FIG. 1A

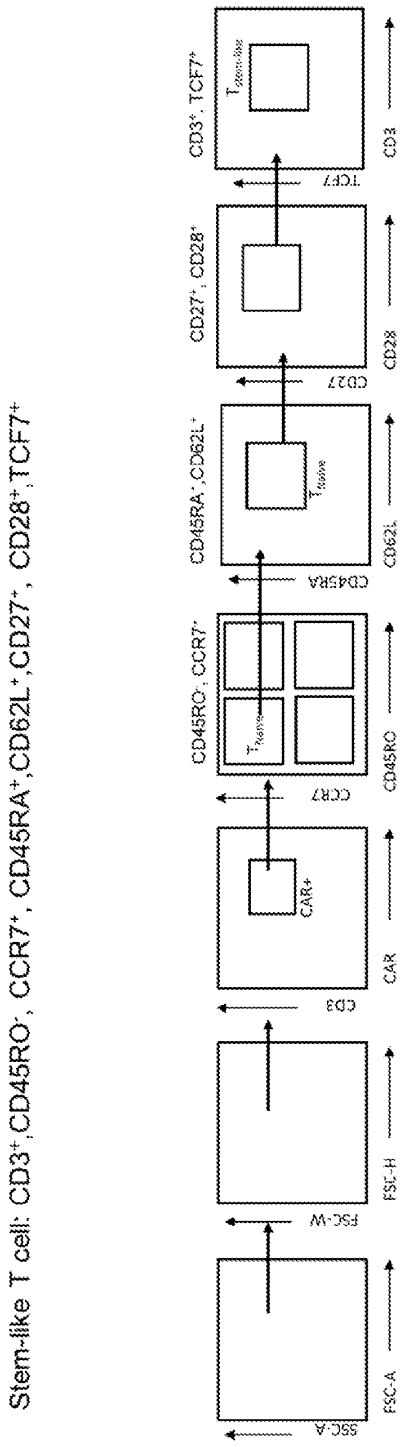
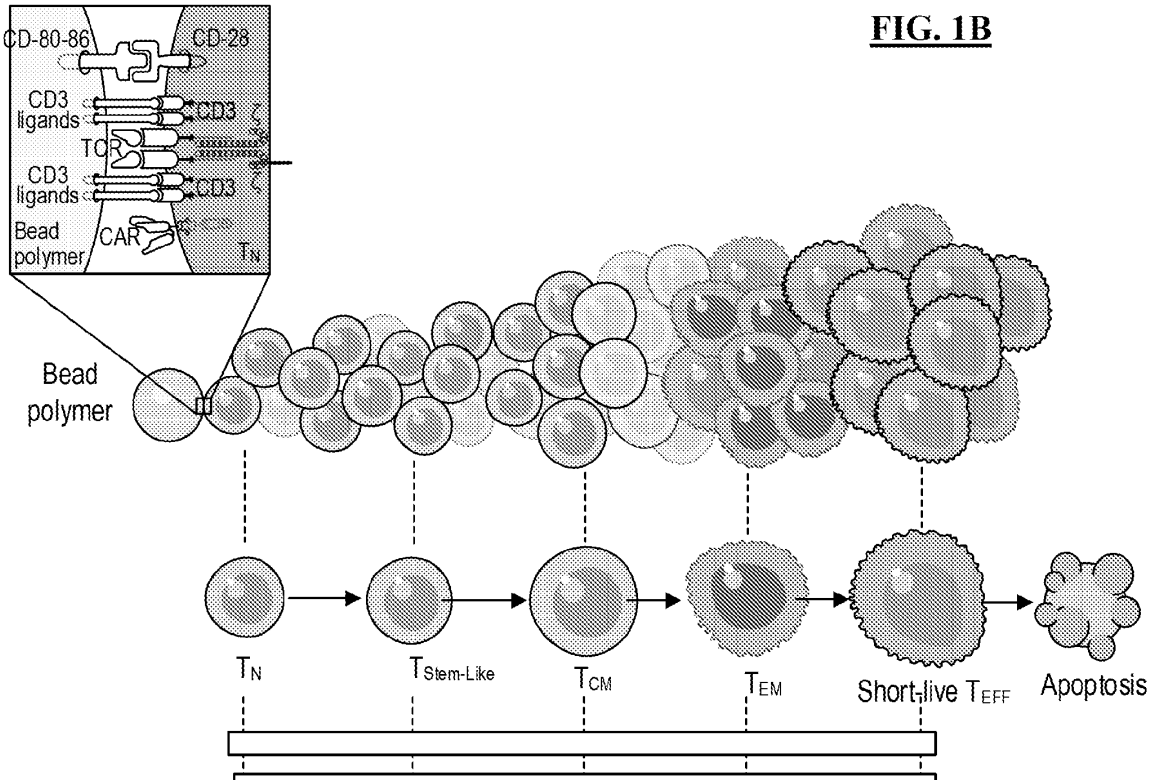

Stem-like T cell: CD3+,CD45RO-, CCR7+, CD45RA+,CD62L+,CD27+, CD28+,TCF7+
SSC-A
FSC-A
FSC-W
FSC-H
CD3
CAR+
CAR
CCR7
CD45RO
CD45RA
CD62L
CD27
CD28
TCF7
CD3
CD45RO-, CCR7+
CD45RA+,CD62L+
CD27+, CD28+
CD3+, TCF7+

FIG. 1B



Multipotency	Senescence
Persistence	Exhaustion
Lymphoid horning	High ROS
Autophagy	Glycolytic metabolism
OXPHOS	Oxidative stress
Low ROS ans low $\Delta\Psi m$	Epigenetic aging
Tissue trafficking	

Phenotype	T_N	$T_{stem-like}$	T_{CM}	T_{EM}
CD27	++++	+++	++	+
CD62L	++++	+++	++	+
TCF7	++++	+++	++	+
CCR7	++++	+++	++	-
CD28	++	+++	+++	+
CD45RA	++	++	-	+/-
CD45RO	-	-	+	+
CD39	-	-	++	+++

