FIG. 1A

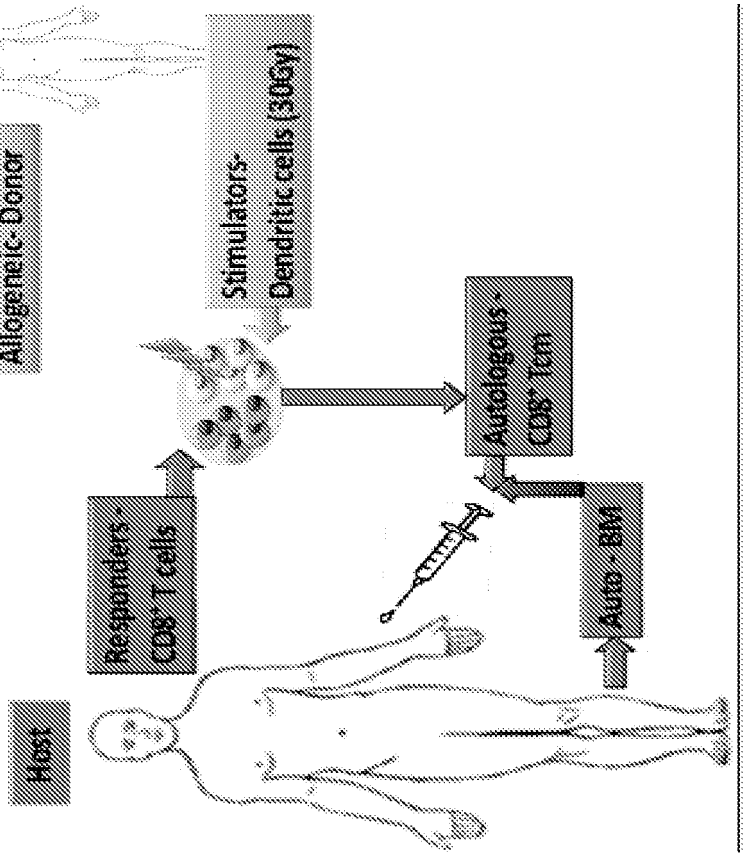

Autologous Human setting
Allogeneic Donor
Stimulators-
Dendritic cells (30Gy)
Responders -
CD8+ T cells
Autologous -
CD8+ Tcm
Host
Auto - BM

FIG. 1B

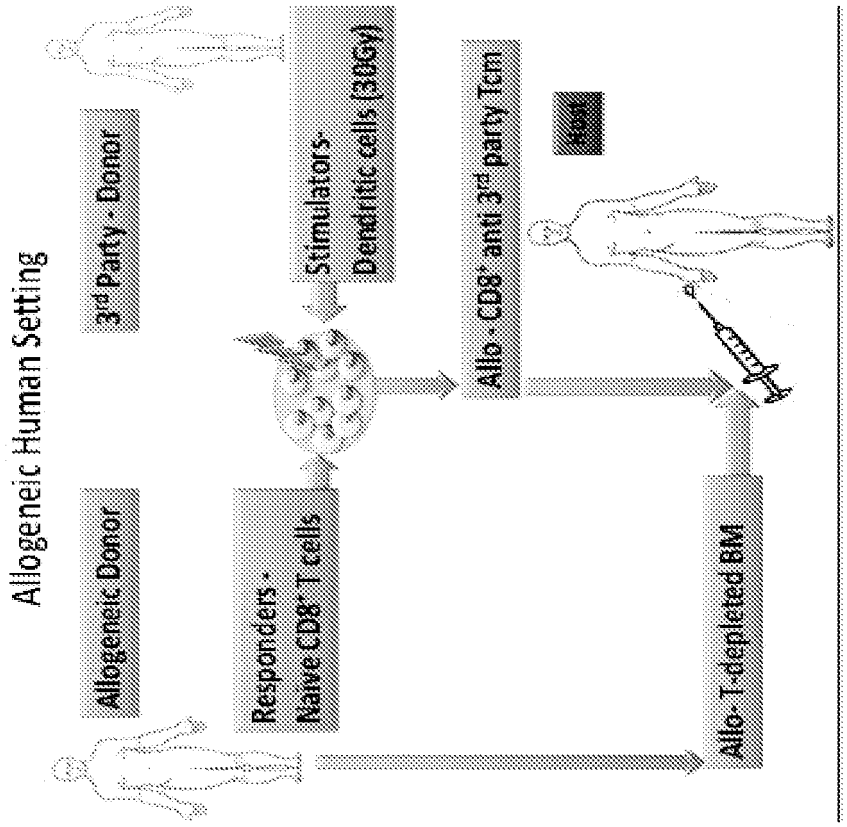

Allogeneic Human Setting
3rd Party - Donor
Stimulators-
Dendritic cells (30Gy)
Allogeneic Donor
Responders -
Naive CD8+ T cells
Allo - CD8+ anti 3rd party Tcm
Host
Allo- T-depleted BM

FIG. 2A

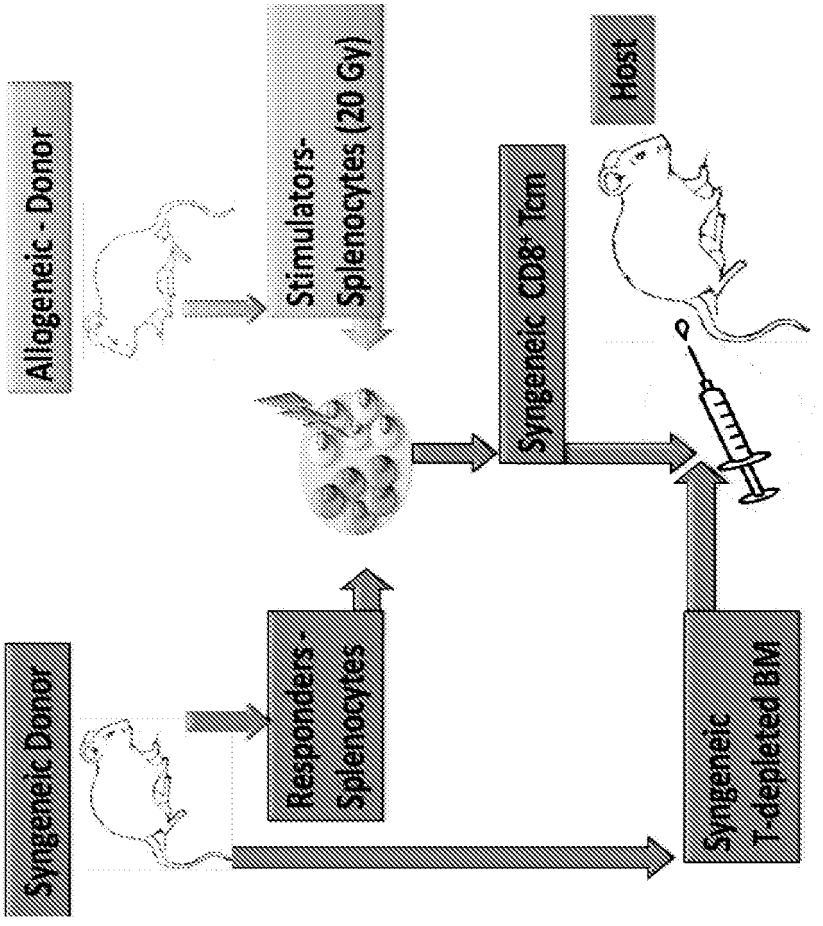

Syngeneic Mouse Setting
Syngeneic Donor
Responders - Splenocytes
Allogeneic - Donor
Stimulators- Splenocytes (20 Gy)
Syngeneic CD8+ Tcm
Host
Syngeneic T-depleted BM

FIG. 2B

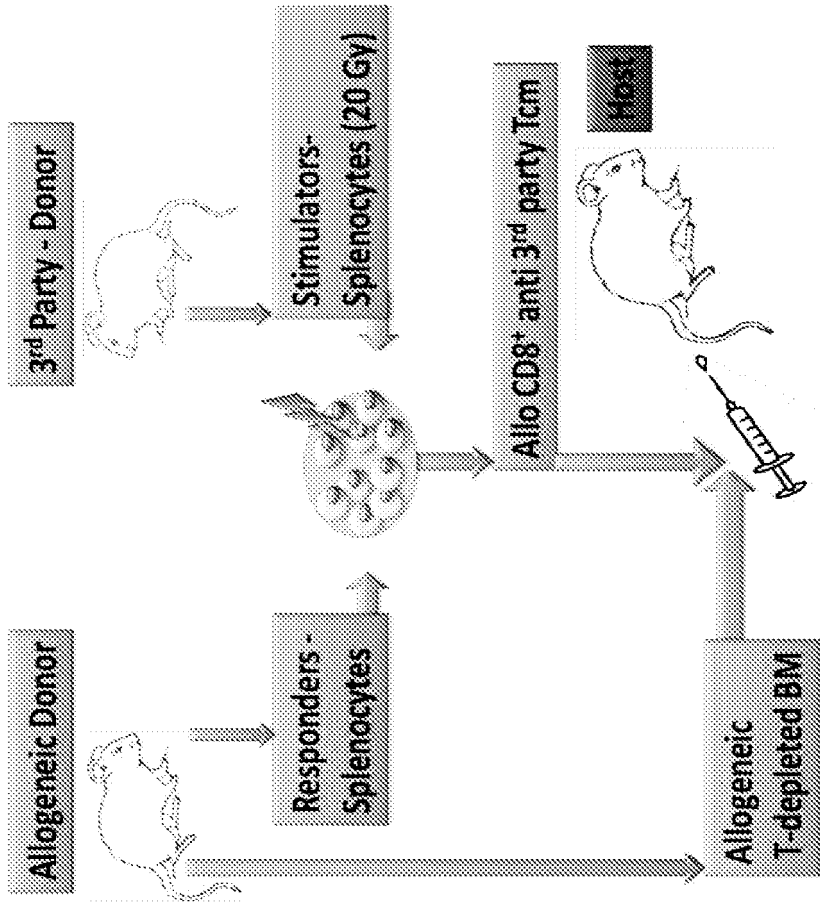

Allogeneic Mouse Setting
Allogeneic Donor
Responders - Splenocytes
3rd Party - Donor
Stimulators- Splenocytes (20 Gy)
Allo CD8+ anti 3rd party Tcm
Host
Allogeneic T-depleted BM

FIG. 3A

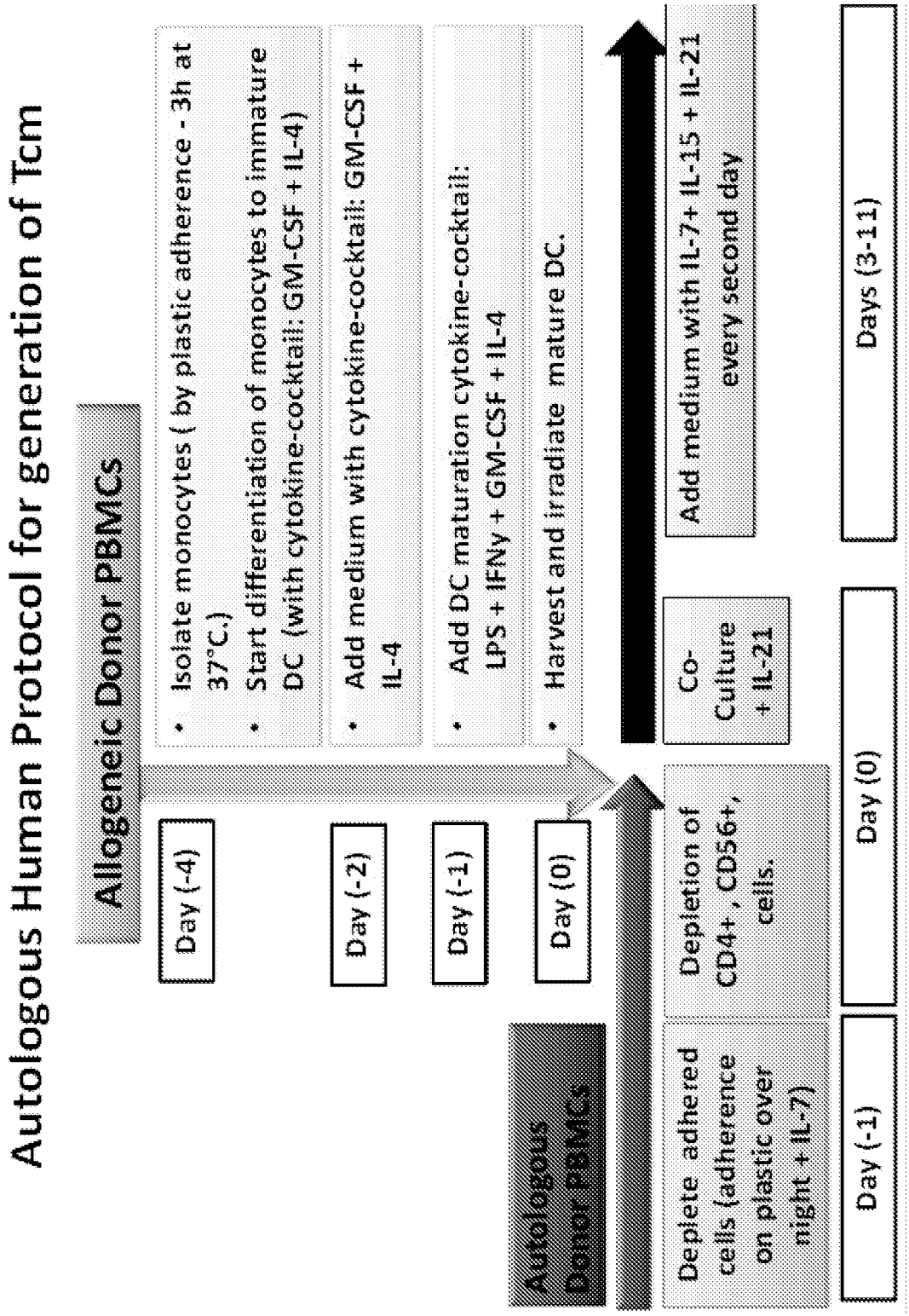

Autologous Human Protocol for generation of Tcm
Allogeneic Donor PBMCs
Day (-4)
Isolate monocytes (by plastic adherence - 3h at 37°C.)
Start differentiation of monocytes to immature DC (with cytokine-cocktail: GM-CSF + IL-4)
Day (-2)
Add medium with cytokine-cocktail: GM-CSF + IL-4
Day (-1)
Add DC maturation cytokine-cocktail: LPS + IFNγ + GM-CSF + IL-4
Day (0)
Harvest and irradiate mature DC.
Autologous Donor PBMCs
Co-Culture + IL-21
Add medium with IL-7+ IL-15 + IL-21 every second day
Depiete adhered cells (adherence on plastic over night + IL-7)
Depletion of CD4+, CD56+, cells.
Day (-1)
Day (0)
Days (3-11)

FIG. 3B

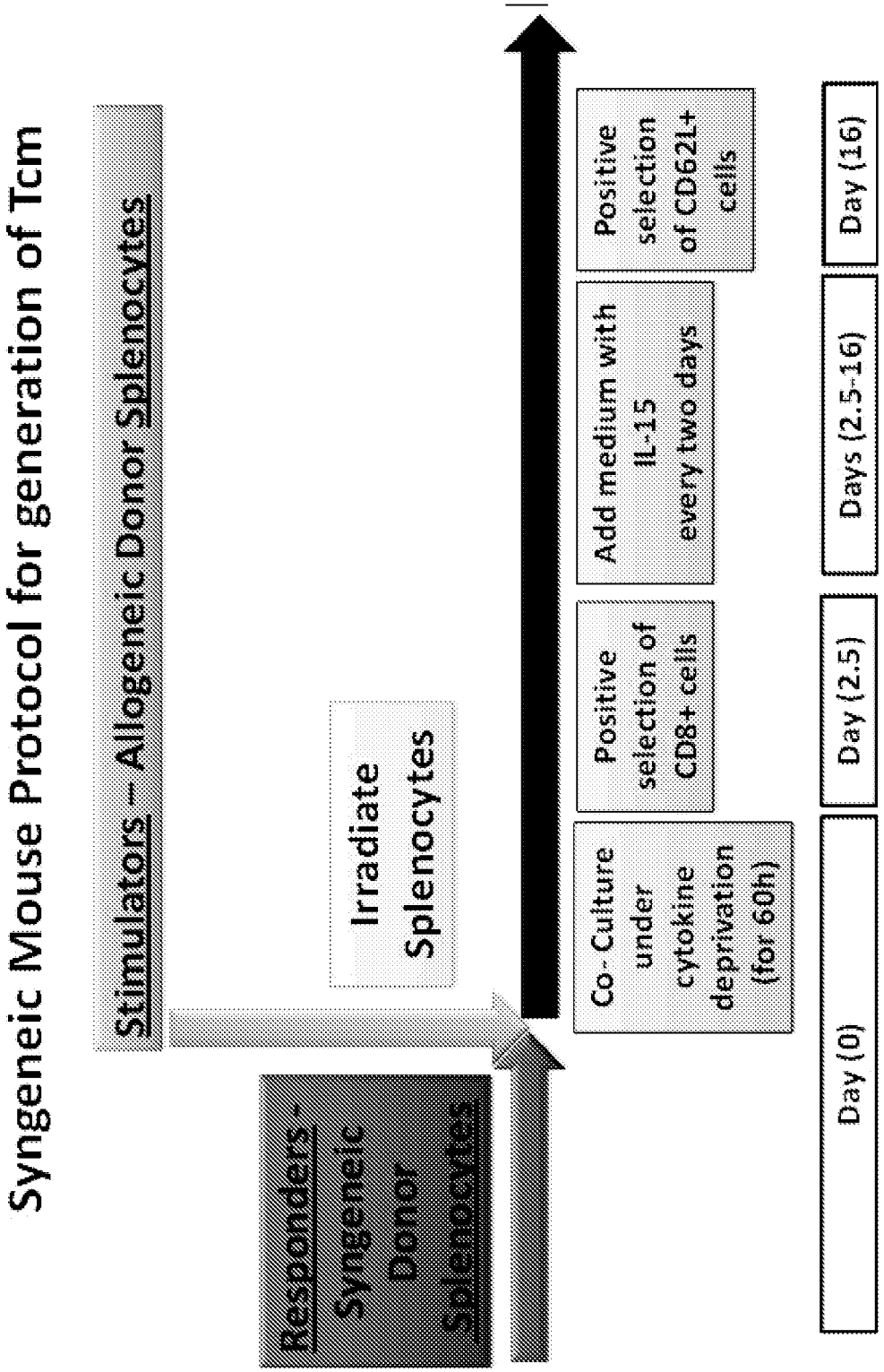

Syngeneic Mouse Protocol for generation of Tcm
Stimulators – Allogeneic Donor Splenocytes
Irradiate Splenocytes
Responders - Syngeneic Donor Splenocytes
Co- Culture under cytokine deprivation (for 60h)
Positive selection of CD8+ cells
Add medium with IL-15 every two days
Positive selection of CD62L+ cells
Day (0)
Day (2.5)
Days (2.5-16)
Day (16)

