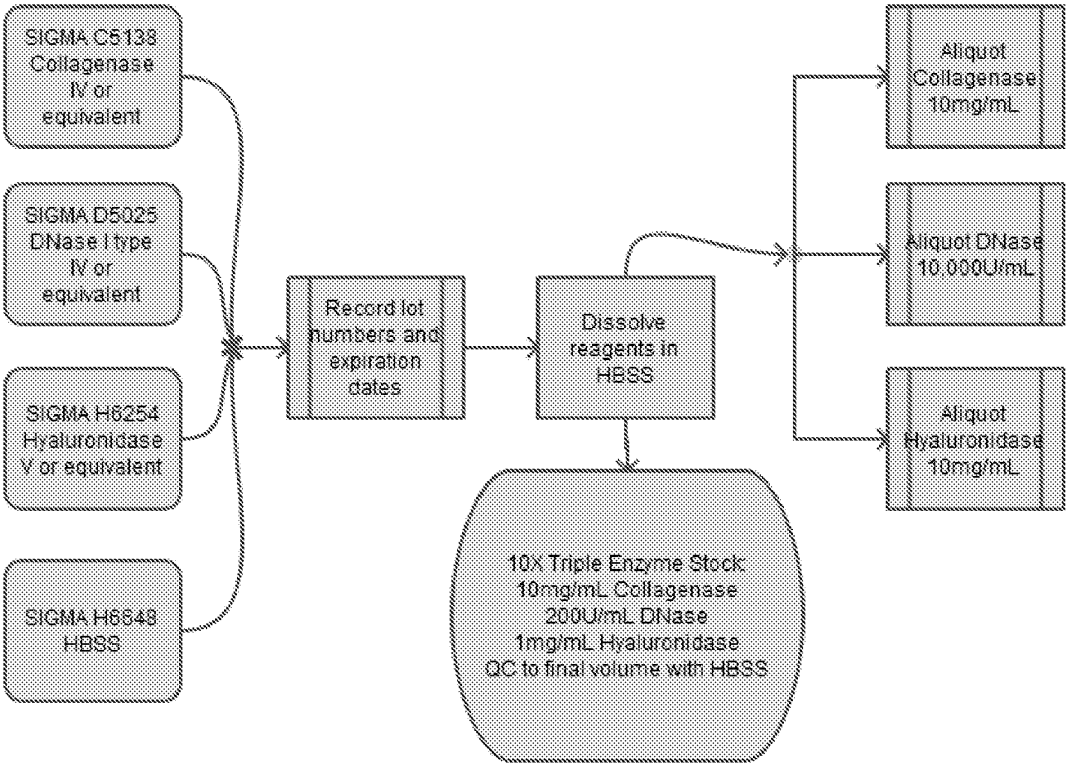

SIGMA C5138
Collagenase
IV or
equivalent
SIGMA D5025
DNase I type
IV or
equivalent
SIGMA H6254
Hyaluronidase
V or equivalent
SIGMA H6648
HBSS
Record lot
numbers and
expiration
dates
Dissolve
reagents in
HBSS
10X Triple Enzyme Stock:
10mg/mL Collagenase
200U/mL DNase
1mg/mL Hyaluronidase
QC to final volume with HBSS
Aliquot
Collagenase
10mg/mL
Aliquot DNase
10,000U/mL
Aliquot
Hyaluronidase
10mg/mL

