

FIG. 1A

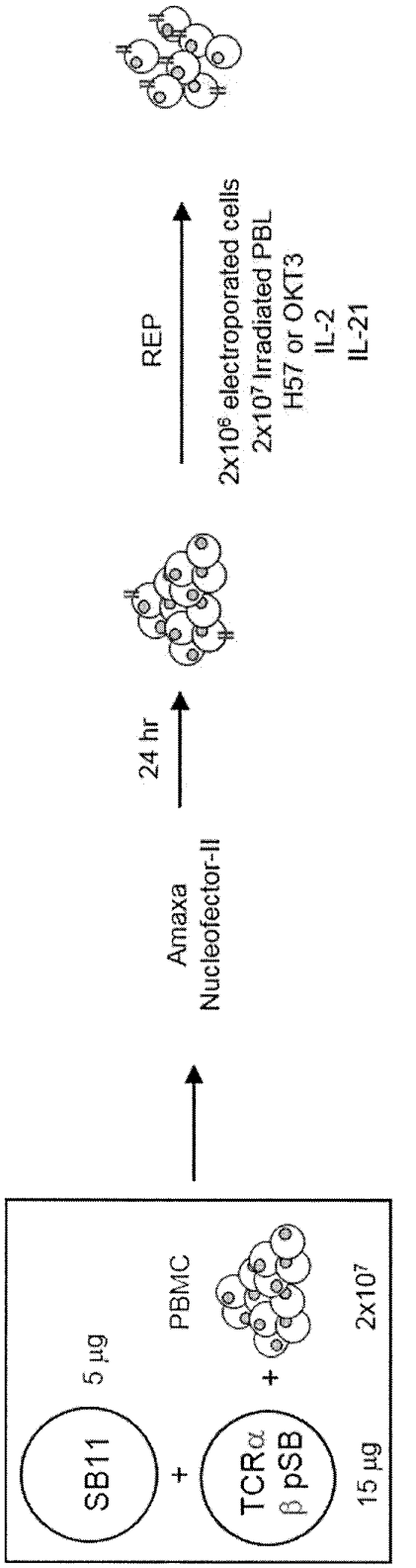

SB11
5 µg
+
TCRα
β pSB
15 µg
PBMC
+
2x10^7
Amaxa
Nucleofector-II
24 hr
REP
2x10^6 electroporated cells
2x10^7 Irradiated PBL
H57 or OKT3
IL-2
IL-21

FIG. 1B

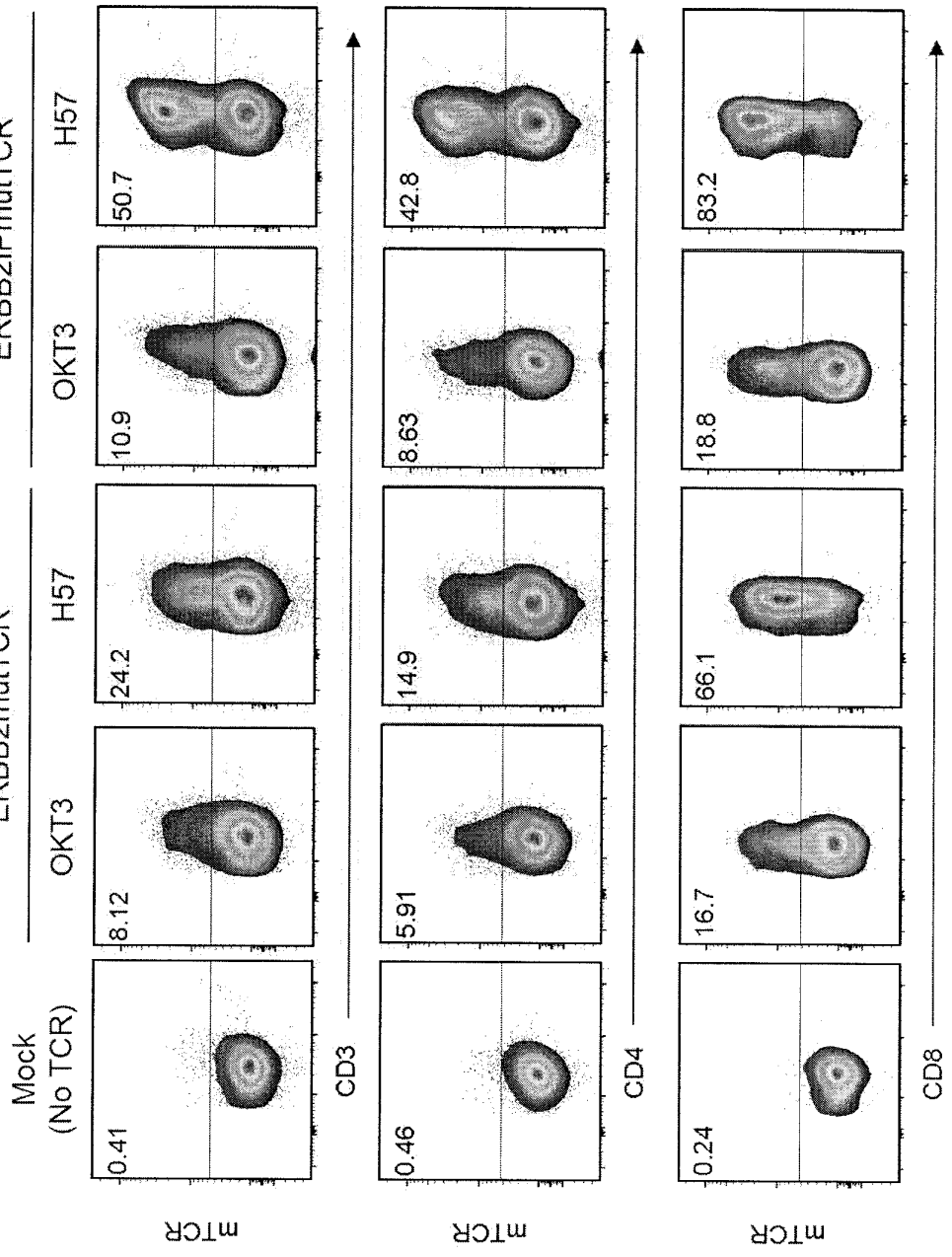

Mock
(No TCR)
ERBB2mutTCR
OKT3
H57
ERBB2IPmutTCR
OKT3
H57
0.41
8.12
24.2
10.9
50.7
0.46
5.91
14.9
8.63
42.8
0.24
16.7
66.1
18.8
83.2
mTCR
CD3
CD4
CD8

FIG. 2

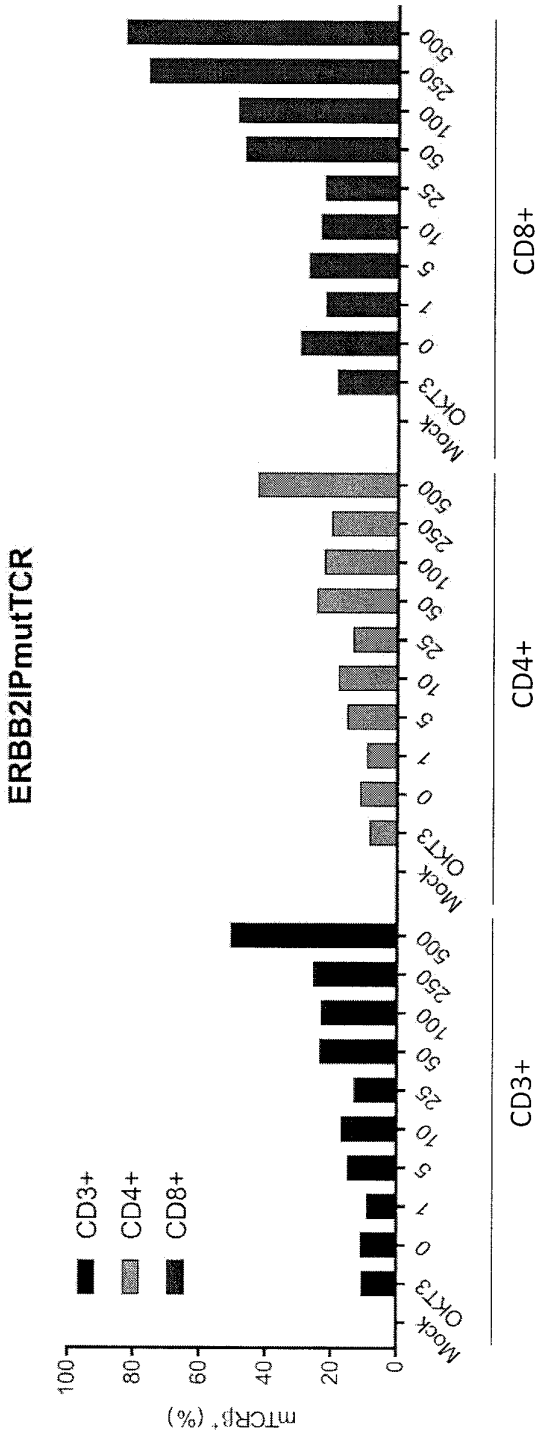

ERBB2IPmutTCR
CD3+
CD4+
CD8+
mTCRβ+ (%)
100
80
60
40
20
0
Mock
OKT3
0
1
5
10
25
50
100
250
500
CD3+
CD4+
CD8+
[H57], ng/mL