FIG. 4A

	Day of Culture	0	7.5	10.5	12.5
CD8+CD3+	AVG	94.1	94.3	93.0	94.8
	SE	0.5	1.1	1.6	1.2
	n	8	5	4	2
CD56+ CD3-	AVG	0.2	1.1	1.4	1.2
	SE	0.0	0.6	0.5	*
	n	2	6	3	1
CD56+CD3+	AVG	*	3.4	4.7	2.6
	SE	*	1.5	3.6	*
	n	*	3.0	2.0	1.0

FIG. 4B

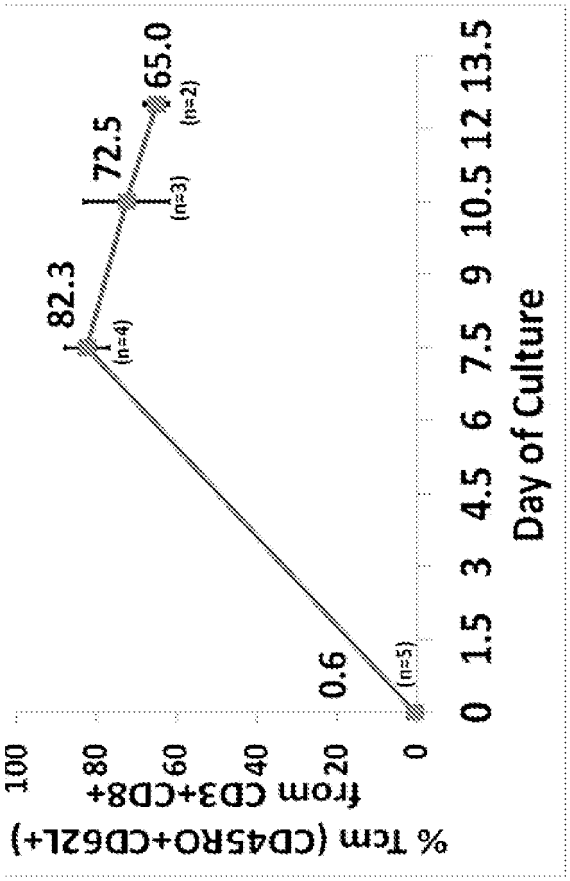


FIG. 4C

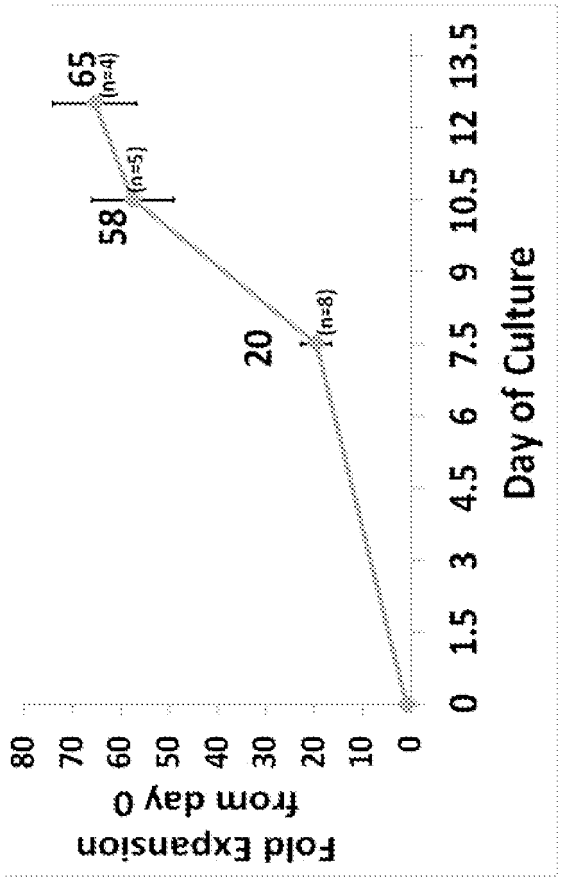


FIG. 5A

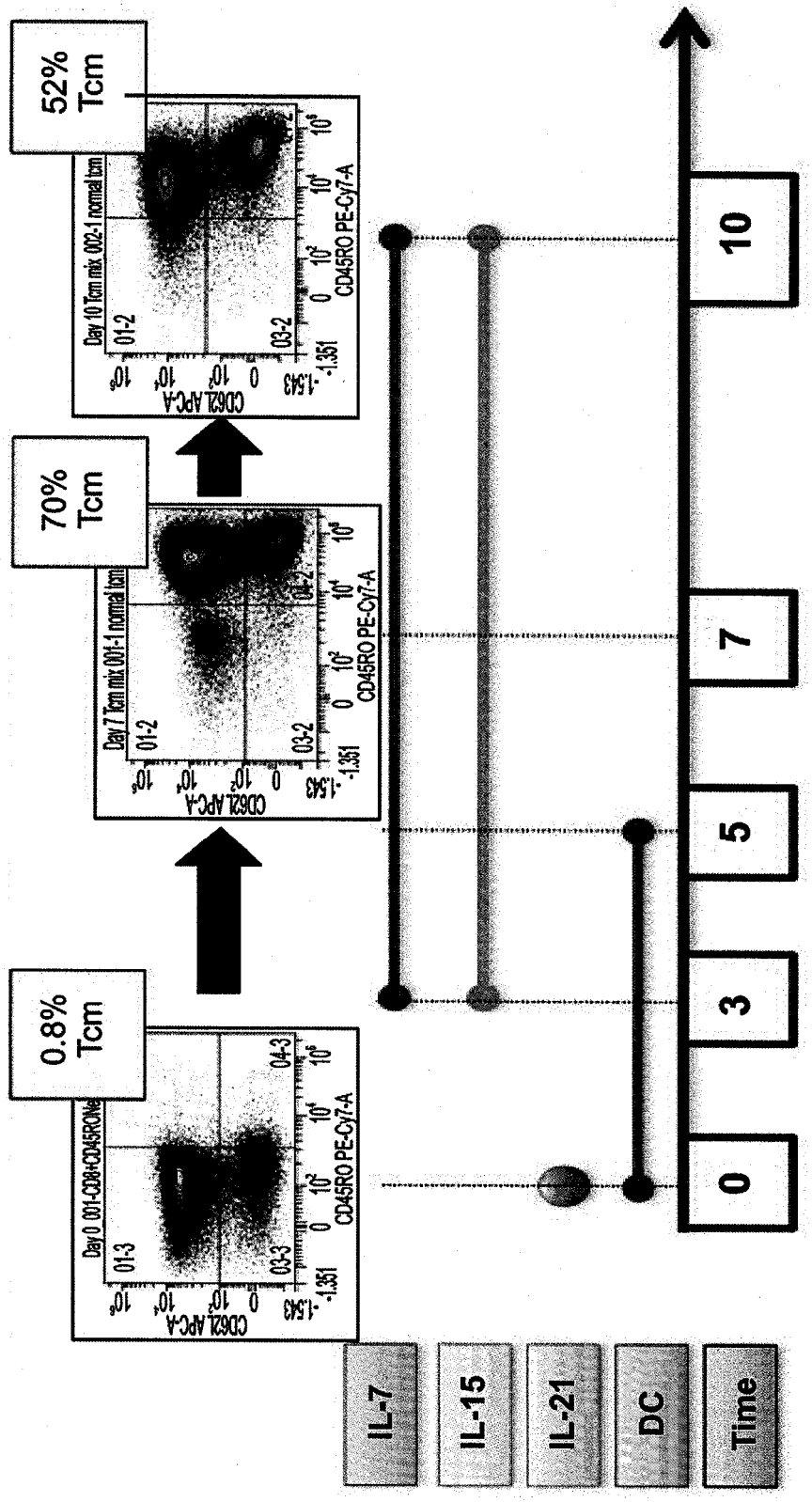

0.8% Tcm
70% Tcm
52% Tcm
Day 0 001-CD8+CD45RONe
Day 7 Tcm mix 001-1 normal tcm
Day 10 Tcm mix 002-1 normal tcm
CD62L APC-A
CD45RO PE-Cy7-A
IL-7
IL-15
IL-21
DC
Time
0
3
5
7
10

FIG. 5B

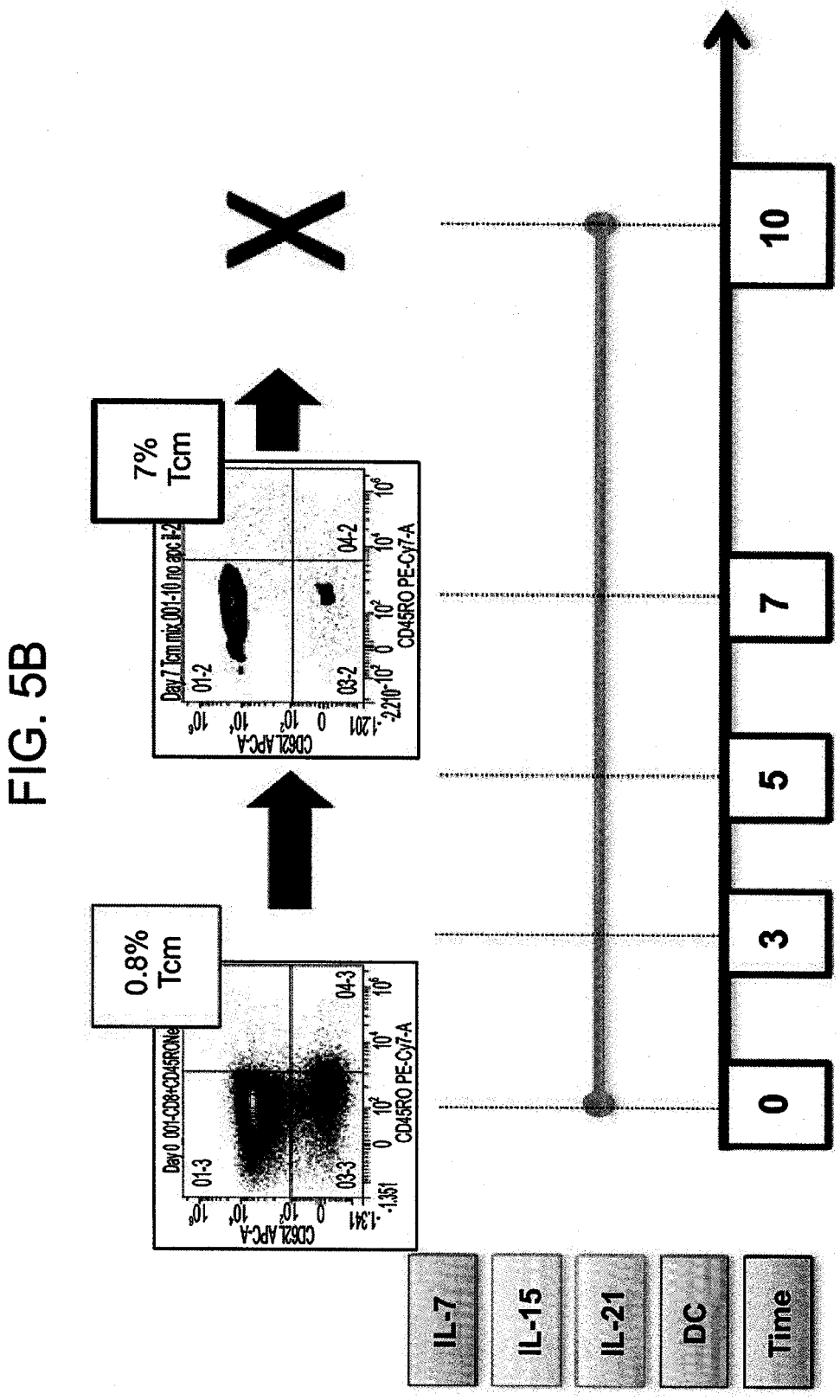

0.8% Tcm
7% Tcm
CD62L APC-A
CD45RO PE-Cy7-A
IL-7
IL-15
IL-21
DC
Time
0
3
5
7
10

FIG. 5C

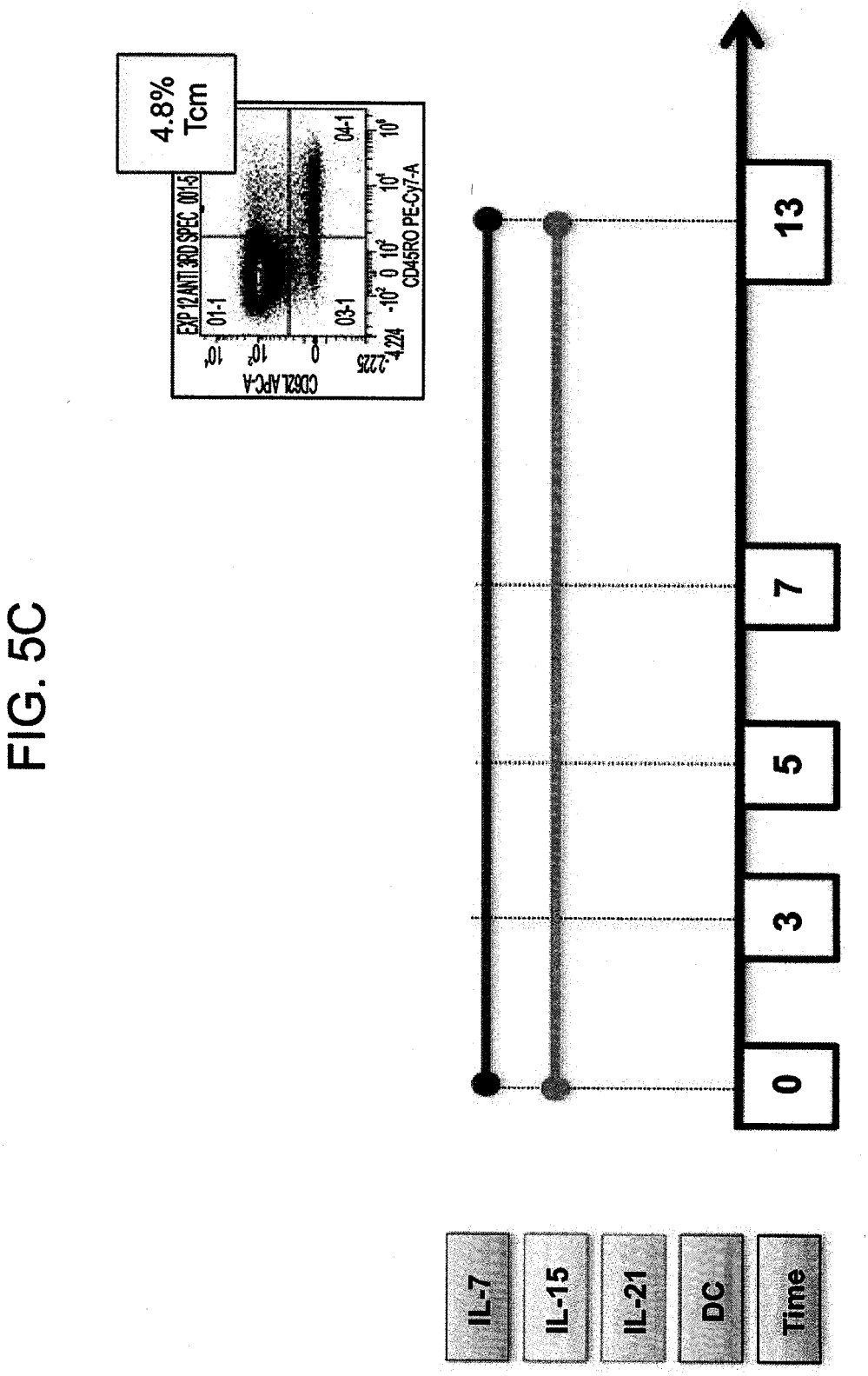

4.8% Tcm
EXP 12 ANTI 3RD SPEC_001-5
01-1
03-1
04-1
CD62L APC-A
CD45RO PE-Cy7-A
-2225
4224
0
3
5
7
13
IL-7
IL-15
IL-21
DC
Time

FIG. 6A

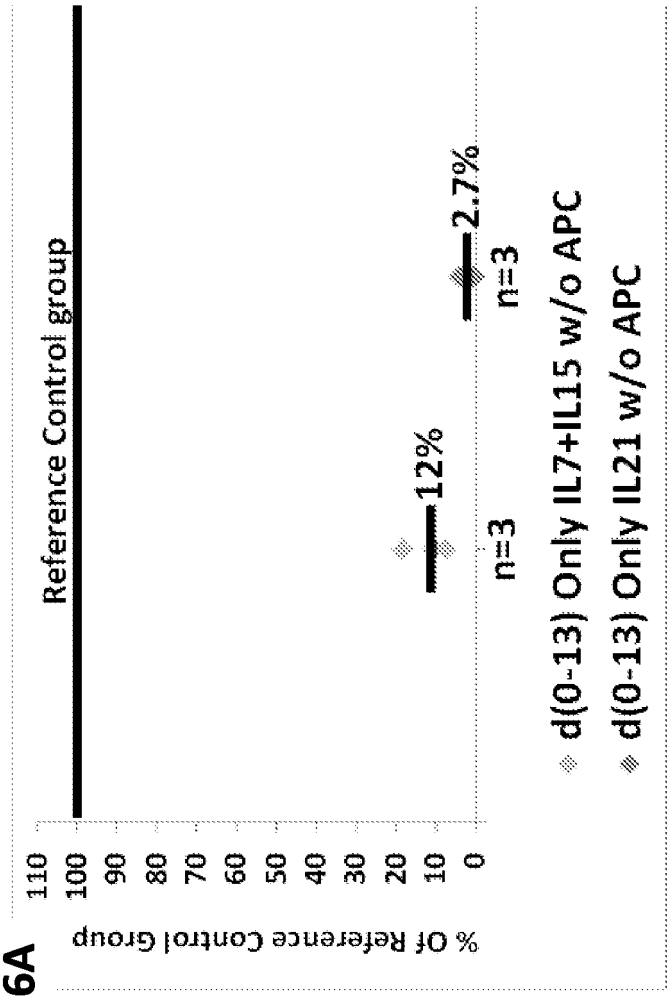

% Of Reference Control Group
110
100
90
80
70
60
50
40
30
20
10
0
Reference Control group
12%
2.7%
n=3
n=3
d(0-13) Only IL7+IL15 w/o APC
d(0-13) Only IL21 w/o APC

