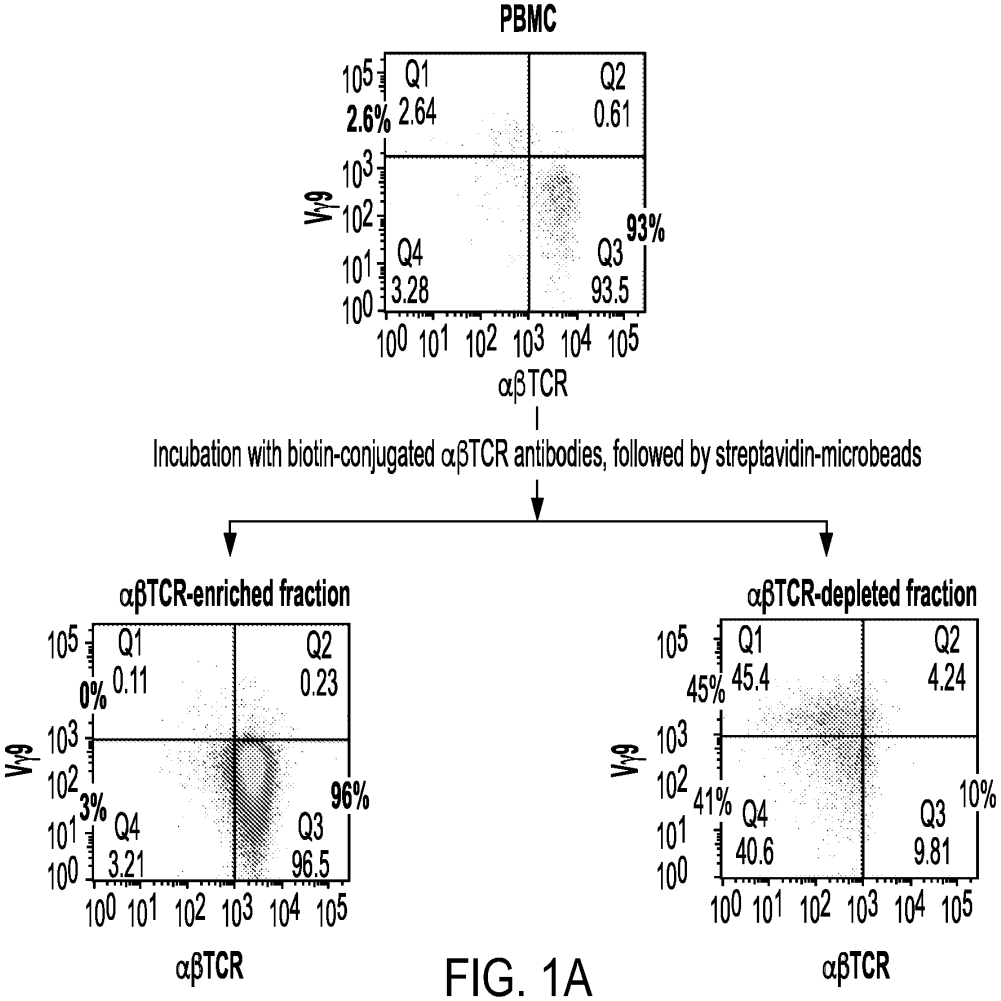

PBMC
Q1
2.64
Q2
0.61
2.6%
Q4
3.28
Q3
93.5
93%
Vγ9
αβTCR
Incubation with biotin-conjugated αβTCR antibodies, followed by streptavidin-microbeads
αβTCR-enriched fraction
Q1
0.11
Q2
0.23
0%
Q4
3.21
Q3
96.5
3%
96%
αβTCR
αβTCR-depleted fraction
Q1
45.4
Q2
4.24
45%
Q4
40.6
Q3
9.81
41%
10%
αβTCR

FIG. 1A

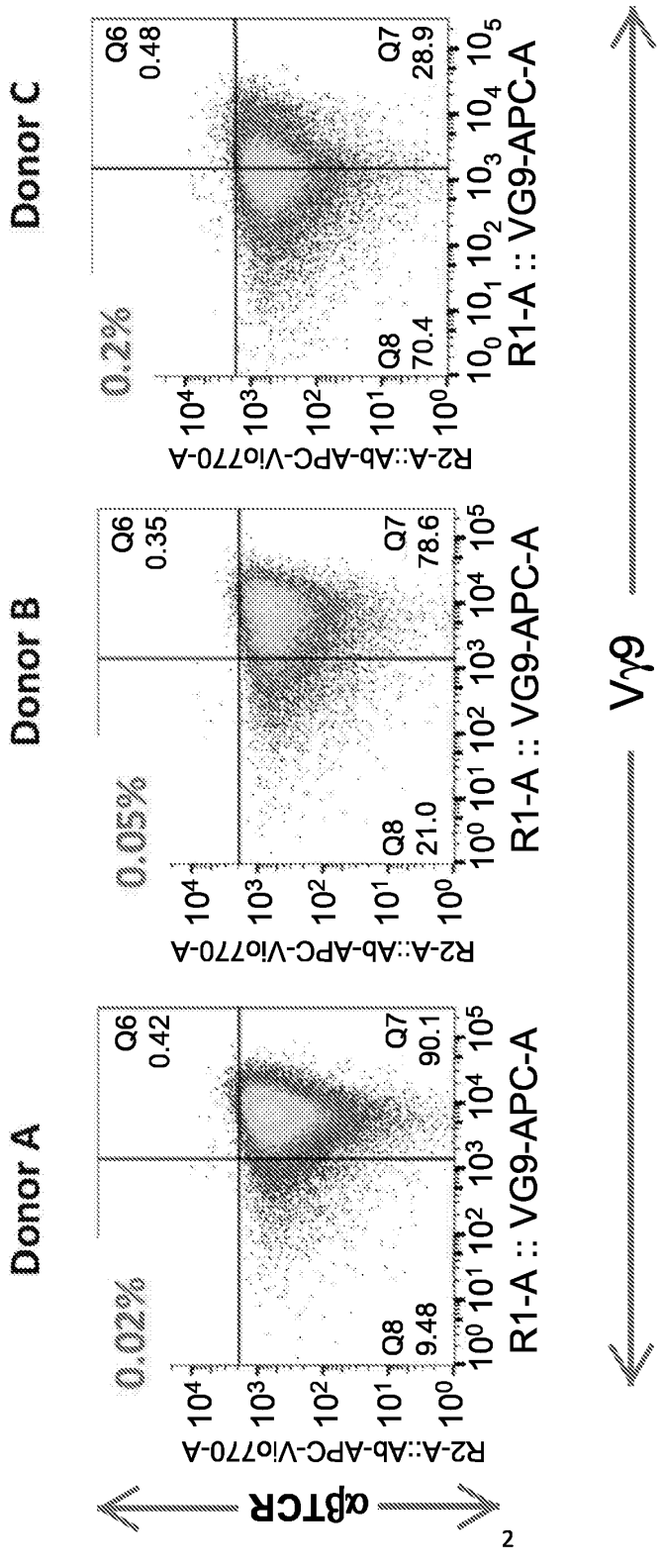

Donor A
Donor B
Donor C
αβTCR
Vγ9
R2-A::Ab-APC-Vio770-A
R1-A :: VG9-APC-A
0.02%
0.05%
0.2%
Q6
0.42
Q7
90.1
Q8
9.48
Q6
0.35
Q7
78.6
Q8
21.0
Q6
0.48
Q7
28.9
Q8
70.4
2

FIG. 1B

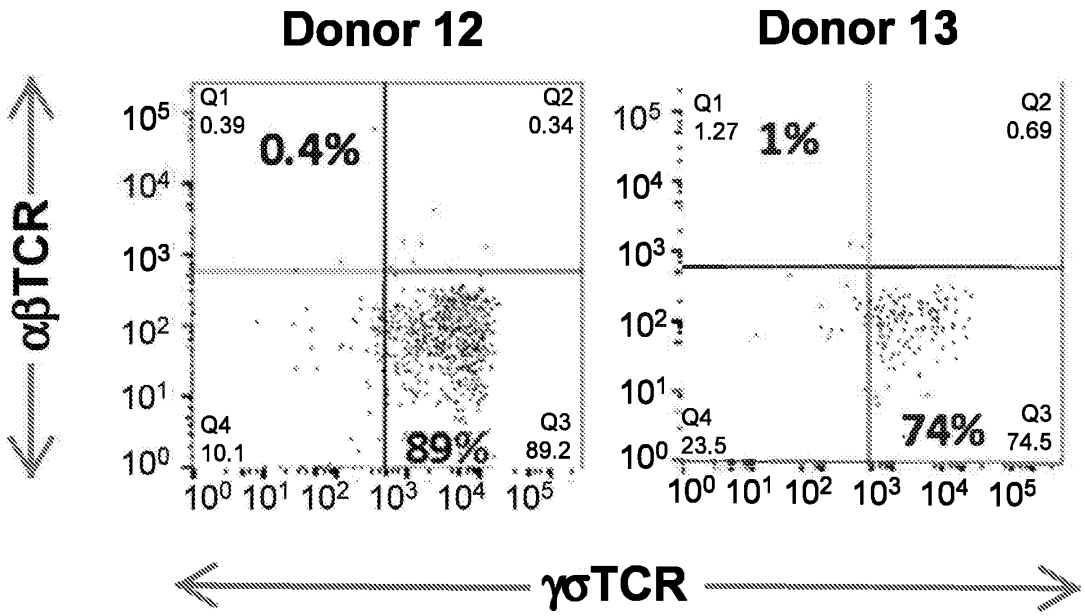

Donor 12
Donor 13
αβTCR
γσTCR
Q1
0.39
0.4%
Q2
0.34
Q4
10.1
89%
Q3
89.2
Q1
1.27
1%
Q2
0.69
Q4
23.5
74%
Q3
74.5
10^0 10^1 10^2 10^3 10^4 10^5