FIG. 1

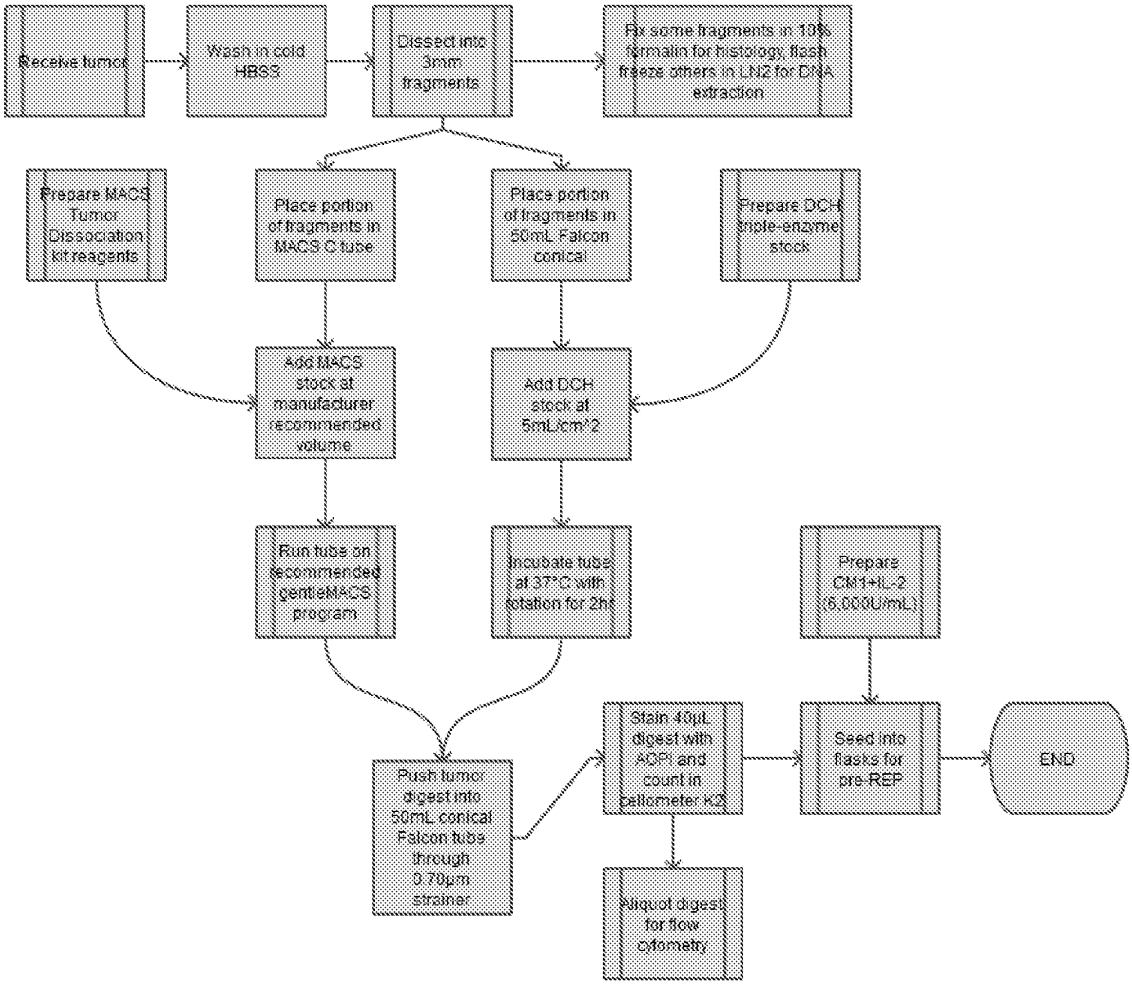
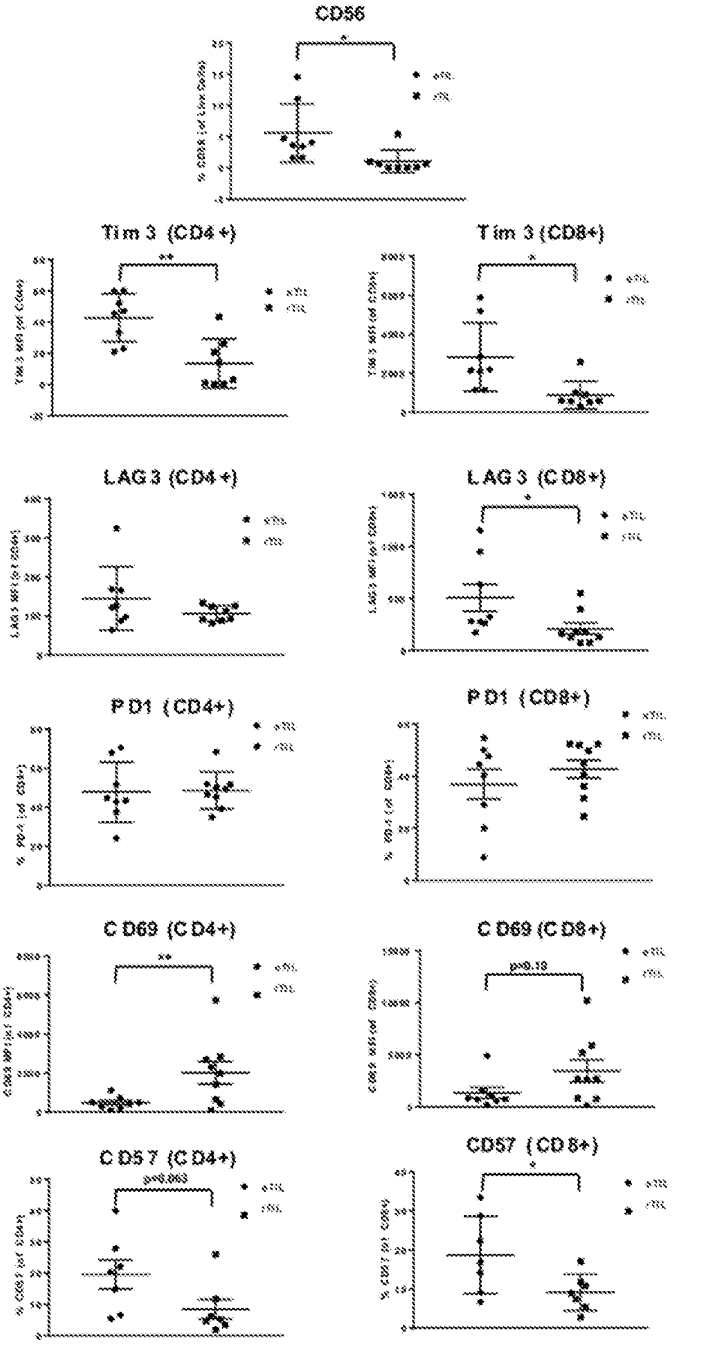
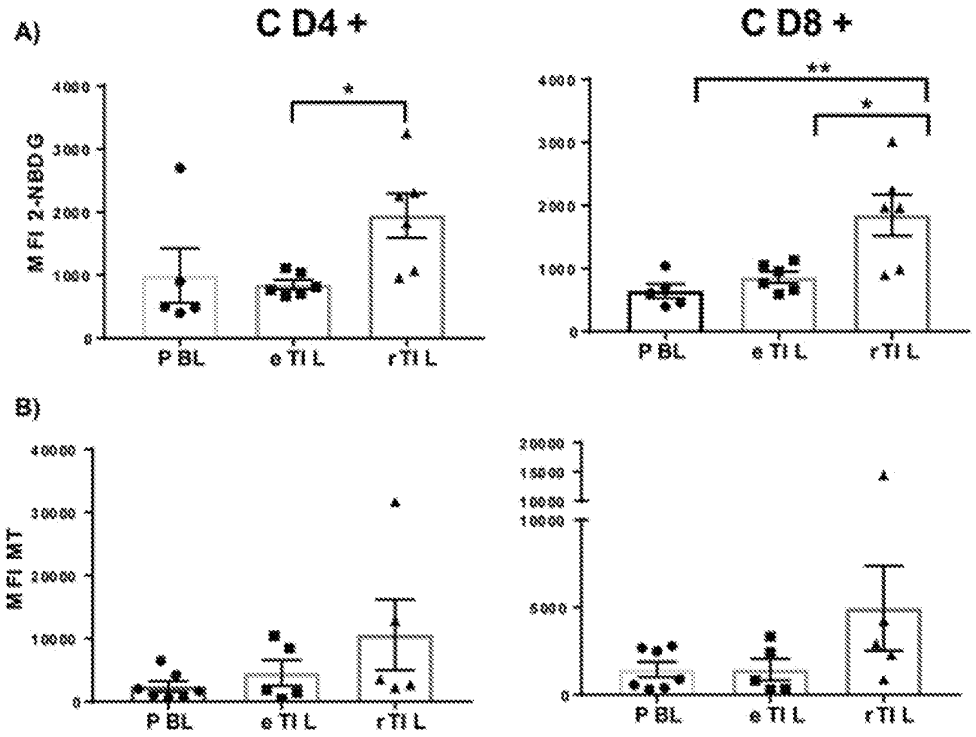

Receive tumor
Wash in cold HBSS
Dissect into 3mm fragments
Fix some fragments in 10% formalin for histology, flash freeze others in LN2 for DNA extraction
Prepare MACS Tumor Dissociation kit reagents
Place portion of fragments in MACS C tube
Place portion of fragments in 50mL Falcon conical
Prepare DCH triple-enzyme stock
Add MACS stock at manufacturer recommended volume
Add DCH stock at 5mL/cm^2
Run tube on recommended gentleMACS program
Incubate tube at 37°C with rotation for 2hr
Prepare CM1+IL-2 (6,000U/mL)
Push tumor digest into 50mL conical Falcon tube through 0.70µm strainer
Stain 40µL digest with ADPI and count in cellometer K2
Seed into flasks for pre-REP
END
Aliquot digest for flow cytometry

FIG. 2



P-values represent the difference between rTIL and eTIL using students unpaired T test; *p<0.05, **p<0.01 p<0.001. p values that are approaching significance are individually indicated above.