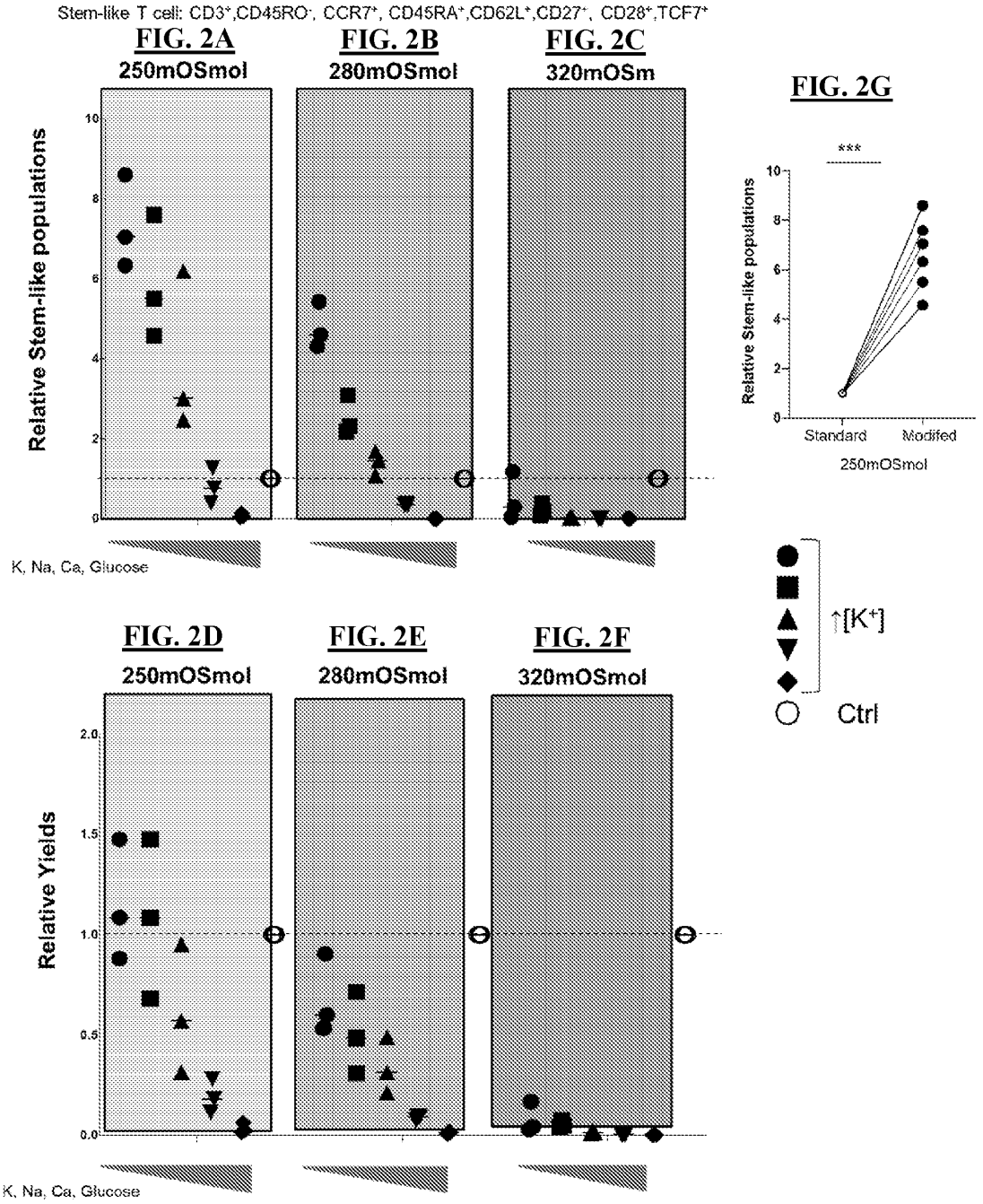

Stem-like T cell: CD3+,CD45RO-, CCR7+, CD45RA+,CD62L+,CD27+, CD28+,TCF7+
FIG. 2A
250mOSmol
FIG. 2B
280mOSmol
FIG. 2C
320mOSm
Relative Stem-like populations
K, Na, Ca, Glucose
FIG. 2G
***
Relative Stem-like populations
Standard
Modifed
250mOSmol
↑[K+]
Ctrl
FIG. 2D
250mOSmol
FIG. 2E
280mOSmol
FIG. 2F
320mOSmol
Relative Yields
K, Na, Ca, Glucose

FIG. 3A

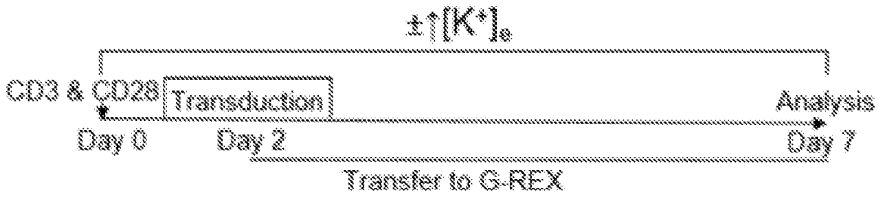

±↑[K+]e
CD3 & CD28
Transduction
Analysis
Day 0
Day 2
Day 7
Transfer to G-REX

FIG. 3B

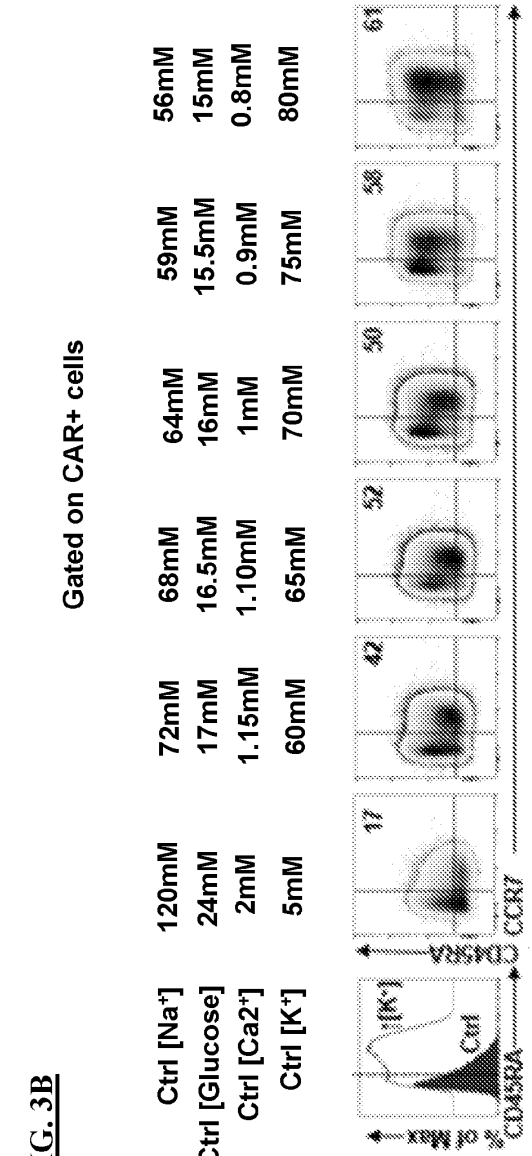

Gated on CAR+ cells
Ctrl [Na+] 120mM 72mM 68mM 64mM 59mM 56mM
Ctrl [Glucose] 24mM 17mM 16.5mM 16mM 15.5mM 15mM
Ctrl [Ca2+] 2mM 1.15mM 1.10mM 1mM 0.9mM 0.8mM
Ctrl [K+] 5mM 60mM 65mM 70mM 75mM 80mM
[K+]
Ctrl
CD45RA
CCR7
17
42
52
50
58
61

FIG. 3C

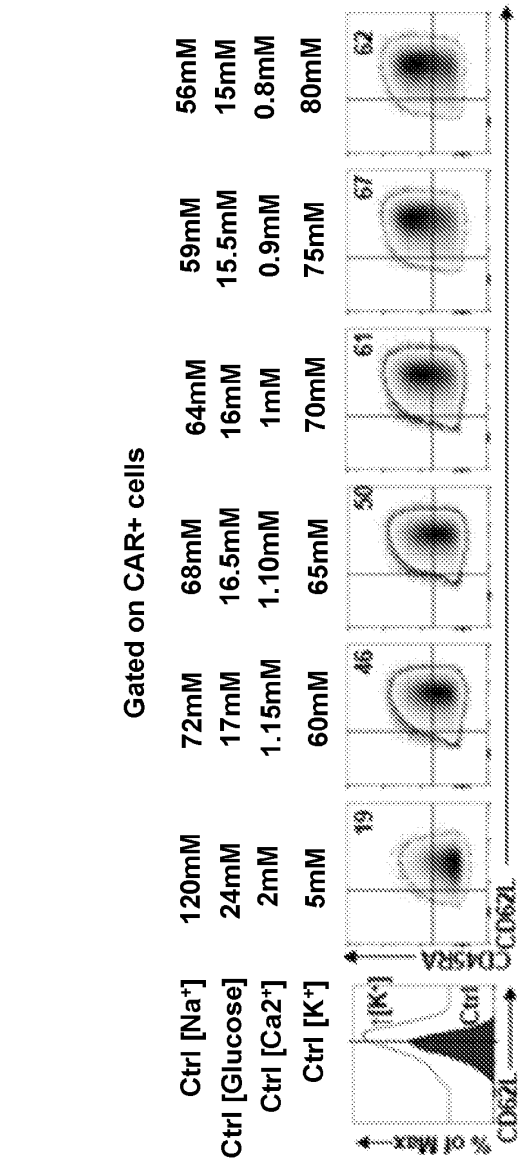

Gated on CAR+ cells
Ctrl [Na+] 120mM 72mM 68mM 64mM 59mM 56mM
Ctrl [Glucose] 24mM 17mM 16.5mM 16mM 15.5mM 15mM
Ctrl [Ca2+] 2mM 1.15mM 1.10mM 1mM 0.9mM 0.8mM
Ctrl [K+] 5mM 60mM 65mM 70mM 75mM 80mM
19
46
50
61
67
62
% of Max
[K+]
Ctrl
CD19 CAR
CD8+