FIG. 1C

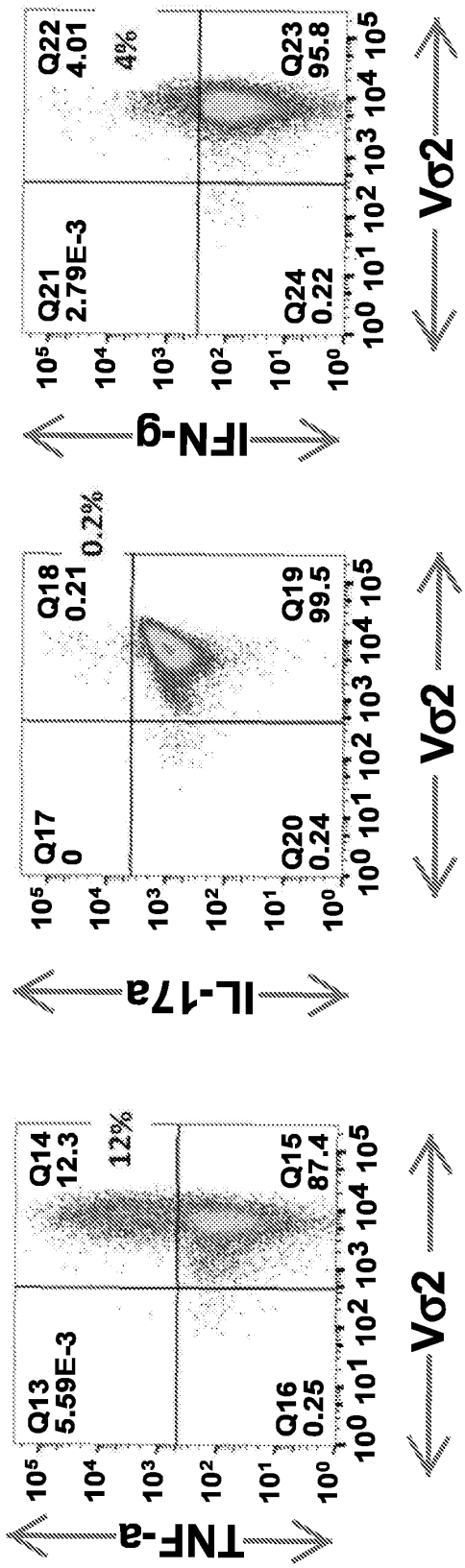

TNF-a
IL-17a
IFN-g
Vσ2
Q13 5.59E-3
Q14 12.3
12%
Q15 87.4
Q16 0.25
Q17 0
Q18 0.21
0.2%
Q19 99.5
Q20 0.24
Q21 2.79E-3
Q22 4.01
4%
Q23 95.8
Q24 0.22

FIG. 1D

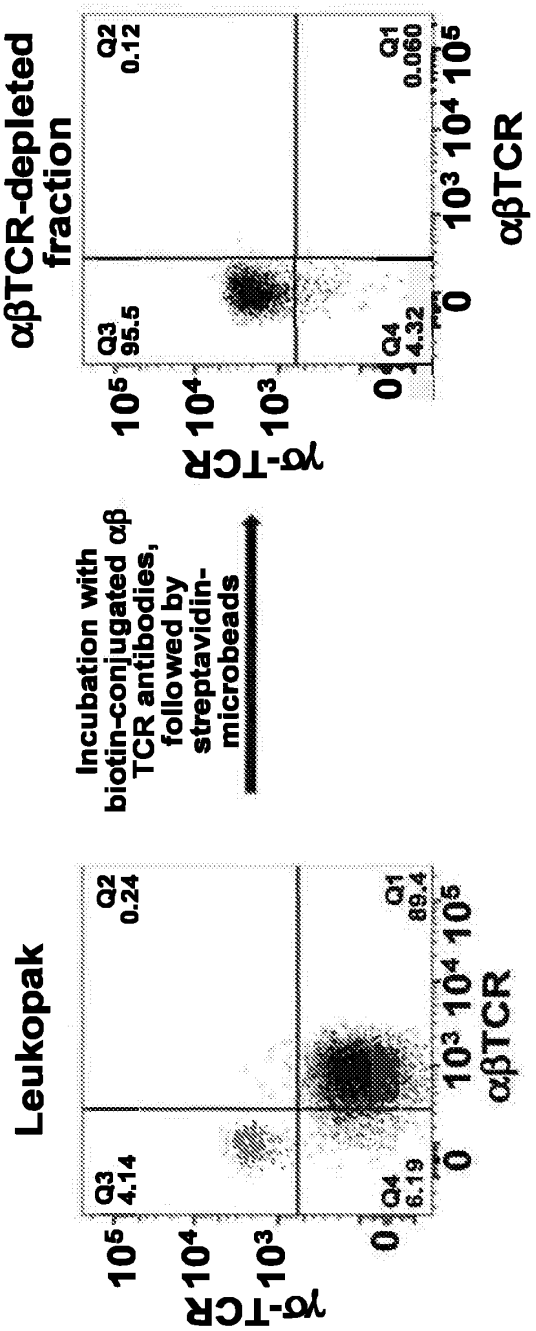

Leukopak
γδ-TCR
αβTCR
Q2 0.24
Q1 89.4
Q3 4.14
Q4 6.19
Incubation with biotin-conjugated αβ TCR antibodies, followed by streptavidin-microbeads
αβTCR-depleted fraction
Q2 0.12
Q1 0.060
Q3 95.5
Q4 4.32

FIG. 1E

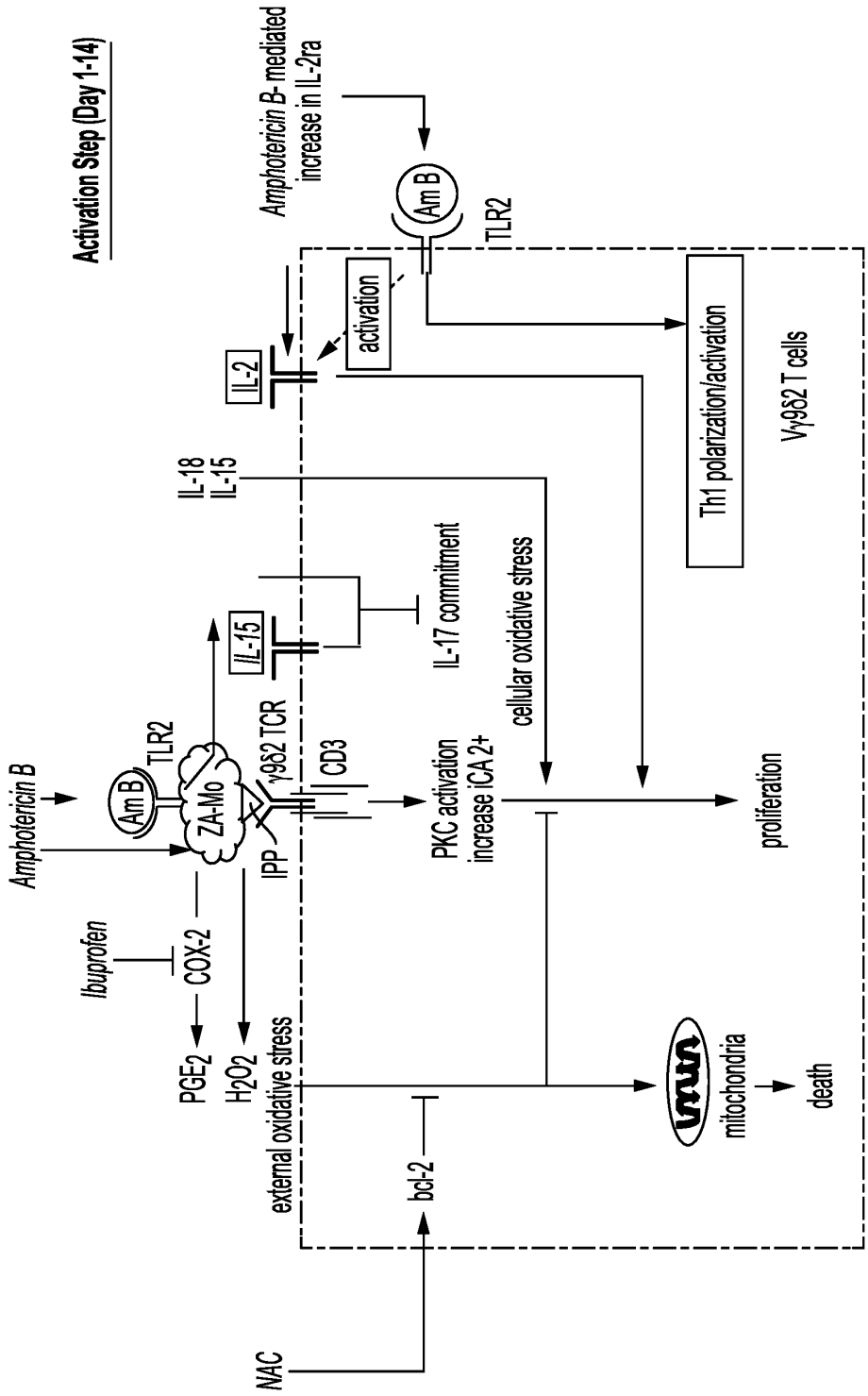

Activation Step (Day 1-14)
Amphotericin B- mediated increase in IL-2ra
Am B
TLR2
activation
IL-2
IL-18
IL-15
Th1 polarization/activation
Vγ9δ2 T cells
IL-17 commitment
cellular oxidative stress
IL-15
Amphotericin B
Am B
TLR2
ZA-Mo
γ9δ2 TCR
CD3
IPP
PKC activation increase iCA 2+
proliferation
Ibuprofen
COX-2
PGE2
H2O2
external oxidative stress
mitochondria
death
bcl-2
NAC

