Process 2A: about 22 days from Steps A - E	Process GEN 3: about 14-18 days from Steps A - E
STEP A Obtain Patient Tumor Sample (optionally can be frozen before Step B; optionally tumor sample can be a core/small biopsy)	**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 7/8 days with media comprising IL-2, OKT-3, and optionally 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 7/8)
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)

FIG. 1A

Process GEN 3: about 14-18 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 1 days to 7/8 days with media comprising IL-2, OKT-3, and optionally antigen-presenting feeder cells)

STEP C
Priming First Expansion to Rapid Second Expansion Transition
(Step B TILs directly move to Step D on day 7/8)

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

STEP F
Final Formulation and/or Transfer to Infusion Bag
(optionally cryopreserve)

FIG. 1B

Embodiment GEN 3.0: about 14-18 days from Steps A-E	**Embodiment GEN 3.1 control: about 14-18 days from Steps A - E**	**Embodiment GEN 3.1 Test/F: about 14-18 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 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 1 days to 7/8 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 7/8 days with media comprising IL-2, and OKT-3)	**STEP B** Priming First Expansion (physical fragmentation of up to 60 fragments per container grown for about 1 days to 7/8 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 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)	**STEP C** Priming First Expansion to Rapid Second Expansion Transition (Step B TILs directly move to Step D on day 7/8)
STEP D Rapid Second Expansion (TILs grown in growth media medium comprising IL-2, OKT-3, and antigen-presenting feeder cells; Days 10-11 scale up and add additional IL-2)	**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 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	**STEP E** Harvest TILS from Step D	**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)	**STEP F** Final Formulation and/or Transfer to Infusion Bag (optionally cryopreserve)

FIG. 1C

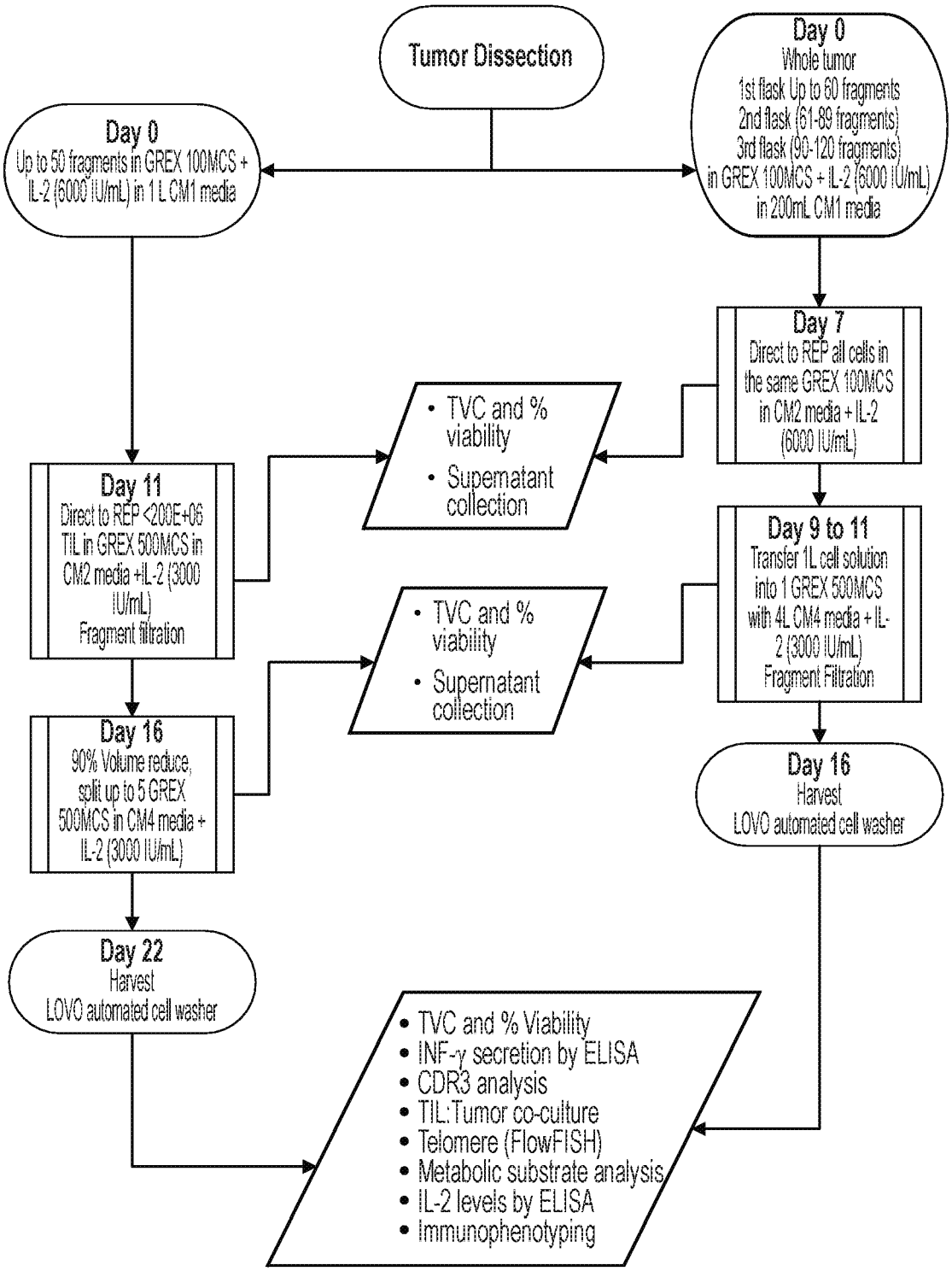
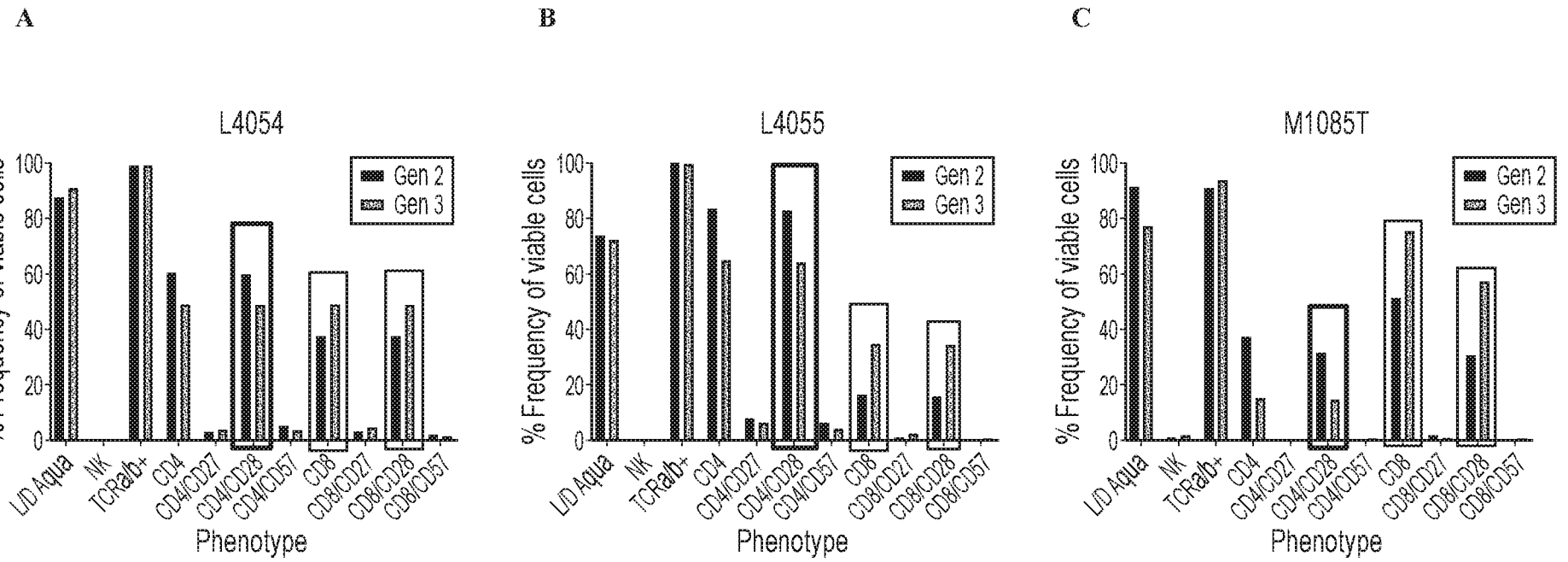

Tumor Dissection
Day 0
Up to 50 fragments in GREX 100MCS + IL-2 (6000 IU/mL) in 1 L CM1 media
Day 0
Whole tumor
1st flask Up to 60 fragments
2nd flask (61-89 fragments)
3rd flask (90-120 fragments)
in GREX 100MCS + IL-2 (6000 IU/mL) in 200mL CM1 media
Day 7
Direct to REP all cells in the same GREX 100MCS 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

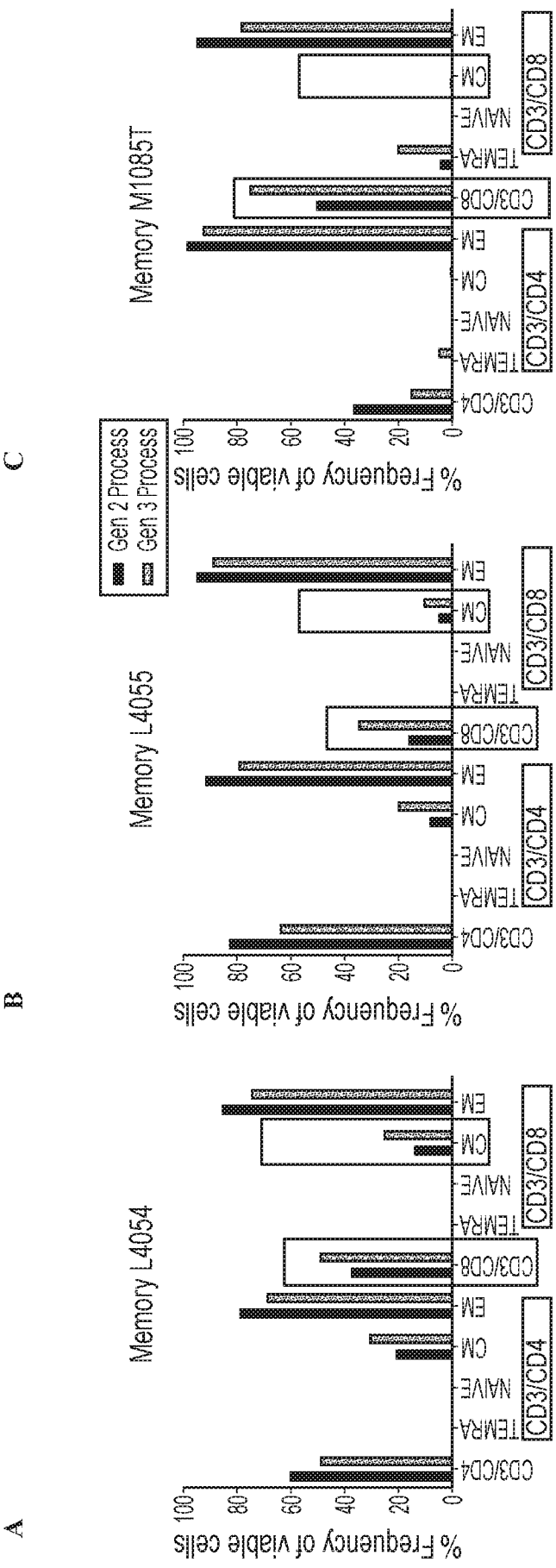
FIG. 2

SUBSTITUTE SHEET (RULE 26)



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

FIG. 3



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

FIG. 4

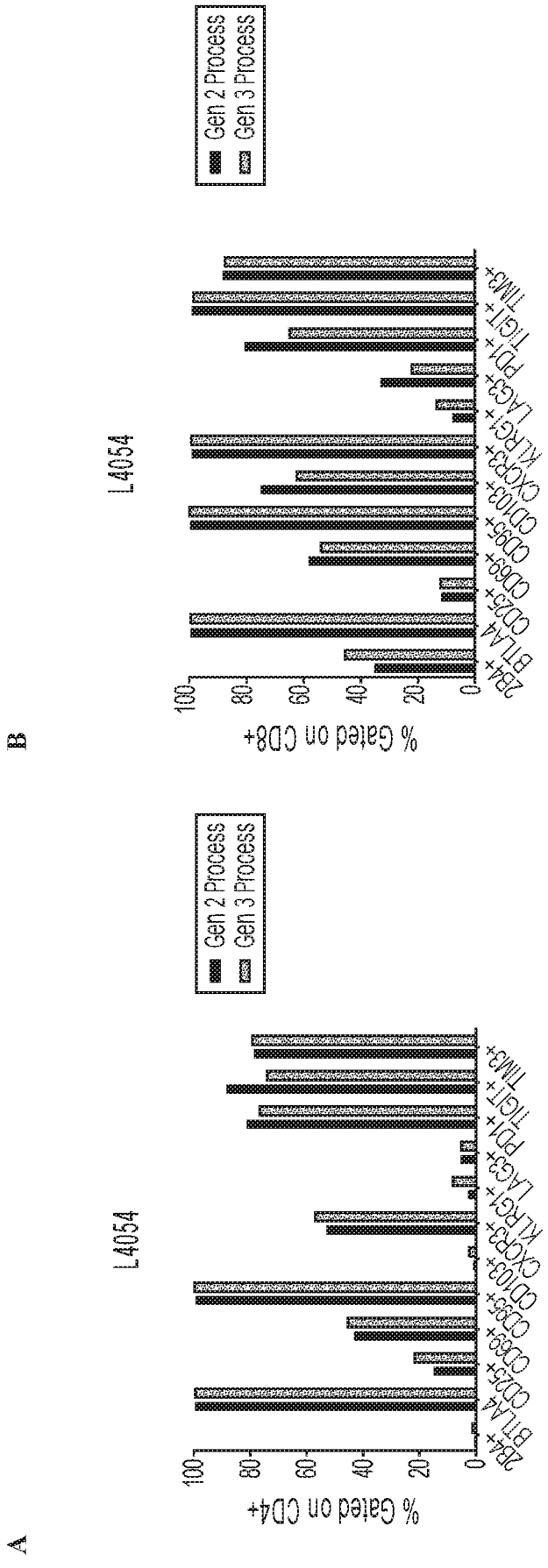

A
% Gated on CD4+
100
80
60
40
20
0
L4054
2B4+
BTLA+
CD25+
CD69+
CD96+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+
Gen 2 Process
Gen 3 Process
B
% Gated on CD8+
100
80
60
40
20
0
L4054
2B4+
BTLA+
CD25+
CD69+
CD96+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+
Gen 2 Process
Gen 3 Process

FIG. 5


A
L4055
% Gated on CD4+
100
80
60
40
20
0
2B4+
BTLA4+
CD25+
CD69+
CD95+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+
Gen 2 Process
Gen 3 Process
B
L4055
% Gated on CD8+
100
80
60
40
20
0
2B4+
BTLA4+
CD25+
CD69+
CD95+
CD103+
CXCR3+
KLRG1+
LAG3+
PD1+
TIGIT+
TIM3+
Gen 2 Process
Gen 3 Process

FIG. 6

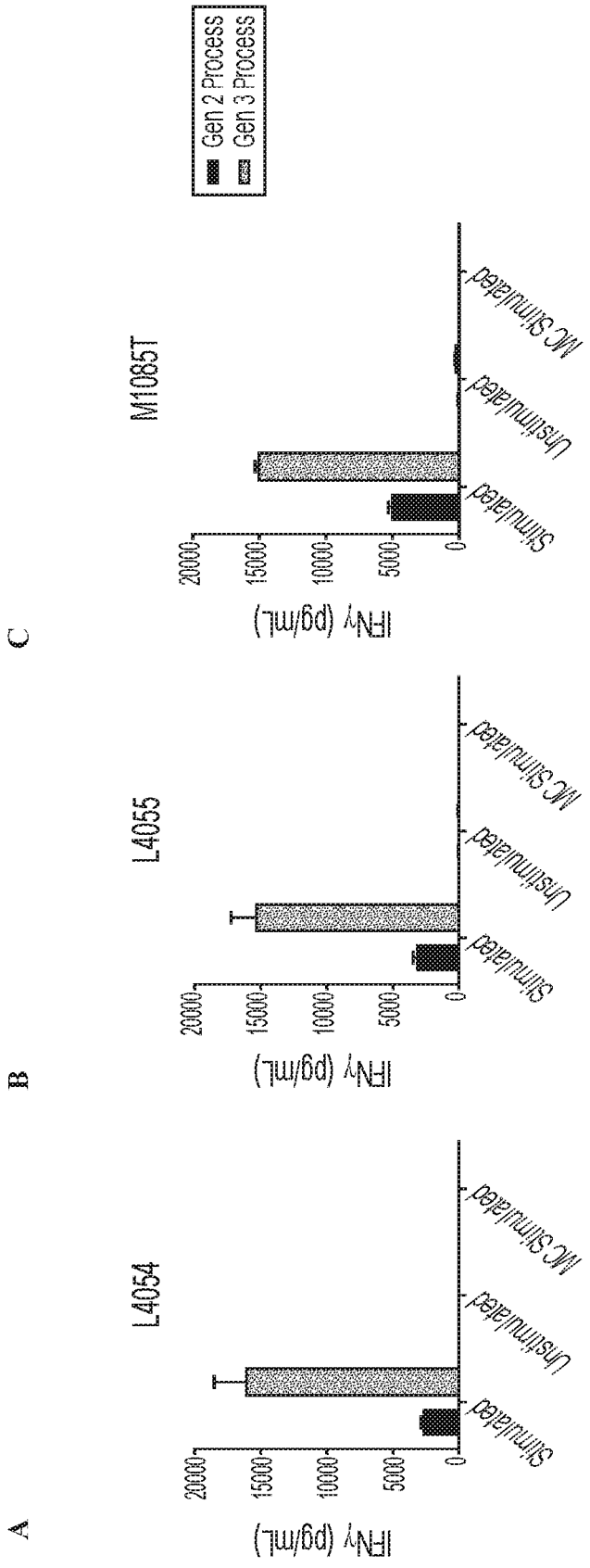

A
L4054
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
B
L4055
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
C
M1085T
IFNγ (pg/mL)
20000
15000
10000
5000
0
Stimulated
Unstimulated
MC Stimulated
Gen 2 Process
Gen 3 Process

FIG. 7

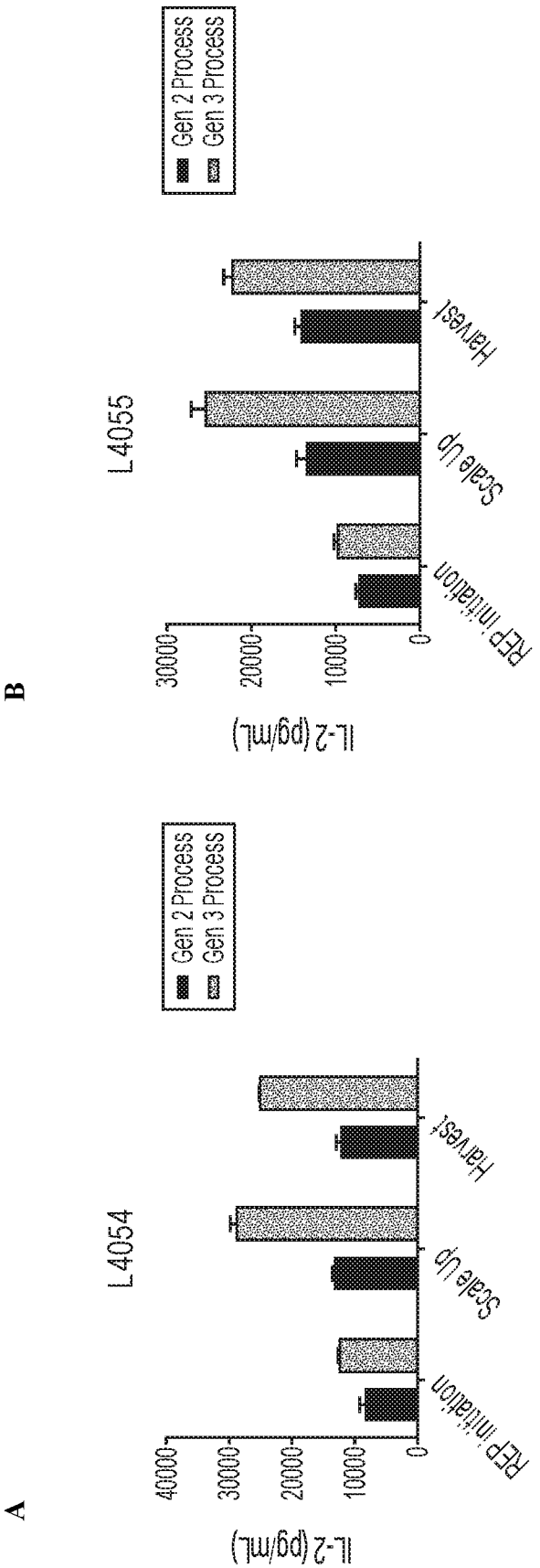

A
L4054
IL-2 (pg/mL)
40000
30000
20000
10000
0
REP Initiation
Scale Up
Harvest
Gen 2 Process
Gen 3 Process
B
L4055
IL-2 (pg/mL)
30000
20000
10000
0
REP Initiation
Scale Up
Harvest
Gen 2 Process
Gen 3 Process

FIG. 8

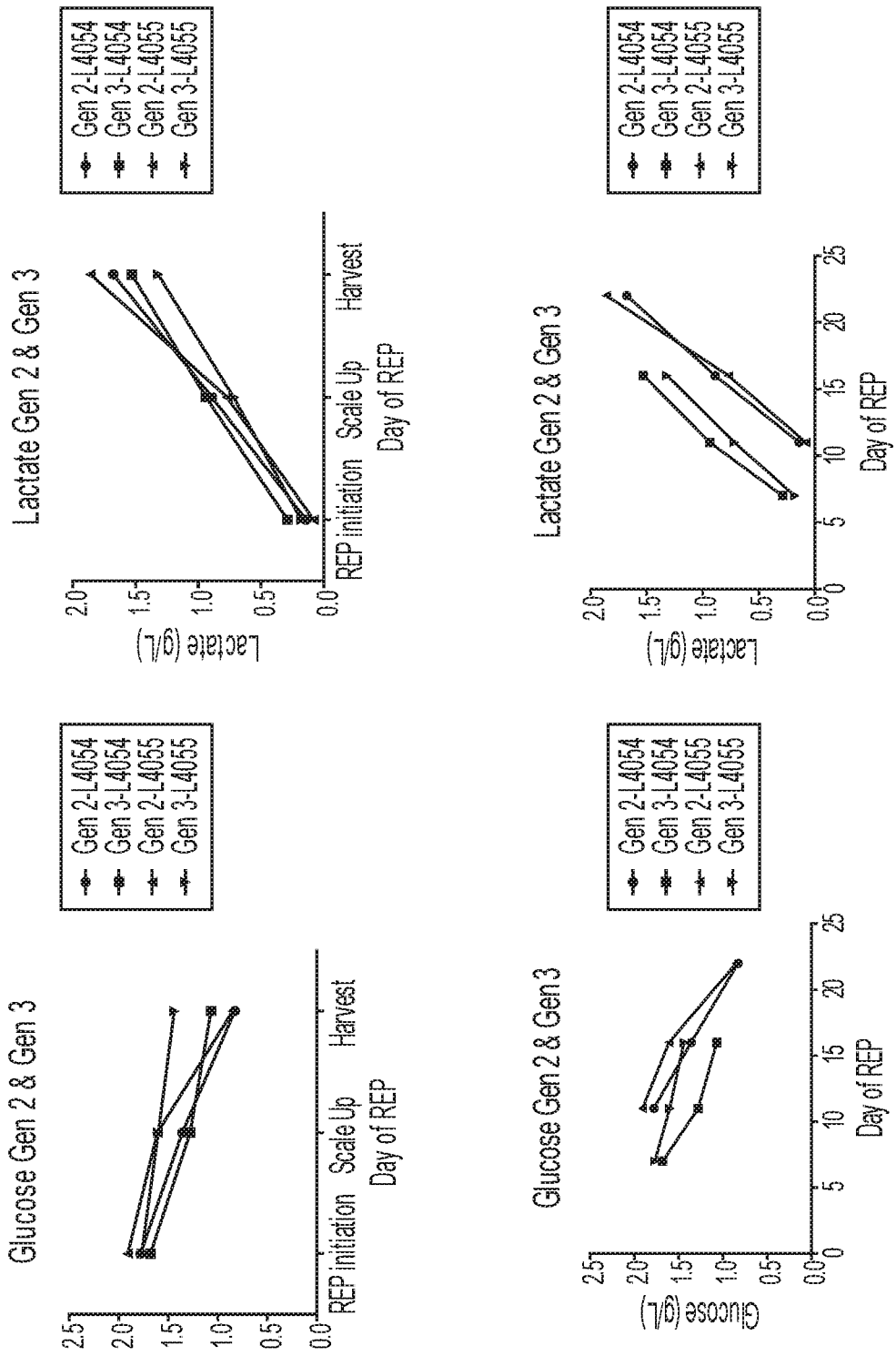
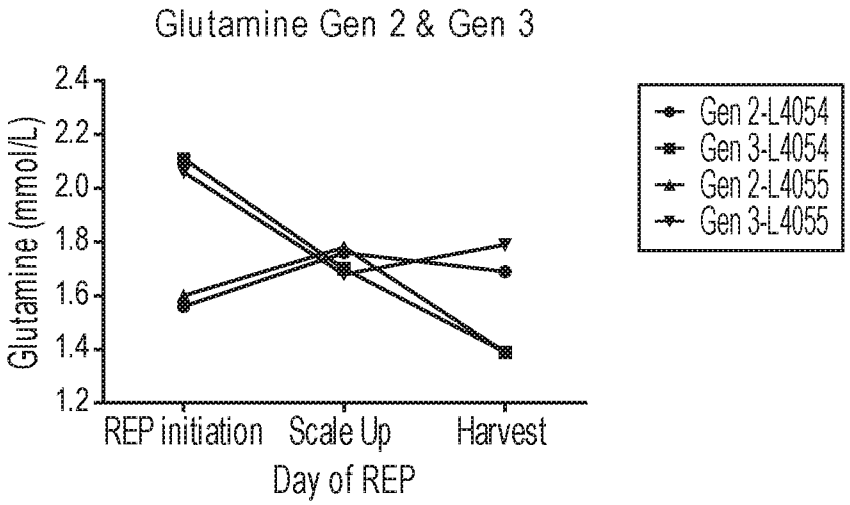

Glucose Gen 2 & Gen 3
Glucose (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
Lactate Gen 2 & Gen 3
Lactate (g/L)
2.0
1.5
1.0
0.5
0.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
A
Glucose Gen 2 & Gen 3
Glucose (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
Lactate Gen 2 & Gen 3
Lactate (g/L)
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055
B

FIG. 9

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

B

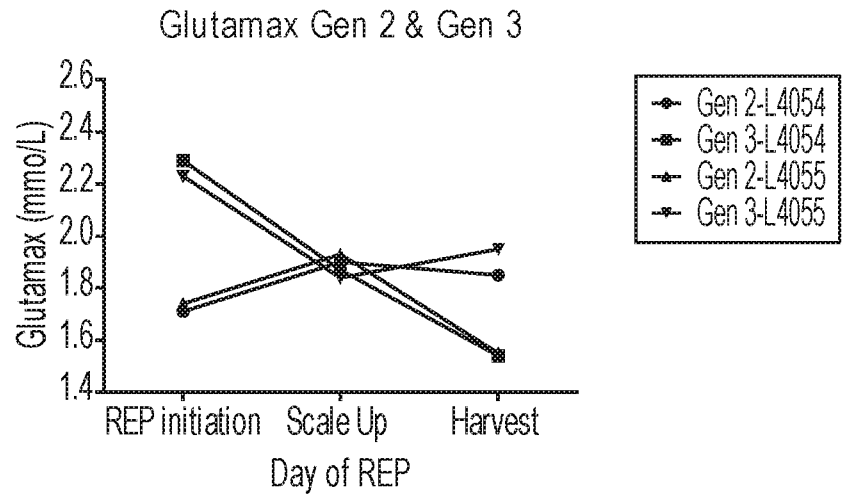

Glutamax Gen 2 & Gen 3
Glutamax (mmol/L)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

FIG. 10

C

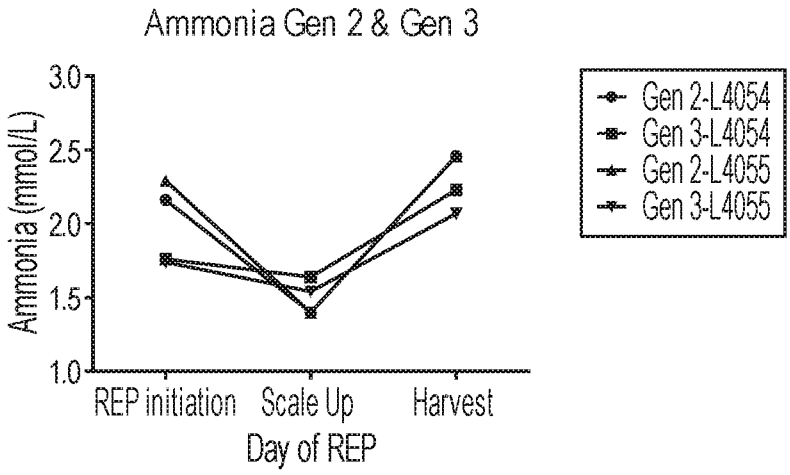

Ammonia Gen 2 & Gen 3
Ammonia (mmol/L)
3.0
2.5
2.0
1.5
1.0
REP initiation
Scale Up
Harvest
Day of REP
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

FIG. 10C

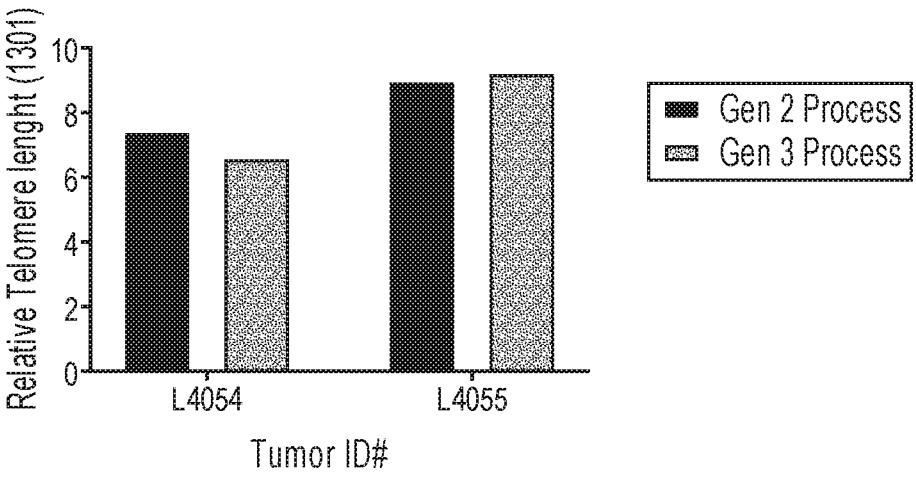

Relative Telomere lenght (1301)
10
8
6
4
2
0
L4054
L4055
Tumor ID#
Gen 2 Process
Gen 3 Process

FIG. 11

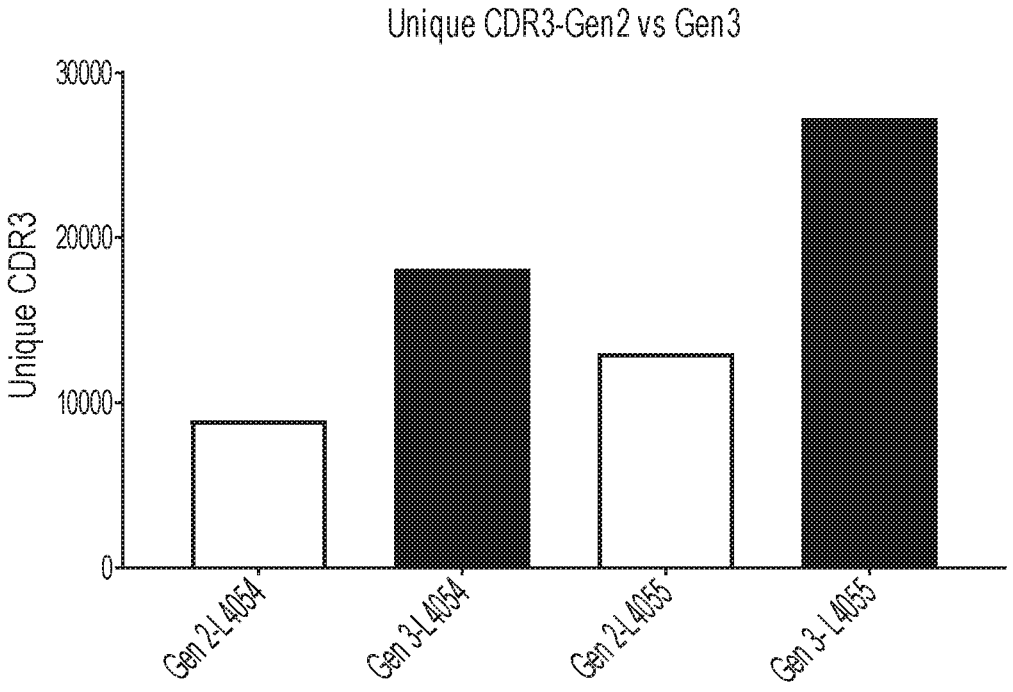
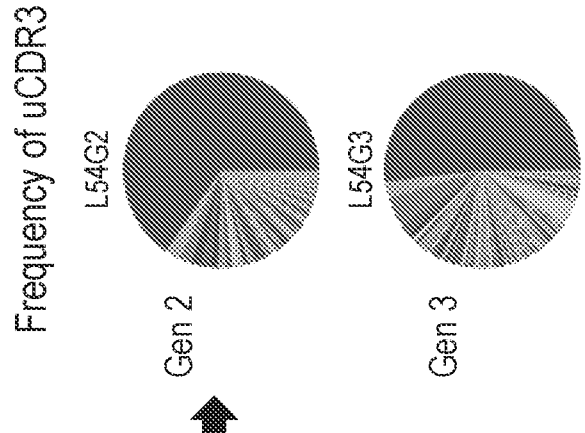

Unique CDR3-Gen2 vs Gen3
Unique CDR3
30000
20000
10000
0
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3-L4055

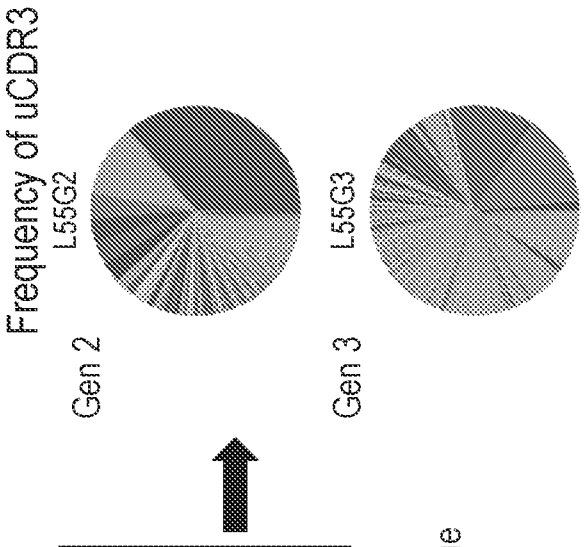
FIG. 12



# 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.

FIG. 13



# 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.

FIG. 14

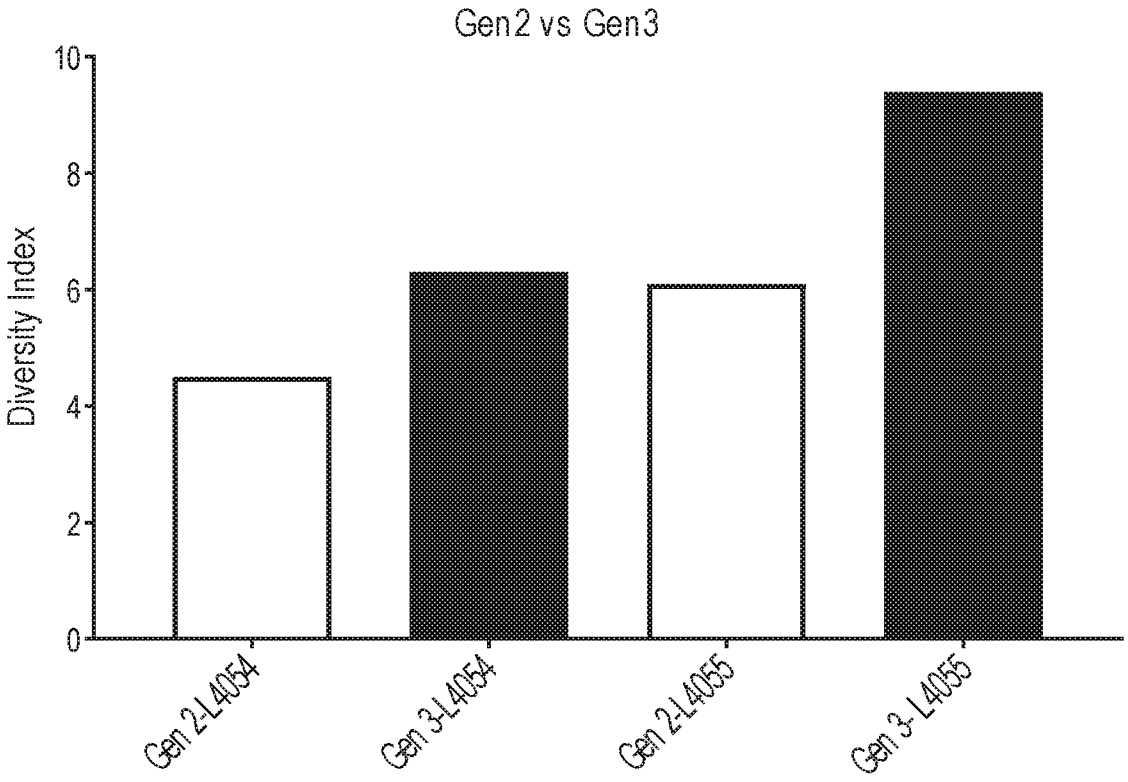

Gen2 vs Gen3
Diversity Index
10
8
6
4
2
0
Gen 2-L4054
Gen 3-L4054
Gen 2-L4055
Gen 3- L4055

FIG. 15

Cell counts Day 7-Gen 3 REP initiation

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%	

FIG. 16

Cell counts Day 11-Gen 2 REP initiation and Gen 3 Scale Up

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	8.17E+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%	

FIG. 17

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	8.67E+09
		Dead	4.56+06	3.61+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%	

FIG. 18

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/S	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

FIG. 19

	S1/S2/All/Live | Freq. of Parent (%)	S1/S2/All/Live/CD14+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/CD3-/NK | Freq. of null (%)	S1/S2/All/Live/CD14-/CD19 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD27 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD28 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD56 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD4/CD57 | Freq. of null (%)
A1 gen2 1st.fcs	87.2	1.77E-03	0.66	0	98.9	60.6	3.35	60.1	0.06	5.34
A2 gen2 2nd.fcs	73.5	0	0.12	0	99.7	83.1	7.94	82.6	0.14	6.18
A3 gen3 1st.fcs	90.4	0	0.197	1.72e-03	98.8	49.1	4.5	48.9	0.18	4.02
A4 gen3 2nd.fcs	72.1	2.56E-03	0.44	0.018	99.3	64.4	6.27	64	0.26	4.3
A5 PBMC.fcs	93.7	6.07	11.6	6.12	70.9	41.1	35.6	40.4	0.041	0.31

	S1/S2/All/Live/CD14-/TCRab/CD4/Q1: CCR7-PE-A-, CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q2: CCR7-PE-A+ CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q3: CCR7-PE-A+ CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q4: CCR7-PE-A-, CD45RA-AF700-A- | Freq. of Parent (%)
	TEMRA	NAIVE	CM	EM
A1 gen2 1st.fcs	0.25	0.073	3.37	96.3
A2 gen2 2nd.fcs	0.16	0.047	2.39	97.4
A3 gen3 1st.fcs	0.2	0.059	2.29	97.5
A4 gen3 2nd.fcs	0.25	0.18	7.12	92.5
A5 PBMC.fcs	0.91	36.1	16.2	46.9

	S1/S2/All/Live/CD14-/TCRab/CD4/Q5: CD62L-B421-A-CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q6: CD62L-B421-A+ CD45RA-AF700-A+ | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q7: CD62L-B421-A+ CD45RA-AF700-A- | Freq. of Parent (%)	S1/S2/All/Live/CD14-/TCRab/CD4/Q8: CD62L-B421-A-CD45RA-AF700-A- | Freq. of Parent (%)
	TEMRA	NAIVE	CM	EM
A1 gen2 1st.fcs	0.056	0.032	21.1	78.8
A2 gen2 2nd.fcs	0.036	0.014	8.3	91.6
A3 gen3 1st.fcs	0.059	0.049	30.7	69.2
A4 gen3 2nd.fcs	0.12	0.072	20.1	79.7
A5 PBMC.fcs	11.4	23.1	16.4	49.1

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

S1/S2/All/Live/CD14-/TCRab/CD8 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD27 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD28 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD56 | Freq. of null (%)	S1/S2/All/Live/CD14-/TCRab/CD8/CD57 | Freq. of null (%)
37.9	3.25	37.3	0.41	2.31
16.1	1.08	15.8	0.67	0.17
49.2	4.83	48.5	0.71	1.53
34.6	2.46	34	1.57	0.5
29.6	25.8	27.4	0.75	2.81

	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 (%)
	TEMRA	NAIVE	CM	EM
A1 gen2 1st.fcs	0.028	0	4.24	95.7
A2 gen2 2nd.fcs	0.042	0.014	4.76	95.2
A3 gen3 1st.fcs	0.056	6.97E-03	3.11	96.8
A4 gen3 2nd.fcs	0.18	0.13	10.2	89.5
A5 PBMC.fcs	8.05	40.9	3.02	48

	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 (%)
	TEMRA	NAIVE	CM	EM
A1 gen2 1st.fcs	0.033	9.37E-03	14.1	85.9
A2 gen2 2nd.fcs	0.071	0.014	5.24	94.7
A3 gen3 1st.fcs	0.077	0.017	25.5	74.4
A4 gen3 2nd.fcs	0.29	0.044	10.6	89.1
A5 PBMC.fcs	22.3	27.1	6.23	44.3

	S1/S2/All/Live/CD14-/TCRgd | Freq. of Parent (%)
A1 gen2 1st.fcs	0
A2 gen2 2nd.fcs	0
A3 gen3 1st.fcs	0
A4 gen3 2nd.fcs	0
A5 PBMC.fcs	0

FIG. 20

	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

FIG. 21

				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	62.40	12.10	99.90	54.00	99.30	13.40	22.10	64.90	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

FIG. 22

	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-Un-stimulated	TIL- Gen 2-Un-stimulated	TIL- Gen 2-Un-stimulated	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-Un stimulated	TIL- Gen 2-Un stimulated	TIL- Gen 2-Un stimulated	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-Un-stimulated	TIL- Gen 2-Un-stimulated	TIL- Gen 2-Un-stimulated	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-Un stimulated	TIL- Gen 2-Un stimulated	TIL- Gen 2-Un stimulated	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-Un-stimulated	TIL- Gen 3-Un-stimulated	TIL- Gen 3-Un-stimulated	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-Un stimulated	TIL- Gen 3-Un stimulated	TIL- Gen 3-Un stimulated	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-Un-stimulated	TIL- Gen 3-Un-stimulated	TIL- Gen 3-Un-stimulated	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-Un stimulated	TIL- Gen 3-Un stimulated	TIL- Gen 3-Un stimulated	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

SUBSTITUTE SHEET (RULE 26)

26/232

FIG. 23A

	1	2	3	4	5	6	7	8	9	10	11	12						Average	Average
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.117	0.116	0.117	0.105	0.108	0.102	450	Gen 2-Stimulated+TIL 1:3	2890.216	2084.946	3055.126	2676.76267	2757.81
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.041	0.043	0.041	0.041	0.043	0.041	570	Gen 2-Stimulated+TIL 1:9	2967.887	2117.46	3072.942	2719.42967	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.076	0.074	0.076	0.064	0.065	0.06	Delta	Gen 2-Stimulated+TIL 1:27	2952.028	2128.412	3035.11	2705.18333	
	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]	Gen 2-Stimulated+TIL 1:81	3261.251	2361.138	3167.212	2929.867	
				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	Gen 3-Stimulated+TIL 1:3	?????	?????	?????	#DIV/0!	#DIV/0!
	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	Gen 3-Stimulated+TIL 1:9	13273.546	>13500.00	13340.37	13306.9575	16089.32
	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	Gen 3-Stimulated+TIL 1:27	16947.994	16962.84	17161.47	17024.1	
	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]	Gen 3-Stimulated+TIL 1:81	17571.427	18246.77	17992.49	17936.8973	
				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	Gen 2-Unstimulated+TIL 1:3	5.474	4.262	5.855	5.197	5.20
	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	Gen 2-Unstimulated+TIL 1:9	<0.000	<0.000	<0.000	#DIV/0!	
	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	Gen 2-Unstimulated+TIL 1:27	<0.000	<0.000	<0.000	#DIV/0!	
	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]	Gen 2-Unstimulated+TIL 1:81	<0.000	<0.000	<0.000	#DIV/0!	
				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	Gen 3-Unstimulated+TIL 1:3	3.435	0.854	2.465	2.25133333	19.74
	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	Gen 3-Unstimulated+TIL 1:9	10.304	<0.000	<0.000	10.304	
	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	Gen 3-Unstimulated+TIL 1:27	30.239	<0.000	31.588	30.9135	
	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]	Gen 3-Unstimulated+TIL 1:81	9.644	4.039	92.74	35.4743333	
				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	Gen 2-Media Control Stimulated+TIL 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
	0.043	0.042	0.042	[illegible]	[illegible]	[illegible]	0.041	0.041	0.042	0.042	0.042	0.043	570	Gen 2-Media Control Stimulated+TIL 1:9	<0.000	<0.000	<0.000	#DIV/0!	
	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	[illegible]	[illegible]	[illegible]	0.114	0.104	0.107	0.101	0.103	0.101	450	Gen 2-Media Control Stimulated+TIL 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
	0.042	0.043	0.042	[illegible]	[illegible]	[illegible]	0.041	0.041	0.042	0.042	0.041	0.042	570	Gen 2-Media Control Stimulated+TIL 1:9	<0.000	<0.000	<0.000	#DIV/0!	
	0.18	0.179	0.181	[illegible]	[illegible]	[illegible]	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	[illegible]	[illegible]	[illegible]	0.114	0.109	0.114	0.106	0.107	0.104	450	Gen 2-Media Control Unstimulated+TIL 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
	0.041	0.041	0.041	[illegible]	[illegible]	[illegible]	0.042	0.042	0.041	0.042	0.041	0.042	570	Gen 2-Media Control Unstimulated+TIL 1:9	<0.000	<0.000	<0.000	#DIV/0!	
	0.132	0.128	0.131	[illegible]	[illegible]	[illegible]	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	Gen 3-Media Control Unstimulated+TIL 1:3	<0.000	<0.000	<0.000	0	30.28
	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	Gen 3-Media Control Unstimulated+TIL 1:9	<0.000	<0.000	60.557	60.557	
	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						

FIG. 23B

	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-Un-stimulated	TIL- Gen 2-Un-stimulated	TIL- Gen 2-Un-stimulated	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-Un stimulated	TIL- Gen 2-Un stimulated	TIL- Gen 2-Un stimulated	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-Un-stimulated	TIL- Gen 2-Un-stimulated	TIL- Gen 2-Un-stimulated	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-Un stimulated	TIL- Gen 2-Un stimulated	TIL- Gen 2-Un stimulated	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-Un-stimulated	TIL- Gen 3-Un-stimulated	TIL- Gen 3-Un-stimulated	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-Un stimulated	TIL- Gen 3-Un stimulated	TIL- Gen 3-Un stimulated	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-Un-stimulated	TIL- Gen 3-Un-stimulated	TIL- Gen 3-Un-stimulated	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-Un stimulated	TIL- Gen 3-Un stimulated	TIL- Gen 3-Un stimulated	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

22.9
22.9

SUBSTITUTE SHEET (RULE 26)

28/232

FIG. 24A

	1	2	3	4	5	6	7	8	9	10	11	12						Average	Average
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.128	0.126	0.132	0.11	0.111	0.107	450	Gen 2-Stimulated+TIL 1:3	3435.858	2933.14	2905.979	3091.659	3261.65
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.041	0.041	0.042	0.042	0.041	0.042	570	Gen 2-Stimulated+TIL 1:9	3676.262	3001.429	2926.474	3201.388333	
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.087	0.085	0.09	0.069	0.069	0.065	Delta	Gen 2-Stimulated+TIL 1:27	3471.282	3097.07	2994.844	3187.732	
	1001.719	995.223	1003.493	1145.286	977.713	968.66	2.922	2.418	3.712	<0.000	<0.000	<0.000	[Concentration]	Gen 2-Stimulated+TIL 1:81	3910.387	3552.941	3234.126	3565.818	
				3435.858	2933.14	2905.979	8.766	7.254	11.136	<0.000	<0.000	<0.000	X dil factor						
B	1.669	1.635	1.668	1.411	1.199	1.177	0.119	0.118	0.126	0.112	0.112	0.114	450	Gen 3-Stimulated+TIL 1:3	?????	?????	?????	#DIV/0!	#DIV/0!
	0.047	0.046	0.047	0.046	0.045	0.047	0.041	0.042	0.044	0.042	0.041	0.042	570	Gen 3-Stimulated+TIL 1:9	13183.826	13421.925	12822.143	13142.63133	15303.54
	1.622	1.589	1.621	1.365	1.154	1.13	0.078	0.076	0.082	0.07	0.07	0.072	Delta	Gen 3-Stimulated+TIL 1:27	16231.22	16700.175	14586.268	15839.221	
	503.563	491.203	503.336	408.474	333.492	325.164	0.931	0.531	1.786	<0.000	<0.000	<0.000	[Concentration]	Gen 3-Stimulated+TIL 1:81	17140.679	17638.195	16007.402	16928.75867	
				3676.262	3001.429	2926.474	8.383	4.779	16.076	<0.000	<0.000	<0.000	X dil factor						
C	0.974	0.957	0.939	0.576	0.528	0.518	0.115	0.116	0.125	0.111	0.114	0.115	450	Gen 2-Unstimulated+TIL 1:3	8.766	7.254	11.136	9.052	27.10
	0.045	0.043	0.045	0.045	0.043	0.045	0.041	0.044	0.042	0.041	0.043	0.043	570	Gen 2-Unstimulated+TIL 1:9	8.363	4.779	16.076	9.746	
	0.929	0.914	0.894	0.531	0.486	0.473	0.074	0.072	0.083	0.07	0.071	0.071	Delta	Gen 2-Unstimulated+TIL 1:27	5.632	<0.000	53.672	29.652	
	256.406	251.388	244.654	128.566	114.706	110.92	0.209	<0.000	1.988	<0.000	<0.000	<0.000	[Concentration]	Gen 2-Unstimulated+TIL 1:81	<0.000	<0.000	59.939	59.939	
				3471.282	3097.07	2994.844	5.632	<0.000	53.672	<0.000	<0.000	<0.000	X dil factor						
D	0.577	0.551	0.56	0.301	0.286	0.27	0.115	0.11	0.119	0.111	0.112	0.11	450	Gen 3-Unstimulated+TIL 1:3	19.738	13.255	20.614	17.869	43.26
	0.044	0.042	0.044	0.042	0.043	0.041	0.042	0.043	0.042	0.042	0.041	0.042	570	Gen 3-Unstimulated+TIL 1:9	13.875	31.096	19.718	21.563	
	0.533	0.509	0.516	0.259	0.243	0.229	0.073	0.067	0.077	0.069	0.07	0.068	Delta	Gen 3-Unstimulated+TIL 1:27	0.172	<0.000	33.321	16.7465	
	129.057	121.737	123.877	48.276	43.863	39.927	<0.000	<0.000	0.74	<0.000	<0.000	<0.000	[Concentration]	Gen 3-Unstimulated+TIL 1:81	77.18	171.968	92.74	35.4743333	
				3910.387	3552.941	3234.126	<0.000	<0.000	59.939	<0.000	<0.000	<0.000	X dil factor						
E	0.361	0.35	0.346	OVRFLW	OVRFLW	OVRFLW	0.143	0.135	0.145	0.115	0.112	0.11	450	Gen 2-Media Control Stimulated+TIL 1:3	<0.000	<0.000	<0.000	#DIV/0!	0.00
	0.042	0.042	0.042	[illegible]	[illegible]	[illegible]	0.041	0.042	0.042	0.041	0.043	0.041	570	Gen 2-Media Control Stimulated+TIL 1:9	<0.000	<0.000	<0.000	#DIV/0!	
	0.319	0.309	0.304	?????	?????	?????	0.102	0.093	0.103	0.074	0.069	0.068	Delta						
	65.323	62.395	61.049	?????	?????	?????	6.579	4.418	6.871	0.076	<0.000	<0.000	[Concentration]						
				?????	?????	?????	19.738	13.255	20.614	0.228	<0.000	<0.000	X dil factor						
F	0.237	0.233	0.239	[illegible]	[illegible]	[illegible]	0.122	0.144	0.125	0.114	0.112	0.111	450	Gen 2-Media Control Stimulated+TIL 1:3	0.228	<0.000	<0.000	0.228	0.11
	0.042	0.043	0.042	[illegible]	[illegible]	[illegible]	0.041	[illegible]	0.042	0.041	0.041	0.042	570	Gen 2-Media Control Stimulated+TIL 1:9	<0.000	<0.000	<0.000	0	
	0.195	0.19	0.197	[illegible]	[illegible]	[illegible]	0.081	0.089	0.084	0.073	0.071	0.069	Delta						
	30.739	29.388	31.253	1464.87	1491.325	1424.683	1.542	3.455	2.191	<0.000	<0.000	<0.000	[Concentration]						
				13183.83	13421.93	12822.14	13.875	31.096	19.718	<0.000	<0.000	<0.000	X dil factor						
G	0.182	0.173	0.183	[illegible]	[illegible]	[illegible]	0.115	0.115	0.121	0.114	0.111	0.114	450	Gen 2-Media Control Unstimulated+TIL 1:3	<0.000	<0.000	<0.000	0	0.00
	0.044	0.041	0.042	[illegible]	[illegible]	[illegible]	0.042	0.041	0.042	0.041	0.041	0.043	570	Gen 2-Media Control Unstimulated+TIL 1:9	<0.000	<0.000	<0.000	0	
	0.138	0.132	0.141	[illegible]	[illegible]	[illegible]	0.073	0.073	0.079	0.073	0.07	0.07	Delta						
	15.598	14.006	16.321	601.156	618.525	540.232	0.006	<0.000	1.234	<0.000	<0.000	<0.000	[Concentration]						
				16231.22	16700.18	14586.27	0.172	<0.000	33.321	<0.000	<0.000	<0.000	X dil factor						
H	0.121	0.117	0.122	0.837	0.857	0.794	0.119	0.125	0.123	0.119	0.12	0.112	450	Gen 3-Media Control Unstimulated+TIL 1:3	<0.000	<0.000	<0.000	0	1.53
	0.045	0.042	0.047	0.043	0.045	0.043	0.041	0.041	0.043	0.045	0.044	0.042	570	Gen 3-Media Control Unstimulated+TIL 1:9	1.357	4.779	<0.000	3.068	
	0.076	0.075	0.074	0.793	0.812	0.75	0.078	0.083	0.079	0.074	0.076	0.071	Delta						
	0.635	0.408	0.17	211.613	217.755	197.622	0.953	2.123	1.256	0.151	0.531	<0.000	[Concentration]						
				17140.68	17638.2	16007.4	77.18	171.968	101.73	1.357	4.779	<0.000	X dil factor						

SUBSTITUTE SHEET (RULE 26)

29/232

FIG. 24B

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

SUBSTITUTE SHEET (RULE 26)

FIG. 25A

	1	2	3	4	5	6	7	8	9	10	11	12							
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.079	0.079	0.081	0.18	0.198	0.191	450	2A-beads+TIL 1:3				Average	Average
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.042	0.042	0.043	0.042	0.043	0.042	570	2A-beads+TIL 1:9	4656.178	5074.253	5029.349	4919.927	5124.412
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.037	0.037	0.037	0.1338	0.156	0.149	Delta	2A-beads+TIL 1:27	4916.763	5266.064	5189.955	5124.261	
	951.995	1012.581	1035.454	1479.442	>1500.000	>1500.000	0.379	0.287	0.472	37.604	44.509	41.927	[Concentration]	2A-beads+TIL 1:81	5249.956	5464.506	5272.686	5329.049	
				4438.326	>4500.000	>4500.000	1.137	0.861	1.417	112.812	133.527	125.78	X dil factor	2B-beads+TIL 1:3	?????	?????	?????	#DIV/0!	
B	1:221	1:22	1:252	1:265	1:361	1:352	0.077	0.077	0.077	0.109	0.115	0.114	450	2B-beads+TIL 1:9				#DIV/0!	
	0.046	0.046	0.046	0.046	0.047	0.047	0.042	0.044	0.042	0.042	0.043	0.042	570	2B-beads+TIL 1:27	15284.891	13019.2	16306.887	14870.33	15061.77
	1:175	1:174	1:206	1:219	1:314	1:304	0.035	0.033	0.035	0.067	0.072	0.072	Delta	2B-beads+TIL 1:81	16086.64	13205.761	16467.237	15253.21	
	496.244	495.812	511.027	517.353	563.806	558.817	<0.000	<0.000	<0.000	10.74	12.637	12.564	[Concentration]						
				4656.178	5074.253	5029.349	<0.000	<0.000	<0.000	96.66	113.737	113.077	X dil factor	2A-Unstimulated+TIL 1:3	112.812	133.527	125.78	124.0397	107.374
C	0.677	0.681	0.681	0.531	0.563	0.555	0.075	0.074	0.075	0.087	0.096	0.091	450	2A-Unstimulated+TIL 1:9	96.66	113.737	113.077	107.8247	
	0.043	0.044	0.044	0.044	0.045	0.043	0.042	0.042	0.042	0.042	0.045	0.042	570	2A-Unstimulated+TIL 1:27	76.848	131.834	113.974	107.552	
	0.634	0.637	0.637	0.488	0.518	0.511	0.033	0.032	0.033	0.045	0.05	0.049	Delta	2A-Unstimulated+TIL 1:81	71.65	82.102	116.487	90.07967	
	245.876	247.384	247.428	182.102	195.039	192.221	<0.000	<0.000	<0.000	2.846	4.883	4.221	[Concentration]						
				4916.763	5266.064	5189.955	<0.000	<0.000	<0.000	76.848	131.834	113.974	X dil factor	2B-Unstimulated+TIL 1:3	317.235	324.939	224.765	288.9797	236.1614
D	0.406	0.405	0.407	0.251	0.257	0.25	0.075	0.075	0.077	0.082	0.081	0.084	450	2B-Unstimulated+TIL 1:9	285.917	302.211	210.37	266.166	
	0.044	0.043	0.044	0.044	0.043	0.042	0.042	0.043	0.043	0.044	0.042	0.044	570	2B-Unstimulated+TIL 1:27	205.324	161.226	93.466	153.3367	
	0.363	0.362	0.363	0.207	0.214	0.208	0.033	0.032	0.035	0.039	0.039	0.04	Delta	2B-Unstimulated+TIL 1:81					
	128.798	128.379	128.798	64.814	67.463	65.095	<0.000	<0.000	<0.000	0.885	1.014	1.438	[Concentration]						
				5249.956	5464.506	5272.686	<0.000	<0.000	<0.000	71.65	82.102	116.487	X dil factor	2A-Media Control 1:3	1.137	0.861	1.417	1.138333	1.138778
E	0.244	0.241	0.241	OVRFLW	OVRFLW	OVRFLW	0.35	0.356	0.275	0.078	0.079	0.079	450	2A-Media Control 1:9					
	0.042	0.042	0.042	[illegible]	[illegible]	[illegible]	0.043	0.043	0.043	0.042	0.042	0.043	570	2A-Media Control 1:27					
	0.201	0.199	0.199	?????	?????	?????	0.307	0.314	0.232	0.036	0.036	0.036	Delta	2A-Media Control 1:81					
	65.323	61.493	61.533	?????	?????	?????	105.745	108.313	74.922	0.079	0.079	<0.000	[Concentration]						
				?????	?????	?????	317.235	324.939	224.765	0.237	0.237	<0.000	X dil factor	2B-Media Control 1:3	0.237	0.953	<0.000	0.595	1.47425
F	0.166	0.165	0.161	[illegible]	[illegible]	[illegible]	0.165	0.17	0.146	0.078	0.079	0.078	450	2B-Media Control 1:9	0.457	4.25	<0.000	2.3535	
	0.042	0.043	0.043	[illegible]	[illegible]	[illegible]	0.043	0.042	0.045	0.042	0.042	0.042	570	2B-Media Control 1:27					
	0.124	0.122	0.119	[illegible]	[illegible]	[illegible]	0.123	0.128	0.101	0.036	0.037	0.035	Delta	2B-Media Control 1:81					
	31.999	31.384	30.079	>1500.000	1346.731	>1500.000	31.769	33.579	23.374	0.051	0.472	<0.000	[Concentration]						
				>13500.000	12120.579	>13500.000	285.917	302.211	210.37	0.457	4.25	<0.000	X dil factor						
G	0.122	0.119	0.117	[illegible]	[illegible]	[illegible]	0.102	0.097	0.092	0.072	0.072	0.07	450						
	0.043	0.042	0.043	[illegible]	[illegible]	[illegible]	0.043	0.044	0.045	0.043	0.045	0.043	570						
	0.078	0.076	0.074	[illegible]	[illegible]	[illegible]	0.058	0.054	0.046	0.029	0.028	0.027	Delta						
	14.956	14.181	13.334	566.107	482.193	603.959	7.605	5.971	3.462	<0.000	<0.000	<0.000	[Concentration]						
				15284.891	13019.2	16306.887	205.324	161.226	93.466	<0.000	<0.000	<0.000	X dil factor						
H	0.077	0.084	0.077	0.572	0.489	0.563	0.098	0.082	0.086	0.074	0.073	0.07	450						
	0.042	0.042	0.042	0.046	0.045	0.046	0.049	0.043	0.043	0.043	0.043	0.043	570						
	0.036	0.042	0.036	0.526	0.443	0.537	0.05	0.038	0.043	0.031	0.03	0.027	Delta						
	<0.000	2.136	0.023	198.6	163.034	203.299	4.534	0.756	2.305	<0.000	<0.000	<0.000	[Concentration]						
				16086.64	13205.761	16467.237	367.254	61.271	186.666	<0.000	<0.000	<0.000	X dil factor						

SUBSTITUTE SHEET (RULE 26)

FIG. 25B

	1	2	3	4	5	6	7	8	9	10	11	12	
A	STD1	STD1	STD1	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				Well ID
	2000	2000	2000										Conc/Dil
B	STD2	STD2	STD2	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				Well ID
	1000	1000	1000										Conc/Dil
C	STD3	STD3	STD3	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				Well ID
	500	500	500										Conc/Dil
D	STD4	STD4	STD4	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				Well ID
	250	250	250										Conc/Dil
E	STD5	STD5	STD5	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				Well ID
	125	125	125										Conc/Dil
F	STD6	STD6	STD6	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				Well ID
	62.5	62.5	62.5										Conc/Dil
G	STD7	STD7	STD7	Empty	Empty	Empty	Empty	Empty	Empty				Well ID
	31.3	31.3	31.3										Conc/Dil
H	STD8	STD8	STD8	Empty	Empty	Empty	Empty	Empty	Empty				Well ID
	0	0	0										Conc/Dil

FIG. 26A

	1	2	3	4	5	6	7	8	9	10	11	12										
A	2.424 0.052 2.471	2.291 0.052 2.246	2.249 0.051 2.183	0.218 0.043 0.175	0.22 0.042 0.177	0.215 0.043 0.172	0.187 0.044 0.143	0.187 0.043 0.145	0.184 0.051 0.133				450 570 Delta	Dilution Factor=100								
	1970.541	2046.905	1987.758	124.53	126.082	121.662	99.302	100.904	92.083				[Concentration]	Gen 3 Rep initiation 1st run	124.530	126.082	121.662	12453.0	12608.2	12166.2	12409.1	
B	2.092 0.049 2.043	2.031 0.049 1.982	2.026 0.049 1.977	0.415 0.043 0.372	0.412 0.043 0.369	0.435 0.043 0.393	0.394 0.043 0.351	0.392 0.044 0.348	0.356 0.044 0.312				450 570 Delta	Gen 3 Scale up 1st run Gen 3 Harvest 1st run	282.400 252.774	280.328 247.768	299.854 250.557	28240.0 25277.4	28032.8 24776.8	29985.4 25055.7	28752.7 25036.6	
	877.734	1051.728	1047.027	282.4	280.328	299.854	264.954	262.893	233.453				[Concentration]	Gen 2 Rep initiation 1st run	79.199	77.702	93.523	7919.9	7770.2	9352.3	8347.5	
C	0.683 0.045 0.638	0.683 0.044 0.639	0.68 0.044 0.637	0.379 0.043 0.336	0.372 0.043 0.33	0.376 0.043 0.333	0.356 0.043 0.313	0.338 0.043 0.295	0.33 0.043 0.287				450 570 Delta	Gen 2 Scale up 1st run Gen 2 Harvest 1st run	136.754 117.564	131.526 130.125	131.682 121.895	13675.4 11756.4	13152.6 13012.5	13168.2 12189.5	13332.1 12319.5	
	510.008	510.619	508.261	252.774	247.768	250.557	233.9443	219.211	212.888				[Concentration]									
D	0.37 0.043 0.328	0.38 0.045 0.335	0.382 0.043 0.338	0.162 0.046 0.116	0.156 0.042 0.114	0.177 0.042 0.135	0.155 0.043 0.112	0.15 0.044 0.106	0.149 0.043 0.106				450 570 Delta	Gen 3 Rep initiation 2nd run Gen 3 Scale up 2nd run Gen 3 Harvest 2nd run	99.302 264.954 233.943	100.904 262.893 219.211	92.083 233.453 212.888	9930.2 26495.4 23394.3	10090.4 26289.3 21921.1	9208.3 23345.3 21288.8	9743.0 25376.7 22201.4	
	246.046	252.035	254.91	79.199	77.702	93.523	76.431	71.588	71.737				[Concentration]									
E	0.216 0.043 0.173	0.219 0.043 0.176	0.22 0.045 0.175	0.235 0.044 0.191	0.227 0.043 0.185	0.228 0.043 0.185	0.253 0.048 0.205	0.231 0.045 0.186	0.222 0.045 0.177				450 570 Delta	Gen 2 Rep initiation 2nd run Gen 2 Scale up 2nd run Gen 2 Harvest 2nd run	76.431 147.807 139.491	71.588 132.695 135.738	71.737 125.539 149.459	7643.1 14780.7 13949.1	7158.8 13269.5 13573.8	7173.7 12553.9 14945.9	7325.2 13534.7 14156.3	
	122.514	124.995	123.909	136.754	131.526	131.682	147.807	132.695	125.539				[Concentration]									
F	0.137 0.047 0.089	0.132 0.043 0.089	0.133 0.044 0.089	0.21 0.043 0.166	0.227 0.044 0.183	0.216 0.044 0.172	0.238 0.043 0.195	0.233 0.043 0.19	0.253 0.045 0.207				450 570 Delta									
	59.482	58.969	59.116	117.564	130.125	121.895	139.491	135.738	149.459				[Concentration]									
G	0.088 0.042 0.046	0.088 0.043 0.046	0.089 0.043 0.046	2.263 0.05 2.213	2.293 0.05 2.243	2.27 0.049 2.221	2.225 0.053 2.172	2.215 0.052 2.163	2.259 0.053 2.206				450 570 Delta									
	28.375	28.237	28.514	2014.9	2081.651	1970.439	1973.799	1920.845	1986.127				[Concentration]									
H	0.05 0.043 0.007	0.048 0.042 0.006	0.048 0.041 0.007	2.272<0.05 2.223	2.297<0.05 2.247	2.253<0.049 2.204	2.244<0.053 2.191	2.253<0.052 2.202	2.248<0.054 2.194				450 570 Delta									
	3.1	2.399	2.573	2023.073	2016.943	1960.16	1986.84	2002.036	1988.165				[Concentration]									

SUBSTITUTE SHEET (RULE 26)

33/232

FIG. 26B

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

FIG. 27

SUBSTITUTE SHEET (RULE 26)

	$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

FIG. 28

SUBSTITUTE SHEET (RULE 26)

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

FIG. 29

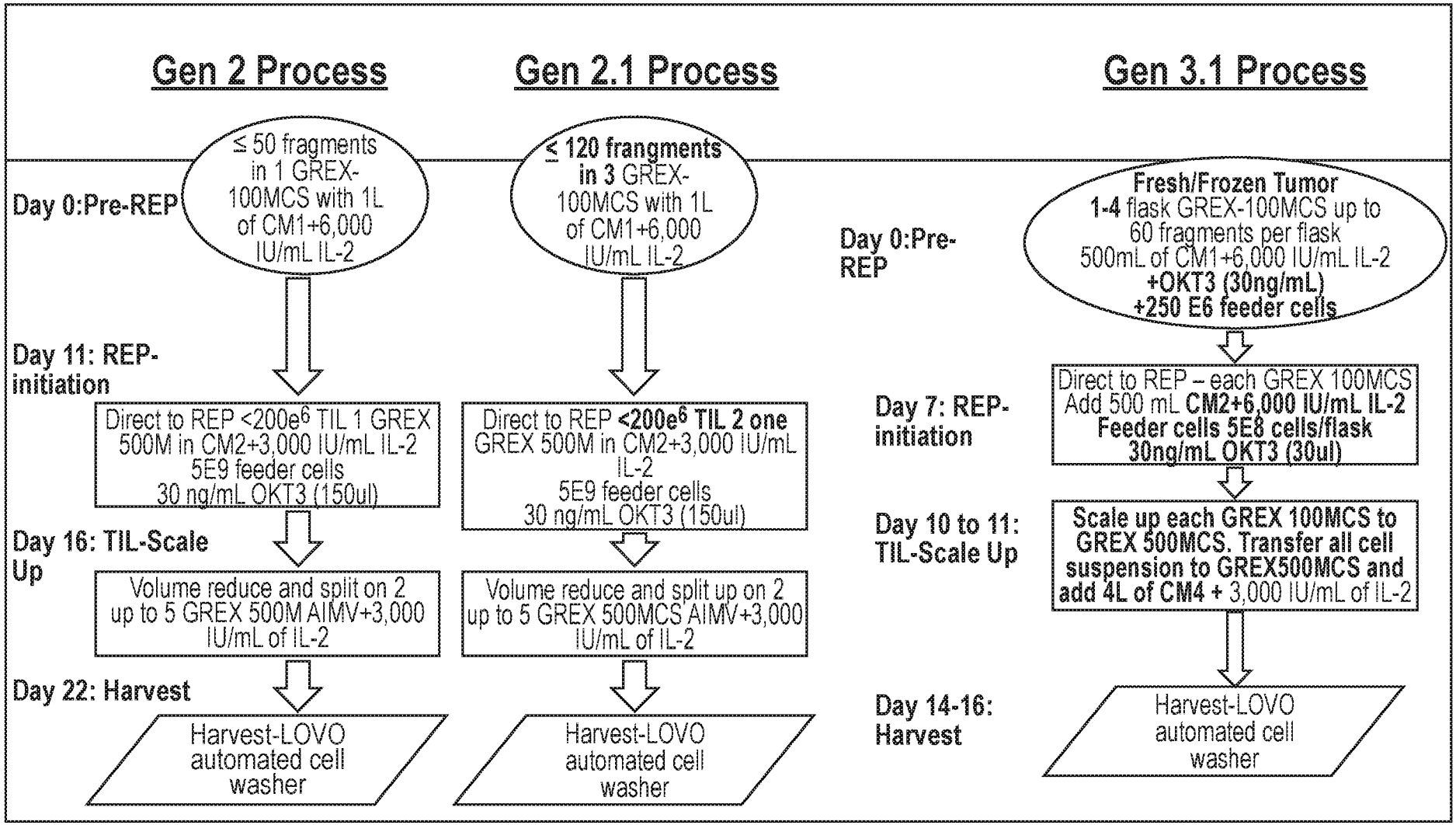

Gen 2 Process
Gen 2.1 Process
Gen 3.1 Process
Day 0:Pre-REP
≤ 50 fragments in 1 GREX-100MCS with 1L of CM1+6,000 IU/mL IL-2
≤ 120 frangments in 3 GREX-100MCS with 1L of CM1+6,000 IU/mL IL-2
Day 0:Pre-REP
Fresh/Frozen Tumor
1-4 flask GREX-100MCS up to 60 fragments per flask
500mL of CM1+6,000 IU/mL IL-2
+OKT3 (30ng/mL)
+250 E6 feeder cells
Day 11: REP-initiation
Direct to REP <200e6 TIL 1 GREX 500M in CM2+3,000 IU/mL IL-2
5E9 feeder cells
30 ng/mL OKT3 (150ul)
Direct to REP <200e6 TIL 2 one GREX 500M in CM2+3,000 IU/mL IL-2
5E9 feeder cells
30 ng/mL OKT3 (150ul)
Day 7: REP-initiation
Direct to REP – each GREX 100MCS
Add 500 mL CM2+6,000 IU/mL IL-2
Feeder cells 5E8 cells/flask
30ng/mL OKT3 (30ul)
Day 16: TIL-Scale Up
Volume reduce and split on 2 up to 5 GREX 500M AIMV+3,000 IU/mL of IL-2
Volume reduce and split up on 2 up to 5 GREX 500MCS AIMV+3,000 IU/mL of IL-2
Day 10 to 11: TIL-Scale Up
Scale up each GREX 100MCS to GREX 500MCS. Transfer all cell suspension to GREX500MCS and add 4L of CM4 + 3,000 IU/mL of IL-2
Day 22: Harvest
Harvest-LOVO automated cell washer
Harvest-LOVO automated cell washer
Day 14-16: Harvest
Harvest-LOVO automated cell washer

FIG. 30

SUBSTITUTE SHEET (RULE 26)

STEP	Gen 2	Gen 2.1	Gen 3.0 Optimized
Pre REP- day 0	≤ 50 fragments/ 1 G-Rex 100MCS - **11 days**	≤ 180 fragments/ 3 G-Rex, Pre-formulated CM1 warmed media 100MCS - **11 days**	***Fresh or Frozen Tumor*** Whole tumor with ≤ 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**

FIG. 31

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

FIG. 32

SUBSTITUTE SHEET (RULE 26)

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.