FIG. 3A

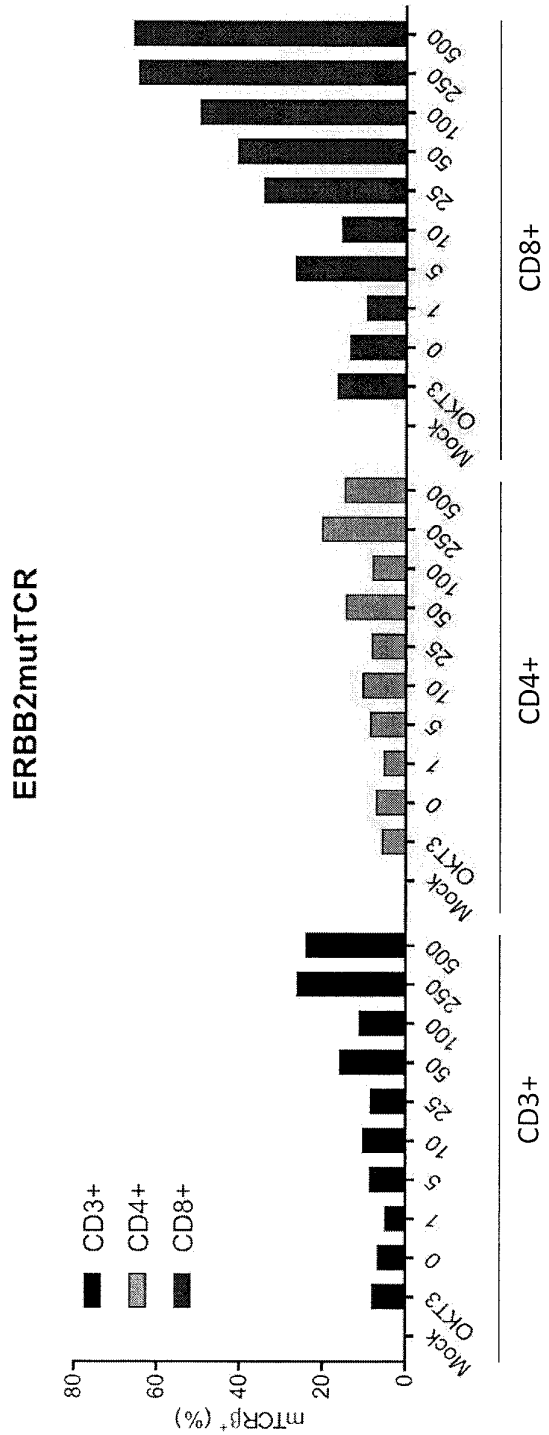

ERBB2mutTCR
CD3+
CD4+
CD8+
mTCRβ+ (%)
0
20
40
60
80
Mock
OKT3
0
1
5
10
25
50
100
250
500
CD3+
CD4+
CD8+
[H57], ng/mL

FIG. 3B

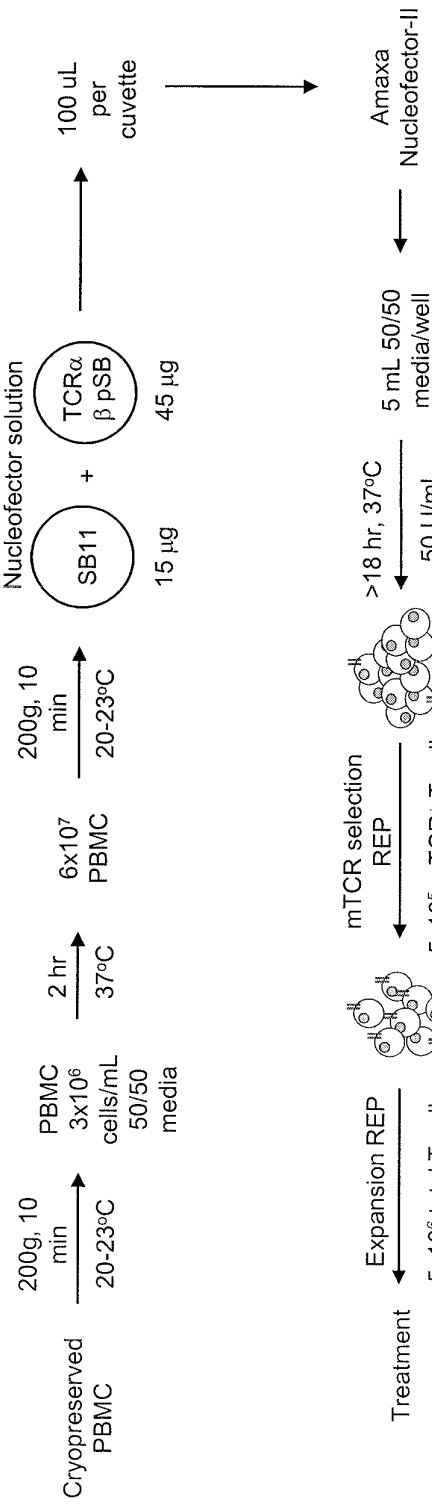

Cryopreserved PBMC
200g, 10 min
20-23°C
PBMC 3x10^6 cells/mL 50/50 media
2 hr
37°C
6x10^7 PBMC
200g, 10 min
20-23°C
300 uL Human T cell Nucleofector solution
SB11
15 µg
+
TCRα β pSB
45 µg
100 uL per cuvette
Amaxa Nucleofector-II
5 mL 50/50 media/well
>18 hr, 37°C
50 U/mL benzonase 1 hr, 37°C
mTCR selection REP
5x10^5 mTCR+ T cells
1x10^8 Irradiated PBL
250 ng/mL H57
50 IU/mL IL-2
30 ng/mL IL-21
Expansion REP
5x10^6 total T cells
5x10^8 Irradiated PBL
30 ng/mL OKT3
3000 IU/mL IL-2
Treatment

FIG. 4A

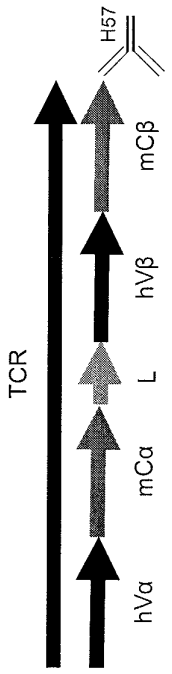


FIG. 4B

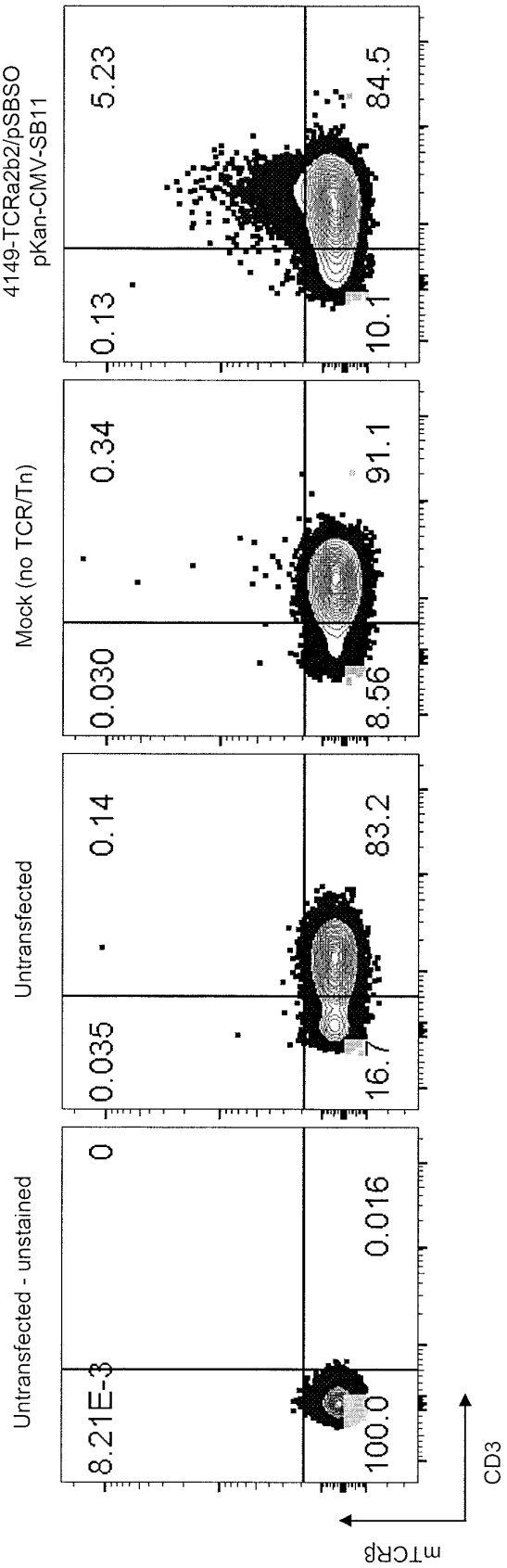


FIG. 5A

FIG. 5B

FIG. 5C

FIG. 5D

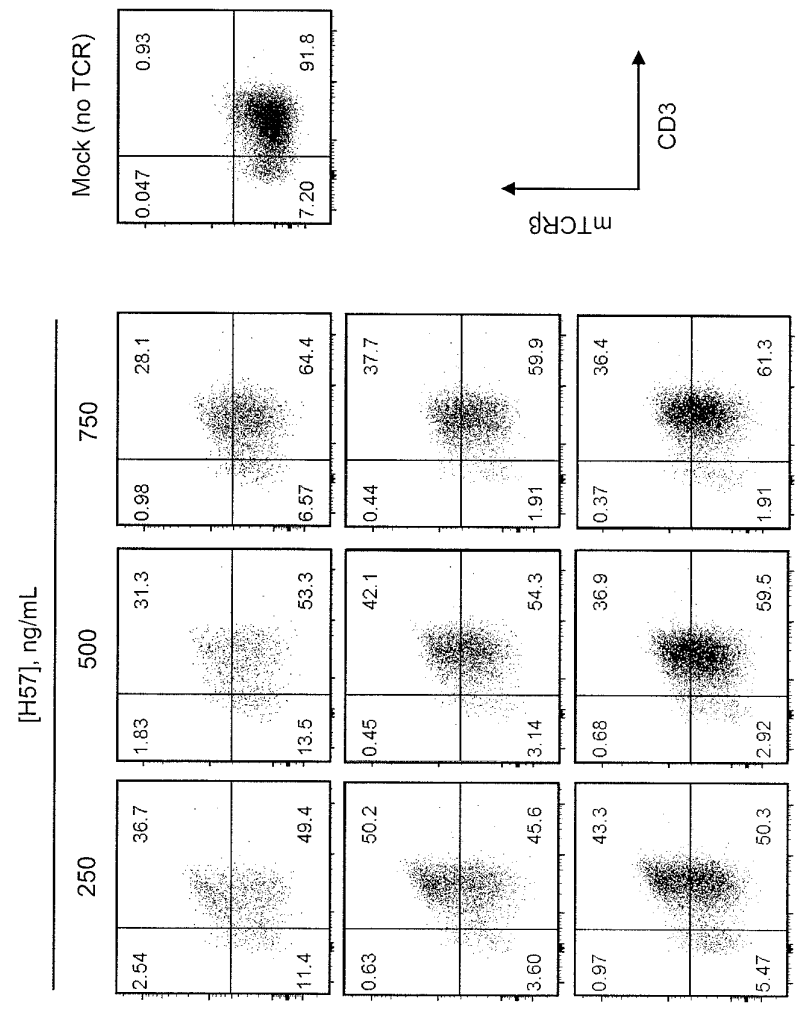

[H57], ng/mL
250
500
750
mTCR+ cells in REP
1x10^5
5x10^5
10x10^5
2.54
36.7
11.4
49.4
1.83
31.3
13.5
53.3
0.98
28.1
6.57
64.4
0.63
50.2
3.60
45.6
0.45
42.1
3.14
54.3
0.44
37.7
1.91
59.9
0.97
43.3
5.47
50.3
0.68
36.9
2.92
59.5
0.37
36.4
1.91
61.3
Mock (no TCR)
0.047
0.93
7.20
91.8
mTCRβ
CD3

