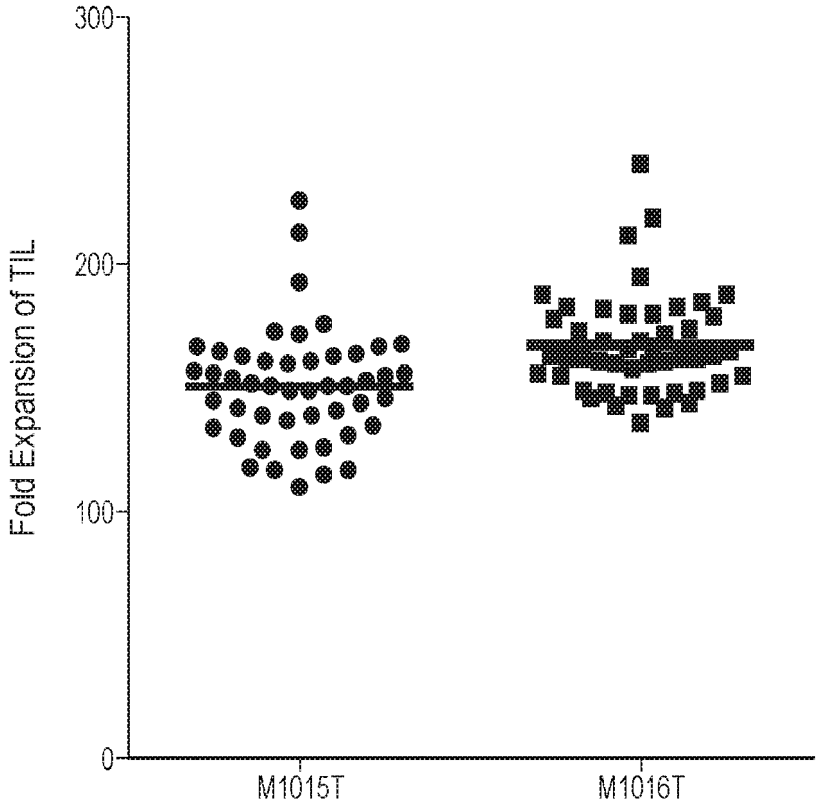


FIG. 1

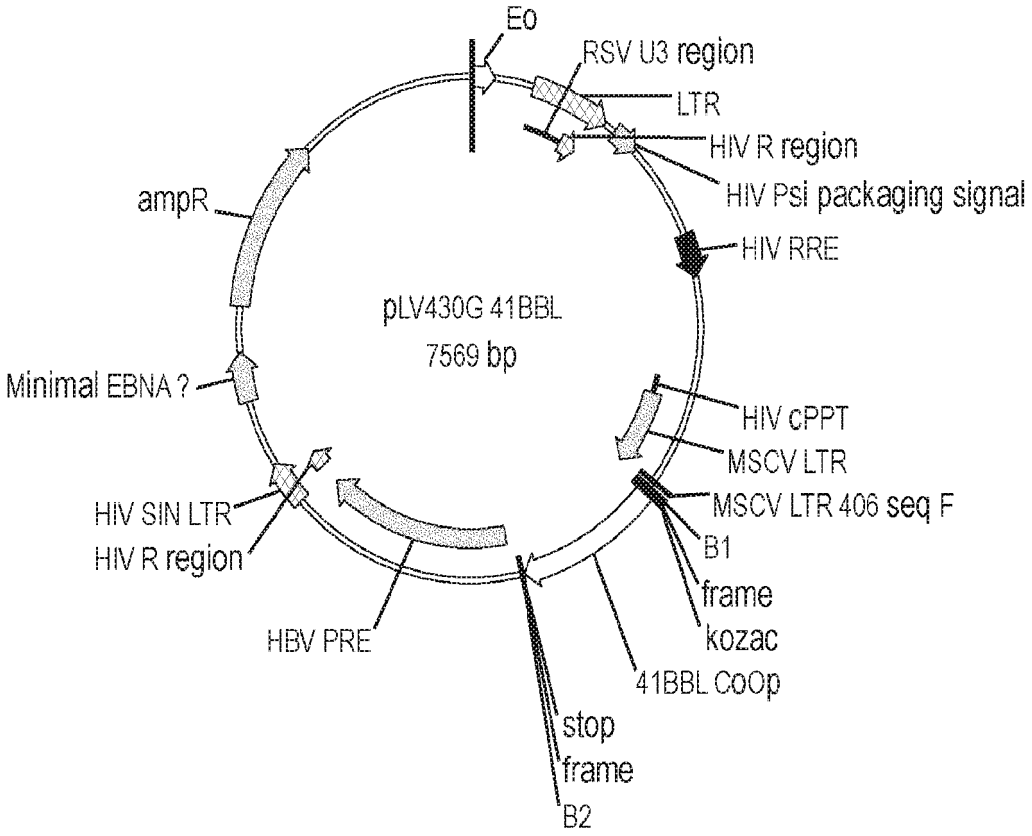

E0
RSV U3 region
LTR
HIV R region
HIV Psi packaging signal
HIV RRE
HIV cPPT
MSCV LTR
MSCV LTR 406 seq F
B1
frame
kozac
41BBL CoOp
stop
frame
B2
HBV PRE
HIV SIN LTR
HIV R region
Minimal EBNA ?
ampR
pLV430G 41BBL
7569 bp

FIG. 2

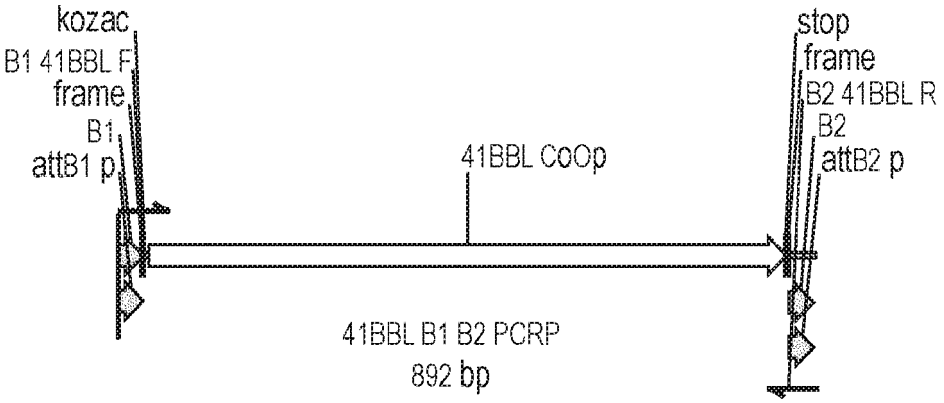

kozac
B1 41BBL F
frame
B1
attB1 p
41BBL COOp
stop
frame
B2 41BBL R
B2
attB2 p
41BBL B1 B2 PCRP
892 bp

FIG. 3

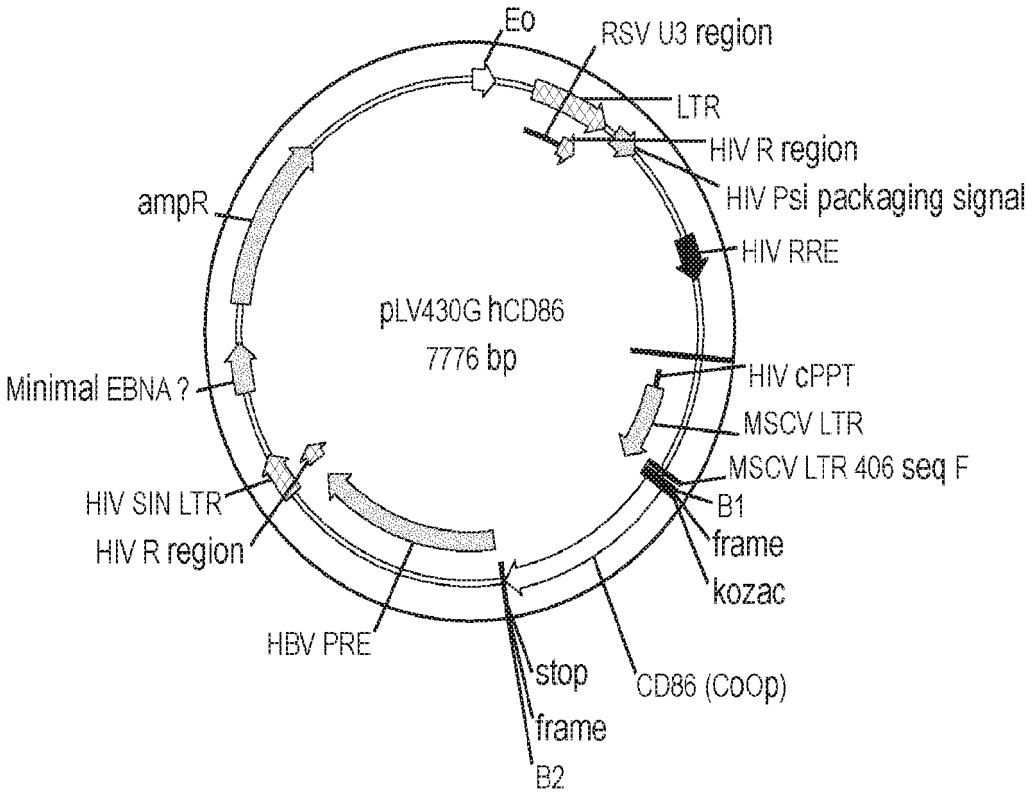

E0
RSV U3 region
LTR
HIV R region
HIV Psi packaging signal
HIV RRE
HIV cPPT
MSCV LTR
MSCV LTR 406 seq F
B1
frame
kozac
CD86 (CoOp)
stop
frame
B2
HBV PRE
HIV R region
HIV SIN LTR
Minimal EBNA ?
ampR
pLV430G hCD86
7776 bp

FIG. 4

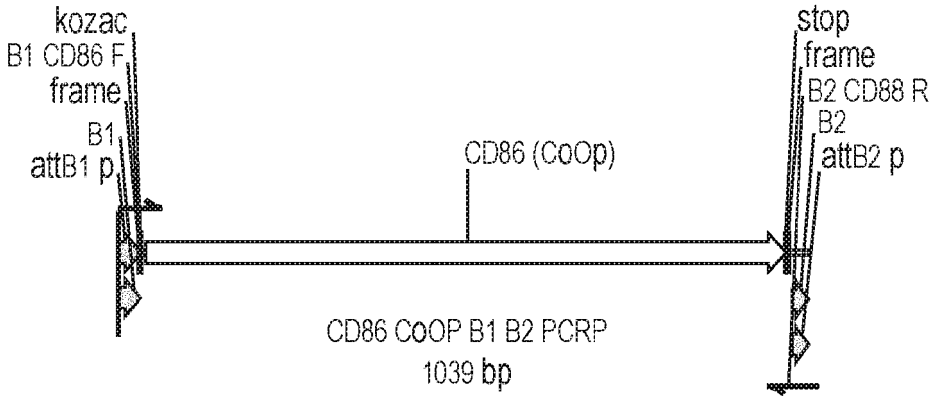

kozac
B1 CD86 F
frame
B1
attB1 p
CD86 (CoOp)
stop
frame
B2 CD88 R
B2
attB2 p
CD86 CoOP B1 B2 PCRP
1039 bp

FIG. 5

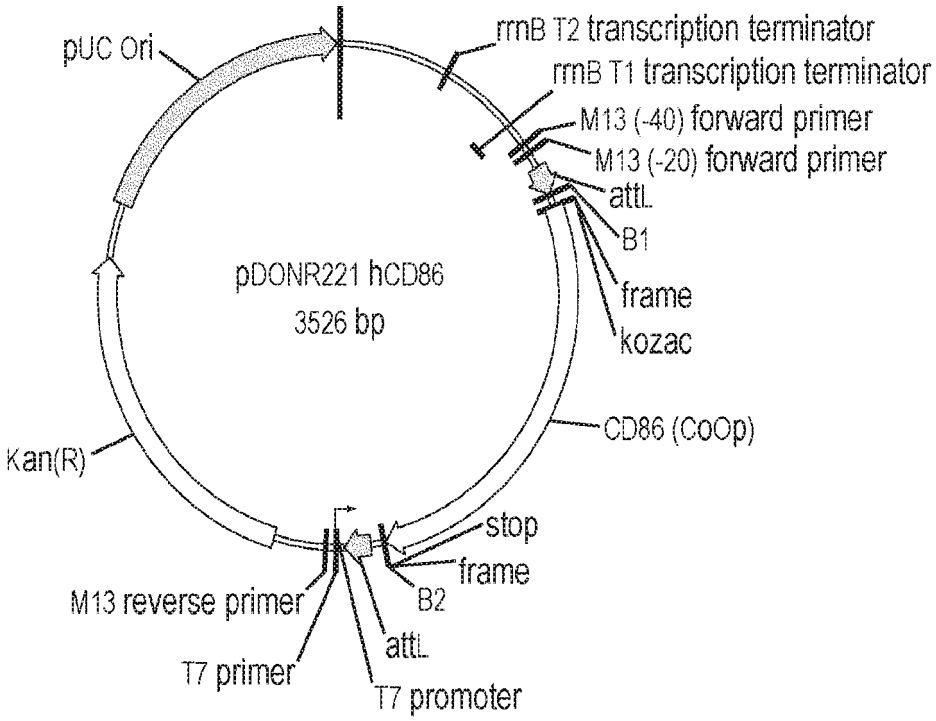

pUC Ori
rrnB T2 transcription terminator
rrnB T1 transcription terminator
M13 (-40) forward primer
M13 (-20) forward primer
attL
B1
frame
kozac
pDONR221 hCD86
3526 bp
CD86 (CoOp)
Kan(R)
stop
frame
B2
attL
M13 reverse primer
T7 primer
T7 promoter

FIG. 6

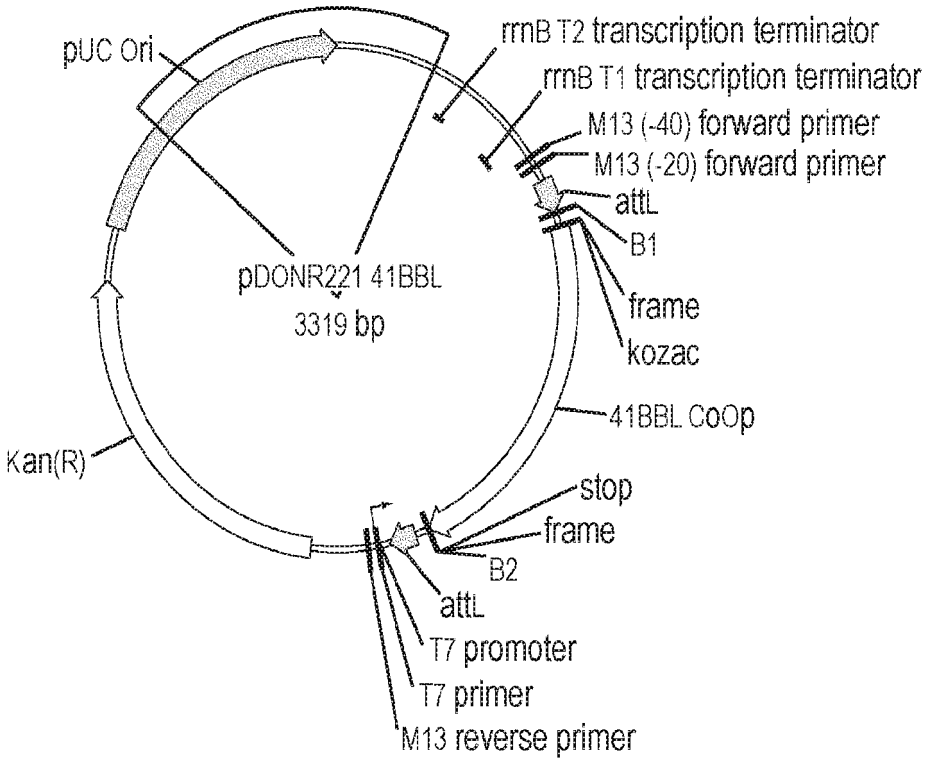

pUC Ori
rrnB T2 transcription terminator
rrnB T1 transcription terminator
M13 (-40) forward primer
M13 (-20) forward primer
attL
B1
pDONR221 41BBL
3319 bp
frame
kozac
41BBL CoOp
Kan(R)
stop
frame
B2
attL
T7 promoter
T7 primer
M13 reverse primer

FIG. 7

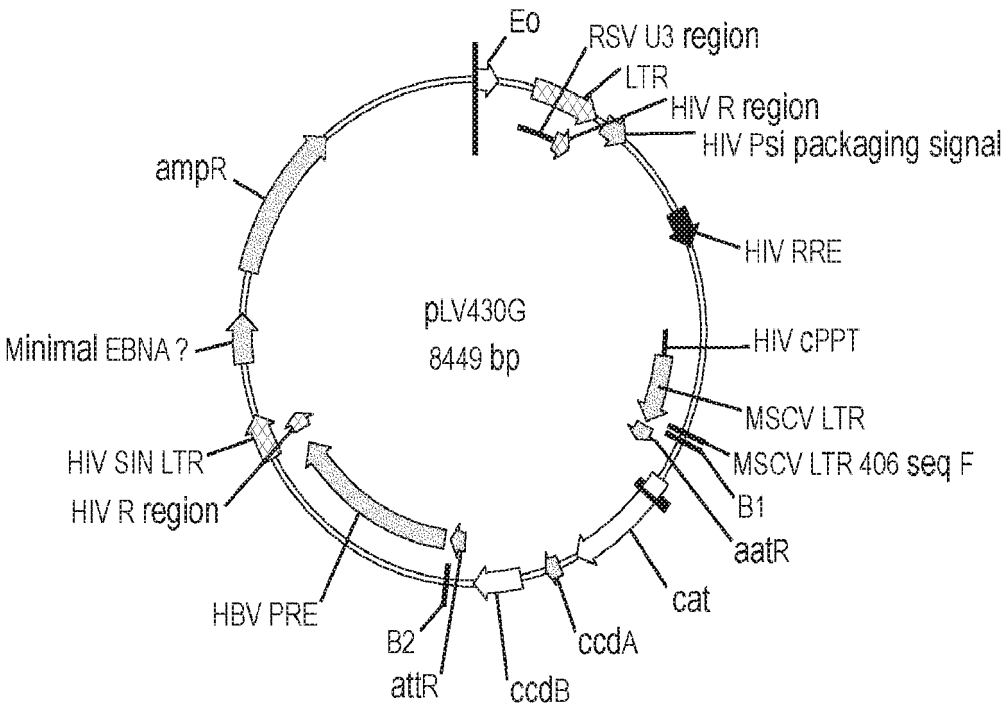

E0
RSV U3 region
LTR
HIV R region
HIV Psi packaging signal
HIV RRE
HIV cPPT
MSCV LTR
MSCV LTR 406 seq F
B1
aatR
cat
ccdA
ccdB
attR
B2
HBV PRE
HIV R region
HIV SIN LTR
Minimal EBNA ?
ampR
pLV430G
8449 bp

FIG. 8

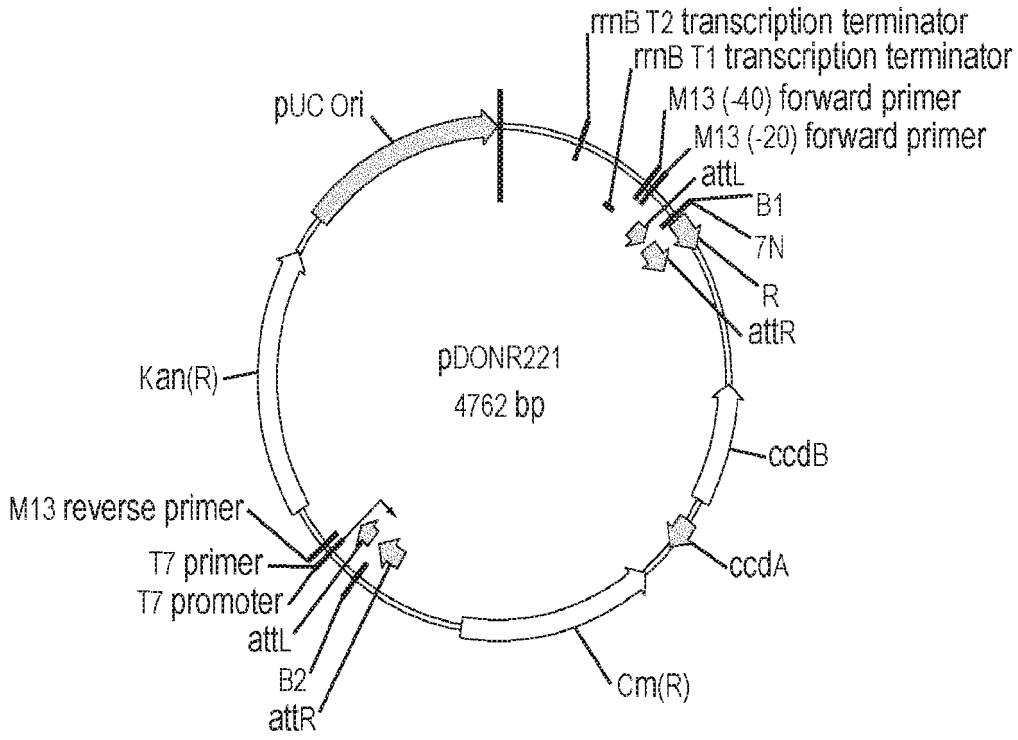

rrnB T2 transcription terminator
rrnB T1 transcription terminator
M13 (-40) forward primer
M13 (-20) forward primer
attL
B1
7N
R
attR
ccdB
ccdA
Cm(R)
attR
B2
attL
T7 promoter
T7 primer
M13 reverse primer
Kan(R)
pUC Ori
pDONR221
4762 bp

FIG. 9

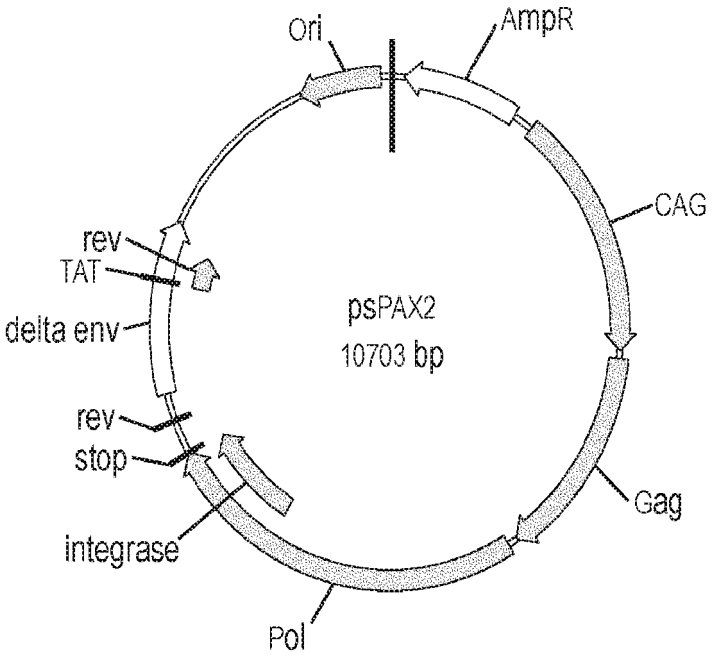

Ori
AmpR
CAG
rev
TAT
delta env
psPAX2
10703 bp
rev
stop
integrase
Gag
Pol

FIG. 10

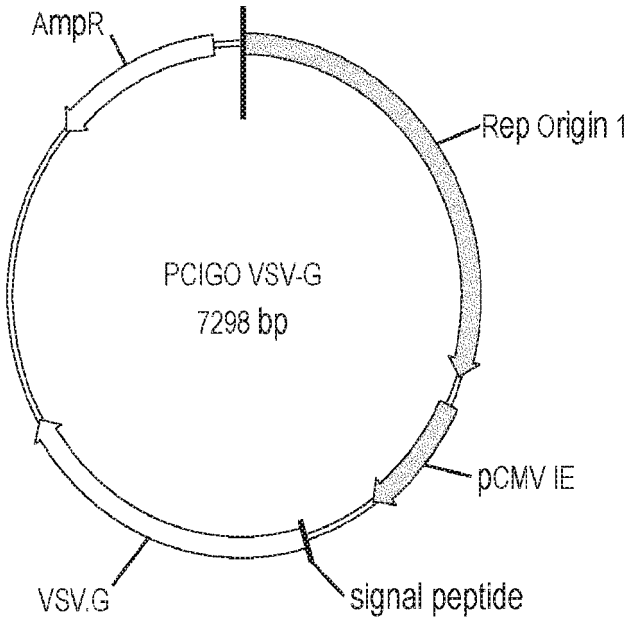

AmpR
Rep Origin 1
PCIGO VSV-G
7298 bp
pCMV IE
VSV.G
signal peptide

FIG. 11

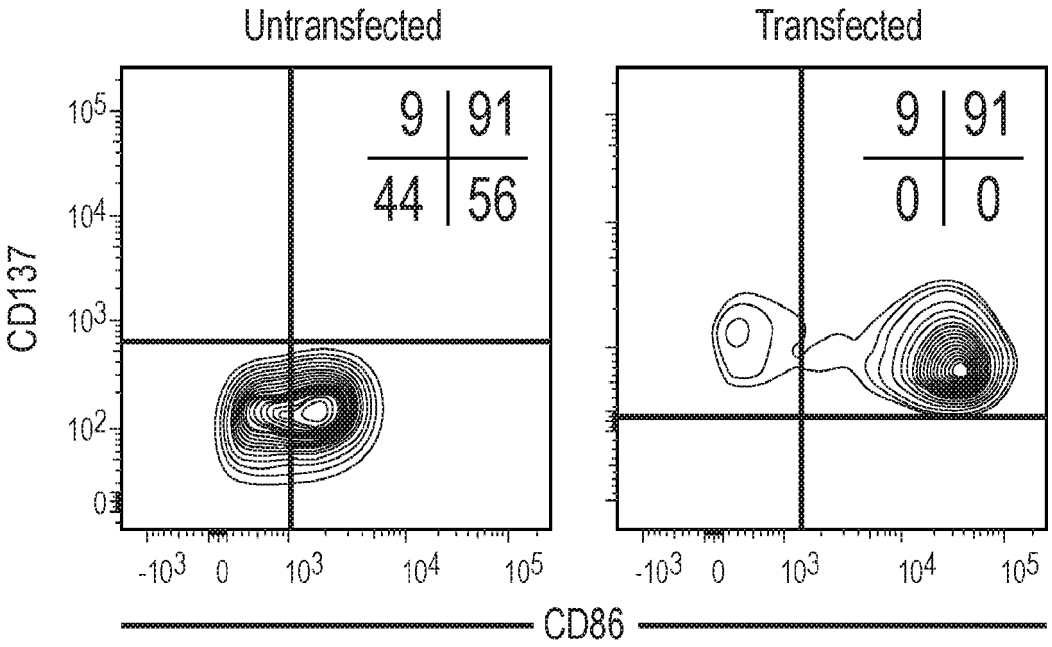

Untransfected
Transfected
9
91
44
56
9
91
0
0
CD137
CD86
10^5
10^4
10^3
10^2
0
-10^3
0
10^3
10^4
10^5

FIG. 12

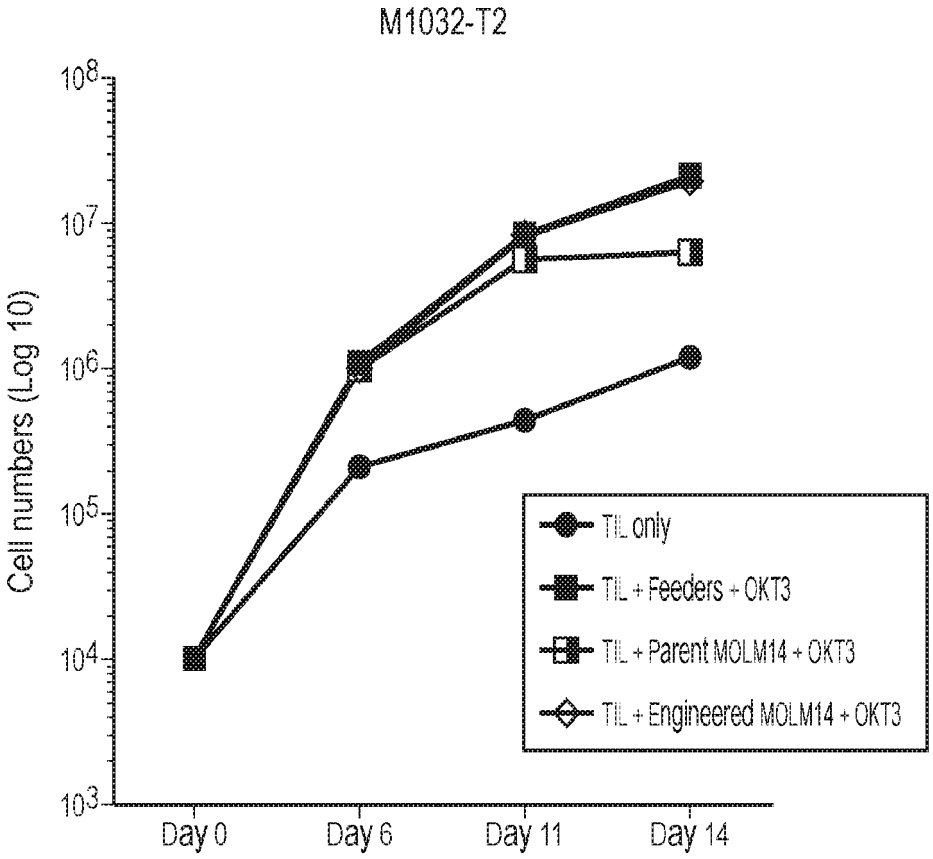

M1032-T2
Cell numbers (Log 10)
10^8
10^7
10^6
10^5
10^4
10^3
Day 0
Day 6
Day 11
Day 14
TIL only
TIL + Feeders + OKT3
TIL + Parent MOLM14 + OKT3
TIL + Engineered MOLM14 + OKT3

FIG. 13

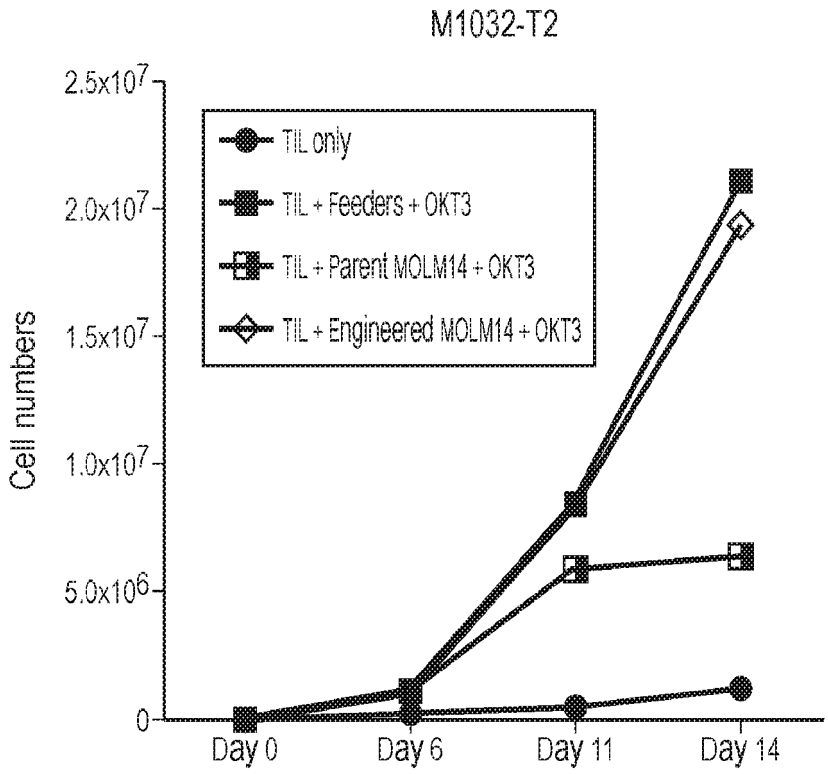

M1032-T2
Cell numbers
2.5x10^7
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0
TIL only
TIL + Feeders + OKT3
TIL + Parent MOLM14 + OKT3
TIL + Engineered MOLM14 + OKT3
Day 0
Day 6
Day 11
Day 14