FIG. 3D

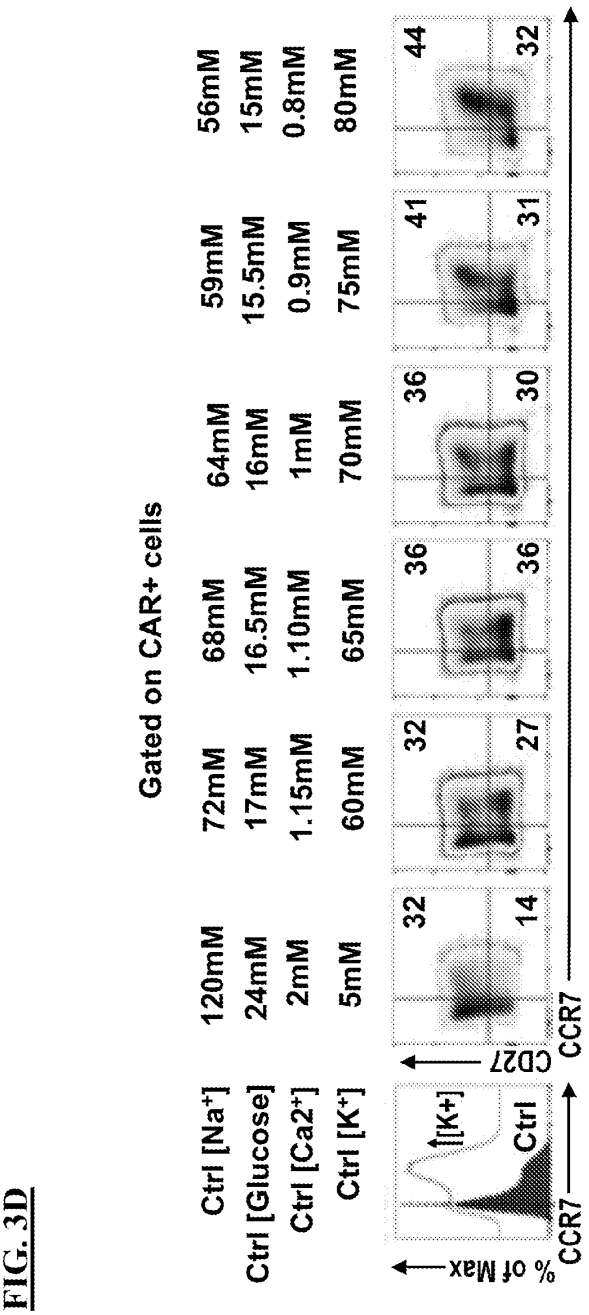
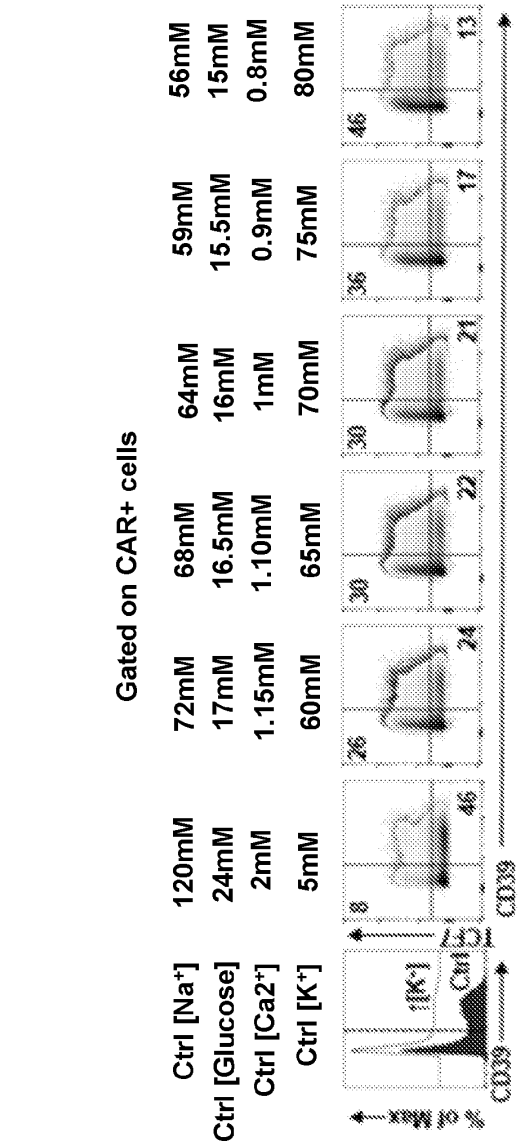

Gated on CAR+ cells
Ctrl [Na+]
Ctrl [Glucose]
Ctrl [Ca2+]
Ctrl [K+]
120mM
24mM
2mM
5mM
72mM
17mM
1.15mM
60mM
68mM
16.5mM
1.10mM
65mM
64mM
16mM
1mM
70mM
59mM
15.5mM
0.9mM
75mM
56mM
15mM
0.8mM
80mM
32
14
32
27
36
36
36
30
41
31
44
32
[K+]
Ctrl
% of Max
CCR7
CD27
CCR7

FIG. 3E


Gated on CAR+ cells
Ctrl [Na+] 120mM 72mM 68mM 64mM 59mM 56mM
Ctrl [Glucose] 24mM 17mM 16.5mM 16mM 15.5mM 15mM
Ctrl [Ca2+] 2mM 1.15mM 1.10mM 1mM 0.9mM 0.8mM
Ctrl [K+] 5mM 60mM 65mM 70mM 75mM 80mM
CD39
Ctrl
[K+]
[CL-]
CD39
8
46
26
24
30
22
30
21
36
17
46
13