FIG. 6B

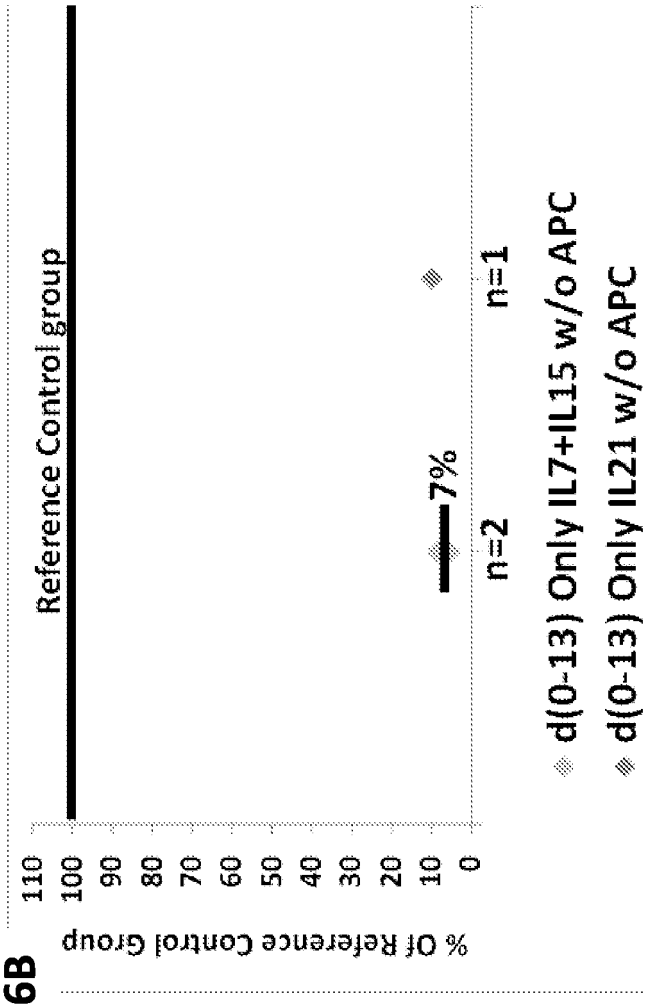

% Of Reference Control Group
110
100
90
80
70
60
50
40
30
20
10
0
Reference Control group
7%
n=2
n=1
d(0-13) Only IL7+IL15 w/o APC
d(0-13) Only IL21 w/o APC

FIG. 7A

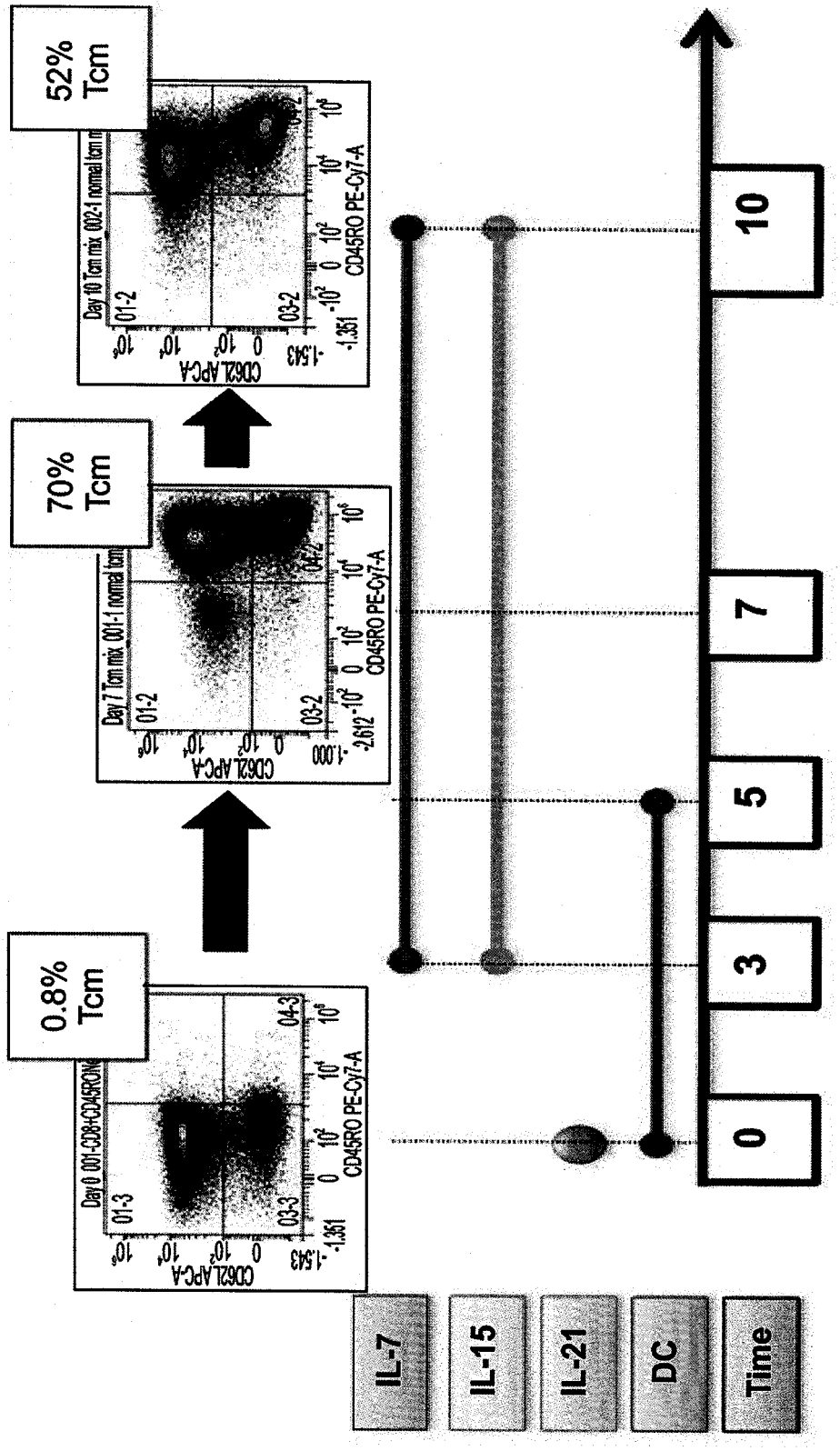

0.8% Tcm
70% Tcm
52% Tcm
Day 0 001-CD8+CD45RO
Day 7 Tcm mix 001-1 normal tcm
Day 10 Tcm mix 002-1 normal tcm
CD62L APC-A
CD45RO PE-Cy7-A
0
3
5
7
10
IL-7
IL-15
IL-21
DC
Time

FIG. 7B

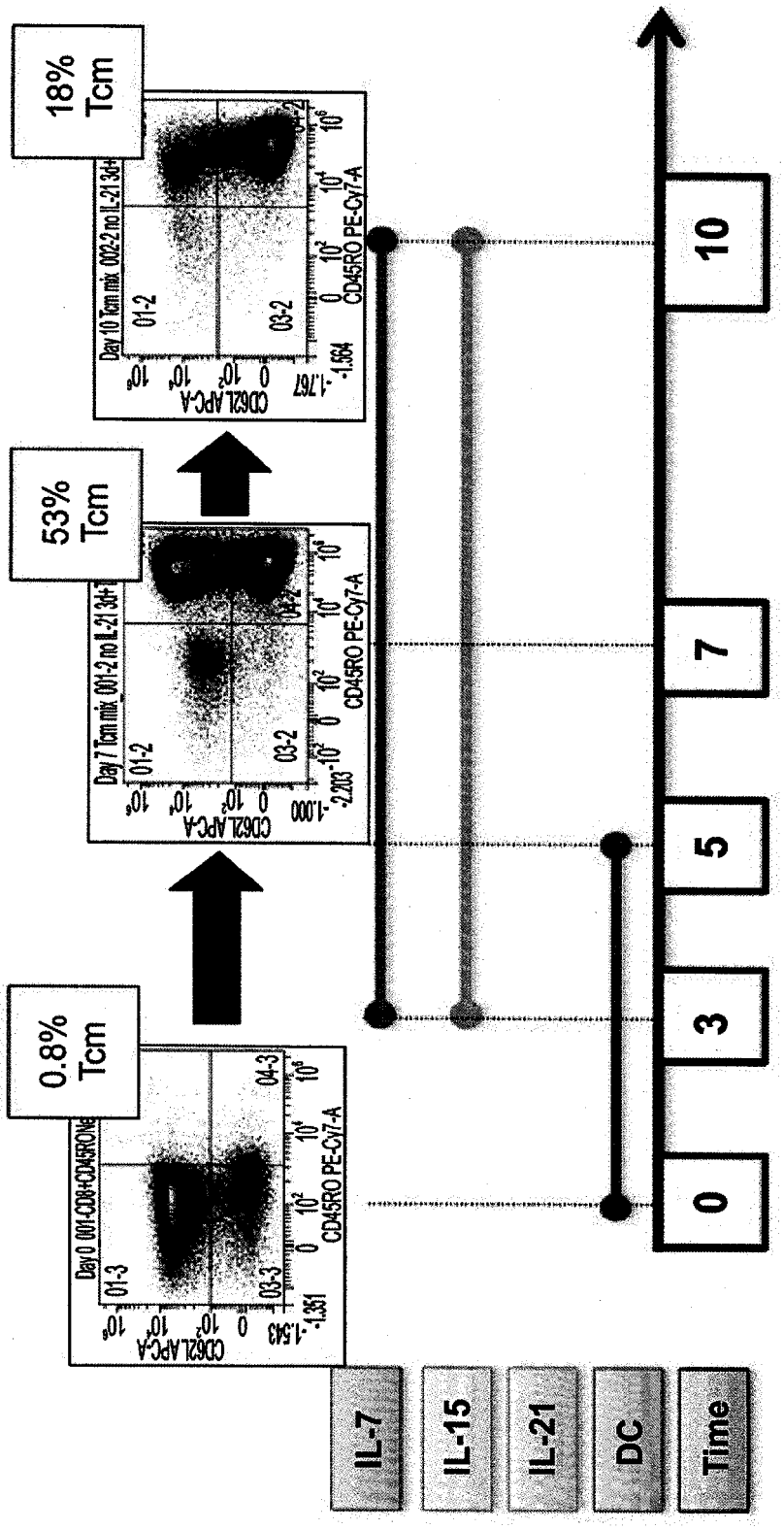

0.8% Tcm
53% Tcm
18% Tcm
Day 0 001-CD8+CD45RONe
Day 7 Tcm mix_001-2 no IL-21 3d+
Day 10 Tcm mix_002-2 no IL-21 3d+
CD62L APC-A
CD45RO PE-Cy7-A
01-3
03-3
04-3
01-2
03-2
04-2
-1.543
-1.351
-1.000
-2.203
-1.767
-1.684
IL-7
IL-15
IL-21
DC
Time
0
3
5
7
10

FIG. 7C

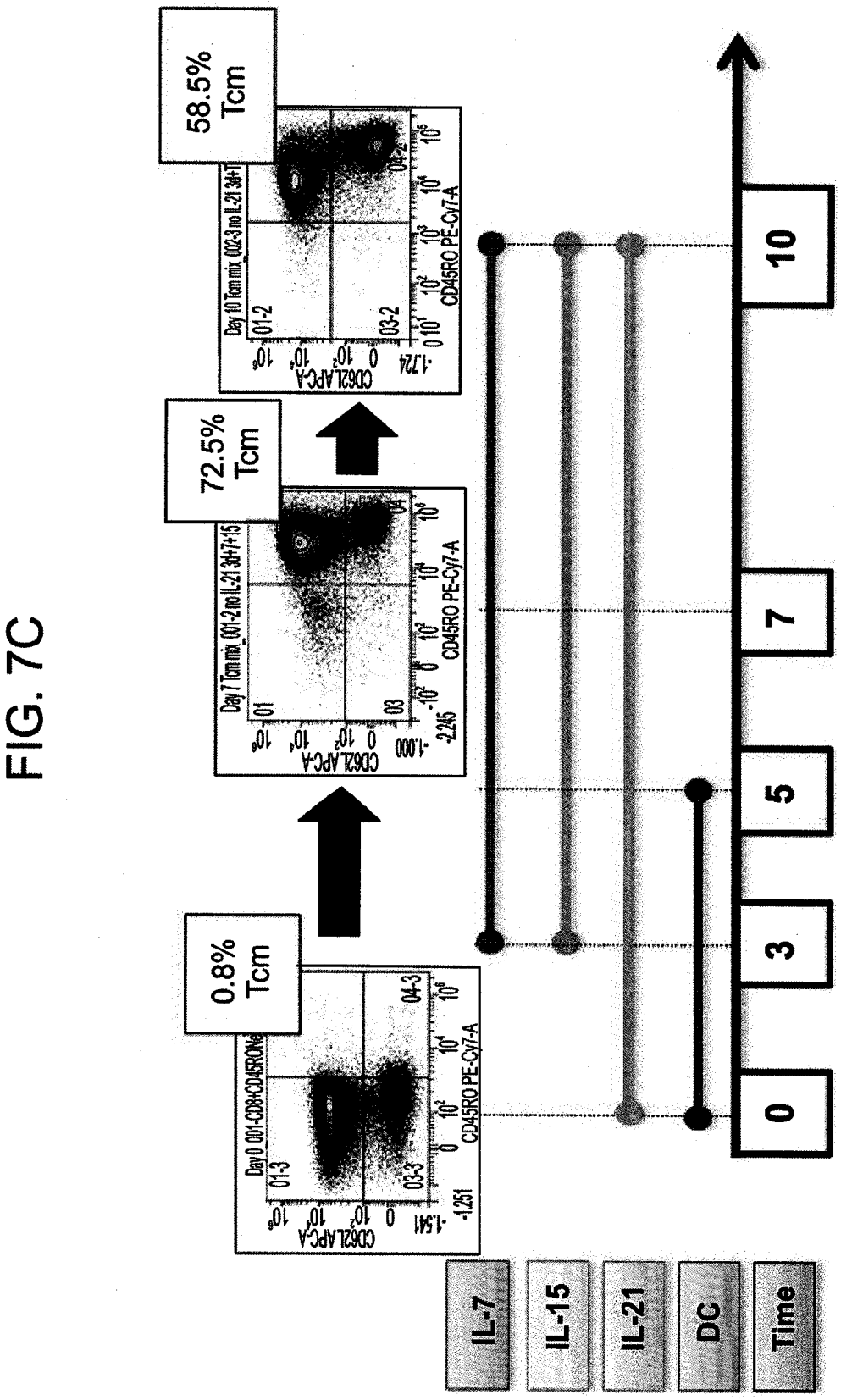

0.8% Tcm
72.5% Tcm
58.5% Tcm
CD62L APC-A
CD45RO PE-Cy7-A
0
3
5
7
10
IL-7
IL-15
IL-21
DC
Time

FIG. 8A

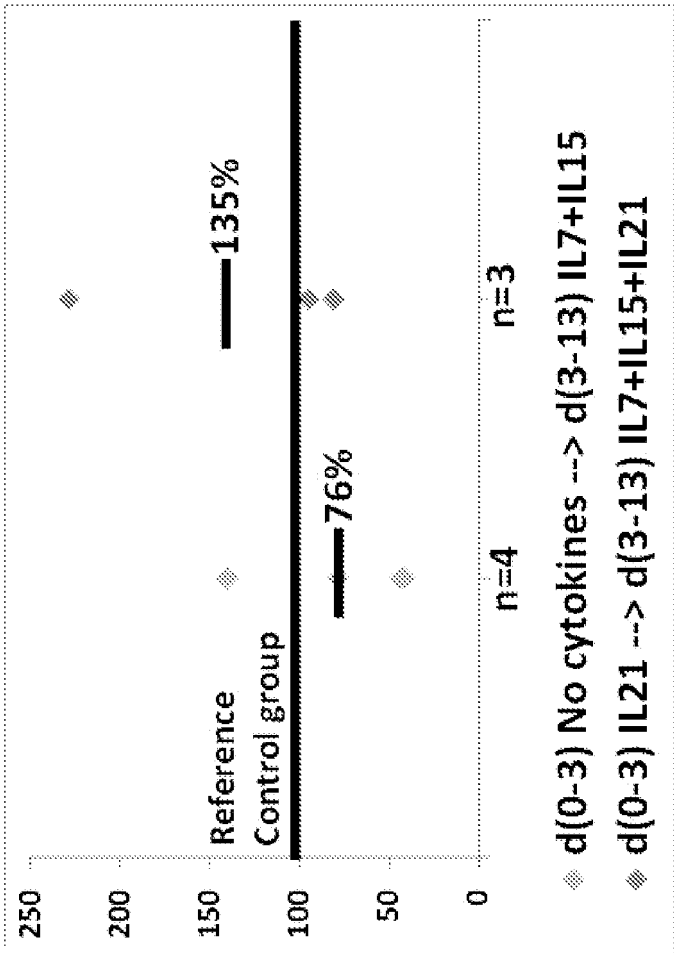

% Of Reference Control Group
250
200
150
100
50
0
Reference
Control group
135%
76%
n=4
n=3
d(0-3) No cytokines --> d(3-13) IL7+IL15
d(0-3) IL21 --> d(3-13) IL7+IL15+IL21