FIG. 14

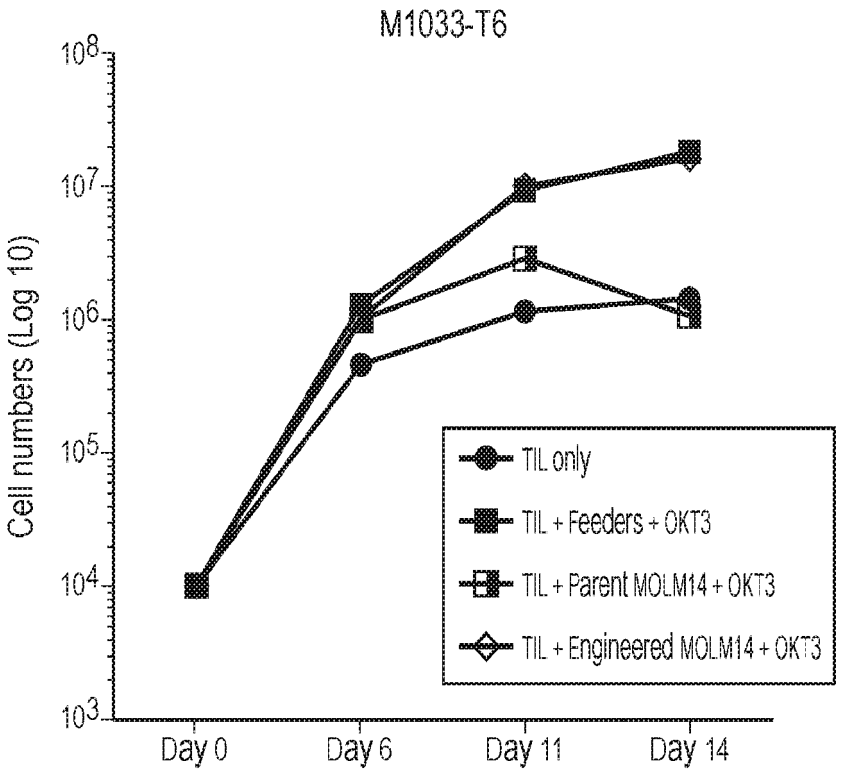

M1033-T6
Cell numbers (Log 10)
10^8
10^7
10^6
10^5
10^4
10^3
Day 0
Day 6
Day 11
Day 14
TIL only
TIL + Feeders + OKT3
TIL + Parent MOLM14 + OKT3
TIL + Engineered MOLM14 + OKT3

FIG. 15

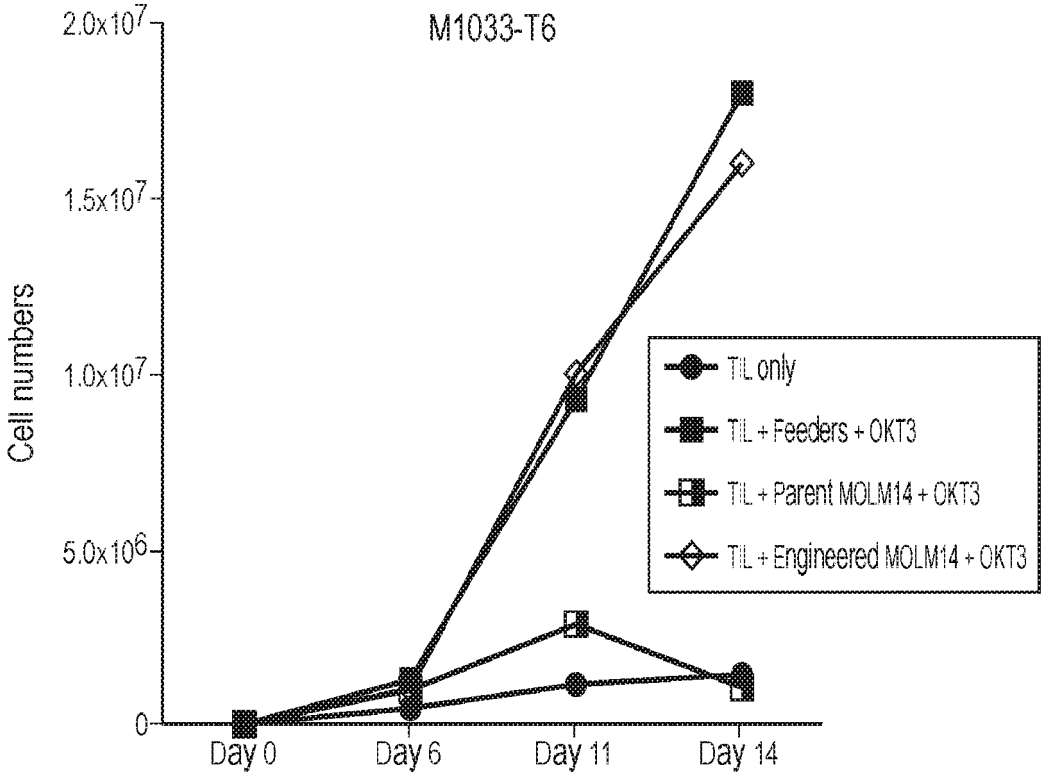

M1033-T6
Cell numbers
2.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0
Day 0
Day 6
Day 11
Day 14
TIL only
TIL + Feeders + OKT3
TIL + Parent MOLM14 + OKT3
TIL + Engineered MOLM14 + OKT3

FIG. 16

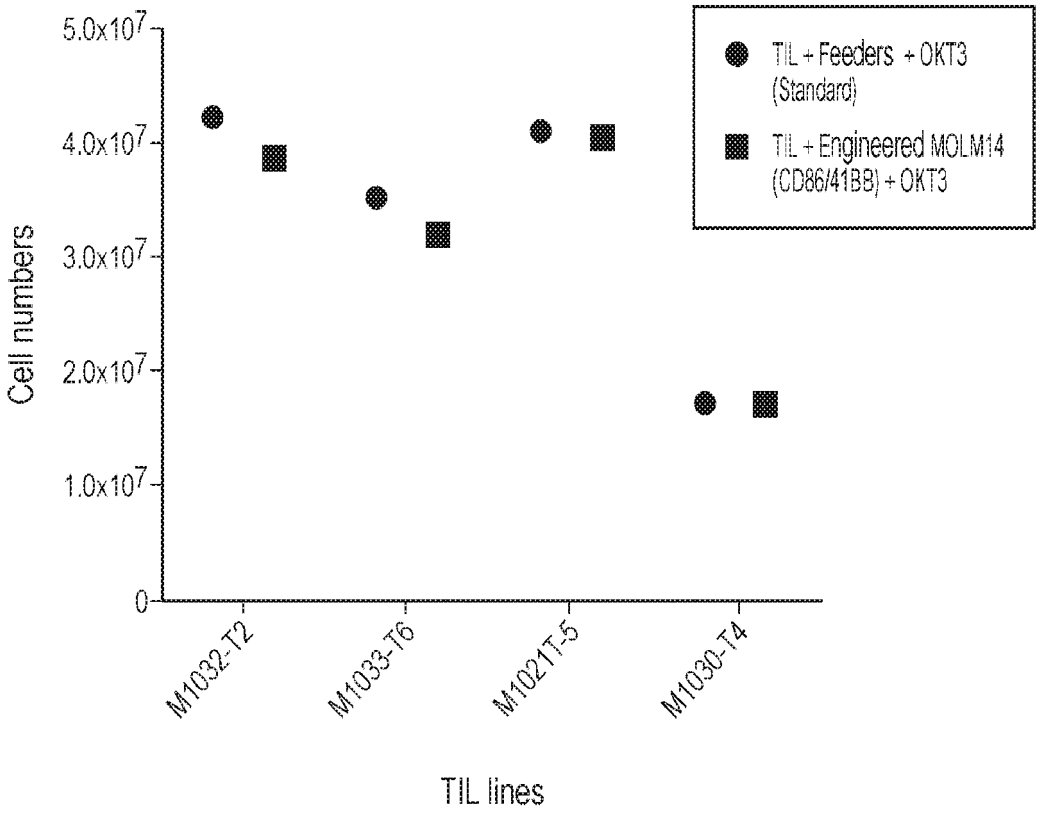

5.0x10^7
4.0x10^7
3.0x10^7
2.0x10^7
1.0x10^7
0
Cell numbers
TIL + Feeders + OKT3 (Standard)
TIL + Engineered MOLM14 (CD86/41BB) + OKT3
M1032-T2
M1033-T6
M1021T-5
M1030-T4
TIL lines

FIG. 17

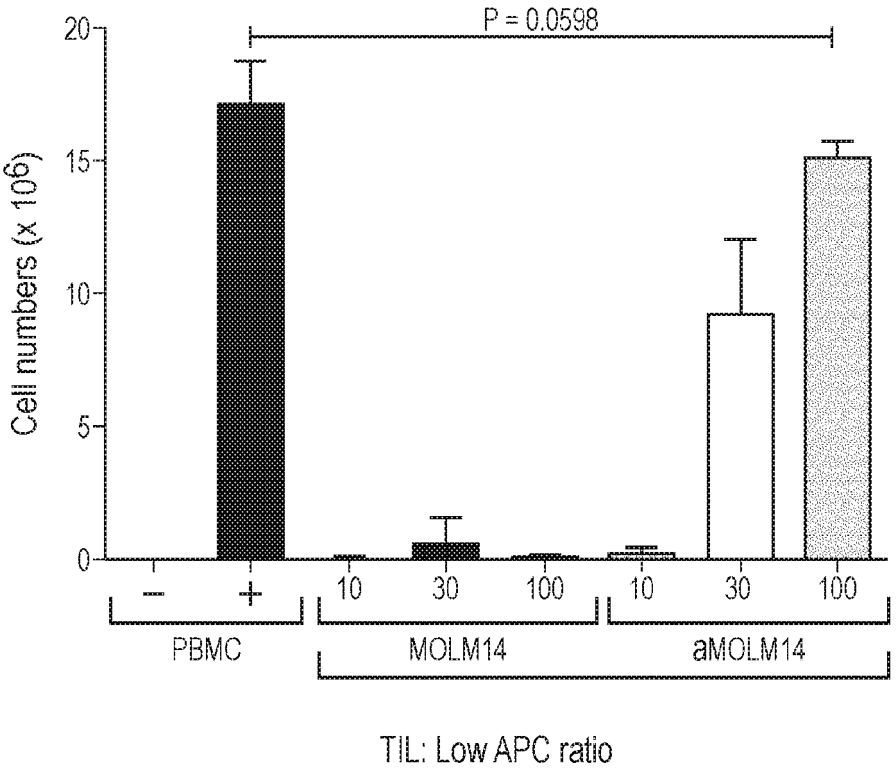

P = 0.0598
20
15
10
5
0
Cell numbers (x 10^6)
–
+
10
30
100
10
30
100
PBMC
MOLM14
aMOLM14
TIL: Low APC ratio

FIG. 18

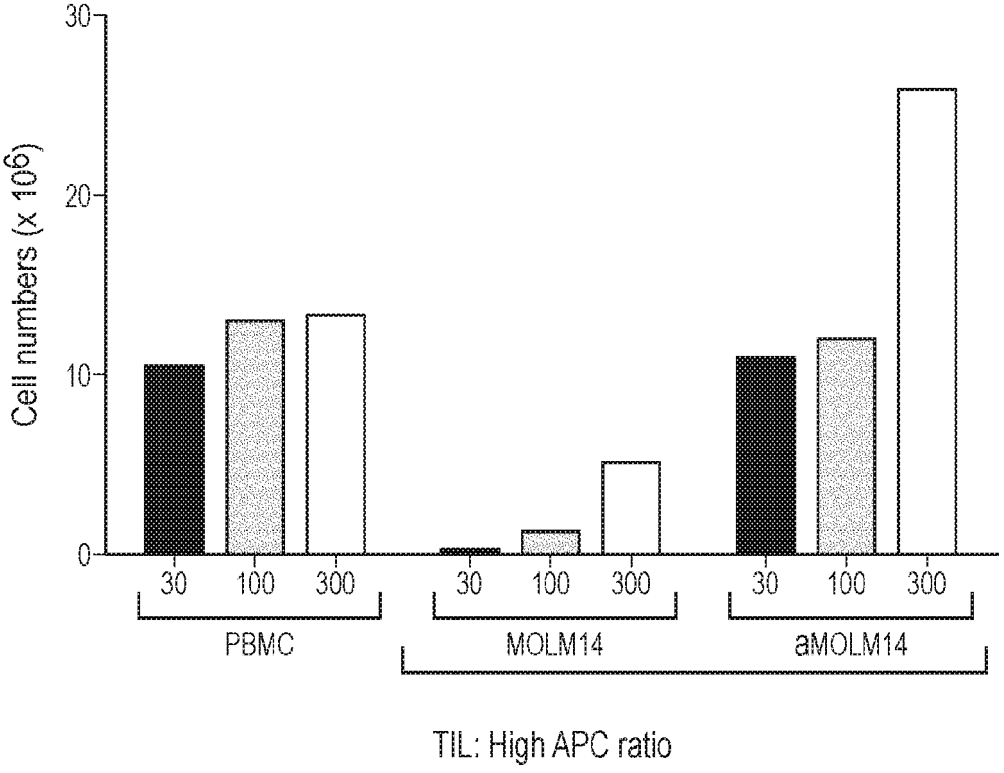

30
20
10
0
Cell numbers (x 10^6)
30
100
300
30
100
300
30
100
300
PBMC
MOLM14
aMOLM14
TIL: High APC ratio

FIG. 19

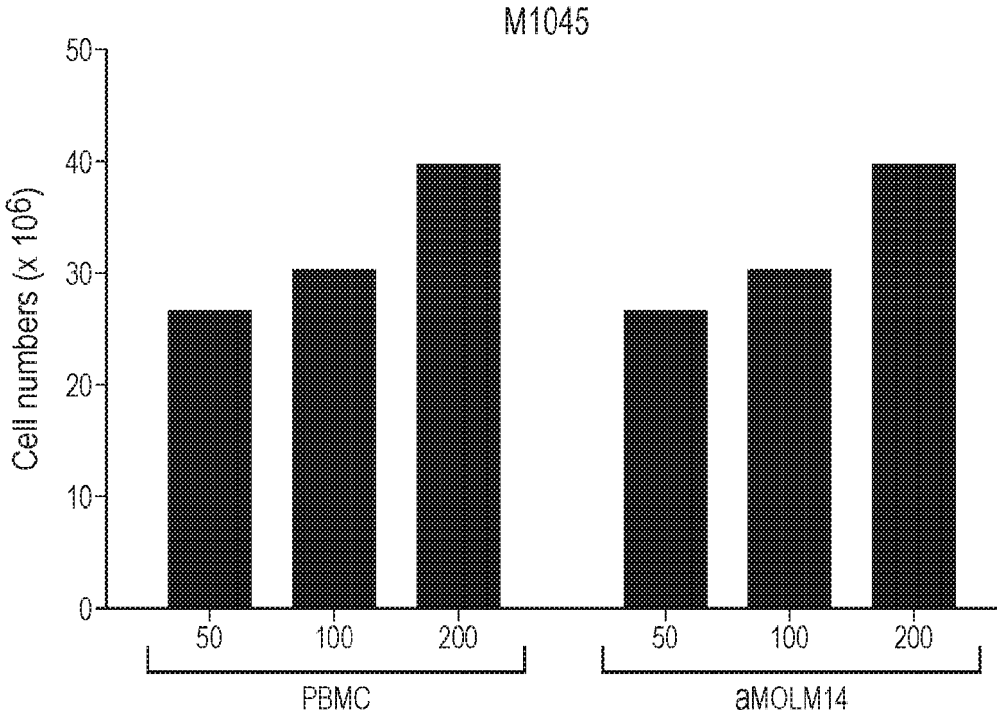

M1045
Cell numbers (x 106)
50
40
30
20
10
0
50
100
200
50
100
200
PBMC
aMOLM14

FIG. 20

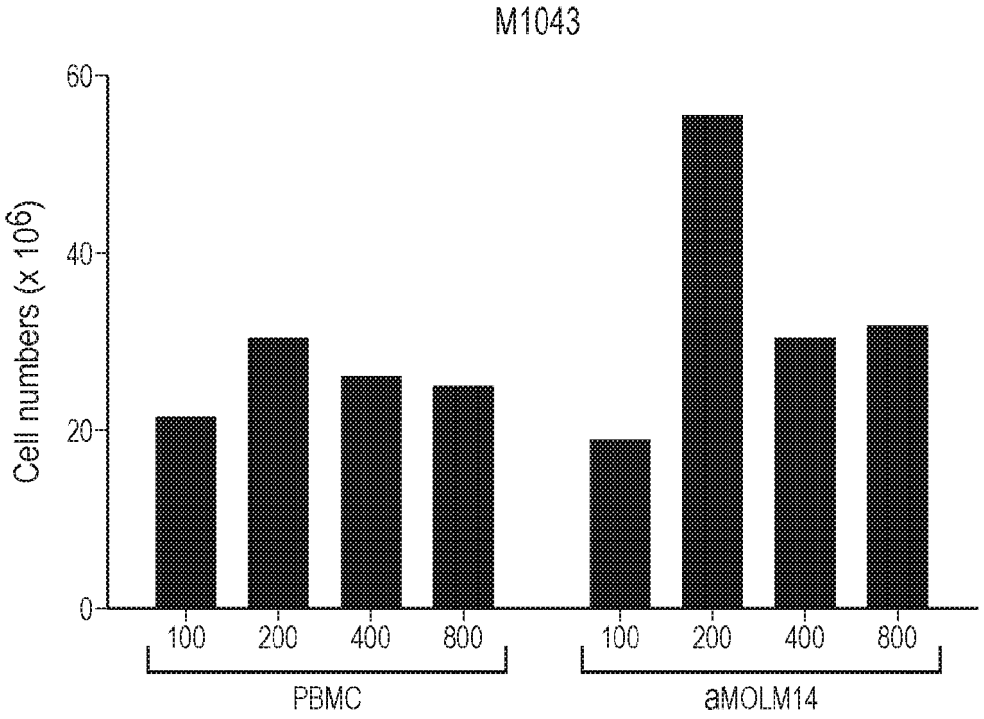
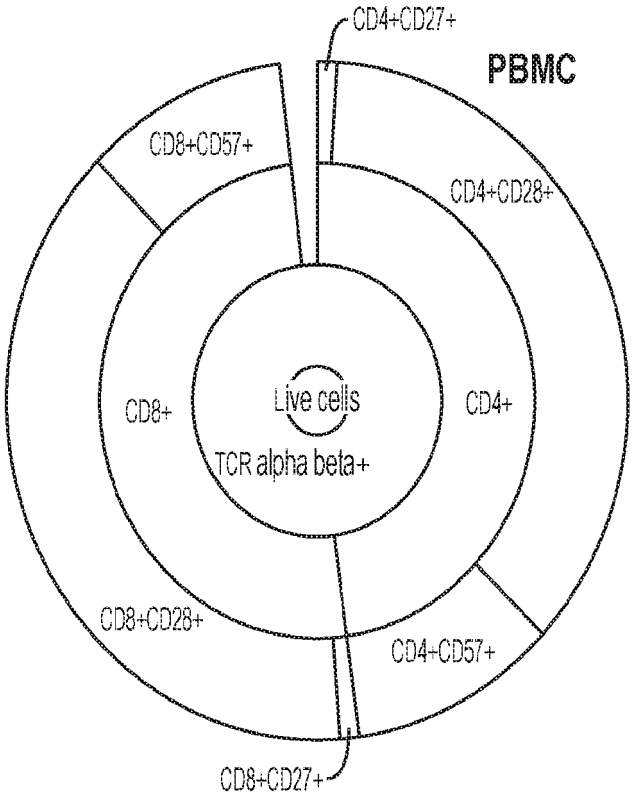
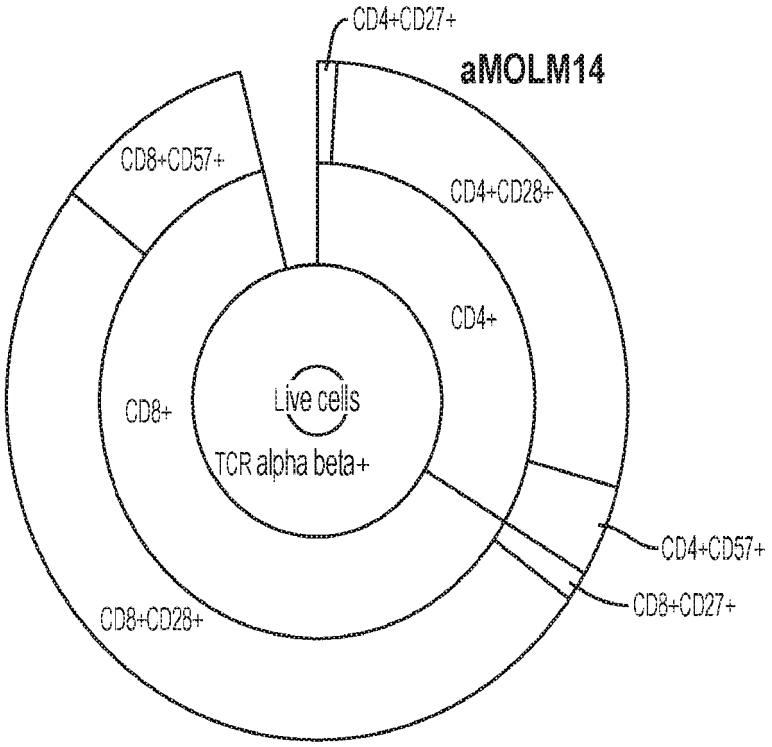

M1043
Cell numbers (x 10^6)
60
40
20
0
100
200
400
800
100
200
400
800
PBMC
aMOLM14

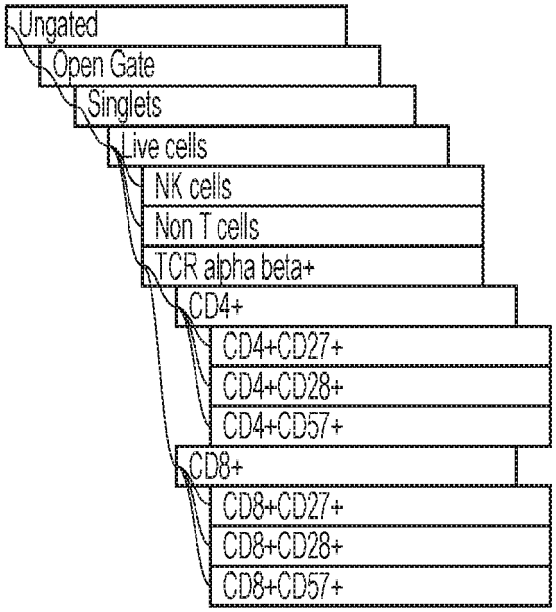
FIG. 21



	Event Count	% of Total	% of Parent
Ungated	10000		
Open Gate	3678	36.78%	36.78% of Ungated
Singlets	3676	36.76%	99.95% of Open Gate
Live cells	3509	35.09%	95.46% of Singlets
NK cells	2	0.02%	0.06% of Live cells
Non T cells	125	1.25%	3.56% of Live cells
TCR alpha beta+	3085	30.85%	87.92% of Live cells
CD4+	1436	14.36%	46.55% of TCR alpha beta+
CD4+CD27+	37	0.37%	2.58% of CD4+
CD4+CD28+	1418	14.18%	98.75% of CD4+
CD4+CD57+	410	4.1%	28.55% of CD4+
CD8+	1579	15.79%	51.18% of TCR alpha beta+
CD8+CD27+	36	0.36%	2.28% of CD8+
CD8+CD28+	1513	15.13%	95.82% of CD8+
CD8+CD57+	405	4.05%	25.65% of CD8+

FIG. 22





Event Count	% of Total	% of Parent
10000		
2848	28.48%	28.48% of Ungated
2845	28.45%	99.89% of Open Gate
2697	26.97%	94.8% of Singlets
3	0.03%	0.11% of Live cells
160	1.6%	5.93% of Live cells
2371	23.71%	87.91% of Live cells
785	7.85%	33.11% of TCR alpha beta+
32	0.32%	4.08% of CD4+
769	7.69%	97.96% of CD4+
119	1.19%	15.16% of CD4+
1475	14.75%	62.21% of TCR alpha beta+
42	0.42%	2.85% of CD8+
1376	13.76%	93.29% of CD8+
291	2.91%	19.73% of CD8+

FIG. 23

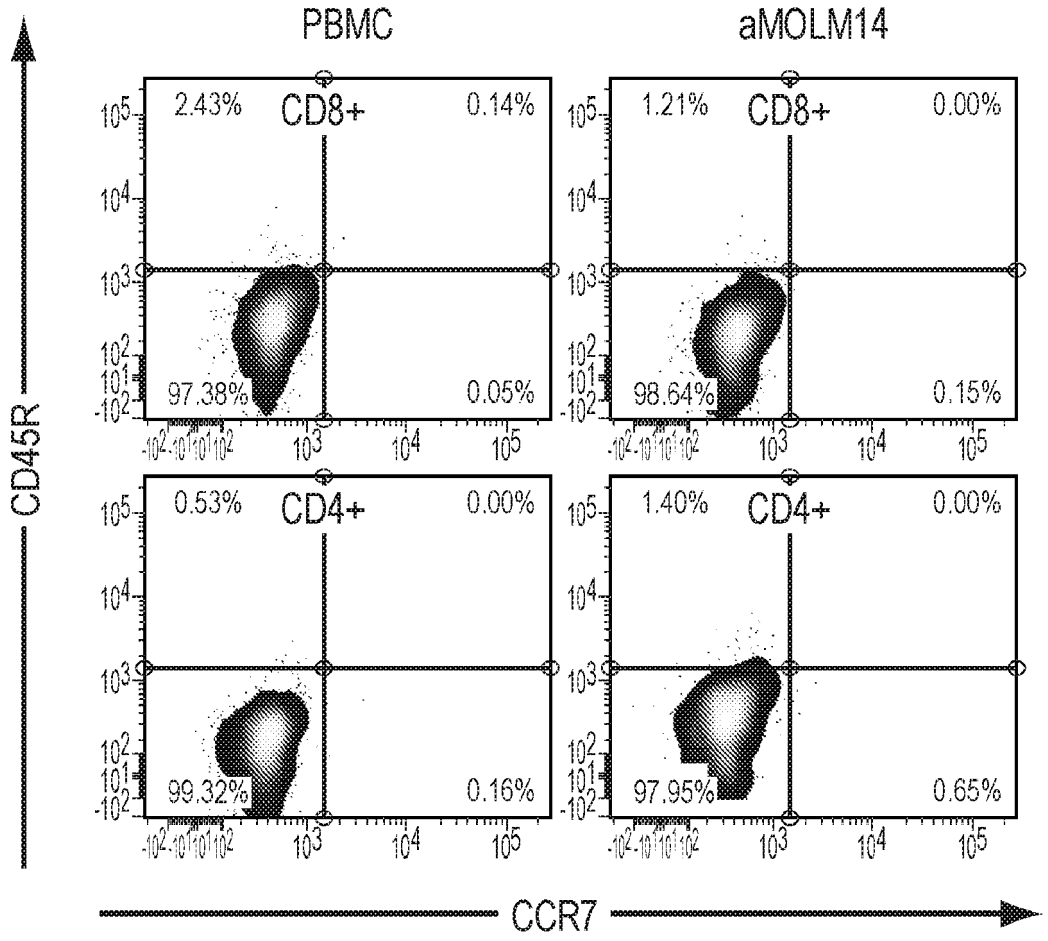

PBMC
aMOLM14
CD8+
CD4+
2.43%
0.14%
97.38%
0.05%
1.21%
0.00%
98.64%
0.15%
0.53%
0.00%
99.32%
0.16%
1.40%
0.00%
97.95%
0.65%
CD45R
CCR7

FIG. 24

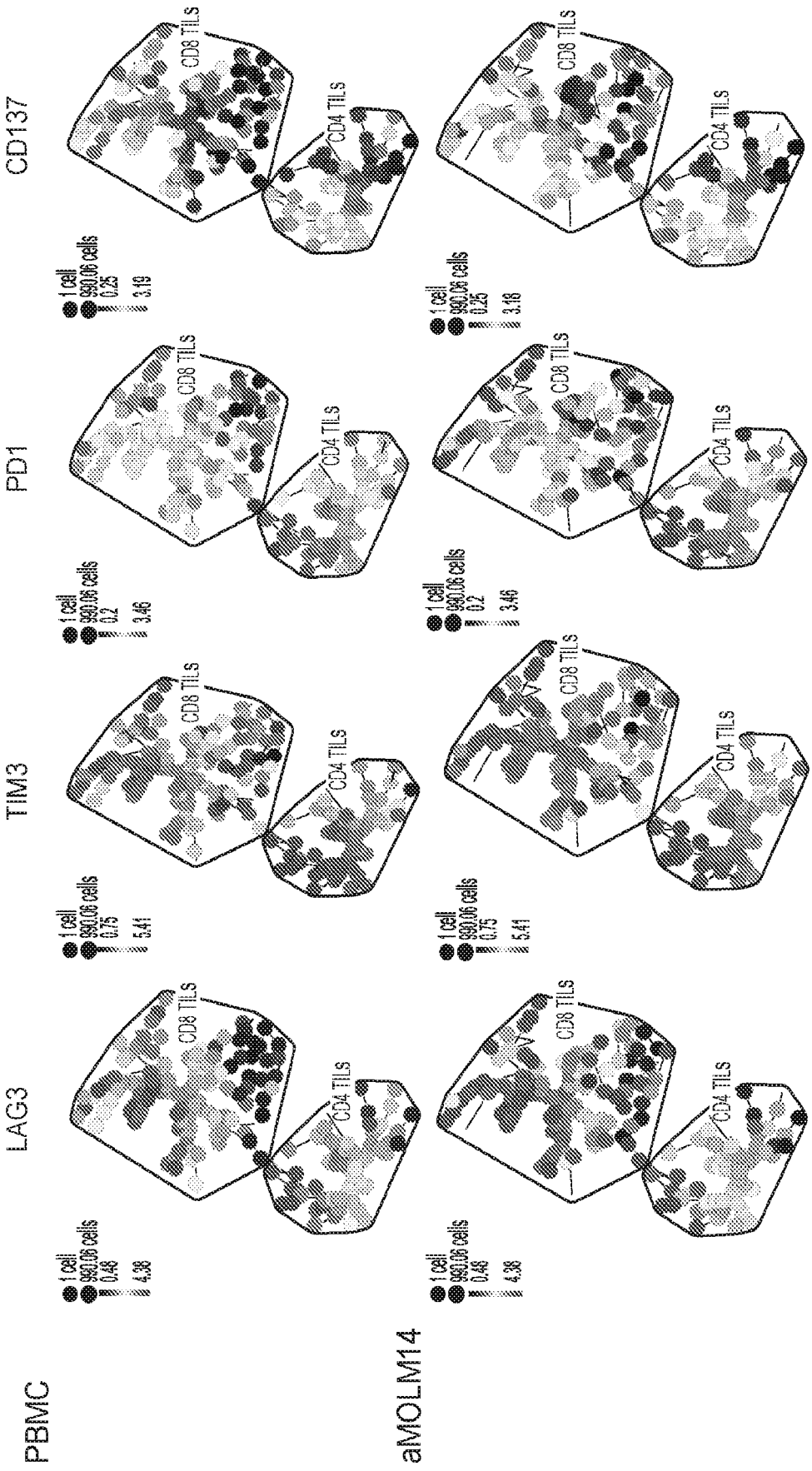

PBMC
aMOLM14
LAG3
TIM3
PD1
CD137
CD8 TILs
CD4 TILs
1 cell
990.06 cells
0.48
4.38
0.75
5.41
0.2
3.46
0.25
3.19
0.25
3.18