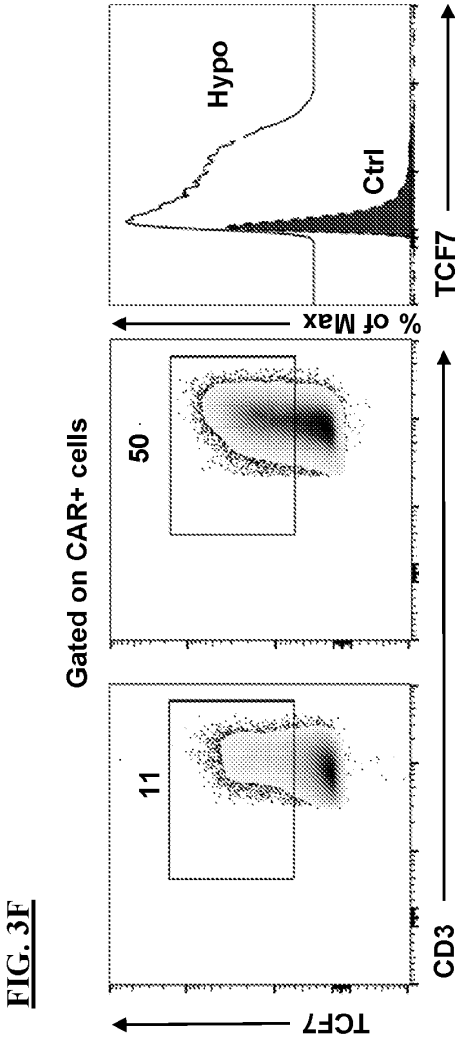


FIG. 3F

FIG. 3G

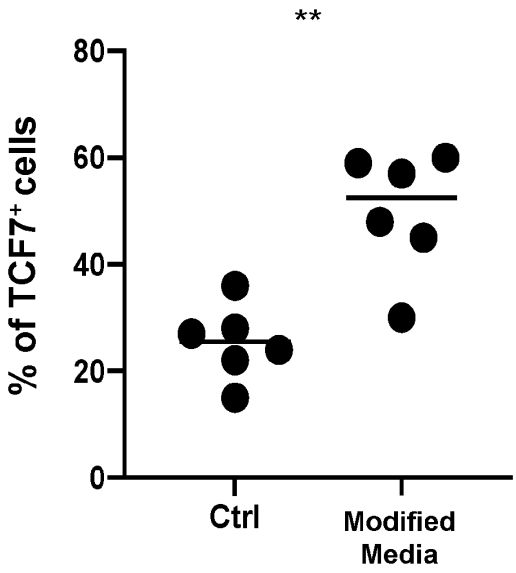


FIG. 4A

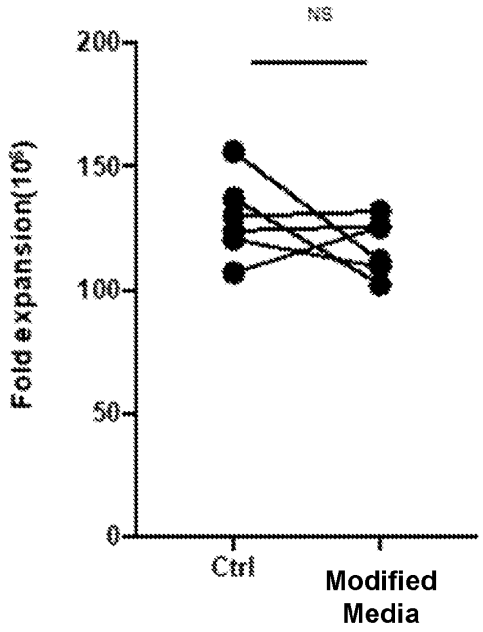
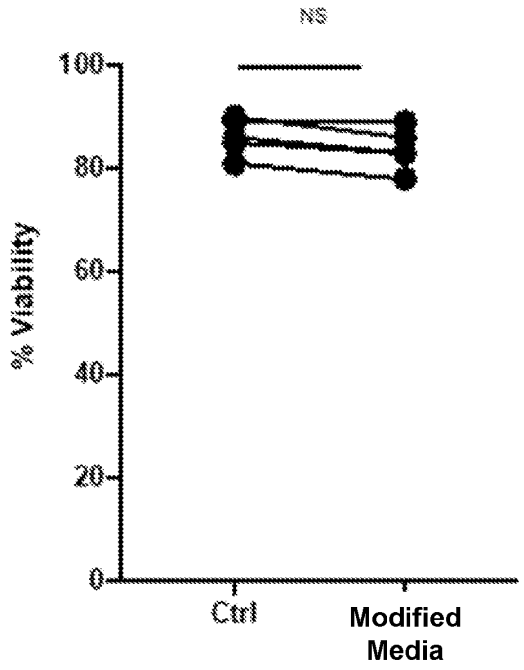


FIG. 4B



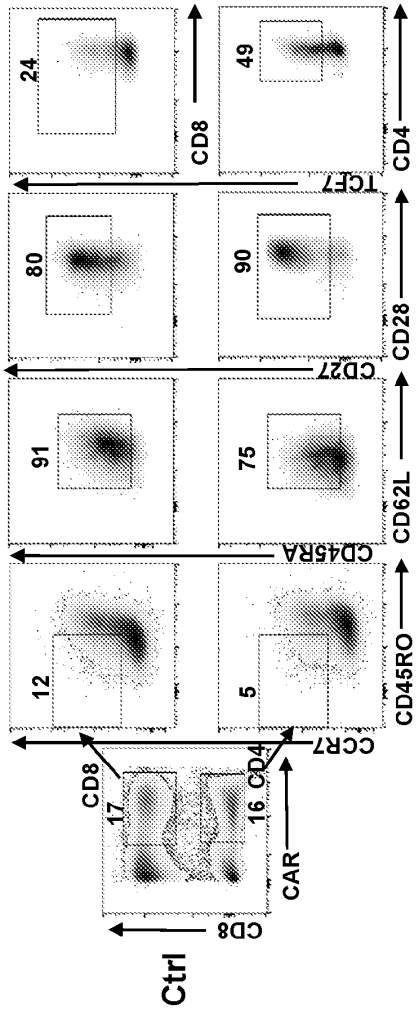

Ctrl
CD8
17
CD4
16
CAR
CD8
CCR7
12
5
CD45RO
CD45RA
91
75
CD62L
CD27
80
90
CD28
TCF7
24
49
CD4

FIG. 5A

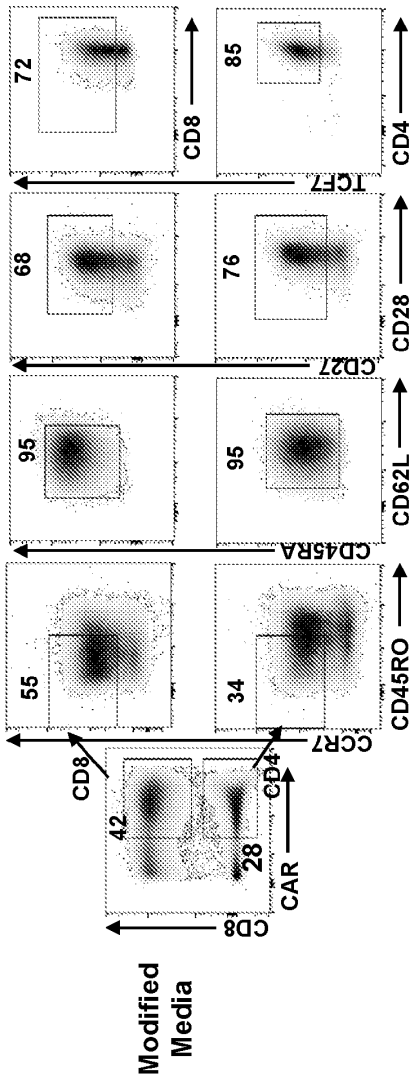

Modified Media
CD8
CAR
CD4
42
28
CCR7
CD45RO
55
34
CD45RA
CD62L
95
95
CD27
CD28
68
76
TCF7
CD4
CD8
72
85