FIG. 8B

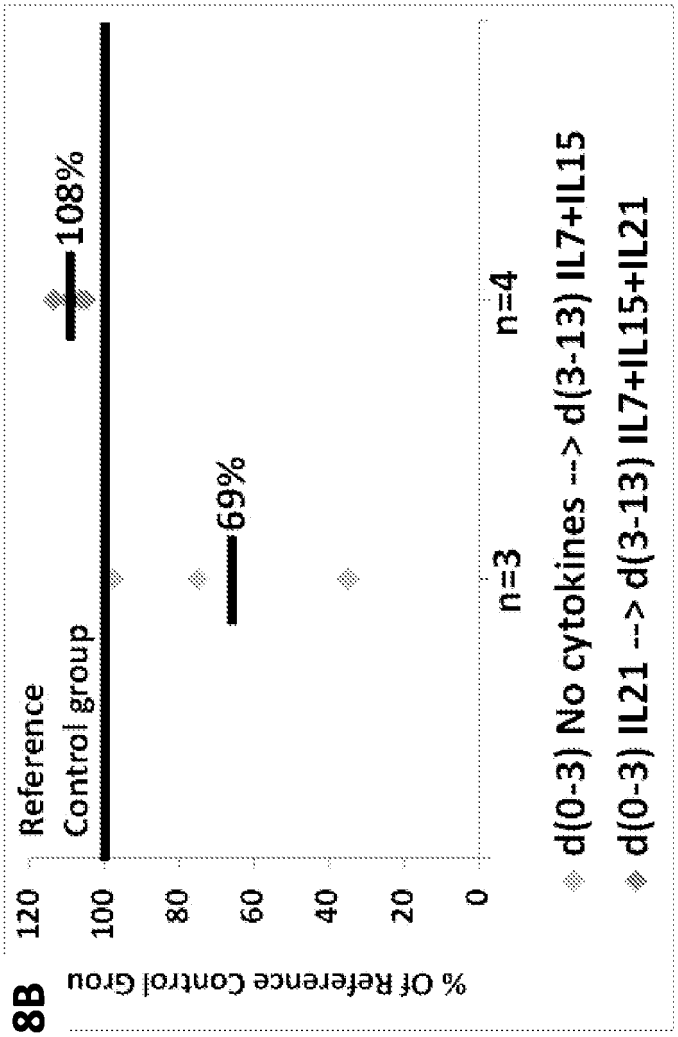

% Of Reference Control Grou
120
100
80
60
40
20
0
Reference
Control group
108%
69%
n=3
n=4
d(0-3) No cytokines --> d(3-13) IL7+IL15
d(0-3) IL21 --> d(3-13) IL7+IL15+IL21

FIG. 9A

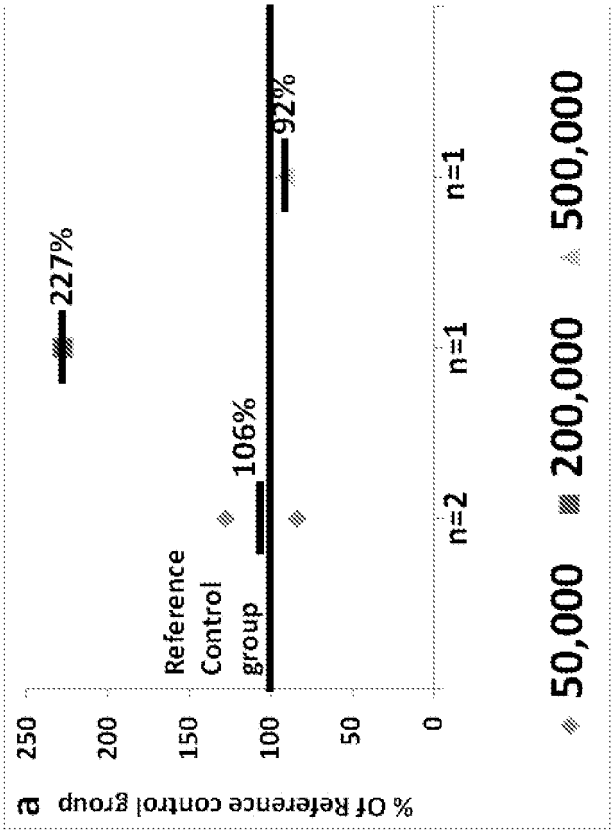
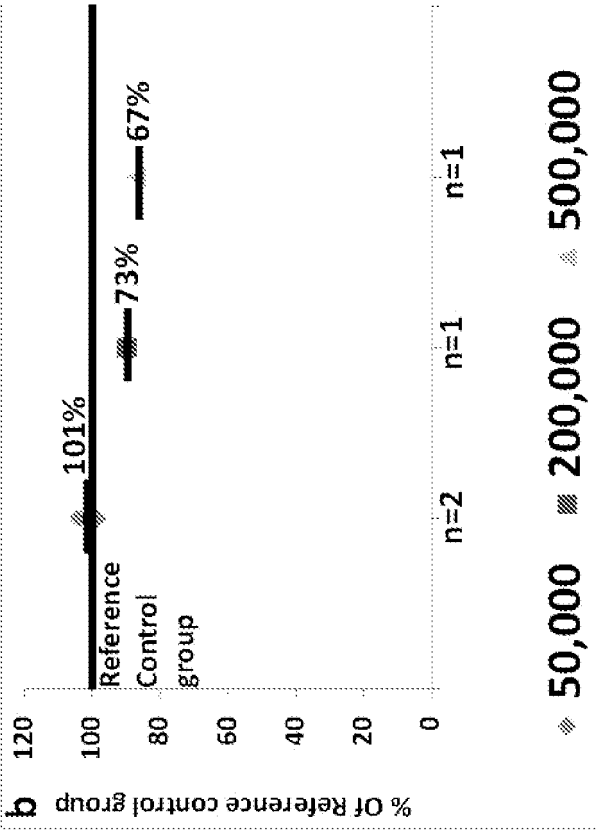

a
% Of Reference control group
250
200
150
100
50
0
227%
106%
92%
Reference Control group
n=2
n=1
n=1
50,000
200,000
500,000

FIG. 9B


b
% Of Reference control group
120
100
80
60
40
20
0
101%
73%
67%
Reference Control group
n=2
n=1
n=1
50,000
200,000
500,000

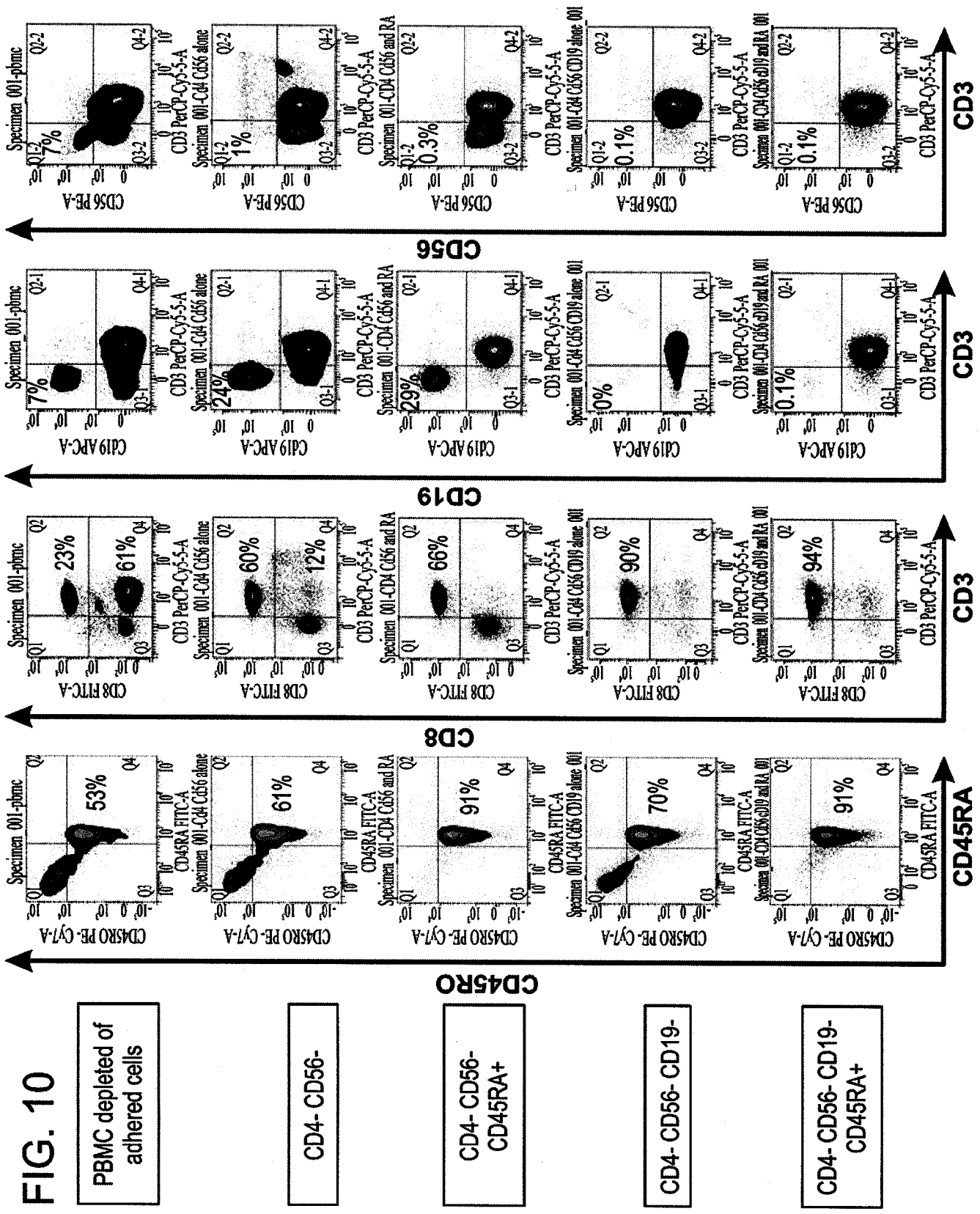

CD45RO
CD8
CD19
CD56
CD3
CD45RA
PBMC depleted of adhered cells
CD4- CD56-
CD4- CD56- CD45RA+
CD4- CD56- CD19-
CD4- CD56- CD19- CD45RA+

FIG. 10

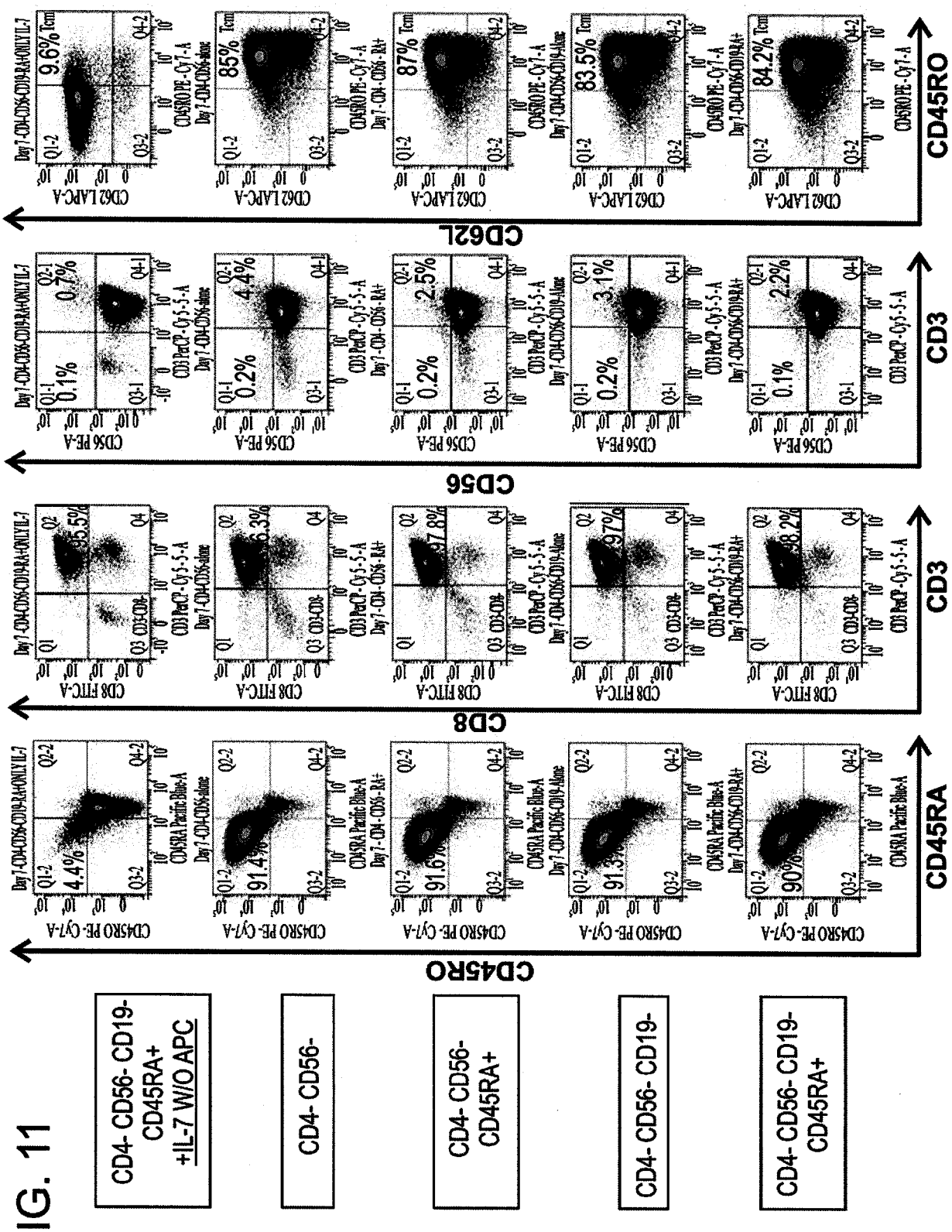

CD45RO
CD8
CD56
CD62L
CD45RA
CD3
CD45RO
CD4- CD56- CD19- CD45RA+ +IL-7 W/O APC
CD4- CD56-
CD4- CD56- CD45RA+
CD4- CD56- CD19-
CD4- CD56- CD19- CD45RA+

FIG. 11

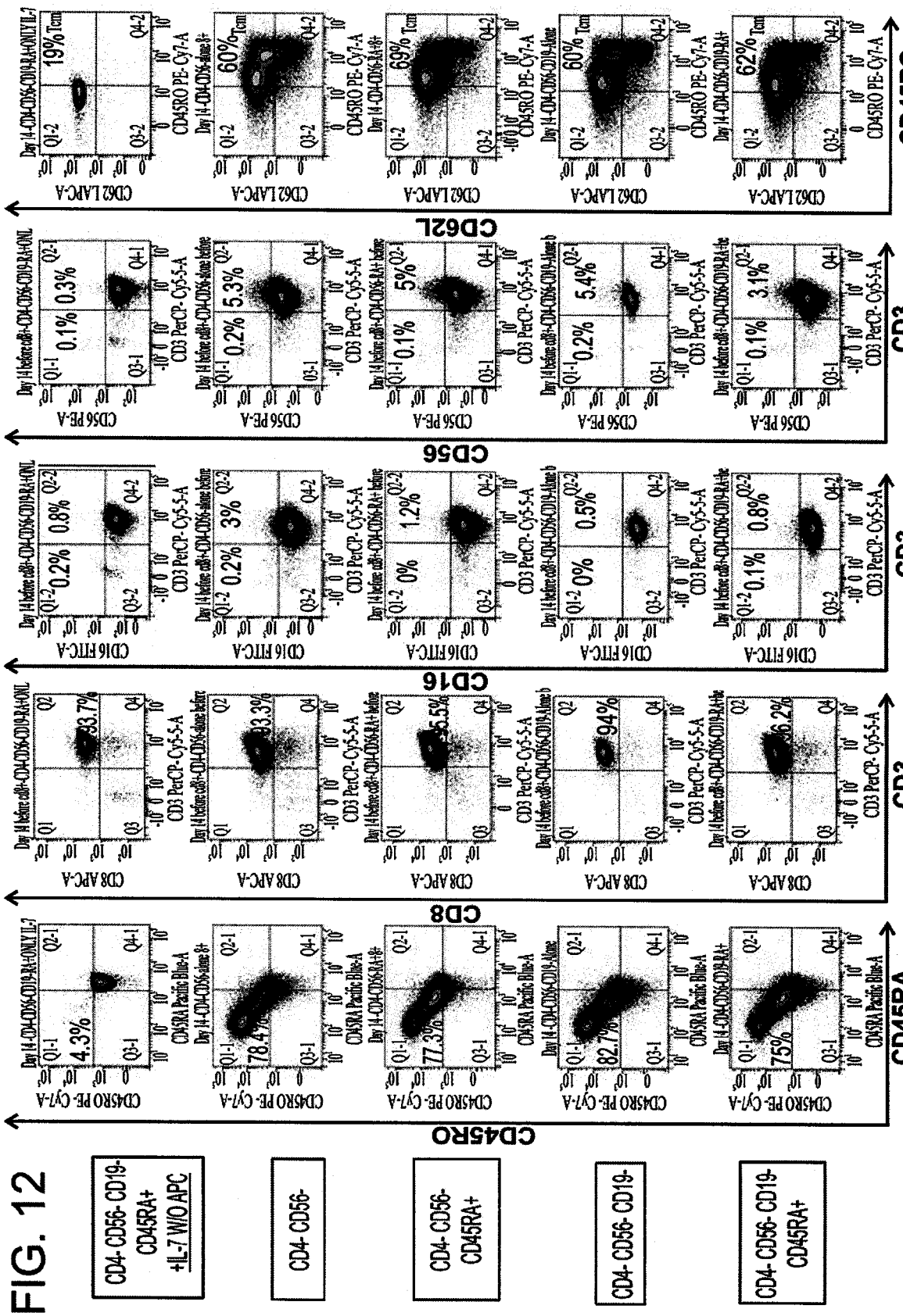

CD45RO
CD8
CD16
CD56
CD62L
CD45RA
CD3
CD4- CD56- CD19- CD45RA+ +IL-7 W/O APC
CD4- CD56-
CD4- CD56- CD45RA+
CD4- CD56- CD19-
CD4- CD56- CD19- CD45RA+