FIG. 2

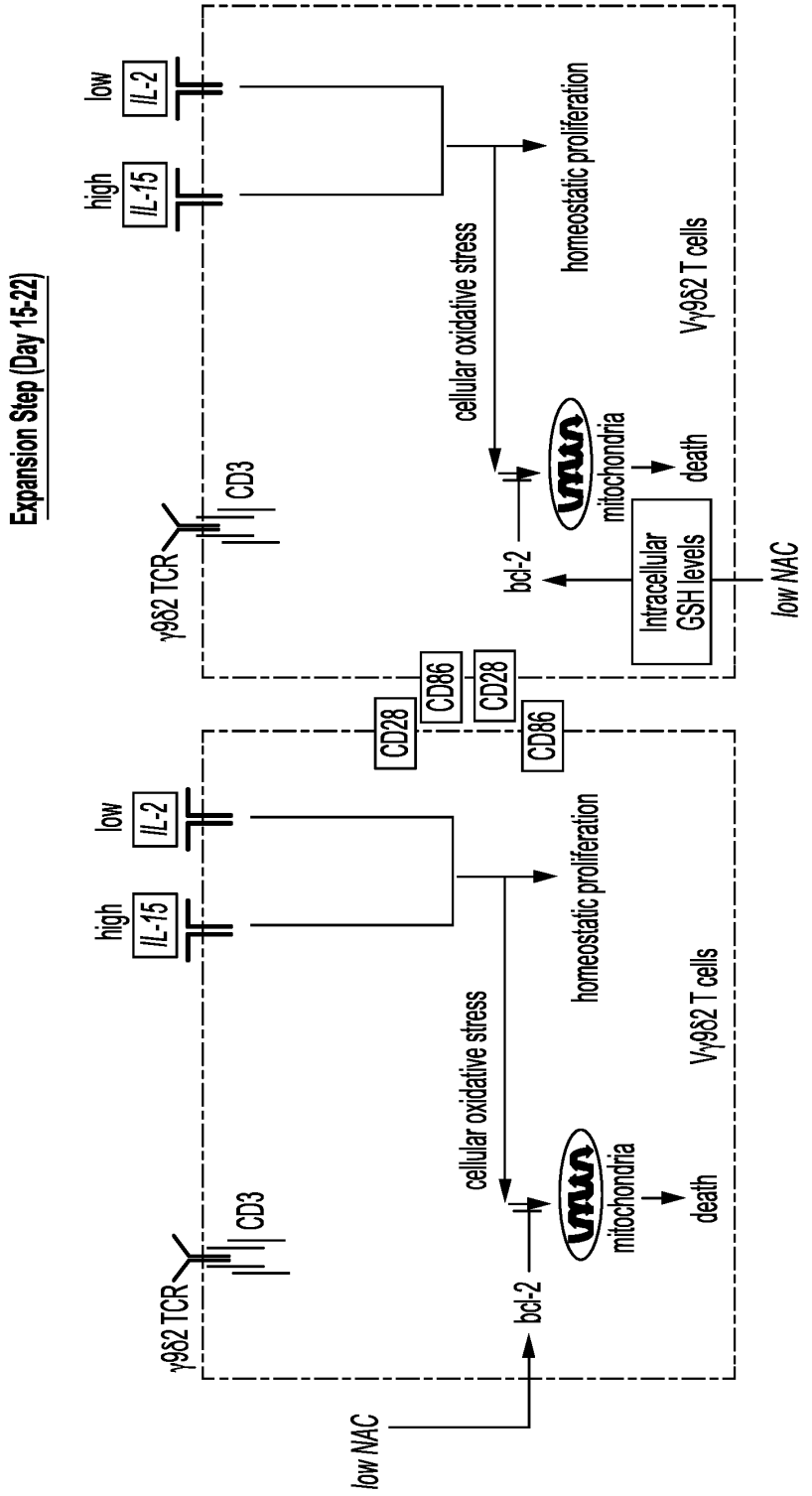

Expansion Step (Day 15-22)
low
IL-2
high
IL-15
homeostatic proliferation
cellular oxidative stress
Vγ9δ2 T cells
γ9δ2 TCR
CD3
mitochondria
death
bcl-2
Intracellular GSH levels
low NAC
CD28
CD86
CD28
CD86
low
IL-2
high
IL-15
homeostatic proliferation
cellular oxidative stress
Vγ9δ2 T cells
γ9δ2 TCR
CD3
mitochondria
death
bcl-2
low NAC

FIG. 3

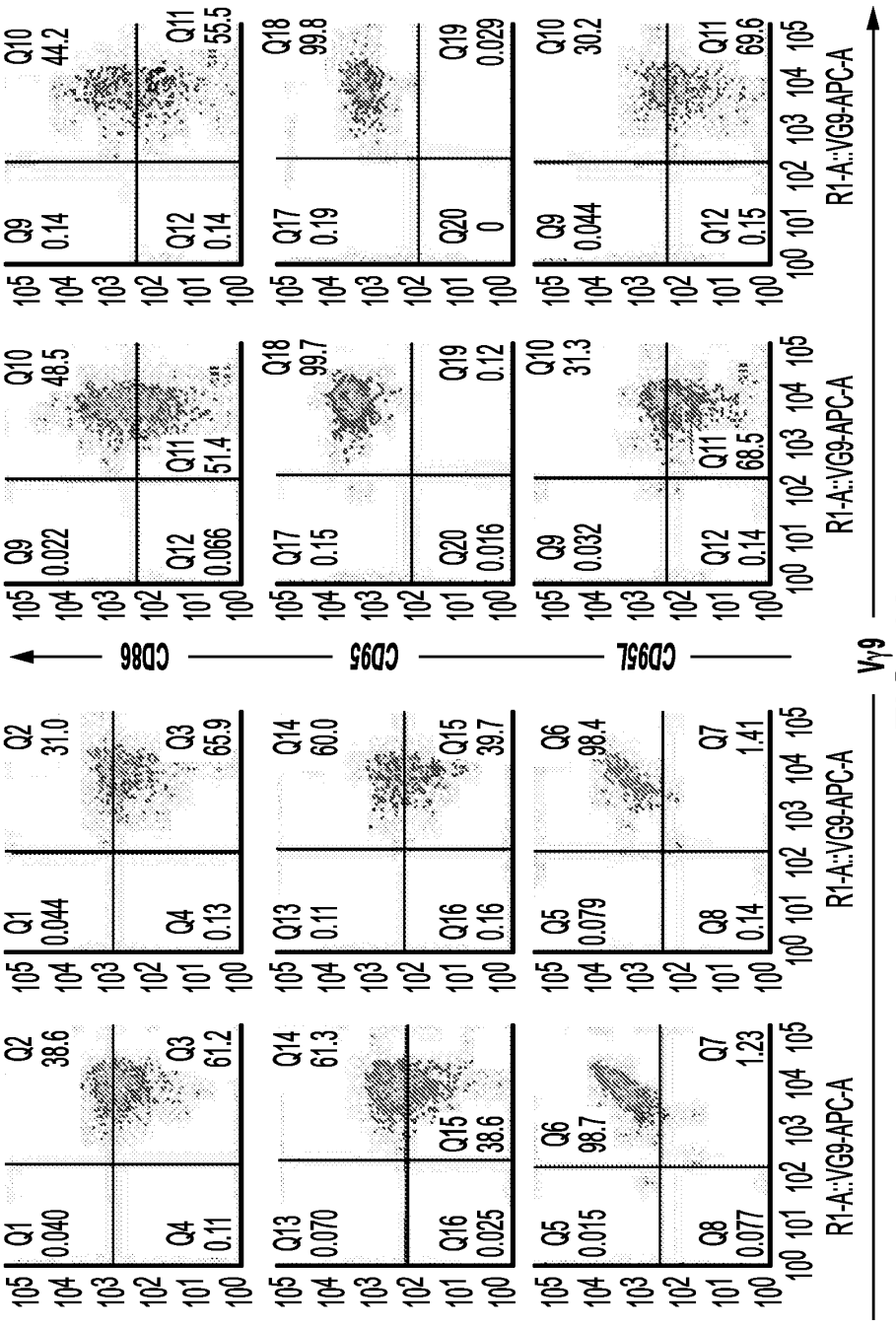

"high density"
"low density"
"high density"
"low density"
CD122
CD80
CD83
CD86
CD95
CD95L
Vγ9
R1-A::Vγ9-APC-A
Q1 0.040
Q2 38.6
Q3 61.2
Q4 0.11
Q5 0.015
Q6 98.7
Q7 1.23
Q8 0.077
Q9 0.022
Q10 48.5
Q11 51.4
Q12 0.066
Q13 0.070
Q14 61.3
Q15 38.6
Q16 0.025
Q17 0.15
Q18 99.7
Q19 0.12
Q20 0.016
Q1 0.044
Q2 31.0
Q3 65.9
Q4 0.13
Q5 0.079
Q6 98.4
Q7 1.41
Q8 0.14
Q9 0.032
Q10 31.3
Q11 68.5
Q12 0.14
Q13 0.11
Q14 60.0
Q15 39.7
Q16 0.16
Q17 0.19
Q18 99.8
Q19 0.029
Q20 0
Q9 0.14
Q10 44.2
Q11 55.5
Q12 0.14
Q9 0.044
Q10 30.2
Q11 69.6
Q12 0.15
Q17 0.19
Q18 99.8
Q19 0.029
Q20 0

