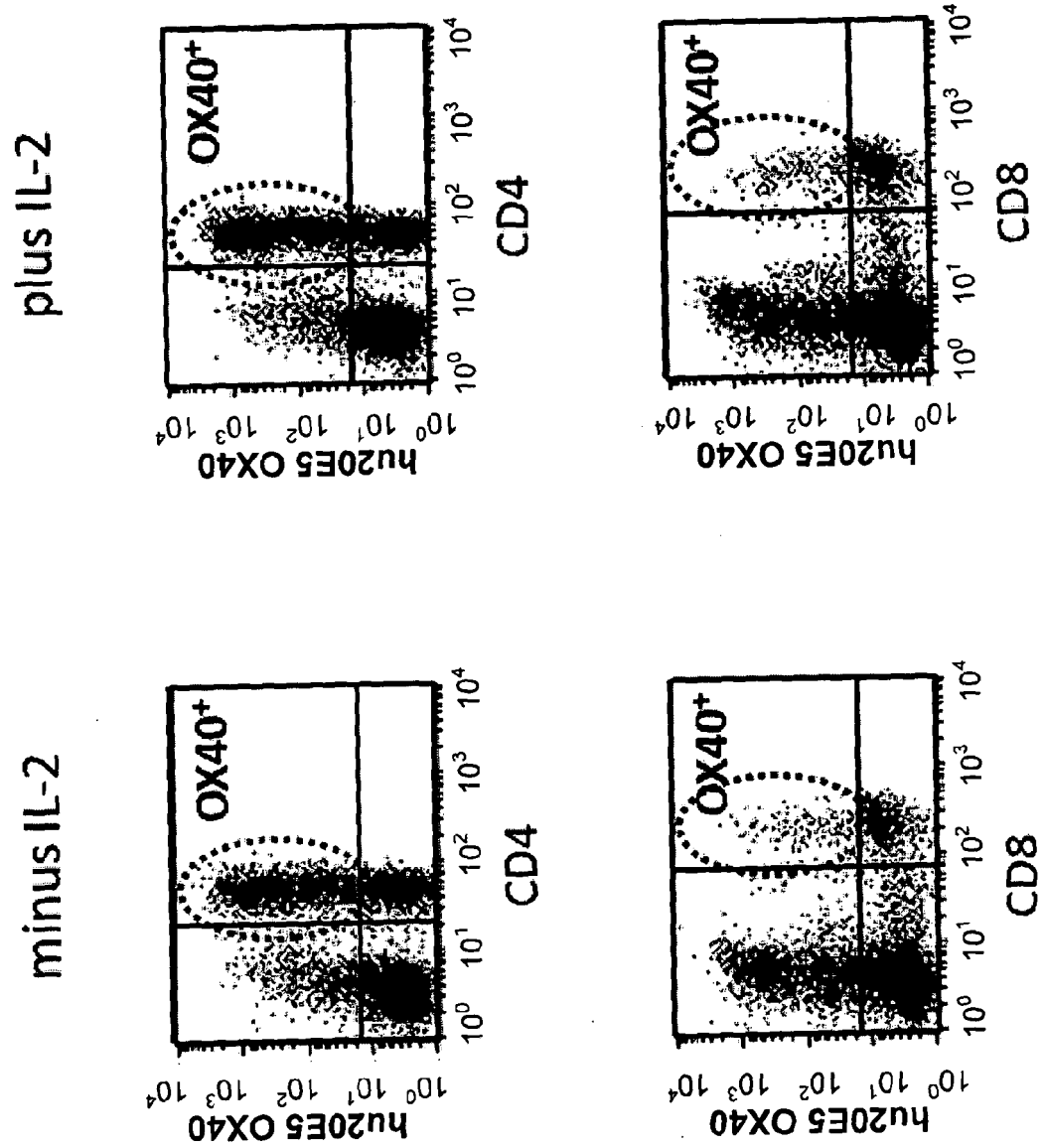

plus IL-2
minus IL-2
OX40+
CD4
CD8
hu20E5 OX40
10^0 10^1 10^2 10^3 10^4

Figure 14

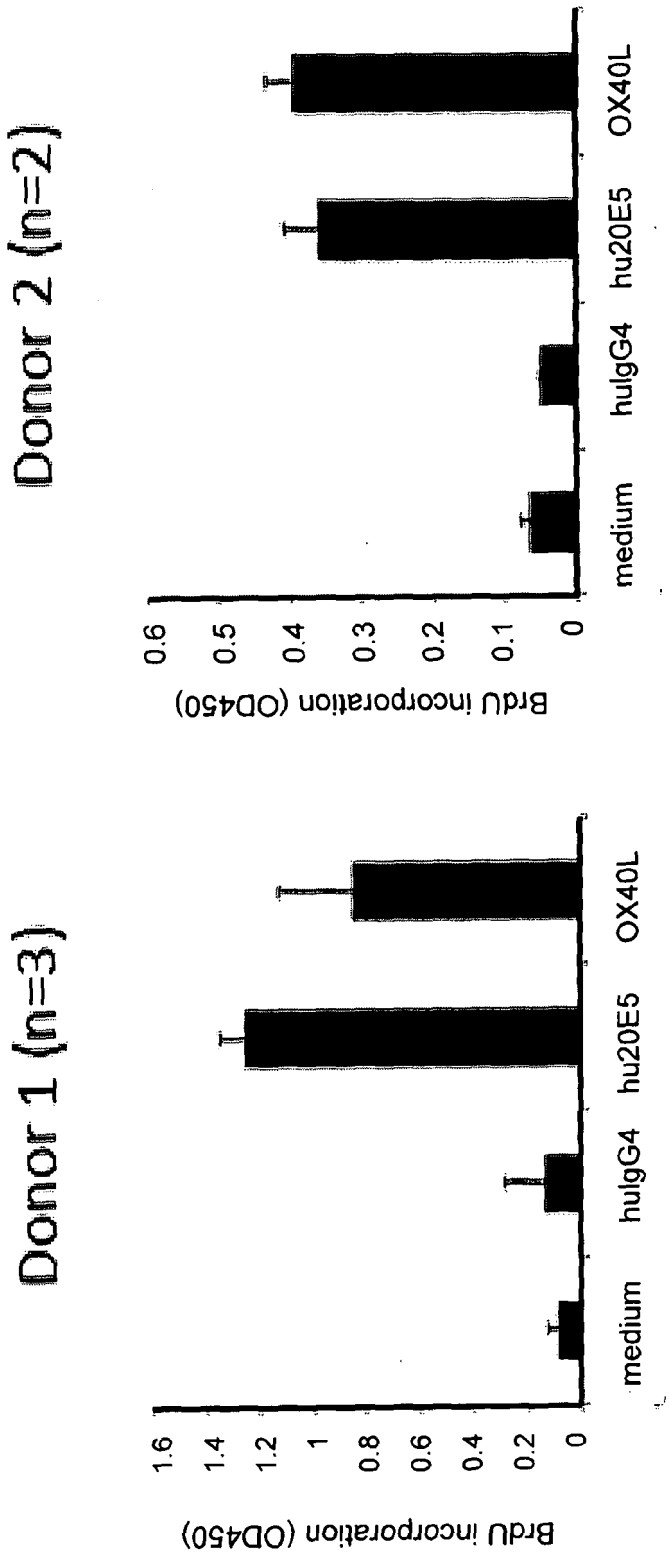
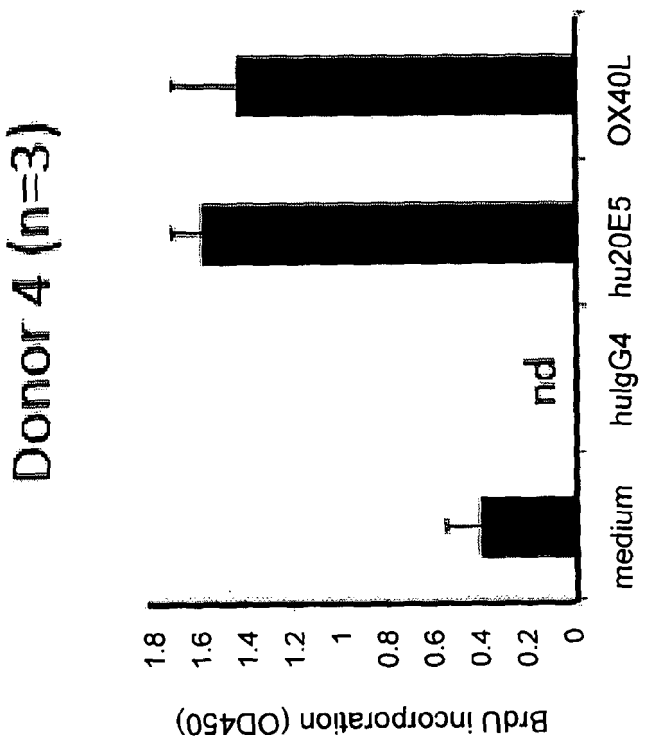

