Figure 1

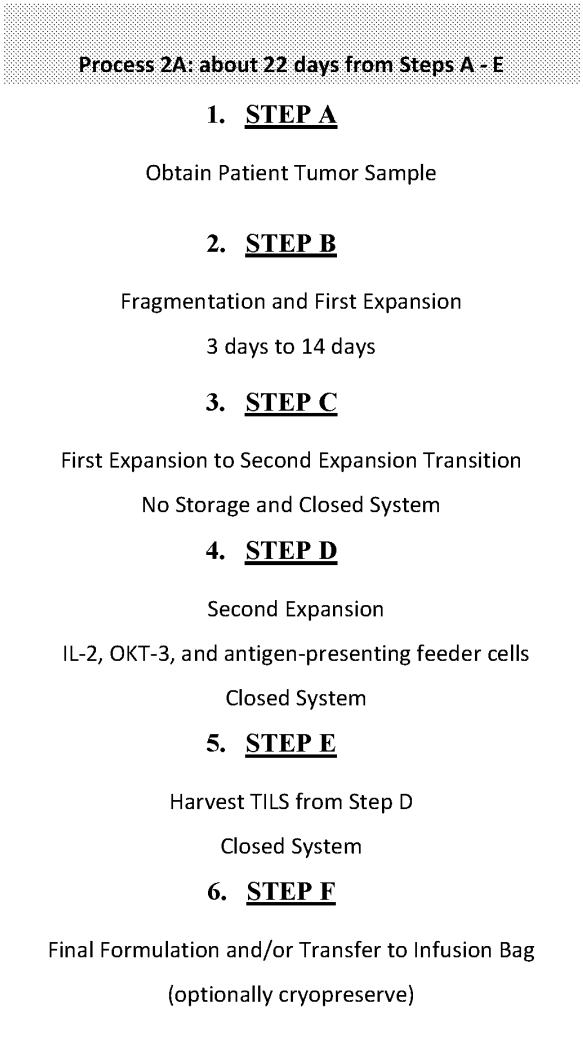

Process 2A: about 22 days from Steps A - E
1. STEP A
Obtain Patient Tumor Sample
2. STEP B
Fragmentation and First Expansion
3 days to 14 days
3. STEP C
First Expansion to Second Expansion Transition
No Storage and Closed System
4. STEP D
Second Expansion
IL-2, OKT-3, and antigen-presenting feeder cells
Closed System
5. STEP E
Harvest TILS from Step D
Closed System
6. STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

Figure 2A

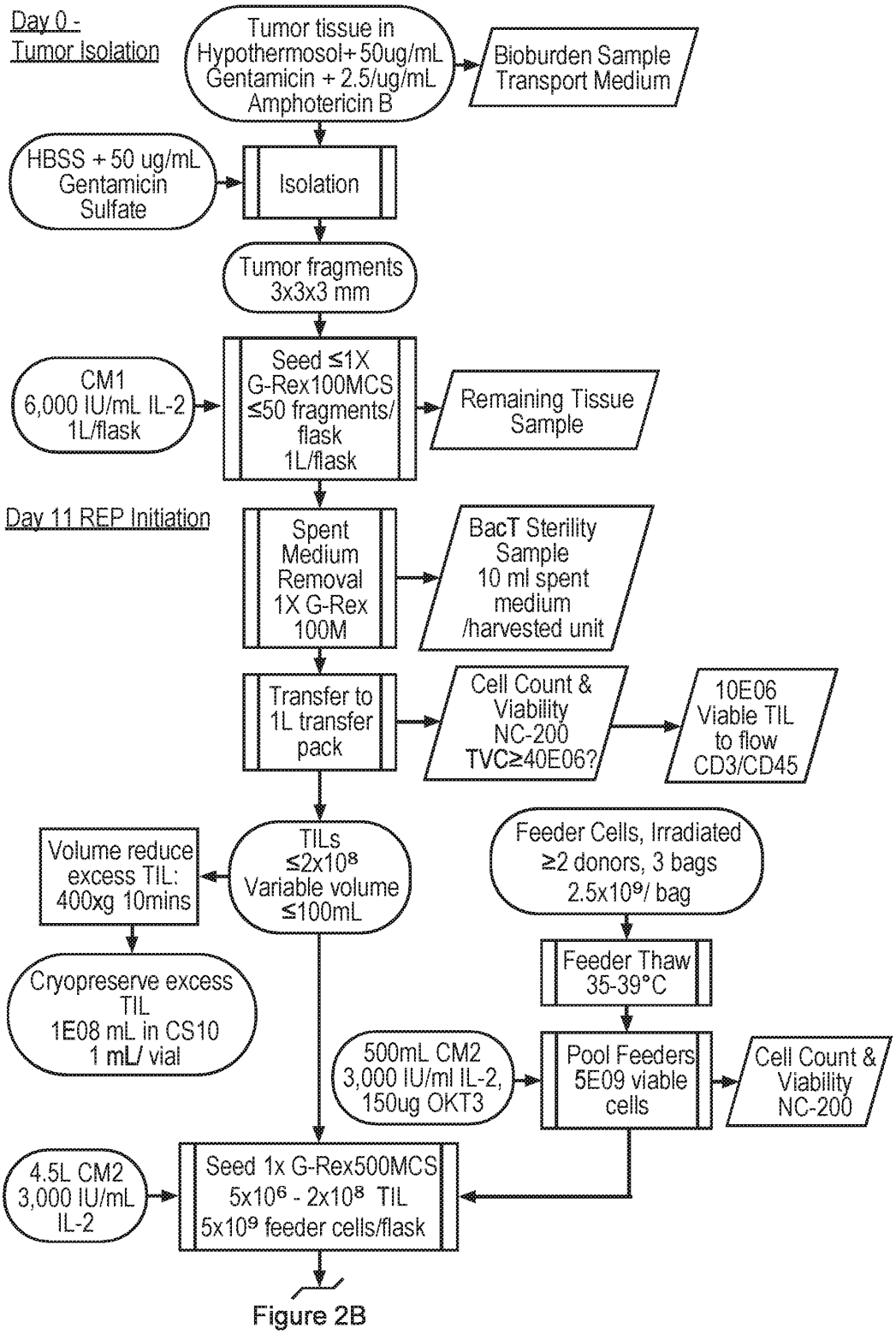


Figure 2B

Figure 2B

Figure 2A

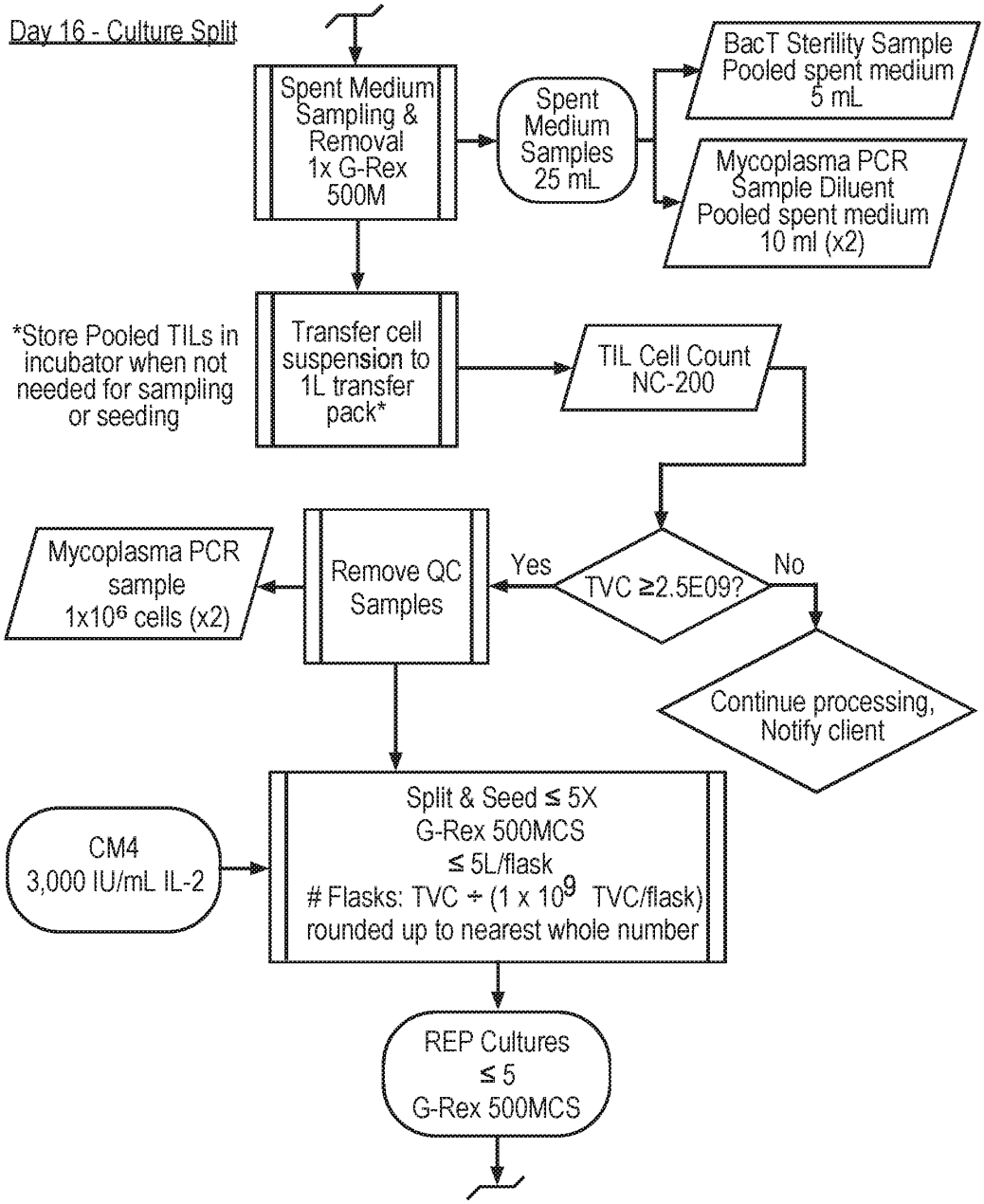
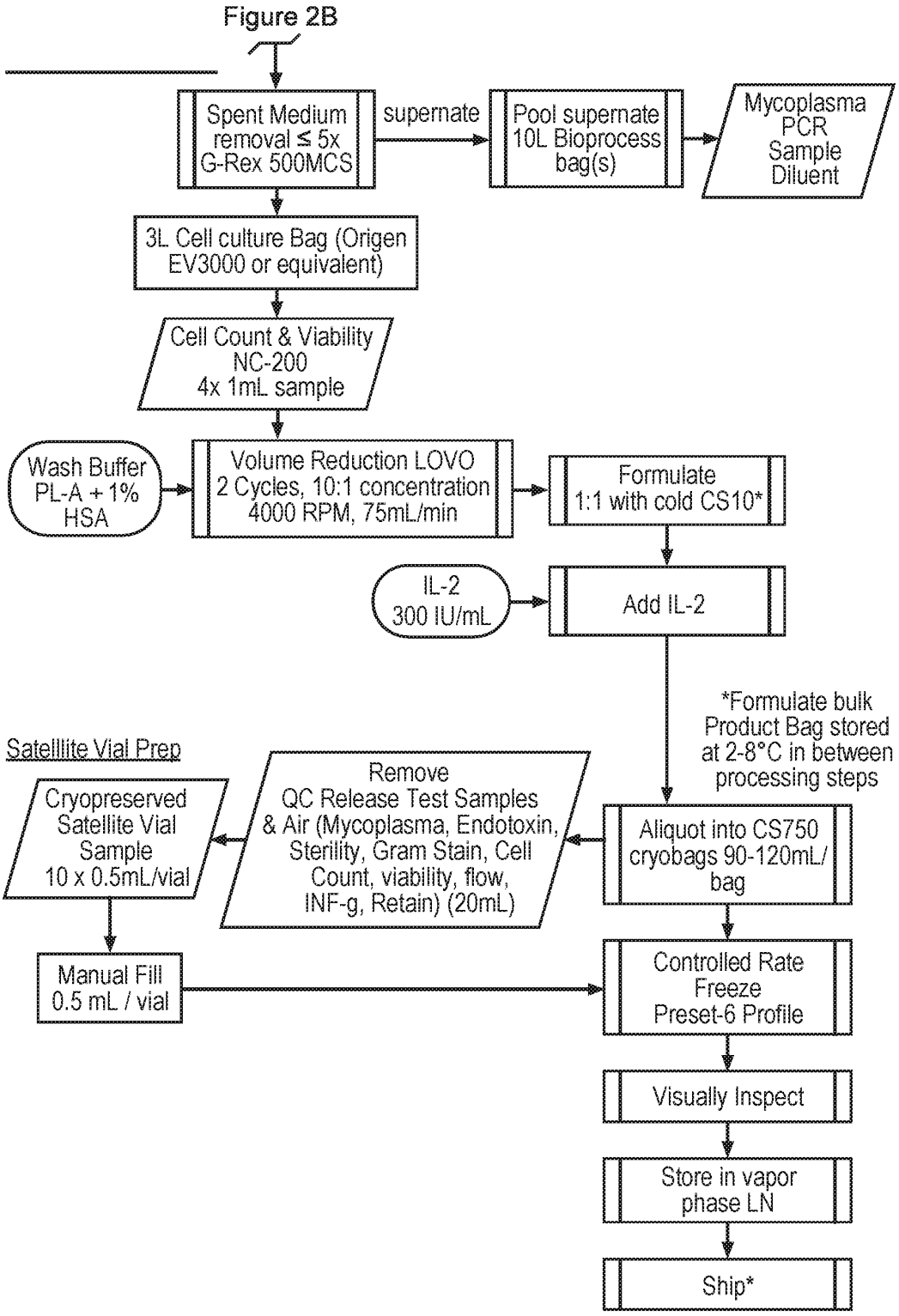


Figure 2C

Figure 2C


Figure 2B
Spent Medium removal ≤ 5x G-Rex 500MCS
supernate
Pool supernate 10L Bioprocess bag(s)
Mycoplasma PCR Sample Diluent
3L Cell culture Bag (Origen EV3000 or equivalent)
Cell Count & Viability NC-200 4x 1mL sample
Wash Buffer PL-A + 1% HSA
Volume Reduction LOVO 2 Cycles, 10:1 concentration 4000 RPM, 75mL/min
Formulate 1:1 with cold CS10*
IL-2 300 IU/mL
Add IL-2
*Formulate bulk Product Bag stored at 2-8°C in between processing steps
Satelllite Vial Prep
Cryopreserved Satellite Vial Sample 10 x 0.5mL/vial
Remove QC Release Test Samples & Air (Mycoplasma, Endotoxin, Sterility, Gram Stain, Cell Count, viability, flow, INF-g, Retain) (20mL)
Aliquot into CS750 cryobags 90-120mL/ bag
Manual Fill 0.5 mL / vial
Controlled Rate Freeze Preset-6 Profile
Visually Inspect
Store in vapor phase LN
Ship*

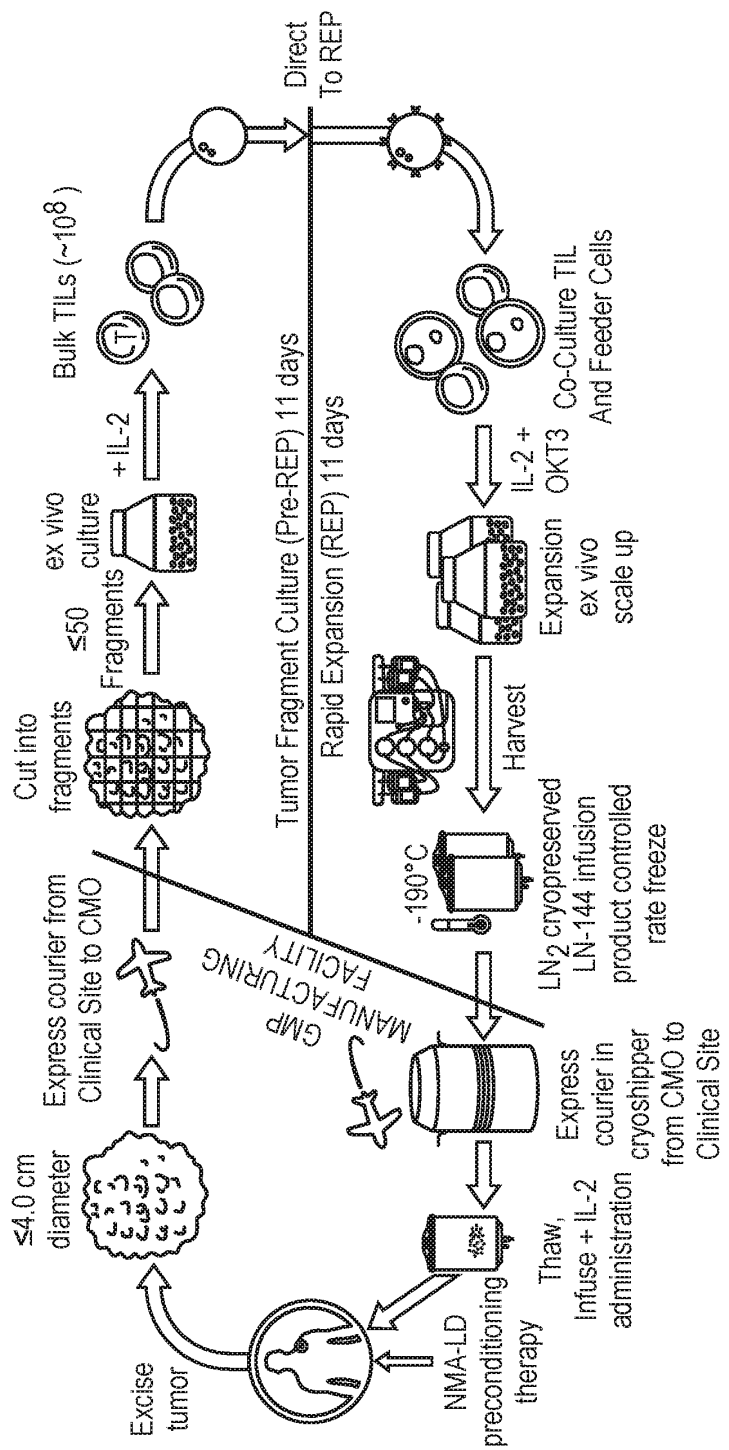

Excise tumor
≤4.0 cm diameter
Express courier from Clinical Site to CMO
Cut into fragments
≤50 Fragments
ex vivo culture
+ IL-2
Bulk TILs (~10^8)
Direct To REP
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
GMP MANUFACTURING FACILITY
NMA-LD preconditioning therapy
Thaw, Infuse + IL-2 administration
Express courier in cryoshipper from CMO to Clinical Site
-190°C
LN2 cryopreserved LN-144 infusion product controlled rate freeze
Harvest
IL-2 + OKT3
Expansion ex vivo scale up
Co-Culture TIL And Feeder Cells

Figure 3

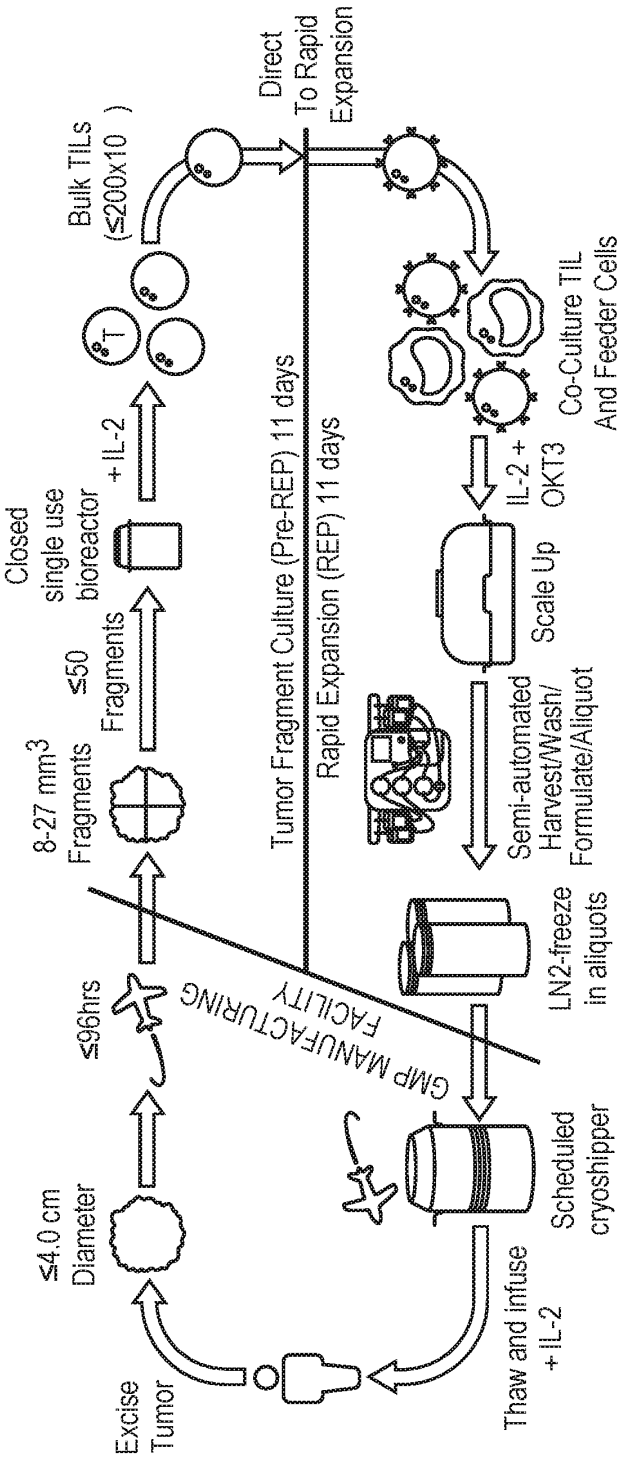

Excise Tumor
≤4.0 cm Diameter
≤96hrs
GMP MANUFACTURING FACILITY
8-27 mm3 Fragments
≤50 Fragments
Closed single use bioreactor
+ IL-2
Bulk TILs (≤200x10)
Tumor Fragment Culture (Pre-REP) 11 days
Rapid Expansion (REP) 11 days
Direct To Rapid Expansion
Co-Culture TIL And Feeder Cells
IL-2 + OKT3
Scale Up
Semi-automated Harvest/Wash/ Formulate/Aliquot
LN2-freeze in aliquots
Scheduled cryoshipper
Thaw and infuse + IL-2

Figure 4

Figure 5

Process 1C: 43-55 Days for Steps A - E	Process 2A: about 22 days from Steps A - E
1. **STEP A** Obtain Patient Tumor Sample	1. **STEP A** Obtain Patient Tumor Sample
2. **STEP B** Fragmentation and First Expansion 11 days to 21 days	2. **STEP B** Fragmentation and First Expansion 3 days to 14 days
3. **STEP C** First Expansion to Second Expansion Transition Optional Storage until Selection	3. **STEP C** First Expansion to Second Expansion Transition No Storage and Closed System
4. **STEP D** Second Expansion IL-2, OKT-3, antigen-presenting feeder cells Optionally repeat one or more times	4. **STEP D** Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells Closed System
5. **STEP E** Harvest TILS from Step D	5. **STEP E** Harvest TILS from Step D Closed System
6. **STEP F** Final Formulation and/or Transfer to Infusion Bag	6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 6

Process Step	Process 1C Embodiment	Process 2A Embodiment	Advantages
Pre-REP	• 4 fragments per 10 GREX-10 flasks • 11-21 day duration	• 40 fragments per 1 GREX-100M flask • 11 day duration	• Increased tumor fragments per flask • Shortened culture time • Reduced number of steps • Amenable to closed system
Pre-REP to REP Transition	• Pre-REP TIL are frozen until phenotyped for selection then thawed to proceed to the REP (~day 30) • REP requires >40×10^6 TIL	• Pre-REP TIL directly move to REP on day 11 • REP requires 25-200×10^6 TIL	• Shortened pre-REP-to-REP process • Reduced number of steps • Eliminated phenotyping selection • Amenable to closed system
REP	• 6 GREX-100M flasks on REP day 0 • 5×10^6 TIL and 5×10^8 PBMC feeders per flask on REP day 0 • Split to 18-36 flasks on REP day 7 • 14 day duration	• 1 GREX-500M flask on day 11 • 25-200×10^6 TIL and 5x10^9 PBMC feeders on day 11 • Split to ≤ 6 GREX-500M flasks on day 16 • 11 day duration	• Reduced number of steps • Shorter REP duration • Closed system transfer of TIL between flasks • Closed system media exchanges
Harvest	• TIL harvested via centrifugation	• TIL harvested via LOVO automated cell washing system'	• Reduced number of steps • Automated cell washing • Closed system • Reduced loss of product during wash
Final Formulation	• Fresh product in Hypothermosol • Single infusion bag • Limited shipping stability	• Cyropreserved product in PlasmaLyte-A + 1% HSA and CS10 stored in LN_2 • Multiple aliquots • Longer shipping stability	• Shipping flexibility • Flexible patient scheduling • More timely release testing
Overall Estimated Process Time	• 43-55 days	• 22 days	• Faster turnaround to patient

Figure 7A

Small Biopsy Process: Version 1 **About 21-33 days from Steps A - E**
1. STEP A Obtain Patient Tumor Sample
2. STEP B First Expansion 1 days to 19 days (with OKT-3 and antigen-presenting feeder cells at day 3)
3. STEP C First Expansion to Second Expansion Transition No Storage and Closed System
4. STEP D Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells 11 to 14 days Closed System
5. STEP E Harvest TILS from Step D Closed System
6. STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 7B

Small Biopsy Process: Version 2 **About 17-24 days from Steps A - E**
1. STEP A Obtain Patient Tumor Sample
2. STEP B First Expansion 3 days to 12 days (with OKT-3 and antigen-presenting feeder cells at any one of days 1-3)
3. STEP C First Expansion to Second Expansion Transition No Storage and Closed System
4. STEP D Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells 3 to 12 days Closed System
5. STEP E Harvest TILS from Step D Closed System
6. STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 7C

Small Biopsy Process: Version 1 About 21-33 days from Steps A - E	Small Biopsy Process: Version 2 (GEN3 based process) About 17-24 days from Steps A - E	Process 2A: About 22 days from Steps A - E
1. **STEP A** Obtain Patient Tumor Sample	1. **STEP A** 1. Obtain Patient Tumor Sample	1. **STEP A** Obtain Patient Tumor Sample
2. **STEP B** First Expansion 1 days to 19 days (with OKT-3 and antigen-presenting feeder cells at day 3)	2. **STEP B** First Expansion 3 days to 12 days (with OKT-3 and antigen-presenting feeder cells at any one of days 1-3)	2. **STEP B** Fragmentation and First Expansion 3 days to 14 days
3. **STEP C** First Expansion to Second Expansion Transition No Storage and Optionally Closed System	3. **STEP C** First Expansion to Second Expansion Transition No Storage and optionally Closed System	3. **STEP C** First Expansion to Second Expansion Transition No Storage and Closed System
4. **STEP D** Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells 11 to 14 days Optionally Closed System	4. **STEP D** Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells 3 to 12 days Optionally Closed System	4. **STEP D** Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells Closed System
5. **STEP E** Harvest TILS from Step D Optionally Closed System	5. **STEP E** Harvest TILS from Step D Closed System	5. **STEP E** Harvest TILS from Step D Closed System
6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	6. **STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figures 8A and 8B

Figure 8A

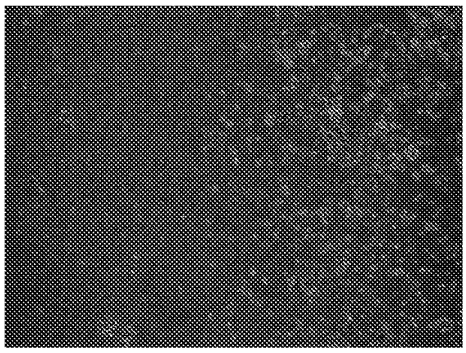
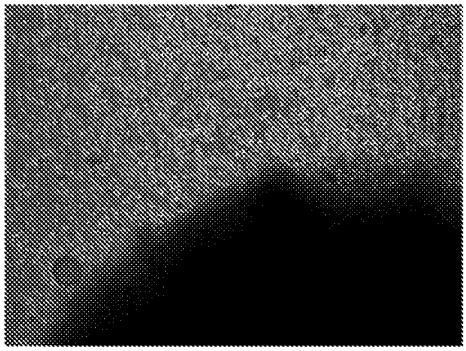


Figure 8B



Figures 9A and 9B

Figure 9A

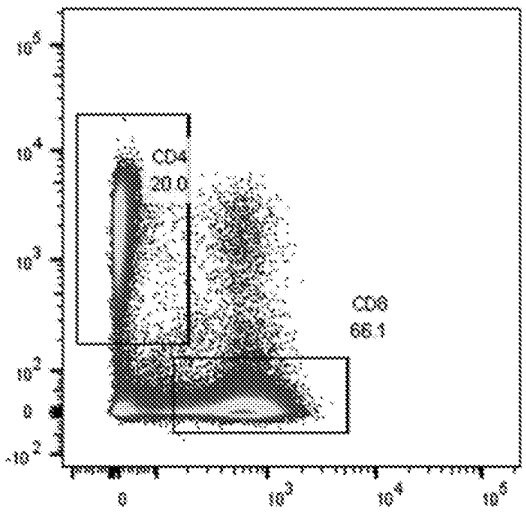
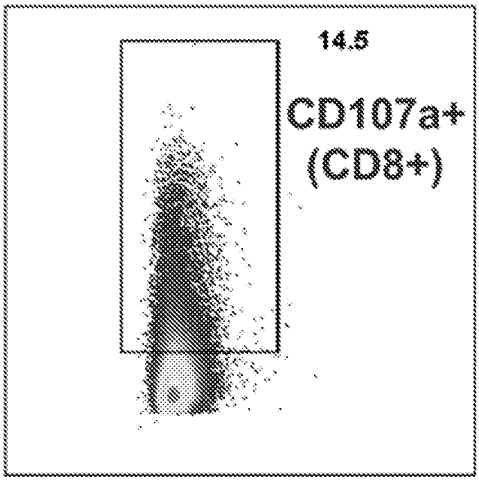


Figure 9B



Figures 9C and 9D

Figure 9C

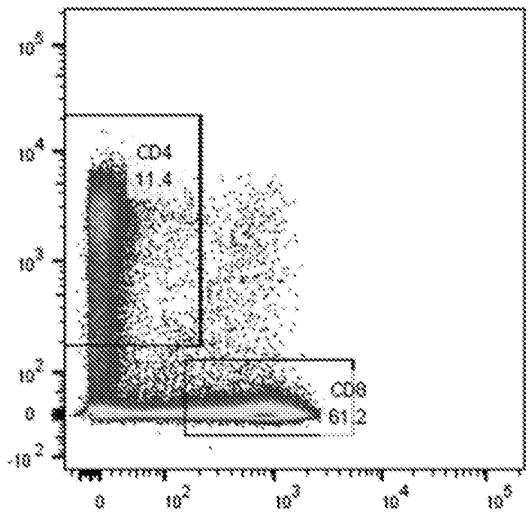
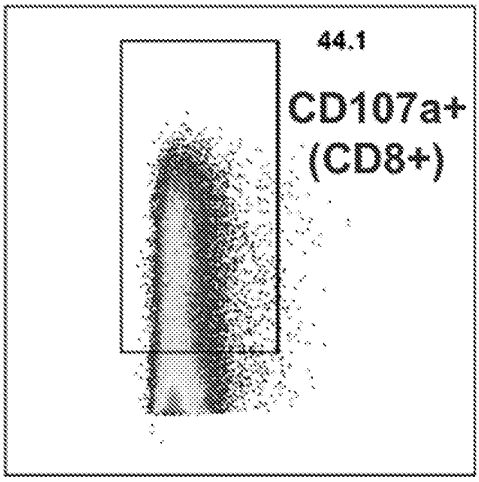


Figure 9D



Figures 10A and 10B

Figure 10A

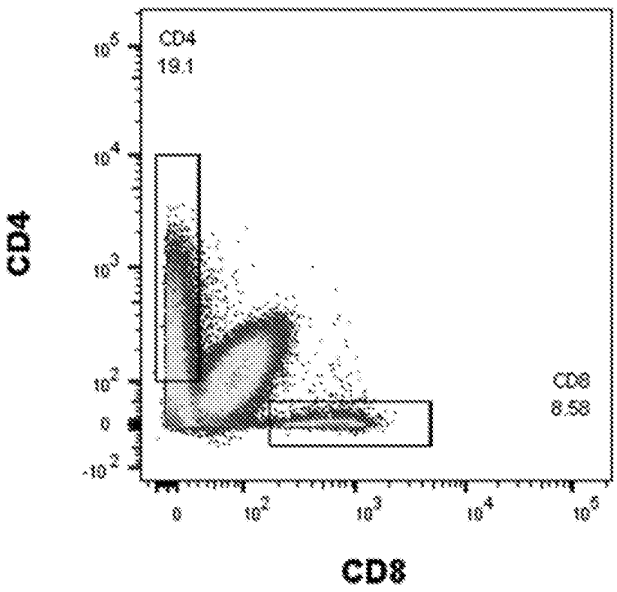
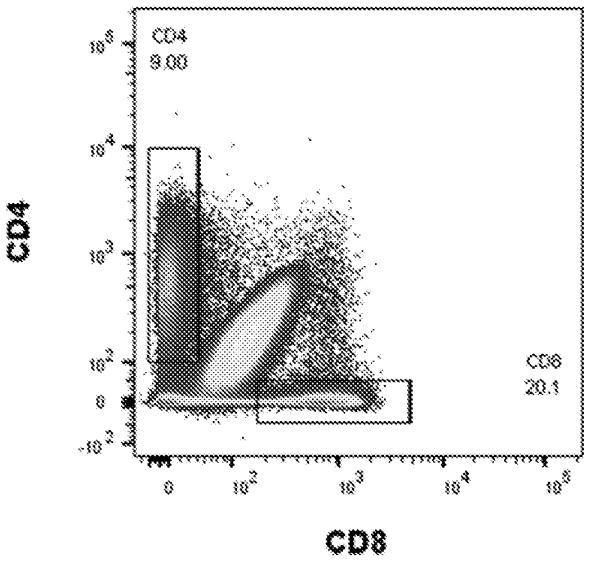


Figure 10B



Figures 11A and 11B

Figure 11A

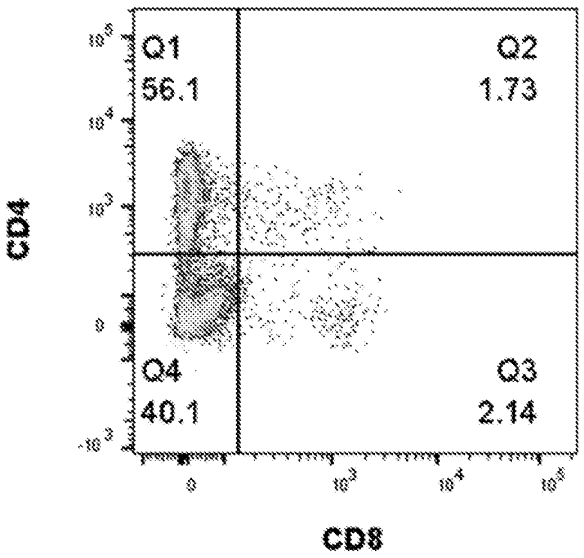


Figure 11B

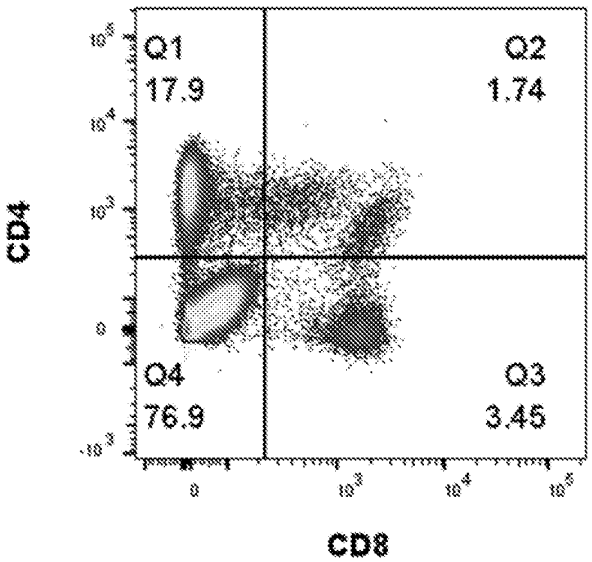
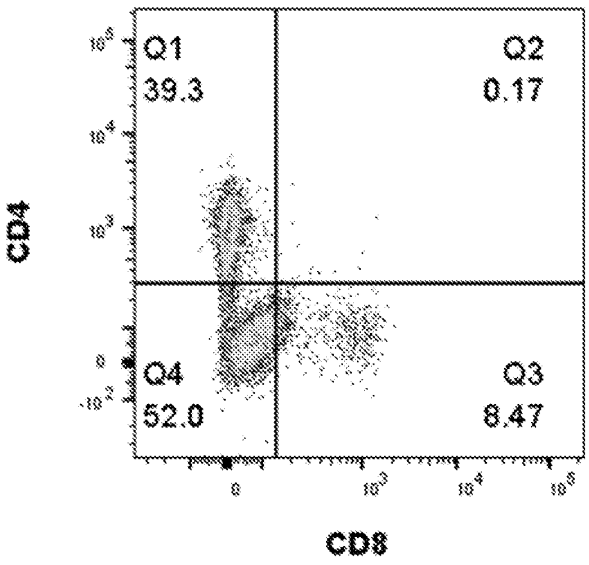


Figure 11C

Figure 11C



Figures 12A and 12B

Figure 12A

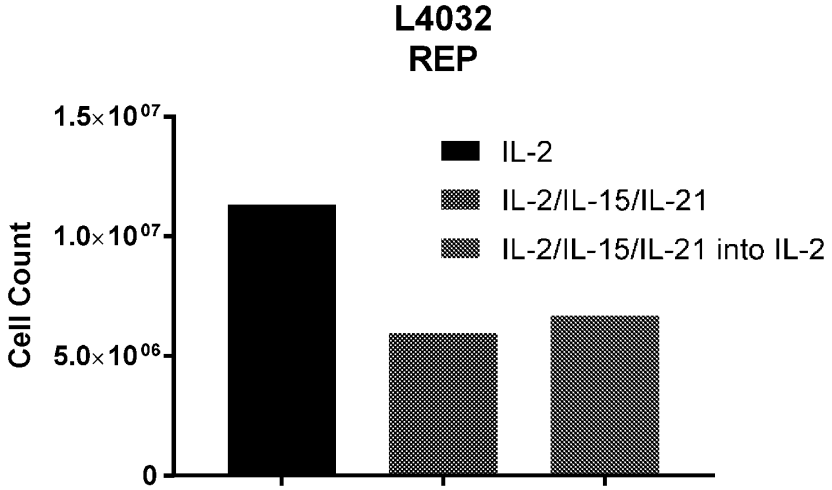
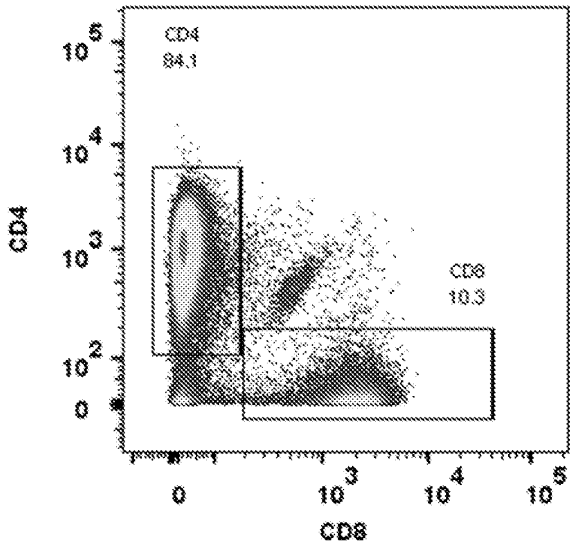


Figure 12B



Figures 13A and 13B

Figure 13A

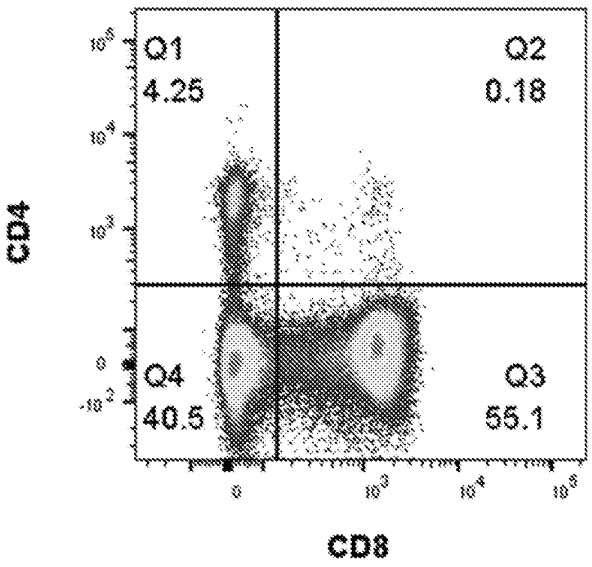


Figure 13B

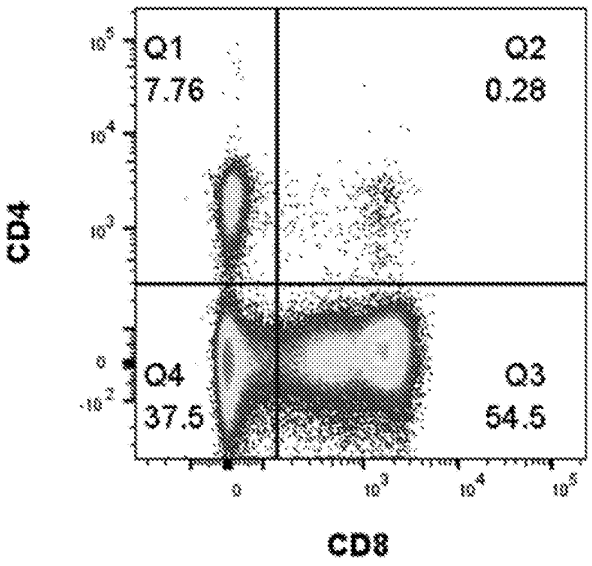


Figure 14

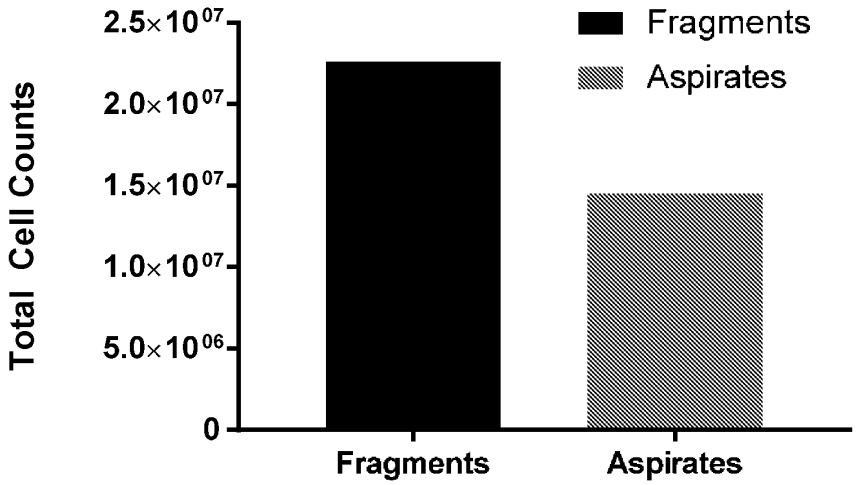
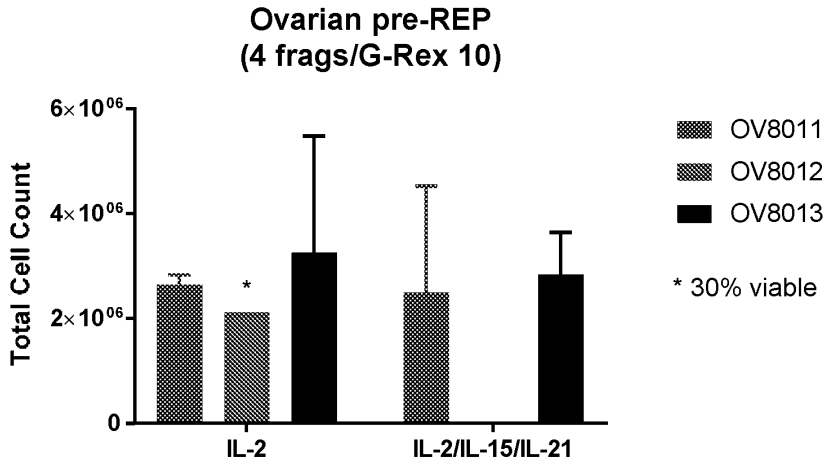

Total Cell Counts
2.5×10^07
2.0×10^07
1.5×10^07
1.0×10^07
5.0×10^06
0
Fragments
Aspirates
Fragments
Aspirates

Figure 15


Ovarian pre-REP
(4 frags/G-Rex 10)
Total Cell Count
6×10^06
4×10^06
2×10^06
0
*
IL-2
IL-2/IL-15/IL-21
OV8011
OV8012
OV8013
* 30% viable

Figures 16A and 16B

Figure 16A

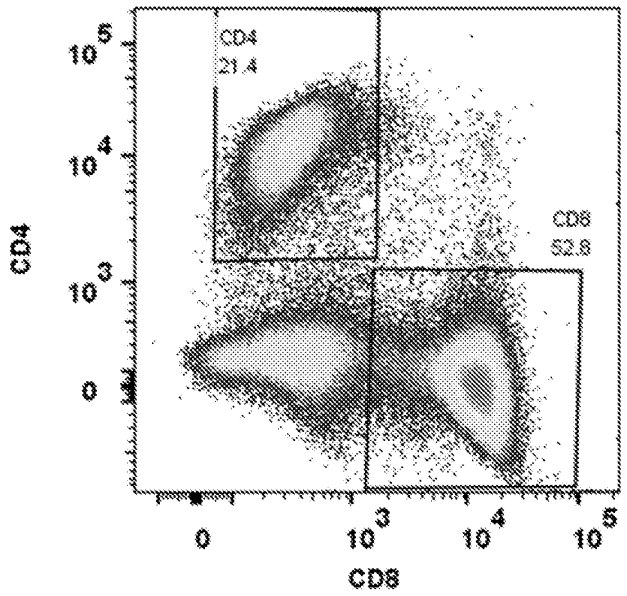
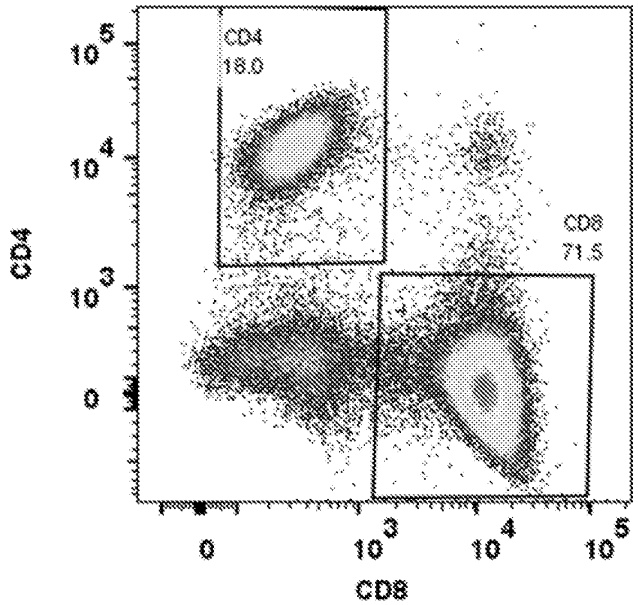