FIG. 12

FIG. 13A

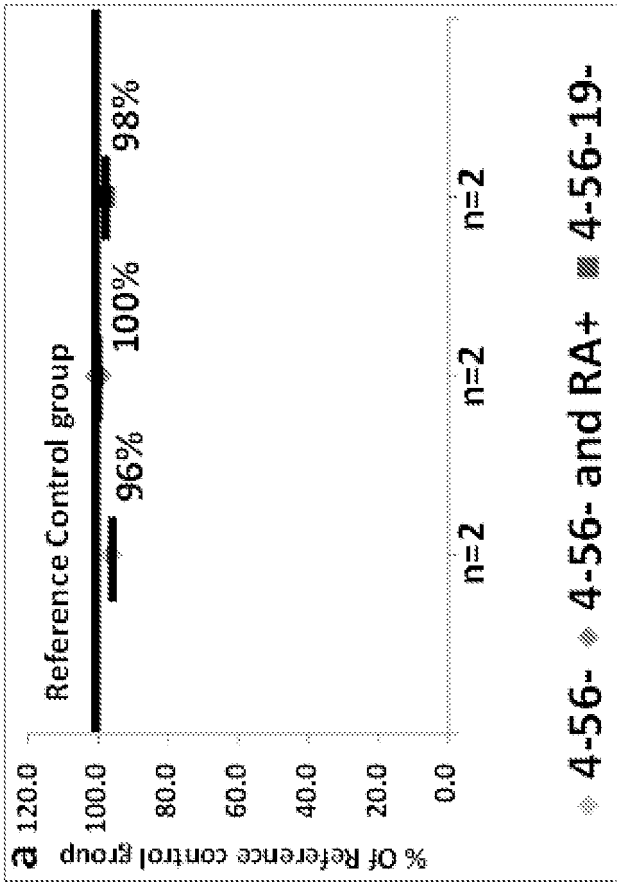
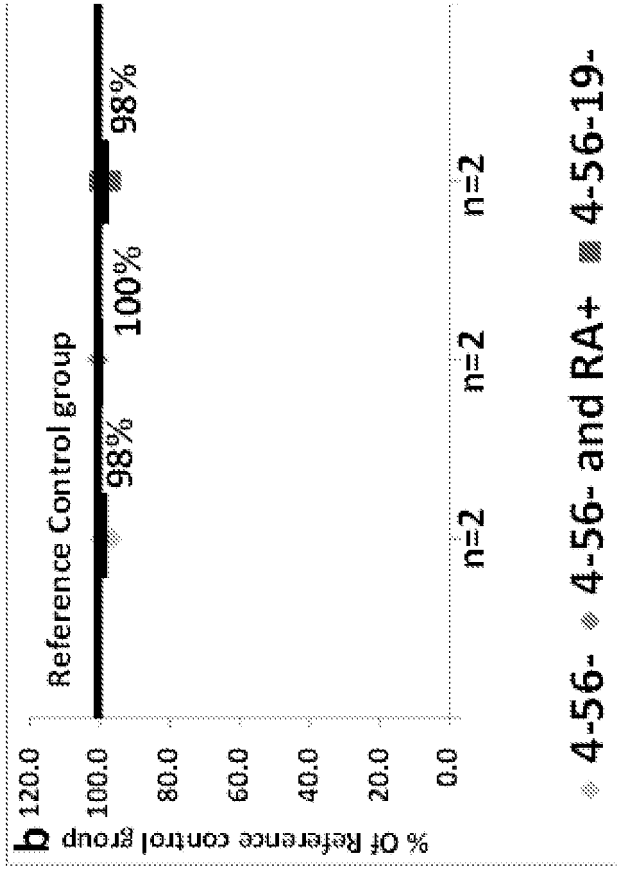


FIG. 13B



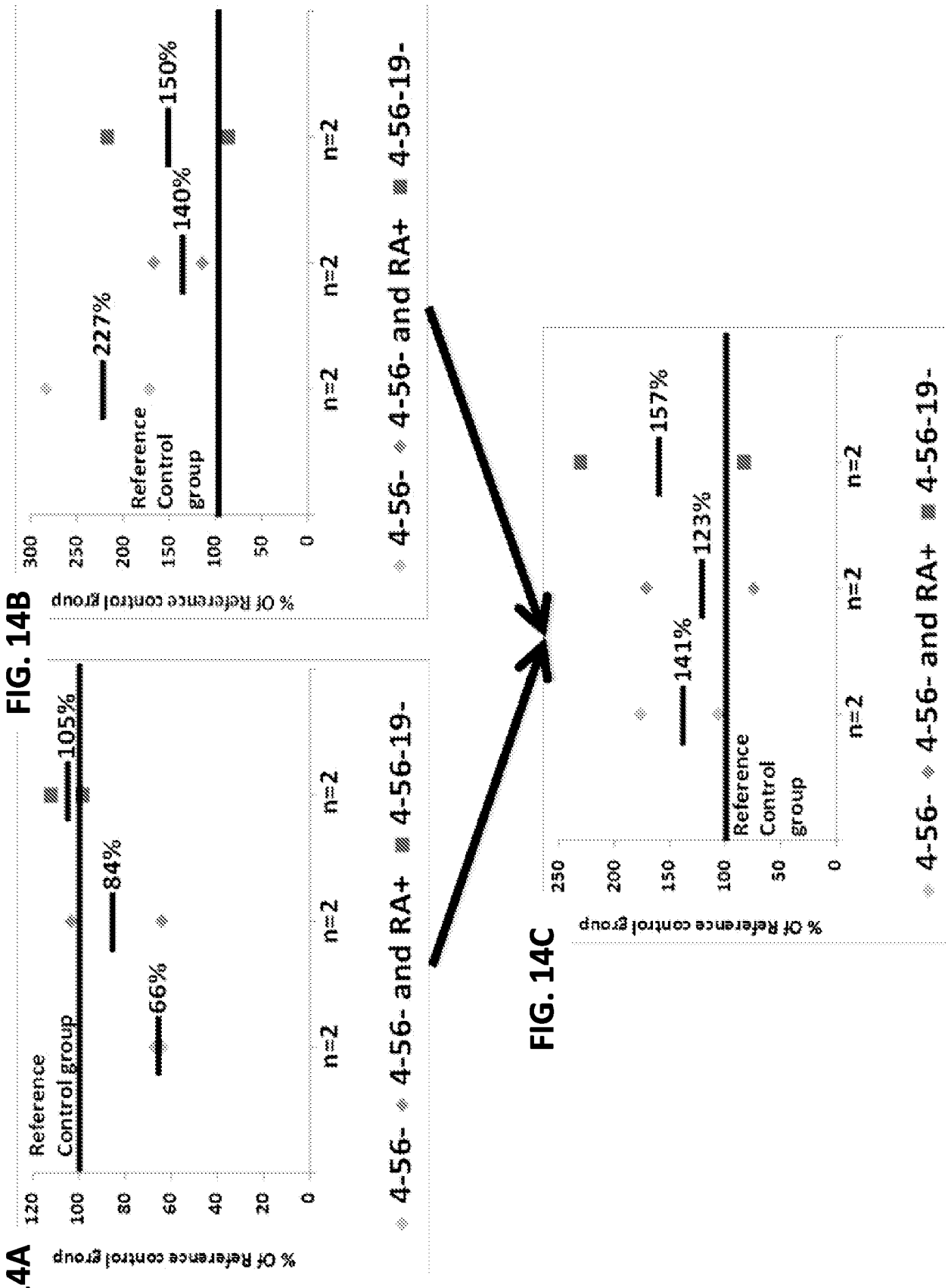


FIG. 14A

FIG. 14B

FIG. 14C

FIG. 15

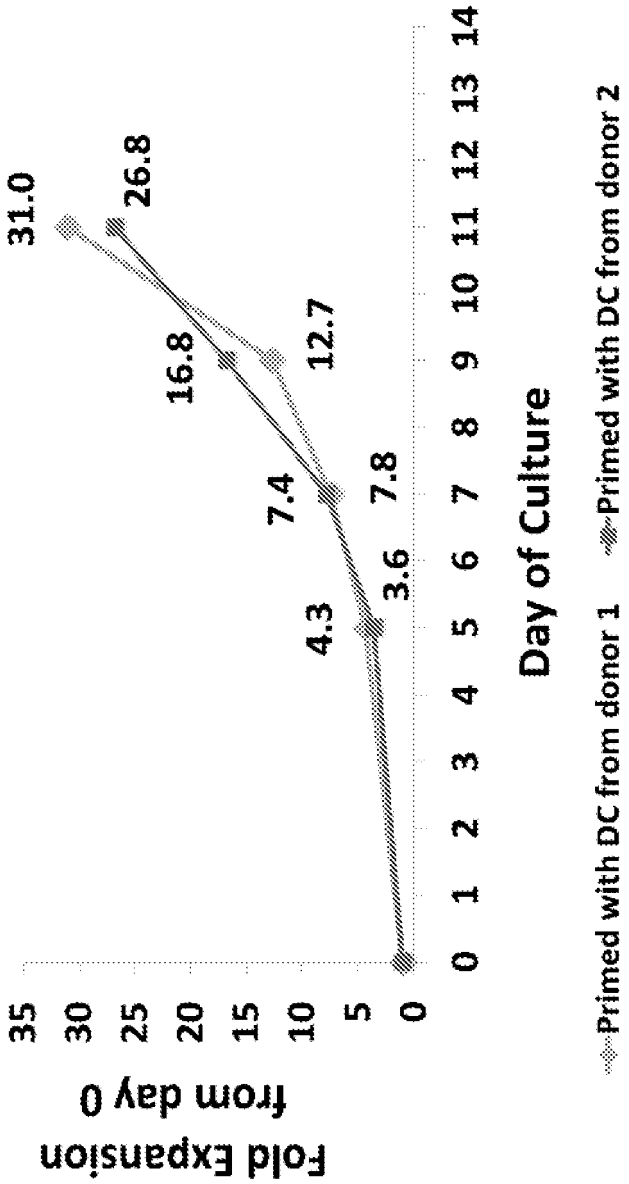
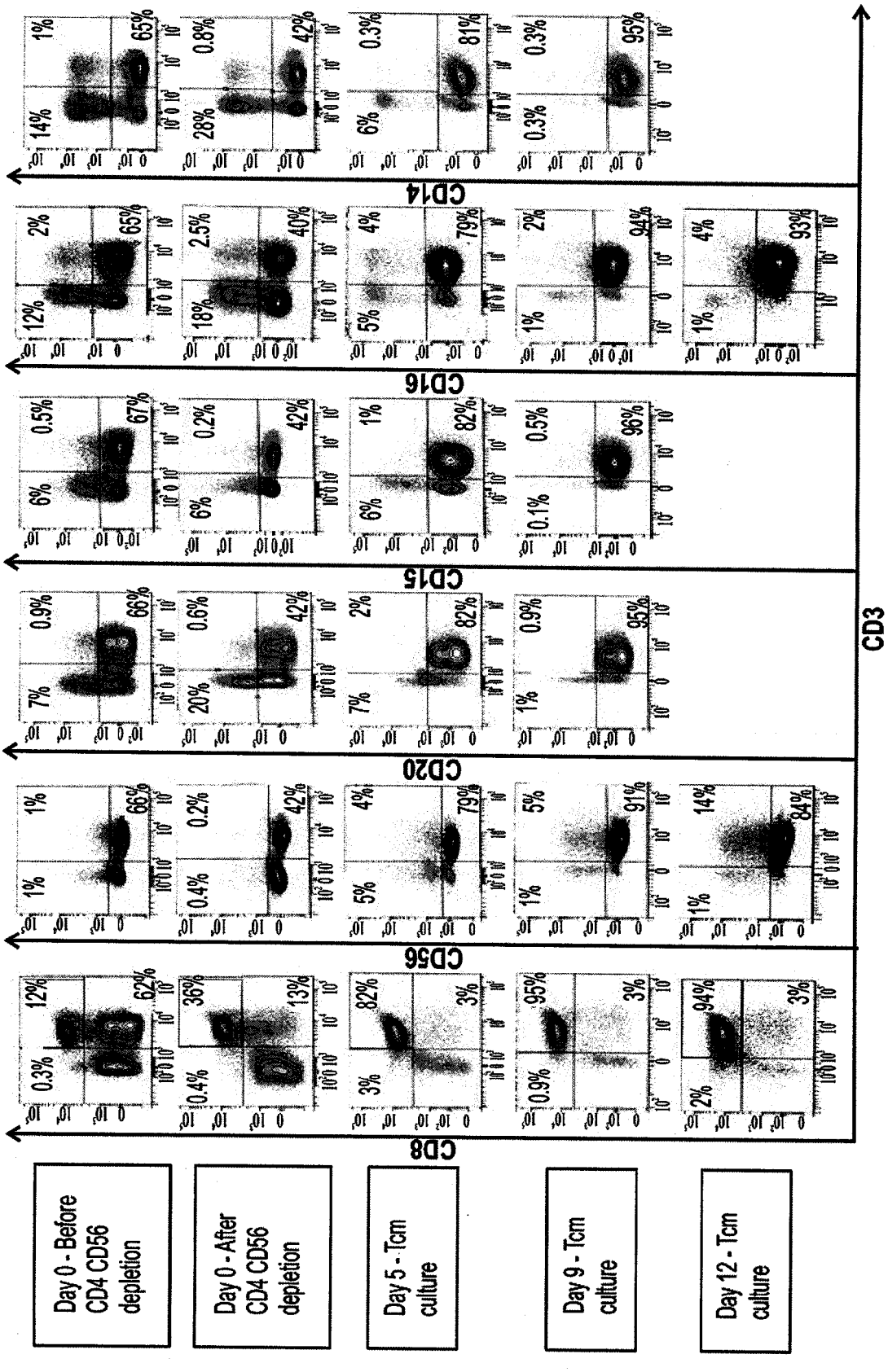

Fold Expansion from day 0
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day of Culture
31.0
26.8
16.8
12.7
7.4
7.8
4.3
3.6
Primed with DC from donor 1
Primed with DC from donor 2

FIG. 16A


Day 0 - Before CD4 CD56 depletion
Day 0 - After CD4 CD56 depletion
Day 5 - Tcm culture
Day 9 - Tcm culture
Day 12 - Tcm culture
CD8
CD56
CD20
CD15
CD16
CD14
CD3

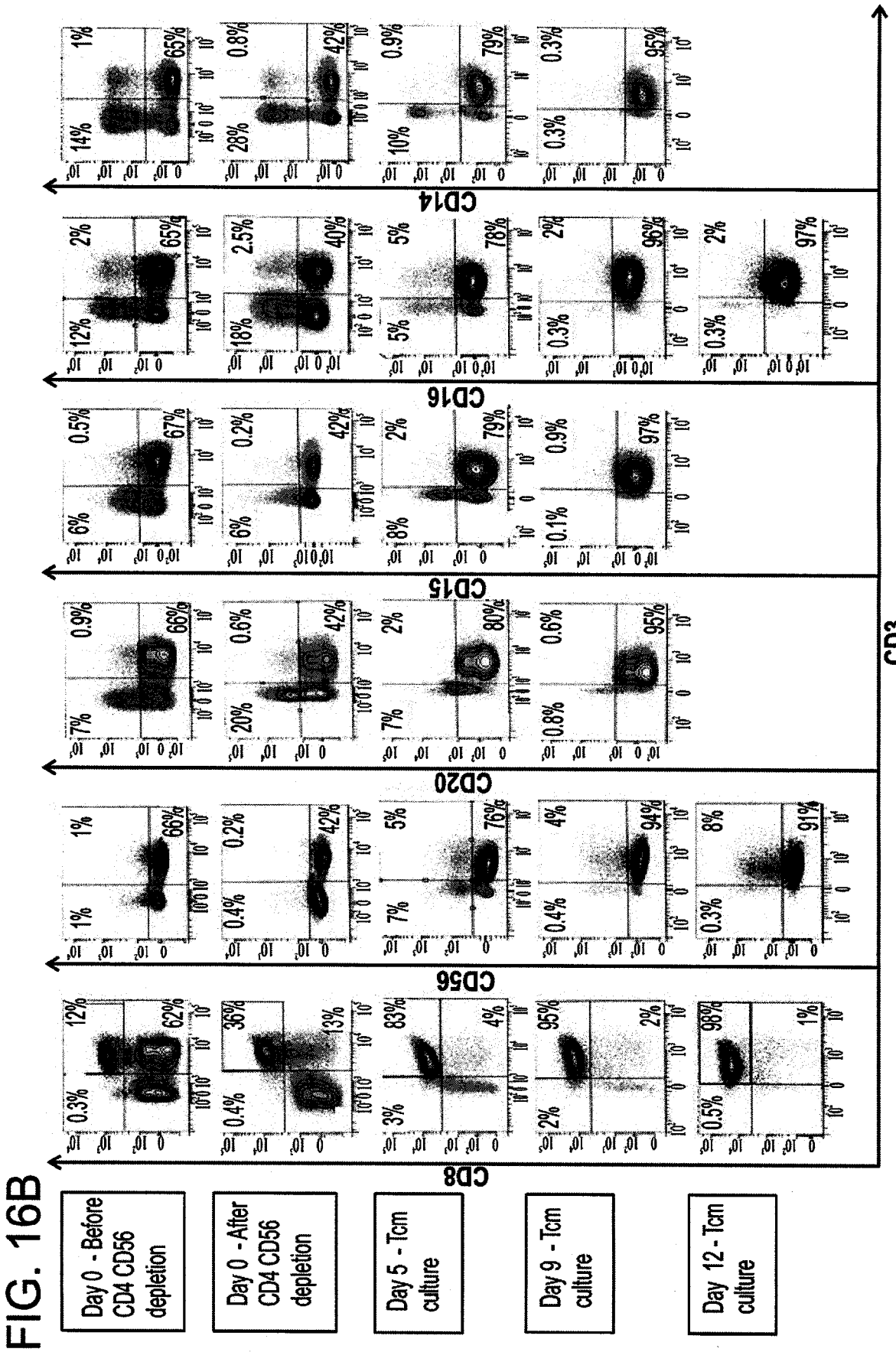

CD3
CD14
CD16
CD15
CD20
CD56
CD8
Day 0 - Before CD4 CD56 depletion
Day 0 - After CD4 CD56 depletion
Day 5 - Tcm culture
Day 9 - Tcm culture
Day 12 - Tcm culture