Donor 1 (n=3)
BrdU incorporation (OD450)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
medium
hulgG4
hu20E5
OX40L
Donor 2 (n=2)
BrdU incorporation (OD450)
0
0.1
0.2
0.3
0.4
0.5
0.6
medium
hulgG4
hu20E5
OX40L

Figure 15



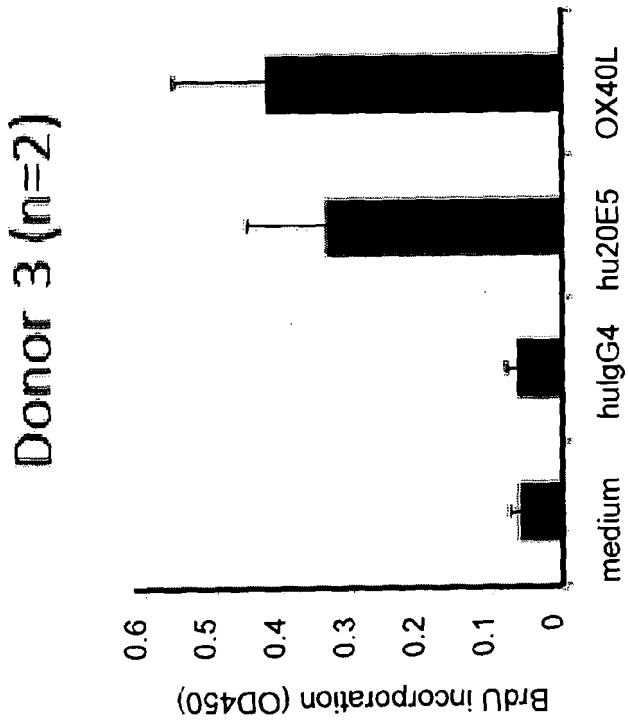


Figure 15 (cont.)

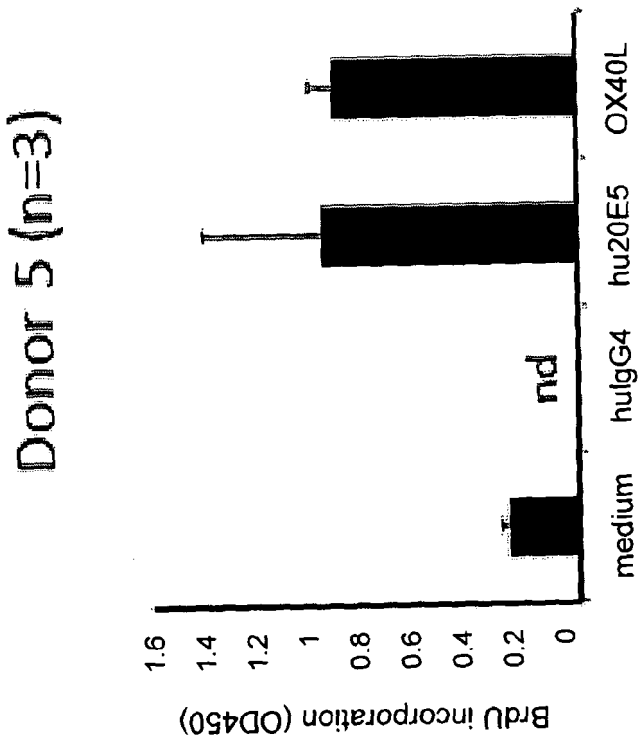
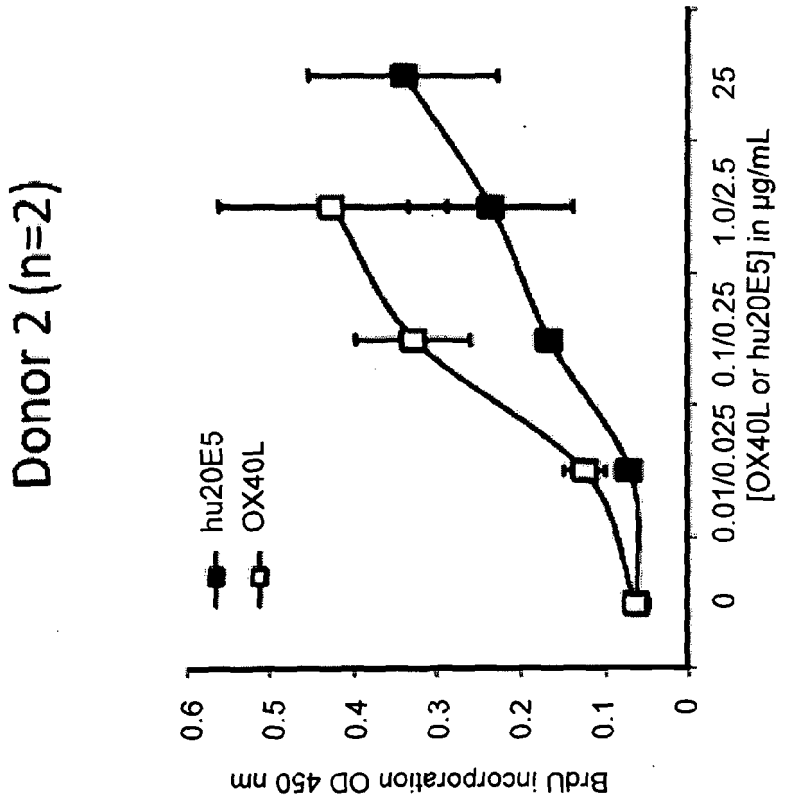


Figure 15 (cont.)