FIG. 5B

FIG. 6

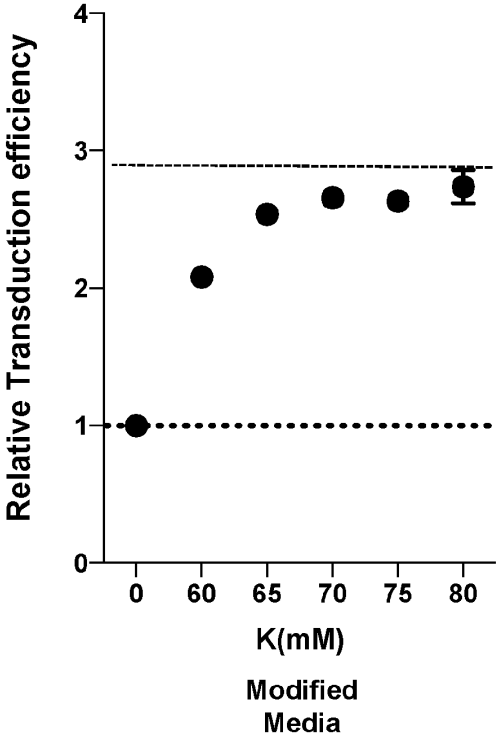

Relative Transduction efficiency
4
3
2
1
0
0
60
65
70
75
80
K(mM)
Modified
Media

FIG. 7

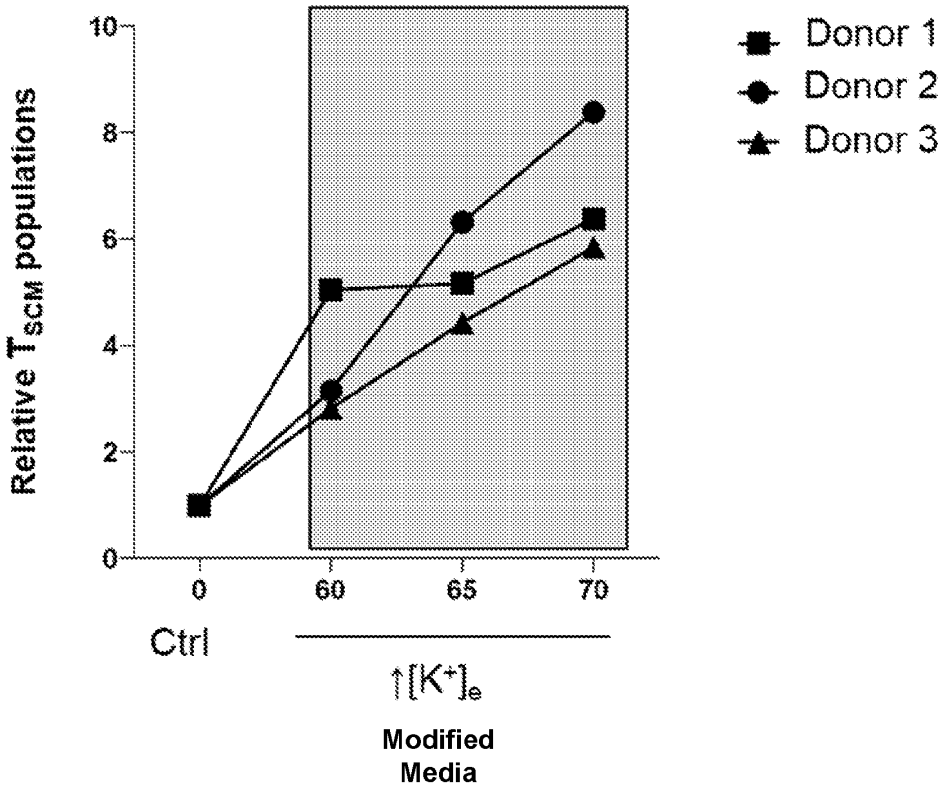

Relative T_{SCM} populations
10
8
6
4
2
0
0
60
65
70
Ctrl
↑[K+]e
Modified Media
Donor 1
Donor 2
Donor 3

FIG. 8A

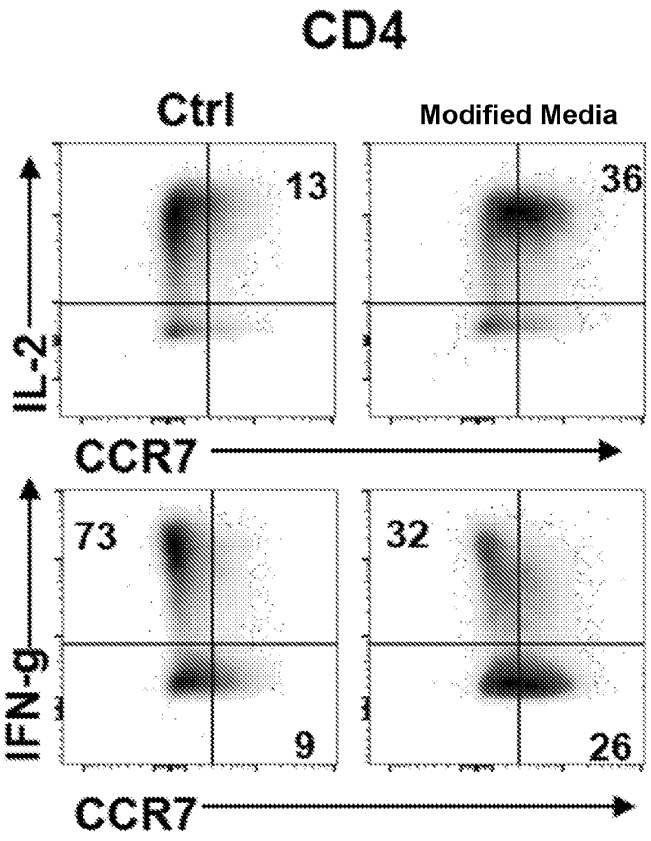


FIG. 8B

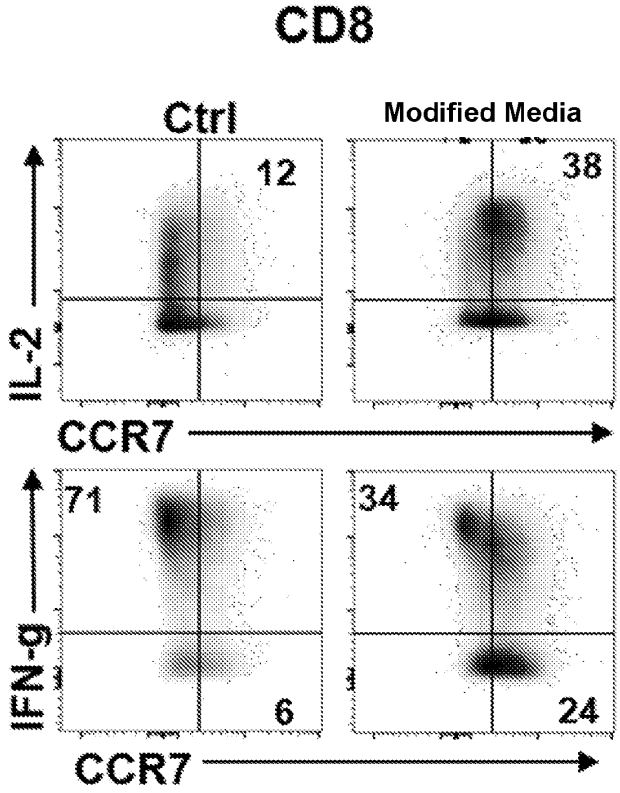
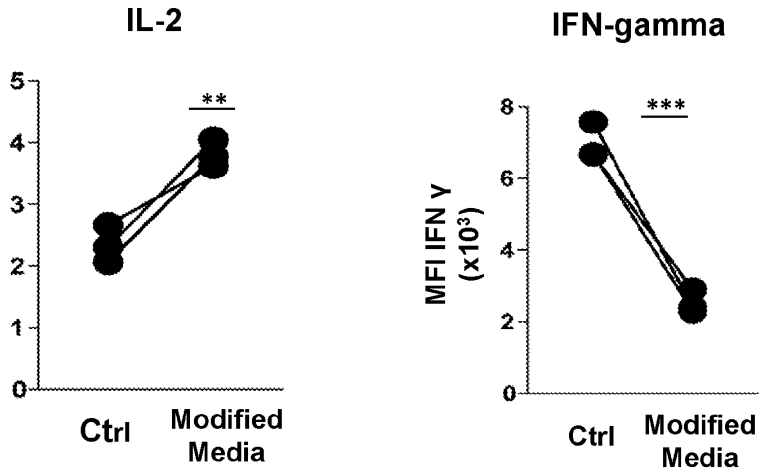


FIG. 8C


IL-2
**
5
4
3
2
1
0
Ctrl
Modified Media
IFN-gamma
***
MFI IFN γ (x10³)
8
6
4
2
0
Ctrl
Modified Media