FIG. 4A

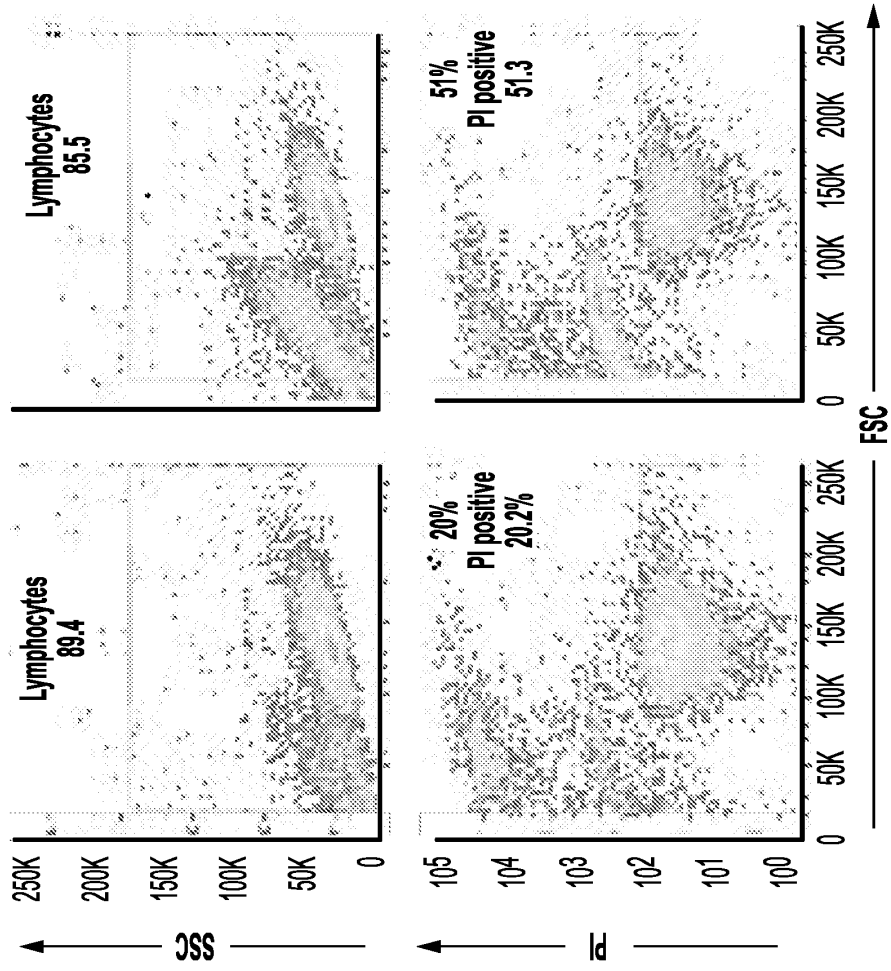

"high density"
Lymphocytes
89.4
20%
PI positive
20.2%
"low density"
Lymphocytes
85.5
51%
PI positive
51.3
SSC
PI
FSC
250K
200K
150K
100K
50K
0
10^5
10^4
10^3
10^2
10^1
10^0

FIG. 4B

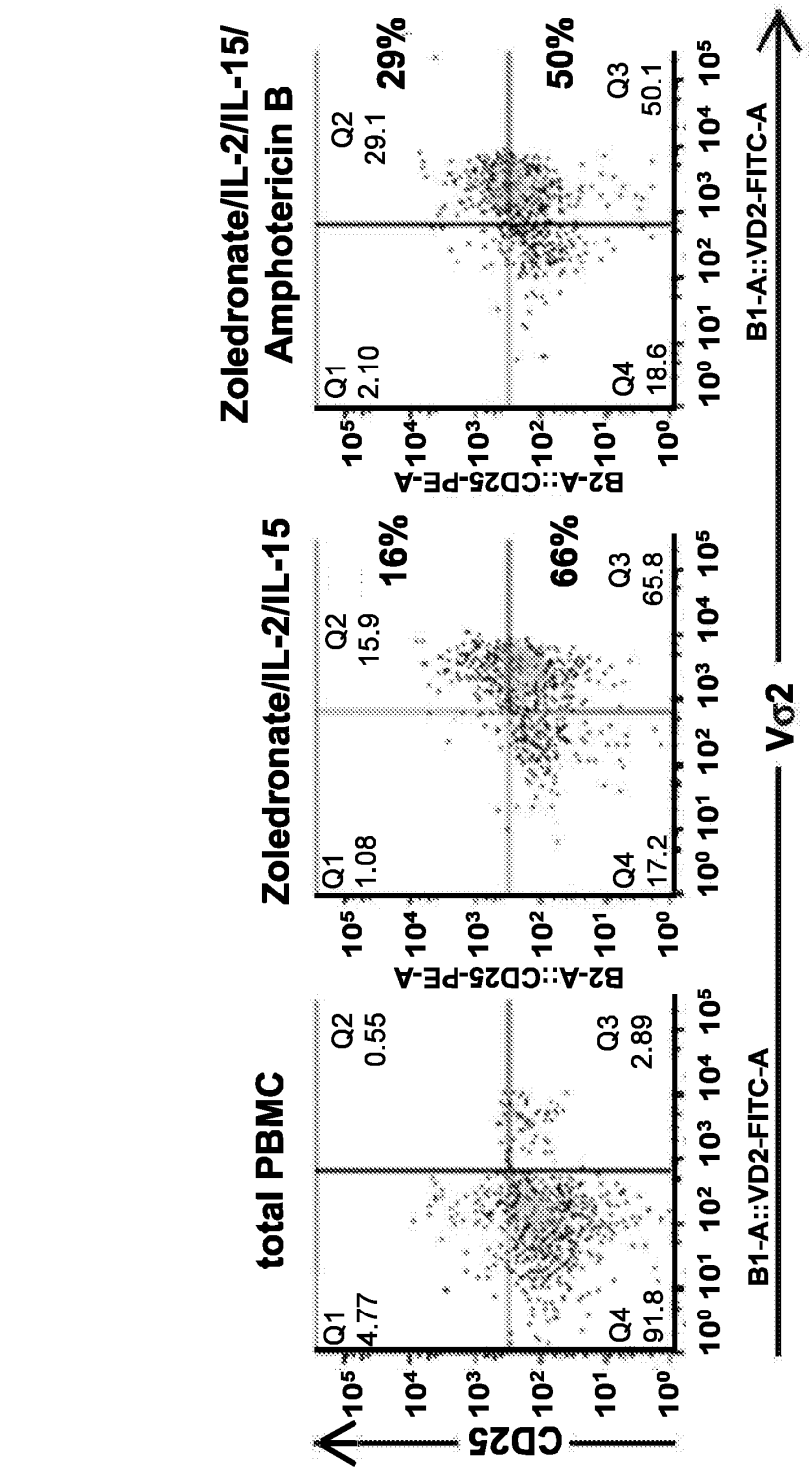

total PBMC
Zoledronate/IL-2/IL-15
Zoledronate/IL-2/IL-15/ Amphotericin B
CD25
B2-A::CD25-PE-A
B1-A::VD2-FITC-A
Vσ2
Q1 4.77
Q2 0.55
Q3 2.89
Q4 91.8
Q1 1.08
Q2 15.9
16%
Q3 65.8
66%
Q4 17.2
Q1 2.10
Q2 29.1
29%
Q3 50.1
50%
Q4 18.6

FIG. 5

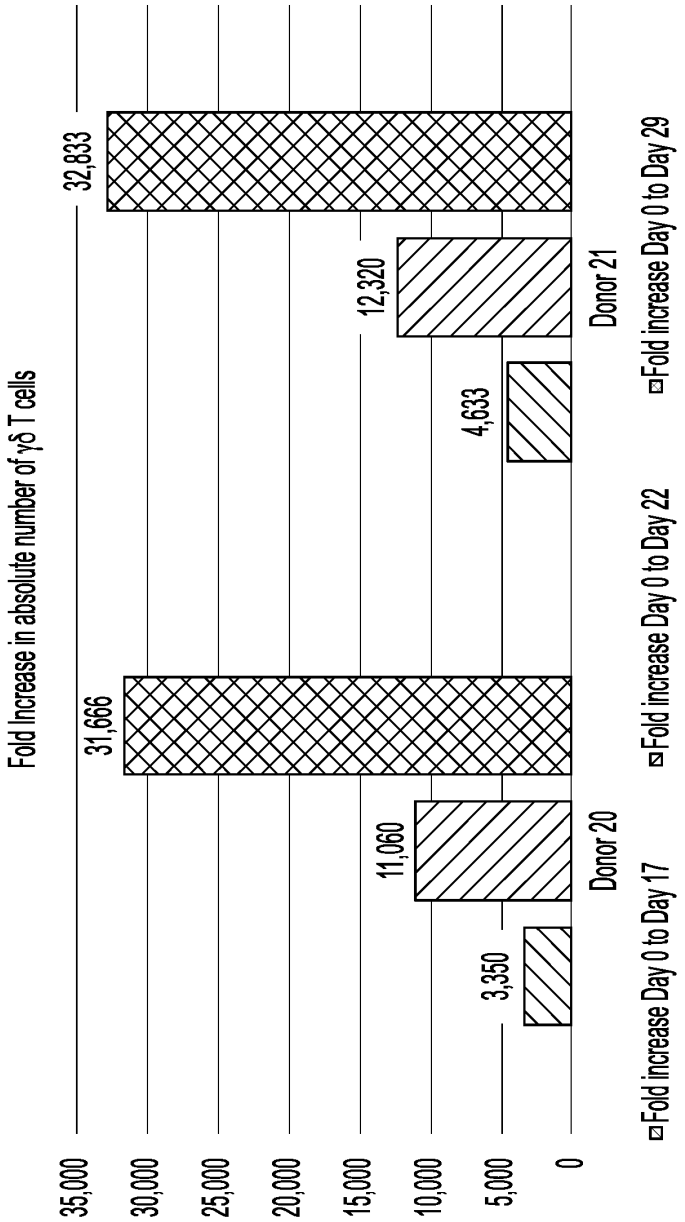

Fold Increase in absolute number of γδ T cells
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
32,833
12,320
4,633
31,666
11,060
3,350
Donor 21
Donor 20
Fold increase Day 0 to Day 29
Fold increase Day 0 to Day 22
Fold increase Day 0 to Day 17