FIG. 16B

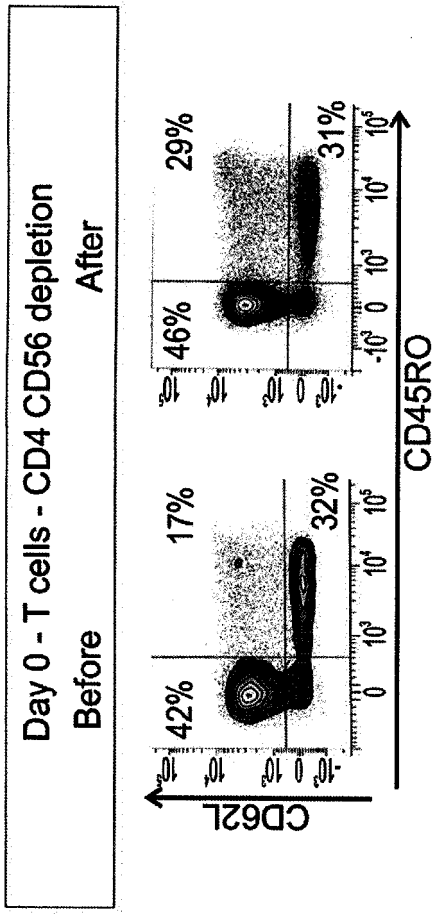


FIG. 17A

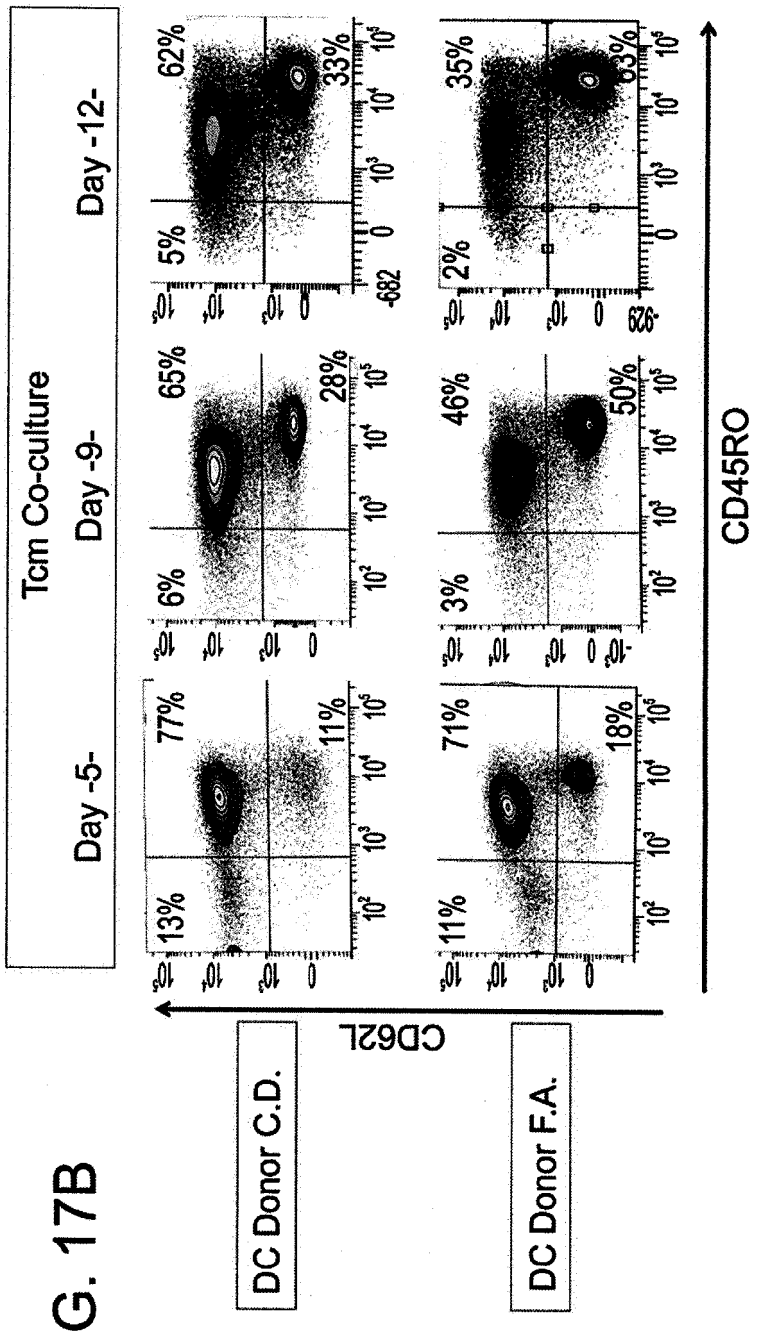
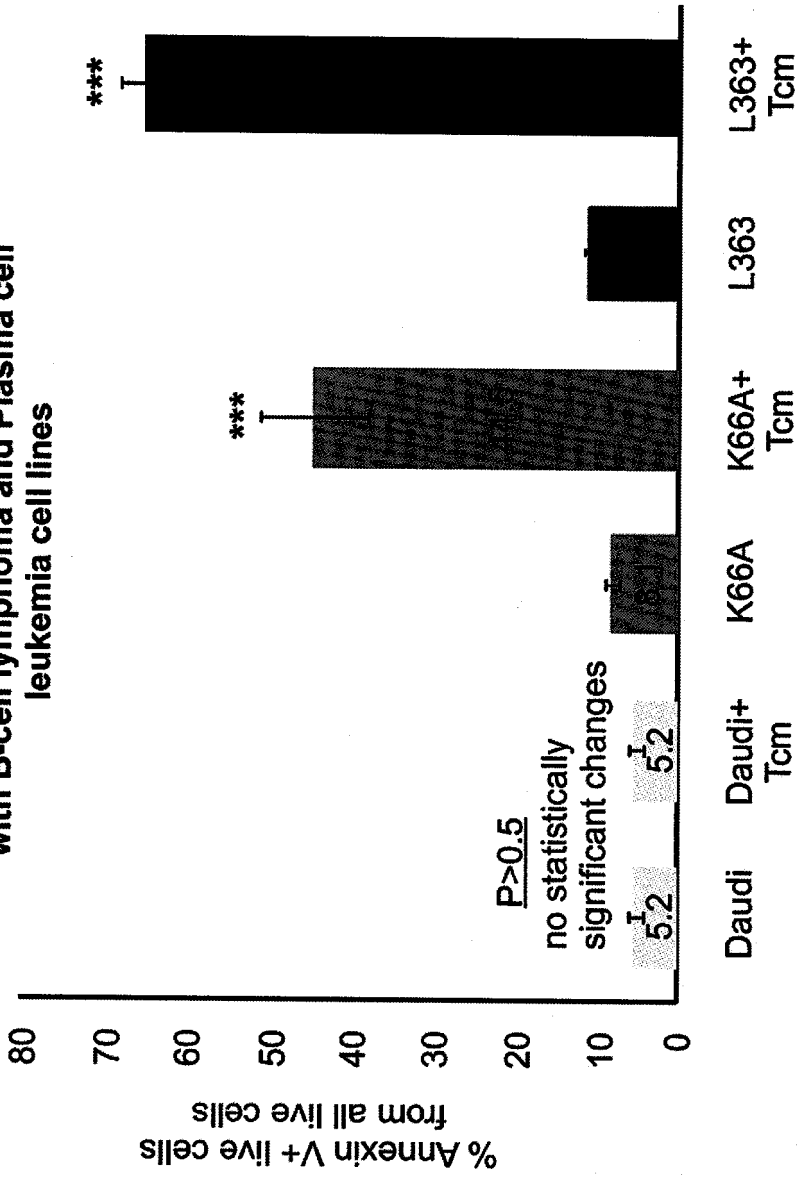


FIG. 17B

FIG. 17C


% Apoptotic cells after 22h of MLR
with B-cell lymphoma and Plasma cell
leukemia cell lines
% Annexin V+ live cells
from all live cells
80
70
60
50
40
30
20
10
0
***
***
P>0.5
no statistically
significant changes
5.2
5.2
Daudi
Daudi+
Tcm
K66A
K66A+
Tcm
L363
L363+
Tcm

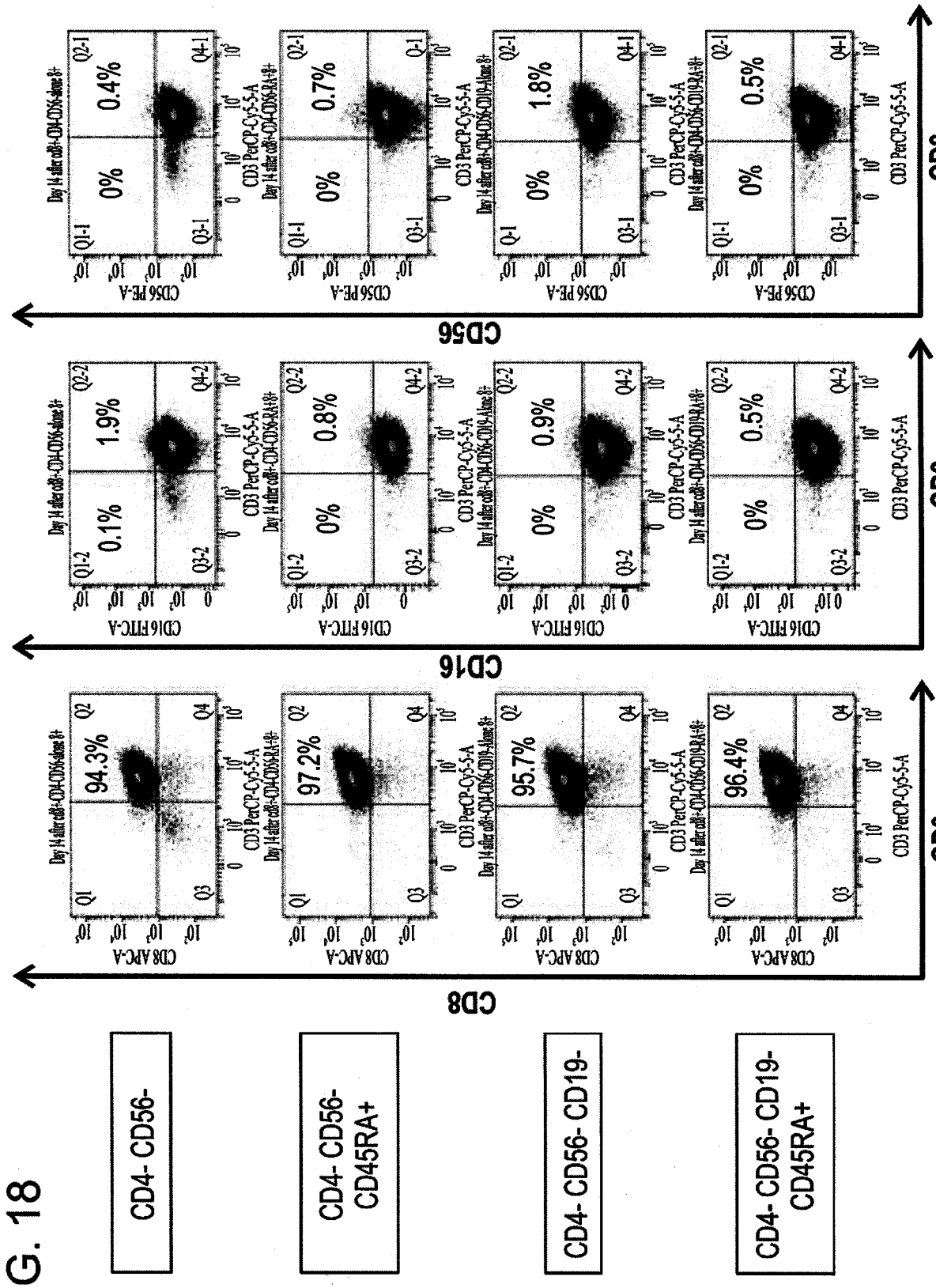

CD8
CD16
CD56
CD3
CD4- CD56-
CD4- CD56- CD45RA+
CD4- CD56- CD19-
CD4- CD56- CD19- CD45RA+

FIG. 18

FIG. 19A

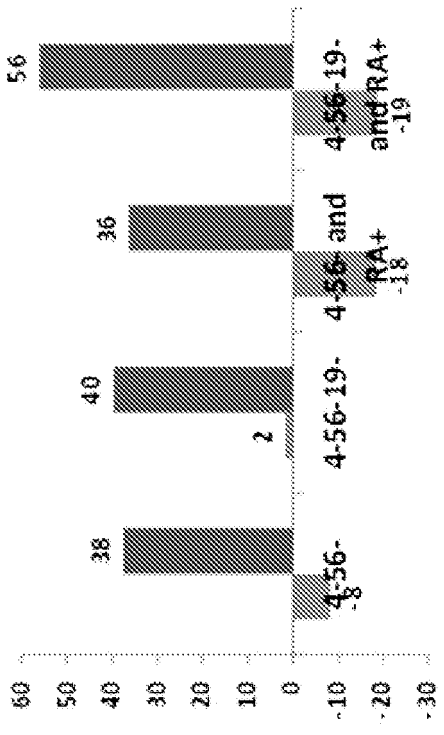


FIG. 19B

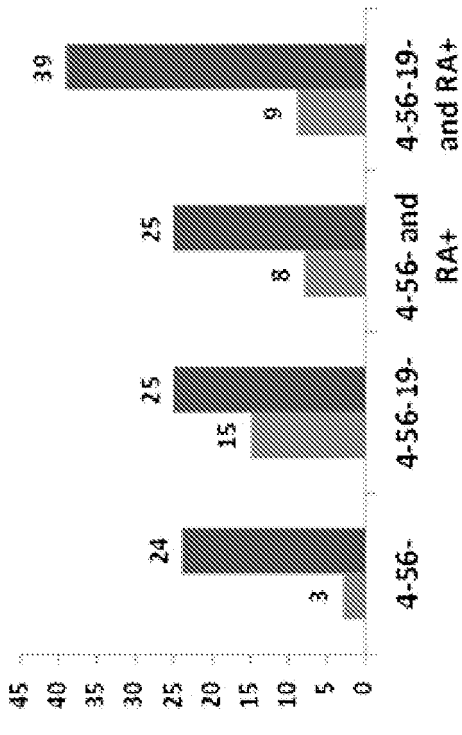


FIG. 19C

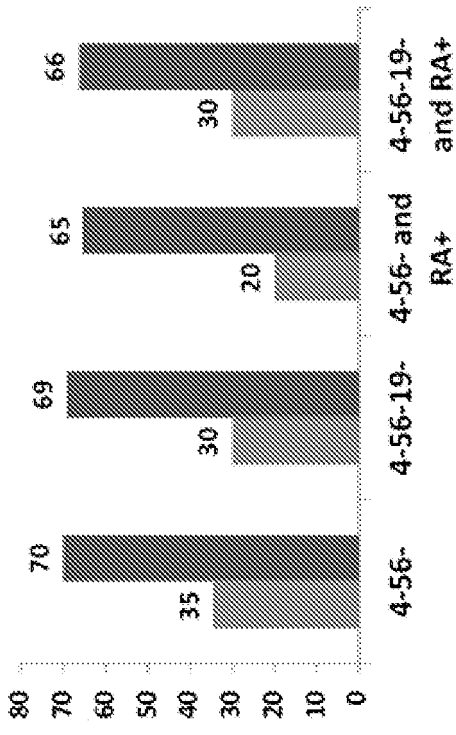


FIG. 19D

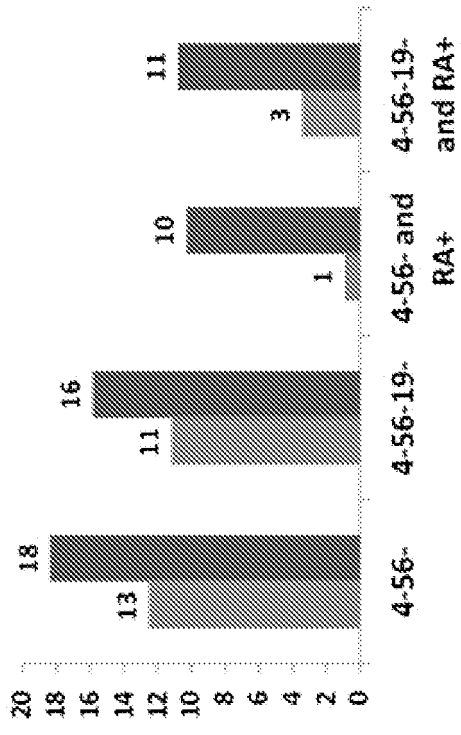


FIG. 20

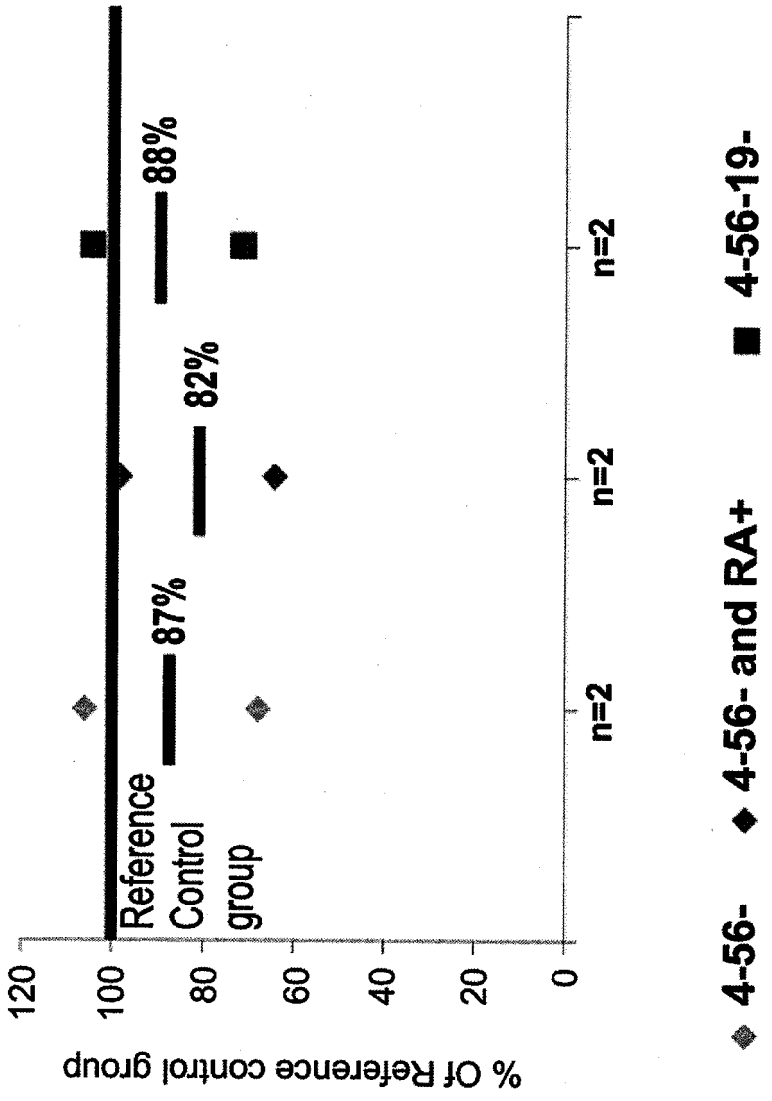

% Of Reference control group
120
100
80
60
40
20
0
Reference Control group
87%
82%
88%
n=2
n=2
n=2
4-56-
4-56- and RA+
4-56-19-