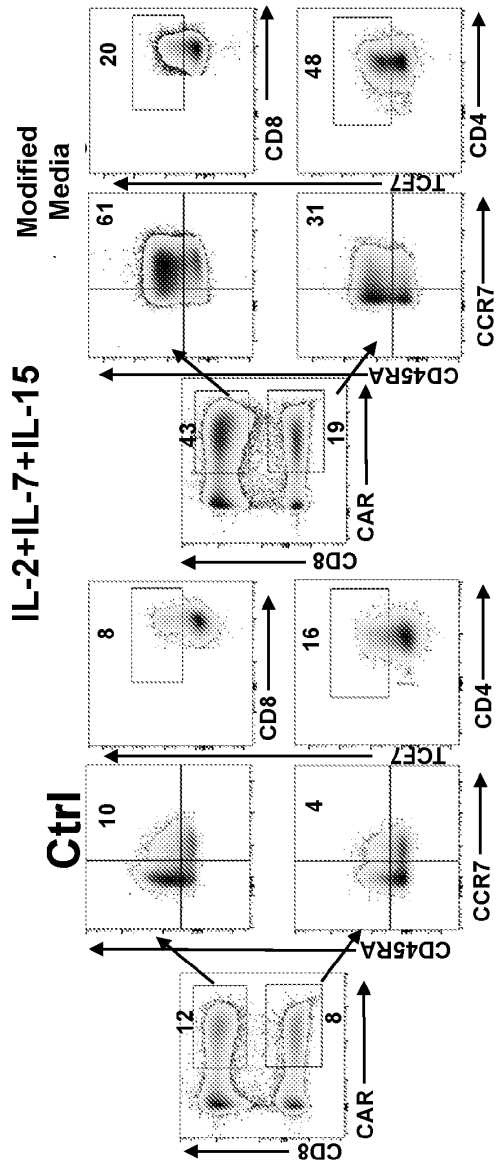

Ctrl
IL-2+IL-7+IL-15
Modified Media
CD8
CAR
CD45RA
CCR7
CD4
TCF7
12
8
10
4
8
16
43
19
61
31
20
48

FIG. 9A

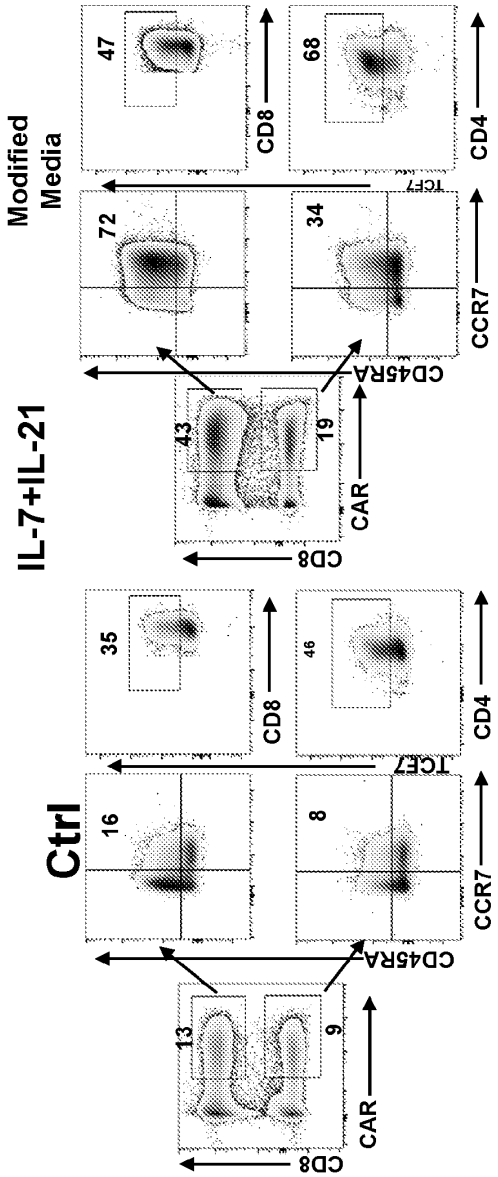

Modified Media
IL-7+IL-21
Ctrl
CD8
CD4
CCR7
CD45RA
CAR
TCF7
47
68
72
34
43
19
35
46
16
8
13
9

FIG. 9B

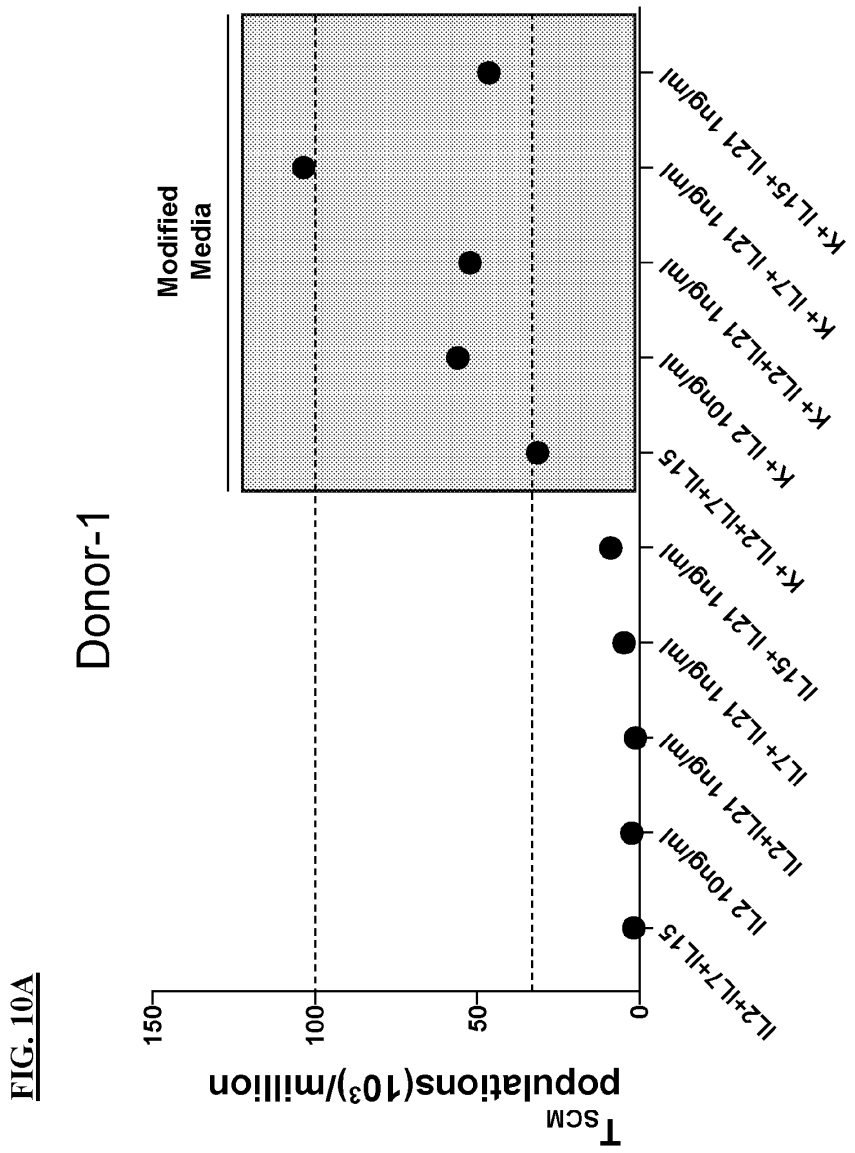

Donor-1
Modified Media
T_{SCM} populations(10^3)/million
150
100
50
0
IL2+IL7+IL15
IL2 10ng/ml
IL2+IL21 1ng/ml
IL7+ IL21 1ng/ml
IL15+ IL21 1ng/ml
K+ IL2+IL7+IL15
K+ IL2 10ng/ml
K+ IL2+IL21 1ng/ml
K+ IL7+ IL21 1ng/ml
K+ IL15+ IL21 1ng/ml