FIG. 6

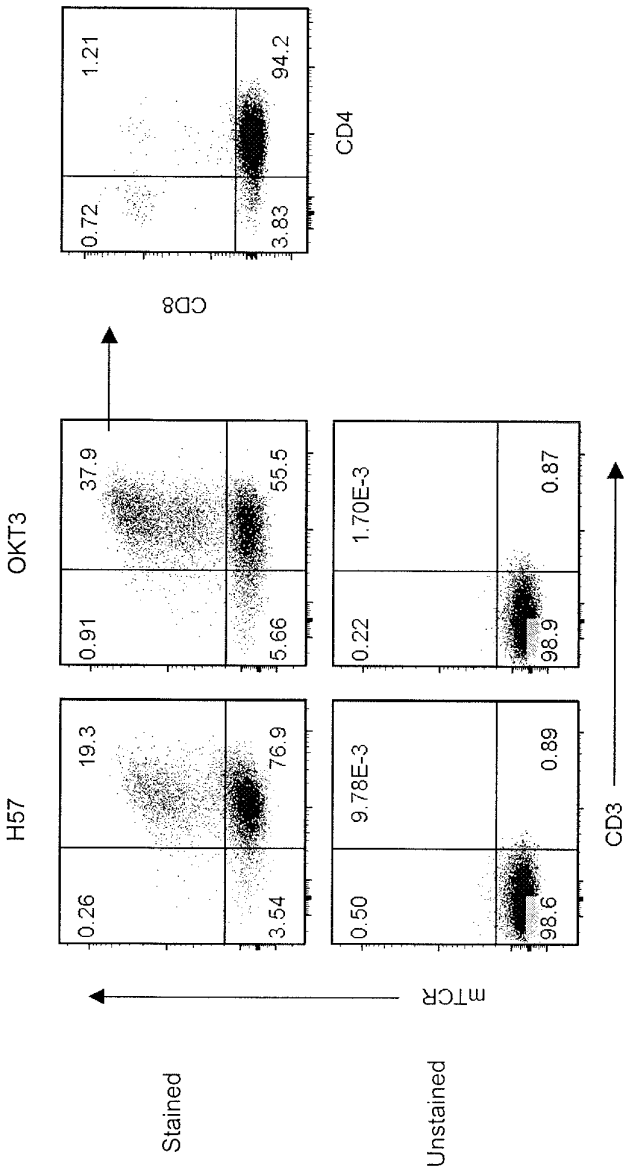

Stained
Unstained
mTCR
CD3
H57
OKT3
CD8
CD4
0.26
19.3
3.54
76.9
0.91
37.9
5.66
55.5
0.50
9.78E-3
98.6
0.89
0.22
1.70E-3
98.9
0.87
0.72
1.21
3.83
94.2

FIG. 7

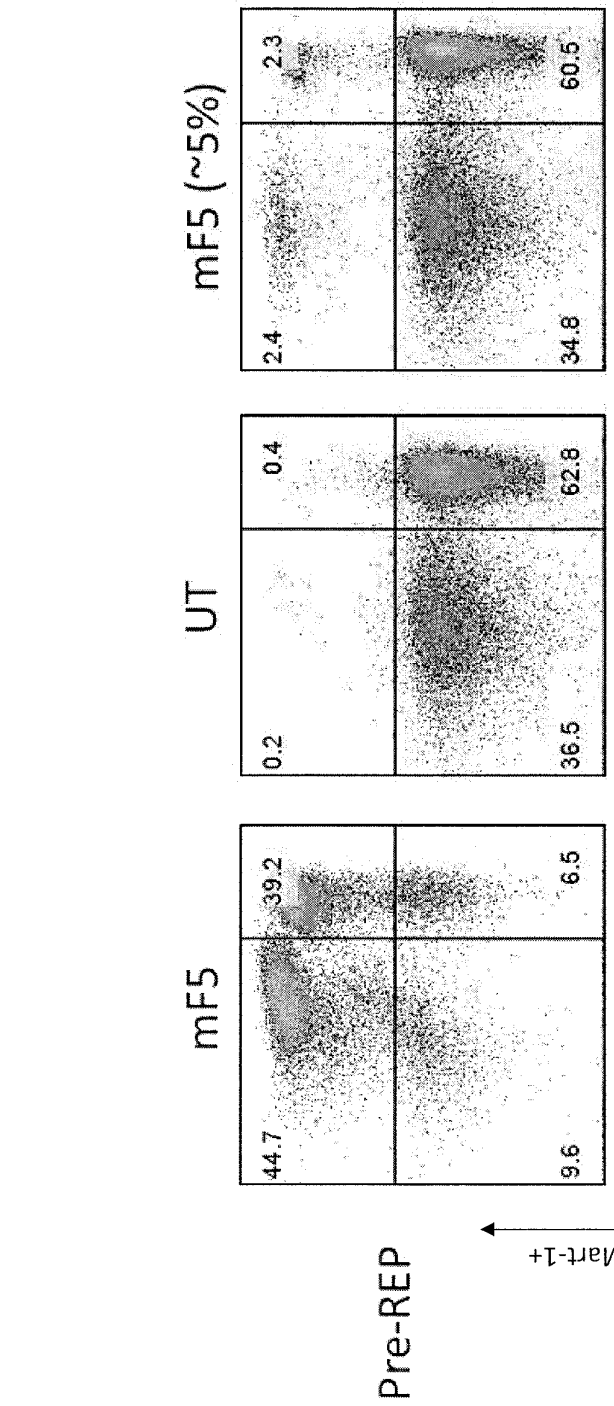


FIG. 8A

FIG. 8B

FIG. 8C

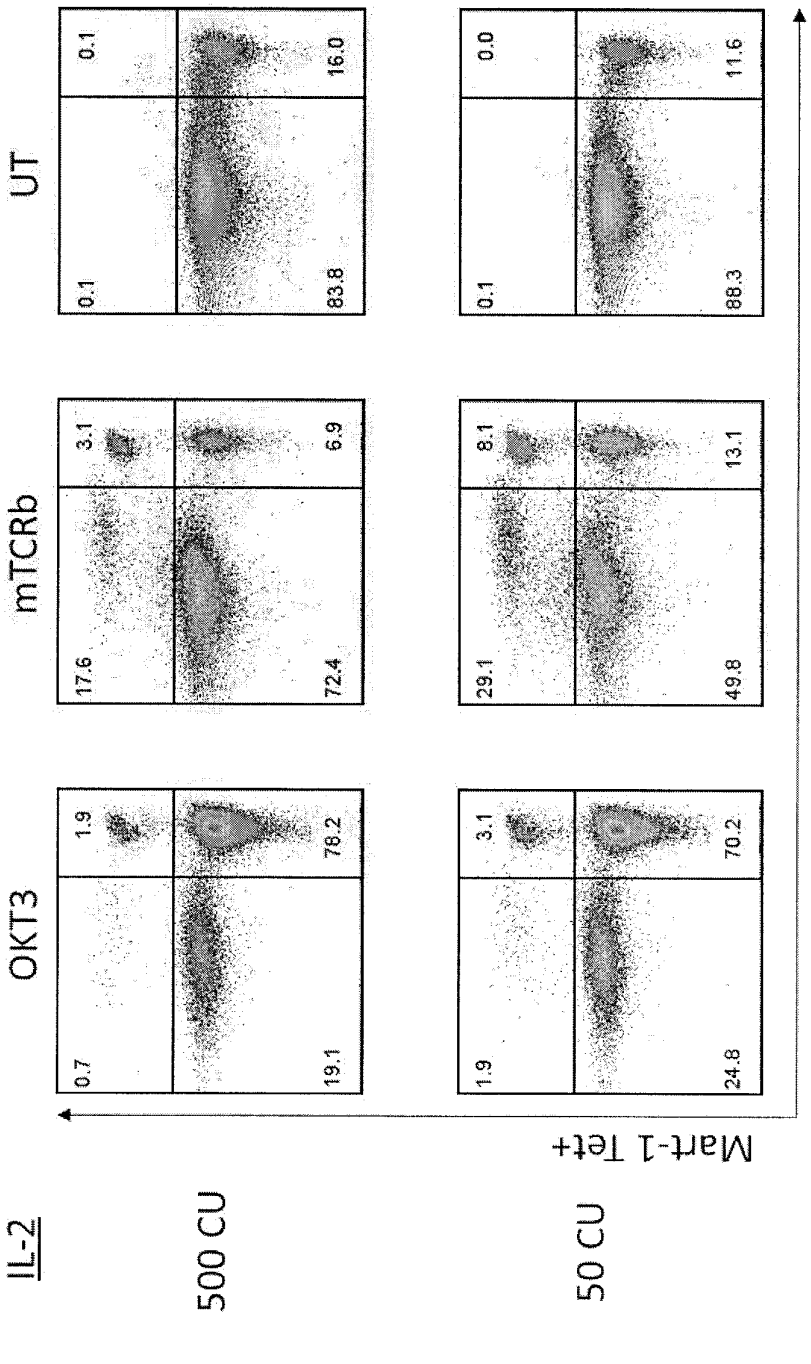

IL-2
500 CU
50 CU
OKT3
mTCRb
UT
0.7
1.9
19.1
78.2
17.6
3.1
72.4
6.9
0.1
0.1
83.8
16.0
1.9
3.1
24.8
70.2
29.1
8.1
49.8
13.1
0.1
0.0
88.3
11.6
Mart-1 Tet+
CD8