FIG. 6

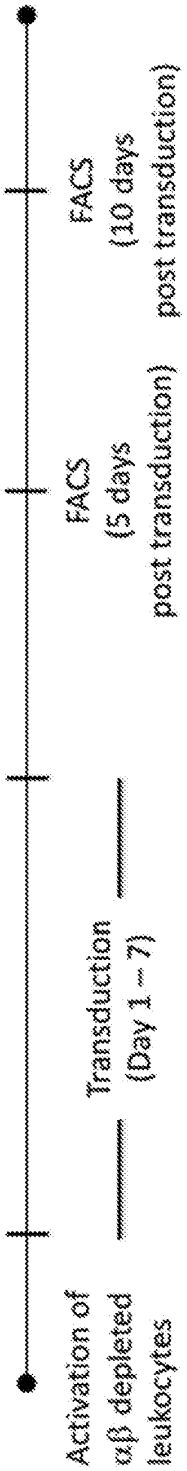

Activation of αβ depleted leukocytes
Transduction (Day 1 – 7)
FACS (5 days post transduction)
FACS (10 days post transduction)

FIG. 7

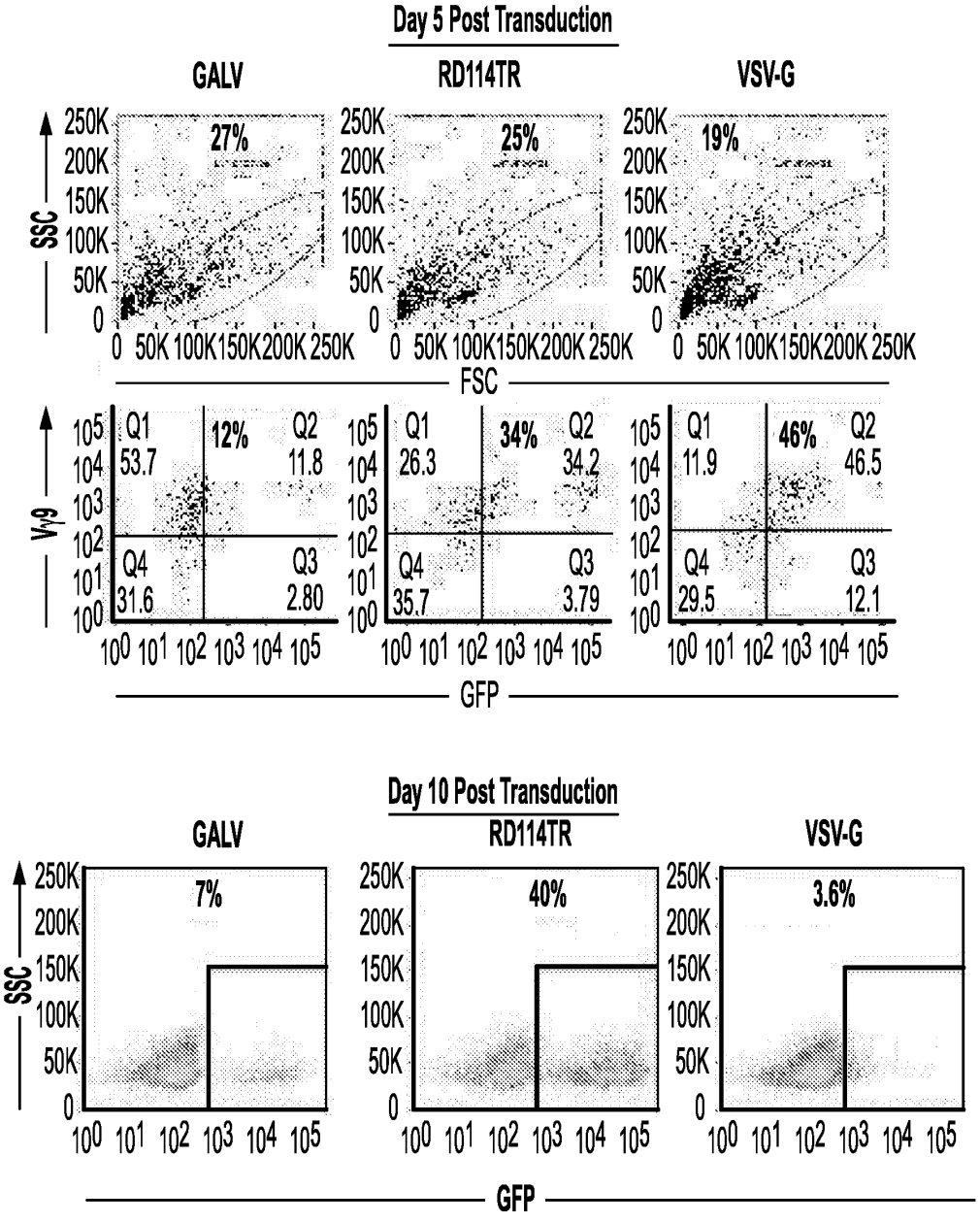

Day 5 Post Transduction
GALV
RD114TR
VSV-G
SSC
FSC
27%
25%
19%
Q1 53.7
Q2 11.8
Q4 31.6
Q3 2.80
12%
Q1 26.3
Q2 34.2
Q4 35.7
Q3 3.79
34%
Q1 11.9
Q2 46.5
Q4 29.5
Q3 12.1
46%
GFP
Day 10 Post Transduction
GALV
RD114TR
VSV-G
7%
40%
3.6%
GFP

FIG. 8A

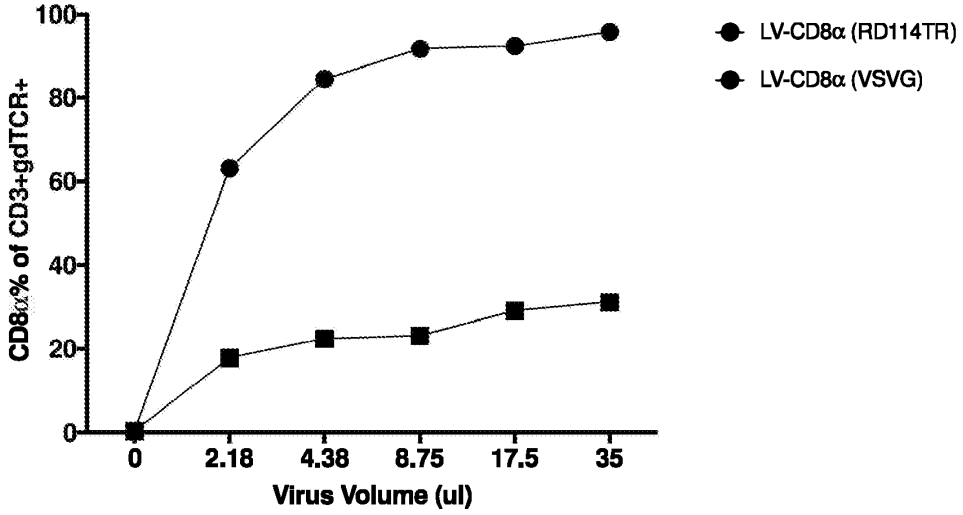

100
80
60
40
20
0
CD8α% of CD3+gdTCR+
0
2.18
4.38
8.75
17.5
35
Virus Volume (ul)
LV-CD8α (RD114TR)
LV-CD8α (VSVG)

FIG. 8B

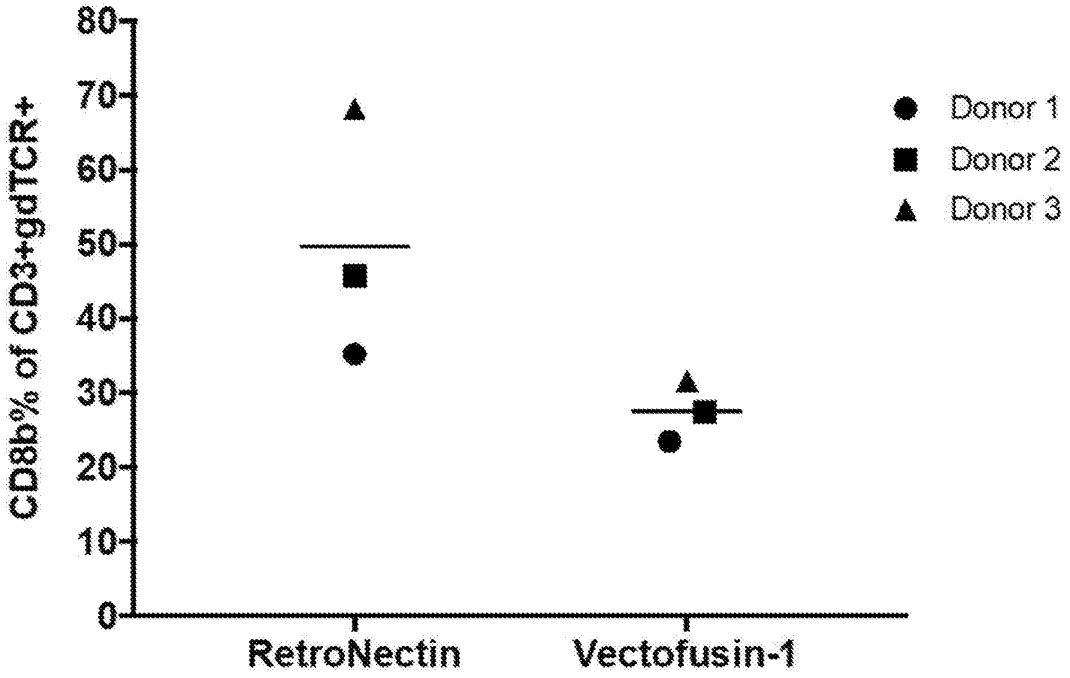

80
70
60
50
40
30
20
10
0
CD8b% of CD3+gdTCR+
Donor 1
Donor 2
Donor 3
RetroNectin
Vectofusin-1