FIG. 25

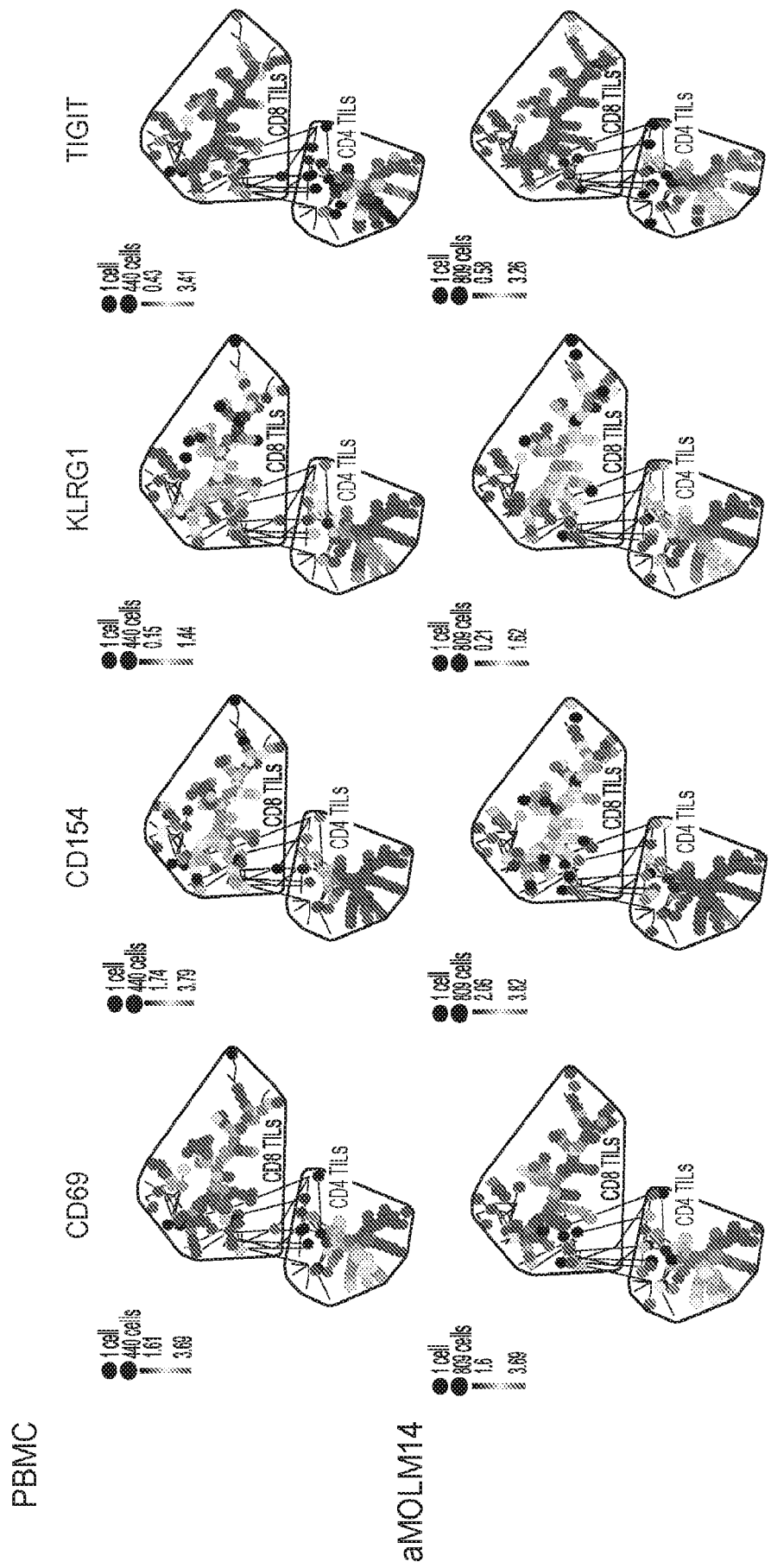

PBMC
aMOLM14
CD69
CD154
KLRG1
TIGIT
CD8 TILs
CD4 TILs
1 cell
440 cells
1.61
3.69
1 cell
809 cells
1.6
3.69
1 cell
440 cells
1.74
3.79
1 cell
809 cells
2.05
3.82
1 cell
440 cells
0.15
1.44
1 cell
809 cells
0.21
1.62
1 cell
440 cells
0.43
3.41
1 cell
809 cells
0.59
3.26

FIG. 26

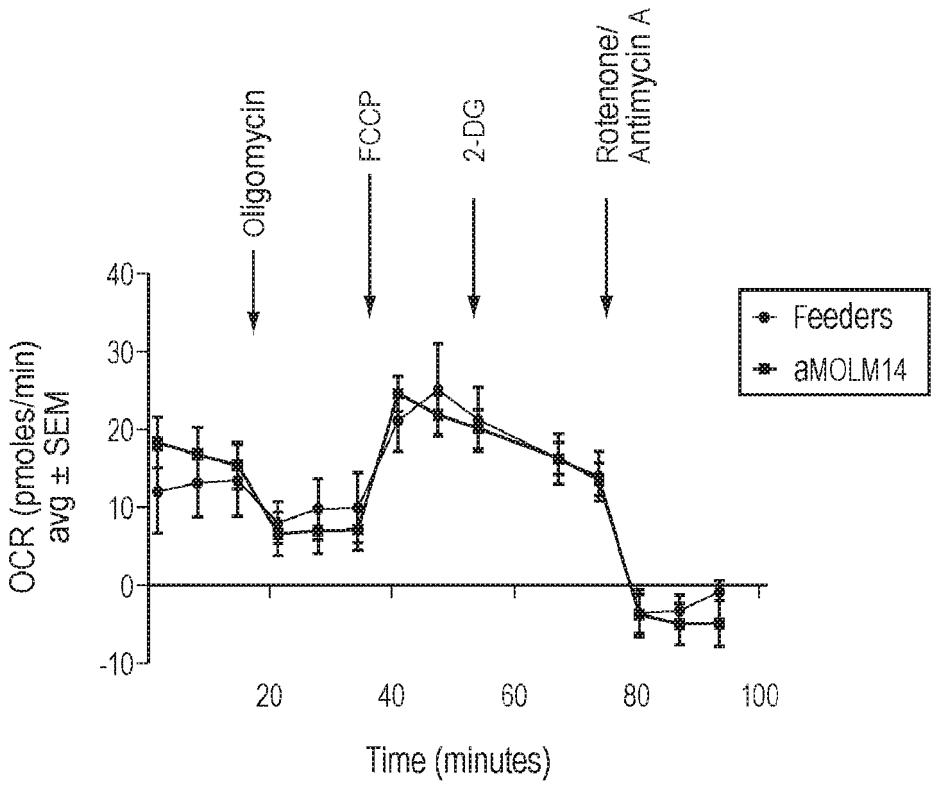

Oligomycin
FCCP
2-DG
Rotenone/
Antimycin A
OCR (pmoles/min)
avg ± SEM
40
30
20
10
0
-10
20
40
60
80
100
Time (minutes)
Feeders
aMOLM14

FIG. 27

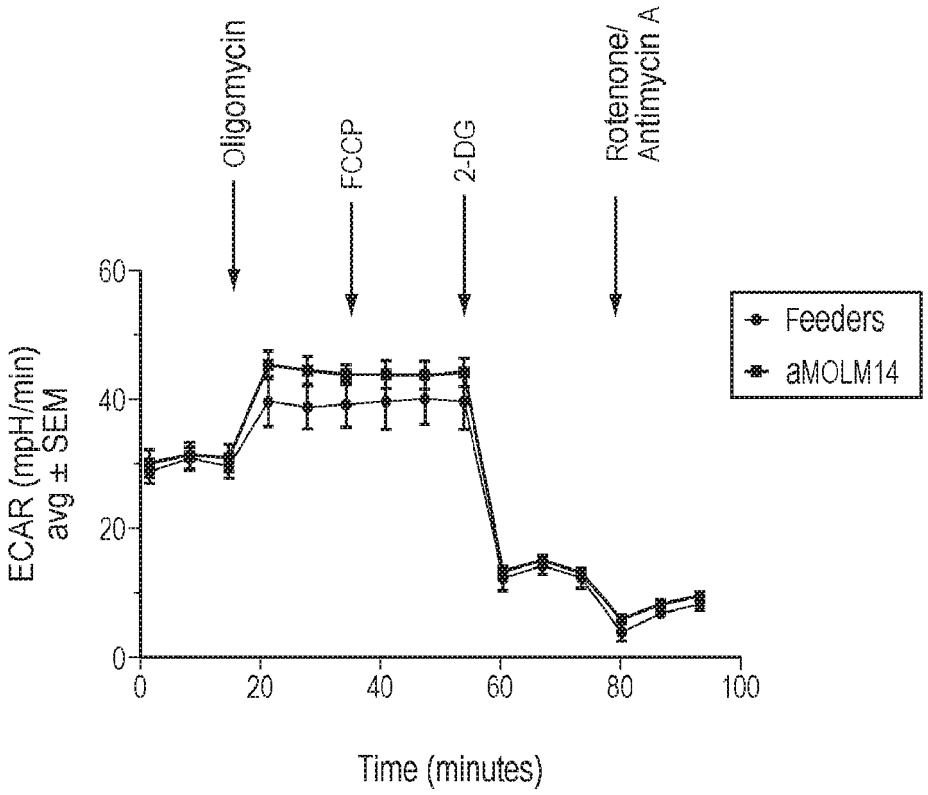

Oligomycin
FCCP
2-DG
Rotenone/
Antimycin A
Feeders
aMOLM14
ECAR (mpH/min)
avg ± SEM
60
40
20
0
0
20
40
60
80
100
Time (minutes)

FIG. 28

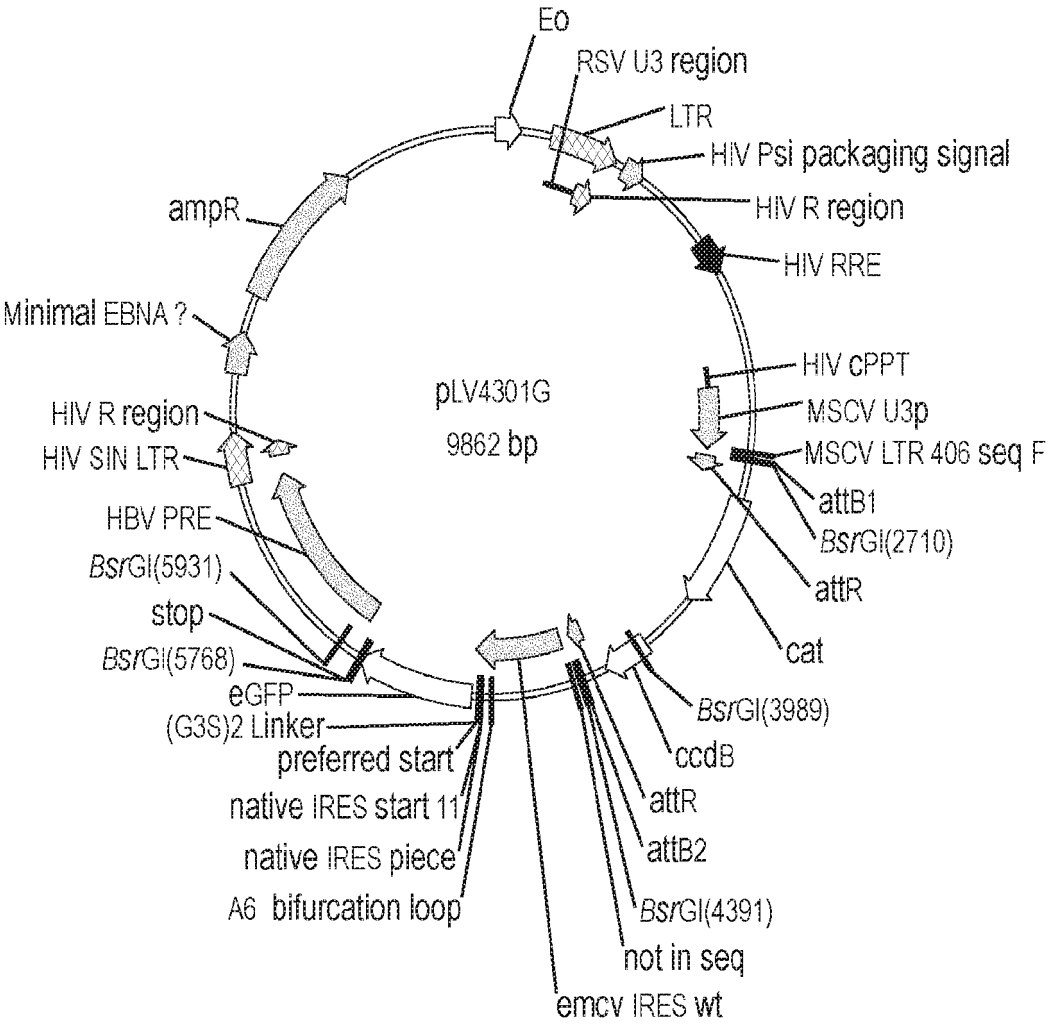

Eo
RSV U3 region
LTR
HIV Psi packaging signal
HIV R region
HIV RRE
HIV cPPT
MSCV U3p
MSCV LTR 406 seq F
attB1
BsrGI(2710)
attR
cat
BsrGI(3989)
ccdB
attR
attB2
BsrGI(4391)
not in seq
emcv IRES wt
A6 bifurcation loop
native IRES piece
native IRES start 11
preferred start
(G3S)2 Linker
eGFP
BsrGI(5768)
stop
BsrGI(5931)
HBV PRE
HIV SIN LTR
HIV R region
Minimal EBNA ?
ampR
pLV4301G
9862 bp

FIG. 29

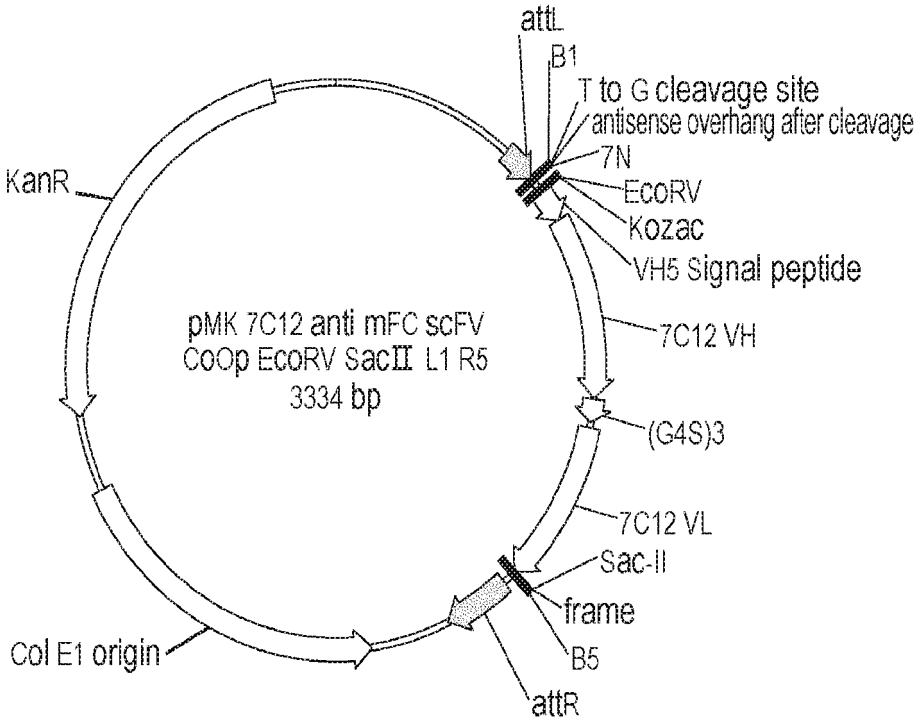

attL
B1
T to G cleavage site
antisense overhang after cleavage
7N
EcoRV
Kozac
VH5 Signal peptide
7C12 VH
(G4S)3
7C12 VL
Sac-II
frame
B5
attR
Col E1 origin
KanR
pMK 7C12 anti mFC scFV
CoOp EcoRV SacII L1 R5
3334 bp

FIG. 30

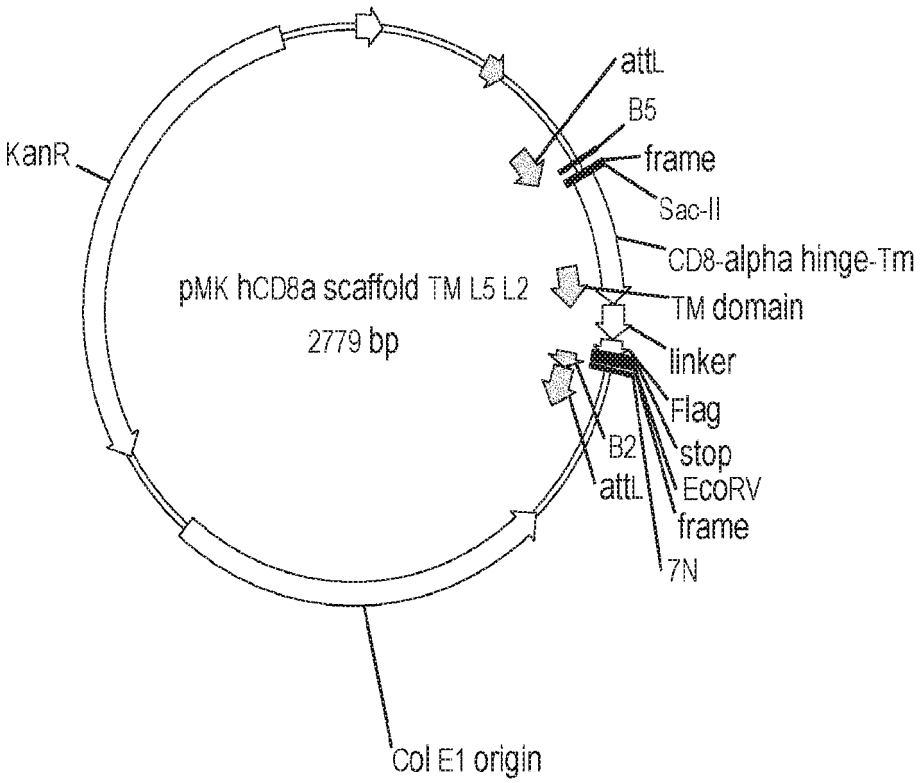

attL
B5
frame
Sac-II
CD8-alpha hinge-Tm
TM domain
linker
Flag
stop
EcoRV
frame
7N
B2
attL
KanR
pMK hCD8a scaffold TM L5 L2
2779 bp
Col E1 origin

FIG. 31

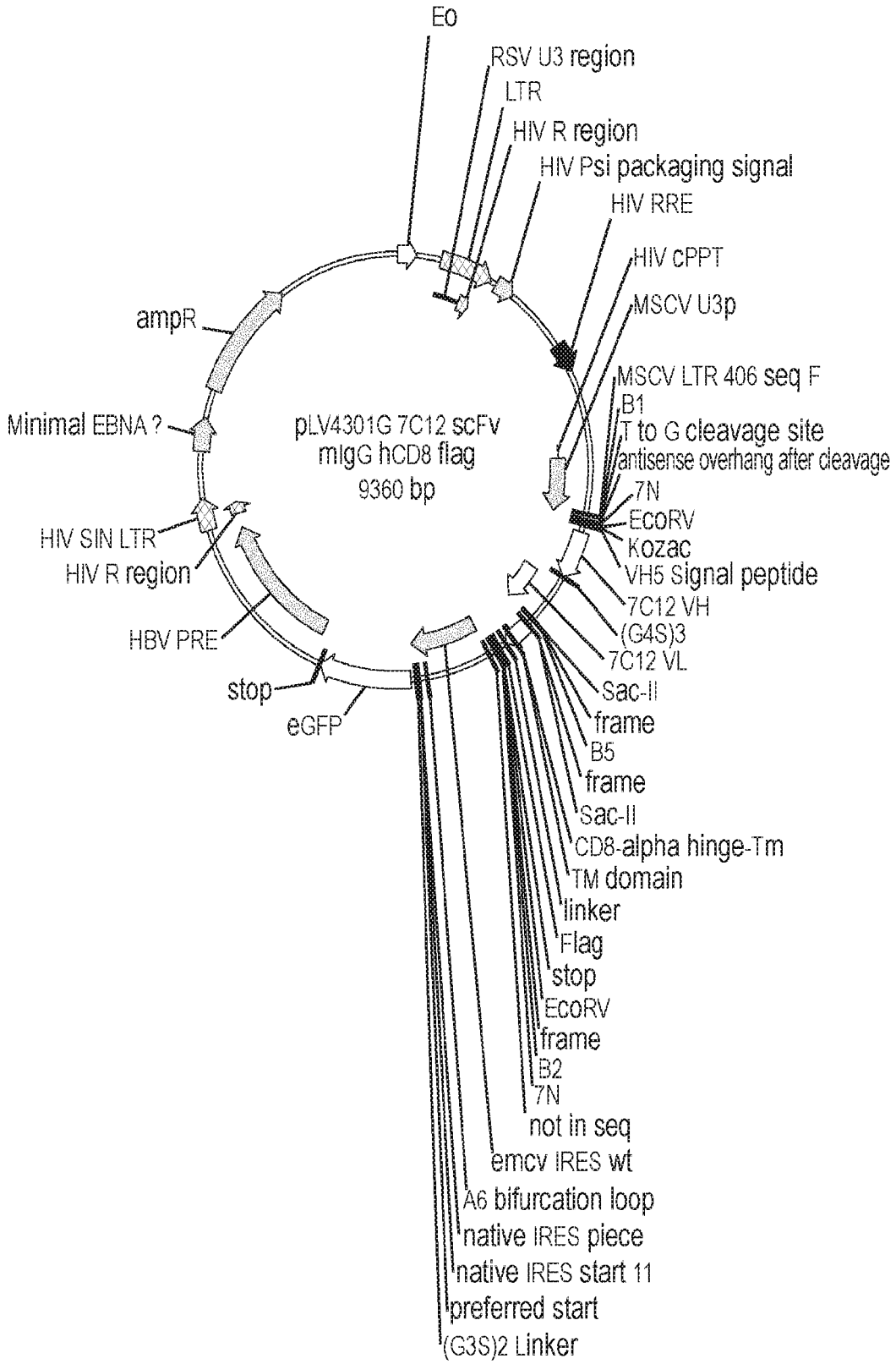

E0
RSV U3 region
LTR
HIV R region
HIV Psi packaging signal
HIV RRE
HIV cPPT
MSCV U3p
MSCV LTR 406 seq F
B1
T to G cleavage site
antisense overhang after cleavage
7N
EcoRV
Kozac
VH5 Signal peptide
7C12 VH
(G4S)3
7C12 VL
Sac-II
frame
B5
frame
Sac-II
CD8-alpha hinge-Tm
TM domain
linker
Flag
stop
EcoRV
frame
B2
7N
not in seq
emcv IRES wt
A6 bifurcation loop
native IRES piece
native IRES start 11
preferred start
(G3S)2 Linker
ampR
Minimal EBNA ?
HIV SIN LTR
HIV R region
HBV PRE
stop
eGFP
pLV4301G 7C12 scFv
mIgG hCD8 flag
9360 bp

FIG. 32

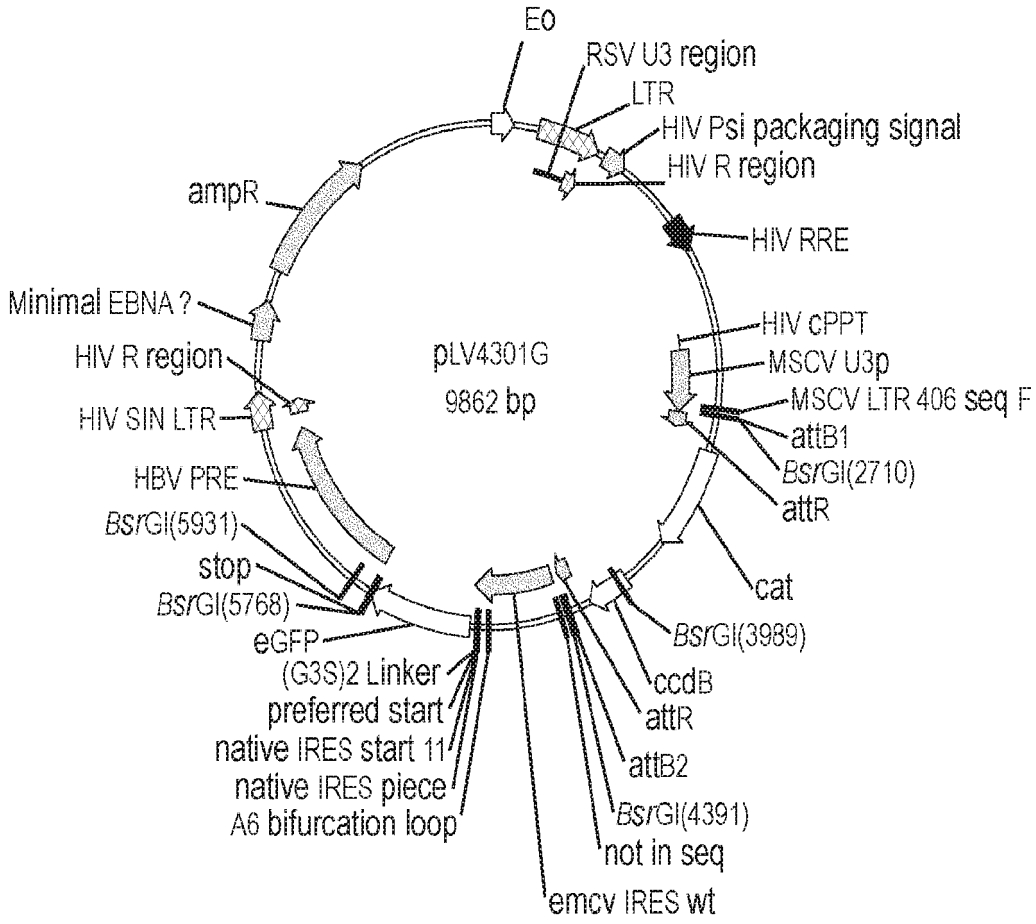

E0
RSV U3 region
LTR
HIV Psi packaging signal
HIV R region
HIV RRE
HIV cPPT
MSCV U3p
MSCV LTR 406 seq F
attB1
BsrGI(2710)
attR
cat
BsrGI(3989)
ccdB
attR
attB2
BsrGI(4391)
not in seq
emcv IRES wt
A6 bifurcation loop
native IRES piece
native IRES start 11
preferred start
(G3S)2 Linker
eGFP
BsrGI(5768)
stop
BsrGI(5931)
HBV PRE
HIV SIN LTR
HIV R region
Minimal EBNA ?
ampR
pLV4301G
9862 bp

FIG. 33

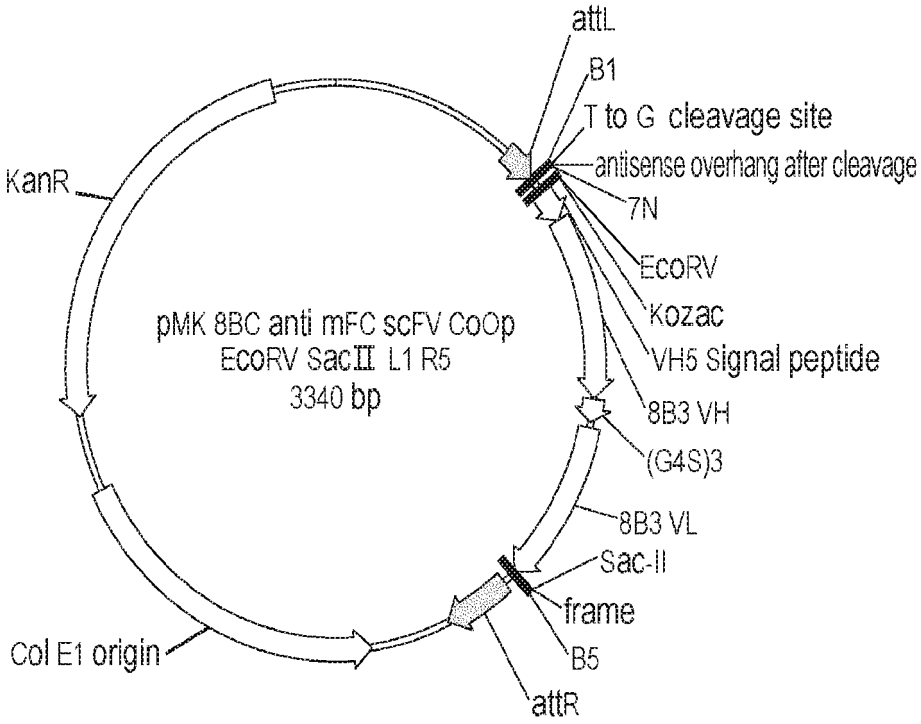

attL
B1
T to G cleavage site
antisense overhang after cleavage
7N
EcoRV
Kozac
VH5 Signal peptide
8B3 VH
(G4S)3
8B3 VL
Sac-II
frame
B5
attR
Col E1 origin
KanR
pMK 8BC anti mFC scFV CoOp
EcoRV SacII L1 R5
3340 bp

FIG. 34

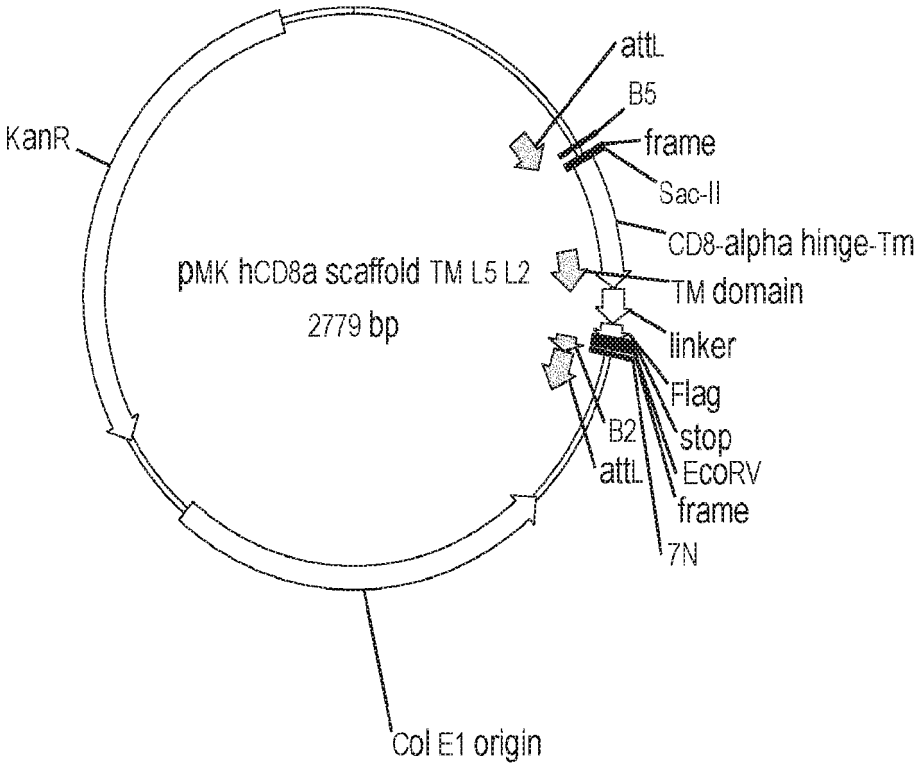

attL
B5
frame
Sac-II
CD8-alpha hinge-Tm
TM domain
linker
Flag
stop
EcoRV
frame
7N
B2
attL
KanR
pMK hCD8a scaffold TM L5 L2
2779 bp
Col E1 origin