Figure 16B



Figures 17A and 17B

Figure 17A

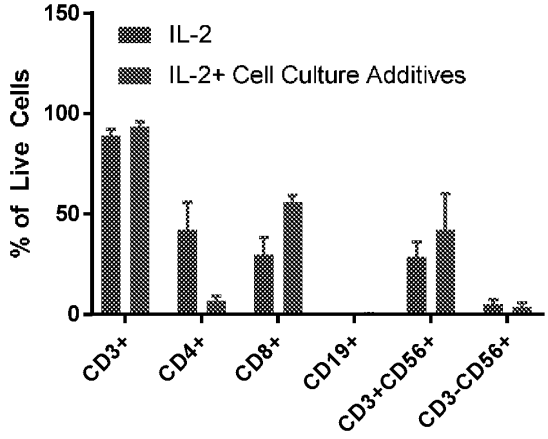
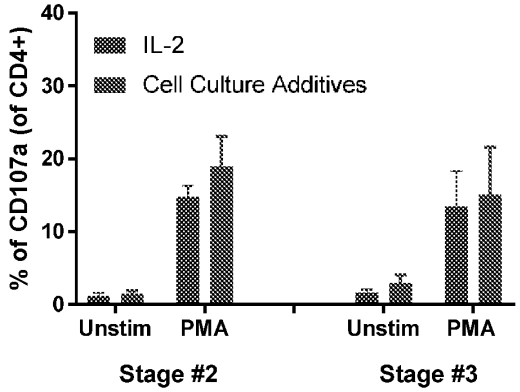


Figure 17B



Figures 17C and 17D

Figure 17C

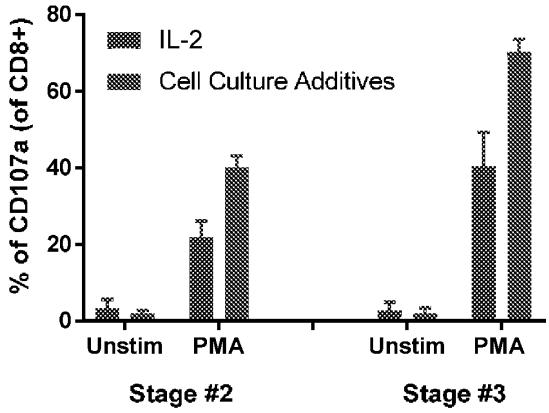


Figure 17D

Tumor ID	Cytokines	Days to reach Stage #1	Acceptance Criteria for Stage #2	Assayed Cells for Stage #2	Acceptance Criteria for Stage #3	Assayed Cells for Stage #3
Pan-17-004	IL-2 #1	27	39x10⁶	116x10⁶	109x10⁶	Media Failure
	IL-2 #2	27		130x10⁶		Media Failure
	Cell Culture Additives #1	14		Media Failure		N/A
	Cell Culture Additives #2	32		102x10⁶		148x10⁶
Pan-17-007	IL-2	18		Did not meet criteria to progress to REP		N/A
	Cell Culture Additives	25		118x10⁶		244x10⁶
Pan-17-008	IL-2	Did not meet criteria for G Rex10		N/A		N/A
	Cell Culture Additives	21		106x10⁶		144x10⁶

Figure 18A

Histology	FNA/Biopsy	Initial Cell Count (FNA's)	Conditions	Days in Culture	Final Cell Counts	Assays
Pancreatic (P7015)	Cores 24 well plate	N/A	**ONE CORE** 1) IL-2 (3 wells) 2) IL-2/IL-15/IL-21 (2 wells) **TWO CORES** 1) IL-2 (1 well) 2) IL-2/IL-15/IL-21 (1 wells)	28 days	**ONE CORE** IL-2 1.2e6 viable cells/well average IL-2/IL-15/IL-21 2e6 viable cells/well average **TWO CORES** No growth	**Phenotypic** FACS **Functional** CD107a
Pancreatic (P7017)	Cores 24 well plate	N/A	**ONE CORE**	N/A	No TIL growth	
Pancreatic (P7019)	Cores G-Rex 10	N/A	**6 CORES**	In progress		
Lung (L4032)	FNA 24 well plate	1.6e6 cells (28% viable)	1) IL-2 (2 wells) 2) IL-2/IL-15/IL-21 (2 wells) + Fragments	13 days	IL-2 4.64e5cells/well average, 76 % viable IL-2/IL-15/IL-21 7.75e5 cells/well average, 94% viable	**Phenotypic** FACS **Functional** CD107a (N/A) **REP** (recently harvested)
Lung (L4033)	**FNA G-Rex 10**	5.5e6 cells (76% viable)	1) IL-2 2) IL-2/IL-15/IL-21 + Fragments	12 days	IL-2 6.e6, 73 % viable IL-2/IL-15/IL-21 No detectable cells (no assay were run on this sample)	**Phenotypic** FACS **Functional** CD107a **REP** (recently harvested)
Lung (L4034)	FNA G-Rex 10	N/A			No TIL growth	No assays

Figure 18B

Histology	FNA/Biopsy	Initial Cell Count (FNA's)	Conditions	Days in Culture	Final Cell Counts	Assays
Colorectal (CC10024)	FNA 24 well plate	8.0e5 viable cells, 42% viable	1) IL-2 (2 wells) 2) IL-2/IL-15/IL-21 (2 wells) Fragments as a control	27 days	No TIL growth	No assays
Ovarian (OV8012)	FNA 24 well plate	3.19e5 viable cells, 28% viable	1) IL-2 (2 wells) 2) IL-2/IL-15/IL-21 (2 wells)		No TIL growth	No assays
Ovarian (OV8013)	FNA 24 well plate	8.3e5 viable cells, 11% viable	1) IL-2 (2 wells) 2) IL-2/IL-15/IL-21 (2 wells)		No TIL growth	No assays
Ovarian (OV8014)	FNA 24 well plate	1.0e5 viable cells, 7% viable	1) IL-2 (1 well) 2) IL-2/IL-15/IL-21 (1 well)		No TIL growth	No assays
Cervical (C2005)	FNA 24 well plate	6.84e5 (13% viable)	1) IL-2 (2 wells) 2) IL-2/IL-15/IL-21 (2 wells) + Fragments	21 days	IL-2 3.45e5 viable cells/well average, 39% viable IL-2/IL-15/IL-21 8.69e5 viable cells/well average, 40% viable	**Phenotypic** FACS **Functional** CD107a
Melanoma (M1101)	FNA G-Rex 10	1.96e6 (65% viable)	IL-2 (1 G-Rex 10) + Fragments	12 days	IL-2 3.0e6, 78 % viable	**Phenotypic** FACS **Functional** CD107a **REP** (recently harvested)
Melanoma (M1107)	FNA G-Rex 10	2.25e6 (69% viable)	IL-2 (2 G-Rex 10) IL-2/IL-15/IL-21 (2 G-Rex) + Fragments			Pre-REP in progress

Figures 19A and 19B

Figure 19A

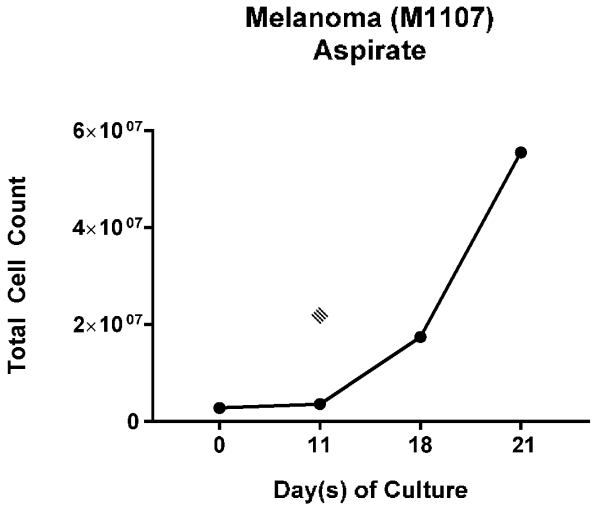


Figure 19B

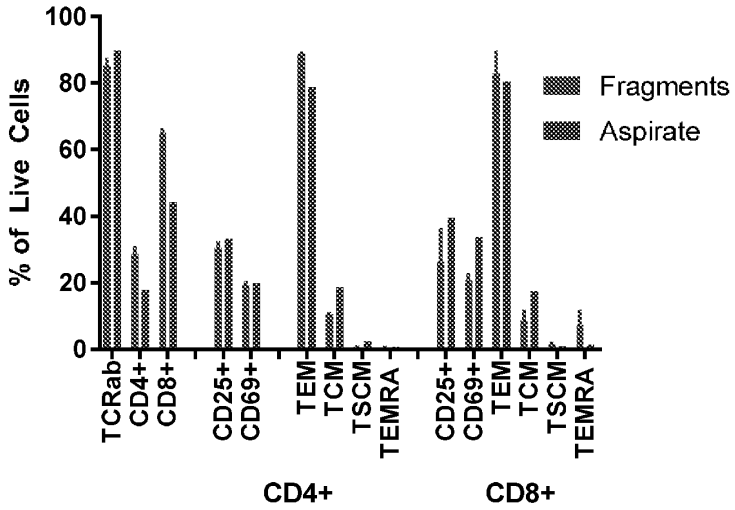


Figure 19C

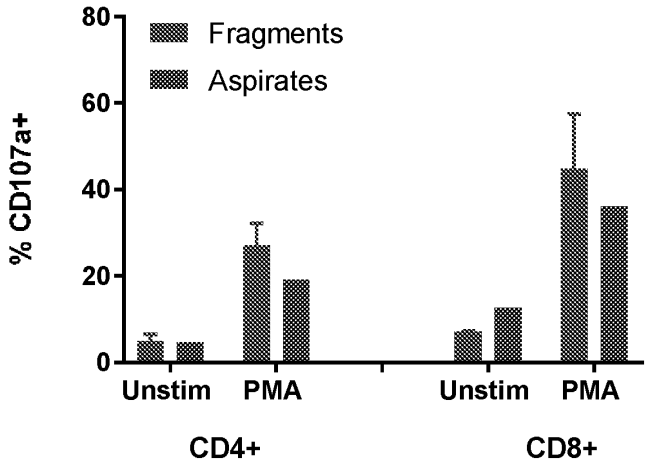
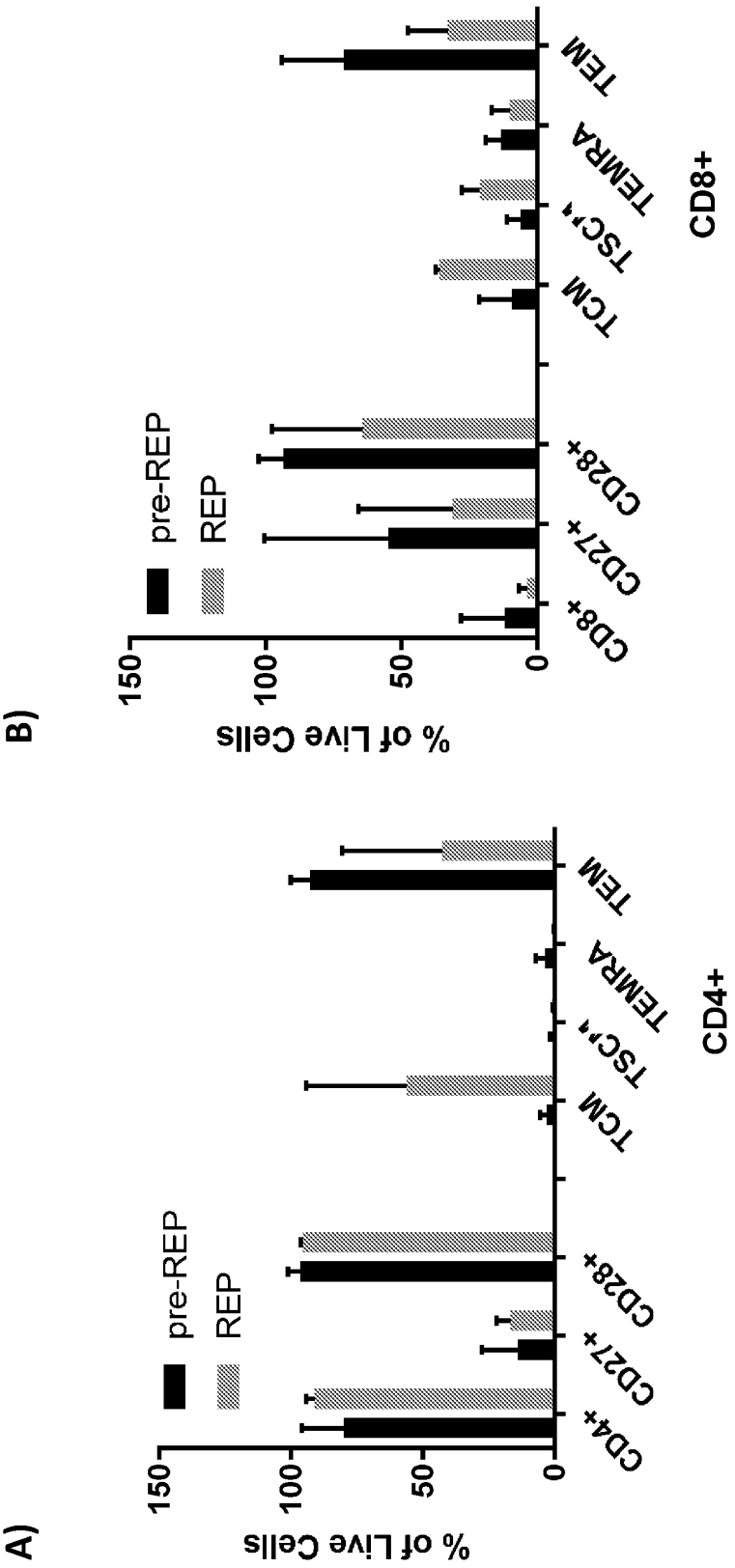

80
60
40
20
0
% CD107a+
Fragments
Aspirates
Unstim
PMA
Unstim
PMA
CD4+
CD8+

Figure 20

Tumor Name	Cell Growth	Analysis
UPMC-3 Reported	24 well plate: 1) IL-2 versus 2) Cytokine Additives	Cell growth, viability, phenotyping, CD107a
UPMC-9 Reported	Expanded poorly	N/A
UPMC-17 Reported	Contaminated culture	N/A
UPMC-26	Expanded poorly	N/A
UPMC-30	pre-REP Day 11: 1.6e6, 40% viable pre-REP Day 18: 3.49e7, 89% viable	Cell growth, viability, phenotyping, CD107a, IFNγ, REP initiation and analysis
UPMC-61	pre-REP Day 11: 1.52e5, 66% viable pre-REP Day 18: 1.14e7, 1.14e7, 40% viable	Cell growth, viability, phenotyping, CD107a, IFNγ, REP initiation and analysis
UPMC-59	Expanded poorly:	N/A
UPMC-61	pre-REP: In progress	

Figure 21


A)
% of Live Cells
150
100
50
0
pre-REP
REP
CD4+
CD4+
CD27+
CD28+
TCM
TSCM
TEMRA
TEM
B)
% of Live Cells
150
100
50
0
pre-REP
REP
CD8+
CD8+
CD27+
CD28+
TCM
TSCM
TEMRA
TEM

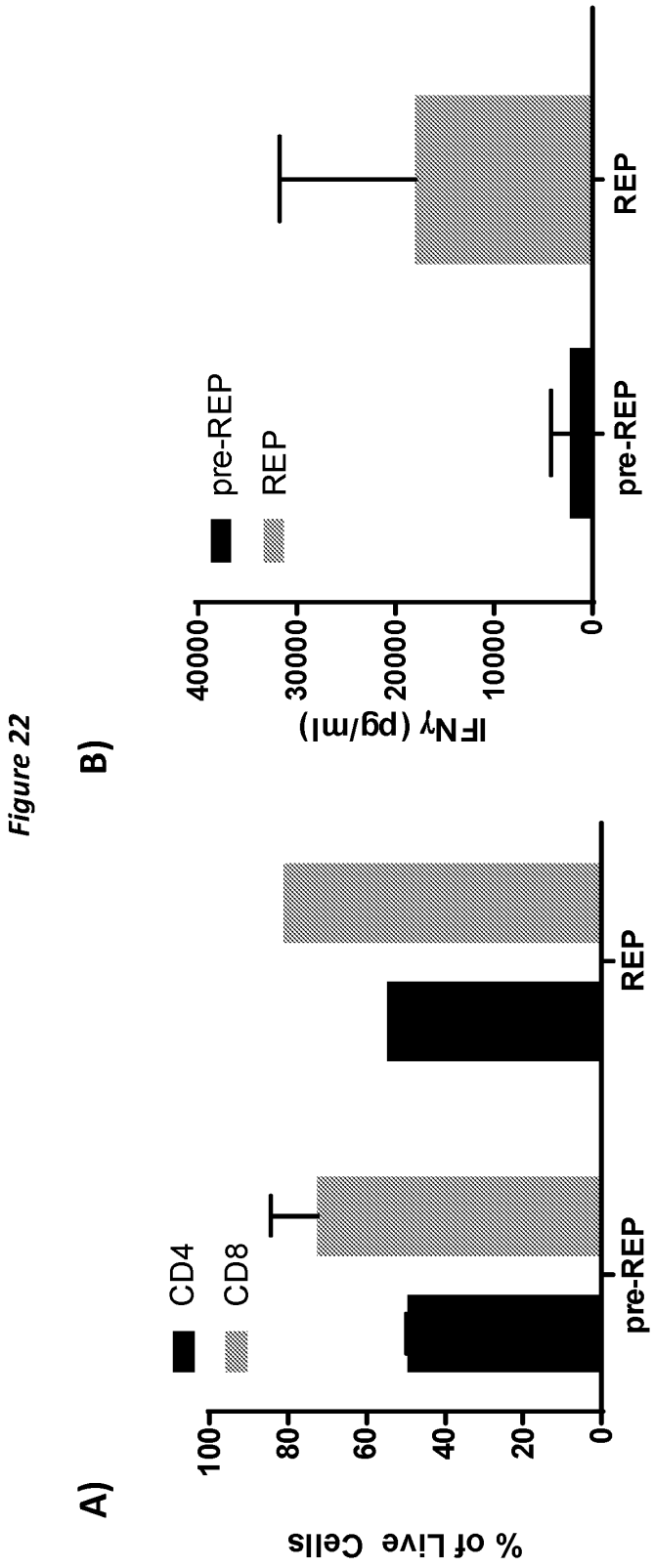

A)
% of Live Cells
100
80
60
40
20
0
CD4
CD8
pre-REP
REP
B)
IFNγ (pg/ml)
40000
30000
20000
10000
0
pre-REP
REP
pre-REP
REP

Figure 22

Figure 23

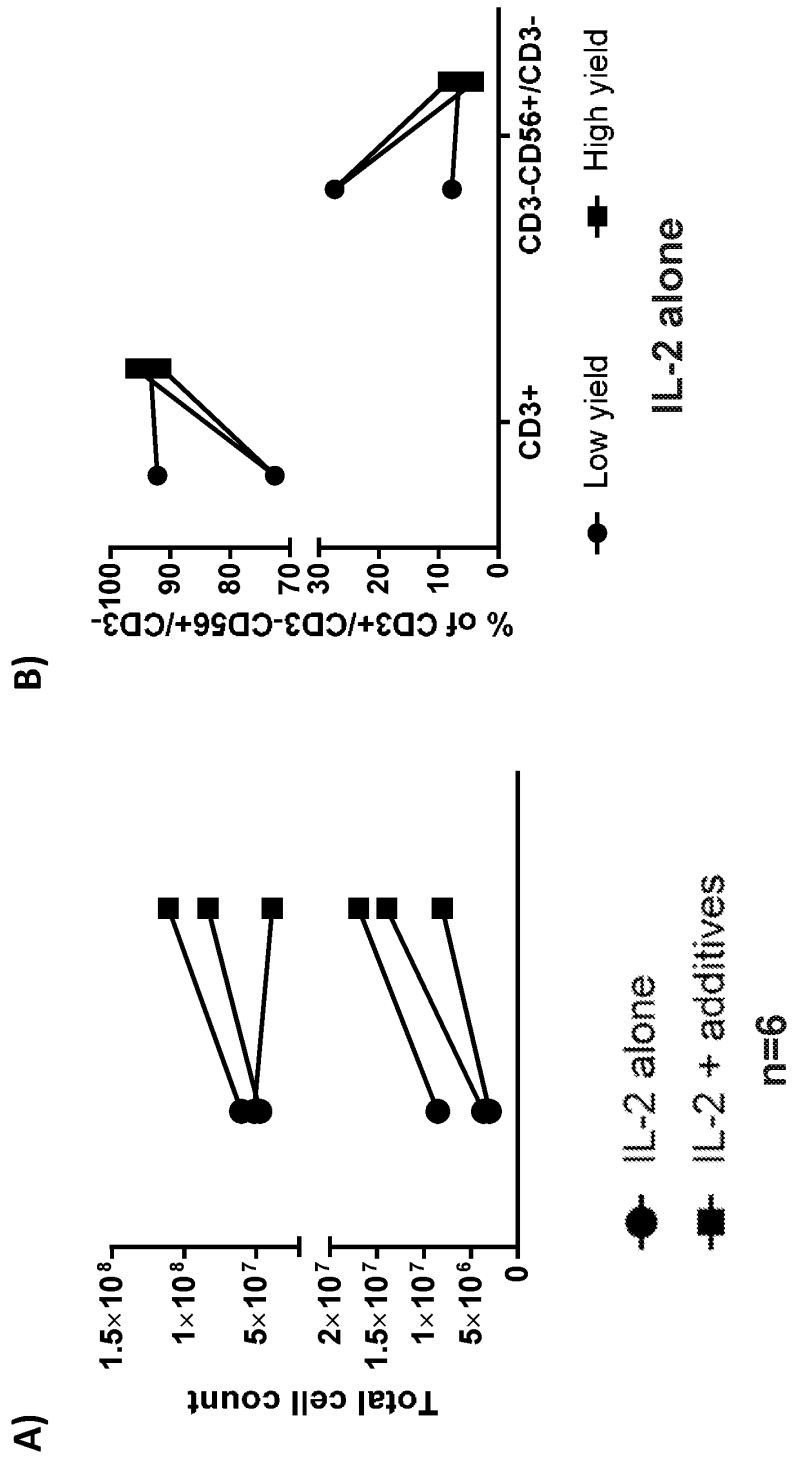
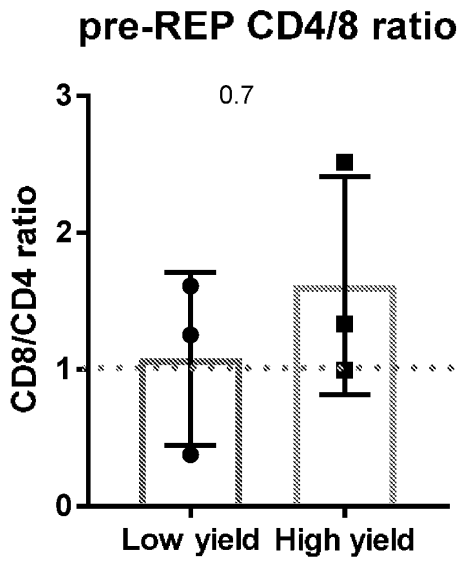

A)
Total cell count
1.5×10^8
1×10^8
5×10^7
2×10^7
1.5×10^7
1×10^7
5×10^6
0
IL-2 alone
IL-2 + additives
n=6
B)
% of CD3+/CD3-CD56+/CD3-
100
90
80
70
30
20
10
0
CD3+
CD3-CD56+/CD3-
Low yield
High yield
IL-2 alone

Figure 24

A)


pre-REP CD4/8 ratio
0.7
CD8/CD4 ratio
3
2
1
0
Low yield
High yield

B)

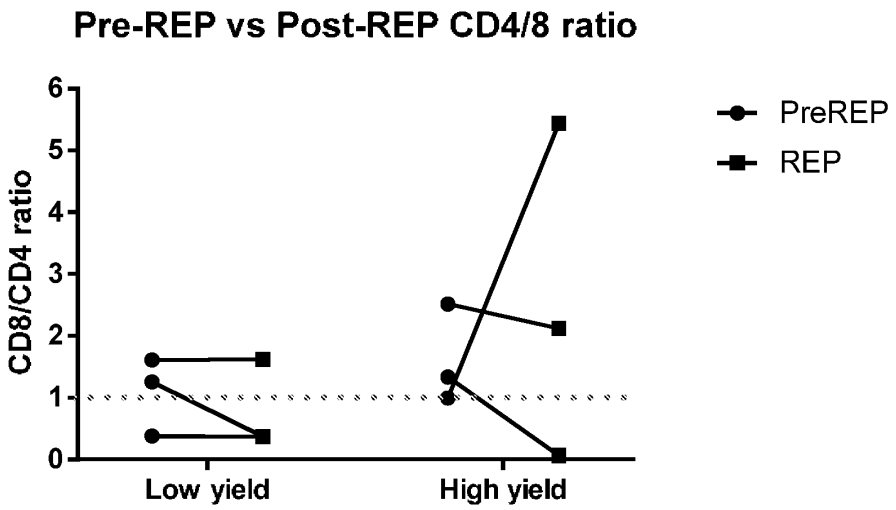

Pre-REP vs Post-REP CD4/8 ratio
CD8/CD4 ratio
6
5
4
3
2
1
0
Low yield
High yield
PreREP
REP

Figure 25

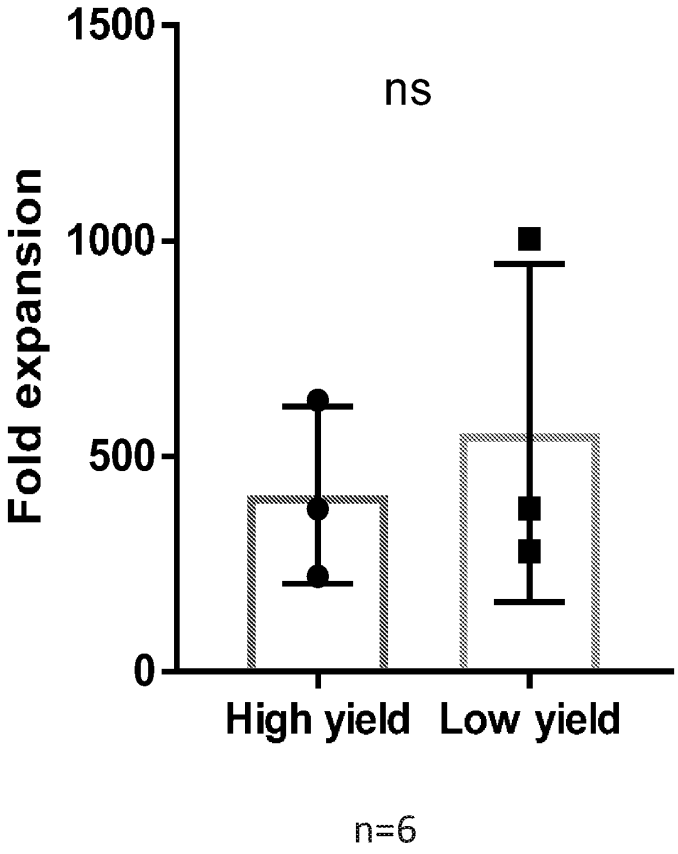


Figure 26

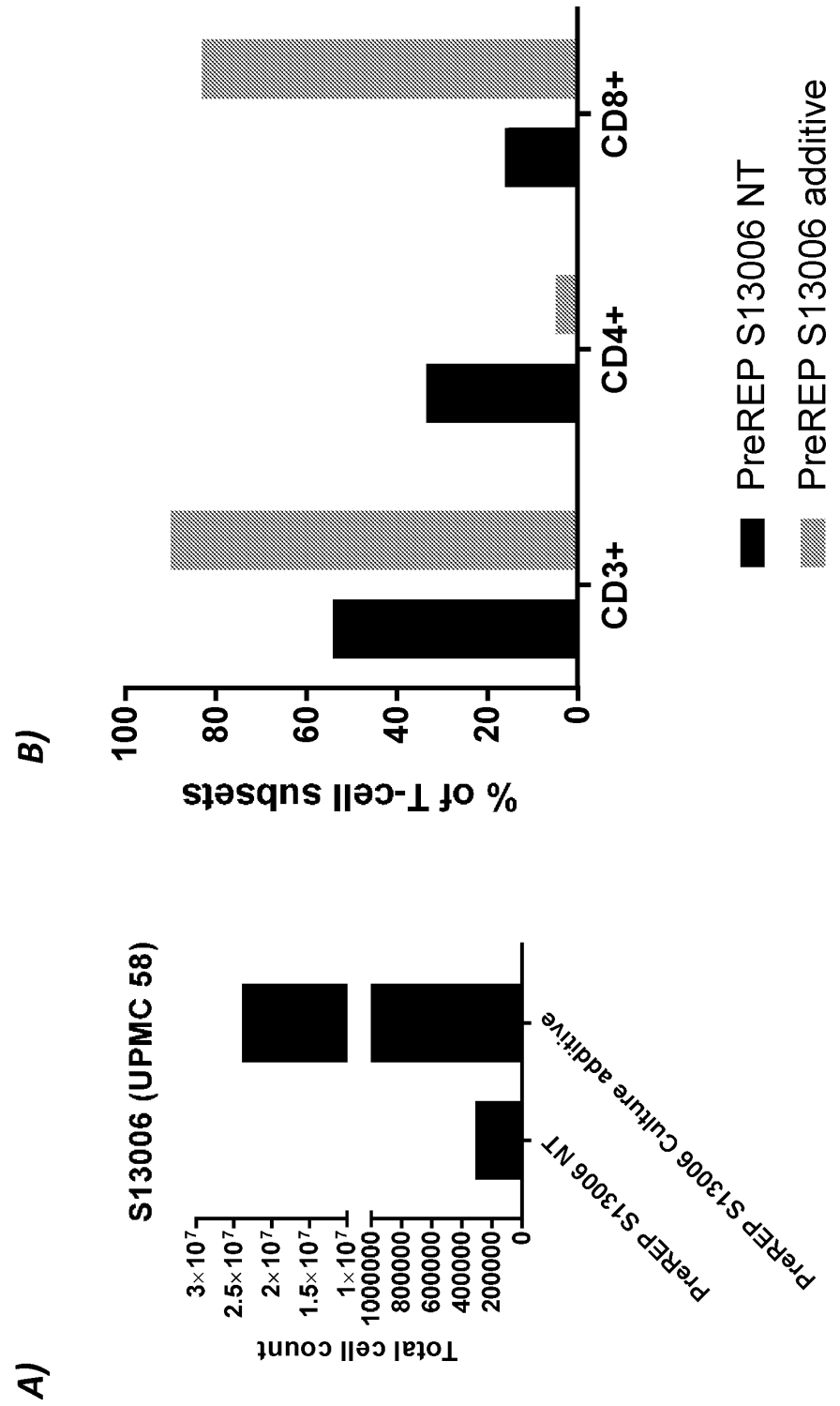

A)
S13006 (UPMC 58)
Total cell count
3×10^7
2.5×10^7
2×10^7
1.5×10^7
1×10^7
1000000
800000
600000
400000
200000
0
PreREP S13006 NT
PreREP S13006 Culture additive
B)
% of T-cell subsets
100
80
60
40
20
0
CD3+
CD4+
CD8+
PreREP S13006 NT
PreREP S13006 additive

Figure 27

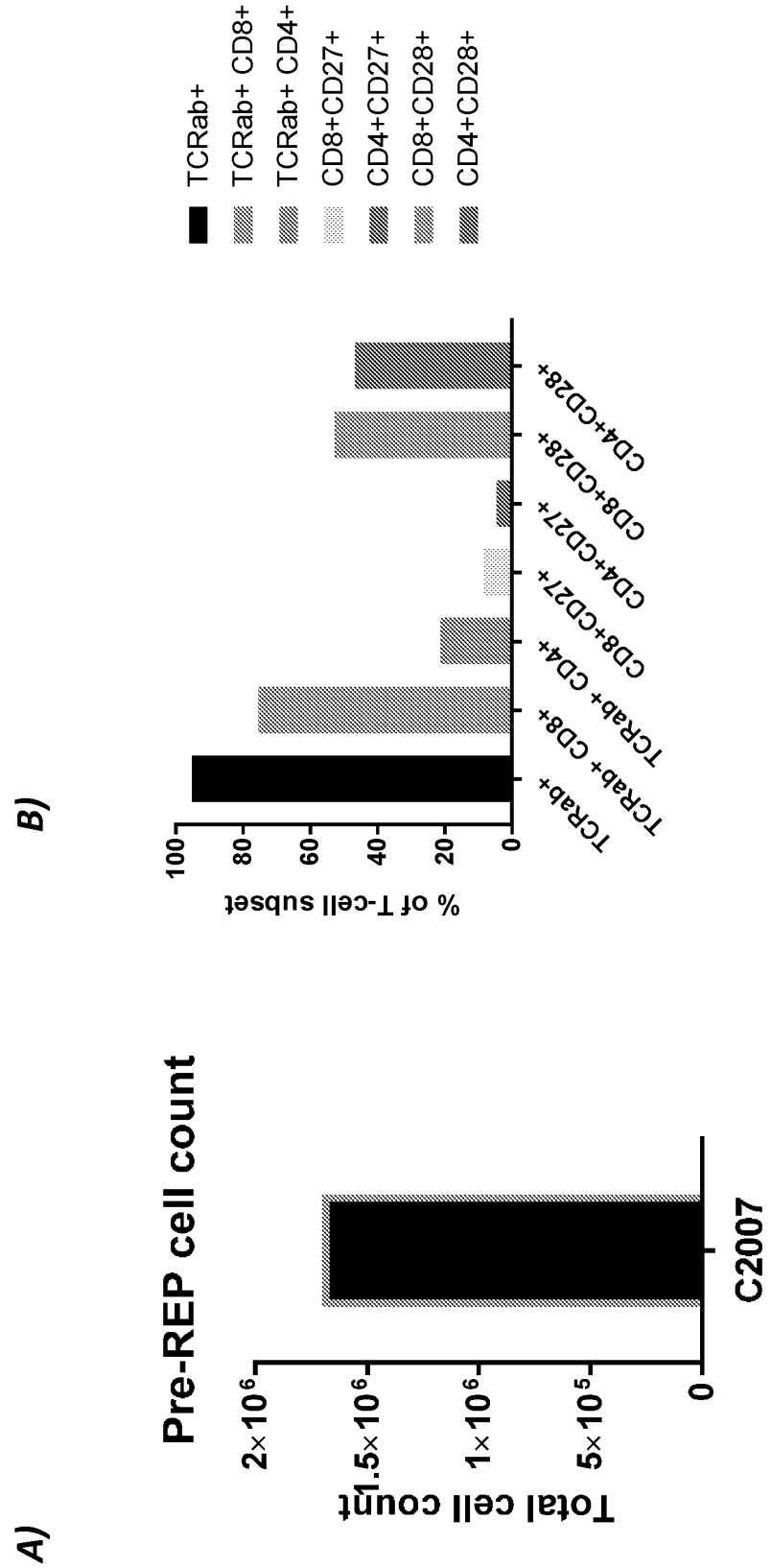
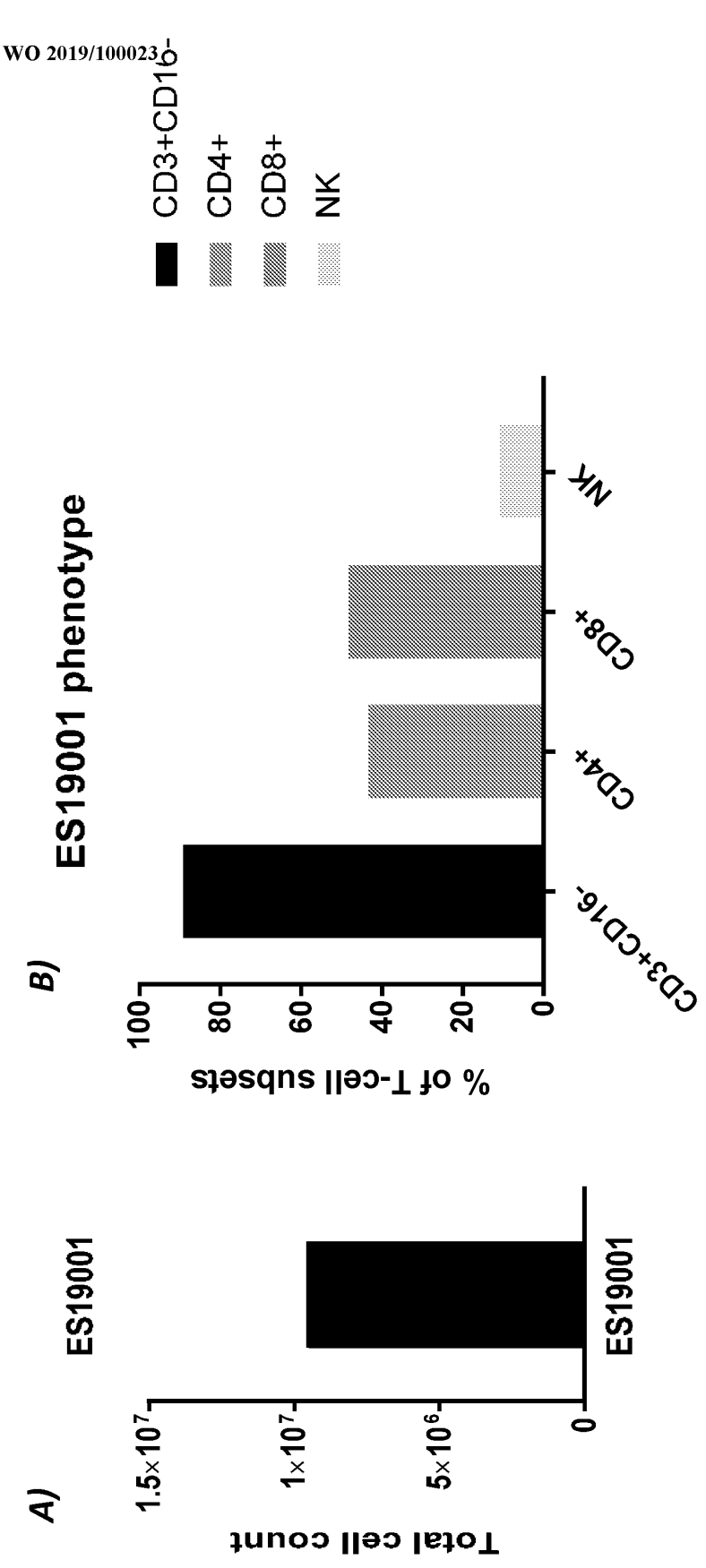

A)
Pre-REP cell count
Total cell count
2×10^6
1.5×10^6
1×10^6
5×10^5
0
C2007
B)
% of T-cell subset
100
80
60
40
20
0
TCRab+
TCRab+ CD8+
TCRab+ CD4+
CD8+CD27+
CD4+CD27+
CD8+CD28+
CD4+CD28+
TCRab+
TCRab+ CD8+
TCRab+ CD4+
CD8+CD27+
CD4+CD27+
CD8+CD28+
CD4+CD28+

Figure 28


A)
ES19001
Total cell count
1.5×10^7
1×10^7
5×10^6
0
ES19001
B)
ES19001 phenotype
% of T-cell subsets
100
80
60
40
20
0
CD3+CD16-
CD4+
CD8+
NK
CD3+CD16-
CD4+
CD8+
NK

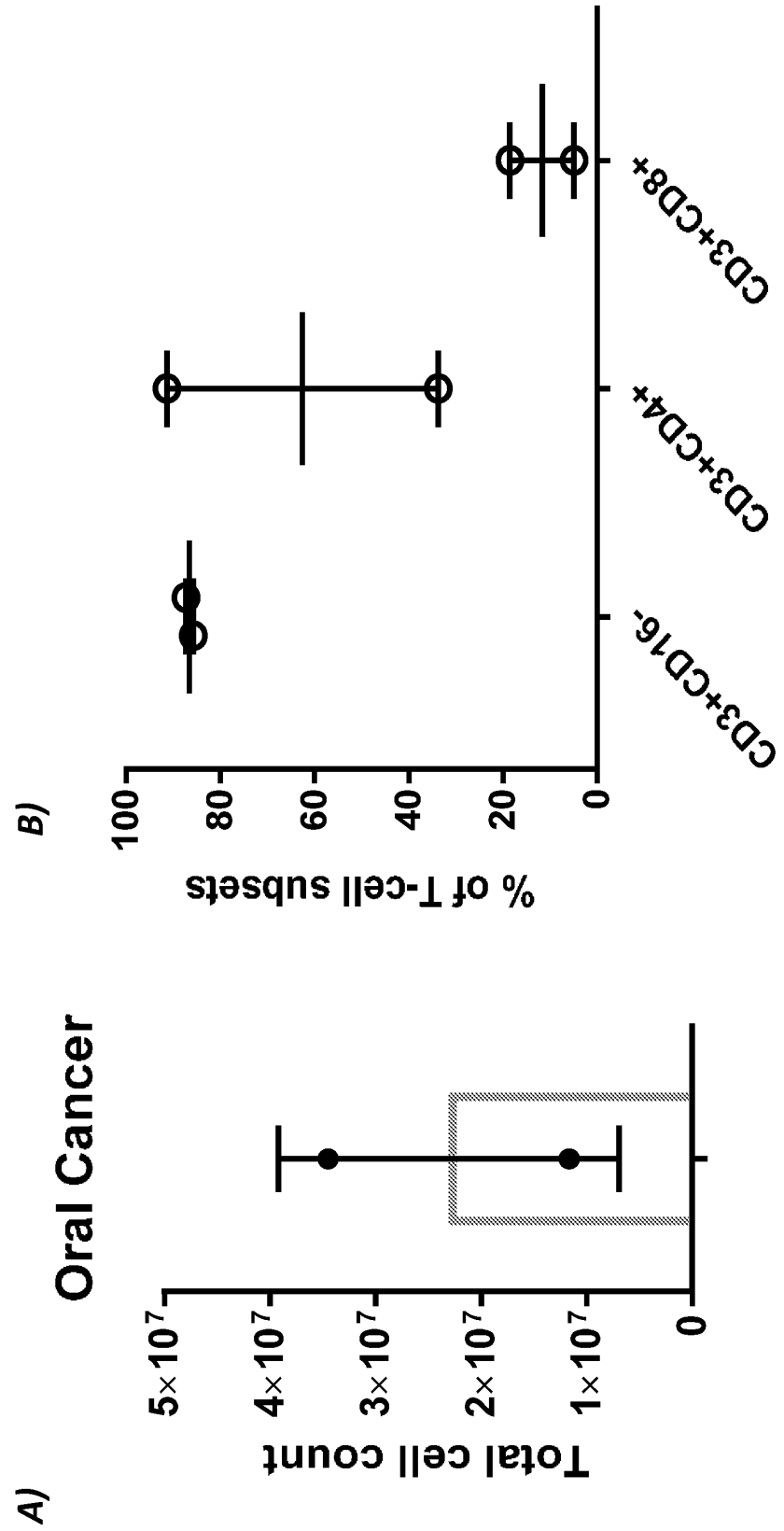

A)
Oral Cancer
Total cell count
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
B)
% of T-cell subsets
100
80
60
40
20
0
CD3+CD16-
CD3+CD4+
CD3+CD8+

Figure 29

Figure 30

Tumor Name	Cell Growth	Analysis
UPMC-3 (P7011) Reported	24 well plate: 1) IL-2 versus 2) Cytokine Additives	Cell growth, viability, phenotyping, CD107a
UPMC-9 (P7017) Reported	Expanded poorly	N/A
UPMC-17 (P7019) Reported	Contaminated culture. Discarded.	N/A
UPMC-26 (P7022)	Expanded poorly	N/A
UPMC-30 (P7028)	pre-REP Day 12: 1.6e6, 40% viable pre-REP Day 21: 3.49e7, 89% viable	Cell growth, viability, phenotyping, CD107a, IFNγ, REP initiation and analysis
UPMC-61 (P7031)	pre-REP Day 12: 1.52e5, 66% viable pre-REP Day 28: 1.14e7, 1.14e7, 40% viable	Cell growth, viability, phenotyping, CD107a, IFNγ, REP initiation and analysis
UPMC-59 (P7033)	Expanded poorly	N/A
UPMC-61 (P7034)	pre-REP: In progress	

Figure 31

A)
IL-2 treated well
Pancreatic Core Biopsies
1.3e6 viable cells/well
1.2e6 viable cells/well average
B)
IL-2/15/21 treated well
2.3e6 viable cells/well
2.0e6 viable cells/well average