Donor 2 (n=2)
hu20E5
OX40L
BrdU incorporation OD 450 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.01/0.025
0.1/0.25
1.0/2.5
25
[OX40L or hu20E5] in µg/mL

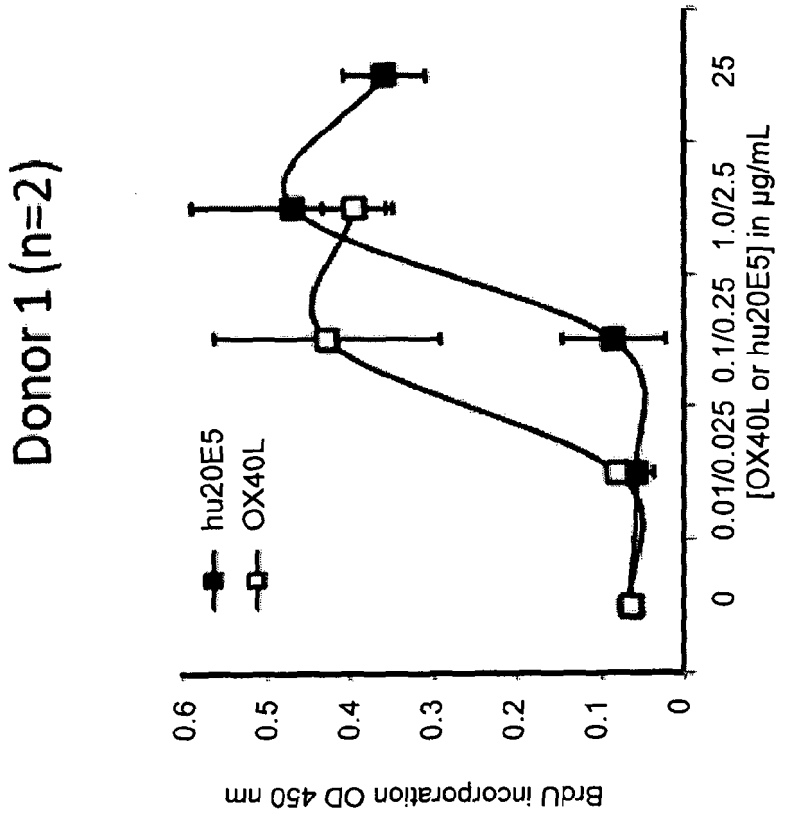
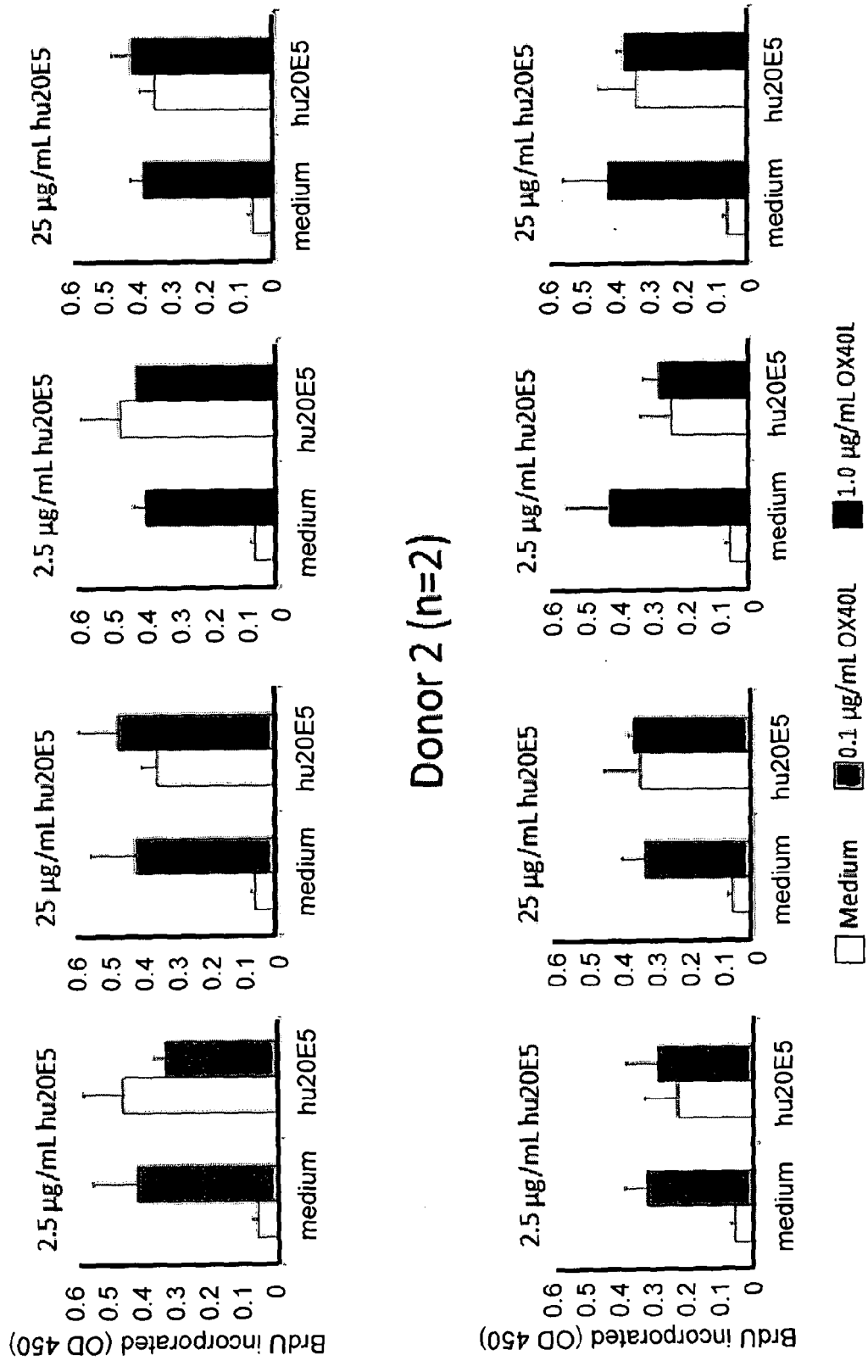

Donor 1 (n=2)
hu20E5
OX40L
BrdU incorporation OD 450 nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.01/0.025
0.1/0.25
1.0/2.5
25
[OX40L or hu20E5] in µg/mL

Figure 16

Figure 17


Donor 1 (n=2)
Donor 2 (n=2)
BrdU incorporated (OD 450)
0.6
0.5
0.4
0.3
0.2
0.1
0
2.5 µg/mL hu20E5
25 µg/mL hu20E5
medium
hu20E5
Medium
0.1 µg/mL OX40L
1.0 µg/mL OX40L