FIG. 21

Autologous Human Protocol for generation of Tcm

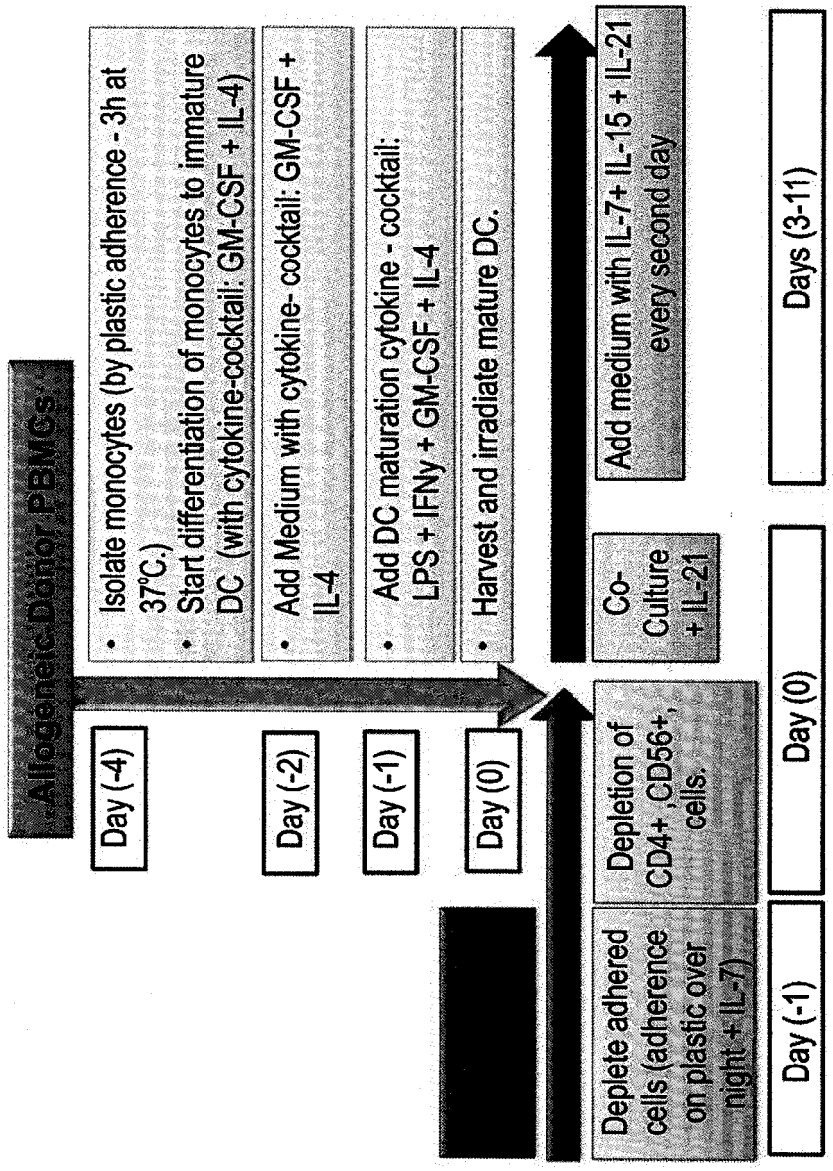
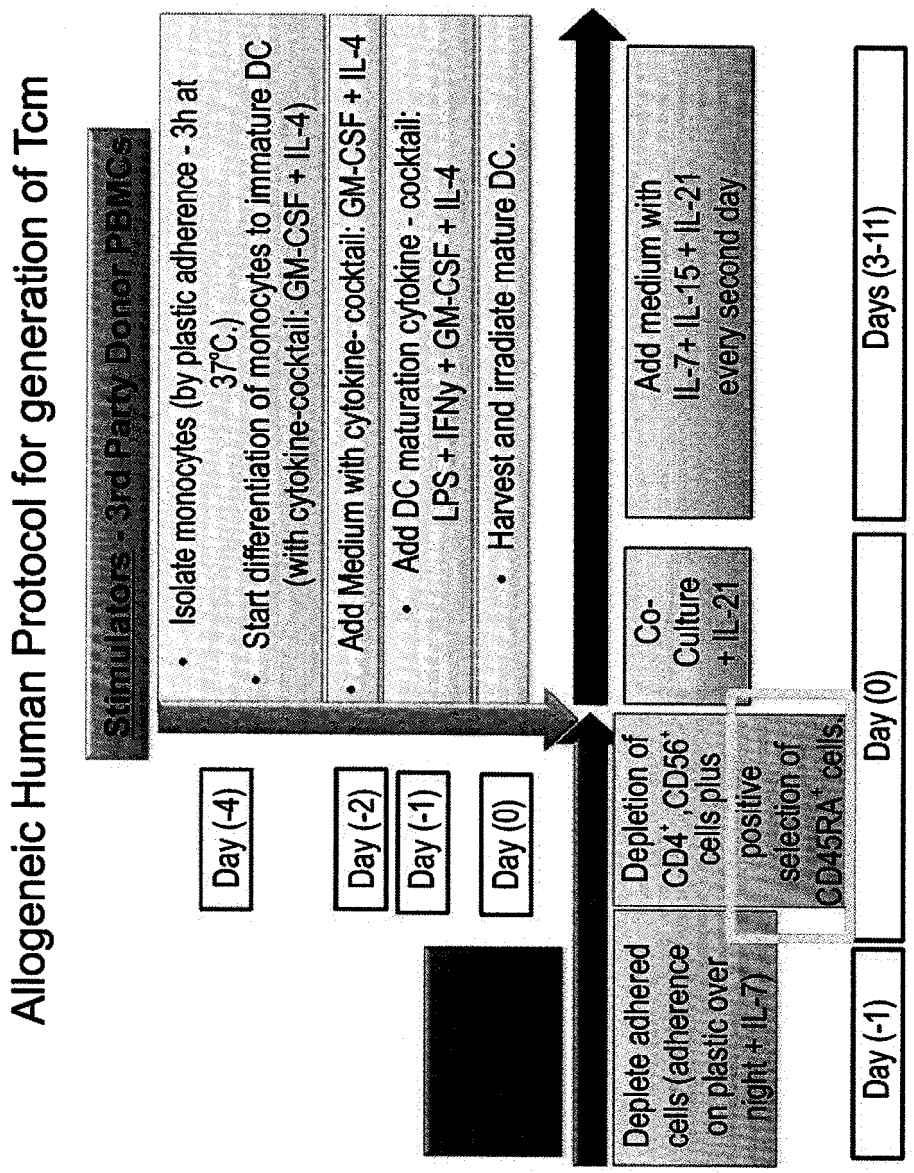


FIG. 22


Allogeneic Human Protocol for generation of Tcm
Stimulators - 3rd Party Donor PBMCs
Isolate monocytes (by plastic adherence - 3h at 37°C.)
Start differentiation of monocytes to immature DC (with cytokine-cocktail: GM-CSF + IL-4)
Add Medium with cytokine- cocktail: GM-CSF + IL-4
Add DC maturation cytokine - cocktail: LPS + IFNγ + GM-CSF + IL-4
Harvest and irradiate mature DC.
Day (-4)
Day (-2)
Day (-1)
Day (0)
Deplete adhered cells (adherence on plastic over night + IL-7)
Depletion of CD4+,CD56+ cells plus positive selection of CD45RA+ cells.
Co-Culture + IL-21
Add medium with IL-7+ IL-15 + IL-21 every second day
Day (-1)
Day (0)
Days (3-11)