FIG. 9

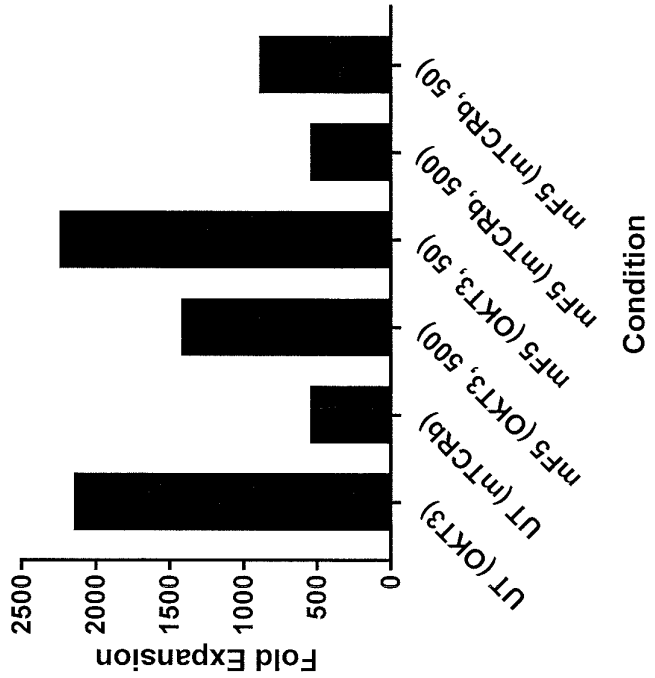


FIG. 10B

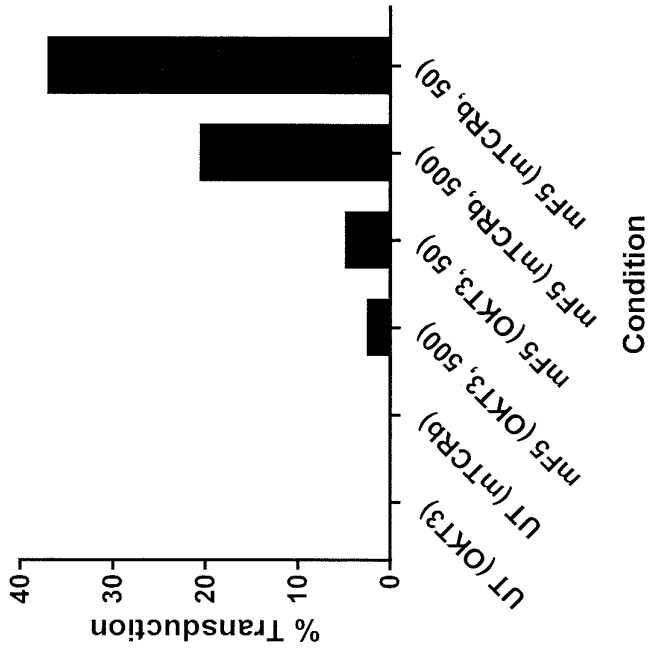


FIG. 10A

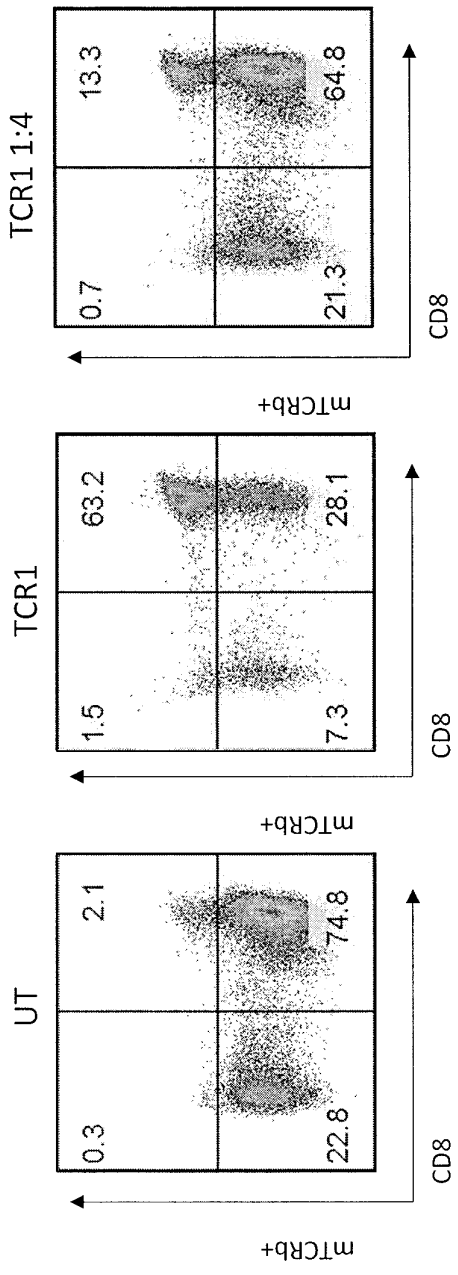


FIG. 11A

FIG. 11B

FIG. 11C

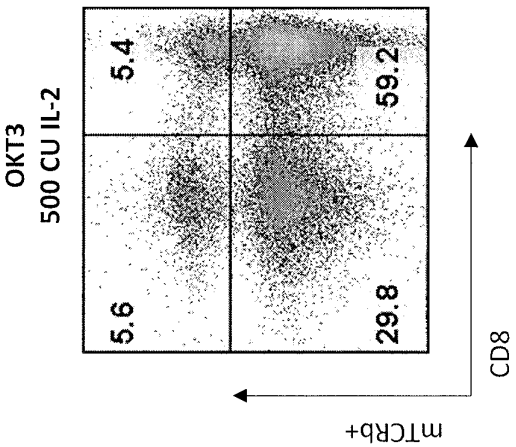


FIG. 12A

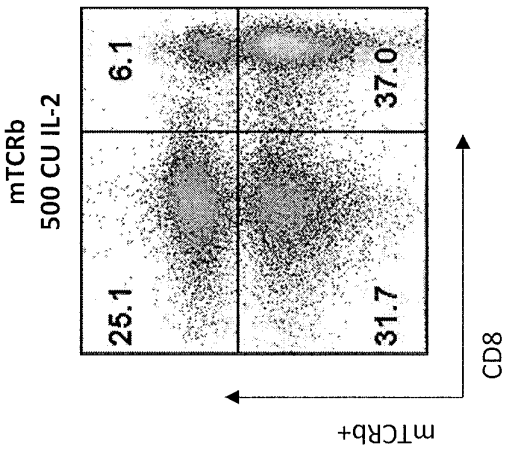


FIG. 12B



FIG. 12C

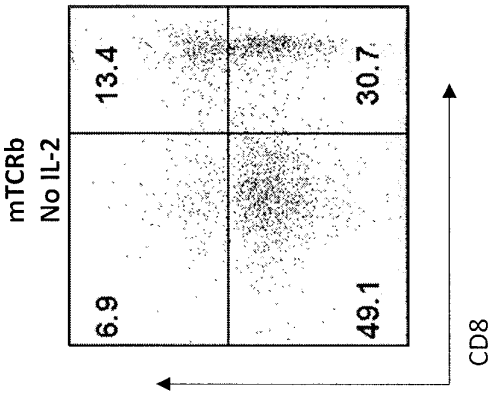


FIG. 12D

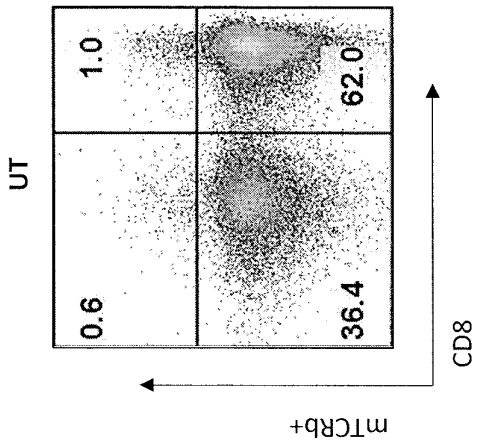


FIG. 12E

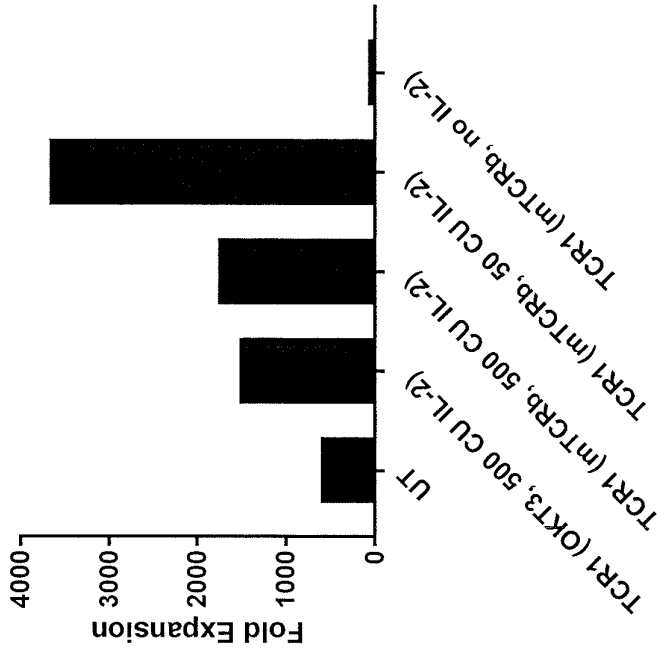


FIG. 13B

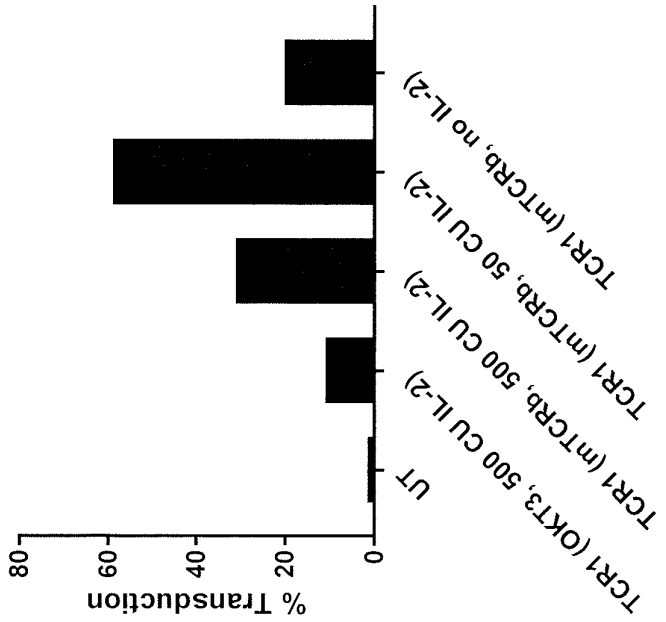