FIG. 3



P-values represent the difference between rTIL and eTIL using ANOVA and students unpaired T test; *p>0.05, **p>0.01

FIG. 4

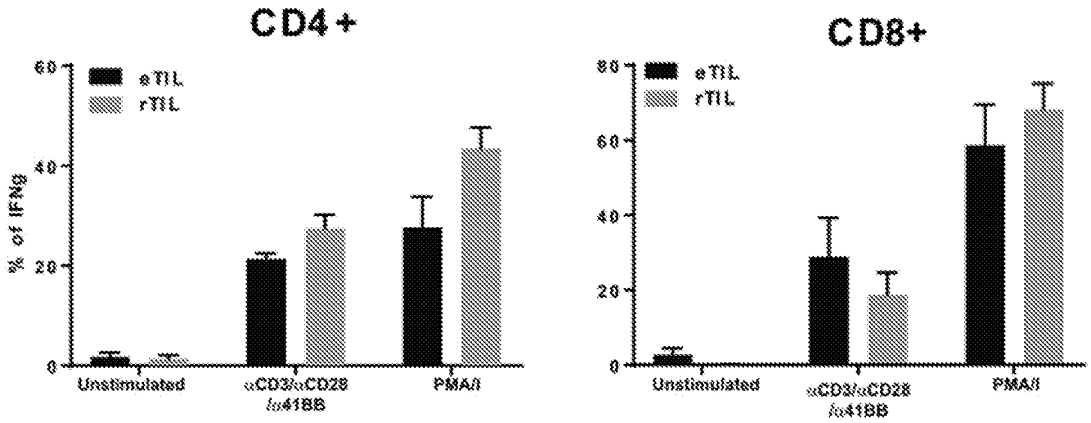

CD4+
eTIL
rTIL
% of IFNg
60
40
20
0
Unstimulated
αCD3/αCD28
/α41BB
PMA/I
CD8+
eTIL
rTIL
80
60
40
20
0
Unstimulated
αCD3/αCD28
/α41BB
PMA/I