FIG. 35

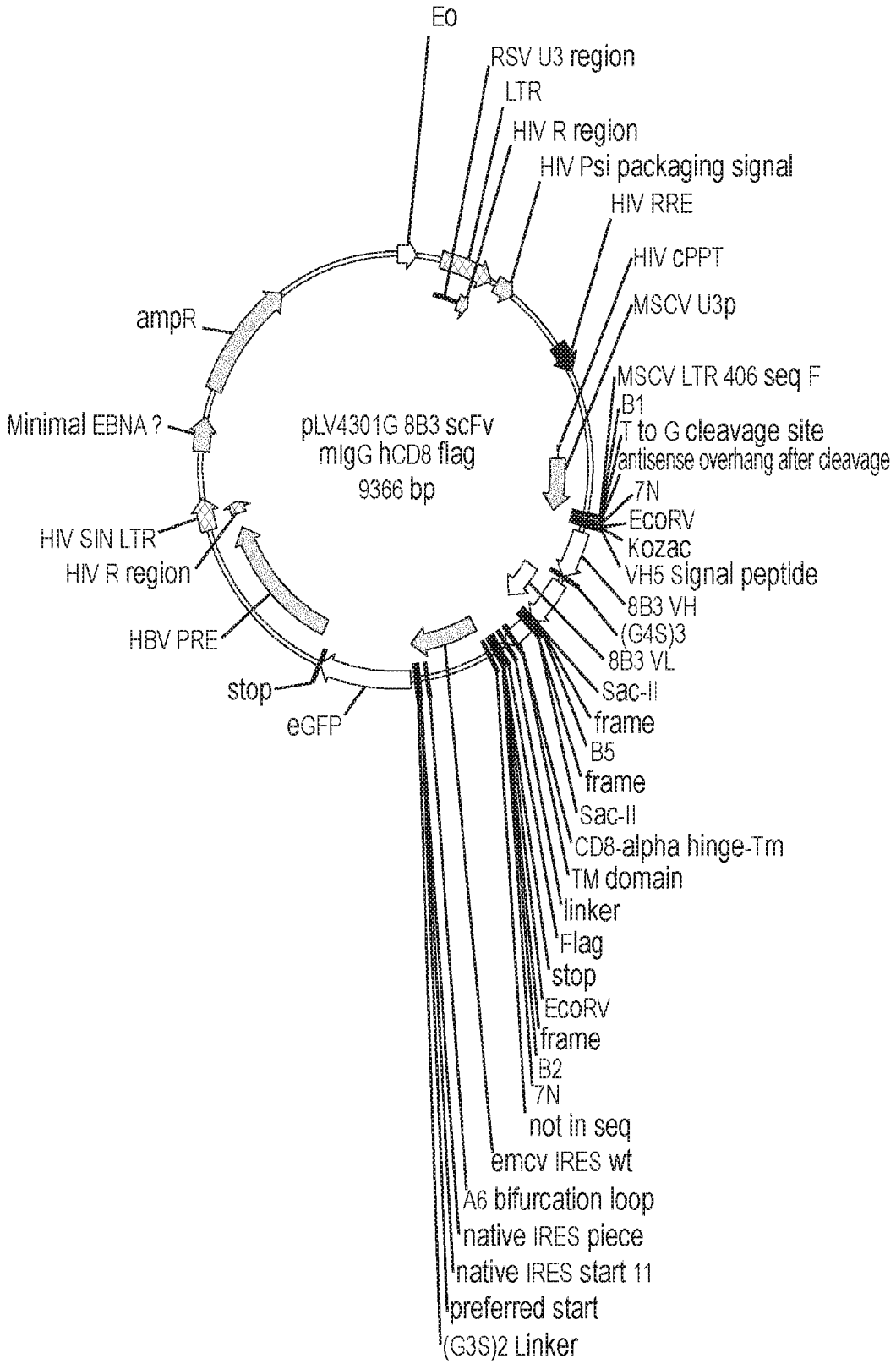

E0
RSV U3 region
LTR
HIV R region
HIV Psi packaging signal
HIV RRE
HIV cPPT
MSCV U3p
MSCV LTR 406 seq F
B1
T to G cleavage site
antisense overhang after cleavage
7N
EcoRV
Kozac
VH5 Signal peptide
8B3 VH
(G4S)3
8B3 VL
Sac-II
frame
B5
frame
Sac-II
CD8-alpha hinge-Tm
TM domain
linker
Flag
stop
EcoRV
frame
B2
7N
not in seq
emcv IRES wt
A6 bifurcation loop
native IRES piece
native IRES start 11
preferred start
(G3S)2 Linker
ampR
Minimal EBNA ?
HIV SIN LTR
HIV R region
HBV PRE
stop
eGFP
pLV4301G 8B3 scFv
mIgG hCD8 flag
9366 bp

FIG. 36

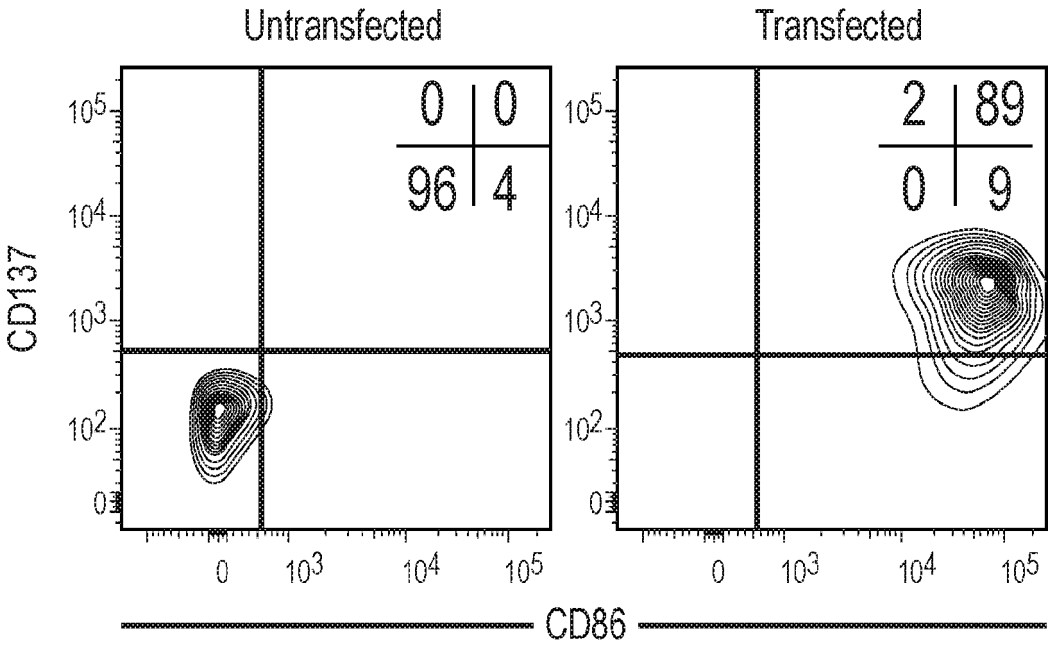

Untransfected
Transfected
0
0
96
4
2
89
0
9
CD137
CD86
10^5
10^4
10^3
10^2
0
0
10^3
10^4
10^5

FIG. 37

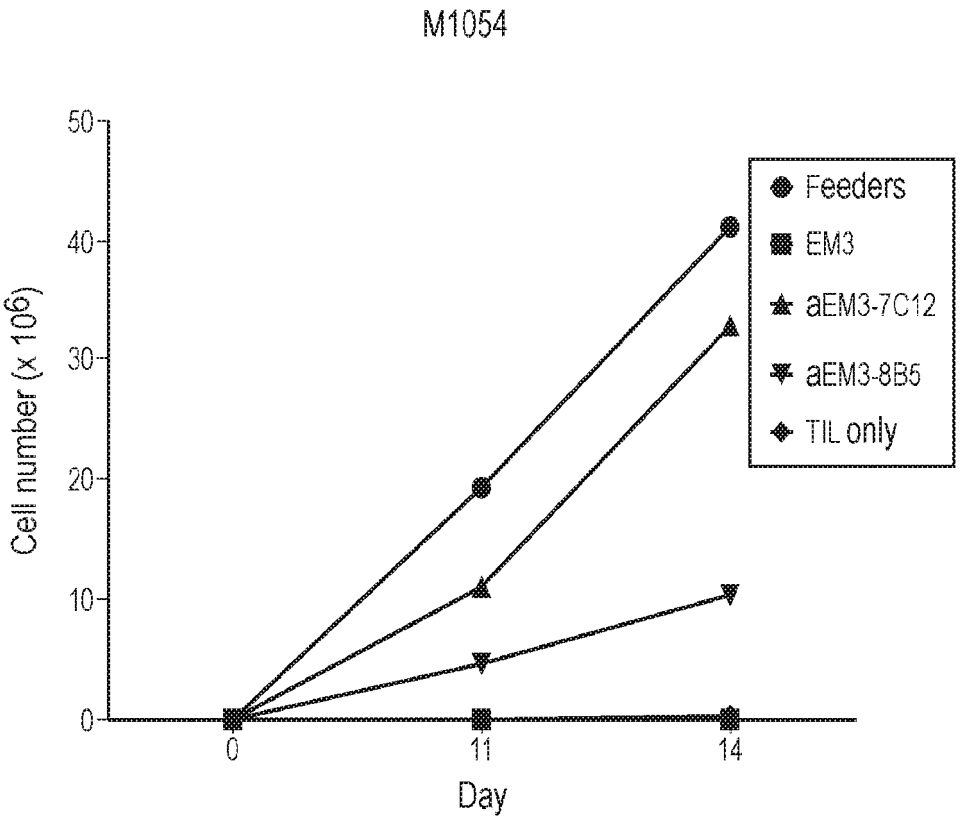

M1054
Cell number (x 10^6)
50
40
30
20
10
0
0
11
14
Day
Feeders
EM3
aEM3-7C12
aEM3-8B5
TIL only

FIG. 38

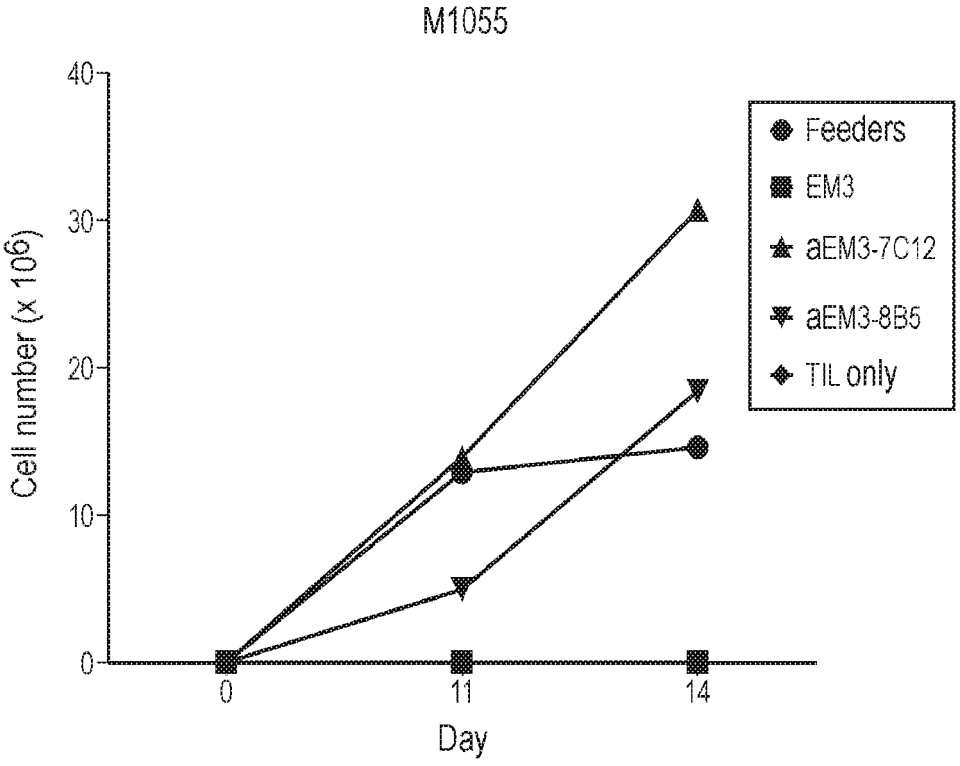

M1055
40
30
20
10
0
Cell number (x 10^6)
0
11
14
Day
Feeders
EM3
aEM3-7C12
aEM3-8B5
TIL only

FIG. 39

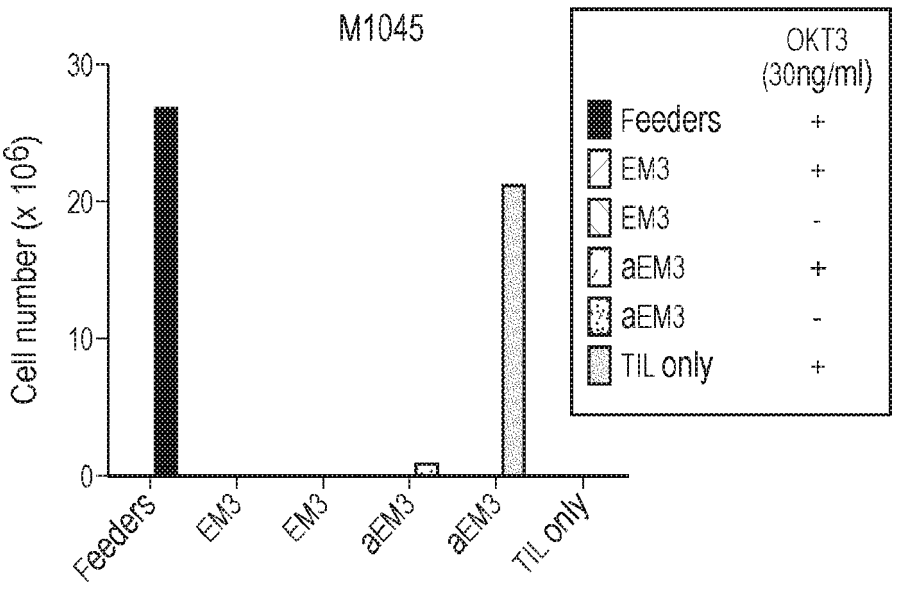

M1045
Cell number (x 10^6)
30
20
10
0
Feeders
EM3
EM3
aEM3
aEM3
TIL only
OKT3 (30ng/ml)
Feeders +
EM3 +
EM3 -
aEM3 +
aEM3 -
TIL only +

FIG. 40

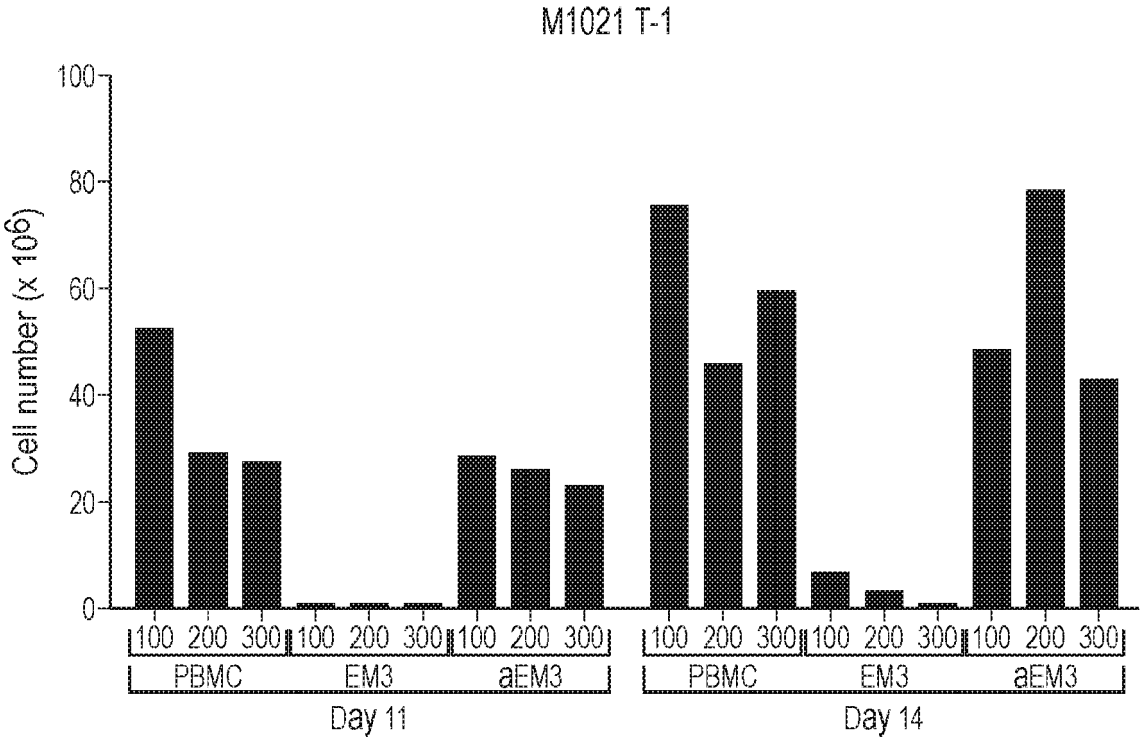

M1021 T-1
Cell number (x 10^6)
100
80
60
40
20
0
100 200 300 100 200 300 100 200 300
PBMC EM3 aEM3
Day 11
100 200 300 100 200 300 100 200 300
PBMC EM3 aEM3
Day 14

FIG. 41

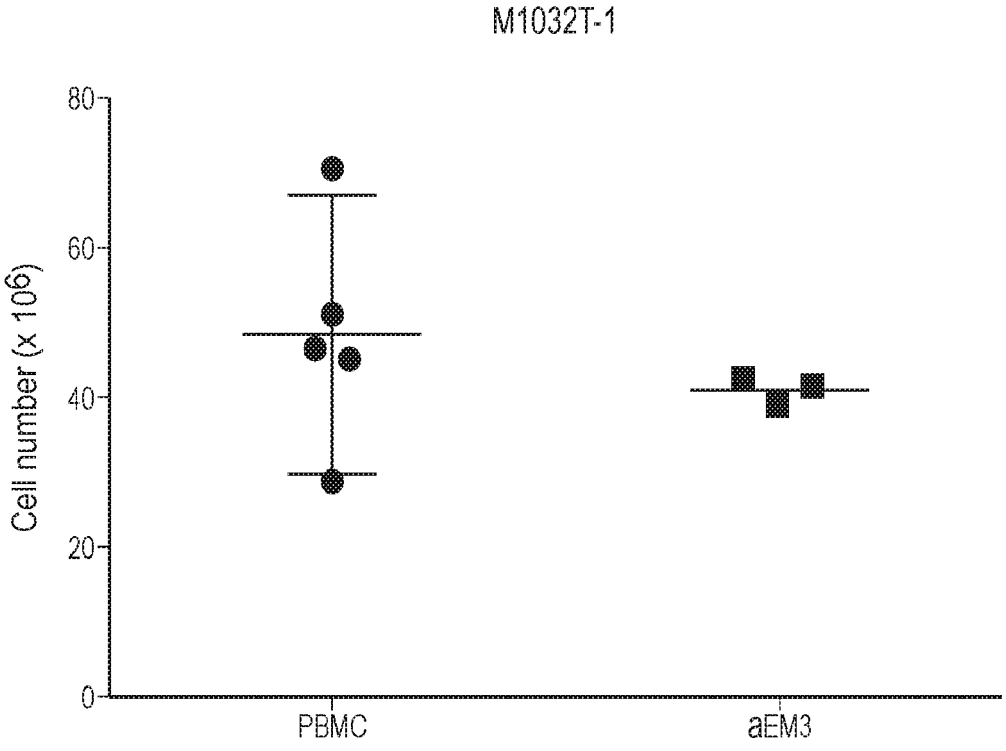


FIG. 42

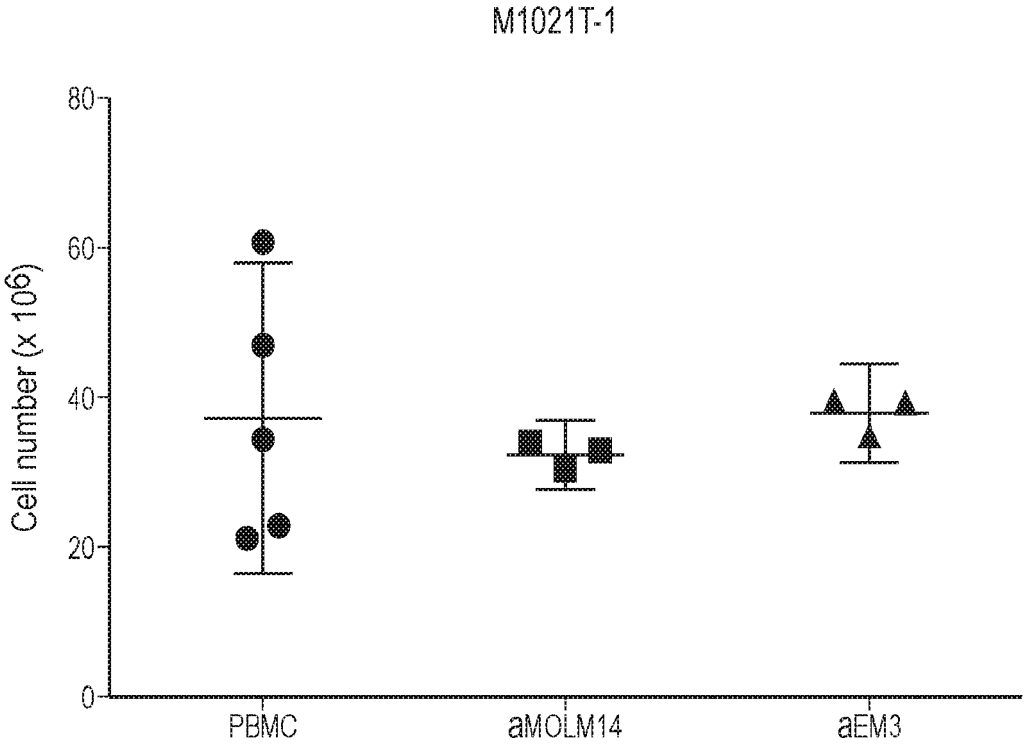
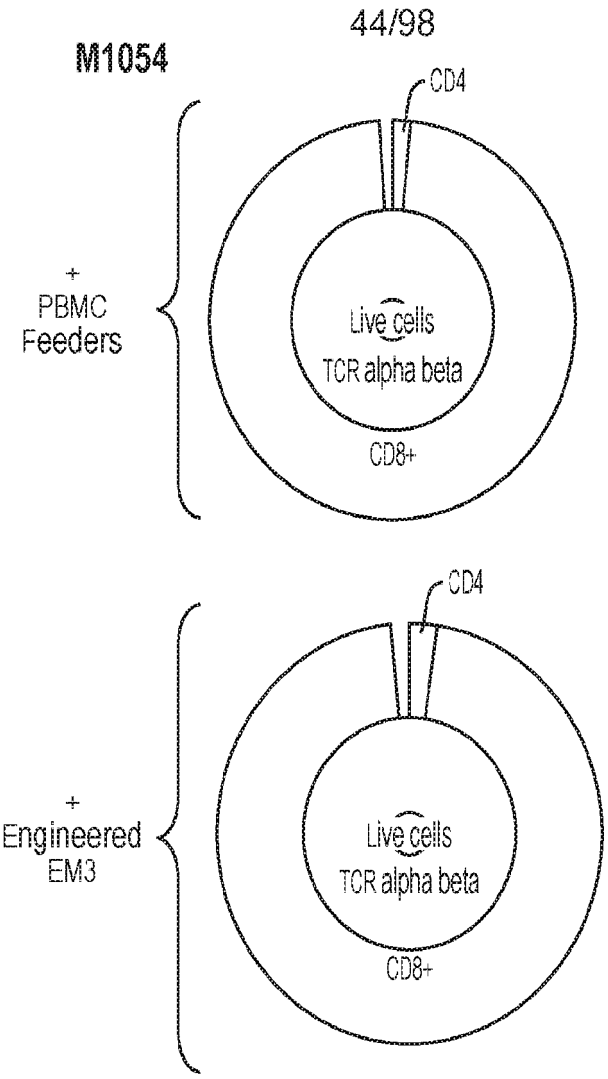
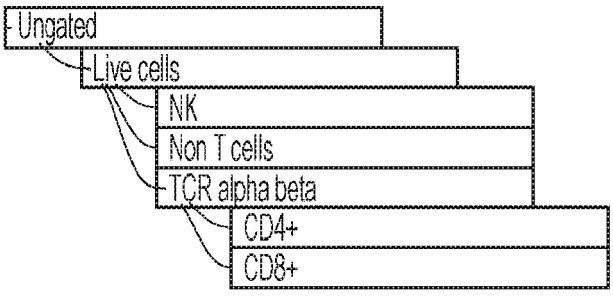
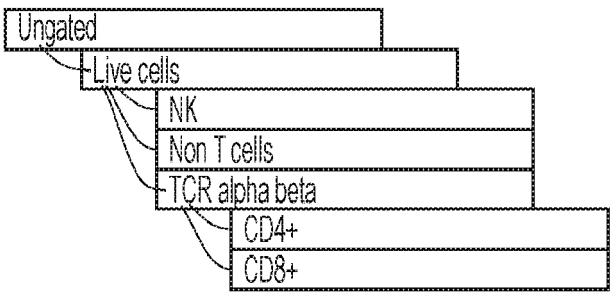


FIG. 43



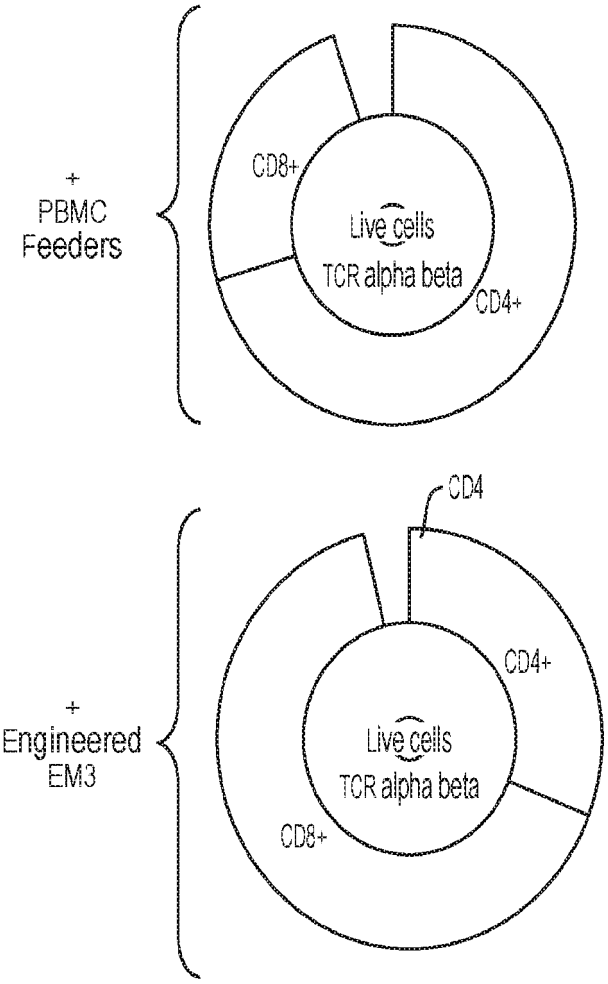


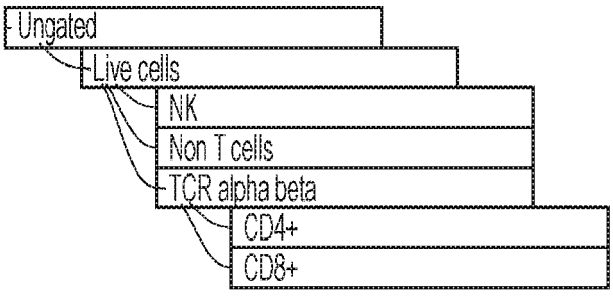
Event Count	% of Total	% of Parent
30000		
21976	73.25%	73.25% of Ungated
58	0.19%	0.26% of Live cells
369	1.23%	1.68% of Live cells
21077	70.26%	95.91% of Live cells
368	1.23%	1.75% of TCR alpha beta
20554	68.51%	97.52% of TCR alpha beta



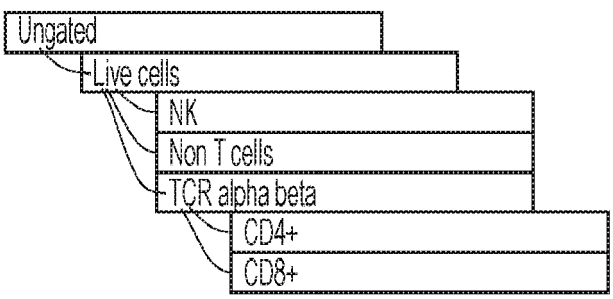
Event Count	% of Total	% of Parent
30000		
22173	73.91%	73.91% of Ungated
1	0%	0% of Live cells
230	0.77%	1.04% of Live cells
21709	72.36%	97.91% of Live cells
532	1.77%	2.45% of TCR alpha beta
20863	69.54%	96.1% of TCR alpha beta

M1055





Event Count	% of Total	% of Parent
30000		
20353	67.84%	67.84% of Ungated
0		
43	0.14%	0.21% of Live cells
20226	67.42%	99.38% of Live cells
14211	47.37%	70.26% of TCR alpha beta
4956	16.52%	24.5% of TCR alpha beta



Event Count	% of Total	% of Parent
30000		
16288	54.29%	54.29% of Ungated
0		
11	0.04%	0.07% of Live cells
16262	54.21%	99.84% of Live cells
5014	16.71%	30.83% of TCR alpha beta
10668	35.56%	65.6% of TCR alpha beta