FIG. 13A

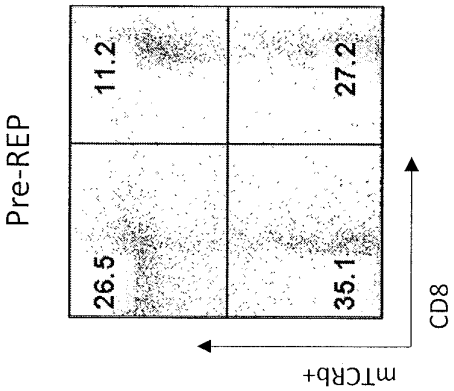
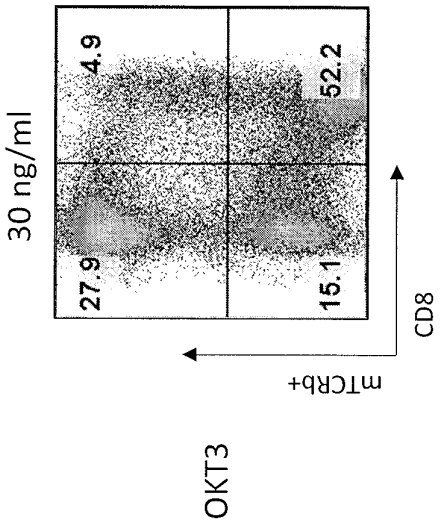


FIG. 14A



OKT3

FIG. 14B

FIG. 14C

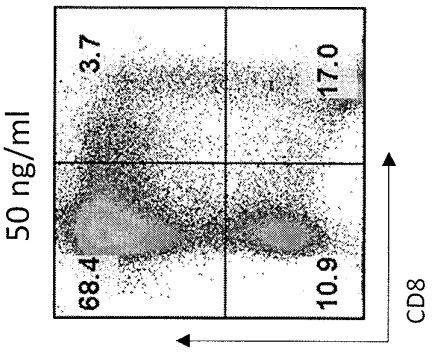


FIG. 14D

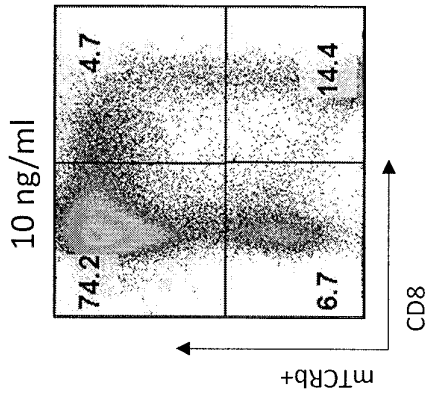


FIG. 14E

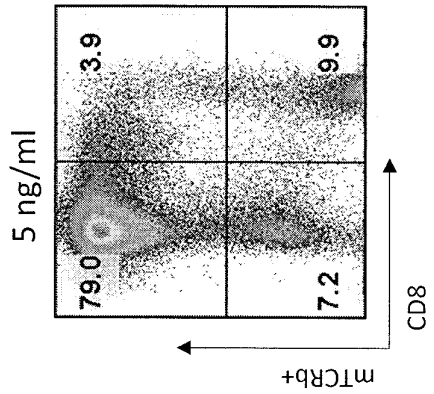


FIG. 14F

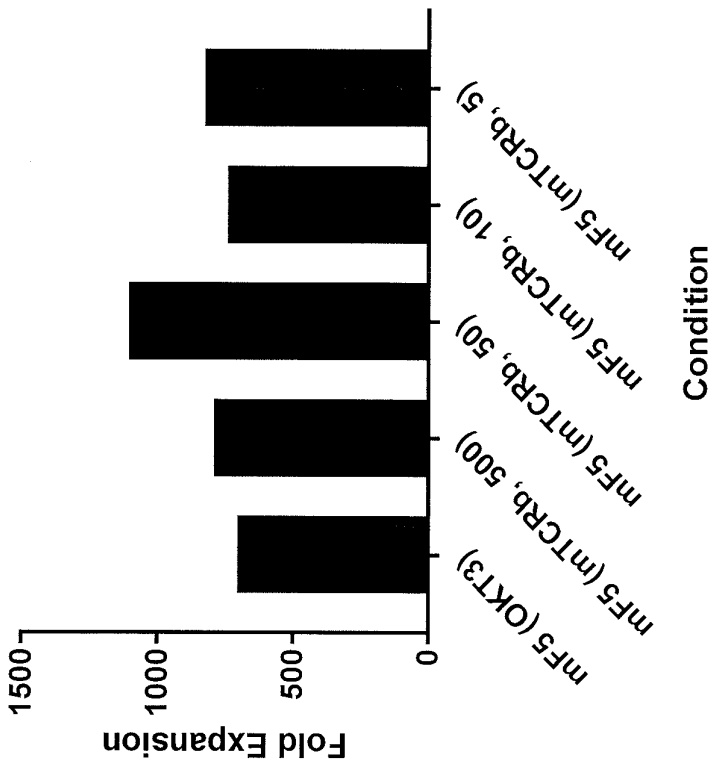
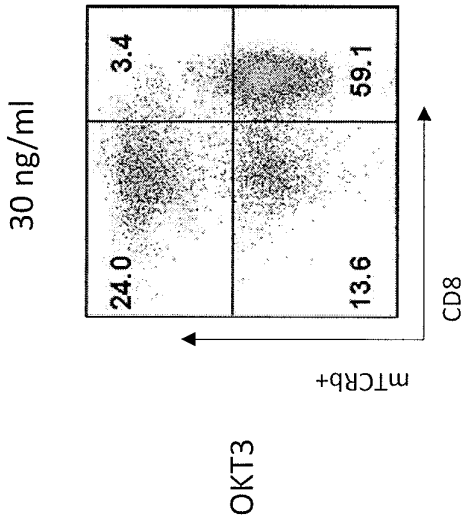

Fold Expansion
1500
1000
500
0
mF5 (OKT3)
mF5 (mTCRb, 500)
mF5 (mTCRb, 50)
mF5 (mTCRb, 10)
mF5 (mTCRb, 5)
Condition