FIG. 5

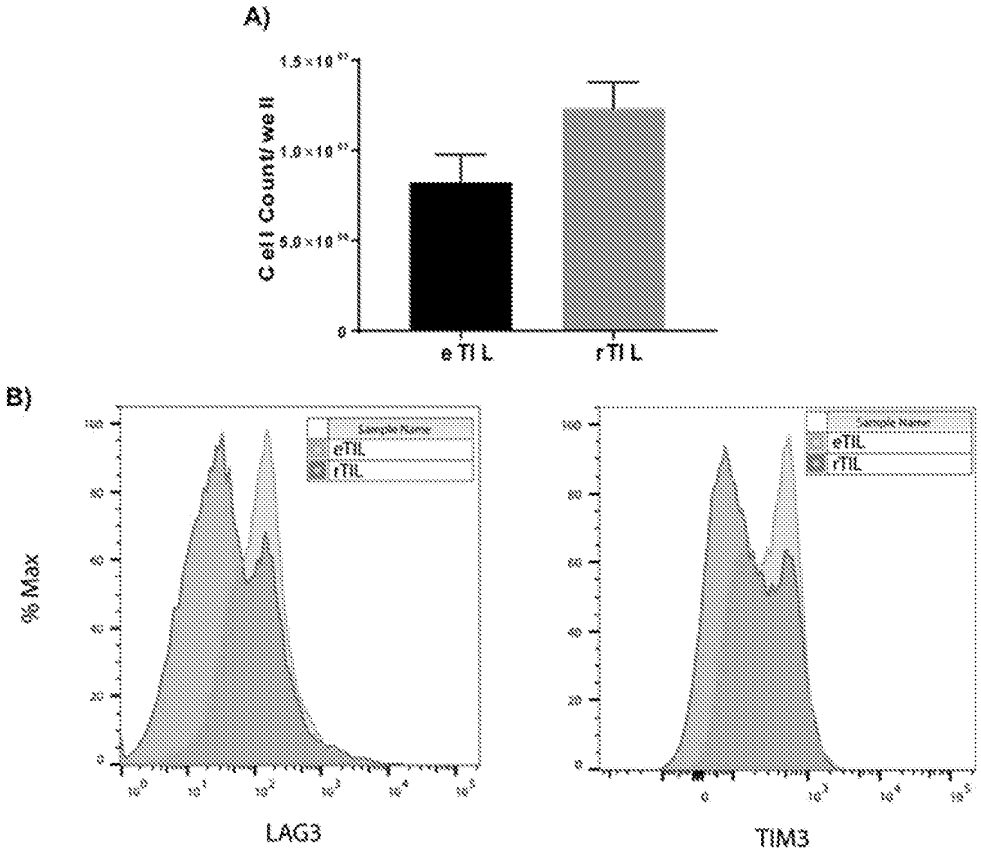

A)
Cell Count/well
eTIL
rTIL
B)
% Max
Sample Name
eTIL
rTIL
LAG3
TIM3

FIG. 6

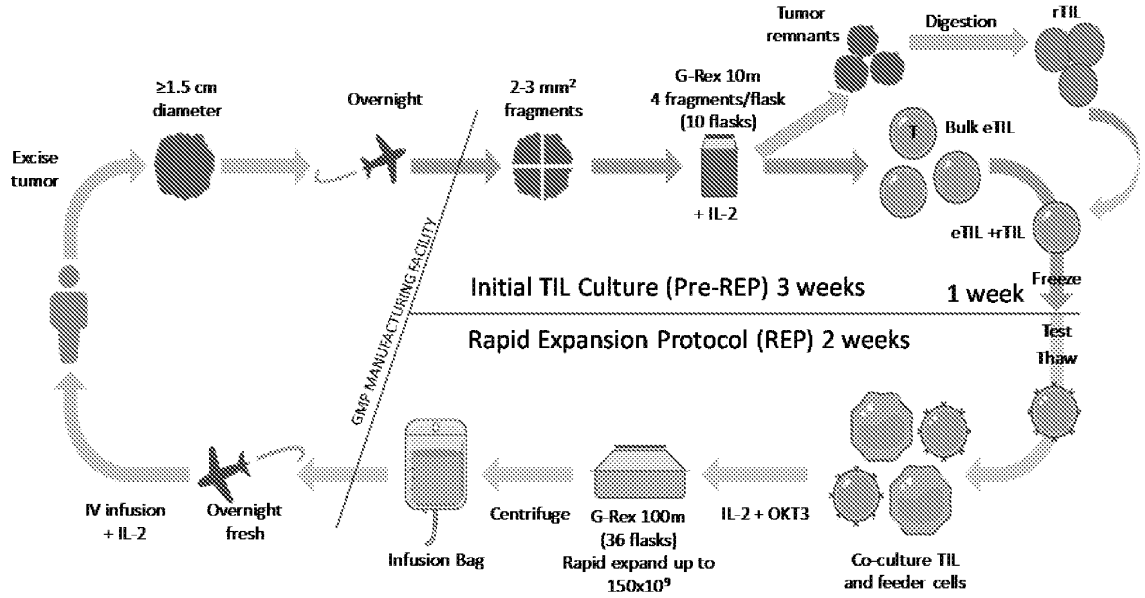

Tumor remnants
Digestion
rTIL
≥1.5 cm diameter
Overnight
2-3 mm² fragments
G-Rex 10m 4 fragments/flask (10 flasks)
Bulk eTIL
Excise tumor
+ IL-2
eTIL +rTIL
Freeze
Initial TIL Culture (Pre-REP) 3 weeks
1 week
Rapid Expansion Protocol (REP) 2 weeks
Test Thaw
GMP MANUFACTURING FACILITY
IV infusion + IL-2
Overnight fresh
Infusion Bag
Centrifuge
G-Rex 100m (36 flasks) Rapid expand up to 150x10⁹
IL-2 + OKT3
Co-culture TIL and feeder cells

FIG. 7

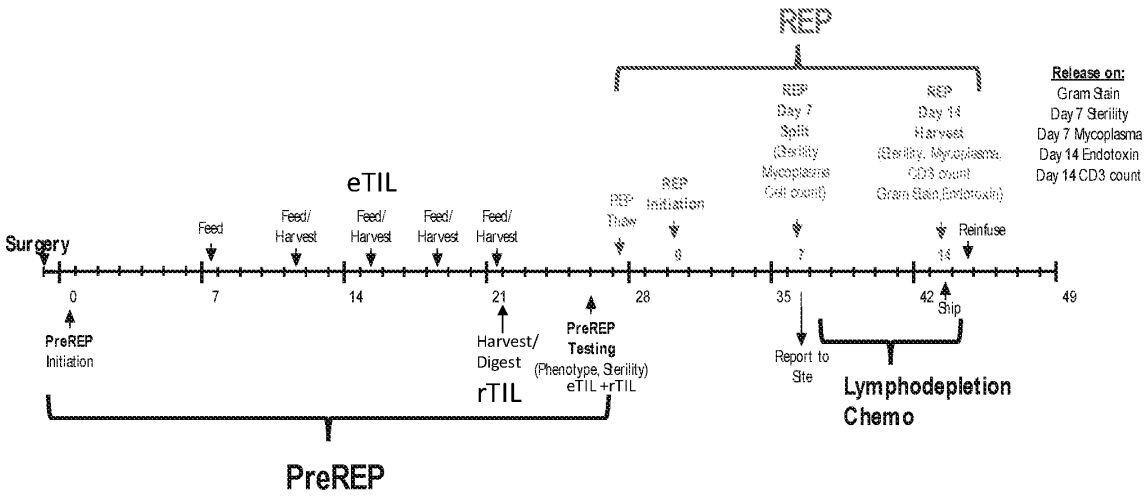

REP
Release on:
Gram Stain
Day 7 Sterility
Day 7 Mycoplasma
Day 14 Endotoxin
Day 14 CD3 count
REP Day 7 Split (Sterility, Mycoplasma, Cell count)
REP Day 14 Harvest (Sterility, Mycoplasma, CD3 count, Gram Stain,Endotoxin)
eTIL
Surgery
Feed
Feed/ Harvest
Feed/ Harvest
Feed/ Harvest
Feed/ Harvest
REP Thaw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