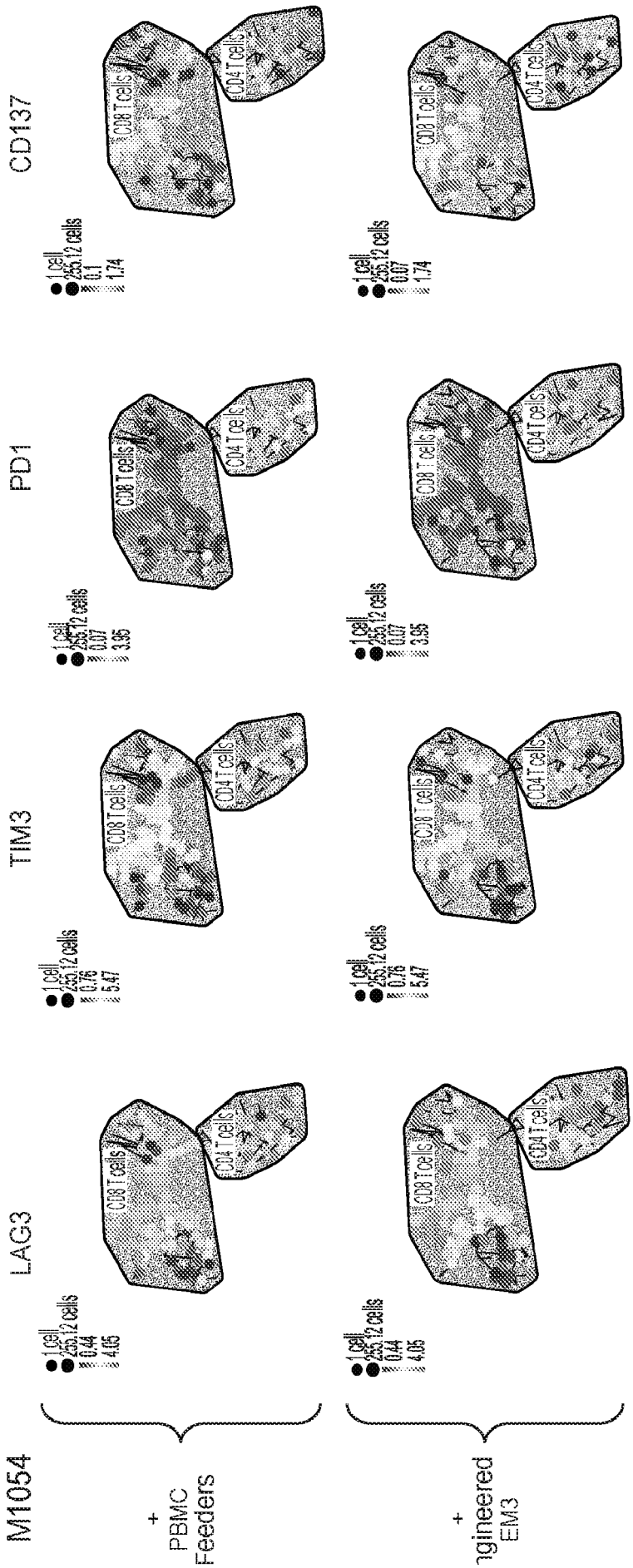

M1054
LAG3
TIM3
PD1
CD137
+ PBMC Feeders
+ ngineered EM3
CD8 T cells
CD4 T cells
1 cell
255.12 cells
0.44
4.05
0.76
5.47
0.07
3.95
0.1
1.74
0.07
1.74

FIG. 46

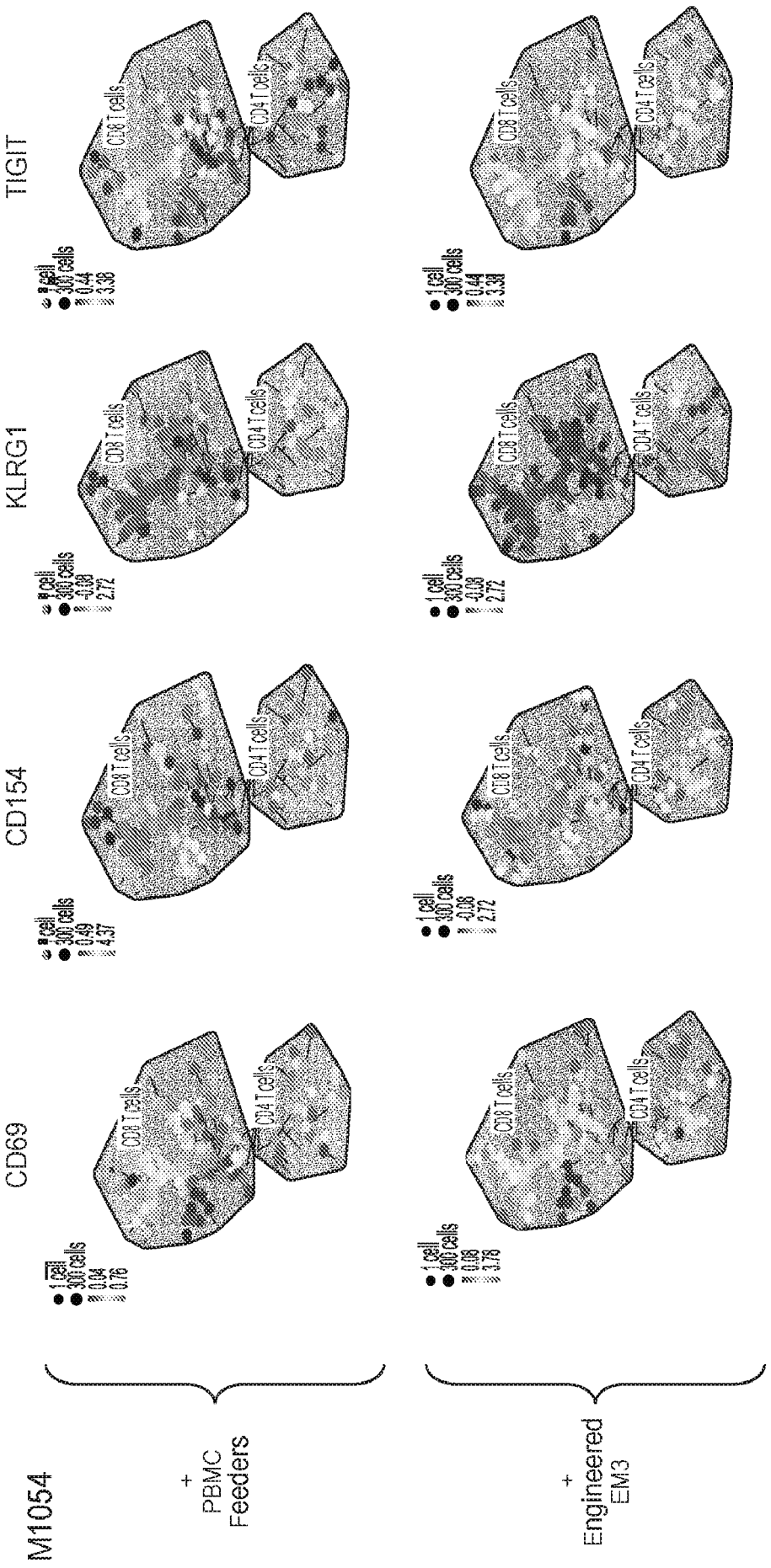

M1054
+ PBMC Feeders
+ Engineered EM3
CD69
CD154
KLRG1
TIGIT
CD8 T cells
CD4 T cells
1 cell
300 cells
0.04
0.76
0.49
4.37
-0.08
2.72
0.06
3.76
0.44
3.36

FIG. 47

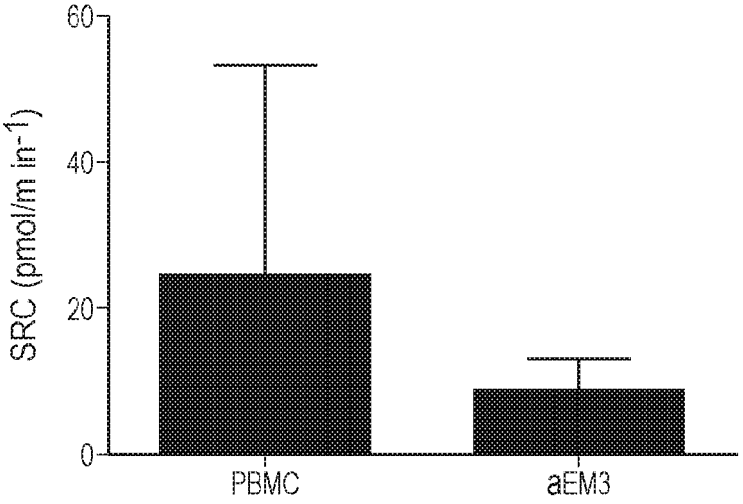


FIG. 48

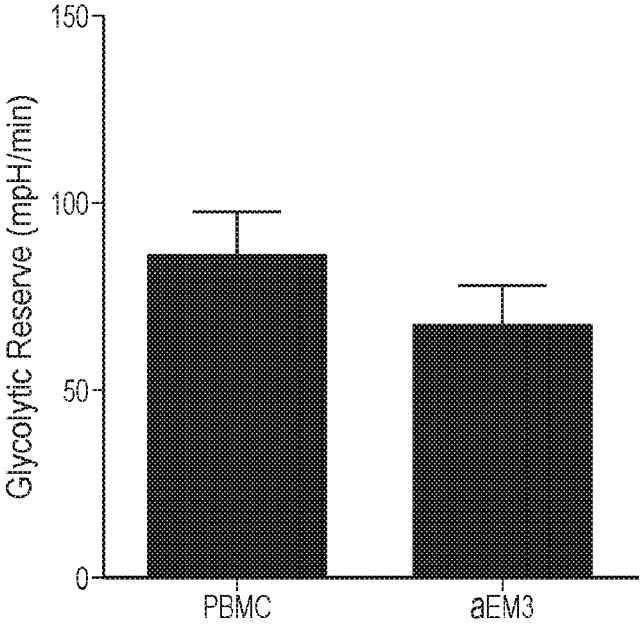


FIG. 49

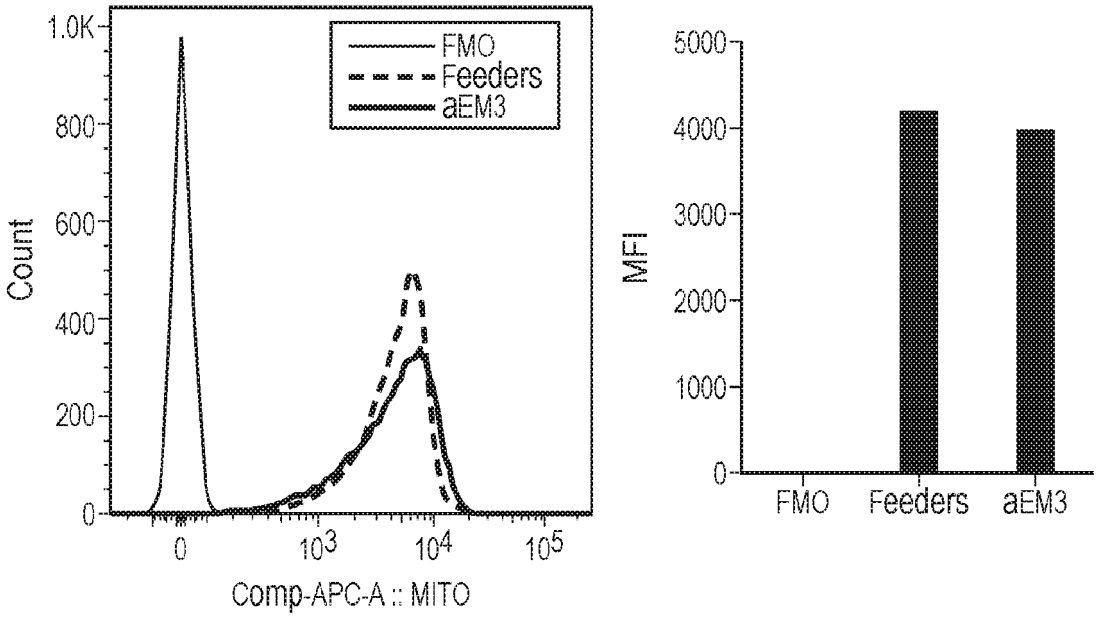

1.0K
800
600
400
200
0
Count
FMO
Feeders
aEM3
0
10^3
10^4
10^5
Comp-APC-A :: MITO
5000
4000
3000
2000
1000
0
MFI
FMO
Feeders
aEM3

FIG. 50

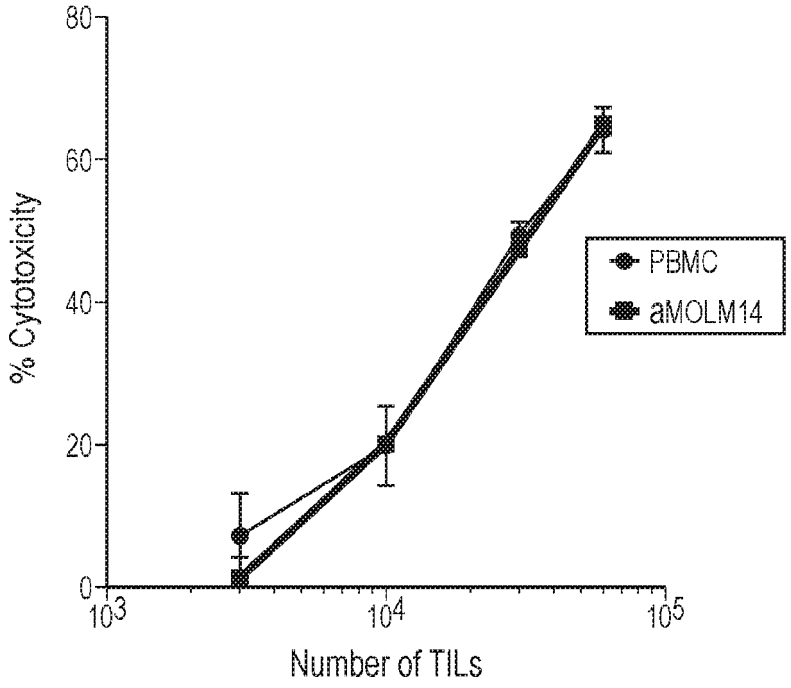

80
60
40
20
0
% Cytotoxicity
10^3
10^4
10^5
Number of TILs
PBMC
aMOLM14

FIG. 51

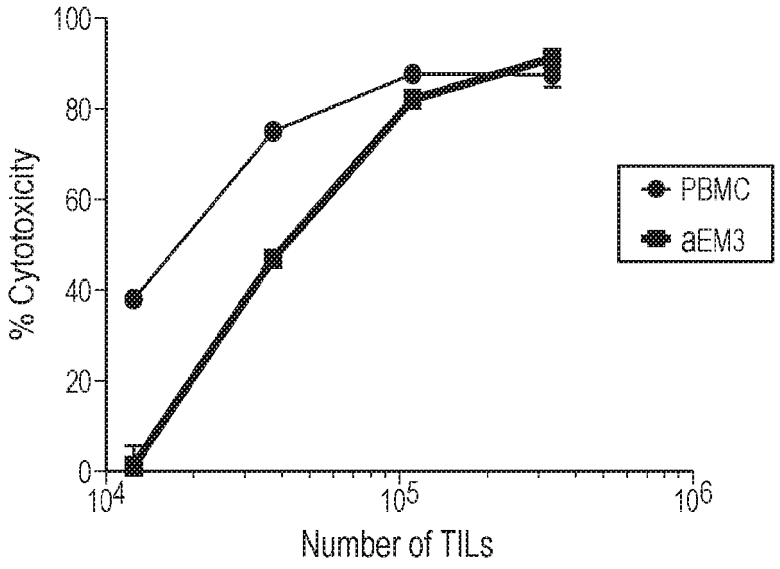

100
80
60
40
20
0
% Cytotoxicity
10^4
10^5
10^6
Number of TILs
PBMC
aEM3

FIG. 52

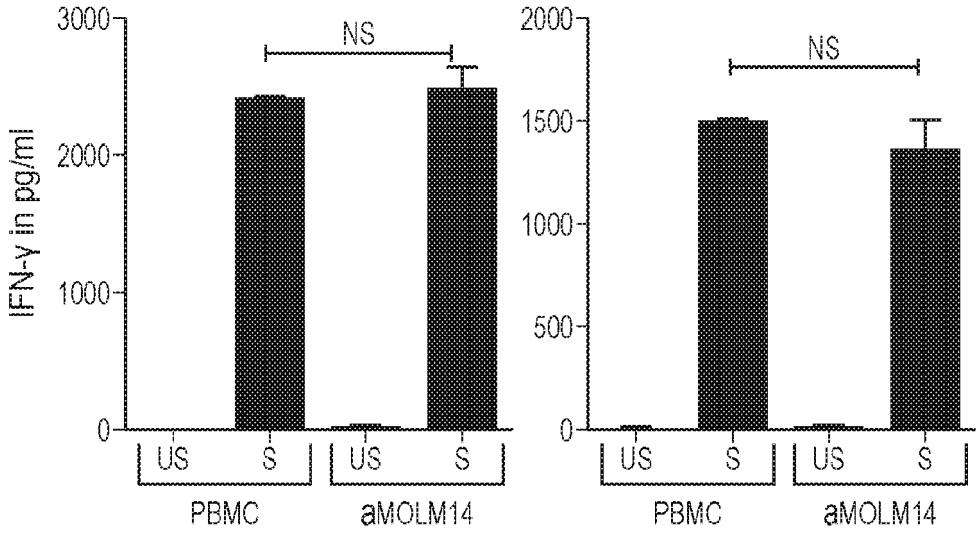

IFN-γ in pg/ml
3000
2000
1000
0
NS
US
S
US
S
PBMC
aMOLM14
2000
1500
1000
500
0
NS
US
S
US
S
PBMC
aMOLM14

FIG. 53

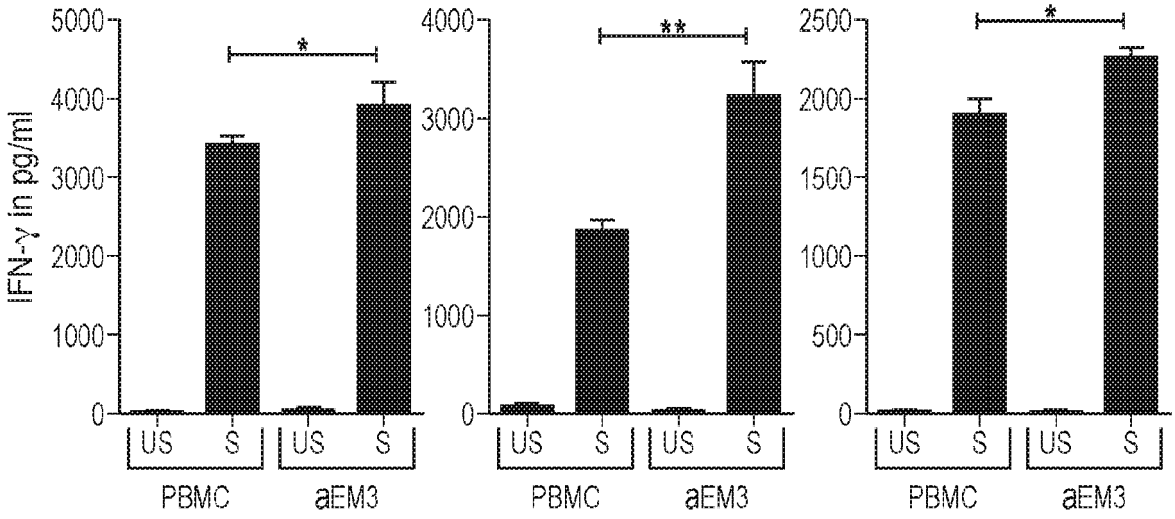

IFN-γ in pg/ml
5000
4000
3000
2000
1000
0
*
US
S
US
S
PBMC
aEM3
4000
3000
2000
1000
0
**
US
S
US
S
PBMC
aEM3
2500
2000
1500
1000
500
0
*
US
S
US
S
PBMC
aEM3

FIG. 54

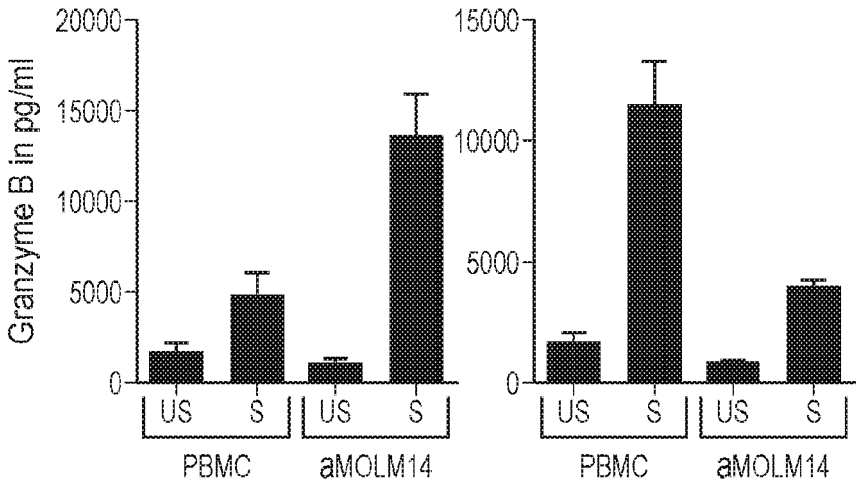

Granzyme B in pg/ml
20000
15000
10000
5000
0
US
S
US
S
PBMC
aMOLM14
15000
10000
5000
0
US
S
US
S
PBMC
aMOLM14

FIG. 55

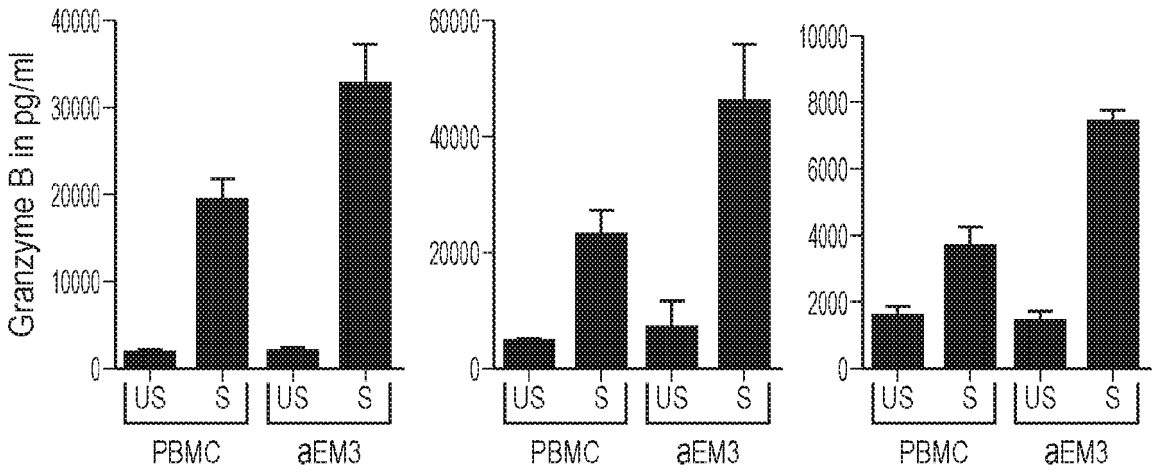

Granzyme B in pg/ml
40000
30000
20000
10000
0
US
S
US
S
PBMC
aEM3
60000
40000
20000
0
US
S
US
S
PBMC
aEM3
10000
8000
6000
4000
2000
0
US
S
US
S
PBMC
aEM3

FIG. 56

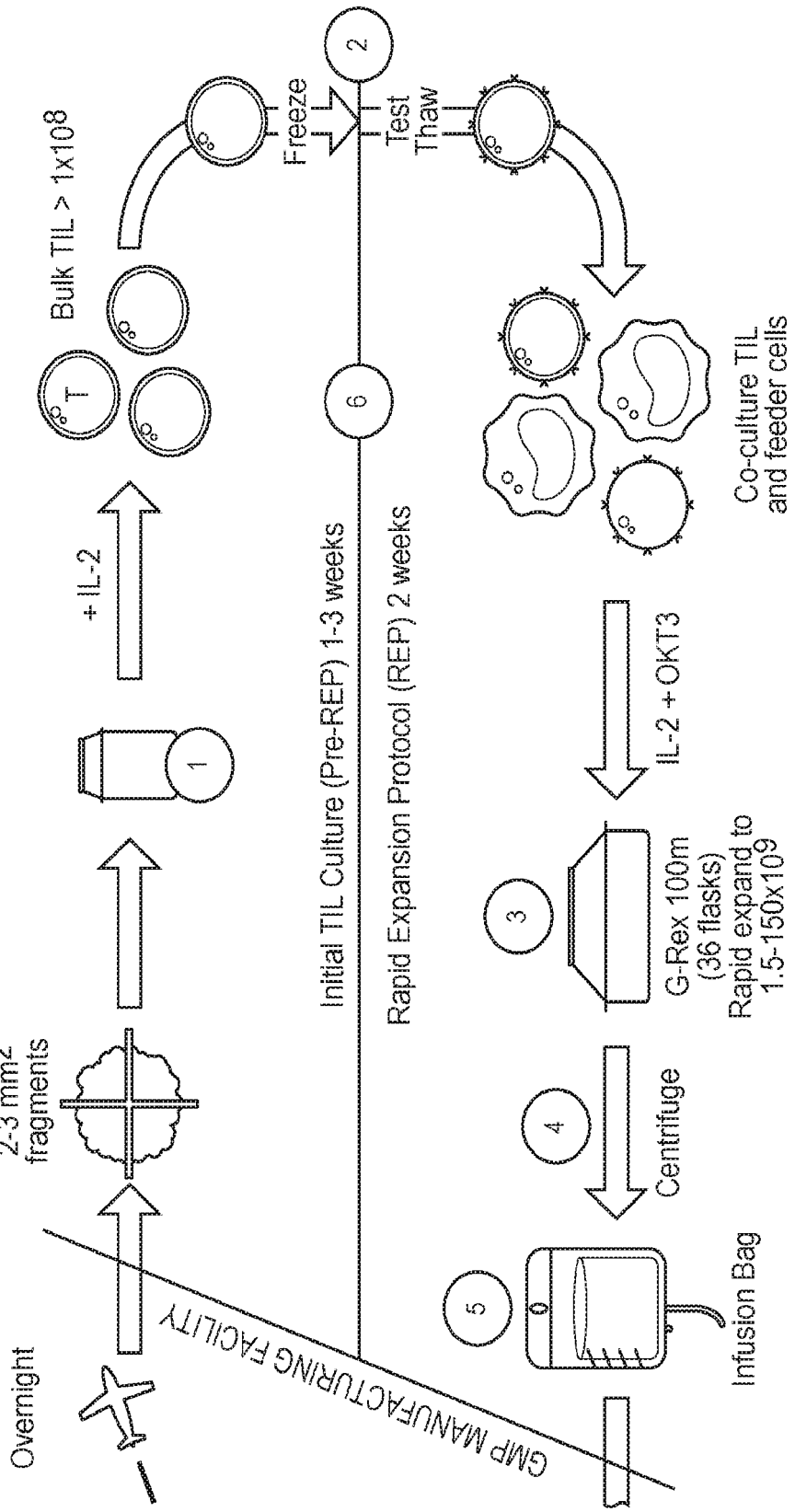

Overnight
GMP MANUFACTURING FACILITY
2-3 mm2 fragments
1
+ IL-2
Bulk TIL > 1x10^8
T
Freeze
2
Test
Thaw
Initial TIL Culture (Pre-REP) 1-3 weeks
6
Rapid Expansion Protocol (REP) 2 weeks
Co-culture TIL and feeder cells
IL-2 + OKT3
3
G-Rex 100m (36 flasks)
Rapid expand to 1.5-150x10^9
4
Centrifuge
5
Infusion Bag

FIG. 57

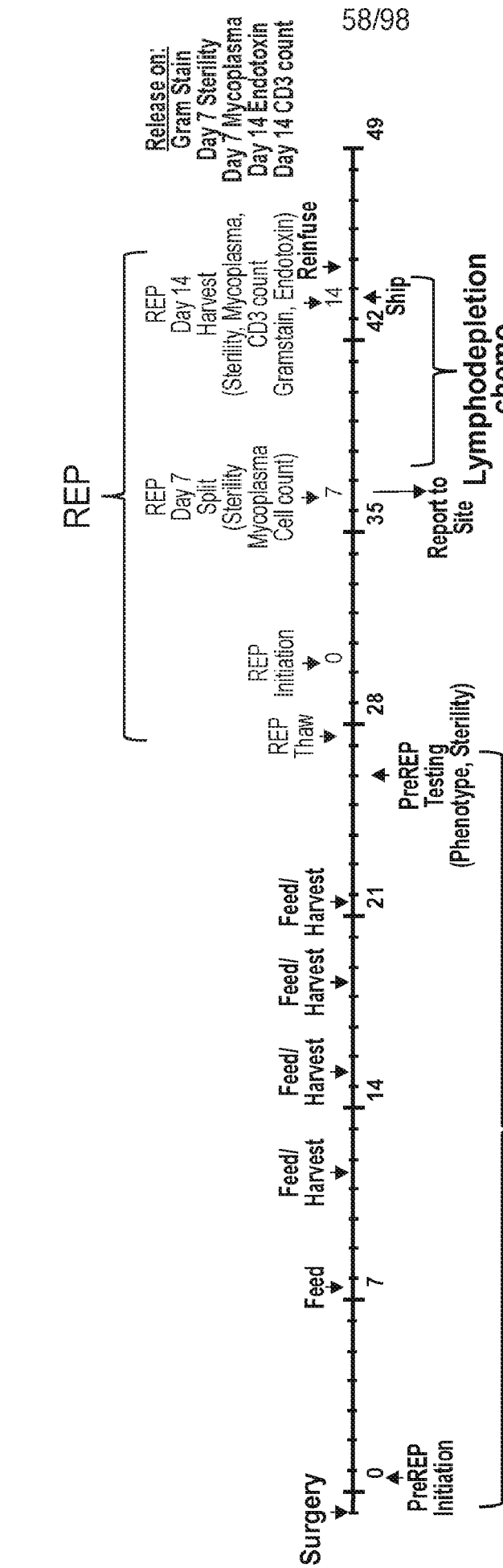

Release on:
Gram Stain
Day 7 Sterility
Day 7 Mycoplasma
Day 14 Endotoxin
Day 14 CD3 count
REP
REP Day 7 Split (Sterility Mycoplasma Cell count)
REP Day 14 Harvest (Sterility, Mycoplasma, CD3 count Gramstain, Endotoxin)
Reinfuse
Ship
Report to Site
Lymphodepletion chemo
REP Initiation
REP Thaw
PreREP Testing (Phenotype, Sterility)
Feed
Feed/ Harvest
Surgery
PreREP Initiation
PreREP
0
7
14
21
28
35
42
49

FIG. 58

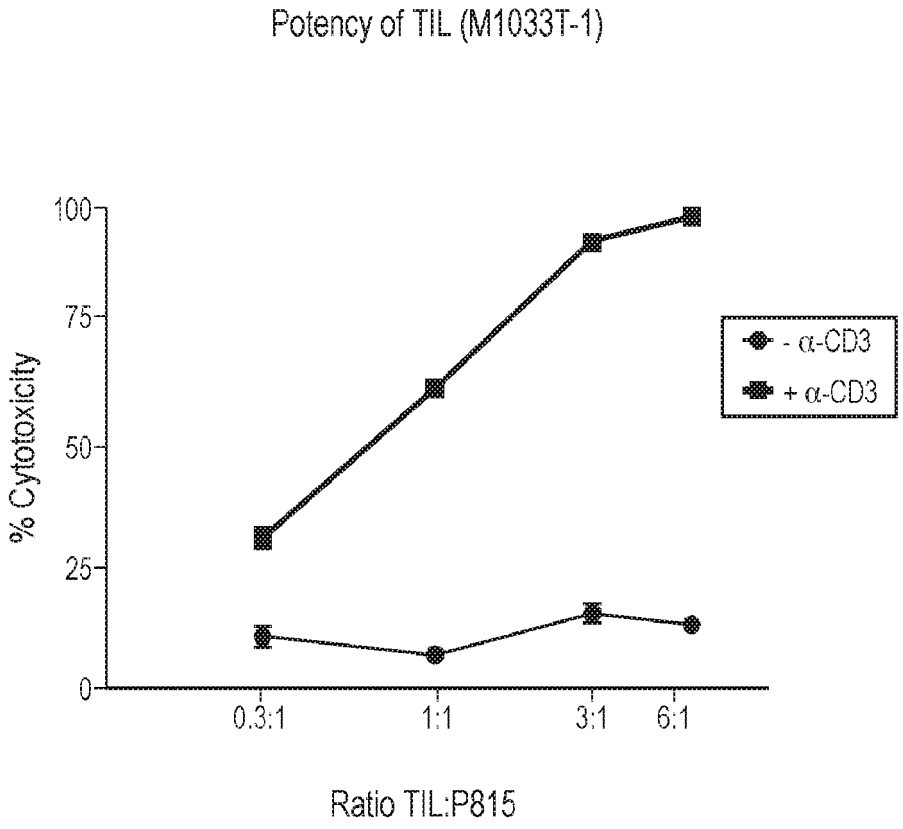

Potency of TIL (M1033T-1)
100
75
50
25
0
% Cytotoxicity
- α-CD3
+ α-CD3
0.3:1
1:1
3:1
6:1
Ratio TIL:P815