Figure 32

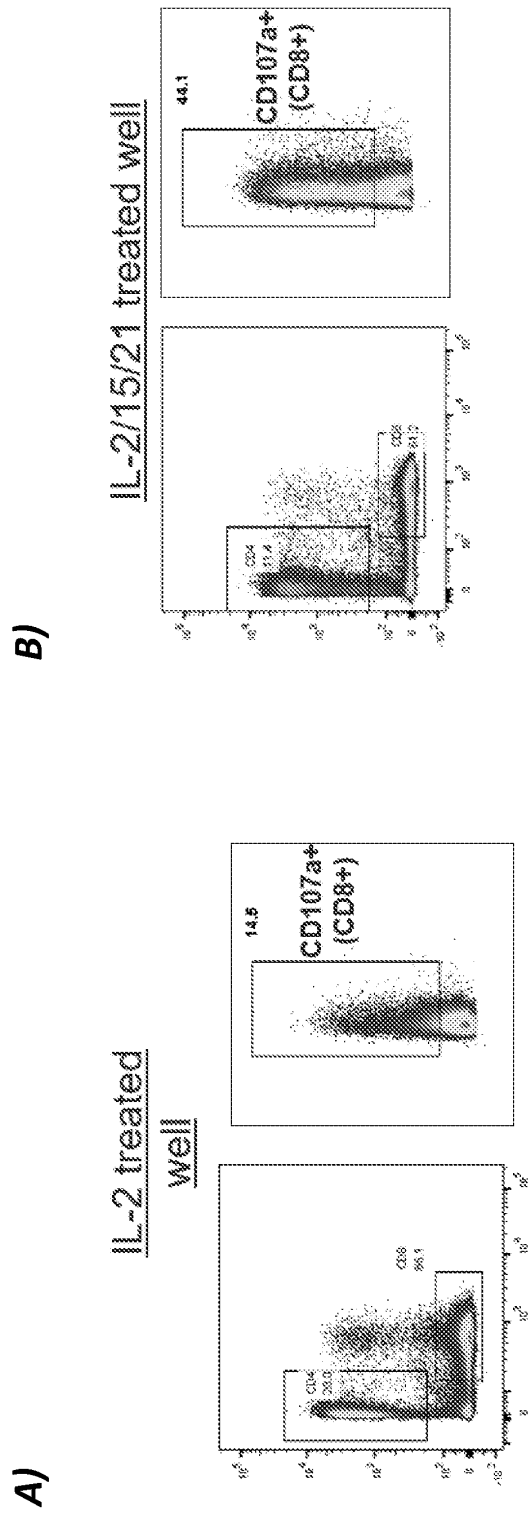

A)
IL-2 treated well
14.5
CD107a+ (CD8+)
B)
IL-2/15/21 treated well
44.1
CD107a+ (CD8+)

Figure 33

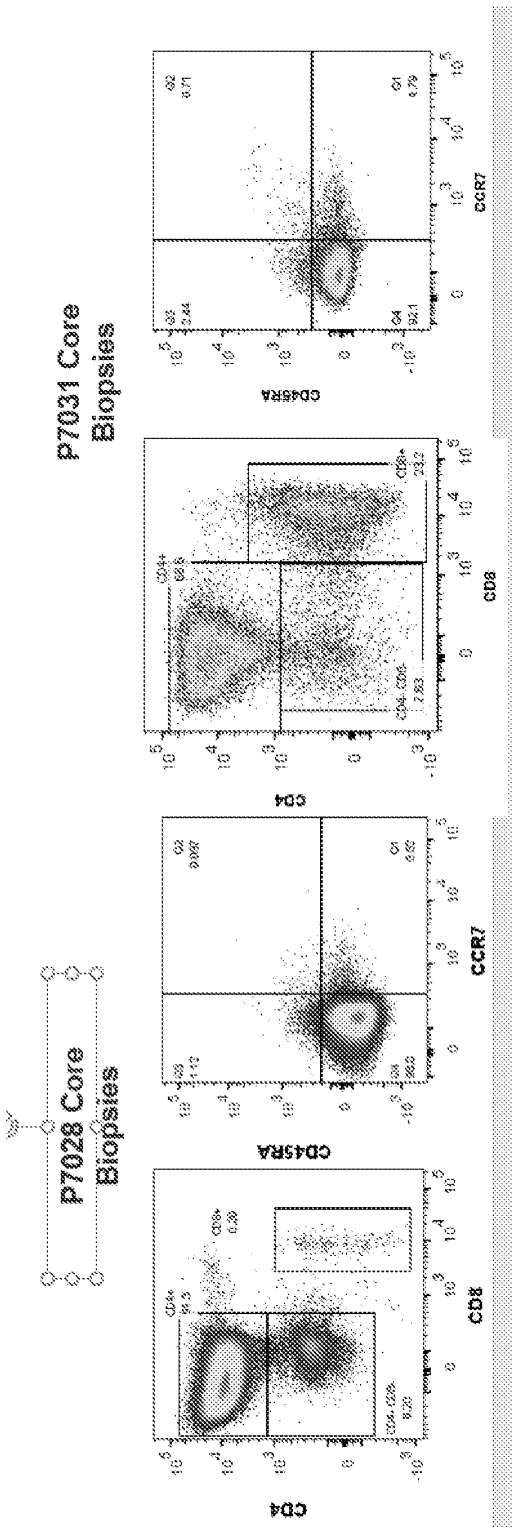

A)
P7028 Core Biopsies
CD4
CD8
CD45RA
CCR7
B)
P7031 Core Biopsies
CD4
CD8
CD45RA
CCR7

Figure 34

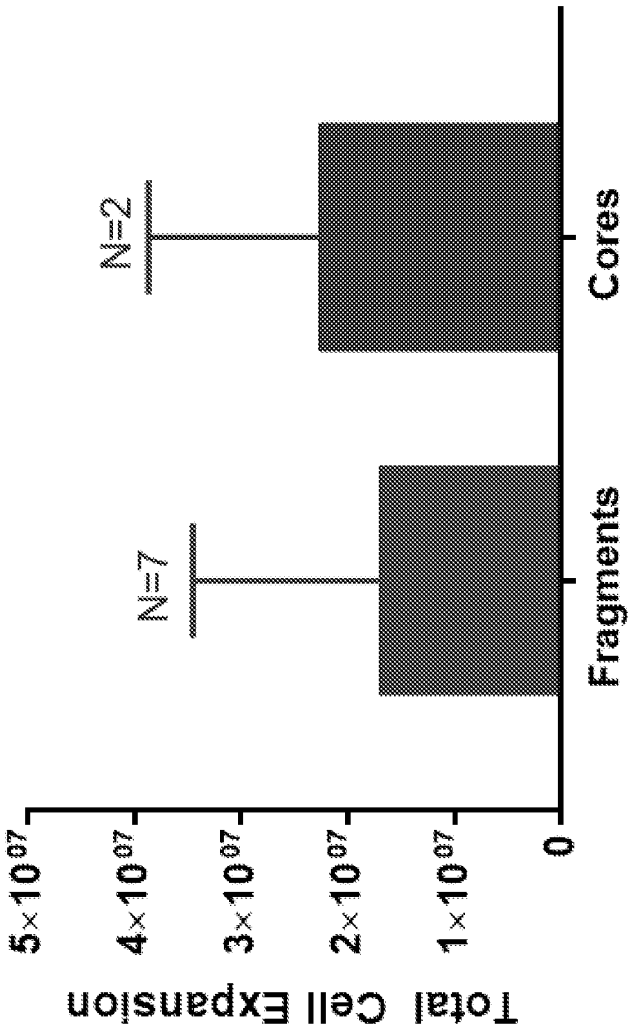

Total Cell Expansion
5×10^07
4×10^07
3×10^07
2×10^07
1×10^07
0
N=7
N=2
Fragments
Cores

Figure 35

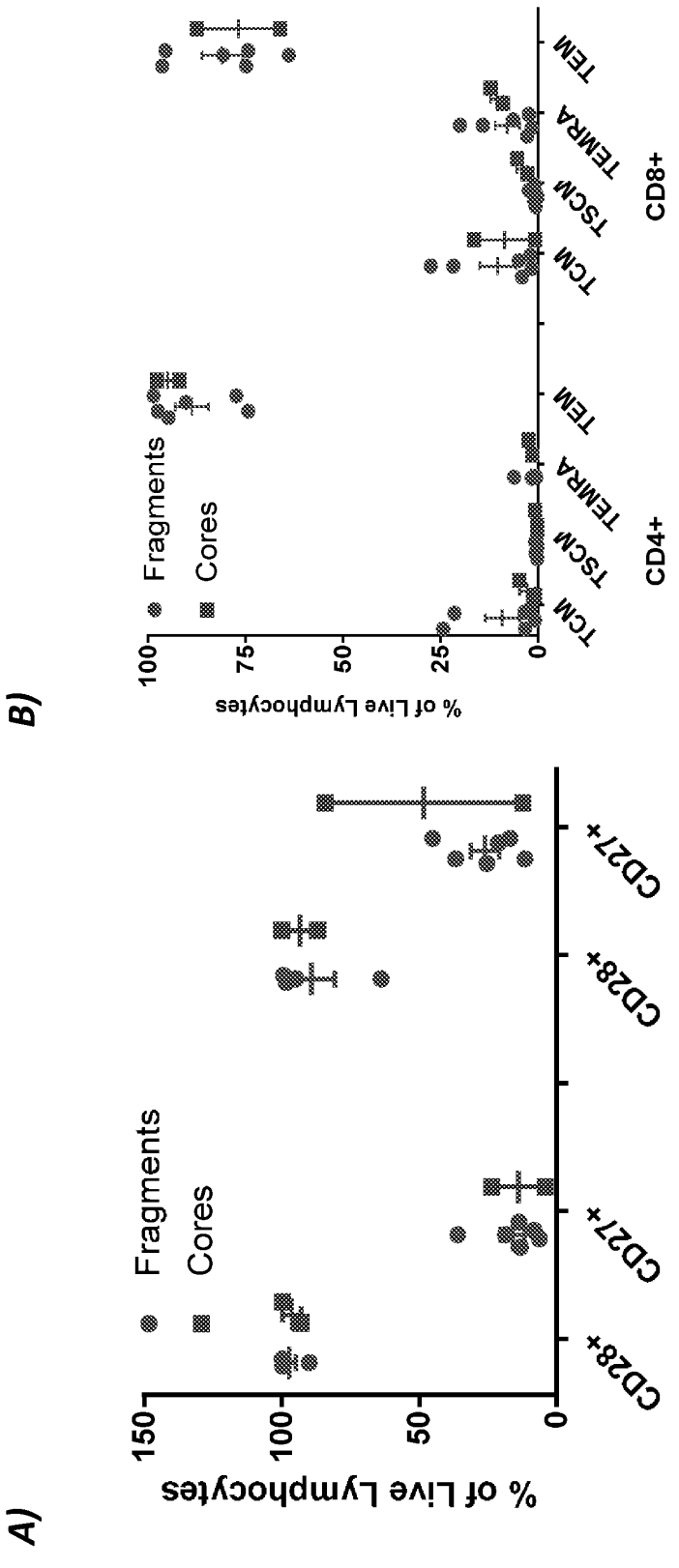

A)
% of Live Lymphocytes
150
100
50
0
Fragments
Cores
CD28+
CD27+
CD28+
CD27+
B)
% of Live Lymphocytes
100
75
50
25
0
Fragments
Cores
TCM
TSCM
TEMRA
TEM
CD4+
TCM
TSCM
TEMRA
TEM
CD8+

Figure 36

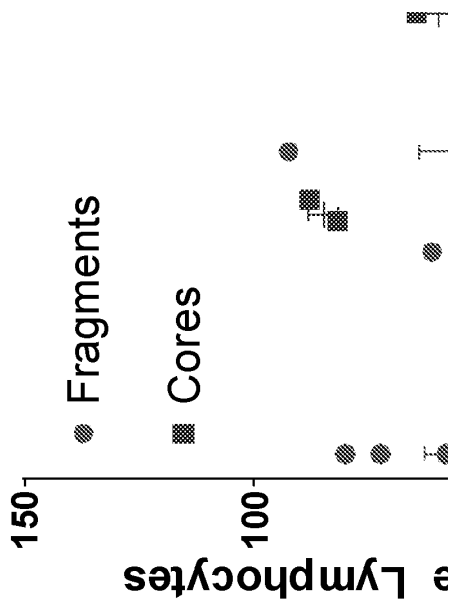


Figure 37

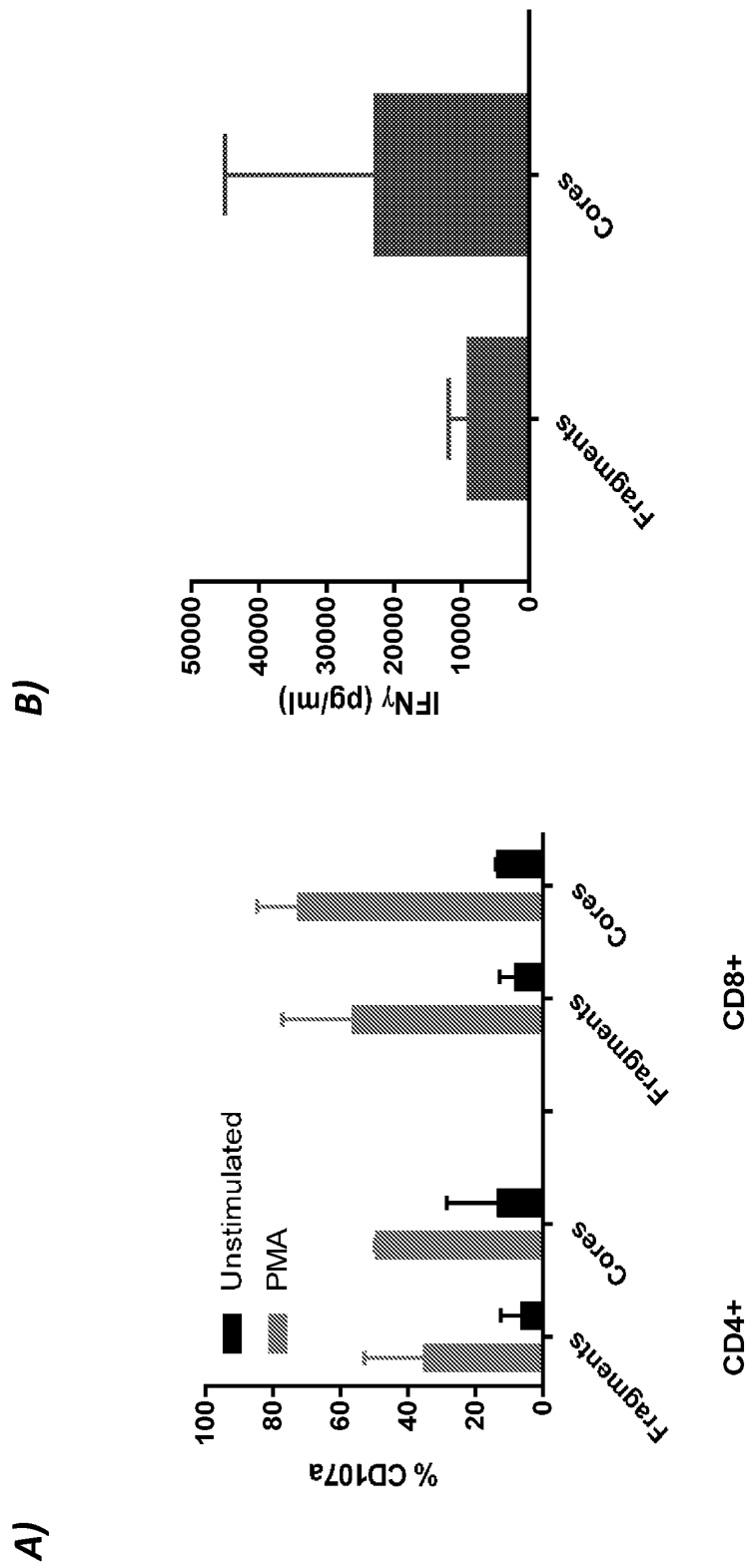
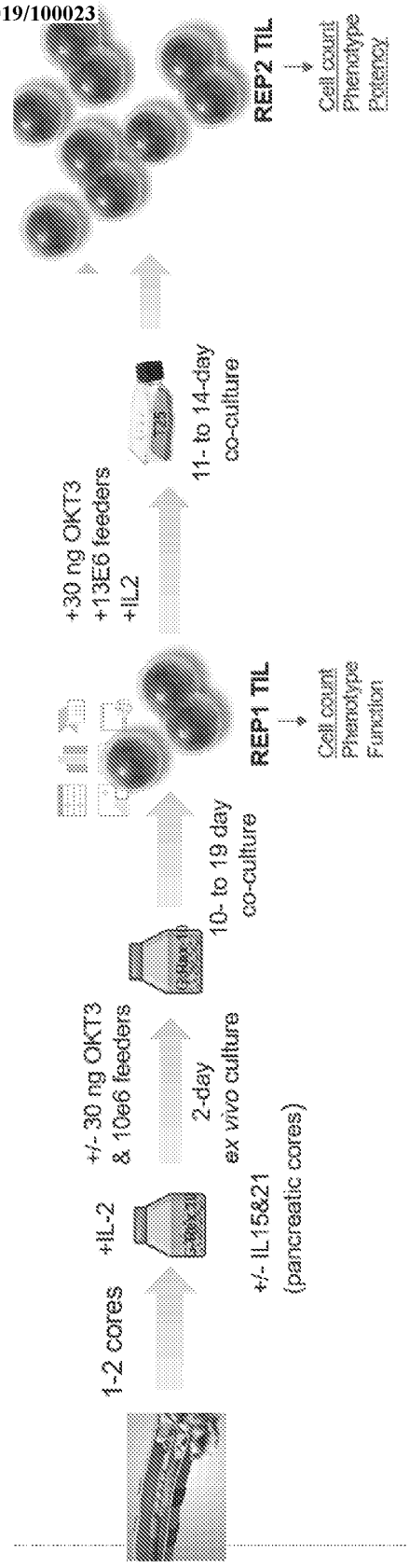

A)
% CD107a
100
80
60
40
20
0
Unstimulated
PMA
Fragments
Cores
Fragments
Cores
CD4+
CD8+
B)
IFNγ (pg/ml)
50000
40000
30000
20000
10000
0
Fragments
Cores

Figure 38A


1-2 cores
+IL-2
+/- 30 ng OKT3
& 10e6 feeders
2-day
ex vivo culture
+/- IL15&21
(pancreatic cores)
10- to 19 day
co-culture
REP1 TIL
Cell count
Phenotype
Function
+30 ng OKT3
+13E6 feeders
+IL2
11- to 14-day
co-culture
REP2 TIL
Cell count
Phenotype
Potency

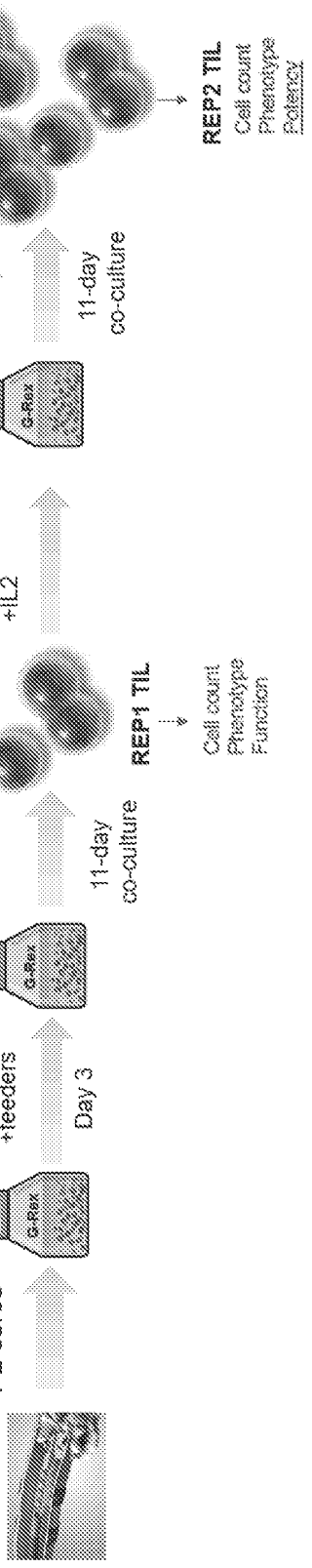
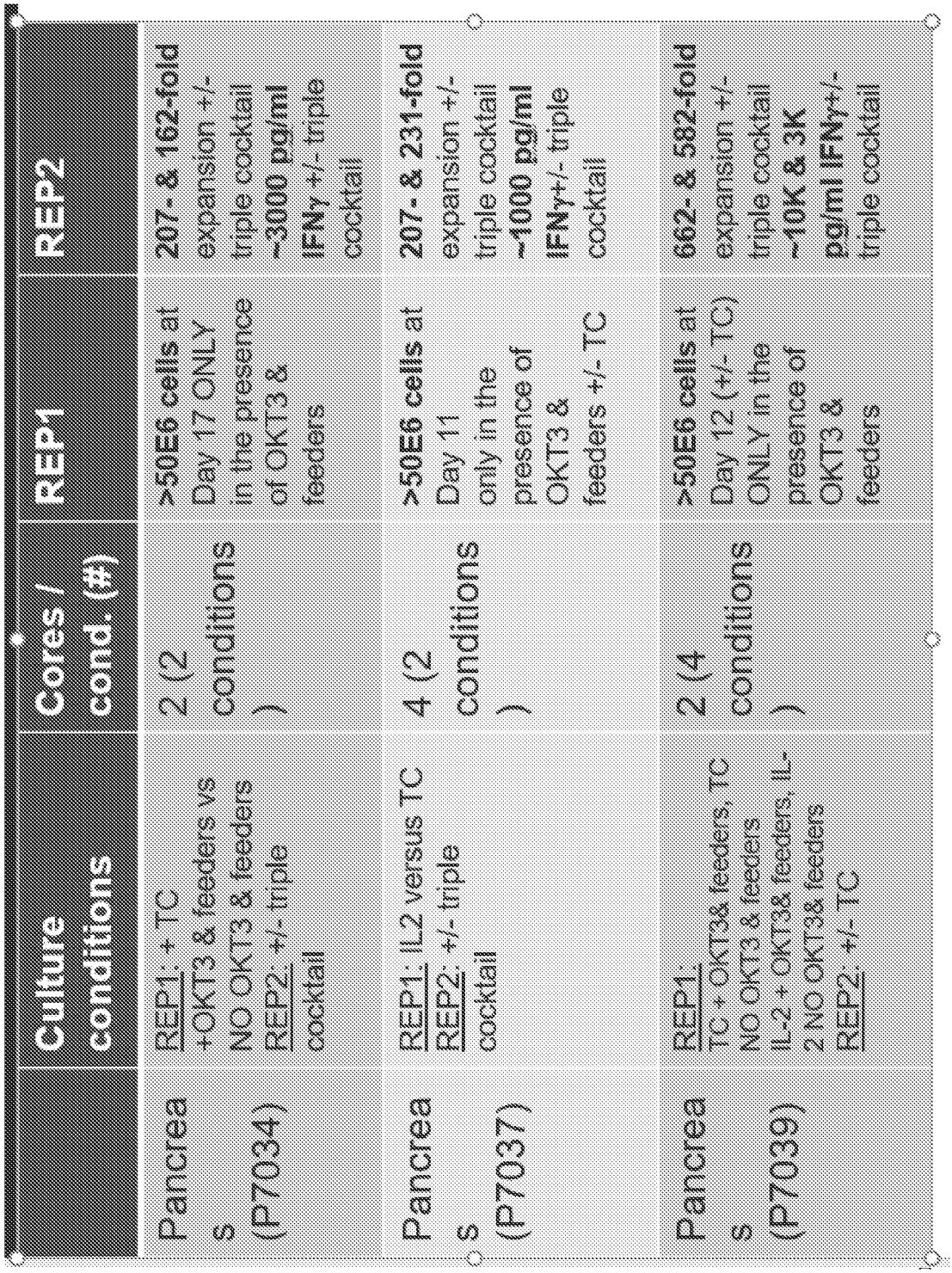

1-2 cores
+IL-2
+OKT3
+feeders
Day 3
11-day
co-culture
REP1 TIL
Cell count
Phenotype
Function
+ OKT3
+ feeders
+IL2
11-day
co-culture
REP2 TIL
Cell count
Phenotype
Potency

Figure 38B

Figure 39



	Culture conditions	Cores / cond. (#)	REP1	REP2
Pancreas (P7034)	REP1: + TC +OKT3 & feeders vs NO OKT3 & feeders REP2: +/- triple cocktail	2 (2 conditions)	**>50E6 cells** at Day 17 ONLY in the presence of OKT3 & feeders	**207- & 162-fold** expansion +/- triple cocktail **~3000 pg/ml IFNγ** +/- triple cocktail
Pancreas (P7037)	REP1: IL2 versus TC REP2: +/- triple cocktail	4 (2 conditions)	**>50E6 cells** at Day 11 only in the presence of OKT3 & feeders +/- TC	**207- & 231-fold** expansion +/- triple cocktail **~1000 pg/ml IFNγ**+/- triple cocktail
Pancreas (P7039)	REP1: TC + OKT3& feeders, TC NO OKT3 & feeders IL-2 + OKT3& feeders, IL-2 NO OKT3& feeders REP2: +/- TC	2 (4 conditions)	**>50E6 cells** at Day 12 (+/- TC) ONLY in the presence of OKT3 & feeders	**662- & 582-fold** expansion +/- triple cocktail **~10K & 3K pg/ml IFNγ**+/- triple cocktail

Figure 40

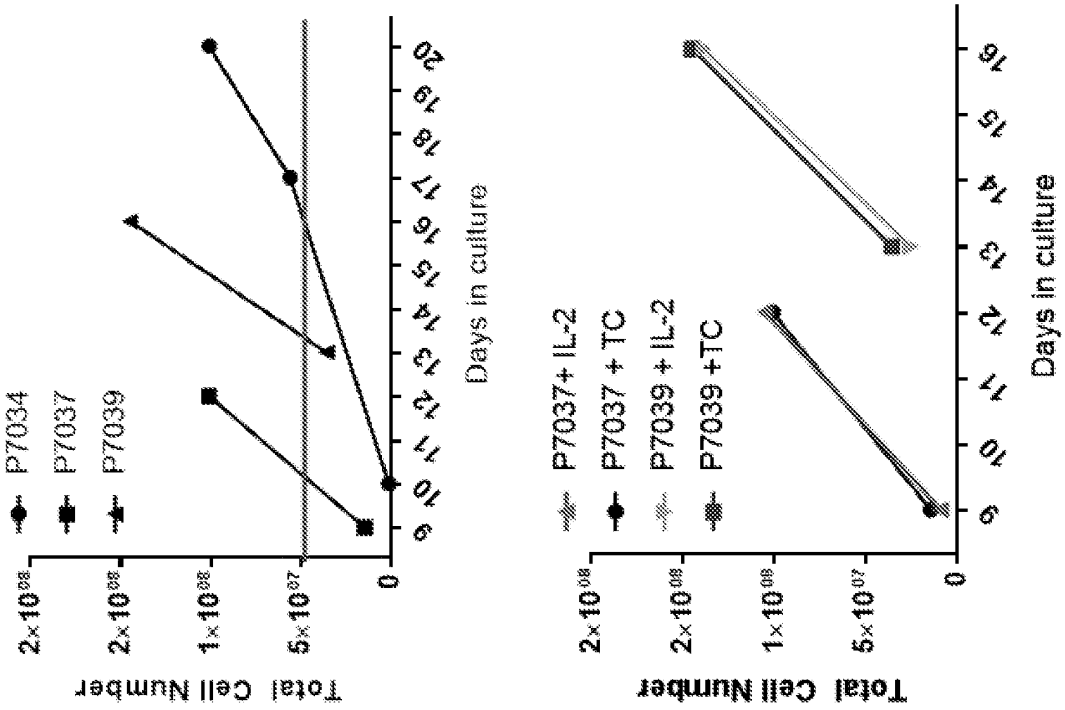

P7034
P7037
P7039
Total Cell Number
2×10^08
2×10^08
1×10^08
5×10^07
0
6
10
11
12
13
14
15
16
17
18
19
20
Days in culture
P7037+ IL-2
P7037 + TC
P7039 + IL-2
P7039 +TC
Total Cell Number
2×10^08
2×10^08
1×10^08
5×10^07
0
6
10
11
12
13
14
15
16
Days in culture

Figure 41

	Culture conditions	Cores / cond. (#)	REP1	REP2
Melanoma (M1114)	REP1: IL2 REP2: IL-2	2	**>50E6 cells** at Day 11	**215-fold** expansion **~1000 pg/ml IFNγ**
Melanoma (M1115)	REP1: IL2 REP2: IL-2	2	**>50E6 cells** at Day 12	**662-fold** expansion **~3000 pg/ml IFNγ**
Melanoma (M1118)	REP1: IL-2 REP2: IL-2	1	**>50E6 cells** at Day 11	**215-fold** expansion **~10,000 pg/ml IFNγ**
** Melanoma (M1121)	REP1: IL2 REP2: IL-2	1	**50E6 cells** at Day 9	**155-fold** expansion **~2000 pg/ml IFNγ**

Figure 42

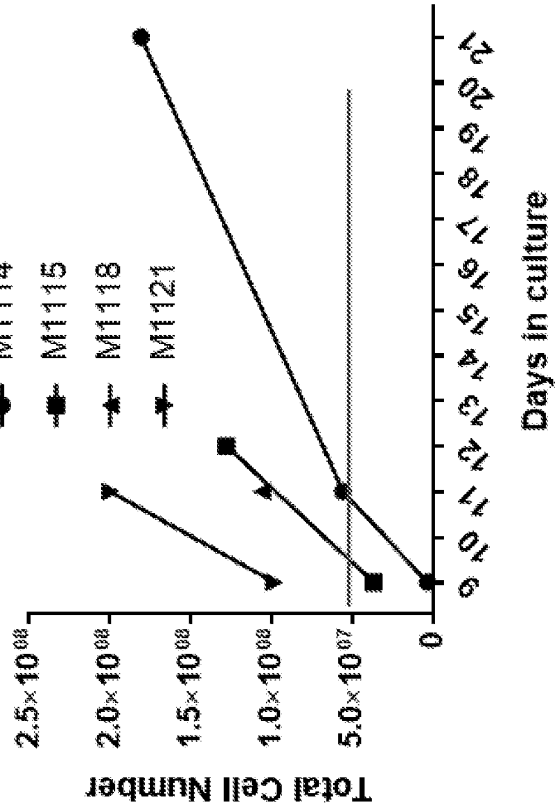

M1114
M1115
M1118
M1121
Total Cell Number
2.5×10^08
2.0×10^08
1.5×10^08
1.0×10^08
5.0×10^07
0
6
10
11
12
13
14
15
16
17
18
19
20
21
Days in culture

Figure 43

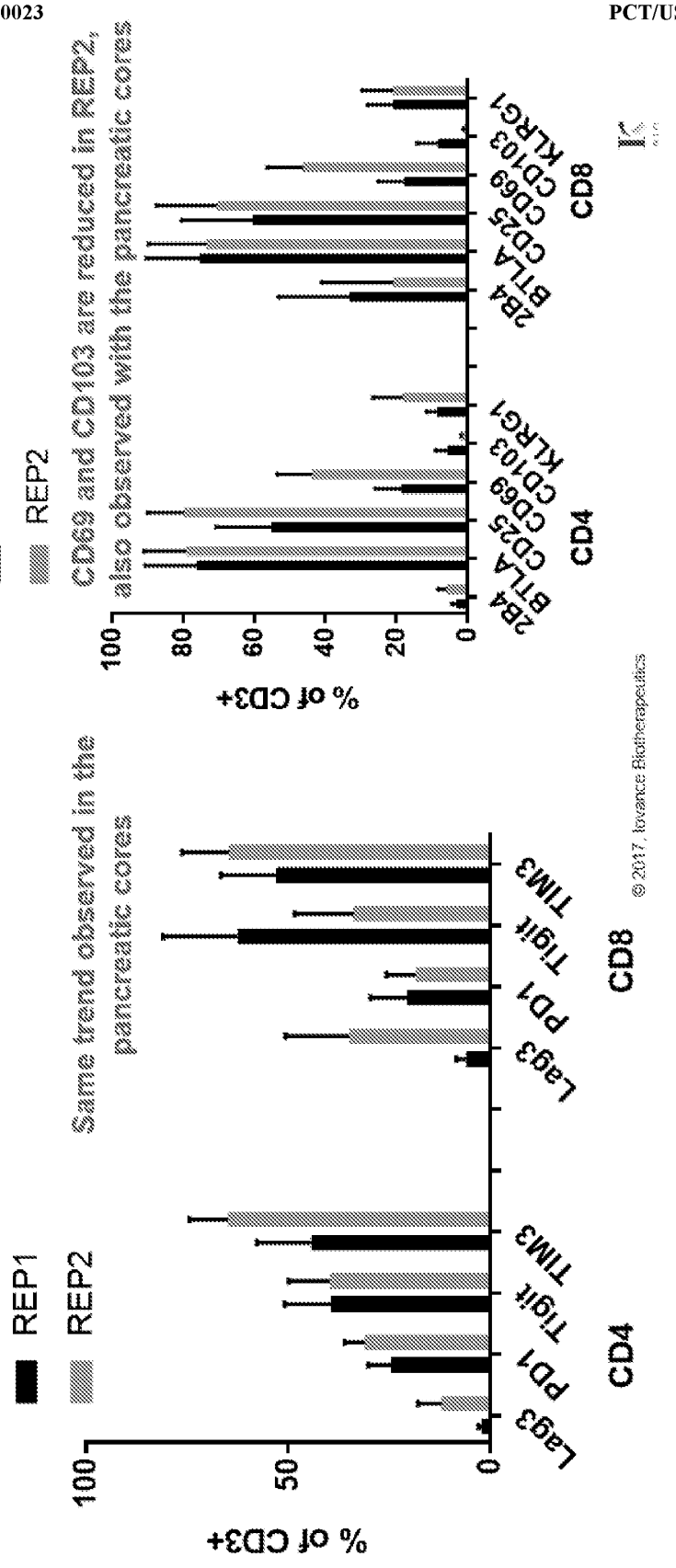

REP1
REP2
Same trend observed in the pancreatic cores
% of CD3+
100
50
0
Lag3
PD1
Tigit
TIM3
CD4
CD8
© 2017, Iovance Biotherapeutics
REP1
REP2
CD69 and CD103 are reduced in REP2, also observed with the pancreatic cores
% of CD3+
100
80
60
40
20
0
2B4
BTLA
CD25
CD69
CD103
KLRG1
CD4
CD8

Figure 44

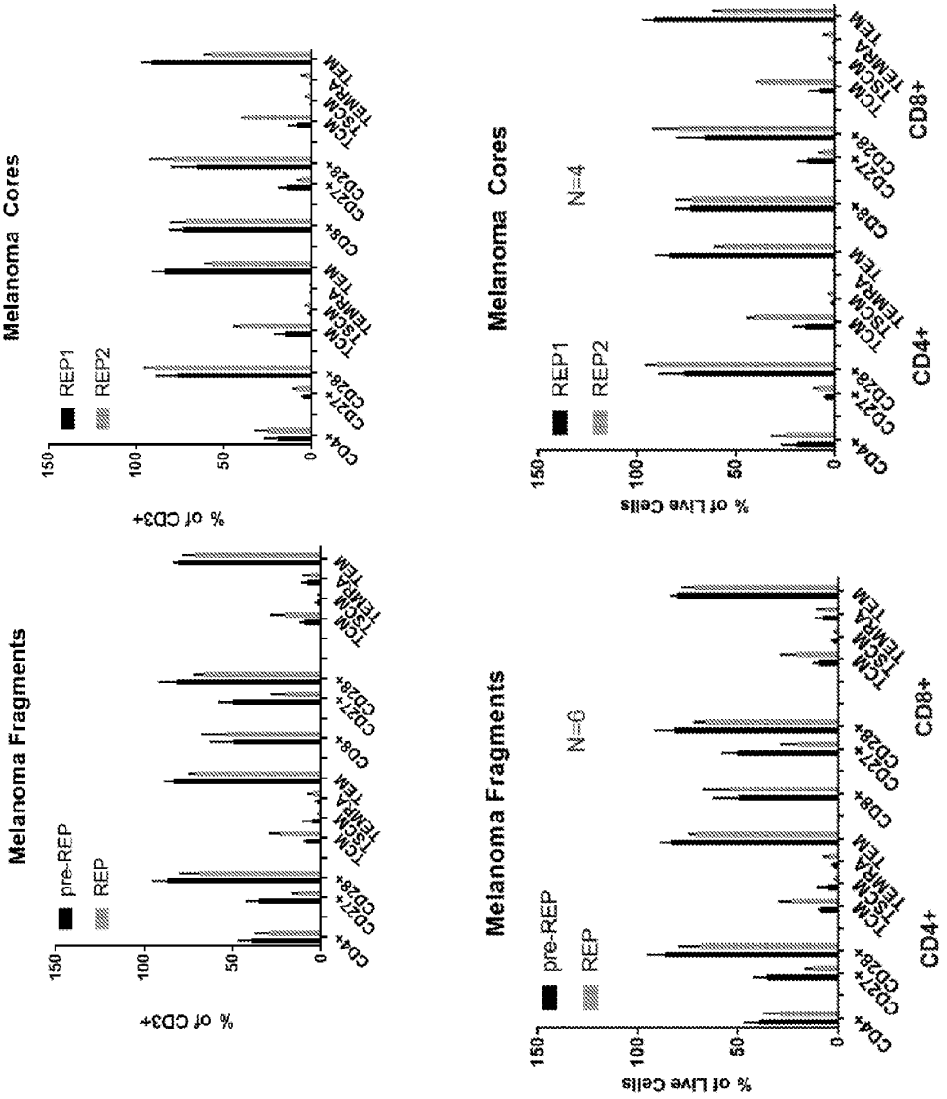

Melanoma Fragments
Melanoma Cores
pre-REP
REP
REP1
REP2
N=6
N=4
% of CD3+
% of Live Cells
CD4+
CD8+
CD4+
CD27+ CD28+
CD8+
TCM
TSCM
TEMRA
TEM

Figure 45

	Culture conditions	Cores / cond. (#)	REP1	REP2
Breast (EP11032)	REP1: IL2 REP2: IL-2	1 vs 2	>50E6 cells at Day 11	173 and 158-fold (1/2 cores) expansion ~ pg/ml IFNg
Ovarian (OV8016)	REP1: IL2 +/- OX40 REP2: IL-2 +/- OX40 (in progress)	2	>50E6 cells at Day 11 (OX40 condition)	In progress

Figure 46

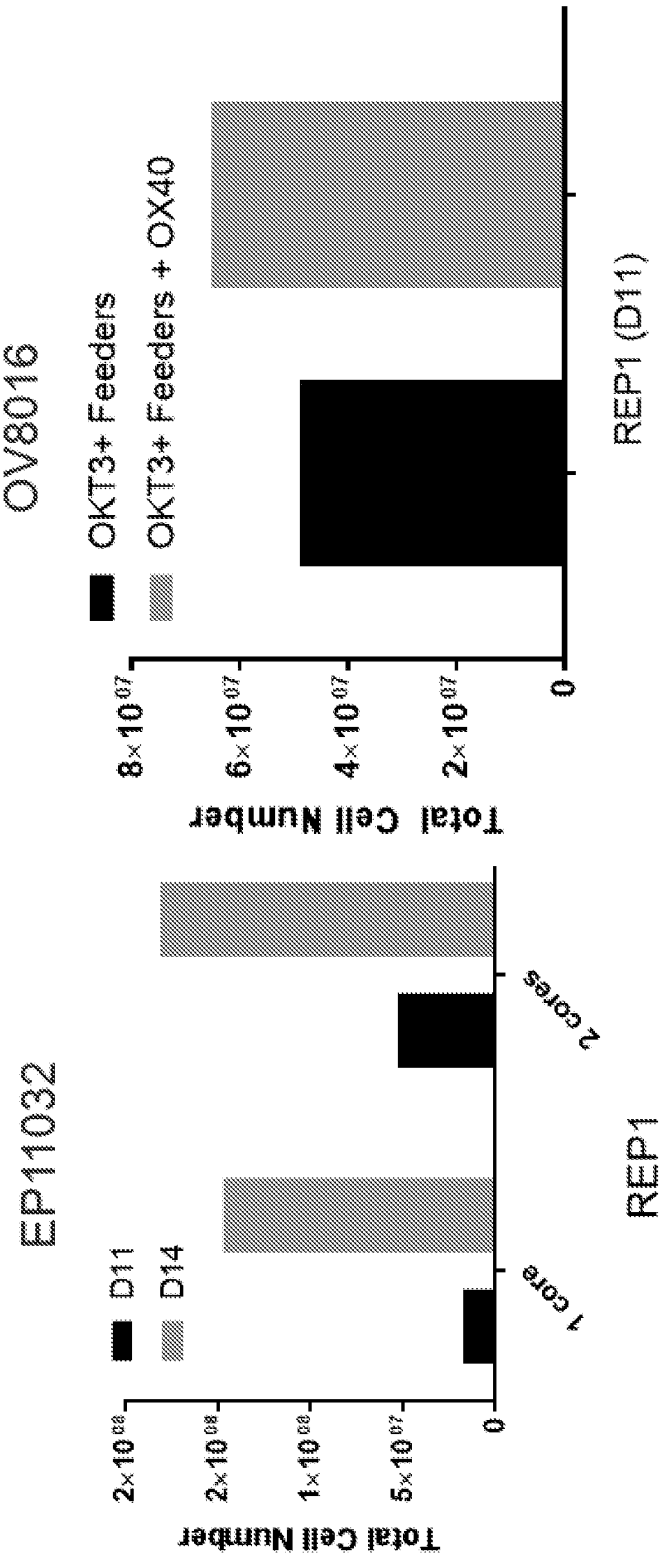
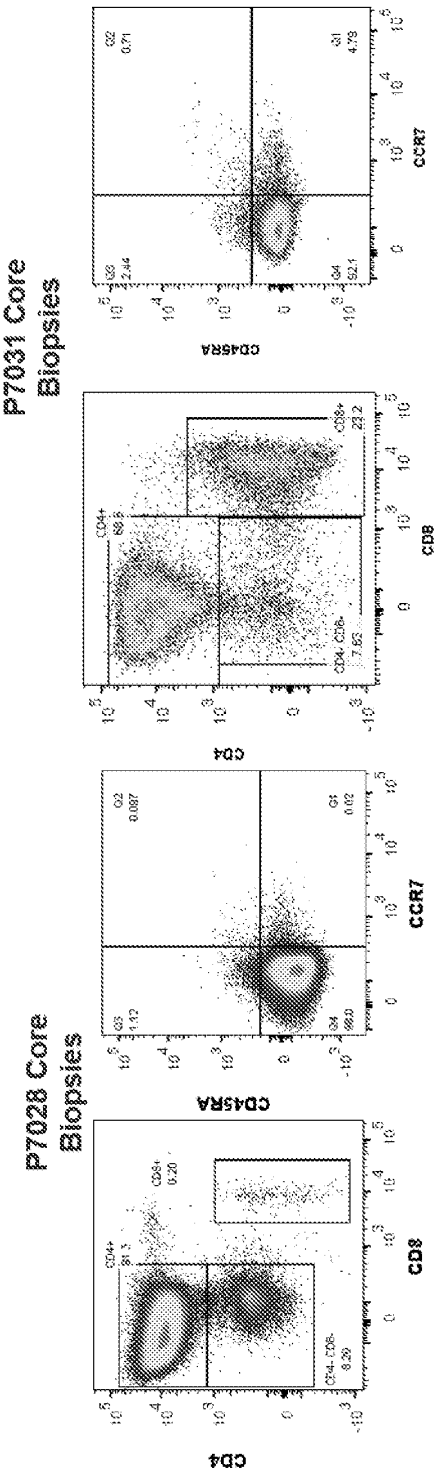

OV8016
OKT3+ Feeders
OKT3+ Feeders + OX40
Total Cell Number
8×10^07
6×10^07
4×10^07
2×10^07
0
REP1 (D11)
EP11032
D11
D14
Total Cell Number
2×10^08
2×10^08
1×10^08
5×10^07
0
1 core
2 cores
REP1

Figure 47


P7028 Core Biopsies
P7031 Core Biopsies
CD4
CD8
CD45RA
CCR7
CD4+
CD8+
CD4+ CD8-
CD4- CD8-

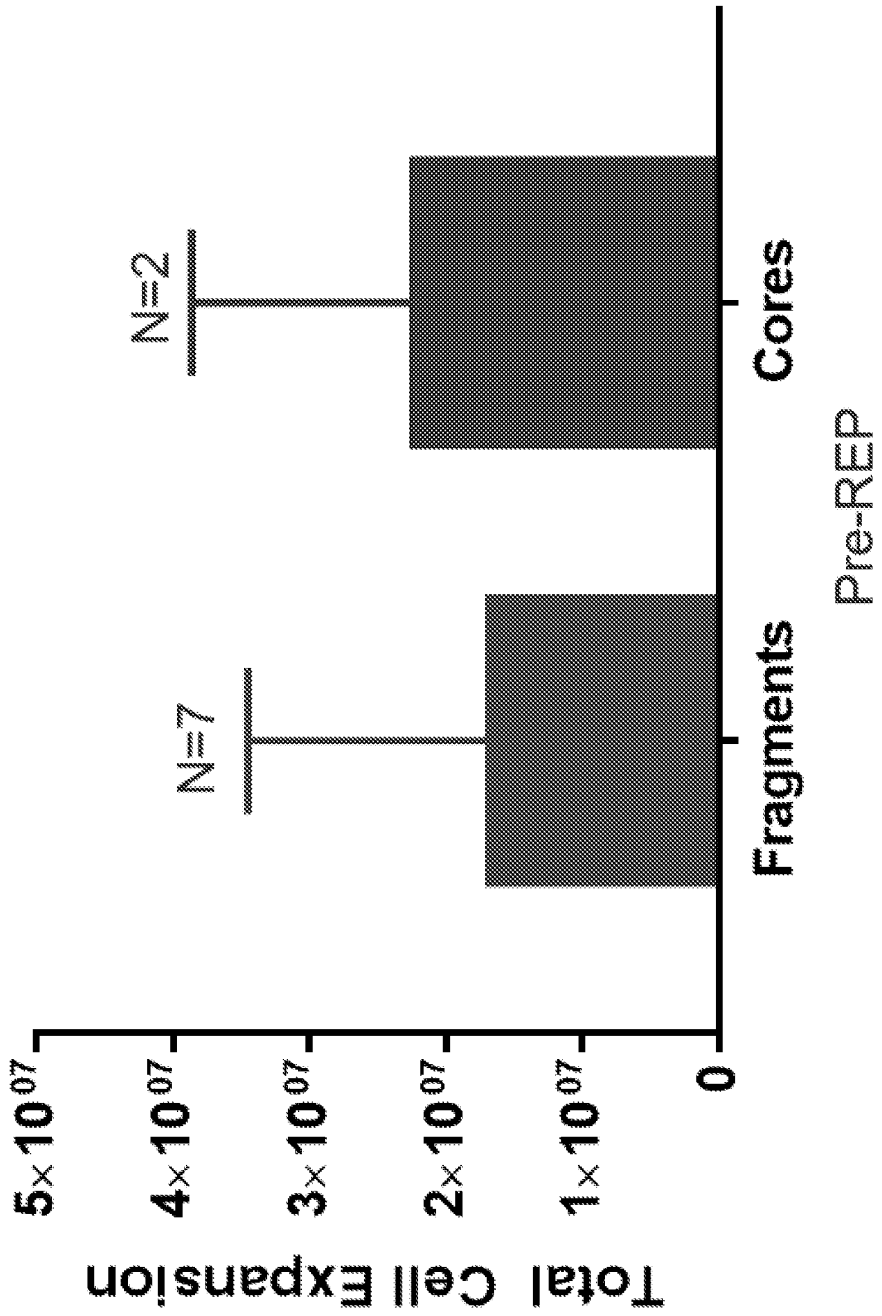

Total Cell Expansion
5×10^07
4×10^07
3×10^07
2×10^07
1×10^07
0
N=7
N=2
Fragments
Cores
Pre-REP