FIG. 8

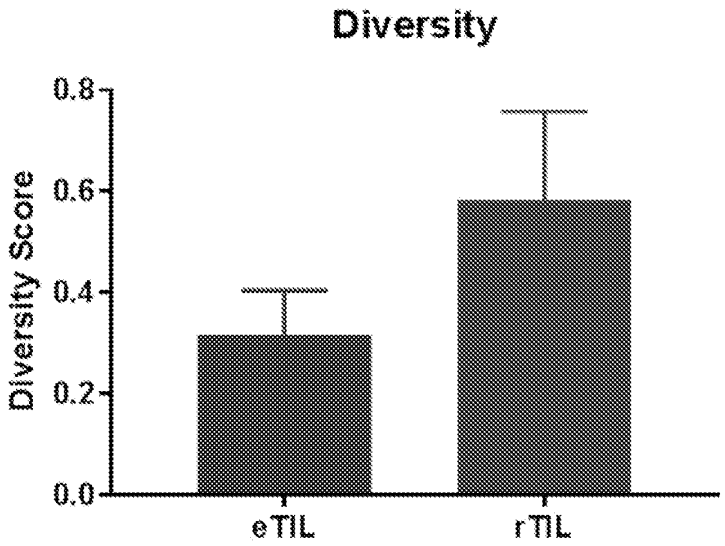


FIG. 9

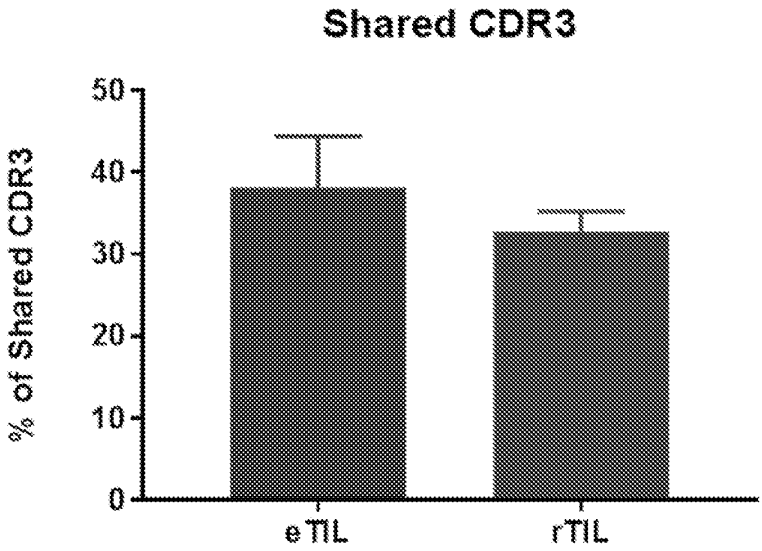


FIG. 10

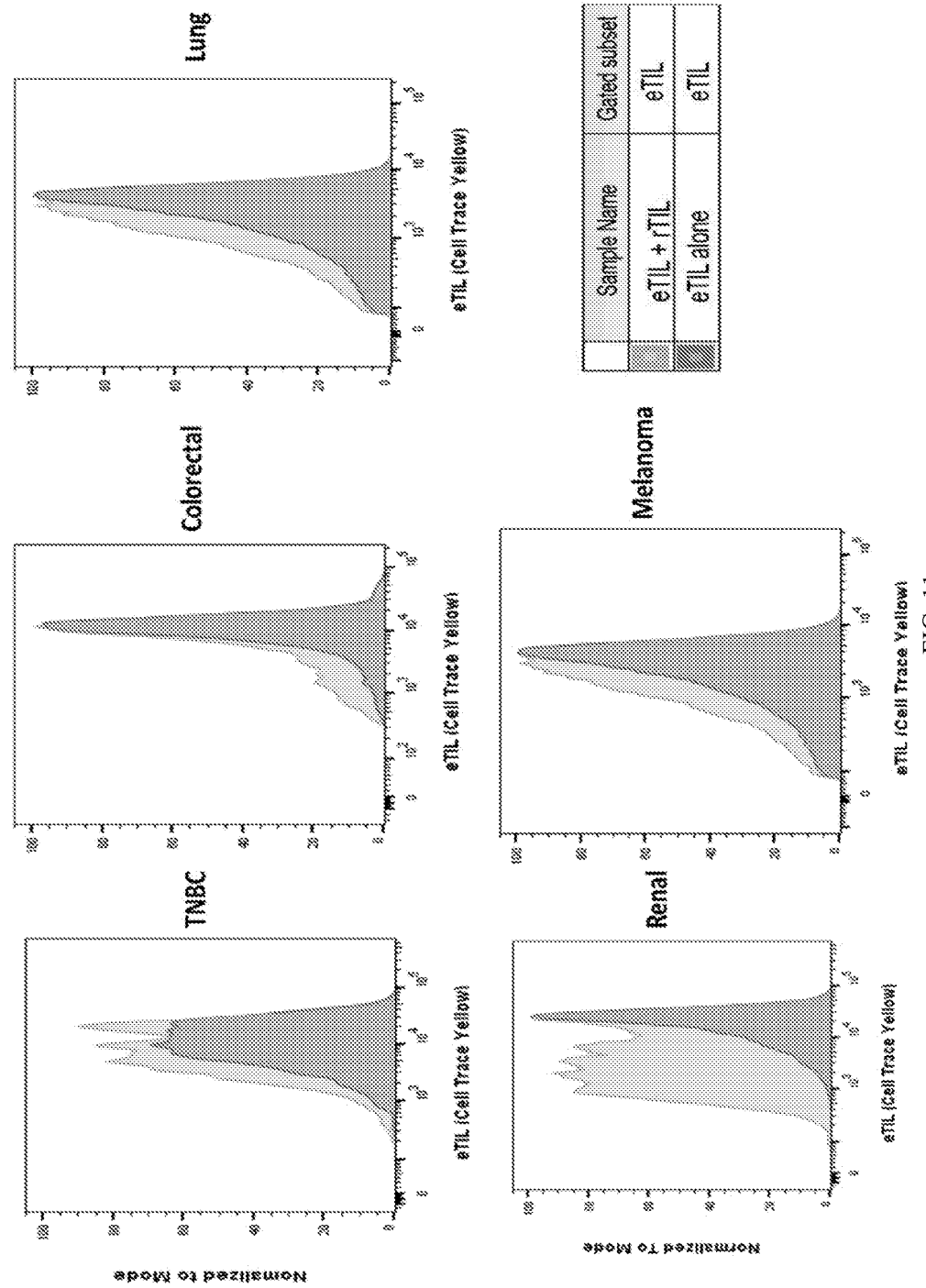

Lung
Colorectal
TNBC
Melanoma
Renal
eTIL (Cell Trace Yellow)
Normalized to Mode
Sample Name
Gated subset
eTIL + rTIL
eTIL
eTIL alone
eTIL