FIG. 59

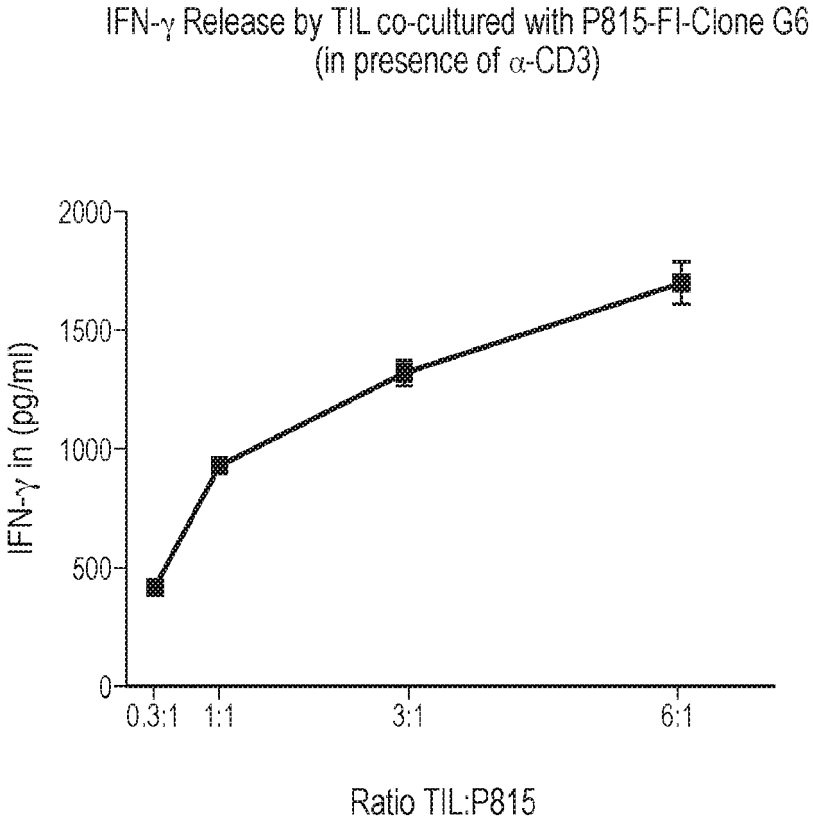

IFN-γ Release by TIL co-cultured with P815-FI-Clone G6
(in presence of α-CD3)
IFN-γ in (pg/ml)
2000
1500
1000
500
0
0.3:1
1:1
3:1
6:1
Ratio TIL:P815

FIG. 60

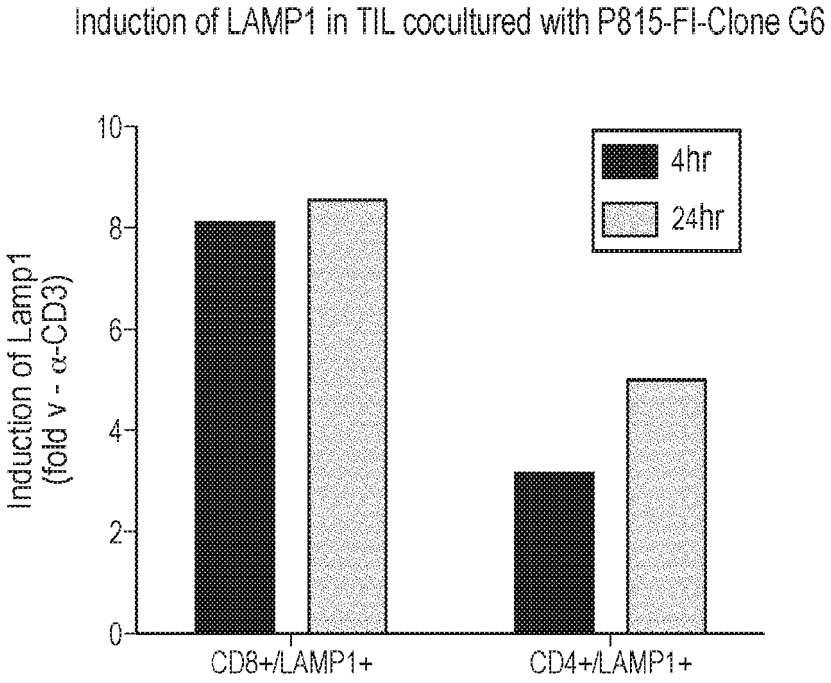

Induction of LAMP1 in TIL cocultured with P815-FI-Clone G6
Induction of Lamp1
(fold v - α-CD3)
10
8
6
4
2
0
4hr
24hr
CD8+/LAMP1+
CD4+/LAMP1+

FIG. 61

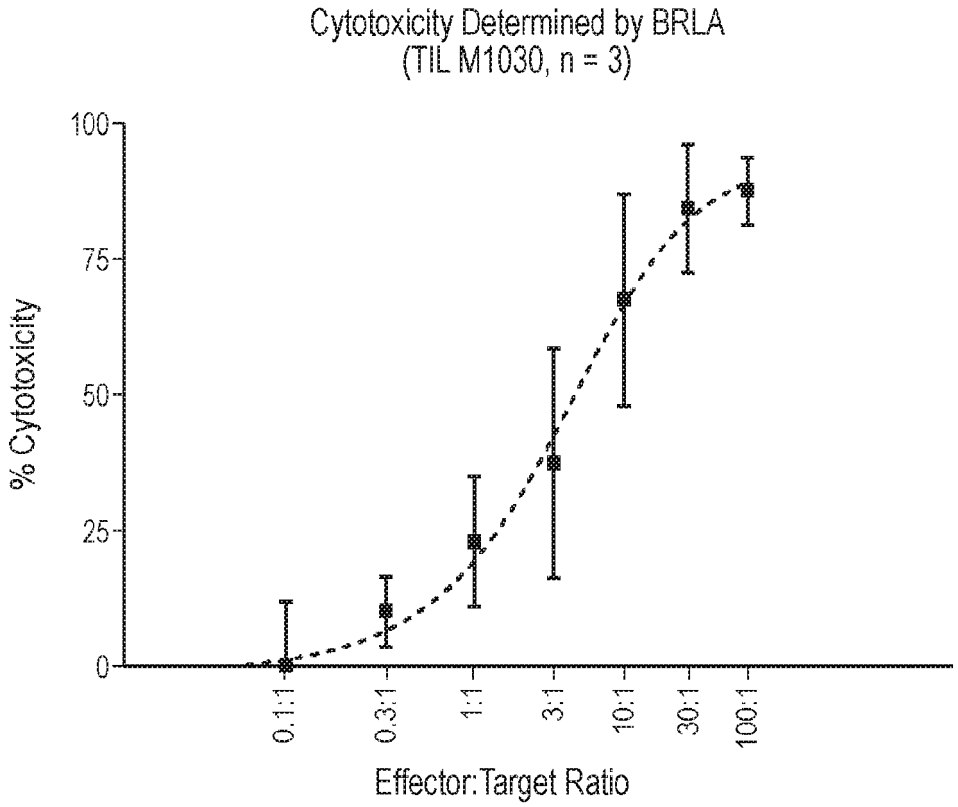

Cytotoxicity Determined by BRLA
(TIL M1030, n = 3)
% Cytotoxicity
100
75
50
25
0
0.1:1
0.3:1
1:1
3:1
10:1
30:1
100:1
Effector:Target Ratio

FIG. 62

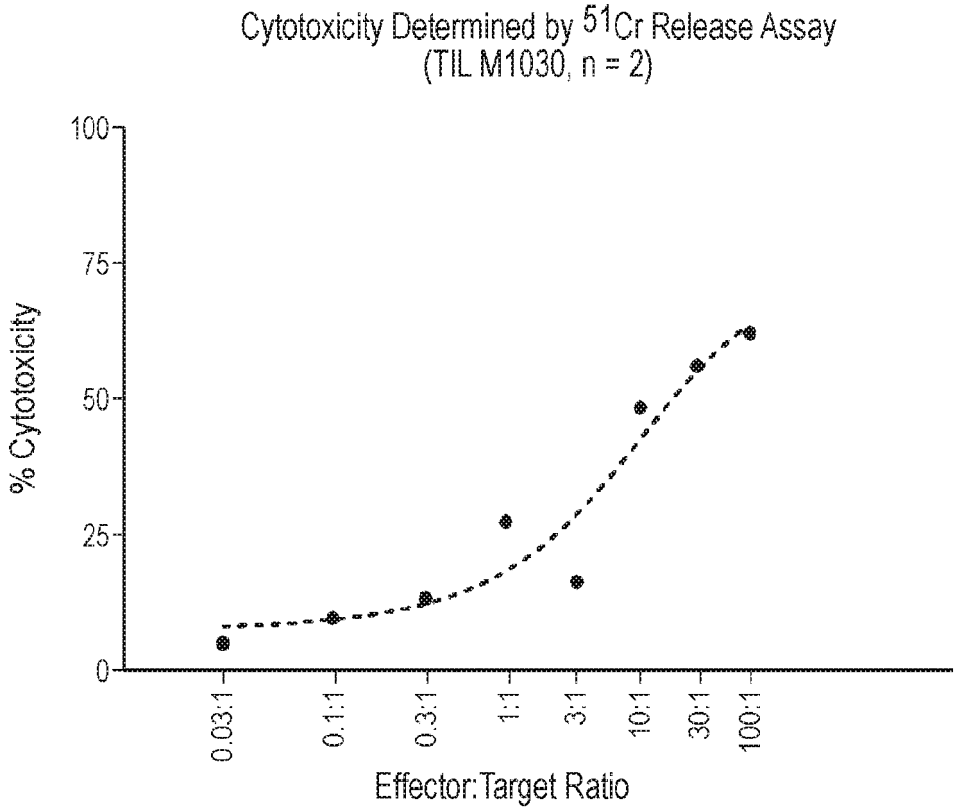

Cytotoxicity Determined by 51Cr Release Assay
(TIL M1030, n = 2)
% Cytotoxicity
100
75
50
25
0
0.03:1
0.1:1
0.3:1
1:1
3:1
10:1
30:1
100:1
Effector:Target Ratio

FIG. 63

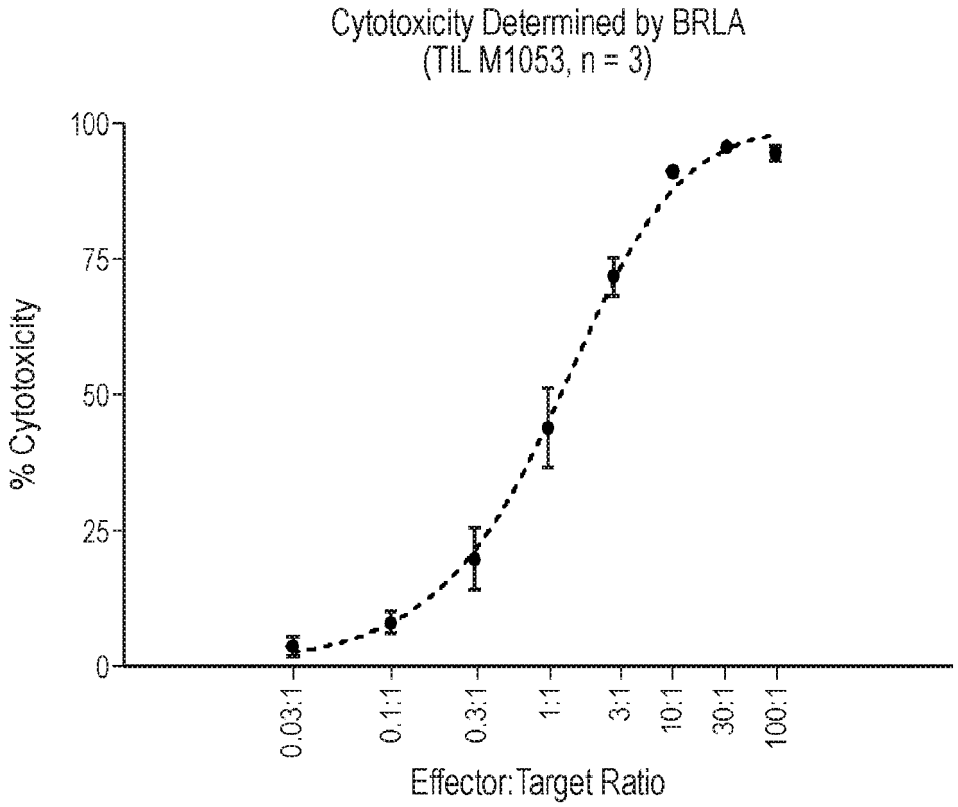

Cytotoxicity Determined by BRLA
(TIL M1053, n = 3)
% Cytotoxicity
100
75
50
25
0
0.03:1
0.1:1
0.3:1
1:1
3:1
10:1
30:1
100:1
Effector:Target Ratio

FIG. 64

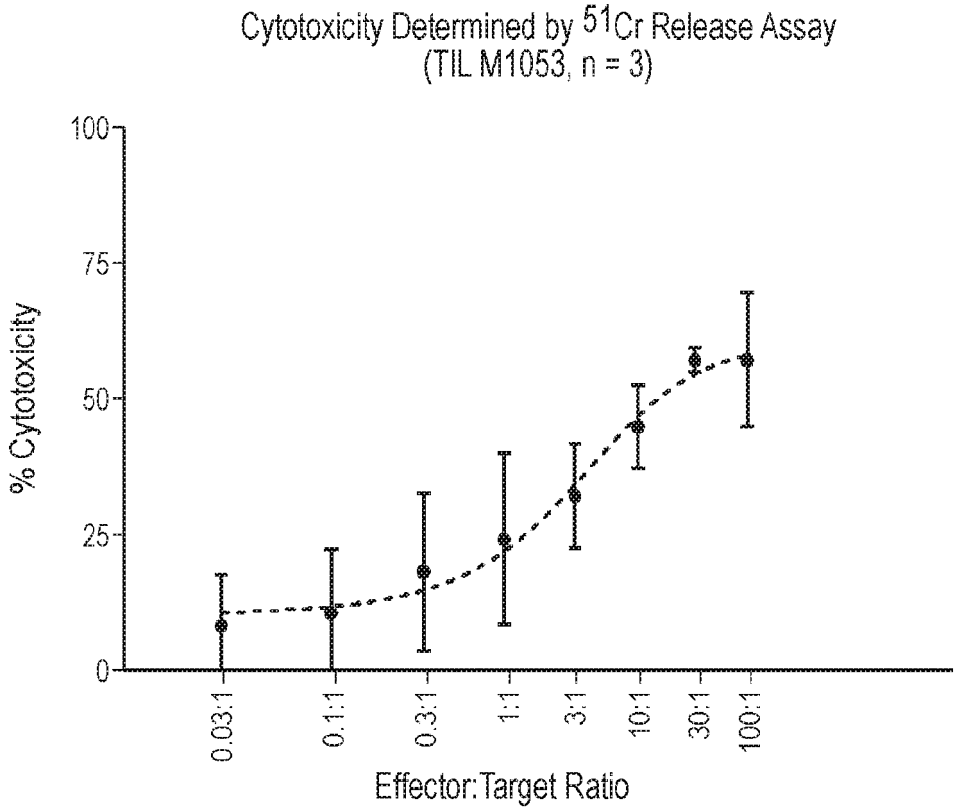

Cytotoxicity Determined by 51Cr Release Assay
(TIL M1053, n = 3)
% Cytotoxicity
100
75
50
25
0
0.03:1
0.1:1
0.3:1
1:1
3:1
10:1
30:1
100:1
Effector:Target Ratio

FIG. 65

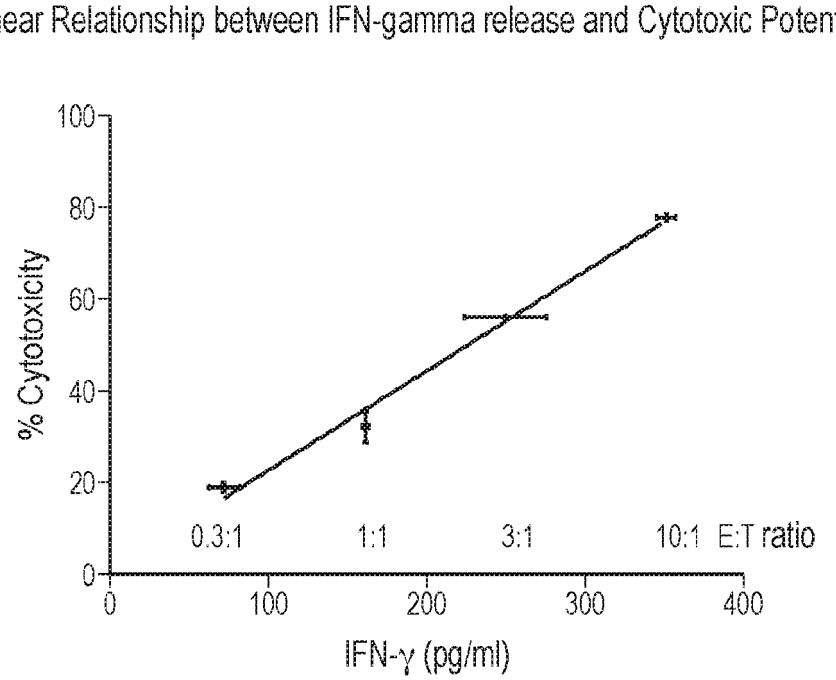

Linear Relationship between IFN-gamma release and Cytotoxic Potential of TIL
% Cytotoxicity
100
80
60
40
20
0
0.3:1
1:1
3:1
10:1
E:T ratio
0
100
200
300
400
IFN-γ (pg/ml)

FIG. 66

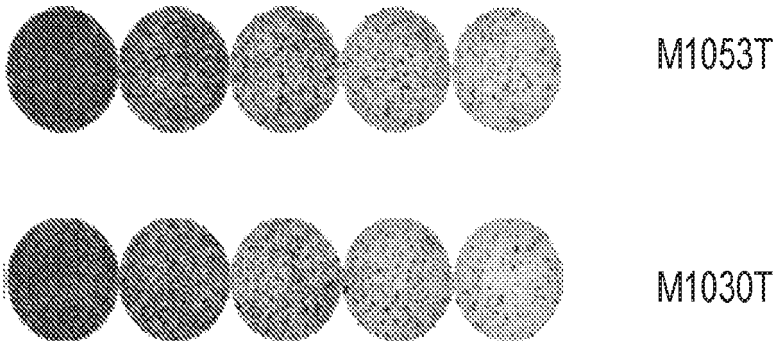


FIG. 67

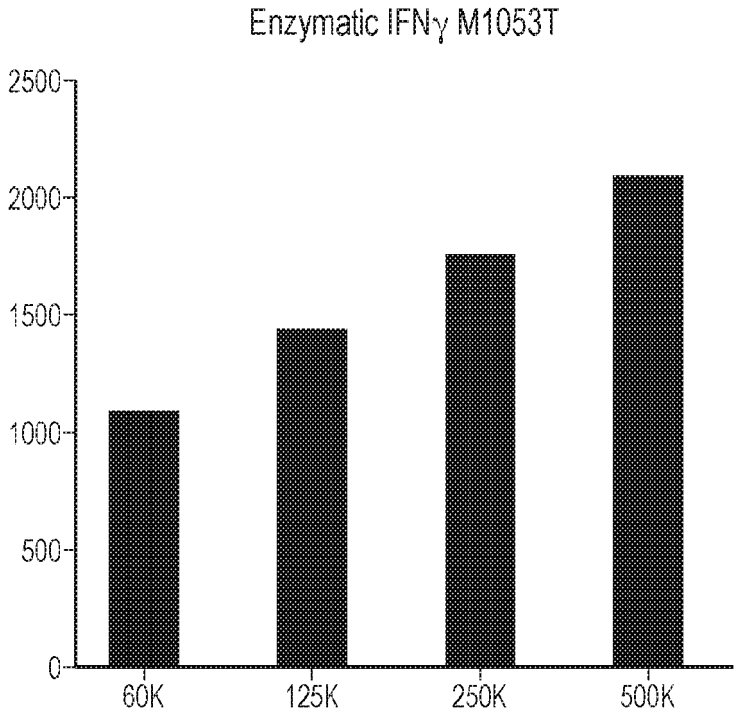

Enzymatic IFNγ M1053T
2500
2000
1500
1000
500
0
60K
125K
250K
500K

FIG. 68

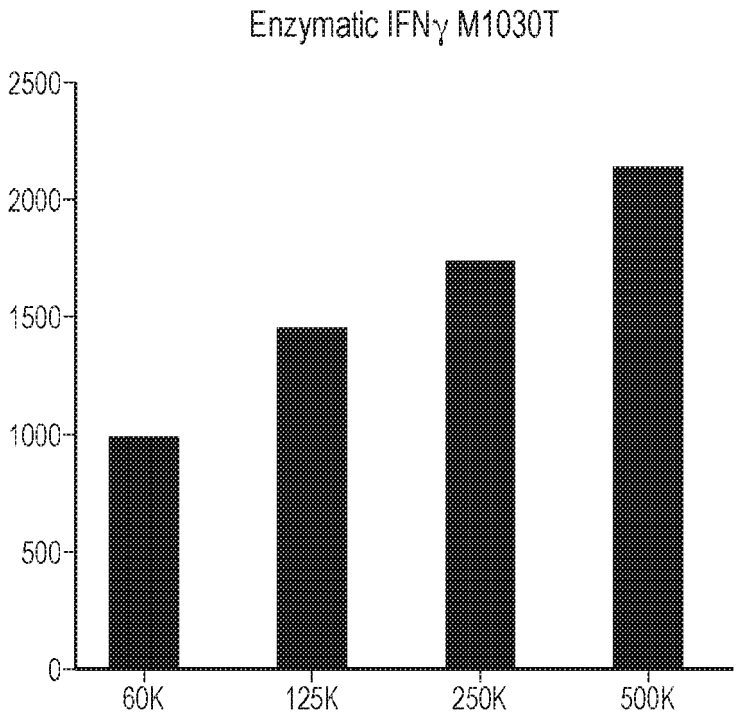

Enzymatic IFNγ M1030T
2500
2000
1500
1000
500
0
60K
125K
250K
500K

FIG. 69

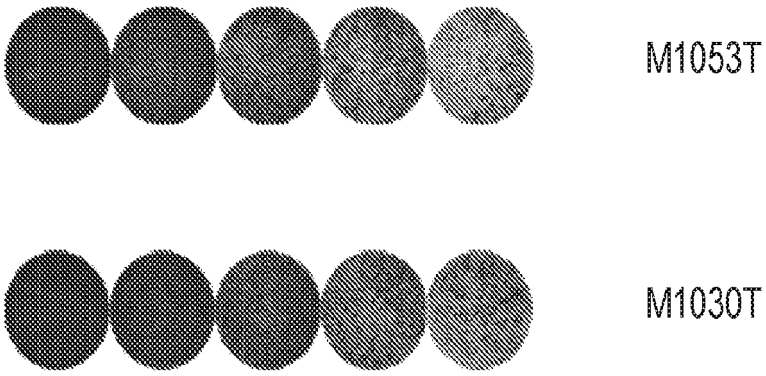


FIG. 70

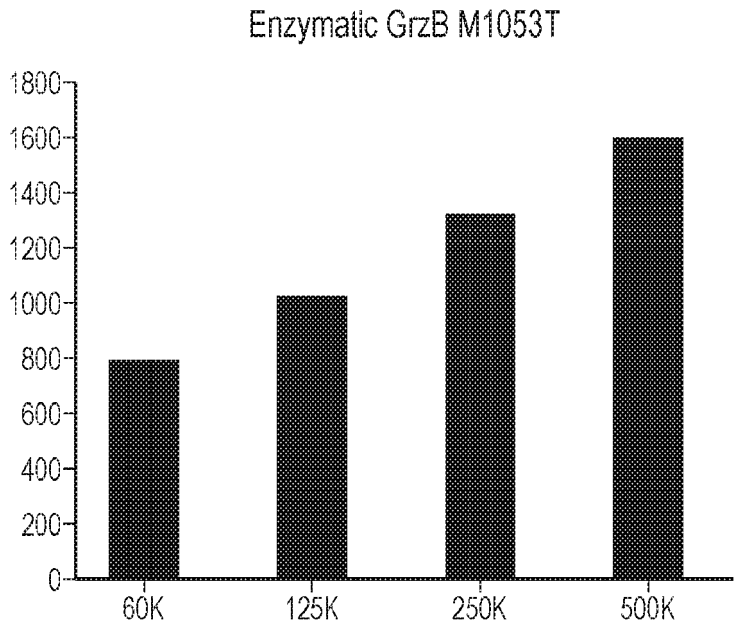

Enzymatic GrzB M1053T
1800
1600
1400
1200
1000
800
600
400
200
0
60K
125K
250K
500K

FIG. 71

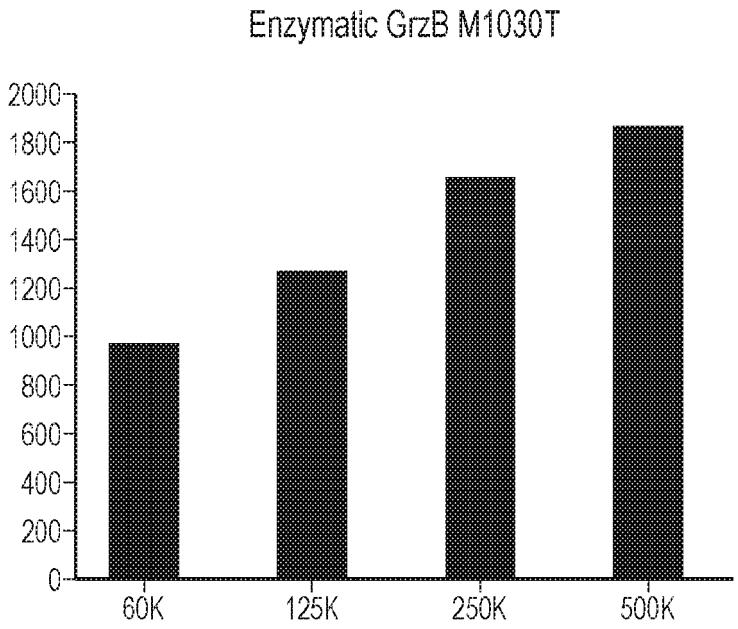

Enzymatic GrzB M1030T
2000
1800
1600
1400
1200
1000
800
600
400
200
0
60K
125K
250K
500K

FIG. 72

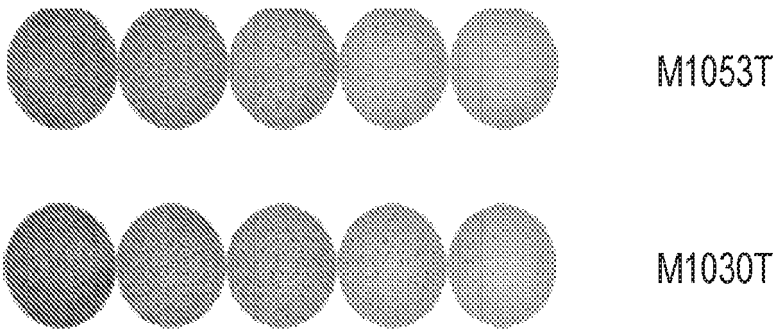


FIG. 73

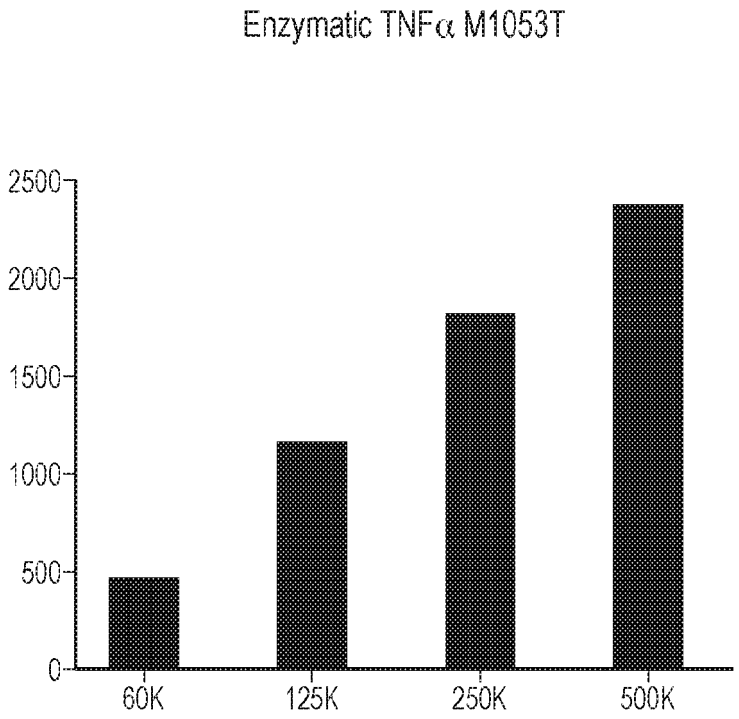

Enzymatic TNFα M1053T
2500
2000
1500
1000
500
0
60K
125K
250K
500K

FIG. 74

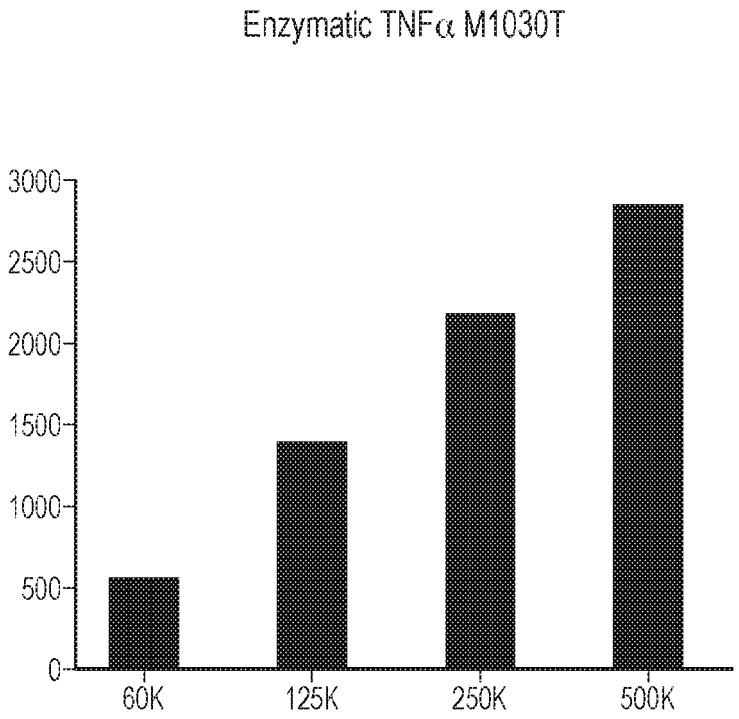

Enzymatic TNFα M1030T
3000
2500
2000
1500
1000
500
0
60K
125K
250K
500K

FIG. 75

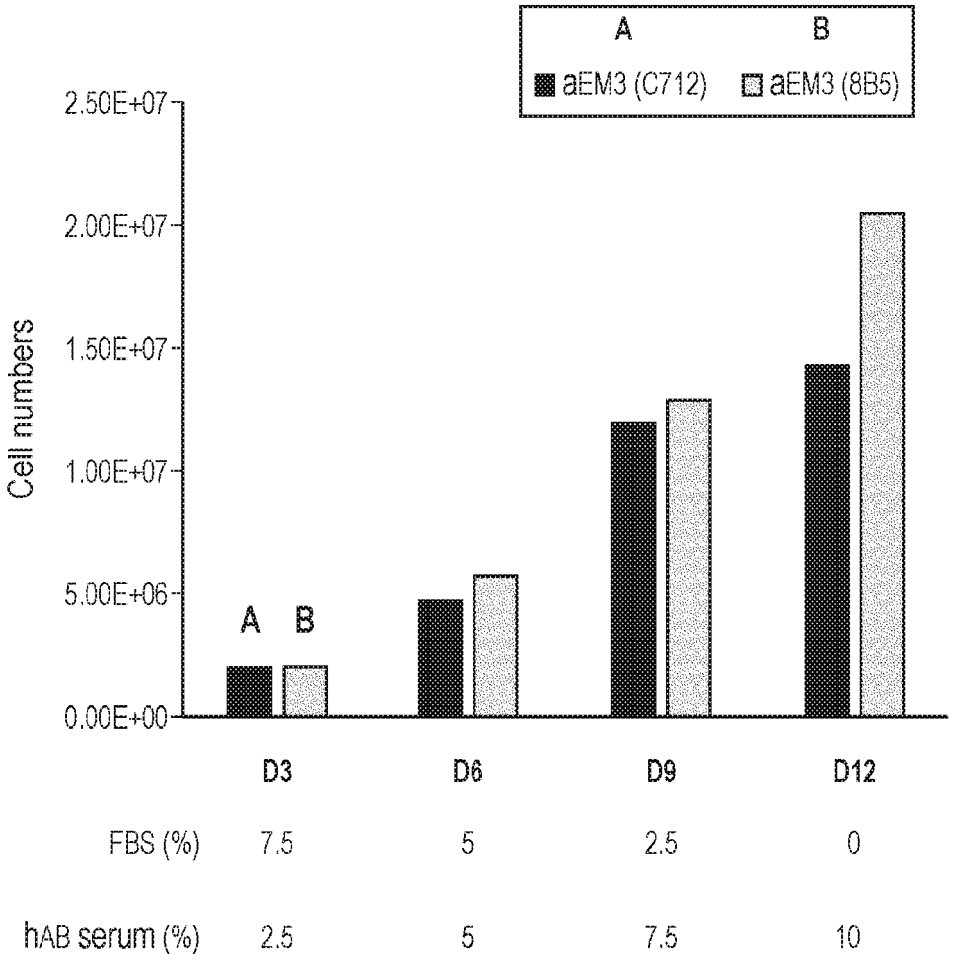

A
B
aEM3 (C712)
aEM3 (8B5)
Cell numbers
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
A B
D3
D6
D9
D12
FBS (%) 7.5 5 2.5 0
hAB serum (%) 2.5 5 7.5 10