FIG. 33

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

FIG. 34

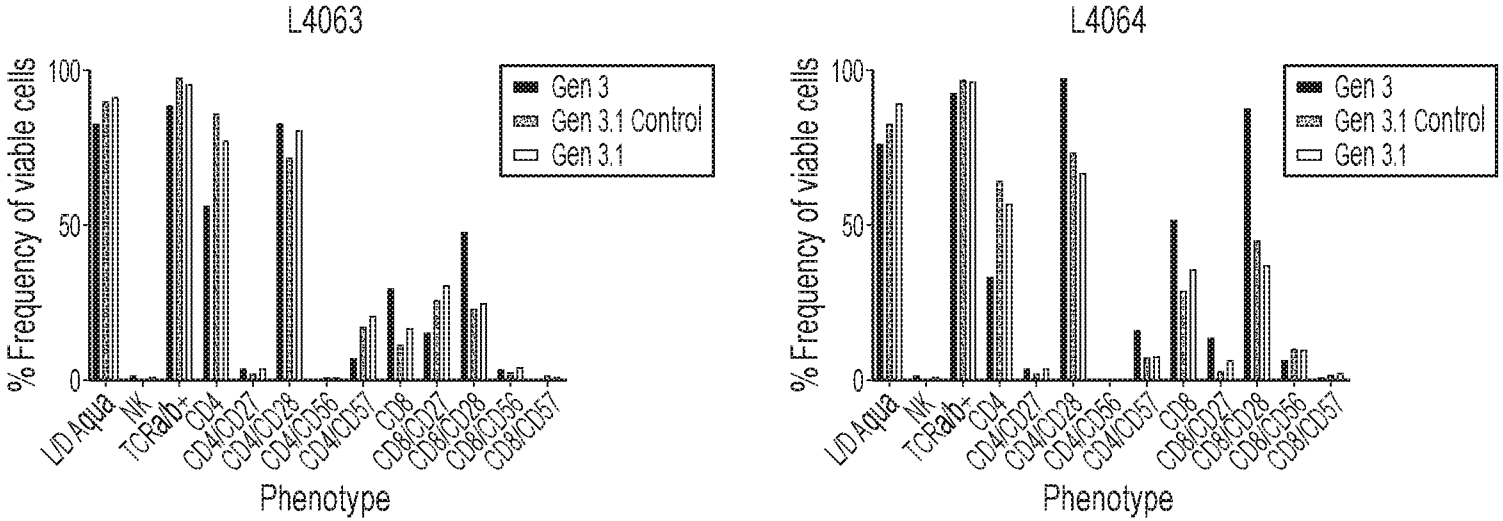
Process	Gen 2	Gen 3
L4054	Standard Media	Standard Media
L4055	Standard Media	Standard Media
M1085T	Standard Media	Standard Media

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

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

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

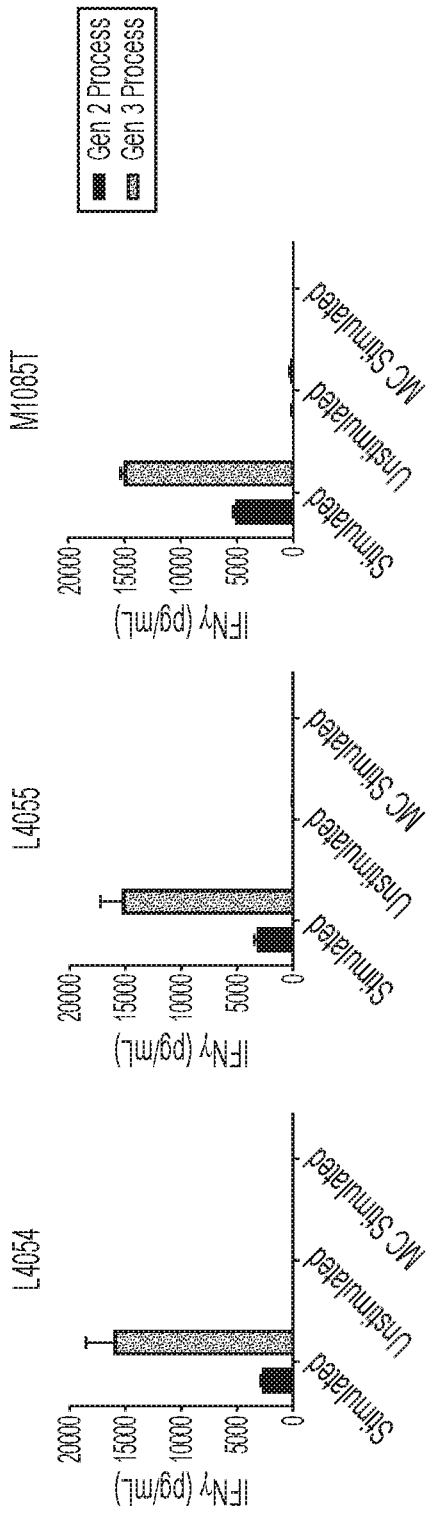
FIG. 35

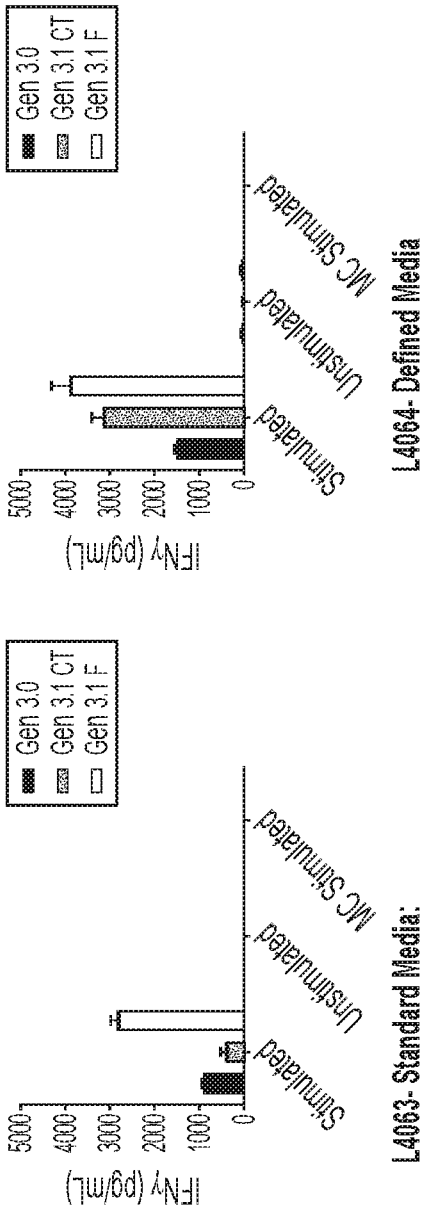


Tumor ID	L4063 Standard media			L4064 Defined media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1	Gen 3.0	Gen 3.1 Control	Gen 3.1
# Fragments	53	53	53	21	21	21

Tumor ID	L4063 in Standard Media			L4064 in CTS Optimizer Media		
Process	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test	Gen 3.0	Gen 3.1 Control	Gen 3.1 Test
Number of fragments	53	53	53	21	21	21
Average TVC per fragment	1.37E+08	3.19E+08	3.53E+08	5.90E+08	7.57E+08	9.29E+08
% viability at Harvest	79.53%	89.43%	94.80%	88.80%	81.90%	84.90%
TVC Harvest	**7.26E+09**	**1.69E+10**	**1.87E+10 ***	**1.24E+10**	**1.59E+10**	**1.95E+10 ***

FIG. 36





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	500 mL	500 mL	500 mL
	IL-2 (6000 IU/mL)	+	+	+
	OKT-3 (30ng/mL)	-	+	+
	Feeders (250 E+06)	-	-	+

FIG. 37

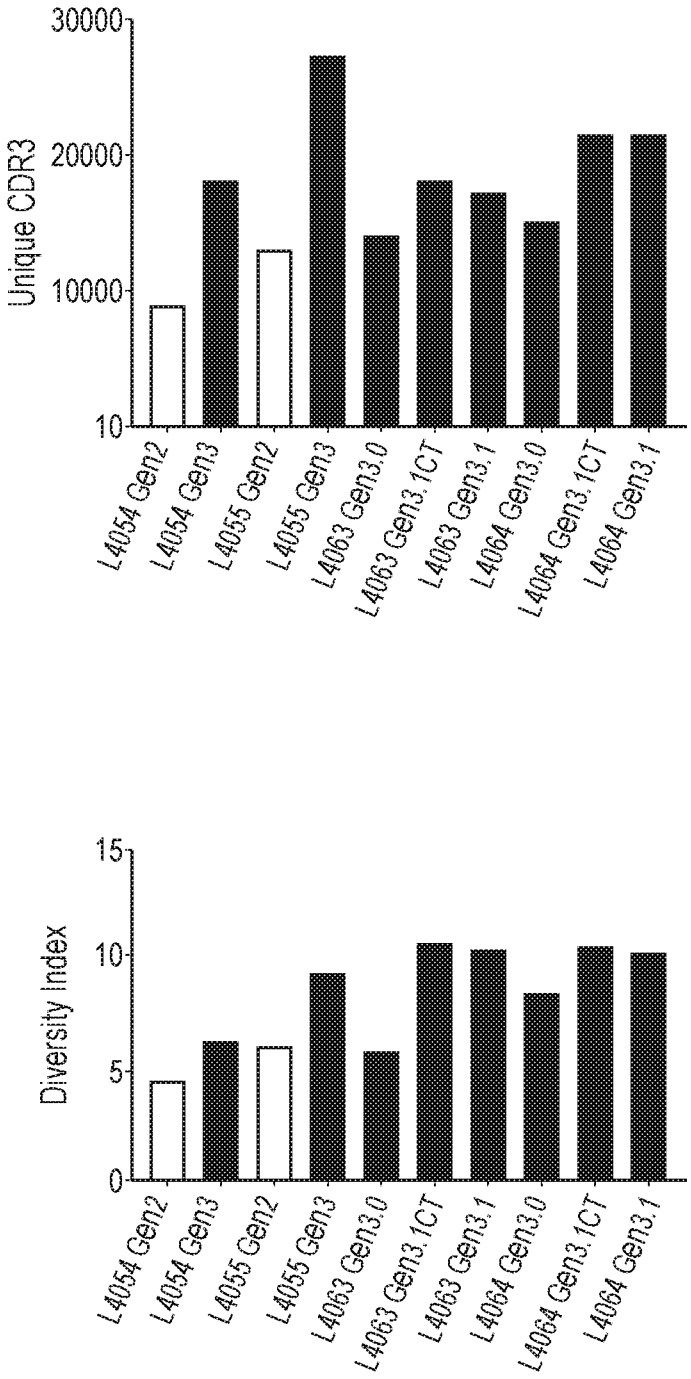

Unique CDR3
30000
20000
10000
10
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064 Gen3.1
Diversity Index
15
10
5
0
L4054 Gen2
L4054 Gen3
L4055 Gen2
L4055 Gen3
L4063 Gen3.0
L4063 Gen3.1CT
L4063 Gen3.1
L4064 Gen3.0
L4064 Gen3.1CT
L4064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

FIG. 38

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	14005	3240 (23)	2959 (21)	1105	1000 (90)	975 (88)
Gen 3.1 control	-	18149	-	-	2438	-
Gen 3.1	-	-	17181	-	-	2332

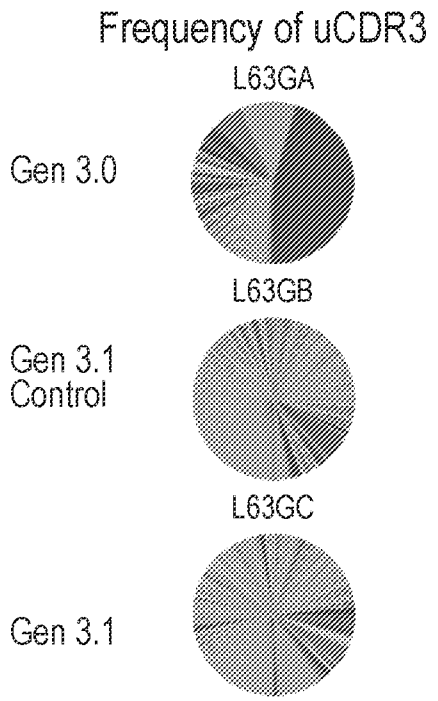


FIG. 39

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

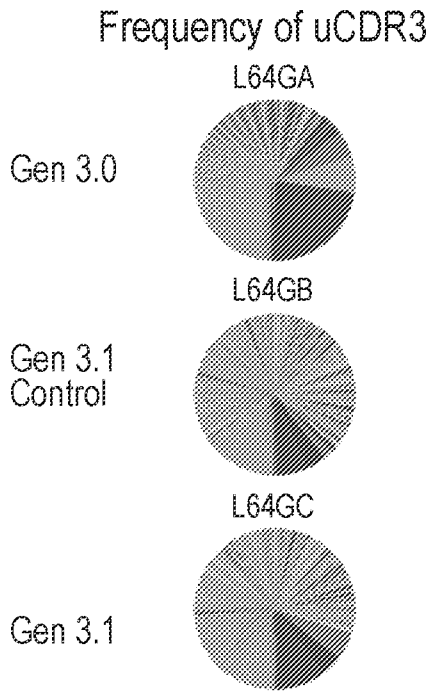


FIG. 40

SUBSTITUTE SHEET (RULE 26)

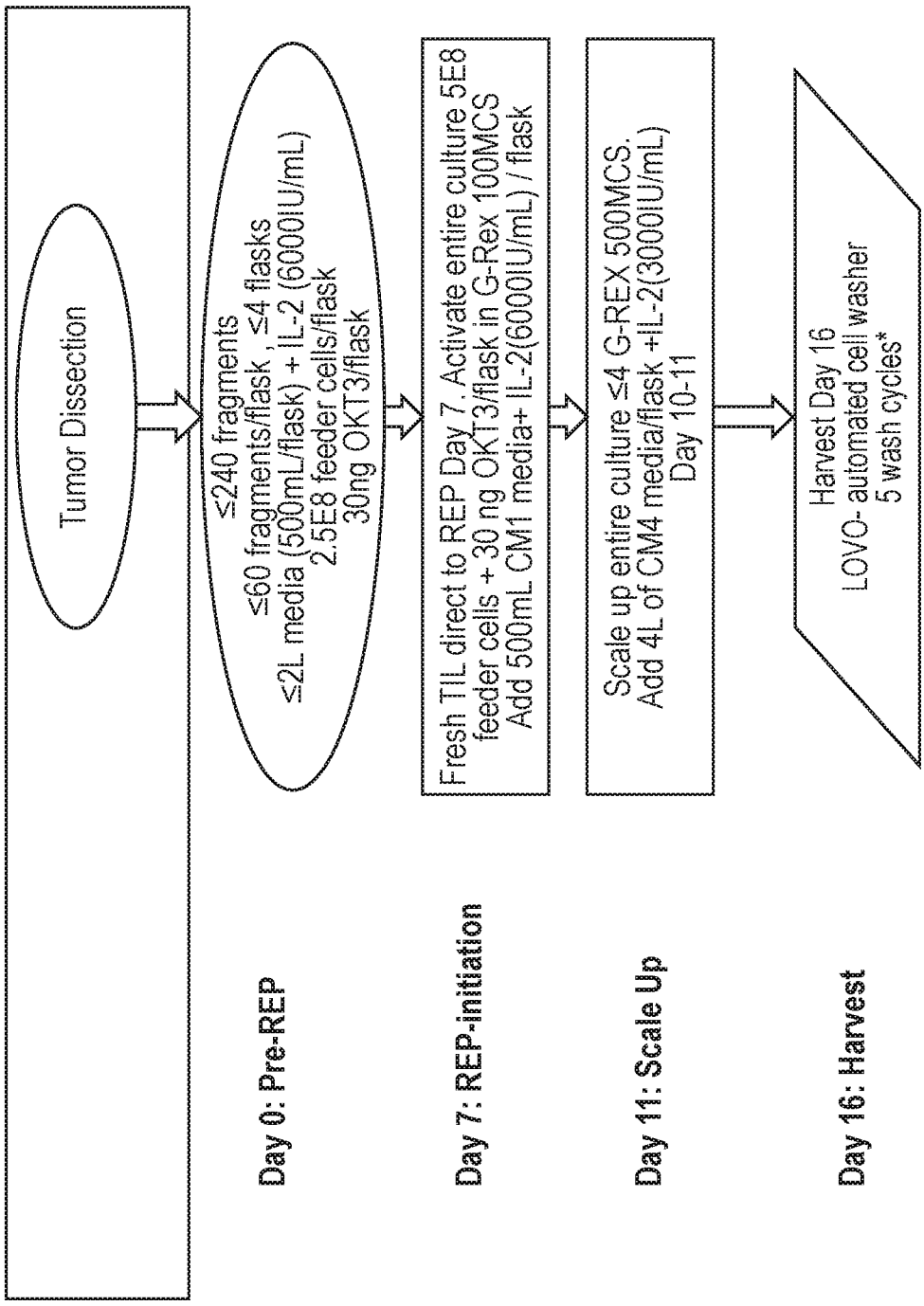

Tumor Dissection
Day 0: Pre-REP
≤240 fragments
≤60 fragments/flask , ≤4 flasks
≤2L media (500mL/flask) + IL-2 (6000IU/mL)
2.5E8 feeder cells/flask
30ng OKT3/flask
Day 7: REP-initiation
Fresh TIL direct to REP Day 7. Activate entire culture 5E8
feeder cells + 30 ng OKT3/flask in G-Rex 100MCS
Add 500mL 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*

FIG. 41

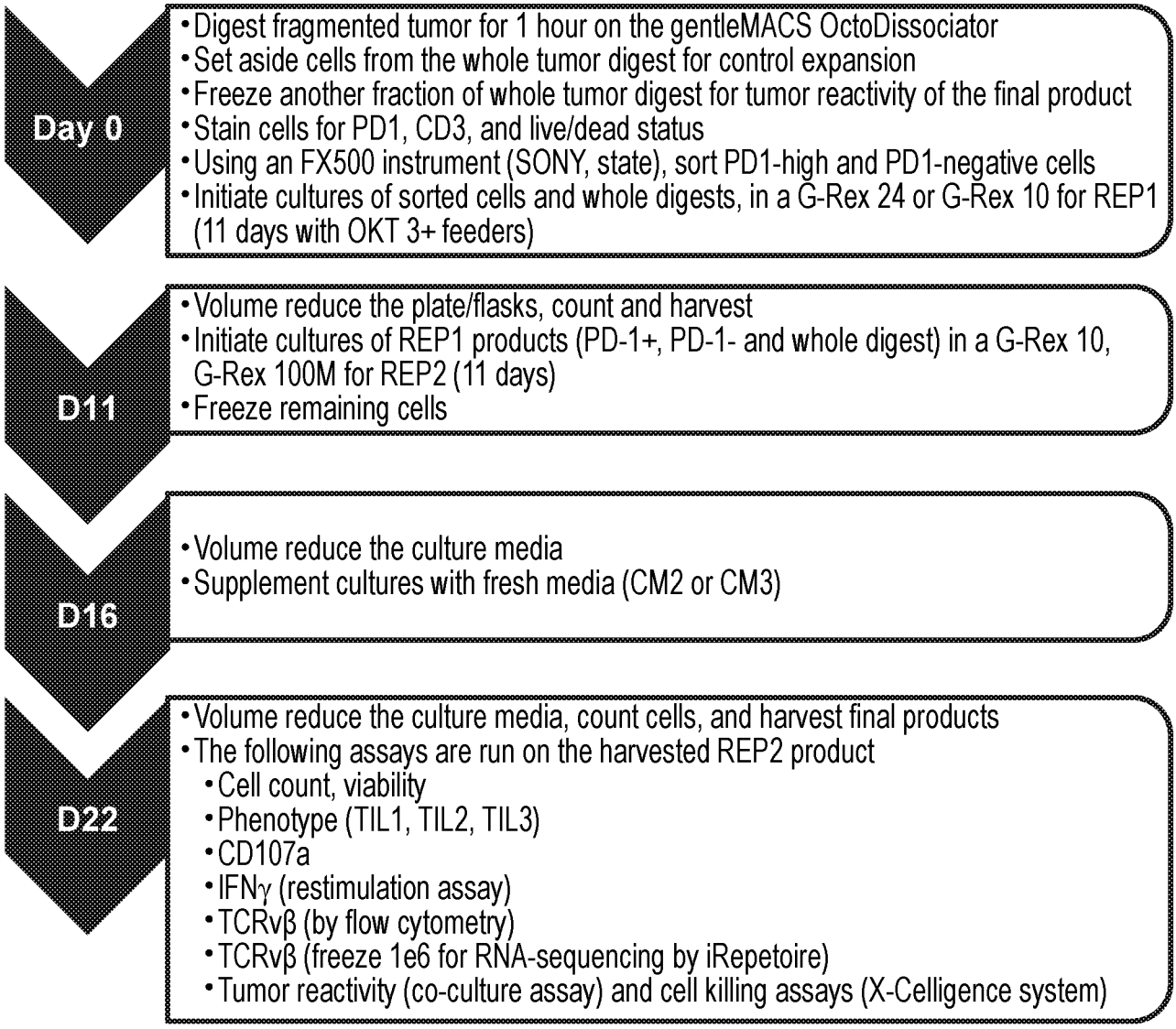

Day 0
• Digest fragmented tumor for 1 hour on the gentleMACS OctoDissociator
• Set aside cells from the whole tumor digest for control expansion
• Freeze another fraction of whole tumor digest for tumor reactivity of the final product
• Stain cells for PD1, CD3, and live/dead status
• Using an FX500 instrument (SONY, state), sort PD1-high and PD1-negative cells
• Initiate cultures of sorted cells and whole digests, in a G-Rex 24 or G-Rex 10 for REP1 (11 days with OKT 3+ feeders)
D11
• Volume reduce the plate/flasks, count and harvest
• Initiate cultures of REP1 products (PD-1+, PD-1- and whole digest) in a G-Rex 10, G-Rex 100M for REP2 (11 days)
• Freeze remaining cells
D16
• Volume reduce the culture media
• Supplement cultures with fresh media (CM2 or CM3)
D22
• Volume reduce the culture media, count cells, and harvest final products
• The following assays are run on the harvested REP2 product
• Cell count, viability
• Phenotype (TIL1, TIL2, TIL3)
• CD107a
• IFNγ (restimulation assay)
• TCRvβ (by flow cytometry)
• TCRvβ (freeze 1e6 for RNA-sequencing by iRepetoire)
• Tumor reactivity (co-culture assay) and cell killing assays (X-Celligence system)

FIG. 42

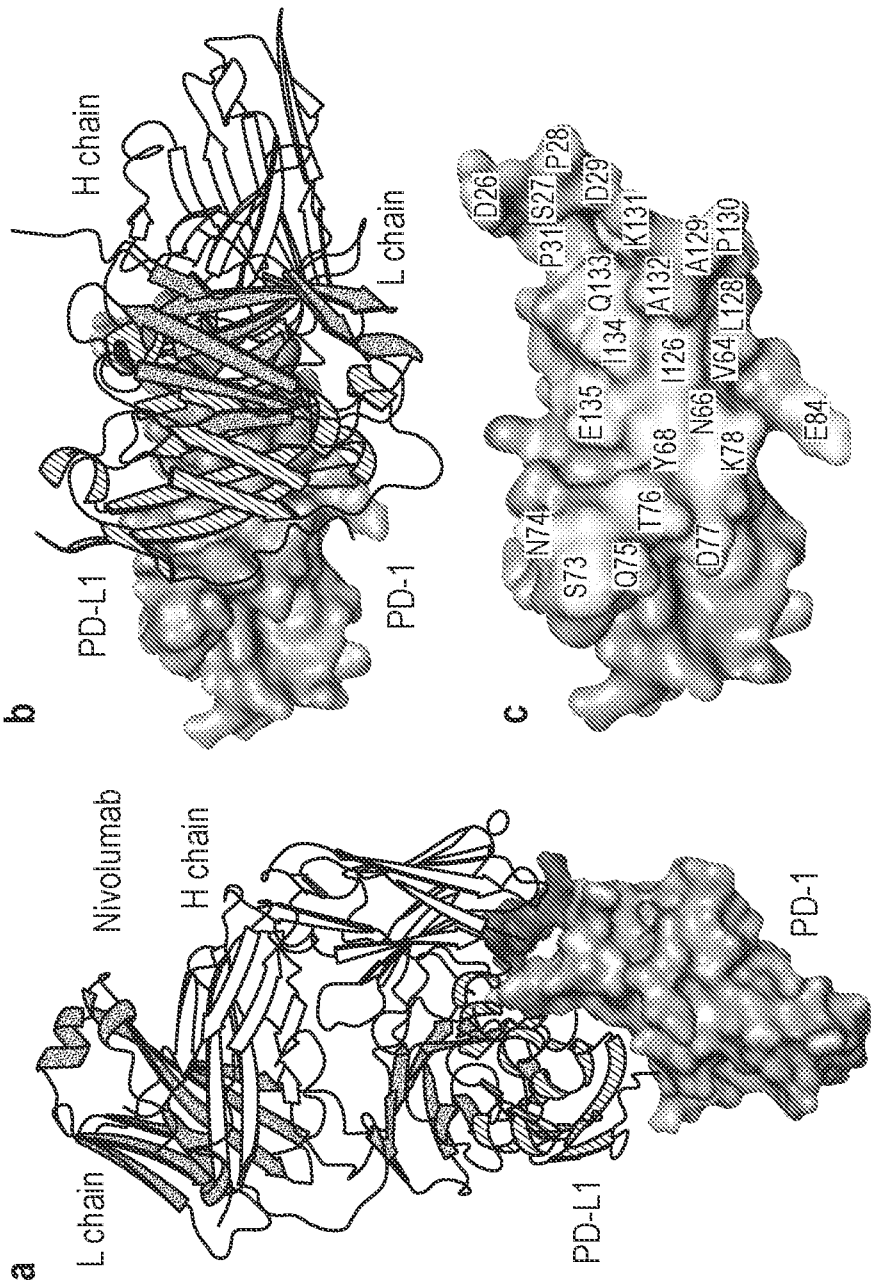

a
L chain
Nivolumab
H chain
PD-L1
PD-1
b
PD-L1
H chain
L chain
PD-1
c
D26
P31
S27
P28
D29
K131
Q133
A132
A129
P130
I134
I126
L128
E135
N66
V64
E84
Y68
K78
N74
T76
S73
Q75
D77

FIG. 43

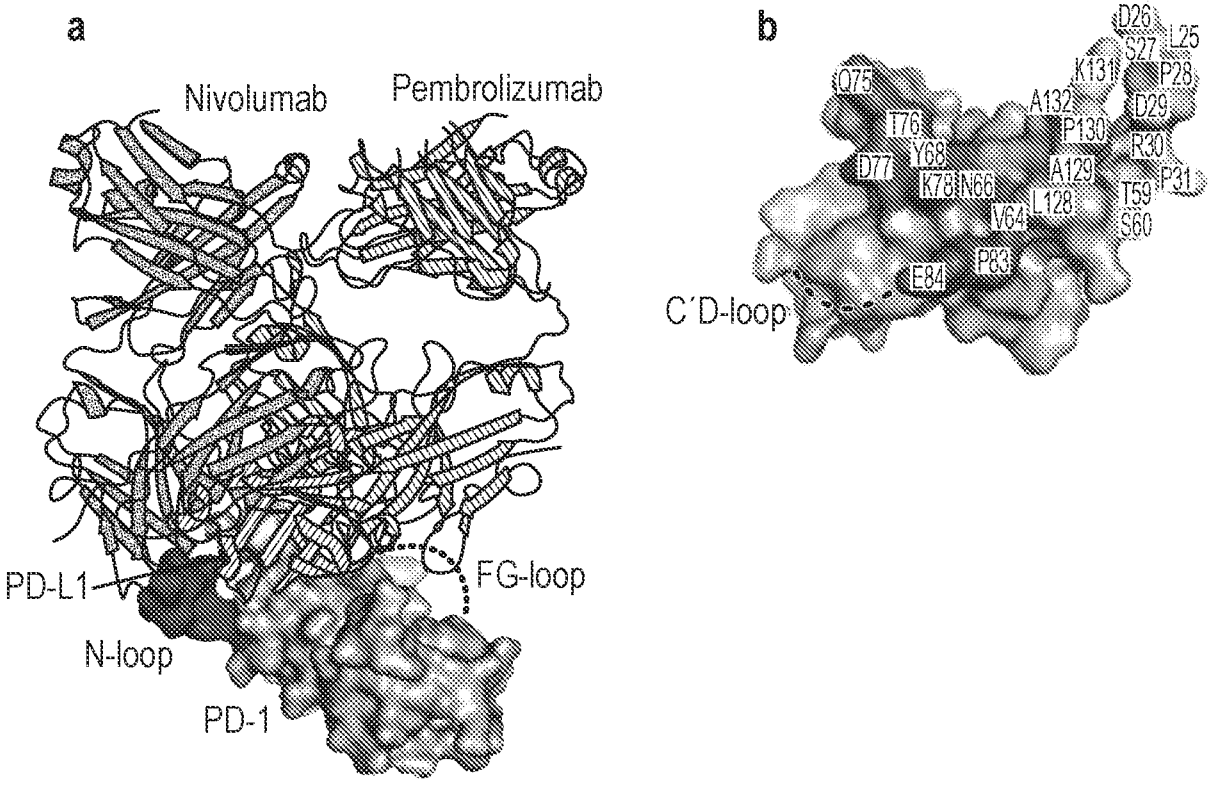

a
Nivolumab
Pembrolizumab
PD-L1
N-loop
PD-1
FG-loop
b
C´D-loop
D26
S27
L25
K131
P28
Q75
A132
D29
T76
P130
R30
Y68
D77
K78
N66
A129
P31
T59
L128
S60
V64
P83
E84

FIG. 44

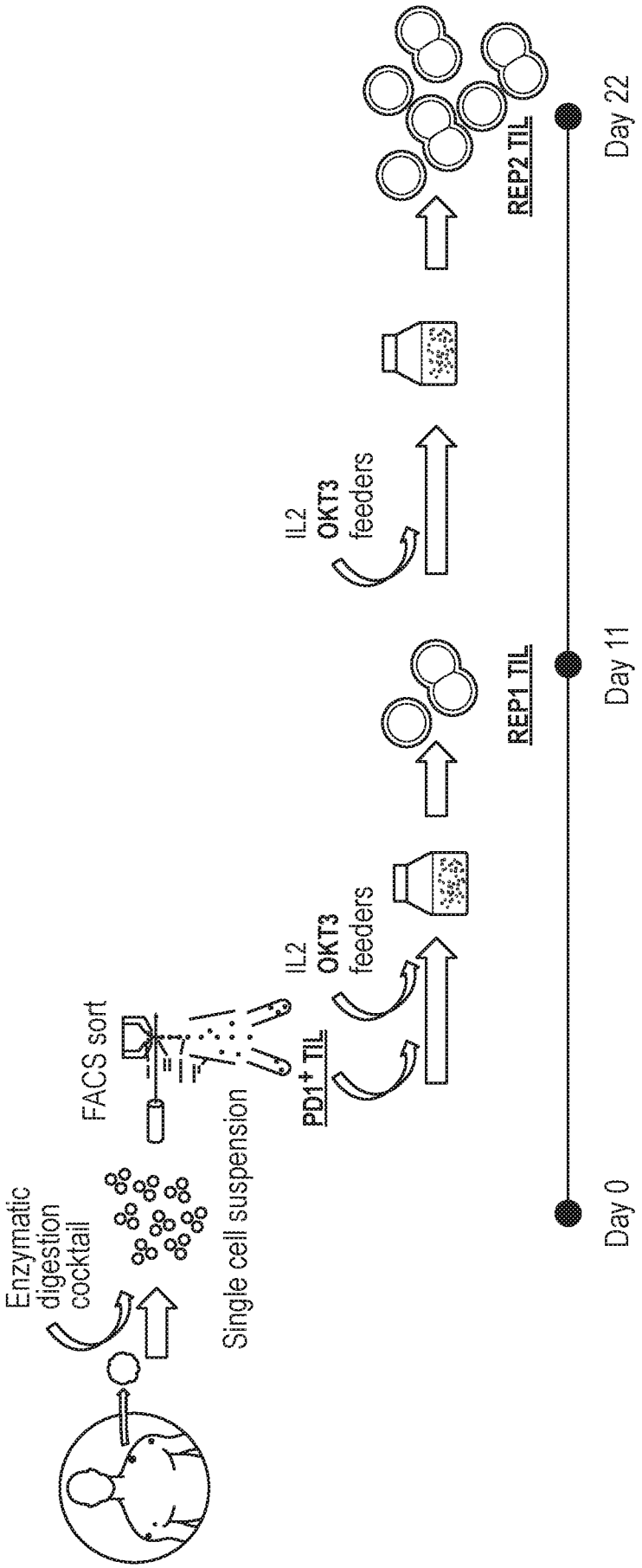

Enzymatic digestion cocktail
Single cell suspension
FACS sort
PD1+ TIL
IL2
OKT3
feeders
REP1 TIL
IL2
OKT3
feeders
REP2 TIL
Day 0
Day 11
Day 22

FIG. 45

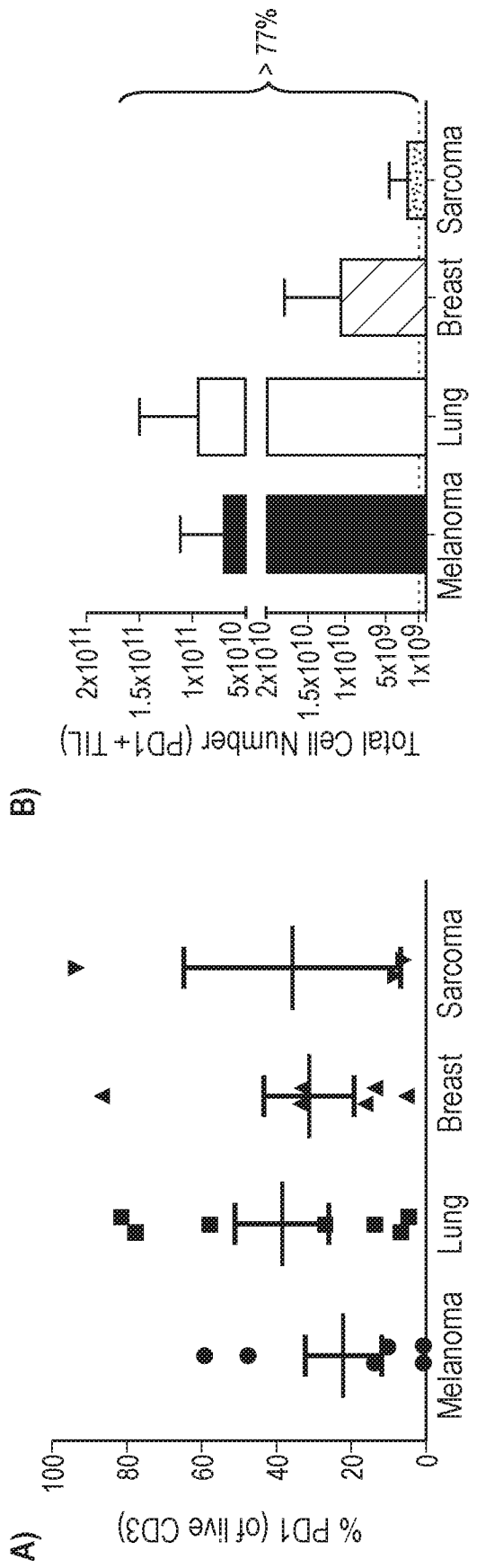

A)
% PD1 (of live CD3)
100
80
60
40
20
0
Melanoma
Lung
Breast
Sarcoma
B)
Total Cell Number (PD1+ TIL)
2x10^11
1.5x10^11
1x10^11
5x10^10
2x10^10
1.5x10^10
1x10^10
5x10^9
1x10^9
> 77%
Melanoma
Lung
Breast
Sarcoma

FIG. 46

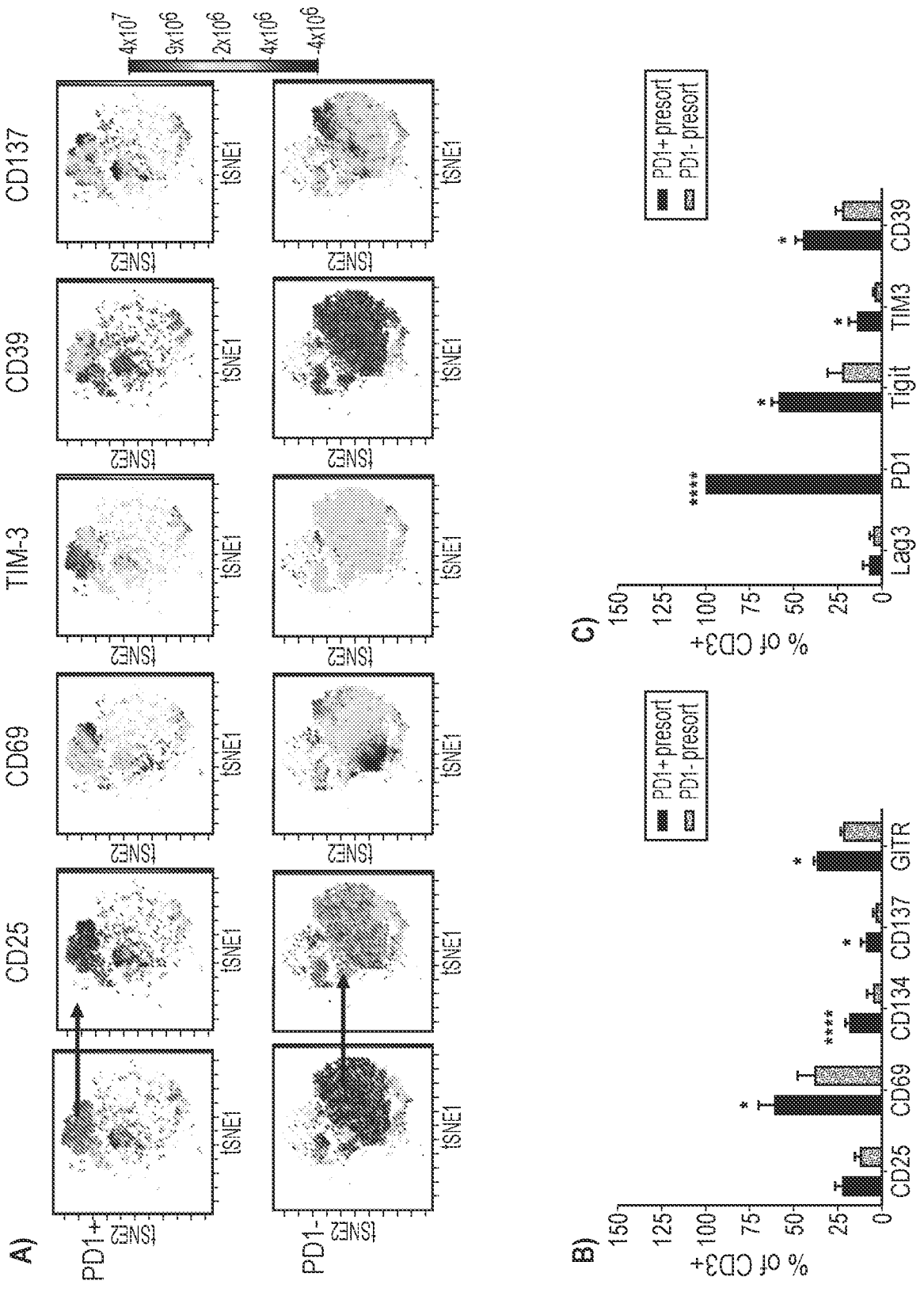

A)
CD25
CD69
TIM-3
CD39
CD137
PD1+
PD1-
tSNE1
tSNE2
4x10^7
9x10^6
2x10^6
4x10^6
4x10^6
B)
% of CD3+
PD1+ presort
PD1- presort
CD25
CD69
CD134
CD137
GITR
C)
% of CD3+
PD1+ presort
PD1- presort
Lag3
PD1
Tigit
TIM3
CD39

FIG. 47

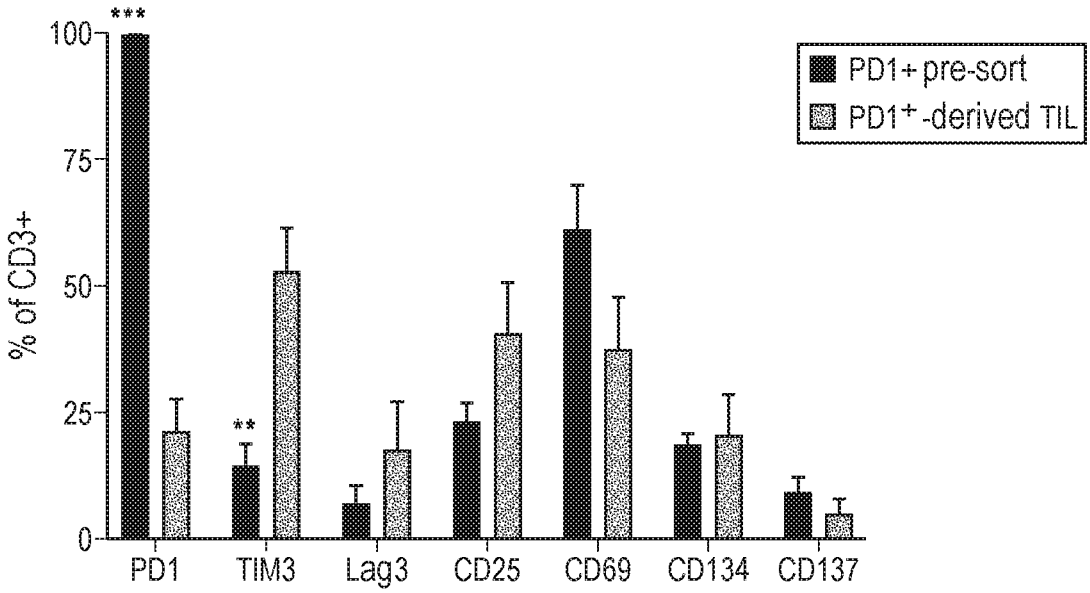

***
**
100
75
50
25
0
% of CD3+
PD1+ pre-sort
PD1+-derived TIL
PD1
TIM3
Lag3
CD25
CD69
CD134
CD137

FIG. 48

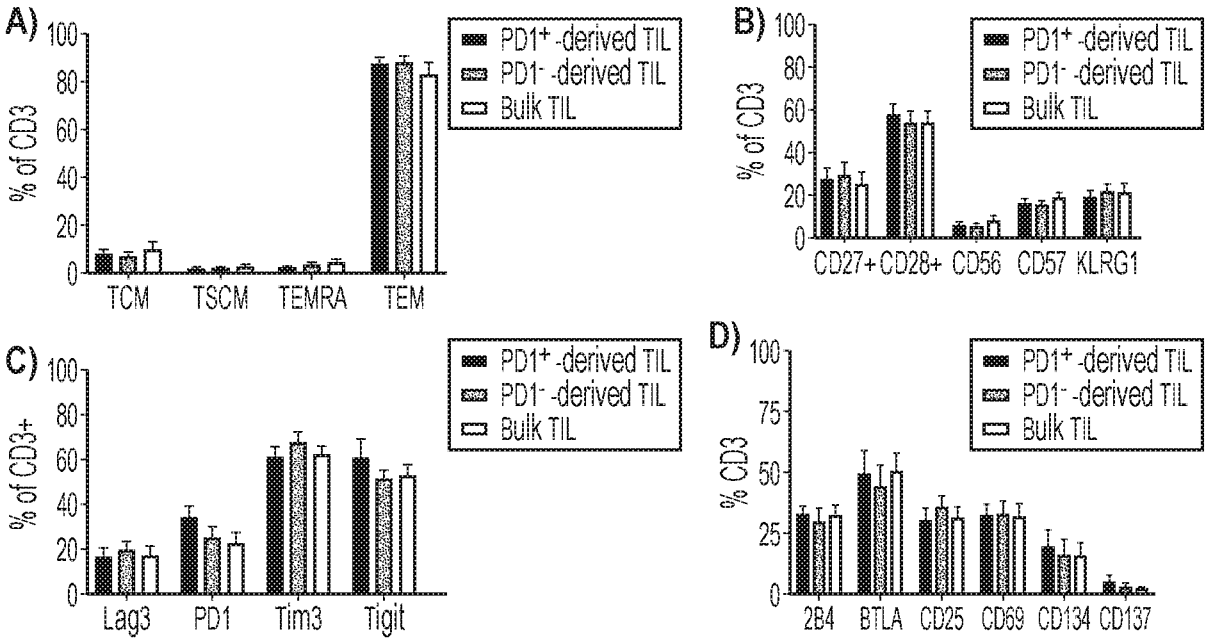
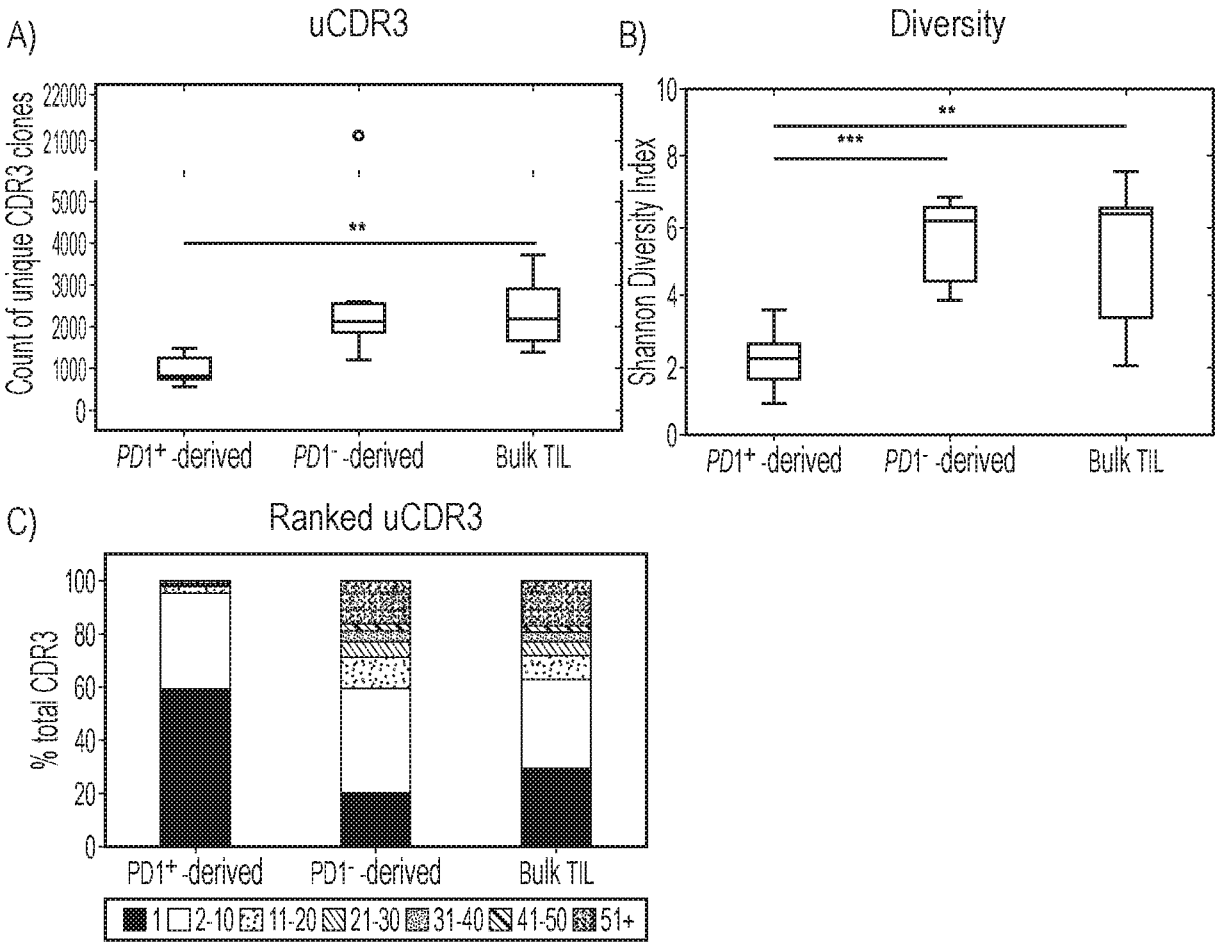

A)
% of CD3
100
80
60
40
20
0
TCM
TSCM
TEMRA
TEM
PD1+ -derived TIL
PD1- -derived TIL
Bulk TIL
B)
% of CD3
CD27+
CD28+
CD56
CD57
KLRG1
C)
% of CD3+
Lag3
PD1
Tim3
Tigit
D)
% CD3
75
50
25
2B4
BTLA
CD25
CD69
CD134
CD137

FIG. 49



C) *Shared clones between bulk TIL and PD1+ -derived*

Tumor	Count of shared uCDR3 clones	Bulk TIL Shared %	PD1+-derived Shared %
Breast	43	0.61	78.95
Breast	76	79.51	88.64
Lung	40	5.26	35.16
Lung	60	4.66	34.57
Melanoma	59	21.14	53.84
Melanoma	39	19.87	71.90

FIG. 50

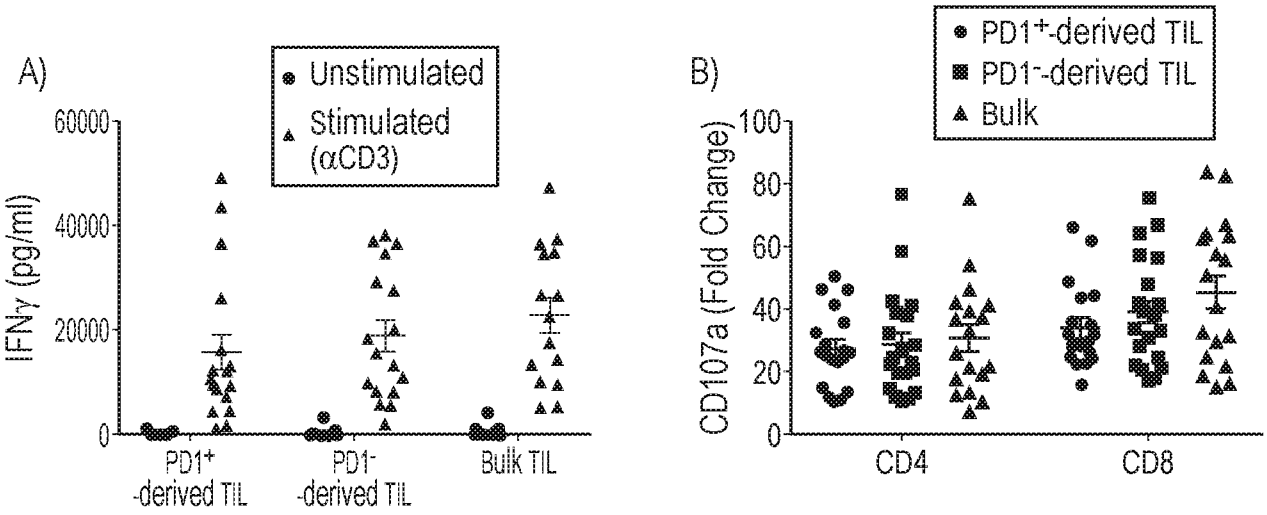
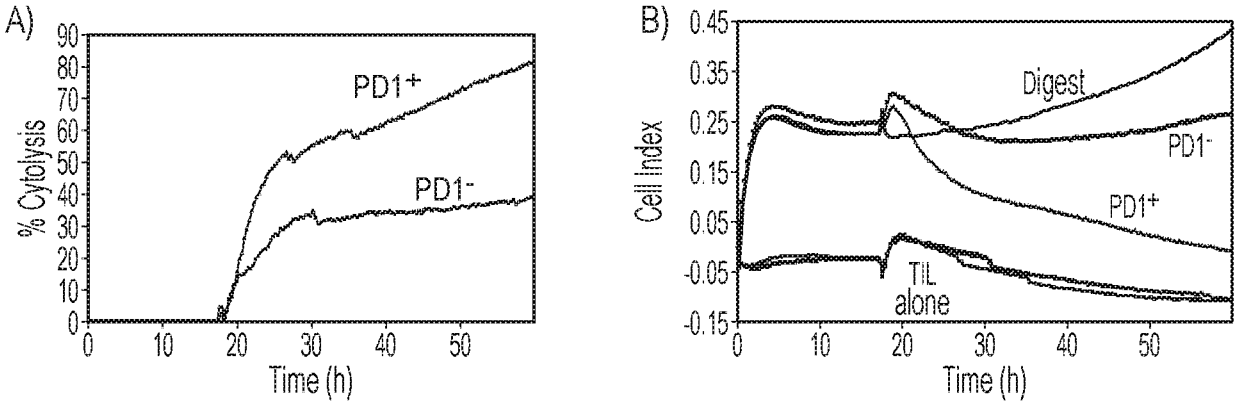

A)
Unstimulated
Stimulated (αCD3)
IFNγ (pg/ml)
60000
40000
20000
0
PD1+ -derived TIL
PD1- -derived TIL
Bulk TIL
B)
PD1+-derived TIL
PD1--derived TIL
Bulk
CD107a (Fold Change)
100
80
60
40
20
0
CD4
CD8

FIG. 51


A)
% Cytolysis
PD1+
PD1-
Time (h)
B)
Cell Index
Digest
PD1-
PD1+
TIL alone
Time (h)

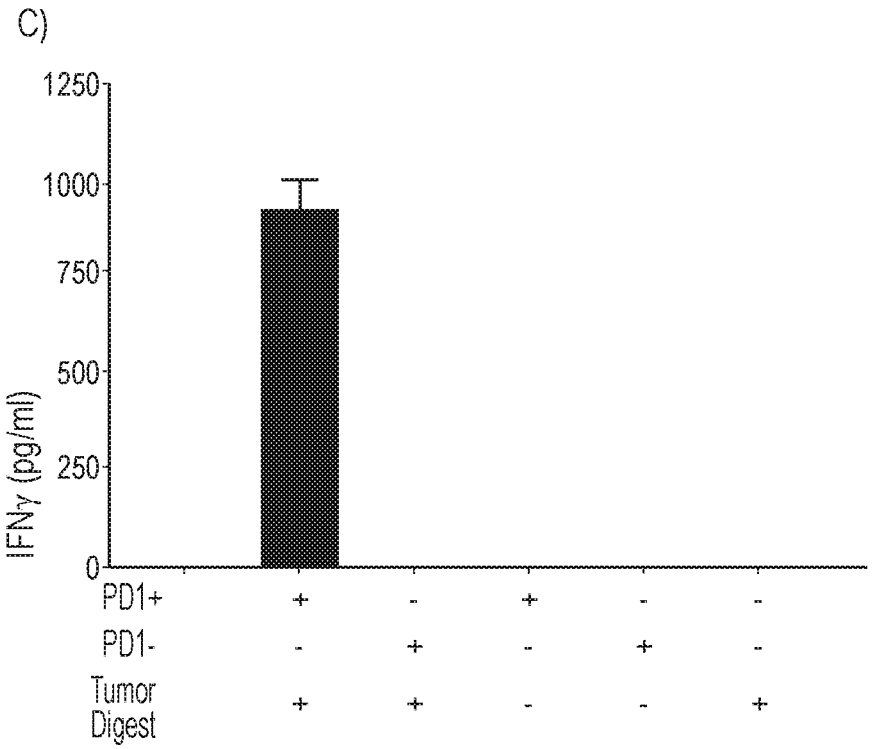

C)
IFNγ (pg/ml)
PD1+
PD1-
Tumor Digest

FIG. 52

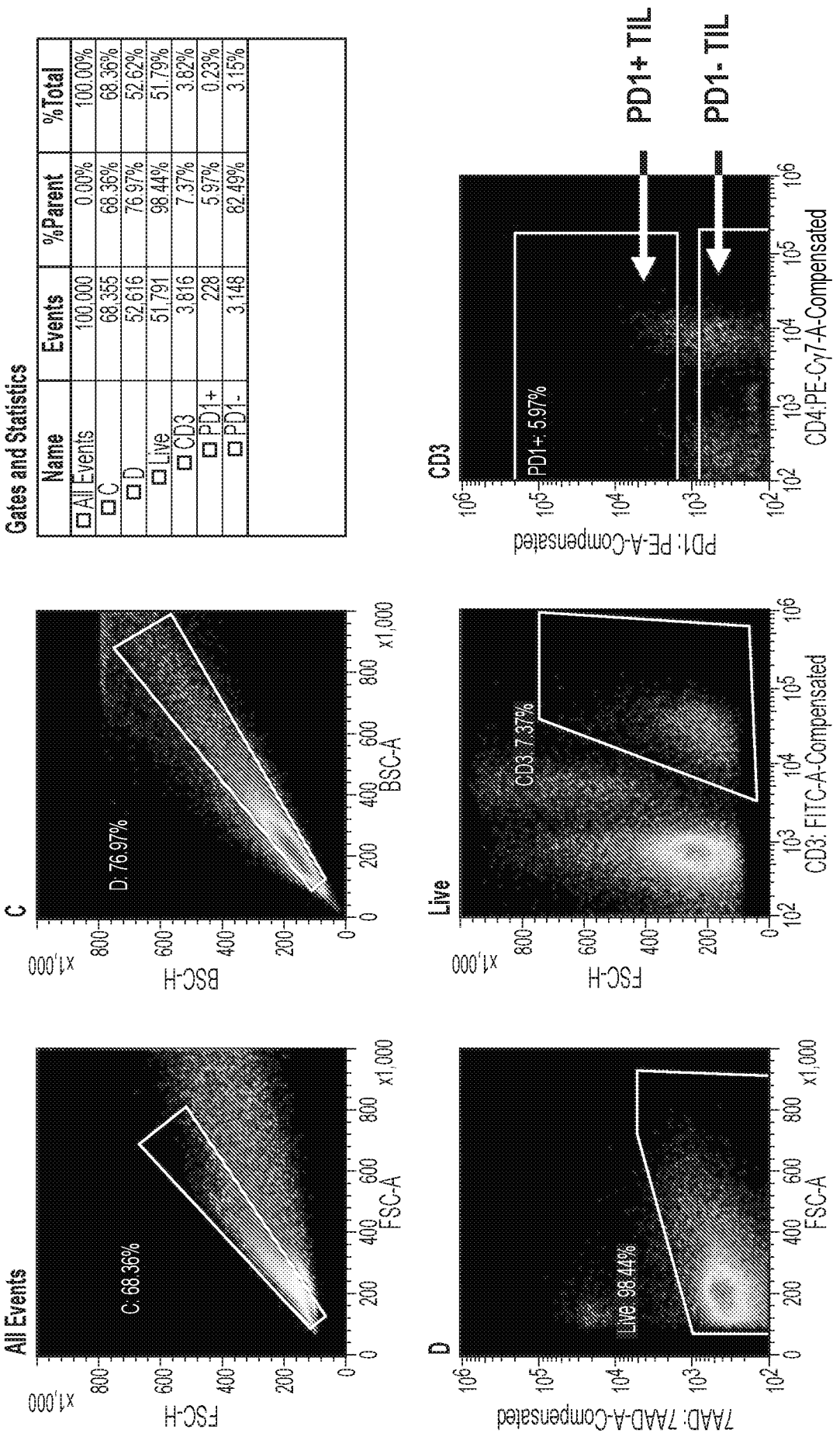

All Events
C: 68.36%
FSC-H
FSC-A
x1,000
C
D: 76.97%
BSC-H
BSC-A
Gates and Statistics
Name | Events | %Parent | %Total
All Events | 100,000 | 0.00% | 100.00%
C | 68,355 | 68.36% | 68.36%
D | 52,616 | 76.97% | 52.62%
Live | 51,791 | 98.44% | 51.79%
CD3 | 3,816 | 7.37% | 3.82%
PD1+ | 228 | 5.97% | 0.23%
PD1- | 3,148 | 82.49% | 3.15%
D
Live: 98.44%
7AAD: 7AAD-A-Compensated
Live
CD3: 7.37%
CD3: FITC-A-Compensated
CD3
PD1+: 5.97%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated
PD1+ TIL
PD1- TIL

FIG. 53

Method 1*: 10ng/ml of Collagenase
1mg/ml of Hyalurodase
500IU/ml of DNaseI

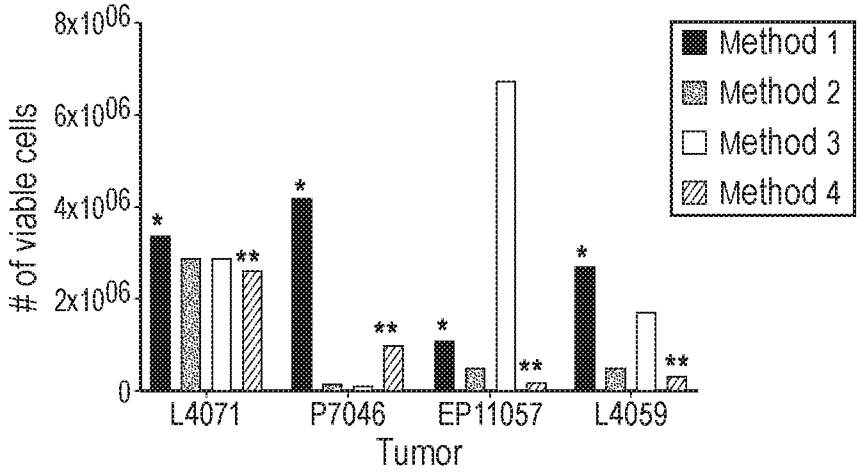
Method 2: 10ng/ml of Collagenase

Method 3: Miltenyi Tumor Dissociation Kit

Method 4:** 170mg/L Collagenase I
56mg/L Collagenase II
170mg/L Collagenase IV
25mg/L Elastase

* NIH Centers for Cancer Research, *Triple Enzyme Mouse Tumor Digestion*. 2006. **https://ccr.cancer.gov/sites/default/files/triple enzyme mouse tumor digestion.pdf**

**Quatromoni et al. An optimized disaggregation method for human lung tumors that preserves phenotype and function of immune cells. J Leukoc Biol. 2015 Jan: 97 (1): 201-209



Method 1 yielded highest cell numbers and greater proportion of T cells.

FIG. 54

Method 1: 0.008DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 2: 0.008DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 3: 0.36 DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 4: 0.36 DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 5: 10ng/ml of Collagenase
1mg/ml of Hyalurodase
500IU/ml of DNaseI
Total Cell Yield
1.5x10^07
1.0x10^07
5.0x10^06
0
GMP1 GMP2 GMP3 GMP4 IOVA
**
GMP1
GMP2
GMP3
GMP4
IOVA
** Likely similar cell numbers to the GMP conditions.
More tumor was used for the IOVA condition because this
condition was being used for the sort. Future
experiments will normalize to tumor weight

FIG. 55

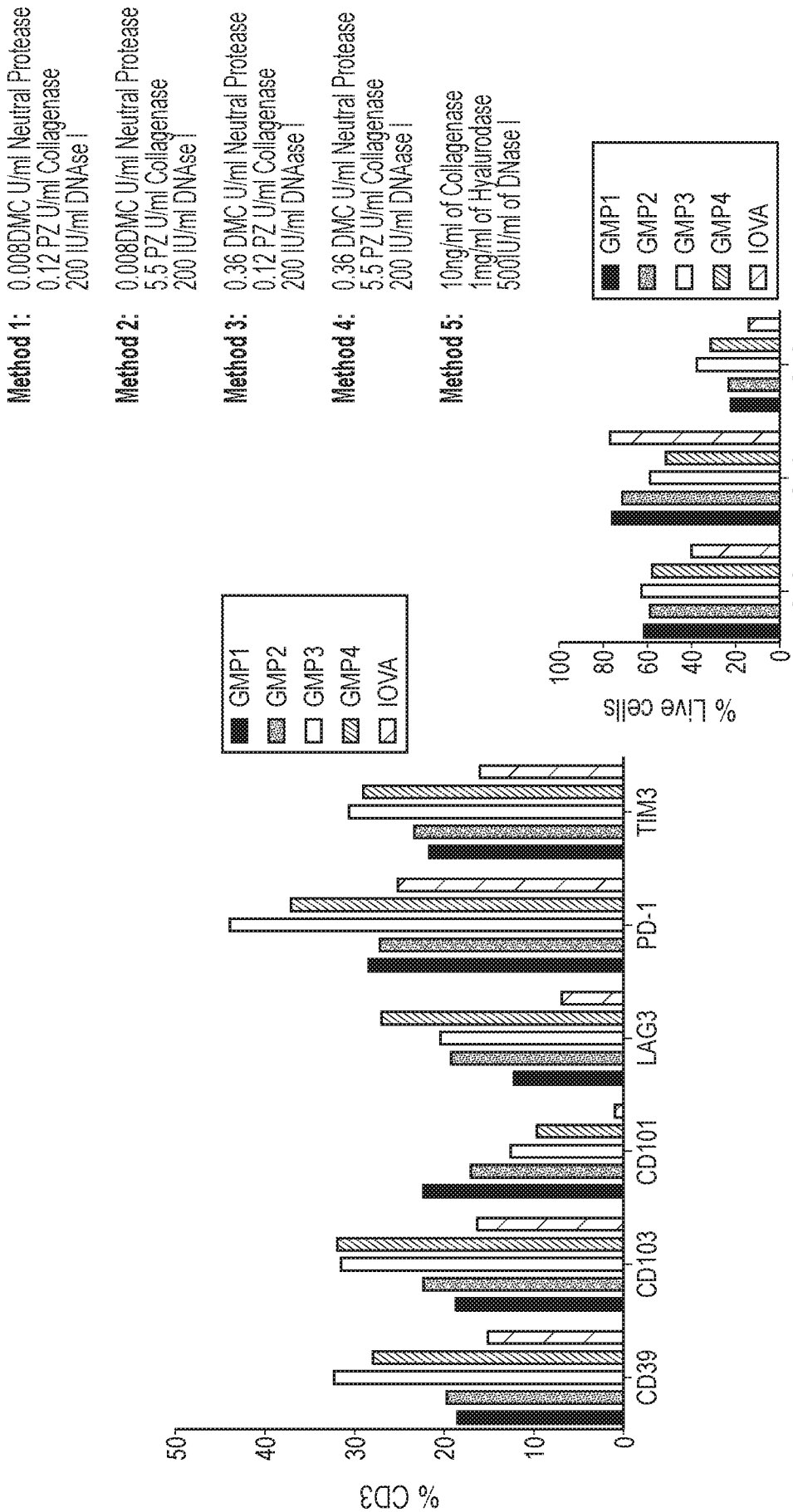

Method 1: 0.008DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 2: 0.008DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 3: 0.36 DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAase I
Method 4: 0.36 DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAase I
Method 5: 10ng/ml of Collagenase
1mg/ml of Hyalurodase
500IU/ml of DNase I
GMP1
GMP2
GMP3
GMP4
IOVA
% Live cells
0
20
40
60
80
100
CD3
CD4
CD8
% CD3
0
10
20
30
40
50
CD39
CD103
CD101
LAG3
PD-1
TIM3

FIG. 56

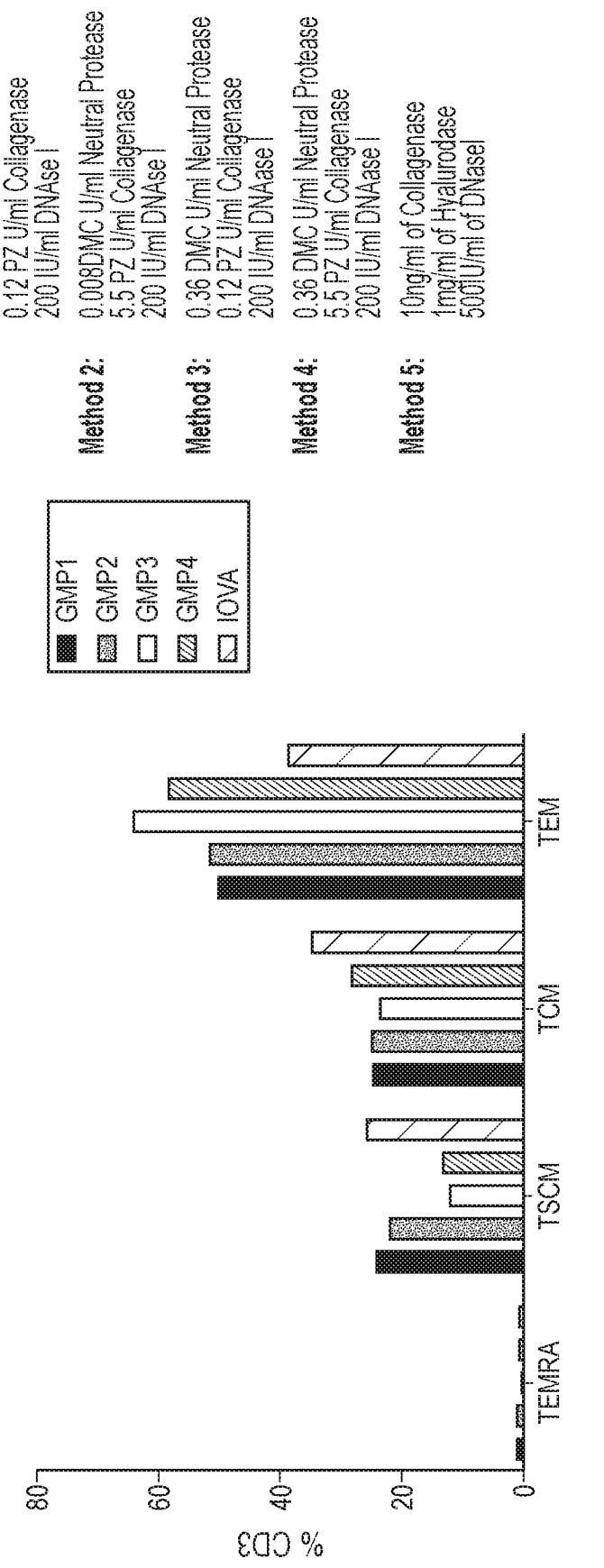

Method 1: 0.008DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 2: 0.008DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAse I
Method 3: 0.36 DMC U/ml Neutral Protease
0.12 PZ U/ml Collagenase
200 IU/ml DNAase I
Method 4: 0.36 DMC U/ml Neutral Protease
5.5 PZ U/ml Collagenase
200 IU/ml DNAase I
Method 5: 10ng/ml of Collagenase
1mg/ml of Hyalurodase
500IU/ml of DNaseI
GMP1
GMP2
GMP3
GMP4
IOVA
% CD3
80
60
40
20
0
TEMRA
TSCM
TCM
TEM

FIG. 57

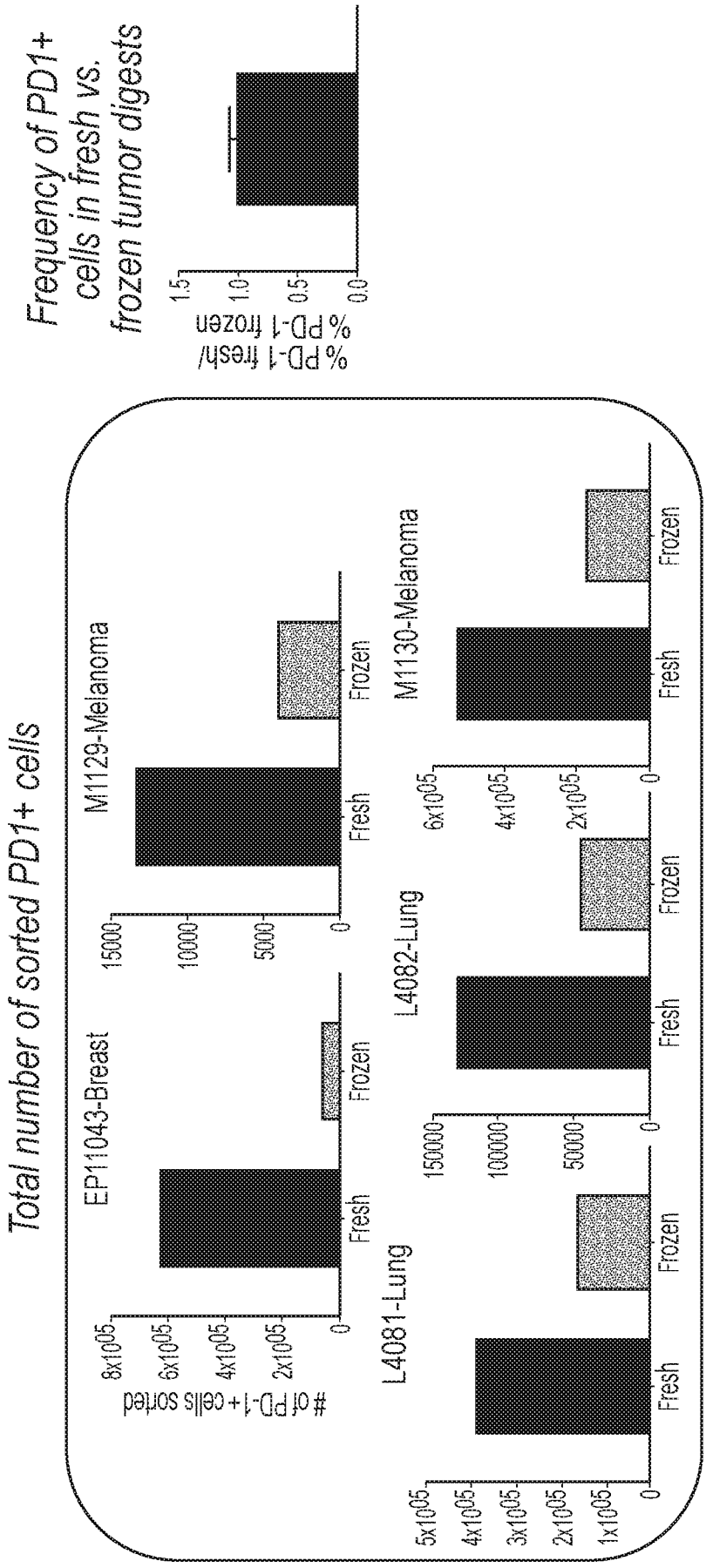

Frequency of PD1+ cells in fresh vs. frozen tumor digests
% PD-1 fresh/ % PD-1 frozen
1.5
1.0
0.5
0.0
Total number of sorted PD1+ cells
# of PD-1+ cells sorted
EP11043-Breast
M1129-Melanoma
L4081-Lung
L4082-Lung
M1130-Melanoma
Fresh
Frozen
0
2x10^05
4x10^05
6x10^05
8x10^05
5000
10000
15000
1x10^05
2x10^05
3x10^05
4x10^05
5x10^05
50000
100000
150000

FIG. 58

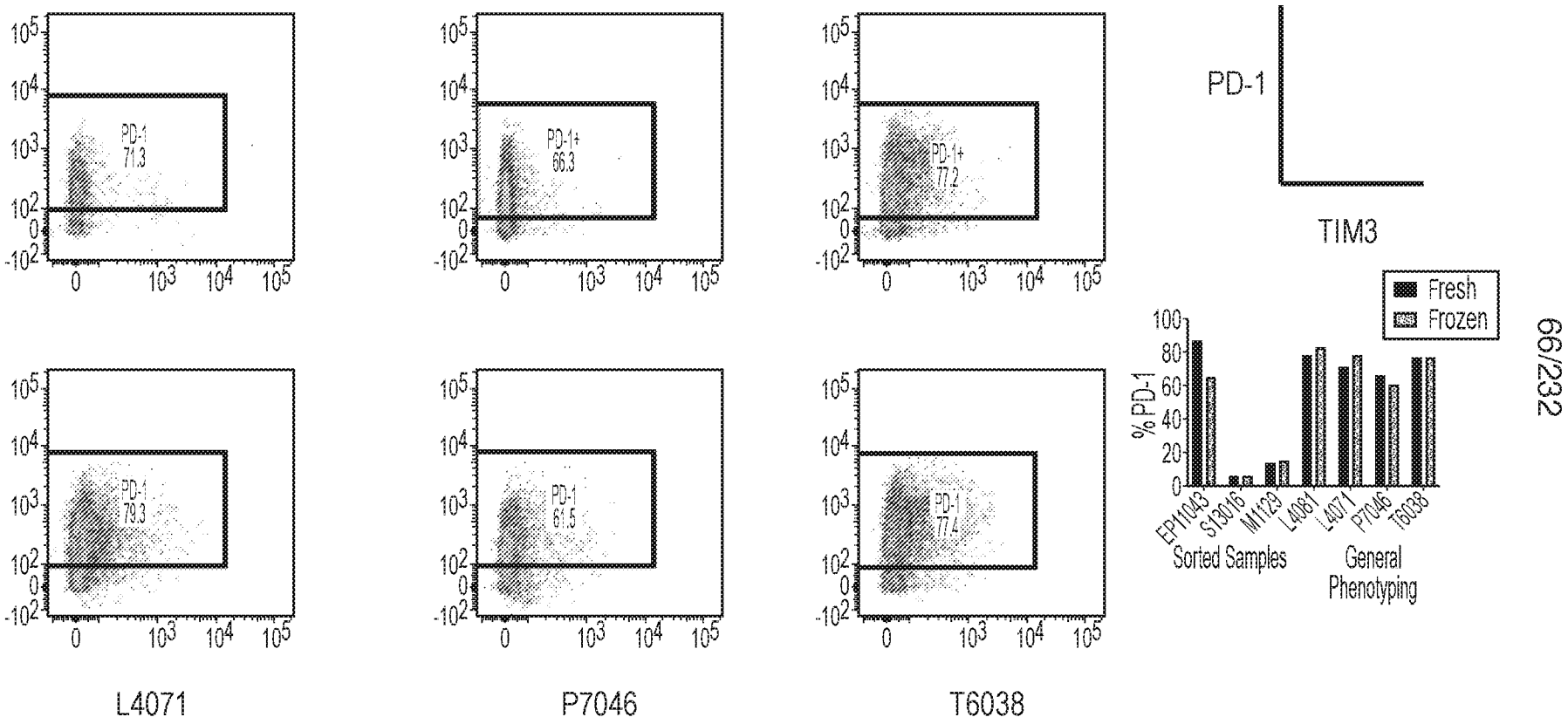


FIG. 59

SUBSTITUTE SHEET (RULE 26)

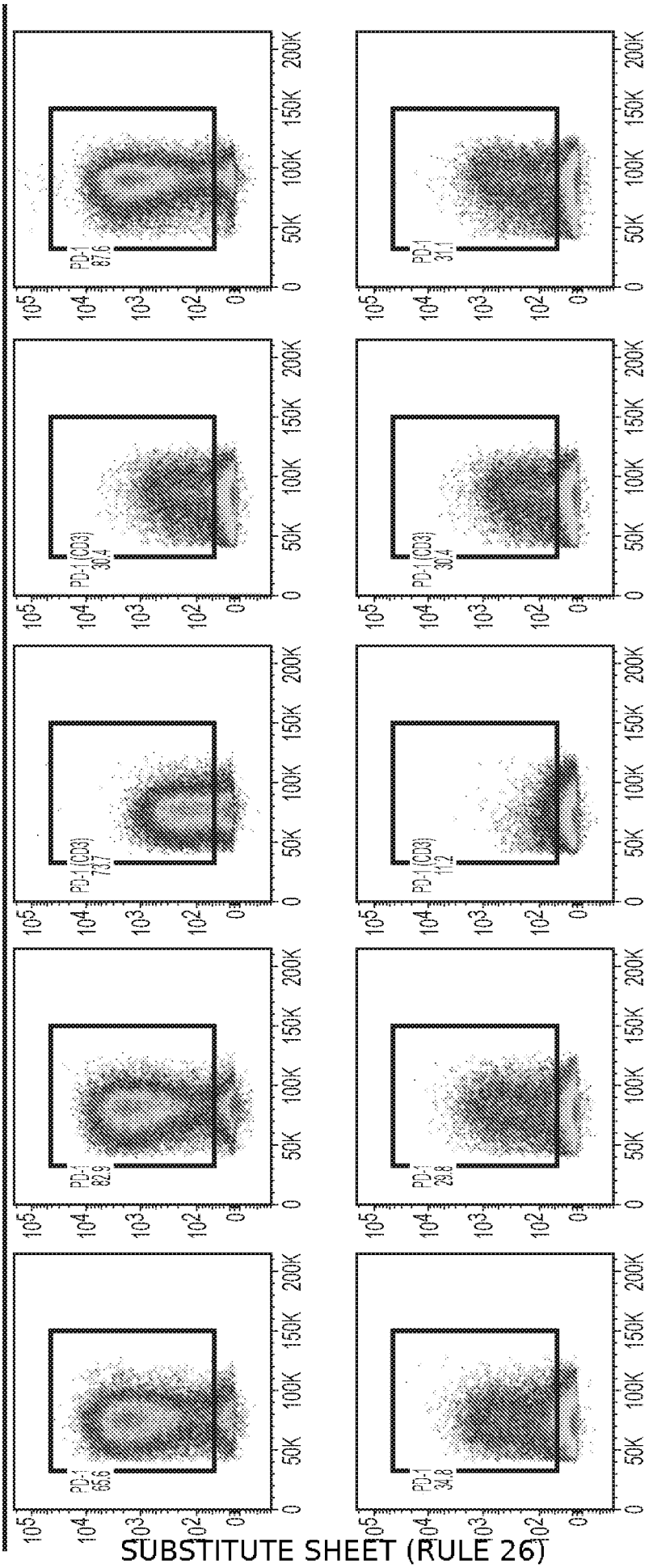

PD-1
65.6
PD-1
82.9
PD-1 (CD3)
73.7
PD-1 (CD3)
30.4
PD-1
87.6
PD-1
34.8
PD-1
29.8
PD-1 (CD3)
11.2
PD-1 (CD3)
30.4
PD-1
31.1
EH12.1
EH12.2H7
M1H4
NAT105
PD1.3.1

FIG. 60

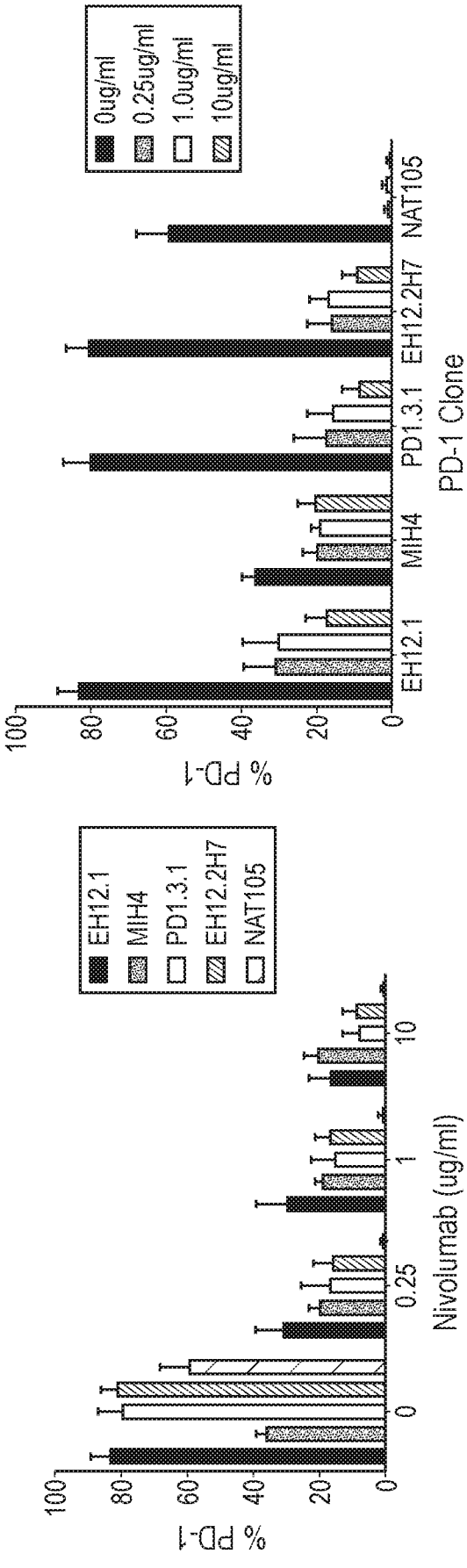
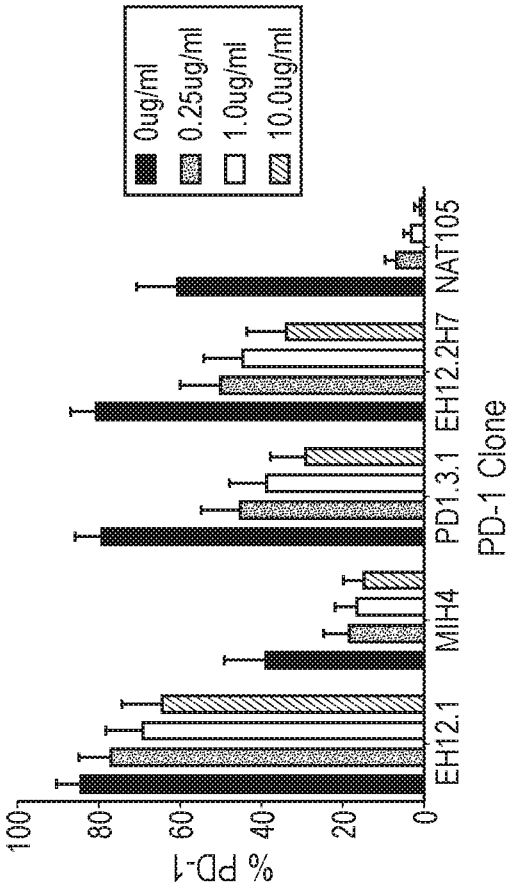

0ug/ml
0.25ug/ml
1.0ug/ml
10ug/ml
% PD-1
100
80
60
40
20
0
EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
PD-1 Clone
EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
% PD-1
100
80
60
40
20
0
0
0.25
1
10
Nivolumab (ug/ml)

FIG. 61


0ug/ml
0.25ug/ml
1.0ug/ml
10.0ug/ml
% PD-1
100
80
60
40
20
0
EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
PD-1 Clone

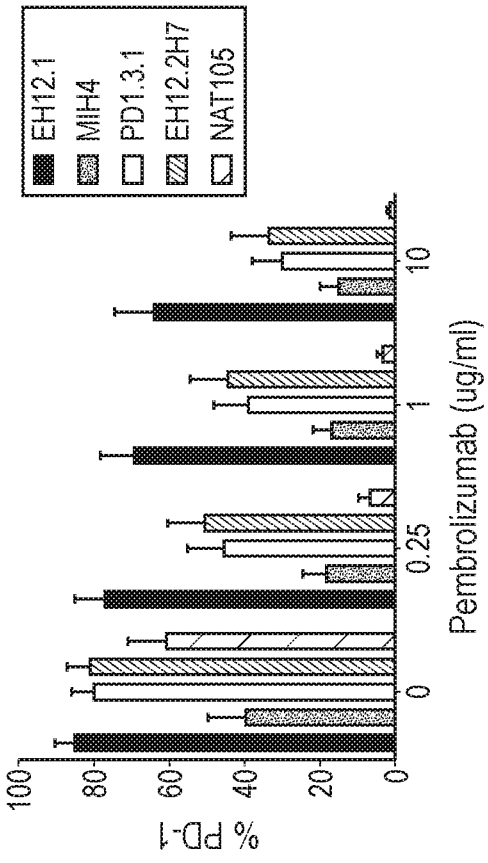

EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
% PD-1
100
80
60
40
20
0
0
0.25
1
10
Pembrolizumab (ug/ml)

FIG. 62

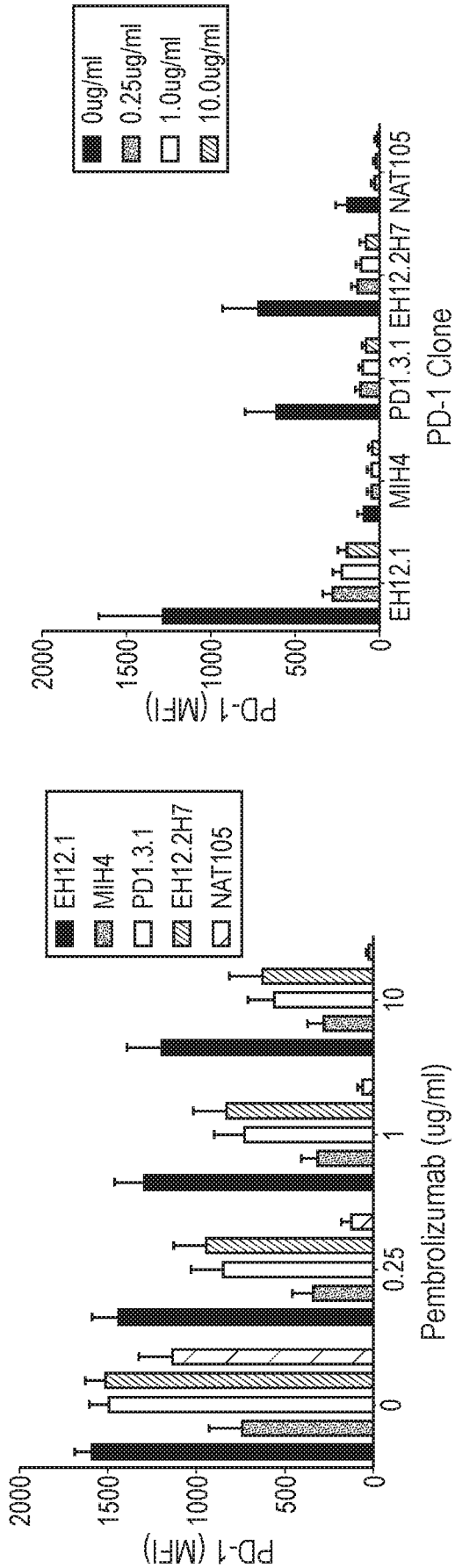

0ug/ml
0.25ug/ml
1.0ug/ml
10.0ug/ml
PD-1 (MFI)
2000
1500
1000
500
0
EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
PD-1 Clone
EH12.1
MIH4
PD1.3.1
EH12.2H7
NAT105
PD-1 (MFI)
2000
1500
1000
500
0
0
0.25
1
10
Pembrolizumab (ug/ml)

FIG. 63

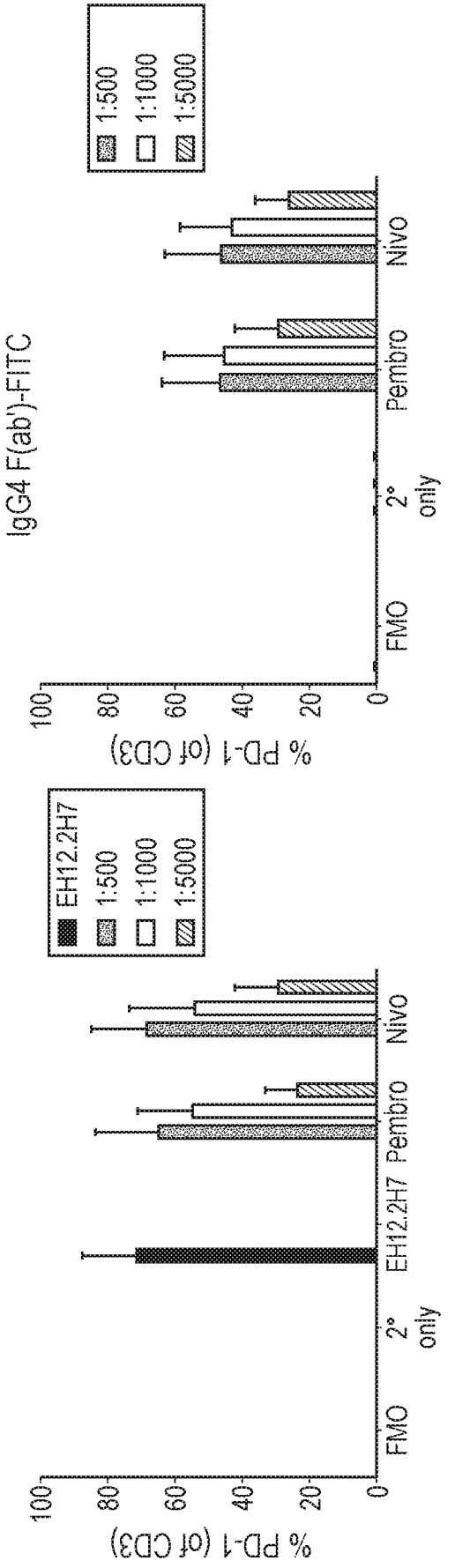
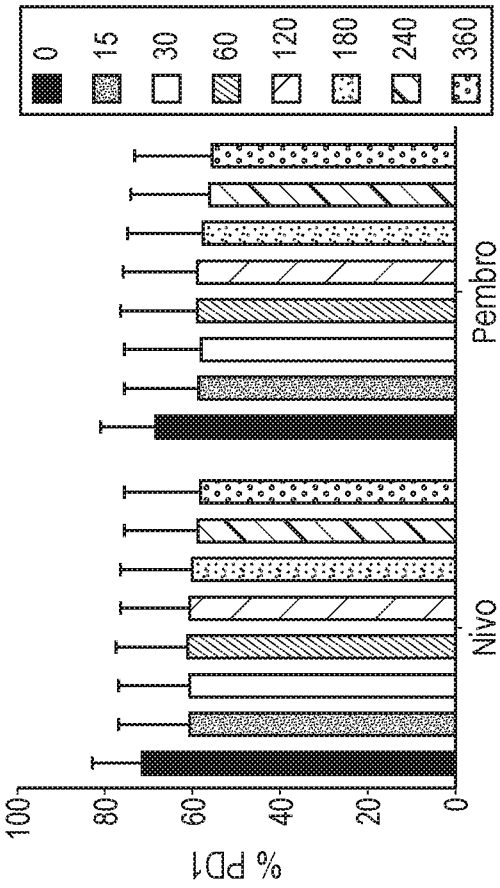

IgG4 F(ab')-FITC
1:500
1:1000
1:5000
% PD-1 (of CD3)
100
80
60
40
20
0
FMO
2° only
Pembro
Nivo
EH12.2H7
1:500
1:1000
1:5000
% PD-1 (of CD3)
100
80
60
40
20
0
FMO
2° only
EH12.2H7
Pembro
Nivo

FIG. 64


0
15
30
60
120
180
240
360
Pembro
Nivo
100
80
60
40
20
0
% PD1

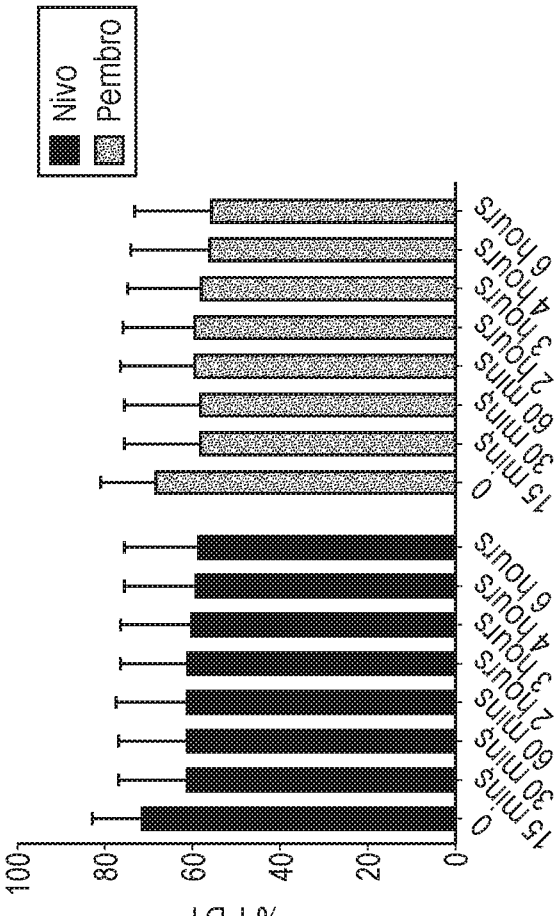

Nivo
Pembro
0
15 mins
30 mins
60 mins
2 hours
3 hours
4 hours
6 hours
100
80
60
40
20
0
% PD1

FIG. 65

	M1121	*M1123*	*M1125*	*M1126*	*M1129*	*M1130*	*EP11032*	*EP11040*	*EP11043*
%PD1+	10.1%	0.66%	47.6%	0.78%	13.9%	59.5%	16.3%	33.5%	87%
# Sorted	30,231	2,000	143,000	3,000	13,393	529,000	23,415	5,693	628,000
Post-sort Purity	NT	86%	62%	35%	73%	96%	NT	96%	94.6%
REP1 seed (#/G-Rex24/10)	30,231	2,000	19,000	3,000	13,393	100,000	23,415	5,693	92,000
REP1 yield PD1+	1.9e7	8.0e5	8.21e5	1.5e7	1.5e7	2.3e7	2.63e7	1.3e6	1.33e6
Fold expansion	**628**	**400**	**43**	**5000**	**1045**	**230**	**1,123**	**228**	**15**
REP2 seed (#/G-Rex10 /100M)	100,000	80,000	100,000	100,000	100,000	100,000	100,000	100,000	100,000
REP2 yield PD1+	1.44e8 (~27e9)	3.0e6 (~3e7)	3.03e7 (~2/5e8)	9.48e7 (~14e9)	1.28e8 (~192e9)	2.38e8 (~203e9)	1.68e8 (~44e9)	1.26e8 (~14e9)	1.7e8 (~1.5e9)
Fold expansion	**1,440**	**37.5**	**303**	**948**	**1280**	**1670**	**1,680**	**1,260**	**1,170**