FIG. 11

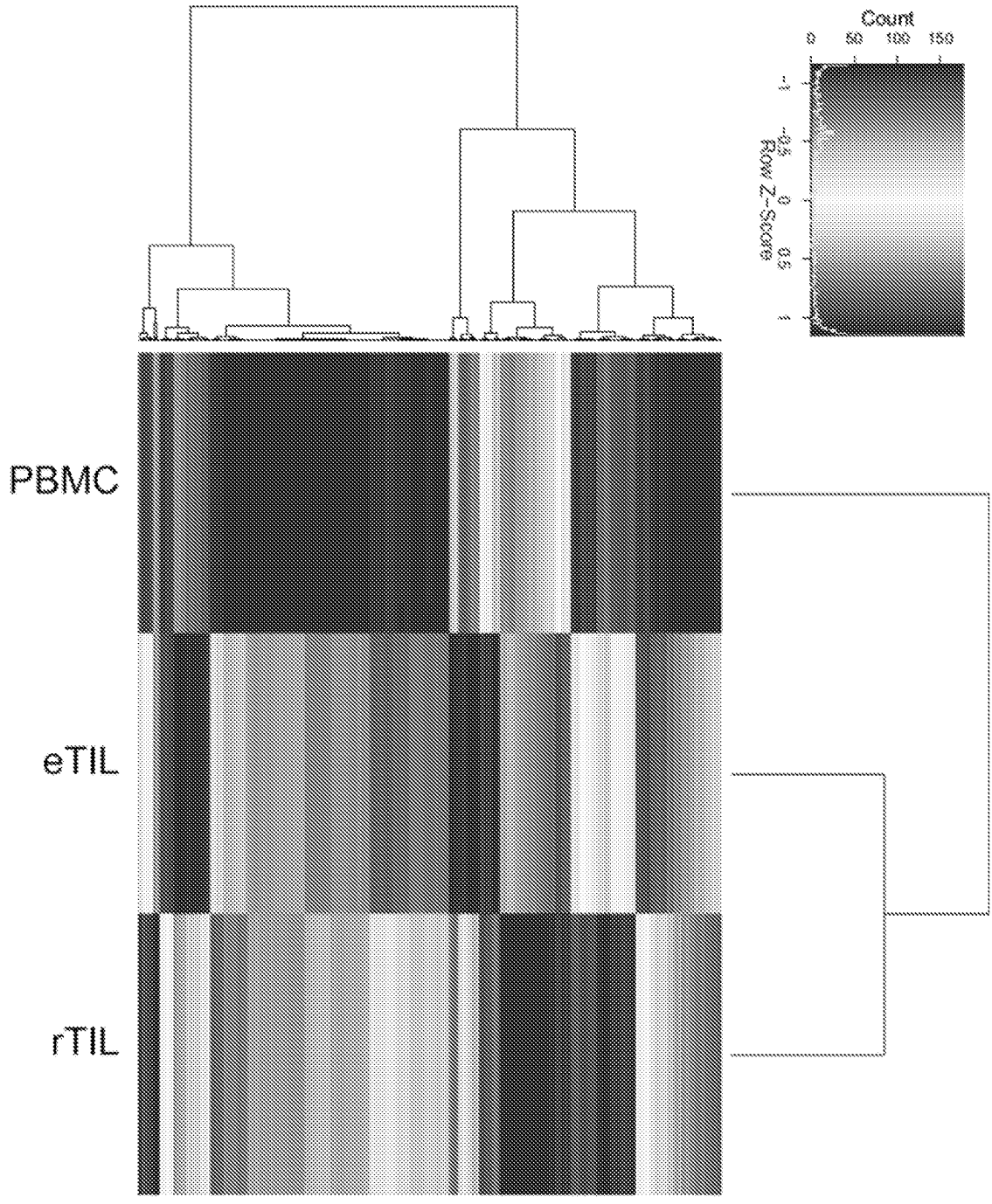


FIG. 12

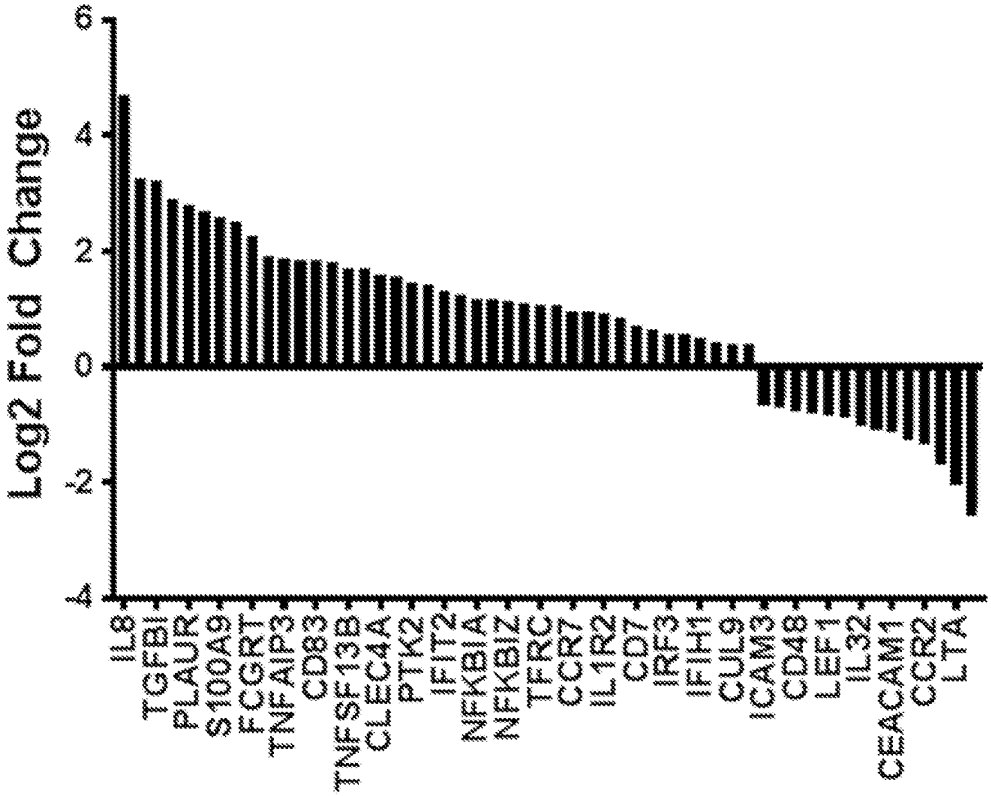

Log2 Fold Change
6
4
2
0
-2
-4
IL8
TGFBI
PLAUR
S100A9
FCGRT
TNFAIP3
CD83
TNFSF13B
CLEC4A
PTK2
IFIT2
NFKBIA
NFKBIZ
TFRC
CCR7
IL1R2
CD7
IRF3
IFIH1
CUL9
ICAM3
CD48
LEF1
IL32
CEACAM1
CCR2
LTA