FIG. 76

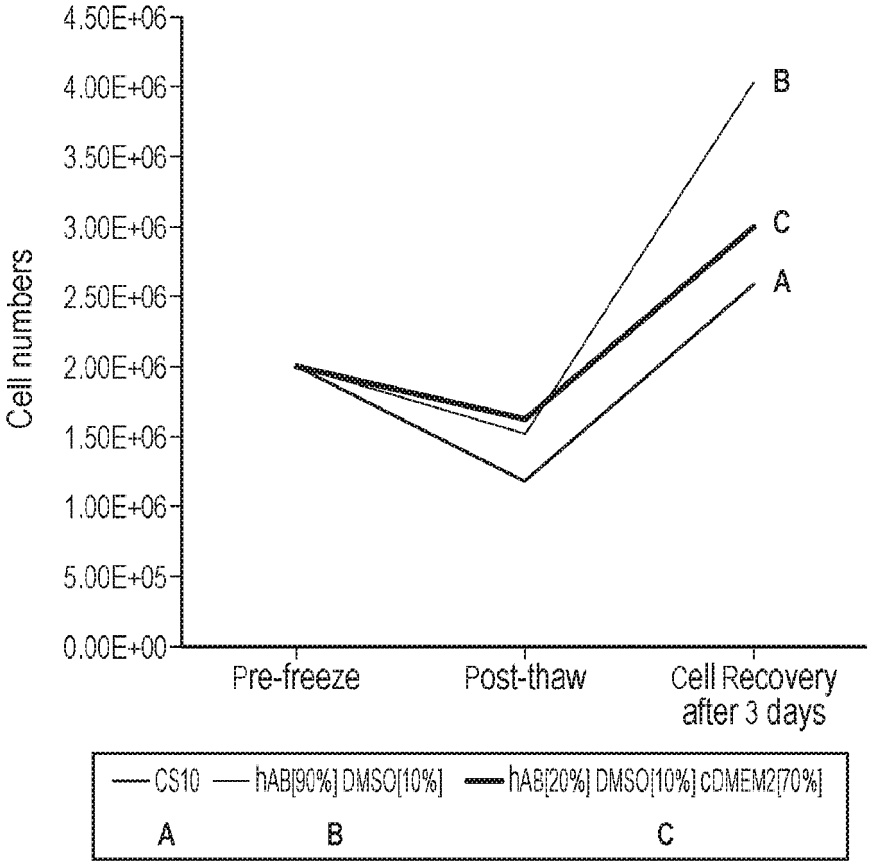

4.50E+06
4.00E+06
3.50E+06
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cell numbers
Pre-freeze
Post-thaw
Cell Recovery after 3 days
B
C
A
CS10
hAB[90%] DMSO[10%]
hAB[20%] DMSO[10%] cDMEM2[70%]
A
B
C

FIG. 77

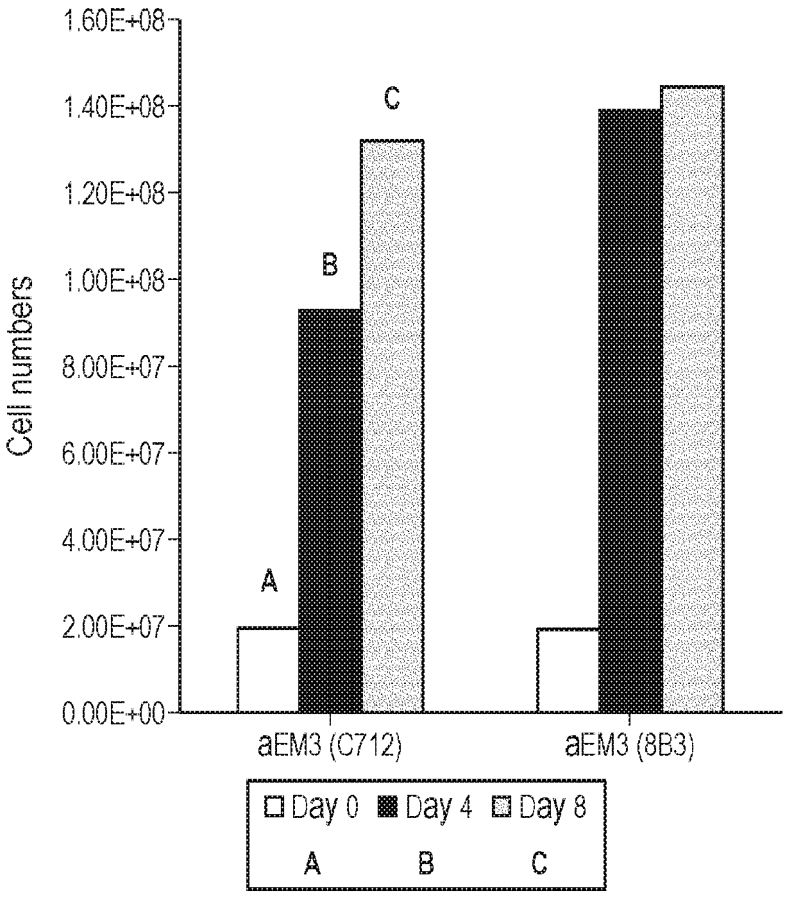

1.60E+08
1.40E+08
1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Cell numbers
A
B
C
aEM3 (C712)
aEM3 (8B3)
Day 0
Day 4
Day 8
A
B
C

FIG. 78

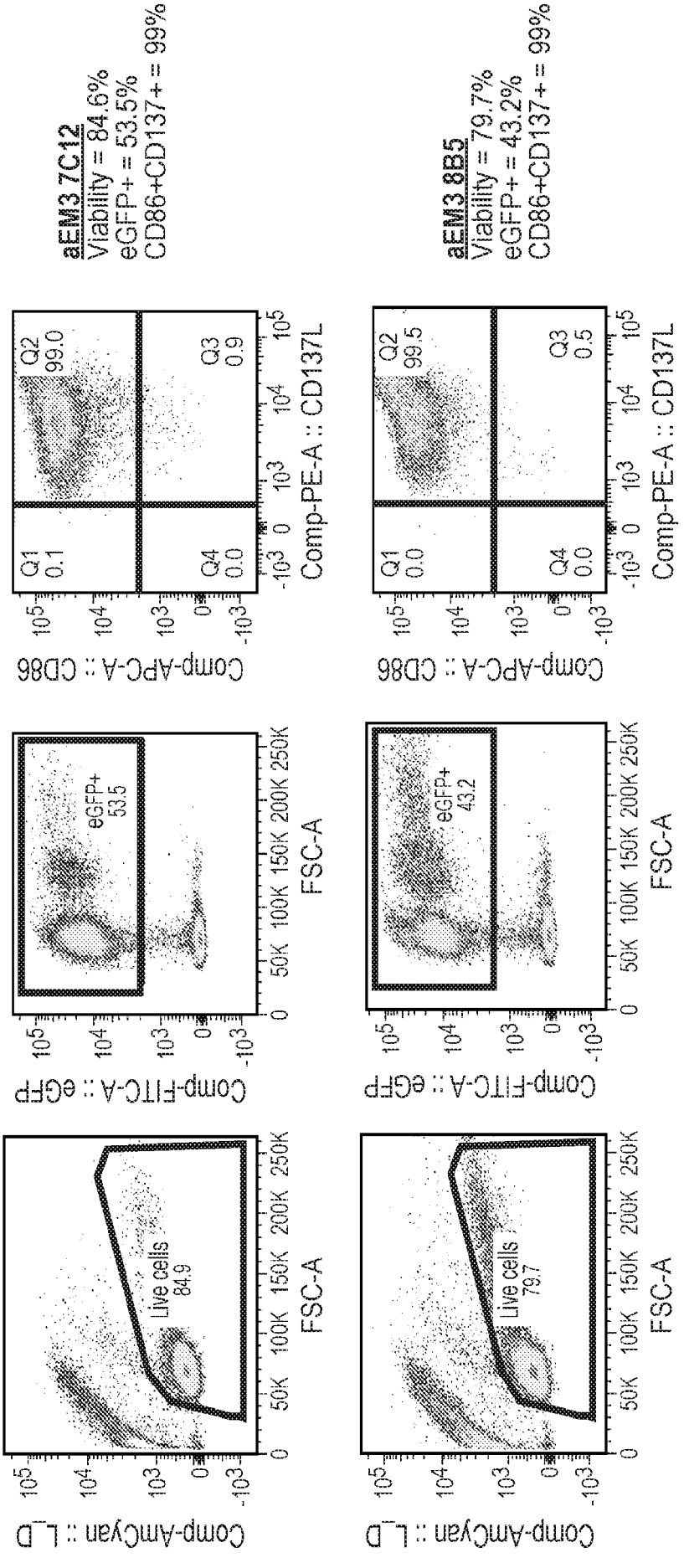

aEM3 7C12
Viability = 84.6%
eGFP+ = 53.5%
CD86+CD137+ = 99%
aEM3 8B5
Viability = 79.7%
eGFP+ = 43.2%
CD86+CD137+ = 99%
Comp-AmCyan :: L_D
Live cells 84.9
Live cells 79.7
FSC-A
Comp-FITC-A :: eGFP
eGFP+ 53.5
eGFP+ 43.2
Comp-APC-A :: CD86
Comp-PE-A :: CD137L
Q1 0.1
Q2 99.0
Q4 0.0
Q3 0.9
Q1 0.0
Q2 99.5
Q4 0.0
Q3 0.5
Flow Panel:
1. L/D Aqua
2. eGFP
3. Anti-CD86 APC
4. Anti-CD137 PE

FIG. 79

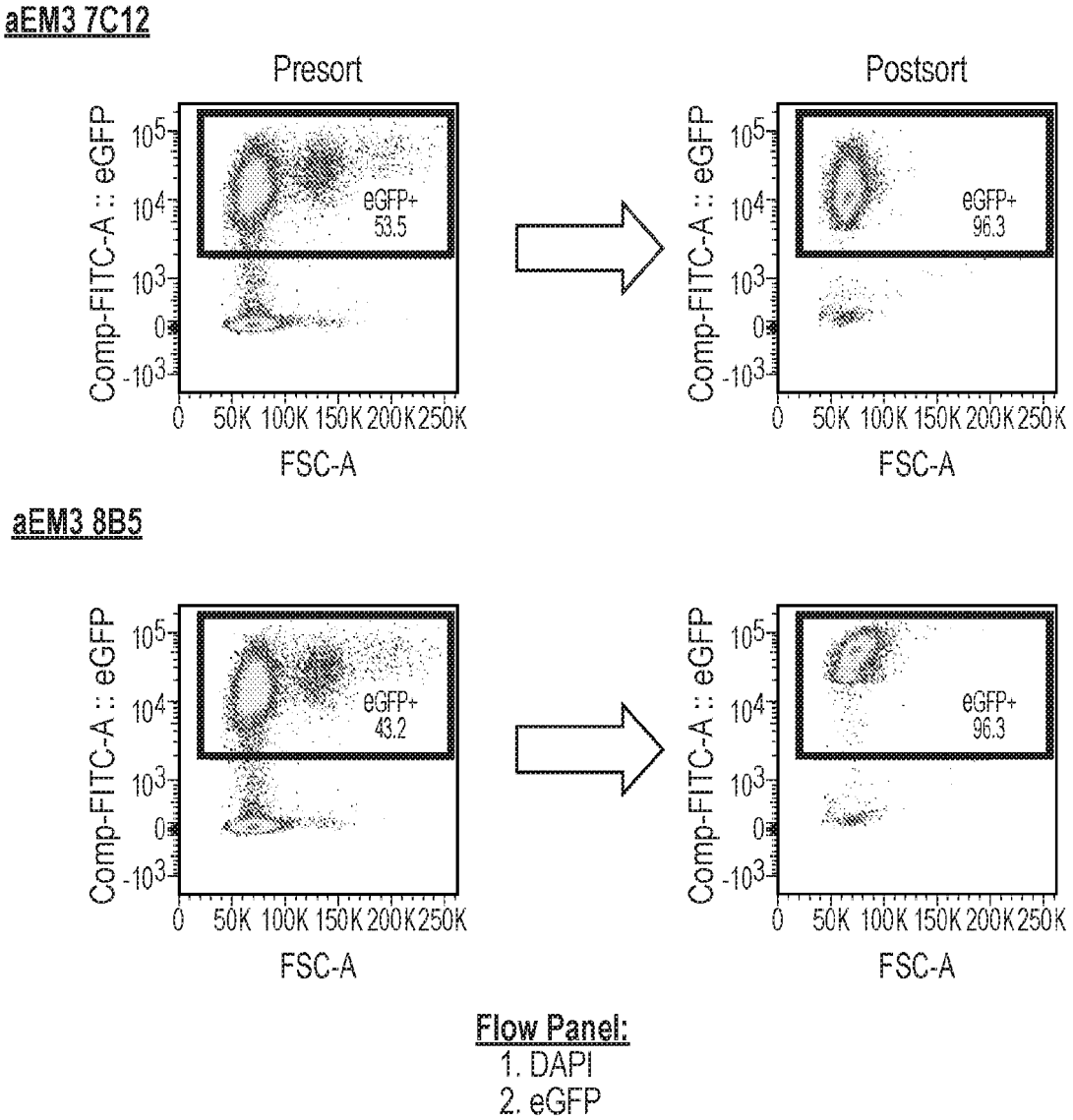

aEM3 7C12
Presort
Postsort
Comp-FITC-A :: eGFP
FSC-A
eGFP+
53.5
eGFP+
96.3
aEM3 8B5
eGFP+
43.2
eGFP+
96.3
Flow Panel:
1. DAPI
2. eGFP

FIG. 80

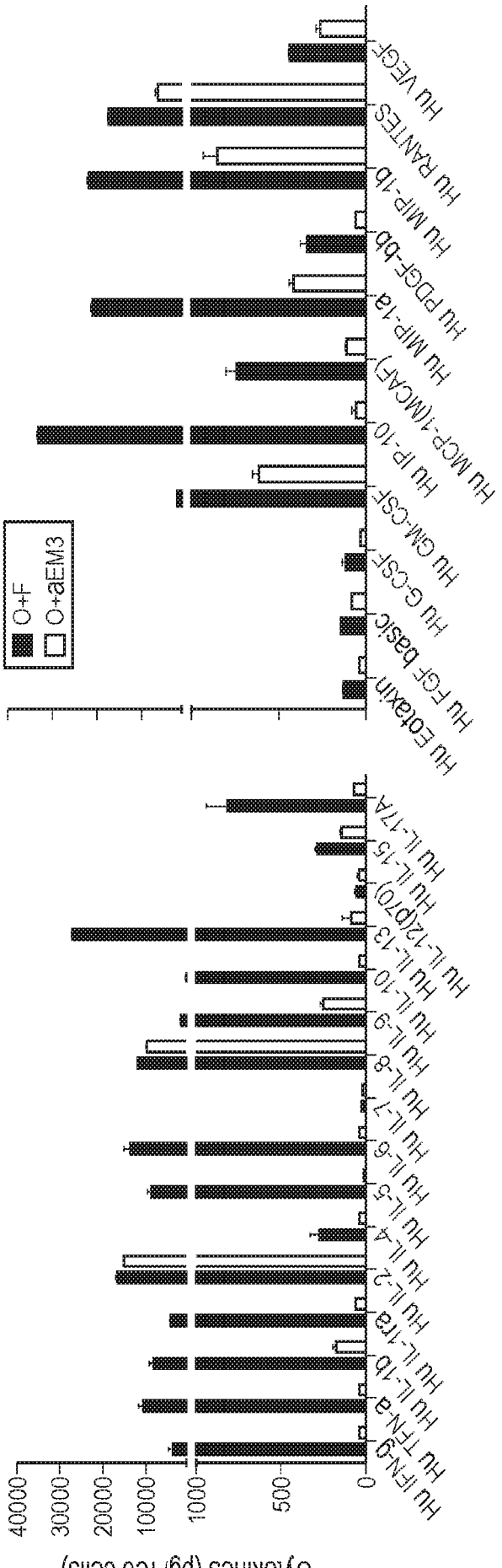

Cytokines (pg/1e6 cells)
40000
30000
20000
10000
1000
500
0
Hu IFN-g
Hu TFN-a
Hu IL-1a
Hu IL-1b
Hu IL-1ra
Hu IL-2
Hu IL-4
Hu IL-5
Hu IL-6
Hu IL-7
Hu IL-8
Hu IL-9
Hu IL-10
Hu IL-13
Hu IL-12(P70)
Hu IL-15
Hu IL-17A
Hu Eotaxin
Hu FGF basic
Hu G-CSF
Hu GM-CSF
Hu IP-10
Hu MCP-1(MCAF)
Hu MIP-1a
Hu PDGF-bb
Hu MIP-1b
Hu RANTES
Hu VEGF
O+F
O+aEM3

FIG. 81

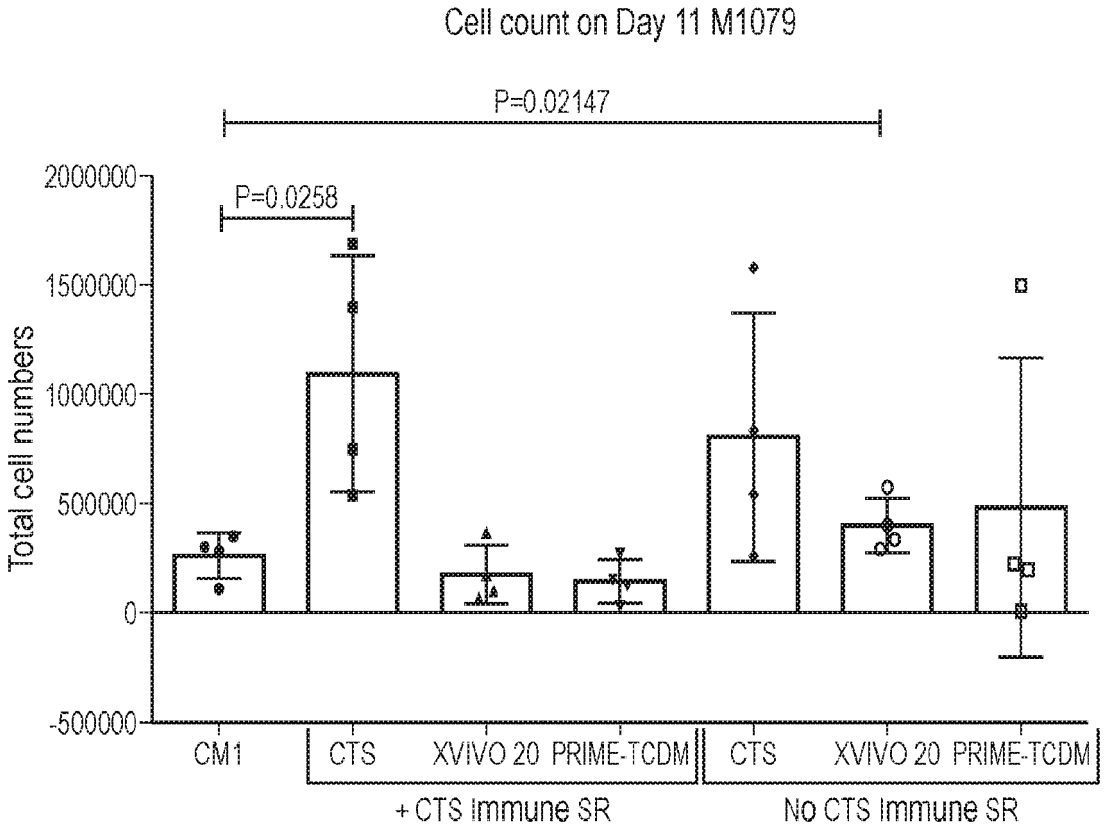
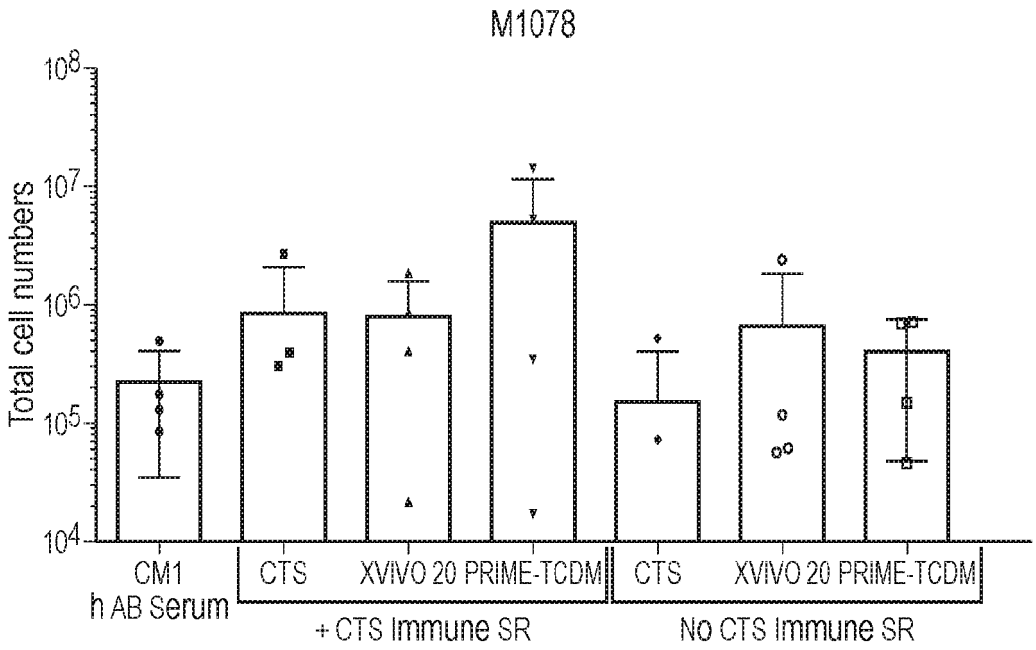

Cell count on Day 11 M1079
P=0.02147
P=0.0258
2000000
1500000
1000000
500000
0
-500000
Total cell numbers
CM1
CTS
XVIVO 20
PRIME-TCDM
CTS
XVIVO 20
PRIME-TCDM
+ CTS Immune SR
No CTS Immune SR

FIG. 82



* For D11, none of the counts Vs. CM1 were statistically relevant based on student 't' test

FIG. 83

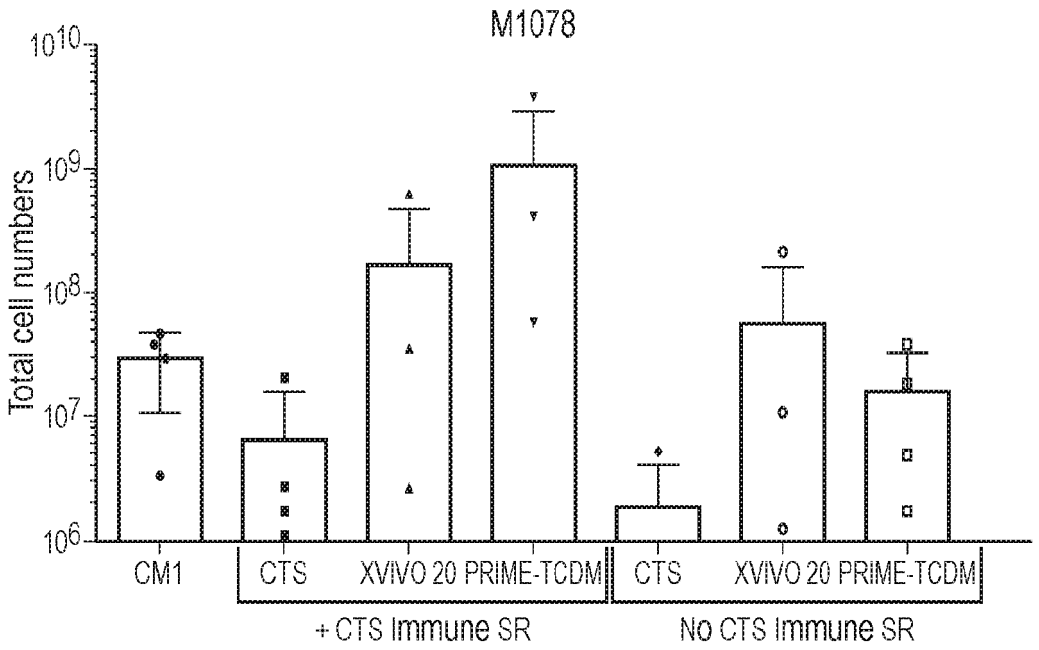

M1078
Total cell numbers
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
CM1
CTS
XVIVO 20 PRIME-TCDM
CTS
XVIVO 20 PRIME-TCDM
+ CTS Immune SR
No CTS Immune SR
* For D22, none of the counts Vs. CM1 were statistically relevant based on student 't' test

FIG. 84

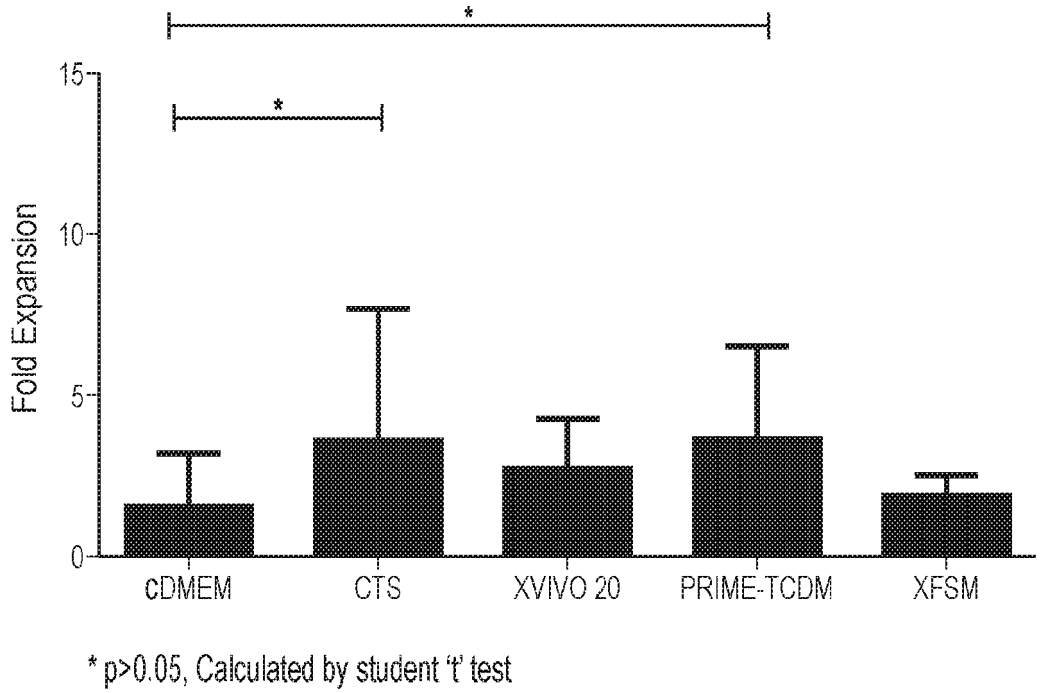

*
*
15
10
5
0
Fold Expansion
cDMEM
CTS
XVIVO 20
PRIME-TCDM
XFSM
* p>0.05, Calculated by student 't' test

FIG. 85

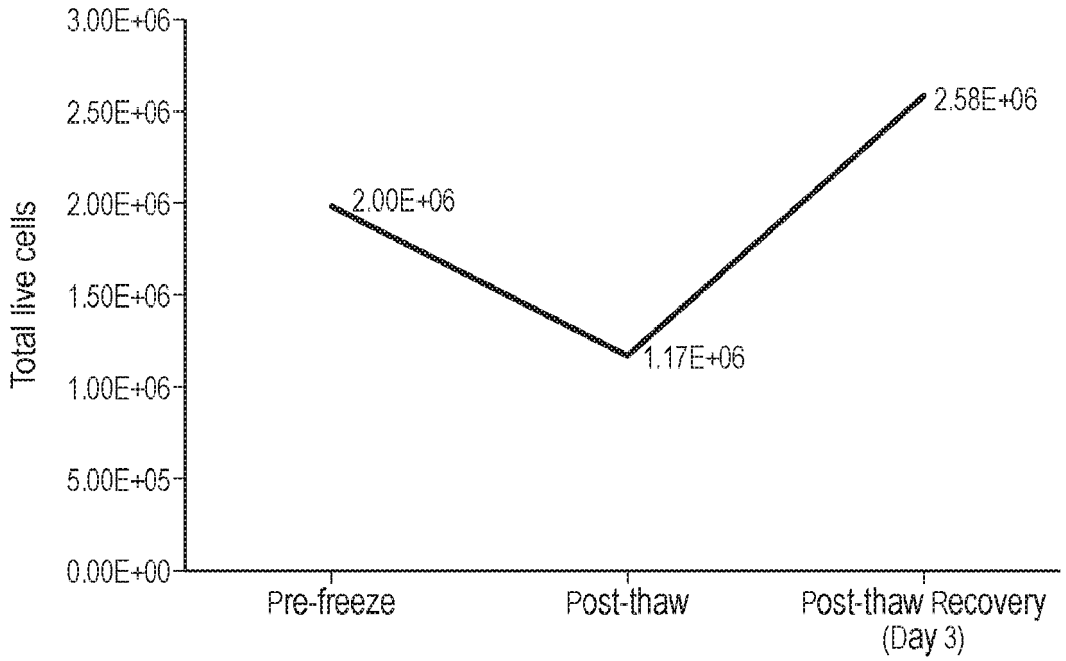

3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Total live cells
2.00E+06
1.17E+06
2.58E+06
Pre-freeze
Post-thaw
Post-thaw Recovery
(Day 3)