FIG. 9A

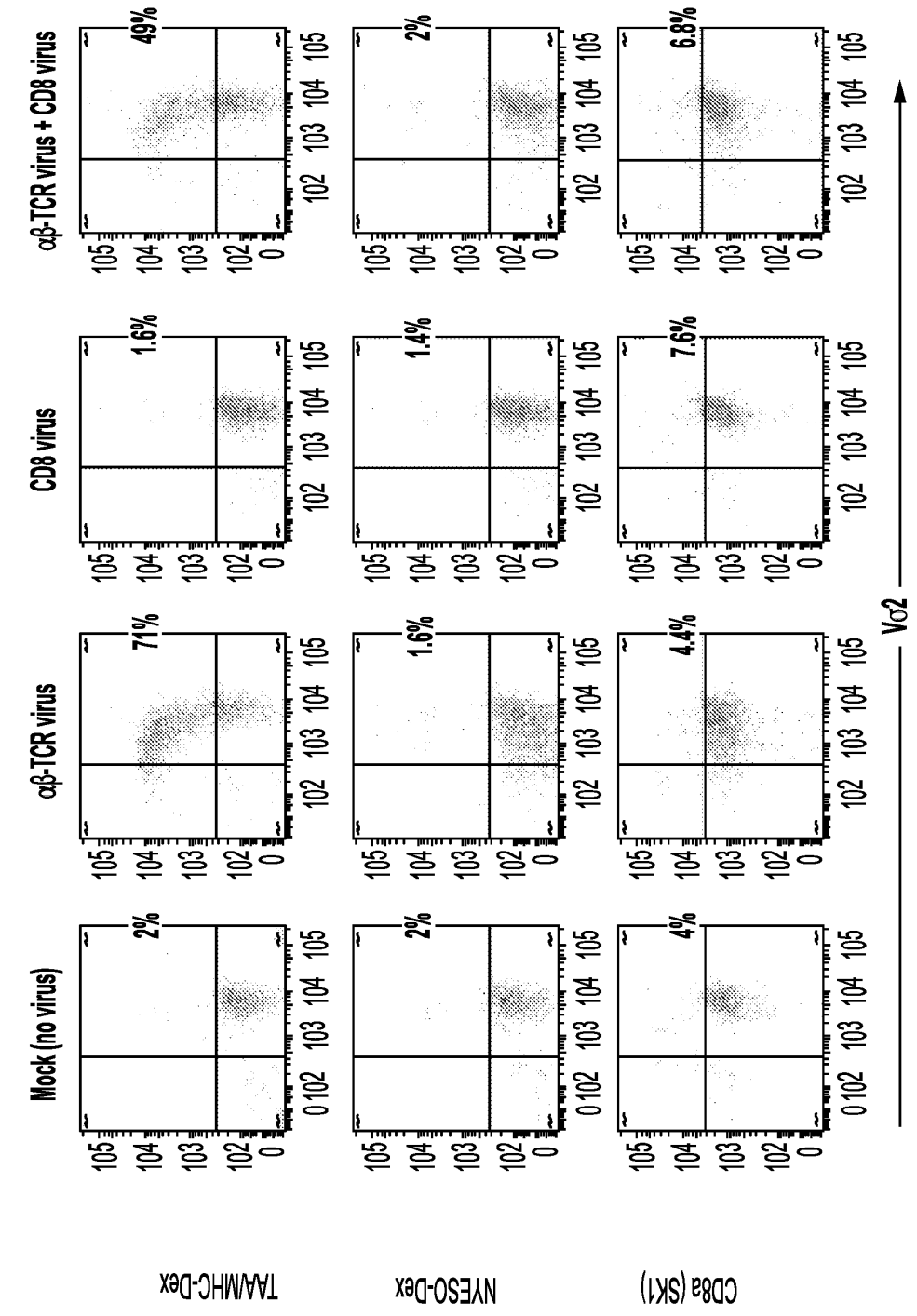

Mock (no virus)
αβ-TCR virus
CD8 virus
αβ-TCR virus + CD8 virus
2%
71%
1.6%
49%
2%
1.6%
1.4%
2%
4%
4.4%
7.6%
6.8%
TAA/MHC-Dex
NYESO-Dex
CD8α (SK1)
Vσ2

FIG. 9B

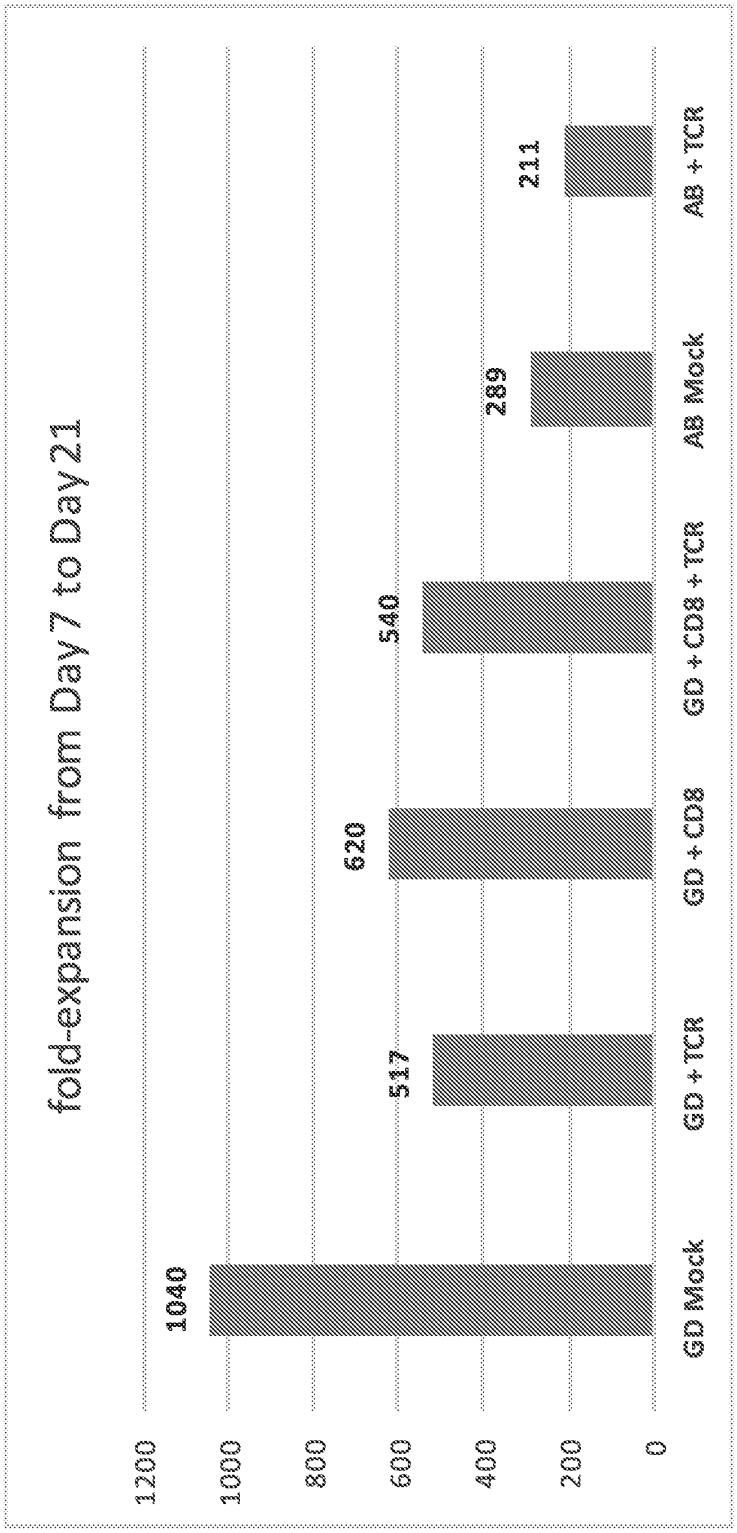

fold-expansion from Day7 to Day21
1200
1000
800
600
400
200
0
1040
517
620
540
289
211
GD Mock
GD + TCR
GD + CD8
GD + CD8 + TCR
AB Mock
AB + TCR