FIG. 13

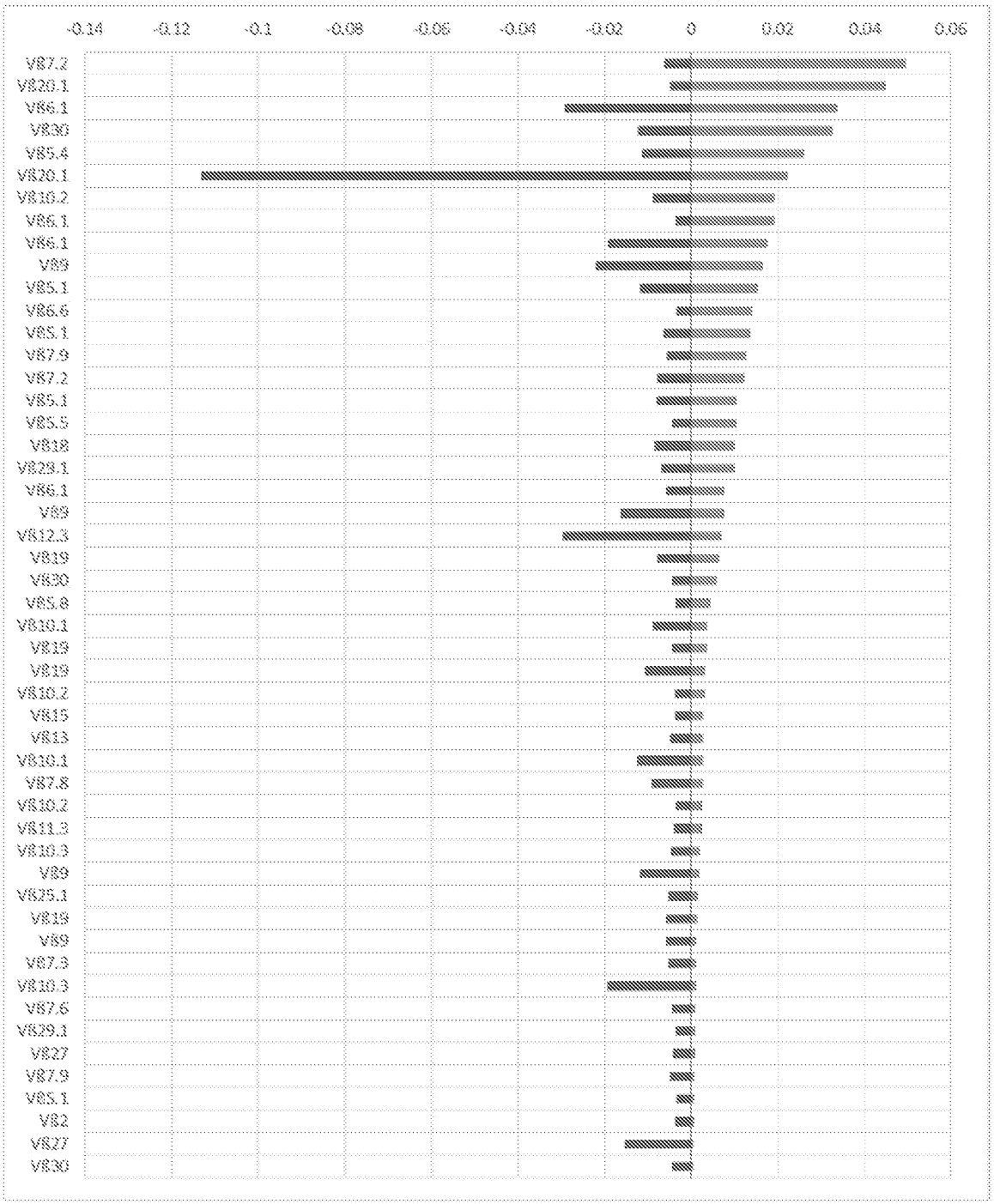

-0.14
-0.12
-0.1
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06
V87.2
V820.1
V86.1
V830
V85.4
V820.1
V810.2
V86.1
V86.1
V89
V85.1
V86.6
V85.1
V87.9
V87.2
V85.1
V85.5
V818
V828.1
V86.1
V89
V812.3
V819
V830
V85.8
V810.1
V819
V819
V810.2
V815
V813
V810.1
V87.8
V810.2
V811.3
V810.3
V89
V825.1
V819
V89
V87.3
V810.3
V87.6
V829.1
V827
V87.9
V85.1
V82
V827
V830