Melanoma patients treated with anti-PD1
Full-scale prep

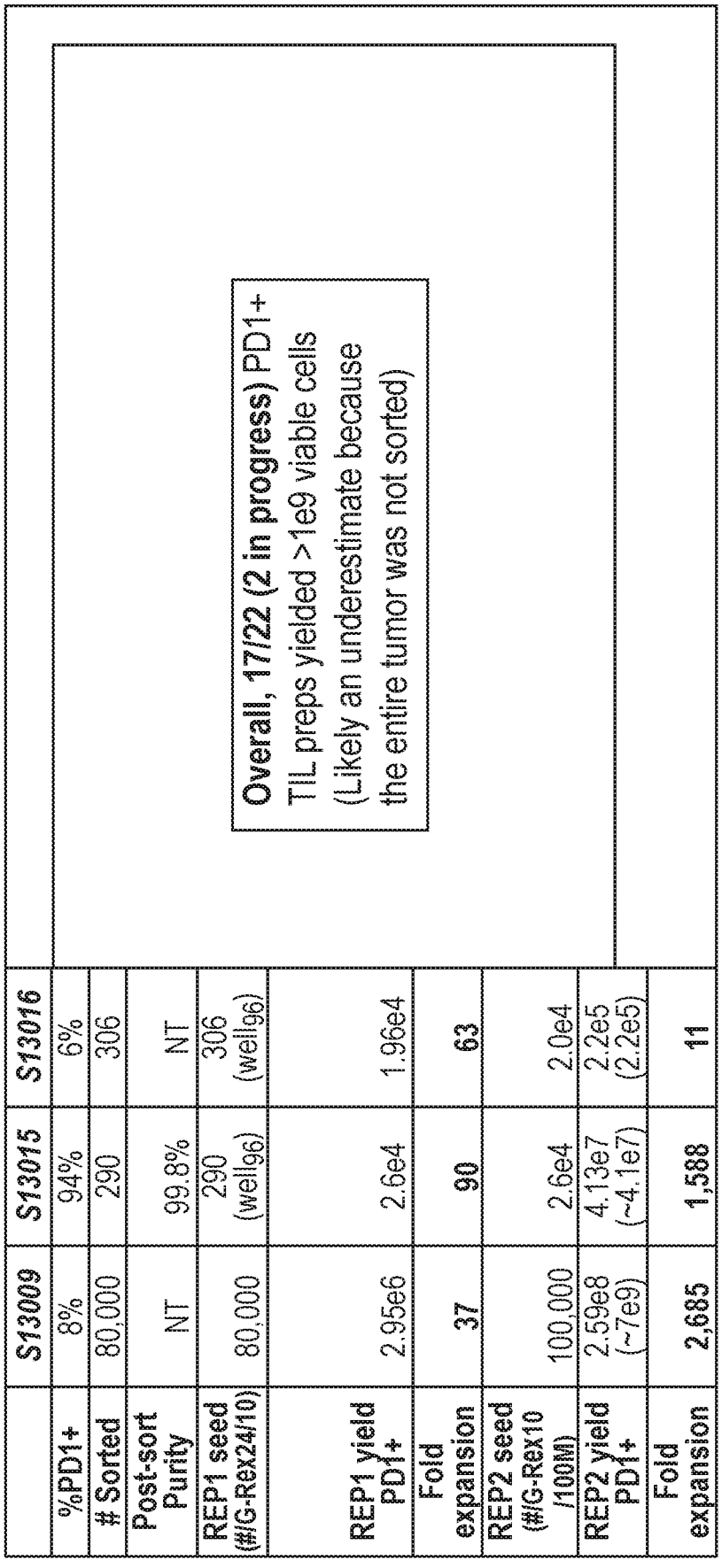
© 2018, Iovance Biotherapeutics

FIG. 66

	T6042	EP11055	EP11061	L4067	L4068	L4073	*L4074*	L4075	L4081	L4082
%PD1+	33%	13.3%	5.2%	58%	4.6%	27%	6.7%	13.5%	78%	81.8%
# Sorted	23,320	33,000	5,500	115,000	32,500	88,426	8,384	11,000	390,000	135,012
Post-sort Purity	90%	72%	65%	95%	59.2%	78%	81%	78%	61%	90%
REP1 seed (#/G-Rex24/10)	23,000	1K/2K/10K 20K	5,500	100,000	32,500	10K/25K/ 50K	8,384	1K/2K/ 5K	50,000	30,000
REP1 yield PD1+	5.54e6	2.6e4/ 5.3e5/ 6.8e5/ 3.28e6	6.71e5	3.0e7	2.32e7	8.5e6/ 3.77e7/ 6.09e7	2.72e6	2.4e4/ 3.4e4/ 2.64e5	1.24e8	6.36e7
Fold expansion	**240**	**26/61/67 164**	**122**	**300**	**73**	**850/1508/ 1218**	**324**	**25/17 /53**	**2480**	**2120**
REP2 seed (#/G-Rex10 /100M)	5.54e6	3.3e6	100,000	100,000	100,000	3.77e7	2.72e6	8.5e5	100,000	100,000
REP2 yield PD1+	1.82e9 (1.82e9)	1.83e9 (~1.83e9)	1.6e8 (~1.0e9)	3.63e8 (~108e9)	1.17e8 (~27e9)	3.64e8 (~2.2e9)	1.97e9 (~2e9)	1.83e8 (~2e8)	1.1e8 (~138e9)	1.99e8 (~396e9)
Fold expansion	**328**	**554**	**1570**	**3,600**	**1170**	**20**	**724**	**2,152**	**1110**	**1990**

FIG. 67

SUBSTITUTE SHEET (RULE 26)



	S13009	S13015	S13016
%PD1+	8%	94%	6%
# Sorted	80,000	290	306
Post-sort Purity	NT	99.8%	NT
REP1 seed (#IG-Rex24/10)	80,000	290 (well96)	306 (well96)
REP1 yield PD1+	2.95e6	2.6e4	1.96e4
Fold expansion	**37**	**90**	**63**
REP2 seed (#IG-Rex10 /100M)	100,000	2.6e4	2.0e4
REP2 yield PD1+	2.59e8 (~7e9)	4.13e7 (~4.1e7)	2.2e5 (2.2e5)
Fold expansion	**2,685**	**1,588**	**11**

FIG. 68

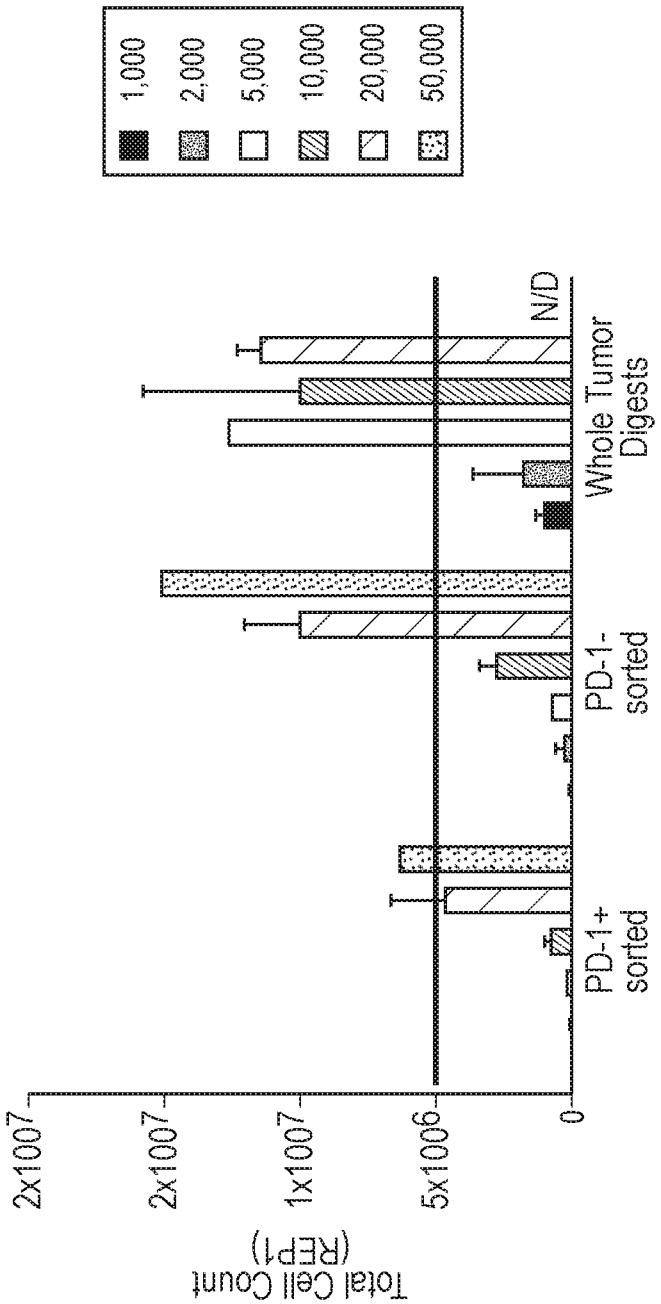

1,000
2,000
5,000
10,000
20,000
50,000
Total Cell Count (REP1)
2x1007
2x1007
1x1007
5x1006
0
PD-1+ sorted
PD-1- sorted
Whole Tumor Digests
N/D

FIG. 69

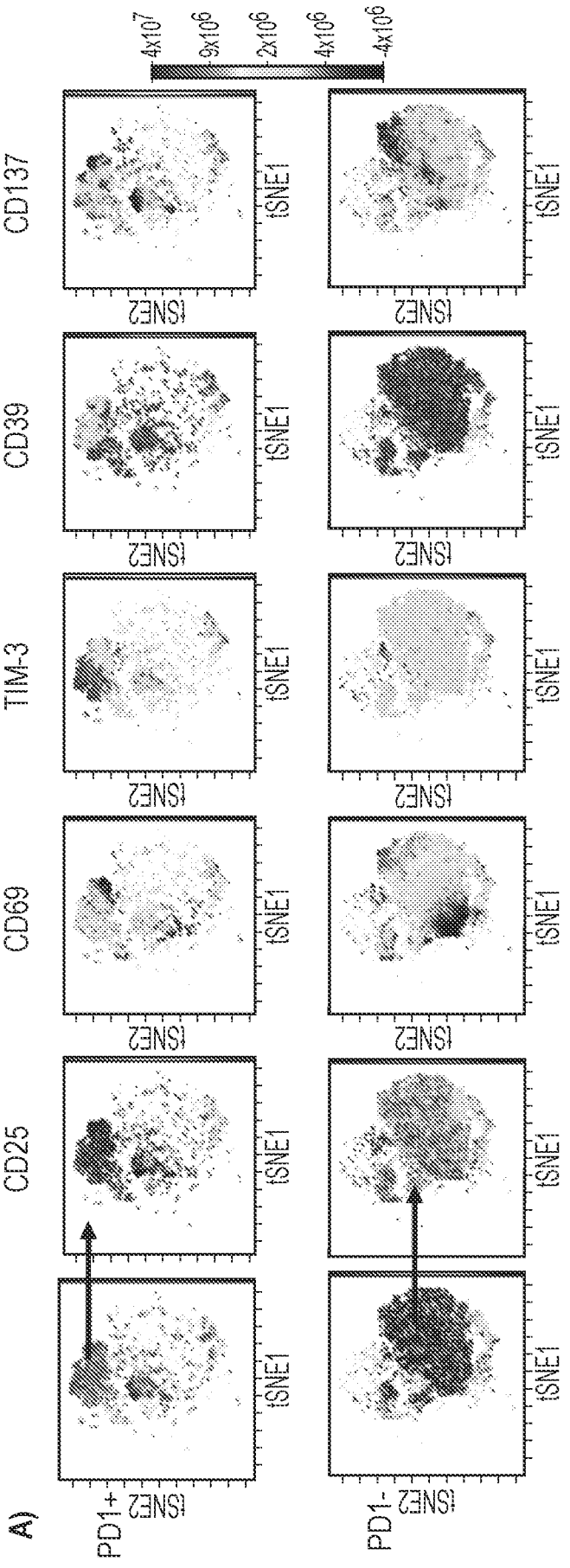

A)
PD1+
PD1-
CD25
CD69
TIM-3
CD39
CD137
tSNE1
tSNE2
4x10^7
9x10^6
2x10^6
4x10^6
4x10^6

FIG. 70

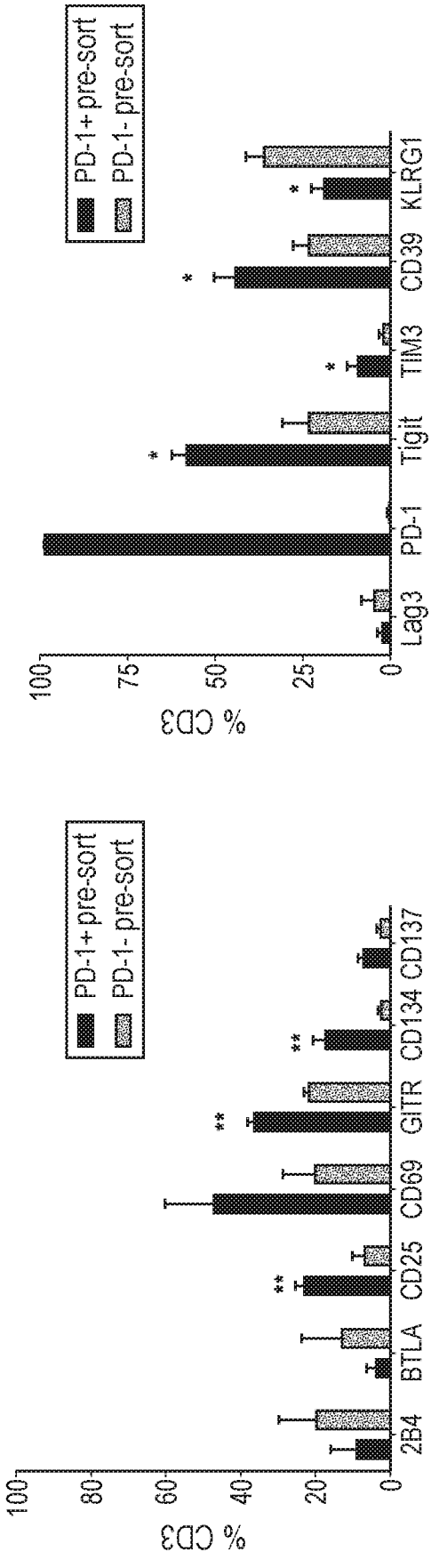

PD-1+ pre-sort
PD-1- pre-sort
% CD3
100
75
50
25
0
Lag3
PD-1
Tigit
TIM3
CD39
KLRG1
*
PD-1+ pre-sort
PD-1- pre-sort
% CD3
100
80
60
40
20
0
2B4
BTLA
CD25
CD69
GITR
CD134
CD137
**

FIG. 71

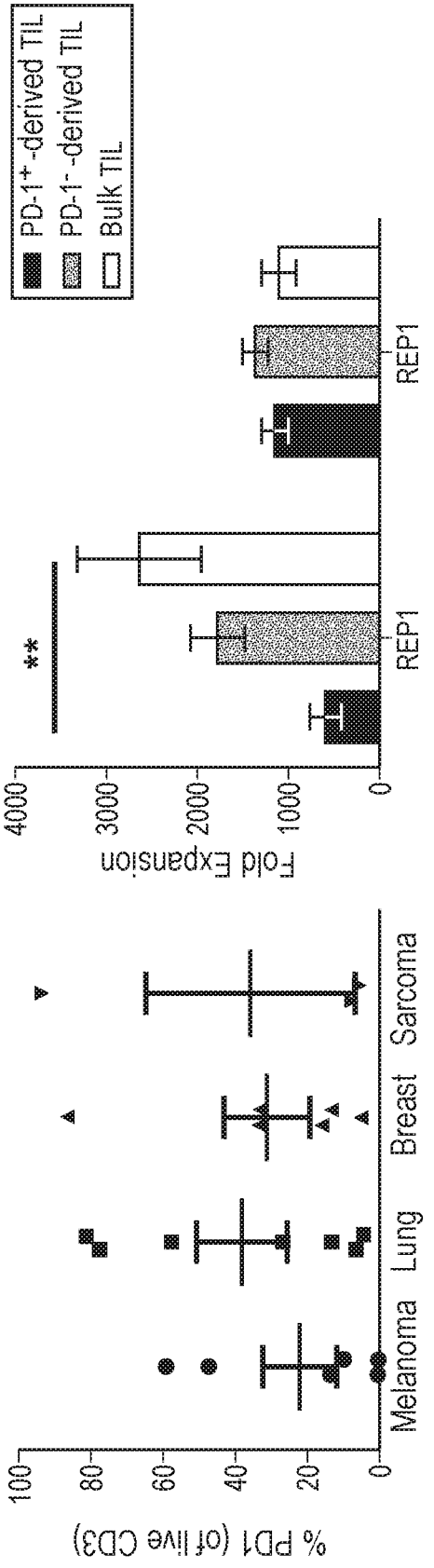

PD-1+-derived TIL
PD-1- -derived TIL
Bulk TIL
Fold Expansion
4000
3000
2000
1000
0
**
REP1
REP1
% PD1 (of live CD3)
100
80
60
40
20
0
Melanoma
Lung
Breast
Sarcoma

FIG. 72

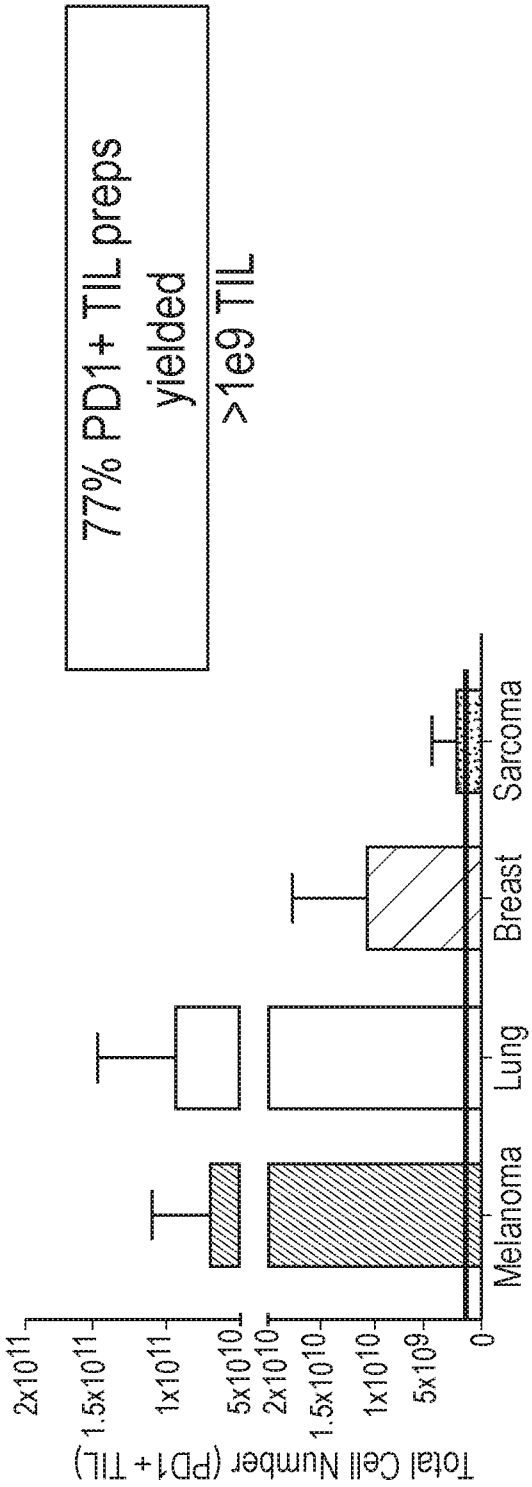

77% PD1+ TIL preps
yielded
>1e9 TIL
Total Cell Number (PD1+ TIL)
2x10^11
1.5x10^11
1x10^11
5x10^10
2x10^10
1.5x10^10
1x10^10
5x10^9
0
Melanoma
Lung
Breast
Sarcoma

FIG. 73

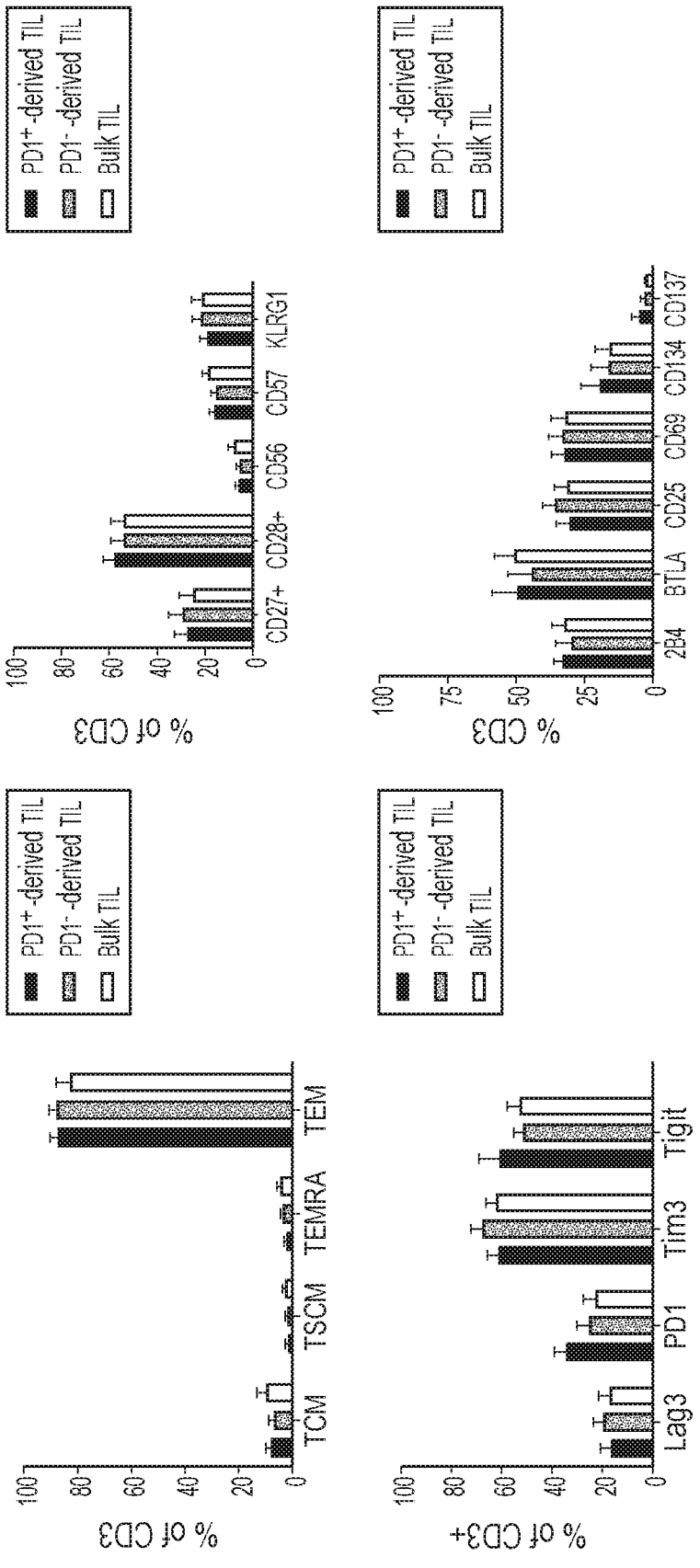

PD1+-derived TIL
PD1--derived TIL
Bulk TIL
% of CD3
CD27+
CD28+
CD56
CD57
KLRG1
% CD3
2B4
BTLA
CD25
CD69
CD134
CD137
TCM
TSCM
TEMRA
TEM
% of CD3+
Lag3
PD1
Tim3
Tigit

FIG. 74

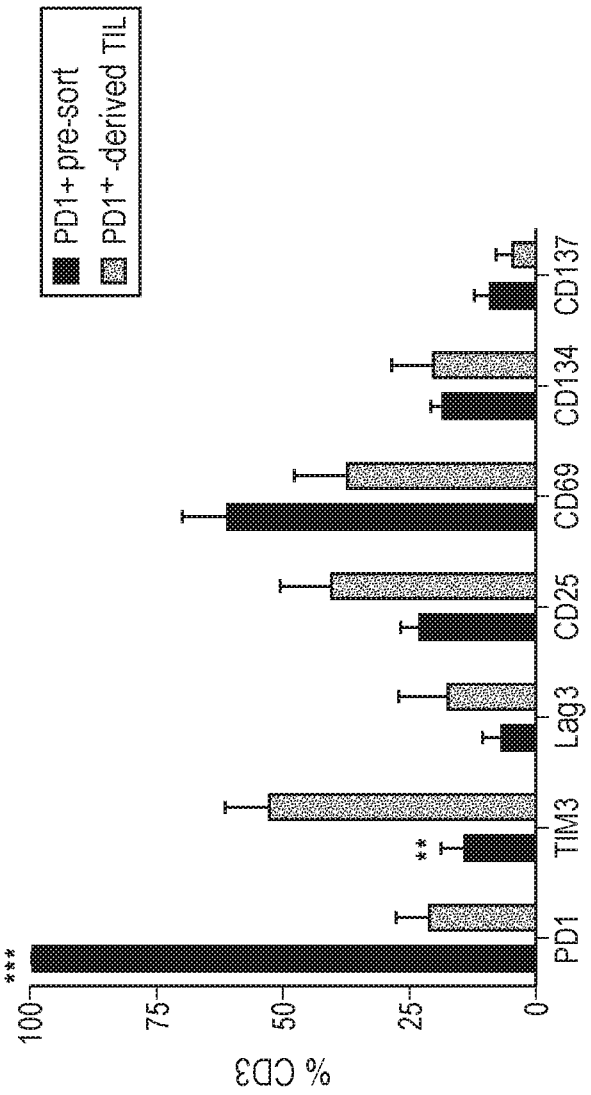

PD1+ pre-sort
PD1+ -derived TIL
% CD3
100
75
50
25
0
***
**
PD1
TIM3
Lag3
CD25
CD69
CD134
CD137

FIG. 75

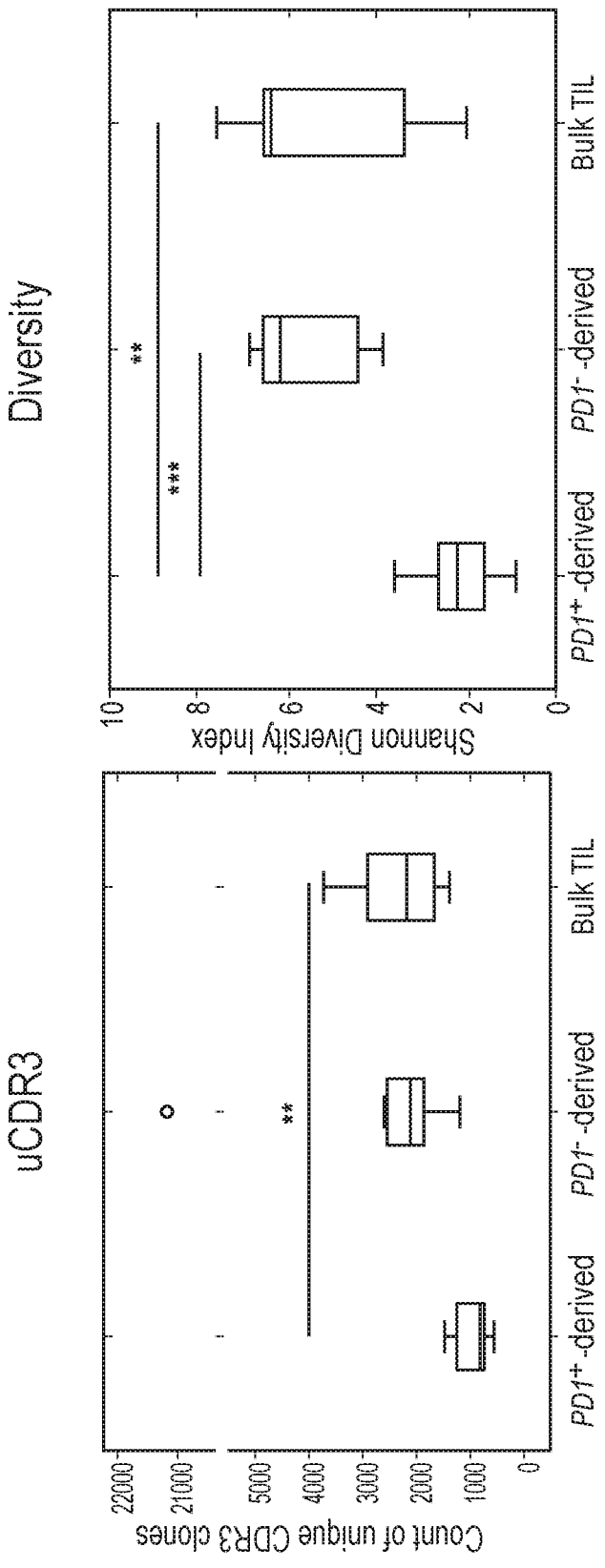

Diversity
Shannon Diversity Index
10
8
6
4
2
0
**
***
PD1+-derived
PD1--derived
Bulk TIL
uCDR3
Count of unique CDR3 clones
22000
21000
5000
4000
3000
2000
1000
0
**
PD1+-derived
PD1--derived
Bulk TIL

FIG. 76

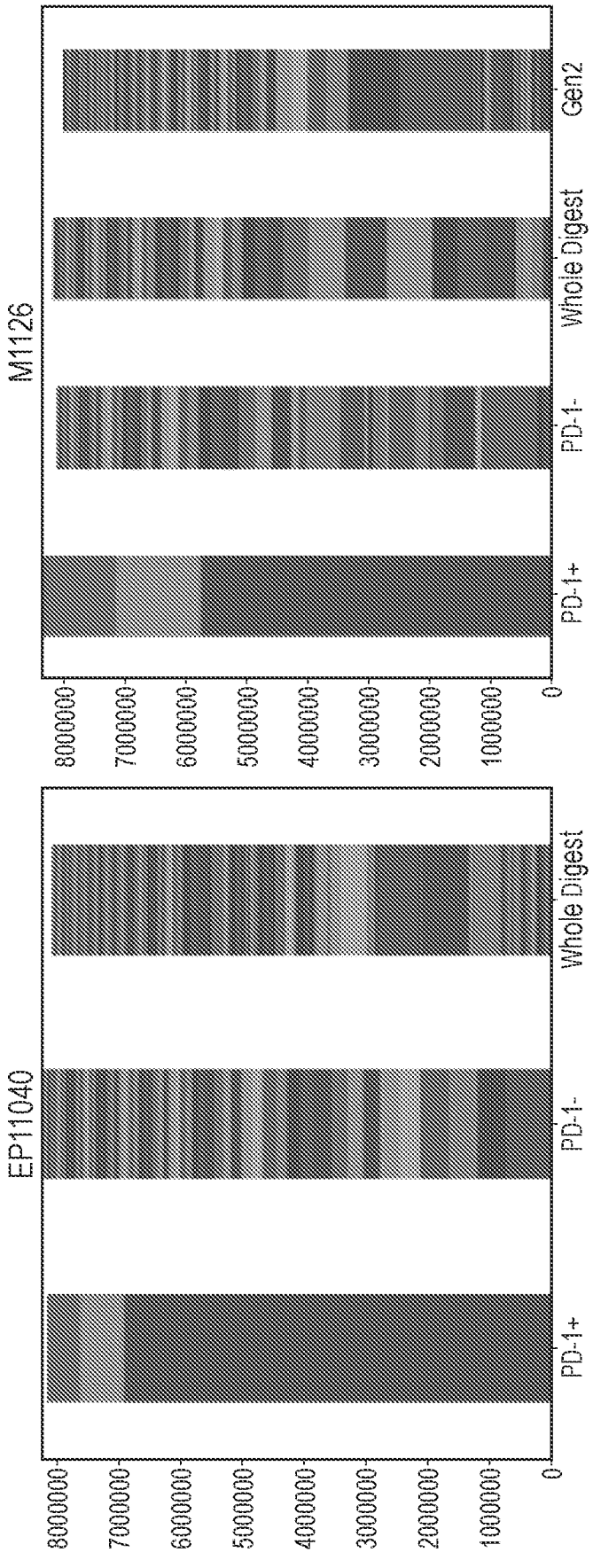

EP11040
M1126
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
PD-1+
PD-1-
Whole Digest
Gen2

FIG. 77

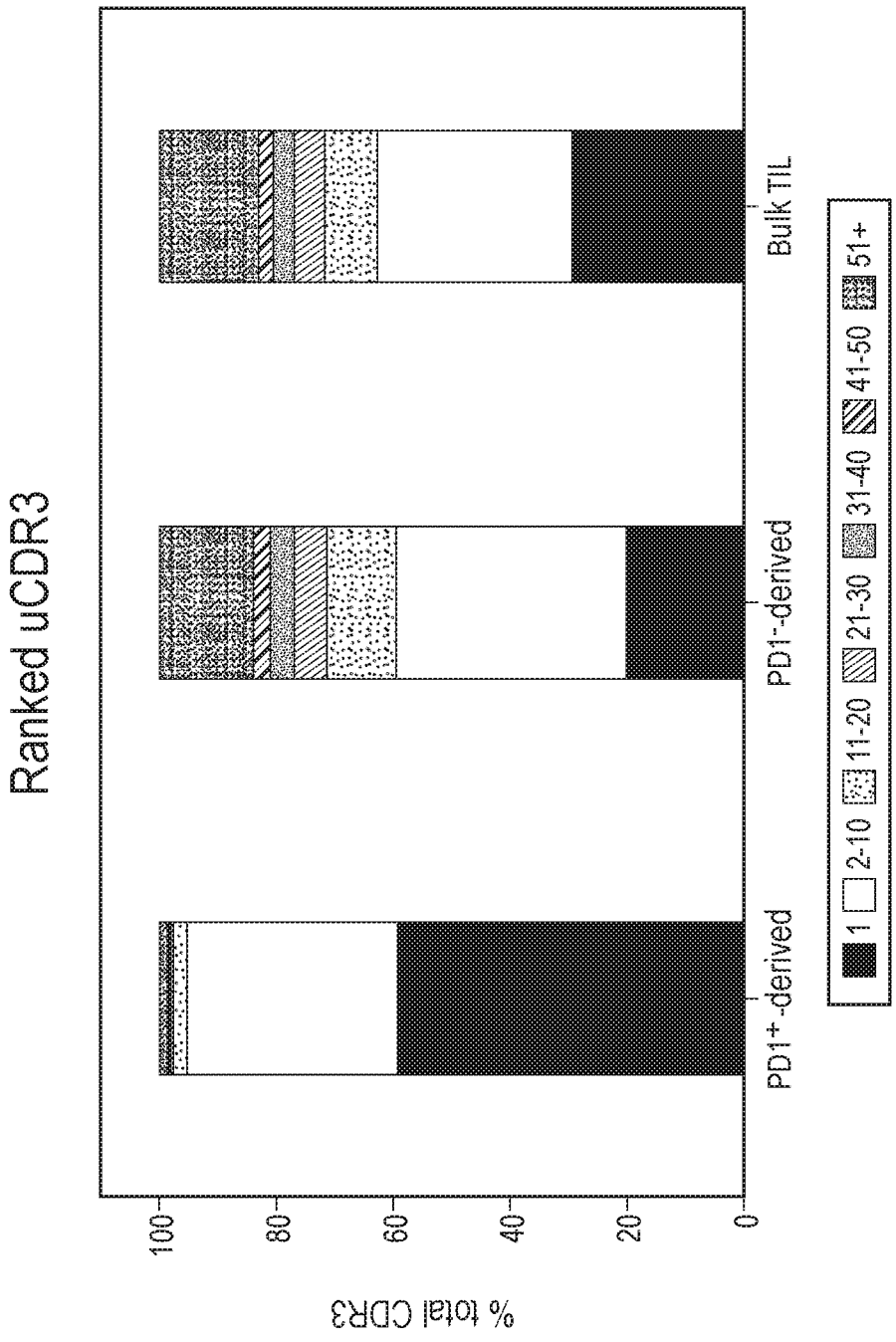

Ranked uCDR3
% total CDR3
100
80
60
40
20
0
PD1+-derived
PD1--derived
Bulk TIL
1
2-10
11-20
21-30
31-40
41-50
51+

FIG. 78

Shared clones between bulk TIL and PD1+ -derived

Tumor	Count of shared uCDR3 clones	Bulk TIL Shared %	PD1+-derived Shared %
Breast	43	0.61	78.95
Breast	76	79.51	88.64
Lung	40	5.26	35.16
Lung	60	4.66	34.57
Melanoma	59	21.14	53.84
Melanoma	39	19.87	71.90

FIG. 79

SUBSTITUTE SHEET (RULE 26)

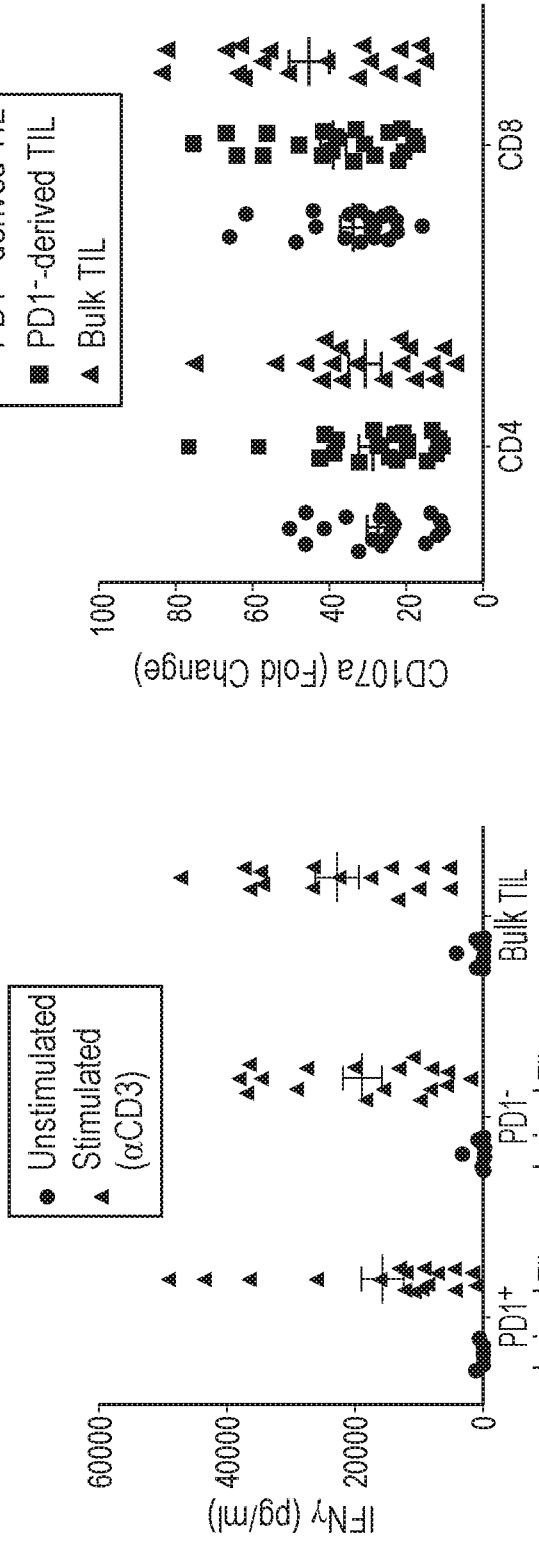
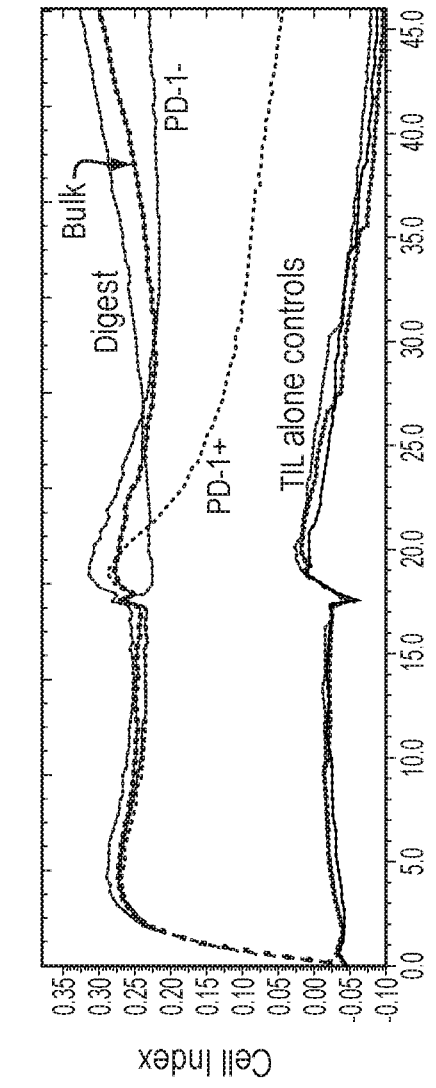

IFNγ (pg/ml)
60000
40000
20000
0
Unstimulated
Stimulated (αCD3)
PD1+ -derived TIL
PD1- -derived TIL
Bulk TIL
CD107a (Fold Change)
100
80
60
40
20
0
PD1+-derived TIL
PD1−-derived TIL
Bulk TIL
CD4
CD8

FIG. 80


Cell Index
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
Time (in Hour)
xCELLigence System
ACEA Biosciences
Bulk
Digest
PD-1-
PD-1+
TIL alone controls

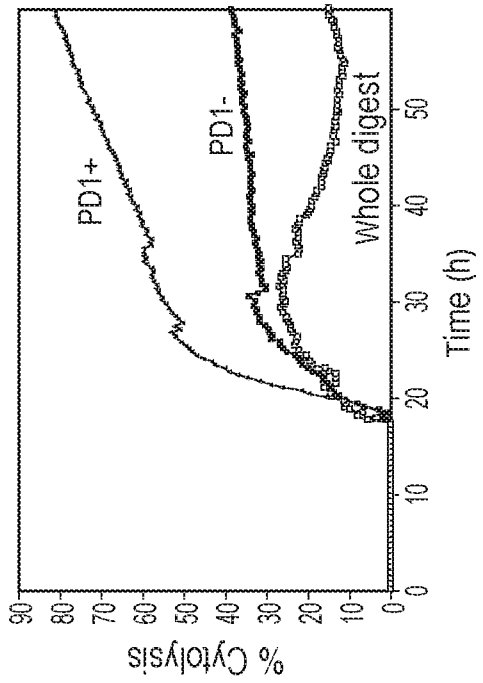

% Cytolysis
0
10
20
30
40
50
60
70
80
90
0
10
20
30
40
50
Time (h)
PD1+
PD1-
Whole digest

FIG. 81

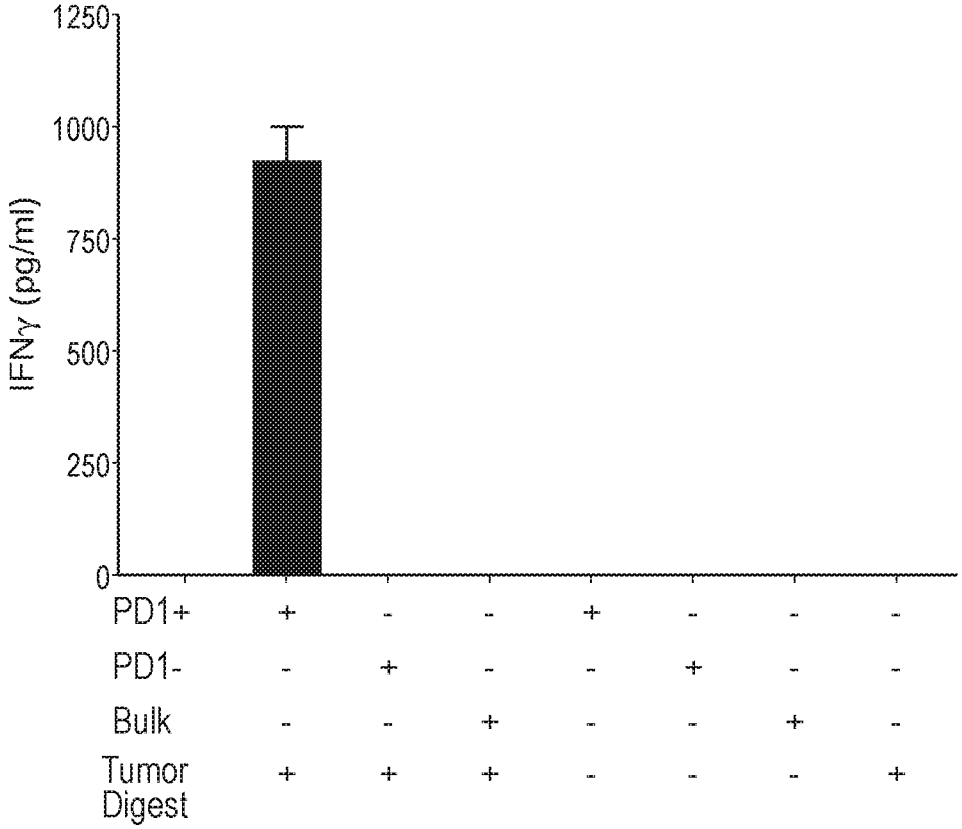


FIG. 82

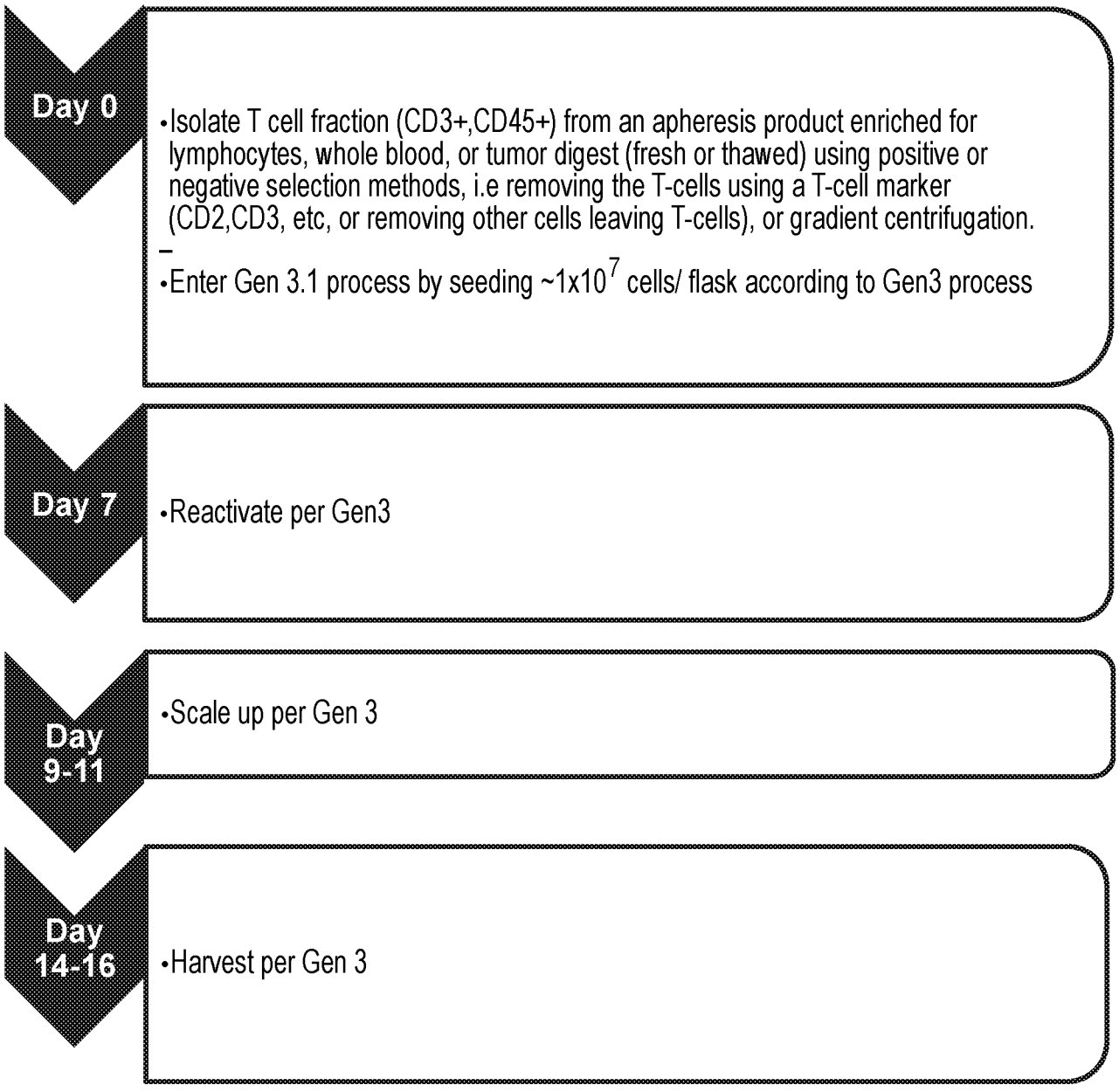

Day 0
•Isolate T cell fraction (CD3+,CD45+) from an apheresis product enriched for lymphocytes, whole blood, or tumor digest (fresh or thawed) using positive or negative selection methods, i.e removing the T-cells using a T-cell marker (CD2,CD3, etc, or removing other cells leaving T-cells), or gradient 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

FIG. 83

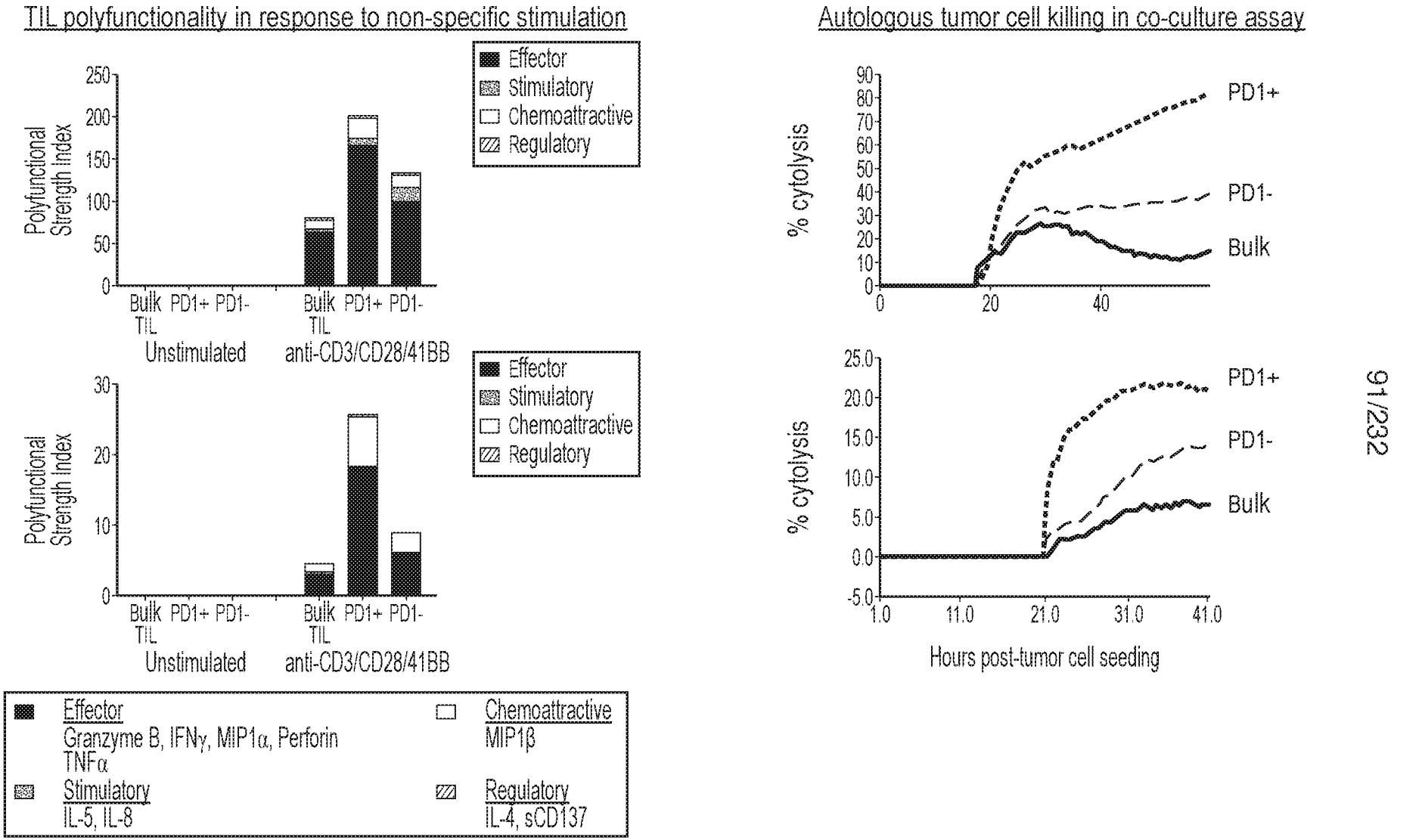

TIL polyfunctionality in response to non-specific stimulation
Melanoma
Lung
Polyfunctional Strength Index
Effector
Stimulatory
Chemoattractive
Regulatory
Bulk TIL
PD1+
PD1-
Unstimulated
anti-CD3/CD28/41BB
Effector
Granzyme B, IFNγ, MIP1α, Perforin TNFα
Stimulatory
IL-5, IL-8
Chemoattractive
MIP1β
Regulatory
IL-4, sCD137
Autologous tumor cell killing in co-culture assay
% cytolysis
PD1+
PD1-
Bulk
Hours post-tumor cell seeding

FIG. 84

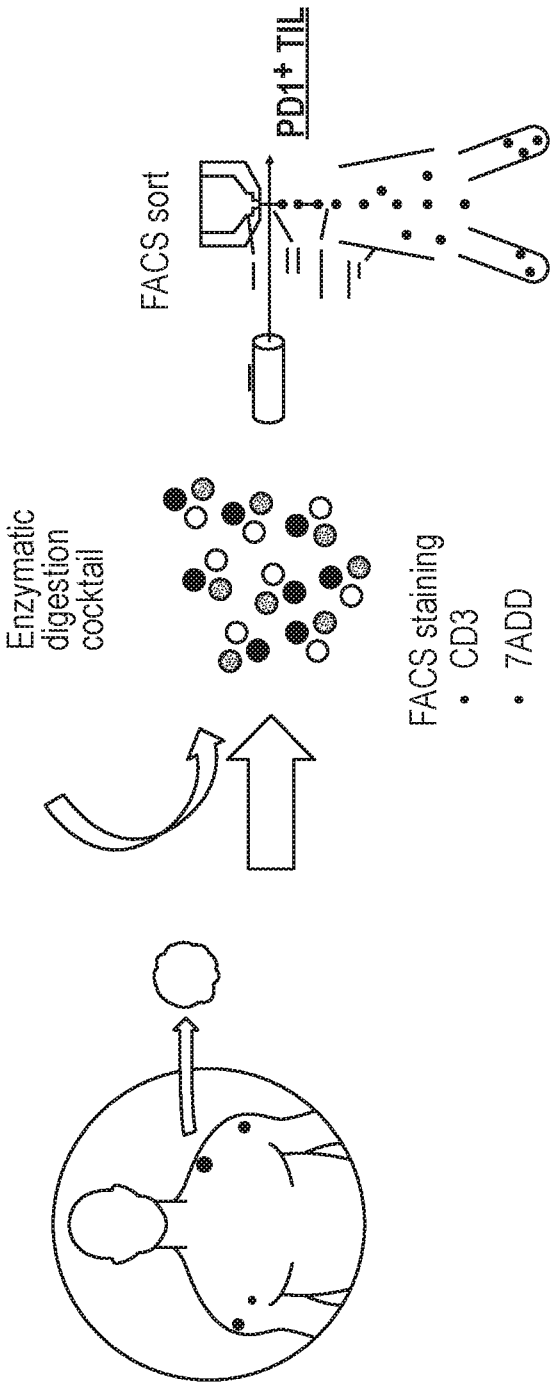

PD1+ TIL
FACS sort
Enzymatic digestion cocktail
FACS staining
• CD3
• 7ADD
• PD1

FIG. 85

	αPD1 clone	% PD1+ in digest	# sorted PD1+ cells	Fold expansion [REP1; REP2]	Total yield (extrapolated)	# of uCDR3	Entropy	Portion Shared (%) [bulk; PD1+]
M1125	M1H4	***47.60%***	143,000	43; 303	(~2.5e8)	1281	3.6	29;36
M1126	M1H4	***0.78%***	3,000	5000; 948	(~14e9)	786	2.3	19.9; 71.9
M1129	M1H4	***13.90%***	13,393	1045; 1280	(~192e9)	18,234	7.4	38.1; 50.9
EP11040	M1H4	***33.50%***	5,693	228; 1260	(~14e9)	823	2.1	0.6; 78.9
EP11043	M1H4	***87%***	628,000	15; 1170	(~1.5e9)	1518	1.4	79.5; 88.6
L4067	M1H4	***58%***	115,000	300; 3600	(~108e9)	566	2.4	5.3; 35.2
L4068	M1H4	***4.60%***	32,500	73; 1170	(~27e9)	806	3.4	4.7; 34.6
L4073	M1H4	***27%***	88,426	1218; 20	(~2.2e9)	45,947	12.5	28.7; 39.5
L4074	M1H4	***6.70%***	8,384	324; 742	(~2e9)	1694	4.5	5.6; 72.2
L4075	M1H4	***13.50%***	11,000	53; 2152	(~2e8)	1416	4.1	17.9; 53.3
M1130	EH12.2H7	***59.50%***	529,000	230; 1670	(~203e9)	2815	6	5.7; 52.9
L4081	EH12.2H7	***78%***	390,000	2480; 1110	(~138e9)	5373	8.7	41.4; 65.5
L4082	EH12.2H7	***81.80%***	135,012	2120; 1990	(~396e9)	4467	6.4	57.8; 56.7
L4089	EH12.2H7	***75%***	826,000	46; 2491	(~950e9)	10093	9.9	58.9; 38.7
H3027	EH12.2H7	***92%***	71,929	31; 1470	(~3.17e9)	808	0.8	0.06; 0.03
L4093	***EH12.2H7***	***79%***	***448,000***	***1336, 483***	***~97e9***	***2886***	***5.7***	***18; 46***
M1135	***EH12.2H7***	***64%***	***48,000***	***5670, 497***	***~99e9***	***2371***	***5.5***	***4.8; 11.6***

FIG. 86

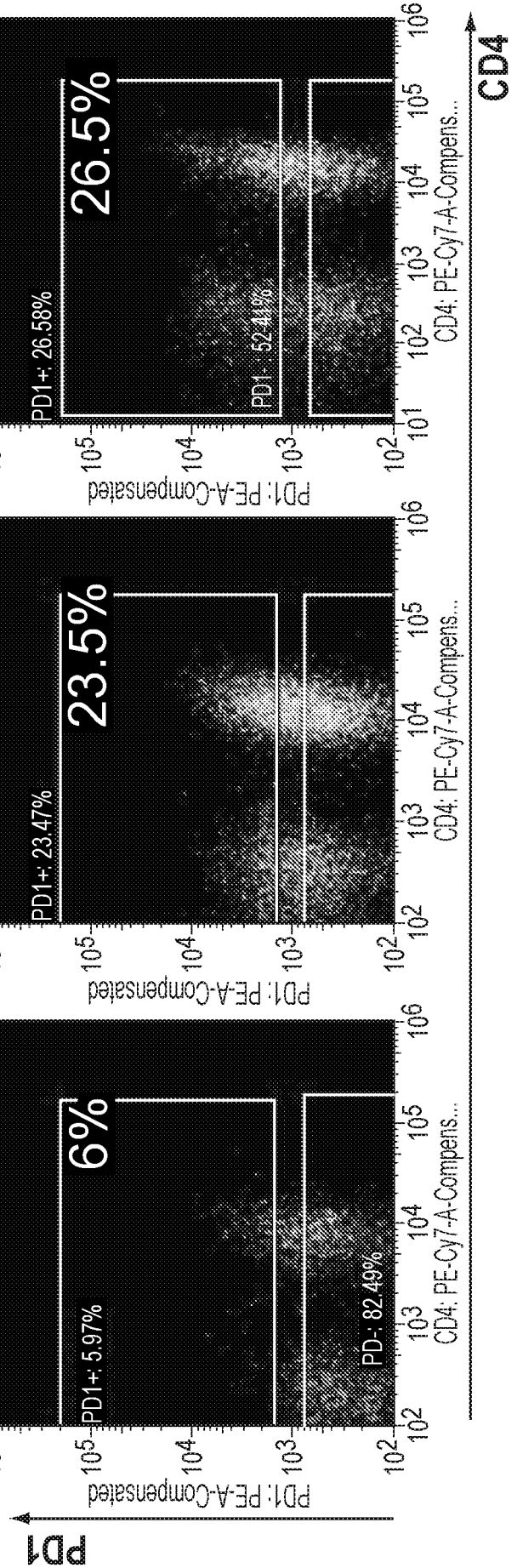

CD3
L4074
6%
PD1+: 5.97%
PD-: 82.49%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compens...
CD3
L4073
23.5%
PD1+: 23.47%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compens...
CD3
T6042
26.5%
PD1+: 26.58%
PD1-: 52.44%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compens...
PD1
CD4

FIG. 87

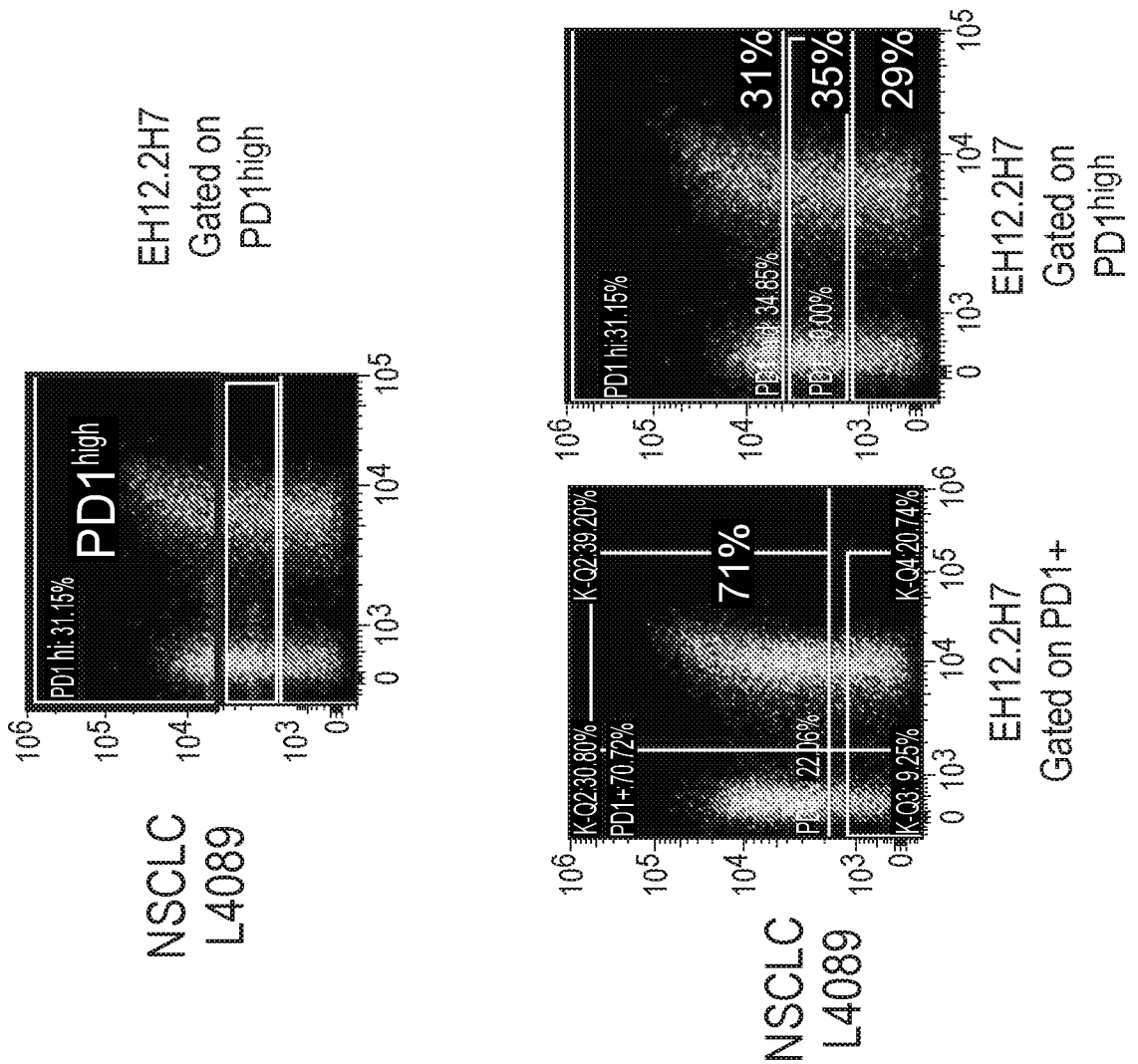

NSCLC
L4089
EH12.2H7
Gated on
PD1high
PD1 hi: 31.15%
PD1high
EH12.2H7
Gated on PD1+
K-Q2:30.80%
PD1+:70.72%
K-Q2:39.20%
71%
K-Q3: 9.25%
K-Q4:20.74%
PD1 hi:31.15%
31%
35%
29%

FIG. 88

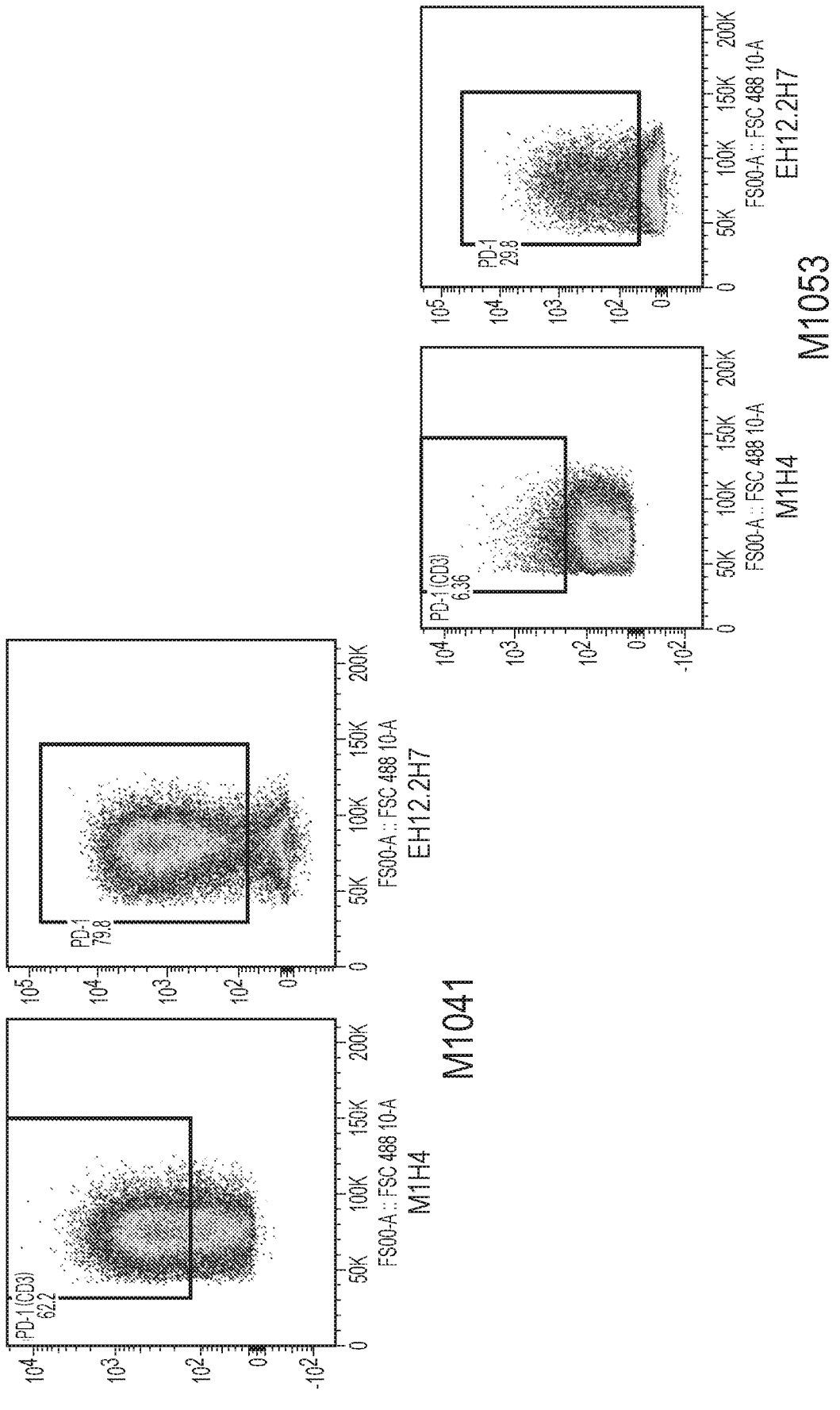

PD-1(CD3)
62.2
M1H4
FS00-A :: FSC 488 10-A
PD-1
79.8
EH12.2H7
M1041
PD-1(CD3)
6.36
M1H4
PD-1
29.8
EH12.2H7
M1053

FIG. 89

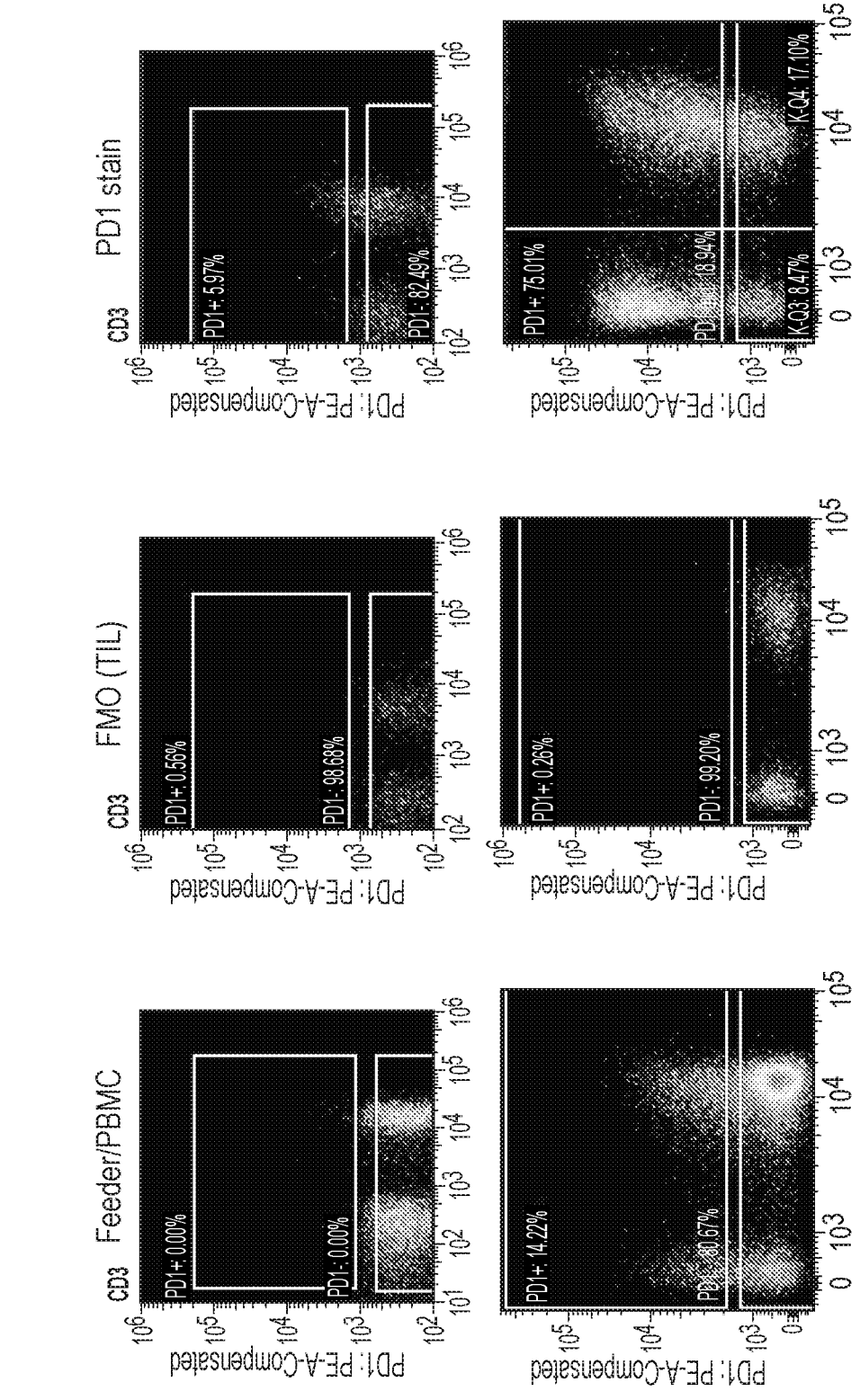

M1H4
L4074
EH12.2H7
H3031
CD3
Feeder/PBMC
FMO (TIL)
PD1 stain
PD1+ 0.00%
PD1- 0.00%
PD1+ 0.56%
PD1- 98.68%
PD1+ 5.97%
PD1- 82.49%
PD1+ 14.22%
PD1- 80.67%
PD1+ 0.26%
PD1- 99.20%
PD1+ 75.01%
PD1- 16.94%
KQ3: 8.47%
KQ4: 17.10%
PD1: PE-A-Compensated

FIG. 90

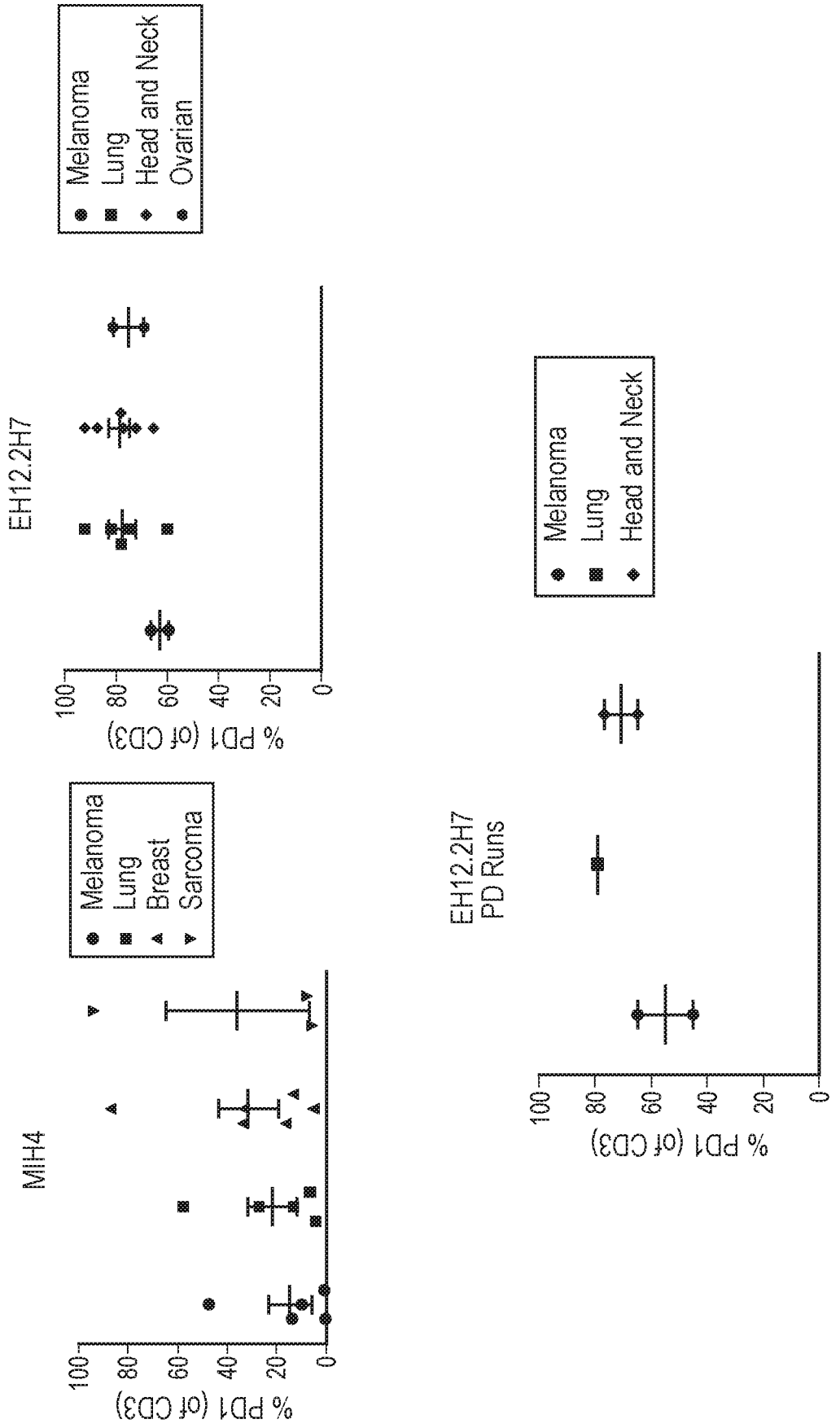

MIH4
% PD1 (of CD3)
100
80
60
40
20
0
Melanoma
Lung
Breast
Sarcoma
EH12.2H7
% PD1 (of CD3)
100
80
60
40
20
0
Melanoma
Lung
Head and Neck
Ovarian
EH12.2H7
PD Runs
% PD1 (of CD3)
100
80
60
40
20
0
Melanoma
Lung
Head and Neck

FIG. 91

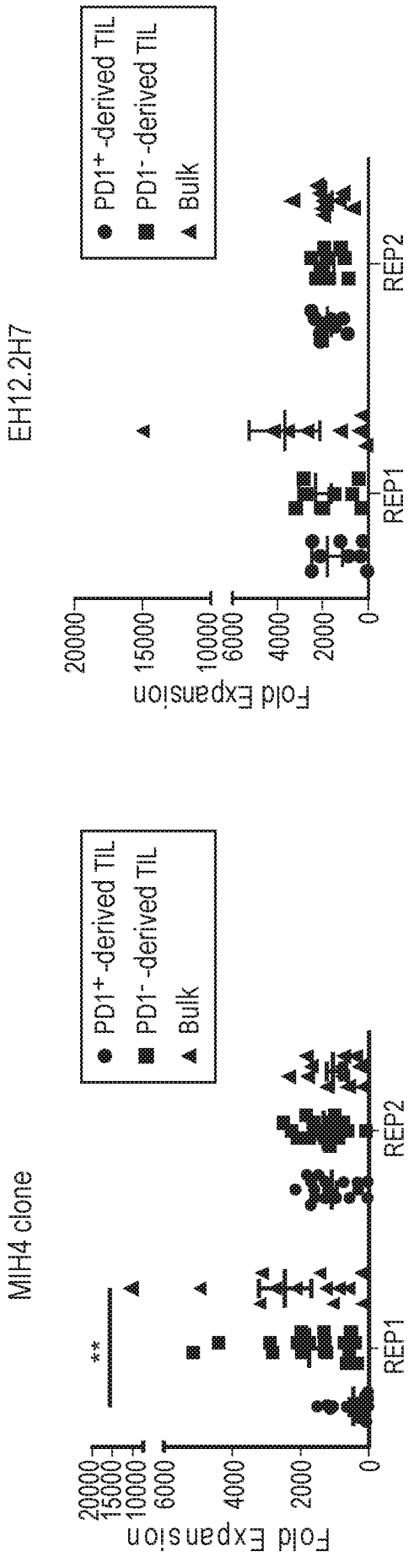

MIH4 clone
EH12.2H7
Fold Expansion
20000
15000
10000
6000
4000
2000
0
**
REP1
REP2
PD1+-derived TIL
PD1--derived TIL
Bulk

FIG. 92

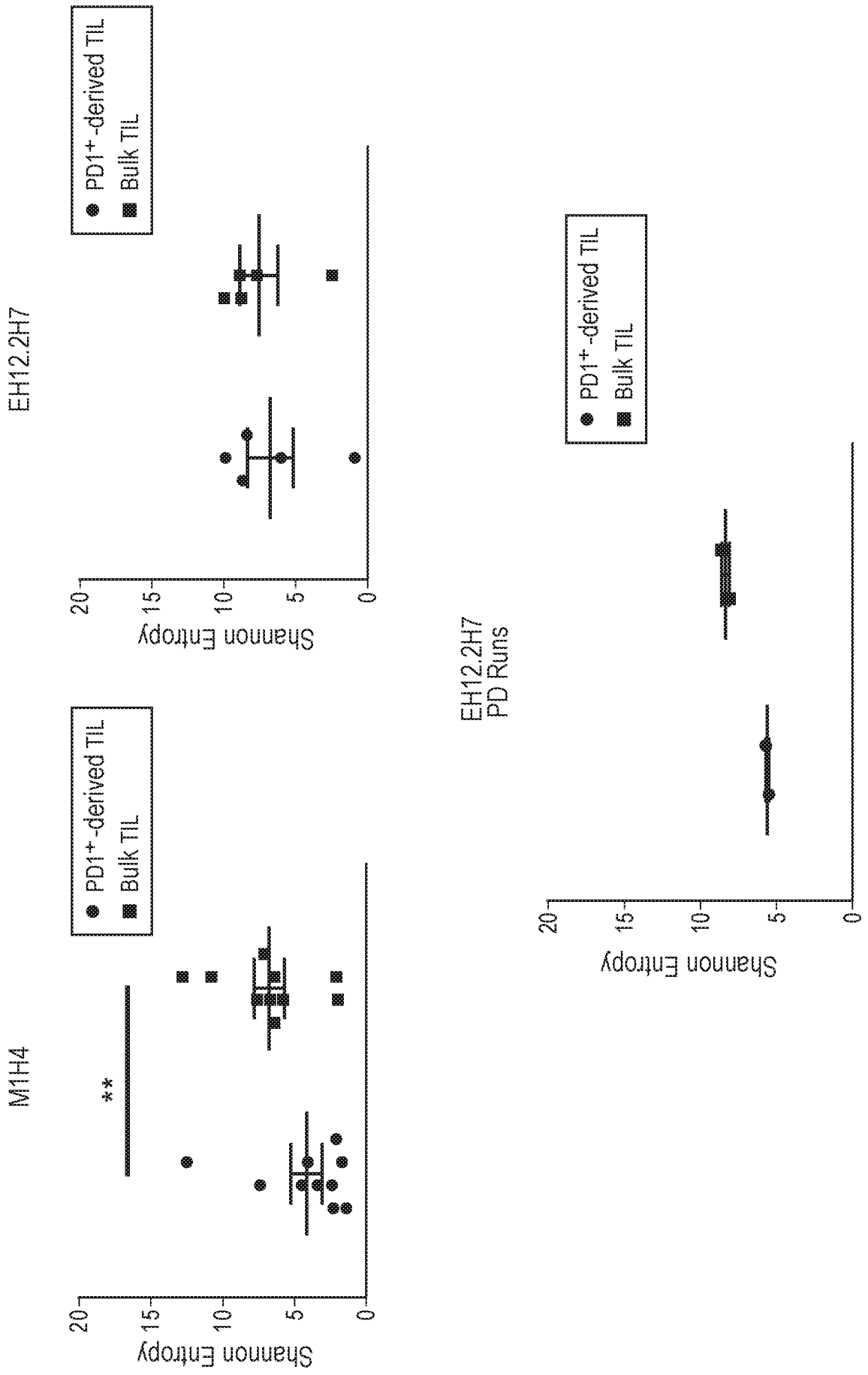

M1H4
**
Shannon Entropy
20
15
10
5
0
PD1+-derived TIL
Bulk TIL
EH12.2H7
Shannon Entropy
20
15
10
5
0
PD1+-derived TIL
Bulk TIL
EH12.2H7
PD Runs
Shannon Entropy
20
15
10
5
0
PD1+-derived TIL
Bulk TIL

FIG. 93

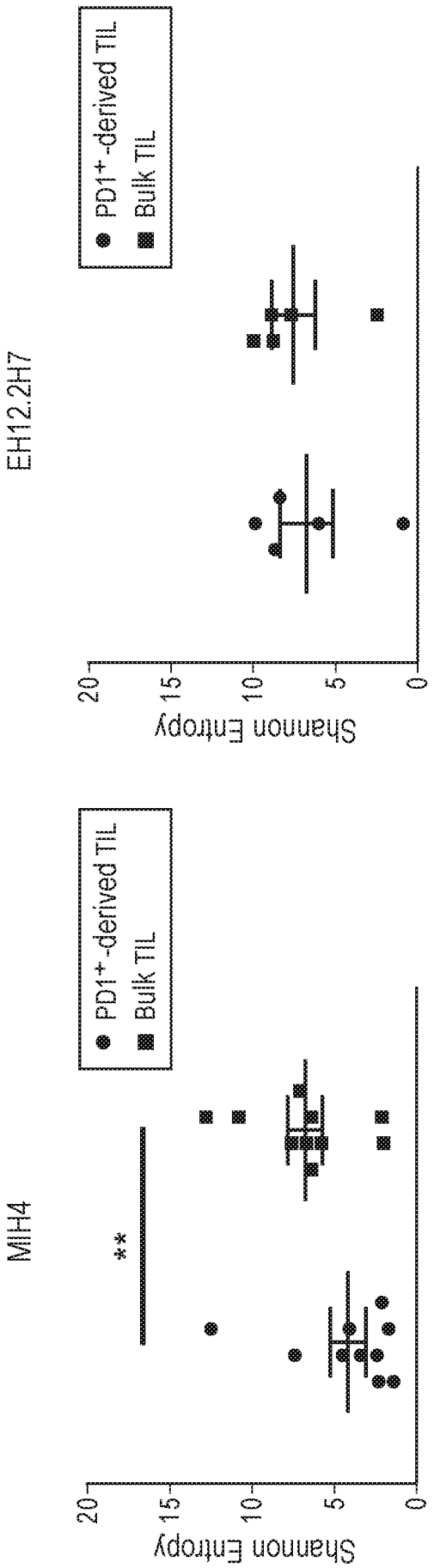

EH12.2H7
PD1+-derived TIL
Bulk TIL
Shannon Entropy
20
15
10
5
0
MIH4
**
PD1+-derived TIL
Bulk TIL
Shannon Entropy
20
15
10
5
0

FIG. 94

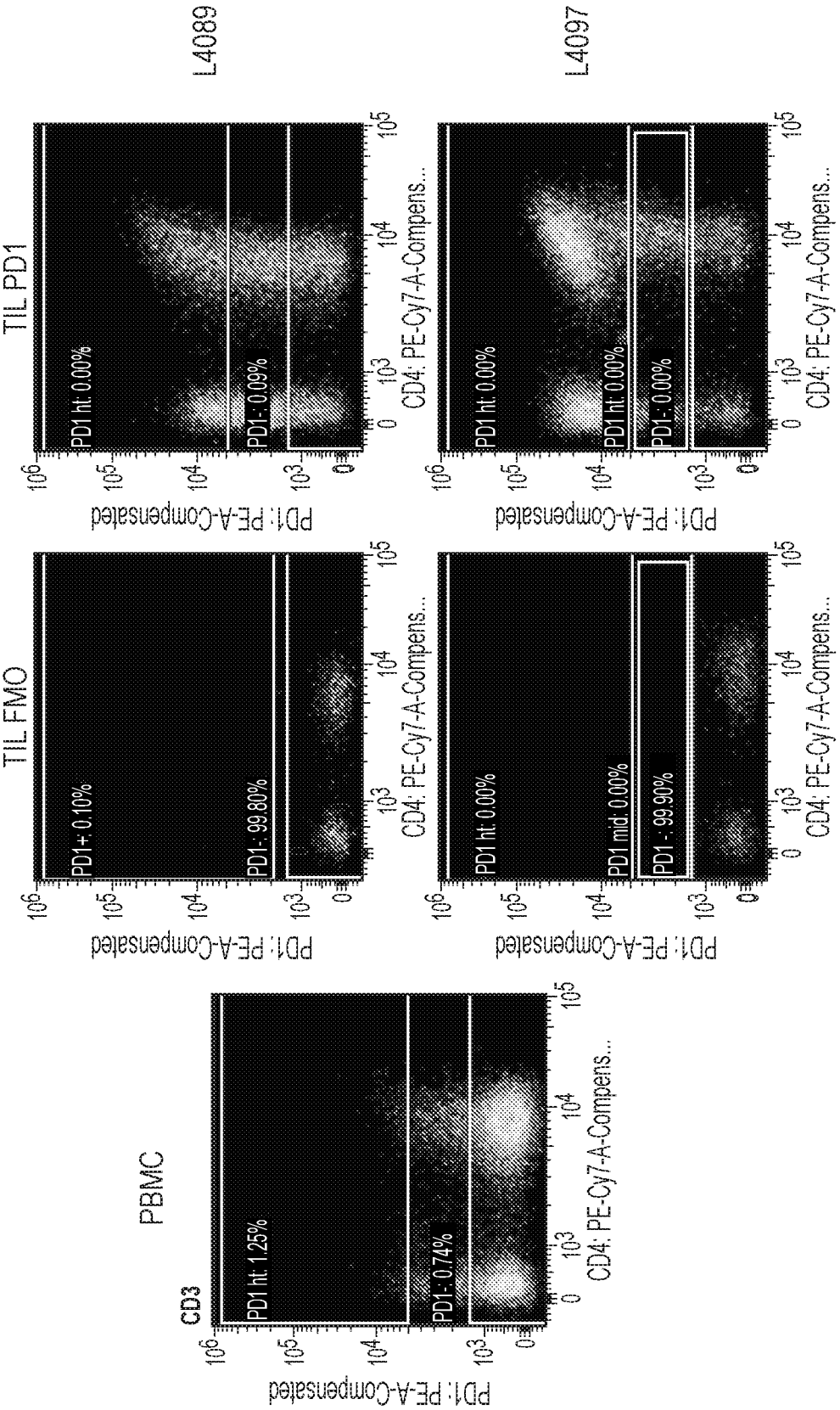

L4089
L4097
TIL PD1
TIL FMO
PBMC
CD3
PD1 ht: 0.00%
PD1-: 0.09%
PD1 ht: 0.00%
PD1-: 0.00%
PD1+: 0.10%
PD1-: 99.80%
PD1 ht: 0.00%
PD1 mid: 0.00%
PD1-: 99.90%
PD1 ht: 1.25%
PD1-: 0.74%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compens...