Figure 48

Figure 49

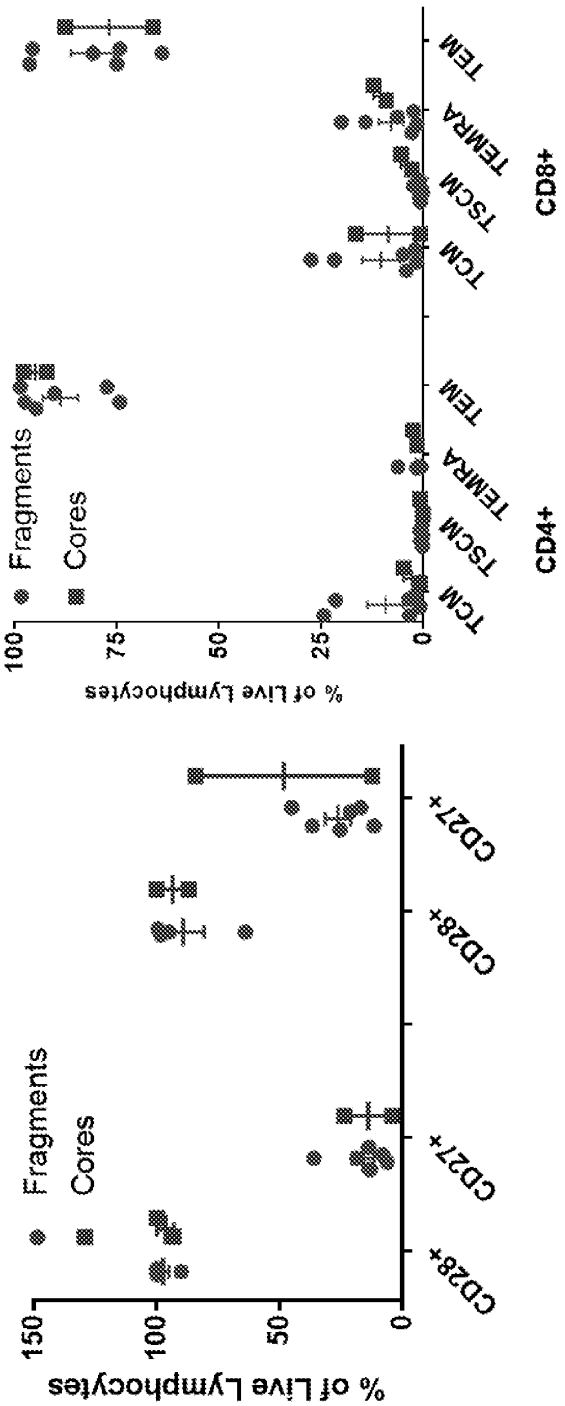

% of Live Lymphocytes
150
100
50
0
Fragments
Cores
CD28+
CD27+
CD28+
CD27+
% of Live Lymphocytes
100
75
50
25
0
Fragments
Cores
TCM
TSCM
TEMRA
TEM
CD4+
TCM
TSCM
TEMRA
TEM
CD8+

Figure 50

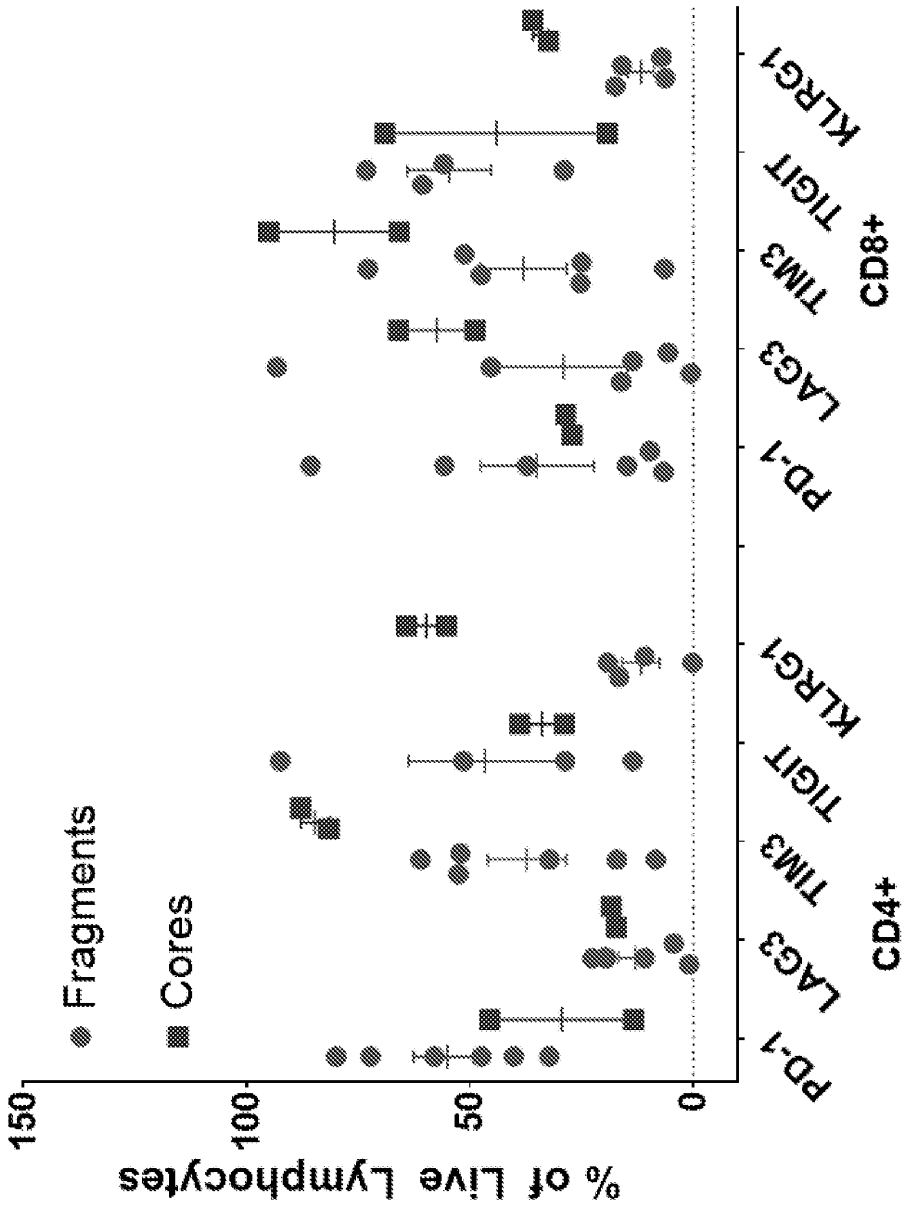

% of Live Lymphocytes
150
100
50
0
Fragments
Cores
PD-1
LAG3
TIM3
TIGIT
KLRG1
CD4+
PD-1
LAG3
TIM3
TIGIT
KLRG1
CD8+

Figure 51

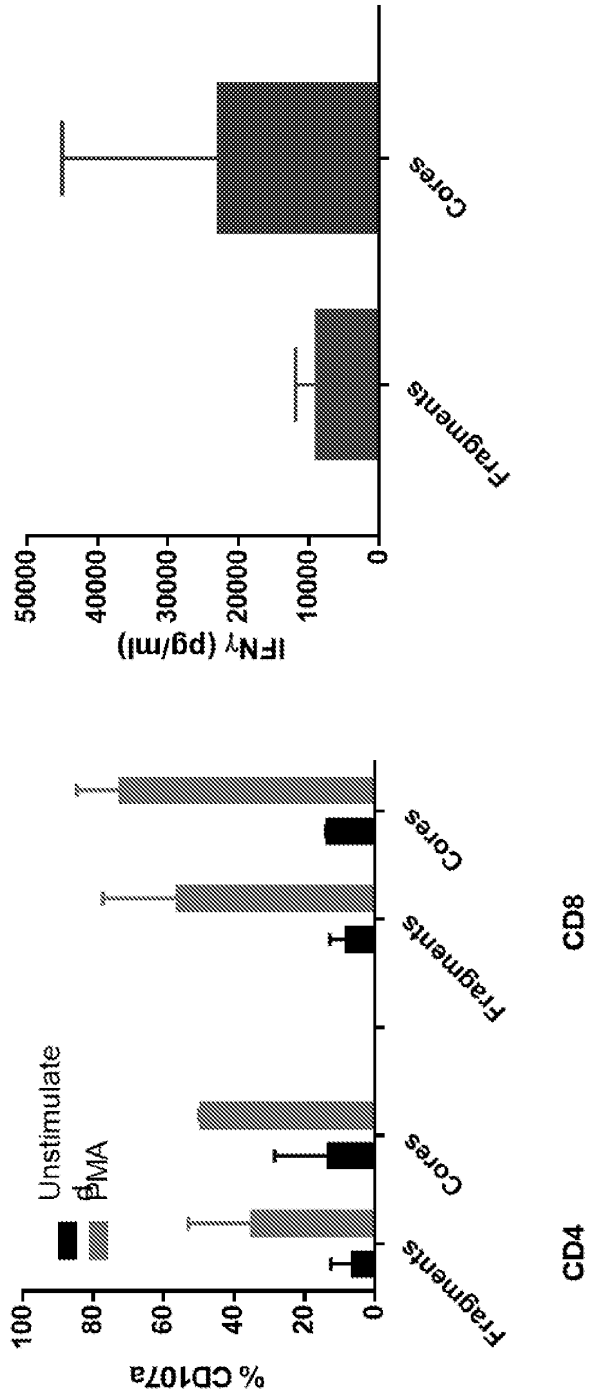

IFNγ (pg/ml)
50000
40000
30000
20000
10000
0
Fragments
Cores
% CD107a
100
80
60
40
20
0
Unstimulate
PMA
Fragments
Cores
Fragments
Cores
CD4
CD8

Figure 52

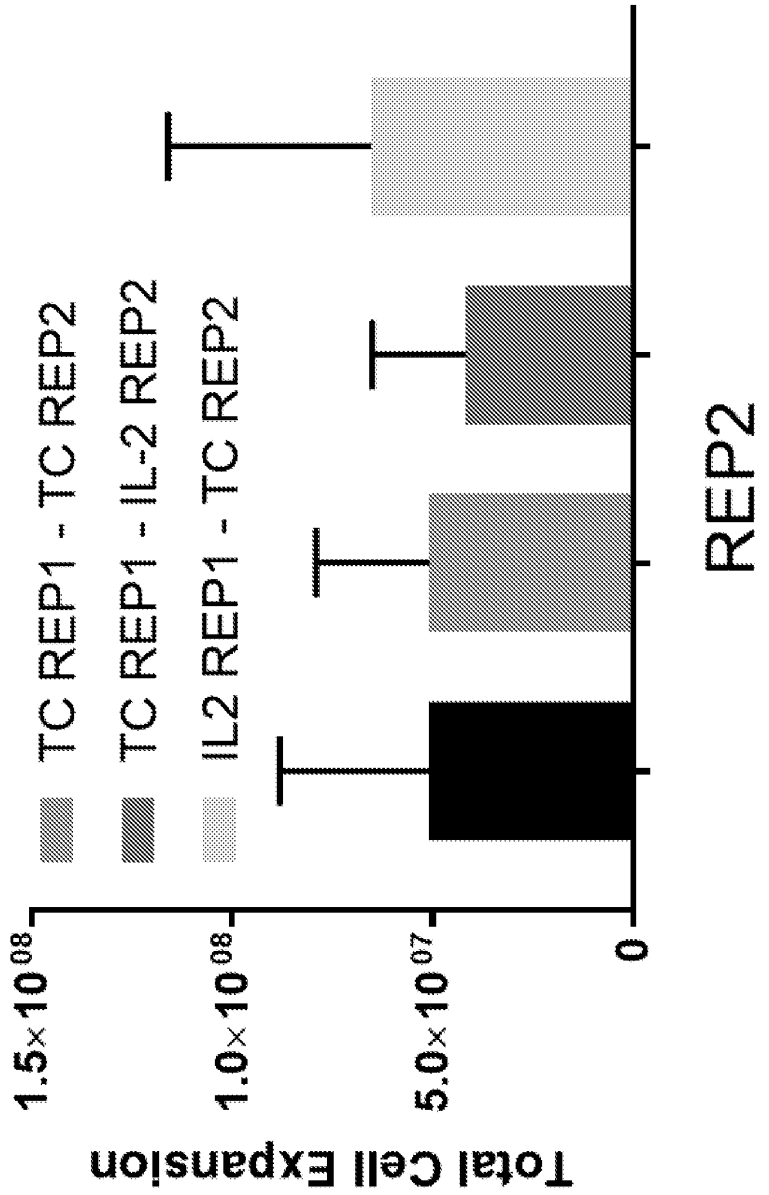

IL-2 REP1 - IL2 REP2
TC REP1 - TC REP2
TC REP1 - IL-2 REP2
IL2 REP1 - TC REP2
Total Cell Expansion
1.5×10^08
1.0×10^08
5.0×10^07
0
REP2

Figure 53

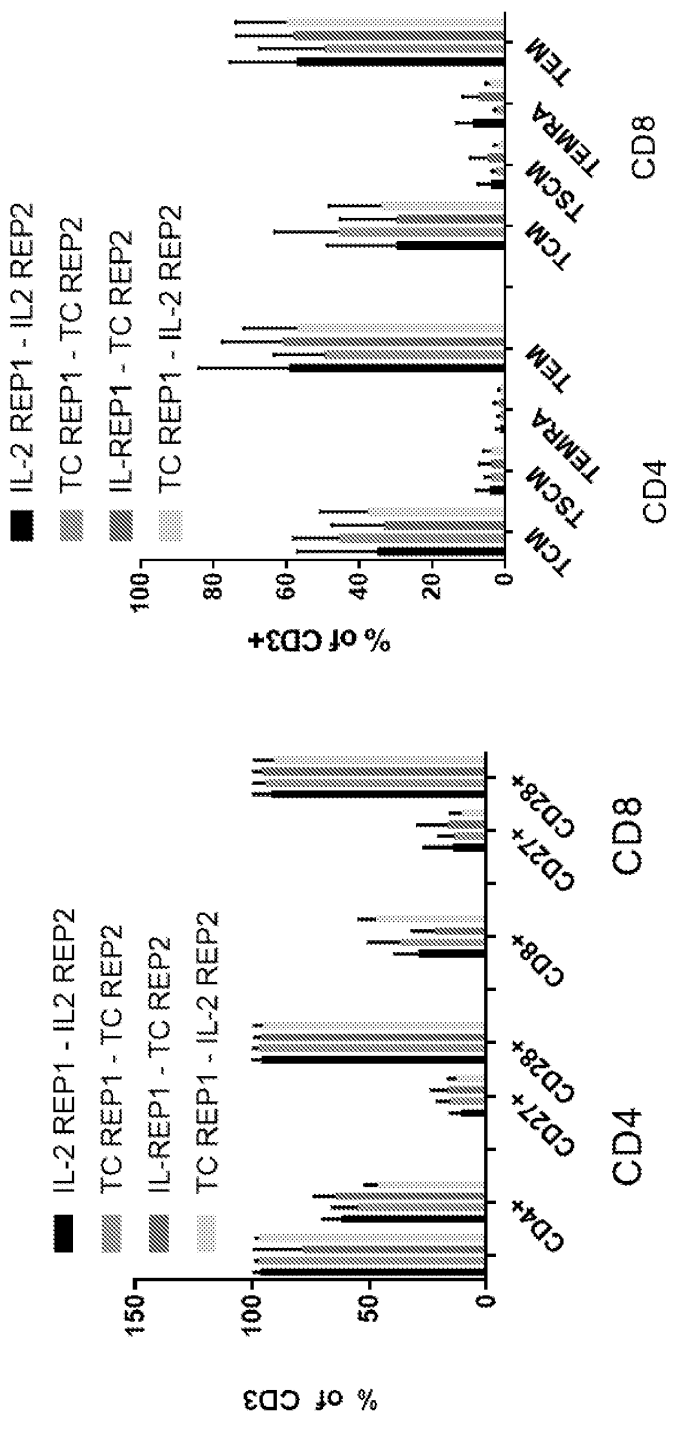

IL-2 REP1 - IL2 REP2
TC REP1 - TC REP2
IL-REP1 - TC REP2
TC REP1 - IL-2 REP2
% of CD3
150
100
50
0
CD4+
CD27+ CD28+
CD8+
CD27+ CD28+
CD4
CD8
% of CD3+
100
80
60
40
20
0
TCM
TSCM
TEMRA
TEM
TCM
TSCM
TEMRA
TEM
CD4
CD8

Figure 54

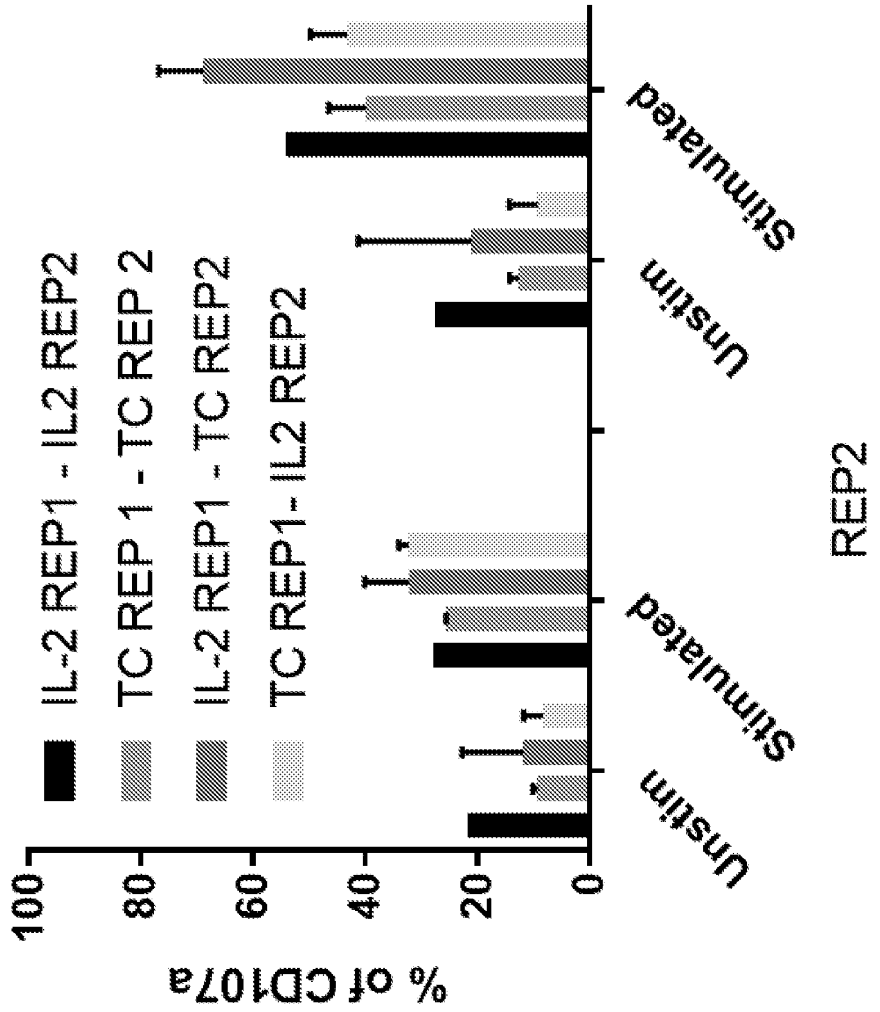

IL-2 REP1 - IL2 REP2
TC REP 1 - TC REP 2
IL-2 REP1 - TC REP2
TC REP1- IL2 REP2
% of CD107a
100
80
60
40
20
0
Unstim
Stimulated
Unstim
Stimulated
REP2

Figure 55

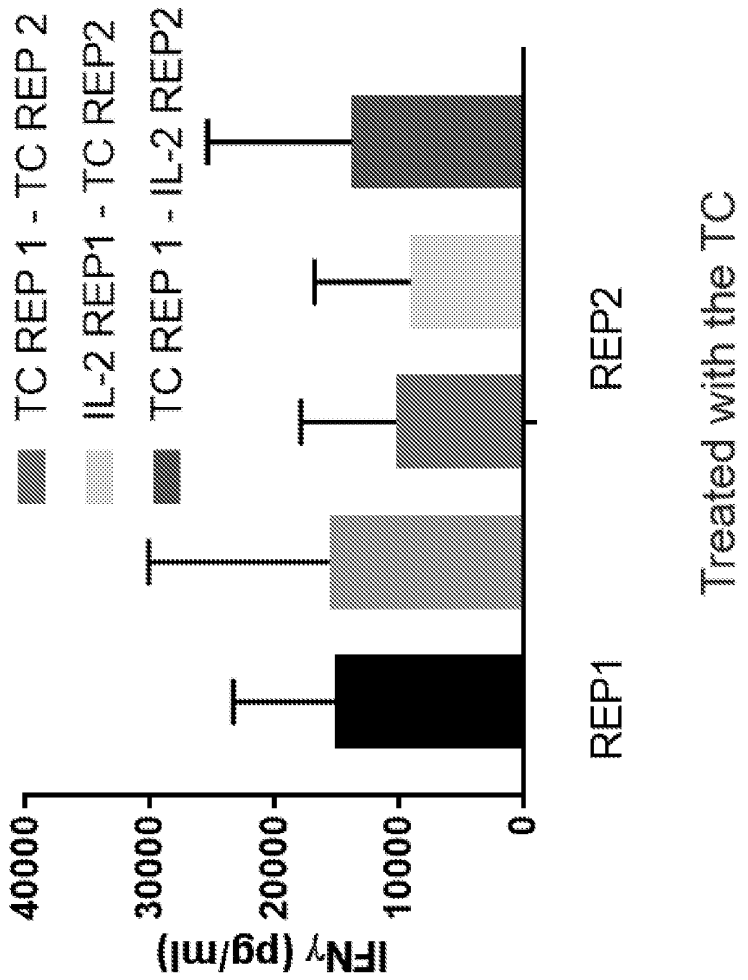

REP1
IL-2 REP1 - IL-REP2
TC REP 1 - TC REP 2
IL-2 REP1 - TC REP2
TC REP 1 - IL-2 REP2
IFNγ (pg/ml)
40000
30000
20000
10000
0
REP1
REP2
Treated with the TC

Figure 56

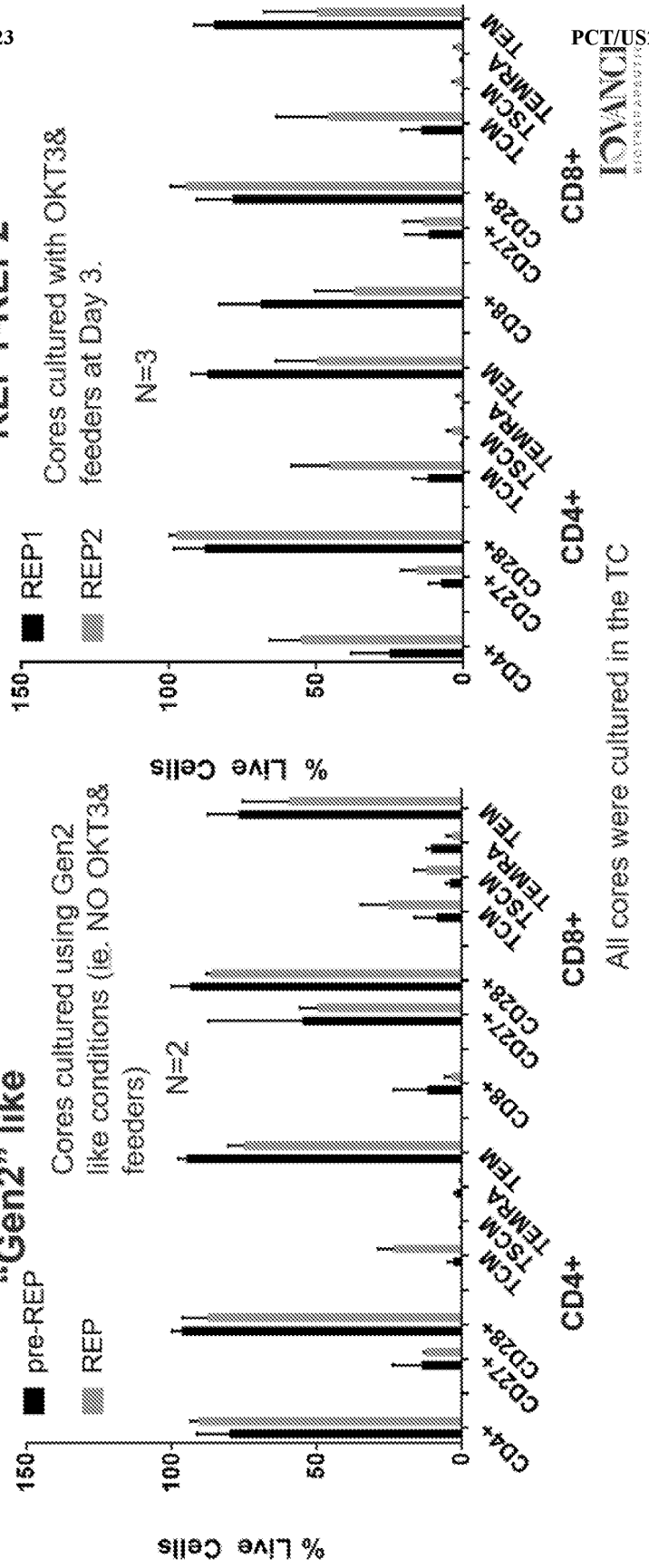
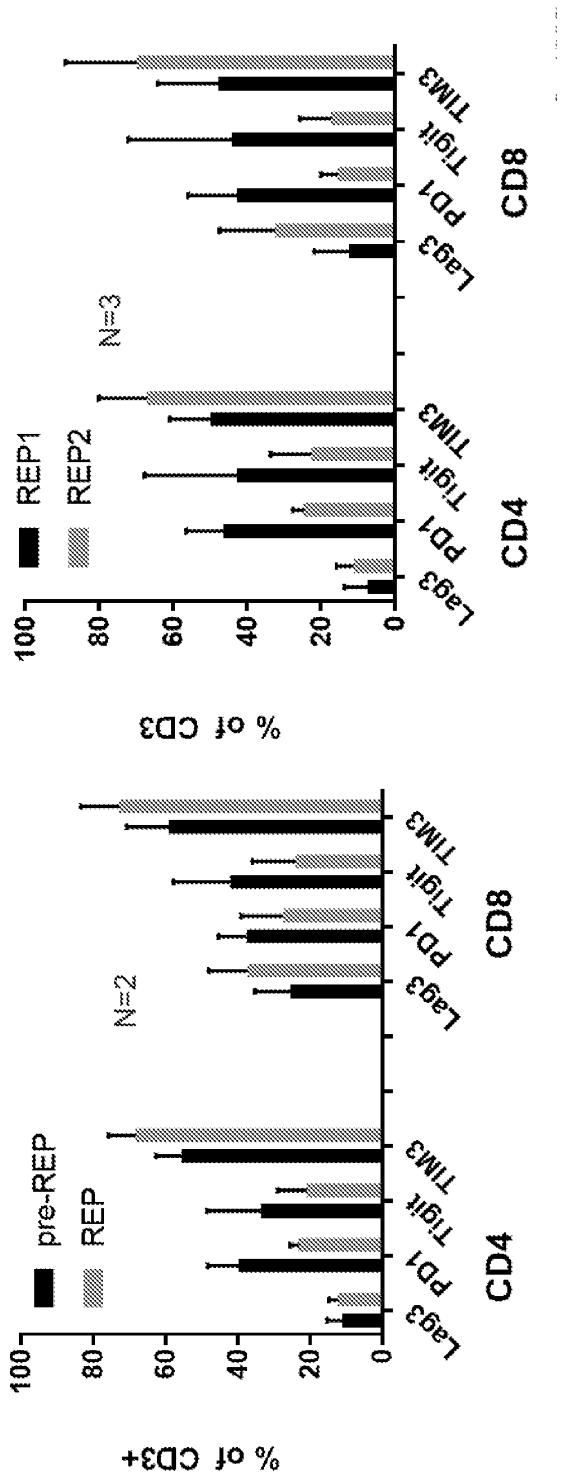
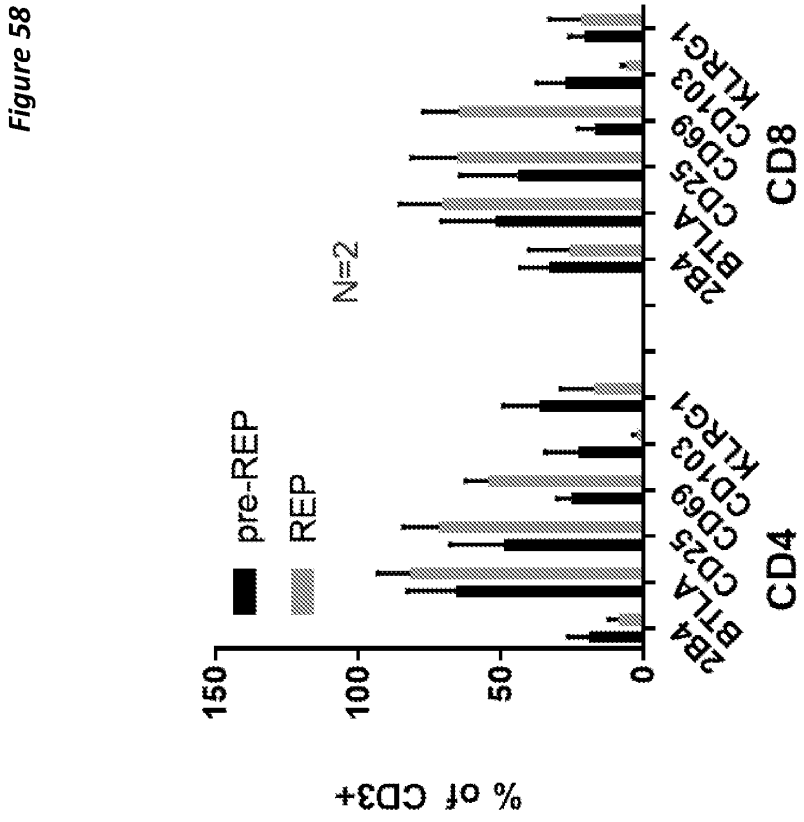

"Gen2" like
pre-REP
REP
Cores cultured using Gen2 like conditions (ie. NO OKT3& feeders)
N=2
% Live Cells
150
100
50
0
CD4+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
CD8+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
REP1-REP2
REP1
REP2
Cores cultured with OKT3& feeders at Day 3.
N=3
All cores were cultured in the TC
IOVANCE

Figure 57


% of CD3+
100
80
60
40
20
0
pre-REP
REP
N=2
Lag3
PD1
Tigit
TIM3
CD4
CD8
% of CD3
REP1
REP2
N=3


% of CD3+
150
100
50
0
pre-REP
REP
N=2
2B4
BTLA
CD25
CD69
CD103
KLRG1
CD4
CD8

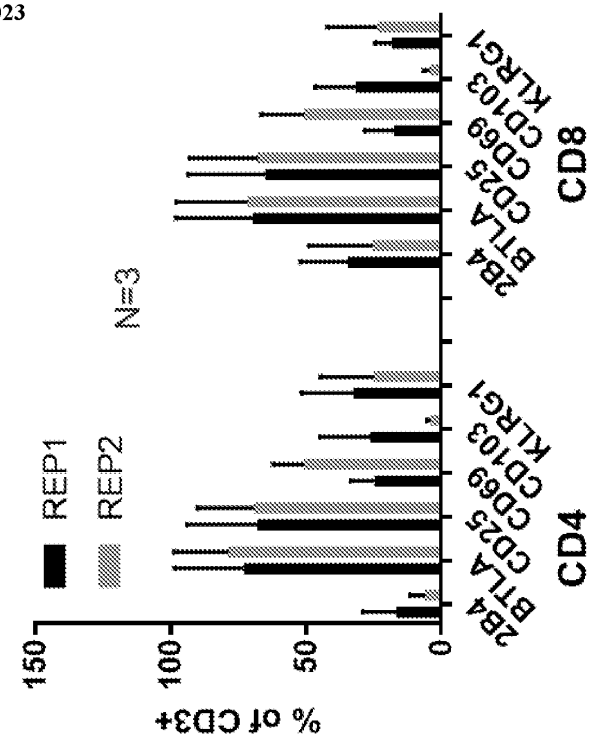

% of CD3+
150
100
50
0
REP1
REP2
N=3
2B4
BTLA
CD25
CD69
CD103
KLRG1
CD4
CD8

Figure 58

Figure 59

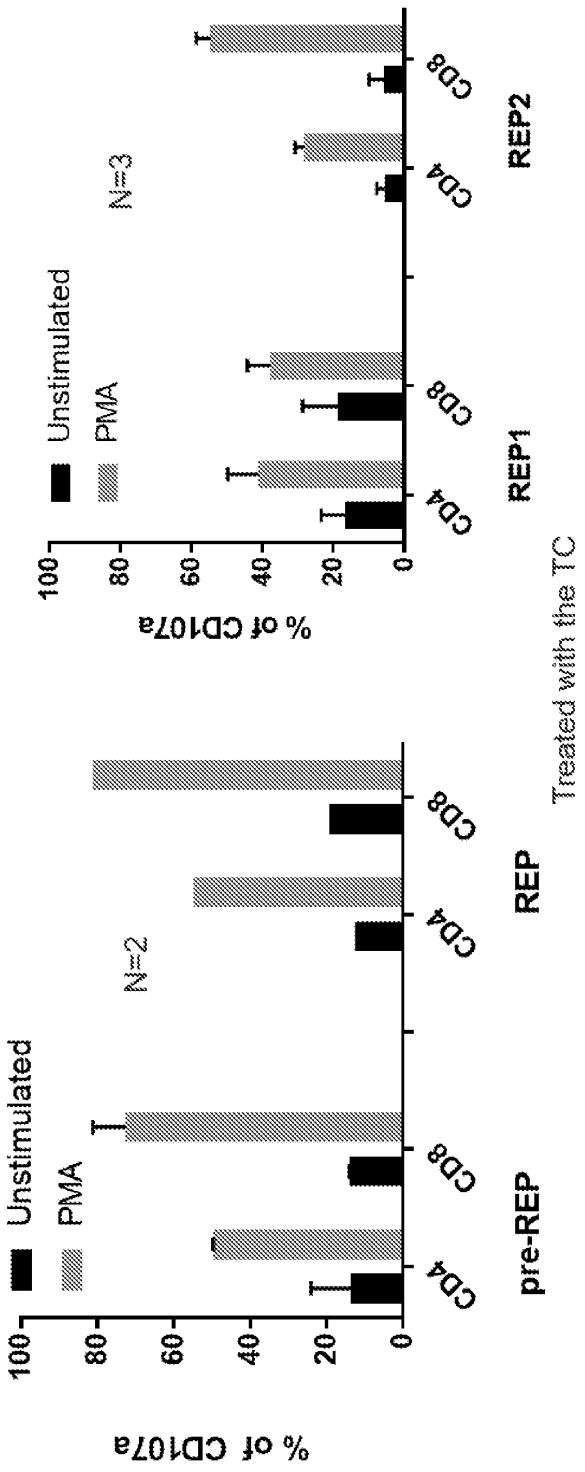

% of CD107a
100
80
60
40
20
0
Unstimulated
PMA
N=2
CD4
CD8
pre-REP
REP
Treated with the TC
N=3
REP1
REP2

Figure 60

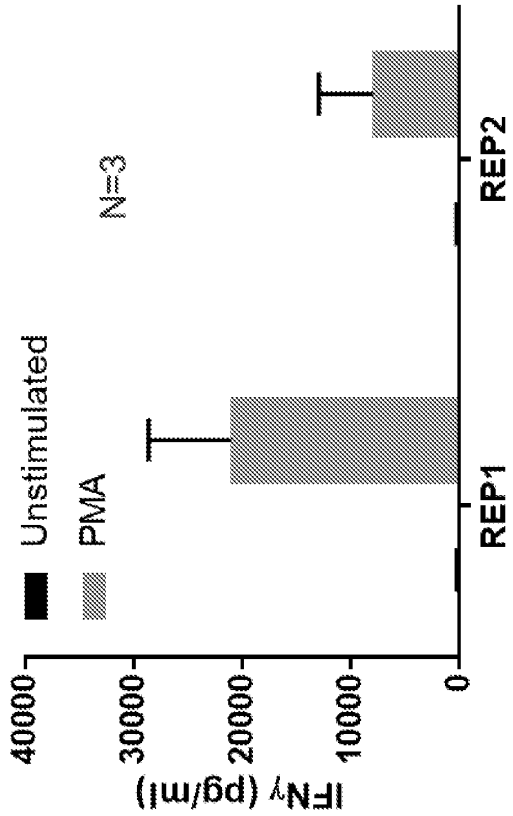

Unstimulated
PMA
N=3
IFNγ (pg/ml)
40000
30000
20000
10000
0
REP1
REP2

Figure 61

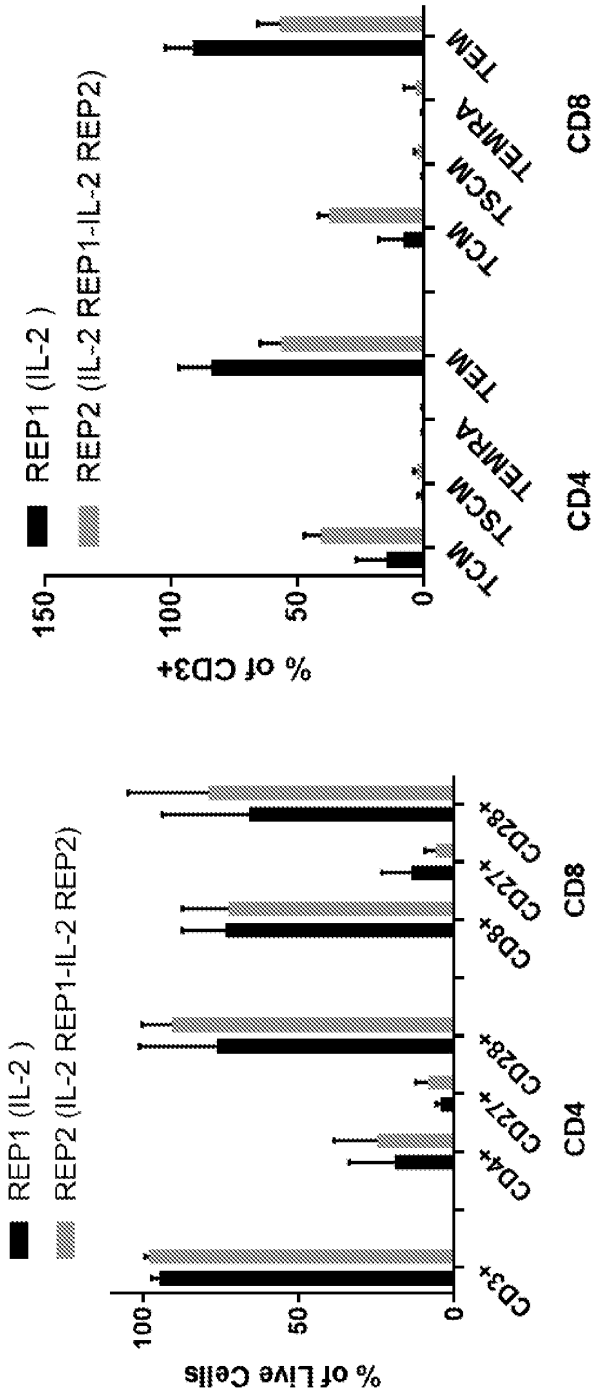

REP1 (IL-2)
REP2 (IL-2 REP1-IL-2 REP2)
% of Live Cells
100
50
0
CD3+
CD4+
CD27+
CD28+
CD8+
CD27+
CD28+
CD4
CD8
% of CD3+
150
100
50
0
TCM
TSCM
TEMRA
TEM
TCM
TSCM
TEMRA
TEM
CD4
CD8

Figure 62

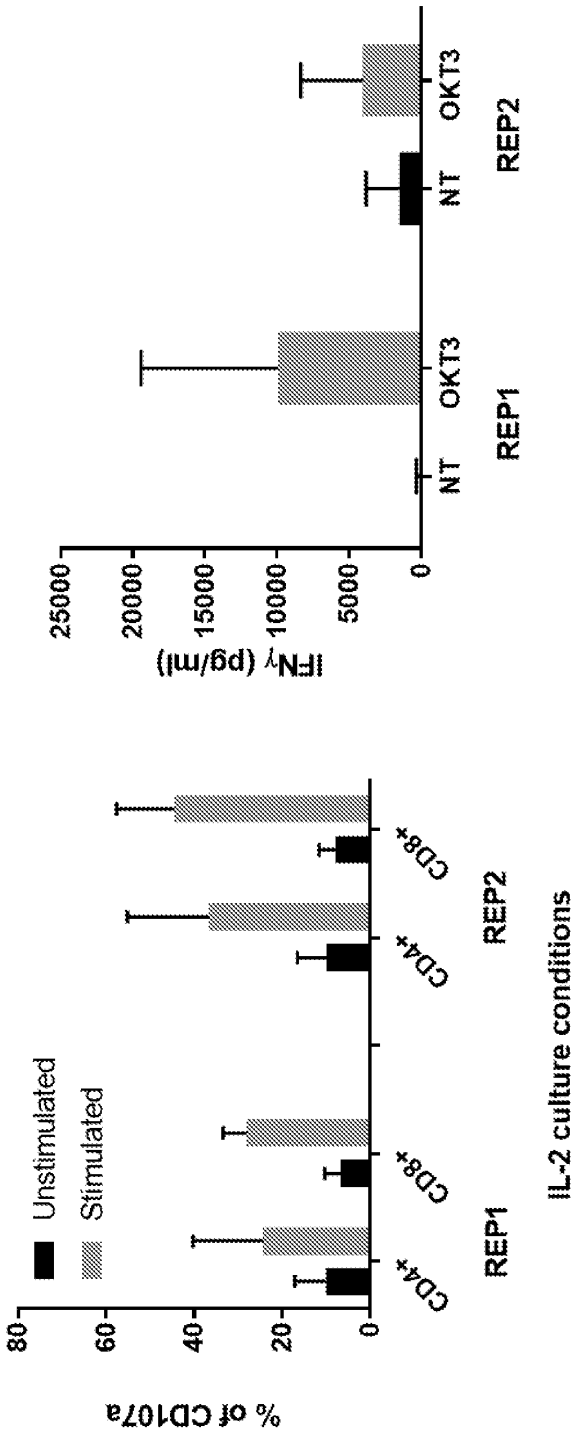

IFNγ (pg/ml)
25000
20000
15000
10000
5000
0
NT
OKT3
NT
OKT3
REP1
REP2
% of CD107a
80
60
40
20
0
Unstimulated
Stimulated
CD4+
CD8+
CD4+
CD8+
REP1
REP2
IL-2 culture conditions

Figure 63

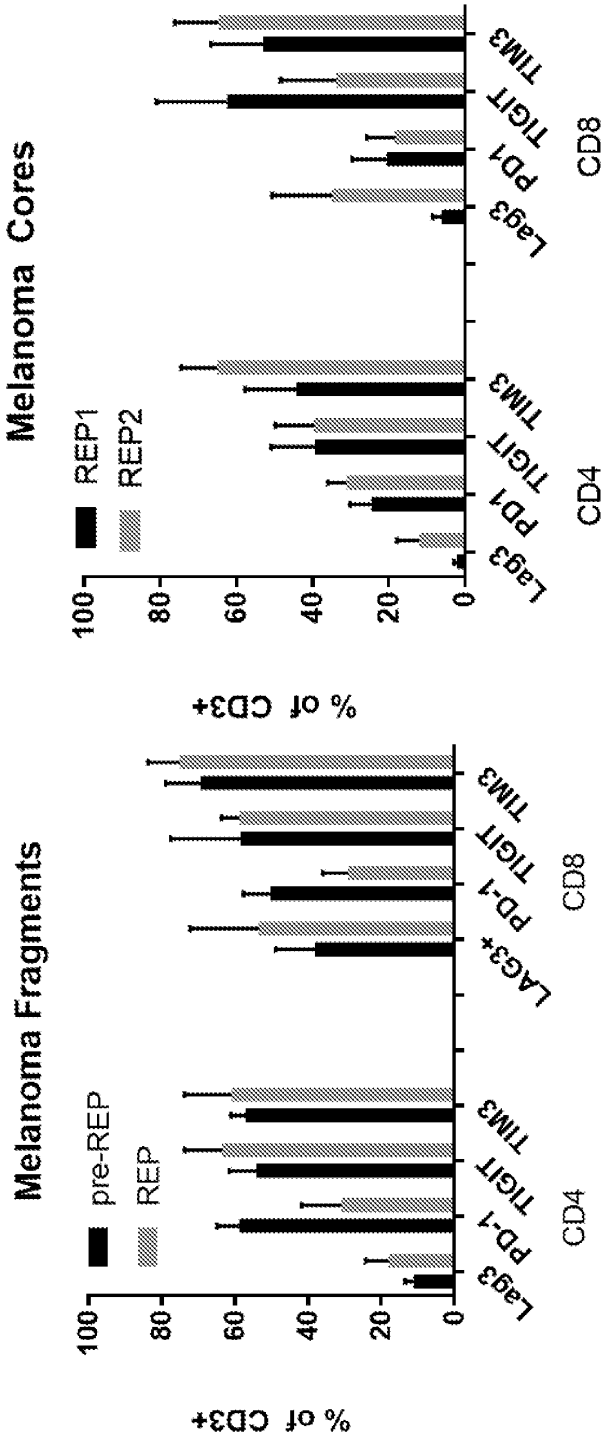

Melanoma Cores
REP1
REP2
% of CD3+
100
80
60
40
20
0
Lag3
PD1
TIGIT
TIM3
CD4
Lag3
PD1
TIGIT
TIM3
CD8
Melanoma Fragments
pre-REP
REP
% of CD3+
100
80
60
40
20
0
Lag3
PD-1
TIGIT
TIM3
CD4
LAG3+
PD-1
TIGIT
TIM3
CD8