FIG. 15



OKT3

FIG. 16A

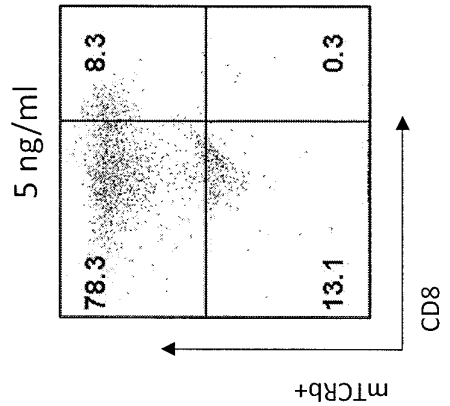


FIG. 16E

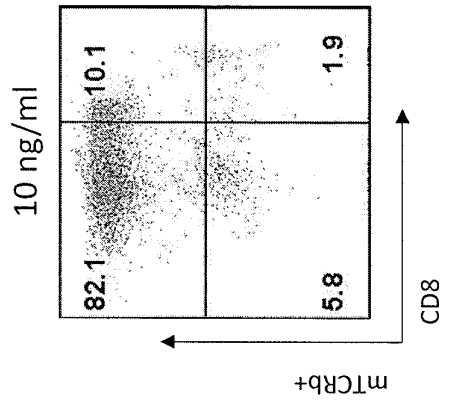


FIG. 16D

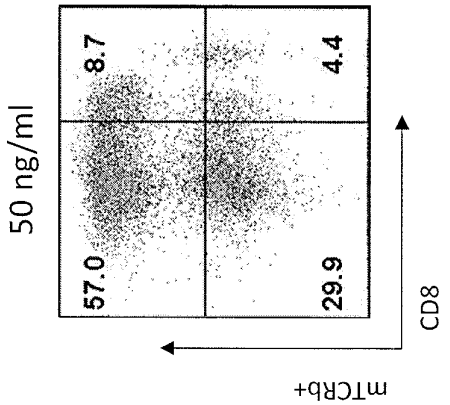


FIG. 16C

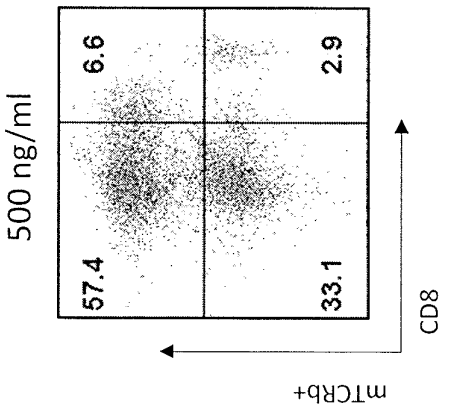


FIG. 16B

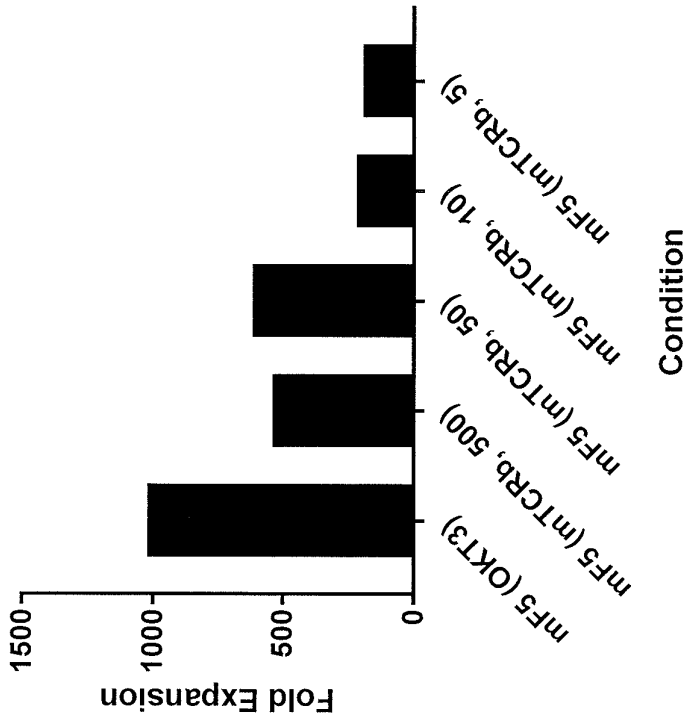

Fold Expansion
1500
1000
500
0
mF5 (OKT3)
mF5 (mTCRb, 500)
mF5 (mTCRb, 50)
mF5 (mTCRb, 10)
mF5 (mTCRb, 5)
Condition

FIG. 17

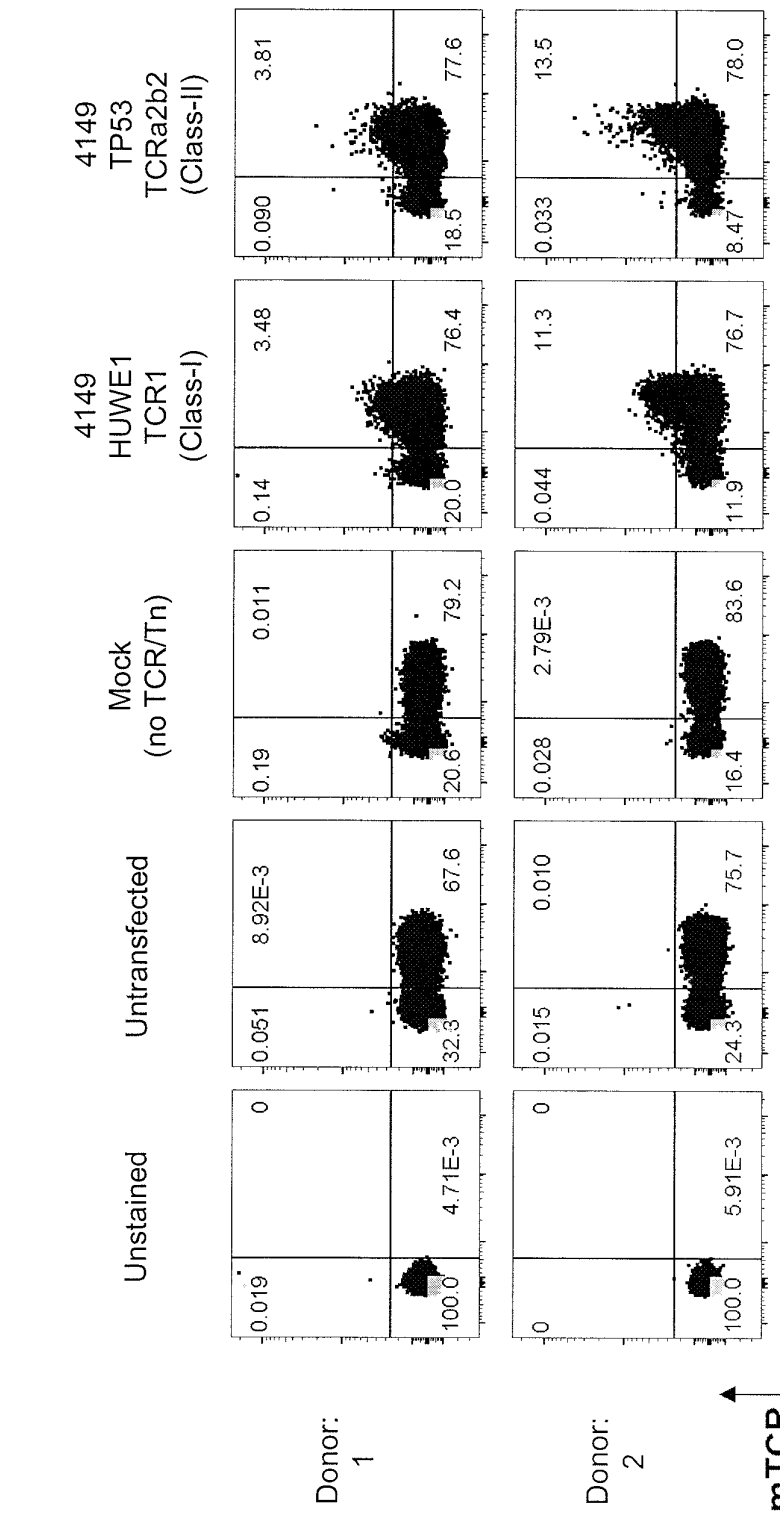

Unstained
Untransfected
Mock
(no TCR/Tn)
4149
HUWE1
TCR1
(Class-I)
4149
TP53
TCRa2b2
(Class-II)
Donor:
1
Donor:
2
mTCR
CD3
0.019
0
100.0
4.71E-3
0.051
8.92E-3
32.3
67.6
0.19
0.011
20.6
79.2
0.14
3.48
20.0
76.4
0.090
3.81
18.5
77.6
0
0
100.0
5.91E-3
0.015
0.010
24.3
75.7
0.028
2.79E-3
16.4
83.6
0.044
11.3
11.9
76.7
0.033
13.5
8.47
78.0