FIG. 95

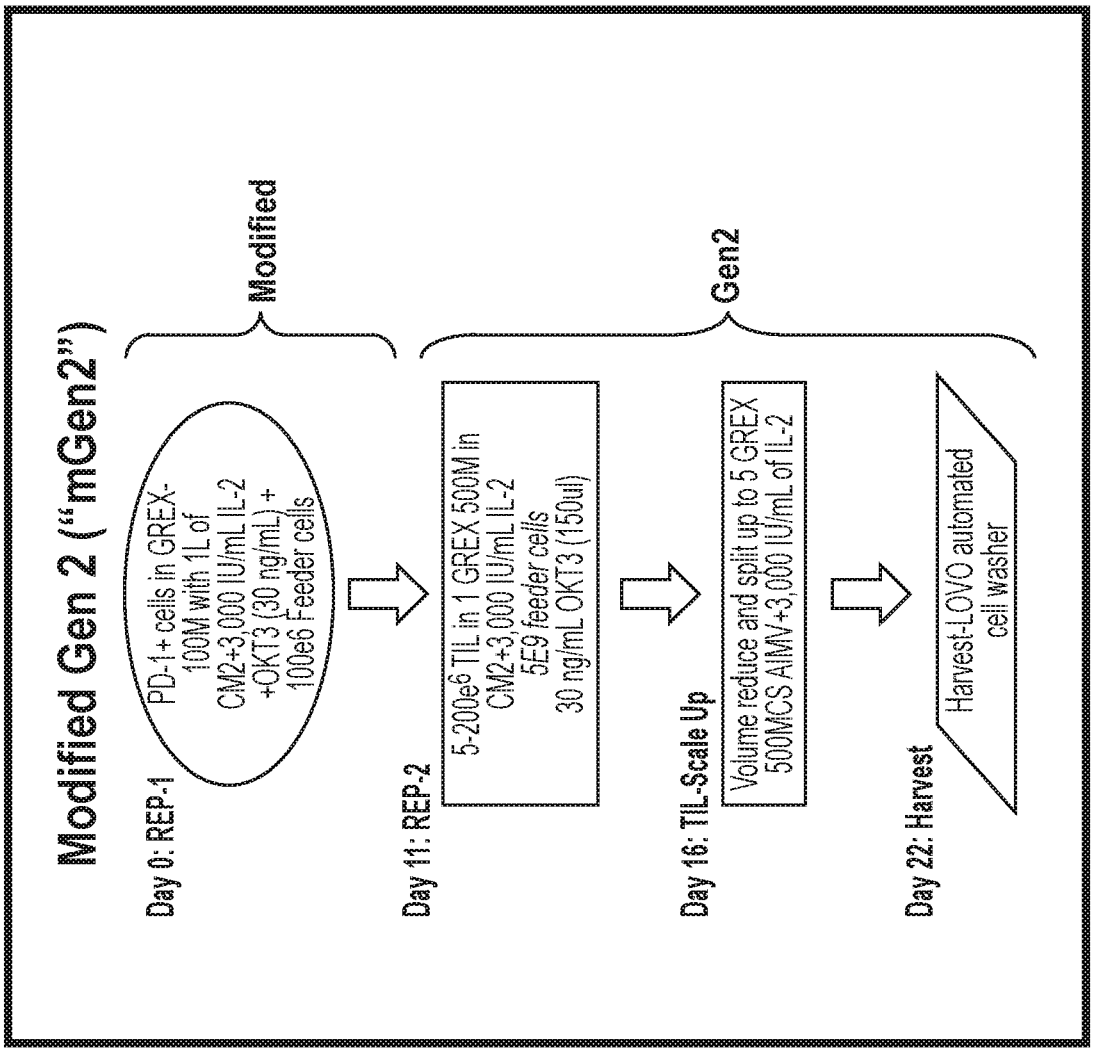
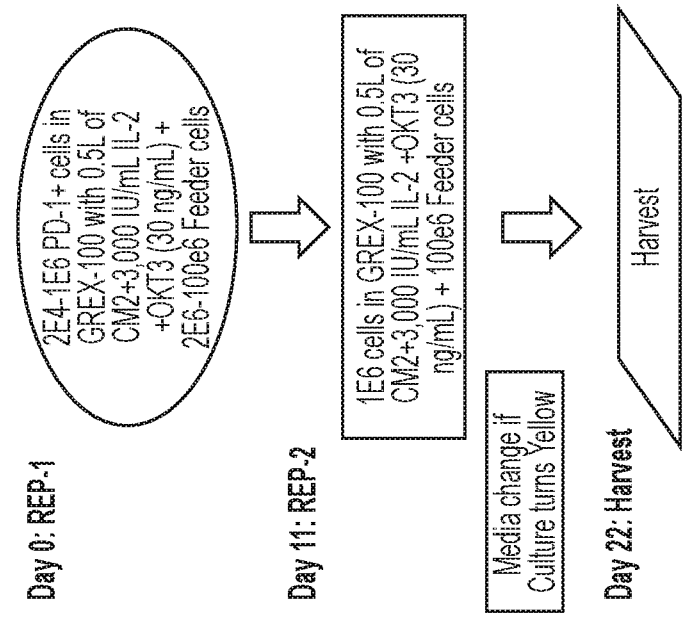

Modified Gen 2 ("mGen2")
Modified
Gen2
Day 0: REP-1
PD-1+ cells in GREX-100M with 1L of CM2+3,000 IU/mL IL-2 +OKT3 (30 ng/mL) + 100e6 Feeder cells
Day 11: REP-2
5-200e6 TIL in 1 GREX 500M in CM2+3,000 IU/mL IL-2 5E9 feeder cells 30 ng/mL OKT3 (150ul)
Day 16: TIL-Scale Up
Volume reduce and split up to 5 GREX 500MCS AIM-V+3,000 IU/mL of IL-2
Day 22: Harvest
Harvest-LOVO automated cell washer

FIG. 96


Day 0: REP-1
2E4-1E6 PD-1+ cells in GREX-100 with 0.5L of CM2+3,000 IU/mL IL-2 +OKT3 (30 ng/mL) + 2E6-100e6 Feeder cells
Day 11: REP-2
1E6 cells in GREX-100 with 0.5L of CM2+3,000 IU/mL IL-2 +OKT3 (30 ng/mL) + 100e6 Feeder cells
Media change if Culture turns Yellow
Day 22: Harvest
Harvest

SUBSTITUTE SHEET (RULE 26)

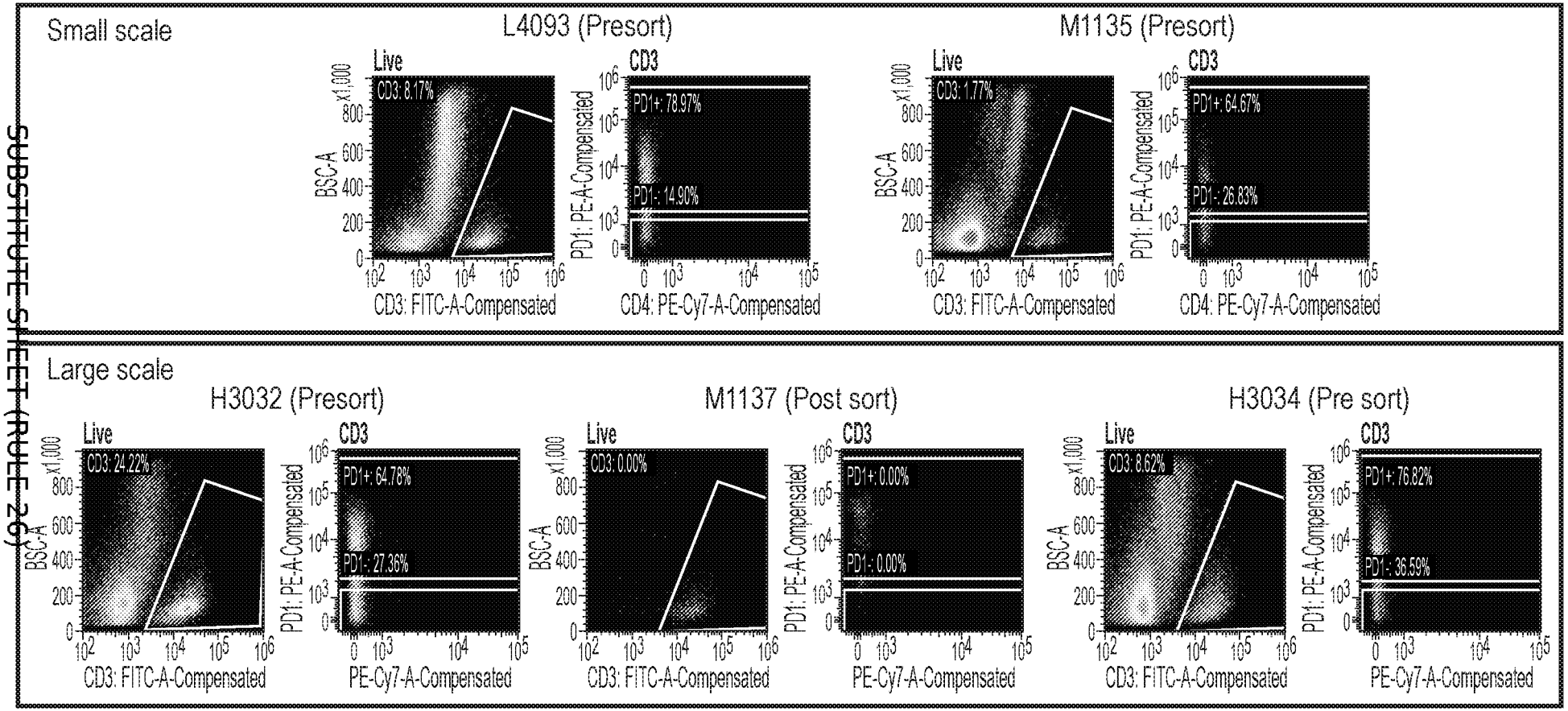


FIG. 97

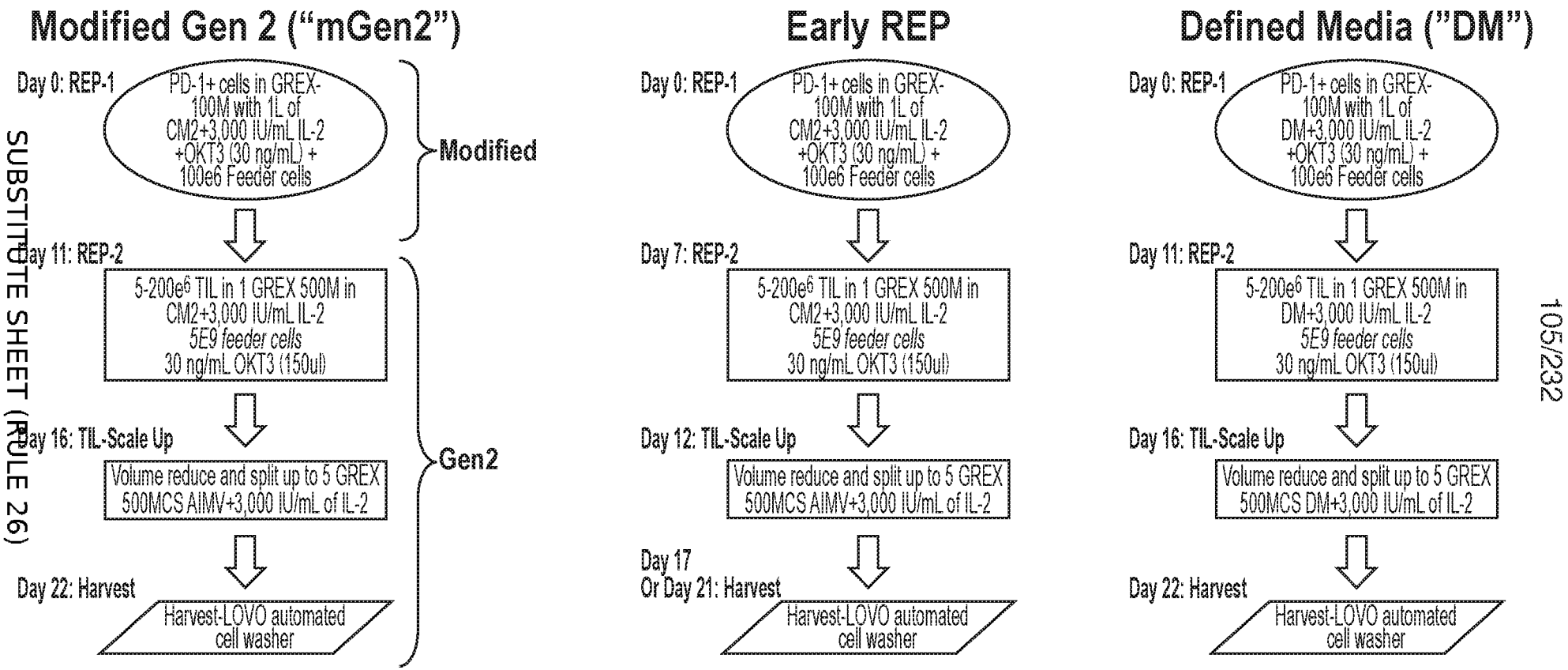


FIG. 98

SUBSTITUTE SHEET (RULE 26)

	Lung (L4093)				Melanoma (M1135)			
Flow Sort Summary								
# Pre-sort (%CD3+PD1+)	5.6e5 (6.5%)				4.88 e4 (1.2%)			
# Sorted (% Yield)	4.5e5 (80.7%)				4.8e4 (98.4)			
*Post-sort Purity (%)	85%				75.7%			
TIL Expansion Summary	***Research PD1+***	***Gen 2 PD1+***	***Gen 2 DM PD1+***	***^ER PD1+***	***Research PD1+***	***Gen 2 PD1+***	***Gen 2 DM PD1+***	***^ER PD1+***
# REP1 seed (G-Rex100/100M)	4.48e5	4.48e5	4.48e5	4.48e5	4.8e4	4.8e4	4.8e4	4.8e4
# REP1 Harvest (Extrapolated)	599e6	560e6	225e6	44e6	34e6	272e6	256e6	46e6
REP 1 Fold expansion	1336	1249	503	99	705	5670	5334	958
# REP2 seed (G-Rex100/500M)	1e6	200e6	200e6	44e6	1e6	200e6	200e6	44e6
# REP2 Harvest (Extrapolated)	24e9	97e9	64e9	55e9	4.3e9	99e9	97e9	37e9
REP 2 Fold expansion	404	483	318	247	1282	497	483	270

#, Total Viable Cells (TVC)
*, Purity was based on FSC/CD3+/PD1+ but not on Viable cells
^, REP1 on Day 7, REP 2 on Day 17

FIG. 99

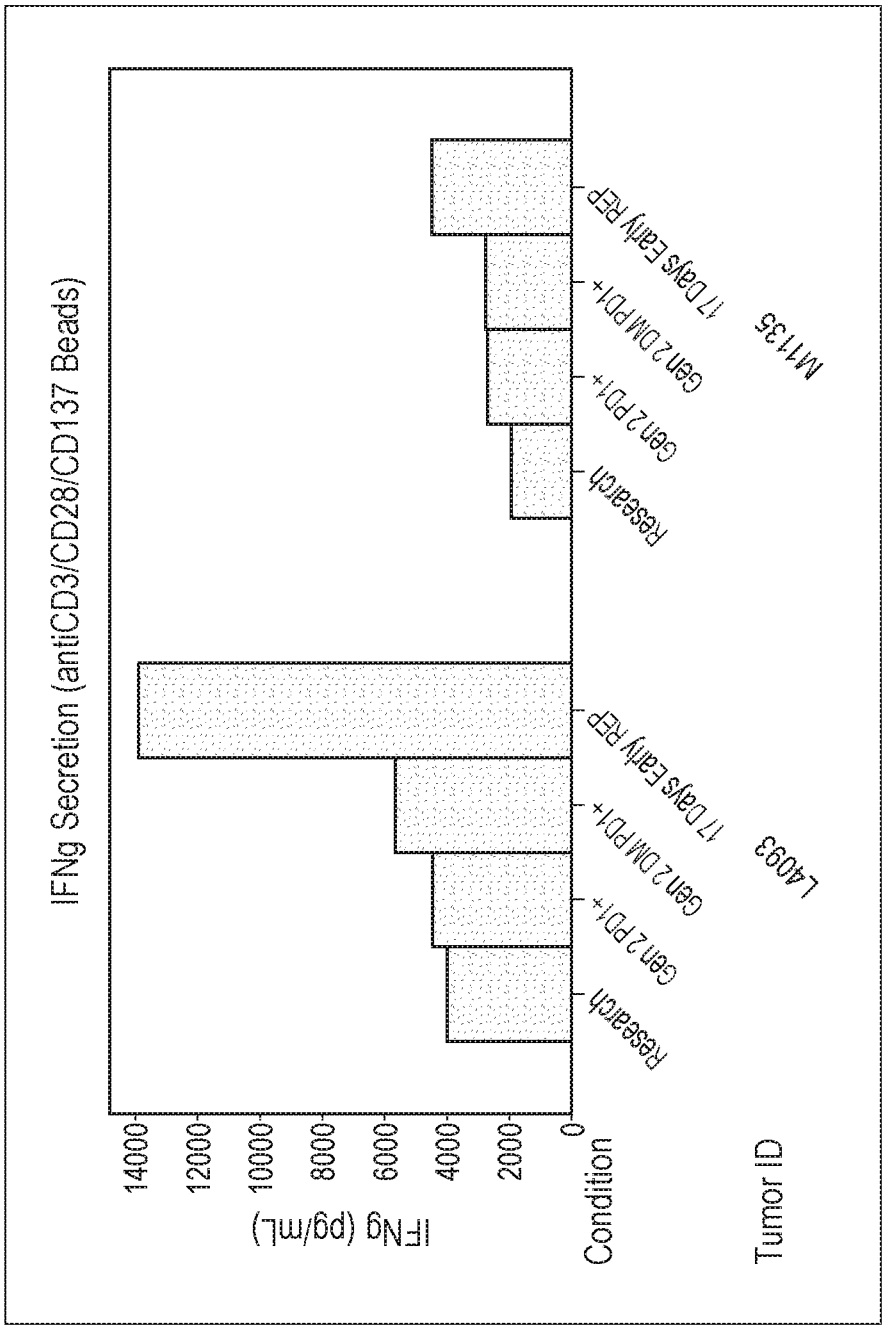

IFNg Secretion (antiCD3/CD28/CD137 Beads)
IFNg (pg/mL)
14000
12000
10000
8000
6000
4000
2000
0
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
17 Days Early REP
Research
Gen 2 PD1+
Gen 2 DM PD1+
17 Days Early REP
Tumor ID
L4093
M1135

FIG. 100

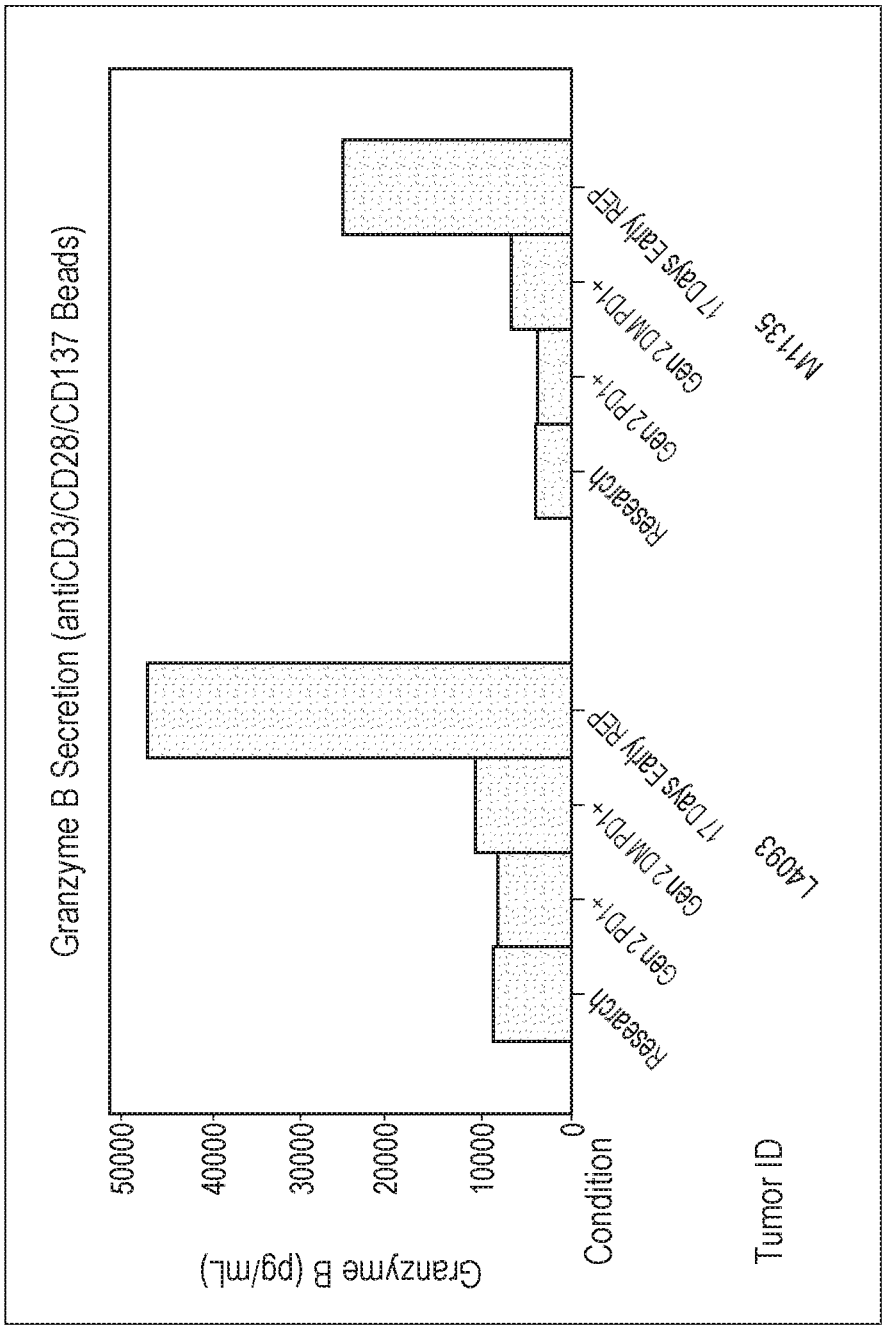

Granzyme B Secretion (antiCD3/CD28/CD137 Beads)
Granzyme B (pg/mL)
50000
40000
30000
20000
10000
0
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
17 Days Early REP
Research
Gen 2 PD1+
Gen 2 DM PD1+
17 Days Early REP
Tumor ID
L4093
M1135

FIG. 101

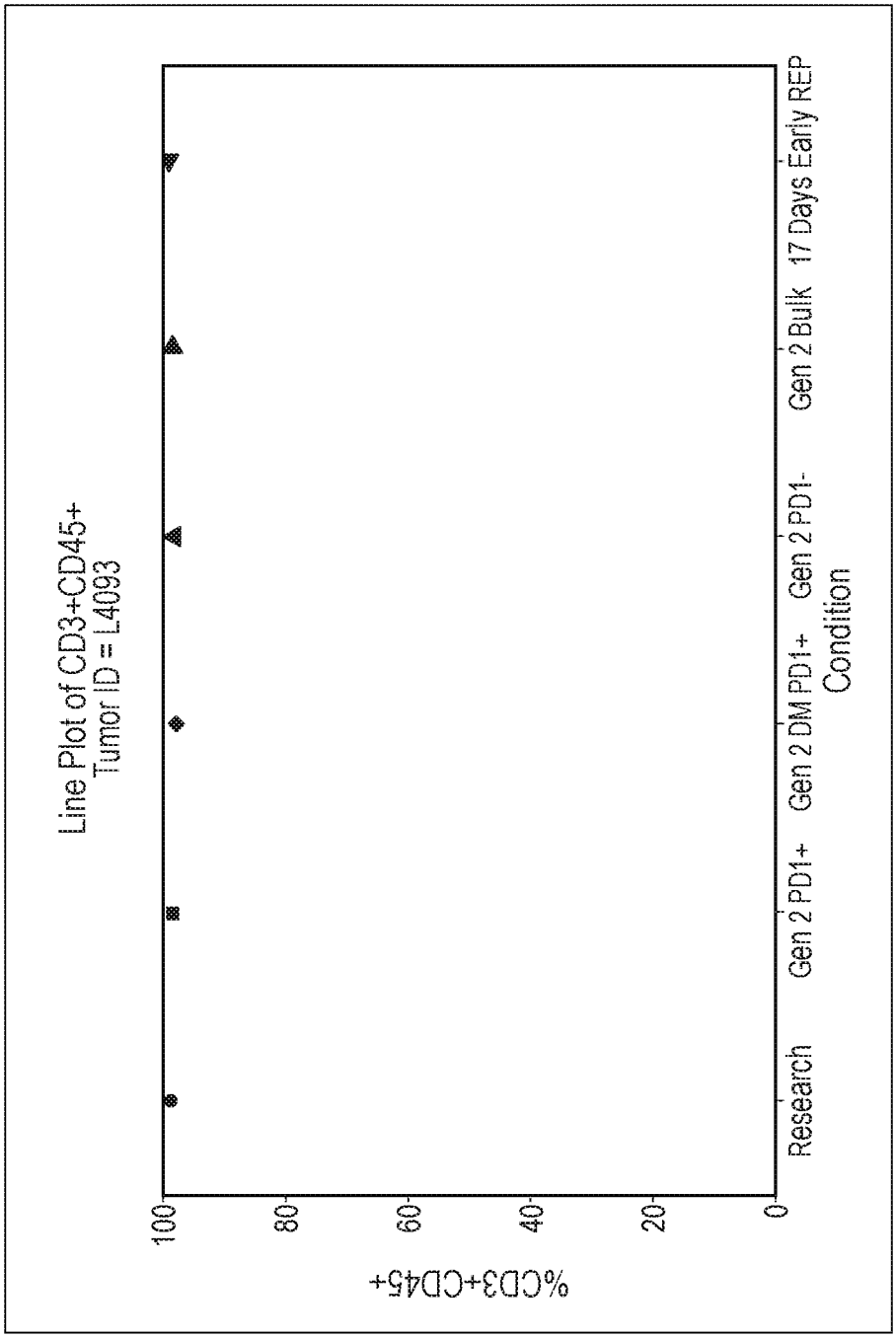

Line Plot of CD3+CD45+
Tumor ID = L4093
%CD3+CD45+
100
80
60
40
20
0
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Condition

FIG. 102

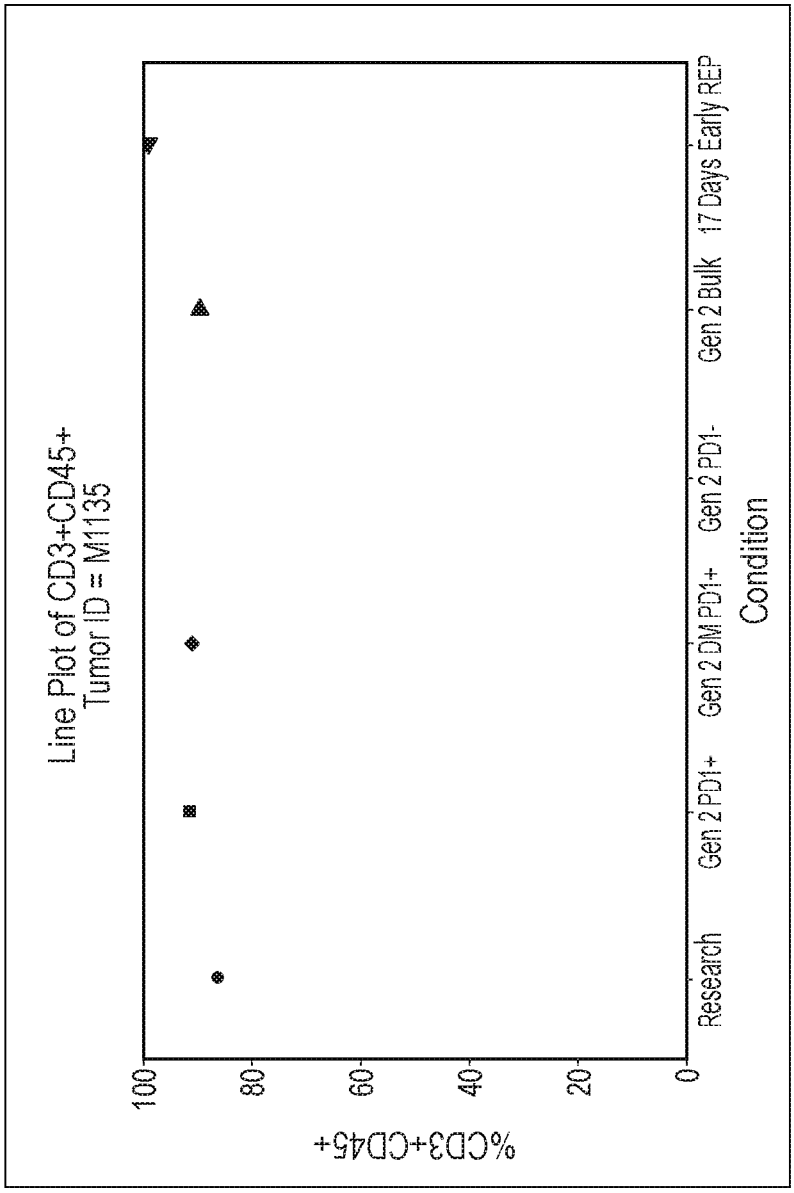

Line Plot of CD3+CD45+
Tumor ID = M1135
%CD3+CD45+
100
80
60
40
20
0
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Condition

FIG. 103

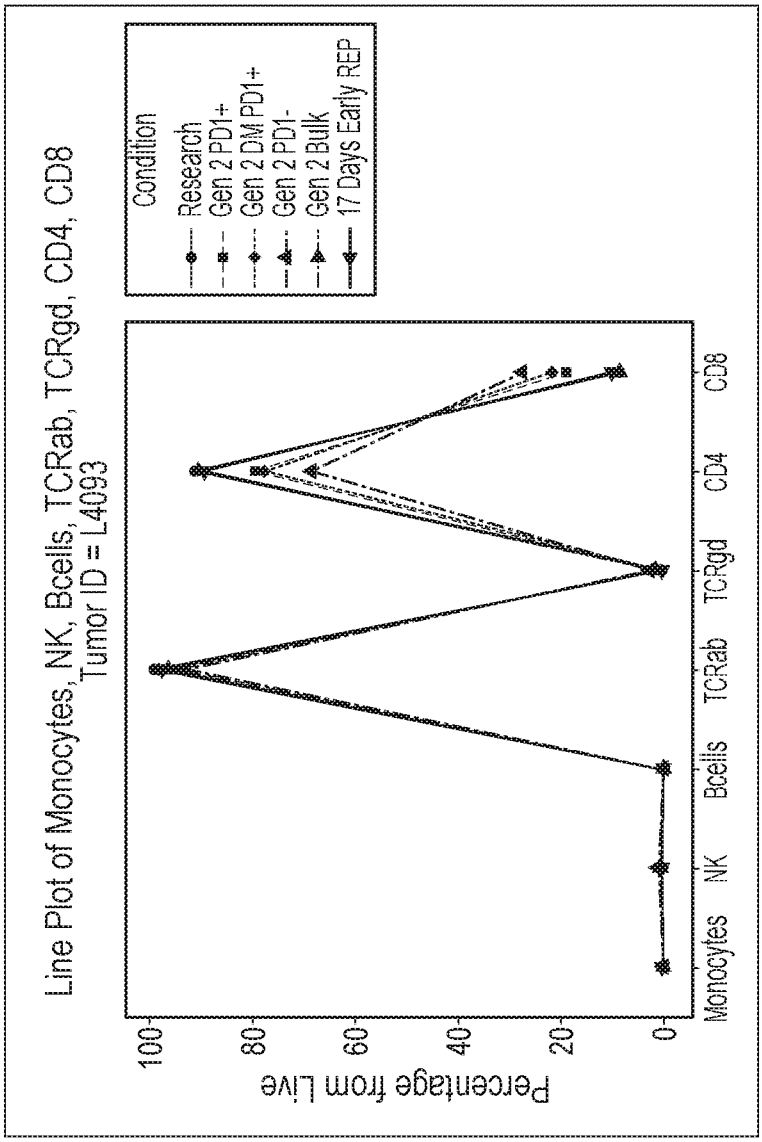

Line Plot of Monocytes, NK, Bcells, TCRab, TCRgd, CD4, CD8
Tumor ID = L4093
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Live
100
80
60
40
20
0
Monocytes
NK
Bcells
TCRab
TCRgd
CD4
CD8

FIG. 104

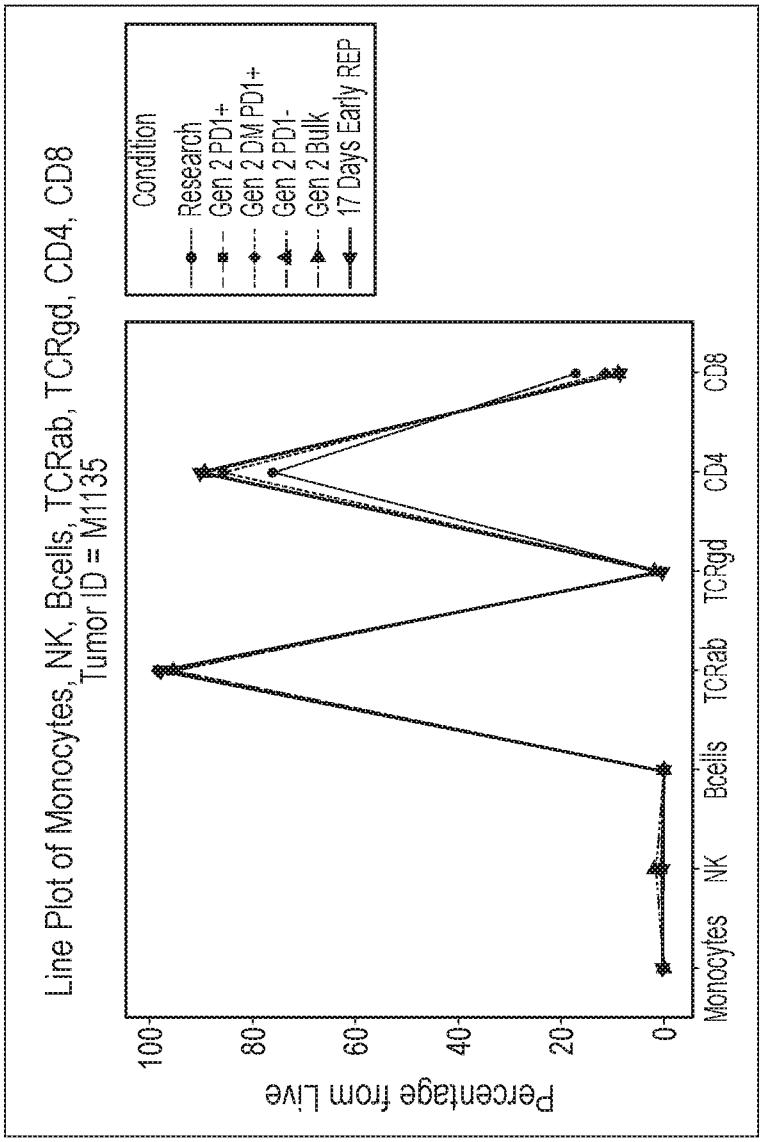

Line Plot of Monocytes, NK, Bcells, TCRab, TCRgd, CD4, CD8
Tumor ID = M1135
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Live
100
80
60
40
20
0
Monocytes
NK
Bcells
TCRab
TCRgd
CD4
CD8

FIG. 105

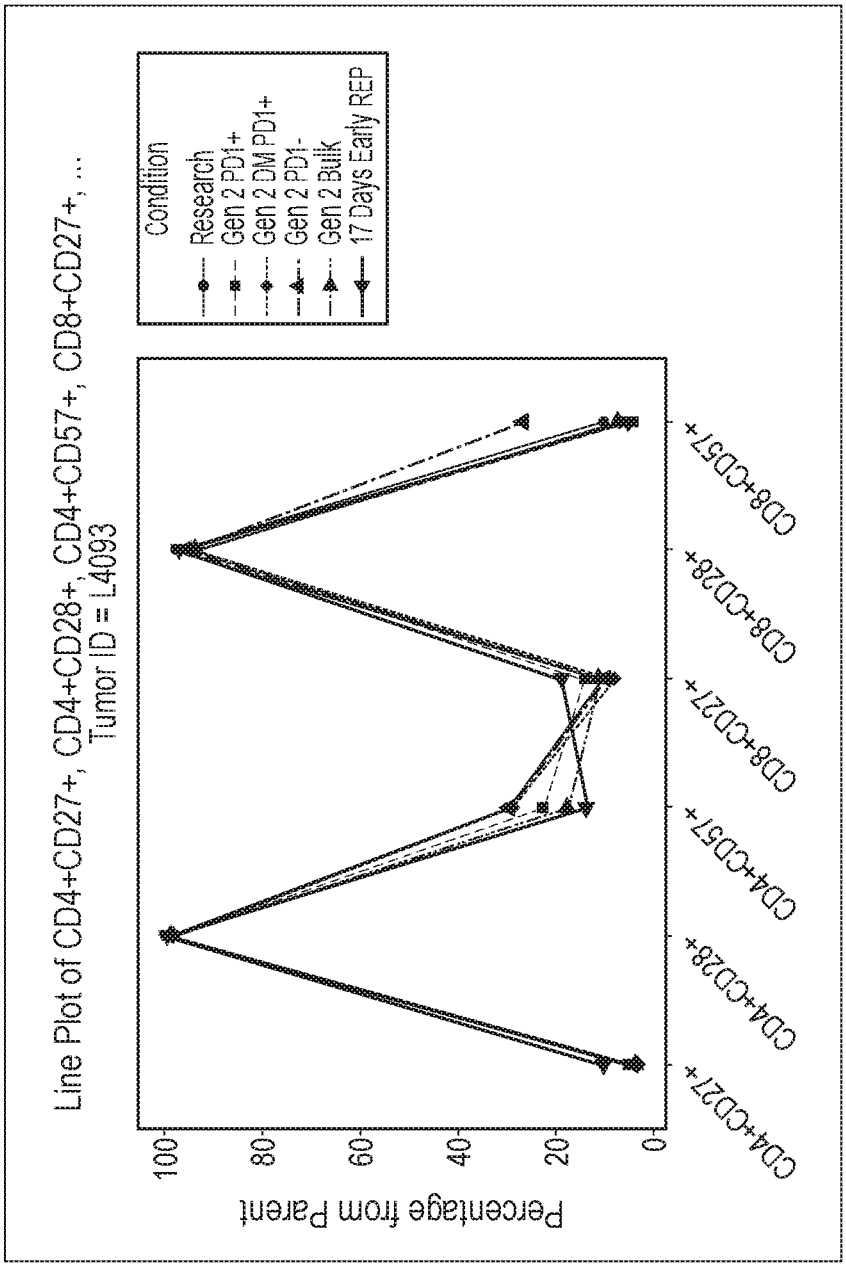

Line Plot of CD4+CD27+, CD4+CD28+, CD4+CD57+, CD8+CD27+, ...
Tumor ID = L4093
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Parent
100
80
60
40
20
0
CD4+CD27+
CD4+CD28+
CD4+CD57+
CD8+CD27+
CD8+CD28+
CD8+CD57+

FIG. 106A

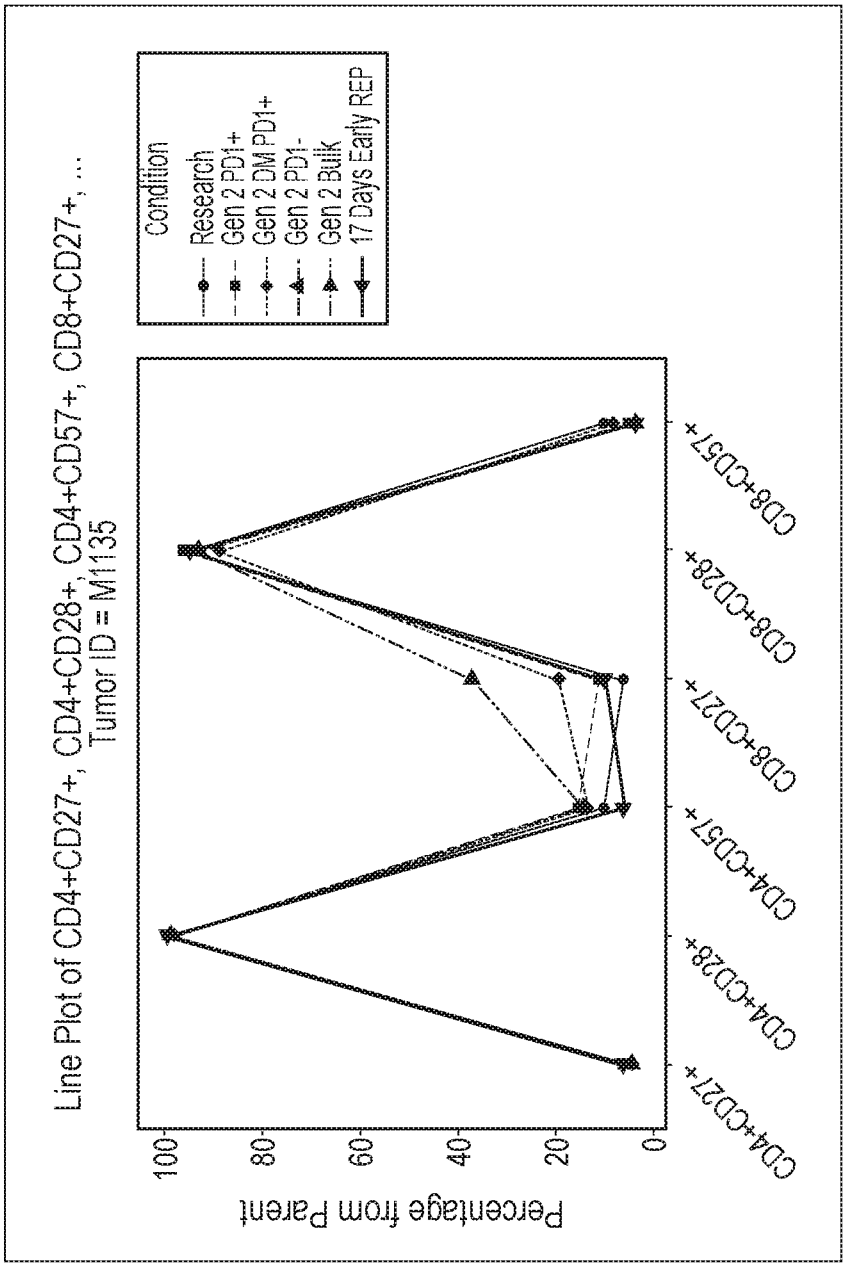

Line Plot of CD4+CD27+, CD4+CD28+, CD4+CD57+, CD8+CD27+, ...
Tumor ID = M1135
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Parent
100
80
60
40
20
0
CD4+CD27+
CD4+CD28+
CD4+CD57+
CD8+CD27+
CD8+CD28+
CD8+CD57+

FIG. 106B

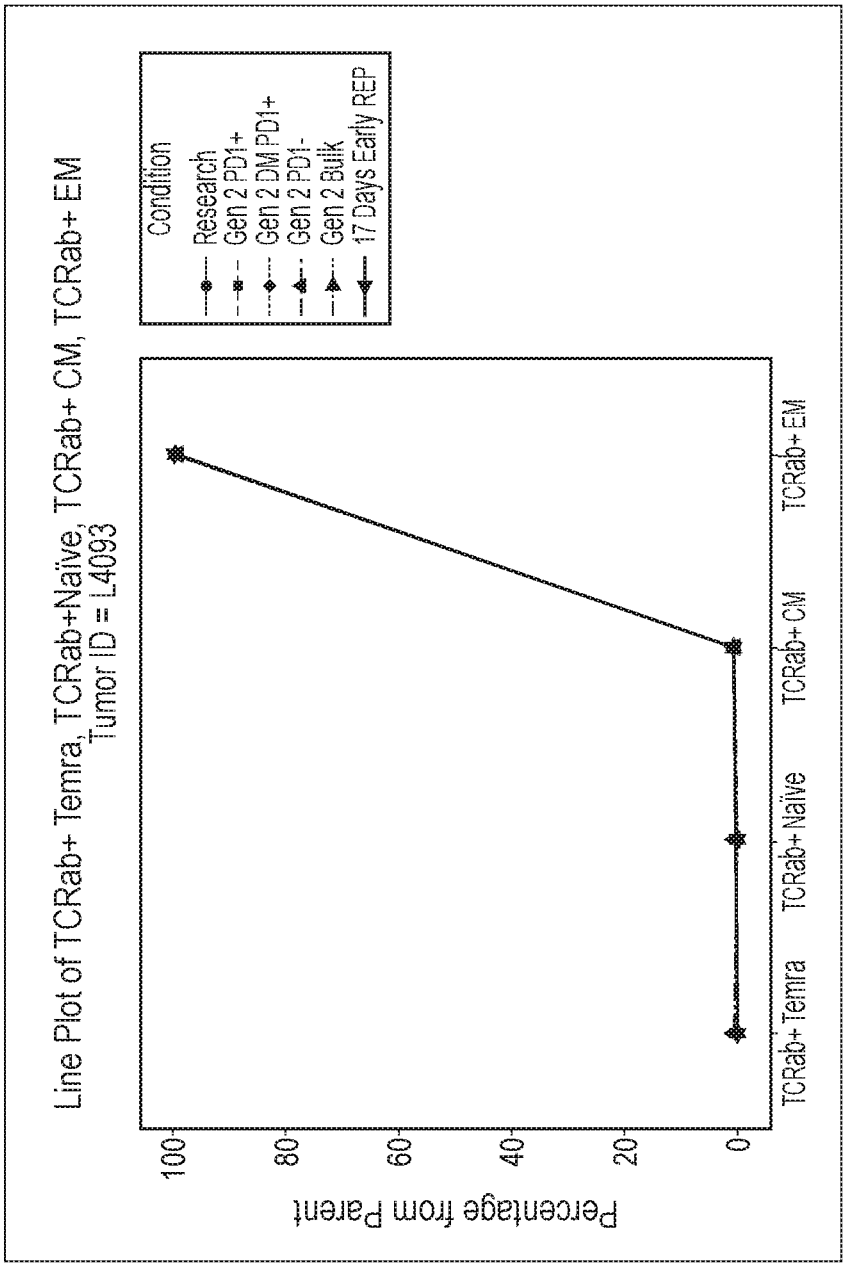

Line Plot of TCRab+ Temra, TCRab+Naïve, TCRab+ CM, TCRab+ EM
Tumor ID = L4093
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Parent
100
80
60
40
20
0
TCRab+ Temra
TCRab+ Naïve
TCRab+ CM
TCRab+ EM

FIG. 107A

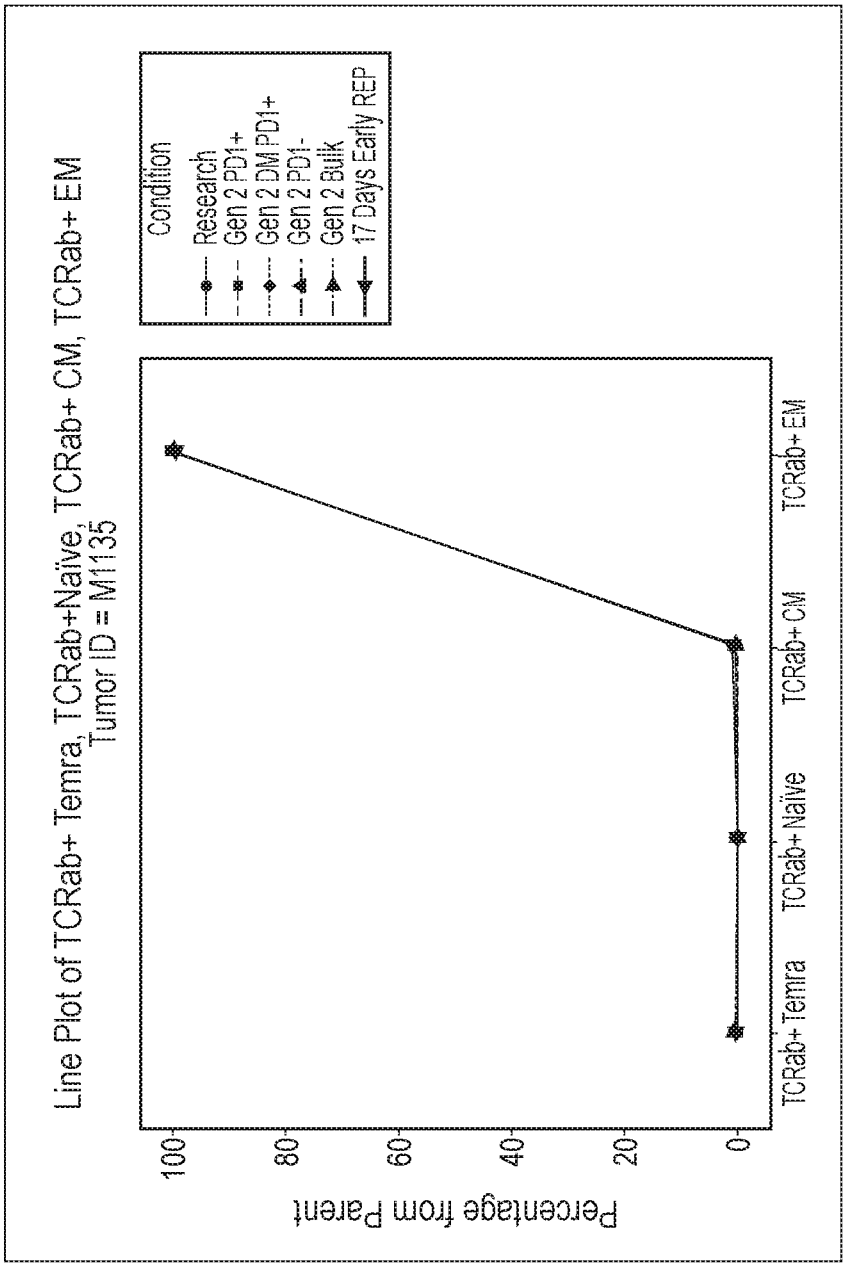

Line Plot of TCRab+ Temra, TCRab+Naïve, TCRab+ CM, TCRab+ EM
Tumor ID = M1135
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from Parent
100
80
60
40
20
0
TCRab+ Temra
TCRab+ Naïve
TCRab+ CM
TCRab+ EM

FIG. 107B

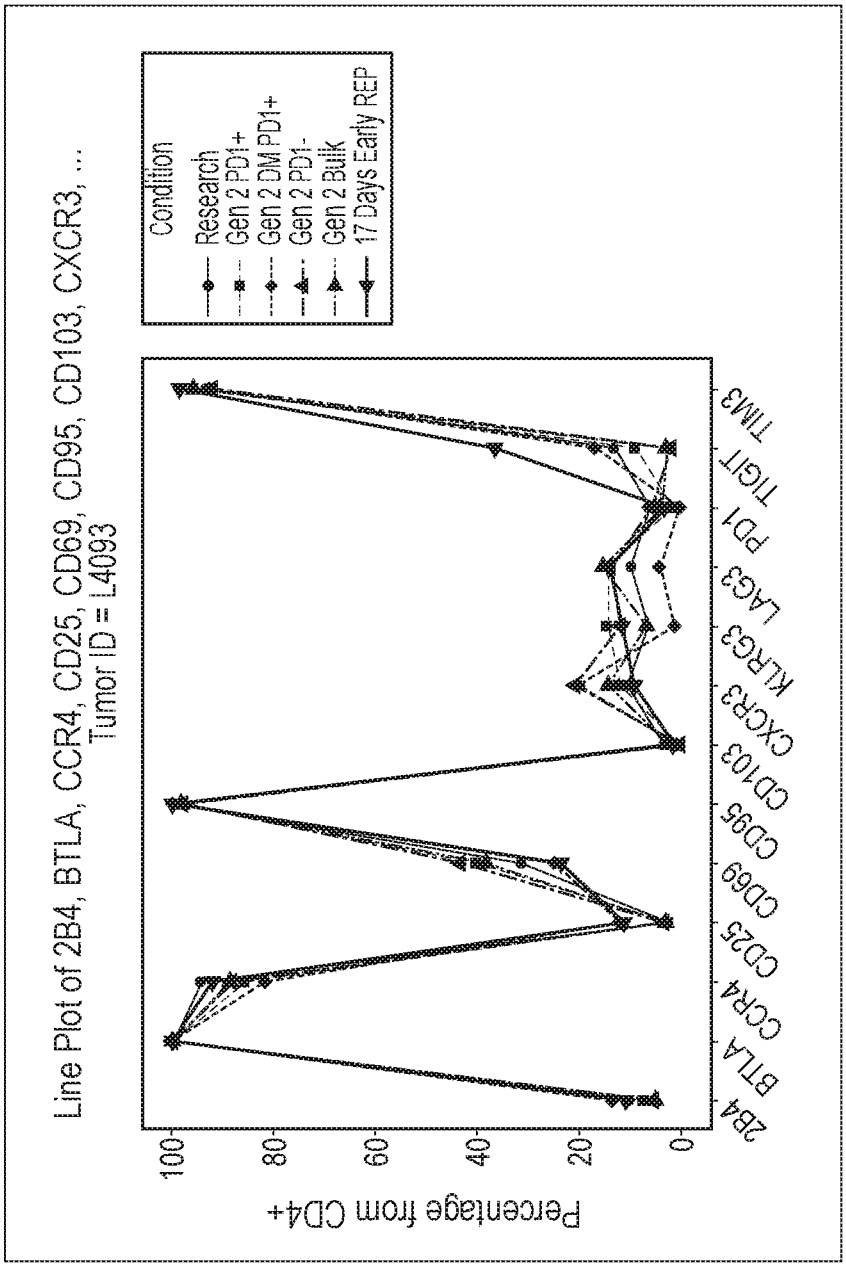

Line Plot of 2B4, BTLA, CCR4, CD25, CD69, CD95, CD103, CXCR3, ...
Tumor ID = L4093
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from CD4+
100
80
60
40
20
0
2B4
BTLA
CCR4
CD25
CD69
CD95
CD103
CXCR3
KLRG3
LAG3
PD1
TIGIT
TIM3

FIG. 108A

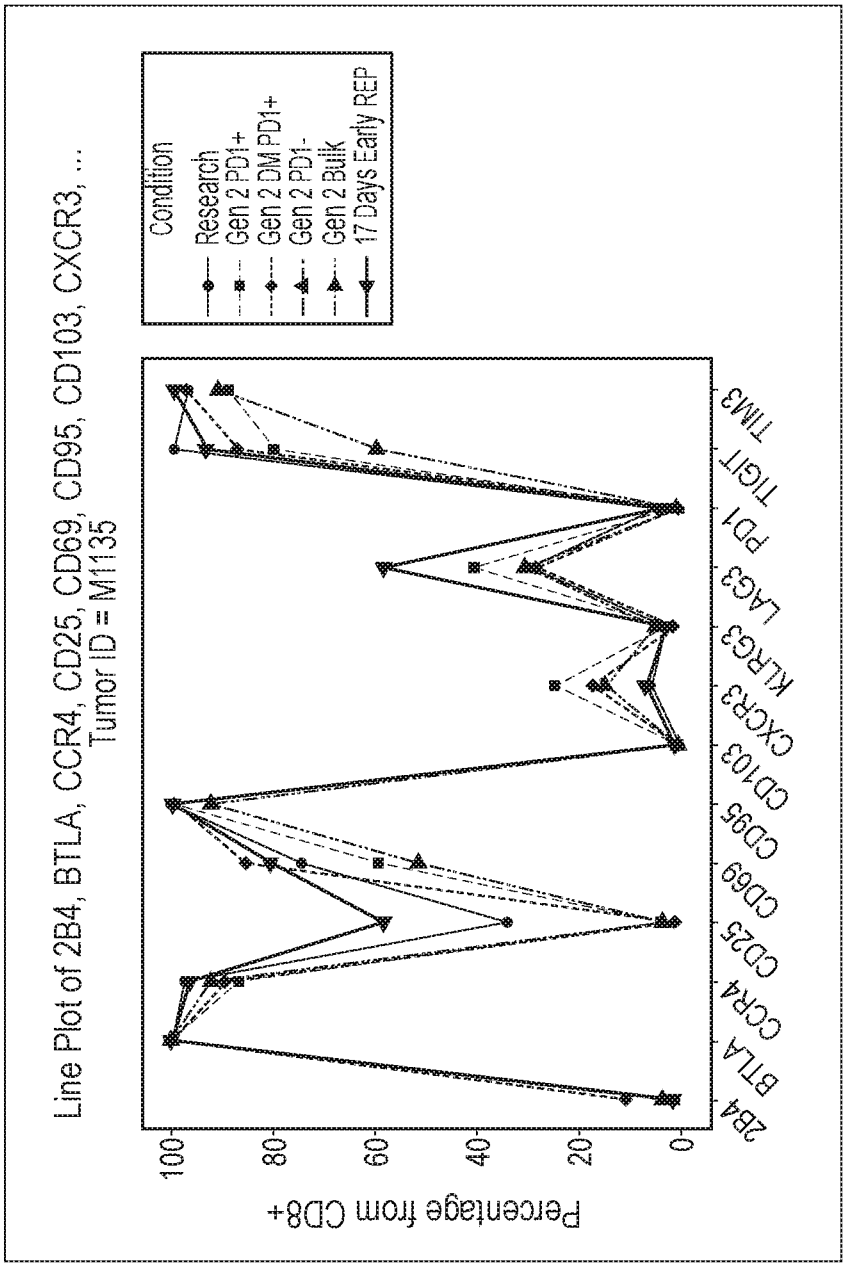

Line Plot of 2B4, BTLA, CCR4, CD25, CD69, CD95, CD103, CXCR3, ...
Tumor ID = M1135
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from CD8+
100
80
60
40
20
0
2B4
BTLA
CCR4
CD25
CD69
CD95
CD103
CXCR3
KLRG3
LAG3
PD1
TIGIT
TIM3

FIG. 108B

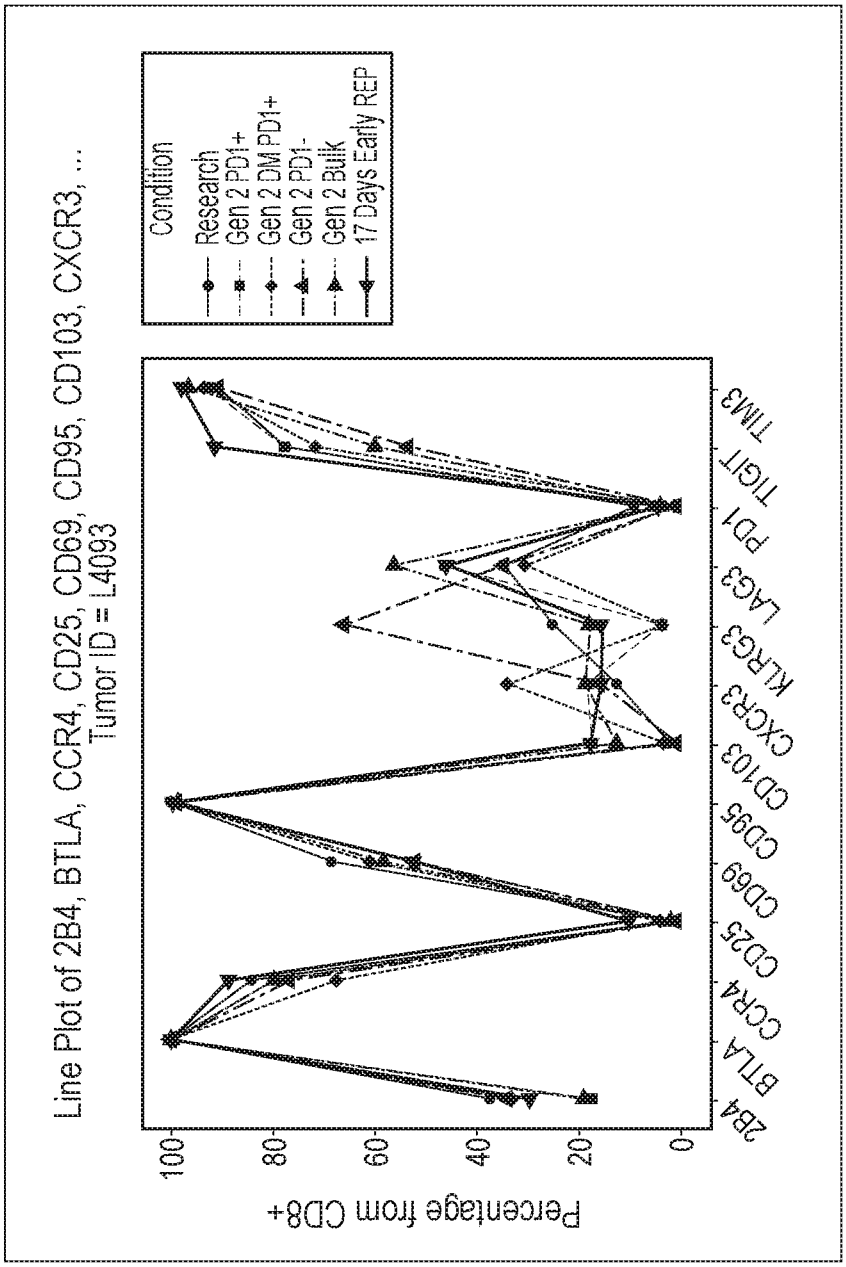

Line Plot of 2B4, BTLA, CCR4, CD25, CD69, CD95, CD103, CXCR3, ...
Tumor ID = L4093
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from CD8+
100
80
60
40
20
0
2B4
BTLA
CCR4
CD25
CD69
CD95
CD103
CXCR3
KLRG3
LAG3
PD1
TIGIT
TIM3

FIG. 109A

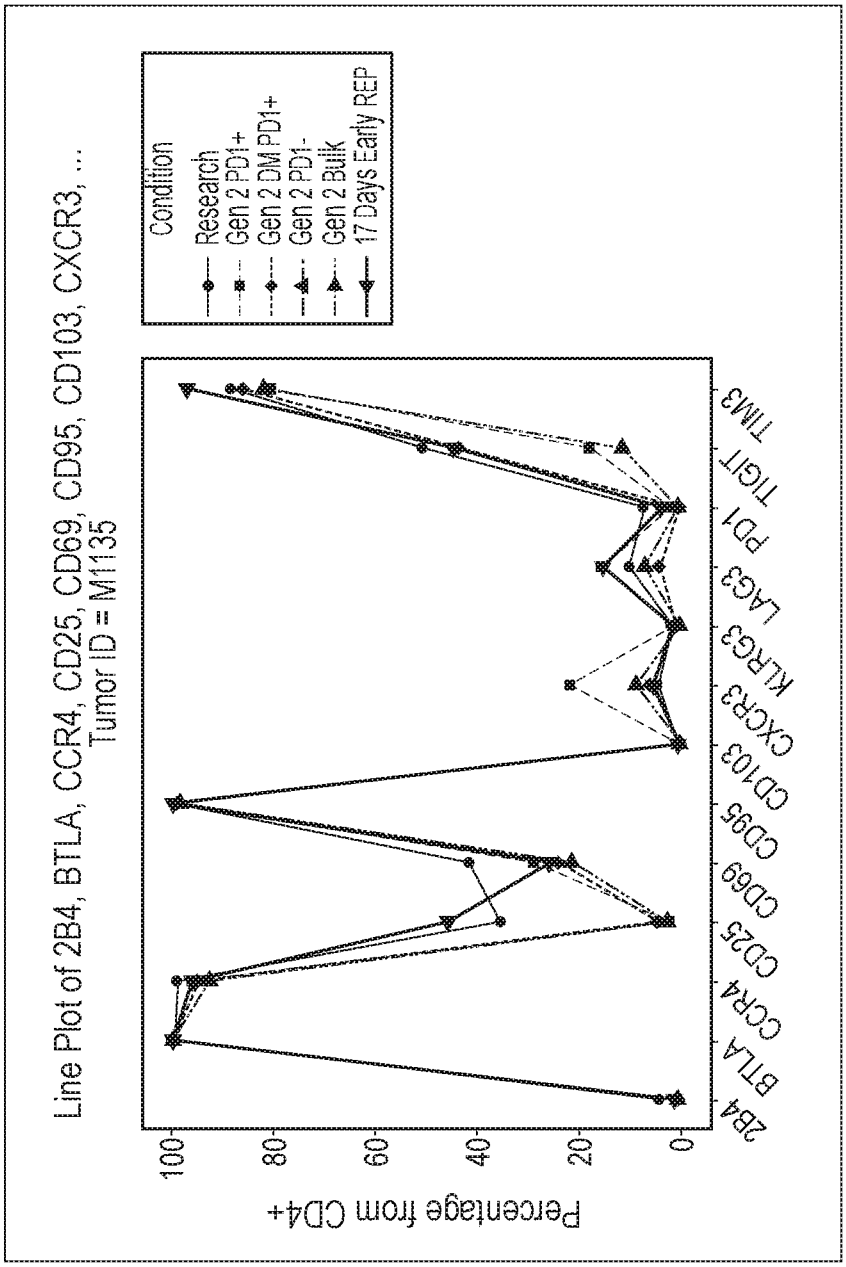

Line Plot of 2B4, BTLA, CCR4, CD25, CD69, CD95, CD103, CXCR3, ...
Tumor ID = M1135
Condition
Research
Gen 2 PD1+
Gen 2 DM PD1+
Gen 2 PD1-
Gen 2 Bulk
17 Days Early REP
Percentage from CD4+
100
80
60
40
20
0
2B4
BTLA
CCR4
CD25
CD69
CD95
CD103
CXCR3
KLRG1
LAG3
PD1
TIGIT
TIM3

FIG. 109B

SUBSTITUTE SHEET (RULE 26)

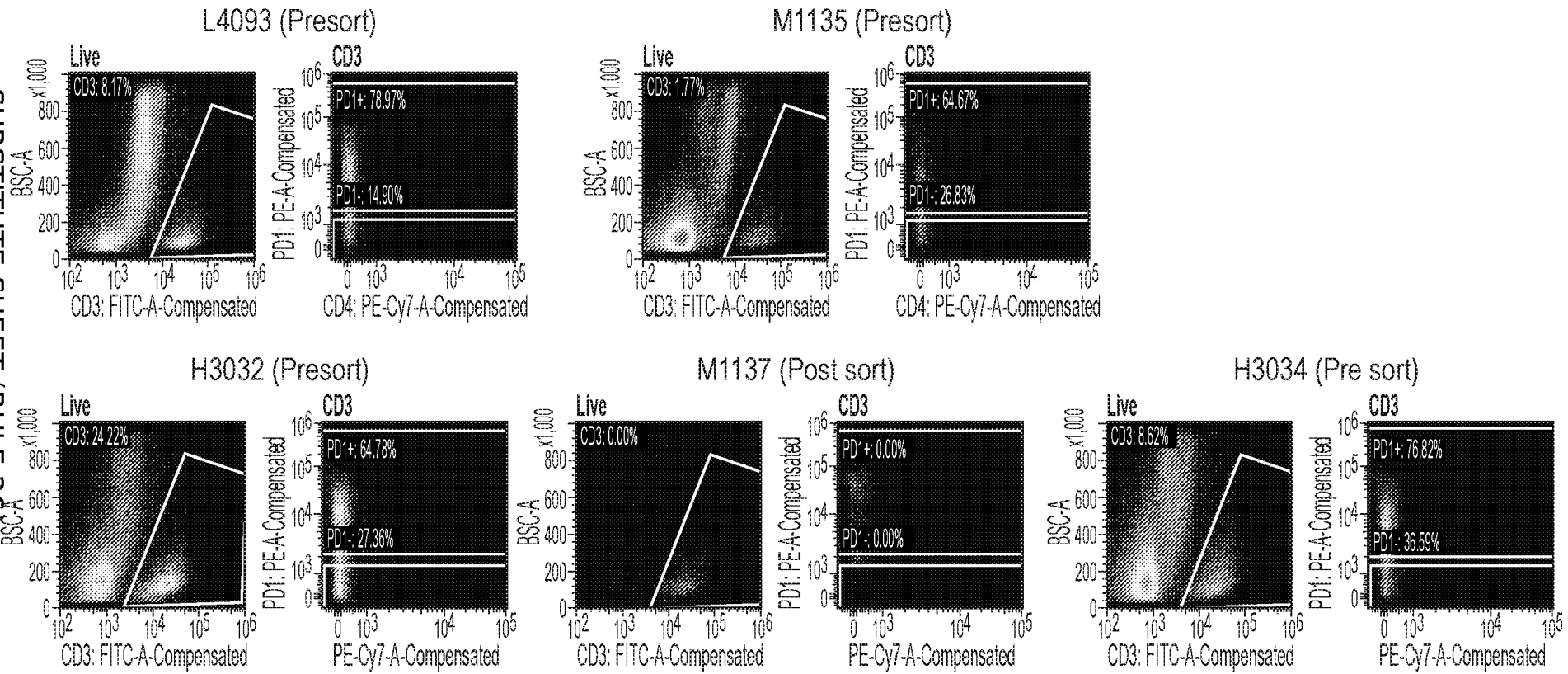


FIG. 110

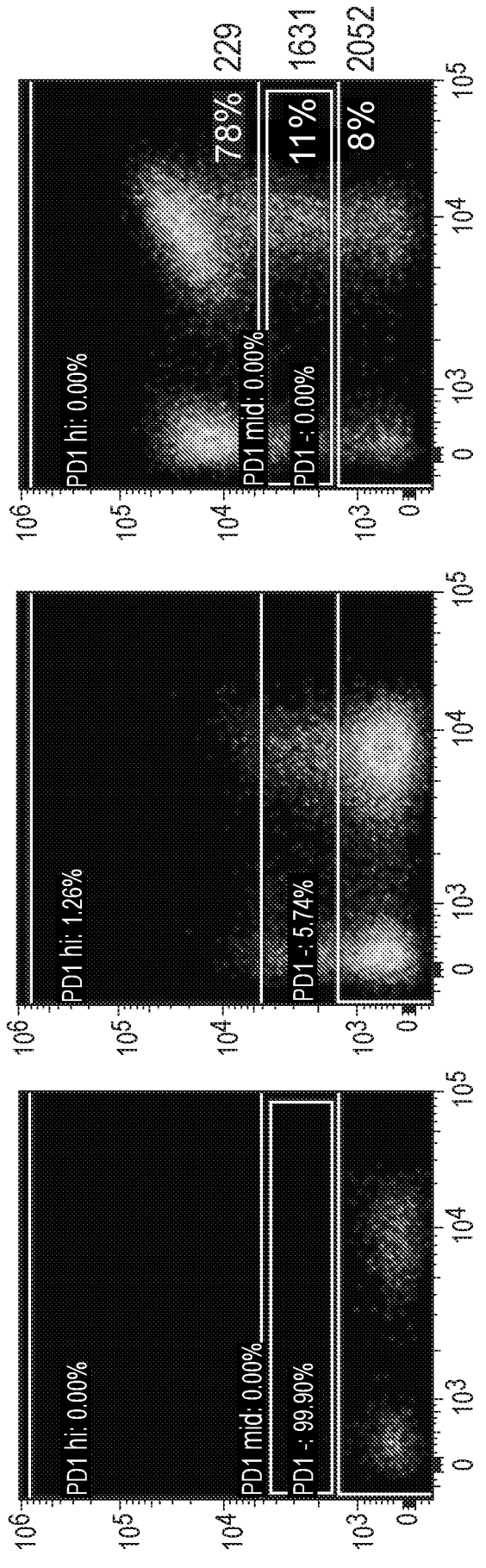

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 99.90%
Feeder Pool
PD1 hi: 1.26%
PD1 -: 5.74%
TIL PD1
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
78%
11%
8%
229
1631
2052

FIG. 111

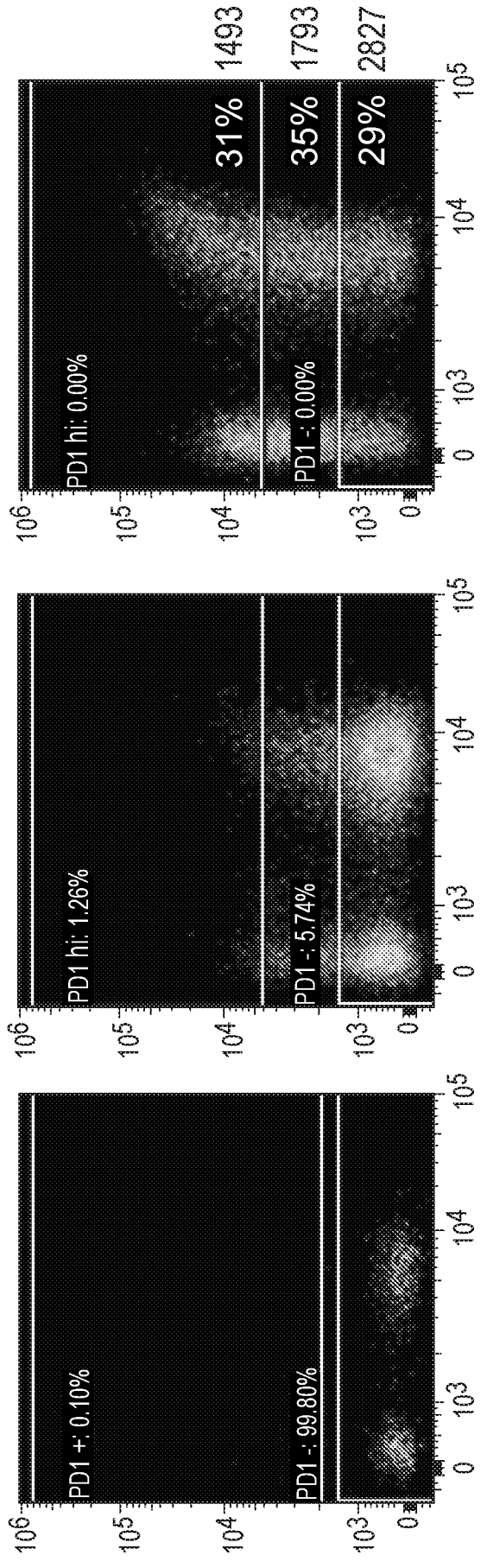

TIL FMO
PD1 +: 0.10%
PD1 -: 99.80%
Feeder Pool
PD1 hi: 1.26%
PD1 -: 5.74%
TIL PD1
PD1 hi: 0.00%
PD1 -: 0.00%
31%
35%
29%
1493
1793
2827
0
10^3
10^4
10^5
10^6

FIG. 112

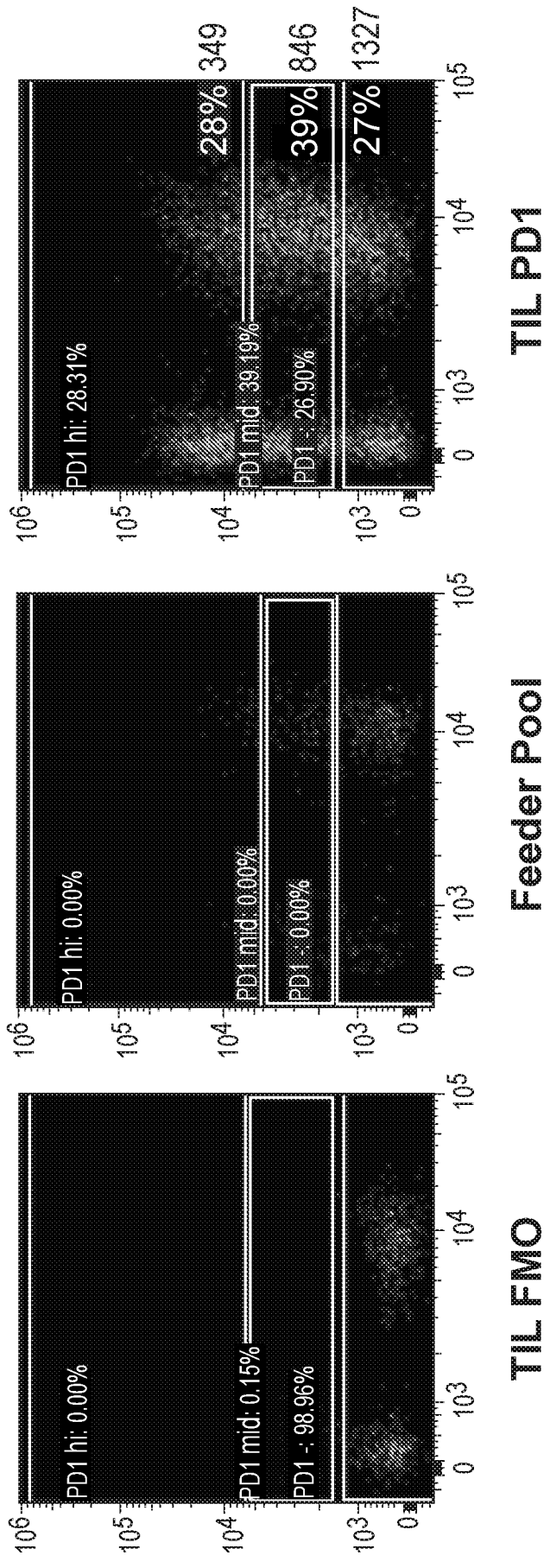

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.15%
PD1 -: 98.96%
Feeder Pool
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
TIL PD1
PD1 hi: 28.31%
PD1 mid: 39.19%
PD1 -: 26.90%
28%
39%
27%
349
846
1327

FIG. 113

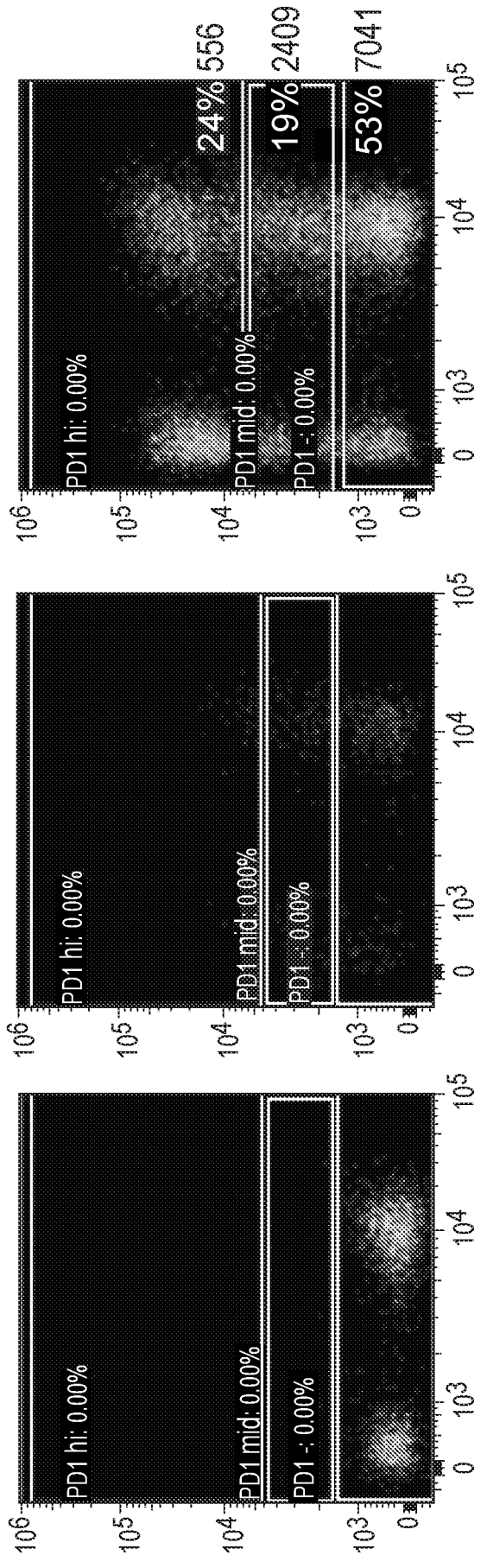

TIL FMO
Feeder Pool
TIL PD1
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
24%
19%
53%
556
2409
7041

FIG. 114

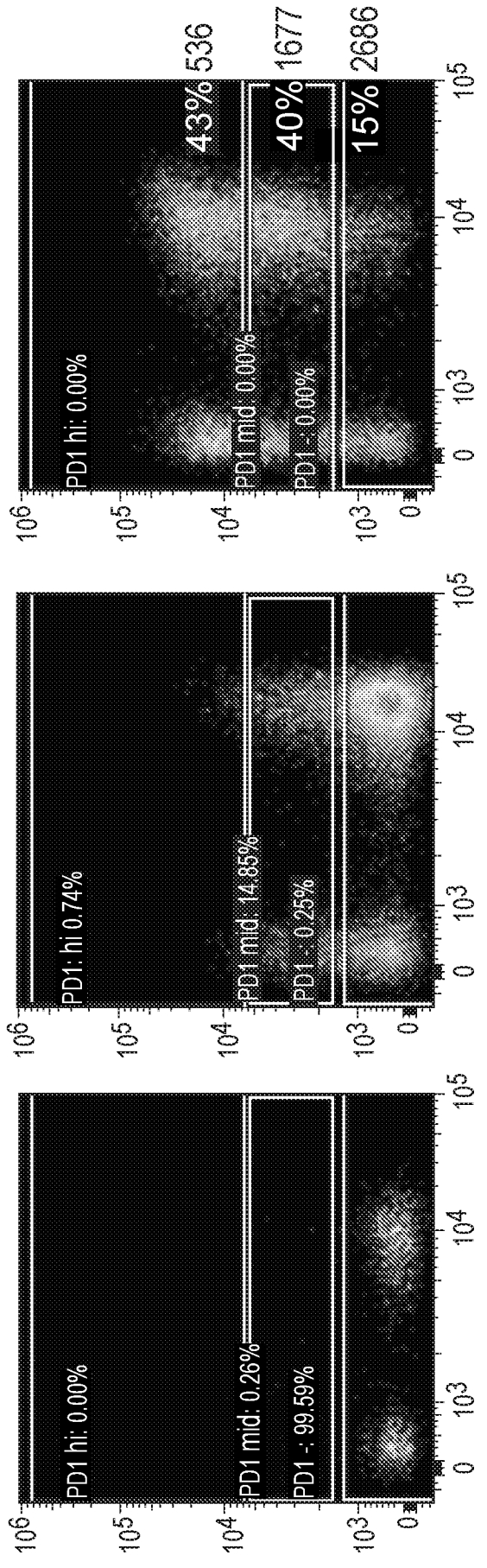

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.26%
PD1 -: 99.59%
Feeder Pool
PD1: hi 0.74%
PD1 mid: 14.85%
PD1 -: 0.25%
TIL PD1
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
43%
536
40%
1677
15%
2686