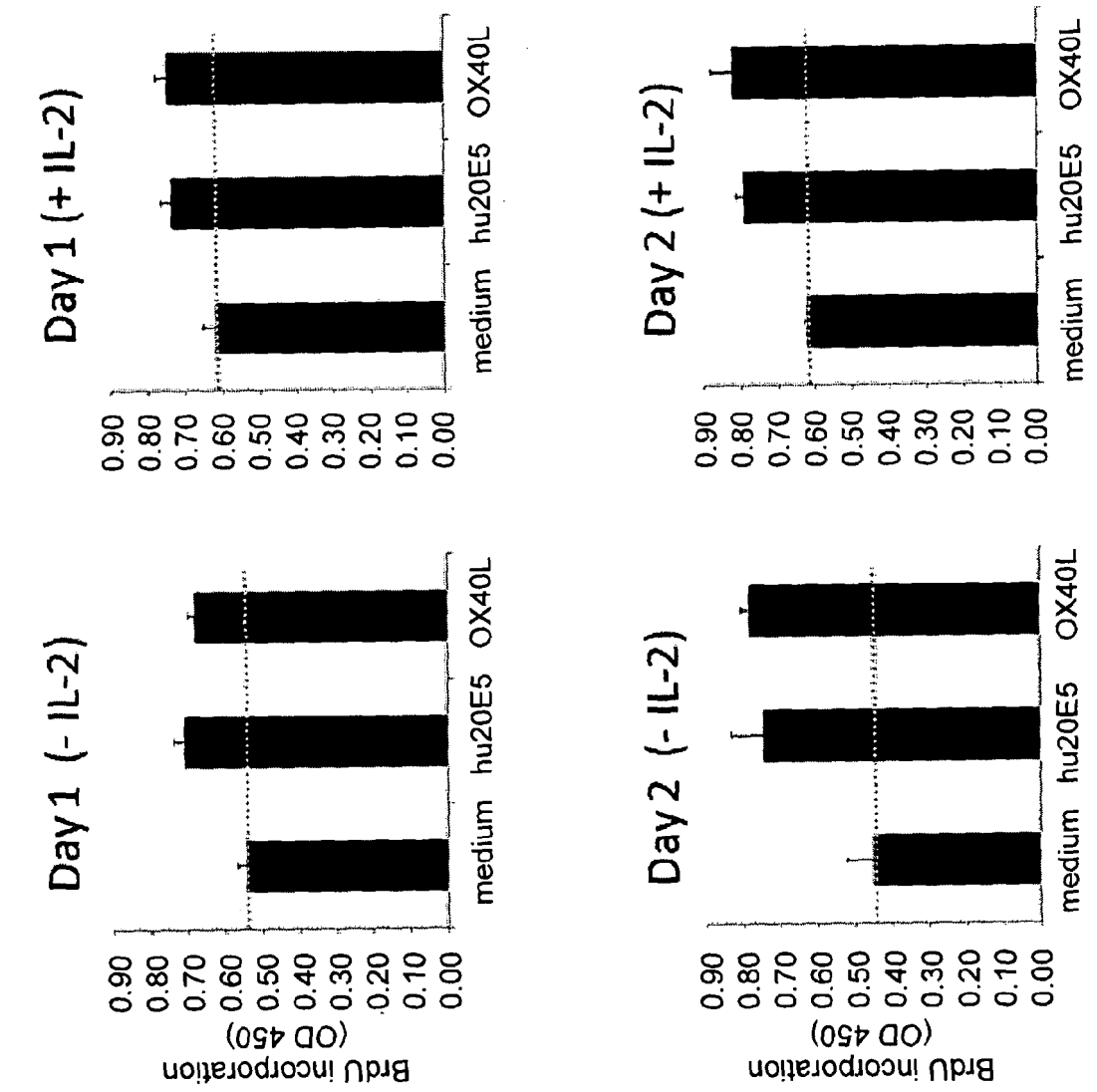

Day1 (- IL-2)
Day1 (+ IL-2)
Day2 (- IL-2)
Day2 (+ IL-2)
BrdU incorporation (OD 450)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
medium
hu20E5
OX40L