FIG. 10

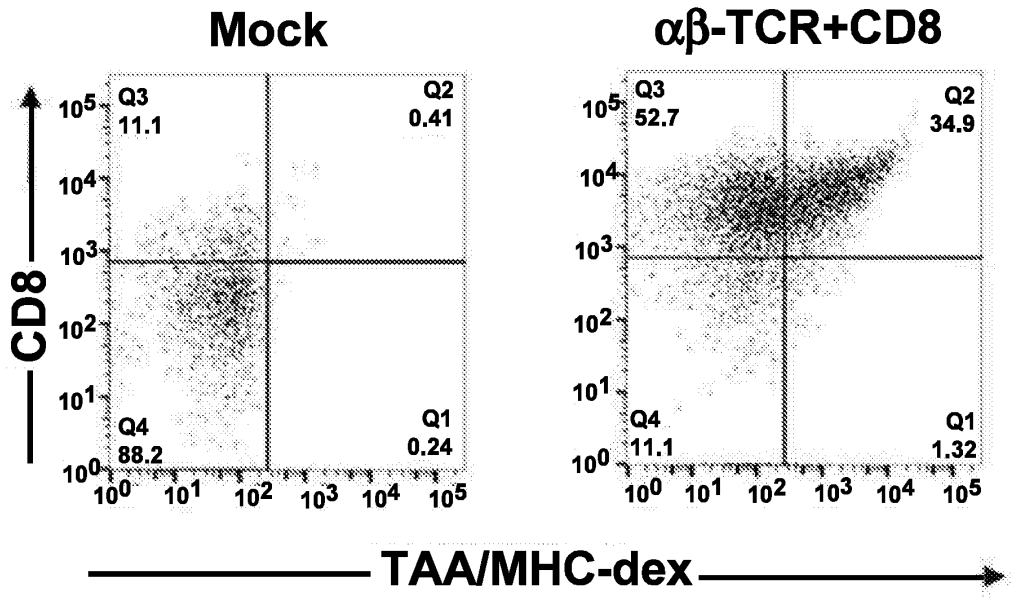

Mock
αβ-TCR+CD8
CD8
TAA/MHC-dex
Q3
11.1
Q2
0.41
Q4
88.2
Q1
0.24
Q3
52.7
Q2
34.9
Q4
11.1
Q1
1.32
10^0
10^1
10^2
10^3
10^4
10^5

FIG. 11A

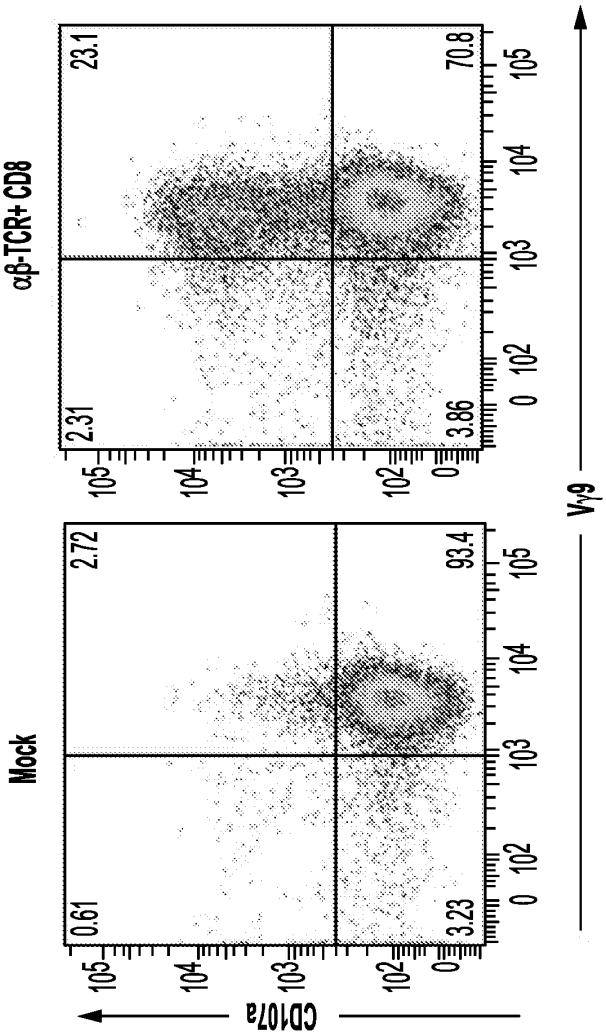

αβ-TCR+ CD8
Mock
CD107a
Vγ9
23.1
70.8
2.31
3.86
2.72
93.4
0.61
3.23
0
10²
10³
10⁴
10⁵

FIG. 11B

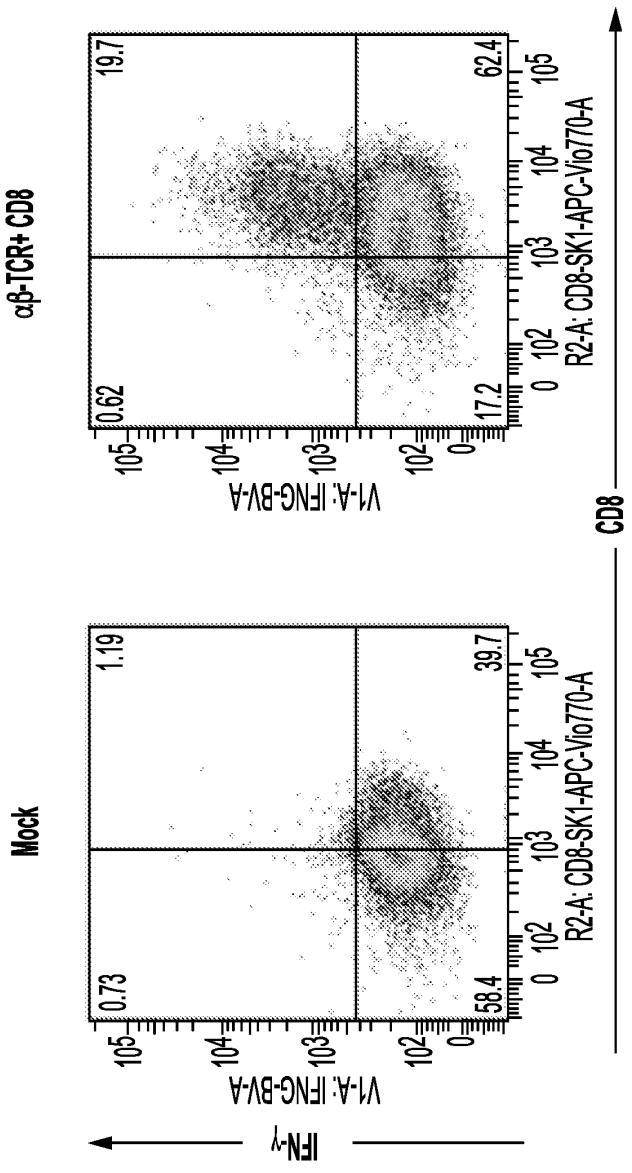

αβ-TCR+ CD8
Mock
IFN-γ
CD8
V1-A: IFNG-BV-A
R2-A: CD8-SK1-APC-Vio770-A
19.7
62.4
0.62
17.2
1.19
39.7
0.73
58.4
0
10²
10³
10⁴
10⁵

FIG. 11C

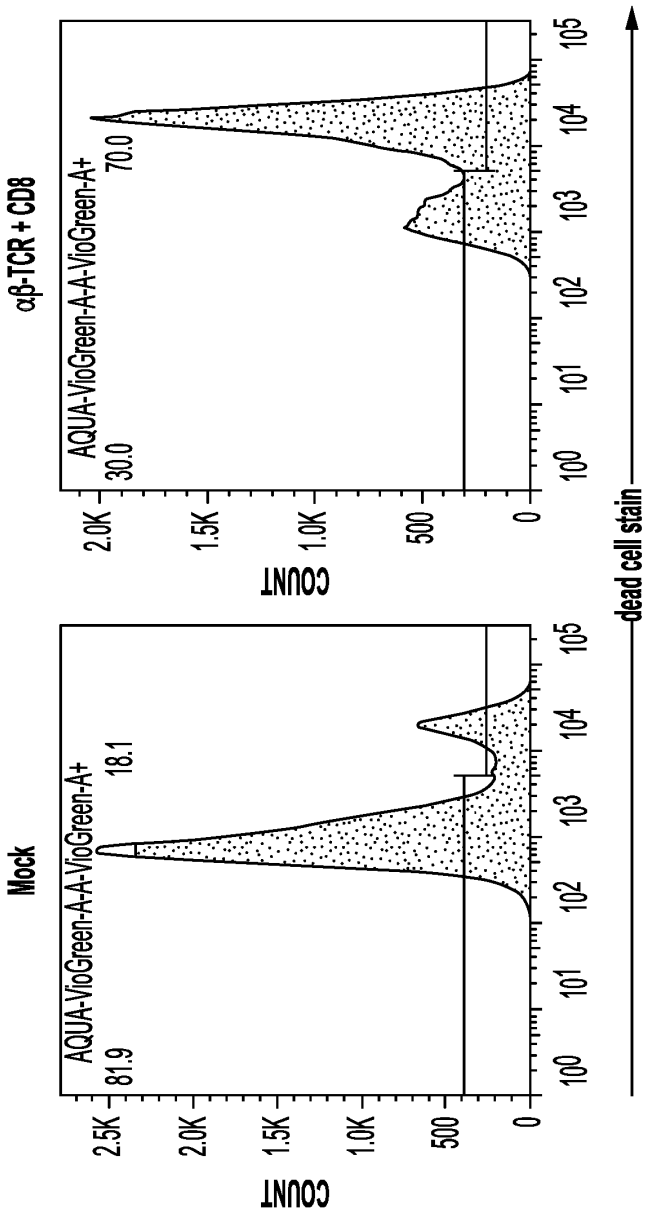

αβ-TCR + CD8
AQUA-VioGreen-A-A-VioGreen-A+
70.0
30.0
COUNT
2.0K
1.5K
1.0K
500
0
Mock
AQUA-VioGreen-A-A-VioGreen-A+
18.1
81.9
2.5K
2.0K
1.5K
1.0K
500
0
COUNT
10^0
10^1
10^2
10^3
10^4
10^5
dead cell stain