FIG. 115

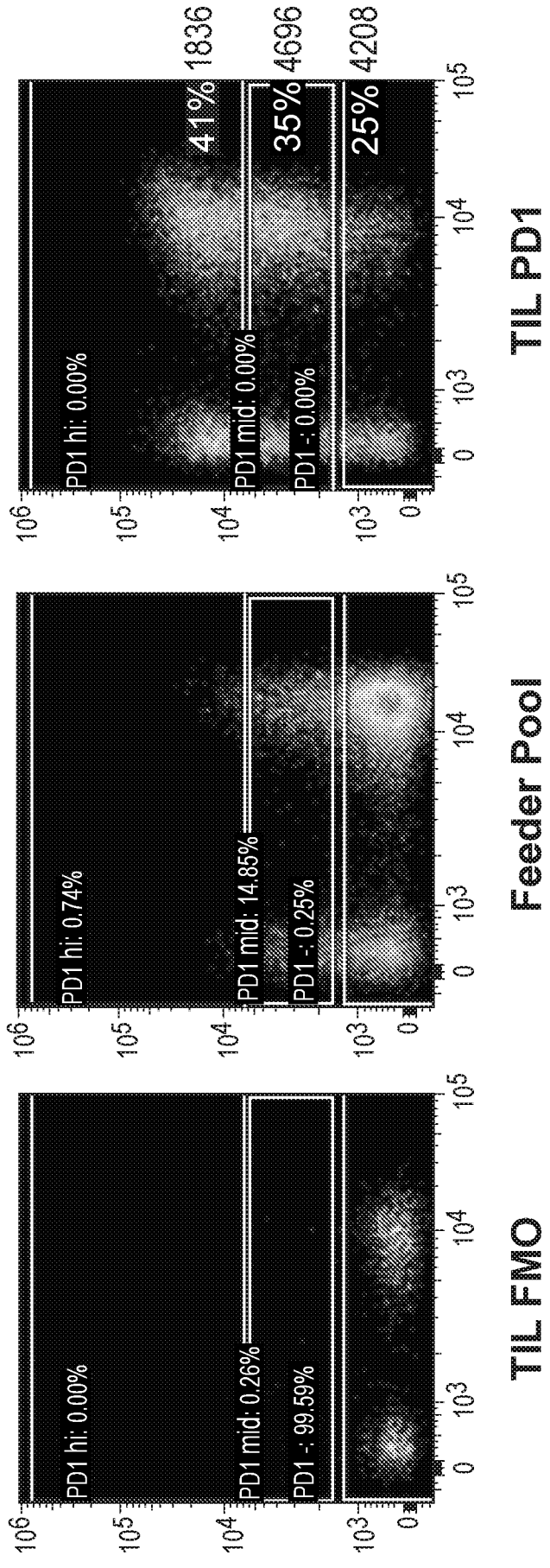

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.26%
PD1 -: 99.59%
Feeder Pool
PD1 hi: 0.74%
PD1 mid: 14.85%
PD1 -: 0.25%
TIL PD1
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
41%
35%
25%
1836
4696
4208
10^6
10^5
10^4
10^3
0
10^3
10^4
10^5

FIG. 116

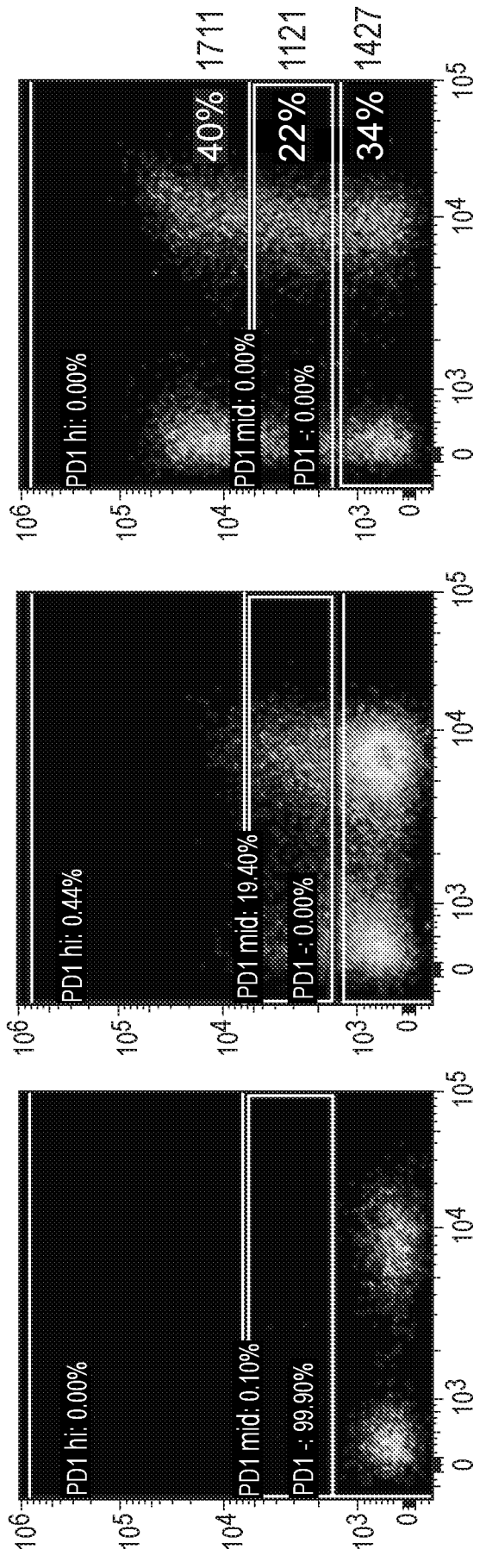

TIL FMO
Feeder Pool
TIL PD1
PD1 hi: 0.00%
PD1 mid: 0.10%
PD1 -: 99.90%
PD1 hi: 0.44%
PD1 mid: 19.40%
PD1 -: 0.00%
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
40%
22%
34%
1711
1121
1427
10^6
10^5
10^4
10^3
0
10^3
10^4
10^5

FIG. 117

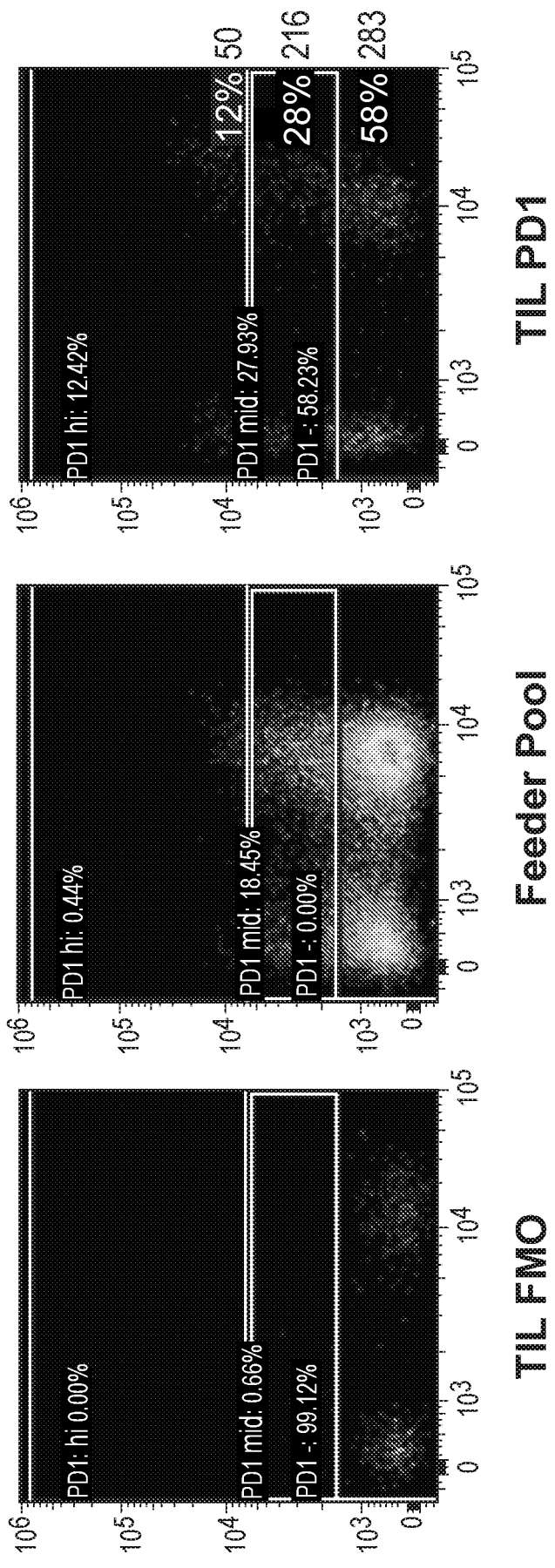

TIL FMO
PD1: hi 0.00%
PD1 mid: 0.66%
PD1 -: 99.12%
Feeder Pool
PD1 hi: 0.44%
PD1 mid: 18.45%
PD1 -: 0.00%
TIL PD1
PD1 hi: 12.42%
PD1 mid: 27.93%
PD1 -: 58.23%
50
216
283
12%
28%
58%
10^6
10^5
10^4
10^3
0
10^3
10^4
10^5

FIG. 118

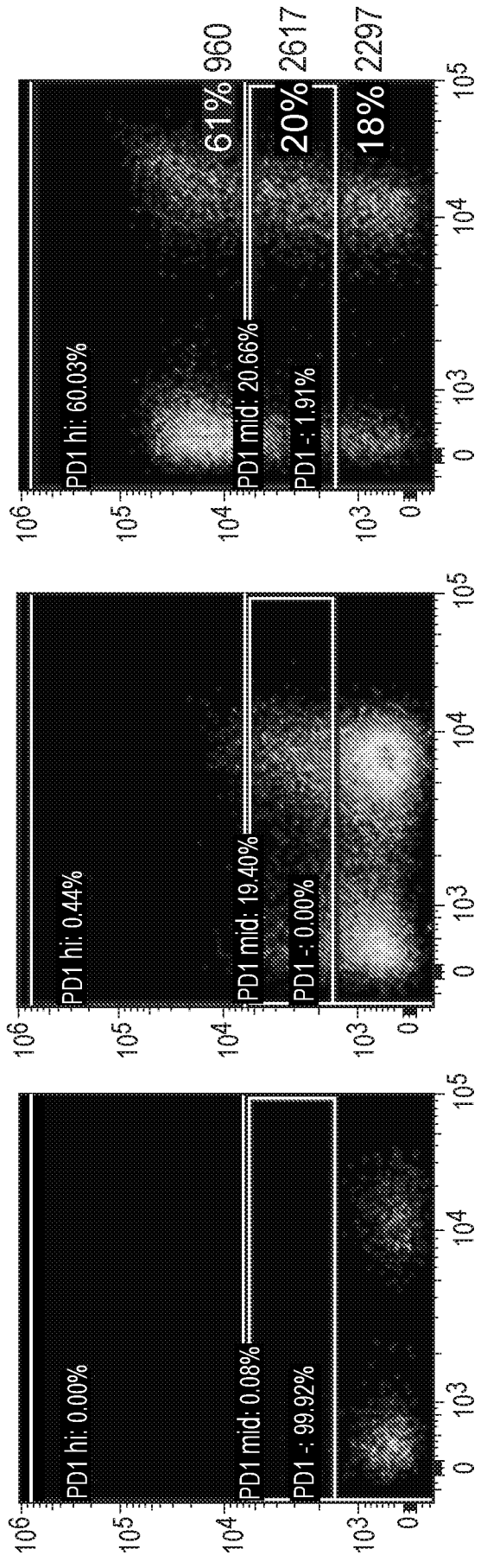

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.08%
PD1 -: 99.92%
Feeder Pool
PD1 hi: 0.44%
PD1 mid: 19.40%
PD1 -: 0.00%
TIL PD1
PD1 hi: 60.03%
PD1 mid: 20.66%
PD1 -: 1.91%
61%
20%
18%
960
2617
2297

FIG. 119

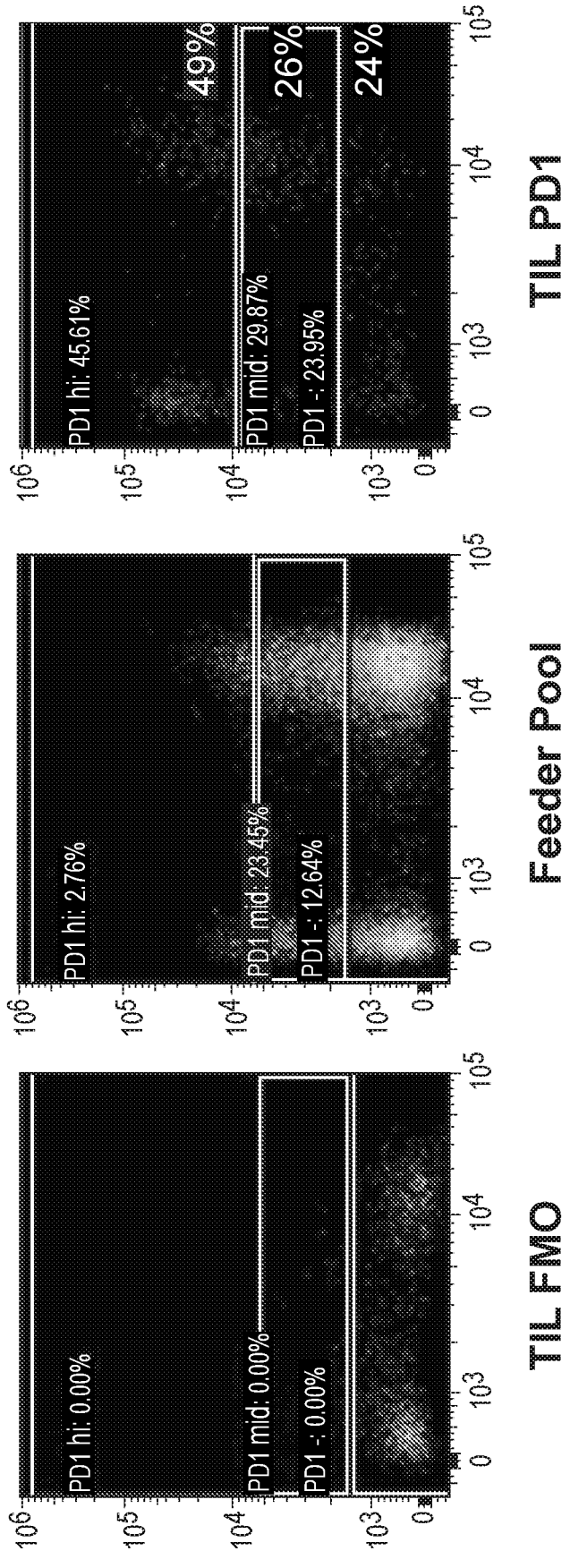

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
Feeder Pool
PD1 hi: 2.76%
PD1 mid: 23.45%
PD1 -: 12.64%
TIL PD1
PD1 hi: 45.61%
PD1 mid: 29.87%
PD1 -: 23.95%
49%
26%
24%

FIG. 120

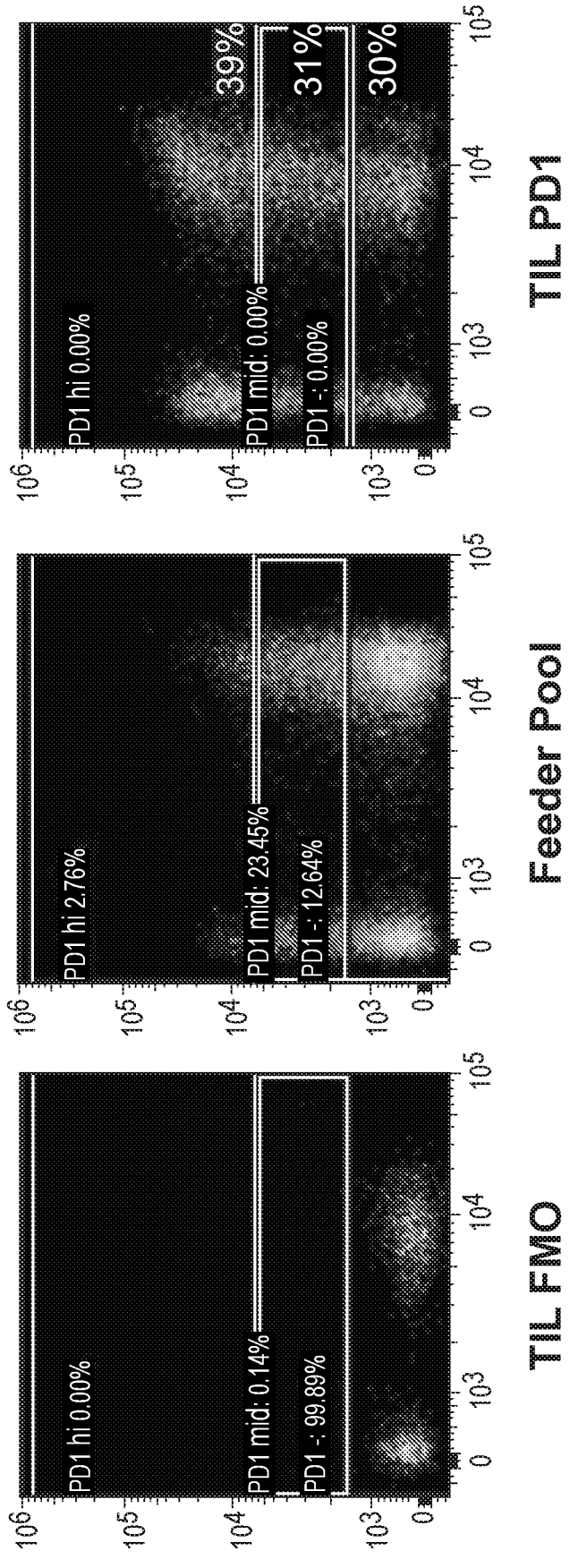

TIL FMO
PD1 hi 0.00%
PD1 mid: 0.14%
PD1 -: 99.89%
Feeder Pool
PD1 hi 2.76%
PD1 mid: 23.45%
PD1 -: 12.64%
TIL PD1
PD1 hi 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%
39%
31%
30%

FIG. 121

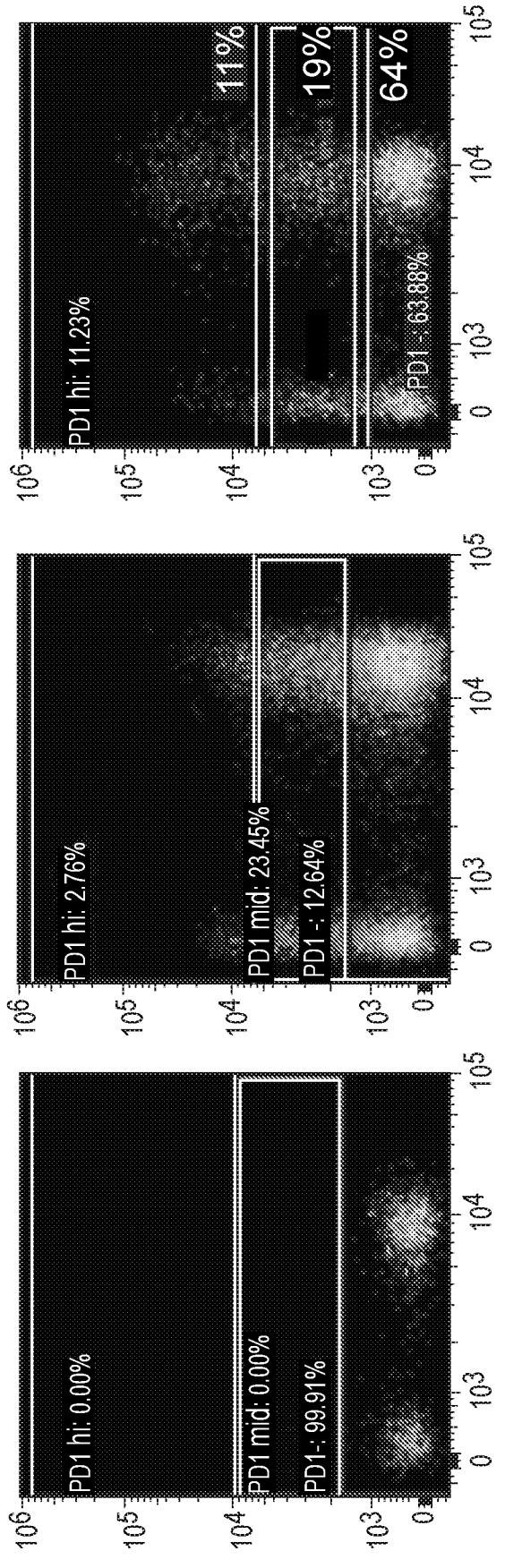

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1-: 99.91%
Feeder Pool
PD1 hi: 2.76%
PD1 mid: 23.45%
PD1-: 12.64%
TIL PD1
PD1 hi: 11.23%
11%
19%
64%
PD1-: 63.88%

FIG. 122

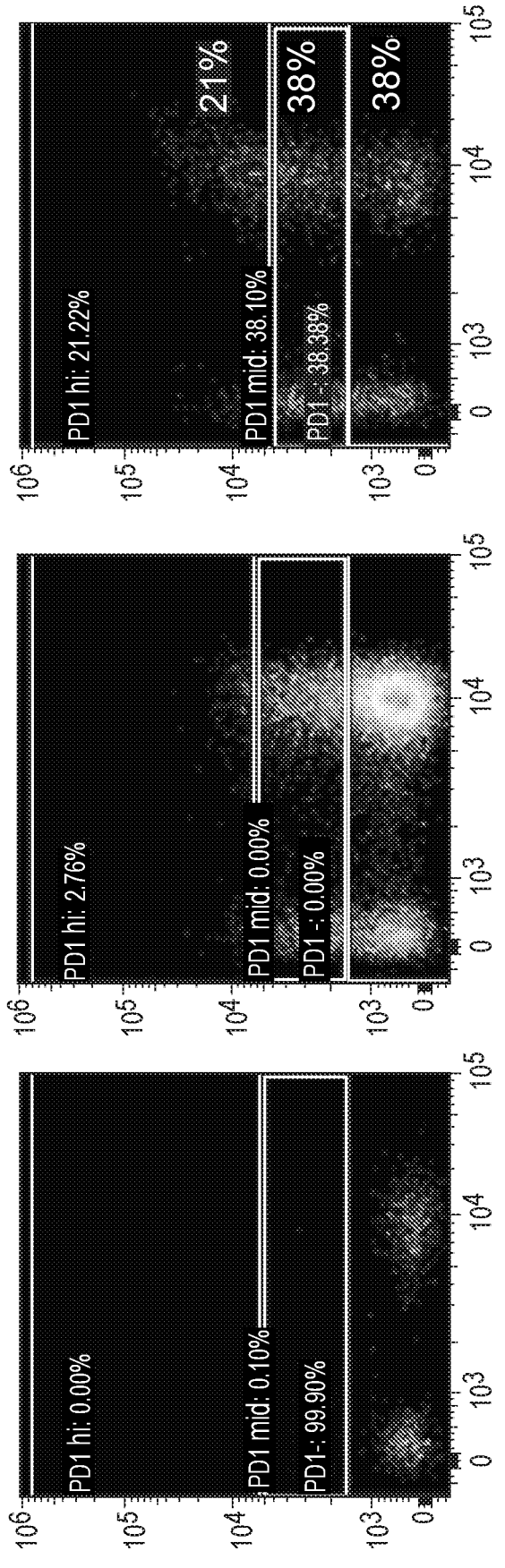

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.10%
PD1-: 99.90%
Feeder Pool
PD1 hi: 2.76%
PD1 mid: 0.00%
PD1 -: 0.00%
TIL PD1
PD1 hi: 21.22%
PD1 mid: 38.10%
PD1 -: 38.38%
21%
38%
38%

FIG. 123

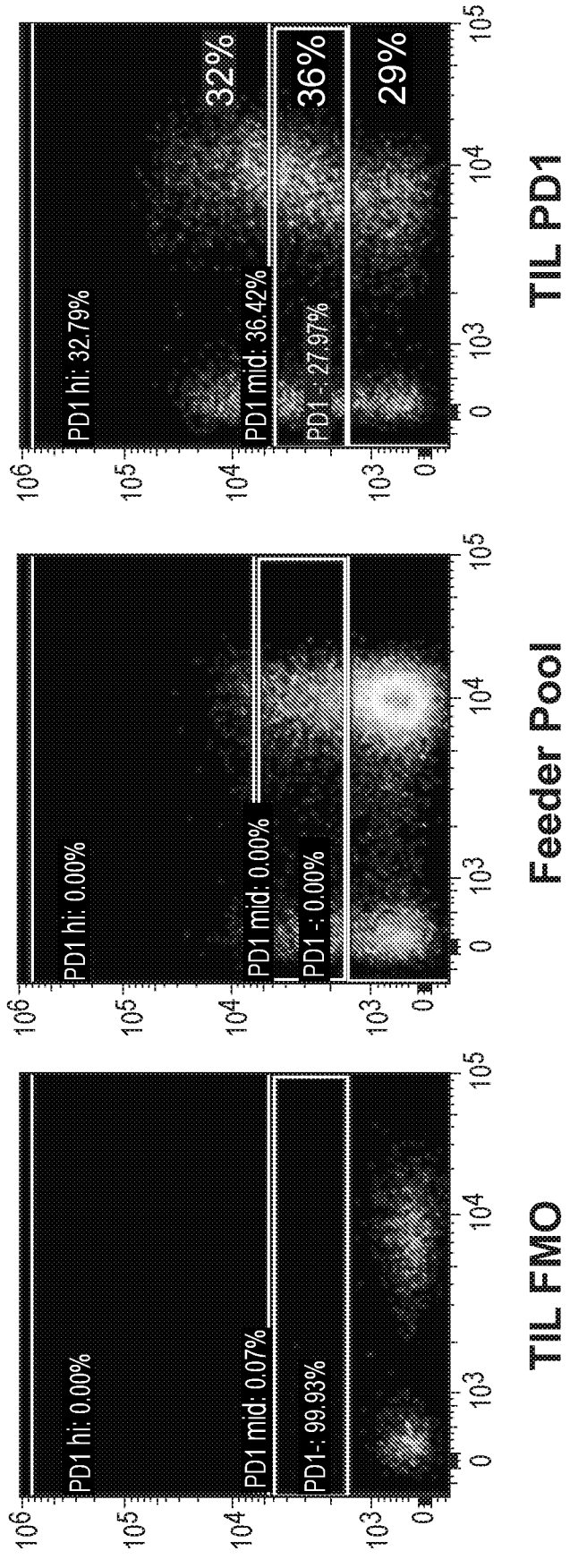

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.07%
PD1-: 99.93%
Feeder Pool
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1-: 0.00%
TIL PD1
PD1 hi: 32.79%
PD1 mid: 36.42%
PD1 -: 27.97%
32%
36%
29%

FIG. 124

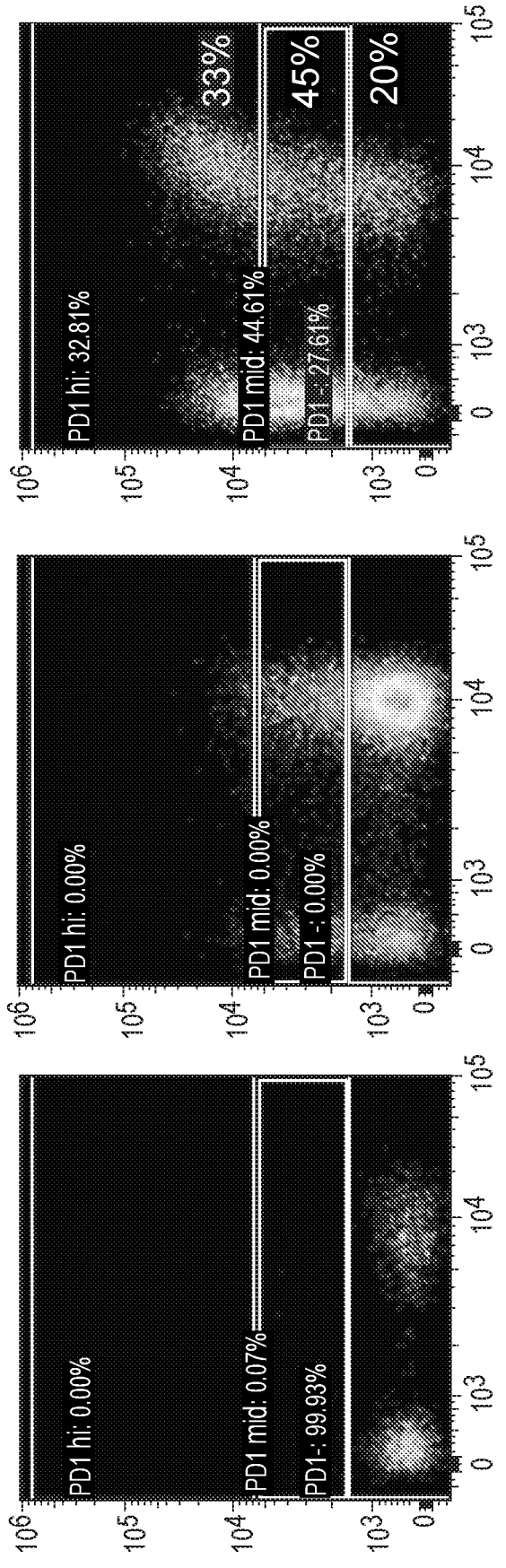

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.07%
PD1-: 99.93%
Feeder Pool
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1-: 0.00%
TIL PD1
PD1 hi: 32.81%
PD1 mid: 44.61%
PD1-: 27.61%
33%
45%
20%

FIG. 125

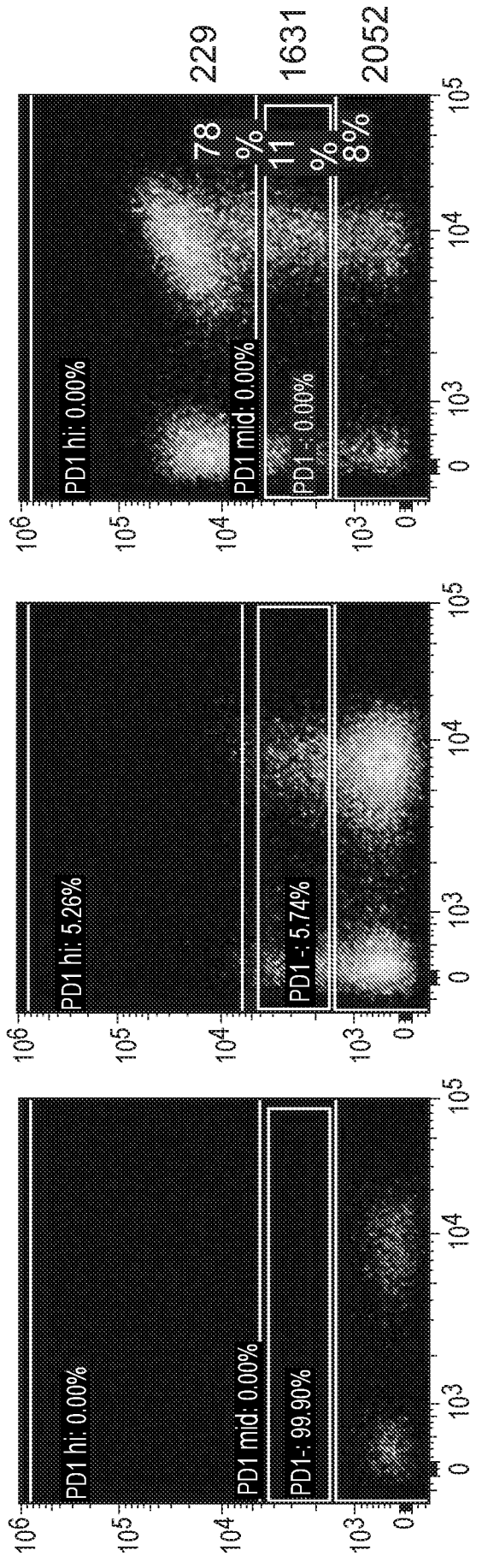

TIL FMO
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1-: 99.90%
Feeder Pool
PD1 hi: 5.26%
PD1+: 5.74%
TIL PD1
229
1631
2052
78 %
11 %
8%
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1 -: 0.00%

FIG. 126

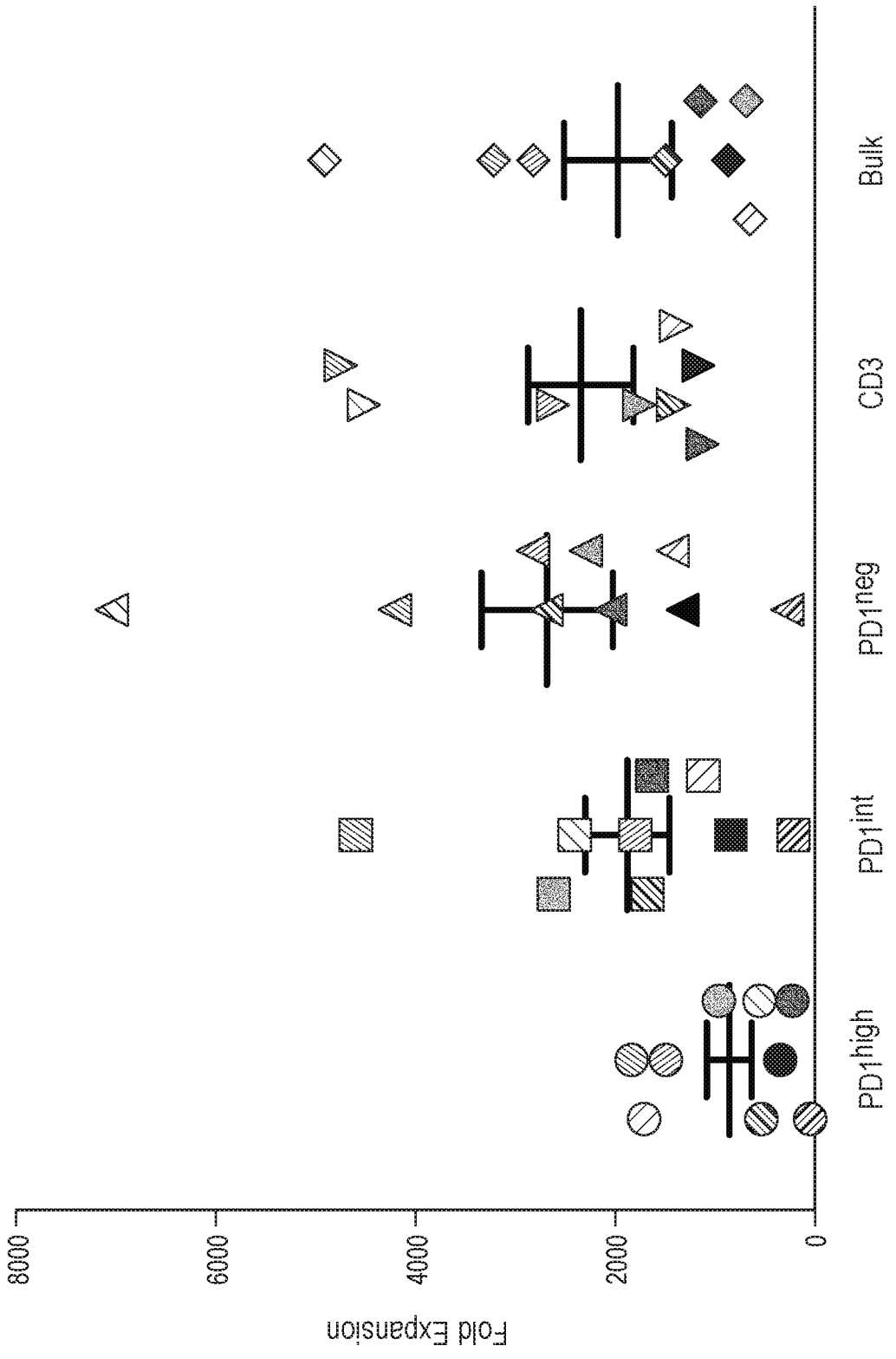
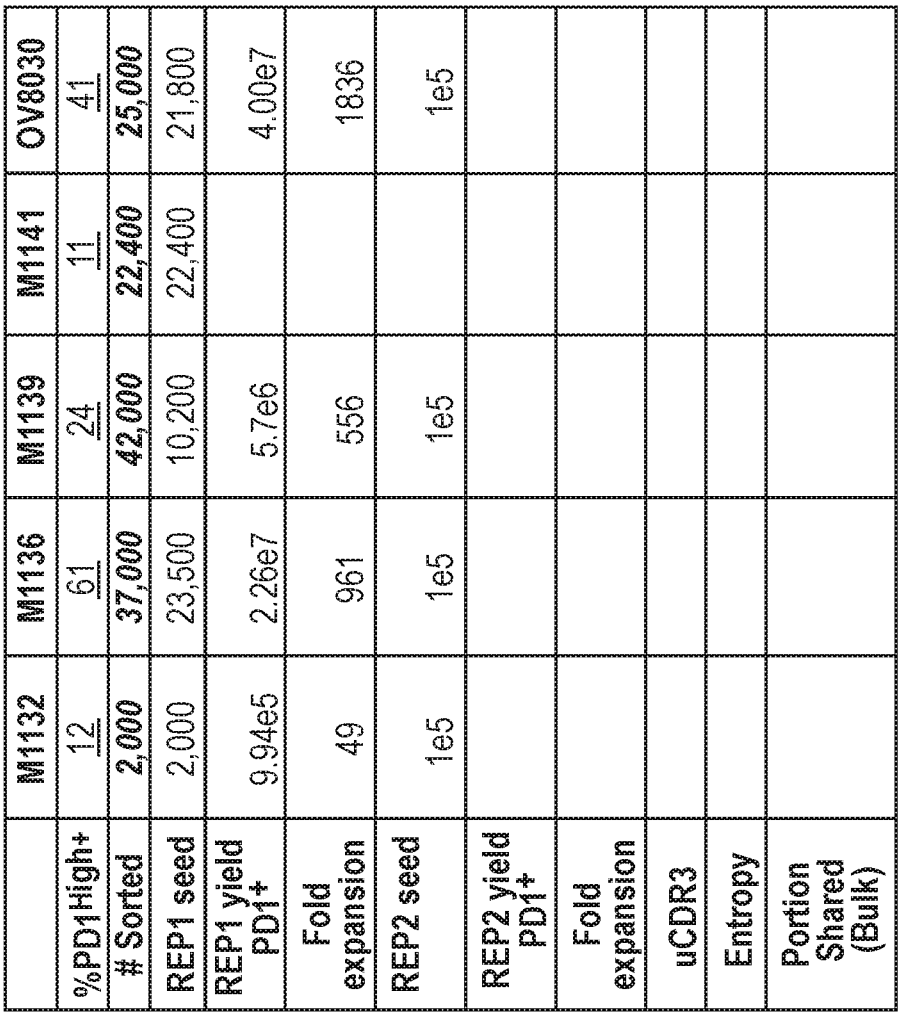

Fold Expansion
8000
6000
4000
2000
0
PD1high
PD1int
PD1neg
CD3
Bulk
REP1

FIG. 127

SUBSTITUTE SHEET (RULE 26)

	L4096	L4097	L4089	L4100	L4101	L4104	L4106	H3035	H3037	H3038
%PD1^{High+}	20%	78%	31%	43%	32%	40%	39%	28%	49%	32%
# Sorted	***10,700***	***62,500***	***20,303***	***46,000***	***99,000***	***70,000***	***20,600***	***25,000***	***25,000***	***3,000***
REP1 seed	8,000	47,000	8,184	33,000	99,000	70,000	18,200	11,000	11,300	3,000
REP1 yield PD1^{+}		1.08e7	1.22e7	1.77e7	99,000	1.2e8		3.85e6	DISCARDED	
Fold expansion		229	1,493	536		1711		350		
REP2 seed		1e5	1e5	1e5		1e5		1e5		
REP2 yield PD1^{+}										
Fold expansion										
uCDR3										
Entropy										
Portion Shared (Bulk)										

FIG. 128



	M1132	M1136	M1139	M1141	OV8030
%PD1High+	12	61	24	11	41
# Sorted	*2,000*	*37,000*	*42,000*	*22,400*	*25,000*
REP1 seed	2,000	23,500	10,200	22,400	21,800
REP1 yield PD1+	9.94e5	2.26e7	5.7e6		4.00e7
Fold expansion	49	961	556		1836
REP2 seed	1e5	1e5	1e5		1e5
REP2 yield PD1+					
Fold expansion					
uCDR3					
Entropy					
Portion Shared (Bulk)					

FIG. 129

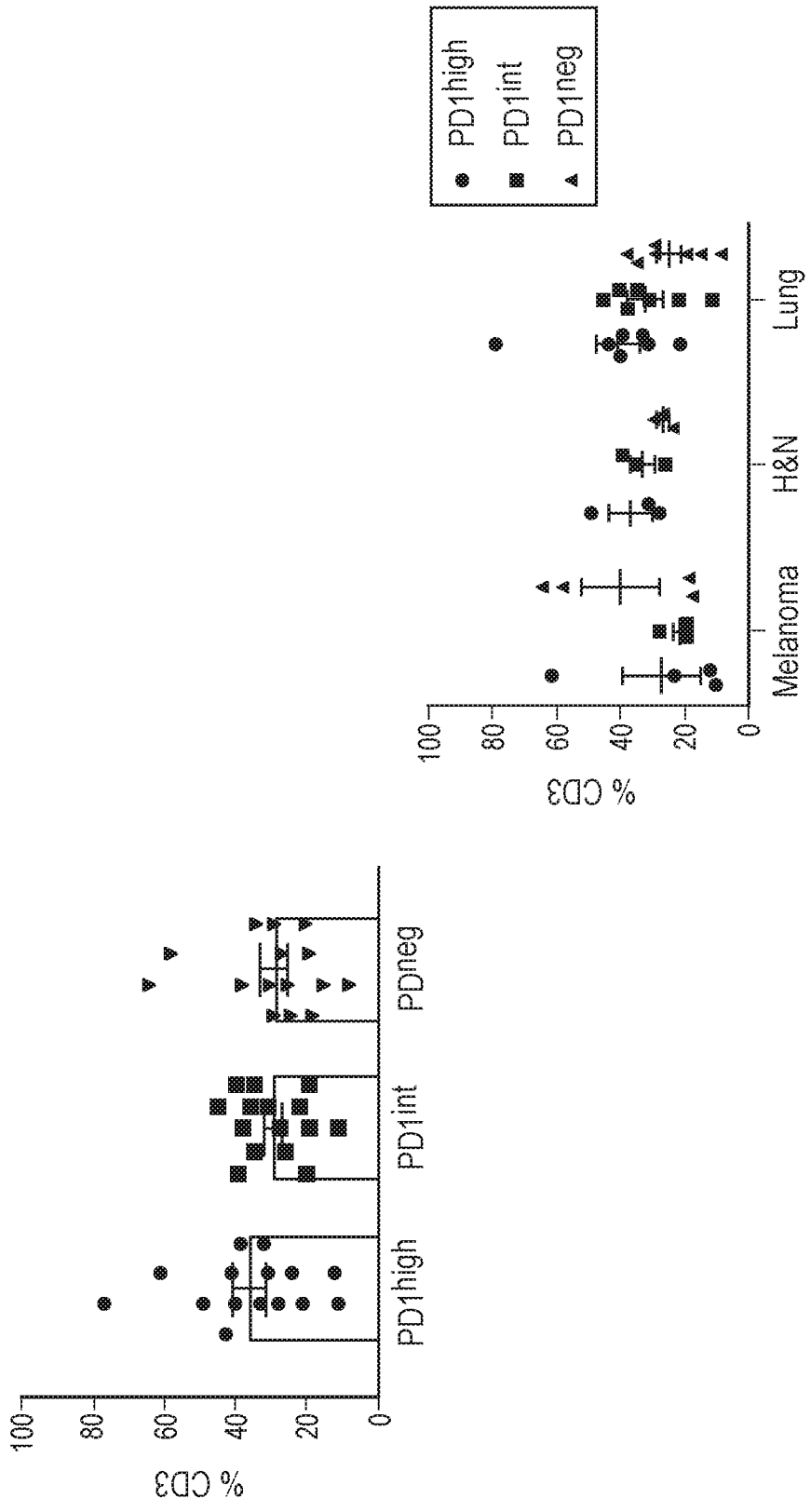

PD1high
PD1int
PD1neg
% CD3
100
80
60
40
20
0
Melanoma
H&N
Lung
% CD3
100
80
60
40
20
0
PD1high
PD1int
PDneg

FIG. 130

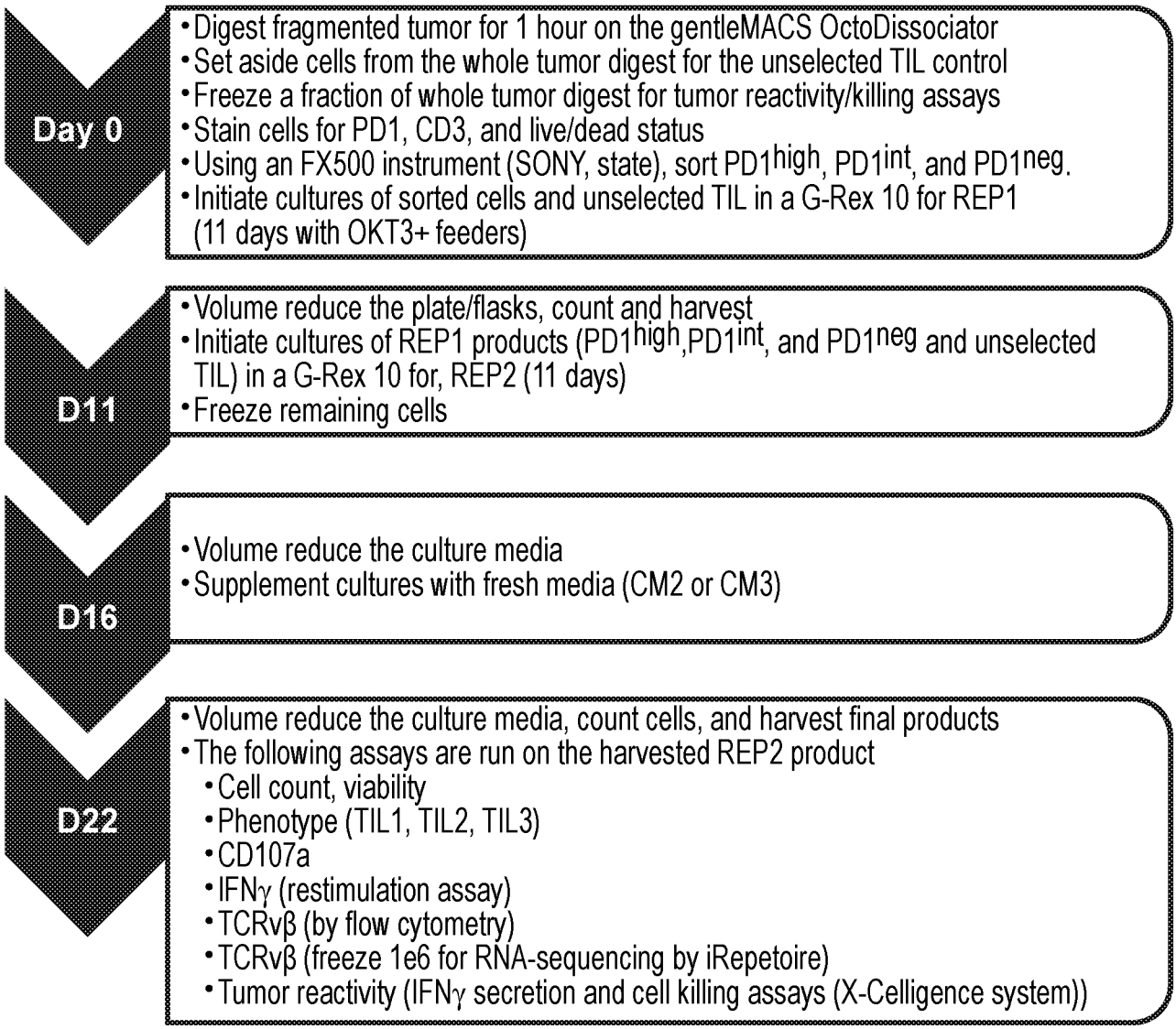

Day 0
• Digest fragmented tumor for 1 hour on the gentleMACS OctoDissociator
• Set aside cells from the whole tumor digest for the unselected TIL control
• Freeze a fraction of whole tumor digest for tumor reactivity/killing assays
• Stain cells for PD1, CD3, and live/dead status
• Using an FX500 instrument (SONY, state), sort PD1high, PD1int, and PD1neg.
• Initiate cultures of sorted cells and unselected TIL in a G-Rex 10 for REP1 (11 days with OKT3+ feeders)
D11
• Volume reduce the plate/flasks, count and harvest
• Initiate cultures of REP1 products (PD1high,PD1int, and PD1neg and unselected TIL) in a G-Rex 10 for, REP2 (11 days)
• Freeze remaining cells
D16
• Volume reduce the culture media
• Supplement cultures with fresh media (CM2 or CM3)
D22
• Volume reduce the culture media, count cells, and harvest final products
• The following assays are run on the harvested REP2 product
• Cell count, viability
• Phenotype (TIL1, TIL2, TIL3)
• CD107a
• IFNγ (restimulation assay)
• TCRvβ (by flow cytometry)
• TCRvβ (freeze 1e6 for RNA-sequencing by iRepetoire)
• Tumor reactivity (IFNγ secretion and cell killing assays (X-Celligence system))

FIG. 131

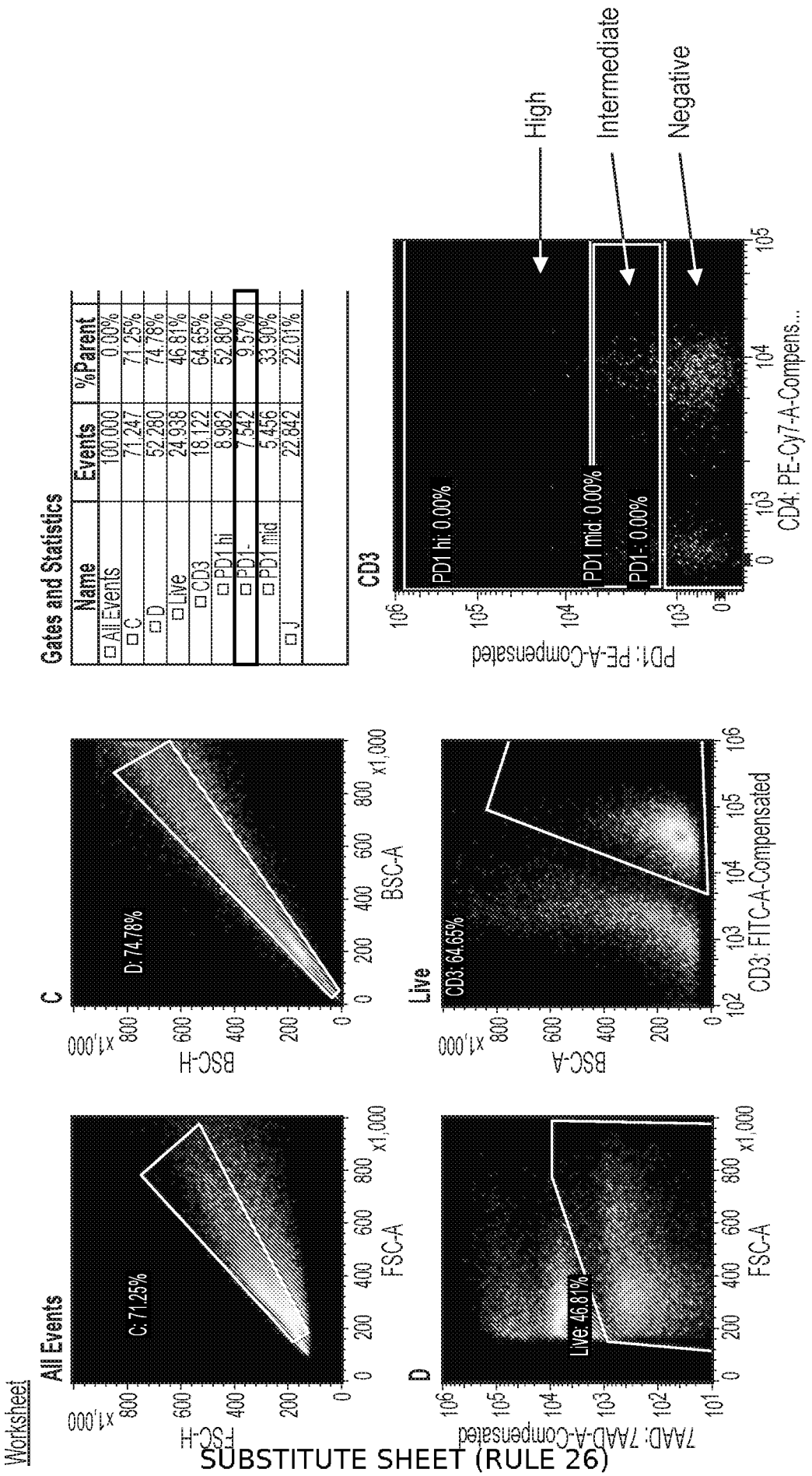

Worksheet
All Events
C: 71.25%
FSC-H
FSC-A
x1,000
C
D: 74.78%
BSC-H
BSC-A
D
Live: 46.81%
7AAD: 7AAD-A-Compensated
Live
CD3: 64.65%
BSC-A
CD3: FITC-A-Compensated
Gates and Statistics
Name | Events | %Parent
All Events | 100,000 | 0.00%
C | 71,247 | 71.25%
D | 52,260 | 74.78%
Live | 24,938 | 46.81%
CD3 | 18,122 | 64.65%
PD1 hi | 8,992 | 52.80%
PD1- | 7,542 | 9.57%
PD1 mid | 5,456 | 33.90%
J | 22,842 | 22.01%
CD3
PD1 hi: 0.00%
PD1 mid: 0.00%
PD1-: 0.00%
High
Intermediate
Negative
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compens...

FIG. 132

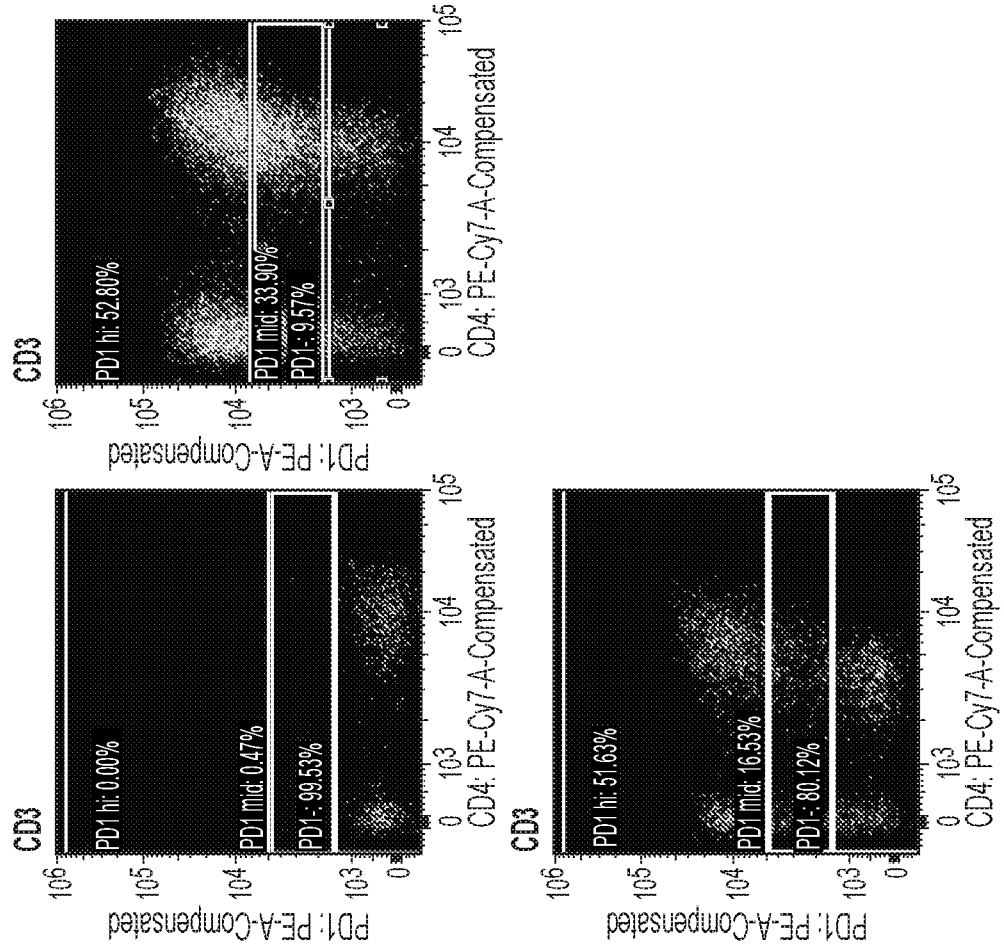
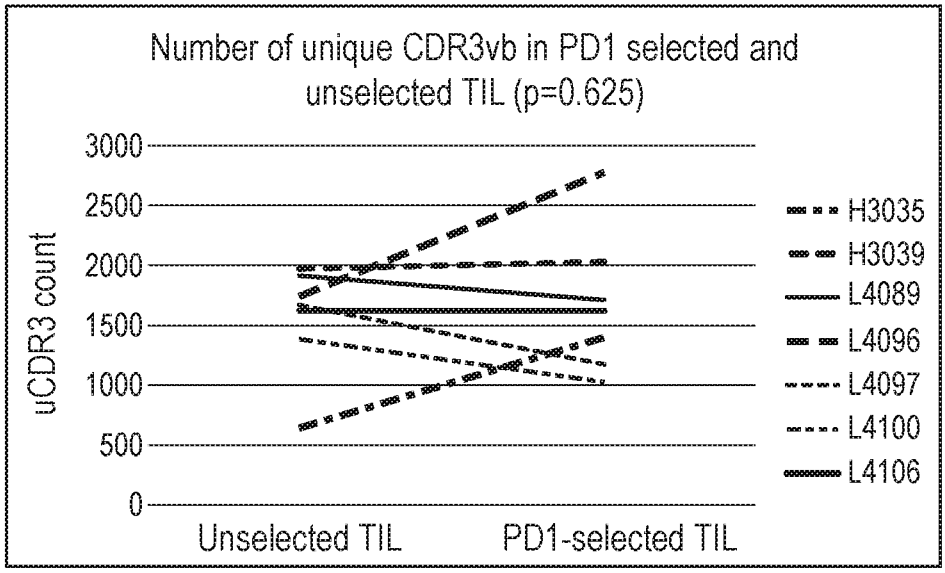

CD3
PD1 hi: 52.80%
PD1 mid: 33.90%
PD1-: 9.57%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated
CD3
PD1 hi: 0.00%
PD1 mid: 0.47%
PD1-: 99.53%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated
CD3
PD1 hi: 51.63%
PD1 mid: 16.53%
PD1-: 80.12%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated

FIG. 133

A.


Number of unique CDR3vb in PD1 selected and unselected TIL (p=0.625)
uCDR3 count
3000
2500
2000
1500
1000
500
0
Unselected TIL
PD1-selected TIL
H3035
H3039
L4089
L4096
L4097
L4100
L4106

B.

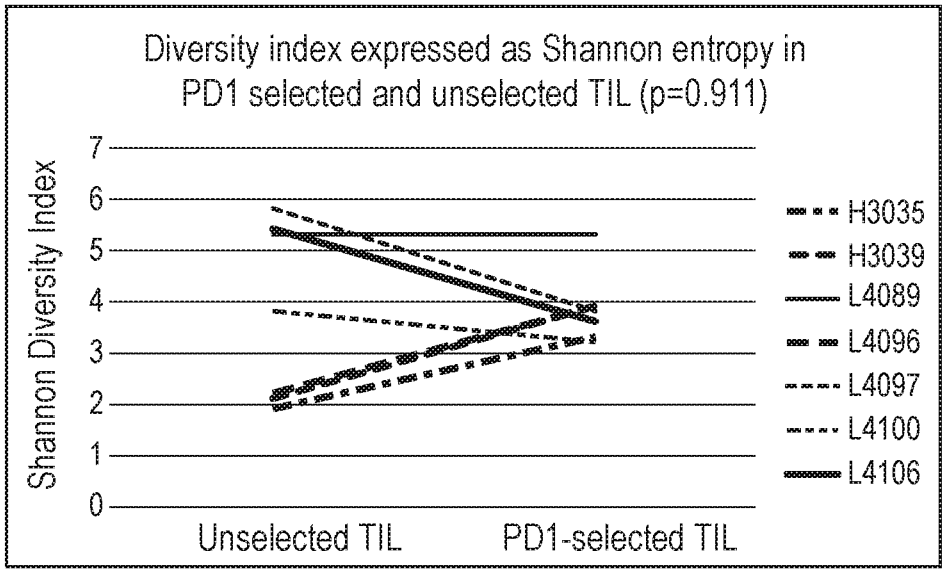

Diversity index expressed as Shannon entropy in PD1 selected and unselected TIL (p=0.911)
Shannon Diversity Index
7
6
5
4
3
2
1
0
Unselected TIL
PD1-selected TIL
H3035
H3039
L4089
L4096
L4097
L4100
L4106

FIG. 134

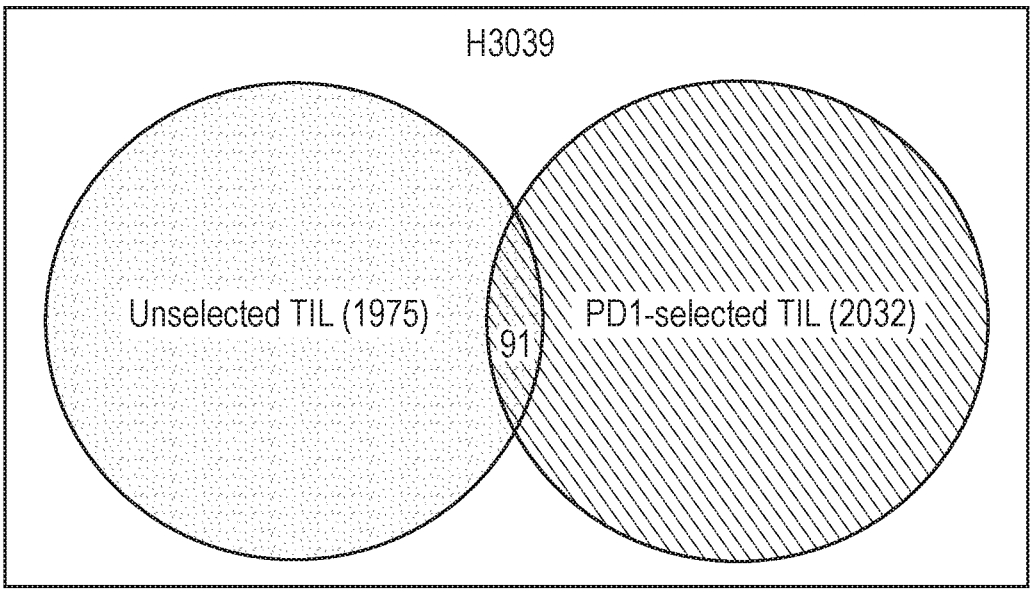


FIG. 135A

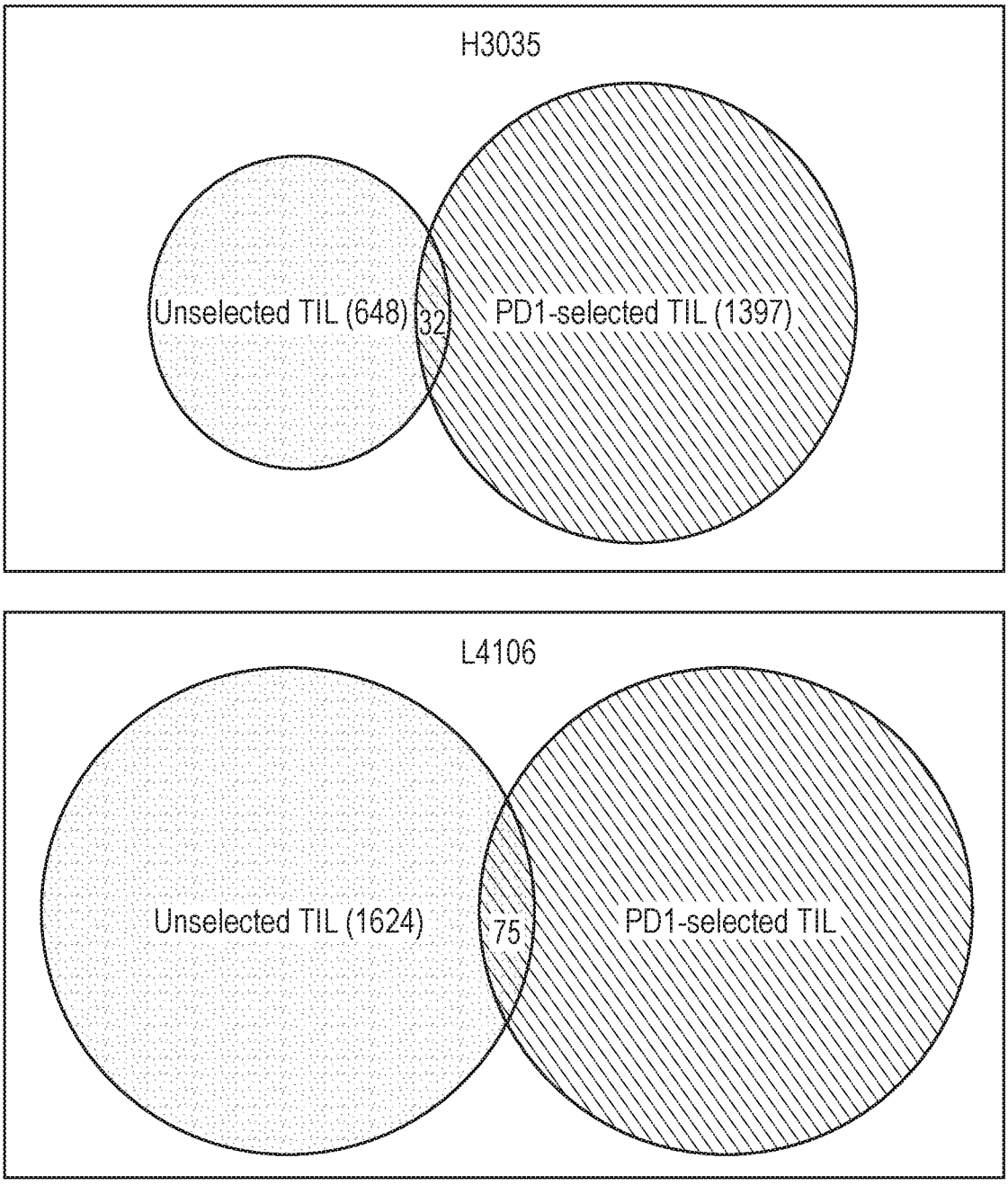

H3035
Unselected TIL (648)
32
PD1-selected TIL (1397)
L4106
Unselected TIL (1624)
75
PD1-selected TIL

FIG. 135B

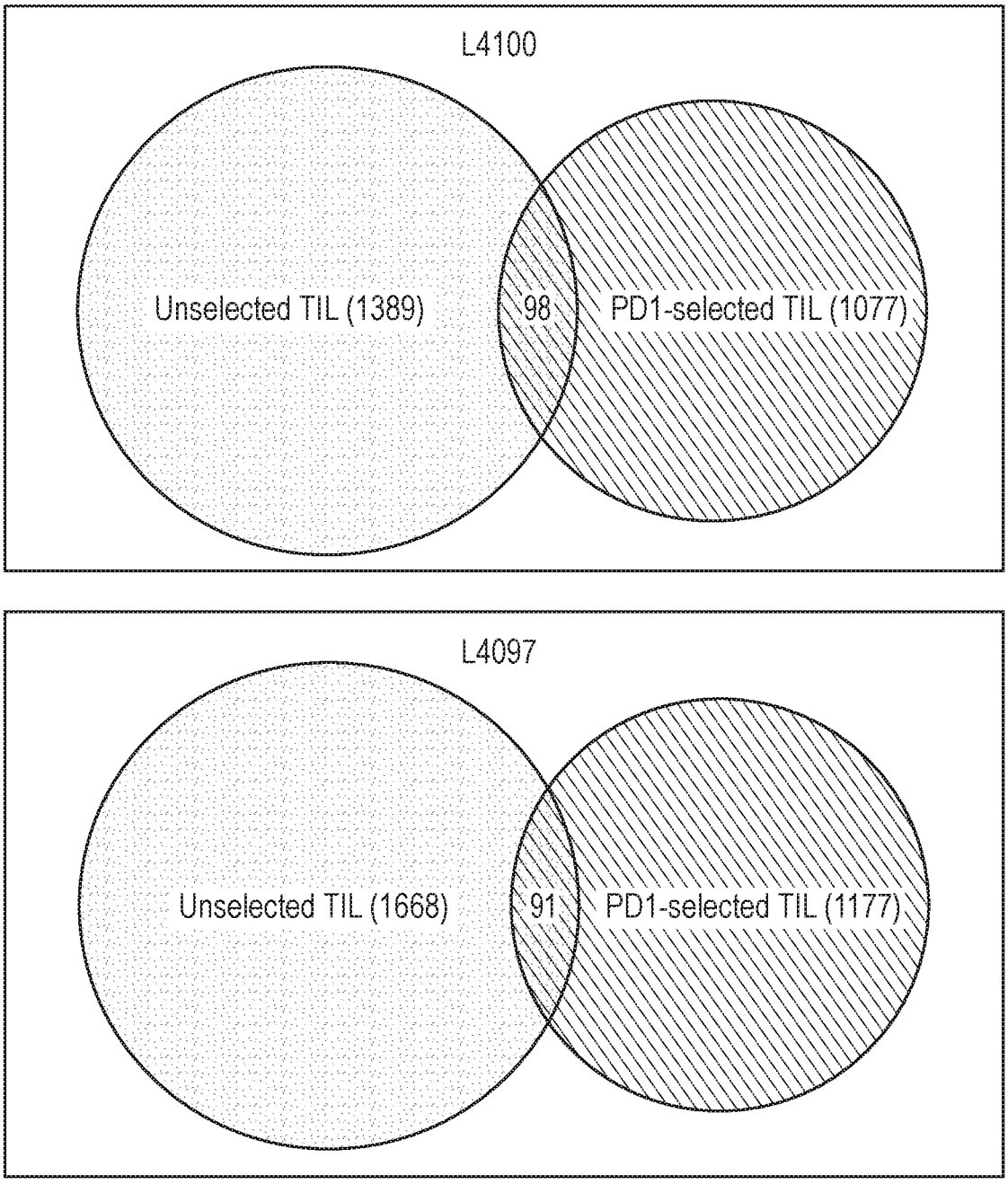

L4100
Unselected TIL (1389)
98
PD1-selected TIL (1077)
L4097
Unselected TIL (1668)
91
PD1-selected TIL (1177)

FIG. 135C

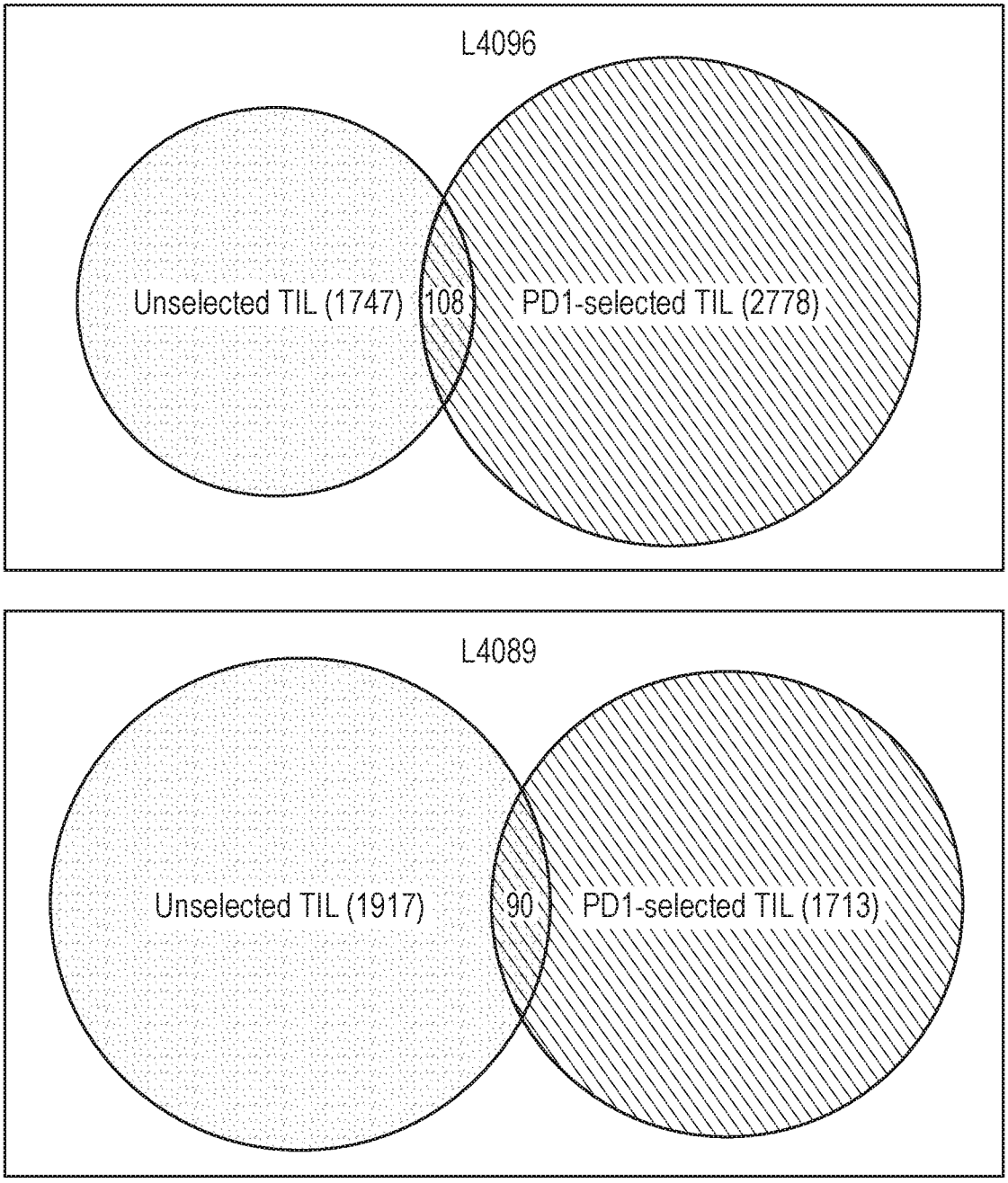

L4096
Unselected TIL (1747)
108
PD1-selected TIL (2778)
L4089
Unselected TIL (1917)
90
PD1-selected TIL (1713)

FIG. 135D

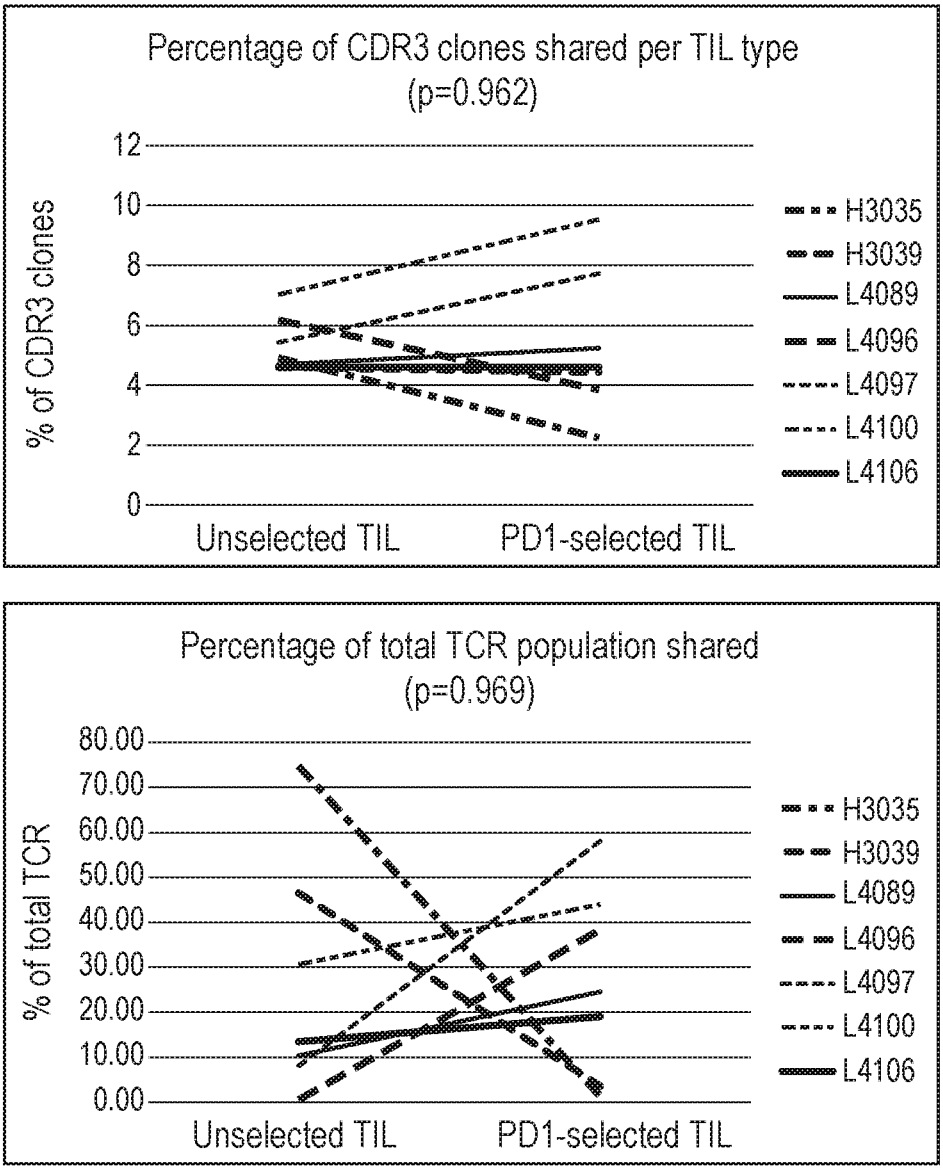

Percentage of CDR3 clones shared per TIL type
(p=0.962)
% of CDR3 clones
12
10
8
6
4
2
0
Unselected TIL
PD1-selected TIL
H3035
H3039
L4089
L4096
L4097
L4100
L4106
Percentage of total TCR population shared
(p=0.969)
% of total TCR
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Unselected TIL
PD1-selected TIL
H3035
H3039
L4089
L4096
L4097
L4100
L4106

FIG. 135E

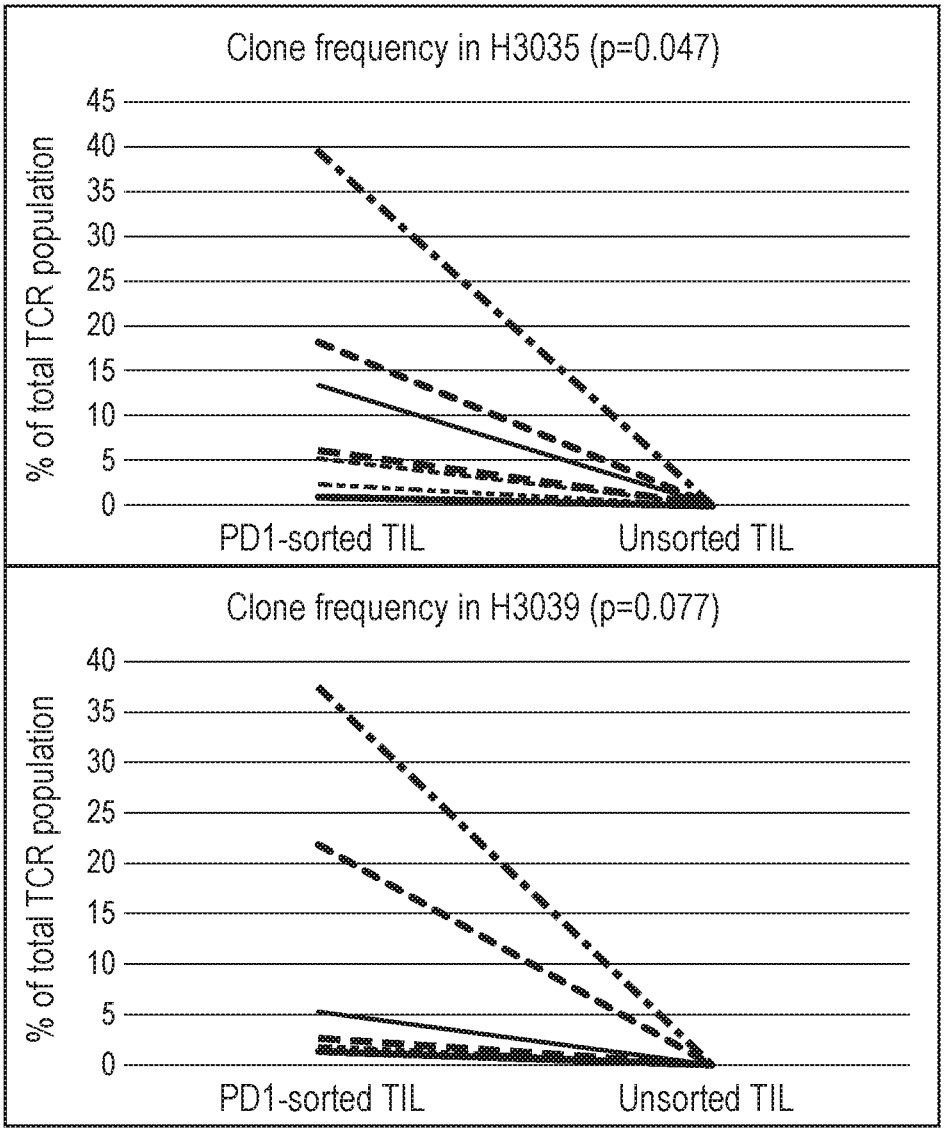

Clone frequency in H3035 (p=0.047)
% of total TCR population
45
40
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL
Clone frequency in H3039 (p=0.077)
% of total TCR population
40
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL

FIG. 136A

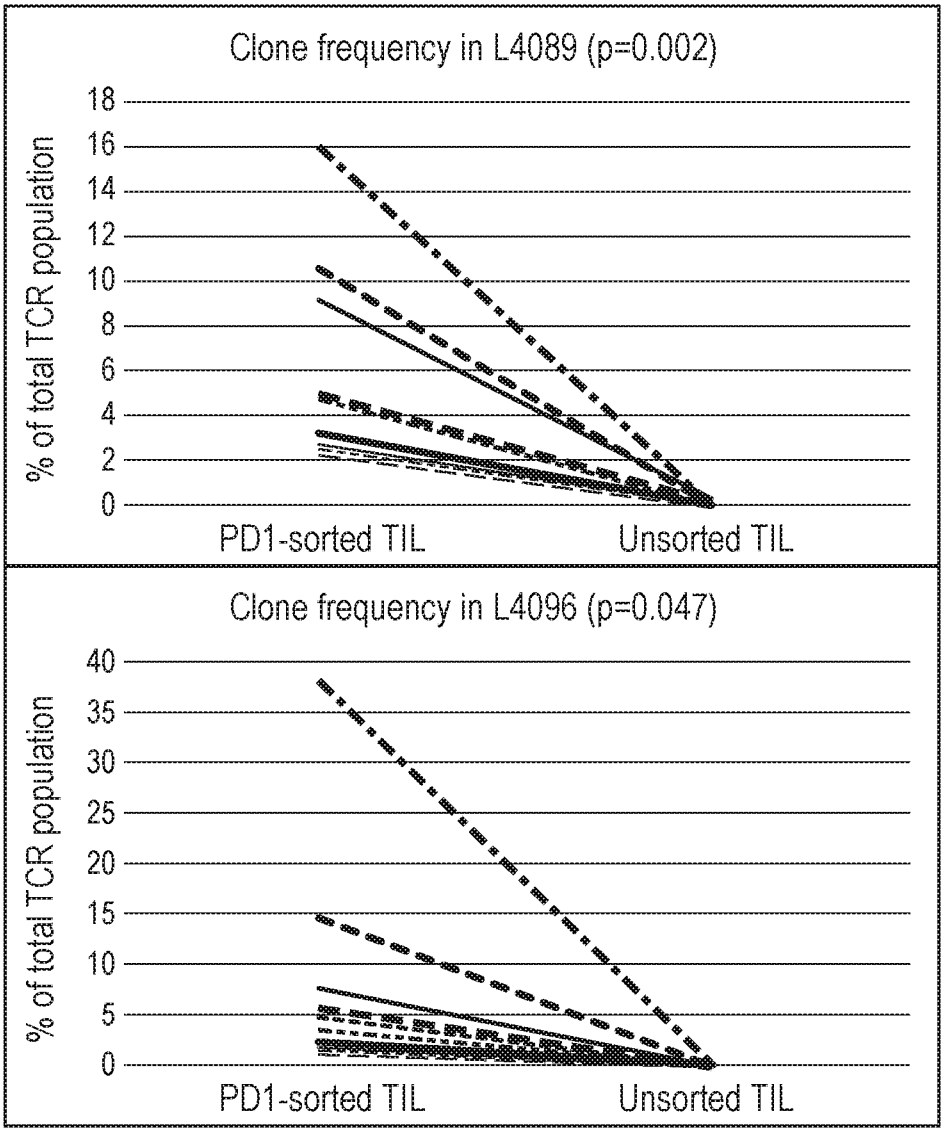

Clone frequency in L4089 (p=0.002)
% of total TCR population
18
16
14
12
10
8
6
4
2
0
PD1-sorted TIL
Unsorted TIL
Clone frequency in L4096 (p=0.047)
% of total TCR population
40
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL

FIG. 136B

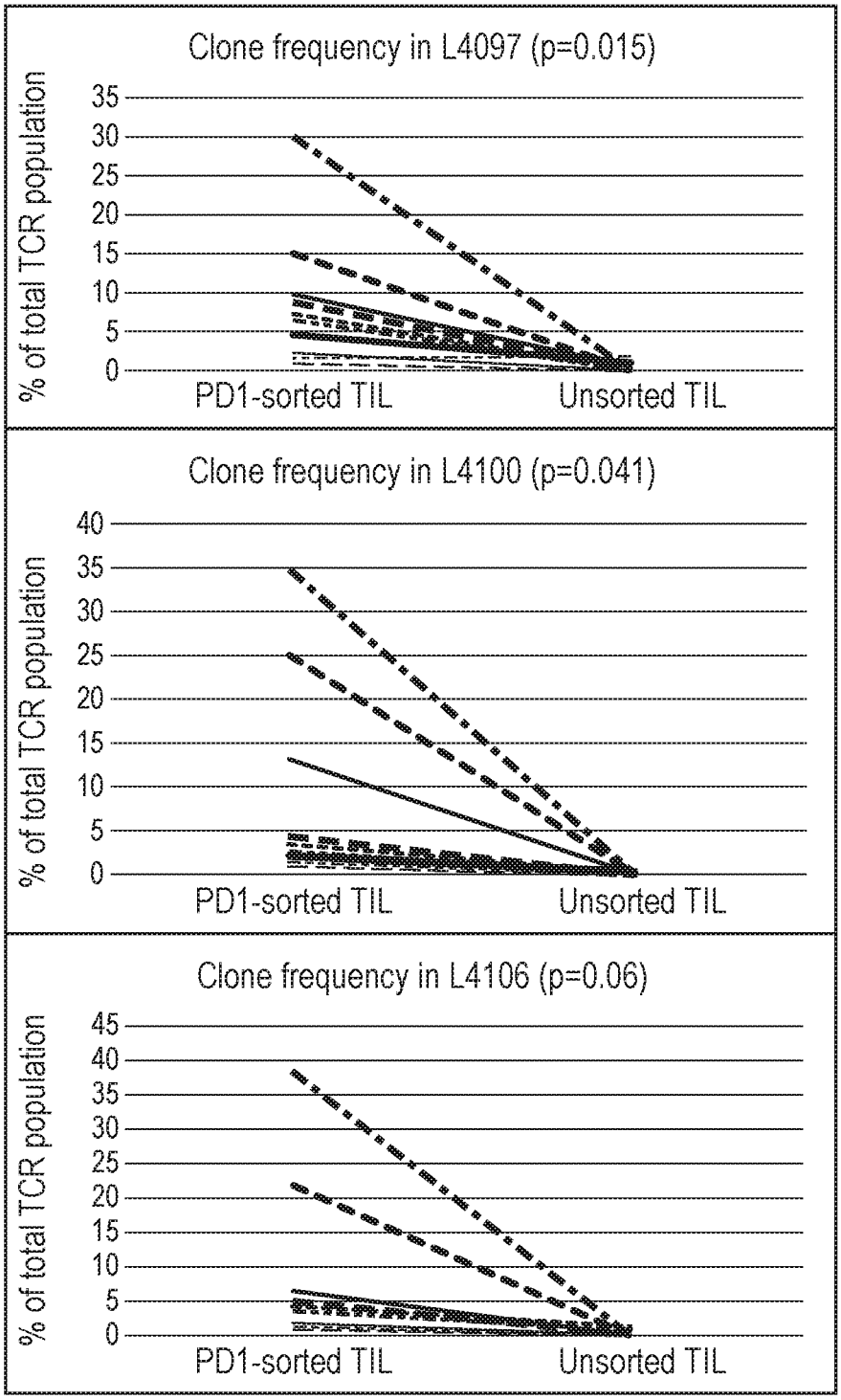

Clone frequency in L4097 (p=0.015)
% of total TCR population
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL
Clone frequency in L4100 (p=0.041)
% of total TCR population
40
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL
Clone frequency in L4106 (p=0.06)
% of total TCR population
45
40
35
30
25
20
15
10
5
0
PD1-sorted TIL
Unsorted TIL

FIG. 136C

Tumor ID	Histology	PD1-selected TIL generated
H3035	HNSCC	Yes
H3036	HNSCC	No Culture Contaminated
H3037	HNSCC	No Culture Contaminated
H3038	HNSCC	Yes
H3039	HNSCC	Yes
L4089	NSCLC	Yes
L4096	NSCLC	Yes
L4097	NSCLC	Yes
L4100	NSCLC	Yes
L4101	NSCLC	Yes
L4104	NSCLC	Yes
L4106	NSCLC	Yes
M1132	Melanoma	Yes
M1136	Melanoma	Yes
M1139	Melanoma	Yes
M1141	Melanoma	Yes
OV8030	Ovarian	Yes
OV8042	Ovarian	Yes
OV8042	Ovarian	Yes
T6049	TNBC	Yes
T6056	TNBC	Yes
T6058	TNBC	Yes
T6060	TNBC	Yes
OC20019	Prostate	Yes
OC20030	Prostate	Yes
C10026	CRC	No Poor digest cell yield
CC10027	CRC	No Poor digest cell yield
CC10028	CRC	Yes
CC10029	CRC	Yes
CC10031	CRC	No Culture Contaminated
CC10034	CRC	Yes
CC10037	CRC	Yes
CC10039	CRC	Yes

FIG. 137

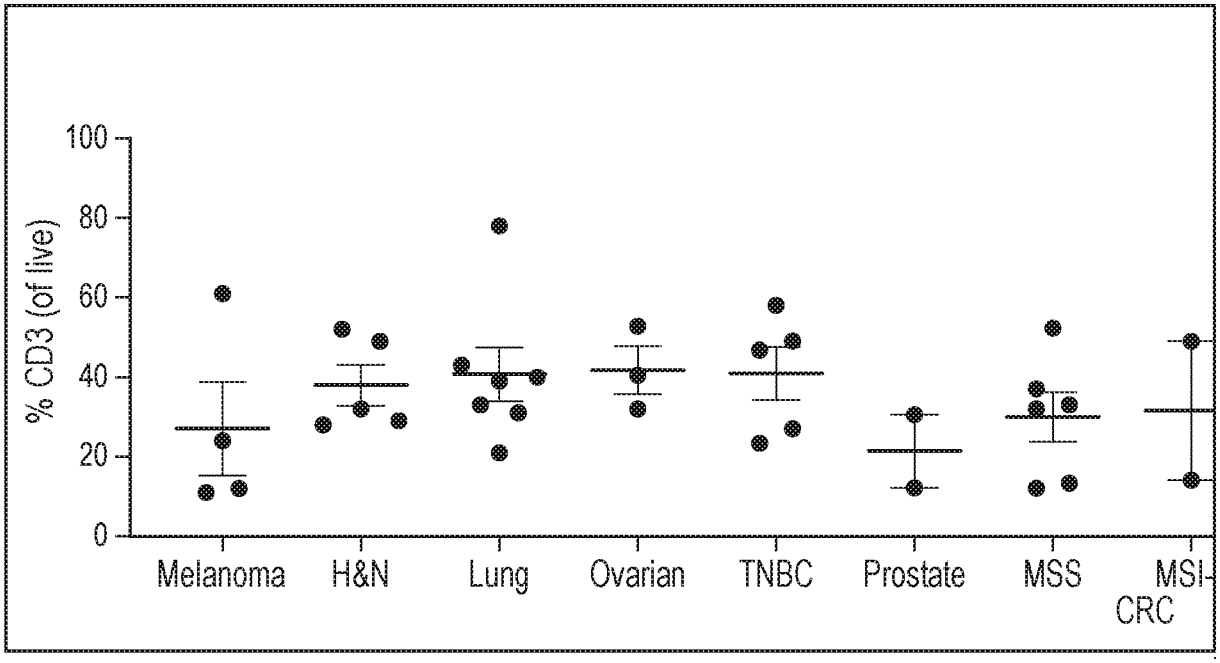

% CD3 (of live)
100
80
60
40
20
0
Melanoma
H&N
Lung
Ovarian
TNBC
Prostate
MSS
MSI-CRC

FIG. 138

Tumor ID	Sample ID	Histology	Date of preparation
H3035	PD1-selected TIL	HNSCC	03/15/19
	Unselected TIL		
H3039	PD1-selected TIL	HNSCC	04/09/19
	Unselected TIL		
L4089	PD1-selected TIL	NSCLC	03/22/19
	Unselected TIL		
L4096	PD1-selected TIL	NSCLC	04/04/19
	Unselected TIL		
L4097	PD1-selected TIL	NSCLC	03/22/19
	Unselected TIL		
L4100	PD1-selected TIL	NSCLC	03/26/19
	Unselected TIL		
L4101	PD1-selected	NSCLC	04/04/19
	Unselected TIL		
L4104	PD1-selected	NSCLC	03/26/19
	Unselected TIL		
L4106	PD1-selected	NSCLC	03/29/19
	Unselected TIL		
M1132	PD1-selected	Melanoma	03/26/19
	Unselected TIL		
M1136	PD1-selected	Melanoma	03/26/19
	Unselected TIL		
M1139	PD1-selected	Melanoma	03/22/19
	Unselected TIL		
M1141	PD1-selected	Melanoma	03/29/19
	Unselected TIL		

FIG. 139

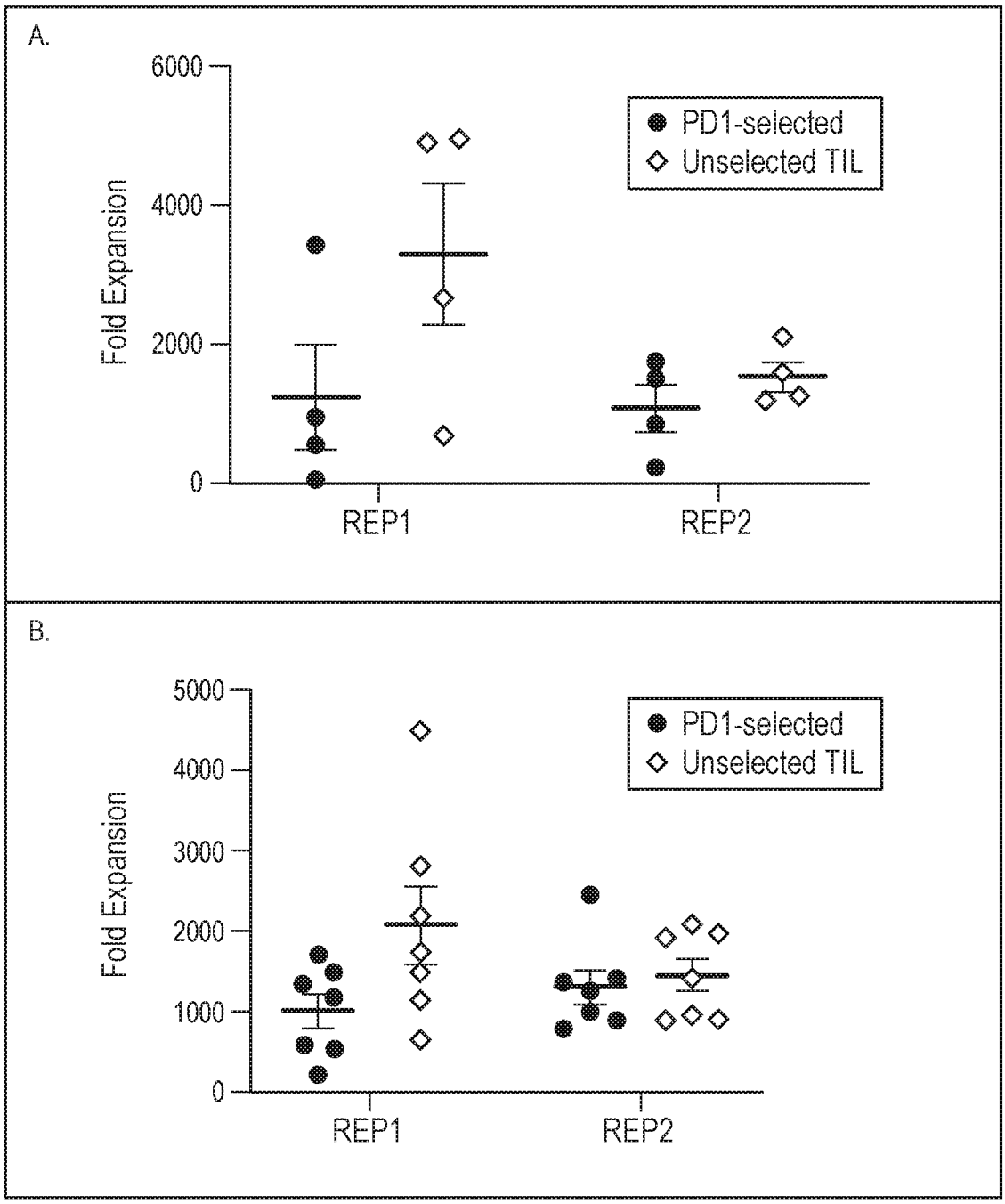

A.
6000
4000
2000
0
Fold Expansion
PD1-selected
Unselected TIL
REP1
REP2
B.
5000
4000
3000
2000
1000
0
Fold Expansion
PD1-selected
Unselected TIL
REP1
REP2

FIG. 140

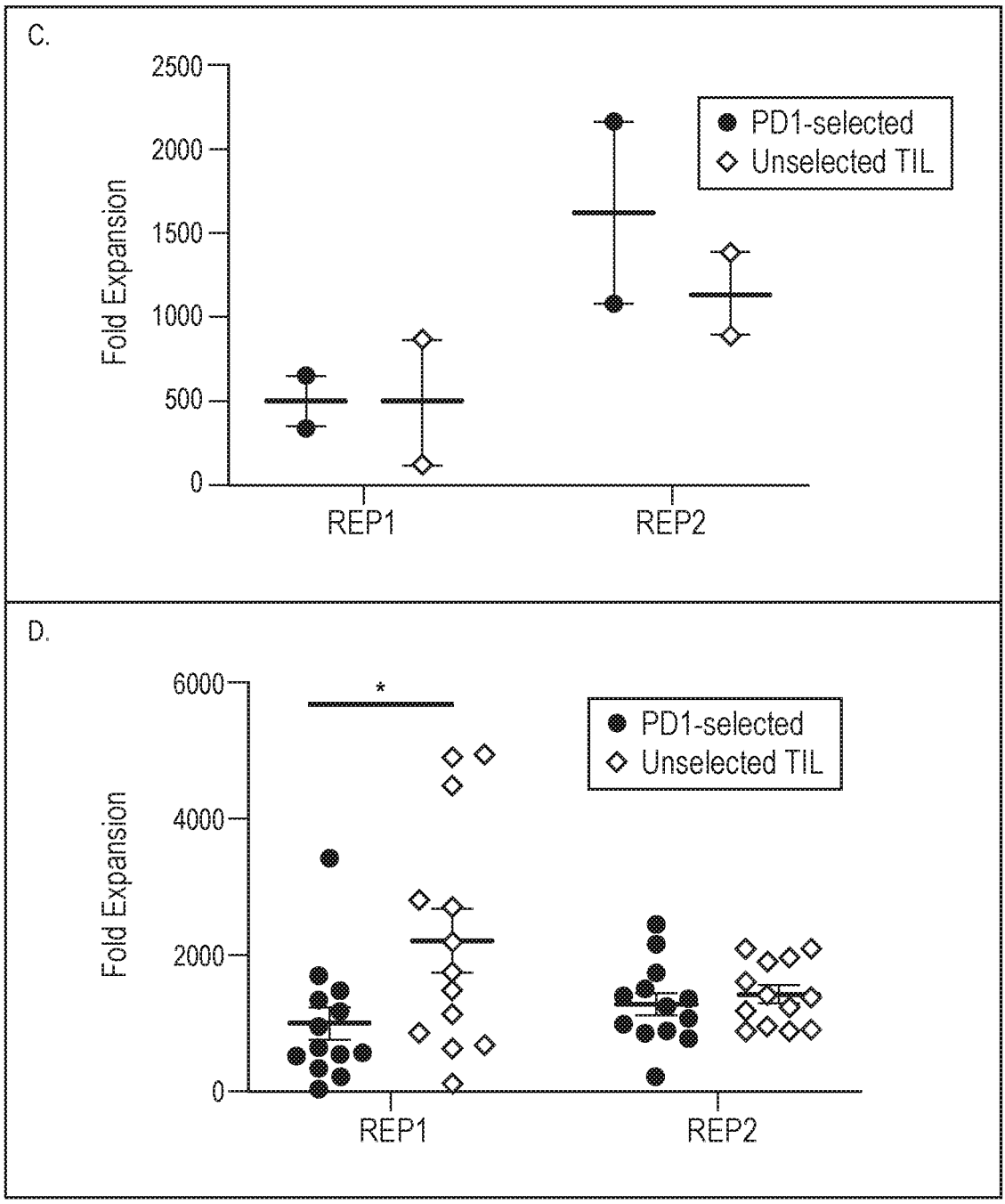

C.
2500
2000
1500
1000
500
0
Fold Expansion
PD1-selected
Unselected TIL
REP1
REP2
D.
6000
4000
2000
0
Fold Expansion
*
PD1-selected
Unselected TIL
REP1
REP2

FIG. 140

Tumor ID	Sample ID	Histology	REP1 Fold Expansion	REP2 Fold Expansion
H3035	PD1-selected TIL	HNSCC	350	2166
	Unselected TIL		869	1385
H3039	PD1-selected TIL	HNSCC	654	1082
	Unselected TIL		123	893
L4089	PD1-selected TIL	NSCLC	1502	2106
	Unselected TIL		2821	1416
L4096	PD1-selected TIL	NSCLC	582	788
	Unselected TIL		1749	919
L4097	PD1-selected TIL	NSCLC	230	1982
	Unselected TIL		1147	2455
L4100	PD1-selected TIL	NSCLC	536	997
	Unselected TIL		1498	900
L4101	PD1-selected	NSCLC	1346	896
	Unselected TIL		2196	962
L4104	PD1-selected	NSCLC	1711	1257
	Unselected TIL		649	1421
L4106	PD1-selected	NSCLC	1179	1367
	Unselected TIL		4500	1912
M1132	PD1-selected	Melanoma	50	234
	Unselected TIL		2670	1263
M1136	PD1-selected	Melanoma	961	867
	Unselected TIL		693	1190
M1139	PD1-selected	Melanoma	557	1751
	Unselected TIL		4908	1613
M1141	PD1-selected	Melanoma	3429	1515
	Unselected TIL		4950	2107

FIG. 141

Tumor ID	Sample ID	Histology	REP1 Fold Expansion	REP2 Fold Expansion
H3035	PD1-selected TIL	HNSCC	350	2166
	Unselected TIL		869	1385
H3039	PD1-selected TIL	HNSCC	654	1082
	Unselected TIL		123	893
L4089	PD1-selected TIL	NSCLC	1502	2106
	Unselected TIL		2821	1416
L4096	PD1-selected TIL	NSCLC	582	788
	Unselected TIL		1749	919
L4097	PD1-selected TIL	NSCLC	230	1982
	Unselected TIL		1147	2455
L4100	PD1-selected TIL	NSCLC	536	997
	Unselected TIL		1498	900
L4101	PD1-selected	NSCLC	1346	896
	Unselected TIL		2196	962
L4104	PD1-selected	NSCLC	1711	1257
	Unselected TIL		649	1421
L4106	PD1-selected	NSCLC	1179	1367
	Unselected TIL		4500	1912
M1132	PD1-selected	Melanoma	50	234
	Unselected TIL		2670	1263
M1136	PD1-selected	Melanoma	961	867
	Unselected TIL		693	1190
M1139	PD1-selected	Melanoma	557	1751
	Unselected TIL		4908	1613
M1141	PD1-selected	Melanoma	3429	1515
	Unselected TIL		4950	2107

FIG. 142

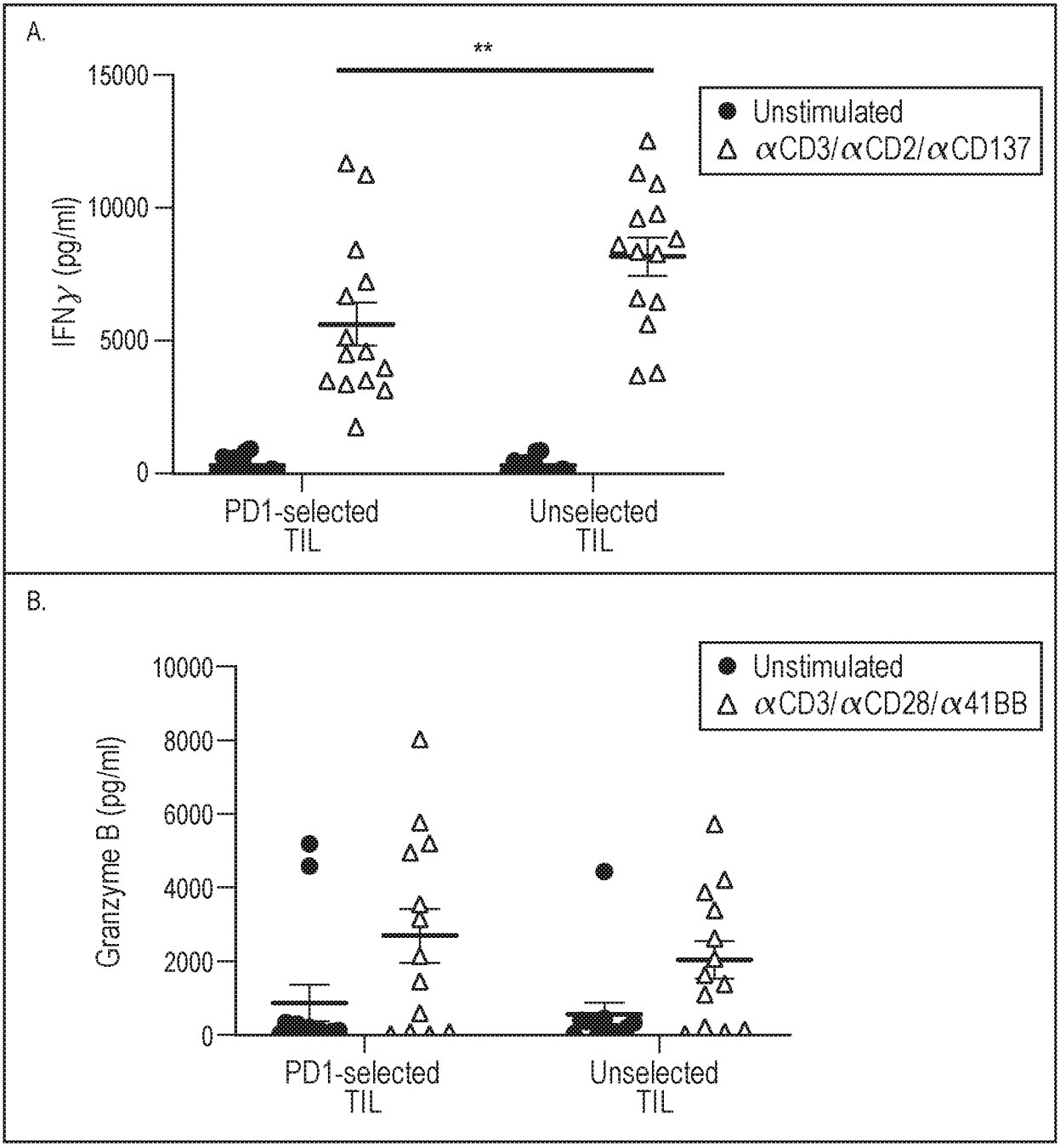

A.
**
15000
10000
5000
0
IFNγ (pg/ml)
Unstimulated
αCD3/αCD2/αCD137
PD1-selected TIL
Unselected TIL
B.
10000
8000
6000
4000
2000
0
Granzyme B (pg/ml)
Unstimulated
αCD3/αCD28/α41BB
PD1-selected TIL
Unselected TIL

FIG. 143

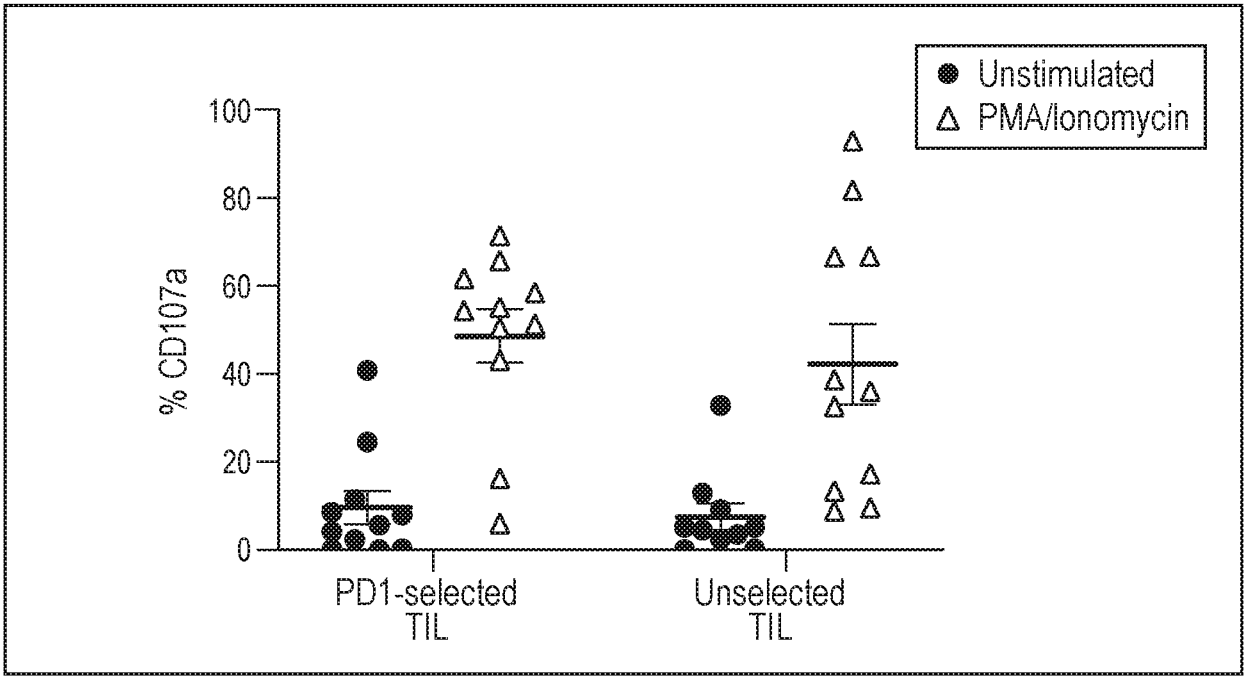

Unstimulated
PMA/Ionomycin
% CD107a
100
80
60
40
20
0
PD1-selected TIL
Unselected TIL

FIG. 144

Tumor ID	Sample ID	Histology	Date of Preparation	REP1 Fold Expansion	REP2 Fold Expansion
H3035	PD1-selected TIL	HNSCC	03/15/19	350	2166
	Unselected TIL			869	1385
H3039	PD1-selected TIL	HNSCC	04/09/19	654	1082
	Unselected TIL			123	893
L4089	PD1-selected TIL	NSCLC	03/22/19	1502	2106
	Unselected TIL			2821	1416
L4096	PD1-selected TIL	NSCLC	04/04/19	582	788
	Unselected TIL			1749	919
L4097	PD1-selected TIL	NSCLC	03/22/19	230	1982
	Unselected TIL			1147	2455
L4100	PD1-selected TIL	NSCLC	03/26/19	536	997
	Unselected TIL			1498	900
L4101	PD1-selected	NSCLC	04/04/19	1346	896
	Unselected TIL			2196	962100
L4104	PD1-selected	NSCLC			
	Unselected TIL				
L4106	PD1-selected	NSCLC	03/29/19	1179	1367
	Unselected TIL			4500	1912
M1132	PD1-selected	Melanoma	03/26/19	50	234
	Unselected TIL			2670	1263
M1136	PD1-selected	Melanoma	03/26/19	961	867
	Unselected TIL			693	1190
M1139	PD1-selected	Melanoma	03/22/19	557	1751
	Unselected TIL			4908	1613
M1141	PD1-selected	Melanoma	03/29/19	3429	1515
	Unselected TIL			4950	2107

FIG. 145

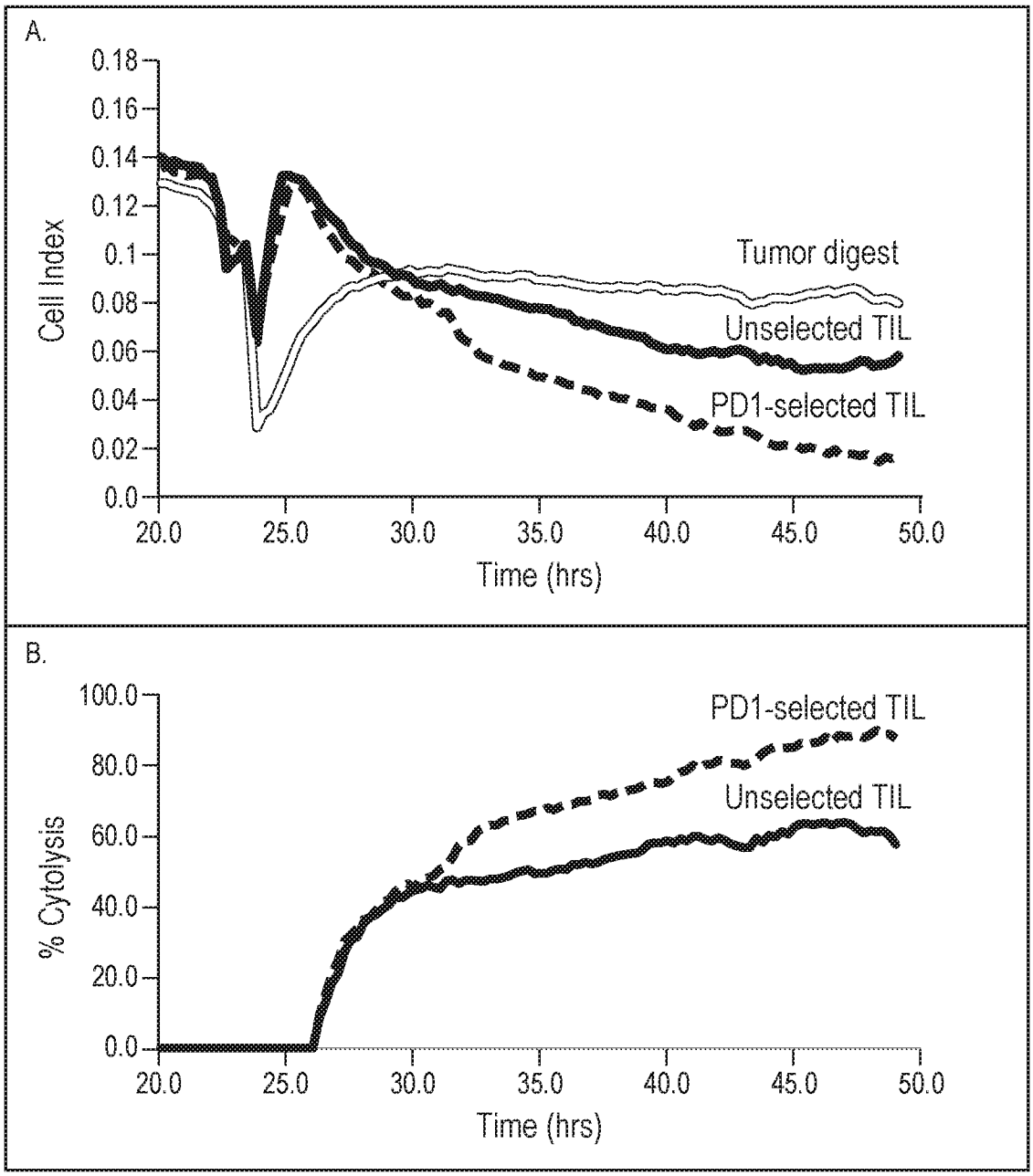

A.
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0.0
Cell Index
Tumor digest
Unselected TIL
PD1-selected TIL
20.0
25.0
30.0
35.0
40.0
45.0
50.0
Time (hrs)
B.
100.0
80.0
60.0
40.0
20.0
0.0
% Cytolysis
PD1-selected TIL
Unselected TIL
20.0
25.0
30.0
35.0
40.0
45.0
50.0
Time (hrs)

FIG. 146

C

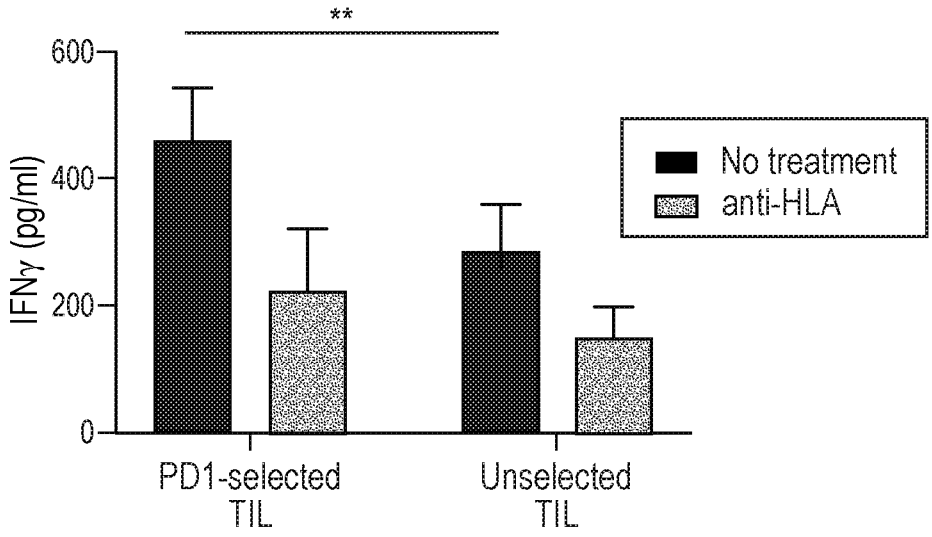

**
600
400
200
0
IFNγ (pg/ml)
No treatment
anti-HLA
PD1-selected TIL
Unselected TIL

FIG. 146C

Tumor ID	Sample ID	Histology	REP1 Fold Expansion	REP2 Fold Expansion
H3035	PD1-selected TIL	HNSCC	350	2166
	Unselected TIL		869	1385
H3039	PD1-selected TIL	HNSCC	654	1082
	Unselected TIL		123	893
L4089	PD1-selected TIL	NSCLC	1502	2106
	Unselected TIL		2821	1416
L4096	PD1-selected TIL	NSCLC	582	788
	Unselected TIL		1749	919
L4097	PD1-selected TIL	NSCLC	230	1982
	Unselected TIL		1147	2455
L4100	PD1-selected TIL	NSCLC	536	997
	Unselected TIL		1498	900
L4101	PD1-selected	NSCLC	1346	896
	Unselected TIL		2196	962
L4104	PD1-selected	NSCLC	1711	1257
	Unselected TIL		649	1421
L4106	PD1-selected	NSCLC	1179	1367
	Unselected TIL		4500	1912
M1132	PD1-selected	Melanoma	50	234
	Unselected TIL		2670	1263
M1136	PD1-selected	Melanoma	961	867
	Unselected TIL		693	1190
M1139	PD1-selected	Melanoma	557	1751
	Unselected TIL		4908	1613
M1141	PD1-selected	Melanoma	3429	1515
	Unselected TIL		4950	2107

FIG. 147

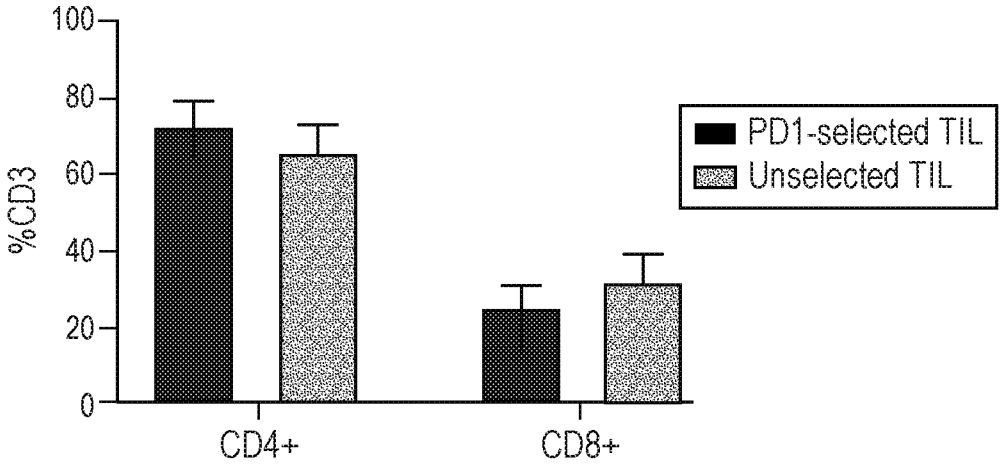


FIG. 148

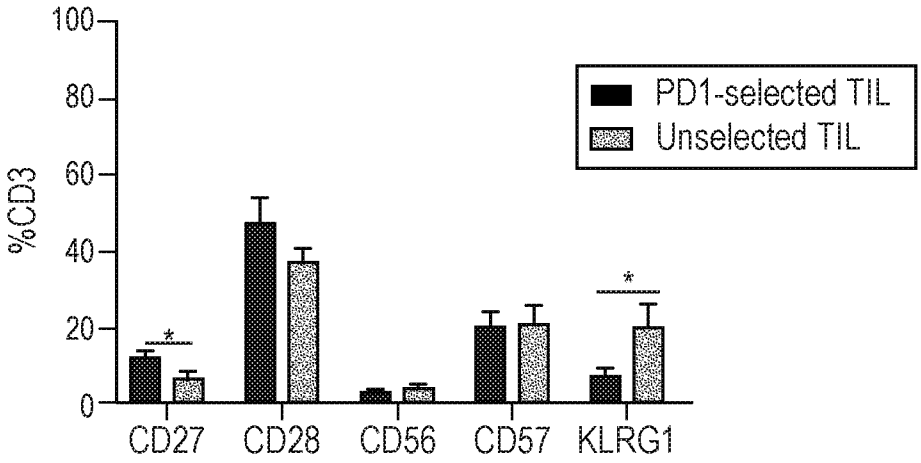

100
80
60
40
20
0
%CD3
PD1-selected TIL
Unselected TIL
*
*
CD27
CD28
CD56
CD57
KLRG1

FIG. 149

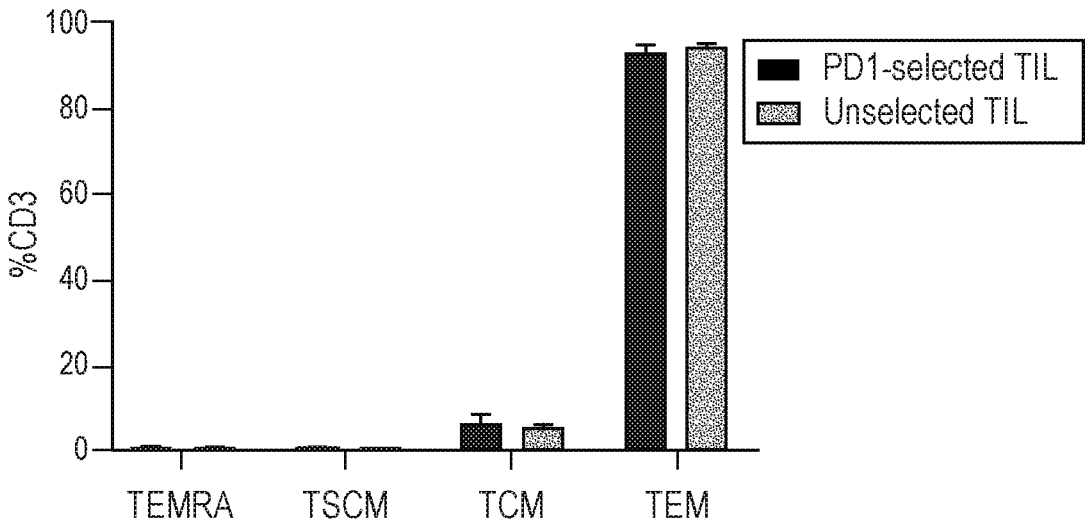

100
80
60
40
20
0
%CD3
TEMRA
TSCM
TCM
TEM
PD1-selected TIL
Unselected TIL

FIG. 150

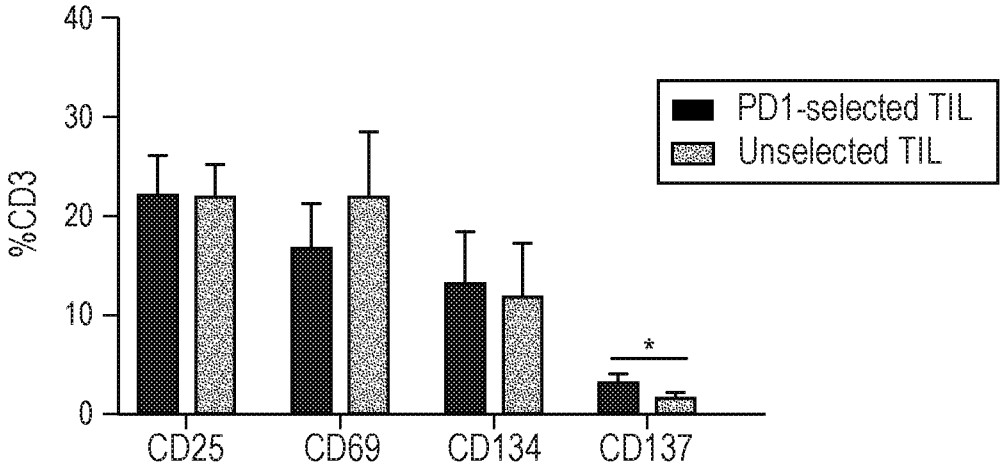

40
30
20
10
0
%CD3
PD1-selected TIL
Unselected TIL
*
CD25
CD69
CD134
CD137

FIG. 151

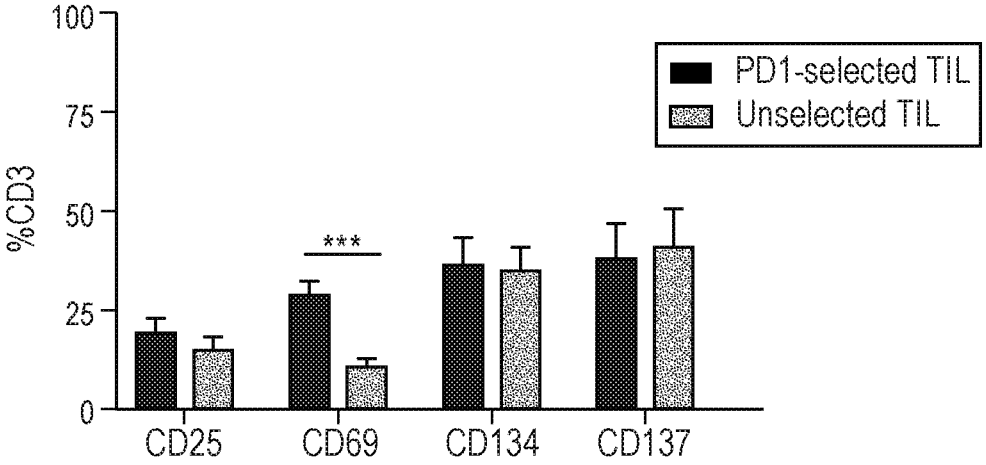

100
75
50
25
0
%CD3
***
PD1-selected TIL
Unselected TIL
CD25
CD69
CD134
CD137

FIG. 152

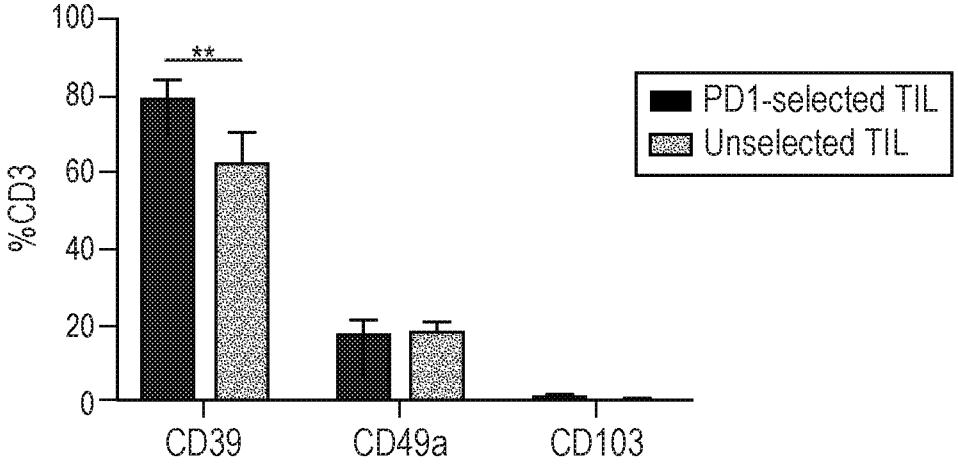

100
80
60
40
20
0
%CD3
**
PD1-selected TIL
Unselected TIL
CD39
CD49a
CD103

FIG. 153

Conditions	Full-Scale Process
REP-1 **TIL** **Feeders** **CM1** **IL-2** **OKT3 (30 ng/mL)** **G-Rex**	**Day 0:** REP-1 PD1 sort count 100e6 1 L 6000 IU/mL 30 ng/mL 100MCS
REP-2 **TIL** **Feeders** **CM2** **IL-2** **OKT3 (30 ng/mL)** **G-Rex**	**Day 11:** REP-2 5e6-200e6 TVC 5e9 5 L 3000 IU/mL 30 ng/mL 500MCS
Scale up	**Day 16:** Volume reduce and split up to 5 G-Rex500MCS in CM4+3000 IU/mL of IL-2
REP-2 Harvest	**Day 22:** REP-2 Harvest

FIG. 154

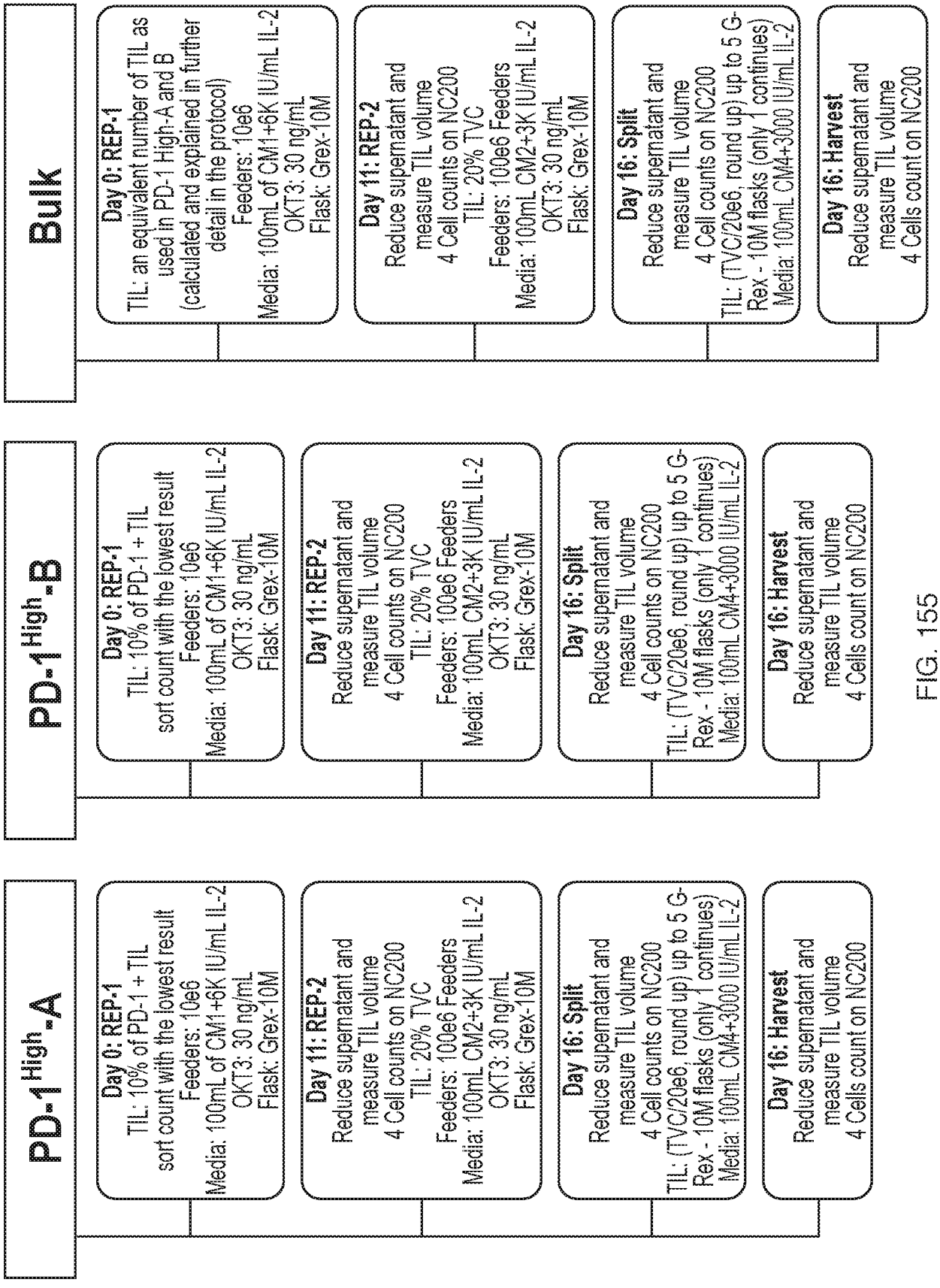

PD-1High-A
Day 0: REP-1
TIL: 10% of PD-1 + TIL
sort count with the lowest result
Feeders: 10e6
Media: 100mL of CM1+6K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 11: REP-2
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: 20% TVC
Feeders: 100e6 Feeders
Media: 100mL CM2+3K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 16: Split
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: (TVC/20e6, round up) up to 5 G-Rex - 10M flasks (only 1 continues)
Media: 100mL CM4+3000 IU/mL IL-2
Day 16: Harvest
Reduce supernatant and
measure TIL volume
4 Cells count on NC200
PD-1High-B
Day 0: REP-1
TIL: 10% of PD-1 + TIL
sort count with the lowest result
Feeders: 10e6
Media: 100mL of CM1+6K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 11: REP-2
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: 20% TVC
Feeders: 100e6 Feeders
Media: 100mL CM2+3K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 16: Split
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: (TVC/20e6, round up) up to 5 G-Rex - 10M flasks (only 1 continues)
Media: 100mL CM4+3000 IU/mL IL-2
Day 16: Harvest
Reduce supernatant and
measure TIL volume
4 Cells count on NC200
Bulk
Day 0: REP-1
TIL: an equivalent number of TIL as
used in PD-1 High-A and B
(calculated and explained in further
detail in the protocol)
Feeders: 10e6
Media: 100mL of CM1+6K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 11: REP-2
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: 20% TVC
Feeders: 100e6 Feeders
Media: 100mL CM2+3K IU/mL IL-2
OKT3: 30 ng/mL
Flask: Grex-10M
Day 16: Split
Reduce supernatant and
measure TIL volume
4 Cell counts on NC200
TIL: (TVC/20e6, round up) up to 5 G-Rex - 10M flasks (only 1 continues)
Media: 100mL CM4+3000 IU/mL IL-2
Day 16: Harvest
Reduce supernatant and
measure TIL volume
4 Cells count on NC200

FIG. 155

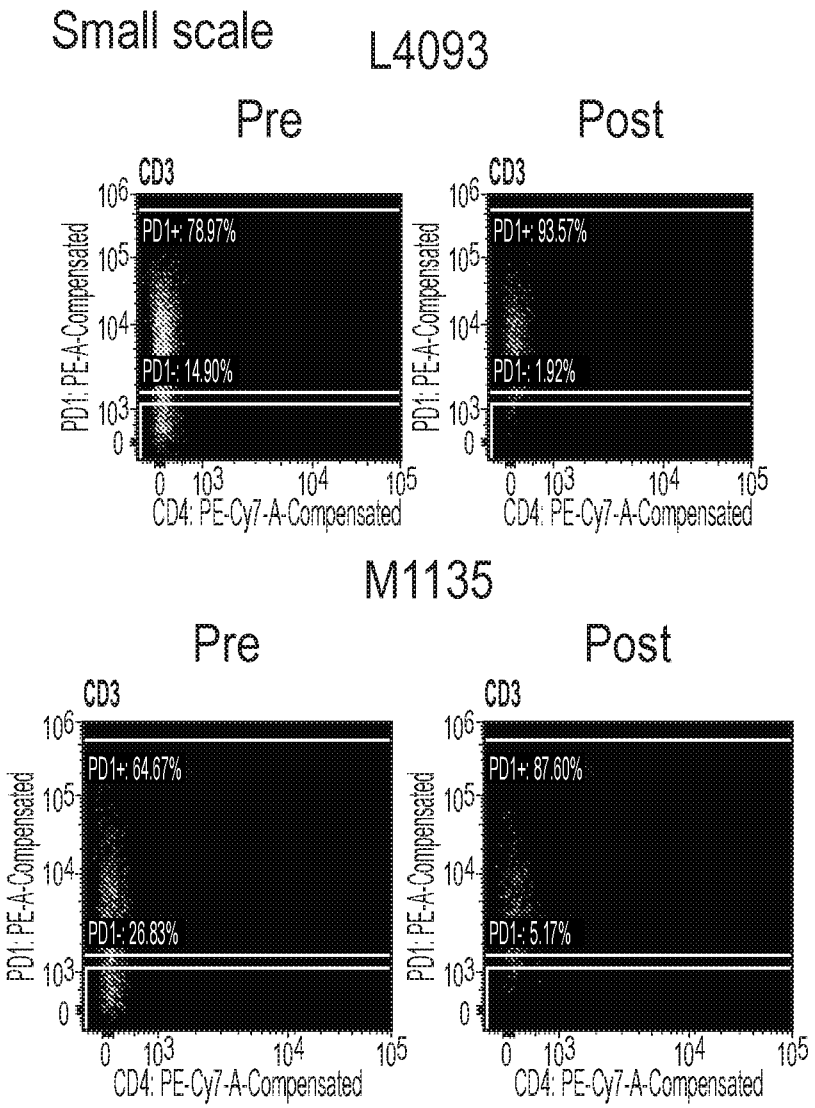

Small scale
L4093
Pre
Post
CD3
PD1+ 78.97%
PD1- 14.90%
PD1+ 93.57%
PD1- 1.92%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated
M1135
Pre
Post
PD1+ 64.67%
PD1- 26.83%
PD1+ 87.60%
PD1- 5.17%

FIG. 156A

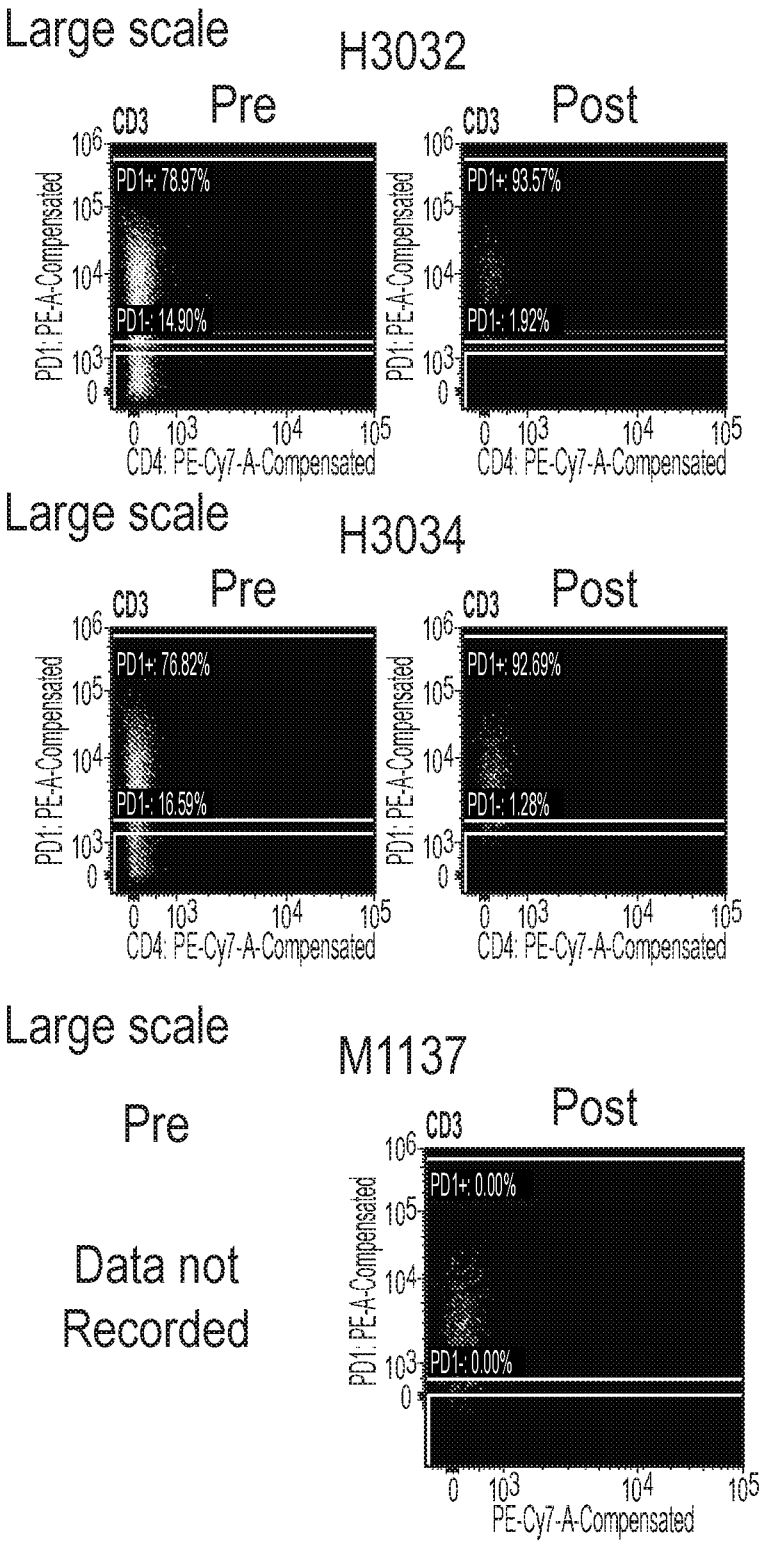
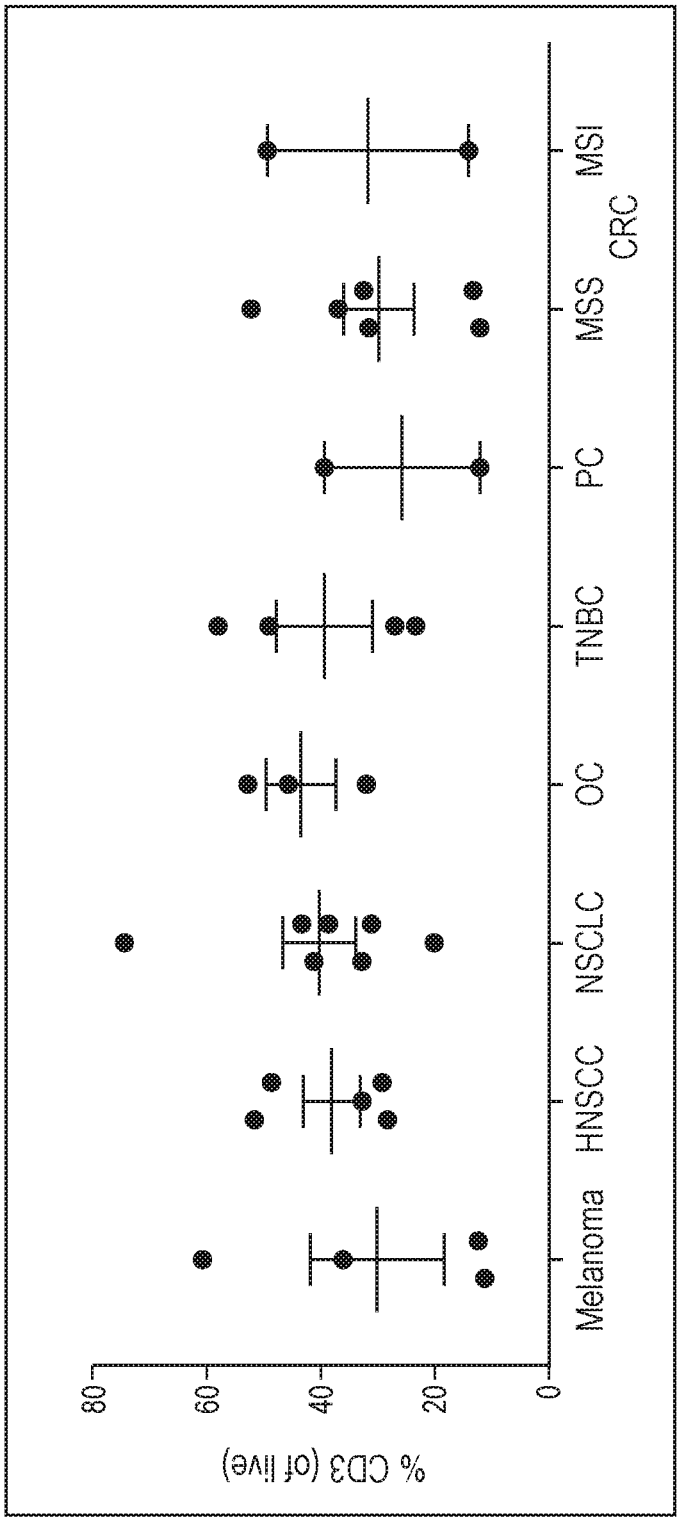

Large scale
H3032
Pre
Post
CD3
PD1+: 78.97%
PD1-: 14.90%
PD1+: 93.57%
PD1-: 1.92%
PD1: PE-A-Compensated
CD4: PE-Cy7-A-Compensated
Large scale
H3034
Pre
Post
PD1+: 76.82%
PD1-: 16.59%
PD1+: 92.69%
PD1-: 1.28%
Large scale
M1137
Pre
Data not Recorded
Post
PD1+: 0.00%
PD1-: 0.00%
PE-Cy7-A-Compensated

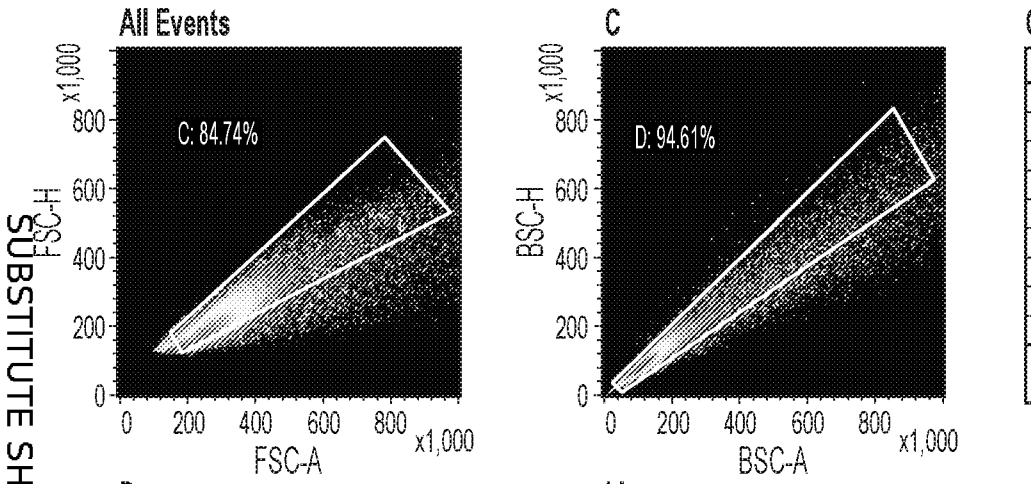
FIG. 156B



Legend: PD-1 expression in multiple histologies. Percentage of PD-1+ TIL in the CD3+ TIL population are plotted for individual samples within each histology. Horizontal lines represent the mean percentages of each subset and vertical lines represent the standard errors.

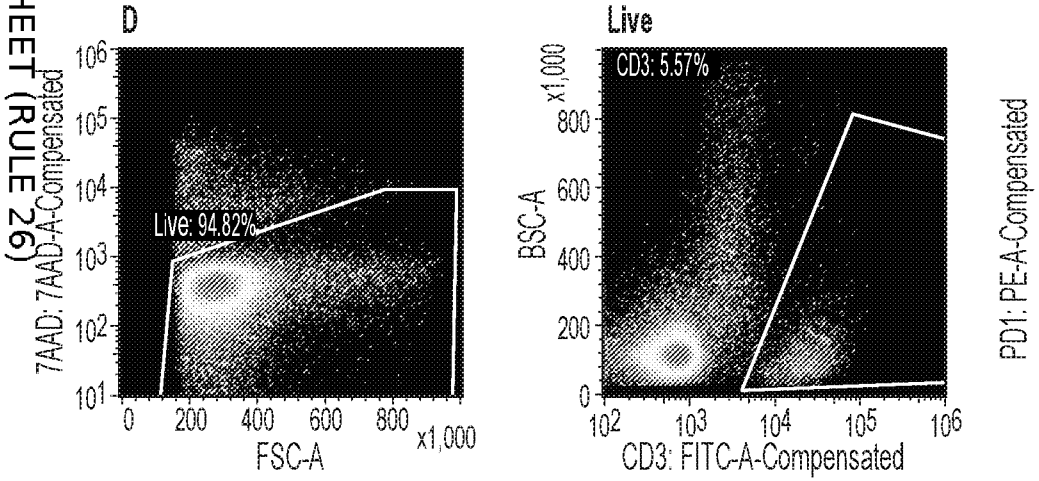
FIG. 157

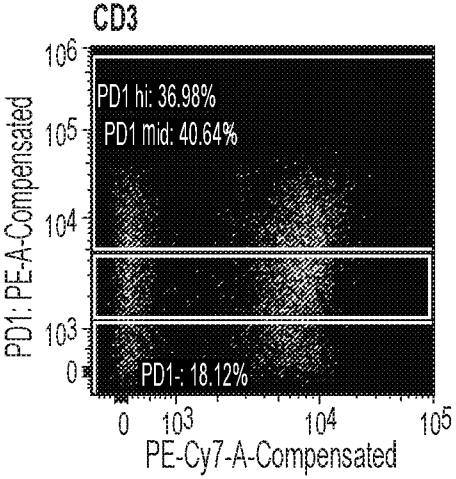
Worksheet

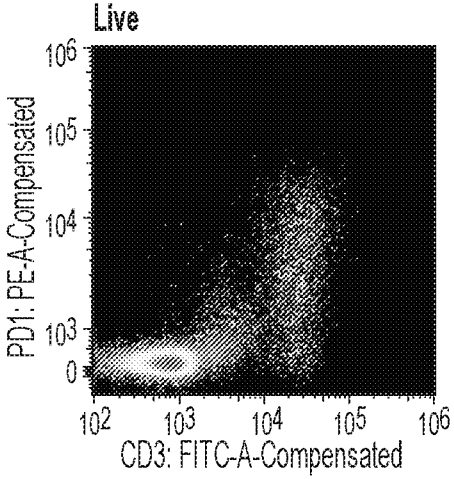


Gates and Statistics Displaying 100,000 / 707,455 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	84,736	84.74%	84.74%
D	80,165	94.61%	80.17%
Live	76,009	94.82%	76.01%
CD3	4,232	5.57%	4.23%
PD1 hi	1,565	36.98%	1.57%
PD1-	767	18.12%	0.77%
PD1 mid	1,720	40.64%	1.72%
J	63,530	63.53%	63.53%



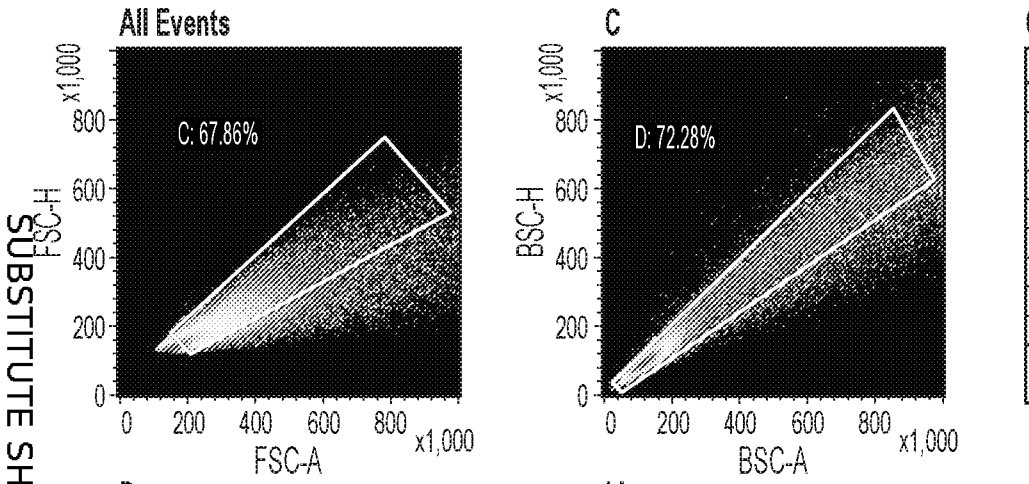




SUBSTITUTE SHEET (RULE 26)

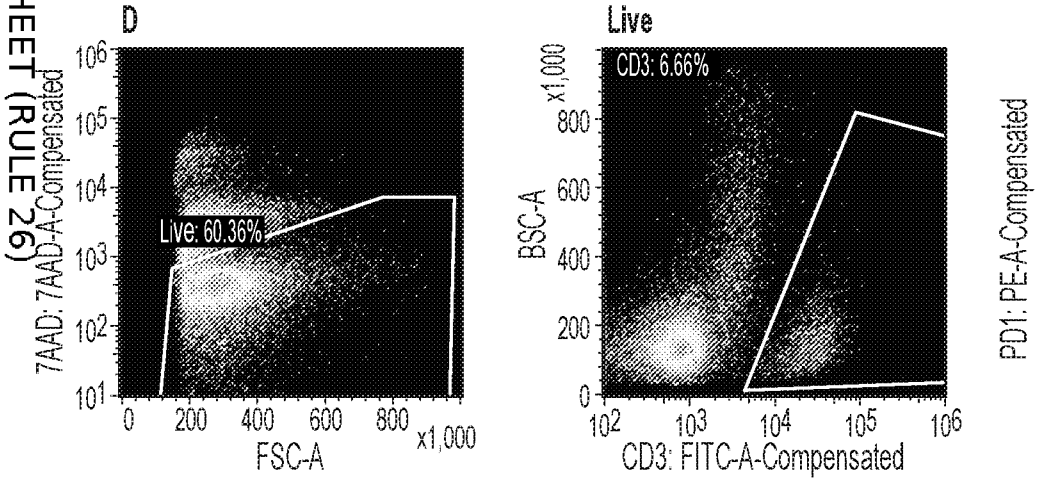
FIG. 158A

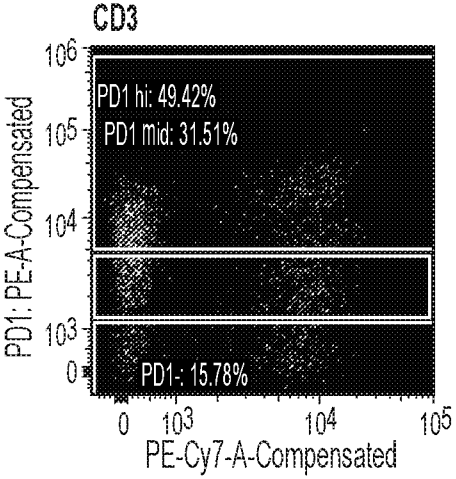
Worksheet

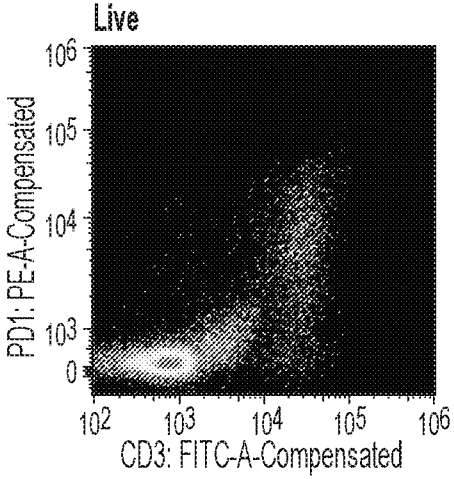


Gates and Statistics — Displaying 100,000 / 1,578,350 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	67,857	67.86%	67.86%
D	49,049	72.28%	49.05%
Live	29,607	60.36%	29.61%
CD3	1,971	6.66%	1.97%
PD1 hi	974	49.42%	0.97%
PD1-	311	15.78%	0.31%
PD1 mid	621	31.51%	0.62%
J	25,031	25.03%	25.03%



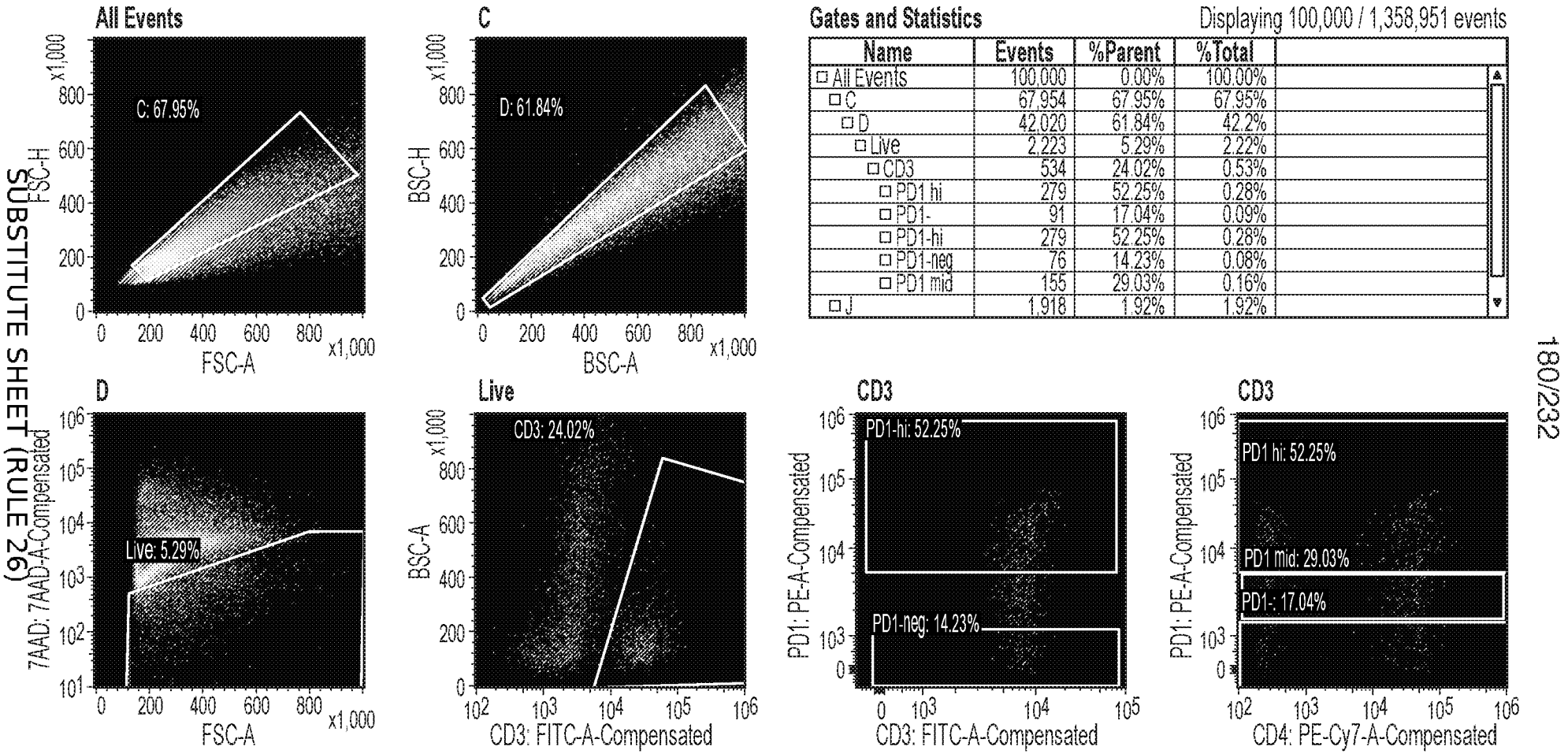




SUBSTITUTE SHEET (RULE 26)

FIG. 158B

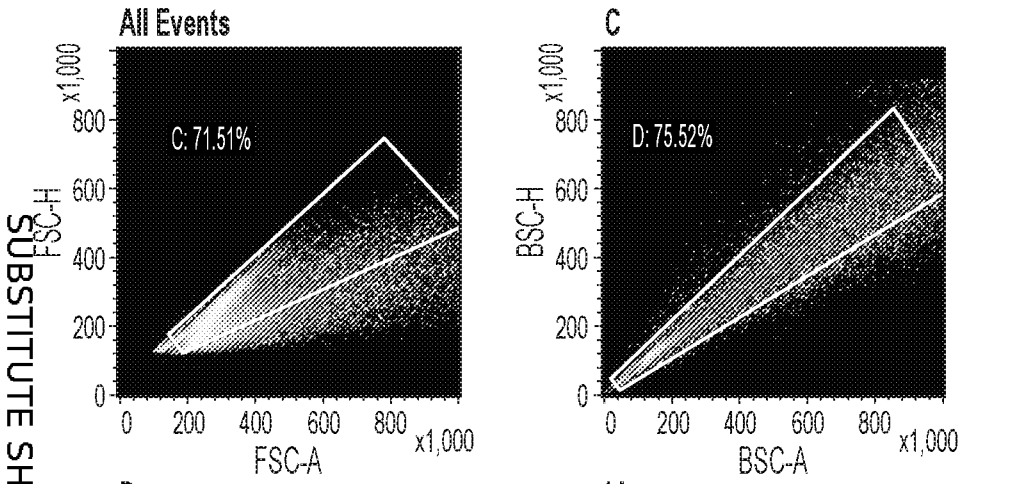
Worksheet



SUBSTITUTE SHEET (RULE 26)

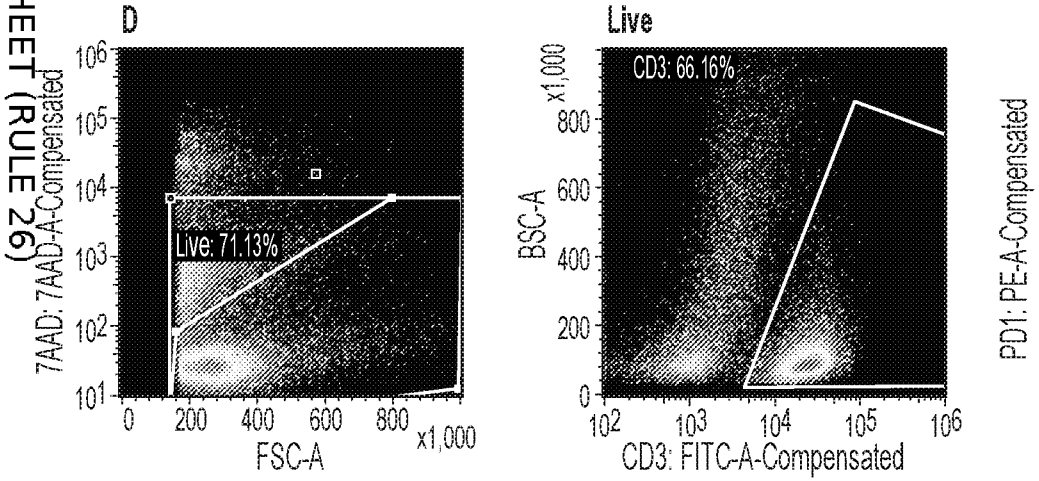
FIG. 158C

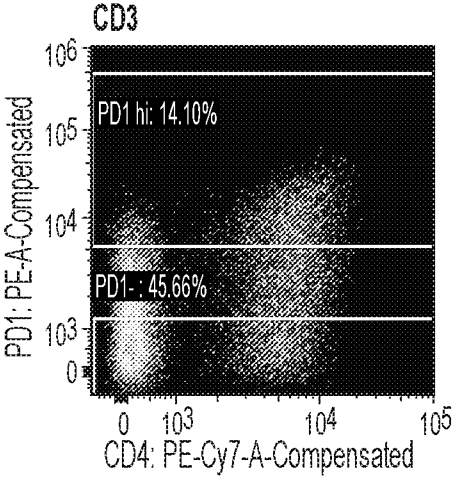
Worksheet

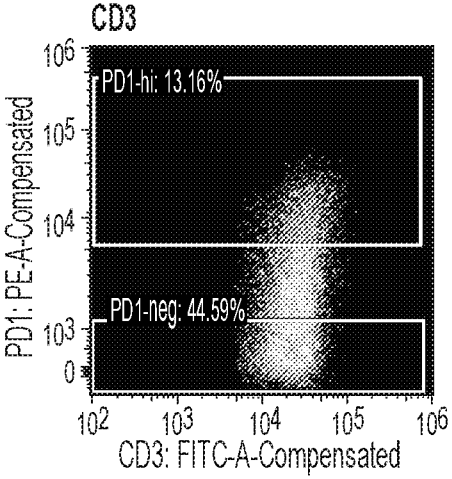


Gates and Statistics Displaying 100,000 / 115,596 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	71,513	71.51%	71.51%
D	54,010	75.52%	54.01%
Live	38,419	71.13%	38.42%
CD3	25,417	66.16%	25.42%
PD1 hi	3,585	14.10%	3.59%
PD1-	11,606	45.66%	11.61%
PD1-hi	3,346	13.16%	3.35%
PD1-neg	11,334	44.59%	11.33%
J	25,739	25.74%	25.74%







SUBSTITUTE SHEET (RULE 26)

FIG. 158D

Worksheet

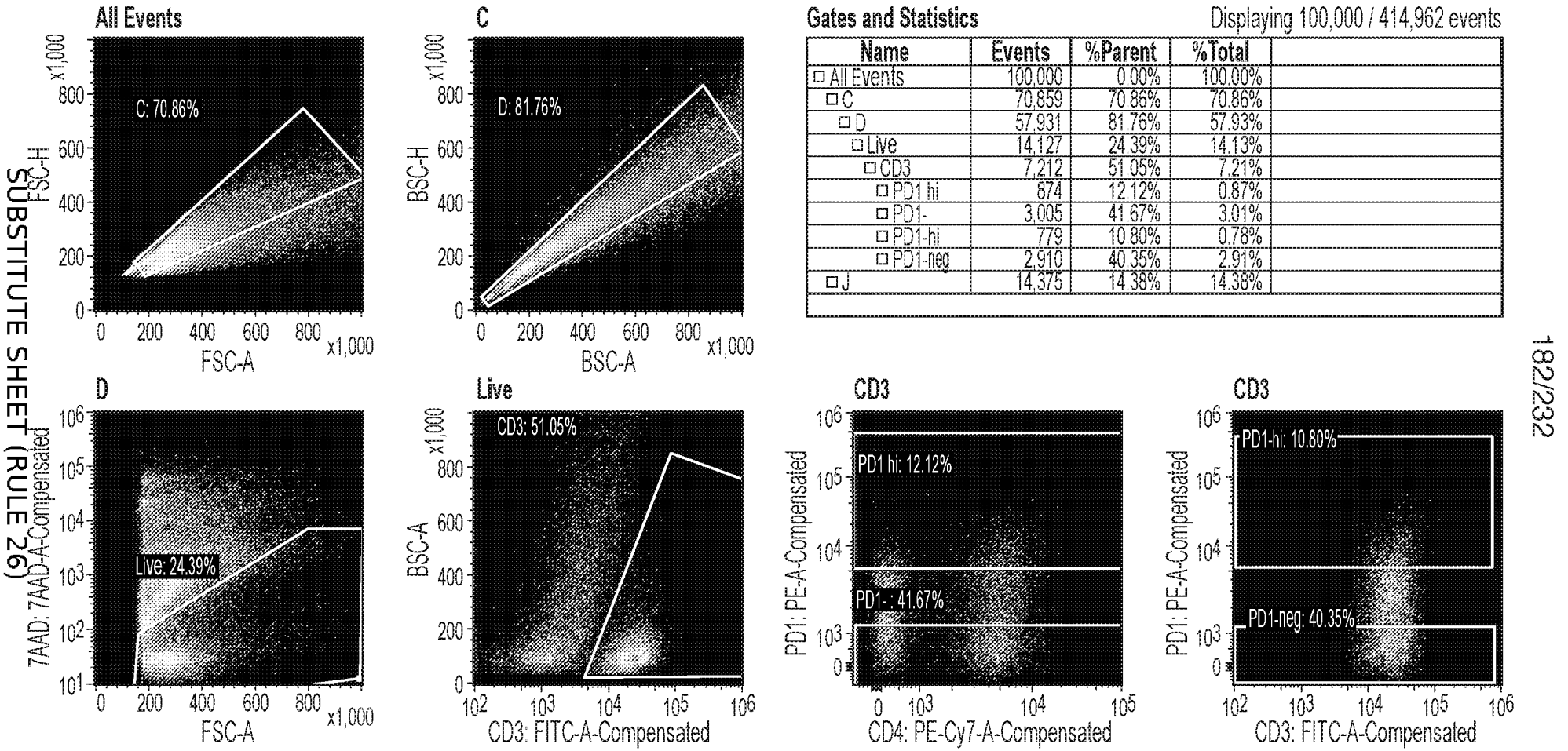
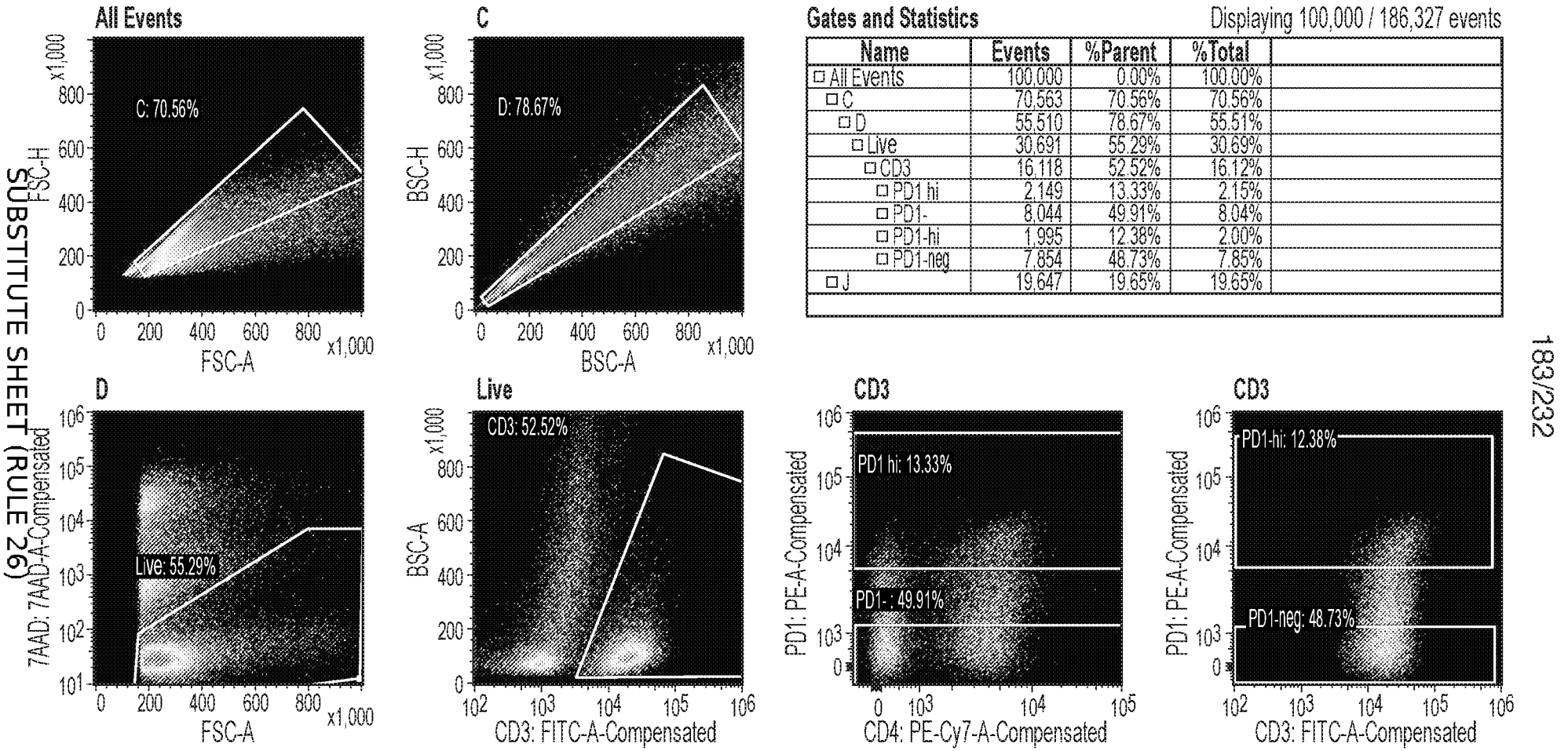