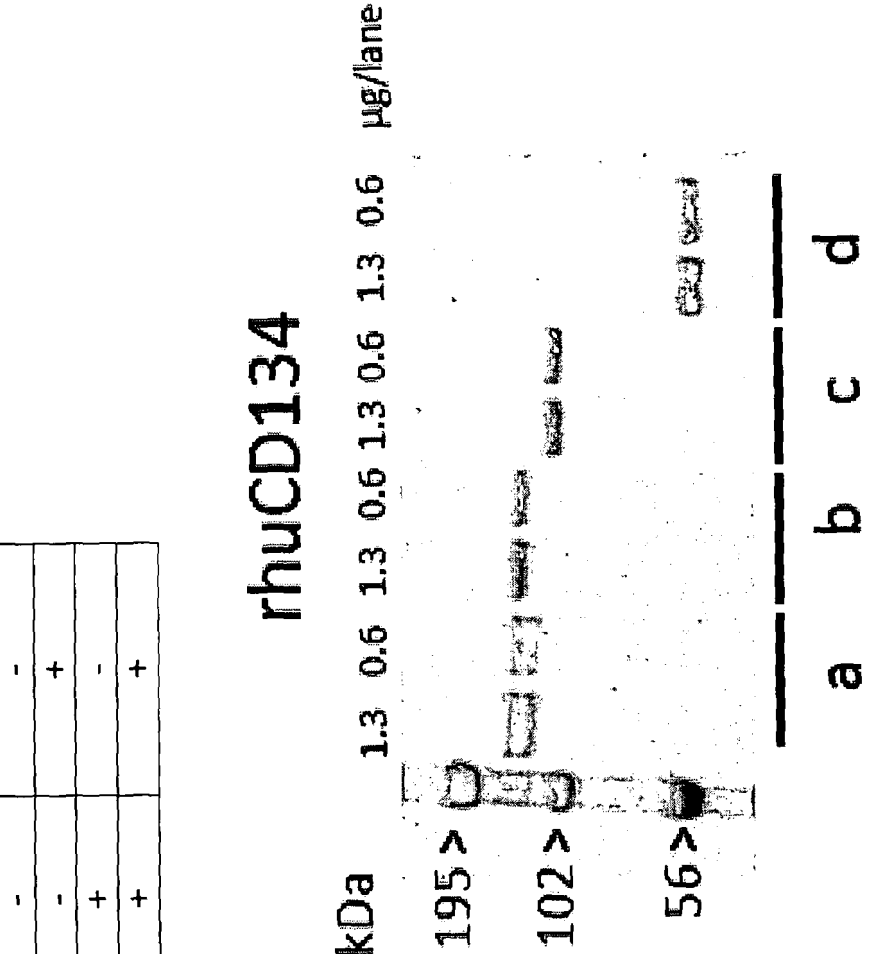
Figure 18

Figure 19

A

	reduction	70 °C heating
a	-	-
b	-	+
c	+	-
d	+	+

B



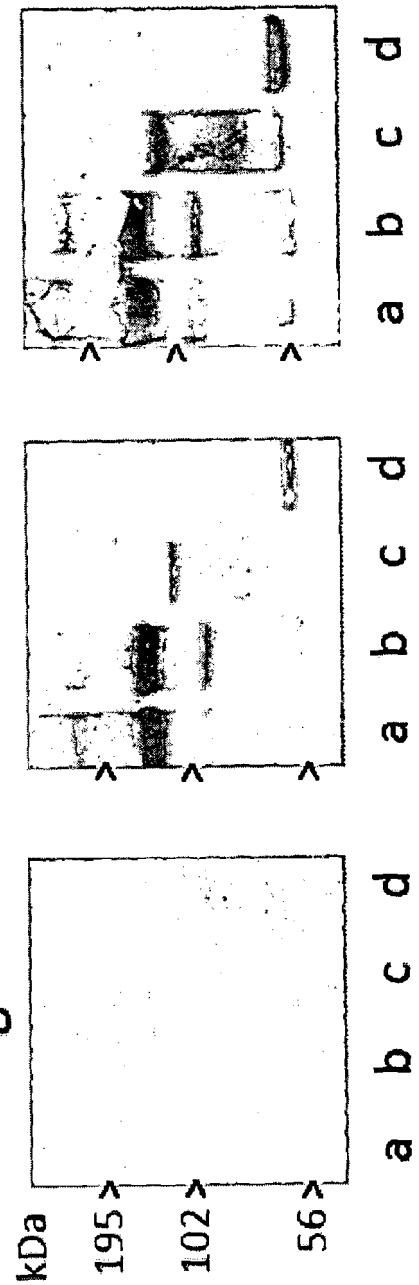
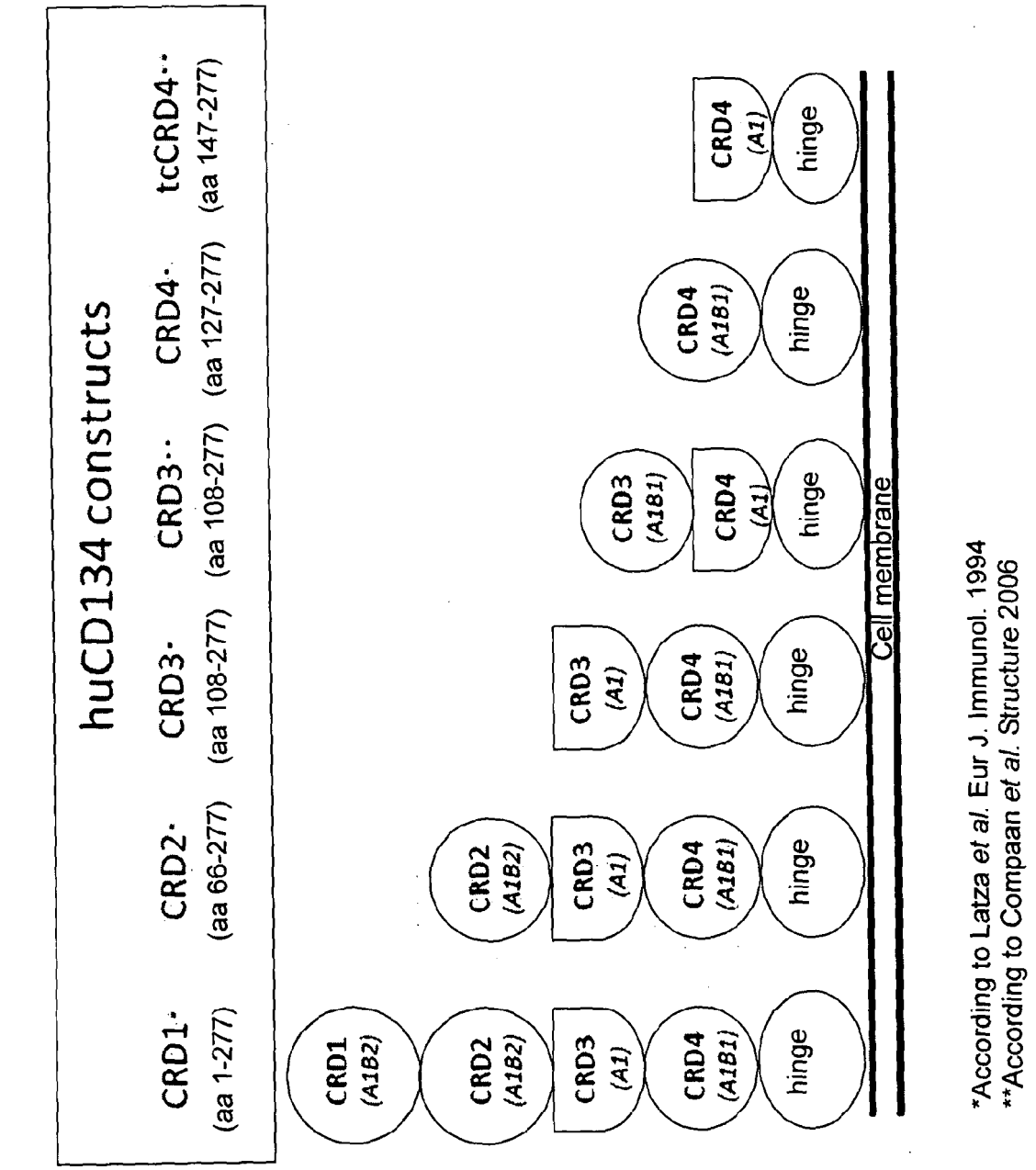


Figure 19 (cont.)



*According to Latza *et al.* Eur J. Immunol. 1994
**According to Compaan *et al.* Structure 2006