Figure 64

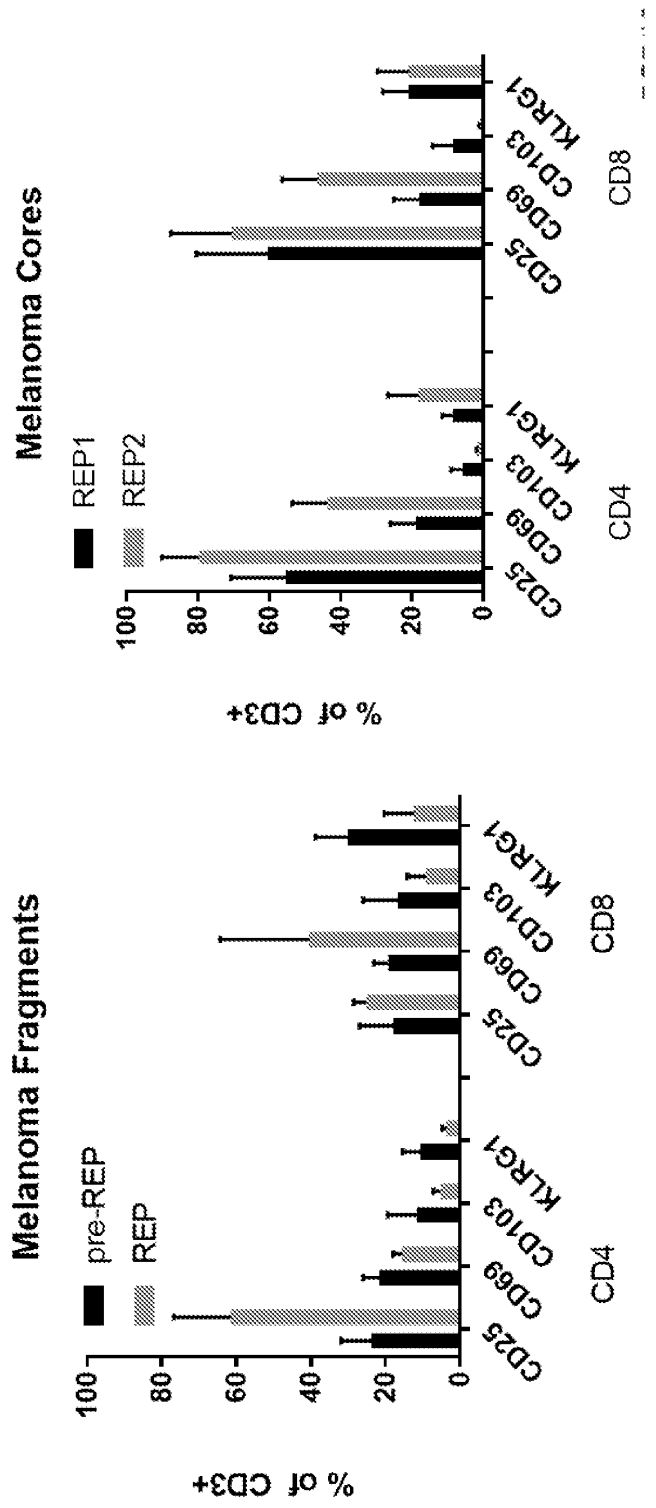
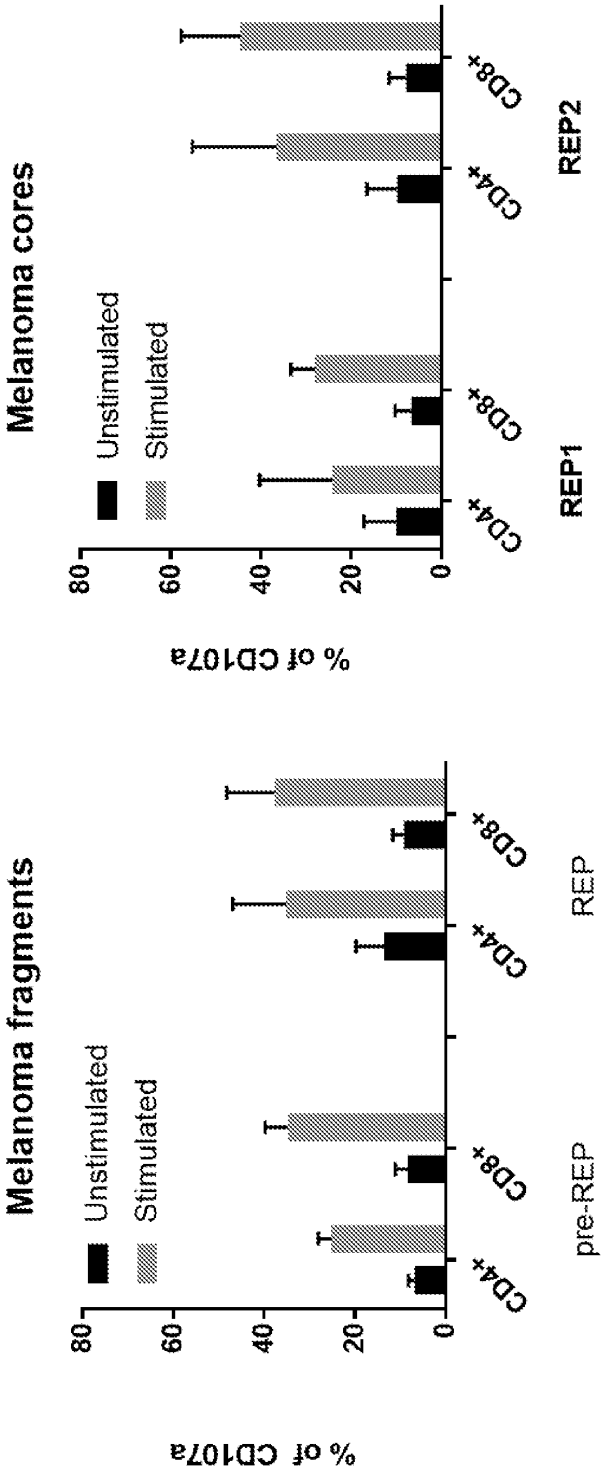

Melanoma Cores
REP1
REP2
% of CD3+
100
80
60
40
20
0
CD25
CD69
CD103
KLRG1
CD4
CD8
Melanoma Fragments
pre-REP
REP
% of CD3+
100
80
60
40
20
0
CD25
CD69
CD103
KLRG1
CD4
CD8

Figure 65


Melanoma fragments
Unstimulated
Stimulated
% of CD107a
0
20
40
60
80
CD4+
CD8+
CD4+
CD8+
pre-REP
REP

Melanoma cores
Unstimulated
Stimulated
% of CD107a
0
20
40
60
80
CD4+
CD8+
CD4+
CD8+
REP1
REP2

Figure 66

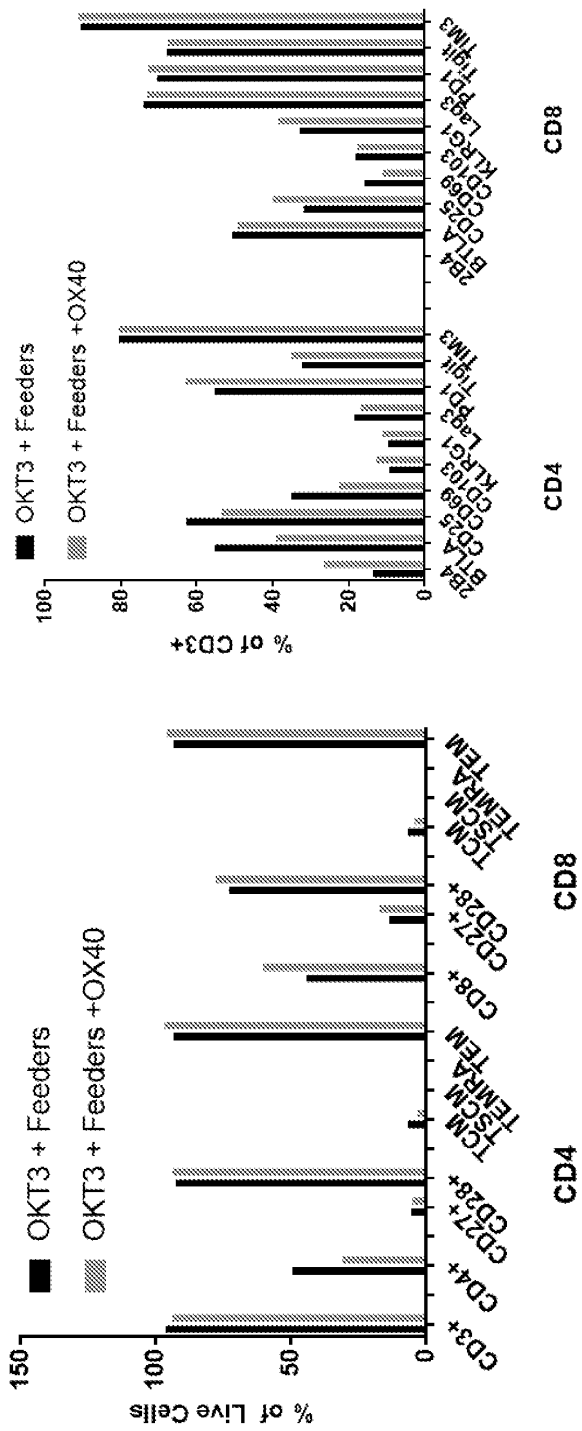

% of Live Cells
150
100
50
0
OKT3 + Feeders
OKT3 + Feeders +OX40
CD3+
CD4+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD8+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD4
CD8
% of CD3+
100
80
60
40
20
0
2B4 BTLA CD25 CD69 CD103 KLRG1 LAG3 PD1 TIGIT TIM3
CD4
CD8

Figure 67

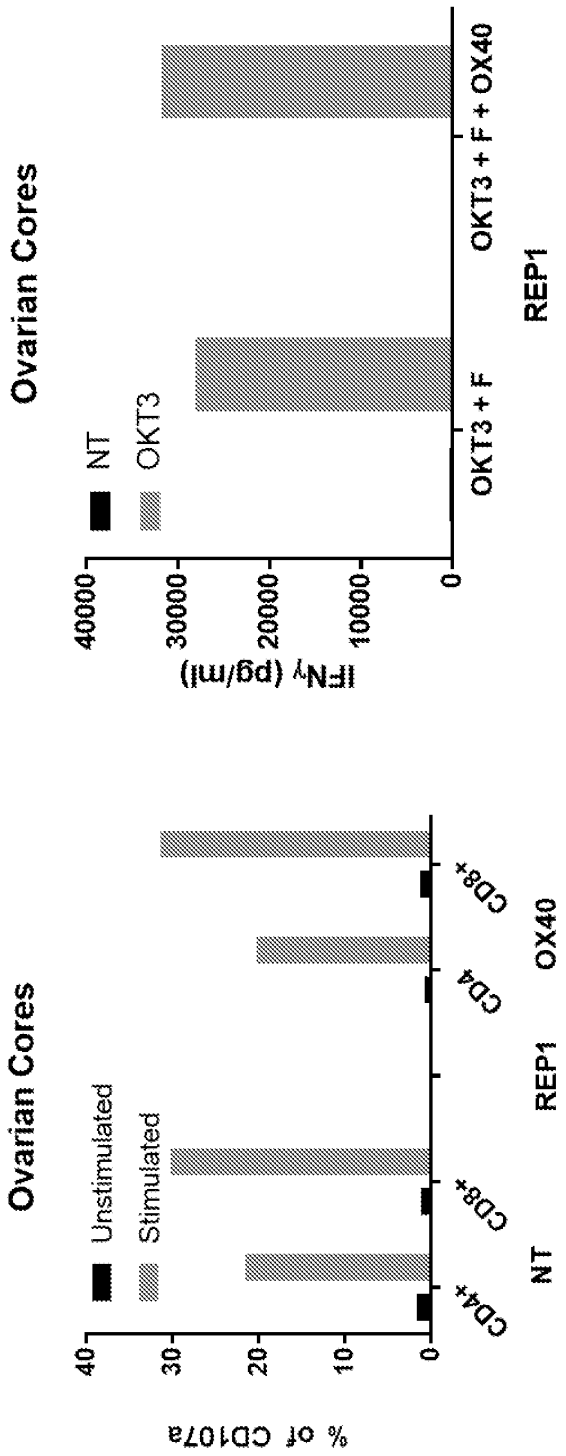

Ovarian Cores
NT
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
OKT3 + F
OKT3 + F + OX40
REP1
Ovarian Cores
Unstimulated
Stimulated
% of CD107a
40
30
20
10
0
CD4+
CD8+
CD4
CD8+
NT
REP1
OX40

Figure 68

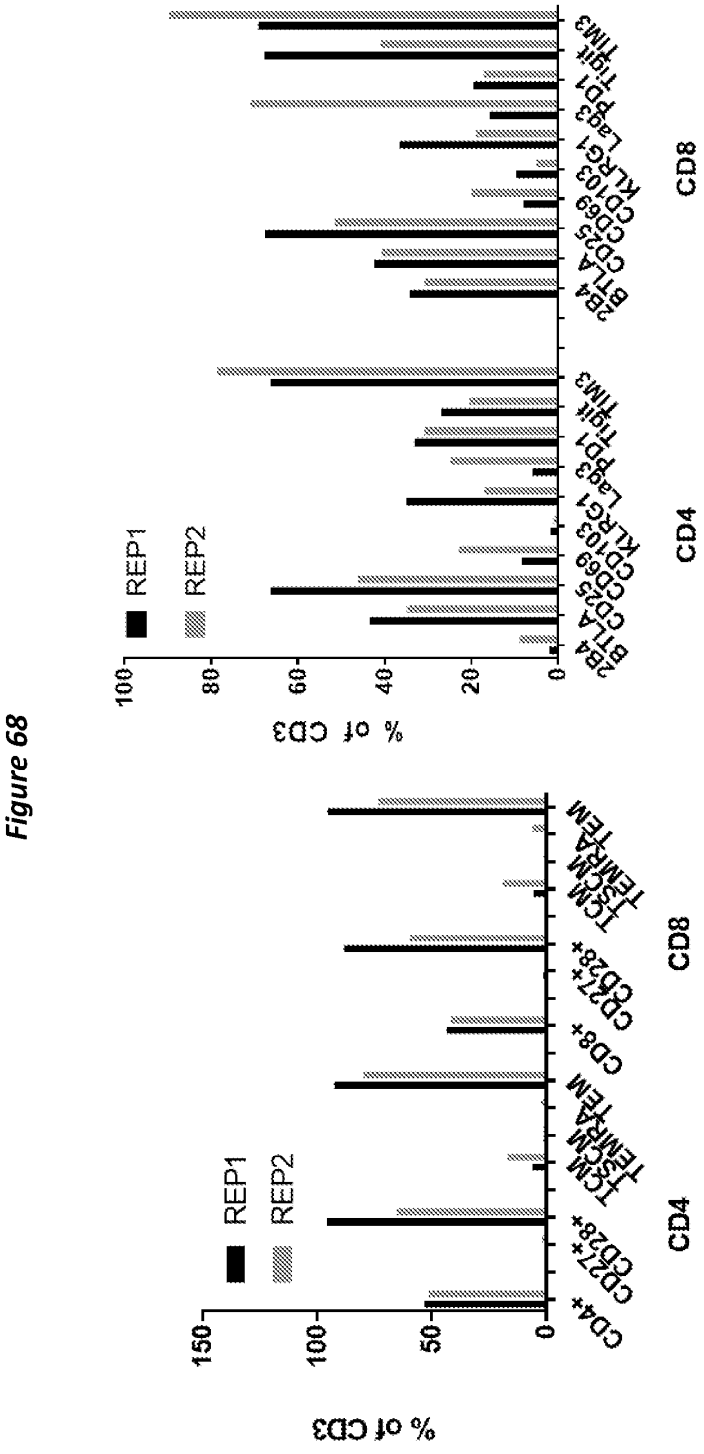
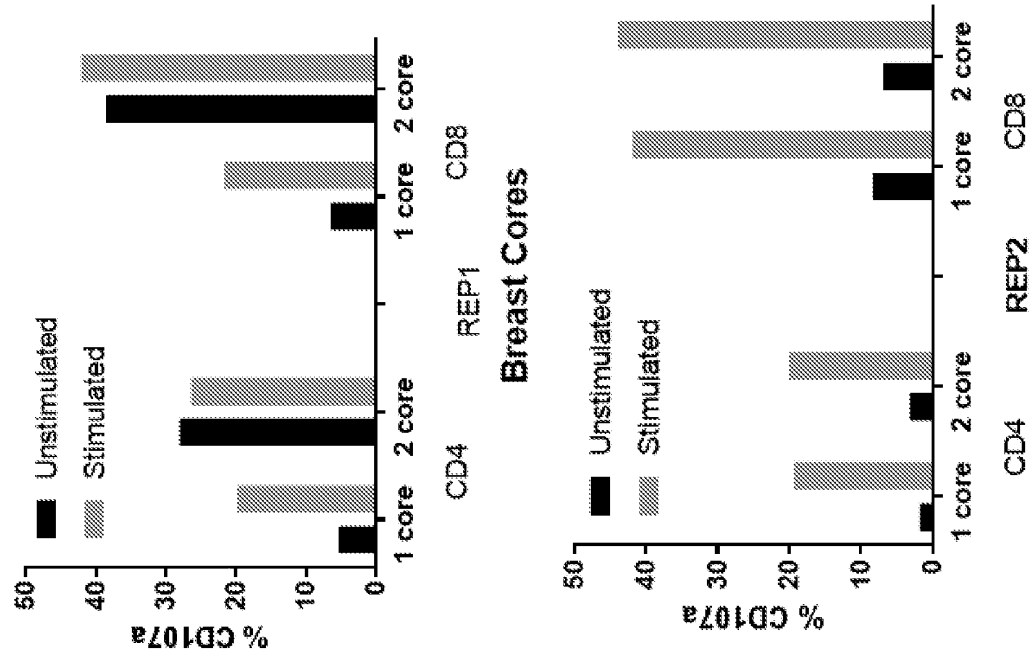

% of CD3
150
100
50
0
REP1
REP2
CD4+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD8+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD4
CD8
% of CD3
100
80
60
40
20
0
REP1
REP2
2B4
BTLA
CD25
CD69
CD103
KLRG1
Lag3
PD1
Tigit
TIM3
2B4
BTLA
CD25
CD69
CD103
KLRG1
Lag3
PD1
Tigit
TIM3
CD4
CD8

Figure 69 A & B


Breast Cores
Unstimulated
Stimulated
% CD107a
0
10
20
30
40
50
1 core
2 core
1 core
2 core
CD4
CD8
REP1
Breast Cores
Unstimulated
Stimulated
% CD107a
0
10
20
30
40
50
1 core
2 core
1 core
2 core
CD4
CD8
REP2

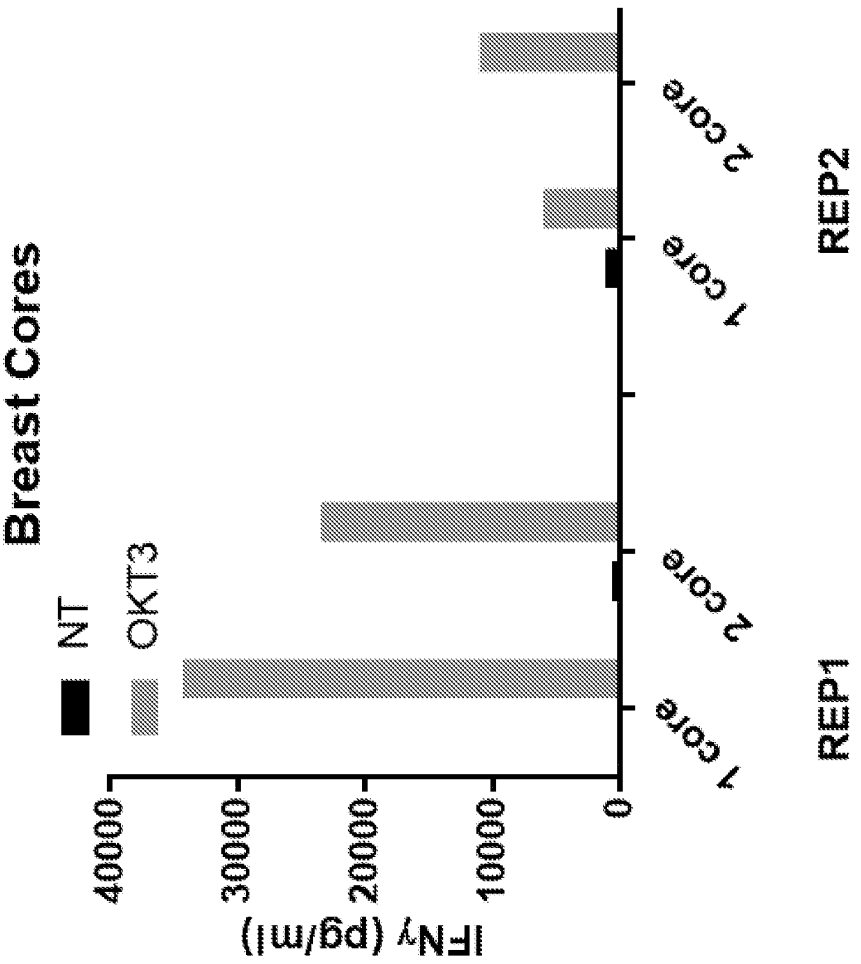

Breast Cores
NT
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
1 core
2 core
1 core
2 core
REP1
REP2

Figure 69C

Figure 70

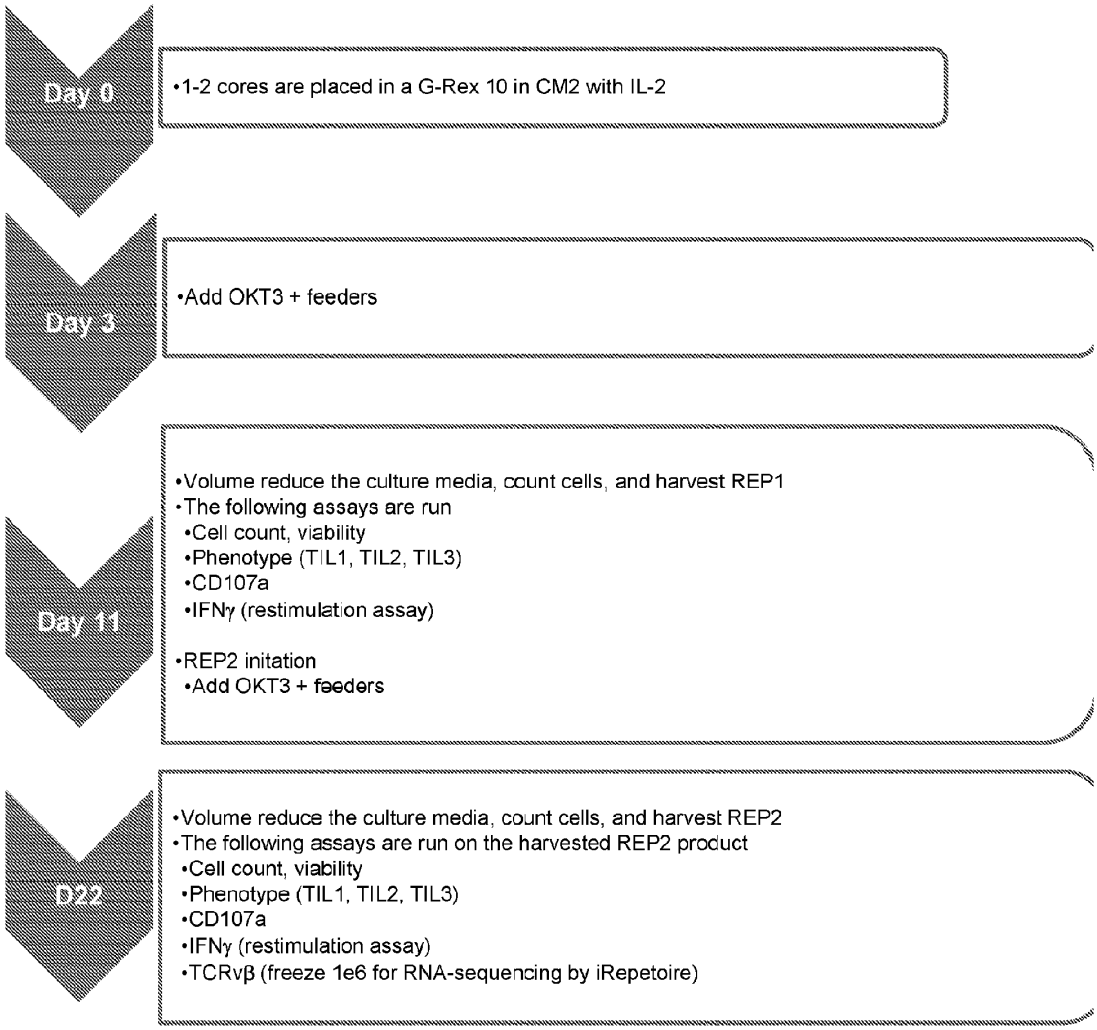

Day 0
•1-2 cores are placed in a G-Rex 10 in CM2 with IL-2
Day 3
•Add OKT3 + feeders
Day 11
•Volume reduce the culture media, count cells, and harvest REP1
•The following assays are run
•Cell count, viability
•Phenotype (TIL1, TIL2, TIL3)
•CD107a
•IFNγ (restimulation assay)
•REP2 initation
•Add OKT3 + feeders
D22
•Volume reduce the culture media, count cells, and harvest REP2
•The following assays are run on the harvested REP2 product
•Cell count, viability
•Phenotype (TIL1, TIL2, TIL3)
•CD107a
•IFNγ (restimulation assay)
•TCRvβ (freeze 1e6 for RNA-sequencing by iRepetoire)

Figure 71

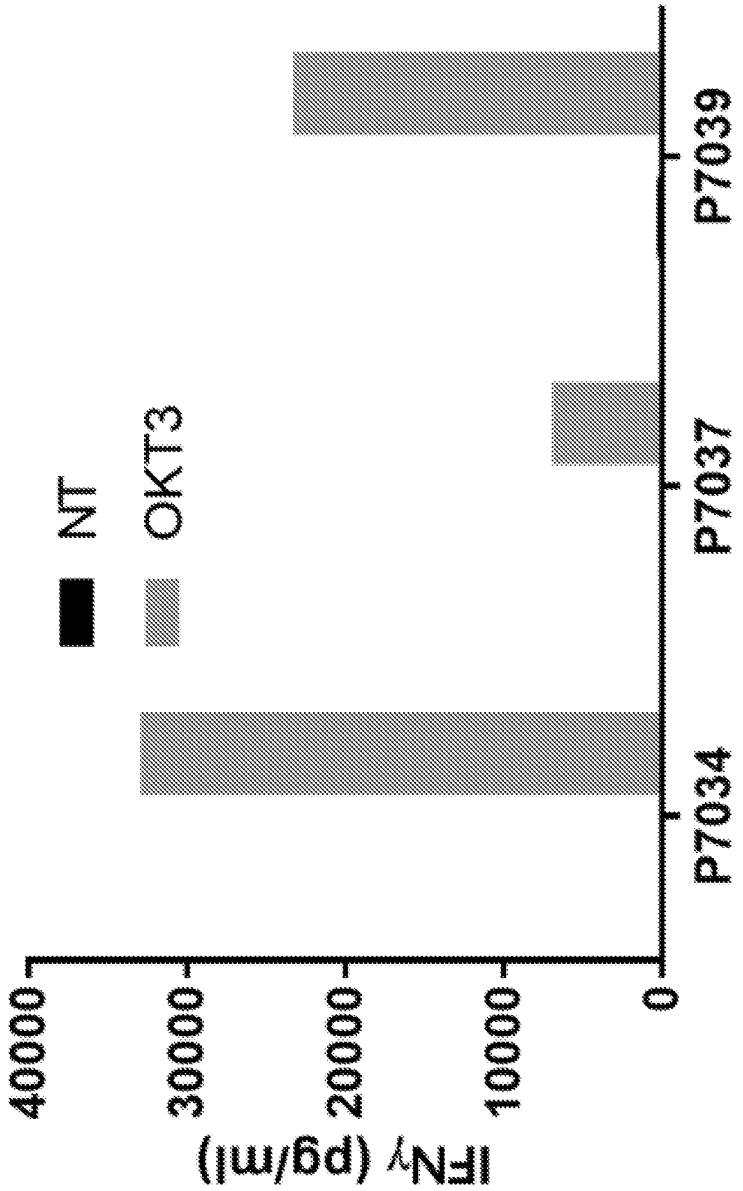

NT
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
P7034
P7037
P7039

Figure 72

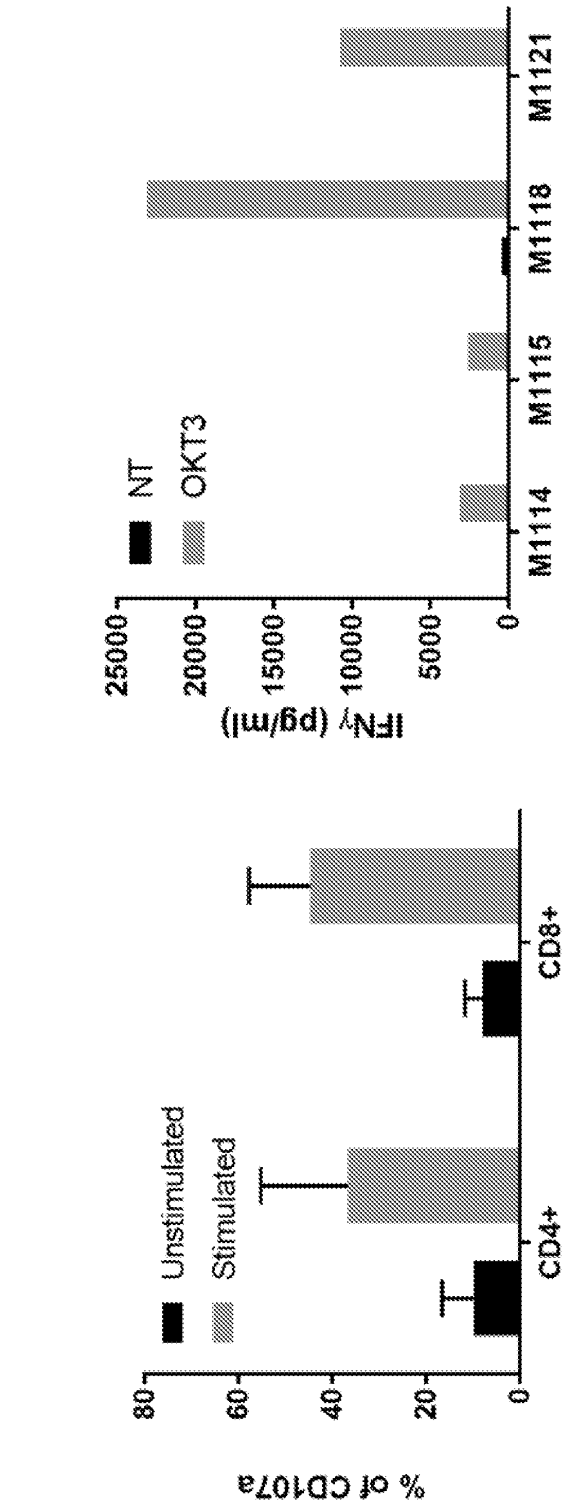

% of CD107a
80
60
40
20
0
Unstimulated
Stimulated
CD4+
CD8+
IFNγ (pg/ml)
25000
20000
15000
10000
5000
0
NT
OKT3
M1114
M1115
M1118
M1121

Figure 73A

	Culture conditions	Cores / cond. (#)	REP1	REP2
Breast (EP11028)	REP1: IL2 REP2: IL-2	1 vs 2	**>50E6 cells** at Day 11 (2 cores)	**173 and 158-fold (1/2 cores) expansion** **~ 34,0000 and 23,000 pg/ml IFNγ**
Breast (EP11045)	REP1: IL-2 (+/- OKT3+feeders) REP2: IL-2 (OKT3+feeders)	2	**>50e6 cells** at Day 11 (OKT3+ feeders)	**1178 and 1920-fold (+/- OKT3+feeders REP1)** **~ 99,000 and 45,000 pg/ml IFNγ**
Ovarian (OV8016)	REP1: IL2 +/- OX40 REP2: IL-2 +/- OX40	2	**>50E6 cells** at Day 11 (OX40 condition)	**1860 and 1540-fold (NO OX40/OX40) expansion** **~ 10,000 and 20,000 pg/ml IFNγ**
Ovarian (OV8031)	REP1: IL2 REP2: IL-2	1(small core)	1.3e6 cells at Day 11	**400-fold expansion** **~ ELISA is in progress**

Figure 73B

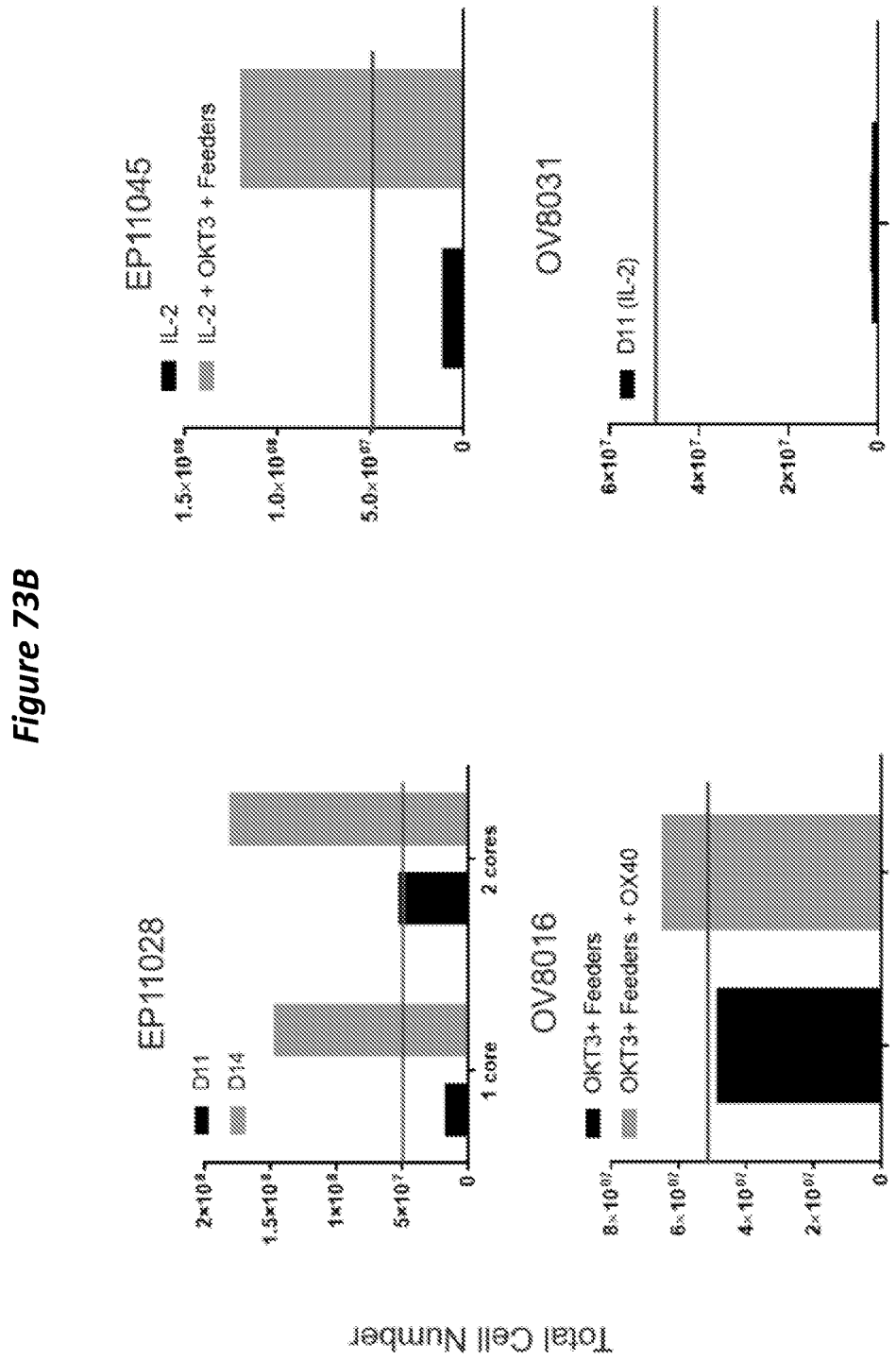

EP11028
D11
D14
2×10^8
1.5×10^8
1×10^8
5×10^7
0
1 core
2 cores
OV8016
OKT3+ Feeders
OKT3+ Feeders + OX40
8×10^7
6×10^7
4×10^7
2×10^7
0
EP11045
IL-2
IL-2 + OKT3 + Feeders
1.5×10^6
1.0×10^6
5.0×10^5
0
OV8031
D11 (IL-2)
6×10^7
4×10^7
2×10^7
0
Total Cell Number

Figure 74

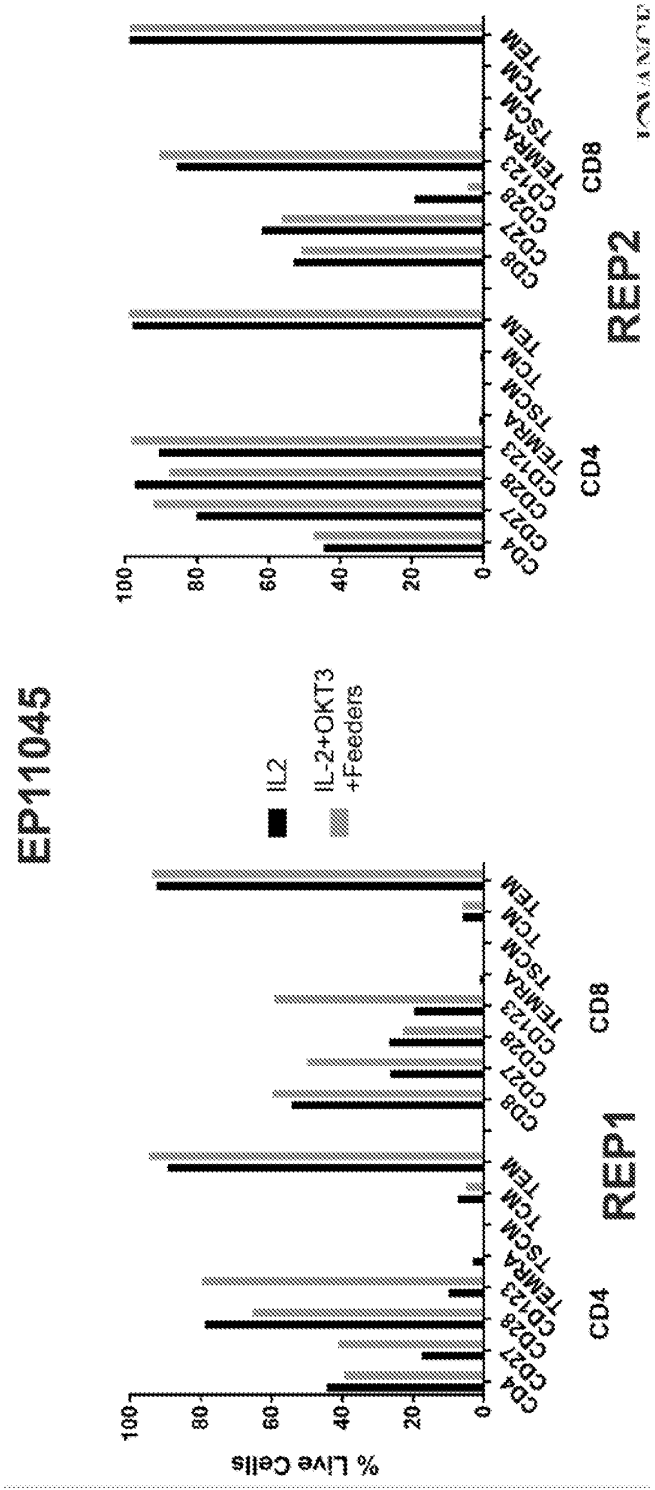

EP11045
IL2
IL-2+OKT3
+Feeders
% Live Cells
100
80
60
40
20
0
CD4
CD8
REP1
REP2
CD4
CD27
CD28
CD123
TEMRA
TSCM
TCM
TEM

Figure 75

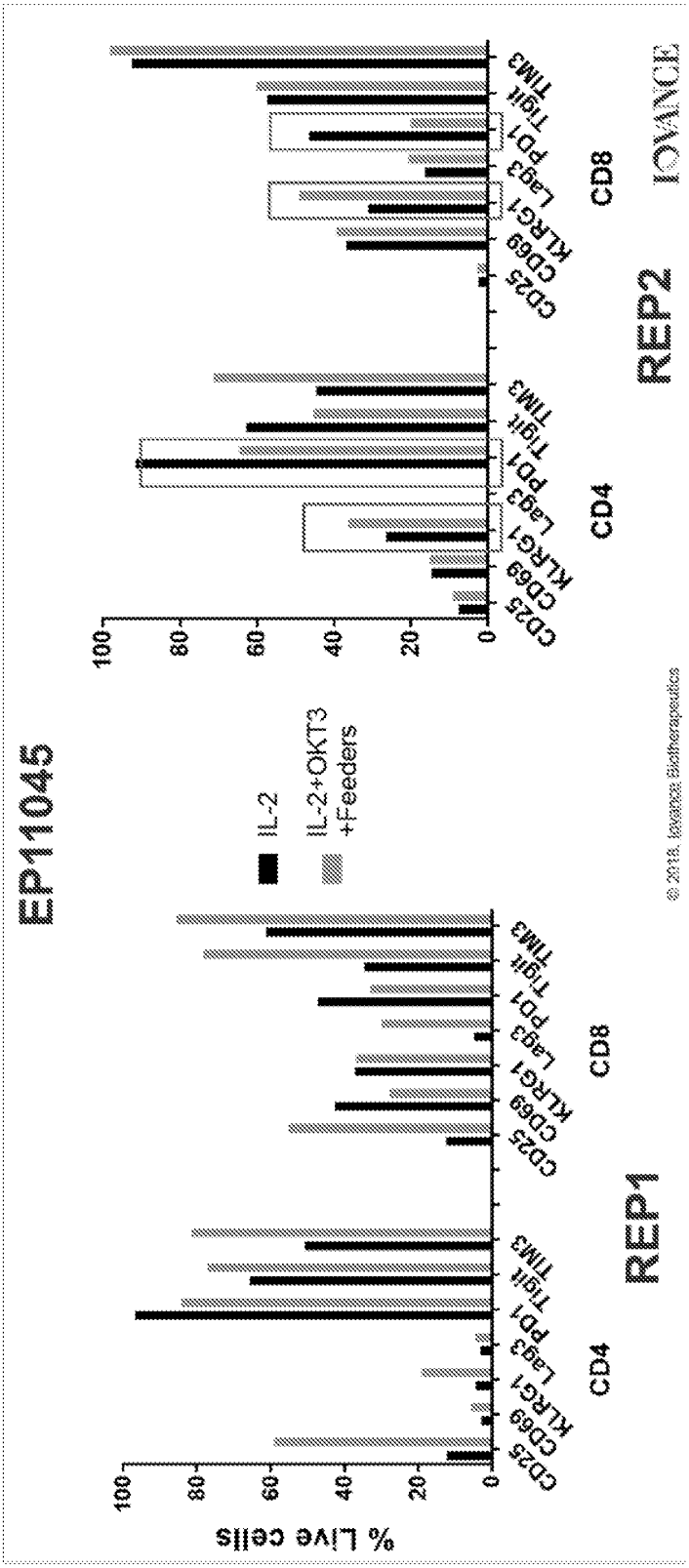

EP11045
IL-2
IL-2+OKT3 +Feeders
% Live cells
100
80
60
40
20
0
CD25
CD69
KLRG1
Lag3
PD1
Tigit
TIM3
CD4
CD8
REP1
REP2
IOVANCE
© 2018, Iovance Biotherapeutics

Figure 76

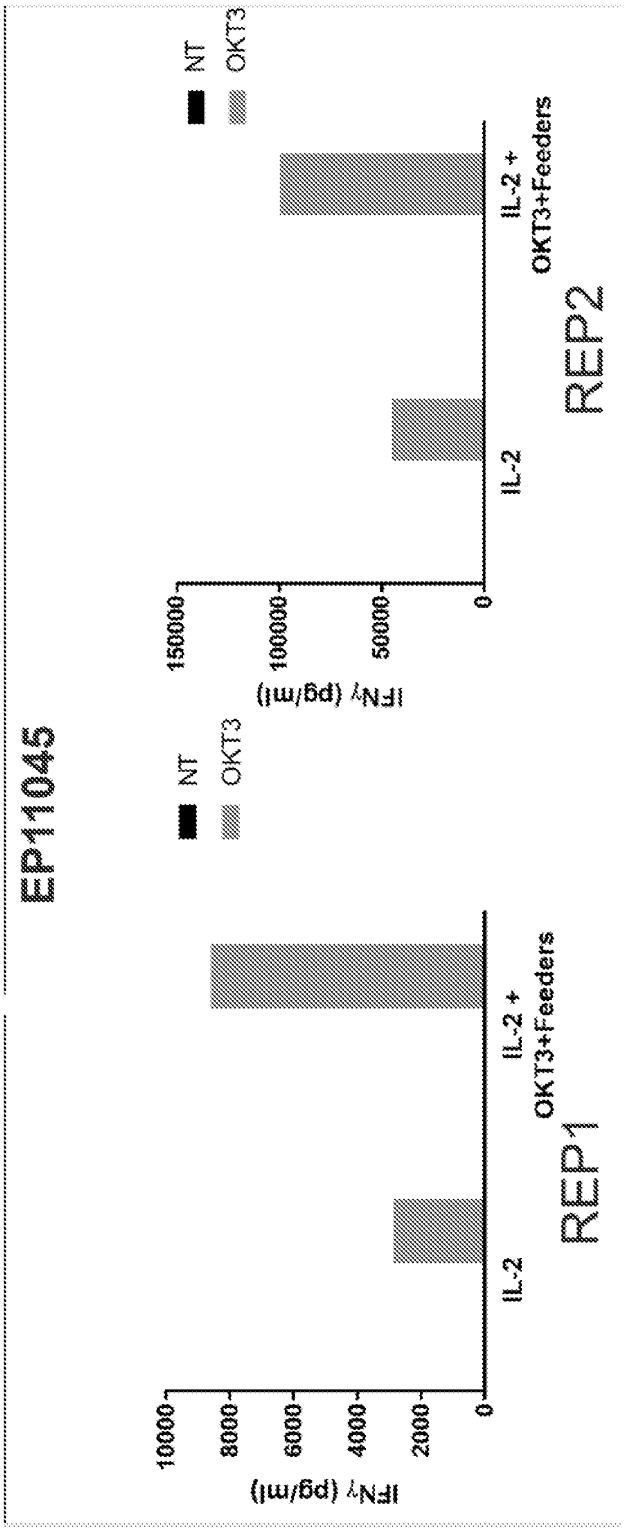

EP11045
NT
OKT3
IFNγ (pg/ml)
10000
8000
6000
4000
2000
0
IL-2
IL-2 + OKT3+Feeders
REP1
NT
OKT3
IFNγ (pg/ml)
150000
100000
50000
0
IL-2
IL-2 + OKT3+Feeders
REP2