FIG. 10A

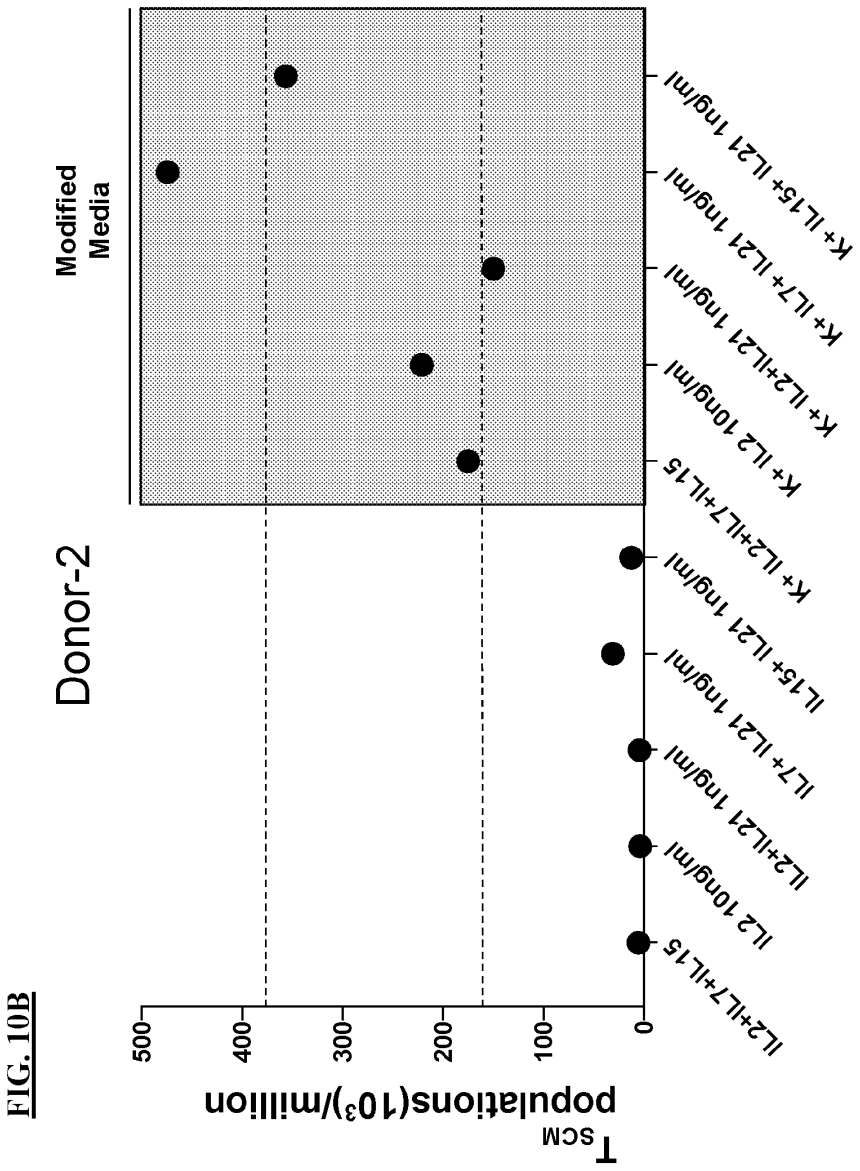

Donor-2
Modified Media
T_{SCM} populations(10^3)/million
500
400
300
200
100
0
IL2+IL7+IL15
IL2 10ng/ml
IL2+IL21 1ng/ml
IL7+ IL21 1ng/ml
IL15+ IL21 1ng/ml
K+ IL2+IL7+IL15
K+ IL2 10ng/ml
K+ IL2+IL21 1ng/ml
K+ IL7+ IL21 1ng/ml
K+ IL15+ IL21 1ng/ml

FIG. 10B

FIG. 11

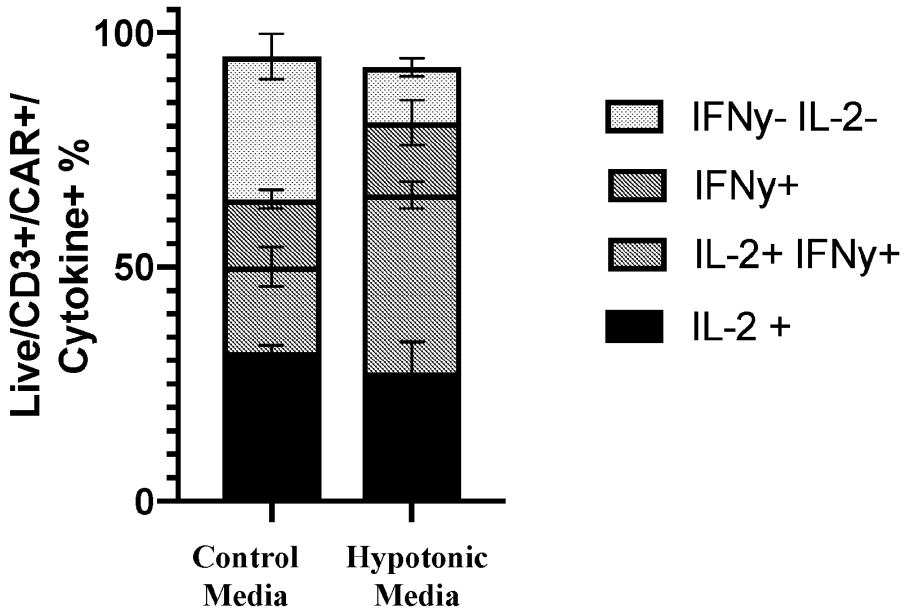

100
50
0
Live/CD3+/CAR+/
Cytokine+ %
Control
Media
Hypotonic
Media
IFNy- IL-2-
IFNy+
IL-2+ IFNy+
IL-2 +

FIG. 12

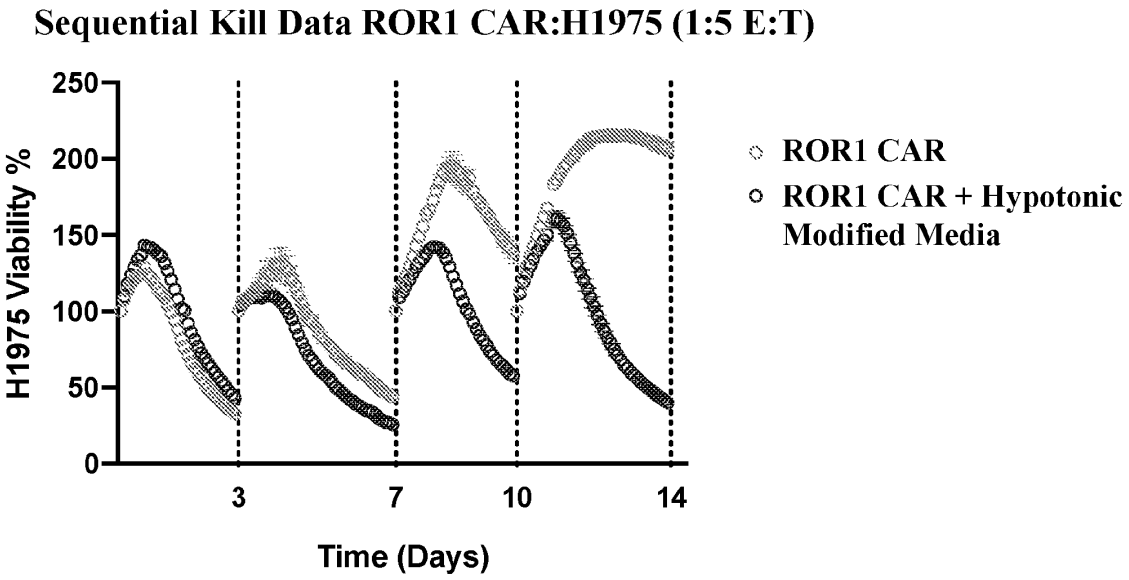

Sequential Kill Data ROR1 CAR:H1975 (1:5 E:T)
H1975 Viability %
250
200
150
100
50
0
3
7
10
14
Time (Days)
ROR1 CAR
ROR1 CAR + Hypotonic Modified Media