FIG. 158E

SUBSTITUTE SHEET (RULE 26)

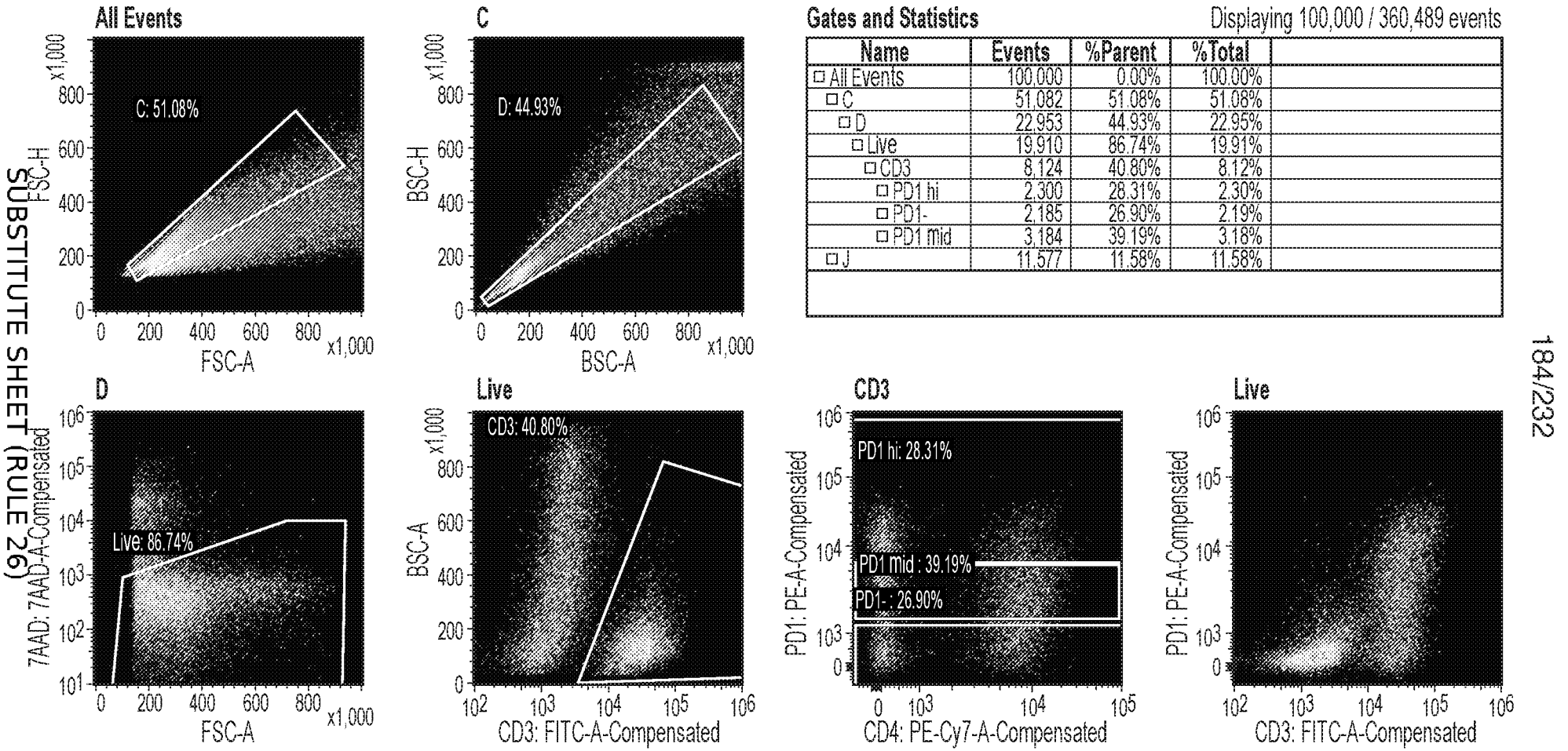
Worksheet



SUBSTITUTE SHEET (RULE 26)

FIG. 158F

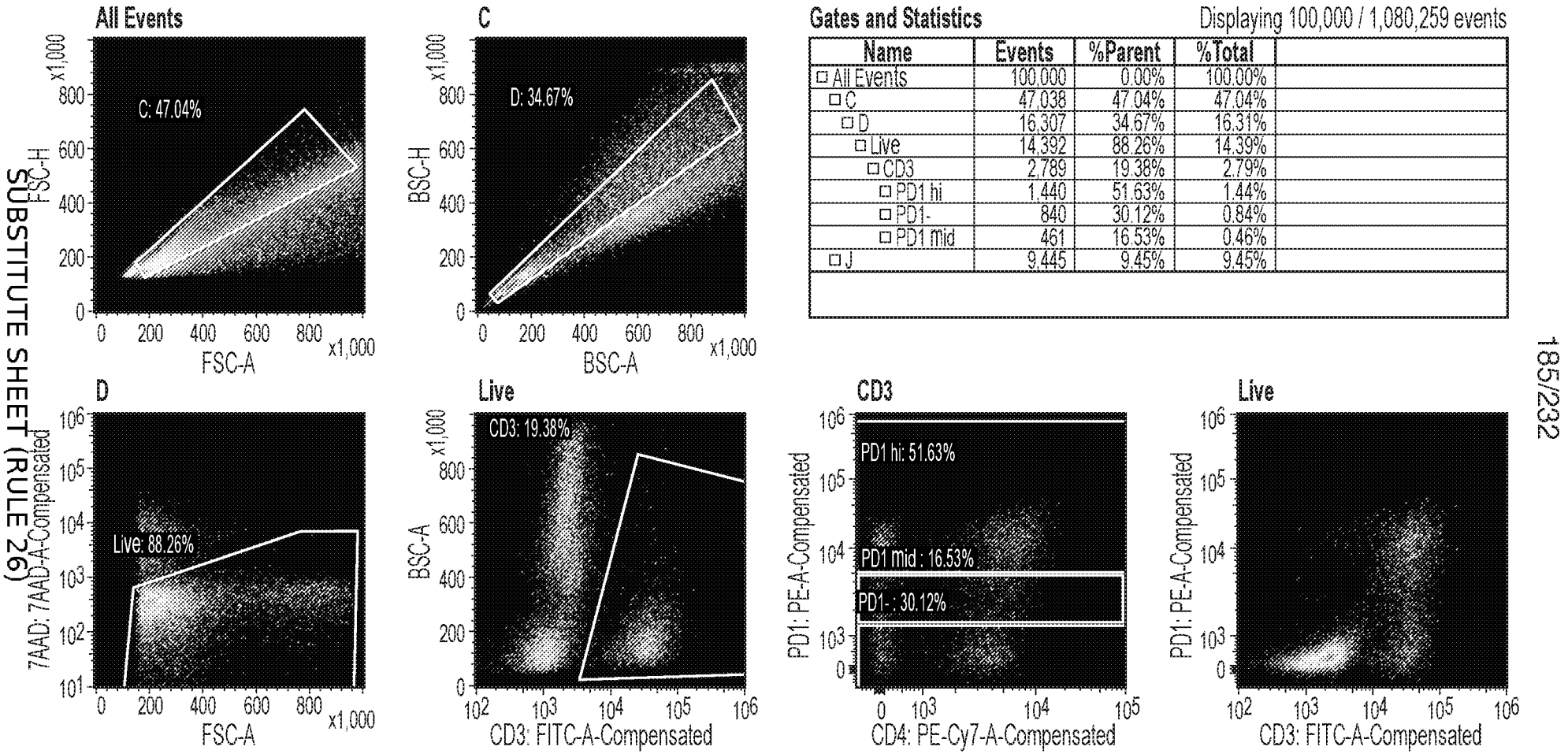
Worksheet



SUBSTITUTE SHEET (RULE 26)

FIG. 158G

Worksheet



SUBSTITUTE SHEET (RULE 26)

FIG. 158H

Worksheet

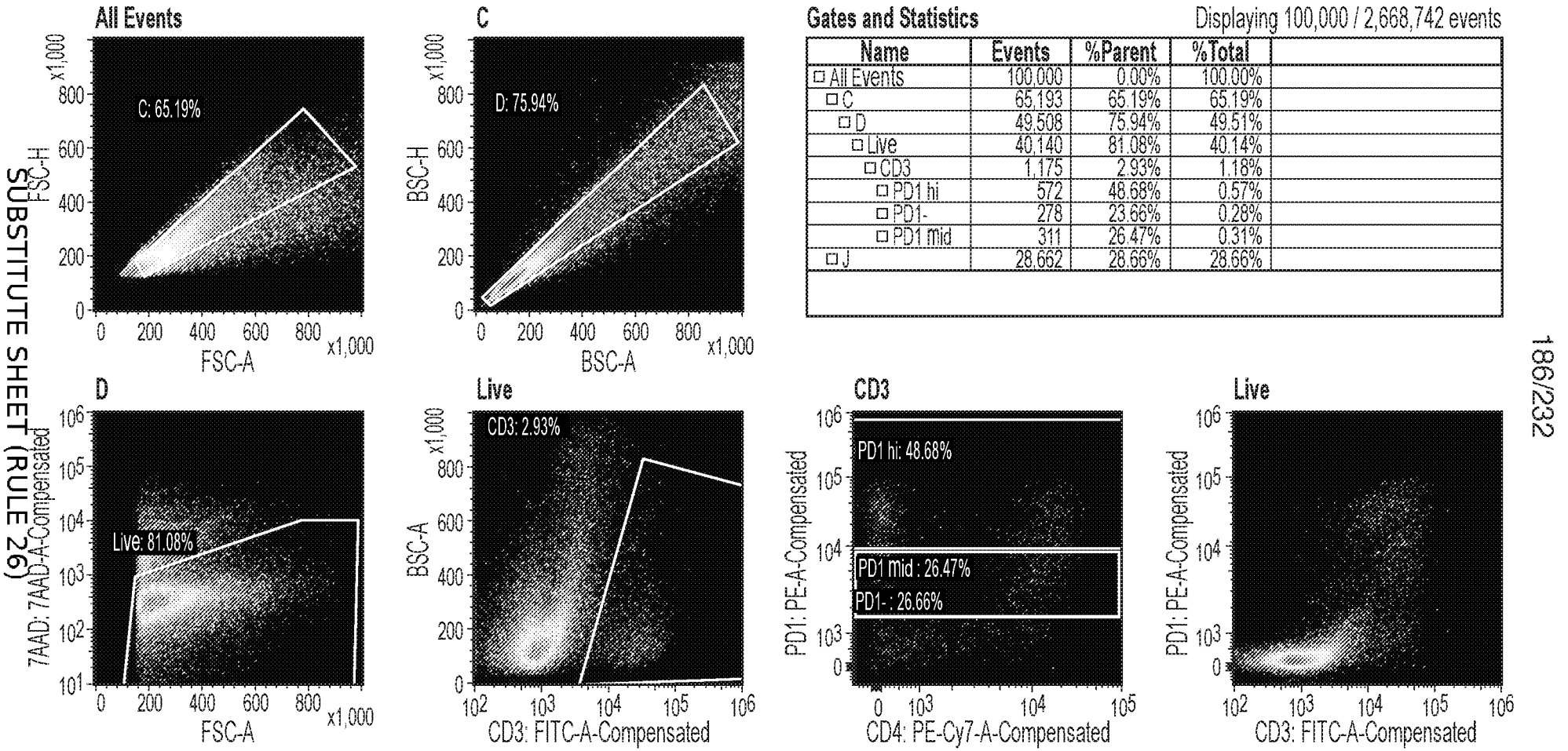
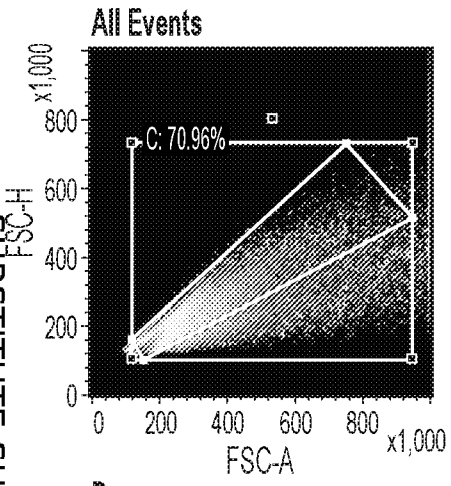
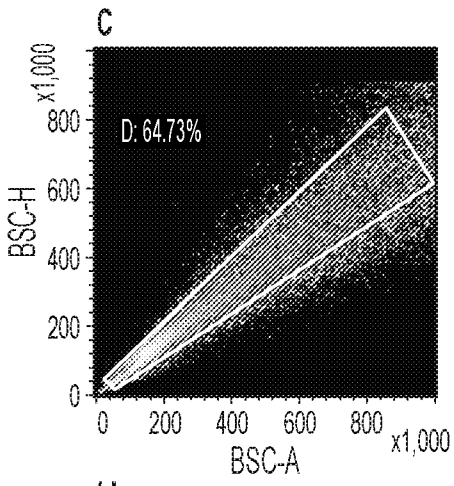


FIG. 158I

SUBSTITUTE SHEET (RULE 26)

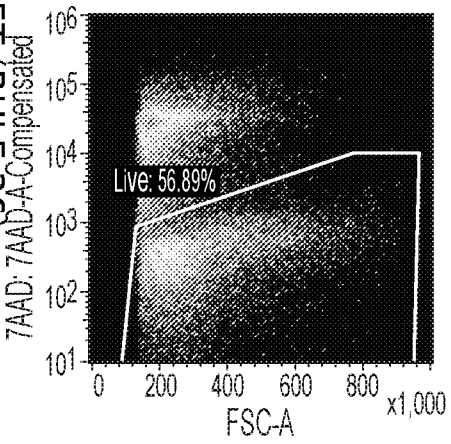
Worksheet

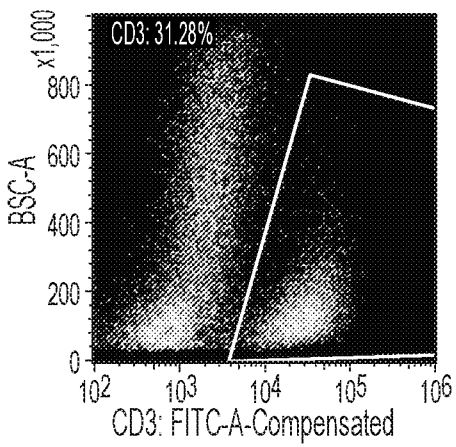


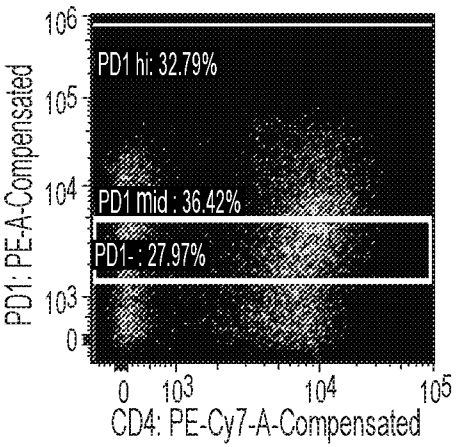


Gates and Statistics Displaying 100,000 / 135,889 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	70,958	70.96%	70.96%
D	45,928	64.73%	45.93%
Live	26,130	56.89%	26.13%
CD3	8,173	31.28%	8.17%
PD1 hi	2,680	32.79%	2.68%
PD1-	2,286	27.97%	2.29%
PD1 mid	2,977	36.42%	2.98%
J	13,976	13.98%	13.98%







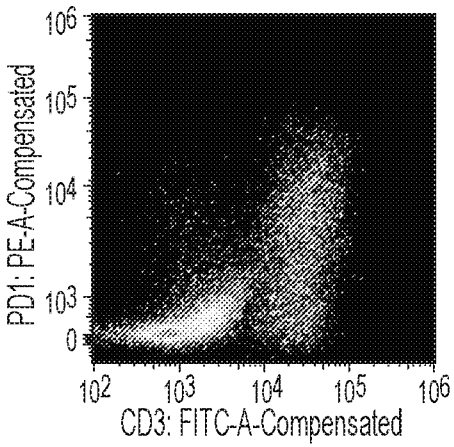
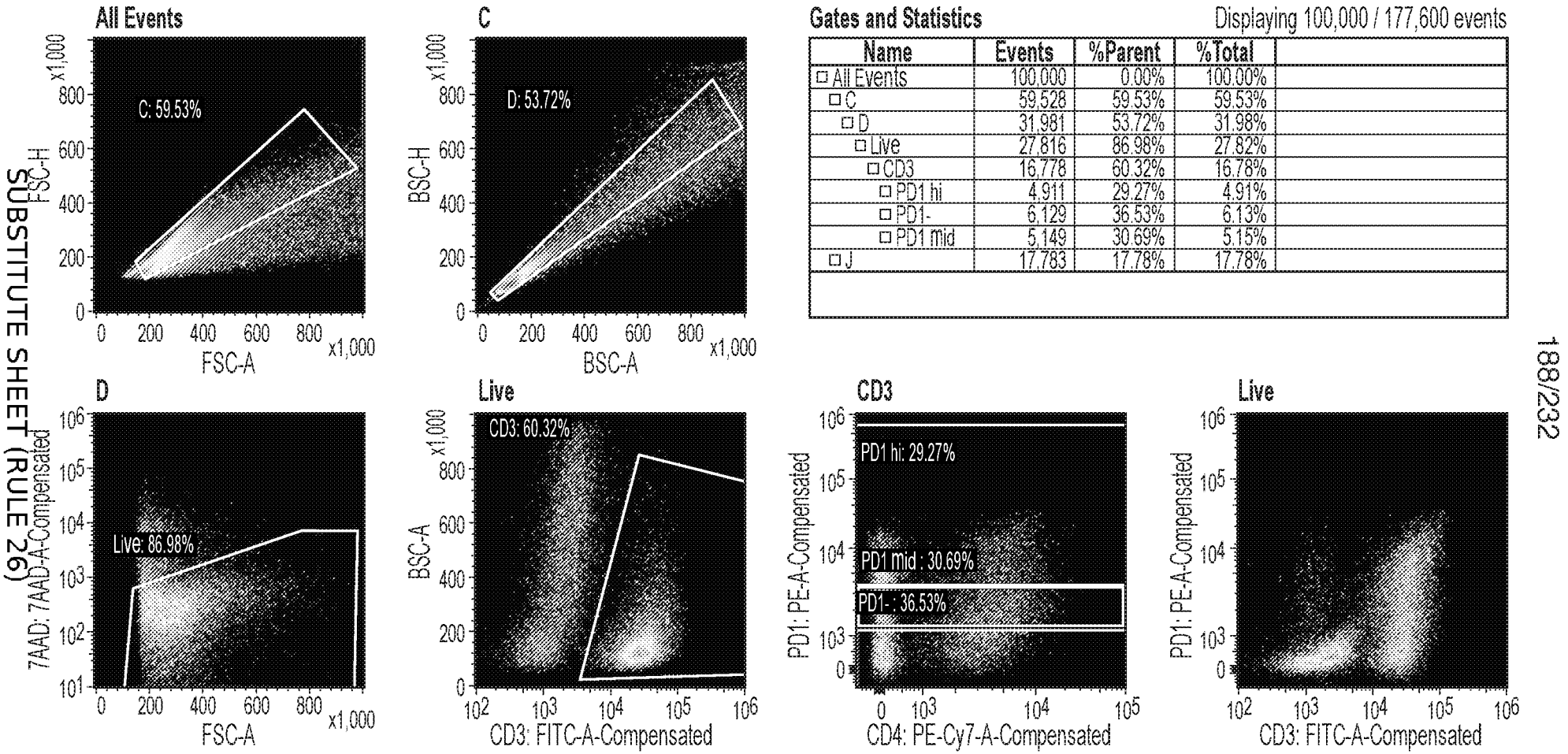


FIG. 158J

SUBSTITUTE SHEET (RULE 26)

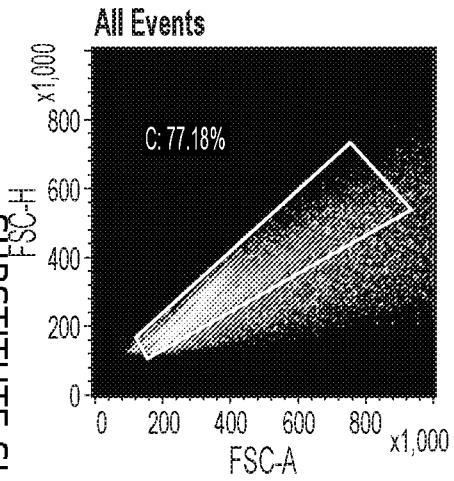
Worksheet

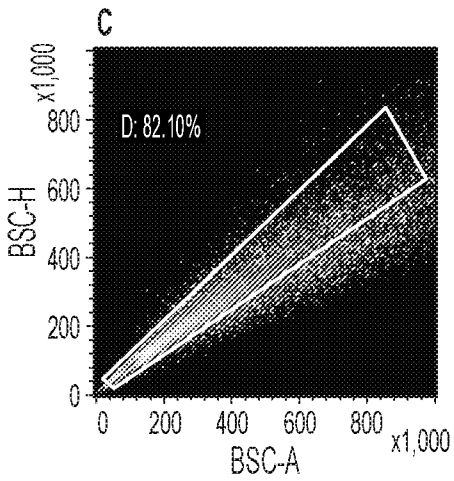


SUBSTITUTE SHEET (RULE 26)

FIG. 158K

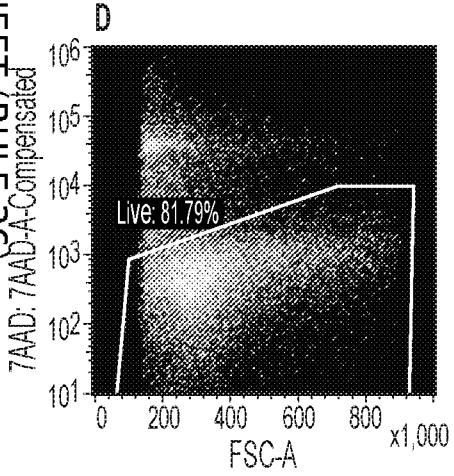
Worksheet

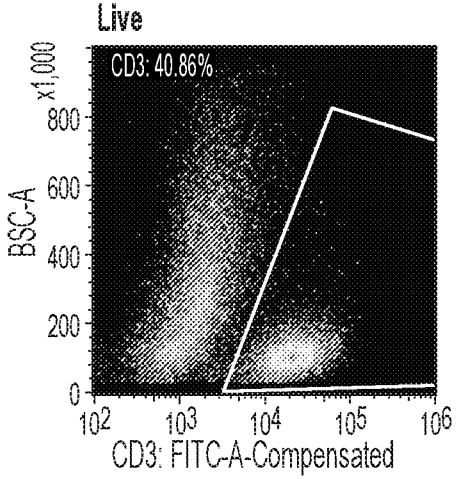


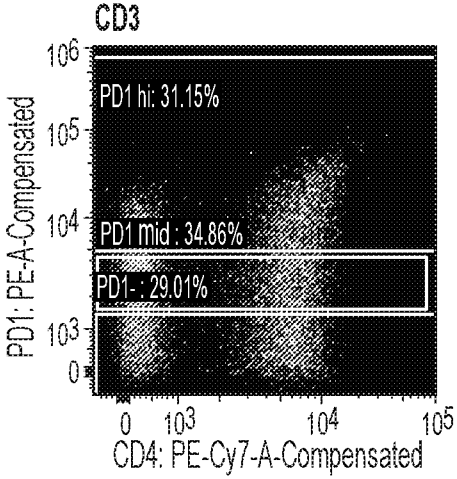


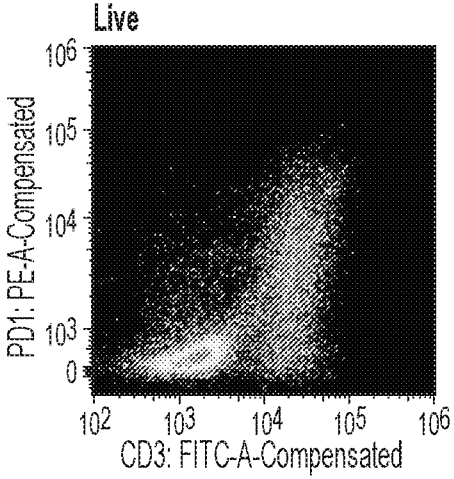
Gates and Statistics

Name	Events	%Parent	%Total
All Events	62,076	0.00%	100.00%
C	47,909	77.18%	77.18%
D	39,331	82.10%	63.36%
Live	32,167	81.79%	51.82%
CD3	13,143	40.86%	21.17%
PD1 hi	4,094	31.15%	6.60%
PD1-	3,813	29.01%	6.14%
PD1 mid	4,582	34.86%	7.38%
J	24,460	39.40%	39.40%





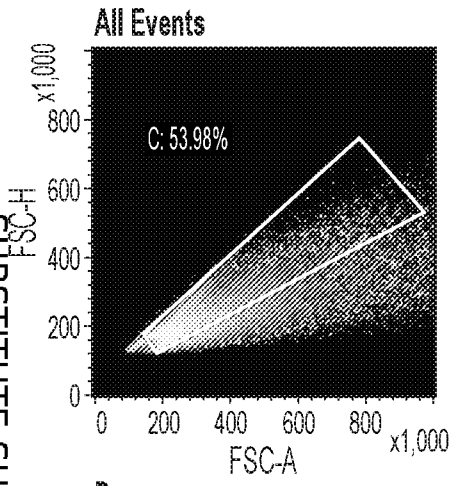


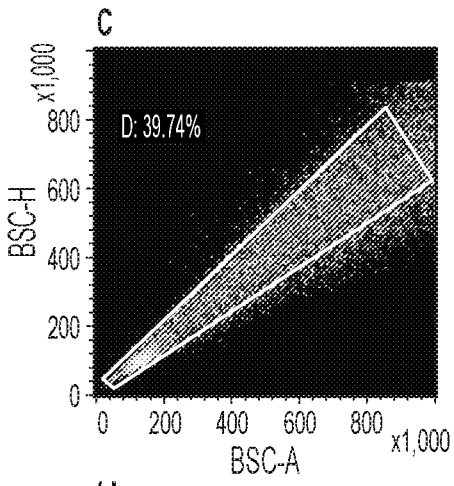


SUBSTITUTE SHEET (RULE 26)

FIG. 158L

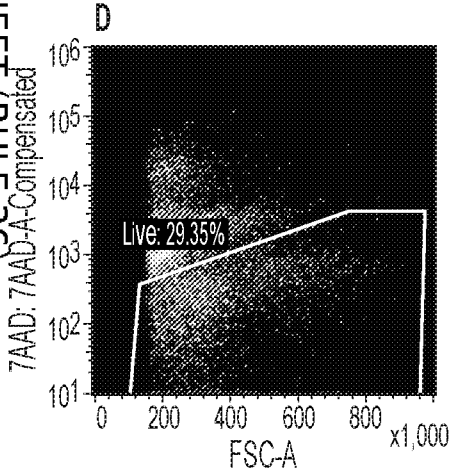
Worksheet

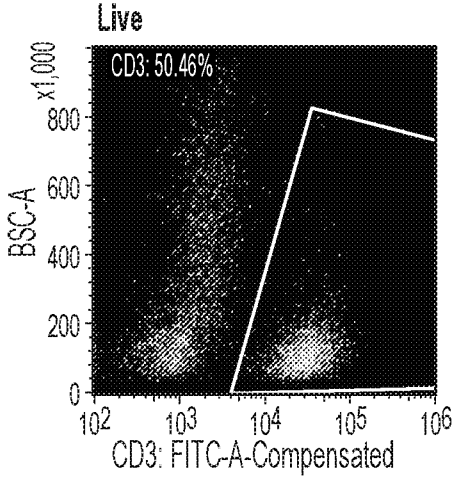


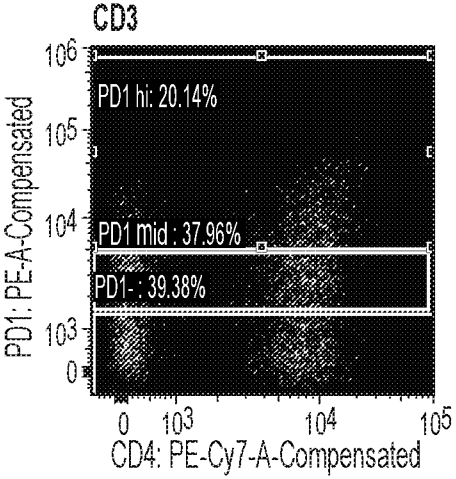


Gates and Statistics Displaying 100,000 / 946,878 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	53,980	53.98%	53.98%
D	21,453	39.74%	21.45%
Live	6,296	29.35%	6.30%
CD3	3,177	50.46%	3.18%
PD1 hi	640	20.14%	0.64%
PD1-	1,251	39.38%	1.25%
PD1 mid	1,206	37.96%	1.21%
J	4,633	4.63%	4.63%







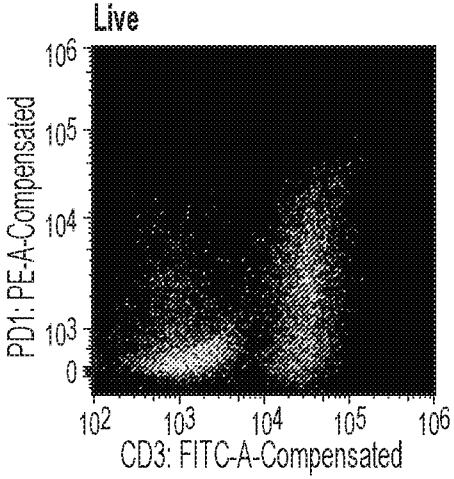
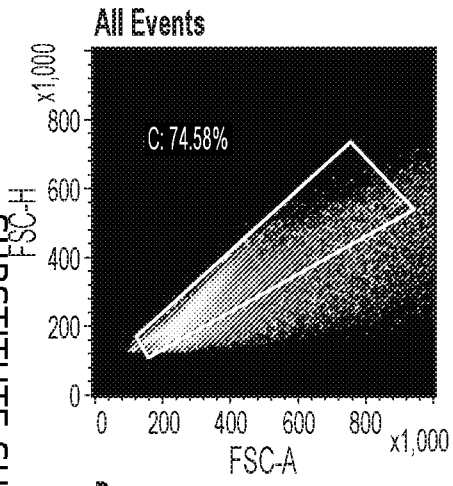
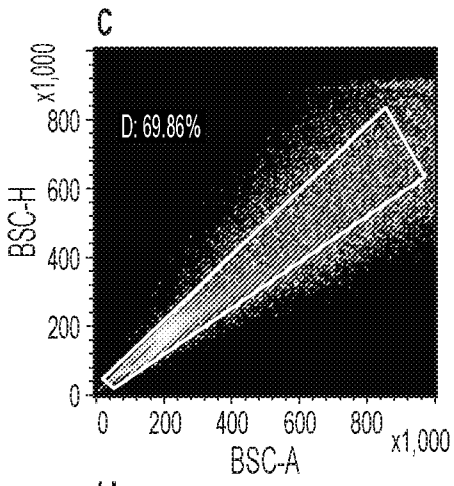


FIG. 158M

SUBSTITUTE SHEET (RULE 26)

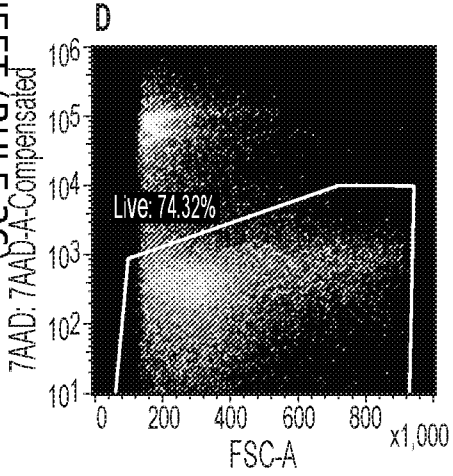
Worksheet

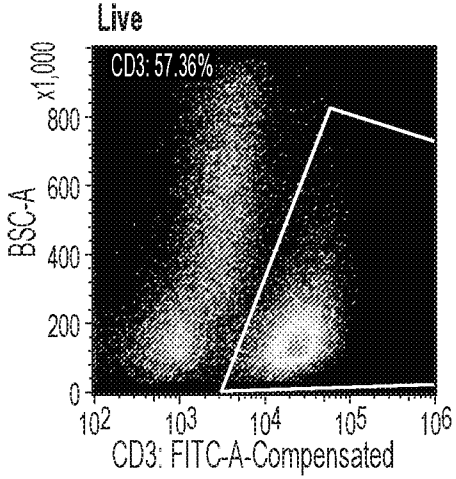


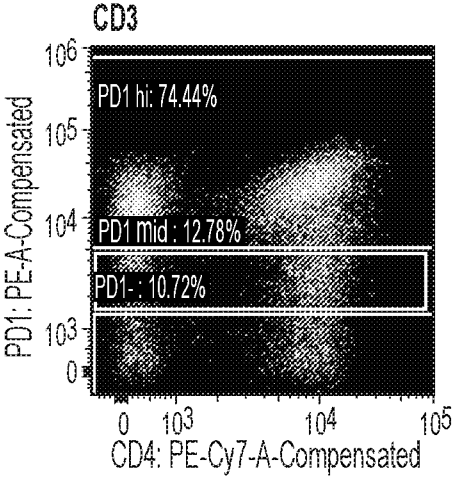


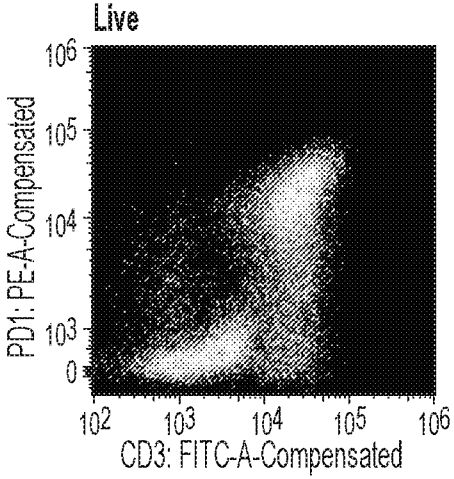
Gates and Statistics Displaying 100,000 / 134,191 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	74,579	74.58%	74.58%
D	52,101	69.86%	52.10%
Live	38,719	74.32%	38.72%
CD3	22,209	57.36%	22.21%
PD1 hi	16,533	74.44%	16.53%
PD1-	2,380	10.72%	2.38%
PD1 mid	2,839	12.78%	2.84%
J	25,248	25.25%	25.25%





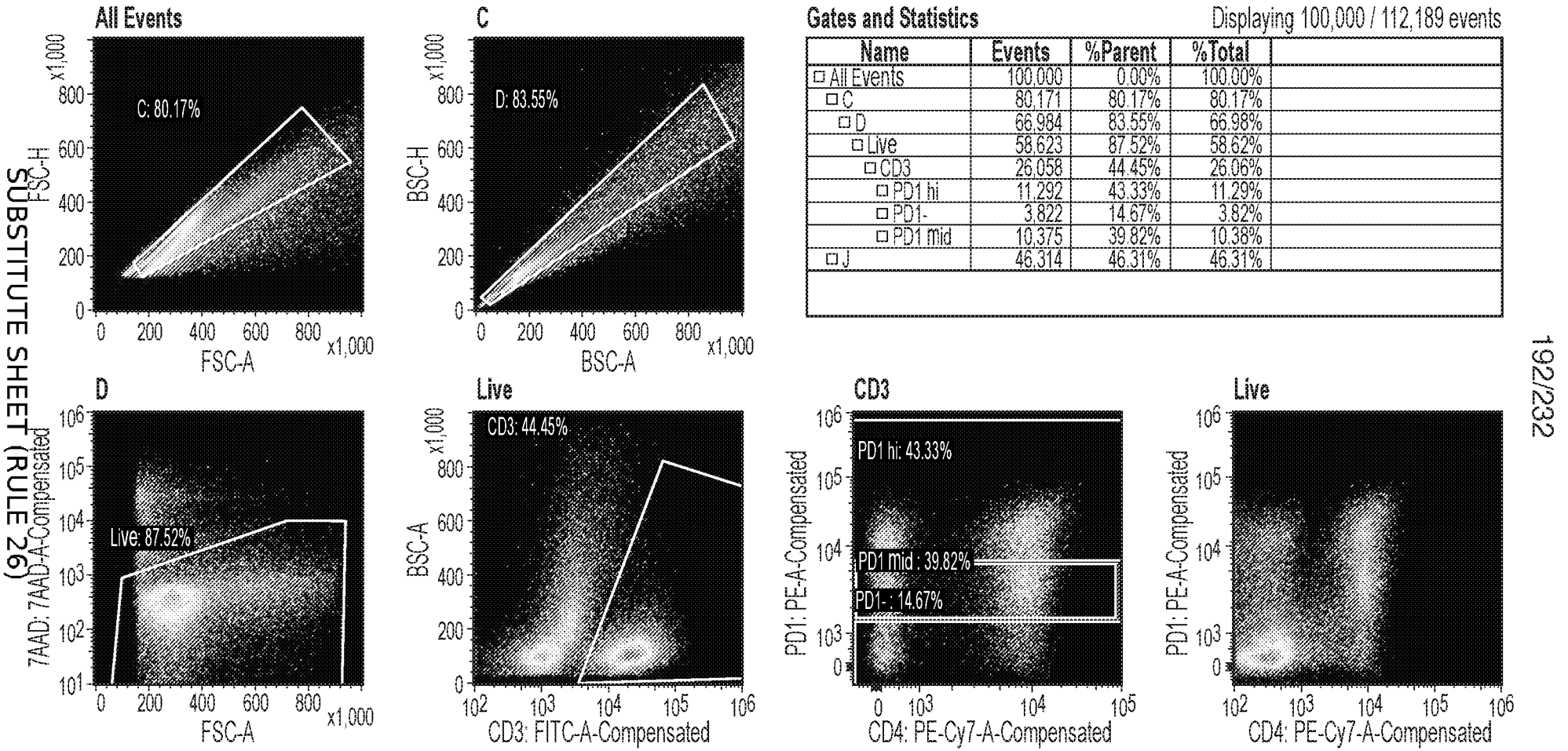




SUBSTITUTE SHEET (RULE 26)

FIG. 158N

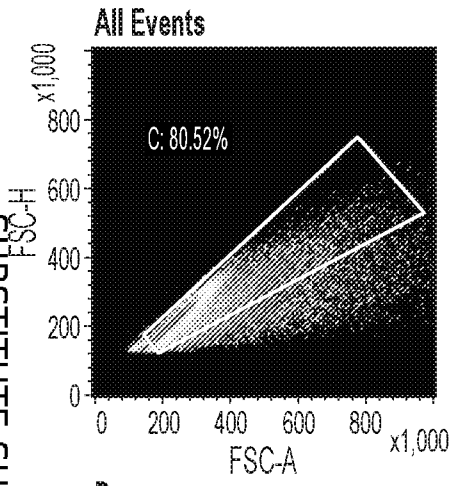
Worksheet

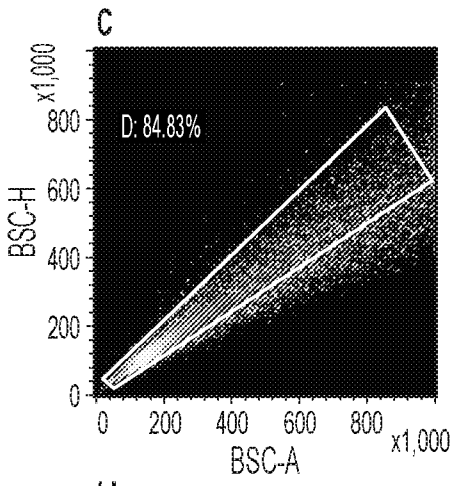


SUBSTITUTE SHEET (RULE 26)

FIG. 158O

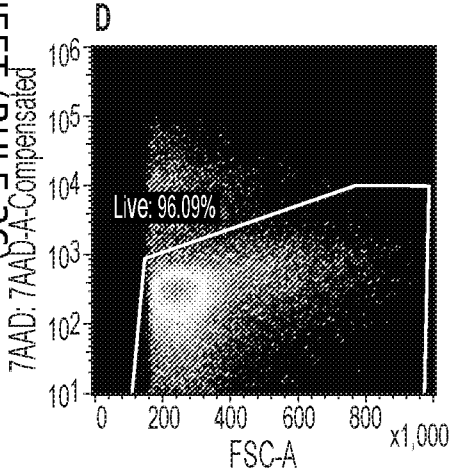
Worksheet

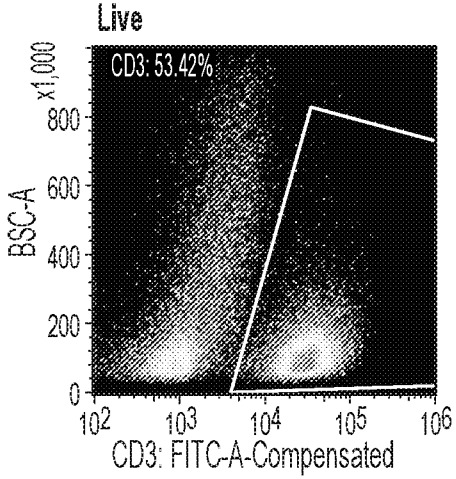


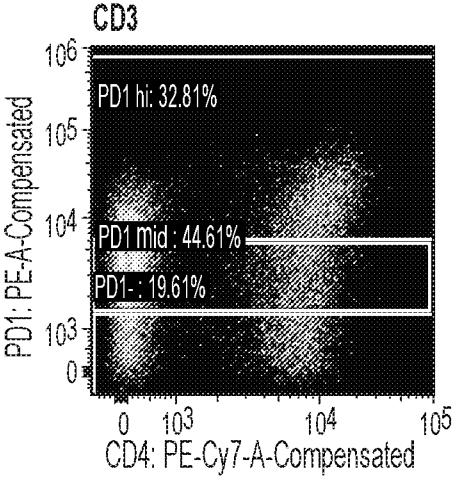


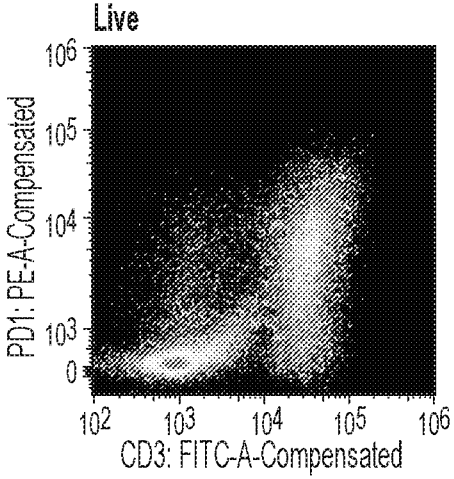
Gates and Statistics

Name	Events	%Parent	%Total
All Events	85,559	0.00%	100.00%
C	68,890	80.52%	80.52%
D	58,442	84.83%	68.31%
Live	56,154	96.09%	65.63%
CD3	30,000	53.42%	35.06%
PD1 hi	9,842	32.81%	11.50%
PD1-	5,882	19.61%	6.87%
PD1 mid	13,382	44.61%	15.64%
J	34,608	40.45%	40.45%









SUBSTITUTE SHEET (RULE 26)

FIG. 158P

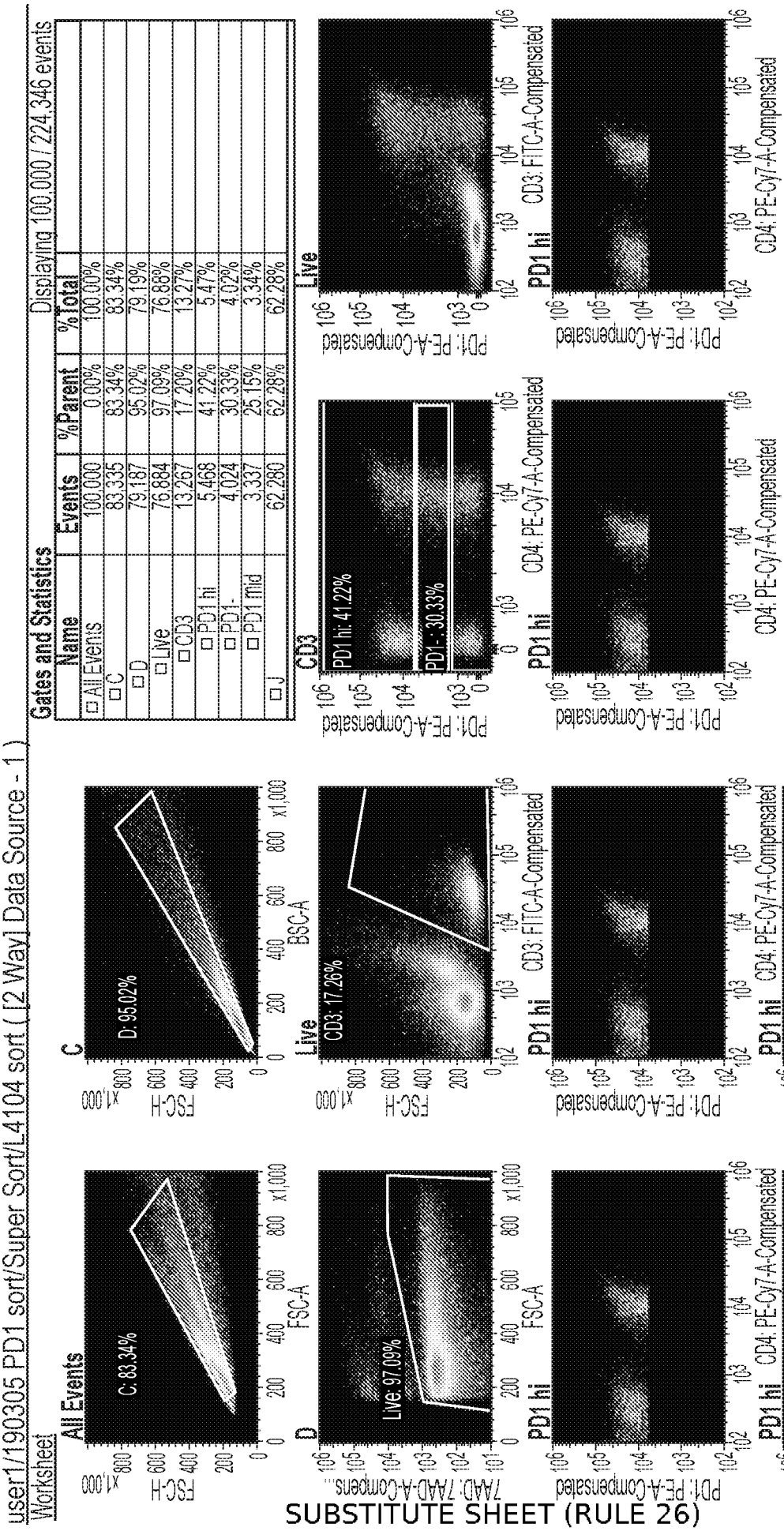
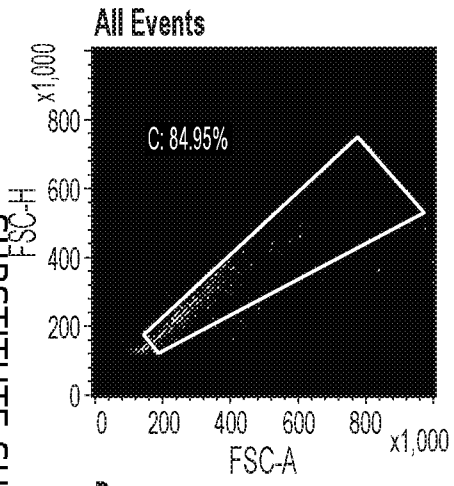
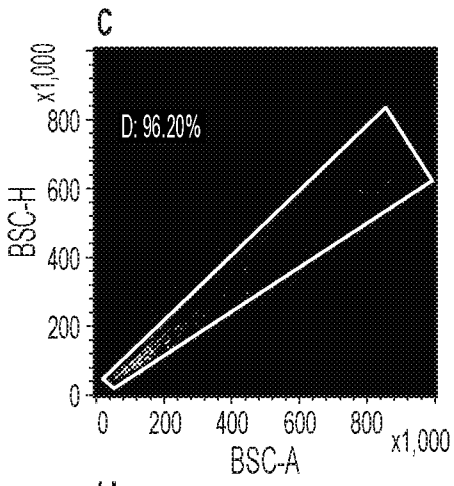

user1/190305 PD1 sort/Super Sort/L4104 sort ([2 Way] Data Source - 1)
Worksheet
All Events
C: 83.34%
FSC-H
FSC-A
C
D: 95.02%
BSC-A
D
Live: 97.09%
7AAD: 7AAD-A-Compens...
Live
CD3: 17.26%
CD3: FITC-A-Compensated
PD1 hi
CD4: PE-Cy7-A-Compensated
PD1: PE-A-Compensated
CD3
PD1 hi: 41.22%
PD1- : 30.33%
Gates and Statistics
Displaying 100,000 / 224,346 events
Name | Events | %Parent | %Total
All Events | 100,000 | 0.00% | 100.00%
C | 83,335 | 83.34% | 83.34%
D | 79,187 | 95.02% | 79.19%
Live | 76,884 | 97.09% | 76.88%
CD3 | 13,267 | 17.20% | 13.27%
PD1 hi | 5,468 | 41.22% | 5.47%
PD1- | 4,024 | 30.33% | 4.02%
PD1 mid | 3,337 | 25.15% | 3.34%
J | 62,280 | 62.28% | 62.28%

FIG. 158Q

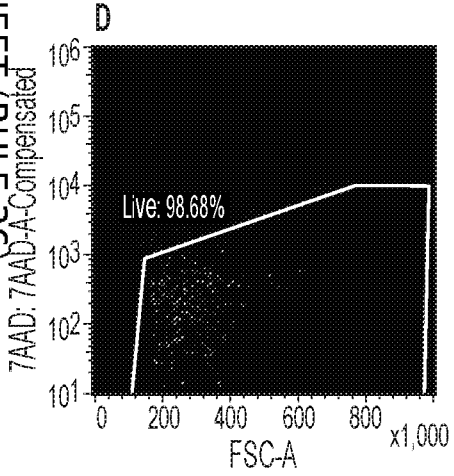
Worksheet

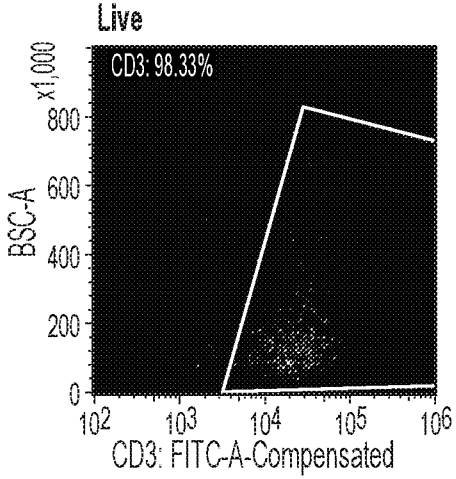


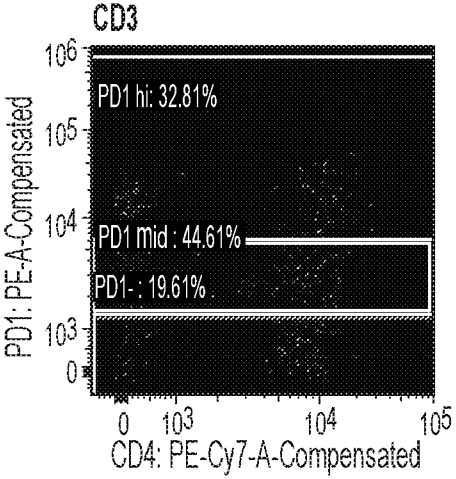


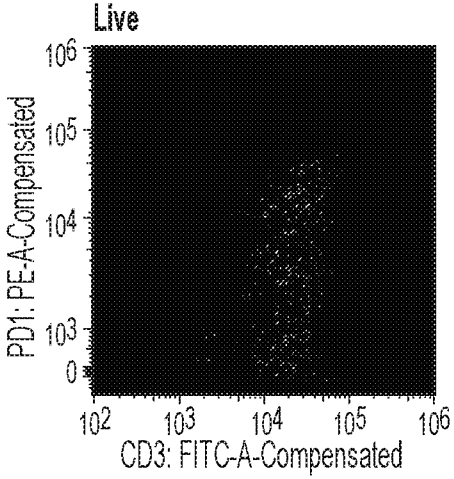
Gates and Statistics

Name	Events	%Parent	%Total
All Events	372	0.00%	100.00%
C	316	84.95%	84.95%
D	304	96.20%	81.72%
Live	300	98.68%	80.65%
CD3	295	98.33%	79.30%
PD1 hi	114	38.64%	30.65%
PD1-	88	29.83%	23.66%
PD1 mid	92	31.19%	24.73%
J	223	59.95%	59.95%





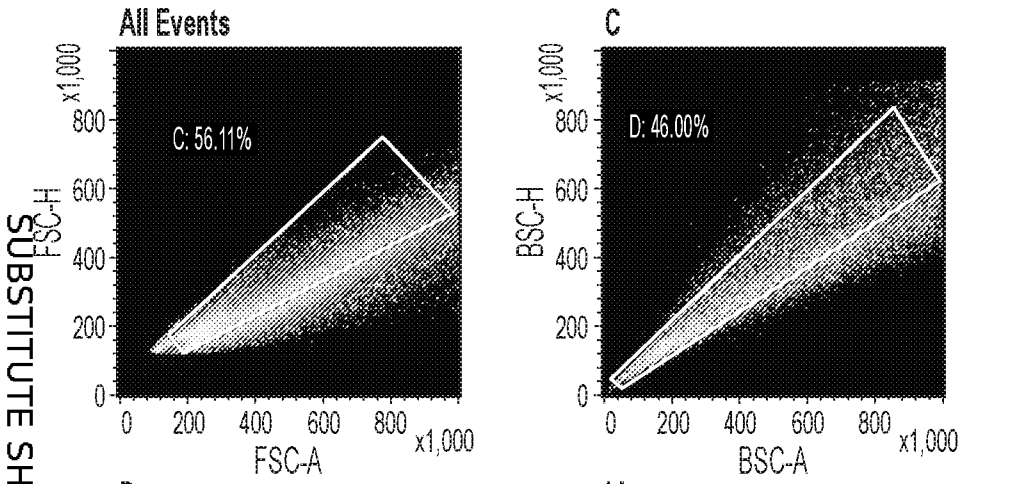




SUBSTITUTE SHEET (RULE 26)

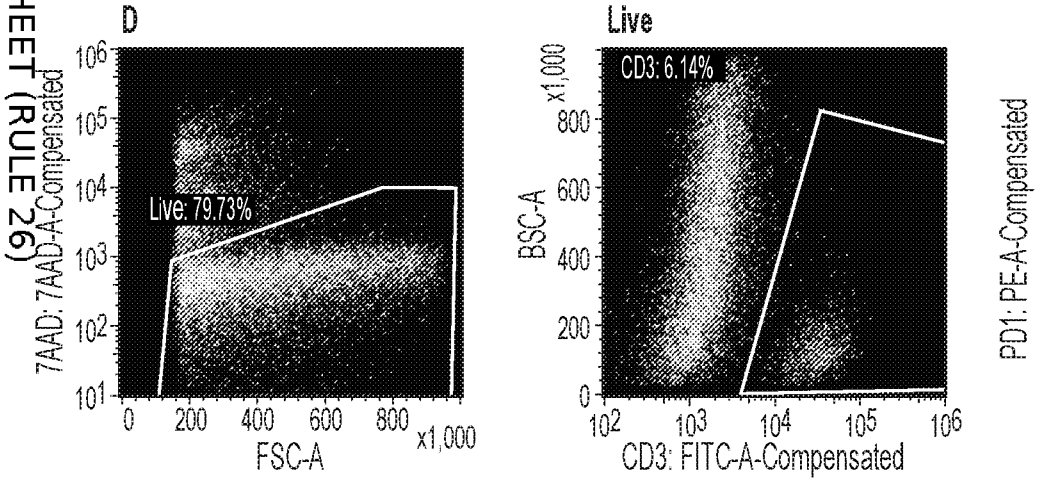
FIG. 158R

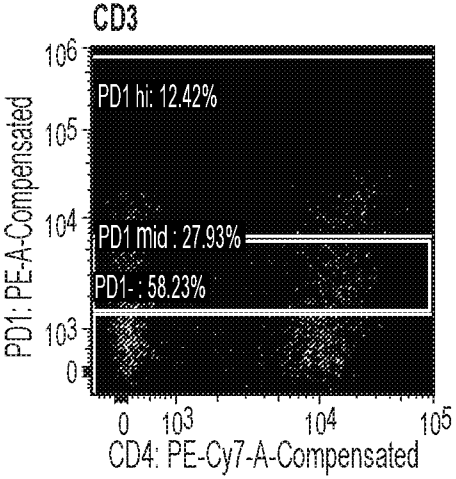
Worksheet

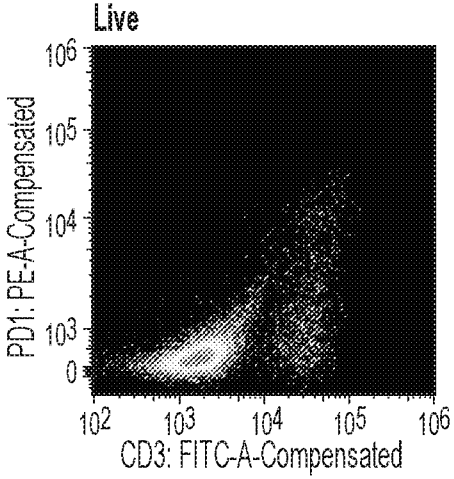


Gates and Statistics Displaying 100,000 / 1,239,610 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	56,105	56.11%	56.11%
D	25,806	46.00%	25.81%
Live	20,576	79.73%	20.58%
CD3	1,264	6.14%	1.26%
PD1 hi	157	12.42%	0.16%
PD1-	736	58.23%	0.74%
PD1 mid	353	27.93%	0.35%
J	12,686	12.69%	12.69%







SUBSTITUTE SHEET (RULE 26)

FIG. 158S

Worksheet

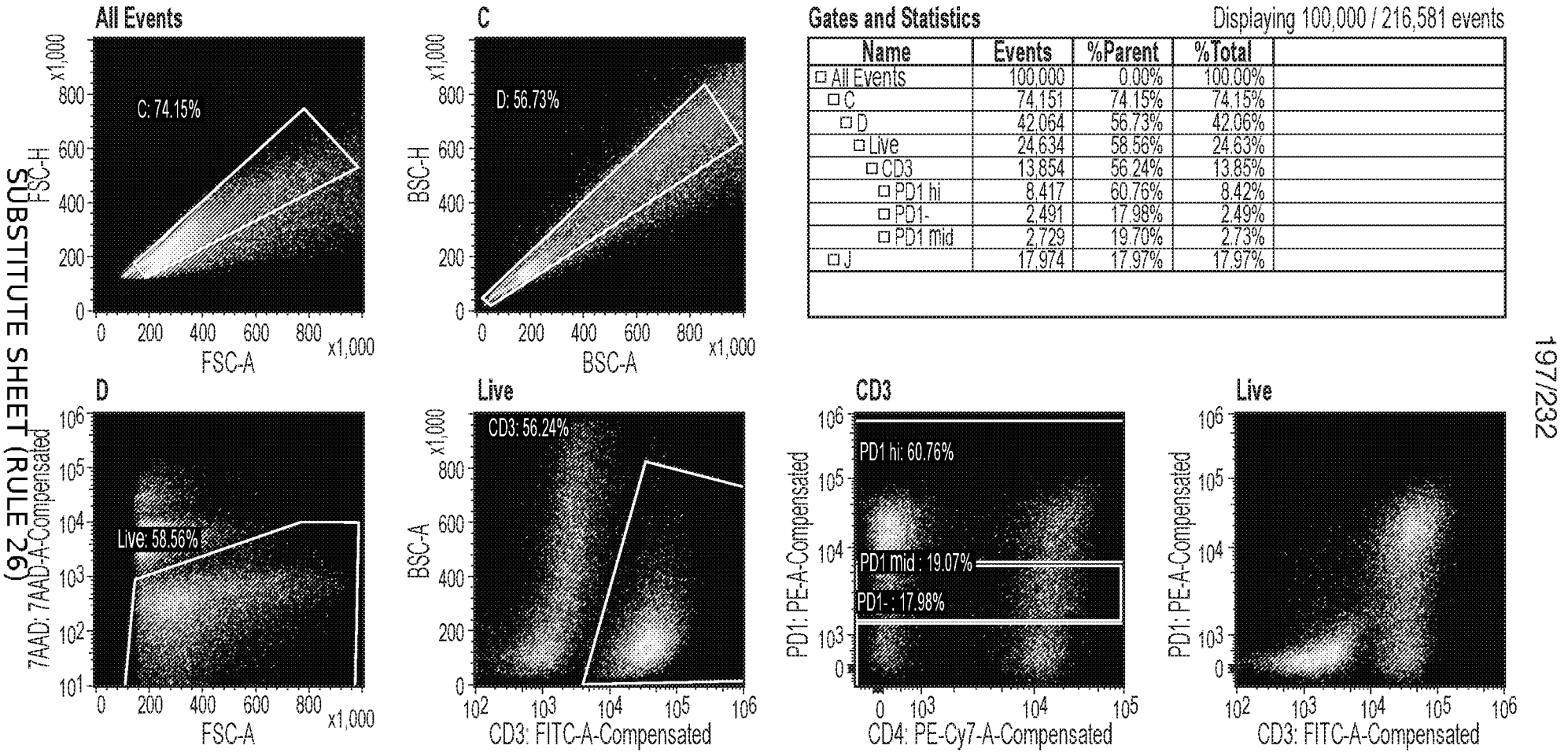
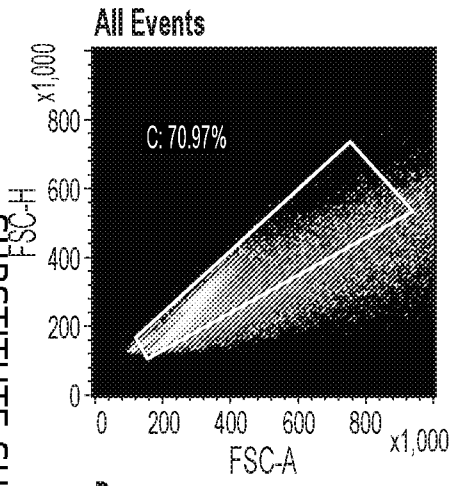
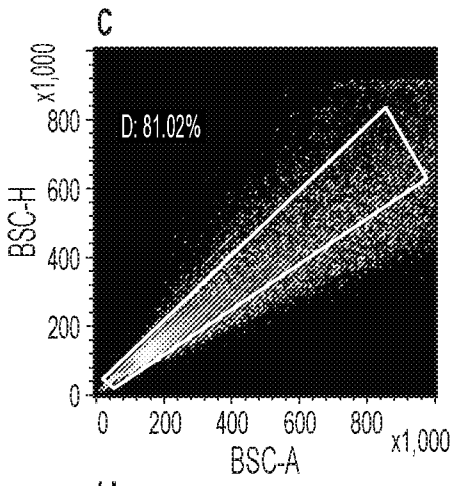


FIG. 158T

SUBSTITUTE SHEET (RULE 26)

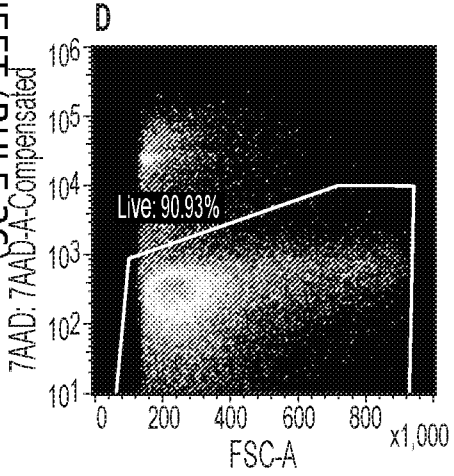
Worksheet

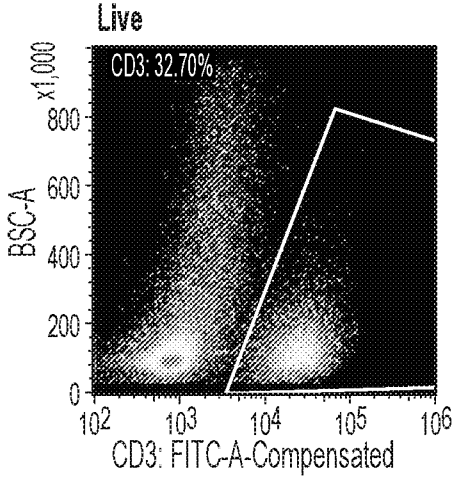


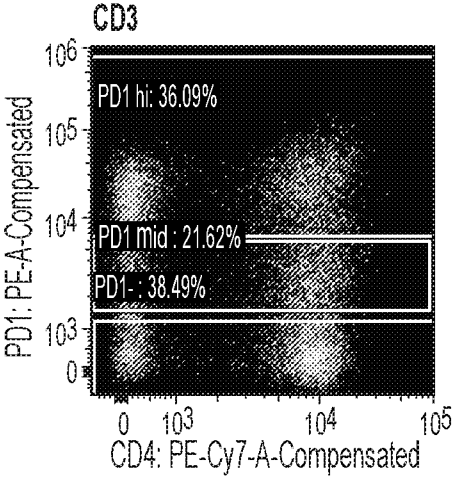


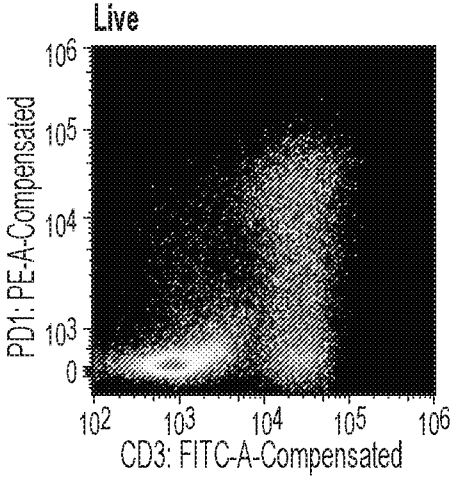
Gates and Statistics Displaying 100,000 / 175,650 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	70,968	70.97%	70.97%
D	57,501	81.02%	57.50%
Live	52,285	90.93%	52.29%
CD3	17,097	32.70%	17.10%
PD1 hi	6,170	36.09%	6.17%
PD1-	6,580	38.49%	6.58%
PD1 mid	3,697	21.62%	3.70%
J	35,775	35.78%	35.78%





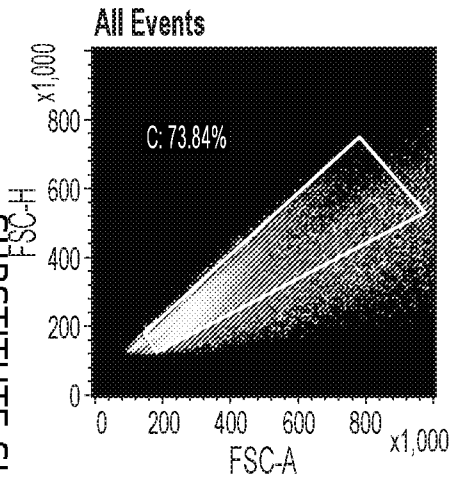


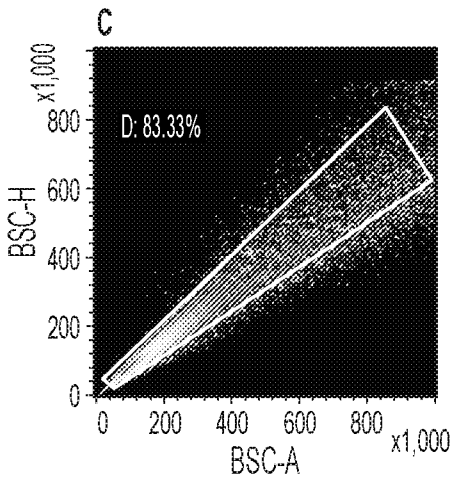


SUBSTITUTE SHEET (RULE 26)

FIG. 158U

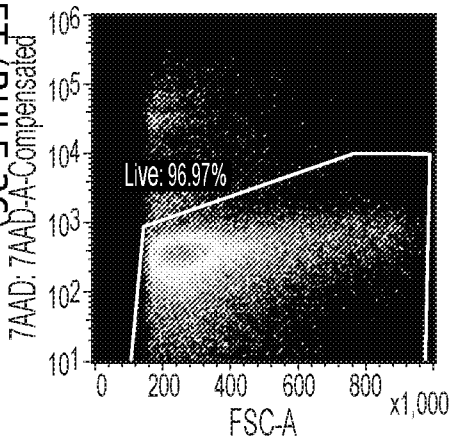
Worksheet

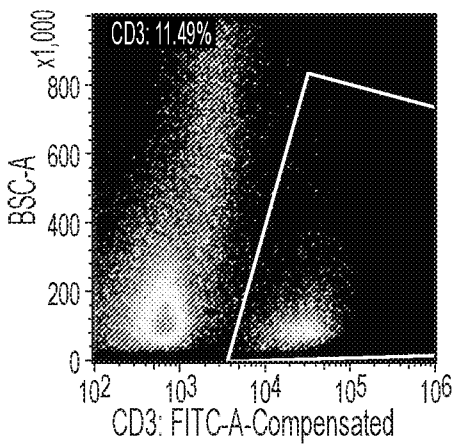


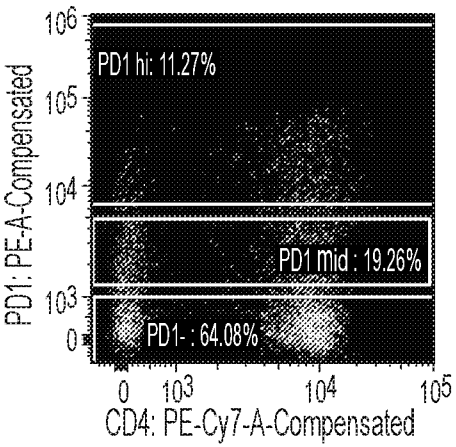


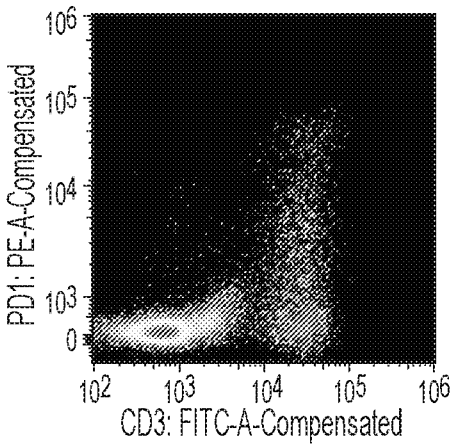
Gates and Statistics Displaying 100,000 / 435,779 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	73,837	73.84%	73.84%
D	61,528	83.33%	61.53%
Live	59,662	96.97%	59.66%
CD3	6,857	11.49%	6.86%
PD1 hi	773	11.27%	0.77%
PD1-	4,394	64.08%	4.39%
PD1 mid	1,321	19.26%	1.32%
J	40,649	40.65%	40.65%





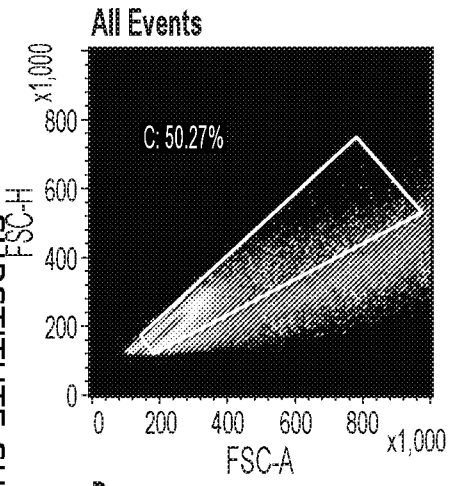


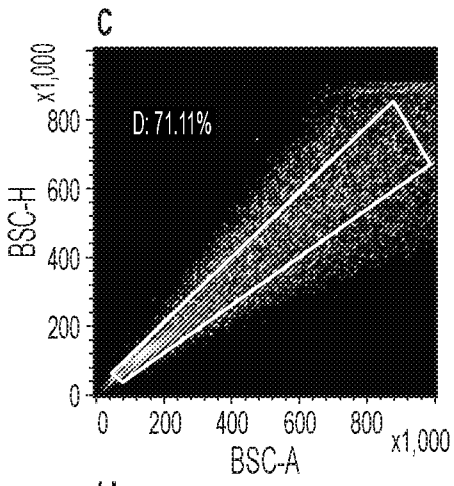


SUBSTITUTE SHEET (RULE 26)

FIG. 158V

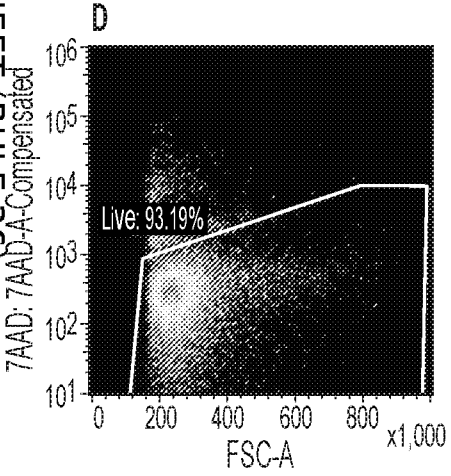
Worksheet

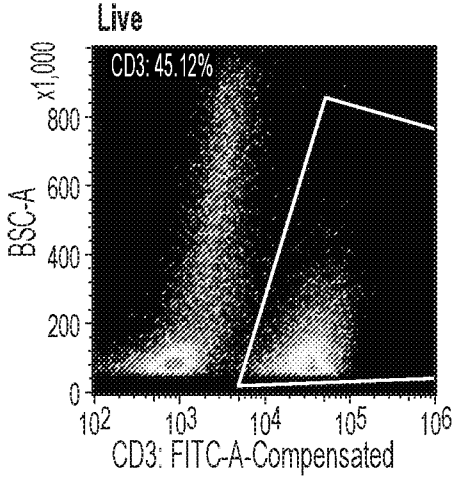


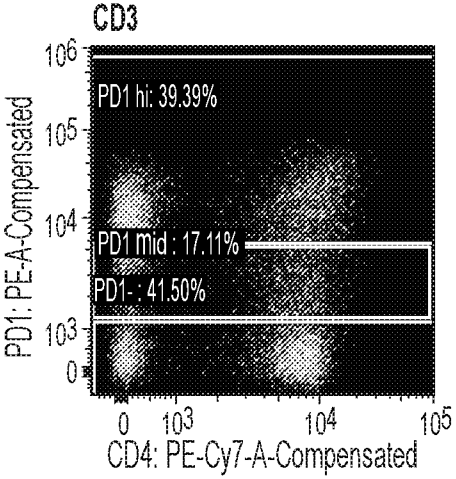


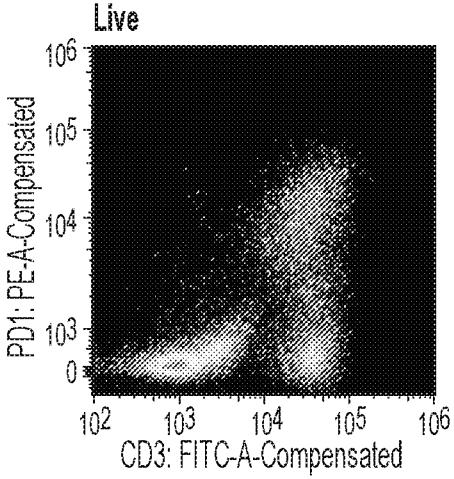
Gates and Statistics Displaying 100,000 / 198,892 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	50,267	50.27%	50.27%
D	35,745	71.11%	35.75%
Live	33,309	93.19%	33.31%
CD3	15,030	45.12%	15.03%
PD1 hi	5,920	39.39%	5.92%
PD1-	6,238	41.50%	6.24%
PD1 mid	2,571	17.11%	2.57%
J	22,093	22.09%	22.09%









SUBSTITUTE SHEET (RULE 26)

FIG. 158W

Worksheet

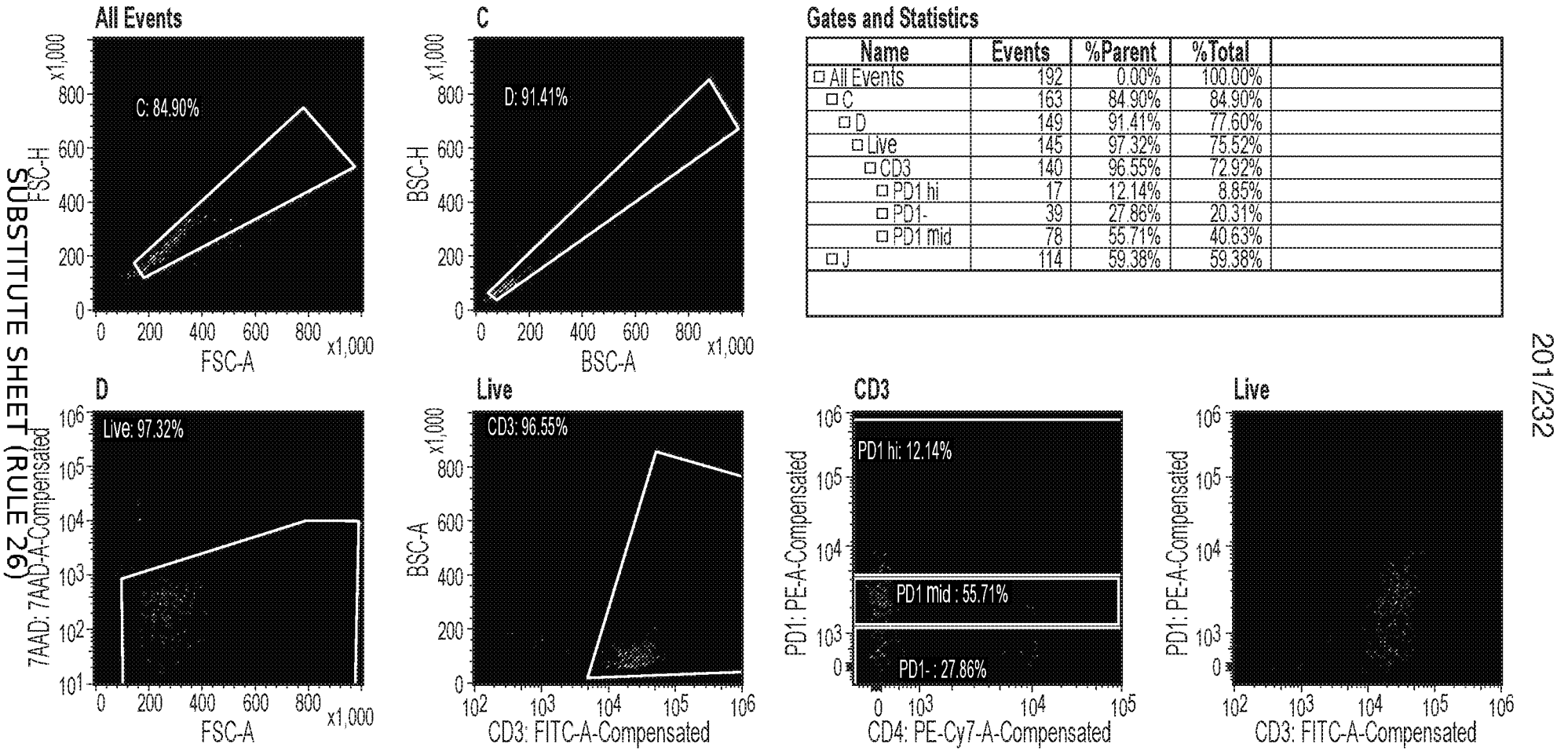
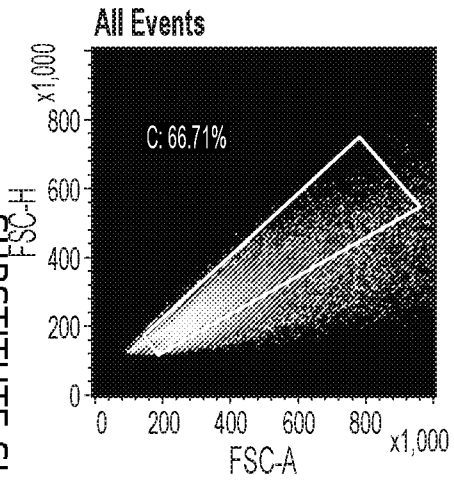
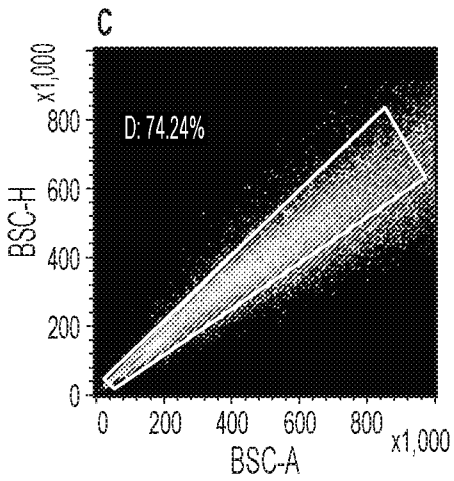


FIG. 158X

SUBSTITUTE SHEET (RULE 26)

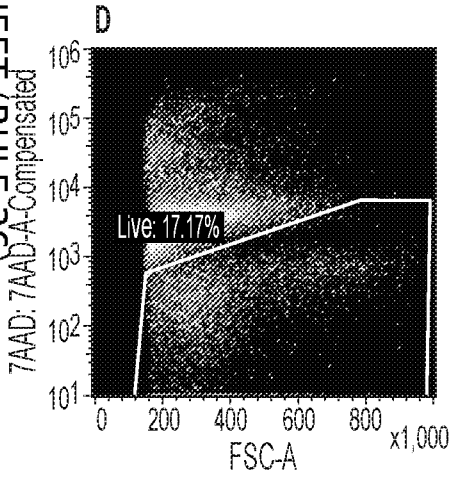
Worksheet

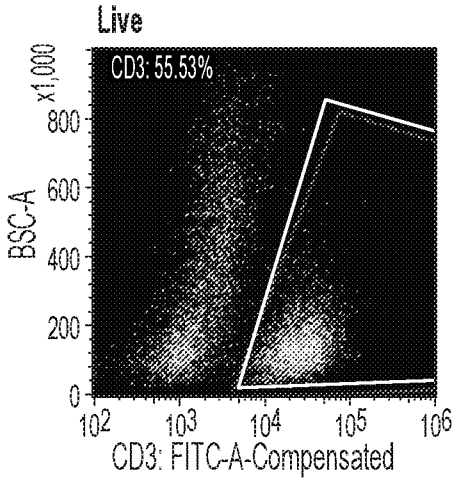


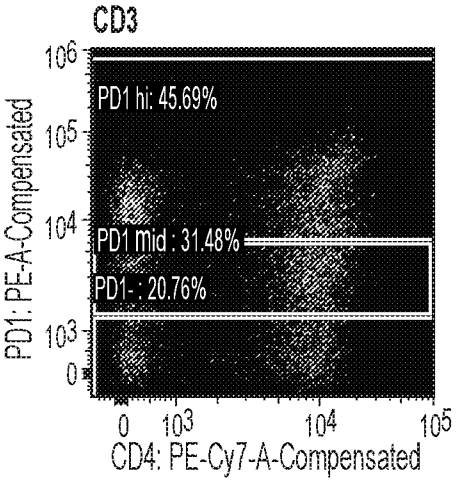


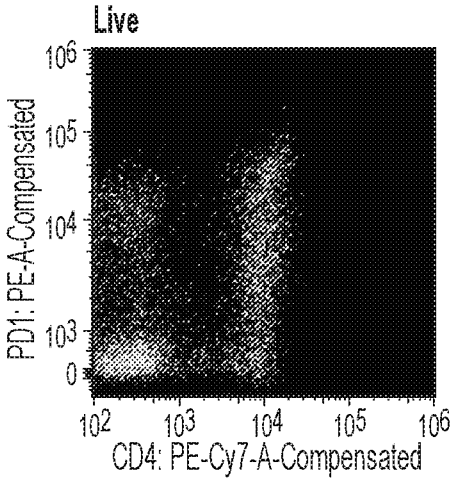
Gates and Statistics Displaying 100,000 / 389,100 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	66,706	66.71%	66.71%
D	49,520	74.24%	49.52%
Live	8,502	17.17%	8.50%
CD3	4,721	55.53%	4.72%
PD1 hi	2,157	45.69%	2.16%
PD1-	980	20.76%	0.98%
PD1 mid	1,486	31.48%	1.49%
J	8,340	8.34%	8.34%