Figure 20

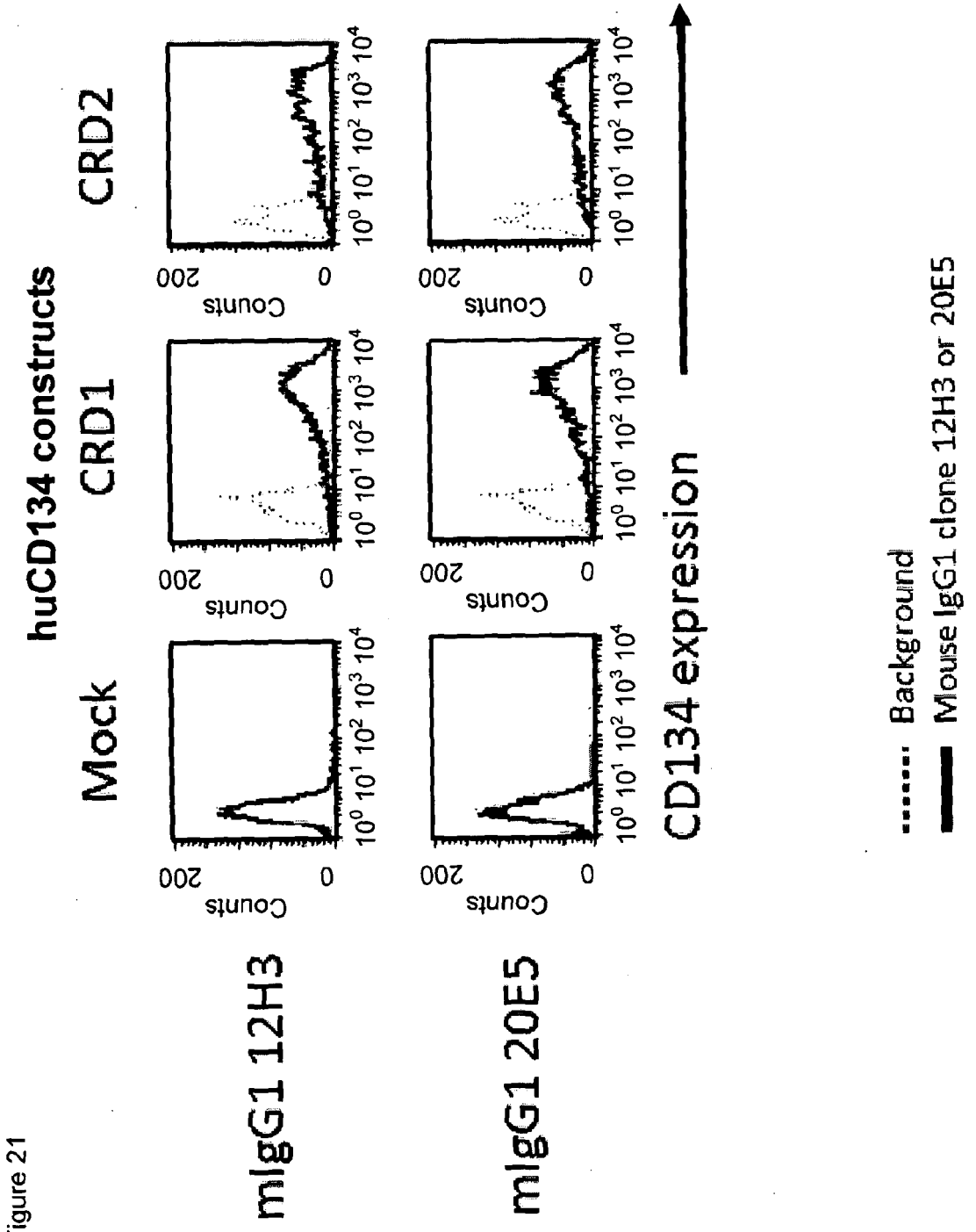

huCD134 constructs
Mock
CRD1
CRD2
mIgG1 12H3
mIgG1 20E5
Counts
200
0
10^0 10^1 10^2 10^3 10^4
CD134 expression
Background
Mouse IgG1 clone 12H3 or 20E5

Figure 21

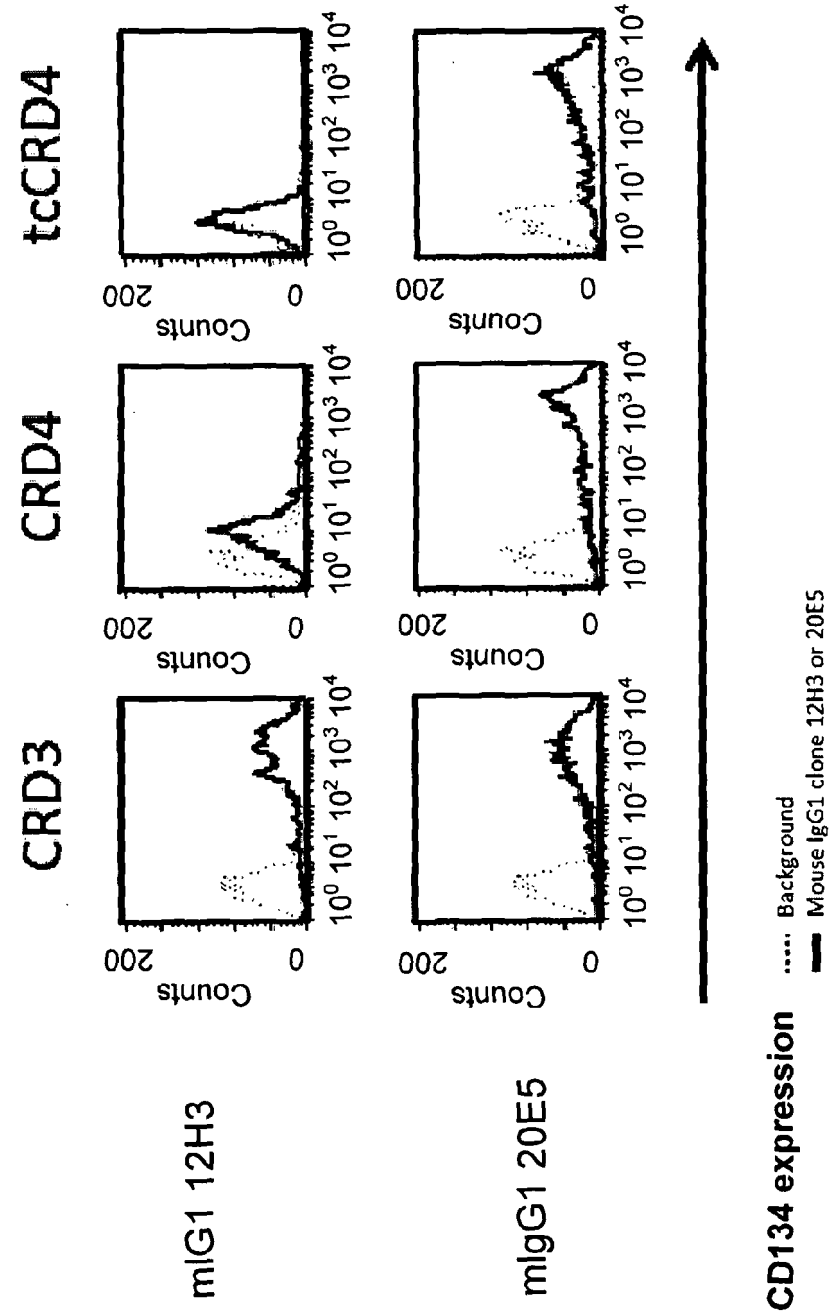


Figure 21 (cont.)