Figure 77

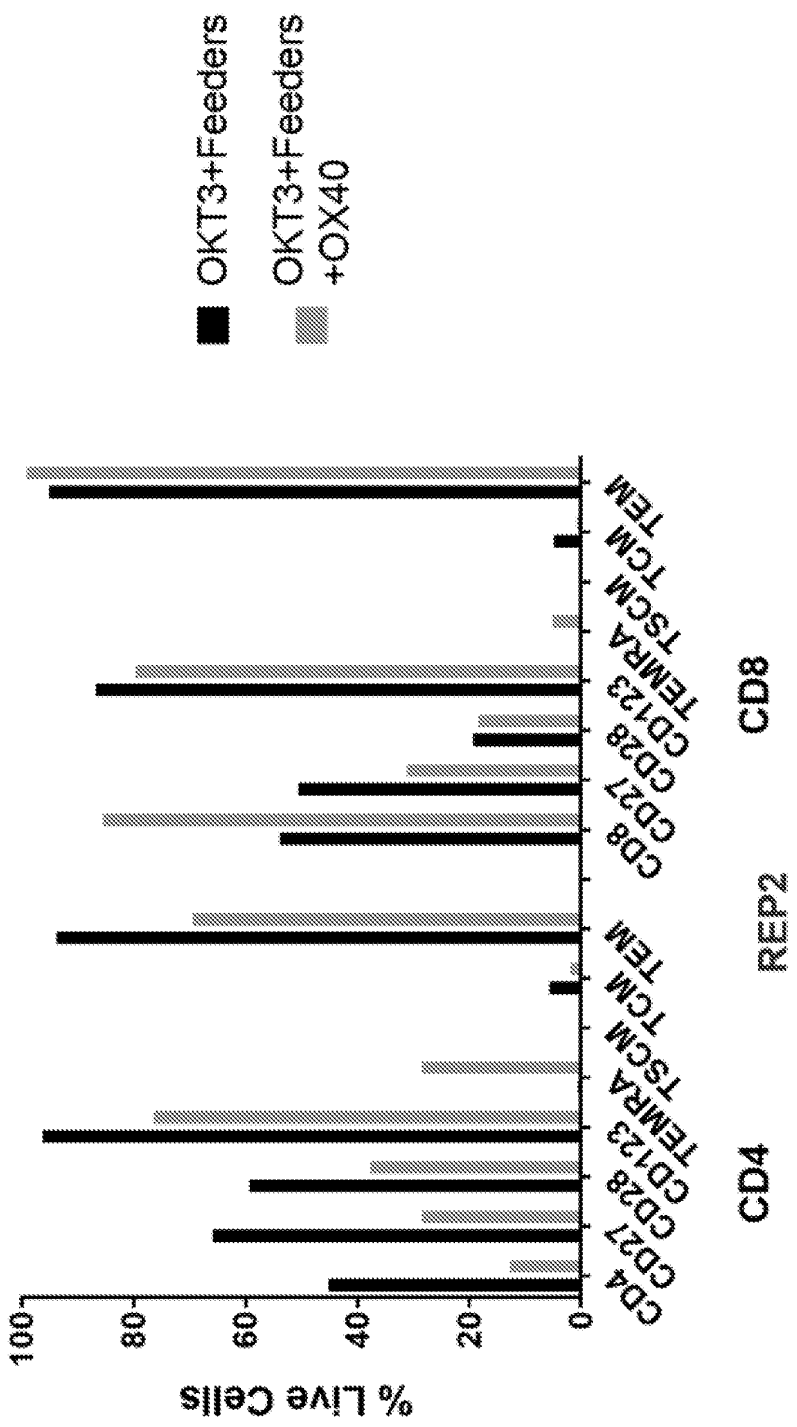

OKT3+Feeders
OKT3+Feeders +OX40
% Live Cells
100
80
60
40
20
0
CD4
CD27
CD28
CD123
TEMRA
TSCM
TCM
TEM
CD8
CD27
CD28
CD123
TEMRA
TSCM
TCM
TEM
CD4
CD8
REP2

Figure 78

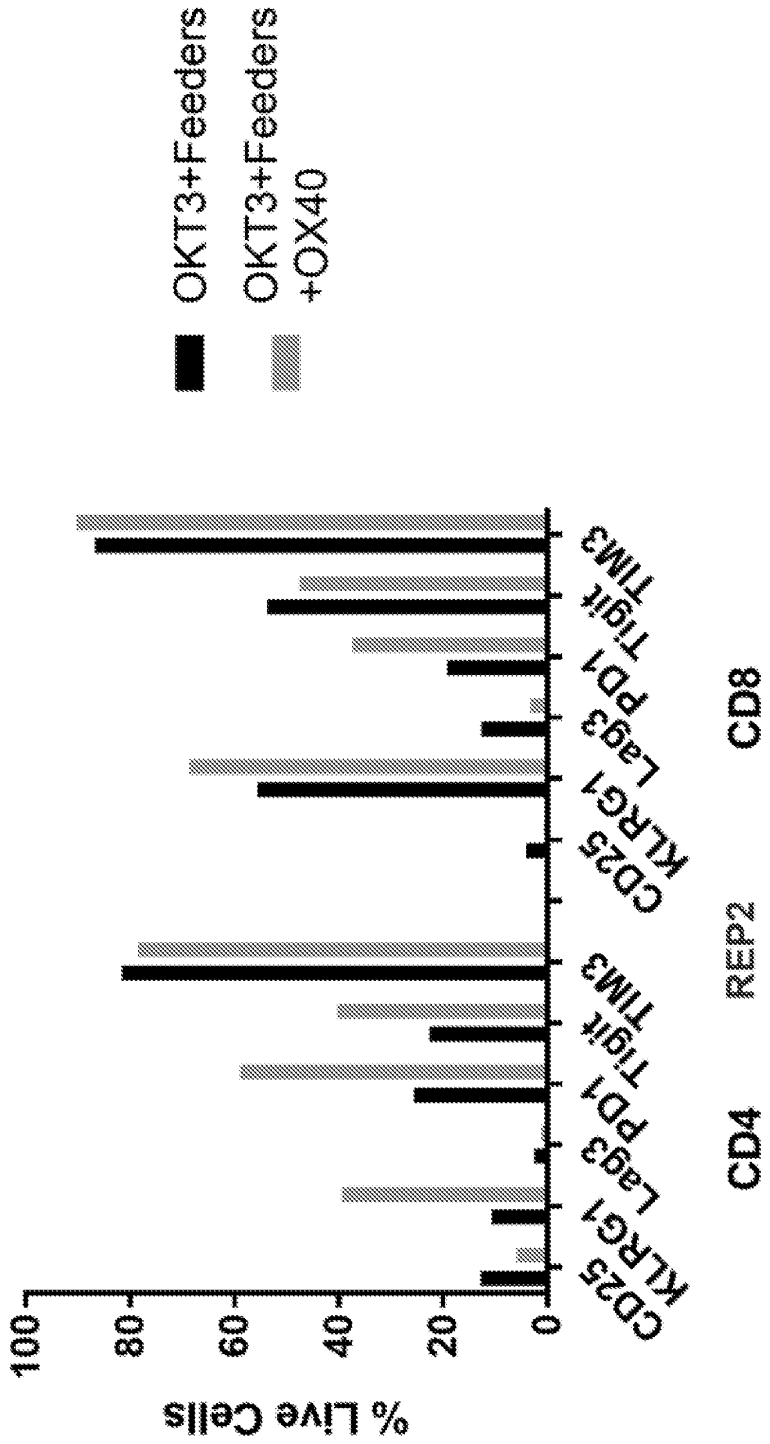

OKT3+Feeders
OKT3+Feeders +OX40
% Live Cells
100
80
60
40
20
0
CD25
KLRG1
Lag3
PD1
Tigit
TIM3
CD4
REP2
CD8

Figure 79

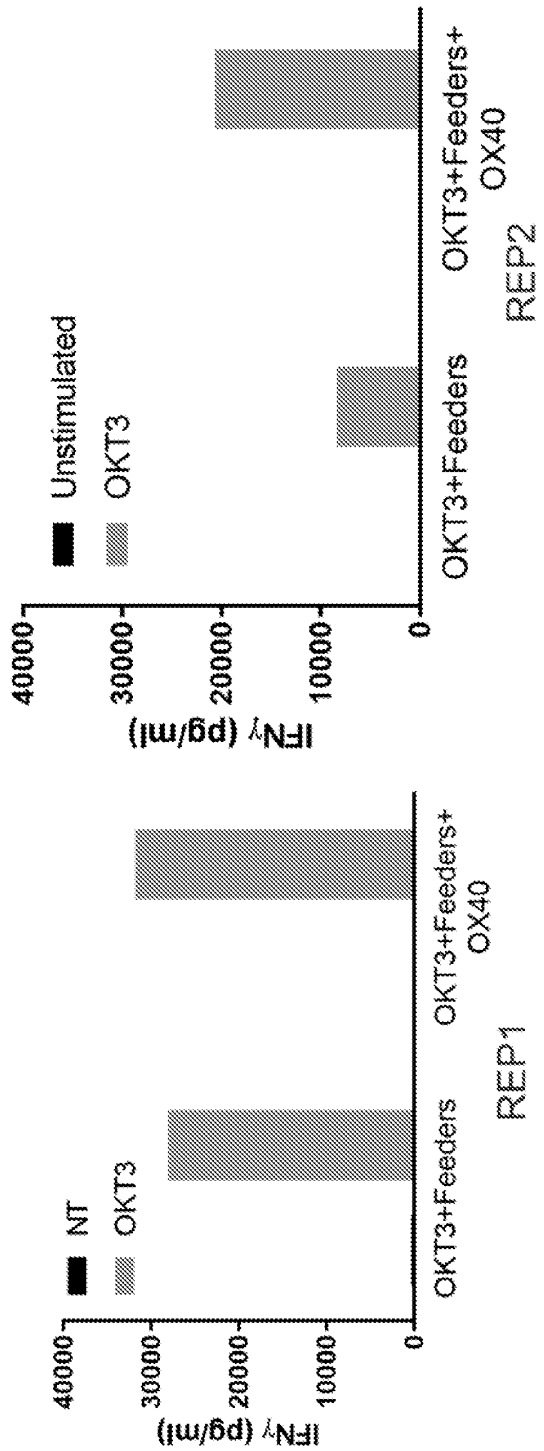

Unstimulated
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
OKT3+Feeders
OKT3+Feeders+ OX40
REP2
NT
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
OKT3+Feeders
OKT3+Feeders+ OX40
REP1

Figure 80

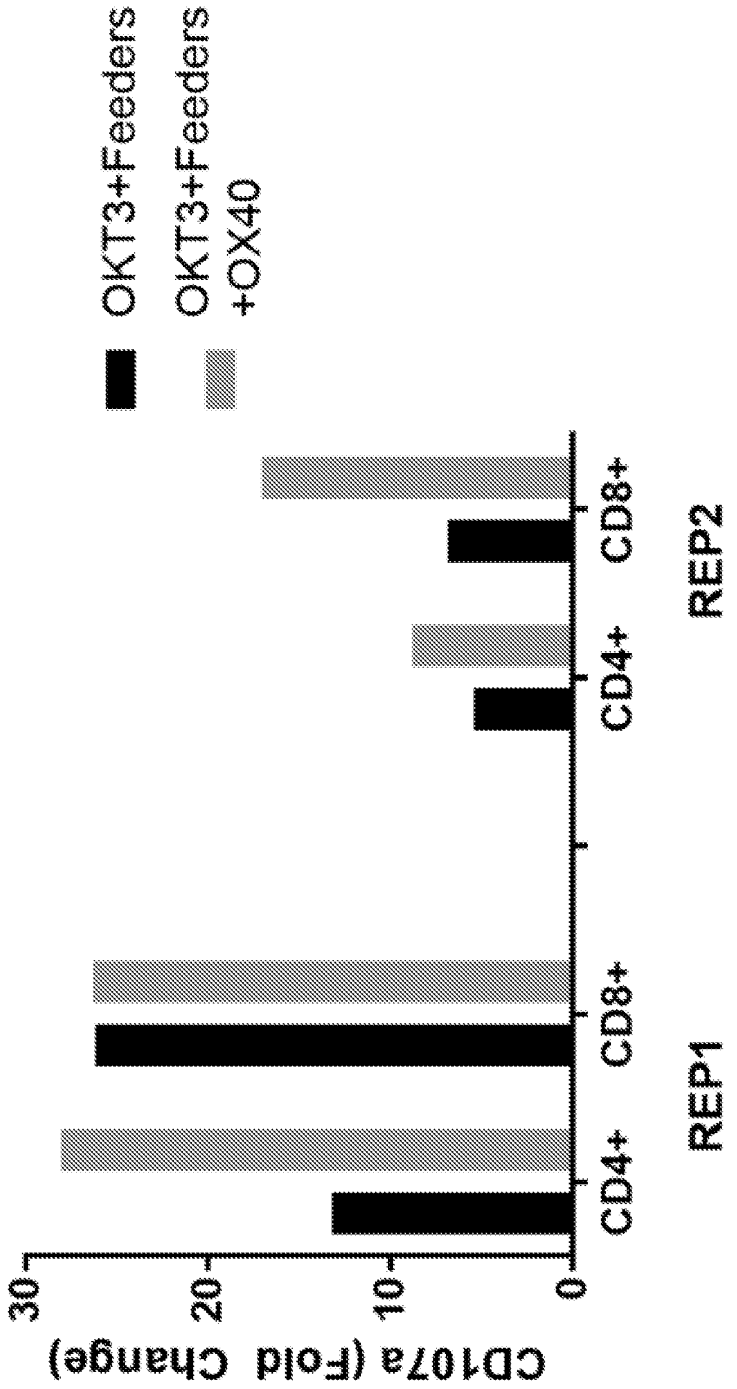

OKT3+Feeders
OKT3+Feeders +OX40
CD107a (Fold Change)
0
10
20
30
CD4+
CD8+
REP1
CD4+
CD8+
REP2

Figure 81

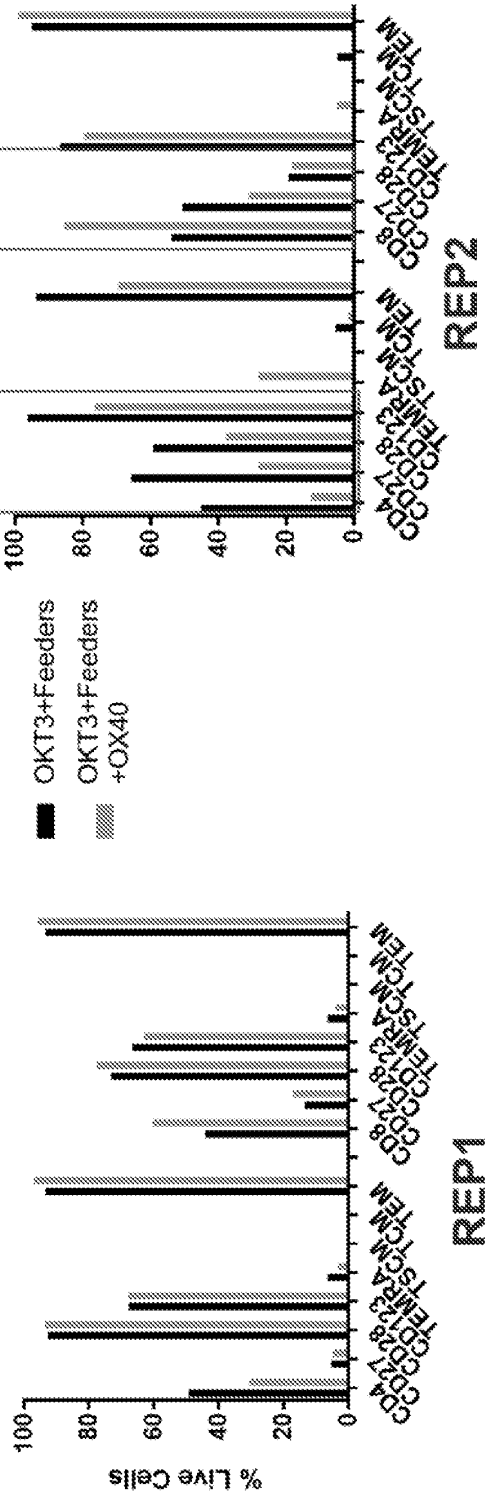

% Live Cells
100
80
60
40
20
0
CD4
CD27
CD28
CD27CD28
CD27CD28CD127
CD27CD28CD127TEMRA
CD27CD28CD127TSCM
CD27CD28CD127TCM
CD27CD28CD127TEM
CD8
CD27
CD28
CD27CD28
CD27CD28CD127
CD27CD28CD127TEMRA
CD27CD28CD127TSCM
CD27CD28CD127TCM
CD27CD28CD127TEM
REP1
REP2
OKT3+Feeders
OKT3+Feeders
+OX40

Figure 82

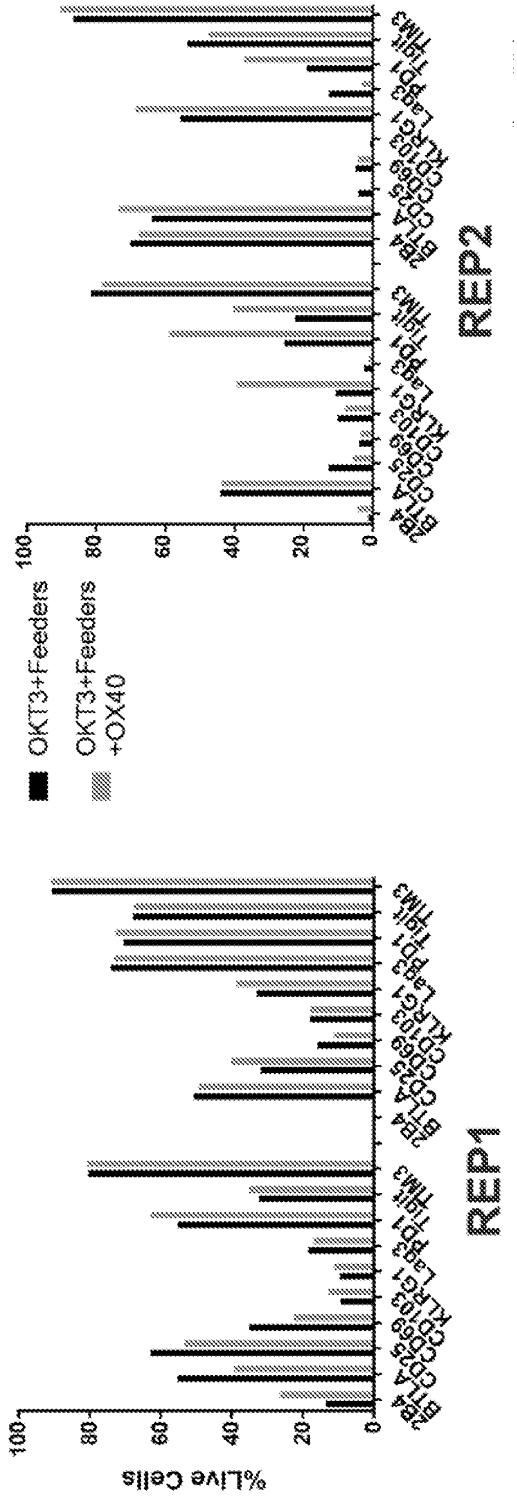

OKT3+Feeders
OKT3+Feeders +OX40
%Live Cells
100
80
60
40
20
0
REP1
REP2
2B4
BTLA
CD25
CD69
CD103
KLRG1
LAG3
PD1
TIGIT
TIM3

Figure 83

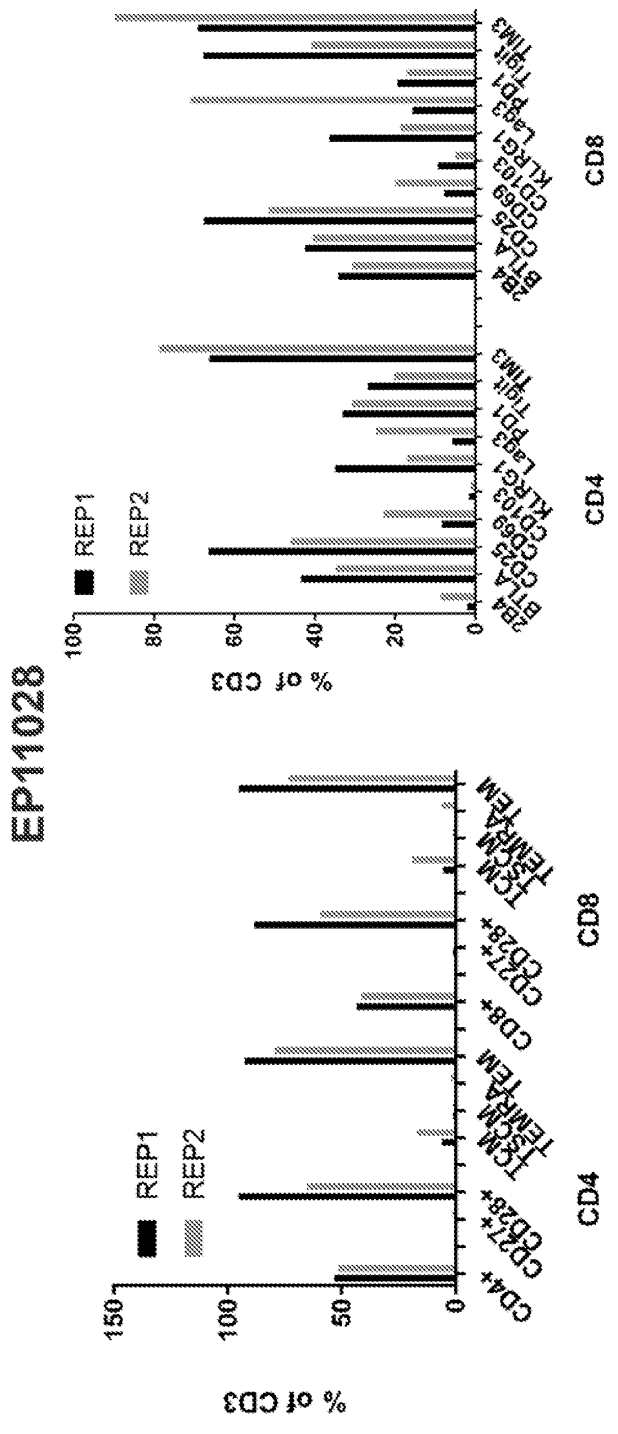

EP11028
% of CD3
REP1
REP2
150
100
50
0
CD4+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD8+
CD27+ CD28+
TCM TSCM TEMRA TEM
CD4
CD8
% of CD3
REP1
REP2
100
80
60
40
20
0
2B4 BTLA CD25 CD69 CD103 KLRG1 Lag3 PD1 Tigit Tim3
2B4 BTLA CD25 CD69 CD103 KLRG1 Lag3 PD1 Tigit Tim3
CD4
CD8

Figure 84

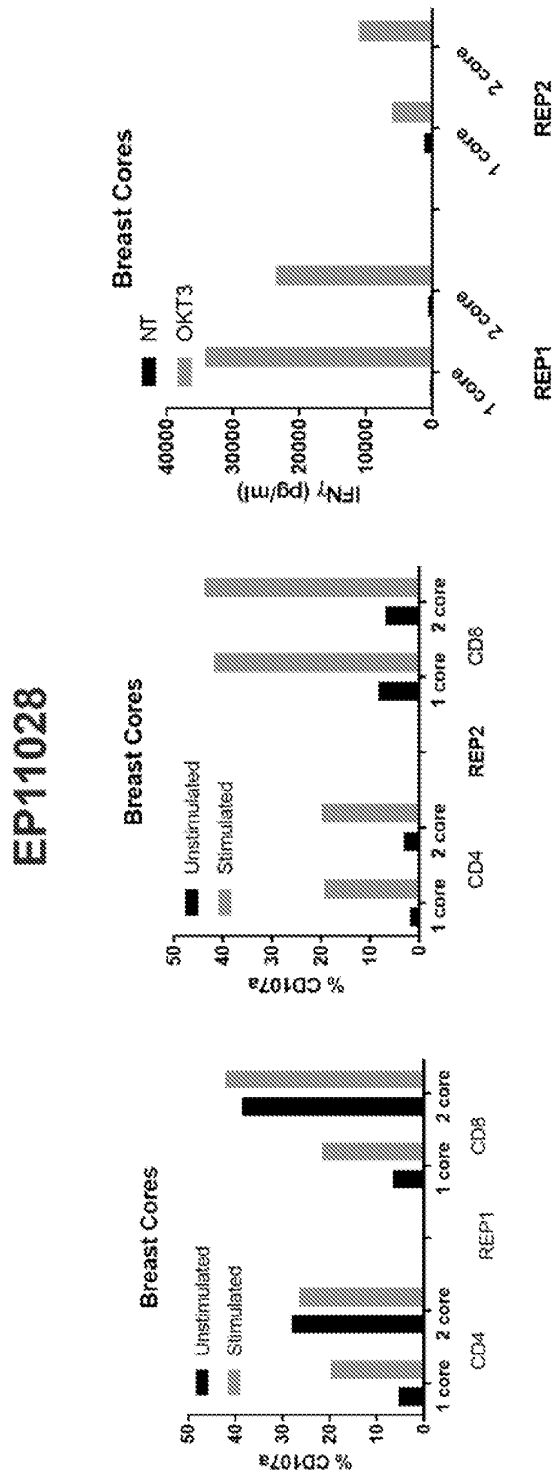

EP11028
Breast Cores
NT
OKT3
IFNγ (pg/ml)
40000
30000
20000
10000
0
1 core
2 core
1 core
2 core
REP1
REP2
Breast Cores
Unstimulated
Stimulated
% CD107a
50
40
30
20
10
0
1 core
2 core
1 core
2 core
CD4
CD8
REP2
Breast Cores
Unstimulated
Stimulated
% CD107a
50
40
30
20
10
0
1 core
2 core
1 core
2 core
CD4
CD8
REP1

Figure 85A

Process 2A: about 22 days from Steps A - E	Process GEN 3: about 14-16 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B)	**STEP A** Obtain Patient Tumor Sample (optionally can be frozen before Step B)
STEP B First Expansion (physical fragmentation to at least 40 fragments per container grown for about 3 days to 14 days with media comprising IL-2)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)
STEP C First Expansion to Second Expansion Transition (Step B TILs directly move to Step D, optionally on Step B day 11)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7)
STEP D Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells in a closed container)	**STEP D** Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)
STEP E Harvest TILS from Step D (TILs harvested via closed system)	**STEP E** Harvest TILS from Step D
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 85B

Small Biopsy GEN 3 Process: about 17-24 days from Steps A - E

STEP A

Obtain Patient Tumor Sample

(optionally can be frozen before Step B)

STEP B

Priming First Expansion

(physical fragmentation of up to 60 fragments per container grown for about 3 days to 12 days with media comprising IL-2, OKT-3, and antigen-presenting feeder cells)

STEP C

Priming First Expansion to Rapid Second Expansion Transition

(Step B TILs directly move to Step D on day 7 to 12)

STEP D

Rapid Second Expansion

(TILs grown in growth media medium comprising IL-2, OKT-3, and 2X antigen-presenting feeder cells; Days 10-12 scale up and add additional IL-2)

STEP E

Harvest TILS from Step D

STEP F

Final Formulation and/or Transfer to Infusion Bag

(optionally cryopreserve)

Figure 85C

Small Biopsy Process: Version 2 (GEN3 based process) **About 17-24 days from Steps A - E**
STEP A Obtain Patient Tumor Sample
STEP B First Expansion 3 days to 12 days (with OKT-3 and antigen-presenting feeder cells at any one of days 1-3)
STEP C First Expansion to Second Expansion Transition No Storage and optionally Closed System
STEP D Second Expansion IL-2, OKT-3, and antigen-presenting feeder cells 3 to 12 days Optionally Closed System
STEP E Harvest TILS from Step D Closed System
STEP F Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

Figure 86

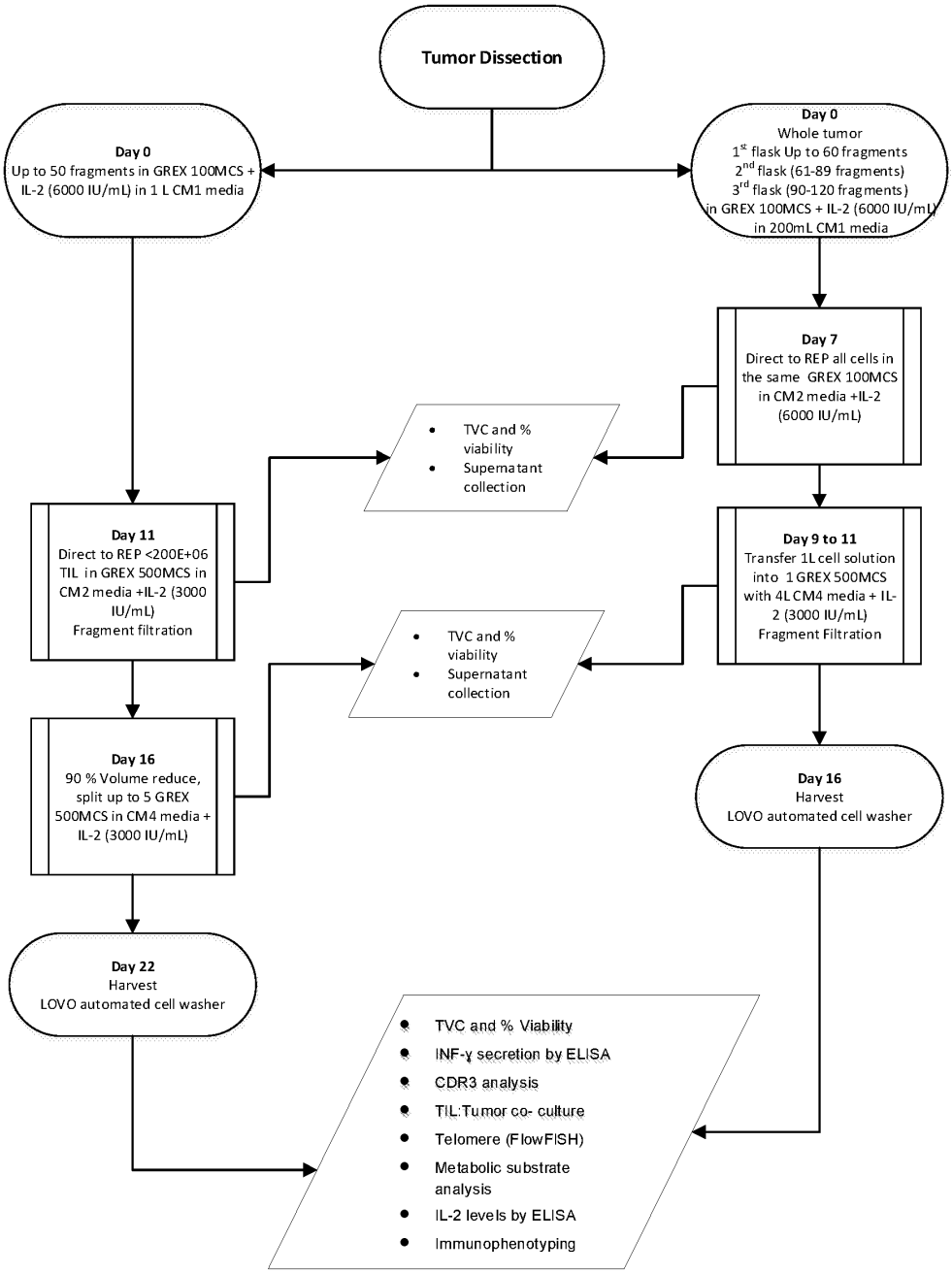
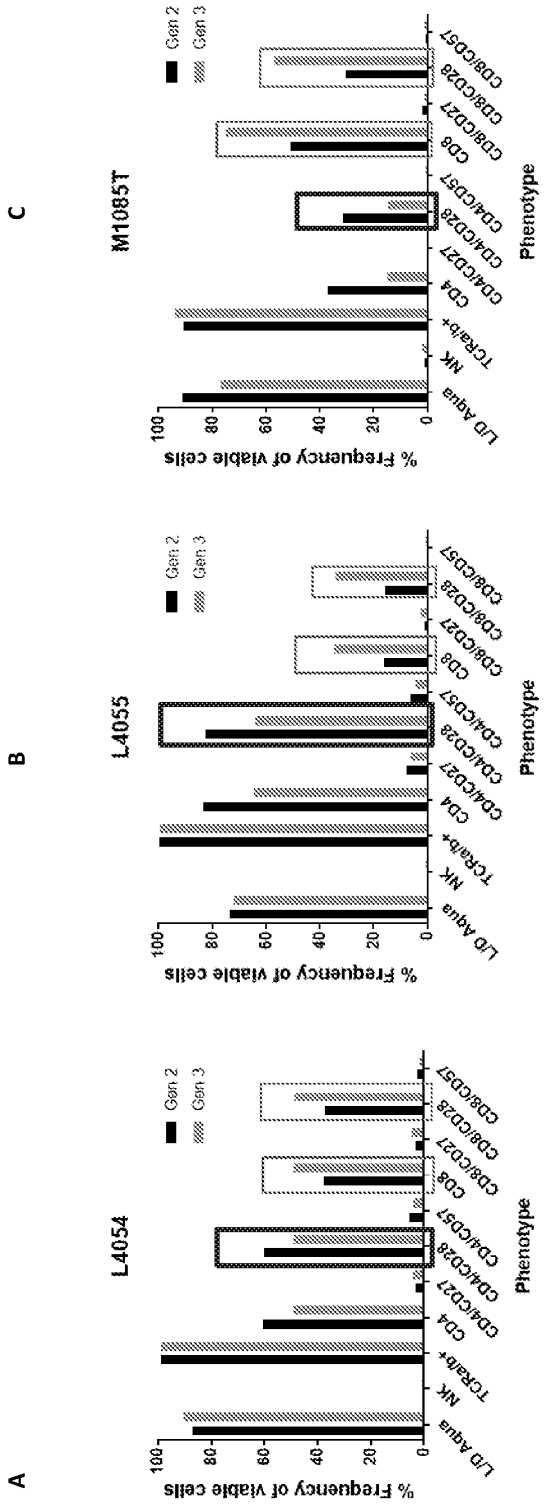
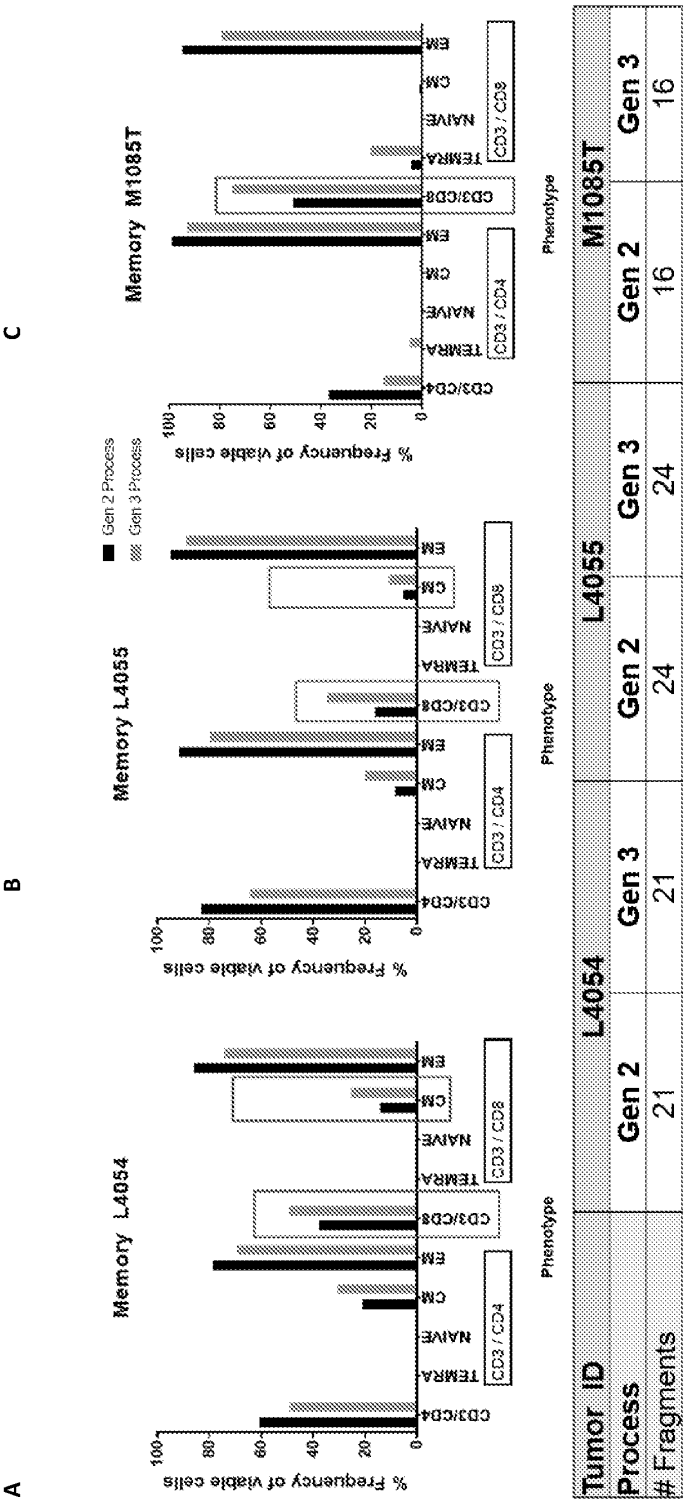

Tumor Dissection
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL) in 200mL CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS in CM2 media +IL-2 (6000 IU/mL)
TVC and % viability
Supernatant collection
Day 11
Direct to REP <200E+06 TIL in GREX 500MCS in CM2 media +IL-2 (3000 IU/mL)
Fragment filtration
Day 9 to 11
Transfer 1L cell solution into 1 GREX 500MCS with 4L CM4 media + IL-2 (3000 IU/mL)
Fragment Filtration
TVC and % viability
Supernatant collection
Day 16
90 % Volume reduce, split up to 5 GREX 500MCS in CM4 media + IL-2 (3000 IU/mL)
Day 16
Harvest
LOVO automated cell washer
Day 22
Harvest
LOVO automated cell washer
TVC and % Viability
INF-γ secretion by ELISA
CDR3 analysis
TIL:Tumor co- culture
Telomere (FlowFISH)
Metabolic substrate analysis
IL-2 levels by ELISA
Immunophenotyping

Figure 87



Tumor ID	L4054		L4055		M1085T	
Process	Gen 2	Gen 3	Gen 2	Gen 3	Gen 2	Gen 3
# Fragments	21	21	24	24	16	16

Figure 88



Tumor ID	L4054		L4055		M1085T	
Process	Gen 2	Gen 3	Gen 2	Gen 3	Gen 2	Gen 3
# Fragments	21	21	24	24	16	16

Figure 89

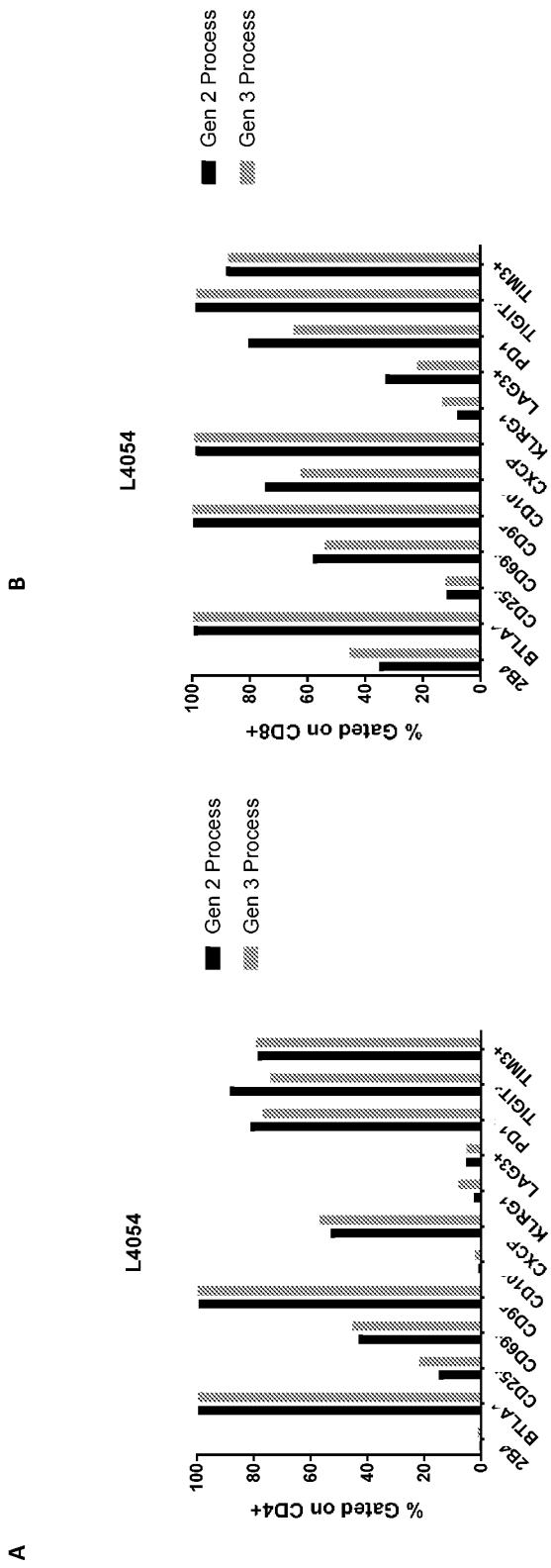

A
L4054
Gen 2 Process
Gen 3 Process
% Gated on CD4+
100
80
60
40
20
0
2Bd
BTLA-
CD25-
CD69-
CD9r
CD1r
CXCP
KLRG1
LAG3+
PD1
TIGIT-
TIM3+
B
L4054
Gen 2 Process
Gen 3 Process
% Gated on CD8+
100
80
60
40
20
0
2Bd
BTLA-
CD25-
CD69-
CD9r
CD1r
CXCP
KLRG1
LAG3+
PD1
TIGIT-
TIM3+

Figure 90

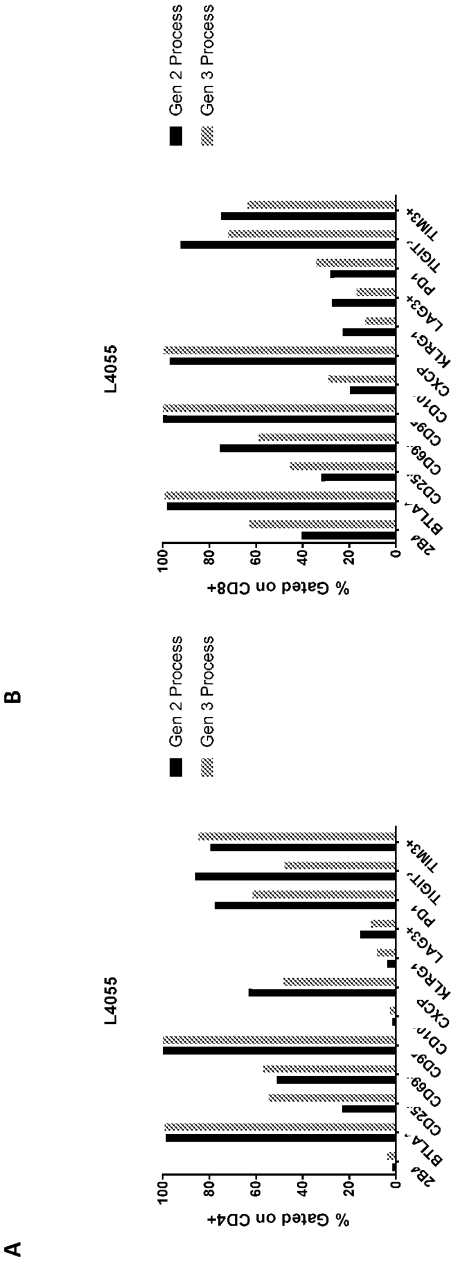

A
L4055
% Gated on CD4+
100
80
60
40
20
0
2Ba
BTLA
CD25
CD69
CD9r
CDrn
CXCP
KLRG1
LAG3+
PD1
TIGIT
TIM3+
Gen 2 Process
Gen 3 Process
B
L4055
% Gated on CD8+
100
80
60
40
20
0
2Ba
BTLA
CD25
CD69
CD9r
CDrn
CXCP
KLRG1
LAG3+
PD1
TIGIT
TIM3+
Gen 2 Process
Gen 3 Process

Figure 91

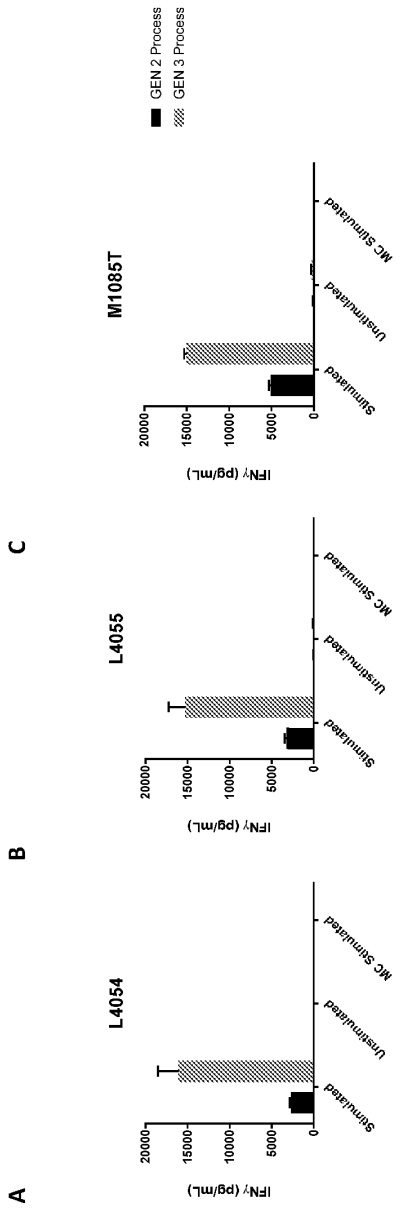

A
L4054
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
B
L4055
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
C
M1085T
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
GEN 2 Process
GEN 3 Process