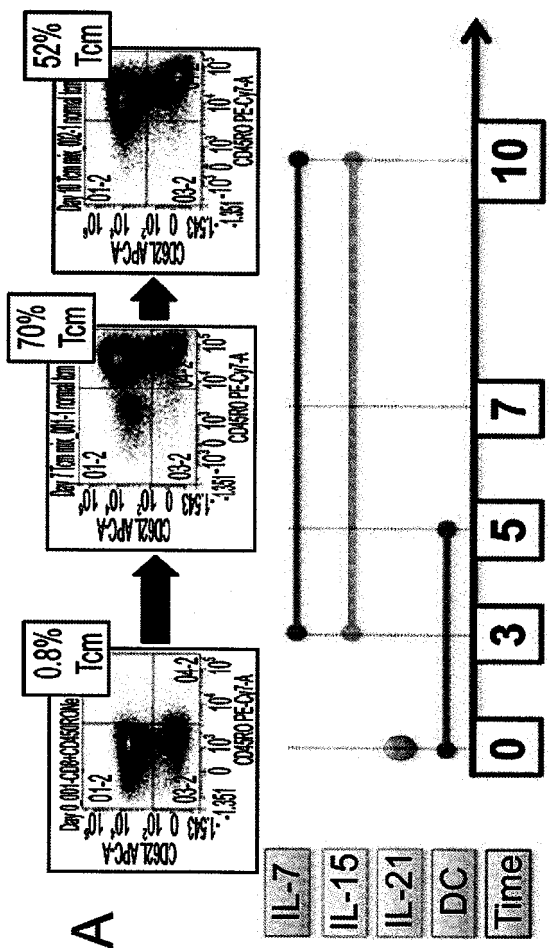


FIG. 23A

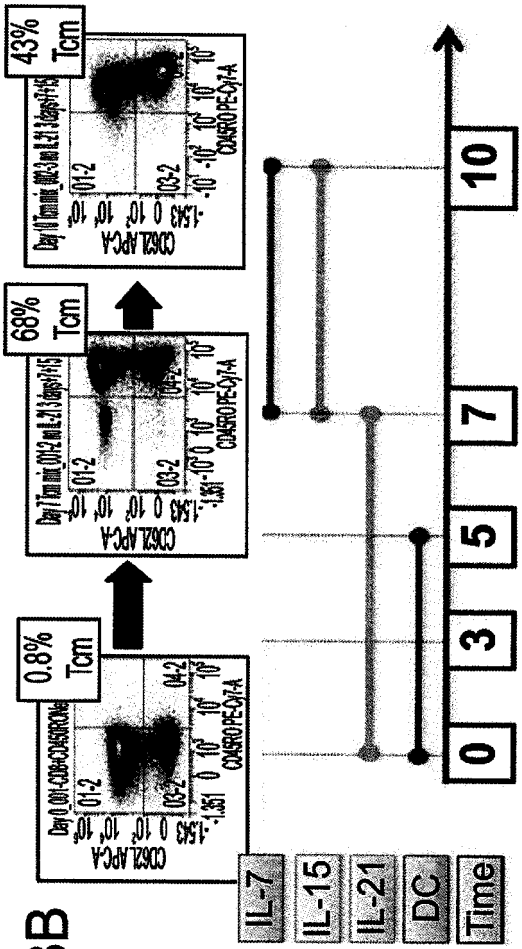


FIG. 23B

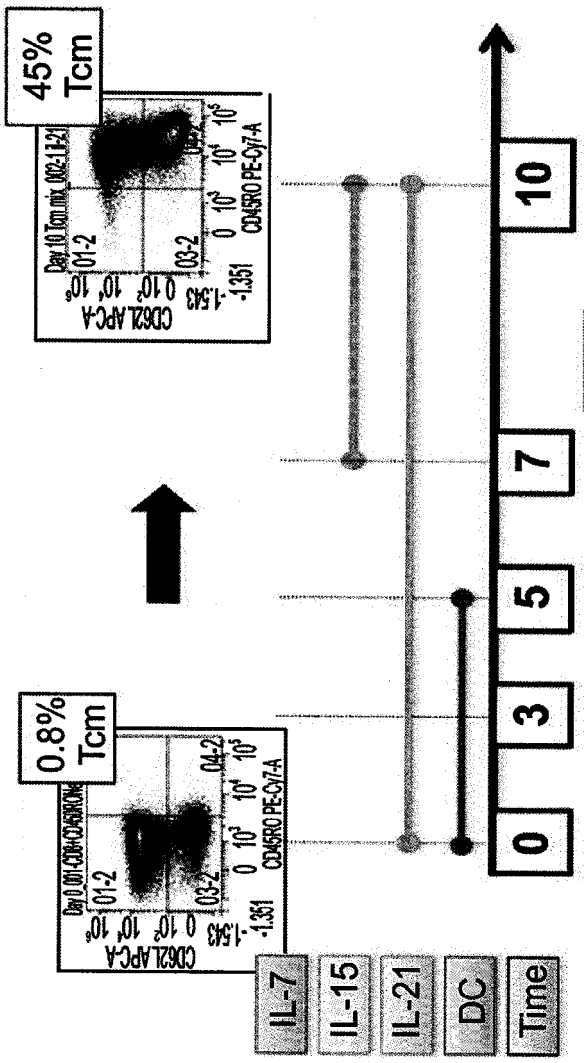


FIG. 23C

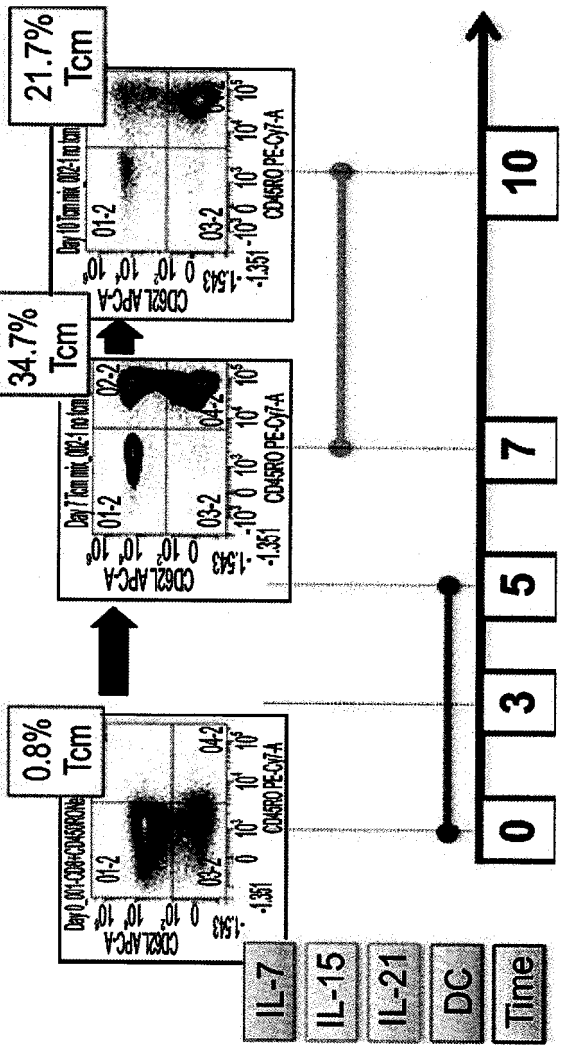


FIG. 23D

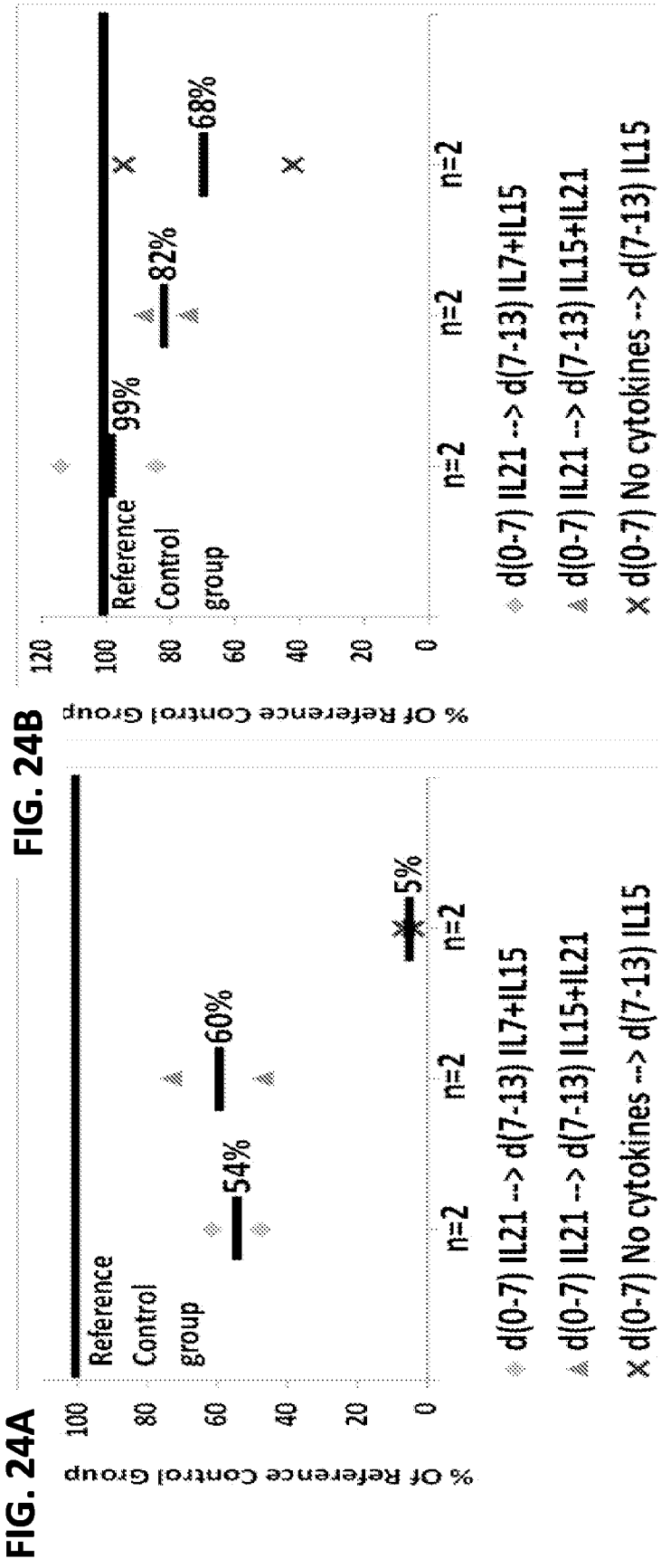


FIG. 24A FIG. 24B

FIG. 25

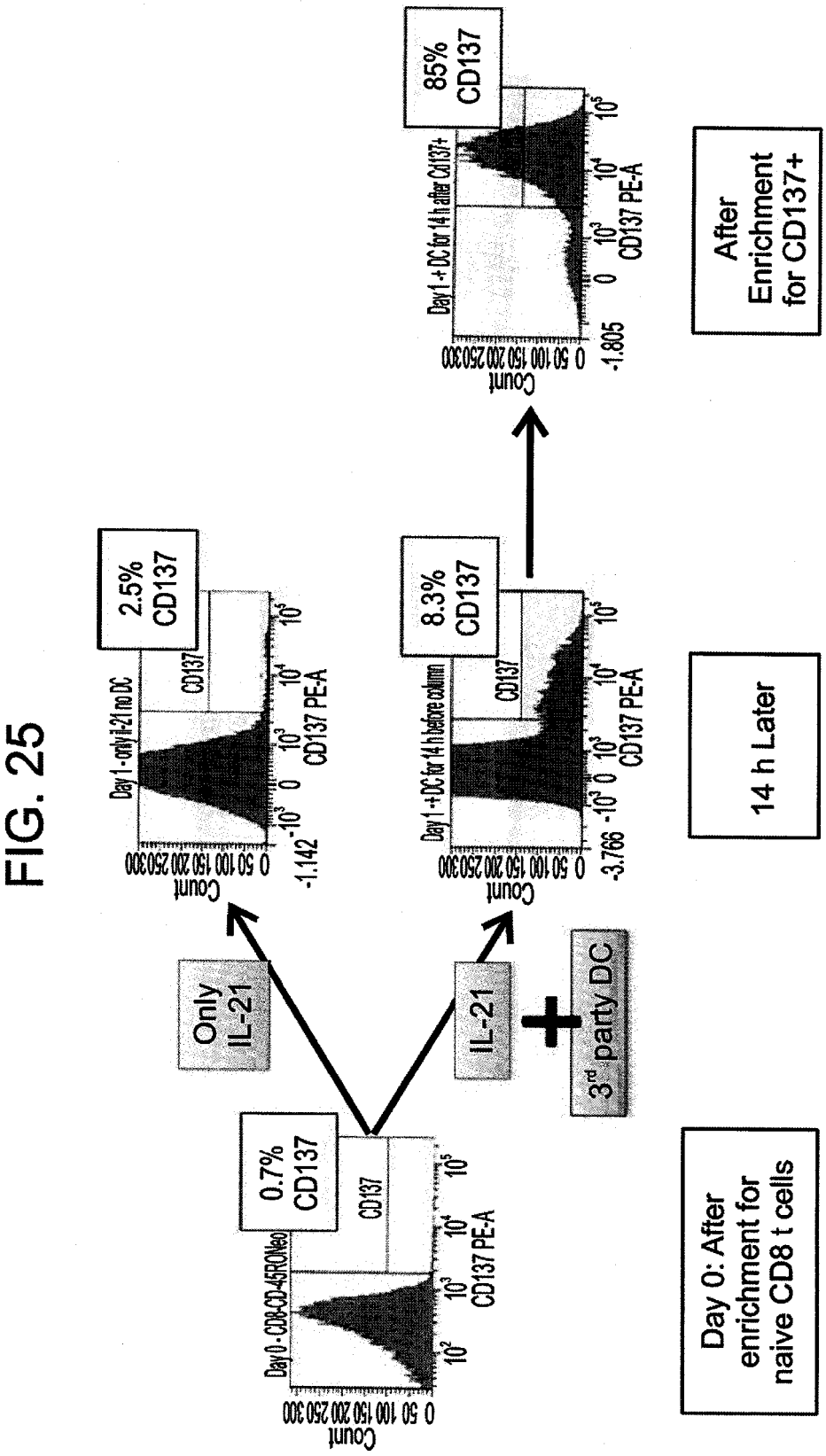

Day 0: After enrichment for naive CD8 t cells
0.7% CD137
Day 0 - CD8-CD-45RONeg
CD137
CD137 PE-A
Count
Only IL-21
IL-21
3rd party DC
14 h Later
2.5% CD137
Day 1 - only IL-21 no DC
-1.142
8.3% CD137
Day 1 - + DC for 14 h before column
-3.766
After Enrichment for CD137+
85% CD137
Day 1 - + DC for 14 h after CD137+
-1.805

FIG. 26

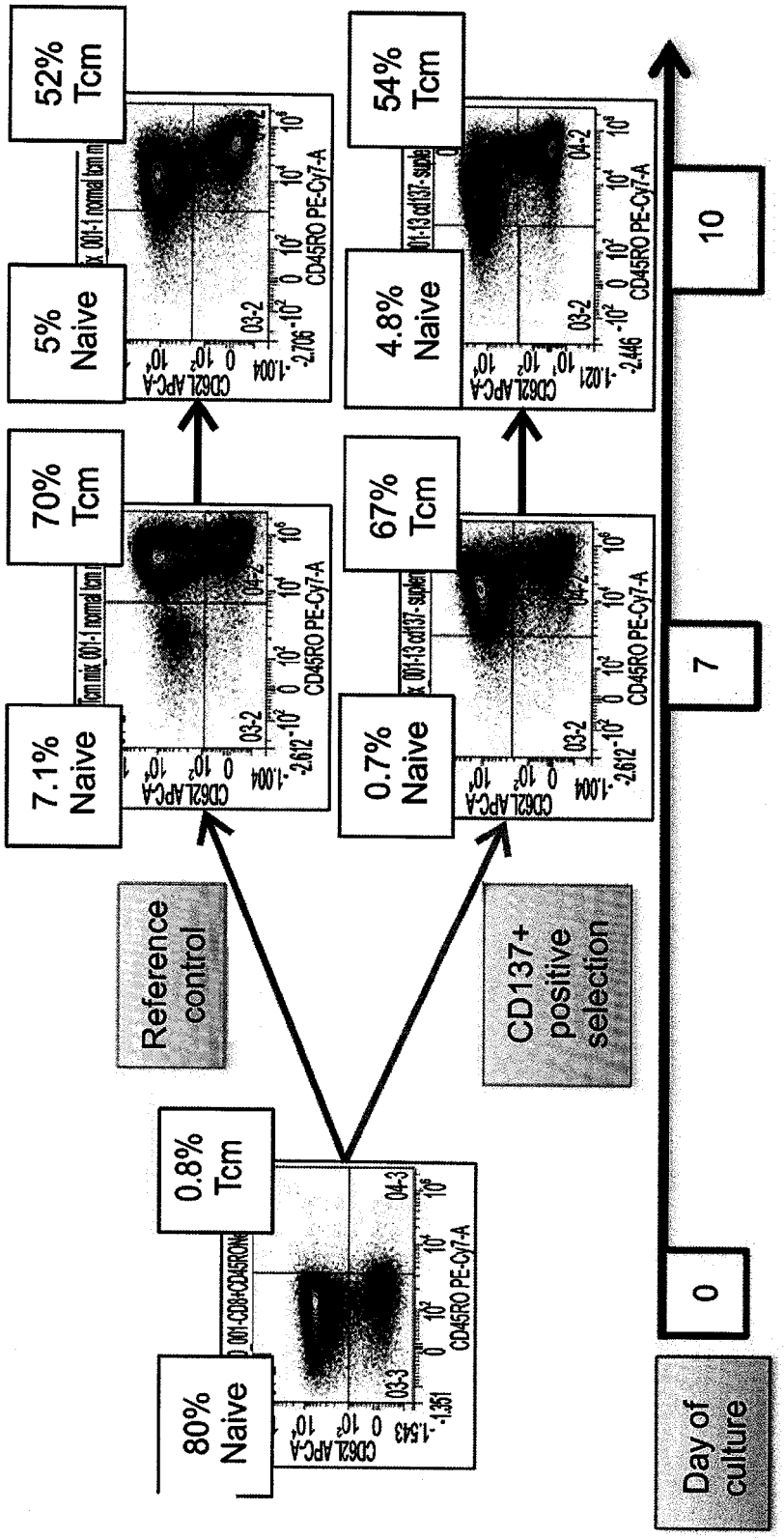

80% Naive
0.8% Tcm
Reference control
7.1% Naive
70% Tcm
5% Naive
52% Tcm
CD137+ positive selection
0.7% Naive
67% Tcm
4.8% Naive
54% Tcm
Day of culture
0
7
10

FIG. 27

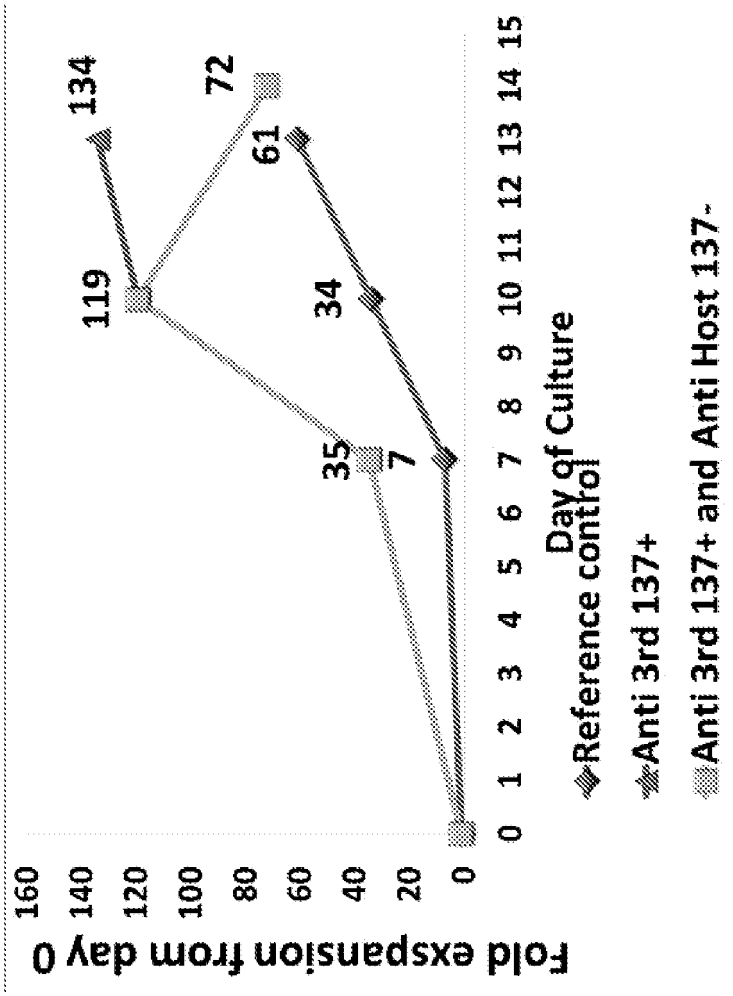

Fold exspansion from day 0
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Day of Culture
Reference control
Anti 3rd 137+
Anti 3rd 137+ and Anti Host 137-
134
119
72
61
34
35
7

FIG. 28

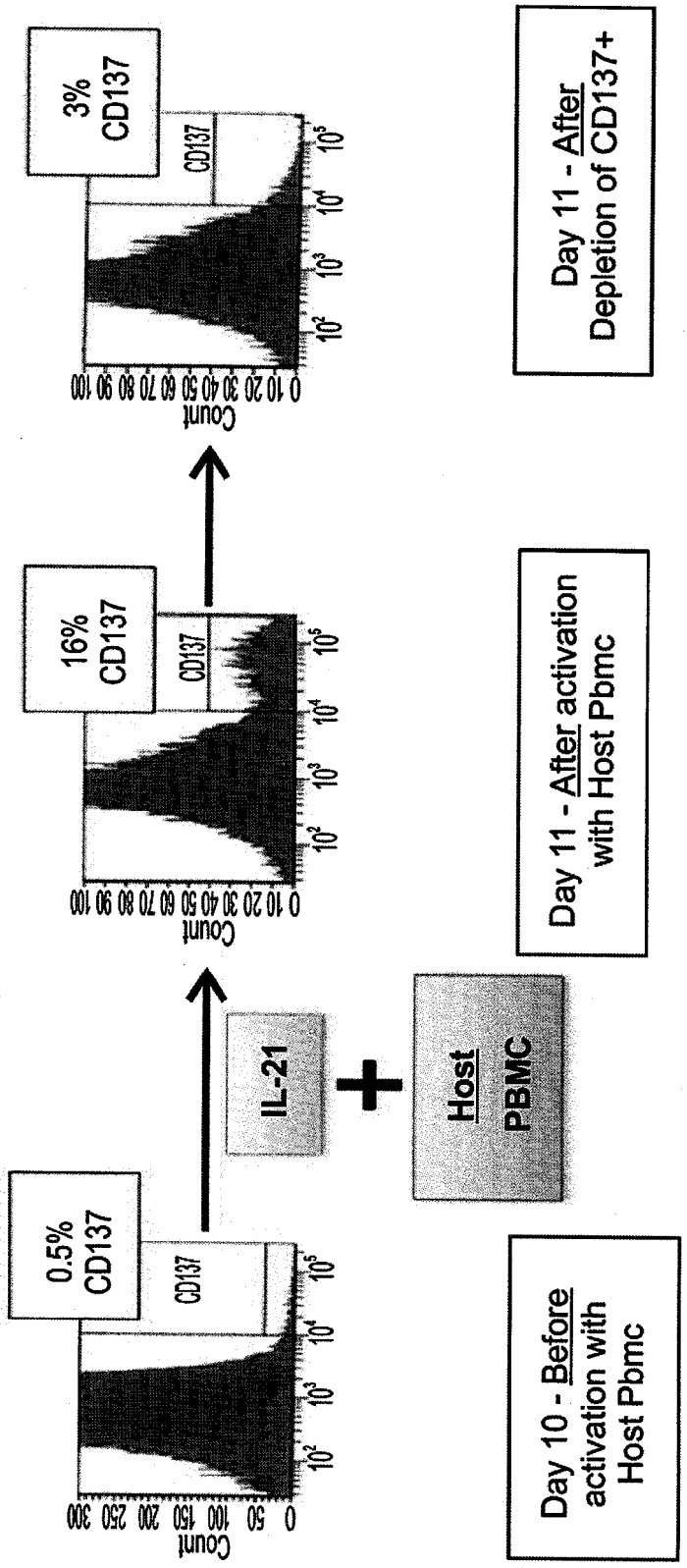
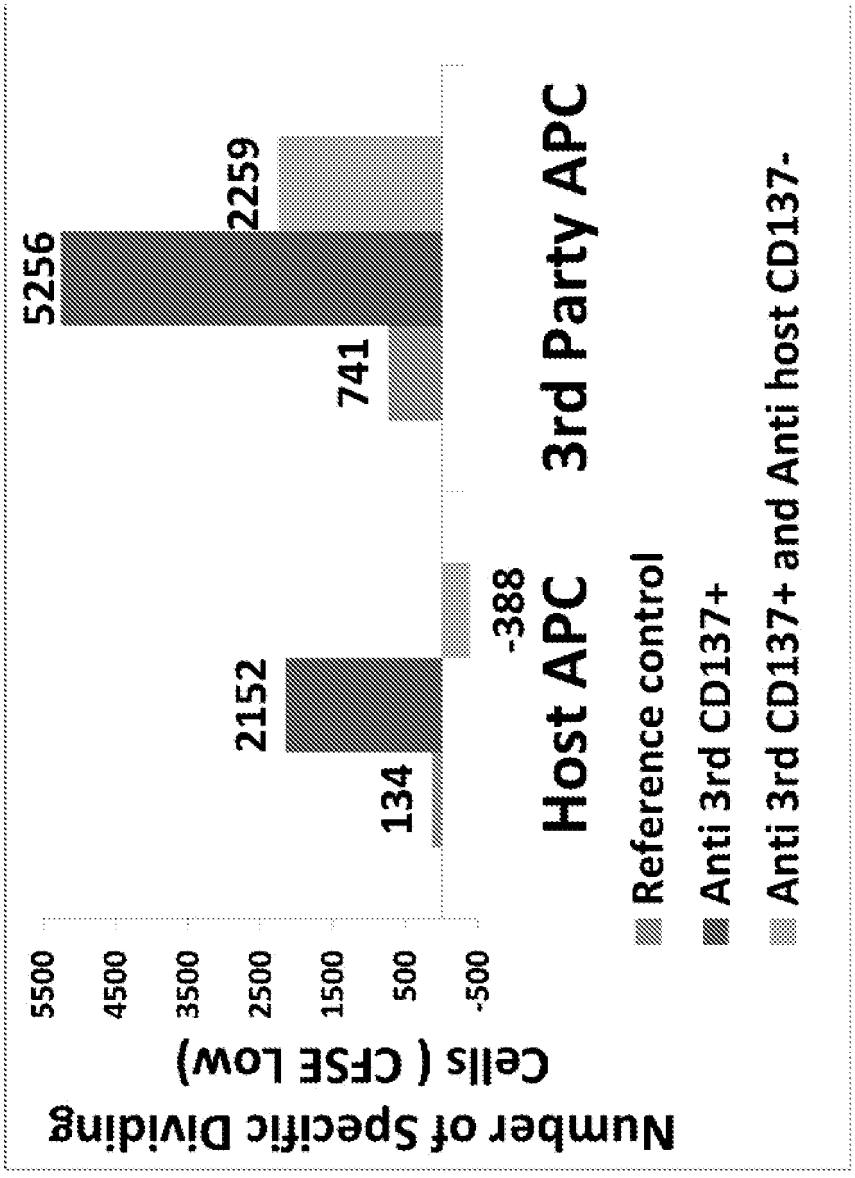

Day 10 - Before activation with Host Pbmc
0.5% CD137
CD137
Count
IL-21
Host PBMC
Day 11 - After activation with Host Pbmc
16% CD137
CD137
Count
Day 11 - After Depletion of CD137+
3% CD137
CD137
Count

FIG. 29


Number of Specific Dividing Cells (CFSE Low)
5500
4500
3500
2500
1500
500
-500
5256
2259
741
2152
134
-388
Host APC
3rd Party APC
Reference control
Anti 3rd CD137+
Anti 3rd CD137+ and Anti host CD137-