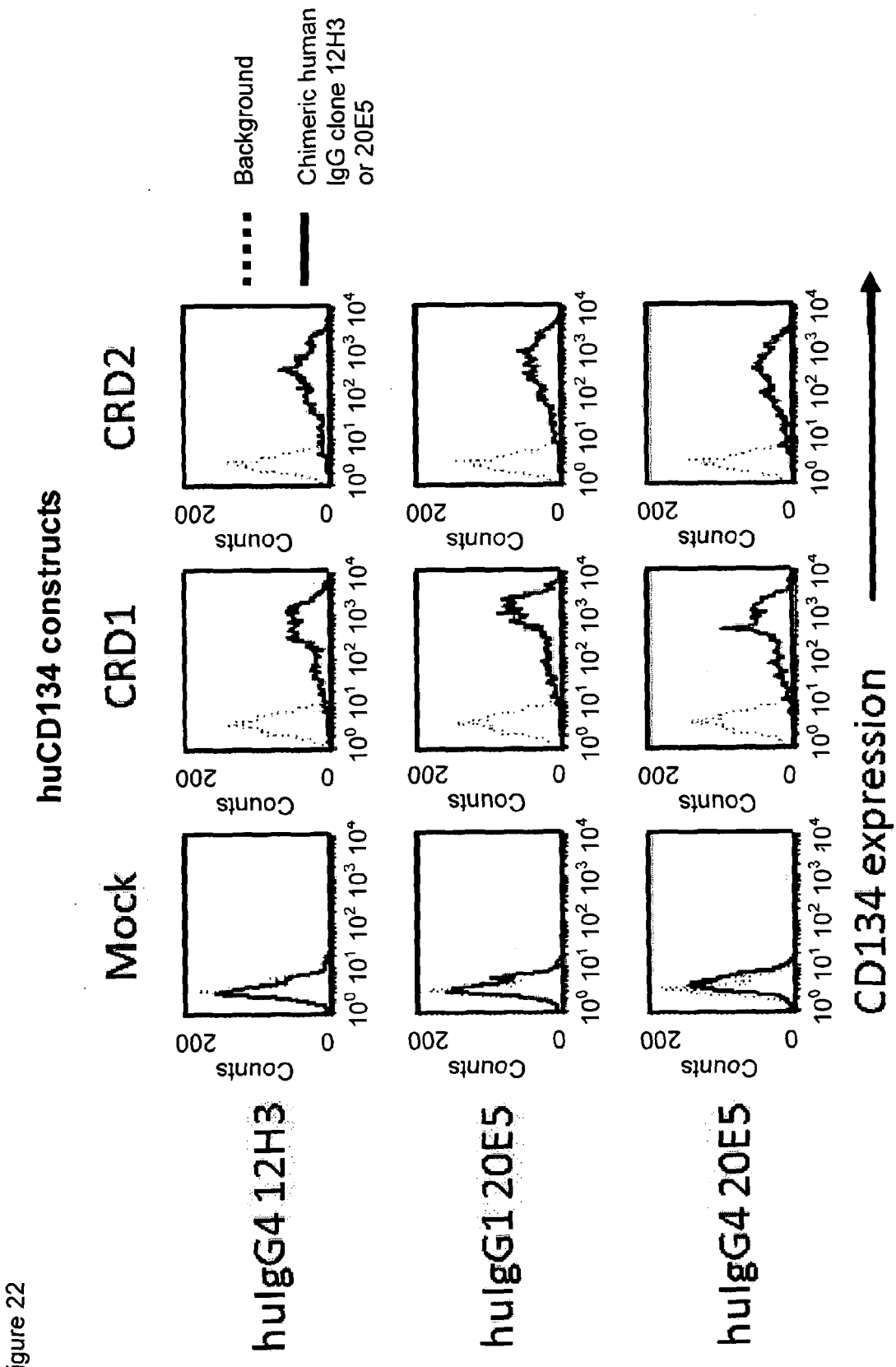

huCD134 constructs
Mock
CRD1
CRD2
Background
Chimeric human IgG clone 12H3 or 20E5
huIgG4 12H3
huIgG1 20E5
huIgG4 20E5
Counts
200
0
10^0 10^1 10^2 10^3 10^4
CD134 expression

Figure 22

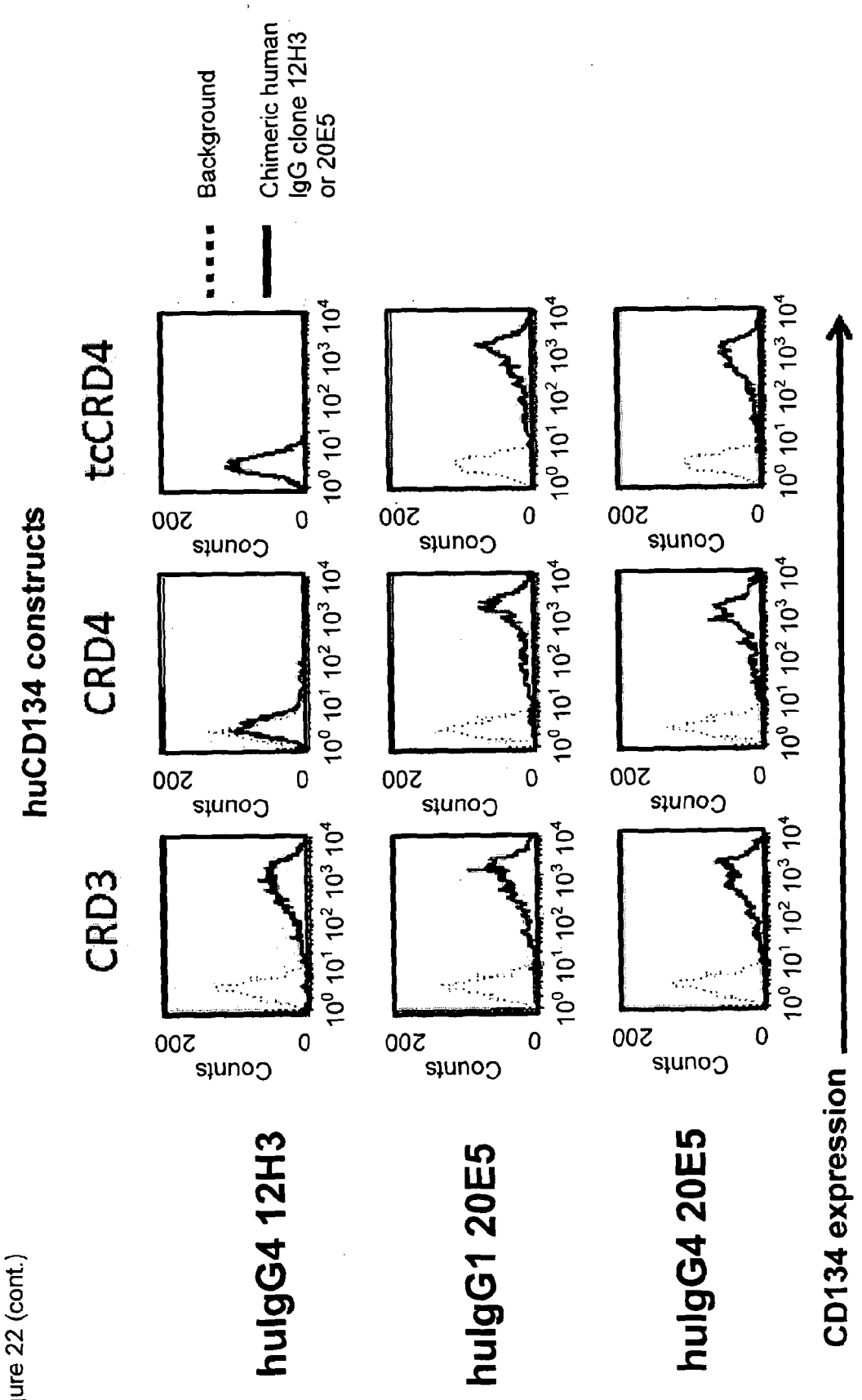


Figure 22 (cont.)