FIG. 11D

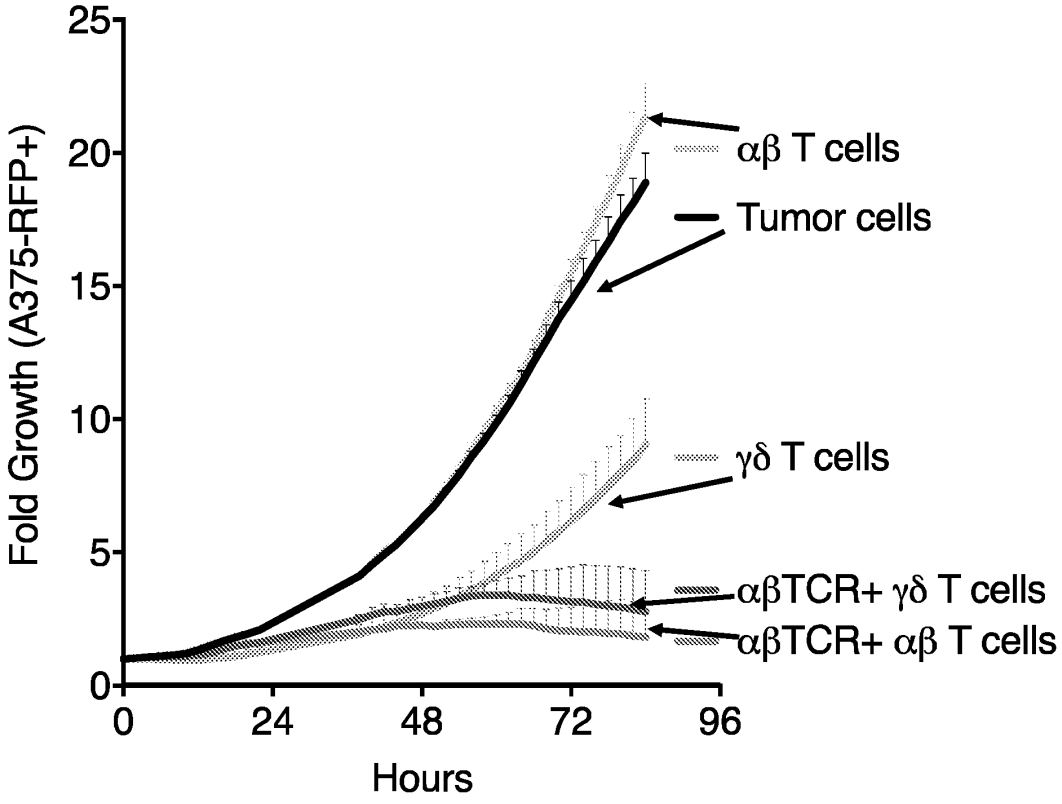

25
20
15
10
5
0
Fold Growth (A375-RFP+)
0
24
48
72
96
Hours
αβ T cells
Tumor cells
γδ T cells
αβTCR+ γδ T cells
αβTCR+ αβ T cells

FIG. 11E

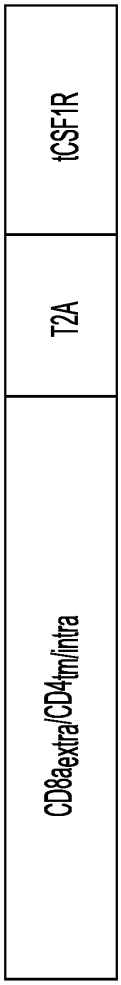


FIG. 12A

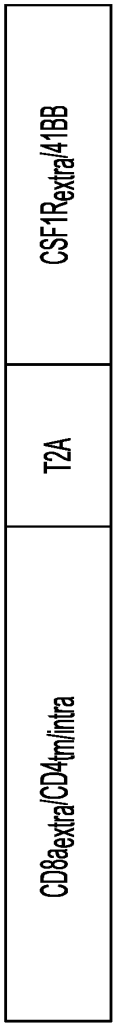


FIG. 12B

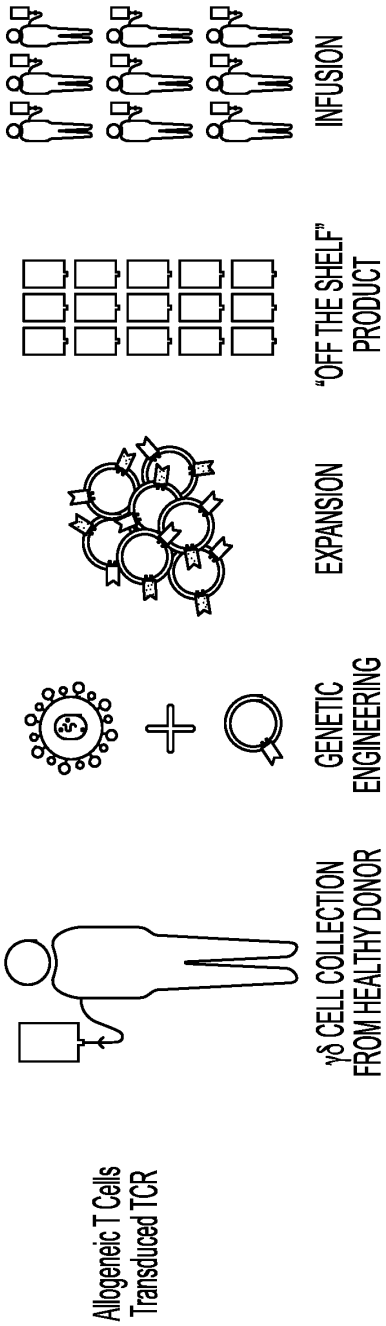

INFUSION
"OFF THE SHELF" PRODUCT
EXPANSION
GENETIC ENGINEERING
γδ CELL COLLECTION FROM HEALTHY DONOR
Allogeneic T Cells Transduced TCR

FIG. 13

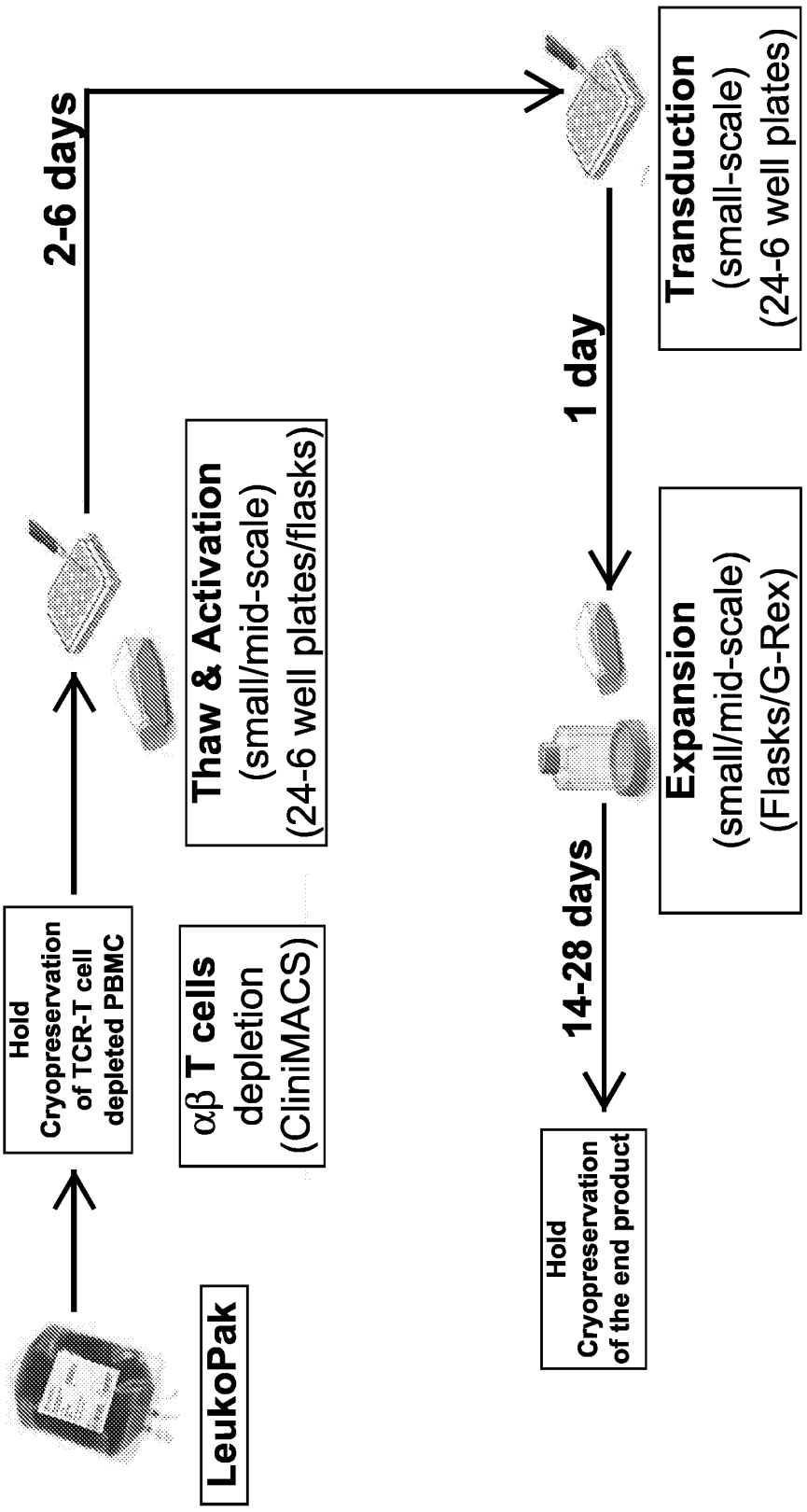

LeukoPak
Hold Cryopreservation of TCR-T cell depleted PBMC
αβ T cells depletion (CliniMACS)
Thaw & Activation (small/mid-scale) (24-6 well plates/flasks)
2-6 days
Transduction (small-scale) (24-6 well plates)
1 day
Expansion (small/mid-scale) (Flasks/G-Rex)
14-28 days
Hold Cryopreservation of the end product

FIG. 14