FIG. 14

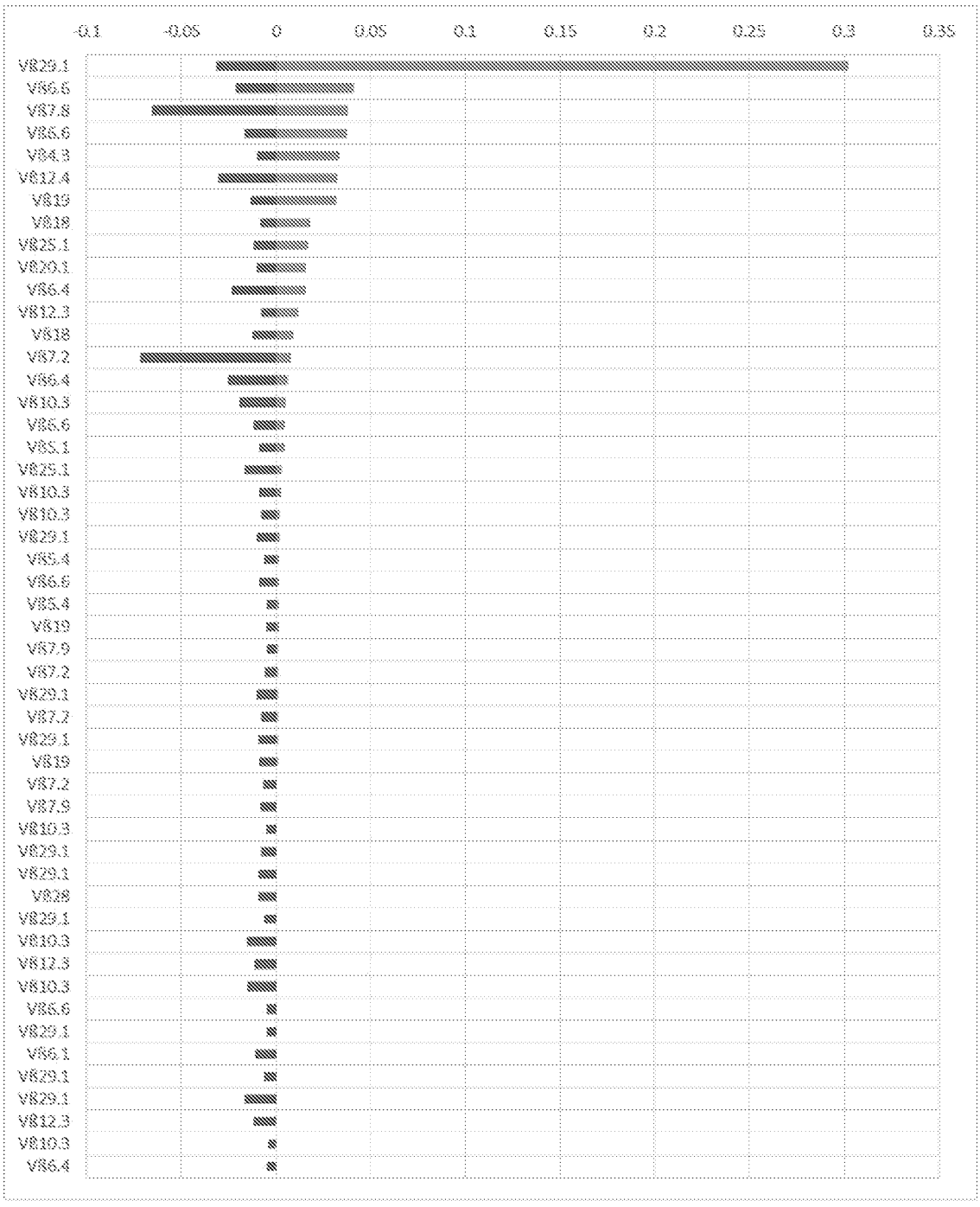

-0.1
-0.05
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
V829.1
V86.6
V87.8
V86.6
V84.3
V812.4
V819
V818
V825.1
V820.1
V86.4
V812.3
V818
V87.2
V86.4
V810.3
V86.6
V85.1
V825.1
V810.3
V810.3
V829.1
V85.4
V86.6
V85.4
V819
V87.9
V87.2
V829.1
V87.2
V829.1
V819
V87.2
V87.9
V810.3
V829.1
V829.1
V828
V829.1
V810.3
V812.3
V810.3
V86.6
V829.1
V86.1
V829.1
V829.1
V812.3
V810.3
V86.4

FIG. 15

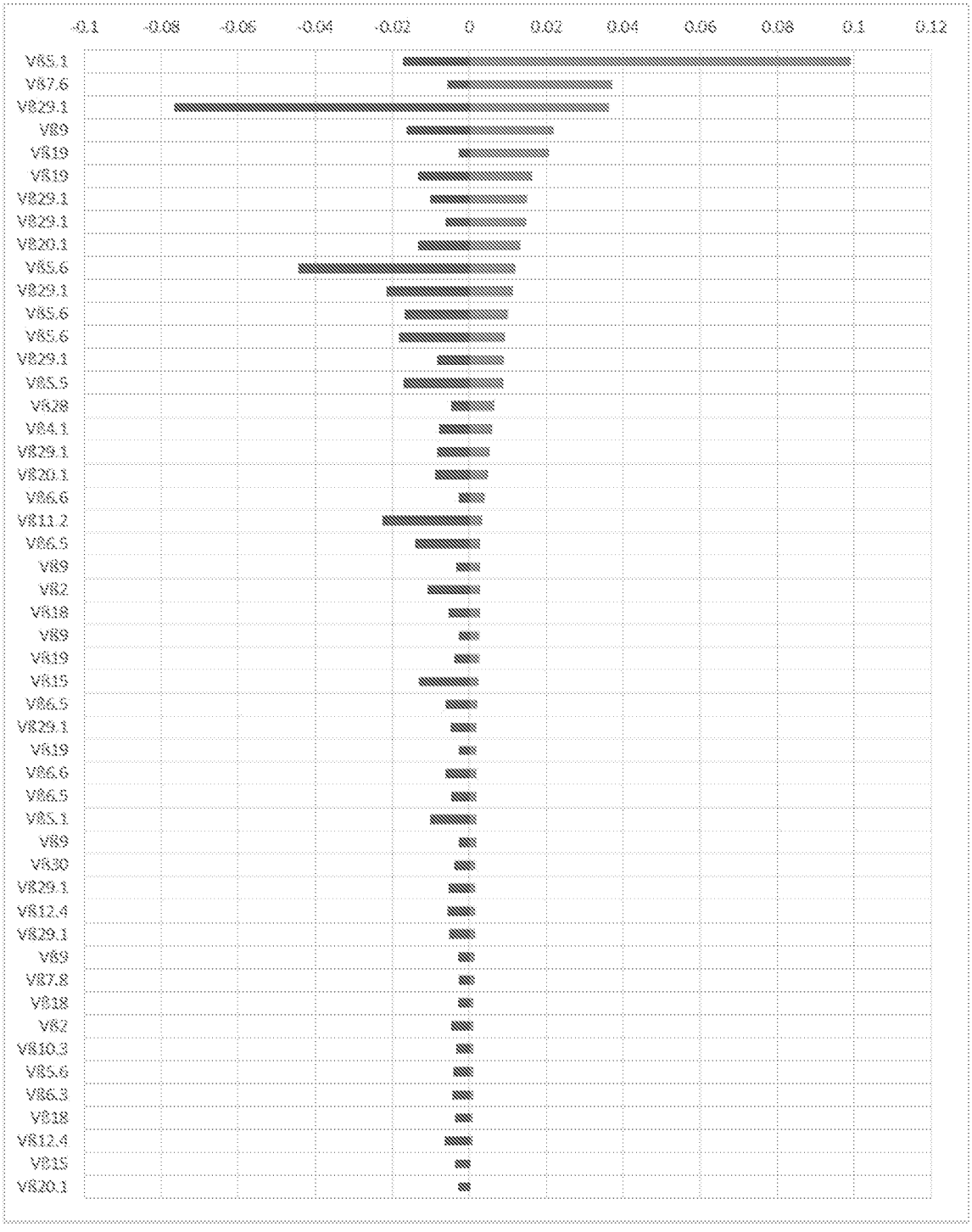

-0.1
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06
0.08
0.1
0.12
V85.1
V87.6
V829.1
V89
V819
V819
V829.1
V829.1
V820.1
V85.6
V829.1
V85.6
V85.6
V829.1
V85.5
V828
V84.1
V829.1
V820.1
V86.6
V811.2
V86.5
V89
V82
V818
V89
V819
V815
V86.5
V829.1
V819
V86.6
V86.5
V85.1
V89
V830
V829.1
V812.4
V829.1
V89
V87.8
V818
V82
V810.3
V85.6
V86.3
V818
V812.4
V815
V820.1

FIG. 16