FIG. 13A

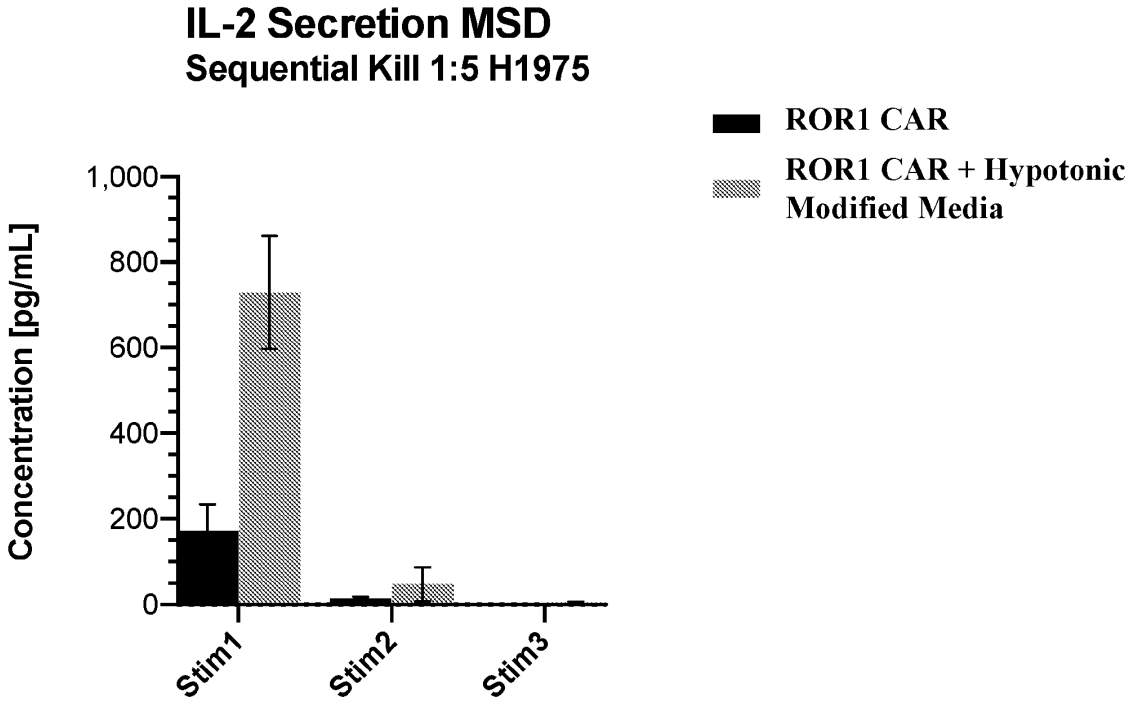


FIG. 13B

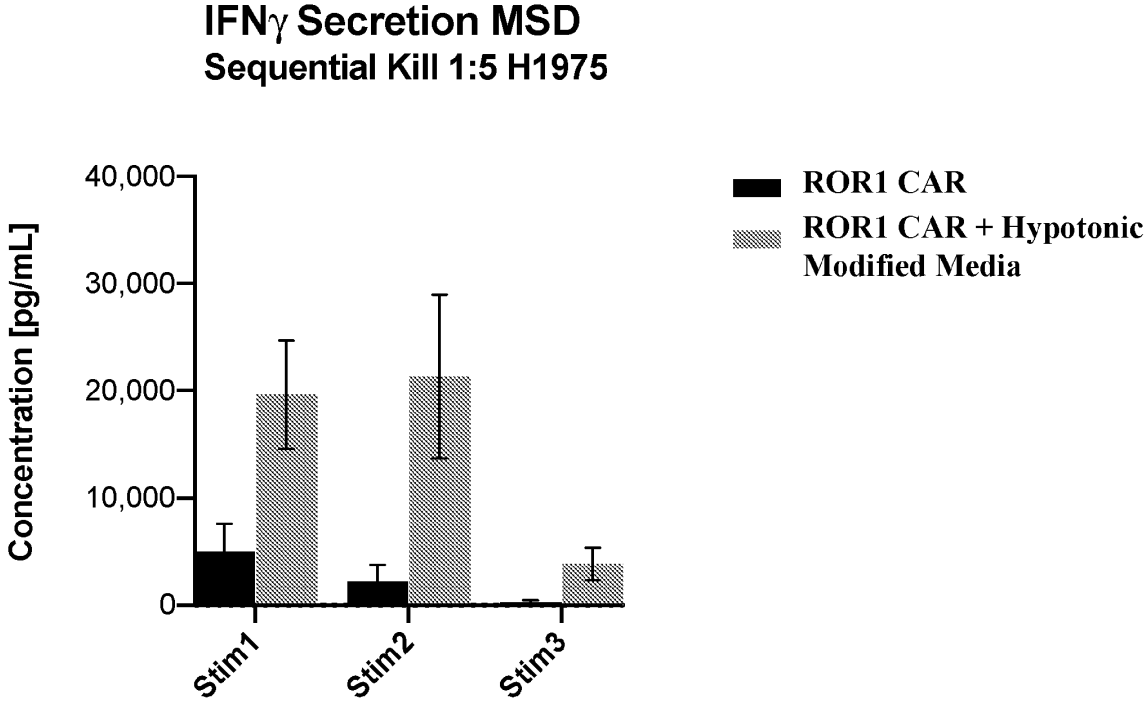
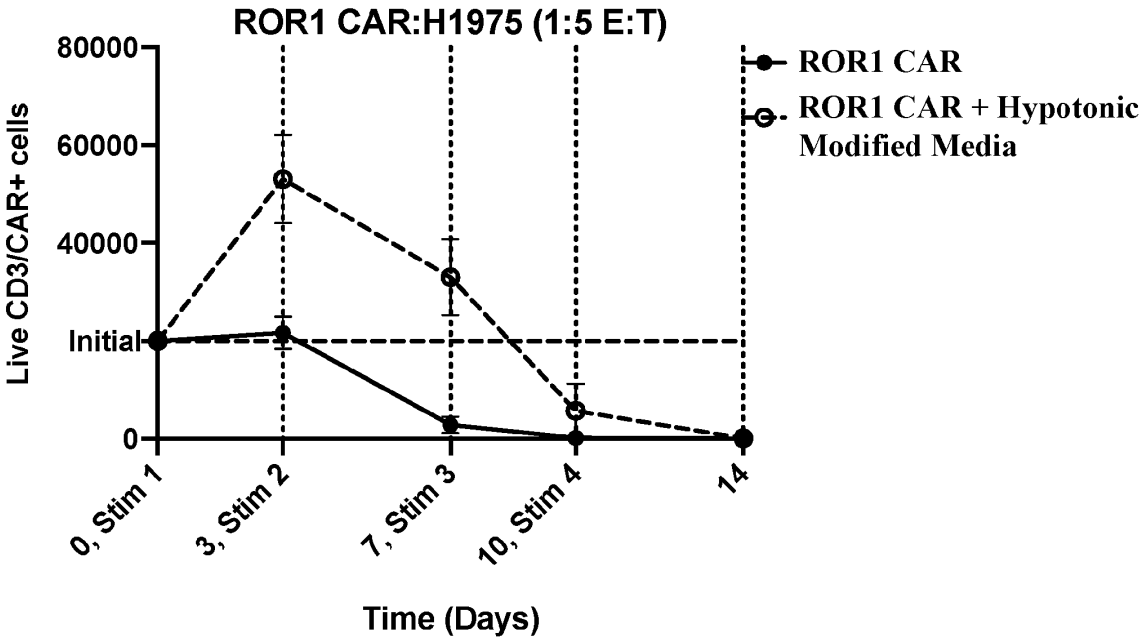


FIG. 14


ROR1 CAR:H1975 (1:5 E:T)
Live CD3/CAR+ cells
80000
60000
40000
Initial
0
0, Stim 1
3, Stim 2
7, Stim 3
10, Stim 4
14
Time (Days)
ROR1 CAR
ROR1 CAR + Hypotonic Modified Media