Figure 92

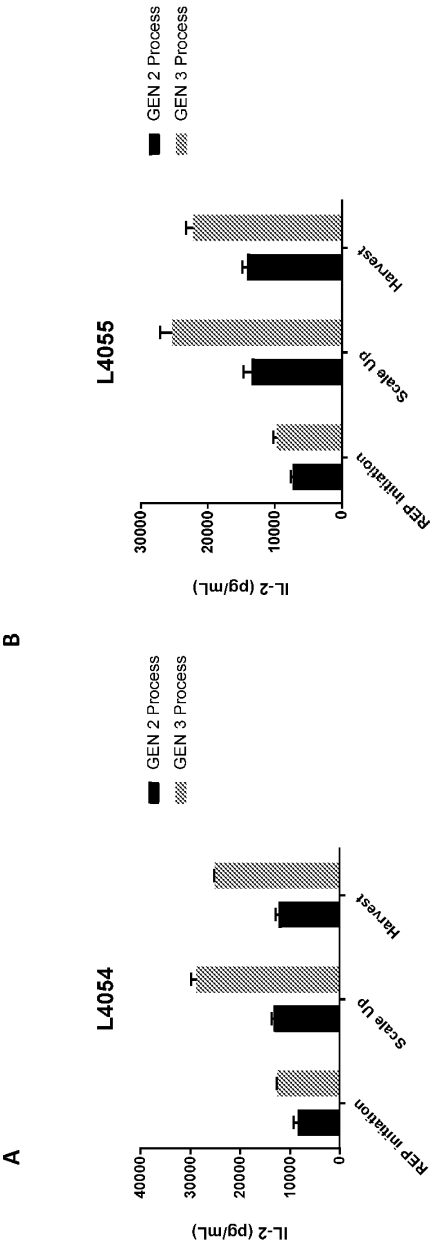

A
L4054
IL-2 (pg/mL)
40000
30000
20000
10000
0
REP Initiation
Scale Up
Harvest
GEN 2 Process
GEN 3 Process
B
L4055
IL-2 (pg/mL)
30000
20000
10000
0
REP Initiation
Scale Up
Harvest
GEN 2 Process
GEN 3 Process

Figure 93

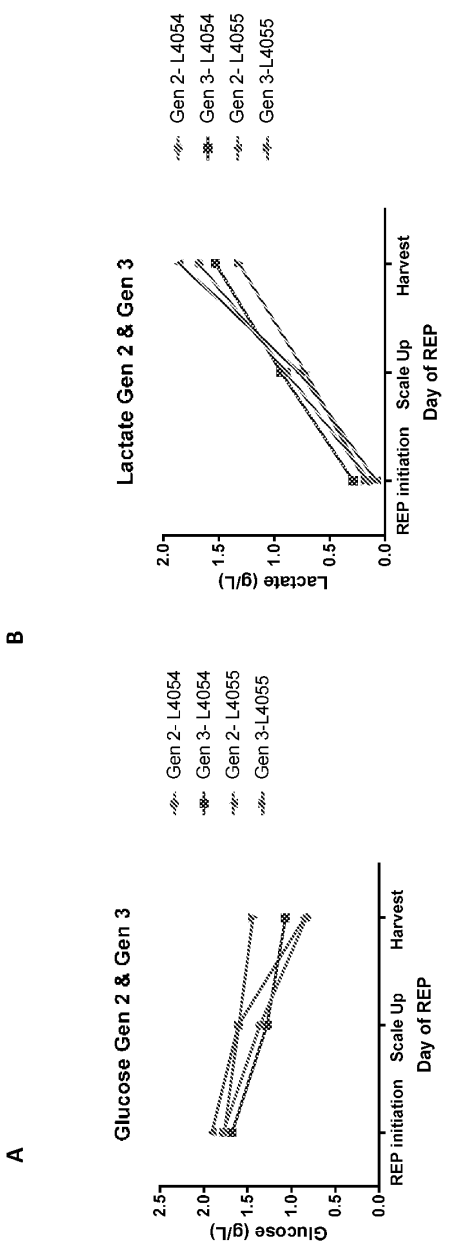
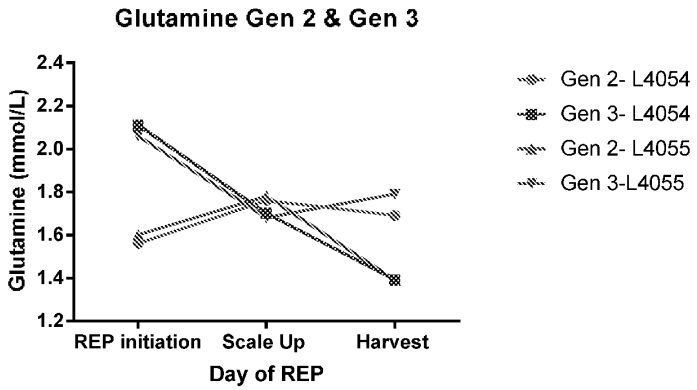
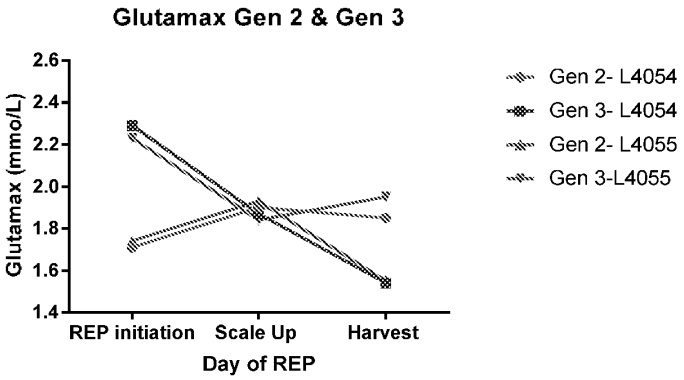

A
Glucose Gen 2 & Gen 3
Glucose (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2- L4054
Gen 3- L4054
Gen 2- L4055
Gen 3-L4055
B
Lactate Gen 2 & Gen 3
Lactate (g/L)
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2- L4054
Gen 3- L4054
Gen 2- L4055
Gen 3-L4055

Figure 94

A


Glutamine Gen 2 & Gen 3
Glutamine (mmol/L)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
REP initiation
Scale Up
Harvest
Day of REP
Gen 2- L4054
Gen 3- L4054
Gen 2- L4055
Gen 3-L4055


Glutamax Gen 2 & Gen 3
Glutamax (mmol/L)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
REP initiation
Scale Up
Harvest
Day of REP
Gen 2- L4054
Gen 3- L4054
Gen 2- L4055
Gen 3-L4055

B

Figure 94

C

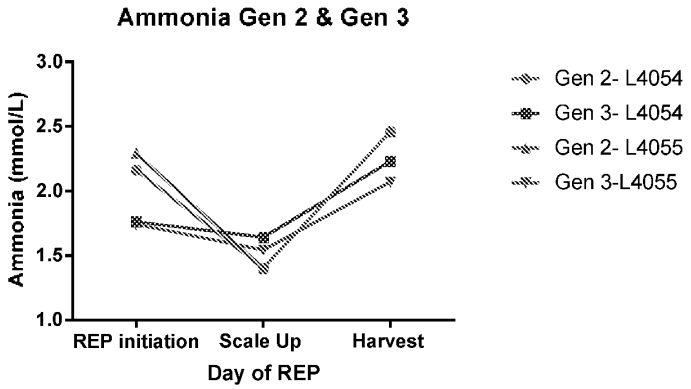

Ammonia Gen 2 & Gen 3
Ammonia (mmol/L)
3.0
2.5
2.0
1.5
1.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2- L4054
Gen 3- L4054
Gen 2- L4055
Gen 3-L4055

Figure 95

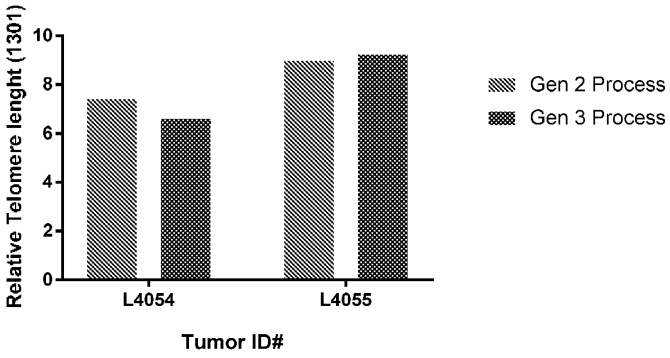

Relative Telomere lenght (1301)
10
8
6
4
2
0
L4054
L4055
Tumor ID#
Gen 2 Process
Gen 3 Process

Figure 96

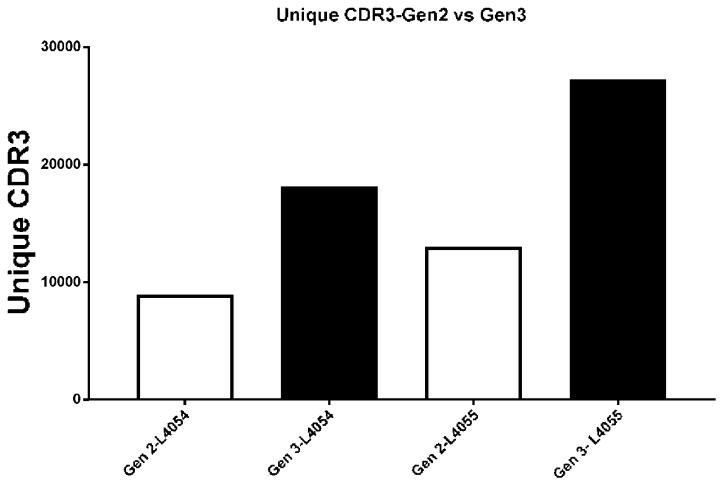
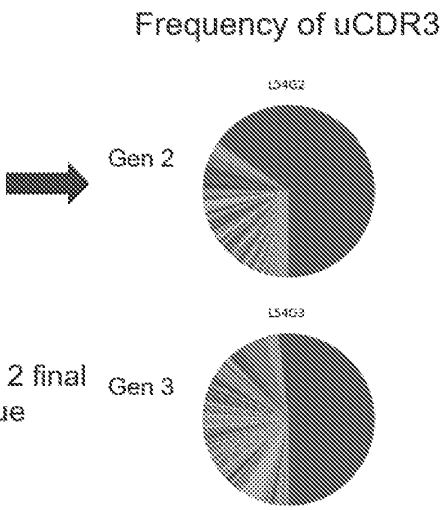

Unique CDR3-Gen2 vs Gen3
Unique CDR3
30000
20000
10000
0
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3- L4055

Figure 97

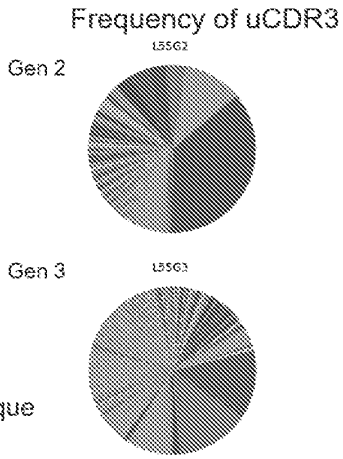
# uCDR3 (% Overlap)	All uCDR3's		Top 80% uCDR3's	
	Gen 2	Gen 3	Gen 2	Gen 3
Gen 2-L4054	8915	4355 **(48.85%)**	205	199 **(97.07%)**
Gen 3-L4054	-	18130	-	223



- 199 sequences are shared between Gen 3 and Gen 2 final product, corresponding to 97.07% of top 80% of unique CDR3 sequences from Gen 2 shared with Gen 3 final product.

Figure 98

# uCDR3 (% Overlap)	All uCDR3's		Top 80% uCDR3's	
	Gen 2	Gen 3	Gen 2	Gen 3
Gen 2-L4055	12996	6599 (50.77%)	1843	1833 (99.45%)
Gen 3-L4055	-	27246	-	2616



- 1833 sequences are shared between Gen 3 and Gen 2 final product, corresponding to 99.45% of top 80% of unique CDR3 sequences from Gen 2 shared with Gen 3 final product.

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Figure 99

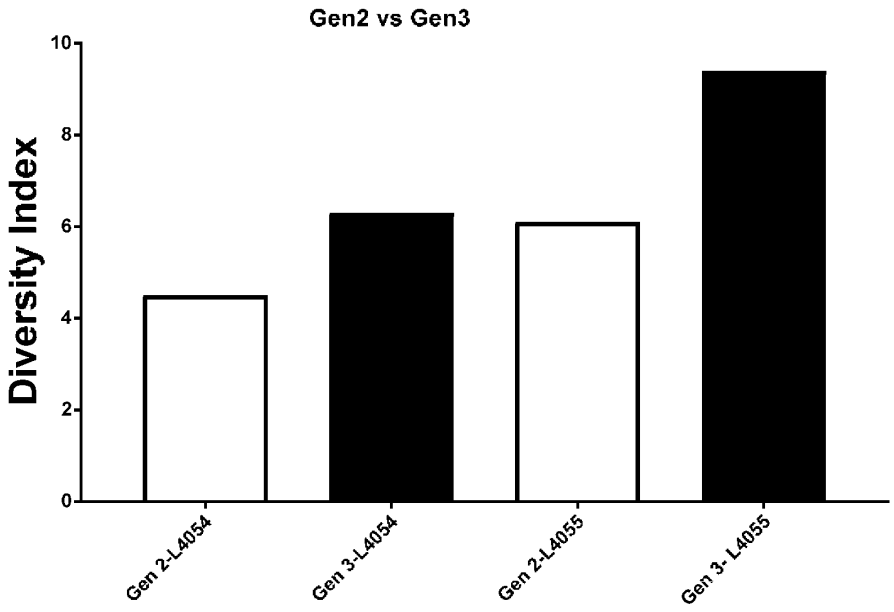

Gen2 vs Gen3
Diversity Index
10
8
6
4
2
0
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3- L4055

Figure 100

Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
188	Total	2.46E+05	2.28E+05	2.31E+05	2.35E+05	4.42E+07
	Live	2.37E+05	2.27E+05	2.26E+05	2.30E+05	4.32E+07
	Dead	9.26E+03	7.03E+02	4.63E+03	4.86E+03	9.14E+05
	% Viability	96.20%	99.70%	98.00%	97.97%	
188	Total	8.26E+04	8.00E+04	7.66E+04	7.97E+04	1.50E+07
	Live	7.60E+04	7.54E+04	6.89E+04	7.34E+04	1.38E+07
	Dead	6.61E+03	4.63E+03	7.73E+03	6.32E+03	1.19E+06
	% Viability	92.00%	94.20%	89.90%	92.03%	
200	Total	2.44E+04	4.20E+04	1.05E+04	2.56E+04	5.13E+06
	Live	1.74E+04	2.80E+04	7.03E+03	1.75E+04	3.50E+06
	Dead	7.03E+03	1.40E+04	3.48E+03	8.17E+03	1.63E+06
	% Viability	71.20%	66.70%	66.90%	68.27%	

Figure 101

ID	Volume (mL)		Count 1 (cells/mL)	Count 3 (cells/mL)	Count 2 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4054 First Round	1000	Total	9.11E+05	1.01E+06	1.08E+06	1.00E+06	1.00E+09
		Live	8.60E+05	9.34E+05	1.01E+06	9.35E+05	9.35E+08
		Dead	5.09E+04	7.49E+04	6.74E+04	6.44E+04	6.44E+07
		% Viability	94.40%	92.60%	93.70%	93.57%	
Gen 3 #L4055 Second Round	1000	Total	8.56E+05	9.04E+05	8.81E+05	8.80E+05	8.80E+08
		Live	8.24E+05	8.67E+05	8.42E+05	8.44E+05	8.44E+08
		Dead	3.17E+04	3.70E+04	3.87E+04	3.58E+04	3.58E+07
		% Viability	96.30%	95.90%	95.60%	95.93%	
Gen 3 #M1085T	200	Total	2.32E+06	2.25E+06		2.29E+06	4.57E+08
		Live	1.68E+06	1.57E+06		1.63E+06	3.25E+08
		Dead	6.41E+05	6.79E+05		6.60E+05	1.32E+08
		% Viability	72.40%	69.90%		71.15%	
Gen 2 #L4054 First Round	142	Total	1.08E+06	9.84E+05	1.00E+06	1.02E+06	1.45E+08
		Live	1.06E+06	9.71E+05	9.79E+05	1.00E+06	1.42E+08
		Dead	2.05E+04	1.26E+04	2.12E+04	1.81E+04	2.57E+06
		% Viability	98.10%	98.70%	97.90%	98.23%	
Gen 2 #L4055 Second Round	96	Total	2.93E+05	3.05E+05	2.64E+05	2.87E+05	2.76E+07
		Live	2.88E+05	2.96E+05	2.55E+05	2.80E+05	2.68E+07
		Dead	4.72E+03	9.14E+03	8.26E+03	7.37E+03	7.08E+05
		% Viability	98.40%	97.00%	96.90%	97.43%	
Gen 2 #M1085T	200	Total	9.10E+04	5.60E+04		7.35E+04	1.47E+07
		Live	8.05E+04	4.21E+04		6.13E+04	1.23E+07
		Dead	1.04E+04	1.39E+04		1.22E+04	2.43E+06
		% Viability	88.50%	75.20%		81.85%	

Figure 102

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4054 First Round -pre LOVO	1243.1	Total	1.40E+07	1.46E+07		1.43E+07	1.78E+10
		Live	1.32E+07	1.35E+07		1.34E+07	1.66E+10
		Dead	8.47E+05	1.12E+06		9.84E+05	1.22E+09
		% Viability	94.00%	92.40%		93.20%	
Gen 3 #L4054 First Round -post LOVO	330	Total	6.35E+07	6.07E+07		6.21E+07	2.05E+10
		Live	5.72E+07	5.44E+07		5.58E+07	1.84E+10
		Dead	6.35E+06	6.27E+06		6.31E+06	2.08E+09
		% Viability	90.00%	89.70%		89.85%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #L4055 Second Round -pre LOVO	1032.6	Total	9.49E+06	1.05E+07		1.00E+07	1.03E+10
		Live	8.90E+06	9.79E+06		9.35E+06	9.65E+09
		Dead	5.85E+05	6.75E+05		6.30E+05	6.51E+08
		% Viability	93.80%	93.60%		93.70%	
Gen 3 #L4055 Second Round -post LOVO	330	Total	3.31E+07	2.82E+07	3.18E+07	3.07E+07	1.01E+10
		Live	2.85E+07	2.46E+07	2.77E+07	2.66E+07	8.76E+09
		Dead	4.56E+06	3.61E+06	4.12E+06	4.09E+06	1.35E+09
		% Viability	86.20%	87.20%	87.10%	86.70%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 3 #M1085T pre-LOVO	5000	Total	2.26E+06	2.64E+06		2.45E+06	1.23E+10
		Live	1.97E+06	2.31E+06		2.14E+06	1.07E+10
		Dead	2.85E+05	3.30E+05		3.08E+05	1.54E+09
		% Viability	87.40%	87.50%		87.45%	
Gen 3 #M1085T - post LOVO pre CS10 addition	150	Total	6.27E+07	5.44E+07		5.86E+07	8.78E+09
		Live	5.50E+07	4.74E+07		5.12E+07	7.68E+09
		Dead	7.70E+06	6.96E+06		7.33E+06	1.10E+09
		% Viability	87.70%	87.20%		87.45%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4054 First Round	913	Total	3.53E+06	4.30E+06		3.92E+06	3.57E+09
		Live	3.35E+06	4.00E+06		3.68E+06	3.36E+09
		Dead	1.75E+05	3.03E+05		2.39E+05	2.18E+08
		% Viability	95.00%	93.00%		94.00%	
Gen 2 #L4055 Second Round	292	Total	1.29E+07	1.36E+07		1.33E+07	3.87E+09
		Live	1.16E+07	1.23E+07		1.20E+07	3.49E+09
		Dead	1.27E+06	1.23E+06		1.25E+06	3.65E+08
		% Viability	90.10%	90.90%		90.50%	
Gen 2 #M1085T	369	Total	6.75E+06	6.98E+06		6.87E+06	2.53E+09
		Live	5.22E+06	5.58E+06		5.40E+06	1.99E+09
		Dead	1.54E+06	1.40E+06		1.47E+06	5.42E+08
		% Viability	77.20%	79.90%		78.55%	

Figure 103

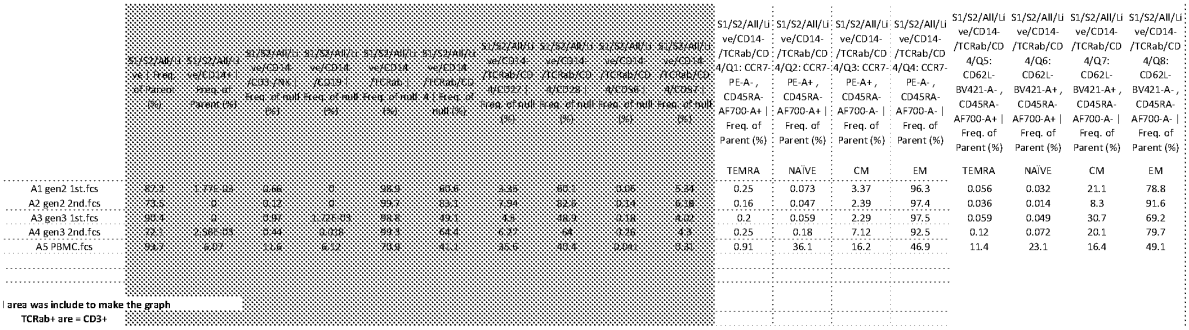
ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4054 First Round -pre LOVO	1385.7	Total	3.55E+07	4.12E+07	4.03E+07	3.90E+07	5.40E+10
		Live	3.26E+07	3.73E+07	3.67E+07	3.55E+07	4.92E+10
		Dead	2.93E+06	3.87E+06	3.59E+06	3.46E+06	4.80E+09
		% Viability	91.70%	90.60%	91.10%	91.13%	
Gen 2 #L4054 First Round -post LOVO	330	Total	1.70E+08	1.79E+08	1.68E+08	1.72E+08	5.69E+10
		Live	1.49E+08	1.58E+08	1.48E+08	1.52E+08	5.01E+10
		Dead	2.16E+07	2.04E+07	2.00E+07	2.07E+07	6.82E+09
		% Viability	87.30%	88.60%	88.10%	87.95%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #L4055 First Round -pre LOVO	1968.2	Total	3.15E+07	2.51E+07	2.97E+07	2.88E+07	5.66E+10
		Live	2.89E+07	2.25E+07	2.72E+07	2.62E+07	5.16E+10
		Dead	2.57E+06	2.61E+06	2.52E+06	2.57E+06	5.05E+09
		% Viability	91.80%	89.60%	91.50%	90.97%	
Gen 2 #L4055 First Round -post LOVO	330	Total	2.33E+08	1.89E+08	1.53E+08	1.92E+08	6.33E+10
		Live	2.03E+08	1.66E+08	1.33E+08	1.67E+08	5.52E+10
		Dead	3.00E+07	2.24E+07	1.94E+07	2.39E+07	7.90E+09
		% Viability	87.10%	88.10%	87.30%	87.50%	

ID	Volume (mL)		Count 1 (cells/mL)	Count 2 (cells/mL)	Count 3 (cells/mL)	Average (cells/mL)	Total Cells Average*Volume
Gen 2 #M1085T-pre LOVO	N/A	Total				#DIV/0!	#DIV/0!
		Live				#DIV/0!	#DIV/0!
		Dead				#DIV/0!	#DIV/0!
		% Viability				#DIV/0!	
Gen 2 #M1085T - post LOVO pre CS10 addition	150	Total	8.51E+07	9.05E+07		8.78E+07	1.32E+10
		Live	7.33E+07	7.79E+07		7.56E+07	1.13E+10
		Dead	1.18E+07	1.26E+07		1.22E+07	1.83E+09
		% Viability	86.10%	86.10%		86.10%	

For L4054 Gen 2, post LOVO count was extrapolated to 4 flasks, because was the total number of the study. 1 flask was contaminated, and the extrapolation was done for total = 6.67E+10

Figure 104



	S1/S2/All/Live/CD14-/TCRab/CD8/Q1: CCR7-PE-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q2: CCR7-PE-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q3: CCR7-PE-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q4: CCR7-PE-A-, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q5: CD62L-BV421-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q6: CD62L-BV421-A+, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q7: CD62L-BV421-A+, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD8/Q8: CD62L-BV421-A-, CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRgd | Freq. of null (%)
	TEMRA	NAÏVE	CM	EM	TEMRA	NAÏVE	CM	EM	
A1 gen2 1st.fcs	0.25	0.073	3.37	96.3	0.056	0.032	21.1	78.8	0
A2 gen2 2nd.fcs	0.16	0.047	2.39	97.4	0.036	0.014	8.3	91.6	0
A3 gen3 1st.fcs	0.2	0.059	2.29	97.5	0.059	0.049	30.7	69.2	0
A4 gen3 2nd.fcs	0.25	0.18	7.12	92.5	0.12	0.072	20.1	79.7	0
A5 PBMC.fcs	0.91	36.1	16.2	46.9	11.4	23.1	16.4	49.1	0

I area was include to make the graph
TCRab+ are = CD3+

Figure 105

	CD3/CD4 CD38	HLA-DR	CCR7- CD45RA+	CCR7+CD45RA+	CCR7+ CD45RA-	CCR7-CD45RA-
GEN 3-Day 16-Harvest	42.4	95.2	4.96	0.7	1.22	93.1
GEN 2-D22-Harvest	50.4	98	0.61	0.13	0.3	99

CD3	CD3/CD8						
CD8	CD38	HLA-DR	CCR7-CD45RA+	CCR7+CD45RA+	CCR7+CD45RA-	CCR7-CD45RA-	
77.6	83.2	90.3	20.5	0.013	0.06	79.5	
54.3	88	83.2	4.24	0.026	1.05	94.7	

Figure 106

			CD4											
	CD3+	CD4+	2B4+	BTLA+	CD103+	CD25+	CD69+	CD95+	CXCR3+	KLRG1+	LAG3+	PD1+	TIGIT+	TIM3+
Gen 2-L4054			0.61	99.50	1.05	14.80	43.20	99.40	52.90	2.65	5.36	81.20	88.40	78.60
Gen 3-L4054			1.11	99.50	2.28	21.70	45.40	99.70	56.90	8.06	5.07	76.70	74.10	79.30
Gen 2-L4055			1.61	98.60	1.56	23.10	51.10	99.80	63.30	3.86	15.30	77.60	86.00	79.50
Gen 3- L4055			3.57	99.30	2.61	54.40	57.00	99.90	48.00	8.02	10.80	61.40	47.50	84.60
			CD8											
Gen 2-L4054		CD8+	2B4+	BTLA4+	CD103+	CD25+	CD95+	CD69+	CXCR3+	KLRG1+	LAG3+	PD1+	TIGIT+	TIM3+
Gen 3-L4054			35.20	99.50	74.90	11.80	99.80	58.30	99.10	8.03	33.10	80.60	99.10	88.30
Gen 2-L4055			45.50	99.60	82.40	12.10	99.90	54.00	99.30	13.40	22.10	84.90	98.50	87.50
Gen 3- L4055			40.60	98.20	19.50	32.00	99.70	75.50	96.80	22.80	27.40	28.10	92.30	75.10
			62.80	99.20	29.20	45.50	99.70	58.80	99.60	13.10	16.60	34.10	71.70	63.60

Figure 107

	1	2	3	4	5	6	7	8	9	10	11	12	
A	STD1	STD1	STD1	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	MC-Stimulated xG2	MC-Stimulated xG2	MC-Stimulated xG2	Well ID
	1000	1000	1000	3	3	3	3	3	3	3	3	3	Conc/Dil
B	STD2	STD2	STD2	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	MC-Stimulated xG2	MC-Stimulated xG2	MC-Stimulated xG2	Well ID
	500	500	500	9	9	9	9	9	9	9	9	9	Conc/Dil
C	STD3	STD3	STD3	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	MC-Unstimulated xG2	MC-Unstimulated xG2	MC-Unstimulated xG2	Well ID
	250	250	250	27	27	27	27	27	27	3	3	3	Conc/Dil
D	STD4	STD4	STD4	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-stimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	TIL- Gen 2-Unstimulated	MC-Unstimulated xG2	MC-Unstimulated xG2	MC-Unstimulated xG2	Well ID
	125	125	125	81	81	81	81	81	81	9	9	9	Conc/Dil
E	STD5	STD5	STD5	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	MC-Stimulated xG3	MC-Stimulated xG3	MC-Stimulated xG3	Well ID
	62.5	62.5	62.5	3	3	3	3	3	3	3	3	3	Conc/Dil
F	STD6	STD6	STD6	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	MC-Stimulated xG3	MC-Stimulated xG3	MC-Stimulated xG3	Well ID
	31.3	31.3	31.3	9	9	9	9	9	9	9	9	9	Conc/Dil
G	STD7	STD7	STD7	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	MC-Unstimulated xG3	MC-Unstimulated xG3	MC-Unstimulated xG3	Well ID
	15.6	15.6	15.6	27	27	27	27	27	27	3	3	3	Conc/Dil
H	STD8	STD8	STD8	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-stimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	TIL- Gen 3-Unstimulated	MC-Unstimulated xG3	MC-Unstimulated xG3	MC-Unstimulated xG3	Well ID
	0	0	0	81	81	81	81	81	81	9	9	9	Conc/Dil

22.6
22.6

	1	2	3	4	5	6	7	8	9	10	11	12	
A	2.866	2.882	2.886	2.797	2.164	2.918	0.117	0.116	0.117	0.105	0.108	0.102	450
	0.05	0.05	0.05	0.05	0.048	0.051	0.041	0.043	0.041	0.041	0.043	0.041	570
	2.816	2.832	2.836	2.746	2.117	2.867	0.076	0.074	0.076	0.064	0.065	0.06	Delta
	994.773	1002.343	1004.079	963.405	694.982	1018.375	1.825	1.421	1.952	<0.000	<0.000	<0.000	[Concentration]
				2890.216	2084.946	3055.126	5.474	4.262	5.855	<0.000	<0.000	<0.000	x dil factor
B	1.659	1.654	1.65	1.181	0.9	1.215	0.107	0.107	0.108	0.106	0.105	0.102	450
	0.046	0.046	0.046	0.045	0.047	0.045	0.042	0.041	0.042	0.043	0.042	0.042	570
	1.613	1.608	1.604	1.136	0.853	1.17	0.066	0.066	0.067	0.062	0.063	0.061	Delta
	499.866	497.826	496.528	329.765	235.273	341.438	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	[Concentration]
				2967.887	2117.46	3072.942	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	x dil factor
C	0.952	0.966	0.94	0.494	0.391	0.505	0.106	0.107	0.107	0.107	0.105	0.103	450
	0.044	0.048	0.043	0.042	0.042	0.043	0.041	0.042	0.042	0.043	0.042	0.042	570
	0.908	0.919	0.896	0.452	0.349	0.462	0.065	0.065	0.065	0.063	0.063	0.062	Delta
	253.244	256.8	249.399	109.334	78.83	112.411	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	[Concentration]
				2952.028	2128.412	3035.11	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	x dil factor
D	0.549	0.541	0.542	0.258	0.218	0.259	0.102	0.101	0.103	0.111	0.102	0.101	450
	0.044	0.044	0.043	0.042	0.041	0.047	0.041	0.042	0.042	0.05	0.042	0.043	570
	0.505	0.498	0.499	0.216	0.176	0.212	0.06	0.059	0.061	0.061	0.06	0.057	Delta
	125.458	123.174	123.508	40.262	29.15	39.101	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	[Concentration]
				3261.251	2361.138	3167.212	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	x dil factor
E	0.334	0.332	0.332	OVRFLW	OVRFLW	OVRFLW	0.114	0.11	0.113	0.103	0.102	0.104	450
	0.043	0.042	0.042	0.07	0.068	0.069	0.041	0.041	0.042	0.042	0.042	0.043	570
	0.291	0.29	0.29	?????	?????	?????	0.073	0.069	0.072	0.062	0.061	0.061	Delta
	61.826	61.537	61.594	?????	?????	?????	1.145	0.285	0.822	<0.000	<0.000	<0.000	[Concentration]
				?????	?????	?????	3.435	0.854	2.465	<0.000	<0.000	<0.000	x dil factor
F	0.222	0.222	0.224	3.84	3.696	3.853	0.114	0.104	0.107	0.101	0.103	0.101	450
	0.042	0.043	0.042	0.058	0.056	0.057	0.041	0.041	0.042	0.042	0.041	0.042	570
	0.18	0.179	0.181	3.782	3.638	3.796	0.073	0.063	0.066	0.059	0.062	0.059	Delta
	30.212	29.82	30.519	1474.838	>1500.000	1482.263	1.145	<0.000	<0.000	<0.000	<0.000	<0.000	[Concentration]
				13273.55	>13500.000	13340.37	10.304	<0.000	<0.000	<0.000	<0.000	<0.000	x dil factor
G	0.174	0.17	0.172	1.996	1.997	2.017	0.114	0.109	0.114	0.106	0.107	0.104	450
	0.041	0.041	0.041	0.048	0.047	0.049	0.042	0.042	0.041	0.042	0.041	0.042	570
	0.132	0.128	0.131	1.948	1.949	1.968	0.073	0.067	0.073	0.063	0.065	0.062	Delta
	16.911	15.874	16.447	627.703	628.253	635.61	1.12	<0.000	1.17	<0.000	<0.000	<0.000	[Concentration]
				16947.99	16962.84	17161.47	30.239	<0.000	31.588	<0.000	<0.000	<0.000	x dil factor
H	0.117	0.111	0.114	0.84	0.867	0.857	0.11	0.11	0.115	0.109	0.11	0.113	450
	0.045	0.043	0.044	0.044	0.044	0.044	0.041	0.042	0.043	0.042	0.043	0.042	570
	0.072	0.068	0.07	0.797	0.822	0.813	0.069	0.068	0.073	0.067	0.067	0.071	Delta
	0.822	0.073	0.405	216.931	225.269	222.13	0.119	0.05	1.145	<0.000	<0.000	0.748	[Concentration]
				17571.43	18246.77	17992.49	9.644	4.039	92.74	<0.000	<0.000	60.557	x dil factor

				Average	Average
Gen 2-Stimulated+TIL 1:3	2890.216	2084.946	3055.126	2676.76267	2757.81
Gen 2-Stimulated+TIL 1:9	2967.887	2117.46	3072.942	2719.42967	
Gen 2-Stimulated+TIL 1:27	2952.028	2128.412	3035.11	2705.18333	
Gen 2-Stimulated+TIL 1:81	3261.251	2361.138	3167.212	2929.867	
Gen 3-Stimulated+TIL 1:3	?????	?????	?????	#DIV/0!	#DIV/0!
Gen 3-Stimulated+TIL 1:9	13273.546	>13500.00	13340.37	13306.9575	16089.32
Gen 3-Stimulated+TIL 1:27	16947.994	16962.84	17161.47	17024.1	
Gen 3-Stimulated+TIL 1:81	17571.427	18246.77	17992.49	17936.8973	
Gen 2-Unstimulated 1:3	5.474	4.262	5.855	5.197	5.20
Gen 2-Unstimulated 1:9	<0.000	<0.000	<0.000	#DIV/0!	
Gen 2-Unstimulated 1:27	<0.000	<0.000	<0.000	#DIV/0!	
Gen 2-Unstimulated 1:81	<0.000	<0.000	<0.000	#DIV/0!	
Gen 3-Unstimulated 1:3	3.435	0.854	2.465	2.25133333	19.74
Gen 3-Unstimulated 1:9	10.304	<0.000	<0.000	10.304	
Gen 3-Unstimulated 1:27	30.239	<0.000	31.588	30.9135	
Gen 3-Unstimulated 1:81	9.644	4.039	92.74	35.4743333	
Gen 2-Media Control Stimulated 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
Gen 2-Media Control Stimulated 1:9	<0.000	<0.000	<0.000	#DIV/0!	
Gen 3-Media Control Stimulated 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
Gen 3-Media Control Stimulated 1:9	<0.000	<0.000	<0.000	#DIV/0!	
Gen 2-Media Control Unstimulated 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
Gen 2-Media Control Unstimulated 1:9	<0.000	<0.000	<0.000	#DIV/0!	
Gen 3-Media Control Unstimulated 1:3	<0.000	<0.000	<0.000	0	30.28
Gen 3-Media Control Unstimulated 1:9	<0.000	<0.000	60.557	60.557	

Figure 108

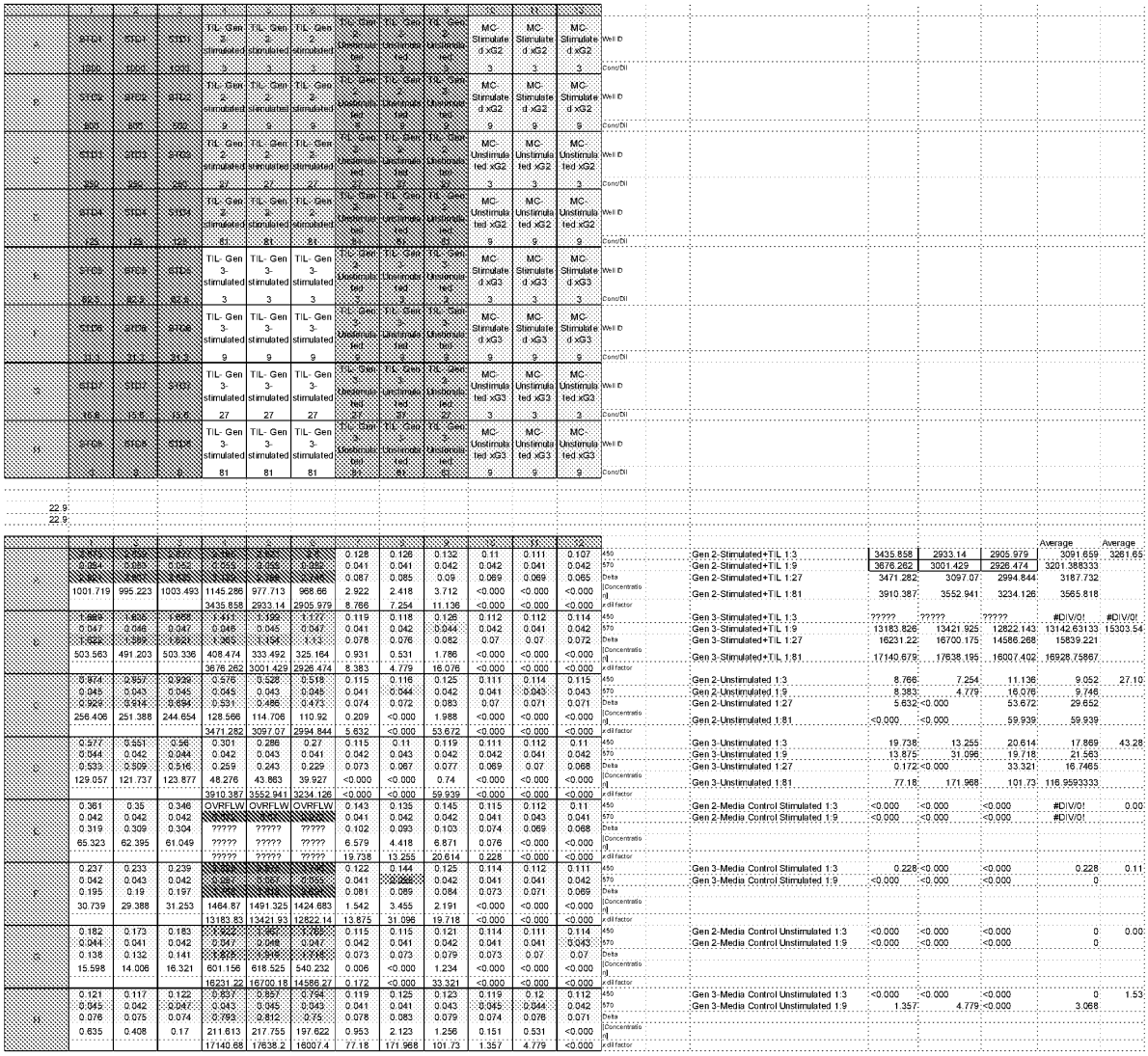


Figure 109

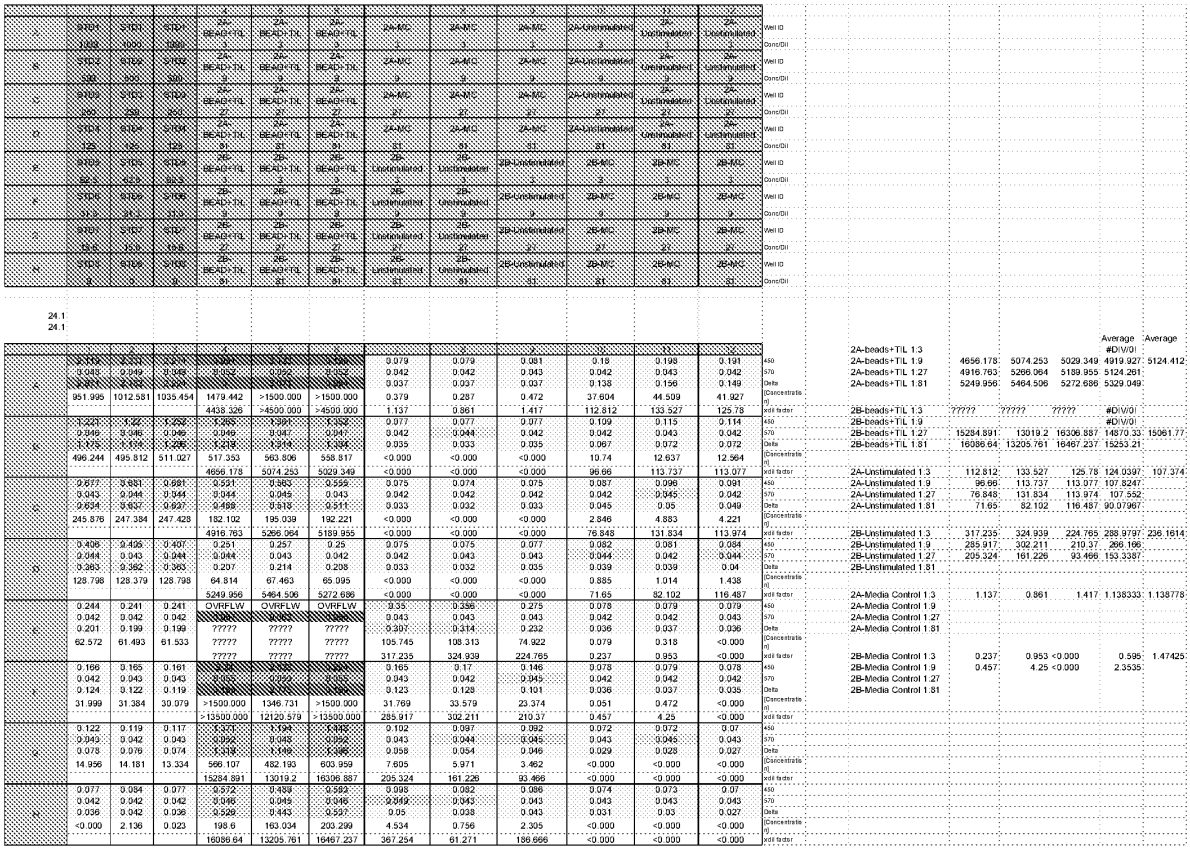
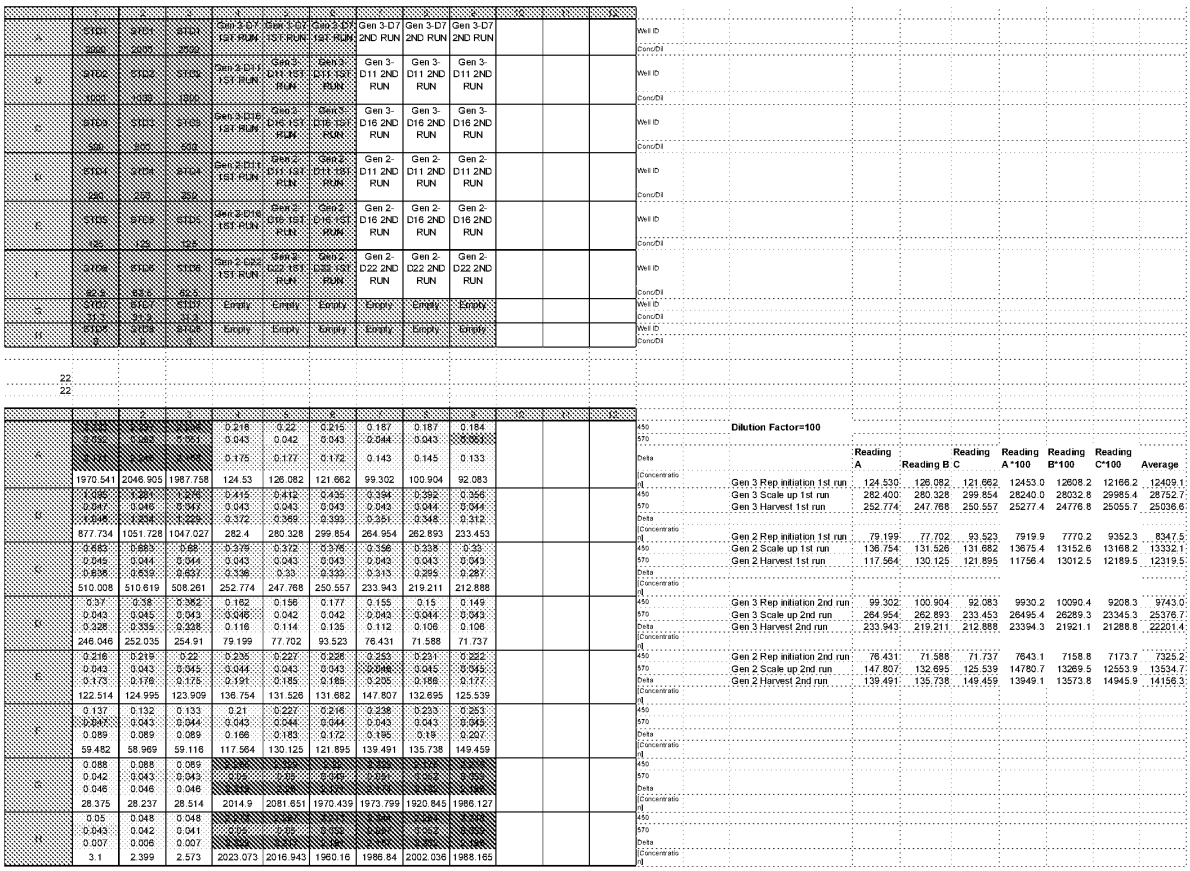