FIG. 86

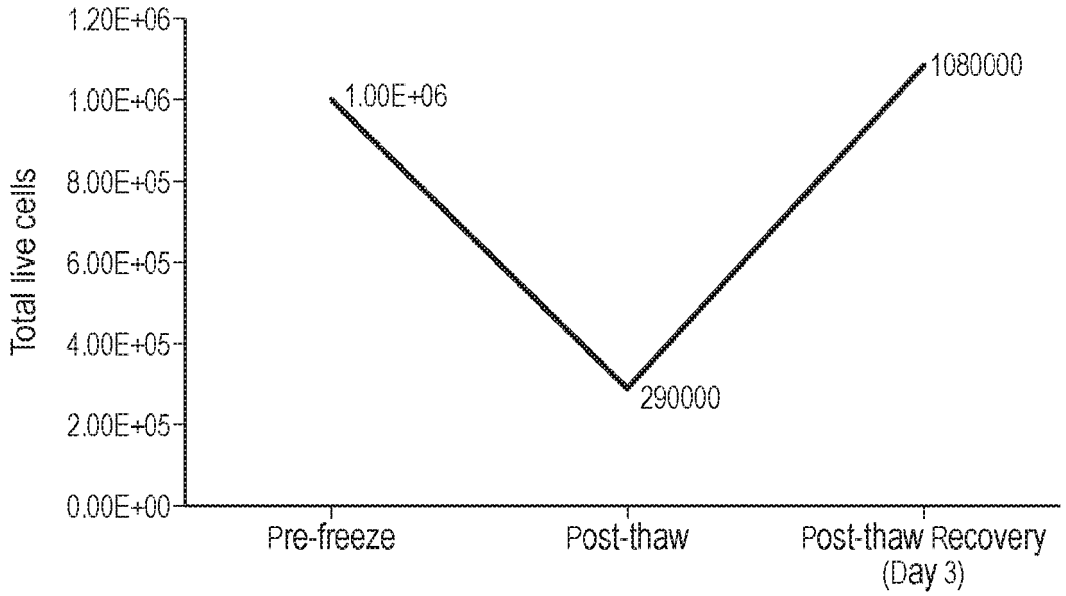

1.20E+06
1.00E+06
8.00E+05
6.00E+05
4.00E+05
2.00E+05
0.00E+00
Total live cells
1.00E+06
290000
1080000
Pre-freeze
Post-thaw
Post-thaw Recovery
(Day 3)

FIG. 87

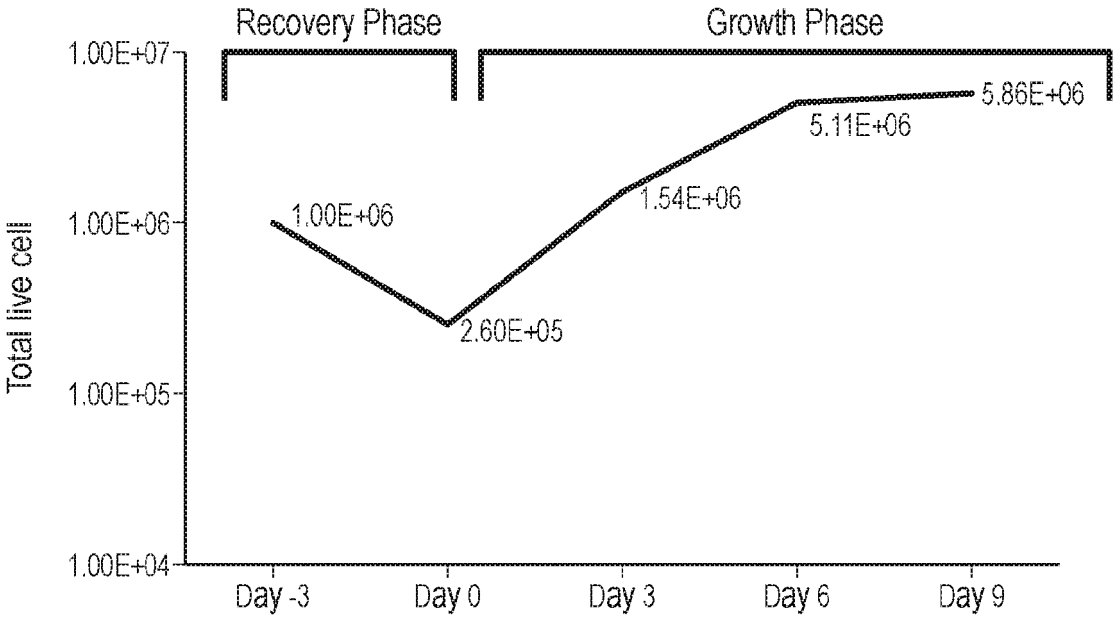

Recovery Phase
Growth Phase
1.00E+07
1.00E+06
1.00E+05
1.00E+04
Total live cell
1.00E+06
2.60E+05
1.54E+06
5.11E+06
5.86E+06
Day -3
Day 0
Day 3
Day 6
Day 9

FIG. 88

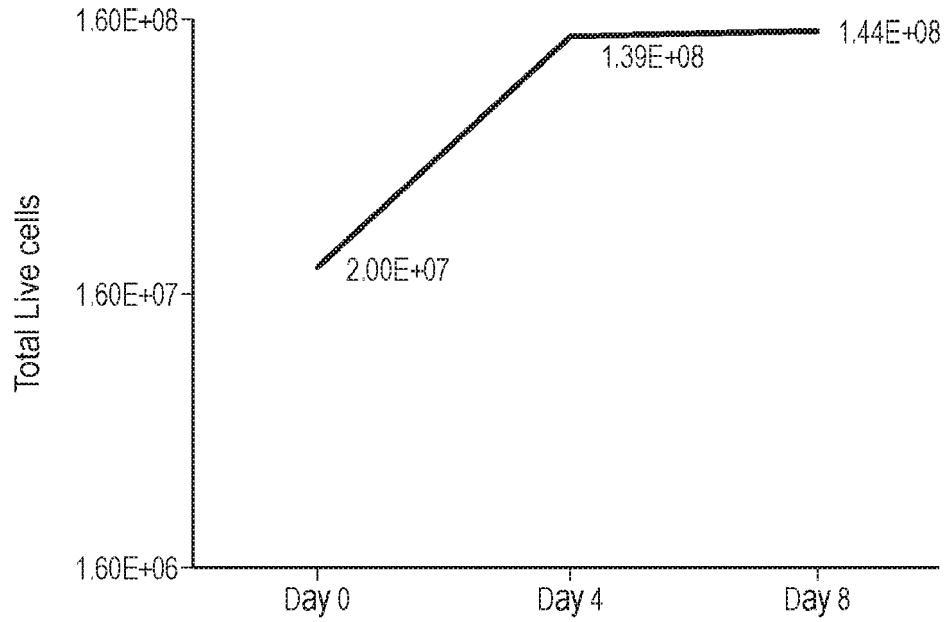

1.60E+08
1.60E+07
1.60E+06
Total Live cells
2.00E+07
1.39E+08
1.44E+08
Day 0
Day 4
Day 8

FIG. 89

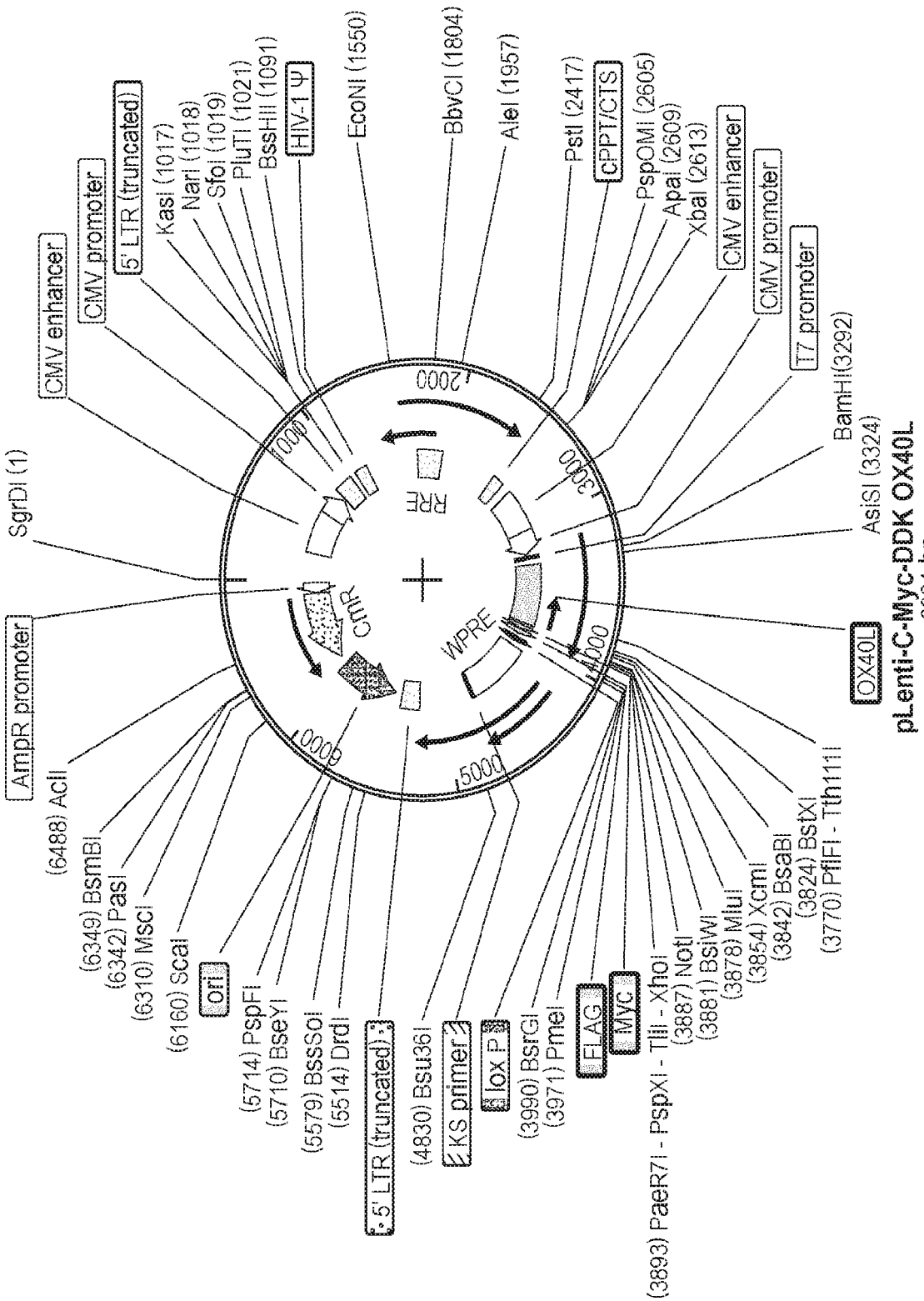

pLenti-C-Myc-DDK OX40L
6924 bp
1000
2000
3000
4000
5000
6000
RRE
WPRE
cPPT/CTS
CMV enhancer
CMV promoter
5' LTR (truncated)
HIV-1 Ψ
T7 promoter
CMV enhancer
CMV promoter
5' LTR (truncated)
AmpR promoter
ori
KS primer 2
lox P
FLAG
Myc
OX40L
(1) SgrDI
(1017) KasI
(1018) NarI
(1019) SfoI
(1021) PluTI
(1091) BssHII
(1550) EcoNI
(1804) BbvCI
(1957) AleI
(2417) PstI
(2605) PspOMI
(2609) ApaI
(2613) XbaI
(3292) BamHI
(3324) AsiSI
(3393) PaeR7I - PspXI - TliI - XhoI
(3770) PflFI - Tth111I
(3824) BstXI
(3842) BsaBI
(3854) XcmI
(3878) MluI
(3881) BsiWI
(3887) NotI
(3971) PmeI
(3990) BsrGI
(4830) Bsu36I
(5514) DrdI
(5579) BsaI
(5710) BseYI
(5714) PspFI
(6160) ScaI
(6310) MscI
(6342) PasI
(6349) BsmBI
(6488) AclI

FIG. 90

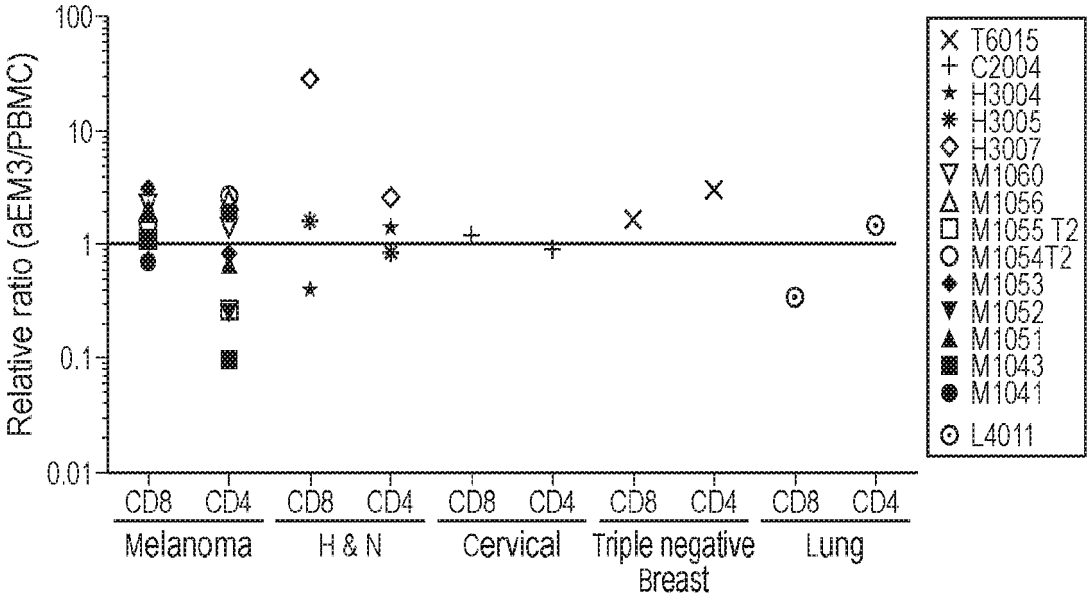

Relative ratio (aEM3/PBMC)
100
10
1
0.1
0.01
CD8
CD4
Melanoma
H & N
Cervical
Triple negative Breast
Lung
T6015
C2004
H3004
H3005
H3007
M1060
M1056
M1055 T2
M1054T2
M1053
M1052
M1051
M1043
M1041
L4011

FIG. 91

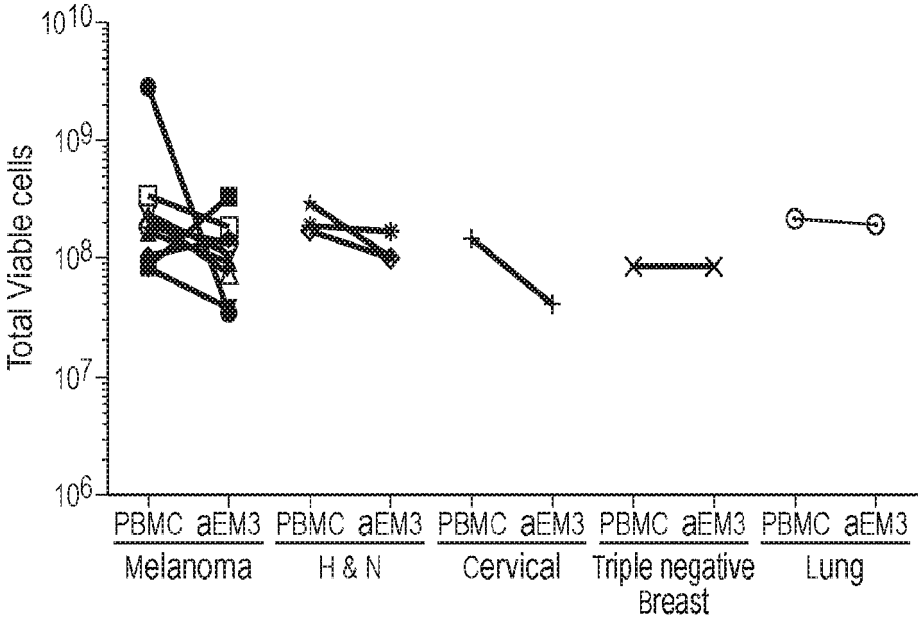

Total Viable cells
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
PBMC aEM3
Melanoma
H & N
Cervical
Triple negative Breast
Lung

FIG. 92

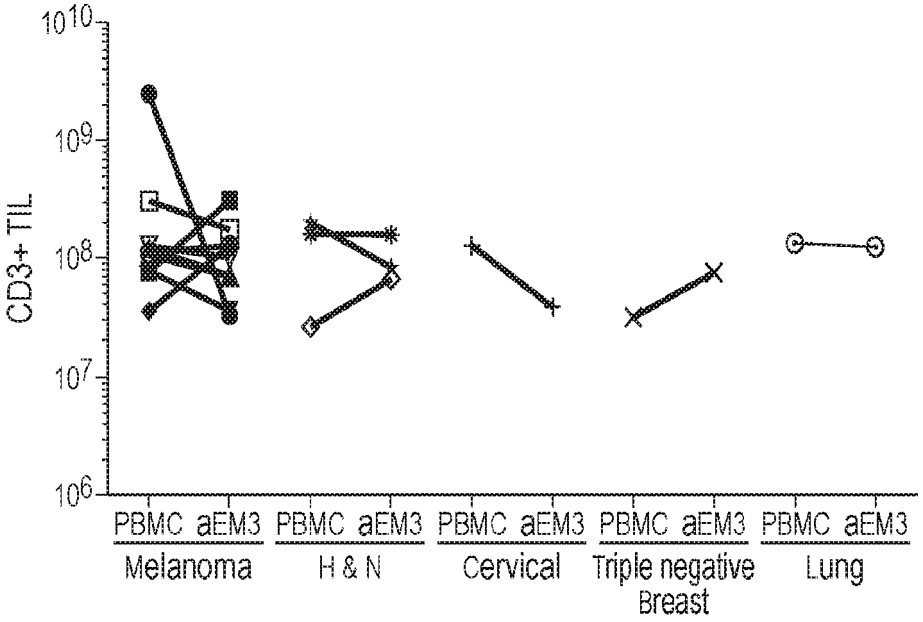

10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
CD3+ TIL
PBMC aEM3
Melanoma
H & N
Cervical
Triple negative Breast
Lung

FIG. 93

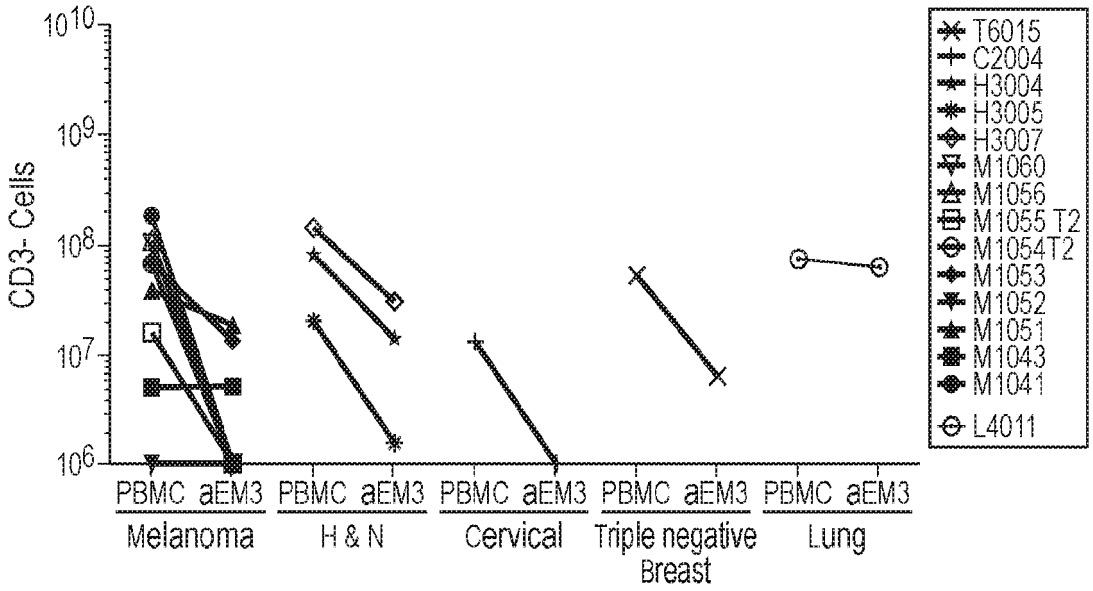

CD3- Cells
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
PBMC aEM3
Melanoma
H & N
Cervical
Triple negative Breast
Lung
T6015
C2004
H3004
H3005
H3007
M1060
M1056
M1055 T2
M1054T2
M1053
M1052
M1051
M1043
M1041
L4011

FIG. 94

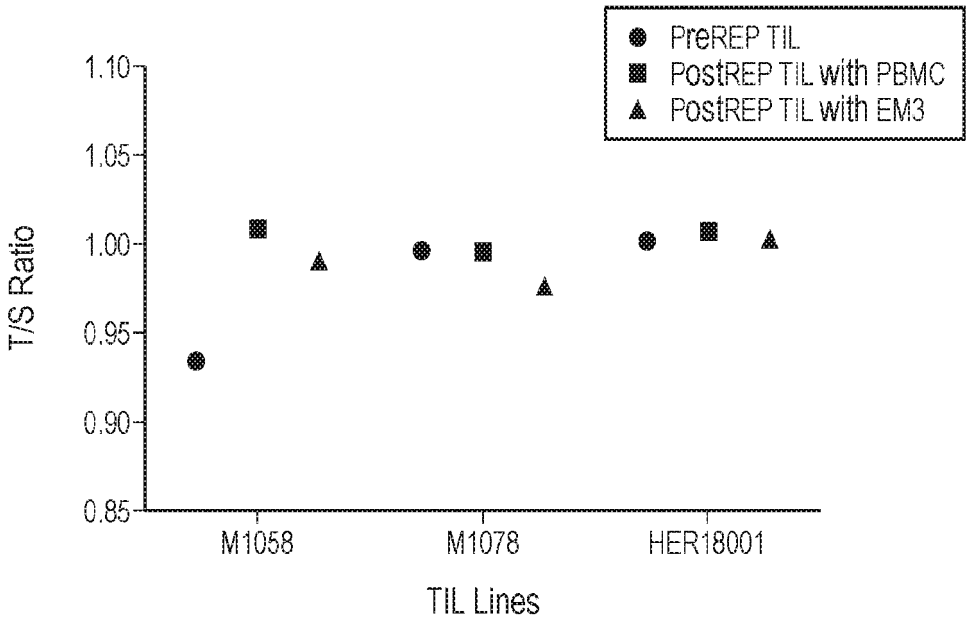

PreREP TIL
PostREP TIL with PBMC
PostREP TIL with EM3
1.10
1.05
1.00
0.95
0.90
0.85
T/S Ratio
M1058
M1078
HER18001
TIL Lines

FIG. 95

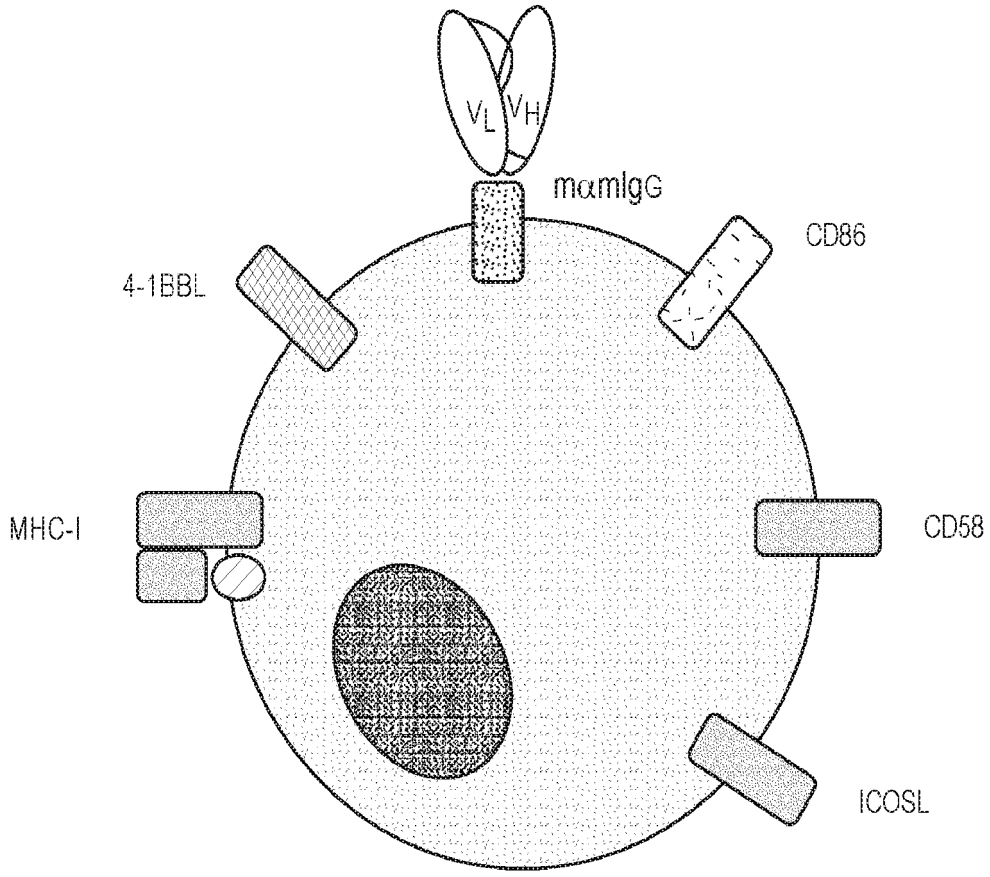


FIG. 96

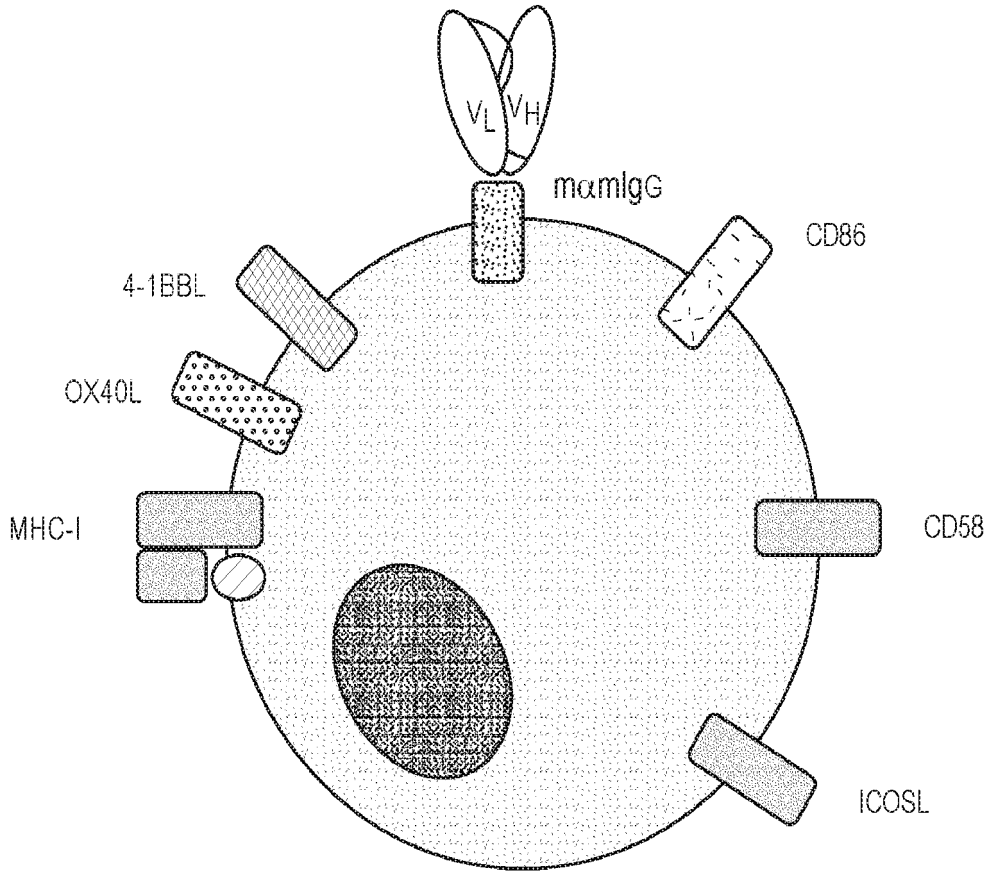


FIG. 97

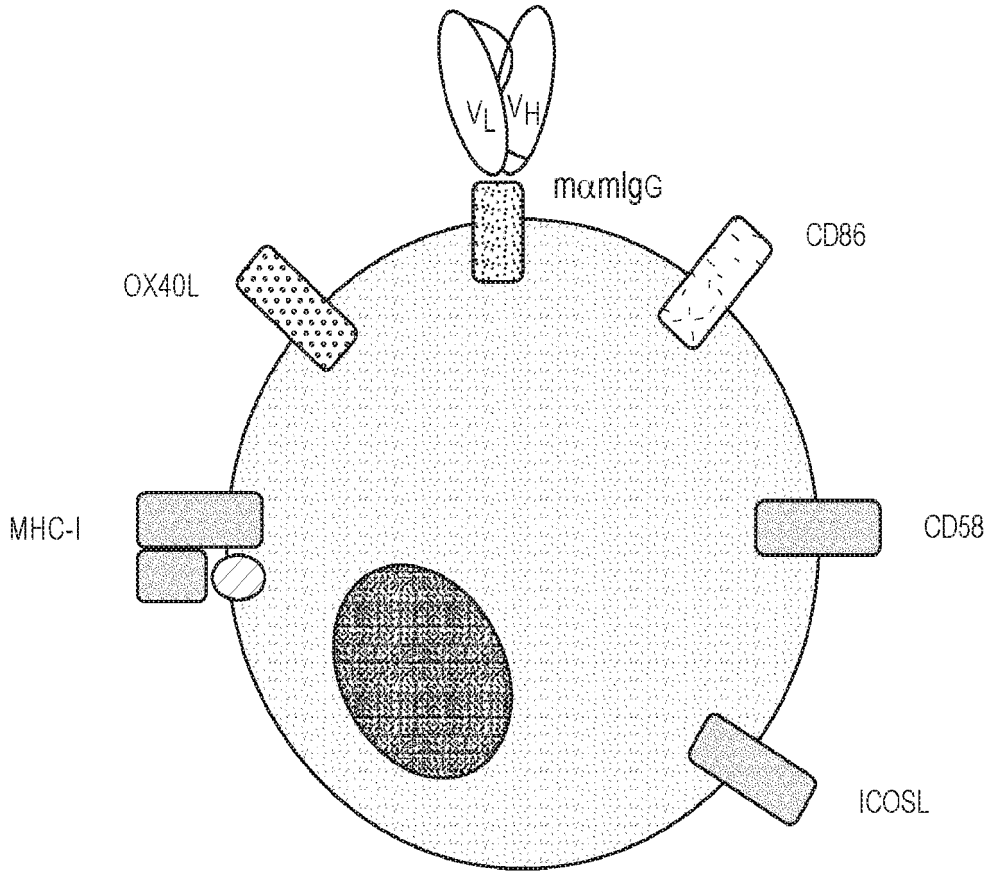


FIG. 98