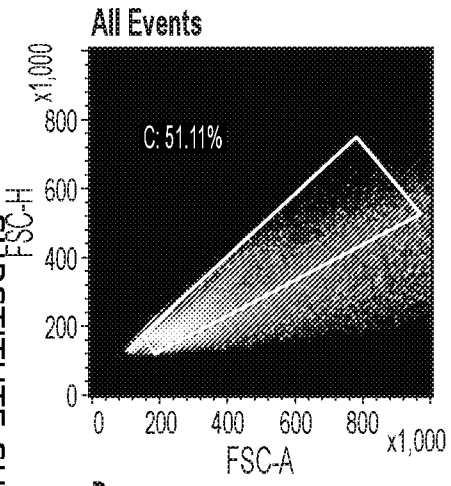


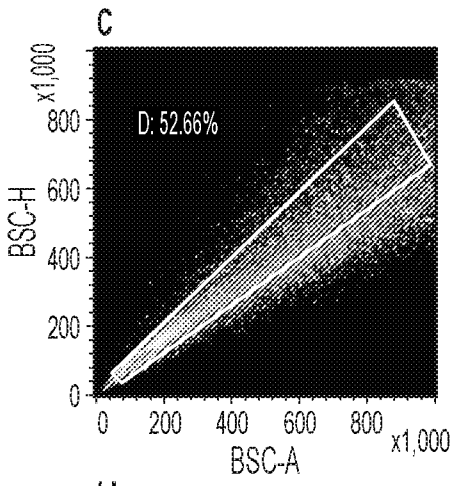


SUBSTITUTE SHEET (RULE 26)

FIG. 158Y

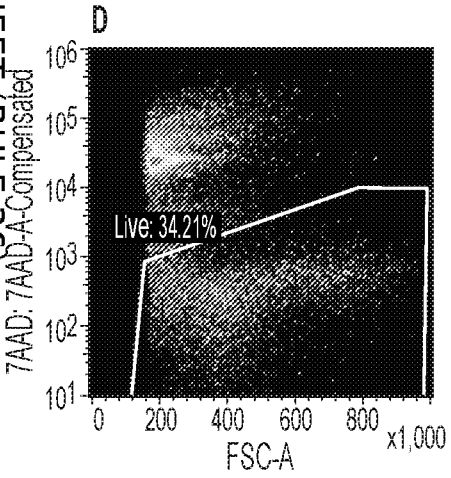
Worksheet

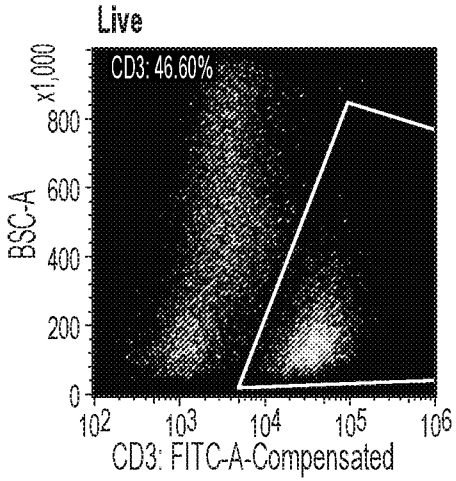


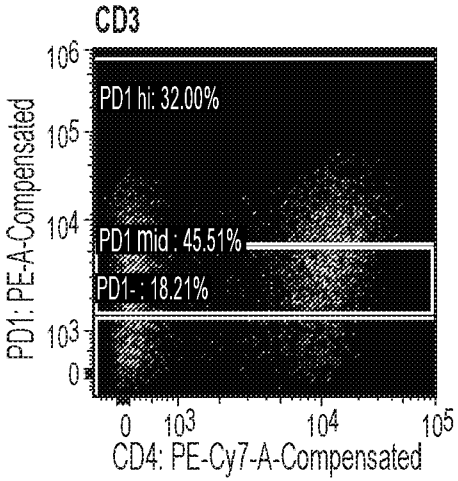


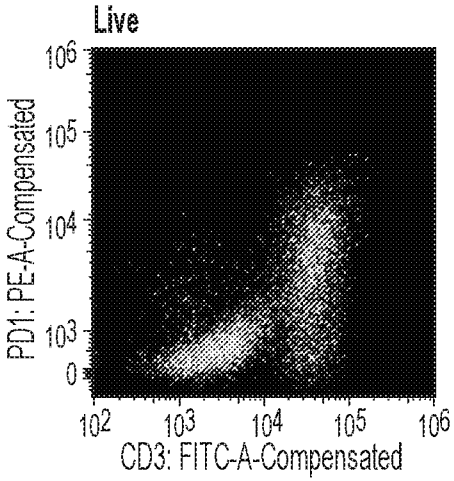
Gates and Statistics — Displaying 100,000 / 693,862 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	51,109	51.11%	51.11%
D	26,916	52.66%	26.92%
Live	9,209	34.21%	9.21%
CD3	4,300	46.69%	4.30%
PD1 hi	1,376	32.00%	1.38%
PD1-	783	18.21%	0.78%
PD1 mid	1,957	45.51%	1.96%
J	10,222	10.22%	10.22%





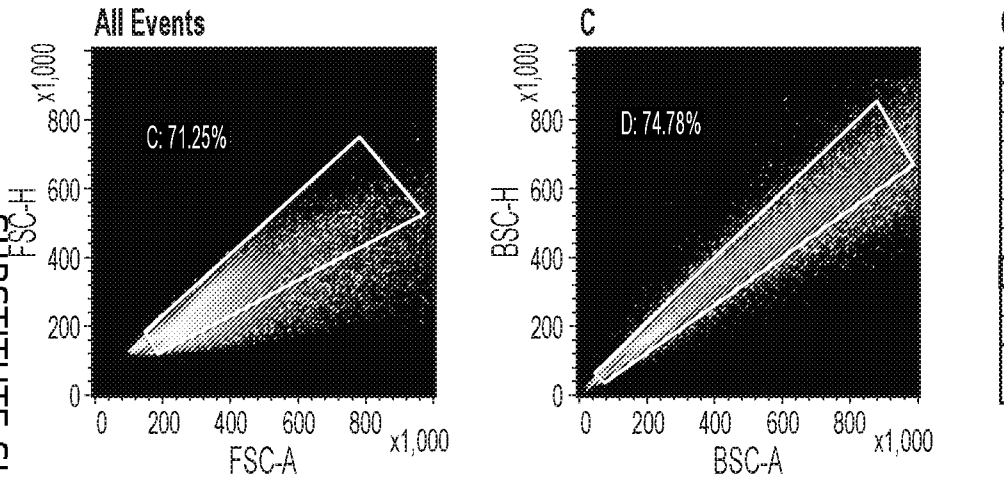




SUBSTITUTE SHEET (RULE 26)

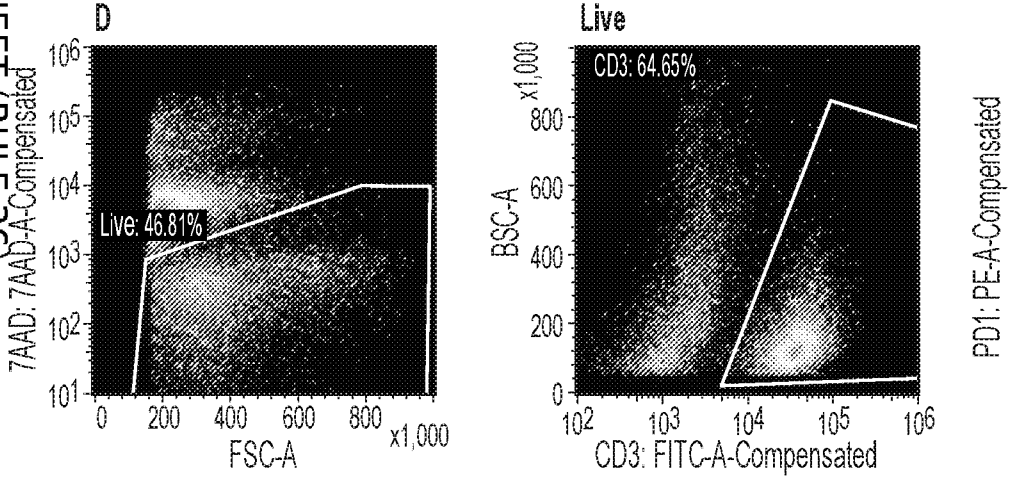
FIG. 158Z

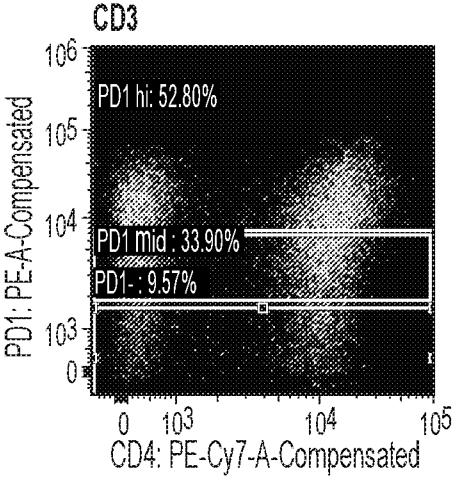
Worksheet



Gates and Statistics Displaying 100,000 / 191,392 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	71,247	71.25%	71.25%
D	53,280	74.78%	53.28%
Live	24,938	46.81%	24.94%
CD3	16,122	64.65%	16.12%
PD1 hi	8,512	52.80%	8.51%
PD1-	1,543	9.57%	1.54%
PD1 mid	5,466	33.90%	5.47%
J	22,012	22.01%	22.01%





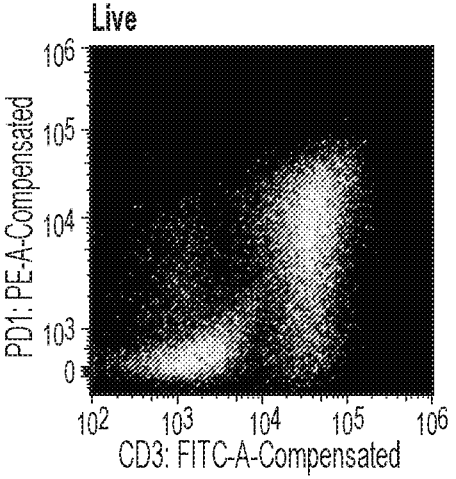
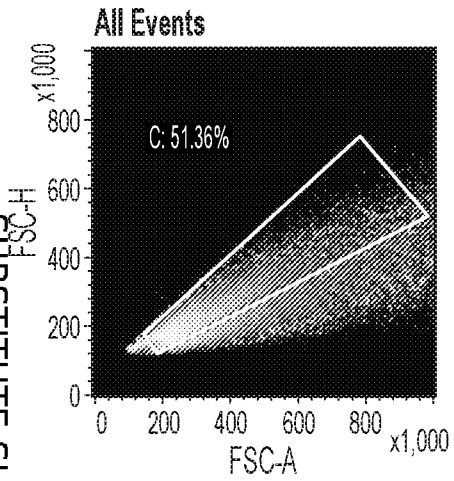
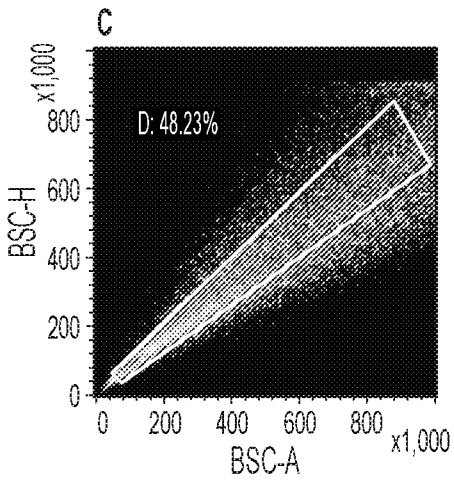


FIG. 158AA

SUBSTITUTE SHEET (RULE 26)

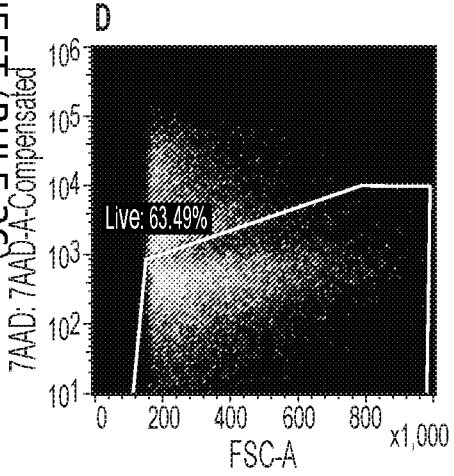
Worksheet

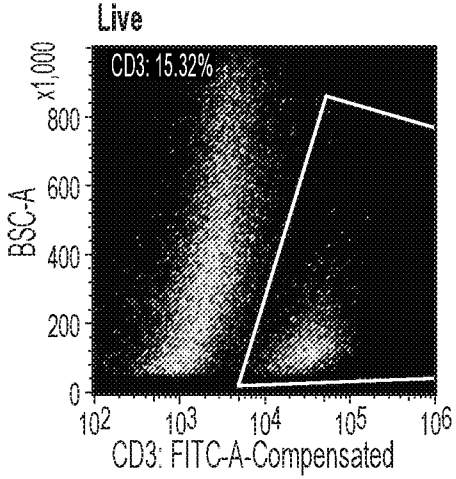


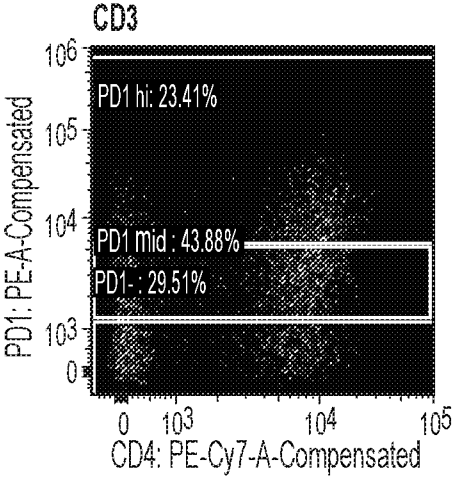


Gates and Statistics Displaying 100,000 / 127,786 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	51,364	51.36%	51.36%
D	24,774	48.23%	24.77%
Live	15,729	63.49%	15.73%
CD3	2,409	15.32%	2.41%
PD1 hi	564	23.41%	0.56%
PD1-	711	29.51%	0.71%
PD1 mid	1,057	43.88%	1.06%
J	9,953	9.95%	9.95%







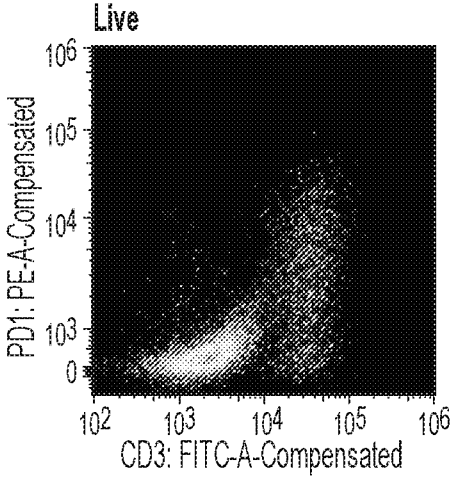
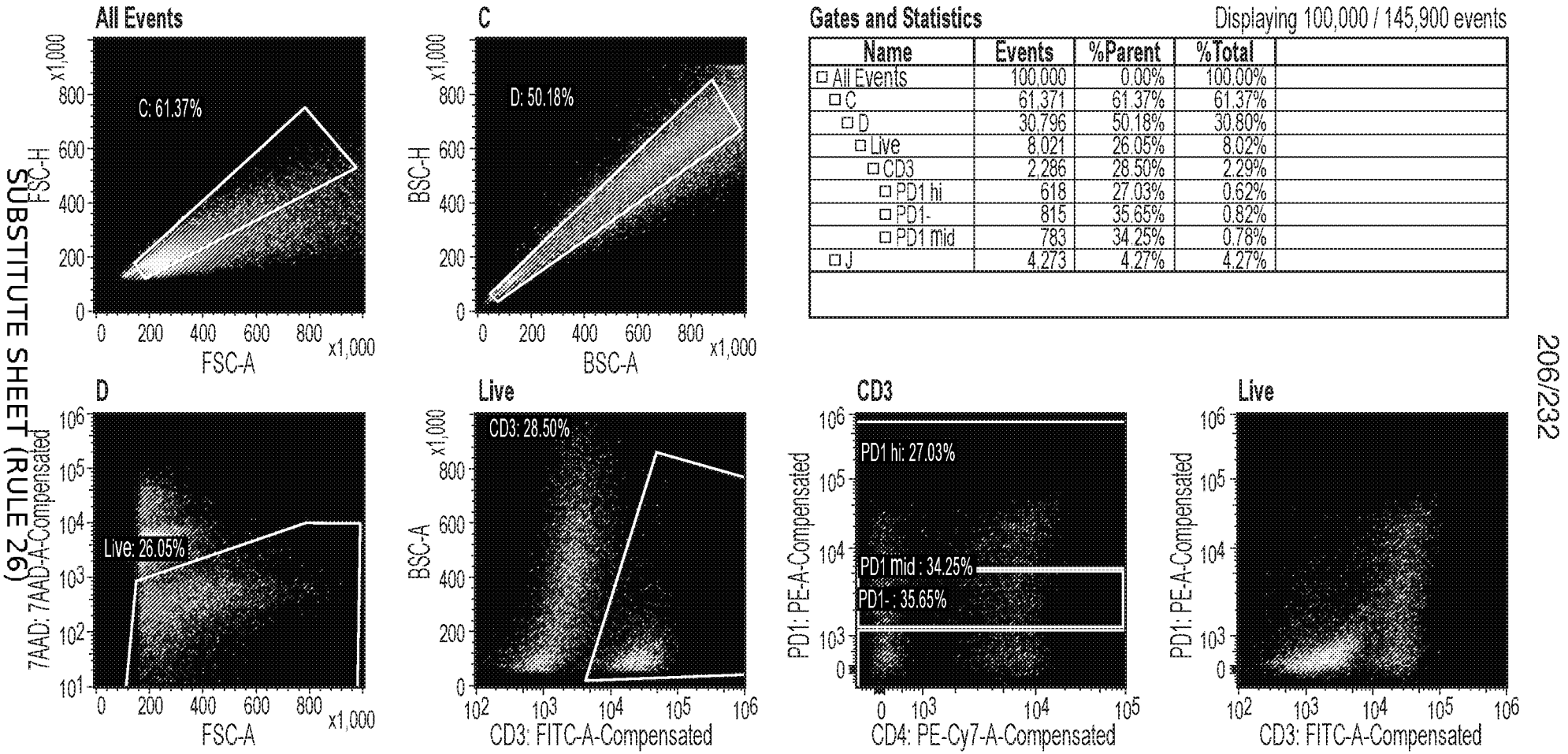


FIG. 158BB

SUBSTITUTE SHEET (RULE 26)

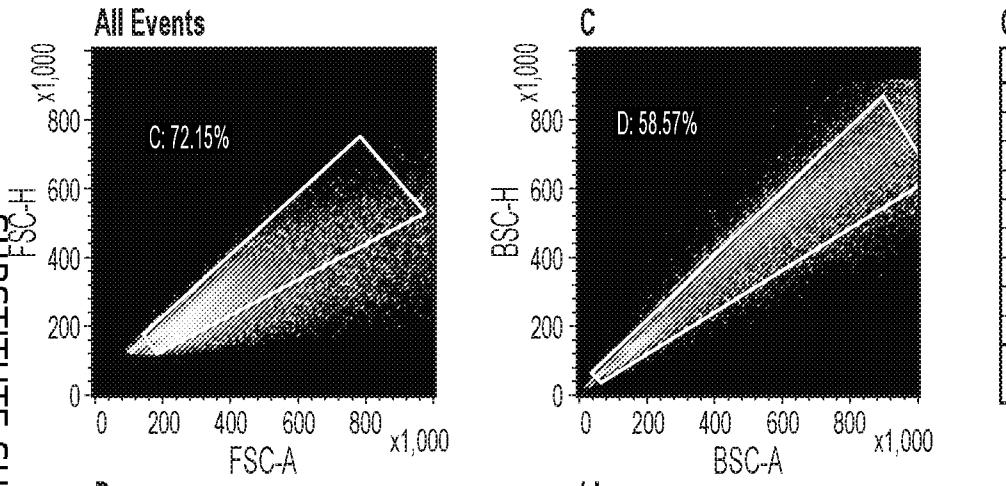
Worksheet



SUBSTITUTE SHEET (RULE 26)

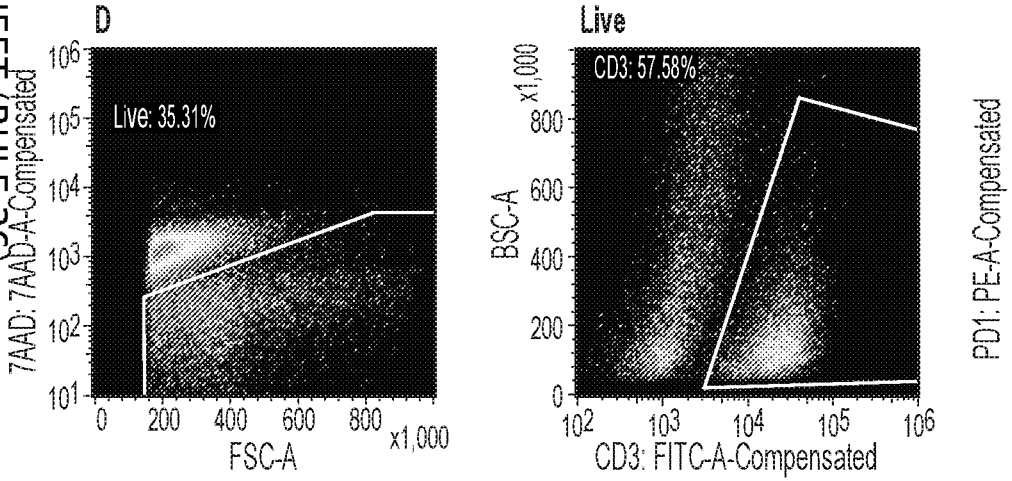
FIG. 158CC

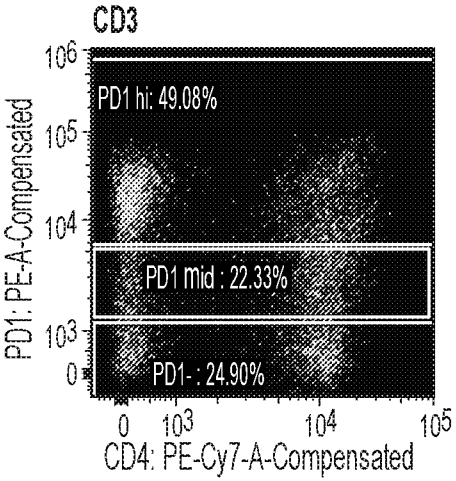
Worksheet



Gates and Statistics Displaying 100,000 / 351,880 events

Name	Events	%Parent	%Total
All Events	100,000	0.00%	100.00%
C	72,145	72.15%	72.15%
D	42,254	58.57%	42.25%
Live	14,921	35.31%	14.92%
CD3	8,592	57.58%	8.59%
PD1 hi	4,217	49.08%	4.22%
PD1-	2,139	24.90%	2.14%
PD1 mid	1,919	22.33%	1.92%
J	11,157	11.16%	11.16%





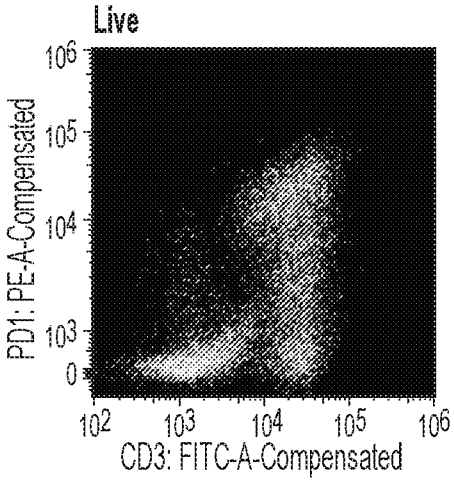
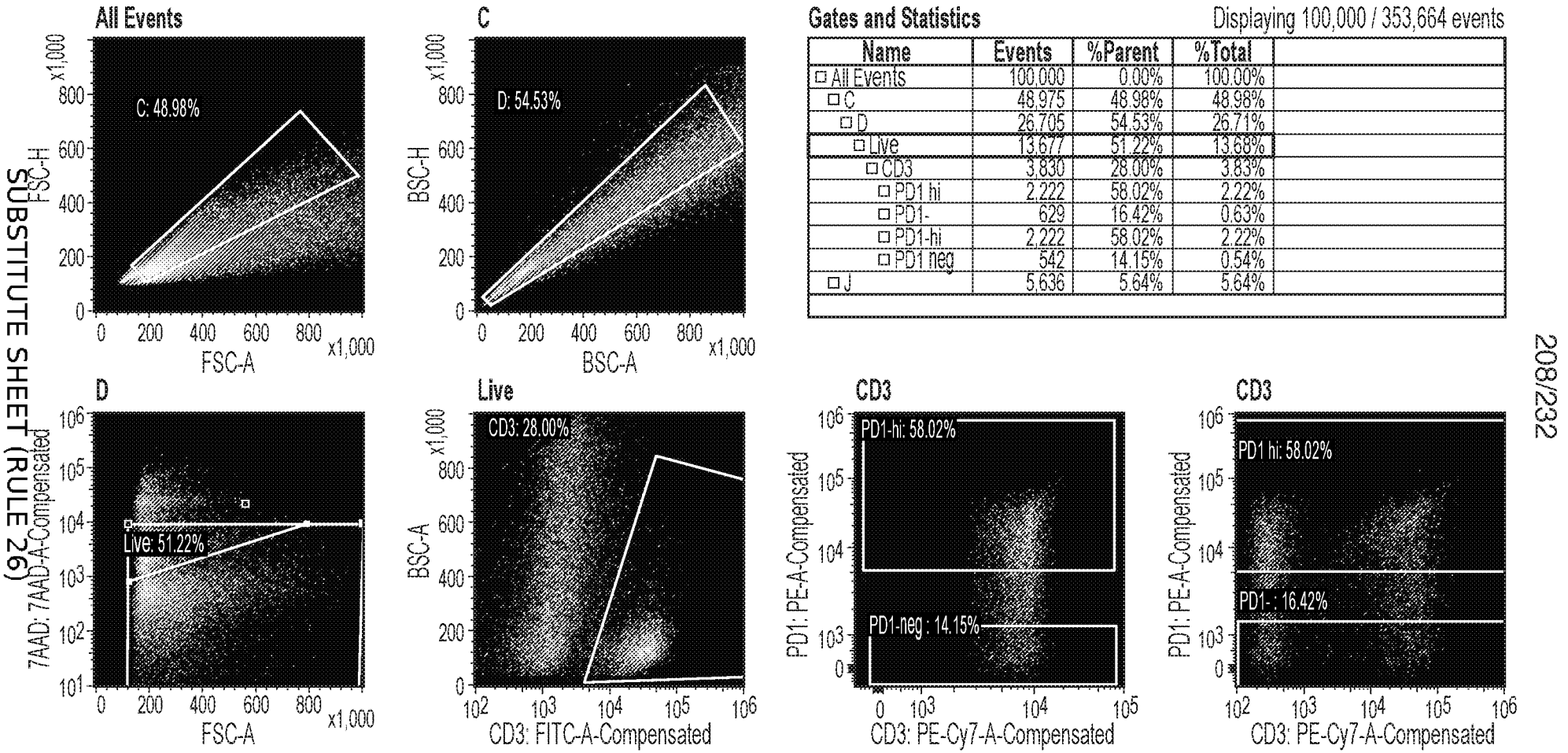


FIG. 158DD

SUBSTITUTE SHEET (RULE 26)

Worksheet



SUBSTITUTE SHEET (RULE 26)

FIG. 158EE

	FSC Single Cells/SSC Single Cells/Live/CD3 | Freq. of Parent	FSC Single Cells/SSC Single Cells/Live/CD3/PD1hi gh | Freq. of Parent
B3 CC10026.fcs	15.4%	32.5%
B4 CC10027.fcs	16.8%	31.6%
B5 PD-1 FMO.fcs	34.4%	0%
B6 PBMC.fcs	16.9%	4.52%
Mean	20.9%	17.2%
SD	9.04%	17.3%

FIG. 158FF

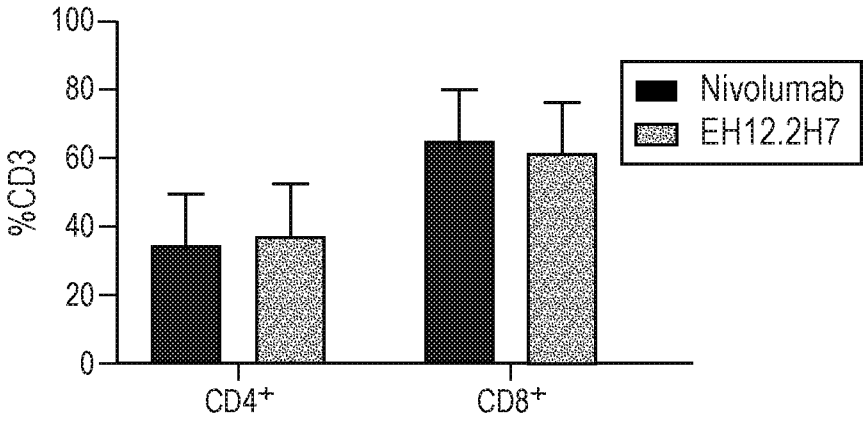


FIG. 159

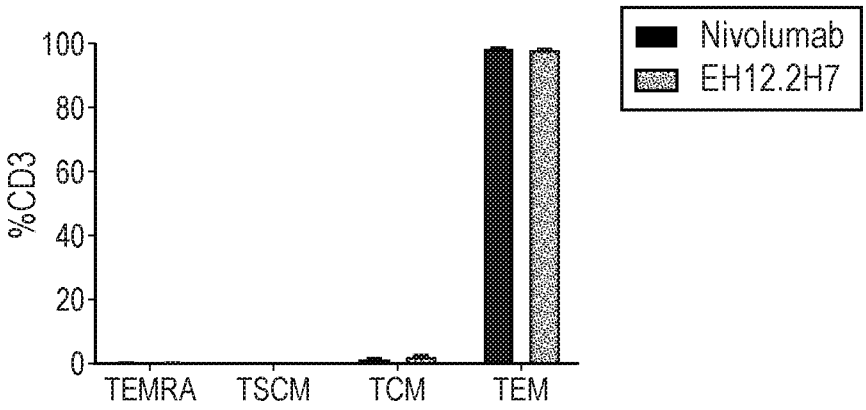


FIG. 160

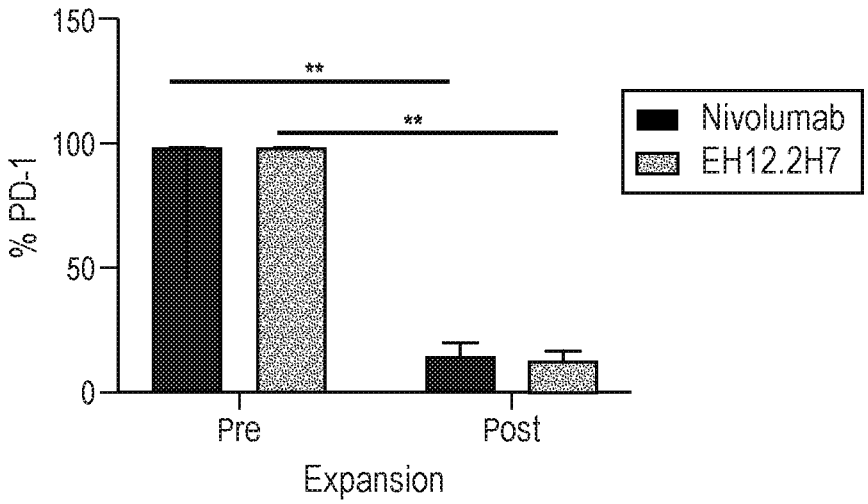


FIG. 161A

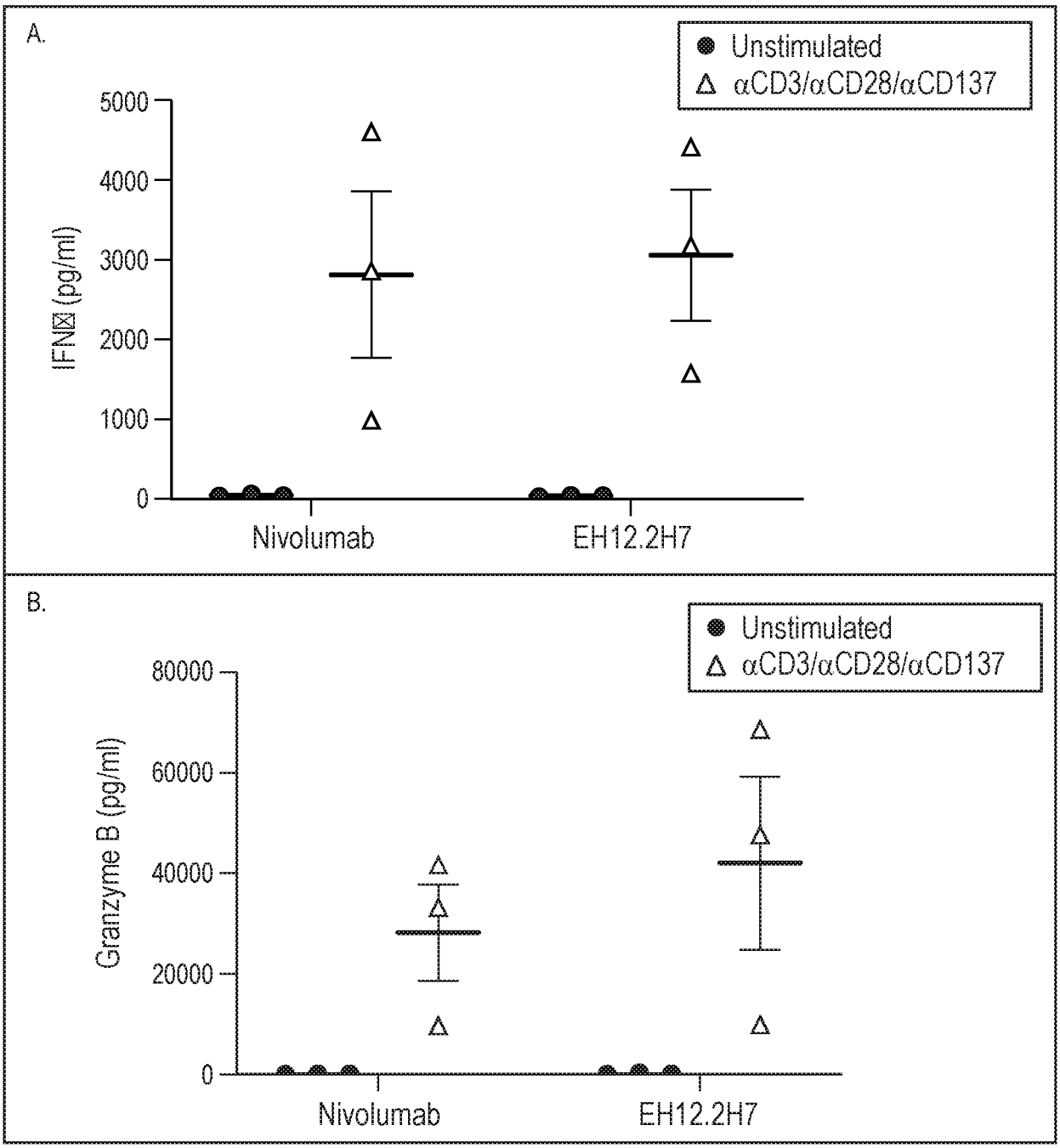

A.
Unstimulated
αCD3/αCD28/αCD137
5000
4000
3000
2000
1000
0
IFN (pg/ml)
Nivolumab
EH12.2H7
B.
Unstimulated
αCD3/αCD28/αCD137
80000
60000
40000
20000
0
Granzyme B (pg/ml)
Nivolumab
EH12.2H7

FIG. 161B

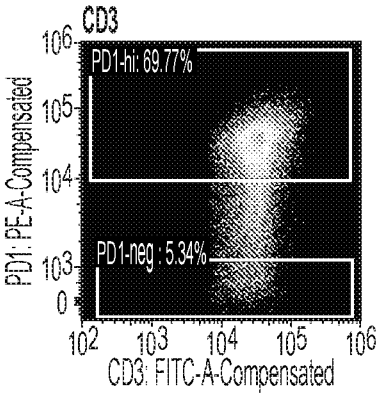
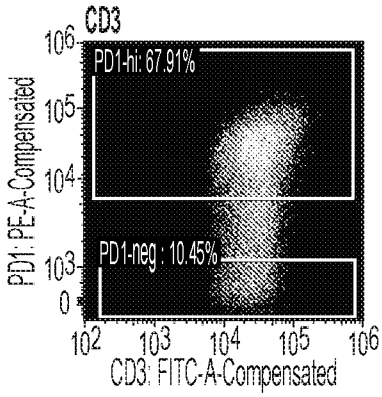
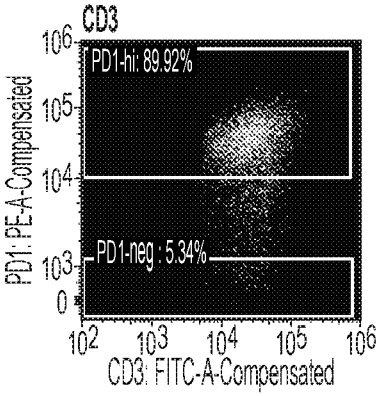
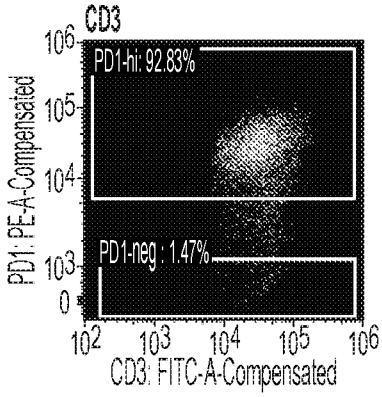
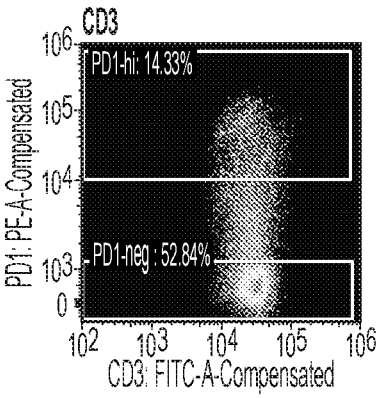
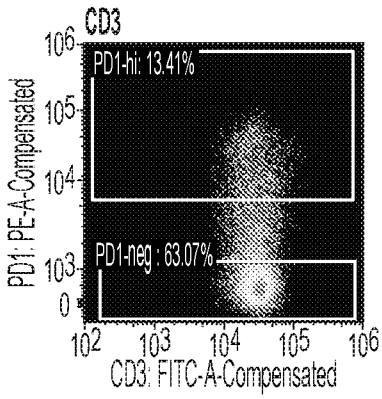
	Nivolumab	EH12.2H7
Ovarian		
Melanoma		
HNSCC		

FIG. 162

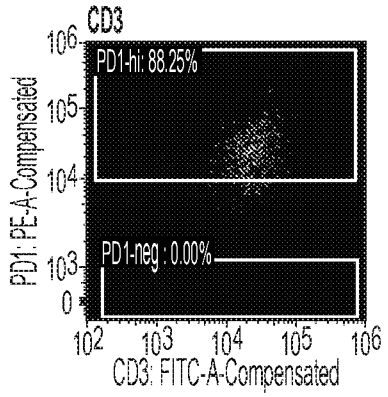
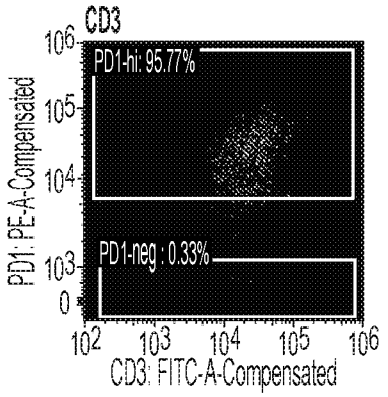
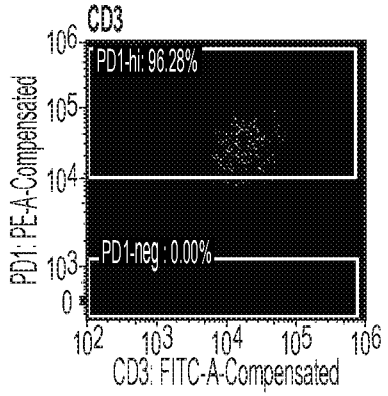
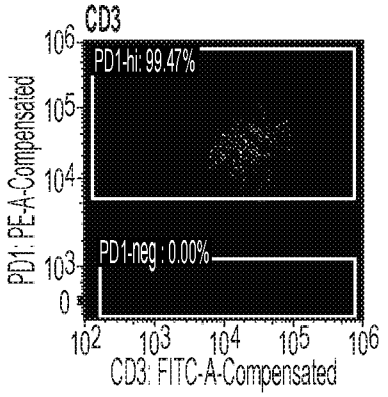
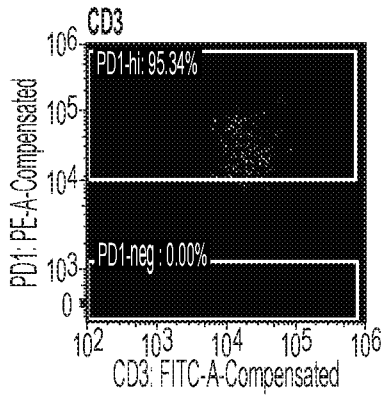
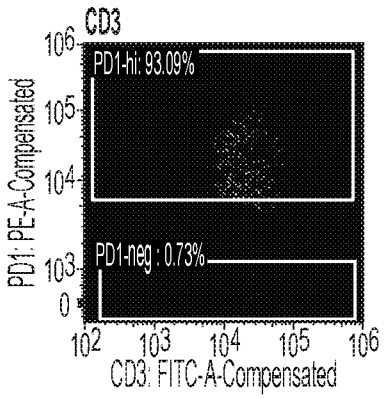
	Nivolumab	EH12.2H7
Ovarian		
Melanoma		
HNSCC		

FIG. 163

SUBSTITUTE SHEET (RULE 26)

Sample Name	Cell Count & Viability												
	Volume (ml)	Count 1		Count 2		Counts 1 and 2 within 10% of Mean?	Count 3		Count 4		Counts 3 and 4 within 10% of Mean?	Average		Total Viable Cells
		Viable Cells/mL	% Viability	Viable Cells/mL	% Viability		Viable Cells/mL	% Viability	Viable Cells/mL	% Viability		Viable Cells/mL	% Viability	
Nivo Stability 120h baseline vol 30 C1	30	4.75E+07	90.9	5.40E+07	89.8	YES	5.56E+07	91.40	5.71E+07	91.40	YES	5.36E+07	90.88	1.61E+09

FIG. 164

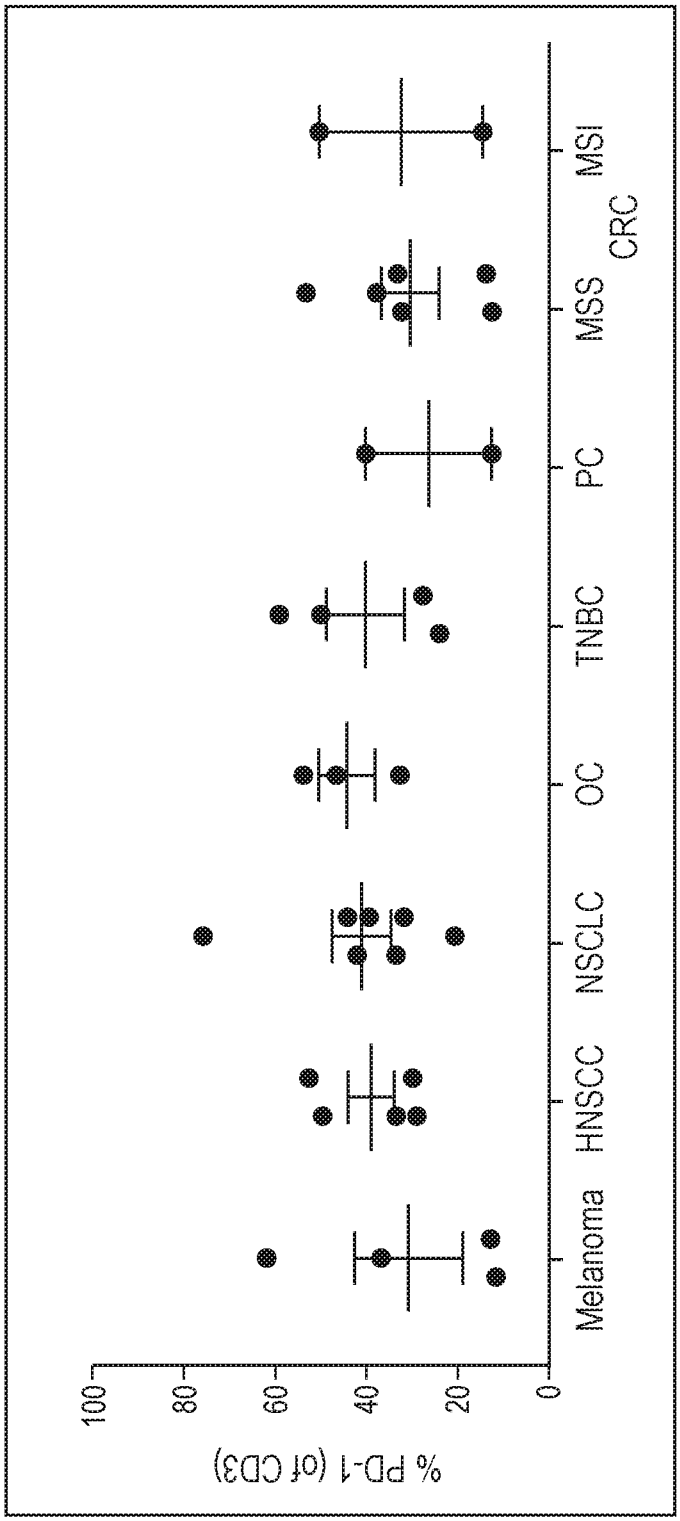

% PD-1 (of CD3)
100
80
60
40
20
0
Melanoma
HNSCC
NSCLC
OC
TNBC
PC
MSS
MSI
CRC

FIG. 165

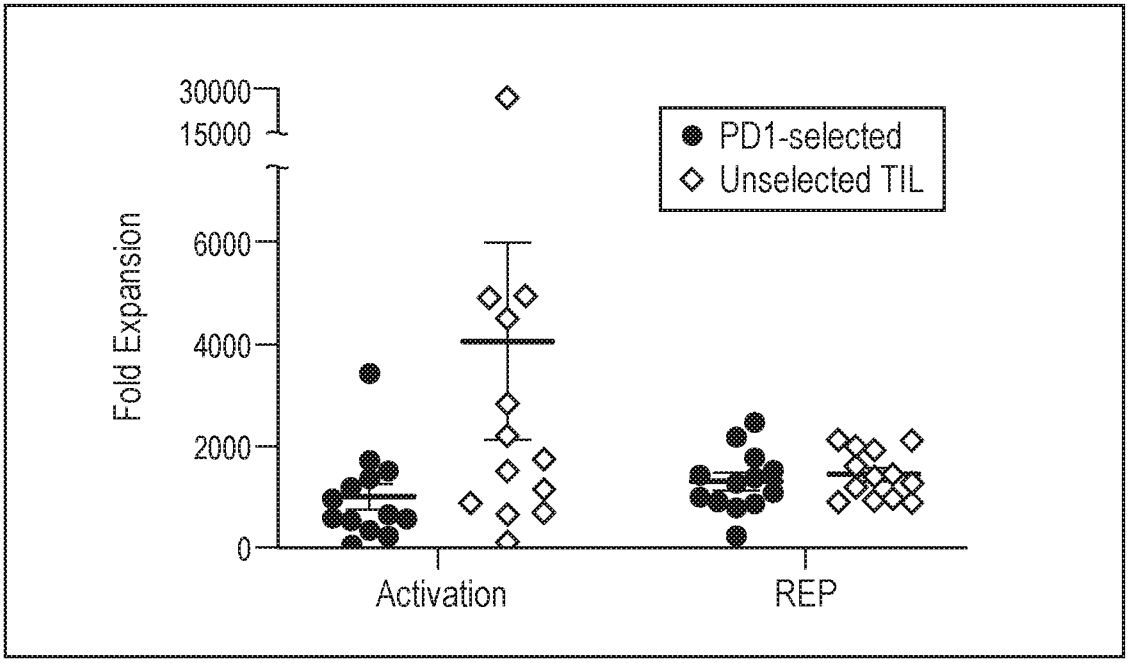

30000
15000
6000
4000
2000
0
Fold Expansion
PD1-selected
Unselected TIL
Activation
REP

FIG. 166

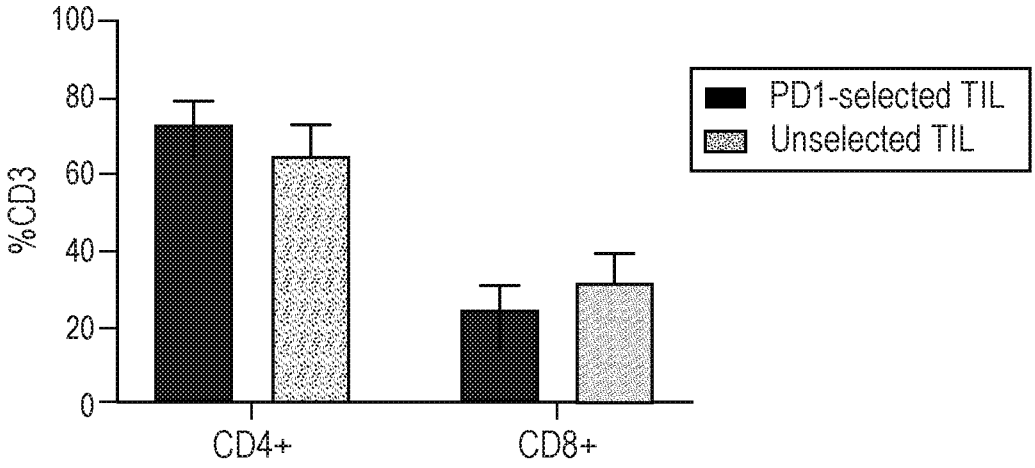


FIG. 167

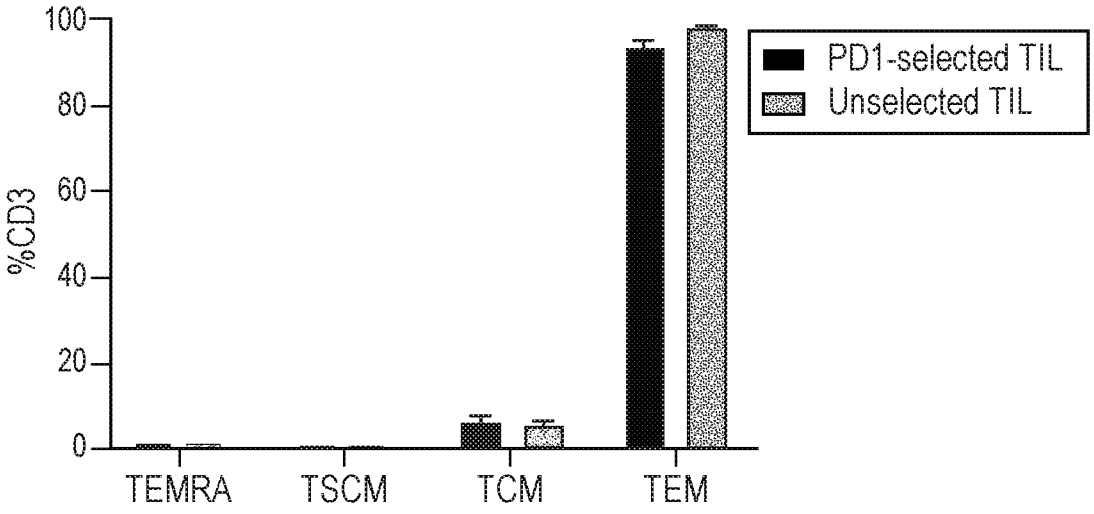

100
80
60
40
20
0
%CD3
TEMRA
TSCM
TCM
TEM
PD1-selected TIL
Unselected TIL

FIG. 168

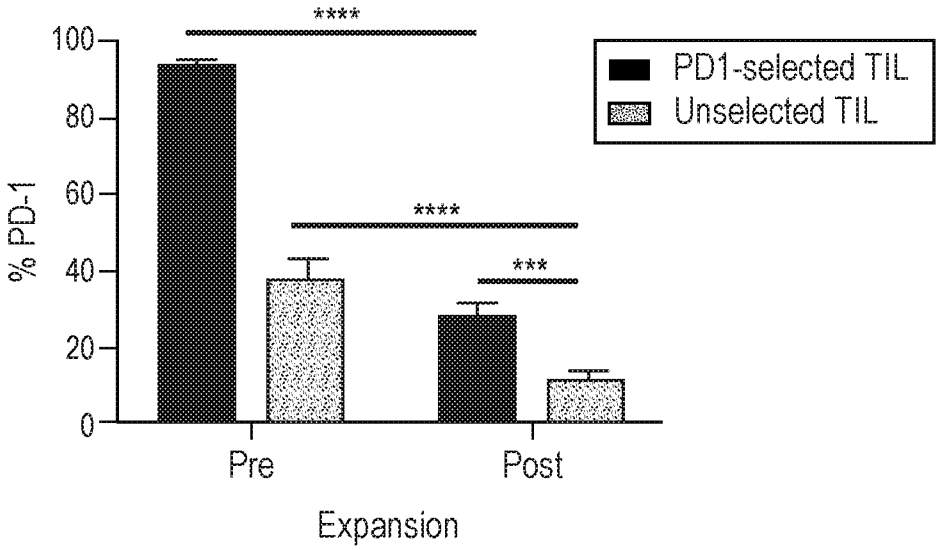

****
100
80
60
40
20
0
% PD-1
PD1-selected TIL
Unselected TIL
****
***
Pre
Post
Expansion

FIG. 169

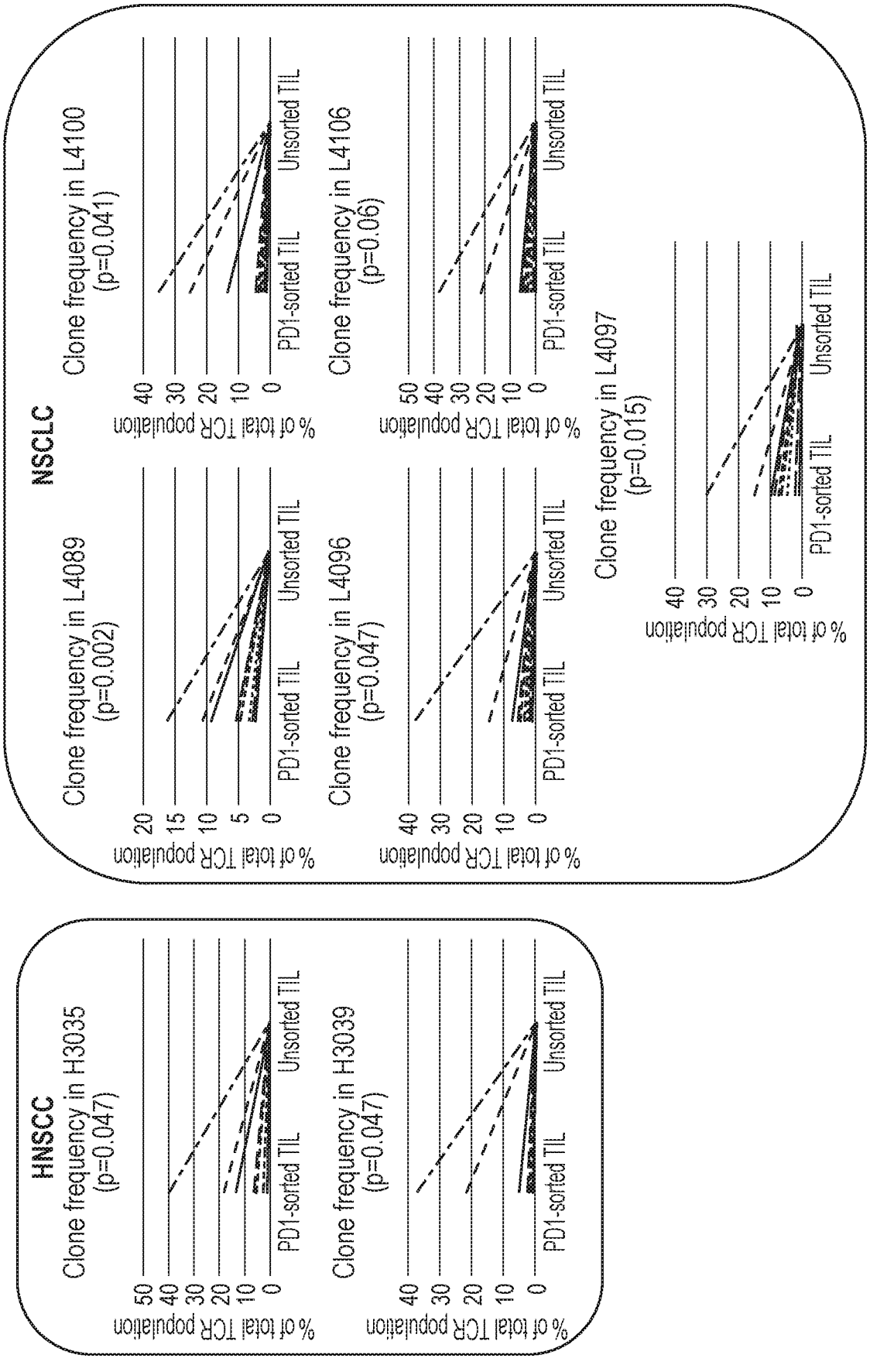

NSCLC
Clone frequency in L4100
(p=0.041)
Clone frequency in L4106
(p=0.06)
Clone frequency in L4089
(p=0.002)
Clone frequency in L4096
(p=0.047)
Clone frequency in L4097
(p=0.015)
% of total TCR population
PD1-sorted TIL
Unsorted TIL
HNSCC
Clone frequency in H3035
(p=0.047)
Clone frequency in H3039
(p=0.047)

FIG. 170

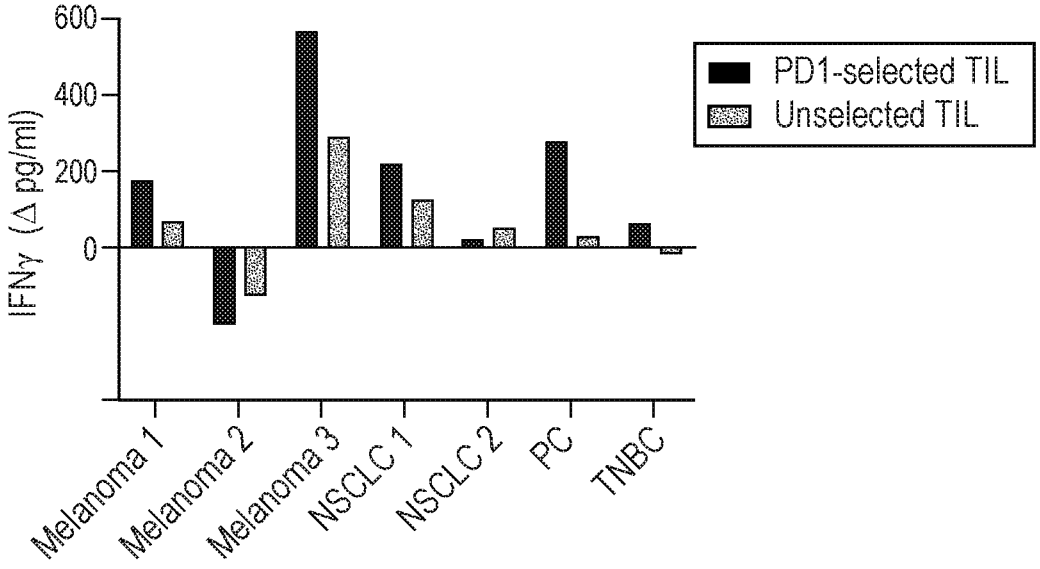

600
400
200
0
IFNγ (Δ pg/ml)
PD1-selected TIL
Unselected TIL
Melanoma 1
Melanoma 2
Melanoma 3
NSCLC 1
NSCLC 2
PC
TNBC

FIG. 171

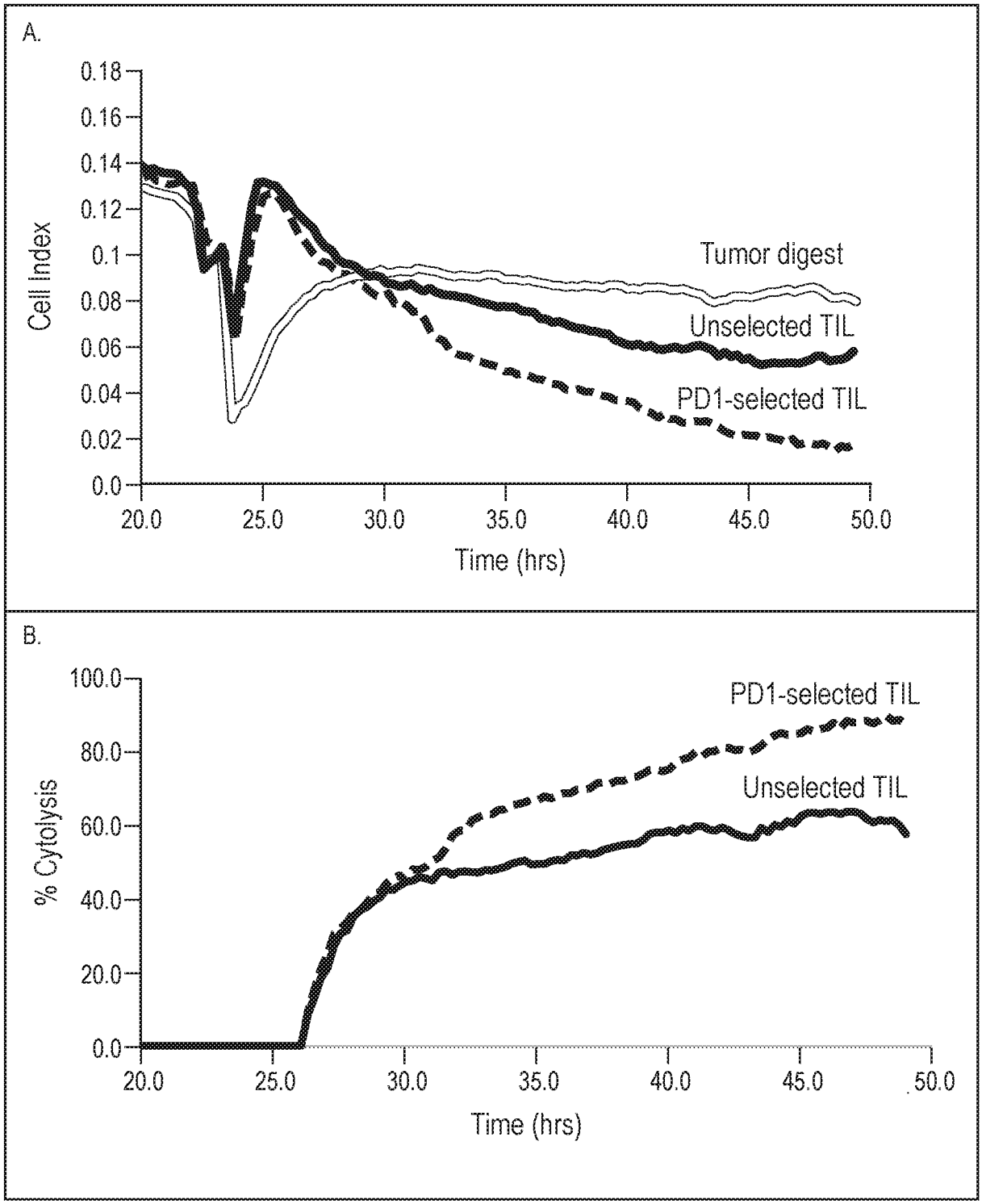

A.
Cell Index
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
Time (hrs)
Tumor digest
Unselected TIL
PD1-selected TIL
B.
% Cytolysis
100.0
80.0
60.0
40.0
20.0
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
Time (hrs)
PD1-selected TIL
Unselected TIL

FIG. 172

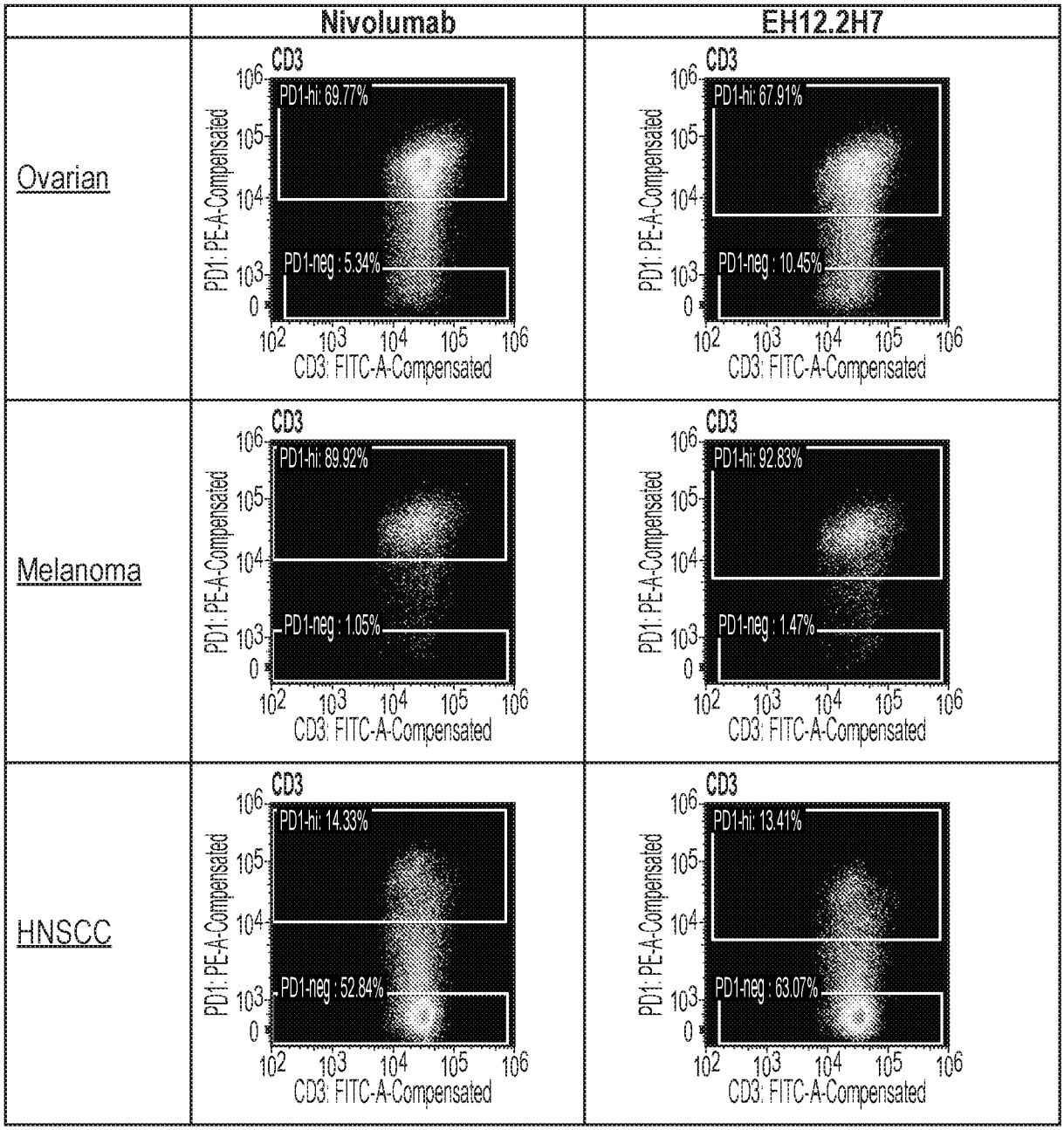

Nivolumab
EH12.2H7
Ovarian
Melanoma
HNSCC
CD3
PD1: PE-A-Compensated
CD3: FITC-A-Compensated
PD1-hi: 69.77%
PD1-neg : 5.34%
PD1-hi: 67.91%
PD1-neg : 10.45%
PD1-hi: 89.92%
PD1-neg : 1.05%
PD1-hi: 92.83%
PD1-neg : 1.47%
PD1-hi: 14.33%
PD1-neg : 52.84%
PD1-hi: 13.41%
PD1-neg : 63.07%

FIG. 172C

Tumor Histology	SSorting anti-PD-1 antibody	% PD-1 (of CD3)	PD-1 Post-sort purity	Activation Fold Expansion	REP Fold Expansion	Total viable cell yield (x 10^9)
Ovarian *	Nivolumab	79.66	98.98	2960	570	114.1
	EH12.2H7	69.91	97.89	4905	461	92.3
Melanoma *	Nivolumab	90.10	96.28	1975	434	86.8
	EH12.2H7	93.57	100.00	1427	559	84.1
HNSCC**	Nivolumab	14.61	95.34	1291	720	88.1
	EH12.2H7	14.60	96.36	1486	612	81.0

FIG. 173

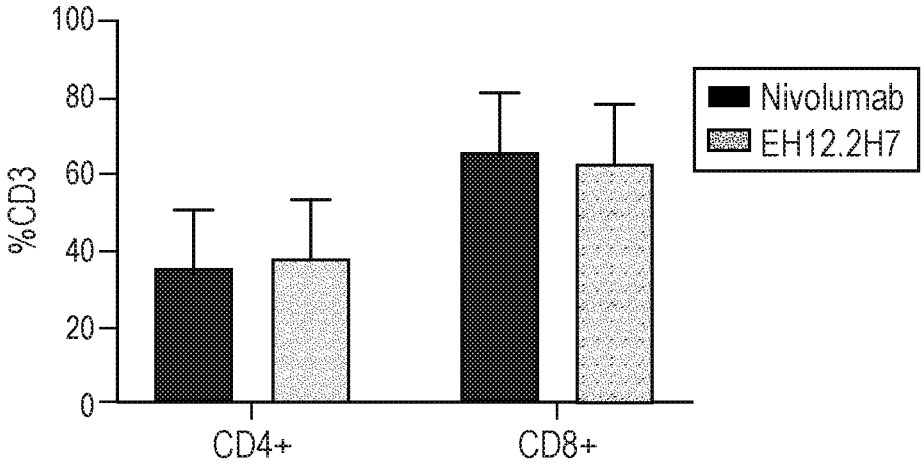


FIG. 174

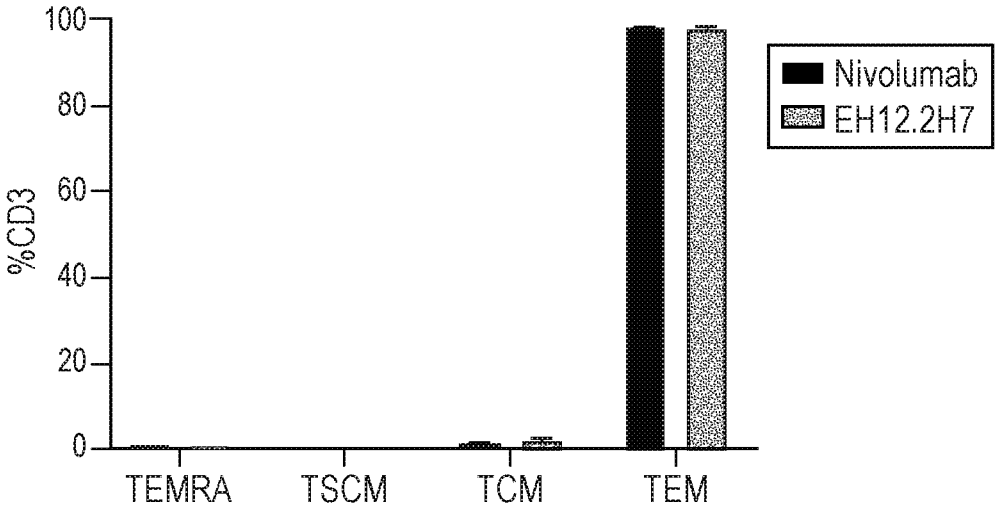


FIG. 175

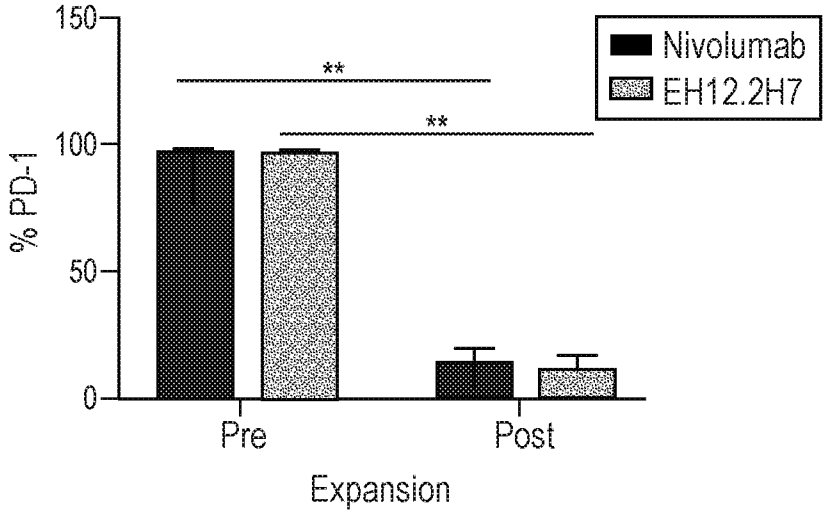


FIG. 176

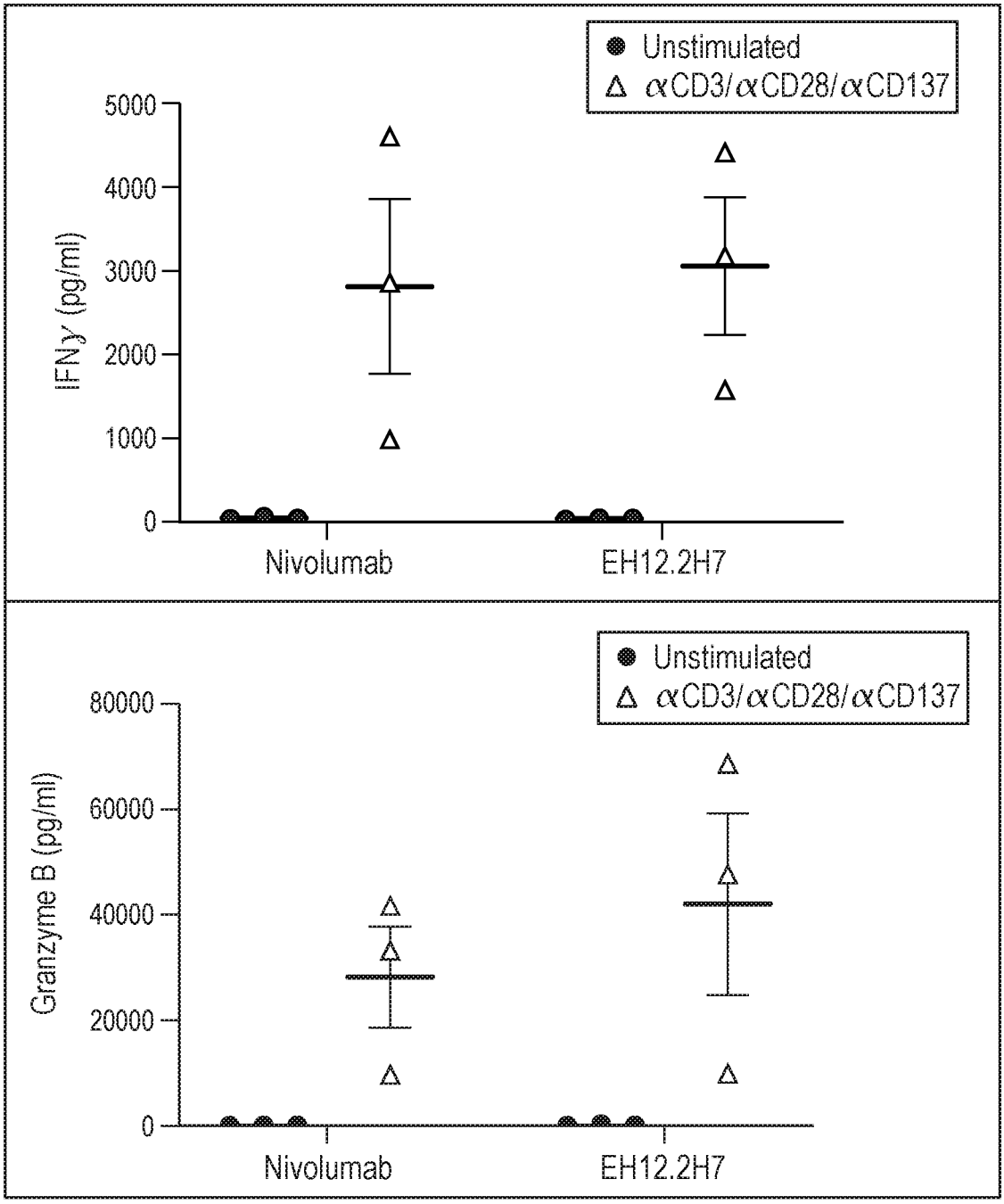

Unstimulated
αCD3/αCD28/αCD137
IFNγ (pg/ml)
5000
4000
3000
2000
1000
0
Nivolumab
EH12.2H7
Unstimulated
αCD3/αCD28/αCD137
Granzyme B (pg/ml)
80000
60000
40000
20000
0
Nivolumab
EH12.2H7

FIG. 177

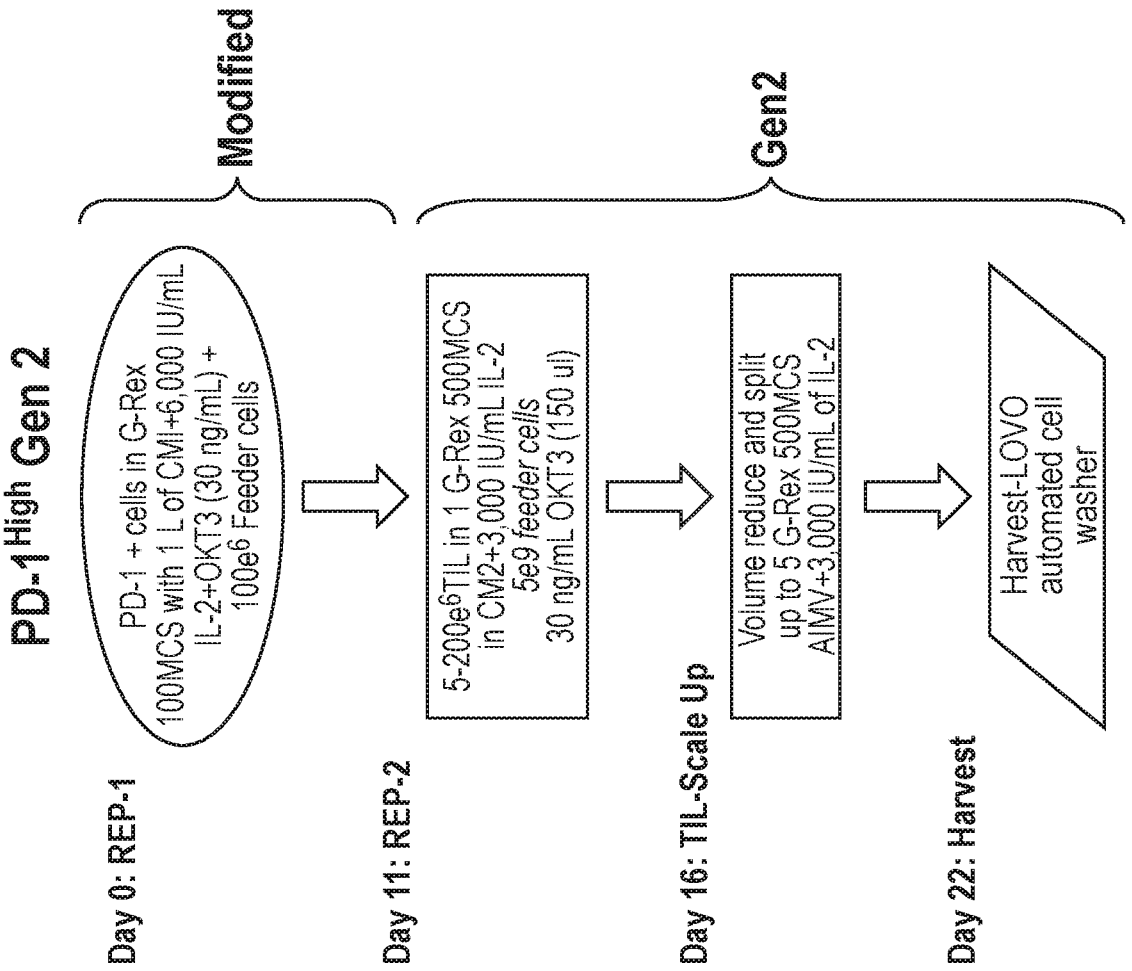

PD-1 High Gen 2
Modified
Gen2
Day 0: REP-1
PD-1 + cells in G-Rex
100MCS with 1 L of CMI+6,000 IU/mL
IL-2+OKT3 (30 ng/mL) +
100e6 Feeder cells
Day 11: REP-2
5-200e6TIL in 1 G-Rex 500MCS
in CM2+3,000 IU/mL IL-2
5e9 feeder cells
30 ng/mL OKT3 (150 ul)
Day 16: TIL-Scale Up
Volume reduce and split
up to 5 G-Rex 500MCS
AIMV+3,000 IU/mL of IL-2
Day 22: Harvest
Harvest-LOVO
automated cell
washer

FIG. 178

SUBSTITUTE SHEET (RULE 26)

Worksheet

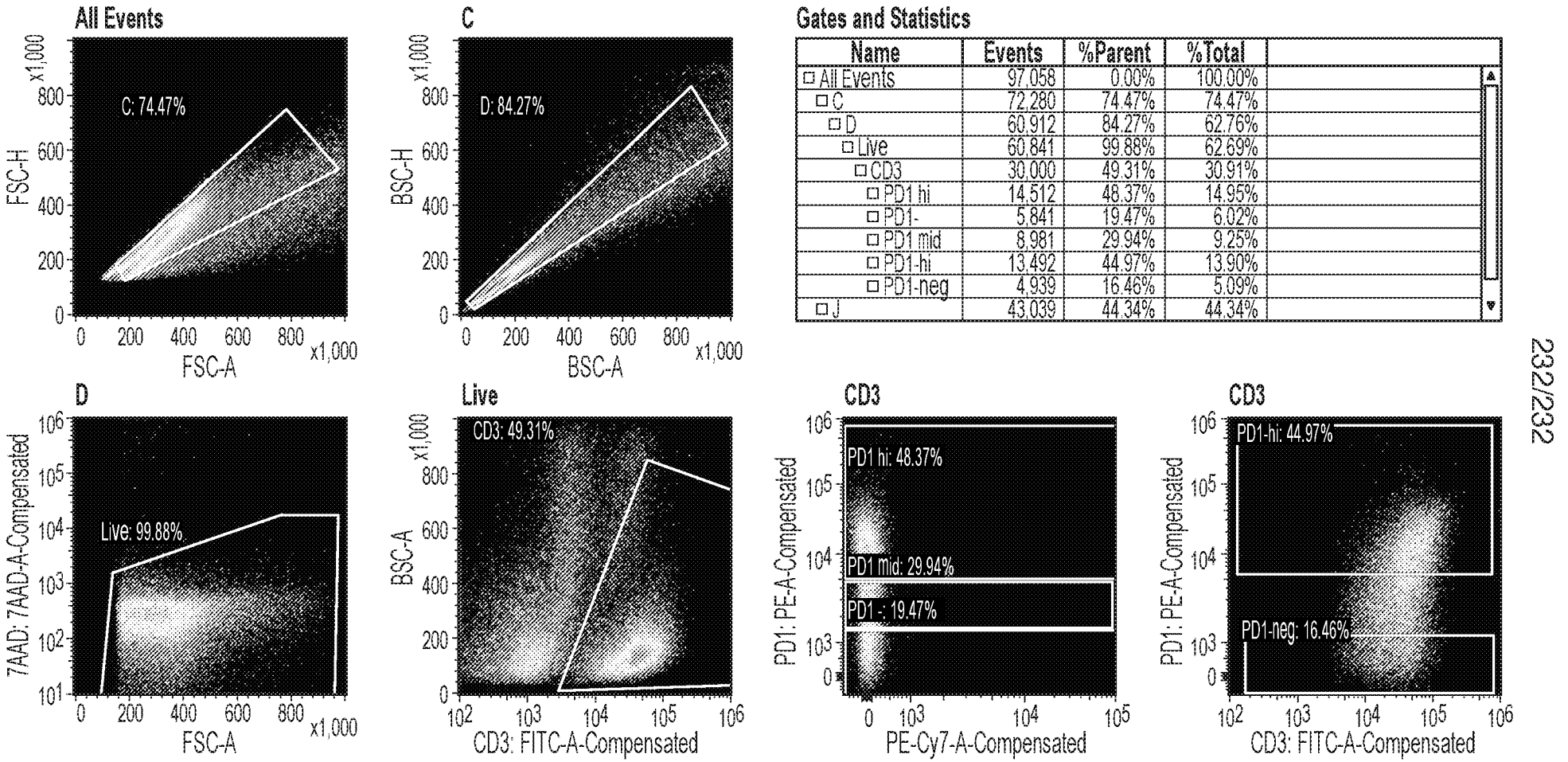


FIG. 179