FIG. 18

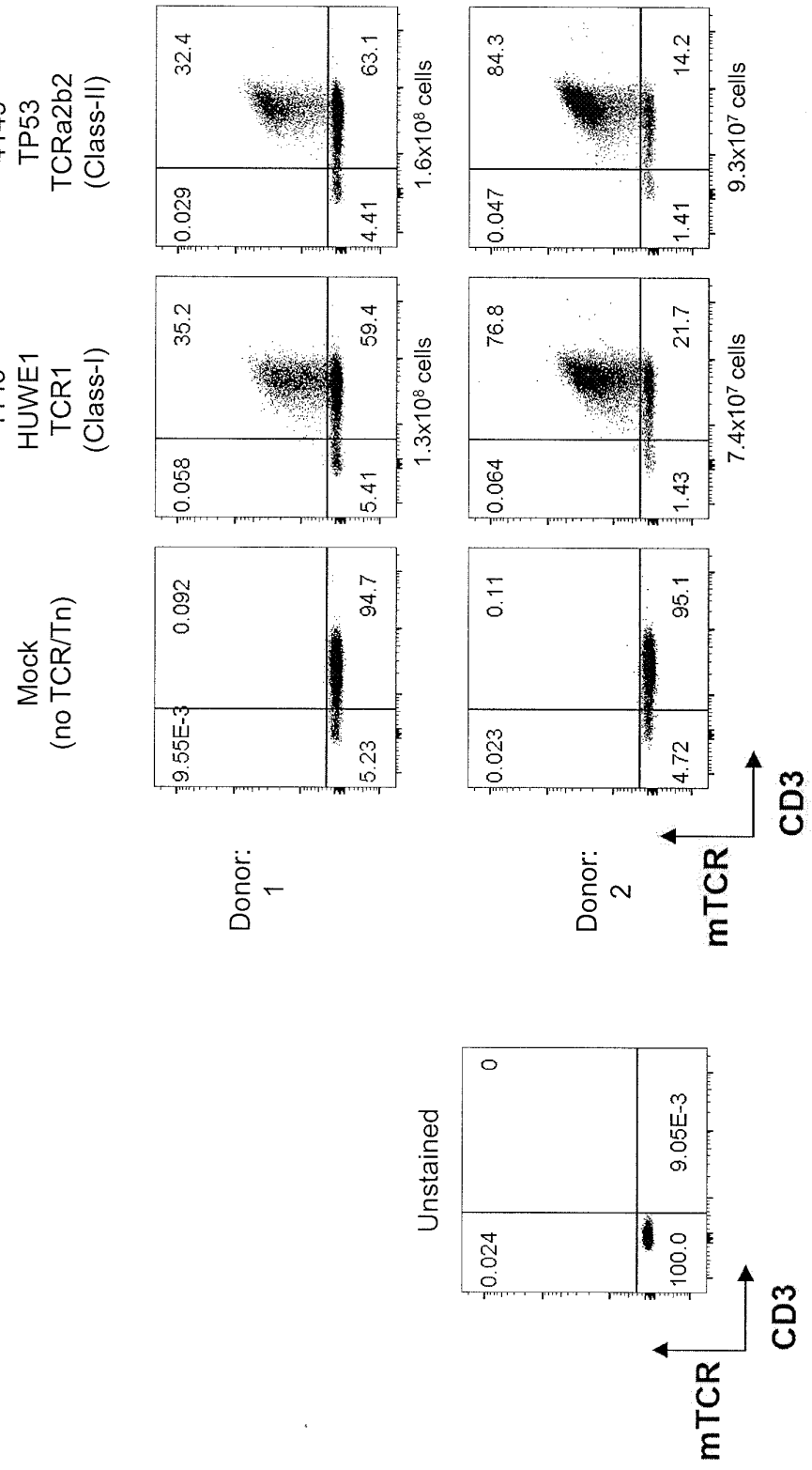


FIG. 19B

FIG. 19A

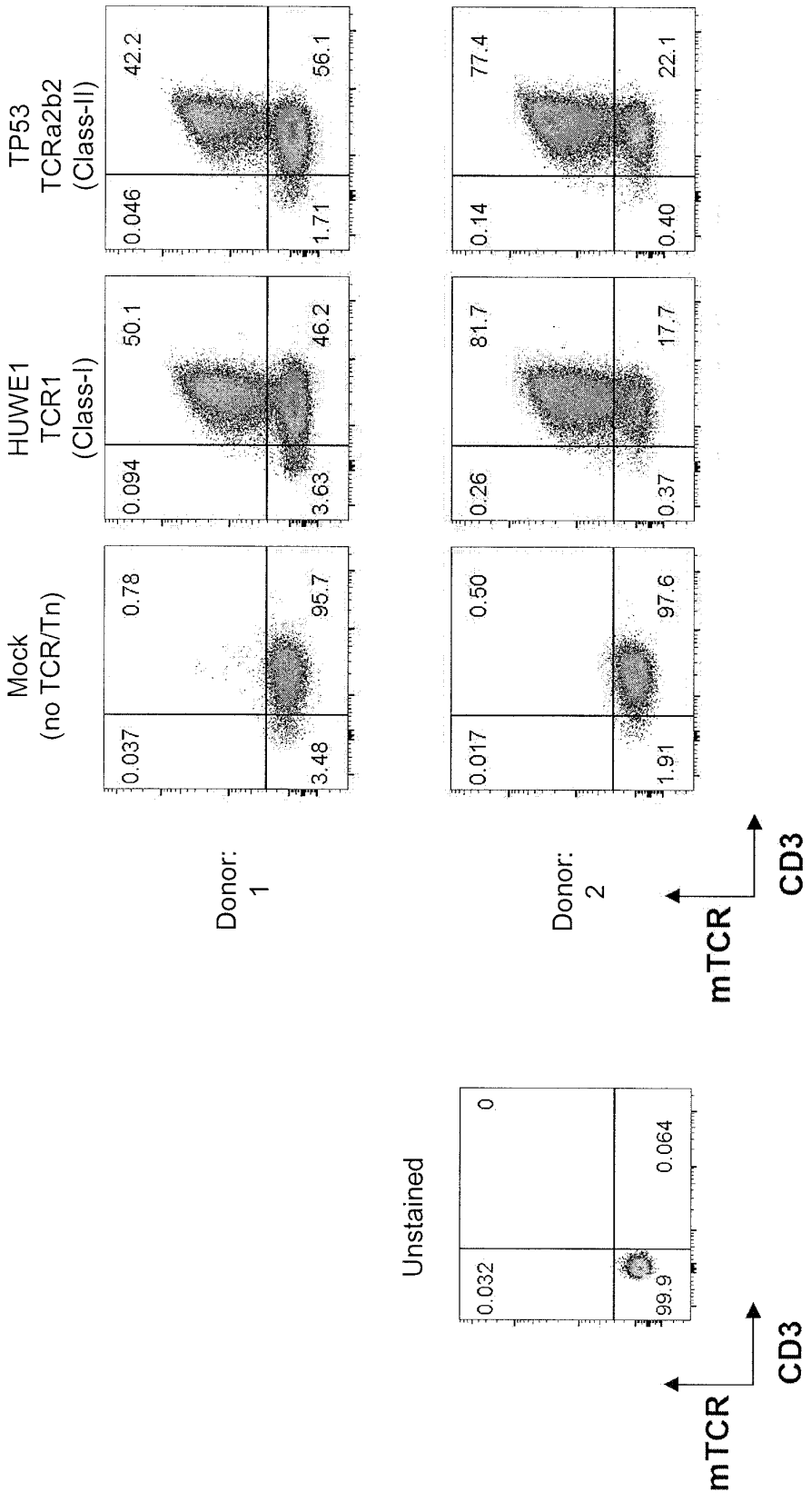


FIG. 20B

FIG. 20A

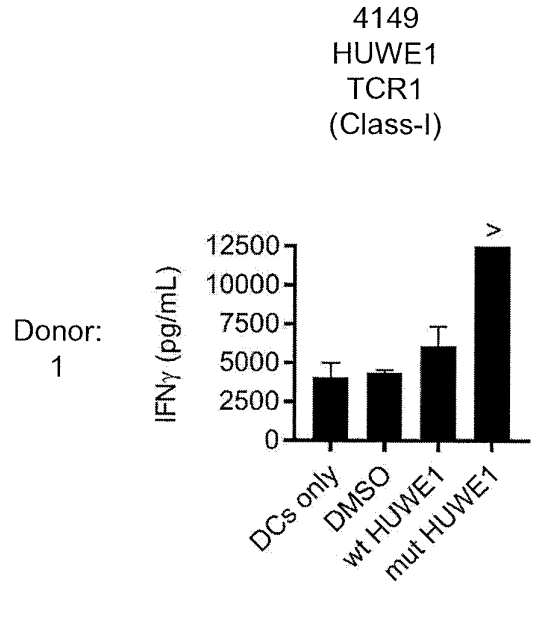


FIG. 21A

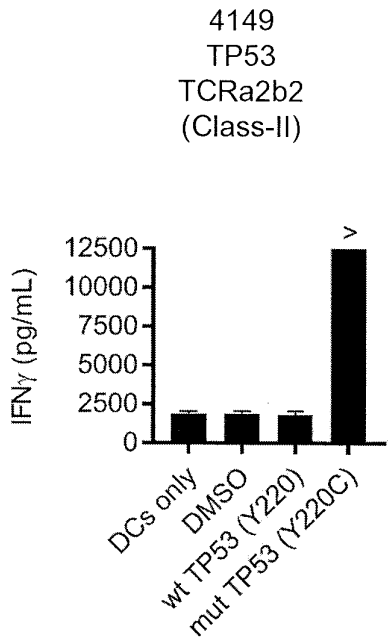


FIG. 21B

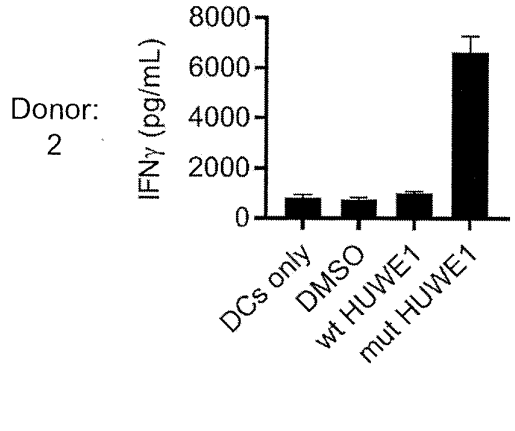


FIG. 21C

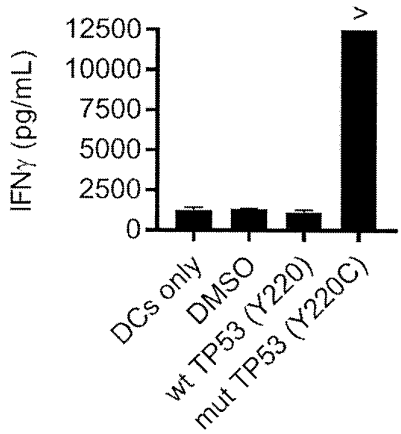


FIG. 21D

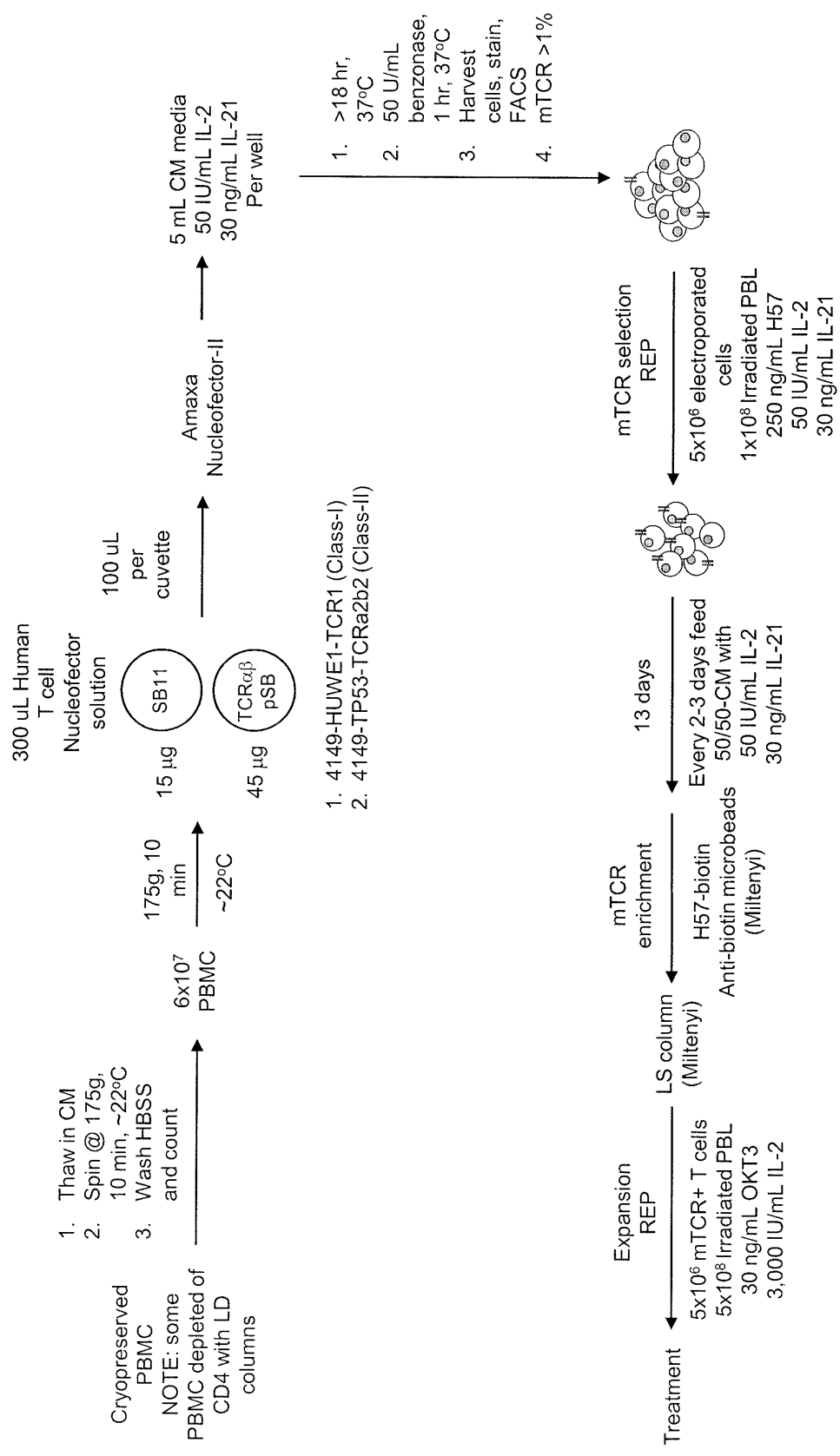

Cryopreserved PBMC
NOTE: some PBMC depleted of CD4 with LD columns
1. Thaw in CM
2. Spin @ 175g, 10 min, ~22°C
3. Wash HBSS and count
6x10^7 PBMC
175g, 10 min
~22°C
300 uL Human T cell Nucleofector solution
15 µg
SB11
45 µg
TCRαβ pSB
1. 4149-HUWE1-TCR1 (Class-I)
2. 4149-TP53-TCRa2b2 (Class-II)
100 uL per cuvette
Amaxa Nucleofector-II
5 mL CM media
50 IU/mL IL-2
30 ng/mL IL-21
Per well
1. >18 hr, 37°C
2. 50 U/mL benzonase, 1 hr, 37°C
3. Harvest cells, stain, FACS