Figure 110



A	1	2	3	Gen 3-D7 1ST RUN	Gen 3-D7 1ST RUN	Gen 3-D7 1ST RUN	Gen 3-D7 2ND RUN	Gen 3-D7 2ND RUN	Gen 3-D7 2ND RUN			
B	4	5	6	Gen 3-D11 1ST RUN	Gen 3-D11 1ST RUN	Gen 3-D11 1ST RUN	Gen 3-D11 2ND RUN	Gen 3-D11 2ND RUN	Gen 3-D11 2ND RUN			
C	7	8	9	Gen 3-D16 1ST RUN	Gen 3-D16 1ST RUN	Gen 3-D16 1ST RUN	Gen 3-D16 2ND RUN	Gen 3-D16 2ND RUN	Gen 3-D16 2ND RUN			
D	10	11	12	Gen 2-D11 1ST RUN	Gen 2-D11 1ST RUN	Gen 2-D11 1ST RUN	Gen 2-D11 2ND RUN	Gen 2-D11 2ND RUN	Gen 2-D11 2ND RUN			
E	13	14	15	Gen 2-D16 1ST RUN	Gen 2-D16 1ST RUN	Gen 2-D16 1ST RUN	Gen 2-D16 2ND RUN	Gen 2-D16 2ND RUN	Gen 2-D16 2ND RUN			
F	16	17	18	Gen 2-D22 1ST RUN	Gen 2-D22 1ST RUN	Gen 2-D22 1ST RUN	Gen 2-D22 2ND RUN	Gen 2-D22 2ND RUN	Gen 2-D22 2ND RUN			
G	19	20	21	Empty	Empty	Empty	Empty	Empty	Empty			
H	22	23	24	Empty	Empty	Empty	Empty	Empty	Empty			

A	1	2	3	0.216	0.22	0.215	0.187	0.187	0.184			
	0.042	0.043	0.043	0.043	0.042	0.043	0.044	0.043	0.043			
	0.174	0.177	0.172	0.175	0.177	0.172	0.143	0.145	0.133			
	1970.541	2046.905	1987.758	124.53	126.082	121.662	99.302	100.904	92.083			
B	1.195	1.281	1.276	0.415	0.412	0.435	0.394	0.392	0.356			
	0.047	0.046	0.047	0.043	0.043	0.043	0.043	0.044	0.044			
	1.048	1.234	1.229	0.372	0.369	0.393	0.351	0.348	0.312			
	877.734	1051.728	1047.027	282.4	280.328	299.854	264.954	262.893	233.453			
C	0.683	0.683	0.68	0.375	0.372	0.376	0.356	0.358	0.33			
	0.045	0.044	0.044	0.043	0.043	0.043	0.043	0.043	0.043			
	0.638	0.639	0.637	0.332	0.33	0.333	0.313	0.295	0.287			
	510.008	510.619	508.261	252.774	247.768	250.557	233.943	219.211	212.888			
D	0.37	0.38	0.362	0.162	0.156	0.177	0.155	0.15	0.149			
	0.043	0.045	0.043	0.046	0.042	0.042	0.043	0.044	0.043			
	0.328	0.335	0.328	0.116	0.114	0.135	0.112	0.106	0.106			
	246.046	252.035	254.91	79.199	77.702	93.523	76.431	71.588	71.737			
E	0.216	0.219	0.22	0.235	0.227	0.228	0.253	0.231	0.222			
	0.042	0.043	0.045	0.044	0.043	0.043	0.046	0.045	0.045			
	0.173	0.176	0.175	0.191	0.185	0.185	0.205	0.186	0.177			
	122.514	124.995	123.909	136.754	131.526	131.682	147.807	132.695	125.539			
F	0.137	0.132	0.133	0.21	0.227	0.216	0.238	0.233	0.253			
	0.043	0.043	0.044	0.043	0.044	0.044	0.043	0.043	0.045			
	0.089	0.089	0.089	0.166	0.183	0.172	0.195	0.19	0.207			
	59.482	58.969	59.116	117.564	130.125	121.895	139.491	135.738	149.459			
G	0.088	0.088	0.089	0.205	0.205	0.205	0.235	0.235	0.235			
	0.042	0.043	0.043	0.05	0.05	0.05	0.045	0.045	0.045			
	0.046	0.046	0.046	0.155	0.155	0.155	0.19	0.19	0.19			
	28.375	28.237	28.514	2014.9	2081.651	1970.439	1973.799	1920.845	1986.127			
H	0.05	0.048	0.048	0.205	0.205	0.205	0.235	0.235	0.235			
	0.043	0.042	0.041	0.05	0.05	0.05	0.045	0.045	0.045			
	0.007	0.006	0.007	0.155	0.155	0.155	0.19	0.19	0.19			
	3.1	2.399	2.573	2023.073	2016.943	1960.16	1986.84	2002.036	1988.165			

Dilution Factor=100

	Reading A	Reading B	Reading C	Reading A*100	Reading B*100	Reading C*100	Average
Gen 3 Rep initiation 1st run	124.530	126.082	121.662	12453.0	12608.2	12166.2	12409.1
Gen 3 Scale up 1st run	282.400	280.328	299.854	28240.0	28032.8	29985.4	28752.7
Gen 3 Harvest 1st run	252.774	247.768	250.557	25277.4	24776.8	25055.7	25036.6
Gen 2 Rep initiation 1st run	79.199	77.702	93.523	7919.9	7770.2	9352.3	8347.5
Gen 2 Scale up 1st run	136.754	131.526	131.682	13675.4	13152.6	13168.2	13332.1
Gen 2 Harvest 1st run	117.564	130.125	121.895	11756.4	13012.5	12189.5	12319.5
Gen 3 Rep initiation 2nd run	99.302	100.904	92.083	9930.2	10090.4	9208.3	9743.0
Gen 3 Scale up 2nd run	264.954	262.893	233.453	26495.4	26289.3	23345.3	25376.7
Gen 3 Harvest 2nd run	233.943	219.211	212.888	23394.3	21921.1	21288.8	22201.4
Gen 2 Rep initiation 2nd run	76.431	71.588	71.737	7643.1	7158.8	7173.7	7325.2
Gen 2 Scale up 2nd run	147.807	132.695	125.539	14780.7	13269.5	12553.9	13534.7
Gen 2 Harvest 2nd run	139.491	135.738	149.459	13949.1	13573.8	14945.9	14156.3

Figure 111

Sample number #	Description	Glucose (g/L)	Lactate (g/L)	Ammonia (mmol/L)	Glutamine (mmol/L)	Glutamax (mmol/L)	Glutamax-Glutamine (mmol/L)
1	Gen 2-Rep initiation First Round #L4054	1.78	0.14	2.16	1.56	1.71	0.16
2	Gen 2-Scale Up First Round #L4054	1.36	0.89	1.40	1.76	1.90	0.15
3	Gen 2-Harvest First Round #L4054	0.83	1.68	2.46	1.69	1.85	0.17
4	Gen 3-REP initiation First Round #L4054	1.68	0.29	1.76	2.11	2.29	0.18
5	Gen 3-Scale Up First Round #L4054	1.28	0.94	1.64	1.70	1.87	0.16
6	Gen 3-Harvest First Round #L4054	1.07	1.53	2.23	1.39	1.54	0.14
7	Gen 2-Rep initiation Second Round #L4055	1.91	0.08	2.29	1.60	1.74	0.14
8	Gen 2-Scale up Second Round #L4055	1.61	0.77	1.40	1.78	1.93	0.15
9	Gen 2-Harvest Second Round #L4055	0.85	1.86	2.46	1.39	1.55	0.16
10	Gen 3-REP initiation Second Round #L4055	1.77	0.18	1.74	2.06	2.23	0.17
11	Gen 3-Scale up Second Round #L4055	1.60	0.72	1.54	1.68	1.84	0.15
12	Gen 3-Harvest Second Round #L4055	1.44	1.32	2.07	1.79	1.95	0.16

Figure 112

	$FIL	S/L/1301 | Geometr	S/L/Pt | Geometric Mean (FL03-A)
A1 gen2L4054 ne	A1 gen2L4054 neg.fcs	34.5	3.24
A2 gen2L4054 ne	A2 gen2L4054 neg.fcs	34	3.19
A3 gen2L4054 po	A3 gen2L4054 pos.fcs	1834	70.6
A4 gen2L4054 po	A4 gen2L4054 pos.fcs	1872	70.3
A5 gen2L4055 ne	A5 gen2L4055 neg.fcs	31.9	4.23
A6 gen2L4055 ne	A6 gen2L4055 neg.fcs	32.4	4.87
A7 gen2L4055 po	A7 gen2L4055 pos.fcs	1941	88.7
A8 gen2L4055 po	A8 gen2L4055 pos.fcs	1933	91.1
B1 gen3L4054 ne	B1 gen3L4054 neg.fcs	31.2	3.45
B2 gen3L4054 ne	B2 gen3L4054 neg.fcs	31.9	3.39
B3 gen3L4054 po	B3 gen3L4054 pos.fcs	2016	61.3
B4 gen3L4054 po	B4 gen3L4054 pos.fcs	2014	76.2
B5 gen3L4055 ne	B5 gen3L4055 neg.fcs	33.7	6.76
B6 gen3L4055 ne	B6 gen3L4055 neg.fcs	35.4	6.7
B7 gen3L4055 po	B7 gen3L4055 pos.fcs	2158	99.4
B8 gen3L4055 po	B8 gen3L4055 pos.fcs	1938	99.5
Mean	-	998	43.3
SD	-	999	41.3

Figure 113

Study	Sample	Sample id	Species	Chain	Reads	CDR3	Unique CDR3	D50
20180511_Study1_FCA	Gen2-L4054	59990	TRB	h	1181732	1181732	8915	0
20180511_Study1_FCA	Gen3-L4054	59991	TRB	h	1145697	1145697	18130	0
20180511_Study1_FCA	Gen2-L4055	59987	TRB	h	1166465	1166465	12996	0.1
20180511_Study1_FCA	Gen3-L4055	59982	TRB	h	1059985	1059985	27246	0.9

Figure 114

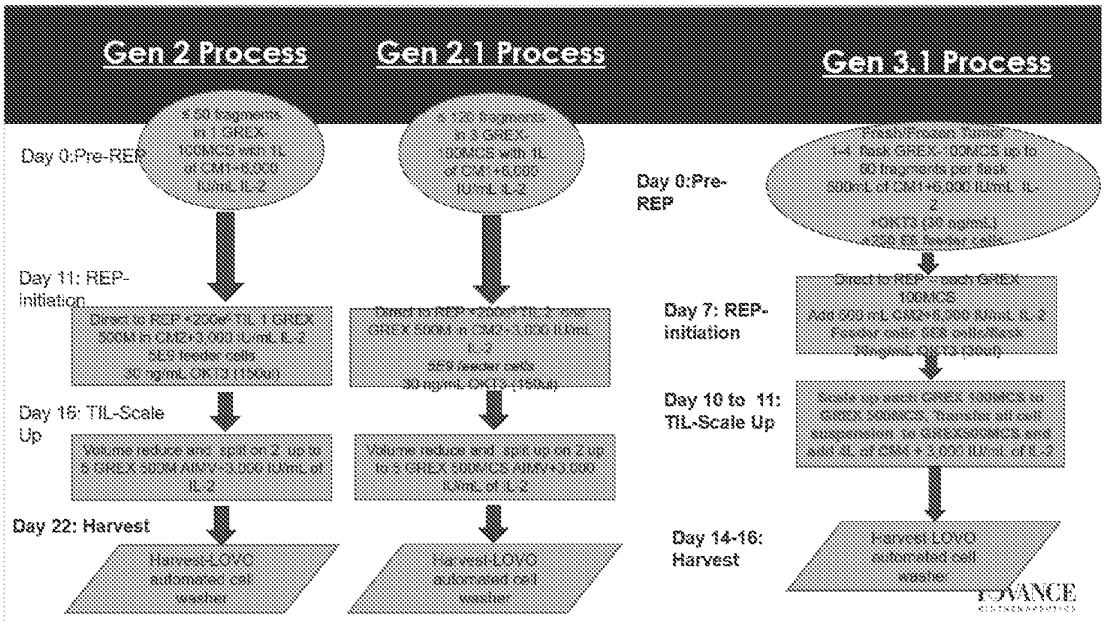

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
Day 11: REP-Initiation
Day 16: TIL-Scale Up
Day 22: Harvest
Harvest-LOVO automated cell washer
Day 0:Pre-REP
Day 7: REP-Initiation
Day 10 to 11: TIL-Scale Up
Day 14-16: Harvest
Harvest-LOVO automated cell washer
IOVANCE

Figure 115

STEP	Gen 2	Gen 2.1	Gen 3.0
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 160 fragments/ 3 G-Rex Pre-formulated CM1 warmed media 100MCS - **11 days**	***Fresh or Frozen Tumor*** Whole tumor with 5 30 fragments up to 60 fragments per 1 G-Rex 100MCS (up to 4 G-Rex), preformulated warmed media - **7 days. Pre REP. Feeders 2.5 E8 cells + OKT-3 (30ng/mL)**
REP Initiation	Direct to REP- Day 11- **<200 E6 TIL** 1 G-Rex 500MCS	Direct to REP- Day 11- **<200 E6 TIL** Pre-formulated CM2 warmed media in one G-Rex 500MCS	Direct to REP- Day **7-all cells TIL- same G-Rex 100MCS (100MCS up to 4 GREX), Standard media or Defined Media (Serum free). Addition Feeders 5 E8 cells +OKT-3 (30ng/mL)**
TIL propagation or Scale up	1 to 5 G-REX 500MCS **Split day 16**	2 to 5 G-REX 500MCS Pre-formulated CM4 warmed media **Split day 16**	From G-REX 100MCS transfer TIL suspension to G-REX 500MCS , up to 4 GREX 500 MCS- Standard media or Defined Media (Serum Free) **Scale up on day 10 or 11**
Harvest	**Harvest day 22,** LOVO-automated cell washer	**Harvest day 22,** LOVO-automated cell washer (5 wash cycle)	**Harvest day 14 or 16** LOVO- automated cell washer (5 wash cycle)
Final formulation	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved Product 300IU/ml IL2- CS10 in LN_2 multiple aliquots	Cryopreserved product 300IU/ml IL-2 CS10 in LN_2 multiple aliquots
Process time	**22 days**	**22 days**	**16 days**

Figure 116

Process Day	Conditions	Gen 3.1
Day 0- pre REP initiation	Media CM1	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL)	+
	Feeders (250 E+06)	+

Process Day	Conditions	Gen 3.1
Day 7- REP initiation	Media CM2	500 mL
	IL-2 (6000 IU/mL)	+
	OKT-3 (30ng/mL) added on Day 7	+
	Feeders Added on Day 7	500 E06
	Total Feeders at Day	750 E+06

Process Day	Conditions	Gen 3.1
Day 9-11 –Scale Up	From G-REX 100MCS transfer TIL suspension to 1 G-REX 500MCS (up to 3 GREX 500MCS)	Yes
Day 16- Harvest	LOVO- automated cell washer	Yes

Figure 117

Process Comparison	Key Process Changes	Benefit
Gen 2 : Gen 2.1	• Initiate process with two flasks instead of one flask • Divide REP initiation feeder layer between 2 G-Rex500MCS Flasks • Pre-formulate media and warm prior to use	• Potential doubling of final cell count (dose) with increased TIL repertoire. • Process redundancy throughout process
Gen 2.1 : Gen 3.1	• Fresh or Frozen tumor • 14-16 day process (from 22 day) • Reduce total feeder layer on process • Feeder layer and OKT3 present at Day 0 • REP initiated with fragments • 100MCS scales to 500MCS • Scales to multiple pre-REP flasks • Standard Media and Defined Media (Serum Free)	• Increased potency • Improved phenotype • Decreased process time • Reduced reagent testing • Decreased process variability • Defined reagents • Increased repertoire • Reduce impurities (feeder) • Comparable or Higher Dose.

Figure 118

Process Comparison	Key Process Changes	Desired Improvement	Criteria for Success	Outcome
Gen 2 : Gen 3.0	• 14-16 days • Initiate REP with fragments up to 4 flask. • 100MCS scales to 500MCS	• Increased potency • Improved phenotype • Decreased process time	• Increase potency as measured by INF-g ✓ • Comparable phenotype ✓ • Comparable Dose ✓ • Comparable purity ✓ (feeder cell) • Maintain clonal diversity ✓	• Potency increased over Gen2 • Improved expression of CD28 on CD8 cells • Maximum capacity of flask reached by day 16 on Gen 3.1 • Reduced feeder cell usage • Increased diversity

Figure 119

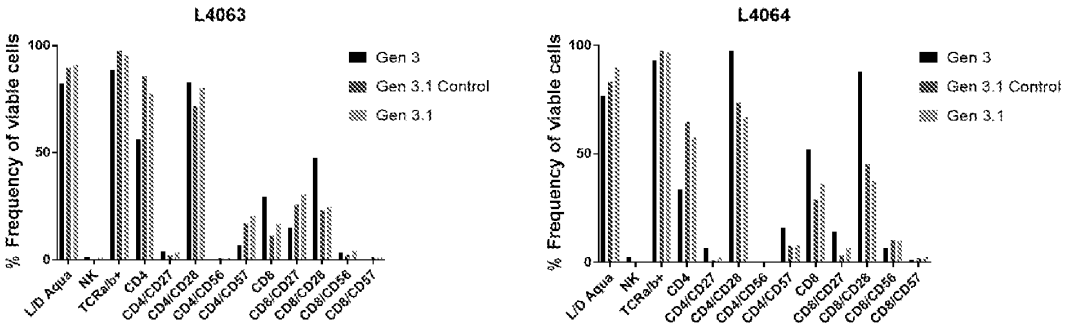
Process	Gen 2	Gen 3
L4054	Standard Media	Standard Media
L4055	Standard Media	Standard Media
M1085T	Standard Media	Standard Media

Process	Gen 3	Gen 3.1 control	Gen 3.1
L4063	Standard Media	Standard Media	Standard Media
L4064	Defined Media	Defined Media	Defined Media

Standard Media:
Pre REP: CM1
REP initiation : CM2
Split or Scale up : CM4

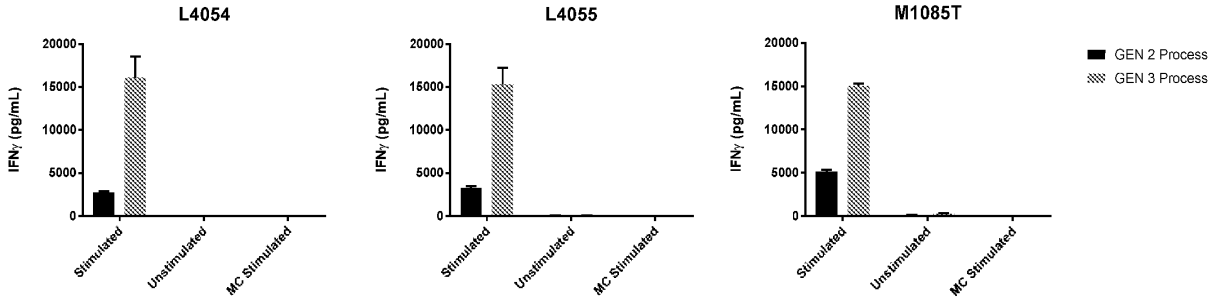
Defined Media:
CTS Optimizer (Serum Free Media) in each day of the process

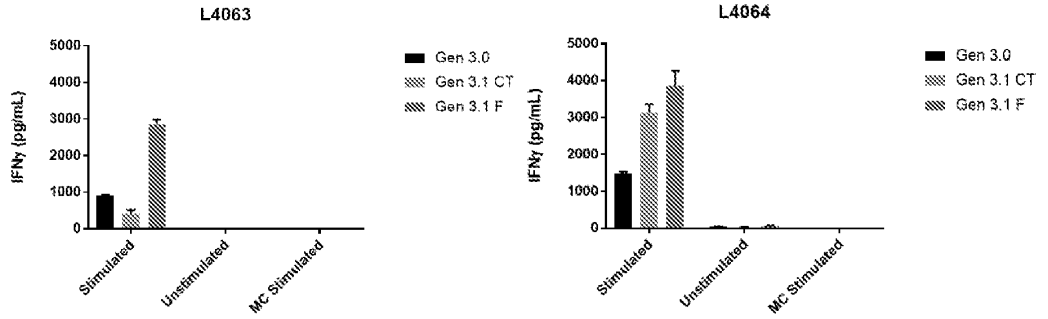
Figure 120



Tumor ID	L4063 Standard media			L4064 Defined Media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.0	Gen 3.1 Control	Gen 3.1
# Fragments	53	53	53	21	21	21

Figure 121





L4063- Standard Media:

L4064- Defined Media

Process Day	Conditions	Gen 3.0	Gen 3.1 CT (control)	Gen 3.1 F
Day 0- pre REP initiation	Media CM1	300 mL	300 mL	300 mL
	IL-2 (6000 IU/mL)	+	+	+
	OKT-3 (30ng/mL)	-	+	+
	Feeders (2E6 E+06)	-	-	+

Figure 122

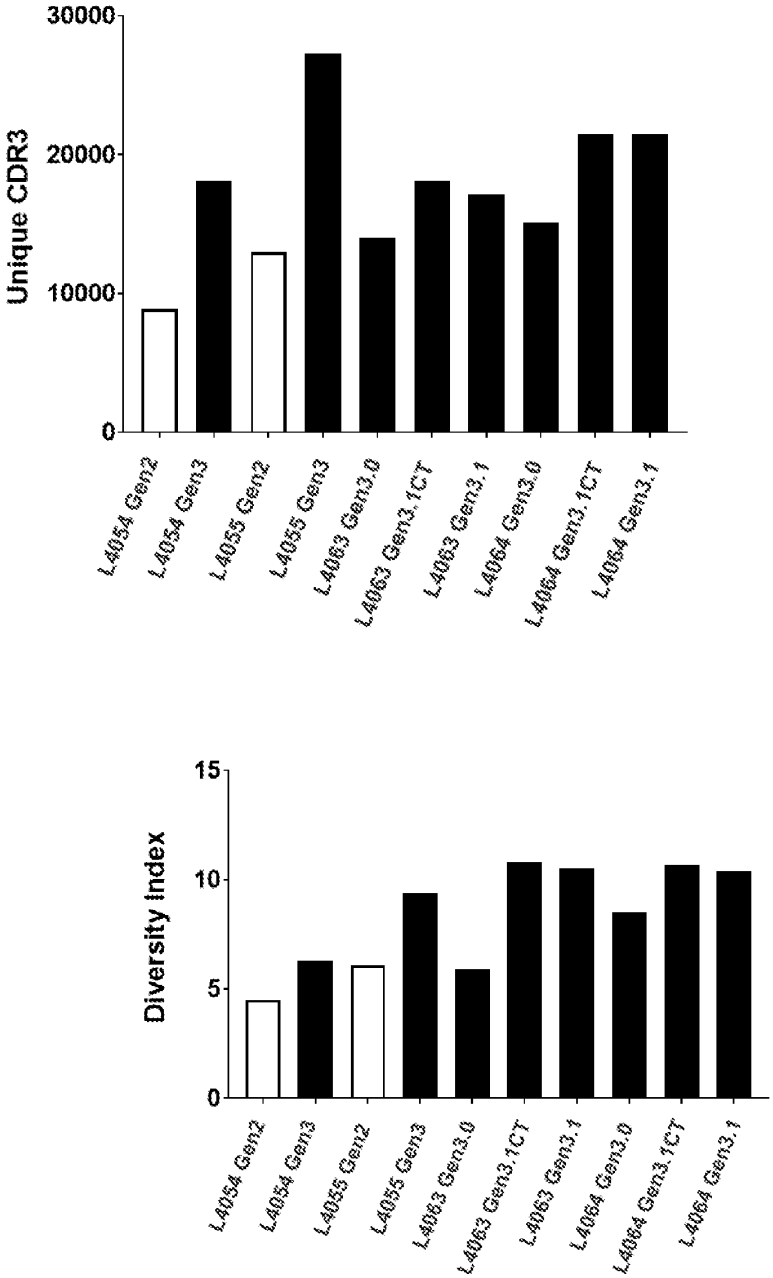

Unique CDR3
30000
20000
10000
0
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064 Gen3.1
Diversity Index
15
10
5
0
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064 Gen3.1

Figure 123

Conditions on Day 0	Gen 3.0	Gen 3.1 (control)	Gen 3.1
Media	500 mL	500 mL	500 mL
IL-2 (6E+06)	+	+	+
OKT-3 (30ng/mL)	-	+	+
Feeders (250 E+06)	-	-	+

Number of uCDR3 (% Overlap)	All uCDR3's			Top 80% uCDR3's		
	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.1	Gen 3.1 control	Gen 3.1
Gen 3.0	14005	3240 (23)	2959 (21)	1105	1000 (90)	975 (88)
Gen 3.1 control	-	18149	-	-	2438	-
Gen 3.1	-	-	17181	-	-	2332

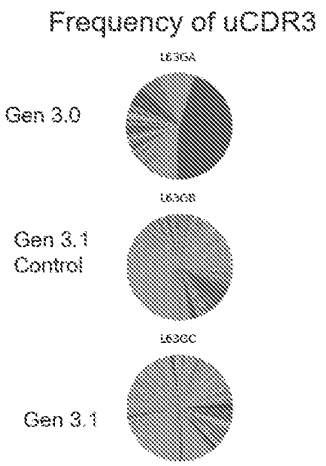


Figure 124

Conditions on Day 0	Gen 3.0	Gen 3.1 (control)	Gen 3.1
Media	500 mL	500 mL	500 mL
IL-2 (6E+06)	+	+	+
OKT-3 (30ng/mL)	-	+	+
Feeders (250 E+06)	-	-	+

Number of uCDR3 (% Overlap)	All uCDR3's			Top 80% uCDR3's		
	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.0	Gen 3.1 Control	Gen 3.1
Gen 3.0	15107	5486 (36)	5541 (37)	2479	2186 (88)	2163 (87)
Gen 3.1 Control	-	21460	-	-	3247	-
Gen 3.1	-	-	21503	-	-	3264

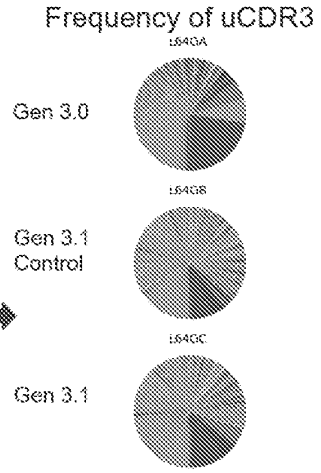


Figure 125

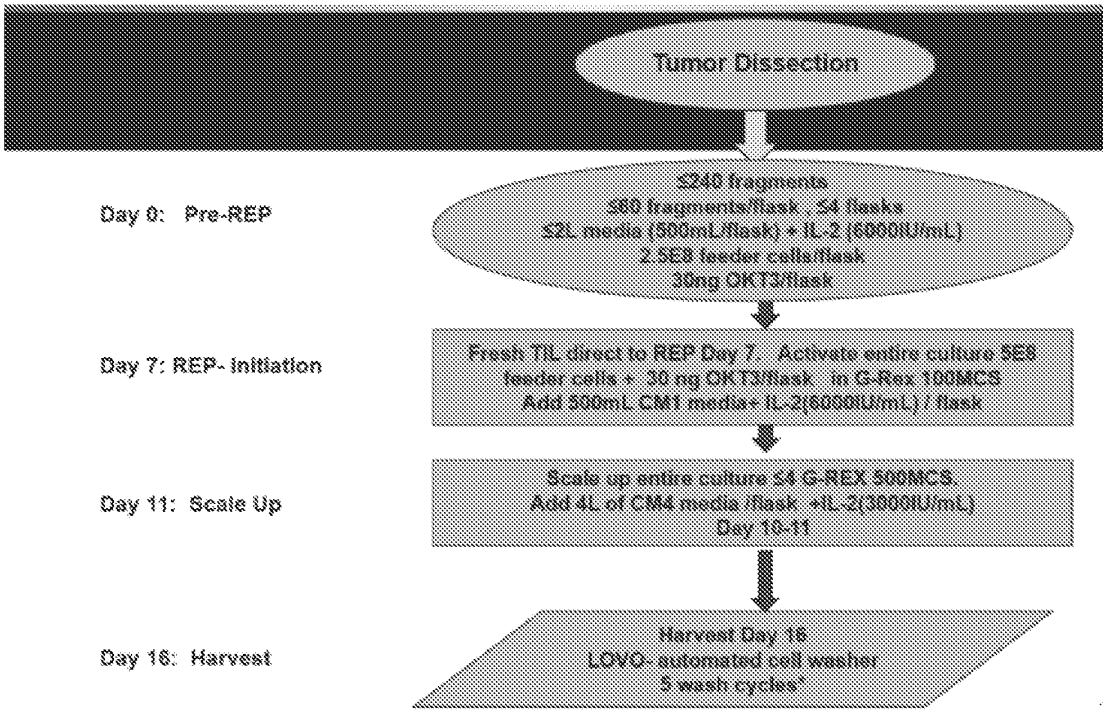

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤80 fragments/flask , ≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP- initiation
Fresh TIL direct to REP Day 7. Activate entire culture 5E8 feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL CM1 media+ IL-2(6000IU/mL) / flask
Day 11: Scale Up
Scale up entire culture ≤4 G-REX 500MCS.
Add 4L of CM4 media /flask +IL-2(3000IU/mL)
Day 10-11
Day 16: Harvest
Harvest Day 16
LOVO- automated cell washer
5 wash cycles*

Figure 126

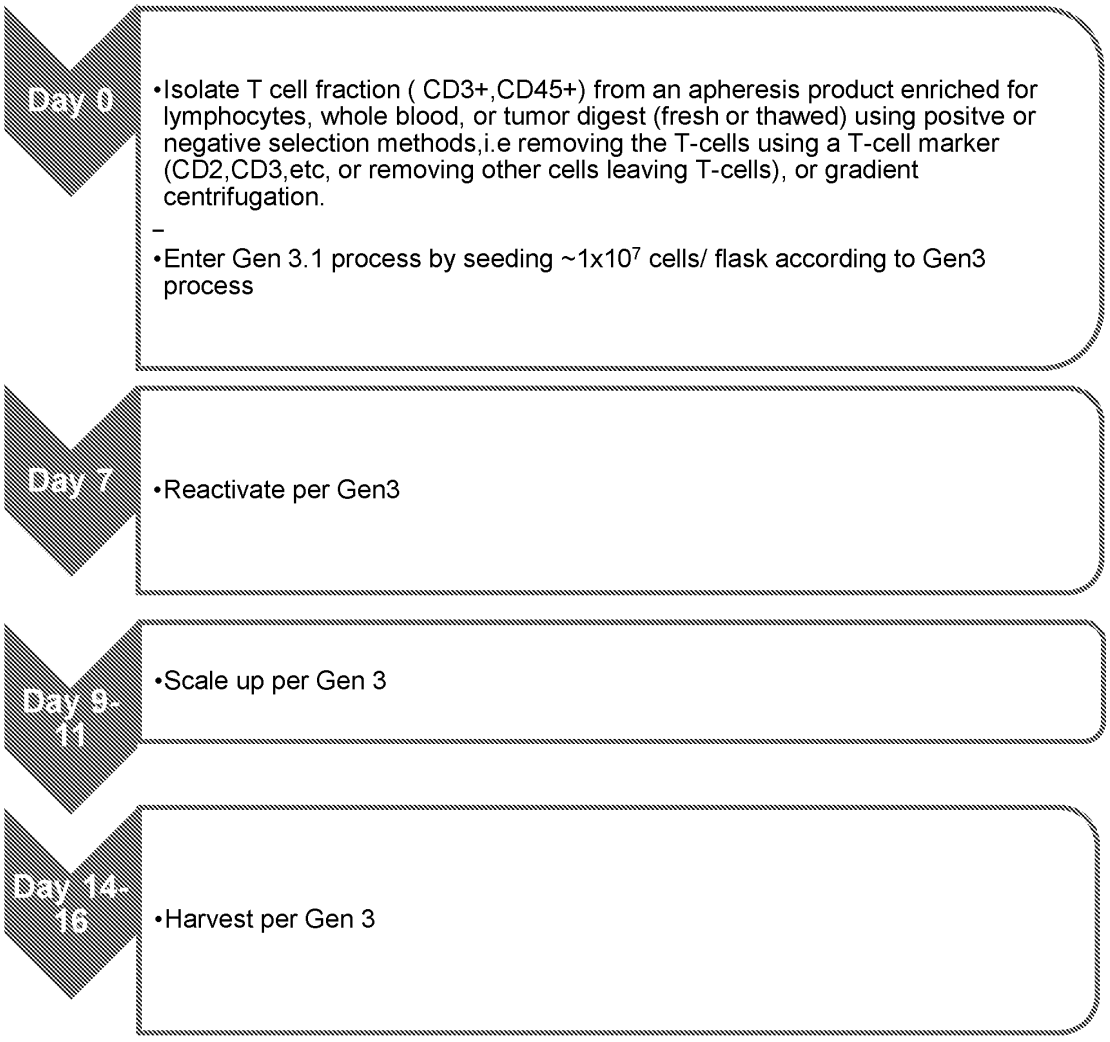

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positve or negative selection methods,i.e removing the T-cells using a T-cell marker (CD2,CD3,etc, or removing other cells leaving T-cells), or gradient centrifugation.
–
•Enter Gen 3.1 process by seeding ~1x10^7 cells/ flask according to Gen3 process
Day 7
•Reactivate per Gen3
Day 9-11
•Scale up per Gen 3
Day 14-16
•Harvest per Gen 3

Figure 127

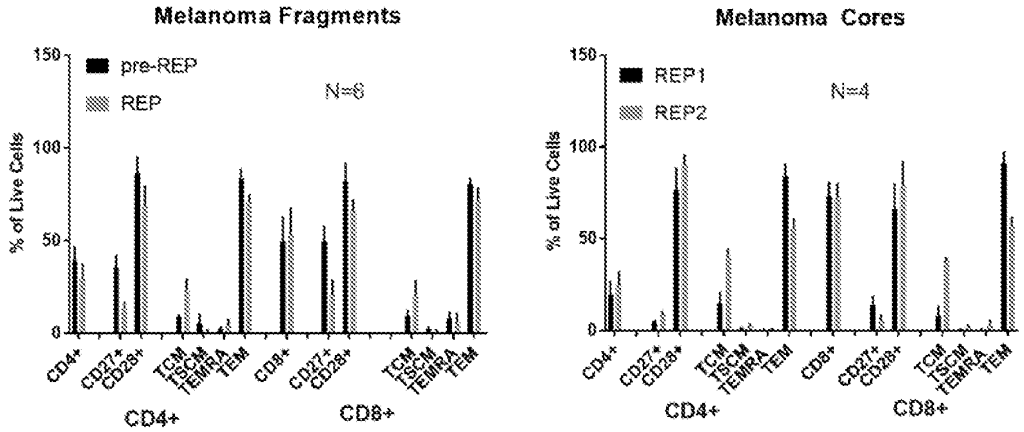

Melanoma Fragments
pre-REP
REP
N=6
% of Live Cells
150
100
50
0
CD4+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
CD8+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
CD4+
CD8+
Melanoma Cores
REP1
REP2
N=4
% of Live Cells
150
100
50
0
CD4+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
CD8+
CD27+ CD28+
TCM
TSCM
TEMRA
TEM
CD4+
CD8+

Figure 128

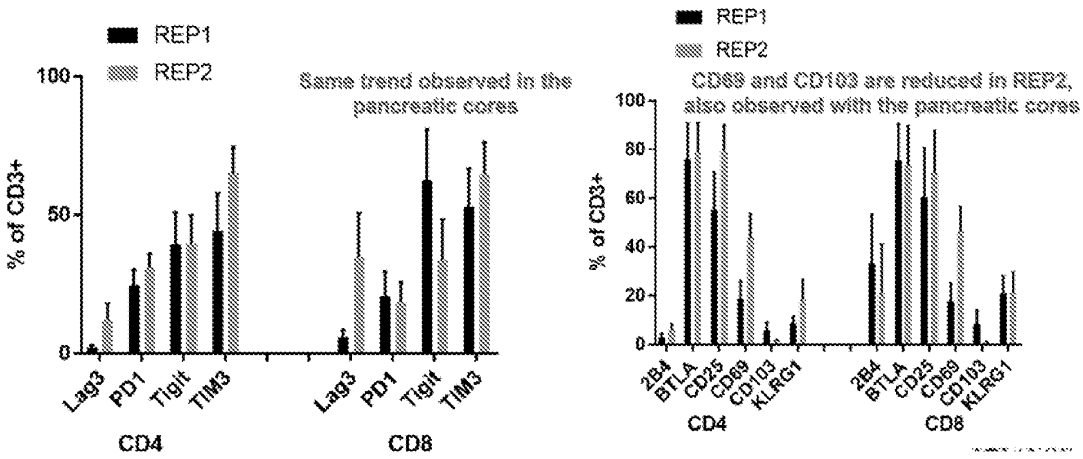

REP1
REP2
Same trend observed in the pancreatic cores
% of CD3+
100
50
0
Lag3
PD1
Tigit
TIM3
CD4
CD8
REP1
REP2
CD69 and CD103 are reduced in REP2, also observed with the pancreatic cores
% of CD3+
100
80
60
40
20
0
2B4
BTLA
CD25
CD69
CD103
KLRG1
CD4
CD8

Figure 129

	Culture conditions	Cores / cond. (#)	REP1	REP2
Lung (L4083)	Day 0: Addition of OKT3+feeders Day 3: Addition of OKT3+feeders	1	>60ES cells at Day 11 in Day 0 condition	1157-fold (Day 0) and 925-fold (1/2 cores) expansion ~ 10,350 and 7,843 pg/ml IFNs (respectively)

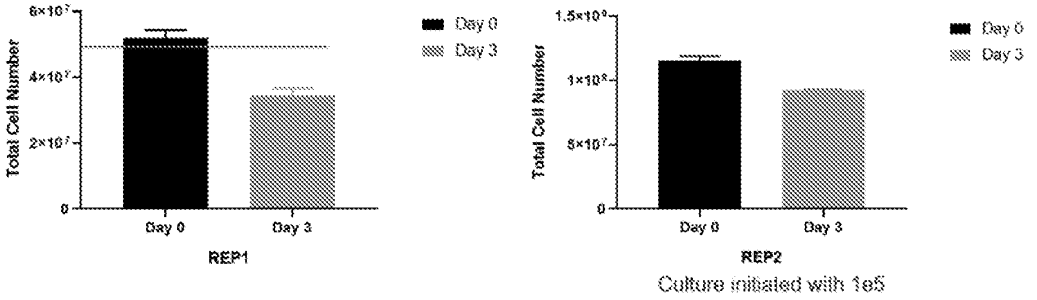


Figure 130

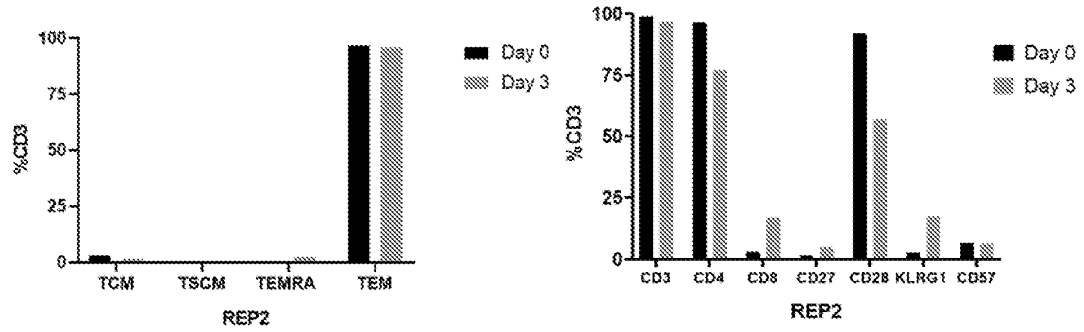

100
75
50
25
0
%CD3
TCM
TSCM
TEMRA
TEM
REP2
Day 0
Day 3
100
75
50
25
0
%CD3
CD3
CD4
CD8
CD27
CD28
KLRG1
CD57
REP2
Day 0
Day 3

Figure 131

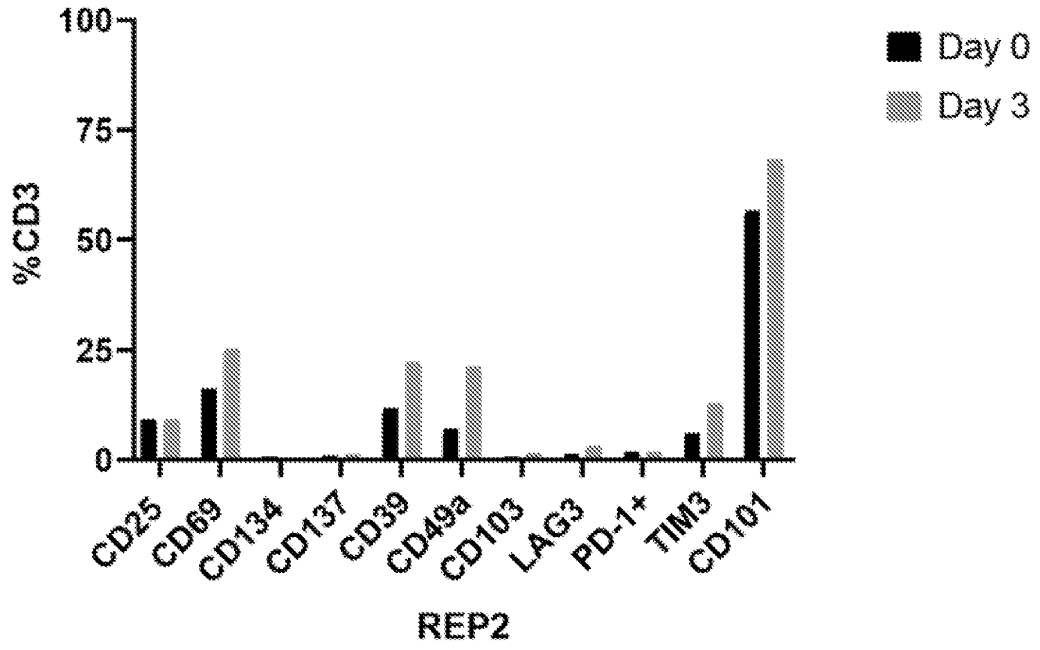

100
75
50
25
0
%CD3
Day 0
Day 3
CD25
CD69
CD134
CD137
CD39
CD49a
CD103
LAG3
PD-1+
TIM3
CD101
REP2

Figure 132

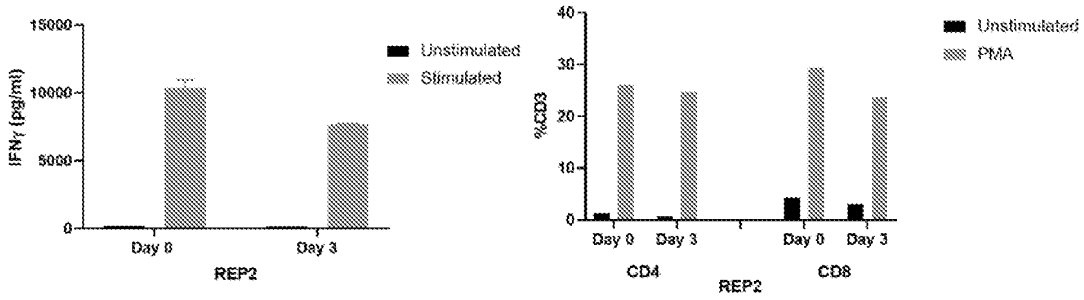
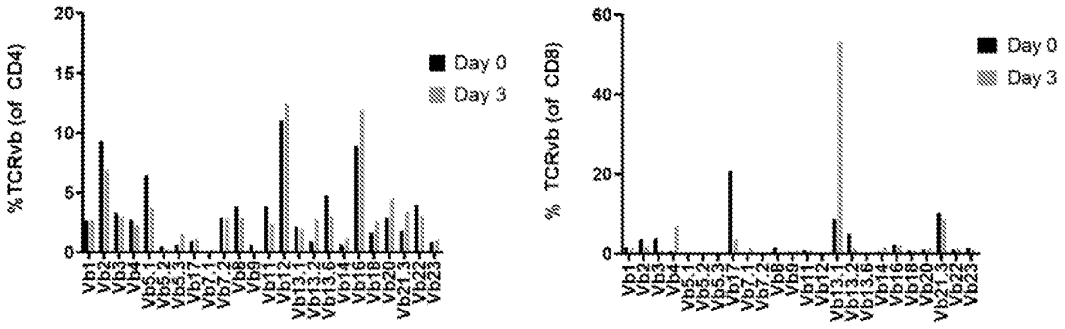

15000
10000
5000
0
IFNγ (pg/ml)
Day 0
Day 3
REP2
Unstimulated
Stimulated
40
30
20
10
0
%CD3
Day 0
Day 3
Day 0
Day 3
CD4
CD8
REP2
Unstimulated
PMA

Figure 133


% TCRvb (of CD4)
20
15
10
5
0
Day 0
Day 3
Vb1
Vb2
Vb3
Vb4
Vb5.1
Vb5.2
Vb5.3
Vb7.1
Vb7.2
Vb8
Vb9
Vb11
Vb12
Vb13.1
Vb13.2
Vb13.6
Vb14
Vb16
Vb18
Vb20
Vb21.3
Vb22
Vb23
% TCRvb (of CD8)
60
40
20
0
Day 0
Day 3
Vb1
Vb2
Vb3
Vb4
Vb5.1
Vb5.2
Vb5.3
Vb7.1
Vb7.2
Vb8
Vb9
Vb11
Vb12
Vb13.1
Vb13.2
Vb13.6
Vb14
Vb16
Vb18
Vb20
Vb21.3
Vb22
Vb23