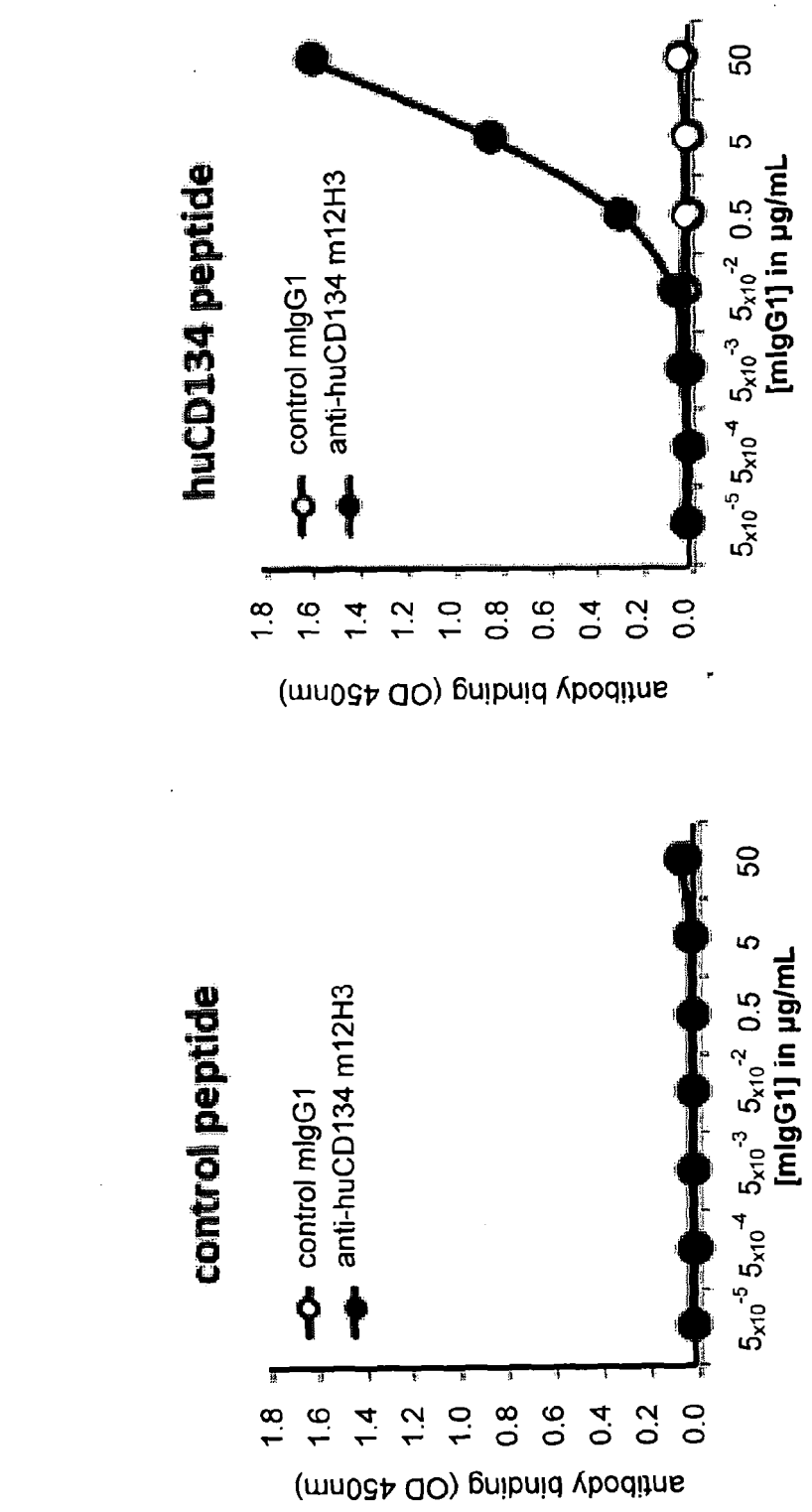

A
control peptide
huCD134 peptide
control mIgG1
anti-huCD134 m12H3
antibody binding (OD 450nm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
5x10-5 5x10-4 5x10-3 5x10-2 0.5 5 50
[mIgG1] in µg/mL

Figure 23

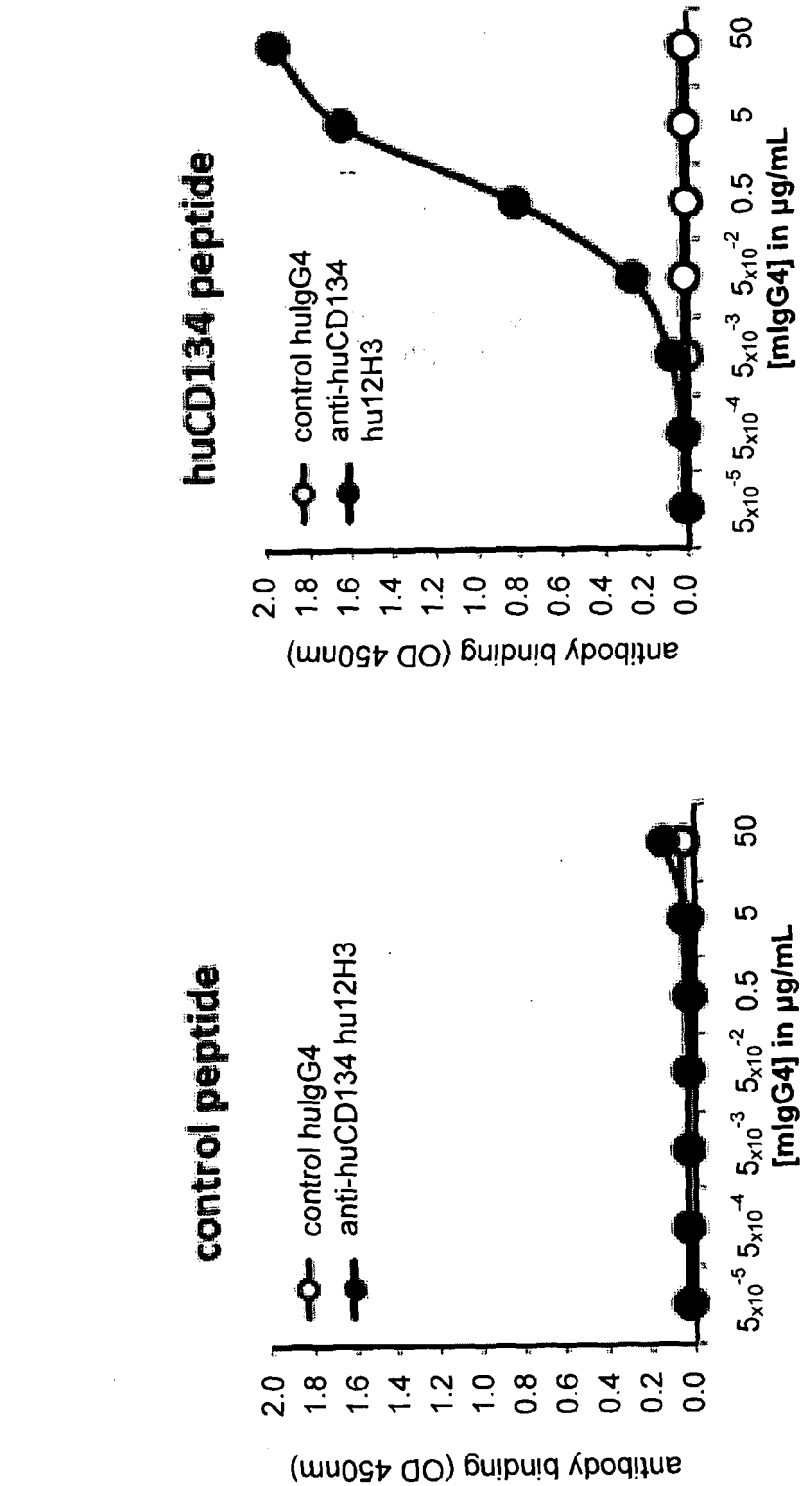


Figure 23 (cont.)