4. mTCR >1%
mTCR selection REP
5x10^6 electroporated cells
1x10^8 Irradiated PBL
250 ng/mL H57
50 IU/mL IL-2
30 ng/mL IL-21
13 days
Every 2-3 days feed
50/50-CM with
50 IU/mL IL-2
30 ng/mL IL-21
mTCR enrichment
H57-biotin
Anti-biotin microbeads
(Miltenyi)
LS column (Miltenyi)
Expansion REP
5x10^6 mTCR+ T cells
5x10^8 Irradiated PBL
30 ng/mL OKT3
3,000 IU/mL IL-2
Treatment

FIG. 22

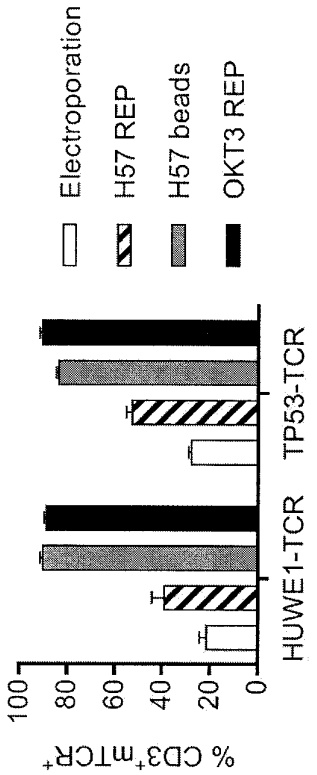


FIG. 23B

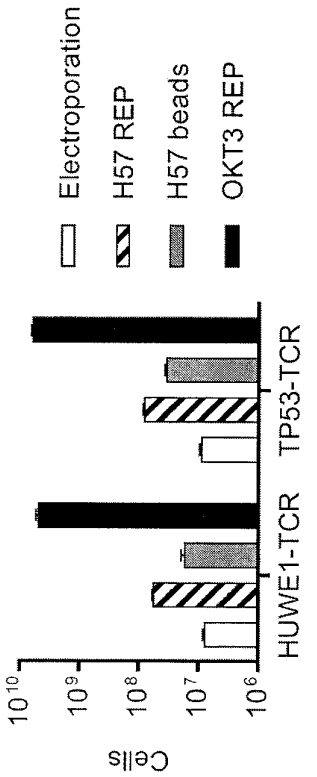


FIG. 23A



FIG. 23E

FIG. 23C

FIG. 23D

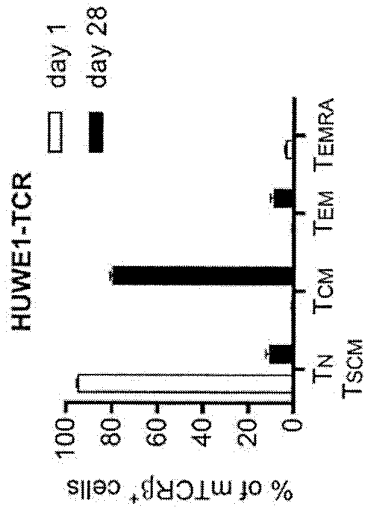


FIG. 24B

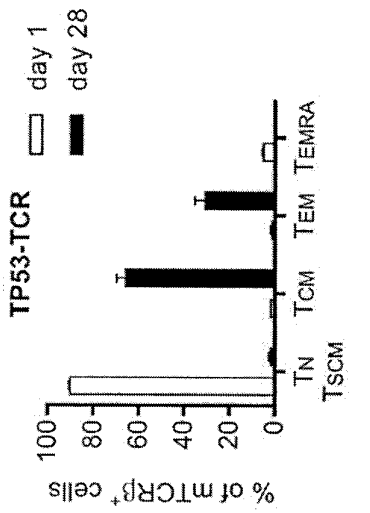


FIG. 24D

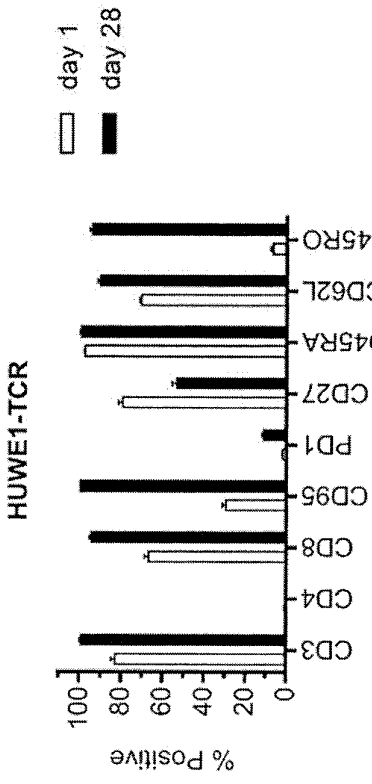


FIG. 24A

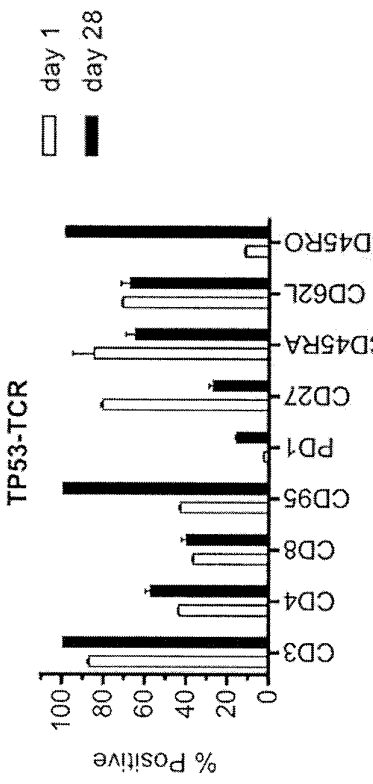


FIG. 24C

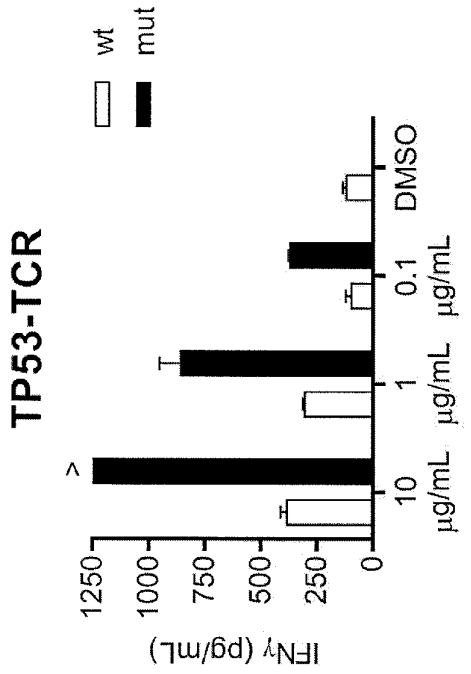


FIG. 25B

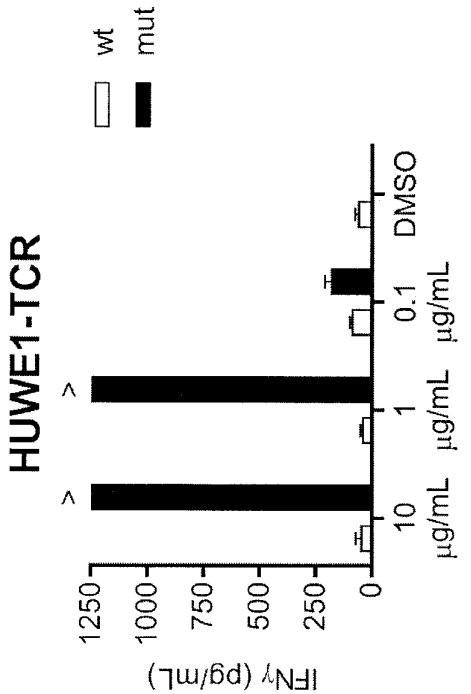


FIG. 25A